Université de Montréal

Synthèse et utilisation de benzotriazepinones comme modulateur du système urotensinergique

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Résumé

Les peptides sont des agents thérapeutiques potentiellement intéressants dû à leur faible toxicité et excellente activité. Cependant, ils ont le désavantage de se métaboliser rapidement, d'avoir une pauvre biodisponibilité et la possibilité d'interagir avec plusieurs sous-types de récepteurs, engendrant des effets secondaires. Afin de contrer ces problèmes, tout en gardant leurs attractivités, des peptides modifiés sont et ont été développés, ils sont appelés peptidomimétiques.

Une classe de peptidomimétique consiste au remplacement complet de la structure peptidique par une petite molécule, qui va mimer la conformation et les fonctions des chaines latérales importantes pour l'activité. De plus, certaines structures comme les benzodiazépines ont été décrites comme des structures privilégiées, du faites de leurs affinités pour de nombreux récepteurs peptidiques. Leurs aza-analogues, les benzotriazépines, ont cependant reçût beaucoup moins d'attention. Leurs synthèses mettent en jeux l'utilisation de réactifs toxiques, des conditions de réactions dures, la nécessité d'avoir des groupements protecteurs ou encore des rendements faibles pour certains substrats.

Le récepteur Urotensine II (UT) est un membre de la classe de la famille des récepteurs couplés aux protéines G, qui interagit à deux ligands peptidiques cycliques endogènes : l'Urotensine II (hUII, H-Glu-Thr-Pro-Asp-c[Cys-Phe-Trp- Lys- Tyr-Cys]-Val-OH) et le peptide apparenté à l'Urotensine II (URP, H-Ala-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH). Aussi appelé système urotensinergique, il joue un rôle déterminant notamment dans la pathogénèse et la progression des maladies cardiovasculaires. Le développement de ligands pouvant différencier les rôles de UII et de URP, afin de sonder leurs voies de signalisations respectives, est primordial pour mieux comprendre cette potentielle cible thérapeutique.

Les travaux présentés dans cette thèse visent deux objectifs principaux, le développement d'une nouvelle méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones, orientée vers une diversification effective, et leurs utilisations comme mime peptidique pour la modulation du système urotensinergique. Pour se faire, une

approche mettant en jeux des alkylations chimiosélectives successives d'une semicarbazone, suivis d'une étape de déprotection/cyclisation, a été développée pour produire une variété de benzotriazepin-2-ones. De plus, il a aussi été mis en évidence la possibilité d'utiliser le cœur achiral des benzotriazépinones comme mime peptidique de conformation tour γ.

Le squelette benzotriazépinique a été employé pour créer des modulateurs allostériques de UT, en mimant la conformation tour γ de la séquence tripeptidique , Bip-Lys-Tyr présent dans l'Urocontrin ([Bip^4]URP) qui est un modulateur allostérique du récepteur urotensinergique. Une série de 27 composés a été synthétisée par notre nouvelle méthodologie développée, afin de mieux comprendre la relation structure/activité dans la modulation de l'UT. Certains composés ont révélé la capacité à moduler sélectivement les effets de hUII et URP sur la contraction de l'aorte de rat de manière *ex-vivo*.

Enfin, dans la perspective d'améliorer l'activité antagoniste, une fonction *N*-benzylamide a été installée en position 8 de la benzotriazepine, pour mimer la phénylalanine présente dans l'Urocontrin. De plus, des analogues photo-réactifs sont poursuivis, par introduction d'un groupement benzophénone, dans le but de se lier de manière covalente au récepteur urotensinergique et de localiser la partie responsable de la modulation de l'activité de hUII et URP.

Pour conclure, l'ensemble de ces travaux représentent une avancée sur l'utilisation des benzotriazépinones en générale et comme mime peptidique possédant notamment une conformation tour γ. Le développement d'une méthodologie orientée sur une diversification efficace a permis le développement de modulateurs achiraux du récepteur UT. Ainsi, ces études ont contribué à l'avancement des connaissances, fondamentales et appliquées, dans les domaines des mimes peptidiques et de la chimie médicinale.

Mots clés : benzotriazépinone, Alkylation chimiosélective, mime peptidique, modulateur UT, système urotensinergique.

Abstract

Peptides are potentially interesting therapeutic agents, due to their low toxicity and excellent activity. They have however disadvantages, such as rapid metabolism, poor bioavailability and potential to react indiscriminately with multiple receptors, which may lead to secondary effects. To address such issues, modified peptide analogs, so-called peptidomimetics, have been developed.

One type of peptidomimetic consists of the complete replacement of the peptide structure by a small molecule that mimics the important backbone and side chain conformation and functions for activity. In addition, certain molecules, such as benzodiazepines have been described as privileged structures, because of their affinity for many peptide receptors. The aza-analogues, benzotriazepines, have received a lot less attention than the parent benzodiazepines, in part due to difficulties in their synthesis, necessitating toxic reagents, harsh reaction conditions, and multiple protecting groups to obtain compound in low yield.

The Urotensin II (UT) receptor is a member of the G protein-coupled receptor family, and interacts with two endogenous cyclic peptide ligands: urotensin II (hUII, H-Glu-Thr-Pro-Asp-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and the urotensin II-related peptide (URP, H-Ala-c[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH). The so-called urotensinergic system has among various physiological roles, significance in the pathogenesis and the progression of cardiovascular diseases. The conception of ligands which can differentiate the signaling and phenotypic outcomes of UII and URP are necessary to more effectively target UT for therapeutic development.

This thesis focuses on two principle objectives: the development of new methods for the synthesis of diverse 1,3,4-benzotriazepin-2-ones, and the application of the latter as peptidomimetics that modulate selectively the endogenous ligands of the urotensinergic system. An approach featuring chemo-selective alkylation of a semicarbazone intermediate has been developed to produce diverse benzotriazepin-2-ones. In addition, the achiral benzotriazepinones were shown to have potential to serve as peptide γ-turn mimics.

The benzotriazepinone scaffold was employed to create achiral allosteric UT modulators by mimicry of the purported γ–turn confirmation of the tripeptide sequence, Bip-Lys-Tyr, in the biologically active UT regulator Urocontrin ([Bip^4]URP). A series of 27 compounds was synthesized by our method to study structure-activity relationships for UT modulation. Certain benzotriazepinones exhibited capacity to selectively modulate the effects of hUII and URP in *ex vivo* studies of rat aorta contraction.

To improve antagonist activity, a *N*-benzylamide was installed at the benzotriazepine 8-position to mimic the phenylalanine residue in Urocontrin. Moreover, photo-reactive analogs have been pursued by introduction of a benzophenone residue towards the goal of binding UT covalently to identify the location responsible for modulation of hUII and URP activity.

In conclusion, advances have been made in the synthesis and application of benzotriazepinones as peptide γ-turn mimics. Methods to diversify effectively the heterocycle have empowered development of achiral modulators of the UT receptor. Fundamental and applied advancements of knowledge have thus been made in the domains of peptide mimicry and medicinal chemistry.

Key Words : benzotriazepinone, chemoselective alkylation, peptide mimicry, UT modulator, urotensinergic system.

Notes

Cette thèse étant rédigée par article, il est important de noter ma contribution sur sa rédaction ainsi que sur les articles présentés.

Le Chapitre 1, comportant l'introduction de cette thèse, a été entièrement rédigé par moi-même, sous la supervision du Pr William D. Lubell et du Pr David Chatenet.

Le Chapitre 2 et l'Article 1 ont été entièrement rédigés par moi-même, sous la supervision du Pr William D. Lubell. Les résultats présentés ont été obtenu lors de mon travail au laboratoire, sous la supervision du Pr William D. Lubell.

Le Chapitre 3 et l'Article 2 ont été entièrement rédigés par moi-même, sous la supervision du Pr William D. Lubell et du Pr David Chatenet, à l'exception de la partie sur l'activation des protéines G_q et G_{12}, rédigée par le doctorant Étienne Billard et le Pr David Chatenet. La librairie des 26 composés benzotriazépiniques a été synthétisée, purifiée et caractérisée lors de mon travail au laboratoire, sous la supervision du Pr William D. Lubell. Les résultats et le traitement des données des tests de contractions aortiques *ex-vivo* ont été obtenus lors de mon travail au laboratoire, sous la supervision du Pr David Chatenet, à partir d'une aorte de rat prélevé par le doctorant Étienne Billard. Les tests d'activation des protéines G_q et G_{12} ont été effectué par le doctorant Étienne Billard.

Le Chapitre 4 et l'Article 3 ont été entièrement rédigés par moi-même, sous la supervision du Pr William D. Lubell. Les résultats présentés ont été obtenu lors de mon travail au laboratoire, sous la supervision du Pr William D. Lubell.

Le Chapitre 5 comportant la conclusion de cette thèse, a été entièrement rédigé par moi-même, sous la supervision du Pr William D. Lubell.

Table des matières

Liste des tableaux

Liste des figures

Liste des schémas

Liste des abréviations

Ac	Acétyle
Ala	Alanine
Ar	Aryle
Asp	Acide Aspartique
Bn	Benzyle
Boc	*tert*-Butyloxycarbonyle
Bip	4,4′-biphenylalanine
br	Broad (en RMN)
BRET	*Bioluminescence resonance energy transfer*
Btz	Benzotrhiazolalanine
Bpa	4-benzoylphenylalanine
°C	Degré Celsius
CCK	Cholecystokinin
Cys	Cystéine
δ	Déplacement chimique en ppm (en RMN)
d	Doublet (en RMN)
DCM	Dichlorométhane
dd	Doublet de doublet (en RMN)
DMEM	*Dulbecco Modified Eagle Medium*
DIEA	N,N-Diisopropyléthylamine
DMF	N,N-Diméthylformamide
DMSO	Diméthylsulfoxyde
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
Et	Éthyle
éq	Équivalent
g	Gramme(s)
GFP	*Green fluorescent protein*
Glu	Acide Glutamique

Gly	Glycine
GPCR	G protein-coupled receptor
h	Heure(s)
HEK	*Human embryonic kidney*
HEPES	*(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)*
His	Histidine
HPLC	*High performance liquid chromatography*
HRMS	*High resolution mass spectrometry*
hUII	*Human urotensin II*
$hHPTH_1$	*Parathyroid hormone-1 receptor*
iPr	iso-Propyle
IR	Infrarouge
J	Constante de couplage (en RMN)
L	Litre
Leu	Leucine
Lys	Lysine
M	Mole(s) par litre
m	Multiplet (en RMN)
Me	Méthyle
Met	Méthionine
MHz	Mégahertz (en RMN)
mp	*Melting point*
Nal	Naphthylalanine
Nap	Naphthylmethyl
NMR	*Nuclear magnetic resonance*
Orn	Ornithine
Pen	Penicillamine
Pep	(phenylethynyl)phenylalanine
Ph	Phényle
Phe	Phénylalanine
ppm	Partie par million (en RMN)

Pro	Proline
Pyr	Pyridine
q	Quadruplet (en RMN)
RCPG	Récepteur couplé aux protéines G
R_f	Facteur de rétention (en chromatographie)
RMN	Résonance magnétique nucléaire
rt	Room temperature
s	Singulet (en RMN)
t	Triplet (en RMN)
*t*Bu	*tert*-Butyle
THF	Tétrahydrofurane
Thr	Thréonine
TLC	*Thin layer chromatography*
TRH	*Thyrotropin-releasing hormone*
Trp	Tryptophane
Tyr	Tyrosine
UII	*Urotensin II*
UT	Récepteur uritensinergique, *Urotensin II receptor*
URP	*Urotensin II-related peptide*
Val	Valine

À notre petite à venir

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Je tiens tout d'abord à remercier mon directeur de recherche, le Professeur William D. Lubell, pour m'avoir acceuillis dans son groupe de recherche, ainsi que pour son soutien et ses idées tout au long de ces années qui m'ont été et me seront d'une aide précieuse pour mon projet et mon avenir professionnel.

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Chapitre 1 : Introduction

1.1. Peptides et Peptidomimétiques

1.1.1. Peptides

Les peptides sont des bio-polymères composés d'une succession d'acides aminés, reliés entre eux par une liaison amide, qui forme leurs structures primaires. Les chaines latérales portées par les acides aminés confèrent aux peptides des propriétés spécifiques, que ce soit ponctuellement au niveau des résidus en eux même, ou globalement au niveau d'une conformation particulière dans l'espace. En effet, les fonctions latérales peuvent varier sur de nombreux critères comme par exemple l'acidité, la basicité, l'hydrophilicité, la lipophilicité ou encore la capacité à pouvoir effectuer des liaisons faibles. Ces spécificités entrainent le peptide à adopter une structure tridimensionnelle spécifique, appelée structure secondaire, correspondant à un niveau d'énergie moindre et pouvant se matérialiser sous formes de trois principaux types de conformations : en hélice, en feuillet ou coudée.

Les peptides sont omniprésents dans les mécanismes biologiques, avec par exemple, l'angiotensine impliquée dans la régulation de la tension artérielle, l'insuline dans la régulation des substrats énergétiques, notamment le glucose, la somatostatine inhibitrice de l'hormone de croissance ou encore l'ocytocine agissant principalement sur les muscles lisses de l'utérus et des glandes mammaires. Lors de dérèglements biologiques, certains peptides naturels comme l'insuline ou l'ocytocine, par exemple, sont respectivement administrées aux patients pour le diabète de type II et lors d'insuffisance des contractions utérines.

Les peptides constituent une classe d'agents thérapeutiques particulièrement attrayante, possédant comme avantages d'avoir une faible toxicité et une excellente activité. Le marché du médicament peptidique est en pleine expansion depuis ces dernières années. Cependant l'utilisation de peptides comme agents thérapeutiques doit faire face à plusieurs contraintes majeures. En effet, les peptides se métabolisent rapidement, ont une pauvre biodisponibilité requérant une administration par intraveineuse et ont la possibilité d'interagir avec plusieurs sous-types de récepteurs et engendrer des effets secondaires. Afin de répondre à ces contraintes et conserver

leurs avantages, dans le but d'optimiser les peptides en tant que médicament, des peptidomimétiques sont et ont été développés.

1.1.2. Peptidomimétiques

Les peptidomimétiques sont des structures chimiques dont le but est de mimer les éléments qui contribuent à l'activité de peptides spécifiques et d'améliorer certaines de leurs propriétés. Le développement de peptidomimétiques biologiquement actif est animé par l'idée de pouvoir concevoir des agents thérapeutiques possédant les avantages inhérents aux structures peptidiques (forte activité et faible toxicité) tout en minimisant, en supprimant, leurs désavantages (rapide métabolisme, pauvre sélectivité et biodisponibilité).

Les mimes peptidiques consistent à mimer les fonctions et/ou la forme des peptides. Une des solutions mise en place, la plus importante, consiste au remplacement d'un ou plusieurs acides aminés dans la chaine peptidique, et peut-être classé en fonction de deux types, le remplacement atome par atome (ex : Aza-peptide)[1] ou par des hétérocycles (ex : aminolactame).[2]

Une autre solution, moins développée, mais qui comprend des exemples intéressants, s'appuie sur le remplacement complet de la structure peptidique par une petite molécule, qui va mimer dans l'espace les chaines latérales importantes pour l'activité. Par exemple, le bicycle **1.2** mime le neurotransmetteur enképhaline (H-Tyr-Gly-Gly-Phe-Met-OH, **1.1**),[3] le dérivé du glucose **1.4** a été élaboré pour imiter la somatostatine,[4] et finalement le cyclohexane **1.6** a été conçu pour mimer l'hormone thyréotrope (TRH) **5** (Figure 1.1).[5] De plus, il existe des "structures privilégiées", décrites pour la première fois par Dr. Ben Evans et les laboratoires Merck, avec l'exemple de la benzodiazepines **1.7**,[6] qui possèdent de fortes affinités pour de nombreux récepteurs peptidiques, probablement dûes à leurs capacités à imiter les conformations adoptées par le peptide lors de sa liaison avec les récepteurs protéiniques.

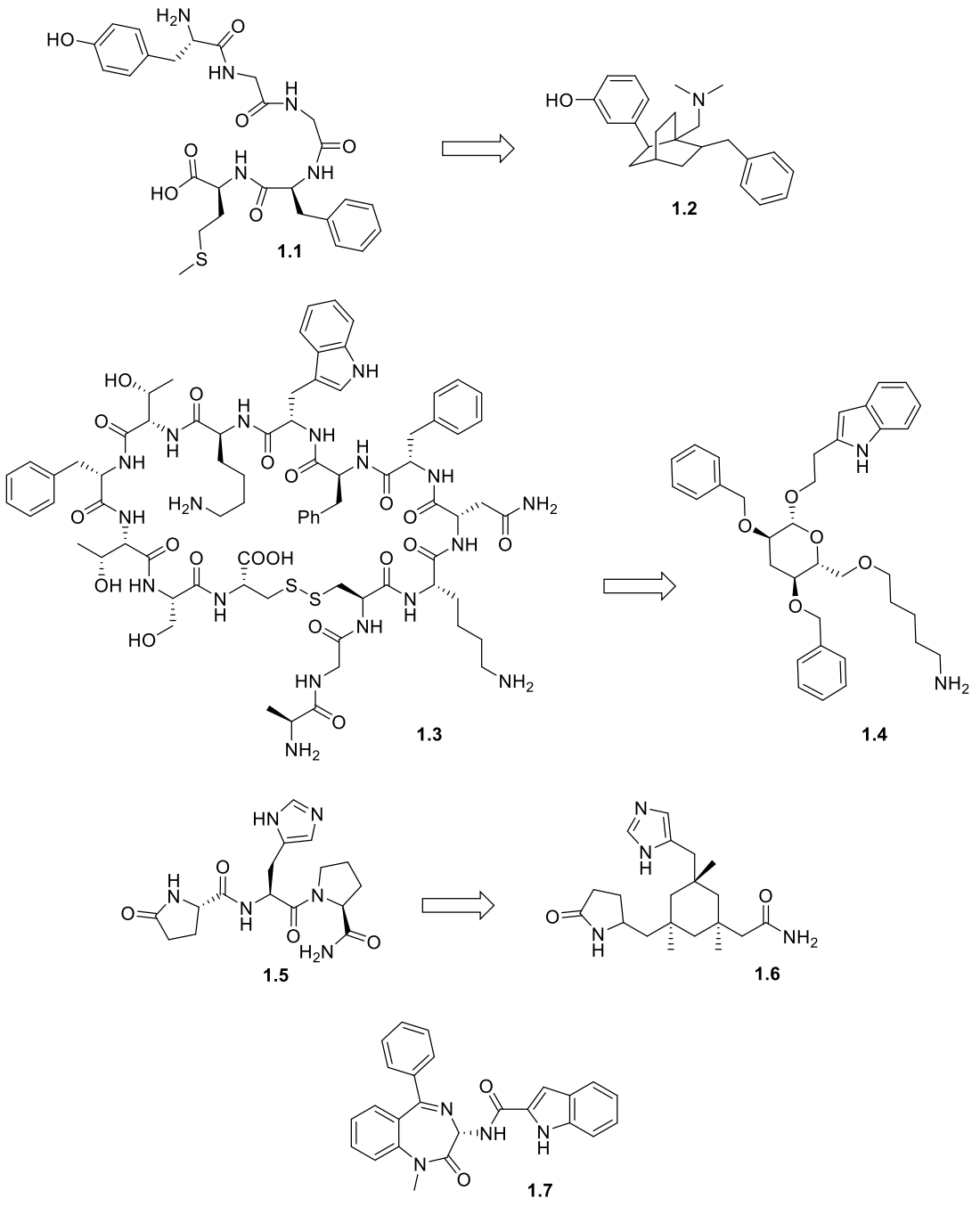

Figure 1.1. Peptidomimétiques **1.2**, **1.4**, **1.6** inspiré par de petites molécules et structure "privilégiée" de la benzodiazépinone **1.7**.

1.2. Des 1,4-benzodiazépin-2-ones aux 1,3,4-benzotriazépin-2-ones

Le squelette benzodiazépinique est une structure privilégiée dans le développement de molécules thérapeutiques. En effet, depuis les années soixante de nombreux

médicaments ont vu le jour basé sur ce système bicyclique, présentant des propriétés sédative, anxiolytique, anti-convulsion, anti-hypnotique, myorelaxante et contre l'amnésie antérograde. Les 1,4-benzodiazepin-2-ones ont la capacité de moduler les récepteurs hormonaux, bloquer les canaux ioniques ou encore inhiber certaines enzymes, comme par exemple CYP2C19 ou CYP3A4.[7]

En comparaison, les 1,3,4-benzotriazépin-2-ones ont reçût beaucoup moins d'attention que leurs analogues diazépiniques. Leurs premières apparitions dans la littérature datent de 1963, avec les premières synthèses décrites par le Dr. Theodore Sulkowski et le Dr Scott Childress.[8] Dans cette publication, ils mettent en avant l'intérêt des benzodiazépinones et l'intérêt certains de synthétiser leurs aza-analogues. Il aura fallu cependant attendre les recherches du Pr. Iain McDonald pour voir apparaitre les premières benzotriazépin-2-ones possédant un intérêt biologique. Ainsi le 5-cyclohexyl-triazépinones **1.8** est un antagoniste du récepteur de l'hormone parathyroïdienne-1,[9] et la benzotriazépin-2-one **1.9** est un antagoniste, oralement actif, du récepteur cholecystokinine-2 (CCK_2, Figure 1.2) Les benzotriazépin-2-ones sont aussi plus généralement décrites comme ayant des propriétés psychostimulante, antidépressive, anorexigène, et anti hypertensive.[10]

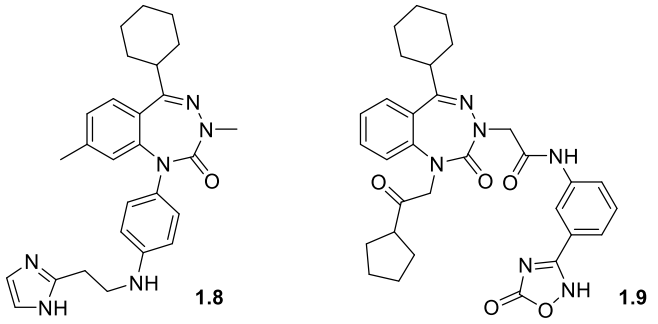

Figure 1.2. Exemples de 1,3,4-benzotriazépin-2-ones d'intérêt thérapeutiques

Les recherches sur l'antagoniste du récepteur cholecystokinine-2 **1.9** sont particulièrement intéressantes puisque l'étude part de la 1,4-benzodiazepin-2-one **1.10**,[11] résultant de l'optimisation de la structure privilégié **1.7**.[6a] Ce composé **1.10** est connu pour être l'un des antagonistes benzodiazépiniques, sélectif de CCK_2, le plus puissant. En inversant le carbone en position 3 du cycle diazépinique avec l'azote de l'urée, la 1,3,4-benzotriazépin-2-one **1.11** obtenue présente la même sélectivité avec

cependant une activité 1000 fois moins importante. Après optimisation, l'activité et la sélectivité du composé benzotriazépinique **1.9** est devenue semblable à celle de la benzodiazépine **1.10** (Figure 1.3). L'avantage de cette structure est de ne posséder aucun centre asymétrique, ce qui est avantageux, d'un point de vue économique, car elle ne nécessite pas de méthodes de synthèses ou d'analyses énantiomèriques.[12] La synthèse de ces composés est donc plus facile et directe.

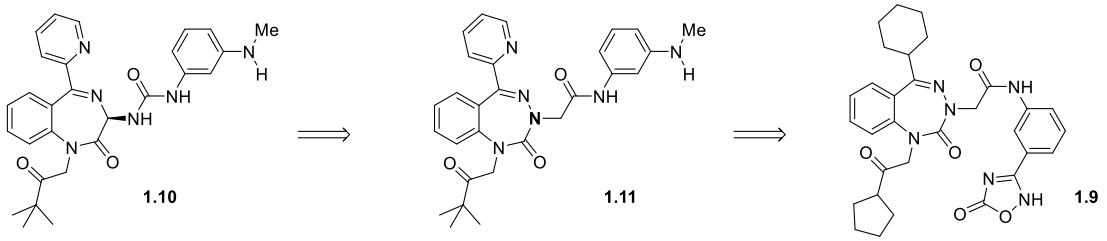

Figure 1.3. Antagoniste sélectif de CCK_2, des diazépines aux triazépines.

1.3. Synthèse des 1,3,4-benzotriazépin-2-ones

Les premières synthèses des 1,3,4-benzotriazépin-2-ones sont décrites par Dr. Schott Childress, avec l'obtention du dérivé **1.14**, sans substituants en positions 1 et 3.[8] Pour ce faire, la cétone **1.12** est convertie en hydrazone **1.13** puis la cyclisation est réalisée en utilisant du phosgène pour obtenir la benzotriazépinone **1.14** avec un rendement de 50%. Elle peut aussi être synthétisée en une étape à partir de la 2-aminobenzophénone **1.12** et le carbazate d' éthyle, en utilisant une condensation et cyclisation thermique, avec un rendement de 30% (Schéma 1.1)

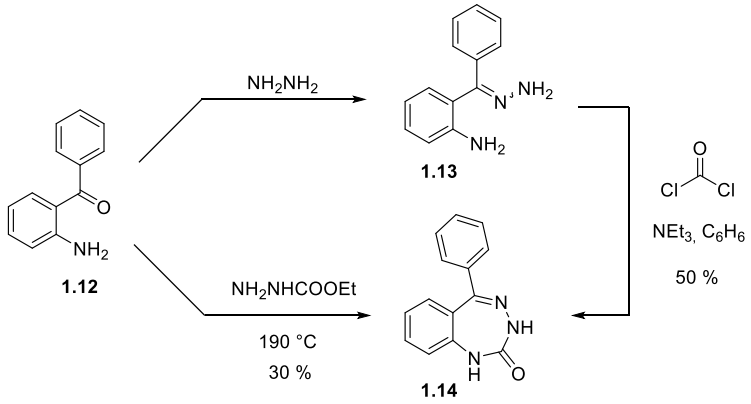

Schéma 1.1. Première méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones.

La synthèse actuelle des composés d'intérêts pharmaceutiques benzotriazépiniques débute par la formation d'une hydrazone substituée, en utilisant une cétone aminé et un dérivé de l'hydrazine, qui est par la suite est cyclisée en utilisant du triphosgène.[9,10] La benzotriazépine **1.19** non substitué en position 1 est conçu en trois étapes à partir de l'anthranilonitrile **1.15** (Schéma 1.2). La première étape consiste à la formation de la 2-aminophényle cétone **1.16**, par une réaction de Grignard sur le nitrile **1.15,** suivis d'une hydrolyse acide, avec un rendement de 70%. La deuxième étape est la synthèse de l'hydrazone substituée **1.18** à partir de la cétone **1.16** et de l'hydrazine **1.17**, en présence de pyridine dans l'éthanol, avec un rendement de 71%. La cyclisation de l'intermédiaire **1.18** pour obtenir la benzotriazépinone **1.19** est accomplie par l'utilisation de triphosgène dans le dichlorométhane en présence de triéthylamine, avec un rendement de 62%.

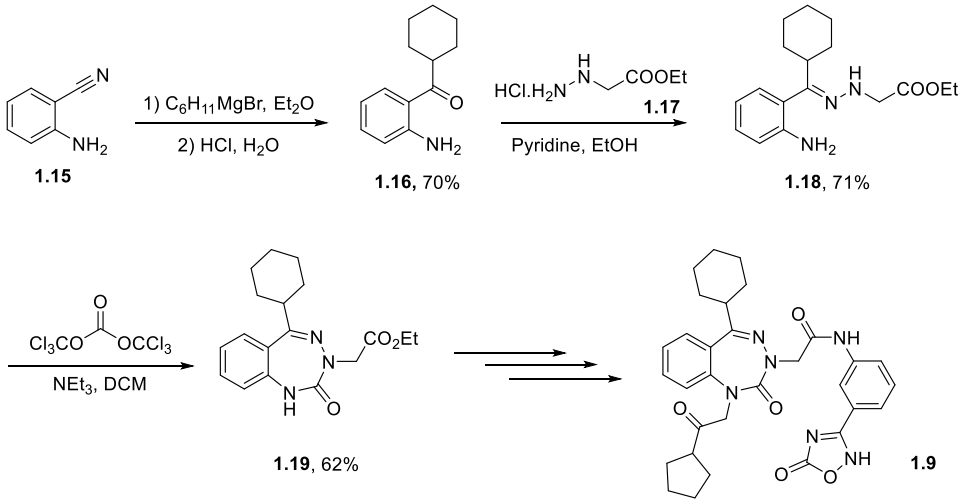

Schéma 1.2. Synthèse de l'intermédiaire benzotriazépinique **1.19** du composé d'intérêt biologique **1.9**.

La formation de 1,3,4-benzotriazépin-2-ones, uniquement non substituée en position-3, est réalisée par l'entremise d'une réaction de cyclisation catalysée au palladium, entre un bromure d'aryle hydrazone et un isocyanate d'aryle.[13] Ainsi la semicarbazone **1.22**, est formée *in-situ,* par l'attaque de l'hydrazone **1.20** sur l'isocyanate **1.21,** puis l'insertion du palladium dans la liaison C-Br suivi d'une élimination réductrice donne la formation de la 1,3,4-benzotriazépin-2-one **1.23** (Schéma 1.3). La substitution de l'isocyanate d'aryle en position para va grandement

influencer le rendement de la réaction, avec un rendement de 82% pour *p*-Cl, 43% pour *p*-H et 37% pour *p*-Me.

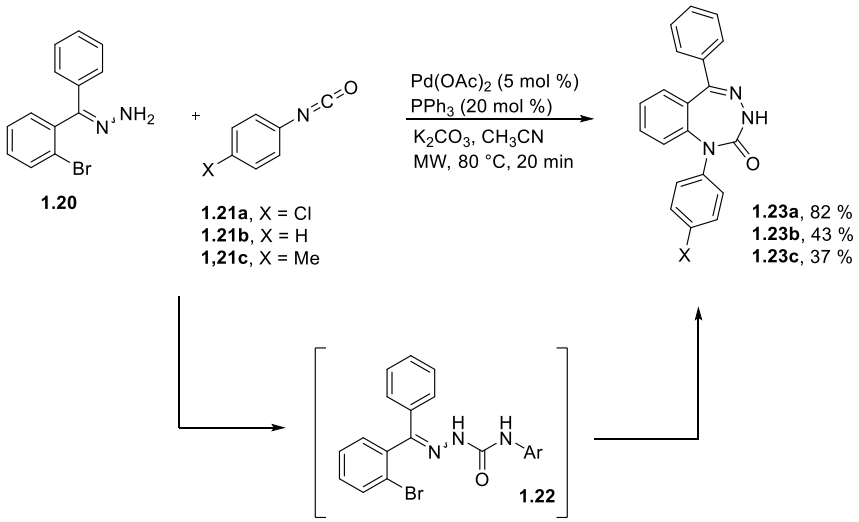

Schéma 1.3. Synthèse de 1,3,4-benzotriazépin-2-ones par cyclisation catalysée au palladium.

Il est intéressant de noter qu'il est possible de diversifier la position 5 des 1,3,4-benzotriazépin-2-ones, substituées en position 1 et 3, par une réaction d' activation de C-H catalysée au cuivre.[14] Pour ce faire, l'alcool aminé **1.24** est convertit en hémiaminal **1.25**, par condensation du benzaldéhyde dans le dichlorométhane en présence de tamis moléculaire et de carbonate de potassium. Après réduction avec du $LiAlH_4$, la benzyle amine **1.26** est obtenue avec un rendement de 75% sur deux étapes. L'oxydation de l'alcool benzylique **1.26**, avec du MnO_2, donne l'aldéhyde **1.27**, avec un rendement de 70%. La 1,3,4-benzotriazépin-2-one **1.28** est synthétisée en deux étapes, avec un rendement de 20%, à partir de l'intermédiaire **1.27**, par réaction avec du triphosgène à –40 °C dans le dichlorométhane puis traitement du carbamate activé obtenu *in situ* avec la méthyle hydrazine. Les conditions d'activation de C-H par catalyse au cuivre permettent l'arylation de la benzotriazépinone **1.28** en position 5. Ainsi, la 1,3,4,-benzotriazépin-2-one **1.29** est obtenue, avec un rendement de 88%, lors de l'utilisation d'iodure de phényle (Schéma 1.4).[14]

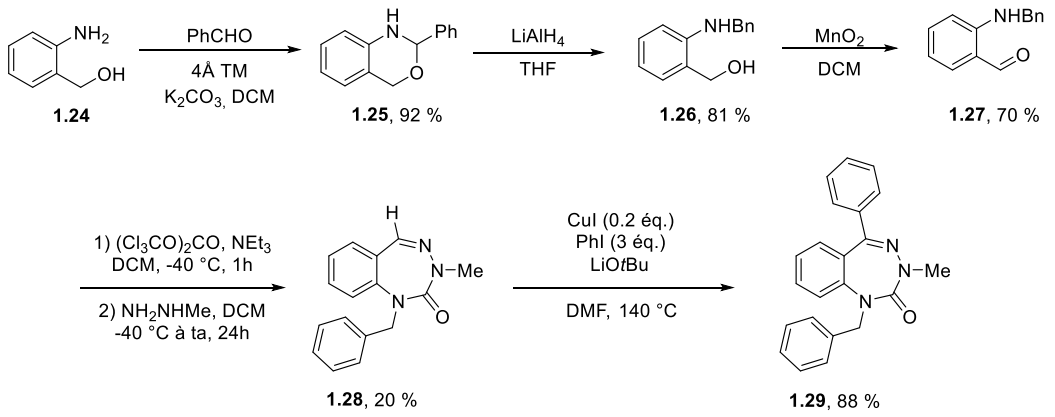

Schéma 1.4. Synthèse de la 1,3,4-benzotriazépinone **1.28** et diversification de la position 5 par arylation C-H catalysée au cuivre.

Les synthèses actuelles des 1,3,4-benzotriazépin-2-ones, bien que variées, possèdent certaines limitations comme l'utilisation de réactifs toxiques tels que le phosgène ou le triphosgène, des conditions de réactions dures, avec des températures élevées, la nécessité d'avoir des groupements protecteurs ou encore des rendements faibles pour certains substrats. De plus la fonctionnalisation, des positions 1, 3 et 5, est possible, mais aucune des conditions décrites actuellement ne permettent d'avoir une synthèse orientée sur une diversification rapide et aisée, à partir d'un substrat commun. Il apparait donc intéressant de développer une nouvelle méthodologie orientée sur une fonctionnalisation efficace, tout en éliminant les groupements protecteurs, les réactifs toxiques et les températures élevées.

1.4. Le système urotensinergique

1.4.1. Le récepteur UT

Le système urotensinergique possède un récepteur couplé aux protéines G (RCPG), nommé urotensine (UT), constitué de sept hélices alpha transmembranaires, reliées entre elles par des boucles intracellulaires et extracellulaires. Ce récepteur est composé de 386 résidus chez l'humain et possède des sites de N-glycosylation dans sa région *N*-terminale au niveau de Asn^{29} et Asn^{33} et des sites potentiels de phosphorylation au niveau de sa queue *C*-terminale (Figure 1.4).

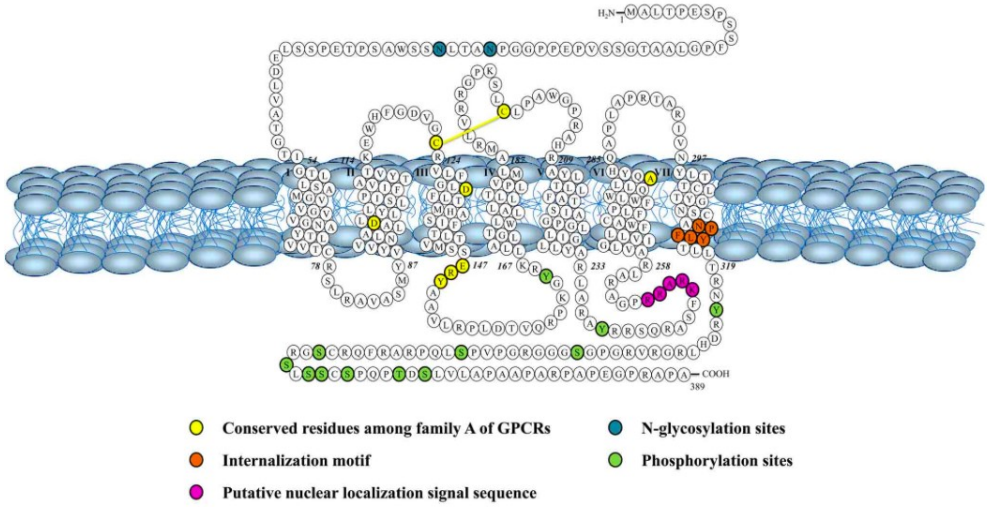

Figure 1.4. Représentation du récepteur urotensine UT.[15]

Chez 1'homme, le récepteur UT est principalement retrouvé dans le système nerveux central et périphérique ainsi que dans le système cardiovasculaire.[16] De plus, deux peptides endogènes sont capables de se lier et d'activer ce récepteur, l'Urotensine II (UII) et l'*Urotensin II-related peptide* (URP).

1.4.2. Les peptides endogènes : hUII et URP

Chez l'homme, l'hUII **1.30** est un undécapeptide cyclique (H-Glu-Thr-Pro-Asp-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) et l'URP **1.31** est un octapeptide cyclique (H-Ala-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH).[17] Ils partagent la même structure au niveau de leur partie cyclique *C*-terminale, et diffèrent donc uniquement sur la partie *N*-terminale (Figure 1.5). Des simulations de dynamique moléculaire et des études par spectroscopie RMN de UII et URP en phase aqueuse, ont démontré qu'ils adoptent des conformations cycliques similaires, réparties à hauteur de 80% sur une conformation ouverte relativement peu organisée et à environ 20% sur une conformation plus repliée, les deux possédants des énergies similaires.[17] Lors de leur liaison avec l'UT, la séquence commune, Trp-Lys-Tyr, primordiale pour l'activité biologique, adopte une conformation de tour γ inverse,[18] c'est-à-dire une structure coudée mettant en jeux trois résidus d'acides aminés, dans laquelle le carbonyle de l'amide du premier résidu forme une liaison hydrogène avec le proton amide du troisième résidu (Figure 1.5).

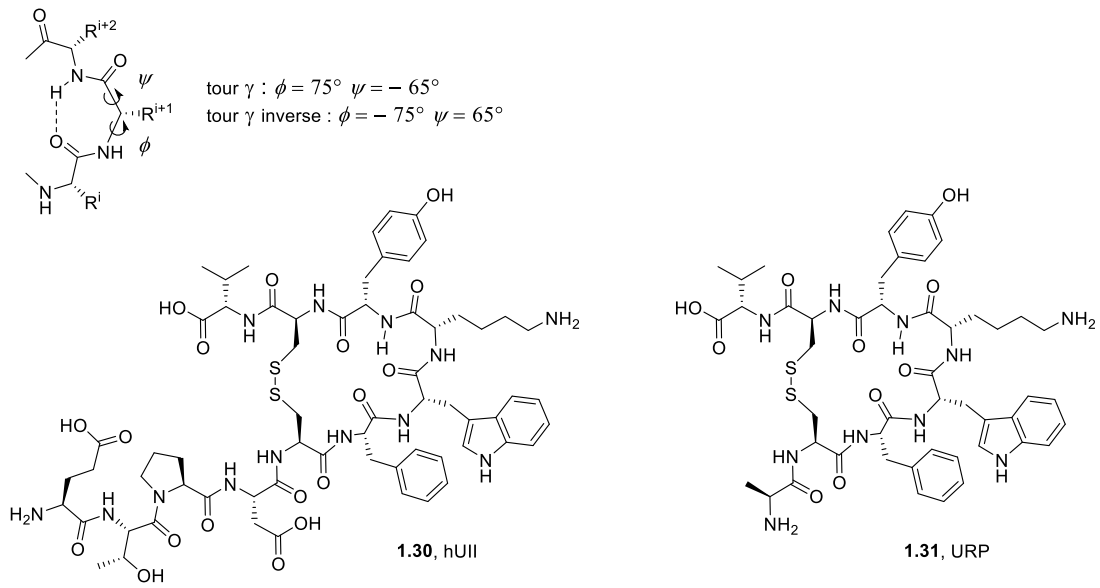

Figure 1.5. Structure du tour γ, de l'Urotensine II et de l'*Urotensin II related peptide*.

L'UII est connu pour être l'un des plus puissants vasoconstricteurs chez les mammifères.[19] L'UII et l'URP sont impliqués dans la génèse et la progression de nombreuses pathologies.[20,21,22] Cependant, bien qu'ils possèdent de nombreux points communs, ils possèdent également des activités biologiques distinctes au niveau de la régulation de la transcription,[23] de la prolifération des astrocytes,[24] ou encore de la contraction des muscles cardiaques.[25] Au cours des dernières années, de nombreuses études ont suggéré que ces deux ligands étaient en fait fonctionnellement sélectifs. En d'autres termes, ces derniers sont capables de stabiliser des conformations distinctes du récepteur ce qui aboutira à une activation différente au niveau intracellulaire.

1.4.3. Modulation de UT

Le développement d'antagonistes de UT apparait comme un choix intéressant pour le développement de nouveaux agents thérapeutiques.[26,27] La modulation de l'UT a ainsi émergé comme une solution nouvelle pour traiter de nombreuses pathologies, comme par exemple l'athérosclérose,[2829] l'hypertension artérielle pulmonaire,[30,31] ainsi que les arrêts cardiaques,[32,33,34] et les insuffisances rénales.[35,36] Ainsi, des analogues peptidiques de l'UII et des petites molécules ont été développés comme agonistes et antagonistes de ce récepteur.

La délétion des trois premiers acides aminés de hUII a ainsi généré un peptide, l'hUII(4-11), qui est le plus court peptide permettant de garder la même activité que le peptide parent **1.30**.[37] A partir ce modèle, des modifications de certains acides aminés de la partie cyclique ont permis de développer des agonistes ainsi que des antagonistes de UT. Le remplacement de la cystéine en position 5 par la pénicilline abouti au peptide agoniste [Pen^5]hUII(4−11) **1.31**,[38,39] qui a lui-même mené aux deux plus puissants agonistes peptidiques connus [Pen^5, Btz^9]hUII(4−11) **1.32** et [Pen^5, (3,4-Cl)Ph^9]hUII(4−11) **1.33**,[40] en substituant la tyrosine en position 9 respectivement par la benzothiazolylalanine et la 3,4-dichloro-phénylalanine (Figure 1.6). Le peptide [Pen^5, D-Trp^7, Orn^8]hUII(4−11) **1.34**, aussi connu sous le nom d'urantide est, quant à lui, un antagoniste compétitif de UT,[40,41] résultant du remplacement de la lysine en position 8 par l'ornithine et de l'inversion de configuration du tryptophane en position 7 (Figure 1.6).

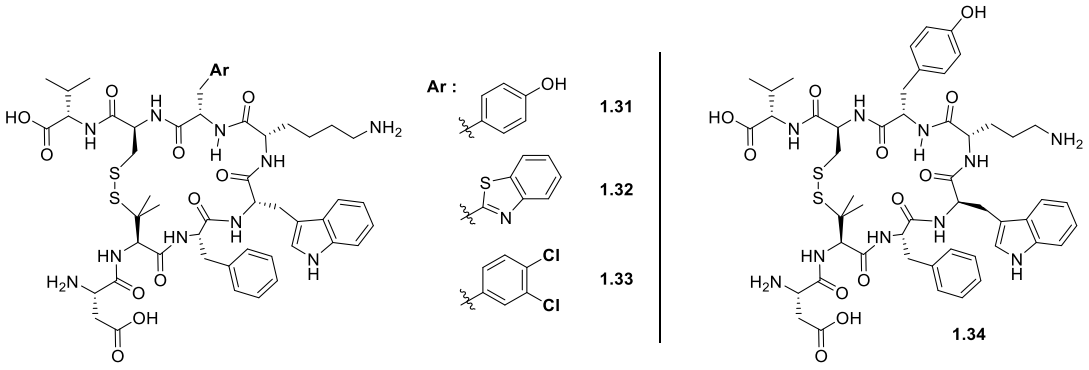

Figure 1.6. Peptides agonistes **1.31-33** et antagoniste **1.34** de l'UT.

Le développement de petites molécules antagonistes du récepteur urotensinergique a donné naissance à des familles de composés de nature diverses.[42] Par exemple, ACT-058362 **1.35**, développé par Actelion Pharmaceuticals, testé en phase clinique 2B, pour le traitement de l'hypertension et de la néphropathie diabétique, est arrêté en 2005 car il ne démontrait pas de changements significatifs dans les paramètres hémodynamiques rénaux, tels que le débit sanguin et de filtration glomérulaire.[17,43] Le composé SAR101099 **1.36** a été amené en phase clinique 1 par Sanofi-Aventis, puis stoppé en 2011 dû à son manque d'efficacité pour le traitement

de la néphropathie diabétique.[17] Enfin, GlaxoSmithKline a développé le composé GSK1440115 **1.37** contre l'asthme puis l'a arrêté en phase clinique 1B, ne fournissant pas l'effet bronchodilatateur escompté chez les patients asthmatiques (Figure 1.7).[17,44]

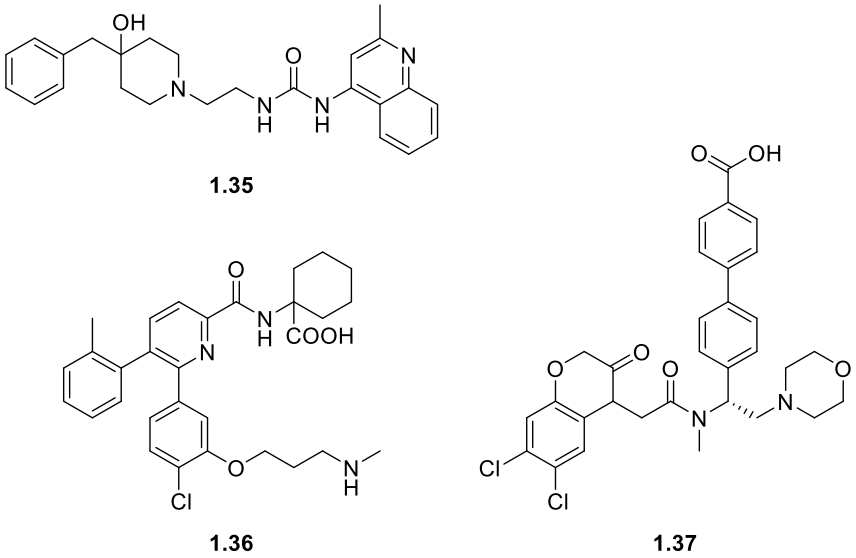

Figure 1.7. Exemples de petites molécules antagoniste de UT.

Ainsi malgré les efforts pour le développement d'agents thérapeutiques efficaces modulant UT, aucune molécule n'est à ce jour sur le marché. Il convient donc de mieux comprendre ce système, et notamment de pouvoir mieux différencier le rôle joué par hUII et URP dans le système urotensinergique et les mécanismes biologiques en découlant, que ce soit dans des conditions saines ou pathologiques.

1.4.4. Modulation sélective de hUII ou URP

Des peptides ont été développés dans le but de pouvoir moduler sélectivement l'action de hUII ou de URP. Ainsi des dérivés de l'URP comme **1.38** ([Bip^4]URP, Bip = 4,4′-biphénylalanine),[45] et **1.39** ([Pep^4]URP, Pep = (phényléthynyl)phénylalanine},[46] (Figure 1.8) permettent de réduire significativement la puissance et l'efficacité de contractions de hUII sans changement notable sur l'effet vasoconstricteur induit par URP. De plus, des dérivés d'azasulfurylpeptide de hUII(4-11) et notamment **1.40** AsNal(2')^7]UII(4-11),[47] (Figure 1.8) bien que moins puissants, ont également permit une distinction des effets entre hUII et URP tout comme **1.38** et

1.39 (Tableau 1.I). Enfin, le peptide cyclique **1.41** *c*[Phe-Trp-Lys-Tyr-Gly-ψ(triazole)-Gly],[48] imitant le corps central de UII, réduit la puissance et l'efficacité des contractions induites par URP, dans un test *ex vivo* sur l'aorte de rat, sans changer celles induites par hUII (Tableau 1.I).

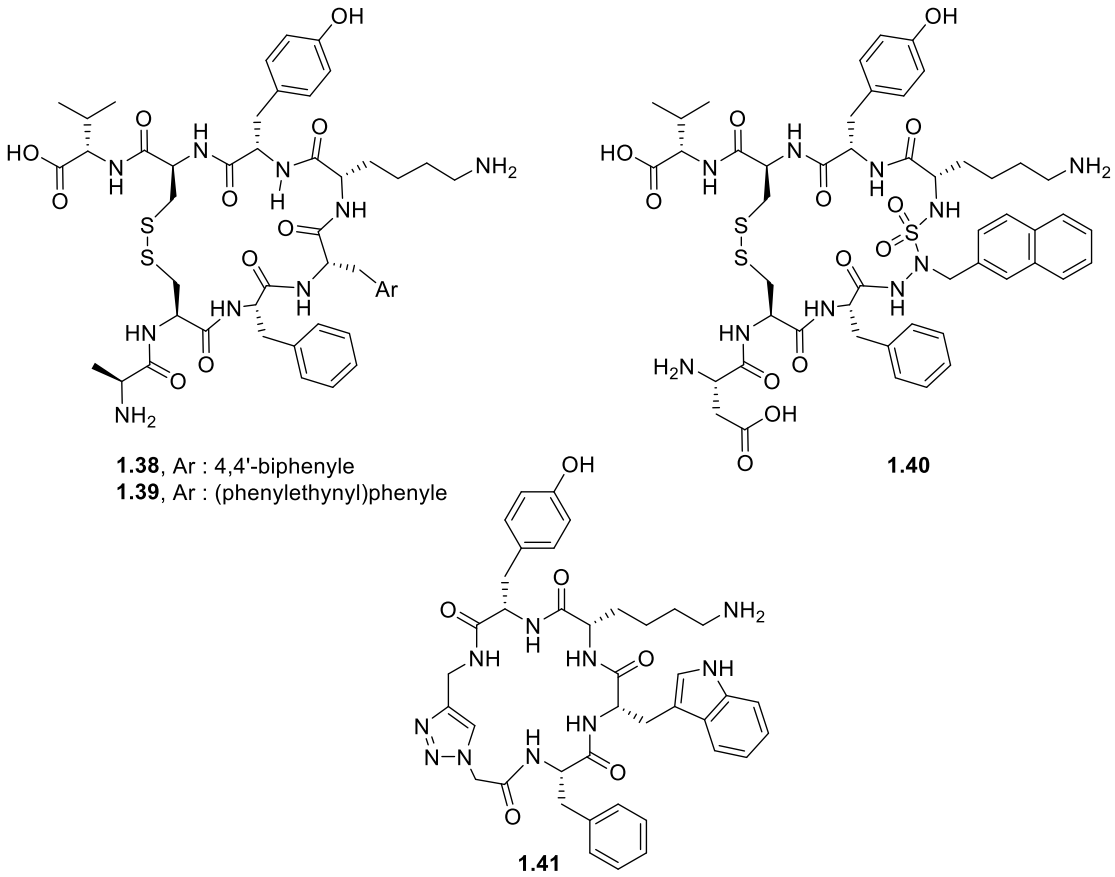

Figure 1.8. Modulateur allostérique peptidique de UT.

Finalement, une librairie de petites molécules basées sur le motif pyrroldiazépinique, comme mime peptidique de la séquence Bip-Lys-Tyr et de sa conformation de tour γ inverse, a été conçue et testée comme modulateur allostérique de UT (Figure 1.9). Par exemple, la pyrrolodiazépine (*R*)- **1.42** agit comme le peptide parent **1.38**, sur des tests *ex vivo* sur l'aorte de rat (Figure 1.9, Tableau 1.I).[49] Cependant, le diastéréoisomère (*S*)- **1.42** ne module pas le maximum de contraction de hUII, ou à la même concentration (*R*)- **1.42** l'inhibe totalement. De plus, la version insaturée **1.43** produit l'effet inverse, en maximisant l'efficacité de hUII, et réduisant faiblement celle de URP (Figure 1.9, Tableau 1.I). La configuration du carbone en

position 5 est donc cruciale pour l'activité de ce mime peptidique comme modulateur allostérique. L'utilisation du squelette pyrrolodiazépinique est prometteuse pour le développement de petites molécules modulatrices du système urotensinergique. Toutefois, l'importance de la configuration engendre une limitation importante pour leurs développements. Il serait donc intéressant de développer d'autres mimes peptidiques basés sur des petites molécules imitant la séquence Bip-Lys-Tyr et la configuration du tour γ.

Tableau 1.I. Comparaison du profile de vasoconstriction de hUII et URP, en absence et en présence des composés **1.38-43**.

		Contraction sur l'aorte de rat (hUII)			Contraction sur l'aorte de rat (URP)		
no.	Conc. (μM)	n	E_{max} (%)[a]	pEC_{50}[b]	n	E_{max} (%)[a]	pEC_{50}[b]
Ø		7	103 ± 2	8.61 ± 0.05	6	118 ± 2	8.09 ± 0 .03
1.38	1	5	61 ± 7**	7.60 ± 0.29*	3	102 ± 5%	7.74 ± 0.12
1.39	1	7	28 ± 3**	8.41 ± 0.47	-	-	-
1.40	10	4	65±6*	7.82 ± 0.24**	5	105±10	8.02 ± 0.25
1.41	25	3	96 ± 15	8.37 ± 0.39	3	54 ± 5**	9.30 ± 0.32**
(*R*)- **1.42**	14	3	0***	0***	3	185 ± 6**	7.71 ± 0.08*
(*R*)- **1.42**	4	3	63 ± 1*	8.46 ± 0.07*	-	-	-
(*S*)- **1.42**	14	3	121±4	8.06 ± 0.09**	4	113±4*	7.99 ± 0.10
1.43	15	4	128±4*	8.19 ± 0.08**	4	110±4*	7.93 ± 0.09

Toutes les données sont exprimée en ± SEM. Les analyses statistiques pour pEC_{50} and E_{max} ont été faites en utilisant le test t non apparié de *Student* *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001 en comparaison avec les données obtenus pour hUII ou URP seul (Ø). Les (n) réplicats ont été fait sur un tissu provenant d'animaux différents. [a] Le maximum d'efficacité (E_{max}) est exprimé en pourcentage de l'amplitude de contraction induite par KCl (40 mM). [b] Les valeurs de pEC_{50} sont définis comme le logarithme négative de la moitié de la concentration (M) maximal nécessaire pour obtenir E_{max}.

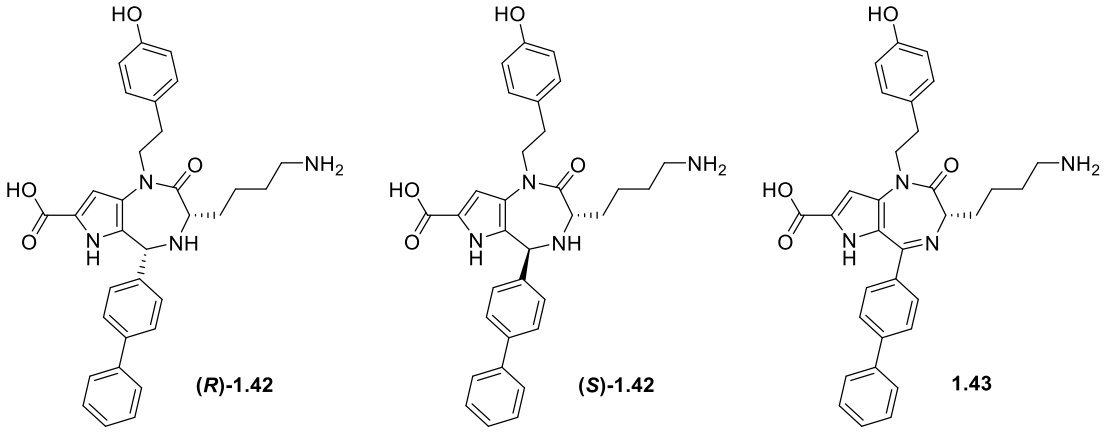

Figure 1.9. Mimes peptidiques pyrrolodiazépiniques **1.42** et **1.43** de la séquence Bip-Lys-Tyr.

1.4.5. Potentiel mime peptidique de tour γ

Les analyses structurales par cristallographie aux rayons X des diazépines **1.44-48** et de la triazépine **1.49** démontrent une forte ressemblance avec les angles dièdres du résidu central d'un tour γ (ϕ = 75°, and ψ = −65°) ou d'un tour γ inverse (ϕ = −75°, and ψ = 65°) (Figure 1.10, Tableau 1.II). Les angles dièdres des pyrrolodiazépinones **1.44** et **1.45** présentent une similarité avec ceux d'une conformation tour γ inverse, (**1.44**, ϕ = –75° ± 18, ψ = 65° ± 8 et **1.45**, ϕ = –75° ± 4, ψ = 65° ± 9), tout comme la benzodiazépinone (*S*)- **1.46**, dont la conformation des angles dièdres est encore mieux respecté [(*S*)- **1.46**, ϕ = –75° ± 3, and ψ = 65° ± 4]. Alors que la benzodiazépinone (*R*)- **1.46** exhibe des angles dièdres proches d'un tour γ (ϕ = 75°, and ψ = −65° ± 3). Les pyrrolobenzodiazépinones (*S*)- **1.47** et (*R*)- **1.47** offre respectivement, tout comme leur analogues benzodiazépiniques (*S*)- **1.46** et (*R*)- **1.46**, des angles dièdres proche de celui de la conformation d'une tour γ inverse et un tour γ (Figure 1.10, Tableau 1.II). Il est à noter cependant l'inversion entre relation de la configuration du carbone en position 3 et la conformation tour γ ou tour γ inverse. Les déviations sont aussi plus importantes [(*S*)- **1.47**, ϕ = 75° ± 14, ψ = –65° ± 8 et (*R*)- **1.47**, ϕ = –75° ± 14, ψ = 65° ± 8] que pour les benzodiazépinones (*S*)- **1.46** et (*R*)- **1.46**. La benzodiazépinone **1.48** présente des angles dièdres d'un tour γ inverse avec une déviation au niveau de l'angle de torsion ϕ (**1.48**, ϕ = –75° ± 7, and ψ = 65° ± 2). La

benzotriazépinone **1.49**, quant à elle, présente, sans avoir de carbone asymétrique, des angles dièdres similaire à ceux d'un tour γ avec une déviation au niveau de l'angle de torsion ψ (**1.49**, ϕ = 75° ± 1, and ψ = −65°± 9) (Figure 1.10, Tableau 1.II).

Les benzotriazépin-2-ones semblent être une structure intéressante pour le développement de mimes peptidiques possédant une conformation de tour γ. En effet, elles permettent d'obtenir une diversification importante en position N1, N3 et C5, en ayant l'avantage d'être achirales. Il serait donc désirable de synthétiser des mimes peptidiques de la séquence Bip-Lys-Tyr, basés sur un squelette benzotriazépinique, et de les tester comme modulateur du système urotensinergique.

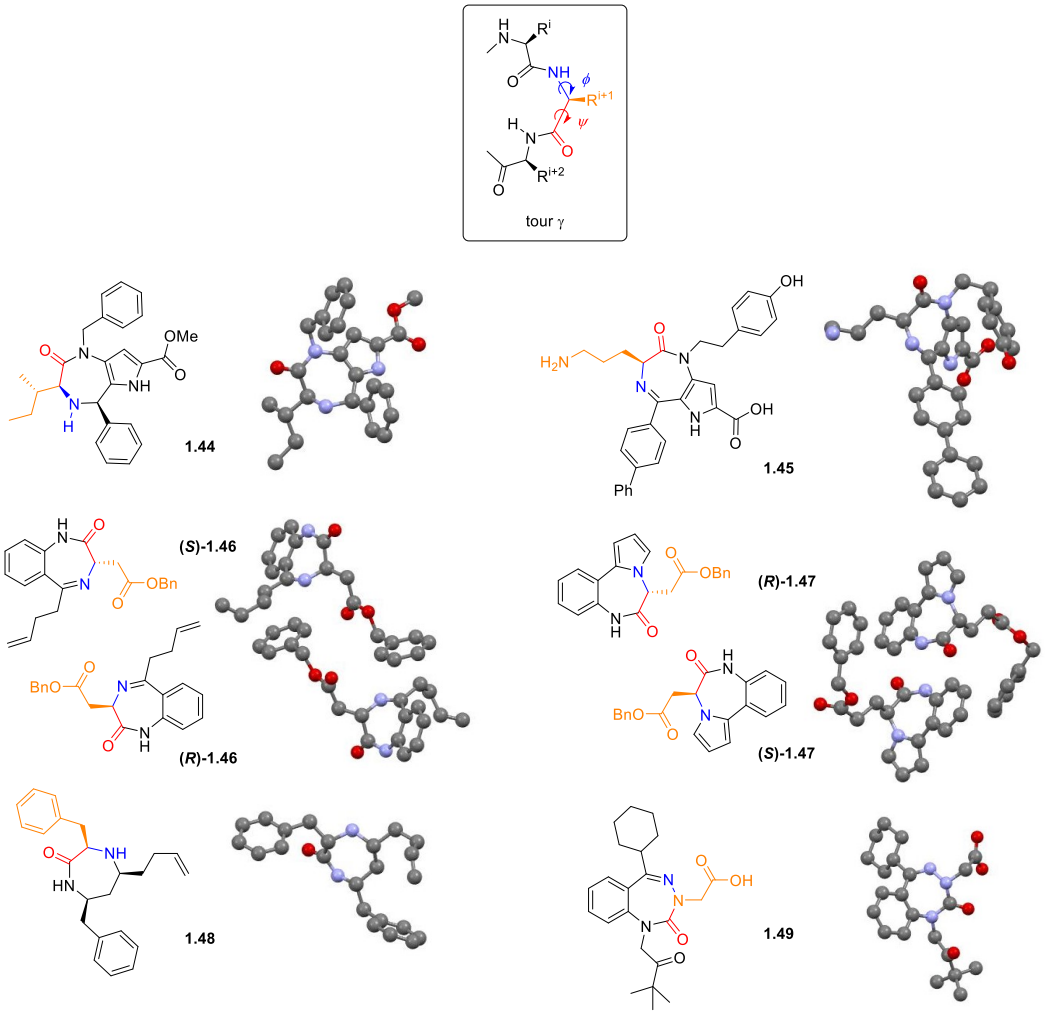

Figure 1.10. Tour γ et structure au rayon X des diazépines **1.44-48** et triazépine **1.49**, les atomes d'hydrogènes et les solvants co-cristallisés ont été retirés pour plus de clarté.

Tableau 1.II. Comparaison des angles dièdre des tours γ avec les analyses structurales des cycles à 7 chainons **1.44-49**.

Type de tour	ϕ	ψ	Réf.
Tour γ	75	–65	50
Tour γ inverse	–75	65	50
1.44	–93	73	51
1.45	–79	74	49
(*R*)-**1.46**	75	–68	52
(*S*)-**1.46**	–72	69	52
(*R*)-**1.47**	–61	57	52
(*S*)-**1.47**	61	–57	52
1.48	–83	67	53
1.49	73	–59	9a

1.5. Description de la thèse

Les 1,3,4-benzotriazépinon-2-ones présentent un intérêt tout particulier étant donné leur relation étroite avec les benzodiazépinones, et leurs possibles capacités communes à mimer les conformations adoptées par certains peptides lors de leurs liaisons avec les récepteurs protéiniques. Cependant les méthodologies de synthèses actuelles présentent certains inconvénients, comme l'utilisation de réactifs toxiques ou de conditions dures.

Dans le cadre de cette thèse, le deuxième chapitre décrit une nouvelle méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones, orientée vers une diversification aisée et n'utilisant pas de température élevés ni de réactifs toxiques. Il est aussi mis en évidence le concept de l'utilisation des 1,3,4-benzotriazépin-2-ones, possédant un chiralité adaptive, comme mime peptidique basé sur une petite molécule et présentant une conformation de tour γ.

Dans le troisième chapitre, ce concept est appliqué à la modulation du récepteur urotensinergique. L'utilisation de la méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones, développée dans le chapitre 2, a permis d'obtenir une librairie de 26 composés benzotriazépiniques, basée sur l'idée de mimer la conformation de tour γ, biologiquement active, du tripeptide Bip-Lys-Tyr présent dans l'Urocontrin ([Bip^4]URP), modulateur allostérique du récepteur urotensinergique.

Ainsi de nombreux composés ont révélé la capacité à moduler sélectivement les effets de hUII et URP sur la contraction de l'aorte de rat de manière *ex-vivo*, démontrant l'utilisation possible du squelette achiral des 1,3,4-benzotriazépin-2-ones comme mime peptidique. De plus, cette étude met en évidence, l'importance du groupement phénol et de son orientation, pour la modulation sélective de hUII et URP, qui pourra servir lors d'études sur des peptides modulateurs du récepteur urotensinergique.

Enfin, dans le quatrième chapitre, une perspective sur l'amélioration de l'activité de ce mime peptidique, pour la modulation des effets de hUII er URP, avec l'incorporation d'un mime de phénylalanine sur le squelette, est présentée. Le développement de molécules photosensibles est exposé dans le but de se lier au récepteur urotensinergique pour mieux comprendre la location responsable de la modulation de ce récepteur et la discrimination sélective entre les activités de hUII et URP.

En résumé, ces travaux de thèse ont contribué à l'avancement des méthodes de synthèse des benzotriazépinones, leur application comme mime de tour γ, et la conception de modulateurs achiraux du récepteur UT. Ces études ont contribué à l'élargissement des connaissances, fondamentales et appliquées, dans les domaines des mimes peptidiques et de la chimie médicinale.

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Chapitre 2 : Développement d'une nouvelle méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones

2.1. Mise en contexte de l'Article 1

Le but de l'Article 1 est de présenter une nouvelle méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones, orientée vers une diversification efficace, qui n'utilise ni de températures élevées, ni multiples plusieurs groupements protecteurs, ni de réactifs toxiques.

2.2. Hypothèse de base : alkylation chimiosélective du cœur triazépinique

Dans le but de répondre à ces critères, une rétrosynthèse a été proposée mettant en jeux deux alkylations chimiosélectives successives, dans un premier temps en position 3 puis dans un second temps en position 1 (Figure 2.1).

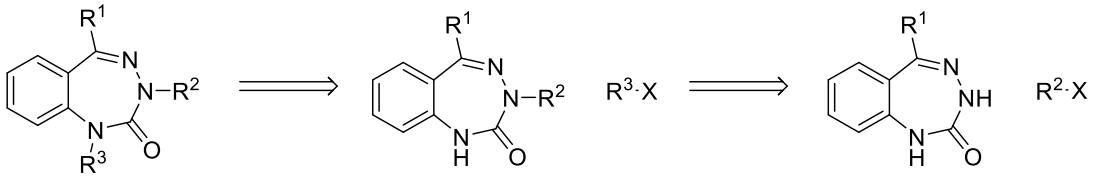


Figure 2.1. Rétrosynthèse pour diversifier le cœur triazépinique.

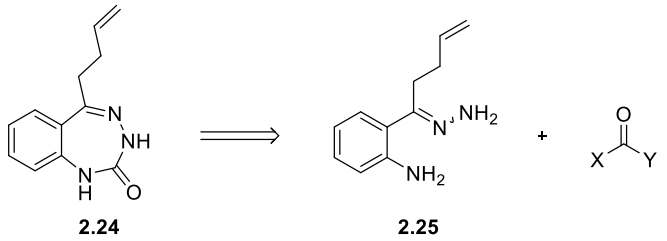
En effet, les 1,3,4-benzotriazépin-2-ones peuvent être conceptuellement vues comme des semicarbazones cycliques, et le groupe du Pr. William D. Lubell a mis en place la synthèse d'aza-peptide par alkylation chimiosélective de semicarbazone. Il aurait été donc intéressant de pouvoir utiliser cette méthodologie pour effectuer une synthèse des 1,3,4-benzotriazépin-2-ones, dirigée vers une diversification simple et efficace.

Dans un premier temps, il est donc important de développer une méthode efficace pour la synthèse des (1H)-(3H)-1,3,4-benzotriazépin-2-ones. Pour ce faire la 1-(2-aminophenyl)pent-4-en-1-one, obtenue à partir de l'anthranilate de méthyle par addition en cascade de vinyl Grignard catalysée par du cuivre, sera utilisée comme produit de départ.

2.3. Synthèse de la (1H)-(3H)-5-(but-3-enyl)-1,3,4-benzotriazépin-2-one

2.3.1. À partir de l'hydrazone comme produit de départ

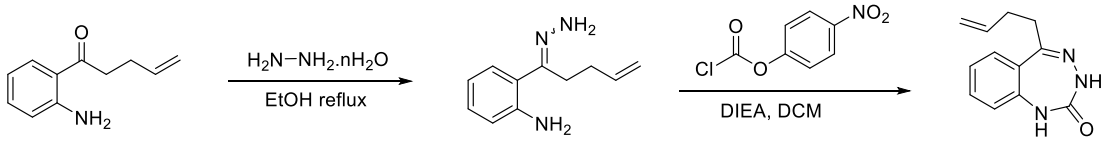
La première rétrosynthèse proposée consistait à cycliser l'hydrazone aminée **2.25** en utilisant un dérivé du phosgène (Figure 2.2).



2.24 **2.25**

Figure 2.2. Rétrosynthèse à partir de l'hydrazone **2.25**.

L'hydrazone aminé **2.25** a été obtenu avec un rendement de 25% à partir de la cétone aminée **2.8**, en la faisant réagir avec de l'hydrazine aqueuse dans l'éthanol à reflux. Les essais de cyclisation ont été effectués dans un milieu dilué, par ajout lent des réactifs dans du dichlorométhane. Différents dérivés du phosgène ont été utilisé, tel que le chloroformate de phényle, le 1,1'-carbonyldiimidazole, le carbonate *N*,*N*′-disuccinimide et le chloroformate de 4-nitrophényle, cependant uniquement l'utilisation de ces deux derniers réactifs ont permis l'obtention de la triazépinone **2.24** mais avec un rendement de seulement 20% (Schéma 2.1). Une analyse HPLC/MS a démontré la formation de produits de dimérisations.



2.8 **2.25**, 25% **2.24**, 20 %

Schéma 2.1. Synthèse de la benzotriazépinone **2.24** à partir de l'hydrazone **2.25**.

Les rendements étant trop faibles pour pouvoir développer une méthodologie efficace, une seconde approche a été envisagée, avec la mise en place du carbonyle à la première étape.

2.3.2. À partir du carbamate activé comme produit de départ

La cyclisation à partir du carbamate activé **2.26** et d'hydrazine a alors été envisagée pour lutter contre les problèmes de dimérisation survenus lors des premiers essais (Figure 2.3).

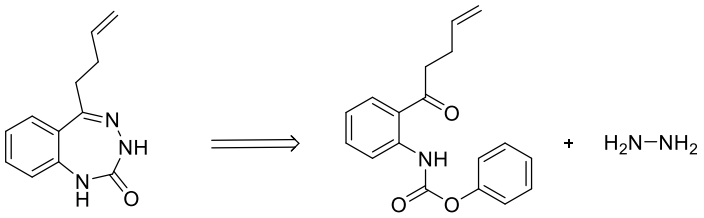


Figure 2.3. Rétrosynthèse à partir du carbamate activé **2.26**.

La cétone aminé **2.8** réagit avec le chloroformate de phényle, dans le dichlorométhane à température ambiante, pour donner le carbamate activé **2.26** avec un rendement quantitatif. Cependant, le traitement du carbamate **2.26** avec de l'hydrazine aqueuse, ne conduit pas à la cyclisation attendue mais à la formation de l'hydrazone semicarbazide **2.27**, avec un rendement de 76% (Schéma 2.2).

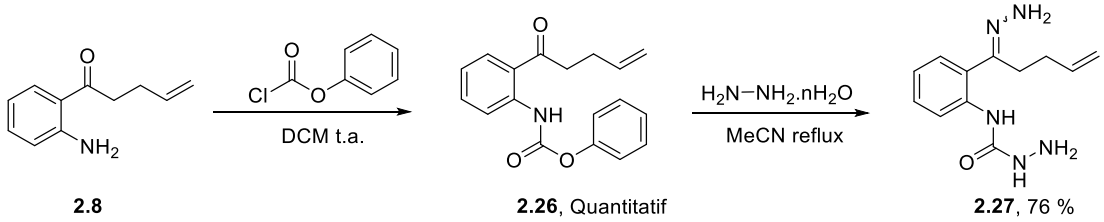


Schéma 2.2. Synthèse et essai de cyclisation du carbamate activé **2.26**.

2.3.3. À partir de la semicarbazone comme produit de départ

Une autre rétro-synthèse a alors été proposée en utilisant la semicarbazone **2.15** comme produit de départ (Figure 2.4). En effet, cette proposition permet d'installer le carbonyle ainsi que le dérivé hydrazinique directement sur la cétone aminée et permettrait ainsi de lutter contre les problèmes de dimérization et de chimiosélectivité.

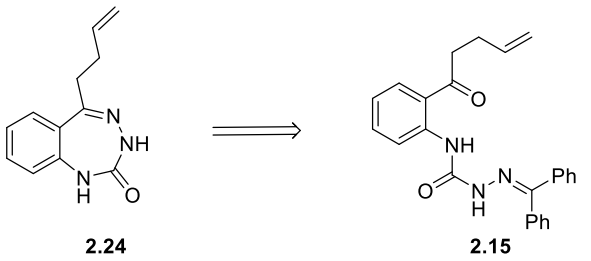


Figure 2.4. Rétrosynthèse à partir de la semicarbazone **2.15**.

La semicarbazone **2.15** est synthétisée à partir de la procédure décrite pour la synthèse des aza-glycine par le groupe du Pr. William D. Lubell.[16,17] Le carbazate activé est formé *in situ* à partir de la benzophénone hydrazone et du 4-nitrophényle chloroformate dans le dichlorométhane à 0 °C, puis traiter avec la cétone aminée **2.8** en présence de DIEA pour donner la semicarbazone **2.15** avec un rendement de 71%. Cependant, les essais de déprotection/cyclisation en milieux acides n'ont pas permis d'obtenir la benzotriazépinone espérée, et une analyse HPLC/MS démontre la formation d'un mélange d'oligomères après traitement de la semicarbazone **2.15** avec du HCl (1M) à 60 °C (Schéma 2.3).

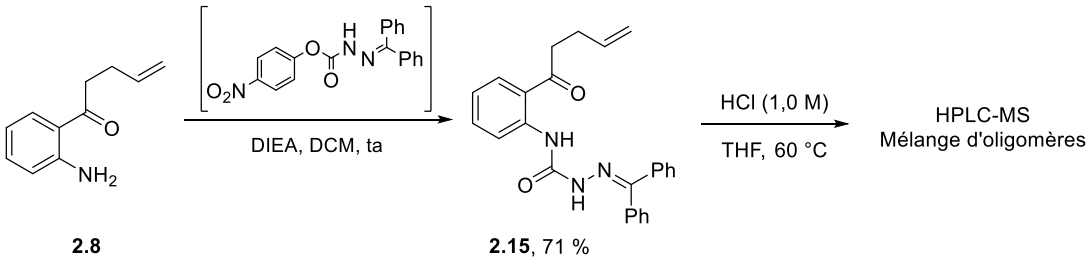


Schéma 2.3. Synthèse et essai de cyclisation de la semicarbazone **2.15**.

2.4. Stratégie de synthèse des (1H, 3H)-5-(but-3-enyl)-1,3,4-benzotriazépin-2-ones N-alkyles

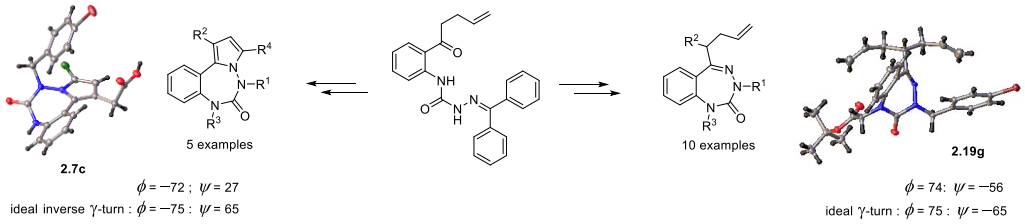
Le développement d'une méthodologie pour la synthèse de la (1H)-(3H)-5-(but-3-enyl)-1,3,4-benzotriazépin-2-one n'a pas abouti aux résultats escomptés. Ainsi, une nouvelle stratégie a été explorée et est présentée dans l'Article 1.

Dans le cadre de cet article, une nouvelle méthodologie de synthèse des 1,3,4-benzotriazépin-2-ones est décrite. La séquence met en jeux des alkylations

chimiosélectives puis une cyclisation en condition acides permettant la synthèse d'analogues diverses de 1,3,4-benzotriazépin-2-one. De plus, les études de rayons-X ont démontré que des 1,3,4-benzotriazépin-2-ones peuvent être considérées comme des mimes peptiques possédant une conformation de tour γ. Ainsi cette nouvelle méthodologie de synthèse pourrait permettre le développement de librairie de mimes peptidiques, basés sur le motif 1,3,4-benzotriazépin-2-onique.

Article 1

Douchez, A.; Lubell, W. D. Chemoselective alkylation for diversity-oriented synthesis of 1,3,4-benzotriazepin-2-ones and pyrrolo[1,2][1,3,4]benzotriazepin-6-ones, potential turn surrogates. *Org. Lett*. **2015**, *17*, 6046-6049.



Chemoselective alkylation for diversity oriented synthesis of 1,3,4-benzotriazepin-2-ones and pyrrolo[1,2][1,3,4]benzotriazepin-6-ones, potential turn surrogates.

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Département de Chimie, Université de Montréal, C.P.6128, Succursale Centre-Ville, Montréal H3C 3J7, Canada

Abstract

1,3,4-Benzotriazepin-2-ones garner interest for medicinal applications, in part due to their relationship with benzodiazepinones. Ten 1,3,4-benzotriazepin-2-ones **2.6** and **2.19**, and six pyrrolo[1,2][1,3,4]benzotriazepin-6-ones **2.7** and **2.23** were prepared in 4-7 steps and 4-60 % overall yields by a divergent strategy from methyl anthranilate employing chemoselective alkylations of common linear and cyclic precursors to diversify three triazepinone ring positions (N1, N3 and C5). X-ray crystallography demonstrated that benzotriazepinone **2.19g** may serve as a γ-turn mimic.

Introduction

1,4-Benzodiazepin-2-ones **2.1** are common targets because of their biological properties and medicinal applications,[1] which may be due in part to their potential to mimic peptide γ-turn secondary structures (Figure 2.5).[2,3,4,5] Although their aza-counterparts have received relatively less attention, 1,3,4-benzotriazepin-2-ones **2.2** possess intriguing biological activity. For example, 5-cyclohexyl triazepinones **2.3** and **2.4** have respectively exhibited activity as a parathyroid hormone-1 receptor antagonist,[6] and an orally active cholecystokinin-2 (CCK_2) antagonist (Figure 2.5).[7] 1,3,4-Benzotriazepin-2-ones have also been claimed to possess psycho-stimulant, antidepressant, anorexigenic, and antihypertensive properties.[8] Although relatively

little is known about 1,3,4-triazepin-2-one conformation, similar to the amino acid component in 1,4-diazepin-2-ones,[9] the aza-phenylglycine residue of 7-chloro-3,5-diphenyl-1,2-dihydro-3H-1,3,4-benzotriazepin-2-one **2.5** was observed by X-ray crystallography to adopt ϕ– and ψ-dihedral angle values close to those of the central residue of an ideal γ-turn.[10]

Since original syntheses from 2-aminobenzophenone,[4] 1,3,4-benzotriazepin-2-ones have been commonly prepared by cyclization of the corresponding hydrazone with a phosgene equivalent, and by a one-pot annulation with a carbazate often at high temperature (e.g., 190°C). Ring closure has also been achieved by palladium-catalyzed cyclisation of aryl isocyanates and 2-haloaryl hydrazones under microwave irradiation,[11] as well as condensation of anthranilic acid hydrazide with isatins, which provided the corresponding spiro[1,3,4-benzotriazepine-2,3'-indole]-2',5(1H,1'H)-diones.[8] Benzotriazepinone skeletons have been alkylated on ring nitrogen, and arylated at C5 using copper-catalysis in solution,[12] and on microelectrode arrays;[13] however, nitrogen protection has been essential for chemoselectivity.

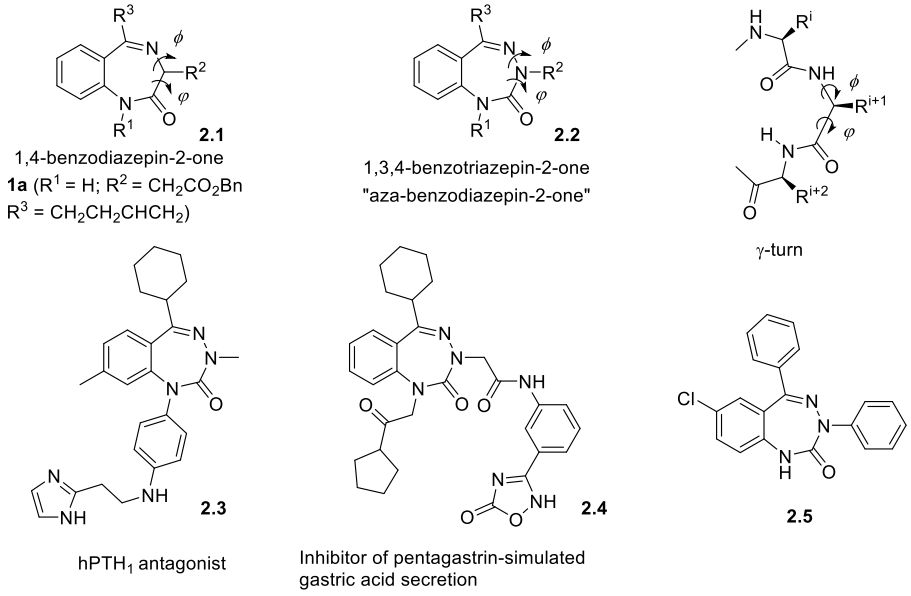


Figure 2.5. Representative benzodiazepin-2-one, benzotriazepin-2-ones and γ-turn structures.

Diversity oriented synthesis of 1,3,4-benzotriazepin-2-ones **2.6** has now been achieved by an approach that avoids nitrogen protection, toxic reagents and harsh

conditions to modify the N1, N3 and C5 positions. Moreover, pyrrolo[1,2][1,3,4]benzotriazepin-6-ones **2.7** have also been synthesized from common linear precursors prepared from 1-(2-aminophenyl)-pent-4-en-1-one **2.8**. This method enhances the utility of amino ketone **2.8**, which has been quantitatively synthesized by a copper-catalyzed cascade addition of vinyl Grignard reagent on methyl anthranilate,[14,15] and used as valuable precursor to make substituted pyrroles **2.9**,[14] quinolines **2.10**,[15] pyrroloquinazolinones **2.11**,[14] 1,4-benzodiazepin-2-ones **2.12**,[5] and pyrrolobenzodiazepin-2-ones **2.13** (Figure 2.6).[5]

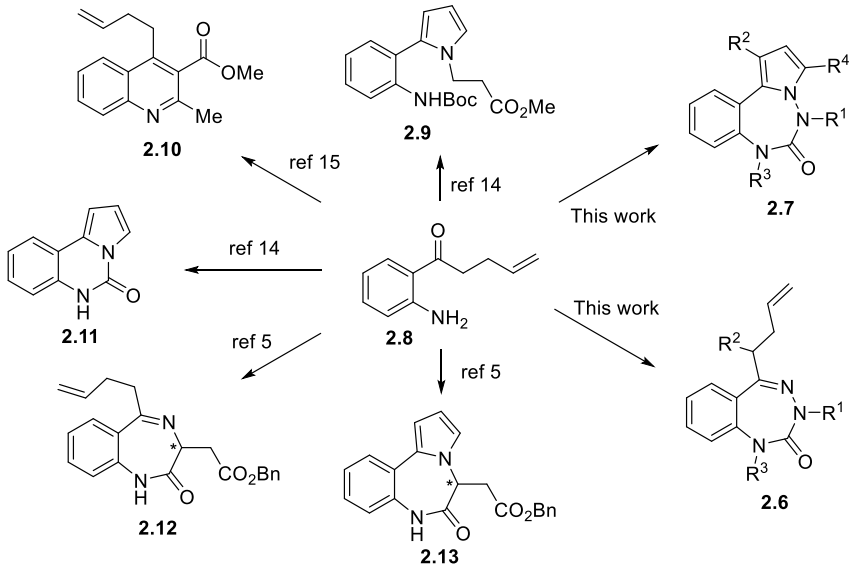


Figure 2.6. Amino ketone **2.8** as precursor for heterocycle synthesis.

Results and Dicsussion

Amino ketone **2.8** was acylated with activated carbazate **2.14**[16] in DCM in the presence of DIEA at room temperature to obtain the key precursor aza-glycinamide **2.15** in 71% yield on multi-gram scale (Scheme 2.1). Chemoselective alkylation of the semicarbazone nitogen of aza-glycinamide **2.15** was achieved using conditions previously developed for submonomer aza-peptide synthesis.[17] Various aza-amino amide analogs **2.16a-h** were thus synthesized from **2.15** using tetraethylammonium hydroxide as base and a diverse set of alkyl halides in THF (Table 2.I).[18] Although reactive and primary alkyl halides reacted at room temperature to give **2.16** in >73% yields, cyclohexylmethyl bromide required heating at reflux to obtain **2.16g** in 52%

yield. 1-Bromo-3-chloropropane (150 mol%) reacted with **2.15** and tetraethylammonium hydroxide (100 mol%) to give aza-chloropropylglycinamide **2.16c** in 79% yield with minimal amounts of cyclic urea **2.17c** from a second intramolecular alkylation of the aniline nitrogen.

Chemoselective alkylation of the ketone moiety of **2.16** without reaction on the aniline nitrogen was achieved using LiHMDS (250 mol%) to generate the dianion, which selectively reacted on carbon with various alkyl halides (200 mol%) in THF at 0°C for 1h to give branched ketones **2.18g-m** in 35-80% yields (Table 2.I). Selective alkylation of the ketone enolate may be due to the relative stability and hindered nature of the lithiated urea, which may interact respectively with the neighboring semicarbazone nitrogen and carbonyl oxygen in five and six membered ring chelates. Incomplete alkylation and difficulty in separating product from starting material may account for the lower yields using methyl and ethyl iodides.

1,3,4-Benzotriazepin-2-ones **2.6a-f** were prepared in 32-99% yields from aza-amino amides **2.16** and **2.18** by semicarbazone cleavage and cyclization under acidic conditions using 1.0 N aq. HCl in THF (Scheme 2.2, Table 2.II). Attempts to prepare benzotriazepinone from unsubstituted semicarbazone **2.15** were unsuccessful using similar conditions; instead, ions corresponding to oligomer were detected by HPLC-MS analysis of the reaction mixture. Semicarbazone alkylation may favor cyclization by lowering the barrier for urea isomerization to the required *E*-isomer.[19] α-Alkyl branched ketones **2.18** reacted slower in the cyclization to **2.6**, likely because the neighboring ketone is engaged in a hydrogen bond with the aniline NH that disfavors the orientation for nuclophilic attack.[20] The favored hydrogen bonded conformer was illustrated in a comparison of **2.16h** and **2.18l** in deuterium exchange NMR experiments using MeOD-d_4 in $CDCl_3$. After 20h, the amount of exchange of the aniline NH proton with deuterium was >95% for **2.16h** but <20% for **2.18l** under the same conditions. Employing EtOH to competitively hydrogen bond with ketone **2.18l** during the cyclization step, the reaction time was reduced and the yield increased; 22h and 53% yield in THF, versus 4h and 63% yield in EtOH.

Scheme 2.1. Synthesis and chemoselective alkylations of semicarbazone **2.15** and ketone **2.16**.

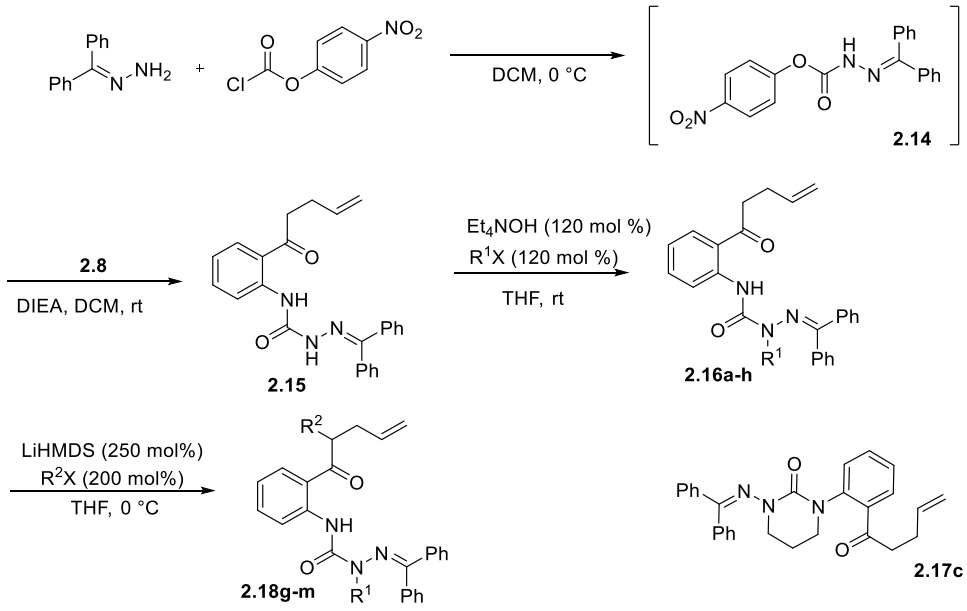


Table 2.I. *N*- and *C*-Alkylation steps to form **2.16** and **2.18**.

entry	R^1X (120 mol %)	yield **2.16** (%)	R^1	R^2X (200 mol %)	yield **2.18** (%)
a	CH_3I	100			
b	$BrCH_2CCH$	73			
c	$Br(CH_2)_3Cl$	79[a]			
d	$Br(CH_2)_4Cl$	88			
e	$Br(CH_2)_3OR$[b]	94			
f	BnBr	78			
g	$BrCH_2C_6H_{11}$	52[c]	$C_6H_{11}CH_2$	*p*-BrBnBr	72
h	*p*-BrBnBr	93	*p*-BrBn	$BrCH_2CO_2Me$	80
i			*p*-BrBn	$BrCH_2CHCH_2$	70
j			*p*-BrBn	$BrCH_2CCH$	68
k			*p*-BrBn	CH_3CH_2I	35
l			*p*-BrBn	CH_3I	47
m			*p*-BrBn	$BrCH_2CO_2tBu$	73

[a] Et_4NOH (100 mol %) R^1X (150 mol %); [b] R = $Si(Ph)_2tBu$; [c] reflux

After cyclization, the aniline nitrogen of triazepinones **2.6** was chemoselectively alkylated using *t*-BuOK (120 mol%) and different alkyl bromides (120 mol%) to give the tri-substituted 1,3,4-benzotriazepin-2-ones **2.19** in 85-100% yields (Table 2.II). The installment of propargyl, bromoaryl, chloroalkyl and carboxylate side chains has been demonstrated with particular interest to further diversify the 1,3,4-benzotriazepin-2-one scaffolds by future employment of such functional groups in orthogonal chemistry: e.g., CuAAC,[21] cross-coupling,[22] nucleophilic displacement,[23] and amide bond forming reactions, respectively. Such chemistry is being explored presently and will be reported in due time.

Scheme 2.2. Cyclisation of **2.16** and **2.18** and N1-alkylation of **2.6**.

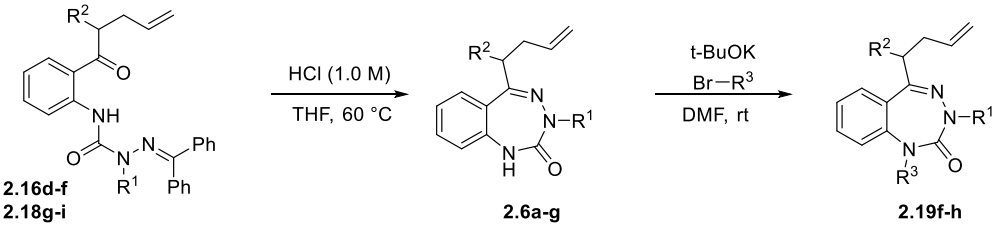


Table 2.II. 1,3,4-Benzotriazepin-2-ones **2.6** and **2.19**.

Entry	yield **2.6** (%)	R^1	R^2	R^3Br	yield **2.19** (%)
a	99	$(CH_2)_4Cl$	H		
b	69	$(CH_2)_3OH$ [a]	H		
c	40	*p*-BrBn	CH_2CO_2H [b]		
d	53[c]	*p*-BrBn	Me		
e	32	$CH_2C_6H_{11}$	*p*-BnBr		
f	90	Bn	H	CH_2CCH	85[d]
g	76	*p*-BrBn	CH_2CHCH_2	CH_2CO_2tBu	100
h		*p*-BrBn	CH_2CHCH_2	CH_2CCH	100

[a] Alcohol from OTBDPS. [b] Acid from CO_2Me. [c] 63% using EtOH. [d] Alkylation was performed in THF.

Pyrrolo[1,2][1,4]benzodiazepin-6-ones have garnered interest because of their biological and medicinal relevance. For example, pyrrolobenzodiazepinone (*S*)-**2.20b** has exhibited activity as a non-nucleoside HIV-1 reverse transcriptase inhibitor (Figure 2.7).[24] To the best of our knowledge, the aza-variant of this ring system has never been reported. Pyrrolobenzotriazepinones **2.7** were thus pursued to further

demonstrate the utility of the alkene function. Olefins **2.16** and **2.18** were respectively oxidized using Lemieux–Johnson conditions to give aldehydes **2.22a-d** in 76-84% yields.[25] Aldehydes **2.22** were then treated with 1.0 M aq. HCl in THF at 60°C to affect semicarbazone cleavage and intramolecular Paal-Knorr condensation. Pyrrolo[1,2-][1,3,4]benzotriazepin-6-ones **2.7** were isolated in 23-67% yields by column chromatography. The sterically hindered semicarbazone **2.22d** gave pyrrole **2.7e** in 30% yield along with a side product in 23% yield having a molecular ion and spectral properties consistent with imine dimer **2.21** (Figure 2.7). Chloro-pyrrole **2.7c** was isolated in 23% yield from **2.22c** using the acidic cyclization conditions and characterized by its four molecular ions corresponding to the bromine and chlorine isotopes, and the pyrrole proton singlet in the NMR spectrum. X-ray analysis of **2.7c** confirmed the structural assignment. Alternatively, by conducting the Paal-Knorr reaction in MeOH in the dark, pyrrolobenzotriazepinone **2.7d** was isolated in 52% yield.

To demonstrate the potential to further diversify pyrrolo[1,2][1,3,4]benzotriazepin-6-one **2.7**, analogous conditions were employed as described above for the alkylation of triazepinone **2.6**. The aniline nitrogen of pyrrolobenzotriazepinone **2.7b** was thus alkylated with potassium *tert*-butoxide and 1-bromo-4-chlorobutane in 84% yield.

Crystals of 1,3,4-benzotriazepin-2-one **2.19g** and the pyrrolo[1,2][1,3,4]benzotriazepin-6-one **7c** were grown by slow diffusion of *n*-hexane into samples in EtOAc, and subjected to X-ray structural analysis (Figure 2.8). Substitution of the diazepinone amino acid component by an aza-residue in triazepinone CCK antagonists has previously been used to amplify selectivity for the CCK_2 over CCK_1 receptors.[7] Better accommodation by the CCK_2 receptor of the achiral triazepinone, may be due to the aza-residue exhibiting adaptive chirality,[26] or a relatively flat geometry.

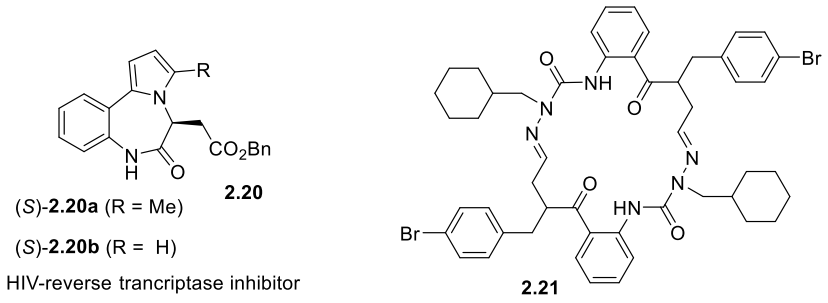
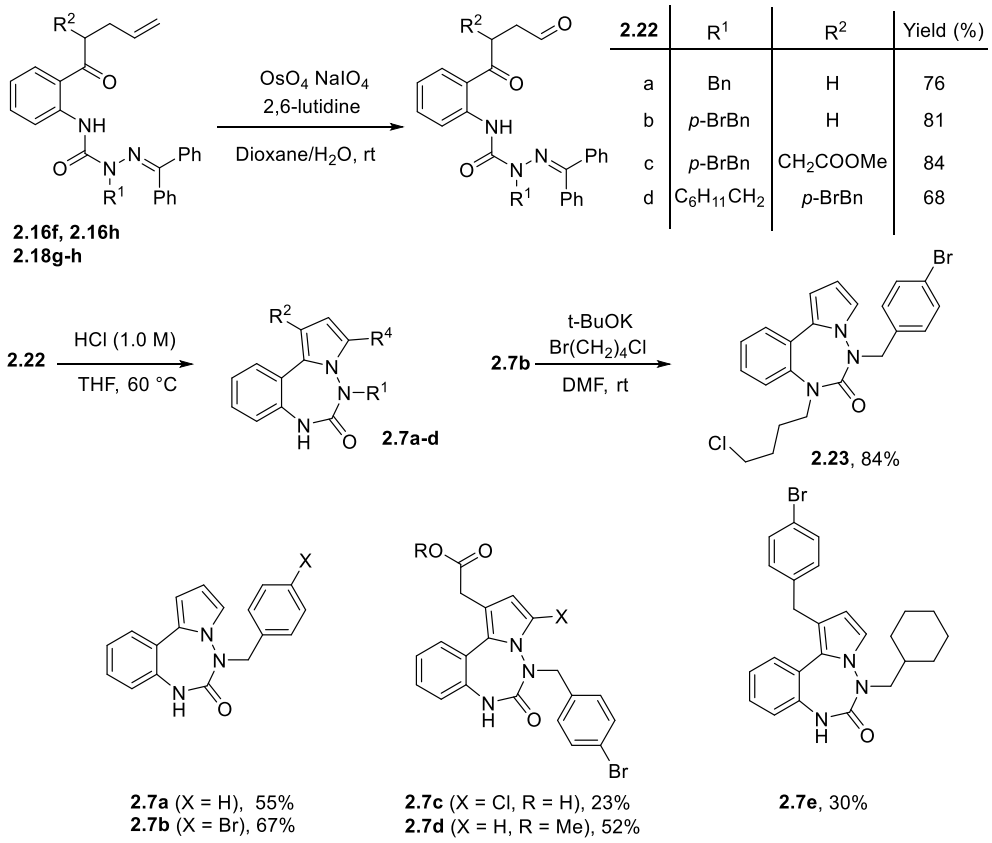


Figure 2.7. Pyrrolobenzodiazepinone **2.20** and macrocyle **2.21**.

Scheme 2.3. Synthesis and alkylation of pyrrolo[1,2][1,3,4]benzotriazepin-6-ones.



Employing X-ray data to probe the differences of their geometry a comparison of ϕ- and ψ-dihedral angle values has been made using tri- and di-substituted 1,3,4-benzotriazepin-2-ones **2.19g** and **2.5**, benzodiazepinones *R*- and *S*-**2.1a**, pyrrolobenzodi- and triazepinones **2.20a** and **2.7c**, as well as the central residues of ideal normal and inverse γ-turns (Figure 2.8, Table 2.III).[27] The addition of an N1 substituent on benzotriazepinone **2.5** had limited influence on the dihedral angles

relative to its di-substituted counterpart **2.19g**. In comparison to the relatively similar dihedral angles of benzodiazepinone **2.1a** and ideal γ-turns (ϕ = 75° ± 3, and ψ = –65° ± 3), benzotriazepinones **2.5** and **2.19g** deviated more significantly about the ψ torsion angle (ϕ = 75° ± 1, and ψ = –65° ± 9). Pyrrolobenzotriazepinone **7c** exhibited a ϕ torsion angle (–75° ± 3) that was more similar to that of an ideal inverse γ-turn than the value in pyrrolobenzodiazepinone *R*-**2.20a** (–75° ± 14); however, the ψ–torsion angle of **7c** differed by 40° away from that of an ideal inverse γ-turn, significantly more than that of its pyrrolobenzodiazepinone counterpart **2.20a** (65° ± 8). The dihedral angle values of the aza-amino acid residue in **2.7c** appear to be more closely related to that of the *i* + 2 residue of a type II' β-turn (ϕ = –80° ± 8, and ψ = 0° ± 27). The aza-amino acid residue nitrogen in the triazepinones adopted non-planar configurations existing out of the plane formed by its three neighboring atoms by ±0.361(1) Å for benzotriazepinone **2.19g** and ±0.015(2) Å for pyrrolobenzotriazepinone **2.7c**. For comparison, the deviation from planarity of the corresponding α-carbons of (*R*)- **2.1a,** (*S*)- **2.1a** and **2.20a** were respectively +0.497(2), –0.500(3) and ±0.441 Å, and illustrate that triazepinones **2.19g** and **2.7c** are chiral albeit flatter than their diazepinone counterparts.

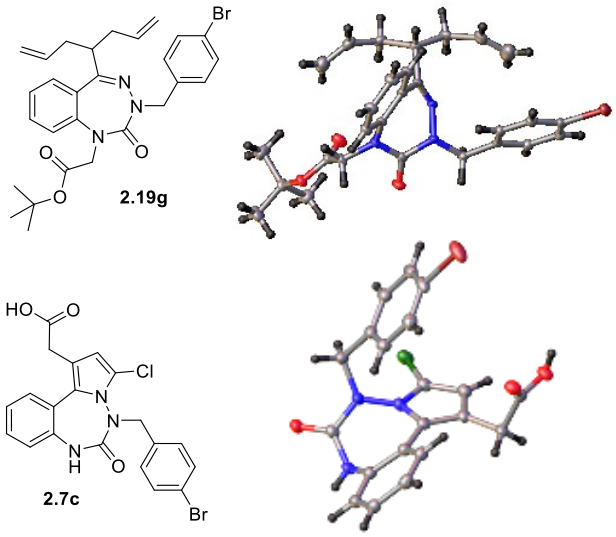


Figure 2.8. X-ray crystal structures of **2.19g** and **2.7c**.

Table 2.III. Crystal analyses with ϕ and ψ dihedral angles compared with ideal γ-turn.

Type of turn	ϕ	ψ	Ref
γ-turn	75	–65	27
Inverse γ-turn	–75	65	27
R-**2.1a**	75	–68	5
S-**2.1a**	–72	69	5
R-**2.20a**	–61	57	5
S-**2.20a**	61	–57	5
2.5	75	–57	10
2.19g	74	–56	-
2.7c	–72	27	-

Conclusion

In conclusion, chemoselective alkylation was key for conception of efficient diversity oriented strategies to make 1,3,4-benzotriazepin-2-one and pyrrolo[1,2][1,3,4]benzotriazepin-6-one analogs. Without nitrogen protection under relatively mild conditions benzotriazepinones were made in 4-7 steps and 4-60 % yields from methyl anthranilate. Using groups suitable for further functionalization, a set of scaffolds was synthesized and shown by X-ray analysis to have potential for γ-turn mimicry. Biological activity of triazepinone library members is under investigation and will be reported in due time.

ASSOCIATED CONTENT

Supporting Information

Experimental details, spectroscopic characterization for all compounds, and x-ray crystallographic data for **2.19g** and **2.7c**. The Supporting Information is available free of charge on the ACS Publications website.

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Notes

The authors declare no competing interest.

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Chapitre 3 : Synthèse de peptidomimétiques de la séquence Bip-Lys-Tyr, basés sur un motif benzotriazépinique, et évaluation de leur capacité à moduler le système urotensinergique.

3.1. Mise en contexte des tests biologiques.

Le test biologique le plus couramment utilisé pour les composés modulateurs du système urotensinergique, et notamment pour la discrimination de hUII ou URP, consiste à mesurer les contractions induites sur l'aorte de rat *ex vivo*. Dans le cadre de mon doctorat, j'ai donc été amené à apprendre puis à effectuer ce test. Ainsi les 1,3,4-benzotriazépin-2-ones, synthétisées et testées dans la cadre de l'article 2, ont été préparées dans le laboratoire du Pr. William D. Lubell puis examinées comme agoniste et antagoniste de hUII et URP dans le laboratoire du Pr. David Chatenet. Afin d'offrir une meilleur compréhension sur cette partie de mon doctorat, une procédure, détaillée et illustrée, introduit l'Article 2.

3.2. Procédure détaillée des tests de contractions aortiques

2.2.1. Appareillage

Les données sont collectées sur un polygraphe GRASS modèle 7E thermostaté à 37°C, composé de 5 cuves de 5mL (Figure 3.1), qui sont oxygénées, surmontées de capteurs de force de traction reliés à un processeur, lui-même relié à une table traçante (Figure 3.2)

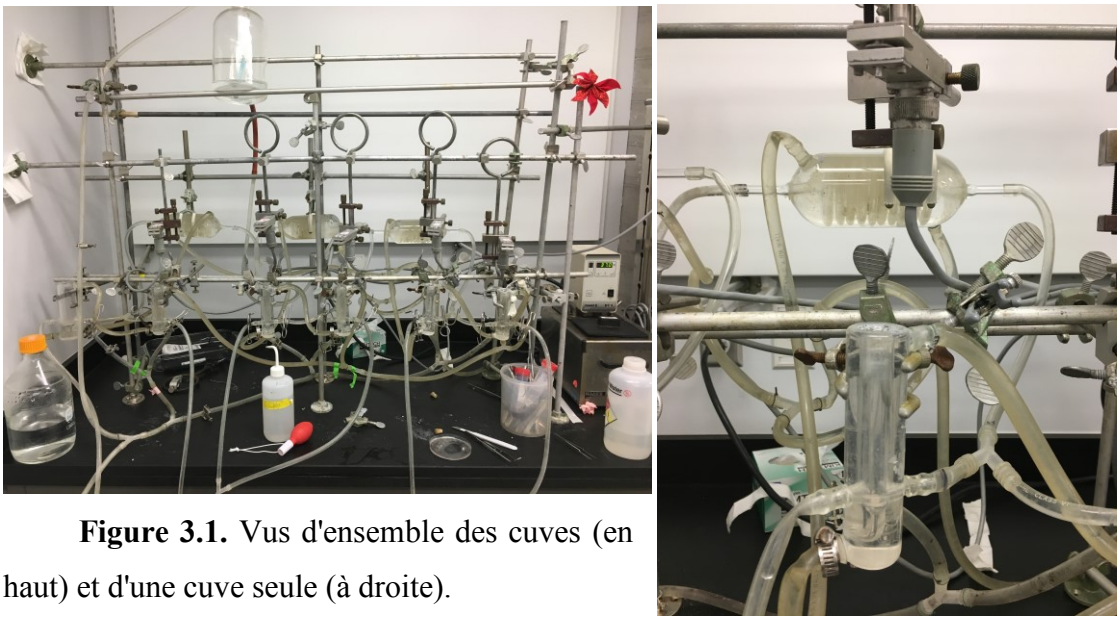


Figure 3.1. Vus d'ensemble des cuves (en haut) et d'une cuve seule (à droite).

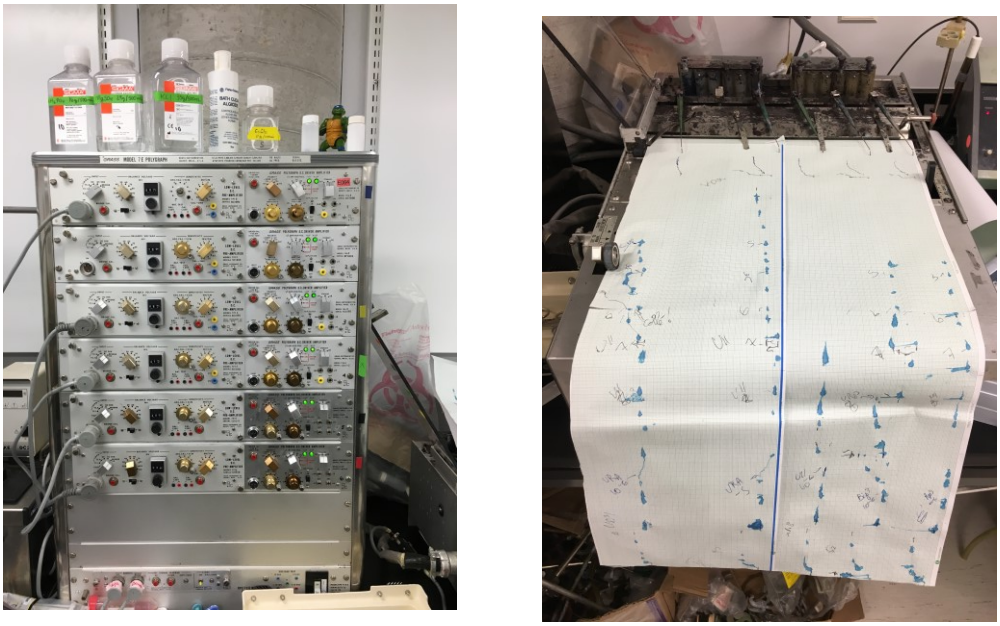


Figure 3.2. Processeur (à gauche) et table traçante (à droite).

Les capteurs de force de traction (Figure 3.3) sont étalonnés avec un poids de 1,0 g, correspondant à la force initiale soumise aux tronçons d'aorte et donc au niveau 0 de la table traçante. Puis un poids de 0,7 g est ajouté, correspondant à une hauteur de 4 cm sur la table traçante (Figure 3.3).

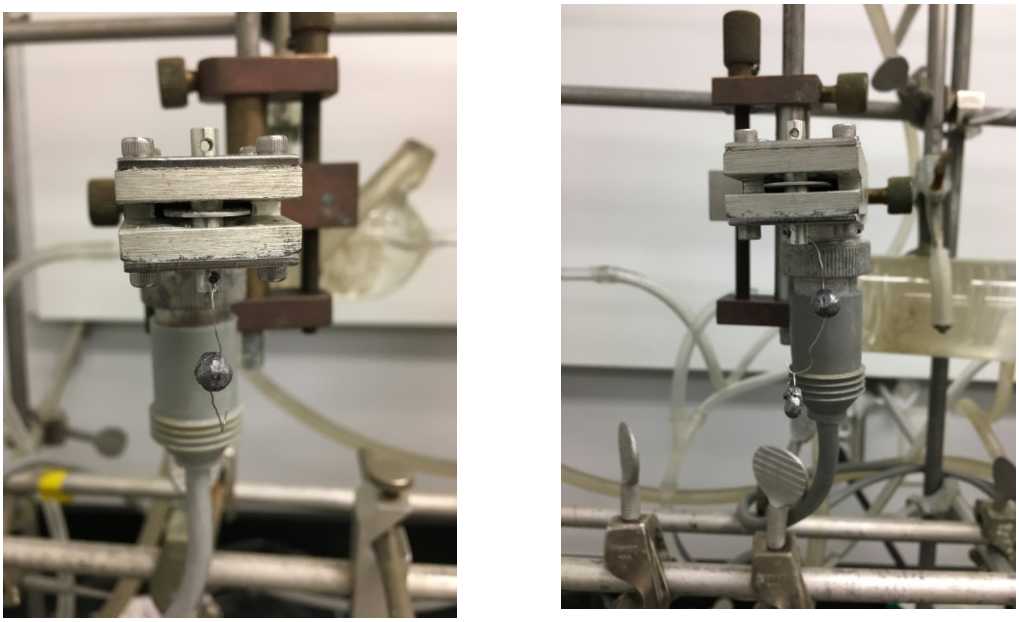


Figure 3.3. Étalonnage du capteur de force de traction.

3.2.2. Préparation de l'aorte de rat

L'aorte de rat a été prélevée, par le doctorant Étienne Billard, sur des rats Sprague−Dawley mâles, pesant entre 250 et 300g, gardés en groupes dans des cages à illumination (12h de lumière – 12h d'obscurité), humidité et température (21 – 23 °C) contrôlées, et ayant un accès libre à de l'eau et de la nourriture. L'aorte est gardée au réfrigérateur et utilisée dans les 24h.

Dans un premier temps, l'aorte doit être nettoyée des tissus adipeux qui l'entoure ainsi que de l'endothélium et des résidus internes qu'elle contient. Pour ce faire, un scalpel et une pince sont employés et il est très important de ne pas entailler l'aorte, au risque de ne plus pouvoir l'utiliser. La partie interne est retirée en exerçant une pression externe douce. Une fois l'aorte propre et rincée, elle est découpée, au niveau de sa partie supérieure, en tronçons de 4mm (Figure 3.4).

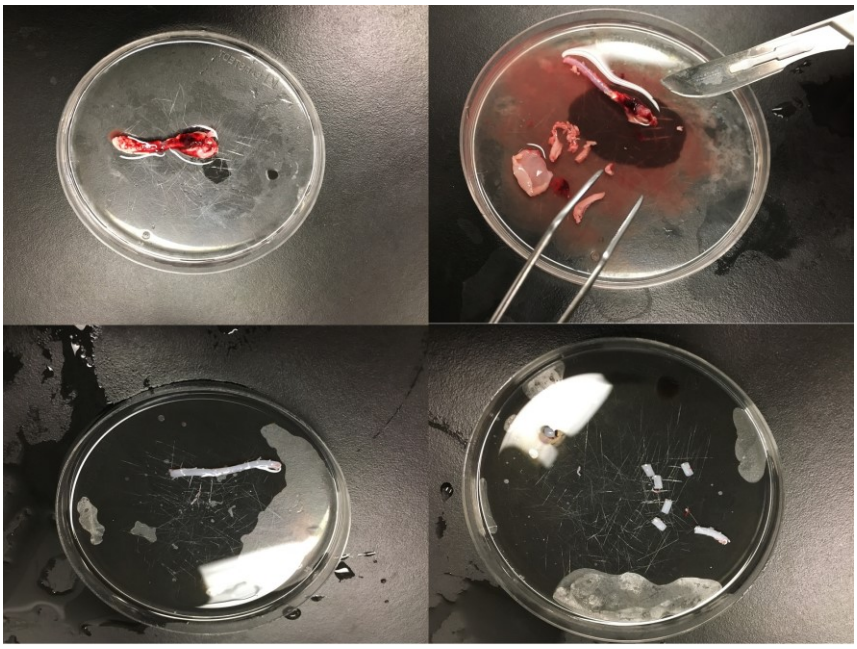


Figure 3.4. Préparation de l'aorte de rat.

3.2.3. Montage de l'aorte sur le système

Les tronçons d'aorte sont enfilés sur deux crochets fermés. L'un est relié à un support fixe et l'autre est relié par une ficelle au capteur de traction. Ils sont ensuite plongés dans les cuves de 5mL, contenant une solution tampon de Krebs-Henseleit (Figure 3.5), puis lavés cinq fois, à intervalle de 15 minutes, avec cette solution. Finalement une traction équivalente à 1,0 g est exercée sur l'aorte.

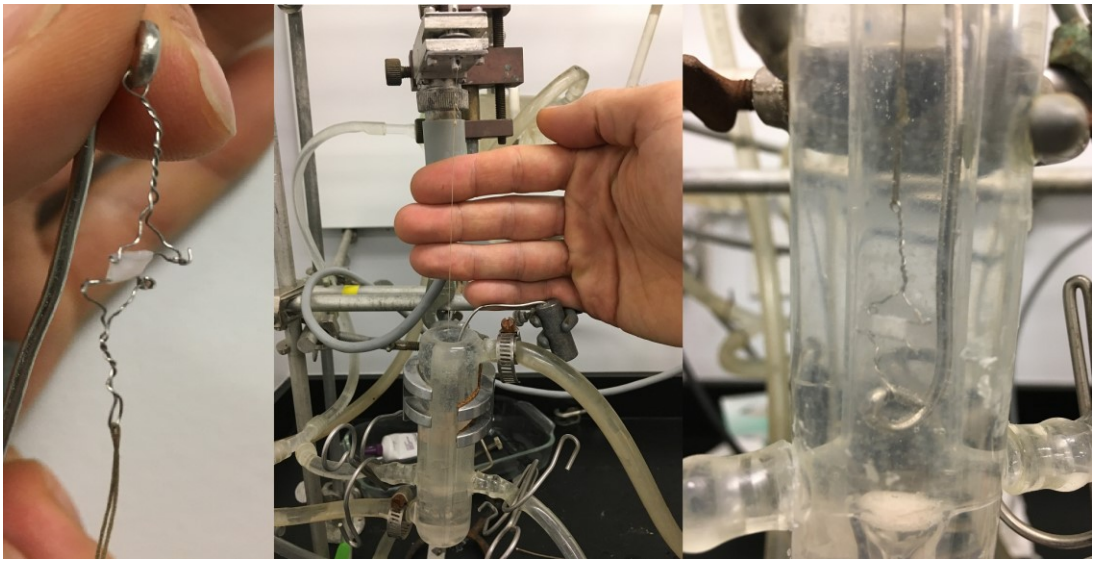


Figure 3.5. Montage des tronçons d'aorte sur le système.

3.2.4. Mesure des contractions induites sur l'aorte

Les composés sont introduits dans la cuve, en solution dans l'eau ou dans un système 10% DMSO dans l'eau, à l'aide d'une micropipette (Figure 3.6). Dans un premier temps, l'aorte est soumise à une addition de KCl, pour obtenir une concentration de 40mM dans la cuve (Figure 3.6, cadre rouge). Ensuite, une solution (10^{-5} M) de 1,3,4-benzotriazépin-2-one est ajoutée dans la cuve et le système est laissé pendant au moins 15 minutes afin d'obtenir l'équilibre et mesurer l'effet agoniste si présent (Figure 3.6, cadre bleu). Finalement, des solutions (de 10^{-11} à 10^{-6} M) de peptides hUII ou URP sont ajoutées graduellement pour construire la courbe dose-réponse (Figure 3.6, cadre vert) et ainsi mesurer l'effet antagoniste. Les mesures de contractions sont mesurés en millimètre puis reporter comme le ratio (en %) entre la mesure exprimé et celle induite par KCl.

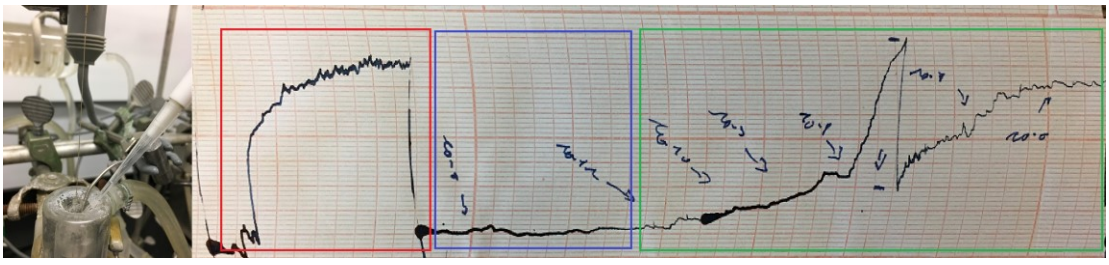


Figure 3.6. Exemple de résultats bruts pour la mesure des contractions induites.

3.3. Technologie BRET

La technologie BRET (Bioluminescence Resonance Energy Transfer) a été utilisé dans cette article pour quantifier l'activation des protéines G_{12} et G_q par hUII et URP en présence de certaines benzotriazépinones. La technologie BRET permet d'identifier si la portion α et la portion $\beta\gamma$ des protéines G sont proches, pour ce faire la portion $\beta\gamma$ possédent une enzyme Renilla luciferase qui va oxyder la coelenterazine et emettre une longeur d'onde à 480 nm, celle-ci va exciter la GFP (Green Fluorescent Protein) attaché à la portion α si celle-ci se trouvent à proximité ($d < 10$ nm), et emettre une fluorescence à 530 nm qui peut-être mesurer.

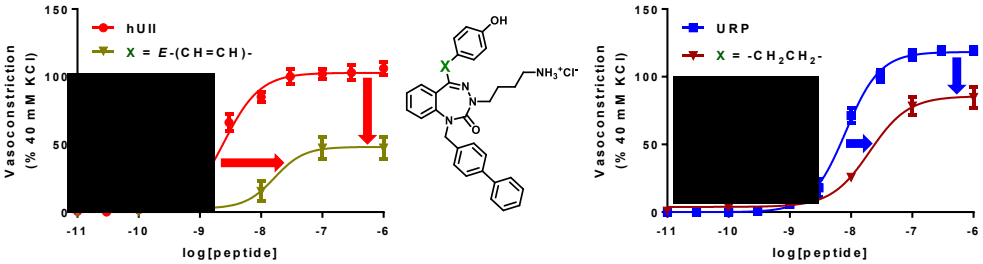
3.4. Mise en contexte de l'article 2

L'article 2 décrit la synthèse de 1,3,4-benzotriazépin-2-ones, suivant la méthodologie développée dans l'article 1, et l'obtention d'une librairie de 26 composés benzotriazépiniques. Ces petites molécules sont basées sur l'idée d'utiliser ce motif comme mime peptidique et imitant la conformation de tour γ. En effet, on retrouve celle-ci dans la séquence biologiquement active Bip-Lys-Tyr, présent dans l'Urocontrin ([Bip^4]URP) qui est un modulateur allostérique du récepteur urotensinergique.

Lors de tests *ex-vivo* sur la contraction induite par hUII ou URP sur l'aorte de rat, certains composés ont montré une activité comme antagoniste sélectif de hUII et URP. Ainsi, l'utilisation du cœur achiral des benzotriazépinones peut servir comme mime peptidique possédant une conformation de tour γ et son utilisation sur la modulation du système urotensinergique pourra servir dans le développement et la compréhension de cette cible biologique.

Article 2

Antoine Douchez ,A.; Billard, E.; Hébert, T. E.; Chatenet, David; Lubel, W. D. Design, synthesis and biological assessment of biased allosteric modulation of the urotensin II receptor using achiral 1,3,4-benzotriazepin-2-one turn mimics. *J. Med. Chem.* **2017**, *60*, 9838–9859.



Design, synthesis and biological assessment of biased allosteric modulation of the urotensin II receptor using achiral 1,3,4-benzotriazepin-2-one turn mimics.

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Abstract

Benzotriazepin-2-ones were designed to mimic the suggested bioactive γ-turn conformation of the Bip-Lys-Tyr tripeptide in Urocontrin ([Bip^4]URP), which modulates the urotensin II receptor (UT) and differentiates the effects of the endogenous ligands urotensin II (UII) and urotensin II-related peptide (URP). Twenty-six benzotriazepin-2-ones were synthesized by acylation of anthranilate-derived amino ketones with an aza-glycine equivalent, chemoselective nitrogen functionalization and ring closure. Several mimics exhibited selective modulatory effects on hUII- and URP-associated vasoconstriction in an *ex-vivo* rat aortic ring bioassay. The C^5 *p*-hydroxyphenethenyl benzotriazepin-2-one **3.20g** decreased hUII potency and efficacy without changing URP induced vasoconstriction. Its saturated phenethyl counterpart **3.23g** decreased URP potency without influencing hUII-

mediated contraction. To our knowledge, **3.20g** and **3.23g** represent the first achiral molecules that modulate selectively hUII and URP biological activities. Effectively synthesized, benzotriaepin-2-one turn mimics offer potential to differentiate the respective roles, signaling pathways, and phenotypic outcomes of hUII and URP in the UT system.

Introduction

Benzodiazepine and benzotriazepine scaffolds offer potential as mimics of peptide turn conformations.[1,2,3] Benzodiazepines have notably achieved wide recognition as "privileged" structures,[4,5,6,7,8] because of their propensity to bind and exhibit activity at various receptor targets. Although their achiral aza-counterparts may be inherently less costly to produce,[9] benzotriazepines have been less frequently employed in medicinal chemistry programs.[10,11,12] For examples, benzotriazepin-2-one **3.1** was employed in the development of achiral orally active cholecystokinin-2 (CCK_2) antagonists,[11,12] and 5-cyclohexyl benzotriazepin-2-one derivative **3.2** exhibited activity as a parathyroid hormone-1 receptor antagonist (Figure 3.7).[10] Benzotriazepin-2-ones have also been claimed to possess psycho-stimulant, antidepressant, anorexigenic, and antihypertensive activities.[13] Benzotriazepin-2-ones merit thus further investigation as scaffolds for generating ligands to biologically relevant receptors.

The urotensin II receptor (UT), a member of the G protein-coupled receptor (GPCR) family, binds, in humans two endogenous cyclic peptide ligands: urotensin II (hUII, H-Glu-Thr-Pro-Asp-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and urotensin II-related peptide (URP, H-Ala-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH).[14] Expressed in the circulatory, excretory, and central nervous systems, UT plays important roles in the physiological regulation of various organ systems, particularly the cardiovascular system.[15] Among various activities, hUII is currently considered as one of the most potent natural vasoconstrictive peptides.[16] Both hUII and URP have been linked to several pathologies.[17,18,19] Modulation of UT, particularly antagonism,[20,21] has thus emerged as an intriguing means for developing therapy to treat atherosclerosis,[2223]

pulmonary arterial hypertension,[24,25] and metabolic syndrome,[26] as well as cardiac,[27,28,29] and renal failure. [30,31] In clinical trials, however, UT antagonist drug candidates have shown limited efficacy against renal ischemia,[32] diabetic nephropathy,[33] and asthma,[34] in humans.[35] Their limited success may be due in part to their inability to discriminate selectively between UII- and URP-mediated biological activities, which have been recently shown to diverge with respect to transcriptional modulation, cell proliferation, and myocardial contractility.[36,37,38]

Differing only in the sequence and length of their *N*-terminal tails, UII and URP have been predicted to adopt similar ring conformations in aqueous solution.[39] The conformers of UII and URP, both were shown to similarly fluctuate between relatively less ordered "open" and more folded geometry of similar energy, albeit the folding of the additional tail sequence of UII augmented the barrier between these states. The different biological activity of UII and URP was thus suggested to result from distinct interactions with UT rather than alternate ring conformations.[38] Contingent on receptor binding,[40,41] their common Trp-Lys-Tyr motif has been proposed to adopt an inverse γ-turn within a specific pocket that has been linked to the molecular pharmacology of both UII and URP.[42]

The concept that the endogenous ligands hUII and URP induce distinct conformational changes of UT that lead to specific signaling pathways and phenotypic activity underlines the necessity for selective modulators to define their independent roles in normal physiology and disease states such as atherosclerosis and heart failure. Towards this goal, peptide-based allosteric modulators derived from URP, such as **3.3** ([Bip^4]URP, Bip = 4,4′-biphenylalanine)[43] and **3.4** ([Pep^4]URP, Pep = (phenylethynyl)phenylalanine} [44] decreased the maximal contractile response without altering significantly the potency of hUII and without a noticeable effect on URP-induced vasoconstriction. Employing the cyclic octapeptide UII(4−11), which is the shortest sequence of the parent peptide that possesses full biological activity,[45] azasulfurylpeptide analogs (e.g., [AsNal(2')^7]UII(4-11), AsNal(2') = azasulfuryl-2-naphthylalanine) were prepared and found to reduce both UII and URP-induced contraction without affecting potency.[46] Urotensin II core mimics *c*[Trp-Lys-Tyr-

Gly-ψ(triazole)-Gly] and *c*[Phe-Trp-Lys-Tyr-Gly-ψ(triazole)-Gly] modulated selectively URP-mediated vasoconstriction without affecting UII-induced contractions.[47] In addition, towards the design of small molecule modulators, pyrrolodiazepinones were conceived to mimic the Bip-Lys-Tyr inverse γ-turn motif of **3.3** and demonstrated potential to differentiate respectively the biological activity of hUII and URP.[48] For example, pyrrolodiazepinone **3.5** reduced significantly the maximum contractile response with slight decrease of the hUII potency, yet only mildly increased the efficacy and slightly decreased the potency of URP.

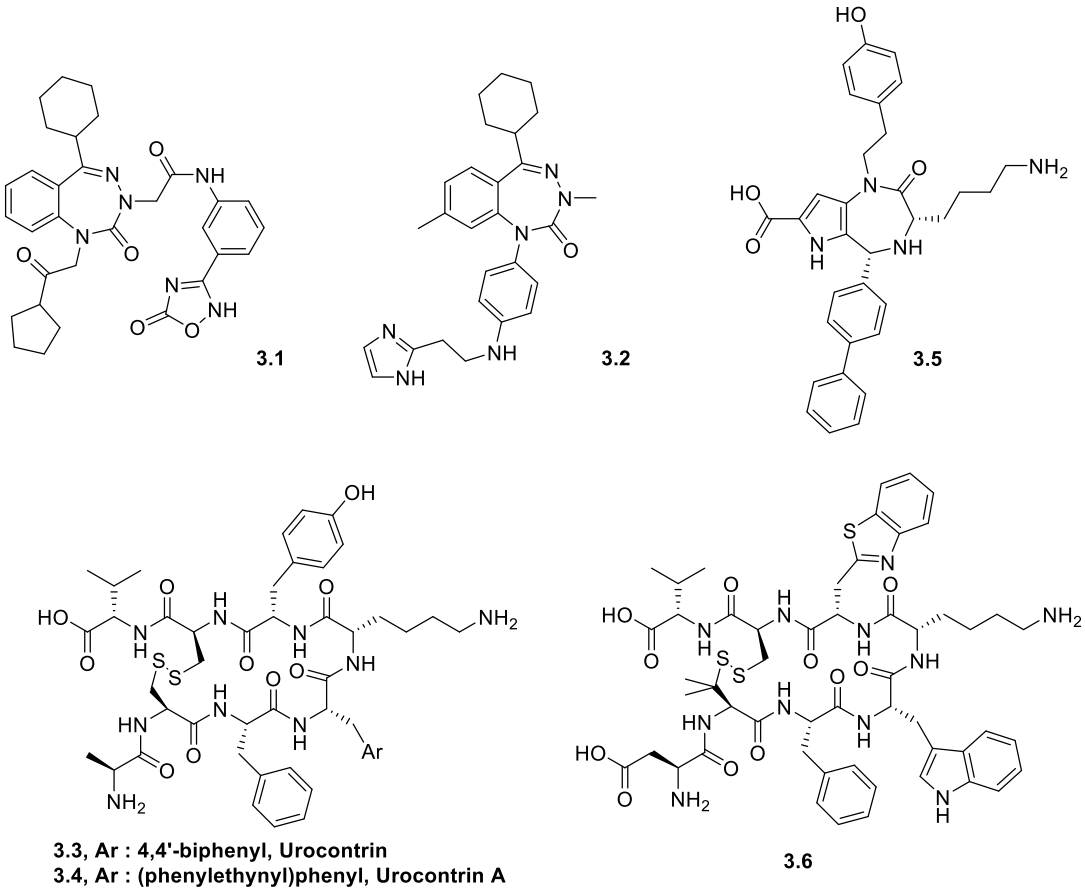


Figure 3.7. Benzotriazepin-2-one, pyrrolodiazepinone, URP and UII(4-11) derivatives.

To further study the structural determinants for differential modulation of the activity of UII and URP at UT, we designed a series of benzotriazepine peptidomimetics based on the tripeptide Bip-Lys-Tyr motif of **3.3** and the structures

of active pyrrolodiazepinone analogs. Considering that replacement of tyrosine by benzothiazolylalanine (Btz) gave a UT super agonist (e.g., **3.6**, H-Asp-*c*[Pen-Phe-Trp-Lys-Btz-Cys]-Val-OH) with high potency,[49] a benzothiazole residue was also installed on certain benzotriazepine candidates. Similar to their pyrrolodiazepinone counterparts, benzotriazepines have exhibited potential to mimic ideal inverse γ-turn geometry.[1,50] Substitution of the chiral α-carbon by nitrogen was however expected to simplify ligand preparation and to offer potential to explore adaptive chirality in ligand binding.[51,52] Examination of the influence of the benzotriazepines on hUII- and URP-induced aortic ring contractions has validated the design strategy, and provided a novel class of achiral small molecule modulators of UT activity.

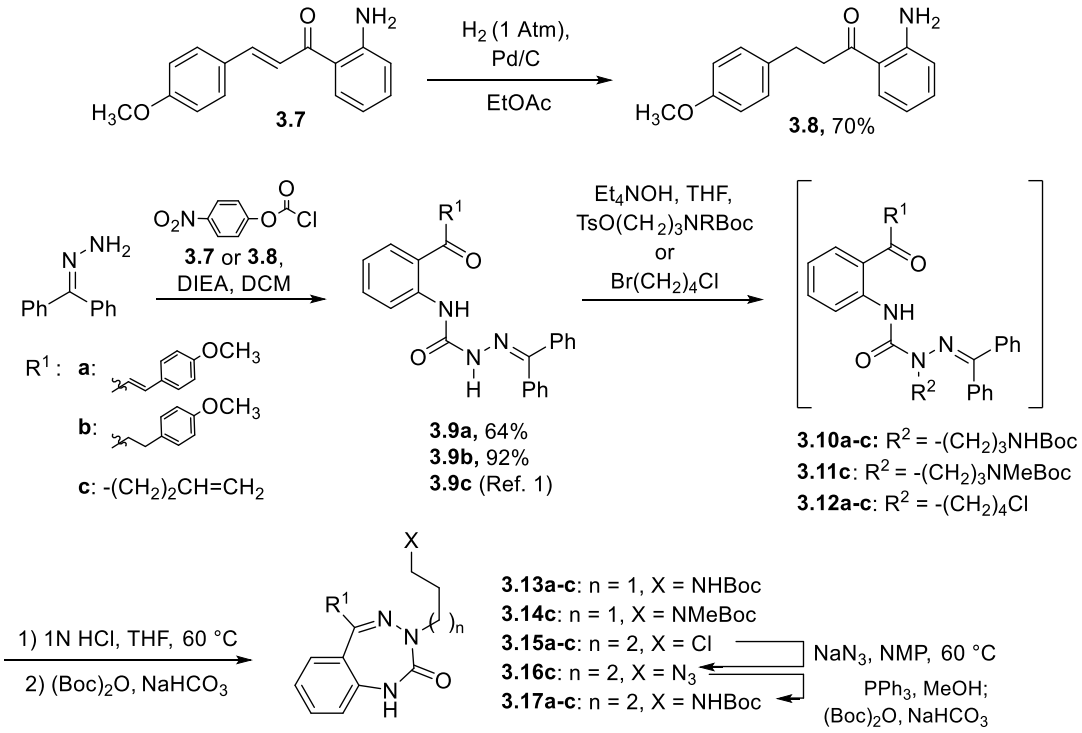
Results and Discussion

Chemistry.

Benzotriazepin-2-ones were designed to mimic the Bip-Lys-Tyr tripeptide sequence of **3.3** and synthesized by routes featuring chemoselective nitrogen alkylation to install pharmacophore mimics of the Bip and Lys side chains (Scheme 3.1-Synthesis of naphthylmethyl benzotriazepin-2-ones tripeptide mimics **3.47-3.49**.Scheme 3.**4**).[53] Biphenylmethyl and 1- and 2-naphthylmethyl groups were examined as surrogates of the Bip residue and introduced by alkylation of the benzotriazepin-2-one N^1-position with the corresponding bromides and *t*-BuOK in DMF.[1] Amino and *N*-(methyl)amino propyl and butyl chains were studied to explore the length and lipophilicity of the Lys residue and installed by chemoselective alkylation of a linear semicarbazide precursor. The Tyr side chain was introduced by an aldol condensation on *o*-aminoacetophenone, followed by alkene reduction. In addition, a benzothiazole side chain was constructed from 1-(2-aminophenyl)-pent-4-en-1-one. This olefin was obtained by copper-catalyzed cascade addition of vinyl Grignard reagent on methyl anthranilate,[54] and employed in an approach featuring a late stage Lemieux-Johnson olefin oxidation,[55,56] followed by oxidative cyclization using 2-aminothiophenol and iodine.[57] In total, twenty-six benzotriazepin-2-ones were prepared to mimic both the Bip-Lys-Tyr and Bip-Lys-Btz motifs.

1-(2-Aminophenyl)-3-(4-methoxyphenyl)propan-1-one (**3.8**) was obtained in 70% yield by hydrogenation of 2'-amino chalcone **7** over palladium-on-carbon (Scheme 3.1).[58,59]

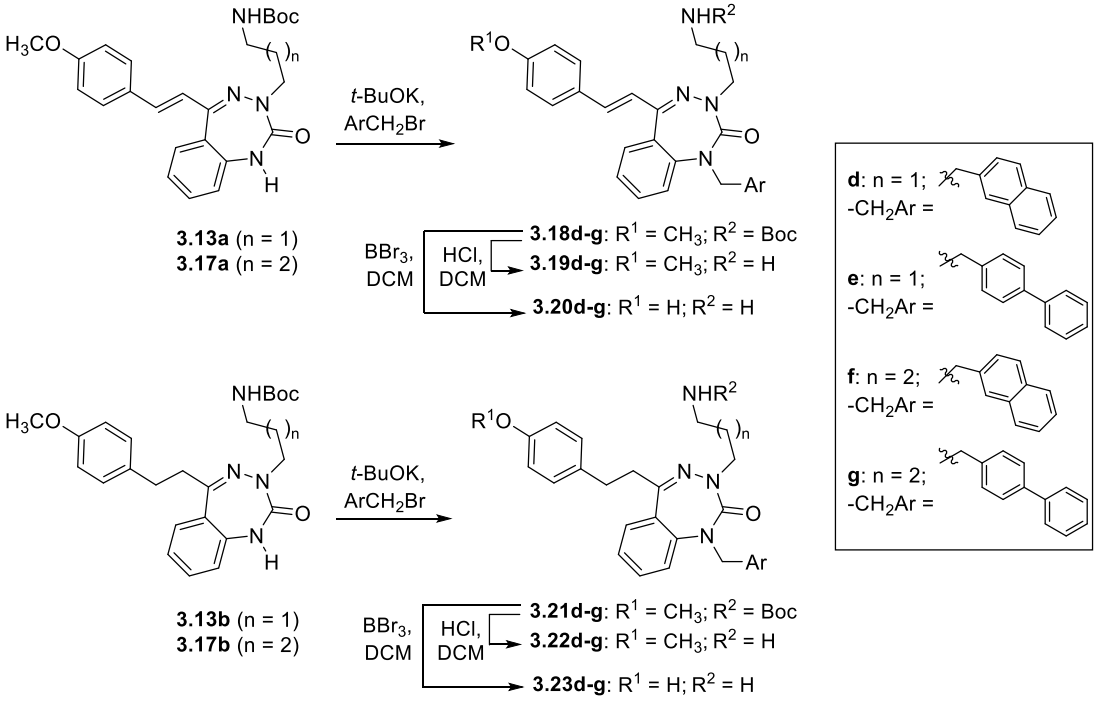
Scheme 3.1. Synthesis of N^1-H-benzotriazepin-2-ones **3.13**, **3.14c** and **3.17**.



Both **3.7** and **3.8** were reacted with the activated carbazate from benzophenone hydrazone and *p*-nitrophenyl chloroformate using conditions previously described for the preparation of semicarbazone **3.9c** to give respectively **3.9a** and **3.9b** in 64% and 92% yield.[1] Amino alkyl benzotriazepinones **3.13**, **3.14** and **3.17** were synthesized from aza-glycinamides **3.9** by a route commencing with chemoselective alkylation of the semicarbazone nitrogen,[60] followed by semicarbazide liberation and cyclization under acidic conditions. To introduce the four-carbon lysine side chain, alkylation was performed using 1-bromo-4-chlorobutane to provide N^3-chlorobutyl benzotriazepin-2-ones **3.15a** and **3.15b** in respectively quantitative and 86% yields. Displacement of the chloride by sodium azide in a 4:1 NMP/H_2O mixture at 60°C, followed by Staudinger reduction,[61] and amine protection with $(Boc)_2O$ furnished benzotriazepin-2-ones **3.17a** and **3.17b,** respectively in 45% and 67% yields.

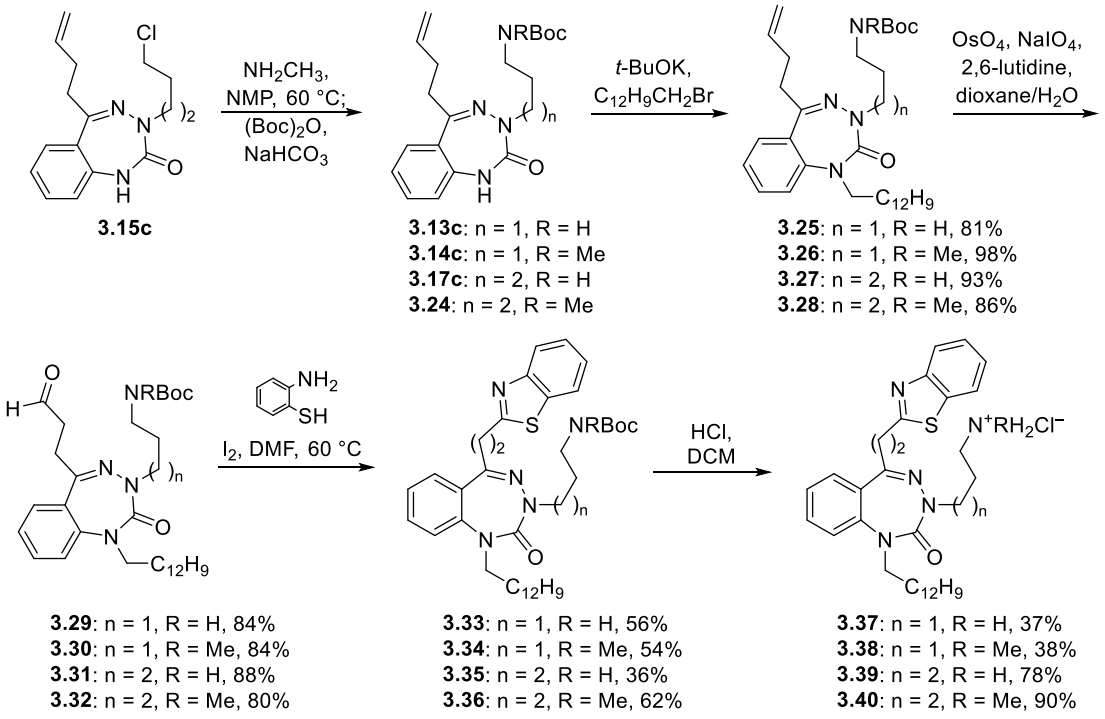
Employing 3-(4-chlorobutyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.15c**),[1] the azide was introduced under the same conditions using DMF instead of NMP, and azide **3.16c** was isolated in 75% yield, prior to Staudinger reduction and Boc protection to provide benzotriazepin-2-one **3.17c** in 71% yield. Moreover, nucleophilic substitution of chloride **3.15c** with methylamine in 4:1 NMP/H_2O at 60°C, followed by the protection with $(Boc)_2O$ gave *N*-methyl amino butyl benzotriazepin-2-one **3.24** in 75% yield (Scheme 3.3). In contrast to reaction with 1-bromo-4-chlorobutane, attempts to prepare benzotriazepin-2-ones by alkylation of semicarbazone using 1-bromo-3-chloropropane led to decomposition during acid mediated cyclization likely due to intramolecular chloride displacement to form pyrazolidine byproducts. The three carbon ornithine side chain was instead incorporated by alkylation with 3-*N*-(Boc)amino and 3-*N*-Boc-*N*-methylamino propyl tosylates, cyclization with HCl in THF at 60°C, and protection of the amine with $(Boc)_2O$ to furnish respectively benzotrazepin-2-ones **3.13a-c** and **3.14c** in 18-37% overall yields.

Scheme 3.2. Benzotriazepin-2-one alkylation and deprotection.



With C^5-*p*-methoxyphenethyl and phenethenyl benzotriazepin-2-ones **3.13a-b** and **3.17a-b** in hand, the N^1-position was respectively alkylated with *p*-phenyl benzyl bromide and 2-(bromomethyl)naphthalene using *t*-BuOK in DMF at room temperature to furnish benzotriazepin-2-ones **3.18d-g** and **3.21d-g** in 46-92% yields (Scheme 3.2). *p*-Methoxyphenyl benzotriazepin-2-ones **3.19d-g** and **3.22d-g** were obtained in 46-99% yields by selective removal of the Boc group using HCl gas in dichloromethane. Alternatively, the phenol was liberated and Boc group removed on treatment of **3.18** and **3.21** with boron tribromide in dichloromethane to provide tripeptide mimics **3.20d-g** and **3.23d-g** in 50-84% yields.

Scheme 3.3. Synthesis of benzothiazole benzotriazepin-2-one mimics **3.37-3.40**.[a]



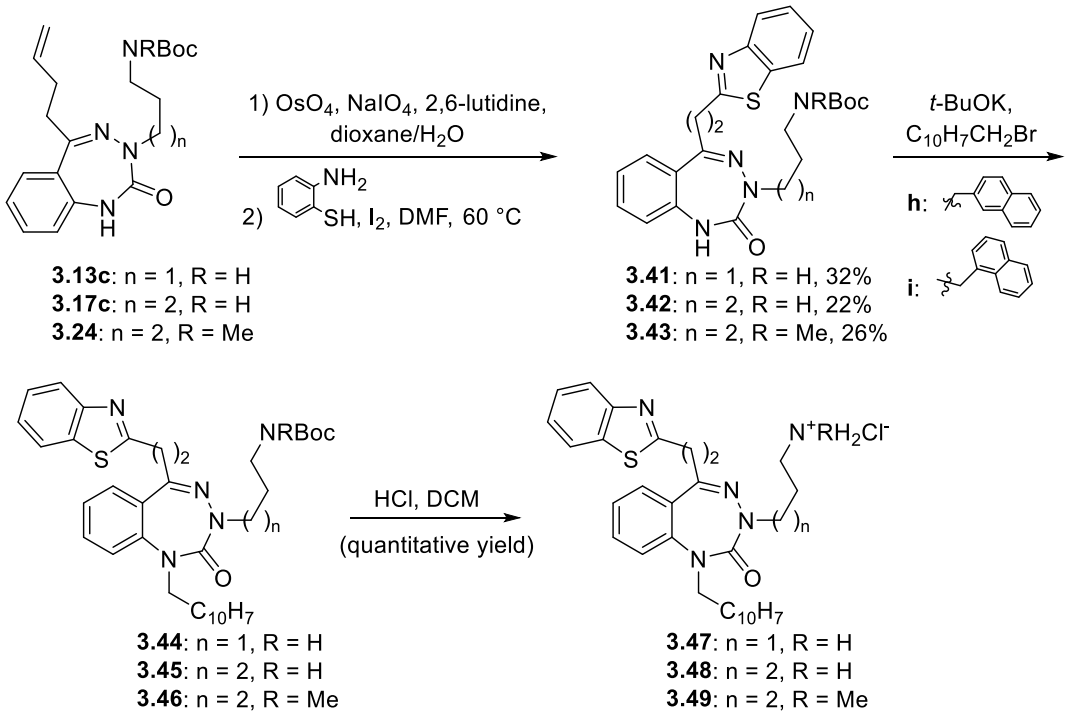
Considering the potency of benzothiazole alanine (Btz) super agonist **3.6**,[49] a 2-benzothiazole ethyl side chain was introduced at the benzotriazepine C^5-position (Scheme 3.3). After installation of a *p*-phenyl benzyl substituent at the N^1-position by alkylation with the corresponding bromide as described above, the C^5 butenyl chains

[a] $C_{12}H_9CH_2$: 4-phenylbenzyl

of **3.25-3.28** were oxidized using osmium tetroxide and sodium periodate to give the corresponding aldehydes **3.29-3.32** in 80-88% yields. Such modified Lemieux-Johnson conditions gave optimal yields at 4 hours; longer reaction times led to side-products likely due to intramolecular cyclisation on route to pyrrolobenzotriazepin-2-one.[1] Benzothiazoles **3.33-3.36** were synthesized in 36-62% yields by treating aldehydes **3.29-3.32** with 2-aminothiophenol followed by iodine in DMF at 60°C. Removal of the Boc protection with HCl gas in dichloromethane gave respectively benzotriazepin-2-one Bip-Orn-Btz tripeptide mimics **3.37** and **3.38** (37% and 38% yields) and Bip-Lys-Btz counterparts **3.39** and **3.40** (78% and 90% yields).

Attempts to diversify the N^1-position after introduction of the benzothiazole moiety at C^5 were prevented by the limited stability of aldehydes from the Lemieux-Johnson oxidation of olefins **3.13c**, **3.17c** and **3.24**; however, benzothiazoles **3.41-3.43** could be synthesized in 22-32% yields by minimizing the oxidation time to one hour and treating crude aldehyde after aqueous extraction with 2-aminothiophenol and iodine. Alkylation of the N^1-position with 1- and 2-(bromomethyl)naphthalenes using *t*-BuOK in DMF, followed by removal of the Boc group from benzotriazepin-2-ones **3.44-3.46**, gave tripeptide mimics **3.47-3.49** in 42-79% yields.

Scheme 3.4. Synthesis of naphthylmethyl benzotriazepin-2-ones tripeptide mimics **3.47-3.49**.



Biology.

With the goal to identify achiral small molecule UT modulators that exhibit selective ability to block UII- and URP-mediated action, benzotriazepin-2-ones were examined in an *ex-vivo* rat aortic ring contraction assay, which has commonly been used to screen for UT ligands.[43,45,46,47] Initially, the benzotriazepin-2-one derivatives were examined for agonist activity and shown to induce rat aortic ring contractions no more than 8% at 10^{-5} M. The potential for benzotriazepin-2-ones to modulate hUII- and URP-induced vasoconstriction was subsequently examined by exposing rat aortic rings to the mimic at 10^{-5} M for at least 15 minutes to establish a binding equilibrium, and by subsequently measuring cumulative concentration-responses on increasing concentrations (10^{-11} to 10^{-6} M) of hUII or URP (Table 3.II, Figure 3.8).

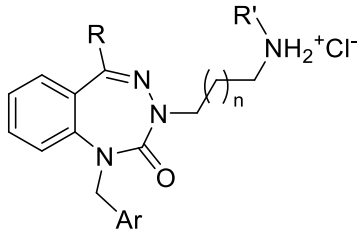


Table 3.I. Summary of the Benzotriazepinones.

R =	Ar =	n = 1		n = 2	
		R' = H		R' = H	
p-methoxyphenethenyl	2-Nap	**3.19d**		**3.19f**	
	Bip	**3.19e**		**3.19g**	
p-hydroxyphenethenyl	2-Nap	**3.20d**		**3.20f**	
	Bip	**3.20e**		**3.20g**	
p-methoxyphenethyl	2-Nap	**3.22d**		**3.22f**	
	Bip	**3.22e**		**3.22g**	
p-hydroxyphenethyl	2-Nap	**3.23d**		**3.23f**	
	Bip	**3.23e**		**3.23g**	
		n = 1		n = 2	
		R' = H	R' = Me	R' = H	R' = Me
Benzothiazole-2-ethyl	Bip	**3.37**	**3.38**	**3.39**	**3.40**
	2-Nap	**3.47h**	**-**	**3.48h**	**3.49h**
	1-Nap	**3.47i**	**-**	**3.48i**	**3.49i**

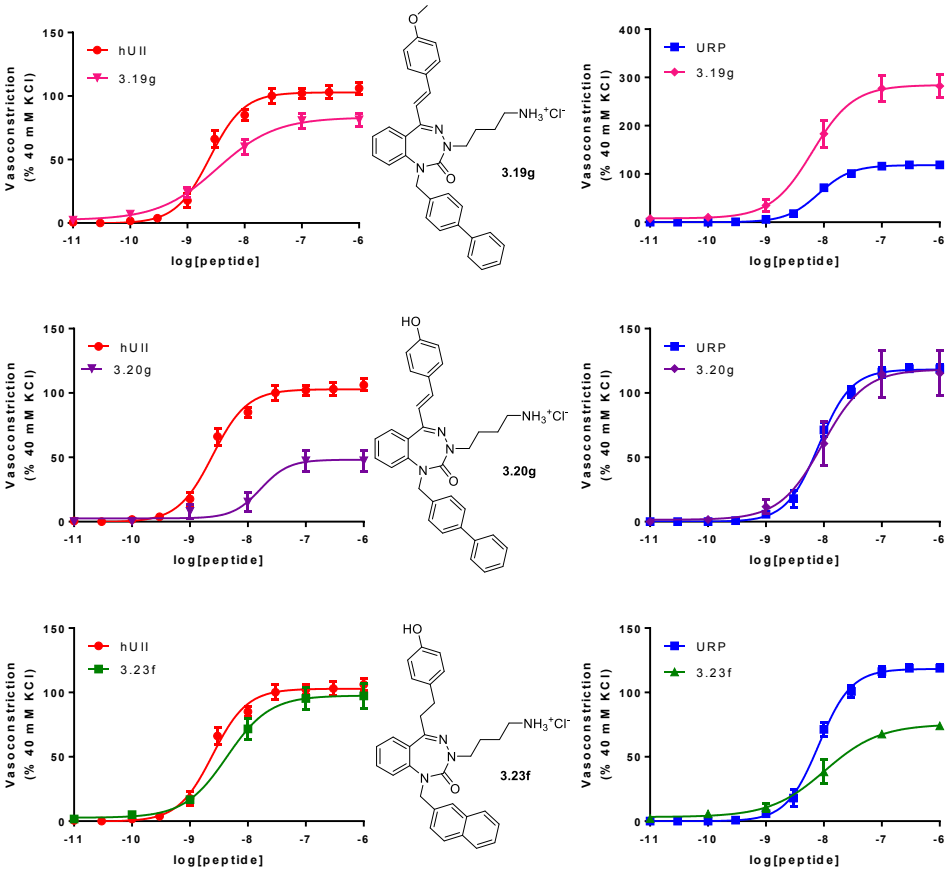


Figure 3.8. Representative modulatory effects of benzotriazepin-2-one {Bip-Lys-Tyr} mimics **3.19g**, **3.20g** and **3.23f** on hUII (red) and URP (blue) mediated vasoconstriction.

Table 3.II. Comparison of vasoactive profiles of hUII and URP in the presence and absence of benzotriazepin-2-one mimics **3.19**, **3.20**, **3.22** and **3.23**.

	Aortic ring contraction (hUII)			Aortic ring contraction (URP)		
no.	n	E_{max} (%)[a]	pEC_{50}[b]	n	E_{max} (%)[a]	pEC_{50}[b]
Ø	7	103 ± 2	8.61 ± 0.05	6	118 ± 2	8.09 ± 0.03
3.3[c]	5	61 ± 7**	7.60 ± 0.29*	3	102 ± 5	7.74 ± 0.12
3.5[d]	3	63 ± 1*	8.46 ± 0.07*	-	-	-
3.19d	3	76 ± 6*	8.30 ± 0.19	3	120 ± 6	7.99 ± 0.09
3.19e	3	106 ± 4	8.49 ± 0.10	3	194 ± 10***	7.87 ± 0.09
3.19f	4	108 ± 6	8.44 ± 0.15	3	110 ± 7	8.07 ± 0.11
3.19g	3	83 ± 4*	8.48 ± 0.13	3	284 ± 15***	8.20 ± 0.12
3.20d	3	59 ± 8**	7.91 ± 0.21*	3	111 ± 7	7.70 ± 0.13*
3.20e	3	51 ± 3***	7.77 ± 0.25***	3	115 ± 3	8.00 ± 0.06
3.20f	3	55 ± 5***	7.82 ± 0.17**	3	114 ± 6	7.86 ± 0.10
3.20g	3	48 ± 4***	7.78 ± 0.31**	3	118 ± 11	8.03 ± 0.18
3.22d	3	55 ± 6***	8.40 ± 0.28	3	41 ± 6***	8.20 ± 0.32
3.22e	3	59 ± 3***	8.49 ± 0.12	3	47 ± 5***	8.11 ± 0.23
3.22f	3	55 ± 5***	8.30 ± 0.25	3	72 ± 6***	8.40 ± 0.23
3.22g	3	82 ± 5*	8.43 ± 0.17	3	84 ± 4***	8.30 ± 0.11
3.23d	3	107 ± 4	8.43 ± 0.10	3	96 ± 6**	8.00 ± 0.12
3.23e	3	95 ± 5	8.35 ± 0.15	3	90 ± 7**	7.97 ± 0.13
3.23f	3	98 ± 5	8.36 ± 0.13	3	75 ± 5***	7.98 ± 0.13
3.23g	3	118 ± 8	8.46 ± 0.18	3	86 ± 5**	7.70 ± 0.11*

[a] The maximum efficacy is expressed as a percentage of the amplitude of the contraction induced by KCl (40 mM). [b] The pEC50 values is defined as the negative logarithm of the half-maximal effective concentration. [c] Effect of **3.3** (1 μM) on UII- and URP-mediated contraction.[43] [d]Effect of **3.5** (4 μM) on UII- and URP-mediated contraction.[48] −, not determined. [e] All values are expressed as mean ± SEM. Statistical analysis for pEC50 and Emax were performed using unpaired Student's t test. [f] *, $P \leq 0.05$ versus values obtained for hUII or URP alone (Ø). [g] **, $P \leq 0.01$ versus values obtained for hUII or URP alone (Ø). [h] ***, $P \leq 0.001$ versus values obtained for hUII or URP alone (Ø).

The *p*-methoxyphenethenyl derivatives **3.19d-g** exhibited various actions on hUII- and URP-mediated vasoconstriction. For instance, triazepinone **3.19d** bearing a 2-naphthyl group modulated the efficiency of UII without affecting the URP vasoactive profile. Triazepinone **3.19e**, bearing a biphenyl moiety, potentiated URP

maximum contraction without altering UII contraction. Introduction of a lysine (**3.19f**) instead of an ornithine side chain abolished the activity of **3.19d**, but enhanced significantly that of **3.19e**: e.g., triazepinone **3.19g**, compared to **3.19e**, increased the efficiency of URP-induced contractions without significant alteration of UII responses. Finally, triazepinones **3.19e** and **3.19g** possessing a biphenyl instead of the 2-naphthyl substituent exhibited a marked influence on URP. Although 2-naphthyl derivatives **3.19d** and **3.19f** were unable to modulate the activity of URP, biphenyl analogs **3.19e** and **3.19g**, both exhibited capacity to increase significantly the efficacy of the maximum contraction of URP (**3.19e**, 194 ± 10% and **3.19g**, 284 ± 15% vs URP, 118 ± 2%). In this series, the length of the alkyl amine chain and the type of aromatic group at the N^1-position, both influenced UII and URP activity.

Removal of the methoxy group in *p*-hydroxyphenethenyl derivatives **3.20d-g** reduced significantly hUII-induced maximal vasoconstriction (E_{max} around 50%) and potency (pEC_{50} around 7.8) without modifying URP contraction. Although triazepinones **3.19e** and **3.19g** modulated URP but had only limited action on UII vasoconstriction, their *p*-hydroxyphenethenyl counterparts **3.20e** and **3.20g** exhibited opposite effects. For example, triazepinone **3.20e** significantly reduced hUII contractile efficiency (**3.20e**, 51 ± 3%; hUII, 103 ± 2%) and potency (**3.20e**, 7.77 ± 0.25; hUII, 8.61 ± 0.05) without significantly affecting URP vasoconstriction (**3.20e**, 115 ± 3%; URP, 118 ± 2%). In the case of *p*-hydroxyphenethenyl derivatives **3.20**, the length of the alkyl amine chain and the type of aromatic group at the N^1-position had limited influence on activity.

Altogether, in the phenethenyl series, the *p*-methoxy and *p*-hydroxy analogs exhibited divergent behavior, *p*-methoxyphenethenyl analog **3.19e** was a selective URP potentiator, and *p*-hydroxyphenethenyl derivatives **3.20e-g** were selective hUII negative regulators.

The saturated *p*-methoxyphenethyl benzotriazepin-2-one derivatives **3.22d-g** modulated the efficacy of hUII and URP without significantly affecting their potency. For example, *p*-methoxyphenethyl benzotriazepin-2-one **3.22d** decreased the maximum contraction efficacy induced by hUII from 103 ± 2% to 55 ± 6% and that

of URP from 118 ± 2% to 41 ± 6% without influencing their potency (pEC_{50}: hUII, 8.40 ± 0.28 *vs* 8.61 ± 0.05 and URP, 8.20 ± 0.32 *vs* 8.09 ± 0.03). Exhibiting similar activity as **3.19g**, triazepinone **3.22g** modulated slightly UII efficiency to contract the rat aortic ring but reduced potently URP-induced vasoconstriction. Throughout this series, the amino propyl analogs were slightly more active than their amino butyl counterparts (Table 3.II). For example propyl analog **3.22e** exhibited enhanced inhibitory activity on both UII and URP relative to its butyl counterpart **3.22g**. The greater flexibility of the methoxyphenethyl chain in **3.22d-g** gave non-selective modulators of UII and URP activity in contrast to the relatively restricted methoxyphenylethenyl counterparts **3.19e** and **3.19g** that potentiated selectively URP activity. For example, in the biphenyl cases, unsaturated **3.19e** increased significantly and selectively the ability for URP to induce contraction (**3.19e**, 194 ± 10% *vs* URP, 118 ± 2%), but saturated derivative **3.22e** reduced the efficacy of both UII (**3.22e**, 59 ± 3% *vs* UII, 103 ± 2%) and URP **3.** (**22e**, 47 ± 5% *vs* URP, 118 ± 2%) to contract aortic ring.

p-Hydroxyphenethyl derivatives **3.23d-g** exhibited marginal effects on potency and efficacy of hUII-induced vasoconstriction. Ornithine mimics **3.23d** and **3.23e** decreased only slightly URP efficacy (**3.23d**, 96 ± 6% and **3.23e**, 90 ± 7% *vs* URP, 118 ± 2%) without affecting potency (pEC_{50}: **3.23d**, 8.00 ± 0.12; **3.23e**, 7.97 ± 0.13 *vs* URP, 8.09 ± 0.03). In contrast, lysine mimics **3.23f** and **3.23g** decreased more significantly the efficacy of URP (**3.23f**, 75 ± 5% and **3.23g**, 86 ± 5% *vs* URP, 118 ± 2%). The potency of URP was respectively unaffected and decreased by biphenyl and 2-naphtyl derivatives **3.23f** (7.98 ± 0.13 *vs* 8.09 ± 0.03) and **3.23g** (7.70 ± 0.11 *vs* 8.09 ± 0.03). Overall, replacement of the methoxy group (compounds **3.22d-g**) by a hydroxyl group completely abrogated the propensity of analogs **3.23d-g** to modulate hUII-induced vasoconstriction. The *p*-hydroxyphenyl moiety that was used to mimic the side chain of the tyrosine residue exhibited important influence on selectivity between hUII and URP. For example, *p*-hydroxyphenethyl and phethenyl derivatives **3.23f** and **3.20f** differentiated ligand activity and exhibited partial agonist activity on URP and hUII, respectively (Figure 3.9, Table 3.II). The orientation of the phenolic

side chain appears to play an important role in binding UT and perturbing the mechanism of action of the native ligands.

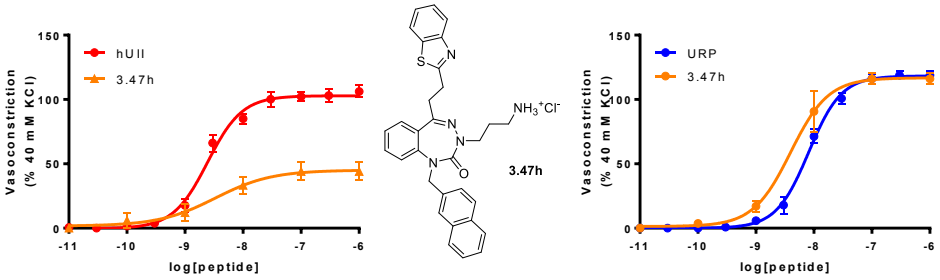


Figure 3.9. Modulation of hUII (red) and URP (blue) mediated vasoconstriction in the presence of benzotriazepin-2-one {Nal(2')-Orn-Btz} mimic **3.47h**.

Table 3.III. Comparison of hUII and URP vasoactive profiles obtained in the presence or absence of benzotriazepin-2-one mimics of the tripeptide {Bip-Lys-Btz} **3.37-3.40** and **3.47-3.49**.

	Aortic ring contraction (hUII)			Aortic ring contraction (URP)		
no.	n	E_{max} (%)[a]	pEC_{50}[b]	n	E_{max} (%)[a]	pEC_{50}[b]
Ø	7	103 ± 2	8.61 ± 0.05	6	118 ± 2	8.09 ± 0 .03
3.3[c]	5	61 ± 7**	7.60 ± 0.29*	3	102 ± 5%	7.74 ± 0.12
3.5[d]	3	63 ± 1*	8.46 ± 0.07*	-	-	-
3.37	3	75 ± 6**	8.85 ± 0.23	3	99 ± 5**	8.43 ± 0.13
3.38	4	76 ± 4**	8.39 ± 0.13	3	110 ± 5	8.31 ± 0.12
3.39	3	68 ± 6**	7.93 ± 0.18**	3	112 ± 6	8.43 ± 0.14
3.40	3	90 ± 6*	8.22 ± 0.17	3	91 ± 5**	7.81 ± 0.44
3.47h	3	45 ± 5***	8.48 ± 0.32	3	117 ± 5	8.40 ± 0.11
3.47i	3	100 ± 4	8.32 ± 0.11	3	101 ± 7	8.24 ± 0.17
3.48h	3	73 ± 5**	8.52 ± 0.19	3	111 ± 5	8.45 ± 0.11
3.48i	3	100 ± 5	8.30 ± 0.13	3	124 ± 6	8.28 ± 0.12
3.49h	3	87 ± 5	8.37 ± 0.16	3	114 ± 5	8.37 ± 0.12
3.49i	3	99 ± 4	8.39 ± 0.11	3	99 ± 6	8.18 ± 0.13

[a] The maximum efficacy is expressed as a percentage of the amplitude of the contraction induced by KCl (40 mM). [b] The pEC50 values is defined as the negative logarithm of the half-maximal effective concentration. [c] Effect of **3.3** (1 μM) on UII- and URP-mediated contraction.[43] [d] Effect of **3.5** (4 μM) on UII- and URP-mediated contraction.[48] −, not determined. [e] All values are expressed as mean ± SEM. Statistical analysis for pEC50 and Emax were performed using unpaired Student's t test. [f] *, $P \leq 0.05$ versus values obtained for hUII or URP alone (Ø). [g] **, $P \leq 0.01$ versus values obtained for hUII or URP alone (Ø). [h] ***, $P \leq 0.001$ versus values obtained for hUII or URP alone (Ø).

Considering the significance of the phenol moiety on activity of the benzotriazepin-2-one ligands, as well as success in enhancing affinity of peptide analogs on replacing Tyr by Btz residues,[45] the *p*-hydroxyphenethyl chain of analogs **3.23e** and **3.23d** was replaced by a benzothiazolylethyl equivalent in triazepinones **3.37** and **3.47h**. In contrast to phenol **3.23e** which modulated selectively URP contractile efficiency, benzotriazepin-2-one **3.37** decreased slightly the efficiency of both UII and URP (Table 3.III). Naphthyl derivative **3.47h** (Figure 3.9) reduced by almost 50% hUII-induced contraction without affecting URP efficiency to contract the rat aortic ring (**3.47h**, 117 ± 5% *vs* URP, 118 ± 2%). Substitution of the biphenyl

group (e.g., **3.37**) by a 2-naphthyl moiety (e.g., **3.47h**) significantly reduced hUII but not URP efficacy. To complete the benzothiazol series, a similar modification, on the length of the side chain that was introduced in derivatives **3.23f** and **3.23g** provided analogs **3.48h** and **3.39**, respectively. In both cases, such modification improved their ability to reduce hUII contractile efficacy while leaving unaffected URP efficiency to contract the aortic ring. Introduction of a benzothiazolylethyl group on benzotriazepin-2-one scaffolds **3.37** and **3.47h** improved their ability to enhance URP potency on rat aortic ring contraction. In the benzothiazolylethyl series, the length of the amino alkyl chain (e.g., propyl, **3.37** and **3.47h**, and butyl, **3.39** and **3.48h**) had a marginal influence on biological activity.

Previous studies have highlighted the critical role played by the shape and orientation of the aromatic side chain.[40,41] In contrast to their 2-naphthyl counterparts **3.47h** and **3.48h**, 1-naphthyl triazepinones **3.47i** and **3.48i** did not reduce the contractile efficacy of hUII and URP but slightly increased hUII potency. The shape and orientation of the aromatic side chain appears again to be critical for modulating hUII- and URP-mediated biological activity.

Finally, *N*-methylamino butyl derivatives **3.38, 3.40, 3.49h,** and **3.49i** were prepared to evaluate the impact of added lipophilicity on the amine.[62,63,64] *N*-Methylation appeared to have little or deleterious effects on the activity of the benzothiazol benzotriazepin-2-ones. The length of the amine chain as well as the orientation and the shape of the aromatic side chains appear to cooperatively mediate the effects observed on hUII contractile efficacy, as previously observed using pyrrolodiazepinone and the UII(4-11)-azasulfuryl peptide UT modulators.[46,48]

In comparison, derivatives **3.20g** and **3.47h** exhibited the same type of activity but were less efficient as **3.5** and **3.3**. For example, inhibition of ≈50% hUII maximal efficacy required concentrations of benzotriazepin-2-ones **3.20g** and **3.47h**, pyrrolodiazepinone **3.5** and **3.3** of 10 μM, 4 μM and 1 μM, respectively (Table 3.II and Table 3.III).

To gain more insight into their mechanisms of action, certain modulators (e.g., **3.19e**, **3.20g**, **3.23f**, and **3.47h**) were examined using BRET-based biosensors, as recently reported,[65] to ascertain their ability to activate the G_q and G_{12} pathways, two signaling features that are involved in UT activation by hUII and URP.[66,67,68] Alone, none of the tested derivatives acted as agonists of either G_q or G_{12} activation (Table 3.IV and Table 3.V). Although **3.20g**, was unable to modulate hUII or URP induced G_q activation, pre-treatment of HEK 293-UT cells with **3.19e**, **3.23f**, and **3.47h** produced significant and similar influences on both hUII and URP efficiency to stimulate G_q activation (Table 3.IV, Figure 3.10). Apart from a probe-dependent action of **3.20g**, which reduced both the efficiency and the potency of URP to activate G_{12} without affecting hUII potency, the other analogs (e.g., **3.19e**, **3.23f**, and **3.47h**) reduced significantly both hUII and URP potency to trigger G_{12} activation (Table 3.V, Figure 3.11). Differences in effects observed *ex vivo* and *in vitro* using an unrelated heterologous cell system may reflect the fact that "cellular and subcellular context determine outputs from signaling biosensors".[69] The observed discrepancies might be related to the use of HEK293-UT cells instead of smooth muscle cells; moreover, β-arrestins 1 and 2, which were not investigated in the present work, may be involved in the contraction of vascular smooth muscle cells.[70,71] . The probe-dependent activity by which benzotriazepinones induce biased UT signaling following UII and URP activation on rat aortic ring contraction merits thus further study with other BRET-based biosensors (*e.g.*, β-arrestin 1 and 2) and more relevant cell lines to further elucidate their mode of action.

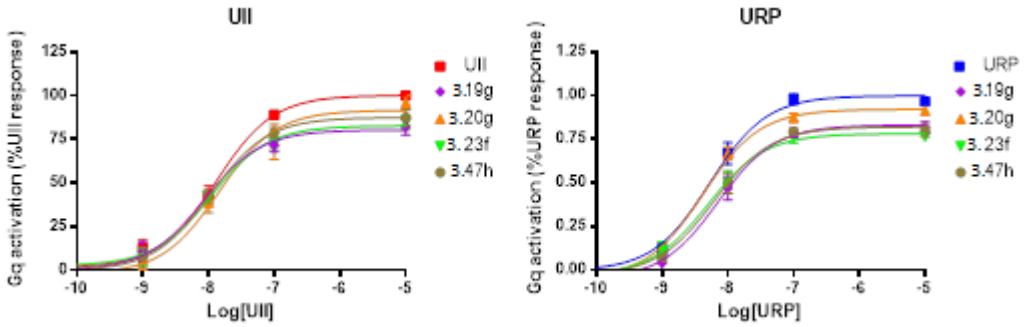


Figure 3.10. Gq activation of hUII and URP profiles obtained in the presence or absence of benzotriazepin-2-one mimics **3.19g**, **3.20g**, **3.23f**, and **3.47h**.

Table 3.IV. Comparison of hUII and URP G_q activation profiles obtained in the presence or absence of benzotriazepin-2-one mimics **3.19g**, **3.20g**, **3.23f**, and **3.47h**.

G_q	UII			URP		
Compounds	N	E_{max} [a]	pEC_{50}	n	E_{max} [a]	pEC_{50}
No treatment	3	100 ± 3	7.88 ± 0.07	3	100 ± 3	8.28 ± 0.08
3.19g	3	80 ± 3**	8.07 ± 0.10	3	83 ± 3*	8.15 ± 0.09
3.20g	3	92 ± 5	7.84 ± 0.13	3	92 ± 2	8.38 ± 0.07
3.23f	3	83 ± 3*	7.96 ± 0.08	3	78 ± 3**	8.30 ± 0.10
3.47h	3	87 ± 3*	7.97 ± 0.08	3	82 ± 3*	8.21 ± 0.08

[a] The maximum efficacy is expressed as a percentage of the activation induced by hUII (10^{-5} M) or URP (10^{-5} M). All values are expressed as mean ± SEM. Statistical analysis were performed using unpaired Student's t test, [b] *, $P \leq 0.05$ versus values obtained for URP. [c] **, $P \leq 0.01$ versus values obtained for URP. [d] ***, $P \leq 0.001$ versus values obtained for URP. Results obtained in G12 recruitment for UII and URP following a 30 min pretreatment with 10^{-5} M of different analogues.

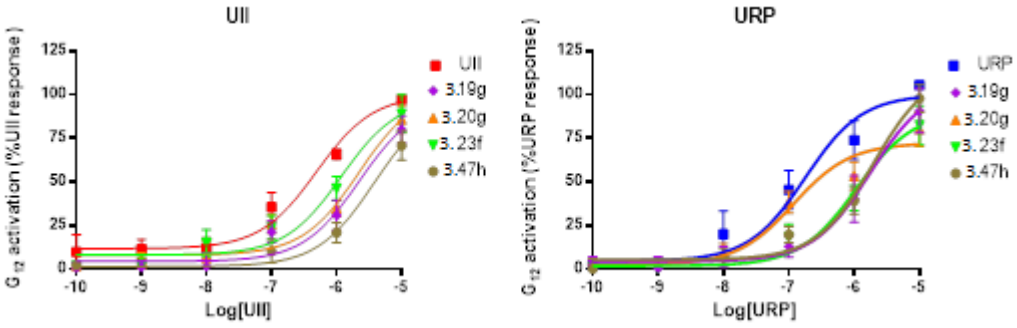


Figure 3.11. G_{12} activation of hUII and URP profiles obtained in the presence or absence of benzotriazepin-2-one mimics **3.19g**, **3.20g**, **3.23f**, and **3.47h**.

Table 3.V. Comparison of hUII and URP G_{12} activation profiles obtained in the presence or absence of benzotriazepin-2-one mimics **3.19g**, **3.20g**, **3.23f**, and **3.47h**.

G_{12}	UII			URP		
Compounds	n	E_{max}	pEC_{50}	n	E_{max}	pEC_{50}
No treatment	7	100 ± 6	6.31 ± 0.17	7	100 ± 7	6.75 ± 0.19
3.19g	7	96 ± 15	5.65 ± 0.22*	7	106 ± 15	5.76 ± 0.23**
3.20g	7	103 ± 13	5.66 ± 0.22*	7	72 ± 6*	6.93 ± 0.21
3.23f	7	98± 10	5.93 ± 0.21	7	90 ± 11	5.96 ± 0.22**
3.47h	7	96 ± 18	5.43 ± 0.25*	7	115 ± 11	5.71 ± 0.18**

[a] The maximum efficacy is expressed as a percentage of the activation induced by hUII (10^{-5} M) or URP (10^{-5} M). All values are expressed as mean ± SEM. Statistical analysis were performed using unpaired Student's t test, [b] *, $P \leq 0.05$ versus values obtained for URP. [c] **, $P \leq 0.01$ versus values obtained for URP. [d] ***, $P \leq 0.001$ versus values obtained for URP. Results obtained in G12 recruitment for UII and URP following a 30 min pretreatment with 10^{-5} M of different analogues.

Conclusion

Benzotriazepin-2-ones were designed to mimic the side chains and conformation of the Bip-Lys-Tyr tripeptide of **3.3**, an allosteric modulator of UT activity. A series of twenty-six analogs were prepared featuring biphenylmethyl and 1- and 2-naphthylmethyl substituents which were installed to mimic the Bip residue. The pharmacological profile of the benzotriazepin-2-ones was evaluated in an *ex vivo* rat aortic ring contraction bioassay and certain analogs were also evaluated for their propensity to activate the G-proteins G_q and G_{12}. The benzotriazepin-2-one scaffold proved effective for the conception of UII and URP modulators, and has indicated the importance of the phenol hydroxyl group and ring orientation for the modulation of the UT system. Saturation of the *p*-hydroxyphenethenyl group to the *p*-hydroxyphenethyl moiety changed the modulator selectively from hUII to URP. The more constrained phenolic side chain modulated selectively vasoconstriction induced by hUII, but its flexible counterpart influenced selectively vasoconstriction induced by URP. *p*-Methoxyphenethenyl benzotriazepin-2-one derivative **3.19g** increased the

efficacy of URP but decreased hUII-mediated contraction. *p*-Hydroxyphenethenyl and *p*-hydroxyphenethyl benzotriazepin-2-one derivatives **3.20g** and **3.23g** exhibited respectively modulator activity that specifically differentiated the vasoconstriction induced by hUII and URP. *p*-Hydroxyphenethenyl analog **3.20g** reduced the efficacy and the potency of hUII without modification of the potency and the efficacy of URP. In contrast to *p*-hydroxyphenethyl benzotriazepin-2-one **3.23d**, which only slightly reduce URP contractile efficacy, its benzothiazole counterpart **3.47h** modulated hUII maximal efficacy without changing its potency. The derivatives **3.20g** and **3.47h** were less potent than the previously reported **3.3** and **3.5** modulators. *p*-Hydroxyphenethyl benzotriazepin-2-one **3.23g** reduced the efficacy of URP without modification of the potency and efficacy of hUII, and represents the first in class non-peptide modulator of URP-mediated activities. To the best of our knowledge, only peptide analogs (*i.e.*, [Pep^{6}]URP and c[Phe-Trp-Lys-Tyr-Gly-ψ(triazole)-Gly]), have exhibited similar behavior.[47,64] Although derivatives **3.19g**, **3.20g, 3.23f**, and **3.47h** biased UT signaling following hUII or URP activation modulating their ability to activate Gq and G12 at the cellular level, such observations were not in correlation with the probe-dependent action on the aortic ring contraction possibly due to the different cellular context of the two assays (HEK cells *vs* smooth muscle cells) or involvement of other signaling pathways mediating hUII and URP- contraction such as β-arrestins. Although their relatively low potency limits our initial efforts to assess mechanism of action, their effective synthesis and the potential of the benzotriazepin-2-ones to discriminate hUII- and URP-associated signaling pathways make these achiral small molecule modulators valuable tools for probing the selective roles of the endogenous ligands in the physiology and associated pathologies of the urotensinergic system.

Experimental Section

Materials and Methods. Dry solvents (DCM, DMF and THF) were obtained by passage through solvent filtration systems (GlassContour Irvine, CA). Hexane was purchased from Fisher Chemical, and fractionally distilled before use. Ethanol, ethyl acetate and methanol were obtained from Fisher Chemical, and along with all reagents from commercial sources were used as received. Melting points are uncorrected, reported in degree Celsius (°C) and obtained on sample that was placed

in a capillary tube using a Mel-Temp melting point apparatus equipped with a thermometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature (298 K) or at 398 K (when specified) in $CDCl_3$ (7.26 ppm/77.16 ppm), DMSO-d_6 (2.50 ppm/39.52 ppm) and CD_3OD-d_4 (3.31 ppm/49.00 ppm) on Brucker AV (300/75, 400/100 and 500/125 MHz) instruments and referenced to internal solvent. Chemicals shifts are reported in parts per million (ppm) and coupling constants (*J*) in Hertz. The abbreviations for peak multiplicities are s (singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), m (multiplet) and br (broad). Certain ^{13}C NMR chemical shifts values were extracted from HMBC and HSQC spectra. Low Resolution Mass Spectrometric analyses were performed on a LCMSD instrument from Agilent technologies in positive electrospray ionization mode (ESI+). High Resolution Mass Spectrometry (HRMS) data were obtained by the Centre Régional de Spectroscopie de Masse de l'Université de Montréal. Purity was defined by analytical HPLC analyses, performed on a Gemini reverse-phase column from Phenomenex (4.6 mm × 150 mm, 5 μm, C18) or a SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18) with a flow rate of 0.5 mL/min using a gradient of acetonitrile (0.1% formic acid) or methanol (0.1% formic acid) in water (0.1% formic acid). All the benzotriazepin-2-one tested for biological activity presented purity higher or equal to 95%.

1-(2-Aminophenyl)-3-(4-methoxyphenyl)propan-1-one (**3.8**). 3-(4-Methoxyphenyl)-1-(2-aminophenyl)-2-propen-1-one (**3.7**, 1g, 4.0 mmol, 1 eq.) was dissolved in EtOAc (25mL), treated with Pd/C (100mg) and stirred overnight under one atmosphere of hydrogen gas. The reaction mixture was filtered over Celite™, and washed with EtOAc (3 x 50mL). The filtrate and washings were evaporated under vacuum. 3-(4-Methoxyphenyl)-1-(2-aminophenyl)propan-1-one (**3.8**) was isolated by chromatography on silica gel (20% EtOAc in hexanes) as yellow oil (713 mg, 70% yield): R_f 0.40 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.31 – 7.21 (m, 1H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.73 – 6.49 (m, 2H), 6.28 (s, 2H), 3.79 (s, 3H), 3.24 (t, *J* = 7.7 Hz, 2H), 2.99 (t, *J* = 7.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.9, 158.1, 150.5, 134.4, 133.7,

131.2, 129.5, 118.0, 117.5, 115.9, 114.1, 55.4, 41.4, 29.9; LRMS (EI) m/z 256 (M + H^+); HRMS (ESI+): m/z for $C_{16}H_{18}NO_2$ $(M + H)^+$, calcd 256.1332, found 256.1337.

Benzhydrylidene aza-glycine *N*'-((*E*)-2-(4-methoxphenylpropenoyl)phenyl)amide (**3.9a**). In a flame-dried round-bottom flask, 4-nitrophenylchloroformate (1.12 g, 5.53 mmol, 1.4 eq.) was dissolved in CH_2Cl_2 (70 mL), cooled to 0°C, treated drop-wise with a freshly prepared solution of benzophenone hydrazone (1.09 g, 5.53 mmol, 1.4 eq.) in CH_2Cl_2 (15 mL) and stirred at 0°C for 2h. A freshly prepared solution of (*E*)-1-(2-aminophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3.8** 1.00 g, 3.95 mmol, 1.0 eq.) and DIEA (1.24 mL, 7.11 mmol, 1.8 eq.) in CH_2Cl_2 (15 mL) was added drop-wise to the reaction mixture at 0°C. The ice bath was removed and the mixture was stirred overnight, washed with H_2O (4 x 50 mL), dried ($MgSO_4$), filtered and evaporated. Amide **3.9a** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as pale yellow solid (1.2 g, 64% yield): m.p. 150-152 °C; R_f 0.27 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.38 (s, 1H), 8.64 (dd, *J* = 8.5, 0.9 Hz, 1H), 8.03 – 7.93 (m, 3H), 7.88 (d, *J* = 15.5 Hz, 1H), 7.73 (s, 1H), 7.67 – 7.37 (m, 10H), 7.37 – 7.28 (m, 2H), 7.13 (ddd, *J* = 8.1, 7.2, 0.9 Hz, 1H), 7.01 – 6.92 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.9, 161.9, 153.4, 148.9, 145.2, 141.0, 137.1, 134.1, 131.9, 130.5, 130.4, 130.0, 129.6, 128.6, 128.5, 127.82, 127.75, 126.2, 124.6, 121.6, 121.2, 120.6, 114.6, 55.6; LRMS (EI) m/z 498 (M + Na^+); HRMS (ESI+): m/z for $C_{30}H_{26}N_3O_3$ $(M + H)^+$, calcd 476.1969, found 476.1975.

Benzhydrylidene aza-glycine *N*'-(2-(4-methoxphenylpropanoyl)phenyl)amide (**3.9b**). Amide **3.9b** was prepared according to the procedure described for the synthesis of amide **3.9a** using 4-nitrophenylchloroformate (718 mg, 3.56 mmol, 1.4 eq.), benzophenone hydrazone (699 mg, 3.56 mmol, 1.4 eq.), aniline **3.8** (650 mg, 2.55 mmol, 1.0 eq.) and DIEA (0.8 mL, 4.59 mmol, 1.8 eq.). Amide **3.9b** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as pale yellow solid (1.1 g, 92% yield): m.p. 99-103 °C; R_f 0.25 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1H), 8.70 (d, *J* = 8.5 Hz, 1H), 8.01 – 7.95 (m, 2H), 7.92 (d, *J* = 8.1 Hz, 1H), 7.73 (s, 1H), 7.62 – 7.47 (m, 4H), 7.47 – 7.35 (m, 3H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 2H), 3.79 (s, 3H), 3.39 (t, *J* = 7.5 Hz, 2H), 3.12 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (75

MHz, $CDCl_3$) δ 203.0, 158.2, 153.5, 149.1, 141.2, 137.0, 134.8, 133.4, 131.9, 130.7, 130.00, 129.98, 129.7, 129.6, 128.61, 128.55, 127.8, 122.2, 121.5, 120.2, 114.1, 55.4, 42.2, 29.5; LRMS (EI) m/z 460 ($M + H^+$); HRMS (ESI+): m/z for $C_{30}H_{28}N_3O_3$ ($M + H)^+$, calcd 478.2125, found 478.2133.

(*E*)-3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.13a**). A solution of aza-glycine **3.9a** (500 mg, 1.05 mmol, 1.0 eq.) in THF (10 mL) was treated with a 40% aqueous solution of tetraethylammonium hydroxide (0.52 mL, 1.26 mmol, 1.2 eq.) and 3-*N*-(Boc)aminopropyl tosylate (414 mg, 1.26 mmol, 1.2 eq. prepared according to ref [72]). The reaction was stirred for 48 h at rt and 6 h at 45°C, diluted with THF (20 mL), treated with HCl (1.0 N, 25 mL) and stirred overnight at 60°C. The volatiles were evaporated and the pH of the resulting aqueous phase was brought to pH = 12 using potassium carbonate, and extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The resulting oil was dissolved in 1:1 THF/H_2O (10 mL), treated with sodium bicarbonate (264 mg, 3.15 mmol, 3 eq.) and di-*tert*-butyl dicarbonate (230 mg, 1.05 mmol, 1 eq.), and stirred overnight at rt. The volatiles were evaporated. The resulting aqueous phase was diluted with H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Benzotriazepinone **3.13a** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as orange oil (175 mg, 37% yield): R_f 0.44 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.37 (m, 4H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.00 – 6.79 (m, 5H), 6.63 (s, 1H), 4.87 (s, 1H), 3.83 (s, 3H), 3.70 (t, *J* = 6.7 Hz, 2H), 3.13 (br dt, *J* = 12.2, 6.3 Hz, 2H), 1.90 (tt, *J* = 11.2, 5.6 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 163.1, 160.6, 156.1, 142.4, 138.4, 132.0, 129.8, 128.9, 128.8, 125.1, 124.0, 123.6, 120.4, 114.4, 79.1, 55.5, 48.6, 38.2, 28.6, 28.0; LRMS (EI) m/z 451 ($M + H^+$); HRMS (ESI+): m/z for $C_{25}H_{31}N_4O_4$ ($M + H)^+$, calcd 451.2340, found 451.2347.

3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.13b**). Benzotriazepinone **3.13b** was prepared according to the procedure described for the synthesis of triazepinone **3.13a** using aza-glycine **3.9b** (500 mg, 1.05 mmol, 1.0 eq.), 40% aqueous solution of tetraethylammonium

hydroxide (0.52 mL, 1.26 mmol, 1.2 eq.), $BocNH(CH_2)_3OTs$ (414 mg, 1.26 mmol, 1.2 eq.), $NaHCO_3$ (264 mg, 3.15 mmol, 3 eq.) and Boc_2O (230 mg, 1.05 mmol, 1 eq.). Benzotriazepinone **3.13b** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as orange oil (104 mg, 22% yield): R_f 0.48 (40% EtOAc in hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 2H), 7.15 – 6.99 (m, 3H), 6.91 – 6.72 (m, 4H), 4.89 (s, 1H), 3.77 (s, 3H), 3.62 (t, *J* = 6.8 Hz, 2H), 3.09 (dd, *J* = 12.3, 6.3 Hz, 2H), 2.99 (dd, *J* = 9.6, 6.5 Hz, 2H), 2.83 (dt, *J* = 9.2, 6.1 Hz, 2H), 1.83 (tt, *J* = 13.4, 6.8 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 163.9, 158.1, 156.1, 142.1, 133.1, 131.9, 129.4, 127.4, 126.1, 123.7, 120.2, 113.9, 79.0, 55.4, 48.2, 38.4, 38.1, 32.8, 28.5, 27.8; LRMS (EI) m/z 453 ($M + H^+$); HRMS (ESI+): m/z for $C_{25}H_{33}N_4O_4$ $(M + H)^+$, calcd 453.2496, found 453.2488.

3-(3-*N*-(Boc)Aminopropyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.13c**). Benzotriazepinone **3.13c** was prepared according to the procedure described for the synthesis of triazepinone **3.13a**, using aza-glycine **3.9c** (1.00 g, 2.51 mmol, 1.2 eq. prepared according to the protocol in ref. 1), 40% aqueous solution of tetraethylammonium hydroxide (0.90 mL, 2.51 mmol, 1.2 eq.), $BocNH(CH_2)_3OTs$ (690 mg, 2.10 mmol, 1 eq.), $NaHCO_3$ (310 mg, 2.51 mmol, 1.2 eq.) and Boc_2O (547 mg, 2.51 mmol, 1.2 eq.). Benzotriazepinone **3.13c** was isolated by chromatography on silica gel (10-30 % EtOAc in hexanes) as yellow oil (260 mg, 28% yield): R_f 0.40 (40% EtOAc in hexanes);^1H NMR (300 MHz, $CDCl_3$) δ 7.45 – 7.31 (m, 2H), 7.12 (ddd, *J* = 8.3, 7.3, 1.1 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.64 (s, 1H), 5.82 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 – 4.94 (m, 2H), 4.88 (br s, 1H), 3.63 (t, *J* = 6.8 Hz, 2H), 3.10 (dt, *J* = 6.1, 6.0 Hz, 2H), 2.81 (dd, *J* = 8.6, 6.6 Hz, 2H), 2.31 (dtt, *J* = 7.5, 6.6, 1.2 Hz, 2H), 1.84 (tt, *J* = 6.7, 6.6 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.6, 156.1, 142.0, 137.1, 132.0, 127.5, 126.1, 123.8, 120.1, 115.7, 79.1, 48.3, 38.2, 35.8, 31.6, 28.6, 27.9; LRMS (EI) m/z 373 ($M + H^+$); HRMS (ESI+): m/z for $C_{20}H_{29}N_4O_3$ $(M + H)^+$, calcd 373.2234, found 373.2239.

3-(3-*N*-Methyl-*N*-(Boc)aminopropyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.14c**). Benzotriazepinone **3.14c** was prepared according to the procedure described for the synthesis of triazepinone **3.13a**, using aza-glycine **9c** (833 mg, 2.10 mmol, 1.2 eq. prepared according to the protocol in ref. 1), 40% aqueous solution of

tetraethylammonium hydroxide (0.75 mL, 2.10 mmol, 1.2 eq.), $BocNCH_3(CH_2)_3OTs$ (600 mg, 1.75 mmol, 1 eq. prepared according to ref [73]), $NaHCO_3$ (176 mg, 2.10 mmol, 1.2 eq.) and Boc_2O (458 mg, 2.10 mmol, 1.2 eq.). Benzotriazepinone **3.14c** was isolated by chromatography on silica gel (10-40% EtOAc in hexanes) as orange oil (120 mg, 18% yield): R_f 0.36 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 2H), 7.11 (ddd, *J* = 8.1, 7.2, 0.9 Hz, 1H), 7.01 (s, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 5.83 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.11 – 4.92 (m, 2H), 3.57 (t, *J* = 7.0 Hz, 2H), 3.18 (t, *J* = 7.0 Hz, 2H), 2.85 – 2.73 (m, 5H), 2.31 (dt, *J* = 7.6, 6.5 Hz, 2H), 1.86 (tt, *J* = 7.2, 6.5 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.8, 155.8, 142.0, 137.2, 131.8, 127.4, 126.3, 123.7, 120.2, 115.6, 79.3, 48.5, 46.9, 35.8, 34.3, 31.6, 28.6, 26.0; LRMS (EI) m/z 387 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{31}N_4O_3$ $(M + H)^+$, calcd 387.2391, found 298.2403.

(*E*)-3-(4-Chlorobutyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.15a**). A solution of aza-glycine **3.9a** (500 mg, 1.05 mmol, 1.0 eq.) in THF (10 mL) was treated with a 40% aqueous solution of tetraethylammonium hydroxide (0.52 mL, 1.26 mmol, 1.2 eq.) and 4-bromo-1-chlorobutane (0,14 mL, 1.26 mmol, 1.2 eq.). The reaction was stirred 24 hours at room temperature, diluted with THF (20 mL), treated with HCl (1.0 M, 25 mL) and stirred overnight at 60°C. The volatiles were evaporated and the resulting aqueous phase was extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Triazepinone **3.15a** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as yellow oil (403 mg, quantitative yield): R_f 0.19 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.37 (m, 4H), 7.17 (dt, *J* = 7.6, 1.1 Hz, 1H), 7.02 – 6.78 (m, 5H), 6.63 (s, 1H), 3.83 (s, 3H), 3.69 (t, *J* = 6.9 Hz, 2H), 3.51 (t, *J* = 6.4 Hz, 2H), 1.94 – 1.67 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4, 163.2, 160.5, 142.4, 138.4, 131.9, 129.7, 128.84, 128.79, 125.2, 124.0, 123.5, 120.4, 114.4, 55.5, 50.4, 45.0, 30.1, 25.2; LRMS (EI) m/z 384 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{23d}lN_3O_2$ $(M + H)^+$, calcd 384.1473, found 384.1468.

3-(4-Chlorobutyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.15b**) was prepared according to the procedure described for the synthesis of benzotriazepinone **3.15a** using aza-glycine **3.9b** (400 mg, 0.84 mmol, 1.0 eq.), 40%

aqueous solution of tetraethylammonium hydroxide (0.41 mL, 1.00 mmol, 1.2 eq.), $Br(CH_2)_4Cl$ (0,12 mL, 1.00 mmol, 1.2 eq.). Benzotriazepinone **3.15b** was isolated by chromatography on silica gel (0-20% EtOAc in hexanes) as yellow oil (275 mg, 86% yield): R_f 0.43 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.30 (m, 2H), 7.15 – 7.05 (m, 3H), 6.87 – 6.76 (m, 3H), 6.69 (s, 1H), 3.78 (s, 3H), 3.60 (t, *J* = 6.8 Hz, 2H), 3.49 (t, *J* = 6.3 Hz, 2H), 2.99 (dd, *J* = 8.9, 5.8 Hz, 2H), 2.84 (dd, *J* = 9.1, 6.0 Hz, 2H), 1.81 – 1.68 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.6, 158.1, 142.0, 133.1, 131.9, 129.5, 127.5, 126.3, 123.8, 120.1, 114.0, 55.4, 50.0, 45.0, 38.4, 32.8, 30.0, 25.0; LRMS (EI) m/z 386 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{25}ClN_3O_2$ $(M + H)^+$, calcd 386.1630, found 386.1634.

(*E*)-3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.17a**)**.** A solution of chloride **3.15a** (410 mg, 1.07 mmol, 1 eq.) in 4:1 NMP/H_2O (10 mL) was treated with NaN_3 (696 mg, 10.70 mmol, 10 eq.). The reaction was stirred for 48 hours at 60°C, diluted with H_2O (40 mL) and extracted with EtOAc (3 X 50 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The resulting oil was dissolved in MeOH (20 mL), treated with PPh_3 (420 mg, 1.60 mmol, 1.5 eq.) and heated at reflux overnight. The volatiles were evaporated and the resulting oil was dissolved in a mixture of 1:1 THF/H_2O (10 mL), treated with $NaHCO_3$ (269 mg, 3.20 mmol, 3 eq.) and Boc_2O (234 mg, 1.07 mmol, 1 eq.). The reaction was stirred for 4 hours at room temperature, the volatiles were evaporated and the resulting aqueous phase was diluted with H_2O (40 mL) and extracted with EtOAc (3 x 50 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Carbamate **3.17a** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as yellow oil (224 mg, 45% yield): R_f 0.42 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 – 7.36 (m, 4H), 7.15 (td, *J* = 7.6, 1.0 Hz, 1H), 7.01 – 6.77 (m, 6H), 4.55 (s, 1H), 3.82 (s, 3H), 3.65 (t, *J* = 7.3 Hz, 2H), 3.11 (dd, *J* = 12.7, 6.4 Hz, 2H), 1.71 (tt, *J* = 7.2, 6.0 Hz, 2H), 1.55 – 1.37 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 163.1, 160.5, 156.1, 142.5, 138.3, 131.9, 129.6, 128.9, 128.8, 125.2, 124.0, 123.4, 120.5, 114.4, 79.1, 55.5, 50.8, 40.5, 28.5, 27.4, 25.1; LRMS (EI) m/z 487 ($M + Na^+$); HRMS (ESI+): m/z for $C_{26}H_{33}N_4O_4$ $(M + H)^+$, calcd 465.2496, found 465.2495.

3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.17b**). Carbamate **3.17b** was prepared according to the procedure described for the synthesis of carbamate **3.17a**, using chloride **3.15b** (246 mg, 0.64 mmol, 1.0 eq.), NaN_3 (416 mg, 6.40 mmol, 10 eq.), PPh_3 (252 mg, 0.96 mmol, 1.5 eq.), $NaHCO_3$ (161 mg, 1.96 mmol, 3 eq.) and Boc_2O (140 mg, 0.64 mmol, 1 eq.). Carbamate **3.17b** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as yellow oil (200 mg, 67% yield): R_f 0.39 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 2H), 7.16 – 7.03 (m, 3H), 6.87 – 6.74 (m, 3H), 6.38 (s, 1H), 4.51 (s, 1H), 3.78 (s, 3H), 3.57 (t, *J* = 8.4 Hz, 2H), 3.09 (br dt, *J* = 12.4, 6.5 Hz, 2H), 2.99 (dd, *J* = 9.2, 6.3 Hz, 2H), 2.83 (dd, *J* = 9.2, 6.5 Hz, 2H), 1.64 (tt, *J* = 15.3, 7.4 Hz, 2H), 1.51 – 1.38 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.1, 163.3, 158.1, 156.1, 141.9, 133.1, 131.9, 129.5, 127.5, 126.3, 123.8, 120.0, 114.0, 79.2, 55.4, 50.5, 40.2 (HSQC), 38.5, 32.9, 28.6, 27.4, 24.9; LRMS (EI) m/z 467 (M + H^+); HRMS (ESI+): m/z for $C_{26}H_{35}N_4O_4$ $(M + H)^+$, calcd 467.2653, found 467.2656.

3-(4-Azidobutyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.16c**). A solution of chloride **15c** (175 mg, 0.57 mmol, 1 eq. prepared according to the protocol in ref. 1) in 4:1 DMF/H_2O (5 mL) was treated with NaN_3 (372 mg, 5,70 mmol, 10 eq.). The reaction was stirred overnight at 60°C, cooled to rt, diluted with EtOAc (20 mL), washed with H_2O (2 x 20 mL), dried ($MgSO_4$), filtered and evaporated. Azide **3.16c** was isolated by chromatography on silica gel (10-20% EtOAc in hexanes) as pale yellow oil (133 mg, 75% yield): R_f 0.48 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.30 (m, 2H), 7.18 (s, 1H), 7.11 (t, *J* = 8.1 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 5.83 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.08 – 4.92 (m, 2H), 3.61 (t, *J* = 7.1 Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 2.81 (dd, *J* = 8.5, 6.7 Hz, 2H), 2.32 (dt, *J* = 14.2, 7.0 Hz, 2H), 1.81 – 1.65 (m, 2H), 1.55 (tt, *J* = 6.9, 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 163.9, 142.1, 137.2, 131.9, 127.4, 126.2, 123.7, 120.2, 115.6, 51.3, 50.1, 35.8, 31.6, 26.2, 24.8; LRMS (EI) m/z 313 (M + H^+); HRMS (ESI+): m/z for $C_{16}H_{21}N_6O$ $(M + H)^+$, calcd 313.1771, found 313.1773.

3-(4-*N*-(Boc)Aminobutyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.17c**). A solution of azide **3.16c** (25 mg, 0.08 mmol, 1 eq.) in methanol (1 mL) was treated with triphenylphosphine (32 mg, 0,12 mmol, 1.2 eq.). The reaction was heated

at reflux for 1 hour, the volatiles were evaporated under vacuum. The resulting residue was dissolved in 1:1 THF/H_2O (1 mL), treated with sodium bicarbonate (20 mg, 0.24 mmol, 3 eq.) and di-*tert*-butyl dicarbonate (20 mg, 0.09 mmol, 1.1 eq.). The reaction was stirred overnight at room temperature. The volatiles were evaporated. The resulting aqueous phase was diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Carbamate **3.17c** was isolated by chromatography on silica gel (30% EtOAc in Hexanes) as colorless oil (22 mg, 71% yield): R_f 0.22 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.28 (m, 2H), 7.10 (ddd, *J* = 8.2, 7.3, 1.1 Hz, 1H), 6.95 (s, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 5.82 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.07 – 4.92 (m, 2H), 4.52 (br s, 1H), 3.57 (t, *J* = 7.4 Hz, 2H), 3.09 (dt, *J* = 6.4, 6.3 Hz, 2H), 2.80 (dd, *J* = 8.5, 6.7 Hz, 2H), 2.30 (dt, *J* = 7.6, 6.5 Hz, 2H), 1.65 (tt, *J* = 7.5, 7.1 Hz, 2H), 1.50 – 1.36 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 163.7, 156.0, 142.1, 137.2, 131.8, 127.4, 126.2, 123.7, 120.2, 115.6, 79.1, 50.4, 40.5, 35.8, 31.6, 28.6, 27.4, 24.9; LRMS (EI) m/z 387 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{31}N_4O_3$ $(M + H)^+$, calcd 387.2391, found 387.2404.

(*E*)-3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (3.18d). In a dried round bottom flask, flushed with argon, benzotriazepinone **3.13a** (64 mg, 0.14 mmol, 1 eq.), potassium *tert*-butoxide (21 mg, 0.17 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (38 mg, 0.17 mmol, 1.2 eq.) were dissolved in DMF (5 mL). The reaction was stirred overnight at rt, diluted with EtOAc (50 mL), washed with H_2O (3 x 50 mL) and brine (25mL), dried ($MgSO_4$), filtered and evaporated. Benzotriazepinone **3.18d** was isolated by chromatography on silica gel (20-30% EtOAc in hexanes) as yellow oil (76 mg, 92% yield): R_f 0.43 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 – 7.52 (m, 4H), 7.44 – 7.03 (m, 10H), 6.92 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 16.4 Hz, 1H), 5.47 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.8 Hz, 1H), 4.75 (br s, 1H), 3.90 – 3.73 (m, 4H), 3.66 – 3.52 (m, 1H), 3.07 (dt, *J* = 12.1, 6.3 Hz, 2H), 1.98 – 1.75 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.23, 164.17, 160.6, 156.1, 144.6, 139.1, 134.4, 133.3, 132.7, 131.2, 129.4, 128.9 (2C), 128.8, 128.3, 127.9, 127.7, 126.4, 126.2, 125.8, 125.6, 124.4, 123.8, 121.1, 114.4, 79.1, 55.5, 50.6, 48.9, 38.3, 28.6, 28.1;

LRMS (EI) m/z 591 (M + H+); HRMS (ESI+): m/z for $C_{36}H_{39}N_4O_4$ (M + H)+, calcd 591.2966, found 591.2994.

(*E*)-3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.18e**). Benzotriazepinone **3.18e** was prepared according to the procedure described for the synthesis of triazepinone **3.18d** by alkylation of benzotriazepinone **3.13a** (80 mg, 0.18 mmol, 1 eq.) using *t*-BuOK (26 mg, 0.21 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (58 mg, 0.21 mmol, 1.2 eq.). Benzotriazepinone **3.18e** was isolated by chromatography on silica gel (20-30% EtOAc in hexanes) as yellow oil (87 mg, 78% yield): R_f 0.67 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.20 (m, 14H), 7.20 – 7.13 (m, 1H), 7.07 (d, *J* = 16.4 Hz, 1H), 6.93 – 6.84 (m, 2H), 6.71 (d, *J* = 16.4 Hz, 1H), 5.31 (d, *J* = 15.5 Hz, 1H), 4.74 (d, *J* = 15.4 Hz, 2H), 3.83 (s, 3H), 3.77 (t, *J* = 7.0 Hz, 1H), 3.63 – 3.50 (m, 1H), 3.06 (dd, *J* = 11.9, 5.9 Hz, 2H), 1.83 (ddt, *J* = 21.2, 14.5, 7.1 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 163.9, 160.6, 156.0, 144.8, 140.6, 140.0, 138.9, 136.2, 131.2, 129.2, 128.84, 128.76 (3C), 128.0, 127.3, 127.2, 127.0, 124.4, 123.6, 121.2, 114.4, 79.0, 55.5, 50.7, 48.9, 38.3, 28.5, 28.1; LRMS (EI) m/z 617 (M + H^+); HRMS (ESI+): m/z for $C_{38}H_{41}N_4O_4$ $(M + H)^+$, calcd 617.3122, found 617.3109.

(*E*)-3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.18f**)**.** Benzotriazepinone **3.18f** was prepared according to the procedure described for the synthesis of triazepinone **3.18d** from alkylation of benzotriazepinone **3.17a** (110 mg, 0.24 mmol, 1 eq.) using *t*-BuOK (34 mg, 0.28 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (62 mg, 0.28 mmol, 1.2 eq.). Benzotriazepinone **3.18f** was isolated by chromatography on silica gel (20-30% EtOAc in hexanes) as yellow oil (120 mg, 83% yield): R_f 0.38 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 – 7.52 (m, 4H), 7.47 – 7.03 (m, 10H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 16.4 Hz, 1H), 5.48 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 15.9 Hz, 1H), 4.50 (s, 1H), 3.86 (s, 3H), 3.83 – 3.71 (m, 1H), 3.51 (ddd, *J* = 13.3, 8.0, 5.5 Hz, 1H), 3.08 (dt, *J* = 12.4, 6.2 Hz, 2H), 1.66 (tt, *J* = 13.6, 7.0 Hz, 2H), 1.55 – 1.31 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.14, 164.10, 160.5, 156.0, 144.6, 139.0, 134.5, 133.3, 132.7, 131.1, 129.5, 128.81 (2C), 128.78, 128.3, 127.9, 127.6,

126.4, 126.1, 125.7, 125.6, 124.2, 123.8, 121.0, 114.4, 79.1, 55.5, 51.0, 50.5, 40.4, 28.5, 27.4, 25.2; LRMS (EI) m/z 605 ($M + H^+$); HRMS (ESI+): m/z for $C_{37}H_{40}N_4O_4$ $(M + H)^+$, calcd 605.3122, found 605.3130.

(*E*)-3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.18g**)**.** Benzotriazepinone **3.18g** was prepared according to the procedure described for the synthesis of benzotriazepinone **3.18d** by alkylation of triazepinone **3.17a** (60 mg, 0.13 mmol, 1 eq.) using *t*-BuOK (19 mg, 0.16 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (44 mg, 0.16 mmol, 1.2 eq.). Benzotriazepinone **3.18g** was isolated by chromatography on silica gel (0-20% EtOAc in hexanes) as yellow oil (66 mg, 80% yield): R_f 0.35 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.19 (m, 14H), 7.16 (t, *J* = 7.5 Hz, 1H), 7.07 (d, *J* = 16.4 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.69 (d, *J* = 16.5 Hz, 1H), 5.31 (d, *J* = 15.5 Hz, 1H), 4.73 (d, *J* = 15.5 Hz, 1H), 4.45 (br s, 1H), 3.84 (s, 3H), 3.82 – 3.67 (m, 1H), 3.54 – 3.39 (m, 1H), 3.06 (dt, *J* = 12.5, 6.2 Hz, 2H), 1.72 – 1.56 (m, 2H), 1.48 – 1.33 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0, 163.9, 160.6, 156.0, 144.9, 140.7, 140.0, 138.8, 136.3, 131.2, 129.4, 128.9, 128.8 (3C), 128.1, 127.3, 127.2, 127.1, 124.3, 123.8, 121.2, 114.4, 79.1, 55.5, 51.1, 50.6, 40.5, 28.6, 27.5, 25.2; LRMS (EI) m/z 653 ($M + Na^+$); HRMS (ESI+): m/z for $C_{39}H_{43}N_4O_4$ $(M + H)^+$, calcd 631.3279, found 631.3281.

(*E*)-3-(3-Aminopropyl)-5-(4-methoxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.19d**). A solution of carbamate **3.18d** (38 mg, 0.06 mmol, 1 eq.) in CH_2Cl_2 (5 mL) was treated with HCl gas bubbles at rt for 2h. The volatiles were evaporated and the residue was purified by chromatography on silica gel (9.5% MeOH and 0.5% NEt_3 in $CHCl_3$) to give an oil, that was dissolved in 0.1 N HCl (5 mL) and freeze-dried to provide hydrochloride salt **3.19d** as yellow solid (23 mg, 80% yield): m.p. 112-116 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.81 – 7.04 (m, 14H), 6.95 (d, *J* = 8.0 Hz, 2H), 6.72 (d, *J* = 15.7 Hz, 1H), 5.46 (br d, *J* = 15.2 Hz, 1H), 4.97 (br s, 1H), 3.84 (s, 3H), 3.77 (br s, 1H), 3.64 (br s, 1H), 2.84 (br s, 2H), 2.03 (br s, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.5, 165.2, 162.9, 145.4, 144.2, 135.4, 134.5, 134.0, 133.5, 130.6, 130.4, 129.6, 129.34, 129.26, 128.7, 128.5, 127.5, 127.2, 126.9, 126.6, 126.2, 123.3, 122.1, 115.5, 56.0, 51.7, 47,1

(HSQC), 38.6, 26.6; LRMS (EI) m/z 491 (M + H^+); HRMS (ESI+): m/z for $C_{31}H_{31}N_4O_2$ $(M + H)^+$, calcd 491.2442, found 491.2427.

(*E*)-3-(3-Aminopropyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.19e**). Hydrochloride salt **3.19e** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.18e** (30 mg, 0.05 mmol, 1 eq.), and after evaporation of the volatiles, obtained as pale yellow solid (22mg, 81% yield): m.p. 108-112°C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.57 – 7.15 (m, 15H), 7.09 (d, *J* = 16.3 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 6.69 (d, *J* = 16.3 Hz, 1H), 5.31 (d, *J* = 15.3 Hz, 1H), 4.79 (d, *J* = 15.3 Hz, 1H), 3.80 (s, 3H), 3.73 (dt, *J* = 13.8, 6.7 Hz, 1H), 3.59 (dt, *J* = 13.2, 6.2 Hz, 1H), 2.84 (t, *J* = 6.9 Hz, 2H), 2.02 (tt, *J* = 14.0, 7.3 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.9, 165.3, 162.5, 145.4, 142.5, 141.6, 141.5, 137.3, 133.0, 130.3, 130.1, 130.0, 129.8, 129.5, 129.3, 128.3, 128.0, 127.8, 126.1, 123.3, 122.9, 115.4, 55.9, 51.6, 48.8, 38.6, 26.7; LRMS (EI) m/z 517 (M + H^+); HRMS (ESI+): m/z for $C_{33}H_{33}N_4O_2$ $(M + H)^+$, calcd 517.2598, found 517.2572.

(*E*)-3-(4-Aminobutyl)-5-(4-methoxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.19f**). Hydrochloride salt **3.19f** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.18f** (23 mg, 0.04 mmol, 1 eq.), and after removal of the volatiles, isolated as yellow solid (19 mg, quantitative yield): m.p. 116-120 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.73 – 7.06 (m, 14H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 16.3 Hz, 1H), 5.44 (d, *J* = 15.5 Hz, 1H), 4.97 (s, 1H), 3.84 (s, 3H), 3.82 – 3.66 (m, 1H), 3.60 – 3.39 (1, 1H), 2.86 (br t, *J* = 5.9 Hz, 2H), 1.89 – 1.46 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.7, 165.6, 162.5, 145.5, 142.2, 135.6, 134.6, 134.1, 132.8, 130.4, 130.1, 130.0, 129.6, 129.2, 128.7, 128.6, 127.4, 127.2, 126.9, 126.6, 125.9, 123.2, 123.0, 115.4, 55.9, 51.54, 51.50, 40.5, 26.0, 25.7; LRMS (EI) m/z 505 (M + H^+); HRMS (ESI+): m/z for $C_{32}H_{33}N_4O_2$ $(M + H)^+$, calcd 505.2598, found 505.2594.

(*E*)-3-(4-Aminobutyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.19g**). Hydrochloride salt **3.19g** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.18g** (42 mg, 0.07 mmol, 1 eq.), and after removal of the volatiles,

obtained as yellow solid (36 mg, 90% yield): m.p. 99-103 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.57 (t, *J* = 7.5 Hz, 1H), 7.52 – 7.23 (m, 12H), 7.20 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 16.3 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 16.2 Hz, 1H), 5.31 (d, *J* = 15.2 Hz, 1H), 4.80 (d, *J* = 15.2 Hz, 1H), 3.91 – 3.66 (m, 4H), 3.48 (dt, *J* = 13.8, 6.3 Hz, 1H), 2.87 (t, *J* = 7.1 Hz, 2H), 1.77 – 1.50 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.4, 165.1, 162.9, 145.6, 144.3, 141.6, 141.5, 137.2, 133.5, 130.5, 130.4, 129.8, 129.7, 129.38, 129.36, 128.3, 128.0, 127.8, 126.3, 123.4, 121.9, 115.5, 55.9, 51.7, 51.2, 40.4, 25.9, 25.6; LRMS (EI) m/z 531 (M + H^+); HRMS (ESI+): m/z for $C_{34}H_{35}N_4O_2$ (M + H)$^+$, calcd 531.2755, found 531.2742.

(*E*)-3-(3-Aminopropyl)-5-(4-hydroxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.20d**)**.** Methyl ether **3.18d** (38 mg, 0.06 mmol, 1 eq.) was dissolved in CH_2Cl_2 (10 mL), cooled to 0°C and treated with BBr_3 (1.0 N in CH_2Cl_2, 0.51 mL, 0.51 mmol, 8 eq.). The cooling bath was removed, and the reaction was let warm to room temperature overnight with stirring, quenched with a solution of saturated $NaHCO_3$ (10 mL), diluted with H_2O (10 mL) and extracted with EtOAc (2 x 25 mL). The organic phases were combined, washed with brine (25 mL), dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$). The resulting oil was dissolved in 0.1 N HCl (2mL) and freeze-dried to afford hydrochloride **3.20d** as yellow solid (23 mg, 70% yield): m.p. 139-143 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.72 – 7.14 (m, 13H), 7.10 (d, *J* = 16.3 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 16.3 Hz, 1H), 5.45 (d, *J* = 15.6 Hz, 1H), 4.97 (br s, 1H), 3.75 (dt, *J* = 14.2, 7.1 Hz, 1H), 3.60 (dt, *J* = 13.5, 6.3 Hz, 1H), 2.84 (t, *J* = 7.1 Hz, 2H), 2.11 – 1.95 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.1, 165.6, 160.5, 145.4, 142.7, 135.6, 134.5, 134.1, 132.8, 130.4, 130.3, 130.0, 129.2, 128.7, 128.5, 128.4, 127.4, 127.2, 126.9, 126.5, 126.0, 123.0, 122.3, 116.8, 51.5, 48.8, 38.6, 26.7; LRMS (EI) m/z 477 (M + H^+); HRMS (ESI+): m/z for $C_{30}H_{29}N_4O_2$ (M + H)$^+$, calcd 477.2285, found 477.2297.

(*E*)-3-(3-Aminopropyl)-5-(4-hydroxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.20e**)**.** Hydrochloride salt **3.20e** was prepared according to the procedure described for the synthesis of **3.20d**

from methyl ether **3.18e** (38 mg, 0.06 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.49 mL, 0.49 mmol, 8 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2mL) and freeze-dried to afford **3.20e** as orange solid (20 mg, 60% yield): m.p. 138-134 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.61 – 7.17 (m, 15H), 7.06 (d, *J* = 16.3 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 16.4 Hz, 1H), 5.32 (d, *J* = 15.2 Hz, 1H), 4.80 (d, *J* = 15.3 Hz, 1H), 3.83 – 3.65 (m, 1H), 3.65 – 3.46 (m, 1H), 2.83 (t, *J* = 6.6 Hz, 2H), 2.00 (tt, *J* = 7.4, 7.1Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.3, 165.3, 160.6, 145.4, 143.4, 141.6, 141.5, 137.3, 133.1, 130.4, 130.2, 130.1, 129.8, 129.3, 128.4, 128.3, 128.0, 127.8, 126.1, 123.3, 121.8, 116.9, 51.6, 48.4 (HSQC), 38.6, 26.7; LRMS (EI) m/z 503 (M + H^+); HRMS (ESI+): m/z for $C_{32}H_{31}N_4O_2$ (M + H)$^+$, calcd 503.2442, found 503.2433.

(*E*)-3-(4-Aminobutyl)-5-(4-hydroxystyryl)-1-(2-naphthylmethyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.20f**)**.** Hydrochloride salt **3.20f** was prepared according to the procedure described for the synthesis of **3.20d** from methyl ether **3.18f** (60 mg, 0.10 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.80 mL, 0.80 mmol, 8 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2 mL) and freeze-dried to afford **3.20f** as orange solid (39 mg, 74% yield): m.p. 134-138 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.75 – 7.15 (m, 13H), 7.11 (d, *J* = 16.3 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 16.4 Hz, 1H), 5.44 (d, *J* = 15.5 Hz, 1H), 4.95 (d, *J* = 15.8 Hz, 1H), 3.77 (dt, , *J* = 12.6, 7.0 Hz, 1H), 3.48 (dt, , *J* = 13.2, 6.4 Hz, 1H), 2.86 (t, *J* = 7.1 Hz, 2H), 1.71 (tt, *J* = 18.7, 6.0 Hz, 2H), 1.58 (tt, *J* = 14.3, 7.1 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 167.1, 165.6, 160.7, 145.6, 143.4, 135.6, 134.6, 134.1, 133.0, 130.5, 130.22, 130.17, 129.2, 128.7, 128.6, 128.4, 127.4, 127.2, 126.9, 126.6, 126.0, 123.0; 121.9, 116.9, 51.6, 51.4, 40.5, 26.0, 25.6; LRMS (EI) m/z 491 (M + H^+); HRMS (ESI+): m/z for $C_{31}H_{31}N_4O_2$ (M + H)+, calcd 491.2442, found 491.2462.

(*E*)-3-(4-Aminobutyl)-5-(4-hydroxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one hydrochloride (**3.20g**). Hydrochloride salt **3.20g** was prepared according to the procedure described for the synthesis of **3.20d** from **3.18g** (20 mg, 0.03 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.2 mL, 0.20

mmol, 6 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2mL) and freeze-dried to afford **3.20g** as orange solid (14 mg, 78% yield): m.p. 111-115 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.61 – 7.13 (m, 15H), 7.02 (d, *J* = 16.3 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 2H), 6.65 (d, *J* = 16.4 Hz, 1H), 5.31 (d, *J* = 15.3 Hz, 1H), 4.80 (d, *J* = 15.5 Hz, 1H), 3.83 – 3.65 (m, 1H), 3.56 – 3.37 (m, 1H), 2.86 (t, *J* = 7.3 Hz, 2H), 1.77 – 1.50 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.8, 165.5, 160.4, 145.6, 142.4, 141.7, 141.5, 137.4, 132.8, 130.6, 130.2, 129.9, 129.8, 129.3, 128.5, 128.3, 128.0, 127.8, 126.0, 123.1, 122.3, 116.8, 51.5, 40.5, 30.7, 26.0, 25.7; LRMS (EI) m/z 517 (M + H^+); HRMS (ESI+): m/z for $C_{33}H_{33}N_4O_2$ (M + H)$^+$, calcd 517.2598, found 517.2588.

3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.21d**). Benzotriazepinone **3.21d** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.13b** (43 mg, 0.10 mmol, 1 eq.) using *t*-BuOK (14 mg, 0.12 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (25 mg, 0.12 mmol, 1.2 eq.). Benzotriazepinone **3.21d** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as pale yellow oil (50 mg, 85% yield): R_f 0.27 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 – 7.57 (m, 4H), 7.48 – 7.24 (m, 5H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.12 – 7.01 (m, 3H), 6.87 – 6.73 (m, 2H), 5.39 (d, *J* = 12.8 Hz, 1H), 4.81 (d, *J* = 28.1 Hz, 2H), 3.77 (s, 3H), 3.69 (s, 1H), 3.56 (s, 1H), 3.12 – 2.93 (m, 4H), 2.79 (d, *J* = 16.9 Hz, 2H), 1.79 (s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 164.1, 158.1, 156.1, 144.5, 134.7, 133.3, 133.1, 132.8, 131.3, 130.2, 129.3, 128.4, 127.77, 127.76, 126.8, 126.5, 126.3, 125.9, 125.7, 124.6, 121.1, 114.0, 79.1, 55.4, 51.1, 48.6, 38.3, 37.9, 33.0, 28.6, 28.0; LRMS (EI) m/z 593 (M + H+); HRMS (ESI+): m/z for $C_{36}H_{41}N_4O_4$ (M + H)+, calcd 593.3122, found 593.3132.

3-(3-*N*-(Boc)Aminopropyl)-5-(4-methoxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.21e**). Benzotriazepinone **3.21e** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.17a** (60 mg, 0.13 mmol, 1 eq.) using *t*-BuOK (19 mg, 0.16 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (44 mg, 0.16 mmol, 1.2 eq.). Benzotriazepinone

3.21e was isolated by chromatography on silica gel (0-20% EtOAc in hexanes) as yellow oil (66 mg, 80% yield): R_f 0.35 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 – 7.47 (m, 2H), 7.47 – 7.42 (m, 2H), 7.42 – 7.29 (m, 5H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.21 – 7.12 (m, 2H), 7.12 – 7.05 (m, 2H), 6.86 – 6.76 (m, 2H), 5.24 (br d, *J* = 13.9 Hz, 1H), 4.86 – 4.62 (br m, 2H), 3.76 (s, 3H), 3.68 (br s, 1H), 3.55 (br s, 1H), 3.12 – 2.93 (br m, 4H), 2.79 (br dt, *J* = 15.8, 7.5 Hz, 2H), 1.77 (br s, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 163.9, 158.1, 156.0, 144.7, 140.7, 140.2, 136.4, 133.1, 131.3, 130.1, 129.3, 128.8, 128.1, 127.3, 127.2, 127.1, 126.5, 124.6, 121.0, 114.0, 79.0, 55.3, 50.9, 48.6, 38.2, 37.8, 32.9, 28.6, 27.9; LRMS (EI) m/z 619 ($M + H^+$); HRMS (ESI+): m/z for $C_{38}H_{43}N_4O_4$ $(M + H)^+$, calcd 619.3279, found 619.3291.

3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.21f**). Benzotriazepinone **3.21f** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.17b** (108 mg, 0.23 mmol, 1 eq.) using *t*-BuOK (34 mg, 0.28 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (62 mg, 0.28 mmol, 1.2 eq.). Benzotriazepinone **3.21f** was isolated by chromatography on silica gel (20-30% EtOAc in hexanes) as colorless oil (76 mg, 54% yield): R_f 0.42 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 – 7.28 (m, 7H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.20 – 7.11 (m, 2H), 7.08 (d, *J* = 8.7 Hz, 2H), 6.88 – 6.72 (m, 2H), 5.25 (br d, *J* = 14.1 Hz, 1H), 4.70 (br d, *J* = 14.5 Hz, 1H), 4.46 (br s, 1H), 3.76 (s, 3H), 3.69 (br s, 1H), 3.43 (br s, 1H), 3.22 – 2.92 (br m, 4H), 2.90 – 2.65 (br m, 2H), 1.76 – 1.48 (br m, 2H), 1.47 – 1.32 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 164.0, 158.1, 156.0, 144.7, 140.8, 140.2, 136.5, 133.2, 131.2, 130.3, 129.4 (2C), 128.8 (2C), 128.2, 127.4, 127.3, 127.1, 126.4, 124.5, 121.1, 114.0, 79.1, 55.4, 50.9, 50.8, 40.5, 37.8, 33.0, 28.6, 27.4, 25.1; LRMS (EI) m/z 607 ($M + H^+$); HRMS (ESI+): m/z for $C_{37}H_{43}N_4O_4$ $(M + H)^+$, calcd 607.3279, found 607.3297.

3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.21g**). Benzotriazepinone **3.21g** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.17b** (92 mg, 0.20 mmol, 1 eq.) using *t*-BuOK (29 mg, 0.24 mmol, 1.2

eq.) and 4-bromomethyl-biphenyl (66 mg, 0.24 mmol, 1.2 eq.). Benzotriazepinone **3.21g** was isolated by chromatography on silica gel (20-30% EtOAc in hexanes) as yellow oil (58 mg, 46% yield): R_f 0.48 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 – 7.28 (m, 9H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.19 – 7.11 (m, 2H), 7.08 (d, *J* = 8.7 Hz, 2H), 6.85 – 6.76 (m, 2H), 5.25 (br d, *J* = 14.1 Hz, 1H), 4.70 (br d, *J* = 14.5 Hz, 1H), 4.46 (s, 1H), 3.76 (s, 3H), 3.69 (br s, 1H), 3.43 (br s, 1H), 3.19 – 2.95 (br m, 4H), 2.77 (br s, 2H), 1.58 (br tt, *J* = 15.0, 8.2 Hz, 2H), 1.48 – 1.31 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 164.0, 158.1, 156.0, 144.7, 140.8, 140.2, 136.5, 133.2, 132.7, 131.2, 129.4, 128.8, 128.2, 127.4, 127.3, 127.1, 126.4, 124.5, 121.1, 114.0, 79.1, 55.4, 50.9, 50.8, 40.5, 37.8, 33.0, 28.6, 27.4, 25.1; LRMS (EI) m/z 633 (M + H^+); HRMS (ESI+): m/z for $C_{39}H_{45}N_4O_4$ $(M + H)^+$, calcd 633.3435, found 633.3444.

3-(3-Aminopropyl)-5-(4-methoxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.22d**). Hydrochloride **3.22d** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.21d** (28 mg, 0.05 mmol, 1 eq.). After purification by chromatography on silica gel (9.5% MeOH and 0.5% NEt_3 in $CHCl_3$), the resulting oil was treated with 0.1 N HCl (5mL) and freeze-dried to give pale yellow solid (13 mg, 80% yield): m.p. 87-91 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 – 7.55 (m, 4H), 7.54 – 7.32 (m, 5H), 7.32 – 7.12 (m, 2H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 5.35 (d, *J* = 15.3 Hz, 1H), 4.85 (s, 1H), 3.68 (s, 3H), 3.65 – 3.48 (m, 2H), 3.03 (dt, *J* = 15.3, 7.3 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 2.62 (t, *J* = 6.2 Hz, 2H), 2.09 – 1.84 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 169.0, 165.5, 159.5, 145.4, 136.0, 134.6, 134.2, 134.0, 132.8, 131.1, 130.2, 129.3, 128.66, 128.63, 127.83, 127.77, 127.3, 127.0, 126.8, 126.1, 122.8, 114.8, 55.6, 52.2, 48.6, 38.6, 38.4, 33.8, 26.6; LRMS (EI) m/z 493 (M + H^+); HRMS (ESI+): m/z for $C_{31}H_{34}N_4O_2$ $(M + H)^+$, calcd 493.2598, found 493.2597.

3-(3-Aminopropyl)-5-(4-methoxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.22e**). Hydrochloride **3.22e** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.21e** (30 mg, 0.05 mmol, 1 eq.), and after removal of the volatiles, obtained as pale yellow solid (22 mg, 81% yield): m.p. 90-94 °C; ^{1}H NMR (300

MHz, CD_3OD) δ 7.53 – 7.21 (m, 11H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.08 – 6.95 (m, 2H), 6.82 – 6.66 (m, 2H), 5.21 (d, *J* = 15.1 Hz, 1H), 4.73 (d, *J* = 15.5 Hz, 1H), 3.68 (s, 3H), 3.64 – 3.46 (m, 2H), 3.06 (dt, *J* = 14.4, 7.2 Hz, 2H), 2.81 (t, *J* = 6.7 Hz, 2H), 2.66 (t, *J* = 7.5 Hz, 2H), 2.08 – 1.89 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.9, 165.4, 159.6, 145.5, 141.8, 141.6, 137.7, 134.0, 132.8, 131.2, 130.3, 129.8, 129.3, 128.3, 128.0, 127.9, 127.8, 126.1, 122.7, 114.9, 55.6, 51.9, 48.6, 38.6, 38.2, 33.6, 26.6; LRMS (EI) m/z 519 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{35}N_4O_2$ $(M + H)^+$, calcd 519.2755, found 519.2747.

3-(4-Aminobutyl)-5-(4-methoxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.22f**). Hydrochloride salt **3.22f** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.21f** (31 mg, 0.05 mmol, 1 eq.), and after removal of the volatiles, obtained as pale yellow solid (28 mg, quantitative yield): m.p. 85-89 °C; ^1H NMR (300 MHz, CD_3OD) δ 7.79 – 7.61 (m, 4H), 7.50 – 7.31 (m, 5H), 7.24 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.17 (td, *J* = 7.2, 1.3 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 2H), 6.82 – 6.68 (m, 2H), 5.33 (br d, *J* = 14.9 Hz, 1H), 4.84 (s, 1H), 3.67 (s, 4H), 3.43 (br s, 1H), 3.03 (dt, *J* = 13.6, 7.2 Hz, 2H), 2.83 (t, *J* = 7.2 Hz, 2H), 2.61 (dt, *J* = 14.7, 7.4 Hz, 2H), 1.76 – 1.44 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.9, 165.5, 159.5, 145.5, 136.0, 134.6, 134.1, 134.0, 132.7, 131.2, 130.2, 129.3, 128.64, 128.62, 127.84, 127.78, 127.3, 126.9, 126.8, 126.0, 122.6, 114.8, 55.6, 52.1, 51.2, 40.4, 38.3, 33.8, 25.9, 25.5; LRMS (EI) m/z 507 ($M + H^+$); HRMS (ESI+): m/z for $C_{32}H_{35}N_4O_2$ $(M + H)^+$, calcd 507.2755, found 507.2730.

3-(4-Aminobutyl)-5-(4-methoxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.22g**). Hydrochloride salt **3.22g** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.21g** (28 mg, 0.05 mmol, 1 eq.), and isolated by chromatography on silica gel (9.5% MeOH and 0.5% NEt_3 in $CHCl_3$) followed by treatment with 0.1 N HCl (5mL), and freeze-dried to give pale yellow solid (12 mg, 46% yield): m.p. 86-90 °C; ^1H NMR (300 MHz, CD_3OD) δ 7.62 – 7.10 (m, 13H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.81 – 6.69 (m, 2H), 5.20 (d, *J* = 15.0 Hz, 1H), 4.73 (d, *J* = 14.8 Hz, 1H), 3.68 (s, 3H), 3.65 (br s, 1H), 3.42 (br s, 1H), 3.04 (dt, *J* = 13.1, 7.4 Hz, 2H), 2.83 (t, *J* = 7.2 Hz, 2H),

2.65 (t, *J* = 7.7 Hz, 2H), 1.77 – 1.41 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.6, 165.4, 159.5, 145.6, 141.8, 141.6, 137.8, 134.1, 132.7, 131.3, 130.3, 129.8, 129.4, 128.3, 128.0, 127.9, 127.8, 126.0, 122.6, 114.9, 55.6, 51.8, 51.3, 40.4, 38.2, 33.7, 26.0, 25.5; LRMS (EI) m/z 533 ($M + H^+$); HRMS (ESI+): m/z for $C_{34}H_{37}N_4O_2$ $(M + H)^+$, calcd 533.2911, found 533.2904.

3-(3-Aminopropyl)-5-(4-hydroxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.23d**). Hydrochloride salt **3.23d** was prepared according to the procedure described for the synthesis of **3.20d** from ether **3.21d** (25 mg, 0.05 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.34 mL, 0.34 mmol, 6 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2 mL) and freeze-dried to afford **3.23d** as pale yellow solid (19 mg, 84% yield): m.p. 116-120 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.83 – 7.60 (m, 4H), 7.55 – 7.33 (m, 5H), 7.26 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 5.34 (d, *J* = 15.7 Hz, 1H), 4.86 (s, 1H), 3.77 – 3.48 (m, 2H), 3.01 (dt, *J* = 13.9, 7.5 Hz, 2H), 2.82 (t, *J* = 6.8 Hz, 2H), 2.59 (dt, *J* = 12.7, 7.0 Hz, 2H), 2.06 – 1.89 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 169.1, 165.5, 156.7, 145.3, 136.0, 134.6, 134.2, 132.8, 132.7, 131.2, 130.3, 130.2, 129.3, 128.7, 128.6, 127.8, 127.3, 127.0, 126.8, 126.1, 122.8, 116.2, 52.2, 48.6, 38.6, 38.5, 33.9, 26.6; LRMS (EI) m/z 479 ($M + H^+$); HRMS (ESI+): m/z for $C_{30}H_{30}N_4O_2$ $(M + H)^+$, calcd 479.2242, found 479.2435.

3-(3-Aminopropyl)-5-(4-hydroxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.23e**). Hydrochloride salt **3.23e** was prepared according to the procedure described for the synthesis of **3.20d** from ether **3.21e** (28 mg, 0.05 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.36 mL, 0.36 mmol, 8 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2 mL) and freeze-dried to afford **3.23e** as yellow solid (12 mg, 50% yield): m.p. 114-118 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.53 – 7.41 (m, 6H), 7.41 – 7.32 (m, 3H), 7.32 – 7.17 (m, 4H), 7.04 – 6.82 (m, 2H), 6.74 – 6.56 (m, 2H), 5.21 (br d, *J* = 14.5 Hz, 1H), 4.75 (br d, *J* = 15.8 Hz, 1H), 3.58 (br d, *J* = 18.4 Hz, 2H), 3.04 (dt, *J* = 15.1, 7.8 Hz, 2H), 2.81 (t, *J* = 7.0 Hz, 2H), 2.62 (t, *J* = 7.9 Hz, 2H), 2.03 – 1.82 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD)

δ 169.0, 165.4, 156.8, 145.4, 141.8, 141.7, 137.7, 132.83, 132.80, 131.2, 130.3, 129.8, 129.4, 128.3, 128.1, 127.9, 127.8, 126.1, 122.7, 116.2, 51.9, 48.6, 38.6, 38.3, 33.8, 26.6; LRMS (EI) m/z 505 ($M + H^+$); HRMS (ESI+): m/z for $C_{32}H_{33}N_4O_2$ $(M + H)^+$, calcd 505.2598, found 505.2588.

3-(4-Aminobutyl)-5-(4-hydroxyphenylethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.23f**). Hydrochloride salt **3.23f** was prepared according to the procedure described for the synthesis of **3.20d** from ether **3.21f** (36 mg, 0.06 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.47 mL, 0.47 mmol, 8 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2 mL) and freeze-dried to afford **3.23f** as pale yellow solid (19 mg, 59% yield): m.p. 93-97 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.78 – 7.61 (m, 4H), 7.49 – 7.33 (m, 5H), 7.26 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.16 (t, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 5.33 (br d, *J* = 15.5 Hz, 1H), 4.89 (br s, 1H), 3.65 (s, 1H), 3.42 (br s, 1H), 3.00 (dt, *J* = 13.0, 7.0 Hz, 2H), 2.83 (t, *J* = 7.1 Hz, 2H), 2.59 (dt, *J* = 15.5, 7.5 Hz, 2H), 1.69 – 1.48 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.7, 165.6, 156.7, 145.4, 136.0, 134.6, 134.2, 132.9, 132.6, 131.3, 130.2, 129.3, 128.7, 128.6, 127.80, 127.76, 127.3, 126.9, 126.8, 126.0, 122.6, 116.2, 52.1, 51.3, 40.5, 38.4, 33.9, 26.0, 25.5; LRMS (EI) m/z 493 ($M + H^+$); HRMS (ESI+): m/z for $C_{31}H_{33}N_4O_2$ $(M + H)^+$, calcd 493.2598, found 493.2604.

3-(4-Aminobutyl)-5-(4-hydroxyphenylethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.23g**). Hydrochloride salt **3.23g** was prepared according to the procedure described for the synthesis of **3.20d** from ether **3.21g** (26 mg, 0.04 mmol, 1 eq.) and BBr_3 (1.0 N in CH_2Cl_2, 0.33 mL, 0.33 mmol, 8 eq.). After purification by silica gel chromatography (10-15% EtOH and 0.5% $HNEt_2$ in $CHCl_3$), the resulting oil was dissolved in 0.1 N HCl (2 mL) and freeze-dried to afford **3.23g** as pale yellow solid (12 mg, 55% yield): m.p. 96-100 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.56 – 7.14 (m, 13H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 5.19 (d, *J* = 15.3 Hz, 1H), 4.74 (d, *J* = 14.3 Hz, 1H), 3.64 br (s, 1H), 3.41 (br s, 1H), 3.02 (br dt, *J* = 13.8, 7.0 Hz, 2H), 2.84 (t, *J* = 7.1 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 1.73 – 1.47 (br m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.7, 165.5, 156.7,

145.5, 141.8, 141.6, 137.7, 132.9, 132.7, 131.3, 130.3, 129.8, 129.4, 128.3, 128.0, 127.9, 127.8, 126.1, 122.6, 116.2, 51.8, 51.3, 40.5, 38.3, 33.8, 26.0, 25.5; LRMS (EI) m/z 519 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{35}N_4O_2$ $(M + H)^+$, calcd 519.2755, found 519.2757.

3-(4-*N*-Boc-*N*-(Methyl)aminobutyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.24**). A solution of chloride **3.15c** (50 mg, 0,16 mmol, 1 eq.) in NMP (1 mL) was treated with an aqueous solution of NH_2CH_3 (40%, 0,14 mL, 1.63 mmol, 10 eq.) and stirred overnight at 60°C. The volatiles were evaporated. The resulting residue was dissolved in 1:1 THF/H_2O (2 mL), treated with $NaHCO_3$ (41 mg, 0.49 mmol, 3 eq.) and Boc_2O (35 mg, 0.16 mmol, 1 eq.), and stirred overnight at rt. The volatiles were evaporated and the resulting aqueous phase was diluted with H_2O (15 mL) and extracted with EtOAc (3 x 15 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. After chromatography on silica gel (35% EtOAc in hexanes), carbamate **3.24** was isolated by as colorless oil (47 mg, 75% yield): R_f 0.19 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 2H), 7.11 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.78 (s, 1H), 5.82 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 – 4.93 (m, 2H), 3.59 (t, *J* = 7.2 Hz, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.80 (dd, *J* = 8.5, 6.7 Hz, 2H), 2.74 (s, 3H), 2.30 (dt, *J* = 7.7, 6.5 Hz, 2H), 1.59 (tt, *J* = 7.9, 6.7 Hz, 2H), 1.52 – 1.35 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.6, 155.9, 142.1, 137.2, 131.8, 127.4, 126.3, 123.7, 120.1, 115.6, 79.2, 50.4, 48.5, 35.8, 34.0, 31.7, 28.6, 25.1, 24.9; LRMS (EI) m/z 401 ($M + H^+$); HRMS (ESI+): m/z for $C_{22}H_{33}N_4O_3$ $(M + H)^+$, calcd 401.2547, found 401.2554.

3-(3-*N*-(Boc)Aminopropyl)-5-(but-3-enyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.25**). Benzotriazepinone **3.25** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.13c** (51 mg, 0.14 mmol, 1 eq.) using *t*-BuOK (20 mg, 0.16 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (41 mg, 0.16 mmol, 1.2 eq.). Benzotriazepinone **3.25** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as colorless oil (60 mg, 81% yield): R_f 0.67 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 – 7.25 (m, 11H), 7.25 – 7.08 (m, 2H), 5.83 (ddt, *J* = 16.8, 10.2, 6.5 Hz, 1H), 5.22 (br s, 1H), 5.14 – 4.93 (m, 2H), 4.74 (br s, 2H), 3.67 (br s, 1H), 3.52 (br s, 1H), 3.03

(dt, *J* = 6.5, 6.0 Hz, 2H), 2.8 (t, *J* = 7.8 Hz, 2H), 2.28 (br tt, *J* = 7.5, 6.8 Hz, 2H), 1.79 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 164.0, 156.0, 144.7, 140.8, 140.3, 137.3, 136.4, 131.4, 130.1, 128.9, 128.2, 127.4, 127.3, 127.1, 126.6, 124.6, 121.0, 115.6, 79.1, 50.9, 48.6, 38.3, 35.2, 31.7, 28.6, 28.0; LRMS (EI) m/z 539 (M + H^+); HRMS (ESI+): m/z for $C_{33}H_{39}N_4O_3$ (M + H)$^+$, calcd 539.3017, found 539.3033.

3-(3-*N*-Boc-*N*-(Methyl)aminopropyl)-5-(but-3-enyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.26**). Benzotriazepinone **3.26** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.13a** (79 mg, 0.20 mmol, 1 eq.) using *t*-BuOK (30 mg, 0.25 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (60 mg, 0.25 mmol, 1.2 eq.). Benzotriazepinone **3.26** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as colorless oil (110 mg, 98% yield): R_f 0.47 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H), 7.49 – 7.27 (m, 9H), 7.20 – 7.09 (m, 2H), 5.84 (ddt, *J* = 16.7, 10.2, 6.5 Hz, 1H), 5.26 (br d, *J* = 16.0 Hz, 1H), 5.09 – 4.95 (m, 2H), 4.71 (br d, *J* = 13.4 Hz, 1H), 3.66 (br s, 1H), 3.43 (br s, 1H), 3.10 (t, *J* = 7.0 Hz, 2H), 2.91 – 2.78 (m, 2H), 2.72 (s, 3H), 2.28 (dt, *J* = 7.5, 7.4 Hz, 2H), 1.80 (br tt, *J* = 6.8, 6.3 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 164.0, 155.8, 144.6, 140.8, 140.2, 137.3, 136.4, 131.2, 130.3, 128.8, 128.2, 127.4, 127.2, 127.1, 126.4, 124.5, 121.1, 115.5, 79.3, 50.8, 48.8, 47.0 35.2, 34.3, 31.7, 28.6, 26.2; LRMS (EI) m/z 553 (M + H^+); HRMS (ESI+): m/z for $C_{34}H_{41}N_4O_3$ (M + H)$^+$, calcd 553.3173, found 553.3191.

3-(4-*N*-(Boc)Aminobutyl)-5-(but-3-enyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.27**). Benzotriazepinone **3.27** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.17c** (20 mg, 0.05 mmol, 1 eq.) using *t*-BuOK (6 mg, 0.05 mmol, 1 eq.) and 4-bromomethyl-biphenyl (13 mg, 0.05 mmol, 1 eq.). Benzotriazepinone **3.27** was isolated by chromatography on silica gel (0-30% EtOAc in hexanes) as pale yellow oil (27 mg, 93% yield): R_f 0.45 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.28 (m, 11H), 7.21 – 7.07 (m, 2H), 5.83 (ddt, *J* = 16.7, 10.2, 6.4 Hz, 1H), 5.25 (br d, *J* = 11.8 Hz, 1H), 5.10 – 4.95 (m, 2H), 4.71 (br d, *J* = 12.6 Hz, 1H), 4.46 (br s, 1H), 3.70 (br s, 1H), 3.42 (br s, 1H), 3.05 (br dt, *J* = 6.6, 6.3 Hz, 2H), 2.84

(t, *J* = 7.8 Hz, 2H), 2.28 (br s, 2H), 1.75 – 1.52 (m, 2H), 1.52 – 1.32 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 164.0, 156.0, 144.7, 140.8, 140.2, 137.3, 136.5, 131.2, 130.2, 128.8, 128.2, 127.4, 127.2, 127.1, 126.4, 124.4, 121.0, 115.5, 79.1, 50.8, 50.7, 40.4, 35.2, 31.7, 28.6, 27.4, 25.0; LRMS (EI) m/z 553 ($M + H^+$); HRMS (ESI+): m/z for $C_{34}H_{41}N_5O_3$ $(M + H)^+$, calcd 553.3173, found 553.3187.

3-(4-*N*-Boc-*N*-(Methyl)aminobutyl)-5-(but-3-enyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.28**). Benzotriazepinone **3.28** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.24** (25 mg, 0.06 mmol, 1 eq.) using *t*-BuOK (9 mg, 0.08 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (19 mg, 0.08 mmol, 1.2 eq.). Benzotriazepinone **3.28** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as white oil (30 mg, 86% yield): R_f 0.65 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H), 7.49 – 7.44 (m, 2H), 7.43 – 7.26 (m, 7H), 7.20 – 7.08 (m, 2H), 5.83 (ddt, *J* = 16.7, 10.2, 6.5 Hz, 1H), 5.25 (br d, *J* = 14.4 Hz, 1H), 5.08 – 4.95 (m, 2H), 4.72 (br d, *J* = 17.6 Hz, 1H), 3.71 (br s, 1H), 3.44 (br s, 1H), 3.12 (br d, *J* = 3.9 Hz, 2H), 2.83 (t, *J* = 7.8 Hz, 2H), 2.70 (s, 3H), 2.27 (br d, *J* = 7.4 Hz, 2H), 1.53 (br q, *J* = 7.2 Hz, 2H), 1.45 – 1.31 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 164.0, 156.0, 144.8, 140.8, 140.2, 137.3, 136.5, 131.2, 130.3, 128.8, 128.3, 127.4, 127.2, 127.1, 126.4, 124.4, 121.0, 115.5, 79.2, 50.8, 50.7, 48.5, 35.2, 33.9, 31.8, 28.6, 25.1, 25.0; LRMS (EI) m/z 567 ($M + H^+$); HRMS (ESI+): m/z for $C_{35}H_{43}N_4NaO_3$ $(M + H)^+$, calcd 589.3149, found 589.3157.

3-((3-*N*-(Boc)Aminopropyl)-1-(4-phenylbenzyl)-2,3-dihydro-2-oxo-1H-benzo[e][1,2,4]triazepin-5-yl)propanal (**3.29**). A solution of olefin **3.25** (55 mg, 0.10 mmol, 1eq.) in 3:1 dioxane/H_2O (2 mL) was treated with 2,6-lutidine (0.04 mL, 0.20 mmol, 2 eq.), $NaIO_4$ (87 mg, 0.40 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.002 mmol, 0.02 mL, 0.02 eq.). The reaction was stirred for 4h at rt, diluted with H_2O (25 mL), and extracted with EtOAc (2 x 25 mL) and $CHCl_3$ (2 x 25 mL). The organic phases were combined, washed with brine, dried ($MgSO_4$), filtered and evaporated. Aldehyde **3.29** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as blue oil (46 mg, 84% yield): R_f 0.26 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.57 – 7.21 (m, 11H), 7.20 – 7.07 (m,

2H), 5.23 (br s, 1H), 4.68 (br s, 2H), 3.55 (br s, 2H), 3.29 – 2.92 (br m, 4H), 2.83 (br s, 2H), 1.73 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.8, 164.0, 163.8, 156.0, 144.3, 140.7, 140.3, 136.4, 131.6, 130.2, 128.9, 128.1, 127.4, 127.3, 127.1, 126.5, 124.7, 121.1, 79.2, 50.9, 48.6, 40.1, 38.2, 28.6, 28.3, 27.9; LRMS (EI) m/z 541 ($M + H^+$); HRMS (ESI+): m/z for $C_{32}H_{37}N_4O_4$ $(M + H)^+$, calcd 541.2809, found 541.2819.

3-((3-*N*-Boc-*N*-(Methyl)aminopropyl)-1-(4-phenylbenzyl)-2,3-dihydro-2-oxo-1H-benzo[e][1,2,4]triazepin-5-yl)propanal (**3.30**). Aldehyde **3.30** was prepared according to the procedure described for the synthesis of **3.29** using olefin **3.26** (110 mg, 0.20 mmol, 1 eq.), 2,6-lutidine (0.07 mL, 0.40 mmol, 2 eq.), $NaIO_4$ (170 mg, 0.80 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.004 mmol, 0.04 mL, 0.02 eq.). Aldehyde **3.30** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as blue oil (92 mg, 84% yield): R_f 0.21 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.58 – 7.21 (m, 11H), 7.19 – 7.08 (m, 2H), 5.25 (br s, 1H), 4.73 (br s, 1H), 3.61 (br s, 1H), 3.42 (br s, 1H), 3.15 (br s, 1H), 3.08 (t, J = 7.1 Hz, 2H), 2.95 (br s, 1H), 2.83 (br s, 2H), 2.71 (s, 3H), 1.74 (br s, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.6, 163.9, 163.8, 155.8, 144.2, 140.7, 140.2, 136.4, 131.5, 130.4, 128.9, 128.1, 127.4, 127.3, 127.1, 126.4, 124.6, 121.2, 79.2, 50.9, 48.9, 46.9, 40.0, 34.3, 28.6, 28.2, 26.1; LRMS (EI) m/z 555 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{39}N_4O_4$ $(M + H)^+$, calcd 555.2966, found 555.2980.

3-((4-*N*-(Boc)Aminobutyl)-1-(4-phenylbenzyl)-2,3-dihydro-2-oxo-1H-benzo[e][1,2,4]triazepin-5-yl)propanal (**3.31**). Aldehyde **3.31** was prepared according to the procedure described for the synthesis of **3.29** using olefin **3.27** (75 mg, 0.14 mmol, 1 eq.), 2,6-lutidine (0.05 mL, 0.27 mmol, 2 eq.), $NaIO_4$ (116 mg, 0.54 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.003 mmol, 0.03 mL, 0.02 eq.). Aldehyde **3.31** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as blue oil (66 mg, 88% yield): R_f 0.32 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.56 – 7.45 (m, 4H), 7.44 – 7.20 (m, 7H), 7.19 – 7.10 (m, 2H), 5.25 (br s, 1H), 4.73 (br s, 1H), 4.52 (br s, 1H), 3.58 (br s, 1H), 3.42 (br s, 1H), 3.11 (br s, 1H), 3.04 (br dt, J = 6.9, 6.0 Hz, 2H), 2.82 (br s, 3H), 1.55 (br tt, J = 7.8, 6.9 Hz, 2H), 1.48 – 1.29 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.8,

163.83, 163.80, 156.1, 144.3, 140.7, 140.2, 136.5, 131.5, 130.3, 128.9, 128.1, 127.4, 127.3, 127.1, 126.4, 124.6, 121.1, 79.1, 50.9, 50.7, 40.4, 40.1, 28.6, 28.2, 27.4, 24.9; LRMS (EI) m/z 555 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{39}N_4O_4$ $(M + H)^+$, calcd 555.2966, found 555.2985.

3-((4-*N*-Boc-*N*-(Methyl)aminobutyl)-1-(4-phenylbenzyl)-2,3-dihydro-2-oxo-1H-benzo[e][1,2,4]triazepin-5-yl)propanal (**3.32**). Aldehyde **3.32** was prepared according to the procedure described for the synthesis of **3.29** using olefin **3.28** (30 mg, 0.05 mmol, 1 eq.), 2,6-lutidine (0.02 mL, 0.11 mmol, 2 eq.), $NaIO_4$ (45 mg, 0.21 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.001 mmol, 0.01 mL, 0.02 eq.). Aldehyde **3.32** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as dark blue oil (24 mg, 80% yield): R_f 0.13 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.56 – 7.44 (m, 4H), 7.44 – 7.21 (m, 7H), 7.19 – 7.09 (m, 2H), 5.23 (br s, 1H), 4.73 (br s, 1H), 3.64 (br s, 1H), 3.42 (br s, 1H), 3.11 (br t, *J* = 6.8 Hz, 3H), 2.96 (br s, 1H), 2.81 (br s, 2H), 2.70 (s, 3H), 1.57 – 1.29 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.7, 163.9, 163.8, 155.9, 144.4, 140.7, 140.2, 136.5, 131.4, 130.4, 128.9, 128.1, 127.4, 127.3, 127.1, 126.3, 124.6, 121.1, 79.2, 50.8, 50.7, 48.6, 40.1, 34.0, 29.8, 28.6, 28.2, 24.9; LRMS (EI) m/z 569 ($M + H^+$); HRMS (ESI+): m/z for $C_{34}H_{41}N_4O_4$ $(M + H)^+$, calcd 569.3122, found 569.3131.

3-(3-*N*-(Boc)Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.33**). A solution of aldehyde **3.29** (45 mg, 0.08 mmol, 1 eq.) in DMF (2 mL) was treated with 2-aminothiophenol (16 mg, 0.12 mmol, 1.5 eq.) and stirred 2h at 60°C. A freshly prepared solution of I_2 (22 mg, 0.08 mmol, 1 eq.) in DMF (1 mL) was added to the reaction mixture at 60°C. The reaction was stirred for 10 min at 60°C, cooled to rt, diluted with EtOAc (50 mL), washed with a solution of 10% $Na_2S_2O_3$ (25 mL) and H_2O (2 x 25 mL), dried ($MgSO_4$), filtered and evaporated. Benzothiazole **3.33** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as brown oil (30 mg, 56% yield): R_f 0.55 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (ddd, *J* = 8.2, 1.1, 0.6 Hz, 1H), 7.83 (ddd, *J* = 7.9, 1.2, 0.6 Hz, 1H), 7.55 – 7.27 (m, 11H), 7.25 – 7.09 (m, 4H), 5.21 (br s, 1H), 4.70 (br s, 1H), 4.62 (br s, 1H), 3.61 (br s, 2H), 3.47 (br s, 3H), 3.28 (br s, 1H), 2.94 (br s, 2H), 1.69 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9,

164.0, 163.8, 152.9, 144.5, 140.7, 140.2, 136.4, 135.1, 131.6, 130.0, 128.8, 128.1, 127.4, 127.3, 127.13, 127.06, 126.6, 126.3, 125.1, 124.7, 122.6, 121.7, 121.1, 79.4, 51.1, 48.5, 38.1, 34.5, 31.0, 28.6, 27.8; LRMS (EI) m/z 646 ($M + H^+$); HRMS (ESI+): m/z for $C_{38}H_{40}N_5O_3S$ $(M + H)^+$, calcd 648.2846, found 648.2852.

3-(3-*N*-Boc-(Methyl)aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.34**). Benzothiazole **3.34** was prepared according to the procedure described for the synthesis of **3.33** using aldehyde **3.30** (90 mg, 0.16 mmol, 1 eq.), 2-aminothiophenol (30 mg, 0.24 mmol, 1.5 eq.), and I_2 (40 mg, 0.16 mmol, 1 eq.). Benzothiazole **3.34** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as brown oil (58 mg, 54% yield): R_f 0.64 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (ddd, *J* = 8.1, 1.1, 0.6 Hz, 1H), 7.82 (ddd, *J* = 7.9, 1.2, 0.5 Hz, 1H), 7.58 – 7.08 (m, 15H), 5.24 (br d, *J* = 11.8 Hz, 1H), 4.71 (br d, *J* = 12.0 Hz, 1H), 3.64 (br s, 1H), 3.46 (br s, 4H), 3.28 (br s, 1H), 3.06 (br s, 2H), 2.66 (s, 3H), 1.73 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 164.0, 163.8, 155.8, 153.3, 144.4, 140.7, 140.2, 136.4, 135.3, 131.4, 130.3, 128.8, 128.1, 127.34, 127.26, 127.1, 127.0, 126.1, 125.0, 124.6, 122.7, 121.7, 121.1, 79.2, 51.0, 48.9, 46.9, 34.4, 34.2, 31.2, 28.6, 26.1; LRMS (EI) m/z 660 ($M + H^+$); HRMS (ESI+): m/z for $C_{39}H_{42}N_5O_3S$ $(M + H)^+$, calcd 660.3003, found 660.3012.

3-(4-*N*-(Boc)Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.35**). Benzothiazole **3.35** was prepared according to the procedure described for the synthesis of **3.33** using aldehyde **3.31** (65 mg, 0.12 mmol, 1 eq.), 2-aminothiophenol (22 mg, 0.18 mmol, 1.5 eq.), and I_2 (30 mg, 0.12 mmol, 1 eq.). Benzothiazole **3.35** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (28 mg, 36% yield): R_f 0.56 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (ddd, *J* = 8.2, 1.1, 0.6 Hz, 1H), 7.83 (ddd, *J* = 7.9, 1.3, 0.6 Hz, 1H), 7.52 – 7.27 (m, 11H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.19 – 7.10 (m, 2H), 5.20 (s, 1H), 4.72 (br s, 1H), 4.41 (br s, 1H), 3.62 (br s, 1H), 3.46 (br s, 4H), 3.28 (s, 1H), 2.96 (br dt, *J* = 6.3, 6.1 Hz, 2H), 1.50 (br tt, *J* = 7.8, 6.6 Hz, 2H), 1.42 (s, 9H), 1.32 (tt, *J* = 7.2, 7.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 163.9, 163.8, 156.0, 153.3, 144.5, 140.7, 140.2, 136.5, 135.3, 131.4, 130.2,

128.8, 128.1, 127.35, 127.27, 127.1, 126.5, 126.1, 125.0, 124.6, 122.7, 121.7, 121.1, 79.1, 51.0, 50.8, 40.4, 34.4, 31.2, 28.6, 27.4, 25.0; LRMS (EI) m/z 660 ($M + H^+$); HRMS (ESI+): m/z for $C_{39}H_{42}N_5O_3S$ $(M + H)^+$, calcd 660.3003, found 660.3019.

3-(4-*N*-Boc-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-one (**3.36**). Benzothiazole **3.36** was prepared according to the procedure described for the synthesis of **3.33** using aldehyde **3.32** (33 mg, 0.06 mmol, 1 eq.), 2-aminothiophenol (11 mg, 0.09 mmol, 1.5 eq.), and I_2 (7 mg, 0.03 mmol, 0.5 eq.). Benzothiazole **3.34** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as brown oil (24 mg, 62% yield): R_f 0.37 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 8.1, 0.5 Hz, 1H), 7.82 (dd, *J* = 7.9, 0.6 Hz, 1H), 7.53 – 7.28 (m, 11H), 7.22 (d, *J* = 8.2 Hz, 2H), 7.19 – 7.08 (m, 2H), 5.21 (s, 1H), 4.69 (s, 1H), 3.65 (s, 1H), 3.45 (s, 4H), 3.27 (s, 1H), 3.18 – 2.98 (m, 2H), 2.67 (s, 3H), 1.53 – 1.29 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 164.0, 163.8, 155.9, 153.3, 144.6, 140.7, 140.2, 136.5, 135.3, 131.4, 130.2, 128.8, 128.1, 127.4, 127.3, 127.1, 126.5, 126.1, 125.0, 124.6, 122.7, 121.7, 121.1, 79.2, 51.0, 50.8, 48.5, 34.5, 34.0, 31.3, 28.6, 25.0, 24.9; LRMS (EI) m/z 674 ($M + H^+$); HRMS (ESI+): m/z for $C_{40}H_{44}N_5O_3S$ $(M + H)^+$, calcd 674.3159, found 674.3176.

3-(3-Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.37**). Hydrochloride salt **3.37** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.33** (28 mg, 0.05 mmol, 1 eq.). After purification by chromatography on silica gel (40% iPrOH and 2% NEt_3 in $CHCl_3$), the resulting oil was dissolved in CH_2Cl_2, treated with HCl (gas), and the volatiles were evaporated to provide hydrochloride **3.37** as brown solid (10 mg, 37% yield): m.p. 86-90 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.04 (d, *J* = 7.9 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 2H), 7.64 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.52 (t, *J* = 5.8 Hz, 2H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.44 – 7.36 (m, 4H), 7.33 (d, *J* = 7.3 Hz, 1H), 7.25 – 7.13 (m, 3H), 6.83 (d, *J* = 2.1 Hz, 1H), 6.63 (d, *J* = 1.5 Hz, 1H), 5.12 (br d, *J* = 15.4 Hz, 1H), 4.80 (br d, *J* = 15.8 Hz, 1H), 3.51 (br s, 1H), 3.43 – 3.35 (m, 3H), 2.64 (tt, *J* = 7.1, 6.5 Hz, 2H), 1.87 – 1.74 (m, 4H); ^{13}C NMR (101 MHz, 100 °C, DMSO) δ 169.7, 163.9, 162.3, 143.1, 139.3,

138.6, 136.1, 131.0, 129.3, 128.2, 127.7, 127.3, 126.7, 126.0, 125.97, 125.94, 125.4, 124.5, 124.3, 124.2, 121.7, 121.3, 120.8, 49.7, 47.3, 36.6, 33.2, 30.3, 24.9; LRMS (EI) m/z 546 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{32}N_5OS$ $(M + H)^+$, calcd 546.2322, found 546.2340.

3-(3-*N*-(Methyl)aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.38**). Hydrochloride salt **3.38** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.34** (62 mg, 0.09 mmol, 1 eq.). After purification by chromatography on silica gel (40% iPrOH and 2% NEt_3 in $CHCl_3$), the oil was dissolved in CH_2Cl_2 (5 mL) and treated with HCl (gas). After the volatiles were evaporated, hydrochloride **3.38** was obtained as brown solid (21 mg, 38% yield): m.p. 90-94 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.67 (s, 2H), 8.05 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.64 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.54 – 7.45 (m, 4H), 7.44 – 7.37 (m, 6H), 7.35 – 7.29 (m, 1H), 7.26 – 7.20 (m, 1H), 7.18 (d, *J* = 8.2 Hz, 2H), 5.11 (br d, *J* = 14.4 Hz, 1H), 4.80 (br d, *J* = 14.6 Hz, 1H), 3.48 (br s, 2H), 3.40 (t, *J* = 6.6 Hz, 2H), 3.31 (br s, 1H), 2.71 (br s, 2H), 2.39 (t, *J* = 5.4 Hz, 3H), 1.85 (tt, *J* = 6.7, 6.2 Hz, 2H); ^{13}C NMR (75 MHz, DMSO) δ 170.6, 164.6, 163.0, 152.7, 143.4, 139.6, 138.8, 136.6, 134.8, 131.6, 129.5, 128.8, 127.9, 127.4, 126.8, 126.5 (2C), 126.1, 124.9, 124.7, 122.2, 122.1, 121.3, 49.8, 47.6, 46.0, 33.6, 32.2, 30.5, 23.6; LRMS (EI) m/z 560 ($M + H^+$); HRMS (ESI+): m/z for $C_{34}H_{34}N_5OS$ $(M + H)^+$, calcd 560.2479, found 560.2482.

3-(4-Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.39**). Hydrochloride salt **3.39** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.35** (26 mg, 0.04 mmol, 1 eq.). After removal of the volatiles under vacuum, hydrochloride **3.39** was obtained as brown solid (16 mg, 78% yield): m.p. 115-119 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.05 (dd, *J* = 8.0, 0.9 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.80 (s, 2H), 7.64 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.58 – 7.35 (m, 10H), 7.35 – 7.28 (m, 1H), 7.26 – 7.14 (m, 3H), 5.11 (br d, *J* = 12.4 Hz, 1H), 4.79 (br d, *J* = 14.9 Hz, 1H), 3.60 – 3.35 (br m, 4H), 3.28 (br s, 2H), 2.59 (br s, 2H), 1.40 (br s, 4H); ^{13}C NMR (75 MHz, DMSO) δ 170.7, 164.1, 163.1, 152.7, 143.5, 139.6, 138.8, 136.6,

134.8, 131.5, 129.7, 128.8, 127.9, 127.4, 126.7, 126.52, 126.49, 126.0, 124.9, 124.7, 122.1, 122.1, 121.2, 50.2, 49.7, 38.5, 33.5, 30.4, 24.5, 24.2; LRMS (EI) m/z 560 (M + H^+); HRMS (ESI+): m/z for $C_{34}H_{34}N_5OS$ $(M + H)^+$, calcd 560.2479, found 560.2500.

3-(4-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(4-phenylbenzyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.40**). Hydrochloride salt **3.40** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.36** (22 mg, 0.03 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as brown solid (15 mg, 90% yield): m.p. 87-91 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.56 (s, 1H), 8.05 (dd, *J* = 7.9, 0.6 Hz, 1H), 7.93 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.65 (dd, *J* = 7.9, 1.4 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.51 – 7.45 (m, 2H), 7.45 – 7.37 (m, 6H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.25 – 7.16 (m, 3H), 5.12 (br d, *J* = 15.2 Hz, 1H), 4.79 (br d, *J* = 13.7 Hz, 1H), 3.59 – 3.37 (br m, 4H), 3.27 (br s, 2H), 2.63 (br s, 2H), 2.38 (t, *J* = 5.5 Hz, 3H), 1.40 (br s, 4H); ^{13}C NMR (75 MHz, DMSO) δ 170.7, 164.2, 162.9, 152.7, 143.5, 139.6, 138.8, 136.6, 134.8, 131.5, 129.7, 128.8, 127.9, 127.4, 126.8, 126.52, 126.49, 126.1, 124.9, 124.7, 122.14, 122.10, 121.2, 54.9, 50.0, 47.8, 33.4, 32.2, 30.4, 24.1, 22.8; LRMS (EI) m/z 574 (M + H^+); HRMS (ESI+): m/z for $C_{35}H_{36}N_5OS$ $(M + H)^+$, calcd 574.2635, found 574.2660.

3-(3-*N*-(Boc)Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.41**). A solution of olefin **3.13c** (250 mg, 0.67 mmol, 1eq.) in 3:1 dioxane/H_2O (42 mL) was treated with 2,6-lutidine (0.24 mL, 1.34 mmol, 2 eq.), $NaIO_4$ (573 mg, 2.69 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.14 mL, 0.01 mmol, 0.02 eq.). The reaction was stirred for 1h at rt, diluted with saturated $Na_2S_2O_4$ (50 mL), and extracted with EtOAc (3 x 50 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The resulting residue was dissolved in DMF (2 mL), treated with 2-aminothiophenol (92 mg, 0.74 mmol, 1.2 eq.), stirred for 1h at 60°C, and treated with a freshly prepared solution of I_2 (170 mg, 0.67 mmol, 1 eq.) in DMF (1 mL). The reaction was cooled to room temperature, diluted with EtOAc (50 mL), washed with a solution of 10% $Na_2S_2O_3$ (50 mL) and H_2O (2 x 50 mL), dried ($MgSO_4$), filtered and evaporated. After purification by chromatography on silica gel (0-1% EtOH in $CHCl_3$), benzothiazole **3.41** was

obtained as brown oil (103 mg, 32% yield): R_f 0.34 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (ddd, *J* = 8.1, 1.2, 0.7 Hz, 1H), 7.83 (ddd, *J* = 7.9, 1.2, 0.6 Hz, 1H), 7.49 – 7.39 (m, 2H), 7.39 – 7.30 (m, 2H), 7.15 – 7.04 (m, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 4.75 (t, *J* = 6.0 Hz, 1H), 3.59 (t, *J* = 6.9 Hz, 2H), 3.49 (t, *J* = 6.9 Hz, 2H), 3.33 (t, *J* = 7.0 Hz, 2H), 3.02 (dt, *J* = 12.0, 5.9 Hz, 2H), 1.72 (tt, *J* = 6.8, 6.6 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 163.5, 163.2, 156.1, 153.2, 141.9, 135.2, 132.2, 127.5, 126.1 (2C), 125.0, 123.9, 122.6, 121.6, 120.2, 79.0, 48.2, 38.1, 35.1, 31.2, 28.6, 27.7; LRMS (EI) m/z 480 ($M + H^+$); HRMS (ESI+): m/z for $C_{25}H_{30}N_5O_3S$ $(M + H)^+$, calcd 480.2069, found 480.2072.

3-(4-*N*-(Boc)Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.42**). Benzothiazole **3.42** was prepared according to the procedure described for the synthesis of **3.41** from olefin **3.17c** (250 mg, 0.65 mmol, 1 eq) using 2,6-lutidine (0.24 mL, 1.30 mmol, 2 eq.), $NaIO_4$ (556 mg, 2.60 mmol, 4 eq.) and OsO_4 (2.5 % solution in *t*-BuOH, 0.13 mL, 0.01 mmol, 0.02 eq.), 2-aminothiophenol (90 mg, 0.72 mmol, 1.1 eq.), and I_2 (165 mg, 0.65 mmol, 1 eq.). Purification by chromatography on silica gel (30% EtOAc in hexanes) gave benzothiazole **3.42** as brown oil (71 mg, 22% yield): R_f 0.32 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (ddd, *J* = 8.1, 1.1, 0.6 Hz, 1H), 7.84 (ddd, *J* = 7.9, 1.2, 0.6 Hz, 1H), 7.52 – 7.39 (m, 2H), 7.39 – 7.29 (m, 2H), 7.10 (td, *J* = 7.7, 1.1 Hz, 1H), 6.83 (dd, *J* = 8.0, 0.7 Hz, 1H), 6.75 (s, 1H), 4.47 (s, 1H), 3.61 – 3.41 (m, 4H), 3.33 (t, *J* = 7.0 Hz, 2H), 2.98 (dt, *J* = 13.8, 6.9 Hz, 2H), 1.53 (tt, *J* = 7.5, 7.2 Hz, 2H), 1.42 (s, 9H), 1.38 – 1.28 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 163.2, 163.0, 156.0, 153.3, 141.8, 135.3, 132.1, 127.5, 126.2, 126.1, 124.9, 123.9, 122.6, 121.7, 120.1, 79.1, 50.5, 40.4, 35.1, 31.2, 28.6, 27.3, 24.8; LRMS (EI) m/z 494 ($M + H^+$); HRMS (ESI+): m/z for $C_{26}H_{32}N_5O_3S$ $(M + H)^+$, calcd 494.2220, found 494.2241.

3-(4-*N*-Boc-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.43**). Benzothiazole **3.43** was prepared according to the procedure described for the synthesis of **3.41** from olefin **3.24** (100 mg, 0.25 mmol, 1 eq) using 2,6-lutidine (0.09 mL, 0.50 mmol, 2 eq.), $NaIO_4$ (214 mg, 1.00 mmol, 4 eq.), OsO_4 (2.5 % solution in *t*-BuOH, 0.05 mL, 0.005 mmol, 0.02 eq.), 2-

aminothiophenol (31 mg, 0.25 mmol, 1.0 eq.), and I_2 (63 mg, 0.25 mmol, 1 eq.). Benzothiazole **3.43** was isolated by chromatography on silica gel (40% EtOAc in hexanes) as brown oil (33 mg, 26% yield): R_f 0.39 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (ddd, *J* = 8.2, 1.1, 0.6 Hz, 1H), 7.83 (ddd, *J* = 8.0, 1.3, 0.6 Hz, 1H), 7.48 – 7.39 (m, 2H), 7.37 – 7.32 (m, 2H), 7.10 (td, *J* = 7.8, 0.8 Hz, 1H), 6.90 (s, 1H), 6.84 (d, *J* = 7.9 Hz, 1H), 3.57 (t, *J* = 7.2 Hz, 2H), 3.49 (t, *J* = 7.0 Hz, 2H), 3.33 (t, *J* = 7.1 Hz, 2H), 3.06 (br s, 2H), 2.71 (s, 3H), 1.50 (br s, 2H), 1.40 (s, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 163.3, 163.2, 155.8, 153.2, 141.9, 135.3, 132.1, 127.4, 126.2, 126.1, 124.9, 123.8, 122.6, 121.6, 120.1, 79.2, 50.5, 48.6, 35.1, 34.1, 31.3, 28.6, 25.0, 24.7; LRMS (EI) m/z 508 ($M + H^+$); HRMS (ESI+): m/z for $C_{27}H_{34}N_5O_3S$ $(M + H)^+$, calcd 508.2377, found 508.2389.

3-(3-*N*-(Boc)Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.44h**). Benzotriazepinone **3.44h** was prepared according to the procedure described for the synthesis of **3.18d** from alkylation of triazepinone **3.41** (17 mg, 0.035 mmol, 1 eq.) using *t*-BuOK (5 mg, 0.042 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (9 mg, 0.042 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (16 mg, 73% yield): R_f 0.50 (50% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.77 – 7.68 (m, 2H), 7.68 – 7.61 (m, 2H), 7.46 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H), 7.43 – 7.24 (m, 6H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 5.35 (br s, 1H), 4.84 (br s, 1H), 4.60 (s, 1H), 3.78 – 3.17 (br m, 6H), 2.96 (br td, *J* = 6.0, 5.6 Hz, 2H), 1.68 (br s, 2H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 164.2, 164.0, 156.0, 153.3, 144.3, 135.3, 134.7, 133.3, 132.8, 131.5, 130.21, 130.16, 128.4, 127.8, 126.8, 126.6, 126.3, 126.1, 126.0, 125.6, 125.0, 124.7, 122.7, 121.7, 121.1, 79.0, 51.6, 48.6, 38.2, 34.4, 31.2, 28.6, 27.8; LRMS (EI) m/z 620 ($M + H^+$); HRMS (ESI+): m/z for $C_{36}H_{38}N_5O_3S$ $(M + H)^+$, calcd 620.2690, found 620.2710.

3-(3-*N*-(Boc)Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.44i**). Benzotriazepinone **3.44i** was prepared according to the procedure described for the synthesis of **3.18d** by alkylation of triazepinone **3.41** (18 mg, 0.038 mmol, 1 eq.) using *t*-BuOK (5 mg,

0.045 mmol, 1.2 eq.) and 1-(bromomethyl)naphthalene (10 mg, 0.045 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (10 mg, 42% yield): R_f 0.48 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.0 Hz, 1H), 7.89 – 7.75 (m, 3H), 7.68 (d, *J* = 8.1 Hz, 1H), 7.53 – 7.23 (m, 9H), 7.09 (ddd, *J* = 10.6, 10.6, 1.4 Hz, 1H), 5.76 (br s, 1H), 5.03 (br s, 1H), 4.60 (s, 1H), 3.61 (br s, 1H), 3.53 (br s, *J* = 1.1 Hz, 1H), 3.16 (br s, 1H), 2.92 (br s, 2H), 2.80 (br s, 3H), 1.59 (br s, 2H), 1.41 (s, *J* = 14.7 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 164.3, 163.8, 156.0, 153.2, 143.9, 135.3, 134.0, 132.2, 131.4, 131.3, 130.3, 129.1, 128.6, 128.0, 126.5, 126.4, 126.1, 126.0, 125.1, 124.9, 124.8, 123.7, 122.6, 121.7, 121.6, 79.0, 49.5, 48.5, 38.1, 34.2, 30.7, 28.6, 27.8; LRMS (EI) m/z 620 (M + H^+); HRMS (ESI+): m/z for $C_{36}H_{38}N_5O_3S$ $(M + H)^+$, calcd 620.2690, found 620.2707.

3-(4-*N*-(Boc)Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.45h**). Benzotriazepinone **3.45h** was prepared according to the procedure described for the synthesis of **3.18d** by alkylation of triazepinone **3.42** (8 mg, 0.016 mmol, 1 eq.) using *t*-BuOK (2 mg, 0.019 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (4 mg, 0.019 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (5 mg, 50% yield): R_f 0.48 (50% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.9 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.77 – 7.68 (m, 2H), 7.68 – 7.61 (m, 2H), 7.46 (ddd, *J* = 7.6, 7.6, 1.2 Hz 1H), 7.43 – 7.27 (m, 6H), 7.18 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 5.36 (br s, 1H), 4.83 (br s, 1H), 4.39 (s, 1H), 3.65 br (s, 1H), 3.46 (br s, 4H), 3.26 (br s, 1H), 2.95 (br s, 2H), 1.49 (br s, 2H), 1.42 (s, 11H) 1.31; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 164.0, 163.9, 156.1, 153.3, 144.3, 135.4, 134.8, 133.4, 132.8, 131.4, 130.3 (2C), 128.4, 127.8, 126.8, 126.5, 126.3, 126.1, 125.9, 125.6, 125.0, 124.6, 122.7, 121.7, 121.1, 79.1, 51.2, 50.8, 49.2, 34.4, 31.2, 28.6, 25.0, 24,9; LRMS (EI) m/z 634 (M + H^+); HRMS (ESI+): m/z for $C_{37}H_{40}N_5O_3S$ $(M + H)^+$, calcd 634.2846, found 634.2862.

3-(4-*N*-(Boc)Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.45i**). Benzotriazepinone **3.45i** was prepared according to the procedure described for the synthesis of **3.18d** by

alkylation of triazepinone **3.42** (8 mg, 0.016 mmol, 1 eq.) using *t*-BuOK (2 mg, 0.019 mmol, 1.2 eq.) and 1-(bromomethyl)naphthalene (4 mg, 0.019 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (7 mg, 70% yield): R_f 0.47 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.87 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.84 – 7.75 (m, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.54 – 7.22 (m, 9H), 7.09 (ddd, *J* = 8.4, 7.2, 1,2 Hz 1H), 5.78 (br d, *J* = 13.5 Hz, 1H), 5.01 (br d, *J* = 11.6 Hz, 1H), 4.38 (s, 1H), 3.66 (br s, 1H), 3.43 (br s, 2H), 3.16 (br s, 1H), 3.01 – 2.84 (br m, 3H), 2.83 – 2.70 (br m, 2H), 1.41 (s, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 164.1, 163.8, 156.0, 153.2, 144.0, 135.3, 134.0, 132.3, 131.4, 131.2, 130.4, 129.0, 128.5, 128.0, 126.43, 126.37, 126.04, 125.96, 125.1, 124.9, 124.8, 123.8, 122.6, 121.7, 121.6, 82.5, 50.7, 49.4, 40.4, 34.2, 30.7, 28.6, 27.4, 25.0; LRMS (EI) m/z 634 ($M + H^+$); HRMS (ESI+): m/z for $C_{37}H_{40}N_5O_3S$ $(M + H)^+$, calcd 634.2846, found 634.2863.

3-(4-*N*-Boc-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.46h**). Benzotriazepinone **3.46h** was prepared according to the procedure described for the synthesis of **3.18d** by alkylation of triazepinone **3.43** (15 mg, 0.030 mmol, 1 eq.) using *t*-BuOK (4 mg, 0.035 mmol, 1.2 eq.) and 2-(bromomethyl)naphthalene (8 mg, 0.035 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (15 mg, 79% yield): R_f 0.51 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 7.5, 0.7 Hz, 1H), 7.83 (dd, *J* = 8.0, 0.7 Hz, 1H), 7.77 – 7.68 (m, 2H), 7.68 – 7.61 (m, 2H), 7.52 – 7.28 (m, 7H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.08 (t, *J* = 7.3 Hz, 1H), 5.38 (br d, *J* = 13.2 Hz, 1H), 4.82 (br d, *J* = 12.1 Hz, 1H), 3.70 (br s, 1H), 3.45 (br s, 4H), 3.26 (br s, 1H), 3.03 (br s, 2H), 2.67 (s, 3H), 1.54 – 1.29 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 164.1, 163.9, 155.9, 153.3, 144.4, 135.3, 134.8, 133.3, 132.8, 131.4, 130.3 (2C), 128.4, 127.8, 126.8, 126.5, 126.3, 126.1, 125.9, 125.6, 125.0, 124.6, 122.7, 121.7, 121.1, 79.2, 51.1, 50.7, 48.5, 34.5, 34.0, 31.3, 28.6, 25.0, 24.9; LRMS (EI) m/z 670 ($M + Na^+$); HRMS (ESI+): m/z for $C_{38}H_{42}N_5O_3S$ $(M + H)^+$, calcd 648.3003, found 648.3030.

3-(4-*N*-Boc-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**3.46i**). Benzotriazepinone

3.46i was prepared according to the procedure described for the synthesis of **3.18d** by alkylation of triazepinone **3.43** (15 mg, 0.030 mmol, 1 eq.) using *t*-BuOK (4 mg, 0.035 mmol, 1.2 eq.) and 1-(bromomethyl)naphthalene (8 mg, 0.035 mmol, 1.2 eq.), and isolated by chromatography on silica gel (30% EtOAc in hexanes) as yellow oil (11 mg, 58% yield): R_f 0.55 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.93 (dd, *J* = 8.1, 0.6 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.25 – 7.52 (m, 9H), 7.08 (ddd, *J* = 8.0, 6.9, 1,2 Hz 1H), 5.80 (br d, *J* = 14.0 Hz, 1H), 5.00 (br d, *J* = 14.8 Hz, 1H), 3.69 (br s, 1H), 3.43 (br s, 1H), 3.14 (br s, 1H), 3.02 (br s, 2H), 2.88 (br s, 1H), 2.79 – 2.62 (m, 5H), 1.54 – 1.27 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 164.3, 163.9, 155.9, 153.2, 144.0, 135.3, 134.0, 132.3, 131.4, 131.2, 130.4, 129.0, 128.6, 128.1, 126.4, 126.4, 126.04, 125.97, 125.1, 124.9, 124.7, 123.8, 122.6, 121.7, 121.6, 79.2, 50.6, 49.4, 48.5, 34.2, 33.9, 30.7, 28.6, 24.9 (2C); LRMS (EI) m/z 670 (M + Na^+); HRMS (ESI+): m/z for $C_{38}H_{42}N_5O_3S$ (M + H)$^+$, calcd 648.3003, found 648.3024.

3-(3-Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.47h**). Hydrochloride salt **3.47h** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.44h** (11 mg, 0.018 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as pale brown solid (10 mg, quantitative yield): m.p. 87-91 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.94 (t, *J* = 9.0 Hz, 2H), 7.62 (ddd, *J* = 15.6, 12.2, 5.4 Hz, 6H), 7.47 (t, *J* = 7.6 Hz, 2H), 7.43 – 7.34 (m, 3H), 7.24 – 7.17 (m, 2H), 5.32 (d, *J* = 11.0 Hz, 1H), 4.87 (br s, 1H), 3.66 – 3.46 (m, 4H), 2.74 (br t, *J* = 6.5 Hz, 2H), 1.91 (br s, 2H), 1.29 (br s, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.5, 166.2, 165.3, 150.7, 145.2, 135.9, 135.2, 134.6, 134.1, 133.1, 131.1, 129.3, 128.64, 128.61, 128.3, 127.8, 127.7, 127.4, 127.1, 127.0, 126.5, 126.2, 123.4, 122.9, 121.9, 52.3, 48.8 (HSQC), 38.5, 31.1, 30.7, 26.5; LRMS (EI) m/z 520 (M + H^+); HRMS (ESI+): m/z for $C_{31}H_{30}N_5OS$ (M + H)$^+$, calcd 520.2166, found 520.2169.

3-(3-Aminopropyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.47i**). Hydrochloride salt **3.47i** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.44i** (12 mg, 0.018 mmol, 1 eq.), and after removal of the volatiles under

vacuum, obtained as pale brown solid (11 mg, quantitative yield): m.p. 84-88°C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.92 (dd, *J* = 7.8, 6.5 Hz, 2H), 7.82 – 7.74 (m, 2H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.63 – 7.36 (m, 8H), 7.27 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.24 – 7.17 (m, 1H), 5.77 (d, *J* = 13.1 Hz, 1H), 5.09 (d, *J* = 14.5 Hz, 1H), 3.57 (d, *J* = 17.5 Hz, 2H), 3.25 (s, 1H), 2.77 (br s, 5H), 1.88 (br tt, *J* = 13.9, 7.0 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.3, 166.3, 165.2, 151.7, 144.8, 135.5, 135.4, 133.5, 132.9, 132.6, 131.3, 130.1, 129.7, 129.6, 128.0, 127.5, 127.3, 126.9, 126.8, 126.4, 126.1, 124.7, 123.6, 123.2, 122.2, 50.3, 48.5, 38.5, 34.7, 30.8, 26.5; LRMS (EI) m/z 520 ($M + H^+$); HRMS (ESI+): m/z for $C_{31}H_{30}N_5OS$ $(M + H)^+$, calcd 520.2166, found 520.2155.

3-(4-Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.48h**). Hydrochloride **3.48h** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.45h** (4 mg, 0.006 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as pale brown solid (4 mg, quantitative yield): m.p. 84-88°C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 – 7.89 (m, 2H), 7.76 – 7.71 (m, 1H), 7.72 – 7.67 (m, 1H), 7.65 (s, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.55 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.50 (ddd, *J* = 8.2, 7.3, 1.1 Hz, 1H), 7.47 – 7.37 (m, 5H), 7.23 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.22 – 7.17 (m, 1H), 5.34 (d, *J* = 14.6 Hz, 1H), 4.85 (br s, 1H), 3.58 (br s, 2H), 3.50 – 3.36 (br m, 3H), 3.25 (br s, 1H), 2.64 (br t, *J* = 6.9 Hz, 2H), 1.61 (br s, 1H), 1.44 (br t, *J* = 10.5 Hz, 2H), 1.30 (s, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 173.3, 166.1, 165.5, 153.9, 145.3, 136.2, 136.0, 134.6, 134.1, 132.8, 131.5, 129.3, 128.64, 128.60, 127.8, 127.7, 127.4, 127.3, 126.9, 126.5, 126.3, 126.1, 123.1, 122.9, 122.7, 52.1, 51.3, 40.3, 34.9, 31.6, 25.9, 25.4; LRMS (EI) m/z 534 ($M + H^+$); HRMS (ESI+): m/z for $C_{32}H_{32}N_5OS$ $(M + H)^+$, calcd 534.2321, found 532.2333.

3-(4-Aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.48i**). Hydrochloride salt **3.48i** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.45i** (4 mg, 0.006 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as pale brown solid (11 mg, quantitative yield): m.p. 89-93°C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.87 – 7.81 (m, 2H), 7.73 (d, *J* = 8.2 Hz,1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.55 – 7.38 (m, 7H),

7.29 (dd, *J* = 7.9, 7.3 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 5.79 (d, *J* = 14.9 Hz, 1H), 5.09 (d, *J* = 14.5 Hz, 1H), 3.64 (s, 2H), 3.39 (s, 1H), 3.23 (s, 1H), 2.77 – 2.68 (m, 3H), 2.69 – 2.61 (m, 2H), 1.63 (br s, 1H), 1.42 (br d, *J* = 5.6 Hz, 2H), ^{13}C NMR (126 MHz, CD_3OD) δ 173.1, 166.1, 165.4, 153.9, 144.7, 136.2, 135.4, 133.5, 132.61 (2C), 131.6, 130.1, 129.63, 129.56, 127.5, 127.34, 127.30, 126.9, 126.3, 126.2, 126.1, 124.7, 123.5, 123.0, 122.9, 51.3, 50.2, 40.3, 34.6, 31.1, 25.9, 25.4; LRMS (EI) m/z 534 ($M + H^+$); HRMS (ESI+): m/z for $C_{32}H_{32}N_5OS$ $(M + H)^+$, calcd 534,2322, found 534.2333.

3-(4-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(2-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.49h**). Hydrochloride salt **3.49h** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.46h** (14 mg, 0.020 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as pale brown solid (13 mg, quantitative yield): m.p. 78-82°C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 – 7.90 (m, 2H), 7.75 – 7.65 (m, 2H), 7.65 – 7.59 (m, 2H), 7.59 – 7.52 (m, 2H), 7.50 – 7.42 (m, 2H), 7.42 – 7.36 (m, 3H), 7.21 (dd, *J* = 12.9, 4.5 Hz, 2H), 5.33 (br d, *J* = 13.6 Hz, 1H), 4.85 (br s, 1H), 3.69 – 3.55 (br m, 2H), 3.55 – 3.45 (br m, 2H), 3.40 (br s, 1H), 3.23 (br s, 1H), 2.68 (t, *J* = 6.9 Hz, 2H), 2.51 (s, 3H), 1.61 (br s, 2H), 1.49 – 1.39 (br m, 2H), ^{13}C NMR (75 MHz, CD_3OD) δ 175.1, 165.9, 165.3, 151.7, 145.3, 136.0, 134.6, 134.1, 132.9, 131.4, 129.3, 128.64, 128.61, 128.0, 127.8, 127.7, 127.3, 127.0, 126.9, 126.5, 126.2, 123.3, 122.8, 122.3, 121.0, 52.2, 51.3, 49.9, 34.7, 33.4, 31.1, 25.3, 24.5; LRMS (EI) m/z 548 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{34}N_5OS$ $(M + H)^+$, calcd 548.2479, found 548.2488.

3-(4-*N*-(Methyl)aminobutyl)-5-(2-(benzo[d]thiazol-2-yl)ethyl)-1-(1-naphthylmethyl)-1H-1,3,4-benzotriazepin-2(3H)-one hydrochloride (**3.49i**). Hydrochloride salt **3.49i** was prepared according to the procedure described for the synthesis of **3.19d** from carbamate **3.46i** (10 mg, 0.015 mmol, 1 eq.), and after removal of the volatiles under vacuum, obtained as brown solid (8 mg, quantitative yield): m.p. 84-88 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.93 (dd, *J* = 11.8, 4.6 Hz, 2H), 7.88 – 7.75 (m, 2H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.61 – 7.33 (m, 8H), 7.28 (dd, *J* = 8.2, 7.1 Hz, 1H), 7.24 – 7.13 (m, 1H), 5.77 (d, *J* = 14.6 Hz, 1H), 5.08 (d, *J* = 14.2 Hz,

1H), 3.61 (s, 1H), 3.47 – 3.18 (m, 3H), 2.83 (t, J = 6.5 Hz, 2H), 2.67 (br t, J = 7.1 Hz, 2H), 2.52 (s, 3H), 1.62 (br s, 1H), 1.41 (br s, 2H), 1.30 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.3, 166.0, 165.3, 152.2, 144.8, 135.4 (2C), 133.4, 132.7, 132.6, 131.6, 130.1, 129.6 (2C), 127.8, 127.5, 127.3, 126.9, 126.7, 126.3, 126.1, 124.7, 123.5, 123.2, 122.4, 51.2, 50.3, 48.2 (HSQC), 34.4, 33.4, 30.8, 25.4, 24.5; LRMS (EI) m/z 548 ($M + H^+$); HRMS (ESI+): m/z for $C_{33}H_{34}N_5OS$ $(M + H)^+$, calcd 548.2479, found 548.2485.

Biology.

Aortic Ring Contraction. Adult male Sprague−Dawley rats (Charles-Rivers, San Diego, CA) weighing 250−300 g were housed in group cages under controlled illumination (12h light − 12h dark cycle), humidity, and temperature (21−23 °C) and were given free access to tap water and rat chow. All experimental procedures were performed in accordance with regulations and ethical guidelines from the Canadian Council for the Care of Laboratory Animals and received approvals from the institutional animal care and use committee of the Institut National de la Recherche Scientifique - Institut Armand-Frappier. On the day of the experiment, rats were sacrified[b] by CO_2 asphyxiation. The thoracic aorta was stored in the fridge, used maximum 24 hours after the death, cleared of surrounding tissue and excised from the aortic arch to the diaphragm. From each vessel, conjunctive tissues were removed, and the clean vessel was cut into 4 mm rings. The endothelium was removed by rubbing gently the vessel intimal surface. All preparations were placed in 5 mL organ baths filled with oxygenated normal Krebs−Henseleit solution. Contractile responses to 40 mM KCl were used as control at the beginning of each experiment. Agonist activity of benzotriazepinone was measured by adding a high concentration of each analog in the organ chamber (10^{-5} M). For antagonist behavior, thoracic aortic rings were first exposed to a sample of benzotriazepinone for at least 15 min (10^{-5} M), to ensure that the peptide reached equilibrium and that no agonistic effect was observed, and then cumulative concentration−response curves to hUII or URP (10^{-11} to 10^{-6} M)

[b] The word "killed" was used in the published article.

were constructed. The amplitude of the contraction induced by each peptide concentration was expressed as a percentage of the KCl-induced contraction.

Gq and G_{12} activation. HEK 293-UT cells were grown in DMEM culture media supplemented with 10% foetal bovine serum, HEPES, sodium pyruvate and G418 (400 µg/mL). Passages were performed when cells reached 80% of confluency. A transfection mix (1 mL) was prepared in OptiMEM by adding 2.5 µg of a Gq-polycistronic or 0.5 µg of a G_{12}-polycistronic BRET biosensor supplemented with a sufficient amount of pBlue Script expression vector to equate 7.5 µg of DNA and 15 µL of TransIT reagent.[74] Next, this mixture was added 30 minutes later to a premixed suspension of $1.5x10^6$ cells in DMEM culture media supplemented with 2.5% foetal bovine serum without antibiotics. Cells were then plated in 96-well plates at a density of 15,000 cells/well. Sixteen hour post-transfection, medium was replaced with DMEM supplemented with 5% FBS, HEPES, sodium pyruvate and G418 (400 µg/mL) and cell growth was resumed for another 24 h. Cells were washed with 120 µL PBS solution supplemented with 0.1 % of glucose, and then incubated with 80 µL of this solution for 2 h. Then, 10 µL of a 1/20 dilution (diluted before use) of coelenterazine 400A (stock at 1 mM in ethanol) in Krebs and 10 µL of the 10x appropriate concentration were added, and the luminescence was evaluated with an Infinite® M1000 PRO. Filters were set at 410 nm and 515 nm for detecting the *Renilla* luciferase II (RlucII, donor) and green fluorescent protein 10 (GFP10, acceptor) light emission, respectively. BRET signals were monitored for 5 min after co-addition of coelenterazine 400A and ligands. BRET ratio was determined by calculating the ratio of the light emitted by GFP10 over the light emitted by the RlucII. BRET signals were normalized to that of URP (10^{-5}M) or hUII (10^{-5}M).

Statistical Analysis. Binding and functional experiments were performed at least in triplicate, and data, expressed as mean ± SEM, were analyzed with the Prism software (Graph Pad Software, San Diego, CA, USA). In all experiments, n represents the total number of animals studied or individual assays performed. EC_{50}, pEC_{50}, pIC_{50}, and E_{max} values were determined from corresponding concentration−response curves obtained through a sigmoidal dose−response fit with variable slope. Statistical comparisons of binding affinities and contractile potencies

were analyzed using unpaired Student's t test, and differences were considered significant when *P < 0.05, **P < 0.01, and ***P < 0.001.

ASSOCIATED CONTENT

The following files are available free of charge.

NMR spectra (^{1}H and ^{13}C) of all new compounds, purity and analytical HPLC chromatograms for all of benzotriazepin-2-ones that were biologically tested, curves of hUII- and URP-induced contractions of rat aortic ring in presence of benzotriazepin-2-one derivatives, curves and tables of G12 and Gq activation and molecular formula strings.

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ABBREVIATIONS

As, Azasulfuryl; Bip, 4,4′-biphenylalanine; Bip, Biphenylmethyl; Boc, tert-butyloxycarbonyl; Btz, Benzotrhiazolalanine; DMF, dimethylformamide; GPCR, G protein-coupled receptor; hUII, human urotensin II; Nal, naphthylalanine; Nap, naphthylmethyl; Orn, ornithine; Pep, (phenylethynyl)phenylalanine; UII, urotensin II; URP, urotensin II-related peptide; UT, urotensin II receptor.

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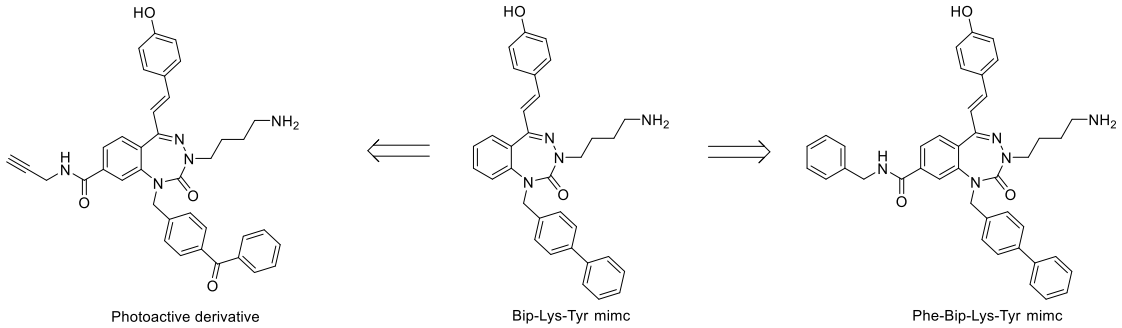
Chapitre 4 : Synthèse de 1,3,4-benzotriazépin-2-one comme mime tetrapeptidiques pour la modulation et la liaison au récepteur urotensinergique.

1. Mise en contexte de l'article 3

Ce troisième article, offre une perspective sur l'amélioration de l'activité de la série benzotriazépinique, développée précédemment, modulant les effets de hUII et URP. En effet, guidé par une étude sur des mimes de hUII, démontrant l'importance de la phénylalanine pour la modulation de UT, l'incorporation d'un mime de phénylalanine sur le squelette benzotriazépinique est présenté. Pour ce faire, une fonction *N*-benzylamide a été installée en position 8 de la benzotriazépine pour mimer la séquence tetrapeptidique Phe-Bip-Lys-Tyr de l'Urocontrin ([Bip^4]URP). De plus le développement de molécules photo-réactives, dérivés de la benzophenone, possédant un groupement propargyle, est exposé. Le but final étant de pouvoir se lier de manière covalente au récepteur urotensinergique, de dégrader le récepteur avec des enzymes, puis de se servir du groupement propargyle pour attacher un auxiliaire de biotin, purifier les fragments peptidiques obtenus avec un colomne avidine et ainsi localiser, par spectrométrie de masse, la partie responsable de la modulation et la discrimination sélective entre les activités de hUII et URP.

Article 3

Douchez, A; Lubel, W. D. Synthesis of 1,3,4-benzotriazepin-2-one tetrapeptide mimics for the modulation and ligation of the urotensin II receptor. *Manuscript in preparation.*



Synthesis of 1,3,4-benzotriazepin-2-one tetrapeptide mimics for the modulation and ligation of the urotensin II receptor.

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Abstract

1,3,4-Benzotriazepin-2-ones have been demonstrated to modulate the urotensin II receptor (UT) and differentiate the effects of the endogenous ligands urotensin II (UII) and urotensin II-related peptide (URP) likely by mimicry of the purported bioactive γ-turn conformation adopted by the Bip-Lys-Tyr tripeptide sequence in Urocontrin ([Bip[4]]URP). Towards the goals of improving the antagonist activity of the Urocontrin mimics and identifying their binding location in UT responsible for modulation of hUII and URP activity, a method for the synthesis of 8-position substituted 1,3,4-benzotriazepin-2-ones has been developed. 1,3,4-Benzotriazepin-2(3H)-on-8-carboxylates (e.g. **4.15**) were synthesized and employed in the preparation of mimics of the Phe-Bip-Lys-Tyr tetrapeptide sequence in Urocontrin to examine the potential for a *N*-benzylamide to mimic the Phe residue. Furthermore, the benzotriazepinon-8-carboxylate scaffold has served in the synthesis of photo-reactive ligands containing a benzophenone residue for tagging the UT receptor.

Introduction

Benzotriazepines are the achiral aza-relatives of benzodiazepine "privileged" structures.[1,2,3,4] Although both heterocycles may exhibit their abundant biological activities through mimicry of natural peptide turn conformations,[5,6,7] benzotriazepines have received significantly less attention than their diazepine cousins,[8] in spite potential advantages such as adaptive chirality for ligand binding,[9,10] and lower costs

to assemble the achiral architecture.[11,12] For example, achiral benzotriazepin-2-ones **4.1** and **4.2** have proven respectively effective as an orally active cholecystokinin-2 antagonist,[10,13] and parathyroid hormone-1 receptor antagonist (Figure 4.1).[14] In addition to claims of their psycho-stimulant, antidepressant, anorexigenic, and antihypertensive activities,[15] benzotriazepine scaffolds can mimic peptide–turn geometry.[1,16] The latter feature has been key for the design of achiral modulators of the urotensinergic system.[17]

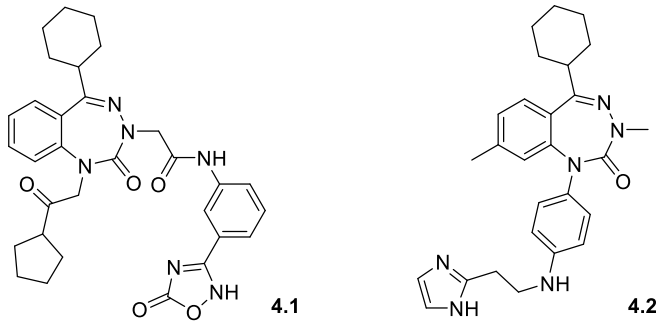


Figure 4.1. Benzotrizepinones **4.1** and **4.2,** CCK_2 and $hPTH_1$ antagonist.

The urotensinergic system is composed of two endogenous cyclic disulfide bridged peptides, urotensin II (hUII, H-Glu-Thr-Pro-Asp-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and urotensin II-related peptide (URP, H-Ala-*c*[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH), which respectively bind to the G protein-coupled receptor (GPCR) urotensin II (UT).[18] Found ubiquitously in vertebrates, UT is primarily expressed in the circulatory, excretory, and central nervous systems.[19] Furthermore, hUII is notably known as the most potent natural vasoconstrictive peptide.[20] Over the past two decades, UT and its ligands have thus garnered significant interest as targets to treat various indications,[21] including atherosclerosis,[22,23] pulmonary arterial hypertension,[24,25] metabolic syndrome,[26] and cardiac[27,28,29] and renal failure.[30,31] In spite of important investments, however, success has eluded efforts to develop drugs targeting UT for renal ischemia,[32] diabetic nephropathy,[33] and asthma,[34] likely due in part to the absence of selectivity in modulating the activity of the two endogenous ligands. Notably the divergent biological activities mediated by UII and URP include transcriptional modulation, [35] cell proliferation,[36] and myocardial contractility.[37]

Sharing a conserved *c*[Cys-Phe-Trp-Lys-Tyr-Cys] sequence, which has been demonstrated by NMR spectroscopy and computational analysis to adopt a similar conformation, UII and URP play distinct roles in different pathologies,[38,39,40] due likely to the differences in the sequence and length of their *N*-terminal tails.[41] The Trp-Lys-Tyr motif has however been shown to be crucial for their biological activity and proposed to adopt an inverse γ-turn within a specific binding pocket.[42,43] With the goal to understand the specific interactions for their UT affinity and respective signaling cascades in normal and pathological conditions, the development of selective modulators of hUII and URP activity has been pursued based on the common elements of their cyclic structures (Figure 4.1). For example, peptides derived from URP, such as Urocontrin (**4.3**, [Bip^4]URP, Bip = 4,4′-biphenylalanine)[44] and Urocontrin A (**4.4**, [Pep^4]URP, Pep = (phenylethynyl)phenylalanine},[45] possess ability to decrease the efficacy but not the potency of hUII and without influencing the efficacy and potency of URP in an *ex-vivo* rat aortic ring contraction bioassay. Azasulfurylpeptide analogs, based on the fully active cyclic octapeptide sequence UII(4−11),[46] (e.g., **4.5**, [AsNal(2')^7]UII(4-11), AsNal(2') = azasulfuryl-2-naphthylalanine),[47] possessed similar but less potent activity as **4.3**. Head-to-tail cyclic urotensin core mimics, *c*[Trp-Lys-Tyr-Gly-w(triazole)-Gly] (**4.6**) and *c*[Phe-Trp-Lys- Tyr-Gly-w(triazole)-Gly] (**4.7**), reduced the potency and the efficacy of URP; however, **4.7** exhibited more pronounced activity, demonstrating the importance of the phenylalanine residue for future UT modulator design.[48]

Small molecule UT modulators include pyrrolodiazepinones (e.g., **4.8**),[49] and benzotriazepinones (e.g., **4.9** and **4.10**),[17] which were designed to mimic the Bip-Lys-Tyr γ-turn motif of **4.3**. Both heterocycle cores demonstrated capacity to differentiate hUII and URP activity. For example, pyrrolodiazepinone **4.8** exhibited the same activity but lower potency as the parent peptide **4.3**. In the benzotriazepinone series, modulator selectivity was contingent of the phenolic side chain: *p*-hydroxyphenethyl and *p*-hydroxyphenethenyl analogs **4.10** and **4.9** blocked respectively URP- and hUII-induced vasoconstriction. Benzotriazepinone **4.9** was however less potent than peptide **4.3** and pyrolodiazepinone **4.8** (Figure 4.2).

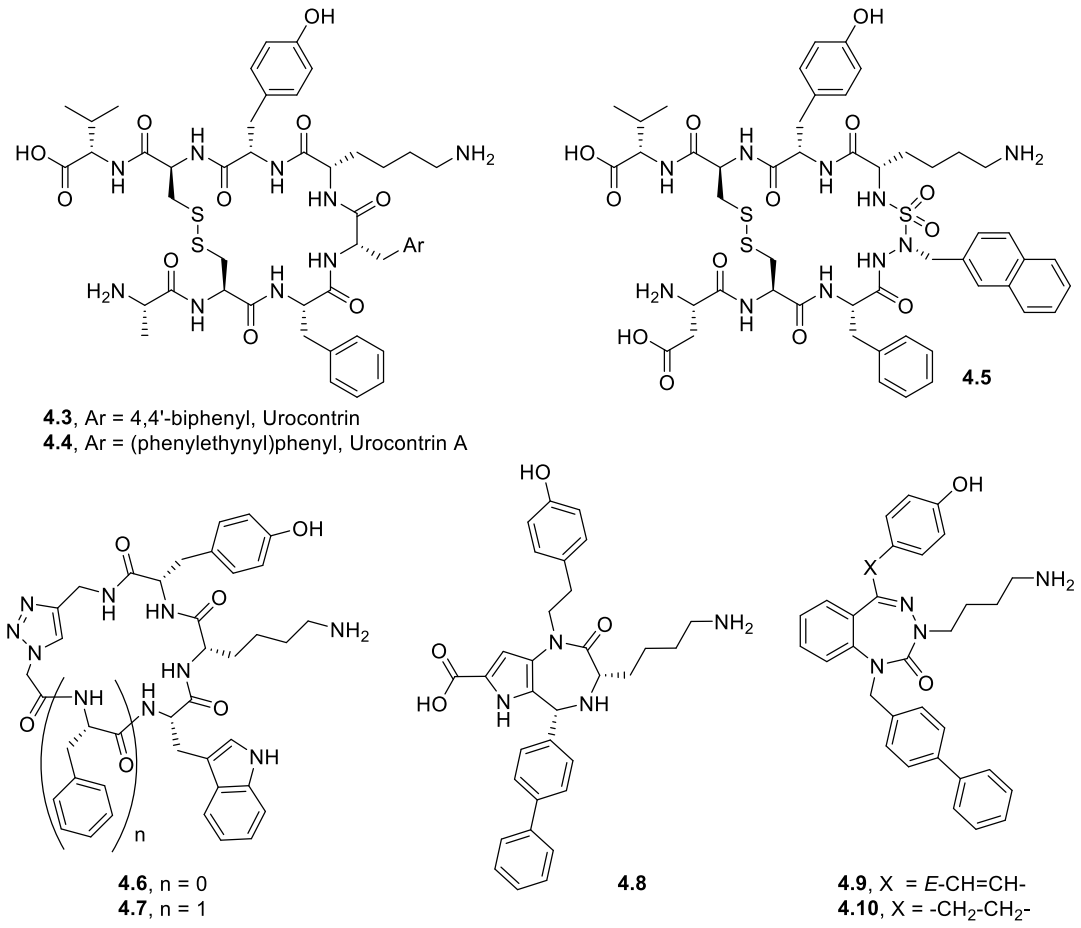


Figure 4.2. Modulators of UT.

Guided by previous studies of peptide and small molecule UT modulators, a *N*-benzylamide has now been installed at the benzotriazepine 8-position to mimic the phenylalanine residue of Urocontrin. In light of the improved activity of the phenylalanine-containing core mimics **4.7** relative to its smaller peptide counterpart **4.7**, mimicry of the Phe residue was pursued in an effort to improve antagonist activity. 1,3,4-Benzotriazepin-2(3H)-on-8-carboxylates (e.g., **4.15**) have thus been synthesized to provide a carboxylate handle for introduction of a fourth substituent onto the heterocycle scaffold.

With 1,3,4-benzotriazepin-2(3H)-on-8-carboxylates in hand, our attention has also turned towards the synthesis of photo-reactive analogues to tag the receptor and identify the binding location of the small molecule modulators. Notably,

benzophenones derivatives of hUII and Urantide {[Pen^5, D-Trp^7, Orn^8]hUII(4−11)} have previously been synthesized and used effectively to identify the binding sites of the endogenous peptide and its potent antagonist.[50,51,52,53] These studies were conducted by treatment of the receptor with ^{125}I-UII-benzophenone derivatives followed by UV trartment to photolabel the receptor. The identification of the ^{125}I-photolabelled receptor complexes was done by following the radioactivity and then digested and purified to identify by mass the ligation site. The results show that the full agonist [Bpa^6]U-II labelled Met^{184}/Met^{185} of the fourth transmembrane domain of the rUT receptor while the weak partial agonist [Pen^5, Bpa^6, D-Trp^7, Orn^8]U-II bound inside the fifth transmembrane domain ot the rUT receptor, supportting the hypothesis that agonist or partial agonist ligands may stabilize different receptor conformations upon binding.[52,53] Based on such success in photo-affinity studies of the peptide ligands, benzophenone moieties were attached to benzotriazepinones **4.9** and **4.10** to favour covalent binding to UT upon UV irradiation for subsequent identification of the location responsible for small molecule modulation of hUII and URP.

Results and Discussion

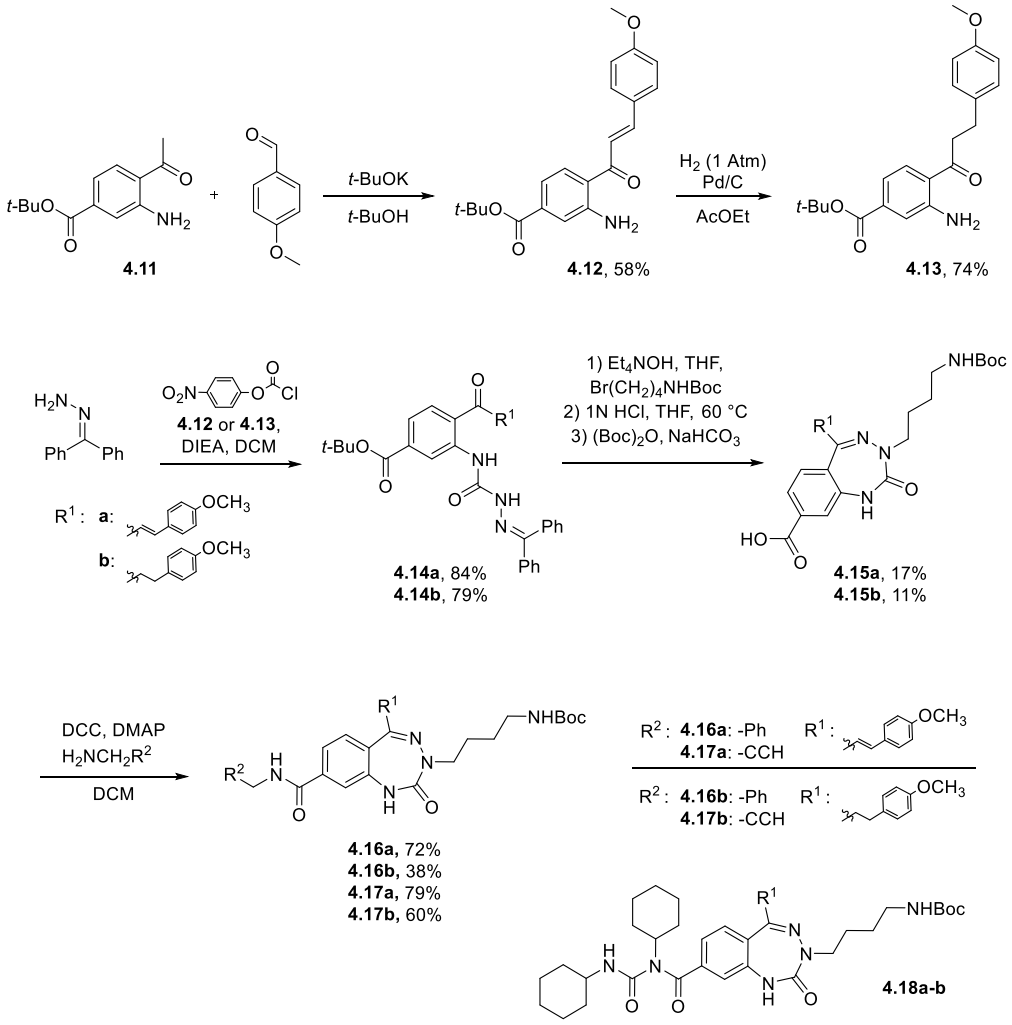
Chemistry

tert-Butyl 4-acetyl-3-aminobenzoate (**4.11**) was employed as starting material for the synthesis of the benzotriazepinone analogs and prepared in four steps from 4-ethylbenzoic acid based on the published procedure (Scheme 4.1).[54] The protected *p*-hydroxyphenethenyl side chain was installed by aldol condensation of *p*-anisaldehyde onto ketone **4.11** using *t*-BuOK in *tert*-butanol which provided chalcone **4.12** on gram scale in 58% yield. Hydrogenation of chalcone **4.12** over palladium-on-carbon gave the corresponding protected *p*-hydroxyphenethyl derivative **4.13** in 74% yield. Anilines **4.12** and **4.13** were respectively acylated, in 84% and 79% yield, using the activated carbazate prepared *in situ* from benzophenone hydrazone and *p*-nitrophenyl chloroformate.[1] Lysine side chain installation and benzotriazepinone annulation were achieved by chemoselective alkylation of semicarbazones **4.14a** and **4.14b** using 4-(Boc)amino-1-bromobutane and tetraethylammonium hydroxide in THF, followed by the semicarbazide liberation and cyclization under acidic conditions, and reprotection

of the amine side chain with di-*tert*-butyldicarbonate to give respectively 1,3,4-benzotriazepin-2-one-8-carboxylic acids **4.15a** and **4.15b** in 17% and 11% overall yields. Benzotriazepinone-8-carboxylic acids **4.15** are versatile orthogonally protected diamino carboxylate scaffolds which may in principle be diversified to prepare ligands to study a variety of receptors as previously demonstrated with related benzodiazepinone amino acid derivatives.[55,56,57,58]

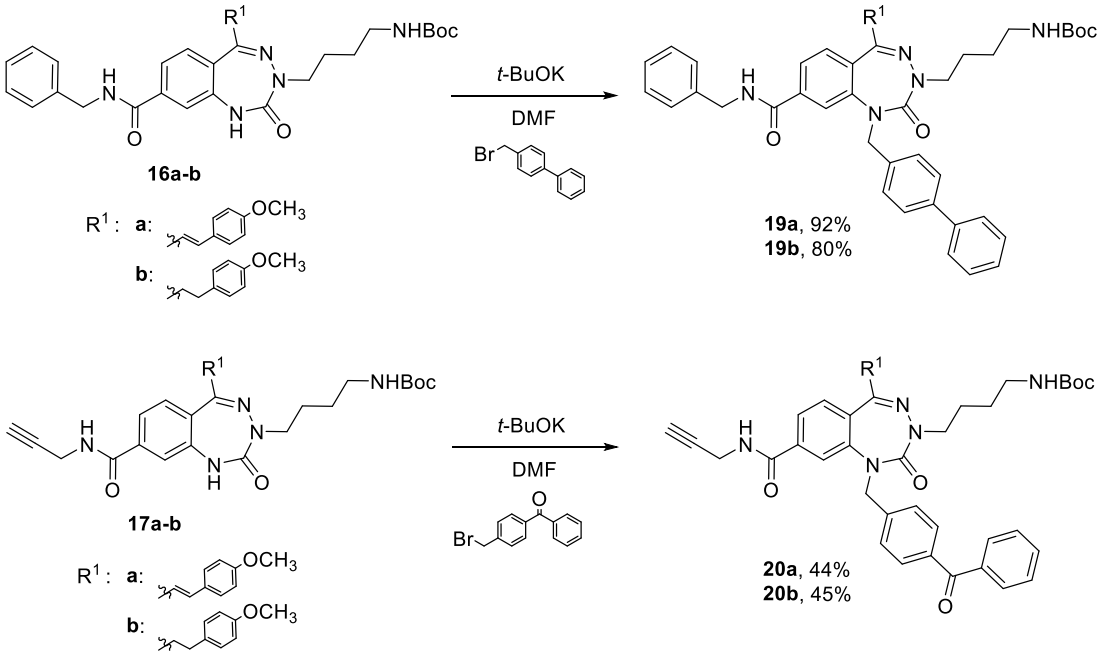
In the contect of the present research, benzotriazepinone-8-carboxylic acids **4.15** were respectively coupled to benzylamine and propargylamine using DCC in the presence of DMAP in dichloromethane to prepare amides **4.16** and **4.17** in 38-88% yields, due in part to the formation of the *N*-acylurea **4.18** (Scheme 4.1). Benzylamide **4.16** was prepared as a mimic of the Phe residue of Urocontrin. Propargylamide **4.17** was synthesized to serve for subsequent attachment of a biotin auxiliary for purification of tagged peptide fragments on an avidin column.[59]

Scheme 4.1. Synthesis of 8-amide-N1-H-benzotriazepin-2-ones **4.16** and **4.17**.



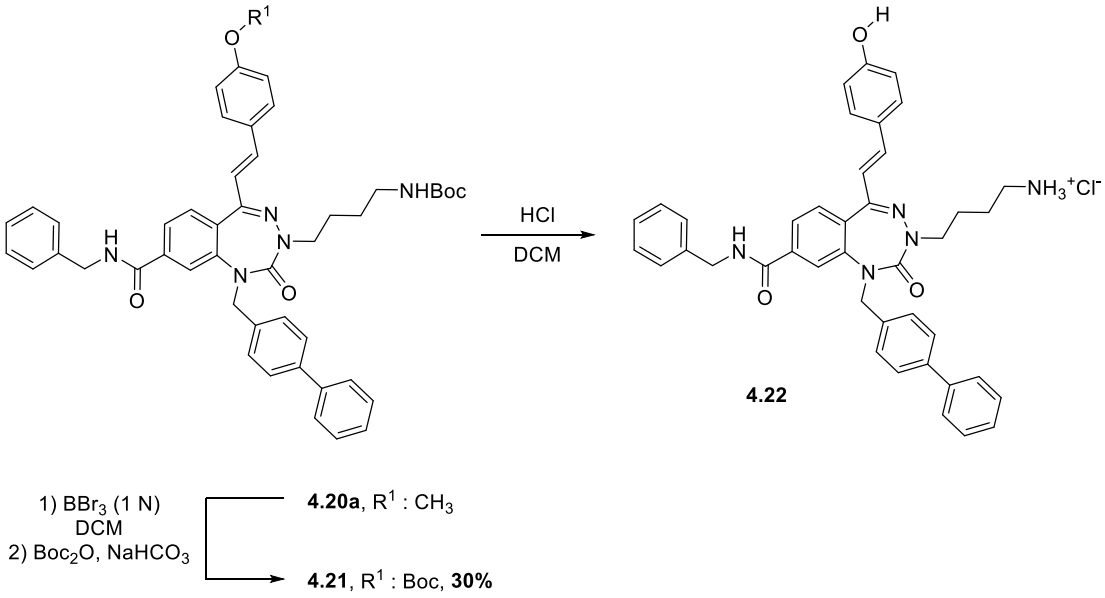
Alkylation of the N^1-position of benzotriazepin-2-ones **4.16** and **4.17** was respectively performed with *p*-phenyl benzyl bromide and 4-(bromomethyl)benzophenone using *t*-BuOK in DMF at room temperature to furnish benzotriazepin-2-ones **4.19a,b** and **4.20a,b** in 92-44 % yields (Scheme 4.2).

Scheme 4.2. Benzotriazepin-2-one alkylation.



Removal of the methyl ether and Boc protecting groups was performed using boron tribromide (1 N) in dichloromethane; however, the reaction mixtures proved difficult to purify.[60] To facilitate the purification, the mixture was treated with di-*tert*-butyldicarbonate and $NaHCO_3$ to protect the amine and phenol groups, which enabled benzotriazepin-2-ones **4.21** to be isolated in 30 % yield. Removal of the Boc protection with HCl gas in dichloromethane gave quantitatively the benzotriazepin-2-one Phe-Bip-Lys-Tyr tetrapeptide mimic **4.22** (Scheme 4.3).

Scheme 4.3. Benzotriazepin-2-one deprotection.



Conclusion

The functionalization of the position 8 of the benzotriazepine scaffold has been achieved by the installation of a carboxylate moiety at the beginning of the synthesis. Benzotriazepinone-8-carboxylic acids **4.15** have been synthesized as orthogonally protected diamino carboxylates for peptidomimetic ligand construction. In a proof-of-concept synthesis, benzotriazepinone **4.22** was prepared to mimic the tetrapeptide Phe-Bip-Lys-Tyr sequence of Urocontrin. Moreover, photo-reactive benzophenone residues have been attached to benzotriazepinones **4.20** towards the goal to identify the UT binding location of small molecule modulators of hUII and URP activity. The biological activity of benzotriazepinone **4.22**, as well as the deprotection of **4.20** and photo-affinity experiments, all are currently under investigation and will be reported in due time.

Experimental Section

Materials and Methods. Dry solvents (DCM, DMF and THF) were obtained by passage through solvent filtration systems (GlassContour Irvine, CA). Hexane was purchased from Fisher Chemical, and fractionally distilled before use. Ethanol, ethyl acetate and methanol were obtained from Fisher Chemical, and along with all

reagents from commercial sources were used as received. Melting points are uncorrected, reported in degree celsius (°C) and were obtained on samples placed in capillary tubes using a Mel-Temp melting point apparatus equipped with a thermometer. ^{1}H and ^{13}C NMR spectra were recorded at room temperature (298 K) and referenced to internal solvent in $CDCl_3$ (7.26 ppm/77.16 ppm), DMSO-d_6 (2.50 ppm/39.52 ppm) and CD_3OD-d_4 (3.31 ppm/49.00 ppm) on Brucker AV (300/75, 400/100 and 500/125 MHz) instruments. Chemical shifts are reported in parts per million (ppm) and coupling constant *J* values are in Hertz. Proton and carbon signals of minor isomers are respectively reported in brackets and parentheses. Abbreviations for peak multiplicities are s (singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), m (multiplet) and br (broad). Certain ^{13}C NMR chemical shifts values were extracted from HMBC and HSQC spectra. Low Resolution Mass Spectrometric analyses were performed on a LCMSD instrument from Agilent technologies in positive electrospray ionization mode (ESI+). High Resolution Mass Spectrometry (HRMS) data were obtained by the Centre Régional de Spectroscopie de Masse de l'Université de Montréal. Purity was ascertained by analytical HPLC analyses, performed on a Gemini reverse-phase column from SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18) with a flow rate of 0.5 mL/min using a gradient of acetonitrile (0.1% formic acid) or methanol (0.1% formic acid) in water (0.1% formic acid).

***tert*-Butyl 3-amino-4-((*E*)-3-(4-methoxyphenyl)acryloyl)benzoate (4.12)**.

tert-Butyl 4-acetyl-3-aminobenzoate (**4.11**, 3.0 g, 12.8 mmol, 1.0 eq., prepared according to reference 53), *p*-anisaldehyde (3.1 mL, 25.6 mmol, 2.0 eq.) and *t*-BuOK (7.2 g, 64.0 mmol, 5.0 eq.) were dissolved in *tert*-butanol (30 mL), stirred for 3 d at rt, diluted with EtOAc (100 mL), washed with H_2O (3 x 100 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated. *tert*-Butyl 3-amino-4-((*E*)-3-(4-methoxyphenyl)acryloyl)benzoate (**4.12**) was isolated by chromatography on silica gel (0-30% Et_2O in hexanes) as yellow solid (2.6 g, 58% yield): m.p. 141-145 °C; R_f 0.31 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 15.5 Hz, 1H), 7.67 – 7.57 (m, 2H), 7.48 (d, *J* = 15.5 Hz, 1H), 7.32 (d, *J* = 1.5 Hz, 1H), 7.27 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.01 – 6.89 (m, 2H), 6.31 (s, 2H),

3.87 (s, 3H), 1.61 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.9, 165.2, 161.7, 150.5, 143.8, 136.6, 130.9, 130.3, 127.9, 121.8, 120.7, 118.6, 116.3, 114.6, 81.7, 55.6, 28.3; LRMS (EI) 354 $(M + H)^+$; HRMS (EI) m/z for $C_{21}H_{24}NO_4$ $(M + H)^+$, calcd 354.1700, found 354.1683.

***tert*-Butyl 4-(3-(4-methoxyphenyl)propanoyl)-3-aminobenzoate (4.13)**.

A solution of olefin **4.12** (200 mg, 0.57 mmol, 1 eq.) in EtOAc/MeOH (5:1, 10 mL) was treated with Pd/C (20 mg) and stirred overnight under a hydrogen balloon. The reaction mixture was filtered over Celite™, and washed with MeOH (50 mL) and EtOAc (50 mL). The filtrate and washings were evaporated under vacuum. *tert*-Butyl 4-(3-(4-methoxyphenyl)propanoyl)-3-aminobenzoate (**4.12**) was isolated by chromatography on silica gel (20% EtOAc in hexanes) as yellow solid (150 mg, 74% yield): m.p. 114-118 °C; R_f 0.50 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 5.3 Hz, 2H), 7.33 – 7.21 (m, 2H), 6.95 (d, *J* = 8.1 Hz, 2H), 6.42 (s, 2H), 3.90 (s, 3H), 3.36 (t, *J* = 7.5 Hz, 2H), 3.09 (t, *J* = 7.6 Hz, 2H), 1.69 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.8, 165.1, 158.2, 150.7, 150.0, 136.8, 133.4, 131.1, 129.5, 118.8, 116.2, 114.1, 81.7, 55.4, 41.7, 29.8, 28.3; LRMS (EI) 356 $(M + H)^+$; HRMS (EI) m/z for $C_{21}H_{26}NO_4$ $(M + H)^+$, calcd 356.1856, found 356.1850.

Benzhydrylidene aza-glycinyl *N*'-((*E*)-2-(4-methoxyphenylpropenoyl)-4-(*tert*-butoxycarbonyl)phenyl)amide (4.14a). In a flame-dried round-bottom flask, 4-nitrophenylchloroformate (1.7 g, 8.49 mmol, 3.0 eq.) was dissolved in CH_2Cl_2 (70 mL), cooled to 0°C, treated drop-wise with a freshly prepared solution of benzophenone hydrazone (1.7 g, 8.49 mmol, 3.0 eq.) in CH_2Cl_2 (15 mL), stirred at 0°C for 2h, and treated drop-wise with a freshly prepared solution of aniline **4.12** (1.00 g, 2.93 mmol, 1.0 eq.) and DIEA (2.6 mL, 14.65 mmol, 5.0 eq.) in CH_2Cl_2 (15 mL). The ice bath was removed and the mixture was stirred 3 days, washed with NaOH (1 N) (4 x 50 mL), dried ($MgSO_4$), filtered and evaporated. Benzhydrylidene aza-glycine **4.14a** was isolated by chromatography on silica gel (10-20% EtOAc in hexanes) as yello oil (1.4 g, 84% yield): R_f 0.26 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.16 (s, 1H), 9.19 (d, *J* = 1.5 Hz, 1H), 8.02 – 7.91 (m, 3H), 7.87 (d, *J* = 15.5 Hz, 1H), 7.73 (dd, *J* = 8.3, 1.7 Hz, 2H), 7.68 – 7.50 (m, 5H), 7.50 –

7.39 (m, 4H), 7.30 (dd, *J* = 7.8, 1.7 Hz, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 3.88 (s, 3H), 1.61 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.7, 165.1, 162.2, 153.2, 149.1, 146.0, 140.7, 137.0, 136.6, 131.9, 130.6, 130.1, 130.0, 129.7, 128.61, 128.57, 127.8, 127.6, 127.3, 122.3 (2C), 121.8, 121.2, 114.7, 81.9, 55.6, 28.3, LRMS (EI) 576 $(M + H)^+$; HRMS (EI) m/z for $C_{35}H_{34}N_3O_5$ $(M + H)^+$, calcd 576.2493, found 576.2498.

Benzhydrylidene aza-glycinyl *N*'-(2-(4-methoxphenylpropanoyl)-4-(*tert*-butoxycarbonyl)phenyl)amide (4.14b). Benzhydrylidene aza-glycine **4.14b** was prepared according to the procedure described for the synthesis of benzhydrylidene aza-glycine **4.14a** using 4-nitrophenylchloroformate (409 mg, 2.03 mmol, 3.0 eq.), benzophenone hydrazone (398 mg, 2.03 mmol, 3.0 eq.), aniline **4.13** (240 mg, 0.68 mmol, 1.0 eq.) and DIEA (0.6 mL, 3.38 mmol, 5.0 eq.). Benzhydrylidene aza-glycine **4.14b** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as pale yellow oil (300 mg, 79% yield): R_f 0.33 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.49 (s, 1H), 9.29 (d, *J* = 1.6 Hz, 1H), 8.07 – 7.86 (m, 3H), 7.76 (s, 1H), 7.65 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.56 (dd, *J* = 8.1, 6.7 Hz, 3H), 7.46 – 7.35 (m, 3H), 7.31 (dd, *J* = 7.7, 1.7 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 6.89 – 6.80 (m, 2H), 3.78 (s, 3H), 3.40 (t, *J* = 7.5 Hz, 2H), 3.12 (t, *J* = 7.5 Hz, 2H), 1.60 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.9, 164.9, 158.3, 153.3, 149.2, 141.1, 137.2, 137.0, 133.2, 131.9, 130.4, 130.04, 130.00, 129.7, 129.6, 128.60, 128.57, 127.8, 124.5, 122.0, 121.4, 114.2, 81.9, 55.4, 42.6, 29.9, 28.2; LRMS (EI) 578 $(M + H)^+$; HRMS (EI) m/z for $C_{35}H_{36}N_3O_5$ $(M + H)^+$, calcd 578.2650, found 578.2651.

(*E*)-3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxylic acid (4.15a). A solution of aza-glycine **4.14a** (590 mg, 1.05 mmol, 1.0 eq.) in THF (10 mL) was treated with a 40% aqueous solution of tetraethylammonium hydroxide (0.75 mL, 2,10 mmol, 2.0 eq.) and 1-*N*-(Boc)amino-4-bromobutane (529 mg, 2.10 mmol, 2.0 eq. prepared according to ref 61). The reaction was stirred for 2 d at rt, diluted with THF (40 mL), treated with HCl (1.0 N, 50 mL) and stirred overnight at 60°C. The volatiles were evaporated and the resulting aqueous phase was extracted with EtOAc (3 x 50 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The resulting oil was dissolved in 1:1 THF/H_2O (10 mL), treated with sodium bicarbonate (265 mg, 3.15 mmol, 3.0 eq.)

and di-*tert*-butyl dicarbonate (230 mg, 1.05 mmol, 1.0 eq.), and stirred overnight at rt. The volatiles were evaporated. The resulting aqueous phase was diluted with H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Benzotriazepinone **4.15a** was isolated by chromatography on silica gel (50% EtOAc in hexanes containing 1% AcOH) as yellow oil (90 mg, 17% yield): R_f 0.54 (80% EtOAc in hexanes containing 1% AcOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.20 – 7.12 (m, 1H), 6.97 (d, *J* = 16.3 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 16.5 Hz, 1H), 4.66 (s, 1H), 3.83 (s, 3H), 3.71 (br dt, *J* = 9.6, 6.2 Hz, 2H), 3.13 (br dt, *J* = 12.3, 5.9 Hz, 2H), 1.75 (tt, *J* = 15.0, 7.7 Hz, 2H), 1.65 – 1.40 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 164.4, 163.0 (HSQC), 160.7, 143.1, 138.7, 129.7, 129.2, 129.0, 128.9, 128.7, 128.4, 124.5, 123.5, 122.4, 114.4, 79.8, 55.5, 51.1, 40.5, 28.6, 27.4, 25.0; LRMS (EI) 509 $(M + H)^+$; HRMS (EI) m/z for $C_{27}H_{33}N_4O_6$ $(M + H)^+$, calcd 509.2395, found 509.2384.

3-(4-*N*-(Boc)Aminobutyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxylic acid (4.15b). Benzotriazepinone **4.15b** was prepared according to the procedure described for the synthesis of triazepinone **4.15a** using aza-glycine **4.14b** (300 mg, 0.53 mmol, 1.0 eq.), 40% aqueous solution of tetraethylammonium hydroxide (0.40 mL, 1.06 mmol, 2.0 eq.), $BocNH(CH_2)_4Br$ (268 mg, 1.06 mmol, 2.0 eq.), $NaHCO_3$ (134 mg, 1.06 mmol, 3.0 eq.) and Boc_2O (116 mg, 0.53 mmol, 1.0 eq.). Benzotriazepinone **4.15b** was isolated by chromatography on silica gel (50% EtOAc in hexanes containing 1% AcOH) as yellow oil (30 mg, 11% yield): R_f 0.44 (80% EtOAc in hexanes containing 1% AcOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.77 (d, *J* = 9.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.4 Hz, 1H), 7.11 – 7.03 (m, 2H), 6.85 – 6.76 (m, 2H), 4.64 (s, 1H), 3.77 (s, 3H), 3.63 (br s, 2H), 3.11 (br dt, *J* = 13.4, 6.9 Hz, 2H), 3.00 (dt, *J* = 8.9, 6.2 Hz, 2H), 2.83 (dt, *J* = 8.7, 6.7 Hz, 2H), 1.66 (tt, *J* = 15.0, 8.2 Hz, 2H), 1.53 – 1.37 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 164.9, 164.5, 158.2, 142.7, 133.7, 132.8, 129.5, 129.2, 128.4, 127.6, 124.8, 122.1, 114.0, 79.8, 55.4, 50.8, 40.5, 38.5, 32.8, 28.6, 27.4, 24.9; LRMS (EI) 511 $(M + H)^+$; HRMS (EI) m/z for $C_{27}H_{35}N_4O_6$ $(M + H)^+$, calcd 511.2551, found 511.2536.

***N*-Benzyl-(*E*)-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxamide (4.16a).** Carboxylic acid **4.15a** (15 mg, 0.03 mmol, 1.0 eq.), benzylamine (0.01 mL, 0.06 mmol, 2 eq.) and DMAP (1 mg, 0.006 mmol, 0.2 eq.) were dissolved in DCM (10 mL), treated with DCC (7 mg, 0.036 mmol, 1.2 eq.) and stirred at rt for 24 h. The volatiles were evaporated and the residue was purified by column chromatography (0-50% EtOAc in hexanes) to provide amide **4.16a** as a yellow oil (13 mg, 72% yield): R_f 0.49 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.46 (m, 2H), 7.46 – 7.24 (m, 8H), 6.97 (d, *J* = 16.4 Hz, 1H), 6.93 – 6.86 (m, 2H), 6.86 – 6.73 (m, 2H), 6.64 (s, 1H), 4.67 (d, *J* = 5.7 Hz, 2H), 4.56 (s, 1H), 3.84 (s, 3H), 3.65 (t, *J* = 7.3 Hz, 2H), 3.09 (dt, *J* = 12.9, 6.8 Hz, 2H), 2.00 – 1.86 (m, 2H), 1.76 – 1.55 (m, 2H), 1.42 (s, 9H).); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 162.8, 162.4, 160.7, 156.2, 156.1, 142.8, 138.6, 137.9, 130.1, 129.0, 128.8, 128.6, 128.1, 128.0, 127.8, 123.7, 121.7, 119.4, 114.4, 79.7, 55.5, 50.9, 44.5, 40.4, 34.1, 28.6, 25.1; LRMS (EI) 598 $(M + H)^+$; HRMS (EI) m/z for $C_{34}H_{40}N_5O_5$ $(M + H)^+$, calcd 598.3024, found 598.3016.

***N*-Benzyl-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxamide (4.16b).** Amide **4.16b** was prepared according to the procedure described for the synthesis of amide **4.16a** using carboxylic acid **4.15b** (14 mg, 0.027 mmol, 1.0 eq.), benzylamine (0.006 mL, 0.054 mmol, 2.0 eq.), DMAP (1 mg, 0.06 mmol, 0.1 eq.) and DCC (7 mg, 0.033 mmol, 1.2 eq.). Amide **4.16a** was isolated by chromatography on silica gel (0-50% EtOAc in hexanes) as yellow oil (6 mg, 38% yield): R_f 0.50 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.41 – 7.26 (m, 7H), 7.10 – 7.04 (m, 2H), 6.83 – 6.77 (m, 2H), 6.57 (s, 1H), 6.44 (s, 1H), 4.66 (d, *J* = 5.6 Hz, 2H), 4.53 (s, 1H), 3.77 (s, 3H), 3.58 (t, *J* = 7.3 Hz, 2H), 3.08 (dt, *J* = 13.0, 6.6 Hz, 2H), 3.00 (dt, *J* = 8.8, 6.2 Hz, 2H), 2.83 (dt, *J* = 9.2, 6.3 Hz, 2H), 1.70 – 1.53 (m, 2H), 1.50 – 1.36 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 162.6 (2C), 158.2, 142.3, 137.9, 137.7, 132.8, 129.5, 129.1 (2C), 129.0, 128.1, 128.0, 127.9, 121.8, 119.1, 114.0, 79.2,

55.4, 50.6, 44.5, 40.4, 38.5, 32.1, 29.9, 29.5, 28.6; LRMS (EI) 600 $(M + H)^+$; HRMS (EI) m/z for $C_{34}H_{42}N_5O_5$ $(M + H)^+$, calcd 600.3186, found 600.3180.

***N*-Propargyl-(*E*)-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxystyryl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxamide (4.17a)**. Amide **4.17a** was prepared according to the procedure described for the synthesis of amide **4.16a** using carboxylic acid **4.15a** (18 mg, 0.035 mmol, 1.0 eq.), propargylamine (0.023 mL, 0.32 mmol, 10.0 eq.), DMAP (1 mg, 0.01 mmol, 0.2 eq.), and DCC (7 mg, 0.033 mmol, 1.2 eq.). Amide **4.17a** was isolated by chromatography on silica gel (0-50% EtOAc in hexanes) as yellow oil (15 mg, 79%): R_f 0.40 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 – 7.50 (m, 1H), 7.43 – 7.38 (m, 3H), 7.01 – 6.92 (m, 2H), 6.92 – 6.84 (m, 2H), 6.79 (d, *J* = 16.4 Hz, 1H), 6.57 (s, 1H), 4.57 (s, 1H), 4.27 (dd, *J* = 5.2, 2.6 Hz, 1.7H), [4.11 (ddd, *J* = 5.3, 2.6, 0.9 Hz, 0.3H)], 3.83 (s, 3H), 3.66 (t, *J* = 7.2 Hz, 2H), 3.10 (br dt, *J* = 13.0, 6.8 Hz, 2H), 2.31 (t, *J* = 2.5 Hz, 0.9H), [2.26 (t, *J* = 2.6 Hz, 0.1H)], 1.71 (tt, *J* = 15.0, 7.6 Hz, 2H), 1.50 – 1.33 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.0, 162.8, 162.3, 160.7, 142.9, 138.6 (2C), 137.1, 130.1, 128.9, 128.6, 128.1, 123.6, 121.7, 119.5, 114.5, 79.3 (2C), 72.4, 55.5, 51.0, 40.5, 30.1, 28.6, 27.4, 25.0; LRMS (EI) 546 $(M + H)^+$; HRMS (EI) m/z for $C_{30}H_{36}N_5O_5$ $(M + H)^+$, calcd 546.2711, found 546.2710.

***N*-Propargyl-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxyphenethyl)-1H-1,3,4-benzotriazepin-2(3H)-on-8-carboxamide (4.17b).** Amide **4.17b** was prepared according to the procedure described for the synthesis of amide **4.16a** using carboxylic acid **4.15b** (14 mg, 0.027 mmol, 1.0 eq.), propargylamine (0.020 mL, 0.27 mmol, 10.0 eq.), DMAP (1 mg, 0.006 mmol, 0.2 eq.) and DCC (33 mg, 0.033 mmol, 1.2 eq.). Amide **4.17b** was isolated by chromatography on silica gel (0-50% EtOAc in hexanes) as yellow oil (9 mg, 60% yield): R_f 0.50 (50% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 1.5 Hz, 1H), 7.10 – 7.01 (m, 2H), 6.85 – 6.74 (m, 3H), 6.44 (s, 1H), 4.56 (s, 1H), 4.25 (dd, *J* = 5.2, 2.6 Hz, 1.6H), [4.11 (ddd, *J* = 5.4, 2.6, 0.9 Hz, 0.4H)], 3.77 (s, 3H), 3.58 (t, *J* = 7.2 Hz, 2H), 3.08 (dt, *J* = 12.9, 6.7 Hz, 2H), 3.03 – 2.94 (m, 2H), 2.82 (dt, *J* = 8.9, 6.4 Hz, 2H), 2.30 (t, *J* = 2.5 Hz, 0.8H), [2.26 (t, *J* = 2.5 Hz, 0.2H)], 1.71 –

1.52 (m, 2H), 1.41 (s, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 164.3, 162.8, 158.2, 156.1, 142.4, 137.0, 132.8, 129.5, 129.1, 127.9, 121.9, 119.1, 114.0, 79.2 (2C), 72.4, 55.4, 50.6, 40.5, 38.4, 32.8, 29.9, 28.6, 27.4, 24.8; LRMS (EI) 548 $(M + H)^+$; HRMS (EI) m/z for $C_{30}H_{38}N_5O_5$ $(M + H)^+$, calcd 548.2867, found 548.2862.

***N*-Benzyl-(*E*)-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide (4.19a).** In a dried round bottom flask, flushed with argon, benzotriazepinone **4.16a** (10 mg, 0.017 mmol, 1 eq.), potassium *tert*-butoxide (2 mg, 0.018 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (5 mg, 0.018 mmol, 1.2 eq.) were dissolved in DMF (5 mL), stirred overnight at rt, and diluted with EtOAc (50 mL). The mixture was transferred to a separatory funnel and washed with H_2O (3 x 50 mL) and brine (25mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. Benzotriazepinone **4.19a** was isolated by chromatography on silica gel (0-40% EtOAc in hexanes) as yellow oil (12 mg, 92% yield): R_f 0.47 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.51 – 7.19 (m, 18H), 7.06 (d, *J* = 16.4 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.62 (d, *J* = 16.4 Hz, 1H), 6.34 (br s, 1H), 5.33 (d, *J* = 15.8 Hz, 1H), 4.75 (d, *J* = 15.0 Hz, 1H), 4.63 (d, *J* = 5.7 Hz, 2H), 4.45 (br s, 1H), 3.84 (s, 3H), 3.78 (br s, 1H), 3.46 (br s, 1H), 3.04 (br dt, *J* = 12.6, 6.3 Hz, 2H), 1.59 (br s, 4H), 1.40 (s, 9H); LRMS (EI) 764 $(M + H)^+$; HRMS (EI) m/z for $C_{47}H_{50}N_5O_5$ $(M + H)^+$, calcd 764.3806, found 764.3812.

***N*-Benzyl-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxyphenethyl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide (4.19b).** Benzotriazepinone **4.19b** was prepared according to the procedure described for the synthesis of triazepinone **4.19a** by alkylation of benzotriazepinone **4.16b** (4 mg, 0.006 mmol, 1 eq.) using *t*-BuOK (1 mg, 0.007 mmol, 1.2 eq.) and 4-bromomethyl-biphenyl (2 mg, 0.007 mmol, 1.2 eq.). Benzotriazepinone **4.19b** was isolated by chromatography on silica gel (0-50% EtOAc in hexanes) as yellow oil (4 mg, 80% yield): R_f 0.53 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.49 – 7.17 (m, 16H), 7.04 (d, *J* = 8.7 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 6.26 (br s, 1H), 5.27 (br d, *J* = 15.1 Hz, 1H), 4.71 (br d, *J* = 17.0 Hz, 1H), 4.62 (d, *J* = 5.0 Hz, 2H), 4.44 (s, 1H), 3.74 (s, 3H), 3.67 (br s, 1H), 3.42 (br s, 1H), 3.03 (br dt, *J* = 13.2, 7.4 Hz, 2H), 2.74

(br d, J = 21.3 Hz, 2H), 1.56 (br s, 4H), 1.40 (s, 9H), LRMS (EI) 766 $(M + H)^+$; HRMS (EI) m/z for $C_{47}H_{52}N_5O_5$ $(M + H)^+$, calcd 766.3963, found 766.3952.

***N*-Propargyl-(*E*)-3-(4-*N*-(Boc)aminobutyl)-5-(4-methoxystyryl)-1-(4-methylbenzophenone)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide (4.20a).** Benzotriazepinone **4.20a** was prepared according to the procedure described for the synthesis of triazepinone **4.19a** by alkylation of benzotriazepinone **4.17a** (12 mg, 0.022 mmol, 1 eq.), using *t*-BuOK (3 mg, 0.024 mmol, 1.1 eq.) and 4-(bromomethyl)benzophenone (7 mg, 0.024 mmol, 1.1 eq.). Benzotriazepinone **4.20a** was isolated by chromatography on silica gel (0-40% EtOAc in hexanes) as yellow oil (5 mg, 44% yield): R_f 0.43 (40% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 – 7.66 (m, 3H), 7.64 (d, J = 8.4 Hz, 2H), 7.55 (ddd, J = 6.8, 4.0, 1.3 Hz, 1H), 7.50 (s, 2H), 7.46 – 7.36 (m, 4H), 7.32 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 16.4 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 16.5 Hz, 1H), 6.36 (br s, 1H), 5.39 (d, J = 15.7 Hz, 1H), 4.81 (d, J = 16.1 Hz, 1H), 4.47 (br s, 1H), 4.26 (dd, J = 5.2, 2.6 Hz, 2H), 3.85 (s, 3H), 3.83 – 3.64 br (m, 1H), 3.59 – 3.38 (br m, 1H), 3.04 (br dt, J = 12.4, 6.2 Hz, 2H), 2.31 (t, J = 2.5 Hz, 1H), 1.61 (s, 4H), 1.43 (s, 9H). LRMS (EI) 740 $(M + H)^+$; HRMS (EI) m/z for $C_{44}H_{46}N_5O_6$ $(M + H)^+$, calcd 740.3448, found 740.3441.

***N*-Propargyl-3-(4-N-(Boc)aminobutyl)-5-(4-methoxyphenethyl)-1-(4-benzoylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide (4.20b).** Benzotriazepinone **4.20b** was prepared according to the procedure described for the synthesis of triazepinone **4.19a** by alkylation of benzotriazepinone **4.17b** (8 mg, 0.015 mmol, 1 eq.) using *t*-BuOK (2 mg, 0.016 mmol, 1.1 eq.) and 4-(bromomethyl)benzophenone (5 mg, 0.016 mmol, 1.1 eq.). Benzotriazepinone **4.20b** was isolated by chromatography on silica gel (0-40% EtOAc in hexanes) as yellow oil (5 mg, 45% yield): R_f 0.57 (40% EtOAc in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 8.3 Hz, 1H), 7.77 – 7.72 (m, 2H), 7.70 (d, J = 8.2 Hz, 2H), 7.63 (s, 1H), 7.58 (t, J = 7.4 Hz, 1H), 7.54 – 7.48 (m, 1H), 7.44 (t, J = 7.8 Hz, 3H), 7.38 (d, J = 8.1 Hz, 1H), 7.31 (d, J = 8.2 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 6.24 (br s, 1H), 5.34 (br s, 1H), 4.78 (br s, 1H), 4.47 (br s, 1H), 4.26 (dd, J = 5.2, 2.6 Hz, 2H), 3.51 (s, 3H), 3.05 (br s, 4H), 2.82 (br d, J = 31.1 Hz, 2H), 2.32 (t, J = 2.6

Hz, 1H), 1.58 (s, 4H), 1.44 (s, 9H); LRMS (EI) 742 $(M + H)^+$. HRMS (EI) m/z for $C_{44}H_{47}N_5O_6Na$ $(M + Na)^+$, calcd 764.3419, found 764.3422.

***N*-Benzyl-(*E*)-3-(4-*N*-(Boc)aminobutyl)-5-(4-*O*-(Boc)hydroxyphenethenyl)-1-(4-methoxystyryl)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide (4.21).** Methyl ether **4.20a** (10 mg, 0.013 mmol, 1 eq.) was dissolved in CH_2Cl_2 (10 mL), cooled to –78°C and treated with BBr_3 (1.0 N in CH_2Cl_2, 0.6 mL, 0.078 mmol, 6 eq.). The cooling bath was removed, and the reaction was let warm to room temperature overnight with stirring, quenched with a solution of saturated $NaHCO_3$ (1 mL), treated with di-*tert*-butyl dicarbonate (3 mg, 0.013 mmol, 1 eq.), and stirred overnight at rt. The volatiles were evaporated. The resulting aqueous phase was diluted with H_2O (25 mL) and extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. Benzotriazepinone **4.21** was isolated by chromatography on silica gel (50% EtOAc in hexanes) as yellow oil (3 mg, 30% yield): R_f 0.54 (40% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.54 – 7.11 (m, 21H), 6.68 (d, *J* = 16.6 Hz, 1H), 6.34 (br s, 1H), 5.35 (d, *J* = 15.6 Hz, 1H), 4.77 (d, *J* = 15.5 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 2H), 4.47 (s, 1H), 3.80 (br s, 1H), 3.50 (br s, 1H), 3.07 (br dt, *J* = 12.9, 7.0 Hz, 2H), 1.60 (s, 13H), 1.43 (s, 9H); LRMS (EI) 850 $(M + H)^+$; HRMS (EI) m/z for $C_{51}H_{56}N_5O_7$ $(M + H)^+$, calcd 850.4174, found 850.4169.

***N*-Benzyl-(*E*)-3-(4-aminobutyl)-5-(4-methoxystyryl)-1-(4-phenylbenzyl)-1H-benzo[e][1,2,4]triazepin-2(3H)-on-8-carboxamide hydrochloride (4.22).** A solution of carbamate **4.21** (3 mg, 0.004 mmol, 1 eq.) in CH_2Cl_2 (5 mL) was treated with HCl gas bubbles at rt for 2h. Hydrochloride salt **4.22** was isolated by evaporation of the the volatiles as pale yellow solid (3 mg, quantitative yield): ^{1}H NMR (400 MHz, MeOD) δ 7.89 (d, *J* = 1.5 Hz, 1H), 7.71 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.44 – 7.06 (m, 16H), 7.02 (d, *J* = 16.3 Hz, 1H), 6.76 (d, *J* = 8.5 Hz, 2H), 6.64 (d, *J* = 16.4 Hz, 1H), 5.34 (d, *J* = 15.3 Hz, 1H), 4.83 (d, *J* = 15.3 Hz, 1H), 4.60 (s, 2H), 3.80 – 3.69 (br m, 1H), 3.52 – 3.41 (br m, 1H), 2.90 – 2.81 (br m, 2H), 1.69 (br tt, *J* = 13.9, 6.8 Hz, 2H), 1.56 (br tt, *J* = 16.0, 8.0 Hz, 2H).LRMS (EI) 650 $(M + H)^+$. HRMS (EI) m/z for $C_{41}H_{40}N_5O_3$ $(M + H)^+$, calcd 650.3126, found 650.3115. 21

ASSOCIATED CONTENT

The following files are available free of charge.

NMR spectra (^{1}H and ^{13}C) of all new compounds, purity and analytical HPLC chromatograms for **4.22**.

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ABBREVIATIONS

As, Azasulfuryl; Bip, 4,4′-biphenylalanine; Bip, Biphenylmethyl; Boc, tert-butyloxycarbonyl; Bpa, 4-benzoylphenylalanine; CCK_2, cholecystokinin-2; DMF, dimethylformamide; $hHPTH_1$, parathyroid hormone-1 receptor; GPCR, G protein-coupled receptor; hUII, human urotensin II; Nal, naphthylalanine; Nap, naphthylmethyl; Orn, ornithine; Pep, (phenylethynyl)phenylalanine; UII, urotensin II; URP, urotensin II-related peptide; UT, urotensin II receptor; UV, ultra-violet.

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Chapitre 5 : Conclusions générales

Les peptides sont des biopolymères composés d'acides aminés reliés entre eux par une liaison amide et sont omniprésents dans les mécanismes biologiques. Ils possèdent un fort potentiel comme agents thérapeutiques car ils ont une faible toxicité et une excellente activité, mais ont le désavantage de se métaboliser rapidement, d'avoir une pauvre biodisponibilité et la possibilité d'interagir avec plusieurs sous-types de récepteurs. Une nouvelle classe de composé à donc vu le jour dans le but de pouvoir employer les propriétés des peptides, comme médicaments, tout en minimisant des inconvénients inhérents à leur utilisation. Ils sont appelés les peptidomimétiques. Une classe de peptidomimétiques, moins développée, remplace la structure peptidique dans son ensemble par une petite molécule, qui va mimer la conformation et les fonctions des chaines latérales. Dans le cadre de cette thèse, nous nous sommes attachés à développer une méthodologie permettant de utiliser les 1,3,4-benzotriazépin-2-ones pour mimer des peptides cycliques.

Pour ce faire, une stratégie synthétique a été developpée de partir d'un composé linéaire semicarbazonique en utilisant des alkylations chimiosélectives. La méthodologie donne des 1,3,4-benzotriazépin-2-ones diverses, en utilisant des conditions douce. De plus, il a été mis en évidence la possibilité d'utiliser le cœur achiral des benzotriazépinones comme mime peptidique de conformation de tour γ.

Explorant la modulation de système urotensinergique, une série de 26 benzotriazépinones a été synthétisée, en utilisant cette méthodologie. Le but de cette série est de mimer la conformation tour γ et la séquence tripeptide Bip-Lys-Tyr présent dans l'Urocontrin ([Bip^4]URP), modulateur allostérique de UT. Afin de déterminer le caractère agoniste et antagoniste de hUII ou URP, les benzotriazépinones ont été examinées sur un test *ex vivo* de contraction aortique de rat. La série a démontré un faible pouvoir agoniste, avec un maximum de contraction de maximum 8% à 10 μM. Nous avons démontré la capacité du squelette benzotriazépinique à servir dans les modulateurs sélectifs de hUII et URP. Le groupement phénol et son orientation ont été important pour la modulation du système urotensinergique. En effet, les benzotriazépinones portant la chaine latérale *p*-hydroxyphenethenyle module sélectivement les contractions induites par hUII,

alors que la version saturé *p*-hydroxyphenethyle, plus flexible, module celles induites par URP. Le cœur achiral des 1,3,4-benzotriazépin-2-ones a été démontré comme un mime peptidique efficace.

Enfin, dans la perspective d'améliorer l'activité antagoniste de ces benzotriazépinones, une fonction carboxylate a été ajoutée en position-8 afin de mimer la conformation et la séquence tétrapeptide Phe-Bip-Lys-Tyr présent dans l'Urocontrin. En effet, une étude récente a démontré l'importance de la phénylalanine pour la modulation du récepteur urotensinergique. De plus, dans le but de pouvoir mieux comprendre la différence de sélectivité, de certaines benzotriazépinones envers hUII ou URP, l'installation d'un groupement benzophénone photosensibles est exposé. L'idée étant de pouvoir se lier de manière covalente au récepteur urotensinergique et permettre la localisation de la partie responsable de la modulation de l'activité de hUII et URP sur UT.

Pour conclure, cette thèse offre une avancée dans la synthèse organique avec le développement d'une méthodologie de synthèse des 1,3,4-benzotriazépinones, orientée vers une fonctionnalisation efficace, basée sur des alkylations chiomosélectives successives. De plus le concept d'utilisation des 1,3,4-benzotriazépin-2-ones, comme peptidomimétiques a été mis en avant et prouvé avec le design de modulateur du système uroentosinergique, basé sur de petite molécules achirales, imitant la séquence biologiquement active Bip-Lys-Tyr et la conformation de tour γ de l'Urocontrin.

De plus, en compléments des précédentes études de peptidomimétiques, basées sur un cœur de petite molécule, l'utilisation du cœur benzotriazépinique permet de pouvoir placer dans l'espace des mimes de chaines secondaires d'un tripeptide. En comparaison avec les autres modèles utilisés (e.g. pyrrolodiazepinone, glucose, cyclohexane), les benzotriazépinones offrent l'avantage de fournir une chiralité adaptive, c'est-à-dire avoir une chiralité basé sur des formes atropoisomèriques dont la barrière d'interconversion est faible à température pièce, rendant potentiellement le coup de dévelopement moins importants. Enfin, l'addition d'un groupement amide en

position 8 de la benzotriazépine permet d'augmenter le nombre de chaines secondaires mimées et ainsi explorer un espace tridimensionnel plus important.

En résumé, la contribution de cette thèse se situe principalement au niveau du dévelopement de peptidomimétiques, basés sur un motif de petite molécule, répondant de manière novatrice aux problémes rencontrés lors de l'utilisation des peptides comme médicament. Cette thèse décrit l'identification d'un cœur pouvant conceptuellement servir de mime tripeptidique, et donnant une approche de synthèse efficace, puis amène la preuve de ce concept en utilisant une cible thérapeutique d'intérêt innovante et, finalement, ouvre sur la perspective d'une augmentation possible des possibilités de fonctionalisation pour en faire un outil encore plus générales. Cette thèse contribue donc au développement des connaissances dans les domaines des mimes peptidiques et de la chimie médicinale, que se soit de manière fondamentale ou appliquée.

Annexes

Supporting information Article 1

Douchez, A.; Lubell, W. D. Chemoselective alkylation for diversity-oriented synthesis of 1,3,4-benzotriazepin-2-ones and pyrrolo[1,2][1,3,4]benzotriazepin-6-ones, potential turn surrogates. *Org. Lett*. **2015**, *17*, 6046-6049.

Supporting Information

Chemoselective alkylation for diversity oriented synthesis of 1,3,4-benzotriazepin-2-ones and pyrrolo[1,2][1,3,4]benzotriazepin-6-ones, potential turn surrogates.

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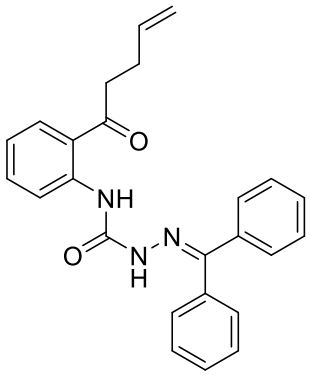
Materials and Methods

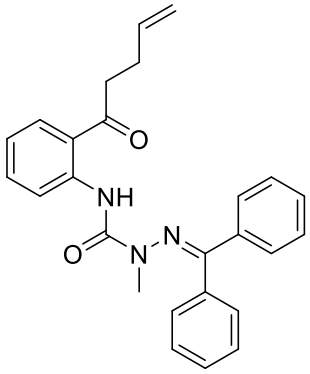
All solvents (DCM, DMF and THF) were used after passage through solvent filtration systems (GlassContour Irvine, CA). All reagents from commercial sources were used as received. Purification by silica gel chromatography was performed on 230-400 mesh silica gel; preparative thin layer chromatography was performed on Silica gel 60 F_{254} (1 mm Glass plates); analytical thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} (Aluminium Sheet) and visualised by UV absorbance. Melting points are uncorrected and were obtained on sample that was placed in a capillary tube using a Mel-Temp melting point apparatus equipped with a thermometer and reported in degree Celsius (°C). Infrared spectra were recorded on a Bruker Alpha P spectrometer and reported in frequency of absorption (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded at room temperature (298 K) in $CDCl_3$ (7.26 ppm/77.16 ppm) and MeOD-d_4 (3.31 ppm/49.00 ppm) on Brucker AV (300/75 and 400/100 MHz) instruments and referenced to internal solvent. Chemicals shifts are reported in parts per million (ppm); coupling constants (*J*) in Hertz; abbreviations for peak multiplicities are s (singlet), d (doublet), t (triplet), q (quadruplet), qu (quintuplet), m (multiplet) and br (broad). Certain ^{13}C NMR chemical shifts values were extracted from HSQC and HMBC spectra. High Resolution Mass Spectrometry (HRMS) data were obtained by the Centre Régional de Spectroscopie de Masse de l'Université de Montréal. X-ray structures were solved on a Brucker Venture Metaljet diffractometer by the Laboratoire de diffraction des rayons X de Université de Montréal.

Synthesis of semicarbazone 2.15

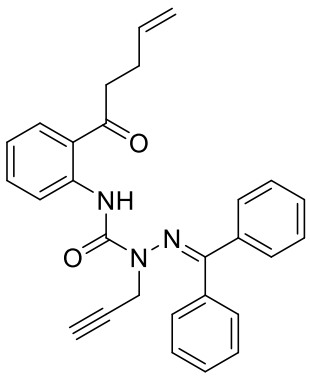


Benzhydrylidene aza-glycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.15**). In a flame dried round-bottom flask, 4-nitrophenylchloroformate (2.57 g, 12.6 mmol, 1.1 eq.) was dissolved in CH_2Cl_2 (200 mL), cooled to 0°C, treated drop-wise with a freshly prepared solution of benzophenone hydrazone (2.47 g, 12.6 mmol, 1.1 eq.) in CH_2Cl_2 (200 mL) and stirred at 0°C for 1h. A freshly prepared solution of 2-pent-4-enoylaniline **2.8** (2.00 g, 11.4 mmol, 1.0 eq., prepared according to reference 15) and DIEA (3 mL, 17.1 mmol, 1.5 eq.) in CH_2Cl_2 (100 mL) was added drop-wise to the reaction mixture at 0°C. The ice bath was removed and the mixture was stirred overnight. The volatiles were evaporated. The residue was purified by silica gel chromatography using 20% ethyl acetate in hexanes as eluent. Evaporation of the collected fractions gave semicarbazone **2.15** as pale yellow solid (3.2 g, 71% yield): m.p. 102-104 °C; R_f 0.46 (20% EtOAc in hexanes); IR 1657, 1686, 3349, 3570 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.67 (s, 1H), 8.72 (dd, *J* = 8.5, 1.1 Hz, 1H), 8.03 – 7.92 (m, 3H), 7.72 (s, 1H), 7.66 – 7.50 (m, 4H), 7.46 – 7.37 (m, 3H), 7.37 – 7.29 (m, 2H), 7.08 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 1H), 6.01 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 1H), 5.17 (ddd, *J* = 17.1, 3.3, 1.6 Hz, 1H), 5.10 – 5.05 (m, 1H), 3.21 (t, *J* = 7.3 Hz, 2H), 2.73 – 2.53 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.0, 153.5, 149.0, 141.3, 137.6, 137.1, 134.8, 131.9, 130.7, 130.0, 129.7, 128.6, 128.6, 127.9, 126.3, 122.2, 121.5, 120.2, 115.7, 39.2, 28.4; LRMS (EI) 398 $(M + H)^+$; HRMS (EI) m/z for $C_{25}H_{24}N_3O_2$ $(M + H)^+$, calcd 398.1863, found 398.1873.

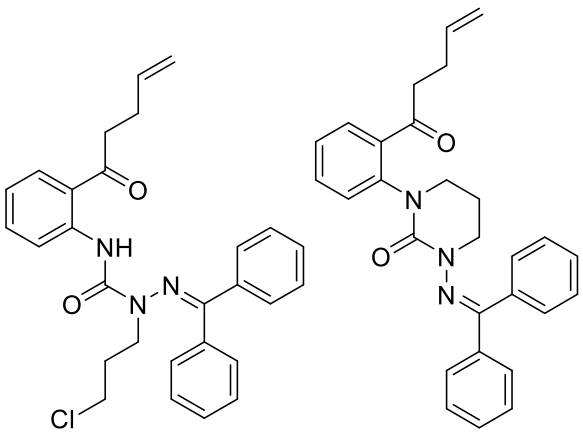
Synthesis of *N*-alkylated semicarbazone 2.16.



Benzhydrylidene aza-alanine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.16a**) A solution of semicarbazone **2.15** (190 mg, 0.480 mmol, 1.0 eq.) in THF (10 mL) was treated with a 40% aqueous solution of tetraethylammonium hydroxide (0.18 mL, 0.6 mmol, 1.2 eq.), stirred for 30 min at room temperature, treated with methyl iodide (0.04 mL, 0.600 mmol, 1.2 eq.) and stirred overnight at room temperature. The volatiles were evaporated. The resulting aqueous phase was diluted with water (25 mL) and extracted with ethyl acetate (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography using ethyl acetate (10%) in hexanes as eluent. Evaporation of the collected fractions gave *N*-(methyl) semicarbazone **2.16a** as pale yellow oil (210 mg, quantitative yield): R_f 0.48 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.51 (s, 1H), 8.72 (dd, *J* = 8.6, 1.0 Hz, 1H), 8.24 – 8.16 (m, 1H), 7.94 – 7.83 (m, 3H), 7.53 (ddd, *J* = 8.6, 7.3, 1.4 Hz, 1H), 7.50 – 7.32 (m, 8H), 7.04 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H), 6.99 – 6.91 (m, 1H), 5.94 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.10 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 5.02 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 3.12 (t, *J* = 7.3 Hz, 2H), 2.84 (s, 3H), 2.56 – 2.45 (m, 2H); ^{13}C NMR (75 MHz, CDCl3) δ 202.3, 156.3, 154.7, 141.6, 138.3, 137.6, 136.5, 134.3, 130.4, 130.0, 129.5, 129.3, 129.1, 128.6, 128.1, 122.5, 121.0, 120.3, 115.4, 39.1, 36.7, 28.3; LRMS (EI) m/z 412 (M + H^+); HRMS (ESI+): m/z for $C_{26}H_{26}N_3O_2$ $(M + H)^+$, calcd 412.2020, found 412.2037.



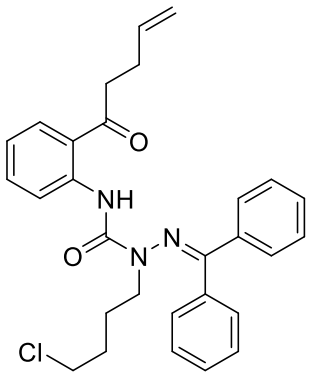
Benzhydrylidene aza-propargylglycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.16b**) was prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **16a** from alkylation of **2.15** (100 mg, 0.25 mmol, 1.0 eq.) using 40% aq Et_4NOH (0.090 mL, 0.30 mmol, 1.2 eq.), and propargyl bromide (40% in toluene, 0.033 mL, 0.30 mmol, 1,2 eq.). Semicarbazone **16b** was isolated by chromatography on silica gel (10% EtOAc in hexanes) as white solid (80 mg, 73% yield): m.p. 114-116 °C; R_f 0.49 (20% EtOAc in hexanes);); IR 687, 753, 1660, 1682, 3191, 3276 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.52 (s, 1H), 8.73 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.97 – 7.82 (m, 3H), 7.60 – 7.37 (m, 9H), 7.06 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H), 5.92 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.08 (ddd, *J* = 17.1, 3.3, 1.6 Hz, 1H), 5.01 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 4.15 (d, *J* = 2.4 Hz, 2H), 3.12 (t, *J* = 7.3 Hz, 2H), 2.53 – 2.42 (m, 2H), 2.08 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.4, 158.9, 156.3, 141.3, 137.9, 137.6, 135.7, 134.5, 130.4 (2C), 129.8, 129.3, 129.2, 128.9, 128.1, 122.7, 121.4, 120.4, 115.5, 78.6, 72.2, 39.2, 35.5, 28.3; LRMS (EI) m/z 458 ($M + Na^+$); HRMS (ESI+): m/z for $C_{28}H_{26}N_3O_2$ $(M + H)^+$, calcd 436.2020, found 436.2038.



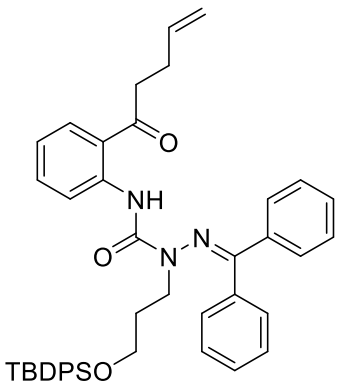
Benzhydrylidene aza-3-chloroproylglycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.16c**) and **3-(Diphenylmethyleneamino)-tetrahydro-1-(2-(pent-4'-enoyl)phenyl)pyrimidin-2(1H)-one** (**2.17c**) were prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **2.16a** by alkylation of **2.15** (1.2 g, 3.0 mmol, 1.0 eq.) using 40% aq Et_4NOH (1.08 mL, 3.0 mmol, 1.0 eq.) and 1-bromo-3-chloropropane (0.43 mL, 4.5 mmol, 1.5 eq.). Semicarbazone **2.16c** was isolated by chromatography on silica gel eluting with 10% EtOAc in hexanes: yellow oil (3,07 g, 79% yield); R_f 0.71 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.31 (s, 1H), 8.71 (dd, *J* = 8.6, 0.9 Hz, 1H), 7.90 – 7.76 (m, 3H), 7.71 – 7.35 (m, 9H), 7.03 (dd, *J* = 7.1, 1.2 Hz, 1H), 5.89 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 (ddd, *J* = 17.2, 3.3, 1.6 Hz, 1H), 4.99 (ddd, *J* = 10.1, 2.9, 1.2 Hz, 1H), 3.51 (t, *J* = 6.9 Hz, 2H), 3.35 (t, *J* = 6.6 Hz, 2H), 3.09 (t, *J* = 7.2 Hz, 2H), 2.47 – 2.40 (m, 2H), 1.82 (qu, *J* = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.4, 160.9, 156.4, 141.5, 137.8, 137.5, 135.8, 134.4, 130.5, 130.4, 129.8, 129.2, 129.0, 128.8, 128.2, 122.5, 121.1, 120.3, 115.4, 44.6, 42.6, 39.1, 30.1, 28.2; LRMS (EI) m/z 474 (M + H^+); HRMS (ESI+): m/z for $C_{28}H_{29}ClN_3O_2$ (M + H)$^+$, calcd 474.1943, found 474.1965.

Pyrimidin-2(1H)-one **2.17c** eluted next using 50% EtOAc in hexanes: yellow oil (239 mg, 18% yield); R_f 0.31 (50% EtOAc in hexanes); IR 637, 698, 762, 1654 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.55 – 7.20 (m, 11H), 7.08 (dd, *J* = 7.9, 0.9 Hz, 1H), 5.87 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.07 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 4.99 (dtd, *J* = 3.1, 2.1, 1.3 Hz, 1H), 3.53 (t, *J* = 5.8 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 7.3 Hz, 2H), 2.42 (q, *J* = 5.2 Hz, 2H), 2.09 – 2.00 (m,

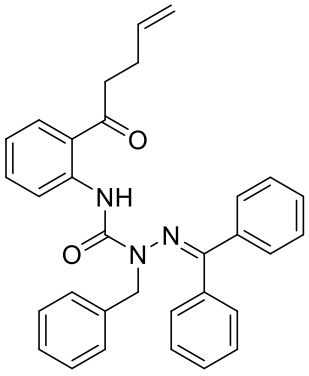
2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 172.3, 153.0, 141.1, 138.5, 137.7, 136.1, 131.6, 130.8, 130.2, 129.4, 129.2, 128.7, 128.22, 128.19, 128.1, 127.7, 126.6, 115.3, 49.9, 49.5, 40.2, 28.2, 22.0; LRMS (EI) m/z 438 (M + H^+); HRMS (ESI+): m/z for $C_{28}H_{28}N_3O_2$ (M + H)$^+$, calcd 438.2176, found 438.2191.



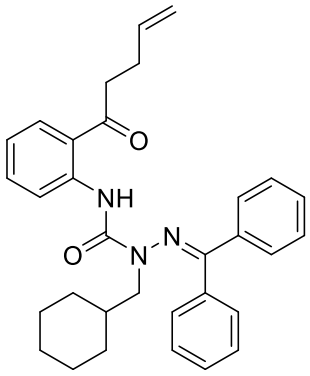
Benzhydrylidene aza-3-chlorobutylglycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (2.16d) was prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **2.16a** by alkylation of **2.15** (2.0 g, 5.0 mmol, 1.0 eq.) using 40% aq. Et_4NOH (0.22 mL, 6.0 mmol, 1.2 eq.) and 1-bromo-4-chlorobutane (0.70 mL, 6.0 mmol, 1.2 eq.). Semicarbazone **2.16d** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as yellow oil (3,07 g, 88% yield): R_f 0.55 (20% EtOAc in hexanes); IR 753, 1443, 1505, 1657, 3213 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.34 (s, 1H), 8.69 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.91 – 7.81 (m, 3H), 7.59 – 7.34 (m, 9H), 7.04 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 5.90 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.07 (ddd, *J* = 17.1, 3.3, 1.6 Hz, 1H), 5.00 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 3.43 – 3.34 (m, 4H), 3.10 (t, *J* = 7.3 Hz, 2H), 2.52 – 2.39 (m, 2H), 1.54 – 1.38 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 158.7, 156.6, 141.6, 138.0, 137.6, 136.2, 134.4, 130.4, 130.38, 129.72, 129.2, 129.0, 128.8, 128.2, 122.6, 121.1, 120.4, 115.5, 45.0, 44.7, 39.2, 29.7, 28.3, 24.1; LRMS (EI) m/z 488 (M + H^+); HRMS (ESI+): m/z for $C_{29}H_{31}ClN_3O_2$ (M + H)$^+$, calcd 488.2099, found 488.2118.



Benzhydrylidene aza-3-*tert*-butyldiphenylsilyloxypropylglycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.16e**) was prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **2.16a** by alkylation of **2.15** (250 mg, 0.630 mmol, 1.0 eq.) using 40% aq Et_4NOH (0,19 mL, 0.755 mmol, 1.2 eq.) and (3-iodopropoxy-*tert*-butyl)diphenylsilane (320 mg, 0.755 mmol, 1.2 eq.). Semicarbazone **2.16e** was isolated by chromatography on silica gel (30% EtOAc in DCM) as yellow oil (412 mg, 94% yield): R_f 0.58 (20% EtOAc in hexanes); IR 699, 1104, 1443, 1505, 1657, 3069 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.30 (s, 1H), 8.74 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.94 – 7.78 (m, 3H), 7.73 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.61 – 7.27 (m, 18H), 7.04 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 5.90 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.07 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 5.00 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 3.55 – 3.40 (m, 4H), 3.10 (t, *J* = 7.3 Hz, 2H), 2.50 – 2.40 (m, 2H), 1.71 – 1.59 (m, 2H), 0.98 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.2, 159.9, 156.5, 141.8, 138.0, 137.7, 136.0, 135.7, 134.9, 134.4, 133.9, 130.4, 129.8, 129.6, 129.3, 128.9, 128.8, 128.1, 127.8, 127.7, 122.5, 120.9, 120.4, 115.4, 61.9, 44.2, 39.1, 29.9, 28.3, 27.0; LRMS (EI) m/z 694 ($M + H^+$); HRMS (ESI+): m/z for $C_{44}H_{47}N_3O_3Si$ $(M + H)^+$, calcd 694.3456, found 694.3460.

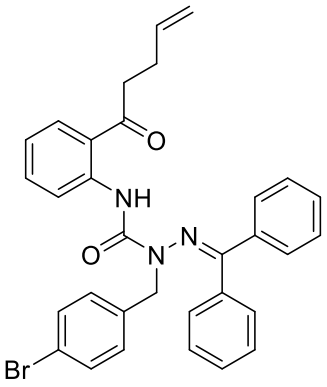


Benzhydrylidene aza-phenylalanine *N*'-(2-(pent-4'-enoyl)phenyl)amide (2.16f) was prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **2.16a** from alkylation of **2.15** (200 mg, 0.500 mmol, 1.0 eq.) using 40% aq Et_4NOH (0,17 mL, 0.6 mmol, 1.2 eq.) and benzyl bromide (0.07 mL, 0.600 mmol, 1.2 eq.). Semicarbazone **2.16f** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as pale yellow solid (250 mg, 78% yield): mp 123-125 °C; R_f 0.61 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.52 (s, 1H), 8.77 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.90 (dd, *J* = 8.1, 1.5 Hz, 1H), (dt, *J* = 6.9, 1.7 Hz, 2H), 7.54 (ddd, *J* = 8.6, 7.3, 1.4 Hz, 1H), 7.47-7.31 (m, 6H), 7.23-7.12 (m, 5H), 7.05 (ddd, *J* = 8.1, 7.3, 1.2 Hz, 1H), 6.93 (dd, *J* = 7.9, 1.6 Hz, 2H), 5.89 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 5.00 (ddt, *J* = 10.2, 1.8, 1.2 Hz, 1H), 4.67 (s, 2H), 3.11 (t, *J* = 7.3 Hz, 2H), 2.45 (qt, *J* = 7.0, 1.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.4, 158.5, 156.8, 141.7, 138.4, 137.7, 136.9, 136.0, 134.5, 130.5, 130.2, 129.7, 129.3, 129.3, 128.7, 128.3, 128.1, 127.4, 126.9, 122.7, 121.2, 120.5, 115.5, 49.6, 39.2, 28.4; LRMS (EI) m/z 510 (M + Na^+); HRMS (ESI+): m/z for $C_{32}H_{30}N_3O_2$ (M + H)$^+$, calcd 488.2333, found 488.2334.



Benzhydrylidene aza-cyclohexylmethylglycine *N*'-(2-(pent-4'-enoyl)phenyl)amide (2.16g) was prepared according to the procedure described for the synthesis of *N*-(methyl)-semicarbazone **2.16a**, excepted the final reaction mixture was heated at reflux using semicarbazone **2.15** (500 mg, 1.260 mmol, 1.0 eq.), 40% aq Et_4NOH (0,45 mL, 1.510 mmol, 1.2 eq.), (bromomethyl)cyclohexane (0.21 mL, 1.510 mmol, 1.2 eq.). Semicarbazone **2.16g** was isolated by chromatography on silica gel (toluene) as yellow oil (325 mg, 52% yield): R_f 0.30 (toluene); IR 750, 1442,

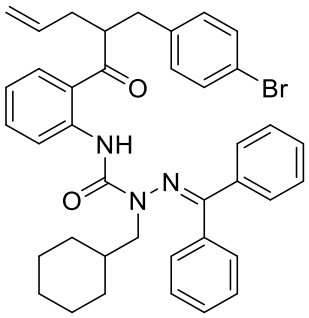
1502, 1657, 2922, 3212 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.39 (s, 1H), 8.72 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.90 – 7.82 (m, 3H), 7.56 – 7.31 (m, 9H), 7.02 (ddd, *J* = 8.1, 7.2, 1.2 Hz, 1H), 5.89 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 4.99 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 3.18 (d, *J* = 7.3 Hz, 2H), 3.10 (t, *J* = 7.3 Hz, 2H), 2.51 – 2.39 (m, 2H), 1.65 – 1.52 (m, 3H), 1.51 – 1.41 (m, 1H), 1.40 – 1.30 (m, 2H), 1.15 – 0.98 (m, 3H), 0.82 – 0.66 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 157.9, 156.8, 141.8, 138.2, 137.7, 136.2, 134.4, 130.4, 130.3, 129.6, 129.3, 128.880, 128.878, 128.1, 122.6, 120.9, 120.4, 115.4, 51.1, 39.2, 34.7, 30.2, 28.3, 26.5, 26.0; LRMS (EI) m/z 516 ($M + Na^+$); HRMS (ESI+): m/z for $C_{32}H_{36}N_3O_2$ $(M + H)^+$, calcd 494.2802, found 494.2818.



Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(pent-4'-enoyl)phenyl)amide (**2.16h**). was prepared according to the procedure described for the synthesis of *N*-(4-bromobenzyl) semicarbazone **2.16a**, using semicarbazone **2.15** (500 mg, 1.260 mmol, 1.0 eq.), 40% aq Et_4NOH (0,45 mL, 1.510 mmol, 1.2 eq4-bromobenzyl bromide (377 mg, 1.510 mmol, 1.2 eq.). Semicarbazone **2.16h** was isolated by chromatography on silica gel (0-10% EtOAc in hexanes) as white solid (660 mg, 93% yield): m.p. 86-90°C; R_f 0.50 (20% EtOAc in hexanes); IR 750, 1511, 1693, 3198 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.51 (s, 1H), 8.74 (dd, *J* = 8.6, 1.1 Hz, 1H), 7.90 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.74 – 7.69 (m, 2H), 7.54 (ddd, *J* = 8.6, 7.3, 1.5 Hz, 1H), 7.50 – 7.27 (m, 8H), 7.22 – 7.15 (m, 2H), 7.06 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 6.83 – 6.77 (m, 2H), 5.89 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 5.06 (ddd, *J* = 17.1, 3.4, 1.6 Hz, 1H), 5.00 (ddd, *J* = 10.2, 3.0, 1.2 Hz, 1H), 4.60 (s, 2H), 3.11 (t, *J* = 7.3 Hz, 2H), 2.51 – 2.36 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.4, 158.9, 156.7, 141.5, 138.1, 137.6, 136.1, 135.8, 134.5, 131.3, 130.5, 130.4, 129.8, 129.3, 129.2, 129.1, 128.7, 128.1,

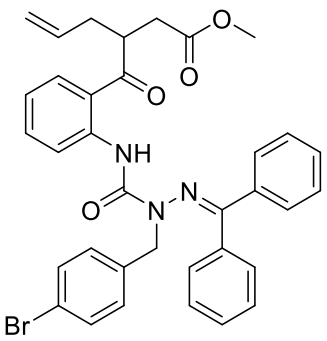
122.7, 121.3, 120.7, 120.4, 115.5, 49.2, 39.2, 28.3; LRMS (EI) 566 $(M + H)^+$; HRMS (EI) m/z for $C_{32}H_{29}BrN_3O_2$ $(M + H)^+$, calcd 566.1438, found 566.1457.

Synthesis of α-alkylated ketone 2.18.

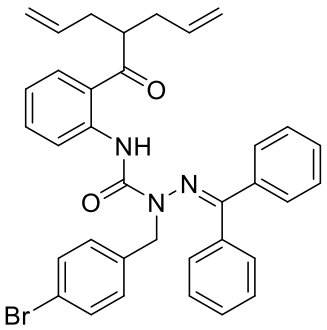


Benzhydrylidene aza-cyclohexylmethylglycine *N*'-(2-(2'-(*p*-bromobenzyl)pent-4'-enoyl)phenyl)amide (2.18g) In a flame dried round bottom flask, under argon, ketone **2.16g** (263 mg, 1.052 mmol, 1.0 eq.) was dissolved in THF (5 mL), cooled to 0°C, treated with a solution of LiHMDS in THF (1.0M, 0.44 mL, 0.443 mmol, 2.5 eq.) followed by a freshly prepared solution of 4-bromobenzyl bromide (260 mg, 0.526 mmol, 2.0 eq.) in the THF (3 mL), stirred at 0°C for 1h, and quenched with 1 mL of water. The volatiles were evaporated. The resulting aqueous phase was diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography (0-10% EtOAc in hexanes) to afford α-branched ketone **2.18g** as yellow oil (252 mg, 72 % yield): R_f 0.33 (toluene); IR 752, 1445, 1503, 1678, 2922, 3210 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.11 (s, 1H), 8.65 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.90 – 7.78 (m, 2H), 7.65 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.55 – 7.32 (m, 9H), 7.32 – 7.26 (m, 2H), 7.08 – 6.85 (m, 3H), 5.73 (ddt, *J* = 17.1, 10.1, 7.0 Hz, 1H), 5.13 – 4.94 (m, 2H), 3.74 (dq, *J* = 8.5, 6.3 Hz, 1H), 3.29 (dd, J = 14.5, 7.6 Hz, 1H), 3.08 (dd, *J* = 12.9, 5.4 Hz, 1H), 3.01 (dd, *J* = 12.1, 6.8 Hz, 1H), 2.71 (dd, *J* = 13.6, 5.5 Hz, 1H), 2.48 (dt, *J* = 13.8, 6.7 Hz, 1H), 2.24 (dt, *J* = 14.0, 6.7 Hz, 1H), 1.64 – 1.54 (m, 3H), 1.51 – 1.43 (m, 1H), 1.41 – 1.33 (m, 2H), 1,12 – 1.05 (m, 3H), 0.81 – 0,73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.1, 158.4, 156.7, 141.7, 138.8, 138.3, 136.1, 135.1, 134.3, 131.5, 131.0, 130.3, 129.9, 129.7, 129.3, 128.90, 128.85, 128.1, 123.1, 121.0, 120.5, 120.2, 117.6, 51.1, 49.1, 37.1, 37.0, 34.7, 30.2, 30.1, 26.5, 26.0, 25.9; LRMS

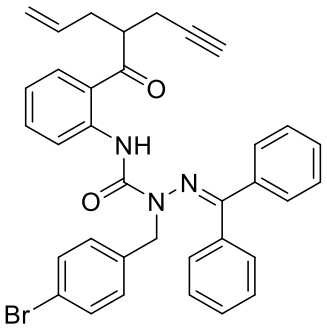
(EI) m/z 685 (M + Na^+); HRMS (ESI+): m/z for $C_{39}H_{41}BrN_3O_2$ (M + H)$^+$, calcd 662.2377, found 662.2377.



Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(methyl 3'-allyl-4'-oxo-4'-phenylbutanoate)amide (**2.18h**) was prepared using the procedure described for the synthesis of **2.18g** by alkylation of **2.16h** (100 mg, 0.177 mmol, 1.0 eq.) using LiHMDS (1.0 M in THF, 0,44 mL, 0.353 mmol, 2.5 eq.) and methyl bromoacetate (0.033 mL, 0.353 mmol, 2.0 eq.). α-Branched ketone **2.18h** was isolated by chromatography on silica gel (0-10% EtOAc in hexanes) as yellow oil (90 mg, 80% yield): R_f 0.33 (20% EtOAc in hexanes); IR 693, 752, 1444, 1504, 1681, 1733, 3224 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.16 (s, 1H), 8.70 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.96 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.71 – 7.63 (m, 2H), 7.55 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.51 – 7.32 (m, 6H), 7.32 – 7.27 (m, 2H), 7.21 – 7.15 (m, 2H), 7.10 (ddd, *J* = 8.3, 7.4, 1.1 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.69 (ddt, *J* = 17.0, 10.2, 7.1 Hz, 1H), 5.08 – 4.99 (m, 2H), 4.60 (dd, *J* = 41.1, 16.6 Hz, 2H), 4.07 – 3.95 (m, 1H), 3.58 (s, 3H), 2.96 – 2.76 (m, 1H), 2.61 – 2.41 (m, 2H), 2.28 – 2.13 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.7, 172.9, 158.4, 156.6, 141.4, 138.1, 136.0, 135.7, 134.5, 134.3, 131.3, 130.30, 130.26, 129.8, 129.2, 129.1, 129.0, 128.7, 128.0, 122.7, 121.6, 120.7 (2C), 118.1, 51.8, 49.2, 43.1, 36.9, 35.1; LRMS (EI) m/z 660 (M + Na^+); HRMS (ESI+): m/z for $C_{35}H_{33}BrN_3O_4$ (M + H)$^+$, calcd 638.1649, found 638.1663.

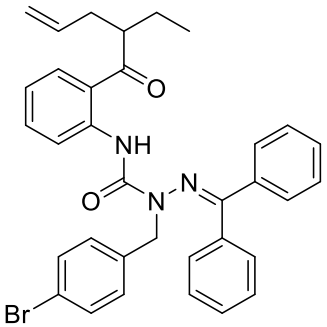


Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(2'-allylpent-4'-enoyl)phenyl)amide (**2.18i**) was prepared using the procedure described for the synthesis of ketone **2.18g** from alkylation of **2.16h** (100 mg, 0.177 mmol, 1eq.) using LiHMDS (1.0 M in THF, 0.44 mL, 0.443 mmol, 2.5 eq.) and allyl bromide (0.03 mL, 0.353 mmol, 2.0 eq.). α-Branched ketone **2.18i** was isolated by chromatography on silica gel (0-10% EtOAc in hexanes) as pale yellow oil (75 mg, 70% yield): R_f 0.58 (30% EtOAc in hexanes); IR 752, 1444, 1503, 1682, 3215 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.26 (s, 1H), 8.69 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.84 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.54 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.50 – 7.32 (m, 6H), 7.32 – 7.27 (m, 2H), 7.23 – 7.14 (m, 2H), 7.08 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H), 6.82 – 6.74 (m, 2H), 5.74 (ddt, *J* = 17.1, 10.1, 7.1 Hz, 2H), 5.07 – 4.94 (m, 4H), 4.61 (s, 2H), 3.60 (qu, *J* = 6.8 Hz, 1H), 2.58 – 2.42 (m, 2H), 2.34 – 2.21 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 158.5, 156.6, 141.3, 138.1, 136.0, 135.8, 135.6, 134.3, 131.3, 130.3, 130.1, 129.8, 129.2, 129.2, 129.0, 128.7, 128.1, 123.6, 121.4, 120.7 (2C), 117.2, 49.2, 46.9, 36.3; LRMS (EI) m/z 606 ($M + H^+$); HRMS (ESI+): m/z for $C_{35}H_{33}BrN_3O_2$ $(M + H)^+$, calcd 606.1751, found 606.1764.



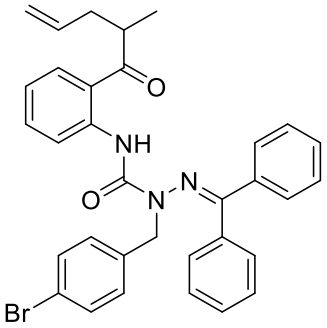
Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(2'-propargylpent-4'-enoyl)phenyl)-amide (**2.18j**) was prepared using the procedure described for the

synthesis of ketone **2.18g** from alkylation of **2.16h** (50 mg, 0.088 mmol, 1.0 eq.) using LiHMDS (1.0 M in THF, 0,22 mL, 0.220 mmol, 2.5 eq.) and propargyl bromide (40% in toluene, 0.02 mL, 0.180 mmol, 2.0 eq.). α-Branched ketone **2.18j** was isolated by chromatography on silica gel (0-10% EtOAc in hexanes) as colorless oil (36 mg, 68% yield): R_f 0.45 (20% EtOAc in hexanes); IR 751, 1443, 1502, 1681, 3295 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.23 (s, 1H), 8.71 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.88 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.78 – 7.62 (m, 2H), 7.56 (ddd, *J* = 8.5, 7.4, 1.4 Hz, 1H), 7.49 – 7.27 (m, 8H), 7.21 – 7.13 (m, 2H), 7.09 (ddd, *J* = 8.1, 7.3, 1.1 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.69 (ddt, *J* = 17.2, 10.1, 7.2 Hz, 1H), 5.14 – 4.91 (m, 2H), 4.74 – 4.48 (m, 2H), 3.76 (qu, *J* = 6.6 Hz, 1H), 2.67 – 2.49 (m, 2H), 2.49 – 2.31 (m, 2H), 1.95 (t, *J* = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.3, 158.4, 156.6, 141.4, 138.1, 136.0, 135.8, 134.6, 134.4, 131.3, 130.4, 130.3, 129.8, 129.3, 129.2, 129.0, 128.7, 128.1, 123.0, 121.5, 120.8, 120.7, 118.0, 82.0, 70.2, 49.2, 46.1, 36.3, 20.7; LRMS (EI) m/z 626 (M + Na^+); HRMS (ESI+): m/z for $C_{35}H_{31}BrN_3O_2$ (M + $H)^+$, calcd 604.1594, found 604.1605.

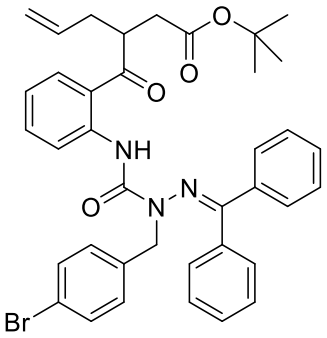


Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(2'-ethylpent-4'-enoyl)phenyl)amide (**2.18k**) was prepared using the procedure described for the synthesis of ketone **2.18g** by alkylation of **2.16h** (50 mg, 0.088 mmol, 1.0 eq.) using LiHMDS (1.0 M in THF, 0.22 mL, 0.220 mmol, 2.5 eq.) and ethyl iodide (0.012 mL, 0.180 mmol, 2.0 eq.). α-Branched ketone **2.18k** was isolated by chromatography on silica gel (0-4% EtOAc in hexanes) as colorless oil (18 mg, 35% yield): R_f 0.57 (20% EtOAc in hexanes); IR 692, 752, 1443, 1503, 1682, 3200 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.31 (s, 1H), 8.68 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.86 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.77 – 7.63 (m, 2H), 7.53 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.48 – 7.27 (m, 8H), 7.22 – 7.14 (m, 2H), 7.08 (ddd, *J* = 8.1, 7.4, 1.1 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.74 (ddt,

J = 17.1, 10.1, 7.0 Hz, 1H), 5.15 – 4.86 (m, 2H), 4.61 (s, 2H), 3.52 – 3.42 (m, 1H), 2.56 – 2.42 (m, 1H), 2.30 – 2.18 (m, 1H), 1.78 (dq, *J* = 14.8, 7.4 Hz, 1H), 1.73 – 1.47 (m, 1H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.8, 158.3, 156.7, 141.2, 138.1, 136.1, 136.0, 135.8, 134.2, 131.3, 130.3, 130.1, 129.8, 129.21, 129.16, 129.0, 128.7, 128.0, 123.9, 121.4, 120.73, 120.67, 116.8, 49.2, 48.6, 36.3, 25.4, 11.8; LRMS (EI) m/z 616 (M + Na^+); HRMS (ESI+): m/z for $C_{34}H_{33}BrN_3O_2$ (M + H)$^+$, calcd 594.1751, found 594.1764.

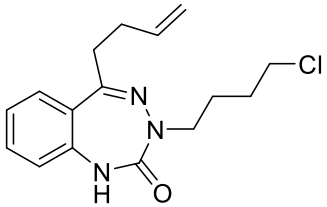


Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(2'-methylpent-4'-enoyl)phenyl)amide (**2.18l**) was prepared using the procedure described for the synthesis of ketone **2.18g** by alkylation of **2.16h** (50 mg, 0.088 mmol, 1.0 eq.) using LiHMDS (1.0 M in THF, 0,22 mL, 0.220 mmol, 2.5 eq.) and methyl iodide (0.011 mL, 0.180 mmol, 2.0 eq.). α-Branched ketone **2.18l** was isolated by chromatography on silica gel (0-4% EtOAc in hexanes) as colorless oil (24 mg, 47%): R_f 0.54 (20% EtOAc in hexanes); IR 700, 750, 1444, 1503, 1681, 3201 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.40 (s, 1H), 8.72 (dd, *J* = 8.5, 0.9 Hz, 1H), 7.88 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.54 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.50 – 7.27 (m, 8H), 7.22 – 7.14 (m, 2H), 7.07 (ddd, *J* = 8.3, 7.4, 1.2 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 5.78 (ddt, *J* = 16.9, 10.0, 7.0 Hz, 1H), 5.02 (ddd, *J* = 9.8, 8.1, 3.2 Hz, 2H), 4.61 (q, *J* = 16.6 Hz, 2H), 3.57 (dt, *J* = 13.5, 6.7 Hz, 1H), 2.53 (dt, *J* = 13.1, 6.5 Hz, 1H), 2.18 (dt, *J* = 14.1, 7.2 Hz, 1H), 1.21 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.7, 158.3, 156.7, 141.6, 138.1, 136.1, 136.0, 135.8, 134.3, 131.3, 130.3, 130.2, 129.8, 129.22, 129.16, 129.0, 128.7, 128.1, 122.5, 121.4, 120.69, 120.68, 116.9, 49.2, 41.6, 38.1, 17.6; LRMS (EI) m/z 602 (M + Na^+); HRMS (ESI+): m/z for $C_{33}H_{31}BrN_3O_2$ (M + H)$^+$, calcd 580.1594, found 580.1599.

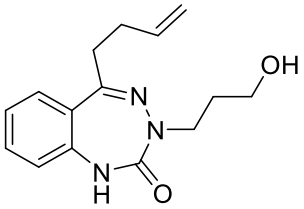


Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(*tert*-butyl 3'-allyl-4'-oxo-4'-phenylbutanoate)amide (**2.18m**) was prepared using the procedure described for the synthesis of **2.18g** by alkylation of **2.16h** (100 mg, 0.177 mmol, 1.0 eq.) using LiHMDS (1.0 M in THF, 0,44 mL, 0.353 mmol, 2.5 eq.) and *tert*-butyl bromoacetate (0.05 mL, 0.353 mmol, 2.0 eq.). α-Branched ketone **2.18m** was isolated by chromatography on silica gel (0-10% EtOAc in hexanes) as white oily solid (88 mg, 73% yield): m.p. 38-40°C; R_f 0.47 (20% EtOAc in hexanes); IR 692, 753, 1150, 1444, 1504, 1683, 1723, 3210 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.31 (s, 1H), 8.73 (dd, *J* = 8.5, 0.9 Hz, 1H), 7.97 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.76 – 7.66 (m, 2H), 7.55 (ddd, *J* = 8.6, 6.9, 1.3 Hz, 1H), 7.51 – 7.32 (m, 6H), 7.32 – 7.23 (m, 2H), 7.17 (dd, *J* = 8.0, 1.4 Hz, 2H), 7.10 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.71 (ddt, *J* = 16.8, 10.0, 7.2 Hz, 1H), 5.05 (dd, *J* = 13.1, 5.6 Hz, 2H), 4.60 (q, *J* = 16.6 Hz, 2H), 4.05 – 3.88 (m, 1H), 2.82 (dd, *J* = 16.8, 9.5 Hz, 1H), 2.52 – 2.38 (m, 2H), 2.26 – 2.10 (m, 1H), 1.33 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.9, 171.5, 158.1, 156.6, 141.6, 138.0, 136.0, 135.8, 134.6, 134.4, 131.3, 130.34, 130.31, 129.8, 129.22, 129.15, 129.0, 128.7, 128.1, 122.7, 121.5, 120.7, 120.6, 117.9, 80.9, 49.1, 43.2, 36.9 (2C), 28.1; LRMS (EI) m/z 702 (M + Na^+); HRMS (ESI+): m/z for $C_{38}H_{39}BrN_3O_4$ $(M + H)^+$, calcd 680.2119, found 680.2139.

Synthesis of benzotriazepin-2-one 2.6.

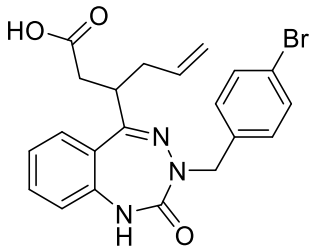


3-(4-Chlorobutyl)-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6a**) Semicarbazone **2.16d** (250 mg, 0.510 mmol, 1.0 eq.) was dissolved in THF (25 mL) and treated with HCl (1.0 M, 25 mL) in a tube that was sealed, heated to 60°C and stirred for 14h. The volatiles were evaporated. The resulting aqueous phase was diluted with water (50 mL) and extracted with EtOAc (2 x 50 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography (20% EtOAc in hexanes) to afford triazepinone **2.6a** as white solid (155 mg, 99 % yield): R_f 0.24 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.32 (m, 2H), 7.12 (ddd, *J* = 8.3, 7.3, 1.1 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.69 (s, 1H), 5.83 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.05 – 4.95 (m, 2H), 3.61 (t, *J* = 6.9 Hz, 2H), 3.50 (t, *J* = 6.3 Hz, 2H), 2.85 – 2.78 (m, 2H), 2.32 (dtt, *J* = 7.8, 6.6, 1.3 Hz, 2H), 1.87-1.65 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 163.7, 142.0, 137.2, 131.9, 127.5, 126.3, 123.8, 120.2, 115.7, 50.00, 45.0, 35.8, 31.7, 30.1, 25.1; LRMS (EI) m/z 306 ($M + H^+$); HRMS (ESI+): m/z for $C_{16}H_{21}ClN_3O$ $(M + H)^+$, calcd 306.1368, found 306.1376.

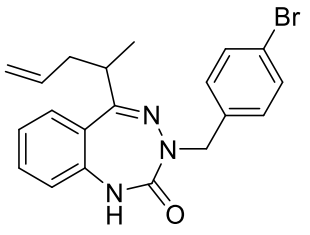


3-(3-Propanol)-5-(but-3-enyl)-)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6b**) was prepared using the procedure described for the synthesis of benzotriazepin-2-one **2.6a** with a reaction time of 24h, from semicarbazone **2.16e** (380 mg, 0.546 mmol, 1.0 eq.), and isolated by chromatography on silica gel (50% EtOAc in hexanes) as white solid (103 mg, 69 % yield): R_f 0.21 (1/1 hexanes/EtOAc); IR 763, 1668, 3233 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 – 7.32 (m, 2H), 7.13 (td, *J* = 7.5 Hz, 1.1, 1H),

6.84 (d, *J* = 7.4 Hz, 1H), 6.59 (s, 1H), 5.82 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.09 – 4.91 (m, 2H), 3.70 (t, *J* = 6.2 Hz, 2H), 3.64 (dt, *J* = 5.6, 5.4 Hz, 2H), 3.02 (t, *J* = 6.2 Hz, 1H), 2.85 – 2.80 (m, 2H), 2.32 (dtt, J 7.8, 6.6, 1.2 = Hz, 2H), 1.97 (tt, *J* = 6.0, 6.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 163.5, 142.1, 137.0, 132.2, 127.6, 125.9, 123.9, 120.2, 115.8, 60.00, 47.5, 35.9, 31.5, 30.4; LRMS (EI) m/z 274 (M + H^+); HRMS (ESI+): m/z for $C_{15}H_{20}N_3O_2$ $(M + H)^+$, calcd 274.1550, found 274.1551

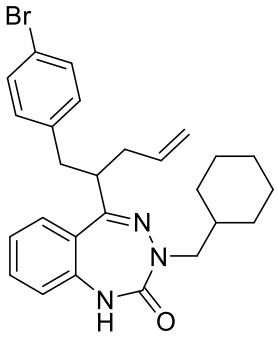


3-(4-Bromobenzyl)-5-(3-hex-5-enoate)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6c**) was prepared using the procedure described for the synthesis of benzotriazepin-2-one **2.6a** from semicarbazone **2.18h** (65 mg, 0.102 mmol, 1.0 eq.). Triazepinone **2.6c** was isolated by chromatography on silica gel (0-50% EtOAc in hexanes) as colorless oil (45 mg, 40 % yield): R_f 0.37 (30% EtOAc in hexanes); IR 770, 1675, 3221 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.45 (dd, J = 8.1, 1.1 Hz, 1H), 7.37 – 7.28 (m, 3H), 7.13 (td, *J* = 7.6, 1.1 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.65 (dd, *J* = 8.0, 0.9 Hz, 1H), 5.56 (ddt, *J* = 17.4, 10.2, 7.3 Hz, 1H), 5.00 – 4.89 (m, 2H), 4.68 (q, *J* = 14.7 Hz, 2H), 3.37 – 3.25 (m, 1H), 2.86 (dd, *J* = 17.3, 10.4 Hz, 1H), 2.59 (dd, *J* = 17.2, 4.4 Hz, 1H), 2.27 (dt, *J* = 13.1, 6.5 Hz, 1H), 2.13 27 (dt, *J* = 14.3, 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 168.2, 164.5, 141.8, 136.7, 134.7, 131.8, 131.3, 130.5, 127.5, 127.4, 124.0, 121.0, 120.1, 118.0, 53.9, 41.3, 38.7, 37.4; LRMS (EI) m/z 442 (M + H^+); HRMS (ESI+): m/z for $C_{21}H_{21}BrN_3O_3$ $(M + H)^+$, calcd 442.0761, found 442.0770.



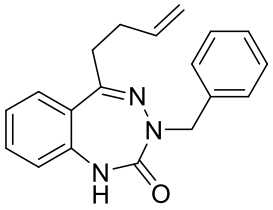
3-(4-Bromobenzyl)-5-(pent-4-en-2-yl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6d**). Samples of semicarbazone **2.18l** (10.0 mg, 0.017 mmol, 1.0 eq.) were respectively

dissolved in THF (1 mL) and EtOH (1 mL) and treated with HCl (1.0 M, 1 mL) in a tube that was sealed, heated to 60°C and stirred until the semicarbazone **2.18l** was observed to have reacted completely by thin layer chromatography (20% EtOAc in hexanes): 22h in THF; 4h in EtOH. The volatiles were evaporated. The residue was purified by preparative thin layer chromatography (20% EtOAc in hexanes) to afford triazepinone **2.6d** as pale yellow solid (3.6 mg, 53% from THF; 4.3 mg, 63% from EtOH): m.p. 54-58°C, R_f 0.37 (20% EtOAc in hexanes), IR (1667, 2918, 3221) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.34 (m, 3H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.20 – 7.07 (m, 3H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.54 (s, 1H), 5.61 (ddt, *J* = 17.1, 10.0, 7.1 Hz, 1H), 5.02 – 4.86 (m, 2H), 4.82 – 4.63 (m, 2H), 2.93 (qt, *J* = 6.8, 6.9 Hz, 1H), 2.37 (dt, *J* = 13.5, 6.6 Hz, 1H), 2.12 (dt, *J* = 14.3, 7.6 Hz, 1H), 1.10 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 163.6, 141.8, 136.9, 136.3, 131.7, 131.3, 130.7, 127.2, 126.9, 124.0, 121.0, 120.1, 116.6, 54.0, 40.1, 39.5, 19.1; LRMS (EI) m/z 398 $(M + H^+)$; HRMS (ESI+): m/z for $C_{20}H_{21}BrN_3O$ $(M + H)^+$, calcd 398.0863, found 398.0872.

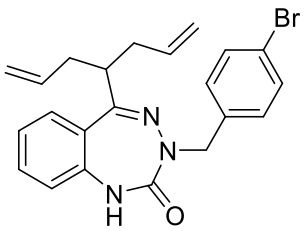


3-(Cyclohexylmethyl)-5-(1-(4-bromophenyl)pent-4-en-2-yl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6e**) was prepared using the procedure described for the synthesis of benzotriazepin-2-one **2.6a** from semicarbazone **2.18g** (100 mg, 0.151 mmol, 1.0 eq.), and isolated by chromatography on silica gel (0-20% EtOAc in hexanes) as white solid (21 mg, 32 % yield): m.p. 87-91°C, R_f 0.43 (20% EtOAc in hexanes); IR 763, 1674, 3221 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 3H), 7.15 – 7.00 (m, 4H), 6.76 (dd, *J* = 8.0, 0.7 Hz, 1H), 6.60 (s, 1H), 5.76 (ddt, *J* = 17.0, 10.1, 7.1 Hz, 1H), 5.10 – 4.97 (m, 2H), 3.49 (ddd, *J* = 34.7, 13.1, 7.1 Hz, 2H), 3.18 – 3.13 (m, 1H), 2.90 (ddd, *J* = 19.8, 13.8, 7.1 Hz, 2H), 2.48 (dt, *J* = 14.1, 7.0 Hz, 1H), 2.27 (dt, *J* = 13.8, 6.8 Hz, 1H), 1.66 – 1.42 (m, 6H), 1.18 – 1.08 (m, 3H),

0.84 – 0.76 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 163.6, 142.3, 139.3, 135.8, 131.6, 131.4, 130.9, 127.3, 127.0, 123.6, 120.0, 119.9, 117.2, 57.0, 47.4, 39.1, 38.5, 36.1, 30.8, 26.7, 25.9; LRMS (EI) m/z 480 (M + H^+); HRMS (ESI+): m/z for $C_{26}H_{31}BrN_3O$ (M + H)$^+$, calcd 480.1645, found 480.1652.



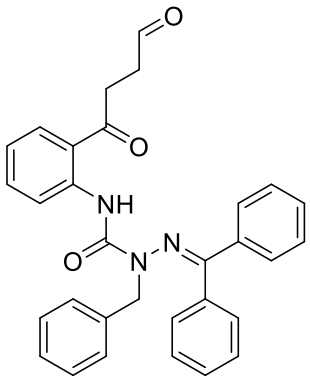
3-Benzyl-5-(but-3-enyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.6f**) was prepared using the procedure described for the synthesis of benzotriazepin-2-one **2.6a** with a reaction time of 14h, from semicarbazone **2.16f** (100 mg, 0.200 mmol, 1.0 eq.), and isolated by chromatography on silica gel (20% EtOAc in hexanes) as white solid (55 mg, 90 % yield): m.p. 65-66°C, R_f 0.34 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.19 (m, 7H), 7.12 (td, *J* = 7.9, 1.1 Hz, 1H), 6.95 (s, 1H), 6.86 (dd, *J* = 8.0, 0.7 Hz, 1H), 5.77 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 4.98 (ddd, *J* = 11.9, 3.3, 1.5 Hz, 1H), 4.95-4.91 (m, 1H), 4.84 (s, 2H), 2.78 – 2.73 (m, 2H), 2.7 (dtt, *J* = 7.8, 6.6, 1.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 163.8, 141.9, 138.0, 137.3, 131.9, 128.5, 128.3, 127.5, 127.0, 126.4, 123.8, 120.3, 115.5, 54.6, 35.8, 31.6; LRMS (EI) m/z 306 (M + H^+); HRMS (ESI+): m/z for $C_{19}H_{20}N_3O$ (M + H)$^+$, calcd 306.1601, found 306.1608.



3-(4-Bromobenzyl)-5-(hepta-1,6-dien-4-yl)-1H-1,3,4-benzotriazepin-2(3H)-one (2.6g) was prepared using the procedure described for the synthesis of benzotriazepin-2-one **2.6a** with a reaction time of 40h, from semicarbazone **2.18i** (150 mg, 0.247 mmol, 1.0 eq.), and isolated by chromatography on silica gel (10% EtOAc in hexanes) as white solid (80 mg, 76%): m.p. 104-107°C, R_f 0.24 (20% EtOAc in hexanes), IR 742, 800, 914, 1668, 3216 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$)

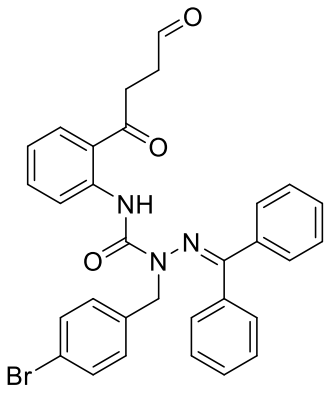
δ 7.41 – 7.32 (m, 3H), 7.27 (dd, *J* = 7.5, 1.2, 1H), 7.16 – 7.08 (m, 3H), 6.89 (s, 1H), 6.84 (dd, *J* = 8.0, 0.9 Hz, 1H), 5.54 (ddt, *J* = 17.0, 10.1, 7.1 Hz, 2H), 4.96 – 4.85 (m, 4H), 4.74 (s, 2H), 2.92 – 2.80 (m, 1H), 2.41 – 2.29 (m, 2H), 2.25 – 2.13 (m, 2H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 163.9, 141.8, 137.0, 135.9, 131.7, 131.4, 130.6, 127.5, 127.3, 123.9, 120.9, 120.1, 116.8, 54.1, 45.6, 37.9; LRMS (EI) m/z 424 (M + H^+); HRMS (ESI+): m/z for $C_{22}H_{23}BrN_3O$ $(M + H)^+$, calcd 424.1019, found 424.1036.

Synthesis of ketoaldehyde 2.22.

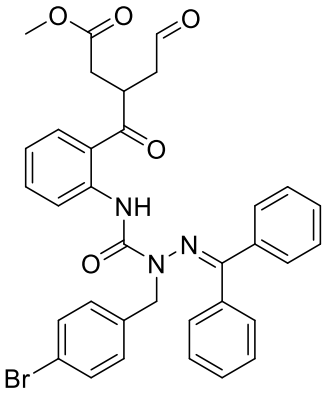


Benzhydrylidene aza-phenylalanine *N*'-(2-(4'-oxo-4'-phenylbutanal)amide (2.22a) homoallylic ketone **2.16f** (80 mg, 0.16 mmol, 1.0 eq.) was dissolved in dioxane (3 mL), treated with water (1 mL), 2,6-lutidine (0.06 mL, 0.33 mmol, 2 eq.), $NaIO_4$ (140 mg, 0.66 mmol, 4 eq.) and OsO_4 (2.5% in *t*-BuOH, 0.03 mL, 0.003 mmol, 0.02 eq.). stirred for 3h at room temperature, diluted with water (25 mL), and extracted with EtOAc (2 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography (20% EtOAc in hexanes) to afford ketoaldehyde **2.22b** as yellow oil (61 mg, 76 % yield): R_f 0.26 (20% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.46 (s, 1H), 9.78 (t, *J* = 0.7 Hz, 1H), 8.81 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.68 – 7.62 (m, 2H), 7.56 (ddd, *J* = 8.6, 7.3, 1.4 Hz, 1H), 7.50 – 7.30 (m, 6H), 7.22 – 7.12 (m, 5H), 7.07 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H), 6.92 (dd, *J* = 7.9, 1.6 Hz, 2H), 4.65 (s, 2H), 3.39 (t, *J* = 6.0 Hz, 2H), 2.77 (td, *J* = 6.0, 0.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.9, 200.5, 158.9, 156.6, 141.9, 138.4, 136.8, 135.9, 135.0, 130.5, 130.2, 129.8, 129.3, 129.2, 128.6, 128.3, 127.9, 127.3, 126.9, 121.8,

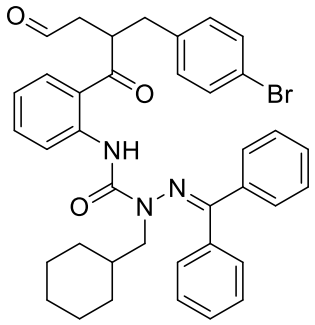
121.2, 120.3, 49.6, 37.6, 32.8; LRMS (EI) m/z 490 ($M + H^+$); HRMS (ESI+): m/z for $C_{31}H_{28}N_3O_3$ $(M + H)^+$, calcd 490.2125, found 490.2121.



Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(4'-oxo-4'-phenylbutanal)amide (**2.22b**) was prepared using the procedure described for the synthesis of ketoaldehyde **2.22a** with a reaction time of 2h, homoallylic ketone **2.16h** (100 mg, -,177 mmol, 1 eq.), 2,6-lutidine (0.064 mL, 0.353 mmol, 2 eq.), $NaIO_4$ (151 mg, 0.706 mmol, 4 eq.) and OsO_4 (2.5% in *t*-BuOH) (0.036 mL, 0.004 mmol, 0.02 eq.), Ketoaldehyde **2.22b** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as brown oil (82 mg, 81%): R_f 0.33 (20% EtOAc in hexanes); IR 693, 751, 1150, 1445, 1505, 1680, 1718, 3225 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.44 (s, 1H), 9.78 (s, 1H), 8.77 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.56 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.51 – 7.34 (m, 6H), 7.34 – 7.27 (m, 2H), 7.22 – 7.14 (m, 2H), 7.07 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H), 6.84 – 6.76 (m, 2H), 4.59 (s, 2H), 3.38 (t, *J* = 6.2 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.8, 200.6, 159.3, 156.5, 141.7, 138.2, 136.0, 135.7, 135.0, 131.3, 130.6, 130.3, 129.9, 129.3, 129.1, 129.1, 128.7, 128.0, 121.9, 121.4, 120.8, 120.3, 49.2, 37.5, 32.7; ; LRMS (EI) m/z 590 ($M + Na^+$); HRMS (ESI+): m/z for $C_{31}H_{27}BrN_3O_3$ $(M + H)^+$, calcd 568.1230, found 568.1232.

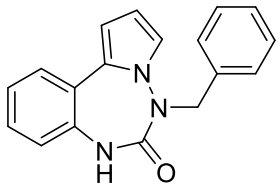


Benzhydrylidene aza-4-bromophenylalanine *N*'-(2-(3'-methyl acetyl-4'-oxo-4'-phenylbutanal)amide (**2.22c**) was prepared using the procedure described for the synthesis of ketoaldehyde **2.22a** with a reaction time of 4.5h, homoallylic ketone **2.18h** (110 mg, 0.172 mmol, 1.0 eq.), 2,6-lutidine (0.067 mL, 0.344 mmol, 2 eq.), $NaIO_4$ (147 mg, 0.689 mmol, 4 eq.) and OsO_4 (2.5% in *t*-BuOH, 0.033 mL, 0.0032 mmol, 0.02 eq.). Ketoaldehyde **2.22c** was isolated by chromatography on silica gel (30% EtOAc in hexanes) as brown oil (92 mg, 84 % yield): R_f 0.21 (30% EtOAc in hexanes); IR 693, 751, 1445, 1504, 1681, 1729, 3234 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.12 (s, 1H), 9.71 (s, 1H), 8.72 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.94 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.56 (ddd, *J* = 8.6, 7.4, 1.4 Hz, 1H), 7.49 – 7.33 (m, 6H), 7.33 – 7.27 (m, 2H), 7.20 – 7.14 (m, 2H), 7.10 (ddd, *J* = 8.2, 7.4, 1.1 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.59 (s, 2H), 4.41 (qu, *J* = 6.6 Hz, 1H), 3.61 (s, 3H), 2.96 (dd, *J* = 18.4, 6.7 Hz, 1H), 2.79 (dd, *J* = 16.4, 6.5 Hz, 1H), 2.68 (dd, *J* = 18.2, 6.5 Hz, 1H), 2.50 (dd, *J* = 16.5, 7.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.0, 199.1, 171.7, 158.5, 156.4, 141.8, 138.0, 135.8, 135.5, 134.8, 131.2, 130.2, 130.1, 129.7, 129.07, 129.00, 128.9, 128.6, 127.9, 121.6, 121.3, 120.7 120.6, 52.0, 49.0, 45.1, 38.0, 35.8; LRMS (EI) m/z 490 ($M + H^+$); HRMS (ESI+): m/z for $C_{34}H_{31}BrN_3O_5$ $(M + H)^+$, calcd 640.1442, found 640,1452.

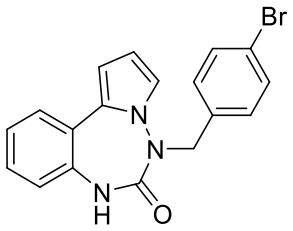


Benzhydrylidene aza-cyclohexylmethylglycine *N*'-(2-(3'-*p*-bromobenzyl-4'-oxo-4'-phenylbutanal)amide (**2.22d**) was prepared using the procedure described for the synthesis of ketoaldehyde **2.22a** with a reaction time of 1h, homoallylic ketone **2.18g** (75 mg, 0.113 mmol, 1.0 eq.), 2,6-lutidine (0.041 mL, 0.226 mmol, 2 eq.), $NaIO_4$ (97 mg, 0.452 mmol, 4 eq.) and OsO_4 (2.5% in *t*-BuOH, 0.022 mL, 0.0022 mmol, 0.02 eq.). Ketoaldehyde **2.22d** was isolated by chromatography on silica gel (20% EtOAc in hexanes) as yellow oil (51 mg, 68 % yield): R_f 0.21 (20% EtOAc in hexanes); IR 692, 750, 1443, 1502, 1655, 1677, 3236 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.07 (s, 1H), 9.66 (s, 1H), 8.73 (dd, *J* = 8.6, 1.0 Hz, 1H), 7.89 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.78 – 7.71 (m, 2H), 7.53 (ddd, *J* = 8.6, 7.3, 1.3 Hz, 1H), 7.50 – 7.43 (m, 4H), 7.42 – 7.30 (m, 6H), 7.04 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H), 6.99 – 6.92 (m, 2H), 4.23 (tt, *J* = 7.6, 5.8 Hz, 1H), 3.26 (dd, *J* = 14.5, 7.5 Hz, 1H), 3.10 (dd, *J* = 14.5, 7.0 Hz, 1H), 3.06 – 2.87 (m, 2H), 2.65 (dd, *J* = 13.8, 7.7 Hz, 1H), 2.49 (dd, *J* = 18.1, 5.2 Hz, 1H), 1.72 – 1.52 (m, 3H), 1.51 – 1.42 (m, 1H), 1.43 – 1.30 (m, 2H), 1.16 – 1.02 (m, 3H), 0.90 – 0.66 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.4, 200.0, 158.2, 156.4, 142.1, 138.1, 137.1, 135.9, 134.7, 131.6, 130.8, 130.1, 130.0, 129.6, 129.1, 128.8, 128.7, 127.9, 121.6, 121.0, 120.6, 120.4, 51.0, 44.7, 43.1, 37.5, 34.6, 30.0, 26.3, 25.8; LRMS (EI) m/z 686 ($M + Na^+$); HRMS (ESI+): m/z for $C_{38}H_{39}BrN_3O_3$ $(M + H)^+$, calcd 664.2169, found 664.2169.

Synthesis of pyrrolobenzotriazepin-6-one 2.7.

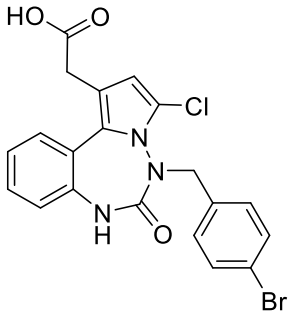


5-Benzyl-5H-pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one (**2.7a**). Ketoaldehyde **2.22a** (50 mg, 0.10 mmol, 1.0 eq.) was dissolved in THF (4 mL) and treated with HCl (1.0 N, 4 mL) in a tube that was sealed, heated to 60°C and stirred overnight. The volatiles were evaporated. The resulting aqueous phase was diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel chromatography (20% EtOAc in hexanes) to afford pyrrolobenzotriazepin-6-one **2.7a** as pale brown solid (16 mg, 55 % yield): R_f 0.42 (20% EtOAc in hexanes); IR 688, 740, 1673, 3239 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.26 – 7.16 (m, 4H), 7.15 – 7.08 (m, 3H), 6.91 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.78 (dd, *J* = 3.0, 1.8 Hz, 1H), 6.51 (s, 1H), 6.16 (dd, *J* = 4.0, 3.0 Hz, 1H), 6.09 (dd, *J* = 4.0, 1.8 Hz, 1H), 4.94 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 136.8, 135.6, 131.0, 128.6, 128.1, 128.0, 127.8, 126.7, 125.1, 123.2, 120.5, 117.9, 108.4, 105.2, 54.4; LRMS (EI) m/z 290 ($M + H^+$); HRMS (ESI+): m/z for $C_{18}H_{16}N_3O$ $(M + H)^+$, calcd 290.1288, found 290.1284.

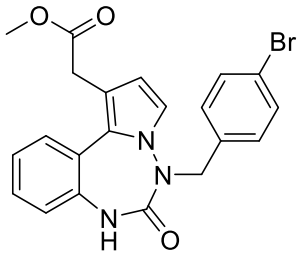


5-(4-Bromobenzyl)-5H-pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one (**2.7b**) was prepared using the procedure described for the synthesis of pyrrolobenzotriazepin-6-one **2.7a** from ketoaldehyde **2.22b** (80 mg, 0.141 mmol, 1.0 eq.), and isolated by chromatography on silica gel (20% EtOAc in hexanes) as white solid (35 mg, 67 % yield): m.p. 182-186°C; R_f 0.28 (20% EtOAc in hexanes); IR 680, 752, 1677, 3234

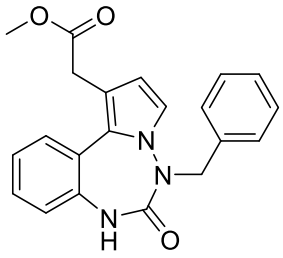
cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.34 – 7.27 (m, 2H), 7.22 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.16 (td, *J* = 7.4, 1.4 Hz, 1H), 7.01 – 6.94 (m, 2H), 6.91 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.77 (dd, *J* = 3.0, 1.8 Hz, 1H), 6.54 (s, 1H), 6.18 (dd, *J* = 4.0, 3.0 Hz, 1H), 6.10 (dd, *J* = 4.0, 1.8 Hz, 1H), 4.88 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 136.6, 134.6, 131.7, 131.0, 129.5, 128.2, 126.8, 125.2, 123.1, 122.0, 120.6, 117.6, 108.6, 105.4, 53.8; LRMS (EI) m/z 368 ($M + H^+$); HRMS (ESI+): m/z for $C_{18}H_{15}BrN_3O$ $(M + H)^+$, calcd 368.0393, found 368.0394.



5-(4-Bromobenzyl)-5H-(5-chloro-3-carboxymethyl)pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one (**2.7c**) was prepared using the procedure described for the synthesis of pyrrolobenzotriazepin-6-one **2.7a**, from ketoaldehyde **2.22c** (60 mg, 0.094 mmol, 1.0 eq.), and isolated by preparative thin layer chromatography (50% EtOAc in hexanes) as white solid (10 mg, 23 % yield): m.p. 165-169 °C; R_f 0.33 (50% EtOAc in hexanes); IR 739, 1687, 3246 cm^{-1}; ^1H NMR (300 MHz, MeOD-d_4) δ 7.28 – 7.06 (m, 5H), 7.01 (dd, *J* = 7.9, 0.9 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.24 (s, 1H), 5.14 (d, *J* = 14.8 Hz, 1H), 4.49 (d, *J* = 14.8 Hz, 1H), 3.42 (d, *J* = 15.9 Hz, 1H), 3.25 (d, *J* = 15.9 Hz, 1H); ^{13}C NMR (75 MHz, MeOD-d_4) δ 174.6 (HSQC), 164.9, 139.2, 135.5, 132.4, 131.5, 130.0, 129.4, 128.4, 125.3, 123.2, 122.8, 122.1, 115.4, 113.2, 109.8, 55.2, 31.8 (HSQC); LRMS (EI) m/z 460 ($M + H^+$); HRMS (ESI+): m/z for $C_{20}H_{16}BrClN_3O_3$ $(M + H)^+$, calcd 462.0037, found 462.0044.



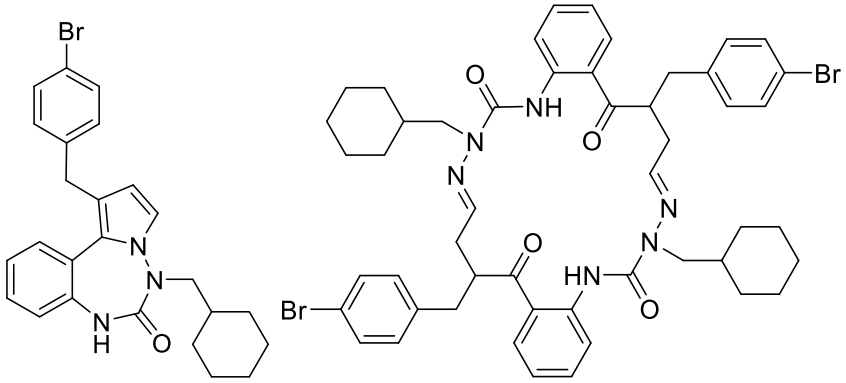
7d



7f

5-(4-Bromobenzyl)-5H-3-(methyl acetate)pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one (**2.7d**) and **5-(benzyl)-5H-3-(methyl acetate)pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one** (**2.7f**). Ketoaldehyde **2.22c** (40 mg, 0.062 mmol, 1.0 eq.) was dissolved in MeOH (4 mL) and treated with HCl (1.0 N, 4 mL) in a tube that was sealed, heated to 60°C and stirred for 2.5h in the dark. The volatiles were evaporated. The resulting aqueous phase was diluted with water (25 mL) and extracted with EtOAc (3 x 25 mL). The organic phases were combined, dried ($MgSO_4$), filtered and evaporated. The residue was purified by preparative thin layer chromatography (40% EtOAc in hexanes). First to elute was pyrrolobenzotriazepin-6-one **2.7f** as yellow oil (5 mg, 22%): R_f 0.45 (40% EtOAc in hexanes); IR 696, 751, 1686, 1732, 3244 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.26 (dd, *J* = 6.7, 1.7 Hz, 1H), 7.24 – 7.18 (m, 4H), 7.12 (dd, *J* = 6.7, 3.0 Hz, 2H), 6.97 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.76 (d, *J* = 3.1 Hz, 1H), 6.27 (s, 1H), 6.20 (d, *J* = 3.1 Hz, 1H), 5.06 (d, *J* = 15.2 Hz, 1H), 4.78 (d, *J* = 15.3 Hz, 1H), 3.70 (s, 3H), 3.55 (d, *J* = 15.7 Hz, 1H), 3.43 (d, *J* = 15.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 163.0, 138.1, 135.6, 128.6, 128.2, 128.0 (2C), 127.9, 127.8, 125.1, 122.4, 120.8, 117.1, 111.2, 109.6, 54.2, 52.1, 32.7; LRMS (EI) m/z 362 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{20}N_3O_3$ $(M + H)^+$, calcd 362.1499, found 362.1505. Second to elute was pyrrolobenzotriazepin-6-one **2.7d** as yellow oil (14 mg, 52 % yield): R_f 0.43 (40% EtOAc in hexanes); IR 645, 755, 1686, 1732, 3259 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (dd, *J* = 7.0, 2.3 Hz, 1H), 7.33 – 7.20 (m, 4H), 7.02 – 6.89 (m, 3H), 6.73 (d, *J* = 3.1 Hz, 1H), 6.25 (s, 1H), 6.19 (d, *J* = 3.1 Hz, 1H), 5.00 (d, *J* = 15.4 Hz, 1H), 4.67 (d, *J* = 15.3 Hz, 1H), 3.69 (s, 3H), 3.54 (d, *J* = 20.9 Hz, 1H), 3.40 (d, *J* = 15.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 162.9, 137.9, 134.6,

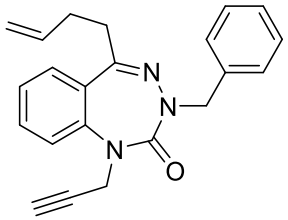
131.7, 129.7, 128.3, 128.0 (2C), 127.8, 125.2, 122.0, 120.9, 116.8, 111.4, 109.8, 53.5, 52.2, 32.7; LRMS (EI) m/z 440 ($M + H^+$); HRMS (ESI+): m/z for $C_{21}H_{19}BrN_3O_3$ $(M + H)^+$, calcd 440.0604, found 440.0622.



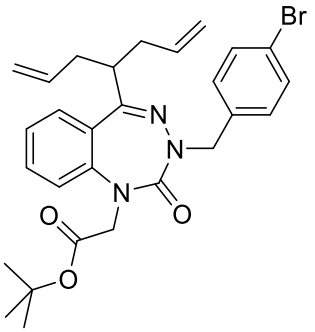
5-(Cyclohexylmethyl)-5H-benzo-(3-(4-bromobenzyl))pyrrolo[1,2][1,3,4]triazepin-6(7H)-one (**2.7e**) and **imine dimer 2.21** were prepared using the procedure described for the synthesis of pyrrolobenzotriazepin-6-one **2.7a**, from ketoaldehyde **2.22d** (90 mg, 0.135 mmol, 1.0 eq.), and isolated isolated by chromatography on silica gel. First to elute (10% EtOAc in hexanes) was imine dimer **2.21** as colorless oil (15 mg, 23 % yield): R_f 0.63 (30% EtOAc in hexanes); IR 751, 1510, 1657, 1691, 3247 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.85 (s, 2H), 8.69 (d, *J* = 8.1 Hz, 2H), 7.52 – 7.32 (m, 8H), 7.19 (d, *J* = 8.4 Hz, 4H), 6.90 (td, *J* = 7.4, 1.0 Hz, 2H), 6.50 (dd, *J* = 9.1, 2.6 Hz, 2H), 4.13 – 3.99 (m, 2H), 3.46 – 3.27 (m, 4H), 3.18 (td, *J* = 12.5, 9.3 Hz, 2H), 3.01 (dd, *J* = 14.9, 7.1 Hz, 2H), 2.90 (dd, *J* = 13.7, 5.9 Hz, 2H), 2.72 (dt, *J* = 13.1, 3.1 Hz, 2H), 1.57 – 1.40 (m, 6H), 1.40 – 1.15 (m, 4H), 1.15 – 0.66 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.9, 153.5, 140.8, 138.5, 135.4, 134.6, 132.0, 131.0, 129.6, 123.9, 120.69, 120.66, 119.6, 48.1, 46.2, 38.9, 38.7, 34.6, 30.8, 30.6, 26.2, 25.7, 25.6; LRMS (EI) m/z 987 ($M + Na^+$); HRMS (ESI+): m/z for $C_{50}H_{56}Br_2N_6NaO_4$ $(M + Na)^+$, calcd 987.2622, found 987.2587. Next to elute (20% EtOAc in hexanes) was triazepinone **2.7e** as white solid (19 mg, 30 % yield): m.p. 165-169 °C; R_f 0.50 (30% EtOAc in hexanes); IR 762, 1678, 3213 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45 – 7.35 (m, 2H), 7.29 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.22 (td, *J* = 7.7, 1.7 Hz, 1H), 7.11 (td, *J* = 7.5, 1.3 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 2H), 6.94 (dd, *J* = 7.9, 1.1 Hz, 1H), 6.86 (d, *J* = 3.1 Hz,

1H), 6.29 (s, 1H), 6.06 (d, *J* = 3.1 Hz, 1H), 4.00 – 3.89 (m, 2H), 3.79 (d, *J* = 16.4 Hz, 1H), 3.37 (dd, *J* = 14.0, 4.6 Hz, 1H), 1.75 – 1.40 (m, 3H), 1.41 – 1.14 (m, 2H), 1.14 – 0.73 (m, 5H), 0.64 (ddd, *J* = 15.2, 12.2, 3.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.3, 140.9, 138.5, 131.6, 130.3, 127.9, 127.6, 127.3, 124.8, 122.8, 120.8, 119.8, 116.9, 116.8, 109.7, 55.5, 35.5, 32.2, 30.5, 30.3, 26.4, 25.9, 25.6; LRMS (EI) m/z 464 ($M + H^+$); HRMS (ESI+): m/z for $C_{25}H_{27}BrN_3O$ $(M + H)^+$, calcd 464.1332, found 464,1336.

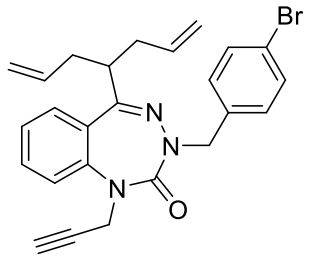
Synthesis of N-alkylated triazepinones 2.19 and 2.23.



3-Benzyl-5-(but-3-enyl)-1-(prop-2-ynyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.19f**). In a flame dried round bottom flask under argon, benzotriazepinone **2.6f** (44 mg, 0.14 mmol, 1.0 eq.), with *t*-BuOK (24 mg, 0.22 mmol, 1.5 eq.) were dissolved in THF (5 mL) at 0°C, stirred for 30 min, and treated with propargyl bromide (80% in toluene, 0.025 mL, 0.22 mmol, 1.5 eq.) at 0°C. The ice bath was removed and the reaction mixture was stirred for 3h at room temperature, diluted with AcOEt (20 mL), washed with water (2 x 20 mL), dried with $MgSO_4$, filtered and evaporated. The residue was purified by silica gel chromatography (20% EtOAc in hexanes) and **2.19f** was isolated as colorless oil (41 mg, 85% yield): R_f 0.39 (20% EtOAc in hexanes); IR 670, 1670, 3291 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 – 7.43 (m, 2H), 7.32 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.25 – 7.11 (m, 6H), 5.73 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.02 (br s, 1H), 4.99 – 4.87 (m, 2H), 4.53 (br s, 1H), 4.43 (d, *J* = 2.3 Hz, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.31 – 2.19 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 163.4, 144.1, 138.0, 137.2, 131.3, 129.7, 128.4, 128.2, 126.8, 126.5, 124.6, 120.2, 115.5, 79.6, 72.8, 54.7, 37.6, 35.2, 31.6; LRMS (EI) m/z 344 ($M + H^+$); HRMS (ESI+): m/z for $C_{22}H_{22}N_3O$ $(M + H)^+$, calcd 344.1757, found 344.1767.

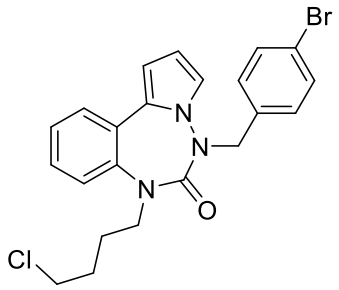


3-(4-Bromobenzyl)-5-(hepta-1,6-dien-4-yl)-1-(*tert*-butyl acetate)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.19g**) was prepared using the procedure described for the synthesis of **2.19f** employing a reaction time of 6h, benzotriazepin-2-one **2.6g** (20 mg, 0.05 mmol, 1.0 eq.), *t*-BuOK (7 mg, 0.06 mmol, 1.2 eq.), DMF (1 mL) and *tert*-butyl bromoacetate (0.009 mL, 0.06 mmol, 1.2 eq.). Benzotriazepinone **2.19g** was isolated by silica gel chromatography (10% EtOAc in hexanes) as white solid (27 mg, quantitative yield): m.p. 101-103 °C; R_f 0.55 (20% EtOAc in hexanes); IR 1227, 1667,1758 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (ddd, *J* = 8.3, 7.1, 1.8 Hz, 1H), 7.33 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.26 – 7.22 (m, 1H), 7.19 (dd, *J* = 7.1, 1.0 Hz, 1H), 7.13 (dd, *J* = 9.6, 1.4 Hz, 1H), 7.07 – 7.00 (m, 2H), 5.65 – 5.35 (br m, 2H), 4.94 – 4.79 (br m, 5H), 4.44 (d, *J* = 14.2 Hz, 1H), 4.18 (d, *J* = 4.9 Hz, 2H), 2.86 (qu, *J* = 6.9 Hz, 1H), 2.54 – 2.36 br (m, 1H), 2.36 – 2.13 (br m, 3H), 1.47 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 167.9, 163.6, 144.6, 137.2, 136.7, 135.6, 131.3, 131.2, 130.7, 130.6, 126.4, 124.6, 120.7, 120.3, 116.7, 116.3, 82.1, 54.4, 51.4, 45.2, 39.8, 36.2, 28.2; LRMS (EI) m/z 560 ($M + Na^+$); HRMS (ESI+): m/z for $C_{28}H_{33}BrN_3O_3$ $(M + H)^+$, calcd 538.1700, found 538.1708.



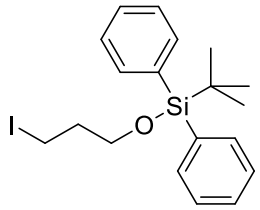
3-(4-Bromobenzyl)-5-(hepta-1,6-dien-4-yl)-1-(prop-2-ynyl)-1H-1,3,4-benzotriazepin-2(3H)-one (**2.19h**) was prepared using the procedure described for the synthesis of **2.19f** employing a reaction time of 6h, benzotriazepin-2-one **2.6g** (20 mg, 0.05 mmol, 1.0 eq.), *t*-BuOK (7 mg, 0.06 mmol, 1.2 eq.), DMF (1 mL) and

propargyl bromide (80% in toluene, 0.007 mL, 0.06 mmol, 1.2 eq.). Benzotriazepinone **2.19h** was isolated by silica gel chromatography (10% EtOAc in hexanes) as colorless oil (23 mg, quantitative yield): R_f 0.51 (20% EtOAc in hexanes); IR 1671, 3294 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 – 7.40 (m, 2H), 7.36 – 7.29 (m, 2H), 7.28 – 7.17 (m, 2H), 7.06 – 6.99 (m, 2H), 5.64 – 5.35 (br m, 2H), 4.96 – 4.80 (br m, 5H), 4.45 (d, *J* = 14.9 Hz, 1H), 4.40 (dd, *J* = 4.2, 2.5 Hz, 1H), 2.86 (qu, *J* = 6.8 Hz, 1H), 2.51 – 2.36 (br m, 1H), 2.27 (t, *J* = 2.5 Hz, 1H), 2.25 – 2.11 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 163.5, 144.0, 137.1, 136.6, 135.3, 131.3, 131.1, 130.8, 130.6, 126.5, 124.7, 120.8, 120.0, 116.9, 116.4, 79.5, 72.8, 54.3, 45.3, 39.7, 37.5, 36.1; LRMS (EI) m/z 462 (M + H^+); HRMS (ESI+): m/z for $C_{25}H_{24}BrN_3O$ $(M + H)^+$, calcd 462.1176, found 462.1189.



7-(4-Chlorobutyl)-5-(4-bromobenzyl)-5H-pyrrolo[1,2][1,3,4]benzotriazepin-6(7H)-one (**2.23**) was prepared using the procedure described for the synthesis of **2.19f** by alkylation of pyrrolobenzotriazepin-6-one **2.7b** (20 mg, 0.045 mmol, 1.0 eq.) using *t*-BuOK (8 mg, 0.065 mmol, 1.2 eq.), DMF (1 mL) and 1-bromo-4-chlorobutane (0.008 mL, 0.065 mmol, 1.2 eq.) over 6h. Triazepinone **2.23** was isolated by silica gel chromatography (10-20% EtOAc in hexanes) as colorless oil (21 mg, 84% yield): R_f 0.38 (20% EtOAc in hexanes); IR 751, 1678 cm^{-1}); ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.33 (td, *J* = 7.7, 1.9 Hz, 1H), 7.28 – 7.22 (m, 3H), 7.22 – 7.16 (m, 1H), 6.91 – 6.80 (m, 3H), 6.18 (dd, *J* = 4.0, 3.0 Hz, 1H), 6.14 (dd, *J* = 4.0, 1.7 Hz, 1H), 4.98 (d, *J* = 15.0 Hz, 1H), 4.56 (d, *J* = 15.0 Hz, 1H), 4.07 (dt, *J* = 13.7, 6.9 Hz, 1H), 3.51 (dt, *J* = 13.7, 6.0 Hz, 1H), 3.34 – 3.18 (m, 2H), 1.69 – 1.52 (m, 1H), 1.53 – 1.32 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.1, 140.1, 135.8 (HMBC), 134.6, 131.6, 130.8, 129.5, 128.0, 127.2, 125.9, 122.0, 121.8, 116.2, 108.1, 103.8, 52.9, 47.7, 44.4, 29.1, 24.8; LRMS (EI) m/z 458 (M + H^+); HRMS (ESI+): m/z for $C_{22}H_{22}BrClN_3O$ $(M + H)^+$, calcd 458.0634, found 458.0629.

Synthesis of (3-iodopropoxy)(*tert*-butyl)diphenylsilane (2.28).



(3-Iodopropoxy)(tert-butyl)diphenylsilane (2.28): Imidazole (1080 mg, 15.87 mmol, 3.0 eq.) was dissolved in DMF (10 mL), treated with 3-chloro-1-propanol (0.44 mL, 5.29 mmol, 1 eq.) followed by *tert*-butyl(chloro)diphenylsilane (1.69 mL, 6.34 mmol, 1.2 eq.). The reaction was stirred for 2 days at r.t., diluted with Et_2O (100 mL), washed with H_2O (3 x 100 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in acetone (10 mL), treated with NaI (4.0 g, 26.5 mmol, 5 eq.) and heated at reflux for 2 days. The volatile was evaporated, treated with H_2O (100 mL) and extracted with Et_2O (2 x 100 mL). The organic phases were combined, washed with H_2O (50 mL), dried ($MgSO_4$), filtered and evaporated to afford **2.28** as yellow oil (2.2 g, quantitative yield): Rf 0.41 (4% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 – 7.66 (m, 4H), 7.54 – 7.37 (m, 6H), 3.76 (t, J = 5.7 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 2.07 (tt, J = 6.8, 5.7 Hz, 2H), 1.11 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.7, 133.7, 129.8, 127.8, 63.3, 36.3, 27.0, 19.4, 3.6; LRMS (EI) m/z 425 ($M + H^+$); HRMS (ESI+): m/z for $C_{19}H_{26}IOSi$ $(M + H)^+$, calcd 425.0792, found 425.0797.

Synthesis of 1-(1-(2-aminophenyl)pent-4-enylidene)hydrazine (2.25).[3]

In a round-bottom flask with reflux condenser 2-pent-4-enoylaniline (**2.8**, 1.8 g, 10.4 mmol, 1.0 eq., prepared according to reference 15) was dissolved in EtOH (2 mL) and treated with aqueous hydrazine (2 mL). The reaction was refluxed 24h, cooled down, diluted with water (30 mL) and extracted with AcOEt (3x40 mL). The organic phases were combined, dried ($MgSO_4$), filtrated and evaportated. The residue was purified by silica gel chromatography using 30% ethyl acetate in hexanes as eluent. Evaporation of the collected fractions gave hydrazazone **2.25** as yellow oil (502 mg, 25% yield): R_f 0.25 and 0.50 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 2H), 7.57 (s, 2H), 7.38 – 7.27 (m, 2H), 7.10 (t, *J* = 7.1 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 5.82 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.04 – 4.94 (m, 2H), 2.83 – 2.74 (m, 2H), 2.29 (dd, *J* = 14.3, 7.6 Hz, 2H); LRMS (EI) m/z 190 ($M + H^+$).

Synthesis of (1H)-(3H)-5-(but-3-enyl)-1,3,4-benzotriazepin-2-one (2.24).[1] In a flame dried round-bottom flask, 4-nitrophenylchloroformate (117 mg, 0.58 mmol, 1.1 eq.) was dissolved in CH_2Cl_2 (10 mL), cooled to 0°C, treated drop-wise with a freshly prepared solution of hydrazone (**2.25**, g, 12.6 mmol, 1.1 eq.) in CH_2Cl_2 (10 mL) and stirred at 0°C for 1h, and DIEA (0.1 mL, 0.58 mmol, 1.1 eq.) was added drop-wise to the reaction mixture at 0°C. The ice bath was removed and the mixture was stirred 60h, diluted with water (40 mL). The organic phase was washed with water (3x40 mL). The aqueous phases were combined and extracted with DCM (25mL). The organic phases were combined, dried ($MgSO_4$), filtrated and evaporated. The residue was purified by silica gel chromatography using a gradient from 0% to 100% ethyl acetate in hexanes as eluent. Evaporation of the collected fractions gave benzotriazepinone **2.24** as pale yellow solid (24 mg, 20% yield): R_f 0.78 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 2H), 7.43 – 7.34 (m, 2H), 7.16 (t, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 5.86 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.07 – 4.99 (m, 2H), 2.87 – 2.79 (m, 2H), 2.39 – 2.29 (m, 2H); LRMS (EI) m/z 216 ($M + H^+$).

Synthesis of 2-(1-pent-4-enoyl)phenyl-1-phenylcarbamate (2.26).[1] In a flame dried round-bottom flask, 2-pent-4-enoylaniline **2.8** (100 mg, 0.57 mmol, 1.0 eq., prepared

[3] Pages ajoutées comme information soutenante du Chapitre 2

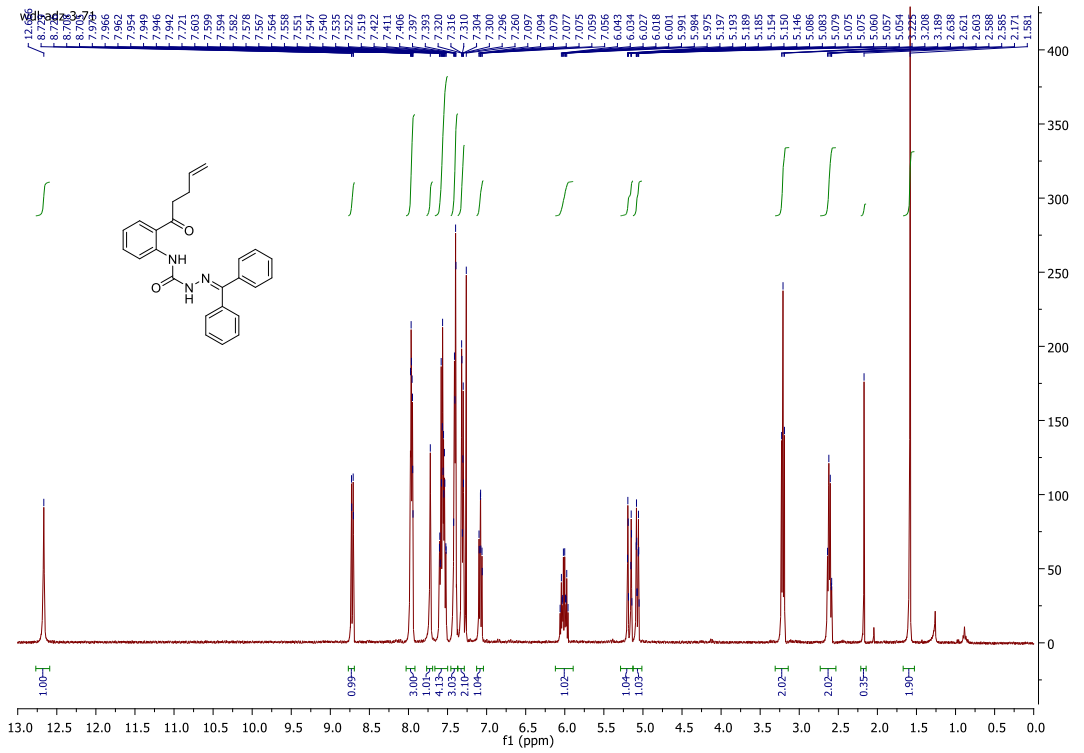
according to reference 15) was dissolved in CH_2Cl_2 (15 mL). The reaction was treated with DIEA (0.1 mL, 0.58 mmol, 1.1 eq.) and then phenylchloroformate (98 mg, 0.58 mmol, 1.1 eq.) was added drop-wise. The mixture was stirred 4h, washed with water (3x25 mL). The aqueous phases were combined and extracted with DCM (10mL). The organic phases were combined, dried ($MgSO_4$), filtrated and evaporated. The residue was purified by silica gel chromatography using a 5% ethyl acetate in hexanes as eluent. Evaporation of the collected fractions gave benzotriazepinone **2.25** as pale pink solid (180 mg, quantitative yield): R_f 0.54 (20% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.64 (s, 1H), 8.55 (d, *J* = 8.4 Hz, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.27 (dd, *J* = 15.3, 7.5 Hz, 3H), 7.16 (t, *J* = 7.6 Hz, 1H), 5.96 (ddt, *J* = 16.8, 10.2, 6.5 Hz, 1H), 5.16 (d, *J* = 17.2 Hz, 1H), 5.09 (d, *J* = 10.2 Hz, 1H), 3.21 (t, *J* = 7.3 Hz, 2H), 2.56 (dd, *J* = 14.0, 7.0 Hz, 2H); LRMS (EI) m/z 296 ($M + H^+$).

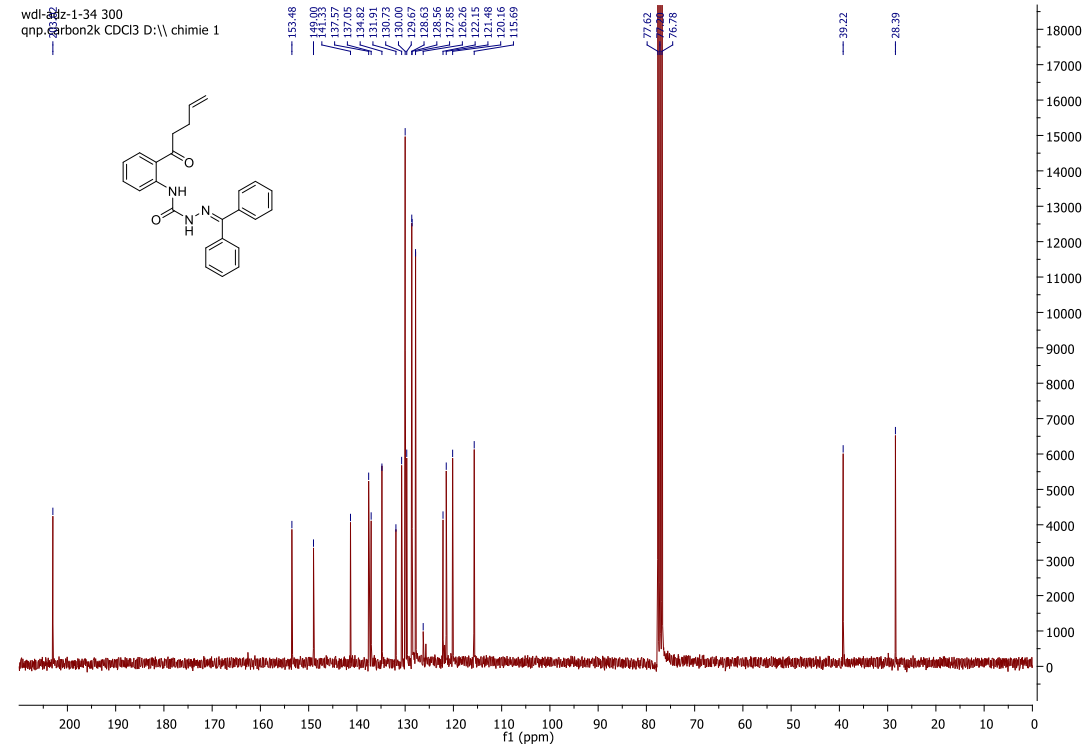
Synthesis of 2-(1-pent-4-enhydrazone)phenyl-1-semicarbazide 2.27.[4] 2-(1-pent-4-enoyl)phenyl-1-phenylcarbamate (**2.26**, 443 mg, 1.5 mmol, 1.0 eq.) was dissolved in THF (40mL), treated with aqueous hydrazine (0.5 mL) and DIEA (0.3 mL, 1.7 mmol, 1.1 eq.). The mixture was stirred 24h at rt and then refluxed 24h. The reaction was cooled down to 0°C and the resulting white precipitate was filtered, washed with cold hexane and dried under strong vaccum to afford **2.27** as white solid (282 mg, 76% yield): Rf 0.66 (10% MeOH in DCM); ^{1}H NMR (400 MHz, DMSO) δ 9.38 (s, 1H), 7.26 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.14 (t, *J* = 6.9 Hz, 1H), 6.90 (t, *J* = 7.0 Hz, 1H), 6.75 (d, *J* = 7.3 Hz, 1H), 5.65 (ddt, *J* = 16.6, 10.2, 6.3 Hz, 1H), 4.87 (dd, *J* = 17.3, 1.9 Hz, 1H), 4.83 (d, *J* = 10.3 Hz, 1H), 4.36 (s, 2H), 4.20 (s, 1H), 2.93 (s, 2H), 2.05 – 1.91 (m, 1H), 1.87 – 1.74 (m, 1H), 1.64 – 1.49 (m, 2H); LRMS (EI) m/z 216 ($M - N_2H_4 + H^+$).

[4] Pages ajoutées comme information soutenante du Chapitre 2

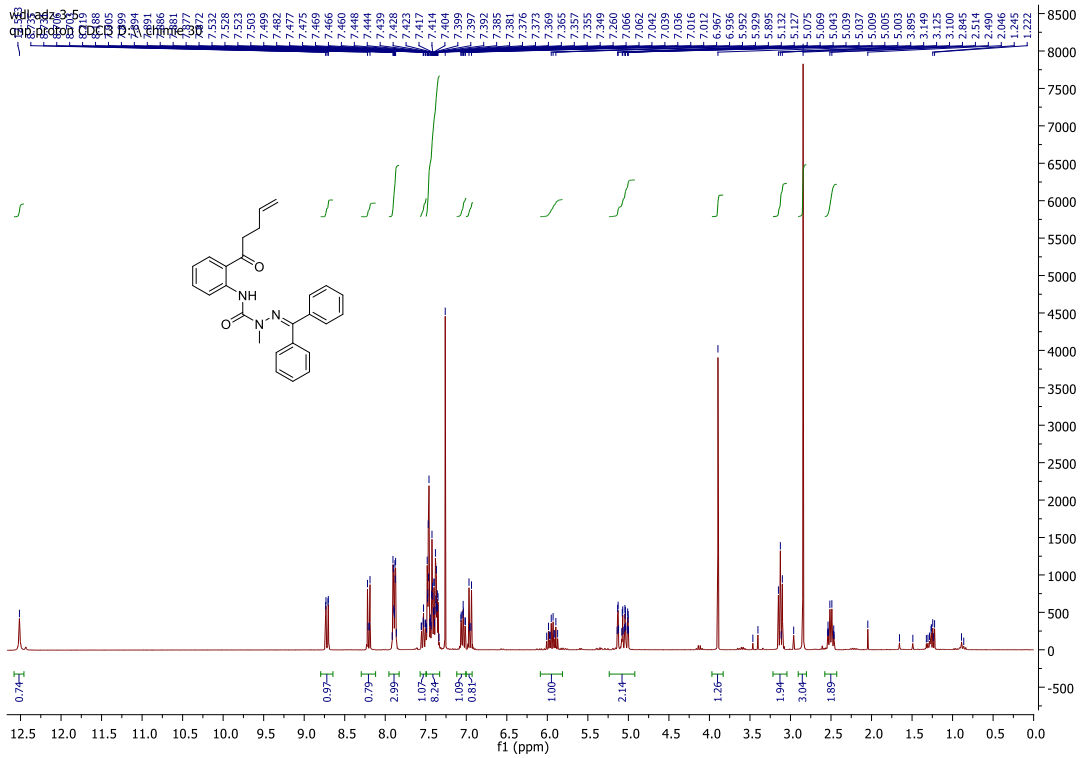
Copies of ^{1}H and ^{13}C NMR Spectra

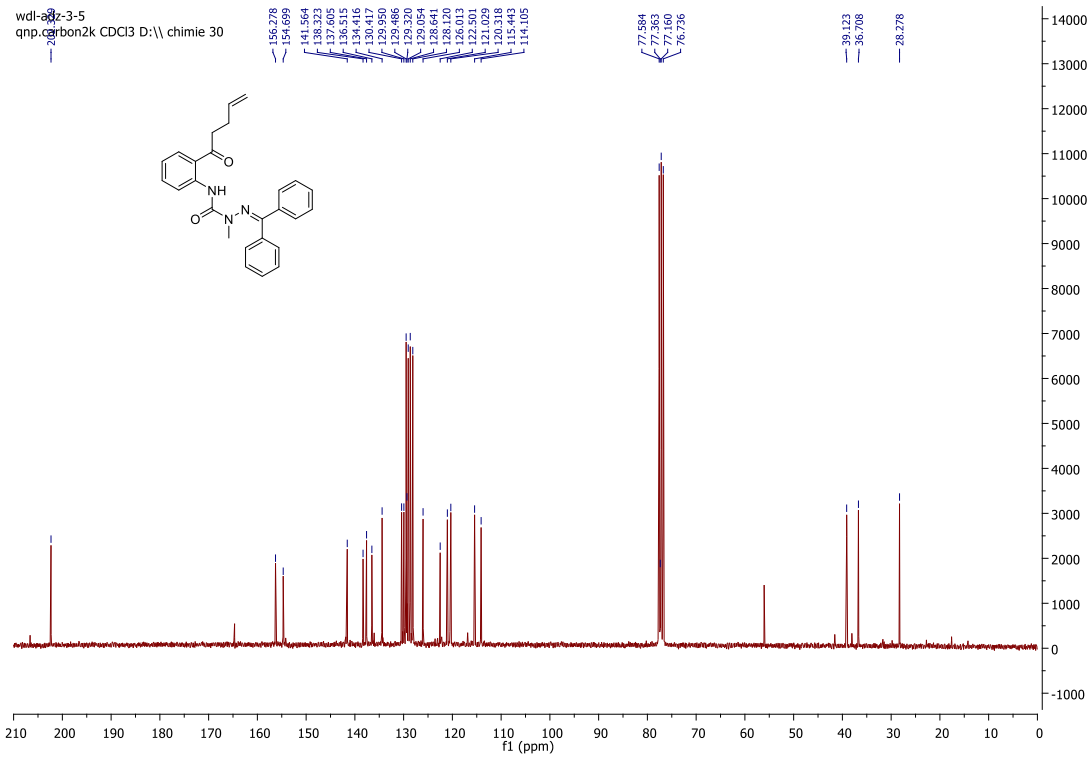
2.15, $CDCl_3$, ^{1}H (400 MHz), ^{13}C (75 MHz).



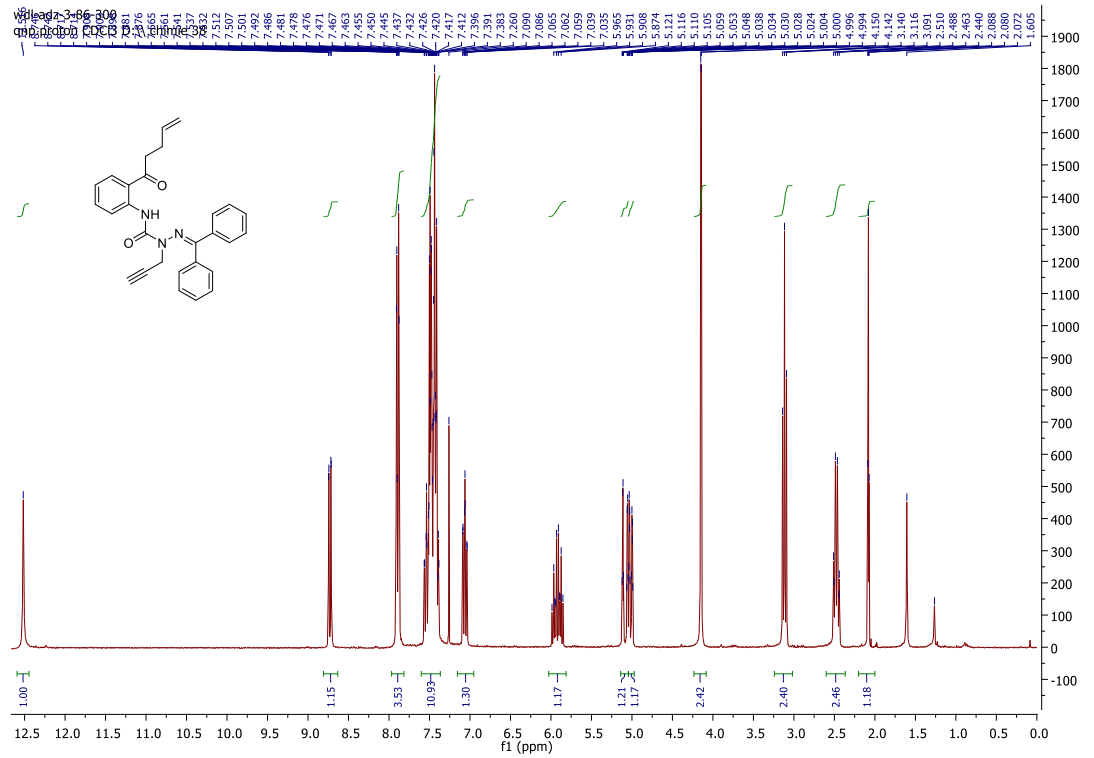


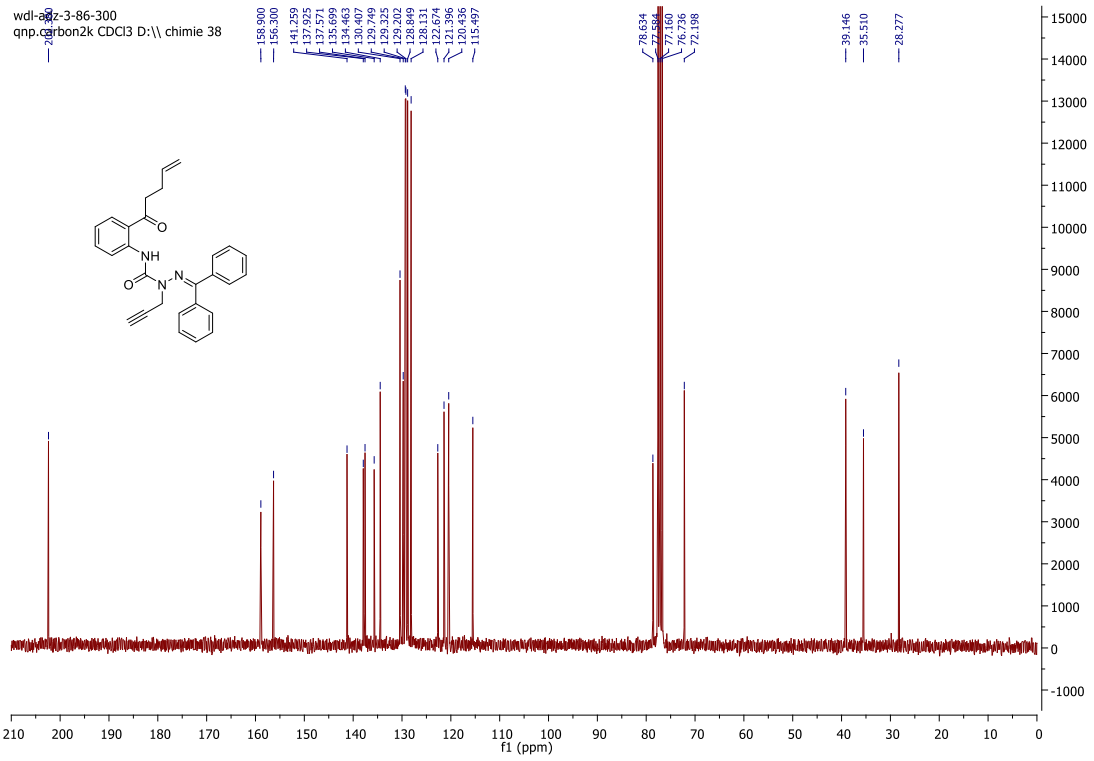
2.16a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



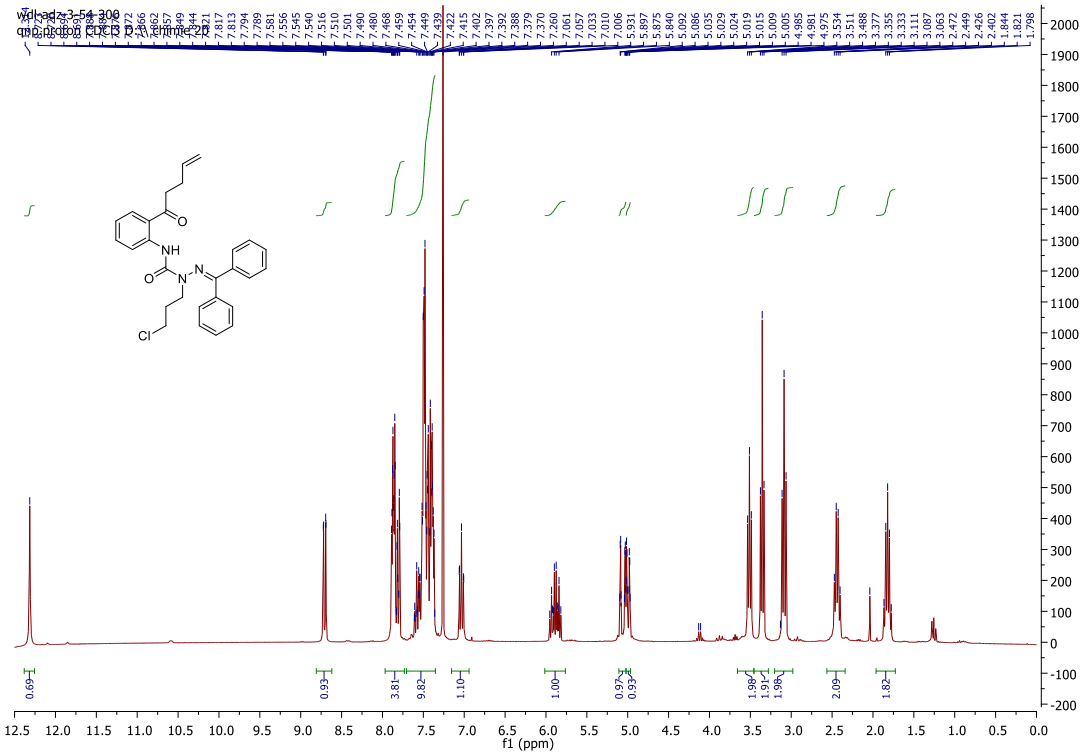


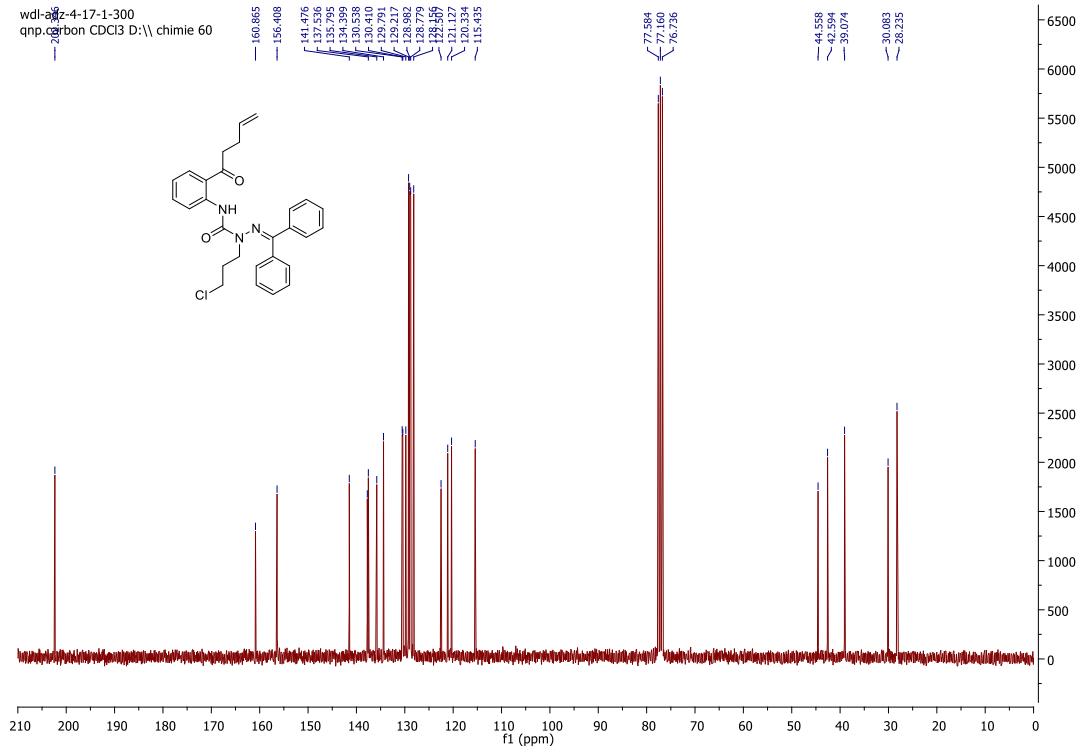
2.16b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



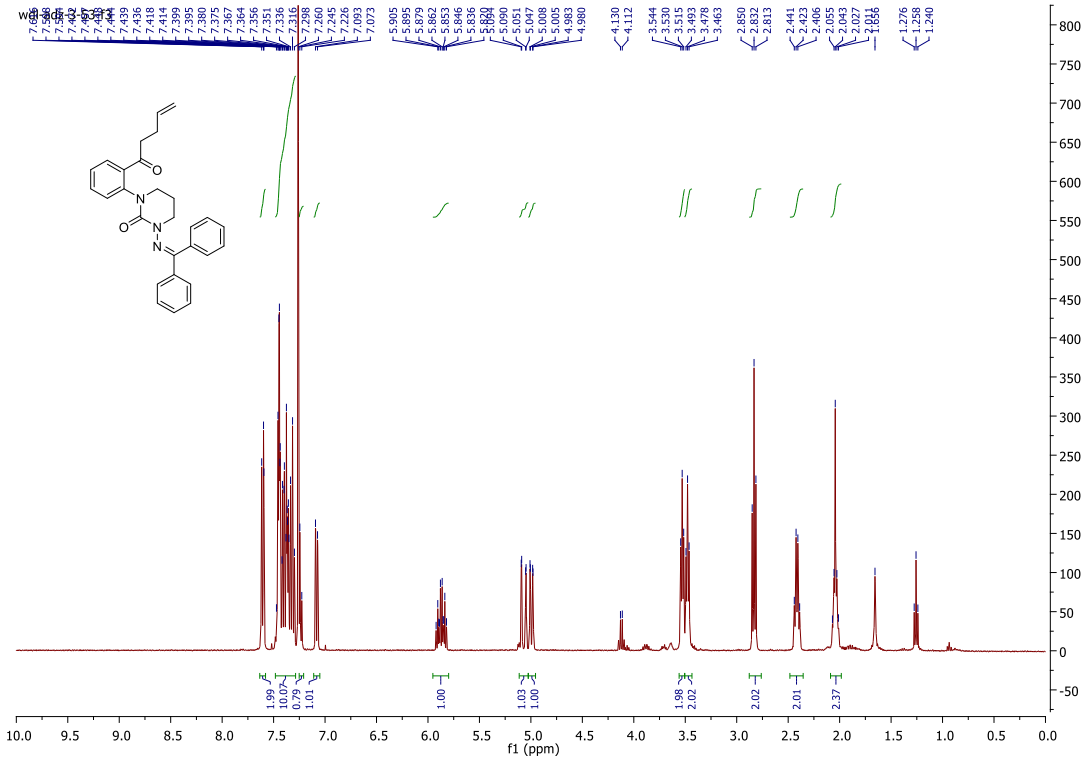


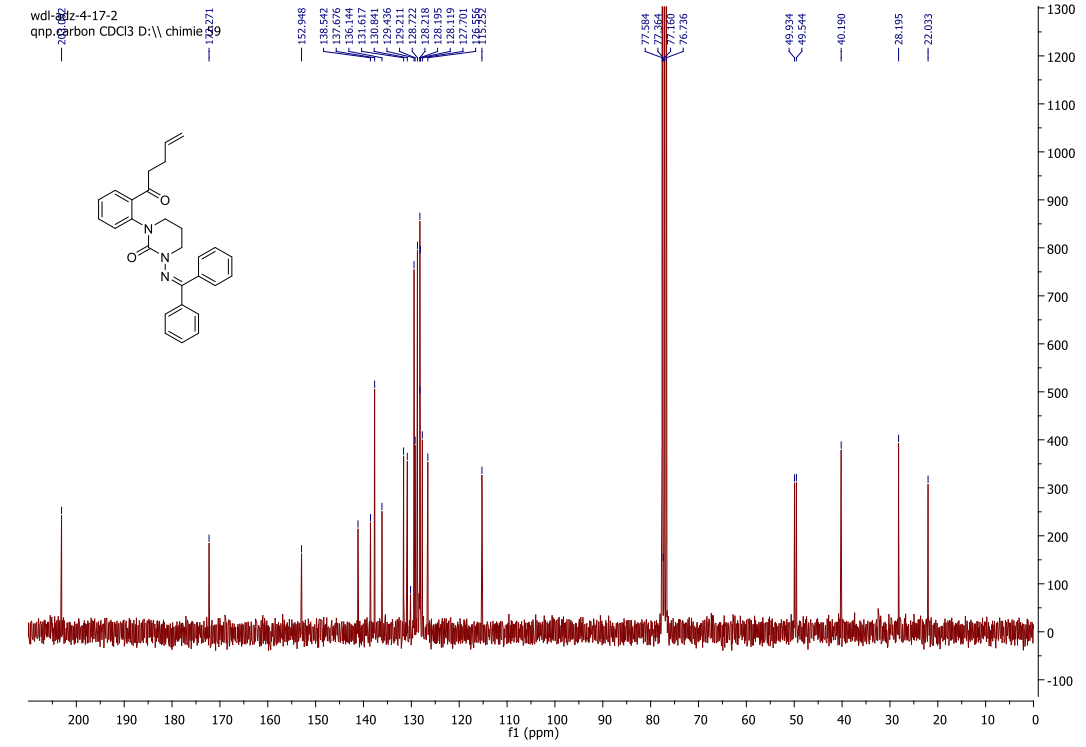
2.16c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



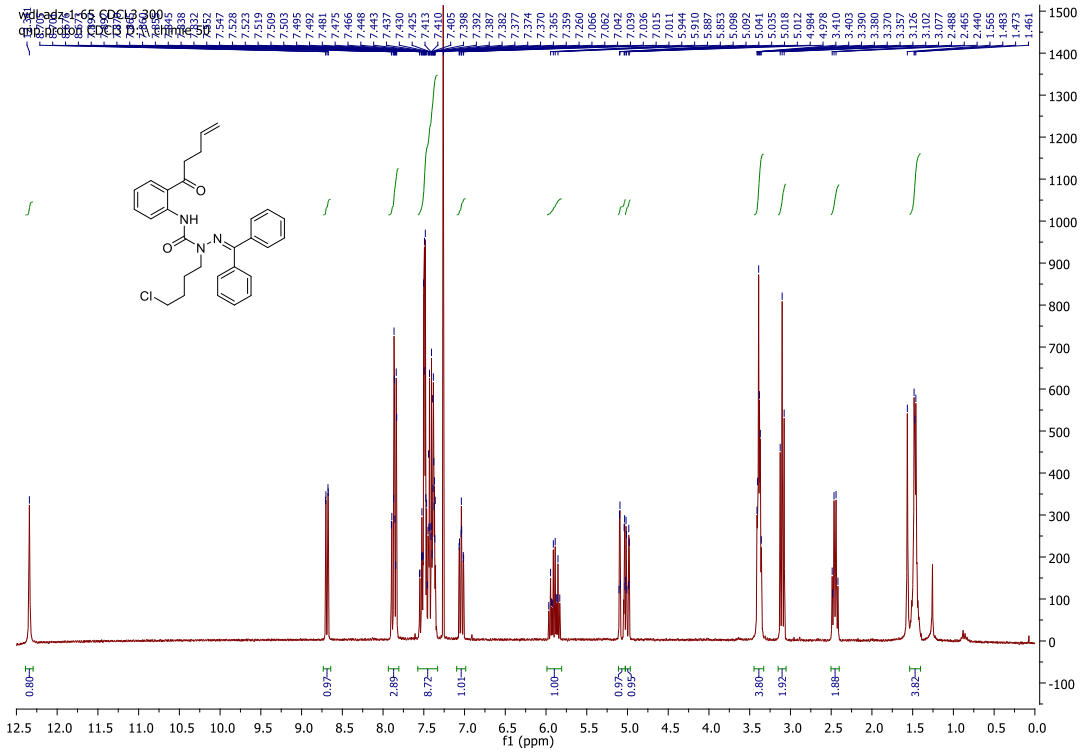


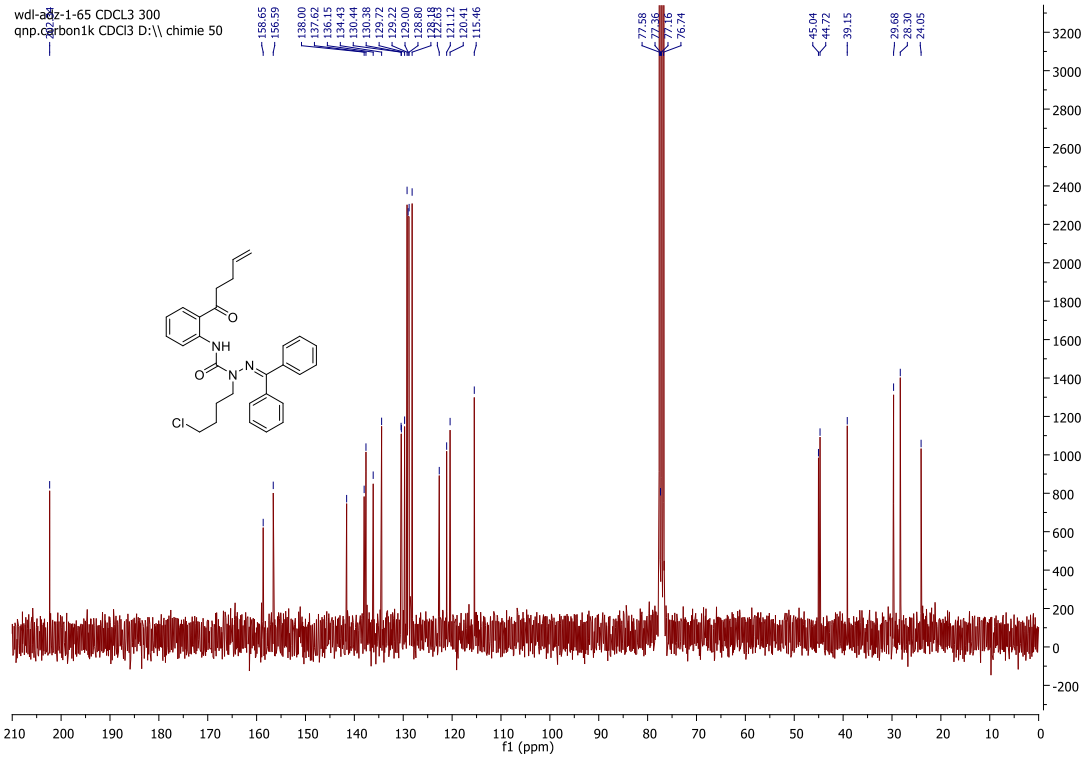
2.17c, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz).



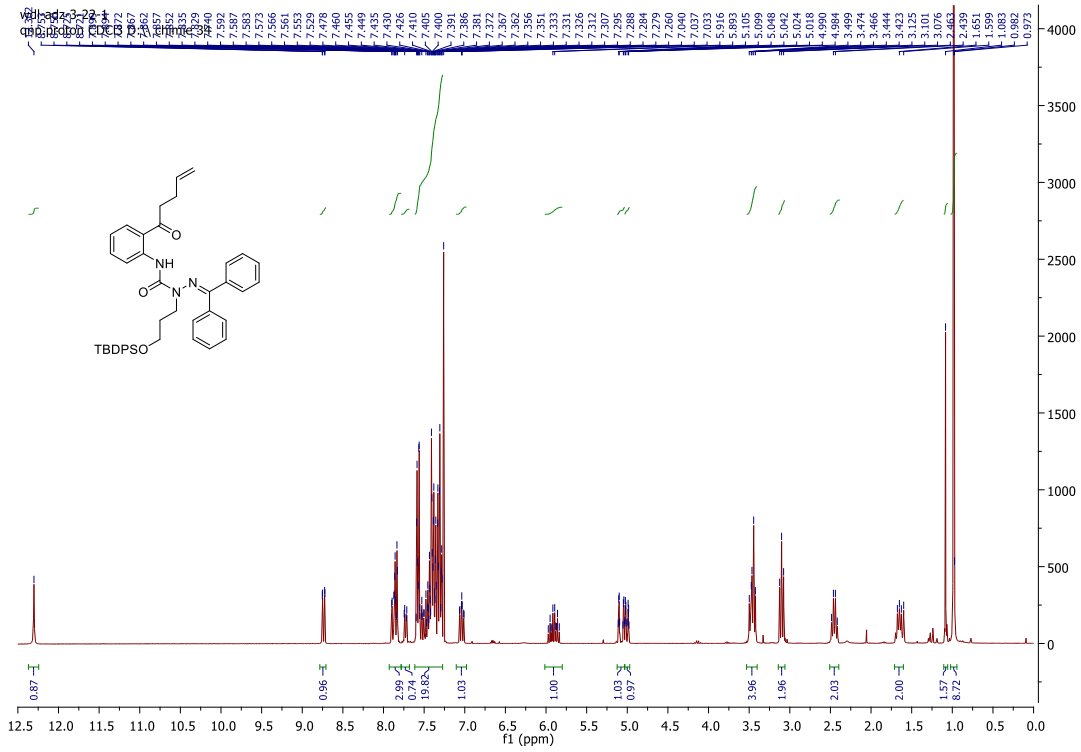


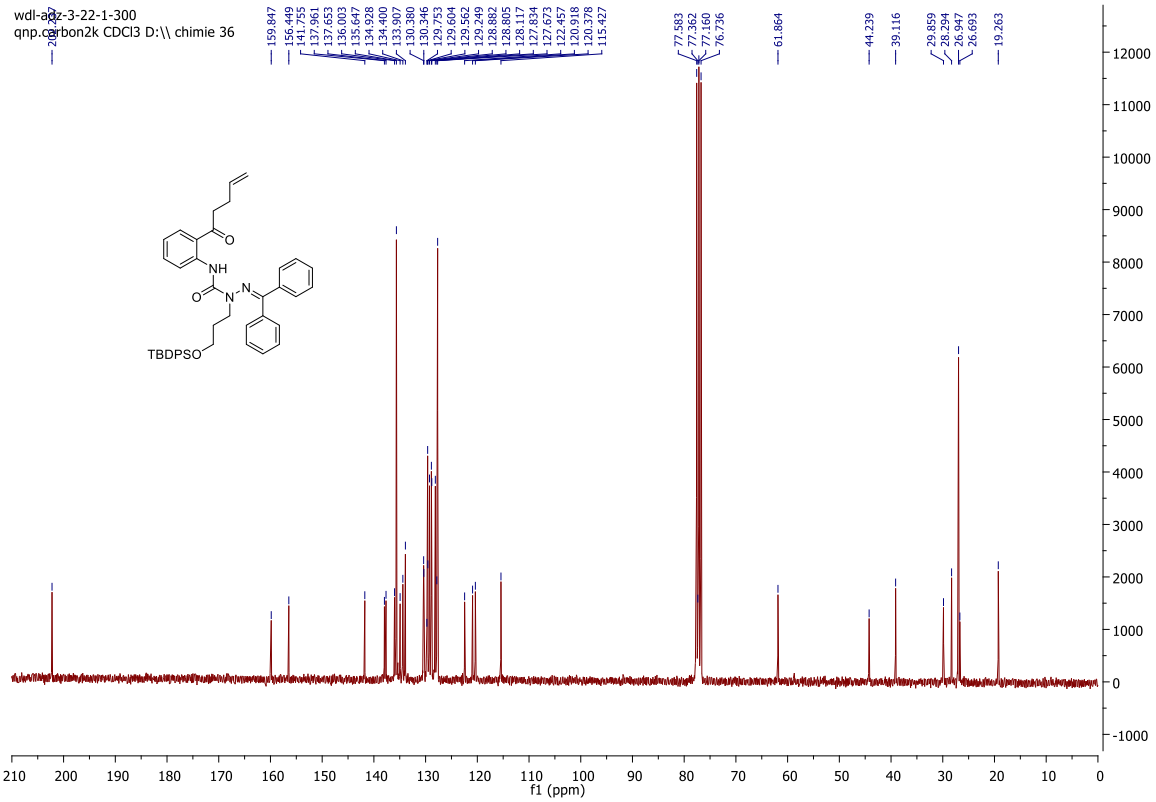
2.16d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



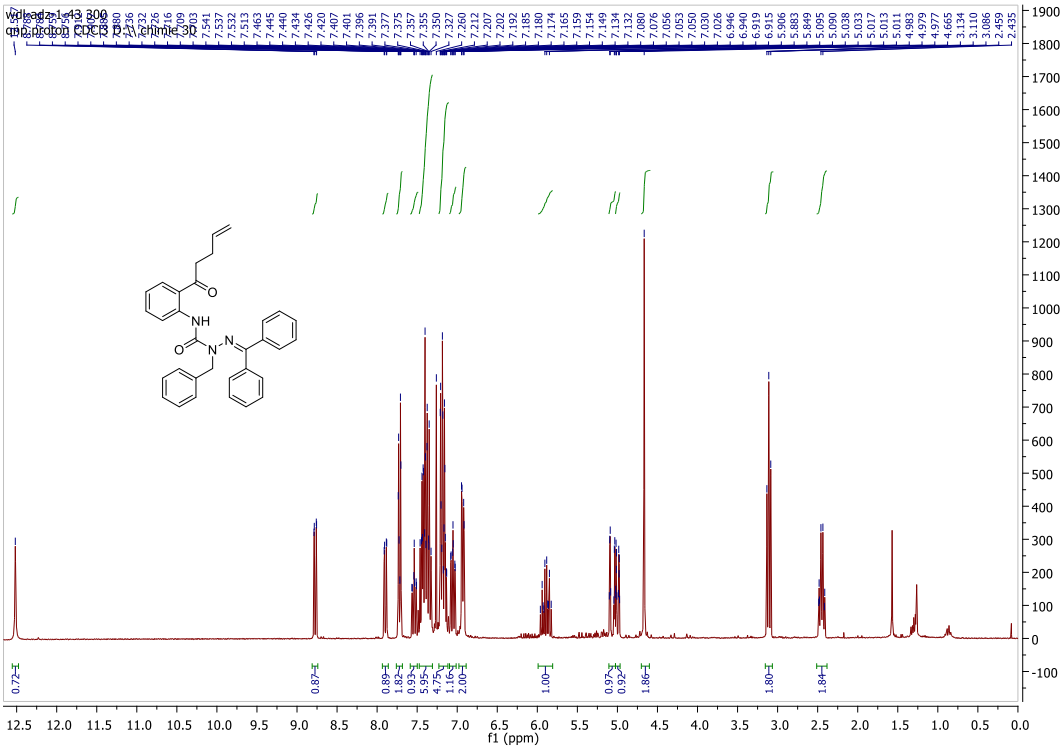


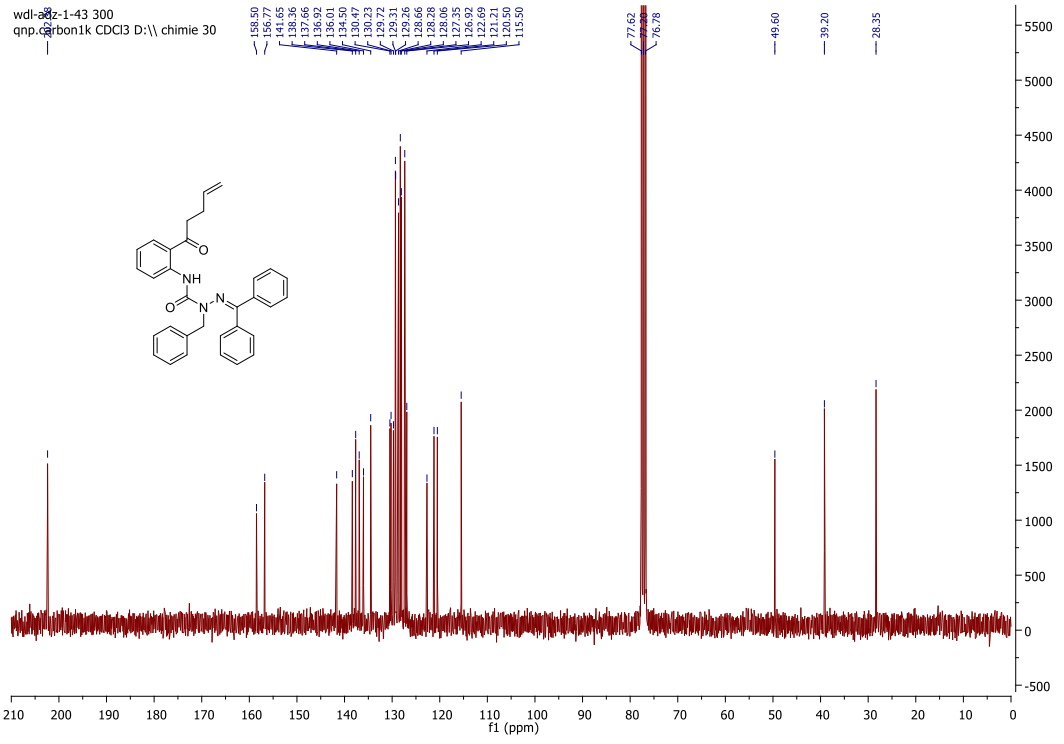
2.16e, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



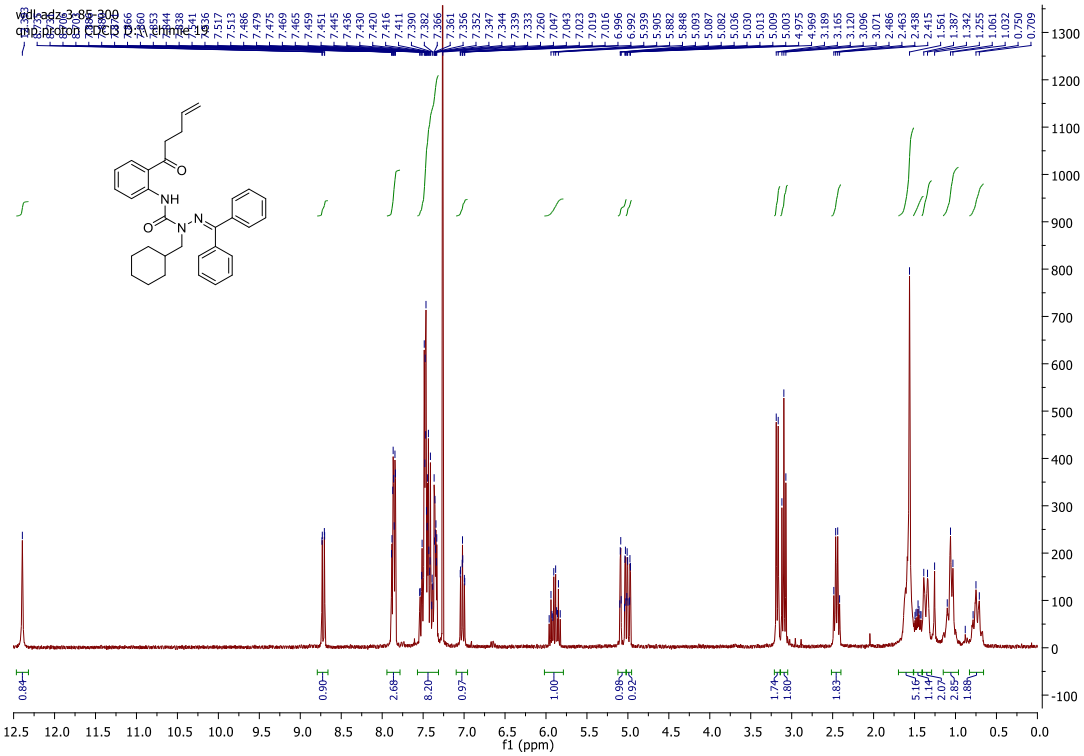


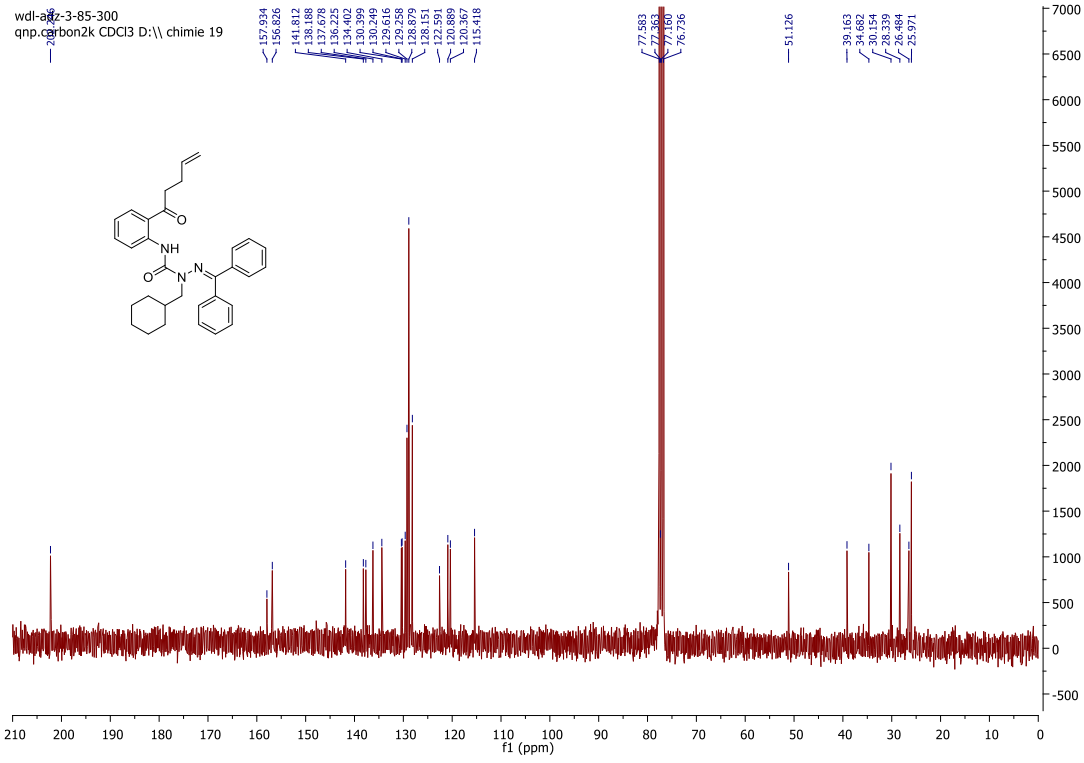
2.16f, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



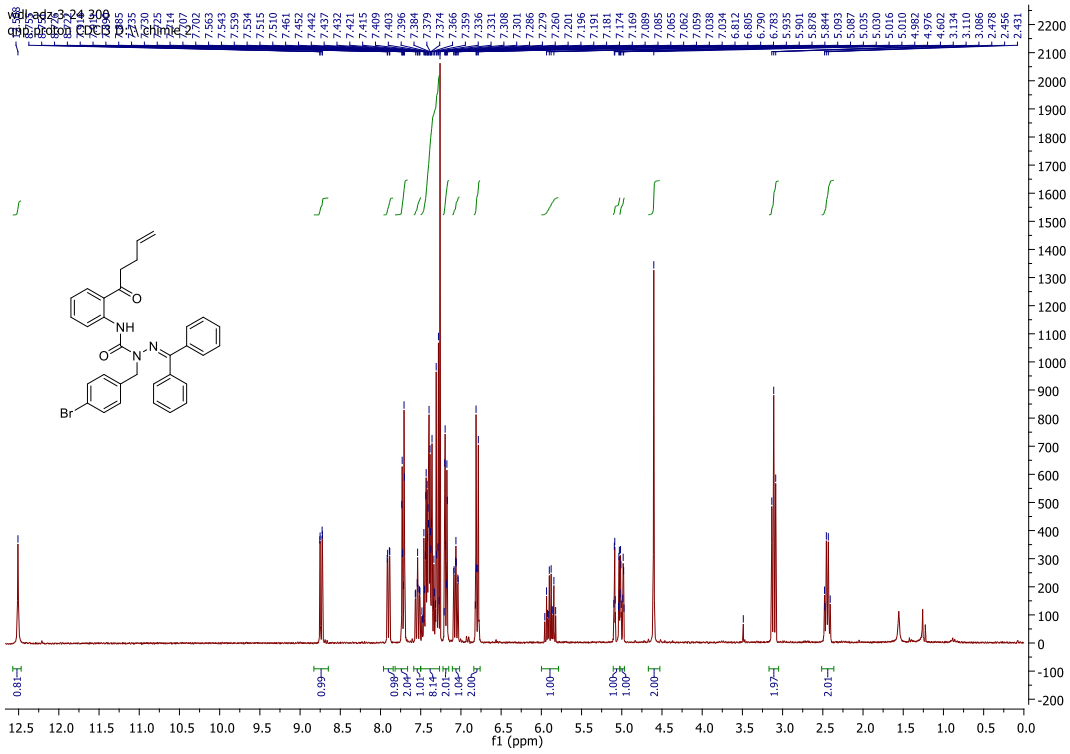


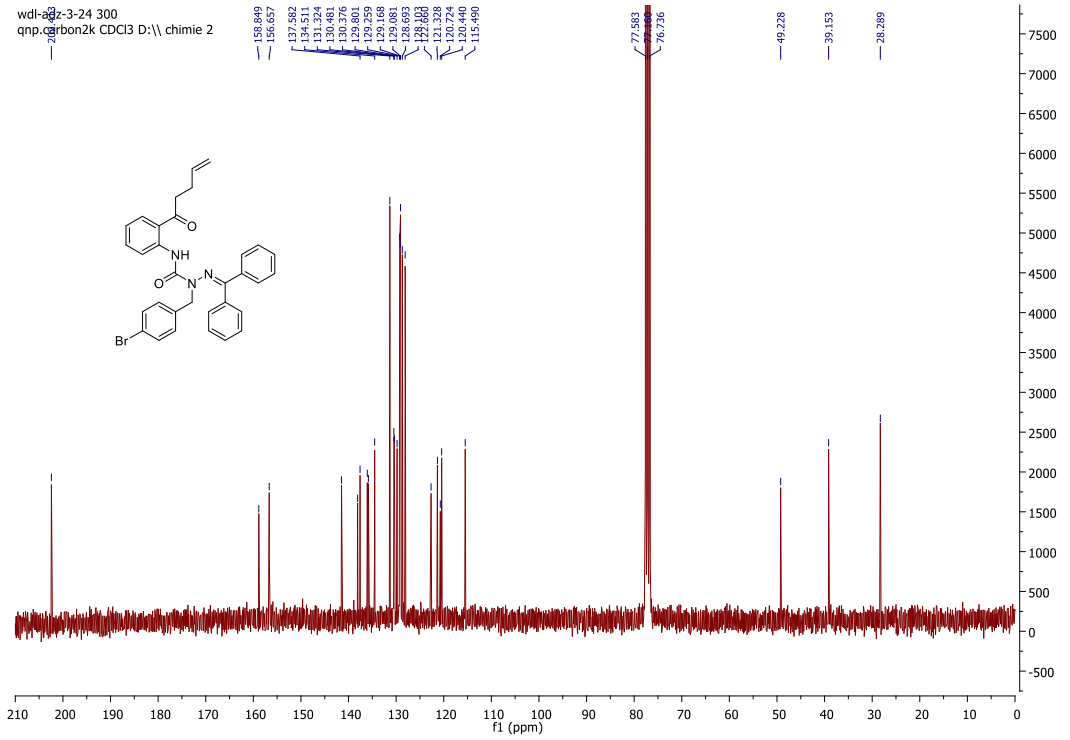
2.16g, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



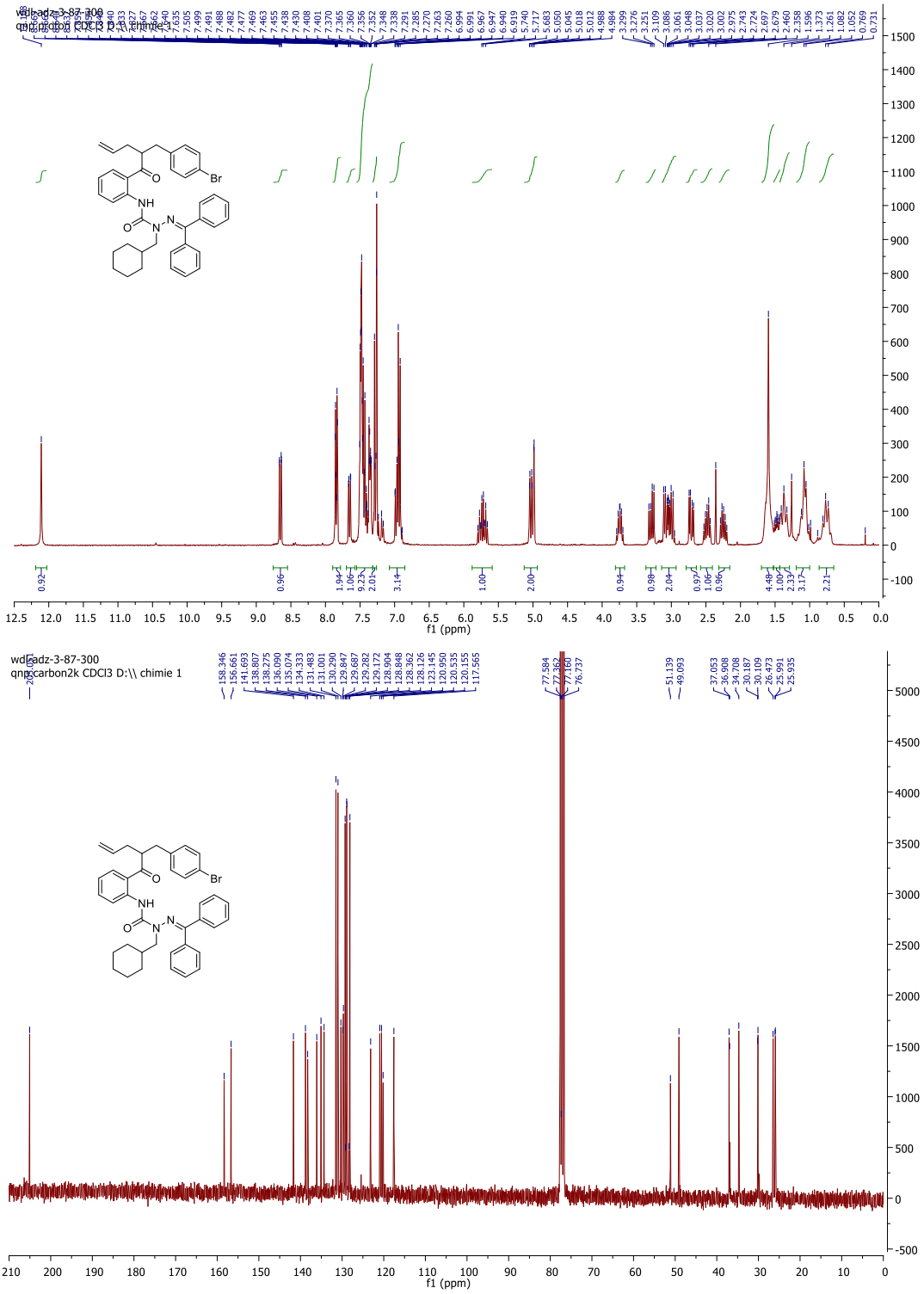


2.16h, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

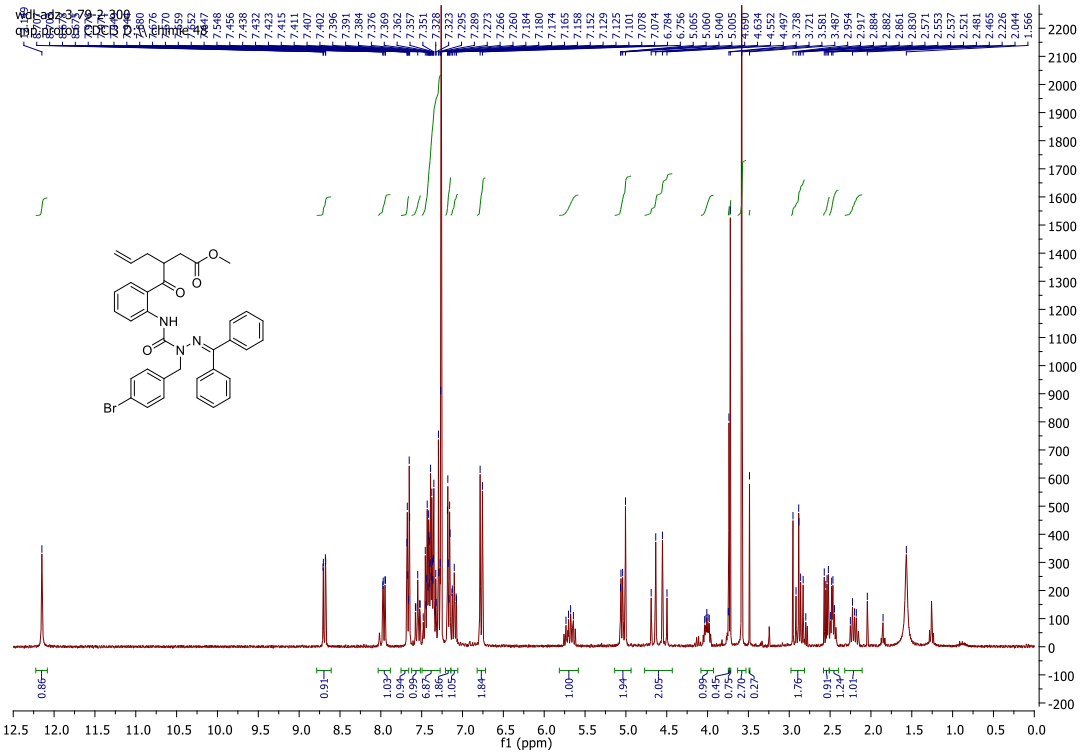


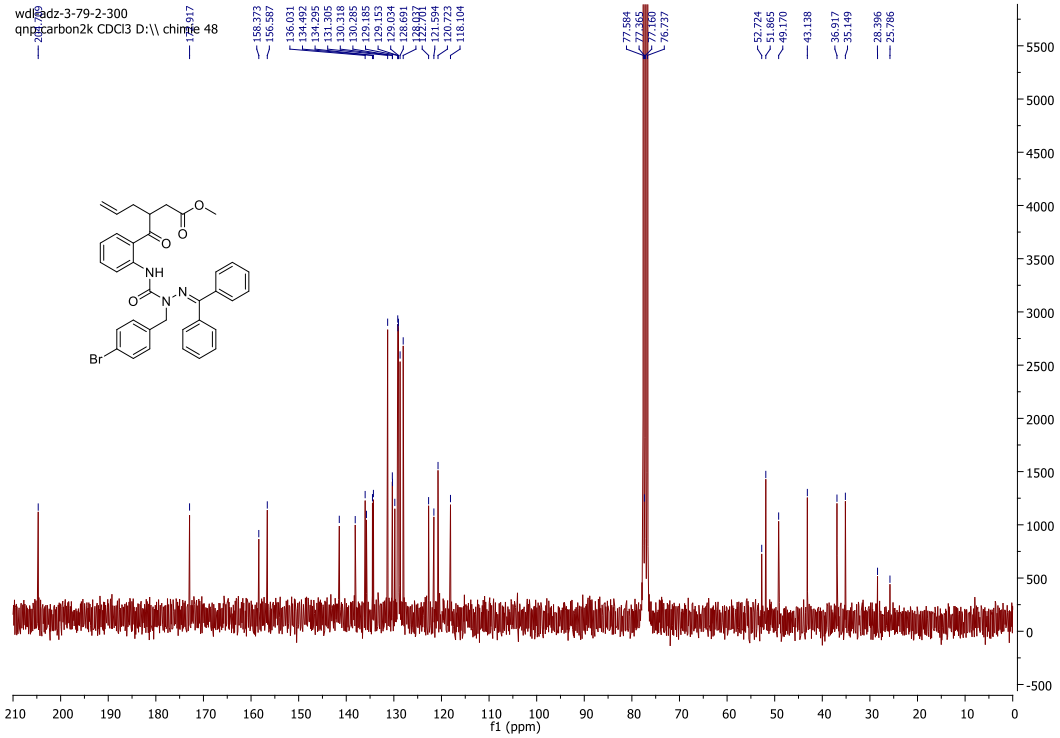


2.18g, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

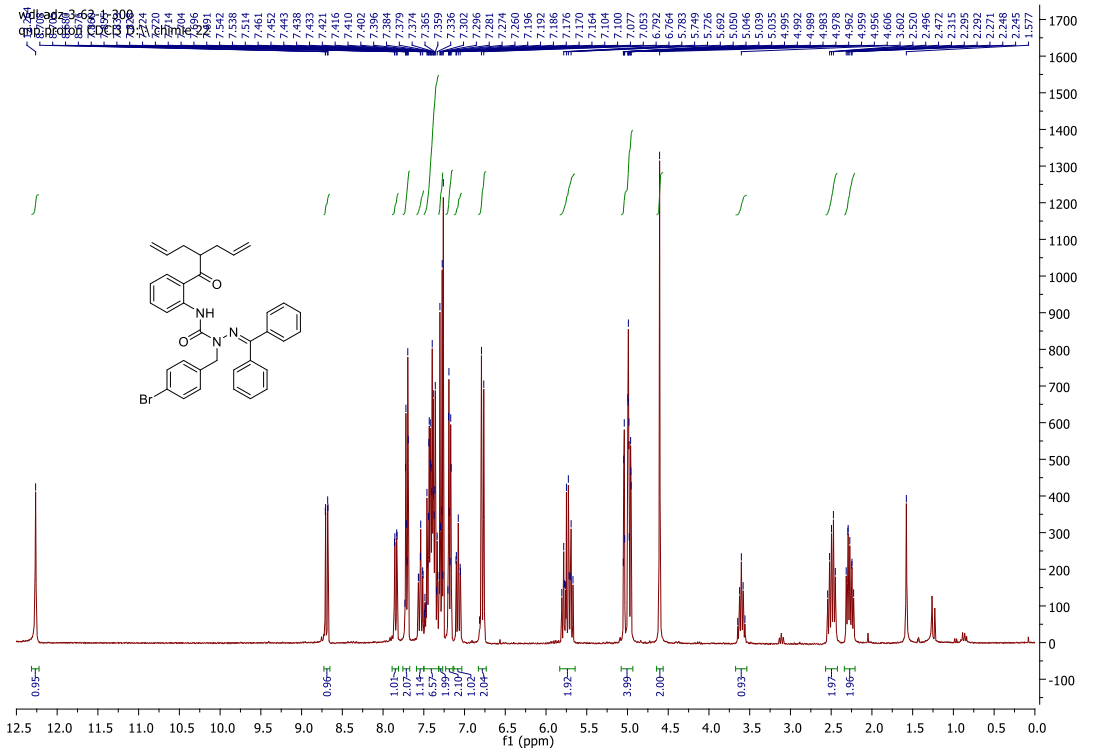


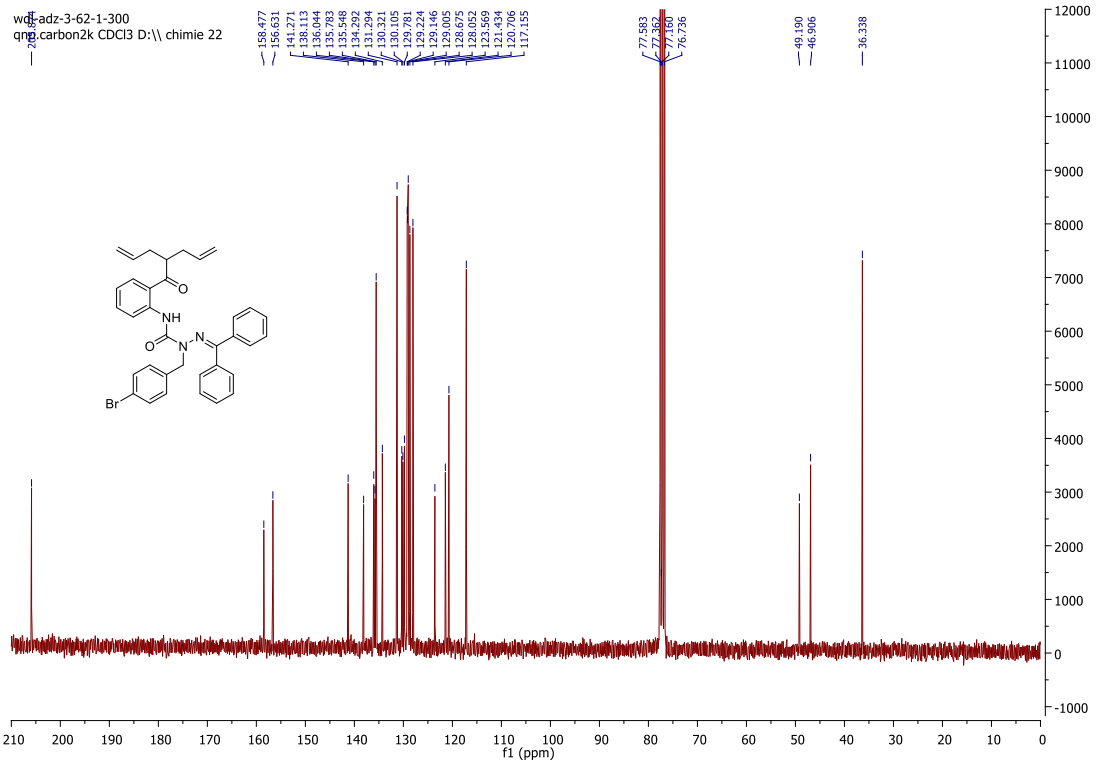
2.18h, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



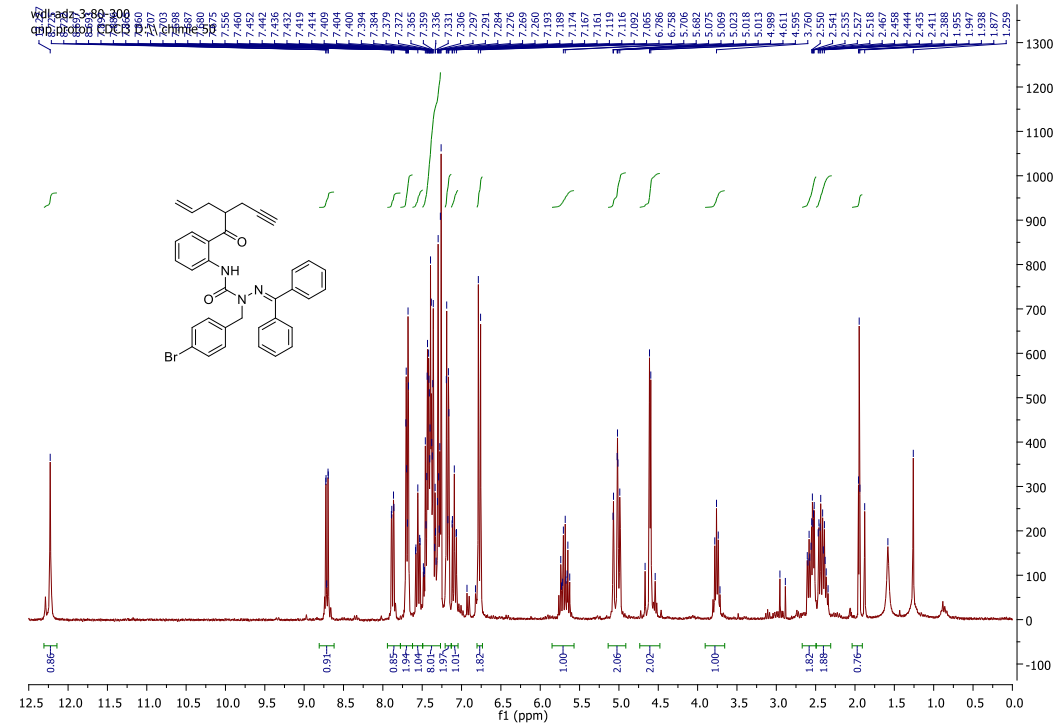


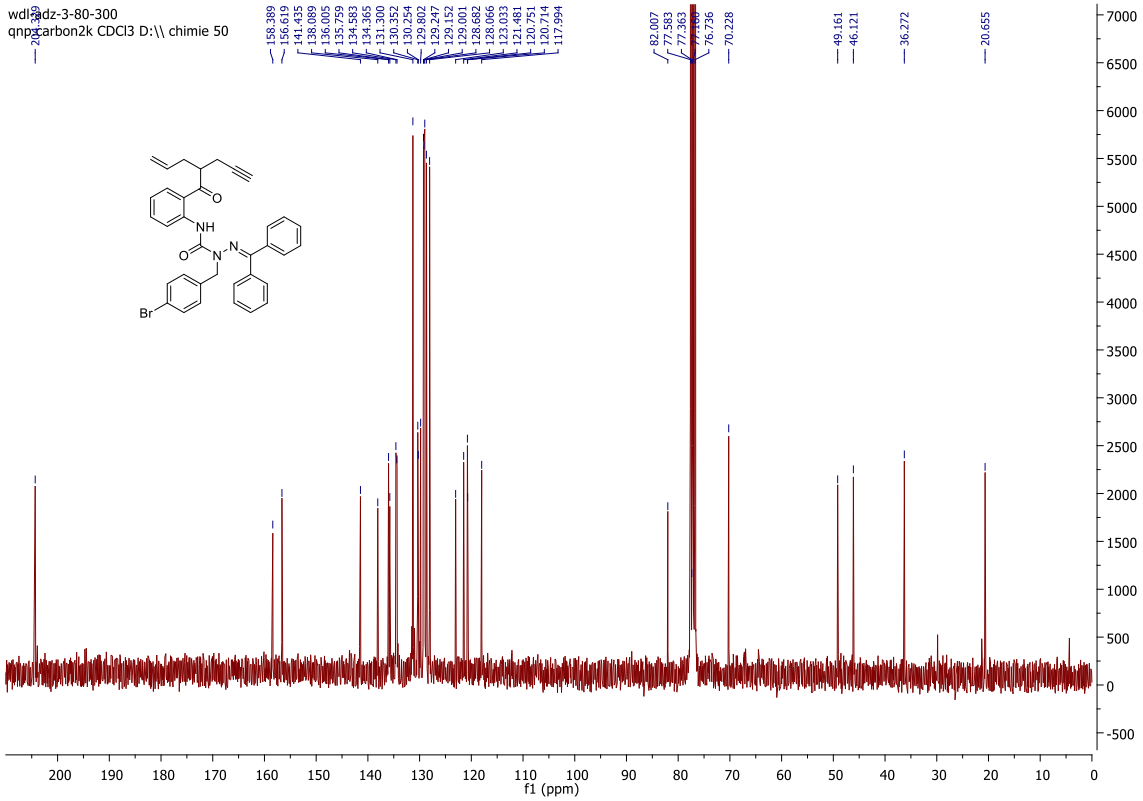
2.18i, **$CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)**.



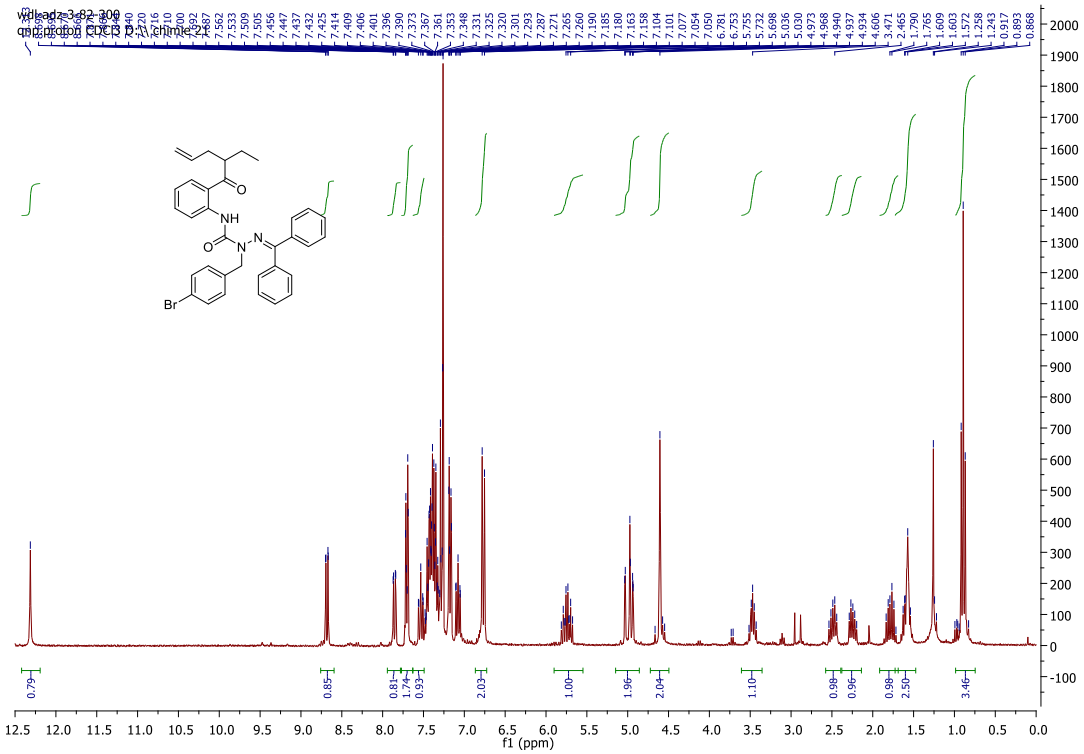


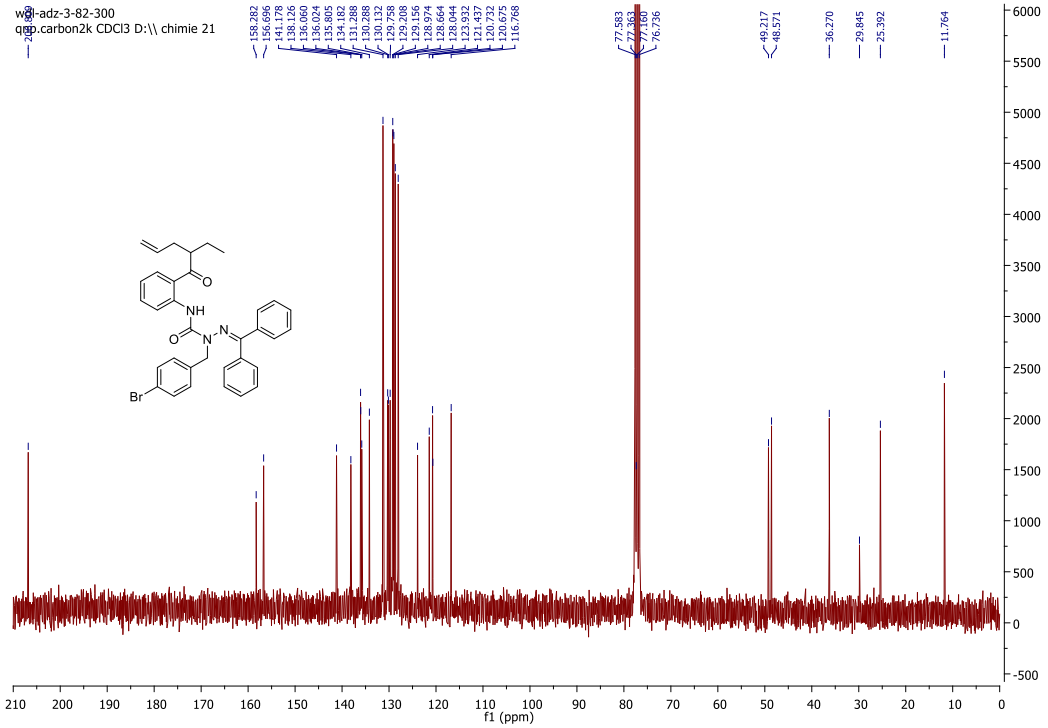
2.18j, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



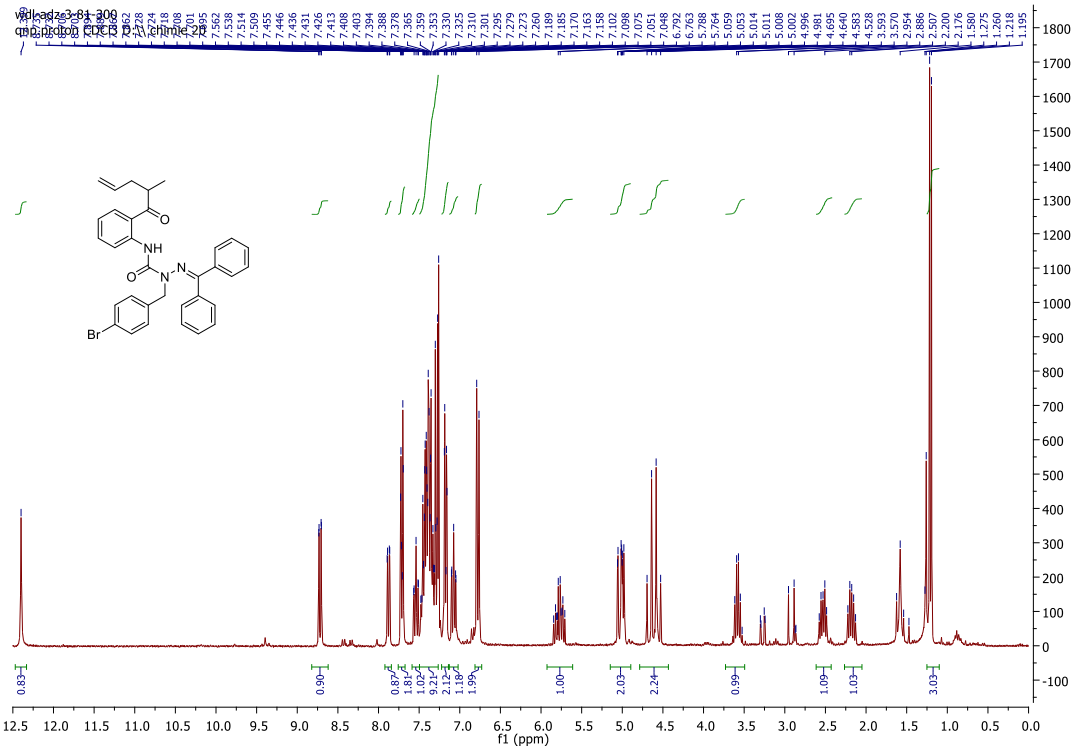


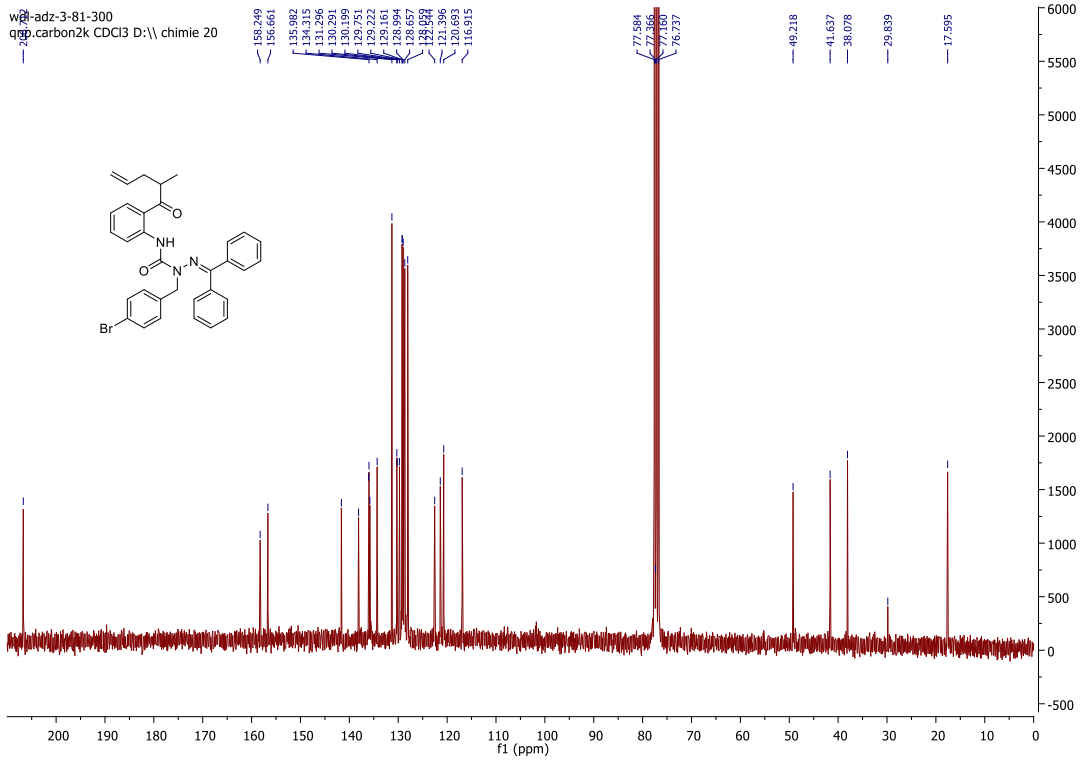
2.18k, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



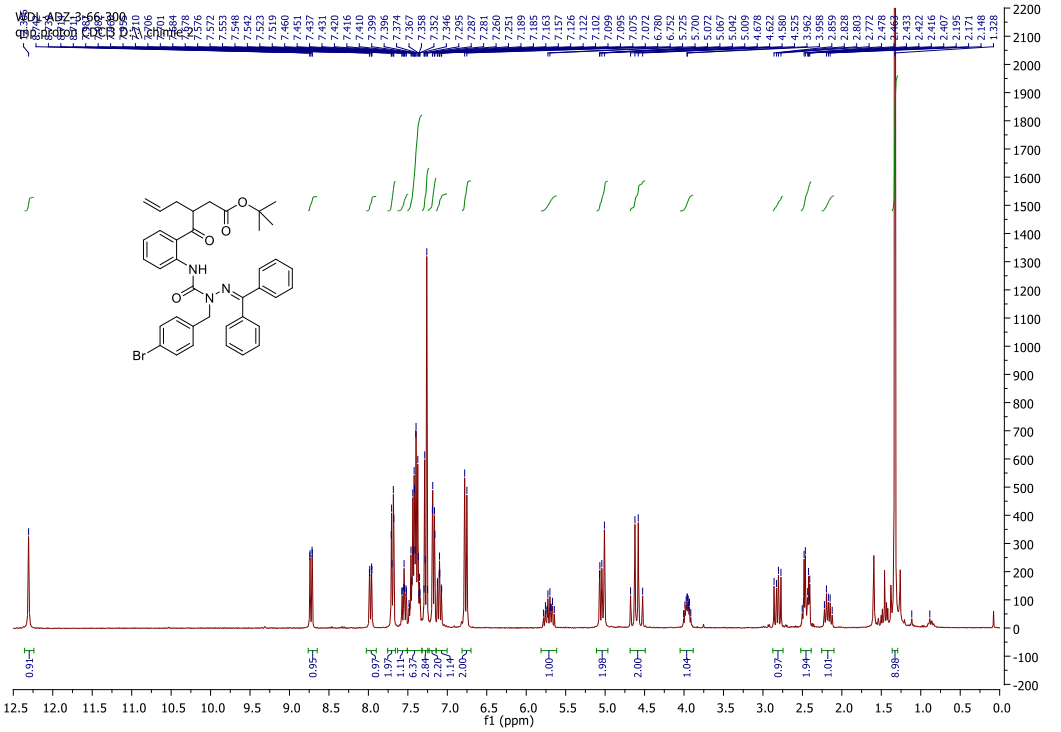


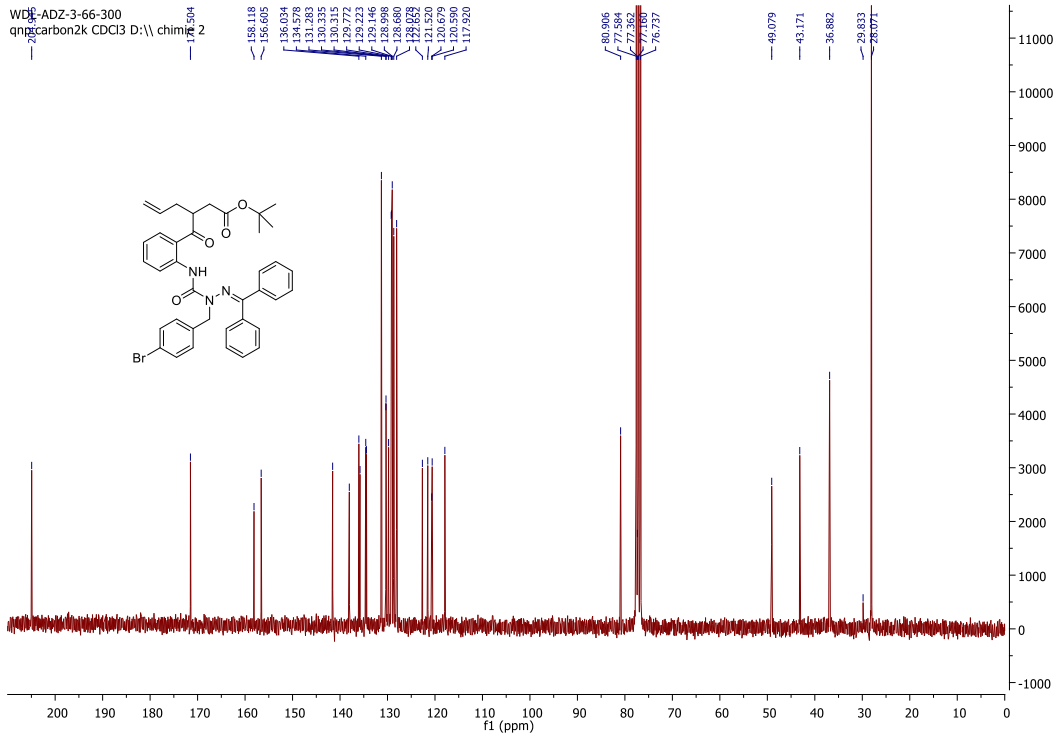
2.18l, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



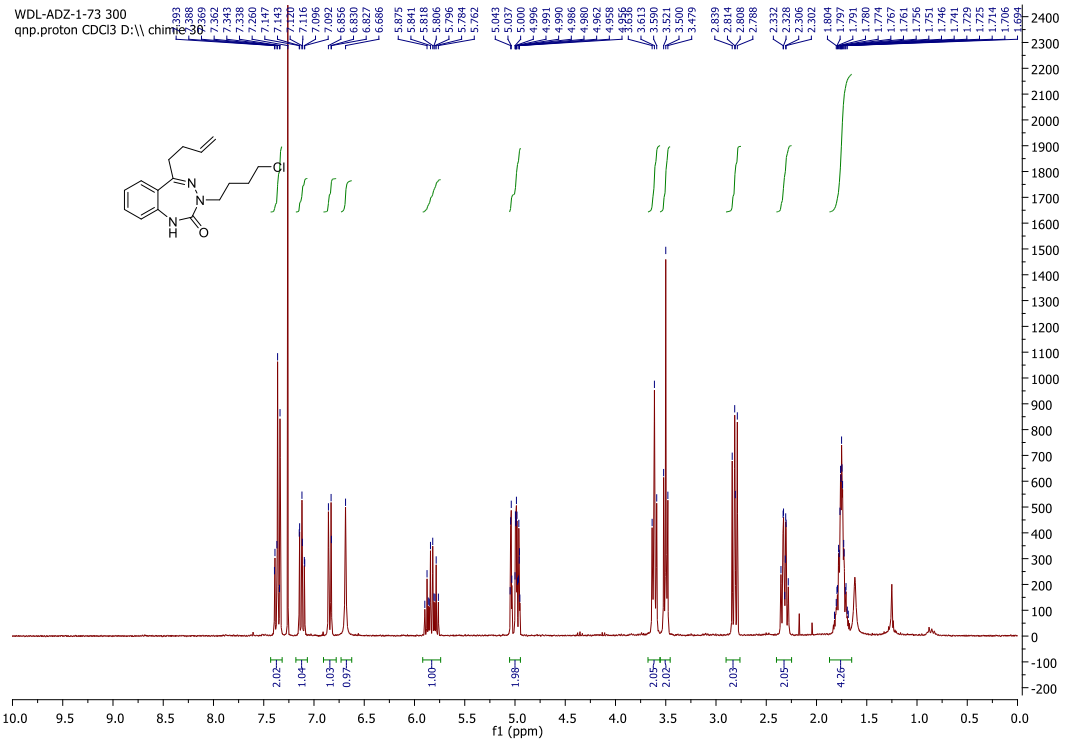


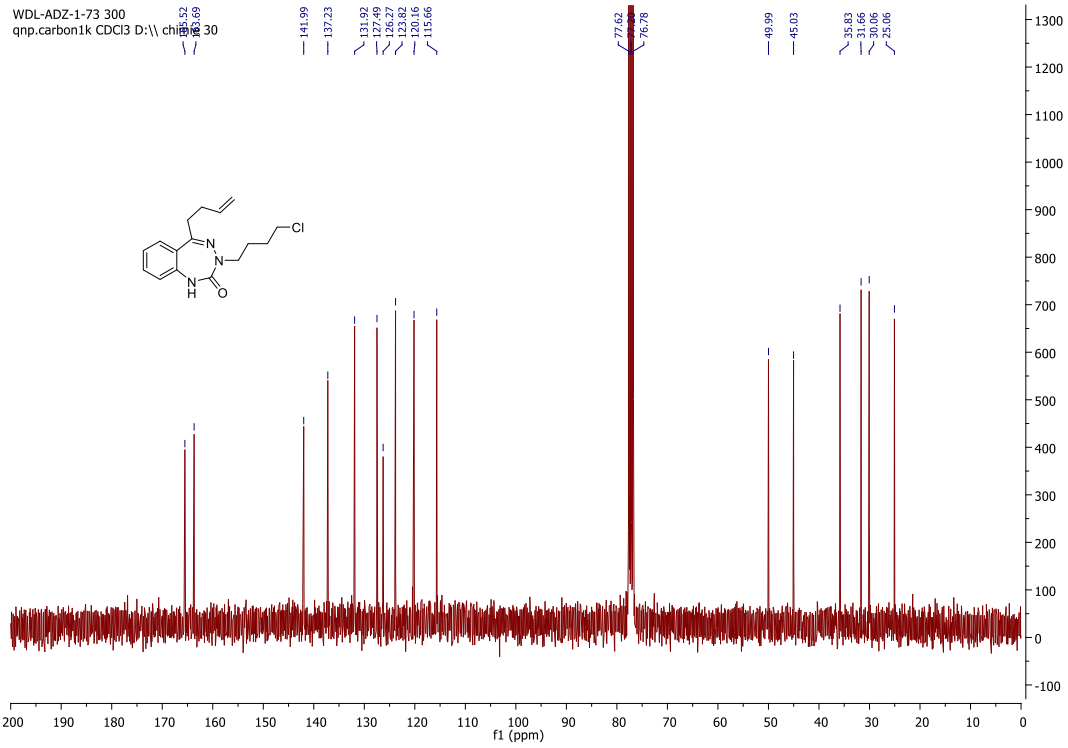
2.18m, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



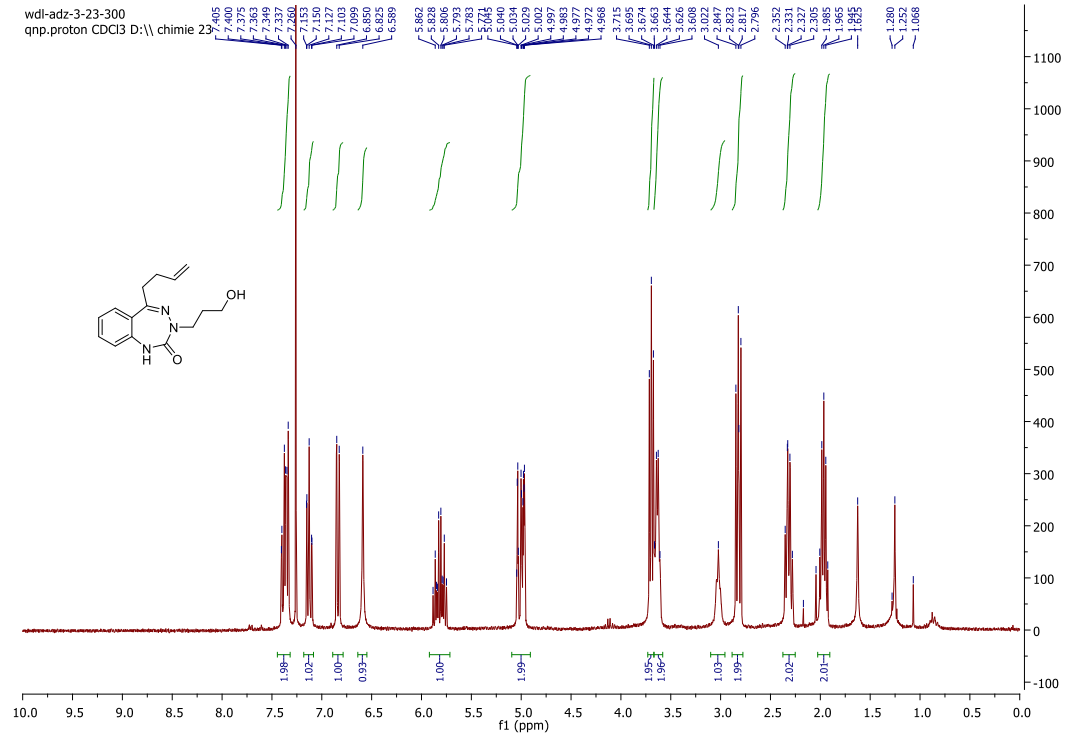


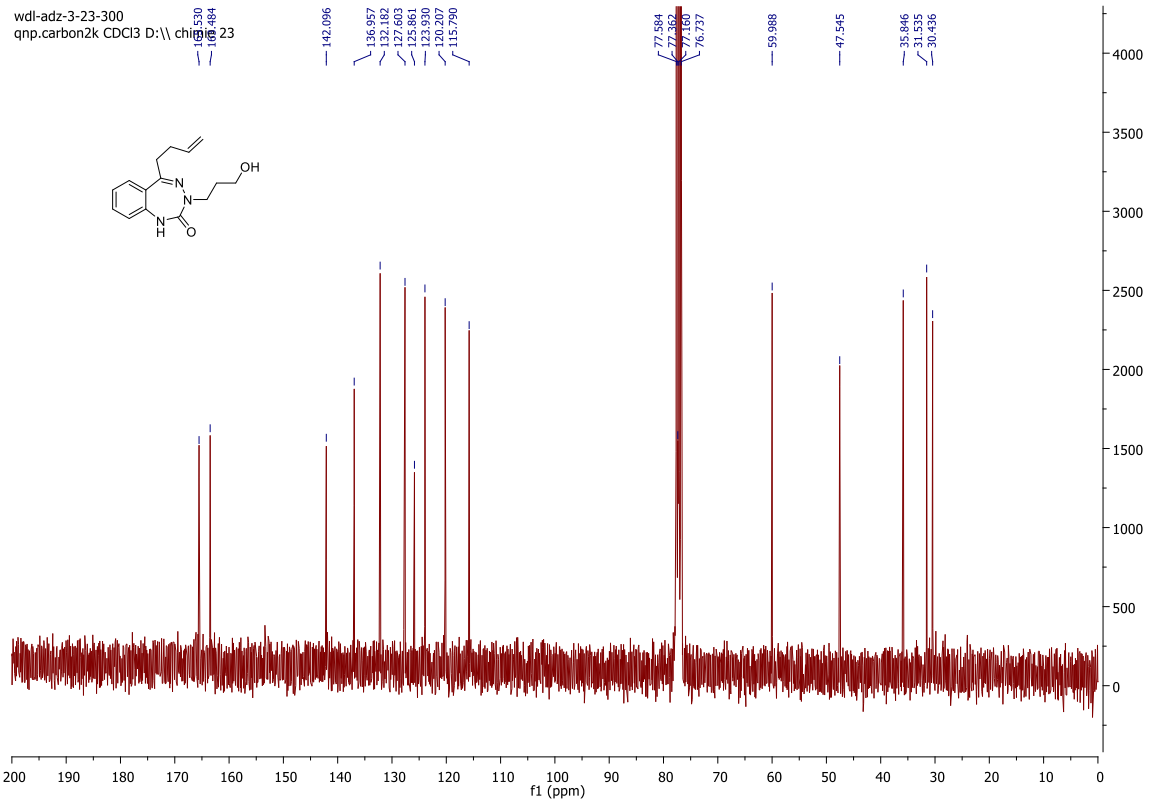
2.6a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



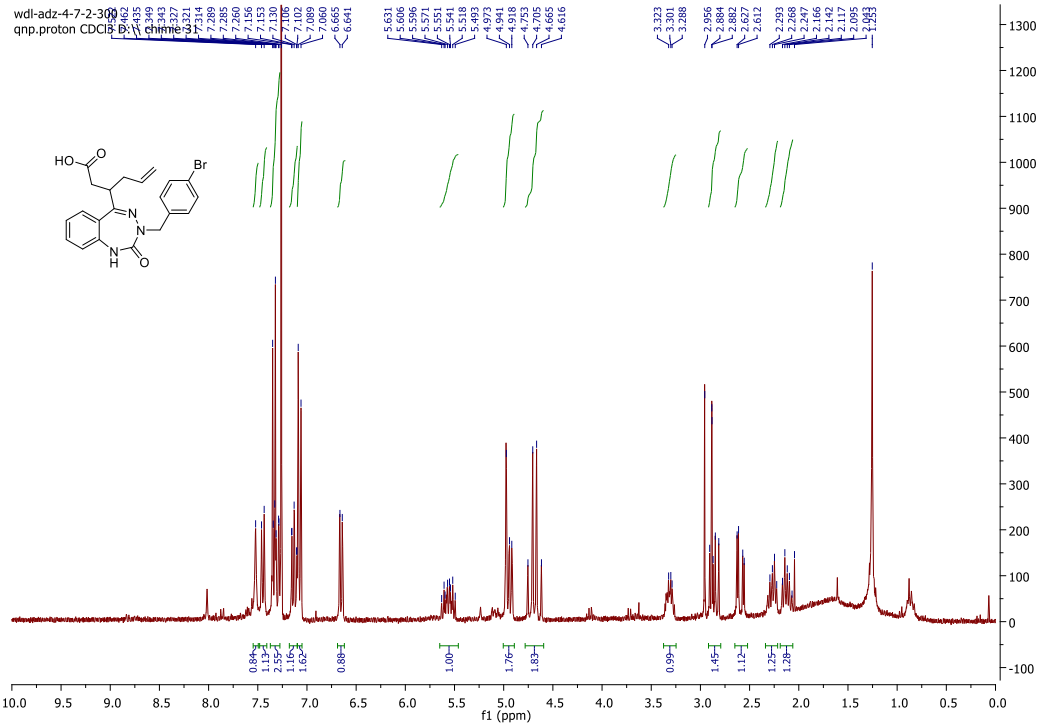


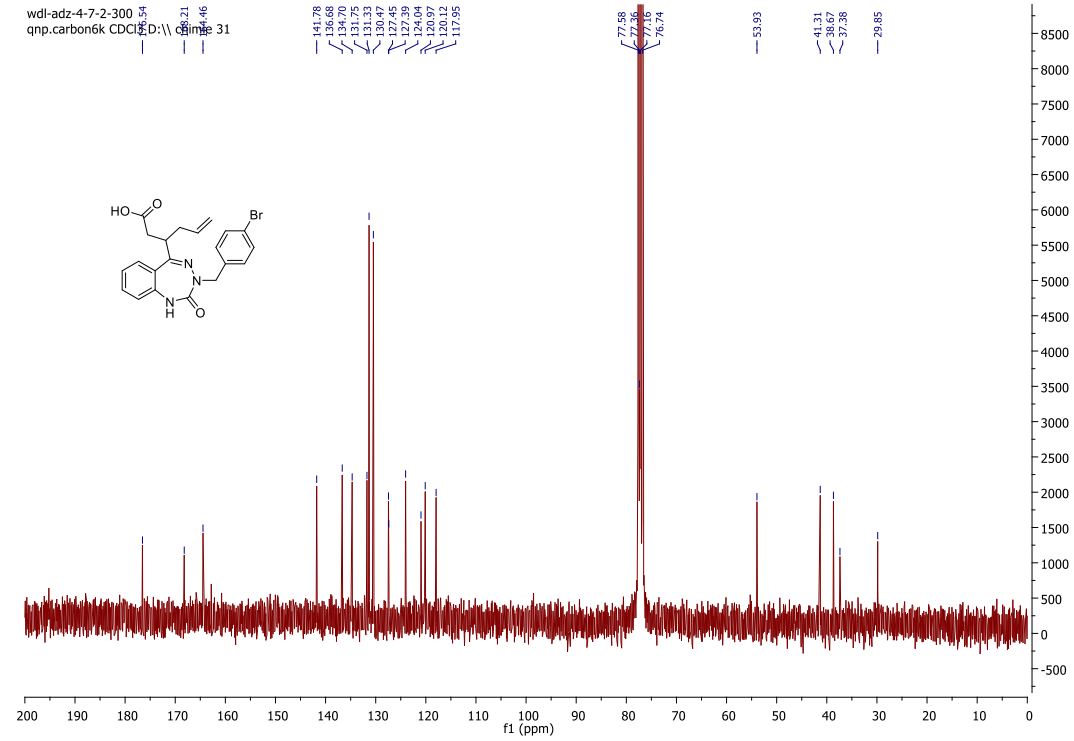
2.6b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



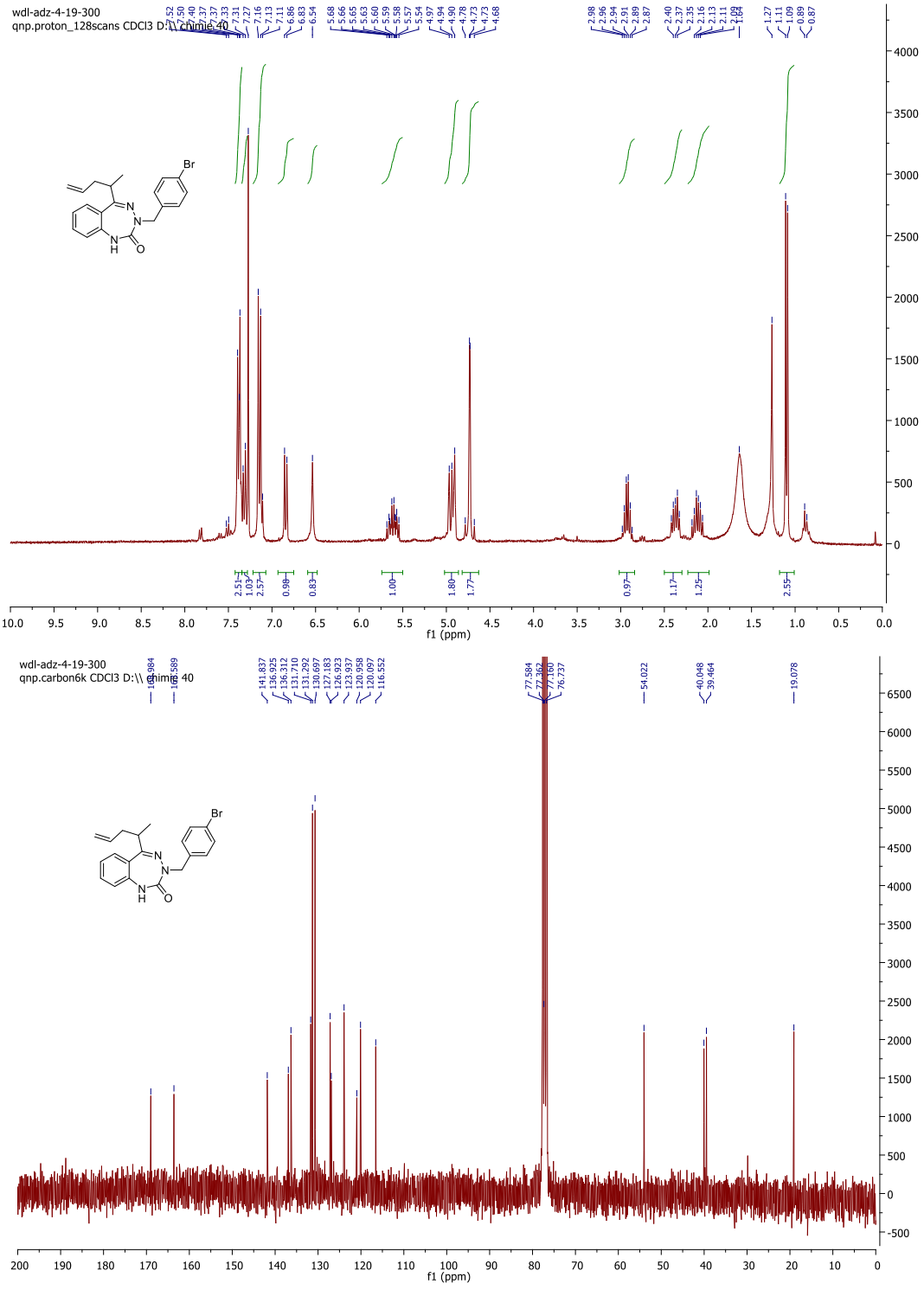


2.6c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

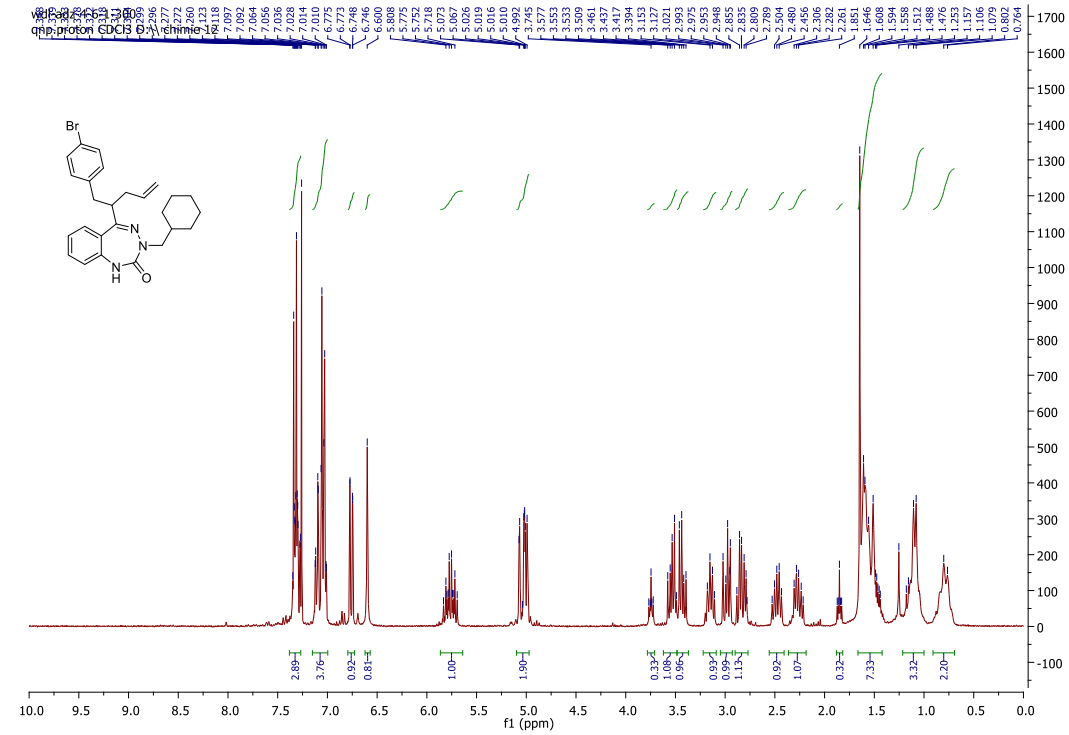


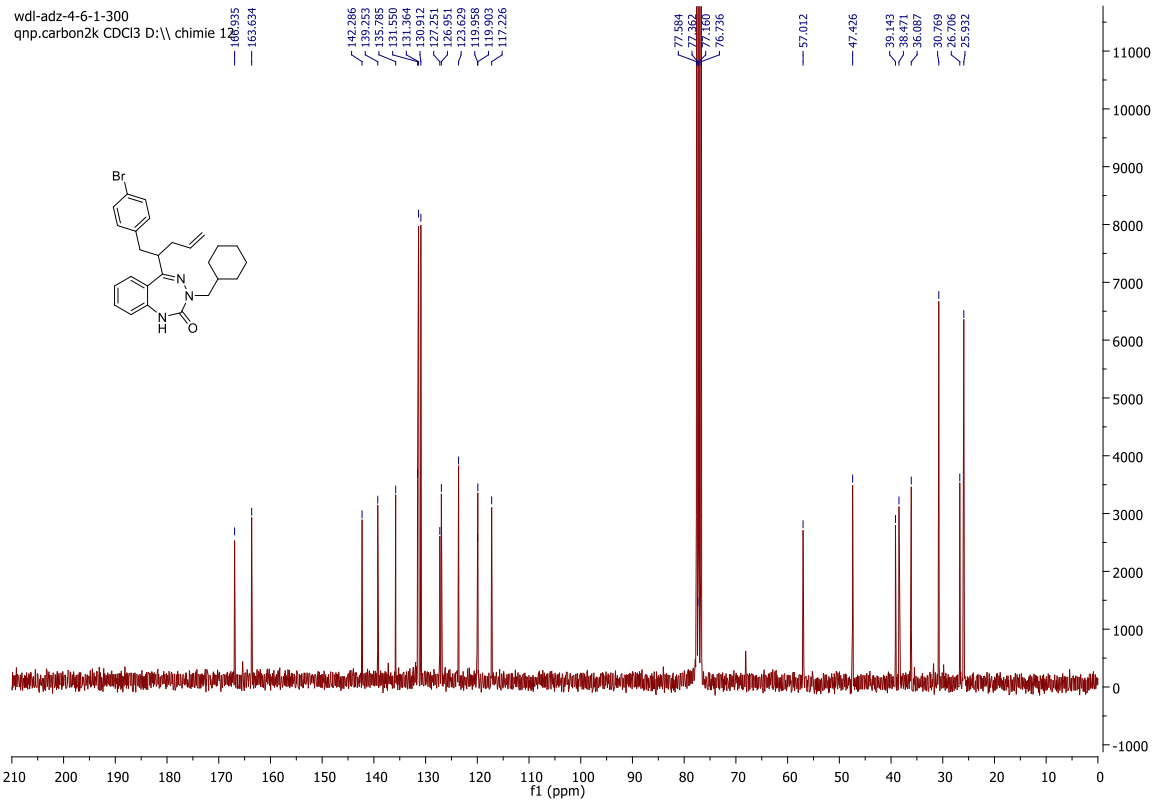


2.6d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

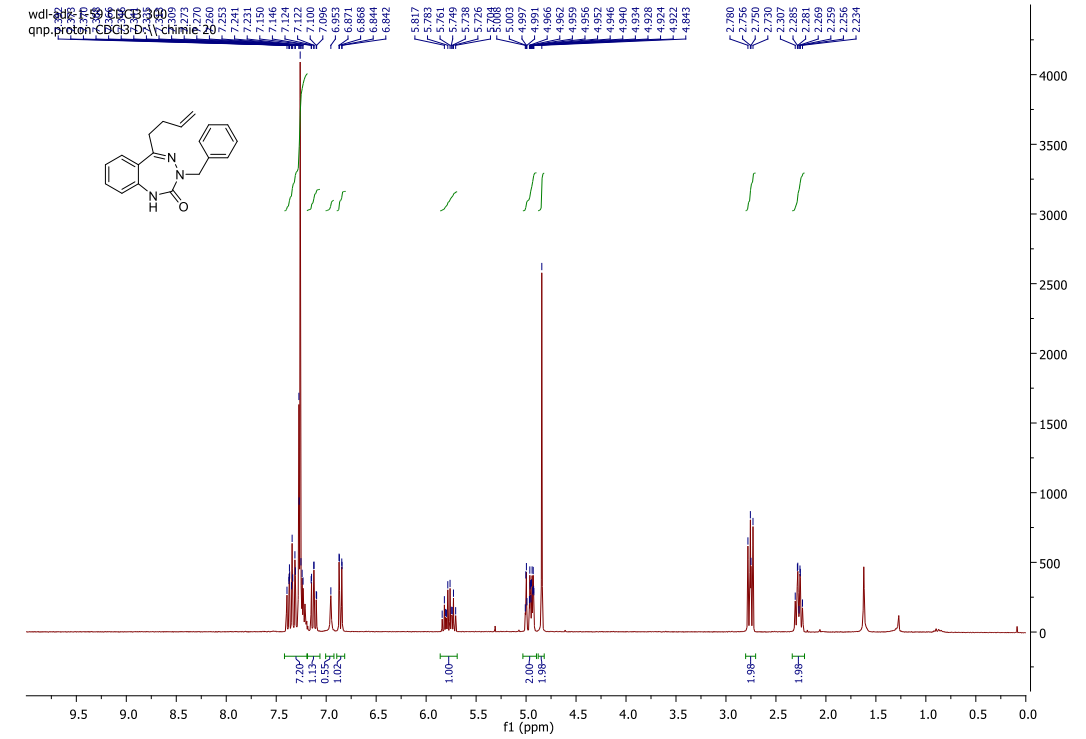


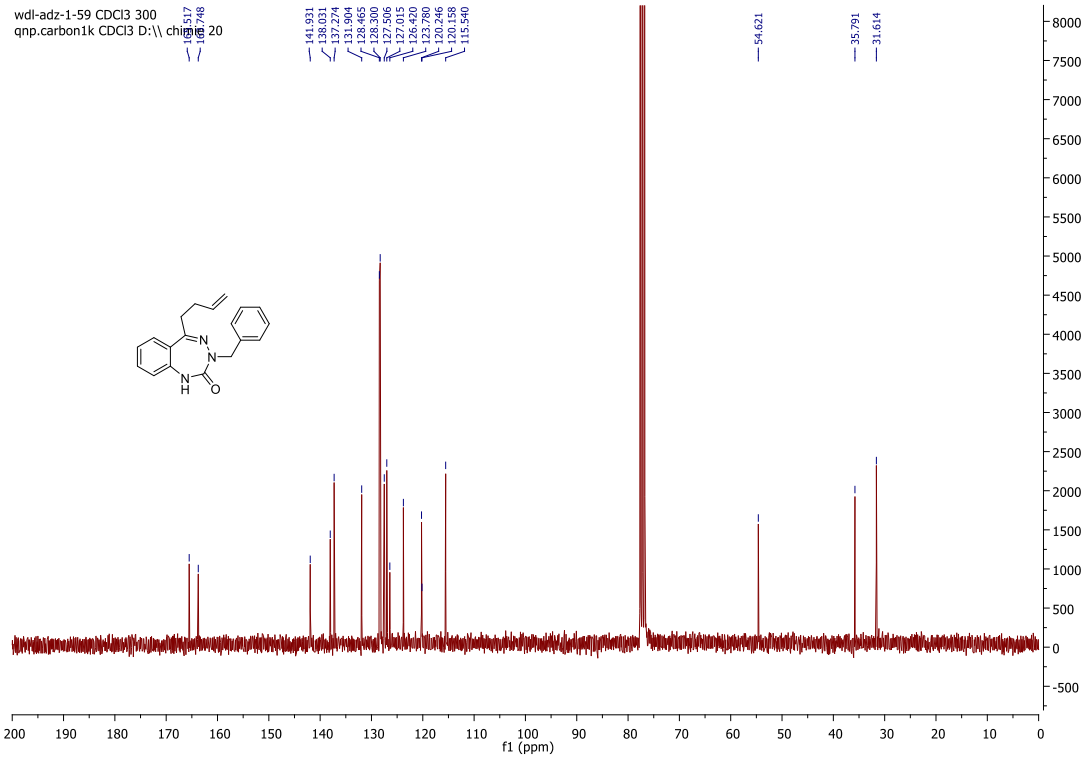
2.6e, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



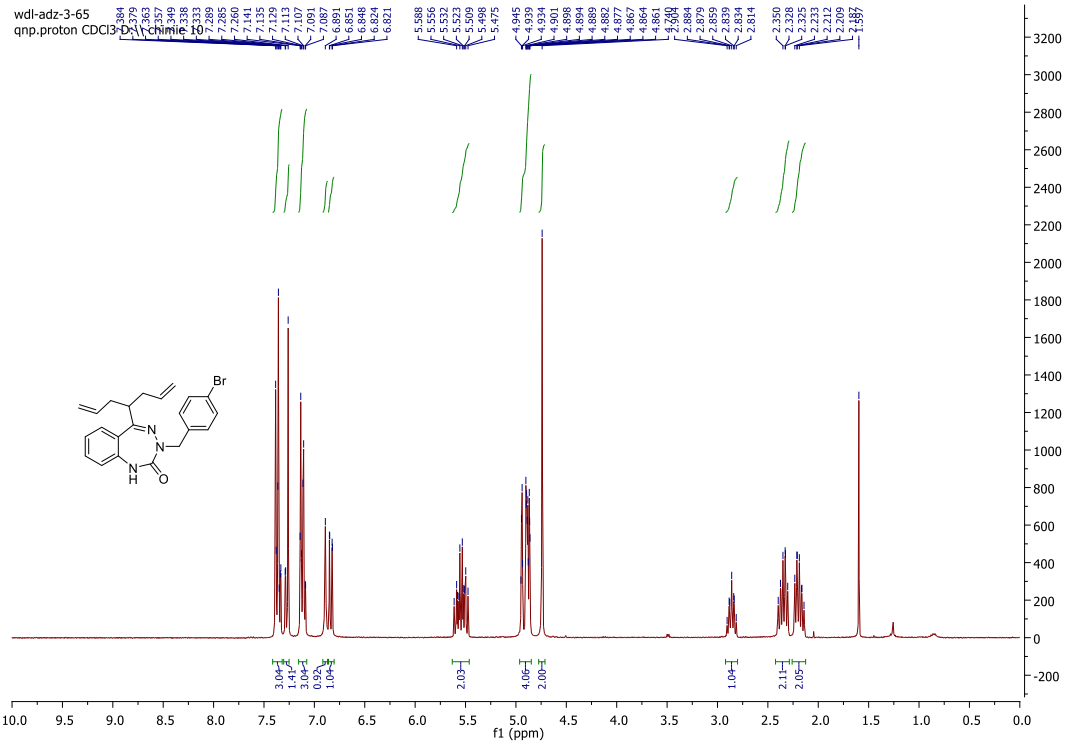


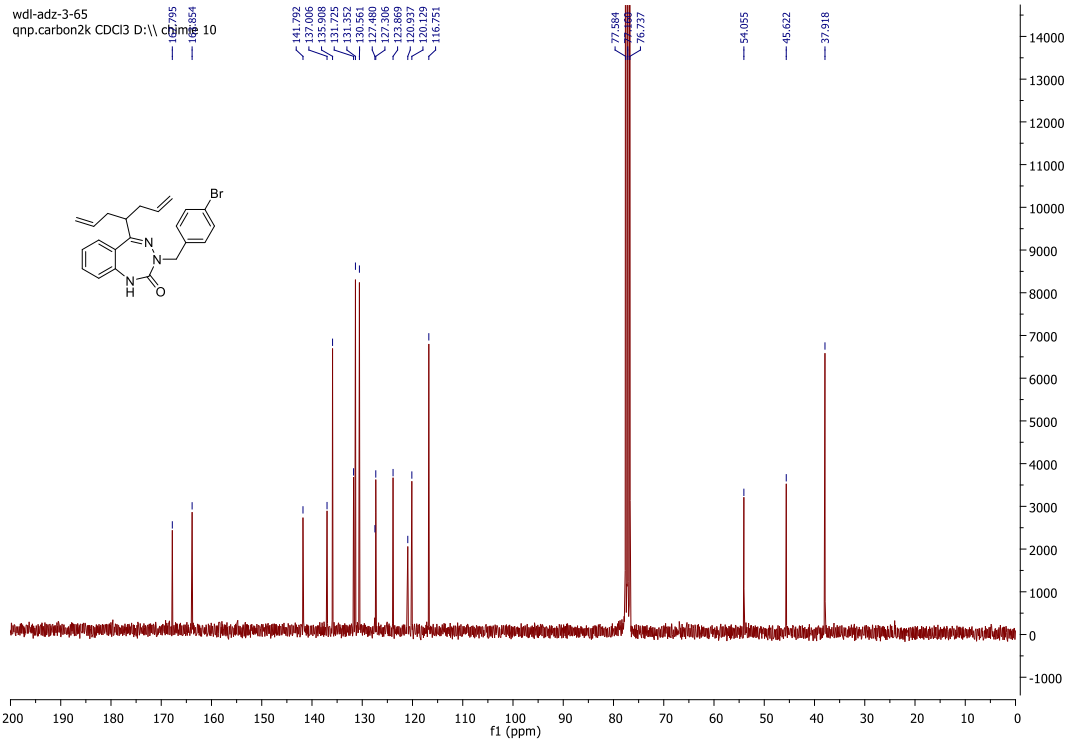
2.6f, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



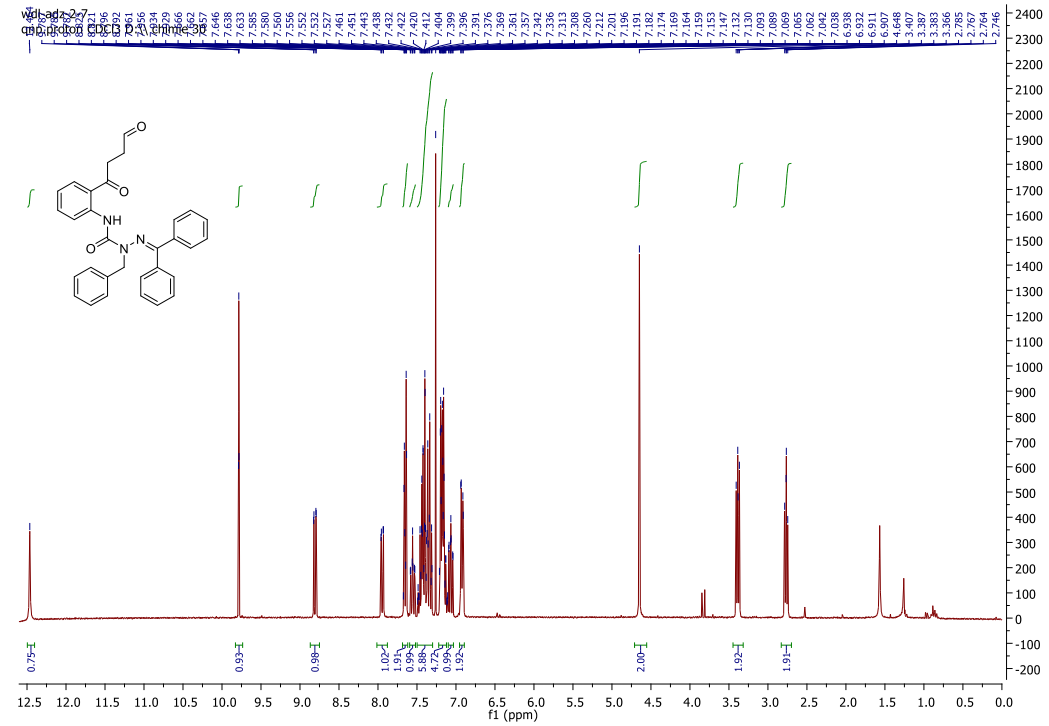


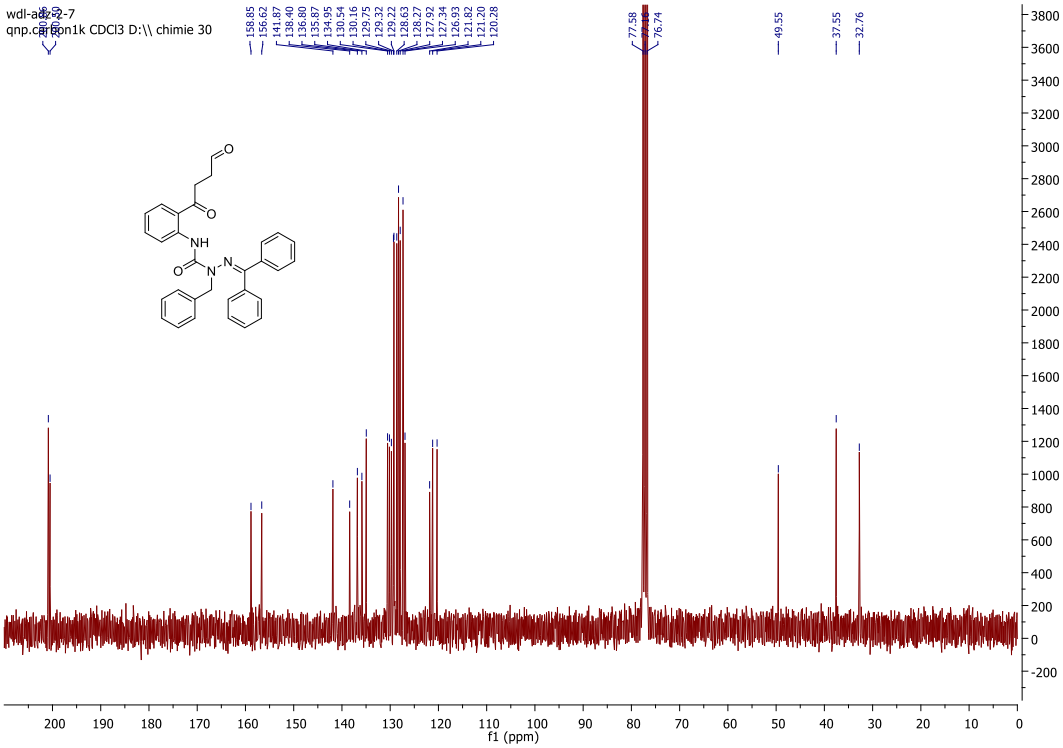
2.6g, **$CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)**.



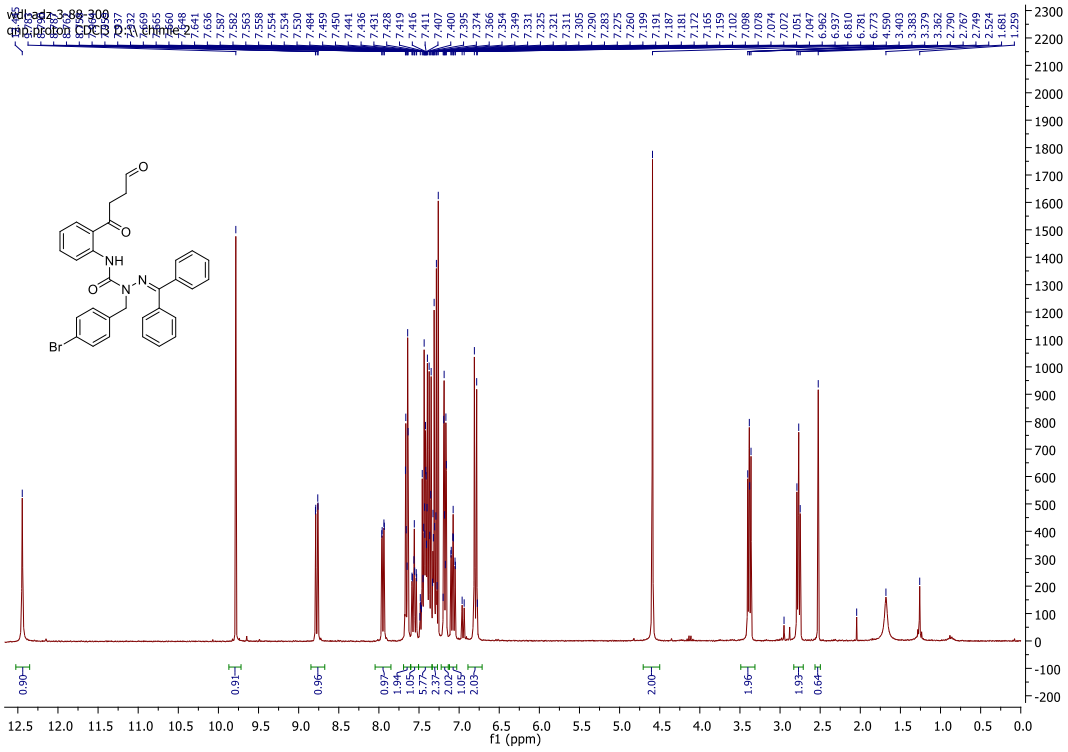


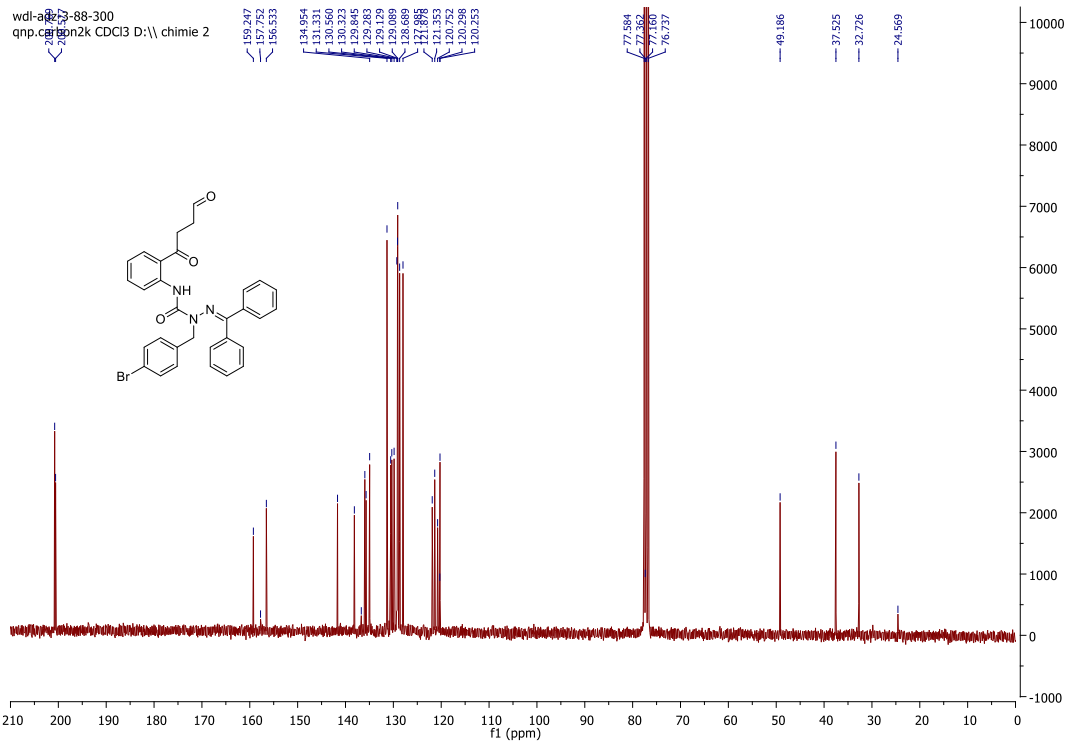
2.22a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



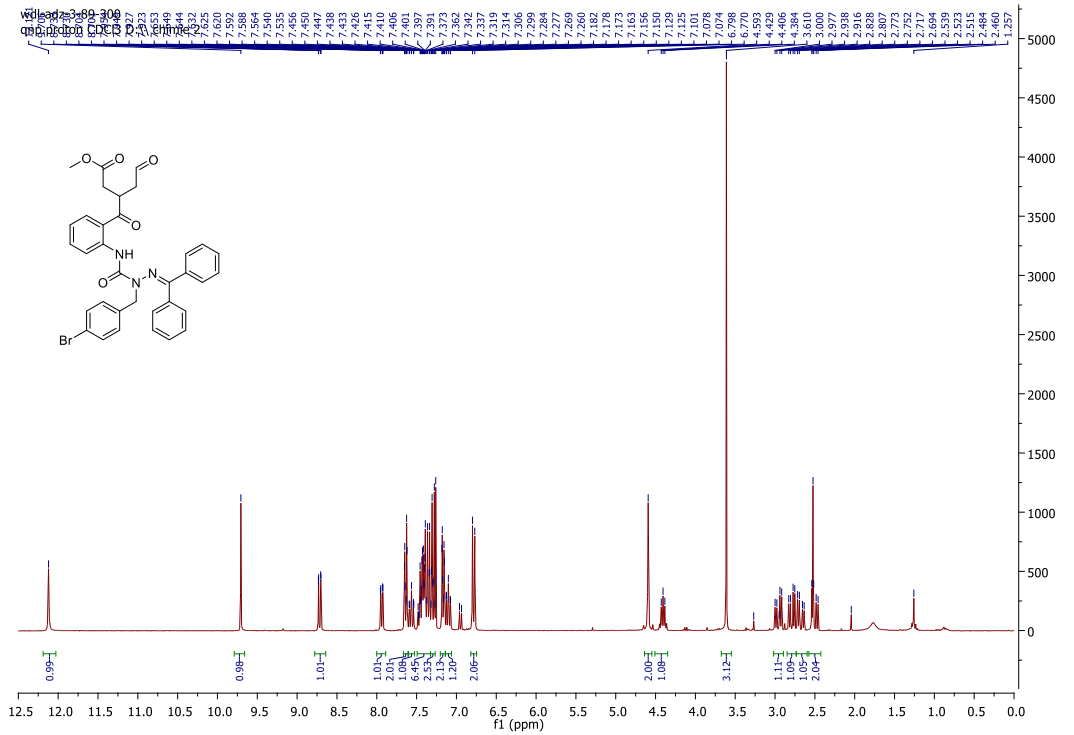


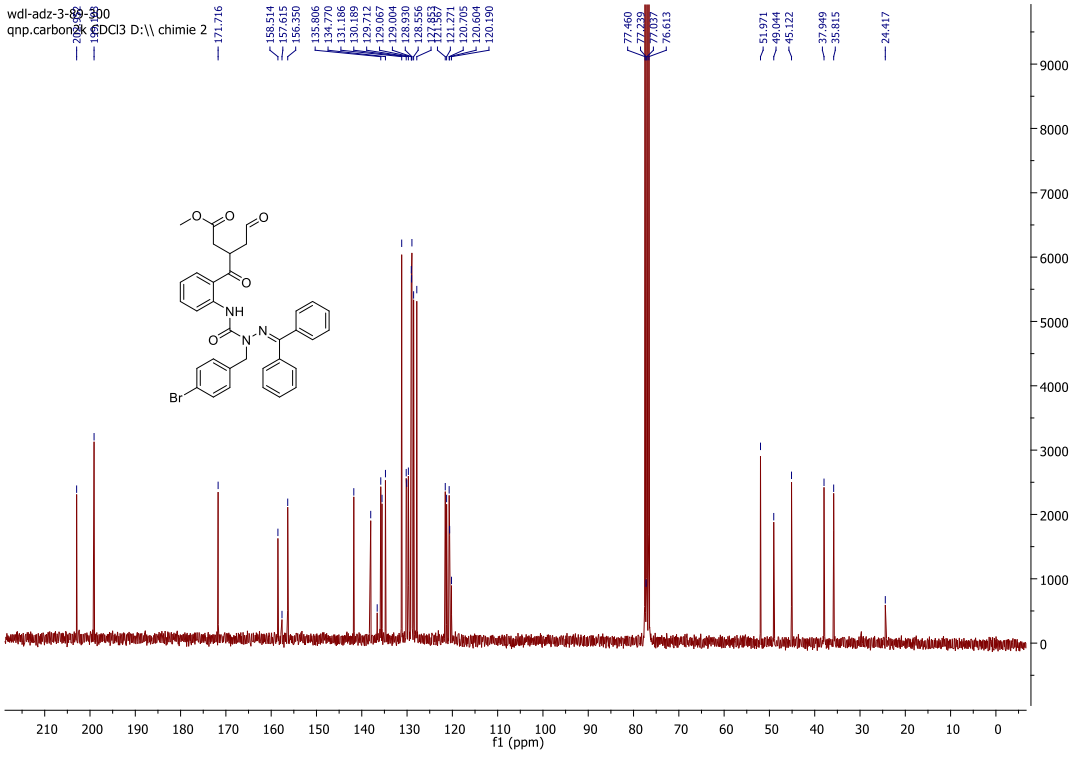
2.22b, **$CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)**.



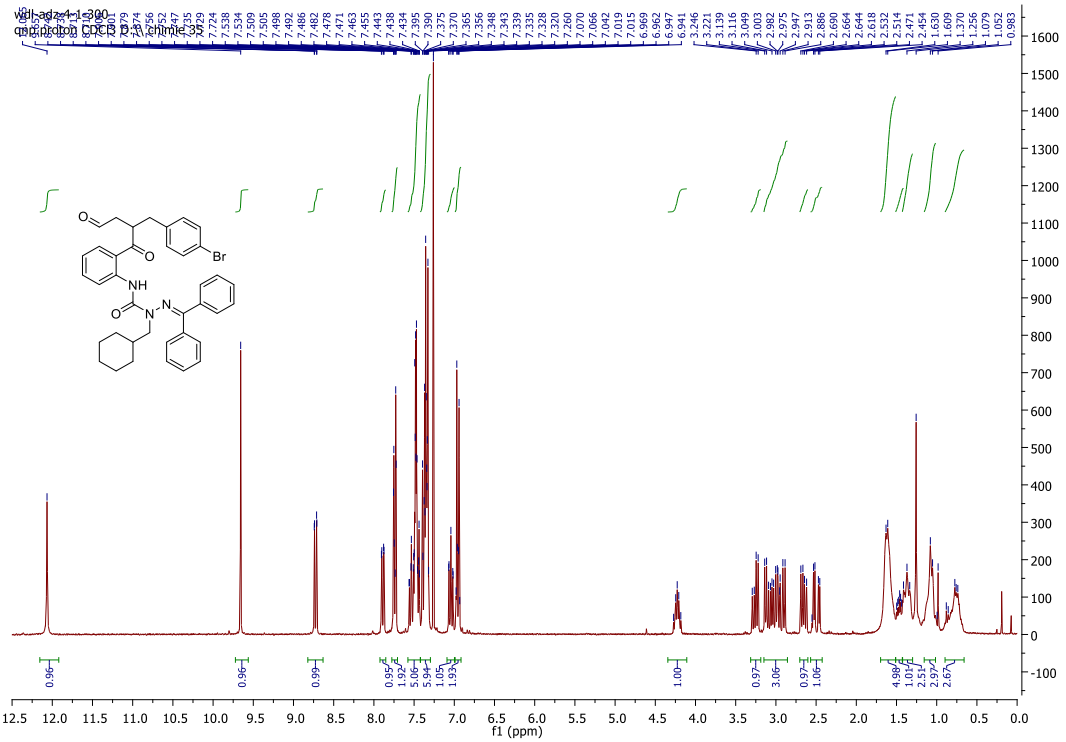


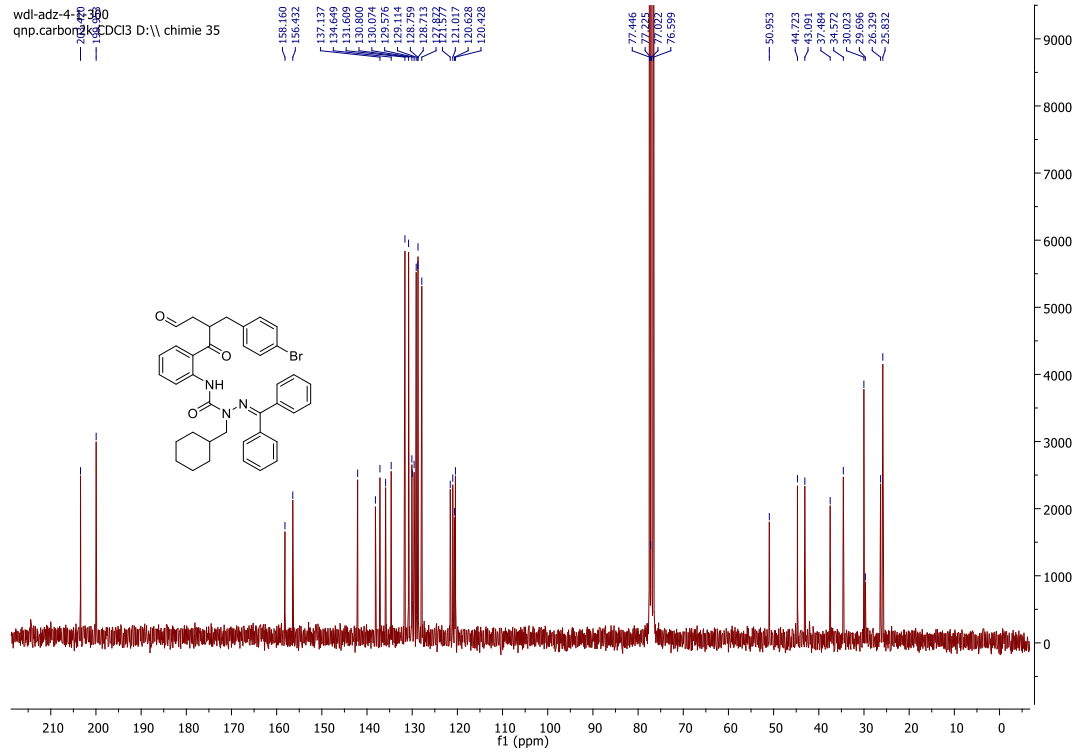
2.22c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



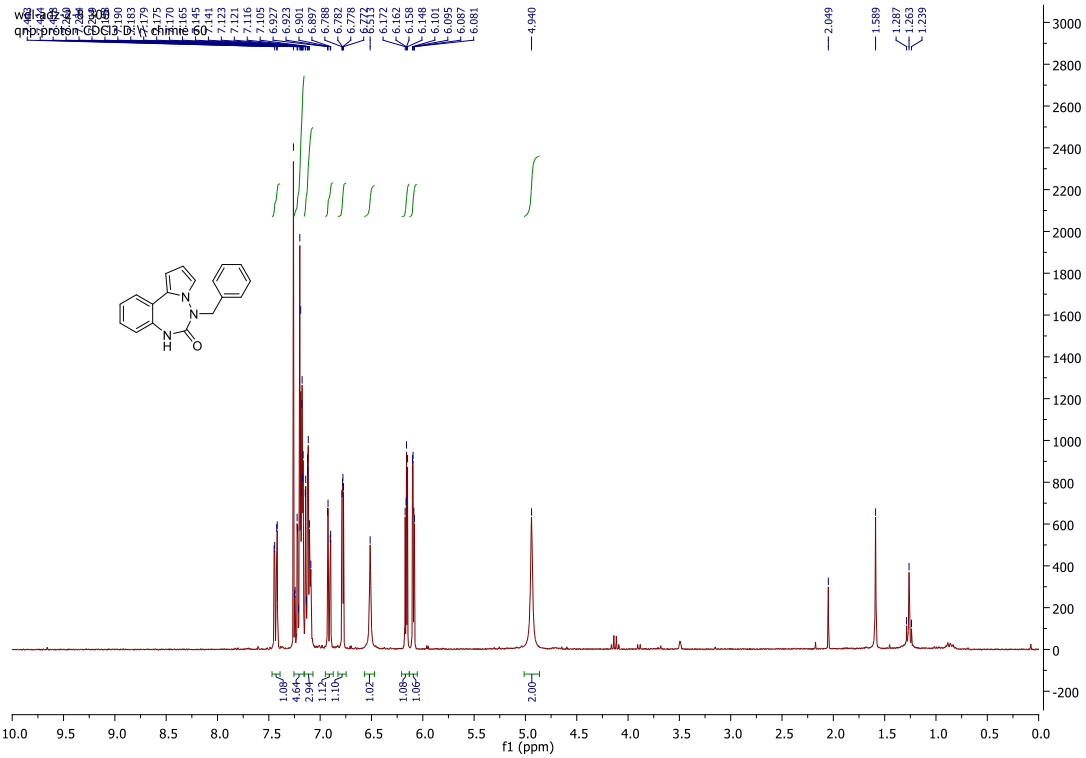


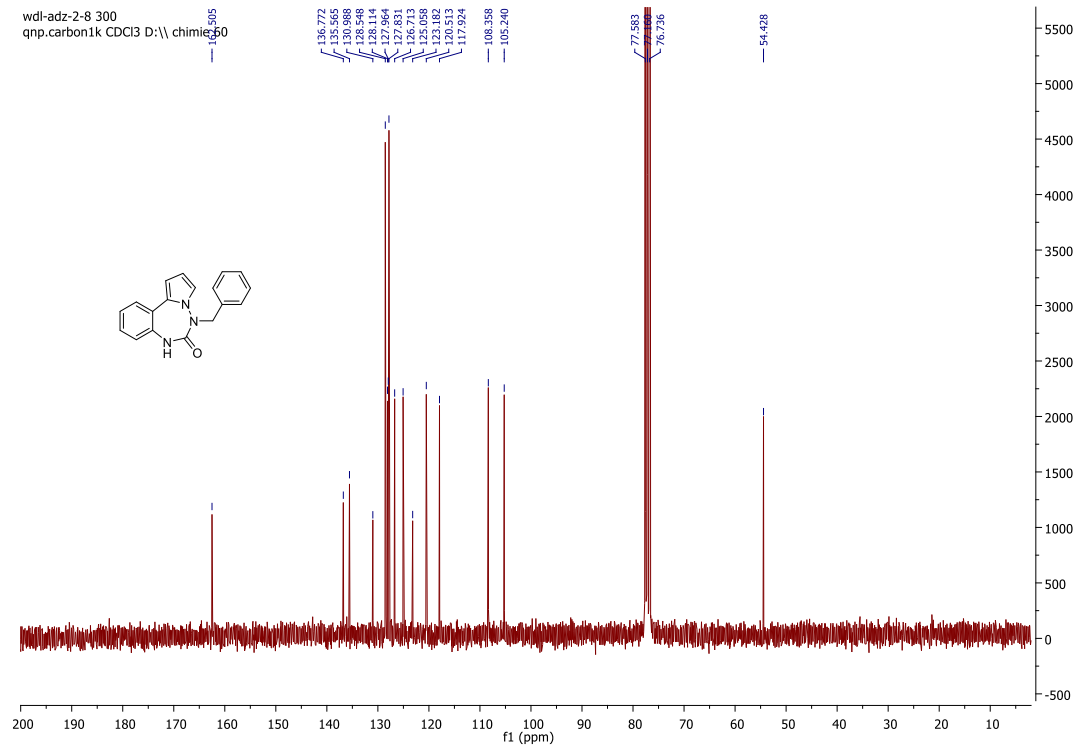
2.22d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



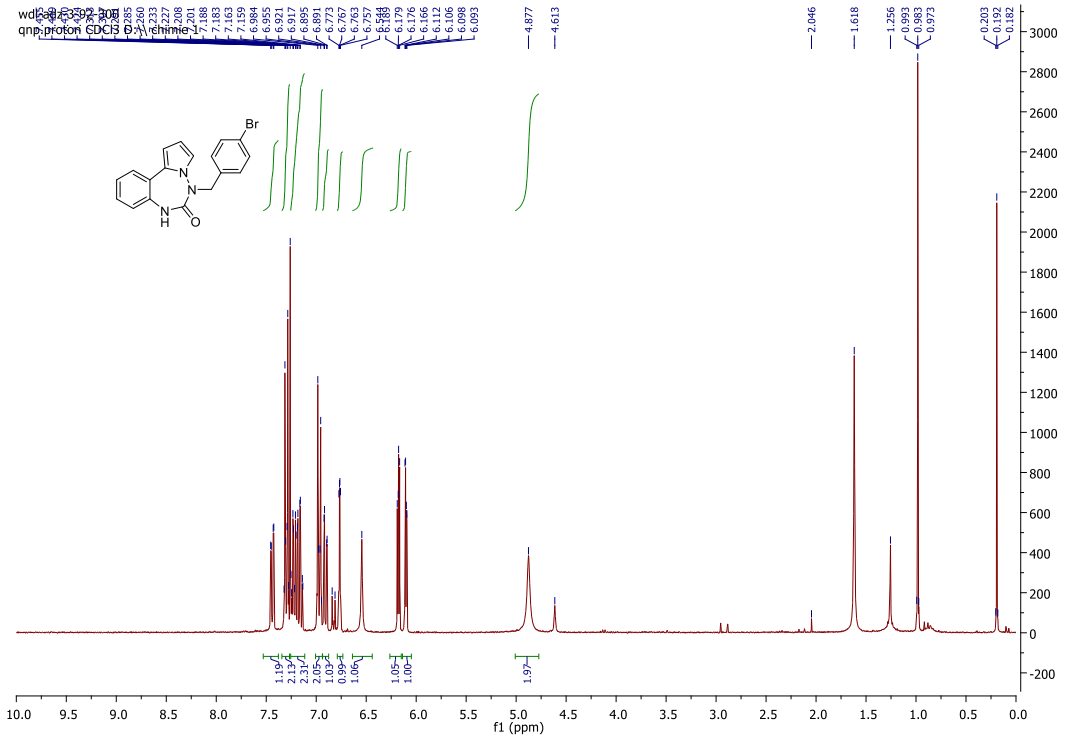


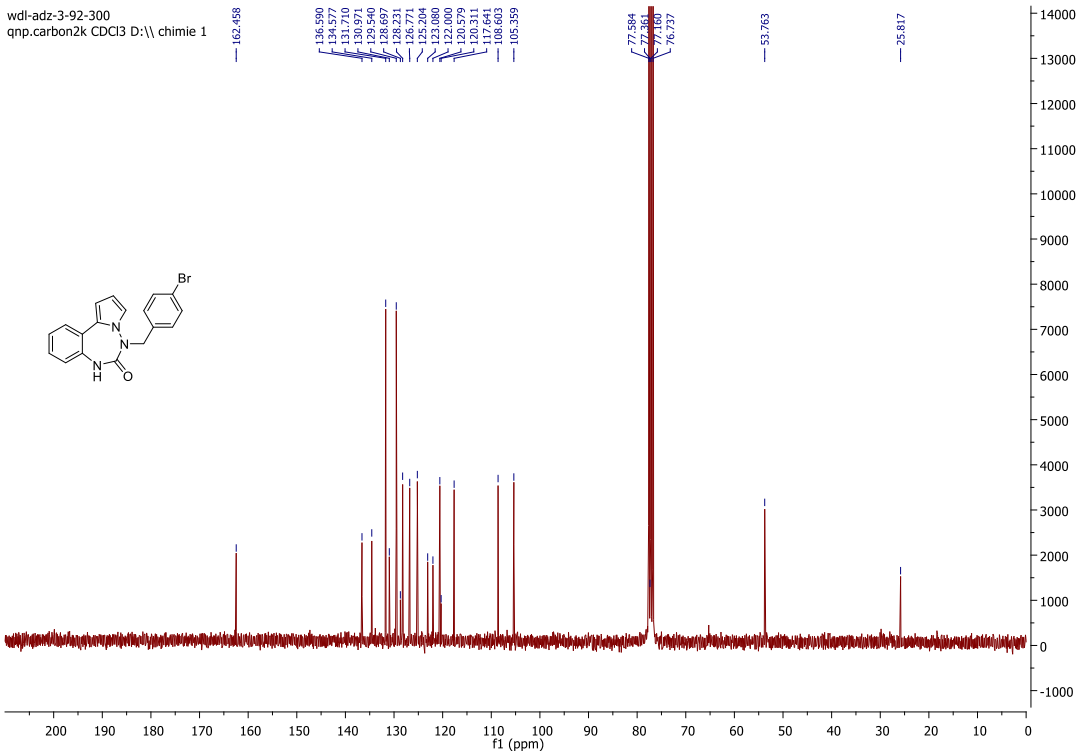
2.7a, **$CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)**.



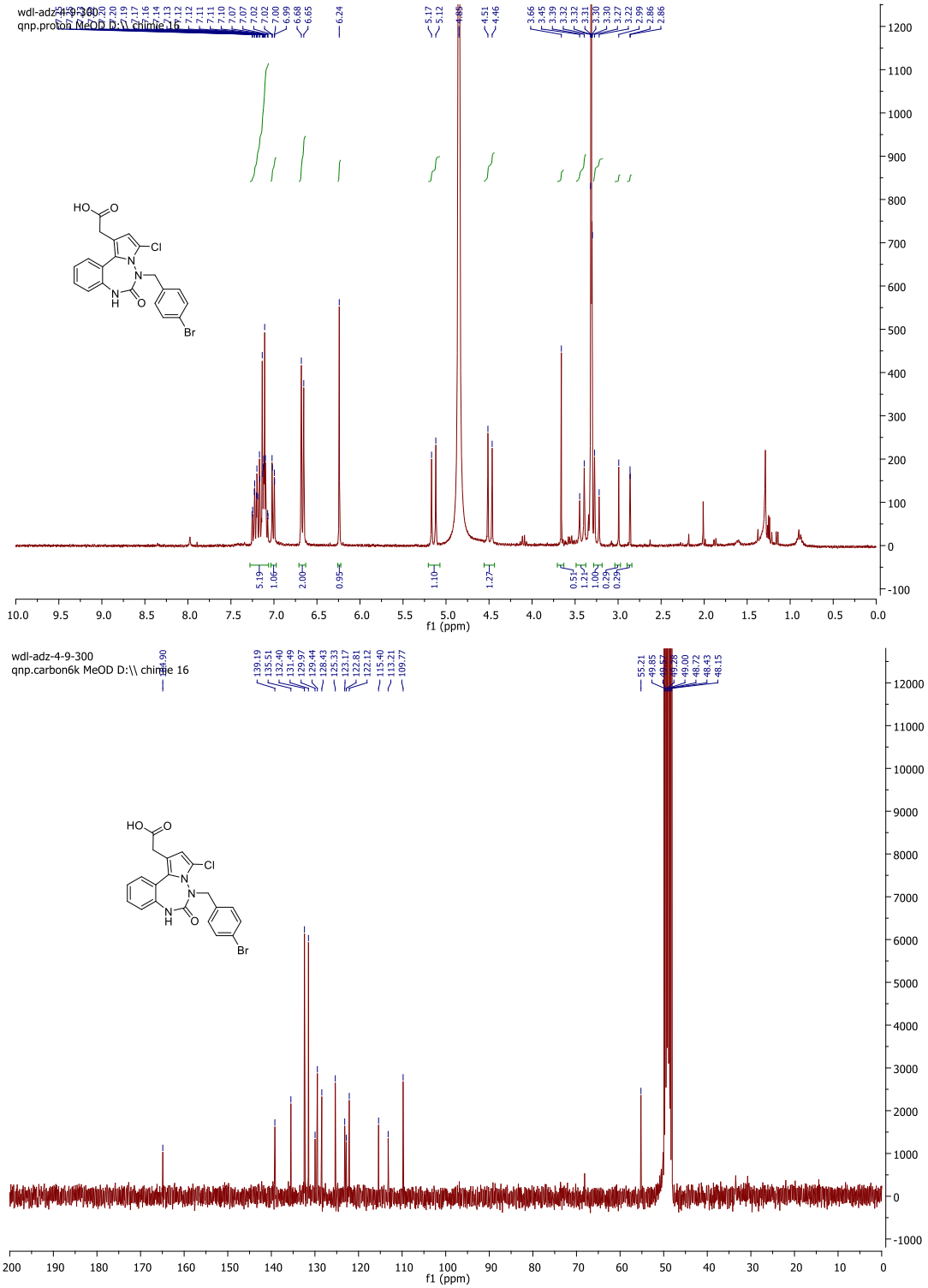


2.7b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

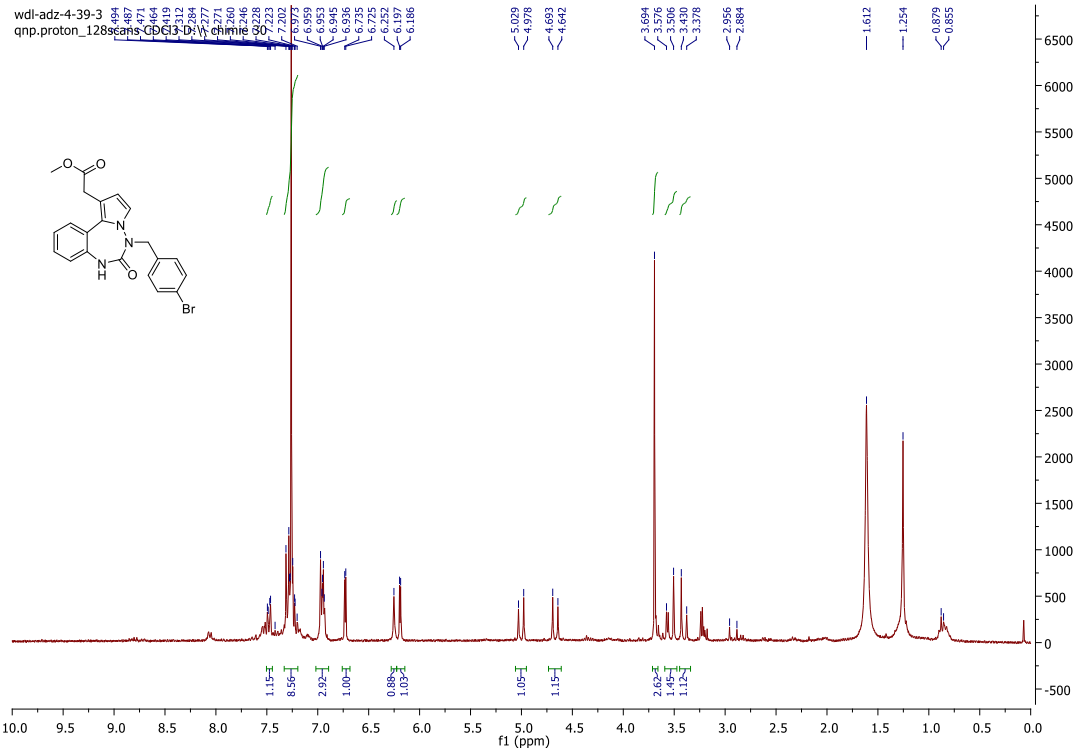


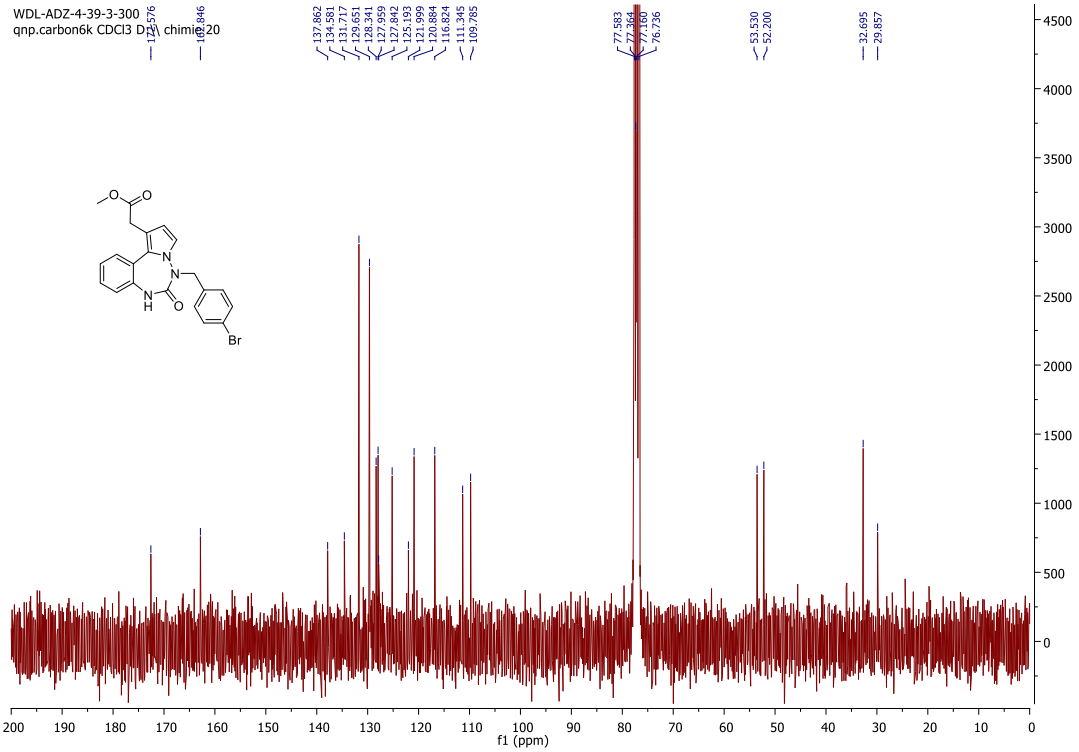


2.7c, MeOD-d$_4$, ^{1}H (300 MHz), ^{13}C (75 MHz).

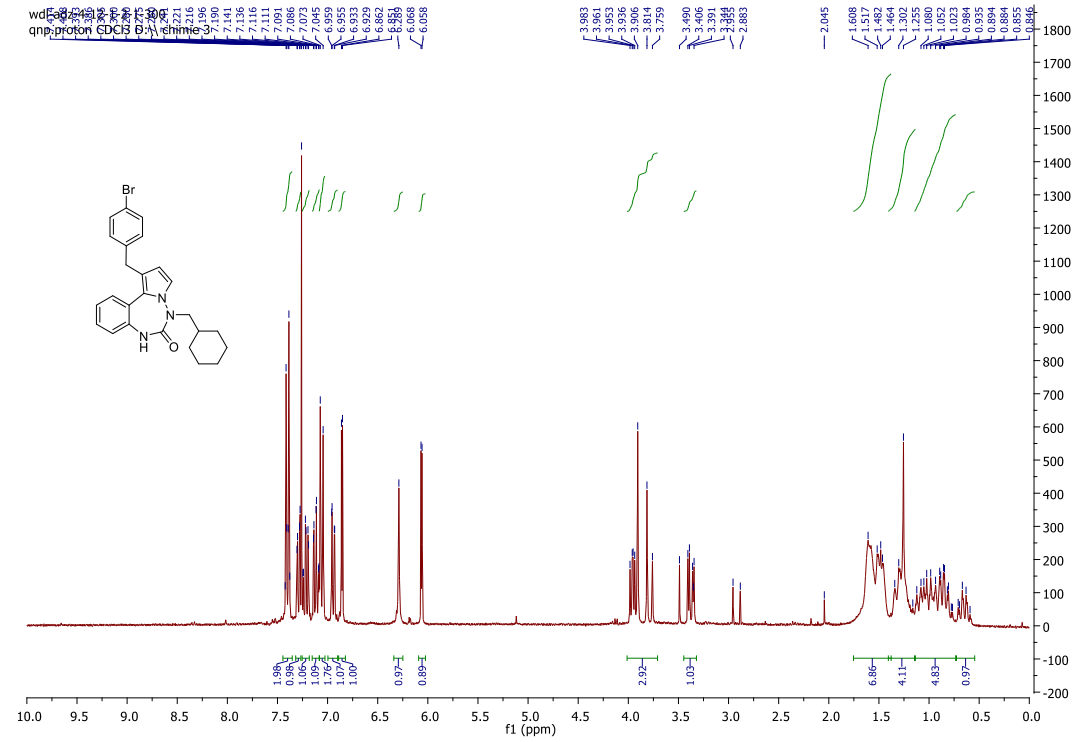


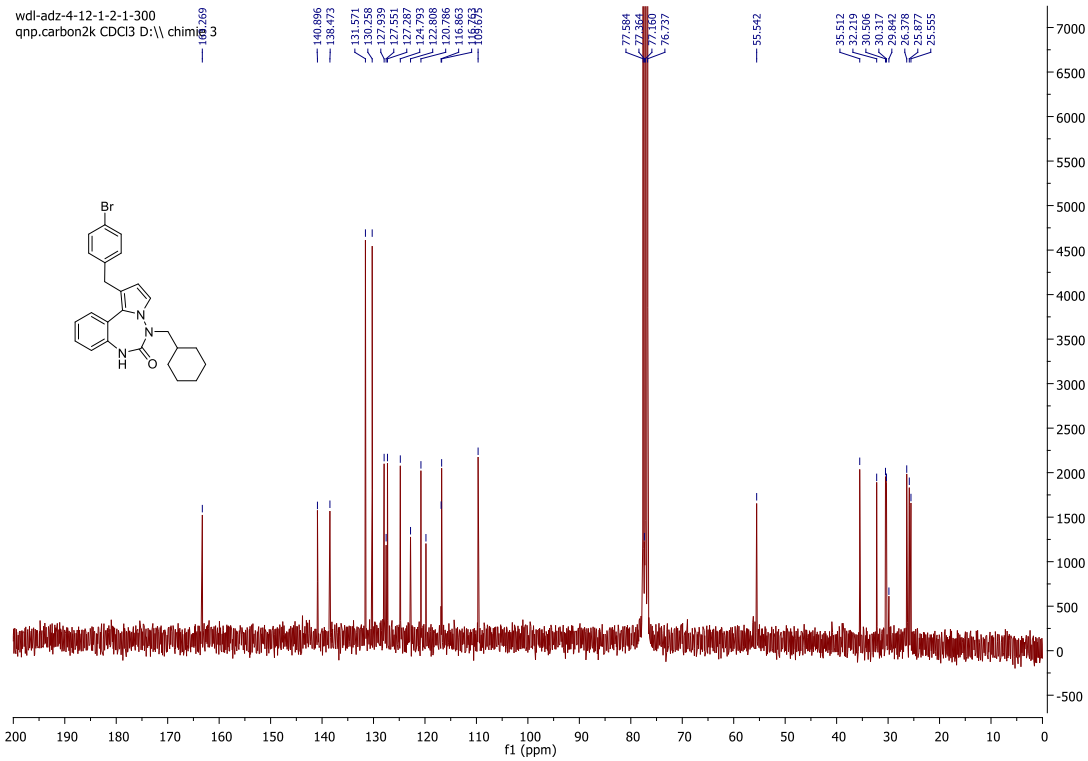
2.7d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



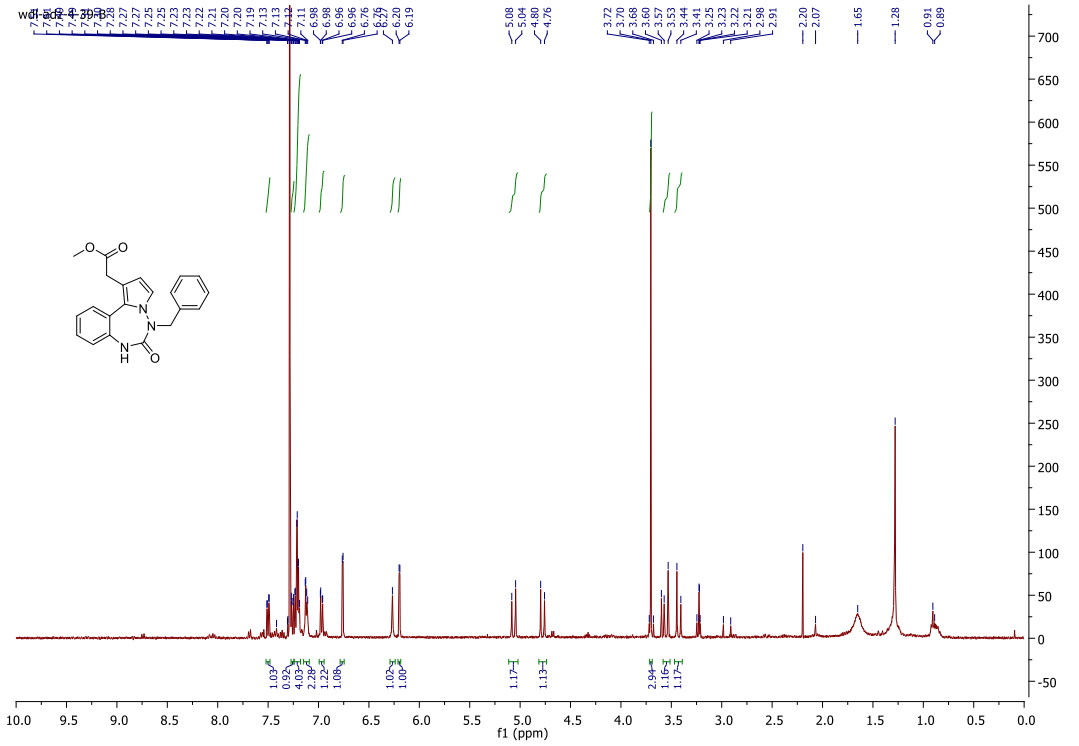


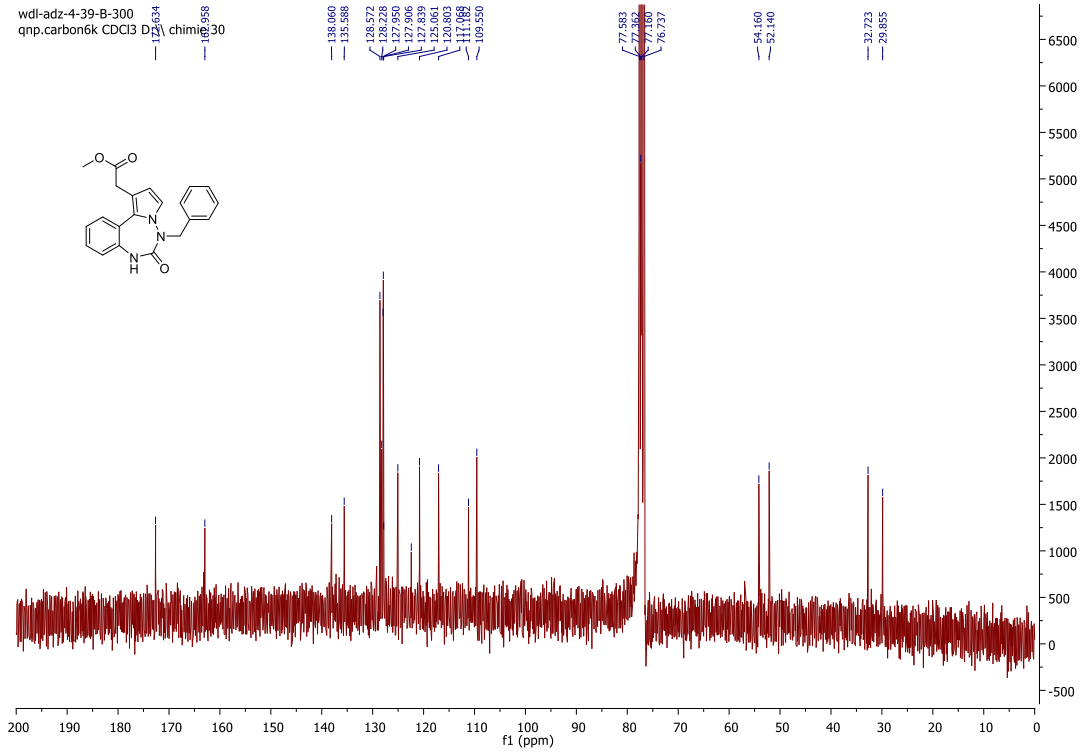
2.7e, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



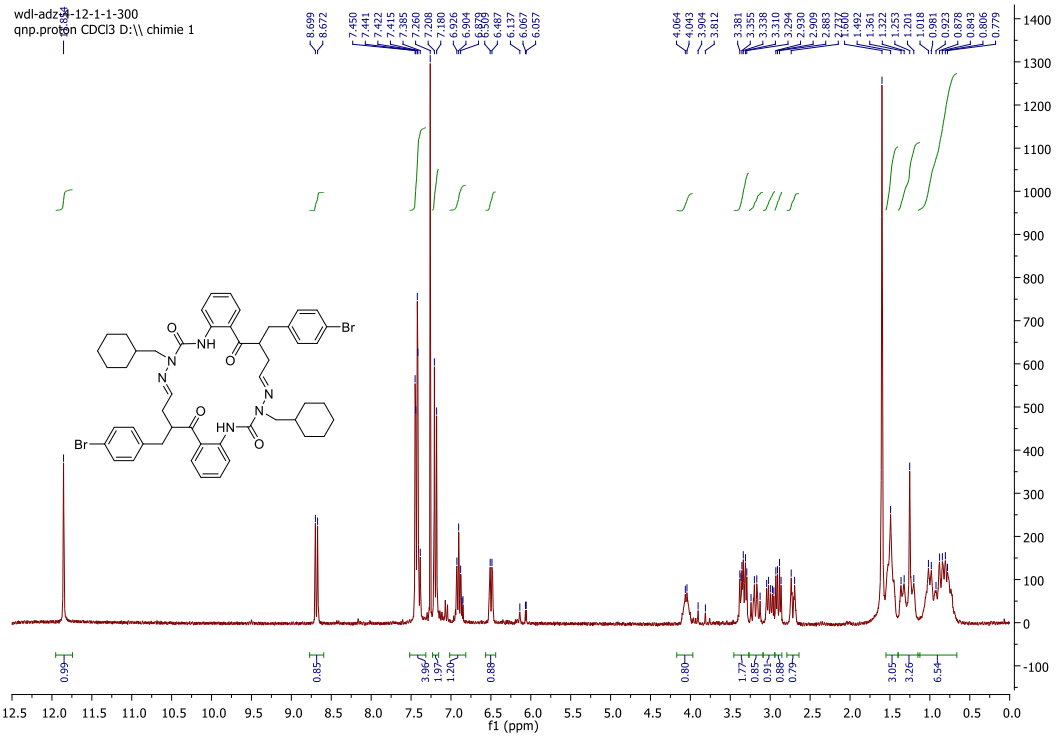


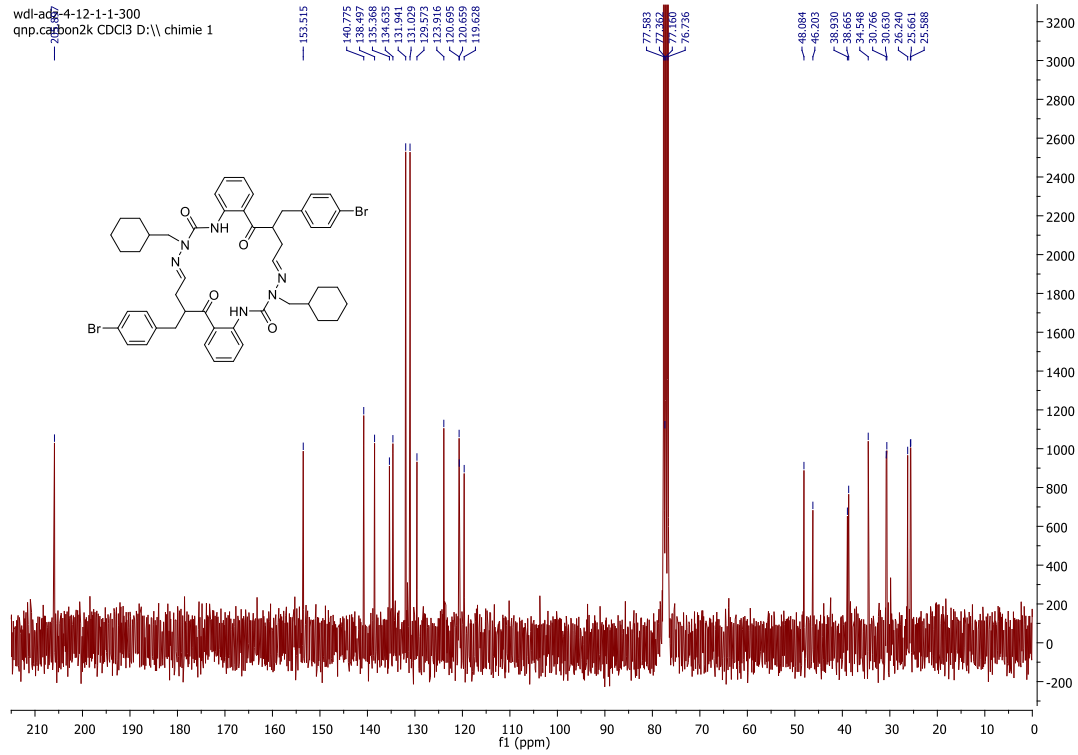
2.7f, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz).



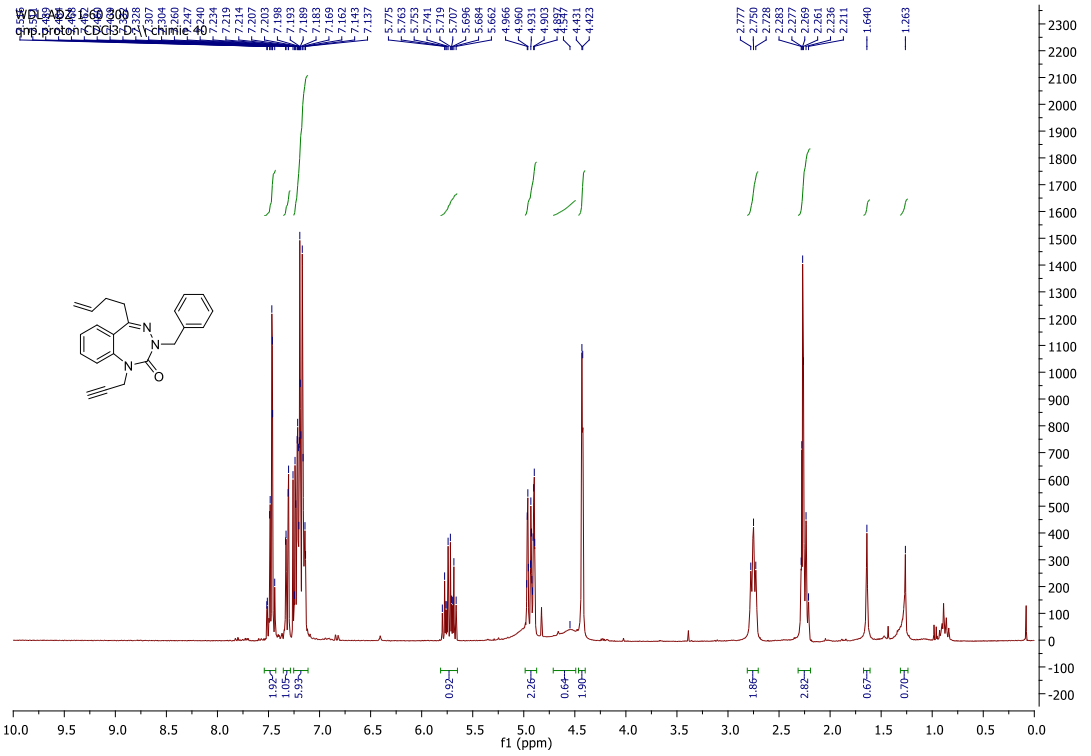


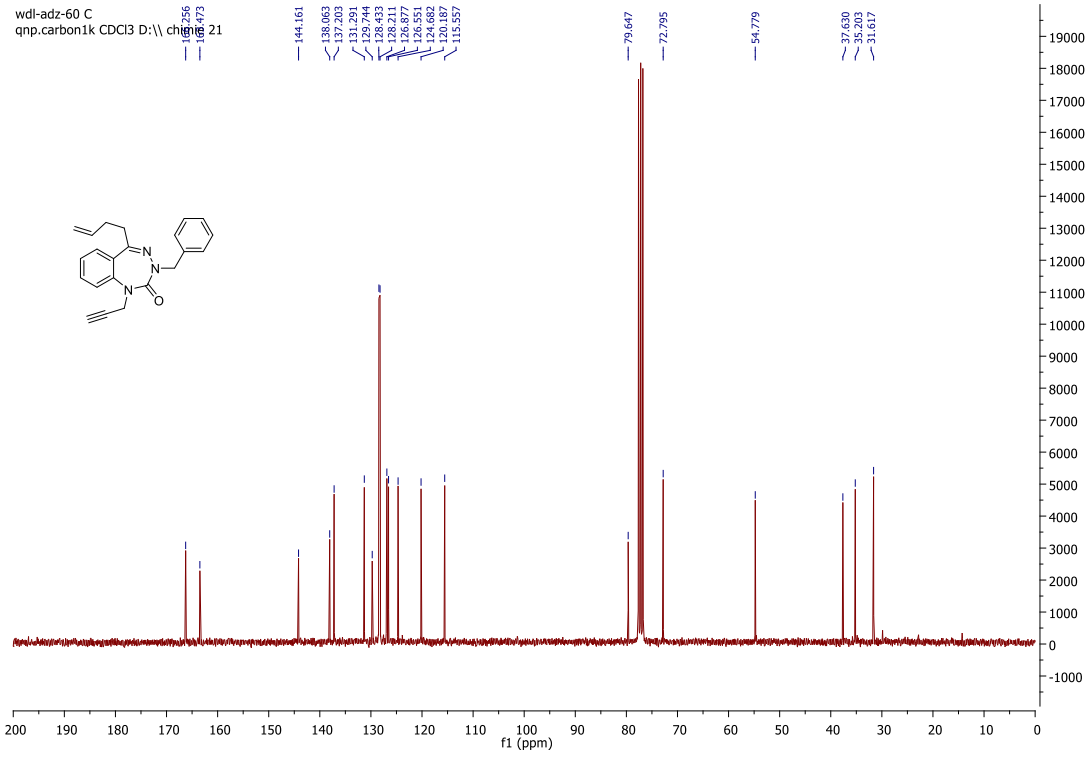
2.21, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



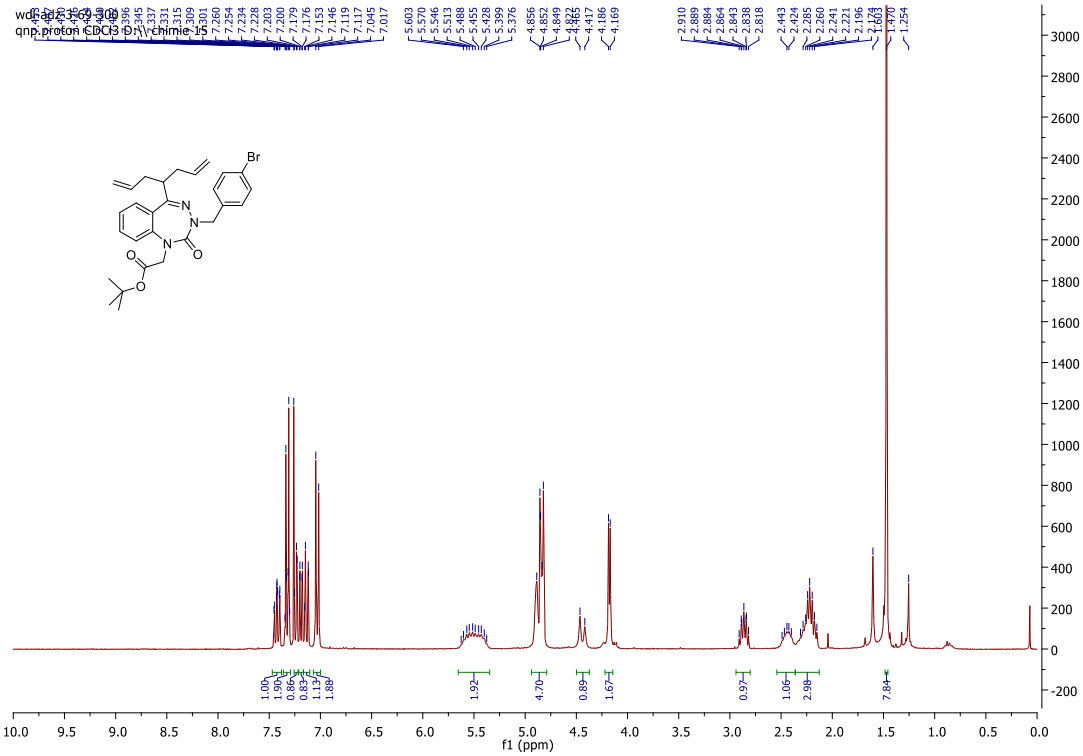


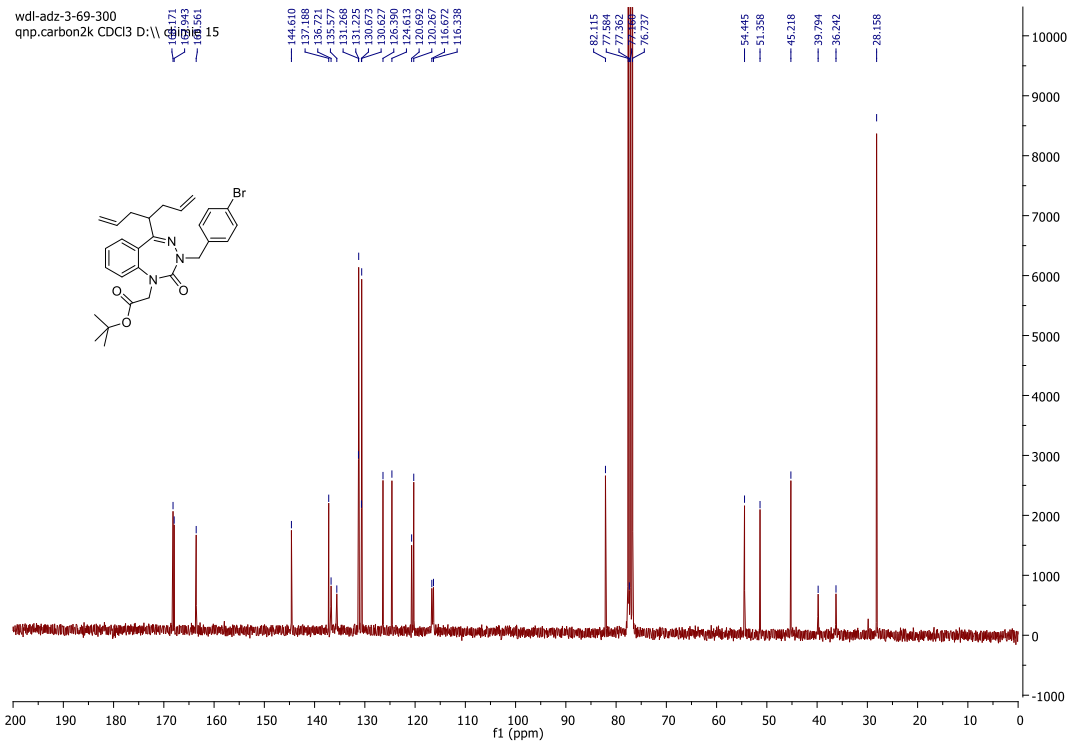
2.19f, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



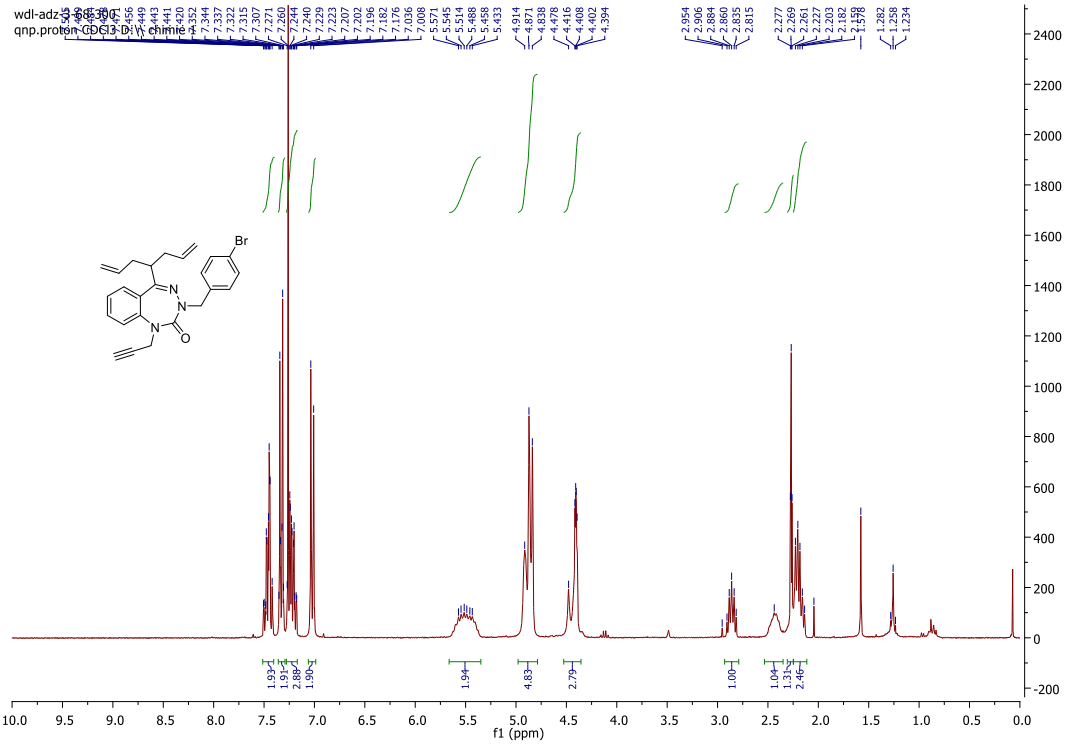


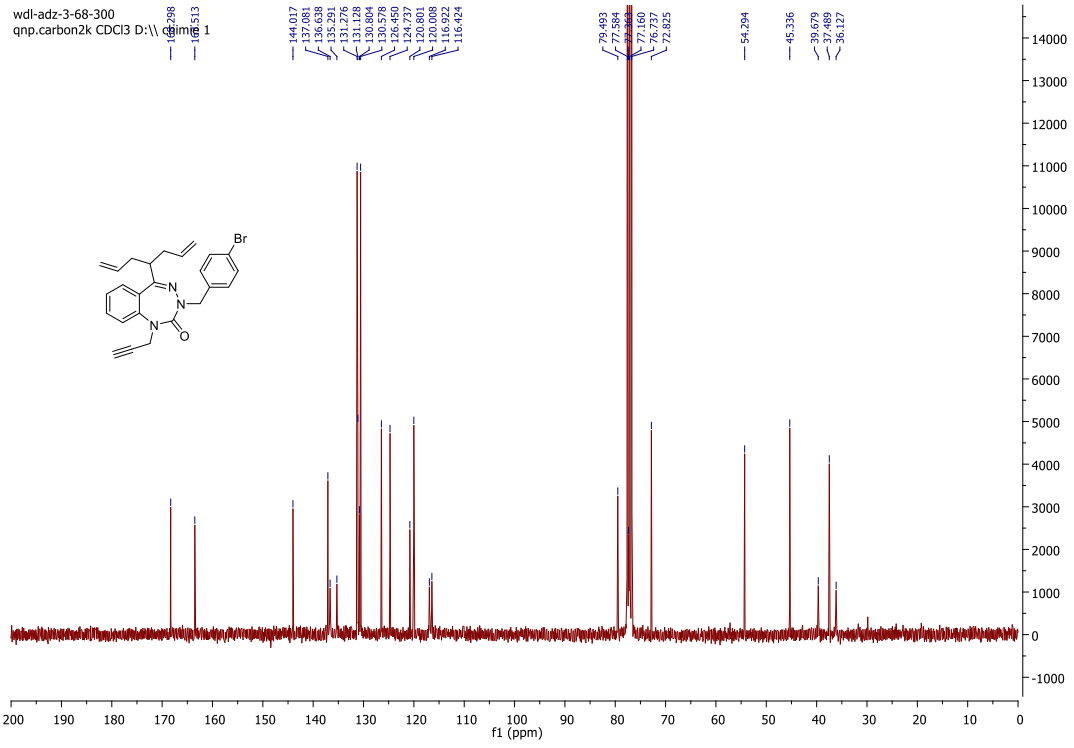
2.19g, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



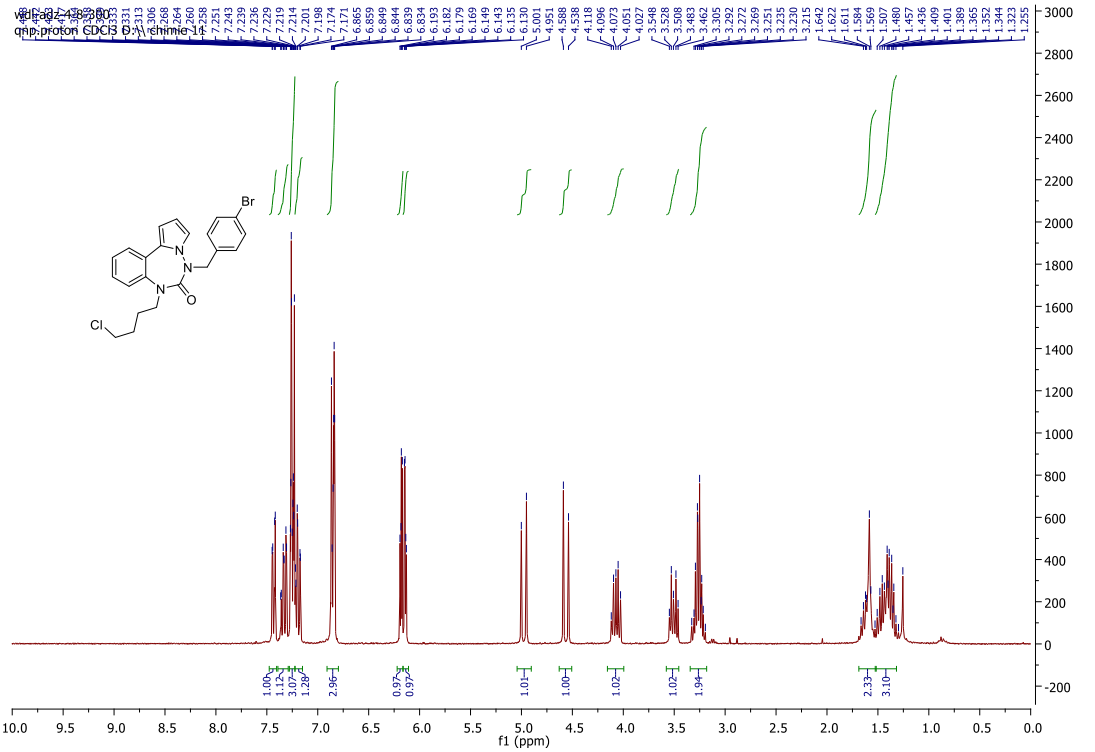


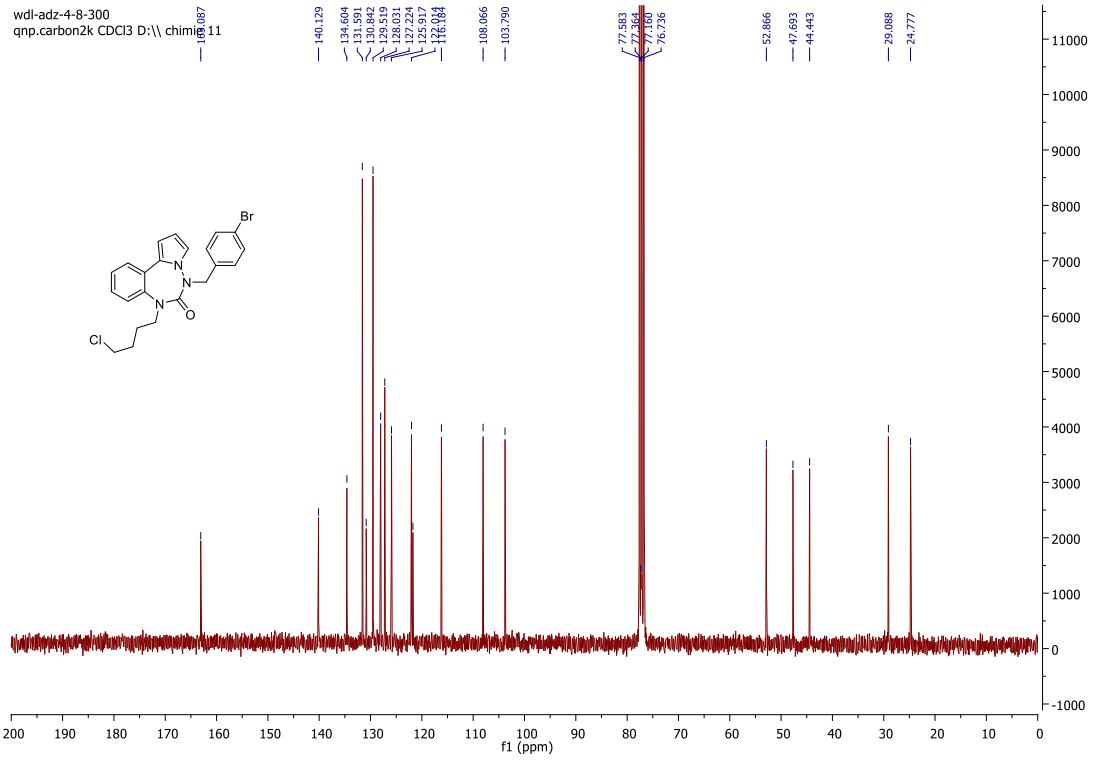
2.19h, **$CDCl_3$**, **^1H (300 MHz)**, **^{13}C (75 MHz)**.



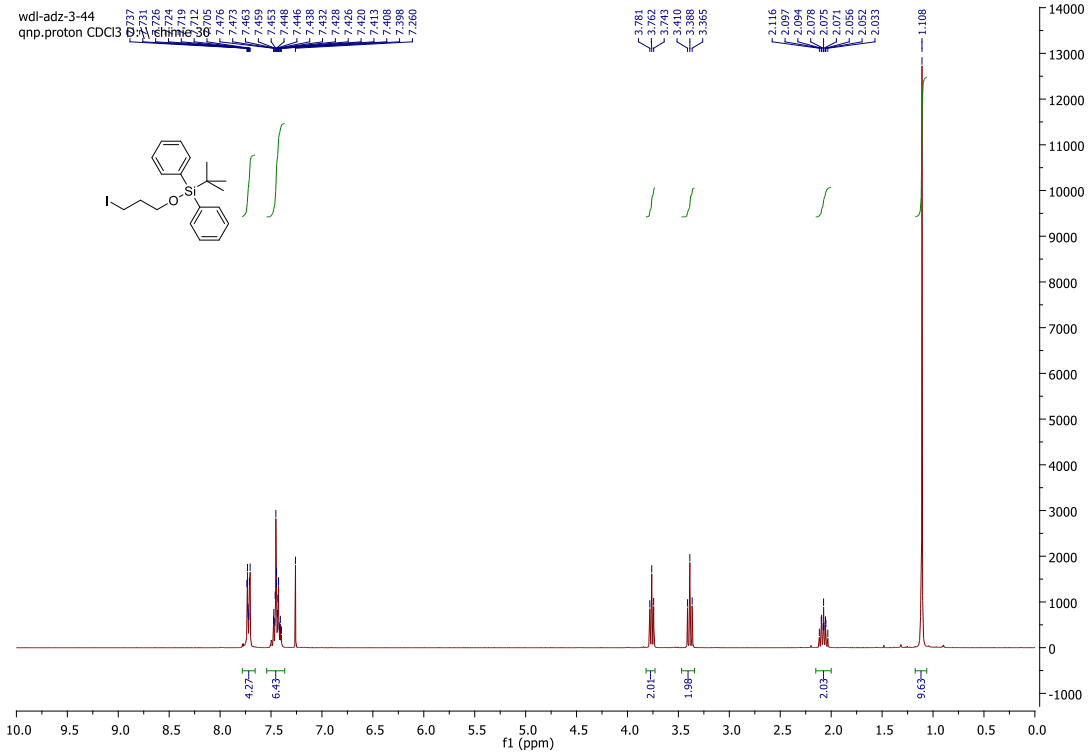


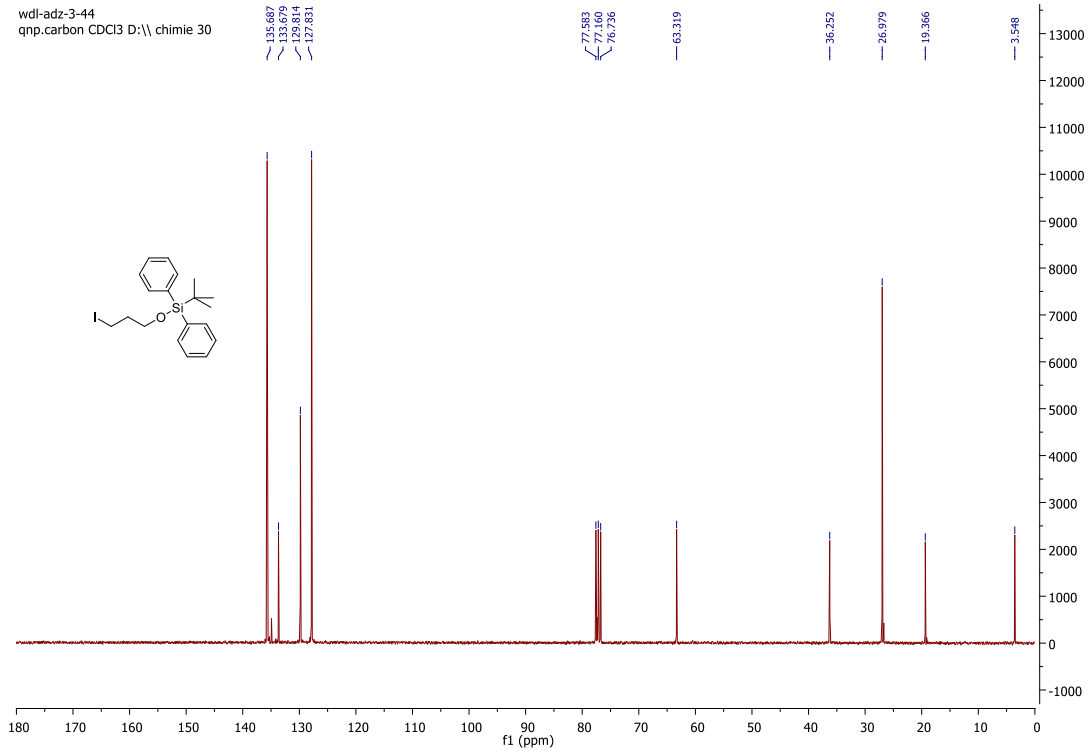
2.23, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).



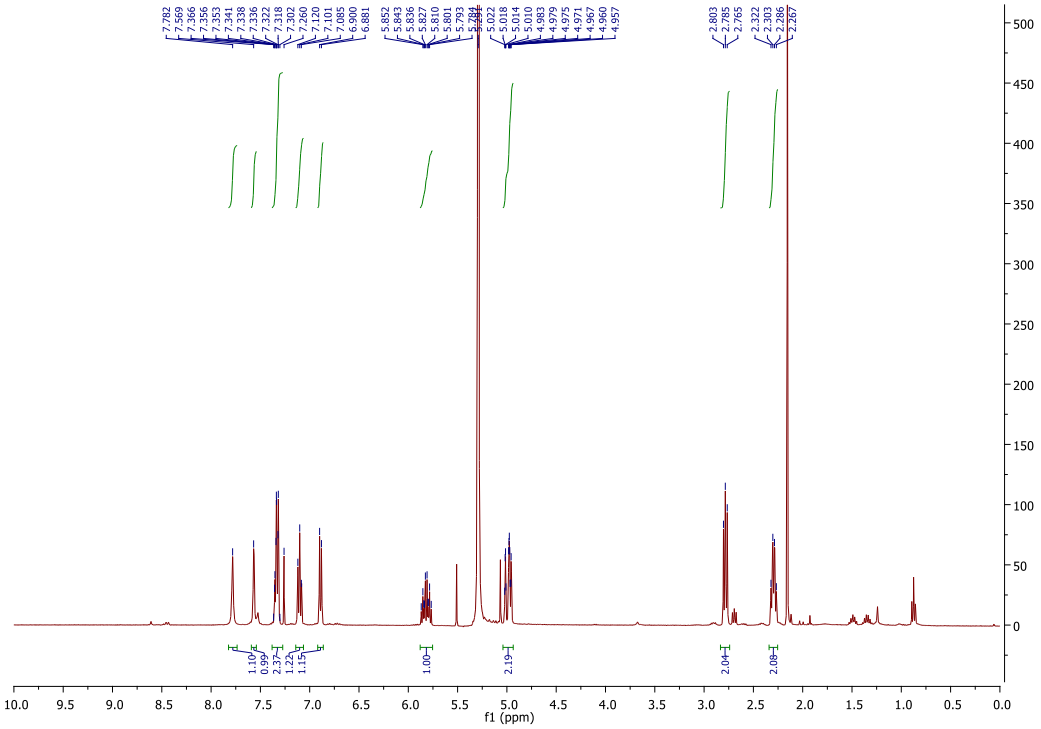


2.28, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz).

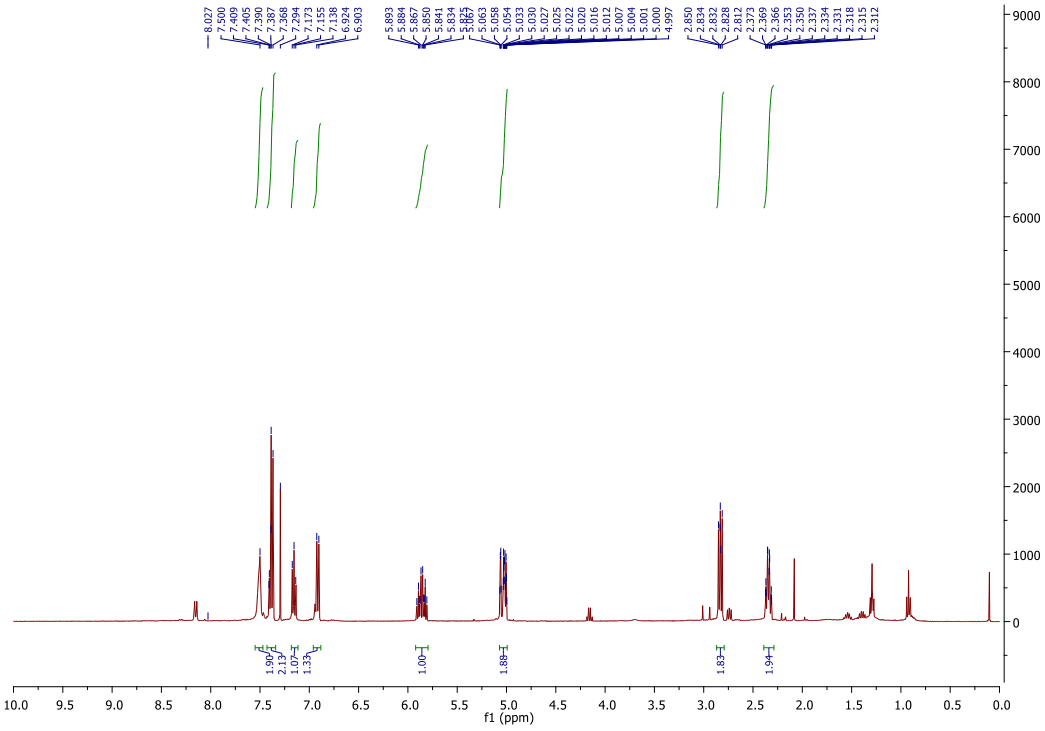




2.25, CDCl3, ^{1}H (400 MHz)[5]

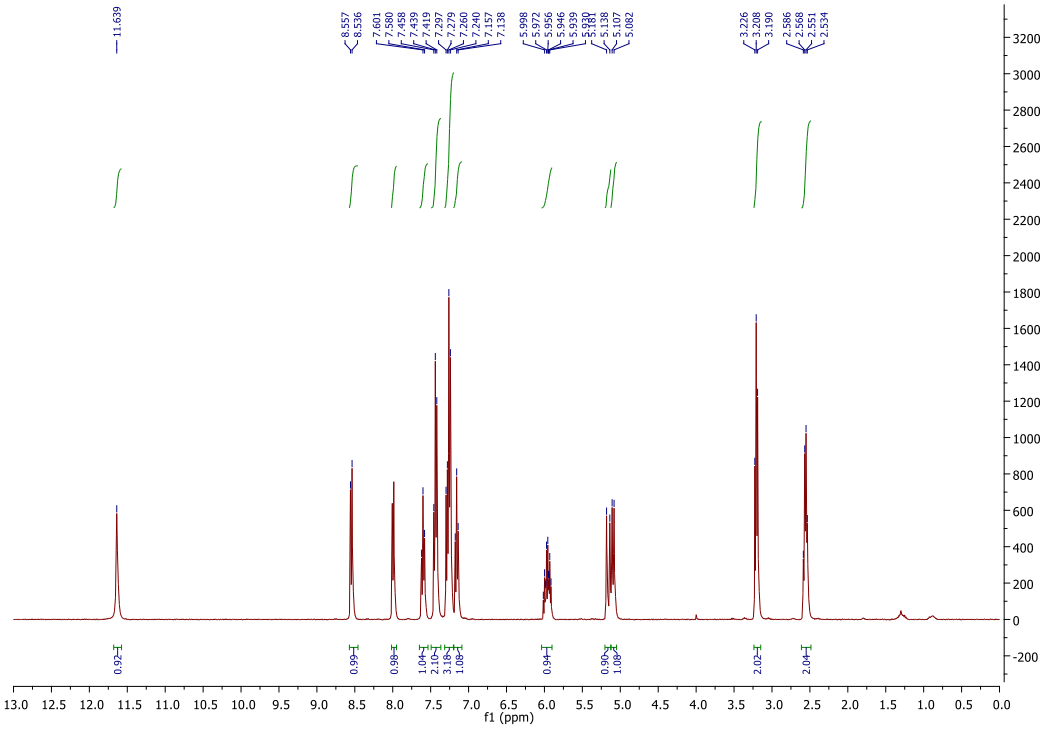


2.24, CDCl3, ^{1}H (400 MHz)[3]

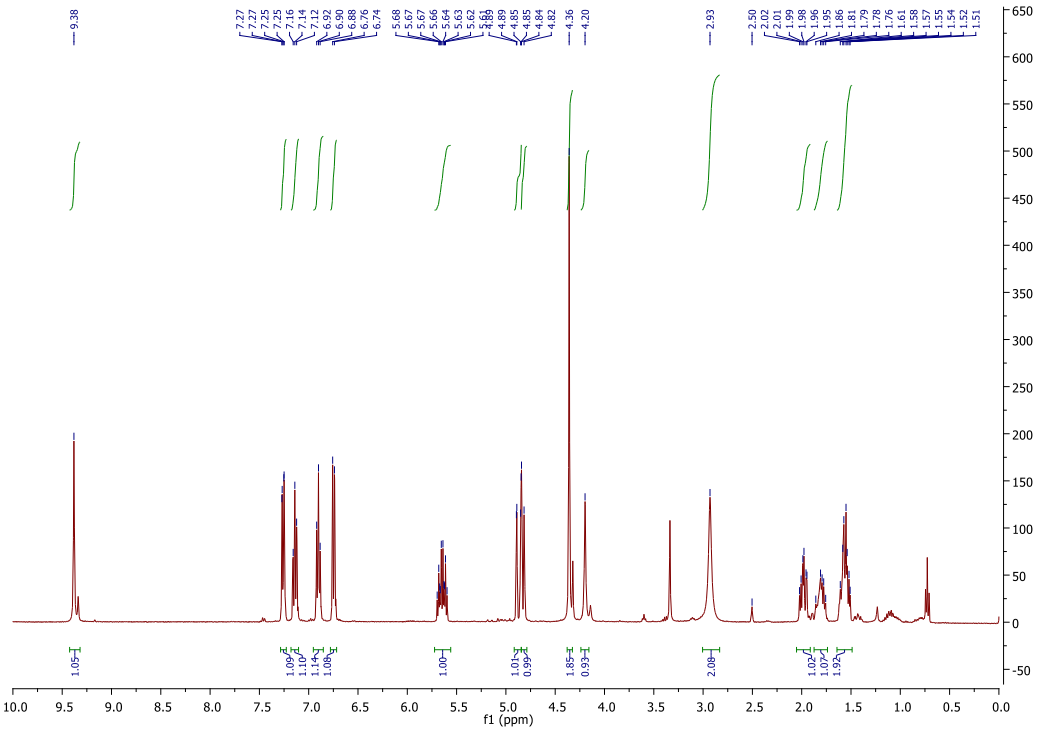


[5] Pages ajoutées comme information soutenante du Chapitre 2

2.26, $CDCl_3$, ^1H (400 MHz)[4]



2.27, DMSO-d_6, ^1H (400 MHz)[6]



[6] Pages ajoutées comme information soutenante du Chapitre 2

Supporting information Article 2

Antoine Douchez ,A.; Billard, E.; Hébert, T. E.; Chatenet, David; Lubel, W. D. Design, synthesis and biological assessment of biased allosteric modulation of the urotensin II receptor using achiral 1,3,4-benzotriazepin-2-one turn mimics. *J. Med. Chem.* **2017**, *60*, 9838–9859.

Supporting Information

Design, synthesis and biological assessment of biased allosteric modulation of the urotensin II receptor using achiral 1,3,4-benzotriazepin-2-one turn mimics.

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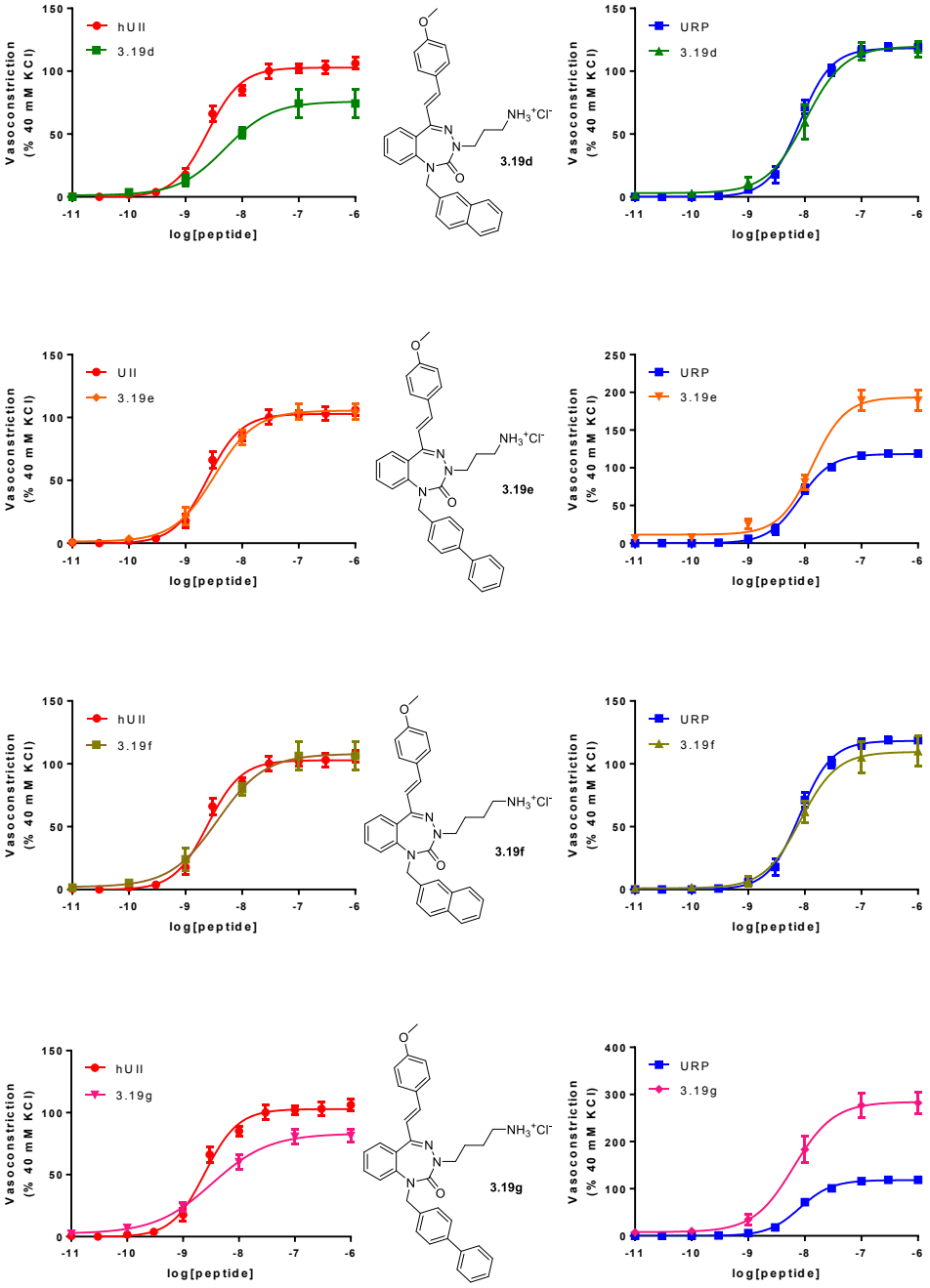
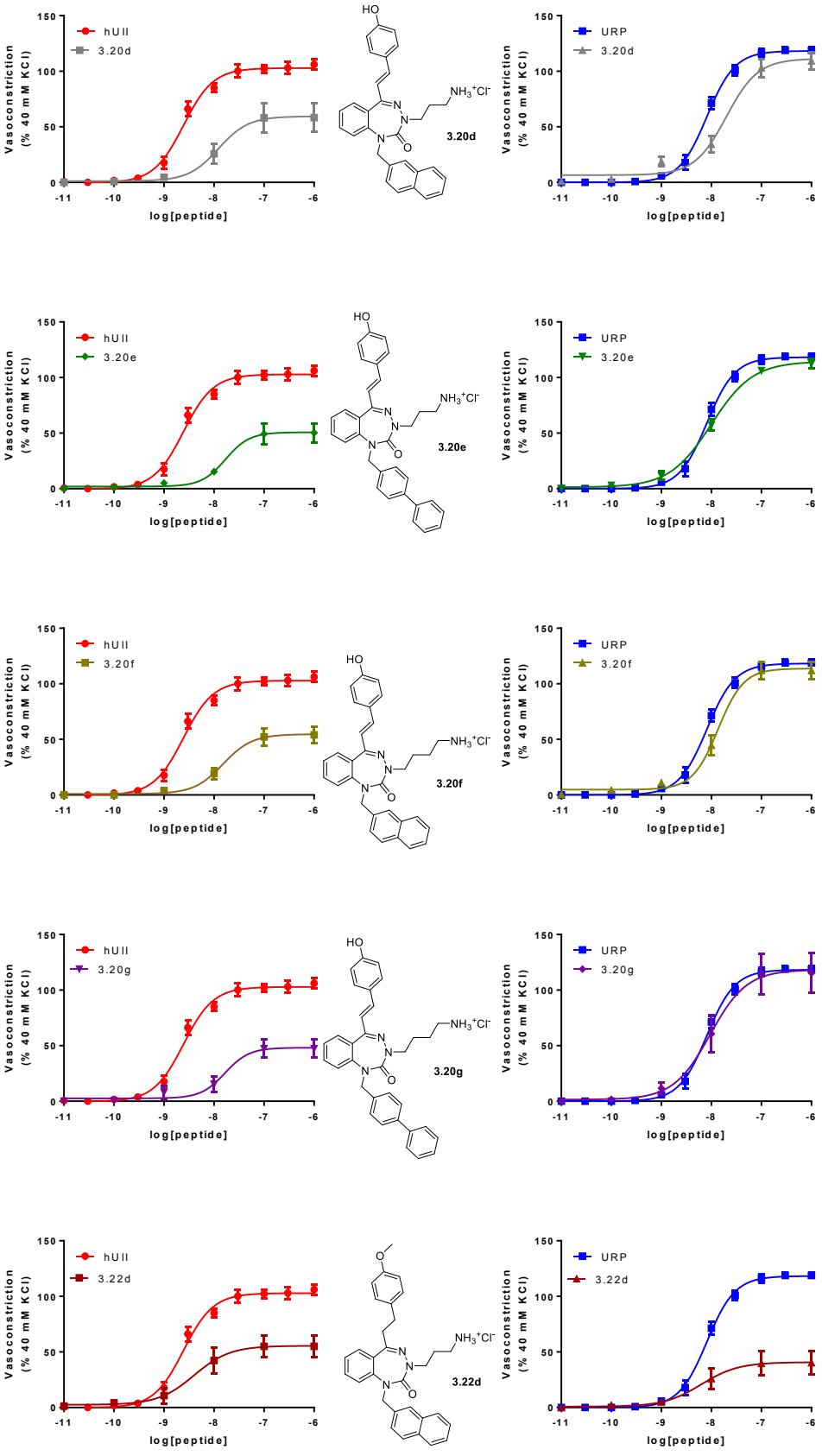
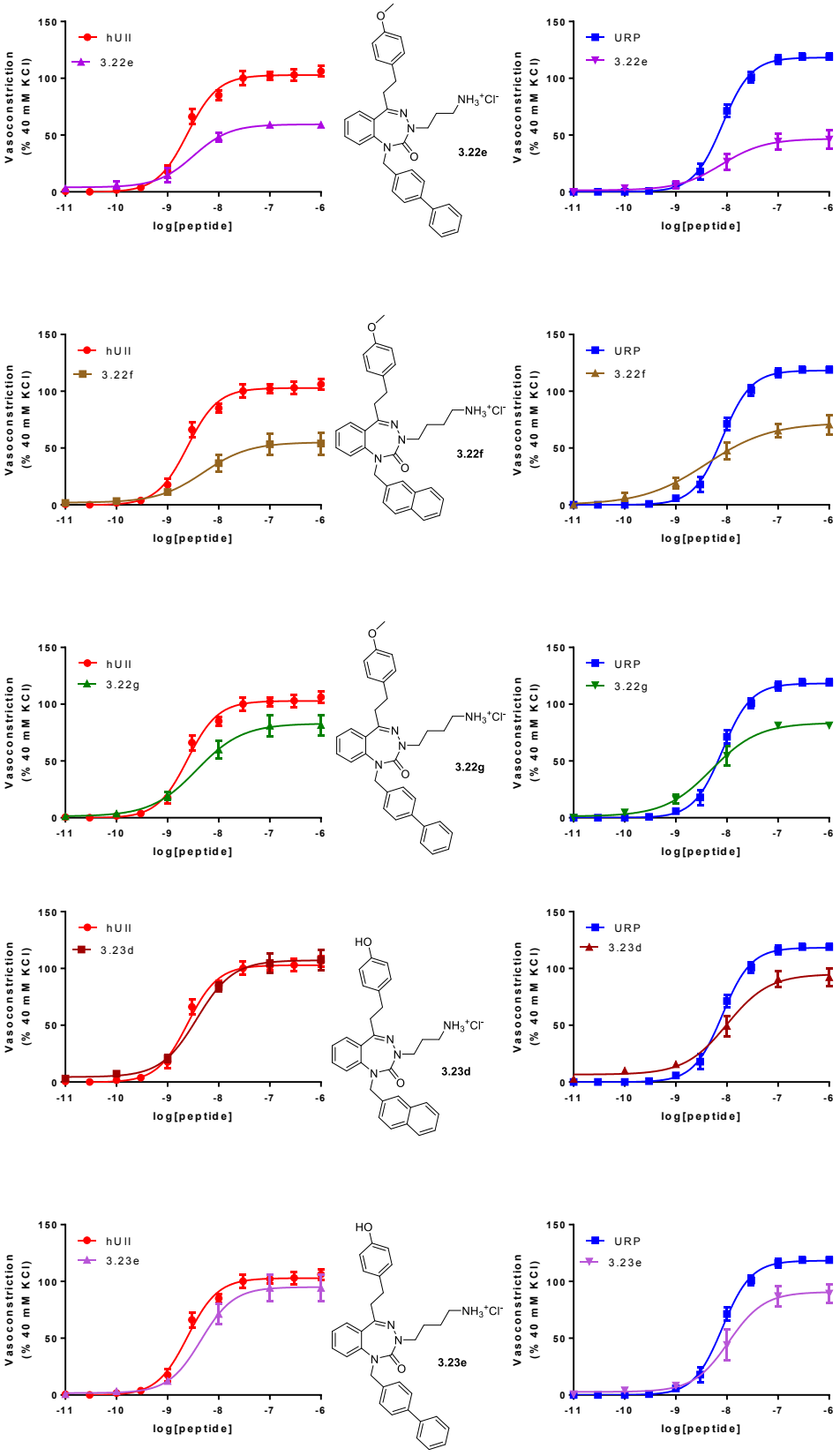
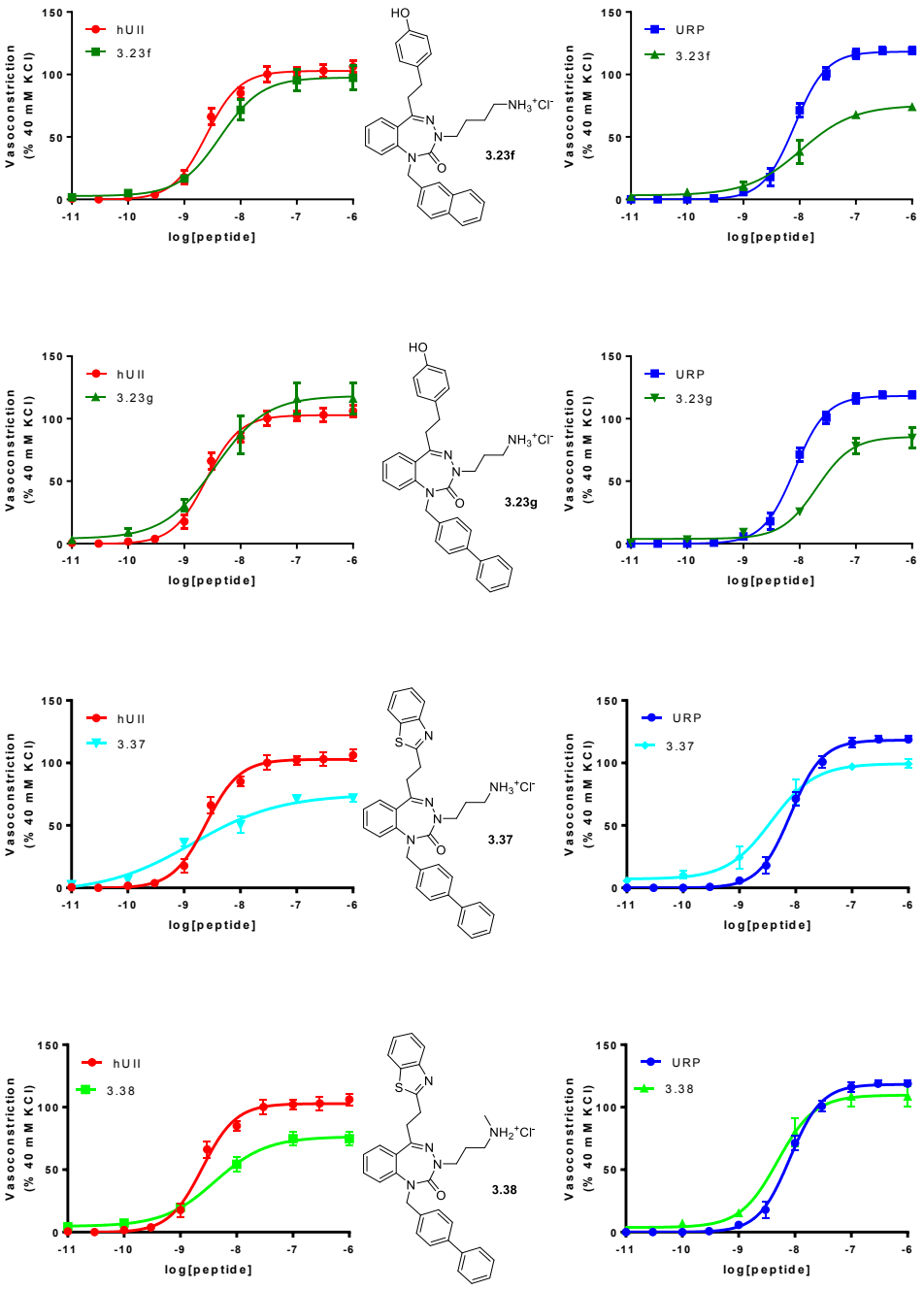
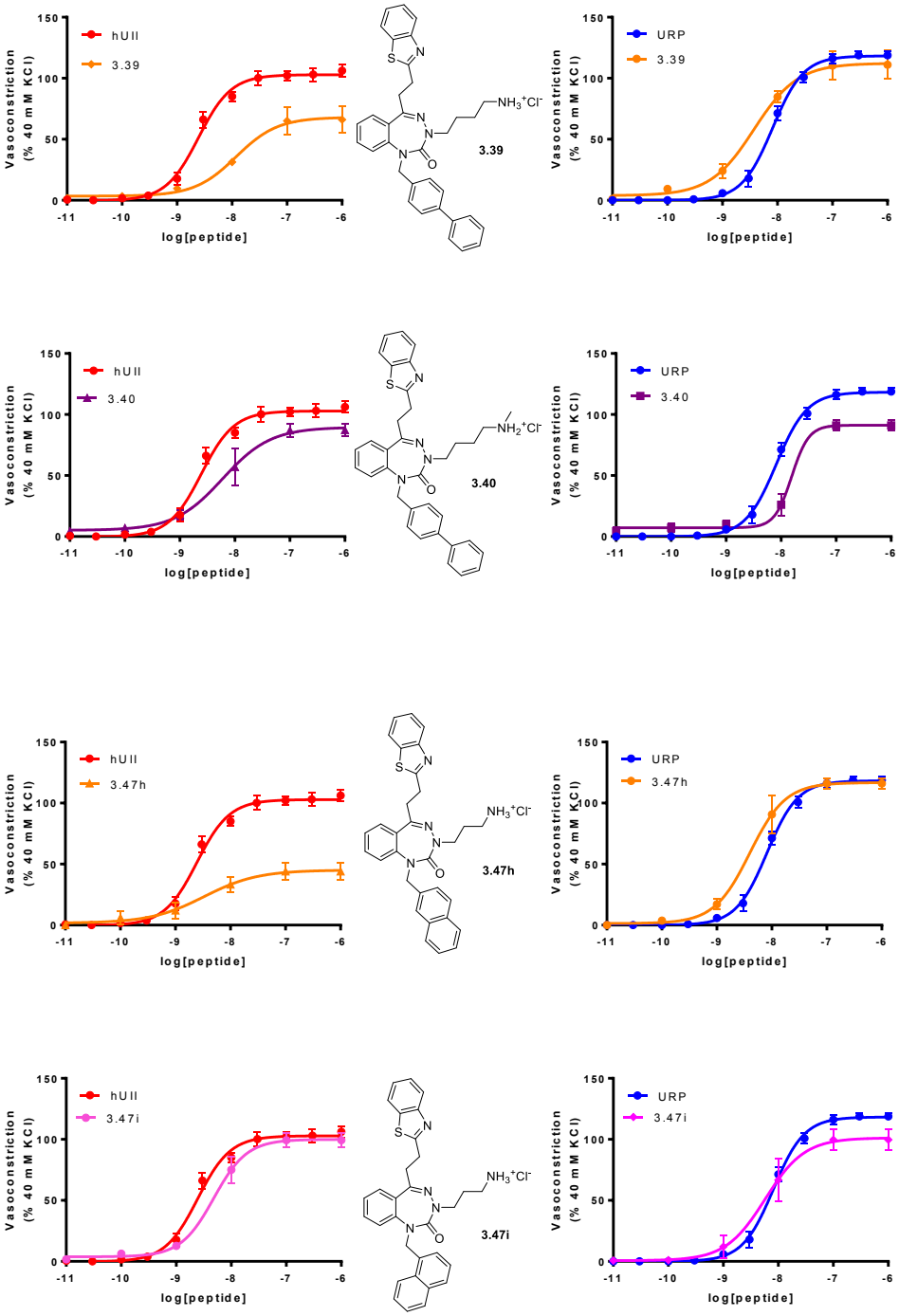
Dose-response curves of hUII- and URP-induced contraction of rat aortic ring in the presence of benzotriazepinone derivatives.

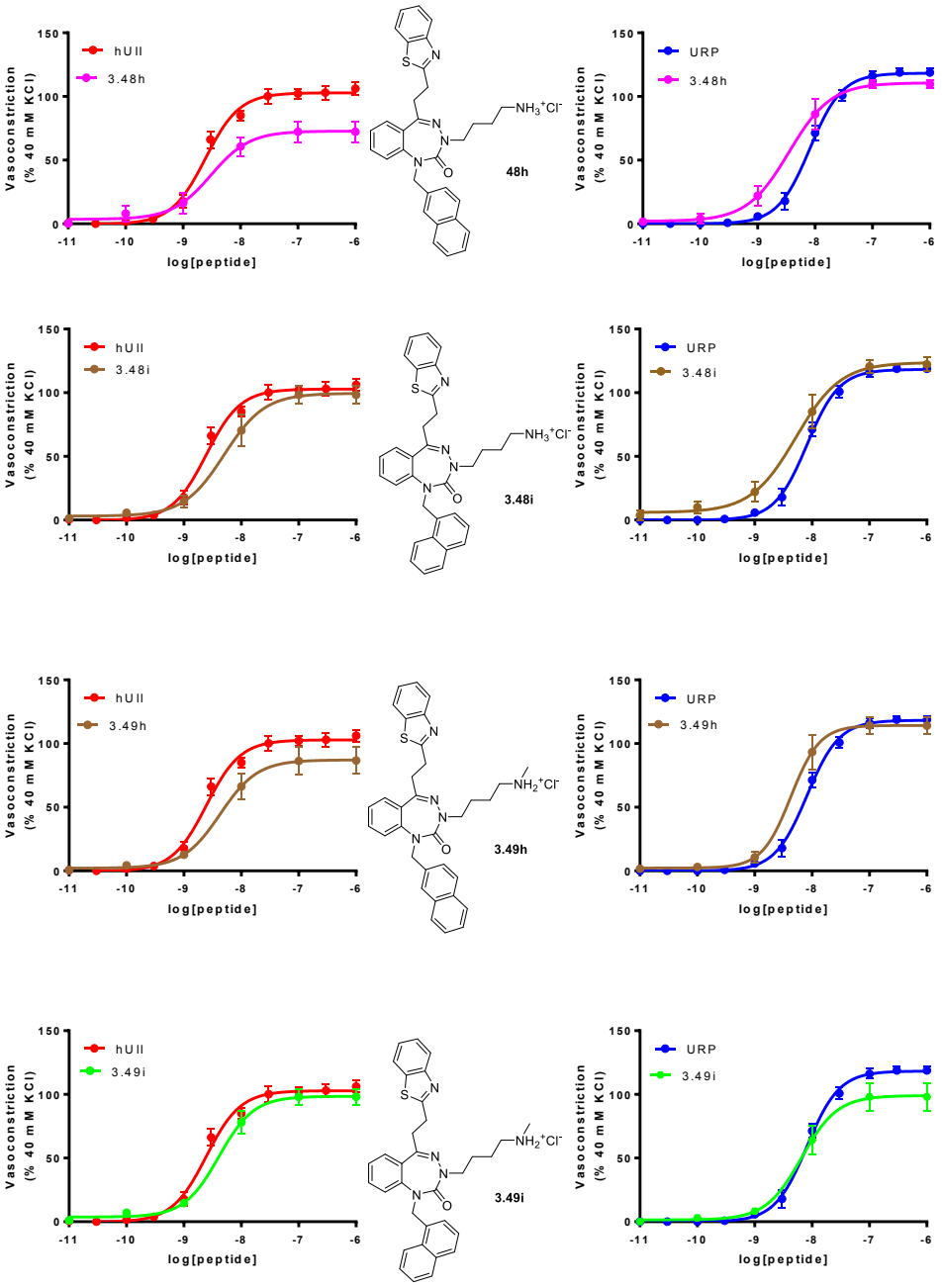



hUII
3.20d
URP
3.20d
3.20d
hUII
3.20e
URP
3.20e
3.20e
hUII
3.20f
URP
3.20f
3.20f
hUII
3.20g
URP
3.20g
3.20g
hUII
3.22d
URP
3.22d
3.22d
Vasoconstriction (% 40 mM KCl)
log[peptide]
HO
NH3+Cl-


hUII
3.22e
URP
3.22e
Vasoconstriction (% 40 mM KCl)
log[peptide]
3.22e
hUII
3.22f
URP
3.22f
3.22f
hUII
3.22g
URP
3.22g
3.22g
hUII
3.23d
URP
3.23d
3.23d
HO
hUII
3.23e
URP
3.23e
3.23e


hUII
3.23f
Vasoconstriction (% 40 mM KCl)
log[peptide]
HO
NH3+Cl-
3.23f
URP
3.23f
hUII
3.23g
3.23g
URP
3.23g
hUII
3.37
3.37
URP
3.37
hUII
3.38
NH2+Cl-
3.38
URP
3.38


hUII
3.39
Vasoconstriction (% 40 mM KCl)
log[peptide]
3.39
URP
3.39
hUII
3.40
3.40
URP
3.40
hUII
3.47h
3.47h
URP
3.47h
hUII
3.47i
3.47i
URP
3.47i


hUII
3.48h
URP
3.48h
Vasoconstriction (% 40 mM KCl)
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48h
hUII
3.48i
URP
3.48i
3.48i
hUII
3.49h
URP
3.49h
3.49h
hUII
3.49i
URP
3.49i
3.49i

Ascertainment of purity by HPLC

Method A: Analytical HPLC, 20 to 90% acetonitrile [0.1% formic acid (FA)] in water (0.1% FA) over 14 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on a Gemini reverse-phase column from Phenomenex (4.6 mm × 150 mm, 5 μm, C18).

Method B: Analytical HPLC, 10 to 90% acetonitrile (0.1% FA) in water (0.1% FA) over 9 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18).

Method C: Analytical HPLC, 20 to 90% acetonitrile (0.1% FA) in water (0.1% FA) over 9 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18).

Method D: Analytical HPLC, 30 to 90% acetonitrile (0.1% FA) in water (0.1% FA) over 9 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18).

Method E: Analytical HPLC, 40 to 90% acetonitrile (0.1% FA) in water (0.1% FA) over 9 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on a SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18).

Method F: Analytical HPLC, 60 to 90% methanol (0.1% FA) in water (0.1% FA) over 14 min followed by 90% methanol (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on a Gemini reverse-phase column from Phenomenex (4.6 mm × 150 mm, 5 μm, C18).

Method G: Analytical HPLC, 20 to 90% methanol (0.1% FA) in water (0.1% FA) over 9 min followed by 90% methanol in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on a SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 μm, C18).

Method H: Analytical HPLC, 40 to 90% methanol (0.1% FA) in water (0.1% FA) over 9 min followed by 90% methanol (0.1% FA) in water (0.1% FA) over 1 min,

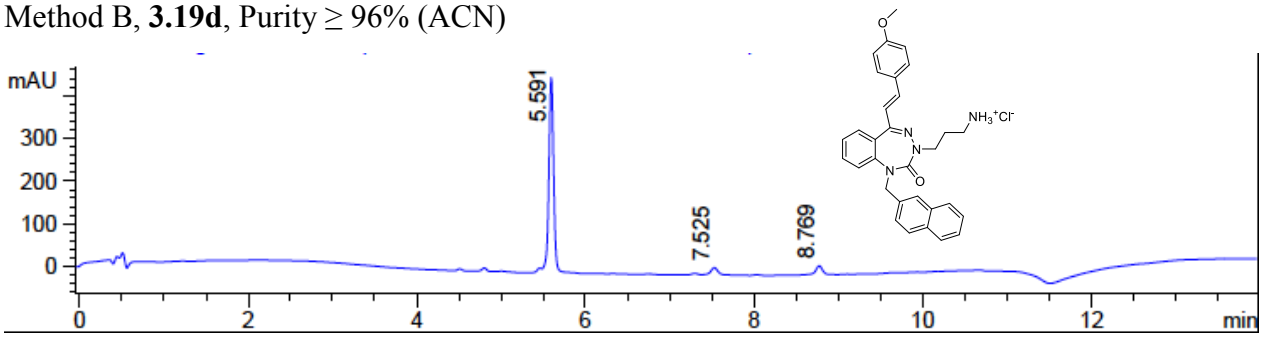
flow rate of 0.5 mL/min on a a SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 µm, C18).

HPLC purity table.

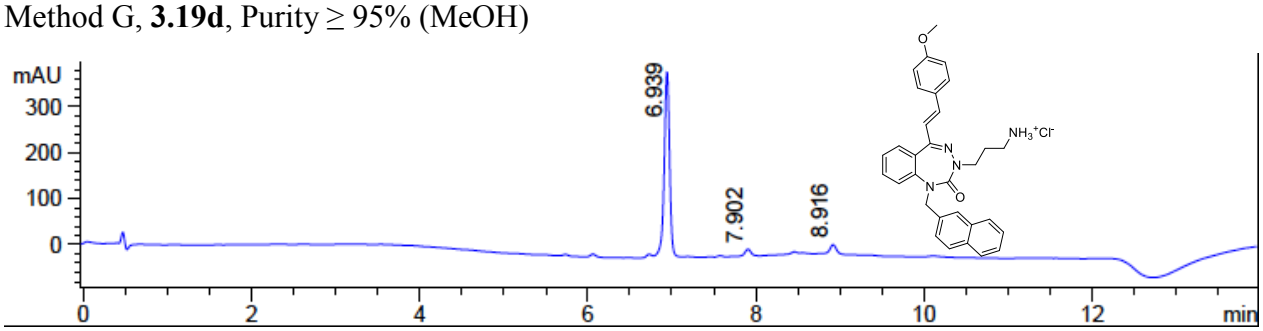
Benzotriazepin-2-one	HPLC Method	t_r (min)	Purity
3.19d	B	5.591	≥ 96%
	G	6.939	≥ 95%
3.19e	B	5.777	≥97%
	G	7.151	≥ 97%
3.19f	B	5.848	≥ 95%
	G	7.290	≥ 96%
3.19g	B	6.048	≥ 96%
	G	7.566	≥ 98%
3.20d	B	5.059	≥ 97%
	G	6.402	≥ 96%
3.20e	B	5.481	≥ 98%
	G	6.856	≥ 95%
3.20f	B	5.196	≥ 96%
	G	6.479	≥ 97%
3.20g	B	5.425	≥ 95%
	G	6.700	≥ 97%
3.22d	B	5.537	≥ 95%
	G	6.891	≥ 95%
3.22e	B	5.755	≥ 99%
	G	7.122	≥ 99%
3.22f	B	5.603	≥ 96%
	G	7.198	≥ 97%
3.22g	B	5.655	≥ 96%
	G	7.029	≥ 96%
3.23d	B	5.044	≥ 96%
	G	6.356	≥ 95%

3.23e	B	5.478	≥ 95%
	G	6.813	≥ 98%
3.23f	B	5.161	≥ 97%
	G	6.548	≥ 98%
3.23g	B	5.398	≥ 98%
	G	6.752	≥ 97%
3.37	A	8.63	≥ 99%
	F	5.60	≥ 99%
3.38	A	8.67	≥ 96%
	F	5.76	≥ 96%
3.39	A	8.72	≥ 97%
	F	6.25	≥ 97%
3.40	A	9.05	≥ 95%
	F	5.99	≥ 97%
3.47h	D	4.242	≥ 99%
	H	6.588	≥ 99%
3.47i	D	3.722	≥ 99%
	H	6.012	≥ 99%
3.48h	E	1.813	≥ 99%
	H	6.239	≥ 99%
3.48i	D	6.322	≥ 95%
	H	6.010	≥ 96%
3.49h	C	6.226	≥ 99%
	H	6.134	≥ 99%
3.49i	A	8.29	≥ 98%
	H	6.112	≥ 99%

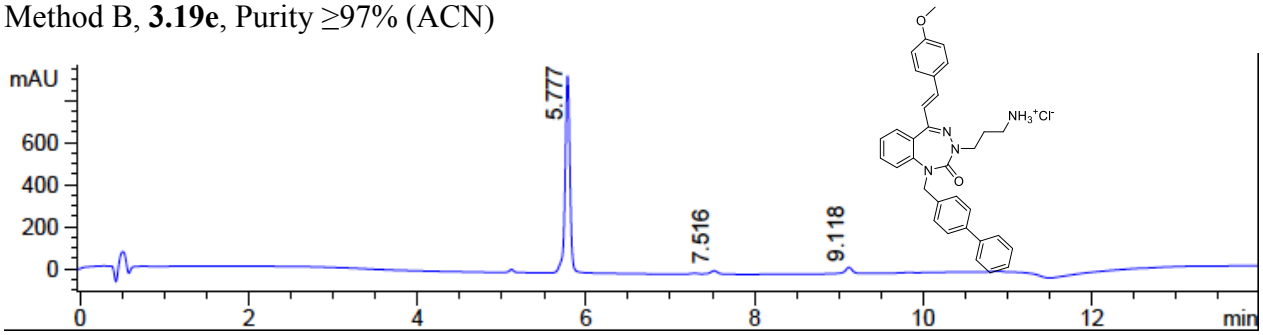
Method B, **3.19d**, Purity ≥ 96% (ACN)



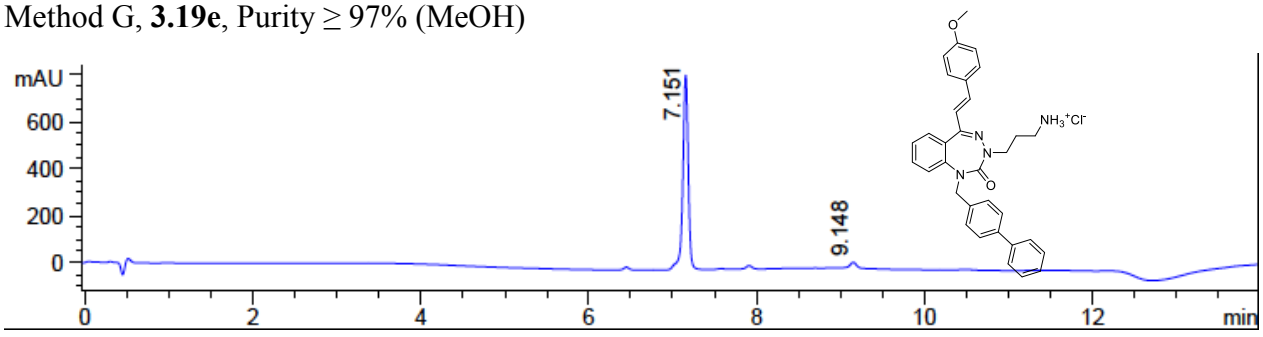
Method G, **3.19d**, Purity ≥ 95% (MeOH)



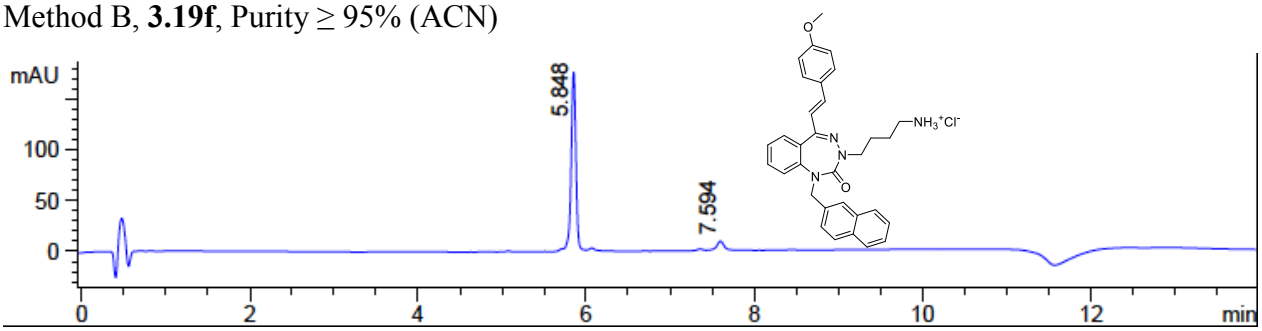
Method B, **3.19e**, Purity ≥97% (ACN)



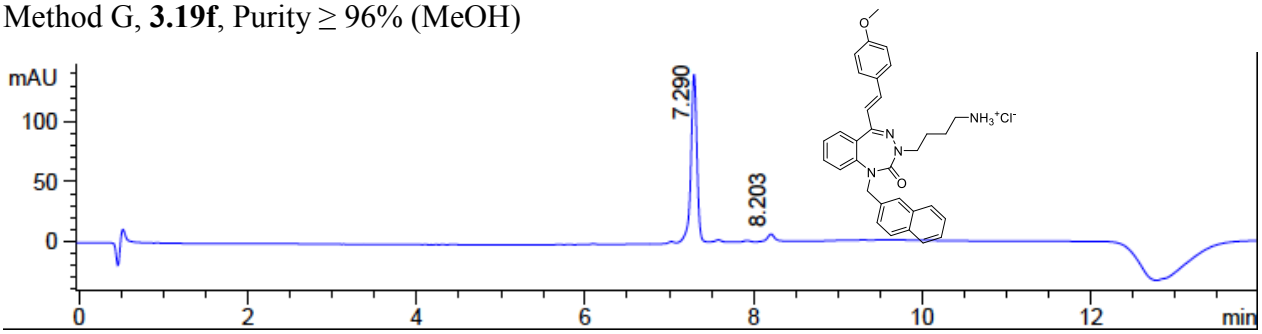
Method G, **3.19e**, Purity ≥ 97% (MeOH)



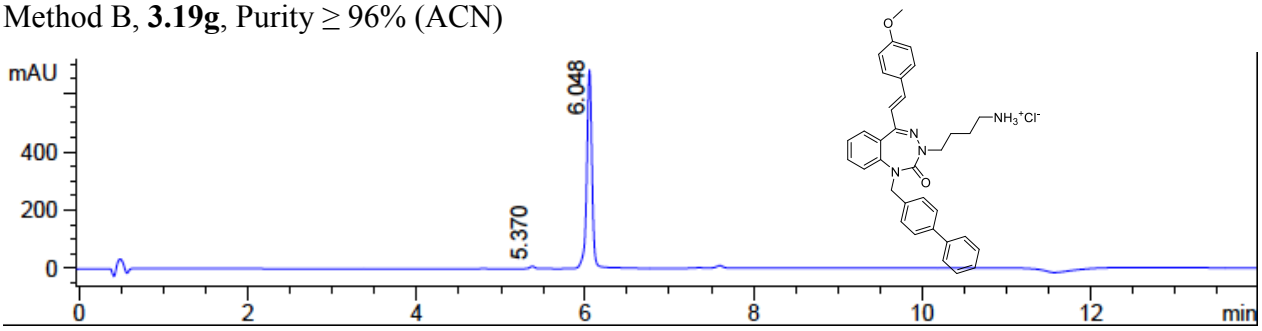
Method B, **3.19f**, Purity ≥ 95% (ACN)



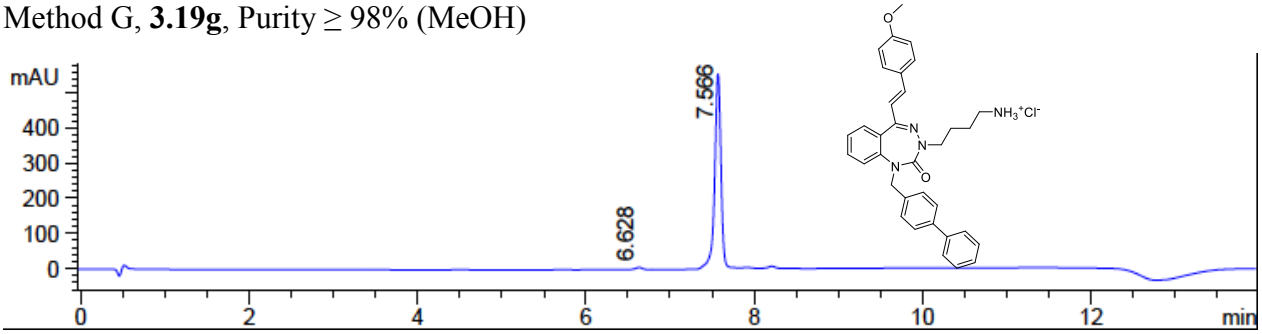
Method G, **3.19f**, Purity ≥ 96% (MeOH)



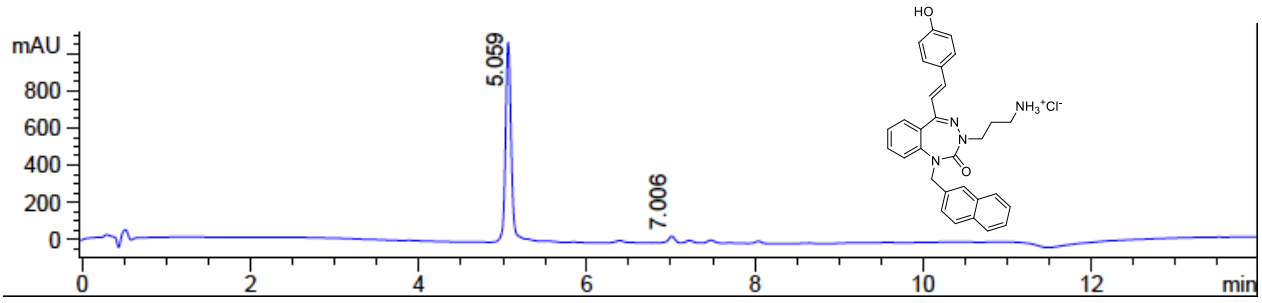
Method B, **3.19g**, Purity ≥ 96% (ACN)



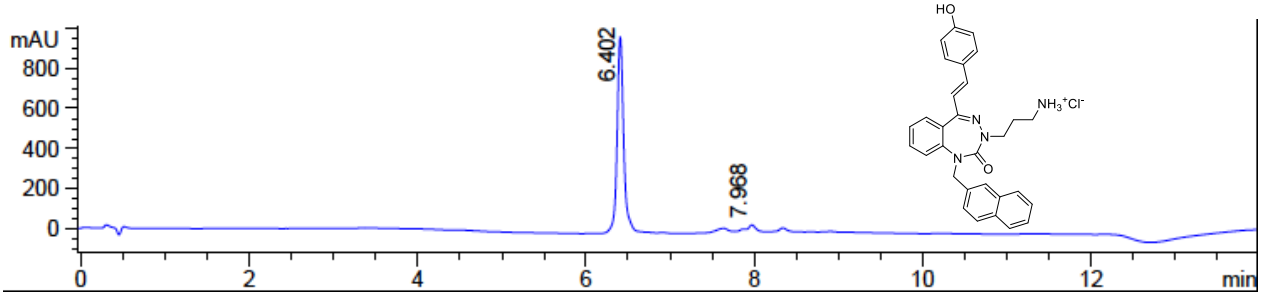
Method G, **3.19g**, Purity ≥ 98% (MeOH)



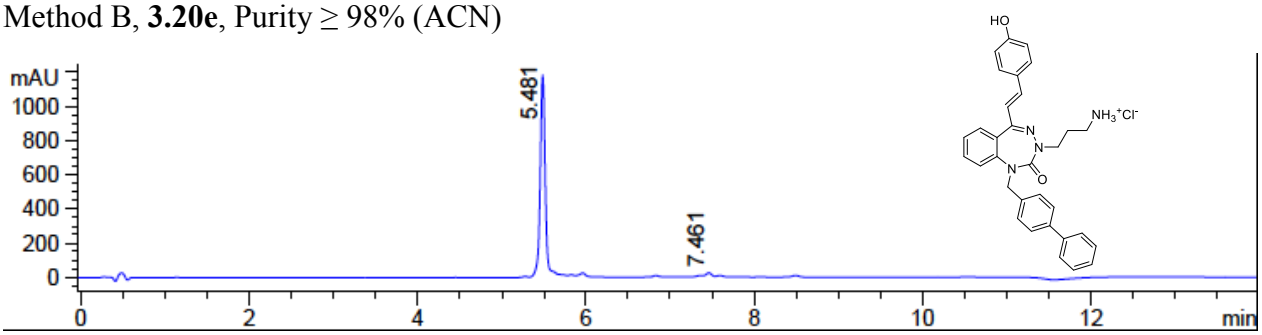
Method B, **3.20d**, Purity ≥ 97% (ACN)



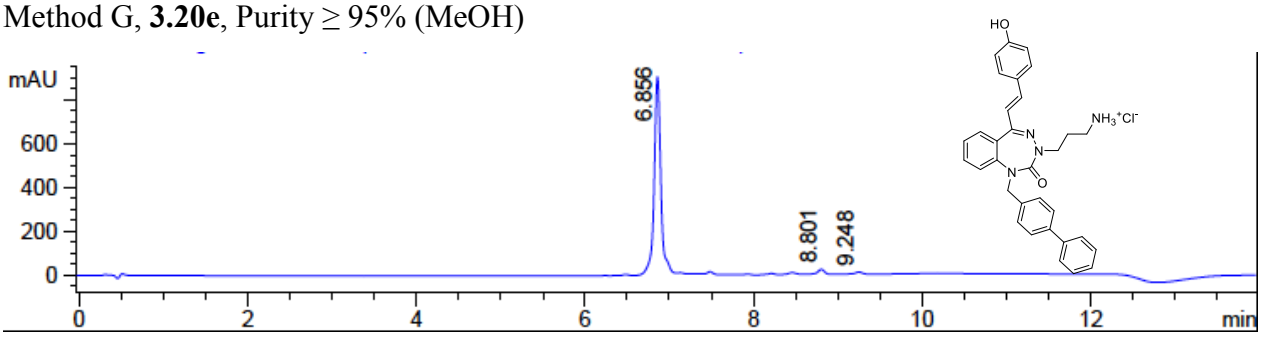
Method G, **3.20d**, Purity ≥ 96% (MeOH)



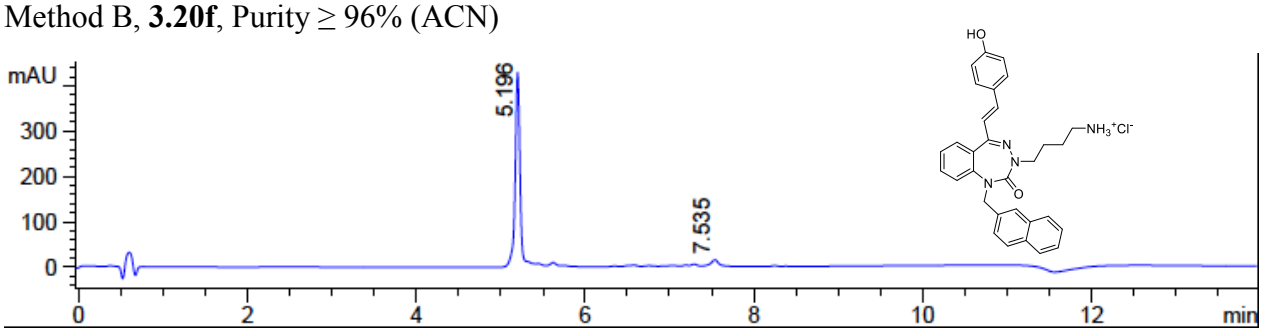
Method B, **3.20e**, Purity ≥ 98% (ACN)



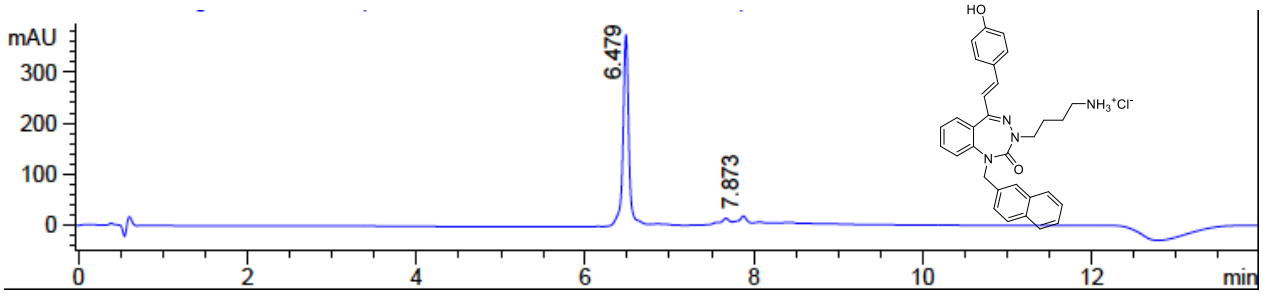
Method G, **3.20e**, Purity ≥ 95% (MeOH)



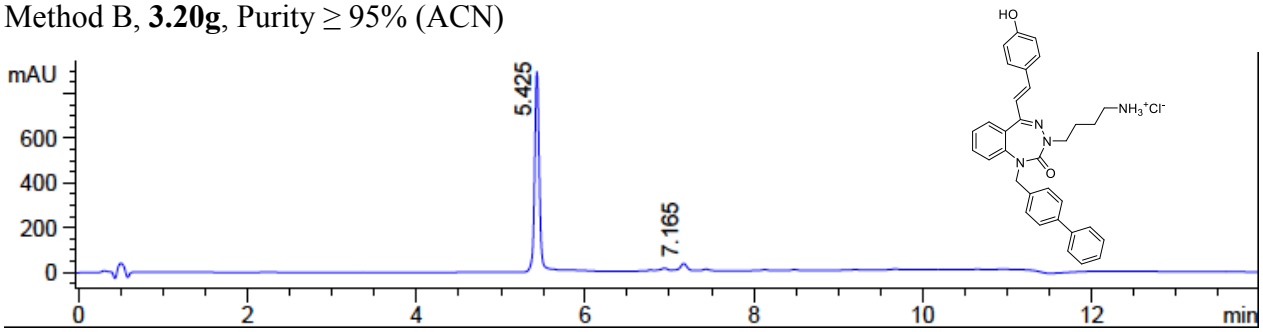
Method B, **3.20f**, Purity ≥ 96% (ACN)



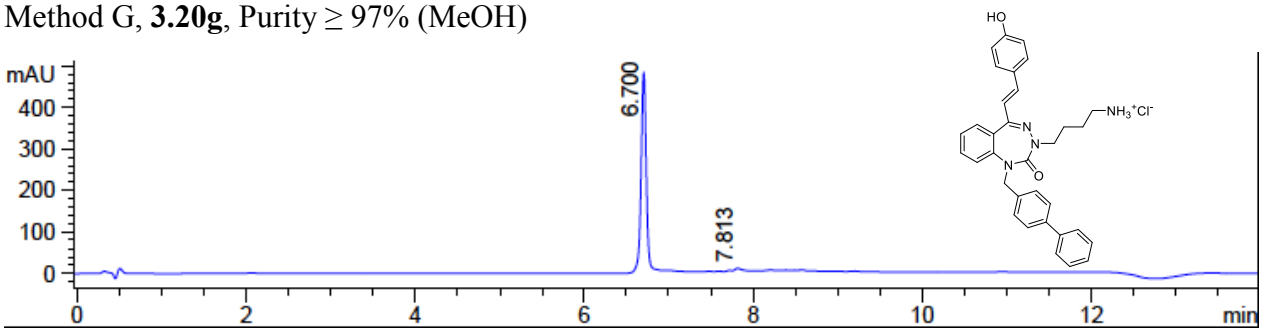
Method G, **3.20f**, Purity ≥ 97% (MeOH)



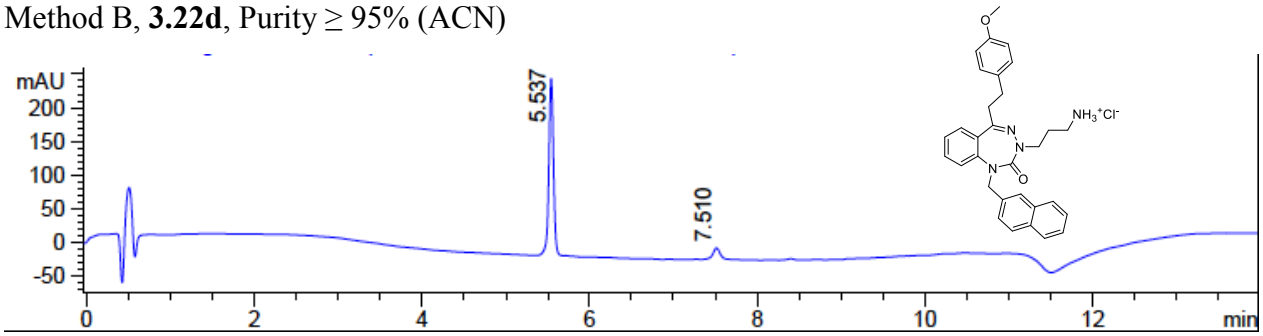
Method B, **3.20g**, Purity ≥ 95% (ACN)



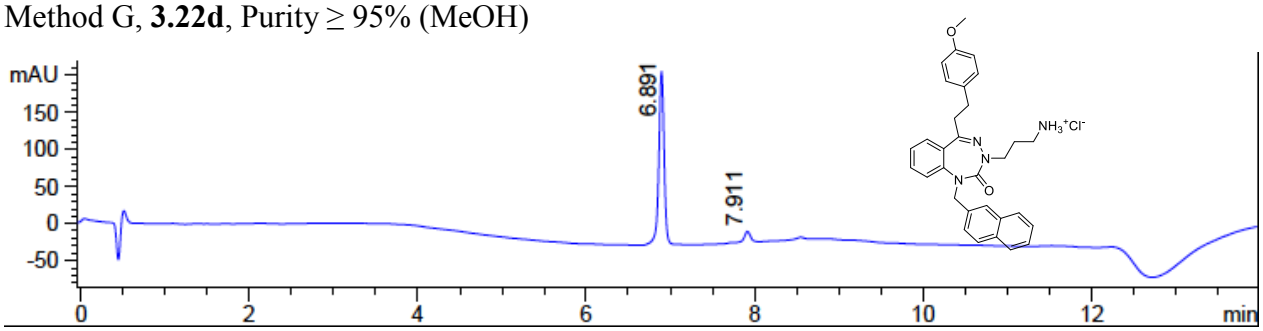
Method G, **3.20g**, Purity ≥ 97% (MeOH)



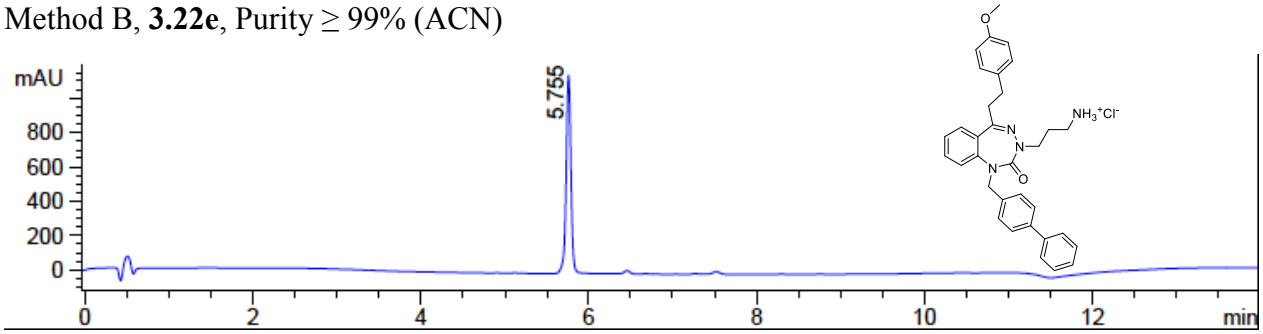
Method B, **3.22d**, Purity ≥ 95% (ACN)



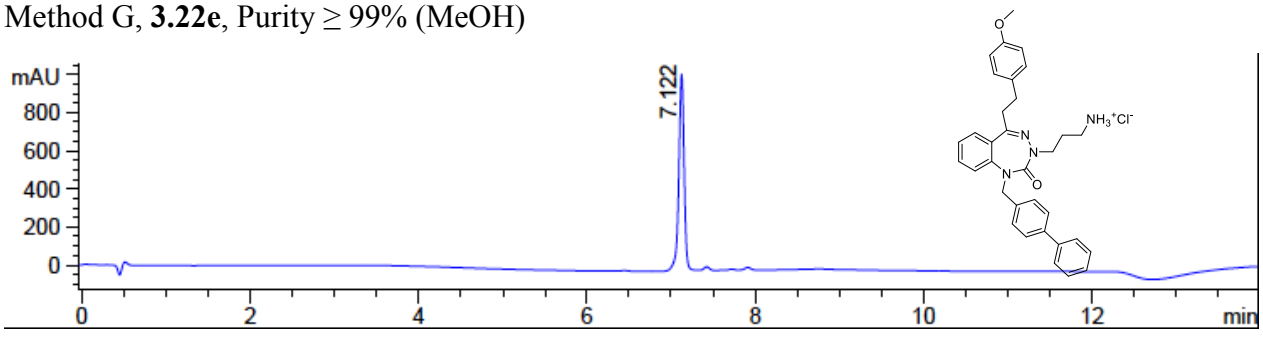
Method G, **3.22d**, Purity ≥ 95% (MeOH)



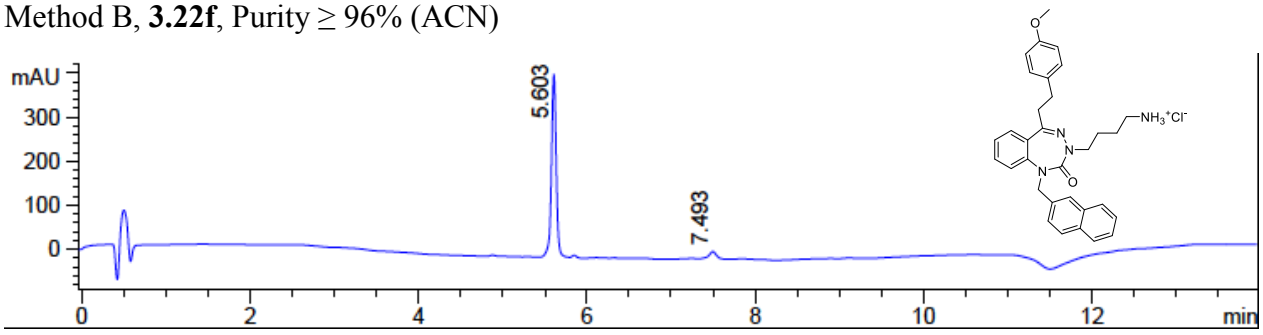
Method B, **3.22e**, Purity ≥ 99% (ACN)



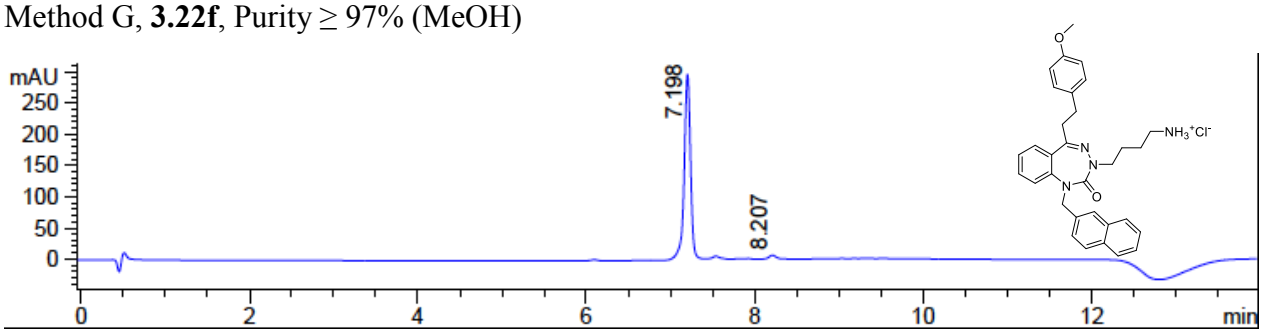
Method G, **3.22e**, Purity ≥ 99% (MeOH)



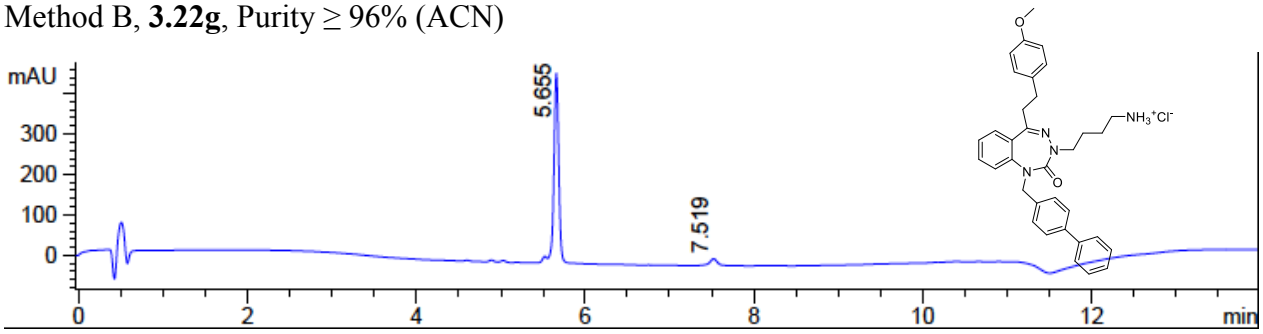
Method B, **3.22f**, Purity ≥ 96% (ACN)



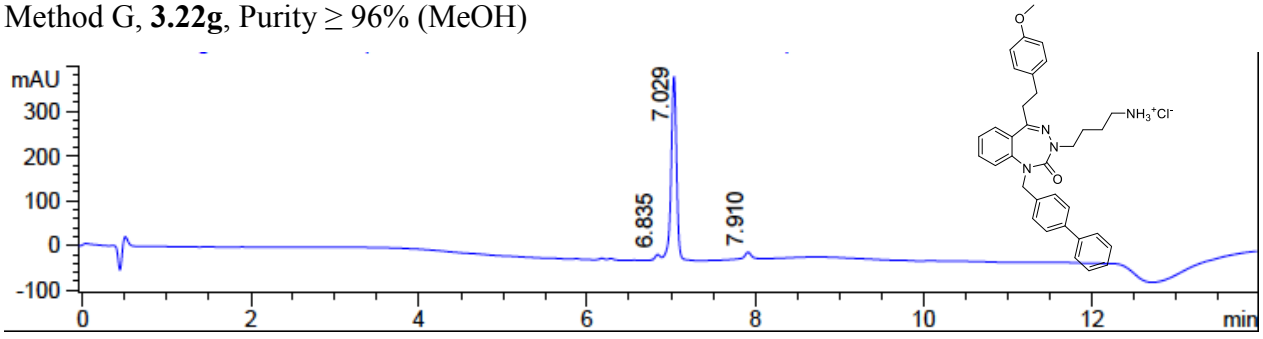
Method G, **3.22f**, Purity ≥ 97% (MeOH)



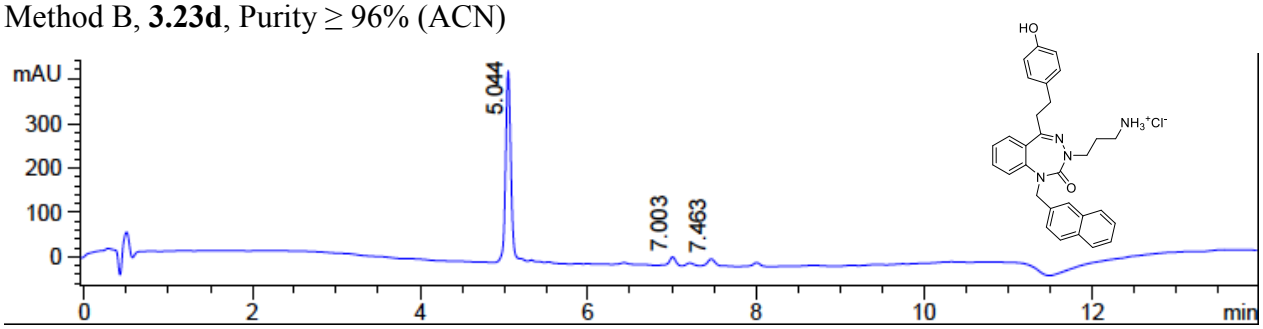
Method B, **3.22g**, Purity ≥ 96% (ACN)



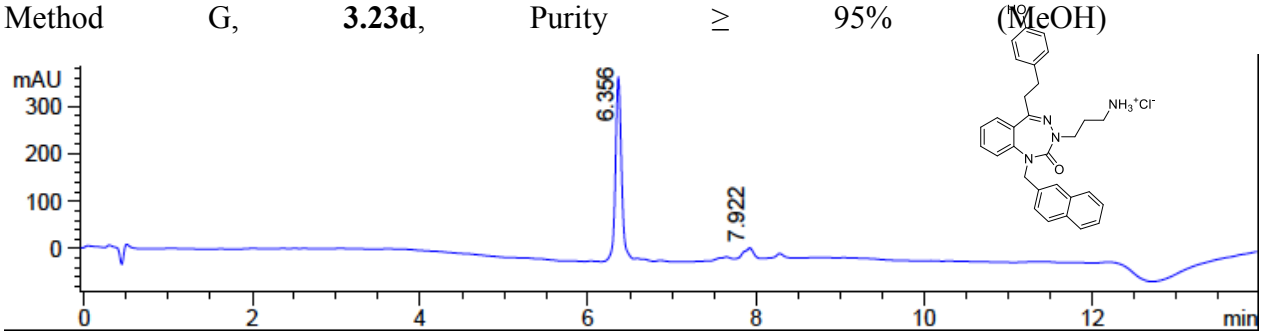
Method G, **3.22g**, Purity ≥ 96% (MeOH)



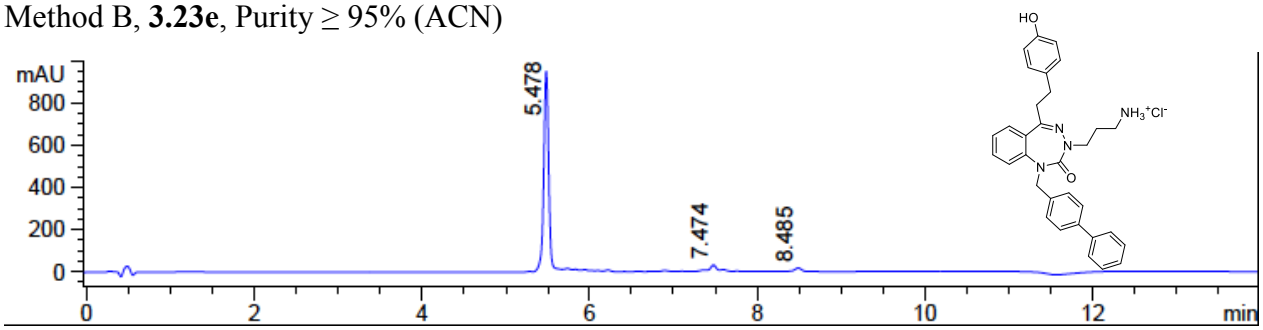
Method B, **3.23d**, Purity ≥ 96% (ACN)



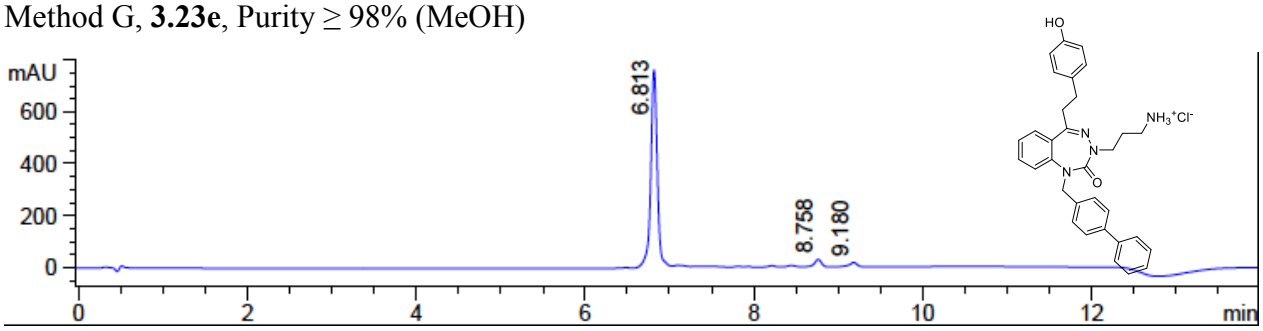
Method G, **3.23d**, Purity ≥ 95% (MeOH)



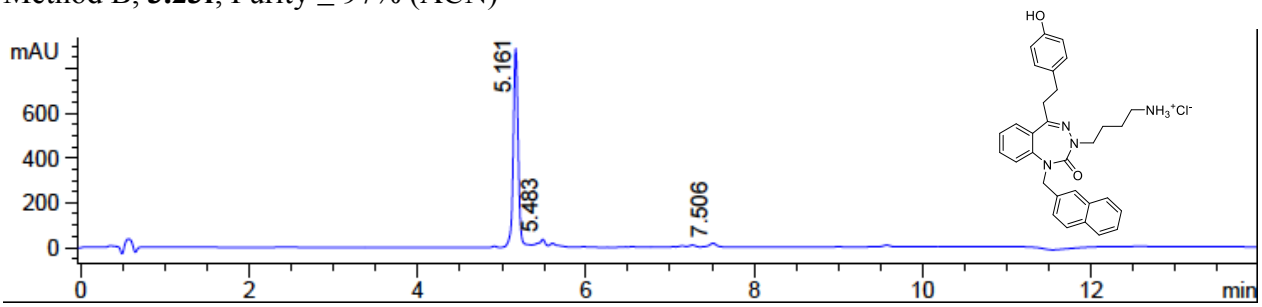
Method B, **3.23e**, Purity ≥ 95% (ACN)



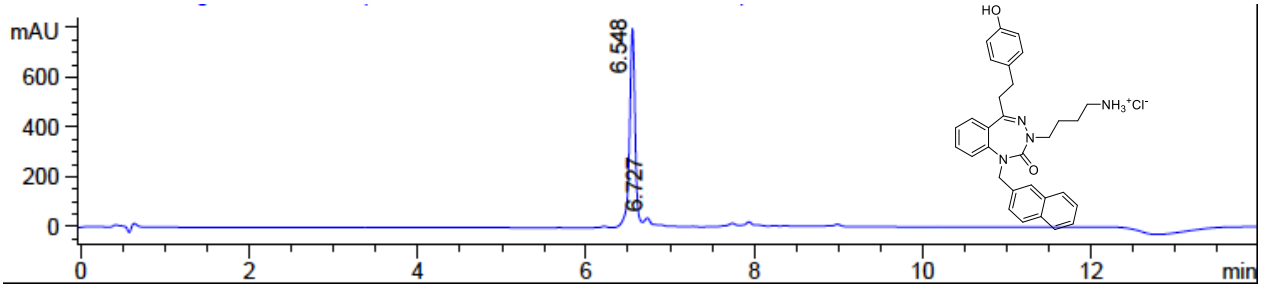
Method G, **3.23e**, Purity ≥ 98% (MeOH)



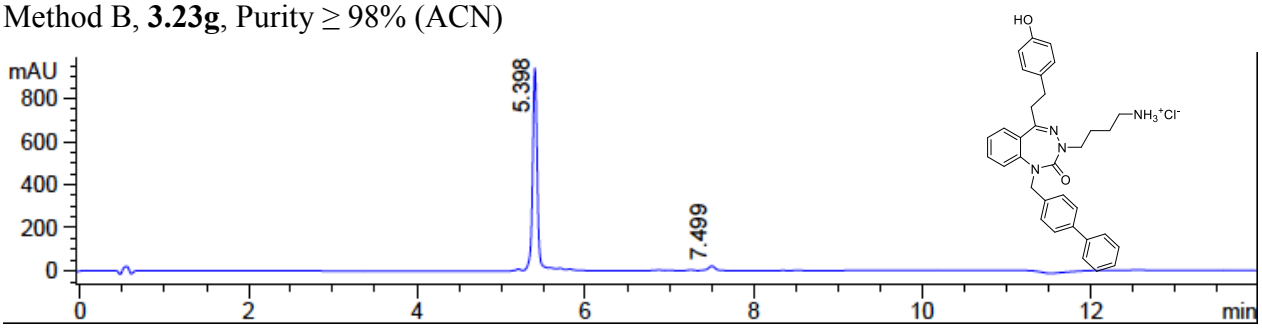
Method B, **3.23f**, Purity ≥ 97% (ACN)



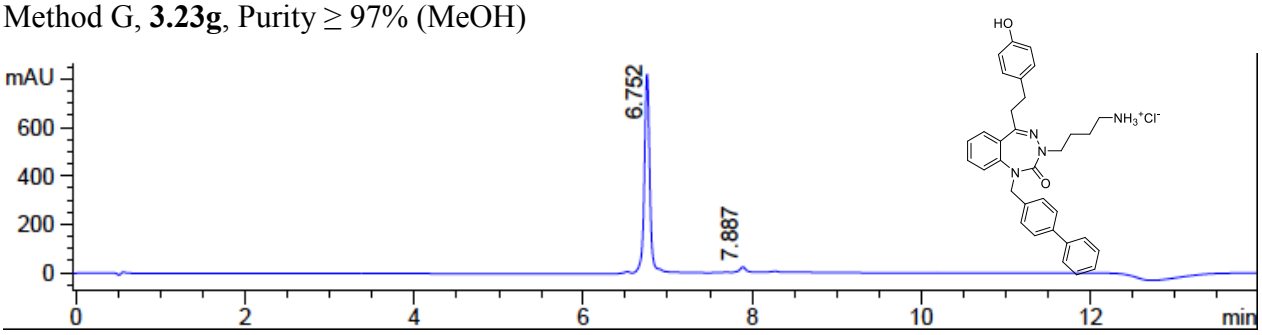
Method G, **3.23f**, Purity ≥ 98% (MeOH)



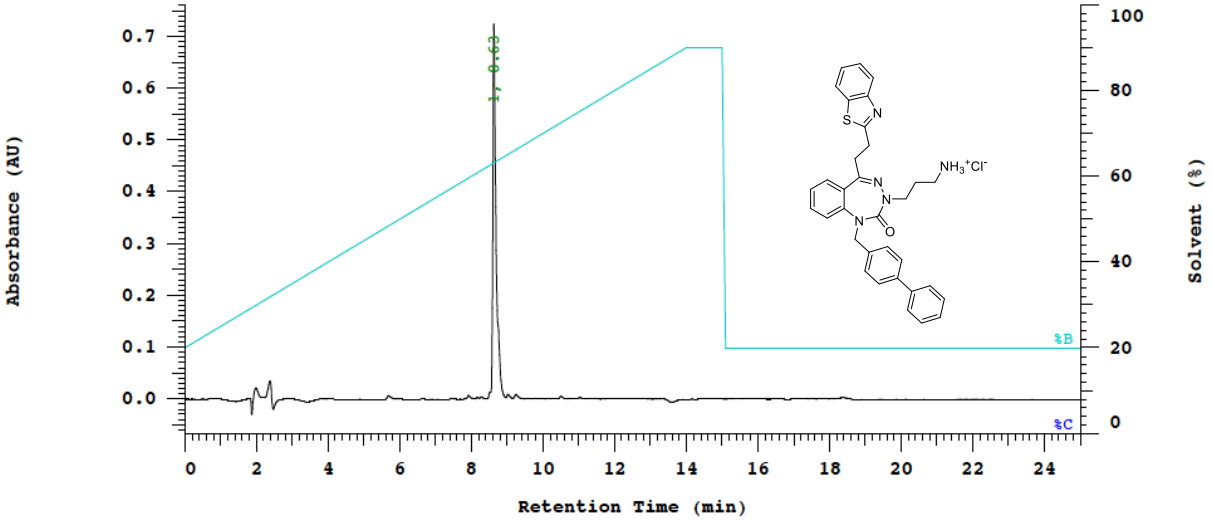
Method B, **3.23g**, Purity ≥ 98% (ACN)



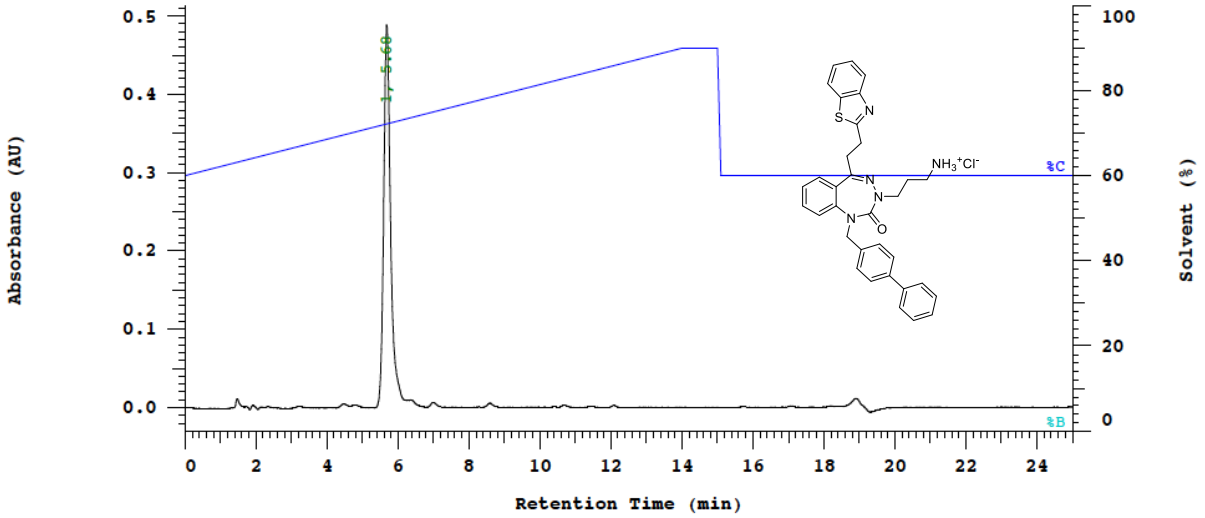
Method G, **3.23g**, Purity ≥ 97% (MeOH)



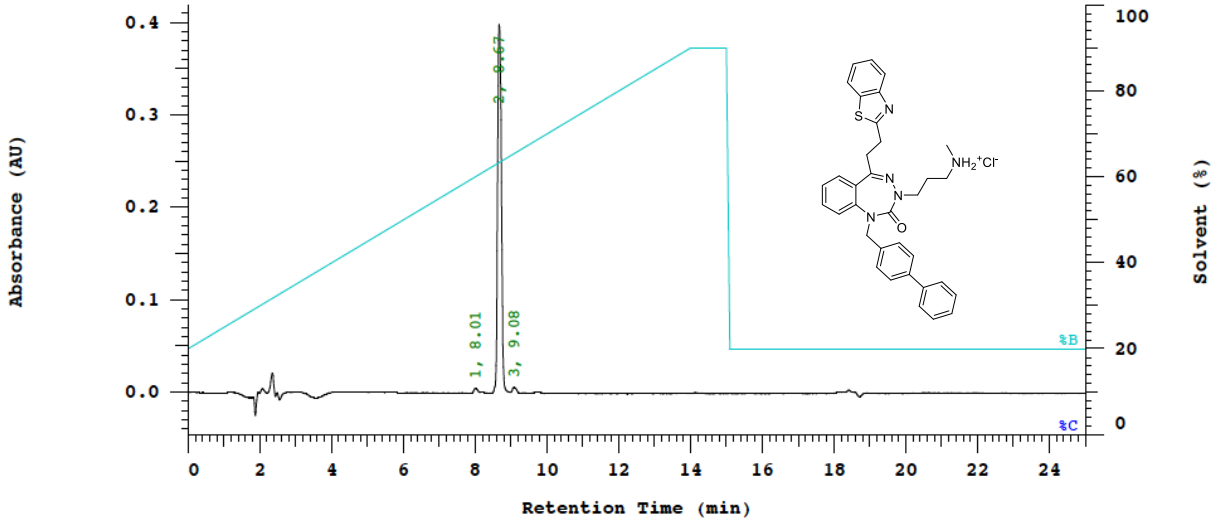
Method A, **3.37**, Purity ≥ 99% (ACN)



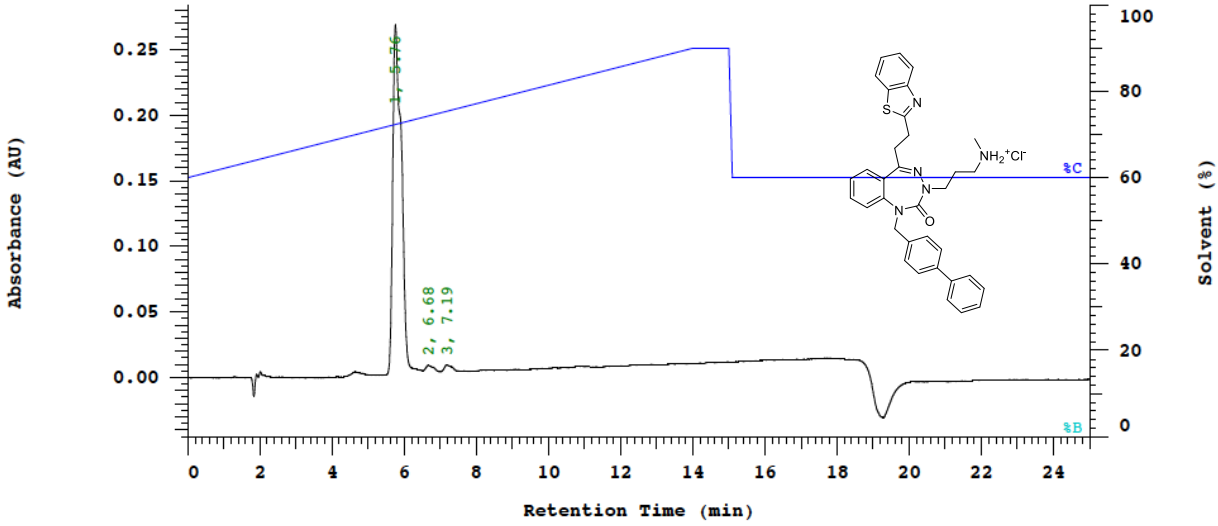
Method F, **3.37**, Purity ≥ 99% (MeOH)



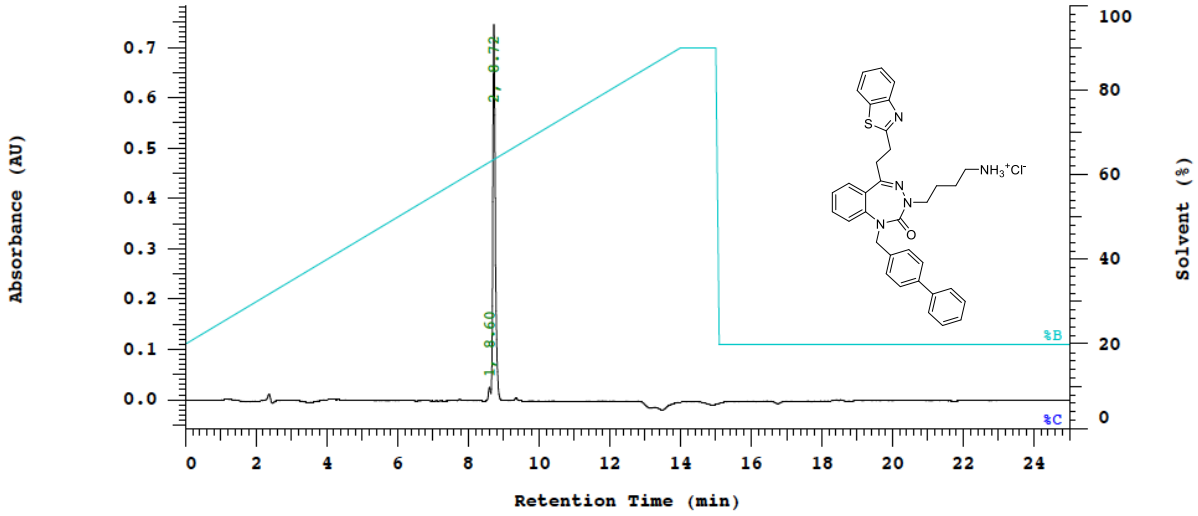
Method A, **3.38**, Purity ≥ 96% (ACN)



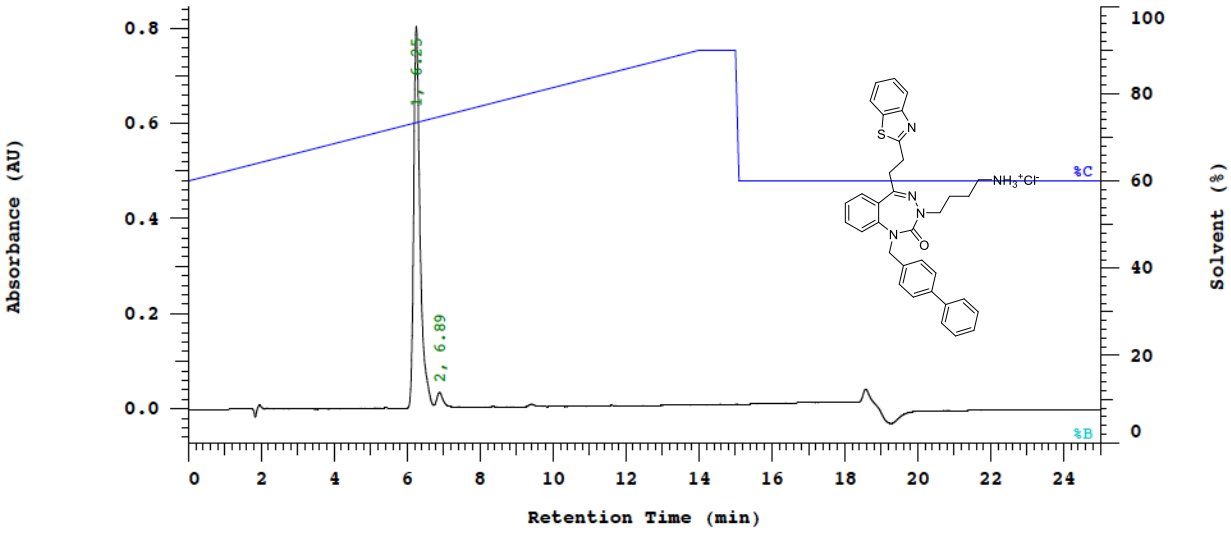
Method F, **3.38**, Purity ≥ 96% (MeOH)



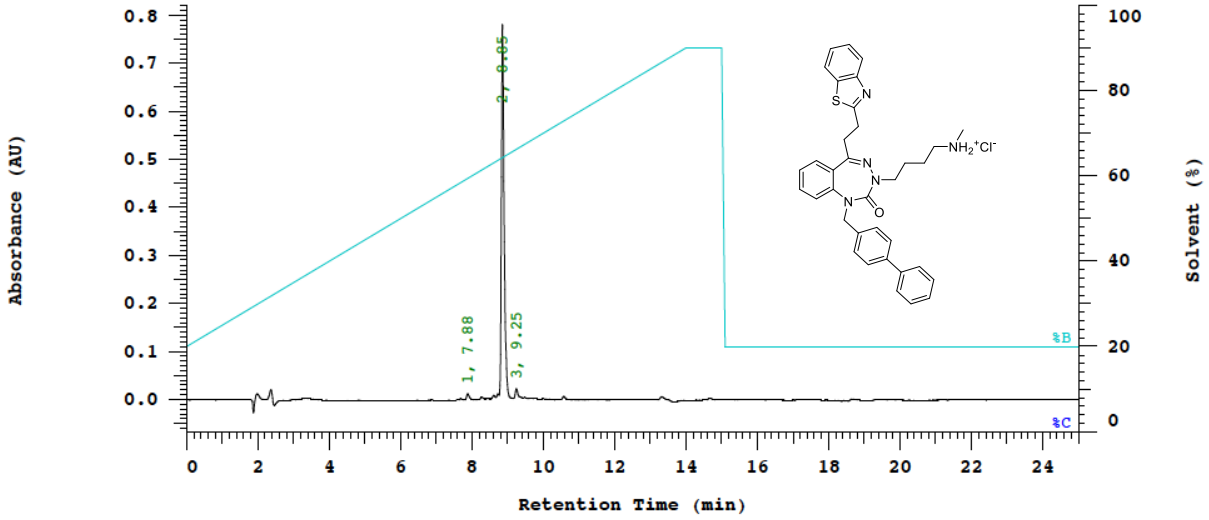
Method A, **3.39**, Purity ≥ 97% (ACN)



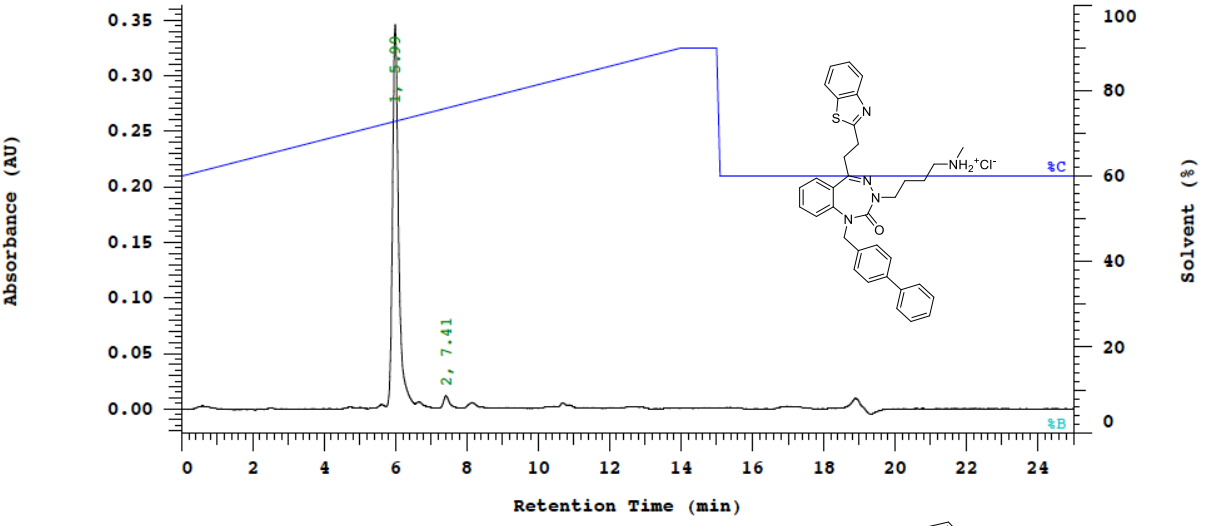
Method F, **3.39**, Purity ≥ 97% (MeOH)



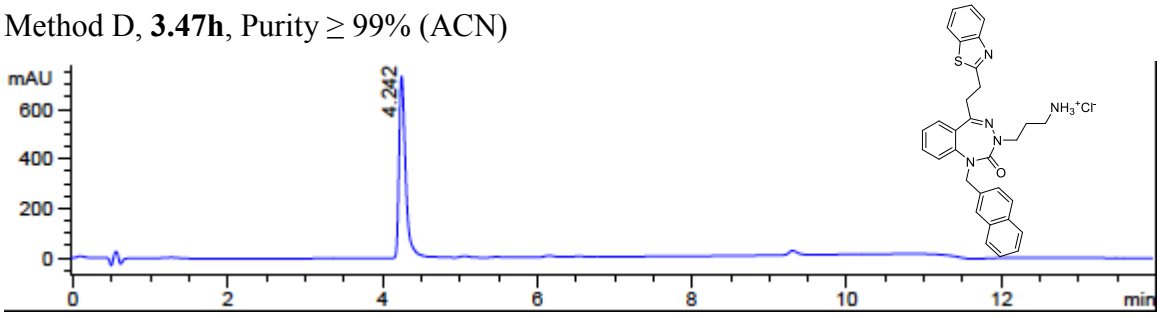
Method A, **3.40**, Purity ≥ 95% (ACN)



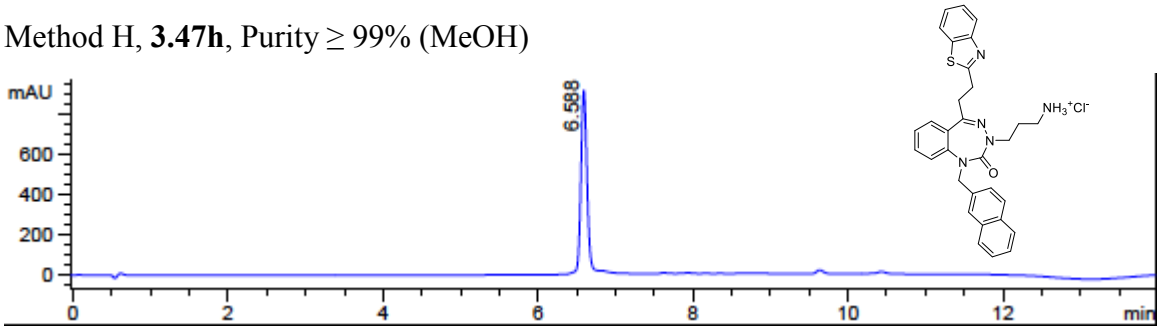
Method F, **3.40**, Purity ≥ 97% (MeOH)



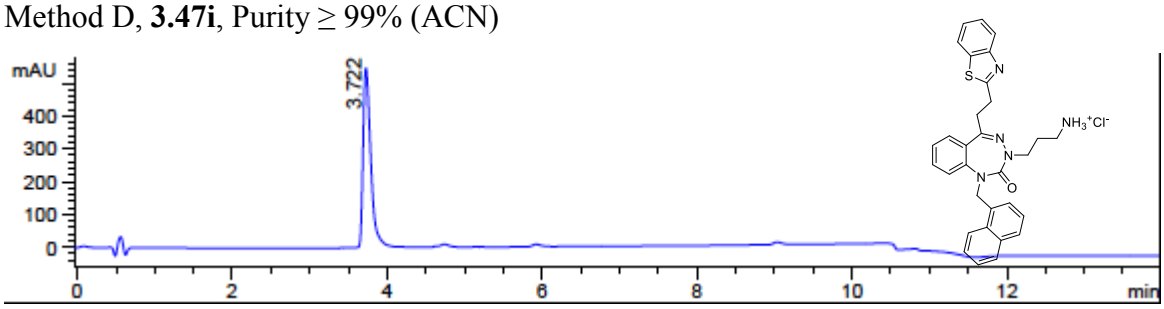
Method D, **3.47h**, Purity ≥ 99% (ACN)



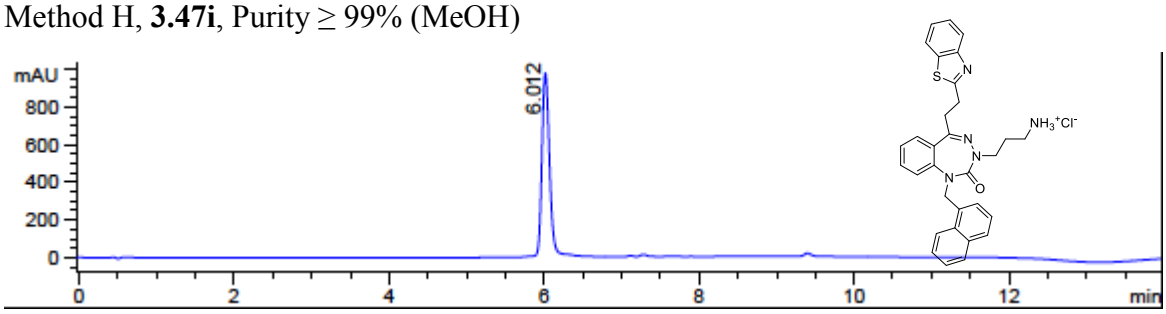
Method H, **3.47h**, Purity ≥ 99% (MeOH)



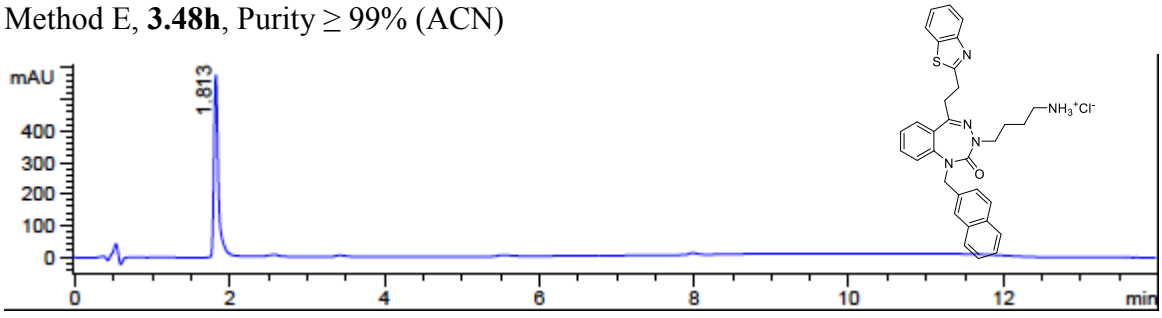
Method D, **3.47i**, Purity ≥ 99% (ACN)



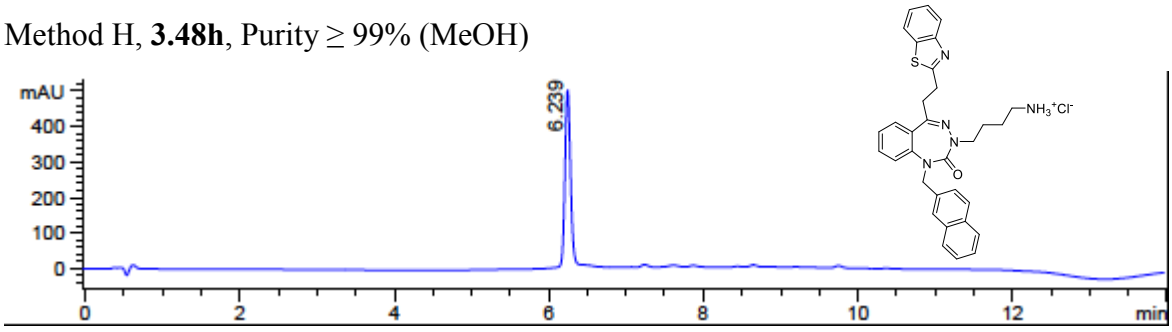
Method H, **3.47i**, Purity ≥ 99% (MeOH)



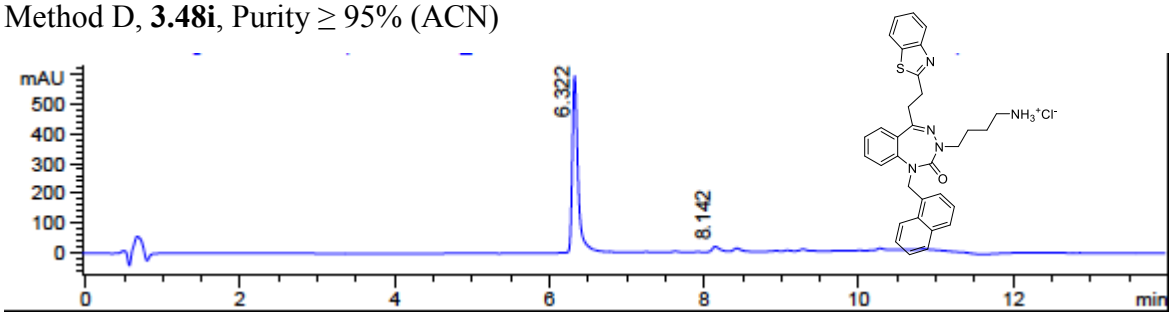
Method E, **3.48h**, Purity ≥ 99% (ACN)



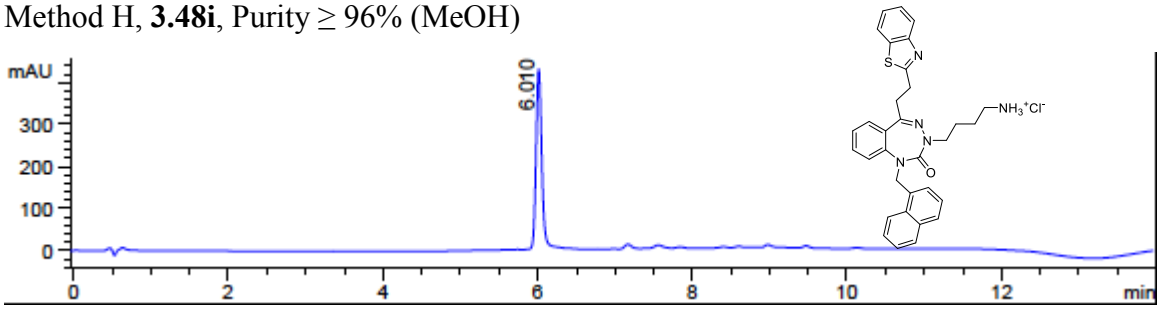
Method H, **3.48h**, Purity ≥ 99% (MeOH)



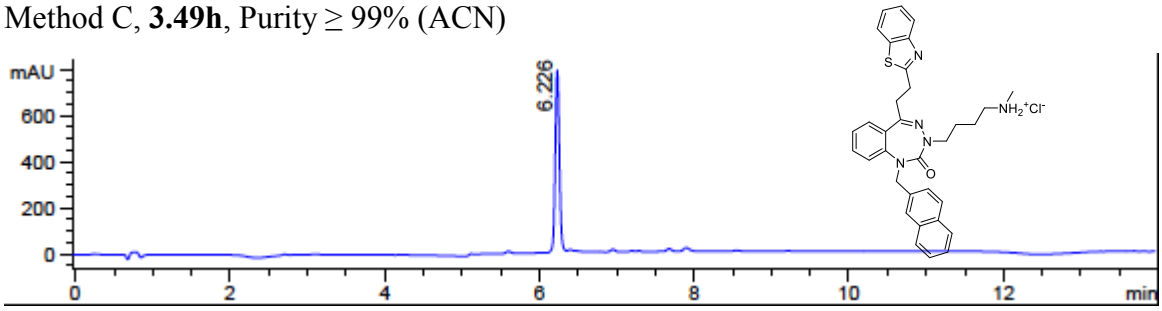
Method D, **3.48i**, Purity ≥ 95% (ACN)



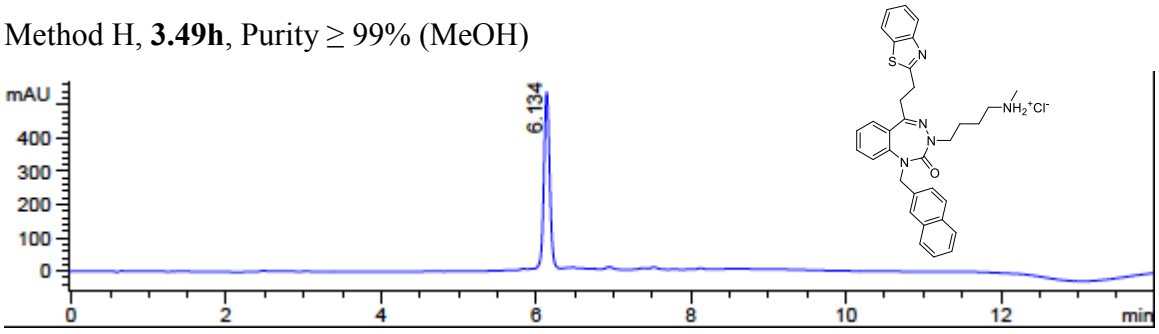
Method H, **3.48i**, Purity ≥ 96% (MeOH)



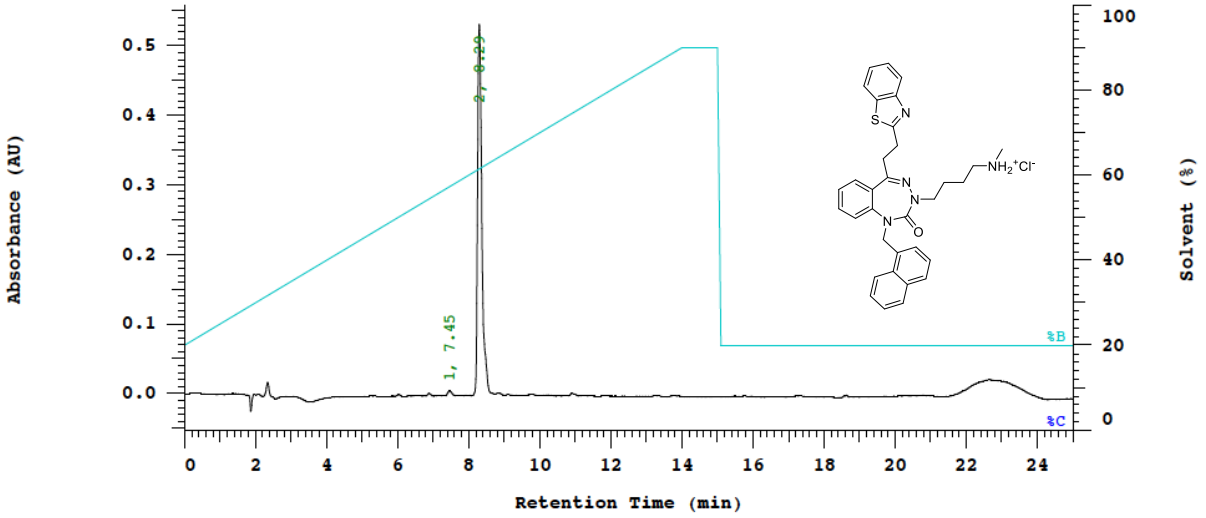
Method C, **3.49h**, Purity ≥ 99% (ACN)



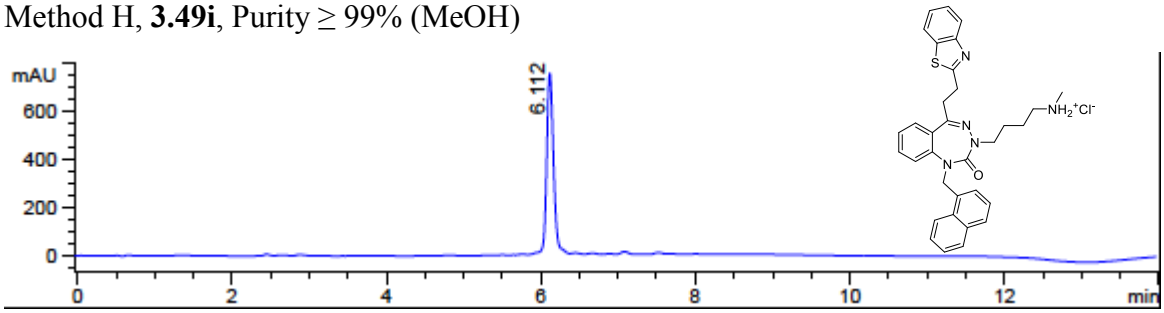
Method H, **3.49h**, Purity ≥ 99% (MeOH)



Method A, **3.49i**, Purity ≥ 98% (ACN)

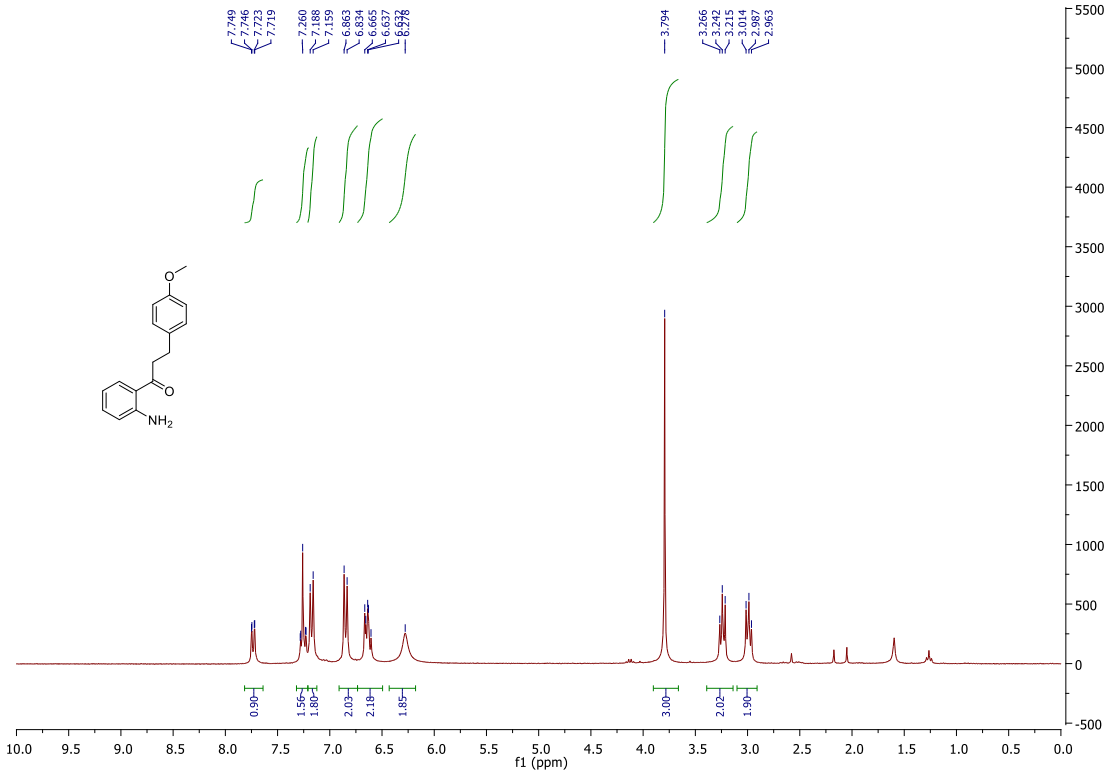


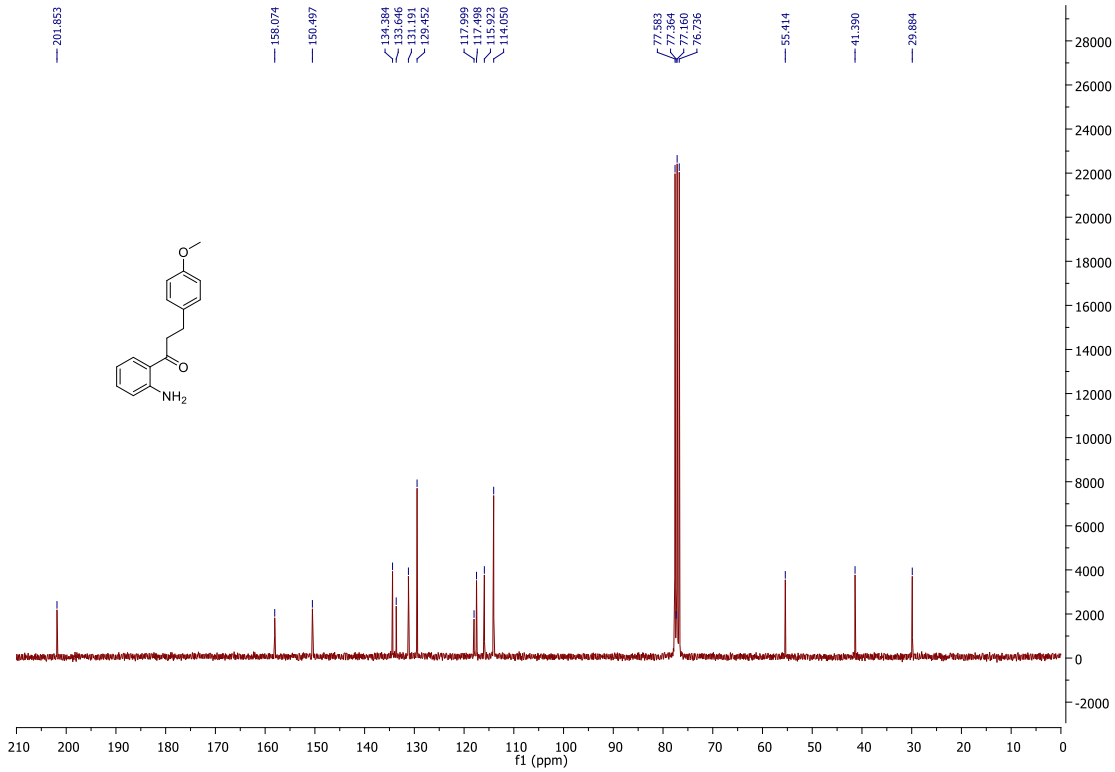
Method H, **3.49i**, Purity ≥ 99% (MeOH)



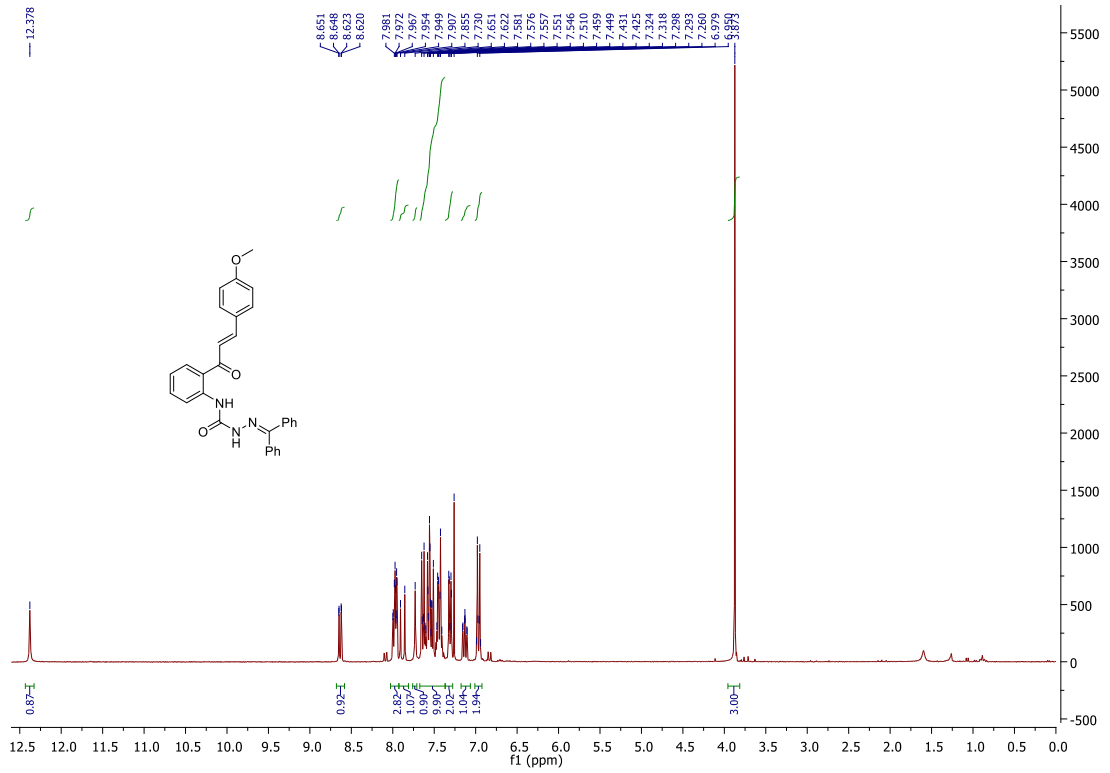
NMR Spectra

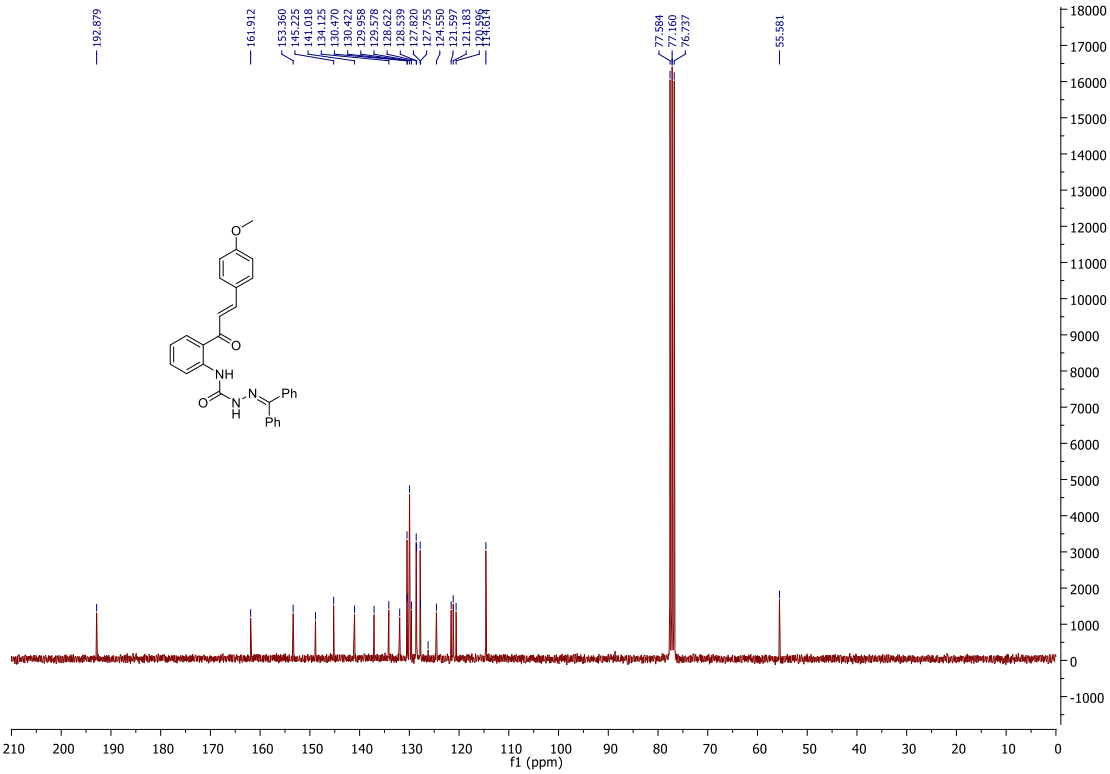
3.8, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



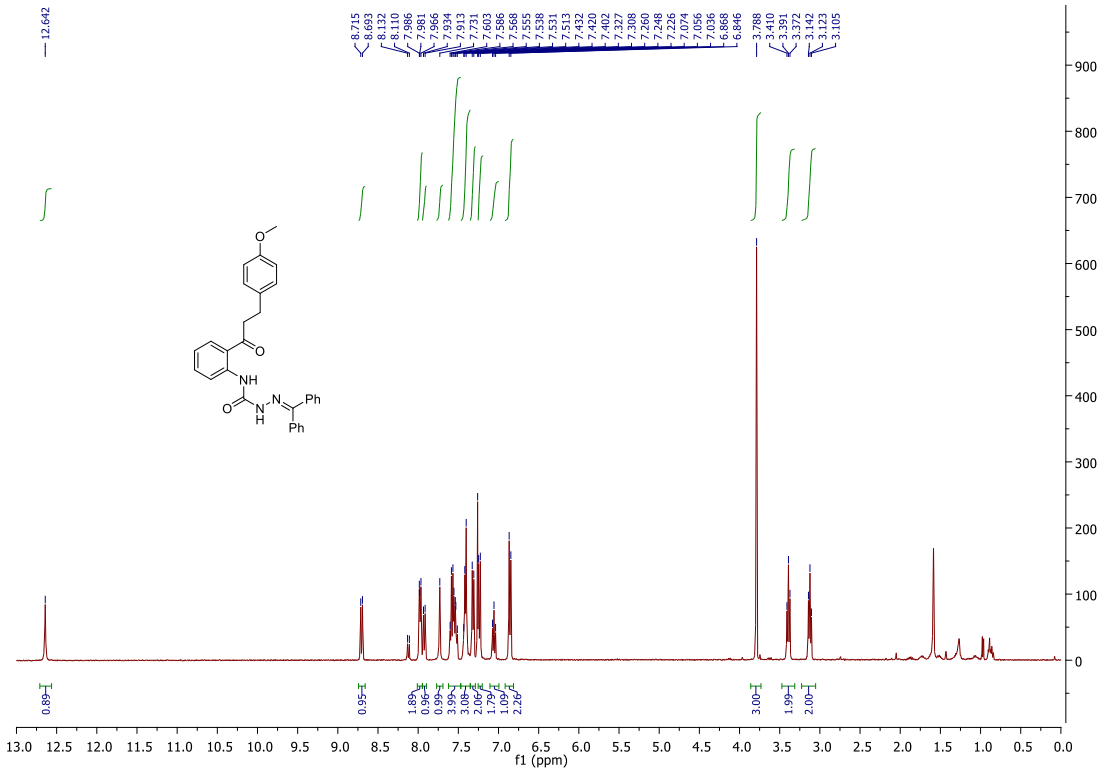


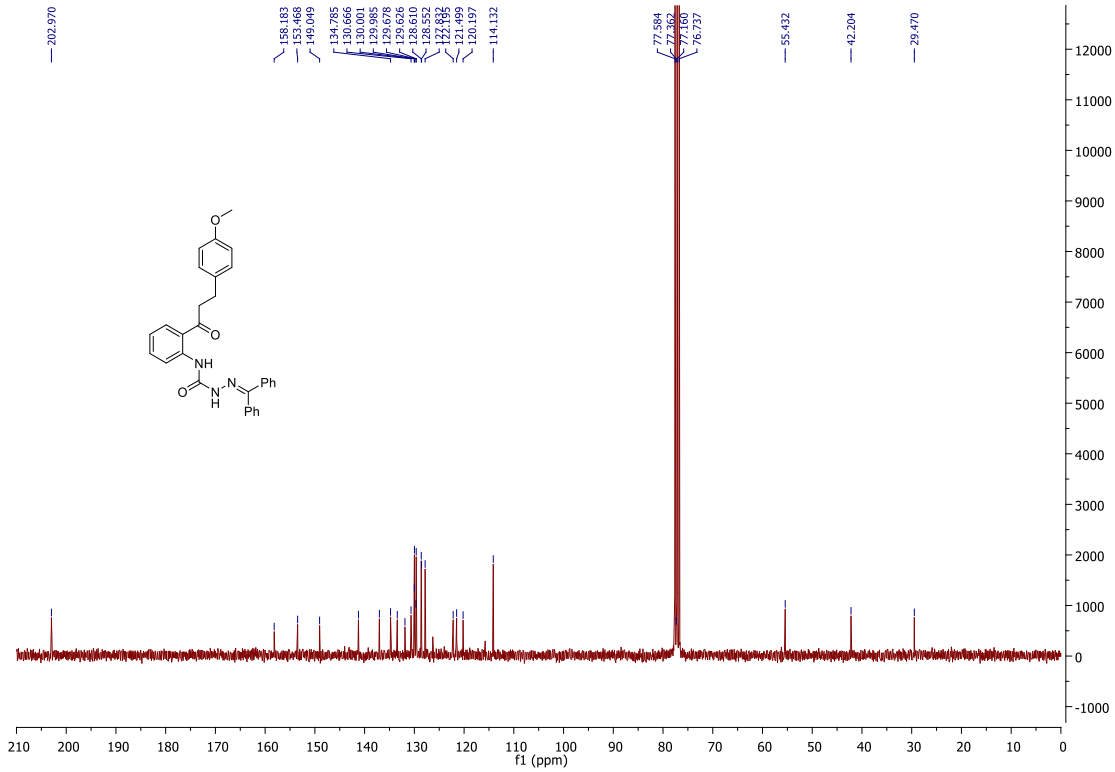
3.9a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



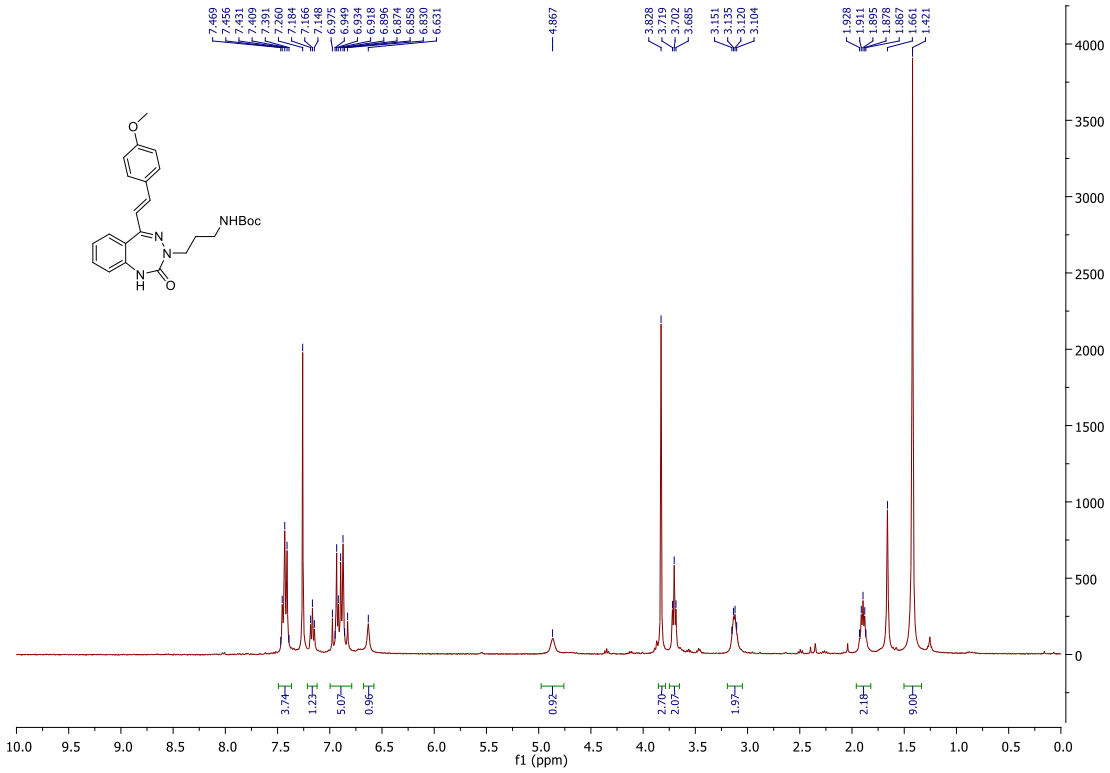


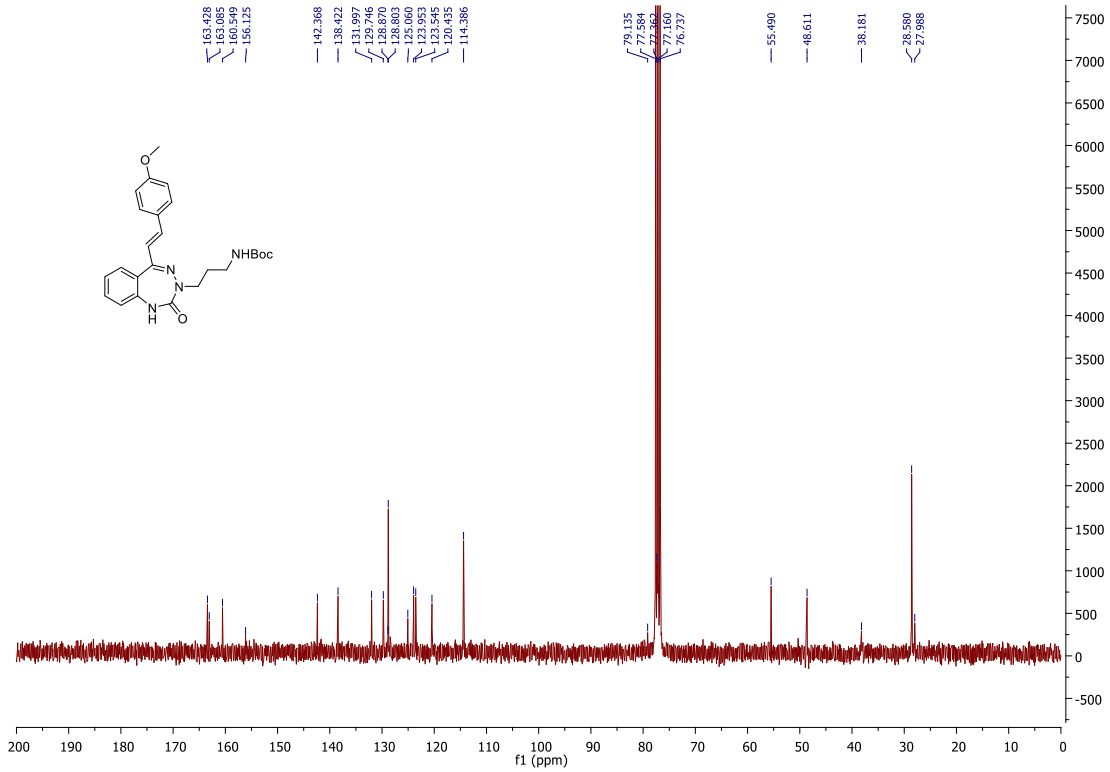
3.9b, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz)



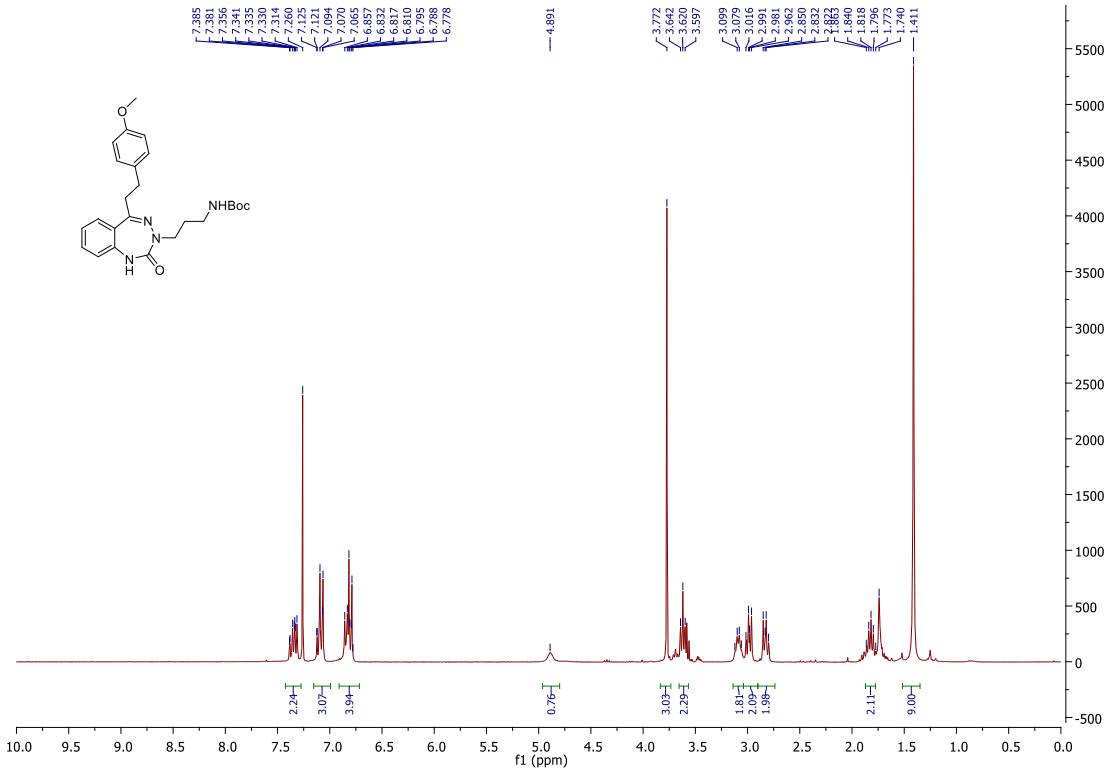


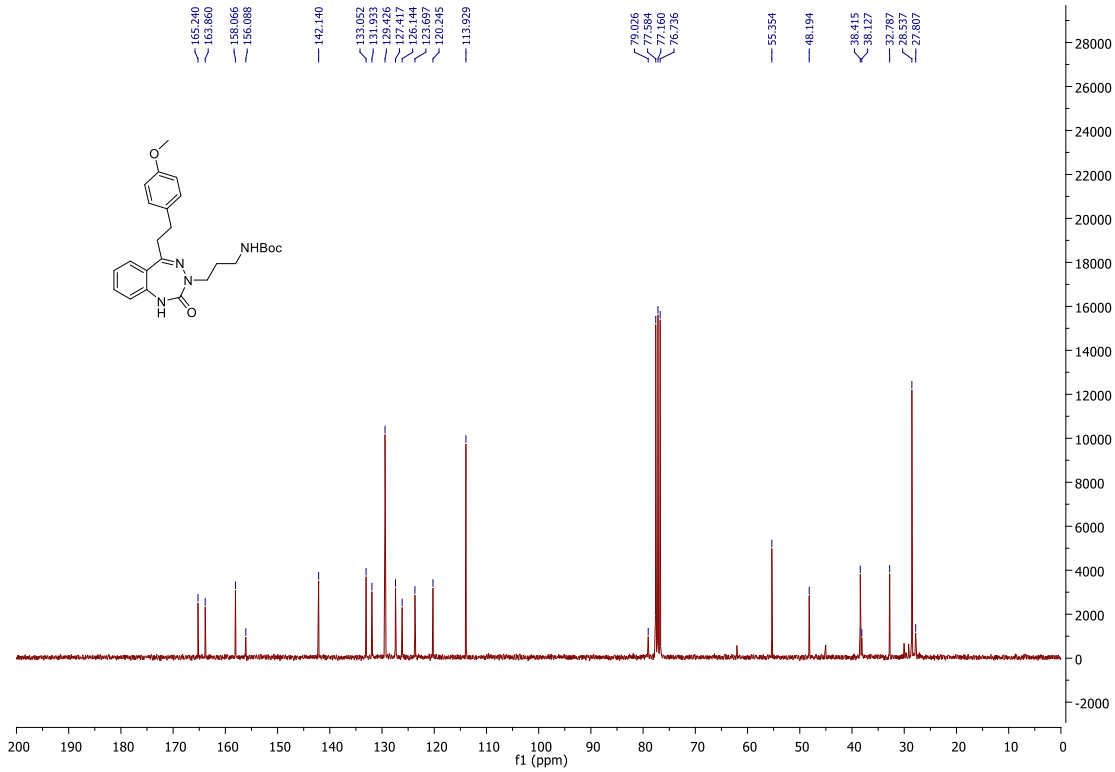
3.13a, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz)



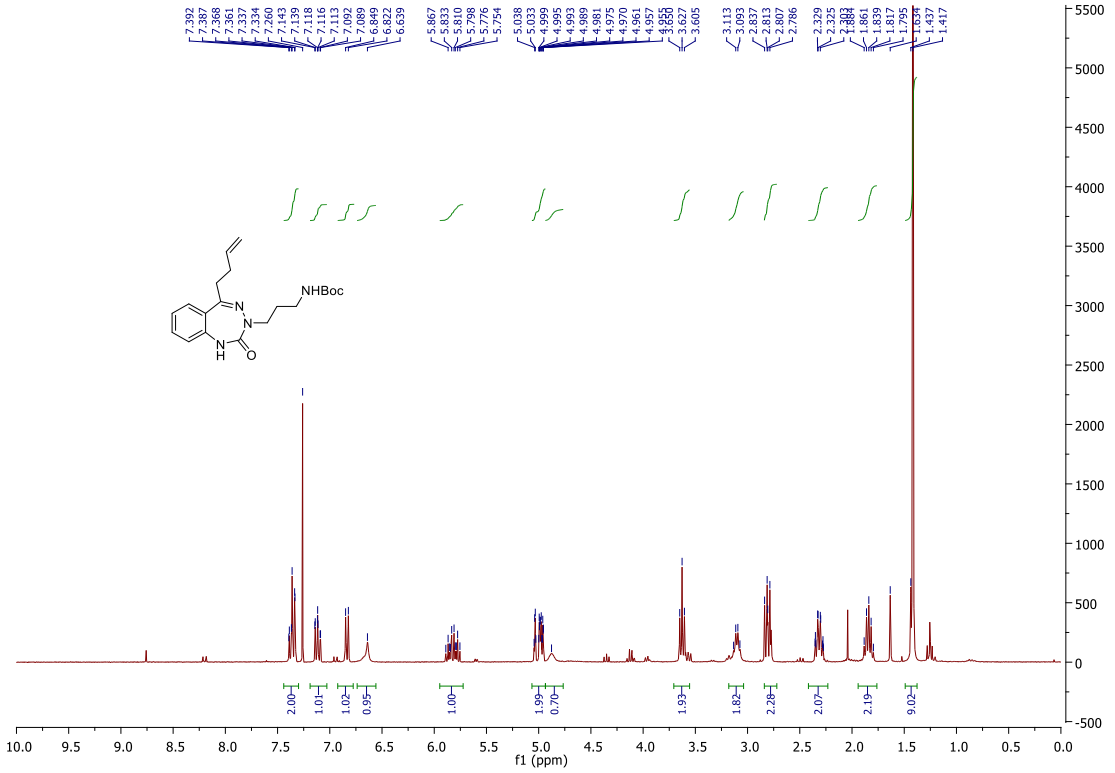


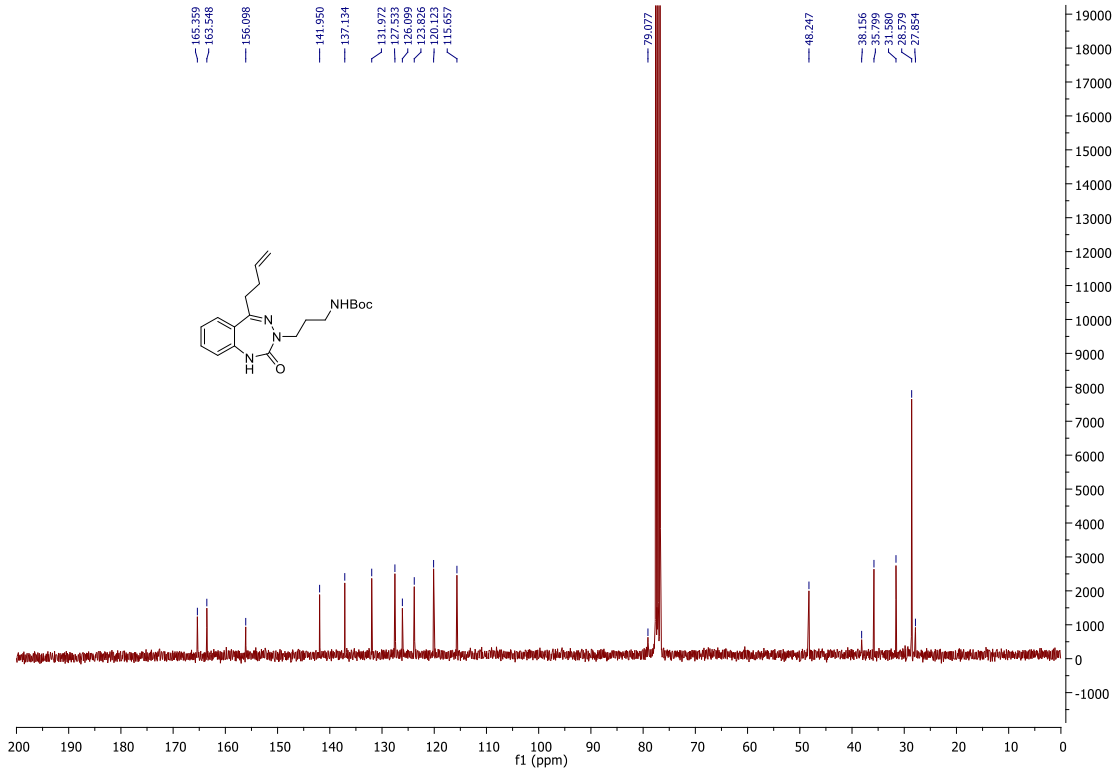
3.13b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



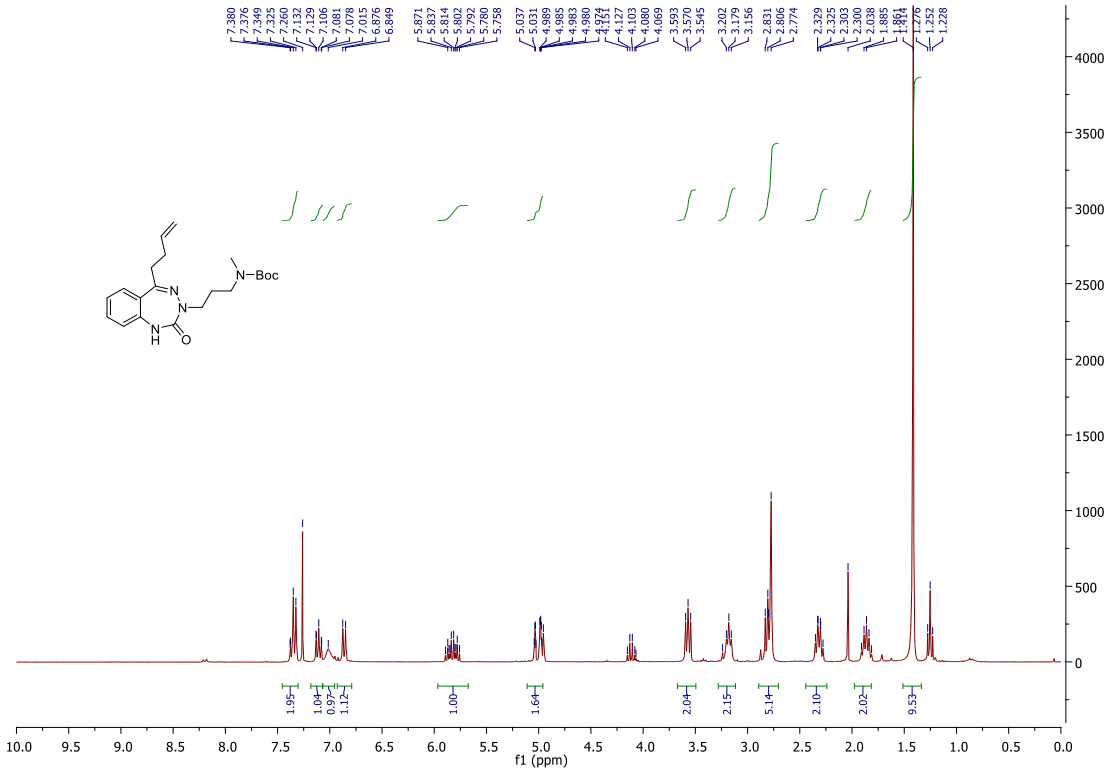


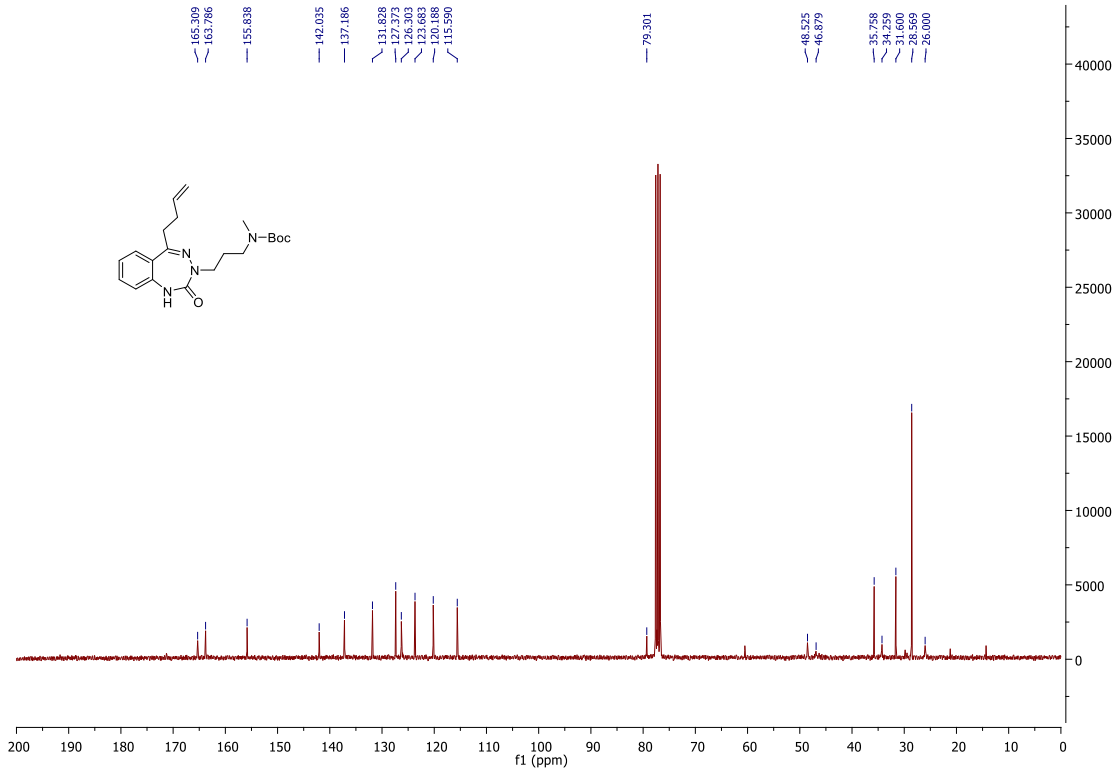
3.13c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



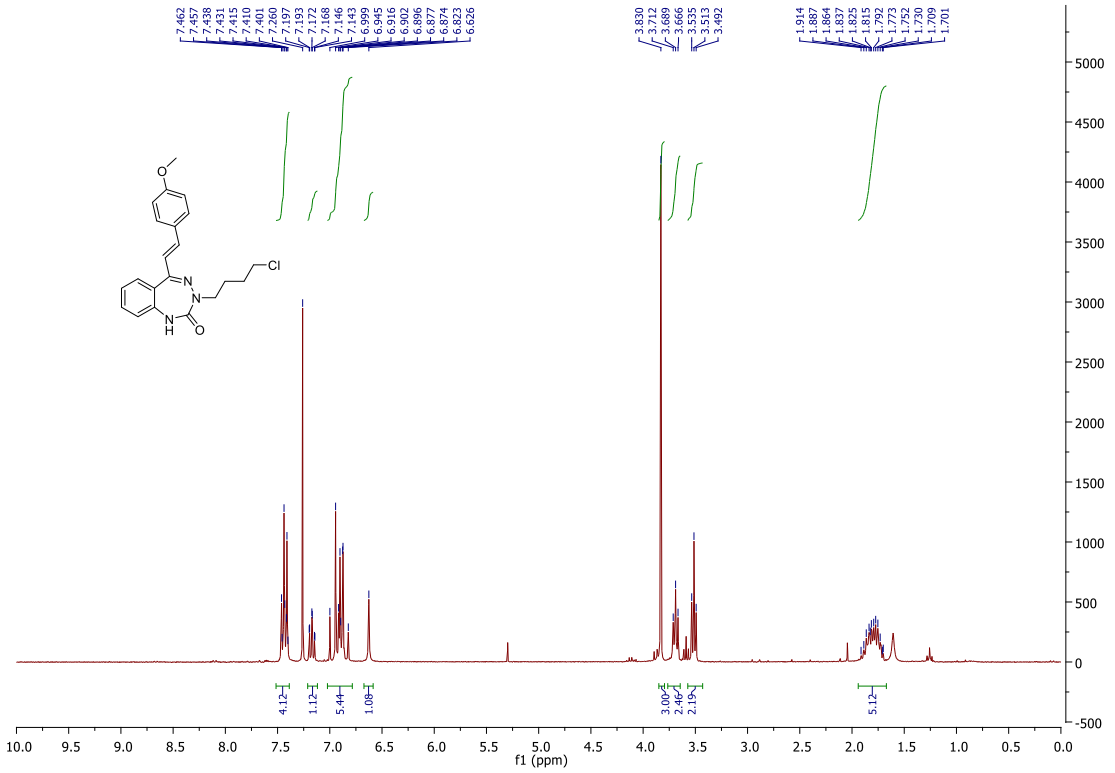


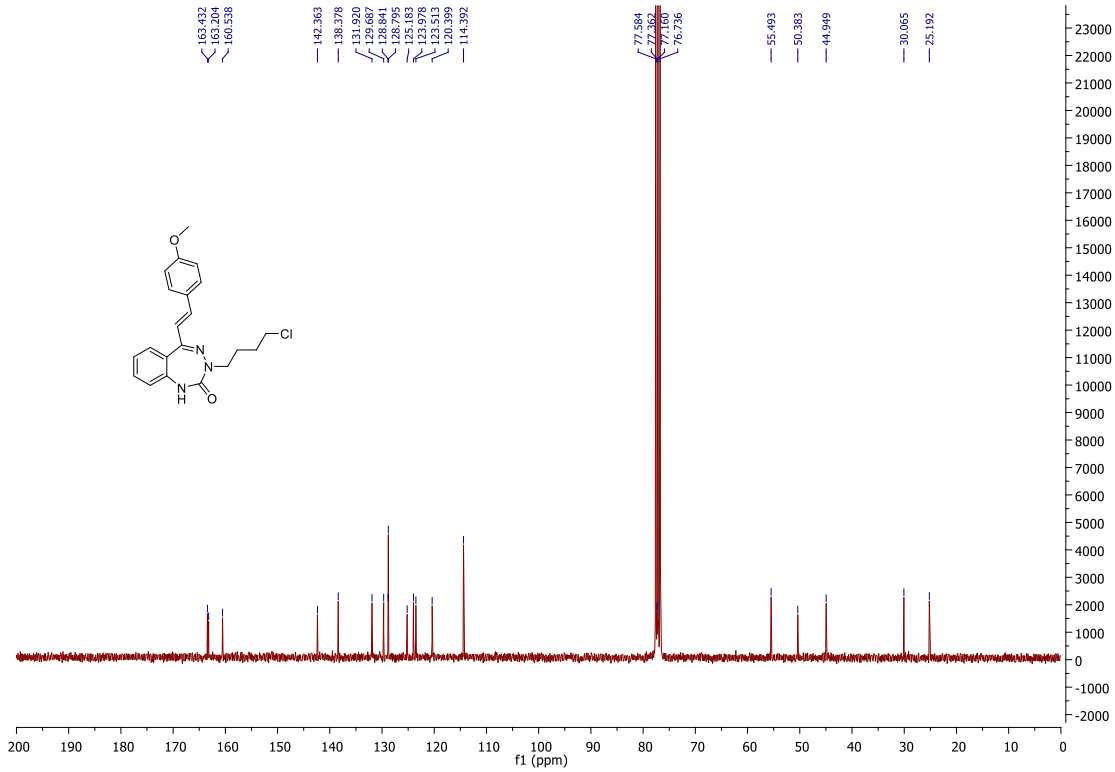
3.14c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



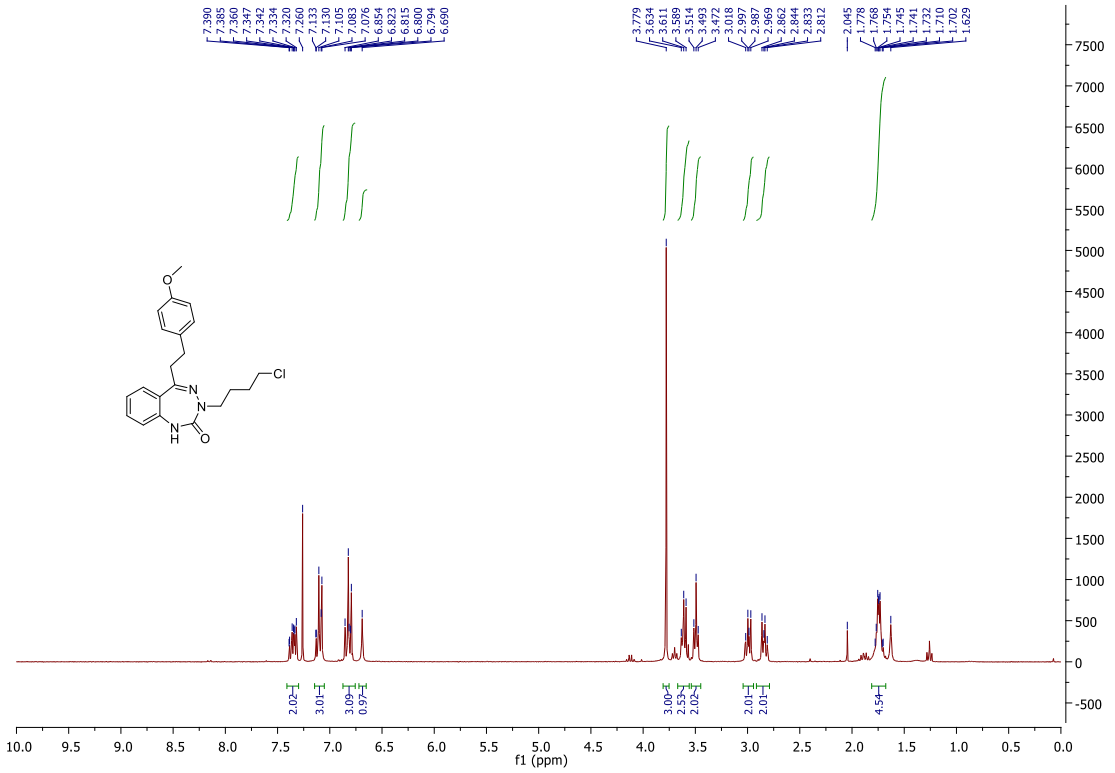


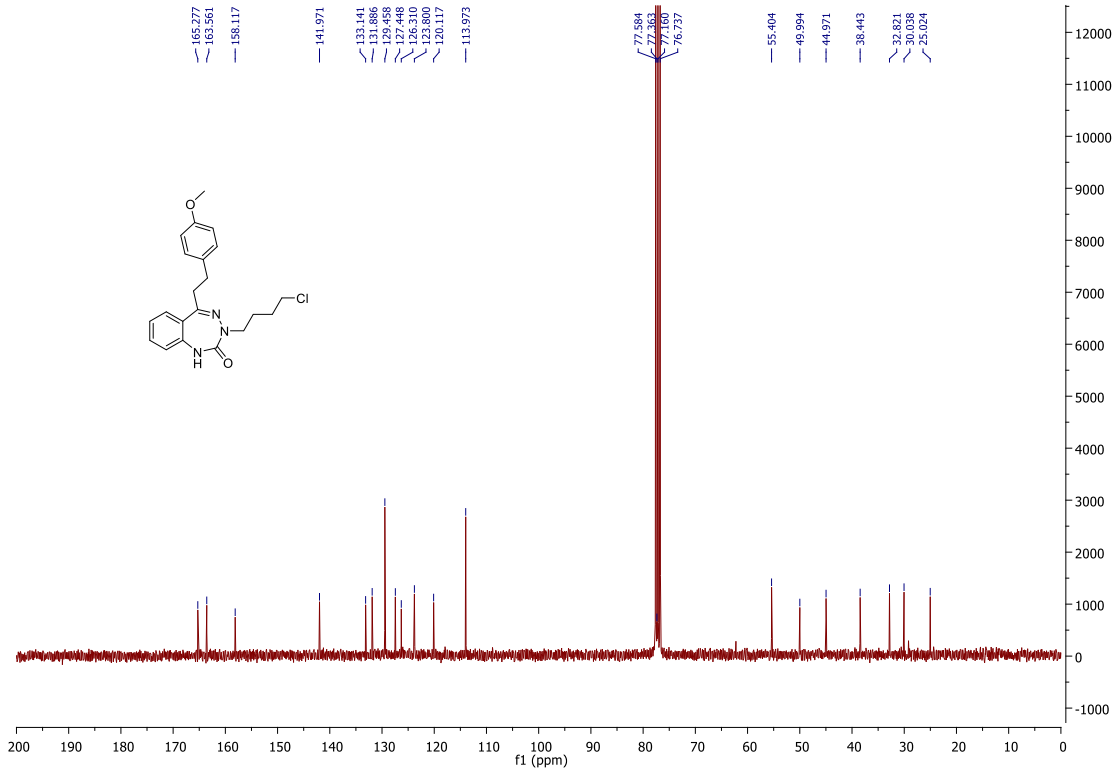
3.15a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



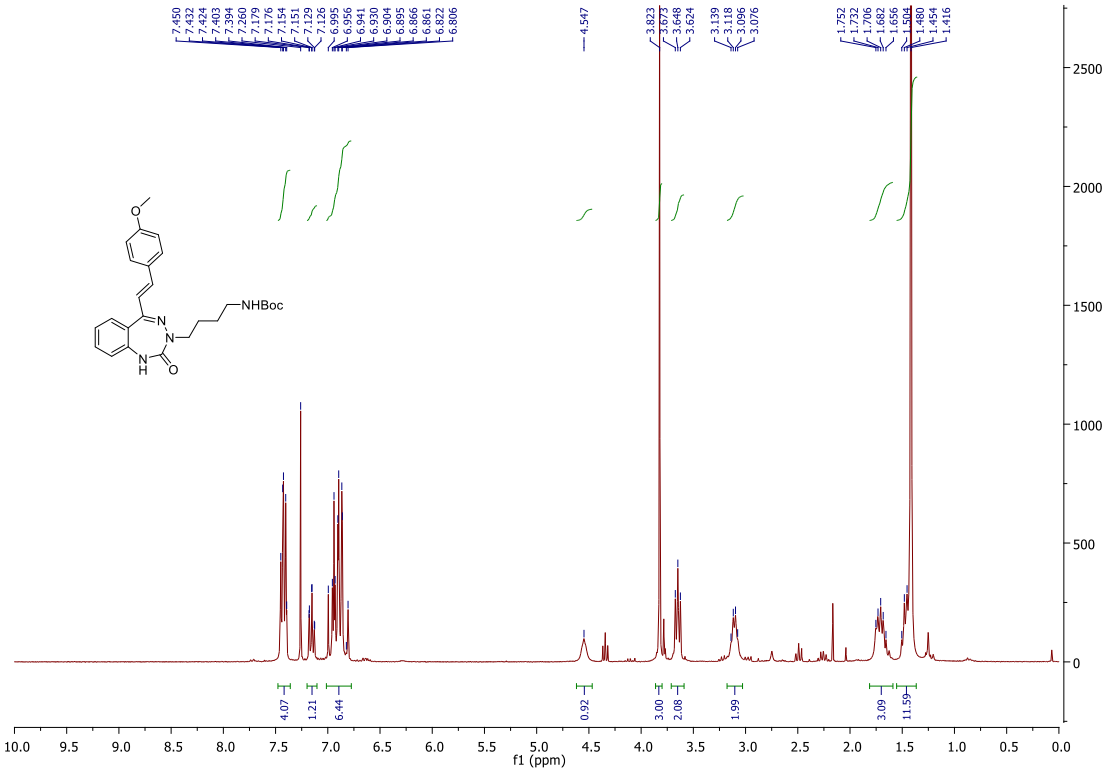


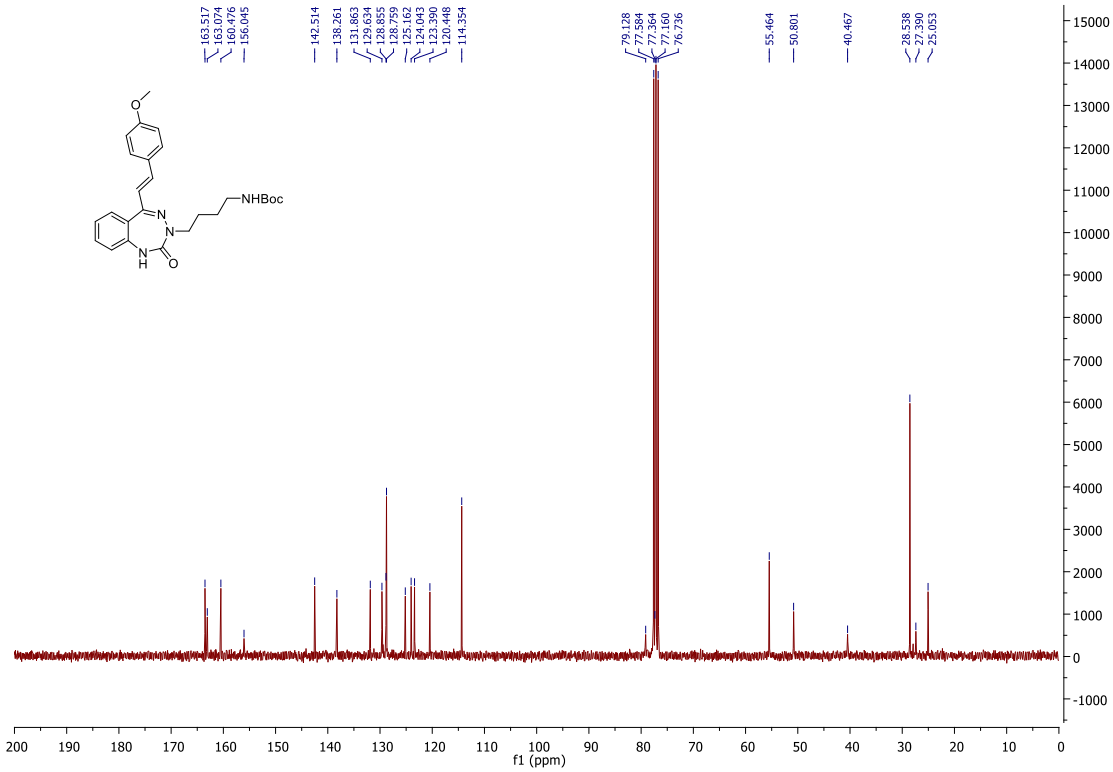
3.15b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



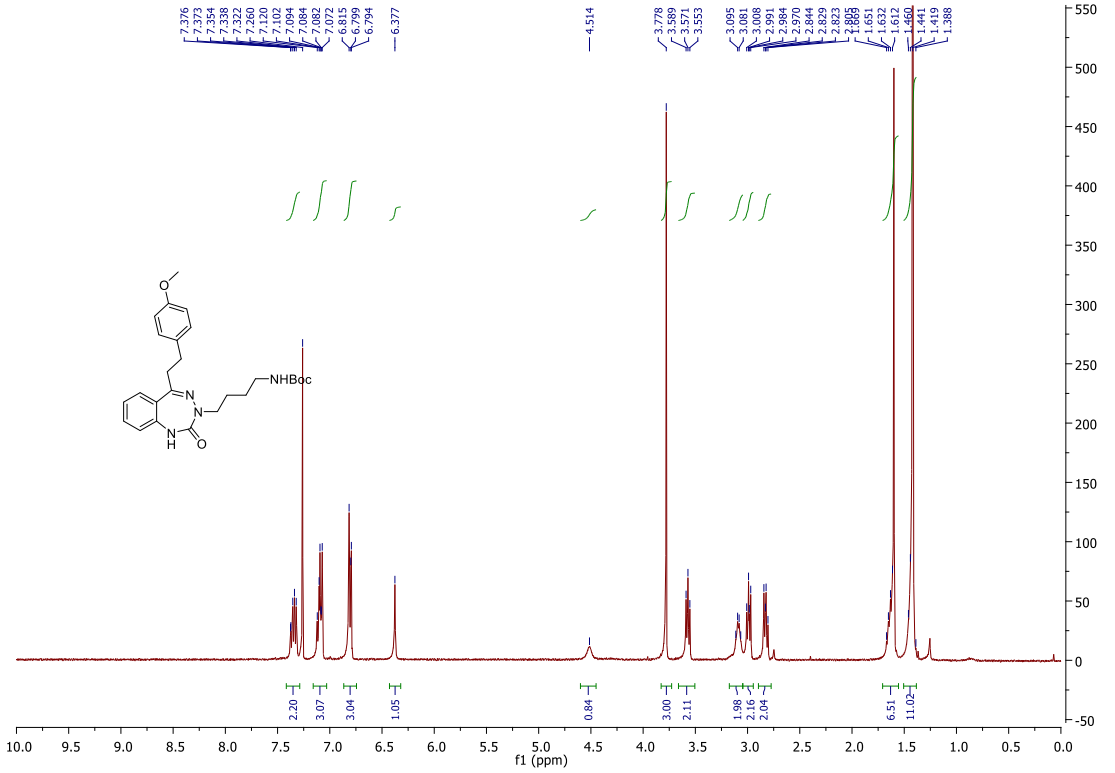


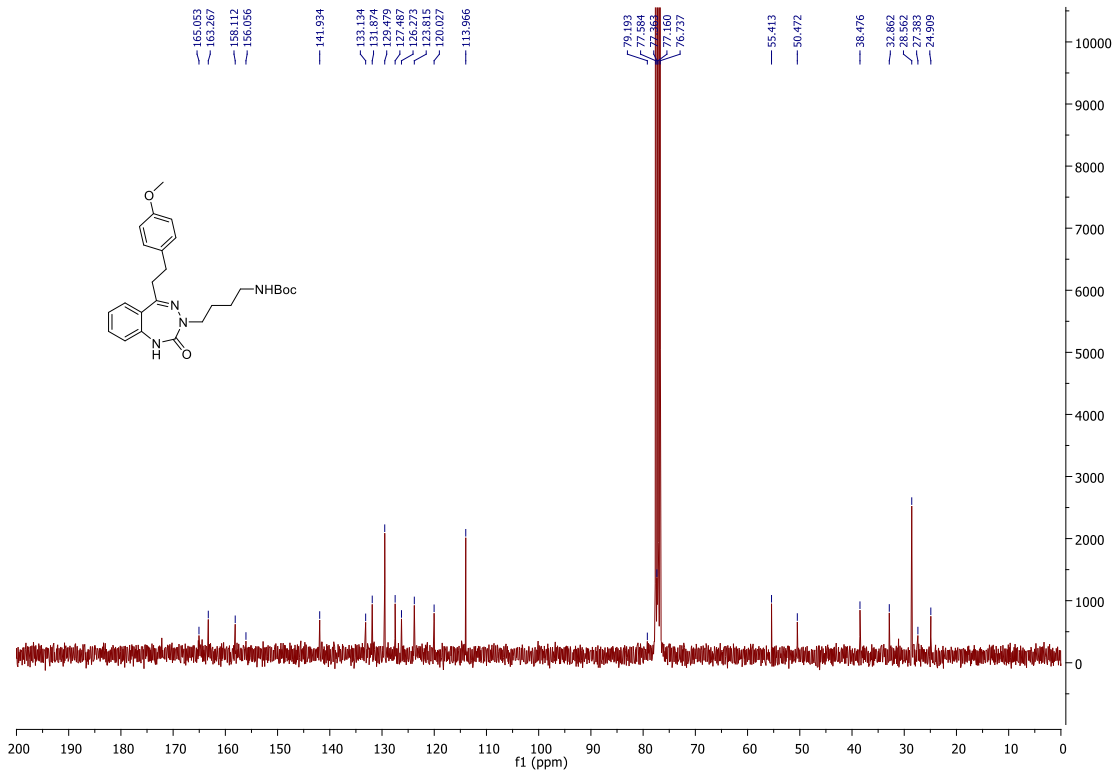
3.17a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



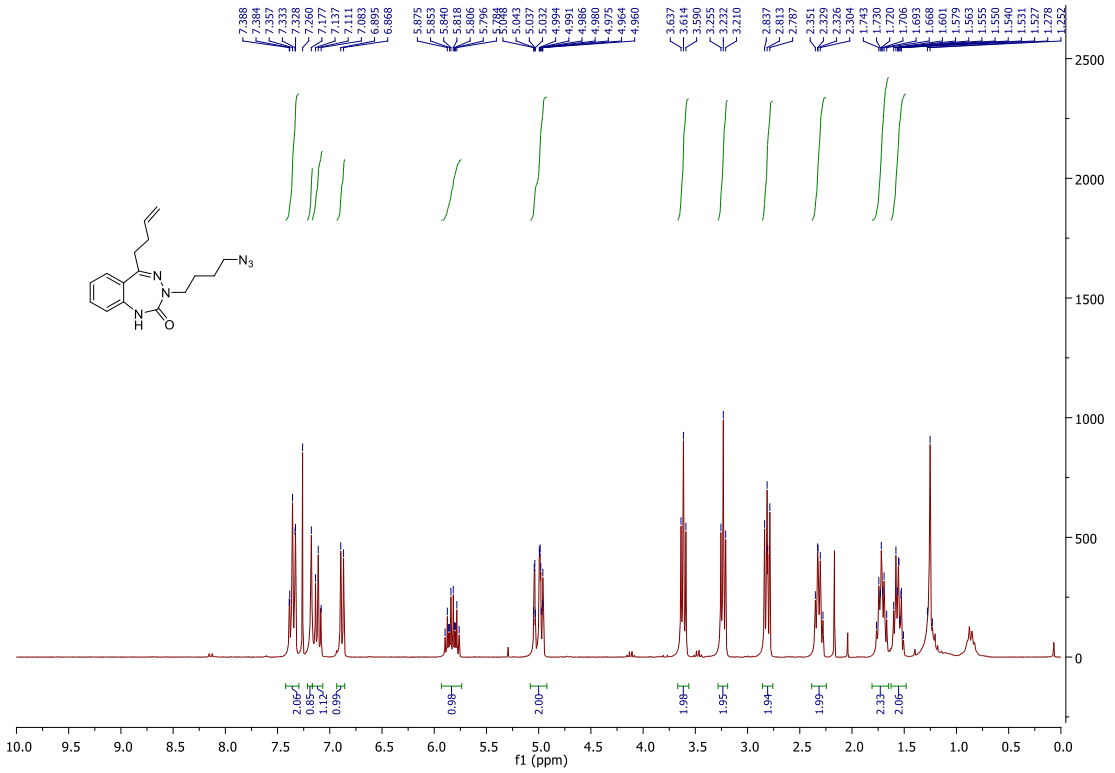


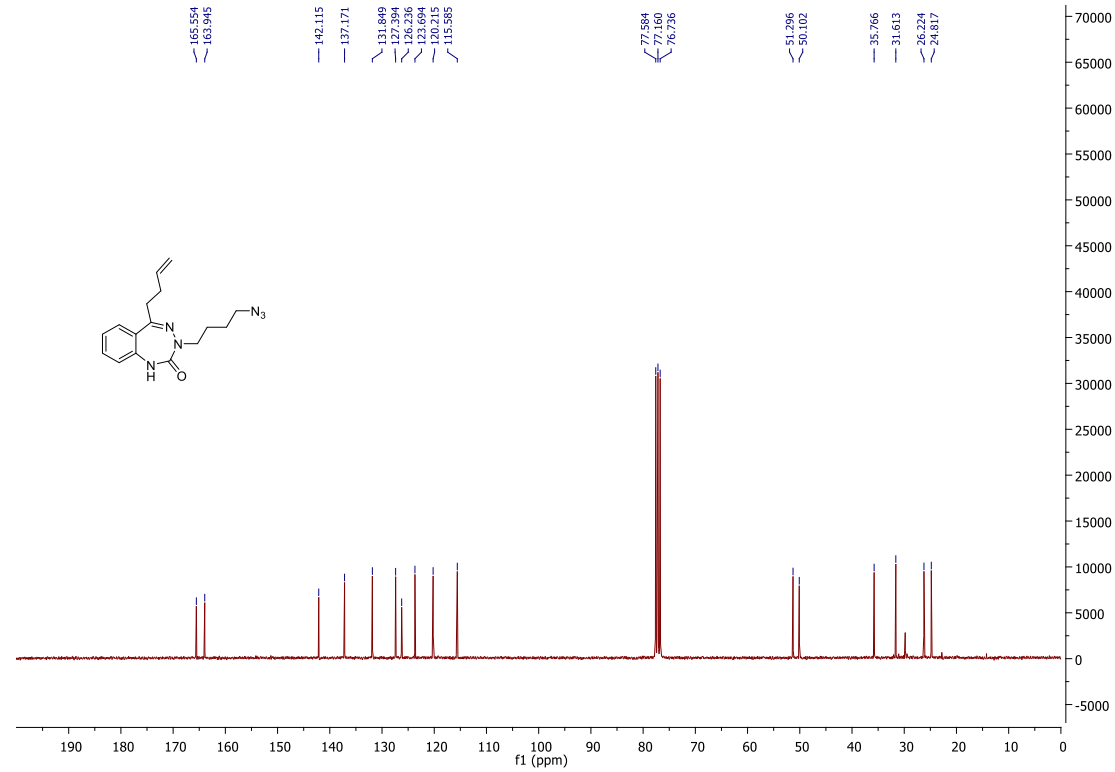
3.17b, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz)



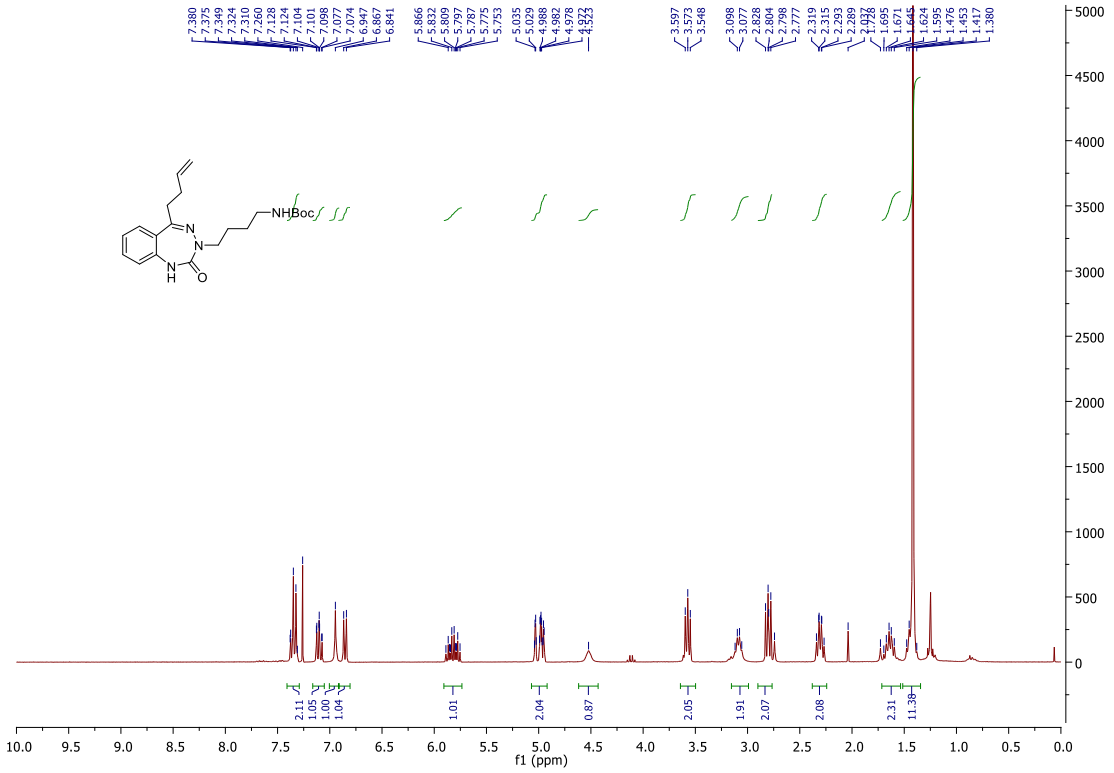


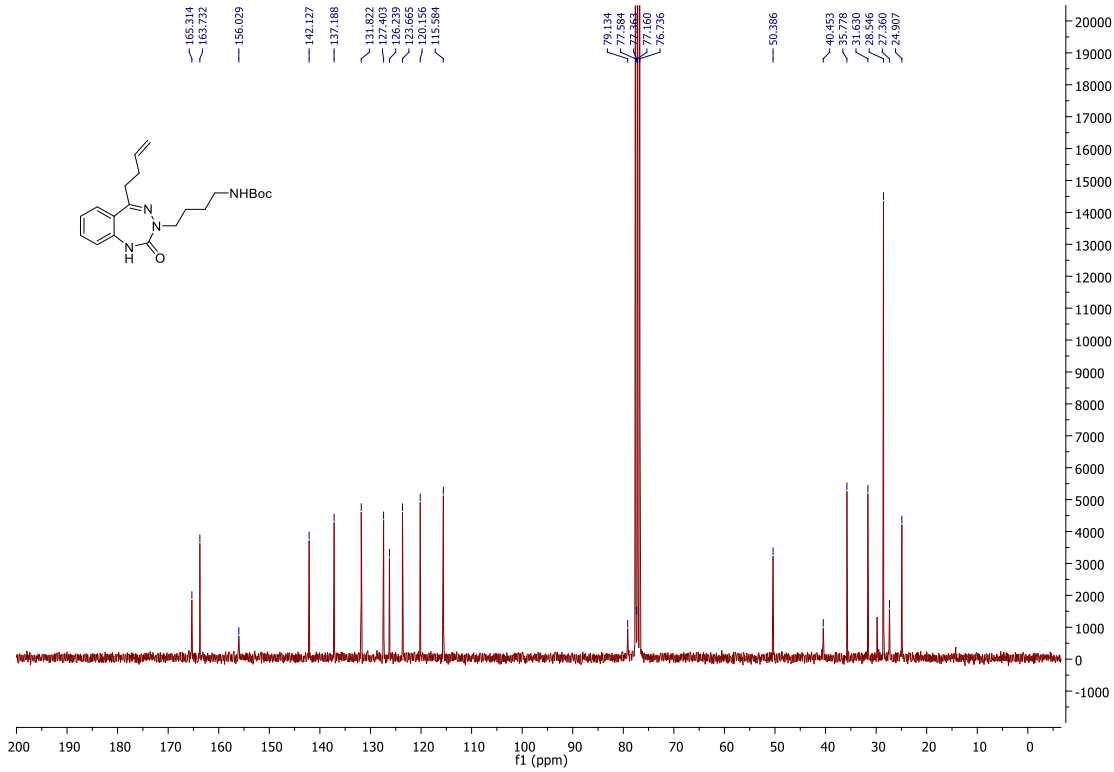
3.16c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



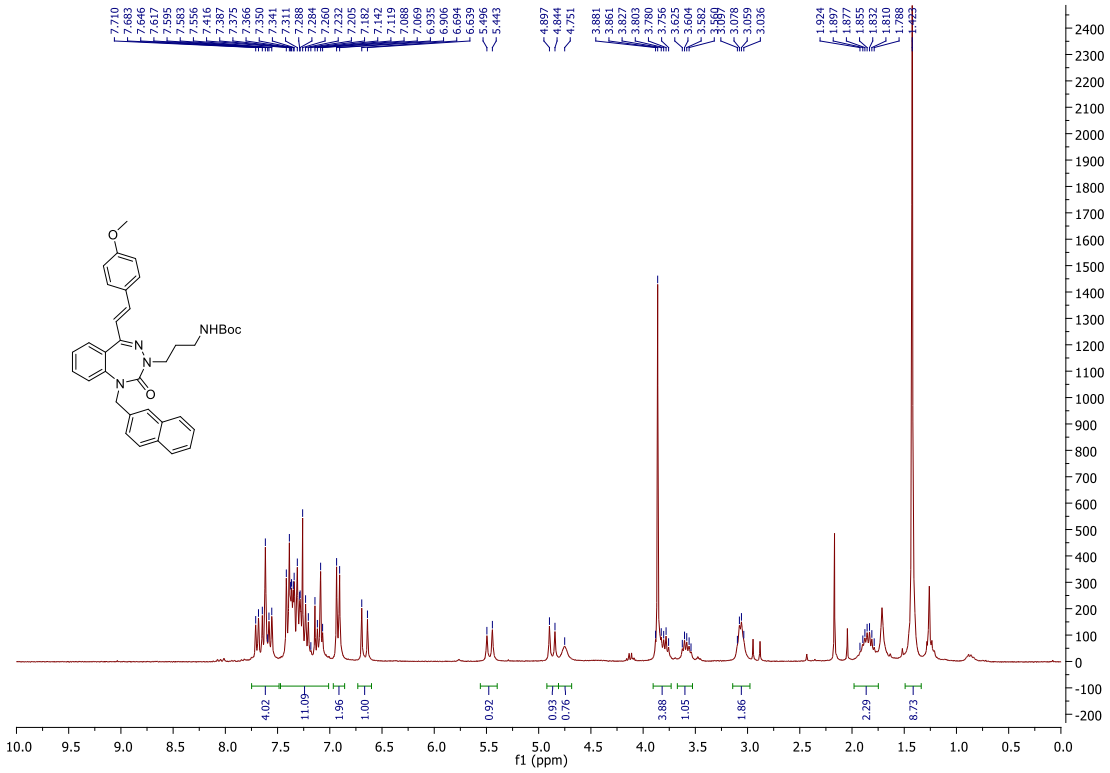


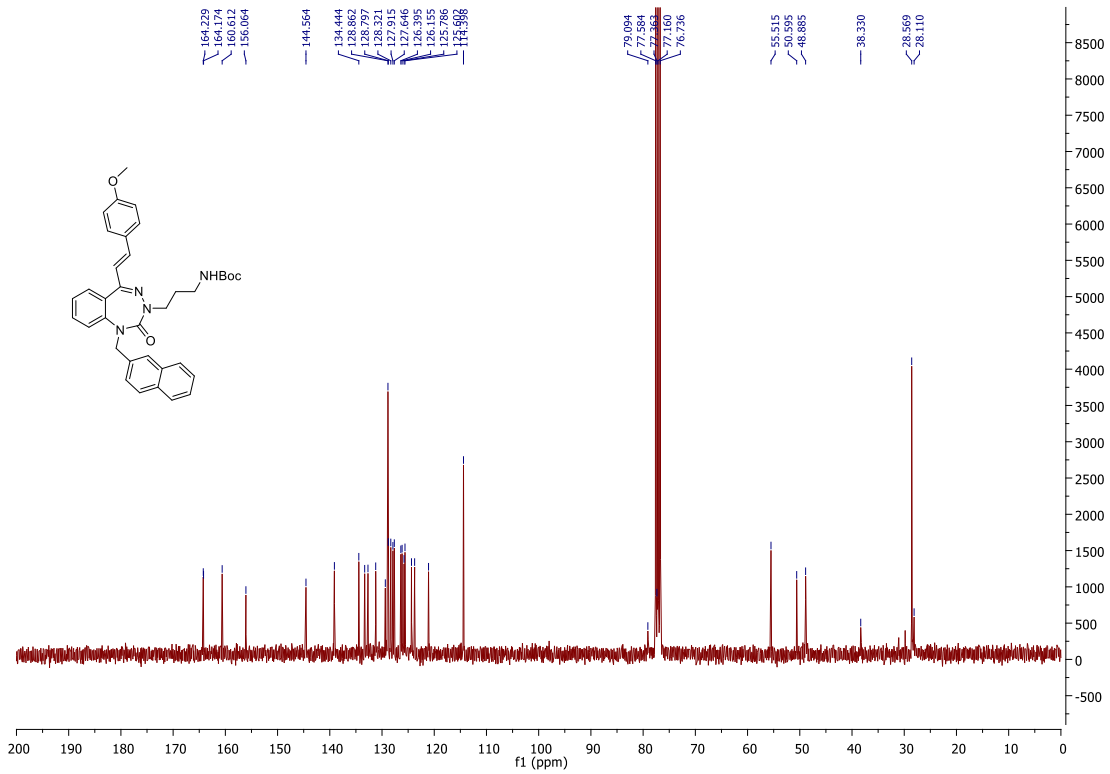
3.17c, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



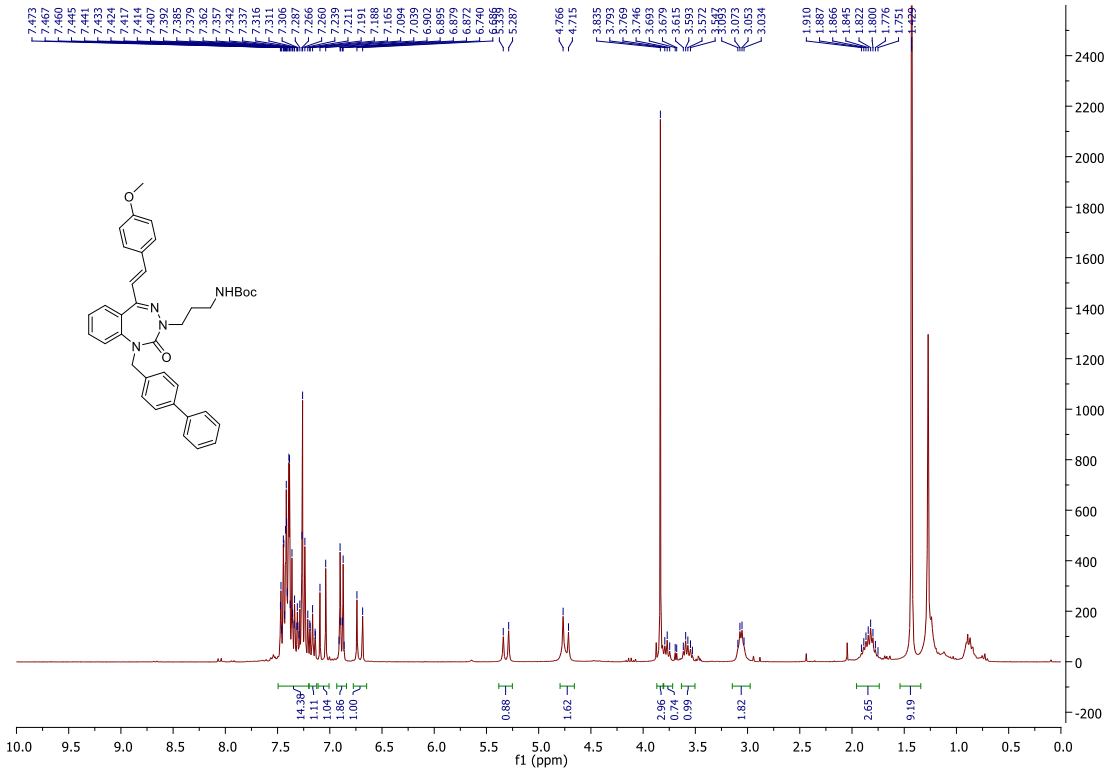


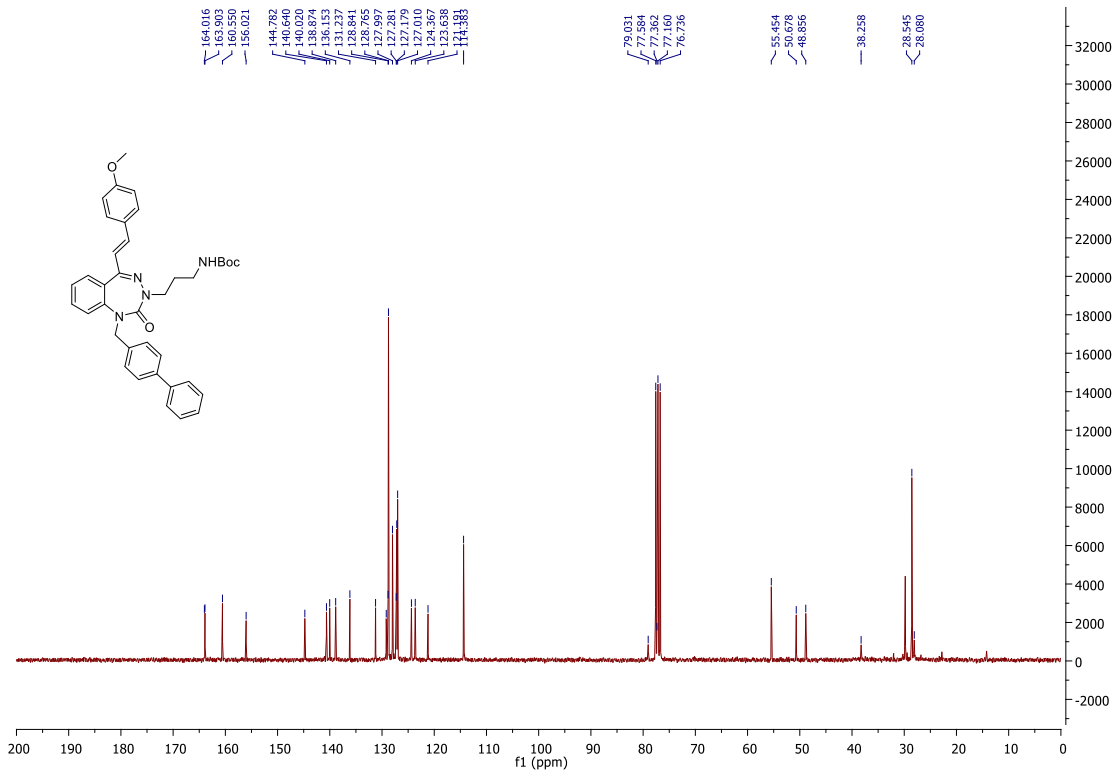
3.18d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



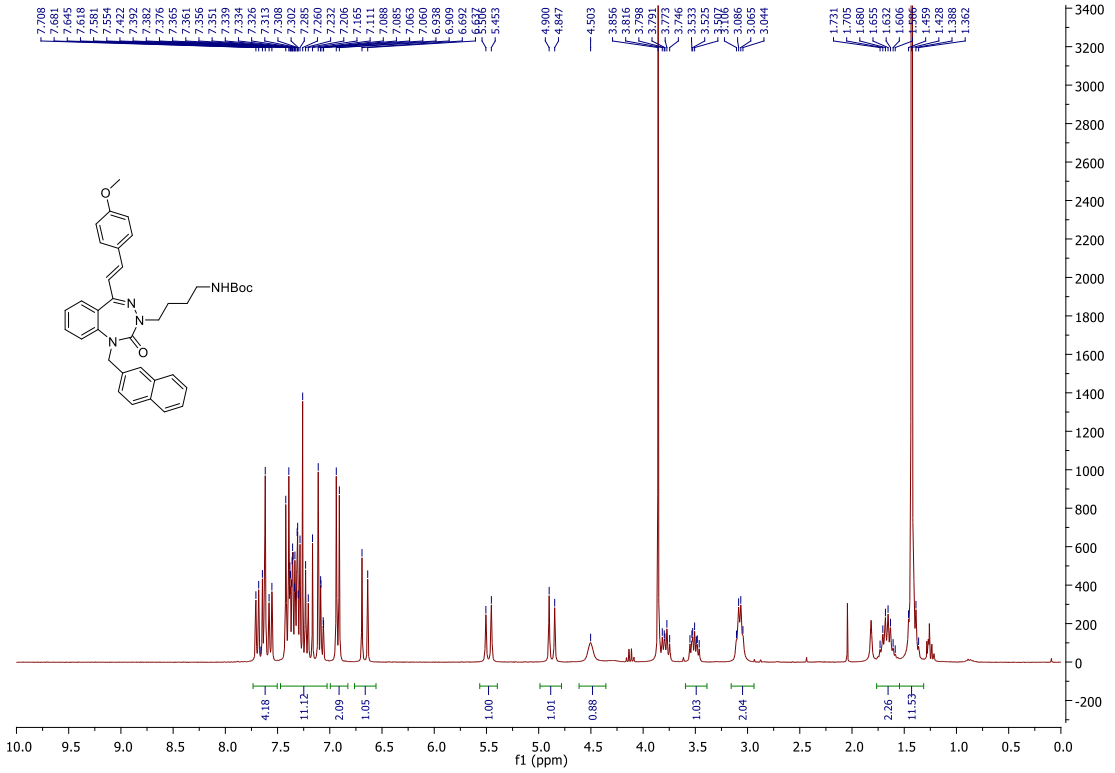


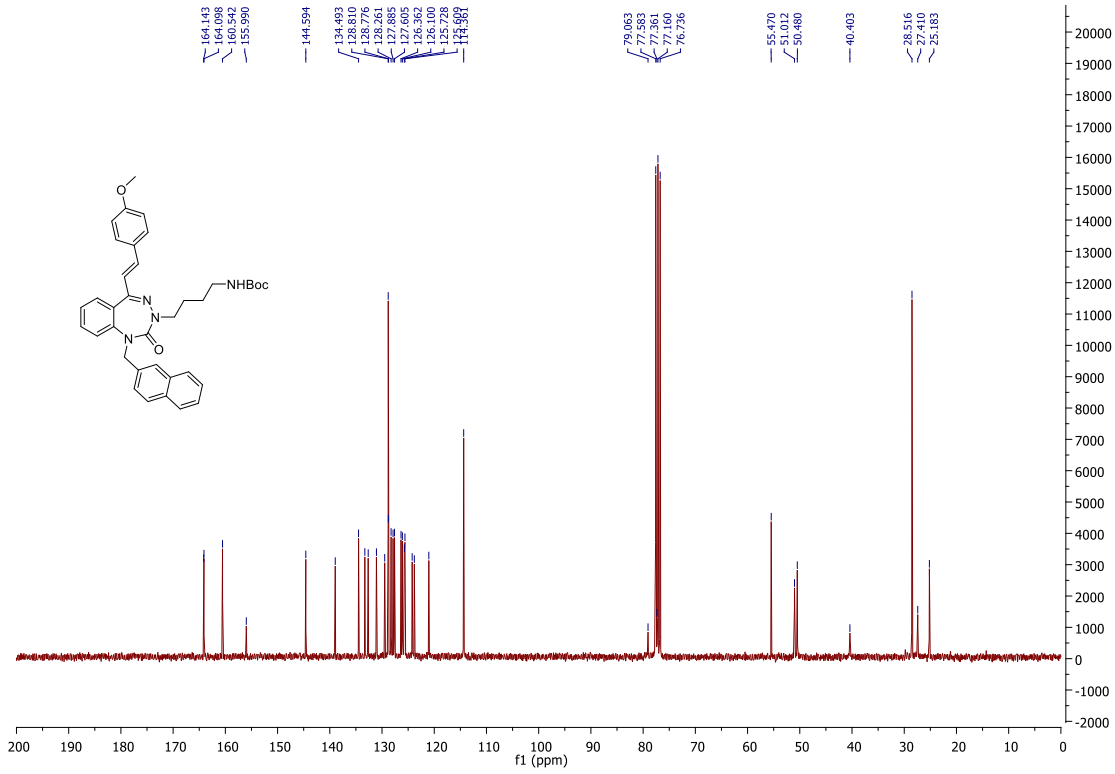
3.18e, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



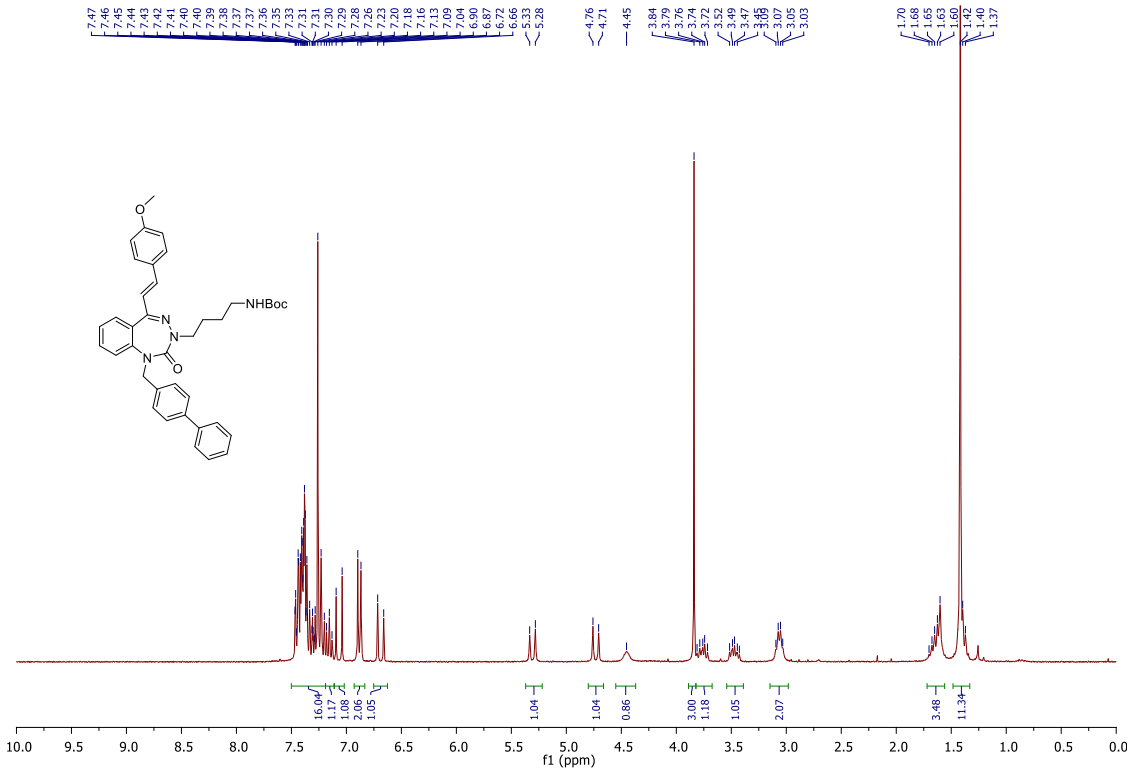


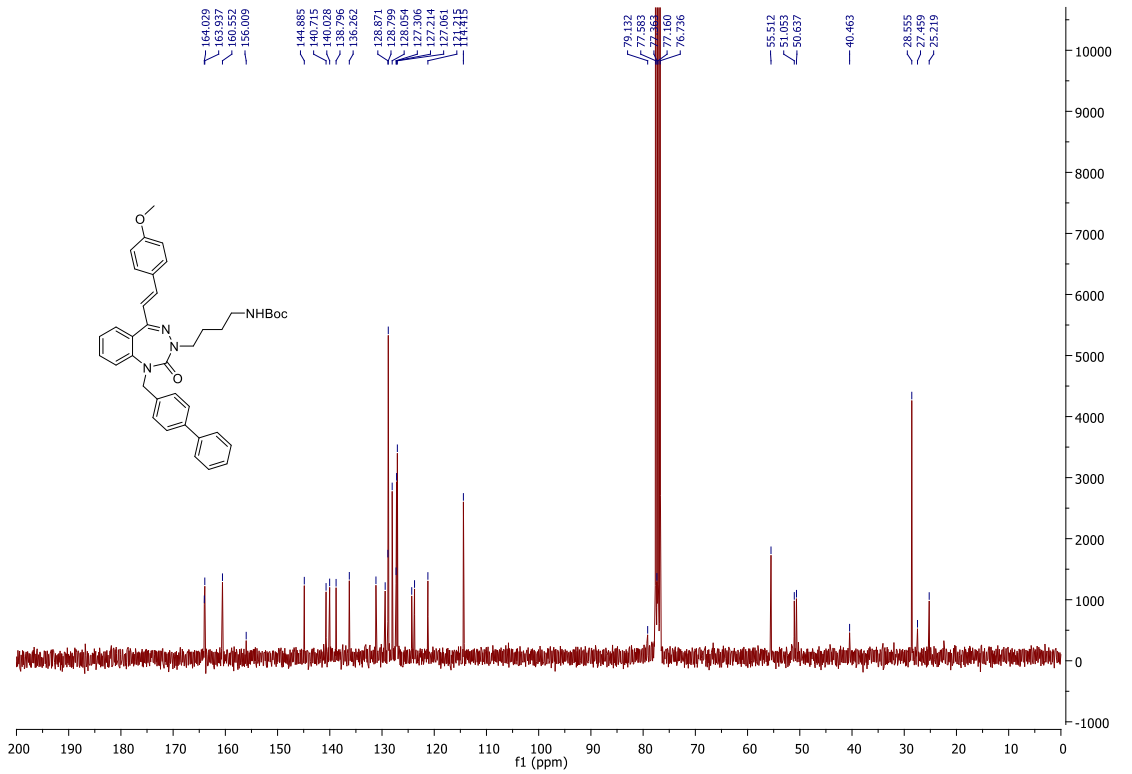
3.18f, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



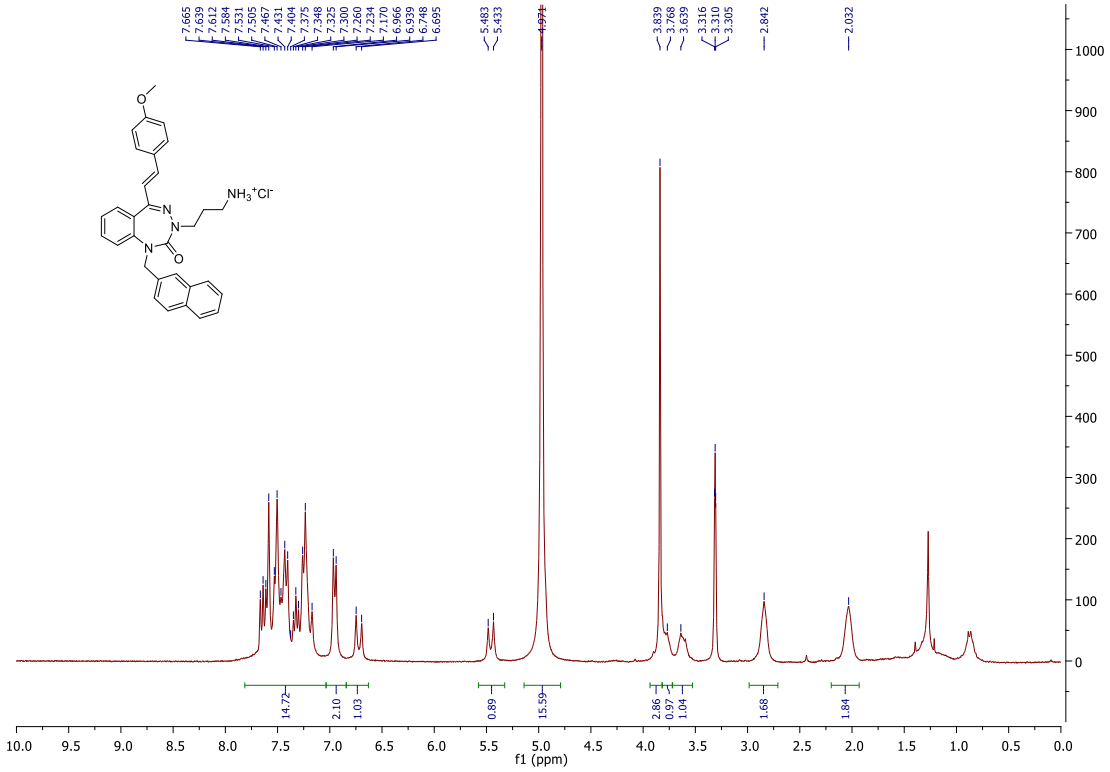


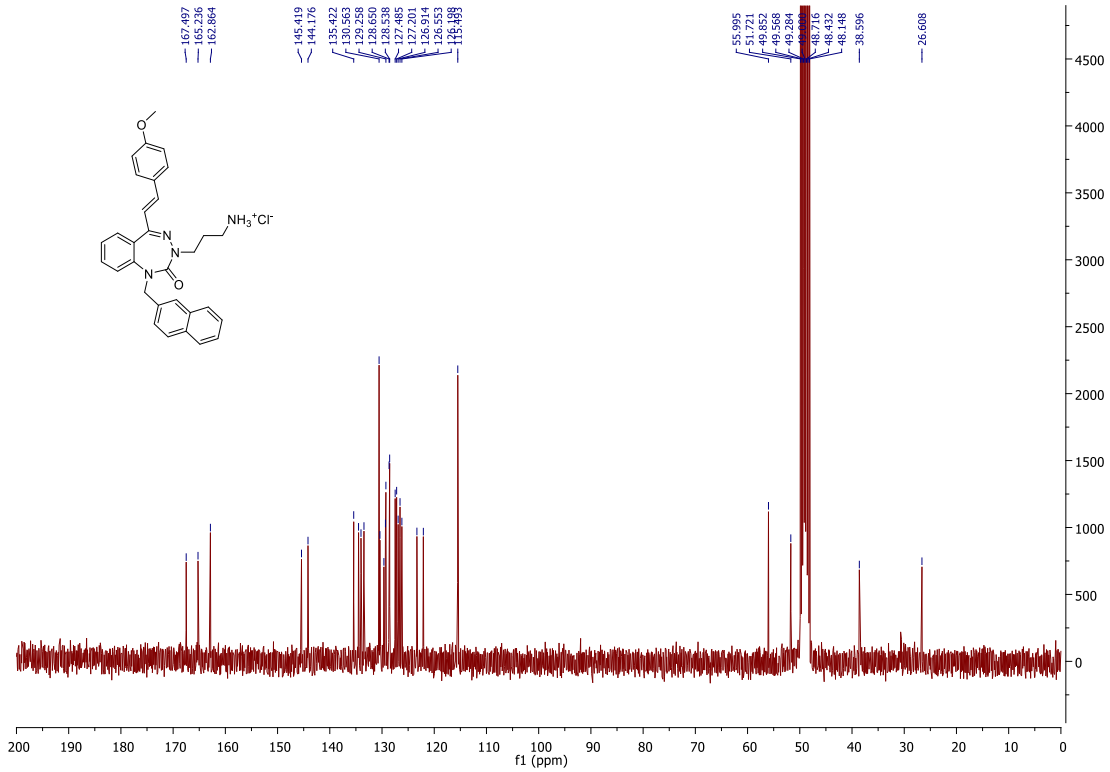
3.18g, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



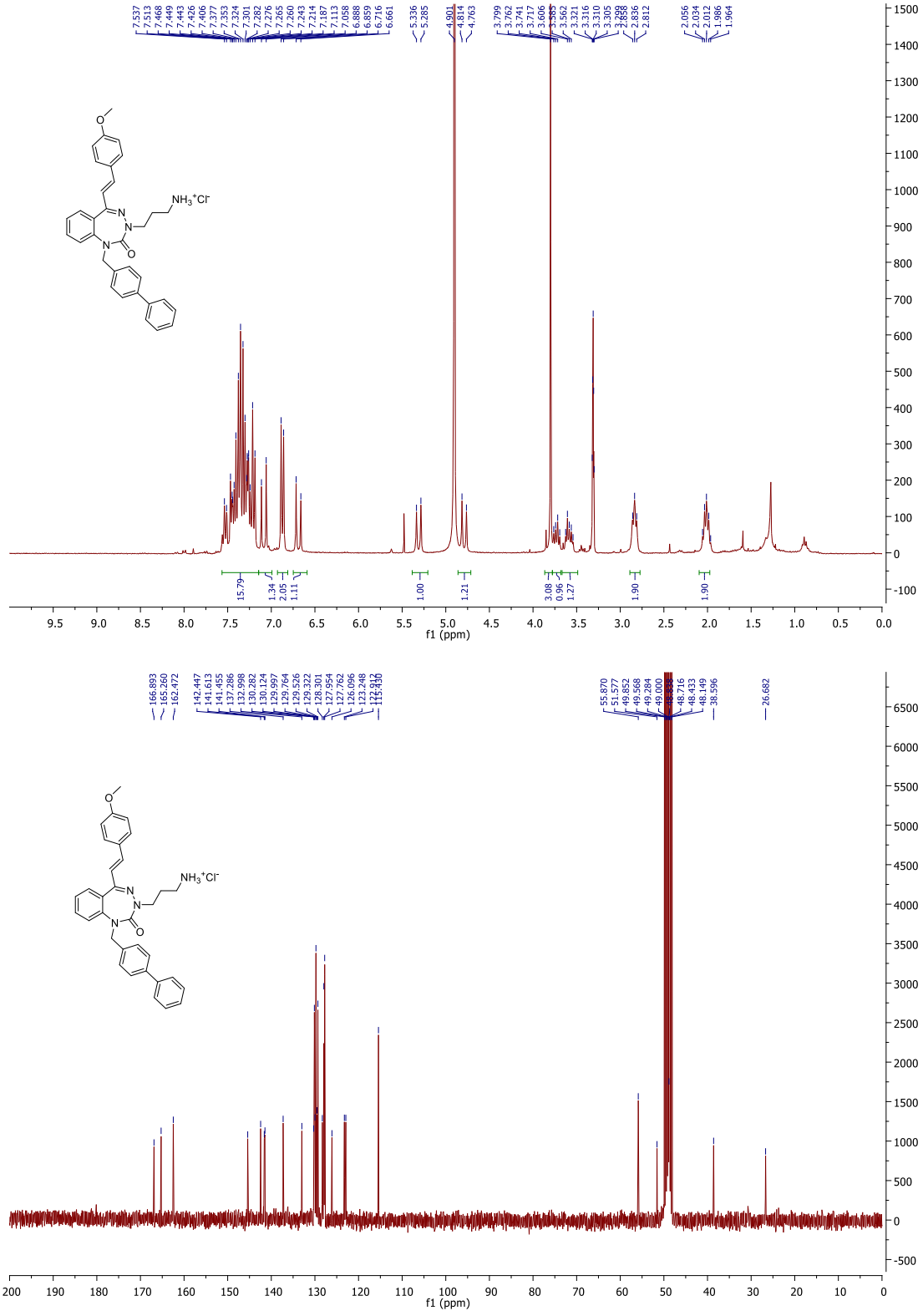


3.19d, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)

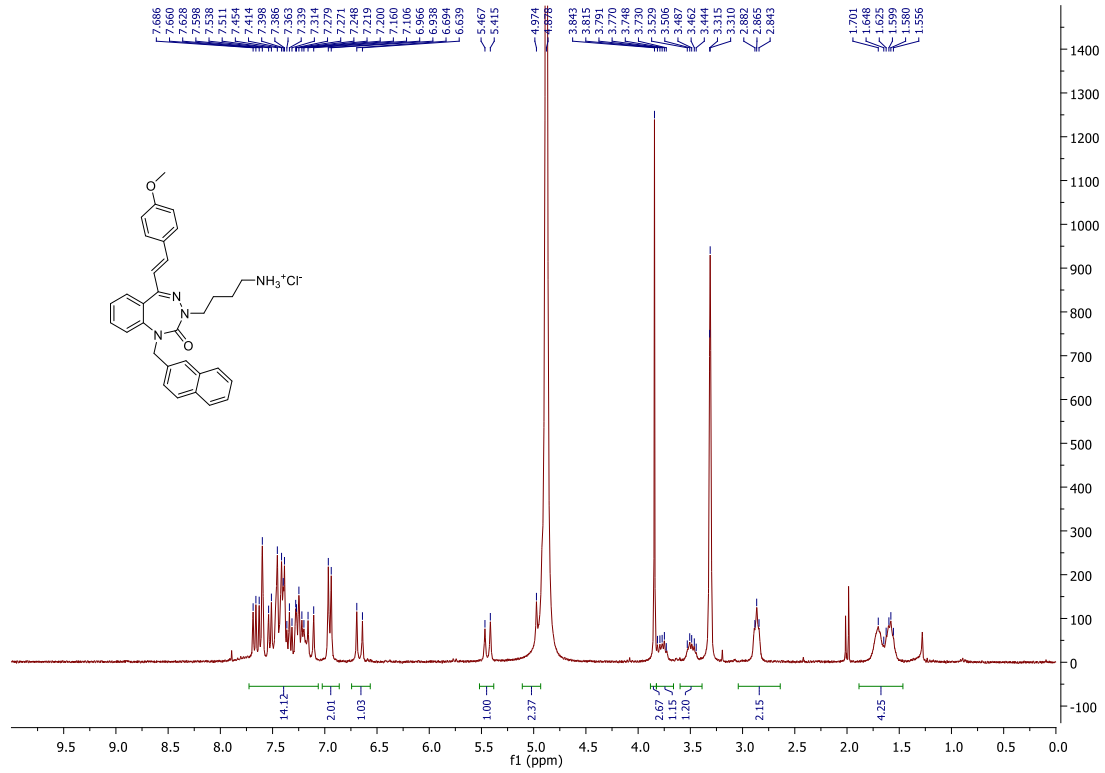


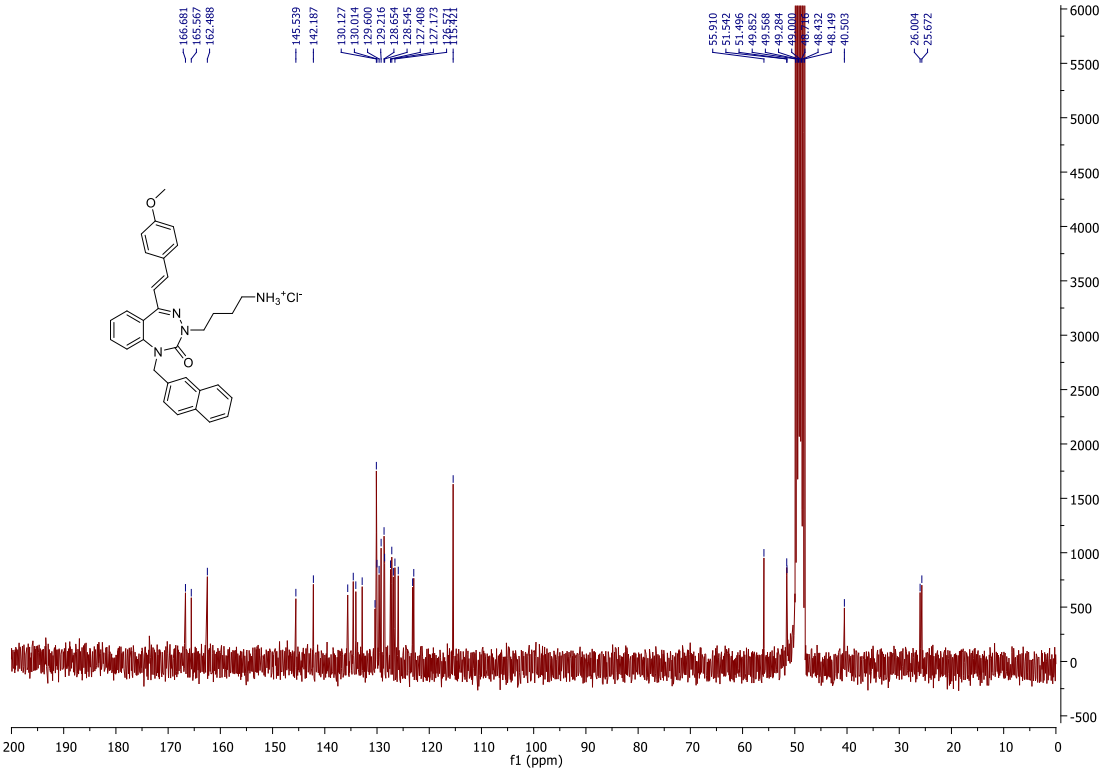


3.19e, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)

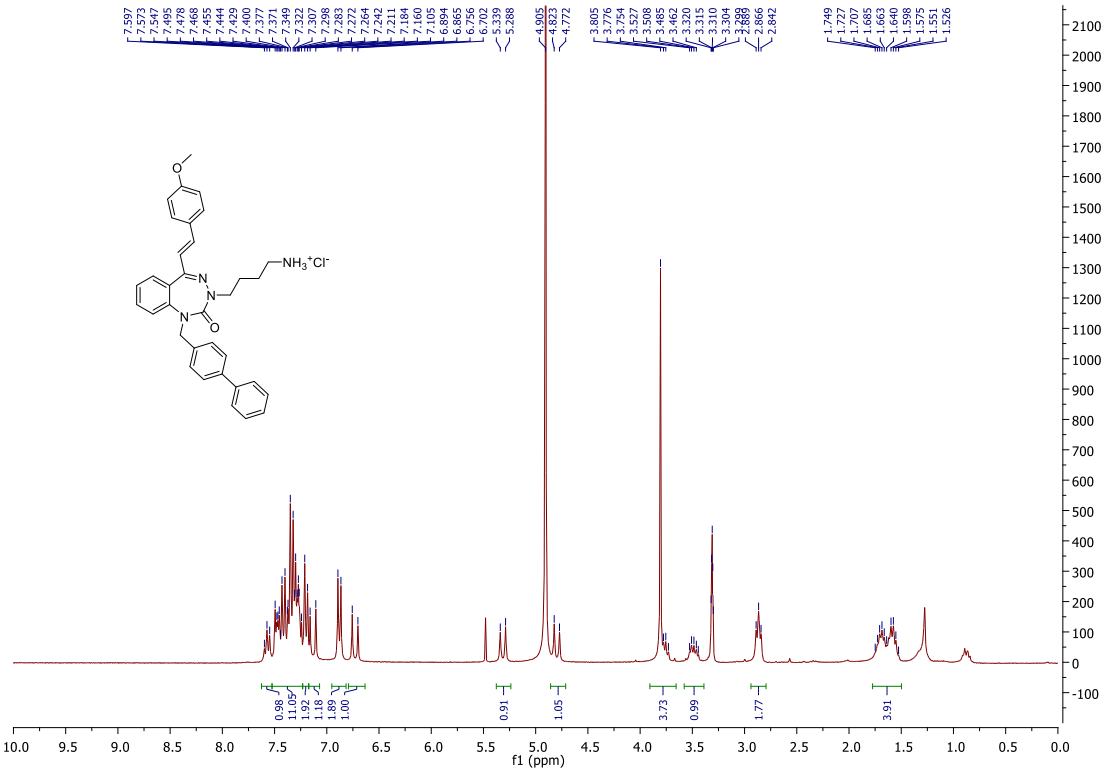


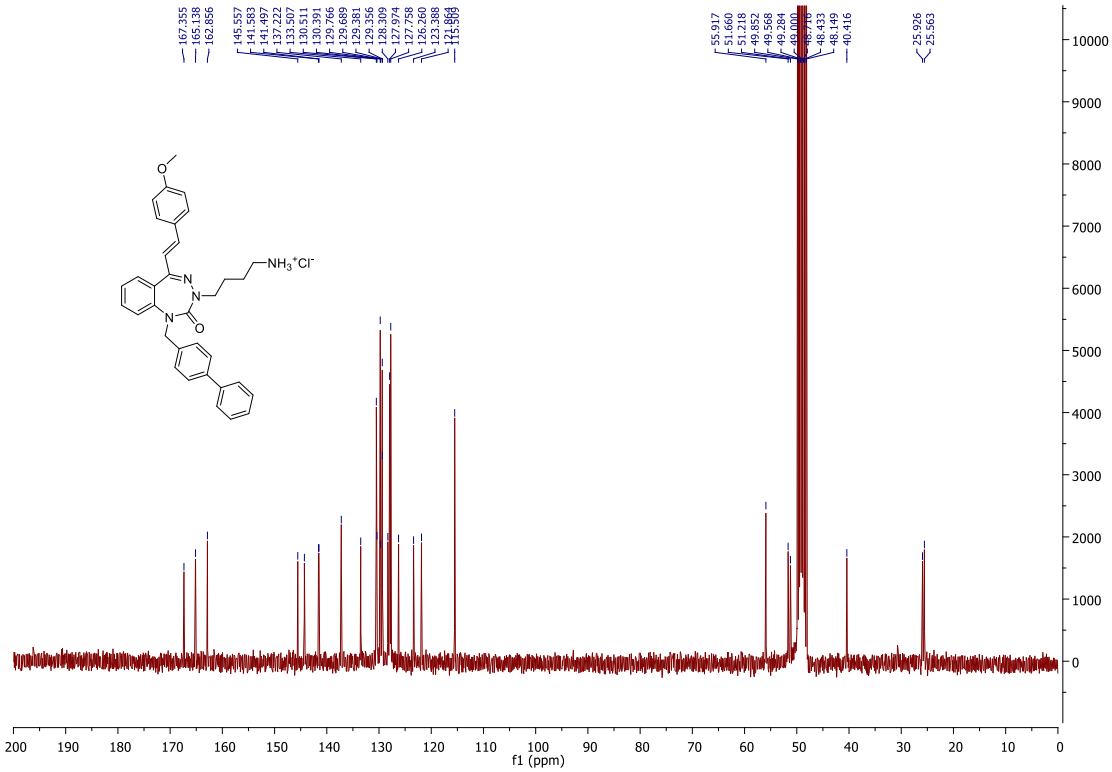
3.19f, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



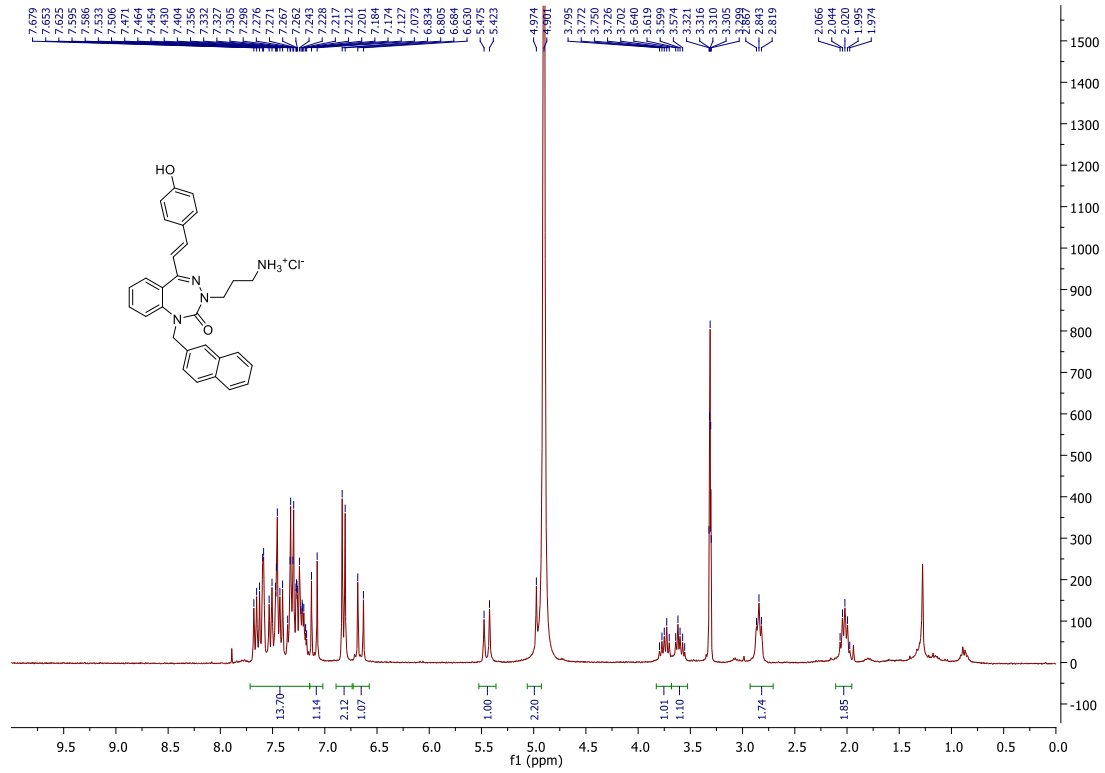


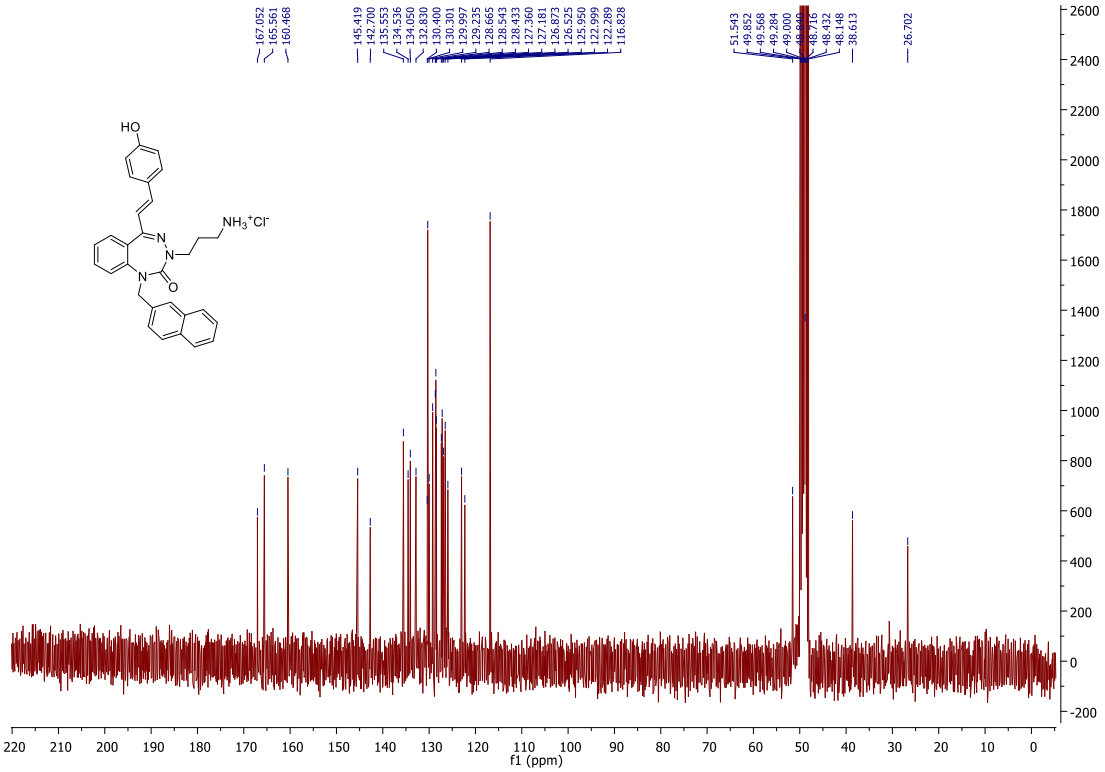
3.19g, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



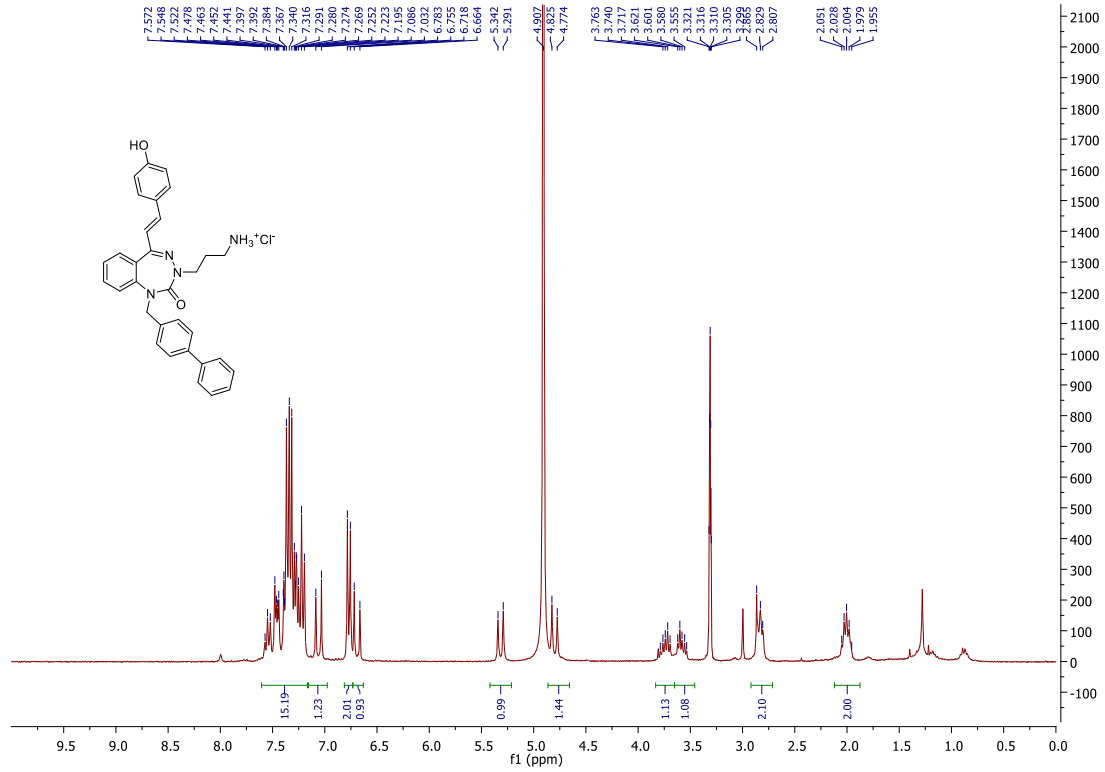


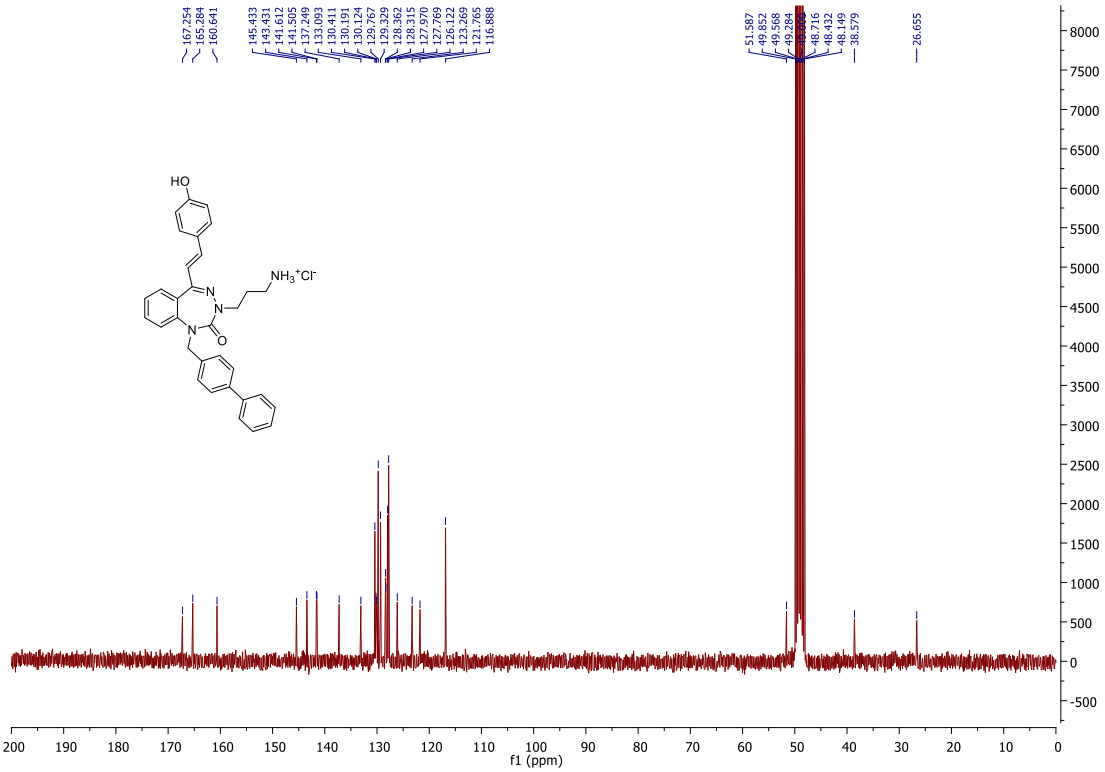
3.20d, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



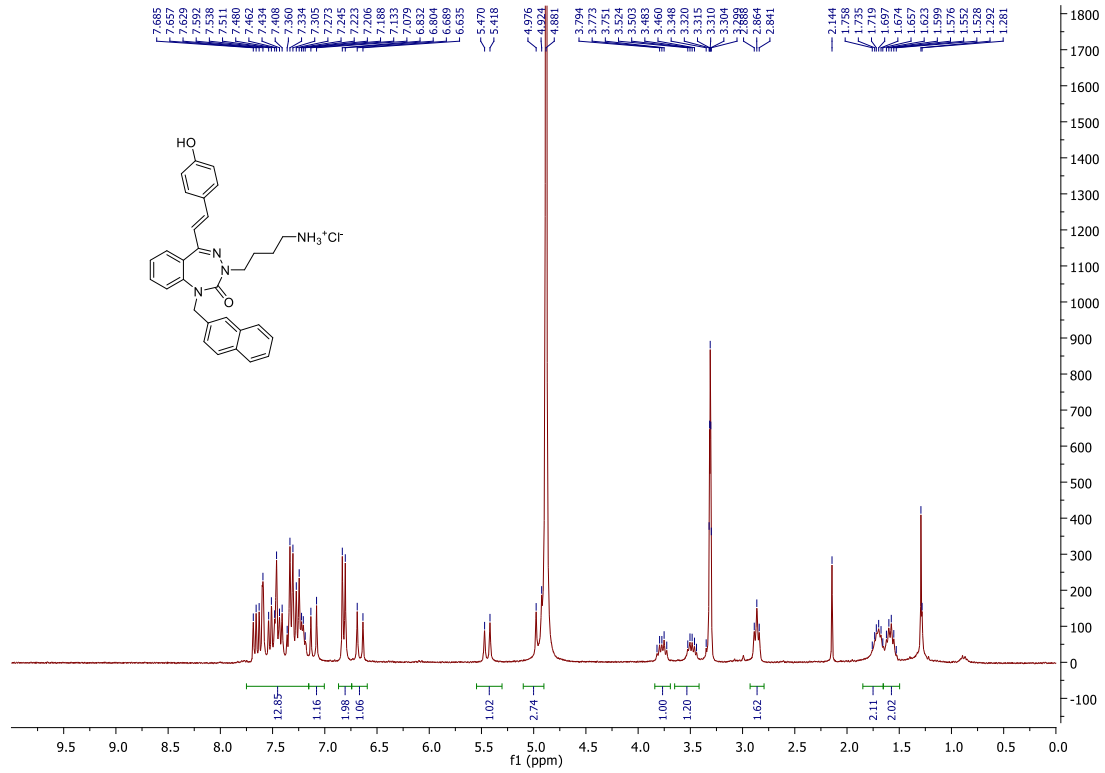


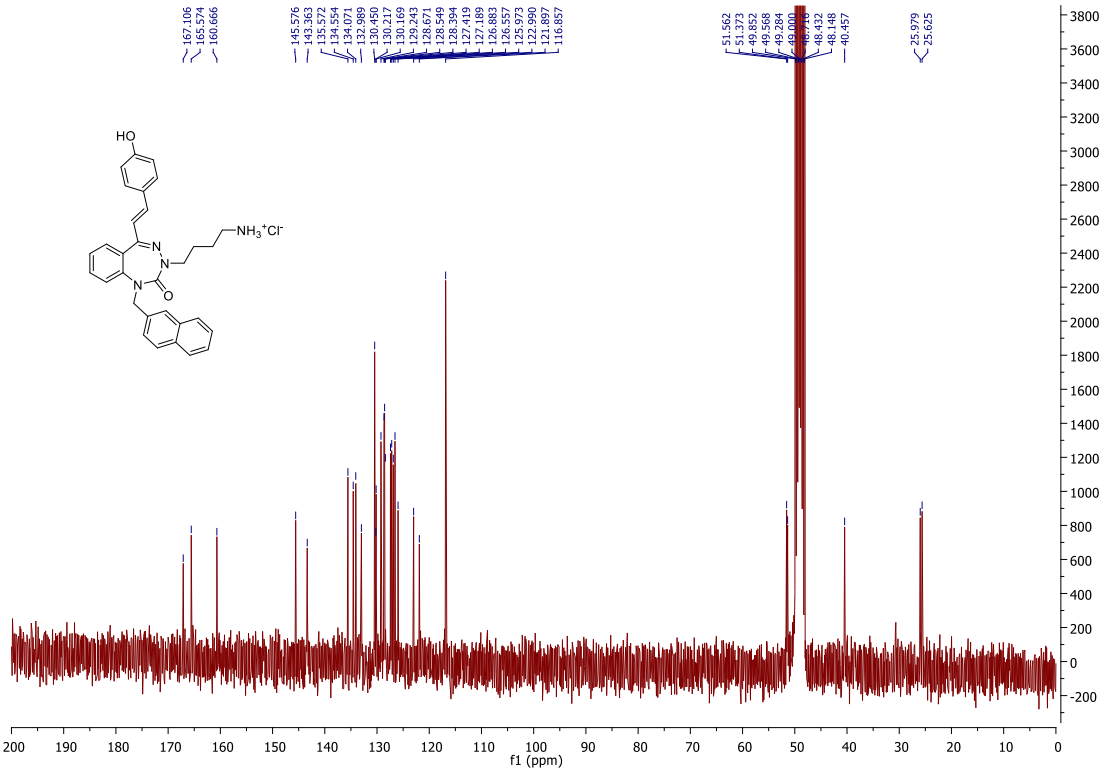
3.20e, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



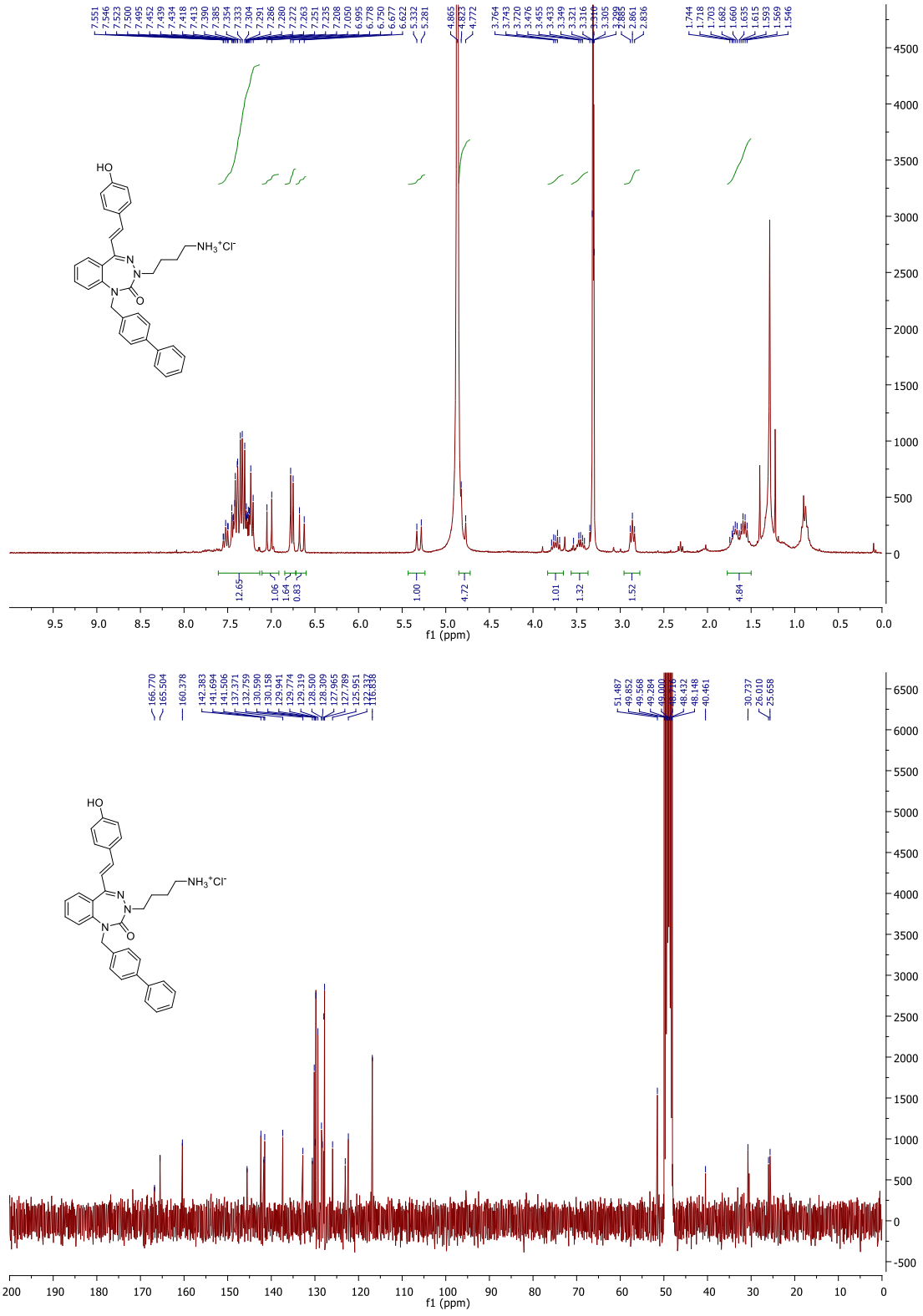


3.20f, CD3OD-d_4, ^{1}H (300 MHz), ^{13}C (75 MHz)

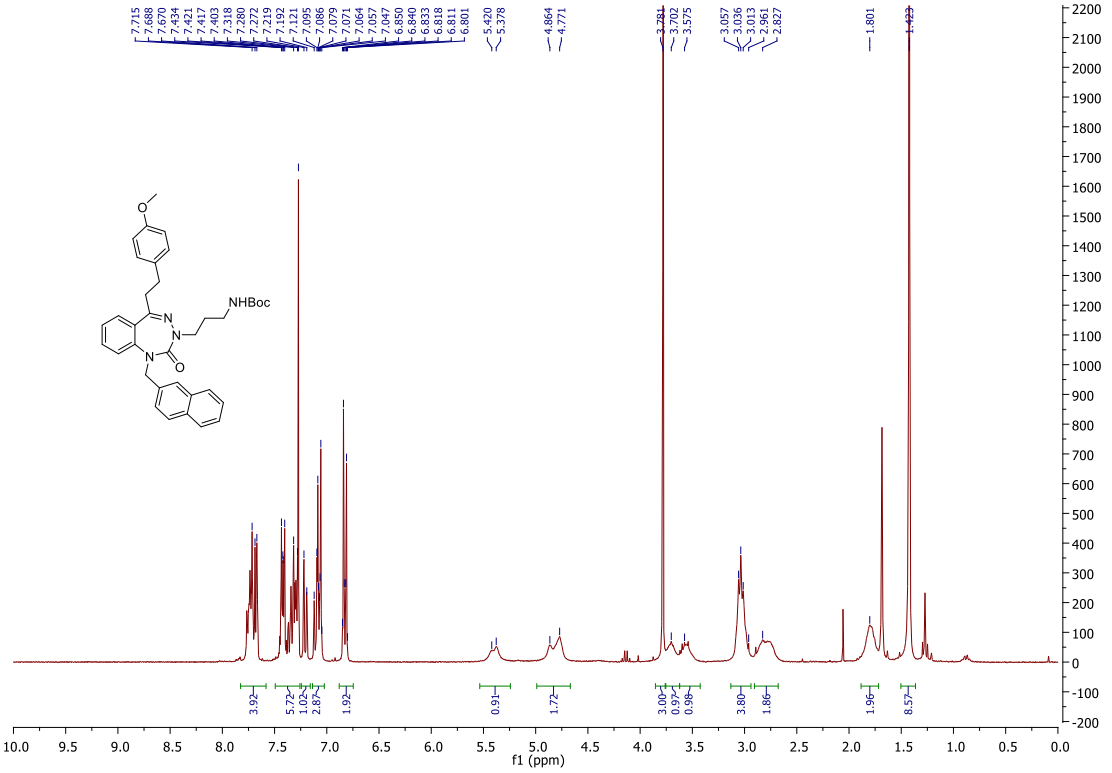


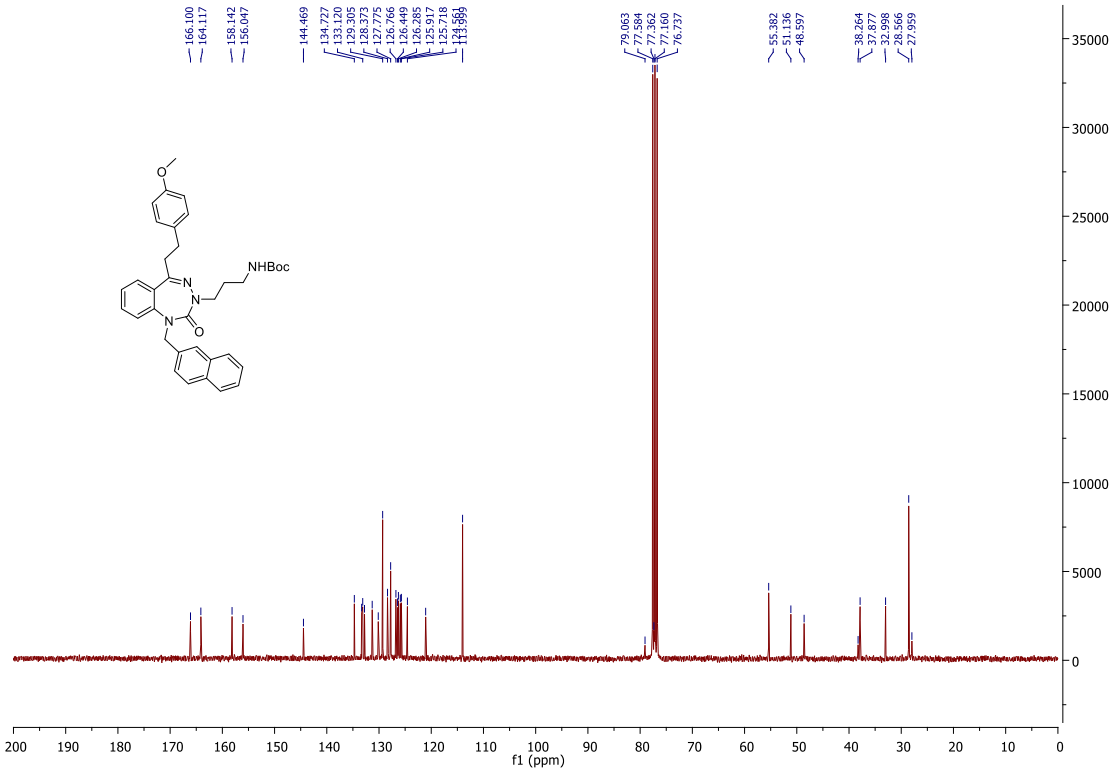


3.20g, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)

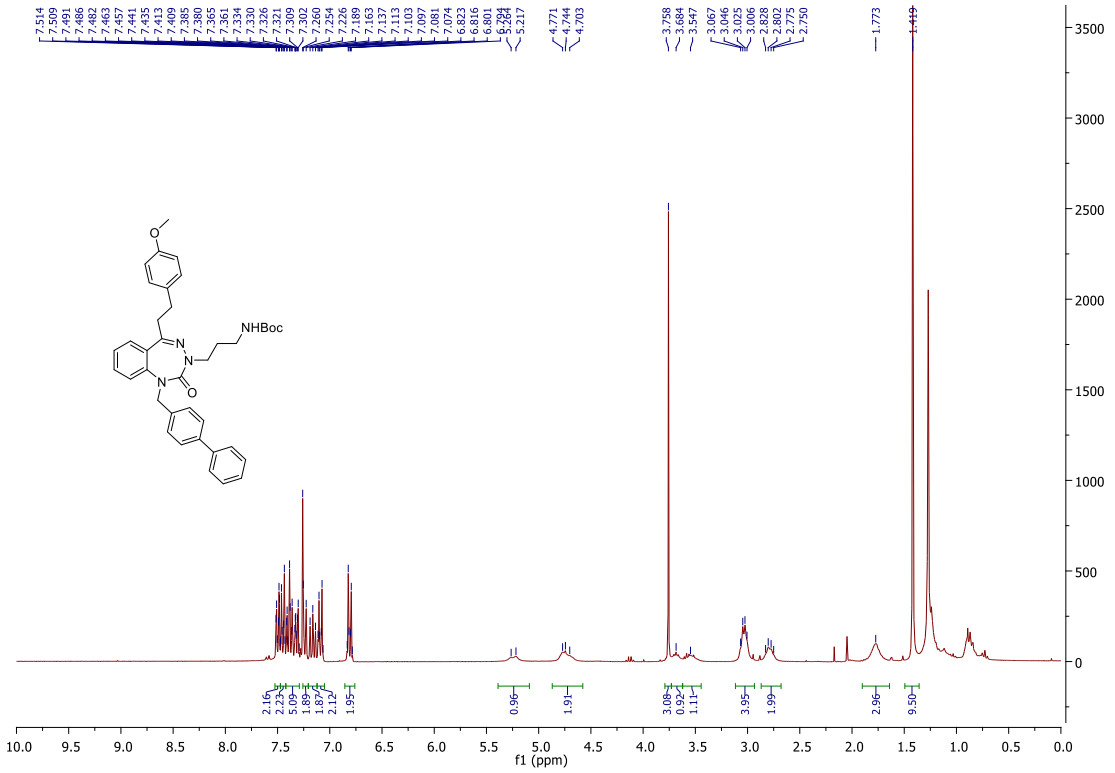


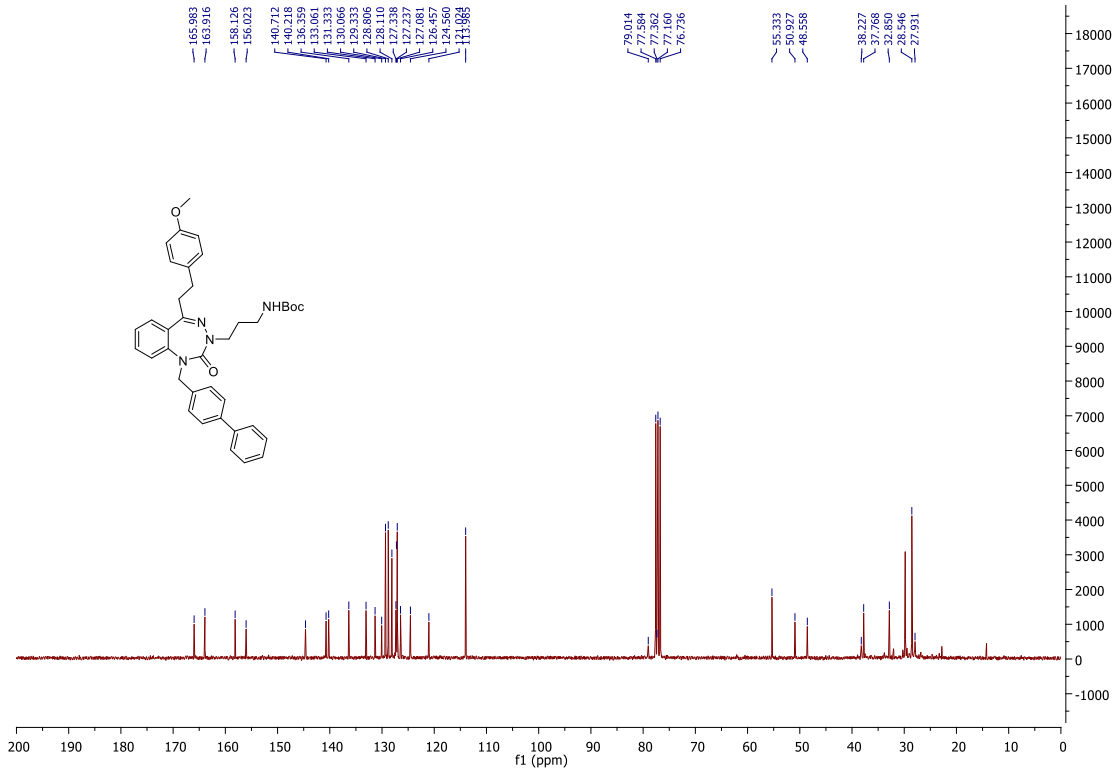
3.21d, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



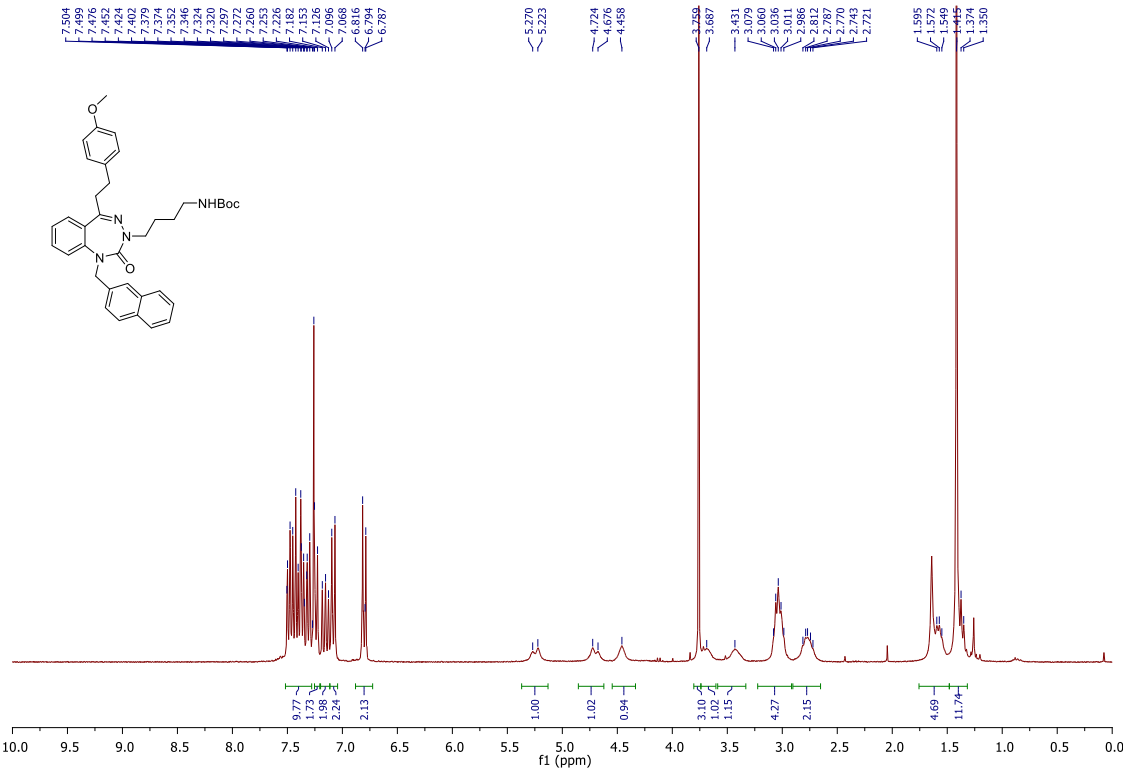


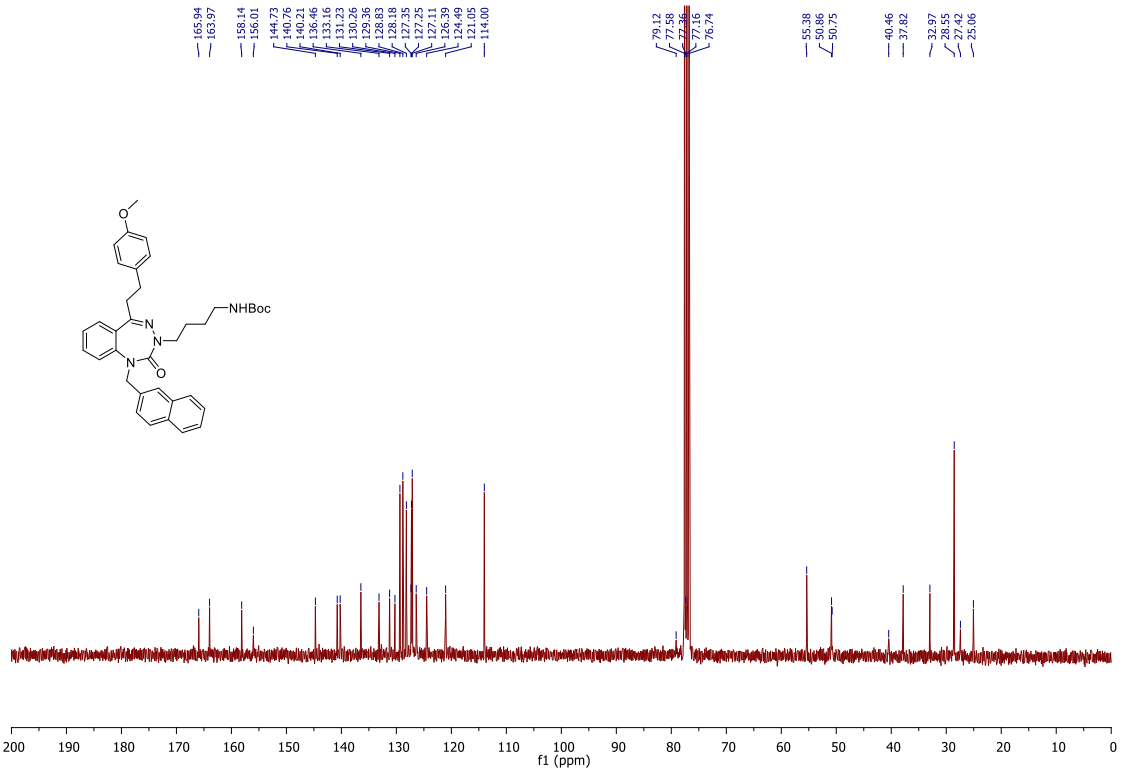
3.21e, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



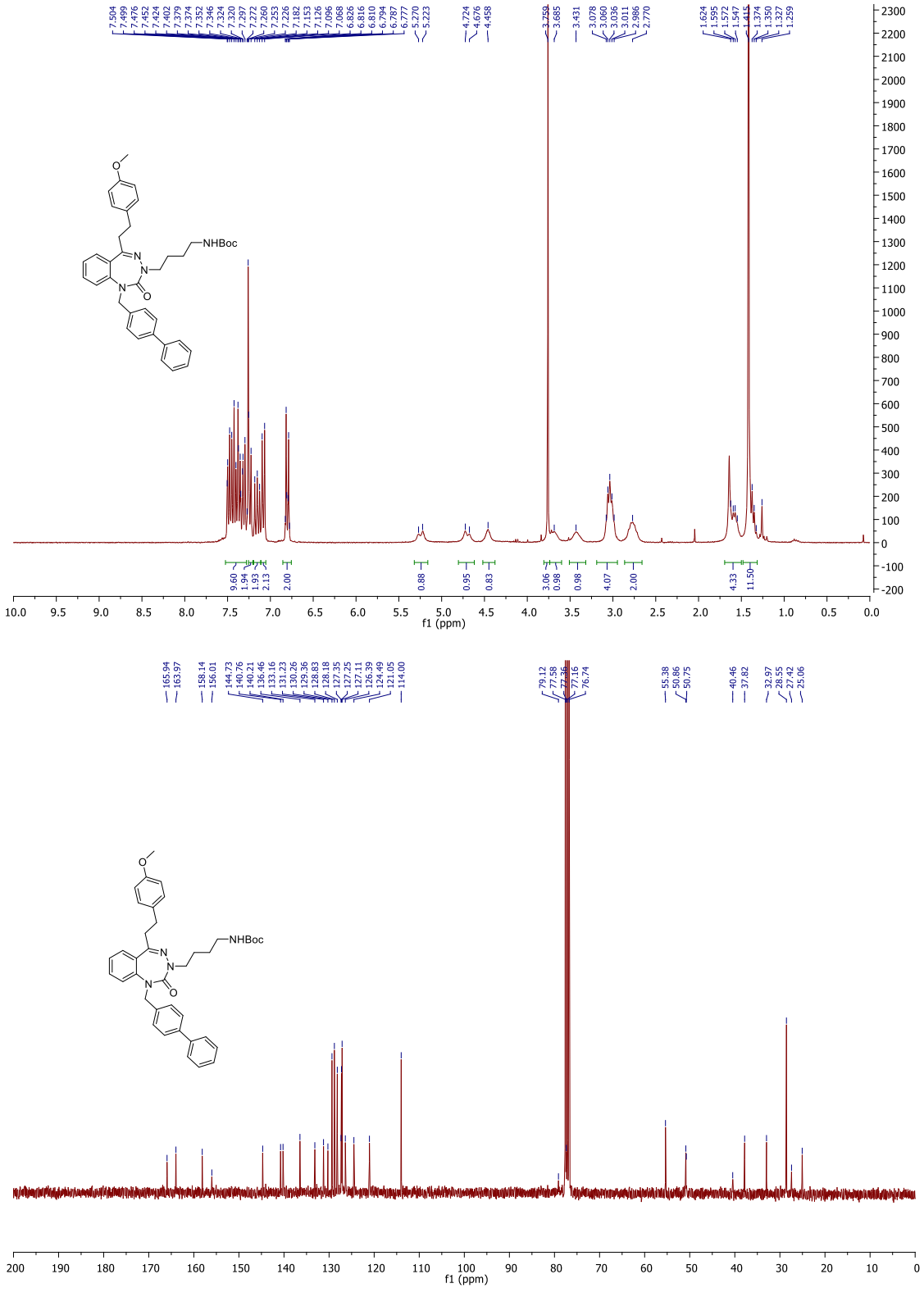


3.21f, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

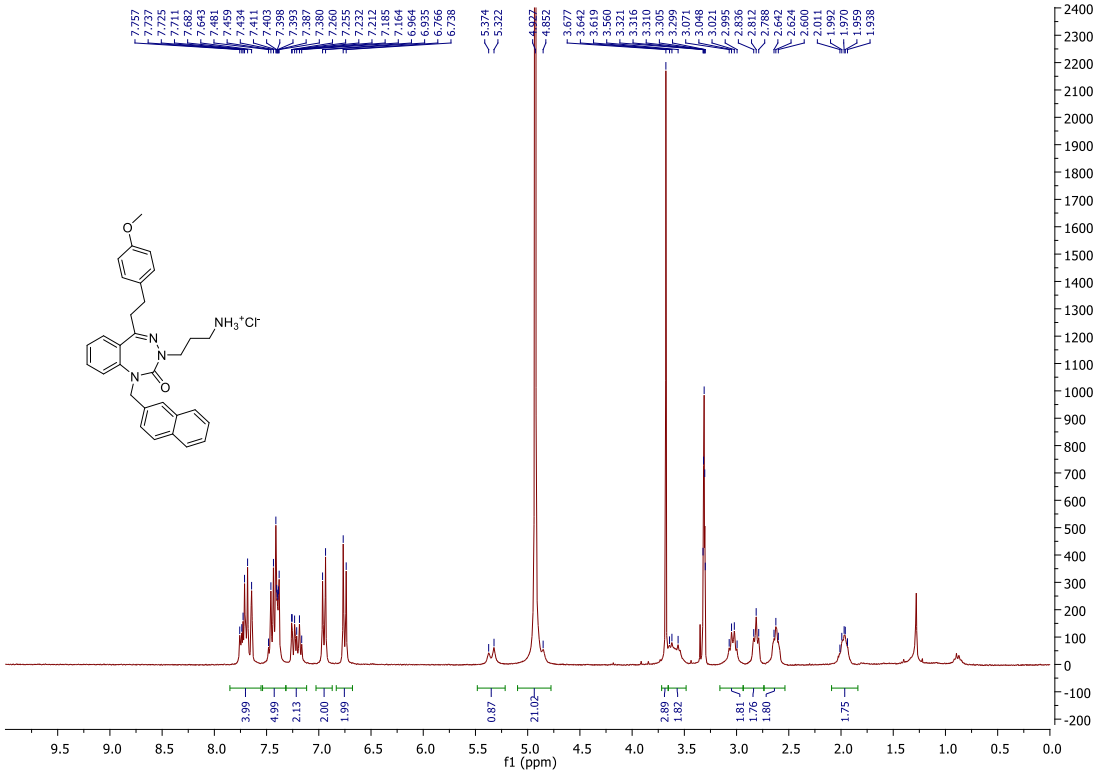


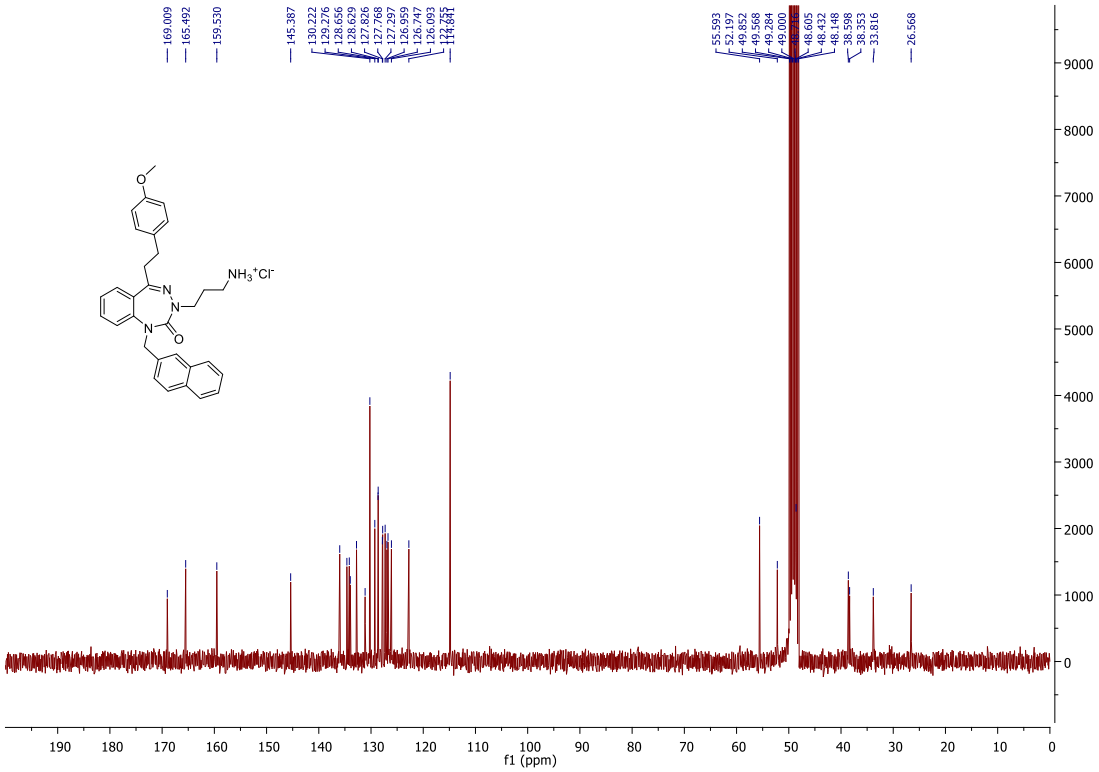


3.21g, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

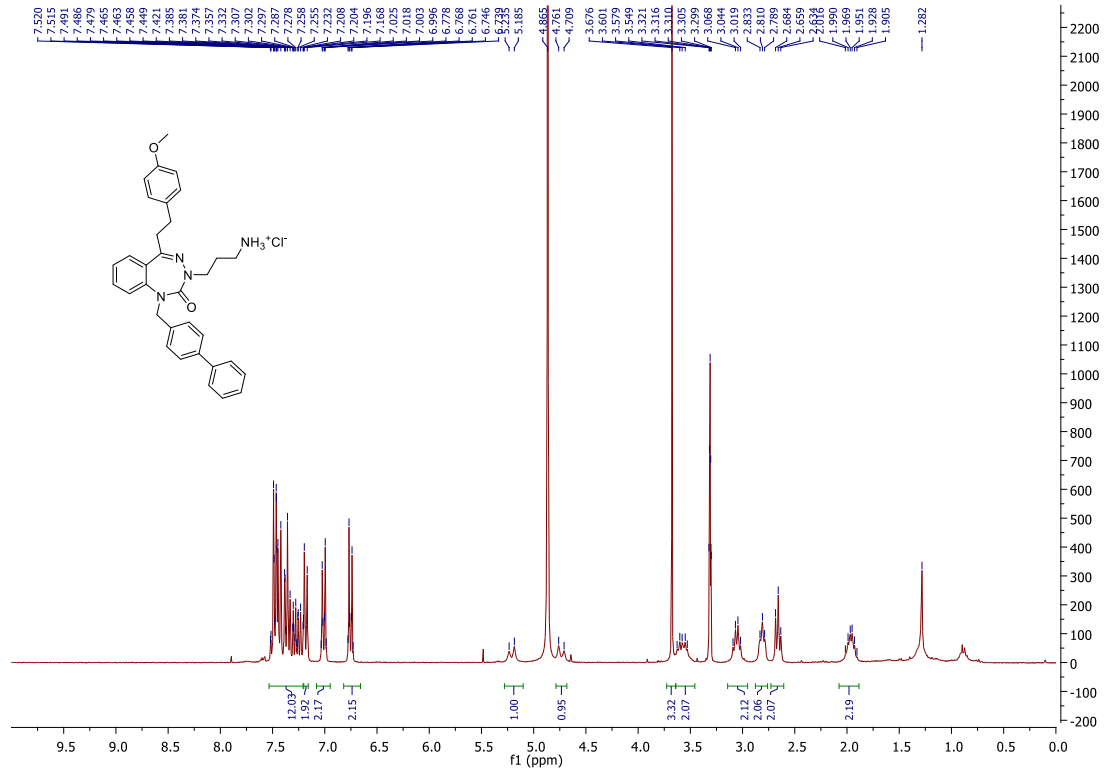


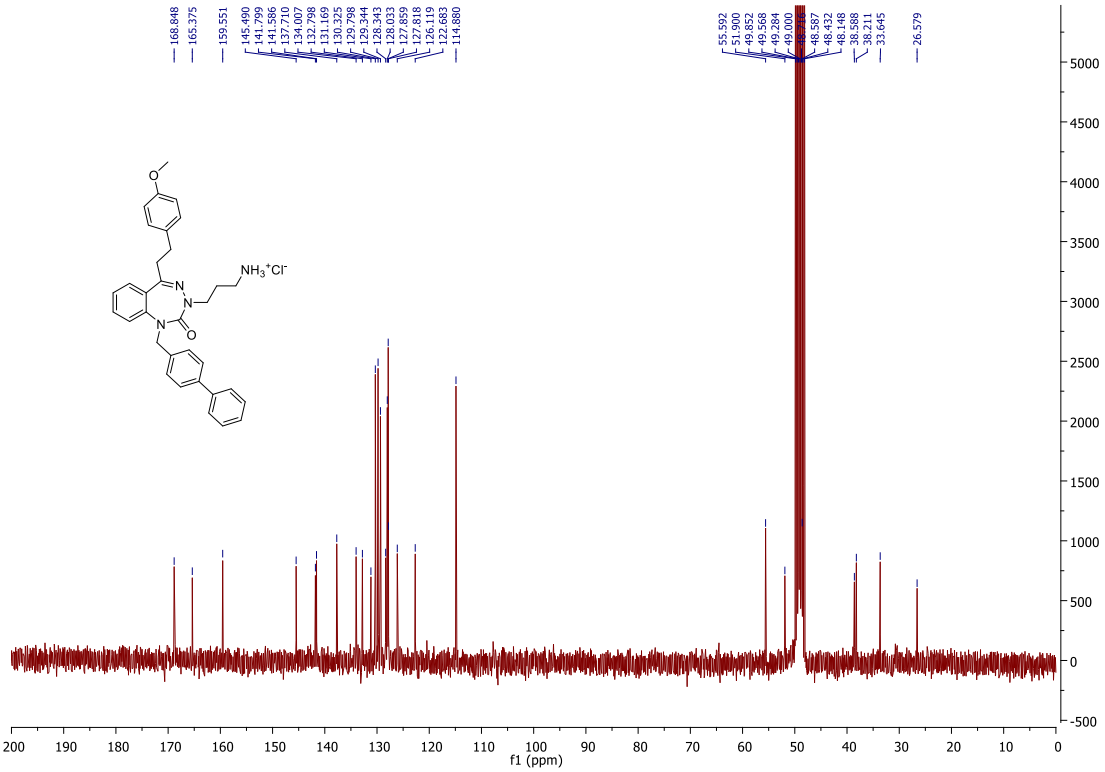
3.22d, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



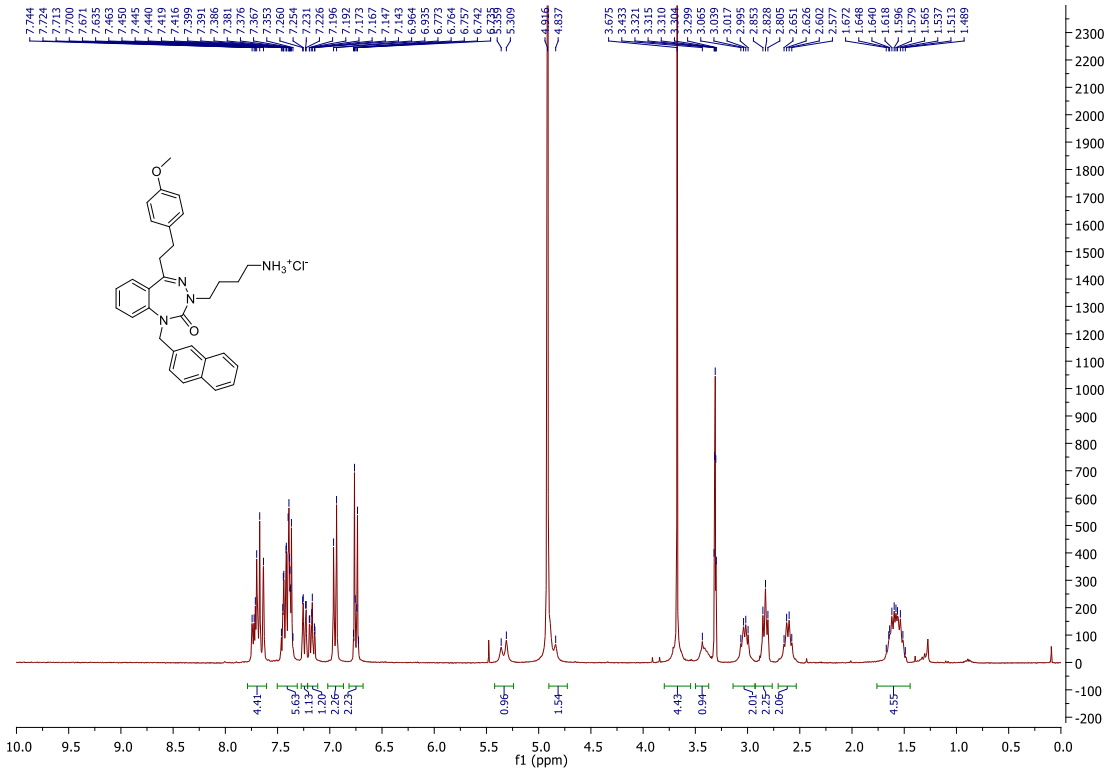


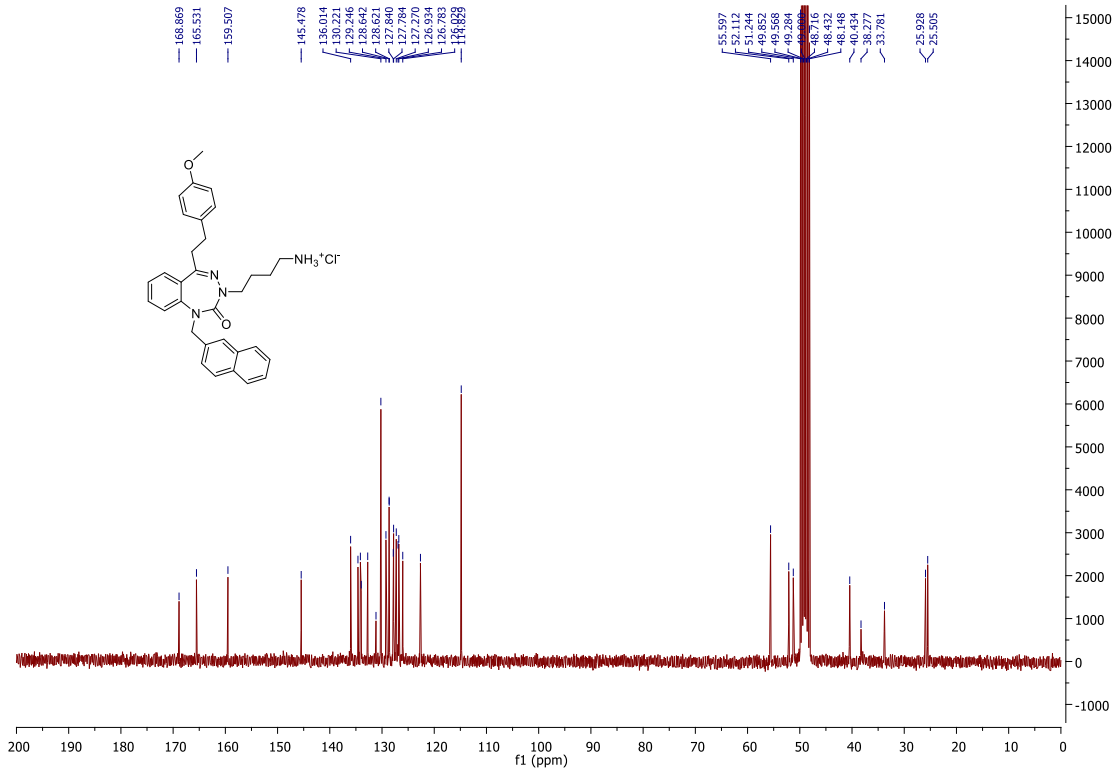
3.22e, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



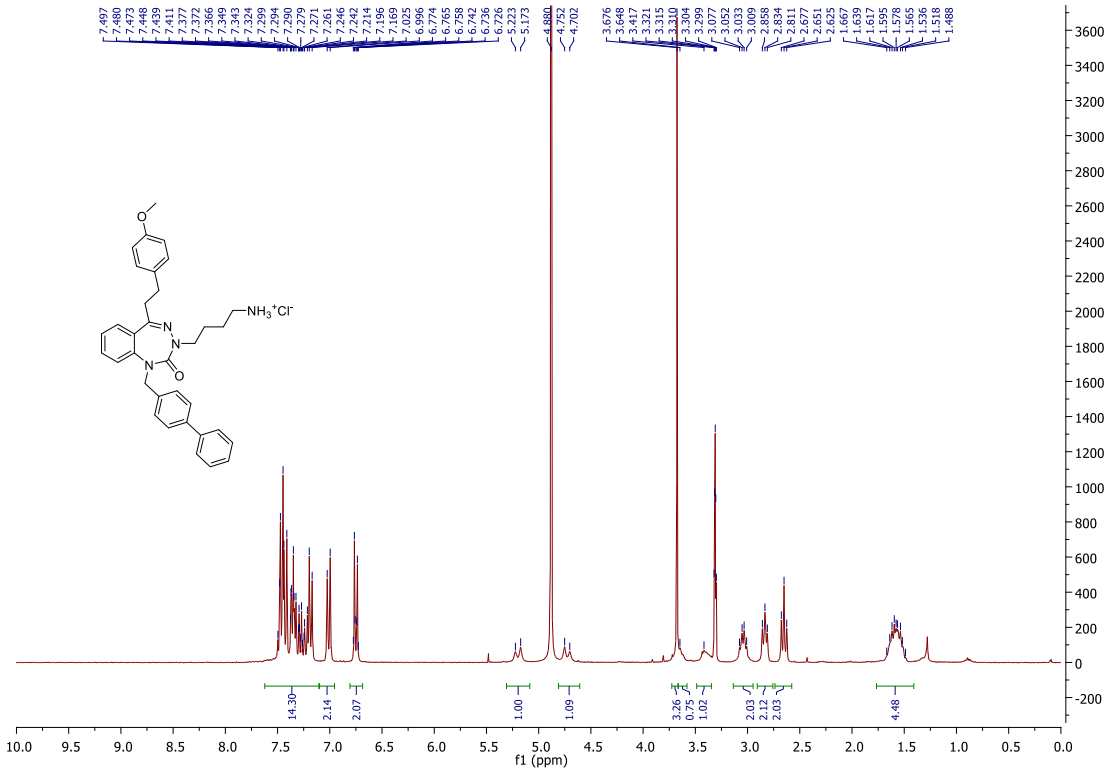


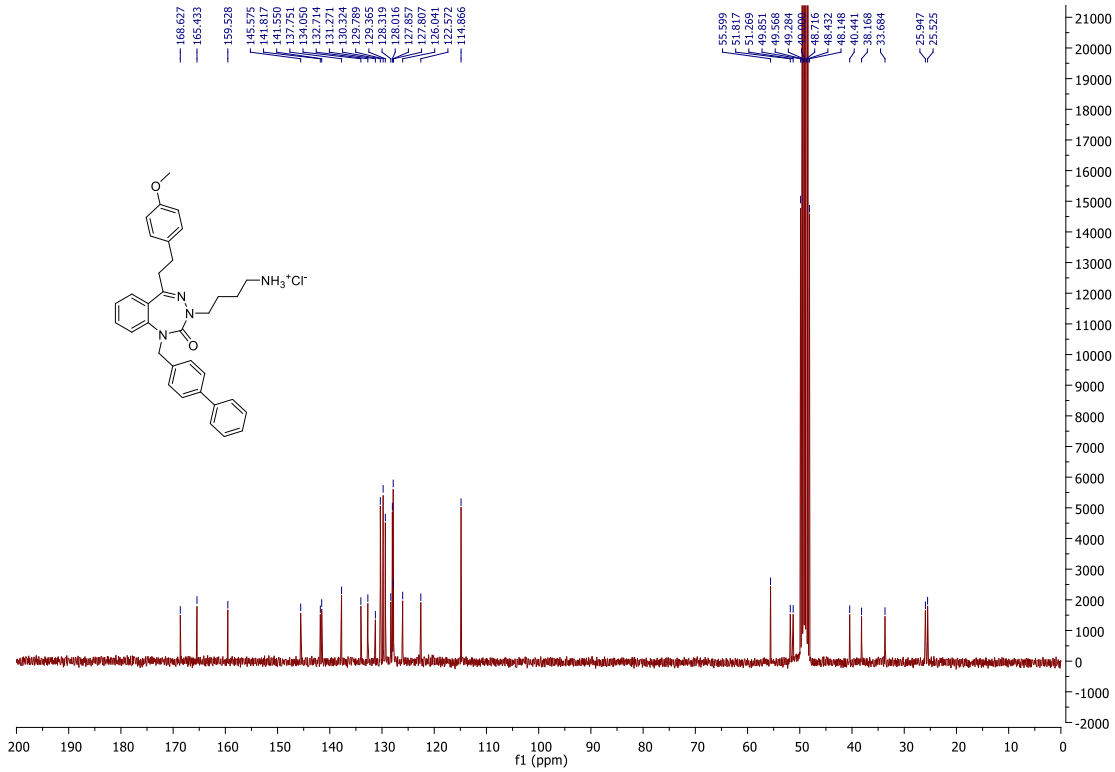
3.22f, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



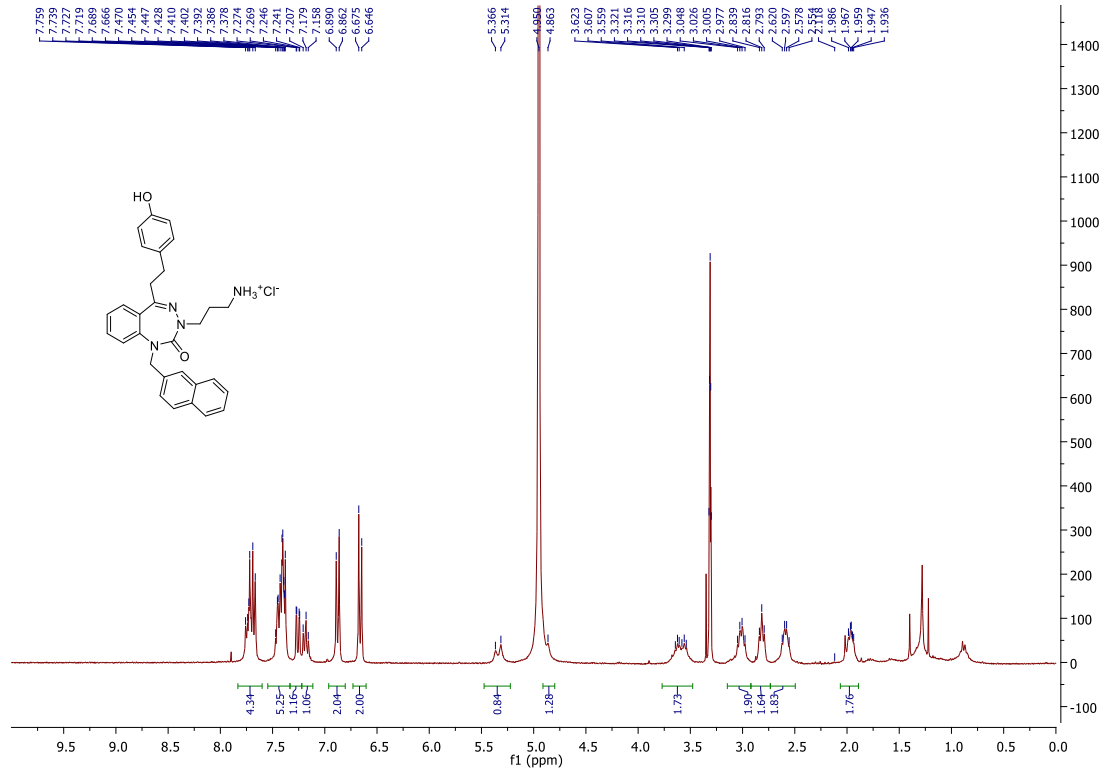


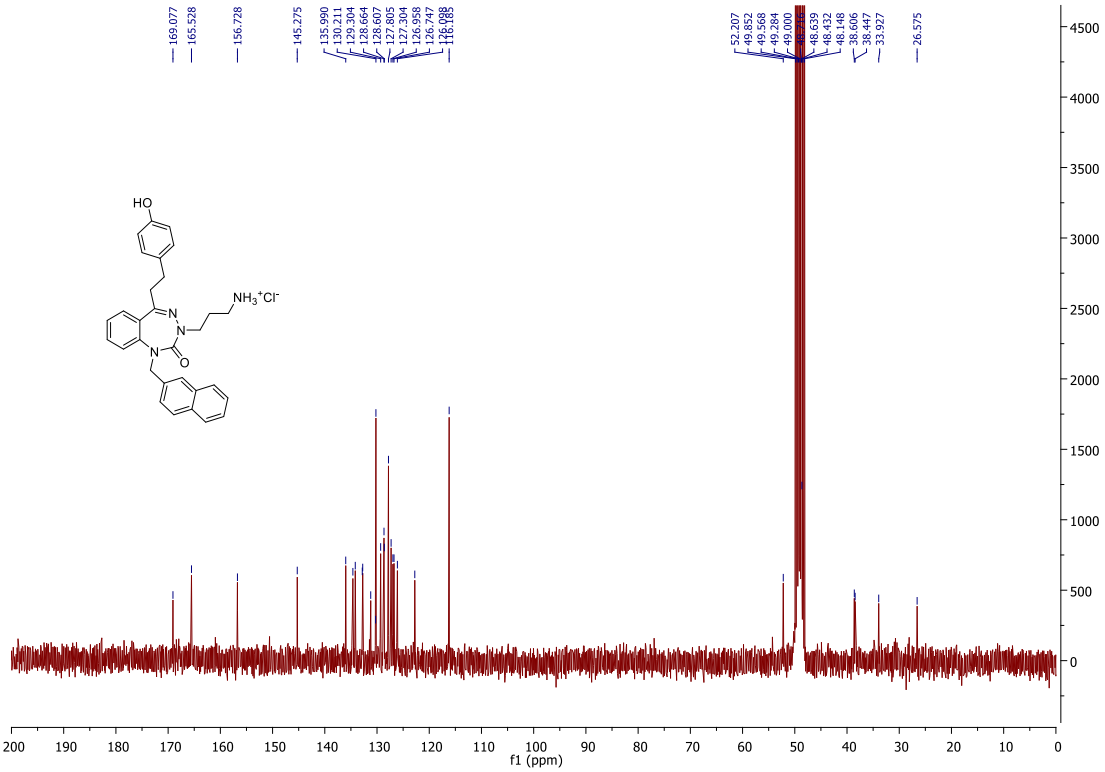
3.22g, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



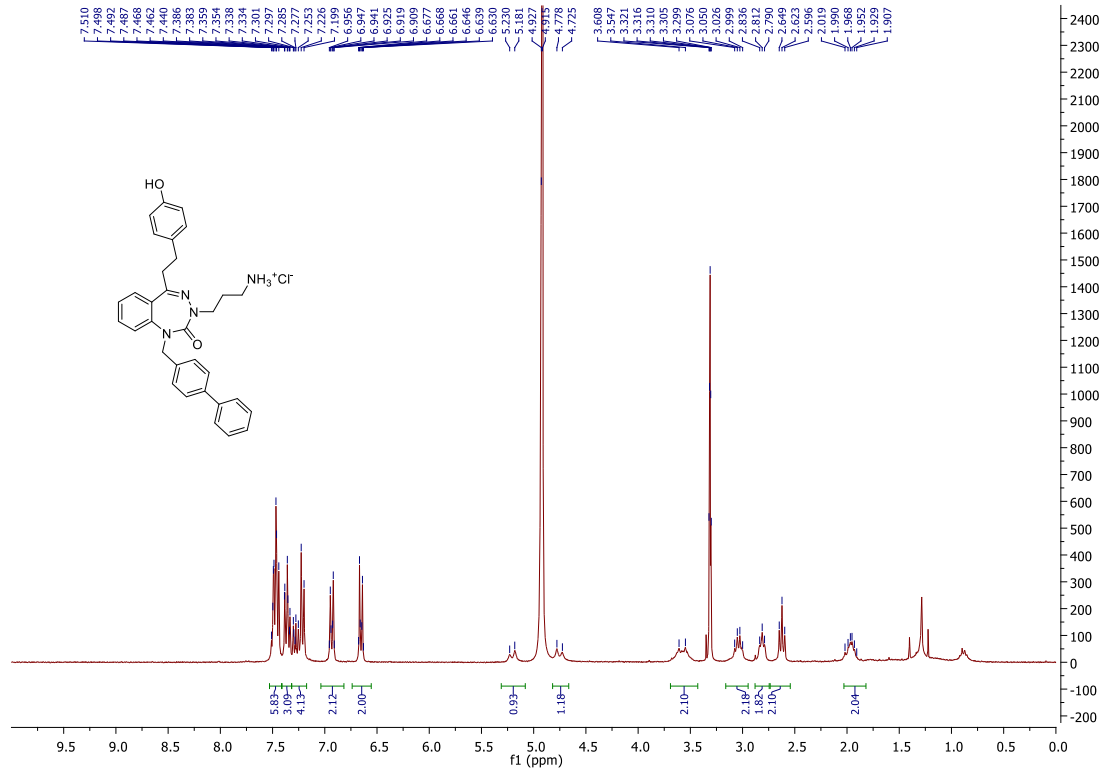


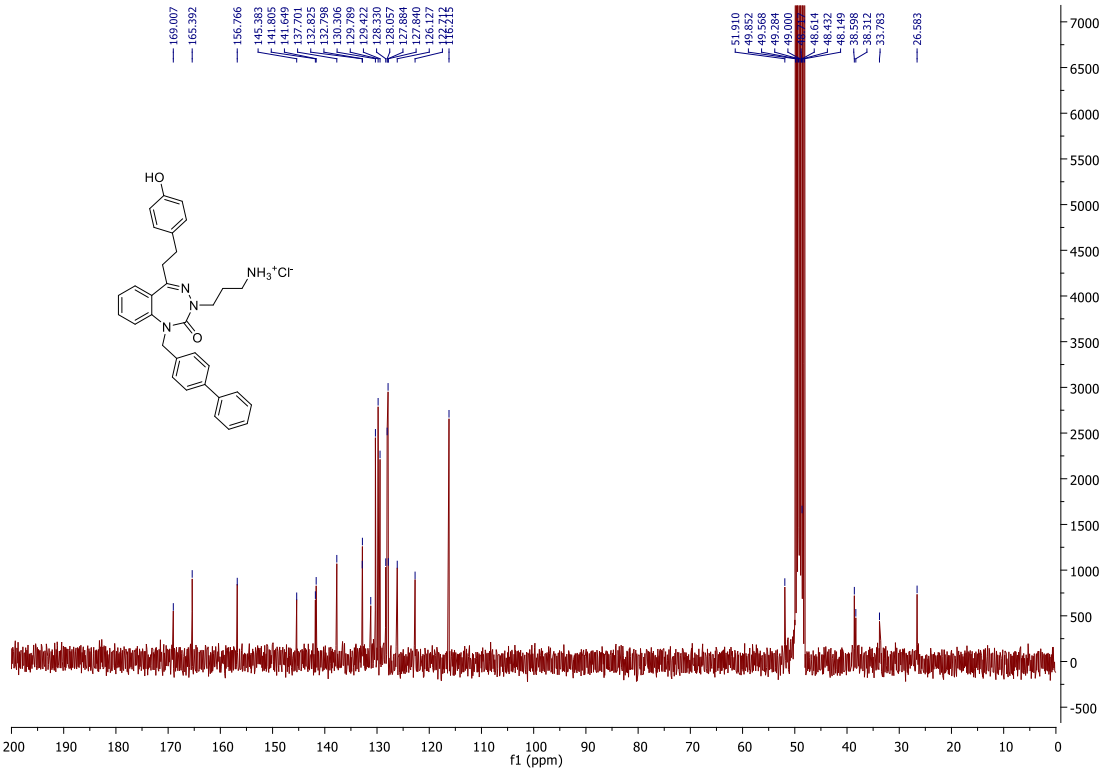
3.23d, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



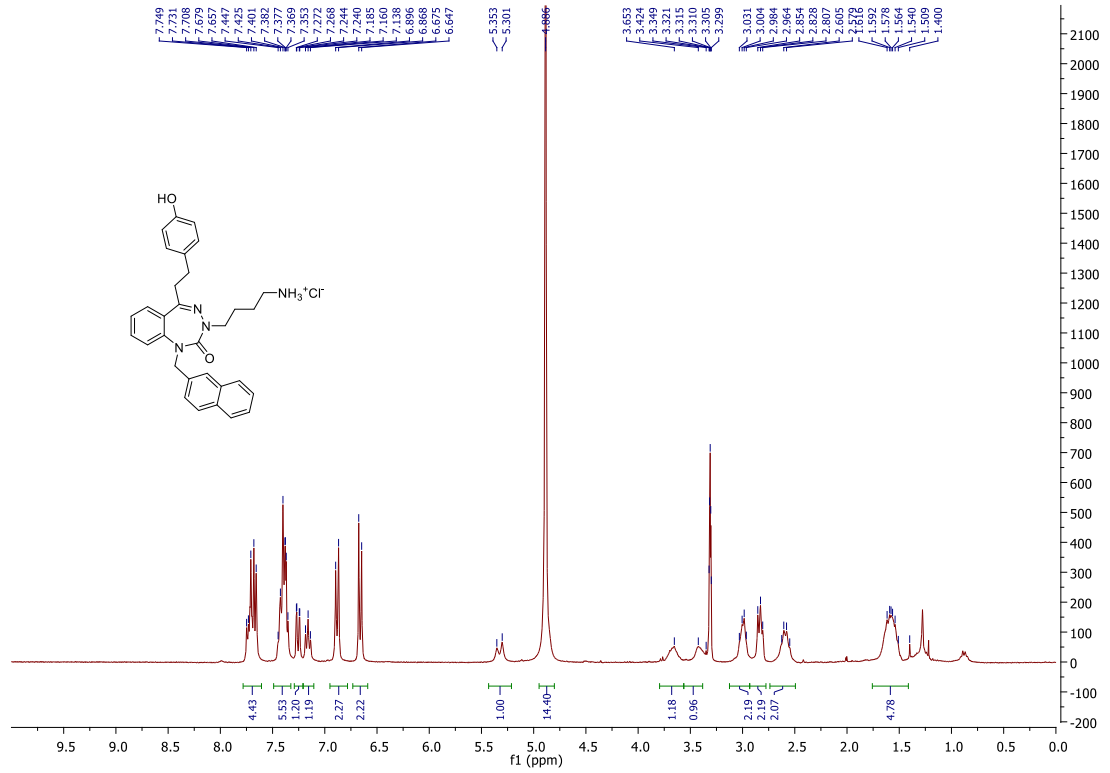


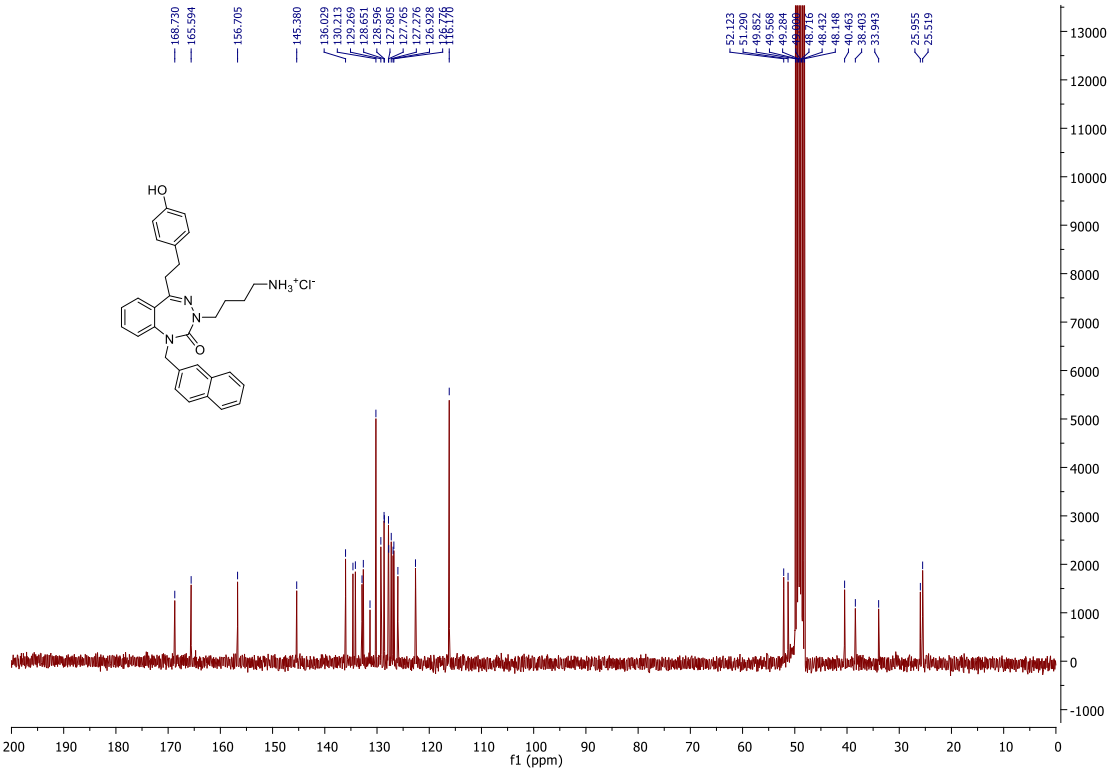
3.23e, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



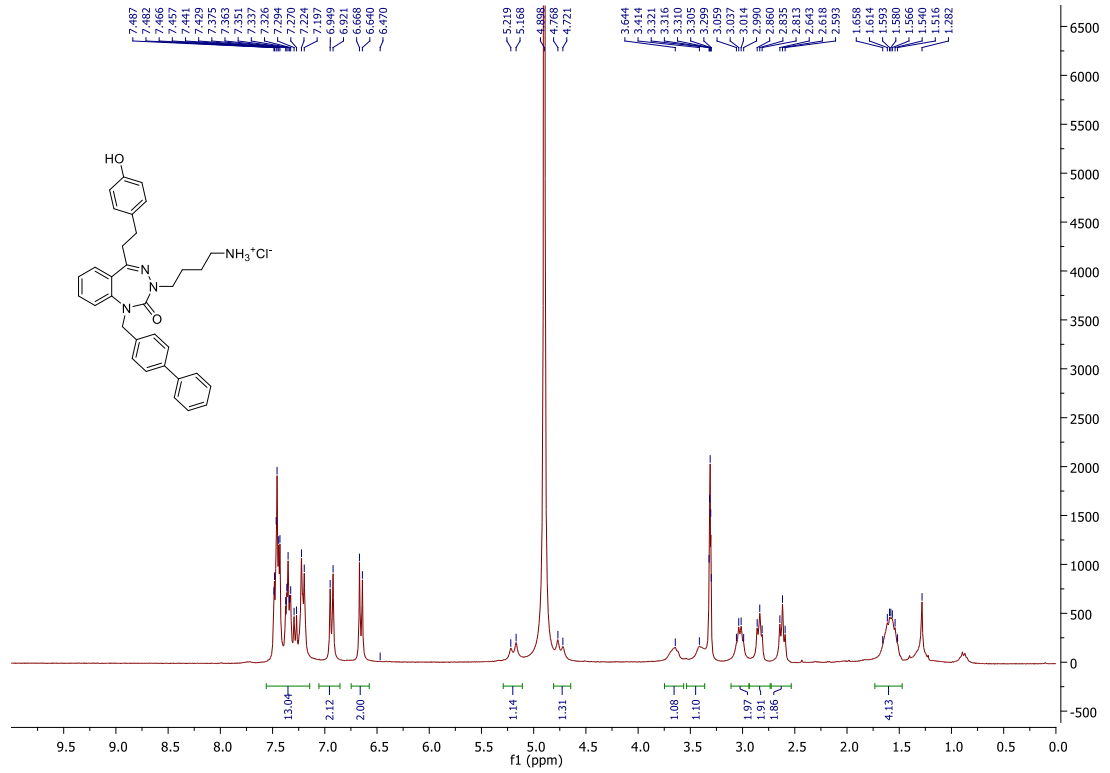


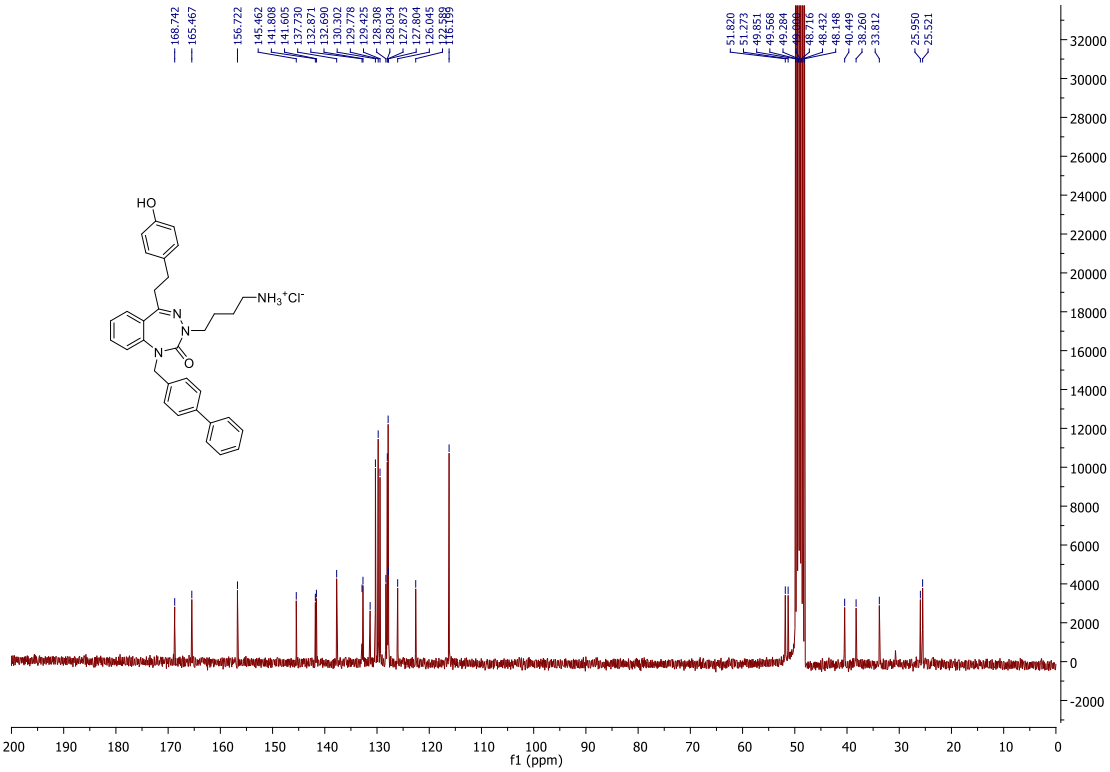
3.23f, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



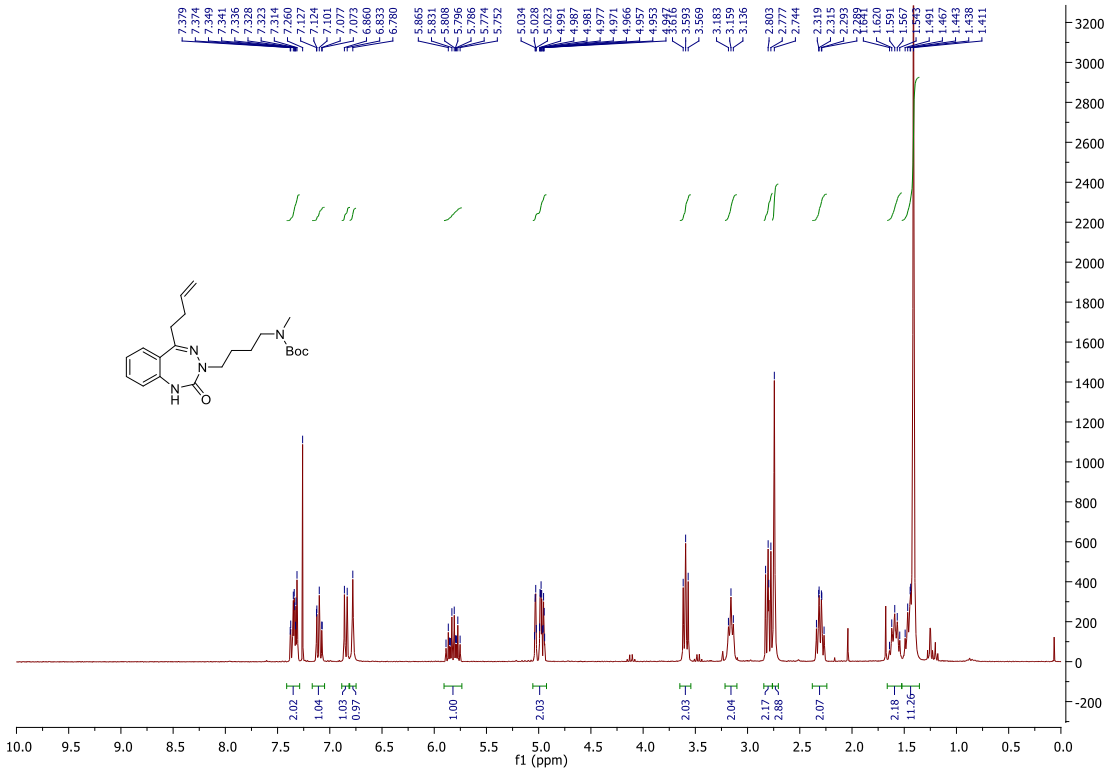


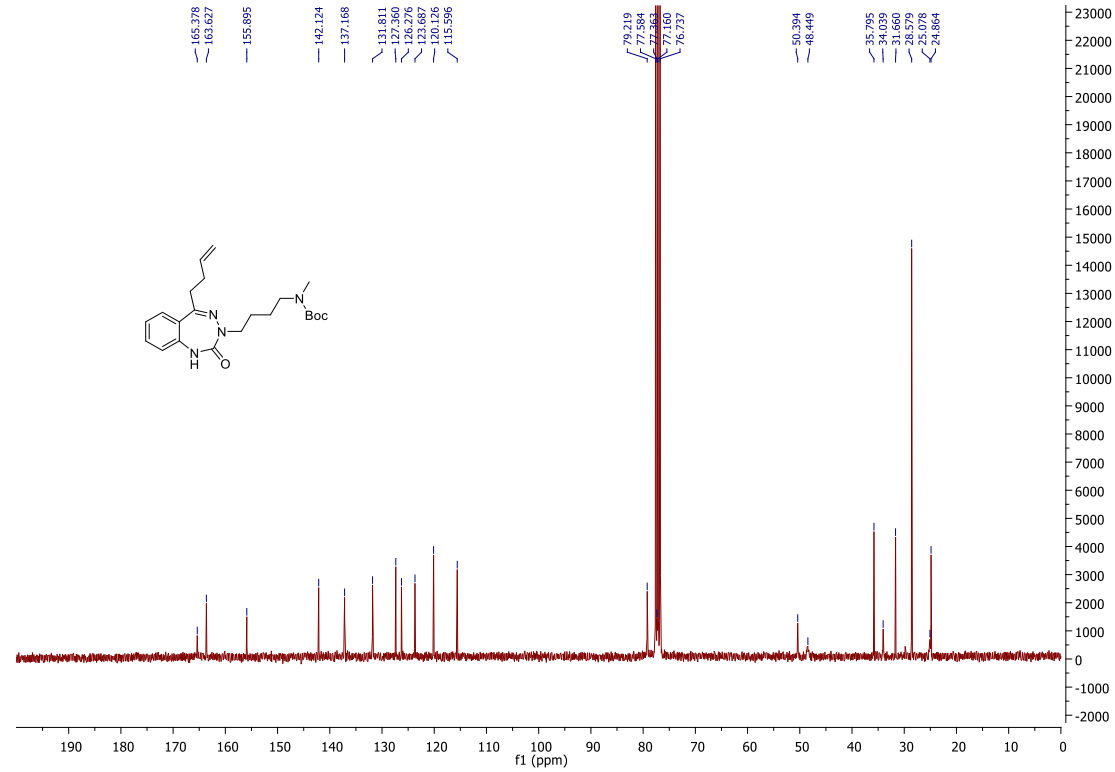
3.23g, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



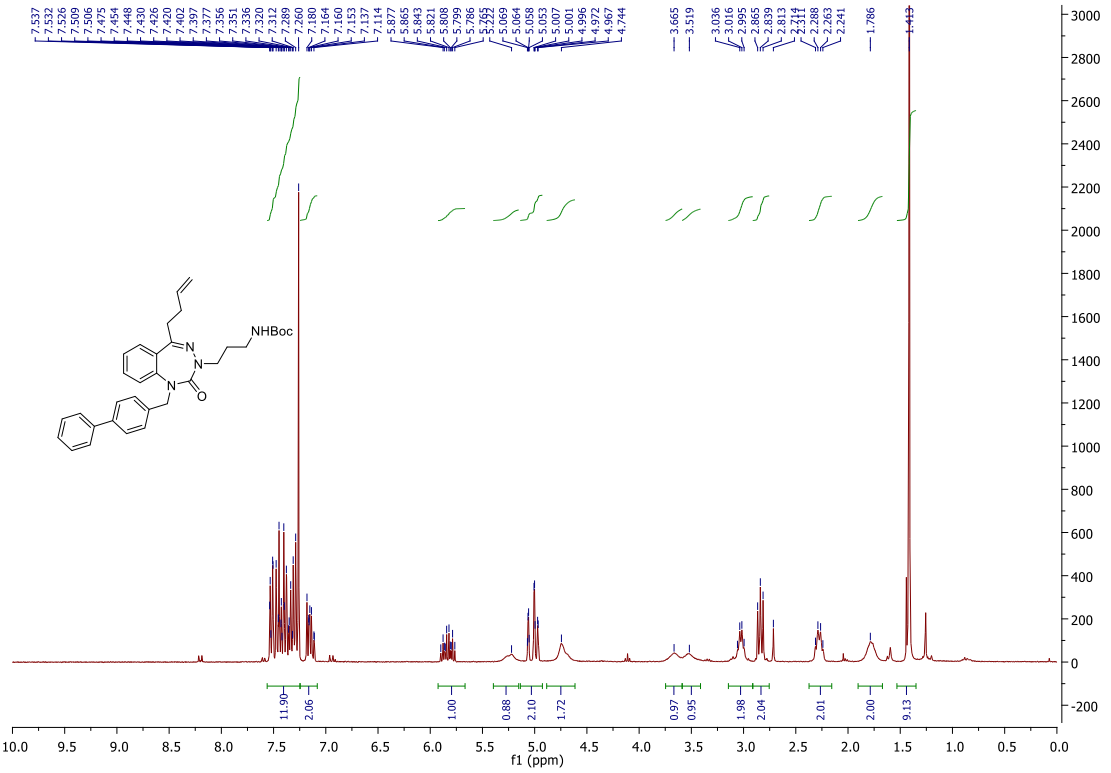


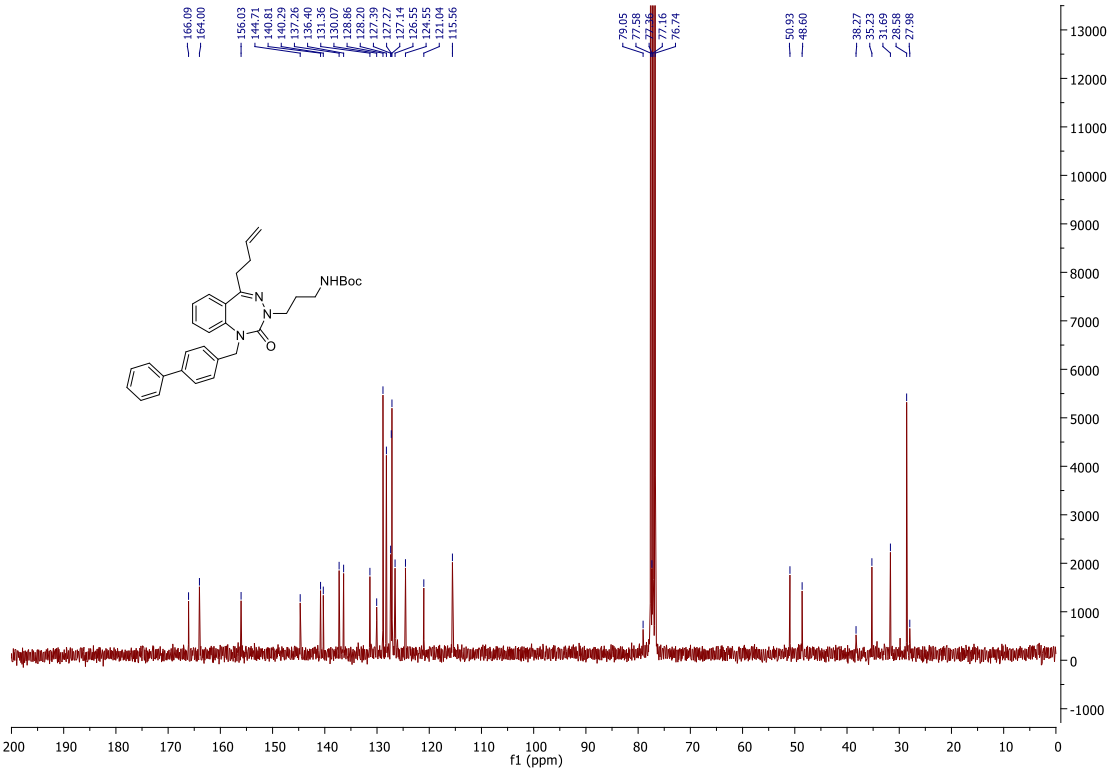
3.24, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



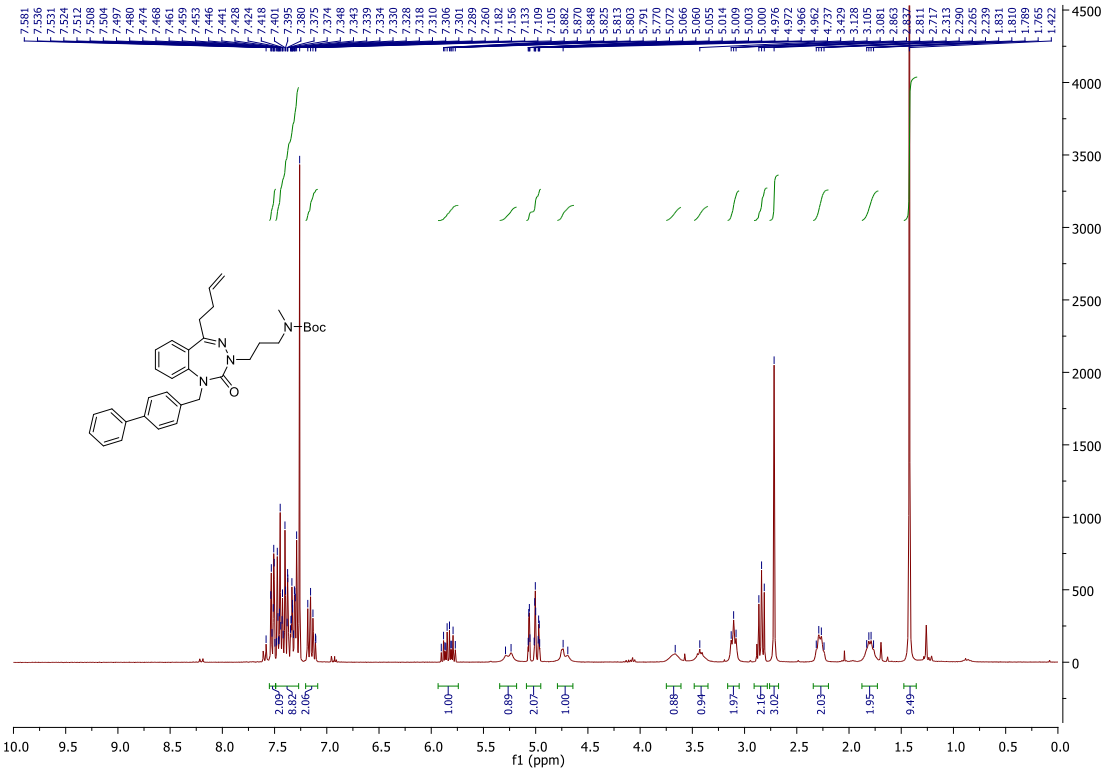


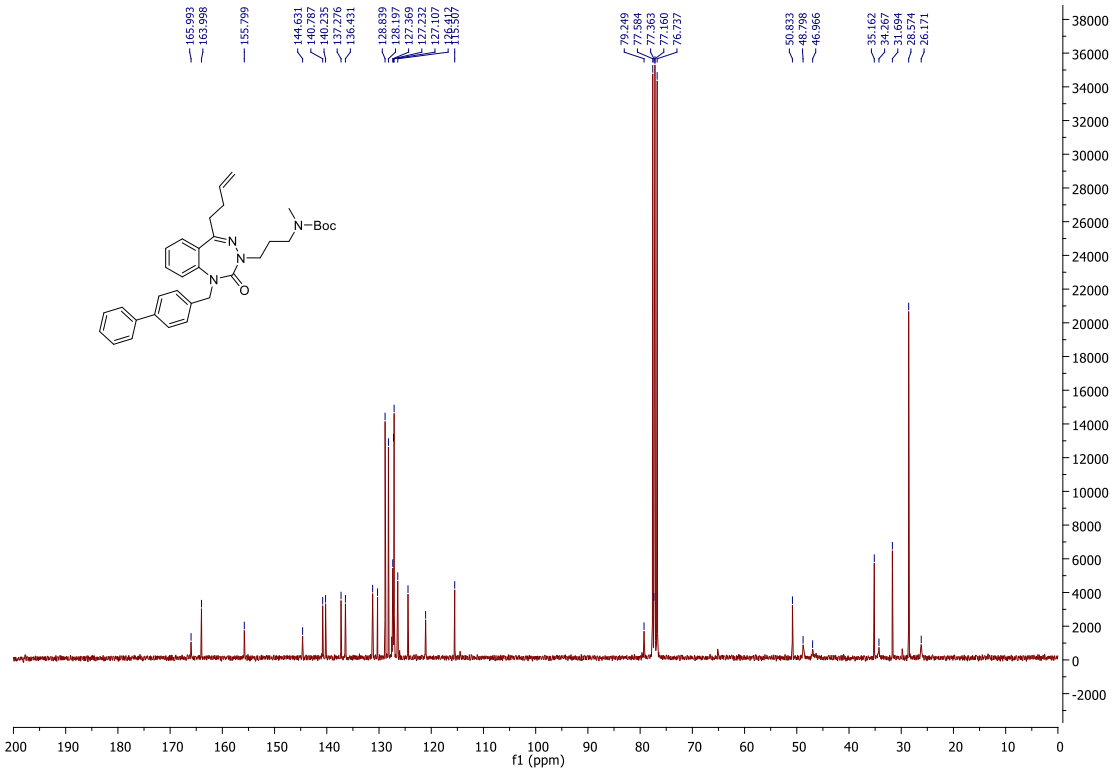
3.25, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



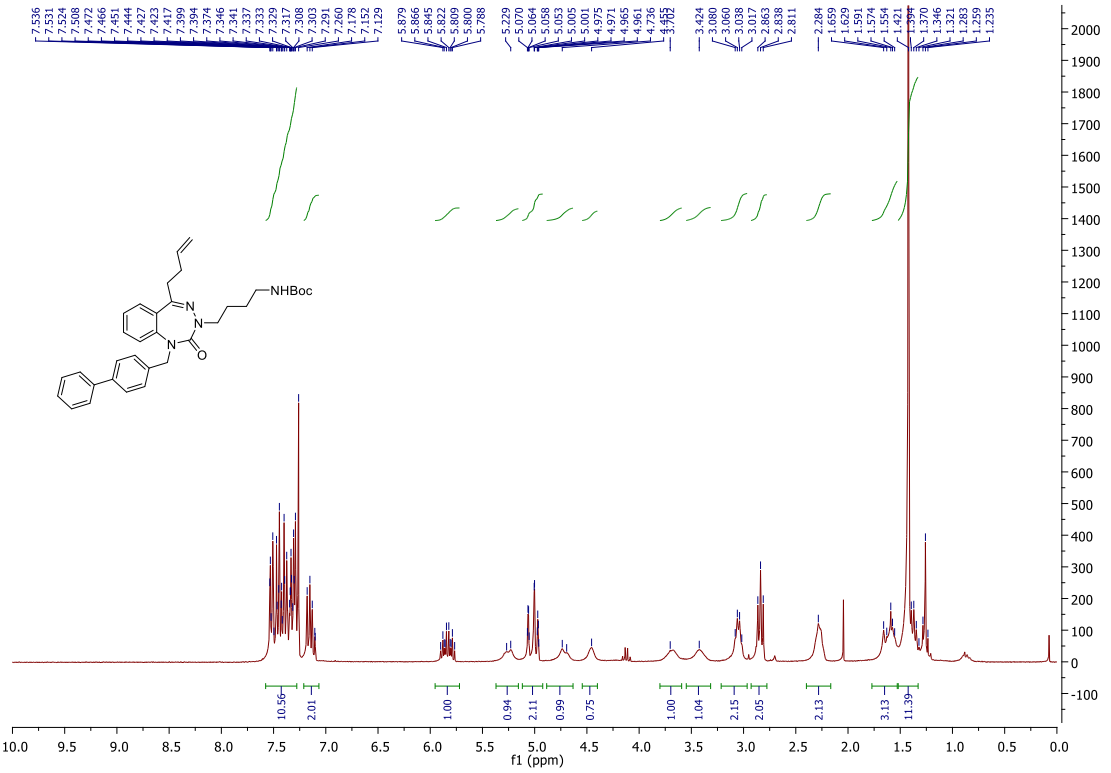


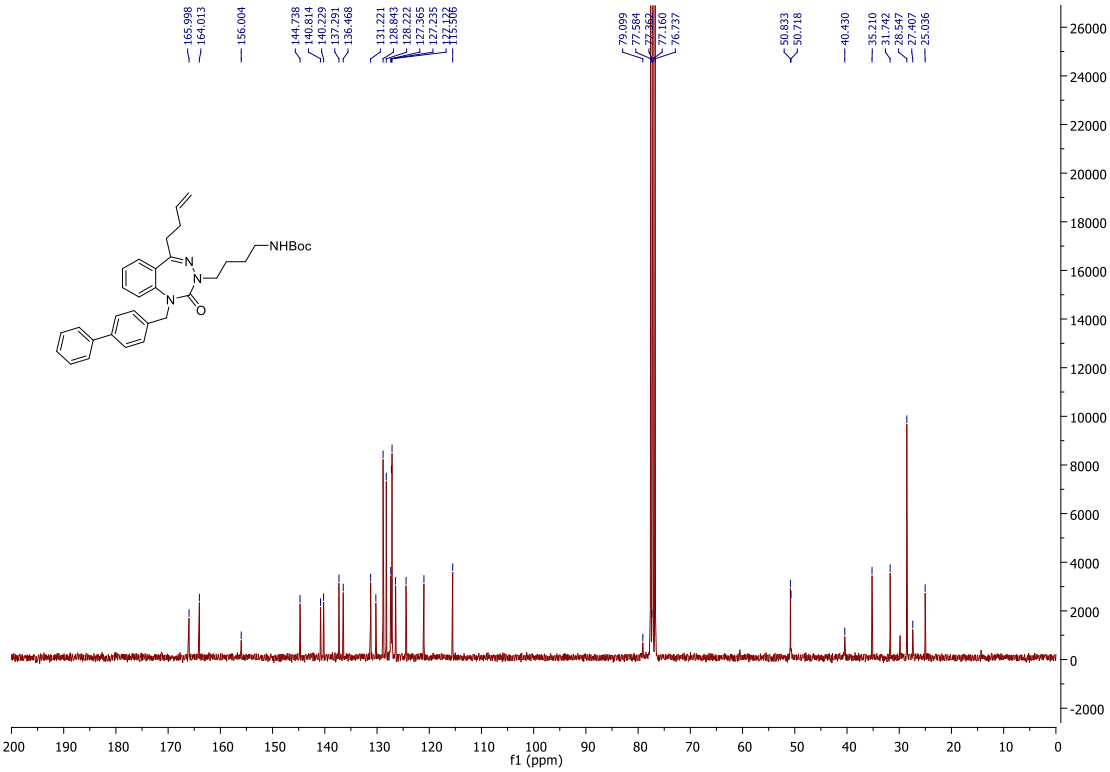
3.26, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



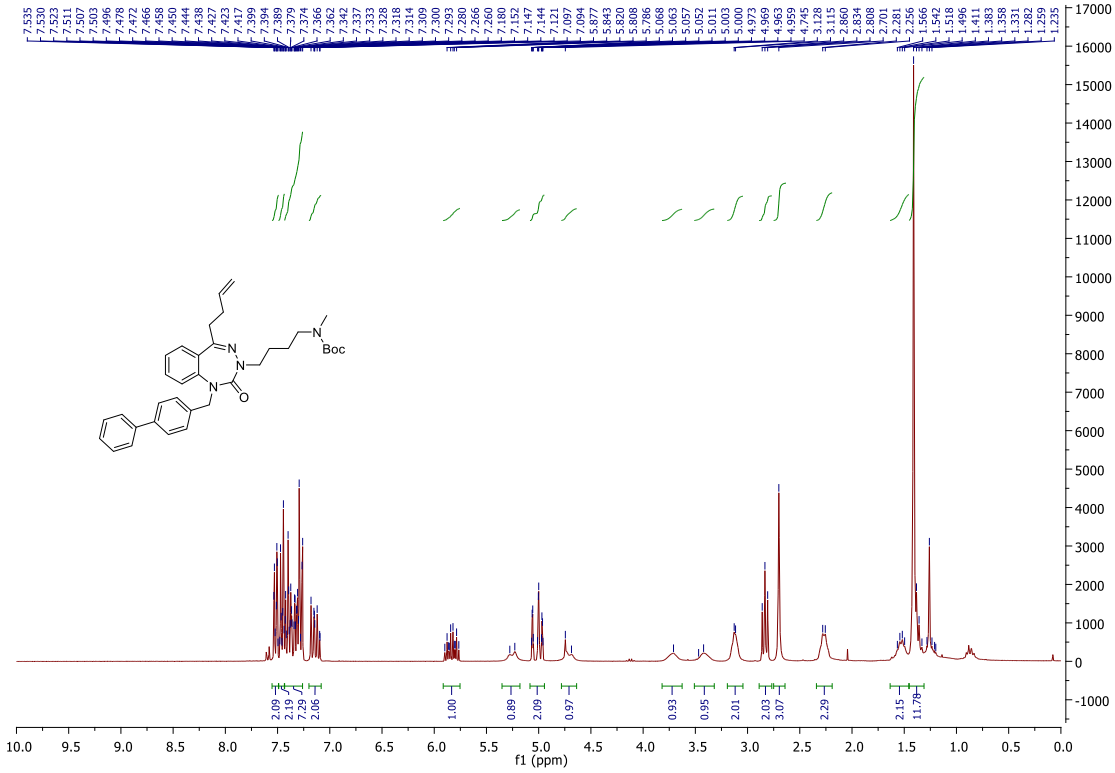


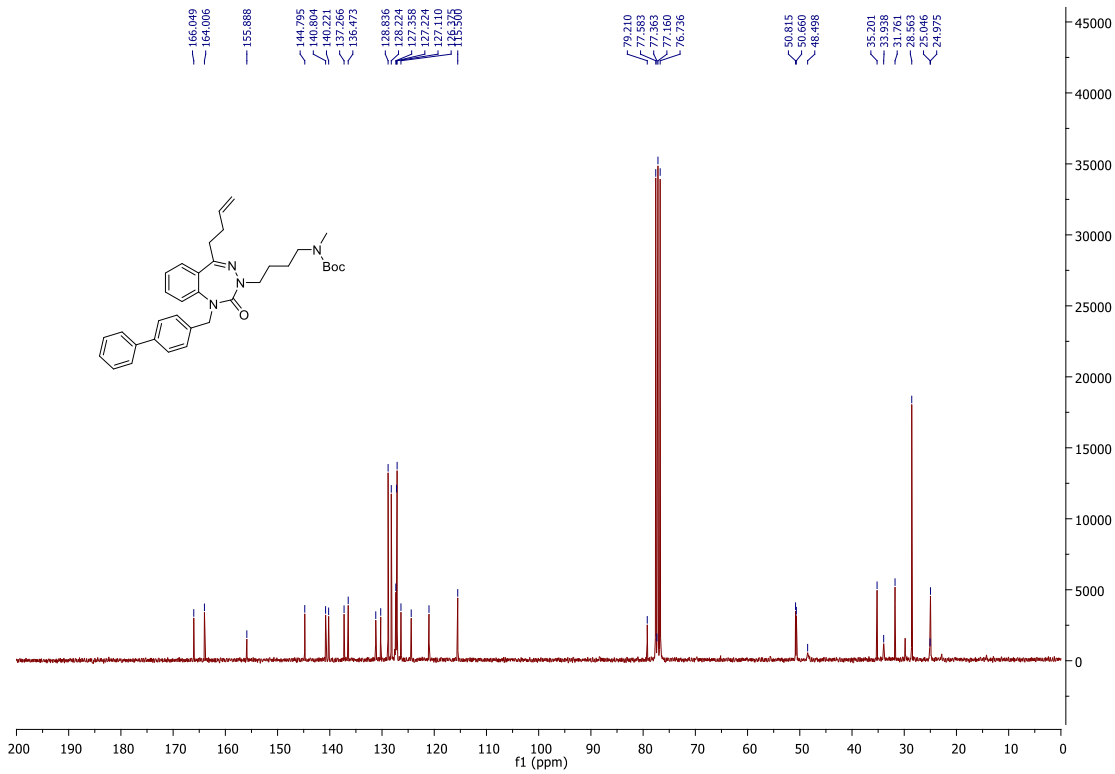
3.27, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



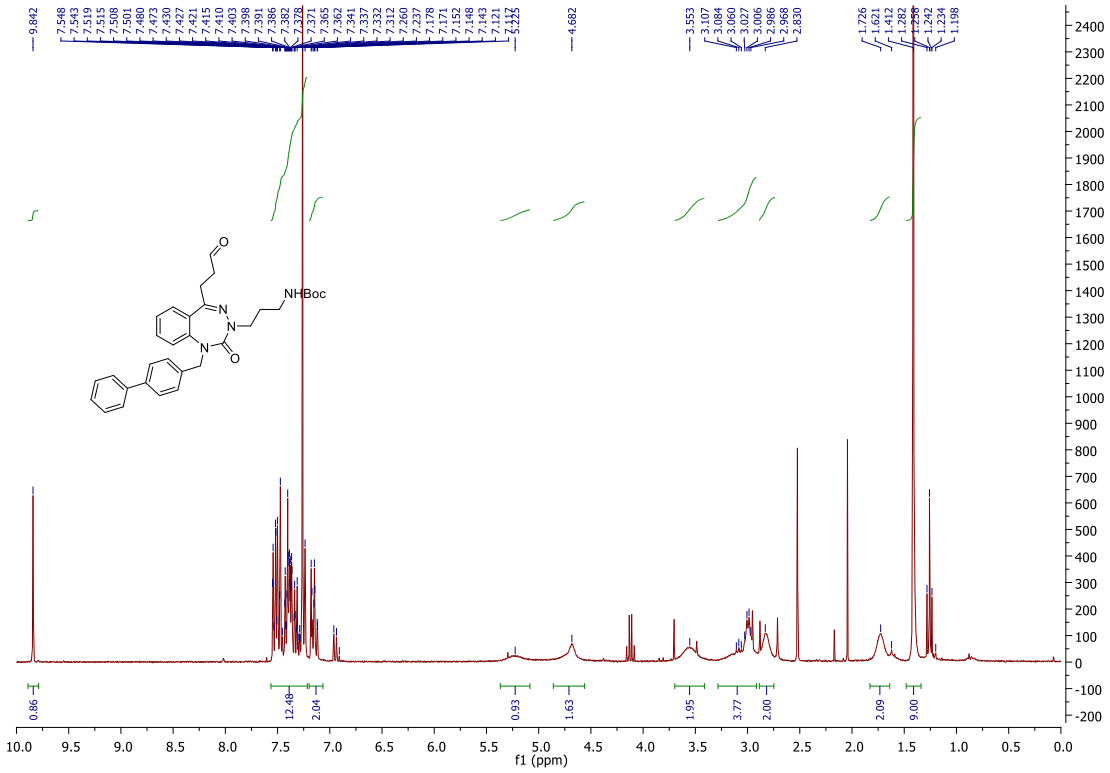


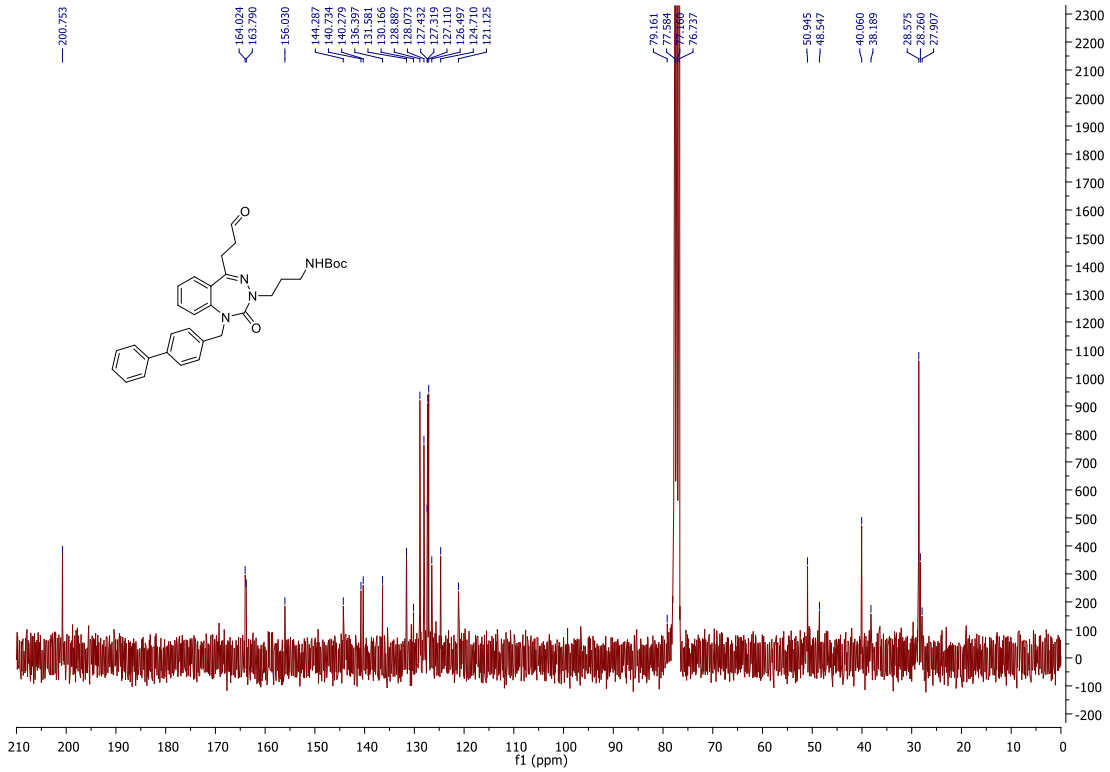
3.28, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



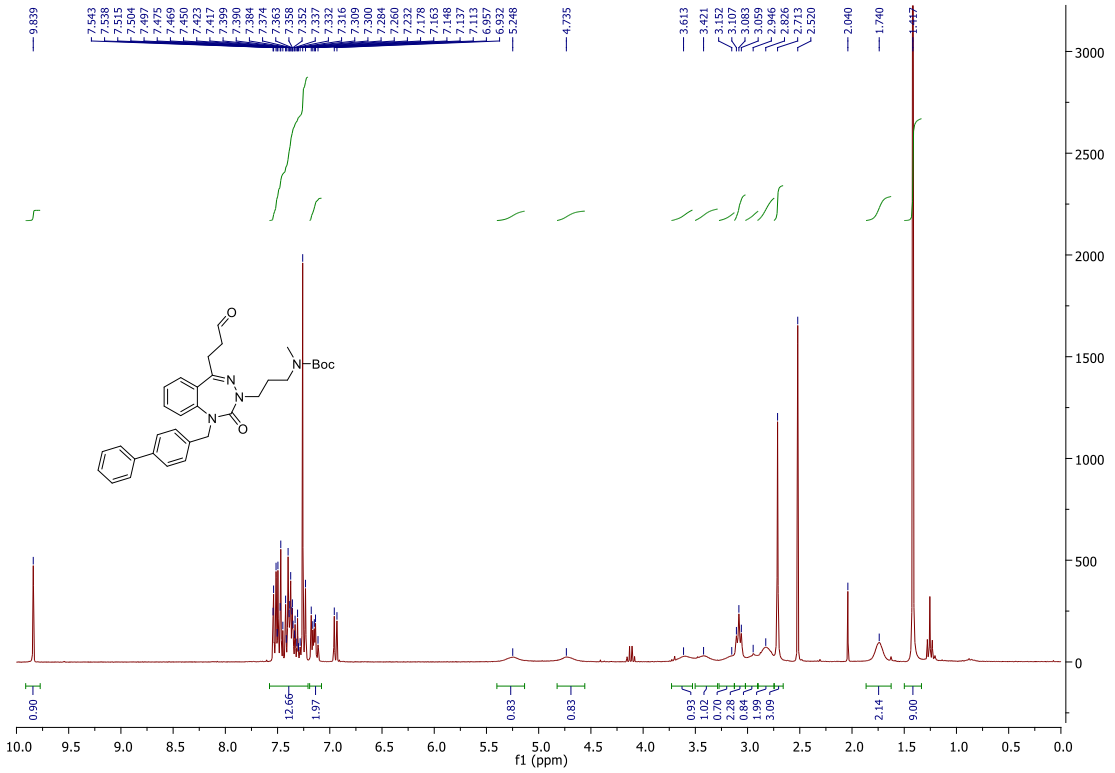


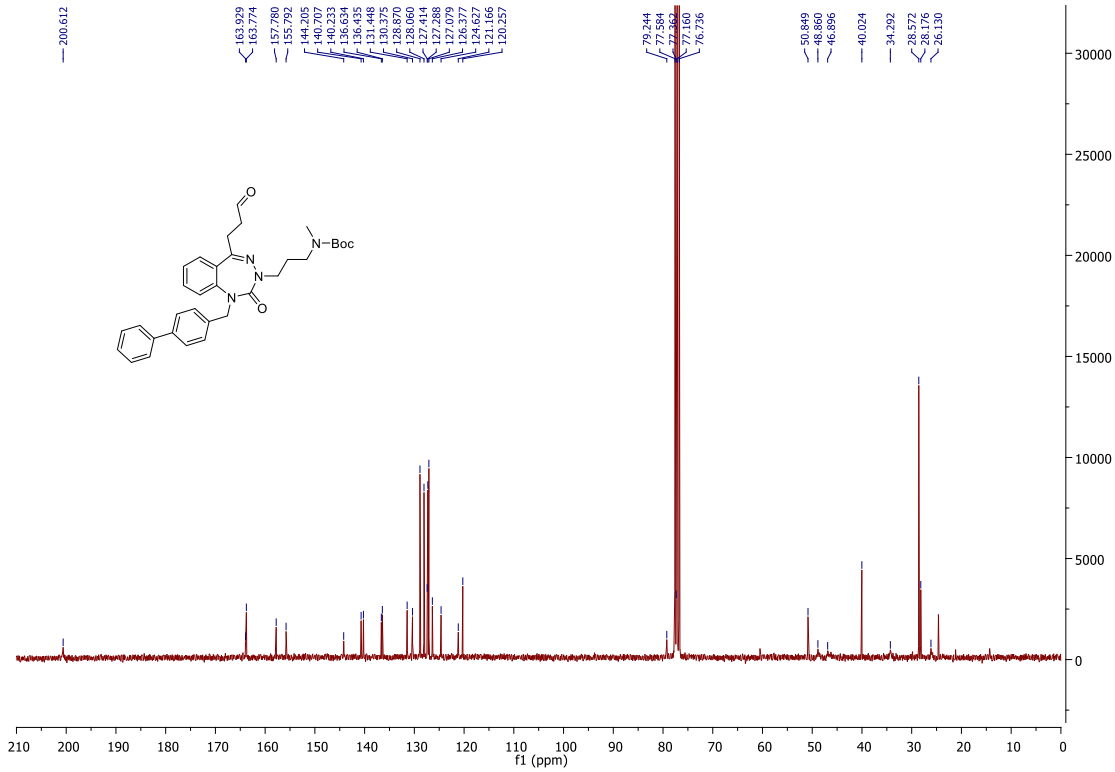
3.29, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



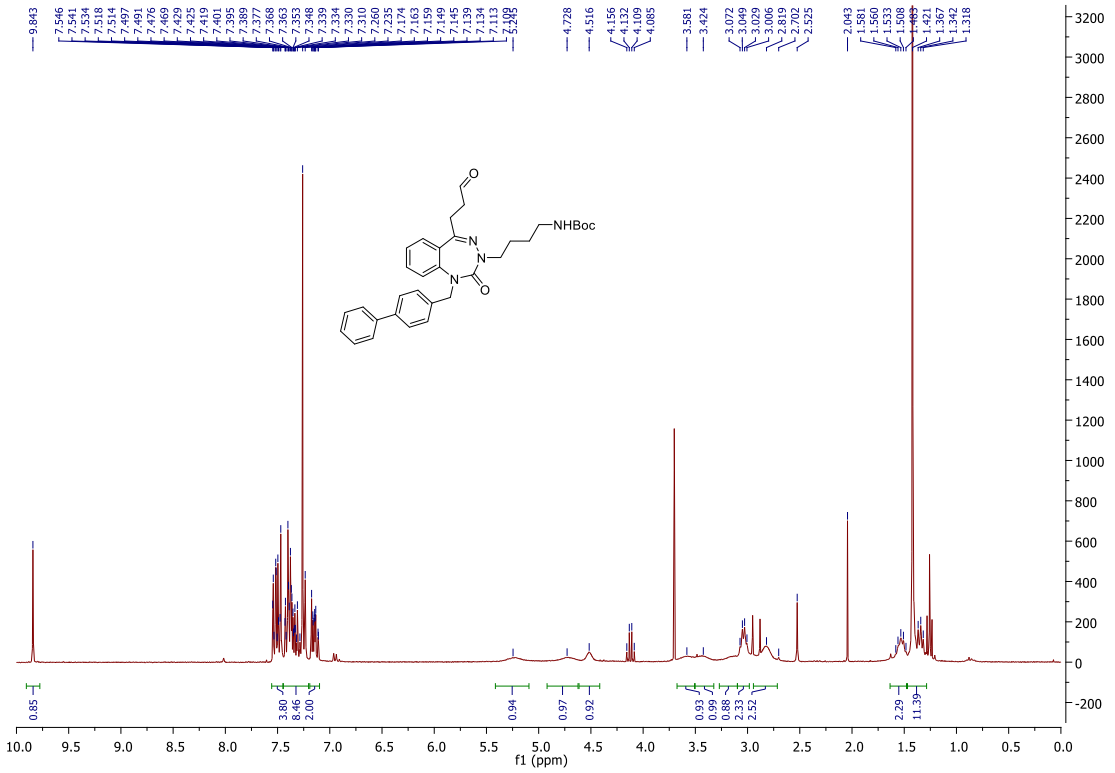


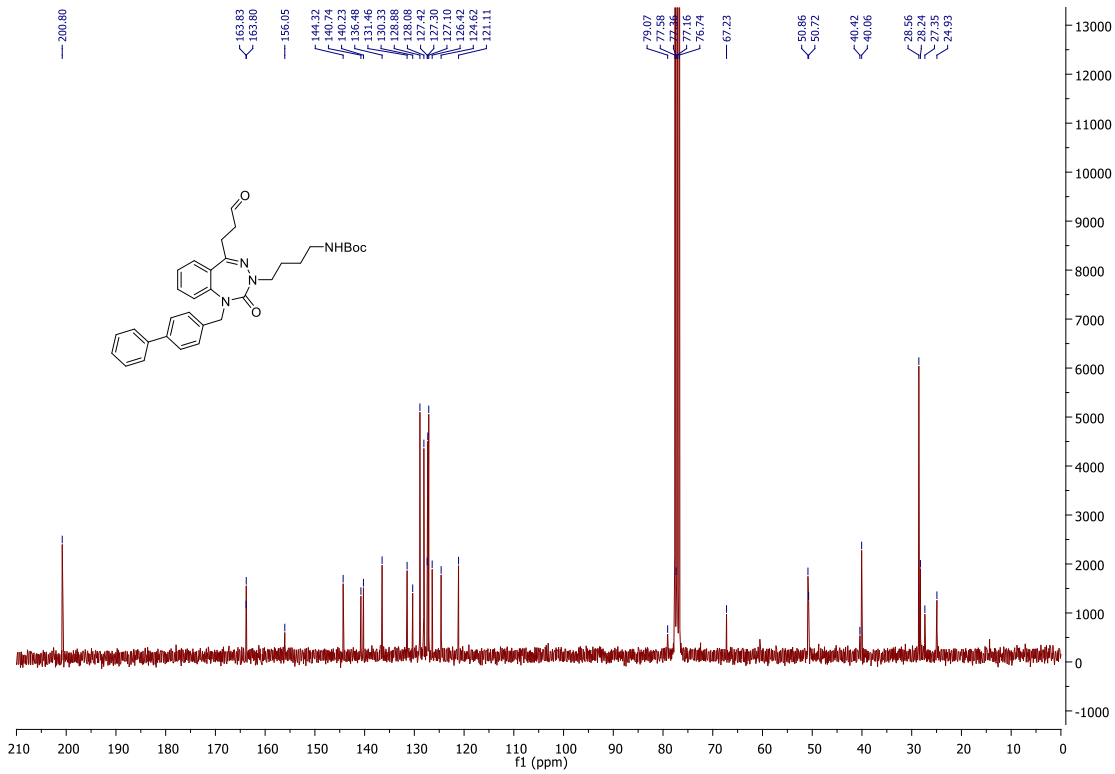
3.30, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



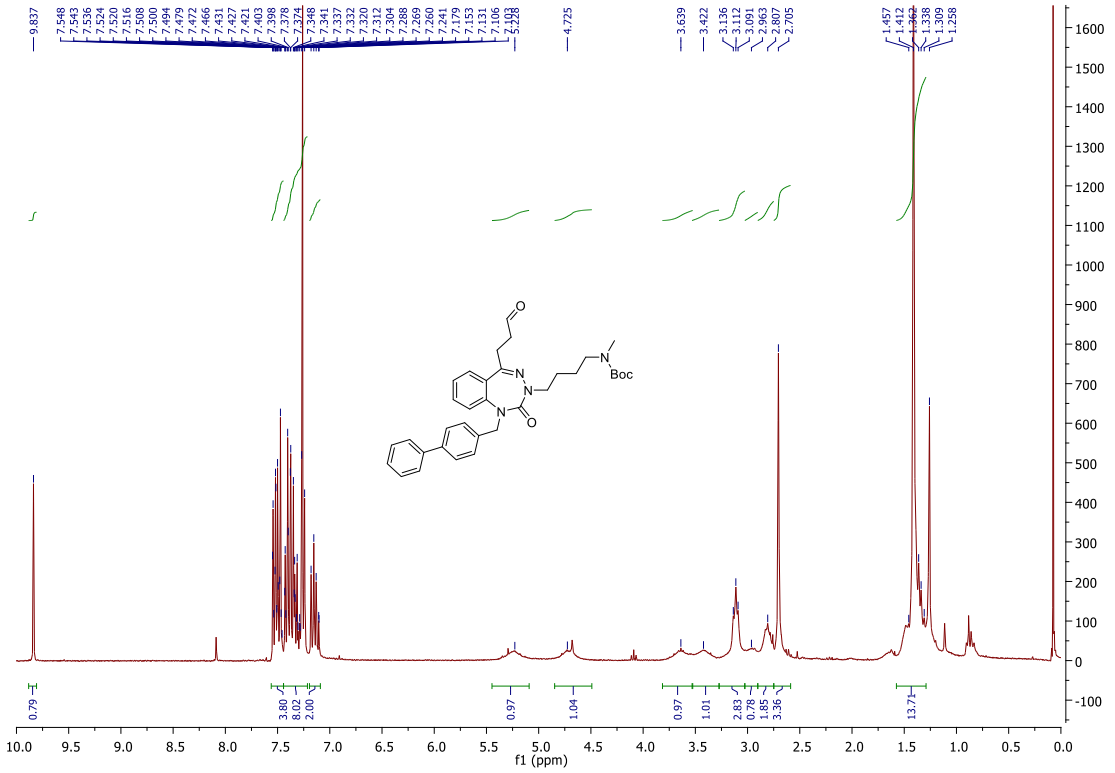


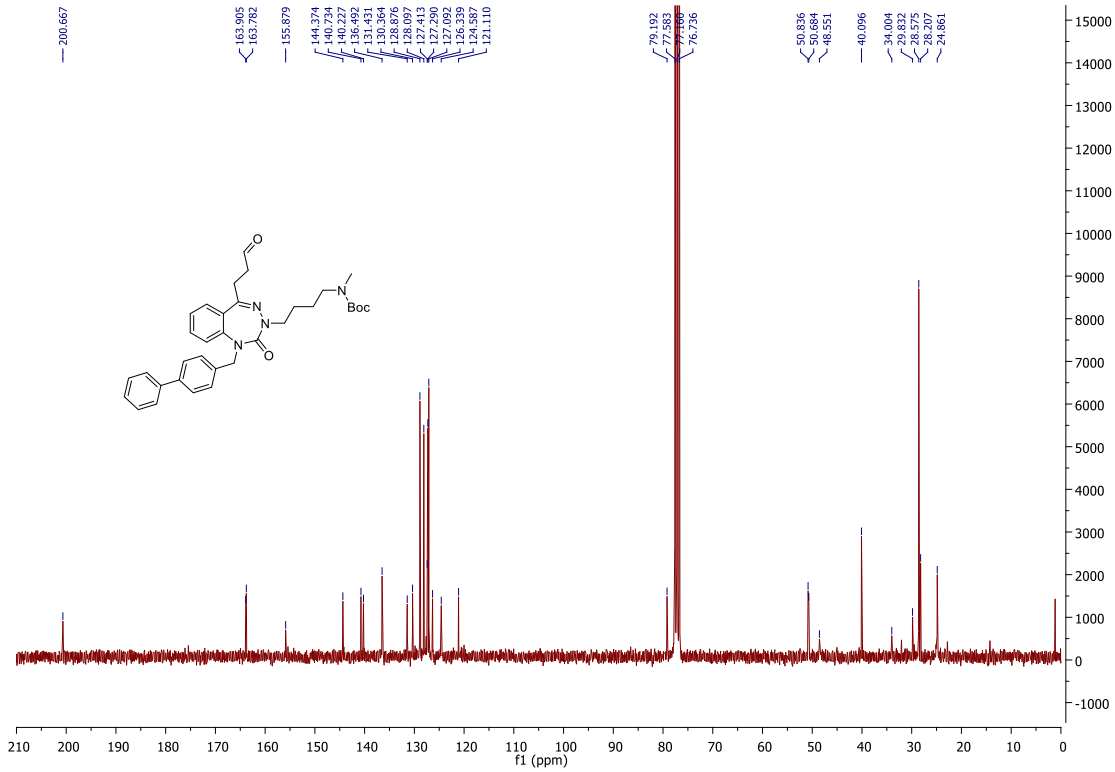
3.31, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



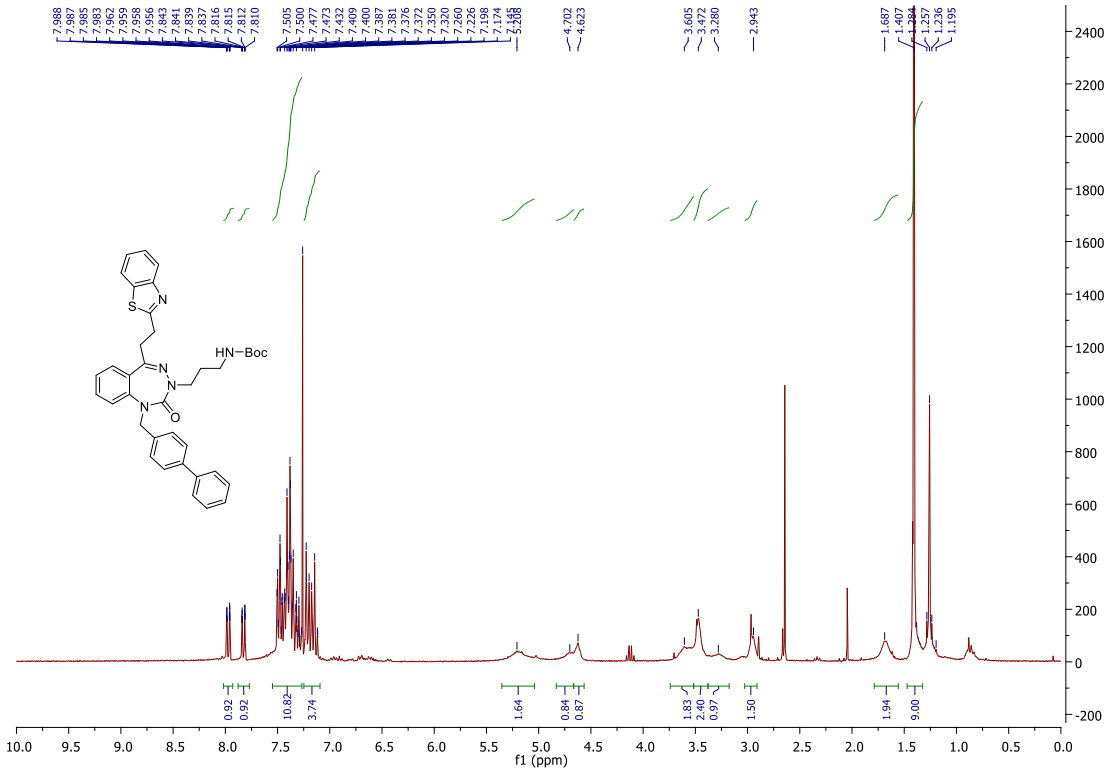


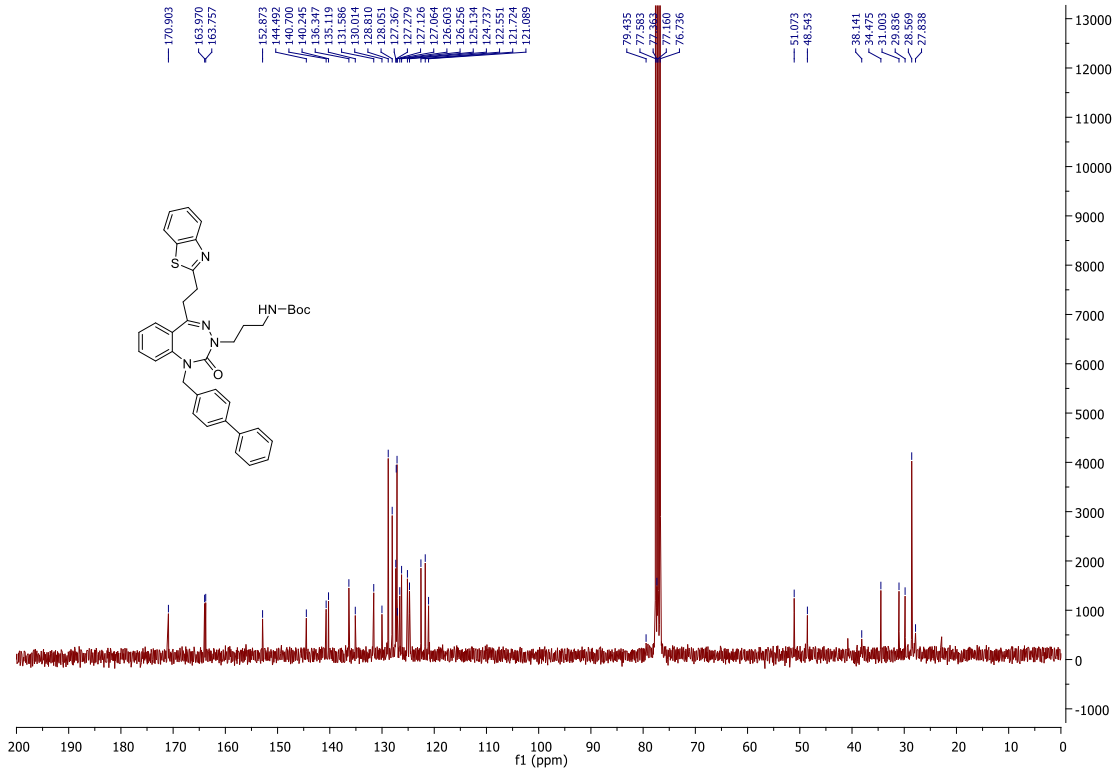
3.32, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



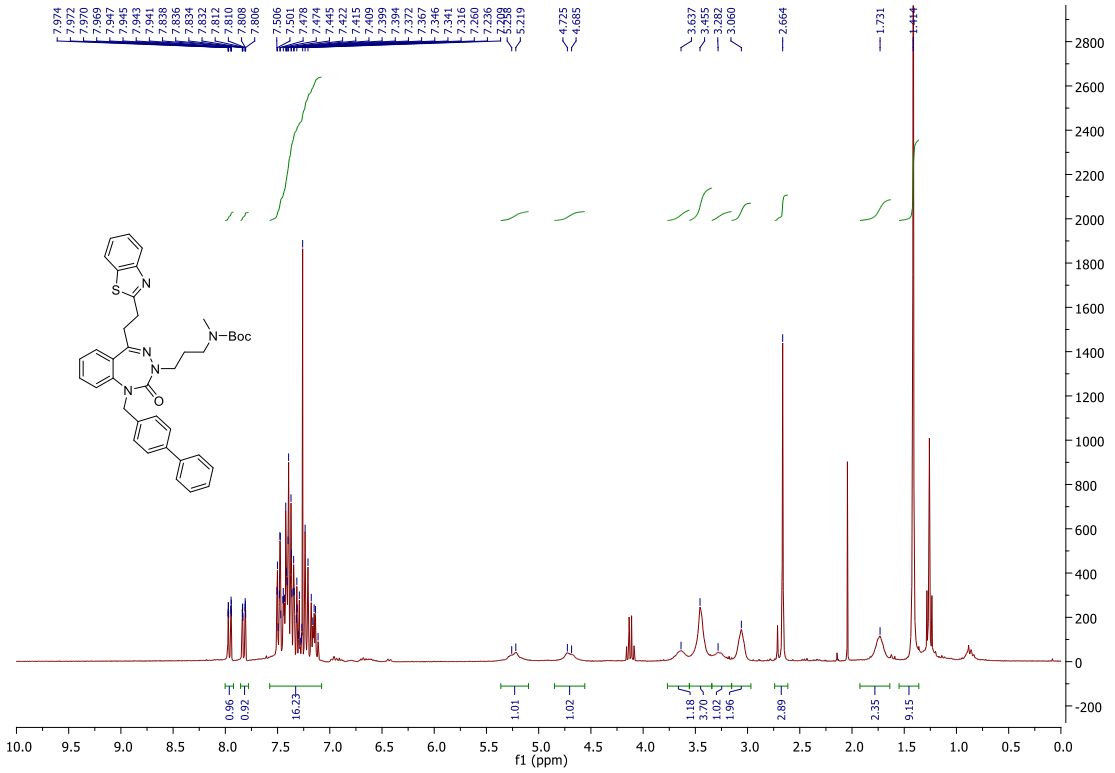


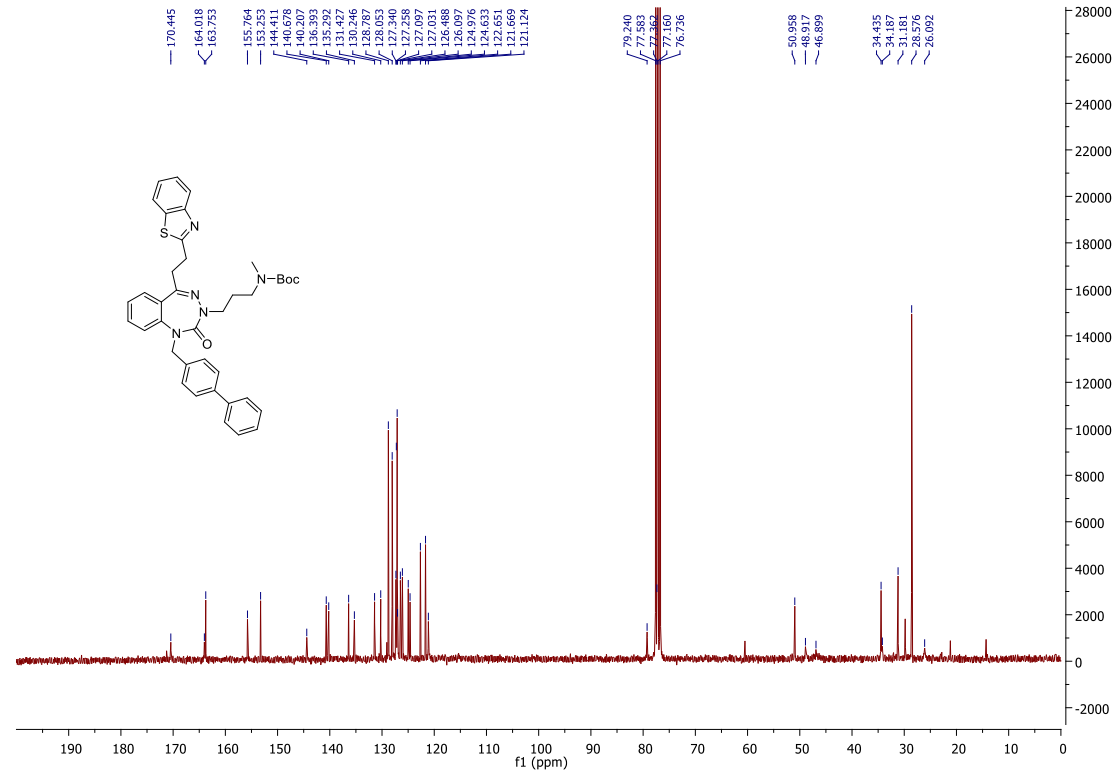
3.33, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



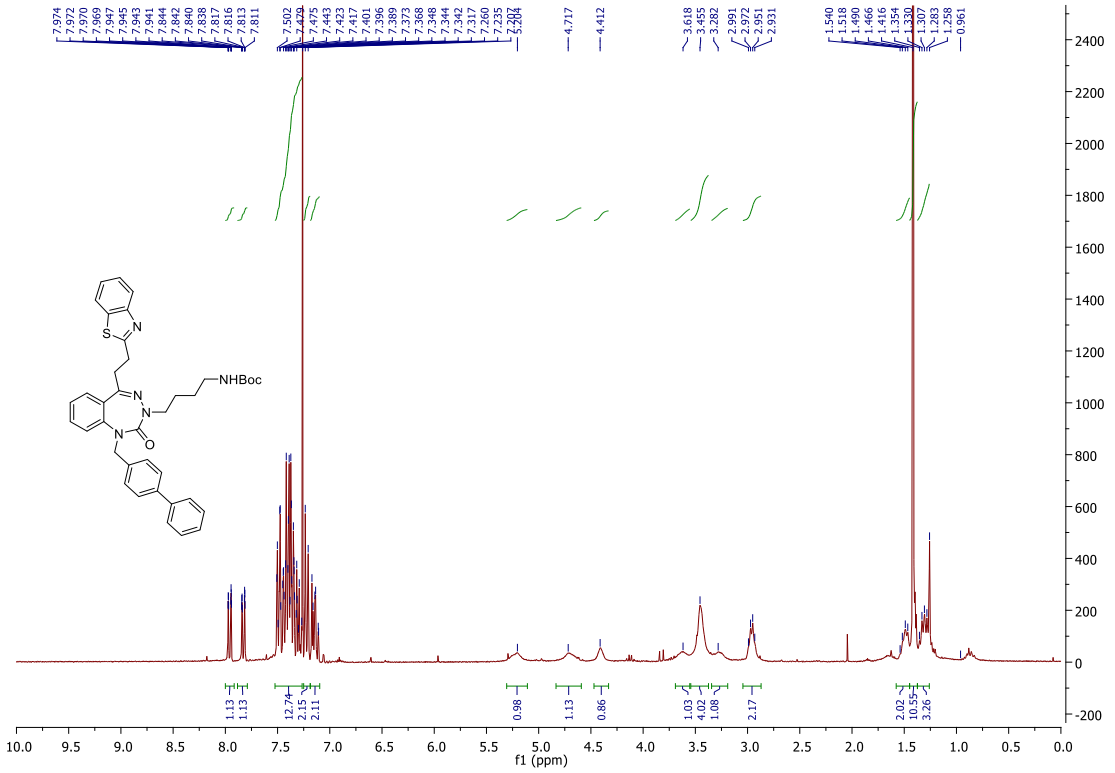


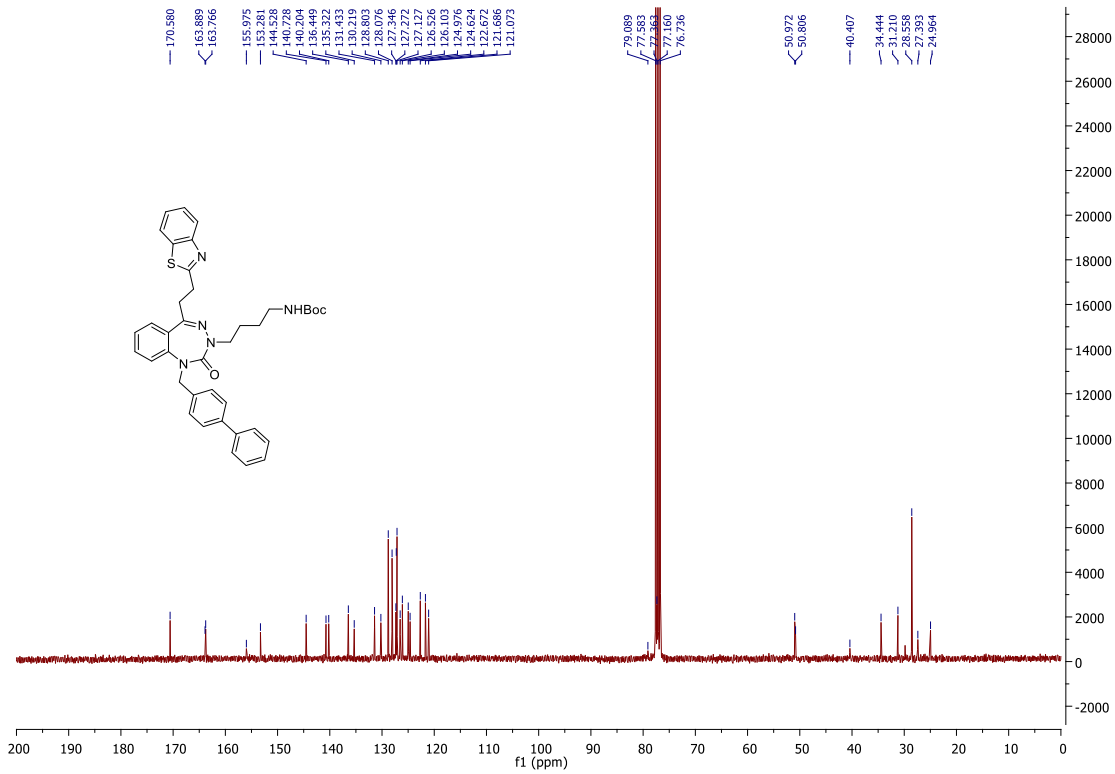
3.34, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



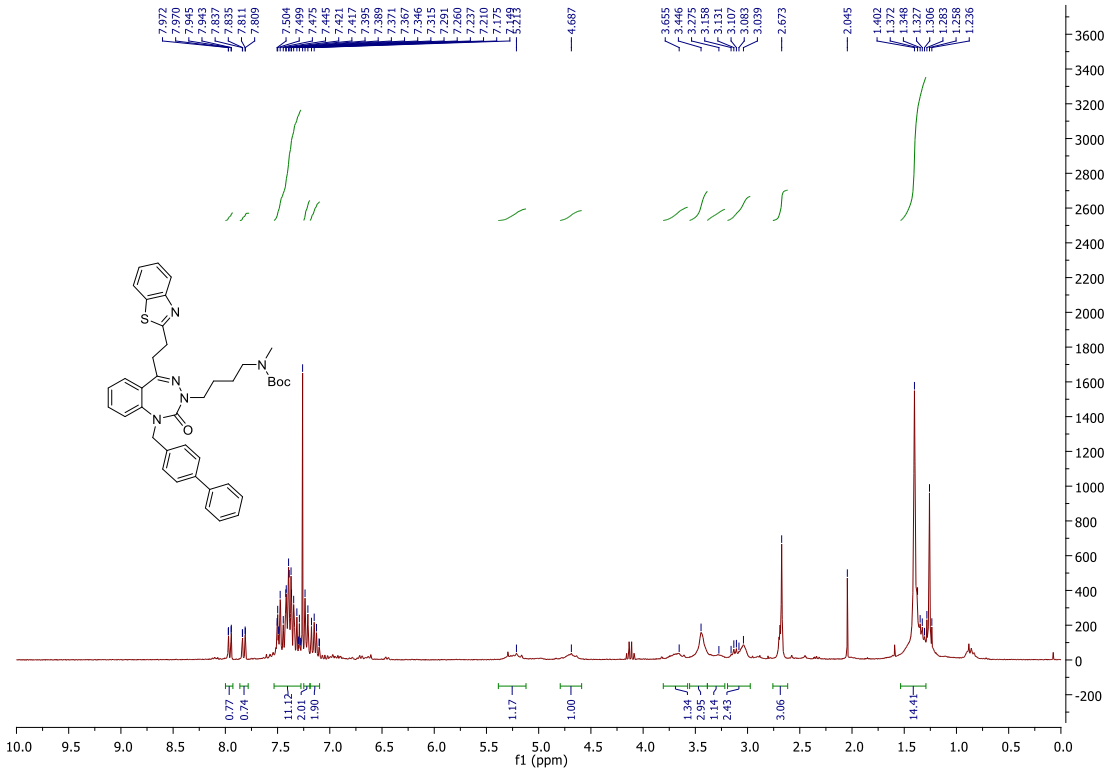


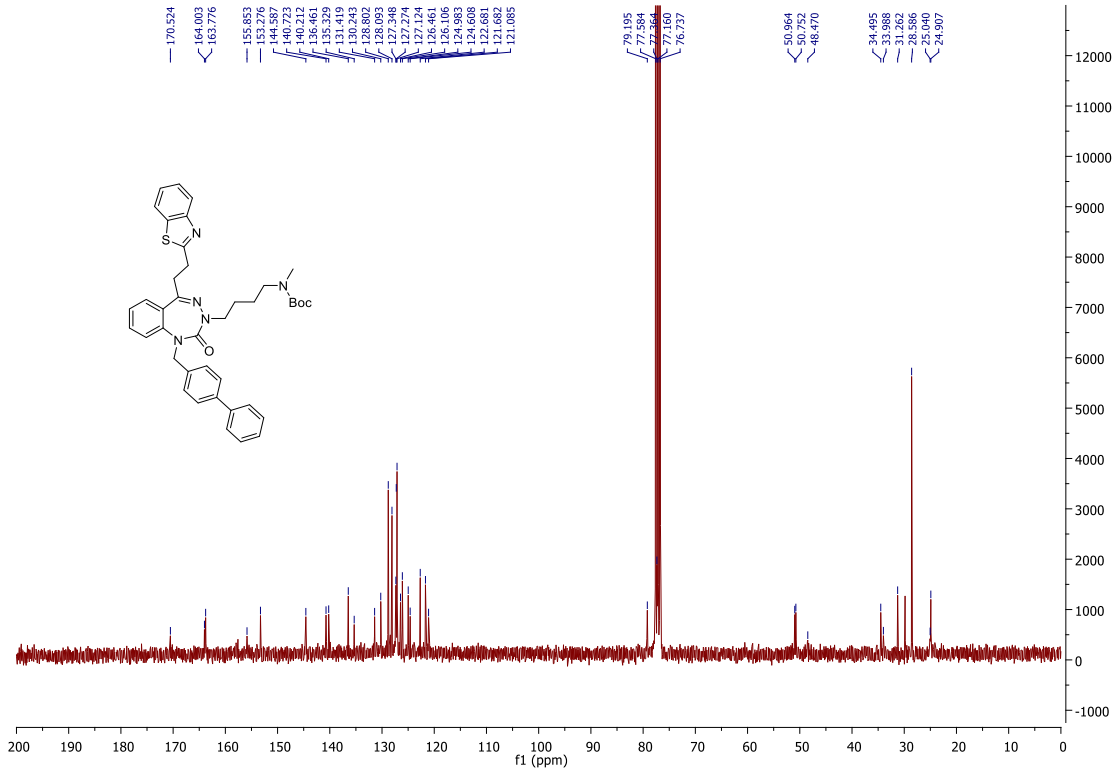
3.35, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



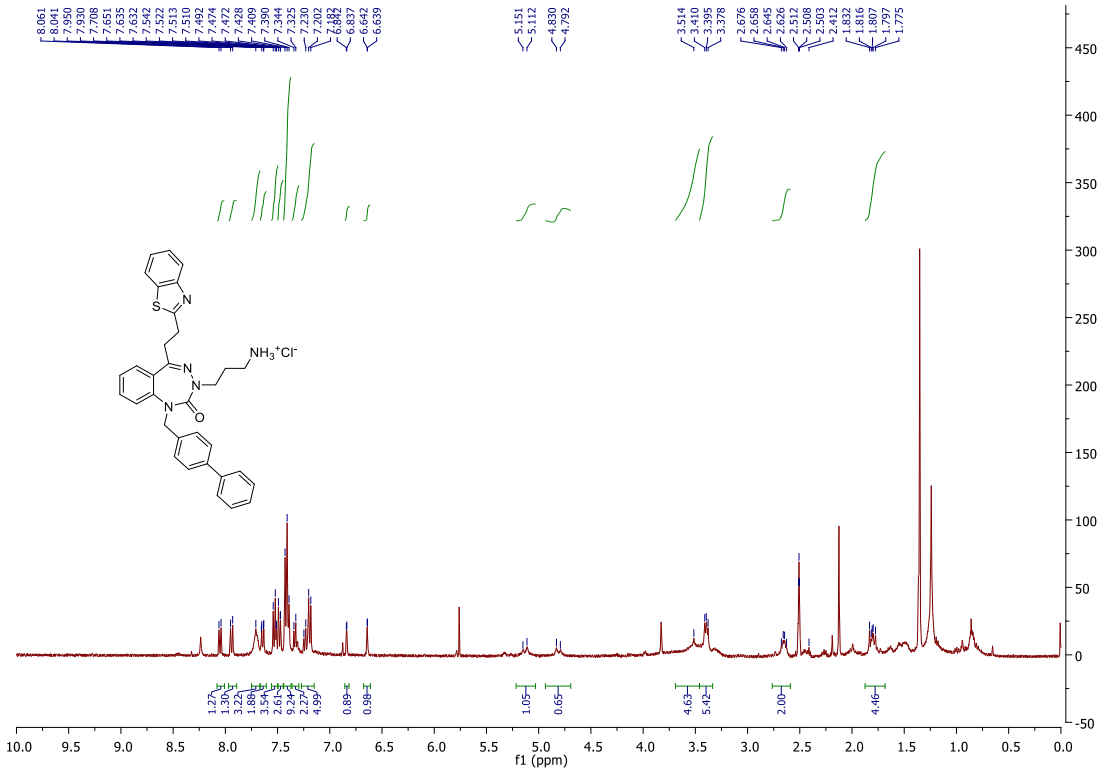


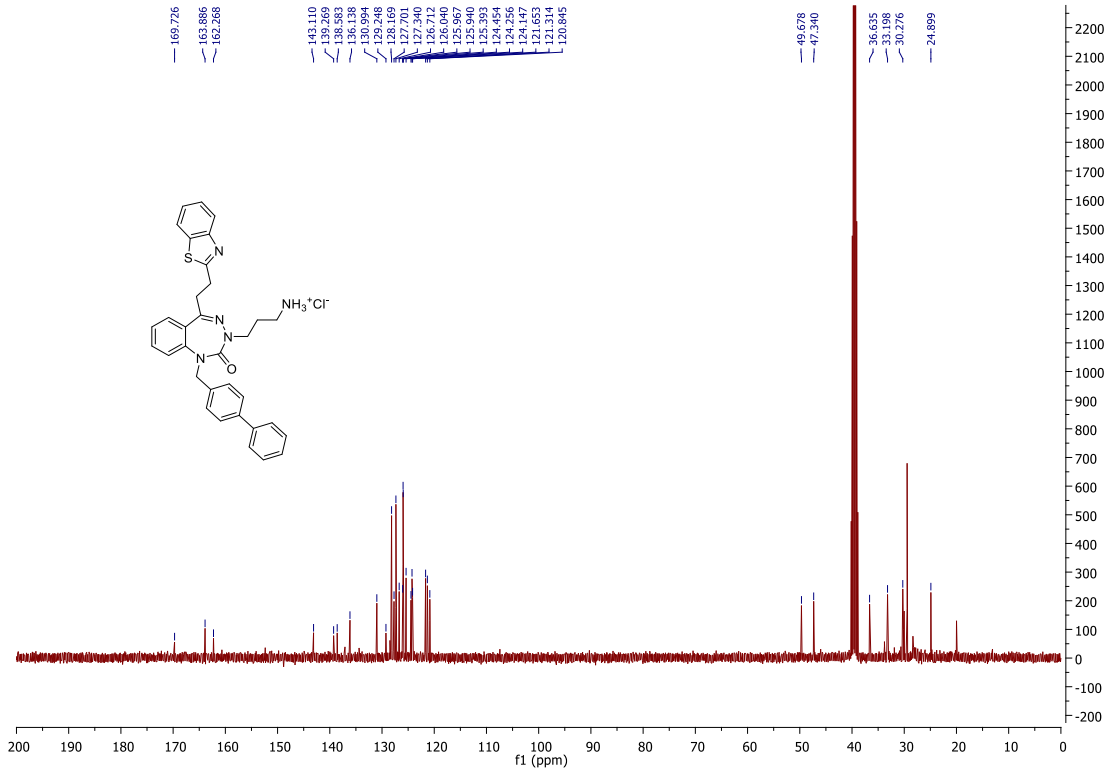
3.36, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



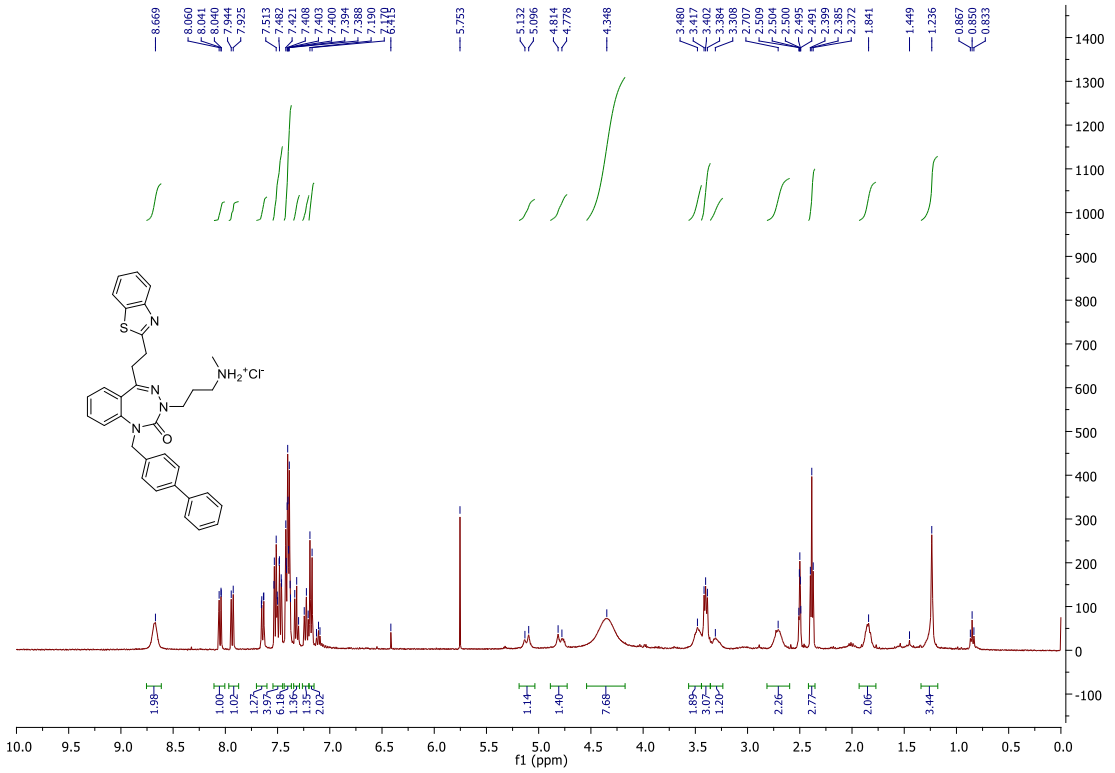


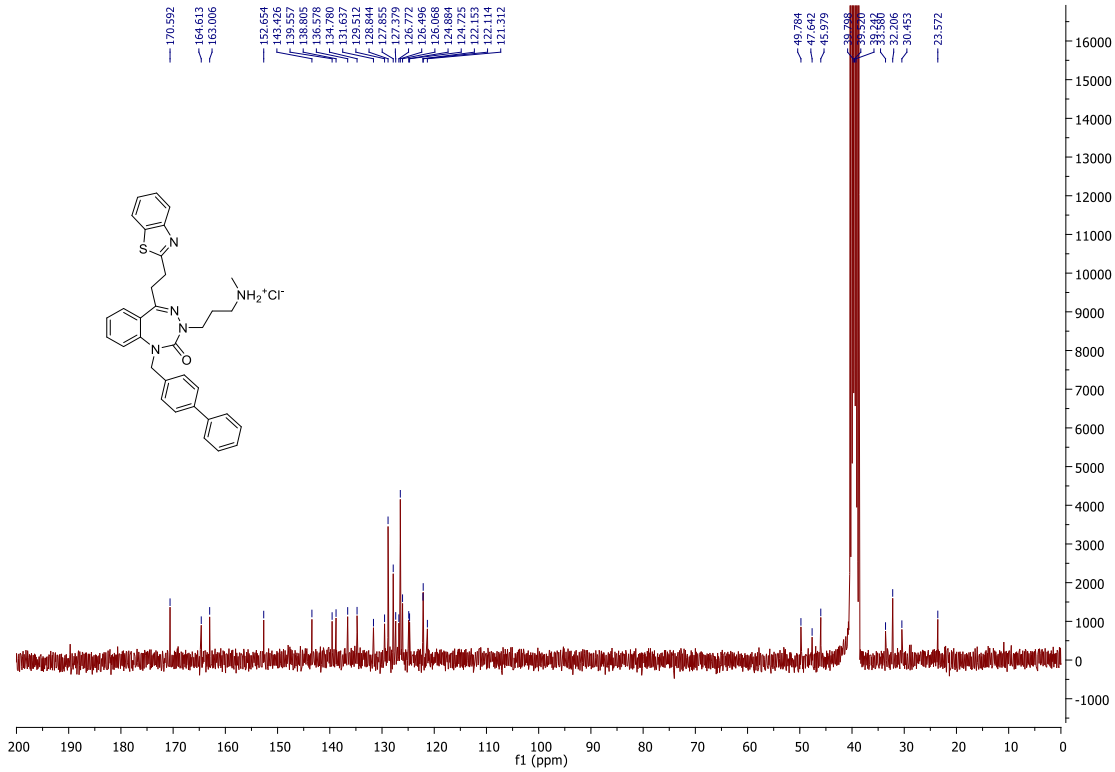
3.37, DMSO-d_6, ^{1}H (400 MHz), ^{13}C (101 MHz, 100°C)



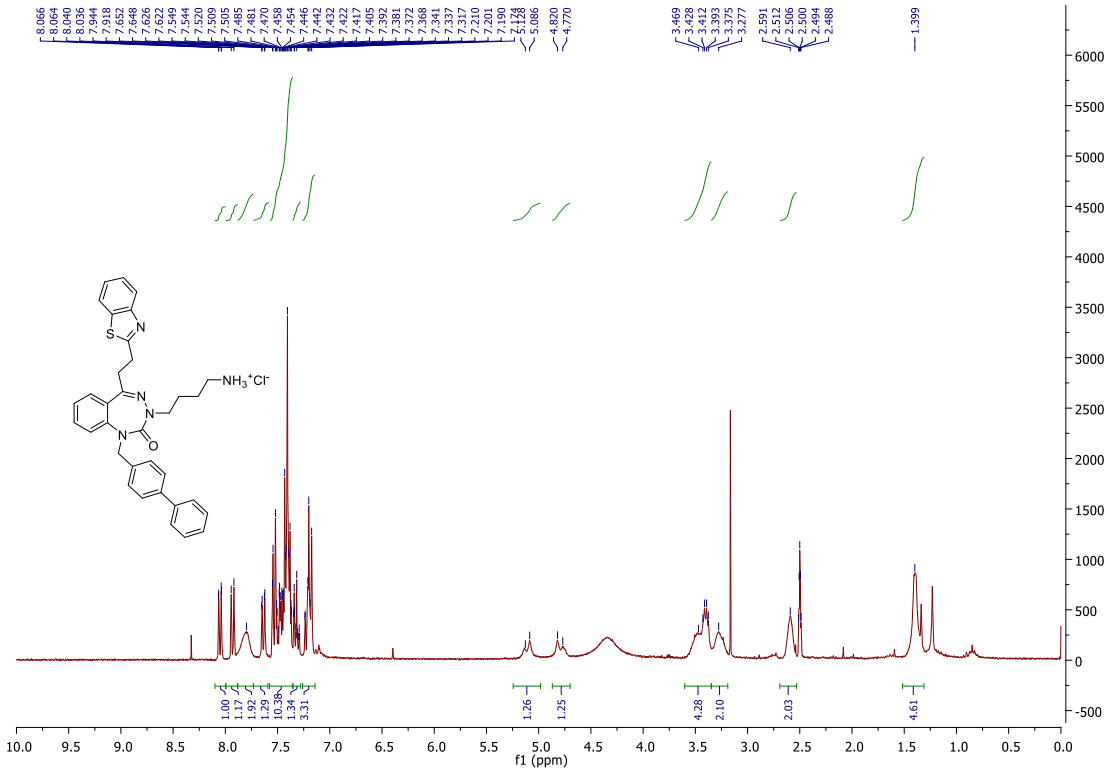


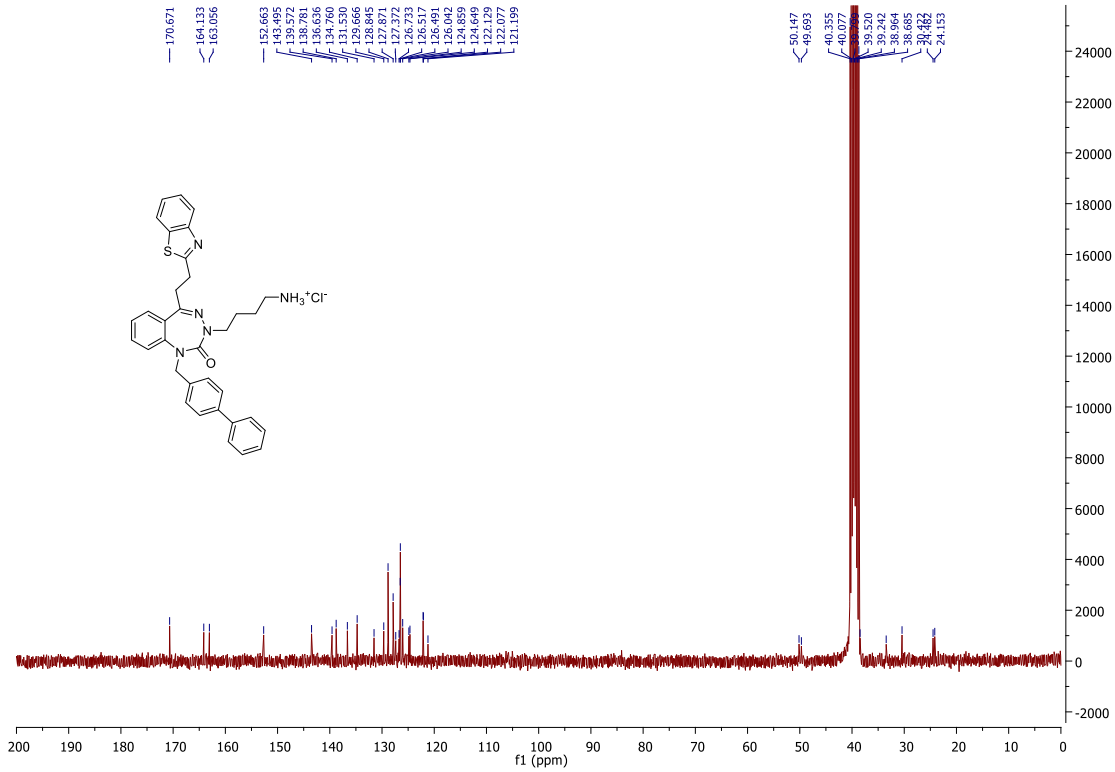
3.38, DMSO-d_6, ^{1}H (400 MHz), ^{13}C (75 MHz)



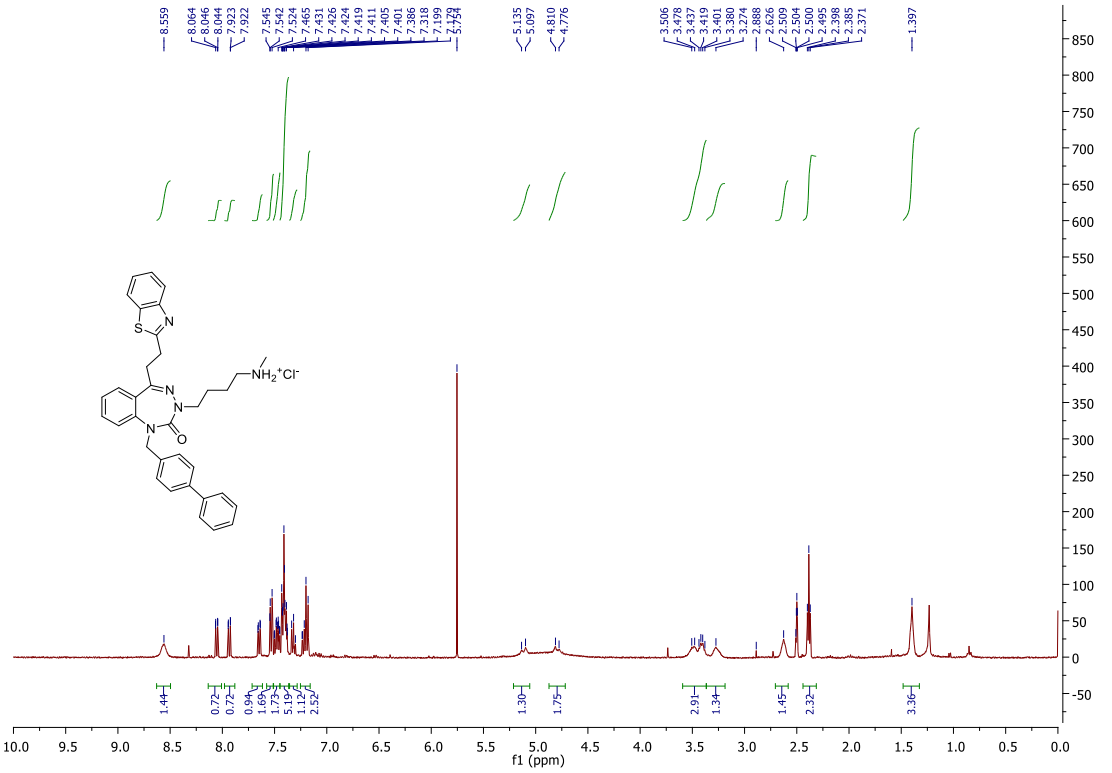


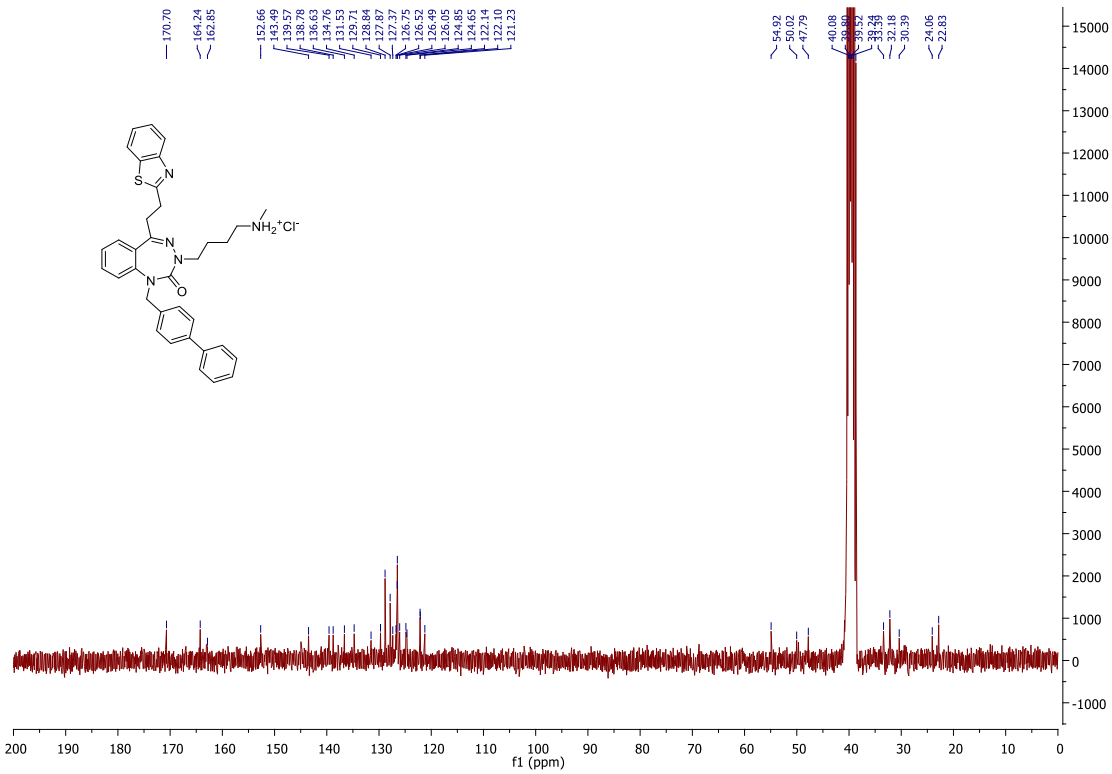
3.39, DMSO-d_6, ^{1}H (300 MHz), ^{13}C (75 MHz)



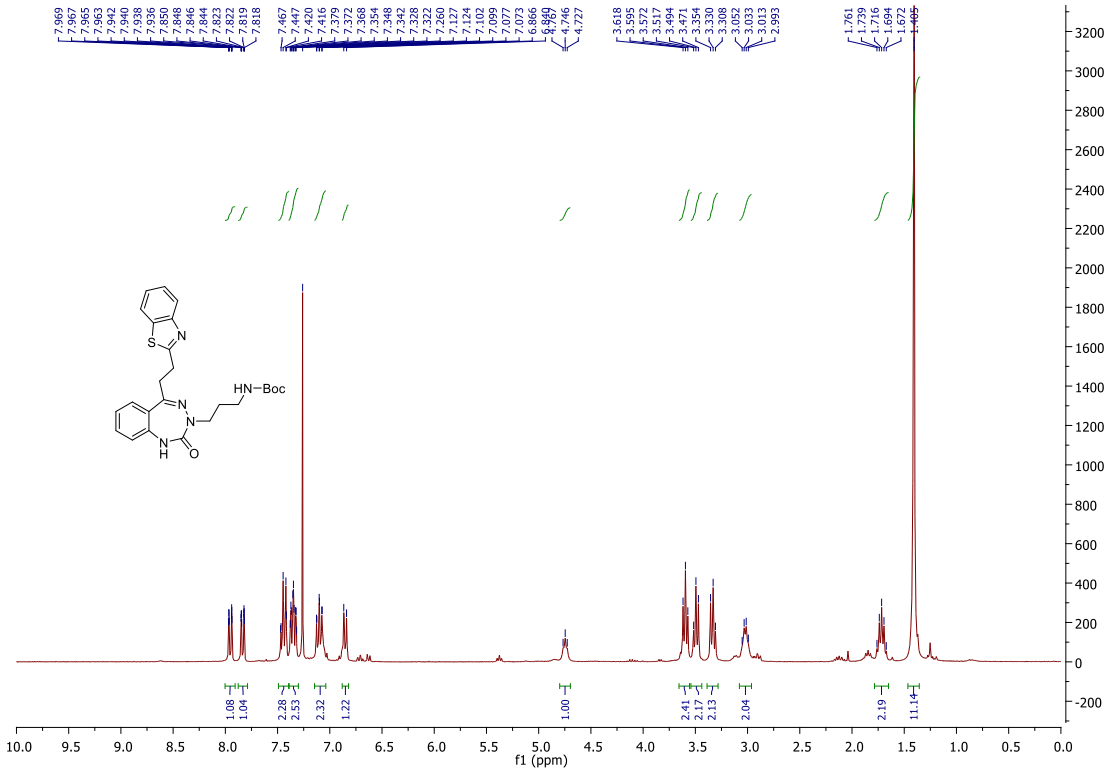


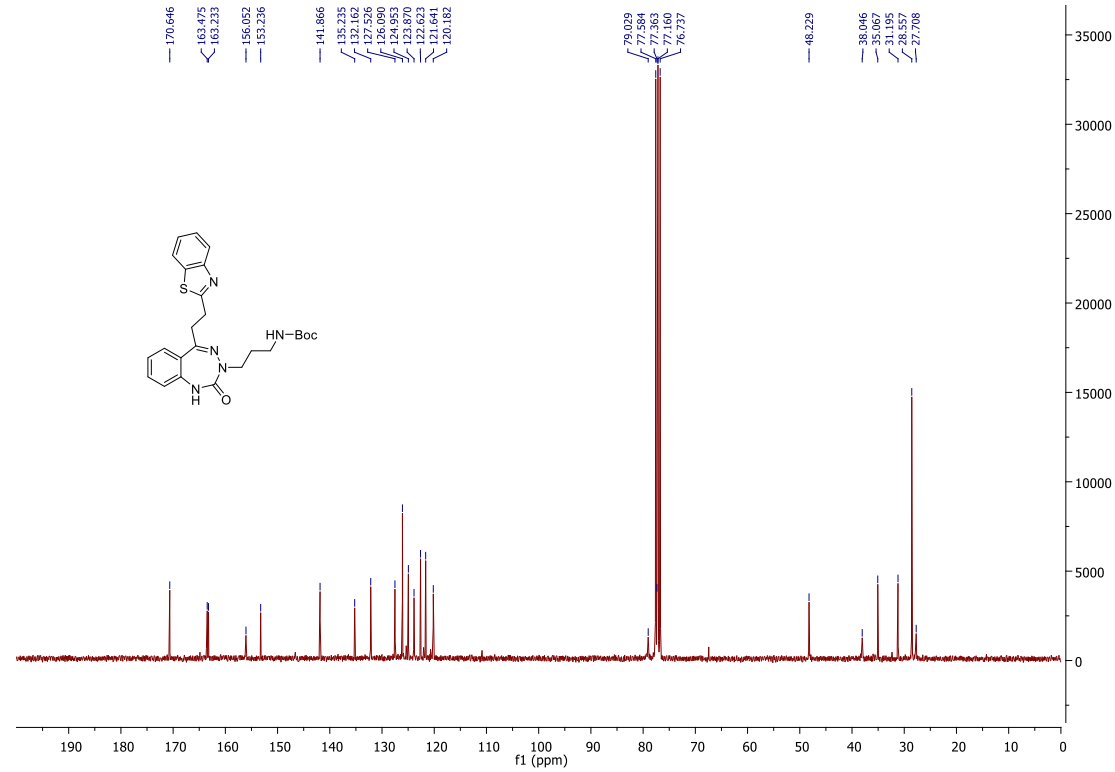
3.40, DMSO-d_6, ^{1}H (400 MHz), ^{13}C (75 MHz)



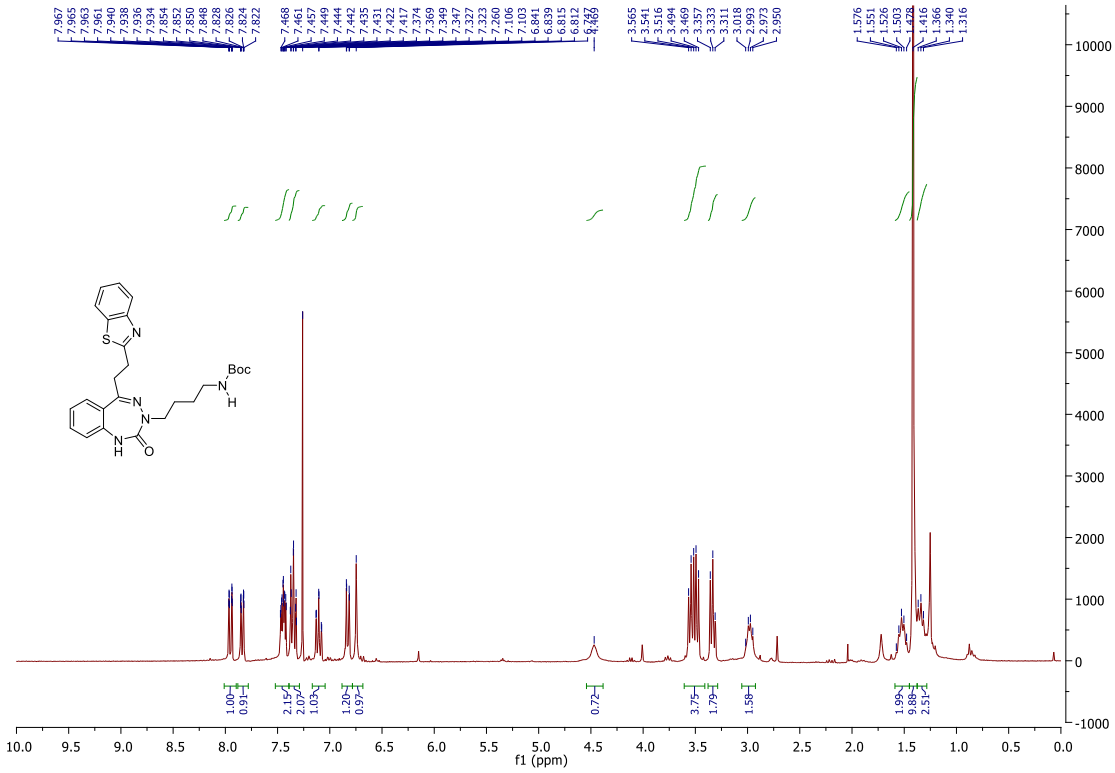


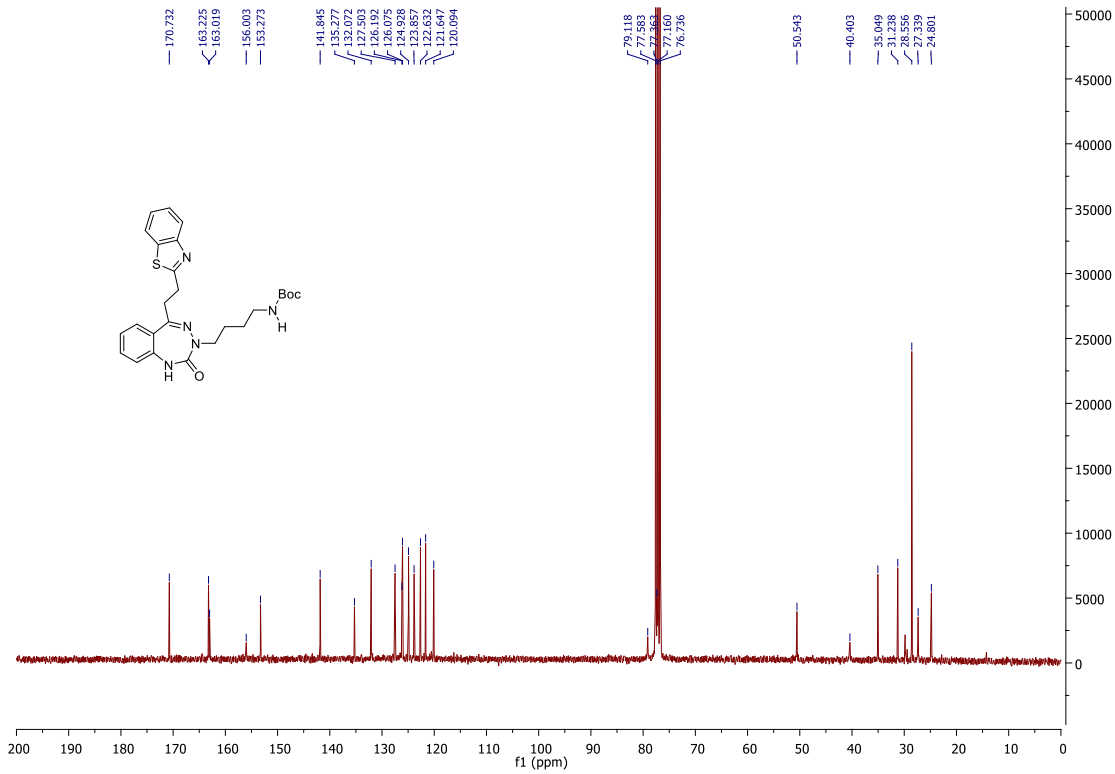
3.41, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



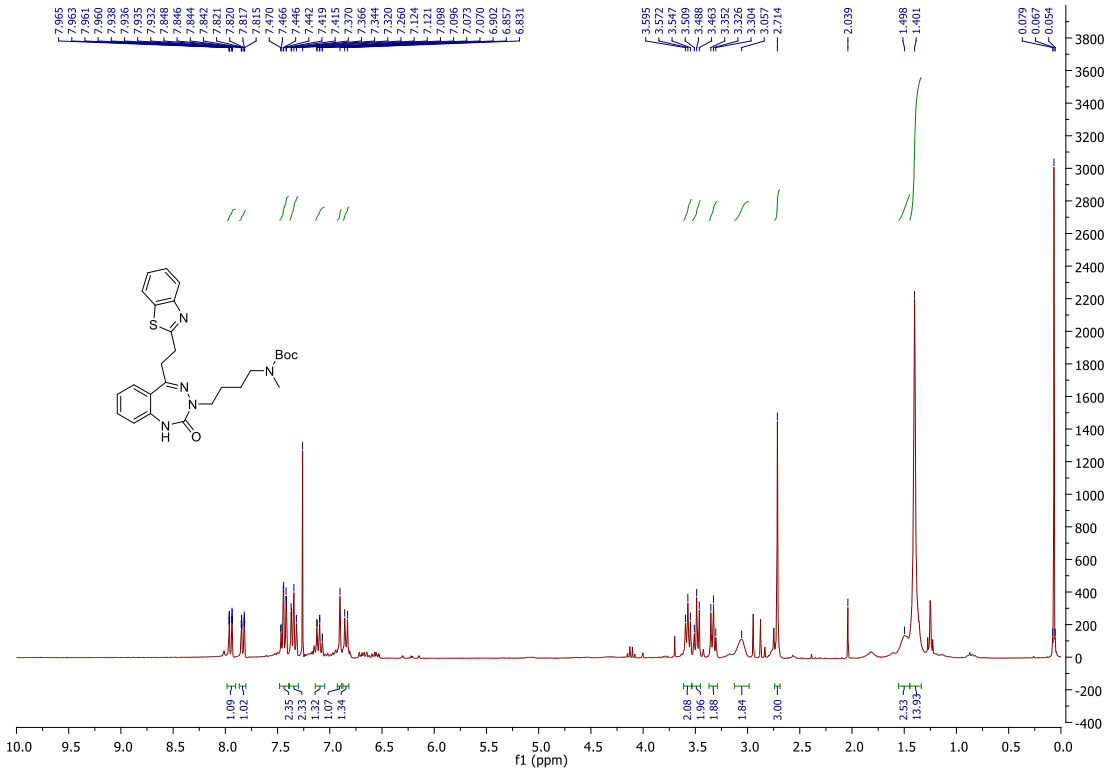


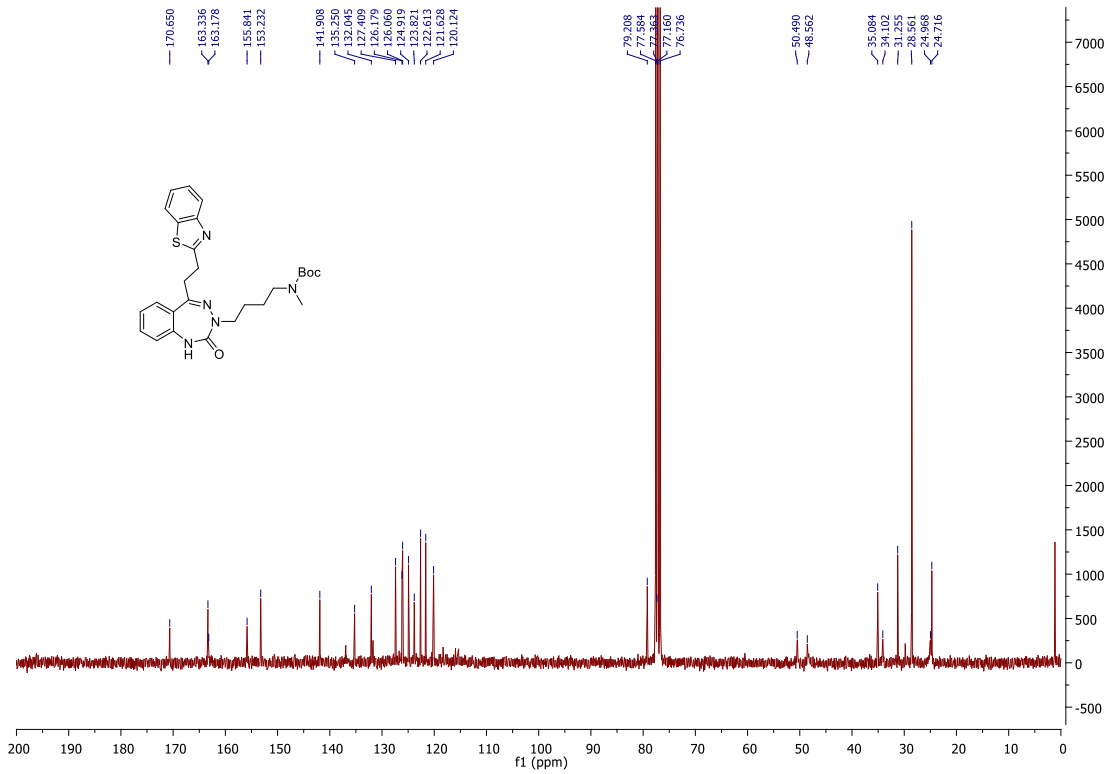
3.42, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



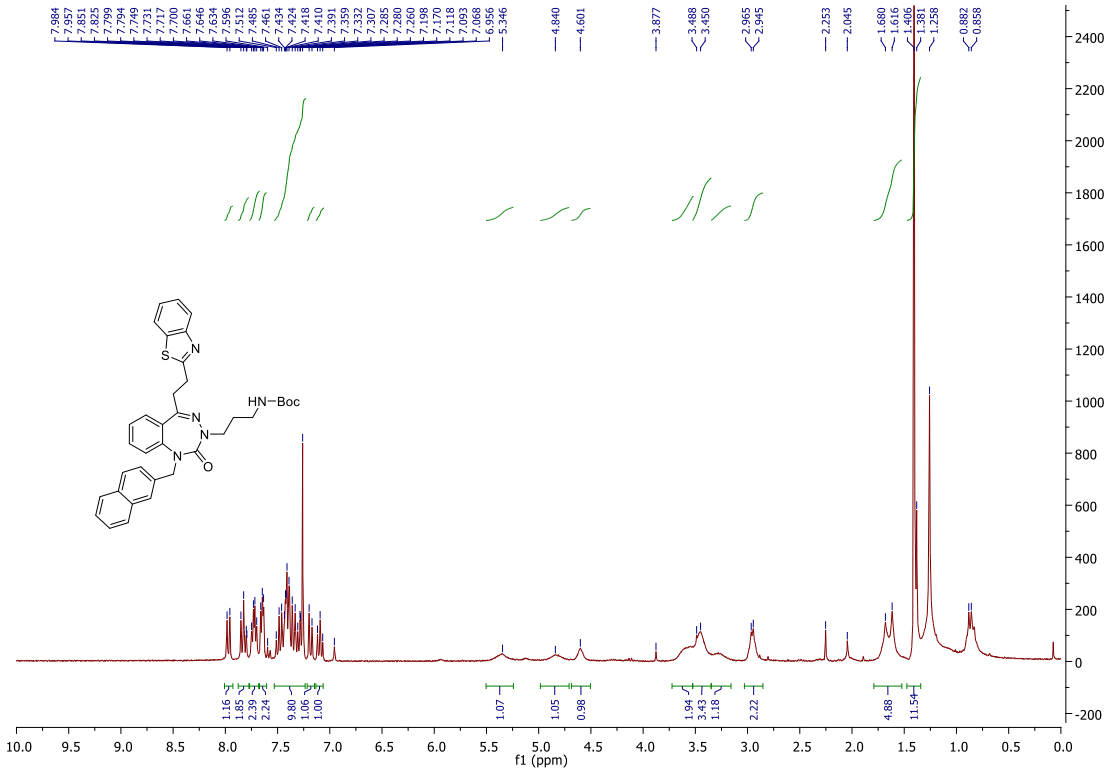


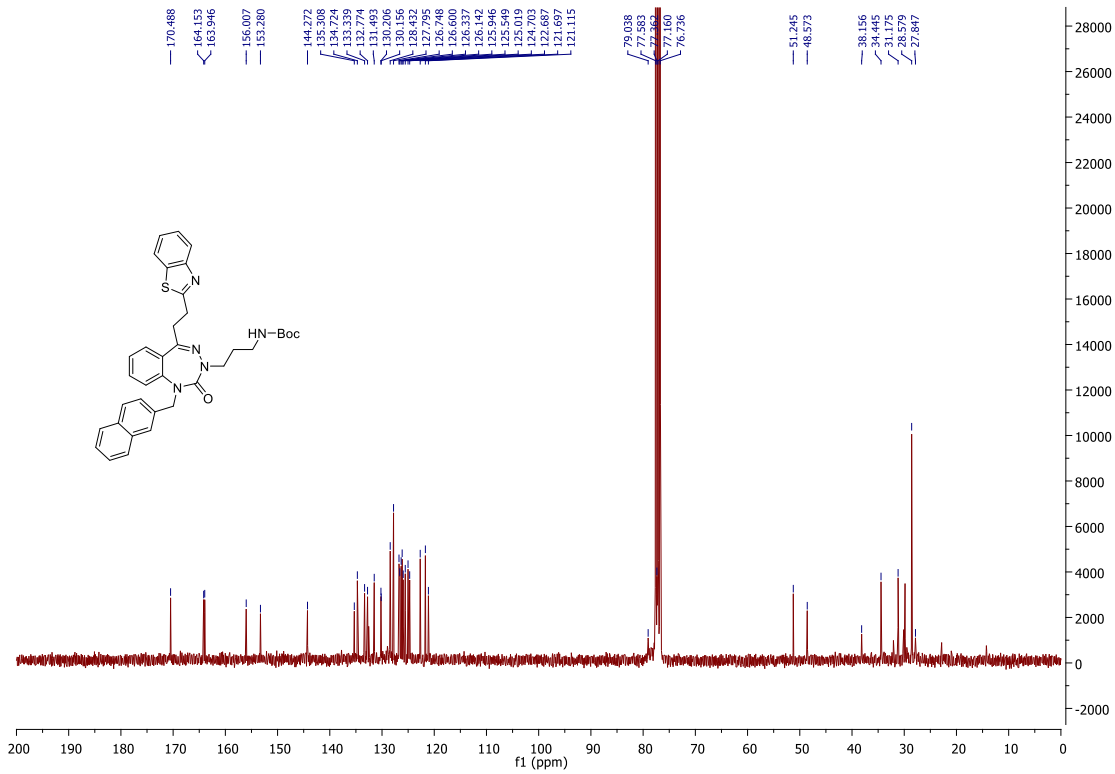
3.43, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



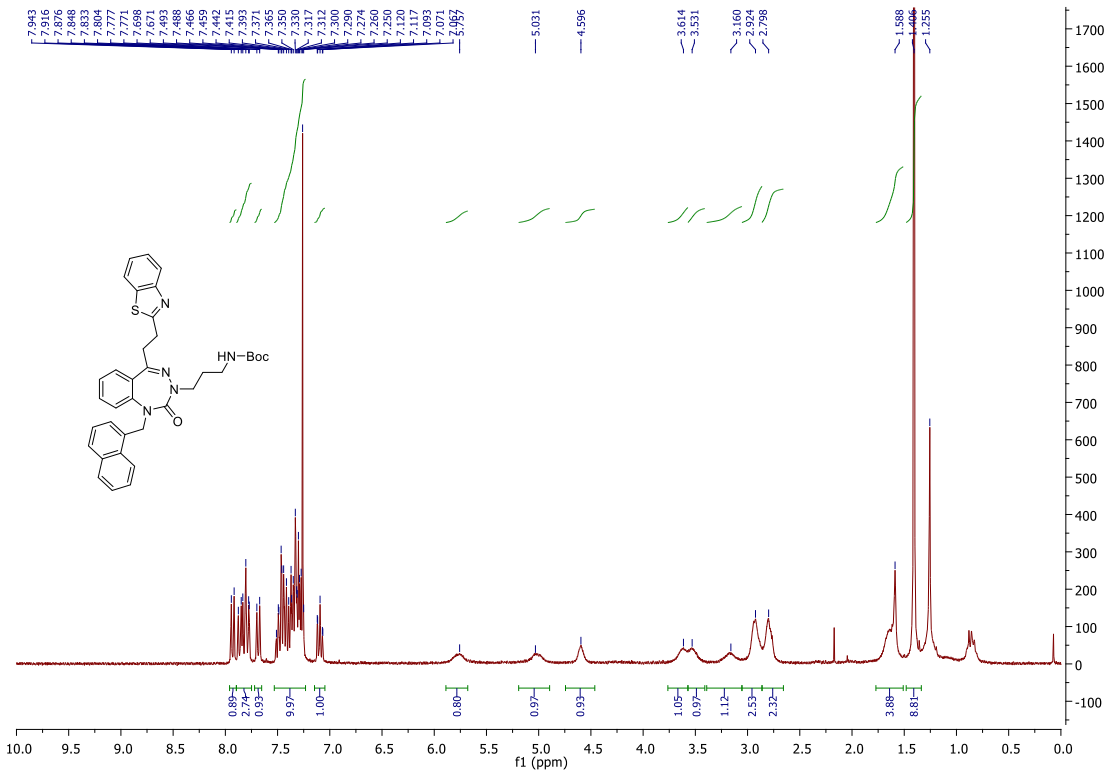


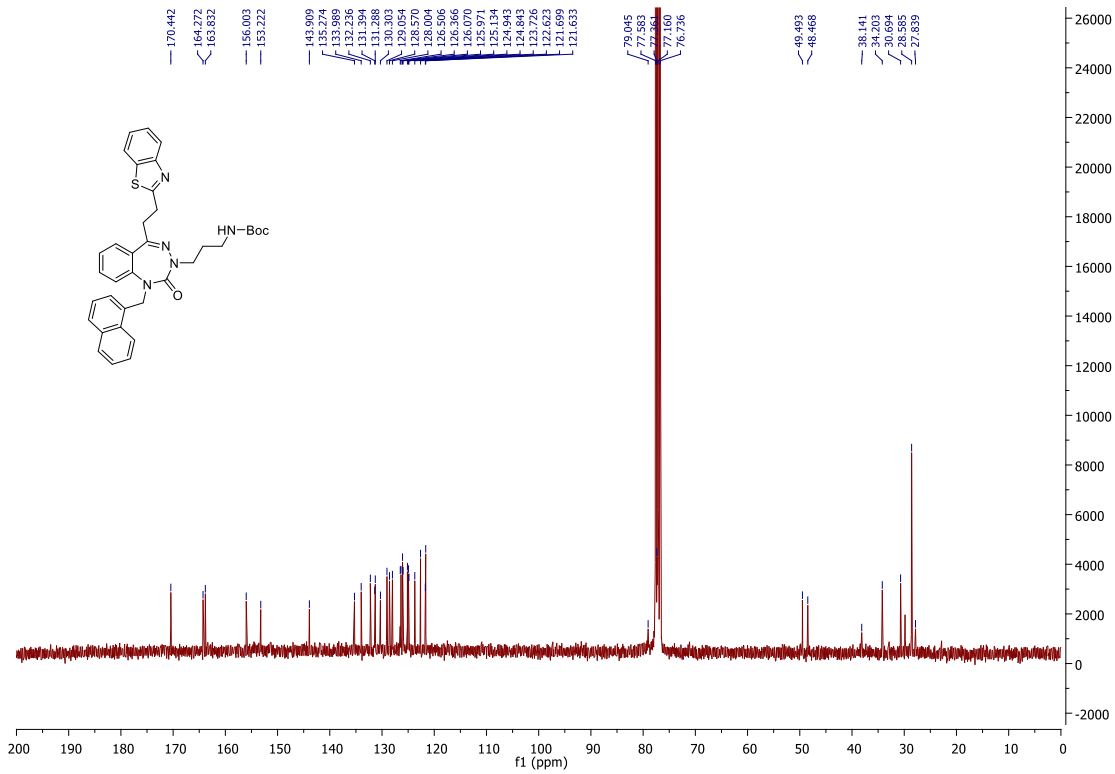
3.44h, $CDCl_3$, ^1H (400 MHz), ^{13}C (75 MHz)



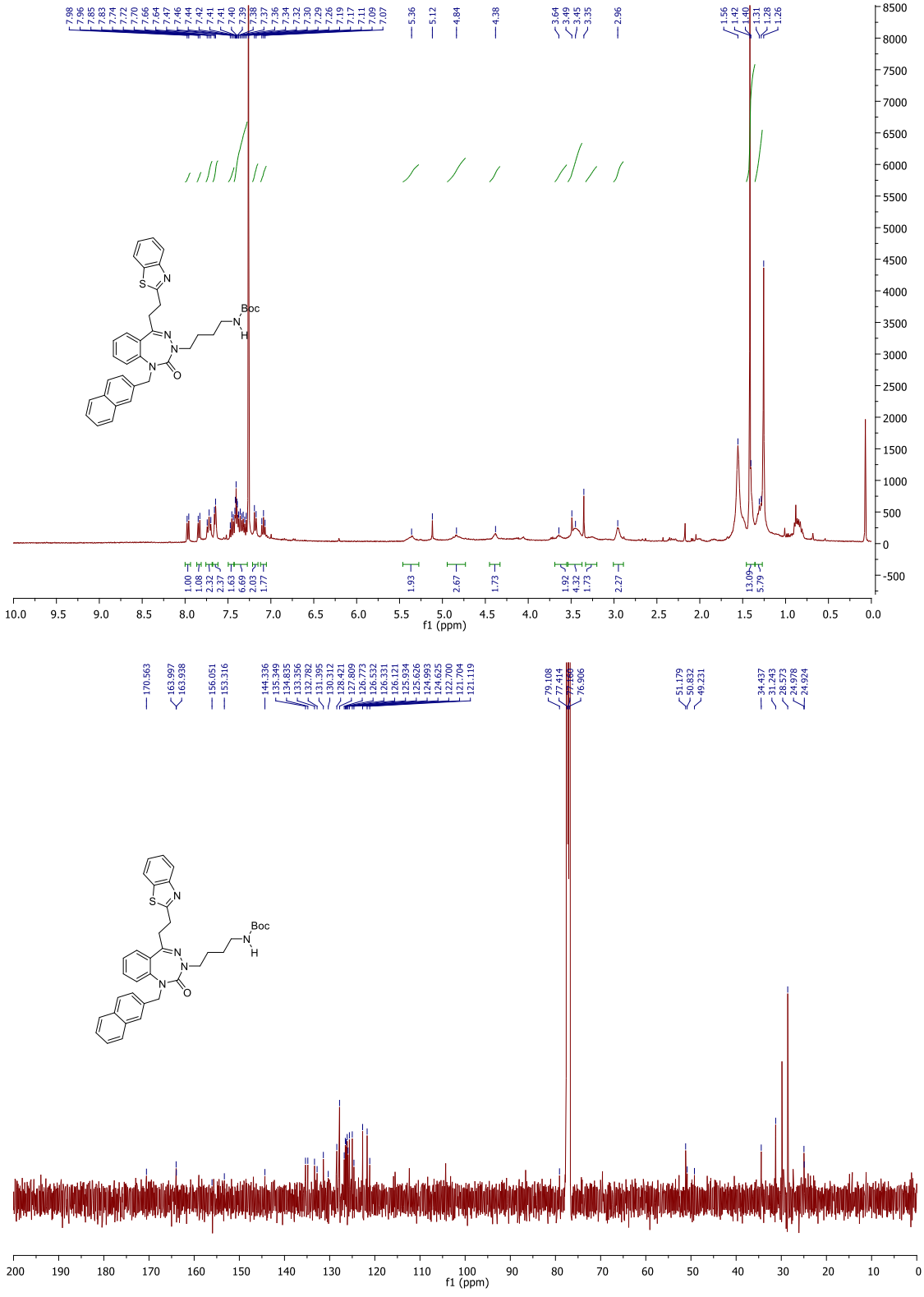


3.44i, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

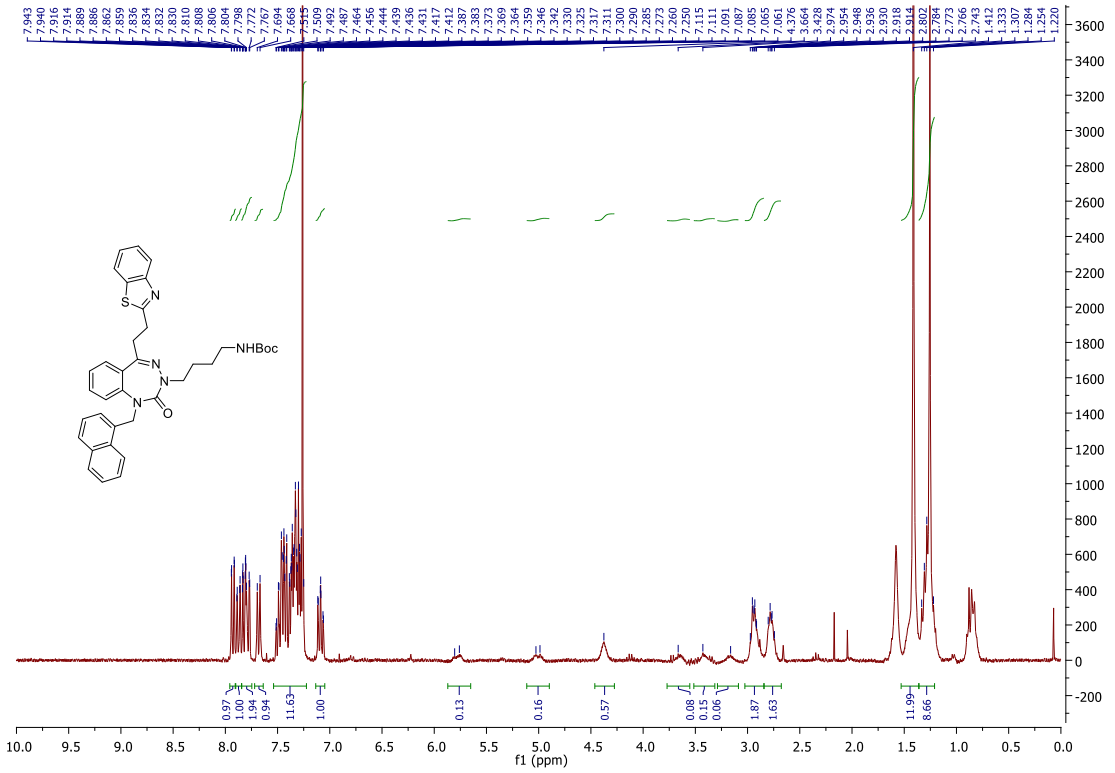


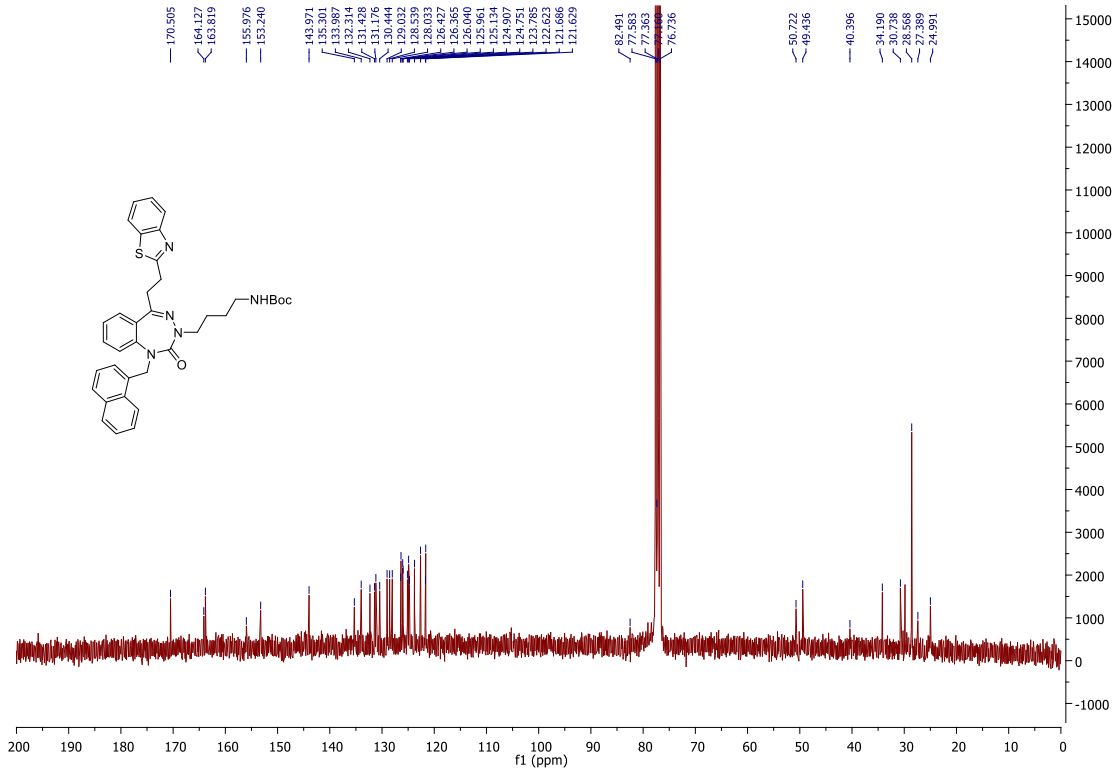


3.45h, $CDCl_3$, ^1H (400 MHz), ^{13}C (126 MHz)

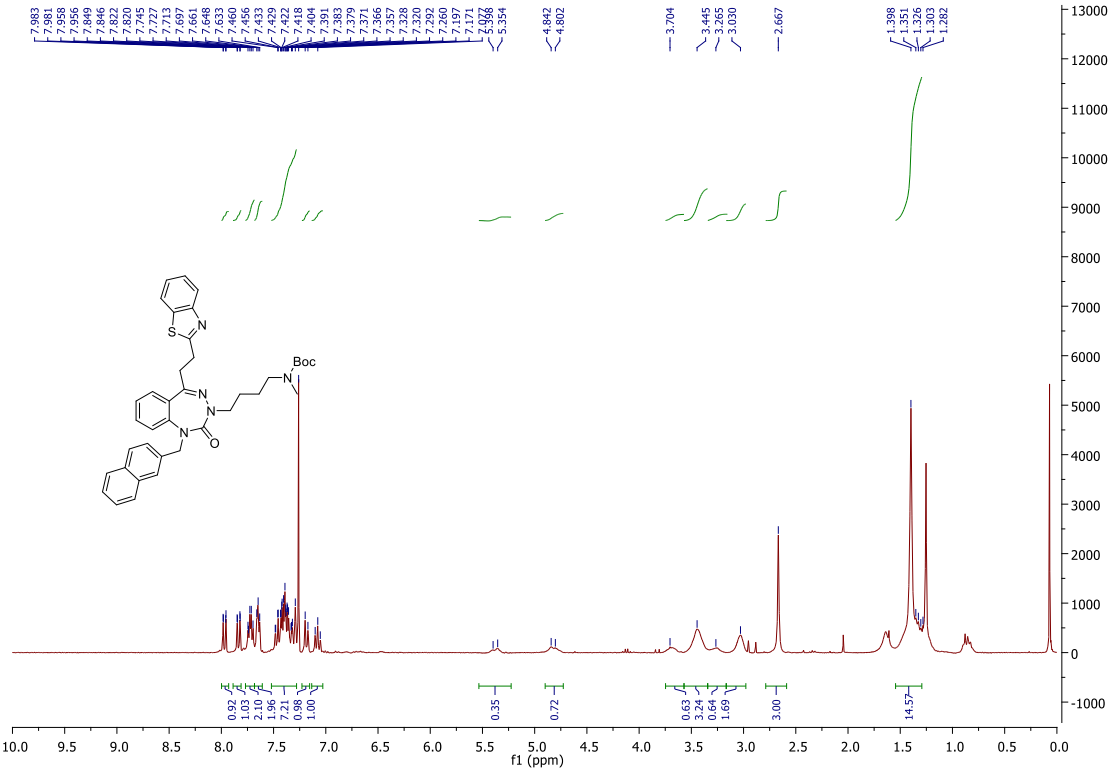


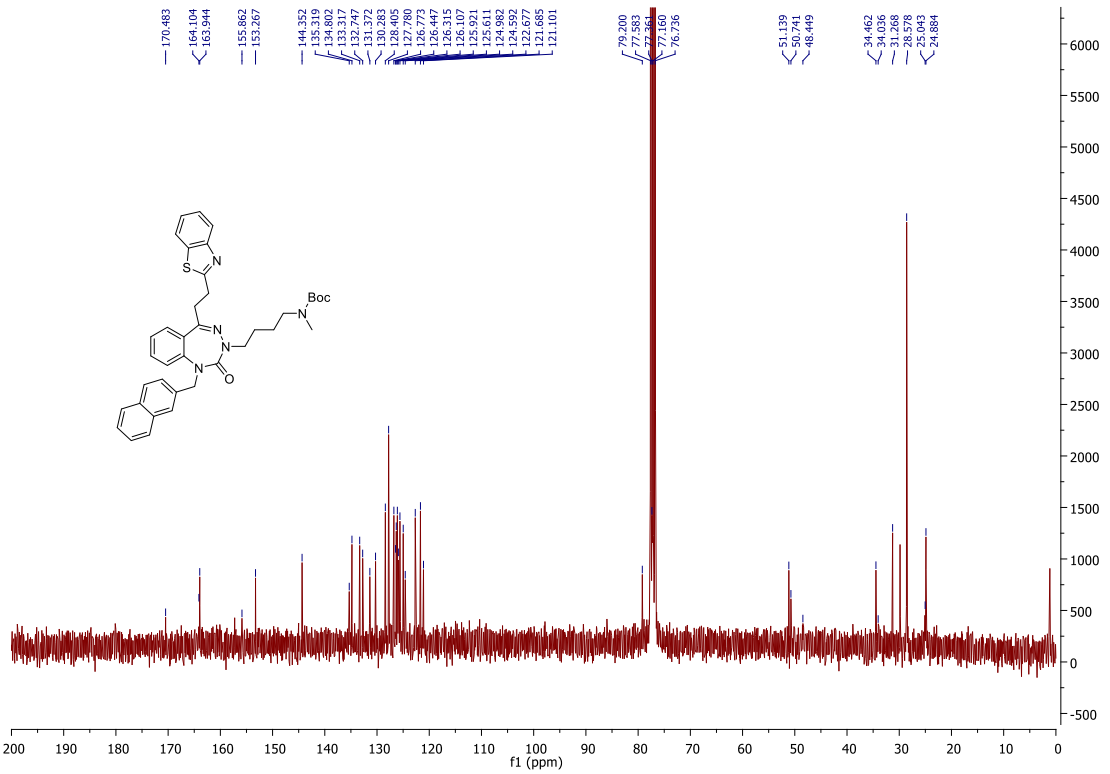
3.45i, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



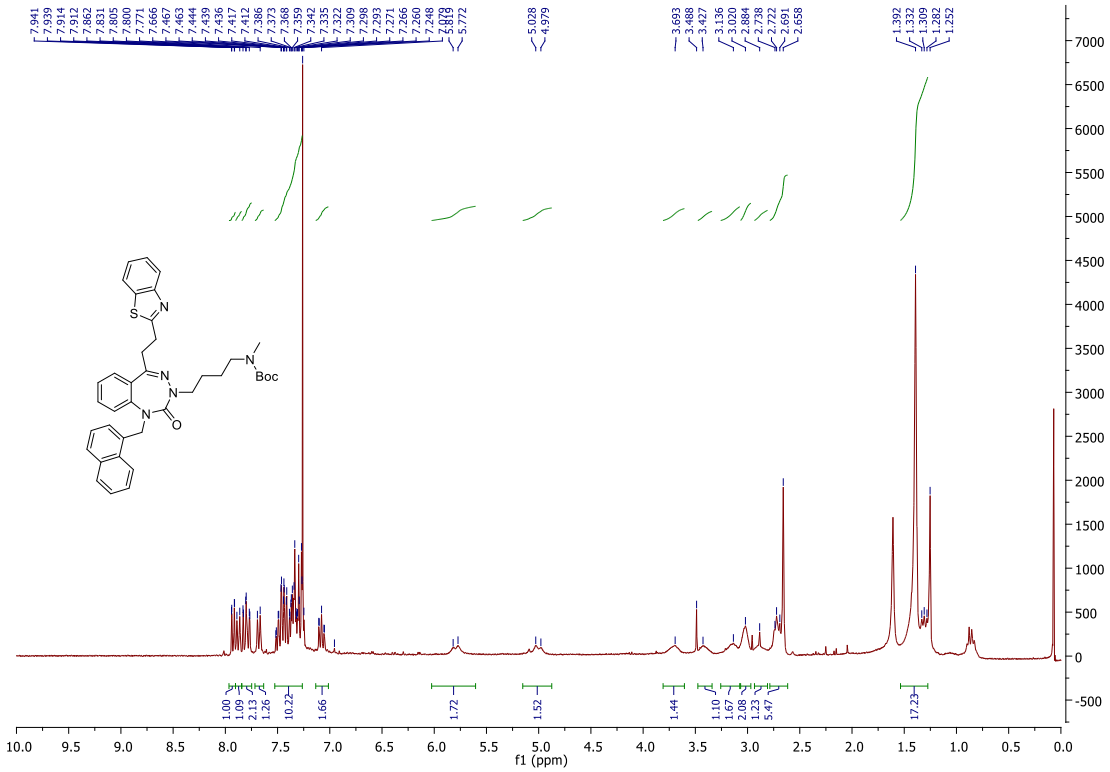


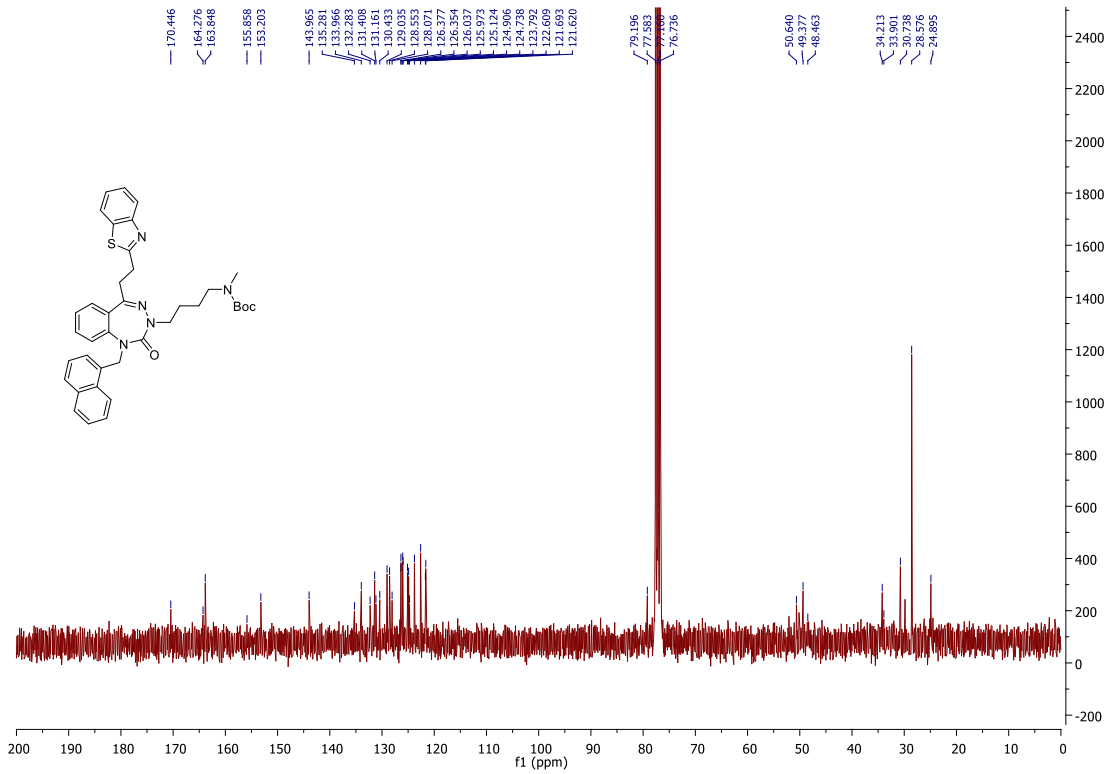
3.46h, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



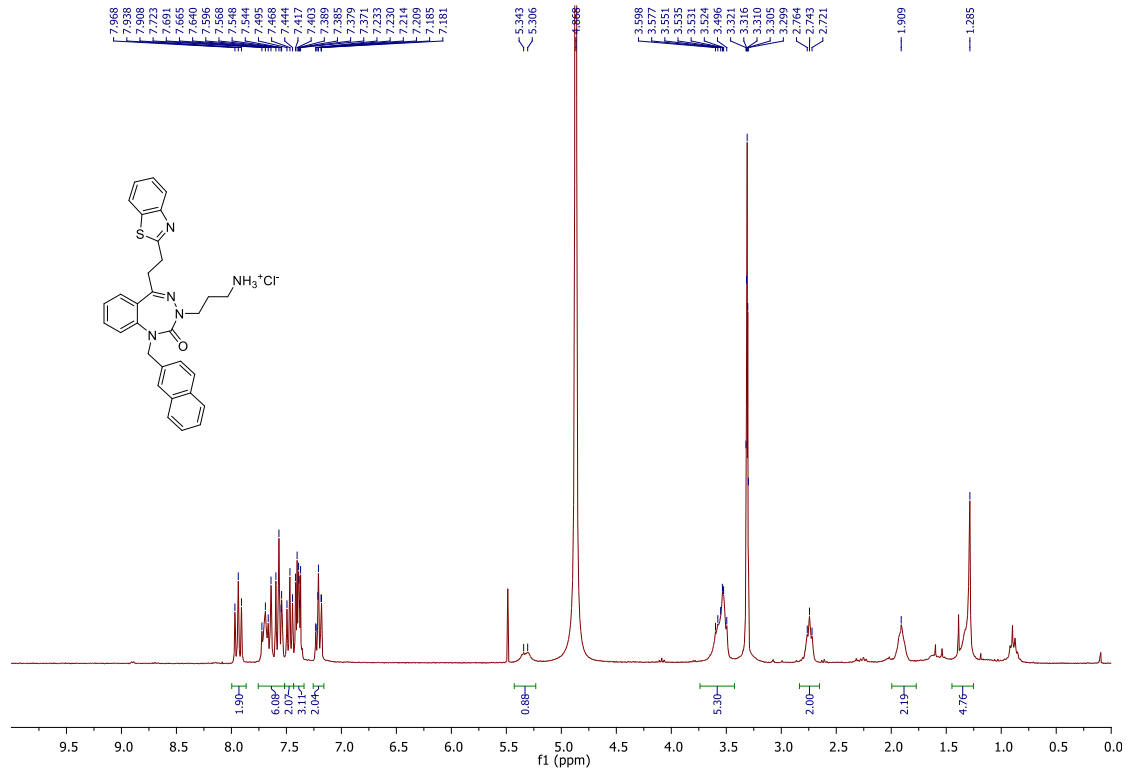


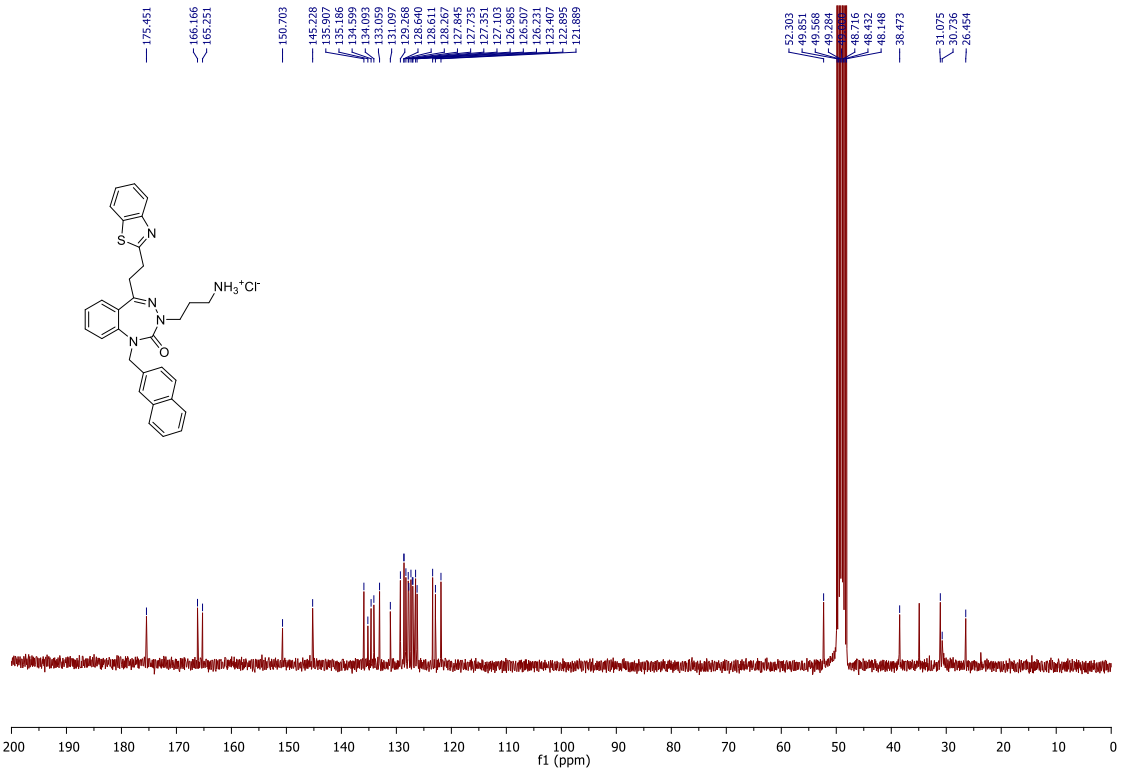
3.46i, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



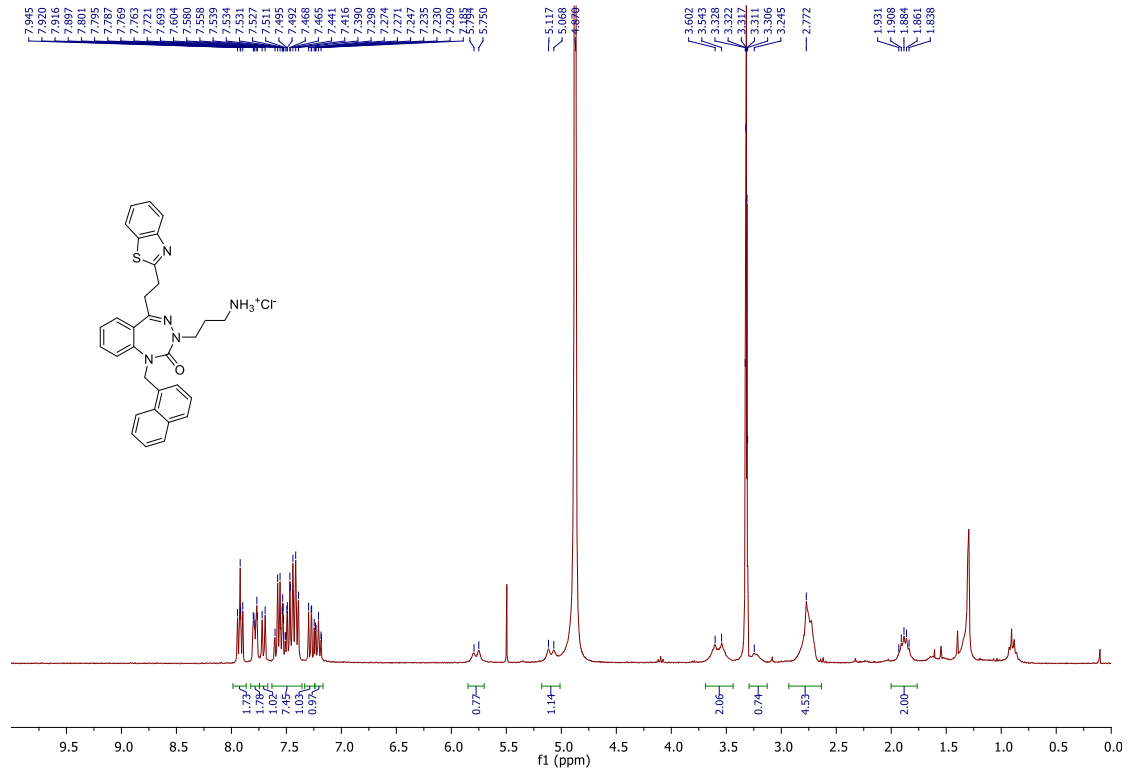


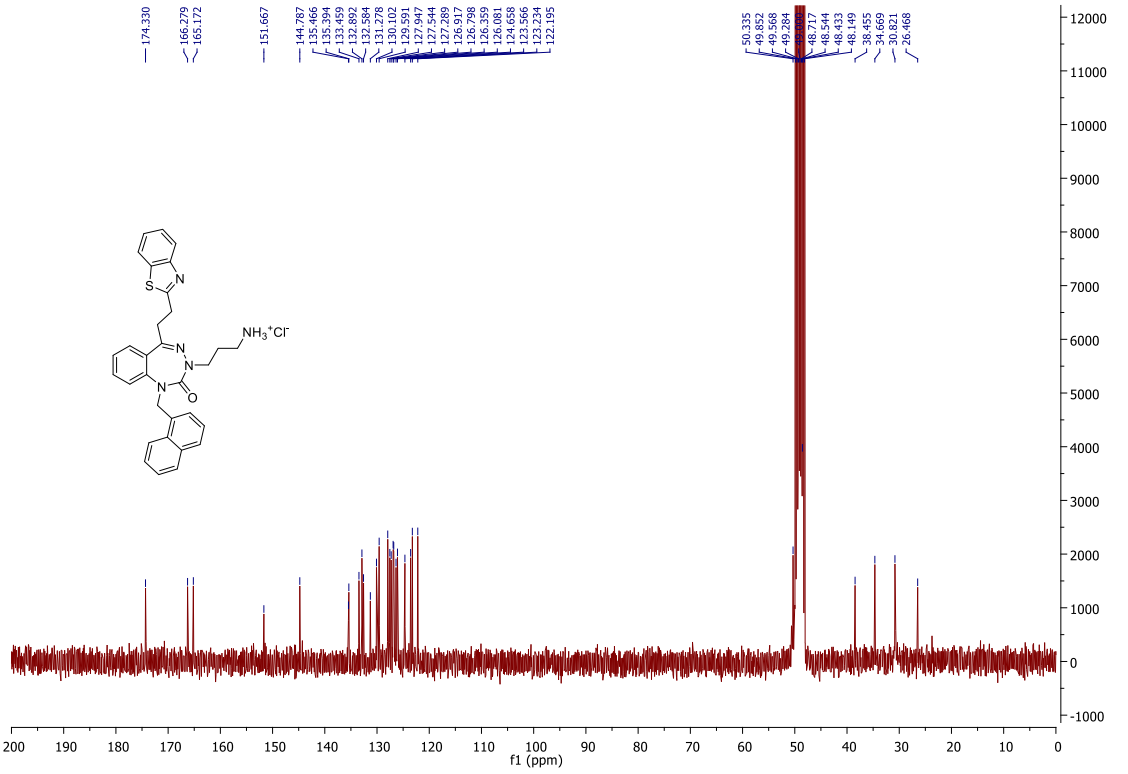
3.47h, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



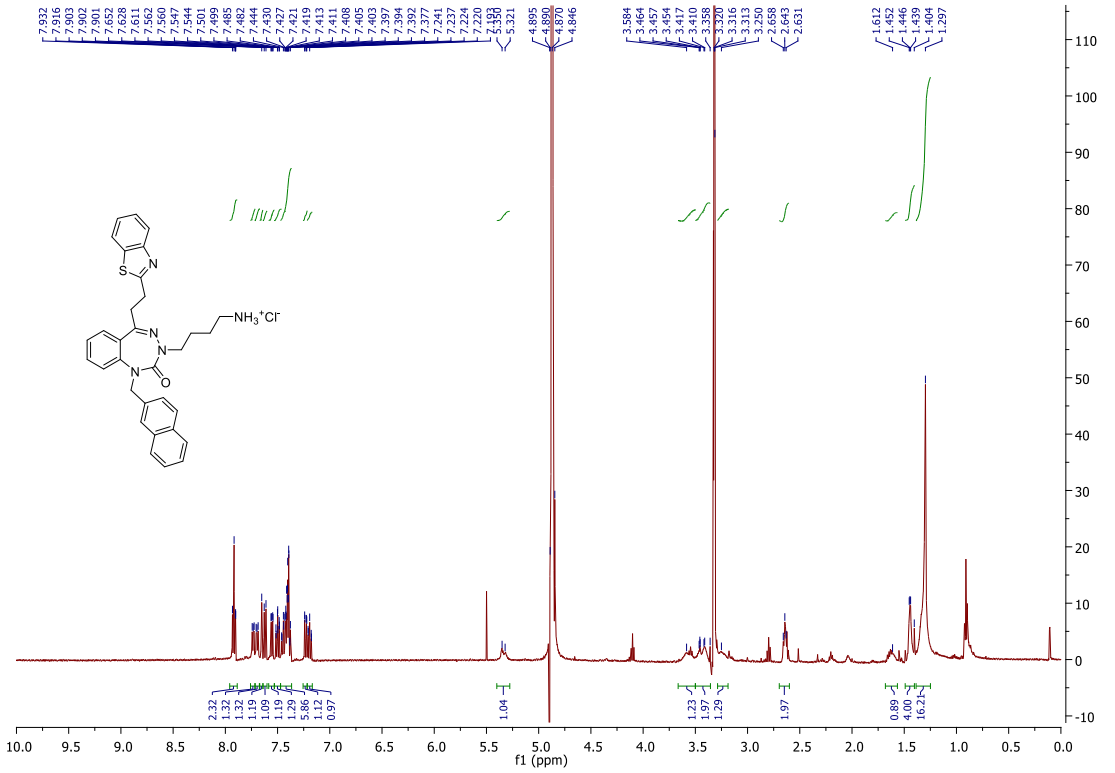


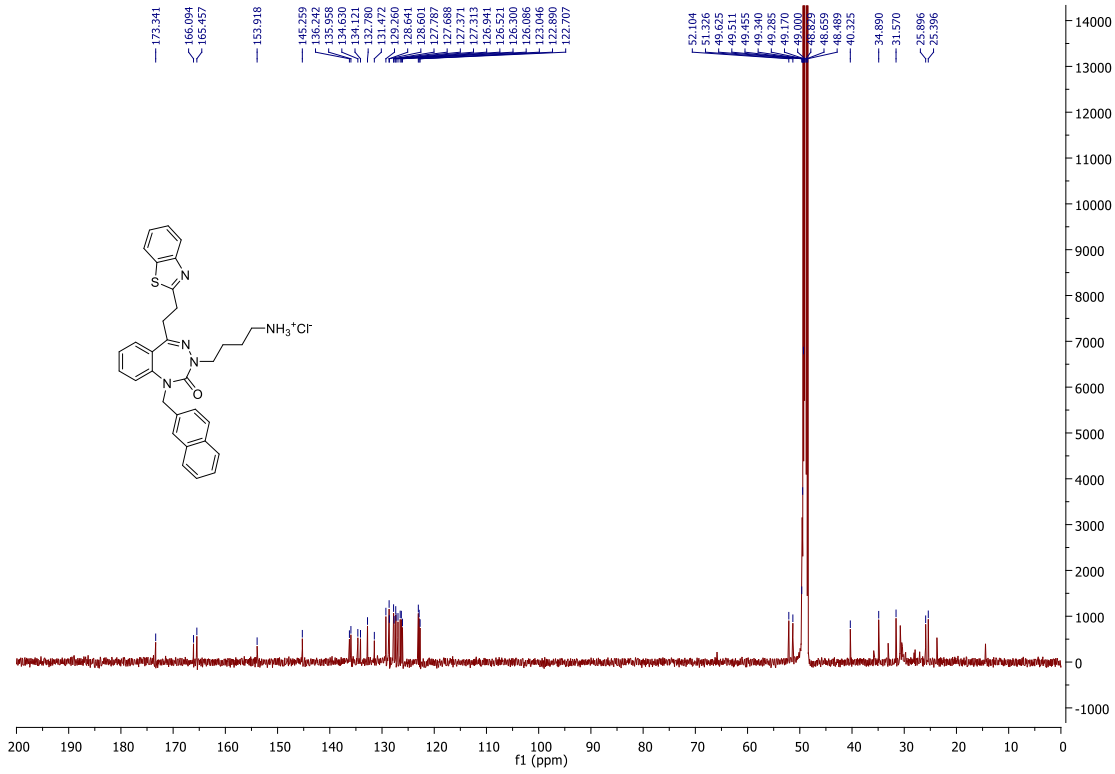
3.47i, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



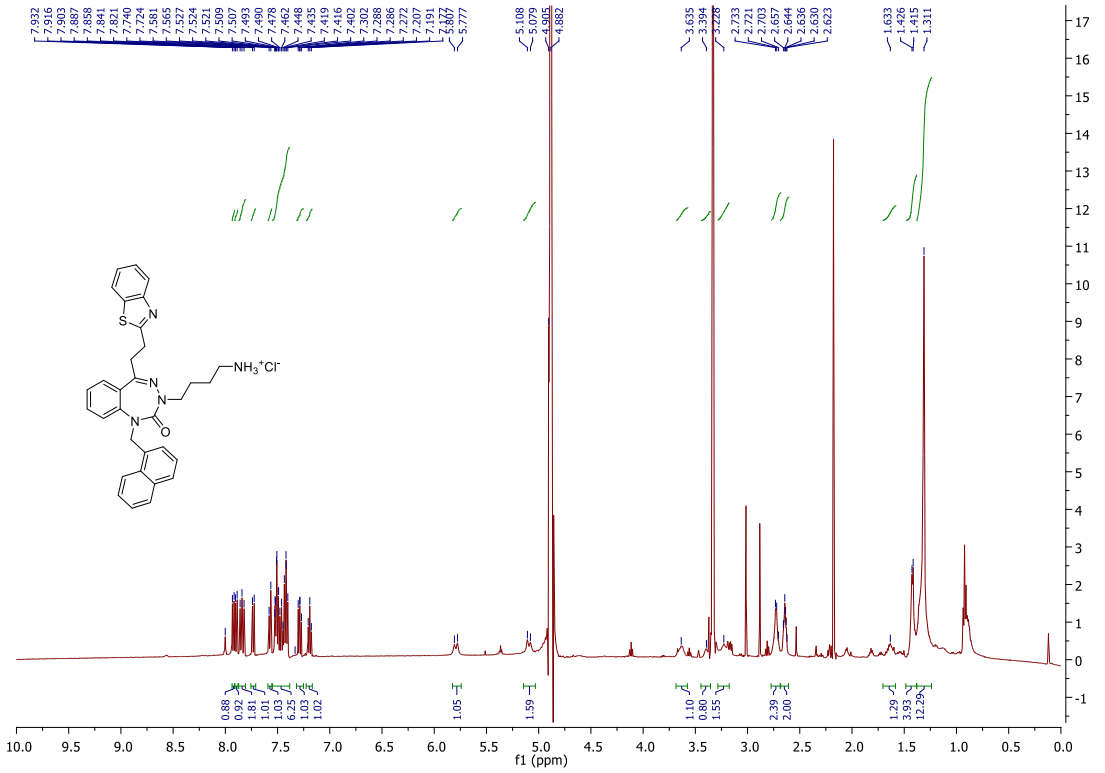


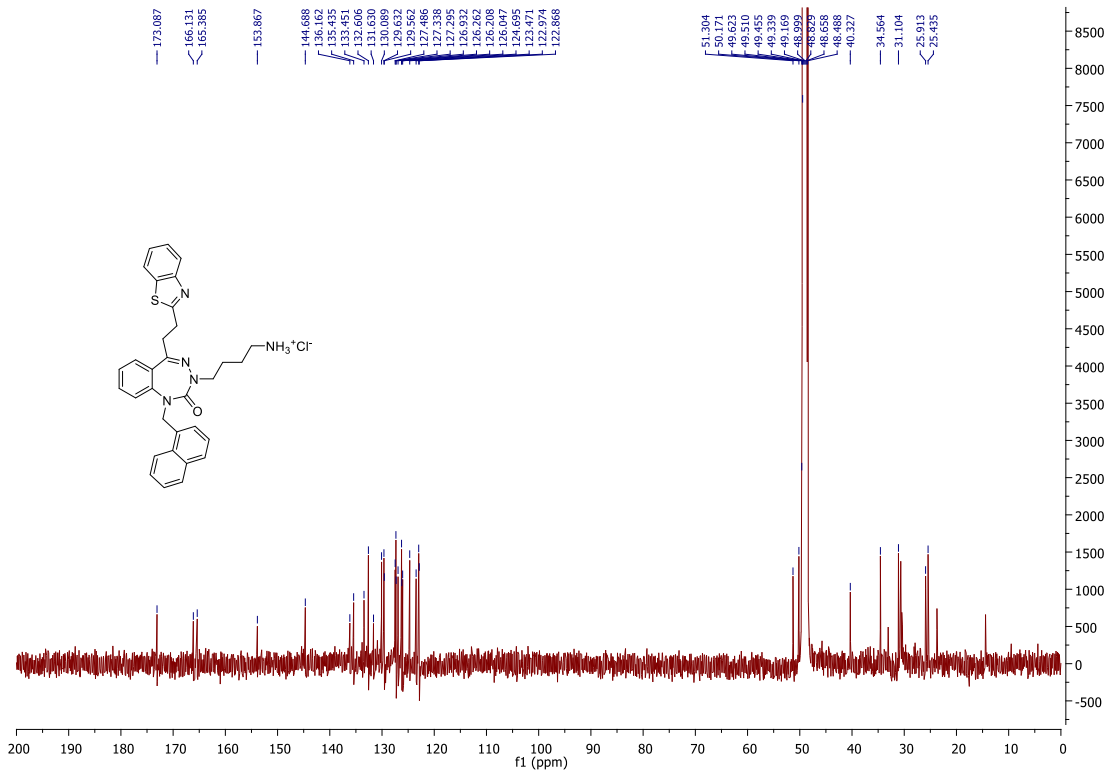
3.48h, CD_3OD-d_4, ^1H (500 MHz), ^{13}C (126 MHz)



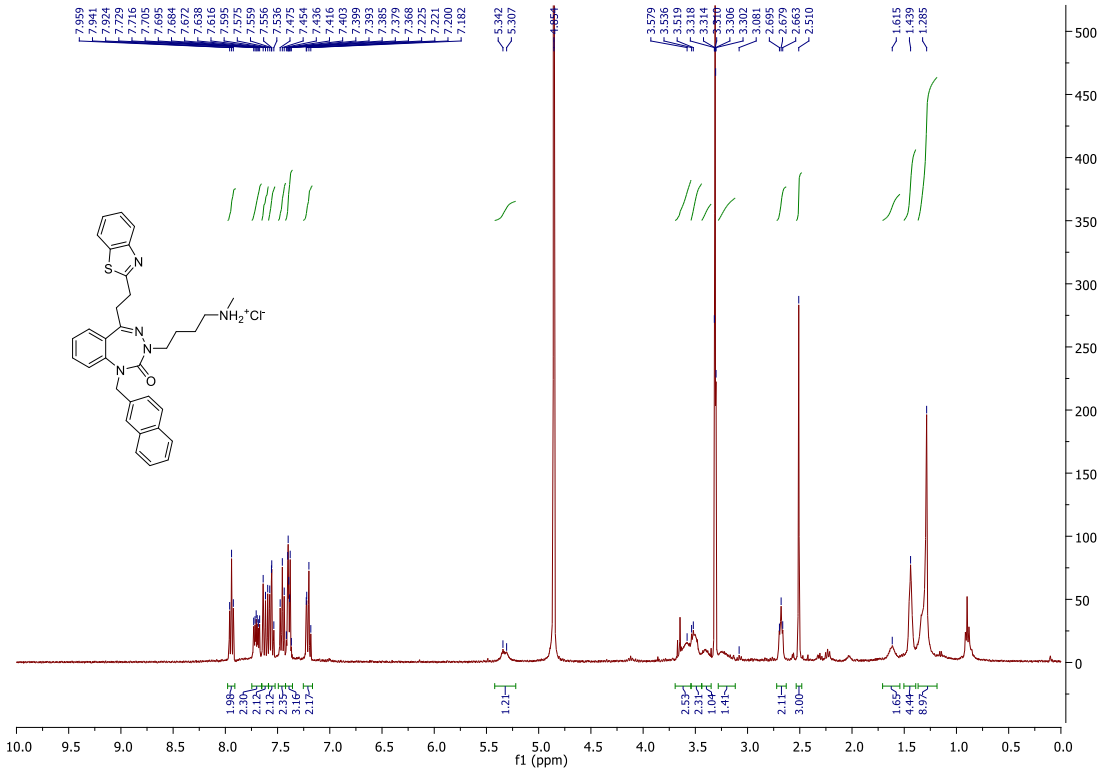


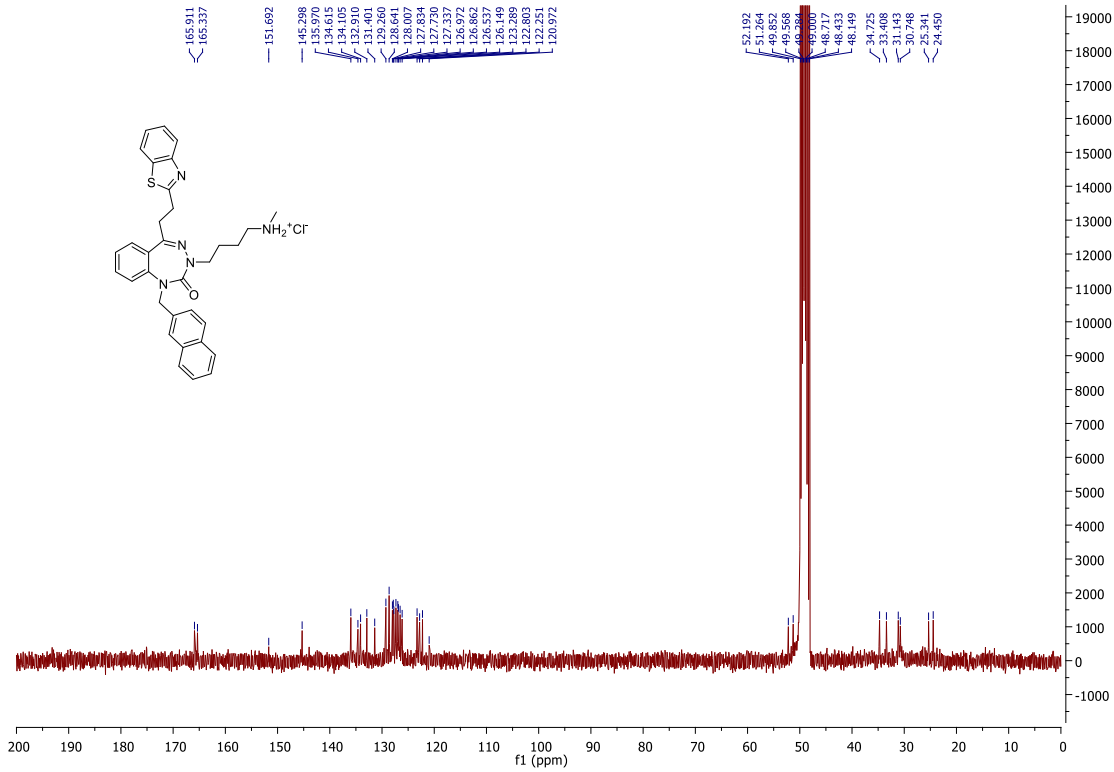
3.48i, CD_3OD-d_4, ^1H (500 MHz), ^{13}C (126 MHz)



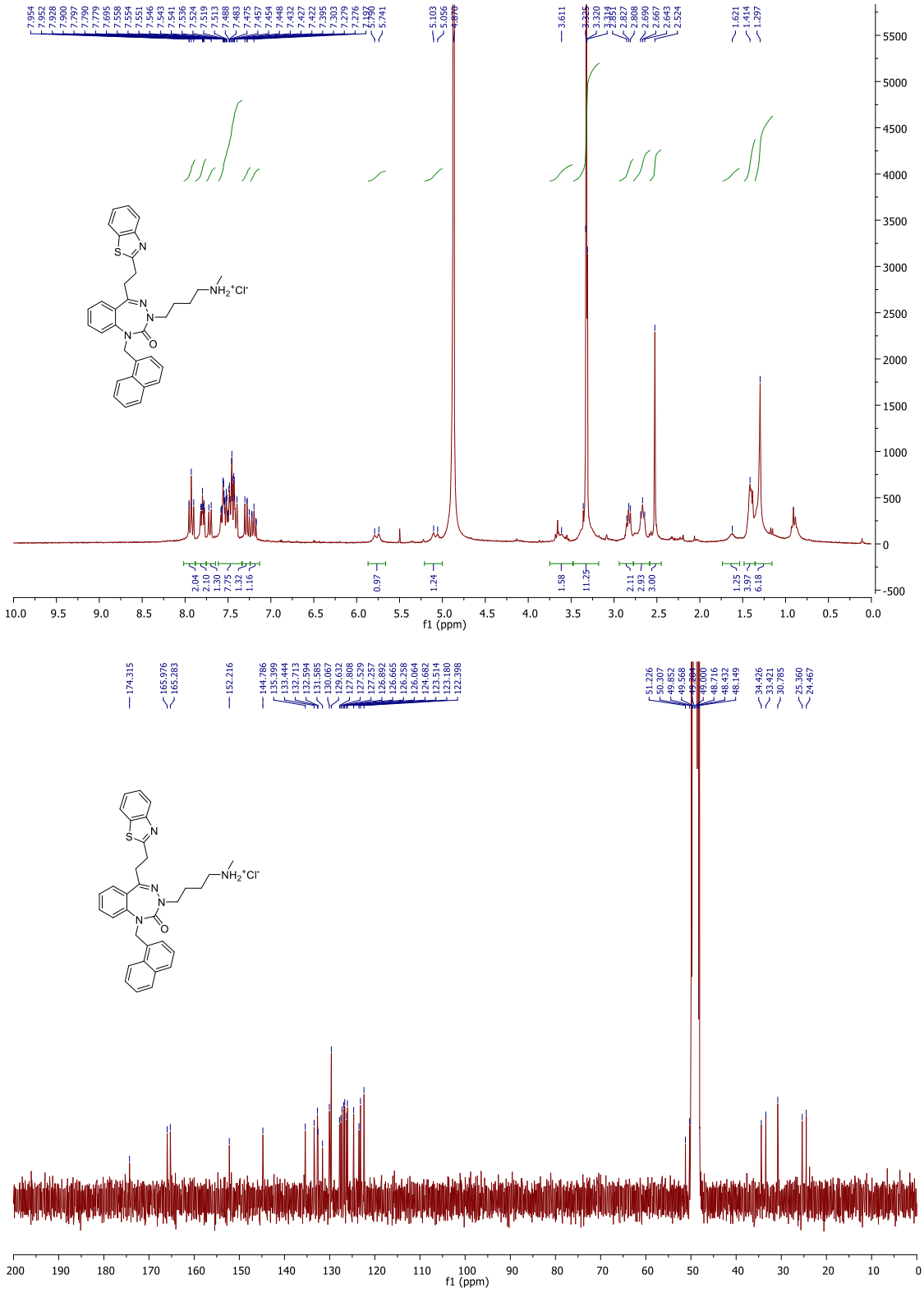


3.49h, CD_3OD-d_4, ^1H (400 MHz), ^{13}C (75 MHz)





3.49i, CD_3OD-d_4, ^1H (300 MHz), ^{13}C (75 MHz)



Supporting information Article 3

Douchez, A; Lubel, W. D. Synthesis of 1,3,4-benzotriazepin-2-one tetrapeptide mimics for the modulation and ligation of the urotensin II receptor.. *Manuscript in preparation.*

Supporting Information

Synthesis of 1,3,4-benzotriazepin-2-one tetrapeptide mimics for the modulation and ligation of the urotensin II receptor.

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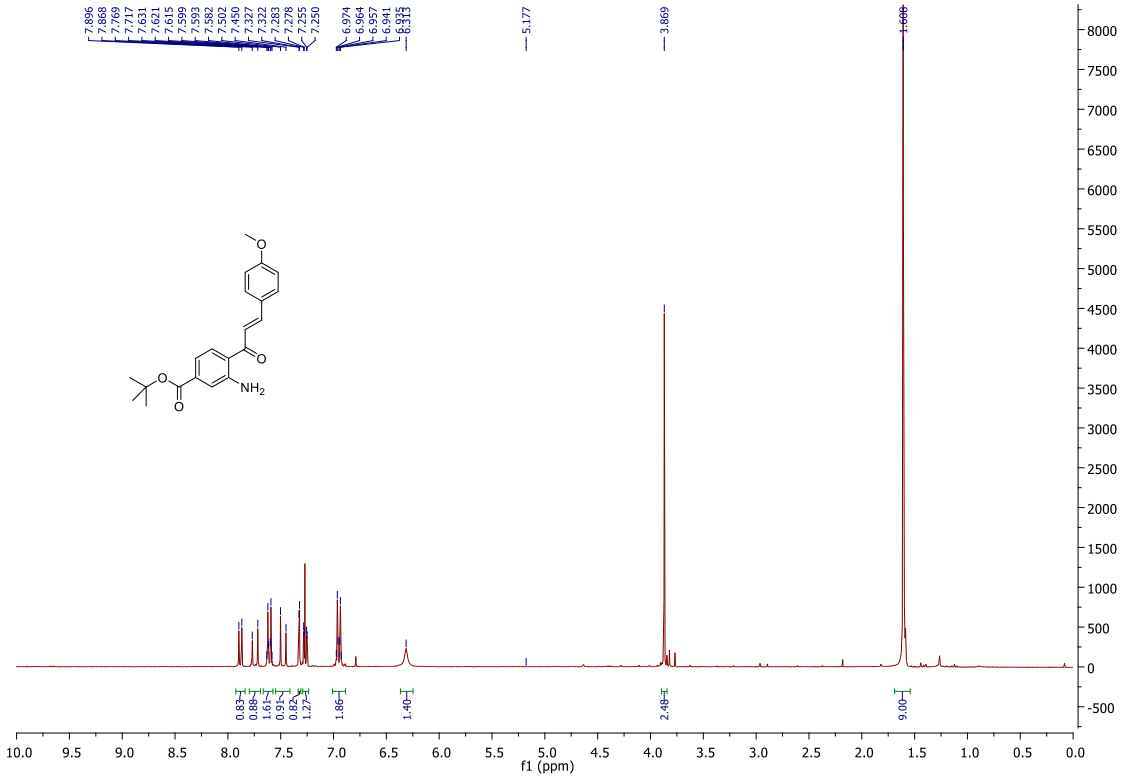
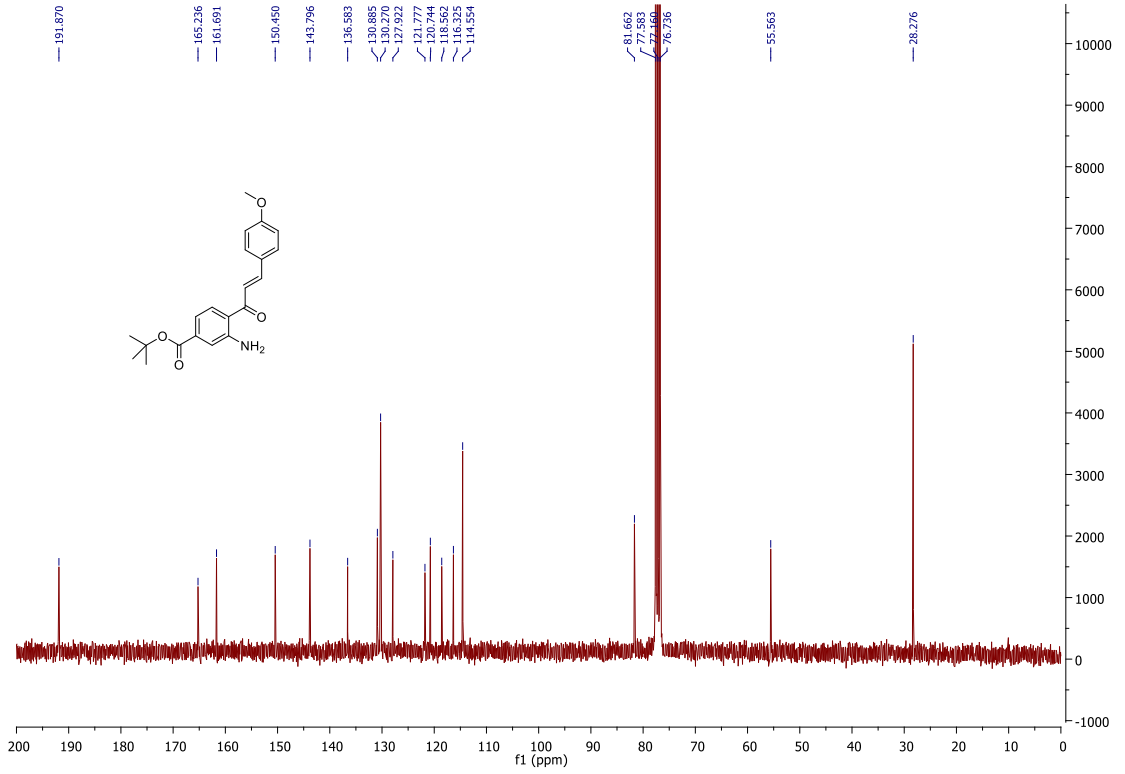
*E-mail : lubell@chimie.umontreal.ca

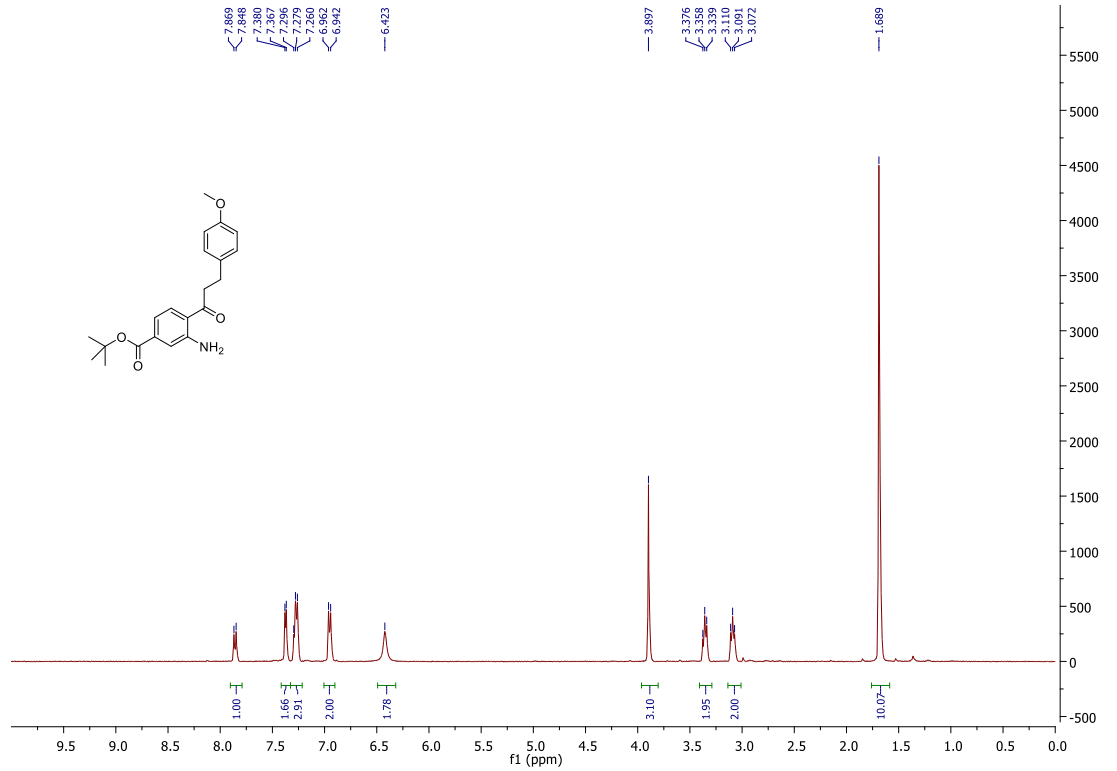
NMR Spectra

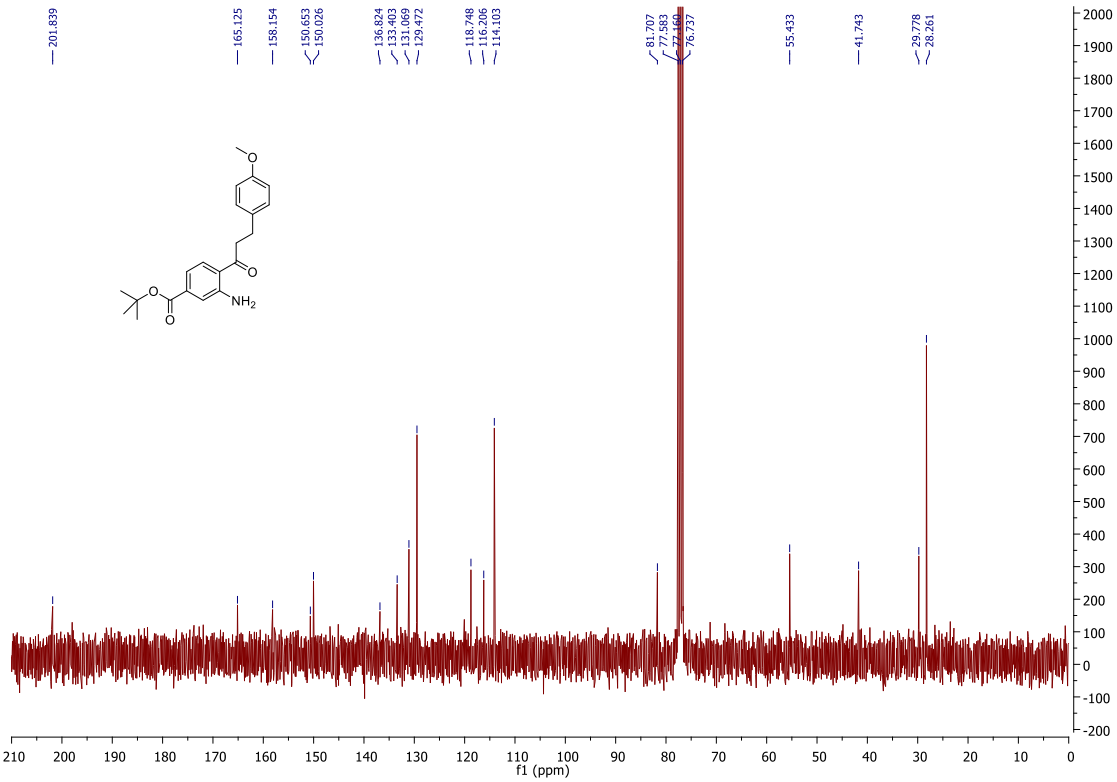
4.12, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



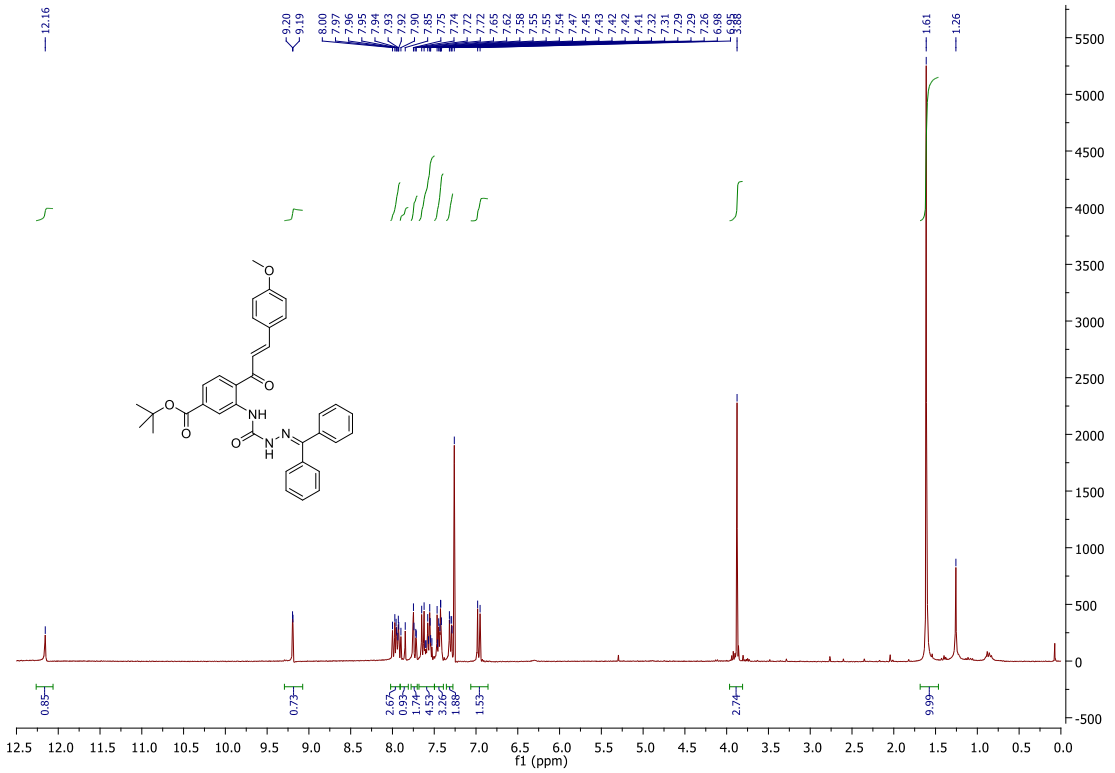


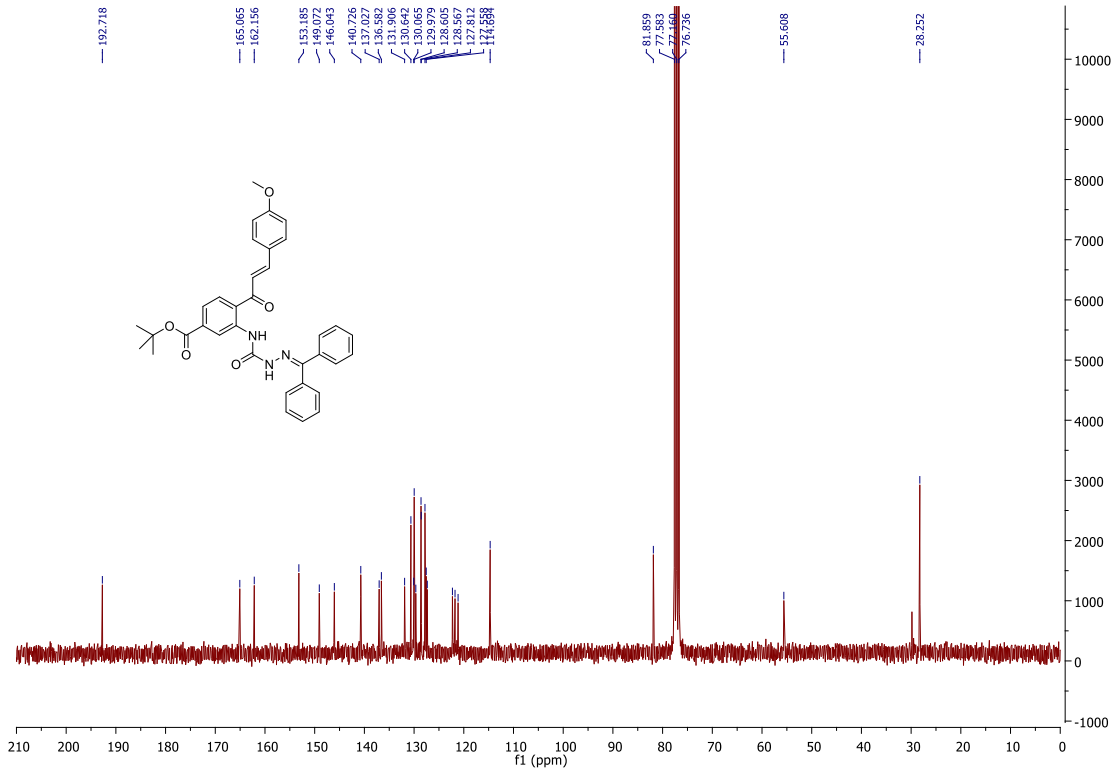
4.13, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



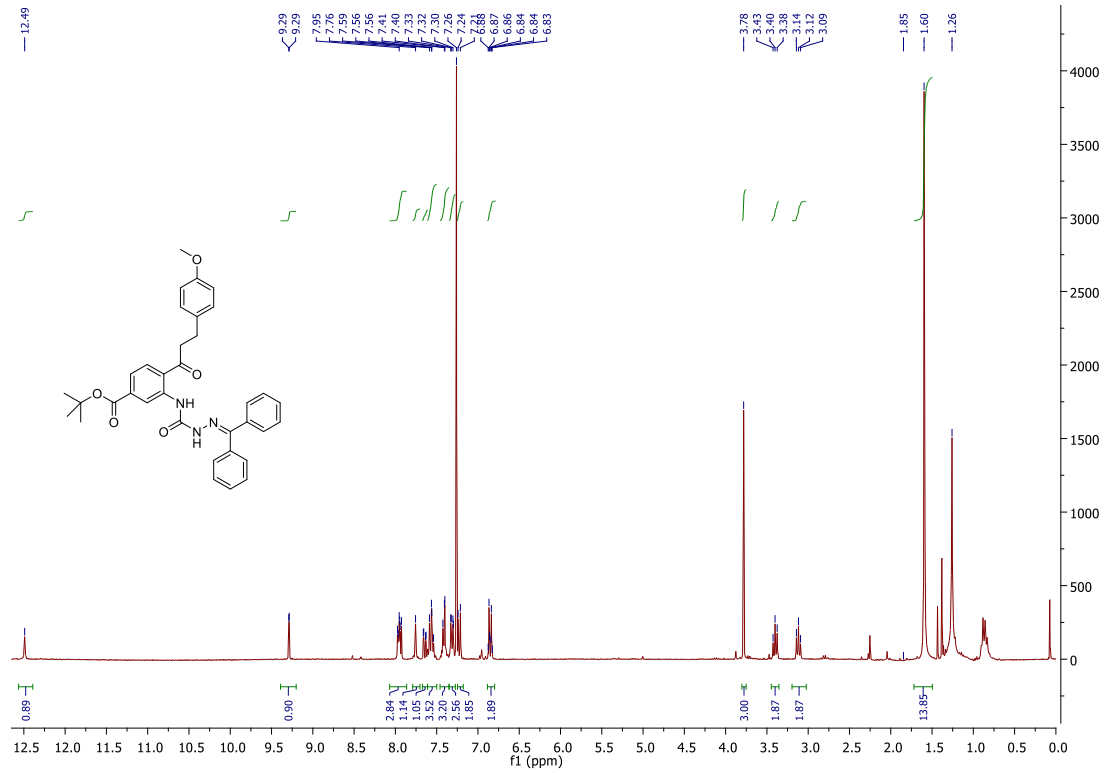


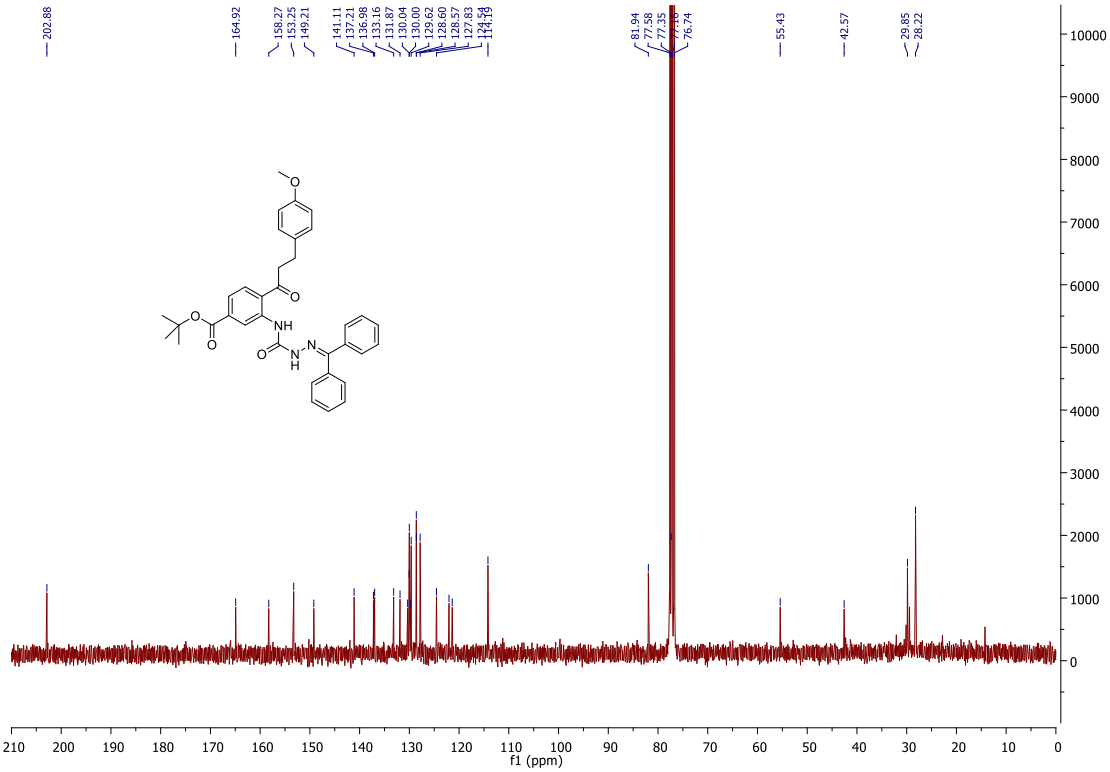
4.14a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



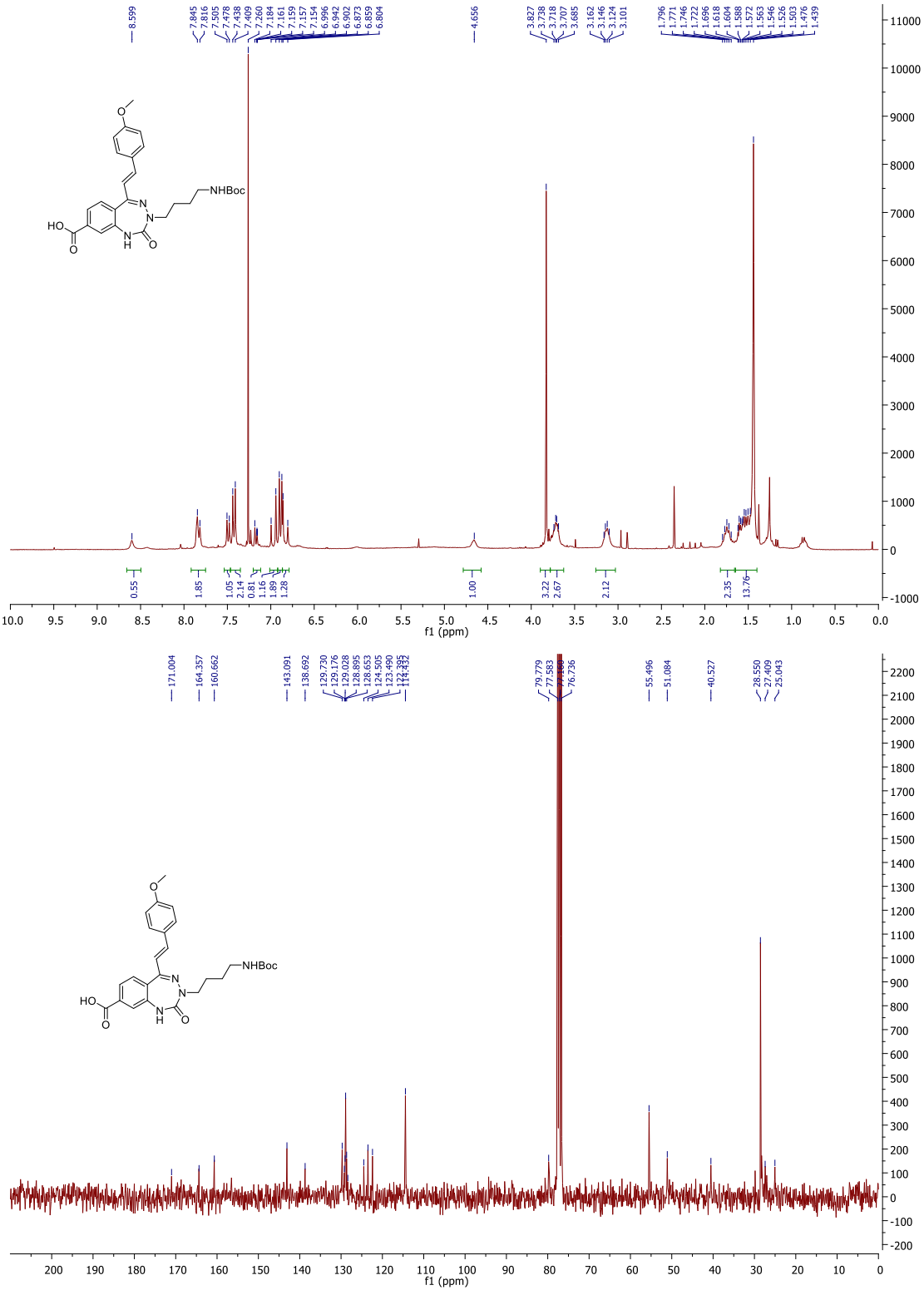


4.14b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

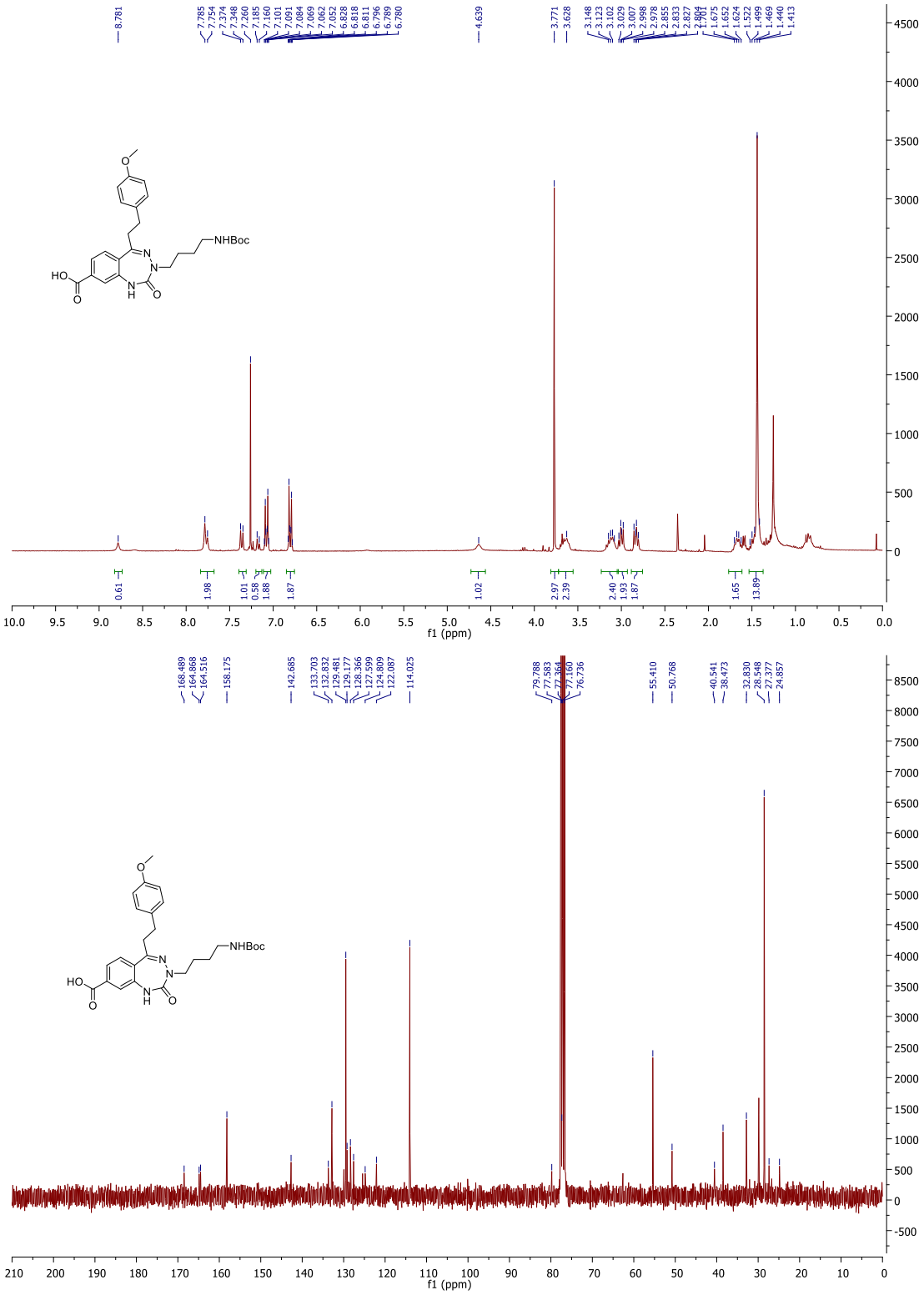




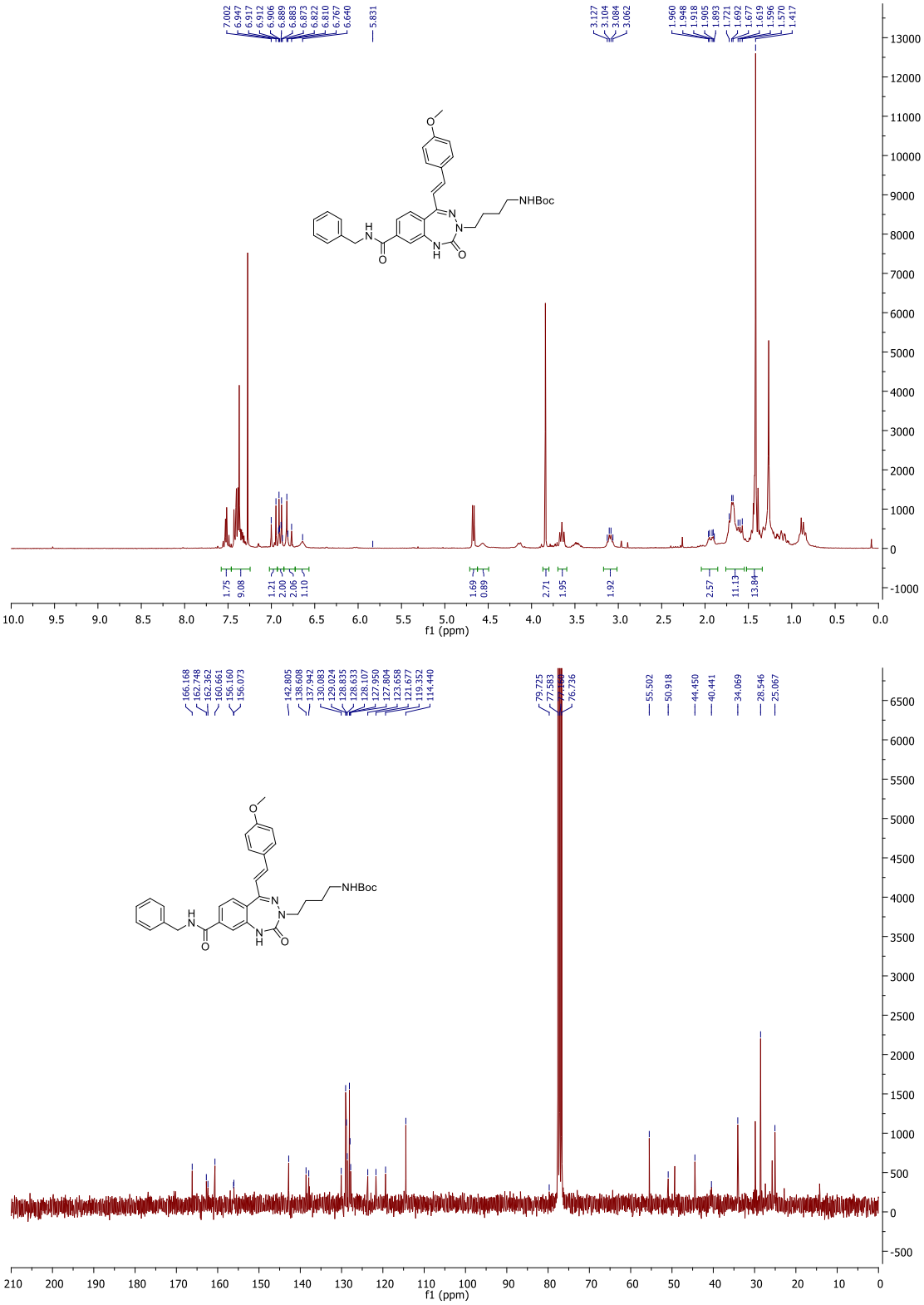
4.15a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



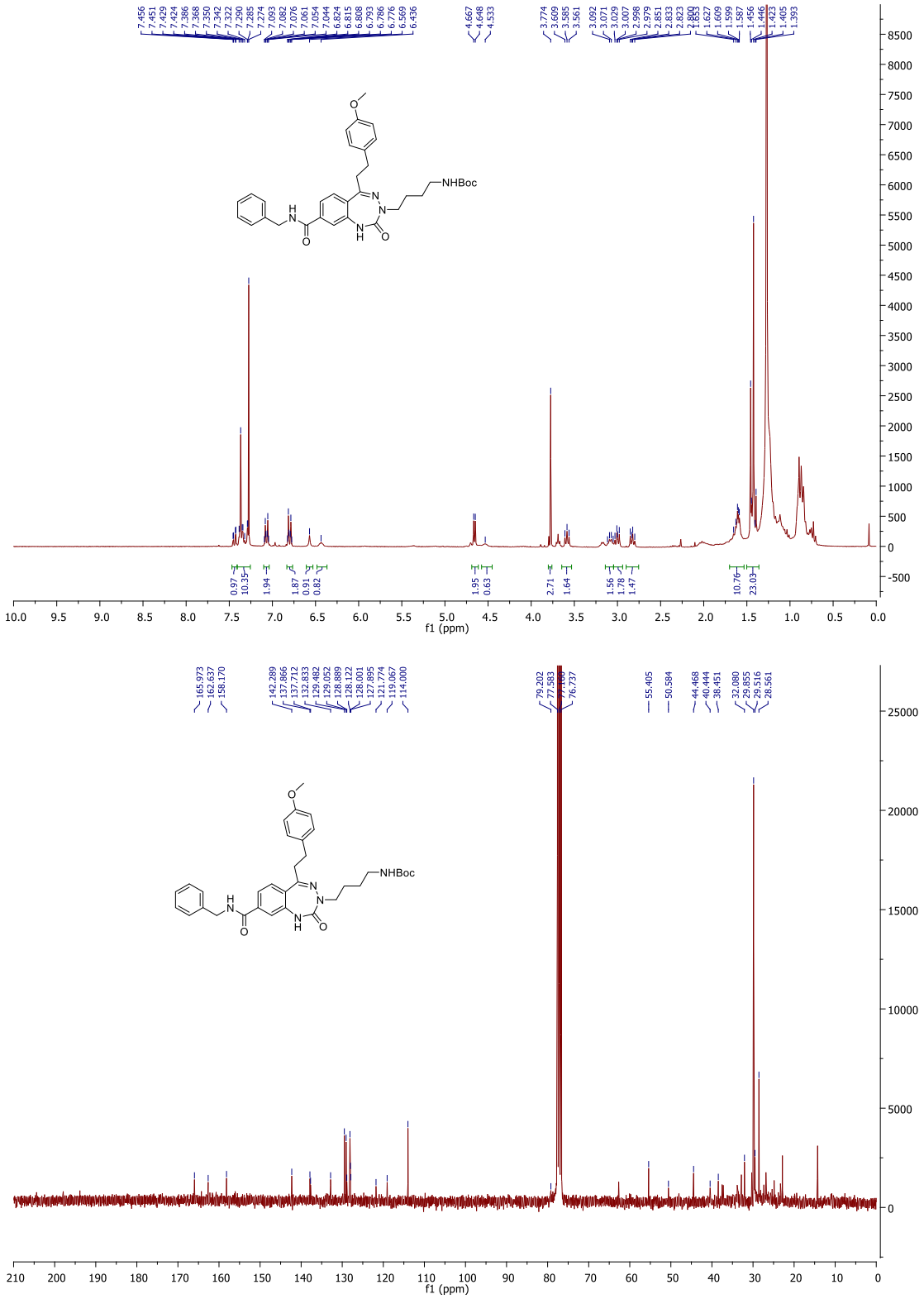
4.15b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



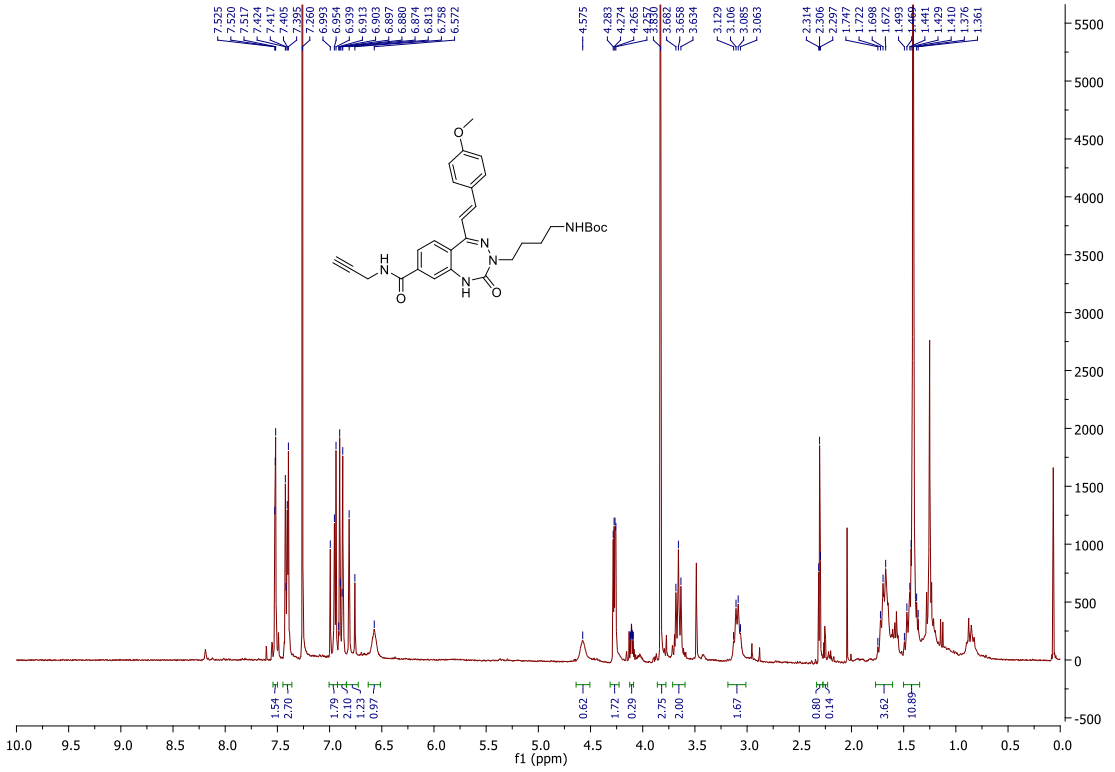
4.16a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

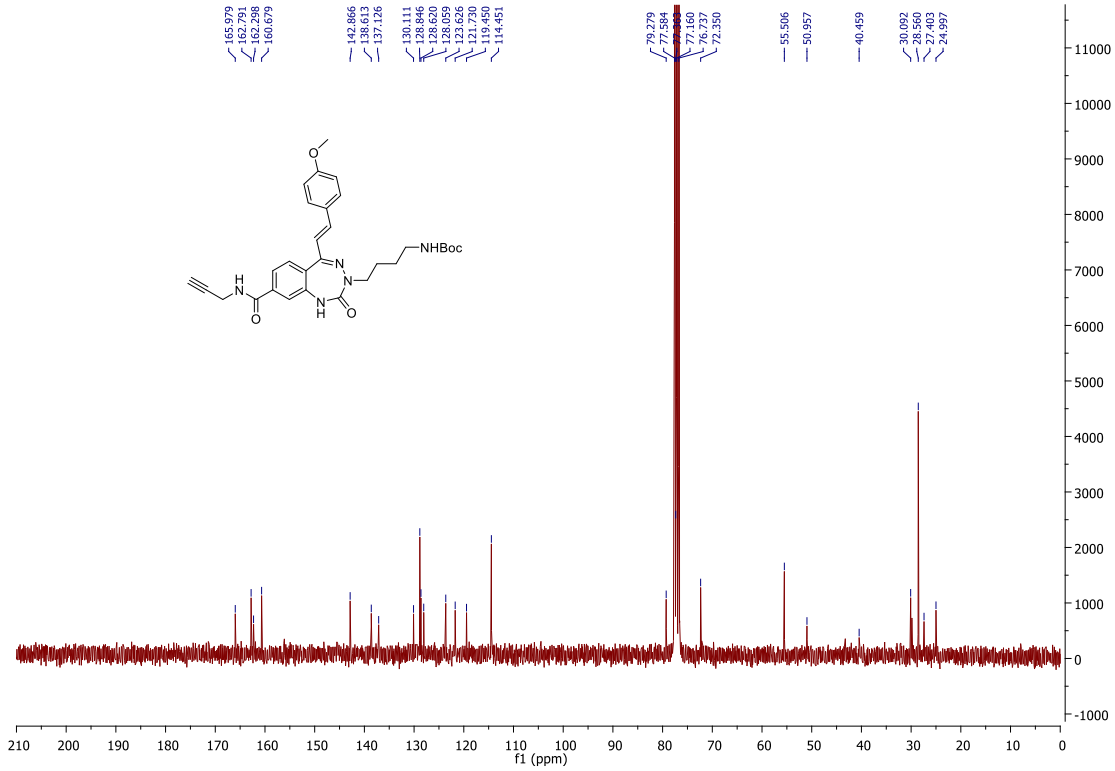


4.16b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

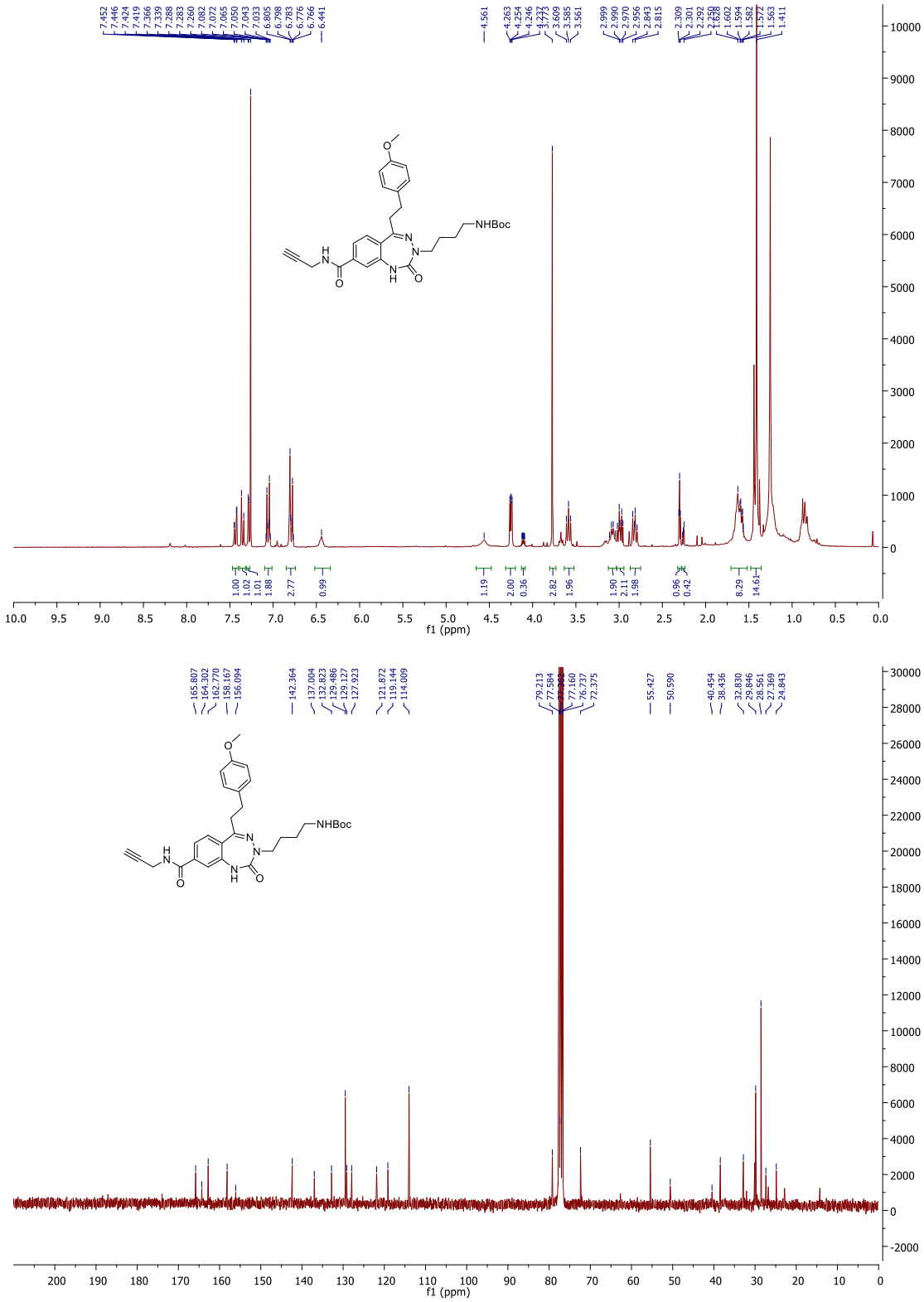


4.17a, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)

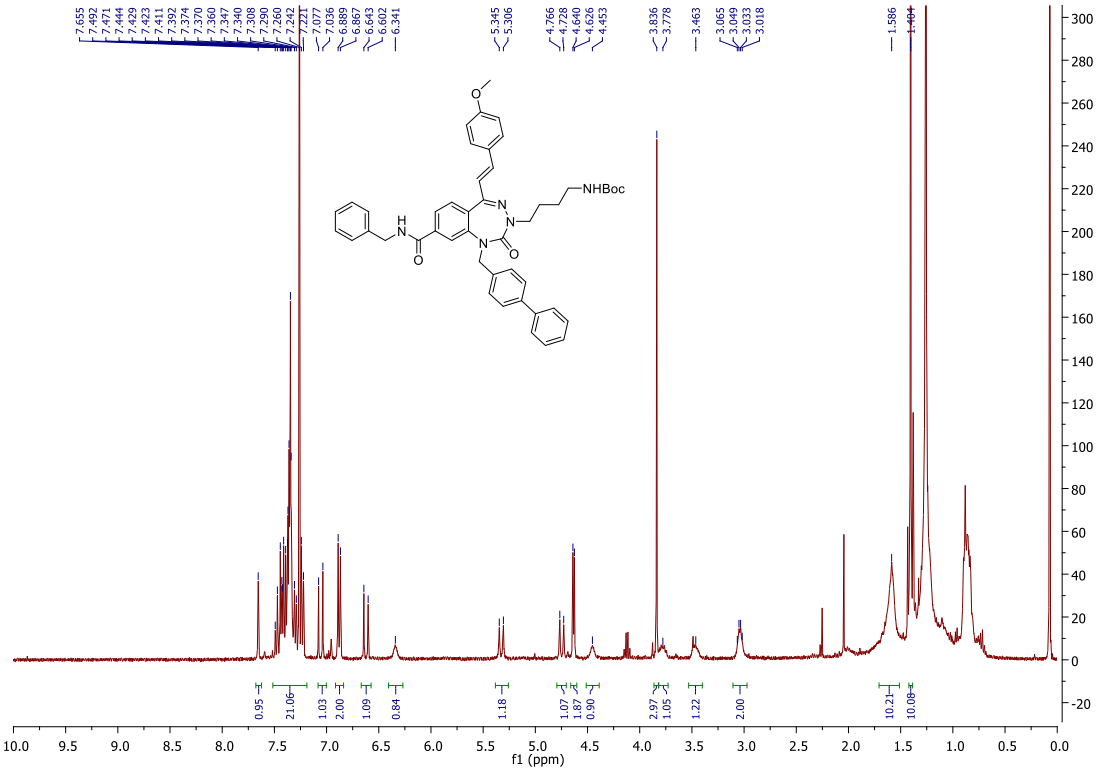




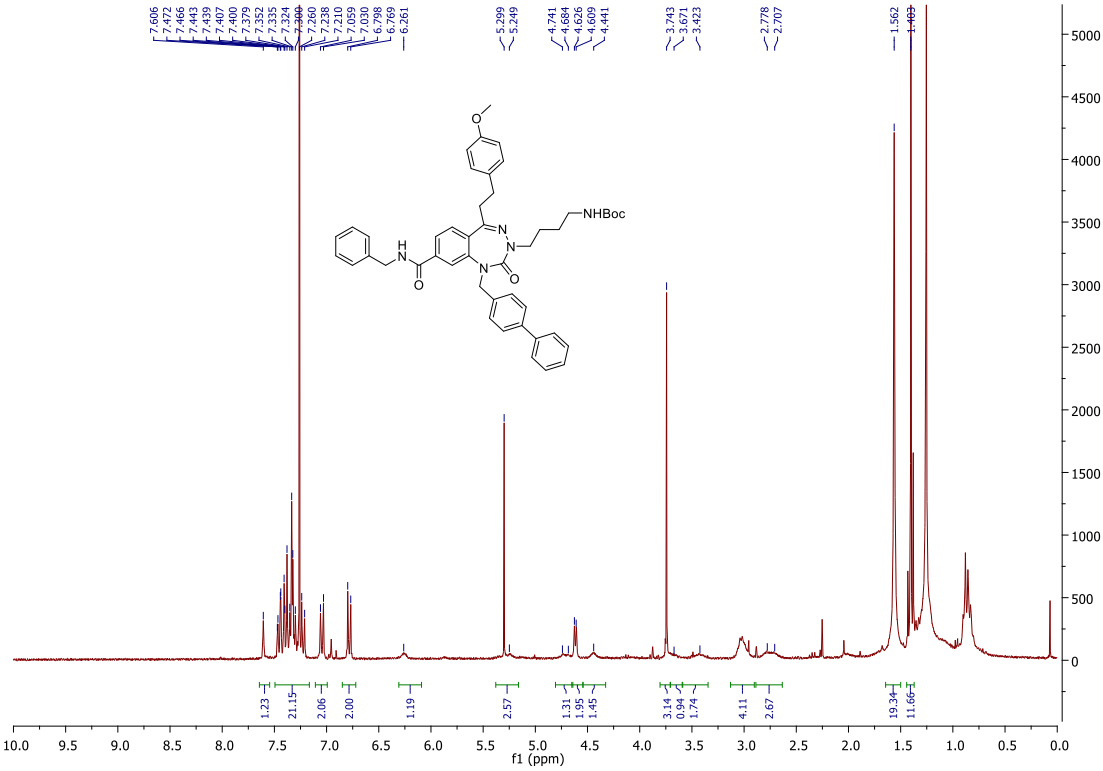
4.17b, $CDCl_3$, ^1H (300 MHz), ^{13}C (75 MHz)



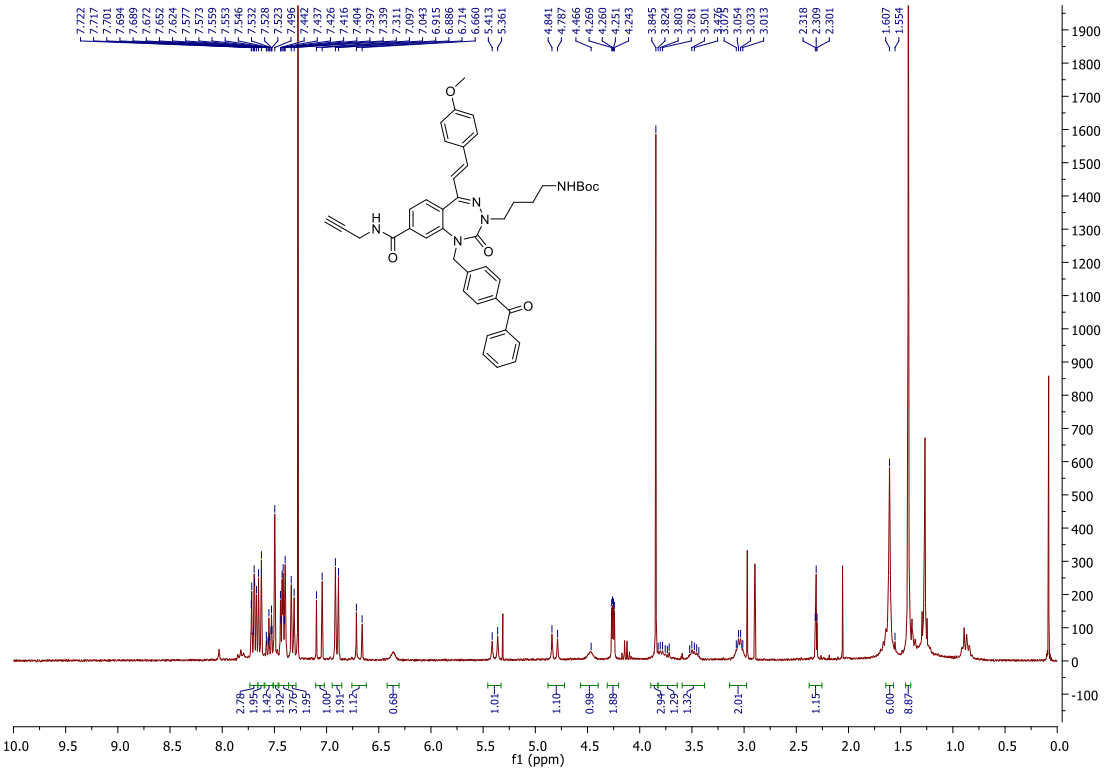
4.19a, $CDCl_3$, ^1H (400 MHz)



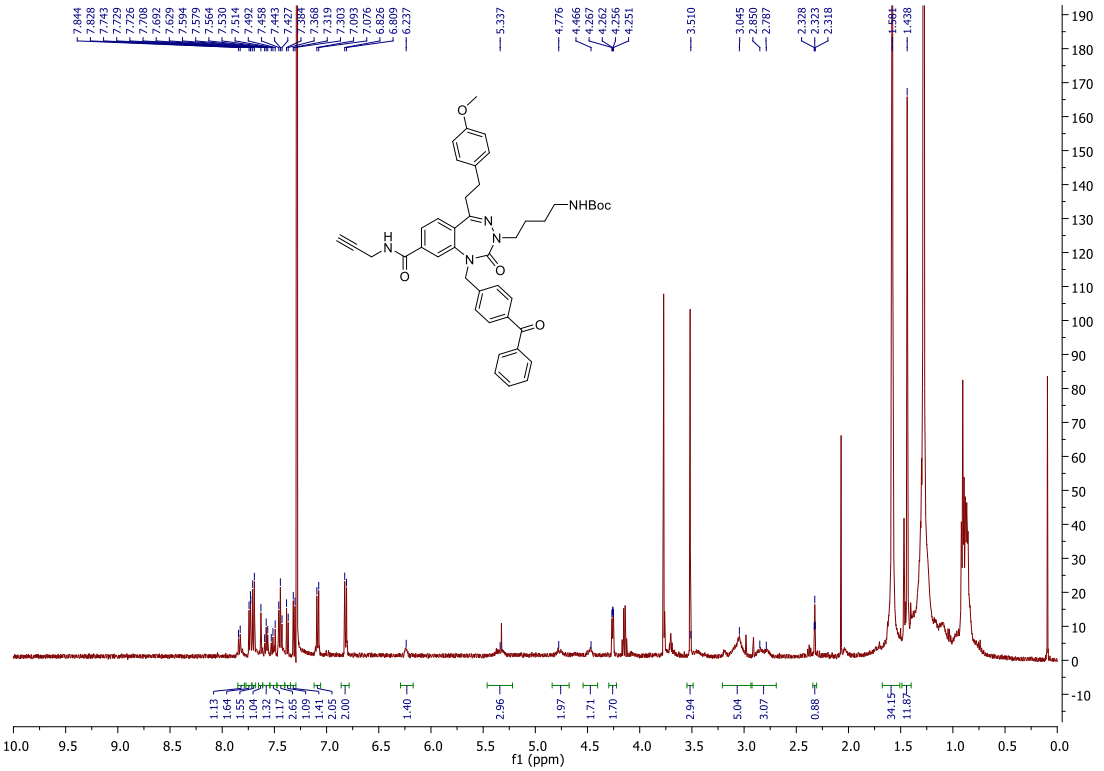
4.19b, $CDCl_3$, ^1H (300 MHz)



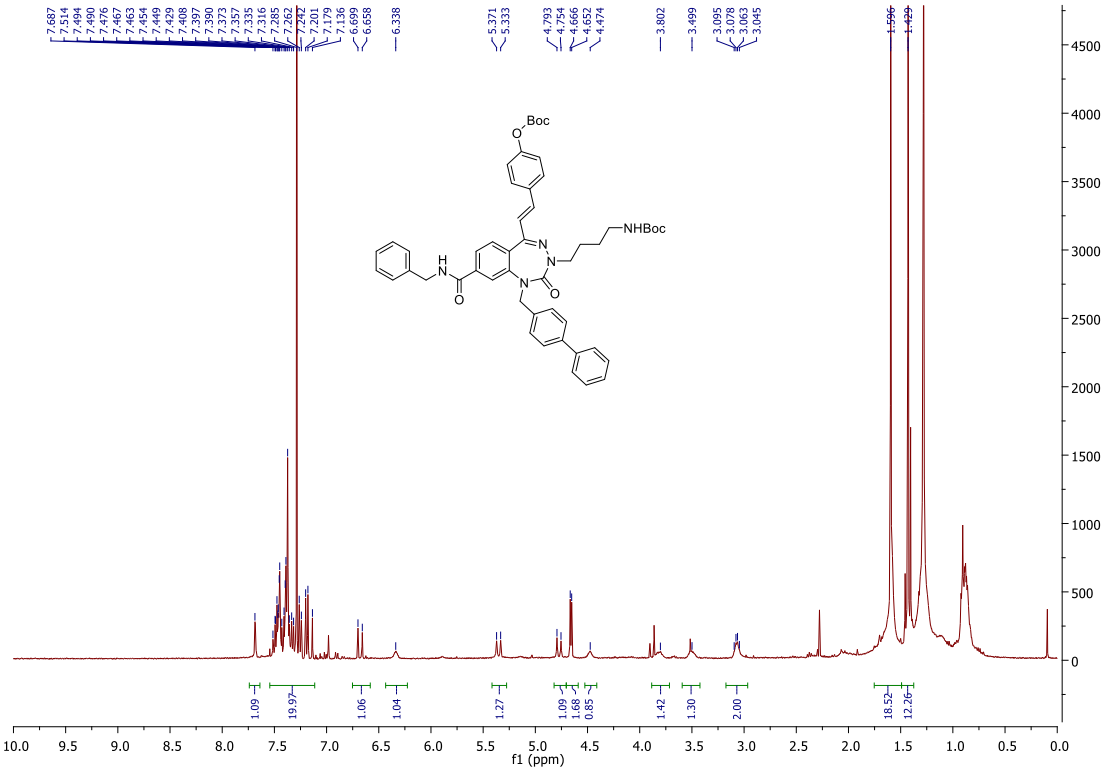
4.20a, $CDCl_3$, ^1H (300 MHz)



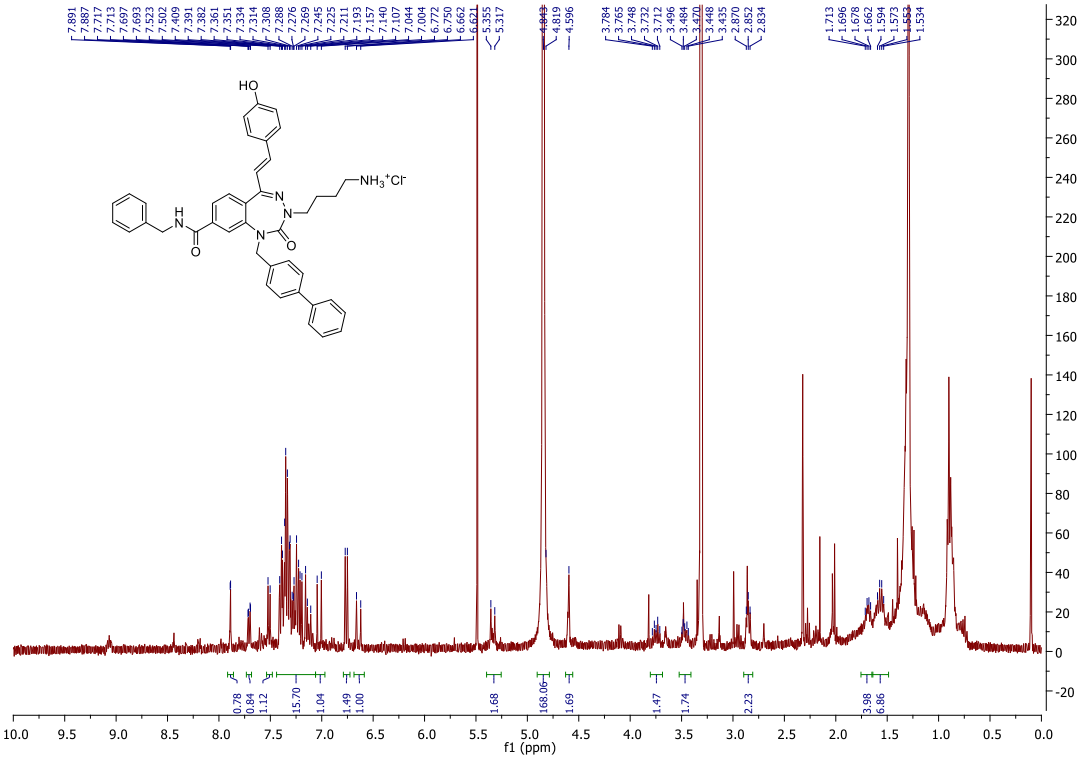
4.20b, $CDCl_3$, ^1H (500 MHz)



4.21, $CDCl_3$, ^1H (400 MHz)



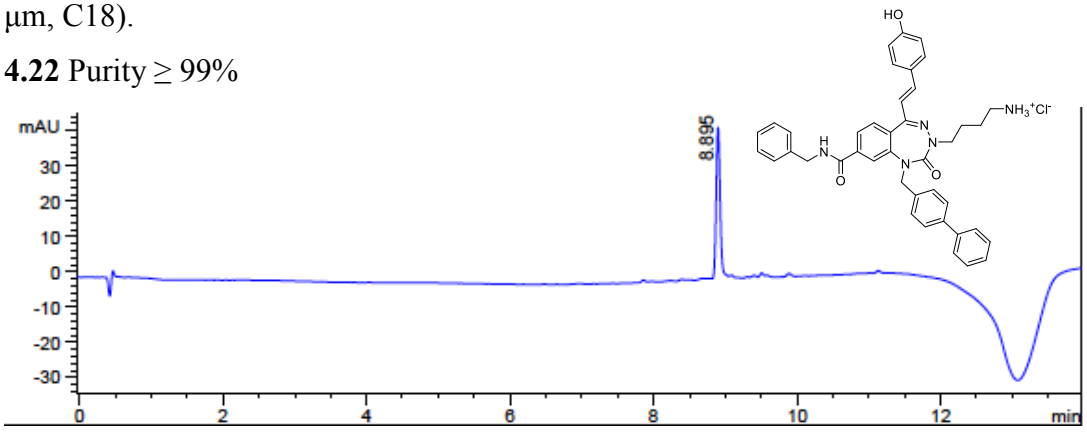
4.22, $CDCl_3$, ^1H (400 MHz)



Purity of 4.22 by HPLC

Analytical HPLC, 10 to 90% methanol (0.1% FA) in water (0.1% FA) over 9 min followed by 90% methanol (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 µm, C18).

4.22 Purity ≥ 99%



Analytical HPLC, 10 to 90% acetonitrile (0.1% FA) in water (0.1% FA) over 9 min followed by 90% acetonitrile (0.1% FA) in water (0.1% FA) over 1 min, flow rate of 0.5 mL/min on SunFire reverse-phase column from Waters (2.1 mm × 50 mm, 3.5 µm, C18).

4.22 Purity ≥ 90%