Université de Montréal

1,4-Diazepin-2-one Synthesis

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Résumé

Une stratégie efficace a été développée pour la synthèse de tours-γ inverses, particulièrement pour la synthèse de 1,4-diazépin-2-ones et de 1,4-pyrrolodiazépin-2-ones. Ces tours-γ ont été formés par couplage d'acides *N*-Boc-α-aminés et d'esters homo-β-aminés comme précurseurs chiraux facilement accessibles, comme décrit au Chapitre 2. Les cétones γ,δ-insaturées, intermédiaires clés, ont été préparées par l'addition en cascade de bromure de vinylmagnésium sur les *N*-Boc-α-aminoacyl β-aminoesters correspondants. Par la suite, les 1,4-diazépin-2-ones ont été obtenues par clivage des groupes protecteurs Boc, neutralisation des fonctions amines résultantes et amination réductrice intramoléculaire. Ces intermédiaires clés cétones γ,δ-insaturés ont été aussi employées dans des réactions d'oxydation d'alcènes, comme le procédé Tsuji-Wacker ou des réactions d'ozonolyse pour produire respectivement les 1,4-diones et 4-cétoaldéhydes correspondantes. Ces composés ont conduit aux 1,4-pyrrolodiazépin-2-ones correspondantes après clivage du Boc, neutralisation de l'amine et cyclisation de type Paal-Knorr. L'accès aux 1,4-diazépin-2-ones possédant un *N*-substitutant supplémentaire en position 1 est décrit dans le Chapitre 3. Les dérivées *N*-alkyl β-propionate de méthyle ont été synthétisées par amination réductrice de quatre différents aldéhydes avec le β-alaninate de méthyle. Les réactions de couplage menées entre ces dérivés alkylés et la phénylalanine ainsi que la lysine, toutes deux protégées sur leurs fonctions amines par des groupements Boc ont conduit à un certain nombre de dipeptides. Les composés cétoniques ont ensuite été obtenus comme précédemment par addition en cascade de bromure de vinylmagnésium sur ces *N*-alkyl α-aminoacyl β-aminoesters. Les 1,4-diazépin-2-ones 1,3,5-trisubstitutées possédant un *N*-substitutant en postion 1 ont alors été préparés de la même manière selon une séquence impliquant le clivage du groupement Boc, la neutralisation de l'amine et amination réductrice cyclisante. La présence de *N*-substituants provoque une accélération sensible de l'étape d'amination réductrice pour fournir les diazépin-2-ones 1,3,5-trisubstituées. Certains résultats préliminaires ont démontré que des 1,4-diazépin-2-one

disubstituées peuvent être obtenues à partir des dérivés trisubstitutés comportant un substituant acide labile avec de bons rendements.

Enfin, l'adaptation de cette méthodologie en phase solide a été menée en étudiant deux stratégies impliquant l'ancrage des substrats par une fonction amide, comme décrit dans le Chapitre 4. Les tentatives employant un linker de type mercaptoéthyle sur une résine polystyrène ont été infructueuses. Alternativement, une 1,4-diazépin-2one a pu être synthétisée en employant une résine aldéhyde de Wang suivant six étapes similaires à celles décrites au Chapitre 3. Le clivage final est effectué par traitement acide, tel que par le TFA, pour fournir un produit brut de haute pureté. Les conformations des tours occasionnées par ces 1,4-diazépin-2-ones ont été déterminées par diffraction de rayons X.

En résumé, ces travaux ont permis d'aboutir à de nouvelles méthodologies en solution et sur support solide permettant l'accès à des dérivés de type diazépin-2-one. Ces méthodologies peuvent de plus potentiellement conduire à des composés de plus grande diversité moléculaire en vue d'éventuelles applications thérapeutiques.

Mots clés : Tours-γ, peptidomimétiques, cétone, résine amide, double addition de réactifs de Grignard vinyliques, antibiotiques.

Abstract

An efficient strategy has been developed for the synthesis of inverse γ-turn mimics, particularly 1,4-diazepin-2-one and 1,4-pyrrolodiazepin-2-one heterocycles. The turn mimics were assembled from the coupling of *N*-Boc-α-amino acids and homo-β-amino esters as inexpensive chiral building blocks, as described in Chapter 2. The key intermediate γ,δ-unsatured ketone was prepared by the cascade addition of vinyl Grignard reagent to the corresponding *N*-Boc-α-aminoacyl β-aminoester. Subsequently, Boc group deprotection, amine neutralization and intramolecular reductive amination yielded the 1,4-diazepin-2-one skeleton. The key intermediate γ,δ-unsatured ketone could also be employed in alkene oxidations by the Tsuji-Wacker process and by ozonolysis to respectively yield 1,4-diones and 4-ketoaldehydes, which upon Boc group removal, amine neutralisation and Paal-Knorr cyclisation yielded 1,4-pyrrolodiazepin-2-ones.

Access to the 1,4-diazepin-2-one possessing an additional *N*-substituent at the 1-position was achieved as described in Chapter 3. Methyl *N*-alkyl β-aminopropionate derivatives were synthesized by reductive amination of the imine generated from methyl β-alaninate and four different aldehydes. Dipeptide formation followed by addition of vinyl Grignard reagent to the *N*-alkylated α-aminoacyl β-aminoester yielded γ-δ-unsatured ketone. Trisubstituted-1,4-diazepin-2-ones possessing a 1-postion *N*-substituent were then prepared by an analogous Boc deprotection / amine neutralisation / reductive amination sequence. The additional *N*-alkyl substituent caused a noticeable acceleration in the reductive amination step to provide the 1,3,5-trisubstituted diazepin-2-ones. Preliminary results demonstrated that disubstituted 1,4-diazepin-2-one can be made from the trisubstituted analog bearing acid labile *N*-substituent in good yield.

Finally, the adaptation of our solution-phase methodology to solid phase was pursued studying two different backbone amide linker strategies, as discussed in Chapter 4. Attempts were unsuccessful in employing a mercaptoethyl linker on polystyrene resin. Alternatively, 1,4-diazepin-2-one was synthesized on Wang aldehyde resin by a 6-step process similar to that described in Chapter 3 featuring cleavage from the resin by treatment

with acid, such as TFA, to provide the crude product of high purity. The turn conformations of 1,4-diazepin-2-ones were determined by x-ray structures analysis.

In summary, this thesis has provided new methodologies for making diazepin-2-one derivatives using solution and solid-phase chemistry and offers potential for synthesizing such γ-turn mimics for future studies in peptide science and medicinal chemistry.

Key Words: γ-turn, peptidomimetics, γ-δ-unsaturated ketone, 1,4-diazepin-2-one, backbone amide linker, cascade addition of vinyl Grignard reagent, antibiotics.

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Abbreviations

$[\alpha]_D$	optical rotation
Ac	acetyl
anhyd	anhydrous
A	alanine
aq	aqueous
Arg	arginine
Ar	aryl
Bn or Bzl	benzyl
Boc	*tert*-butoxycarbonyl
Bop	(1-benzotriazolyl)oxy tris(dimethylamino) phosphonium [PF_6]
Bu	butyl
t-butyl	*t*-butyl
bp	boiling point
br	broad (spectral)
Bz	benzoyl
Cbz	benzyloxycarbonyl
CNS	central nervous system
m-CPBA	*meta*-chloroperbenzoic acid
Cys	cysteine
d	doublet
dd	doublet of doublet
D	aspartic acid

DBU	1,8-diazabicyclo[5.4.0]undec-4-ene
DEAD	diethyl azodicarboxylate
DCC	dicyclohexyl carbodiimide
DIC or DIPC	1,3-diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMF	*N,N*-dimethylformamide
DMPU	*N,N*-dimethylpyrrolidinone
DPPA	diphenyl phosphoryl azide
EADC	ethyl aluminium dichloride
ee	enantiomeric excess
Fmoc	9-fluorenylmethyloxycarbonyl
G	guanine
Glu	glutamic acid
h	hours
HATU	O-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxy 1H-benzotriazole
HRMS	high-resolution mass spectrometry
IBCF	isobutyl chloroformate
IR	infrared
J	coupling constant
LDA	lithium diisopropylamide
LHMDS	lithium hexamethyldisilazane

LHMDS	lithium hexamethyldisilazane
lit.	literature
μ	micro
m	multiplet (spectral)
M	molar
Me	methyl
mg	milligram
min	minutes
MHz	megahertz
mL	millilitre
mmol	millimole
mp	melting point
MS	mass spectrometry
MW	molecular weight
N	asparagine
NMR	nuclear magnetic resonance
NMM	*N*-methylmorpholine
PCC	pyridinium chlorochromate
Ph	phenyl
ppm	part per milliom
R_f	retention factor (in chromatography)
s	singlet
t	triplet
TFA	trifluoroacetic acid

TfOH	triflic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Trt or Tr	trityl
Ts	tosyl

CHAPTER 1

INTRODUCTION

1.1 Peptides as Drugs

Endogenous peptides and proteins such as the enkephalins, angiotensin II, bradykinin, cholecystokinin and insulin were shown to control many of the chemical and physiological processes in the human body. Most exert their biological function at specific receptors, in particular seven transmembrane G-protein coupled receptors (GPCRs).[1,2]

Discovery of biologically active peptides and proteins with relevance in disease processes has led to the discovery of novel therapeutic targets, as well as probes for the development of new drugs.[3] A significant increase over the last decade in the use of peptides as agonists and antagonists for the development of drugs has been observed. However, in most cases the use of native peptides as therapeutic agents has been limited due to poor pharmacokinetics, such as difficulty in crossing the blood-brain barrier, low uptake, poor oral administration, rapid metabolism and facile excretion. Furthermore, the flexibility of the peptide may also reduce receptor specifity.[4,5,6,7] Significant efforts have been committed to the design and synthesis of non-peptidic molecules which display pharmacological activity like the native peptides at their receptors to avoid the above problems. These non-peptidic compounds are referred to peptidomimetics.

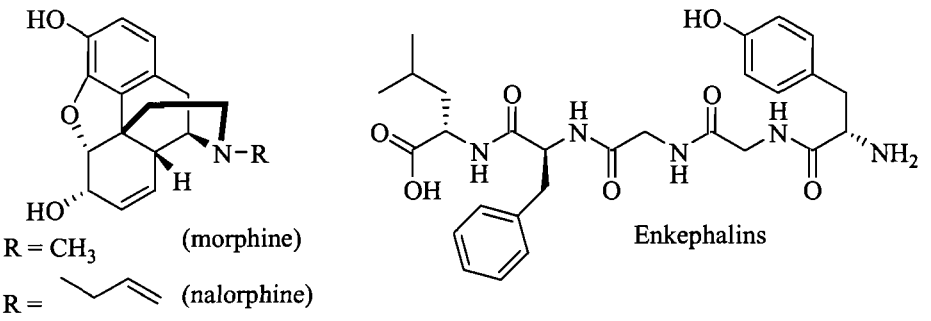


Figure 1.1 Morphine (opiate agonist) **1** and nalorphine (opiate antagonist) **1** compared with enkephalins **2.**

An example of peptide mimicry is that of morphine, which acts as a non-peptide agonist producing a similar agonist effects and binds to the same receptor family compared to the corresponding endogenous peptide enkephalins (Fig 1.1). The possible structural relationship between the morphine skeleton and enkephalins remains unknown.[8] Morphine and related opioids remained the only examples of non-peptide agonists at peptide receptors for an extended period of time. It is only in the past few years that other examples of non-peptide agonists or antagonists at peptide receptors have been reported (Fig. 1.2).[9]

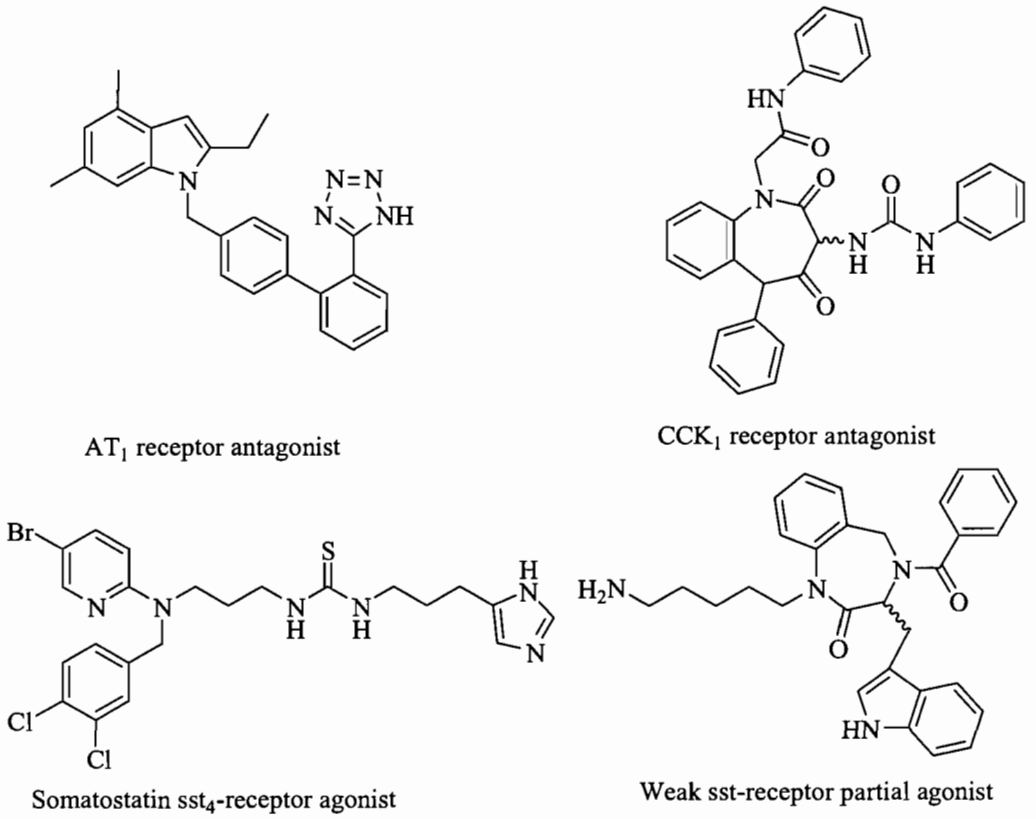


Figure 1.2 Examples of non-peptide agonists and antagonists.[10]

1.2. Peptidomimetics

Peptidomimetics are valuable research tools for the study of molecular recognition by structure-activity relationship (SAR) investigations.[8,11] Constrained structures that mimic the bioactive conformation of a peptide may be more resistant to enzymatic cleavage and exhibit enhanced selectivity compared to the linear flexible peptide.[8,12]

Peptidomimetics can be classified into three types:

Type I: Mimetics which match the peptide backbone. These are peptides which are modified by an amide bond isosteres.

Type II: Small nonpeptidic molecules that bind to a peptide receptor or enzyme.

Type III: Nonpeptidic molecules unrelated to the original peptides which may be considered as ideal mimetics containing the functional groups necessary to serve as topographical mimetics.[13] Several approaches have been described for the development of peptidomimetics, some of them are listed below.

1.2.1. Side-Chain Modifications

The incorporation of amino acids with side chains that do not occur naturally in peptides and proteins may introduce special functional groups, restrict peptide flexibility and increase metabolic stability.[14] Such single residue modifications include enantiopure *N*-alkyl and C^{α}-dialkyl amino acids. For example [1-deamino, D-arg^{8}] vasopressin (DDAVP) is a modified peptide used as a selective antiduretic agent suitable for the treatment of diabetes insipidus.[6]

1.2.2. Backbone Modification

In addition to the backbone modifications caused by single residue substitutions, the replacement of peptide bonds by non-peptide analogs has also been utilised to modify the backbone of peptides. Such isosteric replacements include substitution of the amide by hydroxyethylene, vinyl ester and amine moieties, as well as the replacement of the α-

carbon by nitrogen in azapeptides or by a BH group in borapeptides. The most general modifications are listed in Table 1.1.

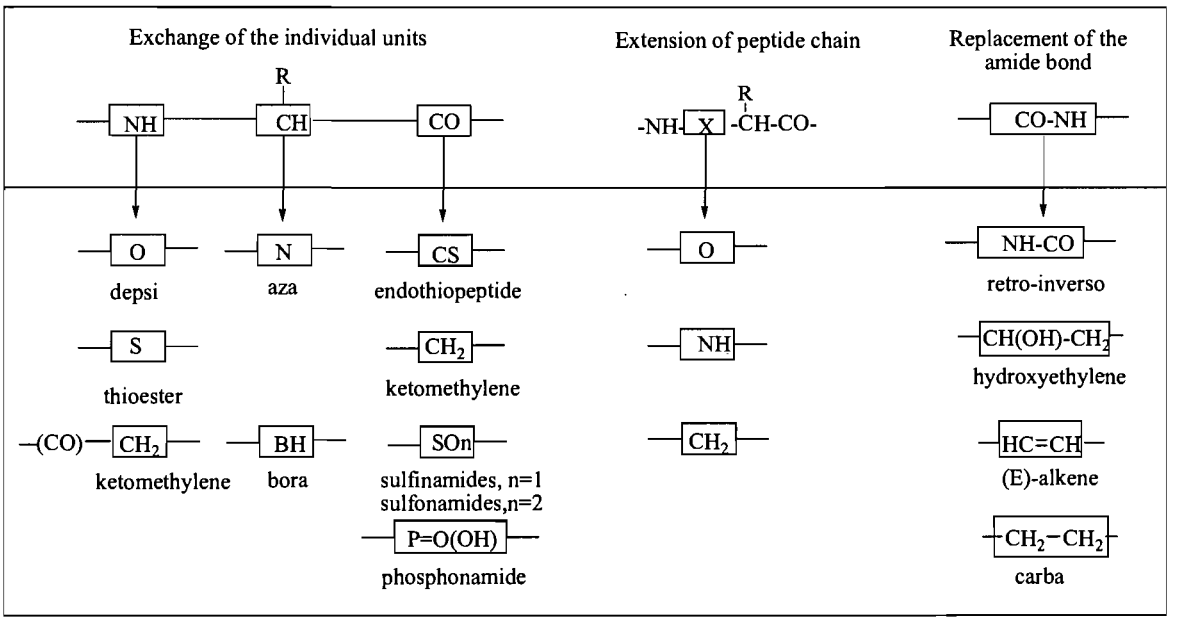


Table 1.1 The most frequent modifications of the peptide backbone.[11]

1.2.3. Restriction by Cyclization

Global restrictions in the conformation of a peptide may be achieved by backbone cyclization. Cyclic peptides may possess relatively rigid conformations, decreased molecular volume, increased metabolic stability, superior bioavailability and higher cell-permeability.[14]

1.2.4. Secondary Structure Mimetics

The secondary structure of a protein refers to the folding of local regions into energy-minimum conformations that are stabilized by non-covalent bonds such as hydrogen bonds and hydrophobic interactions. Hydrogen bonds are formed between the NH group (hydrogen bond donor) and the carbonyl oxygen atom (hydrogen bond acceptor) of peptide amide bonds. Common secondary structural motifs include the: α-helix, β-sheet, and turn conformations. Incorporation of such a moiety will provide additional information about the condition of receptor binding and activation. Secondary structures such as turns are often essential components for peptide and protein biology because they are often located on the protein surface and act as molecular recognition sites. [10,14,15]

1.3. γ-Turn

The γ-turn is a secondary structure that contains three amino acid residues connected by amide bonds. In the γ-turn a hydrogen bond may exist between the backbone carbonyl of the first residue (i) and the amide NH of the third residue (i+2), to form a seven-membered ring (Fig 1.3). The γ-turn has been classified into two families, contingent on the dihedral angles (ϕ andψ), of the central amino acid residue (Fig 1.3).[16-21]

	Classic	Inverse
ϕ_{i+1}	70° to 85°	-70° to -85°
ψ_{i+1}	-60° to -85°	70° to 85°

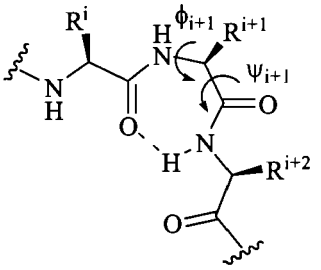


Figure 1.3 Classic and inverse γ-turns with their corresponding angles.

The inverse γ-turn is found more frequently than the classic γ-turn. The inverse γ-turn does not give rise to chain reversal in proteins, inversely to the classic turns. γ-Turns are rare in proteins, however, they are often found in small cyclic peptides (Fig 1.4).[16,18]

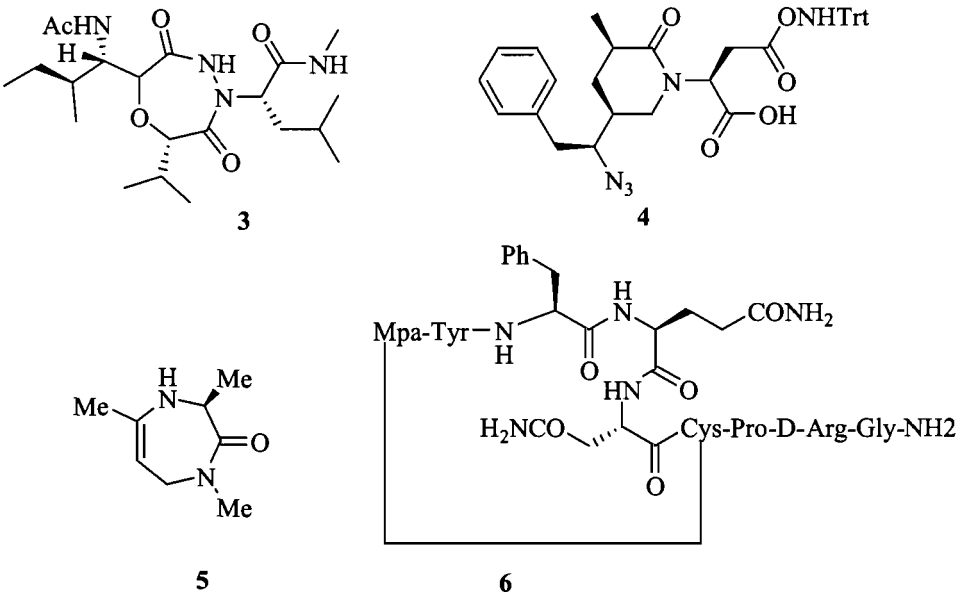


Figure 1.4 Examples of heterocyclic and peptide γ-turn mimetics (**3**[23], **4**[6], **5**[24], **and 6**[6]).

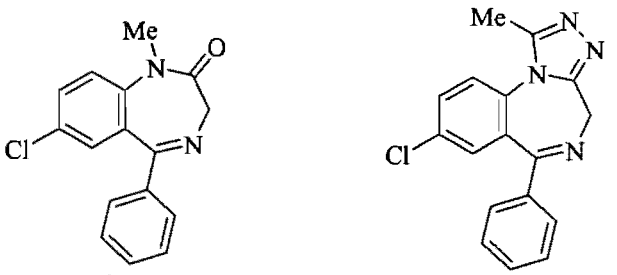
γ-Turns have been shown to play important roles in the control of local peptide conformations and also in biological recognition.[22] For example, the γ-turn present in the RGD (Arg-Gly-Asp) sequence of vitronectin was found to contribute to the specific recognition of integrin receptor $\alpha_v\beta_3$.[25] Several linear and cyclic peptides that exhibit biological activity such as bradykinin, cyclosporin, vasopressin and the cyclic somatostatin analogues have been shown to adopt γ-turns.[24,26]

Some of these molecular scaffolds have been incorporated into biologically active peptides to study the relationship between conformation and activity. For example, the incorporation of an inverse γ-turn in place of residues 3-5 in the peptide hormone oxytocin produced an analog that did not induce contractions of the uterine tissue nor did it act as an inhibitor of oxytocin-induced contractions.[27] However, the incorporation of 2-oxo piperidines as γ-turn mimetics into the bradykinin sequence produced an analog with an activity similar to bradykinin and so this supported the presence of a reverse γ-turn in the bioactive conformation of this peptide.[24]

1.4. 1,4-Diazepinones and Pyrrolodiazepinones

The benzodiazepines (BZDs), which include the diazepam better known as Valium® **7** and alprozalam **8** (Fig 1.5) are an important class of drugs.[28] Benzodiazepines exhibit different therapeutic applications such as antitumor antibiotic, muscle relaxant, HIV Tat antagonist, hypnotic and anxiolytic activities, due to their potential to mimic peptide secondary structures such as γ- and β- turns.[29] Benzodiazepines are known to exert their broad

pharmacological effects through specific binding to a subunit on the γ-aminobutyric acid which acts on the γ-aminobutyric acid-A chloride ion channel ($GABA_A$) receptor.[30] Furthermore, it has been suggested that benzodiazepines, such as diazepam, may be peptidomimetics of endogenous peptide endozepines which are single-chain polypeptides of 86 residues.[31] The seven-membered ring in a benzodiazepine can be formed as a cyclic dipeptide.[32]

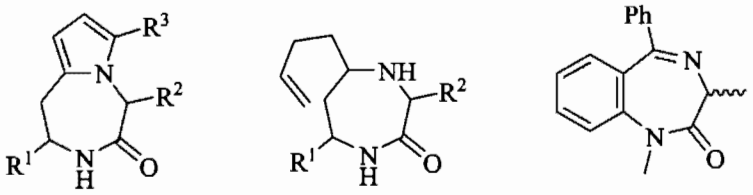


Diazepam
7

Alprazolam
8

Figure 1.5 Structure of diazepam and alprozalam.

1,4-Benzodiazepine heterocycle fused diazepine derivatives such as 1,4-diazepinone and pyrrolobenzodiazepinone derivatives (Fig 1.6) have exhibited widespread biological activities.[33] Examples have been reported for 1,4-diazepinones that act as inhibitors of the lymphocyte function-associated antigen-1(LFA-1)[34] and as anticonvulsant agents.[35] The diazepinone system is also present in the liposidomycin nucleoside antibiotics which inhibit bacterial peptidoglycan synthesis.[36] Furthermore, 1,4-diazepinone derivatives have been incorporated into peptides as constrained dipeptide mimetics.[37]



Pyrrolodiazepinone 1,4-Diazepin-2-one Benzodiazepine

Figure 1.6 Structure relationship between pyrrolodiazepinone, 1,4-diazepin-2-one and benzodiazepine.

The pyrrolobenzodiazepines (PBDs) are a group of naturally occurring antibiotics produced by diverse *Streptomyces* species, which include the anthramycin family, that contain a pyrrolobenzodiazepine structure.[38] Synthetic pyrrolobenzodiazepines analogs have inhibited non-nucleoside reverse transcriptase.[39] Activity was also demonstrated against various leukemia cell lines[40] and as CNS antidepressants[41] (Fig 1.7). The seven-membered 1,4-diazepinone ring adopts a γ-turn conformation,[42,43] as supported by X-ray diffraction.[44]

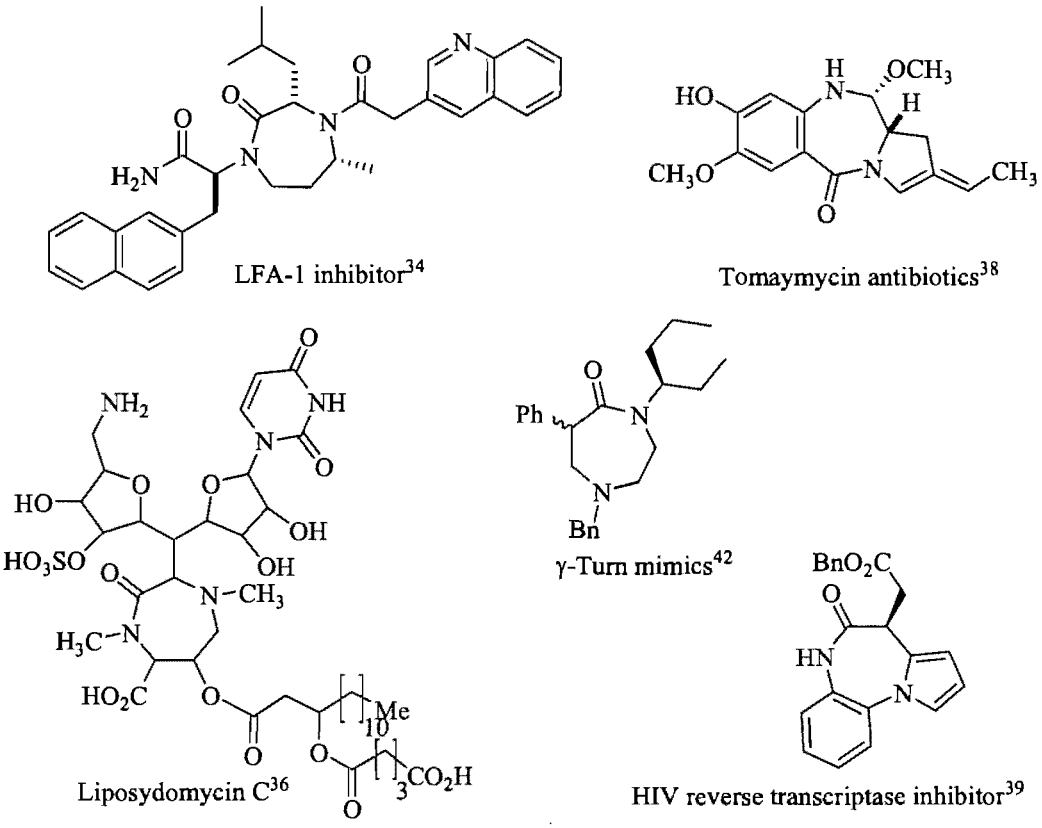


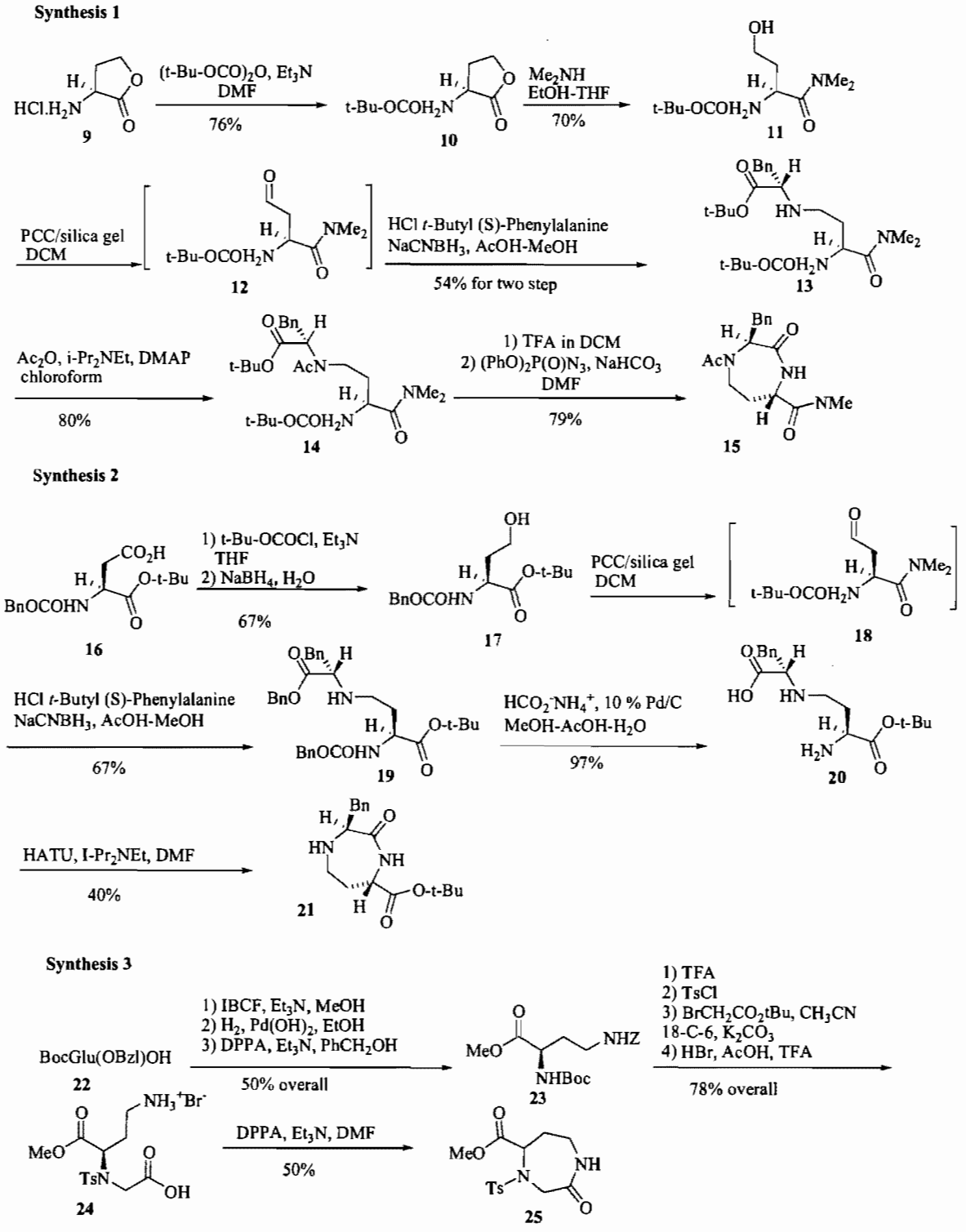
Figure 1.7 Examples of 1,4-diazepinones and 1,4 pyrrolodiazepinones.

1.5. Diazepinone Synthesis

The synthesis of diazepinone ring systems has attracted considerable interest in developing probes for studying structure-activity relationship of biologically relevant receptors. There are a variety of approaches for the synthesis of 1,4-diazepinone ring systems. 1,4-Diazepinones have been synthesized through head-to-tail cyclization of the terminal amine and carboxylic acid moieties of a linear dipeptide consisting of an α-amino and β-amino acid, using an activating agent.

1.5.1 Diazepinones Synthesis by Lactam Formation

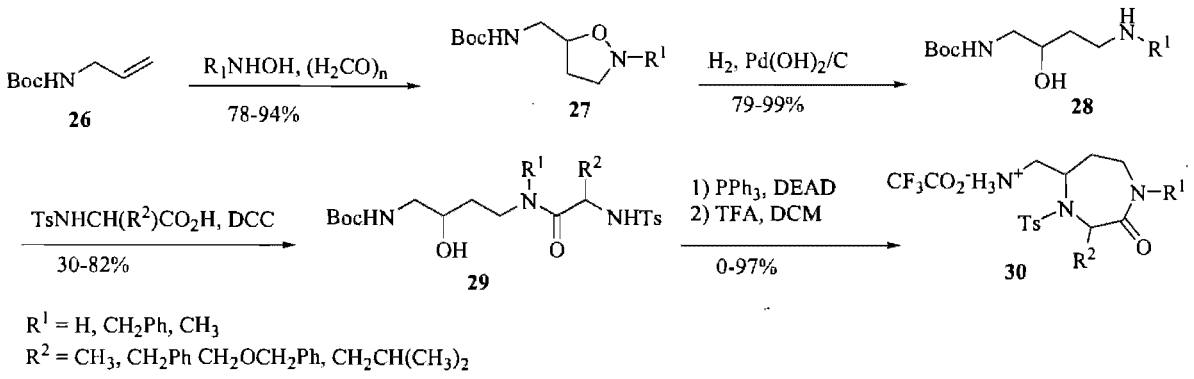
Trisubstituted 1,4-diazepin-3-one was synthesized for use as a novel constrained dipeptide scaffold (Scheme 1.1, synthesis 1). The synthesis began from readily available *N*-protected homoserine lactone **10.** Ring opening of lactone **10** by aminolysis generated the primary alcohol **11** which was oxidized to the corresponding aldehyde **12** using pyridinium chlorochromate (PCC) in the presence of silica gel. The reductive amination of aldehyde **12** with *t*-butyl-(S)-phenylalaninate using sodium cyanoborohydride in methanol afforded the secondary amine **13**. Acylation of the secondary amine **13**, followed by Boc removal and lactam formation yielded 1,4-diazepinone **15**. This acylation of the secondary amine **13** resulted in a mixture of isomers, generated by the *cis-trans* isomerisation around the acetylated tertiary amide bond. A closely related synthesis of diazepinone **15**, was accomplished from *N*-protected aspartic acid **16**, using similar protocols featuring a reductive amination/lactam cyclization sequence (Scheme 1.1, synthesis 2). The yield obtained for diazepinone **21** was low and several side products were obtained in the last step of the synthesis.[37] 1,4-Diazepin-2-one has been prepared in enantiomerically enriched form as a constrained peptide mimic from chiral BocGlu(Obzl)OH **22**. Methylation, debenzylation and modified Curtius reaction yielded diamino acid **23.** Boc removal, *N*-tosylation and *N*-alkylation followed by *t*-butyl removal and lactam formation gave 1,4-diazepin-2-one **25** in moderate yield (Scheme 1.1, synthesis 3).[45]



Scheme 1.1 Diazepinone synthesis by lactam formation.

1.5.2 Diazepinones Synthesis by Mitsunobu Cyclisation

Racemic 1,4-diazepin-2-one has been prepared as a constrained peptide mimic starting from *N*-Boc-allylamine **26**. Dipolar cycloaddition afforded isoxazolidine **27.** Ring opening *N*-acylation yielded the linear precursor **29**. Intramolecular cyclization of **29** was then accomplished under Mitsunobu conditions for the alkylation of sulfonamide **29** to afford 1,4-diazepin-2-one **30**. The cyclization of a tertiary amide was essential for ring formation.

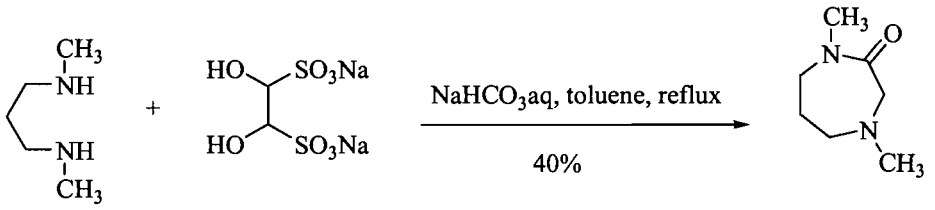


Scheme 1.2 1,4-Diazepin-2-one synthesis by Mitsnubou cyclization.[45]

1.5.3 Diazepinone Synthesis by Parallel Approaches

The liposidomycins are a family of nucleoside antibiotics with complex structures composed of three portions: a diazepinone moiety, an aminopentose moiety and a lipid portion. There have been different procedures describing the synthesis of the diazepinone moiety with the aim of developing liposydomycins analogs as new antibacterial drugs that inhibit the enzymes responsible for the biosynthesis of bacterial peptidoglycan.

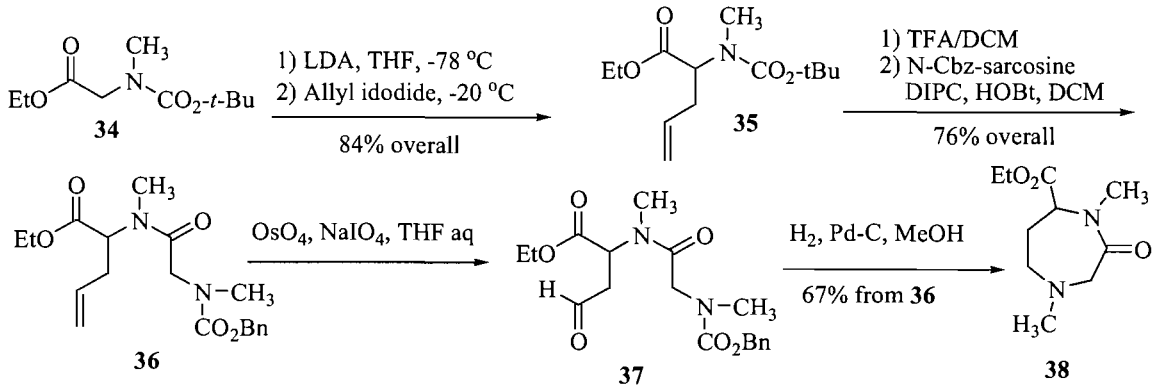
Formation of the *N,N*-dialkyl diazepinone **33** core was accomplished by a one-pot synthesis featuring heating a mixture of *N,N*-dimethylpropane-1,3-diamine **31** and the sodium bisulfite adduct of glyoxal **32** at reflux in toluene (Scheme 1.3). This approach was later abandoned because of the harsh conditions employed that led to epimerization and elimination in the case of more substituted diazepinone.



31 **32** **33**

Scheme 1.3 Synthesis of *N,N*-dimethyl 1,4-diazepin-2-one **33**.

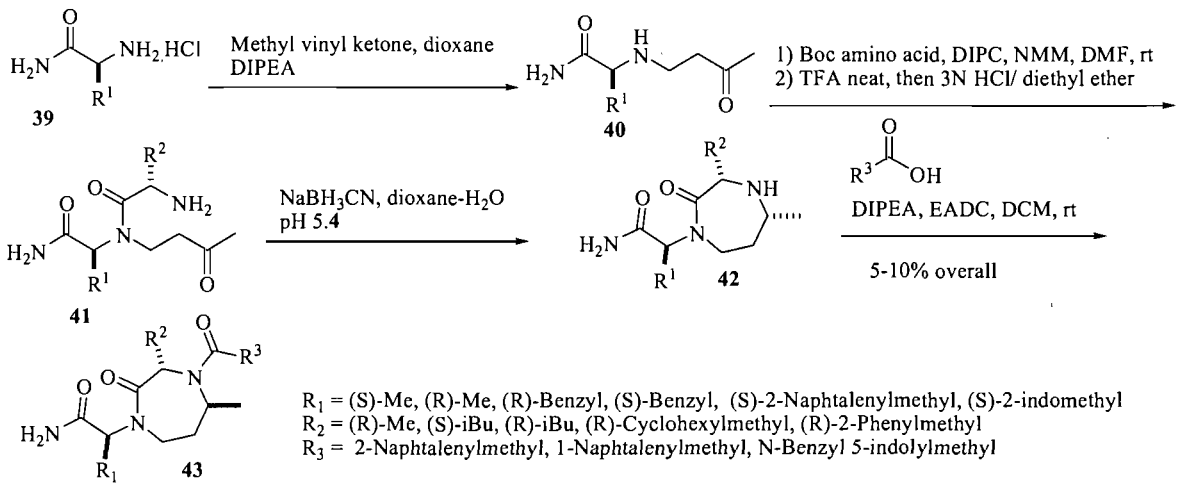
The 7-substitued 1,4-diazepin-2-one **37** was later prepared by a synthetic route featuring annulation by reductive amination (Scheme 1.4). *N*-Boc-sarcosine ethyl ester **34** was converted to its lithium enolate, alkylated with allyl iodide, *N*-deprotected then acylated with *N*-(Cbz)-sarcosine to afford dipeptide **36**. Oxidative cleavage of the olefin gave aldehyde **37**, which was hydrogenated to remove the Cbz group allowing intramolecular iminium ion formation and reductive amination to yield diazepinone **38**.



Scheme 1.4 Synthesis of 1-substitued 1,4-diazepin-2-one **37** .[46]

1.5.4 Diazepinone Library

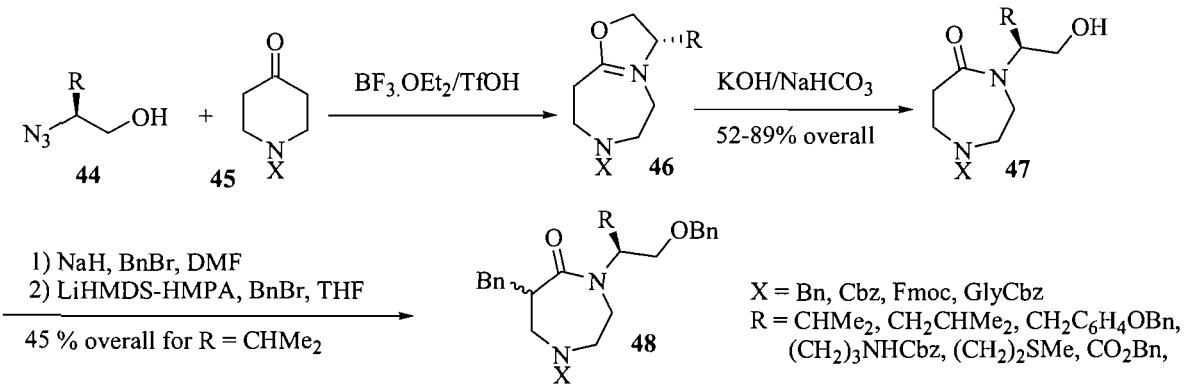
A library of ninety 1,4-diazepin-2-ones was synthesized starting from α-amino amide **39** (Scheme 1.5). Michael addition onto the methyl vinyl ketone gave secondary amine **40**; which was acylated with an *N*-Boc amino acid and *N*-deprotected to yield dipeptide **41**. Intramolecular reductive amination yielded diazepinone **42**, which was acylated to afford diazepinone **43** with overall yield of 5- 10%.



Scheme 1.5 General synthesis of library of 1,4-diazepin-2-one **43**. [34]

1.5.5 1,4-Diazepinones Synthesis By Ring Expansion

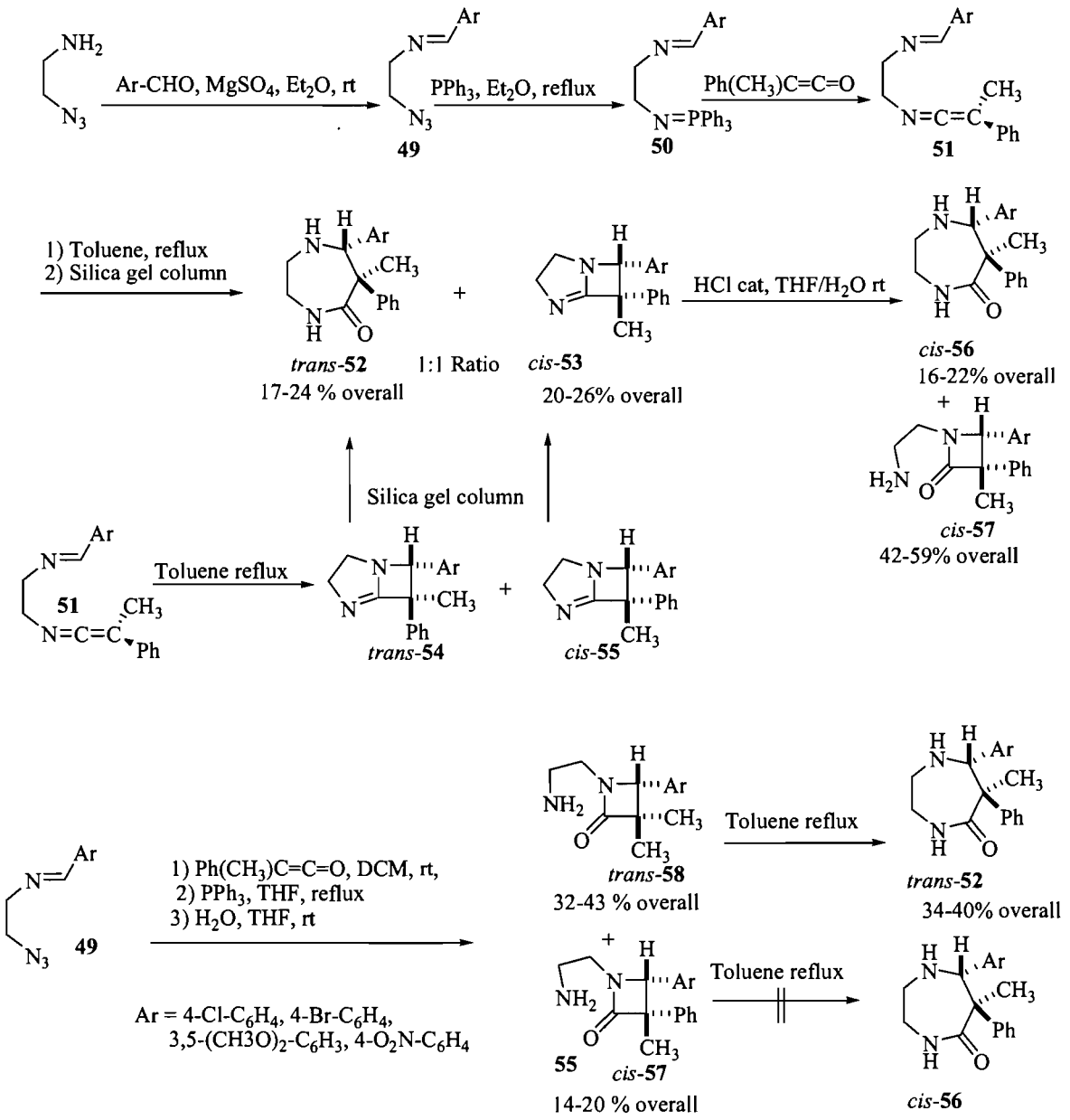
The 1,4-diazepin-5-ones were synthesized by a route featuring ring expansion by an azido-Schmidt reaction (Scheme 1.6). Piperidones **45** reacted in the presence of an acid and with 2-hydroxyalkyl azides **44** to yield a hemiketal intermediate that underwent the azido-Schmidt reaction to furnish cyclic iminium ether **46.** Subsequent hydrolysis produced the desired 1,4-diazepin-5-ones **47**. Enolization and alkylation of 1,4-diazepin-5-one **47** yielded a mixture of diastereoisomers **48.**



Scheme 1.6 Synthesis of 1,4-diazepin-5-ones **47** by azido-Schmidt reaction.[42]

Intramolecular ring expansion has proven to be a versatile method for the synthesis of diazepinones. For example, intramolecular transamidation has been used to prepare 1,4-diazepin-5-ones (Scheme1.7).[43] Heating iminoketimines **51** in toluene at reflux, provided a mixture 1:1 of *cis* and *trans* azeto[1,2-*a*]imidazoles **54** and **55**. Purification of the crude mixture by chromatography on a silica gel column yielded *trans*-diazepinones **52** and *cis*-azeto[1,2-*a*]imidazoles **53**. The *cis*-azeto[1,2-*a*]imidazoles **53** could be converted to *cis*-

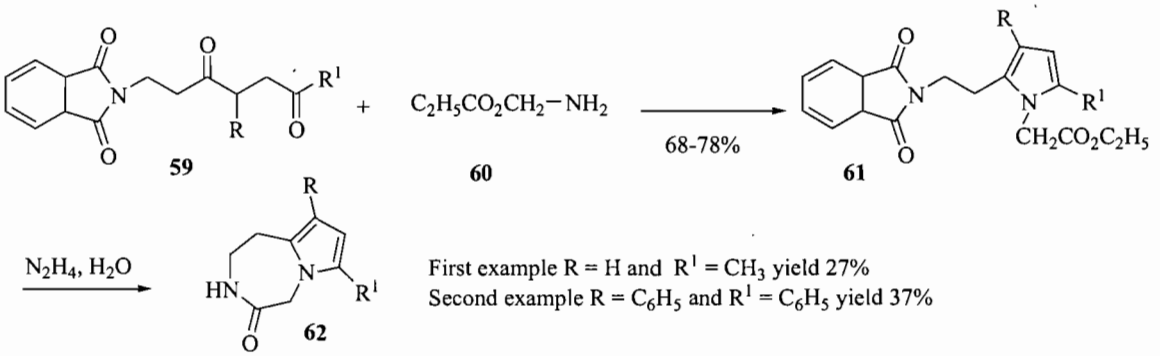
diazepinones **56** with catalytic HCl in THF/H_2O. In an aternative strategy four *N*-aminoethyl-β-lactams, **57** and **58** were prepared by intramolecular 2+2 cycloadditions; however only the *cis* isomer reacted to provide diazepinone **52** by intramolecular transamidation.



Scheme1.7 Synthesis of 1,4-diazepin-5-ones by ring expansion and intramolecular transamidation.[43]

1.5.6 Pyrrolodiazepinones Synthesis

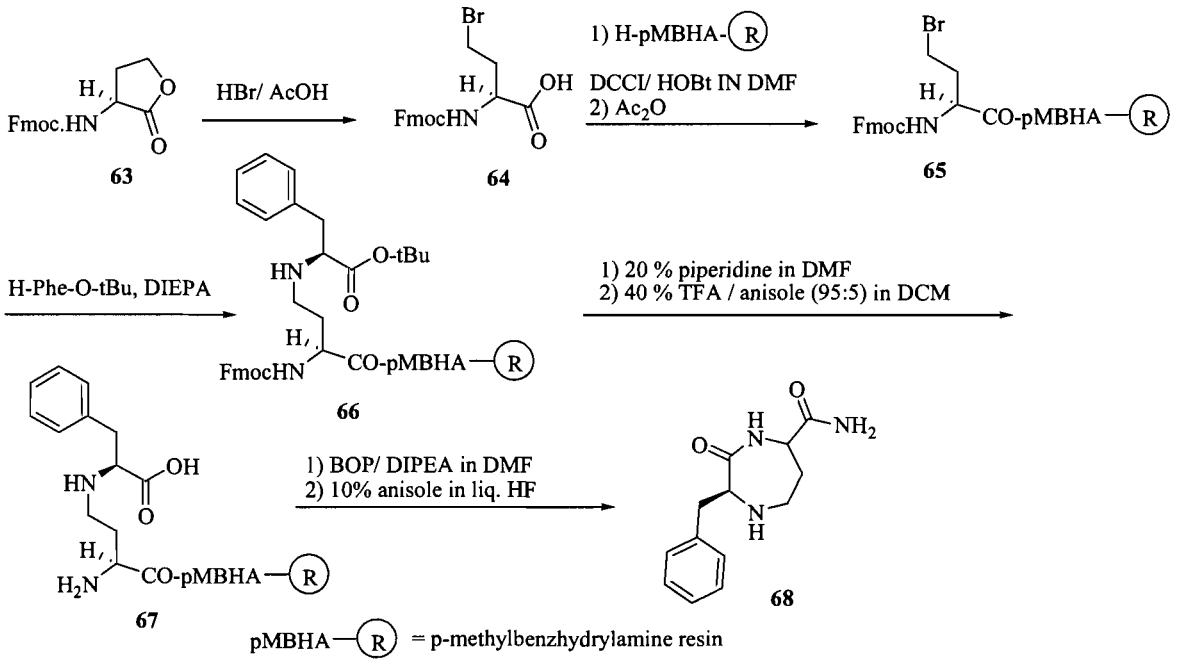
Although pyrrolodiazepinones fused to a third aromatic or heteroaromatic ring have been reported, to the best of our knowledge only one synthesis has been described which affords the parent pyrrolodiazepinone (Scheme 1.8). Paal-Knorr condensation of phtalimidoalkane-1,4-diones **59** and glycine ethyl ester gave (phtalimidoalkyl)pyrrole **60**. Hydrazinolysis removed the phtalimide and intramolecular acylation yielded pyrrolodiazepinones **62**.



Scheme 1.8 Pyrrolodiazepinones synthesis.[47]

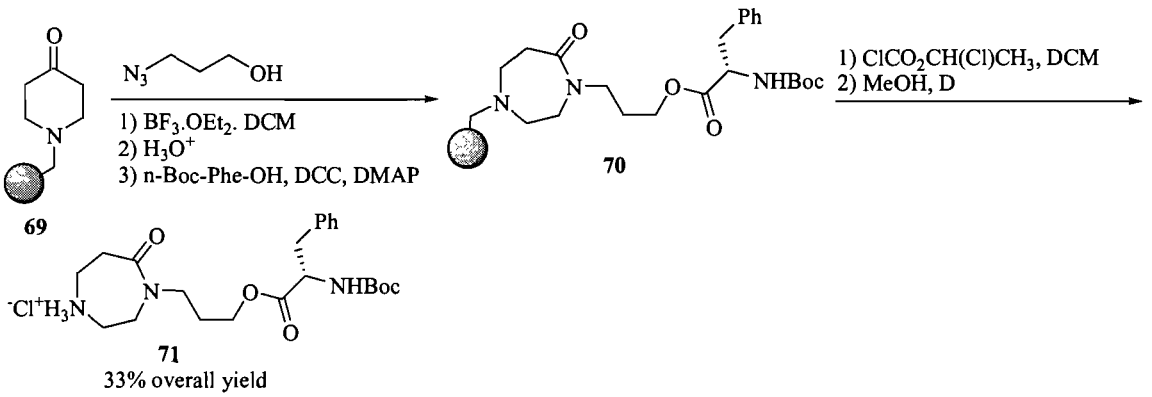
1.5.7 Solid-Phase Synthesis of Diazepinones

The synthesis of diazepinones on solid-phase has attracted attention in order to prepare libraries of these heterocycles. For example, diazepinones were made from a linear precursor **65** generated by *N*-alkylation of the *tert*-butyl phenylalaninate with resin-bound ω-bromo-α-aminobutanoamide **64** and selective removal of the Fmoc and *tert*-butyl groups. Lactam formation and resin cleavage yielded diazepinone **68** (Scheme 1.9).



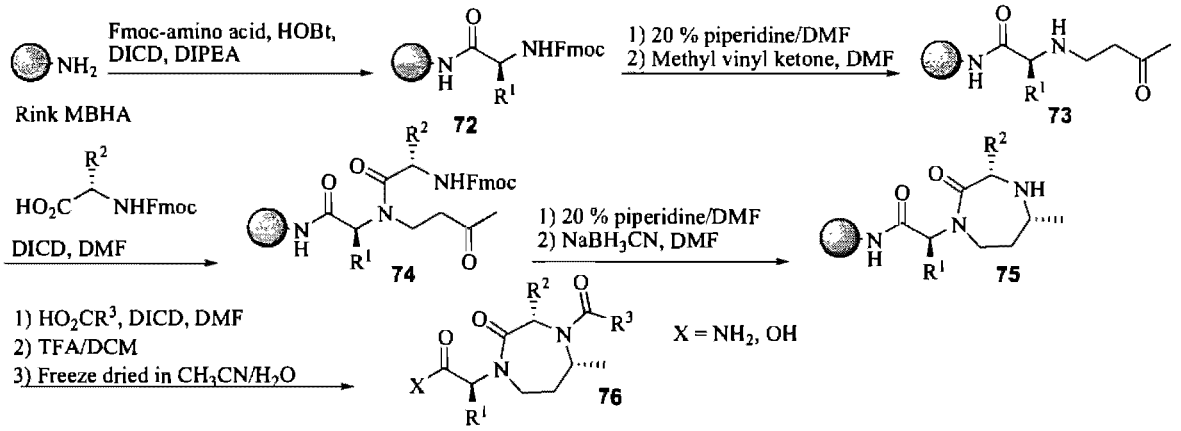
Scheme 1.9 1,4-Diazepin-3-one synthesis on solid support.[48]

Ring expansion of piperidone **69** attached to Merrifield resin was accomplished using 3-azidopropanol in a solide-phase azido-Schmidt reaction (Scheme 1.10). Esterification with *N*-Boc phenylalanine and resin cleavage gave diazepinone **71**.



Scheme 1.10 Solid phase syntheis of diazepinones by ring expansion.[45]

Finally a library of 1,4-diazepin-2-ones were prepared using α-amino acids linked to Rink-amide MBHA resin. Michael addition to methyl vinyl ketone, acylation with a second amino acid and reductive amination gave diazepinone **75**. Acylation of the resulting heterocycle **75** yielded an expanded set of diazepin-2-ones **76** (Scheme 11).



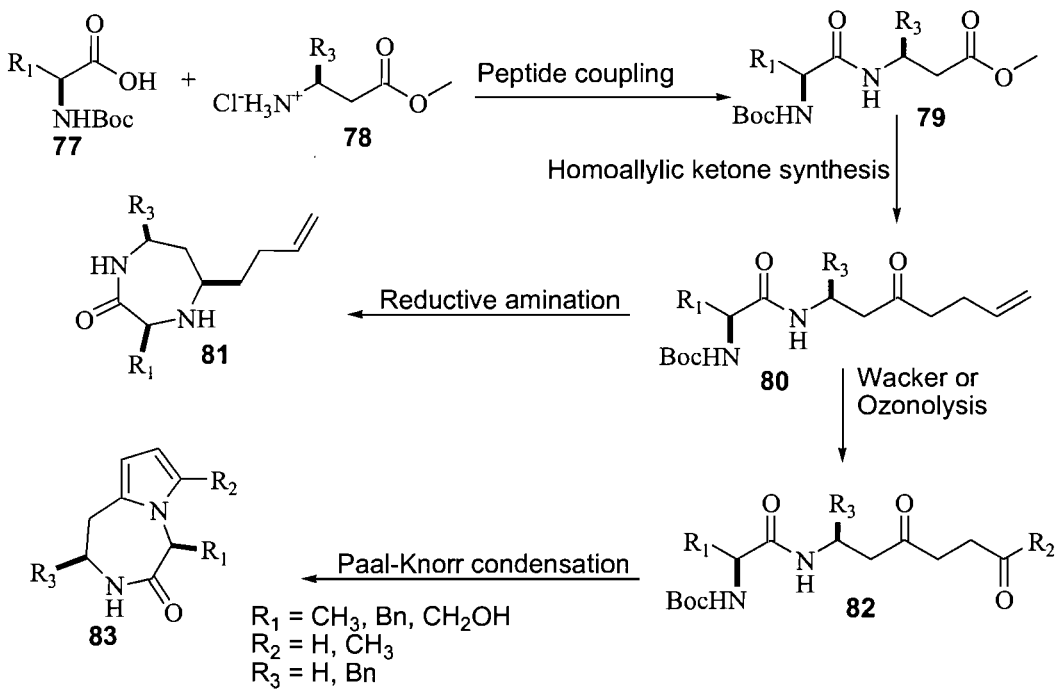
R^1 = (S)-Me, (R)-Me, (R)-Benzyl, (S)-Benzyl, (S)-2-Naphtalenylmethyl, (S)-2-indomethyl
R^2 = (R)-Me, (S)-iBu, (R)-iBu, (R)-Cyclohexylmethyl, (R)-2-Phenylmethyl
R^3 = 2-Naphtalenylmethyl, 1-Naphtalenylmethyl, N-Benzyl 5-indolylmethyl

Scheme 11 Diazepin-2-one library generated by solid-phase synthesis.[34]

1.6 Aims of the Project

This study is part of an ongoing research project with the overall goal of developing new methodologies for the synthesis of diazepinone heterocycles using solid-phase and solution reactions. The solution chemistry utilizes α-amino acids **77** and β-amino esters **78** as inexpensive, commercially available starting materials to provide α-aminoacyl β-amino esters **79** (Scheme 12). The homoallylic ketones **80** were obtained from a Cu-catalyzed

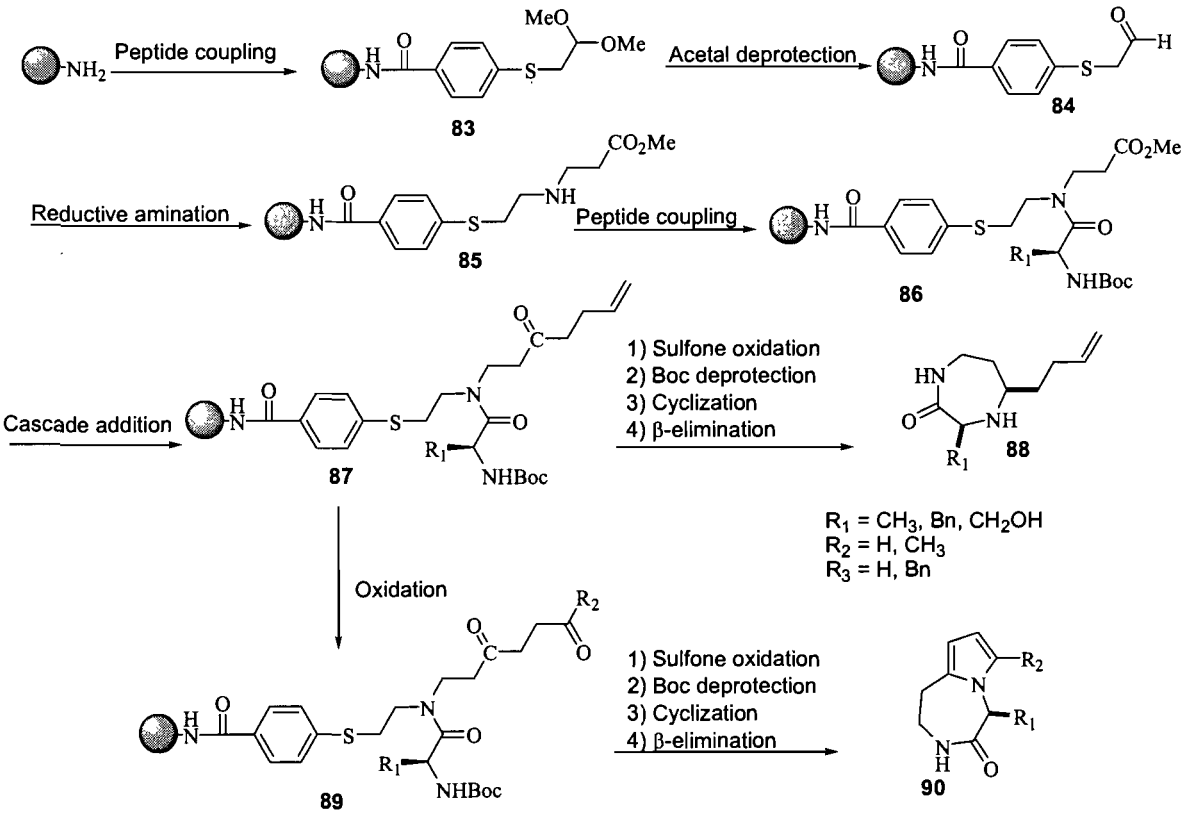
cascade addition of a vinyl Grignard to esters **79**. This methodology developed by Lubell and co-workers, provided pyrroles in two steps from the homoallylic ketones by olefin oxidation of a 1,4-dicarbonyl compound and Paal-Knorr condensation with various primary amines.[49] Homoallylic ketone **80** was then used as a precursor for making diazepinone **81** and pyrrolodiazepinone **83** heterocycles.



Scheme 12 General methodology for making diazepinone and pyrrolodiazepinone.

The 1,4-diazepin-2-one was generated on amino ethylated polystyrene resin (AEPS). Coupling of the resin to *para*-mercaptobenzoic acid derivatives gave polymer supported resin **84**. Acetal deprotection, followed by reductive amination with β-amino esters gave the secondary amine supported resin **85**. The secondary amine **85** was acylated using

activated Boc amino acids. Cascade addition of vinyl Grignard to the resulting tertiary amide **86** yielded homoallylic ketone supported resin **87**. Sulfone oxidation, Boc deprotection, intramolecular reductive amination and β-elimination produced diazepinone **88**. Pyrrolodiazepinone **90** was obtained by oxidation, Boc removal, and intramolecular Paal-Knorr cyclization and β-elimination. The general pathway is presented below in Scheme 13.



Scheme 13 General methodology for making diazepinone and pyrrolodiazepinone using a traceless linker.

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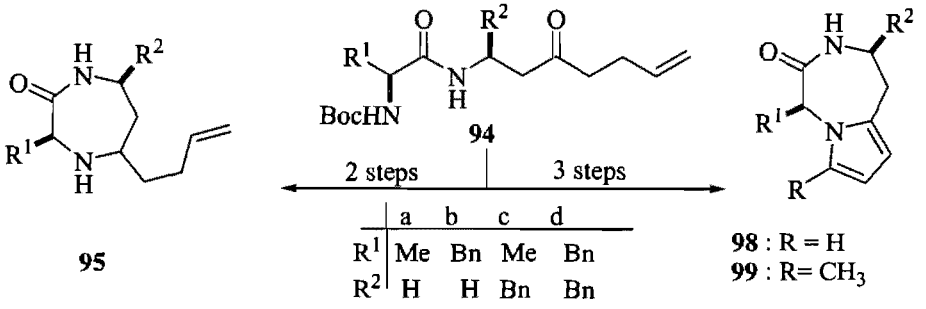
CHAPTER 2

Article : 1,4-Diazepinone and Pyrrolo-Diazepinone Syntheses via Homoallylic Ketones from Cascade Addition of Vinyl Grignard Reagent to α Aminoacyl-β-amino Esters

Iden, H; Lubell, W. D.

Published in: *Org. Lett.* **2006**, 8,16, 3425-3428

2.1 Abstract



1,4-Diazepinone **95** and pyrrolodiazepinones **98** and **99** were synthesized from common homoallylic ketone precursors **94** prepared by copper-catalyzed cascade addition of vinyl Grignard reagent to α-aminoacyl β-amino-esters **93**. Nitrogen deprotection, and intramolecular reductive amination yielded 1,4-diazepinones **95**. Olefin oxidation, Boc removal and intramolecular Paal-Knorr condensation gave pyrrolodiazepinones **98** and **99**. X-ray structures of diazepinones **95c** and **95d** depicted dihedral angles about the α-amino acid moiety similar to the central residue in an ideal reverse γ-turn.

2.2 Introduction

Among the small molecules employed in drug discovery, molecular scaffolds that mimic peptide secondary structures are particularly useful for lead identification and the development of therapeutic agents. In this respect, 1,4-benzodiazepines display a wide range of pharmacological activities likely due to their potential to mimic peptide turn conformations.[1-3] 1,4-Diazepinones have exhibited activity as high-affinity antagonists of the lymphocyte function-associated antigen-1 / immunoglobulin superfamily ICAM-1 interaction.[4] They have shown anticonvulsant activity.[5] The diazepinone system is also present in the liposidomycin nucleoside antibiotics that inhibit bacterial peptidoglycan

synthesis (Figure 1).[6] The pyrrolobenzodiazepine structure is contained in the natural anthramycin family of antitumor antibiotics.[7] Synthetic pyrrolobenzodiazepines have acted as non-nucleoside reverse transcriptase inhibitors[8] and CNS agents;[9] they have also exhibited antiproliferative[10] and antidepressant[11] activity (Figure 1). The synthesis of diazepinone ring systems has attracted considerable interest. Annulation of the diazepinone ring has been accomplished from linear precursors by reductive amination,[12,13] and lactam formation.[14] Ring expansion of *N*-protected-4-piperidones by azido-Schmidt reactions with 2-hyroxyalkyl azides has given *N*-substituted diazepinones.[3] 1,4-Diazepin-5-ones have been recently made by acid-catalyzed hydrolysis of azeto[1,2-a]imidazoles.[15] In addition, a combinatorial library of 1,4-diazepinone derivatives was synthesized by a solid-phase approach.[16]

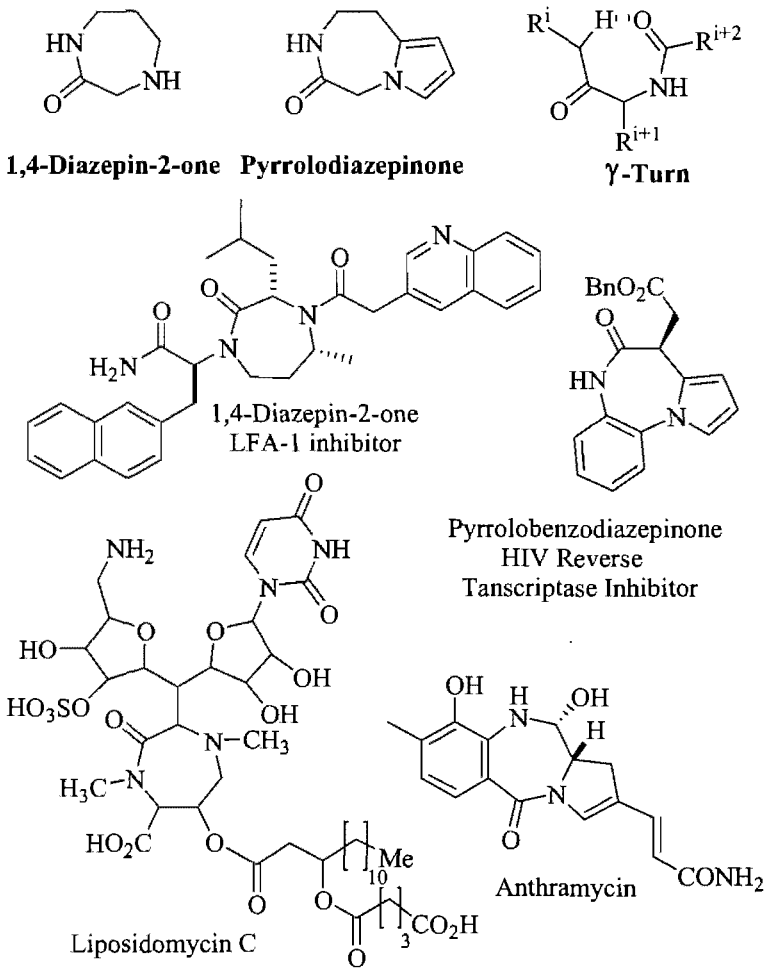


Figure 2.1 Representative 1,4-diazepin-2-one and pyrrolo-diazepinone systems and related biologically active structures.

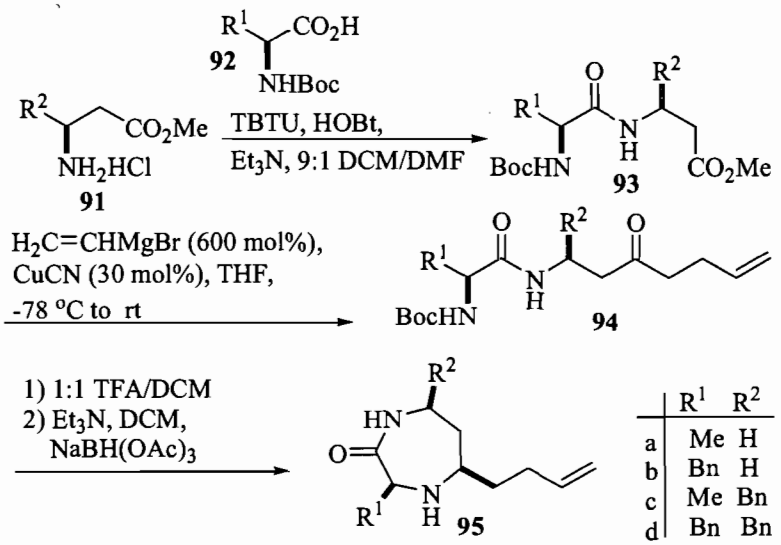
Syntheses of the pyrrolobenzadiazepine structures were reviewed in a recent Note on pyrroloaryldiazepine construction by modification of the four-component Ugi condensation.[17] To the best of our knowledge, only one synthesis of the parent pyrrolodiazepinone has been described featuring a Paal-Knorr condensation / lactam cyclization sequence to form sequentially the pyrrole and diazepinone rings.[18]

Their remarkable biological activity has inspired development of new synthetic methodology for making diazepinone and fused aryl and hetero-aryldiazepinone structures.

Present methods employ expensive starting materials, often give low to moderate yields, and provide compounds of limited diversity. Employing inexpensive amino acids as chiral educts, efficient synthetic approaches for making diazepinone and pyrrolodiazepinone heterocycles were conceived that use a common homoallylic ketone **94.** Four diazepinones **95** and eight pyrrolodiazepinones **98** and **99** (Schemes and Tables 2.1 and 2.2) were synthesized starting from combinations of Ala, Phe, β-Ala and β-homophenylalanine to provide α-aminoacyl β-amino esters **93**. Homoallylic ketones **94** were then made by Cu-catalyzed cascade addition of vinyl Grignard reagent to esters **93**.[19]

2.3 Results and Discussion

α-Aminoacyl β-amino esters **93** were synthesized by coupling β-amino esters **91** to the respective *N*-Boc-α-amino acids **92** using TBTU and HOBt in DCM (Scheme 2.1).[20] Dipeptides **93** were isolated by chromatography on silica gel in 85-92% yields. Homoallylic ketones **94** were synthesized by treating α-aminoacyl-β-amino esters **93** with an excess of freshly prepared vinyl magnesium bromide (600 mol %) in the presence of copper cyanide (30-40 mol%) in THF at -78 °C to rt.[19] After quenching the Grignard reaction at 0 °C, work up and solvent removal under vacuum, homoallylic ketones **94a-c** were isolated by chromatography on silica gel in yields of 60-75% (Table 2.1). Isolation of ketone **94d** was best accomplished by chromatography over silica gel impregnated with silver nitrate, in 45% yield.[21]



Scheme 2.1 Synthesis of Diazepinones **95.**

entry	R^1	R^2	**93** (%)	**94** (%)	**95** (%)
a	CH_3	H	85	75	45
b	CH_2Ph	H	90	60	40
c	CH_3	CH_2Ph	90	65	50
d	CH_2Ph	CH_2Ph	92	45	50

Table 2.1 Yields of isolated product in the synthesis of **95.**

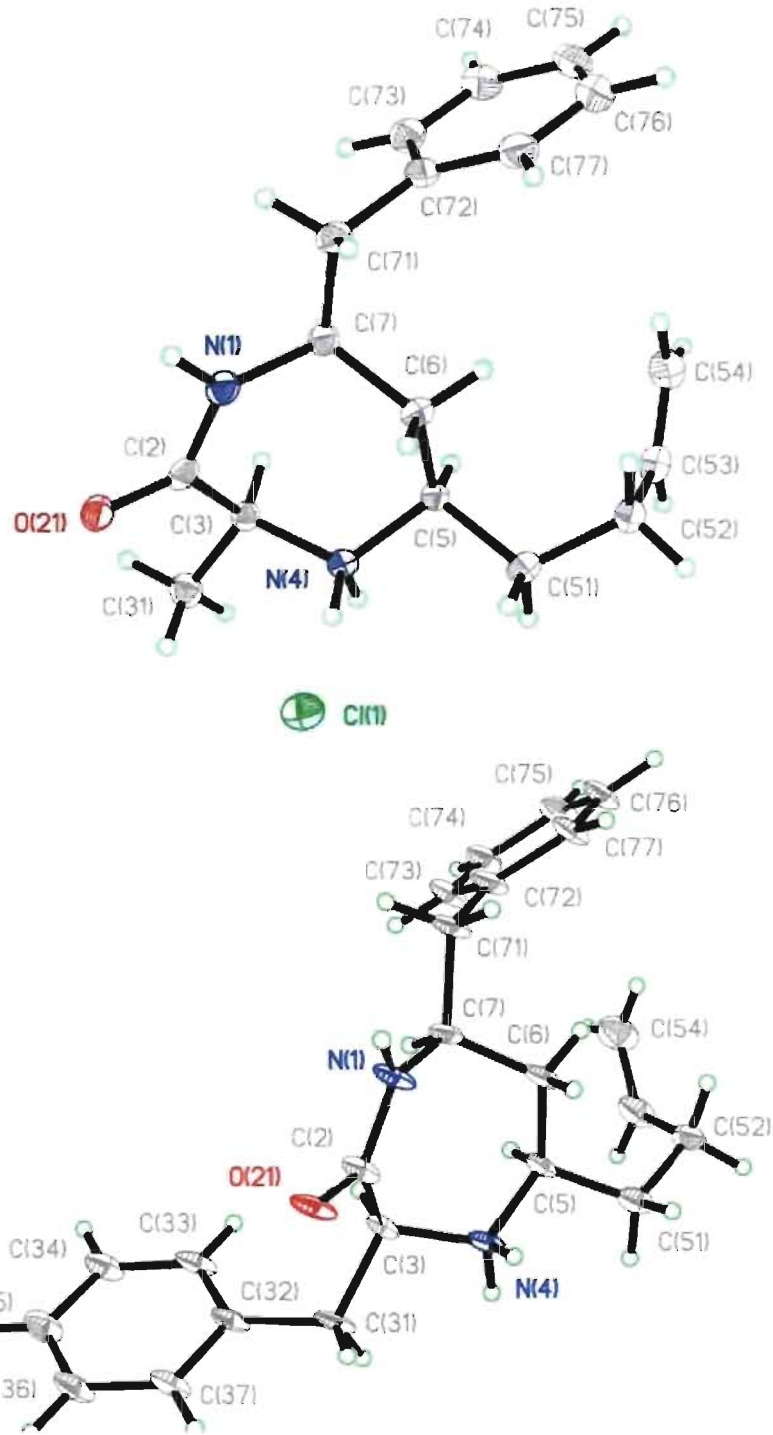


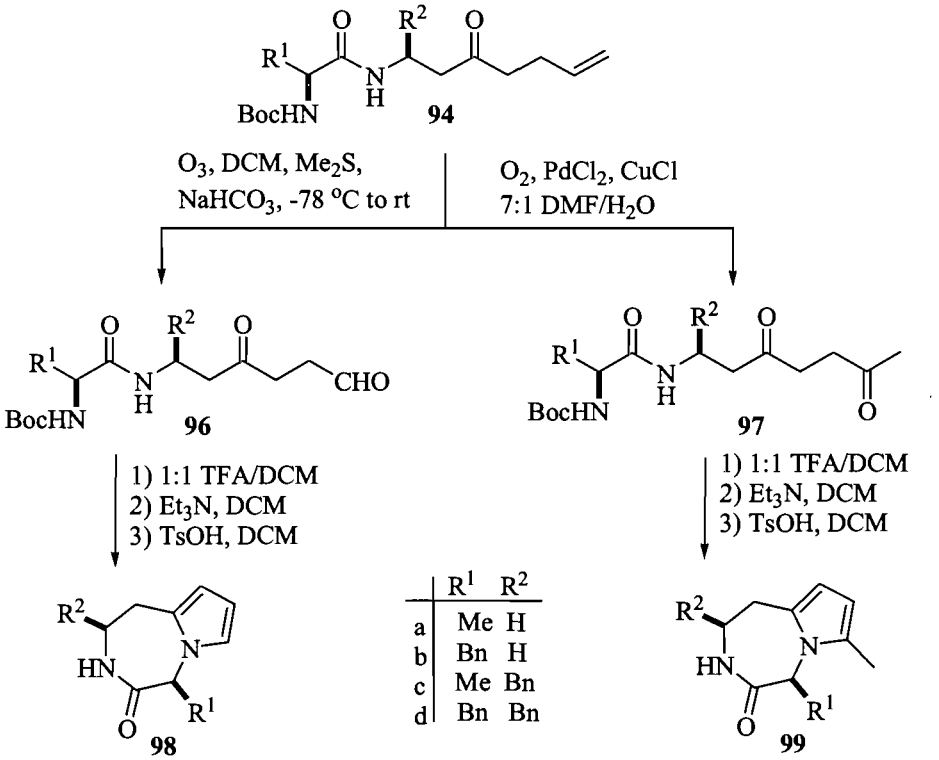
Figure 2.2 X-ray diffraction structures of compounds **95c** (upper) and **95d** (lower).

Diazepinones **95** were synthesized from homoallylic ketones **94** by a route featuring nitrogen deprotection with TFA/DCM (1:1), treatment of the trifluoroacetate salt with triethyl amine at 0 °C and reduction of the imine intermediate with sodium triacetoxyborohydride in dilute DCM.[22] The progress of the imine reduction was followed by LCMS analysis, which showed only one diastereoisomer in the crude product as confirmed by ^{1}H NMR spectroscopy. After work up, diazepinone **95a** was isolated after this sequence from **94a** in 45% yield by preparative HPLC. Diazepinones **95b-d** were purified by precipitation as hydrochoride salts and isolated in 40-50% overall yields from ketones **94b-d** (Scheme 1, Table 1).

The hydrochloride salts of diazepinone **95c** and **95d** were recrystallized from methanol in ethyl acetate to furnish crystals for X-ray analysis. The newly formed stereocentre at the 5-position of diazepinones **95** was found to have a cis relationship with respect to the 3-position substituent as expected based on the literature precedent in which reduction of the cyclic iminium intermediate occurred with hydride attack on the face opposite the ring substituents.[16,23] A survey of the Cambridge structural database indicated that the X-ray structures of **95c** and **95d** represent the first 1,4-diazepin-2-one examples. Upon comparison of the dihedral angle geometry of the α-amino acid portion of diazepinones **95c** (ψ = 70, ϕ = -80) and **95d** (ψ = 67, ϕ = -83) with ideal values of turn conformations, we noted the close resemblance to the dihedral angle geometry of the central residue in a reverse γ-turn conformation (ψ = 60-70, ϕ = -70-85; Figure 2.2).[24]

Pyrrolodiazepinones **98** and **99** were made next from the common homoallylic ketone intermediate **94**. First, 4-ketoaldehydes **96** and 1,4-diketones **97** were obtained from

oxidation of olefin **94** using either ozonolysis or periodate/osmium tetraoxide, and by Tsuji-Wacker oxidation, respectively (Scheme 2.2).



Scheme 2.2 Synthesis of Pyrrolodiazepinones **98** and **99.**

entry	R^1	R^2	**96** (%)	**97** (%)	**98** (%)	**99** (%)
a	CH_3	H	85	85	30	65
b	CH_2Ph	H	85	80	40	45
c	CH_3	CH_2Ph	80	80	55	50
d	CH_2Ph	CH_2Ph	85	80	60	50

Table 2.2 Yields of isolated product in the synthesis of **98** and **99.**

4-Keto aldehydes **96** were isolated in 80-85% yields by chromatographic purification after ozonolysis of homoallylic ketones **94** in CH_2Cl_2 /MeOH (1:1) at -78 °C, and treatment with excess dimethylsulfide in the presence of $NaHCO_3$.[25] Alternatively, oxidative cleavage of the double bond of homoallylic ketones **94** was performed with sodium periodiate in the presence of catalytic amounts of OsO_4[26] to afford aldehydes **96** in 65-85% yield after chromatography. Comparing the two methods for making aldehyde **96**, the ozonolysis was found to be more advantageous, because crude product was obtained in suitable purity for the subsequent Paal-Knorr reaction.

1,4-Diketones **97** were produced by Tsuji-Wacker oxidation[27] employing $PdCl_2$ (0.2 equiv.) and CuCl (2 equiv.), in DMF:H_2O (9:1) at room temperature under an atmosphere of oxygen. After work up and chromatography on silica gel, 1,4-diketones **97** were isolated in 80-85% yields (Scheme 2, Table 2). Yields were improved for olefins **94c** and **94d** by using more $PdCl_2$ (40 mol%) and 2 eq of CuCl in THF/H_2O (4:1) at room temperature under oxygen atmosphere and agitation in a sonicator.

Diazepinones 95 and pyrrolodiazepinones **98** and **99** were respectively prepared from 4-ketoaldehydes **96** and 1,4-diketones **97** by a generally effective albeit moderate yielding route featuring nitrogen deprotection and Paal-Knorr condensation.[28] Acid induced Boc group removal was accomplished using either HCl gas or TFA in CH_2Cl_2, with better success on ketoaldehyde **96** using TFA instead of the HCl conditions. Paal-Knorr cyclization occurred on treatment with triethyl amine and a catalytic amount of toluene sulfonic acid in dilute DCM at 0 °C. After quenching with saturated $NaHCO_3$ solution, pyrrolodiazepinones **99** were isolated by chromatography on silica gel in 45-65% overall

yield from **97**. An improved Paal-Knorr condensation was then developed using basic Amberlyste A-21 ion exchange resin for generating the free amine after Boc deprotection with TFA in DCM.[29]

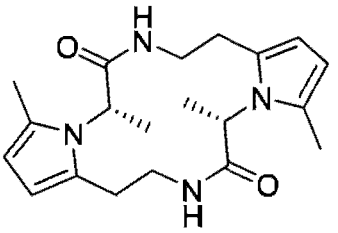


Figure 2.3 Dimer **100.**

The use of higher concentrations of ketoaldehydes **96** and diketones **97** in the Paal-Knorr annulation to form pyrrolodiazepinone **98** and **99** provided mixtures of monomer and dimer as observed by LCMS analyses. Dimer **100** was isolated by preparative HPLC and identified by its doubling in mass, yet relatively simple ^{1}H and ^{13}C NMR spectra due to C_2 symmetry (Figure 2.3).

2.4 Conclusions

Practical, diversity-oriented methodology for the synthesis of diazepinone structures has been developed that features treatment of α-aminoacyl β-amino-esters **93** with excess vinyl Grignard reagent to yield a common homoallylic ketone intermediate.

Diazepinones **95a-d**, pyrrolo- and methylpyrrolodiazepinones **98a-d** and **99a-d**, all were made in 4 to 6 steps and 16 to 31% overall yields from inexpensive amino acid building blocks **91** and **92**. Considering the tolerance of the Cu-catalyzed cascade additions of vinyl Grignard reagent to a variety of *N*-(acyl)amino esters possessing different side-chains,[19] a wealth of peptide-based diazepinone and pyrrolo-diazepinone structures may now be obtained by this practical approach.

2.5 Acknowledgment

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2.6 Supporting Information Available

Experimental details, spectroscopic caracterization for all compounds, and X-ray crystallographic data of **5c** and **5d.** This material is available free of charge via the Internet at http://pubs.acs.org

2.7 Experimental Section

For reactions performed under anhydrous conditions, glassware was either oven or flame-dried and the reaction was performed under a positive pressure of argon. Column chromatography was performed on silica gel (Silicycle; 230-400 mesh) and thin layer chromatography (TLC) was performed on aluminum plates coated with (0.2mm) silica gel F_{254} from EMD Inc or Silicyle Inc, using the solvent system indicated for TLC. Amino acids were obtained from Sigma Aldrich Inc or Avocado Research Chemicals Inc, and were N-protected using standard procedures.[30] β-Phenylalanine was prepared according to the literature protocol[31] and esterified using methanol and acetyl chloride to afford the methyl ester.[32]

Semi-preparative reverse-phase high performance liquid chromatography (RP-HPLC) was carried out on RP-18 column 5μm (250 x 20 mm; TARGA column), at a flow rate of 10 mL/min. The solvent system used in RP-HPLC included the following: solvent A, 0.1% (v/v) TFA in CH_3CN; solvent B, 0.1% (v/v) TFA in H_2O, detector was monitored at 214 nm. Spectral data were collected on Bruker AV300 (300 MHz) and AV400 (400 MHz) instruments. Chemicals shifts (δ_H) were reported in parts per million (ppm) and are referenced to the residual solvent peak $CDCl_3$ (7.26 ppm), unless noted otherwise. ^{13}C were reported in ppm and were referenced to the residual solvent peak, $CDCl_3$ (77 ppm) unless

noted otherwise. Coupling constants *J* are reported in Hz. Melting points were determined with a capillary melting point apparatus and are uncorrected. High Resolution Mass Spectrometry HRMS, Electro Spray Ionisation (ESI), was performed by the Université de Montréal Mass Spectrometry Facility. The reported yields are the actual isolated yields of purified material and are not optimized.

General Procedure (1) for the Synthesis of Dipeptides 93a-d

Triethyl amine (TEA, 3 equiv.) was added over a period of 5 minutes to a suspension of beta amino methyl ester hydrochloride salt (1 equiv.) at 0 °C in DCM / DMF (9:1, c = 0.1 M). The mixture was stirred for 30 minutes, treated with *N*-(Boc)-amino acid (1 equiv.), TBTU (1.5 equiv.), and HOBt (1 equiv.), and stirring was continued overnight. Solvent was evaporated under vaccum, water was added to the residue, and the layers were separated. The aqueous phase was extracted with DCM (3x 50 mL). The combined organic layers were washed with 10% HCl, saturated sodium bicarbonate solution, water and brine, dried over magnesium sulphate, filtered and concentrated under vacuum. The residue was purified by column chromatography as specified.

(2*S*)-Methyl *N*-(Boc)alaninyl-β-alaninate (93a):

The residue was purified on silica gel using the system solvent 4:6 EtOAc/hexane. Evaporation of the collected fractions gave **93a** as a white solid (2.22 g, 90%), mp 76-77 °C. TLC R_f 0.12 (4:6 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.31 (d, 3H, J = 7.1), 1.4 (s, 9H), 2.51 (t, 3H, J = 6.2), 3.43-3.65 (m, 2H), 4.11 (br s, 1H), 5.27-5.29 (br d, 1H), 6.9 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 173.3, 173.1, 155.8, 80.2, 52.1, 50.5,

35.3, 34.1, 28.7, 18.9. HRMS (EI) *m/z* 297.1420 [M+Na$^+$; calcd for $C_{12}H_{22}N_2O_5Na$: 297.1412]. $[\alpha]_D^{20}$ -17.4° (*c* 0.01, DCM).

(2*S*)-Methyl *N*-(Boc)phenylalaninyl-β-alaninate (93b):

The residue was purified on silica gel using 2:8 EtOAc/hexane to afford **93b** (2.12 g, yield 85%) as a white solid, mp 84-85 °C, R_f 0.24 (4:6 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.3-2.52 (m, 2H), 2.96-3.14 (m, 2H), 3.29-3.57 (m, 2H), 3.65 (s, 3H), 4.3 (m, 1H), 5.13 (br d, 1H), 6.33(br s,1H), 7.19-7.31 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 172.8, 171.6, 155.8, 137.1, 129.7, 129.0, 127.3, 80.4, 56.3, 52.1, 39.2, 35.1, 34.0, 28.7. HRMS (EI) *m/z* 373.1722 [M+Na$^+$; calcd for $C_{18}H_{26}N_2O_5Na$: 373.1734]. $[\alpha]_D^{20}$ -4.9° (*c* 0.008, DMF).

(2*S*, 2'*S*)-Methyl *N*-(Boc)alaninyl-β-homophenylalaninate (93c):

The residue was purified on silica gel using 4:6 EtOAc/hexane to afford **93c** (2.14 g, 90% yield) as a white solid, mp 126-127 °C, R_f 0.25 (4:6 EtOAc/hexane), ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, 3H, *J* = 6), 1.5 (s, 9H), 2.55 (t, 2H, *J* = 6), 2.84-3.03 (m, 2H), 3.73 (s, 3H), 4.14 (br m, 1H), 4.50-4.52 (br m, 1H), 5.00 (br s, 1H), 6.75 (d, 1H, *J* = 9), 7.22-7.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 172.9, 172.8, 156.1, 138.3, 130.1, 129.4, 127.6, 80.9, 52.6, 48.1, 40.7, 37.7, 29.2, 19.3. HRMS (EI) *m/z* 387.1890 [M+Na$^+$; calcd for $C_{19}H_{28}N_2O_5Na$: 387.1890]. $[\alpha]_D^{20}$ -42.3° (*c* 0.01, DCM).

(2*S*, 2'*S*)-Methyl *N*-(Boc)phenylalaninyl-β-homophenylalaninate 93d:

The residue was purified on silica gel using 2:8 EtOAc/hexane to afford **93d** (2.65 g, 5,88 mmol) 92% yield as a white solid, mp 119-120 °C, R_f 0.11 (2:8 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.43 (s, 9H), 2.29-2.34 (m, 2H), 2.72-2.90 (m, 2H), 2.97- 3.04 (m, 2H), 3.73 (s, 3H), 4.29-4.3 (br m, 1H), 4.41-4.43 (br m, 1H), 5.08 (br s, 1H), 6.49-6.51 (br d, 1H), 7.12-7.30 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$), 172.2, 170.8, 155.6, 137.8, 137.2, 129.7, 129.6, 129.0, 127.7, 127.3, 127.1, 80.4, 56.4, 52.1, 47.4, 40.1, 39.1, 37.8, 28.7. HRMS (EI) *m/z* 463.2198 [M+Na$^+$; calcd for $C_{25}H_{32}N_2O_5Na$: 463.2203]. $[\alpha]_D^{20}$ -4.8° (*c* 0.008, DCM).

General Procedure (2) for the Synthesis of Homoallylic Ketones 94a-d

A suspension of CuCN (0.3 equiv.) in THF (2mL THF per 1 mmol of CuCN) was cooled to –78 °C, treated dropwise with a vinyl magnesium bromide solution in THF (6 equiv. c = 1 M in THF) over 10 min, followed by a solution of methyl ester **93** (1 equiv.) in THF (2 mL THF per 1 mmol of **93**). The resultant mixture was allowed to stir over night during which time the bath warmed to room temperature. The reaction mixture was cooled to 0 °C, treated with a 1N HCl aqueous solution, and shaken vigorously for 20 minutes. The layers were separated, and the aqueous phase was extracted with Et_2O (3 x 50 mL). The combined extracts were washed with an aqueous solution of saturated sodium bicarbonate (50 mL), pH 6.8 phosphate buffer (50 mL) and brine (2 x 50 mL), dried over magnesium sulfate and concentrated under vacuum. The residue was then purified by column chromatogrophy as specified to provide homoallylic ketone **94**.

(2*S*)-*N*-(Boc)Alanine *N'*-1-(3-oxohept-6-enyl)amide 94a:

The residue was purified on silica gel using 3:2 EtOAc/hexane as eluent to afford olefin **94a** as a white solid (816 mg, 75%): mp 53-55 °C; TLC R_f 0.1 (3:2 EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (d, 3 H, J = 5.1), 1.44 (s, 9 H), 2.29-2.35 (m, 2 H), 2.49-2.54 (m, 2 H), 2.67 (t, 2 H, J = 5.8), 3.45-3.52 (m, 2 H), 4.09 (br m, 1 H), 4.96-5.32 (m, 3 H), 5.73-5.85 (m, 1 H), 6.7 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 209.9, 173.2, 155.7, 137.2, 115.9, 80.4, 50.7, 42.4, 42.3, 34.4, 28.7, 28.0, 19.1. HRMS (EI) *m/z* 321.1776 [$M+Na^+$; calcd for $C_{15}H_{26}N_2O_4Na$: 321.1785]. $[\alpha]_D^{20}$ -20.0° (*c* 0.005, DCM).

(2*S*)-*N*-(Boc)Phenylalanine *N'*-1-(3-oxohept-6-enyl)amide 94b:

The residue was purified on silica gel using 4:6 EtOAc/hexane as eluent. The evaporation of the collected fractions afforded olefin **94b** (750 mg, 70% yield) as a white solid: mp 103-104 °C, R_f 0.24 (4:6 EtOAc/hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.30 (br q, 2 H), 2.41-2.44 (m, 4 H), 3.03 (m, 2 H), 3.40 (m, 2 H), 4.27 (br s, 1 H), 5.03 (m, 3 H), 5.7 (m, 1 H), 6.29 (s, 1 H), 7.18-7.31 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 209.6, 171.4, 155.6, 137.2, 137.1, 129.9, 129.7, 129.0, 127.3, 115.9, 80.5, 56.3, 42.2, 39.2, 34.2, 28.7, 27.9. HRMS (EI) *m/z* 397.2104 [$M+Na^+$; calcd for $C_{21}H_{30}N_2O_4Na$: 397.2098]. $[\alpha]_D^{20}$ -6.2° (*c* 0.008, DMF).

(2*S*, 1'*S*)-*N*-(Boc)Alanine *N'*-1-(1-benzyl-3-oxohept-6-enyl)amide 94c:

The residue was purified on silica gel using 4:6 EtOAc/hexane as eluent. Evaporation of the collected fractions afforded **94c** (664 mg, 60% yield) as a yellow white solid: mp 106-109 °C; R_f 0.28 (4:6 EtOAc/hexane); ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (d, 3 H, J = 6.9), 1.45

(s, 9 H), 2.3 (br s, 2 H), 2.42-2.47 (m, 2 H), 2.59-2.62 (m, 2 H,), 2.82-2.98 (m, 2 H), 4.07 (br s, 1 H), 4.42-4.44 (m, 1 H), 4.96-5.03 (m, 3 H), 5.73-5.78 (m, 1 H), 6.79-6.80 (br m, 1 H), 7.17-7.30 (m, 5 H); ^{13}C NMR (100 MHz, $CDCl_3$), 208.9, 171.7, 154.9, 137.5, 136.4, 128.9, 128.2, 126.3, 115.1, 79.6, 49.9, 46.9, 44.2, 41.9, 39.3, 27.9, 27.1, 18.1. HRMS (EI) *m/z* 411.2254 [M+Na$^+$; calcd for $C_{22}H_{32}N_2O_4Na$: 411.2242]. $[\alpha]_D^{20}$ -21.2° (*c* 0.005, DCM).

(2*S*, 1'*S*)-*N*-(Boc)Phenylalanine *N*'-1-(1-benzyl-3-oxohept-6-enyl)amide 94d:

The residue was purified over silica gel, that was pretreated dropwise and shaken with an aqueous silver nitrate solution (*c* = 3 M, 1g $AgNO_3$ per 10 g of silica) and oven-dried overnight, using 2:8 EtOAc/DCM as eluent. Evaporation of the collected fractions afforded **94d** (750 mg, 45% yield) as a white solid: mp 148-150 °C; R_f 0.13 (3:7 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 2.24-2.2.27 (m, 2 H), 2.31-2.37 (m, 4 H), 2.77-2.86 (m, 2 H), 2.96-2.98 (m, 2 H), 4.27-4.38 (br m, 2 H), 4.99-5.04 (m, 3 H), 5.77-5.79 (m, 1 H), 6.52-6.55 (br d, 1 H), 7.08-7.29 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$), 208.7, 170.0, 154.8, 137.4, 136.4, 136.1, 128.9, 128.8, 128.7, 128.2, 126.5, 126.3, 114.9, 79.7, 55.6, 46.6, 43.6, 41.8, 39.1, 38.3, 27.9, 27.0. HRMS (EI) *m/z* 465.2748 [M+H$^+$; calcd for $C_{28}H_{37}N_2O_4$: 465.2730]. $[\alpha]_D^{20}$ -26.9° (*c* 0.005, DCM).

General Procedure (3) for the Synthesis of 1,4-Diazepinones 95a-d

Homoallylic ketone **94** (1 equiv.) was stirred in a 1:1 TFA/ DCM solution for 30 minutes. After removal of the volatiles under vacuum, the residue was placed for 1 hour under vacum (5 mm Hg) to remove excess TFA. The residue was dissolved in DCM ($c = 10^{-3}$ M), cooled to 0 °C, treated with triethylamine (1.1 mmol), stirred for 20 minutes, treated with 4

Å molecular sieves, stirred 15 minutes, treated with sodium triacetoxyborohydride (2 to 3 equiv.) in two portions and stirred for 3 to 5 days at room temperature under an argon atmosphere. The mixture was filtered and the filtrate concentrated on a rotary evaporator. The resulting residue was then partitioned between saturated aqueous $NaHCO_3$ solution and EtOAc. The aqueous layer was separated and extracted with ethyl acetate (3x 10mL). The combined organic phase were dried over magnesium sulphate, filtered and evaporated to a residue that was purified further as specified.

(3*S*, 5*S*)-5-(But-3-enyl)-3-methyl-1,4-diazepin-2-one 95a :

The residue was purified by reverse-phase HPLC to afford diazepinone **95a** as colorless oil (28 mg, 45% yield): R_f 0.24 (5% MeOH/$CHCl_3$). ^{1}H NMR (300 MHz, MeOD) δ 1.47 (d, 3 H, J = 6.9), 1.61 (m, 2 H), 2.0 (m, 2 H), 2.22 (m, 2 H), 3.30-3.32 (m, 2 H), 3.47-3.52 (m, 2 H), 4.31 (q, 1 H, J = 6.6), 5.08 (m, 2 H), 5.82 (m, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 170.4, 136.7, 115.6, 61.9, 54.6, 48.8, 38.8, 33.0, 29.3, 14.4. HRMS (EI) *m/z* 205.1311 [M+Na^+; calcd for $C_{10}H_{18}N_2ONa$: 205.1313]. $[\alpha]_D^{20}$ 3.9° (*c* 0.007, MeOH).

(3*S*, 5*S*)-3-Benzyl-5-(but-3-enyl)-1,4-diazepin-2-one 95b:

The residue was purified on silica gel using 5% isopropanol in chloroform as eluent. After evaporation of the combined collected fractions, the residue was dissolved in ether and precipitated as its hydrochloride salt by bubbling of HCl gas to afford diazepinone **95b** (14 mg, 40% yield): mp 138-140 °C; R_f 0.32 (5% MeOH/DCM). ^{1}H NMR (400 MHz, MeOD) δ 1.60-1.68 (m, 2 H), 2.04-2.30 (m, 4 H), 3.23-3.26 (br m, 1 H), 3.33-3.35 (s, 2 H), 3.42-3.48 (m, 1 H), 3.50-3.71 (m, 2 H), 4.50-4.53 (m, 2 H), 4.92- 5.16 (m, 2 H), 5.84-5.90 (m, 1

H), 7.21-7.41 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 167.9, 135.9, 135.5, 128.8, 127.7, 126.3, 114.9, 61.4, 58.3, 37.8, 34.1, 32.3, 31.3, 28.6. HRMS (EI) *m/z* 259.1808 [$M+H^+$; calcd for $C_{16}H_{23}N_2O$: 259.1804]. $[\alpha]_D^{20}$ -35.6° (*c* 0.008, DMF).

(3*S*, 5*R*, 7*R*)-7-Benzyl-5-(but-3-enyl)-3-methyl-1,4-diazepin-2-one 95c :

The residue was purified on silica gel using 5% isopropanol in chloroform as eluent. After evaporation of the combined collected fractions, the residue was dissolved in ether and precipitated as a hydrochloride salt by bubbling of HCl gas. The hydrochloride salt was recrystallized from a mixture of methanol and ethyl acetate to afford diazepinone **95c** (16 mg, 50% yield) as a white solid: mp 264-266 °C; R_f 0.2 (3% methanol in DCM). ^1H NMR (300 MHz, MeOD) δ 1.15-1.20 (m, 2 H), 1.45-1.47 (d, 3 H, *J* = 5.6), 1.56 (br m, 1 H), 1.94-1.98 (m, 2 H), 2.15-2.19 (m, 2 H), 2.8-3.32 (m, 2 H), 3.42-3.50 (m, 2 H), 3.98-4.00 (m, 1 H), 4.35-4.37 (m, 1 H), 4.75-4.8 (m, 1 H), 5.68-5.73 (m, 1 H), 7.27-7.35 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$), 170.4, 138.5, 137.2, 130.5, 129.9, 128.1, 116.9, 62.0, 55.6, 53.2, 41.7, 37.3, 33.6, 30.3, 15.4. HRMS (EI) *m/z* 273.1951 [$M+H^+$; calcd for $C_{17}H_{25}N_2O$: 273.1961]. $[\alpha]_D^{20}$ -35.3° (*c* 0.003, MeOH). The X-ray structure of **5c** was solved by direct methods using SHELXS-97, refinement was performed using SHELXL-97: ($C_{17}H_{25}N_2OCl$) M_r = 308.84, orthorombique, space group $P2_12_12_1$, a = 5.3977 (6) Å, b = 12.8158 (13) Å, c = 24.189 (3) Å, V= 1673.3 (3) $Å^3$, Z = 4; ρ_{calcd} = 1.226 $g.cm^{-3}$, μ = 2.016 mm^{-1}, *T* = 150 (2) K, *F*(0,0,0) = 664 , θ_{max} = 59.02° ; 15250 measured reflections, 2356 independent reflections and 1785 reflections with [$I > 2\sigma(I)$] R_1 = 0.0429, ωR_2 = 0.1067, R_{int} = 0.081.

(3*S*, 5*R*, 7*R*)- 5-Butyl-3,7-diphenyl-[1,4]diazepin-2-one 95d.

The residue was purified on silica gel using 5% isopropanol in chloroform as eluent. After evaporation of the combined collected fractions, the residue was dissolved in ether and precipitated as a hydrochloride salt by bubbling of HCl gas. The hydrochloride salt was recrystallized from a mixture of methanol and ethyl acetate to afford diazepinone **95d** (9 mg, 50% yield) as a white solid: mp 175-177 °C; R_f 0.4 (5% MeOH/DCM). ^{1}H NMR (400 MHz, MeOD) δ1.40-1.50 (m, 2H), 1.98-2.22 (m, 4H), 2.76 (q, 1H ,*J* = 9.1), 3.0-3.34(m,2H), 3.43-3.52 (m, 2H), 4.03-4.11 (m, 1H), 4.52-4.55 (m, 1H), 4.78-4.90 (m, 2H), 5.80-5.7 (m, 1H), 7.2-7.4 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$), 167.0, 136.7, 135.4, 135.3, 128.9, 128.7, 128.1, 127.7, 126.3, 126.2, 115.2, 60.5, 58.2, 51.1, 39.8, 35.5, 34.1, 31.8, 28.6. HRMS (EI) *m/z* 349.2274 [M+H$^+$; calcd for $C_{23}H_{29}N_2O$: 349.2278]. $[\alpha]_D^{20}$ -45.5° (*c* 0.007, MeOH).

The X-ray structure of **95d** was solved by direct methods using SHELXS-97, refinement was performed using SHELXL-97: ($C_{23}H_{29}N_2OCl$ (CH_3OH)) M_r = 400.95, Monoclinic, space group C2, a = 24.254 (6) Å, b = 6.643 (13) Å, c = 17.061 (3) Å, V= 2251.4 (7) Å^3, Z = 4; ρ_{calcd} = 1.183 g.cm^{-3}, μ = 1.630 mm^{-1}, *T* = 100 (2) K, *F*(0,0,0) = 664 , θ_{max} = 68.25°; 14342 measured reflections, 3404 independent reflections and 2178 reflections with [$I > 2\sigma(I)$] R_1 = 0.0429, ωR_2 = 0.2786, R_{int} = 0.106.

General Procedure (4) for the Synthesis of Ketoaldehydes 96a-d

Procedure 4a :

To a solution of homoallylic ketone **94** (1 equiv.) in a 3:1 dioxane/water solution, 2,6-lutidine (2 equiv.), OsO_4 (2.5 mol% in 2-methyl-2-propanol, 0.02 equiv., *c* 0.1 M) and $NaIO_4$ (6 equiv.) were added and the reaction was stirred at room temperature for 3 hours. After complete consumption of starting olefin **94** was observed by TLC, water (18 mL) and DCM (36 mL) were added to the mixture and the organic layer was separated. The aqueous layer was extracted with DCM (3x 25 mL). The combined organic layers were washed with brine (2x 25mL) and dried over magnesium sulphate. The volatiles were removed under vacuum and the residue was purified by silica gel column chromatography as specified.

Procedure 4b:

A solution of homoallylic ketone **94** (1 equiv.) in a 1:1 MeOH/DCM solution (c = 0.04 M) was cooled to –78 °C and treated with ozone until a blue colored solution persisted. The reaction mixture was purged at -78 °C with a stream of argon to remove excess ozone, treated with dimethyl sulfide (6 equiv.) and solid sodium bicarbonate (8 equiv.), stirred overnight, after which time the bath temperature had warmed to room temperature. Removal of solvent by rotary evaporation gave a crude product that was sufficiently pure for the next step.

(2*S*)-*N*-(Boc)Alanine *N'*-1-(3,7-dioxoheptanyl)amide 96a :

The residue was purified by silica gel column chromatography using 8:2 EtOAc/hexane as eluent to afford keto aldehyde **96a** (905 mg, yield 85%) as a brown oil: R_f 0.3 (8:2

EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, 3 H), 1.42 (s, 9 H), 2.71 (br t, 4 H), 2.77 (m, 2 H), 3.50 (d, 2 H, J = 5.92), 4.04-4.14 (m, 1 H), 5.14 (s, 1 H), 6.7 (s, 1 H), 9.77 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 207.7, 200.7, 172.5, 155.0, 79.5, 49.80, 41.6, 37.1, 34.4, 33.9, 27.9, 18.3. HRMS (EI) *m/z* 323.1577 [M+Na^+; calcd for $C_{14}H_{24}N_2O_5Na^+$ 323.1574]. $[\alpha]_D^{20}$ -11.9° (*c* 0.01, DCM)

(2*S*)-*N*-(Boc)Phenylalanine *N'*-1-(3,7-dioxoheptanyl)amide 96b:

The crude product was purified on silica gel using the solvent system 7:3 EtOAc/hexane to afford keto aldehyde **96b** (854 mg, 85% yield) as a white solid: mp 106-107 °C, R_f 0.11 (7:3 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9 H), 2.62-2.76 (m, 4 H), 2.74-2.76 (br q, 2 H), 3.04 (d, J = 6.6, 2 H), 3.41-3.45 (m, 2 H), 4.28 (br d, 1 H), 5.10 (br d, 1 H), 6.33 (s, 1 H) 7.13-7.30 (m, 5 H), 9.77 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 207.4, 199.9, 170.8, 154.9, 136.4, 128.9, 128.2, 126.4, 79.7, 55.5, 41.4, 38.4, 37.1, 34.4, 33.7, 27.9. HRMS (EI) *m/z* 399.1890 [M+Na^+; calcd for $C_{20}H_{28}N_2O_5Na$: 399.1875]. $[\alpha]_D^{20}$ 2.8° (*c* 0.005, DCM).

(2*S*, 1'*S*)-*N*-(Boc)Alanine *N'*-1-(1-denzyl-3,7-dioxoheptanyl)amide 96c:

The crude product was purified on silica gel using the solvent system 6:4 EtOAc/hexane to afford keto aldehyde **96c** (80 mg, 80% yield) as a white solid: mp 125-127 °C; R_f 0.19 (6:4 EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27-1.29 (d, 3 H, J = 6.5), 1.46 (s, 9 H), 2.66-2.71 (m, 4 H), 2.74-2.87 (m, 2 H), 2.89-2.98 (m, 2 H), 4.1 (br s, 1 H), 4.44-4.47 (br d, 1 H), 4.99-5.01 (br m, 1 H), 6.72-6.75 (br d, 1 H), 7.2-7.35 (m, 5 H), 9.8 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.6, 201.2, 173.0, 156.2, 138.6, 138.6, 130.1, 129.5, 127.6. 80.9,

51.2, 48.3, 45.5, 40.5, 38.3, 36.1, 29.2. HRMS (EI) *m/z* 413.2047 [M+Na^+; calcd for $C_{21}H_{30}N_2O_5Na$: 413.2047]. $[\alpha]_D^{20}$ -49.2° (*c* 0.005, DCM).

(2*S*, 1'*S*)-*N*-(Boc)Phenylalanine *N*'-1-(1-benzyl-3,7-dioxoheptanyl)amide 96d:

The crude product was purified on silica gel using the solvent system 6:4 EtOAc/hexane to afford keto aldehyde **96d** (85 mg, 85% yield) as a white solid: mp 125-126 °C; R_f 0.2 (4:6 EtOAc/hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9 H), 2.40-2.42 (m, 1 H), 2.54-2.59 (m, 3 H), 2.70-2.74 (br q, 2 H), 2.77-2.88 (m, 2 H), 3.00 (br m, 2 H), 4.28 (m, 1 H), 4.40 (m, 1 H), 5.04 (br s, 1 H), 6.49-6.50 (br d, 1 H), 7.12-7.31 (m, 10 H), 9.78 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$), 207.1, 199.8, 170.1, 155.0, 137.3, 136.4, 129.0, 128.8 128.2, 126.4, 126.3, 79.8, 55.5, 46.8, 43.7, 39.1, 38.2, 36.9, 34.8, 28.0. HRMS (EI) m/z 489.2365 [M+Na^+; calcd for $C_{27}H_{34}N_2O_5Na$: 489.2370]. $[\alpha]_D^{20}$ -22.8° (*c* 0.006, DCM)

General Procedure for the Synthesis of Diketones 97a-d

Olefin **94** (1 equiv.) was added as a solid with stirring to a mixture of $PdCl_2$ (2 equiv.) and CuCl (0.3 equiv.) in a 7:1 DMF/water solution (c = 0.375M) under an oxygen atmosphere. After complete consumption of olefin **94** was observed (typically 2 days, as monitored by TLC), the reaction mixture was treated with 10 mol% of aqueous citric acid solution. The layers were separated and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with water (100 mL) and brine (2 x 50 mL), dried over magnesium sulfate, filtered and concentrated under vacuum. The residue was purified by column chromatography as specified.

(2*S*)-*N*-(Boc)Alanine *N'*-1-(3,6-dioxoheptyl)amide 97a:

The residue was purified by column chromatography using 7:3 EtOAc/hexane as eluent. Evaporation of the collected fractions gave dione **97a** (895 mg, 85% yield) as a white solid: mp 83-85 °C; TLC R_f 0.11 (7:3 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.32 (d, 3 H, J = 7.2), 1.41 (s, 9 H), 2.16 (s, 3 H), 2.60-2.63 (m, 2 H), 2.68-2.73 (m, 4 H), 3.55 (q, 2 H, J = 6.0), 4.1 (br s, 1 H), 5.2 (br s, 1 H), 6.7 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 208.4, 206.9, 172.4, 154.9, 79.4, 49.8, 41.7, 36.7, 35.9, 33.9, 29.4, 27.9, 18.3. HRMS (EI) *m/z* 337.1734 [M+Na$^+$; calcd for $C_{15}H_{26}N_2O_5Na$: 337.1727].

$[\alpha]_D^{20}$ = 8.0° (*c* 0.005, DCM).

(2*S*)-*N*-(Boc)Phenylalanine *N'*-1-(3,6-dioxoheptyl)amide 97b:

The residue was purified on silica gel using 7:3 EtOAc/hexane as eluent. The evaporation of the collected fractions afforded dione **97b** (834 mg, 80% yield) as a yellow solid: mp 103-104 °C; R_f 0.37 (7:3 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 9 H), 2.17 (s, 3 H), 2.48-3.03 (m, 6 H), 3.02-3.04 (d, 2 H, J = 6.2), 3.41-3.44 (m, 2 H), 4.29-4.30 (br m, 1 H), 5.16-5.18 (br s, 1 H), 6.40 (s, 1 H), 7.18-7.29 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 208.2, 206.9, 170.7, 155.0, 136.5, 129.0, 128.2, 126.4, 79.6, 55.5, 41.6, 38.4, 36.7, 35.9, 33.8, 29.4, 27.9. HRMS (EI) *m/z* 413.2047 [M+Na$^+$; calcd for $C_{21}H_{30}N_2O_5Na$: 321.1776]. $[\alpha]_D^{20}$ -8.5° (*c* 0.005, DCM).

(2*S*, 1'*S*)-*N*-(Boc)Alanine *N'*-1-(1-benzyl-3,6-dioxoheptyl)amide 97c :

The residue was purified on silica gel using 7:3 EtOAc/hexane as eluent. The evaporation of the collected fractions afforded dione **97c** (833 mg, 80% yield) as a yellow solid: mp

125-128 °C; R_f 0.37 (60% EtOAc/hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, J = 7.0, 3 H), 1.43 (s, 9 H),1.37 (s, 1 H), 2.12 (s, 3 H), 2.52-2.86 (m, 5 H), (m, 2 H), 4.00 (br s, 1 H), 4.34-4.38 (m, 1 H), 5.23 (br s, 1 H), 6.65 (br s, 1 H), 7.12-7.25 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$), 209.3, 208.0, 172.9, 156.0, 138.7, 130.1, 129.4, 127.5, 80.6, 51.1, 48.4, 45.4, 40.7, 37.8, 37.6, 30.7, 29.2, 19.4. HRMS (EI) *m/z* 405.2384 [M+H$^+$; calcd for $C_{22}H_{33}N_2O_5$: 405.2377]. $[\alpha]_D^{20}$ -41.6° (*c* 0.005, DCM).

(2*S*, 1'*S*)-*N*-(Boc)Phenylalanine *N*'-1-(1-benzyl-3,6-dioxoheptyl)amide 97d :

The residue was purified on silica gel using 7:3 EtOAc/hexane as eluent. The evaporation of the collected fractions afforded dione **97d** (800 mg, 80% yield) as a yellow white solid: mp 138-140 °C; R_f 0.11 (4:3 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9 H), 2.20 (s, 3 H), 2.35-2.39 (m, 4 H), 2.48-2.52 (m, 2 H), 2.57-2.84 (m, 2 H), 3.00 (br s, 2 H), 4.28-4.38 (m, 2 H), 5.04 (br s, 1 H), 6.52 (br s, 1 H), 7.13-7.28 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$), 207.9, 206.7, 189.1, 170.0, 154.8, 137.4, 136.5, 129.0, 128.9, 128.2, 126.4, 126.3, 79.6, 55.6, 46.9, 43.7, 39.1, 38.3, 36.4, 36.3, 29.5, 27.9. HRMS (EI) *m/z* 481.2697 [M+H$^+$; calcd for $C_{28}H_{37}N_2O_5$: 481.2676]. $[\alpha]_D^{20}$ -24.2° (*c* 0.005, DCM)

General Procedure for the Synthesis of Pyrrolodiazepinones 98a-d and 99a-d

Method A:

Ketoaldehyde **96**, or diketone **97** (1 equiv.), was treated with a 1:1 TFA/ DCM solution for 30 minutes. The volatiles were removed under vacuum and the residue was placed under vacuum (5 mmHg) for 1 hour to remove excess TFA. The residue was dissolved in DCM (*c* 5.10^{-4} M). The mixture was cooled to 0 °C, treated with triethylamine (1.1 equiv.) and

stirred for 20 minutes. The ice bath was removed. Toluene sulfonic acid (0.02 mmol) was added to the mixture, which was stirred overnight and then treated with a saturated aqueous sodium bicarbonate solution. The layers were separated. The aqueous phase was extracted with DCM (3 x 10 mL). The combined organic laters were dried over magnesium sulphate, filtered and concentrated on a rotary evaporator to a residue that was purified by silica gel column chromatography as specified.

Method B:

The preparation of pyrrolodiazepinones **98** and **99** was performed as described above for method A, with the exception that after treatment of ketoaldehyde **7** with TFA/DCM for 5 minutes and evaporation of the excess of TFA, the residue was dissolved in DCM (*c* 5 $x10^{-4}$ M) and treated with Amberlyste A-21 (10 to 15 mmol). The pH was monitored using pH paper until the solution was pH 6 to 7. After filtration to remove the supported base, the mixture was shaken for 3 hours or until the disappearance of the starting material (as monitored by TLC).

_(S)_-2,3-Dihydro-5-methyl-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 98a :

The residue was purified on silica gel using 2:8 EtOAc/DCM as eluent to afford pyrrolodiazepinone **98a** (7mg, 30%) as a colorless oil: R_f 0.17 (2:8 EtOAc/DCM). ^1H NMR (400 MHz, acetone-d_6) δ 1.61 (d, 3H, *J* = 7.3), 2.52-2.09 (m, 2 H), 3.06-3.07 (m, 1 H), 3.84-3.89 (m ,1 H), 4.59 (q, 1 H, *J* = 7.29), 5.62 (br s, 1 H), 5.74-5.75 (m, 1 H), 6.02 (t, 1 H, *J* = 3.2), (dd, 1 H, *J* =1.7). ^{13}C NMR (100 MHz, acetone-d_6) δ 170.8, 129.9, 116.7, 108.4, 106.0, 55.1, 36.7, 25.2, 16.6. HRMS (EI) *m/z* 165.1029 [$M+H^+$; calcd for $C_9H_{13}N_2O$: 165.1022]. $[\alpha]_D^{20}$ -106.8° (*c* 2.5.10^{-3}, DCM).

(S)-5-Benzyl-2,3-dihydro-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 98b :

The residue was purified on silica gel using 3:7 EtOAc/hexane as eluent to afford pyrrolodiazepinone **98b** (10 mg, 40% yield) as a colorless oil: R_f 0.17 (3:7 EtOAc/hexane). ^{1}H NMR (400 MHz, acetone-d_6) 2.1 (s, 2H), 2.15-2.33 (m, 2H), 3.69-3.72 (br d, 2H), 4.61-4.63 (m, 1H), 5.60 (s, 1H), 6.0 (s, 1H), 6.7 (s, 1H), 6.83 (s, 2H), 7.05-7.14 (m, 4H). ^{13}C NMR (100 MHz, acetone-d_6), 170.07, 137.67, 132.9, 128.42, 127.65, 125.92, 117.23, 108.78, 105.63, 61.0, 41.36, 38.61, 23.69. HRMS (EI) *m/z* 503.2412 [2M+Na^+; calcd for $C_{30}H_{32}N_4O_2Na^+$: 503.2417]. $[\alpha]_D^{20}$ -132.7° (*c* 0.008, DCM).

(2*S*,5*S*)-2-Benzyl-2,3-dihydro-5-methyl-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 98c:

The residue was purified on silica gel using 4:6 EtOAc/hexane as eluent to afford pyrrolodiazepinone **98c** (14 mg, 55%) as a colorless oil: R_f 0.24 (4:6 EtOAc/hexane). ^{1}H NMR (400 MHz, acetone-d_6) δ 1.60 (d, 3 H, *J* = 7.0), 1.83-2.92 (m, 4 H), 3.14-3.18 (m, 1 H), 4.05-4.07 (br m, 1 H), 5.053 (t, 1H, *J* = 6.8), 5.88 (s, 1 H), 5.97 (t, *J* = 3.2, 1 H), 6.55 (br s, 1 H), 6.7 (d, *J* = 1.2, 1 H), 7.28-7.36 (m, 5 H). ^{13}C NMR (100 MHz, acetone-d_6) δ 170.0, 137.5, 129.1, 128.3, 128.2, 126.3, 117.5, 107.7, 106.3, 54.5, 53.8, 41.5, 30.0, 15.8. HRMS (EI) *m/z* 255.1498 [M+Na^+; calcd for $C_{16}H_{19}N_2O$: 255.1491]. $[\alpha]_D^{20}$ -17.3° (*c* 0.007, DCM).

(2*S*, 5*S*)-2,5-Dibenzyl-2,3-dihydro-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 98d :

The residue was purified on silica gel using 4:6 EtOAc/hexane as eluent to afford pyrrolodiazepinone **98b** (17 mg, 60%) as an colorless oil R_f 0.4 (4:6 EtOAc/hexane). ^{1}H NMR (400 MHz, acetone-d_6) δ 2.89-3.02 (m, 4 H), 3.27-3.31 (m, 2 H), 3.85-3.89 (m, 1 H),

5.0 (q, J = 3.3, 1H), 5.73-5.74 (m, 1H), 5.77 (s, 1H), 6.28 (br s, 1H), 6.55 (s, 1H), 6.98-7.36 (m, 10 H). ^{13}C NMR (100 MHz, acetone-d_6) δ 169.4, 137.0, 129.2, 128.8, 128.7, 128.2, 127.7, 126.4, 126.1, 121.1, 107.4, 105.9, 64.1, 55.6, 42.2, 39.0, 30.1, 29.2. HRMS (EI) *m/z* 331.1809 [M+Na$^+$; calcd for $C_{22}H_{23}N_2O$: 331.1804]. $[\alpha]_D^{20}$ 16.4° (*c* 0.0075, DCM).

(*S*)-2,3-Dihydro-5,7-dimethyl-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 99a :

The residue was purified on silica gel using 2:8 hexane/diethyl ether as eluent to afford pyrrolodiazepinone **99a** (36 mg, 65% yield) as a white solid: mp 114-116 °C; R_f 0.26 (diethyl ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (d, 3 H, J = 7.3), 2.23 (s, 3 H), 3-3.2 (m, 2 H), 3.41-3.53 (m, 2 H), 4.94 (q, 1 H, J = 7.3), 5.81 (s, 1 H), 5.84 (d, 1 H, J = 2.8), 7.25 (br s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 129.9, 128.8, 106.2, 105.7, 56.5, 43.8, 26.5, 21.3, 12.8. HRMS (EI) *m/z* 179.1789 [M+H$^+$; calcd for $C_{10}H_{15}N_2O$: 179.1186]. $[\alpha]_D^{20}$ 16.4° (*c* 0.001, EtOH).

(*S*)-5-Benzyl-7-methyl-2,3-dihydro-1H-pyrrolo[1,2-d][1,4]diazepin-4-one (99b):

The residue was purified on silica gel using 20% hexane in ether as eluent to afford pyrrolodiazepinone **99b** (32 mg, 50% yield) as an oil: R_f 0.16 (1:1 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3 H), 3.04-3.22 (br d, 2 H), 3.25-3.53 (m, 4 H), 4.86-4.9 (m, 1H), 5.6 (s, 1 H), 5.88 (d, 1 H, J = 2.3), 6.84-7.21 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.8, 136.3, 130.5, 129.8, 126.1, 128.0, 126.7, 106.0, 105.4, 63.3, 44.6, 41.9, 26.6, 11.3. HRMS (EI) *m/z* 277.1305 [M+Na$^+$; calcd for $C_{16}H_{18}N_2ONa$: 277.1311]. $[\alpha]_D^{20}$ 48.1° (*c* 0.008, DCM).

(2*S*,5*S*)-2-Benzyl-2,3-dihydro-5,7-dimethyl-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 99c :

The residue was purified on silica gel using 2:8 hexane/diethyl ether as eluent to afford pyrrolodiazepinone **99c** (38 mg, 55% yield) as an oil: R_f 0.38 (diethyl ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (d, 3 H, J = 6), 2.22 (s, 3 H), 2.75-2.78 (m, 1 H), 2.78-3.04 (m, 3 H), 3.75-3.80 (m, 1 H), 4.95 (q, 1 H, J = 7.4), 5.80 (s, 2 H), 5.84-8.85 (d, 1 H, J = 2.7), 7.22-7.39 (m, 5 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 135.6, 128.9, 128.8, 128.5, 128.4, 127.1, 106.0, 105.7, 56.3, 56.0, 43.3, 32.1, 21.4, 11.8. HRMS (EI) *m/z* 291.1468 [M+Na$^+$; calcd for $C_{17}H_{20}N_2ONa$: 291.1459]. $[\alpha]_D^{20}$ 59.0° (*c* 0.017, DCM).

(2*S*,5*S*)-2,5-Dibenzyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-g][1,4]diazepin-4(5H)-one 99d :

The residue was purified on silica gel using 4:6 EtOAc/hexane as eluent to afford pyrrolodiazepinone **99d** (36 mg, 50% yield) as an oil: R_f 0.4 (4:6 EtOAc/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3 H), 2.75-2.80 (m, 2 H), 2.99-3.07 (m, 2 H), 3.28-3.38 (m, 2 H), 3.78-3.80 (br d, 1 H), 4.86-4.89 (br d, 1 H), 5.60 (s, 1 H), 5.87 (s, 1 H), 5.98 (s, 1 H), 7.21-7.40 (m, 10 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 136.2, 135.5, 130.5, 129.1, 129.0, 128.8, 128.2, 128.0, 127.1, 126.7, 106.3, 150.4, 62.8, 56.6, 43.3, 41.8, 32.3, 11.2. HRMS (EI) *m/z* 345.1961 [M+H$^+$; calcd for $C_{23}H_{25}N_2O$: 345.1967] $[\alpha]_D^{20}$ - 47.7° (*c* 0.002, DCM)

Macrocycle 100 (dimmer):

As described for the synthesis of pyrrolodiazepinones **98a-d** and **99a-d**, with the exception that the concentration of solution after Boc removal was $5x10^{-2}$ M. The residue was purified by reverse-phase HPLC to afford macrocycle **100** as a solid (6 mg, 20% yield), R_f 0.26 (diethyl ether). ^1H NMR (400 MHz,$CDCl_3$), 1.66 (d, 6H, *J*= 7.4), 2.2 (s, 6H), 2.38-2.42 (m, 2H), 2.72 -2.80 (m, 4H), 3.08-3.15 (m, 2H), 4.2-4.27 (m, 2H), 4.74 (q, 2H, *J* = 7.2), 5.62 (s, 2H), 5.80 (d, 2H, *J* =2.7). ^{13}C NMR (100 MHz, $CDCl_3$), 170.8, 128.8, 127.2, 106.8, 105.1, 53.2, 35.1, 28.1, 15.9, 12.4.

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CHAPTER 3

1,3,5-Trisubstituted 1,4-Diazepin-2-ones

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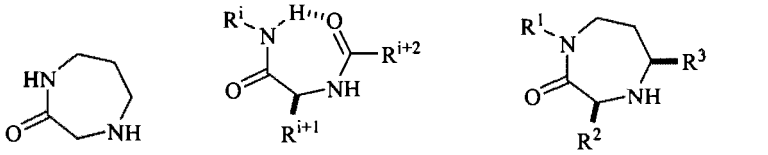
3.1 Abstract

Six 1,3,5-trisubstituted 1,4-diazepin-2-ones were synthesized by a sequence featuring the cascade addition of vinyl Grignard to *N*-[Boc-aminoacyl]-*N*-alkyl-β-amino esters, followed by Boc group removal and annulation by a reductive amination process. Relative to the parent sequence employing *N*-Boc-aminoacyl β-amino esters to make 3,5-disubstituted heterocycle, the additional *N*-alkyl substituent caused a noticeable acceleration and gave relatively higher yield in the annulation reaction to provide 1,4-diazepin-2-one.

3.2 Introduction

Mimicry of peptide conformations by privileged organic structures has proven effective for identifying candidates for drug development.[1] For example, 1,4-diazepin-2-ones have been suggested to function as privileged structures in medicinal chemistry,[2] because the seven-member diazepinone ring may mimic β- and γ-turn secondary structures.[3,4] In X-ray structural analyses of 1,4-diazepinones,[5] the conformation of the α-amino acid moiety in the ring system mimicked the dihedral angle geometry of an ideal reverse γ-turn (ψ = 60 to 70°, ϕ = –70 to –85°, Figure 3.1).[6] γ-Turn conformations have been suggested to be populated by many biologically active peptides, such as angiotensin II, bradykinin, Leu-enkephalin, oxytocin and urotensin.[7,8] Furthermore, biologically active 1,4-diazepinones include antagonists of the lymphocyte function-associated antigen-1/ immunoglobulin superfamily ICAM-1 interaction,[9] the liposidomycin family of nucleoside antibiotics that inhibit bacterial peptidoglycan synthesis,[10] as well as anticonvulsants.[11] The development of

methodology for the synthesis of 1,4-diazepin-2-ones is thus well merited with potential for creating new biologically active peptide mimics.



1,4-Diazepin-2-one γ-Turn 1,3,5-Trisbstituted-1,4-diazepin-2-one

Figure 3.1 Structural relationship between γ-turn and 1,4-diazepin-2-ones.

The diazepinone ring has been typically formed from linear precursors by intramolecular reductive amination,[12] lactam formation,[13,14] and transamidation reactions.[15] For example, employing a reductive amination reaction, a library of 1,4-diazepinones was generated by a solid-phase approach.[16] A sequence employing Ugi multicomponent and Mitsunobu annulation reactions has also delivered libraries of 1,4-diazepin-1- and -5-ones. [17]

We recently reported a general method for the synthesis of enantiopure 3,5-disubstituted 1,4-diazepin-2-ones from inexpensive amino acids.[5] This process involved the cascade addition of vinyl magnesium bromide to an α-aminoacyl β-amino ester to provide a γ,δ-unsaturated ketone intermediate.[18] Nitrogen deprotection and intramolecular reductive amination provided the 1,4-diazepinone.[5]

In light of the relationship between 1,4-diazepin-2-ones and γ-turn conformations, the display of substituents on the 1-, 3- and 5-positions of the heterocycle could respectively mimic the pharmacophores at the i, i + 1 and i + 2 residues in the γ-turn (Figure 1). Methodology for introducing substituents at the 3- and 5-positions was previously achieved by the employment of different α-amino acid starting materials and by the modification of

the olefin of the γ,δ-unsaturated ketone, respectively.[5] Employing α-aminoacyl β-amino esters possessing *N*-alkyl substituents, we have now elaborated our synthesis methodology to introduce functionality at the N_1-position of the 1,4-diazepin-2-one. Moreover, the presence of the *N*-alkyl substituent was noted to accelerate ring closure in the reductive amination step and improve the yield of diazepinone.

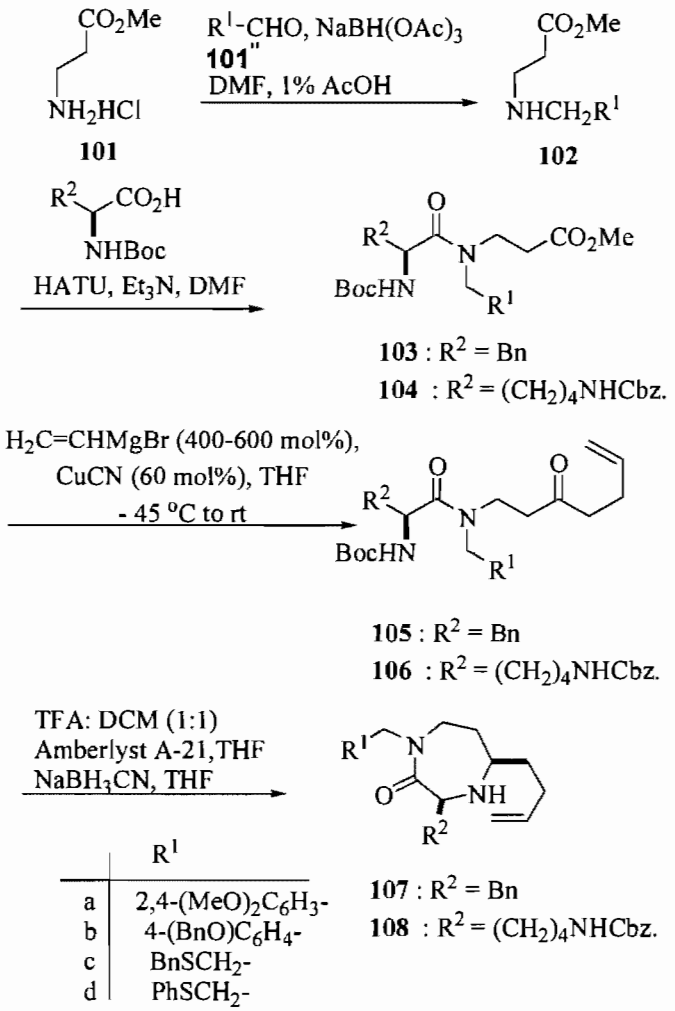
3.3 Results and Discussion

α-Aminoacyl β-amino esters possessing *N*-alkyl substituents were initially prepared from coupling of *N*-Boc-α-amino acids to *N*-alkyl β-amino esters. Methyl *N*-alkyl β-aminopropionate derivatives **102a-d** were synthesized by reductive amination of the imine generated from methyl *β*-alaninate **101** and the respective aldehyde **101''** with sodium triacetoxy borohydride and 1% acetic acid in DMF.[19] *N*-Alkyl β-amino esters **102a-d** were isolated from the reductive amination procedure in 70-93% yield after purification by chromatography on silica gel (Scheme 1). The requisite aldehydes were usually obtained from commercial sources. Benzylthioacetaldehyde was prepared by oxidation of 2-benzylthioethanol using CrO_3•pyridine.[20]Alternatively, benzyl and phenylthioacetaldehydes were prepared by alkylation of the respective thiol with 3-bromo-1,1-dimethoxyethane, followed by acetal hydrolysis using 2N HCl at 80 °C for 24 h.[21] 2,4-Dimethoxy- and 4-benzyloxybenzyl amino esters **102a** and **102b** exhibited identical spectral data as previously described.[22, 23]

Dipeptides **103a-d** and **104a,b** were respectively prepared by coupling either Boc-Phe or α-Boc-(ε-Cbz)-Lys to secondary amino esters **2** using HATU in DMF,[24] and isolated by silica gel chromatography in 55-90% yield. Lysine was employed as the α-amino acid,

because of the importance of constrained ε-amino alkyl amino acids,[25] as well as to examine the influence of a remote carbamate group on the synthesis method. Homoallylic ketones **105a-d** and **106a,b** were synthesized in 35-60% yields by respectively treating α-aminoacyl β-amino esters **103a-d** and **104a,b** with an excess of freshly prepared vinyl magnesium bromide in the presence of a catalytic amount of CuCN in THF at -45 °C followed by warming to rt.[18]

Diazepinones **107a-d** and **108a,b** were respectively synthesized from γ,δ-unsaturated ketones **105a-d** and **106a,b** by removal of the Boc group using a 1:1 TFA:DCM solution, free-basing of the amine TFA salt using Amberlyste A-21 ion exchange resin,[26] and reductive amination of the imine intermediate with sodium cyanoborohydride in THF (Scheme 3.1). We observed only one diastereoisomer by LCMS for diazepinones **107a,b,d** and **108a,b**; diazepinone **107b** was observed to consist of a 30:1 mixture of diastereoisomers. The relative configuration of the newly formed stereocentre in diazepinones **107a-d** and **108a,b** was assigned to have a *cis*-relationship based on our previous studies of 1,4-diazepin-2-ones.[5] The *cis* assignment was confirmed by a NOESY experiment in which strong NOE was observed between the C_3 and C_5 protons of diazepinone **107b.**



	R1
a	2,4-$(MeO)_2C_6H_3$-
b	4-$(BnO)C_6H_4$-
c	$BnSCH_2$-
d	$PhSCH_2$-

Scheme 3.1 1,3,5-Trisubstituted diazepin-2-one synthesis.

[a]% Yields	a	b	c	d
Amide				
103	77	82	90	77
104	55	63		
Ketone				
105	66	50(78)	60	35
106	62	70		
Diazepine				
107	68	85	65	66
108	71	78		

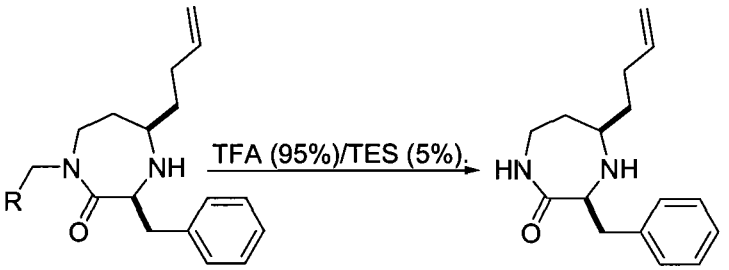
[a]% Yields refer to isolated yields, yield in parentheses accounts for recovered starting material

Table 3.1 Product Yields.

In our previous synthesis of 3,5-disubstituted 1,4-diazepin-2-ones from α-aminoacyl β-amino esters, the final amine deprotection / free-basing / reductive amination sequence gave at best 50% overall yield.[5] A significant improvement in overall yield was obtained from performing this sequence on the corresponding tertiary amides **103** and **104** to form 1,3,5-trisubstituted 1,4-diazepin-2-ones **107** and **108**. For example, a 45% augmentation in overall yield was obtained from performing the Boc removal / reductive amination sequence on *N*-(4-benzyloxylbenzyl) amide **107b** instead of the corresponding secondary amide. The increase in yield was accompanied by a noticeable acceleration in the reductive-amination step, which had usually occurred in 3 to 7 days in the absence of the *N*-alkyl substituent.[5] In the presence of *N*-alkyl substituent, the reductive amination was typically complete after 24 h. The *N*-alkylation caused likely steric effects that destabilized the *trans* amide isomer and augmented the population of the *cis* conformer, that was necessary for ring formation.[27,28] Characterization of tertiary amides indicated mixtures of *cis* and *trans* amide isomers identified by multiple signals due to slow amide isomerization on the NMR time scale. In addition, the trisubstituted diazepinone products were less polar than their disubstituted counterparts and easier to purify by silica gel chromatography, which may also account for the better yields in the annulation sequence.

The removal of the 1-position substituent was also explored using trisubstituted diazepinones **107** in order to provide an alternative strategy for making 3,5-disubstituted diazepinone **95b.** Attempts failed to remove the mercaptoethyl moieties by oxidation of the sulfur to the sulfone followed by β-elimination. The presence of olefin and secondary amine groups in diazepinones **107c** and **107d** prevented selective sulfur oxidation such that multiple products were formed using conditions such as hydrogen peroxide in the presence

of catalytic amounts of ammonium heptamolybdate tetrahydrate,[29] Oxone,[30] and *m*-CPBA.[31]



107b : R = 4-BnO-C_6H_4-
109 : R = 4-HO-C_6H_4-

95b

Equation 3.1 4-Benzyloxybenzylamide cleavage under acidic conditions.

Efforts were next committed to the removal of the 4-benzyloxybenzyl group from diazepinone **107b**. Attempts failed to remove the 4-benzyloxybenzyl protecting group employing oxidative conditions at room temperature, such as CAN (ceric ammonium nitrate)[32] and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[33] and only starting material **107b** was recovered. Trace amounts of desired diazepinone **95b** and 1-hydroxybenzyl diazepinone **109** were observed by LCMS to form at 100 °C using CAN in acetonitrile:water; however, decomposition ensued on prolonged exposure of diazepinone **107b** to these conditions.

Alternatively, deprotection of the 4-benzyloxybenzyl group was successfully accomplished under acidic conditions. Starting material remained unaffected using trifluoroacetic acid (TFA) in DCM (1:1) at room temperature after 2 days. Employment of a 95:5 mixture of TFA/triethylsilane (TES)[34] at room temperature for 1 day gave some diazepinone **95b** along with its 1-hydroxybenzyl counterpart **109**. After stirring under the TFA/TES conditions at

80° C for 3 days, diazepinones **109** and **95b** were respectively isolated in 25% and 70% yields after purification by column chromatography (Eq 3.1).

3.4 Conclusion

1,4-Diazepin-2-ones with substituents at the 1-, 3- and 5-positions of the heterocycle have been synthesized by an effective method featuring the copper-catalyzed cascade addition of vinyl Grignard reagent to *N*-alkyl *N*-Boc-α-aminoacyl β-amino esters **103a-d** and **104a,b**. Trisubstituted diazepinones **107a-d** and **108a,b** were respectively synthesized from phenylalanine and lysine to provide diversity at the 3-position. Furthermore, a variety of 1-position substituents were introduced by reductive aminations of different aldehydes onto the β-amino ester precursor. A cursory study of the removal of the 1-position thioethyl and benzyl amide substituents demonstrated that the 4-benzyloxybenzyl group may be cleaved under acid conditions to provide an alternative route to 3,5-disubstituted 1,4-diazepin-2-ones. A study to extend this approach for generating a library of diazepinones is currently in progress in our laboratory.

3.5 Acknowledgment

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3.6 Experimental Section

α-Amino acid derivatives were *N*-protected using di-*tert*-butyl dicarbonate in dioxane:water 1:1.[35] Reagent-grade solvents were purified and dried by passage through solvent filtration systems prior to use. For reactions performed under anhydrous conditions, glassware was either oven- or flame-dried and the reaction was performed under a positive pressure of argon. Column chromatography was performed on silica gel (230-400 mesh) and thin layer chromatography (TLC) was performed on glass plates coated with (0.2 mm) silica gel, using the indicated solvent system. Proton (and carbon) NMR spectral data were collected at 300 MHz (75 MHz) and 400 MHz (100 MHz). Proton chemicals shifts (δ) were reported in parts per million (ppm) and referenced to the residual solvent peak at 7.26 ppm in $CDCl_3$, unless otherwise noted. The ^{13}C NMR spectra were reported in ppm, and referenced to the residual solvent peak at 77 ppm in $CDCl_3$, unless otherwise noted. Coupling constants *J* are reported in Hz. The NMR spectra of tertiary amides **103-106** exhibited usually a mixture of amide isomers, the minor isomer signals for which are denoted by an asterisk. Melting points were determined with a capillary melting point apparatus and are uncorrected. High Resolution Mass Spectrometry (HRMS) was performed by Electrospray Ionisation (ESI).

General Protocol 1, Synthesis of Secondary Amine 102.

A 100 mL flask was charged with 1% acetic acid in DMF (*c* 0.8 M), β-alanine methyl ester hydrochloride salt (12 mmol, 200 mol%) and a magnetic stir bar. To the flask, $NaBH(OAc)_3$ (12 mmol, 200 mol%) was added generating a white turbid suspension, that was stirred for 10 minutes and treated dropwise with a solution of the respective aldehyde

(6 mmol, 100 mol%, *c* 0.4 M) in DMF. The suspension was stirred for 1 hour. Completion of the reaction was monitored by TLC examining the disappearance of aldehyde (10% EtOAc in hexanes). The volatiles were then removed on a rotary evaporator. The residue was partitioned between saturated aqueous $NaHCO_3$ solution (50 mL) and ether (100 mL). The aqueous phase was separated and extracted with ether (3 x 50 mL). The combined organic phase was dried over magnesium sulphate, filtered and evaporated to a residue that was purified on silica gel using the specified solvent as eluant, as described below, to afford secondary amino esters **102a-d** as oils.

Methyl 3-*N*-(2,4-dimethoxybenzyl)aminopropanoate (102a).

Amine **102a** was prepared from aldehyde **101"a** (6 mmol, 1.08 g) according to the general protocol 1 and isolated in 85% yield after purification on silica gel using 5% MeOH/DCM as eluant: R_f 0.28 (5% MeOH/DCM). ^{1}H NMR δ 2.56 (t, J = 6.5, 2H), 2.87 (t, J = 6.5, 2H), 3.68 (s, 3H), 3.75 (s, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 6.44 (br m, 2H), 7.13 (d, J = 7.9, 1H). ^{13}C NMR δ 174.0, 161.1, 159.5, 131.3, 120.8, 104.6, 99.4, 56.2, 56.1, 52.4, 49.4, 44.9, 35.1. HRMS (EI) *m/z* 254.1397 $[M+H]^+$; calcd for $C_{13}H_{20}NO_4$: 254.1387.

Methyl 3-*N*-(4-benzyloxybenzyl)aminopropanoate (102b).

Amine **102b** was prepared from aldehyde **101"b** (6 mmol, 0.996 g) according to the general protocol 1 and isolated in 93% yield after purification on silica gel using 5% MeOH/DCM as eluant: R_f 0.38 (5% MeOH/DCM). ^{1}H NMR δ2.58 (m, 2H), 2.91 (t, J = 6.5, 2H), 3.68 (s, 3H), 3.71 (s, 2H), 5.06 (s, 2H), 6.93-7.45 (m, 9H). ^{13}C NMR δ 174.1,

158.8, 137.9, 132.5, 130.3, 129.4, 128.8, 128.3, 115.8, 70.9, 53.8, 52.7, 45.0, 34.6. HRMS (EI) *m/z* 300.1597 $[M+H]^+$; calcd for $C_{18}H_{21}NO_3$: 300.1594.

Methyl 3-(2-(benzylthio)ethylamino)propanoate (102c).

Amine **102c** was prepared from aldehyde **101"c** (6 mmol, 0.997 g) according to the general protocol 1 and isolated in 80% yield after purification on silica gel using 3% MeOH/EtOAc as eluant: R_f 0.11 (3% MeOH/EtOAc). ^{1}H NMR δ 2.51 (t, *J* = 6.4, 2H), 2.59 (t, *J* = 6.8, 2H), 2.77 (t, *J* = 6.4, 2H), 2.86 (t, *J* = 6.8, 2H), 3.70 (s, 3H), 3.73 (s, 2H), 7.28-7.33 (m, 5H). ^{13}C NMR δ 172.7, 138.0, 128.5, 128.2, 126.7, 51.3, 47.7, 44.3, 35.7, 34.1, 31.1. HRMS (EI) *m/z* 254.1209 $[M+H]^+$; calcd for $C_{13}H_{19}NO_2S$: 254.1211.

Methyl 3-(2-(phenylthio)ethylamino)propanoate (102d).

Amine **102d** was prepared from aldehyde **101"d** (6 mmol, 0.913 g) according to the general protocol 1 in 70% yield, after purification on silica gel using 3% MeOH/EtOAc as eluant: R_f 0.11 (3 % MeOH/EtOAc). ^{1}H NMR δ 2.52 (t, *J* = 6.8, 2H), 2.78 (t, *J* = 6.8, 2H), 2.85 (t, *J* = 6.4, 2H), 3.01 (t, *J* = 6.8, 2H), 3.08 (t, *J* = 6.8, 2H), 3.32-3.33 (m, 1H), 7.20-7.42 (m, 5H). ^{13}C NMR δ 172.7, 135.3, 129.3, 128.6, 125.9, 51.3, 47.7, 44.2, 34.1, 33.7. HRMS (EI) *m/z* 240.1053 $[M+H]^+$; calcd for $C_{12}H_{17}NO_2S$: 240.1057.

General Protocol 2, Synthesis of Tertiary Amides 103a-d and 104a,b.

N-Boc-α-Amino acid (*N*-(Boc)-Phe or *N*-(Boc)-ω-(Cbz)-Lys, 2.2 mmol) and HATU (2.2 mmol) were placed in a 100 mL round bottom flask, dissolved in DMF (*c* 0.4 M), cooled to 0 °C, treated with DIEA (4.4 mmol), stirred for 10 min under argon and treated with a solution of secondary amine **102** (2 mmol) in DMF (*c* 0.8 M). The reaction mixture was stirred overnight at room temperature. The solvent was concentrated on a rotary evaporator, and the reduced volume was partitioned between an aqueous HCl solution (10%, 50 mL) and ether (50 mL). The aqueous phase was separated and extracted with ether (3 x 20 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (2 x 30 mL) and brine (2 x 50 mL), dried over magnesium sulphate, filtered and evaporated to a residue, that was purified on silica gel using the specified solvent as eluant to afford respectively tertiary amide **103a-d**, **104a** or **104b**.

(2*S*)-Methyl-*N*-(2,4-dimethoxybenzyl)-*N*-[(Boc)-phenylalaninyl]-β-alaninate (103a)

A colorless oil was prepared from amine **102a** (2 mmol, 0.506 g) according to the general protocol 2 and isolated in 77% yield after purification on silica gel eluting with 40% EtOAc /hexane, (R_f 0.4, 40% EtOAc/hexane). ^{1}H NMR (1.6:1 isomer ratio, 400 MHz, CD_3OD) δ 1.39 (s, 9H), 1.4* (s, 9H), 2.4* (m, 2H), 2.45 (m, 2H), 2.90-2.93 (m, 2H), 2.95-2.95* (m, 2H), 3.42-3.50 (m, 2H), 3.59*(s, 3H), 3.60 (s, 3H), 3.74 (s, 6H), 3.78 (s, 6H), 4.24-4.70 (m, 2H), 5.0 (br d, 1H), 6.41-7.20 (m, 8H). ^{13}C NMR (2:1 isomer ratio, 100 MHz, CD_3OD) δ 172.4*, 172.0, 171.9, 171.3*, 160.7, 160.2*, 158.2, 158.1*, 155.6*, 155.2, 136.7*, 136.5, 129.5*, 129.3, 128.9, 126.2, 126.0*, 116.4*, 115.8, 103.9*, 103.8, 97.9, 97.4*, 78.8*, 78.7,

54.2, 51.7, 46.7, 42.6*, 42.2*, 41.4, 38.8*, 38.2, 32.2, 31.1, 27.1. $[\alpha]_D^{20}$ –7.1° (*c* 0.008 M , $CHCl_3$). HRMS (EI) *m/z* 523.2425 [M+Na^+; calcd for $C_{27}H_{36}N_2O_7Na$: 523.2429].

(2*S*)-Methyl *N*-(4-(benzyloxy)-benzyl)-*N*-[(Boc)phenylalaninyl]-β-alaninate (103b).

A white solid (mp 82-83 °C) was prepared from amine **102b** (2 mmol, 0.6 g) according to the general protocol 2, purified on silica gel eluting with 40% EtOAc/hexane (R_f 0.34, 40% EtOAc/hexane), and isolated in 82% yield. ^{1}H NMR (1.3:1 isomer ratio, 400 MHz, CD_3OD) δ 1.36 (s, 9H), 1.40* (s, 9H), 2.44-2.48 (t, *J* = 8 Hz, 2H), 2.86-2.2.91 (m, 2H), 2.92-2.93* (m, 2H), 3.44-3.51 (m, 2H), 3.57* (s, 3H), 3.59 (s, 3H), 4.39-4.86 (m, 2H), 4.79-4.82 (br t, 1H), 5.01 (s, 2H), 6.86-7.40 (m, 14H). ^{13}C NMR (1:1 isomer ratio, 100 MHz, CD_3OD) δ 172.5*, 172.3, 171.9, 171.3*, 156.0, 157.8*, 155.7*, 155.5, 136.9, 136.8, 136.4*, 128.9, 128.8, 128.6, 128.3, 127.8, 127.2, 126.8, 126.2*, 126.1, 114.5, 114.3*, 78.9*, 78.8, 69.2, 51.6, 50.5*, 50.4, 41.9, 38.3, 32.1, 31.1, 27.1. $[\alpha]_D^{20}$ –7.1° (*c* 0.012 M, $CHCl_3$). HRMS (EI) *m/z* 547.2807 [M+H]$^+$; calcd for $C_{32}H_{38}N_2O_6$: 547.2803.

(2*S*)-Methyl *N*-(2-benzylthio)-ethyl-*N*-[(Boc)-phenylalaninyl]-β-alaninate (103c).

A white solid (mp 73-74 °C) was prepared from amine **102c** (2 mmol, 0.506 g) according to the general protocol 2, purified on silica gel eluting with 25% EtOAc/hexane (R_f 0.2, 25% EtOAc/hexane), and isolated in 90% yield. ^{1}H NMR (1.7:1 ratio of amide isomers, 400 MHz, CD_3OD) δ 1.39 (s, 9 H), 1.41*(s, 9 H), 2.28-2.5 (m, 4 H), 2.85-2.98 (m, 2 H), 3.2 (br, 1 H), 3.33-3.42 (m, 2 H), 3.51 (m, 1 H), 3.67 (s, 3 H), 3.74 (s, 2 H), 3.75* (s, 2 H), 4.60-4.71 (m, 1 H), 6.83-7.35 (m, 10 H). ^{13}C NMR (1.7:1 ratio of amide isomers, 100 MHz, CD_3OD) δ 172.3*, 171.9, 171.3, 155.5, 138.3, 138.1*, 136.4, 128.8, 128.4, 128.4, 127.8,

127.7, 126.4, 126.3, 126.2*, 78.8, 51.5*, 50.5, 47.1, 42.9*, 42.3, 38.2, 35.1*, 34.9*, 32.2, 31.2, 28.4, 27.0*, 26.9. $[\alpha]_D^{20}$ 3.23° (*c* 0.015 M, $CHCl_3$). HRMS (EI) *m/z* 501.2418 $[M+H]^+$; calcd for $C_{27}H_{36}N_2O_5S$: 501.2422.

(2*S*)-Methyl *N*-(2-phenylthio)-ethyl-*N*-[(Boc)-phenylalaninyl]-β-alaninate (103d).

A colorless oil was prepared from amine **102d** (2 mmol, 0.478 g) according to the general protocol 2, purified on silica gel using 25% EtOAc/hexane (R_f 0.26, 25% EtOAc/hexane), and isolated in 77% yield. ^1H NMR (1:1 amide isomer ratio, 300 MHz, CD_3OD) δ 1.39 (s, 9H), 1.43* (s, 9H), 1.86 (m, 2H), 2.48-3.01 (m, 4H), 3.23-3.37(m, 3H), 3.49-3.54 (br t, 1H), 3.63 (s, 3H), 4.49-4.72 (m, 1H), 6.75-7.45 (m, 10H). ^{13}C NMR (1:1 isomer ratio, 75 MHz, CD_3OD) δ 173.7, 173.1, 172.5*, 156.7, 137.6*, 137.2, 136.6*, 135.6, 130.8, 130.1, 129.9*, 129.7, 129.6, 129.2*, 129.0, 127.4, 127.3*, 126.5, 80.0*, 79.9, 52.8*, 51.7, 47.1, 44.6*, 43.7, 39.3, 33.6*, 32.5, 31.7*, 30.3, 28.2. $[\alpha]_D^{20}$ 5.44° (*c* 0.016 M, $CHCl_3$). HRMS (EI) *m/z* 487.2277 $[M+H]^+$; calcd for $C_{26}H_{34}N_2O_5S$: 487.2261.

(2*S*)-Methyl-*N*-[ε-*N*-Cbz-α-*N*-(Boc)-lysinyl]-*N*-(2,4-dimethoxybenzyl)-β-alaninate (104a).

A colorless oil was prepared from amine **102a** (2 mmol, 0.506 g) according to the general protocol 2, purified on silica gel eluting with 50% EtOAc/hexane (R_f 0.24, 50% EtOAc/hexane), and isolated in 55% yield. ^1H NMR (1:1 isomer ratio, 400 MHz, CD_3OD) δ 1.44-1.45 (br, 17H), 2.41-2.54 (m, 2H), 3.08-3.01 (m, 2H), 3.49-3.53 (m, 2H), 3.55 (s, 3H), 3.57* (s, 3H), 3.78* (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 4.89 (s, 2H), 5.07 (s, 2H), 6.47-7.35 (m, 8H). ^{13}C NMR (1.5:1 isomer ratio, 100 MHz, CD_3OD) δ 173.0, 172.1, 160.9,

158.3, 157.0, 136.7, 129.4, 127.7, 127.2, 127.0, 115.9, 103.9*, 103.7, 97.8, 97.4*, 78.6, 65.5, 54.1, 50.4, 47.9, 47.7, 42.1, 41.5, 39.7, 32.4, 31.9, 31.1, 28.8, 27.0, 22.2*, 22.0. $[\alpha]_D^{20}$ –20.4° (*c* 0.02 M, $CHCl_3$). HRMS (EI) *m/z* 616.3230 $[M+H]^+$; calcd for $C_{32}H_{45}N_3O_9$: 616.3229.

(2*S*)-Methyl *N*-[ω-Cbz-N-(Boc)lysinyl]-*N*-(4-(benzyloxy)benzyl)-β-alaninate (104b).

A white solid (mp 77-79 °C) was prepared from amine **102b** (2 mmol, 0.6 g) according to the general protocol 2, purified on silica gel eluting with 30% EtOAc/toluene (R_f 0.2, 30% EtOAc/toluene), and isolated in 63% yield. ^1H NMR (1:1 isomer ratio, 400 MHz, CD_3OD) δ 1.26-1.66 (br, 17H), 2.53-2.57 (m, 2H), 2.72-2.8* (m, 2H), 3.06-3.55 (m, 2H), 3.55-3.63 (m, 2H), 3.68 (s, 3H), 4.46-4.69 (m, 2H), 4.89 (s, 2H), 5.07 (s, 2H), 6.75-7.42 (m, 14). ^{13}C NMR (1:1 isomer ratio, 100 MHz, CD_3OD) δ 173.4*, 173.3, 172.0, 171.5*, 158.1, 157.9*, 157.1, 156.0*, 136.9, 136.7, 129.0, 128.6, 128.4, 127.9, 127.7, 127.2, 127.1, 127.0, 126.8, 114.5, 114.3*, 78.7, 69.2, 65.5, 50.5, 50.3, 42.2, 42.1*, 39.7, 39.6, 32.4, 31.1, 28.7, 26.7, 22.2, 22.1*. $[\alpha]_D^{20}$ –7.9° (*c* 0.026 M, $CHCl_3$). HRMS (EI) *m/z* 662.3438 $[M+H]^+$; calcd for $C_{37}H_{47}N_3O_8$: 662.3436.

General Protocol 3, Synthesis of Ketones 105a-d and 106a,b.

A suspension of CuCN (0.3 mmol, 60 mol%) in THF (2 mL of THF per 1 mmol CuCN) was cooled to –45 °C, treated with vinyl magnesium bromide (3 mmol, 600 mol%, *c* 1 M) over 10 min, stirred for 1 h, treated with a solution of the corresponding ester **103a-d**, **104a** or **104b** (0.5 mmol, 100 mol%) in THF (0.1 M), stirred for 2 h at –45 °C, and warmed to room temperature for an additional 30 min. The reaction mixture was cooled to 0 °C,

treated with a saturated ammonium chloride solution (30 mL) and shaken vigorously for 20 min. The layers were separated, and the aqueous phase was extracted with Et_2O (3 x 50 mL) or EtOAc (3 x 50 mL). The combined extracts were washed with saturated sodium bicarbonate solution (50 mL), pH 6.8 phosphate buffer (50 mL) and brine (2 x 50 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by column chromatography with an eluant of EtOAc in hexane as specified for each compound. Evaporation of the collected fractions afforded the respective ketone **105a-d, 106a** or **106b.**

(2*S*)-*N*'-(2,4-Dimethoxybenzyl)-*N*'-1-(3-oxohept-6-enyl)-*N*-(Boc)-phenylalaninamide (105a).

A colorless oil was prepared from ester **103a** (0.5 mmol, 0.25 g) according to the general protocol 3, purified on silica gel eluting with 30% EtOAc/hexane (R_f 0.2, 30% EtOAc/Hexane), and isolated in 66% yield: ^{1}H NMR (1.3:1 isomer ratio, 400 MHz, CD_3OD) δ 1.39 (s, 9H), 1.42* (s, 9H), 2.16-2.53 (m, 6H), 2.89-2.92 (m, 2H), 3.33-3.41(m, 2H), 3.42-3.76* (m, 2H), 3.77 (s, 6H), 4.86 (s, 2H), 4.93-5.03 (m, 3H), 5.76-5.80 (m, 1H), 6.41-7.23 (m, 8H). ^{13}C NMR (2:1 isomer ratio, , 100 MHz, CD_3OD) δ 210.0, 209.2*, 173.7*, 173.2, 161.9, 161.4*, 159.4, 159.2*, 156.8, 156.5*, 137.9, 137.8*, 130.5, 130.4*, 130.2, 130.1*, 129.8, 129.0, 128.9, 127.4, 127.2, 117.6, 117.0, 115.1, 99.0*, 98.6, 80.0*, 79.9, 55.4*, 55.3, 42.5*, 42.2, 41.9, 41.6, 40.7, 39.9*, 28.2, 28.1*. $[\alpha]_D^{20}$ –7.82° (*c* 0.011 M, $CHCl_3$). HRMS (EI) *m/z* 525.2957 [M+H$^+$; calcd for $C_{30}H_{40}N_2O_6$: 525.2959].

(2*S*)-*N*-(Boc)Phenylalanine-*N'*-(4-(benzyloxy)benzyl)-*N'*-1-(3-oxohept-6-enyl)amide (105b).

A colorless oil was prepared from ester **103a** (0.5 mmol, 0.273 g) according to the general protocol 3, and purified on silica gel eluting with 30% EtOAc/hexane (R_f 0.29, 30% EtOAc/hexane). First to elute was the starting ester **103b** (0.076 g, 28%). Second to elute was ketone **105b** (0.143 g, 50%): ^{1}H NMR (1:1 isomer ratio, 400 MHz, CD_3OD) δ 1.37 (s, 9H), 1.42* (s, 9H), 2.17-2.42 (m, 2H), 2.56-2.40* (m, 2H), 2.42-2.48 (m, 2H), 2.56-2.60* (m, 2H), 2.83-2.95 (m, 2H), 3.02-3.08* (m, 2H), 3.32-3.50 (m, 2H), 4.33-4.55 (m, 2H), 4.73-4.80 (m, 1H), 4.90 (s, 2H), 4.93-4.99 (br t, 2H), 5.04 (s, 2H), 5.71-5.83 (m, 1H), 6.90-7.42 (m, 14H). ^{13}C NMR (1:1 isomer ratio, 100 MHz, CD_3OD) δ 208.8, 208.0*, 172.5, 172.2*, 158.0*, 157.7, 155.7*, 155.5, 137.0, 136.8, 136.7, 136.5, 129.0, 128.9, 128.4, 127.9, 127.8, 127.1, 126.8, 126.2, 126.0*, 114.5*, 114.2, 113.9, 78.8, 69.3*, 69.2, 51.7, 51.5, 41.1*, 41.0, 40.4, 39.4, 38.2, 27.0. $[\alpha]_D^{20}$ –1.1° (*c* 0.01 M, $CHCl_3$). HRMS (EI) *m/z* 571.3169 $[M+H]^+$; calcd for $C_{35}H_{42}N_2O_5$: 571.3167.

(2*S*)-*N*-(Boc)-Phenylalanin-*N'*-((2-benzylthio)ethyl)-*N'*-1-(3-oxohept-6-enyl)amide (105c).

A colorless oil was prepared from ester **103c** (0.5 mmol, 0.25 g) according to the general protocol 3, purified on silica gel eluting with 25% EtOAc/hexane (R_f 0.34, 25% EtOAc/hexane), and isolated in 60% yield. ^{1}H NMR (1.7:1 isomer ratio, 400 MHz, CD_3OD) δ 1.38 (s, 9H), 1.40* (s, 9H), 2.26-2.31 (br q, 2H), 2.43-2.53 (m, 6H), 2.82-3.01 (m, 2H), 3.01-3.20 (m, 2H), 3.32-3.38 (m, 1H), 3.74 (s, 2H), 3.78* (s, 2H), 4.58-4.66 (m, 1H), 4.97-5.15 (m, 2H), 5.71-5.77* (m, 1H), 5.78-5.88 (m, 1H), 7.15-7.37 (m, 10H). ^{13}C

NMR (2:1 isomer ratio, 100 MHz, CD_3OD) δ 209.1, 208.4*, 172.7, 172.5*, 155.9, 155.8*, 138.7, 138.5*, 137.1, 137.0, 136.8, 129.3, 129.2*, 129.0, 128.8, 128.7*, 128.2, 128.1*, 126.8, 126.7*, 126.6, 114.3, 79.2, 51.9, 51.7*, 46.2, 42.4*, 41.8, 41.5*, 41.4, 41.0, 40.5, 38.6, 35.5*, 35.3, 27.4*, 26.2. $[\alpha]_D^{20}$ 2.7° (*c* 0.04 M, $CHCl_3$). HRMS (EI) *m/z* 525.2786 $[M+H]^+$; calcd for $C_{30}H_{40}N_2O_4S$: 525.2782.

(2*S*)-*N*-(Boc)-Phenylalanin-*N'*-((2-phenylthio)-ethyl)-*N'*-1-(3-oxohept-6-enyl)-amide (105d).

A colorless oil was prepared from ester **103d** (0.5 mmol, 0.243 g) according to the general protocol 3, purified on silica gel eluting with 25% EtOAc/hexane (R_f 0.34, 25% EtOAc/hexane), and isolated in 35% yield. ^1H NMR (1.2:1 isomer ratio, 400 MHz, CD_3OD) δ 1.40 (s, 9H), 1.43* (s, 9H), 2.24-2.27 (m, 2H), 2.41-2.56 (m, 4H), 2.80-2.97 (m, 4H), 3.18-3.57 (m, 4H), 5.7-5.86 (m, 1H), 4.95-5.04 (m, 2H), 5.78-5.81 (m, 1H), 7.0-7.89 (m, 10H). ^{13}C NMR (1.8:1 isomer ratio, 100 MHz, CD_3OD) δ 208.7, 207.9*, 172.5, 172.4*, 155.6, 155.5*, 136.7, 136.6*, 136.4, 136.1*, 135.4, 134.5*, 129.5, 128.9, 128.7*, 128.5, 128.4*, 128.0, 127.9*, 127.8, 126.3*, 126.2, 126.1, 125.3, 114.0, 78.8*, 77.7, 59.8, 51.7*, 51.5, 46.2, 42.3*, 41.1*, 41.0, 39.7, 38.2*, 30.6*, 29.1, 27.0. $[\alpha]_D^{20}$ 4.1° (*c* 0.008 M, $CHCl_3$). HRMS (EI) *m/z* 511.2634 $[M+H]^+$; calcd for $C_{29}H_{38}N_2O_4S$: 511.2625.

(2*S*)-*N*-(Boc)-*Nε*-(Cbz)-Lysin-*N'*-(2,4-dimethoxybenzyl)-*N'*-1-(3-oxohept-6-enyl)-amide (106a).

A colorless oil was prepared from ester **104a** (0.5 mmol, 0.308 g) according to the general protocol 3, purified on silica gel eluting with 40% EtOAc/hexane (R_f 0.15, 40%

EtOAc/hexane), and isolated in 62% yield. ^{1}H NMR (1.1:1 isomer ratio, 400 MHz, CD_3OD) δ 1.29-1.45 (m, 17H), 2.22-2.26 (t, J = 6.9, 2H), 2.43-2.45 (m, 2H), 2.55-2.67* (m, 2H), 3.08-3.10 (m, 2H), 3.48* (m, 2H), 3.62-3.64*(m, 2H), 3.76* (s, 3H), 3.78 (s, 3H), 3.8 (s, 3H), 4.54 (br s, 2H), 4.65-4.74 (m, 1H), 4.86 (s 2H), 4.93-4.98 (m, 2H), 5.06 (s, 2H), 5.77-5.79 (m, 1H), 6.92-7.3 (m, 8H). ^{13}C NMR (2.7:1 isomer ratio, 100 MHz, CD_3OD) δ 209.0, 208.2*, 173.2*, 172.9, 172.0, 160.8, 160.2*, 158.3, 158.1*, 157.06, 156.1*, 155.8, 136.7, 129.4, 129.3*, 127.7, 127.2, 127.0, 116.5*, 116.0, 113.9, 103.9*, 103.7, 97.8, 97.5*, 78.7, 78.6*, 65.5, 54.2, 54.1*, 50.5, 42.9, 41.2*, 41.1*, 41.0, 40.8, 40.6, 39.9*, 39.5, 31.9, 31.3*, 28.8, 27.0, 22.2*, 22.1. $[\alpha]_D^{20}$ –17.6° (*c* 0.012 M, $CHCl_3$). HRMS (EI) *m/z* 640.3591 $[M+H]^+$; calcd for $C_{35}H_{49}N_3O_8$: 640.3592.

(2*S*)-*N*-(Boc)-*Nε*-(Cbz)-Lysin-*N'*-(4-(benzyloxy)benzyl)-*N'*-1-(3-oxohept-6-enyl)-amide (106b).

A colorless oil was prepared from ester **104b** (0.5 mmol, 0.331 g) according to the general protocol 3, purified on silica gel eluting with 40% EtOAc/hexane (R_f 0.17, 40% EtOAc/hexane), and isolated in 70% yield. ^{1}H NMR (1:1 isomer ratio, 400 MHz, CD_3OD) δ 1.24-1.66 (m, 17H), 2.23-2.26 (br t, 2H), 2.39-2.49 (m, 2H), 2.60-2.72* (m, 2H), 2.84 (br s, 1H) 3.04-3.13 (br d, 2H), 4.34-4.67 (m, 2H), 4.88 (s, 2H), 4.93-4.98 (t, J = 9.76, 2H), 5.05 (s, 4H), 5.73-5.83 (m, 1H), 6.71-7.40 (m, 14 H). ^{13}C NMR (1.3:1 isomer ratio, 100 MHz, CD_3OD) δ 209.0, 208.2*, 173.4*, 173.2, 158.1, 157.8*, 157.2, 157.1*, 156.2, 156.0*, 136.9, 136.8, 136.7, 129.2, 128.7, 128.5, 127.9, 127.8, 127.7, 127.2, 127.1, 127.0, 126.8, 78.7, 69.2, 65.5, 50.6, 50.4*, 41.5, 41.1*, 41.0, 40.5, 39.8, 39.6, 39.5, 31.3, 28.7, 27.0, 22.2*,

22.1. $[\alpha]_D^{20}$ –9.5° (*c* 0.042 M , $CHCl_3$). HRMS (EI) *m/z* 686.3802 $[M+H]^+$; calcd for $C_{40}H_{51}N_3O_7$: 686.3800.

General Protocol 4, Synthesis of 1,3,5-Trisubstituted 1,4-Diazepinones 107a-d and 108a,b.

Ketone **105a-d**, **106a** or **106b** (0.095 mmol, 100 mol%) was dissolved in a 1:1 TFA/DCM solution (4 mL), stirred for 10 min, and evaporated to a residue. The residue was dissolved in THF (5.10^{-3} M), treated with supported free tertiary amine Amberlyste A-21 resin (1.9 mmol, 2000 mol%, prewashed with methanol, THF, DCM and dried under high vaccum for 2 h), stirred for 2 h, filtered and treated with a sodium cyanoborohydride solution in THF (0.475 mmol, 500 mol%, *c* 1 M). The reaction mixture was stirred overnight under argon atmosphere. After completion of the reaction was indicated by TLC, the solvent was concentrated on a rotary evaporator, and the remainder was partitioned between a saturated $NaHCO_3$ solution (20 mL) and ethyl acetate (20 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 10 mL). The combined organic phase was dried over magnesium sulphate, filtered and evaporated to a residue, which was purified further as specified below.

(3*S*,5*S*)-3-Benzyl-5-but-3-enyl-1-(2,4-dimethoxybenzyl)-[1,4]diazepin-2-one (107a).

A colorless oil was prepared from ketone **105a** (0.095 mmol, 0.05 g) according to the general protocol 4, purified on silica gel eluting with 20% EtOAc/hexane (R_f 0.22, 20% EtOAc/Hexane), and isolated in 68% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 1.18-1.34 (m, 3H), (m, 1H), 1.78 (m, 2H), (m, 1H), 2.83-2.89 (q, *J* = 8, 1H), 3.28-3.33 (m, 2H), 3.57-3.60

(m, 2H), 3.81 (s, 6H), 4.51-4.68 (q, J = 16, 2H), 4.7-4.84 (m, 2H), 5.56-5.57 (m 1H), 6.46-6.49 (m, 2H), 7.25-7.35 (m, 6H).^{13}C NMR (100 MHz, $CDCl_3$), δ 174.4, 159.8, 158.1, 138.9, 137.5, 130.8, 129.0, 128.1, 126.0, 118.0, 114.4, 103.9, 97.9, 60.6, 59.8, 55.0, 55.9, 46.9, 45.2, 37.8, 35.7, 35.3, 29.4. $[\alpha]_D^{20}$ –7.6° (*c* 0.018, $CHCl_3$). HRMS (EI) *m/z* 409.2491 [M+H$^+$; calcd for $C_{25}H_{32}N_2O_3$: 409.2486].

(3*S*,5*S*)-3-Benzyl-1-(4-benzyloxybenzyl)-5-(but-3-enyl)-1,4-diazepin-2-one (107b).

A colorless oil was prepared from ketone **105b** (0.095, 0.054 g) according to the general protocol 4, purified on silica gel eluting with 30% EtOAc/hexane (R_f 0.24, 30% EtOAc/hexane), and isolated in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28-1.34 (m, 3H), 1.46 (m, 1H), 1.76 (m, 2H), 2.3 (m, 1H), 2.89 (q, J = 4.0, 1H), 3.31-3.58 (m, 2H), 3.56-3.62 (m, 2H), 4.57-4.59 (q, J = 8, 2H), 4.69-4.89 (m, 2H), 5.08 (s, 2H), 5.51-5.61 (m, 1H), 6.95-6.97 (d, J = 8, 2H), 7.24-7.47 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.3, 157.8, 138.6, 137.4, 136.6, 129.7, 129.3, 129.2, 129.0, 128.2, 127.6, 127.1, 126.1, 115.1, 114.5, 69.7, 60.7, 59.8, 50.4, 46.4, 37.7, 35.6, 35.2, 29.4. $[\alpha]_D^{20}$ –41.8° (*c* 0.079, CH_2Cl_2). HRMS (EI) *m/z* 455.2707 [M+H]$^+$; calcd for $C_{30}H_{34}N_2O_2$: 455.2693.

(3*S*,5*S*)-3-Benzyl-1-((2-benzylthio)ethyl)-5-(but-3-enyl)-1,4-diazepin-2-one (107c).

A colorless oil was prepared from ketone **105c** (0.095 mmol, 0.048 g) according to the general protocol 4, purified on silica gel eluting with 10% EtOAc/$CHCl_3$ (R_f 0.22, 10% EtOAc/$CHCl_3$), and isolated in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28-1.37 (m, 4H), 1.65-1.67 (m, 4H), 2.6-2.64 (m, 2H), 3.22-3.24 (m, 2H), 3.50-3.6. (m, 4H), 3.79 (s, 2H), 4.67-4.85 (m, 2H), 5.55-5.57 (m, 1H), 7.25-7.38 (m, 10H). ^{13}C NMR (100 MHz,

$CDCl_3$), δ 174.2, 138.5, 138.0, 137.4, 129.0, 128.6, 128.2, 126.7, 126.2, 114.6, 60.6, 59.8, 48.1, 48.0, 37.6, 35.8, 35.7, 35.6, 35.6, 29.4, 28.9. $[\alpha]_D^{20}$ –37.6° (*c* 0.03, EtOH). HRMS (EI) *m/z* 409.2326 $[M+H]^+$; calcd for $C_{25}H_{32}N_2OS$: 409.2308.

(3*S*,5*S*)-3-Benzyl-1-((2-phenylthio)ethyl)-5-(but-3-enyl)-1,4-diazepin-2-one (107d). A colorless oil was prepared from ketone **105d** (0.095 mmol, 0.047 g) according to the general protocol 4, purified on silica gel eluting with 20% EtOAc/hexane (R_f 0.22, 20% EtOAc/hexane), and isolated in 66% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 1.28-1.39 (m, 2H), 1.41-1.76 (m, 4H), 2.48 (br s, 1H), 2.81-2.85 (m, 1H), 3.1-3.29 (m, 4H), 3.44-3.47 (m, 4H), 4.68-4.84 (m, 2H), 5.52-5.59 (m, 1H), 7.19-7.42 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.3, 138.5, 137.4, 135.4, 129.0, 128.7, 128.4, 128.2, 126.2, 125.6, 114.6, 60.5, 59.8, 49.1, 48.9, 37.5, 35.8, 35.6, 30.7, 29.3. $[\alpha]_D^{20}$ –23.16° (*c* 0.043, $CHCl_3$). HRMS (EI) *m/z* 395.2171 $[M+H]^+$; calcd for $C_{24}H_{30}N_2OS$: 395.2152.

(3*S*,5*S*)-1-(2,4-Dimethoxybenzyl)-3-(4-*N*-(Cbz)-aminobutyl)-5-(but-3-enyl)-1,4-diazepin-2-one (108a).

A colorless oil was prepared from ketone **106a** (0.095 mmol, 0.061 g) according to the general protocol 4, purified on silica gel eluting with 70% EtOAc/hexane (R_f 0.21, 70% EtOAc/hexane), and isolated in 71% yield. ^1H NMR (300 MHz, $CDCl_3$) δ1.40-1.65 (m, 10H), 2.13 (m, 2H), 2.74 (br d, 1H), 3.20-3.42 (m, 4H), 3.52 (m, 1H), 3.80 (s, 6H), 4.42-4.47 (d, J = 14.5, 1H), 4.61-4.66 (d, J = 14.7, 1H), 4.97 (br t, 2H), 5.10 (s, 2H), 5.79-5.80 (m, 1H), 6.44 (br d, 2H), 7.21-7.38 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 175.5, 161.0, 159.3, 157.3, 139.1, 137.6, 132.0, 129.3, 128.9, 128.8, 119.1, 115.7, 105.1, 99.2, 67.3, 61.0,

60.2, 56.2, 47.8, 46.3, 41.6, 37.0, 36.1, 33.0, 31.1, 30.6, 30.5, 24.4. $[\alpha]_D^{20}$ –8.7° (*c* 0.013, $CHCl_3$). HRMS (EI) *m/z* 524.3119 $[MH]^+$; calcd for $C_{30}H_{41}N_3O_5$: 524.3110.

(3*S*,5*S*)-1-(4-Benzyloxybenzyl)-3-(4-*N*-(Cbz)-aminobutyl)-5-(but-3-enyl)-1,4-diazepin-2-one (108b).

A colorless oil was prepared from ketone **106b** (0.095 mmol, 0.065 g) according to the general protocol 4, purified on silica gel eluting with 70% EtOAc/hexane (R_f 0.24, 70% EtOAc/hexane), and isolated in 78% yield. ^1H NMR (300 MHz, $CDCl_3$) δ1.27-1.61 (m, 10H), 2.08-2.13 (m, 2H), 3.20-3.40 (m, 4H), 3.57 (br q, 1H), 4.40-4.45 (d, *J* =14.4, 1H), 4.62 (d, *J* =14.4, 1H), 4.95-5.04 (m, 2H), 5.05 (s, 4H), 5.10 (s, 2H), 5.76 (m, 1H), 6.92 (d, *J* = 8.4, 2H), 7.19 (d, *J* = 8.7, 2H), 7.28-7.469 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$), δ 174.7, 159.0, 157.3, 138.8, 137.8, 137.5, 130.8, 130.7, 130.5, 129.4, 129.3, 129.1, 128.8, 128.3, 116.0, 115.8, 70.9, 67.4, 61.1, 60.2, 51.5, 47.2, 41.5, 36.5, 35.5, 32.7, 31.0, 30.6, 24.3. $[\alpha]_D^{20}$ -8.1° (*c* 0.095, $CHCl_3$). HRMS (EI) *m/z* 570.3344 $[M+H]^+$; calcd for $C_{35}H_{43}N_3O_4$: 570.3326.

(3*S*,5*S*)-3-Benzyl-5-but-3-enyl-1,4-diazepin-2-one 95b and 3-benzyl-5-but-3-enyl-1-(4-hydroxy-benzyl)-[1,4]diazepin-2-one (109).

4-Benzyloxybenzyl amide **107b** (12 mg, 0.026 mmol) was placed into a 10 mL three necked flask, equipped with a water condenser, treated with 3 mL of a mixture of TFA/TES (95:5), and heated with an oil bath to a bath temperature of 80 °C for 72 h. The progress of the reaction was followed by LCMS analysis, by removing aliquots from the mixture and injecting directly onto a Gemini column (C18 110 A, 50x4.60 mm, 5μ) and analyzed with

an eluant of 20-80% acetonitrile:H_2O buffered with 0.1% TFA. After 72h, a 75:25 ratio was observed of three peaks eluting at retention times of 3.49 and 4.24 min corresponding to 3,5-disubstituted diazepinone **95b** and 4-hydroxybenzylamide **109**. The solution was cooled to room temperature, and concentrated under vacuum to a residue, that was purified on silica gel using 50% EtOAc/hexane as eluent, to afford diazepinone **109** (2.4 mg, 25% yield) as a colorless oil, R_f 0.25 (50% EtOAc/hexanes). ^1H NMR (400 MHz, $CDCl_3$) δ 1.29-1.47 (m, 2H), 1.6-1.93 (m, 4H), 2.02-2.22 (m, 4H), 3.20-3.24 (br d, 1H), 3.75-3.82 (br q, 1H), 4.50-4.62 (m, 2 H), 5.02-5.10 (m, 2H), 5.75-5.83 (m, 1H), 6.70-7.39 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.3, 165.7, 156.5, 135.8, 135.6, 129.0, 128.8, 127.8, 126.5, 126.2, 114.8, 114.6, 60.4, 58.6, 43.9, 34.7, 32.0, 30.1, 28.6. HRMS (EI) *m/z* 365.2228 [$M+H^+$; calcd for $C_{23}H_{28}N_2O2_3$: 365.2224]. $[\alpha]_D^{20}$ –7.0° (*c* 0.014, $CHCl_3$). Second to elute was diazepinone **95b**[5] on changing the solvent system to 5% methanol/DCM. After evaporation of the combined collected fractions, the residue was dissolved in 10% HCl solution followed by freeze drying to afford diazepinone **95b**[5] (5.0 mg, 70% yield): mp 138-140 °C; R_f 0.32 (5% MeOH/DCM), $[\alpha]_D^{20}$ –35.0° (*c* 0.008, DMF). HRMS (EI) *m/z* 259.1808 [$M+H^+$; calcd for $C_{16}H_{23}N_2O$: 259.1804]. Ref 5 : mp 138-140 °C; $[\alpha]_D^{20}$ –35.6° (*c* 0.008, DMF).

3.7 References

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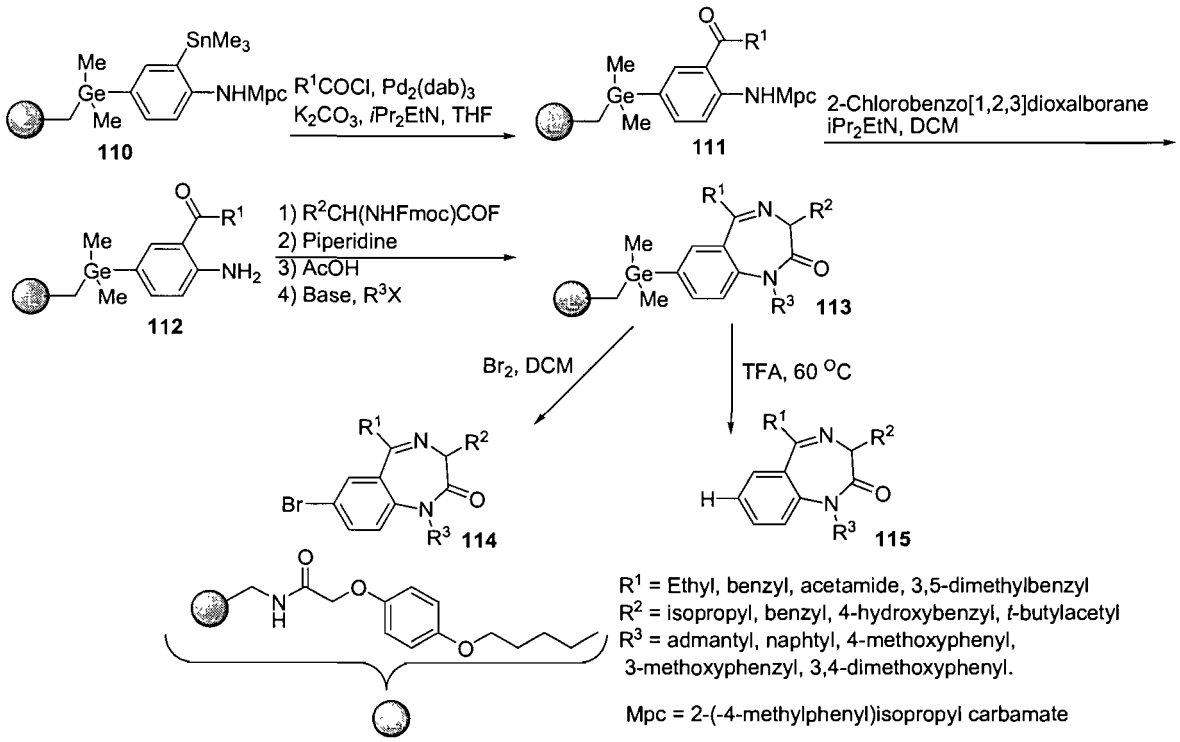
Chapter 4

Concluding Statements and Future Work

4.1. Introduction

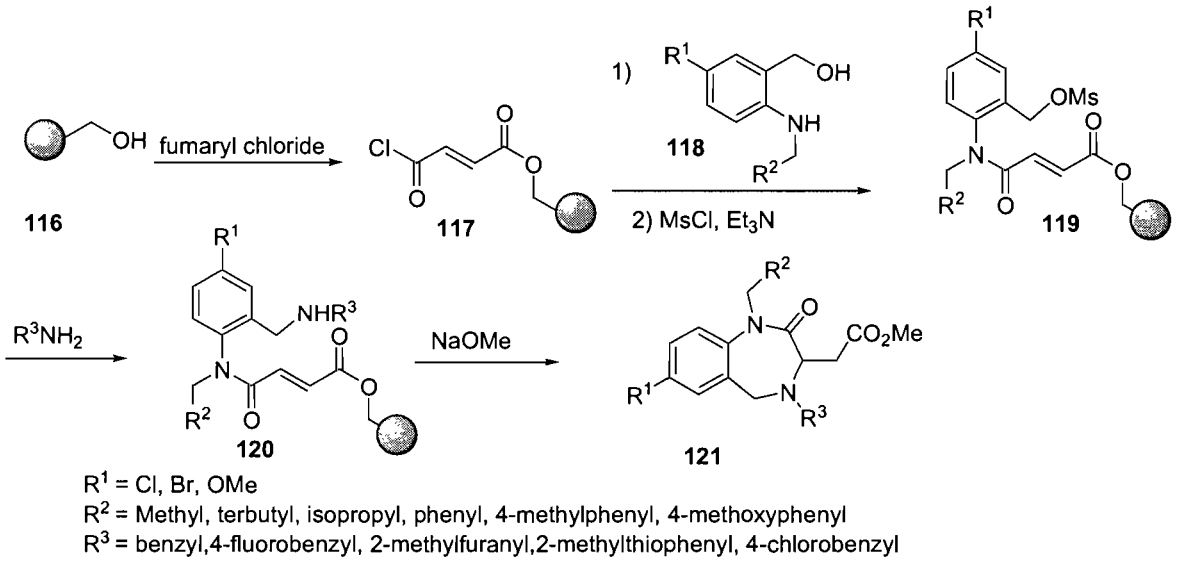
Since the first report of solid phase peptide synthesis by Merrifield in 1963,[1] the solid-phase-synthesis method has become a common practice in the academic and industrial settings, because it can serve as an efficient and powerful tool for preparing organic molecules.[2] The solid-phase method offers many advantages over the conventional solution-phase approaches, primarily because of the convenient work-up and purification of intermediates during synthesis.[3]

Attractive targets due to their well-known biological properties, the 1,4-benzodiazepin-2-ones were among the first classes of small molecules to be synthesized on solid supports.[4] Many syntheses of similar skeletons on solid support have since been reported.[4,5] For example the synthesis of a benzodiazepin-2-one library has been achieved using an acid labile traceless linker composed of supported germanium **110** (Scheme 4.1).[6] Palladium-catalyzed cross coupling of resin bound aryl stanane **110** with an acyl chloride, aniline deprotection, followed by acylation with a series of Fmoc amino acids, Fmoc deprotection, and cyclization afforded resin supported benzodiazepin-2-one **113**. Benzodiazepinone **114** and bromobenzodiazepinone **115** were respectively cleaved from the resin by treatment with trifluoroacetic acid and bromine.



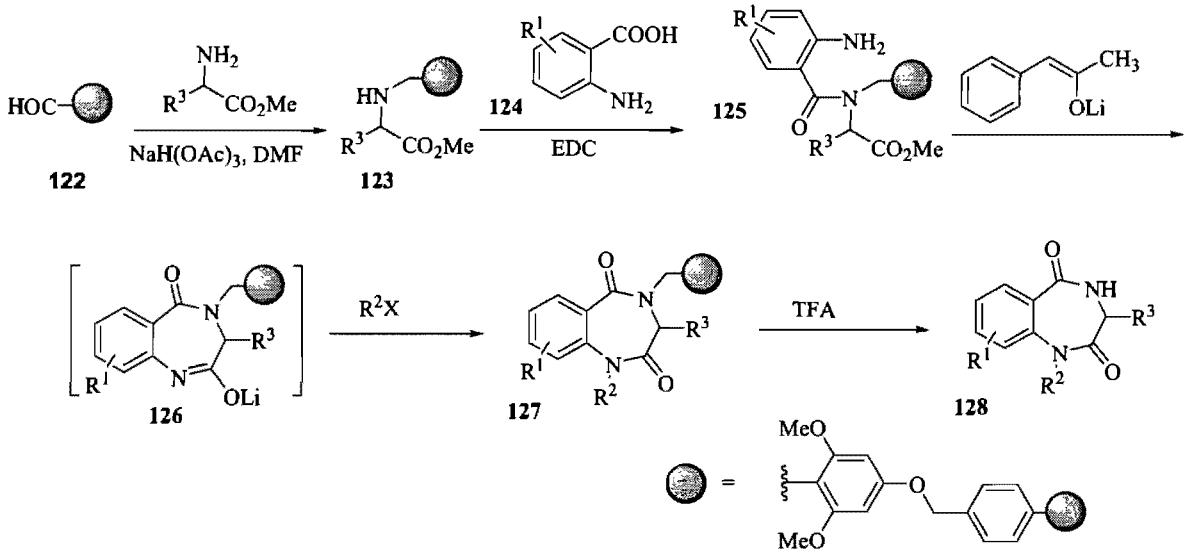
Scheme 4.1 Synthesis of 1,4-benzodiazepine derivatives using germanium-linker.

A second benzodiazepinone synthesis strategy involved ring closure between *C3* and *N4* by a conjugate addition on a resin bound fumarate.[7] Wang resin **116** was treated with fumaryl chloride to furnish the supported acyl chloride **117.** Mesylate **119** was prepared by acylation of aminobenzyl alcohol **118** first with the fumaryl chloride resin **117,** followed by methanesulfonyl chloride. 1,4-Benzodiazepin-2-ones **121** were then produced by intermolecular displacement of mesylate **119** with different primary amines, intramolecular *7-exo-trig* cyclization by conjugate addition, and cleavage by transesterification with sodium methoxide (Scheme 4.2)



Scheme 4.2 Synthesis of tetrahydro-1,4-benzodiazepin-2-one by intramolecular *7-exo-trig* cyclization.

In adopting our solution-phase synthesis to solid support, a sulfide safety-catch linker was examined for the synthesis of 1,4-diazepin-2-ones (Scheme 4.4), because of its successful use in the solid-phase synthesis of other heterocycles. The β-sulfide group can be activated on oxidation to a sulfone and cleaved by β-elimination under basic conditions.[8] Such a strategy has been utilized for the synthesis of peptides,[8,9] carboxylic acids,[10] esters,[11] amines,[12] nucleic acids[13] and heterocycles such as diketopiperazines,[10] benzopurans[14] and pyrrolopyrimidines.[15] In addition, desulfurisation by breaking the C-S bond was considered for making *N*-ethyl diazepinone. In addition to the thioethyl amide linker, alkoxybenzyl amide linkers were explored, because they had already proven suitable for solid-phase diazepine synthesis. For example, a library of 1,4-diazepin-2,5-diones (2058 compounds) was prepared by a strategy employing a 2,4,6-trialkoxy benzyl linker (Scheme 4.3).[6]

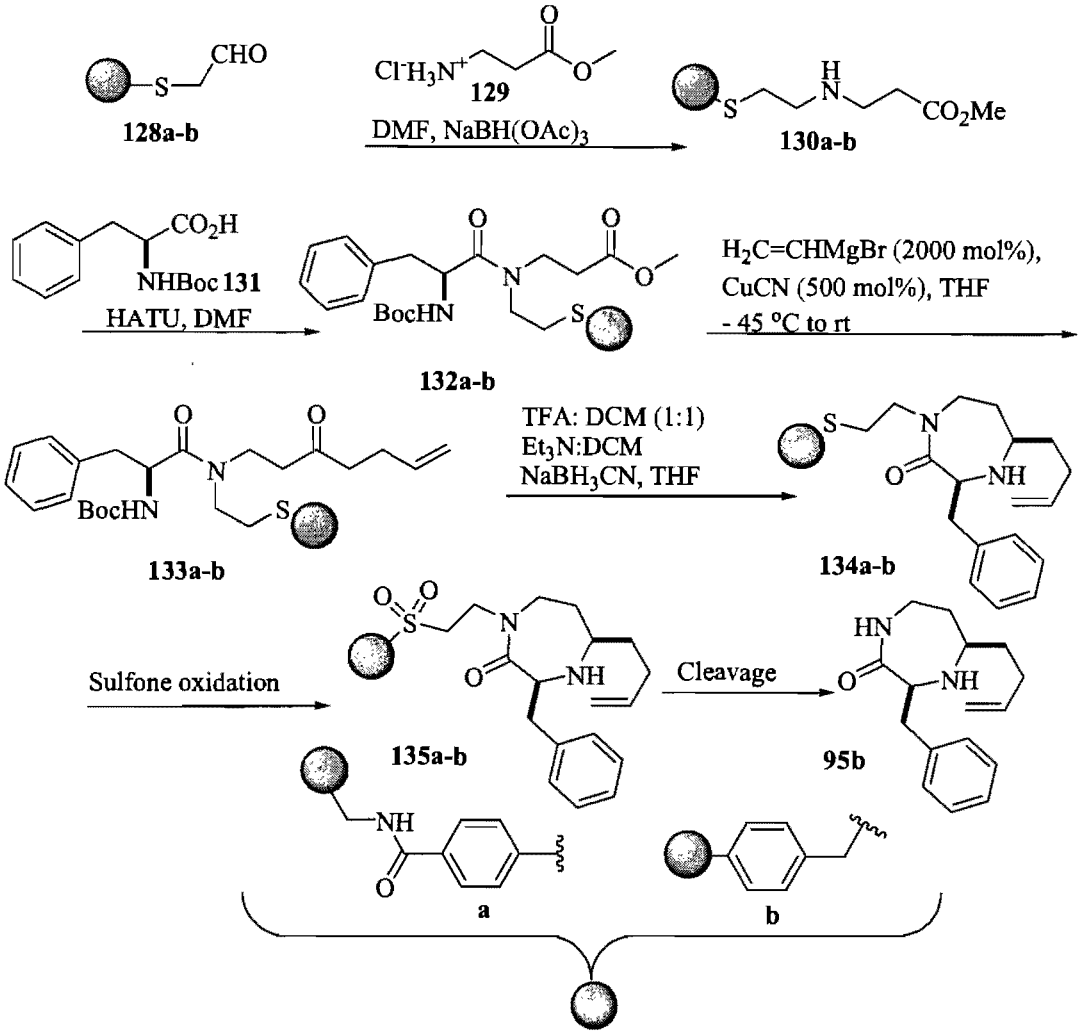


Scheme 4.3 1,4-Benzodiazepin-2,5-dione synthesis using 2,4,6-trialkoxybenzyl linker.

α-Amino esters were linked to benzaldehyde support **122** by a reductive amination employing sodium triacetoxybrohydride in DMF to provide amine **123**, which was acylated with various substituted anthranilic acids **124** using EDC as activating agent. Treatment of the resulting dipeptide **125** with the lithium salt of acetanilide followed by alkylation afforded the alkyl substituted 1,4-diazepin-2,5-dione **127**, which was cleaved from the resin on treatment with TFA:Me_2S:H_2O (90:5:5). In light of the succes of solid phases synthesis for preparing librairies of 1,4-benzodiazepin-2-one derivatives, the adaptation of our solution-phase methodology to a solid-phase strategy was envisioned to prepare diazepinones librairies. Two strategies were pursued using different linker techinques. Initially, a thioethyl linker was explored to attach the β-amino ester to the support with the objective of cleaving the heterocycle by an oxidation/β-elimination sequence. Subsequently, an acid labile benzyl linker was used to make the 1,4-diazepin-one core.

4.2. Attempted Solid-Phase Synthesis of 1,4- Diazepin-2-one Using Thioethyl and 4-Alkoxybenzyl Linkers

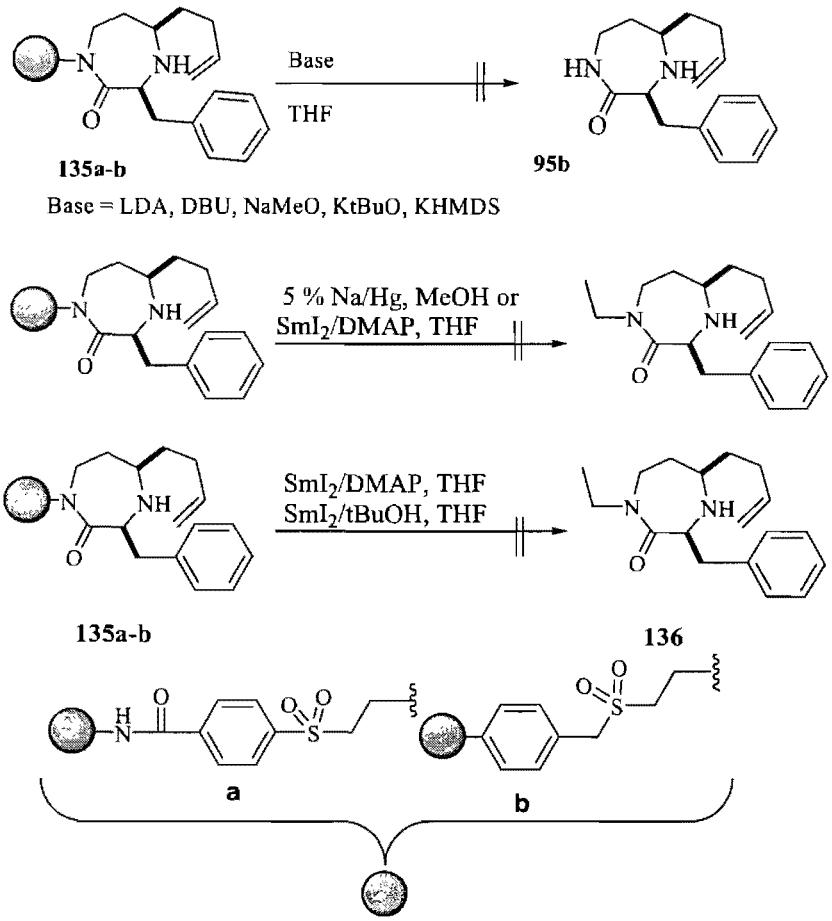
Resin-bound aldehyde **128a** was prepared from amino ethyl polystyrene resin and 4-(dimethoxyethylthio)benzoic acid using diisopropylcarbodimide and HOBt followed by acetal deprotection using dioxane/2N HCl.[8] A second resin-bound aldehyde **128b** was prepared by thiol alkylation with 2-bromo-1,1-dimethoxyethane, followed by acetal hydrolysis. Resin-bound aldehydes **128a** and **128b** were characterized by IR spectroscopy, which showed an aldehyde stretching band at 1722 cm^{-1} for **128a** and at 1718 cm^{-1} for **128b**. Aldehyde loadings of 0.2 mmol/g and 0.7 mmol/g were determined by elemental analysis of resins **128a** and **128b** by measurement of sulfur content using elemental analysis. Resin-bound amines **130a-b** were synthesized by the reductive amination of the imine from β-alaninate **129** and the respective aldehyde resin **128a-b** employing $NaBH(OAc)_3$ in a 1% acetic acid in DMF solution.[6]



Scheme 4.4 Proposed approach for the solid-phase synthesis of 1,4- diazepin-2-one employing thioethyl linkers.

Completion of the reaction was ascertained when the aldehyde stretch was no longer observed at respectively 1722 and 1718 cm^{-1} for resins **128a** and **128b** using IR analysis; moreover, a new stretching band appeared at 1738 and 1729 cm^{-1} corresponding to the respective methyl esters. Acylation of the resulting secondary amine **130a-b** with *N*-Boc-phenylalanine using HATU in DMF provided tertiary amide **132a-b**.[16] In the FTIR spectra, a new streching band at 1709 and 1736 cm^{-1} was observed. Attempts to cleave the

dipeptide from the resin to determine loading and purity were, however, unsuccessful using trifluoromethane sulfonate. Acylation was examined qualitatively by Boc group deprotection using TFA: DCM (1:1) and a ninhydrin test[17] which showed a positive blue colour. In the ^{1}H MAS-NMR (magic angle spinning-NMR) spectrum singlets were observed at ~ 1.4 and 3.45 ppm which corresponded to the Boc *tert*-butyl group and methyl ester, and may be used to assess the reaction conversion from the starting amino ester with signal at 3.45 ppm. Resin bound homoallylic ketone **133a-b** was synthesized by treating tertiary amide **132a-b** with freshly prepared vinyl magnesium bromide (2000 mol%) in the presence of copper cyanide (500 mol%) in THF at -45 °C.[18] Ketone formation was indicated by the weak carbonyl C=O streching band at 1709 cm^{-1} in the FT-IR spectrum of resin **133a-b** in compressed in KBr tablets. Moreover, large stretching bonds observed at 3429 and 3431 cm^{-1} indicated the formation of tertiary alcohol. Supported 1,4-diazepin-2-one **134a-b** was prepared from a sequence of reactions initiated by Boc group removal using either TFA:DCM (1:1) or $SiCl_4$ (*c* 1M, 2000 mol%) and phenol (3M, 6000 mol%) in DCM.[19] Intramolecular imine formation was achieved by neutralization of the TFA salt using triethylamine; imine reduction was then achieved using sodium cyanoborohydride in THF buffered with 1% acetic acid. The progress of the sequence was monitored by ninhydrin test which showed the disappearance of the primary amine. In addition, FT-IR spectroscopy indicated the ketone C=O stretching band at 1709 cm^{-1} had disappeared.

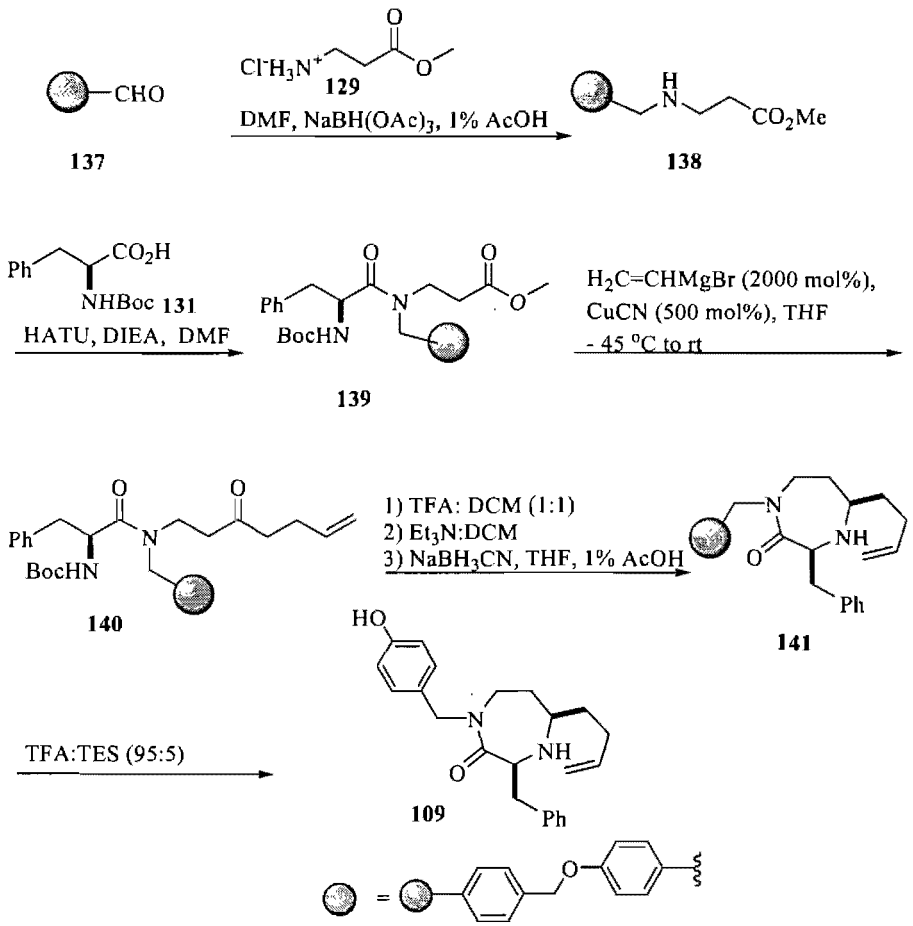


Equation 4.1 Attempted Cleavage of Diazepinone.

For the cleavage of the diazepinone product, sulfone resins **135a-b** were pursued by oxidation of the sulfur using either Oxone in DCE/DMF 4:1 or *m*-CPBA in DCM.[8] The IR spectroscopy showed a superposition of the sulfone band with alkane band this result suggesting that the sulfur may have not been completely oxidized to sulfone. Attempts failed to liberate diazepinone **95b** from oxidized resins **135a-b** in THF using respectively LDA, DBU, NaMeO, KtBuO, and KHMDS (600 mol%) to effect β-elimination. Similarly, attempts failed to liberate diazepinone **136** by desulfurization employing respectively 5% Na/Hg[20] in methanol, SmI_2/DMAP in THF and SmI_2/tBuOH in THF[21] In all cases, no

product was observed by LCMS analysis (Eq. 4.1). Finally, using SmI_2/DMAP on resin **135b** trace amounts of a peak having correct mass for **95b** were observed by LCMS analysis. The presence of free amine, the possibility of incomplete sulfur oxidation and the poor swelling property of the linker in polar solvents such as methanol, all may have inhibited the release of the desired compound from the linker.

Resin-bound aldehyde **137** was prepared by alkylation of Merrifield resin with 4-methoxybenzaldehyde employing NaOH in DMF at 80 °C.[22] Conversion of the aldehyde **137** to supported secondary amine **138** was achieved by a reductive amination of the imine produced on treatmeant of the resin with β-alanine methyl ester using sodium triacetoxyborohydride in DMF containing a catalytic amount of acetic acid.[6] The reductive amination reaction was monitored by a dinitrophenyl hydrazine test which showed colourless beads after disappearence of aldehyde[23] and by IR spectroscopy following the disappearance of the aldehyde stretch at 1681 cm^{-1}. A loading of 1.0 mmol/g was determined by elemental analysis of resin **138** and by measurement of nitrogen content.



Scheme 4.5 Diazepinone synthesis using a 4-alkoxybenzaldehyde resin.

The *N*-(Boc)-Phenylalanine was coupled to secondary amine **138** using HATU and DIEA in DMF. The progress of the reaction was monitored by chloranil test which involves reacting of the secondary amine **138** with equal amounts of solutions of acetaldehyde and tetrachloro-*p*-benzoquinone. Upon heating the colour changed from green to blue due to the presence of a complex between the secondary amine **138**, acetaldehyde and tetrachloro-*p*-benzoquinone.[23] After acylation no change in the colour was observed by the chloranil test indicate of the fornation of amide. Homoallylic ketone **140** was prepared by a similar method as described for ketone **133**, in which the vinyl magnesium bromide and copper

cyanide were mixed in THF for at least 1 hour at -45 °C, and then added over 15 minutes to the mixture containing the swollen ester resin **139** in THF.[18] Ketone resin **140** exhibited a sharp band at 1707 cm^{-1} corresponding to carbonyl bond in its FTIR-spectrum. Supported diazepinone **141** was prepared by the same method as described for diazepinone **134,** initiated by Boc group removal using either TFA:DCM (1:1) or $SiCl_4$ (*c* 1M, 2000 mol%) and phenol (*c* 3M, 6000 mol%) in DCM. Intramolecular imine formation was achieved by neutralization of the TFA salt using triethylamine; imine reduction was performed using sodium cyanoborohydride in THF buffered with 1% acetic acid. The progress of the sequence was monitored by the Kaiser test,[17] which showed the disappearance of the primary amine by changing the blue colour of beads to colourless. Diazepinone **142,** was cleaved from the solid support using TFA:TES 95:5,[24] and by using 4M HCl in dioxane. The crude product was obtained in 80% purity as esteemed by HPLC analysis after cleavage from the supported 1,4-diazepin-2-one **141** using TFA:TES 95:5. Purification of the crude product on silica gel chromatography afforded diazepinone **109** in 18% overall yield from the supported amine **138**.

4.3 Conclusion and Perspectives

Solid-phase synthesis of 1,4-diazepin-2-one has been pursued using two different strategies for linking the amide to the solid phase. The thioethyl linker strategies may have provided diazepinone; however, an effective cleavage strategy for removing the 1,4-diazepin-2-one from the solid supports could not be achieved. Diazepinone was prepared by an approach using 4-alkoxybenzaldehyde resin **137**. Furthermore, potential may now exist to make diazepinones on solid support possessing greater diversity at the 5-7 positions by employment of substituted β-amino esters and vinyl Grignard reagents.[26] In summary, an effective acid labile linker strategy has been developed for the synthesis of enantiopure 1,4-diazepin-2-one from inexpensive amino acid building blocks. This method offers potential for synthesis of libraries of diazepinones with interesting pharmaceutical properties.

4.4 General Procedure for The Synthesis of 1,4-Diazepinone.

A typical procedure for the generation of 1,4-diazepinone follows.

Synthesis of secondary amine resin 130a-b and 138. The reductive amination of aldehyde derived resin was performed as follows: to a polyethylene 20 mL tube charged with 1 g of resin (**128a-b** and **137**) was added 6 equiv of β-alanine methyl ester hydrochloride and 6 equiv of sodium triacetoxyborohydride dissolved in a minimum volume of DMF buffered with 1% acetic acid, the reaction mixture was vigorously shaken. Completion of the reaction was monitored by IR spectroscopy after 20 min, by analysis of an aliquot of the resin, which was removed and rinsed with DMF (3 x 5 mL), CH_3OH (3 x 5 mL) and DCM (3x 5 mL). Completion of the reaction was verified by DNPH test, (IR data, 1738, 1729 and 1728 cm^{-1}).

Loading : 0.2 mmol/g for resin **128a** according to sulfur microanalysis found C, 87.35; H, 7.43: N, 0.80, S, 0.94, 0.5 mmol/g for resin **128b** according to sulfur microanalysis found C, 87.25; H, 7.47: N, 1.29, S, 2.55 and 1.0 mmol/g for resin **138** according to nitrogen microanalysis found C, 85.03; H, 7.67: N, 1,38.

Synthesis of tertiary amide resin 132a-b and 139.

N-(Boc)Phenylalanine or what (400 mol%), was dissolved in a minimum amount of DMF, cooled to 0 °C, treated with DIEA (800 mol%) followed by HATU (400 mol%), stirred for 5 min under argon, and the mixture was added to swollen secondary amine resin (**128a-b** and **138**, 500 mg) in DMF (6 mL). The reaction mixture was shaken for 12 hours at room temperature, filtered, and the resin was washed with DMF (3 x 20 mL), THF (3 x 20 mL),

MeOH (3 x 20 mL) and DCM (3 x 20 mL). Completion of the reaction was verified by chloranil test, (IR data, 1736, 1734 and 1737 cm^{-1})

Homoallylic ketone resin 133a-b and 140.

In a flame-dried, three-neck round bottom flask with a mechanical stirrer and an argon inlet, resin (500 mg, **132a-b** and **139**) was swollen in THF (6 mL), treated with copper cyanide (500 mol%), cooled to -45 °C, treated dropwise with a freshly prepared solution of vinyl magnesium bromide (2000 mol%, *c* 1M in THF), stirred over night during which time the bath temperature warmed to room temperature. The resin mixture was cooled to -45 °C, treated with with saturated ammonium chloride solution shaken vigorously for 20 minutes, filtered then washed with water (3 x 60 mL), DMF (3 x 20 mL), THF (3 x 20 mL), CH_3OH (3 x 20 mL) and DCM (3 x 20 mL) and then dried under *vaccuo* to give ketone resin **(133a-b and 140).** The formation of ketones was confirmed by DNPH test, (IR data, 1709 cm^{-1} weak signal and 1707 cm^{-1}). In order to prevent aggregation ether or ethyle acetate with saturated ammonium chloride solution were added in the first step of washing.

Diazepinone resin 134a-b and 141: Ketone resin **133a-b and 140** (250 mg) was first exposed for 5 min to a solution of silicon tetrachloride 1M (2000 mol%) and phenol 3 M (6000 mol%) in DCM to effect Boc deprotection, which was qualitatively indicated by a positive blue colour Kaiser test. The resin was then rinsed with DCM:Et_3N 9:1 (3 x 20 mL), DCM (3 x 20 mL) and THF (3 x 20 mL). The deprotected resin was swollen in THF (4 mL) buffered with 1% acetic acid and treated with sodium cyanoborohydride (600 mol%). The reaction was continued until the Kaiser Test showed colourless beads. The resin was

rinsed with THF (3 x 20 mL), DMF (3 x 20 mL) and DCM (3 x 20 mL). In the IR spectrum of resin **134a-b** and **141**, the ketone stretch was no longer observed at 1709 and 1707 cm^{-1}.

Sulfone Resin 135a-b: In three-neck round bottom flask with a mechanical stirrer, diazepinone resin **134a-b** (250 mg) was swollen in DMF/H_2O (3.2 mL: 0.8 mL) for 15 min at room temperature, treated with Oxone (0.275 mg, 1500 mol%), and stirred at room temperature for 18 h. The resin was filtered, washed with DMF (3 x 20 mL), water (3x 20mL), THF (3 x 20 mL), and DCM (3 x 20 mL) and dried in *vaccuo*.

(3*S*,5*S*)-3-Benzyl-5-but-3-enyl-1,4-diazepin-2-one (95b) :

Diazepinone resin **135b** (100 mg) was swollen in THF (4 mL) for 15 minutes in a 10 mL polyethylene tube. DMPU (0.17 mL, 8.4 mmol), and a solution of SmI_2 (2.2 mL, *c* 0.1 M in THF, 3.7 mmol) were added and the reaction mixture was allowed to stir at room temperature for 18 h at room temperature. The resin was filtered and the solution phase was collected and concentrated in *v*accuo. Only trace of compound **95b** was observed by LCMS analysis. R*f* 0.32 (5% MeOH/DCM). LRM [$M+H^+$; calcd for $C_{16}H_{22}N_2O$: 259.2]

(3*S*,5*S*)-3-Benzyl-5-but-3-enyl-1-(4-hydroxy-benzyl)-[1,4]diazepin-2-one (109).

Diazepinone resin **141** (150 mg) was swollen in DCM (4 mL) in a 10 mL polyethylene tube, treated with 4mL of a mixture of TFA/TES (95:5) at room temperature for 24 h. The resin was filtred and washed with TFA (2 x 5 mL) and DCM (2 x 5 mL). The filtrate was concentrated under vacuum to a residue, that was purified by flash column chromatography using 50% EtOAc/hexane as eluent to afford diazepinone **109** (10 mg, 18% overall yield) as colorless oil, R_f 0.25 (50% EtOAc/hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 1.29-1.47 (m,

2H), 1.6-1.93 (m, 4H), 2.02-2.22 (m, 4H), 3.20-3.24 (br d, 1H), 3.75-3.82 (br q, 1H), 4.50-4.62 (m, 2 H), 5.02-5.10 (m, 2H), 5.75-5.83 (m, 1H), 6.70-7.39 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 174.3, 165.7, 156.5, 135.8, 135.6, 129.0, 128.8, 127.8, 126.5, 126.2, 114.8, 114.6, 60.4, 58.6, 43.9, 34.7, 32.0, 30.1, 28.6. HRMS (EI) *m/z* 365.2228 [$M+H^+$; calcd for $C_{23}H_{28}N_2O_2$: 365.2224]. $[\alpha]_D^{20}$ –7.0° (*c* 0.014, $CHCl_3$).

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CHAPTER 5

CONCLUSION

5.1 Conclusion

Inverse γ-turn conformations have been implicated in the biological activity of peptides such as Leu-enkephalin, angiotensin II, bradykinin, urotensin-II and oxytocin.[1] The introduction of 1,4-diazepin-2-ones into peptide mimic libraries may provide active candidates in structure-activity relationship studies because of their potential to mimic γ-turns. The development of a new synthetic approach for building 1,4-diazepin-2-one heterocycles has been achieved from *N*-substituted and unsubstituted α-aminoacyl β-amino esters by a sequence featuring copper catalyzed cascade addition of vinyl Grignard to the corresponding ester, Boc removal, amine neutralisation and finally reductive amination. Alkene double bond oxidation of the homoallylic ketone followed by Boc removal and neurtralisation of the amine with supported base (Amberlyste A-21) yielded pyrrolodiazepinones. A small family of diazepinones and pyrrolodiazepinones has been synthesized to demonstrate this methodology.

The seven membered heterocyclic mimetics were synthesized from two buildings blocks: a *N*-Boc-α-amino acid and β-amino-ester. The reductive amination was slow in the case of dipeptide; however, conversion of the secondary into tertiary amide caused a noticeable acceleration in the cyclisation step to provide 1,3,5-trisubstituted 1,4-diazepin-2-ones. This approach allowed for the introduction of new diversity at N_1, by employing acid labile nitrogen susbstituent, the 1,3,5-trisubstituted 1,4-diazepin-2-one, could be converted to its 3,5-disubstituted counterpart. For the application of this methodology to solid phase we have focused on phenyl, benzylthioethyl and acid labile linkers.

The efficiency of phenyl and benzylthioethyl linkers was evaluated in solution and solid suppport, LCMS analysis showed traces of 1,4-diazepin-2-one after release from benzylthioethyl linker. In addition we demonstrated that the use of acid labile linker proved a more promising approach to prepare 1,3,5-trisubstituted and 3,5-disubstituted 1,4-diazepin-2-ones as confirmed in the last chapter. In the future this approach may allow for the preparation of of γ-turn mimics libraries libraries for styding peptide chemistry and biology.

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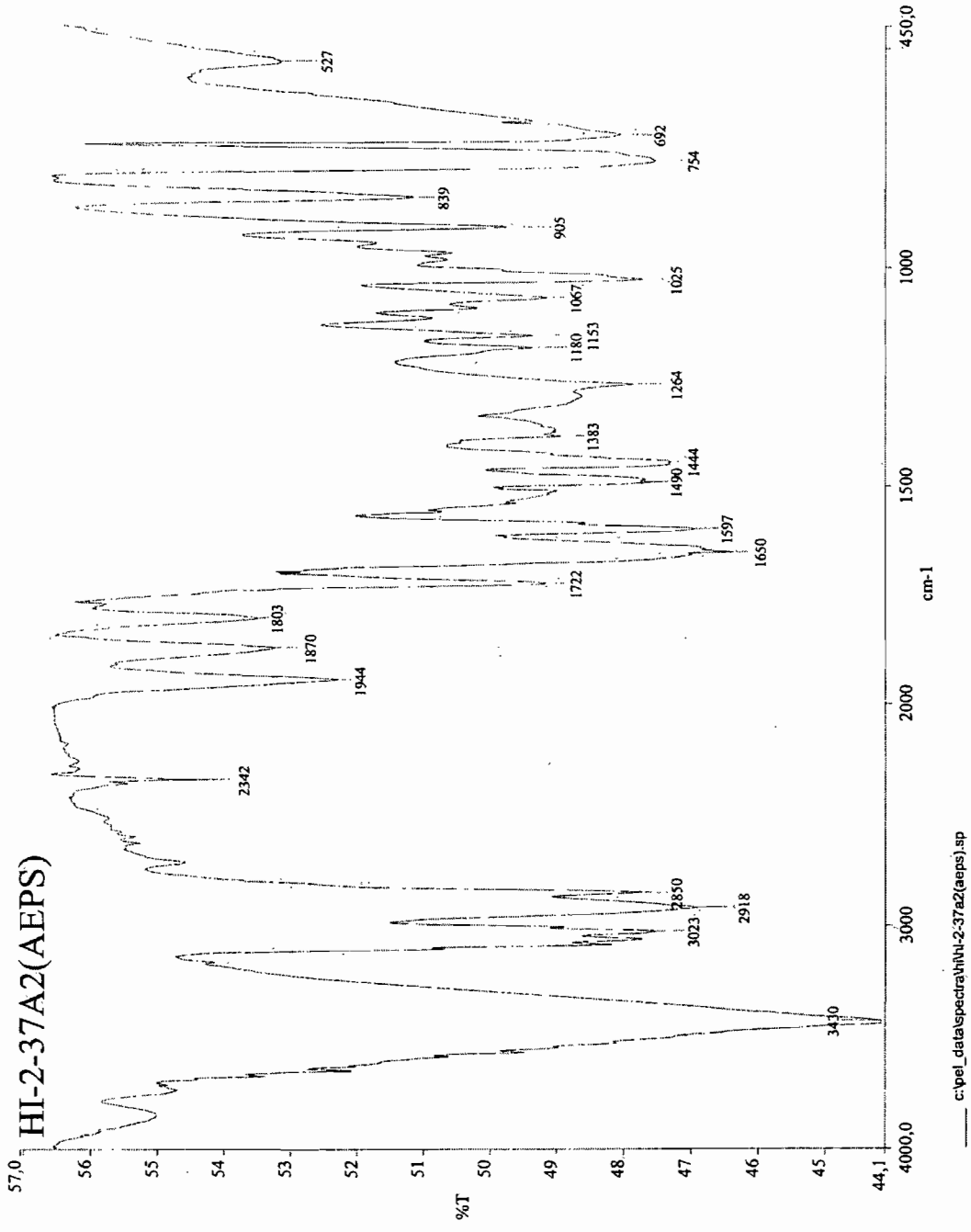
Appendix 1

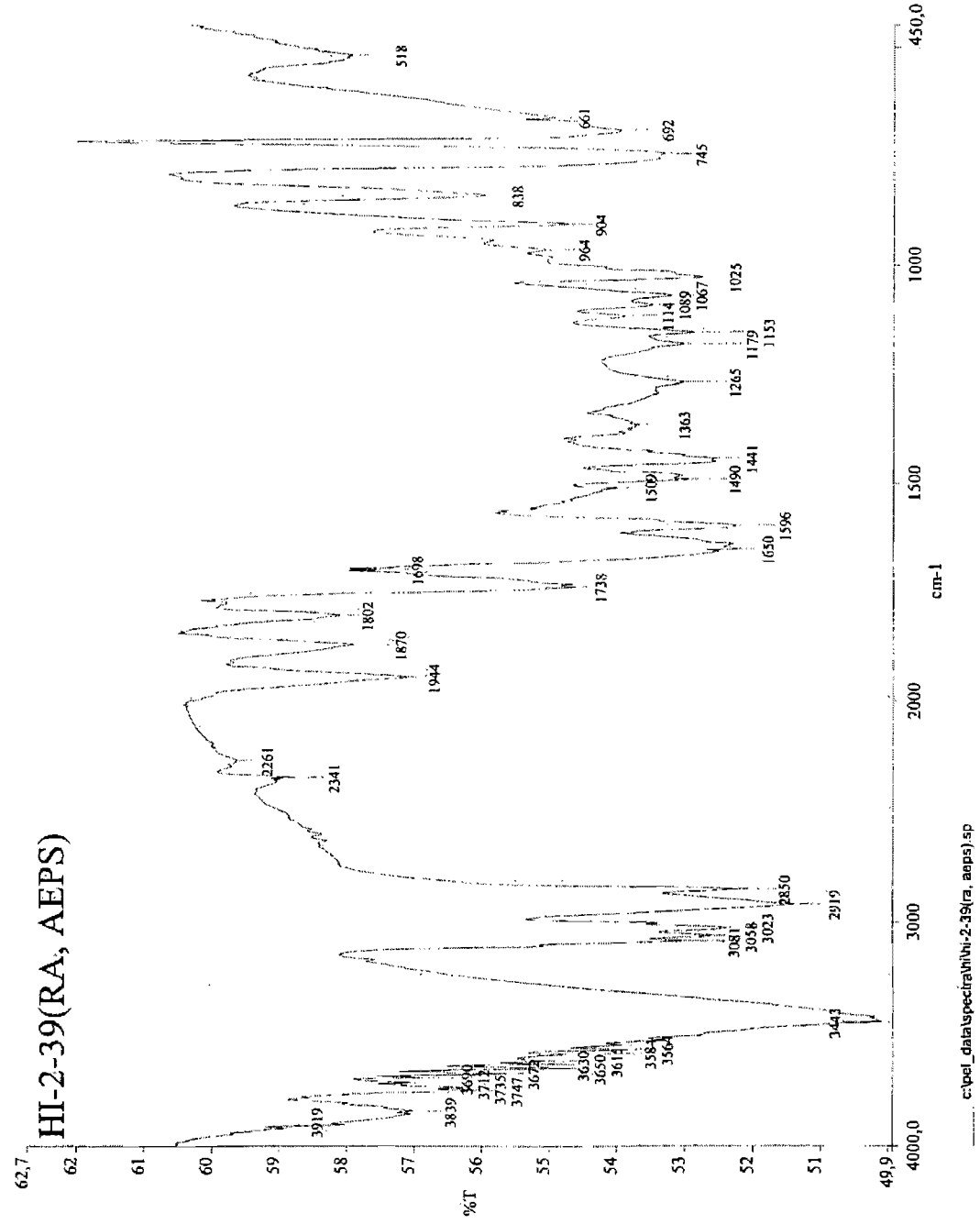
IR spectral data of resins related to chapter 4

Appendix 1

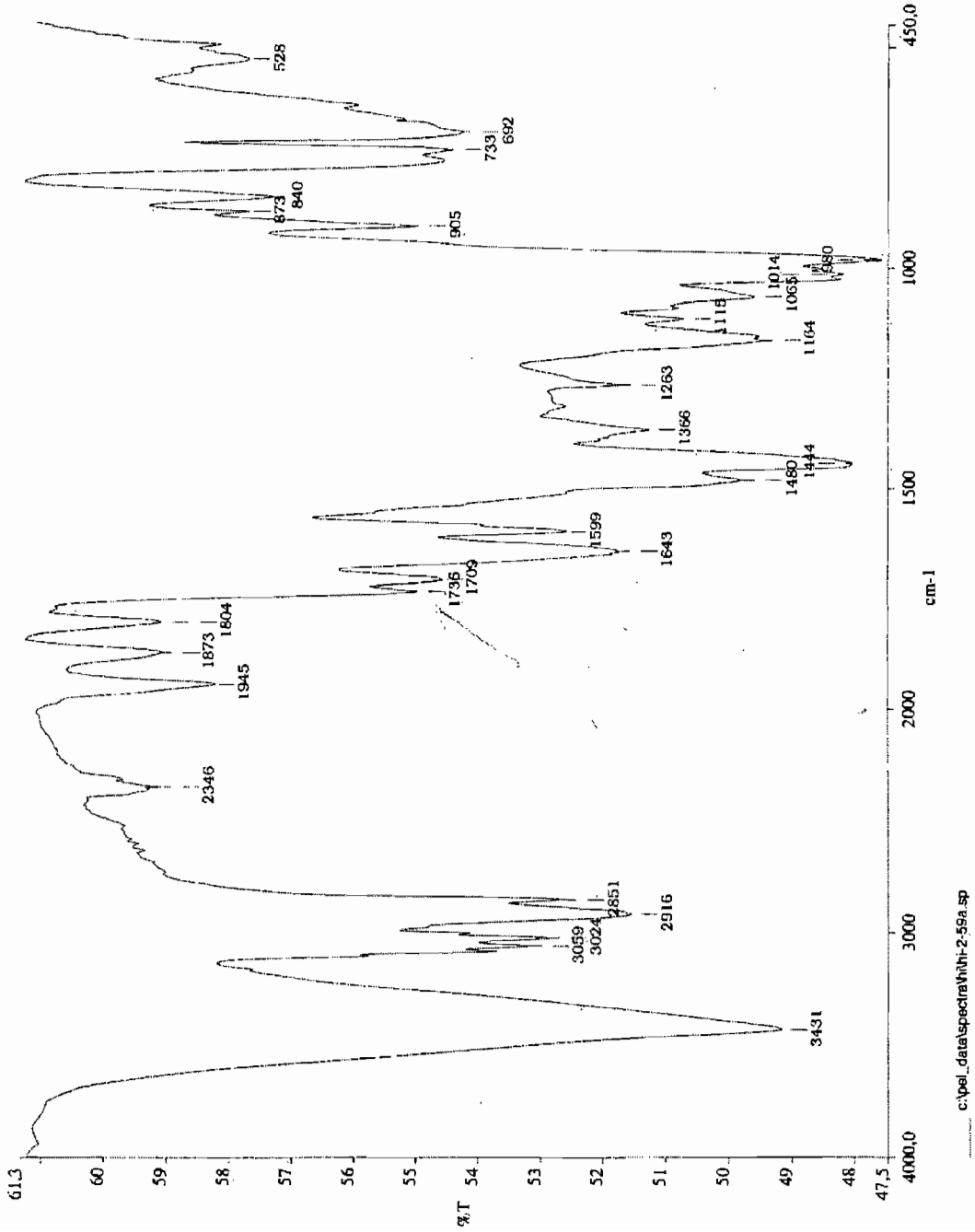
IR spectrum of resin **128a**



IR spectrum of resin **130a**

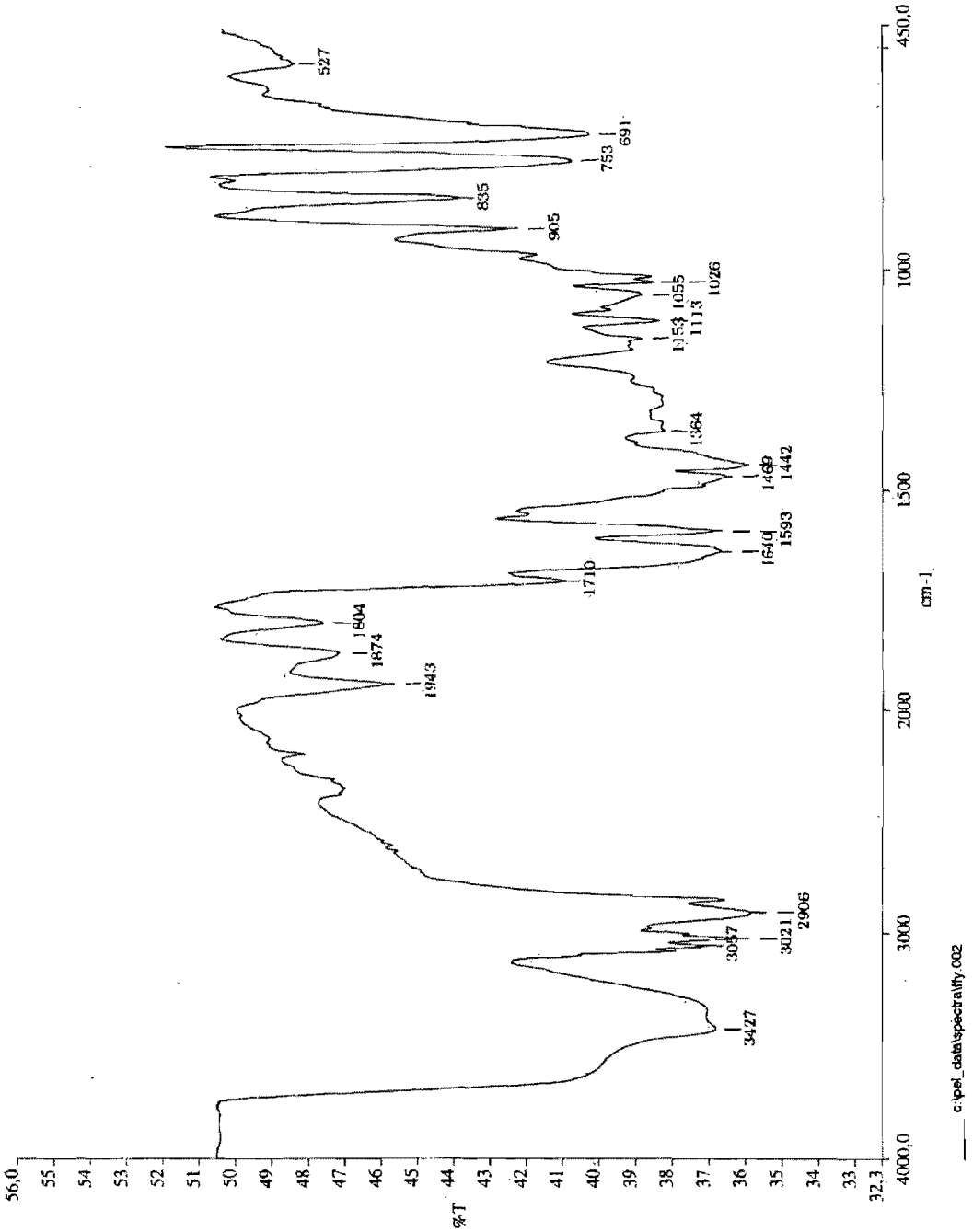


IR spectrum of resin **132a**

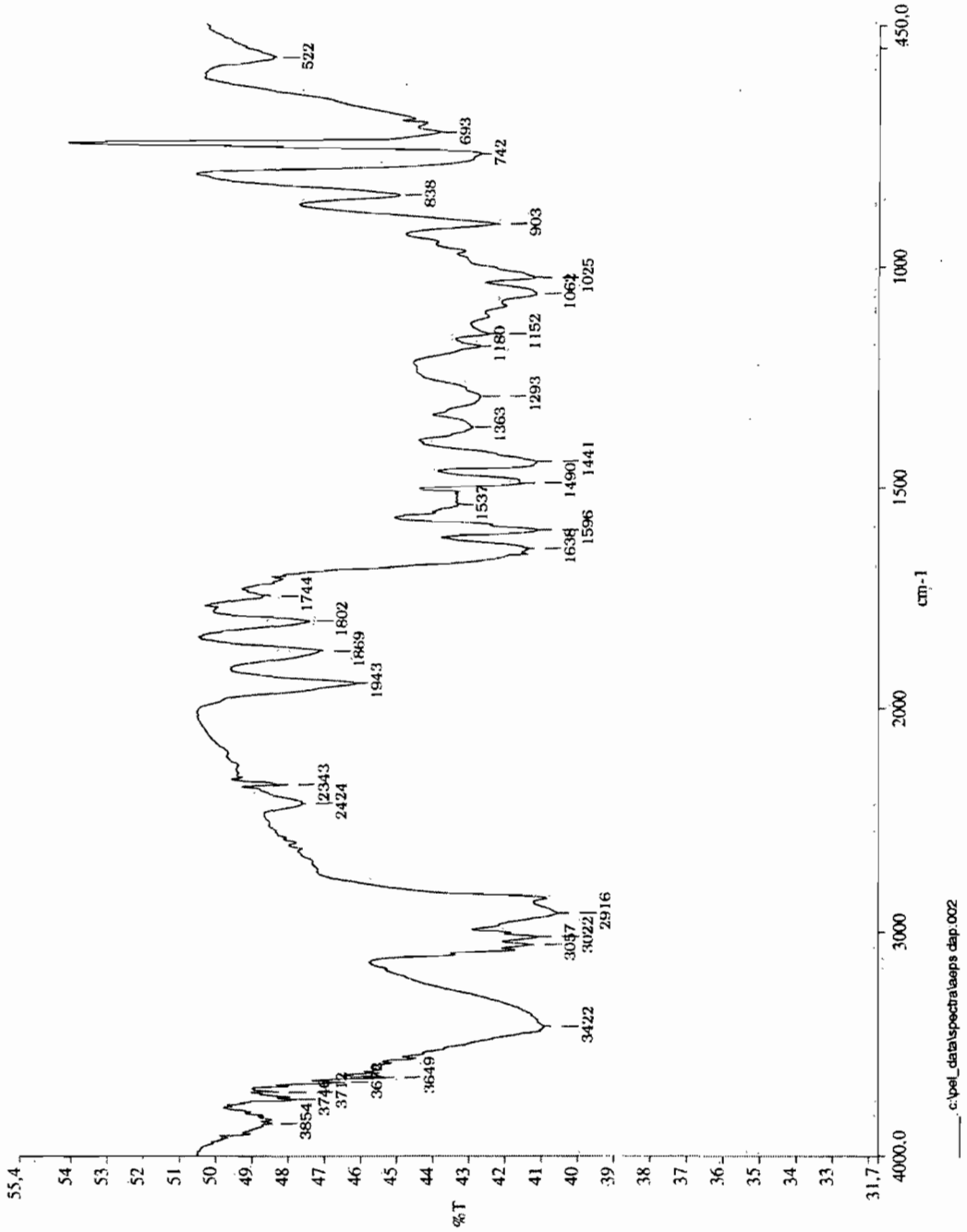


Appendix 1

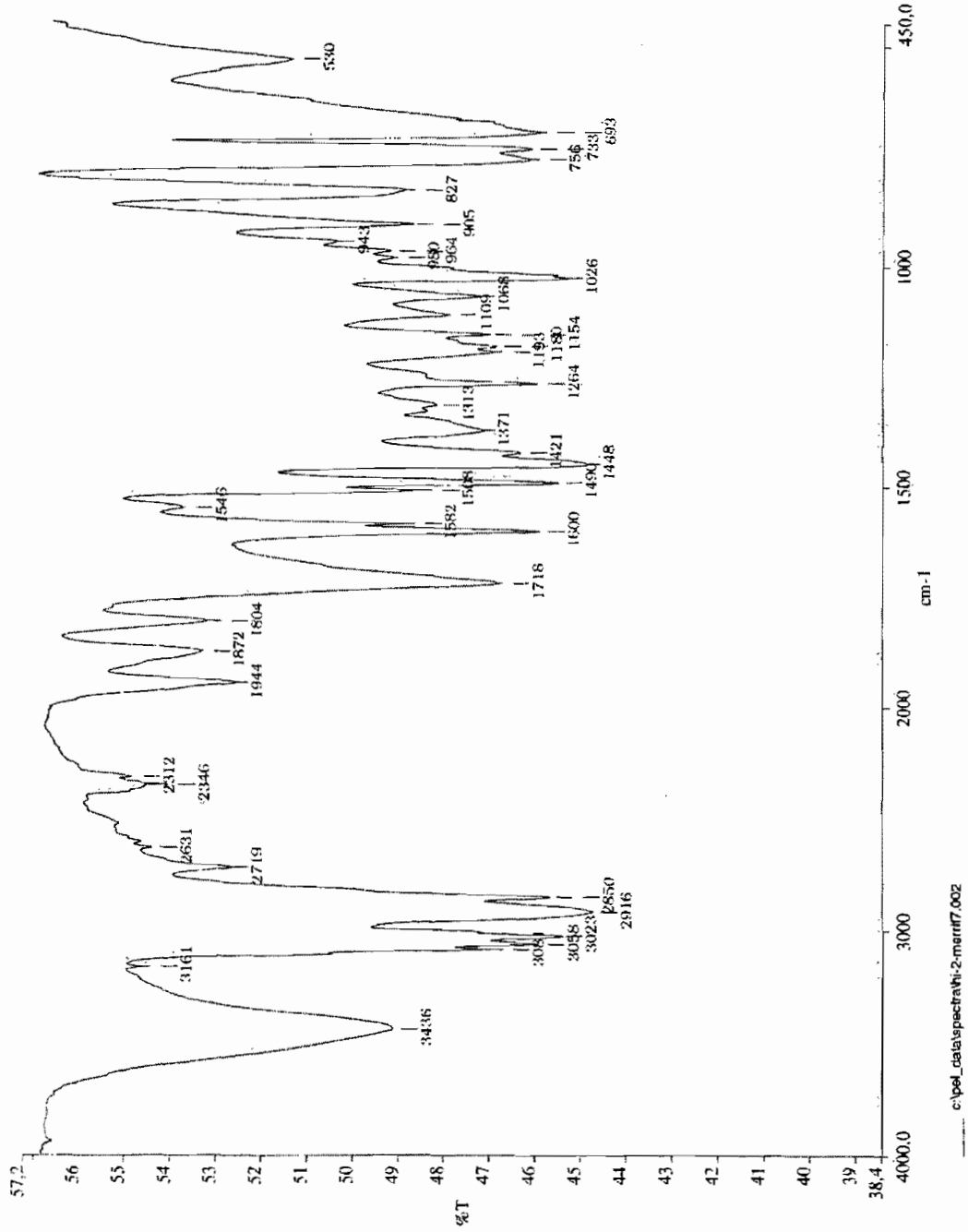
IR spectrum of resin **133a**



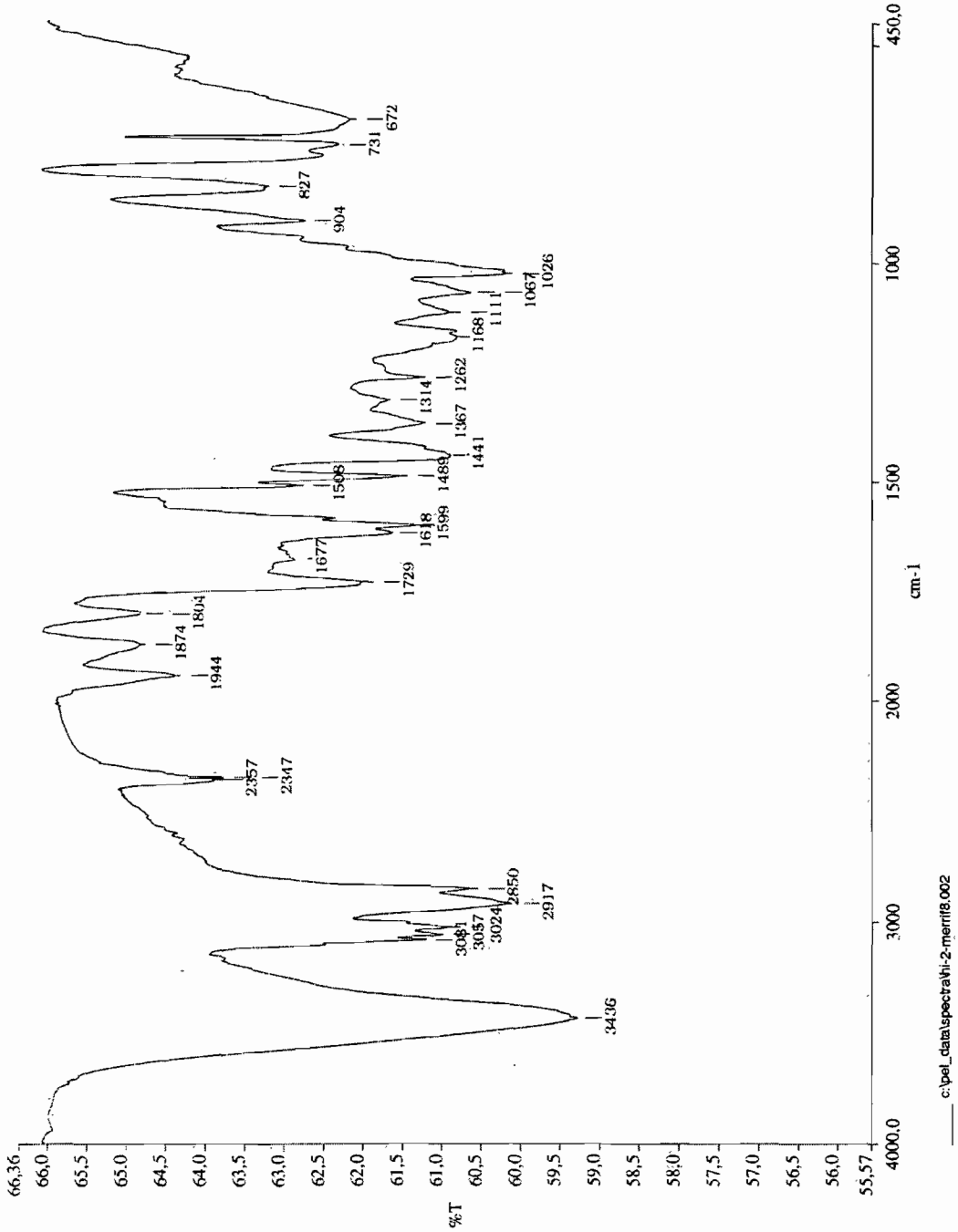
IR spectrum of resin **134a**



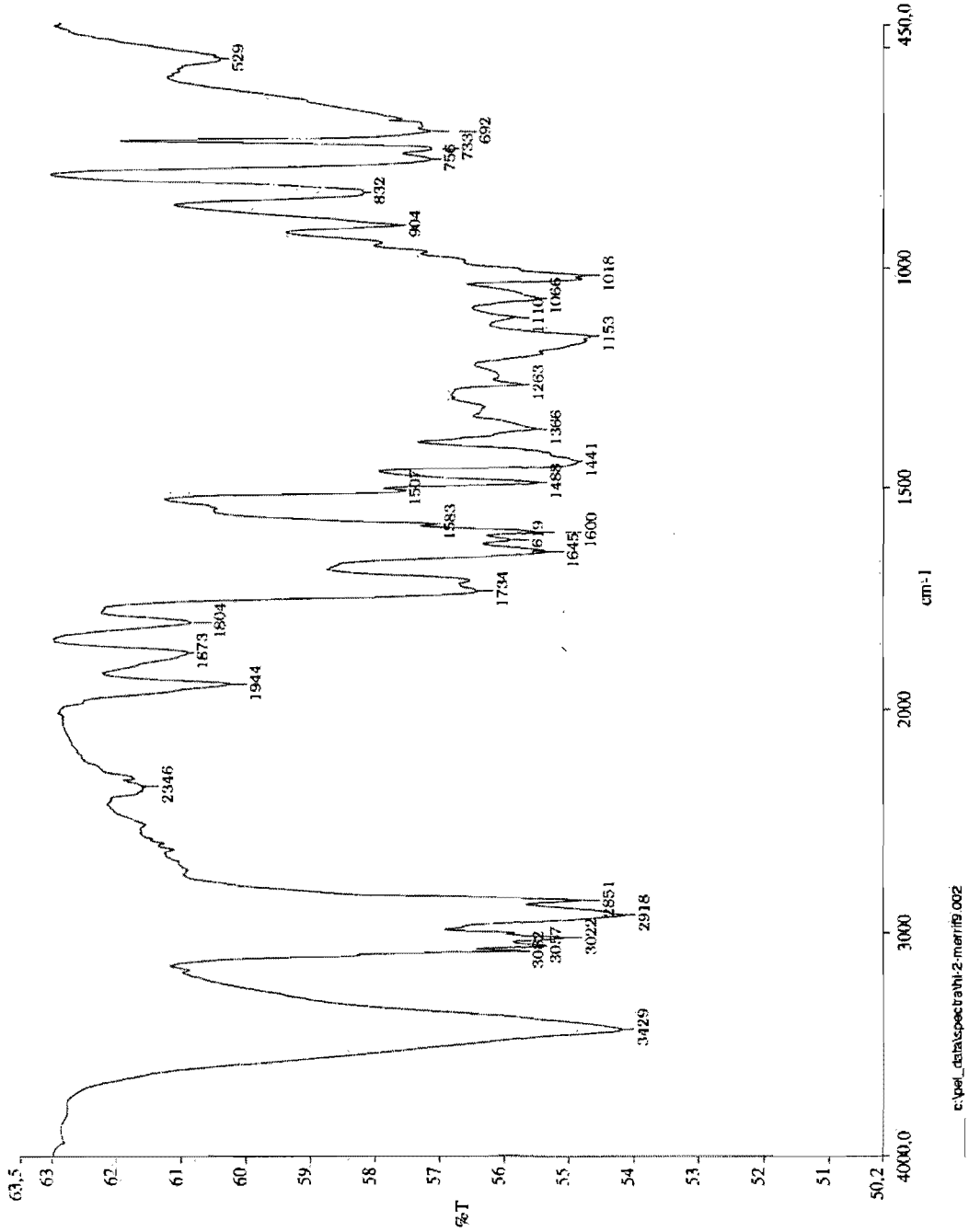
IR spectrum of resin **128b**



IR spectrum of resin **130b**

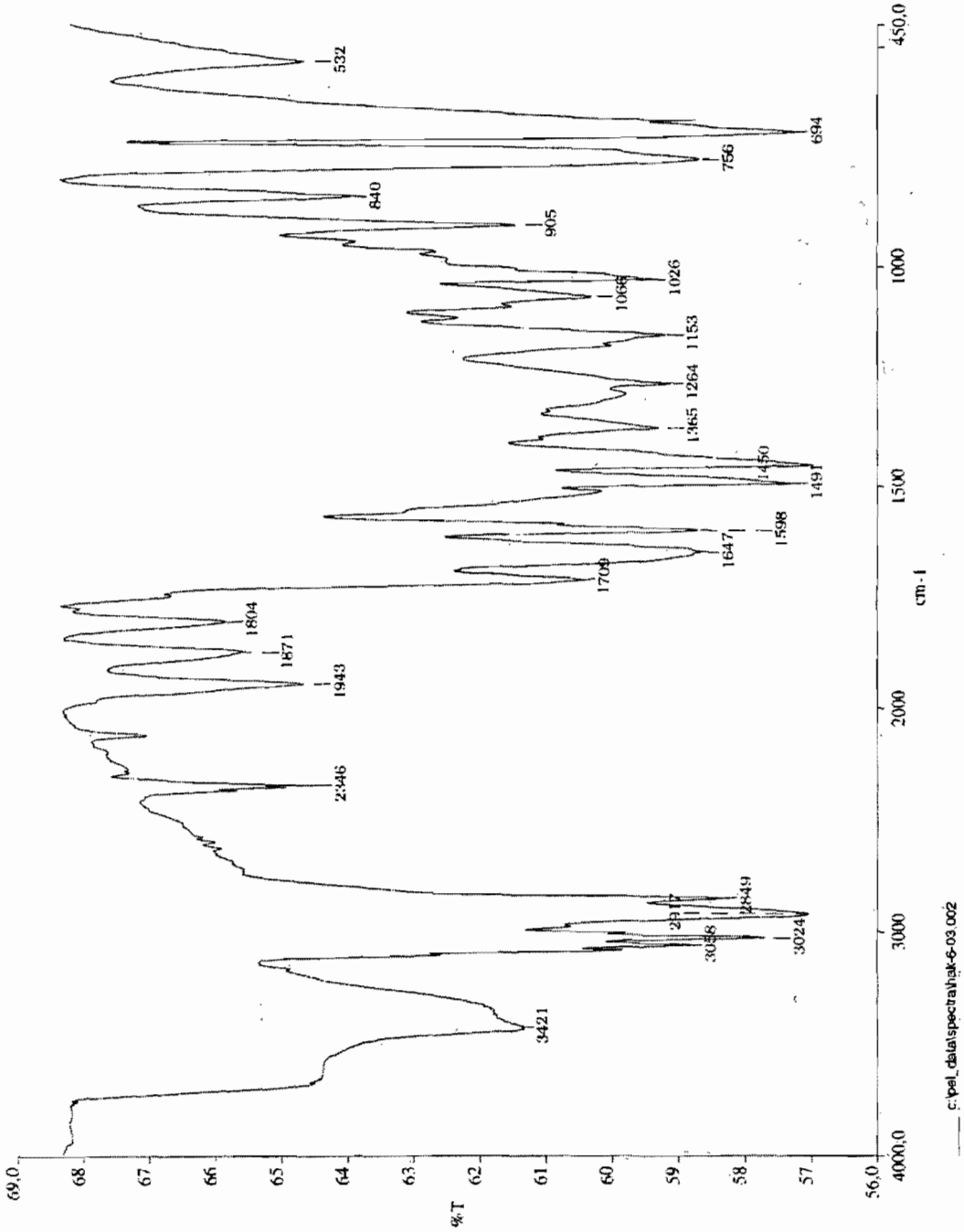


IR spectrum of resin **132b**

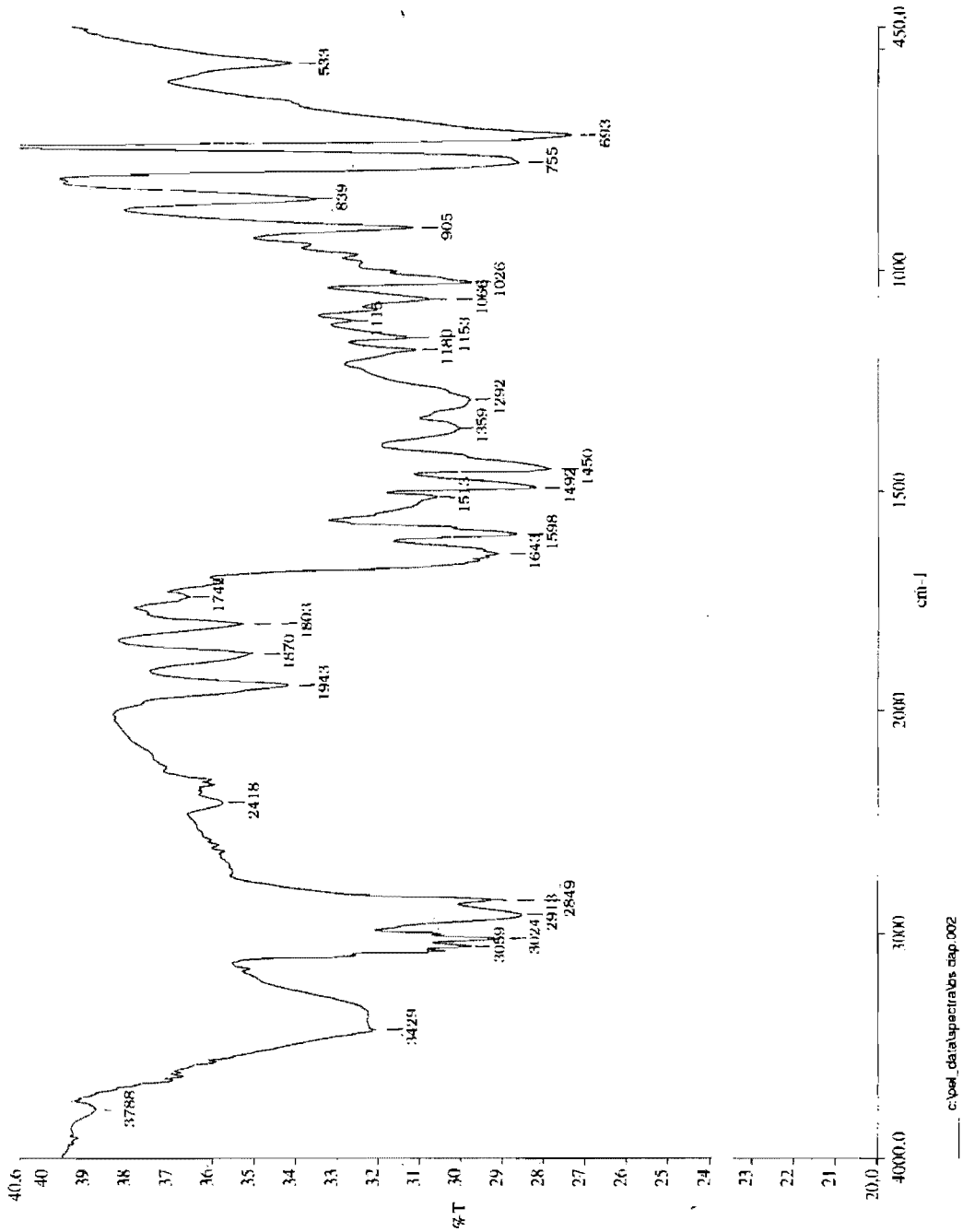


Appendix 1

IR spectrum of resin **133b**



IR spectrum of resin **134b**



Appendix 1

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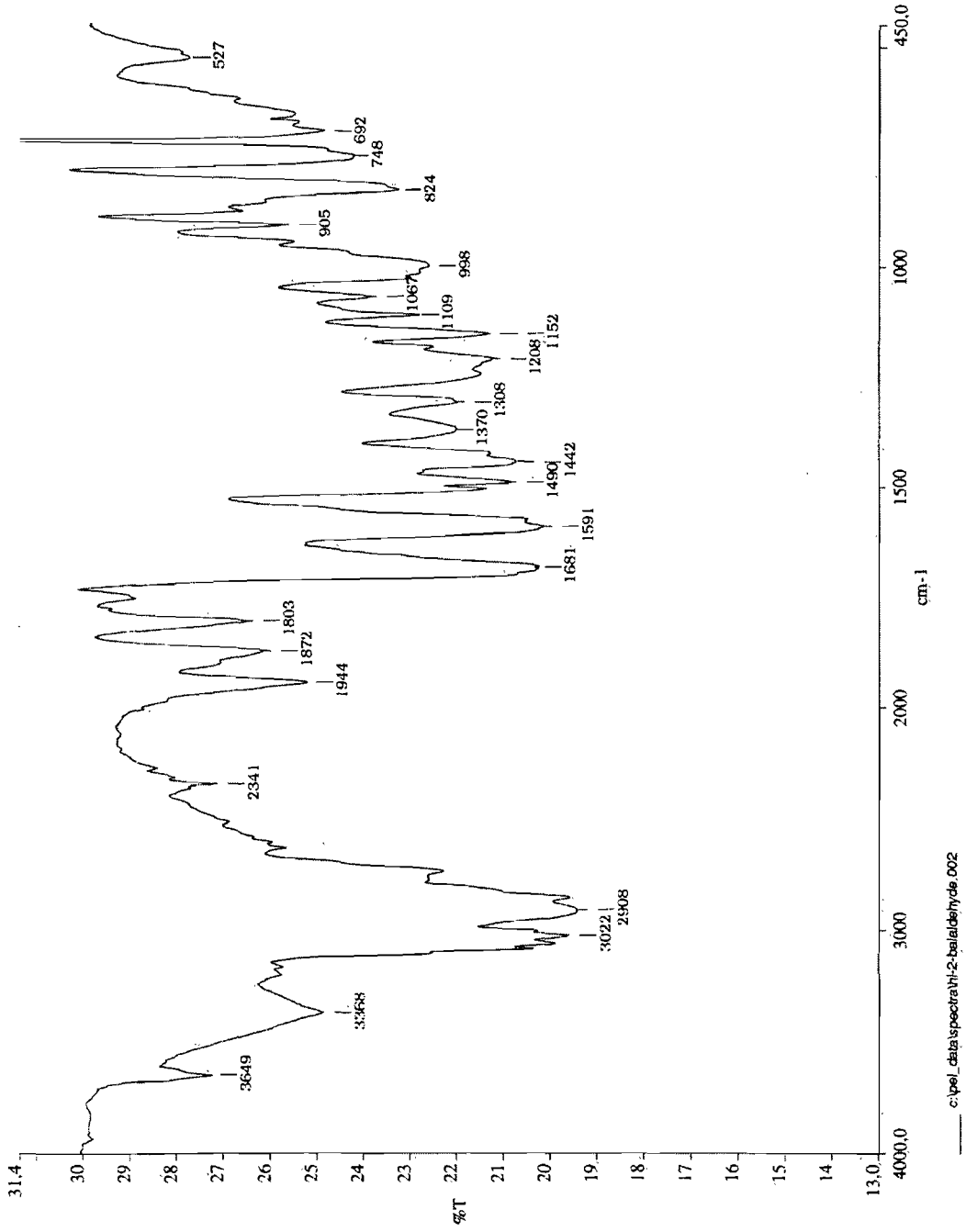
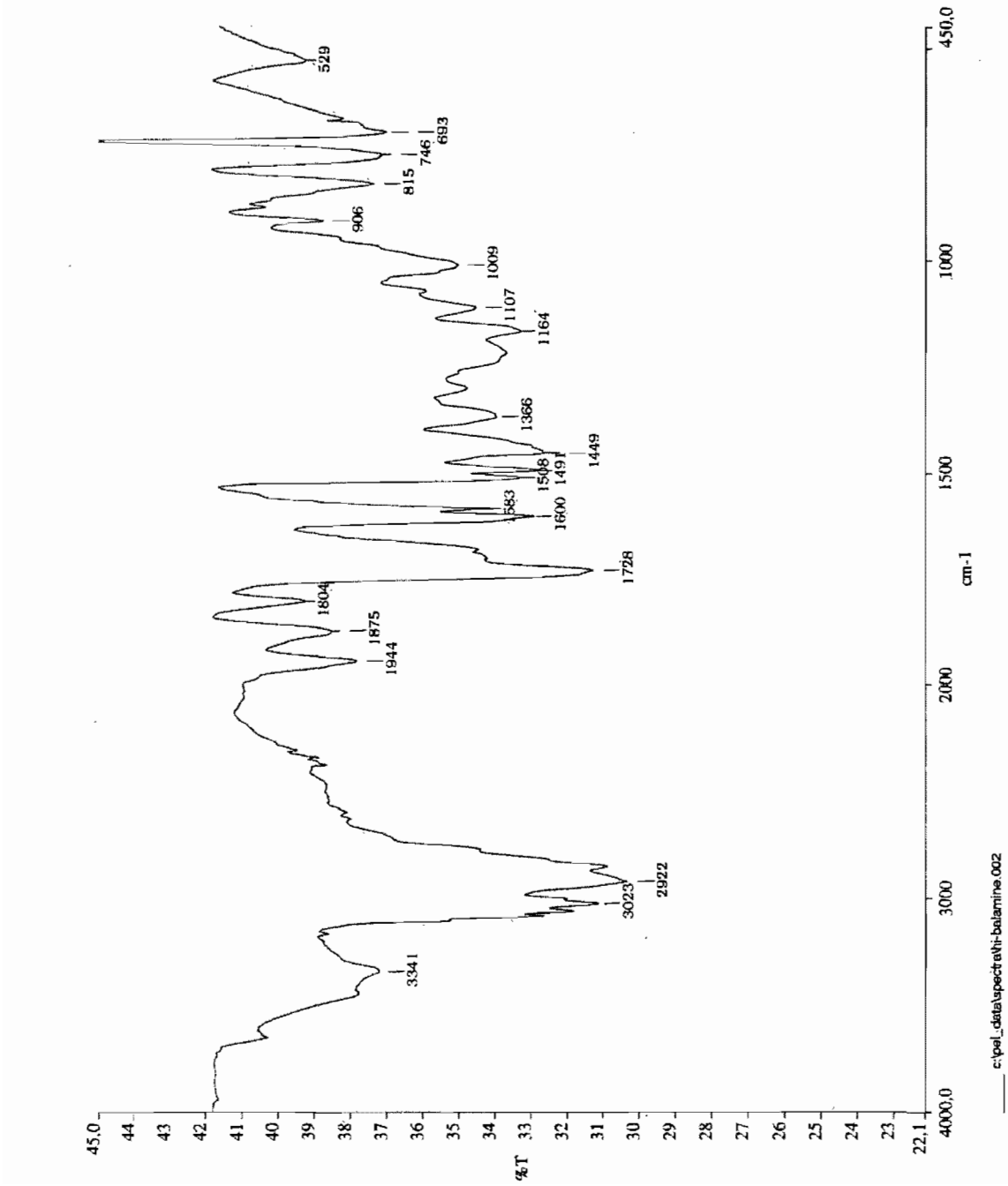


IR spectrum of resin **138**



Appendix 1

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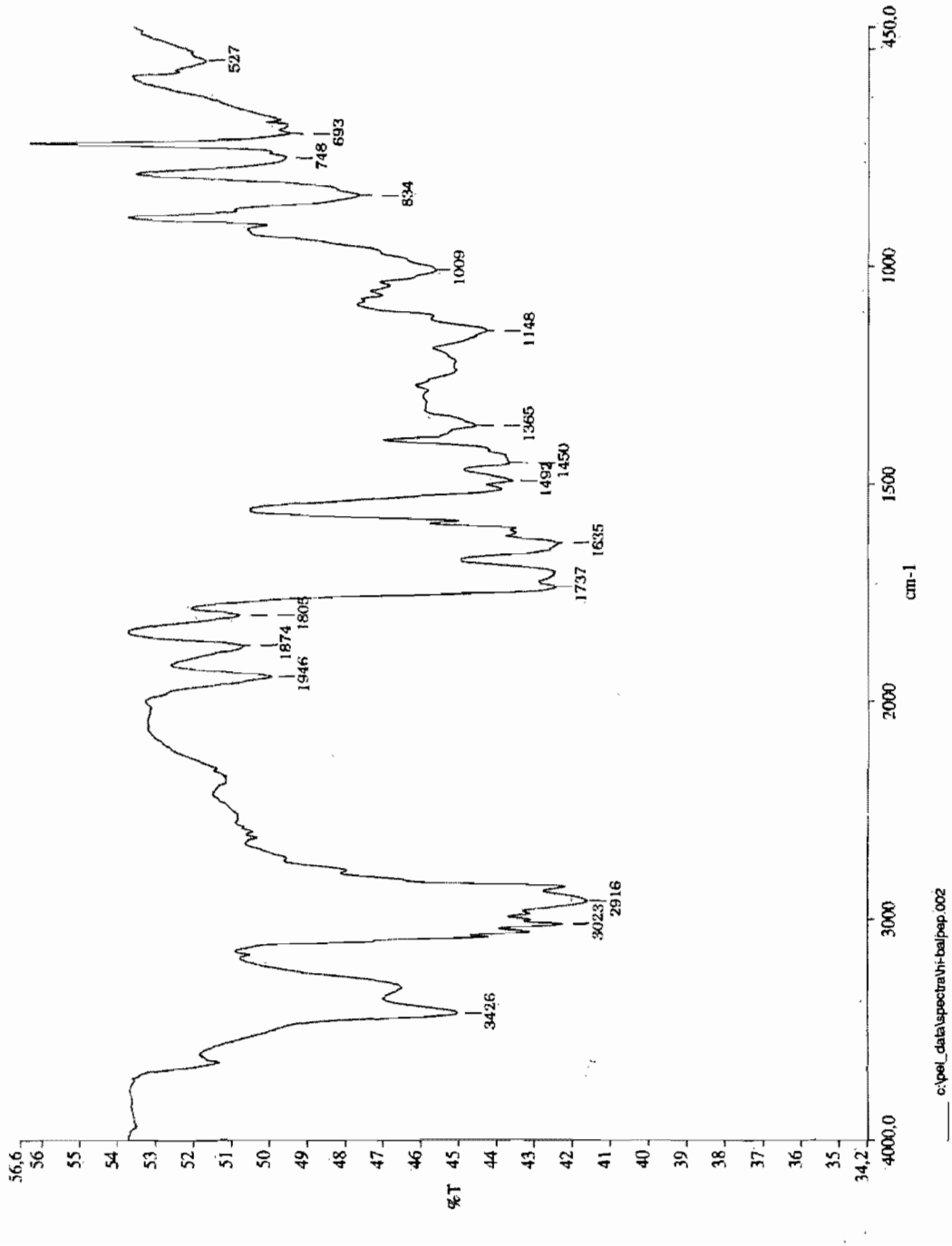

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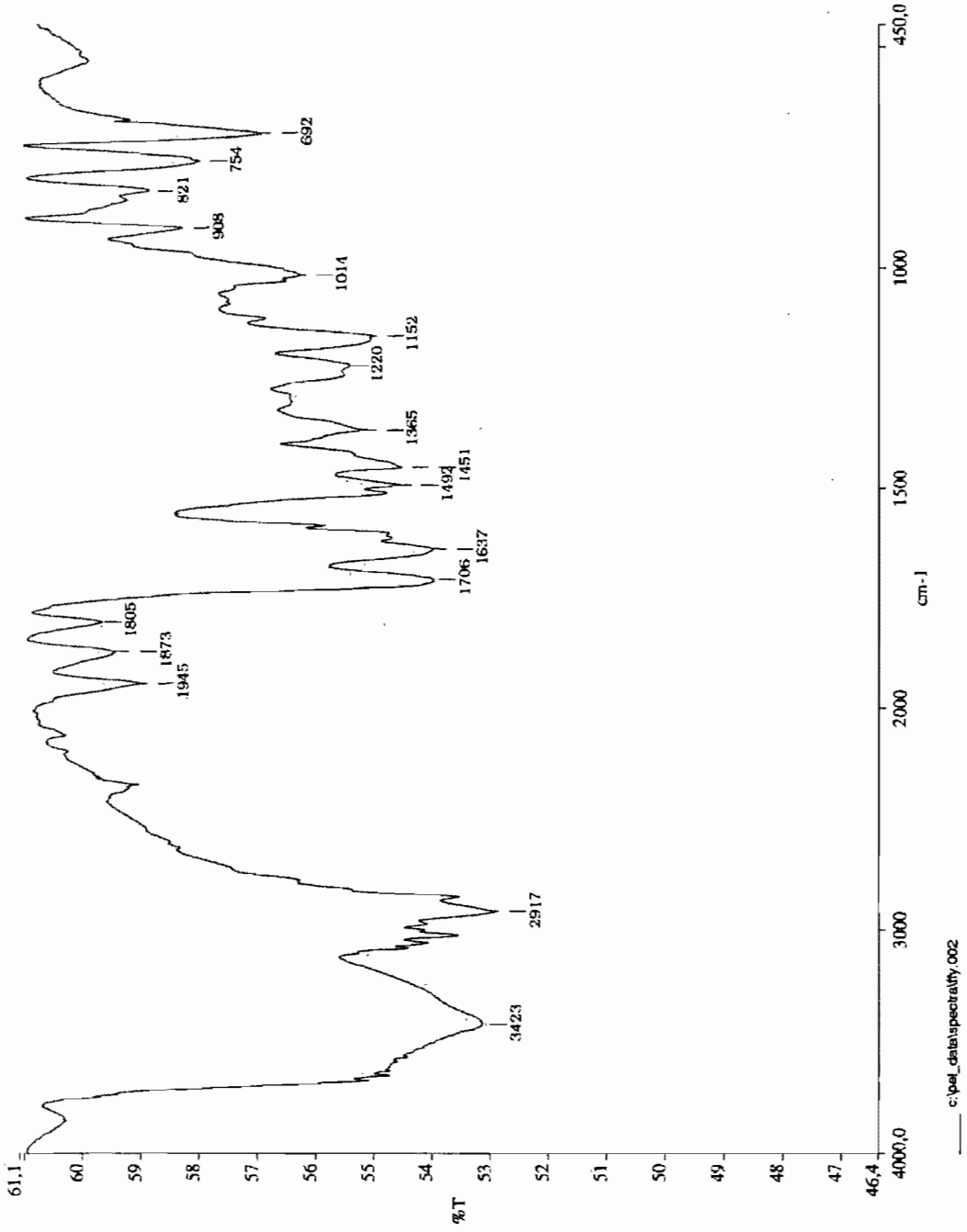

IR spectrum of resin **141**