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Studies toward the Total Synthesis of Natural and Unnatural Aeruginosins

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Abstract

We have demonstrated the usefulness of *tert*-butylsulfonyl (*N*-Bus) protecting group in amino acid and peptide chemistry. It is formed in a 2-step procedure involving reaction of an amine with *tert*-butylsulfinyl chloride, followed by oxidation with *m*-CPBA to obtain the corresponding *tert*-butyl-sulfonamides in excellent yields. The *N*-Bus group can be cleaved to regenerate the corresponding amino salt in 0.1 N TfOH/DCM/anisole at 0 ^{o}C for 10 h.

A variety of *N*-Bus protected amino acids and other common amino acids can be used to form dipeptides and tripeptides. With the exception of the *N*-Fmoc group, the conditions required for the *N*-Bus group cleavage also cleaved the *N*-Boc, *N*-Cbz and *O*-Bn groups. Selective and orthogonal deprotection of *N*-Boc, *N*-Cbz, *N*-Fmoc and *O*-Bn groups could be achieved in the presence of the *N*-Bus protecting group.

The new unnatural amino acids *(3R, 2R)* 3–methyl-D-leucine (β-Me-Leu) and its 2-methyl regioisomer were synthesized by ring opening of an *N*-Ts aziridine intermediate with excess $LiMe_2Cu$. The 1:1.2 mixture of regioisomers were each converted to the corresponding methyl leucines, then coupled to D-phenyllactic acid, followed by coupling with 2-carboxyperhydroindole 4-amidino-benzamide core in the presence of DEPBT. Further elaboration led to linear peptidic unnatural analogues of known aeruginosins such as chlorodysinosin A. The two analogues were also evaluated in enzymatic assays for their inhibitory activity against thrombin and trypsin.

The presumed 3-sulfated aeruginosin 205B and its β–anomer were successfully synthesized from 5 subunits: 3-chloroleucine, D-phenyllactic acid, D-xylose, 2-carboxy-6-hydroxyoctahydroindole, and agmatine. Comparison of

^{1}H and ^{13}C NMR reported data with that of synthetic aeruginosin 205B revealed a disturbing discrepancy with regard to the position of the presumed 3'-sulfate on the D-xylopyranosyl unit. We synthesized methyl α-D-xylopyranosides with sulfates at each of the hydroxyl groups and conclusively demonstrated the the presence of a C-4'-sulfate by comparison of the ^{1}H and ^{13}C NMR spectroscopic data. Thus, the structure of aeruginosin 205B should be revised.

One of the key steps in the synthesis is glycoside formation of the axially oriented C-6 hydroxyl group in the Choi subunit. The 2-thiopyridyl carbonate was a suitable method for anomeric activation, followed by treatment with AgOTf and tetramethylurea in ether-DCM solution to give the desired α-anomer, which was easily separable from the β-anomer by column chromatography.

Keywords: *tert*-butylsulfonyl (*N*-Bus) group, amino acid protection, β–methyl-D-leucinyl aeruginosin, *(3R, 2R)* 3–methyl-D-leucine (β-Me-Leu), aeruginosin 205B, axially oriented glycoside synthesis

Résumé

Nous avons démontré l'utilité du groupement protecteur *tert*-butylsulfonyle (*N*-Bus) pour la chimie des acides aminés et des peptides. Celui-ci est préparé en deux étapes, impliquant la réaction d'une amine avec le chlorure de *tert*-butylsulfinyle, suivie par l'oxydation par du *m*-CPBA, pour obtenir les *tert*-butylsulfonamides correspondants avec d'excellents rendements. Le groupement *N*-Bus peut être clivé par traitement avec 0.1 N TfOH/DCM/anisole à 0°C en 10h pour régénérer le sel d'ammonium.

Une variété d'acides aminés *N*-Bus protégés ainsi que d'autres aminoacides peuvent alors être utilisés pour préparer divers dipeptides et tripeptides. A l'exception du groupe *N*-Fmoc, les conditions de déprotection du groupe *N*-Bus clivent également les groupements *N*-Boc, *N*-Cbz et *O*-Bn. Une déprotection sélective et orthogonale des groupes *N*-Boc, *N*-Cbz, *N*-Fmoc et *O*-Bn est également possible en présence du groupe protecteur *N*-Bus.

Le nouvel acide aminé non-naturel *(3R, 2R)* 3–méthyl-D-leucine (β-Me-Leu) et son régioisomère 2-méthyle ont été synthétisés par ouverture d'une *N*-Ts aziridine en présence d'un excès de $LiMe_2Cu$. Chacun des régioisomères du mélange (1:1,2) a été converti en la méthylleucine correspondante, puis couplé à l'acide D-phényllactique puis au motif 2-carboxyperhydroindole 4-amidinobenzamide en présence de DEPBT. Des élaborations ultérieures ont conduit à des analogues peptidiques non-naturels d'aeruginosines telles que la chlorodysinosine A. Les deux analogues ont ensuite été évalués pour leur activité inhibitrice de la thrombine et la trypsine.

La présumée aeruginosine 3-sulfate 205B et son anomère β ont été synthétisés avec succès à partir de 5 sous-unités : la 3-chloroleucine, l'acide

D-phénylactique, le D-xylose, le 2-carboxy-6-hydroxyoctahydroindole et l'agmatine. La comparaison des données RMN ^{1}H et ^{13}C reportées avec celles obtenues avec l'aeruginosine synthétique 205B révèle une différence majeure pour la position du groupe présumé 3'-sulfate sur l'unité D-xylopyranosyle. Nous avons alors synthétisés les dérivés méthyl-α-D-xylopyranosides avec un groupement sulfate à chacune des positions hydroxyles, afin de démontrer sans ambiguïté la présence du sulfate en position C-4' par comparaison des données spectroscopiques RMN ^{1}H et ^{13}C. La structure de l'aeruginosine 205B a alors été révisée.

Une des étapes-clés de cette synthèse consiste en la formation du glycoside avec le groupe hydroxyle en C-6 orienté en axial sur la sous-unité Choi. Le 2-thiopyridylcarbonate s'est avéré une méthode efficace pour l'activation anomérique. Le traitement par AgOTf et la tétraméthylurée en solution dans un mélange éther-DCM permet d'obtenir l'anomère α désiré, qui peut alors être aisément séparé de l'anomère β par chromatographie

Mots clés: groupe *tert*-butylsulfonyle (*N*-Bus), protection d'acides aminés, β–méthyl-D-leucinyl aeruginosine, *(3R,2R)* 3–méthyl-D-leucine (β-Me-Leu), aeruginosine 205B, synthèse de glycosides orientés en axial

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Abbreviations

$[\alpha]_D$	specific rotation
Ac	acetyl
Bn	benzyl
Boc	*tert*-butoxylcarbonyl
Bus	*tert*-butylsulfonyl
δ	chemical shift in ppm
Cbz	carbobenzyloxy
COSY	correlation spectroscopy
^oC	degrees celsius
dd	doublet doublet
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DEPBT	3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
DMAP	dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide, methyl sulfoxide
dt	doublet of triplets
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
Et	ethyl
eq (equiv.)	equivalent
ESI/MS	electrospray ionisation-mass spectrometric

Fmoc	9-fluorenylmethoxycarbonyl
g	gram
h	hour(s)
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrum
Hz	hertz
IR	infrared
J	coupling constant
m	multiplet
μ	micro 10^{-6}
μL	microliter
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
mg	milligram
min.	minute
MHz	megahertz
mL	milliliter
mmol	millimole
MOM	methoxymethyl
Ms	mesyl
MS	molecular sieves
NMO	4-methylmorpholine *N*-oxide
OTf	trifluoromethanesulfonate, triflate
Ph	phenyl
PMB	*para*-methoxybenzyl
PTC	phenylthiocarbamide
PTSA	*para*-toluenesulfonic acid

ppm	parts per million
PPTS	pyridinium p-toluenesulfonate
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
Py.	pyridine
q	quartet
R.T. (r.t.)	room temperature
s	singlet
sec	second(s)
t	triplet
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
t-Bu	*tert*-butyl
TEA (NEt_3)	triethylamine
tert	tertiary
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMU	tetramethyl urea
Ts	tosyl

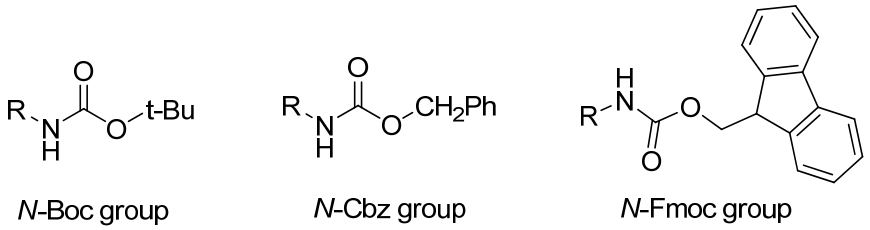
Chapter One

Tert-Butylsulfonyl (Bus), a New Protecting Group for Amino Acids and Peptides

1-1 Introduction

1-1-1 Common amino acid protecting groups: Cbz, Boc, Fmoc…

N-Protecting groups are of primary importance in the utilization of amino acids and nitrogen containing organic molecules in synthesis.[1-3] Among the more popular *N*-protecting groups that can also benefit from an orthogonal deprotection strategy are the *N*-Boc, *N*-Cbz and *N*-Fmoc groups (Figure 1).[4,5]



N-Boc group *N*-Cbz group *N*-Fmoc group

Figure 1. Structures of the *N*-Boc, *N*-Cbz and *N*-Fmoc groups.

1-1-1-1 *N*-Boc group

The Boc group is used extensively in peptide synthesis for amine protection,[6] due to its stability under basic conditions and its inertness to many other nucleophilic reagents.

One of the more common methods for Boc introduction is to treat the amine with Boc anhydride (Boc_2O) in aqueous sodium hydroxide. It has the advantage that the product is easily isolated.[7] For sterically hindered amino acids, a superior method is to utilize Boc_2O with tetramethylammonium hydroxide pentahydrate ($Me_4NOH.5H_2O$) in acetonitrile.[8] Both of these procedures afford excellent yields.

The *N*-Boc group is sensitive to acidic conditions, such as 3 M HCl in ethyl acetate, [9] acetyl chloride (AcCl) in methanol,[10] 10% H_2SO_4 in dioxane and 50% trifluoroacetic acid (TFA) in dichloromethane.[11] The *N*-Boc group can also be deprotected by a catalytic amount of cerium(IV) ammonium nitrate (CAN) in

acetonitrile.[12]

1-1-1-2 *N*-Cbz group

The *N*-Cbz group is similar to the *N*-Boc group in its stability under basic conditions, however unlike *N*-Boc group, is also stable under mild acidic conditions.

The reagent dibenzyl dicarbonate $(PhCH_2OCO)_2O$ with sodium hydroxide or triethylamine (Et_3N) as base in dioxane-water mixture[13,14], has been used to prepare *N*-Cbz compounds. Better yields were reported than with benzyl chloroformate ($PhCH_2OCOCl$)[15].

The most common method to cleave the *N*-Cbz group is to use Pd over activated carbon (Pd-C) as a catalyst under hydrogen gas atmosphere.[15] The *N*-Cbz group is also readily cleaved under strongly acidic conditions such as hydrogen bromide (HBr) in acetic acid (AcOH),[16] 50% trifluoroacetic acid (TFA) solution,[17] or trifluoromethanesulfonic acid (CF_3SO_3H).[18] This group is also unstable under strongly basic condition such as 40% potassium hydroxide in the methanol-water solution[19] and with sodium (Na)-ammonia (NH_3) in a dissolving metal reduction. [20]

1-1-1-3 *N*-Fmoc group

A major advantage of the Fmoc protecting group is that it has excellent acid stability, under which *N*-Boc and *N*-Cbz groups can be removed. It can be readily cleaved by simple amines to liberate the amine as its free base.[21]

The most common method of introducing Fmoc is using 9-fluorenyl-methoxycarbonyl chloride (Fmoc-Cl) with sodium bicarbonate ($NaHCO_3$) as a base in dioxane.[22] Diisopropylethylamine is reported to suppress dipeptide formation during Fmoc introduction with Fmoc-Cl.[23] Another reagent that also could be used is 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) in

acetonitrile-water solution.[24,25] The advantage of Fmoc-OSu is that little or no oligopeptides are formed when amino acid derivatives are prepared.[26]

The *N*-Fmoc group is cleaved under mild conditions with an amine base, such as 20% piperidine in DMF, to afford dibenzofulvene and free amine.[26] *N*-Fmoc is generally considered to be stable to hydrogenation, but it has been shown that under certain conditions it can be hydrogenated by Pd-C as a catalyst under hydrogen gas in the presence of acetic acid in methanol.[27]

1-1-2 *Tert*-Butylsulfonyl (Bus), as a new protecting group for amines

Sulfonyl groups have had a long history in the protection of amines and other nitrogeneous functionalities.[28] Their stability within a wide array of reaction conditions is one of the most attractive features of sulfonamides. Unfortunately due to the harsh conditions, which are required for their removal, *N*-sulfonyl groups are not as versatile in highly functionalized or sensitive substrates.

In recent years, *N*-sulfonyl protecting groups have been improved by addition of new aryl varieties, including the heteroarene-2-sulfonyl chlorides such as BtsCl and ThsCl groups (Figure 2) introduced by Vedejs[29]; and the *N*-o-nitrophenylsulfonyl chlorides such as DNsCl and NsCl groups (Figure 2), which were introduced by Fukuyama[30]. Those are stable in acidic conditions and amenable to mild removal, such as *N*-Bts or *N*-Ths groups were removed by treatment with zinc (Zn) in acetic acid-ethanol mixture or aluminum-mercury (Al-Hg) in ether-water solution at room temperature,[29] deprotection of *N*-DNs group was performed by treatment with thioglycolic acid ($HSCH_2CO_2H$) and triethylamine in dichloromethane,[30] and *N*-Ns group was deprotected with thiophenol (PhSH) and cesium carbonate (Cs_2CO_3) in acetonitrile.[30]

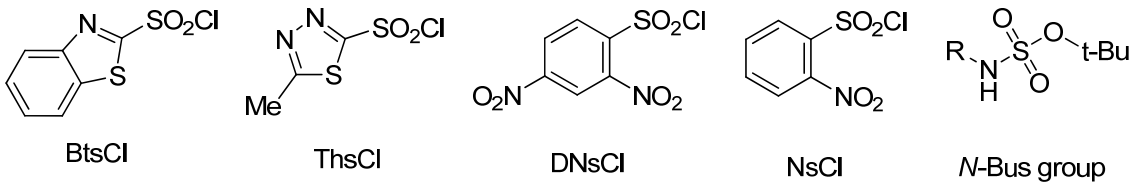


Figure 2. Structures of the BtsCl, ThsCl, DNsCl, NsCl and *N*-Bus groups.

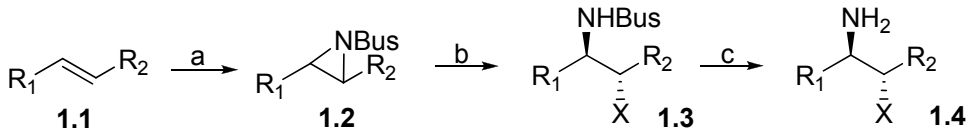
In 1997 Sun and Weinreb reported the use of the *N*-*tert*-butylsulfonyl group (Figure 2) as a new protecting group for amines.[31] *tert*-Butylsulfonyl chloride is quite unstable and does not normally undergo nucleophilic displacement at sulfur with amines.[32] The preparation of *N*-*tert*-butylsulfonamides consists of a 2-step procedure, which involves: 1) reaction of an amine with the commercially available *tert*-butylsulfinyl chloride; 2) oxidation of resulting *tert*-sulfinamide with a variety of oxidants, including $KMnO_4$,[33] *m*-CPBA,[31] $RuCl_3/NaIO_4$,[31] $H_2O_2/LiOH$[34] and NaOCl/PTC;[35] to afford the corresponding product in excellent overall yield. Sun and Weinreb showed that the *N*-Bus group is stable to a variety of organolithium and Grignard reagents and also exhibited stability toward strong bases. The *N*-Bus group can be cleaved with TFA/anisole at room temperature to regenerate the amine salt in good yields.

1-1-3 *Tert*-butylsulfonamide as a new functional group

Only limited use has been made of this versatile functionality since its initial report in 1997 by Sun and Weinreb.[31]

1-1-3-1 As an effective nitrogen source of aziridine

Sharpless and coworkers have shown that *tert*-butylsulfonamide is an effective nitrogen source for catalytic aziridination of olefins (Scheme 1).[36]

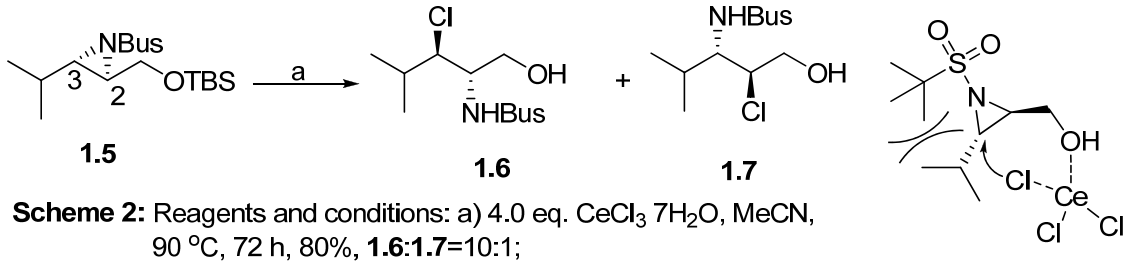


Scheme 1: Reagents and conditions: a) 1.2 eq. Bus-NClNa, PTAB (phenyltrimethylammonium tribromide) 10 mol%, MeCN, r.t.,10 h; b) nucleophilic reagent; c) 20 eq. anisole, TfOH, DCM, r.t.;

With simple unfunctionalized olefins **1.1**, aziridines **1.2** which often are the only reaction products, are generally obtained in high yields. Product isolation is simple, and purification does not require chromatography. The aziridination of the olefins is stereospecific. For example, *cis*- and *trans*-*β*-methylstyrene afford exclusively the *cis*- and *trans*-aziridines, respectively. Substantial amounts of the rearranged allylic sulfonamide were obtained in the case of exocyclic olefins, such as methylenecyclohexane. Nucleoplilic attack led to *tert*-butylsulfonamides **1.3**. The corresponding amines **1.4** were formed under reasonably mild acidic conditions, after cleavage of the Bus-group.

1-1-3-2 As a protecting group in regioseletive opening of aziridines

During the total synthesis of chlorodysinosin A, the regioselective opening of an aziridine intermediate **1.5** with $CeCl_3$ (Scheme 2),[37] was only possible with the *N*-Bus protecting group on the aziridine.

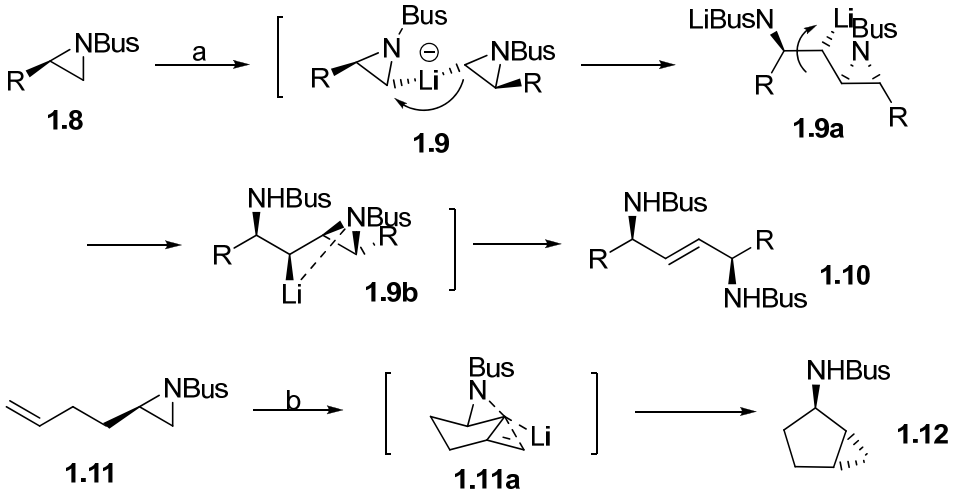


Scheme 2: Reagents and conditions: a) 4.0 eq. $CeCl_3$ $7H_2O$, MeCN, 90 °C, 72 h, 80%, **1.6**:**1.7**=10:1;

The ratio of the products **1.6** and **1.7** was more than 10 to 1. A steric clash between the isopropyl group and the *tert*-butyl group on the *N*-Bus group could

be the reason for the high regioselectivity of $CeCl_3$-mediated opening of the Bus-protected, and thus providing an open path for S_N^2 attack at C3. The less sterically demanding *N*-Ts and *N*-trifluoromethylsulfonyl groups led to a competitive reaction at C2. This hypothesis is supported by the fact that cleavage of the aziridine with the smallest *N*-sulfonyl group (SO_2CF_3), showed the lowest regioselectivity at C3. The corresponding 3-chlorosulfonamide was obtained with the same region- selectivity and yield by $CeCl_3$-mediated opening of hydroxymethyl aziridine, suggesting that the steric bulk of the TBS group was not responsible for the selectivity.

Lithiation and electrophile trapping of terminal *N*-Bus aziridine **1.8**,[38] led to an α-lithiated aziridine **1.9**, which underwent rapid dimerization to afford 2-ene-1,4-diamine **1.10** (Scheme 3).



Scheme 3: Reagents and conditions: a) lithium 2,2,6,6-tetramethylpiperidide (LTMP); b) lithium dicyclohexylamide (LiNCy2)

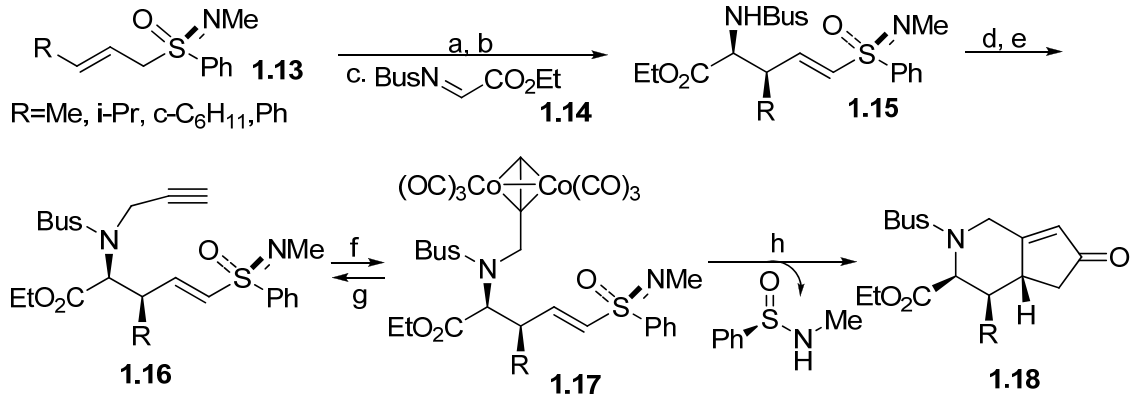
The aziridine **1.8** was transformed to the α-lithiated aziridine **1.9**, which could occur via a 1,2-metallated shift. In the process, a nucleophilic attack can occur on **1.8**. Subsequent syn-elimination, driven by the steric demand of the aziridine substituents and the *N*-Bus groups, leads to the formation of the *E*-alkene isomer **1.10** in highly selectivity. Compared to the analogous epoxide

dimerization reactions, the improved *E*/*Z* selectivity is attributed to the extra steric demand of the Bus groups. If a pendant alkene is present, as in compound **1.11**, the *N*-Bus-α-lithiated unsaturated aziridine **1.11a** affords a 2-aminobicyclo-[3.1.0]hexane **1.12**.

1-1-3-3 As a functional group on imines

There are also applications involving addition reactions on *N*-Bus imines, as exemplified by the asymmetric synthesis of fused bicyclic amino acids (Scheme 4).[39]

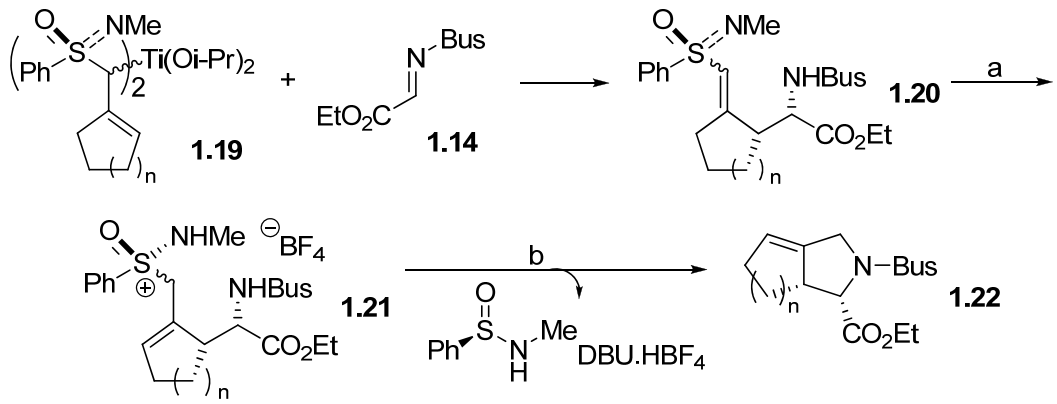
The *N*-*tert*-butylsulfonyl imino ester **1.14** was used in one of the key steps of the synthesis. Firstly, starting material **1.13** was transformed to enantiomerically a pure bis(allylsulfoximine)titanium complex, which reacted with imino ester **1.14**, through a highly selective allylation to give the enantio- and diastereo- merically pure product **1.15**.



Scheme 4: Reagents and conditions: a) n-BuLi; b) ClTi(Oi-Pr)$_3$; c) 14; d) Cs_2CO_3, DMF; e) propargyl bromide; f) $Co_2(CO)_8$, THF; g) $(NH_4)_2Ce(NO_3)_6$, acetone; h) NMO

Another example is the asymmetric synthesis of fused bicyclic proline **1.22**.[40] The key steps of this synthesis are: 1) a highly regio- and diastereo-selective amino alkylation of the cyclic bis(allylsulfoximine) titanium complex **1.19** with the *tert*-butylsulfonyl imino ester **1.14**; 2) a novel migratory

cyclization of the *α*-amino alkenyl sulfoxonium salt **1.21** (Scheme 5).



Scheme 5: Reagents and conditions: a) Me_3OBF_4 in DCM; b) DBU, THF;

1-2 Results and Discussion

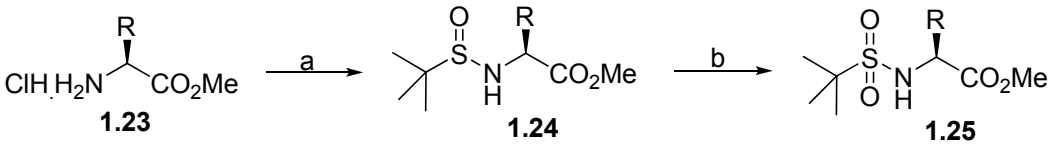
1-2-1 *Tert*-Butylsulfonyl (Bus), as a new protecting group for amino acids

To date, there are no reports of the use of *N*-Bus protecting group in amino acid and peptide chemistry.[1-5] In this chapter, we report our efforts in the preparation of a variety of *N*-Bus derivatives of common amino acids and peptides. We further show the combination of *N*-Bus and other common *N*-protecting groups, and also show the prospects of performing orthogonal deprotections.

1-2-2 *N*-Bus formation and cleavage

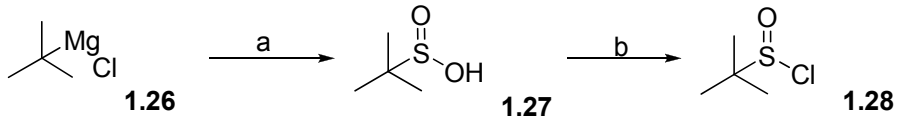
1-2-2-1 Formation of the *N*-Bus group of amino acid esters

tert-Butylsulfonyl chloride is quite unstable and cannot be used directly in the sulfonation of amines. Therefore, the straightforward and well-documented 2-step procedure was chosen. [31] The amine **1.23** was reacted with the *tert*-butylsulfinyl chloride **1.28** to afford the corresponding *tert*-butylsulfonamides **1.24**. The reagents and conditions are shown in Scheme 6.[31]



Scheme 6: Reagents and conditions: a) *tert*-butylsulfinyl chloride **1.28**, Et_3N, DCM, 0 ^oC, 1 h; b) *m*-CPBA, DCM, r.t., 1 h;

Due to the low purity of commercially available *tert*-butylsulfinyl chloride **1.28**, it was prepared by a 2-step procedure.[37] The Grignard reagent from *tert*-butylmagnesium chloride **1.26**, was reacted with the excess SO_2 gas, followed by the acylation of the resulting sulfinic acid **1.27** with thionyl chloride to afford *tert*-butylsulfinyl chloride **1.28** as a light yellow oil (Scheme 7).



Scheme 7: Reagents and conditions: a) SO_2, THF, 0 ^oC, 3 h; b) thionyl chloride, THF, r.t., 4 h;

Neat *tert*-butylsulfinyl chloride **1.28** decomposes readily. However, a solution of **1.28** in dry DCM can be stored in a freezer for prolonged periods (more than 6 months without decomposing).

The resulting sulfinamide **1.24** can be oxidized with a variety of oxidants. For our purposes, *m*-CPBA was proved to be a much milder and efficient oxidant. Since the thiohydryl group of cysteine is also oxidized by *m*-CPBA under these conditions, the Bus group cannot be used as a protecting group for cysteine.

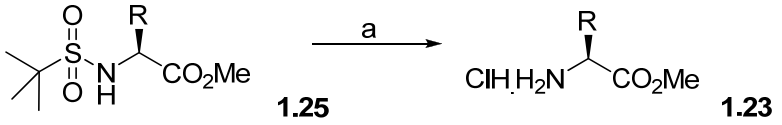
The results of the *N*-Bus group formation for a series of amino acid esters are shown in Table 1.

1-2-2-2 Cleavage of the *N*-Bus group of amino acid esters

A catalytic amount of CAN was found to act as an efficient catalyst for

removal of the *N*-Boc group from an amino, hydroxyl, or mercapto functionality in organic compounds, under the mild and neutral reaction conditions.[12] In contrast, the *N*-Bus group was completely unreactive when refluxed with 1 eq. CAN for 24 h.

Various methods for the Bus cleavage were attempted, however the best way to cleave and to regenerate the corresponding amine salt was found to be with 0.1 N TfOH/DCM/anisole at 0 °C for 10 h (Scheme 8).

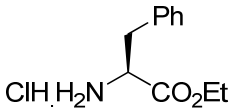
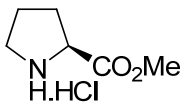


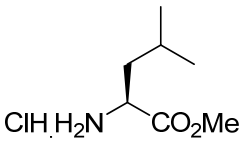
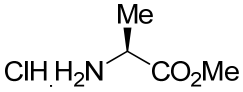
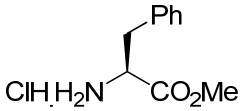
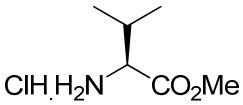
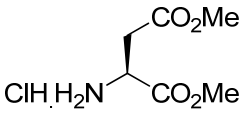
Scheme 8: Reagents and conditions: a) CF_3SO_3H, DCM, anisole, 0 °C, 10 h;

During the workup either a strong base resin, such as DOWEX Monosphere 550A a hydroxide form anion exchange resin, or saturated aqueous $NaHCO_3$ was used to neutralize the excess trifluoromethanesulfonic acid (TfOH) and to remove anisole from the reaction mixture. However, since some free amino acids are volatile, the amino acid esters were isolated as HCl salts.

The results of the *N*-Bus group cleavage for a series of amino acid esters are shown in Table 1.

Table 1 Bus-group formation and cleavage for amino acid esters

Entry	Compound	*N*-Bus Formation[b]		*N*-Bus cleavage	
		Compound	Yield%[a]	Compound	Yield%[a]
1		**1.25a**	84	**1.23a**	89
2		**1.25b**	82	**1.23b**	90

3		**1.25c**	82	**1.23c**	79
4		**1.25d**	77	**1.23d**	85
5		**1.25e**	88	**1.23e**	85
6		**1.25f**	77	**1.23f**	85
7		**1.25g**	72	**1.23g**	84

a) yields of isolated pure product; b) the total yields over two steps

The results show that the 2-step formation of *N*-Bus derivatives of the amino acids some common and *N*-Bus cleavage with TfOH/anisole could be achieved in excellent overall yields.

1-2-2-3 Retention of the stereochemistry during the protection and deprotection

It is very important to retain the stereochemistry of the amino acid during the protection and deprotection steps. The stereochemistry of the amino acid esters, obtained after the Bus-group protection and deprotection, was analyzed by transforming them to the corresponding Mosher amides and NMR analysis. The ^{1}H NMR and ^{19}F NMR spectrum showed that the chirality of the amino acids was unaffected.

HPLC was used for the further verification. The *de* % of the alanine Mosher amide and phenylalanine Mosher amide were >99.9%.

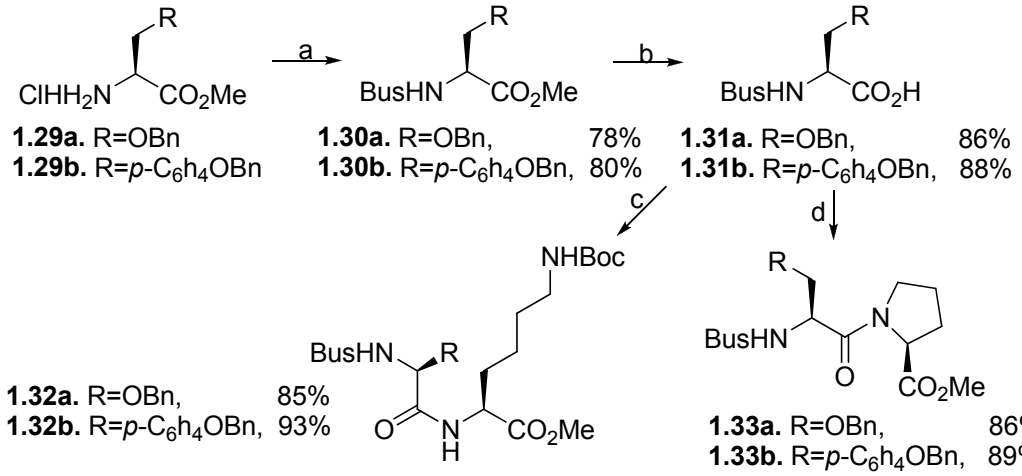
In conclusion, the process of Bus-groups formation and cleavage does not affect the stereochemistry of the amino acids.

1-2-3 Forming dipeptides and tripeptides with the Bus protecting group

1-2-3-1 Formation of amino acid esters dipeptides

The formation of peptides using the *N*-Bus protected amino acids was studied next.

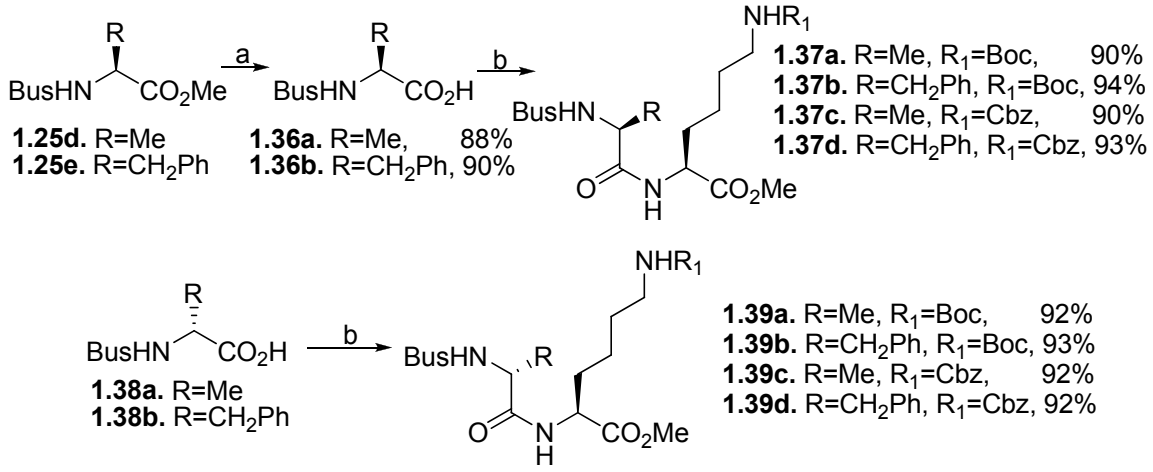
N-Bus-*O*-benzyl-L-serine methyl ester **1.30a** and *N*-Bus-*O*-benzyl-L-tyrosine methyl ester **1.30b** were formed as previously described, then hydrolyzed using aqueous lithium hydroxide to afford carboxylic acids **1.31a** and **1.31b**, respectively. These were coupled with the primary amine of N^6-Boc-L-lysine methyl ester **1.34** by using EDC, 2,6-lutidine and HOBt as coupling reagents to afford dipeptides **1.32a** and **1.32b** in 85% and 93% yield respectively (Scheme 9).



Scheme 9: Reagents and conditions: a) i) *tert*-butylsulfinyl chloride, Et_3N, DCM, 0 ^oC, 1 h; ii) *m*-CPBA, DCM, r.t., 1h; b) $LiOH.H_2O$, MeOH/H_2O, 4 ^oC, 10 h; c) EDC, HOBt, 2,6-lutidine, N_6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34**, DMF/DCM 1:4, r.t., 4 h; d) L-proline methyl ester **1.23b**, EDC, HOBt, 2,6-lutidine, DMF/DCM 1:4, r.t., 4 h;

The carboxylic acids **1.31a** and **1.31b** were also coupled under the same conditions with L-proline methyl ester **1.23b** to form corresponding dipeptides **1.33a** and **1.33b** in high yields (Scheme 9).

Next *N*-Bus-L-alanine methyl ester **1.25d** and *N*-Bus-L-phenylalanine methyl ester **1.25e** were also hydrolyzed to the free acids **1.36a** and **1.36b** respectively, then coupled with N^6-Boc-L-lysine methyl ester **1.34** and N^6-Cbz-L-lysine methyl ester **1.35** to form dipeptides **1.37a-d** in excellent yields (Scheme 10).

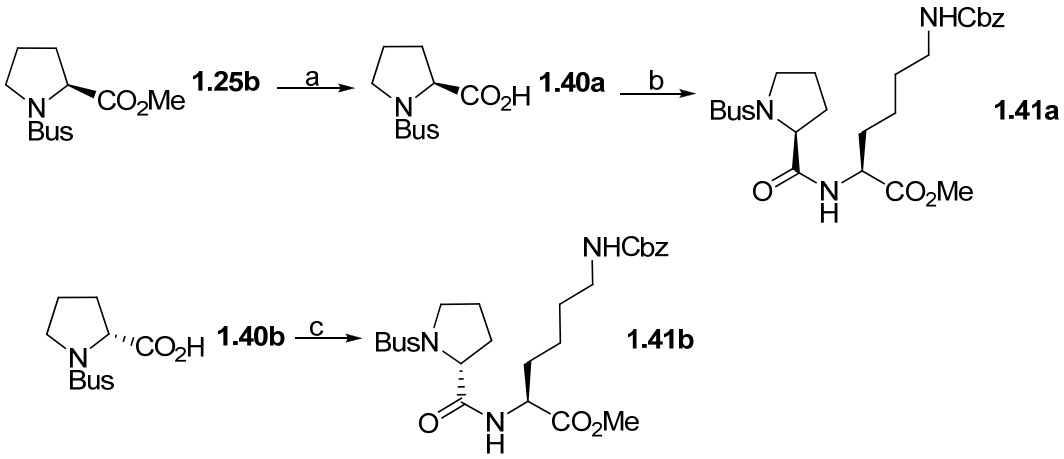


Scheme 10: Reagents and conditions: a) $LiOH.H_2O$, $MeOH/H_2O$, 4 ^oC, 10 h; b) EDC, HOBt, 2.6-lutidine, N_6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34** or N_6-benzyloxycarbonyl-L-lysine methyl ester **1.35**, DMF/DCM 1:4, r.t., 4 h;

D-Amino acids were also utilized in preparation of *N*-Bus-D-alanine **1.38a** and *N*-Bus-D-phenylalanine **1.38b**. Following the same procedure, the carboxylic acids **1.38a** and **1.38b** were also coupled with N^6-Boc-L-lysine methyl esters **1.34** and N^6-Cbz-L-lysine methyl esters **1.35** to form dipeptides **1.39a-d** in excellent yields (Scheme 10).

In order to study an *N*-Bus protected secondary amine, *N*-Bus-prolines **1.40a** and **1.40b** were used. These were obtained from corresponding proline methyl esters using the standard method. Following the same procedure, the

N-Bus carboxylic acids **1.40a** and **1.40b** were coupled with N^6-Cbz-L-lysine methyl esters **1.35** to form a pair of diastereomeric dipeptides **1.41a** and **1.41b** in 91% and 92% yield, respectively (Scheme 11).



Scheme 11: Reagents and conditions: a) $LiOH.H_2O$, $MeOH/H_2O$, 4 °C,10 h, 90%; b) HOBt, N_6-Cbz-L-lysine methyl ester **1.35**, 2.6-lutidine, EDC, DMF/DCM 1:4, r.t., 4 h, 92%; c) **1.35**, EDC, HOBt, 2.6-lutidine, DMF/DCM 1:4, r.t., 4 h, 92%;

1-2-3-2 Retention of the stereochemistry during the formation of the dipeptide

HPLC was utilized to check the *de*% of dipeptides **1.37** and **1.39**. The corresponding peptides, **1.37a** and **1.39a** differing in one chiral center, were used as tests. The 4 pairs were utilized to validate that the stereochemistry was retained during dipeptide formation as shown in Table 2.

Table 2 HPLC results and methods of the peptides

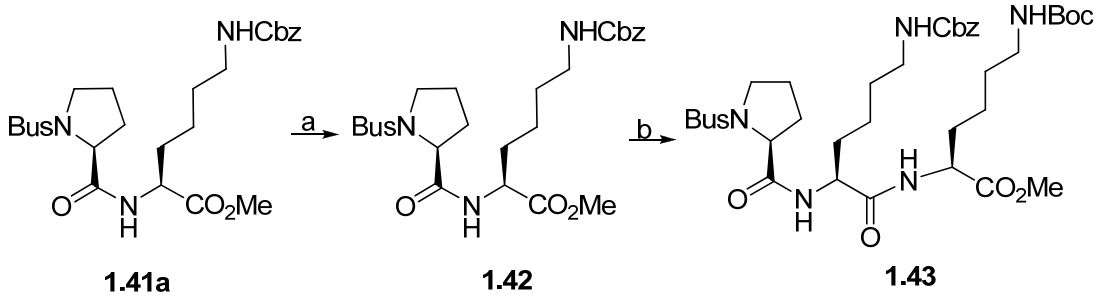
Entry	Compound	*De* %	Compound	*De* %	HPLC method
1	**1.37a**	>99.9%	**1.39a**	>99.9%	a
2	**1.37b**	>99.9%	**1.39b**	99.86%	b
3	**1.37c**	95.66%	**1.39c**	96.24%	c
4	**1.37d**	95.50%	**1.39d**	92.22%	d

a) AS-RH 150*4.6 mm, 25% MeCN in H_2O (0.1%TFA), 0.5 mL/min;
b) AS-RH 150*4.6 mm, 35% MeCN in H_2O (0.1%TFA), 0.5 mL/min;
c) OJ-R 150*4.6 mm, 30% MeCN in H_2O (0.05%TFA), 0.5 mL/min;
d) C-18 250*4.6 mm, 60% MeCN in H2O (0.1%TFA), 0.5 mL/min.

In conclusion, little or no erosion of stereochemistry was observed during peptide formation using the *N*-Bus protected amino acids.

1-2-3-3 Formation of amino acid ester tripeptides

To form a tripeptide, N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34** and *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.41a** were used as examples. First, proline-lysine peptide methyl ester **1.41a** was hydrolyzed using LiOH×H_2O to free acid **1.42**. Then, N^6-Boc-L-lysine methyl ester **1.34** was used to couple with proline-lysine acid **1.42** using 2.6-lutidine, EDC and HOBt as coupling reagents to form tripeptide **1.43** in excellent yield (Scheme 12).



1.41a **1.42** **1.43**

Scheme 12: Reagents and conditions: a) LiOH.H_2O, MeOH/H_2O, 4 °C, 10 h, 93%; b) EDC, N_6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34**, 2.6-Lutidine, HOBt, r.t., DMF/DCM 1:4, 4 h, 90%;

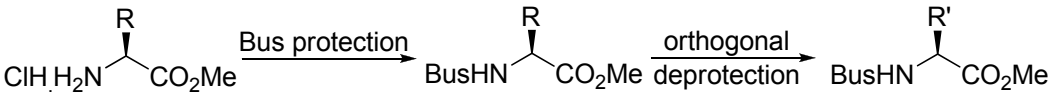
1-2-4 Retaining the Bus group, and use in the orthogonal deprotection

The deprotection of the *N*-Bus derivatives to regenerate the corresponding amino salt was realized by using 0.1 N TfOH/DCM/anisole at 0 °C for 10 h.

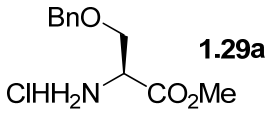
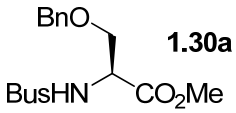
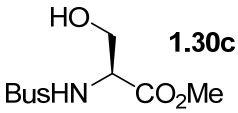
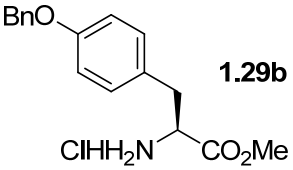
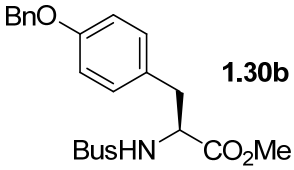
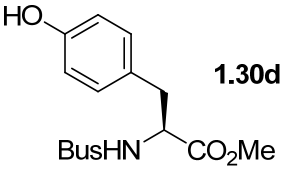
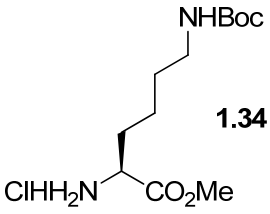
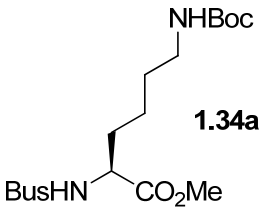
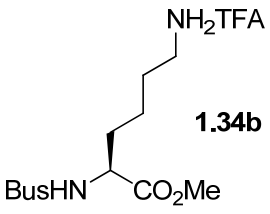
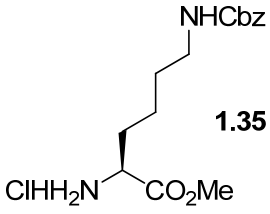
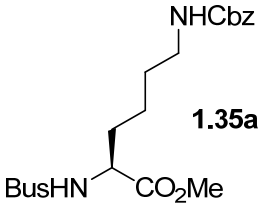
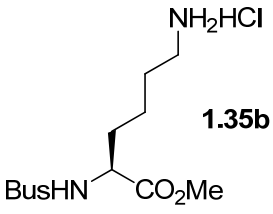
Unfortunately these conditions also cleaved *N*-Boc, *N*-Cbz and benzyl ether groups. However selective deprotection of *N*-Boc, *N*-Cbz, *N*-Fmoc and benzyl ether groups could be achieved in the presence of the *N*-Bus protecting group.

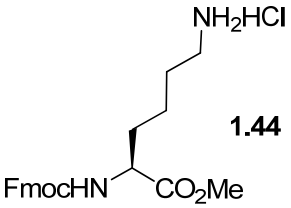
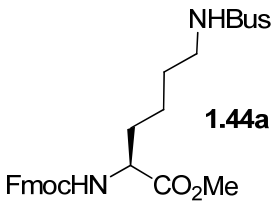
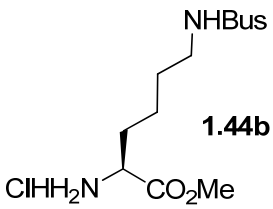
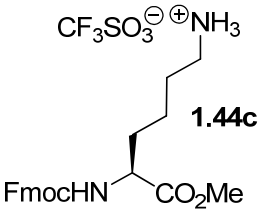
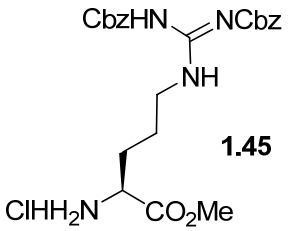
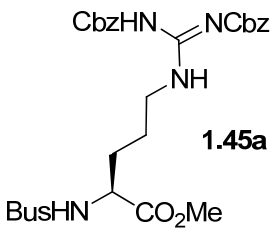
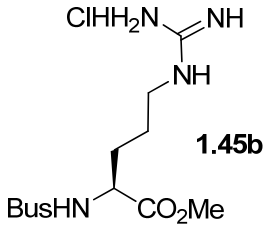
The formation of *N*-Bus derivatives of *N*-protected amino acids and orthogonal deprotection in presence of *N*-Bus group was also studied (Table 3).

Table 3 Protection and orthogonal deprotection of *N*-Bus derivatives



R=*N*-protected amino acid, R'=*N*-unprotected amino acid

Entry	Compound	Bus-Formation		Orthogonal Cleavage	
		Compound	Yield %[a]	Compound	Yield %[a]
1	 **1.29a**	 **1.30a**	78	 **1.30c**	97[b]
2	 **1.29b**	 **1.30b**	80	 **1.30d**	97[b]
3	 **1.34**	 **1.34a**	80	 **1.34b**	97[c]
4	 **1.35**	 **1.35a**	76	 **1.35b**	97[b]

5	 **1.44**	 **1.44a**	81	 **1.44b** 92[d]  **1.44c** 89[e]
6	 **1.45**	 **1.45a**	76	 **1.45b** 95[b]

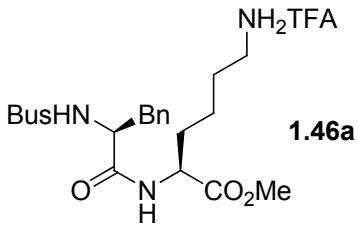
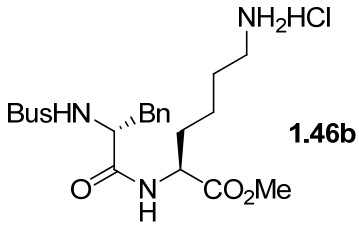
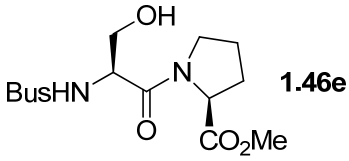
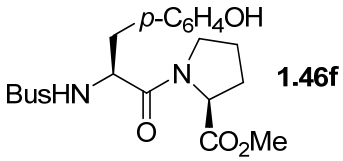
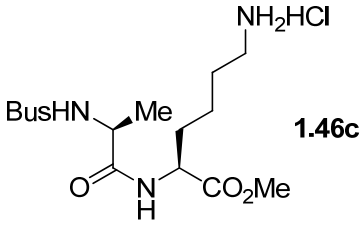
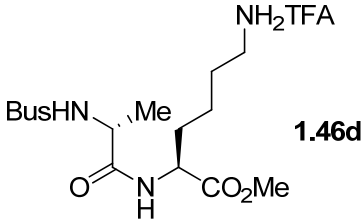
a) yields of isolated pure product; b) 20% wt.% $Pd(OH)_2$, H_2, MeOH, r.t., 2 or 3 h; c) 5 eq. TFA, DCM, r.t., 8 h; d) 5% piperidine, DMF, r.t., 8 h; e) CF_3SO_3H, DCM, anisole, 4 °C, 6 h.

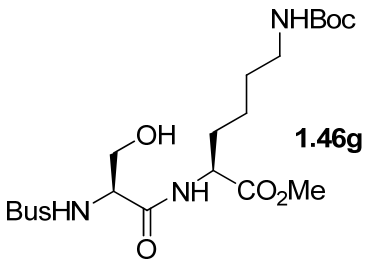
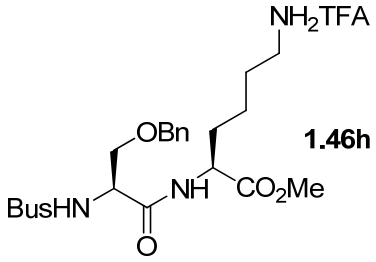
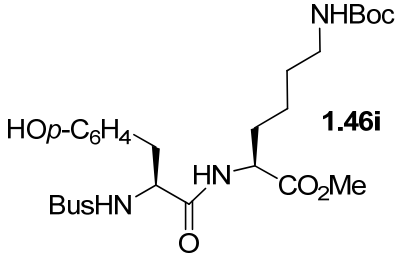
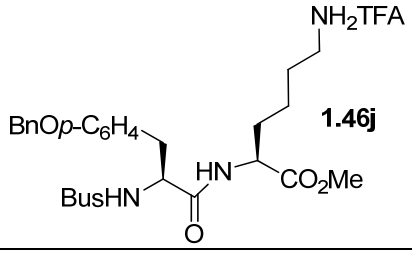
In conclusion, the *N*-Bus formation of the *N*-Boc, *N*-Cbz, *N*-Fmoc and benzyl ether protected functionalized amino acids was achieved in good overall yields. Selective deprotection of other *N*-protecting groups in the presence of the *N*-Bus group was also realized in excellent yields.

The *N*-Fmoc is stable during the deprotection of the *N*-Bus derivatives to regenerate the corresponding amino salt. As an example, the compound **1.44a** N^6-Bus-*N*-Fmoc-L-Lys-OMe was used to afford free amine *N*-Fmoc-L-Lys-OMe compound **1.44c**, by using 0.1 N TfOH/DCM/anisole at 4 °C for 6 h with an excellent yield.

The selective and orthogonal deprotection of the *N*-protecting groups on the dipeptides in the presence of *N*-Bus group was also studied. The results are shown in Table 4.

Table 4 Orthogonal deprotection of *N*-Bus group peptides

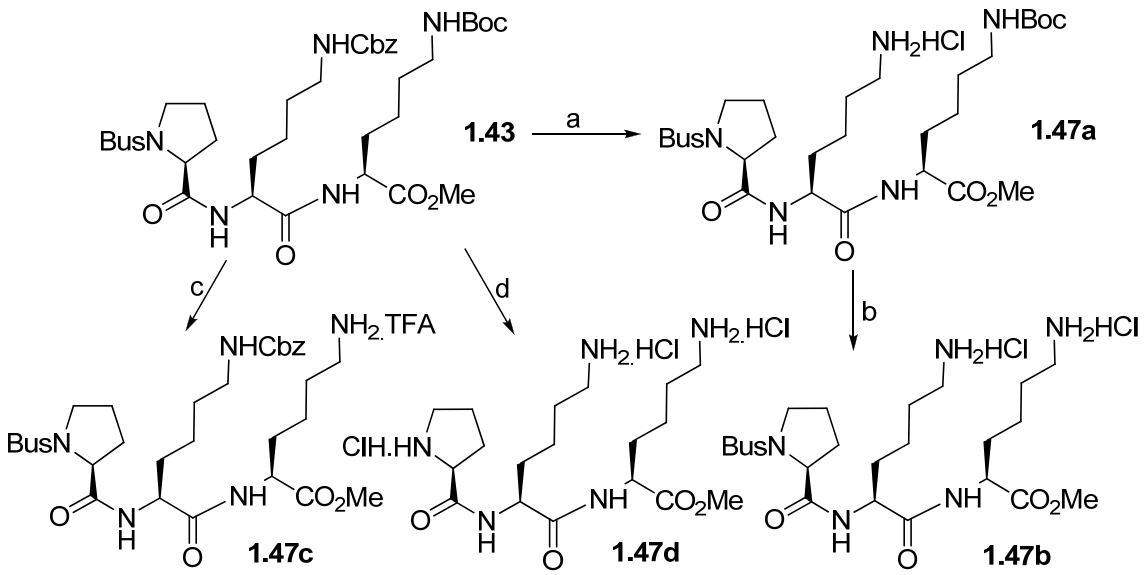
Entry	Start Material	cleavage Product	Method	Yield%[a]
1	**1.37b**	 **1.46a**	10% TFA, DCM, r.t., 2 h,	94
2	**1.39d**	 **1.46b**	20 wt.% $Pd(OH)_2$, H_2, r.t., 2 h, MeOH,	95
3	**1.33a**	 **1.46e**	20 wt.% $Pd(OH)_2$, H_2, r.t., 3 h, MeOH,	89
4	**1.33b**	 **1.46f**	20 wt.% $Pd(OH)_2$, H_2, r.t., 3 h, MeOH,	89
5	**1.37c**	 **1.46c**	20 wt.% $Pd(OH)_2$, H_2, r.t., 2 h, MeOH,	89
6	**1.39a**	 **1.46d**	10% TFA, DCM, r.t., 2 h,	90

7	**1.32a**	 **1.46g**	20 wt.% $Pd(OH)_2$, H_2, r.t., 1 h, MeOH,	86
8	**1.32a**	 **1.46h**	10% TFA, DCM, r.t., 2 h	92
9	**1.32b**	 **1.46i**	20 wt.% $Pd(OH)_2$, H_2, r.t., 1 h, MeOH,	87
10	**1.32b**	 **1.46j**	10% TFA, DCM, r.t., 2 h	94

a) yields of isolated pure product

Thus, selective and orthogonal deprotections of *N*-Boc, *N*-Cbz, and benzyl ether groups in the presence of the *N*-Bus dipeptides were also achieved in excellent yields.

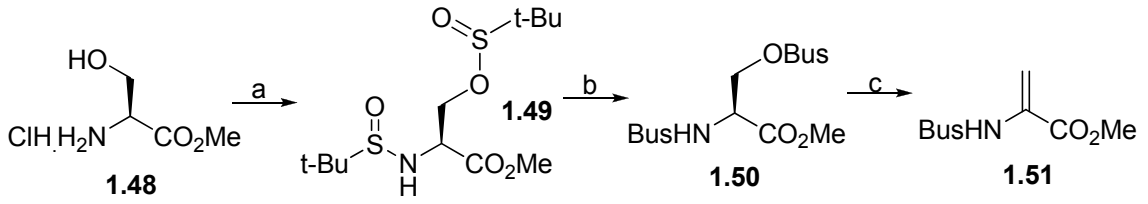
The results of orthogonal deprotection the *N*-protecting groups on the triipeptide are shown in Scheme 13. Using different methods, the selective and orthogonal deprotection steps of *N*-Boc, *N*-Cbz and *N*-Bus of *N*-Bus tripeptides could be realized in excellent yields.



Scheme 13: Reagents and conditions: a) 20 wt.% $Pd(OH)_2/C$, H_2, MeOH, r.t., 1 h, 98%; b) 1.25 M HCl, MeOH, r.t., 4 h, 99%; c) TFA/DCM 1:10, r.t., 4 h, 93%; d) CF_3SO_3H, DCM, anisole, 0 °C, 12 h, 82%;

1-2-5 *O*-Bus as a leaving group:

When 1.0 equivalent serine methyl ester hydrochloride **1.48** was treated with 2.5 equivalents *tert*-butylsulfinyl chloride **1.28** and 3.0 equivalents *m*-CPBA, *N*-Bus-*O*-Bus-L-serine methyl ester **1.50** was isolated (Scheme 14). The *O*-Bus group underwent facile elimination to form a double bond even in the mild and neutral reaction conditions of tetrabutylammonium azide. With the *O*-Bus group of compound **1.50** being a good leaving group and an acidic proton present, the elimination occurs faster than the S_N^2 substitution.



Scheme 14: Reagents and conditions: a) 2.5 eq. compound **1.28**, 5 eq. Et_3N, DCM, 0 °C, 1 h; b) 3 eq. *m*-CPBA, DCM, r.t., 1 h; 60% over 2 steps; c) THF, 2.4 eq. $Bu_4N^+ N_3^-$, r.t., 2 h. 62%

1-2-6 Conclusion:

The *N*-Bus protecting group in amino acid and peptide chemistry is formed in a 2-step procedure involving reaction of an amine with the commercially available *tert*-butylsulfinyl chloride, followed by oxidation of the resulting sulfinamide with a variety of oxidants to afford the corresponding *tert*-butyl-sulfonamides in excellent overall yields. The *N*-Bus group can be cleaved to regenerate the corresponding amino salt in 0.1 N TfOH/DCM/anisole at 0 ^{o}C for 10 h.

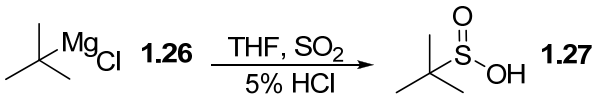
A variety of *N*-Bus protected amino acids and other common amino acids can be used to form dipeptide and tripeptides.

Our studies showed that the conditions required for the *N*-Bus group cleavage, also cleaved the *N*-Boc, *N*-Cbz and *O*-Bn groups. The *N*-Fmoc is stable during the deprotection of the *N*-Bus derivatives to regenerate the corresponding amine salt with an excellent yield. However, selective and orthogonal deprotection of *N*-Boc, *N*-Cbz, *N*-Fmoc and *O*-Bn groups could be achieved in the presence of the *N*-Bus protecting group.

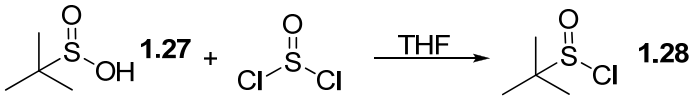
1-3 Experimental

General: Solvents were distilled under positive pressure of dry argon before use and dried by standard methods. THF, ether, DCM and toluene were dried by the SDS (*Solvent Delivery System*). All commercially available reagents were used without further purification. All reactions were performed under argon atmosphere and monitored by thin-layer chromatography. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate, ninhydrine or potassium permanganate. IR, Perkin-Elmer FTIR Paragon 1000. Low- and high- resolution mass spectra were recorded using

fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded in a 1 dm cell at 20 °C (PerkinElmer 343). Flash column chromatography was performed using (40-60 μm) silica gel at increased pressure. NMR (^{1}H, ^{13}C, ^{19}F) spectra were recorded on Bruker AV-300 and AV-400 spectrometers. When necessary, assignments were aided by DEPT, COSY, NOESY, and HMBC and HMQC correlation experiments.



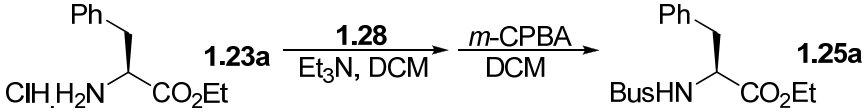
***Tert*-butylsulfinic acid (1.27):** Excess sulfur dioxide was bubbled quickly through a solution of *tert*-butylmagnesium chloride **1.26** (1.0 M in THF, 100 mL) at 0 °C in a fume hood allowing for adequate venting of excess gas. When the solution became saturated with SO_2, (then excess SO_2 was sent out from the solution and the pH of the bubble was <6), the SO_2 bubble was stopped. After stirring at 0 °C under the argon atmosphere for 3 h, the reaction mixture was cautiously diluted with ice-cold 5% aqueous HCl (100 mL). After effervescence subsided, the aqueous layer was extracted with 100 mL×3 of DCM. The organic extracts were combined, dried over $MgSO_4$, and concentrated under reduced pressure to afford *tert*-butylsulfinic acid **1.27** (11.37 g, 93%) as a colorless solid. The material was used without further purification: ^{1}H NMR, (400M Hz, $CDCl_3$) δ 1.20 (s, 9H).



***Tert*-butylsulfinyl chloride (1.28):** To a solution of *tert*-butylsulfinic acid **1.27** (11.0 g, 90 mmol) in THF (50 mL), thionyl chloride (7.88 mL, 108 mmol) in THF (10 mL) was added dropwise at R.T.. After 4 h, the solvent was removed under reduced pressure and excess thionyl chloride was evaporated under

reduced pressure at 40 °C. The residue was then distilled at 61-62 °C at aspirator pressure to give *tert*-butylsulfinyl chloride **1.28** (9.97 g, 79%) as a light yellow oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 1.40 (s, 9H).

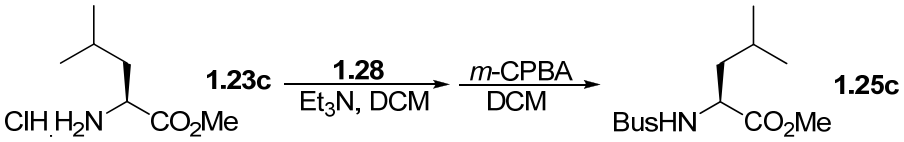
General Procedure for Formation of *tert*-Butylsulfonamides.



***N*-Bus-L-Phe-OEt (1.25a):** A solution of L-phenylanine ethyl ester hydrochloride **1.23a** (58 mg, 0.25 mmol) in 3 mL of DCM was cooled to 0 °C and treated with Et_3N (0.35 mL, 2.5 mmol), followed by dropwise addition of *tert*-butylsulfinyl chloride **1.28** (62 μL, 0.5 mmol) in 1 mL of DCM. The reaction mixture was stirred at 0 °C until TLC showed consumption of the starting material (1 h). Upon completion, 5 mL of saturated aqueous $NaHCO_3$ were added, and the layers separated (note: acidic washes should be avoided as *tert*-butylsulfinamides **1.24a** are known to be unstable at low pH). The organic layer was then dried over Na_2SO_4, and concentrated under reduced pressure. Flash column chromatography (EtOAc/hexane 3:2) gave pure sulfinamide **1.24a** which was directly taken up in 5 mL of DCM, and treated with *m*-CPBA (58 mg, 0.34 mmol) at 0 °C to R.T.. After the oxidation was complete by TLC (at R.T. for 1 h), the mixture was diluted with a mixture of saturated aqueous $NaHCO_3$ (5 mL) and saturated aqueous Na_2SO_3 (5 mL). The aqueous layer was extracted with DCM (2×10 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:1) to afford *tert*-butylsulfonyl-L-phenylalanine ethyl ester **1.25a** (66 mg, 84% over 2 steps), as a colorless solid, m.p. 71-73 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ

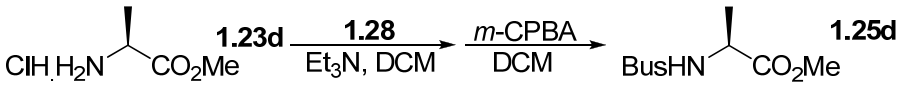
7.31-7.20 (m, 5H), 4.80 (d, 1H, *J*=10.3 Hz), 4.21-4.16 (m, 3H), 3.10-3.04 (m, 2H), 1.25-1.21 (m, 12H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.2, 135.8, 129.8, 128.7, 127.4, 61.8, 60.2, 58.9, 39.7, 24.0, 14.2; $[\alpha]_D$ +6.7 (c 1.0, $CHCl_3$); HRMS for $C_{15}H_{23}NO_4S$ calculated ($M+H^+$) 314.14206, found 314.14119.

***N*-Bus-L-Pro-OMe (1.25b):** The general procedure was followed using L-proline methyl ester hydrochloride **1.23b** (166 mg, 1.0 mmol), Et_3N (1.39 mL, 10.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.25 mL, 2.0 mmol) in 5 mL of DCM and DCM (5 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (233 mg, 1.35 mmol) and DCM (10 mL). The residue was purified by flash column chromatography (EtOAc/hexane 1:3) to afford *tert*-butylsulfonyl-L-proline methyl ester **1.25b** (203 mg, 82% over 2 steps), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 4.45 (d, 1H, *J*=6.6 Hz), 3.58-3.55 (m, 4H), 3.38-3.36 (m, 1H), 2.10-2.07 (m, 1H), 1.88-1.80 (m, 3H), 1.24 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.0, 61.0, 60.9, 51.8, 49.9, 30.5, 24.7, 23.9; $[\alpha]_D$ -66.9 (c 1.0, $CHCl_3$); HRMS for $C_{11}H_{23}NO_4S$ calculated ($M+H^+$) 250.11076, found 250.11106.

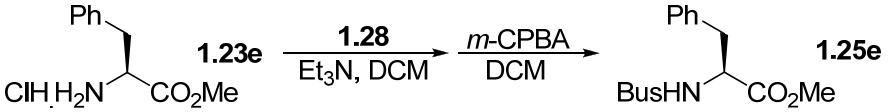


***N*-Bus-L-Leu-OMe (1.25c):** The general procedure was followed using L-leucine methyl ester hydrochloride **1.23c** (182 mg, 1.0 mmol), Et_3N (1.39 mL, 10.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.25 mL, 2.0 mmol) in 5 mL of DCM and DCM (5 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:2), which was oxidized with *m*-CPBA (233

mg, 1.35 mmol) and DCM (10 mL). The residue was purified by flash column chromatography (EtOAc/hexane 1:3) to afford *tert*-butylsulfonyl-L-leucine methyl ester **1.25c** (219 mg, 82% over 2 steps), as a colorless solid, m.p. 56-57 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.21 (d, 1H, *J*=9.9 Hz), 3.88-3.82 (m, 1H), 3.53 (s, 3H), 1.60-1.54 (m, 1H), 1.38-1.21 (m, 1H), 1.15 (s, 9H), 0.75-0.72 (m, 6H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.5, 59.5, 55.4, 51.9, 42.6, 24.0, 23.7, 22.4, 21.4; $[\alpha]_D$ -16.0 (c 1.0, $CHCl_3$); HRMS for $C_{11}H_{23}NO_4S$ calculated ($M+H^+$) 266.14206, found 266.14225.

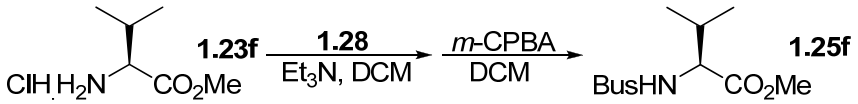


***N*-Bus-L-Ala-OMe (1.25d):** The general procedure was followed using L-alanine methyl ester hydrochloride **1.23d** (209 mg, 1.5 mmol), Et_3N (2.09 mL, 15.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.37 mL, 3.0 mmol) in 8 mL of DCM and DCM (8 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (350 mg, 2.025 mmol) and DCM (20 mL). The residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-L-alanine methyl ester **1.25d** (257 mg, 77% over 2 steps), as a colorless solid, m.p. 69-70 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.07 (d, 1H, *J*=9.5 Hz), 4.08-4.04 (m, 1H), 3.68 (s, 3H), 1.38 (d, 3H, *J*=7.2 Hz), 1.30 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.7, 59.9, 52.8, 52.6, 24.0, 20.2; $[\alpha]_D$ -12.4 (c 1.0, $CHCl_3$); HRMS for $C_8H_{14}NO_4S$ calculated ($M+H^+$) 224.09511, found 224.09507.

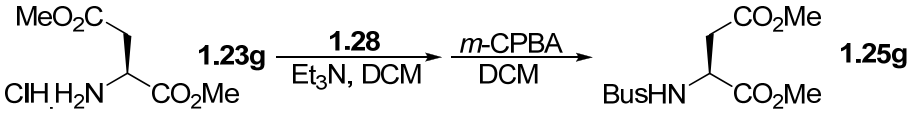


***N*-Bus-L-Phe-OMe (1.25e):** The general procedure was followed using L-phenylalanine methyl ester hydrochloride **1.23e** (86 mg, 0.4 mmol), Et_3N

(0.14 mL, 1.0 mmol), *tert*-butylsulfinyl chloride **1.28** (55 uL, 0.44 mmol) in 0.5 mL of DCM and DCM (4 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:2), which was oxidized with *m*-CPBA (93 mg, 0.54 mmol) and DCM (3 mL). The crude residue was purified by flash column chromatography (EtOAc/hexane 13:7) to afford *tert*-butylsulfonyl-L-phenyl-alanine methyl ester **1.25e** (105 mg, 88% over 2 steps), as a colorless solid, m.p. 101-103 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.34-7.27 (m, 3H), 7.19-7.17 (m, 2H), 4.49 (d, 1H, *J*=10.3 Hz), 4.34-4.28 (m, 1H), 3.76 (s, 3H), 3.15-3.03 (m, 2H), 1.20 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.7, 135.8, 129.7, 128.8, 127.5, 60.3, 58.8, 52.7, 39.9, 24.0; $[\alpha]_D$ +12.1 (c 1.0, $CHCl_3$); HRMS for $C_{14}H_{21}NO_4S$ calculated ($M+H^+$) 300.12641, found 300.12591.



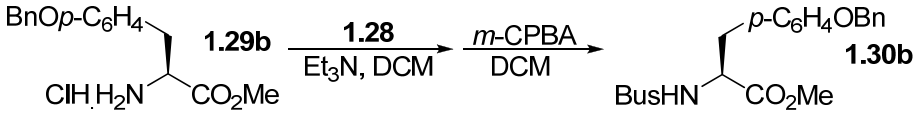
***N*-Bus-L-Val-OMe (1.25f):** The general procedure was followed using L-valine methyl ester hydrochloride **1.23f** (1.26 g, 7.5 mmol), Et_3N (2.61 mL, 18.75 mmol), *tert*-butylsulfinyl chloride **1.28** (1.02 mL, 8.25 mmol) in 25 mL of DCM and DCM (25 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (1.75 g, 10.125 mmol) and DCM (30 mL). The residue was purified by flash column chromatography (EtOAc/hexane 13:7) to afford *tert*-butylsulfonyl-L-valine methyl ester **1.25f** (1.46 g, 77% over 2 steps), as a colorless solid, m.p. 70-72 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.14 (d, 1H, *J*=10.2 Hz), 3.71-3.67 (m, 1H), 3.53 (s, 3H), 1.90-1.85 (m, 1H), 1.14 (s, 9H), 0.78 (d, 3H, *J*=6.8 Hz), 0.73 (d, 3H, *J*=7.3 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 62.3, 59.7, 51.9, 31.7, 23.8, 18.8, 17.6; $[\alpha]_D$ -8.9 (c 1.0, $CHCl_3$); HRMS for $C_{10}H_{21}NO_4S$ calculated ($M+H^+$) 252.12641, found 252.12660.



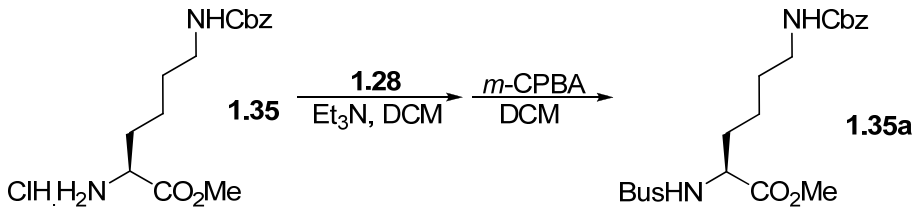
***N*-Bus-L-Asp-diOMe (1.25g):** The general procedure was followed using L-aspartic dimethyl ester hydrochloride **1.23g** (198 mg, 1 mmol), Et_3N (0.35 mL, 2.5 mmol), *tert*-butylsulfinyl chloride **1.28** (0.14 mL, 1.1 mmol) in 2 mL of DCM and DCM (15 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 9:1), which was oxidized with *m*-CPBA (233 mg, 1.35 mmol) and DCM (10 mL). The residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-L-aspartic dimethyl ester **1.25g** (201 mg, 72% over 2 steps), as a colorless solid, m.p. 77-78 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.14 (d, 1H, *J*=9.6 Hz), 4.34-4.32 (m, 1H), 3.75 (s, 3H), 3.67 (s, 3H), 3.02 (dd, 1H, *J*=4.6 Hz, *J*=17.2 Hz), 2.85 (dd, 1H, *J*=4.7 Hz, *J*=17.2 Hz), 1.36 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.3, 171.2, 60.5, 53.5, 53.1, 52.3, 38.6, 24.1; $[\alpha]_D$ +21.3 (c 1.0, $CHCl_3$); HRMS for $C_{10}H_{19}NO_6S$ calculated ($M+H^+$) 282.10058, found 282.10097.

***N*-Bus-*O*-Bn-L-Ser-OMe (1.30a):** The general procedure was followed using *O*-benzyl-L-serine methyl ester hydrochloride **1.29a** (1.13 g, 4 mmol), Et_3N (1.39 mL, 10.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.55 mL, 4.4 mmol) in 5 mL of DCM and DCM (40 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (932 mg, 5.4 mmol) and DCM (30 mL). The residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-*O*-benzyl-L-serine methyl ester **1.30a** (1.02 g, 78% over 2 steps), as a colorless solid, m.p. 88-89 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.35-7.25 (m, 5H),

4.98 (d, 1H, *J*=9.8 Hz), 4.52 (dd, 2H, *J*=12.2 Hz, *J*=25.3 Hz), 4.28-4.24 (m, 2H), 3.87 (dd, 1H, *J*=3.2 Hz, *J*=9.4 Hz), 3.75 (s, 3H), 3.70 (dd, 1H, *J*=3.5 Hz, *J*=9.4 Hz), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.9, 137.4, 128.4, 127.9, 127.6, 73.2, 71.1, 60.1, 57.3, 52.7, 24.0; $[\alpha]_D$ +2.4 (c 1.0, $CHCl_3$); HRMS for $C_{15}H_{23}NO_5S$ calculated ($M+H^+$) 330.13697, found 330.13723.

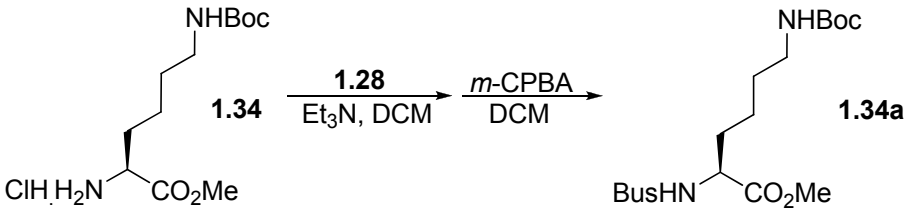


***N*-Bus-*O*-Bn-L-Tyr-OMe (1.30b):** The general procedure was followed using *O*-benzyl-L-tyrosine methyl ester hydrochloride **1.29b** (1.13 g, 3.5 mmol), Et_3N (1.22 mL, 8.75 mmol), *tert*-butylsulfinyl chloride **1.28** (0.48 mL, 3.85 mmol) in 4 mL of DCM and DCM (40 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:2), which was oxidized with *m*-CPBA (699 mg, 4.05 mmol) and DCM (50 mL). The residue was purified by flash column chromatography (EtOAc/hexane 1:1) to afford *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine methyl ester **1.30b** (1.14 g, 80% over 2 steps), as a colorless solid, m.p. 107-108 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.44-7.32 (m, 5H), 7.16 (d, 2H, *J*=8.5 Hz), 6.94 (d, 2H, *J*=8.6 Hz), 5.08 (d, 1H, *J*=10.3 Hz), 5.04 (s,2H), 4.32-4.26 (m, 1H), 3.74 (s, 3H), 3.08-3.02 (m, 2H), 1.21 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.8, 157.9, 136.9, 130.6, 129.5, 128.1, 127.9, 127.4, 115.0, 69.8, 60.0, 59.0, 52.4, 38.6, 23.7; $[\alpha]_D$ +11.8 (c 1.0, $CHCl_3$); HRMS for $C_{21}H_{27}NO_5S$ calculated ($M+H^+$) 406.16827, found 406.16843.



***N*-Bus-*N*^{6}-Cbz-L-Lys-OMe (1.35a):** The general procedure was followed

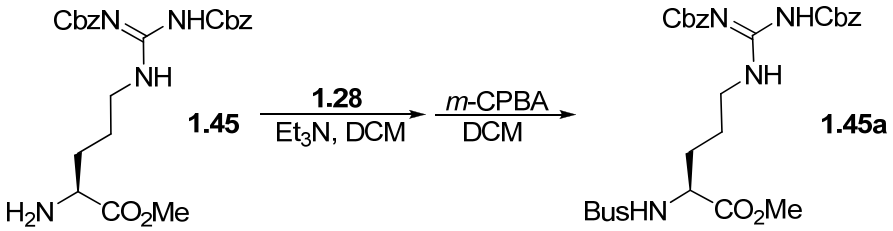
using N^6-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (1.46 g, 4.4 mmol), Et_3N (1.53 mL, 11.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.60 mL, 4.84 mmol) in 5 mL of DCM and DCM (40 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (812 mg, 5.94 mmol) and DCM (30 mL). The residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.35a** (1.02 g, 76% over 2 steps), as a colorless solid, m.p. 82-83 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.25-7.21 (m, 5H), 5.38 (d, 2H, *J*=9.5 Hz), 5.00 (s, 2H), 3.98-3.94 (m, 1H), 3.64 (s, 3H), 3.08 (d, 2H, *J*=6.2 Hz), 1.72-1.62 (m, 2H), 1.44-1.38 (m, 4H), 1.27 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.2, 156.5, 136.6, 128.3, 127.83, 127.81, 66.2, 59.8, 56.9, 52.3, 40.2, 33.0, 29.0, 23.9, 22.2; $[\alpha]_D$ +2.4 (c 1.0, $CHCl_3$); HRMS for $C_{19}H_{30}N_2O_6S$ calculated ($M+H^+$) 415.18973, found 415.19077.



***N*-Bus-N^6-Boc-L-Lys-OMe (1.34a):** The general procedure was followed using N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (950 mg, 3.2 mmol), Et_3N (1.12 mL, 8.0 mmol), *tert*-butylsulfinyl chloride **1.28** (0.44 mL, 3.52 mmol) in 5 mL of DCM and DCM (30 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 4:1), which was oxidized with *m*-CPBA (746 mg, 4.32 mmol) and DCM (30 mL). The residue was purified by flash column chromatography (EtOAc/hexane 7:13) to afford *tert*-butylsulfonyl-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34a** (978 mg, 80% over 2 steps), as a colorless solid, m.p. 84-85 °C: ^{1}H NMR, (400 MHz,

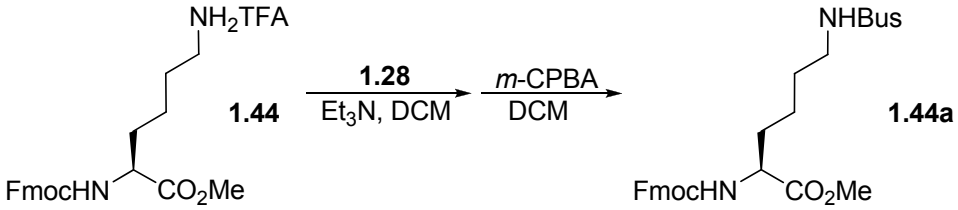
$CDCl_3$) δ 5.00 (d, 1H, *J*=9.8 Hz), 4.73 (s, 1H), 4.04-3.98 (m, 1H), 3.71 (s, 3H), 3.05 (s, 2H), 1.75-1.66 (m, 2H), 1.46-1.39 (m, 2H), 1.37 (s, 9H), 1.31 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.1, 155.8, 78.4, 59.6, 56.9, 52.1, 39.7, 33.0, 29.1, 28.1, 23.8, 22.3; $[\alpha]_D$ +1.4 (c 1.0, $CHCl_3$); HRMS for $C_{16}H_{32}N_2O_6S$ calculated ($M+Na^+$) 403.18733, found 403.18793.

***N*-Bus-*O*-Bus-L-Ser-OMe (1.50):** The general procedure was followed using L-serine methyl ester hydrochloride **1.48** (934 mg, 6 mmol), Et_3N (4.18 mL, 30 mmol), *tert*-butylsulfinyl chloride **1.28** (1.86 mL, 15 mmol) in 15 mL of DCM and DCM (40 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:2), which was oxidized with *m*-CPBA (3.11 g, 18 mmol), DCM (25 mL). The residue was purified by flash column chromatography (EtOAc/hexane 1:4) to afford *tert*-butylsulfonyl-*O*-*tert*-butyl-sulfonyl-L-serine methyl ester **1.50** (1.30 g, 60% over 2 steps), as a colorless solid, m.p. 61-62 °C: ^1H NMR, (400 MHz, $CDCl_3$) δ 5.15 (d, 1H, *J*=9.7 Hz), 4.54 (dd, 1H, *J*=5.4 Hz, *J*=10.8 Hz), 4.45-4.39 (m, 2H), 3.79 (s, 3H), 1.40 (s, 9H), 1.36 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 169.4, 69.4, 60.5, 60.0, 56.5, 53.4, 24.4, 24.1; $[\alpha]_D$ +14.5 (c 1.0, $CHCl_3$); HRMS for $C_{12}H_{25}NO_7S_2$ calculated ($M+H^+$) 360.11452, found 360.11443.



***N*-Bus-N_δ,N_ω-di-Cbz-L-Arg-OMe (1.45a):** The general procedure was followed using N_δ,N_ω-dibenzyloxycarbonyl-L-arginine methyl ester **1.45** (319

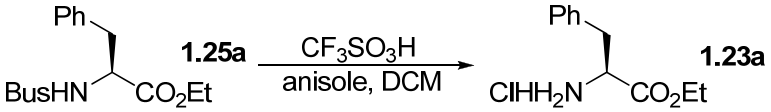
mg, 0.7 mmol), Et_3N (0.13 mL, 0.91 mmol), *tert*-butylsulfinyl chloride **1.28** (95 µL, 0.77 mmol) in 1 mL of DCM and DCM (15 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 1:1), which was oxidized with *m*-CPBA (163 mg, 0.95 mmol) and DCM (10 mL). The residue was purified by flash column chromatography (EtOAc/hexane 1:3) to afford *tert*-butylsulfonyl-N_δ,N_ω-dibenzyloxycarbonyl-L-arginine methyl ester **1.45a** (307 mg, 76% over 2 steps), as a mucous soft solid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 9.26 (s, 1H), 7.39-7.26 (m, 10H), 5.22 (s, 2H), 5.14 (d, 1H, J=10.0Hz), 5.12 (s, 2H), 4.16-4.14 (m, 1H), 4.05-4.00 (m, 1H), 3.62 (s, 3H), 1.78-1.66 (m, 4H), 1.30 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.0, 163.7, 160.6, 155.7, 136.9, 134.6, 128.8, 128.33, 128.28, 128.0, 127.8, 68.9, 66.9, 59.9, 56.8, 52.4, 44.9, 30.4, 24.6, 24.0; $[\alpha]_D$ +6.4 (c 1.0, $CHCl_3$); HRMS for $C_{27}H_{36}N_4O_8S$ calculated ($M+H^+$) 577.23266, found 577.23372.



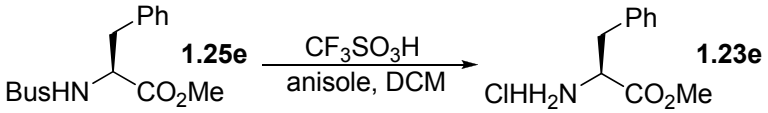
***N*^{6}-Bus-*N*-Fmoc-L-Lys-OMe (1.44a):** The general procedure was followed using *tert*-butylsulfinyl chloride **1.28** (68 µL, 0.55 mmol) in 0.5 mL of DCM, *N*-9-fluorenylmethoxycarbonyl-L-lysine methyl ester hydrochloride **1.44** (248 mg, 0.5 mmol), Et_3N (0.17 mL, 1.25 mmol) and DCM (12 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 1:1), which was oxidized with *m*-CPBA (117 mg, 0.675 mmol) and DCM (10 mL). The residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford N^6-*tert*-butylsulfonyl-*N*-9-fluorenylmethoxy-carbonyl-L-lysine methyl ester **1.44a** (204 mg, 81% over 2 steps), as a colorless solid, m.p. 49-51 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J*=7.5 Hz), 7.63-7.60 (m, 2H), 7.40-7.37

(m, 2H), 7.32-7.28 (m, 2H), 5.65 (d, 2H, *J*=8.2 Hz), 4.64-4.61 (m, 1H), 4.38-4.33 (m, 3H), 4.23-4.19 (m, 1H), 3.73 (s, 3H), 1.86-1.78 (m, 1H), 1.73-1.65 (m, 1H), 1.61-1.51 (m, 2H), 1.47-1.40 (m, 2H), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.1, 156.2, 144.0, 143.9, 141.3, 127.8, 127.2, 125.3, 120.0, 67.1, 59.9, 53.8, 52.5, 47.2, 44.4, 32.0, 30.6, 24.4, 22.2; $[\alpha]_D$ +6.5 (c 1.0, $CHCl_3$); HRMS for $C_{26}H_{34}N_2O_6S$ calculated ($M+H^+$) 503.22103, found 503.22105.

General Procedure for Cleavage of *tert*-Butylsulfonamides. A.

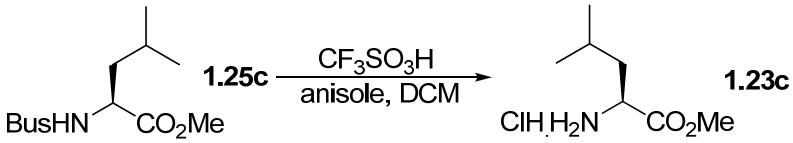


Cleavage of *N*-Bus-L-Phe-OEt (1.23a): To a solution of anisole (0.65 mL, 6.0 mmol) and *tert*-butylsulfonyl-L-phenylalanine ethyl ester **1.25a** (90 mg, 0.3 mmol) in DCM (9 mL) was slowly added trifluoromethanesulfonic acid (0.2 N in DCM, 9 mL) at 0 °C. The solution was stirred at 0 °C for 2 h, then warmed to 4 °C for 10 h (TLC monitoring, EtOAc/hexane 1:1), 15 mL H_2O was added. The aqueous layer was neutralized with saturated aqueous $NaHCO_3$ at 0 °C until pH=7.5, then extracted with DCM (2×10 mL). The organic extracts were combined and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to give L-phenylalanine ethyl ester hydrochloride salt **1.23a** (61 mg, 89%), as a colorless oil.



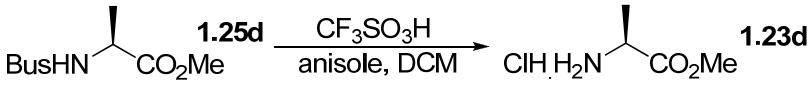
Cleavage of N-Bus-L-Phe-OMe (1.23e): The general procedure was followed using *tert*-butylsulfonyl-L-phenylalanine methyl ester **1.25e** (60 mg, 0.2 mmol), trifluoromethanesulfonic acid (0.2 N in DCM, 6 mL), anisole (0.43

mL, 4.0 mmol), and DCM (6 mL). The aqueous layer was frozen and lyophilized to afford L-phenylalanine methyl ester hydrochloride salt **1.23e** (37 mg, 85%), as a colorless oil.

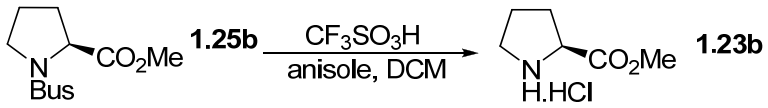


Cleavage of *N*-Bus-L-Leu-OMe (1.23c): The general procedure was followed using *tert*-butylsulfonyl-L-leucine methyl ester **1.25c** (80 mg, 0.3 mmol), trifluoromethanesulfonic acid (0.2 N in DCM, 9 mL), anisole (0.65 mL, 6 mmol), and DCM (9 mL). The aqueous layer was frozen and lyophilized to afford L-leucine methyl ester hydrochloride salt **1.23c** (43 mg, 79%), as a colorless oil.

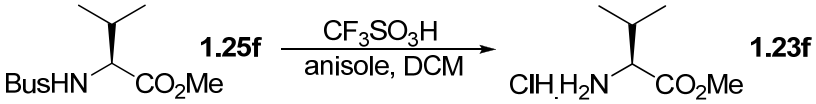
General Procedure for Cleavage of *tert*-Butylsulfonamides. B.



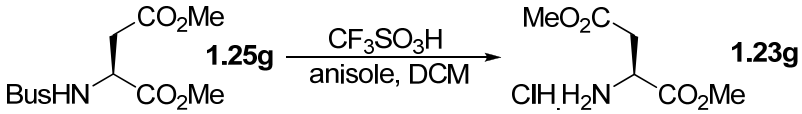
Cleavage of *N*-Bus-L-Ala-OMe (1.23d): To a solution of anisole (0.22 mL, 2.0 mmol) and *tert*-butylsulfonyl-L-alanine methyl ester **1.25d** (45 mg, 0.2 mmol) in DCM (3 mL) was slowly added trifluoromethanesulfonic acid (0.2 N in DCM, 3 mL) at 0 °C. The solution was stirred at 0 °C for 2 h, then warmed to 4 °C for 10 h (TLC monitoring, EtOAc/hexane 2:3), and 6 mL H_2O was added. The aqueous layer was neutralized with DOWEX Monosphere 550A hydroxide form anion exchange resin at 0 °C until pH=8.5, then 6 mL MeOH was added and the resin was filtered. The filtrate was combined and acidified with 1 M HCl aqueous (3 mL). The aqueous layer was frozen and lyophilized to afford L-alanine methyl ester hydrochloride salt **1.23d** (24 mg, 85%), as a colorless oil.



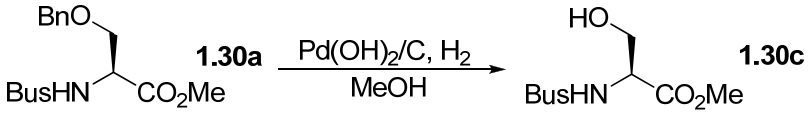
Cleavage of *N*-Bus-L-Pro-OMe (1.23b): The general procedure was followed using *tert*-butylsulfonyl-L-proline methyl ester **1.25b** (50 mg, 0.2 mmol), trifluoromethanesulfonic acid (0.2 N in DCM, 3 mL), anisole (0.22 mL, 2 mmol), and DCM (3 mL). The aqueous layer was frozen and lyophilized to afford L-proline methyl ester hydrochloride salt **1.23b** (30 mg, 90%), as a colorless oil.



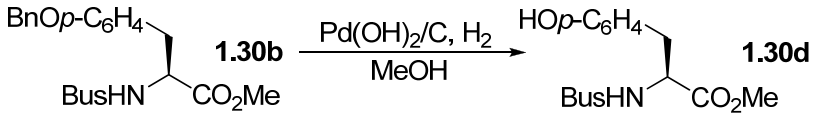
Cleavage of *N*-Bus-L-Val-OMe (1.23f): The general procedure was followed using *tert*-butylsulfonyl-L-valine methyl ester **1.25f** (50 mg, 0.2 mmol), trifluoromethanesulfonic acid (0.2 N in DCM, 3 mL), anisole (0.22 mL, 2.0 mmol), and DCM (3 mL). The aqueous layer was frozen and lyophilized to afford L-valine methyl ester hydrochloride salt **1.23f** (28 mg, 85%), as a colorless oil.



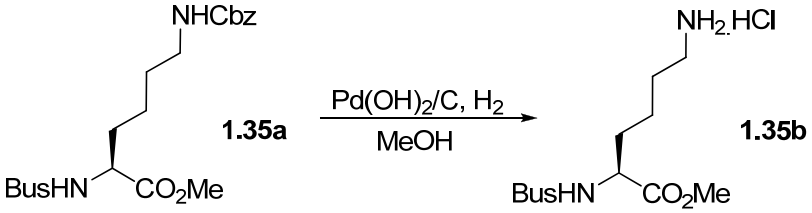
Cleavage of *N*-Bus-L-Asp-diOMe (1.23g): The general procedure was followed using *tert*-butylsulfonyl-L-aspartic dimethyl ester **1.25g** (56 mg, 0.2 mmol), trifluoromethanesulfonic acid (0.2 N in DCM, 3 mL), anisole (0.22 mL, 2.0 mmol), and DCM (3 mL). The acid aqueous layer was frozen and lyophilized to afford L-aspartic dimethyl ester hydrochloride salt **1.23g** (33 mg, 84%), as a colorless oil.



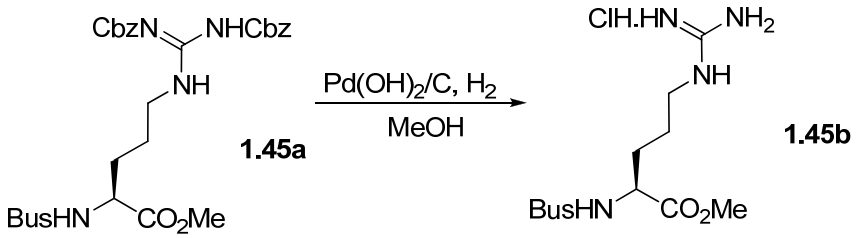
Cleavage Bn of *N*-Bus-*O*-Bn-L-Ser-OMe (1.30c): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-serine methyl ester **1.30a** (99 mg, 0.3 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 2 h (TLC monitoring, EtOAc/ hexane 3:7). The reaction mixture was filtered over celite, and filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-serine methyl ester **1.30c** (70 mg, 97%), as a colorless solid, m.p. 77-79 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.66 (d, 1H, *J*=9.5 Hz), 4.18-4.16 (m, 1H), 3.96 (d, 1H, *J*=11.2 Hz), 3.86 (d, 1H, *J*=11.2 Hz), 3.77 (s, 3H), 3.23 (s, 1H), 1.35 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.4, 64.5, 60.5, 59.2, 53.0, 24.2; $[\alpha]_D$ +0.99 (c 1.0, $CHCl_3$); ESI/MS for $C_8H_{17}NO_5S$ calculated ($M+H^+$) 240, found 240.



Cleavage Bn of *N*-Bus-*O*-Bn-L-Tyr-OMe (1.30d): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine methyl ester **1.30b** (122 mg, 0.3 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 3 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite, and filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-tyrosine methyl ester **1.30d** (92 mg, 97%), as a colorless solid, m.p. 141-143 °C: ^{1}H NMR, (400 MHz, CD_3OD) δ 7.08 (d, 2H, *J*=8.5 Hz), 6.74 (d, 2H, *J*=8.5 Hz), 4.60 (s, 1H), 4.02-3.99 (m, 1H), 3.72 (s, 3H), 3.03-2.98 (m, 1H), 2.80-2.74 (m, 1H), 1.34 (d, 1H, *J*=7.1 Hz), 1.09 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 174.9, 157.6, 131.9, 129.0, 116.4, 61.3, 61.0, 53.0, 39.2, 24.2; $[\alpha]_D$ -23.8 (c 1.0, MeOH); ESI/MS for $C_{14}H_{21}NO_5S$ calculated ($M+H^+$) 316, found 316.

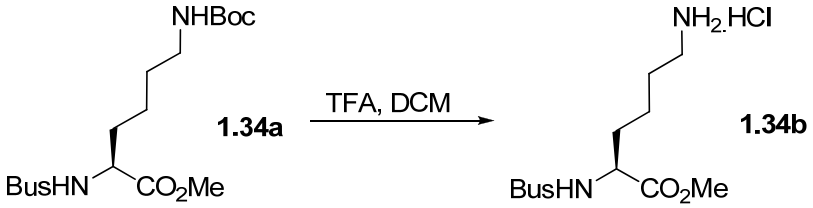


Cleavage Cbz of *N*-Bus-*N*^{6}-Cbz-L-Lys-OMe (1.35b): To a solution of *tert*-butylsulfonyl-*N*^{6}-benzyloxycarbonyl-L-lysine methyl ester **1.35a** (125 mg, 0.3 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 2 h (TLC monitoring, EtOAc/ hexane 1:1). The reaction mixture was filtered over celite and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to afford *tert*-butylsulfonyl-L-lysine methyl ester hydrochloride salt **1.35b** (92 mg, 97%), as a colorless oil: ^{1}H NMR, (400 MHz, CD_3OD) δ 4.02-4.00 (m, 1H), 3.75 (s, 3H), 2.98-2.94 (m, 2H), 1.84-1.77 (m, 1H), 1.76-1.69 (m, 3H), 1.64-1.53 (m, 2H), 1.35 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 174.7, 61.0, 58.3, 53.1, 40.6, 34.0, 28.0, 24.5, 23.8; $[\alpha]_D$ -15.0 (c 1.0, MeOH); ESI/MS for $C_{11}H_{25}ClN_2O_4S$ calculated ($M+H^+$-HCl) 281, found 281.

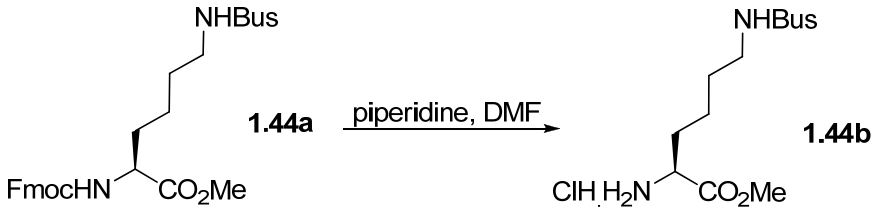


Cleavage Cbz of *N*-Bus-*N*$_{\delta}$,*N*$_{\omega}$-di-Cbz-L-Arg-OMe (1.45b): To a solution of *tert*-butylsulfonyl-*N*$_{\delta}$,*N*$_{\omega}$-dibenzyloxycarbonyl-L-arginine methyl ester **1.45a** (58 mg, 0.1mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (3 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to

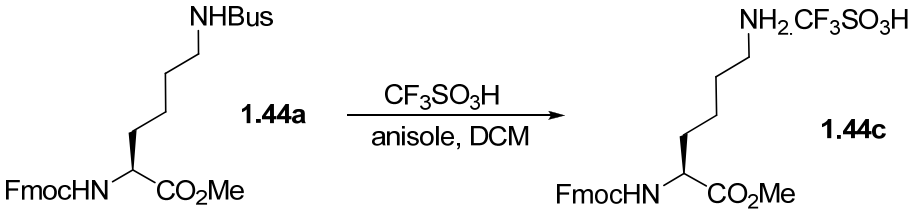
afford *tert*-butylsulfonyl-L-arginine methyl ester hydrochloride salt **1.45b** (29 mg, 95%), as a colorless oil: ^{1}H NMR, (400 MHz, CD_3OD) δ 4.07-3.98 (m, 1H), 3.76 (s, 3H), 3.26-3.22 (m, 2H), 1.86-1.79 (m, 1H), 1.77-1.72 (m, 3H), 1.36 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 174.4, 158.7, 61.1, 58.1, 53.1, 41.9, 31.9, 26.4, 24.5; $[\alpha]_D$ -9.6 (c 1.0, MeOH); ESI/MS for $C_{11}H_{25}ClN_4O_4S$ calculated ($M+H^+$-HCl) 309, found 309.



Cleavage Boc of *N*-Bus-*N*^{6}-Boc-L-Lys-OMe (1.34b): To a solution of *tert*-butylsulfonyl-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.34a** (95 mg, 0.25 mmol) in DCM (5 mL), trifluoroacetic acid (56 µL, 0.75 mmol) was added dropwise and stirred at R.T. for 8 h (TLC monitoring, EtOAc/hexane 1:1). Solvent was evaporated, then 15 mL H_2O was added and neutralized with saturated aqueous $NaHCO_3$ at 0 ^{o}C until pH=7.5, then extracted with DCM (2×10 mL). The organic extracts were combined and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to afford *tert*-butylsulfonyl-L-lysine methyl ester hydrochloride salt **1.34b** (77 mg, 97%), as a colorless oil: ^{1}H NMR, (400 MHz, CD_3OD) δ 4.02-4.00 (m, 1H), 3.75 (s, 3H), 2.98- 2.94 (m, 2H), 1.84-1.77 (m, 1H), 1.76-1.69 (m, 3H), 1.64-1.53 (m, 2H), 1.35 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 174.7, 61.0, 58.3, 53.1, 40.6, 34.0, 28.0, 24.5, 23.8; $[\alpha]_D$ -15.0 (c 1.0, MeOH); ESI/MS for $C_{11}H_{25}ClN_2O_4S$ calculated ($M+H^+$-HCl) 281, found 281.



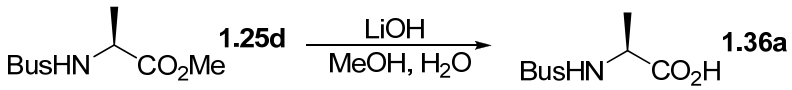
Cleavage Fmoc of *N*^{6}-Bus-*N*-Fmoc-L-Lys-OMe (1.44b): To a solution of 5% piperidine in DMF (1 mL) was added *N*^{6}-*tert*-butylsulfonyl-*N*-9-fluorenyl-methoxycarbonyl-L-lysine methyl ester **1.44a** (50 mg, 0.1 mmol) at R.T.. The reaction mixture was stirred at R.T. for 8 h (TLC monitoring, EtOAc/hexane 2:3), after which time it was diluted with 50 mL of EtOAc. The reaction mixture was washed with 2×50 mL of saturated aqueous Na_2CO_3 and 50mL of brine. The organic layer was dried over Na_2SO_4, and solvent was evaporated. The crude product was purified by flash column chromatography (EtOAc/hexane 2:3). The organic extracts were combined and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to afford *N*^{6}-*tert*-butylsulfonyl-L-lysine methyl ester hydrochloride salt **1.44b** (26 mg, 92%), as a colorless oil: ^{1}H NMR, (400 MHz, CD_3OD) δ 4.07-4.04 (m, 1H), 3.85 (s, 3H), 3.18- 3.15 (m, 2H), 1.99-1.92 (m, 2H), 1.61-1.49 (m, 4H), 1.36 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 171.1, 60.8, 54.1, 53.8, 45.0, 31.9, 31.2, 24.8, 22.9; $[\alpha]_D$ +13.3 (c 1.0, MeOH); ESI/MS for $C_{11}H_{25}ClN_2O_4S$ calculated ($M+H^+$-HCl) 281, found 281.



Cleavage Bus of *N*^{6}-Bus-*N*-Fmoc-L-Lys-OMe (1.44c): To a solution of *N*^{6}-*tert*-butylsulfonyl-*N*-9-fluorenylmethoxycarbonyl-L-lysine methyl ester **1.44a** (25 mg, 0.05 mmol) and anisole (0.11 mL, 1.0 mmol) in DCM (1.5 mL) was slowly added trifluoromethanesulfonic acid (0.2 N in DCM, 1.5 mL) at 0 °C.

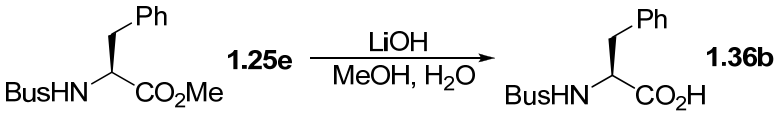
The solution was stirred at 4 °C for 6 h (TLC monitoring, EtOAc/hexane 2:3), the reaction was finished. Solvents were evaporated. The residue was taken up in the 20 mL H_2O and washed with 3×20 mL Et_2O. The water layer was frozen and lyophilized to afford *N*-9-fluorenylmethoxycarbonyl-L-lysine methyl ester trifluoromethanesulfonic acid salt **1.44c** (24 mg, 89%), as a colorless oil: ^{1}H NMR, (400 MHz, CD_3OD) δ 7.80 (d, 2H, *J*=7.5 Hz), 7.67 (t, 2H, *J*=6.7 Hz), 7.40(t, 2H, *J*=7.3 Hz), 7.32(t, 2H, *J*=7.5 Hz), 4.42-4.32 (m, 2H), 4.24-4.16 (m, 2H), 3.72 (s, 3H), 2.92 (t, 2H, *J*=7.2 Hz), 1.92–1.82 (m, 1H), 1.78–1.65 (m, 3H), 1.52–1.42 (m, 2H); ^{13}C NMR, (100 MHz, CD_3OD) δ 174.5, 158.8, 145.3, 145.2, 142,7, 128.9, 128.3, 126.34, 126.32, 121.0, 120.3, 68.0, 55.2, 52.9, 48.5, 40.6, 32.0, 28.0, 23.9; $[\alpha]_D$ –9.5(c 1.0, MeOH); ESI/MS for $C_{23}H_{27}F_3N_2O_6S$ calculated ($M+H^+$-CF_3SO_3H) 383, found 383.

General Procedure for Hydrolysis of *tert*-Butylsulfon amides.

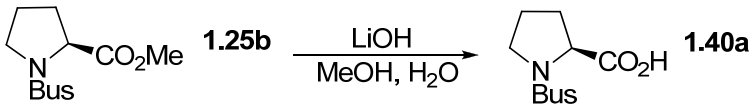


Hydrolysis of *N*-Bus-L-Ala-OMe (1.36a): To a solution of *tert*-butyl-sulfonyl-L-alanine methyl ester **1.25d** (669 mg, 3.0 mmol) in MeOH (21 mL) and H_2O (7 mL) was slowly added $LiOH\times H_2O$ (627 mg, 15 mmol) at 0 °C. The solution was stirred at 4 °C for 10 h (TLC monitoring, EtOAc/hexane 3:2), and solvents were evaporated. Then the residue was taken up in the 30 mL H_2O and acidified with 1 M HCl aqueous to pH=1, the aqueous layer was extracted with EtOAc (4×50 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to give *tert*-butylsulfonyl-L-alanine **1.36a** (552 mg, 88%), as a colorless solid, m.p. 161-162 °C: ^{1}H NMR, (400 MHz, CD_3OD) δ 4.03 (dd, 1H, *J*=7.3 Hz, *J*=14.6 Hz), 1.43 (d, 3H, *J*=7.3 Hz), 1.35 (s,

9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 176.4, 60.8, 54.1, 24.5, 20.4; $[\alpha]_D$ -19.9 (c 1.0, MeOH); HRMS for $C_7H_{15}NO_4S$ calculated ($M+Na^+$) 232.06140, found 232.06111.

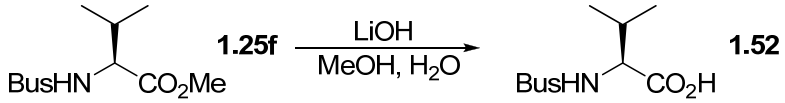


Hydrolysis of *N*-Bus-L-Phe-OMe (1.36b): The general procedure was followed using *tert*-butylsulfonyl-L-phenylalanine methyl ester **1.25e** (500 mg, 1.67 mmol), LiOH×H_2O (351 mg, 8.36 mmol), MeOH (21 mL) and H_2O (7 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-phenylalanine **1.36b** (425 mg, 90%), as a colorless solid, m.p. 203-205 °C: ^{1}H NMR, (400 MHz, CD_3OD) δ 7.30-7.22 (m, 5H), 4.04 (dd, 1H, *J*=4.5 Hz, *J*=10.0 Hz), 3.15 (dd, 1H, *J*=4.5 Hz, *J*=13.5 Hz), 2.87 (dd, 1H, *J*=10.0 Hz, *J*=13.5 Hz), 1.07 (s, 9H); ^{13}C NMR, (100 MHz, CD_3OD) δ 175.8, 138.6, 130.9, 129.6, 128.1, 61.0, 60.9, 40.2, 24.2; $[\alpha]_D$ -21.5 (c 1.0, MeOH); HRMS for $C_{13}H_{19}NO_4S$ calculated ($M+Na^+$) 308.09270, found 308.09191.

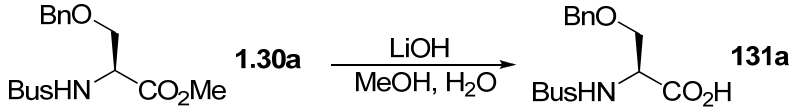


Hydrolysis of *N*-Bus-L-Pro-OMe (1.40a): The general procedure was followed using *tert*-butylsulfonyl-L-proline methyl ester **1.25b** (250 mg, 1.0 mmol), LiOH×H_2O (210 mg, 5.0 mmol), MeOH (15 mL) and H_2O (5 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-proline **1.40a** (214 mg, 90%), as a colorless solid, m.p. 135-137 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 9.85 (s, 1H), 4.55 (d, 1H, *J*=6.3 Hz), 3.67-3.65 (m, 1H), 3.46-3.45 (m, 1H), 2.27-2.21 (m, 1H), 2.07-1.93 (m, 3H), 1.36 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 178.5, 61.6,

61.5, 50.3, 31.0, 25.1, 24.4; $[\alpha]_D$ -67.3 (c 1.0, $CHCl_3$); HRMS for $C_9H_{17}NO_4S$ calculated ($M+H^+$) 236.09511, found 236.09521.

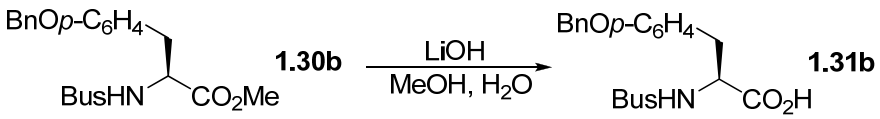


Hydrolysis of *N*-Bus-L-Val-OMe (1.52): The general procedure was followed using *tert*-butylsulfonyl-L-valine methyl ester **1.25f** (25 mg, 0.1 mmol), LiOH×H_2O (21 mg, 0.5 mmol), MeOH (3 mL) and H_2O (1 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-valine **1.52** (21 mg, 89%), as a colorless solid, m.p. 101-104 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 5.01 (d, 1H, *J*=10.2 Hz), 3.95 (dd, 1H, *J*=4.8 Hz, *J*=10.3 Hz), 2.20-2.16 (m, 1H), 1.36 (s, 9H), 1.04 (d, 3H, *J*=6.8 Hz), 0.94 (d, 3H, *J*=6.9 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 177.2, 62.5, 60.7, 32.1, 24.3, 19.5, 17.6; $[\alpha]_D$ -3.2 (c 1.0, $CHCl_3$); HRMS for $C_9H_{19}NO_4S$ calculated ($M+Na^+$) 260.0927, found 260.09235.



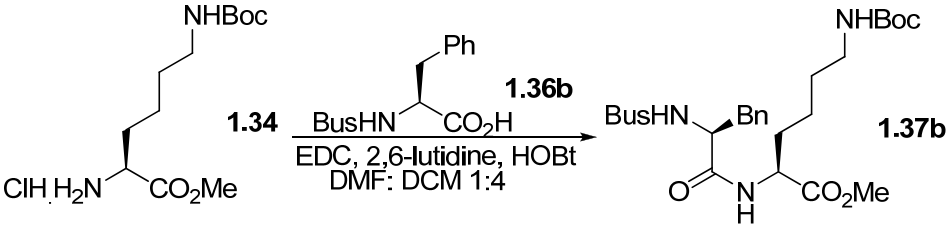
Hydrolysis of *N*-Bus-*O*-Bn-L-Ser-OMe (1.31a): The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-serine methyl ester **1.30a** (495 mg, 1.5 mmol), LiOH×H_2O (315 mg, 7.5 mmol), MeOH (15 mL) and H_2O (5 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford *tert*-butylsulfonyl-*O*-benzyl-L-serine **1.31a** (408 mg, 86%), as a colorless solid, m.p. 58-60 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.33-7.28 (m, 5H), 6.95 (s, 1H), 5.22 (d, 1H, *J*=9.3 Hz), 4.55 (s, 2H), 4.30-4.27 (m, 1H), 3.93 (dd, 1H, *J*=3.4 Hz, *J*=9.5 Hz), 3.73 (dd, 1H, *J*=3.6 Hz, *J*=9.5 Hz), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 174.6, 137.3, 128.7, 128.2, 127.9, 73.6, 71.1, 60.5, 58.3, 24.2; $[\alpha]_D$ -4.7 (c 1.0,

$CHCl_3$);HRMS for $C_{14}H_{21}NO_5S$ calculated ($M+H^+$) 316.12132, found 316.12180.



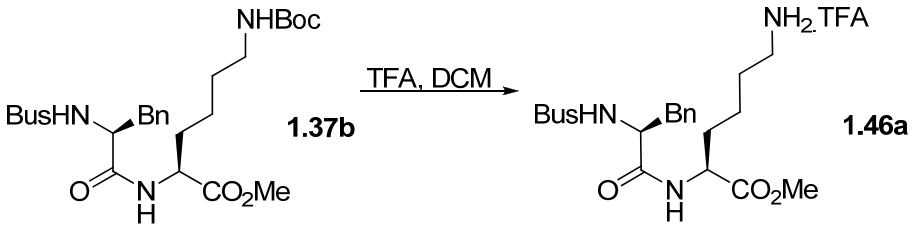
Hydrolysis of *N*-Bus-*O*-Bn-L-Tyr-OMe (1.31b): The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine methyl ester **1.30b** (609 mg, 1.5 mmol), $LiOH\times H_2O$ (315 mg, 7.5 mmol), MeOH (15 mL) and H_2O (5 mL). The organic extracts were combined, dried over Na_2SO_4, concentrated under reduced pressure to give *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine **1.31b** (518 mg, 88%), as a colorless solid, m.p. 148-149 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.43-7.30 (m, 5H), 7.17 (d, 2H, *J*=8.4 Hz), 6.93 (d, 2H, *J*=8.4 Hz), 5.04 (s, 2H), 5.02 (d, 1H, *J*=13.0 Hz), 4.25 (s, 1H), 3.14 (dd, 1H, *J*=4.7 Hz, *J*=13.9 Hz), 2.98 (dd, 1H, *J*=7.7 Hz, *J*=13.8 Hz), 1.20 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 176.7, 158.2, 137.0, 130.9, 128.7, 128.1, 128.0, 127.6, 115.2, 70.1, 60.5, 58.9, 38.6, 23.9; $[\alpha]_D$ +3.7 (c 1.0, $CHCl_3$); HRMS for $C_{20}H_{25}NO_5S$ calculated ($M+H^+$) 392.15262, found 392.15377.

General Procedure for Peptide Coupling:



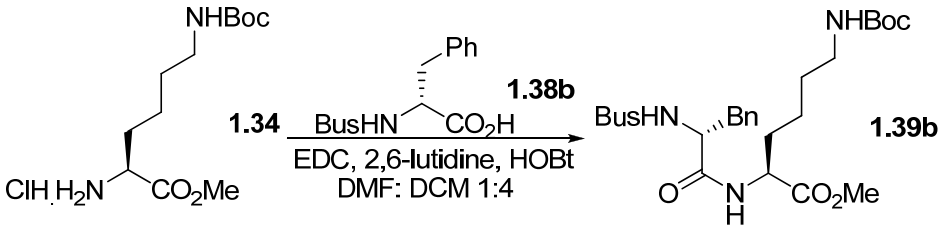
***N*-Bus-L-Phe-N^6-Boc-L-Lys-OMe (1.37b):** A solution of *tert*-butyl-sulfonyl-L-phenylalanine **1.36b** (143 mg, 0.5 mmol) in 8mL of DCM and 2 mL of DMF was cooled to 0 °C, then EDC (144 mg, 0.75 mmol), HOBt (101 mg, 0.75 mmol), N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34**

(148 mg, 0.5 mmol) and 2,6-lutidine (0.17 mL, 1.5 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h, then at R.T. until MS showed consumption of the starting material (4 h). Upon completion, 20 mL of DCM was added and washed successively with the saturated aqueous $NaHCO_3$ (2×30 mL), 1 M HCl aqueous (2×30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:1) to afford *tert*-butylsulfonyl-L-phenylalanine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.37b** (247 mg, 93%), as a colorless solid, m.p. 131-132 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.26-7.16 (m, 5H), 6.95 (d, 1H, *J*=7.5 Hz), 5.51 (d, 1H, *J*=9.9 Hz), 4.91 (s, 1H), 4.56-4.54 (m, 1H), 4.09-4.07 (m, 1H), 3.66 (s, 3H), 3.11-3.02 (m, 4H), 1.82-1.71 (m, 1H), 1.66-1.60 (m, 1H), 1.45-1.38 (m, 11H), 1.28-1.21 (m, 2H), 1.12 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 172.4, 156.1, 136.5, 129.9, 128.6, 127.1, 78.9, 60.1, 59.8, 52.4, 52.1, 40.1, 39.1, 31.7, 29.2, 28.5, 23.8, 22.2; $[\alpha]_D$ -15.9 (c 1.0, $CHCl_3$); HRMS for $C_{25}H_{41}N_3O_7S$ calculated ($M+H^+$) 528.27380, found 528.27291.

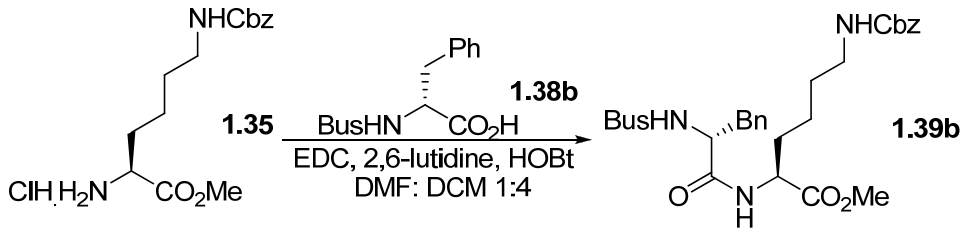


Cleavage Boc of *N*-Bus-L-Phe-N^6-Boc-L-Lys-OMe (1.46a): To a solution of *tert*-butylsulfonyl-L-phenylalanine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.37b** (53 mg, 0.1 mmol) in DCM (5 mL), trifluoroacetic acid (0.5 mL, 6.7 mmol) was added dropwise and stirred at 0 °C (TLC monitoring, EtOAc/hexane 1:1). Solvent was evaporated to afford *tert*-butylsulfonyl-L-phenylalanine-L-lysine methyl ester trifluoroacetic acid salt **1.46a** (51 mg, 94%), as a colorless oil: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.38-7.30 (m, 4H), 7.27-7.24 (m,

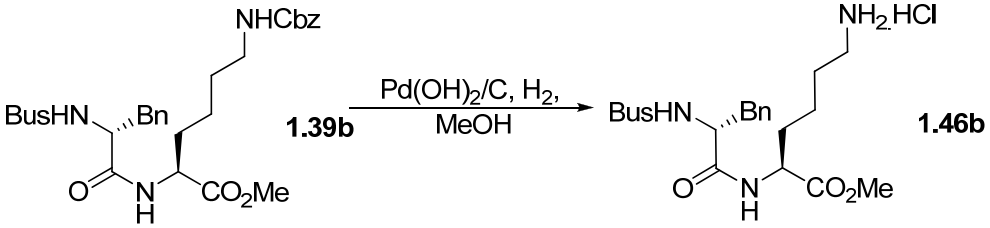
1H), 4.56-4.53 (m, 1H), 4.11-4.07 (m, 1H), 3.73 (s, 3H), 3.09 (dd, 1H, *J*=4.4 Hz, *J*=13.7 Hz), 2.96-2.92 (t, 2H, *J*=7.5 Hz), 2.82 (dd, 1H, *J*=10.6 Hz, *J*=13.5 Hz), 2.02-1.91 (m, 1H), 1.83-1.60 (m, 3H), 1.58-1.49 (m, 2H), 1.06 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.4, 175.3, 173.8, 138.8, 131.1, 129.6, 128.1, 61.7, 60.9, 53.0, 52.9, 40.7, 40.0, 32.2, 27.8, 24.1, 23.5; $[\alpha]_D$ -20.9 (c 1.0, MeOH); ESI/MS for $C_{22}H_{34}F_3N_3O_7S$ calculated ($M+H^+$) 428, found 428.



***N*-Bus-D-Phe-N^6-Boc-L-Lys-OMe (1.39b):** The general procedure was followed using *tert*-butylsulfonyl-D-phenylalanine **1.38b** (14 mg, 0.05 mmol), N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (15 mg, 0.05 mmol), EDC (14 mg, 0.08 mmol), HOBt (10 mg, 0.08 mmol), 2,6-lutidine (17 μL, 0.15 mmol), DCM (0.8 mL) and DMF (0.2 mL). The product (33 mg) was checked directly by HPLC and purified by flash column chromatography (EtOAc/ hexane 1:1) to afford *tert*-butylsulfonyl-D-phenylalanine-N^6-*tert*-butyloxy carbonyl-L-lysine methyl ester **1.39b** (24 mg, 92%), as a colorless soft powder: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.35-7.25 (m, 5H), 6.68 (d, 1H, *J*=7.4 Hz), 4.96 (d, 1H, *J*=9.0 Hz), 4.72 (tr, s, 1H), 4.56 (dd, 1H, *J*=7.6 Hz, *J*=12.4 Hz), 4.16(dd, 1H, *J*=7.2 Hz, *J*=16.1 Hz), 3.73 (s, 3H), 3.19-2.98 (m, 4H), 1.81-1.71 (m, 1H), 1.69-1.59 (m, 1H), 1.45-1.41 (m, 11H), 1.31-1.17 (m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 171.2, 156.2, 136.4, 129.8, 129.0, 127.5, 79.3, 60.5, 60.0, 52.7, 52.3, 40.2, 40.0, 31.8, 29.5, 28.6, 24.1, 22.3; $[\alpha]_D$ +17.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{25}H_{41}N_3O_7S$ calculated ($M+H^+$) 528, found 528.

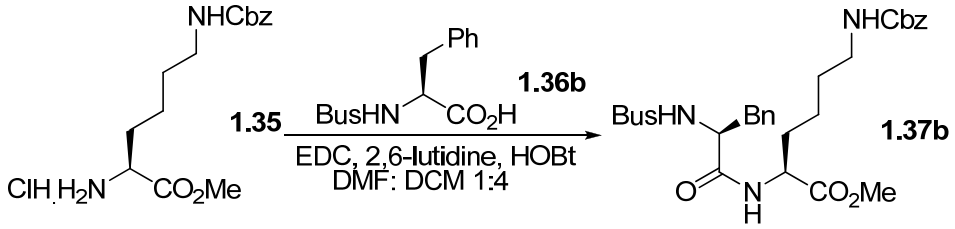


***N*-Bus-D-Phe-N^6-Cbz-L-Lys-OMe (1.39d):** The general procedure was followed using *tert*-butylsulfonyl-D-phenylalanine **1.38b** (143 mg, 0.5 mmol) N^6-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (166 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), HOBt (101 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), DCM (8 mL) and DMF (2 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-D-phenylalanine-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.39d** (257 mg, 92%), as a colorless solid, m.p. 57-59 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.31-7.21 (m, 5H), 7.02 (d, 1H, *J*=7.9 Hz), 5.58 (d, 1H, *J*=9.3 Hz), 5.32-5.29 (m, 1H), 5.14-5.03 (m, 2H), 4.59-4.54 (m, 1H), 4.18-4.12 (m, 1H), 3.66 (s, 3H), 3.19-3.10 (m, 3H), 3.03-2.98 (m, 1H), 1.77-1.73 (m, 1H), 1.63-1.60 (m, 1H), 1.43-1.29 (m, 2H), 1.25-1.22 (m, 2H), 1.10 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 171.6, 156.7, 136.7, 136.6, 129.7, 128.7, 128.4, 128.0, 127.9, 127.2, 66.5, 60.3, 60.2, 52.4, 52.0, 40.4, 39.5, 31.4, 29.1, 23.8, 22.0; $[\alpha]_D$ +21.1 (c 1.0, $CHCl_3$); ESI/MS for $C_{28}H_{39}N_3O_7S$ calculated ($M+H^+$) 562, found 562.



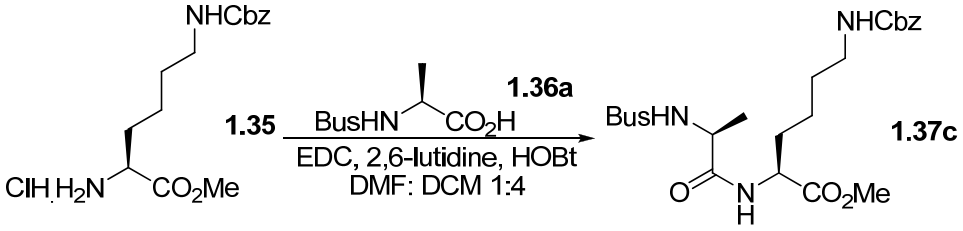
Cleavage Cbz of *N*-Bus-D-Phe-N^6-Cbz-L-Lys-OMe (1.46b): To a solution of *tert*-butylsulfonyl-D-phenylalanine-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.39d** (56 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over

activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to afford *tert*-butylsulfonyl-D-phenylalaninel-L-lysine methyl ester hydrochloride salt **1.46b** (44 mg, 95%), as a colorless oil: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.34-7.32 (m, 4H), 7.28-7.26 (m, 1H), 4.40 (dd, 1H, *J*=4.6 Hz, *J*=8.8 Hz), 4.14-4.09 (m, 1H), 3.74 (s, 3H), 3.11 (dd, 1H, *J*=5.6 Hz, *J*=11.5 Hz)， 2.93-2.86 (m, 3H), 1.94-1.82 (m, 1H), 1.79-1.64 (m, 3H), 1.40-1.33 (m, 2H), 1.13 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 174.8, 174.7, 138.6, 131.0, 129.7, 128.2, 62.0, 61.0, 53.4, 53.0, 40.8, 40.6, 32.2, 28.0, 24.3, 23.5; $[\alpha]_D$ +27.8 (c 1.0, MeOH); ESI/MS for $C_{20}H_{34}ClN_3O_5S$ calculated (M-HCl+H$^+$) 428, found 428.

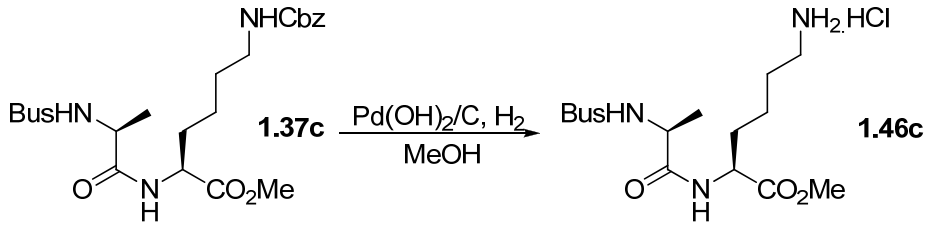


***N*-Bus-L-Phe-*N*^{6}-Cbz-L-Lys-OMe (1.37d):** The general procedure was followed using *tert*-butylsulfonyl-L-phenylalanine **1.36b** (14 mg, 0.05 mmol), EDC (14 mg, 0.08 mmol), HOBt (10 mg, 0.08 mmol), 2,6-lutidine (0.017 mL, 0.15 mmol), *N*^{6}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (16 mg, 0.05 mmol), DCM (0.8 mL) and DMF (0.2 mL). The crude product (34 mg) was checked directly by HPLC, then purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-L-phenyl-alanine-*N*^{6}-benzyloxy-carbonyl-L-lysine methyl ester **1.37d** (26 mg, 93%), as a colorless solid, m.p. 136-138 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.37-7.22 (m, 5H), 6.77 (d, 1H, *J*=7.8 Hz), 5.23 (d, 2H, *J*=7.0 Hz), 5.24-5.04 (m, 2H), 4.63-4.60 (m, 1H), 4.13-4.07 (m, 1H), 3.73 (s, 3H), 3.20-3.08 (m, 4H),

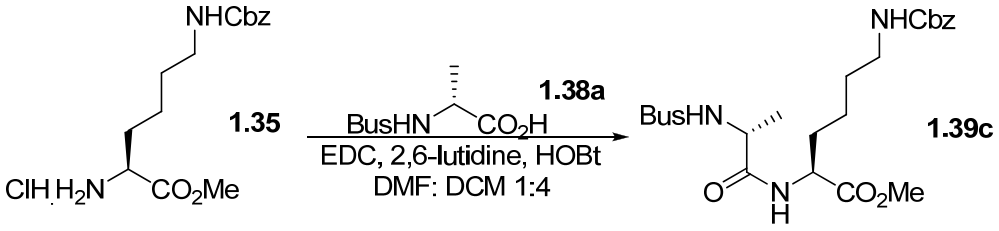
1.93-1.85 (m, 1H), 1.73-1.62 (m, 1H), 1.57-1.43 (m, 2H), 1.32-1.26 (m, 2H), 1.16 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 171.3, 156.9, 136.8, 136.4, 130.0, 128.8, 128.7, 128.3, 128.2, 127.4, 127.1, 66.8, 60.4, 59.9, 52.6, 52.1, 40.5, 39.3, 31.7, 29.2, 23.9, 22.1; $[\alpha]_D$ -13.8 (c 1.0, $CHCl_3$); HRMS for $C_{28}H_{39}N_3O_7S$ calculated ($M+H^+$) 562.25815, found 562.25834.



***N*-Bus-L-Ala-*N*^{6}-Cbz-L-Lys-OMe (1.37c):** The general procedure was followed using *tert*-butylsulfonyl-L-alanine **1.36a** (209 mg, 1.0 mmol), EDC (288 mg, 1.5 mmol), HOBt (202 mg, 1.5 mmol), 2,6-lutidine (0.35 mL, 3.0 mmol), *N*^{6}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (331 mg, 1.0 mmol), DCM (16 mL) and DMF (4 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-L-alanine-*N*^{6}-benzyloxycarbonyl-L-lysine methyl ester **1.37c** (437 mg, 90%), as a thick colorless liquid. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.33-7.28 (m, 5H), 7.10 (d, 1H, *J*=7.8 Hz), 5.53 (d, 1H, *J*=9.4 Hz), 5.43-5.40 (m, 1H), 5.12-5.03 (m, 2H), 4.58-4.53 (m, 1H), 4.09-4.02 (m, 1H), 3.70 (s, 3H), 3.20-3.08 (m, 2H), 1.90-1.82 (m, 1H), 1.74-1.65 (m, 1H), 1.56-1.45 (m, 2H), 1.41-1.37 (m, 5H), 1.33 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.60, 172.57, 156.9, 136.7, 128.5, 128.09, 128.07, 66.6, 60.0, 53.6, 52.5, 52.1, 40.4, 31.5, 29.2, 24.1, 22.2, 19.8; $[\alpha]_D$ -17.5 (c 1.0, $CHCl_3$); HRMS for $C_{22}H_{35}N_3O_7S$ calculated ($M+H^+$) 486.22685, found 486.22712.

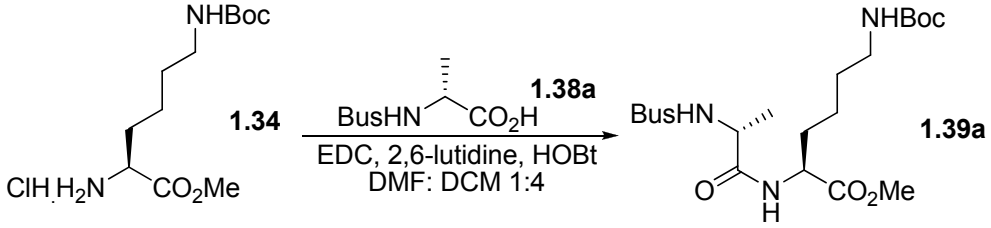


Cleavage Cbz of *N*-Bus-L-Ala-*N*^{6}-Cbz-L-Lys-OMe (1.46c): To a solution of *tert*-butylsulfonyl-L-alanine-*N*^{6}-benzyloxycarbonyl-L-lysine methyl ester **1.37c** (49 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite and acidified with 1 M HCl aqueous (10 mL). The aqueous layer was frozen and lyophilized to afford *tert*-butylsulfonyl-L-alanine-L-lysine methyl ester hydrochloride salt **1.46c** (35 mg, 90%), as a thick colorless oil. ^{1}H NMR, (400 MHz, MeOH-d_4) δ 4.48 (dd, 1H, *J*=6.8 Hz, *J*=9.8 Hz), 4.11-4.04 (m, 1H), 3.73 (s, 3H), 2.96-2.92 (t, 2H, J=7.5 Hz), 2.00-1.89 (m, 1H), 1.81-1.63 (m, 3H), 1.56-1.47 (m, 2H), 1.40 (d, 3H, *J*=7.2 Hz), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.9, 173.8, 60.9, 54.9, 53.1, 53.0, 40.7, 32.1, 27.9, 24.6, 23.6, 19.9; $[\alpha]_D$ -50.6 (c 1.0, MeOH); ESI/MS for $C_{14}H_{30}ClN_3O_5S$ calculated (M-HCl +H$^+$) 352, found 352.



***N*-Bus-D-Ala-*N*^{6}-Cbz-L-Lys-OMe (1.39c):** The general procedure was followed using *N*^{6}-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (33 mg, 0.1 mmol), *tert*-butylsulfonyl-D-alanine **1.38a** (21 mg, 0.1 mmol), EDC (29 mg, 0.15 mmol), HOBt (20 mg, 0.15 mmol), 2,6-lutidine (0.035 mL, 0.3 mmol), DCM (1.6 mL) and DMF (0.4 mL). The product (61 mg) was checked

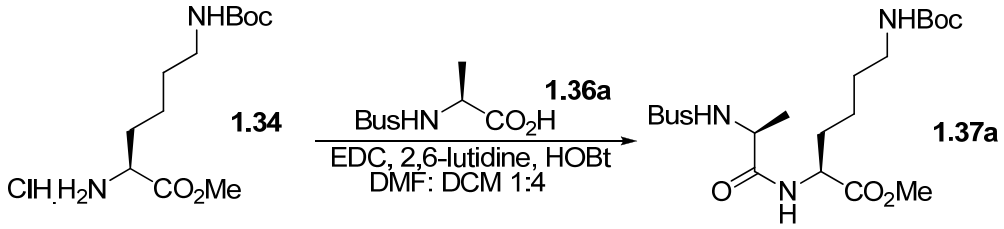
directly by HPLC and purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-D-alanine-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.39c** (45 mg, 92%), as a thick colorless liquid. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.36-7.28 (m, 5H), 7.09 (d, 1H, *J*=7.9 Hz), 5.37 (d, 1H, *J*=8.9Hz), 5.19 (tr, t, 1H), 5.09 (s, 2H), 4.59-4.54 (m, 1H), 4.13-4.06 (m, 1H), 3.72 (s, 3H), 3.20-3.14 (m, 2H), 1.93-1.83 (m, 1H), 1.78-1.68 (m, 1H), 1.57-1.48 (m, 2H), 1.45 (d, 3H, *J*=7.1 Hz), 1.40-1.36 (m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.7, 172.6, 156.9, 136.7, 128.6, 128.2, 66.7, 60.3, 54.0, 52.6, 52.2, 40.5, 31.5, 29.3, 24.3, 22.2, 20.3; $[\alpha]_D$ +24.8 (c 1.0, $CHCl_3$); ESI/MS for $C_{22}H_{35}N_3O_7S$ calculated ($M+H^+$) 486, found 486.



***N*-Bus-D-Ala-N^6-Boc-L-Lys-OMe (1.39a) :** The general procedure was followed using N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **34** (297 mg, 1.0 mmol), *tert*-butylsulfonyl-D-alanine **1.38a** (209 mg, 1.0 mmol), EDC (288 mg, 1.5 mmol), 2,6-lutidine (0.35 mL, 3.0 mmol), HOBt (202 mg, 1.5 mmol), DCM (16 mL) and DMF (4 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 1:1) to afford *tert*-butylsulfonyl-D-alanine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.39b** (415 mg, 92%), as a colorless solid, m.p. 92-94 ^{o}C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 5.83 (d, 1H, *J*=8.5 Hz), 5.03 (s, 1H), 4.39 (br, d, 1H), 3.98 (br, t, 1H), 3.56 (s, 3H), 2.92 (br, m, 2H), 1.71 (br, m, 1H), 1.58 (br, m, 1H), 1.31 (br, m, 3H), 1.30 (br, m, 3H), 1.27 (s, 9H), 1.23 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.8, 172.4, 156.0, 78.6, 59.7, 53.6, 52.1, 51.9, 39.8, 31.2, 29.0, 28.2, 23.9, 22.2, 20.2; $[\alpha]_D$ +24.6 (c 1.0, $CHCl_3$); ESI/MS for

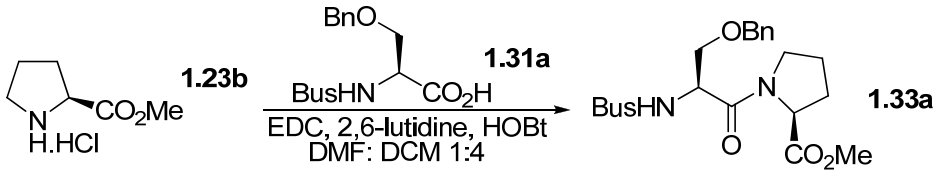
$C_{19}H_{37}N_3O_7S$ calculated ($M+H^+$) 452, found 452.

Cleavage Boc of *N*-Bus-D-Ala-*N*^{6}-Boc-L-Lys-OMe (1.46d): To a solution of *tert*-butylsulfonyl-D-alanine-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.39a** (45 mg, 0.1 mmol) in DCM (5 mL), trifluoroacetic acid (0.5 mL, 6.7 mmol) was added dropwise and stirred at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). Solvent was evaporated to afford *tert*-butylsulfonyl-D-alanine-L-lysine methyl ester trifluoroacetic acid salt **1.46d** (42 mg, 89%), as a colorless oil: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 4.44 (dd, 1H, J=4.8 Hz, J=9.2 Hz), 4.05 (dd, 1H, J=7.2 Hz, J=14.4 Hz), 3.73 (s, 3H), 2.95-2.91 (t, 2H, J=7.5 Hz), 1.97-1.89 (m, 1H), 1.82-1.60 (m, 3H), 1.53-1.44 (m, 2H), 1.42 (d, 3H, J=7.2 Hz), 1.38 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.8, 174.8, 173.6, 61.1, 55.4, 53.3, 52.9, 40.6, 32.0, 28.0, 24.6, 23.6, 20.1; $[\alpha]_D$ +23.4 (c 1.0, MeOH); ESI/MS for $C_{16}H_{30}F_3N_3O_7S$ calculated (M-TFA+H^+) 352, found 352.

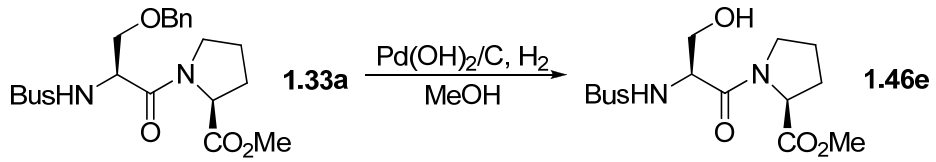


***N*-Bus-L-Ala-*N*^{6}-Boc-L-Lys-OMe (1.37a):** The general procedure was followed using *N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (30 mg, 0.1 mmol), *tert*-butylsulfonyl-L-alanine **1.36a** (21 mg, 0.1 mmol), EDC (29 mg, 0.15 mmol), HOBt (20 mg, 0.15 mmol), 2,6-lutidine (35 µL, 0.3 mmol), DCM (1.6 mL) and DMF (0.4 mL). The crude product (56 mg)

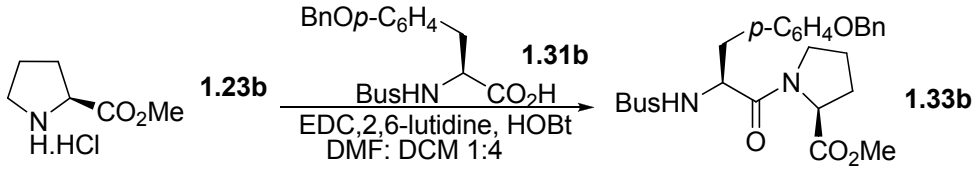
was checked directly by HPLC, and then purified by flash column chromatography (EtOAc/hexane 1:1) to afford *tert*-butylsulfonyl-L-alanine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.37a** (41 mg, 90%), as a colorless soft solid . ^{1}H NMR, (400 MHz, $CDCl_3$) δ 6.99 (d, 1H, *J*=9.4 Hz), 5.40 (d, 1H, *J*=9.4 Hz), 4.91 (br, t, 1H), 4.60-4.57 (br, m, 1H), 4.09-4.06 (br, t, 1H), 3.73 (s, 3H), 3.09-3.07 (br, m, 2H), 1.92-1.84 (br, m, 1H), 1.77-1.67 (br, m, 1H), 1.44 (br, d, 14H), 1.40 (br, s, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.7, 172.5, 156.4, 79.3, 60.2, 53.8, 52.6, 52.2, 40.1, 31.6, 29.4, 28.6, 24.3, 22.4, 20.0; $[\alpha]_D$ -18.0 (c 1.0, $CHCl_3$); HRMS for $C_{19}H_{37}N_3O_7S$ calculated ($M+Na^+$) 474.22444, found 474.22551.



***N*-Bus-*O*-Bn-L-Ser-L-Pro-OMe (1.33a):** The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-serine **1.31a** (158 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), L-proline methyl ester hydrochloride **1.23b** (83 mg, 0.5 mmol), HOBt (101 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), DCM (8 mL), DMF (2 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:7) to afford *tert*-butylsulfonyl-*O*-benzyl-L-serine-L-proline methyl ester **1.33a** (184 mg, 86%), as a colorless liquid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.31-7.21 (m, 5H), 5.49-5.45 (m, 1H), 4.58-4.39 (m, 4H), 3.68-3.55 (m, 6H), 2.32 (s, 1H), 2.20-2.04 (m, 1H), 1.99-1.86 (m, 3H),1.81-1.71 (m, 1H), 1.33 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.1, 169.5, 137.7, 128.3, 127.6, 127.5, 73.5, 71.1, 60.0, 59.0, 55.4, 52.2, 46.9, 29.0, 24.8, 24.1; $[\alpha]_D$ -43.3 (c 1.0, $CHCl_3$); HRMS for $C_{20}H_{30}N_2O_6S$ calculated ($M+H^+$) 427.18973, found 427.19101.

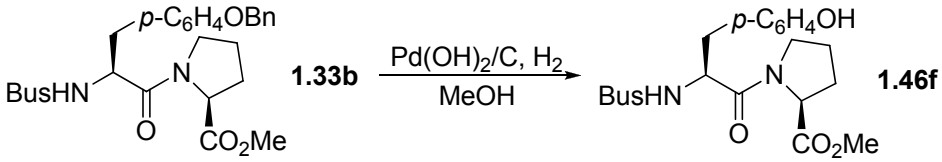


Cleavage Bn of *N*-Bus-*O*-Bn-L-Ser-L-Pro-OMe (1.46e): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-serine-L-proline methyl ester **1.33a** (43 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (3 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 3 h (TLC monitoring, EtOAc/ hexane 1:1). The reaction mixture was filtered over celite, and the filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-serine-L-proline methyl ester **1.46e** (30 mg, 89%), as a colorless liquid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.45 (d, 1H, *J*=9.4 Hz), 4.62-4.59 (m, 1H), 4.42-4.36 (m, 1H), 3.86-3.365 (m, 7H), 2.28-2.19 (m, 1H), 2.05-1.94 (m, 3H), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.2, 170.5, 64.5, 60.2, 59.2, 56.7, 52.9, 47.3, 29.0, 24.9, 24.2; $[\alpha]_D$ -67.0 (c 1.0, $CHCl_3$); ESI/MS for $C_{13}H_{24}N_2O_6S$ calculated ($M+H^+$) 337, found 337.

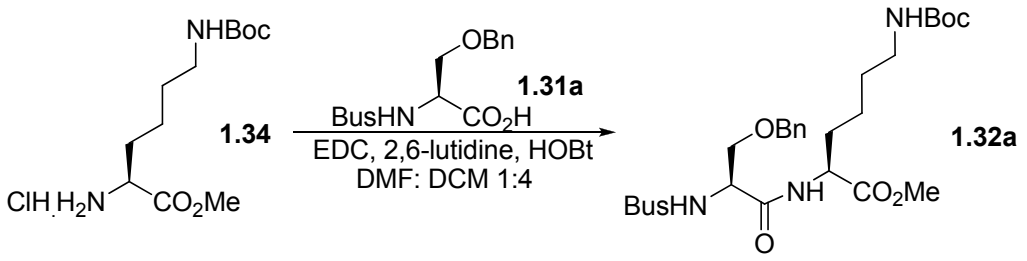


***N*-Bus-*O*-Bn-L-Tyr-L-Pro-OMe (1.33b):** The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine **1.31b** (196 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), L-proline methyl ester hydrochloride **1.23b** (83 mg, 0.5 mmol), HOBt (101 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), DCM (8 mL) and DMF (2 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:7) to afford *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-L-proline methyl ester **1.33b** (223 mg, 89%), as a colorless solid, m.p. 45-47 ^{o}C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.39-7.27 (m, 5H), 7.22-7.10 (m, 2H), 6.90-6.88 (m, 2H), 5.63 (d, 1H, *J*=9.9 Hz), 5.02 (s, 2H),

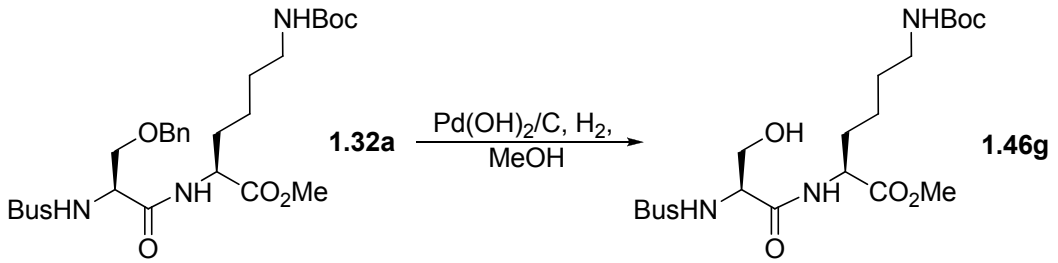
3.66-3.64 (m, 4H), 3.33-3.30 (m, 1H), 3.06-3.01 (m, 1H), 2.89-2.83 (m, 1H), 2.17-2.12 (m, 1H),1.93-1.92 (m, 1H), 1,27-1.19 (m, 2H), 1.13 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.2, 171.1, 157.6, 137.0, 131.0, 128.8, 128.4, 127.8, 127.3, 114.8, 69.8, 59.7, 58.8, 57.6, 52.1, 46.6, 38.5, 28.9, 24.7, 23.6; $[\alpha]_D$ -43.3 (c 1.0, $CHCl_3$); HRMS for $C_{26}H_{34}N_2O_6S$ calculated ($M+H^+$) 503.22103, found 503.22170.



Cleavage Bn of *N*-Bus-*O*-Bn-L-Tyr-L-Pro-OMe (1.46f): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-L-proline methyl ester **1.33b** (50 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (3 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 3 h (TLC monitoring, EtOAc/ hexane 1:1). The reaction mixture was filtered over celite, and the filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-tyrosine-L-proline methyl ester **1.46f** (37 mg, 89%) as a colorless solid, m.p. 70-72 ^{o}C: ^{1}H NMR, (400MHz, $CDCl_3$) δ 7.09 (d, 2H, *J*=8.1 Hz), 6.75 (d, 2H, *J*=8.1 Hz), 5.09 (d, 1H, *J*=9.8 Hz), 4.58-4.55 (m, 1H), 4.30-4.24 (m, 1H), 3.72-3.63 (m, 4H), 3.40-3.37 (m, 1H), 3.01 (dd, 1H, *J*=5.8 Hz, *J*=13.8 Hz), 2.82 (dd, 1H, *J*=7.3 Hz, *J*=14.0 Hz), 2.39 (s, 1H), 2.21-1.95 (m, 2H), 1.33 (d, 2H, *J*=16.4 Hz), 1.20 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.5, 171.4, 155.6, 131.2, 127.3, 115.6, 60.1, 59.2, 57.5, 52.5, 46.9, 38.9, 29.1, 25.0, 24.1, 23.9; $[\alpha]_D$ -38.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{19}H_{28}N_2O_6S$ calculated ($M+H^+$) 413, found 413.

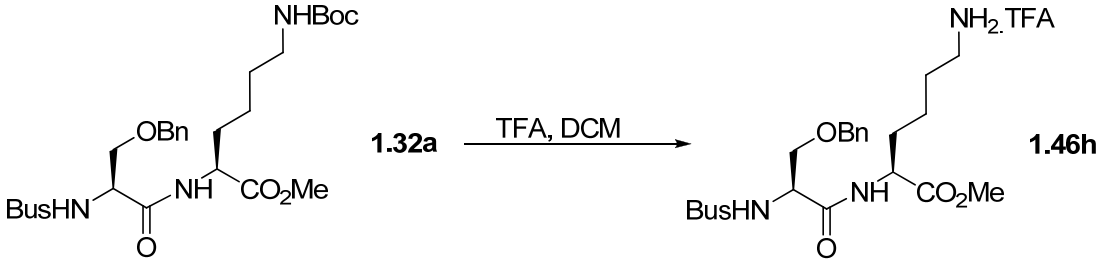


***N*-Bus-*O*-Bn-L-Ser-N^6-Boc-L-Lys-OMe (1.32a):** The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-serine **1.31a** (158 mg, 0.5 mmol), *N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (148 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), HOBt (101 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), DCM (8 mL) and DMF (2 mL). The product was purified by flash column chromatography (EtOAc/hexane 3:7) to afford *tert*-butylsulfonyl-*O*-benzyl-L-serine-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.32a** (238 mg, 85%), as a colorless thick liquid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.30-7.21 (m, 5H), 5.44 (dd, 1H, *J*=8.2 Hz, *J*= 26.5 Hz), 4.76-4.74 (m, 1H), 4.55-4.45 (m, 3H), 4.16-4.13 (m, 1H), 3.88-3.82 (m, 1H), 3.65-3.62 (m, 3H), 2.97-2.95 (m, 2H), 1.80-1.76 (m, 1H), 1.62-1.59 (m, 1H), 1.37 (s, 11H), 1.34 (s, 9H), 1.24-1.20 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.2, 169.7, 156.0, 137.2, 128.4, 127.9, 127.7, 78.9, 73.5, 71.1, 70.8, 60.4, 52.3, 52.2, 40.0, 31.8, 29.2, 28.4, 24.1, 22.3; $[\alpha]_D$ +6.3 (c 1.0, $CHCl_3$); HRMS for $C_{26}H_{43}N_3O_8S$ calculated ($M+Na^+$) 580.26631, found 580.26710.



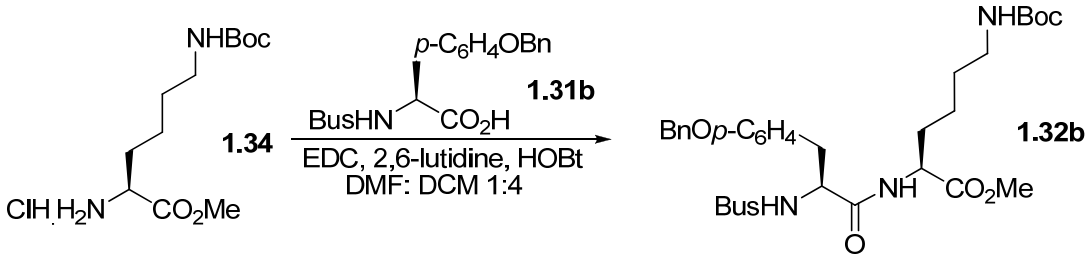
Cleavage Bn of *N*-Bus-*O*-Bn-L-Ser-*N*^{6}-Boc-L-Lys-OMe (1.46g): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-serine-*N*^{6}-*tert*-butyloxycarbonyl-L-

lysine methyl ester **1.32a** (56 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (3 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 1 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite, and filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-serine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.46g** (40 mg, 86%), as a colorless thick liquid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.36 (d, 1H, *J*=7.5 Hz), 5.84 (d, 1H, *J*=8.7 Hz), 5.74 (d, 1H, *J*=8.7 Hz), 4.89 (br, m, 1H), 4.62-4.55 (br, m, 1H), 4,11 (br, m, 1H), 4.01 (br, m, 1H), 3.91 (br, m, 1H), 3.80 (br, m, 1H), 3.73 (s, 3H), 3.07 (d, 1H, *J*=5.3 Hz), 1.93-1.85 (br, m, 1H), 1.78-1.68 (br, m, 1H),1.57-1.34 (br, m, 20H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.9, 170.9, 156.4, 79.4, 64.1, 60.5, 59.4, 52.7, 52.5, 52.2, 40.2, 31.4, 29.4, 28.6, 24.3, 22.5; $[\alpha]_D$ -5.5 (c 1.0, $CHCl_3$); ESI/MS for $C_{19}H_{37}N_3O_8S$ calculated ($M+H^+$) 468, found 468.

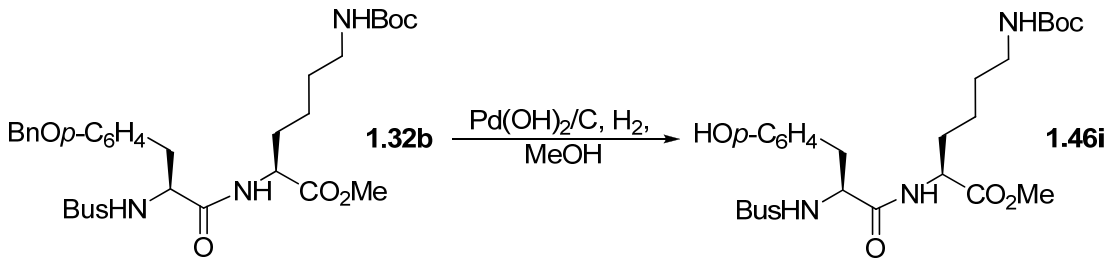


Cleavage Boc of *N*-Bus-*O*-Bn-L-Tyr-N^6-Boc-L-Lys-OMe (1.46h): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-serine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.32a** (56 mg, 0.1 mmol) in DCM (5 mL), trifluoroacetic acid (0.5 mL, 6.7 mmol) was added dropwise and stirred at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). Then the solvent was evaporated to afford *tert*-butylsulfonyl-*O*-benzyl-L-serine-L-lysine methyl ester trifluoroacetic acid salt **1.46h** (53 mg, 92%), as a colorless solid, m.p. 37-39 ^{o}C: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.35-7.29 (m, 5H), 4.57 (s, 2H), 4.53-4.45 (m, 1H), 4.24-4.23 (br, t, 1H), 3.73-3.69 (m, 4H), 2.90-2.79 (m, 2H), 1.95-1.86 (br, m,

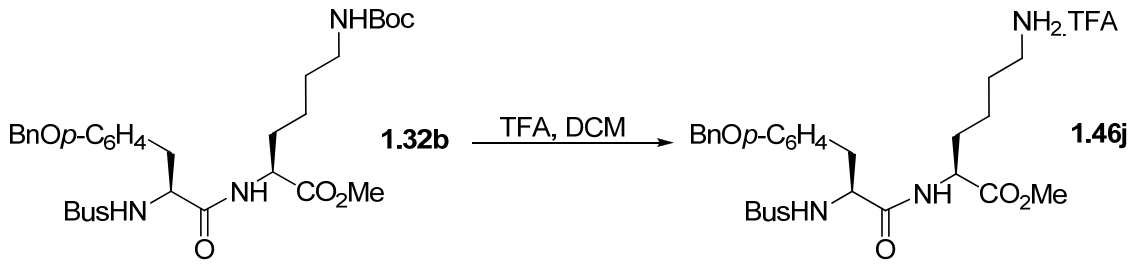
1H), 1.76-1.59 (m, 3H),1.47-1.41 (m, 2H), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 173.6, 172.9, 139.3, 129.5, 129.0, 189.9, 74.4, 71.9, 61.2, 59.3, 53.2, 52.9, 40.6, 32.2, 27.9, 24.5, 23.5; $[\alpha]_D$ -11.6 (c 1.0, MeOH); ESI/MS for $C_{23}H_{36}F_3N_3O_8S$ calculated (M-TFA+H^+) 458, found 458.



***N*-Bus-*O*-Bn-L-Tyr-N^6-Boc-L-Lys-OMe (1.32b):** The general procedure was followed using *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine **1.31b** (196 mg, 0.5 mmol), N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (148 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), HOBt (101 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), DCM (8 mL) and DMF (2 mL). The product was purified by flash column chromatography (EtOAc/hexane 3:7) to afford *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.32b** (295 mg, 93%), as a colorless solid, m.p. 160-161 ^{o}C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.42-7.31 (m, 5H), 7.16 (d, 2H, *J*=8.5 Hz), 6.90 (d, 2H, *J*=8.6 Hz), 6.75 (d, 1H, *J*=8.2 Hz), 5.04 (s, 3H), 4.82 (m, 1H), 4.66-4.57 (m, 1H), 4.07-4.03 (m, 1H), 3.70 (m, 3H), 3.16-2.87 (m, 4H), 1.84-1.81 (m, 1H), 1.70-1.64 (m, 1H), 1.48-1.42 (m, 11H), 1.29-1.25 (m, 2H), 1.11 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.4, 171.2, 158.0, 156.2, 137.0, 131.0, 128.7, 128.5, 128.1, 127.5, 115.2, 79.1, 70.1, 60.3, 59.8, 52.5, 52.2, 40.2, 38.5, 31.9, 29.4, 28.6, 23.9, 22.3; $[\alpha]_D$ -7.0 (c 1.0, $CHCl_3$); HRMS for $C_{32}H_{47}N_3O_8S$ calculated (M+Na^+) 656.29761, found 656.29837.

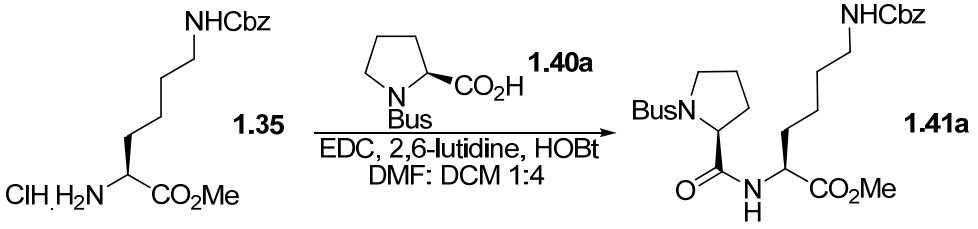


Cleavage Bn of *N*-Bus-*O*-Bn-L-Tyr-*N*^{6}-Boc-L-Lys-OMe (1.46i): To a solution of *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.32b** (63 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (3 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 1 h (TLC monitoring, EtOAc/hexane 1:1). The reaction mixture was filtered over celite, filtrate was concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-tyrosine-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.46i** (47 mg, 87%), as a white powder, m.p. 71-73 ^{o}C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.07 (d, 2H, *J*=7.8 Hz), 6.93 (d, 1H, *J*=7.1 Hz), 6.88 (d, 2H, *J*=7.8 Hz), 4.89 (br, r, 1H), 4.62 (br, d, 1H), 4.49 (d, 1H, *J*=10.2 Hz), 4.25-4.24 (br, m, 1H), 3.73 (s, 3H), 3.50 (br, dd, 1H), 3.16-3.08 (m, 1H), 1.92-1.85 (m, 1H), 1.73-1.62 (m, 1H), 1.49-1.42 (m, 11H), 1.42-1.30 (m, 9H), 0.92-0.83 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.1, 170.2, 156.8, 156.7, 131.1, 125.6, 116.1, 80.4, 60.6, 59.1, 52.8, 52.4, 40.8, 38.3, 31.7, 30.7, 28.6, 24.2, 21.7; $[\alpha]_D$ -9.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{25}H_{41}N_3O_8S$ calculated ($M+H^+$) 544, found 544.



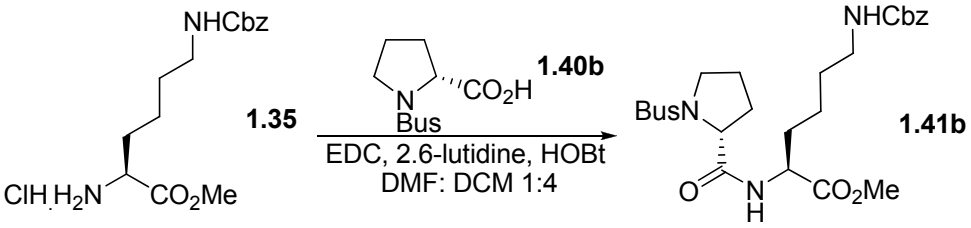
Cleavage Boc of *N*-Bus-*O*-Bn-L-Tyr-*N*^{6}-Boc-L-Lys-OMe (1.46j): To a

solution of *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.32b** (63 mg, 0.1 mmol) in DCM (5 mL), trifluoroacetic acid (0.5 mL, 6.7 mmol) was added dropwise and stirred at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:1). Then the solvent was evaporated to afford *tert*-butylsulfonyl-*O*-benzyl-L-tyrosine-L-lysine methyl ester trifluoroacetic acid salt **1.46j** (77 mg, 94%), as a colorless solid, m.p. 89-91 °C: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.43-7.27 (m, 5H), 7.24 (d, 2H, *J*=8.6 Hz), 6.93 (d, 2H, *J*=8.6 Hz), 6.75 (d, 1H, *J*=8.2 Hz), 5.07 (s, 2H), 4.53 (dd, 1H, *J*=4.5 Hz, *J*=10.0 Hz), 4.01 (dd, 1H, *J*=4.6 Hz, *J*=10.4 Hz), 3.70 (m, 3H), 3.01 (dd, 1H, *J*=4.6 Hz, *J*=13.8 Hz), 2.94-2.91 (m, 2H), 2.77-2.71 (m, 1H), 1.98-1.89 (m, 1H), 1.77-1.60 (m, 3H), 1.54-1.46 (m,2H), 1.37-1.28 (m, 2H), 1.03 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.3, 173.7, 159.2, 138.9, 132.0, 130.9, 129.6, 128.9, 128.6, 116.1,70.9, 61.7, 60.9, 52.9, 52.8, 40.6, 39.2, 32.1, 27.7, 24.1, 23.4; $[\alpha]_D$ -28.4 (c 1.0, MeOH); ESI/MS for $C_{29}H_{40}F_3N_3O_8S$ calculated (M-TFA+H^+) 534, found 534.

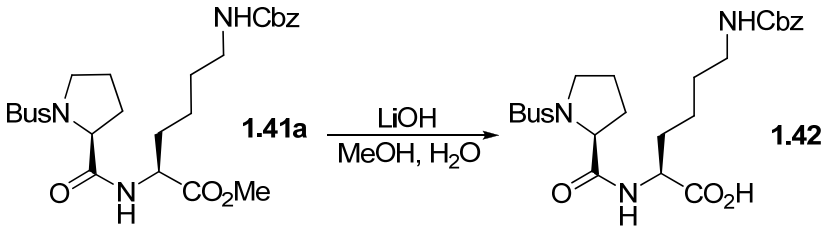


***N*-Bus-L-Pro-N^6-Cbz-L-Lys-OMe (1.41a):** The general procedure was followed using N^6-benzyloxycarbonyl-L-lysine methyl ester hydrochloride **1.35** (332 mg, 1.0 mmol) with *tert*-butylsulfonyl-L-proline **1.40a** (238 mg, 1.0 mmol), EDC (288 mg, 1.5 mmol), HOBt (203 mg, 1.5 mmol), 2,6-lutidine (0.35 mL, 3.0 mmol), DCM (8 mL) and DMF (2 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 2:3) to afford *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine methyl ester **1.41a** (469 mg, 92%), as a colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.38-7.31 (m, 5H), 6.80 (d, 1H,

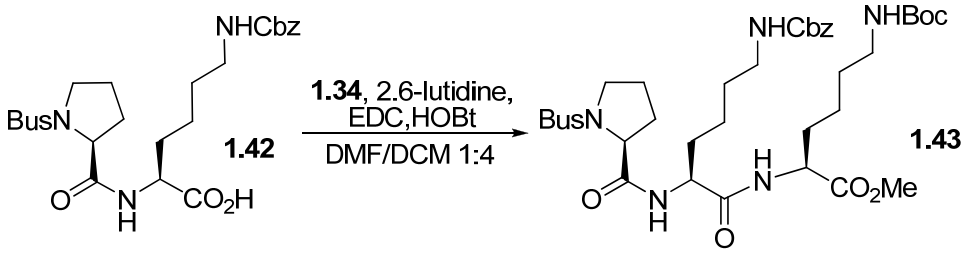
J=7.2 Hz), 5.08 (m, 2H), 4.99 (br, t, 1H), 4.58-4.53 (m, 1H), 4.50 (dd, 1H, *J*=3.9Hz, *J*=7.9 Hz), 3.76-3.70 (m, 4H), 3.44-3.38 (m, 1H), 3.25-3.12 (m, 2H), 2.23-2.08 (m, 2H), 2.05-1.85 (m, 3H), 1.78-1.69 (m, 1H), 1.58-1.51 (m, 2H), 1.41-1.33 (m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.6, 171.9, 156.8, 136.8, 128.7, 128.34, 128.28, 66.8, 62.9, 61.9, 52.7, 52.3, 50.8, 40.7, 32.0, 30.6, 29.4, 25.6, 24.8, 22.4; $[\alpha]_D$ -30.6 (c 1.0, $CHCl_3$); HRMS for $C_{24}H_{37}N_3O_7S$ calculated ($M+H^+$) 512.24250, found 512.24303.



***N*-Bus-D-Pro-*N*^{6}-Cbz-L-Lys-OMe (1.41b):** The general procedure was followed using *N*^{6}-benzyloxy-carbonyl-L-lysine methyl ester hydrochloride **1.35** (166 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), *tert*-butylsulfonyl-D-proline **1.40b** (120 mg, 0.5 mmol), HOBt (101 mg, 0.75 mmol), DCM (8 mL) and DMF (2 mL). The crude product was purified by flash column chromatography (EtOAc/hexane 3:2) to afford *tert*-butylsulfonyl-D-proline-*N*^{6}-benzyloxycarbonyl-L-lysine methyl ester **1.41b** (236 mg, 92%), as a colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.28-7.21 (m, 5H), 6.96 (d, 1H, *J*=8.0 Hz), 5.36 (br, t, 1H), 5.01 (s, 2H), 4.52-4.44 (m, 2H), 3.65-3.59 (m, 4H), 3.47-3.41 (m, 1H), 3.15-3.07 (m, 2H), 2.11-2.02 (m, 2H), 1.98-1.82 (m, 3H), 1.69-1.61 (m, 1H), 1.51-1.41 (m, 2H), 1.32 (m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.4, 171.9, 156.6, 136.7, 128.4, 128.0, 127.9, 66.4, 62.8, 62.0, 52.3, 51.8, 50.4, 40.3, 31.5, 30.8, 28.9, 25.0, 24.7, 22.1; $[\alpha]_D$ +54.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{24}H_{37}N_3O_7S$ calculated ($M+H^+$) 512, found 512.

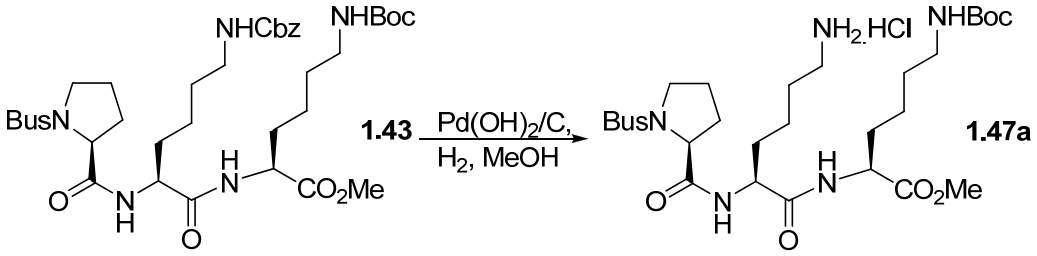


Hydrolyzation of *N*-Bus-L-Pro-*N*^{6}-Cbz-L-Lys-OMe (1.42): The general procedure was followed using *tert*-butylsulfonyl-L-proline-*N*^{6}-benzyloxy-carbonyl-L-lysine methyl ester **1.41a** (409 mg, 0.8 mmol), LiOH×H_2O (168 mg, 4.0 mmol), MeOH (24 mL) and H_2O (8 mL). The organic extract was combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford *tert*-butylsulfonyl-L-proline-*N*^{6}-benzyloxycarbonyl-L-lysine **1.42** (371 mg, 93%), as a colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.37-7.30 (m, 5H), 7.11 (d, 1H, *J*=7.2 Hz), 5.27-5.25 (br, t, 1H), 5.15-5.06 (m, 2H), 4.54-4.50 (m, 2H), 3.73-3.66 (m, 1H), 3.45-3.39 (m, 1H), 3.24-3.15 (m, 2H), 2.16-2.11 (m, 2H), 2.04-1.97 (m, 1H), 1.95-1.86 (m, 2H), 1.80-1.71 (m, 1H), 1.55-1.49 (m, 2H), 1.37 (s, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 174.9, 172.7, 157.0, 136.6, 128.7, 128.3, 128.1, 66.9, 62.8, 61.9, 52.4, 50.9, 40.6, 31.5, 30.8, 29.4, 25.6, 24.7, 22.3; $[\alpha]_D$ -24.5 (c 1.0, $CHCl_3$); HRMS for $C_{23}H_{35}N_3O_7S$ calculated ($M+H^+$) 498.22685, found 498.22749.



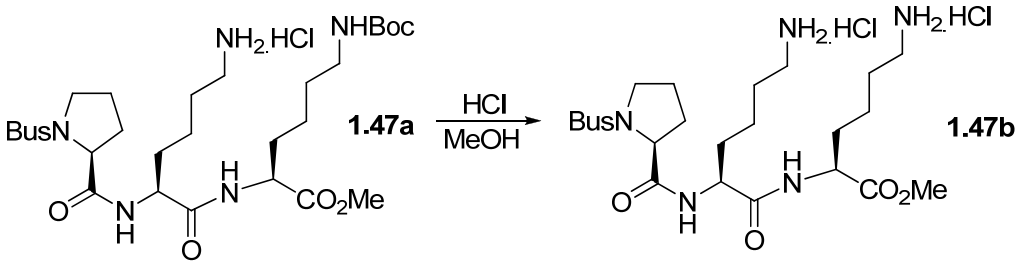
***N*-Bus-L-Pro-*N*^{6}-Cbz-L-Lys-*N*^{6}-Boc-L-Lys-OMe (1.43):** The general procedure was followed using *N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.34** (148 mg, 0.5 mmol), EDC (144 mg, 0.75 mmol), 2,6-lutidine (0.17 mL, 1.5 mmol), HOBt (101 mg, 0.75 mmol), *tert*-butyl-

sulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine **1.42** (249 mg, 0.5 mmol), DCM (8 mL) and DMF (2 mL). The product was purified by flash chromatography (EtOAc/hexane 7:3) to afford *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine-N^6-*tert*- butyloxycarbonyl-L-lysine methyl ester **1.43** (333 mg, 90%), as a colorless oil.^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.33-7.28 (m, 5H), 7.09 (br, s, 1H), 6.99 (br, s, 1H), 5.32 (br, s, 1H), 5.06 (s, 2H), 4.86 (br, s, 1H), 4.49 (br, m, 3H), 3.72-3.65 (br, m, 4H), 3.52-3.46 (br, m, 1H), 3.17 (br, d, 2H), 3.07 (br, d, 2H), 2.20-2.12 (m, 2H), 2.01-1.98 (m, 3H), 1.96-1.87 (m, 1H), 1.82-1.63 (m, 2H), 1.60-1.24 (m, 22H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.9, 172.3, 171.5, 156.8, 156,3, 136.8, 128.6, 128.2, 128.1, 79.2, 66.6, 63.2, 62.2, 53.0, 52.5, 52.4, 52.2, 50.8, 40.5, 40.2, 31.9, 31.6, 31.3, 31.1, 29.8, 29.5,29.3, 28.6, 25.4, 24.8, 22.8, 22.3; $[\alpha]_D$ -31.3 (c 1.0, $CHCl_3$); HRMS for $C_{35}H_{57}N_5O_{10}S$ calculated ($M+H^+$) 740.38989, found 740.39049.

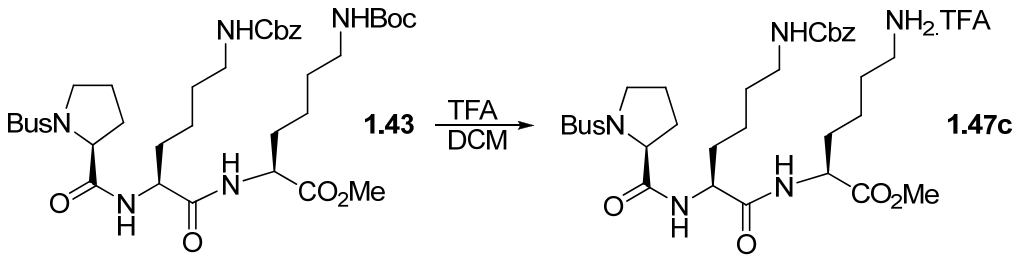


Cleavage Cbz of *N*-Bus-L-Pro-N^6-Cbz-L-Lys-N^6-Boc-L-Lys-OMe (1.47a): To a solution of *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **43** (73.9 mg, 0.1 mmol) in MeOH (5 mL), 20 wt.% $Pd(OH)_2$ over activated carbon (5 mg, cat.) was added and hydrogenated at 1 atm at R.T. for 1 h (TLC monitoring, EtOAc/hexane 4:1). The reaction mixture was filtered over celite and acidified with 1 M HCl aqueous (10 mL) carefully to pH=6∼7. The aqueous layer was frozen and lyophilized to afford *tert*-butylsulfonyl-L-proline-L-lysine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride salt **1.47a** (63 mg, 98%),

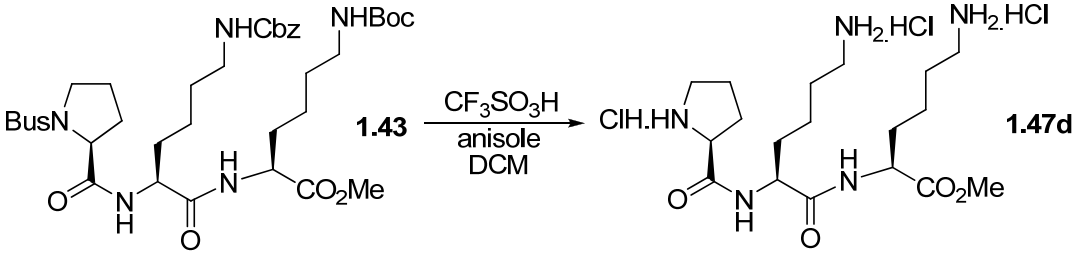
as a colorless oil. ^{1}H NMR, (400 MHz, MeOH-d_4) δ 4.51 (dd, 1H, J=3.2 Hz, J=8.2 Hz), 4.45-4.38 (m, 2H), 3.73 (s, 3H), 3.68-3.61 (m, 2H), 3.06-3.02 (t, 2H, J=7.5 Hz), 2.98-2.94 (t, 2H, J=7.5 Hz), 2.34-2.26 (m, 1H), 2.13-1.82 (m, 5H), 1.79-1.68 (m, 4H), 1.56-1.47 (m, 4H), 1.45 (s, 10H), 1.41 (s, 10H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.7, 174.2, 156.4, 80.0, 64.0, 63.0, 54.0, 52.9, 52.0, 41.2, 40.7, 32.6, 32.2, 30.6, 28.9, 28.1, 26.2, 25.0, 24.3, 23.5; $[\alpha]_D$ -52.2 (c 1.0, MeOH); ESI/MS for $C_{27}H_{52}ClN_5O_8S$ calculated (M-HCl+H$^+$) 606, found 606.



Cleavage Boc of *N*-Bus-L-Pro-L-Lys-*N*^{6}-Boc-L-Lys-OMe (1.47b): To a solution of *tert*-butylsulfonyl-L-proline-L-lysine-*N*^{6}-*tert*-butyloxycarbonyl-L-lysine methyl ester hydrochloride **1.47a** (58 mg, 0.09 mmol) in MeOH (1 mL), 1.25 M HCl in MeOH solution 1 mL was added and stirred at R.T. for 4 h. Solvent was evaporated to afford *tert*-butylsulfonyl-L-proline-L-lysine-L-lysine methyl ester hydrochloride salt **1.47b** (51 mg, 99%) as a colorless oil. ^{1}H NMR, (400 MHz, MeOH-d_4) δ 4.53-4.50 (m, 1H), 4.44-4.36 (m, 2H), 3.74 (s, 3H), 3.65-3.60 (m, 2H), 2.99-2.93 (m, 4H), 2.36-2.27 (m, 1H), 2.08-2.93 (m, 2H), 1.90-1.89 (m, 3H), 1.81-1.72 (m, 6H), 1.57-1.48 (m, 4H), 1.41 (s, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.7, 174.2, 173.9, 64.0, 63.1, 54.5, 53.6, 53.0, 52.0, 40.7, 32.7, 32.3, 31.9, 28.1, 28.0, 26.2, 25.1, 23.9, 23.7; $[\alpha]_D$ -45.6 (c 1.0, MeOH); ESI/MS for $C_{22}H_{45}Cl_2N_5O_6S$ calculated (M-2HCl+H$^+$) 506, found 506.

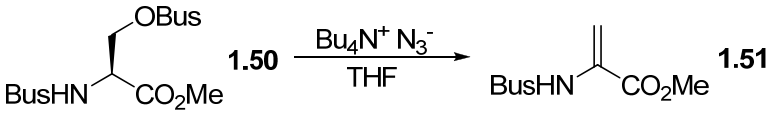


Cleavage Boc of *N*-Bus-L-Pro-N^6-Cbz-L-Lys-N^6-Boc-L-Lys-OMe (1.47c): To a solution of *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.43** (74 mg, 0.1 mmol) in DCM (5 mL), trifluoroacetic acid (0.5 mL, 6.7 mmol) was added dropwise and stirred at R.T. for 4 h (TLC monitoring, 100% EtOAc). Solvent was evaporated to afford *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine-L-lysine methyl ester trifluoroacetic acid salt **1.47c** (70 mg, 93%), as a colorless oil.^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.36-7.30 (m, 5H), 5.07 (s, 2H), 4.50 (dd, 1H, J=2.8 Hz, J=8.2 Hz), 4.47-4.39 (m, 1H), 4.30-4.24 (m, 1H), 3.71(s, 3H), 3.68-3.62 (m, 1H), 3.59-3.53 (m, 1H), 3.16-3.12 (m, 2H), 2.97-2.93 (t, 2H, J=7.5 Hz), 2.33-2.21 (m, 1H), 2.08-1.83 (m, 5H), 1.80-1.65 (m, 4H), 1.62-1.42 (m, 6H), 1.39 (m, 9H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.7, 175.1, 174.5, 159.0, 138.5, 129.6, 129.1, 128.9, 67.5, 63.9, 62.9, 55.1, 53.3, 52.9, 52.0, 41.6, 40.6, 32.5, 32.4, 32.1, 30.5, 28.0, 26.3, 25.0, 24.2, 23.9; $[\alpha]_D$ -36.4 (c 1.0, MeOH); ESI/MS for $C_{32}H_{50}F_3N_5O_{10}S$ calculated (M-TFA $+H^+$) 640, found 640.



Cleavage the whole protecting groups of *N*-Bus-L-Pro-N^6-Cbz-L-

Lys-N^6-Boc-L-Lys-OMe (1.47d): To a solution of *tert*-butylsulfonyl-L-proline-N^6-benzyloxycarbonyl-L-lysine-N^6-*tert*-butyloxycarbonyl-L-lysine methyl ester **1.43** (74 mg, 0.1mmol) and anisole (0.11 mL, 1.0 mmol) in DCM (1.5 mL) was slowly added trifluoromethanesulfonic acid (0.2 N in DCM, 1.5 mL) at 0 °C. The solution was stirred at 0 °C for 48 h (TLC monitoring, 100% EtOAc), 6 mL H_2O and 4 mL DCM was added. The aqueous layer was neutralized with DOWEX anion exchange resin at 0 °C until pH=9.5. Then 6 mL MeOH was added and filtered. At last the solution was combined and acidified with 1 M HCl aqueous (3 mL). The acid layer was frozen and lyophilized to afford L-proline-L-lysine-L-lysine methyl ester hydrochloride salt **1.47d** (41 mg, 82%), as a colorless oil.^{1}H NMR, (400 MHz, MeOH-d_4) δ 4.45-4.37 (m, 3H), 3.73 (s, 3H), 3.45-3.33 (m, 2H), 3.01-2.94 (br, m, 4H), 2.57-2.47 (m, 1H), 2.17-2.01 (m, 3H), 1.99-1.87 (m, 2H), 1.84-1.67 (m, 6H), 1.62-1.46 (m, 4H); ESI/MS for $C_{18}H_{38}Cl_3N_5O_4$ calculated (M-3HCl+H^+) 386, found 386.



Elimination OBn of *N*-Bus-*O*-Bus-L-Ser-OMe (1.51): A solution of *tert*-butylsulfonyl-*O*-*tert*-butylsulfonyl-L-serine methyl ester **1.50** (54 mg, 0.15 mmol) in 2 mL of THF was treated with Bu_4N^+ N_3^- (102 mg, 0.36 mmol) at R.T.. The solution was stirred at R.T. for 2 h (TLC monitoring, EtOAc/hexane 1:4). Solvent was evaporated, and the residue was purified by flash column chromatography (EtOAc/hexane 1:4) to afford *tert*-butylsulfonyl-2-amino-acrylate methyl ester **1.51** (14 mg, 62%), as a colorless liquid: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 6.62 (s, 1H), 5.84 (s, 1H), 5.69 (s, 1H), 3.86 (s, 3H), 1.43 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 164.3, 132.5, 106.8, 62.5, 53.5, 29.8, 24.6; HRMS for $C_8H_{15}NO_4S$ calculated (M+Na^+) 244.06140, found 244.06116.

HPLC method of Mosher ester: 1) alanine Mosher ester: Column: AD-RH 150*4.6 mm. Comment: 0.1% TFA and 25% MeCN in H_2O solution. Flow: 0.5 mL/min. 2) phenylalanine Mosher ester: Column: AD-RH 150*4.6 mm. Comment: 0.1% TFA and 45% MeCN in H_2O solution. Flow: 0.5 mL/min.

1-4 References:

1. A. I. Llobet, M. Alvarez, and F. Albericio, *Chem. Rev.,* **2009**, *109*, 2455.
2. P. Ribiere, V. Declerck, J. Martinez, and F. Lamaty, *Chem. Rev.,* **2006**, *106*, 2249.
3. G. Theodoridis, *Tetrahedron*, **2000**, *56*, 2339.
4. P. J. Kocienski, *Protecting Groups*, G. Thieme, New York, 2000.
5. T. W. Greene, P. G. M. Wuts, *Protecting Groups in Organic Synthesis*, J. Wiley & Sons., New York, 1999.
6. M. Bodanszky, *Principles of Peptide Chemistry,* **1984**, 99.
7. D. S. Tarbell, Y. Yamamoto, and B. M. Pope, *Proc. Natl. Acad. Sci.,* **1972**, *69*, 730.
8. E. M. Khalil, N. L. Subasinghe, and R. L. Johnson, *Tetrahedron Lett.,* **1996**, *37*, 3441.
9. G. L. Stahl, R. Walter and C. W. Smith, *J. Org. Chem.,* **1978**, *43*, 2285.
10. A. Nudelman, Y. Bechor, E. Falb, B. Fischer, and B. A. Wexler, *Synth. Commun.,* **1998**, *28*, 471.
11. R. A. Houghten, A. Beckman, and J. M. Ostresh, *Int. J. Pept. Protein Res.,* **1986**, *27*, 653.
12. J. R. Hwu, M. L. Jain, S. C. Tsay, and G. H. Hakimelahi, *Tetrahedron Lett.,* **1996**, *37*, 2035.
13. W. Graf, O. Keller, G. Wersin, and E. Wuensch, *Peprides,* **1986**: *Proc. 19th Eur. Pept. Symp.,* **1987**, 73.

14. G. Sennyey, G. Barcelo, and J. P. Senet, *Tetrahedron Lett.,* **1986**, *27*, 5375.

15. M. Bergmann, and L. Zervas, *Ber.,* **1932**, *65*, 1192.

16. D. Ben-Ishai and A. Berger, *J. Org. Chem.,* **1952**, *17*, 1564.

17. A. R. Mitchell and R. B. Merrifield, *J. Org. Chem.,* **1976**, *41*, 2015.

18. H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc. Chem. Commun.,* **1974**, 107.

19. S. R. Angle and D. O. Arnaiz, *Tetrahedron Lett.,* **1989**, *30*, 515.

20. I. Schon, T. Szirtes, T. Uberhardt, A. Rill, A. Csehi, and B. Hegedus, *Int. J. Pept. Protein Res.*, **1983**, *22*, 92.

21. L. A. Carpino, D. Sadat-Aalaee, and M. Beyermann, *J. Org. Chem.,* **1990**, *55*, 1673.

22. L. A. Carpino and G. Y. Han, *J. Org. Chem.,* **1972**, *37*, 3404.

23. F. M. F. Chen and N. L. Benoiton, *Can. J. Chem.,* **1987**, *65*, 1224.

24. R. C. de L. Milton, E. Becker, S. C. F. Milton, J. E. H. Baxter, and J. F. Elsworth, *Int. J. Pept. Protein Res.,* **1987**, *30*, 431.

25. L. Lapatsanis, G. Milias, K. Froussios, and M, Kolovos, *Synthesis,* **1983**, 671.

26. E. Atherton and R. C. Sheppard, *The Peptides,* **1987**, *9*, 1.

27. E. Atherton, C. Bury, R. C. Sheppard, and B. J. Williams, *Tetrahedron Lett.,* **1979**, *20*, 3041.

28. T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis,* J. Wiley & Sons., New York, **1991**, 379.

29. E. Vedejs, S. Lin, A. Klapars, and J. Wang, *J. Am. Chem. Soc.,* **1996**, 118, 9796.

30. a) T. Fukuyama, C. K. Jow, and M. Cheung, *Tetrahedron Lett.,* **1995**, *36*, 6373; b)T. Fukuyama, M. Cheung, C. K. Jow, Y. Hidai, and T. Kan, *Tetrahedron Lett.,* **1997**, *38*, 5831.

31. P. Sun, S. M. Weinreb, and M. Shang, *J. Org. Chem.,* **1997**, *62,* 8604.

32. J. F. King, J. Y. L. Lam, and V. Dave, *J. Org. Chem.,* **1995**, *60*, 2831.

33. W. Oppolzer, M. Wills, C. Starkemann, and G. Bernardinelli, *Tetrahedron Lett.,* **1990**, *31*, 4117.

34. V. Martinez-Merino, M. J. Gil, J. M. Zabalza, and A. Gonzalez, *Heterocycles,* **1995**, *41*, 2737.

35. J. F. King, J. Y. L. Lam, and V. Dave, *J. Org. Chem.,* **1995**, *60*, 2831.

36. A. V. Gontcharov, H. Liu, and K. B. Sharpless, *Org. Lett.,* **1999**, *1*, (5), 783.

37. S. Hanessian, J. R. Del Valle, Y. Xue, and N. Blomberg, *J. Am. Chem. Soc.,* **2006**, *128*, 10491.

38. D. M. Hodgson, P. G. Humphreys, S. M. Miles, C. A. J. Brierley, and J. G. Ward, *J. Org. Chem.,* **2007**, *72*, 10009.

39. M. Gunter and H. J. Gais, *J. Org. Chem.,* **2003**, *68*, 8037.

40. S. Koep, H. J. Gais, and G. Raabe, *J. Am. Chem. Soc.,* **2003**, *125*, 13243.

Chapter Two

Total Synthesis of a β-Methyl-D-leucinyl Unnatural Aeruginosin Hybrid

2-1 Introduction

2-1-1 Aeruginosin family

The aeruginosin family is a relatively new class of potent serine protease inhibitors.[1] In the last two decades, more than 20 aeruginosins have been isolated from blue-green algae or marine sponges originating from geographically distinct aquatic sources.[1-7] Their structures have been determined by chemical, spectroscopic, and X-ray crystallographic methods.[2]

The aeruginosins are a new class of linear peptides, that for the most part contain a characteristic central core (P_2), which is a 6-mono- or 5,6-dihydroxy-2-carboxyoctahydroindole core unit (named as Choi or OH-Choi subunit respectively).[1,3] Almost all members are also composed of three subunits: an N-terminal hydroxy or acidic group (P_4), a bulky hydrophobic amino acid (P_3), and a C-terminal guanidine-containing group (P_1)(Figure 3)[4-6].

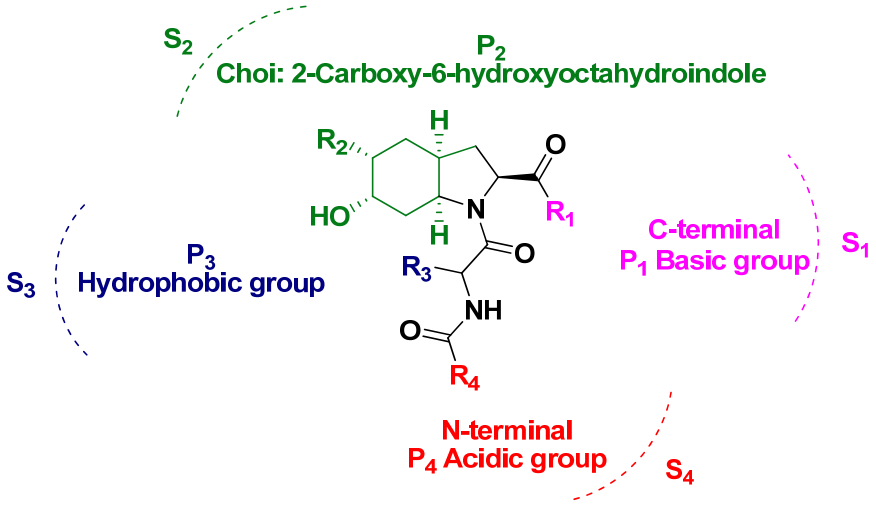


Figure 3. Generalized structure of the aeruginosins.

The members of the aeruginosin family exhibit very different degrees of inhibitory activity against serine proteases, and the profile of their activity has been explained by a high degree of pharmacophoric and structural homology

within the family. This array of structural and functional features specifically, a basic P_1 subunit with three hydrophobic P_2, P_3 and P_4 main pharmacophoric subunits, is responsible for their affinity to the catalytic and optimal binding pockets of trypsin, thrombin, and other serine proteases, involved in the blood coagulation cascade.

Due to the structural novelty and the biological activity of the aeruginosins, the family members have also garnered considerable attention from synthetic organic chemists. The 2-carboxyperhydroindole core has received the most attention with respect to conceptually diverse synthetic approaches. To date, the total syntheses of seven aeruginosins have been completed, and four of these involving revisions about their originally proposed structures.[2,4,7]

2-1-2 Biological activity

The biological activities associated with the aeruginosin family of natural products have mainly been in relation to inhibitors of serine proteases such as thrombin, Factor VIIa, and Factor Xa. [8-11] These proteases are implicated in either intrinsic or extrinsic pathways, and eventually leading to blood clots in humans. Some of the aeruginosins have also been evaluated against the cysteine protease papain or other enzymes.

2-1-2-1 Inhibition of coagulation cascade factors

Blood coagulation is a very complex process, but still an ordered event. The process involves two components. One is cellular such as blood platelets and leukocytes. The other one is called as proteinaceous, which includes the coagulation factors and cofactors. The basic events involve two steps: 1) primary hemostasis, which is the aggregation of platelet and formation of a primary platelet plug, 2) secondary hemostasis, where plasma coagulation

factors are activated and generate fibrin, then the fibrin intertwines and reinforces the aggregated platelets, to form a strong fibrin clot.[9-12] There are also two pathways, which are respectively defined as an intrinsic contact activation system and an extrinsic tissue factor system. Both pathways are responsible for the biochemical coagulation cascade, which leads to the generation of fibrin. The details are shown as Figure 4.[10]

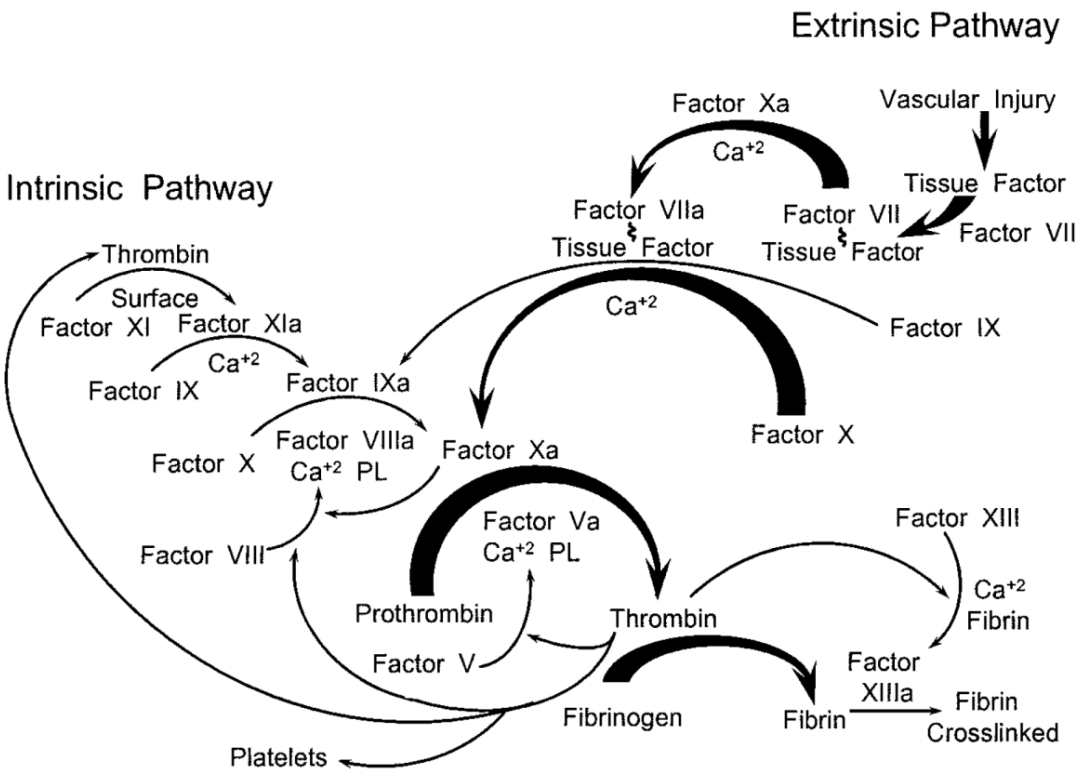


Figure 4. Schematic overview of the blood coagulation cascade.

Both pathways involve a stepwise activation of proteases, including thrombin. Thrombin is a multifunctional serine protease,[13] and responsible for the final cleavage of fibrinogen and the formation of fibrin. Along both pathways, the coagulation process is accelerated by the positive- feedback mechanisms. In recent years, the extrinsic system has been considered to be the major pathway to thrombinogenesis, triggered by tissue factors.[10]

Several of the aeruginosins exhibit high inhibitory potency against blood coagulation factors, which has made them attractive small-molecule targets in the search for new anticoagulants, although they may not be suitable as such for direct use in humans. Overall, the aeruginosins exhibit in vitro inhibitory activities against thrombin with micromolar to nanomolar IC_{50} values. Until now two compounds, chlorodysinosin A[5,14] and oscillarin[4] (thrombin IC_{50}=0.0057 μM and 0.028 μM, respectively), are the most potent members of the aeruginosin family, which have been isolated to date. Their structures and data are depicted in Figure 5. Total synthesis and structure confirmation of the chlorodysinosin A[5,14] and oscillarin[4] have been reported by Hanessian and coworkers. A total synthesis of dysinosin A[15] was also reported by the Hanessian group.

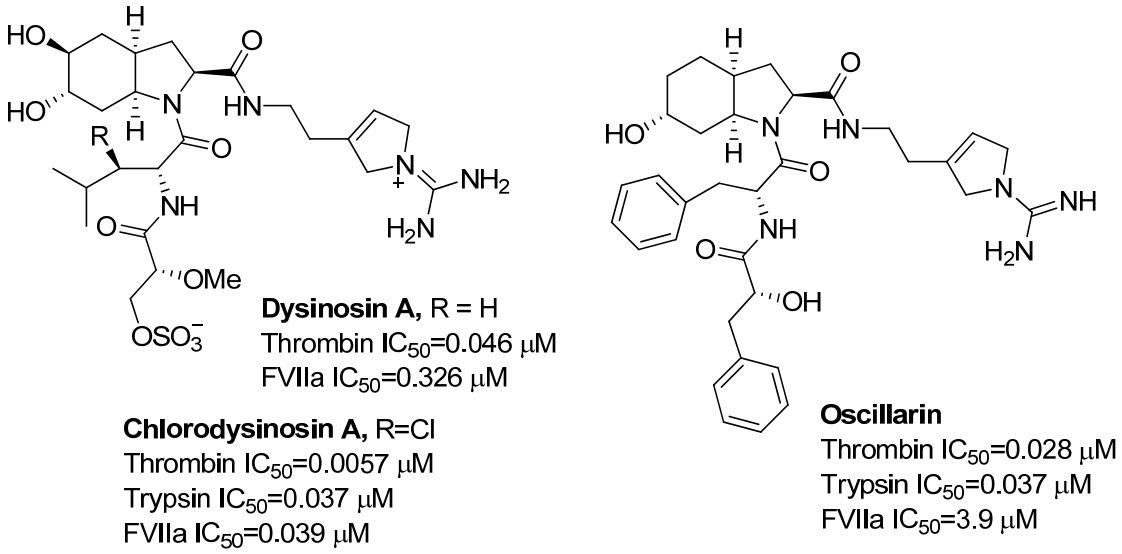


Figure 5. Structures and enzymatic activities of the natural aeruginosins dysinosin A, chlorodysinosin A, and oscillarin.

2-1-2-2 Inhibition of trypsin

Trypsin-like substrate specificity is responsible for the high inhibitory activities against serine proteases. These enzymes are involved in important

physiological processes, and their relevance in the complex blood coagulation cascade has been well established.[16,17] Trypsin-like proteases are known to cleave substrates, which contain positively charged amino acid residues in the P_1 position. Due to the fact that the aeruginosins contain a basic C-terminal arginine mimetic in the active sites of these proteases, some members of the family bind well. Trypsin is a digestive enzyme, which is frequently employed as a marker for inhibition of trypsin-like serine proteases. Ingeneral inhibition of trypsin should be avoided and selectivity is a prime consideration.

2-1-2-3 Other biological activities

The aeruginosins have also been evaluated for activity against other of enzyme targets. Several of the naturally occurring aeruginosins have been considered as inhibitors of plasmin, which is a trypsin-like serine protease involved in the fibrinolytic system. Because plasmin has also been implicated in angiogenesis and metastasis during the progression of cancer,[18] the aeruginosin family has been regarded as the potential agents for development as anticancer agents.

So far, no inhibitory activity of the aeruginosins has been found against the digestive serine proteases chymotrypsin and elastase. However, some of the aeruginosins have also been tested against the cysteine protease papain,[19] but only weak activity has been demonstrated. Only one aeruginosin has been evaluated against the metalloprotease neprolysine, but no inhibition was detected at 20.0 μgmL^{-1}.[20]

2-1-3 Effect of the chlorine substituent

The most recently isolated chlorodysinosin A exhibits the highest in vitro inhibitory activities against thrombin (Factor IIA) and Factor VIIA (IC_{50}=

0.0057 and 0.039 μM, respectively) among the 20 or so normally occurring aeruginosins.[5] Another member, dysinosin A (IC_{50} thrombin, 0.046 μM; Factor VIIA, 0.326 μM), lacks the chlorine in the (*2S,3R*)-3-chloroleucine subunit. The dramatically different activities of these two compounds is remarkable in view of the simple replacement of a hydrogen by a chlorine atom in the same amino acid unit.[15] Both the X-ray co-crystal structure data and molecular modeling studies[5] of chlorodysinosin A[5] and dysinosin A[3] have identified the possible reasons for this remarkable "chlorine effect". The amino acid residues in the enzyme, do not undergo major positional or conformational changes in the presence of their inhibitors. Our laboratory has ascribed a more restricted χ^1 angle as the reason of this unexpected "chlorine effect". Due to the possible position of the chlorine atom in a favorable orientation within the hydrophobic S_3 pocket, an entropic gain associated with the loss of water molecules may present a distinct advantage (Figure 6).[5]

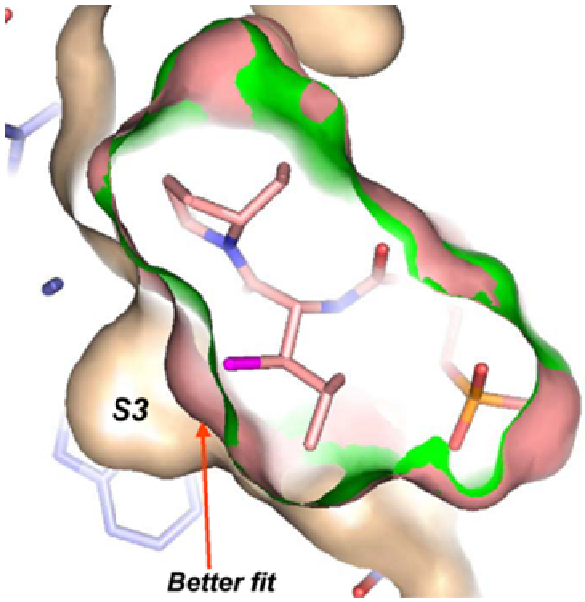


Figure 6. Contour diagram of the S_3 pocket and the P_3 side-chains from an overlay of the co-crystal structures of chlorodysinosin A[3] (pink) and dysinosin A[5] (green) with thrombin.

To map the structure-activity relationships of the P_1, P_2, P_3, and the N-terminal subunits against thrombin, a series of unnatural aeruginosin hybrids were synthesized.[21] When incorporating a *β*-substituent on the D-leucinyl P_3 subunit instead of the chlorine, a pronounced increase in activity was found, which also established the previously acknowledged importance of a hydrophobic interaction originally observed with the *(3R)*-chloro-D-leucine unit in chlorodysinosin A.

2-1-4 β-Methyl-D-leucinyl analog of aeruginosin

According to the contour diagram in Figure 6,[3,5] it appears that the S_3 subsite can accommodate β-substituents of the D-leucinyl subunit, and even larger ones as potential aeruginosin hybrids.

Due to the presence of the α-proton in D-*(3R)*-chloroleucine, in chloro-dysinosin A, there is a possibility of β-elimination under basic conditions. The methyl group is very similar to the chlorine atom in molecular size, and a D-*(3R)*-β-methylleucine analog would not be subject to elimination. To validate such this notion, the new unnatural amino acid was synthesized and incorporated in a hybrid type analog.

Among a series of unnatural chloroaeruginosin hybrids, which have been previously synthesized, compound **2.1** (Figure 7) was found to be the most potent (thrombin IC_{50}=0.0016 μM). [21] Thus, deleting the hydroxyl group from oscillarin bicyclic core was not detrimental to acitivity against thrombin.

Compound **2.2**, in which the β-substituent of the P_3 D-leucinyl subunit is a methyl group, was the target compound (Figure 7). The regioisomer **2.3** was also prepared as a model compound for comparison of their thrombin inhibitory acitivities.

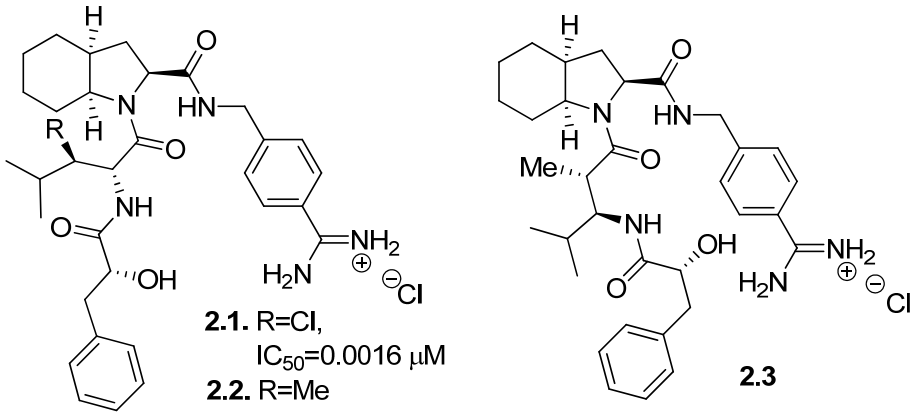


Figure 7. Structures of chloroaeruginosin **2.1** and target comounds.

2-2 Synthesis of ”methyl-leucine” aeruginosin hybrids

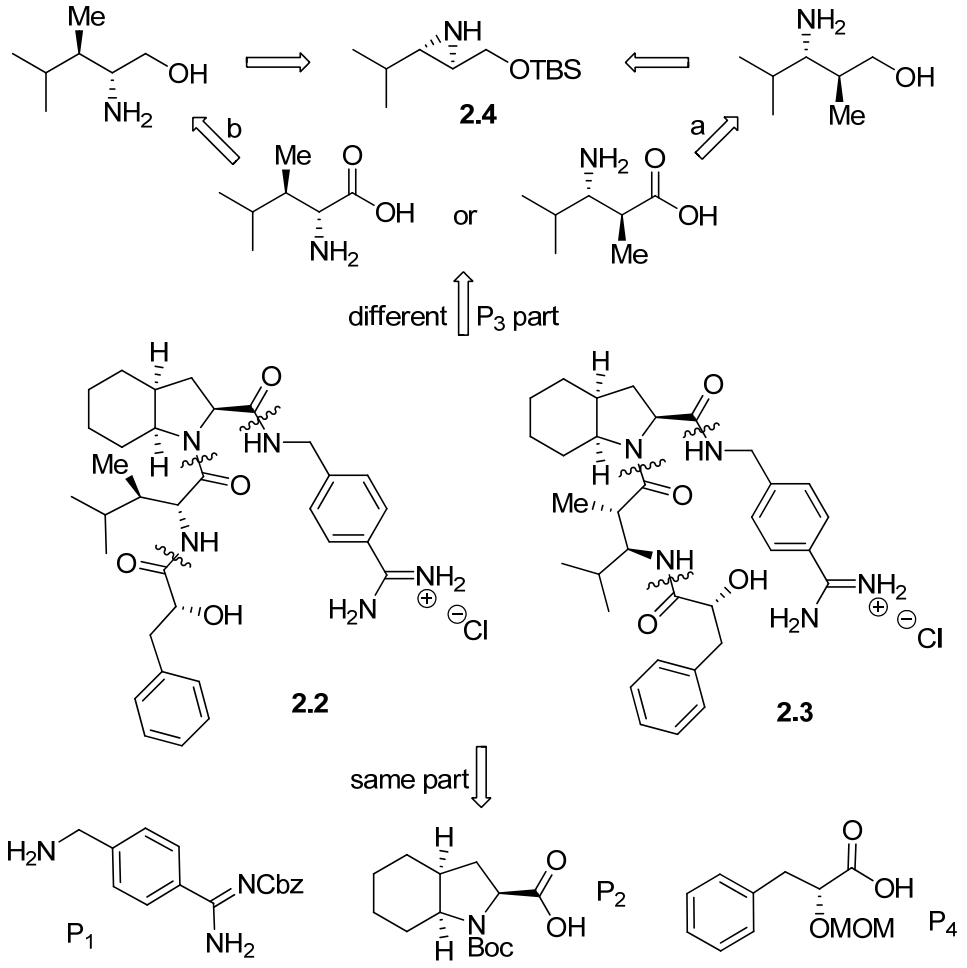


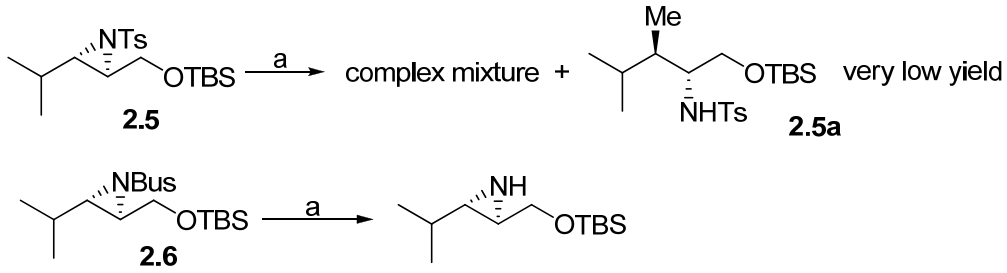
Figure 8. Retrosynthesis of the target compound.

Based on the method of synthesis of chloroaeruginosin **2.1**,[21] we envisaged the disconnections shown in the Figure 8. The two hybrid molecules **2.2** and **2.3** were formed by coupling four subunits. The C-terminal guanidine-containing group (P_1) is 4-amidino-benzamide, the P_2 subunit is the 2-carboxyperhydroindole core, and the N-terminal subunit (P_4) is D-phenyllactic acid. Aziridine **2.4** is the starting material, which could be opened by a methyl nucleophile, then followed by oxidation to carboxylic acid, to give β-methyl-D-leucine and its regioisomer (P_3).

2-2-1 Synthesis of *(3R, 2R)*-3-methyl-D-leucine (β-Me-Leu) and its 2-methyl regioisomer (P_3 subunit)

2-2-1-1 Aziridine opening

The aziridine **2.4**, was prepared as previously reported,[5] and was protected as *N*-Ts aziridine **2.5** and *N*-Bus aziridine **2.6**. Trimethylaluminum ($AlMe_3$) was the first reagent to be used as the source of the methyl nucleophile (Scheme 15).



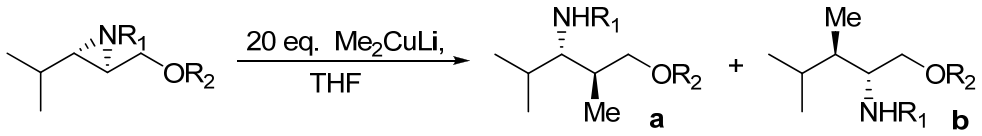
Scheme 15: Reagents and conditions: a) $AlMe_3$, 75 °C, toluene;

Initial attempts to open the corresponding TBS-protected *N*-Ts aziridine **2.5** using $AlMe_3$ led to a complex mixture and a poor yield of the desired 3-methyl regioisomer. Only cleavage of the Bus-group occurred in the case of the *N*-Bus

aziridine **2.6**.

Flame-dried copper(I) iodide (CuI) was treated with methyllithium and lithium bromide (MeLi.LiBr) complex to form $LiMe_2Cu$. Reaction of **2.5** with $LiMe_2Cu$ led to a mixture of 2-methyl and 3-methyl regioisomers in good combined yield. In addition to the *N*-Ts aziridine and *N*-Bus aziridine, the free and protected hydroxyl groups of the *N*-Ts aziridine, were also reacted in order to obtain a better ratio of the desired 3-methyl regioisomer as shown in Table 5.

Table 5 Reaction of *N*-sulfonylaziridines with lithium dimethylcopper(II)



compound	R_1	R_2	Temperature	Time (hr)	Ratio[a] **a** : **b**	Total Yield (%)
2.5	Ts	TBS	-10 °C - 0 °C	16	1.8 : 1	89
2.7	Ts	H	-10 °C - 0 °C	16	1 : 0	96
2.5	Ts	TBS[b]	-10 °C– r.t.	16	2.3 : 1	nd
2.8	Ts	TBDPS	-10 °C – r.t.	24	3 : 1	nd
2.9	Ts	TES	-10 °C – r.t.	24	1.6 : 1	nd
2.10	Ts	TIPS	-10 °C – r.t.	24	1.2 : 1	84
2.11	Bus	TIPS	-10 °C – r.t.	24	2 : 1	53

a) Determined by NMR on the crude reaction mixture after workup

b) $Yb(OTf)_3$ was added as catalyst (20 mol%)

Using excess $LiMe_2Cu$, the TIPS-protected *N*-Ts aziridine **2.10** led to a 1:1.2 mixture of the two regioisomers **2.10a** and **2.10b**. The other protecting groups, such as TBS, TES, TBDPS and *N*-Bus all resulted in lower ratios, and the

undesired 2-methyl regioisomer **a** was the predominant product. In the presence of the Lewis acid $Yb(OTf)_3$, which was added as catalyst,[22] the ratio of the undesired 2-methyl regioisomer was also increased.

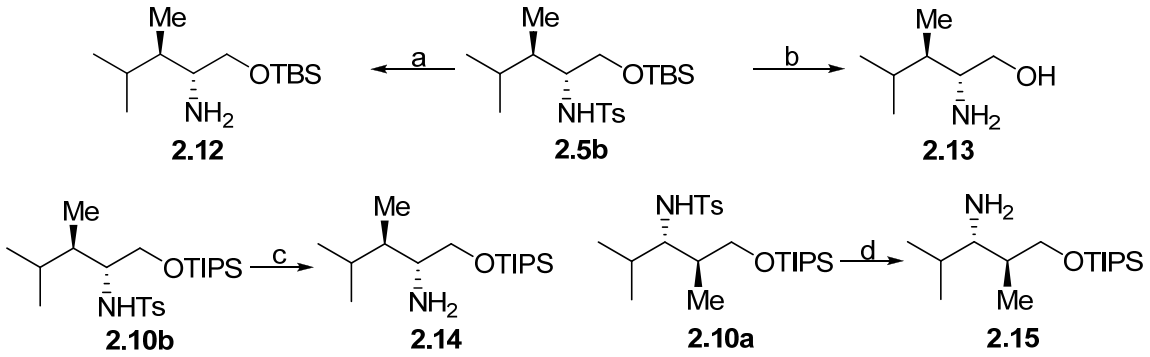
2-2-1-2 *N*-Ts group cleavage

Compound **2.5b** was used as the model to test three different conditions, for cleavage the *N*-Ts group, including acidic, free radical and basic conditions.

First, compound **2.5b** was treated with 2 equivalents of phenol in 30% hydrogen bromide (HBr) in acetic acid solution and heated to 60 °C,[23] for 12 h. MS analysis showed the TBS-group was cleaved, and the Ts-group remained intact even after heating to reflux for 36 h. The result was the same with 3 equivalents of phenol in 48% hydrogen bromide aqueous at 90 °C.[24,25]

Compound **2.5b** was heated to reflux with 20 equivalents of samarium (II) iodide (SmI_2) and 120 equivalents of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) in THF solution under argon atmosphere. [26] However all starting material remained unreacted after heating to reflux for 24 h.

Compound **2.5b** in THF with 6 equivalents of sodium in liquid ammonia at -78 °C for 1 h resulted in the cleavage of the Ts-group (Scheme 16).

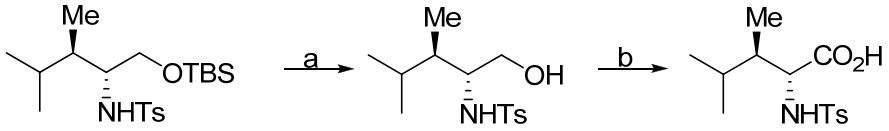


Scheme 16: Reagents and conditions: a) 20 mg s.m., Na/NH_3, -78 °C, 30 min., 83%; b) 100 mg s.m., Na/NH_3, -78 °C, 1 h, 74%; c) 50 mg s.m., Na/NH_3, -78 °C, 1 h, 88% d) 100 mg s.m., Na/NH_3, -78 °C, 1 h, 83%;

In cases involving small scale reactions and short reaction times, the TBS-group was found to be stable during the reaction. When scaling up a reaction, the mixture of compounds **2.12** and **2.13** was obtained. Compound **2.12** was totally transformed to **2.13** in longer reaction times. The TIPS-group was stable in the Birch reaction conditions, so the analog **2.10a** and **2.10b** was converted to free amines **2.14** and **2.15**, respectively (Scheme 16).

2-2-1-3 *(3R, 2R)*-β-Methyl-D-leucine

As shown in Scheme 17, compound **2.5b** was treated with an HF solution to form compound **2.16**, which was oxidized to the β-methyl-*N*-Ts-D-leucine **2.16a** in the presence of H_5IO_6 and CrO_3.[5] Unfortunately, the yield of the oxidation was low and the acid was difficult to purify.



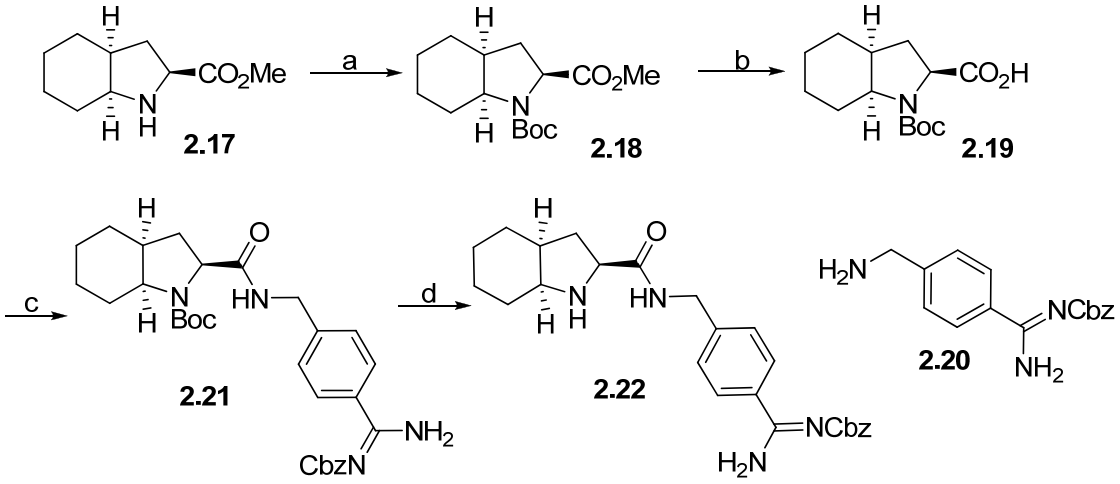
2.5b **2.16** **2.16a**

Scheme 17: Reagents and conditions: a) HF solution, MeCN, r.t., 1 h, 96%; b) cat. CrO_3, 0.4 M H_5IO_6 in wet MeCN, 0 °C, 30 min., 47%;

2-2-2 Synthesis of the intermediates

2-2-2-1 Formation of the P_1 and P_2 subunit

Methyl octahydroindole-2-carboxylate **2.17**[21] was first protected as the Boc-group. After saponification with LiOH and acidification, the free acid **2.19** was coupled with P_1 subunit (benzamidine **2.20**), in the presence of PyBOP and 2.6-lutidine as coupling reagents, to afford the coupling product **2.21**. Finally, the *N*-Boc was cleaved by trifluoroacetic acid (TFA) to give the free amine **2.22** as the final segment of the P_1 and P_2 subunit. The conditions, reagents and yields are shown in Scheme 18.

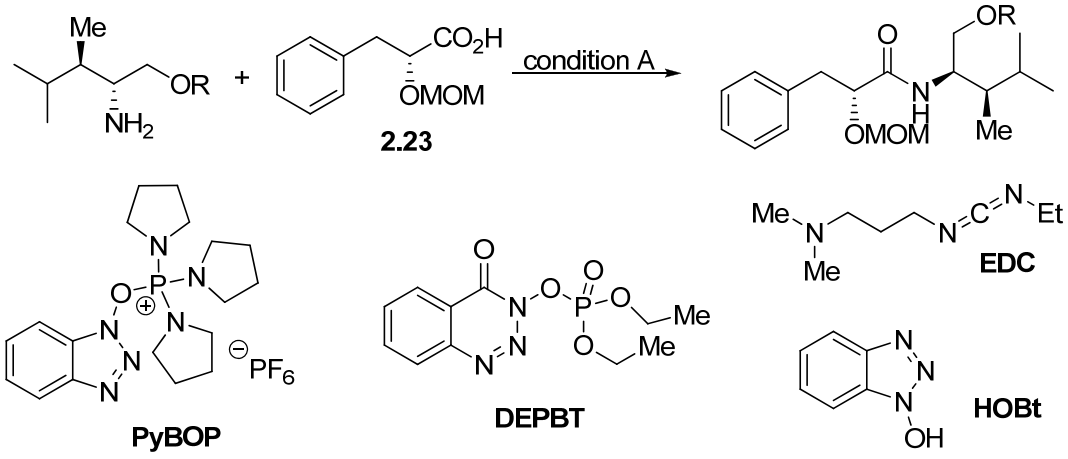


Scheme 18: Reagents and conditions: a) Boc_2O, Et_3N, MeOH, r.t., 26 h, 95%; b) LiOH, THF/H_2O 5:3, r.t., 18 h, 85%; c) PyBOP, **2.20**, 2,6-lutidine, r.t.,18 h, 91%; d) TFA/DCM 1:9, r.t., 1 h, 99%;

2-2-2-2 Formation of the P_3 and P_4 subunit

In the coupling reaction involving the free amine of P_3 subunit with MOM-D-phenyllactic acid **2.23**[21] (P_4 subunit), using 2.6-lutidine as base, three different kinds of coupling reagents were tested: 1) EDC and HOBt, 2) DEPBT, 3) PyBOP. For the P_3 subunit, compounds **2.12**, **2.13** and **2.14** were used as the starting materials. The results are shown in Table 6.

Table 6 Coupling reaction of P_3 and P_4 subunit



P_3 subunit		Coupling reagent	Time	Poduct	
Compound	R	condition A	(h)	Compound	Yield[a](%)
2.13	H	PyBOP	24	**2.24**	Not found
2.13	H	DEBPT	20	**2.24**	57
2.14	TBS	EDC and HOBt	16	**2.24a**	64
2.15	TIPS	EDC and HOBt	16	**2.24b**	56
2.15	TIPS	DEBPT	40	**2.24b**	86

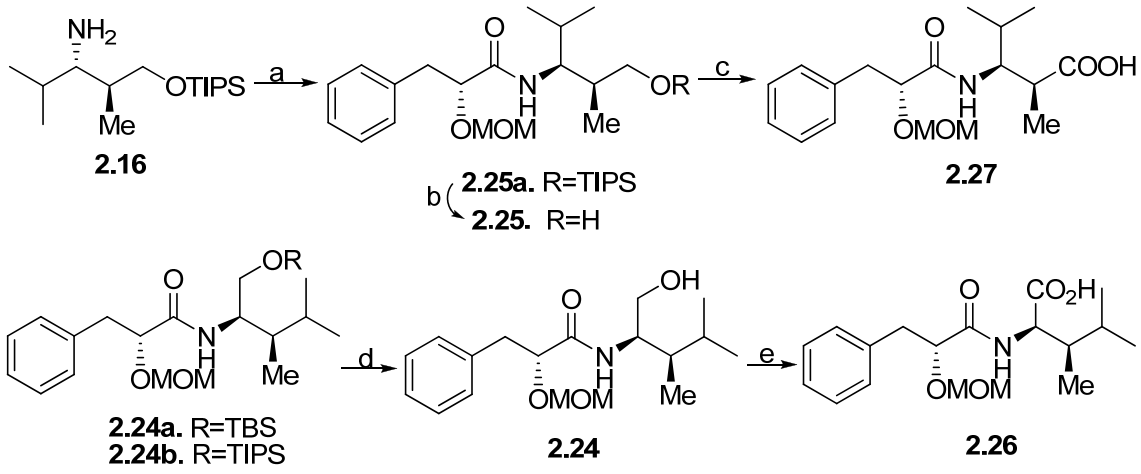
a) Yields of isolated pure product

Coupling reagent PyBOP was not effective. However, when the R-group is small (as TBS in the compound **2.14**), EDC and HOBt are suitable reagents for the coupling reaction. When the R-group is large (as TIPS in the compound **2.15**), DEBPT was satisfactory, although the reaction was slow. The 2-methyl regioisomer **2.16** of the P_3 subunit was treated under the same conditions.

2-Methyl-*O*-TIPS analog **2.16** was used as the starting material for coupling with the P_4 acid **2.23** in the presence of DEPBT to afford the coupling product **2.25a**, in an acceptable yield (Scheme 19).

Hydrogen fluoride (HF) stock solution was used in wet acetonitrile (MeCN) solution to easily cleave the TBS- and TIPS- hydroxyl protecting groups to afford compounds **2.24** and **2.25** in excellent yields, respectively (Scheme 19).

Oxidation of the primary alcohol was effected upon treatment with 0.4 M periodic acid (H_5IO_6)[5] in wet acetonitrile (MeCN) and a catalytic amount of chromium (VI) oxide (CrO_3) at 0 °C to afford the carboxylic acid. Following the same method, compounds **2.24** and **2.25** were oxidized to afford the carboxylic acids **2.26** and **2.27** (Scheme 19), as the final segment of the P_3 and P_4 subunit.

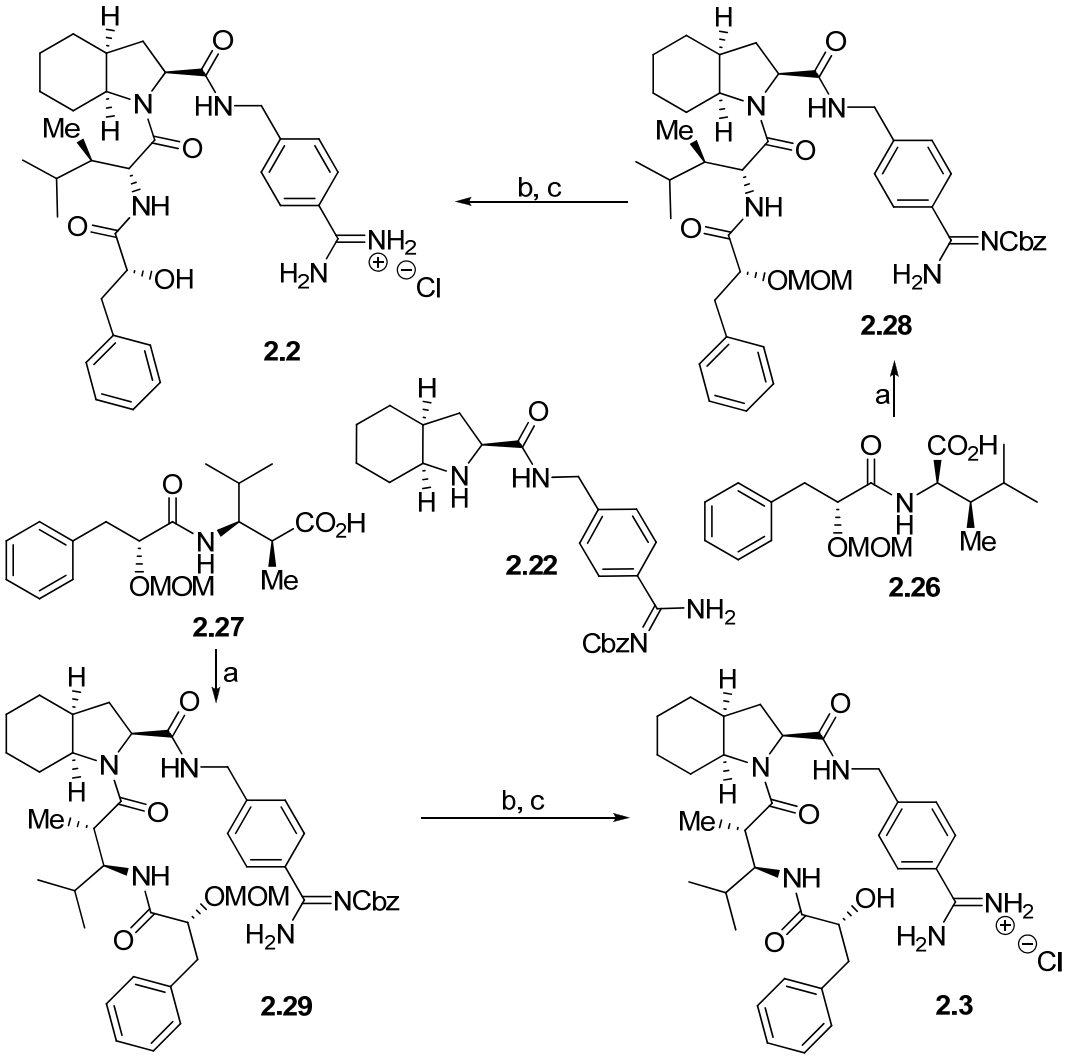


Scheme 19: Reagents and conditions: a) DEPBT, 2,6-lutidine, **2.23**, DCM, 0 °C to r.t., 48 h, 52%; b) HF stock solution, MeCN, r.t., 4 h, 86%; c) 0.4 M H_5IO_6 in wet MeCN, cat. CrO_3, MeCN, 0 °C, 30 min., 82%; d) HF stock solution, MeCN, r.t., **2.24a** 1 h 99%, **2.24b** 6 h 98%; e) 0.4 M H_5IO_6 in wet MeCN, cat. CrO_3, MeCN, 0 oC, 30 min., 82%;

2-2-3 Synthesis of the β-methyl-D-leucinyl aeruginosin hybrid

The carboxylic acids **2.26** and **2.27** were coupled with free amine **2.22**, to form compounds **2.28** and **2.29**, as shown in Scheme 20.

The Cbz-group was cleaved using Pd over activated carbon (Pd/C) as a catalyst under hydrogen gas atmosphere, followed by cleavage of the MOM group using a solution of >99.9% trifluoroacetic acid (TFA) and dichloro-methane (DCM) (9:1). The final target compound methyl-aeruginosin hybrid **2.2** and its isomer **2.3** were purified by RP-HPLC, (85 min. gradient of 20-80% CH_3CN in 0.05% aqueous TFA) to afford products as the TFA salt, which was transformed to the hydrochloride salt (1.25 M hydrochloric acid in methanol solution), as shown in Scheme 20.



Scheme 20: Reagents and conditions: a) DEPBT, 2,6-lutidine, **2.22**, DCM, r.t., 20 h, **2.28** 74%, **2.29** 69%; b) Pd/C 10 wt.%, H_2, MeOH, r.t., 10 h; c) TFA/DCM 9:1, r.t., 3 h, **2.2** 57% 2 steps, **2.3** 59% 2 steps;

2-3 Biological results

Methyl-aeruginosin hybrid **2.2** and its isomer **2.3** were evaluated in enzymatic assays for their inhibitory activity against thrombin and trypsin. The results are presented as IC_{50} values as below.

A. β-methyl-D-leucinyl aeruginosin hybrid **2.2**

Thrombin IC_{50}=0.022 μM (n=2, 0.016 μM and 0.028 μM)

Trypsin IC_{50}=0.02 μM (n=1)

B. Isomer **2.3**

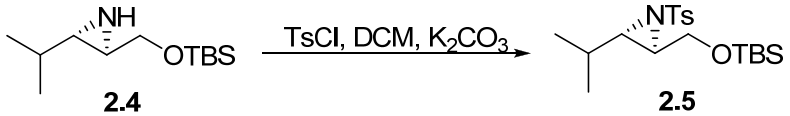
Thrombin IC_{50}=17.6 μM (n=1)

Trypsin IC_{50}=2.89 μM (n=1)

The β–methyl analog **2.2** is less active than the β–chloro one **2.1**. As expected, the 2–methyl analog **2.3** is much less active than its isomer **2.2**.

2-4 Experimental

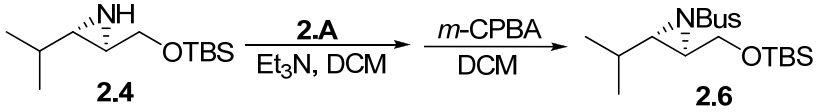
General: Solvents were distilled under positive pressure of dry argon before use and dried by standard methods. THF, ether, DCM and toluene were dried by the SDS (*Solvent Delivery System*). All commercially available reagents were used without further purification. All reactions were performed under argon atmosphere and monitored by thin-layer chromatography. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate, ninhydrine or potassium permanganate. IR, Perkin-Elmer FTIR Paragon 1000. Low- and high-resolution mass spectra were recorded using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded in a 1 dm cell at 20 °C (PerkinElmer 343). Flash column chromatography was performed using (40-60 μm) silica gel at increased pressure. NMR (^{1}H, ^{13}C) spectra were recorded on Bruker AV-300 and AV-400 spectrometers. When necessary, assignments were aided by DEPT, COSY, NOESY, and HMBC and HMQC correlation experiments.



2.4 **2.5**

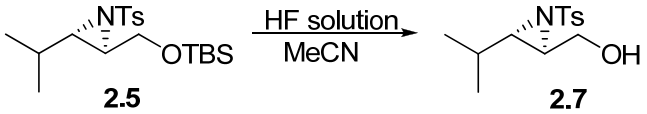
Compound 2.5: To a solution of **2.4** (459 mg, 2.0 mmol) in DCM (10.0 mL) at 0 °C was added K_2CO_3 (1.38 g, 10.0 mmol) under argon atmosphere. After stirring for 5 min., TsCl was added to the reaction mixture at 0 °C, then warmed

to R.T.. After stirring at R.T. for 16 h, the reaction mixture was filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (toluene/hexane 1:1 to toluene/EtOAc 1:5) to give **2.5** (679 mg, 89%), as a colorless solid, m.p. 46-48 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.79 (d, 2H, *J*=8.3 Hz), 7.23 (d, 2H, *J*=8.0 Hz), 4.02 (dd, 1H, *J*=5.0 Hz, *J*=11.0 Hz), 3.82 (dd, 1H, *J*=7.0 Hz, *J*=11.0 Hz), 2.77-2.73 (m, 1H), 2.52 (dd, 1H, *J*=4.5 Hz, *J*=8.5 Hz), 2.35 (s, 3H), 1.64-1.56 (m, 1H), 0.88 (d, 3H, *J*=6.8 Hz), 0.83-0.81 (m, 12H), -0.01 (s, 6H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 143.8, 137.5, 129.4, 127.6, 61.1, 53.7, 49.0, 29.6, 25.8, 21.5 20.3, 19.8, 18.1, -5.3; $[\alpha]_D$ -5.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{19}H_{33}NO_3SSi$ calculated ($M+H^+$) 384, found 384.



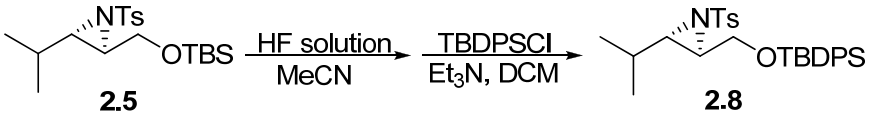
Compound 2.6: A solution of **2.4** (1.0 g, 4.4 mmol) in 40 mL of DCM was cooled to 0 °C and treated with Et_3N (1.53 mL, 11.0 mmol), followed by dropwise addition of *tert*-butyl sulfinyl chloride **2.A** (0.60 mL, 4.8 mmol) in 4.8 mL of DCM. The reaction mixture was stirred at 0 °C until TLC showed consumption of the starting material (1 h). Upon completion, 40 mL of saturated aqueous $NaHCO_3$ was added, and the layers separated (note: acidic washes should be avoided as *tert*-butyl sulfinamides are known to be unstable at low pH). The aqueous layer was extracted with DCM (3×50 mL). The organic layer was then dried over Na_2SO_4, and concentrated under reduced pressure. Flash column chromatography (EtOAc/hexane 1:9) afforded pure sulfinamide, which was directly taken up in 30 mL of DCM, and treated with *m*-CPBA (2.56 g, 14.9 mmol). After the oxidation was complete by TLC (2 h), the reaction mixture was diluted with a mixture of saturated aqueous $NaHCO_3$ (25 mL) and saturated aqueous Na_2SO_3 (25 mL). The aqueous layer was extracted with DCM

(3×50 mL). The organic extract was combined, dried over Na_2SO_4 and concentration under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 1:19) to afford **2.6** (1.36 g, 88% over 2 steps), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 4.04 (m, 1H), 3.72 (dd, 1H, *J*=7.1 Hz, *J*=10.9 Hz), 2.75 (m, 1H), 2.53 (m, 1H), 1.94-1.87 (m, 1H), 1.44 (s, 9H), 1.16 (d, 3H, *J*=6.6 Hz), 0.96 (d, 3H, *J*=6.8 Hz), 0.88 (s, 9H), 0.08 (s, 6H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 61.8, 59.8, 52.0, 46.6, 31.3, 28.3, 25.6, 24.0, 23.5, 20.4, 18.0; $[\alpha]_D$ +31.5 (c 1.0, $CHCl_3$); HRMS for $C_{16}H_{35}NO_3SSi$ calculated ($M+H^+$) 350.21797, found 350.21788.



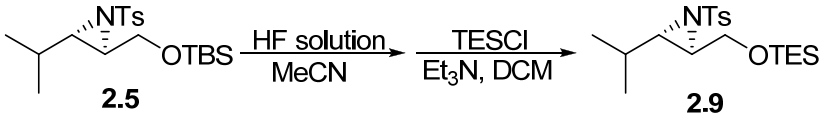
2.5 **2.7**

Compound 2.7: To a solution of **2.5** (58 mg, 0.15 mmol) in MeCN (1 mL) at 0 °C was added HF stock solution (0.79 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The product **2.7** (39 mg, 95%) is unstable in the silica gel which was sufficiently pure for use in the following step: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J*=8.2 Hz), 7.33 (d, 2H, *J*=8.1 Hz), 4.15 (dd, 1H, *J*=2.9 Hz, *J*=13.3 Hz), 4.00 (dd, 1H, *J*=8.7 Hz, *J*=13.5 Hz), 2.98-2.94 (m, 1H), 2.79 (dd, 1H, *J*=4.7 Hz, *J*=7.8 Hz), 2.45 (s, 3H), 1.54-1.46 (m, 1H), 1.27 (s, 1H), 0.89 (d, 3H, *J*=6.8 Hz), 0.77 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 144.1, 137.7, 129.6, 127.8, 61.7, 54.4, 49.3, 29.9, 21.8, 20.7, 20.0, 18.1, 12.1; ESI/MS for $C_{13}H_{19}NO_3S$ calculated ($M+H^+$) 270, found 270.



2.5 **2.8**

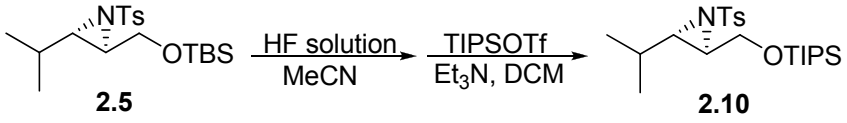
Compound 2.8: To a solution of **2.5** (77 mg, 0.2 mmol) in MeCN (3.6 mL) at 0 °C was added HF stock solution (3.2 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure and used directly. A solution of the crude **2.7** in DCM (8 mL) was cooled on ice to 0 °C and treated with NEt_3 (366 μL, 0.9 mmol) and TBDPSCl (0.18 mL, 0.72 mmol), then warmed to R.T. After stirring for 10 h, the reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl aqueous (20 mL). The aqueous phase extracted with DCM (2×20 mL). The organic phase was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:19) to afford **2.8** (98 mg, 96%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.84 (d, 2H, *J*=8.3 Hz), 7.67 (d, 4H, *J*=6.6 Hz), 7.48-7.40 (m, 6H), 7.27 (d, 2H, *J*=7.8 Hz), 4.09 (dd, 1H, *J*=5.2 Hz, *J*=11.2 Hz), 3.92 (dd, 1H, *J*=6.6 Hz, J=11.2 Hz), 2.94-2.90 (m, 1H), 2.55 (dd, 1H, *J*=4.5 Hz, *J*=8.6 Hz), 2.43 (s, 3H), 1.83-1.73 (m, 1H), 1.09 (s, 9H), 1.00-0.98 (t, 6H, *J*=6.8 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 144.0, 137.6, 134.0, 130.0, 129.6, 129.3, 127.8, 61.7, 54.3, 49.3, 29.8, 21.8, 20.7, 20.0, 18.1, 17.9; ESI/MS for $C_{29}H_{37}NO_3SSi$ calculated ($M+H^+$) 508, found 508.



2.5 **2.9**

Compound 2.9: To a solution of compound **2.5** (77 mg, 0.2 mmol) in MeCN

(3.6 mL) at 0 ◦C was added HF stock solution (3.2 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure and used directly. A solution of the crude **2.7** in DCM (8 mL) was cooled on ice to 0◦C and treated with NEt_3 (627 μL, 0.9 mmol) and TESCl (0.11 mL, 0.64 mmol), then warmed to R.T.. After stirring for 6 h, the reaction mixture was diluted with DCM (10 mL) and washed with 1 M HCl aqueous (20 mL). The aqueous phase extracted with DCM (2×20 mL). The organic phase was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:19) to give **2.9** (73 mg, 94%), as a colorless oil: ^{1}H NMR, (300 MHz, $CDCl_3$) δ 7.82 (d, 2H, *J*=8.3 Hz), 7.27 (d, 2H, *J*=8.1 Hz), 4.03 (dd, 1H, *J*=5.0 Hz, *J*=11.0 Hz), 3.83 (dd, 1H, *J*=7.0 Hz, *J*=11.0 Hz), 2.81-2.75 (m, 1H), 2.55 (dd, 1H, *J*=4.5 Hz, *J*=8.5 Hz), 2.40 (s, 3H), 1.74-1.58 (m, 1H), 0.93-0.84 (m, 15H), 0.55 (dd, 6H, *J*=8.2 Hz, *J*=16.2 Hz); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 138.7, 132.8, 125.4, 123.8, 62.3, 56.0, 51.5, 33.5, 26.2, 25.0, 24.6, 12.4, 10.2; ESI/MS for $C_{19}H_{33}NO_3SSi$ calculated ($M+H^+$) 384, found 384.



2.5 **2.10**

Compound 2.10: To a solution of **2.5** (575 mg, 1.50 mmol) in MeCN (9.0 mL) at 0 ◦C was added HF stock solution (7.9 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (40 mL) and extracted with DCM (3×50 mL). The combined

organic phases were dried over Na_2SO_4 and concentrated under reduced pressure and used directly. A solution of the crude **2.7** in DCM (20 mL) was cooled on ice to 0 °C and treated with NEt_3 (627 μL, 4.5 mmol) and TIPSOTf (1.0 mL, 3.75 mmol). After stirring at 0 °C for 4 h, the reaction mixture was diluted with DCM (30 mL) and washed with 1 M HCl aqueous (40 mL). The aqueous phase extracted with DCM (2×50 mL). The organic phase was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:19) to afford **2.10** (609 mg, 95%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J*=8.3 Hz), 7.30 (d, 2H, *J*=8.0 Hz), 4.14 (dd, 1H, *J*=4.7 Hz, *J*=10.7 Hz), 3.92 (dd, 1H, *J*=7.3 Hz, *J*=10.7 Hz), 2.84-2.80 (m, 1H), 2.59 (dd, 1H, *J*=4.5 Hz, *J*=8.5 Hz), 2.43 (s, 3H), 1.71-1.62 (m, 1H), 1.04 (s, 18H), 1.03 (s, 3H), 0.95 (d, 3H, *J*=6.8 Hz), 0.88 (d, 3H, *J*=6.6 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 144.1, 137.7, 129.6, 127.8, 61.7, 54.4, 49.3, 29.9, 21.8, 20.7, 20.0, 18.1, 12.1; $[\alpha]_D$ -3.5 (c 1.0, $CHCl_3$); HRMS for $C_{22}H_{39}NO_3SSi$ calculated ($M+H^+$) 426.24927, found 426.25097.

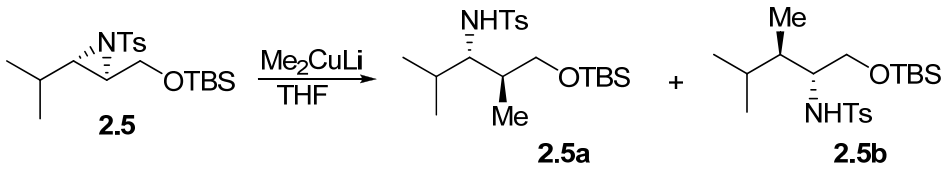
2.6 **2.6a**

Compound 2.6a: To a solution of **2.6** (1.33 g, 3.8 mmol) in MeCN (18.0 mL) at 0 °C was added HF stock solution (15.8 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (60 mL) and extracted with DCM (3×80 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:3) to afford **2.6a** as a colorless oil (823 mg, 92%): ^{1}H NMR,

(300 MHz, $CDCl_3$) δ 4.10 (br, dd, 1H), 3.85 (dd, 1H, *J*=6.7 Hz, *J*=10.1 Hz), 3.23 (s, 1H), 2.90-2.86 (m, 1H), 2.81-2.79 (t, 1H, *J*=3.8 Hz), 1.88-1.80 (m, 1H), 1.50 (s, 9H), 1.03 (d, 3H, *J*=5.1 Hz), 0.91 (d, 3H, *J*=5.2 Hz); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 63.1, 61.8, 49.7, 49.0, 28.7, 24.3, 19.7, 18.1, 12.5; $[\alpha]_D$ -3.7 (c 1.0, $CHCl_3$); HRMS for $C_{10}H_{21}NO_3S$ calculated ($M+H^+$) 236.13149, found 236.13176.

2.6a **2.11**

Compound 2.11: A solution of **2.6a** (612 mg, 2.6 mmol) in DCM (20 mL) was cooled on ice to 0 °C and treated with NEt_3 (1.09 mL, 7.8 mmol) and TIPSOTf (1.75 mL, 6.5 mmol). After stirring at 0 °C for 4 h, the reaction mixture was diluted with DCM (60 mL) and washed with 1 M HCl aqueous (80 mL). The aqueous phase extracted with DCM (2×80 mL). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:24) to afford compound **2.11** (916 mg, 90%), as a colorless oil: ^1H NMR, (400 MHz, $CDCl_3$) δ 4.13 (br, dd, 1H), 3.79 (dd, 1H, *J*=7.2 Hz, *J*=10.7 Hz), 2.77-2.73 (m, 1H), 2.55 (dd, 1H, *J*=4.6 Hz, *J*=6.8 Hz), 1.97-1.88 (m, 1H), 1.46 (s, 9H), 1.03 (s, 3H), 1.13 (d, 3H, *J*=6.6 Hz), 1.05 (s, 14H), 1.04 (s, 7H), 0.95 (d, 3H, *J*=6.8 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 62.6, 60.2, 52.7, 46.9, 28.7, 24.4, 20.8, 19.5, 18.1, 12.0; $[\alpha]_D$ +31.5 (c 1.0, $CHCl_3$); HRMS for $C_{19}H_{41}NO_3SSi$ calculated ($M+H^+$) 392.26492, found 392.26553.



2.5 **2.5a** **2.5b**

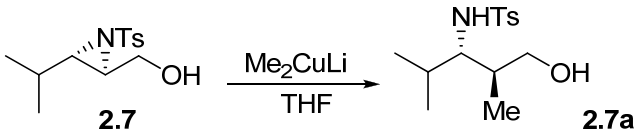
Compounds 2.5a and 2.5b：Flame-dried CuI (1.91 g, 10 mmol) was

suspended in fresh dry THF (40 mL), and the solution was cooled to -10 °C. MeLi.LiBr (20 mmol) 1.5 M in Et_2O (13.3 mL) was added dropwise and stirred at -10 °C until everything was transparent to colorless solution. Then a solution of **2.5** (192 mg, 0.5 mmol) and activated 4Å molecular sieves in THF (5 mL) was added. Stirring was continued for 2.5 h, then the solution was warmed to R.T.. After stirring for 72 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL) and extracted with $CHCl_3$ (4×100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/ hexane 1:49) to afford **2.5a** (114 mg, 57%) as a colorless oil and **2.5b** (63 mg, 32%) as a colorless oil. The ratio of **2.5a**:**2.5b** is 1.8:1 and total yield is 89%.

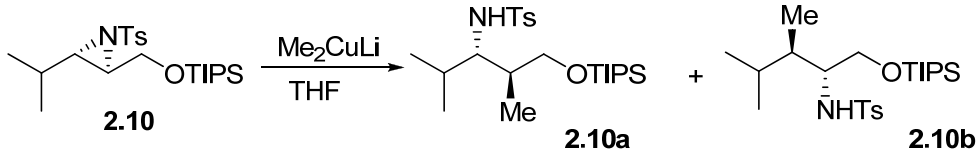
Compound 2.5a: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.69 (d, 2H, *J*=8.3 Hz), 7.22 (d, 2H, J=8.1 Hz), 5.68 (d, 1H, *J*=8.3 Hz), 3.64 (dd, 1H, *J*=3.8 Hz, *J*=10.5 Hz), 3.33 (dd, 1H, *J*=4.5 Hz, *J*=10.3 Hz), 3.04-3.01 (m, 1H), 2.37 (s, 3H), 1.79-1.70 (m, 2H), 0.88 (s, 9H), 0.83(d, 6H, *J*=6.8 Hz), 0.76 (d, 3H, *J*=7.0 Hz), 0.01 (d, 6H, *J*=1.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 142.6, 139.7, 129.4, 126.9, 65.6, 64.2, 35.6, 31.9, 26.0, 21.6, 20.3, 19.4, 18.1, 16.4, -5.5; $[\alpha]_D$ -10.3 (c 1.0, $CHCl_3$); HRMS for $C_{20}H_{37}NO_3SSi$ calculated ($M+H^+$) 400.23362, found 400.23390.

Compound 2.5b: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J*=8.3 Hz), 7.29 (d, 2H, *J*=8.4 Hz), 4.83 (d, 1H, *J*=8.9 Hz), 3.51 (dd, 1H, *J*=2.6 Hz, *J*=10.3 Hz), 3.22 (dd, 1H, *J*=3.3 Hz, *J*=10.3 Hz), 3.12-3.07 (m, 1H), 2.43 (s, 3H), 1.96-1.90 (m, 1H), 1.57-1.52 (m, 1H), 0.88 (d, 3H, *J*=6.9 Hz), 0.83 (s, 9H), 0.73 (d, 3H, *J*=7.0 Hz), 0.68 (d, 3H, *J*=6.8 Hz), -0.06(d, 6H, *J*=14.5 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 143.3, 138.5, 129.8, 127.2, 61.4, 57.4, 39.8, 27.3, 25.9, 21.8, 21.7, 18.3, 16.4, 10.4, -5.5; $[\alpha]_D$ +8.3 (c 1.0, $CHCl_3$); HRMS for

$C_{20}H_{37}NO_3SSi$ calculated ($M+H^+$) 400.23362, found 400.23389.



Compound 2.7a: Flame-dried CuI (167 mg, 0.88 mmol) was suspended in fresh dry THF (28 mL), and the solution was cooled to -10 °C. MeLi.LiBr (1.76 mmol) 1.5 M in Et_2O (1.17 mL) was added dropwise and stirred at -10 °C, until everything was transparent to colorless solution. The reaction mixture was cooled to -10 °C, then a solution of **2.7** (17 mg, 0.044 mmol) and activated 4Å molecular sieves in THF (0.8 mL) was added at -10 °C. Stirring was continued for 1 h, then the solution was warmed to 0 °C. After stirring for 10 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (15 mL) and extracted with $CHCl_3$ (3×20 mL), then the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 3:7) to give **2.7a** (12 mg, 97%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J*=8.3 Hz), 7.28 (d, 2H, *J*=7.9 Hz), 4.87 (d, 1H, *J*=9.5 Hz), 3.97 (dd, 1H, *J*=3.0 Hz, *J*=11.6 Hz), 3.49 (dd, 1H, *J*=3.4 Hz, *J*=11.4 Hz), 3.17-3.11 (m, 1H), 2.54 (s, 1H), 2.42 (s, 3H), 1.86-1.81 (m, 1H), 1.68-1.60 (m, 1H), 0.95 (d, 3H, *J*=7.0 Hz), 0.74 (d, 3H, *J*=6.9 Hz), 0.51(d, 3H, *J*=6.8 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 143.4, 138.4, 129.8, 127.3, 62.3, 57.8, 39.9, 27.5, 21.9, 21.7, 18.1, 16.7, 12.0, 10.7; HRMS for $C_{14}H_{23}NO_3S$ calculated ($M+H^+$) 286.14714, found 286.14719.



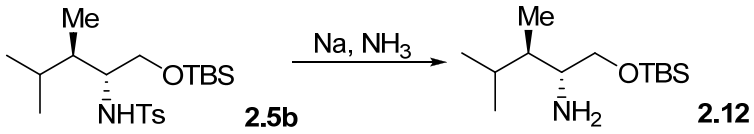
Compounds 2.10a and 2.10b: Flame-dried CuI (1.33 g, 7.0mmol) was

suspended in fresh dry THF (28 mL), and the solution was cooled to -10 °C. MeLi.LiBr (14.0 mmol) 1.5 M in Et_2O (9.3 mL) was added dropwise and stirred at -10 °C, until everything was transparent to colorless solution. The reaction mixture was cooled to -35 °C, then a solution of **2.10** (149 mg, 0.35 mmol) and activated 4Å molecular sieves in THF (5 mL) was added at -35 °C. Stirring was continued for 2.5 h, then the solution was warmed to R.T.. After stirring for 48 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (80 mL) and extracted with $CHCl_3$ (4×80 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:24) to afford **2.10a** (71 mg, 46%) as a colorless oil and **2.10b** (59 mg, 38%) as a colorless oil. The ratio of **2.10a**:**2.10b** is 1.2:1 and total yield is 84%.

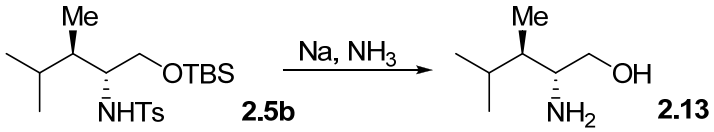
Compound 2.10a：^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.73 (d, 2H, *J*=8.3 Hz), 7.24 (d, 2H, *J*=8.1 Hz), 5.77 (d, 1H, *J*=8.3 Hz), 3.51 (dd, 1H, *J*=2.6 Hz, *J*=10.3 Hz), 3.22 (dd, 1H, *J*=3.3 Hz, *J*=10.3 Hz), 3.07-3.02 (m, 1H), 2.40 (s, 3H), 1.86-1.76 (m, 2H), 1.06-1.05 (m, 21H), 0.87 (dd, 6H, *J*=2.0 Hz, *J*=6.8 Hz), 0.81 (d, 3H, *J*=7.0 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 142.6, 139.7, 129.4, 127.0, 66.1, 64.3, 35.8, 31.9, 21.6, 20.4, 19.6, 18.1, 16.5, 11.9; $[\alpha]_D$ -9.9 (c 1.0, $CHCl_3$); HRMS for $C_{23}H_{43}NO_3SSi$ calculated ($M+H^+$) 442.28057, found 442.28079.

Compound 2.10b：^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J*=8.2 Hz), 7.27 (d, 2H, *J*=8.3 Hz), 4.80 (d, 1H, *J*=8.5 Hz), 3.66 (dd, 1H, *J*=3.0 Hz, *J*=10.1 Hz), 3.45 (dd, 1H, *J*=3.3 Hz, *J*=10.2 Hz), 3.15-3.09 (m, 1H), 2.41 (s, 3H), 1.92-1.84 (m, 1H), 1.65-1.56 (m, 1H), 0.97 (s, 21H), 0.86 (d, 3H, *J*=6.9 Hz), 0.76 (d, 3H, *J*=7.0 Hz), 0.66 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 143.4, 138.4, 129.8, 127.3, 62.3, 57.8, 39.9, 27.5, 21.9, 21.7, 18.1, 16.7, 12.0, 10.7; $[\alpha]_D$ +14.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{23}H_{43}NO_3SSi$ calculated

(M+H$^+$) 442, found 442.

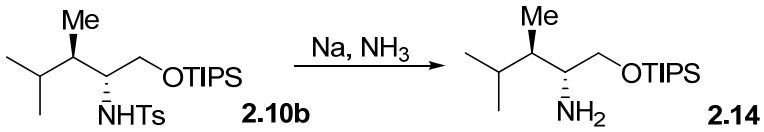


Compound 2.12: To 5 mL of liquid ammonia was added Na (8 mg, 0.36 mmol) at -78 °C. After stirring, to the dark-blue solution **2.5b** (24 mg, 0.6 mmol) in fresh dry THF (2 mL) was added dropwise. The cooling bath was removed and stirring was continued for 30 min.. The reaction mixture was quenched by addition of solid NH_4Cl carefully. After NH_3 was evaporated, the residue was partitioned between water 5 mL and DCM 10 mL. The aqueous layer was extracted with DCM (2×10 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The residue was purified by flash column chromatography (MeOH/DCM 1:24) to give **2.12** (12 mg, 83%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 3.74 (d, 1H, *J*=8.0 Hz), 3.36 (dd, 1H, *J*=8.0 Hz, *J*=9.7 Hz), 2.73 (s, 1H), 2.24 (s, 2H), 1.99-1.91 (m, 1H), 1.35-1.20 (m, 1H), 0.92 (d, 3H, *J*=6.8 Hz), 0.90 (s, 9H), 0.80 (d, 3H, *J*=6.8 Hz), 0.75 (d, 3H, *J*=6.9 Hz), 0.06 (d, 6H, *J*=3.0 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 66.3, 55.4, 41.6, 27.9, 26.1, 21.8, 18.5, 16.6, 10.4, -5.1, -5.2; $[\alpha]_D$ -10.0 (c 1.0, $CHCl_3$); ESI/MS for $C_{13}H_{31}NOS$ calculated (M+H$^+$) 246, found 246.



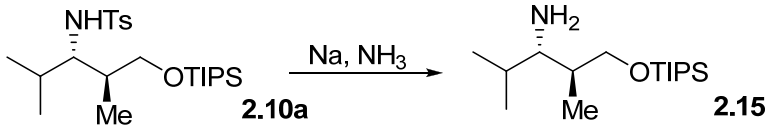
Compound 2.13: To 15 mL of liquid ammonia was added Na (58 mg, 2.5 mmol) in small pieces at -78 °C. After stirring, to the dark-blue solution, **2.5b** (100 mg, 0.25 mmol) in fresh dry THF (5 mL) was added dropwise. The cooling bath was removed and stirring was continued for 1 h. The reaction mixture was

quenched by addition of solid NH_4Cl carefully. After NH_3 was evaporated, the residue was partitioned between water 30 mL and DCM 35 mL. The aqueous layer was extracted with DCM (2×30 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:1) to afford **2.13** (24 mg, 74%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 3.68 (br, d, 1H), 3.26-3.21 (t, 1H, *J*=9.5 Hz), 2.69 (s, 1H), 2.17 (s, 3H), 1.96-1.85 (m, 1H), 1.30-1.19 (m, 1H), 0.93 (d, 3H, *J*=6.8 Hz), 0.79 (d, 3H, *J*=6.8 Hz), 0.74 (d, 3H, *J*=6.9 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 64.6, 55.3, 28.0, 21.7, 16.5, 10.2; $[\alpha]_D$ -14.4 (c 1.0, $CHCl_3$); ESI/MS for $C_7H_{17}NO$ calculated ($M+H^+$) 132, found 132.



Compound 2.14: To 15 mL of liquid ammonia was added Na (17 mg, 0.72 mmol) at -78 °C. After stirring, to the dark-blue solution **2.10b** (53 mg, 0.25 mmol) in fresh dry THF (5 mL) was added dropwise. The cooling bath was removed and stirring was continued for 1 h. The reaction mixture was quenched by addition of solid NH_4Cl carefully. After NH_3 was evaporated, the residue was partitioned between water 20 mL and DCM 25 mL. The aqueous layer was extracted with DCM (2×20 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:49) to afford **2.14** (30 mg, 88%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 3.85 (dd, 1H, *J*=3.1 Hz, *J*=9.6 Hz), 3.46 (dd, 1H, *J*=8.2 Hz, *J*=9.5 Hz), 2.78 (br, t, 1H), 2.21 (br, s, 2H), 2.03-1.95 (m, 1H), 1.36-1.23 (m, 1H), 1.11-1.07 (m, 21H), 0.94 (d, 3H, *J*=6.8 Hz), 0.82 (d, 3H, *J*=6.8 Hz), 0.77 (d, 3H, *J*=6.9 Hz); ^{13}C NMR, (100

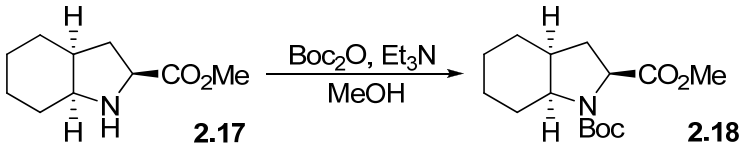
MHz, $CDCl_3$) δ 66.5, 55.8, 41.5, 27.9, 21.8, 18.2, 16.6, 12.1, 10.4; $[\alpha]_D$ -5.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{16}H_{37}NOSi$ calculated ($M+H^+$) 288, found 288.



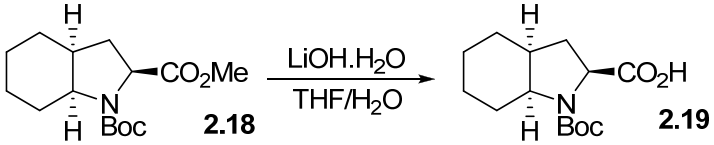
Compound 2.15：To 25 mL of liquid ammonia was added Na (31 mg, 1.32 mmol) in small pieces at -78 °C. After stirring, to the dark-blue solution **2.10a** (97 mg, 0.25 mmol) in fresh dry THF (7 mL) was added dropwise. The cooling bath was removed and stirring was continued for 1 h. The reaction mixture was quenched by addition of solid NH_4Cl carefully. After NH_3 was evaporated, and the residue was partitioned between water 30 mL and DCM 35 mL. The aqueous layer was extracted with DCM (2×30 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:49 to MeOH/DCM 1:9) to give **2.15** (53 mg, 83%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 3.80-3.71 (m, 2H), 2.47 (dd, 1H, *J*=4.5 Hz, *J*=6.8 Hz), 2.14 (s, 2H), 1.88-1.80 (m, 1H), 1.71-1.63 (m, 1H), 1.13-1.04 (m, 21H), 0.96 (dd, 6H, *J*=5.3 Hz, *J*=5.1 Hz), 0.87 (d, 3H, J=6.8 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 66.9, 59.8, 38.9, 29.7, 20.9, 18.2, 16.0, 15.3, 12.2; $[\alpha]_D$ +1.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{16}H_{37}NOSi$ calculated ($M+H^+$) 288, found 288.

Compound 2.16：To a solution of **2.5b** (20 mg, 0.05 mmol) in MeCN (0.9 mL) at 0 °C was added HF stock solution (0.79 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated

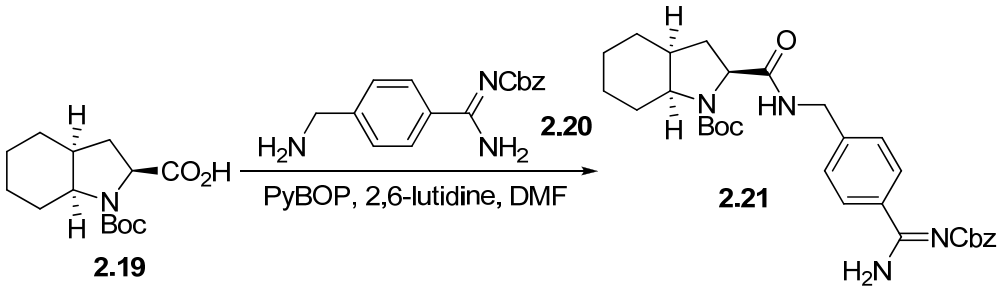
aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford **2.16** (16 mg, 96%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, *J*=8.3 Hz), 7.31 (d, 2H, *J*=7.9 Hz), 5.06 (d, 1H, *J*=8.2 Hz), 3.57 (d, 1H, *J*=3.3 Hz,), 3.20-3.13 (m, 1H), 2.43 (s, 3H), 2.02 (s, 1H), 1.70-1.62 (m, 1H), 1.43-1.45 (m, 1H), 0.80 (d, 3H, *J*=6.8 Hz), 0.74 (d, 3H, *J*=7.0 Hz), 0.54 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 140.9, 135.0, 127.1, 124.6, 59.6, 55.2, 37.8, 25.2, 18.9, 18.8, 14.2, 8.0; $[\alpha]_D$ +11.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{14}H_{23}NO_3S$ calculated ($M+H^+$) 286, found 286.



Compound 2.18: To a solution of methyl octahydroindole-2-carboxylate **2.17** (0.10 g, 0.46 mmol) in MeOH (2 mL) at 0 °C were added NEt_3 (220 μL, 1.6 mmol) and Boc_2O (0.11 g, 0.50 mmol), then warmed to R.T.. After stirring for 26 h, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:19 to EtOAc/ hexane 1:9) to afford **2.18** (124 mg, 95%), as a white powder: ^{1}H NMR, (300 MHz, $CDCl_3$) δ (rotamers) 4.05 (br, m, 1H), 3.60 (br, m, 1H), 3.55 (s, 3H), 2.10 (br, m, 1H), 2.08-0.79 (m, 19H); ^{13}C NMR, (75 MHz, $CDCl_3$) δ (rotamers) 59.0, 58.5, 56.8, 51.8, 51.6, 36.8, 36.2, 32.2, 31.4, 28.2, 28.0, 27.6, 27.2, 25.6, 23.4, 20.3; $[\alpha]_D$ -32.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{15}H_{25}NO_4$ calculated ($M+H^+$) 284, found 284.

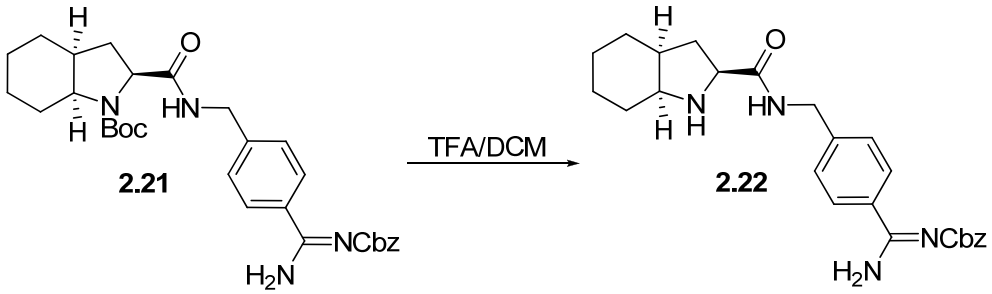


Compound 2.19: A solution of **2.18** (1.2 g, 4.3 mmol) in THF/H_2O (5:3, 160 mL) was treated with LiOH.H_2O (0.72 g, 17.0 mmol). After stirring at R.T. for 18 h, the reaction mixture was washed with Et_2O. The aqueous phase was cooled on ice to 0 °C, acidified with 5% citric acid aqueous until pH=3-4 and extracted with DCM (3×100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to afford carboxylic acid **2.19** (0.98 g, 85%) as a colorless solid, m.p. 130-132 °C, which was sufficiently pure for use in the following step: ^{1}H NMR, (400 MHz, DMSO-d_6) δ (rotamers) 12.49 (br, s, 1H), 4.03 (br, m, 1H), 3.63 (br, m, 1H), 2.24 (br, m, 1H), 2.06 (br, m, 1H), 1.95-1.76 (m, 2H), 1.67-1.52 (m, 3H), 1.43-1.01 (m, 13H); ^{13}C NMR, (100 MHz, DMSO-d_6) δ (rotamers) 174.6, 174.0, 153.0, 152.4, 78.4, 78.3, 58.8, 58.5, 56.8, 56.4, 36.2, 35.7, 31.7, 31.0, 28.1, 27.9, 27.5, 27.0, 25.3, 23.3, 23.2, 20.1, 20.0; $[\alpha]_D$ -23.2 (c 1.0, MeOH); ESI/MS for $C_{15}H_{25}NO_4$ calculated ($M+H^+$) 270, found 270.



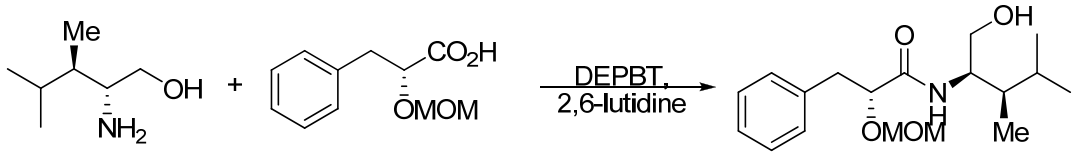
Compound 2.21: To a solution of carboxylic acid **2.19** (71 mg, 0.26 mmol) in DMF (5 mL) at 0 °C was added PyBOP (0.21 g, 0.40 mmol) with stirring for 10 min., then benzamidine **2.20** (0.11 g, 0.40 mmol) and 2,6-lutidine (92 μL, 0.79 mmol) were added, and warmed to R.T.. After stirring for 18 h, the reaction mixture was diluted with DCM (50 mL), washed with 0.5 M HCl aqueous (50 mL), 5% aqueous Na_2CO_3 (50 mL), brine (50 mL), and H_2O (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by

flash column chromatography (EtOAc/hexane 1:1 to EtOAc/hexane 2:1) to give **2.21** (126 mg, 91%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 9.50 (br, s, 1H), 7.85-7.70 (m, 2H), 7.47-7.24 (m, 8H), 5.3 (s, 0.3H), 5.2 (s, 1.7H), 4.53-4.39 (m, 2H), 4.25 (m, 1H), 3.84 (m, 1H), 2.30-2.00 (m, 3H), 1.90 (m, 1H), 1.75-1.55 (m, 3H), 1.51-1.10 (m, 13H); ^{13}C NMR, (100 MHz $CDCl_3$) δ (rotamers) 172.9, 167.8, 165.2, 164.3, 155.2, 151.2, 142.9, 136.5, 134.9, 133.1, 128.59, 128.56, 128.3, 128.14, 128.18, 128.9, 127.7, 127.3, 80.3, 67.8, 67.1, 60.3, 58.1, 42.7, 36.5, 28.5, 28.3, 25.8, 23.7, 20.4; $[\alpha]_D$ –19.4 (c 1.0, MeOH); ESI/MS for $C_{30}H_{38}N_4O_5$ calculated ($M+H^+$) 535, found 535.



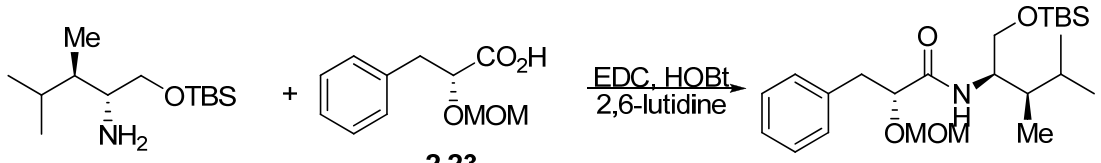
Compound 2.22: Compound **2.21** (0.57 g, 1.1 mmol) was treated with >99.9% TFA/DCM (1:9, 10 mL). After stirring at R.T. for 1 h, the reaction mixture was diluted with EtOAc (50 mL) and concentrated under reduced pressure. The crude residue was suspended in 5% Na_2CO_3 aqueous (40 mL) and extracted with DCM (3×40 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure to afford **2.22** (473 mg, 99%), as a colorless oil, which was sufficiently pure for use in the following step: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 9.45 (br, s, 1H), 8.28 (t, 1H, *J*=6.2 Hz), 7.84 (AA' part of AA'XX', 2H), 7.44-7.39 (m, 2H), 7.35-7.24 (m, 3H), 7.19 (XX' part of AA'XX', 2H), 5.17 (s, 2H), 4.46-4.30 (m, 2H), 3.67 (dd, 1H, *J*=5.1 Hz, *J*=10.6 Hz), 3.21 (br, m, 1H), 2.20 (br, m, 1H), 1.91 (br, m, 1H), 1.70 (br, m, 1H), 1.55-1.35 (m, 5H), 1.30-1.13 (m, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ

(rotamers) 176.2, 176.1, 167.7, 164.3, 142.63, 142.61, 136.5, 133.2, 128.2, 127.9, 127.71, 127.69, 127.1, 66.8, 58.6, 57.1, 42.3, 42.1, 37.8, 35.2, 29.0, 27.3, 23.3, 21.5; $[\alpha]_D$ +8.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{25}H_{30}N_4O_3$ calculated ($M+H^+$) 435, found 435.

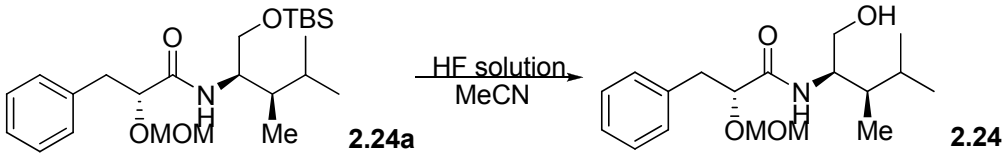


2.13 **2.23** **2.24**

Compound 2.24: To a solution of MOM-D-phenyllactic acid **2.23** (35 mg, 0.17 mmol) in DCM (5 mL) at 0 °C were added DEPBT (75 mg, 0.25 mmol) with stirring at 0 °C for 10 min., then a solution of 2,6-lutidine (58 μL, 0.50 mmol) and **2.13** (18 mg, 0.14 mmol) in DCM (1.5 mL) was added, and then slowly warmed to R.T.. After stirring for 20 h, the reaction mixture was diluted with DCM (15 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), 1 M HCl aqueous (20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (100% EtOAc) to afford **2.24** (26 mg, 57%), as a colorless solid, m.p. 62-64 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.30-7.20 (m, 5H), 6.59 (d, 1H, *J*=8.4 Hz), 4.66 (d, 1H, *J*=6.4 Hz), 4.52 (d, 1H, *J*=6.4 Hz), 4.31 (dd, 1H, *J*=3.8 Hz, *J*=7.4 Hz), 3.79-3.72 (m, 1H), 3.71 (dd, 1H, *J*=3.0 Hz, *J*=11.4 Hz), 3.60 (dd, 1H, *J*=5.2 Hz, *J*=11.2 Hz), 3.22 (s, 3H), 3.19 (dd, 1H, *J*=3.8 Hz, *J*=14.2 Hz), 2.98 (dd, 1H, *J*=7.2 Hz, *J*=14.0 Hz), 2.55 (s, 1H), 1.53-1.42 (m, 2H), 0.86 (d, 3H, *J*=6.8 Hz), 0.76 (d, 3H, *J*=6.8 Hz), 0.73 (d, 3H, *J*=6.8 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.2, 137.2, 130.0, 128.4, 126.8, 96.8, 79.3, 63.7, 56.2, 54.2, 39.0, 27.9, 21.7, 16.5, 10.7; $[\alpha]_D$ +80.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{18}H_{29}NO_4$ calculated ($M+H^+$) 324, found 324.

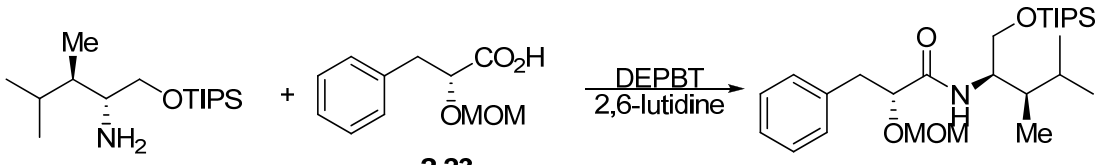


Compound 2.24a: To a solution of MOM-D-phenyllactic acid **2.23** (18 mg, 0.084 mmol) in DCM (3 mL) at 0 °C were added EDC (20 mg, 0.11 mmol) and HOBt (14 mg, 0.11 mmol) with stirring at 0 °C for 10 min., then a solution of **2.14** (17 mg, 0.07 mmol) and 2,6-lutidine (16 μL, 0.14 mmol) in DCM (2 mL) was added, and then slowly warmed to R.T.. After stirring for 72 h, the reaction mixture was diluted with DCM (15 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), 1 M HCl aqueous (20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:9) to give **2.24a** (20 mg, 64%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.29-7.24 (m, 4H), 7.23-7.19 (m, 1H), 6.72 (d, 1H, *J*=9.7 Hz), 4.57 (d, 1H, *J*=6.6 Hz), 4.52 (d, 1H, *J*=6.6 Hz), 4.36 (dd, 1H, *J*=3.7 Hz, *J*=7.6 Hz), 3.85-3.78 (m, 1H), 3.73 (dd, 1H, *J*=2.6 Hz, *J*=10.3 Hz), 3.57 (dd, 1H, *J*=2.8 Hz, *J*=10.3 Hz), 3.20 (dd, 1H, *J*=3.6 Hz, *J*=10.5 Hz), 3.11 (s, 3H), 2.97 (dd, 1H, *J*=7.5 Hz, *J*=14.1 Hz), 1.57-1.49 (m, 1H), 1.48-1.40 (m, 1H), 0.87 (s, 9H), 0.83 (d, 3H, *J*=6.8 Hz), 0.73 (dd, 6H, *J*=3.3 Hz, *J*=6.8 Hz), 0.01 (d, 6H, *J*=11.0 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.7, 137.5, 130.0, 128.4, 126.7, 95.8, 87.2, 63.3, 56.0, 52.5, 39.0, 38.5, 27.4, 26.0, 21.9, 16.2, 14.3, 10.3, -5.3, -5.4; $[\alpha]_D$ +103.6 (c 1.0, $CHCl_3$); ESI/MS for $C_{24}H_{43}NO_4Si$ calculated ($M+H^+$) 438, found 438.



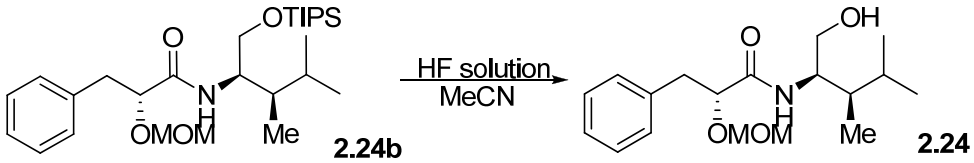
Compound 2.24: To a solution of **2.24a** (19 mg, 0.044 mmol) in MeCN (0.9 mL) at 0 °C was added HF stock solution (0.79 mL, prepared by mixing 43 mL

MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (100% EtOAc) to give **2.24** (14 mg, 99%), as a colorless solid.



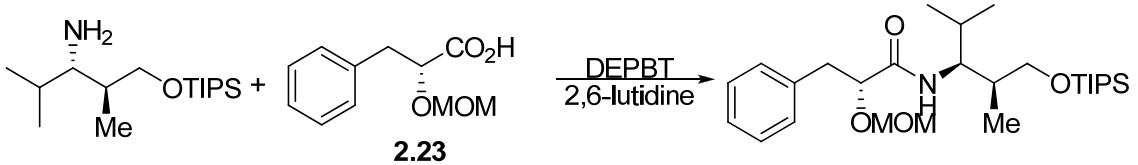
Compound 2.24b: To a solution of MOM-D-phenyllactic acid **2.23** (23 mg, 0.11 mmol) in DCM (3 mL) at 0°C were added DEPBT (48 mg, 0.16 mmol) with stirring at 0°C for 10 min., a solution of **2.15** (26 mg, 0.09 mmol) and 2,6-lutidine (38 μL, 0.32 mmol) in DCM (2 mL) was added, then slowly warmed to R.T.. After stirring for 40 h, the reaction mixture was diluted with DCM (15 mL), washed with saturated aqueous $NaHCO_3$ (20 mL), 1 M HCl aqueous (20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/ hexane 1:9) to afford **2.24b** (37 mg, 86%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.28-7.25 (m, 4H), 7.23-7.21 (m, 1H), 6.72 (d, 1H, *J*=9.3 Hz), 4.56 (dd, 1H, *J*=6.6 Hz, *J*=20.4 Hz), 4.36 (dd, 1H, *J*=3.6 Hz, *J*=7.3 Hz), 3.91-3.77 (m, 2H), 3.73-3.71 (m, 1H), 3.21 (dd, 1H, *J*=3.6 Hz, *J*=14.1 Hz), 3.09 (s, 9H), 2.99 (dd, 1H, *J*=7.4 Hz, *J*=14.1 Hz), 1.62-1.54 (m, 1H), 1.48-1.40 (m, 1H), 1.12-0.98 (m, 21H), 0.84 (d, 3H, *J*=6.9 Hz), 0.76 (d, 3H, *J*=6.9 Hz), 0.72 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.8, 137.5, 130.0, 128.4, 126.7, 96.1, 78.5, 63.7, 56.0, 52.9, 39.2, 38.6, 27.6, 22.0, 18.2, 16.3, 12.1, 10.6; $[\alpha]_D$ +89.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{27}H_{49}NO_4Si$ calculated

$(M+H^+)$ 480, found 480.



2.24b 2.24

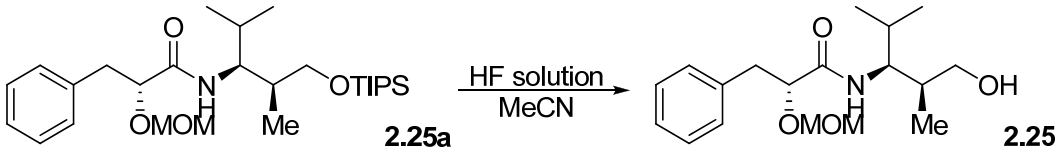
Compound 2.24: To a solution of **2.24b** (36 mg, 0.075 mmol) in MeCN (0.9 mL) at 0 °C was added HF stock solution (0.79 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 6 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (20 mL) and extracted with DCM (3×25 mL), and the combined organic phases were dried over Na_2SO_4, then concentrated under reduced pressure. The crude residue was purified by flash column chromatography (100% EtOAc) to give **2.24** (24 mg, 98%) as a colorless solid.



2.16 2.23 2.25a

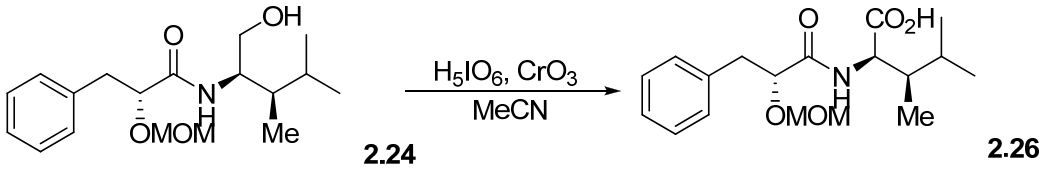
Compound 2.25a: To a solution of MOM-D-phenyllactic acid **2.23** (45 mg, 0.22 mmol) in DCM (6 mL) at 0 °C were added DEPBT (97 mg, 0.32 mmol) with stirring at 0 °C for 10 min., a solution of 2,6-lutidine (75 μL, 0.65 mmol) and **2.16** (52 mg, 0.18 mmol) in DCM (4 mL) was added, and then slowly warmed to R.T.. After stirring for 48 h, the reaction mixture was diluted with DCM (25 mL), washed with saturated aqueous $NaHCO_3$ (30 mL), 1 M HCl aqueous (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/ hexane 1:9) to afford **2.25a** (45 mg, 52%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.30-7.26 (m 4H), 7.24-7.18 (m, 1H), 6.98 (d, 1H, *J*=10.1 Hz),

4.52 (d, 1H, *J*=6.6 Hz), 4.39 (d, 1H, *J*=6.6 Hz), 4.33 (dd, 1H, *J*=3.1 Hz, *J*=9.4 Hz), 3.79 (dd, 1H, *J*=4.6 Hz, *J*=10.1 Hz), 3.72-3.65 (m, 1H), 3.56 (dd, 1H, *J*=4.1 Hz, *J*=10.1 Hz), 3.25 (dd, 1H, *J*=3.1 Hz, *J*=14.0 Hz), 2.98 (s, 3H), 2.86 (dd, 1H, *J*=9.5 Hz, *J*=14.0 Hz), 1.96-1.82 (m, 2H), 1.16-1.09 (m, 21H), 0.95 (dd, 6H, *J*=2.5 Hz, *J*=6.8 Hz), 0.90 (d, 3H, J=6.7Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.9, 138.1, 129.8, 128.4, 126.6, 95.9, 78.9, 65.6, 57.5, 55.7, 40.0, 35.9, 31.0, 20.3, 19.1, 18.3, 18.2, 15.7, 12.1; $[\alpha]_D$ +36.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{27}H_{49}NO_4Si$ calculated ($M+H^+$) 480, found 480.

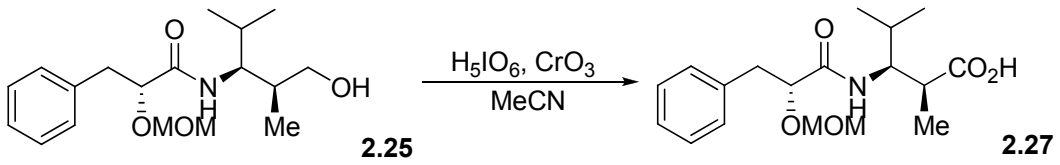


Compound 2.25：To a solution of **2.25a** (43 mg, 0.09 mmol) in MeCN (0.9 mL) at 0 ◦C was added HF stock solution (0.79 mL, prepared by mixing 43 mL MeCN, 4.5 mL H_2O, and 7.5 mL 48% aqueous HF solution), then warmed to R.T.. After stirring for 4 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$ (25 mL) and extracted with DCM (3×30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:1) to afford **2.25** (25 mg, 86%), as a colorless solid, m.p. 59-60 ◦C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.33-7.27 (m, 4H), 7.26-7.21 (m, 1H), 6.69 (d, 1H, *J*=9.8 Hz), 4.62 (d, 1H, *J*=6.5 Hz), 4.52 (d, 1H, *J*=6.5 Hz), 4.39 (dd, 1H, *J*=3.4 Hz, *J*=8.3 Hz), 3.75-3.69 (m, 1H), 3.37 (dd, 1H, *J*=3.0 Hz, *J*=11.8 Hz), 3.32-3.25 (m, 2H), 3.14 (s, 3H), 2.95 (dd, 1H, *J*=8.3 Hz, *J*=14.1 Hz), 2.89 (s, 1H), 2.08-1.99 (m, 1H), 1.52-1.43 (m, 1H), 1.01 (d, 3H, *J*=6.9 Hz), 0.92 (d, 3H, *J*=6.8 Hz), 0.85 (d, 3H, *J*=6.9 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 173.0, 137.4, 129.9, 128.5, 126.9, 96.3, 78.8, 64.8, 56.1, 55.1, 39.2, 37.4, 27.8, 20.8, 15.5, 15.2; $[\alpha]_D$ +78.6 (c 1.0, $CHCl_3$); ESI/MS for $C_{18}H_{29}NO_4$

calculated ($M+H^+$) 324, found 324.

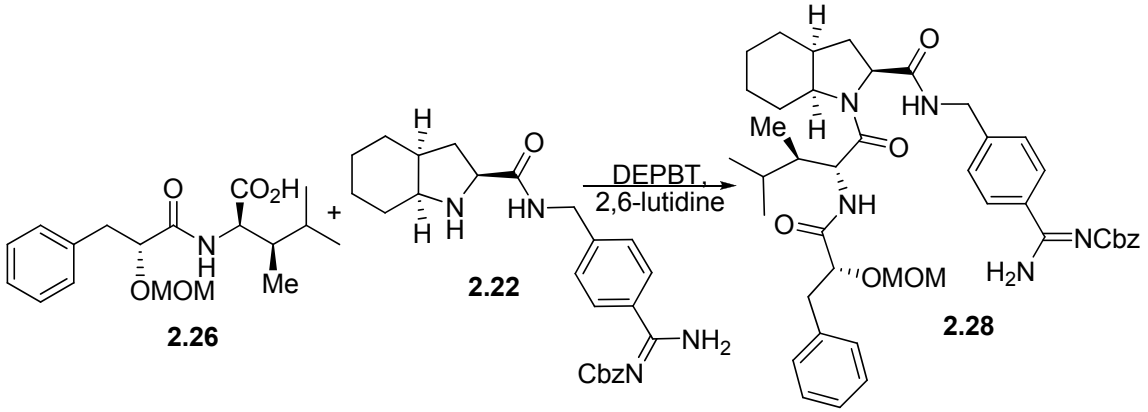


Compound .26: A solution of **2.24** (23 mg, 0.07 mmol) in MeCN (2.5 mL) was cooled to 0 °C and a solution of 1.0 mg CrO_3 dissolved in 420 μL of 0.4 M H_5IO_6 in wet MeCN (MeCN/H_2O 99.3:0.7) was added. After stirring at 0 °C for 30 min., the reaction mixture was quenched at same temperature by adding 2.4 mL saturated aqueous $NaHCO_3$. After MeCN was evaporated, the aqueous mixture was treated with 1 mL of 0.5 M aqueous NaOH, and washed with DCM (15 mL). The aqueous layer was acidified with 0.5 M HCl aqueous (10 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give carboxylic acid **2.26** (19 mg, 82%), as a colorless oil, which was sufficiently pure for use in the following step. ^1H NMR, (400 MHz, $CDCl_3$) δ 10.4 (s, 1H), 7.31-7.22 (m, 5H), 7.03 (d, 1H, *J*=8.8 Hz), 4.65-4.58 (m, 3H), 4.40 (dd, 1H, *J*=3.6 Hz, *J*=6.9 Hz), 3.22-3.14 (m, 4H), 2.99 (dd, 1H, *J*=6.9 Hz, *J*=14.0 Hz), 1.62-1.50 (m, 2H), 0.95 (d, 3H, J=6.6 Hz), 0.78 (dd, 6H, *J*=6.6 Hz, *J*=11.4 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 176.2, 171.8, 136.9, 130.0, 128.4, 126.9, 96.1, 77.9, 56.2, 55.0, 42.1, 38.9, 28.6, 21.5, 18.2, 11.8; $[\alpha]_D$ +45.5 (c 1.0, $CHCl_3$); ESI/MS for $C_{18}H_{27}NO_5$ calculated ($M+H^+$) 338, found 338.



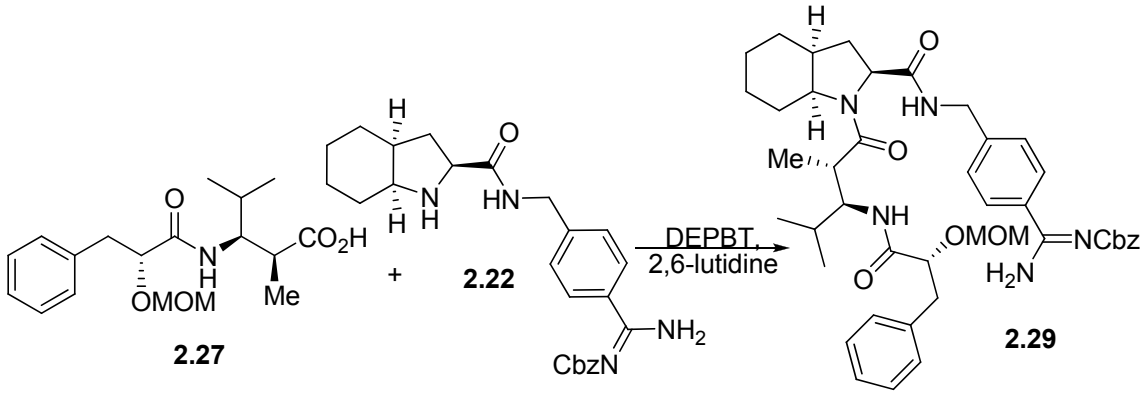
Compound 2.27: A solution of alcohol **2.25** (24 mg, 0.075 mmol) in MeCN (2.5 mL) was cooled to 0 °C and a solution of 1.0 mg CrO_3 dissolved in 450 μL

of 0.4 M H_5IO_6 in wet MeCN (MeCN/H_2O, 99.3:0.7) was added. After stirring at 0 °C for 30 min., the reaction mixture was quenched by adding 2.4 mL saturated aqueous $NaHCO_3$. After MeCN was evaporated, the aqueous mixture was treated with 1 mL 0.5 M aqueous NaOH, and washed with DCM (15 mL). The aqueous layer was acidified with 0.5 M HCl aqueous (10 mL) and extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give carboxylic acid **2.27** (21 mg, 82%), as a colorless oil, which was sufficiently pure for use in the following step. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 10.6 (s, 1H), 7.44 (d, 1H, *J*=10.2 Hz), 7.35-7.25 (m, 4H), 7.23-7.18 (m, 1H), 4.59 (d, 1H, *J*=6.7 Hz), 4.48 (d, 1H, *J*=6.7 Hz), 4.39 (dd, 1H, *J*=3.1 Hz, *J*=8.7 Hz), 3.82-3.76 (m, 1H), 3.26 (dd, 1H, *J*=3.1 Hz, *J*=14.0 Hz), 3.12 (s, 3H), 2.93-2.83 (m, 2H), 1.85-1.76 (m, 1H), 1.07 (d, 3H, *J*=7.1 Hz), 0.98 (d, 3H, *J*=6.6 Hz), 0.91 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 180.4, 172.5, 137.6, 129.9, 128.5, 126.8, 96.4, 78.9, 56.6, 56.1, 40.1, 39.5, 31.6, 20.0, 19.5, 15.6; $[\alpha]_D$ +20.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{18}H_{27}NO_5$ calculated ($M+H^+$) 338, found 338.



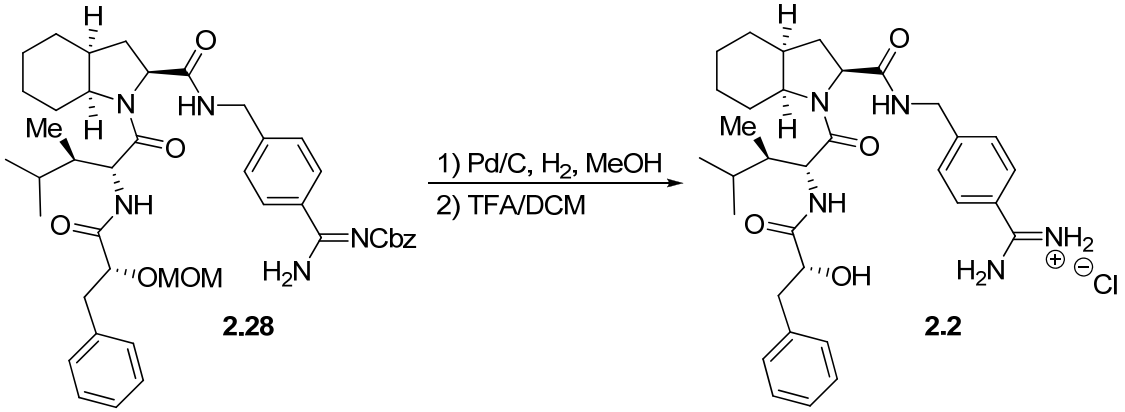
Compound 2.28: To a solution of **2.22** (29 mg, 0.085 mmol) and carboxylic acid **2.26** (44 mg, 0.10 mmol) in DCM (2 mL) at 0 °C were added fresh *recrystallized* DEPBT (45 mg, 0.15 mmol), and 2,6-lutidine (35 μL, 0.30 mmol), and then warmed to R.T.. After stirring for 20 h, the reaction mixture was

diluted with DCM (20 mL), washed with 0.5 M HCl aqueous (20 mL), 5% Na_2CO_3 aqueous (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/toluene 1:3 to EtOAc/hexane 3:1 to 100% EtOAc to MeOH/$CHCl_3$ 1:49) to afford **2.28** as a colorless oil (47 mg, 74%). ^{1}H NMR, (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 7.81 (d, 2H, *J*=8.0 Hz), 7.56-7.52 (m, 1H), 7.46 (d, 2H, *J*=6.8 Hz), 7.38-7.14 (m, 9H), 7.07 (d, 2H, *J*=6.8 Hz), 6.79 (d, 1H, *J*=6.8 Hz), 5.23 (s, 2H), 4.76 (dd, 1H, *J*=7.6 Hz, *J*=16.0 Hz), 4.62-4.56 (m, 1H), 4.38 (d, 1H, *J*=6.8 Hz), 4.32 (d, 1H, *J*=6.8 Hz), 4.29 (d, 1H, *J*=4.4 Hz), 4.25 (d, 1H, *J*=4.4 Hz), 3.92-3.89 (br, t, 1H), 3.68 (dd, 1H, *J*=3.4 Hz, *J*=7.8 Hz), 3.04 (s, 3H), 2.98 (dd, 1H, *J*=3.2 Hz, *J*=14.2 Hz), 2.74 (dd, 1H, *J*=7.9 Hz, *J*=14.1 Hz), 2.32-2.23 (m, 4H), 1.84-1.15 (m, 9H), 0.88 (d, 3H, *J*=6.9 Hz), 0.77 (dd, 6H, *J*=1.9 Hz, *J*=6.9 Hz); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 174.2, 173.7, 172.3, 170.5, 165.2, 144.9, 138.5, 138.4, 134.3, 131.0, 129.6, 129.3, 129.21, 129.17, 129.16, 128.9, 128.5, 127.8, 97.0, 78.6, 68.2, 61.8, 60.5, 56.6, 54.4, 43.7, 42.7, 39.7, 38.8, 32.0, 28.9, 27.8, 26.7, 25.0, 21.9, 21.2, 16.1, 9.6; $[\alpha]_D$ +9.6 (c 1.0, $CHCl_3$); ESI/MS for $C_{43}H_{55}N_5O_7$ calculated ($M+H^+$) 754, found 754.



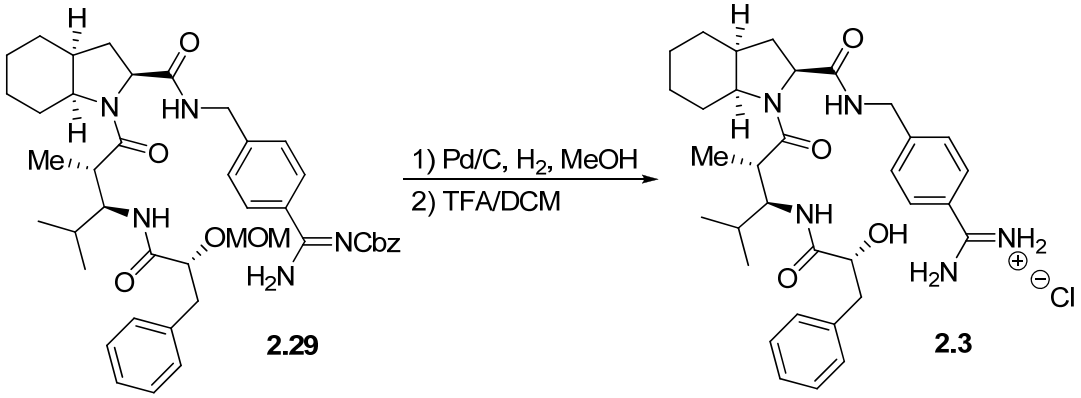
Compound 2.29: To a solution of **2.22** (20 mg, 0.06 mmol) and carboxylic acid **2.27** (31 mg, 0.072 mmol) in DCM (2 mL) at 0 °C were added fresh *recrystallized* DEPBT (31 mg, 0.11 mmol), and 2,6-lutidine (24 μL, 0.21 mmol),

and then warmed to R.T.. After stirring for 20 h, the reaction mixture was diluted with DCM (20 mL), washed with 0.5 M HCl aqueous (20 mL), 5% Na_2CO_3 aqueous (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/toluene 1:3 to EtOAc/hexane 3:1 to 100% EtOAc to MeOH/$CHCl_3$ 1:99) to afford **2.29** as a colorless oil (31 mg, 69%). ^{1}H NMR, (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 8.32 (d, 1H, *J*=9.9 Hz), 7.83 (d, 2H, *J*=8.2 Hz), 7.53-7.17 (m, 14H), 5.22 (s, 2H), 4.61-4.34 (m, 5H), 3.76-3.66 (m, 2H), 3.22 (dd, 1H, *J*=3.1 Hz, *J*=14.0 Hz), 3.02 (s, 3H), 2.90 (dd, 1H, *J*=8.8 Hz, *J*=14.0 Hz), 2.79 (dd, 1H, *J*=3.4 Hz, *J*=7.1 Hz), 2.67-2.58 (m, 1H), 2.32-2.19 (m, 1H), 2.03-1.95 (m, 1H), 1.85-1.18 (m, 10H), 0.97 (d, 3H, *J*=7.1 Hz), 0.82 (dd, 6H, *J*=6.7 Hz, *J*=12.3 Hz); ^{13}C NMR, (100 MHz, MeOH-*d*$_4$) δ 176.3, 174.43, 174.37, 170.4, 154.3, 144.9, 138.9, 138.4, 134.6, 131.0, 129.6, 129.4, 129.2, 129.1, 128.7, 127.7, 97.3, 79.8, 68.1, 61.8, 59.80, 59.76, 56.5, 43.8, 40.4, 39.0, 38.7, 33.2, 32.0, 29.5, 26.8, 25.0, 21.15, 21.10, 20.3, 16.9,; $[\alpha]_D$ -12.0 (c 1.0, $CHCl_3$); ESI/MS for $C_{43}H_{55}N_5O_7$ calculated ($M+H^+$) 754, found 754.



Compound 2.2: To a solution of **2.28** (34 mg, 0.045 mmol) in MeOH (2 mL) was added Pd/C 10 wt.% (6 mg) and the reaction mixture was stirred under H_2 (1 atm) at R.T. for 10 h. The Pd/C catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford benzamidine (18 mg)

as a colorless oil, which was used directly. The crude benzamidine was treated with >99.9% TFA/DCM (9:1, 2 mL) and 1 drop of H_2O. After stirring at R.T. for 3 h, the reaction mixture was diluted with EtOAc (15 mL) and concentrated under reduced pressure. The crude residue was purified by RP-LC-MS (85 min. gradient of 20-80% CH_3CN in 0.05% aqueous TFA) to afford final **2.2** (18 mg, 57%, over two steps) as the TFA salt. The TFA salt of the product was transformed to the hydrochloride salt (16 mg) and analyzed. ^{1}H NMR, (400 MHz, MeOH-d_4) δ 8.61 (s, 1H), 7.74 (d, 2H, J=6.8 Hz), 7.56 (d, 2H, J=6.9 Hz), 7.37 (br, d, 1H), 7.21 (br, m, 5H), 4.57 (d, 2H, J=12.5 Hz), 4.43-4.36 (br, m, 2H), 4.15 (br, m, 1H), 3.96 (br, m, 1H), 3.01 (d, 1H, J=13.6 Hz), 2.82 (br, dd, 1H), 2.38 (br, m, 1H), 2,15 (br, m, 2H), 2.04 (br, dd, 1H), 1.80-1.58 (br, m, 5H), 1.50 (br, d, 1H), 1.41 (br, m, 1H), 1.29 (d, 2H, J=15.0 Hz), 0.80 (d, 3H, J=5.9 Hz), 0.73 (t, 6H, J=7.1 Hz); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 175.4, 174.6, 172.4, 168.2, 147.2, 138.7, 131.0, 129.15, 129.12, 129.0, 128.0, 127.6, 73.8, 61.8, 60.4, 54.1, 43.6, 42.9, 41.3, 38.7, 32.1, 28.9, 27.6, 26.6, 24.9, 21.8, 21.2, 16.0, 9.5; $[\alpha]_D$ +70.7 (c 0.10, MeOH); HRMS (Not salt, Free amine) for $C_{33}H_{45}N_5O_4$ calculated ($M+H^+$) 576.35443, found 576.35492.



Compound 2.3: To a solution of **2.29** (30 mg, 0.04 mmol) in MeOH (2 mL) was added Pd/C 10 wt.% (6 mg) and the reaction mixture was stirred under H_2 (1 atm) at R.T. for 10 h. The Pd/C catalyst was removed by filtration and the

filtrate was concentrated under reduced pressure to afford the benzamidine (19 mg) as a colorless oil, which was used directly. The crude benzamidine was treated with >99.9% TFA/DCM (9:1, 2 mL) and 1 drop of H_2O. After stirring at R.T. for 3 h, the reaction mixture was diluted with EtOAc (15 mL) and concentrated under reduced pressure. The crude residue was purified by RP-LC-MS (85 min. gradient of 20-80% CH_3CN in 0.05% aqueous TFA) to afford final **2.3** (16 mg, 59%, over two steps) as the TFA salt. The TFA salt of the product was transformed to the hydrochloride salt (15 mg) and analyzed. ^{1}H NMR, (400 MHz, MeOH-d_4) δ 9.22 (s, 1H), 8.72 (s, 1H), 7.76 (d, 2H, J=7.9 Hz), 7.61 (d, 2H, J=7.9 Hz), 7.26-7.22 (br, m, 4H), 7.19-7.15 (br, m, 1H), 4.66 (d, 1H, J=16.2 Hz), 4.39-4.35 (m, 2H), 4.26 (d, 1H, J=7.5 Hz), 3.95-3.89 (br, m, 1H), 3.75 (br, m, 1H), 3.05 (d, 1H, J=14.2 Hz), 2.90-1.96 (br, m, 1H), 2.76 (d, 1H, J=8.4 Hz, J=14.0 Hz), 2.41 (br, m, 1H), 2.11 (t, 2H, J=9.2 Hz), 2.00-1.95 (br, m, 1H), 1.85-1.73 (m, 4H), 1.54-1.50 (m, 1H), 1.43-1.23 (m, 3H), 1.00 (d, 3H, J=6.4 Hz), 0.95 (d, 3H, J=6.6 Hz), 0.85 (d, 3H, J=6.6 Hz); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 176.3, 176.1, 174.8, 168.5, 147.4, 139.7, 130.8, 129.3, 129.24, 129.19, 128.1, 127.5, 74.4, 62.0, 59.7, 59.0, 43.7, 41.5, 39.4, 39.1, 32.6, 32.1, 29.8, 26.9, 25.0, 21.2, 19.2, 16.6; $[\alpha]_D$ +24.7 (c 0.10, MeOH); HRMS (Not salt, Free amine) for $C_{33}H_{45}N_5O_4$ calculated ($M+H^+$) 576.35443, found 562.35369.

General Procedure for Recrystallization of DEPBT: DEPBT (5 g) was dissolved in DCM (50 mL), and then filtered out the undissolved solid, which is probably the acid. The solution was washed with saturated aqueous Na_2CO_3 (3×30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was redissolved in very small amount of hot EtOAc (reflux, a few drops), and then petroleum ether (20 mL) was added. The solution was stand

at R.T. for 1 h, then removed the solution, and washed with petroleum ether (2 mL) to afford recrystallized DEPBT (4 g), as a colorless crystalline solid. The recrystallized DEPBT was stored in the 0 ^{o}C under the argon atmosphere.

2-5 References:

1. M. Murakami, Y. Okita, H. Matsuda, T. Okino, and K. Yamaguchi, *Tetrahedron Lett.,* **1994**, *35*, 3129.
2. K. Ersmark, J. R. Del Valle, and S. Hanessian, *Angew. Chem. Int. Ed.,* **2008**, *47*, 1202.
3. A. R. Carroll, G. K. Pierens, G. Fechner, P. de Almeida Leone, A. Ngo, M. Simpson, E. Hyde, J. N. A. Hooper, S.-L. Bostrom, D. Musil, and R. J. Quinn, *J. Am. Chem. Soc.,* **2002**, *124*, 13340.
4. S. Hanessian, M. Tremblay, and J. F. W. Petersen, *J. Am. Chem. Soc.,* **2004,** *126*, 6064.
5. S. Hanessian, J. R. Del Valle, Y. Xue, and N. Blomberg, *J. Am. Chem. Soc.,* **2006,** *128*, 10491.
6. J. L. Rios Steiner, M. Murakami, and A. Tulinsky, *J. Am. Chem. Soc.,* **1998,** *120*, 597.
7. a) N. Valls, M. López-Canet, M. Vallribera, and J. Bonjoch, *J. Am. Chem. Soc.,* **2000**, *122*, 11248; b) P. Wipf and J. L. Methot, *Org. Lett.,* **2000**, *2*, 4213. c) N. Valls, M. López-Canet, M. Vallribera, and J. Bonjoch, *Chem. Eur. J.,* **2001**, *7*, 3446. d) N. Valls, M. Vallribera, S. Carmeli, and J. Bonjoch, *Org. Lett.,* **2003**, *5*, 447.
8. Hemostasis and Thrombosis. *Basic Principles and Clinical Practice,* Eds. R. W. Colman, J. L. Hirsh, J. V. Marder, A. W. Clowes, J. N. George, Lippincott Williams & Wilkins, Philadelphia, 2001, p. 3.
9. J. D. Kulman and E. W. Davie, *Encyclopedia of Biological Chemistry,* **2004**,

3, 457.

10. E. W. Davie, *J. Biol. Chem.,* **2003**, *278*, 50819.
11. B. Dahlbäck, *Lancet,* **2000**, *355*, 1627.
12. R. W. Colman, A. W. Clowes, J. N. George, J. L. Hirsch, and J. V. Marder, *Overview of Homeostasis*, 4th ed. (Eds.: R. W. Colman, J. L. Hirsh, J. V. Marder, A. W. Clowes, J. N. George), Lippincott Williams & Wilkins, Philadelphia, 2001, p. 3.
13. E. W. Davie and J. D. Kulman, *Semin. Thromb. Hemostasis*, **2006**, *32*, 3.
14. G.H. Goetz, G.G. Harrigan, J.J. Likos, and T.P. Kasten, *PCT WO03/ 051831*, 2003.
15. S. Hanessian, R. Margarita, A. Hall, S. Johnstone, M. Tremblay, and L. Parlanti, *J. Am. Chem. Soc.,* **2002,** *124*, 13342.
16. T. J. Martin, *Highlights Bioorg. Chem.,* **2004**, 239.
17. J. D. Kulman and E. W. Davie, *Encycl. Biol. Chem.,* **2004**, *3*, 457.
18. A. Noel, C. Gilles, K. Bajou, L. Devy, and F. L. Kebers, *Invasion Metastasis,* **1997**, *17*, 221.
19. K. Ishida, Y. Okita, H. Matsuda, T. Okino, and M. Murakami, *Tetrahedron,* **1999**, *55*, 10971.
20. R. Banker and S. Carmeli, *Tetrahedron,* **1999**, *55*, 10835.
21. S. Hanessian, K. Ersmark, X. Wang, J. R. Del Valle, N. Blomberg, Y. Xue, and O. Fjellstrom, *Bioorg. & Medicinal Chem. Lett.,* **2007**, *17*, 3480.
22. M. Meguro, N. Asao, and Y. Yamamoto, *Tetrahedron Lett.,* **1994**, *35*, 7395.
23. B. E. Haskell and S. B. Bowlus, *J. Org. Chem.,* **1984**, *49*, 953.
24. R. C. Roemmele and H. Rapoport, *J. Org. Chem.,* **1988**, *53*, 2367.
25. R. S. Compagnone and H. Rapoport, *J. Org. Chem.,* **1986**, *51*, 1713.
26. E. Vedejs and S. Lin, *J. Org. Chem.,* **1994**, *59*, 1602.

Chapter Three

Total Synthesis and Structural Revision of the Presumed Aeruginosin 205B

3-1 Introduction

3-1-1 Revision of proposed and misassigned structures of aeruginosins through total synthesis

The aeruginosins are classified as natural products produced by the cyanobacterium *Microcystis aeruginosa* that incorporate a new azabicyclic amino acid, 2-carboxy-6-hydroxyoctahydroindole (Choi). To date, the total syntheses of seven aeruginosins have been completed, four of these involving revisions to the originally proposed structures.[1-14]

3-1-1-1 Revision of aeruginosin 298A

In 1994, aeruginosin 298A was isolation from from *Microcystis* aeruginosa by Murakami and coworkers.[1] The structure of aeruginosin 298A (**3.1a** in Figure 9)[1,2] was initially elucidated through 2D NMR studies. In Murakami's initial report,[1] a linear peptide containing four subunits was proposed: a reduced arginine (L-Argol), a 2-carboxy-6-octahydroindole (L-Choi) core, a L-leucine, and an hydroxyphenyllactic acid (D-Hpla), based on degradation and derivatization of the acid hydrolyzate.

In 1998, Tulinsky and coworkers[2] published an X-ray crystallographic structure of a ternary complex of **3.1a** bound to a hirugen–thrombin complex, thus providing an absolute stereochemical configuration. The crystal structure of the ternary complex revealed some unexpected interactions between **3.1a** and the binding pocket of thrombin. L-Leu was found to occupy the hydrophobic D-enantiomorphic S3 subsite. In addition, the Hpla subunit was also found to interact with the S3 subsite, which was expected to accommodate the L-Leu subunit. These key observations cast some doubt on the stereochemical assignments initially made for aeruginosin 298A, and would not be resolved until the proposed structure (**3.1a**) was revised through total synthesis by the

groups of Bonjoch[3,4] and Wipf[5] independently in 2000 (see revised structure **3.1b** in Figure 9).

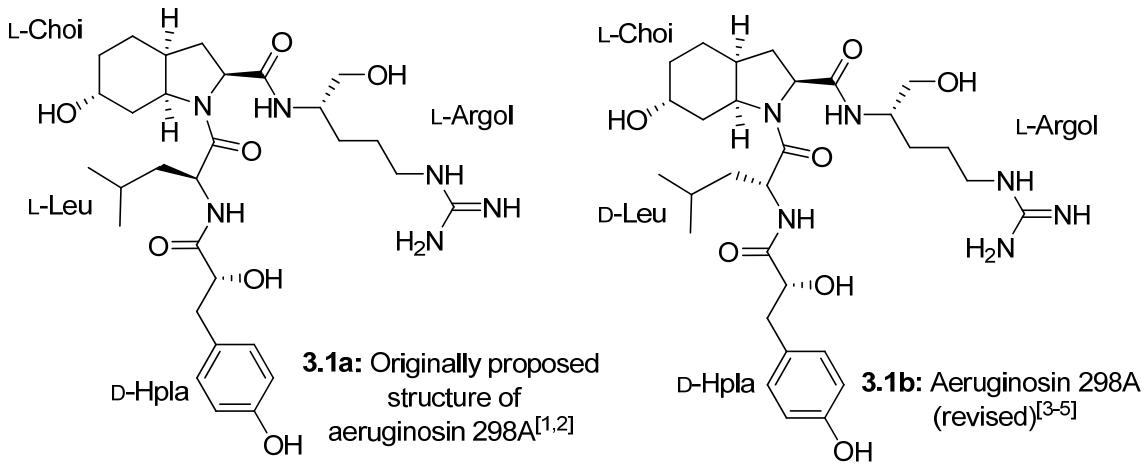


Figure 9. Originally proposed and revised structures of aeruginosin 298A.

3-1-1-2 Revision of oscillarin

In 1997, researchers at Boehringer Mannheim GmbH (now Roche Diagnostics) in Germany disclosed the isolation and characterization of a new compound **3.2a** (Figure 10) called oscillarin, from algal cultures of *Oscillatoria agardhii*, and originally isolated from Lake Kasumigaura in Japan.[6]

The structure and absolute configuration were proposed on the basis of NMR data and a partially resolved co-crystal complex with trypsin. Oscillarin was proposed to comprise a D-phenyllactic acid (D-Pla), a D-Phe subunit, a L-Choi subunit and a cyclic guanidine containing P1 subunit. The structure was later revised to be **3.2b** (Figure 10) without any change in the original data.[7] The revised structure of oscillarin **3.2b** was confirmed by total synthesis, and by high-resolution X-ray crystallography of a thrombin–oscillarin complex by Hanessian and coworkers.[8] Oscillarin comprises a D-phenyllactic acid (D-Pla), a D-phenylalanine, a L-Choi coin, and an unique heterocyclic motif, described as 1-(*N*-amidino-Δ^3-pyrrolino)ethyl subunit (Adc).

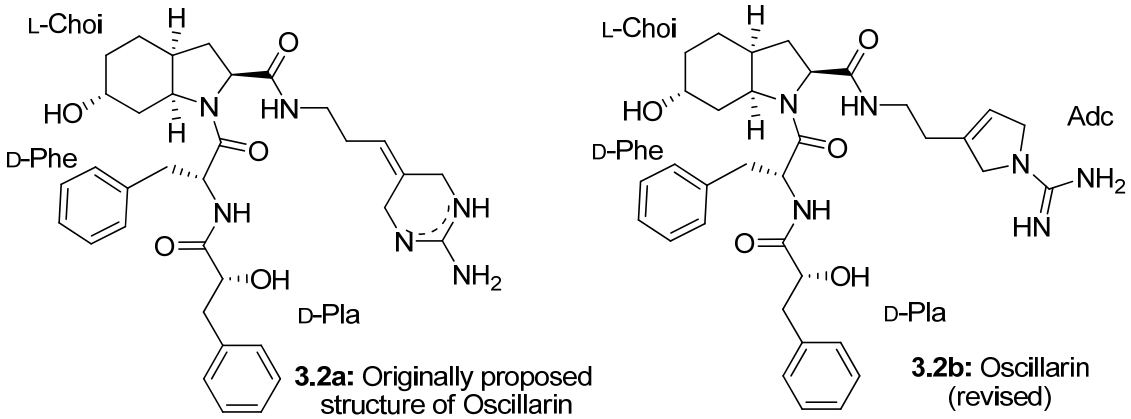


Figure 10. Originally proposed and revised structures of oscillarin.

3-1-1-3 Total synthesis of dysinosin A and chlorodysinosin A

The unique Adc heterocyclic motif is also present in dysinosin A **3.3a** (Figure 11),[9,10] whose isolation and characterization were reported by Quinn and coworkers in 2002.[10] Dysinosin A, isolated from the extracts of sponge belonging to the family *Dysideidae,* is a functionally novel aeruginosin. Its structural elucidation was achieved through a combination of NMR and degradation studies, while the stereochemical assignments were confirmed by the X-ray structure of a ternary complex of dysinosin A, thrombin, and hirugen.[10] The familiar perhydroindole core structure of Dysinosin A harbors an additional hydroxy group at C5, resulting in a trans-diaxial orientation. The N-terminal residues are D-Leu and a sulfated glyceric acid derivative not previously found in the other aeruginosins. A total synthesis of dysinosin A was reported by Hanessian and coworkers.[11]

In 2003, the Pharmacia Corporation[12] disclosed the isolation and characterization of a chlorinated dysinosin A derivative **3.3b** (Figure 11) later, named as chlorodysinosin A following its total synthesis by Hanessian and coworkers.[13] It contains 3-chloroleucine (Cleu), an amino acid unknown in the

natural-product literature. The basic structure of **3.3b** was first deduced by NMR spectroscopy and degradation studies, and its structure and absolute configuration have recently been confirmed by total synthesis and a high resolution X-ray structure with thrombin.[13]

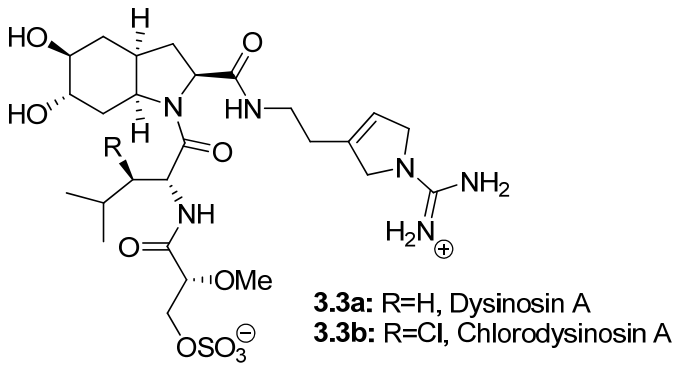
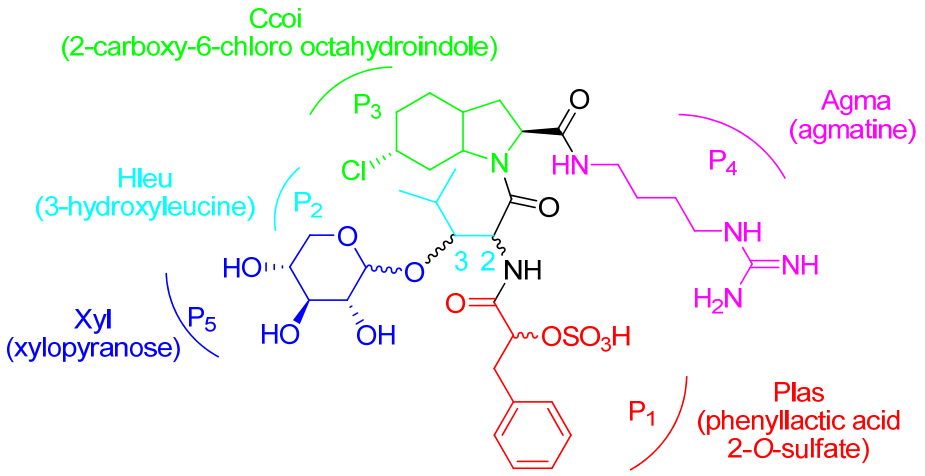


Figure 11. Structure of dysinosin A and chlorodysinosin A.

3-1-2 Original structure of aeruginosins 205A and B

In 1997, Murakami and coworkers[15] isolated new aeruginosins from the genus *Microcystis,* designed as 205A and 205B. Two compounds were isolated from the cyanobacterium *Oscillatoria agardhii*, which was collected from Lake Kasumigaura in Japan. These glycopeptides are potent inhibitors of trypsin (IC_{50}=0.07 μg/mL) and thrombin (IC_{50}=1.5 μg/mL 205A, IC_{50}=0.17 μg/mL 205B).[15] A characteristic isotopic and fragmentation pattern in positive-ion FAB mass spectrum indicated that there is a chlorine atom and a sulfate group in the molecules.

Extensive spectroscopic studies based on previous reports that related to aeruginosins,[1,14,16] suggested the presence of five subunits: 1) P_1 subunit is Plas (phenyllactic acid 2-*O*-sulfate), 2) P_2 subunit is Hleu (3-hydroxyleucine), 3) P_3 subunit is Ccoi (2-carboxy-6-chloro octahydroindole), 4) P_4 subunit is Agma (agmatine), and 5) P_5 subunit is Xyl (xylopyranose), assembled as a linear peptide array, and shown in expressions **3.4a** and **3.4b** (Figure 12).



3.4a: Aeruginosins 205A L-Plas, *(2R, 3S)*-Hleu;
3.4b: Aeruginosins 205B D-Plas, *(2S, 3R)*-Hleu;

Figure 12. Originally proposed structures of aeruginosins 205A and B.

The absolute stereochemistry of these aeruginosins was determined by acid hydrolysis and HPLC analysis of appropriate derivatives.[15]

Hydrolysis of the peptides and derivatization of the Plas residues as their methyl esters showed an L- and D-Plas configuration for aeruginosins 205A and 205B, respectively.

Although the relative stereochemistry of the Ccoi residue was determined from the NMR coupling constants and by NOE analysis, however the absolute stereochemistry of the Ccoi subunit remained undisclosed.

The *(2R,3S)*- and *(2S,3R)*-stereochemistry of Hleu, respectively, were assigned after derivatization with Marfey's reagent and comparison of HPLC retention times with the authentic three of the four possible diastereomers of the same compound. Since the retention time did not match, the stereochemistry was assigned by exclusion.

An assignment of D-xylose arose from GC analysis of the degradation fragment on a chiral column.

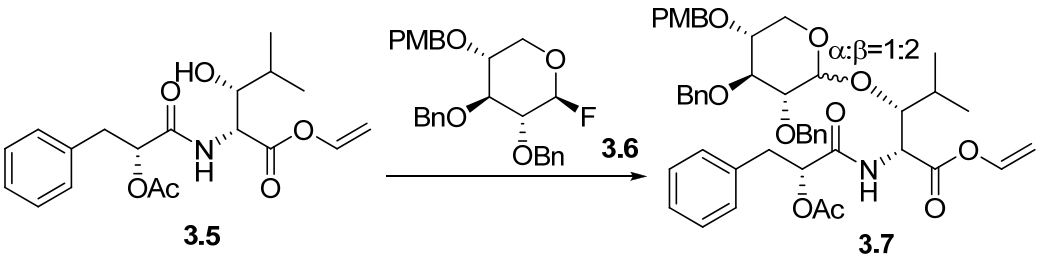
205A and 205B were the only known glycopeptidic aeruginosins at the time of their discovery, and they are among a very small group of sugar-containing natural products isolated from cyanobacteria.[9,17-22] The elucidation of their structures also resulted in the identification of two new structural units named Ccoi and Plas, respectively. The third residue, Hleu, is also quite rare among natural products.

3-1-3 Revised structure of aeruginosins 205A and 205B

There has been an ongoing debate over the definitive structures of aeruginosins. In spite of a seemingly definitive evidence for the original and revised structures of **3.4a** and **3.4b**, recent developments in the total synthesis of other members of this family of glycopeptides have cast some doubt on the structural and stereochemical assignments.

3-1-3-1 The first revision of aeruginosins 205A and 205B

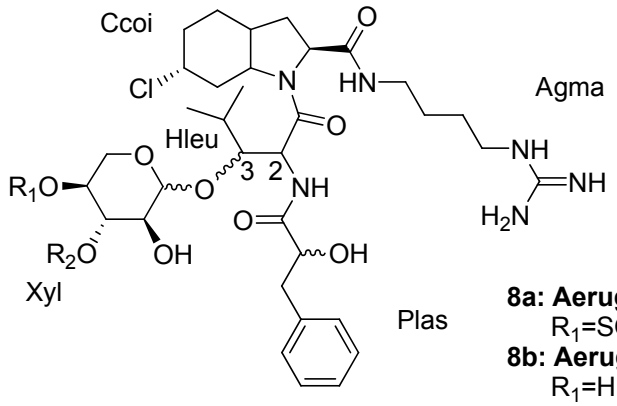
In 2003, Toyooka, Nemoto and coworkers[23] synthesized glycopeptides model compounds **3.7a** and **3.7b** (α and β isomers)(Scheme 21).



Scheme 21: Reagents and conditions: $SnCl_2$, $AgClO_4$, MS 4A, Et_2O, -20 °C to r.t., 72%;

Based on the comparison of NMR data and the composition of other members of aeruginosin family, the authors revised the position of the sulfate group, which was originally thought to be on the Plas residue, to the 3- or

4-position of the xyl residue, while maintaining the Ccoi subunit intact. The proposed revised structures of aeruginosins 205A and B are showed as **3.8a** and **3.8b** in the Figure 13.

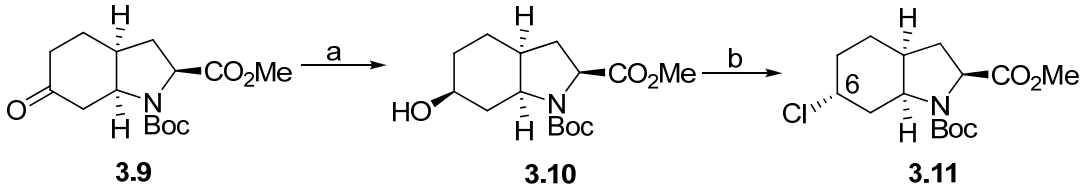


8a: Aeruginosins 205A
R_1=SO_3H, R_2=H, L-Plas, *(2R, 3S)*-Hleu;
8b: Aeruginosins 205B
R_1=H, R_2=SO_3H, D-Plas, *(2S, 3R)*-Hleu;

Figure 13. First revision of aeruginosins 205A and B by Toyooka/Nemoto.[23]

3-1-3-2 The synthesis of the supposed Ccoi core

The position of the chlorine atom on aeruginosins 205A and 205B has been called into question by definitive synthesis of the Ccoi subunit,[24] which was the original proposed structure by Murakami, Toyooka and their respective coworkers. Recently, Valls, Bonjoch and coworkers[24] reported the synthesis of the supposed Ccoi core (Scheme 22).



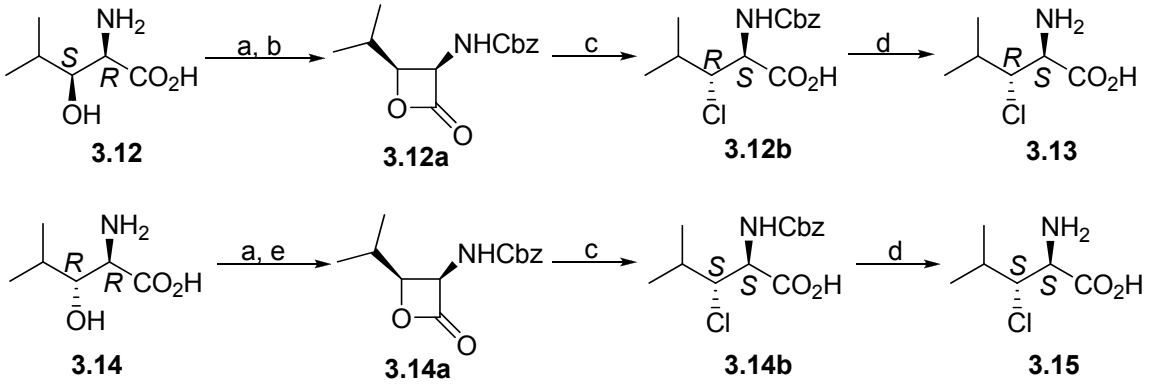
Scheme 22: Reagents and conditions: a) $NaBH_4$, $CeCl_3$, 91%; b) CCl_4, Ph_3P, MeCN, r.t., 88%;

When the NMR data of the synthetic Ccoi subunit was compared with the data of isolated natural products, some significant differences in the chemical shifts of the protons and carbons close to the 6-chlorine atom were found. For

example, in the ^{1}H NMR spectrum the H-6 methine proton of synthetic Ccoi shows a signal peak at δ=4.60, while in the spectrum of the natural products the resonance for this proton is recorded at δ=3.83. There are also a number of differences in the ^{13}C NMR spectrum, such as the C-6 methine carbon of synthetic Ccoi shows a peak at δ=59.1, while in the spectrum of the natural products the resonance for this carbon is recorded at δ=68.7. These results seemed to indicate that the chlorine atom of aeruginosins 205A and 205B is not on the 2-carboxyperhydroindole (Choi) core.

3-1-3-3 The second revision of aeruginosins 205A and 205B

In 2006, Valls and Bonjoch reported the synthesis of β-chloroleucine derivatives **3.13** and **3.15**, which consisted of the chlorine promoted ring opening of hydroxyleucine β-lactones **3.12** and **3.14** (Scheme 23).



Scheme 23: Reagents and conditions: a) Cbz-Suc, **3.12** 92%, **3.14** 92%; b) *O*-benzotriazole-*N,N,N′,N′*-tetramethyluronium-hexafluorophosphate (HBTU), **3.12** 83%; c) LiCl, THF, reflux, 90 h, **3.12** 46%, **3.14** 35%;d) H_2, Pd/C, EtOH, **3.12** 96%, **3.14** 85%; e) benzo-triazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate **(**PyBOP), **3.14** 95%;

The reported occurrence of a 3-chloroleucine in chlorodysinosin A[25] and a Choi subunit (as above discussion in B)[24] prompted Valls and Bonjoch to suspect that the same subunits might also be present in aeruginosin 205A and 205B. Thus, not only the originally proposed structures of aeruginosins 205 A

and B (**3.4a** and **3.4b**), but also the first revised structures **3.8a** and **3.8b** were incorrect. Furthermore, the authors proposed the structures of aeruginosins 205A and 205B to be as **3.16a** and **3.16b** (shown in Figure 14),[25] in which the sulfated xylose residue (*O*-sulfated D-xylopyranosyl ring) must be residing on the C-6 methine carbon of a 2-carboxy-6-hydroxyocta-hydroindole (Choi) subunit instead of the originally assumed Ccoi; and the chlorine atom was assumed to be part of a 3-chloroleucine (Cleu) residue as in chlorodysinosin A[13] instead of Hleu as originally proposed.

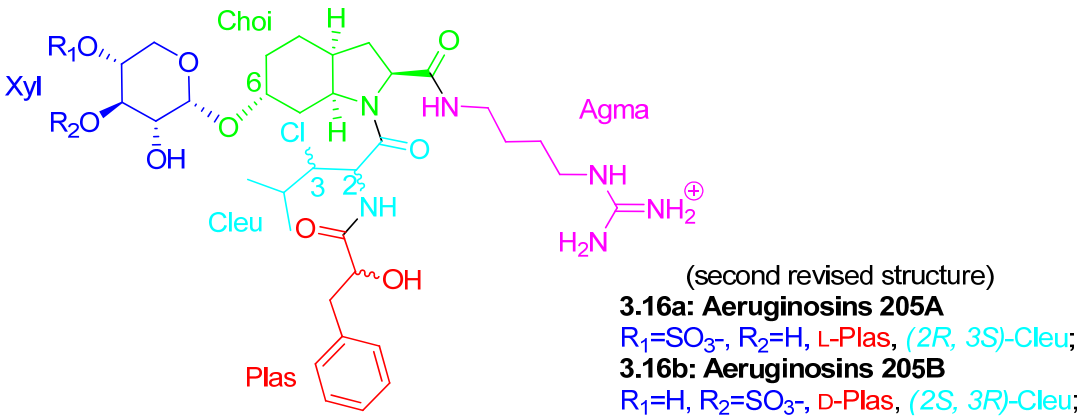


Figure 14. Second revision of aeruginosins 205A and B by Valls/Bonjoch.[25]

3-2 Synthesis of the presumed aeruginosin 205B

Based on the above information it seemed most likely that the structure of aeruginosin 205A and 205B should be revised as shown **3.16a** and **3.16b**. We therefore undertook the total synthesis of **3.16b** to validate this conclusion.

Based on the previous synthesis of compounds in aeruginosins' family, we envisaged the disconnections to the 5 subunits (Figure 15). These are 1) Agma subunit, an agmatine; 2) Choi subunit, a 2-carboxy-6-hydroxyoctahydroindole; 3) Xyl subunit, a D-xylose; 4) Cleu subunit, 3-chloroleucine; and 5) Pla subunit, a D-phenyllactic acid.

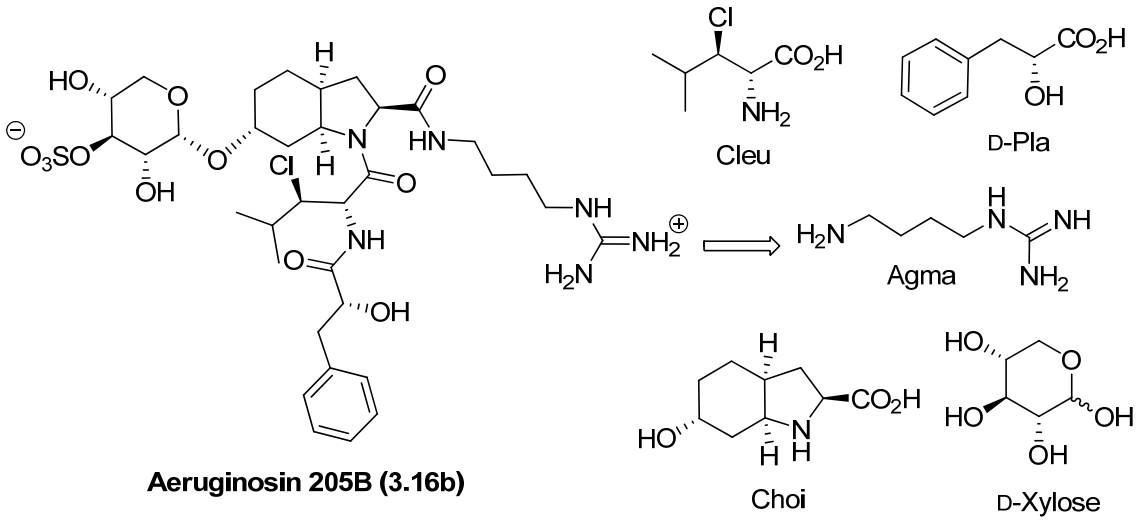


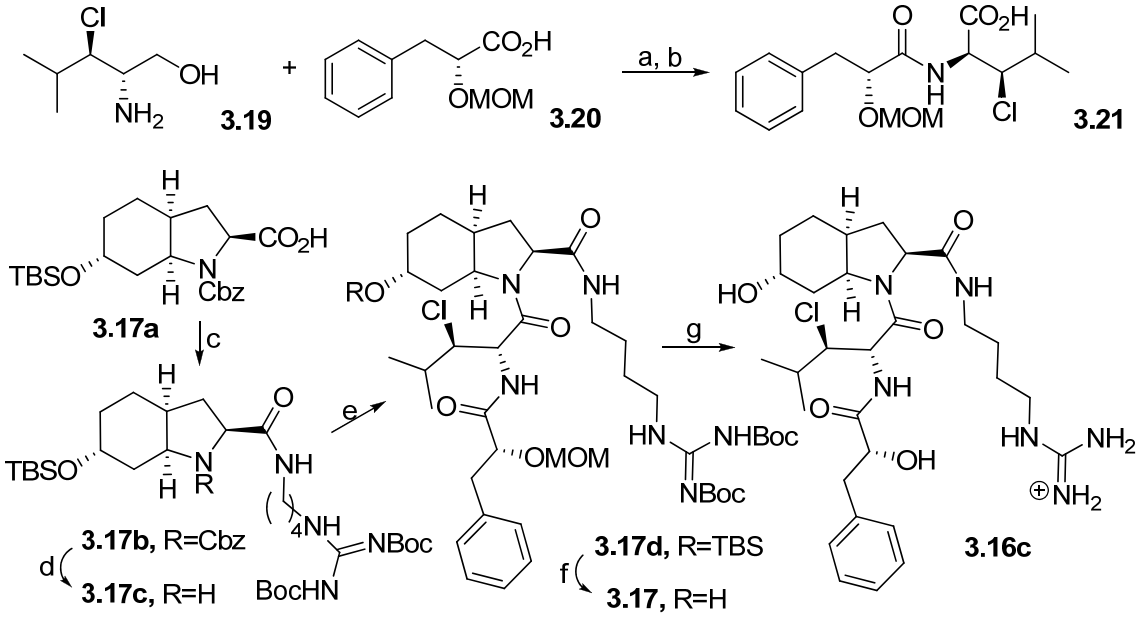
Figure 15. Retrosynthesis of the target compound.

3-2-1 Synthesis plan for aeruginosin 205B

3-2-1-1 Original attempt

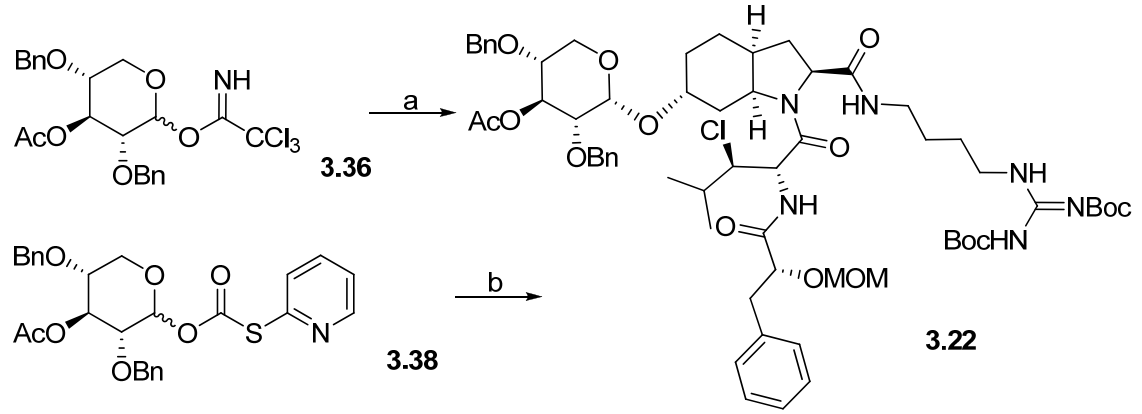
Initially, the previously published method to synthesize the aeruginosin family[14,26] was used to obtain the protected aglycone subunit **3.17** of aeruginosin 205B (Scheme 24) by postdoctoral associates Dr. Karolina Ersmark and Dr. Juan R. Del Valle.

First the Choi subunit **3.17a** was coupled with the Agma subunit **3.18** to afford compound **3.17c**. Next, the Cleu subunit **3.19** was coupled with Pla subunit **3.20**, followed by deprotection and oxidation to give free acid **3.21**. Finally, compound **3.17c** was coupled with free acid **3.21** to afford the protected aeruginosin 205B aglycone **3.17d**. Because of steric hindrance, the yield of the coupling reaction was low. Cleavage of the TBS ether group led to intermediate **3.17** ready to be coupled with the Xyl subunit to get our target. At the same time, the sequential deprotected of the *O*-MOM and *N*-Boc groups with TFA afforded the intended glycone **3.16c**, which inhibited the enzyme thrombin at IC_{50}=0.31 μM.



Scheme 24: Reagents and conditions: a) PyBOP, 2,6-lutidine, DCM, r.t., 27 h, 60%; b) cat. CrO_3 0.4 M H_5IO_6, wet MeCN, 0 °C, 45 min., 85%; c) *N,N*-bisBoc-agmatine **3.18**, PyBOP 2,6-lutidine,DCM, r.t., 16 h, 84%; d) H_2, $Pd(OH)_2/C$, EtOAc, r.t., 20 h, 95%; e) **3.21**, DEPBT, 2,6-lutidine, DCM, r.t., 4.5 h, 37%; f) HF, CH_3CN/H_2O, 0 °C, 8.5 h, 89%; g) TFA/DCM 1:1, r.t., 5 h, 61%;

Two coupling methods[27, 28] between compound **3.17** and Xyl subunit were studied to afford the product **3.22** (scheme 25).



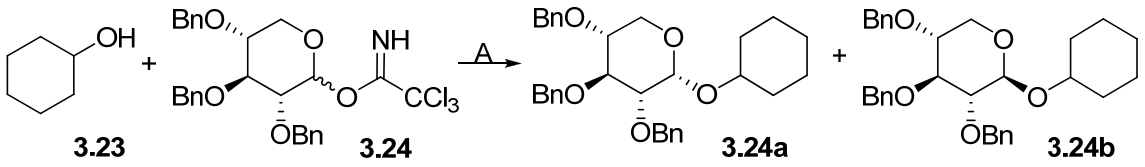
Scheme 25: Reagents and conditions: a) **3.17**, MS 4A, Et_2O/DCM,10:1, cat. TfOH, r.t., 30 min, 11%, α:β =1:4; b) **3.17**, 1.4 eq. TMU, 9 eq. AgOTf, MS 4A, Et_2O/DCM, 10:1, r.t., 36 h, 33%, α:β =1:1.5;

Due to the hindrance, the coupling reaction is too hard to finish. However, the ratios of α– and β–regioisomers and the yields of glycoside synthesis were unsatisfactory, so this route was abandoned.

3-2-1-2 Coupling of Xyl subunit and Choi subunit

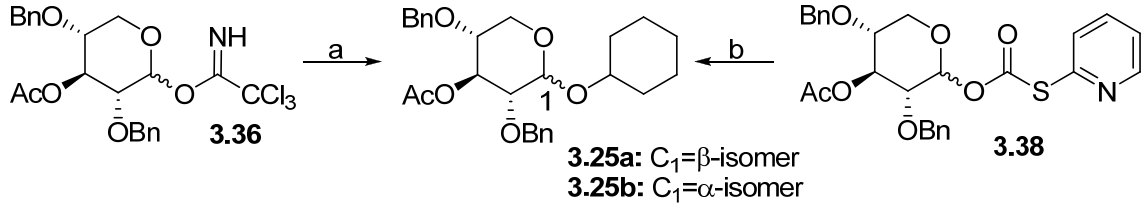
To solve the glycoside problem, as a second option, the coupling between the Xyl subunit and the Choi subunit was studied as a model.

Firstly, two different solvent systems were tested in the glycosylation of cyclohexanol **3.23** with the trichloroacetimidate derivative of 2,3,4-tri-*O*-benzyl-D-xylopyranose **3.24** (Scheme 26). The results showed that the presence of Et_2O can increase the ratio of the desired α-regioisomer **3.24a**.



Scheme 26: Reagents and conditions: cat. TfOH, MS 4A, r.t., 30 min.;

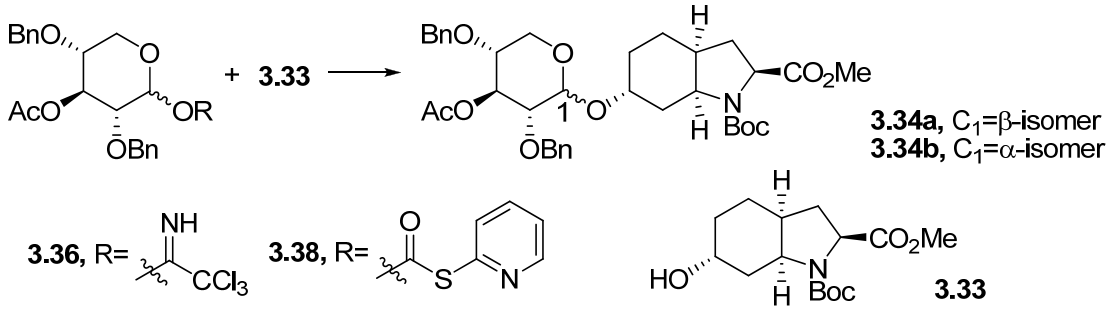
Secondly, two glycosylation methods, using anomeric trichloroacetimidate[27] and 2-pyridyl thiocarbonate derivatives[28], were also attempted by using the 3-acetate derivatives **3.36** and **3.38** as models (Scheme 27).



Scheme 27: Reagents and conditions: a) **23**, cat. TfOH, MS 4A, DCM, r.t., 30 min., 88%, **3.25a**:**3.25b**=1:1.7; b) **23**, MS 4A, 1.4 eq. TMU, 9 eq. AgOTf, 36 h, DCM, 82%, **3.25a**:**3.25b**=1:2.4;

Excellent yields of the α– and β–anomers (**3.25a** and **3.25b**) were obtained, favoring the α-anomer (**3.25b**), especially when **3.38** was used as the glycosyl donor. Glycosylation reactions were done between the Xyl donors **3.36** and **3.38**, and the Choi subunit led to excellent yields with the acceptable ratios, as shown in Table 7.

Table 7 Coupling reaction between the Xyl subunit and the Choi subunit



Xyl	Reagents and Conditions	Et_2O/DCM	α/β (**3.34b/3.34a**)	Yield[a](%)
31	cat. TfOH, MS 4A, r.t., 0.5 h	6:1	1:1.4	91
31	cat. TfOH, MS 4A, r.t., 0.5 h	8:1	1:1.2	90
31	cat. TfOH, MS 4A, r.t., 0.5 h	10:1	1:1.1	82
33	1) 1.4 eq. TMU, MS 4A, 12 h, 2) 9 eq. AgOTf, 36 h,	10:1	1:1.2	95

a) Yields of isolated pure product

Due to the hindrance of the Choi subunit, the α-anomer (**3.34b**), which is the desired intermediate, is not favoring. Increasing the α/β ratio to 1:1.2, we therefore decided to adopt the 2-pyridyl thiocarbonate method[28] and use Et_2O/DCM 10:1 as the solvent system in view of the excellent yield and acceptable α/β–ratio.

3-2-1-3 Final plan

Due to the successful result of coupling between the Xyl subunit and the Choi subunit, the synthesis plan was changed to another sequence of coupling (Figure 16). Firstly, the Choi subunit was coupled with the Xyl subunit, followed by the Agma subunit to get the subunit **B**. Secondly, the Cleu subunit was coupled with Pla subunit, followed by deprotection and oxidation to afford subunit **C**. Finally the subunit **B** was coupling with the subunit **C**. After sulfation and deprotection, the intended target aeruginosin 205B was obtained.

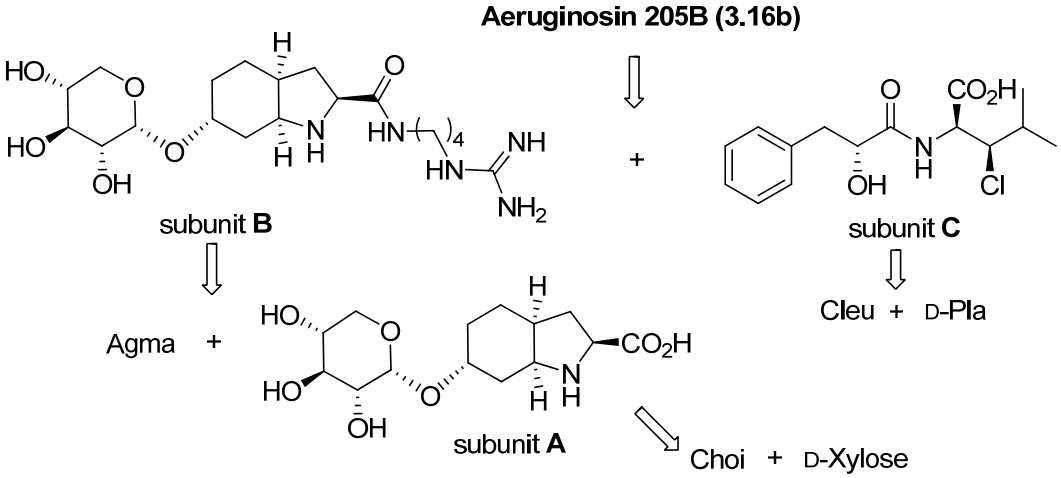
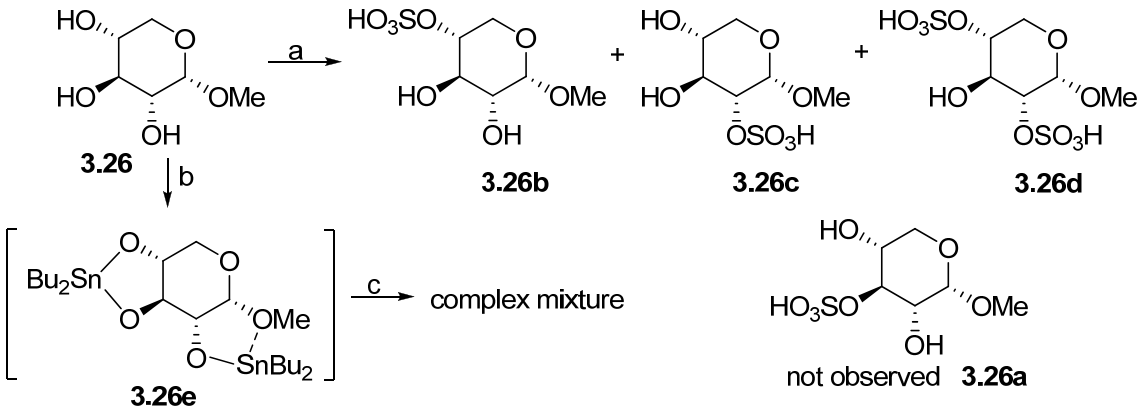


Figure 16. Final plan of retrosynthesis of the target compound.

3-2-1-4 Sulfation

Methyl α-D-xylopyranoside **3.26**[29] was used as a model in search of a method for selective 3-*O*-sulfation.

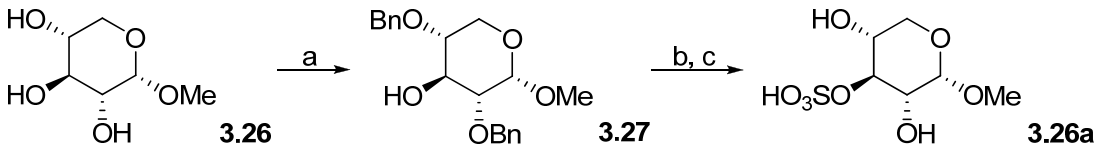
Sulfur trioxide pyridine complex, is a common sulfation reagent for the secondary alcohols of the sugars.[30, 31] In the case of **3.26**, NMR studies result showed that a mixture of 2-monosulfated (**3.26c**) 4-monosulfated (**3.26b**) and 2,4-disulfated (**3.26d**) products was obtained. 3-Monosulfated as in **3.26a** was not observed (Scheme 28).



Scheme 28: Reagents and conditions: a) 3 eq. SO_3/pyridine, pyridine, 50 °C, 5 days; b) toluene, 2.1 eq. Bu_2SnO, MS, refluxe, 10 h; c) 1.1 eq. SO_3/NMe_3, DMF/THF 3:2, r.t., 10 h;

The use of bis(tributyltin) oxide reagent[32, 33] to activate hydroxyl groups through stannylene acetals such as **3.26e** was expected to give the single product **3.26a**. Unfortunately, a complex mixture was obtainedunpo sulfation (Scheme 28).

To get the single 3-monosulfation product **3.26a**, the 2,4-hydroxy groups of **3.26** were first protected by Bn-groups to get the major di-benzyl product **3.27**[34], which was sulfated, followed by deprotection to form the single sulfate ester **3.26a**. The reagents and conditions are shown in Scheme 29.



Scheme 29: Reagents and conditions: a) BnCl, 2 eq. NaH, 100 °C, 4 h, 62%; b) pyridine 50 eq. SO_3/pyridine, 50 °C , 48 h; c) $Pd(OH)_2/C$, H_2, MeOH, r.t., 4h, 96%;

3-2-1-5 Choice of protecting group

From the analysis of the structure and previous experience in the synthesis of the aeruginosin family, the stability of the final compound was considered in the choice of orthogonally compatible protecting groups.

Firstly in basic conditions: Due to the presence of the α-proton and the β-chlorine atom on the Cleu subunit, the elimination of the β-chlorine atom was a possibility under the basic conditions. Hence, strong bases should be avoided in the final deprotection.

Secondly in acidic conditions: The final product is a monosulfate, and most sulfates are reported to be unstable to mineral acids.[35, 36] The compound **3.26a,** which is the 3-*O*-sulfated product of methyl α-D-xylopyranoside **3.26**, was used as the model, and the results showed that the sulfates can be purified via the silica gel chromatography with 5% methanol in DCM, but not stable under pH<3 conditions.

According to these results, hydrogenation was considered as a safe deprotection process, and new protecting groups, which should remain intact until the last step, were considered (Figure 17). For example: 1) The Cbz-groups on the guanidine on the Agma subunit was used in place of Boc-groups. 2) The Bn-group on the alcohol on the Pla subunit was to be used instead of MOM-group. 3) The two final free alcohols on the Xyl subunit were also to be protected with Bn-groups.

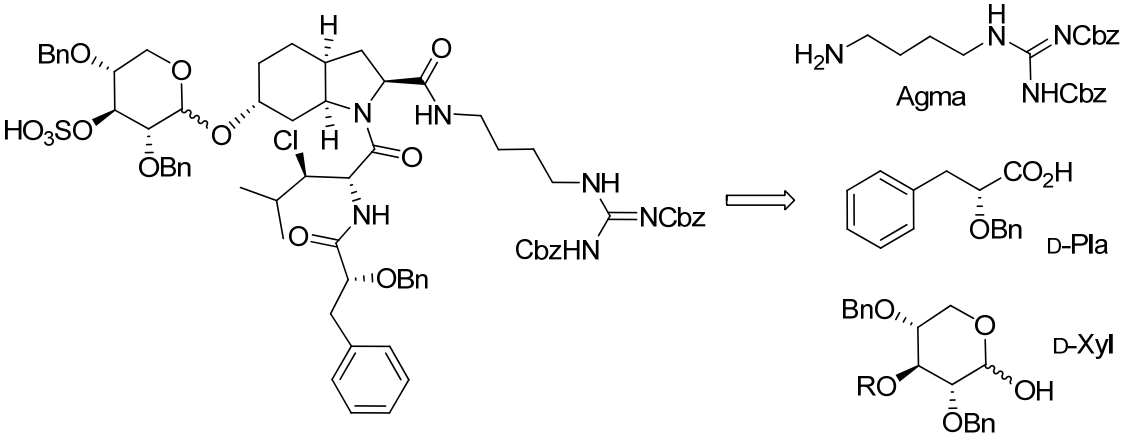
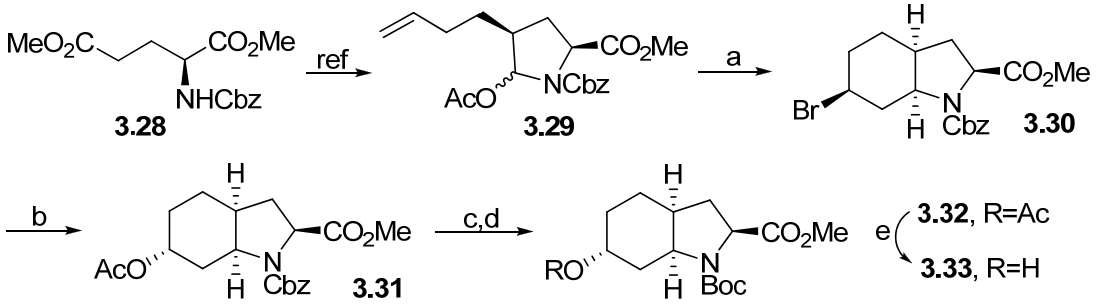


Figure 17. Protecting groups in the total synthesis.

3-2-2 Synthesis of the A-C subunits

3-2-2-1 The Choi subunit

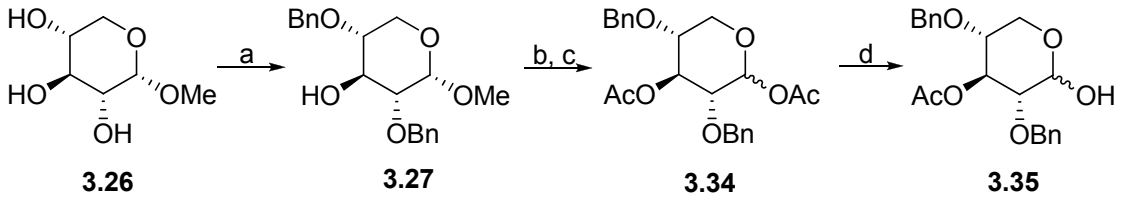
Following our previous studies,[8] *N*-Cbz-L-glutamic acid dimethyl ester **3.28** was converted to intermediate **3.29**. Azonia-Prins reaction led to compound **3.30** in greater yield than previously reported.[8] During this reaction, dry DCM as solvent, 2 equivalents of tin(IV) bromide ($SnBr_4$) and extending the reaction time to 30min led to a higher yield (90%). The bromine atom was substituted in an S_N2 reaction with Bu_4NOAc to form **3.31**, which was followed by Cbz-group deprotection, Boc-group protection and Ac-group cleavage to give the *N*-Boc Choi derivative **3.33**. The structure and stereochemistry of **3.33** was ascertained by X-ray crystallography. The details are shown as Scheme 30.



Scheme 30: Reagents and conditions: a) $SnBr_4$, DCM, -78 °C, 30 min, 90%; b) Bu_4NOAc, toluene, 40 °C, 2.5 h, 82%; c) Pd/C, H_2, MeOH, r.t., 5 h, 95%; d) Boc_2O, Et_3N, DCM, r.t., 26 h, 88%; e) MeONa, MeOH, r.t., 10 h, 90%;

3-2-2-2 The Xyl subunit

The 2,4-hydroxyl groups of methyl α-D-xylopyranoside **3.26**[29] were selectively protected as Bn-ethers[34] to give **3.27**, which was hydrolyzed under acidic conditions and acetylated to afford **3.34**. After selective deacetylation, 3-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose **3.35** was obtained. The reagents and conditions are shown in Scheme 31.

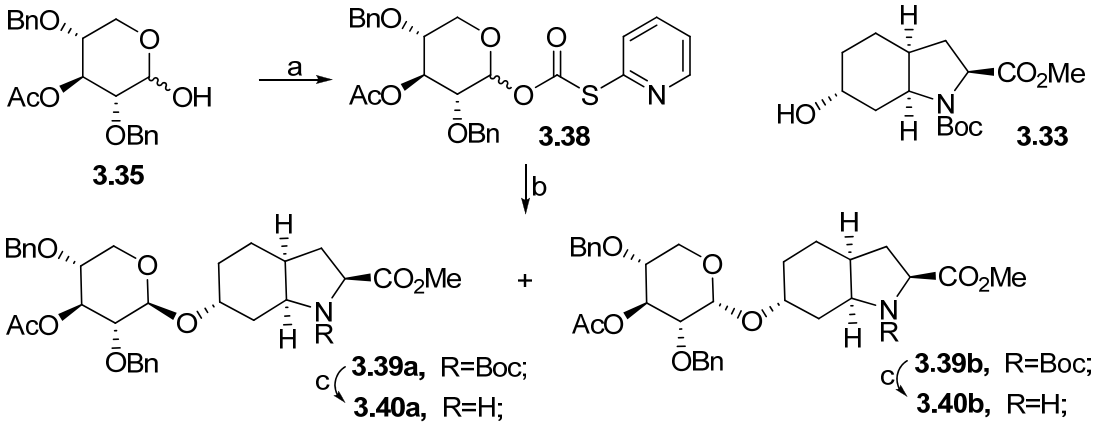


3.26 **3.27** **3.34** **3.35**

Scheme 31: Reagents and conditions: a) BnCl, NaH, 100 ^{o}C, 4 h, 62%; b) 1,4-dioxane/AcOH 1:1, 1 M H_2SO_4, 60 ^{o}C , 72 h; c) Ac_2O, DMAP, Et_3N, DCM, r.t., 3 h, 98% over 2 steps; d) benzylamine, THF, r.t., 48 h, 83%;

3-2-2-3 Subunit A

As the discussed in section **3-2-1-2**, *N*-Boc Choi **3.33** was glycosylated with **3.38** to form compound **3.39**, which is a 1.2:1 mixture of β- and α-anomers (**3.39a** and **3.39b**). These were separated by flash column chromatography and their structures determined by NMR data of **3.40a** and **3.40b** (Scheme 32). The ratio of the desired α-anomer was lower compared to cyclohexanol possibly due to the axial disposition of the hydroxyl group in **3.33**.

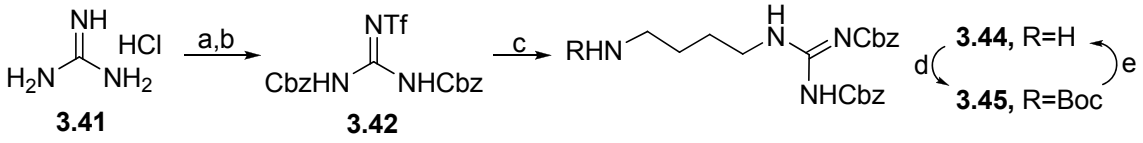


Scheme 32: Reagents and conditions: a) 3 eq. di(S-2-pyridyl) thiocarbonate **3.37**, 3 eq. Et_3N, DCM, r.t., 24 h, 86%; b) **3.33**, MS 4A, 1.4 eq. TMU, Et_2O/DCM 10:1, 9 eq. AgOTf, r.t., 36 h, 95%, **3.39a**:**3.39b**1.2:1; c) TFA/DCM 1:9, r.t., 3 h, **3.40a** 95%; **3.40b** 92%;

3-2-2-4 The Agma subunit

Following the literature[37], guanidine hydrochloride **3.41** was transformed into the Goodman's reagent **3.42**, which was reacted with 10 equivalents of

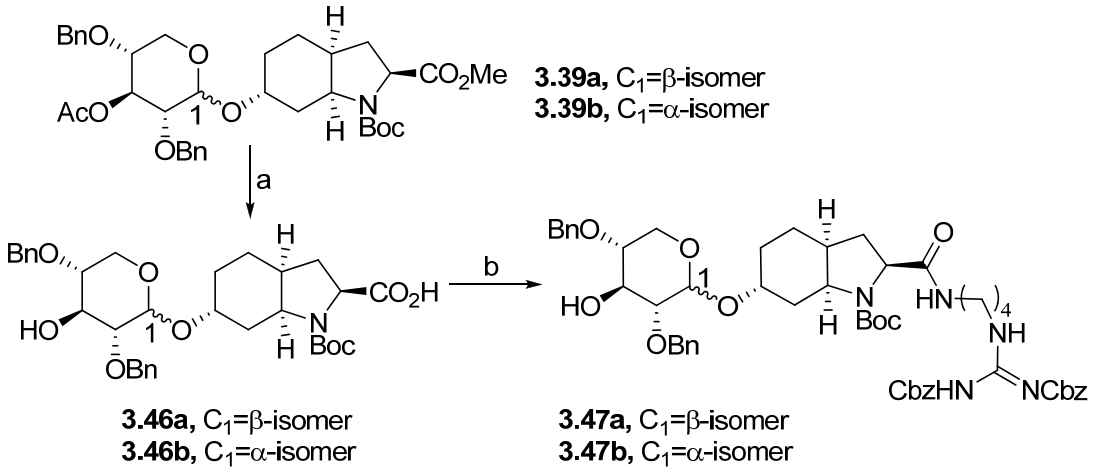
1,4-diaminobutane **3.43** to form the Agma subunit **3.44**. Protection as N-Boc gave an easily purifiable product **3.45**, as shown in Scheme 33.



Scheme 33: Reagents and conditions: a) 5 eq. NaOH, 3 eq. CbzCl, H_2O/DCM 1:2, 20 h, 0 °C, 86%; b) 2 eq. NaH, 0 °C, 1 eq. Tf_2O, chlorobenzene, r.t., 9 h, 67%; c) $CHCl_3$, 10 eq. 1,4-diaminobutane **3.43**, r.t., 2 h, 94%; d) 1.4 eq. Boc_2O, 2 eq. NEt_3, DCM, 0 °C to r.t., 10 h, 92%; e) 4 M HCl, 1,4-dioxane, 99%;

3-2-2-5 Subunit B

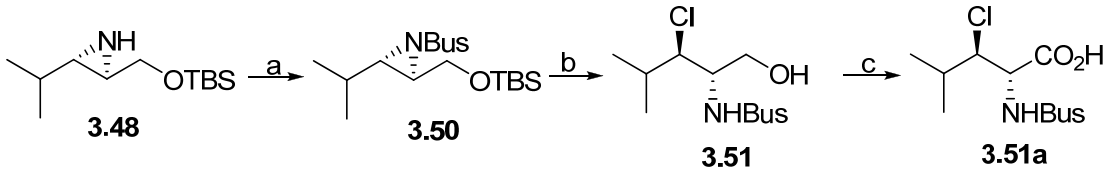
Compounds **3.46a** and **3.46b**, which were obtained by saponification of the compounds **3.39a** and **3.39b** respectively, were coupled with the Agma subunit **3.44** to afford compounds **3.47a** and **3.47b**, in the good yield (Scheme 34). The structure and stereochemistry of compound **3.46b** was also established by X-ray crystallography.



Scheme 34: Reagents and conditions: a) 3 eq. LiOH, THF/H_2O 5:3, r.t., 24 h, **3.46a** 99%, **3.46b** 99%; b) 1.5 eq. **3.39**, 5 eq. 2,6-lutidine, 1.5 eq. PyBOP, DCM, 0 °C to r.t., 10 h, **3.47a** 83%, **3.47b** 84%;

3-2-2-6 The Cleu subunit

The Cleu subunit was prepared as previously described[13] from aziridine **3.48**. First the reaction with *tert*-butylsulfinyl chloride **3.49**, followed by oxidation with *m*-CPBA gave **3.50**. Then regioselectivie opening with $CeCl_3$ as the chloride source afforded the major isomer **3.51**, which was oxidized with H_5IO_6 in the presence of CrO_3 catalyst[13] to afford *N*-Bus-3-chloroleucine **3.51a**. The detailed reagents and conditions are shown in Scheme 35.

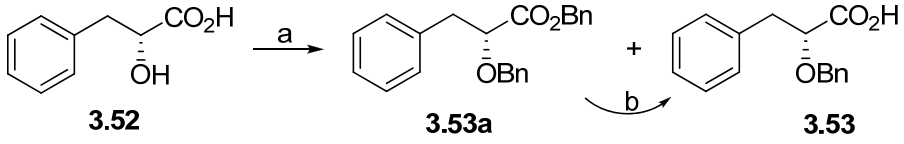


3.48 **3.50** **3.51** **3.51a**

Scheme 35: Reagents and conditions: a) **i**) 1.2 eq. *tert*-butyl sulfinyl chloride **3.49**, 2.5 eq. Et_3N, 0 °C, DCM; **ii**) *m*-CPBA, DCM, r.t., 2 h, 85% over 2 steps; b) 4.0 eq. $CeCl_3.7H_2O$, MeCN, 90 °C, 72 h, 80%; c) cat. CrO_3, 2.4 eq. 0.4M H_5IO_6, MeCN, 0 °C, 1 h, 57%;

3-2-2-7 The Pla subunit

D-(+)-3-Phenyllactic acid **3.52** was treated with NaH and BnBr to obtain *O*-Bn-D-(+)-3-phenyllactic acid **3.53** as the major product in addition to the ester **3.53a**. After saponification, the transesterification by-product **3.53a** was transformed back to **3.53** (Scheme 36).



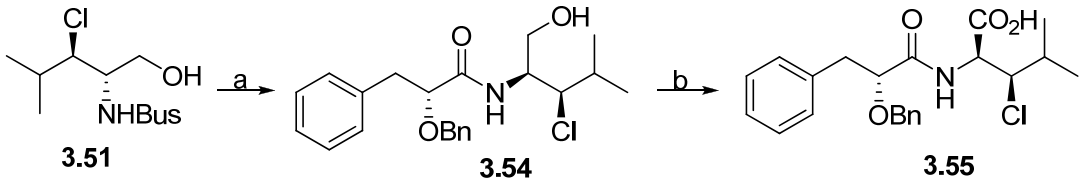
3.52 **3.53a** **3.53**

Scheme 36: Reagents and conditions: a) 2.5 eq. NaH, 1.5 eq. BnBr, 1.0 eq. Bu_4NI, THF, 0 °C to r.t., 10 h, **3.53a** 33%, **3.53** 62%; b) 3 eq. $LiOH.H_2O$, THF/H_2O 7:3, r.t., 4 h, 86%;

3-2-2-8 Subunit C

The Cleu intermediate **3.51** was treated with TfOH to cleave the *N*-Bus

group, then the free amine salt was coupled in presence of DEPBT and 2,6-lutidine with *O*-Bn-D-(+)-3-phenyllactic acid **3.53** to afford **3.54**. The carboxylic acid **3.55** was obtained by oxidation of intermediate **3.54** with H_5IO_6 and CrO_3 catalyst. The details are shown in Scheme 37.

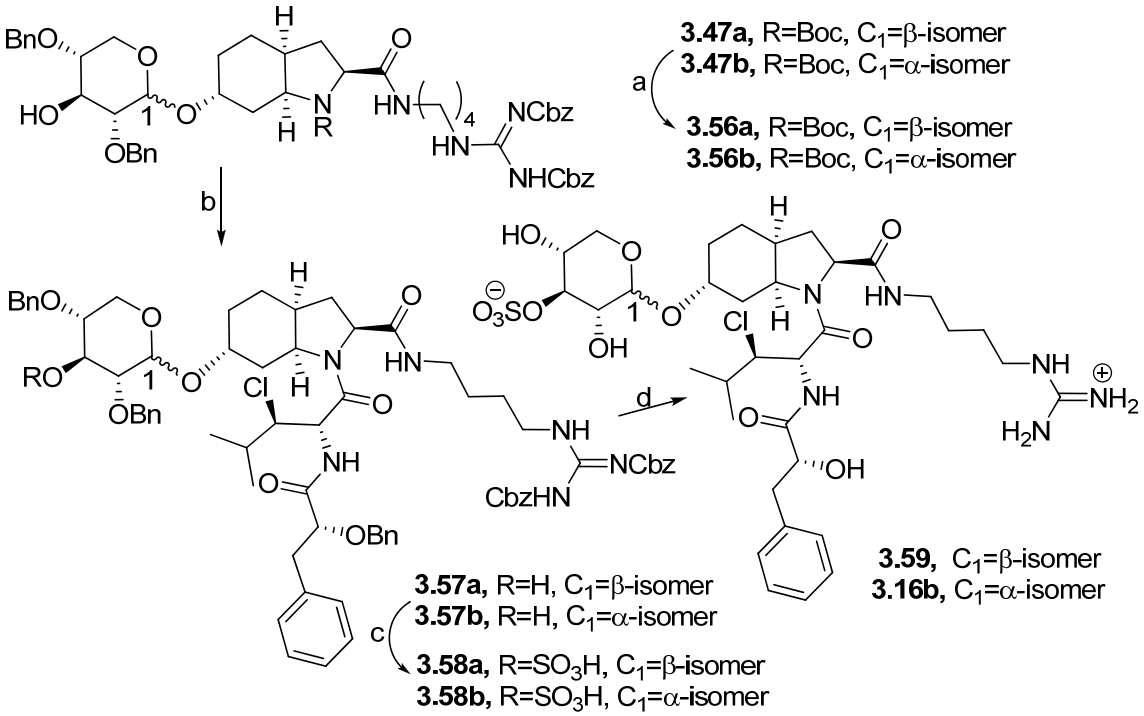


Scheme 37: Reagents and conditions: a) i) 20 eq. anisole, 5 eq. TfOH, DCM, r.t., 12h; ii) 5 eq. 2,6-lutidine, 1.1 eq. *O*-Bn-D-(+)-3-phenyllactic acid **3.53**,1.6 eq. DEPBT, DCM, 0 °C to r.t., 27 h, 87% over 2 steps; b) 2.4 eq. 0.4M H_5IO_6, cat. CrO_3, wet MeCN, 0 °C, 45 min., 87%;

3-2-3 Synthesis of aeruginosin 205B

As shown in Scheme 38, free amines **3.57a** and **3.57b**, obtained from **3.42a** and **3.42b** respectively, were coupled with the subunit **C** carboxylic acid **55** to form corresponding **3.57a** and **3.57b** in presence of 2,6-lutidine and DEPBT. The compounds **3.57a** and **3.57b** were sulfated with 50 equivalents sulfur trioxide (SO_3) pyridine complex at 50 °C for 2 days. The *O*-Bn and *N*-Cbz groups in **3.58a** and **3.58b** were cleaved in presence of excess palladium hydroxide ($Pd(OH)_2$, 20 wt.% Pd on carbon) and hydrogen in methanol to form target compounds **3.59** and **3.16b** respectively. The progress of the deprotection was checked by MS each hour to avoid the over-reduction of the D-Pla subunit.

Finally, HPLC was used to purify the compounds **3.59** and **3.16b**. Due to the stability of the sulfates on these two compounds, the whole HPLC system was treated in the neutral condition, and no acidic buffer (0.5% Formate Acid) was presence in the flow.



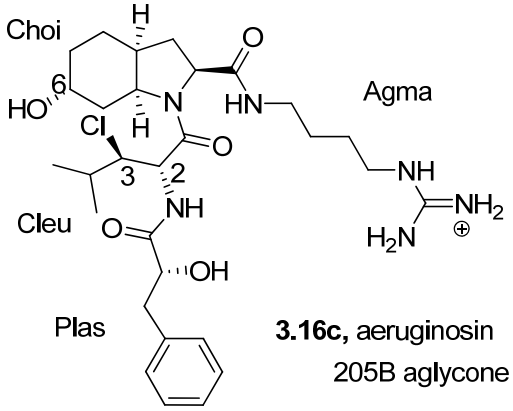
Scheme 38: Reagents and conditions: a) TFA/DCM 1:9, r.t., 2 h, **56a** 96%, **56b** 91%; b) **57a** 3 eq. recrystallized DEPBT, 6 eq. 2,6-lutidine, 1.7 eq. **55**, DCM, 0 °C, 44 h, 69%; **57b** 5 eq. recrystallized DEPBT, 9 eq. 2,6-lutidine, 2.2 eq. **55**, DCM, 0 °C, 68 h, 60%; c) 50 eq. SO_3/pyridine, 50 °C, pyridine, 48 h, **58a** 88%, **58b** 85%; d) 20 wt.% $Pd(OH)_2/C$, H_2, MeOH, r.t., **59** 6 h, 85%; **16b** 5.5 h, 84%;

3-3 Comparison of NMR data

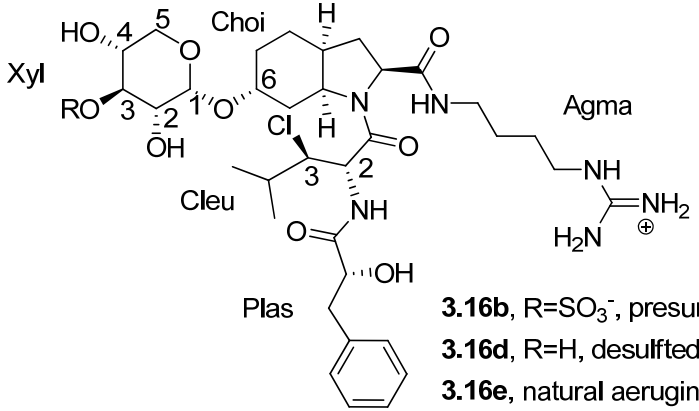
3-3-1 Comparison of NMR data of aeruginosin 205B aglycone, synthetic aeruginosin 205B and natural aeruginosin 205B

To confirm the presumed structure of aeruginosin 205B **3.16b**, comparison of ^{1}H and ^{13}C NMR of synthetic aeruginosin 205B aglycone **3.16c**, synthetic aeruginosin 205B **3.16b**, desulfated synthetic aeruginosin 205B **3.16d** and natural aeruginosin 205B **3.16e**[15] has been done. The chemical shift values for the Cleu, Pla, Agma, Choi and Xyl subunit of each compound were listed group by group in Table 8.

Table 8 ^{1}H and ^{13}C NMR data for each subunit of synthetic aeruginosin 205B aglycone **3.16c** (500 Hz), synthetic presumed aeruginosin 205B **3.16b** (700 Hz) and desulfated presumed aeruginosin 205B **3.16d** (700 Hz) with reported data[15] for natural aeruginosin 205B **3.16e** in DMSO-d_6[a]



3.16c, aeruginosin 205B aglycone



3.16b, R=SO_3^-, presumed aeruginosin 205B
3.16d, R=H, desulfted aeruginosin 205B
3.16e, natural aeruginosin 205B

		3.16c	**3.16d**	**3.16b**	**3.16e**	**3.16c**	**3.16d**	**3.16b**	**3.16e**
Subunit		^{1}H	^{1}H	^{1}H	^{1}H	^{13}C	$^{13}C^{b}$	^{13}C	^{13}C
Cleu	1	--	--	--	--	169.1		167.5	167.3
	2	4.88	4.91	4.93	4.90	51.0	51.4	51.1	51.1
	3	3.92	3.99	3.97	3.98	69.5	69.3	69.0	68.9
	4	1.62	1.67	1.66	1.69	27.3	30.0	27.5	27.5
	5,5′	0.83,0.84	0.85,0.86	0.84,0.86	0.85,0.88	20.3,14.7	21.2,15.9	20.9,15.4	20.8,15.4
	NH	7.52		7.67	7.62	--	--	--	--

Table 8 (contd.)		**3.16c**	**3.16d**	**3.16b**	**3.16e**	**3.16c**	**3.16d**	**3.16b**	**3.16e**
Subunit		^{1}H	^{1}H	^{1}H	^{1}H	^{13}C	^{13}C^{b}	^{13}C	^{13}C
Pla	1	--	--	--	--	173.8		172.4	172.4
	2	4.19	4.19	4.19	4.19	72.0	72.1	71.8	71.9
	3a,3b	2.82,2.93	2.79,2.96	2.78,2.94	2.78, 2.93	39.8	40.4	40.0	40.0
	4	--	--	--	--	139.1		138.1	137.9
	5,9	7.22	7.23	7.22	7.23	130.9	130.0	129.8	129.6
	6,8	7.25	7.26	7.24	7.25	129.0	128.3	127.9	127.9
	7	7.18	7.19	7.17	7.18	127.2	126.4	126.1	126.0
	2-OH	5.92		3.16	--	--	--	--	--
Choi	1	--	--	--	--	172.8		171.2	171.2
	2	4.17	4.19	4.19	4.19	60.0	60.2	59.8	59.8
	3,3′	1.80,1.97	1.82,2.01	1.85,2.01	1.84,2.02	30.6	31.1	30.8	30.8
	3a	2.18	2.23	2.26	2.24	35.7	36.3	35.9	35.8
	4,4′	1.41,2.01	1.47,2.14	1.49,2.13	1.48,2.13	18.5	19.9	19.5	19.4
	5	1.42	1.53	1.54	1.54	25.9	25.2	24.9	24.7
	6	3.89	3.84	3.86	3.83	64.1	68.8	67.3	68.7
	7,7′	1.62,2.02	1.58,2.22	1.58,2.32	1.59,2.23	33.2	29.0	28.8	28.7
	7a	4.26	4.31	4.35	4.23	54.7	54.6	54.0	54.2

Table 8 (contd.)		**3.16c**	**3.16d**	**3.16b**	**3.16e**	**3.16c**	**3.16d**	**3.16b**	**3.16e**
Subunit		^{1}H	^{1}H	^{1}H	^{1}H	^{13}C	^{13}C[b]	^{13}C	^{13}C
Agma	1	2.99,3.09	2.98,3.08	3.00,3.07	2.97,3.11	37.9	38.3	38.0	37.9
	2	1.38	1.44	1.41	1.40	26.0	26.4	26.3	26.2
	3	1.42	1.45	1.44	1.44	26.0	25.5	25.9	25.8
	4	3.06	3.09	3.03	3.09	40.3	40.8	40.3	40.4
	1-NH	7.96		8.83	7.92		--	--	--
	4-NH	7.63		8.05	7.44		--	--	--
	C=N			--	--	158.5		157.5	156.6

a. Recorded at MH_Z (^{1}H) and MH_Z (^{13}C); b. Recorded from HMQC

Part of Xyl subunit of synthetic **3.16b**, desulfated **3.16d** and natural **3.16e** in DMSO-d_6[a]

Table 8 (contd.)		**3.16d**	**3.16b**	**3.16e**	**3.16d**	**3.16b**	**3.16e**
Subunit		^{1}H	^{1}H	^{1}H	^{13}C[b]	^{13}C	^{13}C
Xyl	1	4.96	5.09	4.93	95.8	94.2	95.2
	2	3.24	3.39	3.28	72.5	70.7	72.1
	3	3.27	4.14	3.58	70.5	80.5	71.7
	4	3.42	3.45	3.93	73.9	68.6	74.9
	5	3.30,3.39	3.35,3.45	3.37,3.67	62.4	61.4	59.6

a. Recorded at MH_Z (^{1}H) and MH_Z (^{13}C); b. Recorded from HMQC

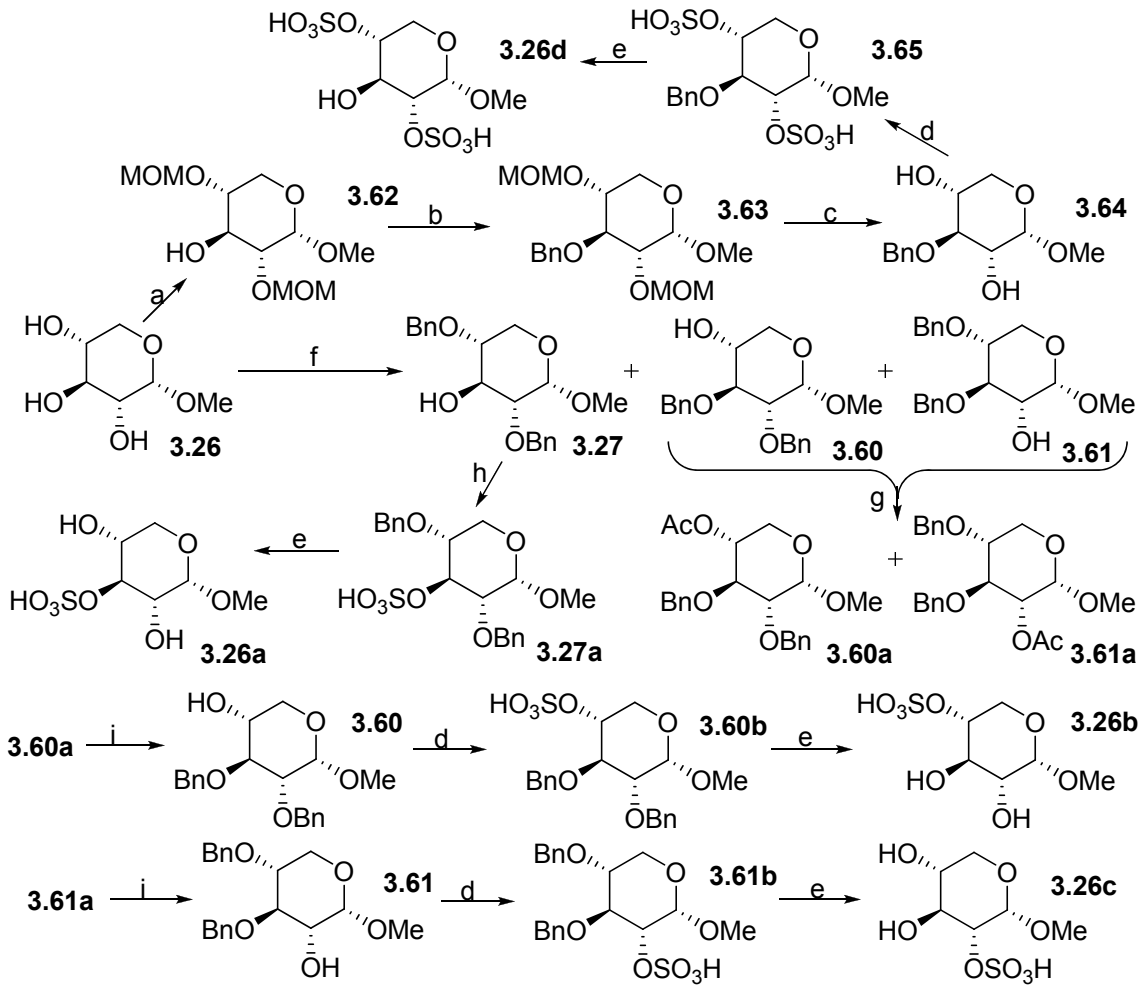
Thus similarity of chemical shift values for the Cleu, Pla, Agma and Choi subunit listed in Table 8, indicate that the structure of the natural product should be revised to include a Cleu , Pla and Choi subunit, rather than a Hleu, Plas and Ccoi subunit as originally proposed by Murakami.[15]

Comparison of ^{1}H and ^{13}C NMR shows virtual identity with allowance made for the absence of the Xyl subunit in the synthetic aeruginosin 205B aglycone **3.16c**. The 1H and 13C spectra characteristics matched those published for aeruginosin 205B **3.16e** except for the absence of resonances due to the sulfated D-xylopyranosyl moiety. Thus, the reported values for the Pla and Cleu subunits were agree to those in the synthetic sample of 205B aglycone **3.16c**. The same was also true for the Choi subunit with allowance made for C-6, which carries a glycosidic moiety in the natural product. The agreement also extends to the synthetic aeruginosin 205B **3.16b**, and desulfated synthetic aeruginosin 205B **3.16d**, according to the ^{1}H and ^{13}C chemical shift at C-6 of the Choi sununit.

Comparison of ^{1}H and ^{13}C NMR data of the Xyl subunit of synthetic aeruginosin 205B **3.16b**, desulfated synthetic aeruginosin 205B **3.16d** and natural aeruginosin 205B **3.16e**, indicates that the ^{1}H and ^{13}C chemical shifts do not match at the C-3 position. Therefore the sulfate group cannot be on C-3 of the Xyl subunit (Table 8, the part of Xyl subunit). To validate this conclusion, we prepared methyl α-D-xylopyranosides with sulfate group on each hydroxyl group individually.

3-3-2 NMR Comparison of model sulfate esters

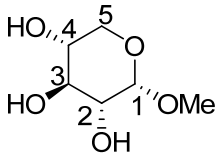
Model compounds with and without the *O*-sulfated group were synthesized. Firstly, Methyl α-D-xylopyranoside **3.26**, 2-*O*-sulfate **3.26c**, 3-*O*-sulfate **3.26a**, 4-*O*-sulfate **3.26d** and 2,4-*O*-disulfate **3.26d** were prepared (Scheme 39).



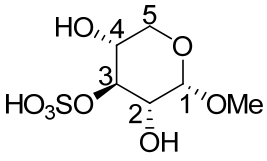
Scheme 39: Reagents and conditions: a) 1) 2.2 eq. Bu_2SnO, MS, toluene/MeOH, 70 ^oC, 10 h, 2) 2.2 eq. MOMCl, 1 eq. Bu_4NI, toluene, r.t., 10 h, 47%; b) 1.5 eq. NaH, 1.5 eq. BnBr, 1 eq. Bu_4NI, r.t., 10 h, 89%; c) TFA/DCM 1:2, r.t., 3 h, 82%; d) pyridine, 25 eq. SO_3/pyridine, 50 ^oC, 10 h, **3.65** 87%, **3.60b** 92%, **3.61b** 91%; e) 20 wt.% $Pd(OH)_2/C$, H_2, r.t., 3h, **3.26a** 96%, **3.26b** 96%, **3.26c** 97%, **3.26d** 96%; f) BnCl, 2 eq. NaH, 100 ^oC, 3 h, **3.27** 62%, **3.60**+**3.61** 12%; g) 1.5 eq. Ac_2O, cat. DMAP, 2 eq. Et_3N, r.t., 3 h, **3.60a** 35%, **3.61a** 48%; h) 50 eq. SO_3/pyridine, pyridine, 24 h, 50 ^oC, 87%; i) 2 eq. NaOMe, MeOH, r.t., 2 h, **3.60** 96%, **3.61** 96%

Compound **3.26** was protected as Bn-ethers to give **3.27**, **3.60** and **3.61**, which were sulfated and cleaved *O*-Bn groups to afford **3.26a-c** respectively. Compound **3.26** was also selectively protected as MOM-ethers to give **3.62**, which subjected Bn-protection, MOM-cleavage and sulfation, followed by *O*-Bn group cleavage to afford **3.26d**. ^1H and ^{13}C NMR data are shown in Table 9.

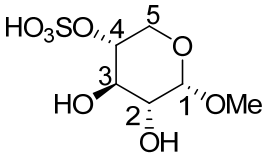
Table 9 ^{1}H and ^{13}C NMR data for compounds **3.26**, **3.26a-d** (Xyl subunit), aeruginosin 205A and B in DMSO-d_6



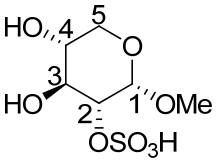
3.26



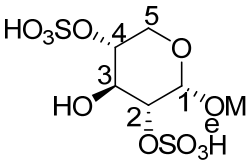
3.26a



3.26b



3.26c



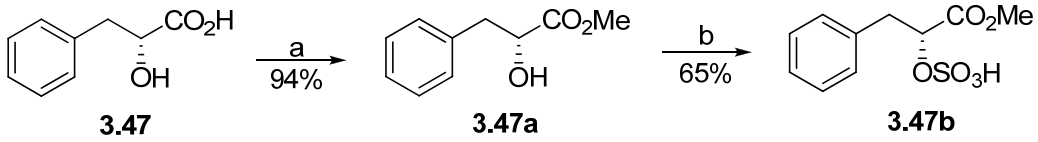
3.26d

	3.26	**3.26a**	**3.26b**	**3.26c**	**3.26d**	**205B**	**205A**	**3.26**	**3.26a**	**3.26b**	**3.26c**	**3.26d**	**205B**	**205A**
	^{1}H	^{1}H	^{1}H	^{1}H	^{1}H	^{1}H	^{1}H	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C
1	4.47	4.52	4.48	4.72	4.79	4.93	4.95	100.2	99.8	99.8	98.3	97.9	95.2	95.1
2	3.17	3.35	3.25	3.81	3.83	3.28	3.28	72.0	70.4	71.7	76.2	76.0	72.1	72.1
3	3.29	4.11	3.46	3.46	3.53	3.58	3.58	73.5	79.8	71.9	71.8	69.4	71.7	71.8
4	3.24	3.46	3.90	3.32	3.93	3.93	3.93	70.0	68.6	74.8	70.3	74.9	74.9	75.0
5	3.25 3.39	3.25 3.48	3.30 3.66	3.22 3.43	3.26 3.76	3.37 3.67	3.37 3.67	61.7	61.5	59.3	61.2	59.0	59.6	59.5
OMe	3.24	3.26	3.25	3.23	3.24			54.6	54.7	54.7	54.7	54.8		

a. Recorded at MH_Z (^{1}H) and MH_Z (^{13}C);

Conclusion: The similarity in the chemical shifts of the protons and carbons reported for aeruginosin 205B and 205A with those recorded for **3.26b** shows that sulfate unit is not on C-3, but on C-4 of the Xyl subunit.

Secondly, compound **3.47** subjected esterification to afford D-Phenyllactic acid methyl ester **3.52a**, and followed by sulfation to give *O*-sulfated product **3.52b**. The reagents and conditions are shown in Scheme 40. Both **3.52a** and **3.52b** were used as the Pla subunit models. ^{1}H and ^{13}C NMR data are shown in Table 10.



3.47 **3.47a** **3.47b**

Scheme 40: Reagents and conditions: a) 2 eq. trimethylsilyldiazomethane, hexanes/MeOH, r.t., 1 h, 94%; b) 1) 2 eq Bu_2SnO, MS, toluene, 70 °C, 10 h; 2) 2 eq. SO_3/NMe_3, toluene/DMF, r.t., 10 h, 65%;

Table 10 ^{1}H and ^{13}C NMR data for compounds **3.52a** and **3.52b** (Pla subunit) in DMSO-d_6 (400 MHz)

	3.52a	**3.52b**	**205B**	**3.52a**	**3.52b**	**205B**
	^{1}H	^{1}H	^{1}H	^{13}C	^{13}C	^{13}C
1	--	--	--	174.0	171.3	172.4
2	4.25	4.68	4.19	71.3	74.9	71.9
3	2.83,2.96	2.95,3.00	2.78,2.93	40.1	38.3	40.0
Ph	7.18-7.23 7.26-7.29	7.16-7.22 7.24-7.28	7.18,7.23 7.25	126.3,128.1 129.4,137.7	126.6,128.2, 129.3, 36.3,	126.0,127.9 129.6,137.9
OMe	3.61	3.50		51.5	51.3	

a. Recorded at MH_Z (^{1}H) and MH_Z (^{13}C);

Conclusion: The similarity in the chemical shifts of the protons and carbons on 3-position of **3.52a** and aeruginosin 205B shows that sulfate group is not on the D-Pla subunit.

3-3-3 Conclusion

According to the ^{1}H and ^{13}C NMR data reported[15] for natural 205B and synthetic **3.16b**, the *O*-sulfated group must be present on the 4-OH of Xyl subunit, but not on the D-Pla subunit or on the 3-OH of the D-Xyl subunit as originally proposed by Toyooka[23]. Furthermore, the chlorine atom is not situated at C-6 of the Choi subunit as originally proposed. The third revised structure and stereochemistry of aeruginosin 205B based on the work described in this thesis is shown in the Figure 18. The structure of aeruginosin 205A is the same as 205B except for the presence of *(2R, 3S)*-Cleu and L-Plas instead of *(2S, 3R)*-Cleu and D-Plas. The revision should also be valid for 205A.

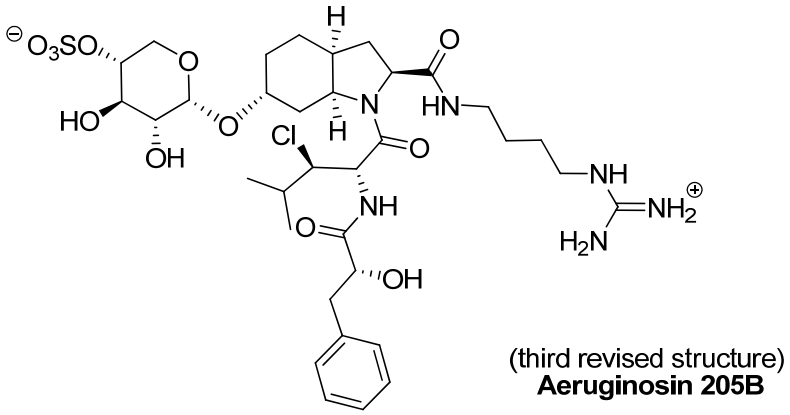
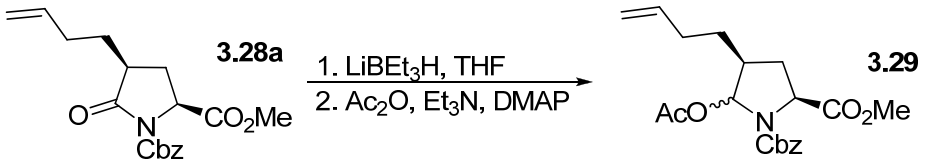


Figure 18. Third revision of aeruginosin 205B.

3-4 Experimental

General: Solvents were distilled under positive pressure of dry argon before use and dried by standard methods. THF, ether, DCM, and toluene were dried by the SDS (*Solvent Delivery System*). All commercially available reagents were used without further purification. All reactions were performed under argon atmosphere and monitored by thin-layer chromatography. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate, ninhydrine or potassium permanganate. IR, Perkin-Elmer FTIR

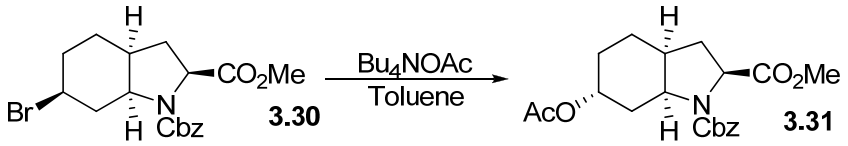
Paragon 1000. Low- and high-resolution mass spectra were recorded using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded in a 1 dm cell at 20 °C (PerkinElmer 343). Flash column chromatography was performed using (40-60 μm) silica gel at increased pressure. NMR (^{1}H, ^{13}C) spectra were recorded on Bruker AV-300, AV-400, AV-500 and AV-700 spectrometers. When necessary, assignments were aided by DEPT, COSY, NOESY, and HMBC and HMQC correlation experiments.



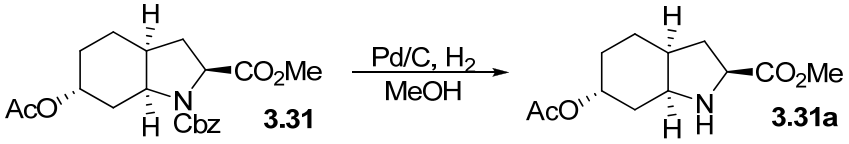
Compound 3.29: To a solution of **3.28a** (500 mg, 1.5 mmol) in dry THF (15 mL) under argon atmosphere was added dropwise $LiBEt_3H$ (1.7 mmol) 1 M in THF (1.7 mL) at -78 °C. After stirring at -78 °C for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (7.5 mL) carefully, then warmed to R.T., and 30% H_2O_2 aqueous (7.5 mL) was added. After stirring for 30 min., the reaction mixture was extracted with EtOAc (4×50 mL), then the organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was redissolved in DCM (20 mL), washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was redissolved in dry DCM (5 mL) and cooled at 0 °C. To the solution was added Ac_2O (430 μL, 4.5 mmol), DMAP (15 mg, 0.15 mmol) and Et_3N (421 μL, 3.0 mmol), and warmed to R.T.. After stirring for 4 h, the reaction mixture was diluted with DCM (20mL), washed with 0.5 M HCl aqueous (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:9) to afford **3.29** (454 mg, 80%)(two products mixture as 1:1.5), as a colorless oil: ^{1}H NMR,

(400 MHz, $CDCl_3$) δ 7.35-7.17 (m, 5H), 6.73 (d, 0.4H, *J*=4.5 Hz), 6.7 (d, 0.6H, *J*=4.6 Hz), 5.78-5.67 (m, 1H), 5.21-4.95 (m, 4H), 4.30 (dd, 1H, *J*=10.5 Hz, *J*=18.4 Hz), 3.76 (s, 2H), 3.57 (s, 1H), 2.46-2.39 (m, 1H), 2.28-2.15 (m, 1H), 2.10-2.01 (m, 4H), 1.83-1.70 (m, 1H), 1.62-1.52 (m, 1H), 1.40-1.31 (m, 1H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.3, 172.0, 169.9, 153.6, 153.4, 137.6, 136.0, 128.5, 128.23, 128.19, 128.1, 127.8, 115.5, 83.1, 82.5, 67.6, 67.5, 59.1, 59.1, 52.4, 52.2, 43.0, 42.4, 33.7, 32.7, 31.7, 27.3, 27.2, 21.11, 21.06; ESI/MS for $C_{20}H_{25}NO_6$ calculated (M-OAc+H^+) 316, found 316.

Compound 3.30: To a solution of **3.29** (319 mg, 0.85 mmol) in dry DCM (5 mL) under argon atmosphere was added dropwise $SnBr_4$ (223 μL, 1.7 mmol) in fresh dry DCM (6 mL) at -78 °C. After stirring for 30 min., the reaction mixture was quenched with aqueous 10% Na_2CO_3 (30 mL), then warmed to R.T., and extracted with DCM (3×30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 1:4) to give **3.30** (300 mg, 89%), as a colorless oil: ^1H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 7.42-7.24 (m, 5H), 5.21-4.97 (m, 2H), 4.38-4.23 (m, 1H), 4.07-4.00 (m, 0.5H), 3.98-3.70 (m, 3H), 3.56 (s, 1.5H), 2.85-2.75 (m, 0.5H), 2.70-2.60 (m, 0.5H), 2.40-2.30 (m, 1H), 2.28-1.92 (m, 4H), 1.90-1.65 (m, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (rotamers) 173.0, 172.8, 154.0, 153.4, 136.3, 136.1, 128.4, 128.3, 128.0, 127.9, 127.7, 67.1, 67.0, 59.1, 59.0, 57.8, 57.4, 52.3, 52.0, 46.8, 38.9, 38.3, 35.4, 34.7, 32.2, 31.9, 31.7, 31.1, 25.4, 25.3; $[\alpha]_D$ +5.2 (c 1.0, $CHCl_3$); ESI/MS for $C_{16}H_{23}BrNO_4$ calculated (M+H^+) 396, found 396.



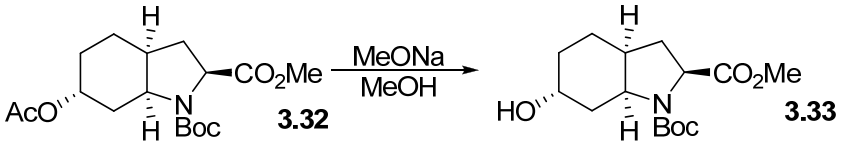
Compound 3.31: To a solution of **3.30** (320 mg, 0.8 mmol) in dry toluene (9 mL) at 40 °C was added Bu_4NOAc (3.65 g, 12 mmol). After stirring at 40 °C for 2.5 h, the reaction mixture was diluted by addition hexane (40 mL) and cooled to R.T.. The reaction mixture was washed with H_2O (40 mL) and brine (40 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 3:17) to afford **3.31** (245 mg, 82%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 7.36-7.28 (m, 5H), 5.23-4.99 (m, 3H), 4.39-4.31 (m, 1H), 4.27-4.21 (m, 0.5H), 4.17-4.11 (m, 0.5H), 3.77 (s, 1.5H), 3.57 (s, 1.5H), 2.46-2.30 (m, 2H), 2.25-2.18 (m, 1H), 2.06 (s, 1.5H), 2.05 (s, 1.5H), 2.03-1.88 (m, 2H), 1.78-1.69 (m, 2H), 1.64-1.54 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (rotamers) 173.8, 173.6, 170.5, 170.4, 154.6, 153.9, 136.9, 136.6, 128.6, 128.5, 128.1, 128.05, 127.98, 127.6, 69.4, 69.2, 67.2, 67.0, 59.4, 59.3, 54.5, 54.1, 52.5, 52.3, 36.8, 36.5, 35.8, 32.7, 31.7, 31.2, 30.7, 23.8, 21.4; ESI/MS for $C_{20}H_{25}NO_6$ calculated ($M+H^+$) 376, found 376.



Compound 3.31a: To a solution of **3.31** (99 mg, 0.25 mmol) in dry MeOH (5 mL) was added Pd/C 10 w.t.% (20 mg) and the suspension was stirred under H_2 (1 atm) at R.T. for 5 h. The Pd/C catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:39) to give **3.31a** (57 mg, 95%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.03-4.95 (m, 1H),

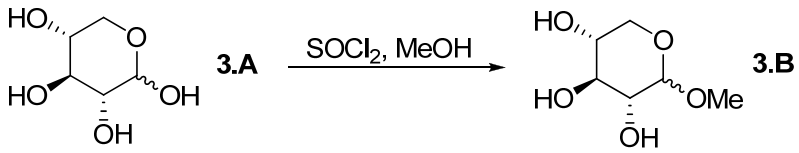
3.79 (dd, 1H, *J*=5.7 Hz, *J*=10.3 Hz), 3.72 (s, 3H), 3.66 (s, 1H), 3.31 (dd, 1H, *J*=4.9 Hz, *J*=9.9 Hz), 2.36 (br, m, 1H), 2.22-2.15 (m, 1H), 2.05-1.89 (m, 5H), 1.85-1.78 (m, 1H), 1.74-1.56 (m, 4H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 175.8, 170.6, 69.7, 58.4, 52.3, 37.2, 35.2, 32.9, 29.3, 24.9, 21.5; ESI/MS for $C_{12}H_{19}NO_4$ calculated ($M+H^+$) 242, found 242.

Compound 3.32: To a solution of **3.31a** (145 mg, 0.6 mmol) in dry DCM (15 mL) at 0 °C was added NEt_3 (167 μL, 1.20 mmol) and Boc_2O (196 mg, 0.9 mmol), then warmed to R.T.. After stirring for 26 h, the reaction mixture was diluted with DCM (30 mL) and washed with brine (30 mL). The aqueous phase extracted with DCM (2×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 3:17) to afford **3.32** (180 mg, 88%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.07 (br, m, 1H), 4.24-4.18 (m, 1H), 4.16-4.10 (m, 0.65H), 3.97 (br, m, 0.35H), 3.71 (s, 3H), 2.36-2.29 (br, m, 2H), 2.18-2.11 (m, 1H), 2.02 (s, 3H), 1.99-1.88 (m, 2H), 1.73-1.62 (m, 2H), 1.56-1.47 (m, 2H), 1.37 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 174.1, 170.4, 153.3, 80.1, 69.3, 59.5, 53.6, 52.1, 35.8, 32.5, 30.8, 28.4, 23.8, 21.3, 20.1; $[\alpha]_D$ -47.5 (c 1.0, $CHCl_3$); ESI/MS for $C_{17}H_{28}NO_6$ calculated ($M+H^+$) 342, found 342.

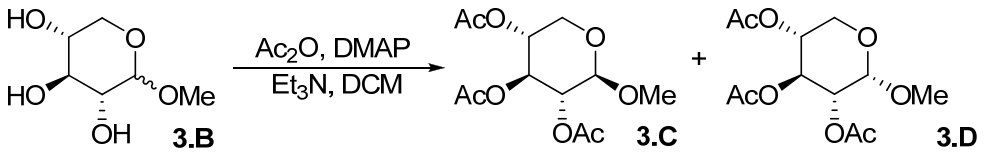


Compound 3.33: A solution of **3.32** (51 mg, 0.15 mmol) in 1.8 mL of MeOH was treated with 0.5 M NaOMe (1.1 mmol) in MeOH 2.1 mL. After stirring at R.T.

for 6 h, the reaction mixture was diluted with saturated aqueous NH_4Cl (1 mL), and concentrated under reduced pressure. The residue was redissolved in EtOAc (20 mL), filtered and concentration under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 2:3) to afford **3.33** (40 mg, 90%), the solid was recrystallized from DCM, to give a colorless crystalline solid, m.p. 153-155 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 4.20-4.11 (br, m, 3H), 3.72 (s, 3H), 2.32 (br, m, 2.5H), 2.16-2.09 (m, 2.5H), 1.98-1.38 (m, 12H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 174.2, 153.4, 80.0, 66.0, 59.6, 53.7, 52.1, 36.0, 33.8, 32.6, 28.4, 26.5, 19.7; $[\alpha]_D$ -31.8 (c 1.0, $CHCl_3$); ESI/MS for $C_{15}H_{25}NO_5$ calculated ($M+H^+$) 300, found 300.



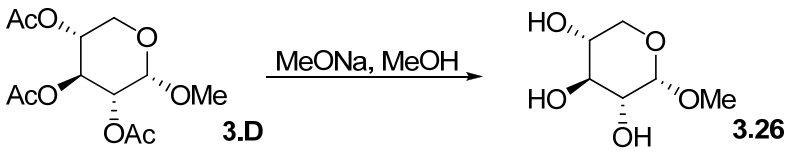
Methyl D-xylopyranoside 3.B: $SOCl_2$ (0.25 ml, 3.45 mmol) was added carefully at 0 °C into dry MeOH, then the reaction mixture was allowed to warm up to R.T. slowly. After stirring for 30 min., D-xylose **3.A** (1.2 g, 8.0 mmol) was added, and the reaction mixture was heated to reflux. After refluxing for 15 h, the reaction mixture was cooled to R.T., and neutralized by solid $NaHCO_3$ carefully. After MeOH was evaporated, the crude residue was purified by flash column chromatography (MeOH/DCM 3:17) to afford **3.B** (1.28 g, 98%, a colorless oil), as a α/β=7:3 mixture: ^{1}H NMR, (400 MHz, MeOH-d_4)(α and β mixture) δ 4.63 (d, 0.7H, J=3.6 Hz), 4.12 (d, 0.3H, J=7.6 Hz), 3.88 (dd, 0.3H, J=5.3 Hz, J=11.4 Hz), 3.59-3.53 (m, 1H), 3.51-3.45 (m, 2H), 3.43-3.30 (m, 4H), 3.23-3.14 (m, 0.7H); ^{13}C NMR, (100 MHz, MeOH-d_4)(α and β mixture) δ 106.1, 101.5, 77.8, 75.2, 74.9, 73.6, 71.5, 71.2, 67.0, 62.8, 57.4, 55.7; Chemical Formula: $C_6H_{12}O_5$.



Methyl 2,3,4-tri-*O*-acetyl-D-xylopyranosides 3.C (β) and 3.D (α) : Methyl D-xylopyranoside **3.B** (0.98 g, 6.0 mmol) was dissolved in dry DCM (50 mL), and cooled at 0 °C, then Ac_2O (2.8 mL, 30.0 mmol), DMAP (73 mg, 0.6 mmol), and Et_3N (5.0 mL, 36 mmol) were added. After stirring at 0 °C for 6 h, the reaction mixture was washed with 1 M HCl aqueous (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 3:17) to give the β-isomer **3.C** (507 mg, 29%), as a colorless solid, m.p. 100-105 °C, and the α-isomer **3.D** (1.18 g, 68%), as a colorless solid, m.p. 76-81 °C.

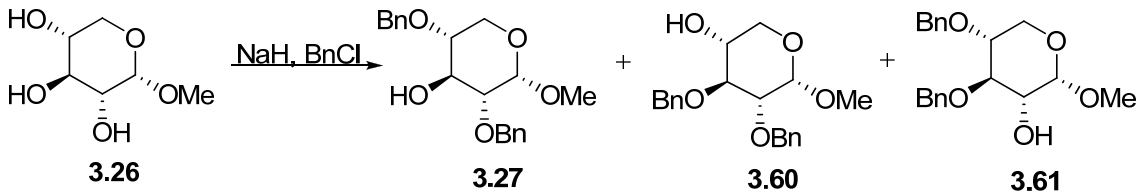
Methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside 3.C: ^{1}H NMR, (400VMHz, $CDCl_3$) δ 5.05 (t, 1H, *J*=8.6 Hz), 4.87-4.76 (m, 2H), 4.30 (d, 1H, *J*=6.9 Hz), 4.01 (dd, 1H, *J*=5.1 Hz, *J*=11.8 Hz), 3.35 (s, 3H), 3.28 (dd, 1H, *J*=5.1 Hz, *J*=6.7 Hz), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 169.9, 169.7, 169.3, 101.5, 71.4, 70.7, 68.8, 61.9, 56.5, 20.6; $[\alpha]_D$ -56.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{12}H_{18}O_8$ calculated (M-OAc+H^+) 259, found 259.

Methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside 3.D: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 5.42 (t, 1H, *J*=9.7 Hz), 4.92 (ddd, 1H, *J*=6.0 Hz, *J*=9.6 Hz, *J*=10.5 Hz), 4.83 (d, 1H, *J*=3.6 Hz), 4.79 (dd, 1H, *J*=3.6 Hz, *J*=10.0 Hz), 3.75 (dd, 1H, *J*=6.0 Hz, *J*=10.9 Hz), 3.54 (t, 1H, *J*=10.8 Hz), 3.35 (s, 3H), 2.03 (s, 3H), 1.99 (s, 6H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.3, 170.1, 170.0, 96.9, 71.1, 69.6, 69.4, 58.2, 55.4, 20.8; $[\alpha]_D$ +117.9 (c 1.0, $CHCl_3$); ESI/MS for $C_{12}H_{18}O_8$ calculated (M-OAc+H^+) 259, found 259.



3.D 3.26

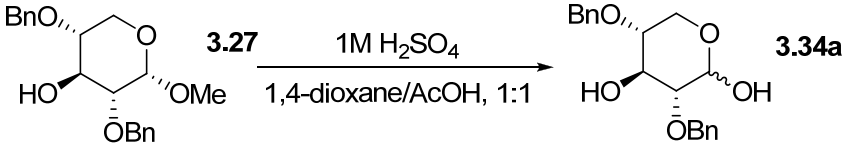
Methyl α-D-xylopyranoside 3.26: A solution of methyl 2,3,4-tri-*O*-acetyl-α-D-xylopyranoside **3.D** (3.5 g, 12.0 mmol) in dry fresh MeOH (40 mL) was treated with small pieces of Na (414 mg, 18.0 mmol) carefully. After stirring at R.T. for 1 h, the reaction mixture was diluted with solid NH_4Cl, filtered and concentrated under reduced pressure. The crude residue was redissolved in DCM (40 mL), filtered and concentration under reduced pressure. The residue was purified by flash column chromatography (MeOH/DCM 3:17) to afford **3.26** (1.9 g, 97%), as a colorless solid, m.p. 81-82 °C: ^{1}H NMR, (300 MHz, MeOH-d_4) δ 5.54 (s, 1H), 5.38 (d, 1H, *J*=6.2 Hz), 4.62 (d, 1H, *J*=3.7 Hz), 3.60–3.49 (m, 2H), 3.49–3.41 (m, 2H), 3.41–3.35 (m, 4H); ^{13}C NMR, (75 MHz, MeOH-d_4) δ 101.5, 75.2, 73.6, 71.5, 62.8, 55.7; $[\alpha]_D$ +153.4 (c 1.0, MeOH); Chemical Formula: $C_6H_{12}O_5$.



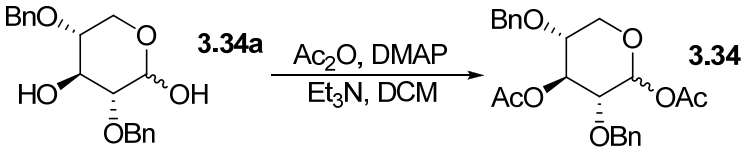
3.26 3.27 3.60 3.61

Compounds 3.27, 3.60+3.61: To a solution of methyl α-D-xylopyranoside **3.26** (879 mg, 5.35 mmol) in dry BnCl (20 mL) at 0 °C was added NaH (428 mg, 10.7 mmol) carefully, and then heated to 100 °C. After stirring at 100 °C for 10 h, the reaction mixture was cooled to R.T., and quenched with AcOH (0.06 mL). Then solvent was evaporated at 90 °C to afford yellow oil, which was purified by flash column chromatography (EtOAc/hexane 1:4) to give the major product **3.27** (1.14 g, 62%), as a colorless solid, m.p. 77-78 °C. Then the mixture of products **3.60** and **3.61** (221 mg, 12%), as a colorless oil, was obtained by flash column chromatography (EtOAc/hexane 1:4).

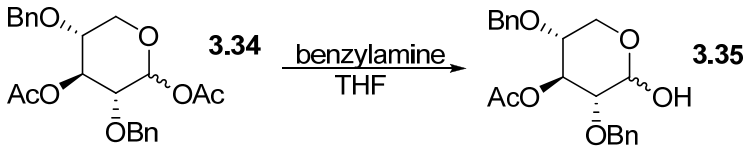
Methyl 2,4-di-*O*-benzyl-α-D-xylopyranoside 3.27: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.43-7.29 (m, 10H), 4.79 (dd, 2H, *J*=5.5 Hz, *J*=12.1 Hz), 4.69 (t, 2H, *J*=11.7 Hz), 4.60 (d, 1H, *J*=3.5 Hz), 4.09 (t, 1H, *J*=8.1 Hz), 3.66-3.63 (m, 1H), 3.53-3.51 (m, 2H), 3.39 (dd, 1H, *J*=3.6 Hz, *J*=9.6 Hz), 3.37 (s, 3H), 3.09 (s, 1H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 138.4, 138.2, 128.43, 128.39, 127.98, 127.87, 127.71, 97.8, 79.4, 77.7, 73.1, 73.0, 72.8, 59.5, 55.1; $[\alpha]_D$ +63.0 (c 1.0, $CHCl_3$); ESI/MS for $C_{20}H_{24}O_5$ calculated ($M+H^+$) 345, found 345.



2,4-Di-*O*-benzyl-α,β-D-xylopyranose 3.34a: To a solution of methyl 2,4-di-*O*-benzyl-α-D-xylopyranoside **3.27** (2.15 g, 6.25 mmol) in 1,4-dioxane (18 mL) was added 3 M H_2SO_4 aqueous (18 mL) and AcOH (18 mL), then the reaction mixture was heated to 60 °C. After refluxing for 72 h, the reaction mixture was cooled to R.T., diluted with H_2O (200 mL), and extracted with EtOAc (3×200 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The oil was purified by flash column chromatography (EtOAc/hexane 3:7) to afford **3.34a** (2.04 g, 99%, a colorless oil), as a α/β=1:1 mixture: ^{1}H NMR, (400 MHz, $CDCl_3$)(α and β mixture) δ 7.40-7.29 (m, 10H), 5.18 (d, 0.5H, *J*=3.6 Hz), 4.97 (d, 0.5H, *J*=11.5 Hz), 4.79-4.56 (m, 4H), 4.01 (t, 0.5H, *J*=9.1 Hz), 3.97 (dd, 0.5H, *J*=5.2 Hz, *J*=11.5 Hz), 3.79-3.66 (m, 1H), 3.57-3.44 (m, 1.5H), 3.41 (dd, 0.5H, *J*=3.6 Hz, *J*=9.3 Hz), 3.27 (dd, 0.5H, *J*=10.1 Hz, *J*=11.6 Hz), 3.21 (dd, 0.5H, *J*=7.5 Hz, *J*=9.1 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$)(α and β mixture) δ 138.5, 138.4, 137.9, 128.82, 128.74, 128.34, 128.30, 128.24, 128.15, 128.13, 128.10, 128.03, 128.00, 97.9, 91.2, 79.7, 77.7, 74.6, 73.9, 73.4, 73.3,73.03, 72.98, 72.94, 64.2, 61.7; ESI/MS for $C_{19}H_{22}O_5$ calculated ($M+H_2O$) 348, found 348.

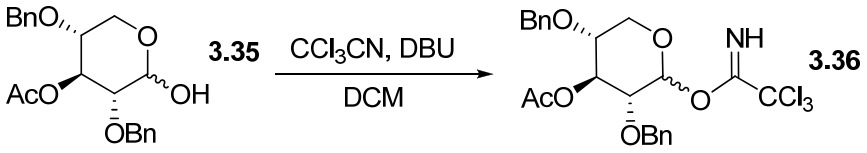


1,3-Di-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose 3.34: 2,4-di-*O*-benzyl-α,β-D-xylopyranose **3.34a** (1.31 g, 3.95 mmol) was dissolved in dry DCM (50 mL), and cooled at 0 °C, then Ac_2O (1.12 mL, 11.85 mmol), DMAP (48 mg, 0.4 mmol), and Et_3N (2.2 mL, 15.8 mmol) were added, then warmed to R.T.. After stirring for 3 h, the reaction mixture was washed with 1 M HCl aqueous (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/ hexane 1:4) to afford **3.34** (1.61 g, 98%, a colorless oil), as a α/β=1:1 mixture: ^{1}H NMR, (400 MHz, $CDCl_3$)(α and β mixture) δ 7.38-7.32 (m, 10H), 7.32-7.27 (m, 10H), 6.26 (d, 1H, *J*=3.6 Hz), 5.63 (d, 1H, *J*=7.6 Hz), 5.43 (t, 1H, *J*=9.6 Hz), 5.25 (t, 1H, *J*=8.9 Hz), 4.73-4.50 (m, 8H), 3.99 (dd, 1H, *J*=5.0 Hz, *J*=11.6 Hz), 3.80 (dd, 1H, *J*=5.8 Hz, *J*=11.2 Hz), 3.70 (t, 1H, *J*=10.9 Hz), 3.61-3.44 (m, 5H), 2.17 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$)(α and β mixture) δ 170.2, 169.9, 169.8, 169.1, 137.99, 137.97, 137.93, 137.63, 128.66, 128.63, 128.59, 128.16, 128.14, 128.00, 127.96, 127.92, 127.88, 127.83, 94.7, 89.7, 78.2, 76.1, 75.4, 75.2, 74.5, 74.4, 73.2, 72.91, 72.88, 64.5, 62.0; ESI/MS for $C_{23}H_{26}O_7$ calculated (M+H_2O) 432, found 432.



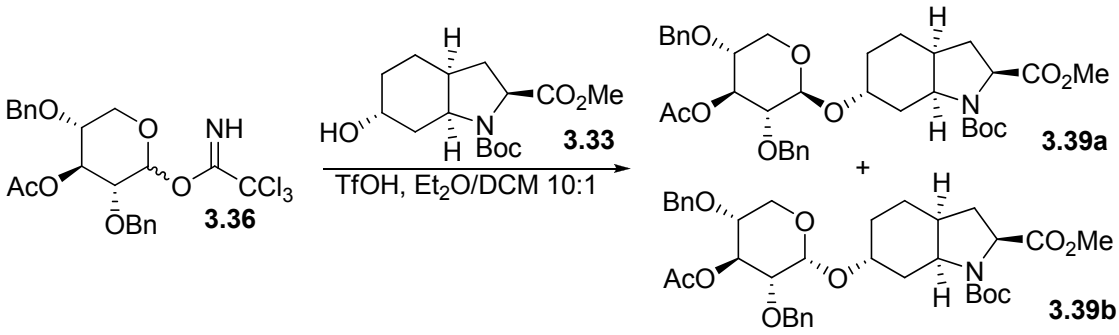
3-*O*-Acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose 3.35: 1,3-di-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose **3.34** (2.3 g, 5.55 mmol) was dissolved in dry THF (30 mL), and benzylamine (2.42 mL, 22.2 mmol) was added at R.T..

After stirring for 48 h, the reaction mixture was concentrated under reduced pressure. The crude residue was redissolved in DCM (30 mL), and washed with 1 M HCl aqueous (30 mL). The aqueous phase was extracted with DCM (2×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:3) to give **3.35** (1.72 g, 83%, a colorless oil), as a α/β=1:1 mixture: ^{1}H NMR, (400 MHz, $CDCl_3$)(α and β mixture) δ 7.38-7.27 (m, 20H), 5.44 (t, 1H, *J*=8.9 Hz), 5.19 (t, 2H, *J*=9.3 Hz), 4.88 (d, 1H, *J*=11.9 Hz), 4.71 (d, 1H, *J*=7.5 Hz), 4.68-4.52 (m, 7H), 4.19 (s, 1H), 3.97 (dd, 1H, *J*=5.3 Hz, *J*=11.6 Hz), 3.90 (dd, 1H, *J*=10.5 Hz, *J*=10.9 Hz), 3.72 (dd, 1H, *J*=5.2 Hz, *J*=11.4 Hz), 3.61 (s, 1H), 3.60-3.48 (m, 2H), 3.46 (dd, 1H, *J*=3.4 Hz, *J*=9.1 Hz), 3.34-3.25 (m, 2H), 2.04 (s, 3H), 1.97 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$)(α and β mixture) δ 170.3, 138.4, 138.1, 138.0, 137.6, 128.7, 128.58, 128.57, 128.47, 128.2, 128.02, 127.98, 127.8, 98.0, 91.1, 80.2, 77.4, 75.6, 75.3, 74.9, 74.1, 72.9, 72.8, 72.6, 72.4, 63.9, 60.3, 21.2, 21.1; ESI/MS for $C_{21}H_{24}O_6$ calculated (M+H_2O) 390, found 390.



Compound 3.36: 3-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose **3.35** (104 mg, 0.28 mmol) was dissolved in dry DCM (10 mL), then CCl_3CN (93 μL, 0.93 mmol) and DBU (13 μL, 0.08 mmol) were added at R.T.. After stirring for 2.5 h, the solvent was evaporated. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:4) to give **3.36** (131 mg, 91%, a light yellow oil), as a α/β=3.5:1 mixture, which is not stable by keeping and used directly after made: ^{1}H NMR, (400 MHz, $CDCl_3$)(α and β mixture) δ 8.74 (s, 0.3H), 8.61 (s, 1H), 7.39-7.28 (m, 13H), 6.41 (d, 1H, *J*=3.5 Hz), 5.93 (d, 0.3H,

J=6.5 Hz), 5.54 (t, 1H, *J*=9.6 Hz), 5.29 (t, 0.3H, *J*=7.9 Hz), 4.86 (d, 0.3H, *J*= 11.8 Hz), 4.70-4.55 (m, 4.9H), 4.15 (q, 0.3H, *J*=7.1 Hz), 4.11-4.07 (m, 0.3H), 3.86 (d, 1H, *J*=2.4 Hz), 3.84 (d, 1H, *J*=5.3 Hz), 3.68-3.58 (m, 2.6H), 2.04 (s, 3H), 1.98 (s, 0.9H); ^{13}C NMR, (100 MHz, $CDCl_3$)(α and β mixture) δ 170.0, 161.6, 160.9, 137.93, 137.88, 137.81, 137.78, 128.7, 128.57, 128.56, 128.15, 128.12, 128.08, 128.0, 127.95, 127.91, 127.7, 98.4, 93.9, 91.2, 91.0, 77.1, 76.6, 75.3, 74.5, 74.2, 73.14, 73.11, 72.75, 72.73, 72.66, 64.0, 62.3, 21.2, 21.1; ESI/MS for $C_{23}H_{24}Cl_3NO_6$ calculated (M-O(C=NH)CCl_3) 355, found 355.

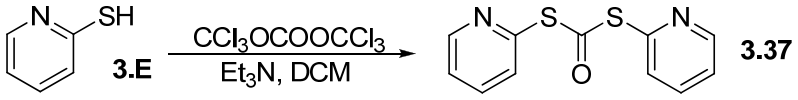


Compounds 3.39a and 3.39b: To a solution of **3.33** (129 mg, 0.4 mmol) and 4Å molecular sieves (400 mg, powdered, activated) in DCM (1 mL) was added a solution of **3.36** (289 mg, 0.6 mmol) in Et_2O (10 mL) under argon atmosphere. After stirring at R.T. for 30 min., TfOH (4 μL, 0.04 mmol) was added dropwise. After addition was completed, the solution was stirring for 30 min., then neutralized with solid $NaHCO_3$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 3:17) to obtain the β-isomer **3.39a** (126 mg, 44%), as a colorless oil, and the α-isomer **3.39b** (114 mg, 40%), as a colorless oil.

Compound 3.39a: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 7.35-7.25 (m, 10H), 5.13 (t, 1H, *J*=9.3 Hz), 4.83 (d, 1H, *J*=11.8 Hz), 4.63-4.51 (m, 3H), 4.44 (br, m, 1H), 4.31-4.21 (br, m, 1.5H), 4.11 (br, m, 1.5H), 4.00-3.87 (br, m, 1.5H), 3.73 (s, 3H), 3.55-3.48 (br, m, 1H), 3.30-3.23 (m, 2H), 2.53-2.44 (br, m,

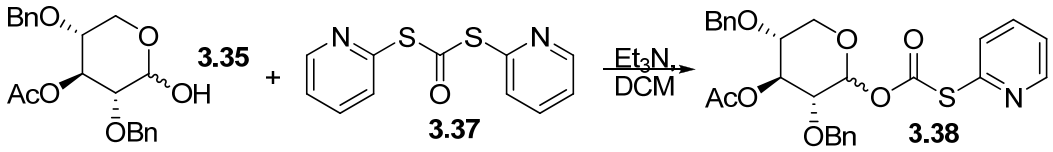
0.5H), 2.41-2.25 (br, m, 1.5H), 2.19-2.10 (br, m, 1H), 2.08-1.87 (m, 5H), 1.88-1.76 (br, d, 1H), 1.71-1.60 (br, m, 1H), 1.50-1.36 (br, m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (rotamers) 174.2, 174.0, 170.1, 154.0, 153.1, 138.33, 138.26, 138.15, 138.01, 128.5, 128.4, 128.0, 127.97, 127.94, 127.92, 127.7, 101.5, 101.2, 79.7, 79.4, 79.0, 75.6, 75.5, 74.9, 74.6, 74.2, 72.8, 72.1, 63.8, 63.7, 59.5, 58.9, 54.1, 53.8, 52.2, 52.0, 36.4, 35.8, 32.6, 32.4, 32.3, 31.8, 28.5, 28.4, 22.9, 22.7, 21.1, 19.8; $[\alpha]_D$ -30.9 (c 1.0, $CHCl_3$); HRMS for $C_{36}H_{47}NO_{10}$ calculated ($M+H^+$) 654.32727, found 654.32596.

Compound 3.39b: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 7.34-7.27 (m, 10H), 5.47-5.42 (br, t, 1H), 5.14-5.18 (br, m, 1H), 4.89-4.82 (br, m, 1H), 4.56 (q, 3H, *J*=12.0 Hz), 4.28-4.11 (br, m, 2H), 3.99 (br, m, 1H), 3.74 (s, 3H), 3.64 (d, 2H, *J*=8.9 Hz), 3.53 (t, 1H, *J*=8.4 Hz), 3.47 (dd, 1H, *J*=3.1 Hz, *J*=9.9 Hz), 2.58-2.53 (br, m, 0.6H), 2.45-2.40 (br, m, 0.4H), 2.36-2.10 (br, m, 3H), 2.03-1.92 (br, m, 5H), 1.70-1.67 (br, m, 1H), 1.59-1.41 (br, m, 11H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (rotamers) 174.4, 174.1, 170.2, 154.0, 153.0, 138.5, 138.1, 128.52, 128.48, 128.44, 128.29, 128.14, 127.94, 127.79, 127.57, 127.45, 93.9, 93.1, 79.9, 79.5, 76.3, 75.0, 73.15, 73.04, 72.95, 72.89, 72.3, 72.0, 70.3, 69.3, 60.0, 59.8, 59.5, 59.1, 53.8, 53.5, 52.2, 52.0, 36.4, 35.7, 32.7, 31.8, 29.8, 29.4, 28.5, 28.4, 25.7, 25.6, 21.2, 21.1, 20.2, 20.1; $[\alpha]_D$ +21.4 (c 1.0, $CHCl_3$); HRMS for $C_{36}H_{47}NO_{10}$ calculated ($M+H^+$) 654.32727, found 654.32635.



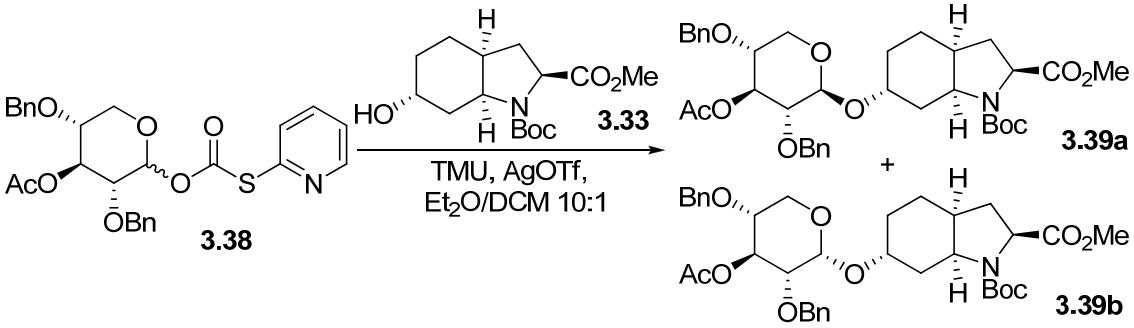
Di(*S*-2-pyridyl) Thiocarbonate (3.37): To a solution of triphosgene (505 mg, 1.7 mmol) and 2-mercaptopyridine **3.E** (1.1 g, 10 mmol) in DCM (50 mL) at 0 °C was added dropwise Et_3N (1.5 mL, 10.8 mmol). The reaction mixture was stirred for 1 h, then warmed to R.T. and stirred for 2 h. The reaction

mixture was concentrated, treated with cold saturated aqueous $NaHCO_3$ (50 mL) at 0 °C, and extracted with EtOAc (2×50 mL). The organic layer was washed with H_2O (50 mL) and brine (50 mL), dried over $NaSO_4$, filtered and concentrated under reduced pressure to obtain the crude product as a yellow solid. The crude residue was purified by flash column chromatography (EtOAc/ hexane 3:7) to afford **3.37** (1.04 g, 84%), as a pale yellow needle-shaped crystals, m.p. 42-44 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (ddd, 2H, *J*=0.8 Hz, *J*=1.9 Hz, *J*=4.8 Hz), 7.69 (td, 2H, *J*=1.9 Hz, *J*=7.9 Hz), 7.63 (dt, 2H, *J*=1.1 Hz, *J*=7.9 Hz), 7.26 (ddd, 2H, *J*=1.3 Hz, *J*=4.8 Hz, *J*=7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.6, 150.6, 150.5, 137.5, 130.5, 124.2; ESI/MS for $C_{11}H_9ON_2S_2$ calculated ($M+H^+$) 250, found 250.



3-*O*-Acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose 2-Thiopyridyl Carbonate 3.38: A mixture of di(*S*-2-pyridyl) thiocarbonate **3.37** (201 mg, 0.81 mmol), 3-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose **3.35** (101 mg, 0.27 mmol), and Et_3N (113 μL, 0.81 mmol) in DCM (10 mL) was stirred at R.T. for 24 h. Concentration and purification by flash column chromatography (EtOAc/hexane 1:3) gave **3.38** (119 mg, 86%, a light yellow oil), as a α/β=1:3.2 mixture: ^{1}H NMR, (400 MHz, $CDCl_3$)(α and β mixture) δ 8.63-8.59 (m, 4H), 8.49-8.48 (m, 1H), 7.76-7.70 (m, 9H), 7.66-7.59 (m, 2H), 7.38-7.27 (m, 52H), 7.12 (ddd, 1H, *J*=1.7Hz, *J*=4.8 Hz, J=6.7 Hz), 6.28 (d, 1H, *J*=3.5 Hz), 5.79 (d, 3H, *J*=6.8 Hz), 5.32 (t, 1H, *J*=9.6 Hz), 5.24 (t, 3H, *J*=8.2 Hz), 4.77 (d, 4H, *J*= 11.8 Hz), 4.67-4.51 (m, 12H), 4.03 (dd, 3H, *J*=4.3 Hz, *J*=11.4 Hz), 3.80 (dd, 1H, *J*=5.6 Hz, *J*=11.2 Hz), 3.67-3.48 (m, 12H), 2.03 (s, 3H), 1.99 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$)(α and β mixture) δ 169.93, 169.88, 167.9, 167.6,

151.0, 150.33, 150.28, 149.6, 137.8, 137.7, 137.58, 137.54, 137.51, 137.49, 137.43, 129.9, 129.4, 128.56, 128.53, 128.09, 128.06, 128.01, 127.84, 127.81, 127.7, 123.87, 123.85, 121.2, 119.7, 97.0, 92.8, 76.8, 75.9, 74.9, 74.4, 74.2, 73.1, 72.9, 72.8, 72.6, 72.4, 64.0, 62.2, 21.1, 21.0; HRMS for $C_{23}H_{24}Cl_3NO_6$ calculated ($M+H^+$) 510.1581, found 510.15856.



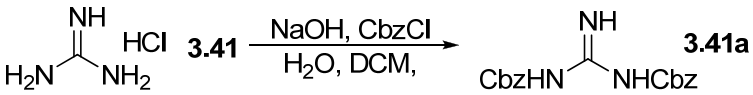
Compounds 3.39a and 3.39b: A mixture of 3-*O*-acetyl-2,4-di-*O*-benzyl-α,β-D-xylopyranose 2-thiopyridyl carbonate **3.38** (120 mg, 0.24 mmol), **3.33** (50 mg, 0.17 mmol), 1,1,3,3-tetramethylurea (30 μL, 0.24 mmol) and 4Å activated molecular sieves in Et_2O (20 mL) and DCM (2 mL) was stirred at R.T. for 10 h, and then cooled at 0 °C. Silver triflate (400 mg, 1.53 mmol) was added to the reaction mixture, and the stirring was continued 24 h at R.T. in the dark. The suspension was treated with a few drops of pyridine, filtered over celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 2:3) to give **3.39a** (58 mg, 53%), as a colorless oil, and **3.39b** (47 mg, 43%), as a colorless oil.

Compound 3.40a: Compound **3.39a** (39 mg, 0.06 mmol) was treated with >99.9% TFA/DCM (1:9, 3 mL). After stirring at R.T. for 3 h, the reaction

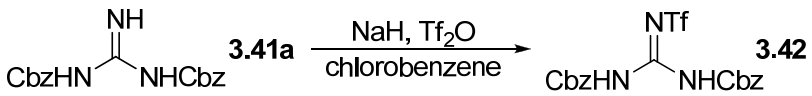
mixture was diluted with EtOAc (20 mL) and concentrated under reduced pressure. The crude residue was suspended in saturated aqueous Na_2CO_3 (10 mL) and extracted with DCM (3×10 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM to MeOH/DCM 1:24) to afford **3.40a** (32 mg, 95%), as a light yellow oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.35-7.26 (m, 10H), 5.13 (t, 1H, *J*=9.4 Hz), 4.82 (d, 1H, *J*=11.9 Hz), 4.60-4.48 (m, 4H), 4.03-3.89 (m, 3H), 3.77 (s, 3H), 3.52 (dt, 1H, *J*=5.4 Hz, *J*=9.9 Hz, *J*=9.7 Hz), 3.46 (dd, 1H, *J*=5.0 Hz, *J*=10.0 Hz), 3.29-3.24 (m, 2H), 2.30-2.23 (m, 1H), 2.11-2.03 (m, 3H), 1.97-1.93 (m, 3H), 1.86-1.66 (m, 3H), 1.45-1.31 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ176.1, 170.2, 138.5, 138.1, 128.61, 128.47, 128.11. 128.05, 127.88, 127.84, 127.77, 102.4, 79.2, 75.6, 75.1, 74.4, 74.1, 72.9, 72.4, 63.9, 58.43, 58.38, 52.4, 37.3, 35.4, 34.8, 29.9, 29.4, 25.0, 21.2; $[\alpha]_D$ -9.3 (c 1.0, $CHCl_3$); ESI/MS for $C_{31}H_{39}NO_8$ calculated ($M+H^+$) 554, found 554.

Compound 3.40b: Compound **3.39b** (27 mg, 0.042 mmol) was treated with >99.9% TFA/DCM (1:9, 3 mL). After stirring at R.T. for 3 h, the mixture was diluted with EtOAc (20 mL) and concentrated under reduced pressure. The crude residue was suspended in saturated aqueous Na_2CO_3 (10 mL) and extracted with DCM (3×10 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:24) to give **3.40b** (22 mg, 92%), as a light yellow oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.38-7.28 (m, 10H),

5.42 (t, 1H, *J*=9.6 Hz), 4.88 (d, 1H, *J*=3.6 Hz), 4.61-4.52 (m, 4H), 3.93 (dd, 1H, *J*=5.6 Hz, *J*=10.5 Hz), 3.84-3.76 (m, 4H), 3.70-3.61 (m, 2H), 3.54-3.43 (m, 2H), 3.40 (dd, 1H, *J*=3.6 Hz, *J*=10.0 Hz), 1.79-1.70 (m, 2H), 1.52-1.43 (m, 1H), 1.36-1.30 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ176.1, 170.2, 138.26, 138.24, 128.6, 128.0, 127.93, 127.86, 95.2, 77.3, 76.3, 73.2, 73.0, 72.5, 72.4, 59.9, 58.54, 58.45, 52.4, 37.4, 35.7, 32.6, 31.2, 25.5, 21.3; $[\alpha]_D$ +37.2 (c 1.0, $CHCl_3$); ESI/MS for $C_{31}H_{39}NO_8$ calculated ($M+H^+$) 554, found 554.

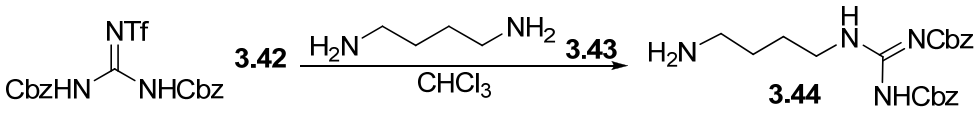


Compound 3.41a: To a solution of guanidine hydrochloride **3.41** (960 mg, 10 mmol) and NaOH (2.0 g, 50 mmol) in H_2O (10 mL) was added DCM (20 mL), then the reaction mixture was cooled to 0 °C. CbzCl (4.3 mL, 30 mmol) was added dropwise with stirring over a period of 45 min.. After addition was completed, the reaction mixture was stirring at 0 °C for 20 h, and diluted with DCM (10 mL). The layers were separated, and the aqueous layer was extracted with DCM (25 mL). The combined organic phases were washed with H_2O (2×50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was recrystallized from MeOH to give **3.41a** (2.8 g, 86%), as a colorless crystals, m.p. 148-150 °C: ^{1}H NMR, (400 MHz, DMSO-d_6) δ 10.88 (br, s, 1H), 8.67 (br, s, 2H), 7.40-7.25 (m, 10H), 5.10 (s, 4H); ESI/MS for $C_{17}H_{17}N_3O_4$ calculated ($M+H^+$) 328, found 328.

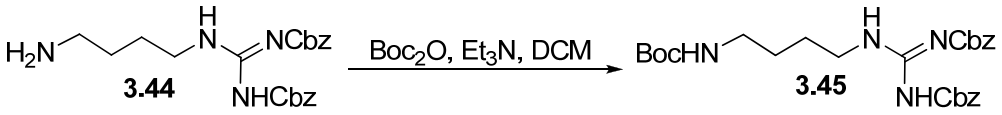


Compound 3.42: A solution of crystals **3.41a** (1.65 g, 5.0 mmol) in chlorobenzene (50 mL) was cooled on ice to 0 °C, and treated with NaH (400 mg, 60 dispersion in mineral oil) under argon atmosphere. After stirring at 0 °C for

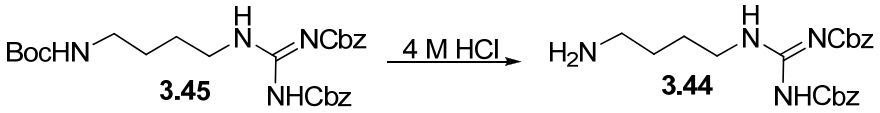
2 h, the reaction mixture was cooled to -45 °C, and Tf_2O (0.82 mL, 5 mmol) was added, then allowed to warm up to R.T. and stirred for 10 h. After solvent was evaporated, the residue was redissolved in a mixture of EtOAc (100 mL) and 2 M $NaHSO_4$ aqueous (50 mL), then the phases were separated. The organic phase was washed with H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM/Et_2O 19:1) to afford Goodman's reagent **3.42** (1.56 g, 67%), as a pale oil that was crystallized under reduced pressure, m.p. 73-75 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 10.35 (br, s, 2H), 7.46-7.42 (m, 10H), 5.29 (s, 4H); ESI/MS for $C_{18}H_{16}F_3N_3O_6S$ calculated ($M+H^+$) 460, found 460.



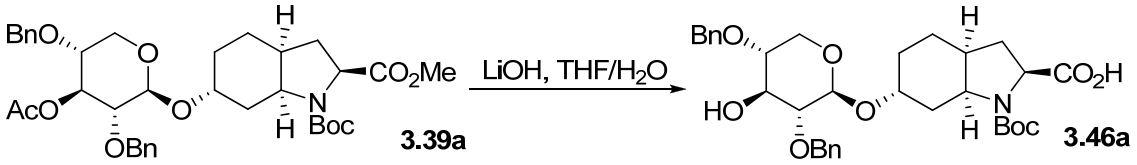
Compound 3.44: A solution of Goodman's reagent **3.42** (1.17 g, 2.55 mmol) in $CHCl_3$ (40 mL) was added dropwise to a vigorously stirred solution of 1,4-diaminobutane **43** (2.25 g, 25.5 mmol) in $CHCl_3$ (30 mL) at R.T. over 2 h. After addition, the reaction mixture was stirred for 30 min.. then diluted with $CHCl_3$ (30 mL) and washed with 10% $NaHCO_3$ aqueous (2×100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/$CHCl_3$ 1:9) to give **3.44** (0.96 g, 94%), as a colorless wax: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.40-7.23 (m, 10H), 5.24 (s, 1H), 5.15 (s, 2H), 5.12 (s, 2H), 3.40 (t, 2H, *J*=6.1 Hz), 2.67 (t, 2H, *J*=6.1 Hz), 1.61-1.54 (m, 2H), 1.49-1.42 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 163.6, 155.9, 153.7, 136.7, 134.6, 128.7, 128.6, 128.4, 128.3, 128.0, 127.8, 68.0, 67.0, 41.4, 40.8, 30.3, 26.2; ESI/MS for $C_{21}H_{26}N_4O_4$ calculated ($M+H^+$) 399, found 399.



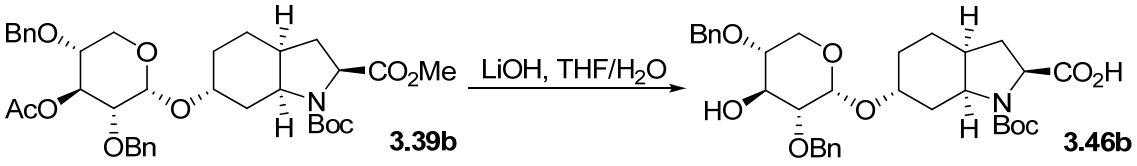
Compound 3.45: To a solution of **3.44** (300 mg, 0.75 mmol) in dry DCM (15 mL) at 0 °C was added NEt_3 (209 μL, 1.50 mmol) and Boc_2O (231 mg, 1.06 mmol), then warmed to R.T.. After stirring for 10 h, the reaction mixture was diluted with DCM (15 mL) and washed with brine (30 mL). The aqueous phase was extracted with DCM (3×30 mL). The organic phase was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:4) to afford **3.45** (344 mg, 92%), as a colorless solid, m.p. 63-65 °C: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.75 (s, 1H), 8.32 (t, 1H, *J*=5.2 Hz), 7.39-7.24 (m, 10H), 5.15 (s, 2H), 5.12 (s, 2H), 4.87 (br, t, 1H), 3.40 (dd, 2H, *J*=6.7 Hz, *J*=12.6 Hz), 3.11 (br, dd, 2H), 1.59-1.53 (m, 2H), 1.52-1.46 (m, 2H), 1.43 (s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 163.6, 155.95, 155.88, 153.7, 136.7, 134.6, 128.7, 128.6, 128.34, 128.28, 128.0, 127.8, 78.9, 68.0, 67.0, 40.6, 39.9, 28.3, 27.2, 26.2; ESI/MS for $C_{26}H_{34}N_4O_6$ calculated ($M+H^+$) 499, found 499.



Compound 3.44：A solution of **3.45** (136 mg, 0.27 mmol) was treated with 4 M HCl in 1,4-dioxane (2 mL) at R.T. for 6 h. Then the reaction mixture was diluted with $CHCl_3$ (10 mL) and washed with 10% $NaHCO_3$ aqueous (15 mL) carefully, dried over Na_2SO_4 and concentrated under reduced pressure to obtain Cbz-protected agmatine **3.44**, as a colorless wax (108 mg, 99%), which was sufficiently pure for use in the following step.

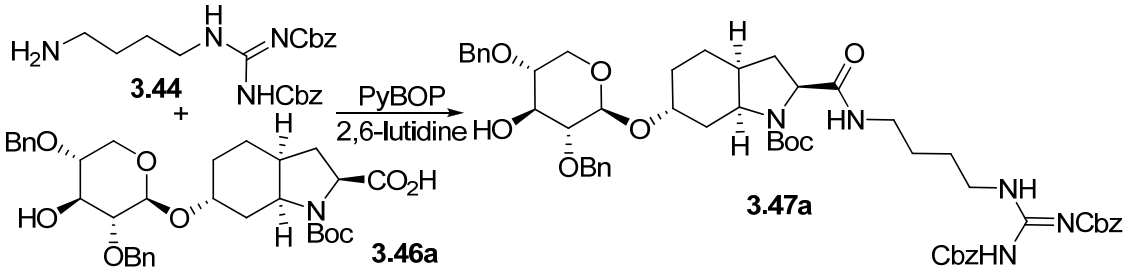


Compound 3.46a: A solution of **3.39a** (85 mg, 0.13 mmol) in THF/H_2O (5:3, 3.2 mL) was treated with LiOH×H_2O (16 mg, 0.39 mmol). After stirring at R.T. for 24 h, the reaction mixture was cooled on ice to 0 °C and acidified with 5% citric acid aqueous to pH=3-4. The organic phase was separated and the aqueous phase was extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to afford carboxylic acid **3.46a** (77 mg, 99%), as a colorless oil, which was sufficiently pure for use in the following step. ^{1}H NMR, (400 MHz, $CDCl_3$) δ (rotamers) 7.38-7.30 (m, 10H), 4.94 (d, 1H, *J*=11.4Hz), 4.77 (d, 1H, *J*=11.9 Hz), 4.72 (d, 1H, *J*=11.4 Hz), 4.65 (d, 1H, *J*=11.8 Hz), 4.41 (d, 1H, *J*=7.4 Hz), 4.32 (s, 1H), 4.17 (s, 1H), 4.08 (s, 1H), 3.91 (s, 1H), 3.70 (t, 1H, *J*=8.9 Hz), 3.55-3.49 (m, 1H), 3.28-3.19 (m, 2H), 2.38-2.28 (br, m, 1.5H), 2.20-2.13 (br, m, 1.5H), 2.08-1.99 (br, m, 1H), 1.79-1.76 (br, dd, 1H), 1.56-1.53 (br, dd, 2H), 1.46 (br, s, 9H), 1.28 (br, s, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (major rotamer) 138.43, 138.36, 128.71, 128.67, 128.09, 128.02, 101.6, 81.3, 77.4, 75.8, 74.7, 73.3, 73.0, 64.0, 59.3, 54.7, 36.0, 32.6, 29.9, 28.6, 23.4, 20.1; $[\alpha]_D$ -43.5 (c 1.0, $CHCl_3$); HRMS for $C_{33}H_{43}NO_9$ calculated ($M+Na^+$) 620.283, found 620.28556.



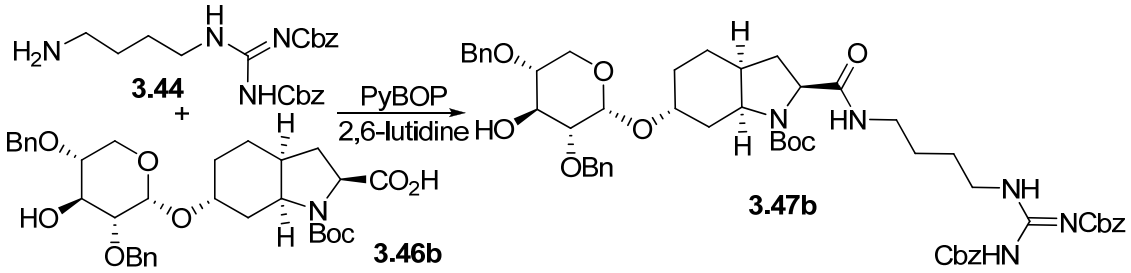
Compound 3.46b: A solution of **3.39b** (118 mg, 0.18 mmol) in THF/H_2O (5:3, 4.8 mL) was treated with LiOH×H_2O (23 mg, 0.54 mmol). After stirring at R.T. for 24 h, the reaction mixture was cooled on ice to 0 °C and acidified with

5% citric acid aqueous to pH=3-4. The organic phase was separated and the aqueous phase was extracted with DCM (3×20 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to afford **3.46b** (107 mg, 99%). The crude solid was recrystallized from MeCN to give as a colorless crystalline solid, m.p. 143-145 °C, which was sufficiently pure for use in the following step. ^{1}H NMR, (700 MHz, $CDCl_3$, 50 °C) δ (rotamers) 7.37-7.35 (m, 7.5H), 7.32-7.28 (m, 2.5H), 5.03 (s, 1H), 4.84 (s, 1H), 4.80 (d, 1H, *J*=11.7 Hz), 4.68 (dd, 3H, *J*=6.5 Hz, *J*=11.8 Hz), 4.30 (s, 1H), 4.16 (s, 1H), 4.07 (t, 1H, *J*=9.0 Hz), 3.93 (s, 1H), 3.63 (dd, 1H, *J*=5.1 Hz, *J*=10.3 Hz), 3.56-3.49 (m, 2H), 3.40(dd, 1H, *J*=3.5 Hz, *J*=9.6 Hz), 2.37-2.31 (m, 1H), 2.19-2.13 (m, 2H), 1.72-1.67 (m, 1H), 1.65-1.59 (m, 2H), 1.57-1.48 (m, 1H), 1.44 (s, 9H), 1.35-1.30 (m, 2H); ^{13}C NMR, (175 MHz, $CDCl_3$, 50 °C) δ (major rotamer) 138.6, 138.4, 130.3, 129.9, 128.7, 128.1, 94.3, 79.8, 78.0, 73.6, 73.1, 72.7, 70.8, 60.3, 59.5, 54.5, 36.1, 32.1, 29.99, 29.92, 29.91, 29.73, 29.55, 29.51, 29.43, 29.39, 29.33, 28.6, 27.5, 27.4, 25.9, 25.7, 22.9, 20.5, 14.3; $[\alpha]_D$ +15.5 (c 1.0, $CHCl_3$); HRME for $C_{33}H_{43}NO_9$ calculated ($M+NH_4^+$) 615.32761, found 615.32741.



Compound 3.47a: To a solution of **3.46a** (60 mg, 0.1 mmol) in DCM (2 mL) at 0 °C was added PyBOP (78 mg, 0.15 mmol) with stirring for 10 min., and then a solution of Cbz-protected agmatine **3.44** (60 mg, 0.15 mmol) in DCM (3 mL) and 2,6-lutidine (58 μL, 0.5 mmol) were added, then allowed to warm to R.T. slowly. After stirring for 10 h, the reaction mixture was diluted with DCM (20

mL), washed with 0.5 M HCl aqueous (15 mL) quickly, saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM/EtOAc/hexane 1:8:11 to DCM/EtOAc/hexane 1:12:7) to afford **3.47a** (82 mg, 84%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (major rotamer) 11.76 (br, s, 1H), 8.34 (t, 1H, *J*=5.1 Hz), 7.42-7.28 (m, 20H), 5.17 (d, 4H, *J*=17.2 Hz), 4.95(d, 1H, *J*=11.4 Hz), 4.74 (dd, 2H, *J*=11.7 Hz, *J*=17.4 Hz), 4.63 (d, 1H, *J*=11.9 Hz), 4.41 (d, 1H, *J*=7.4 Hz), 4.20 (t, 1H, *J*=8.0 Hz), 4.08 (br, s, 1H), 3.91 (dd, 1H, *J*=5.1 Hz, *J*=11.6 Hz), 3.69 (t, 1H, *J*=8.9 Hz), 3.53-3.43 (m, 3H), 3.33-3.19 (m, 4H), 2.61 (br, s, 1H), 2.26-2.15 (br, m, 3H), 2.08-1.96 (br, m, 3H), 1.76-1.72 (br, d, 1H), 1.66-1.49 (br, m, 5H), 1.43 (br, s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (major rotamer) 172.7, 163.8, 156.2, 155.4, 154.0, 138.5, 138.4, 136.9, 134.8, 128.9, 128.8, 128.7, 128.64, 128.61, 128.55, 128.3, 128.07, 128.04, 127.98, 101.3, 81.3, 80.3, 77.3, 75.8, 74.6, 73.2, 72.9, 68.3, 67.3, 64.0, 60.4, 55.2, 40.8, 39.1, 36.0, 33.0, 29.9, 28.6, 27.0, 26.5, 23.3, 20.3; $[\alpha]_D$ -27.8 (c 1.0, $CHCl_3$); HRMS for $C_{54}H_{67}N_5O_{12}$ calculated ($M+H^+$) 978.4859, found 978.48615.

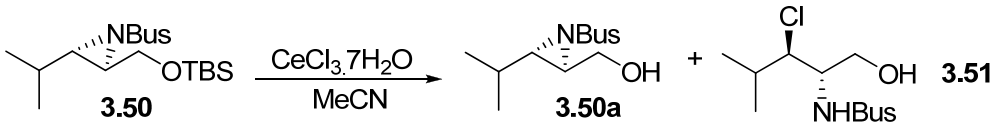


Compound 3.47b: To a solution of **3.46b** (107 mg, 0.18 mmol) in DCM (6 mL) at 0 °C, was added PyBOP (141 mg, 0.27 mmol) with stirring for 10 min., and then a solution of Cbz-protected agmatine **3.44** (108 mg, 0.27 mmol) in DCM (8 mL) and 2,6-lutidine (105 μL, 0.9 mmol) were added, then allowed to warm to R.T. slowly. After stirring for 10 h, the reaction mixture was diluted

with DCM (20 mL), washed with 0.5 M HCl aqueous (15 mL) quickly, saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/DCM 1:4 to EtOAc/DCM 2:3) to afford **3.47b** (148 mg, 84%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ (major rotamer) 11.77 (br, s, 1H), 8.36 (t, 1H, *J*=5.2 Hz), 7.45-7.28 (m, 20H), 5.16 (d, 4H, *J*=13.5 Hz), 5.03 (d, 1H, *J*=3.4 Hz), 4.84 (d, 0.8H, *J*=11.6 Hz), 4.79 (d, 1.2H, *J*=11.7 Hz), 4.66 (dd, 2H, *J*=6.6 Hz, *J*=11.7 Hz), 4.20-4.14 (br, m, 2H), 4.07 (t, 1H, *J*=8.8 Hz), 3.92 (br, s, 1H), 3.64-3.60 (br, m, 1H), 3.57-3.43 (m, 4H), 3.39 (dd, 1H, *J*=3.4 Hz, *J*=9.6 Hz), 3.36-3.19 (m, 2H), 2.34-2.20 (br, m, 2H), 2.15-2.01 (br, m, 2H), 1.66-1.50 (br, d, 9H), 1.40 (br, s, 9H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (major rotamer) 172.8, 163.8, 156.1, 154.8, 153.9, 138.4, 138.2, 136.8, 134.7, 128.9, 128.8, 128.57, 128.56, 128.52, 128.50, 128.2, 128.08, 128.03, 128.00, 127.95, 127.91, 93.9, 80.2, 79.4, 77.7, 73.4, 72.8, 72.3, 70.7, 68.2, 67.2, 60.9, 60.0, 54.8, 40.7, 39.1, 36.0, 30.2, 29.8, 28.5, 26.9, 26.5, 25.8, 20.5; $[\alpha]_D$ +10.9 (c 1.0, $CHCl_3$); HRMS for $C_{54}H_{67}N_5O_{12}$ calculated ($M+H^+$) 978.4859, found 978.48704.

Compound 3.50: A solution of **3.48** (460 mg, 2 mmol) in 40 mL of DCM was cooled to 0 ^{o}C and treated with Et_3N (0.7 mL, 5 mmol), followed by dropwise addition of *tert*-butylsulfinyl chloride **3.49** (0.3 mL, 2.4 mmol) in 2.4 mL of DCM. The reaction mixture was stirred at 0 ^{o}C until TLC showed consumption of the starting material (1 h). Upon completion, 40 mL of saturated aqueous $NaHCO_3$ was added, and the layers separated (note: acidic washes should be avoided as *tert*-butylsulfinamides are known to be unstable at low pH). The aqueous layer was extracted with DCM (3×50 mL). The organic layer was combined, dried

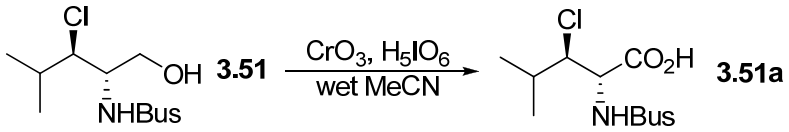
over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (EtOAc/hexane 1:9) gave pure sulfinamide, which was taken up directly in 30 mL of DCM, and treated with *m*-CPBA (466 mg, 2.7 mmol). After oxidation was complete by TLC (2 h), the reaction mixture was diluted with a mixture of saturated aqueous $NaHCO_3$ (25 mL) and saturated aqueous Na_2SO_3 (25 mL). The aqueous layer was extracted with DCM (3×50 mL). The organic extract was combined, dried over Na_2SO_4 and concentration under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 1:19) to afford **3.50** (594 mg, 85% over 2 steps), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 4.04 (m, 1H), 3.72 (dd, 1H, *J*=7.1 Hz, *J*=10.9 Hz), 2.75 (m, 1H), 2.53 (m, 1H), 1.94-1.87 (m, 1H), 1.44 (s, 9H), 1.16 (d, 3H, *J*=6.6 Hz), 0.96 (d, 3H, *J*=6.8 Hz), 0.88 (s, 9H), 0.08 (s, 6H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 61.8, 59.8, 52.0, 46.6, 31.3, 28.3, 25.6, 24.0, 23.5, 20.4, 18.0; $[\alpha]_D$ +31.5 (c 1.0, $CHCl_3$); ESI/MS for $C_{16}H_{35}NO_3SSi$ calculated ($M+H^+$) 350, found 350.



Compounds 3.50a and 3.51：A solution of **50** (700 mg, 2.0 mmol) in 30 mL MeCN was heated to reflux and treated with $CeCl_3.7H_2O$ (3.35 g, 6.0 mmol) in portions over 36 h (best results were obtained, when 1 equiv. was added every 12 h). The reaction mixture was then refluxed for an additional 36 h, cooled to R.T. and filtered over Celite pad with EtOAc rinsing. After concentration of the filtrate under reduced pressure, the crude residue could be readily crystallized from DCM/hexane to give pure by-product **3.50a**. Alternatively, the crude material was purified by flash column chromatography (EtOAc/hexane 3:7) to afford **3.51** as a white solid (428 mg, 79%).

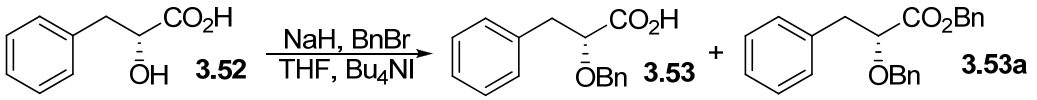
Compound 3.50a：^{1}H NMR, (300 MHz, $CDCl_3$) δ 4.10 (br, dd, 1H), 3.85 (dd, 1H, *J*=6.7 Hz, *J*=10.1 Hz), 3.23 (s, 1H), 2.90-2.86 (m, 1H), 2.81-2.79 (t, 1H, *J*=3.8 Hz), 1.88-1.80 (m, 1H), 1.50 (s, 9H), 1.03 (d, 3H, *J*=5.1 Hz), 0.91 (d, 3H, *J*=5.2 Hz); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 63.1, 61.8, 49.7, 49.0, 28.7, 24.3, 19.7, 18.1, 12.5; $[\alpha]_D$ -3.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{10}H_{21}NO_3S$ calculated ($M+H^+$) 236, found 236.

Compound 3.51：^{1}H NMR, (400 MHz, $CDCl_3$) δ 4.48 (d, 1H, *J*=10.0 Hz), 4.00- 3.76 (m, 4H), 2.14 (sept, 1H, *J*= 6.8 Hz), 2.01 (br, s, 1H), 1.42 (s, 9H), 1.07 (2d, 6H, *J*= 6.4 Hz, *J*= 6.4 Hz); ^{13}C NMR, (100MHz, $CDCl_3$) δ 71.7, 61.8, 60.7, 60.6, 60.5, 60.0, 57.4, 31.3, 29.7, 24.3, 24.1, 20.2, 19.0 ; $[\alpha]_D$ -4.6 (c 1.0, $CHCl_3$); ESI/MS for $C_{10}H_{22}ClNO_3S$ calculated ($M+H^+$) 272, found 272.



Compound 3.51a: A solution of **3.51** (49 mg, 0.18 mmol) in MeCN (1 mL) was cooled to 0 °C and a solution of 1 mg CrO_3 dissolved in 1.1 mL of 0.4 M H_5IO_6 in wet MeCN (MeCN/H_2O 99.3:0.7) was added. After stirring for 1 h, the reaction mixture was quenched with 1.2 mL saturated aqueous $NaHCO_3$. The solvent was evaporated, and the aqueous mixture was treated with 400 μL 0.5 M NaOH aqueous, and washed with Et_2O (5 mL). The aqueous layer was acidified with 1 M HCl aqueous (5 mL) and extracted with DCM (3×10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography ($CHCl_3$/MeOH 10:1) to afford carboxylic acid **3.51a**, as a colorless oil (30 mg, 57%). ^{1}H NMR, (400 MHz, MeOH-d_4) δ 3.02 (d, 1H, *J*=4.1 Hz), 2.74 (dd, 1H, *J*=3.0 Hz, *J*=7.6 Hz), 2.02 (td, 1H, *J*=6.5 Hz, *J*=13.5 Hz), 1.45 (s, 9H), 1.21 (d, 3H, *J*=6.5 Hz), 1.04 (d, 3H, *J*=6.7 Hz); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 174.4,

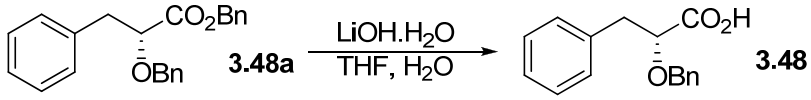
61.9, 55.2, 47.4, 29.8, 24.5, 21.7, 21.0; $[\alpha]_D$ +42.3 (*c* 1.0, MeOH). HRMS for $C_{10}H_{20}ClNO_4S$ calculated $(M-H)^-$ 284.07288, found 284.07270.



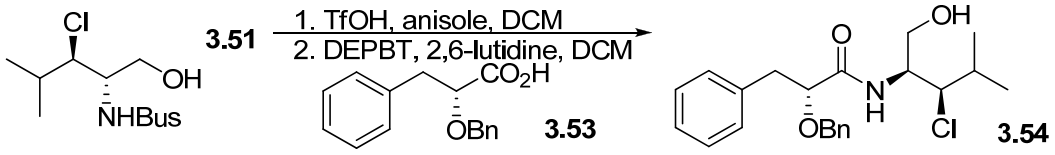
Compounds 3.53 and 3.53a： A solution of D-(+)-3-phenyllactic acid **3.52** (400 mg, 2.4 mmol) in THF (25 mL) was cooled on ice under argon atmosphere to 0◦C, and treated with NaH (240 mg, 60 dispersion in mineral oil), then allowed to warm up to R.T.. After stirring for 2 h, BnBr (0.43 mL, 3.6 mmol) and Bu_4NI (369 mg, 1.0 mmol) were added, then stirring was continued for 10 h. The reaction mixture was quenched by H_2O (2 mL) carefully and concentrated under reduced pressure. Then the residue was redissolved in a mixture of DCM (35 mL) and H_2O (25 mL). The phases were separated and the organic phase was washed with H_2O (25 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:19) to give **3.53a** (271 mg, 33%), as a colorless oil. The aqueous layer was acidified carefully by concentrated aqueous HCl to pH=1 and then extracted with DCM (3×50 mL). The organic phases were combined, dried over Na_2SO_4 and concentration under reduced pressure. The residue was purified by flash column chromatography (MeOH/DCM 1:19) to afford carboxylic acid **3.53** (379 mg, 62%), as a colorless solid, m.p. 47-49 ◦C.

Compound 3.53： ^{1}H NMR, (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.37-7.28 (m, 8H), 7.22-7.20 (m, 2H), 4.74 (d, 1H, *J*=11.8 Hz), 4.46(d, 1H, *J*=11.8 Hz), 4.24 (dd, 1H, *J*=4.1 Hz, *J*=8.5 Hz), 3.22 (dd, 1H, *J*=4.0 Hz, *J*=14.0 Hz), 3.11 (dd, 1H, *J*=8.6 Hz, J=14.0 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 177.3, 137.0, 136.9, 129.7, 128.5, 128.1, 127.0, 78.8, 72.9, 39.2; $[\alpha]_D$ +59.0 (c 1.0, $CHCl_3$); ESI/MS for $C_{16}H_{16}O_3$ calculated $(M+H_2O)$ 274, found 274.

Compound 3.53a: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.45-7.40 (m, 3H), 7.39-7.35 (m, 2H), 7.34-7.30 (m, 6H), 7.29-7.26 (m, 2H), 7.24-7.21 (m, 2H), 5.19 (s, 2H), 4.74 (d, 1H, *J*=11.8 Hz), 4.45 (d, 1H, *J*=11.8 Hz), 4.26 (dd, 1H, *J*=5.4 Hz, *J*=8.0 Hz), 3.21-3.11 (m, 2H),; ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.1, 137.4, 127.0, 135.6, 129.6, 128.7, 128.5, 128.43, 128.42, 128.0, 127.9, 126.8, 79.4, 72.6, 66.8, 39.4; $[\alpha]_D$ +43.7 (c 1.0, $CHCl_3$); ESI/MS for $C_{23}H_{22}O_3$ calculated ($M+H_2O$) 364, found 364.

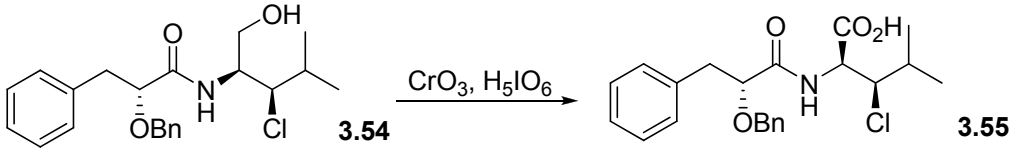


Compound 3.53: To a solution of **3.53a** (118 mg, 0.34 mmol) in THF (3.5 mL) and H_2O (1.5 mL) was added LiOH×H_2O (43 mg, 1.0 mmol) at R.T., and then stirred for 4 h (TLC monitoring, EtOAc/hexanes 1:9). After solvent was evaporated, 15 mL H_2O was added and extracted with DCM (20 mL), then acidified dropwise with concentrated HCl aqueous to pH=1. The aqueous layer was extracted with DCM (3×20 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:19) to give **3.53** (75 mg, 86%), as a colorless solid, m.p. 47-49 °C.



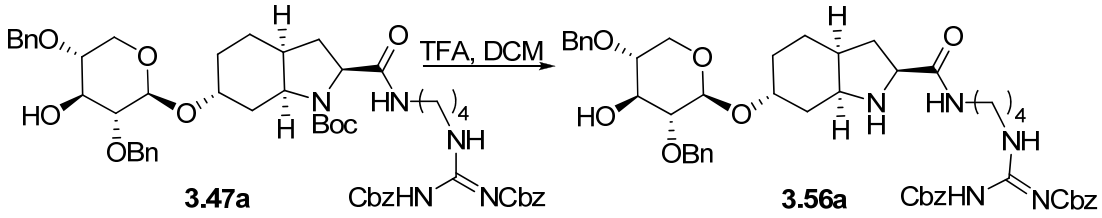
Compound 3.54: A solution of **3.51** (340 mg, 1.25 mmol) in DCM (63 mL) was cooled to 0 °C and treated with anisole (2.7 mL, 25 mmol) and TfOH (0.56 mL, 6.25 mmol). The solution was stirred at R.T. for 12 h, after which the volatiles were removed under reduced pressure. The residue was taken up in water (15 mL), washed with Et_2O (2×15 mL), frozen and lyophilized to afford

triflate salt of amine, which was used directly. A solution of crude amine salt in DCM (10 mL) was cooled to 0 °C. 2,6-Lutidine (0.73 ml, 6.25 mmol), carboxylic acid **53** (352 mg, 1.38 mmol), and DEPBT (598 mg, 2.0 mmol) were added, then allowed to warm up to R.T. slowly. After stirring for 27 h, the reaction mixture was diluted with DCM (30 mL), washed with 0.5 M HCl aqueous (30 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/Hexane/DCM 4:15:1) to afford **3.54** (422 mg, 87% over 2 steps), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.38-7.27 (m, 10H), 7.14 (d, 1H, *J*=9.5 Hz), 4.54 (dd, 2H, *J*=11.6 Hz, *J*=28.3 Hz), 4.26-4.20 (m, 1H), 4.17 (dd, 1H, *J*=3.6 Hz, *J*=7.0 Hz), 3.98 (dd, 1H, *J*=4.5 Hz, *J*=11.3 Hz), 3.78-3.72 (m, 2H), 3.23 (dd, 1H, *J*=3.6 Hz, *J*=14.1 Hz), 3.01 (dd, 1H, *J*=7.1 Hz, *J*=14.1 Hz), 1.76-1.68 (m, 1H), 0.99 (dd, 6H, *J*=3.6 Hz, *J*=6.6 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.9, 137.0, 136.9, 129.9, 128.6, 128.25, 128.17, 128.13, 126.7, 80.6, 73.0, 68.5, 62.2, 52.6, 38.6, 30.2, 20.8, 17.1; $[\alpha]_D$ +60.3 (c 1.0, $CHCl_3$); HRMS for $C_{22}H_{28}ClNO_3$ calculated ($M+H^+$) 390.18305, found 390.18235.



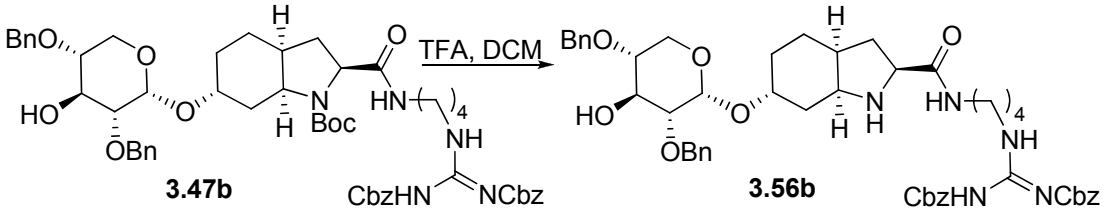
Compound 3.55: A solution of **3.54** (300 mg, 0.77 mmol) in MeCN (5 mL) was cooled to 0 °C and a solution of 8 mg CrO_3, which was dissolved in 4.6 mL of 0.4 M H_5IO_6 in wet MeCN (MeCN/H_2O 99.3:0.7), was added. After stirring at 0 °C for 45 min., the reaction mixture was quenched by adding 5mL saturated aqueous $NaHCO_3$. After MeCN was evaporated, the aqueous mixture was treated with 2 mL 0.5 M NaOH aqueous and washed with Et_2O (15 mL). The aqueous layer was acidified with concentrated HCl aqueous carefully at 0 °C,

and extracted with DCM (3×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give **3.55** (271 mg, 87%), as a colorless oil, which was sufficiently pure for use in the following step, and was purified by RP-LC-MS (C4 column, 25 min., gradient of 73-80% CH_3CN in 0.1% aqueous FA) to afford pure **3.55** (217 mg, 70%): ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.03 (s, 1H), 7.48 (d, 1H, *J*=9.0 Hz), 7.33-7.27 (m, 8H), 7.25-7.22 (m, 2H), 5.07 (dd, 1H, *J*=4.3 Hz, *J*=9.0 Hz), 4.60 (d, 1H, *J*=11.4 Hz), 4.45 (d, 1H, *J*=11.4 Hz), 4.22 (dd, 1H, *J*=3.4 Hz, *J*=7.4 Hz), 3.51 (dd, 1H, *J*=4.4 Hz, *J*=8.4 Hz), 3.21 (dd, 1H, *J*=3.4 Hz, *J*=14.1 Hz), 2.99 (dd, 1H, *J*=7.4 Hz, *J*=14.1 Hz), 2.16-2.04 (m, 1H), 1.12 (d, 3H, *J*=6.6 Hz), 1.06 (d, 3H, *J*=6.5 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 172.7, 172.4, 136.9, 136.8, 130.0, 128.7, 128.5, 128.4, 128.3, 127.0, 80.4, 73.3, 69.6, 54.3, 39.0, 31.9, 20.4, 20.0; $[\alpha]_D$ +37.7 (c 1.0, $CHCl_3$); HRMS for $C_{22}H_{26}ClNO_4$ calculated ($M+H^+$) 404.16231, found 404.16164.



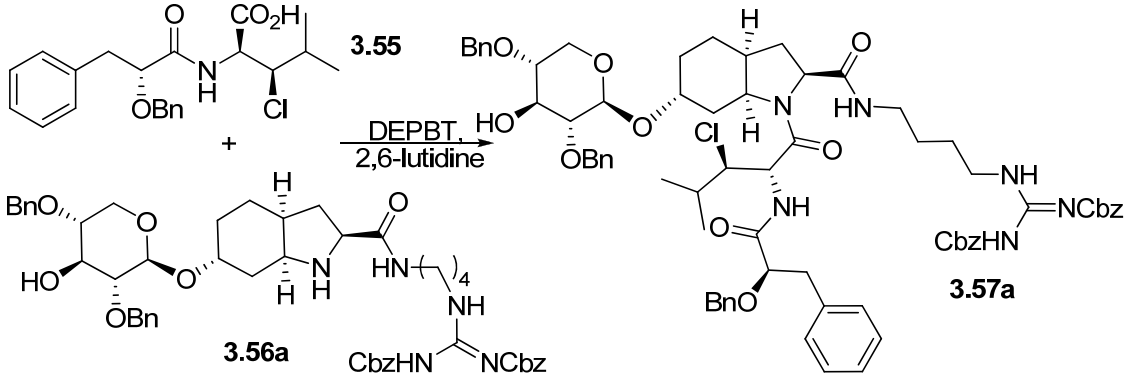
Compound 3.56a: Compound **3.47a** (103 mg, 0.11 mmol) was treated with >99.9% TFA/DCM (1:9, 3 mL) at R.T.. After stirring for 2 h, the reaction mixture was diluted with EtOAc (20 mL) and concentrated under reduced pressure. The crude residue was suspended in saturated aqueous Na_2CO_3 (20 mL) and extracted with DCM (3×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:49 to MeOH/ DCM 1:19) to give **3.56a** (89 mg, 96%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.77 (s, 1H), 8.35 (t, 1H, *J*=5.2 Hz), 7.65 (t, 1H, *J*=6.0 Hz),

7.42-7.28 (m, 20H), 5.16 (d, 4H, *J*=14.6 Hz), 4.95(d, 1H, *J*=11.4 Hz), 4.77 (d, 1H, *J*=11.9 Hz), 4.69 (d, 1H, *J*=11.4 Hz), 4.64 (d, 1H, *J*=11.9 Hz), 4.49 (d, 1H, *J*=7.5 Hz), 4.02-3.97 (m, 1H), 3.93 (dd, 1H, *J*=5.1 Hz, *J*=11.6 Hz), 3.71 (t, 1H, *J*=5.6 Hz), 3.67 (d, 1H, *J*=8.9 Hz), 3.55-3.43 (m, 4H), 3.32-3.22 (m, 4H), 2.37-2.26 (m, 1H), 1.98-1.88 (br, m, 3H), 1.77-1.67 (m, 3H), 1.64-1.53 (br, m, 4H), 1.42-1.26 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 175.7, 163.8, 156.1, 154.0, 138.5, 138.3, 136.8, 134.7, 128.9, 128.8, 128.58, 128.57, 128.53, 128.50, 128.2, 128.07, 128.02, 127.96, 127.93, 101.8, 81.2, 77.2, 75.7, 74.5, 74.2, 73.2, 68.3, 67.2, 63.9, 58.8, 57.7, 40.8, 38.5, 37.1, 35.9, 35.2, 29.4, 27.2, 26.5, 25.6; $[\alpha]_D$ -12.9 (c 1.0, $CHCl_3$); HRMS for $C_{49}H_{59}N_5O_{10}$ calculated ($M+H^+$) 878.43347, found 878.43666.



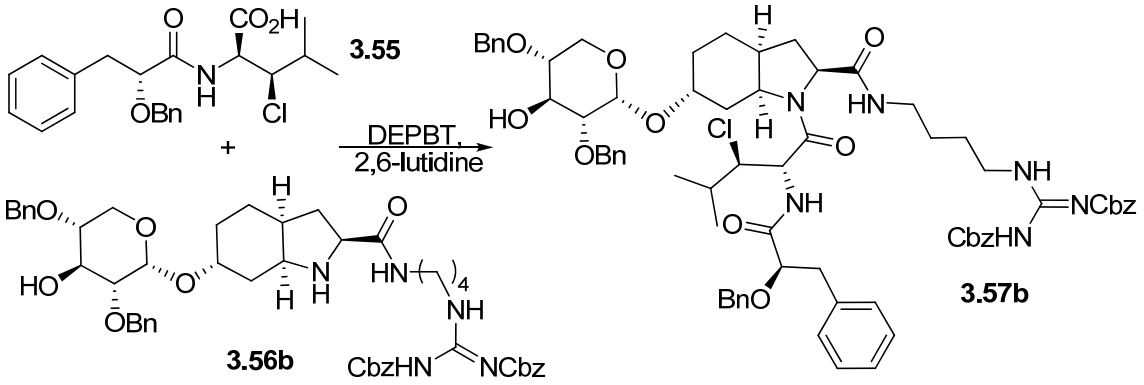
Compound 3.56b: Compound **3.47b** (104 mg, 0.11 mmol) was treated with >99.9% TFA/DCM (1:9, 3 mL) at R.T.. After stirring for 2 h, the reaction mixture was diluted with EtOAc (20 mL) and concentrated under reduced pressure. The crude residue was suspended in saturated aqueous Na_2CO_3 (20 mL) and extracted with DCM (3×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:49 to MeOH/ DCM 1:19) to give **3.56b** (84 mg, 91%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.77 (s, 1H), 8.35 (t, 1H, *J*=5.2 Hz), 7.66 (t, 1H, *J*=5.5 Hz), 7.41-7.28 (m, 20H), 5.15 (d, 4H, *J*=10.4 Hz), 4.89 (d, 1H, *J*=3.4 Hz), 4.77 (d, 1H, *J*=11.8 Hz), 4.70 (d, 2H, *J*=5.3 Hz), 4.66 (d, 1H, *J*=11.8 Hz), 4.04 (t, 1H, *J*=9.2 Hz), 3.82-3.76 (br, m, 1H), 3.72 (dd, 1H, *J*=4.1 Hz, *J*=11.0 Hz), 3.61 (d,

2H, *J*=8.2 Hz), 3.51-3.43 (m, 4H), 3.35 (dd, 1H, *J*=3.4 Hz, *J*=9.6 Hz), 3.32-3.26 (m, 2H), 2.61 (s, 1H), 2.35-2.28 (m, 1H), 1.99-1.90 (br, m, 1H), 1.87-1.68 (br, m, 3H), 1.67-1.52 (br, m, 5H), 1.48-1.39 (br, m, 1H), 1.35-1.25 (br, m, 1H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 175.5, 163.8, 156.1, 154.0, 138.4, 138.3, 136.8, 134.7, 128.9, 128.8, 128.64, 128.60, 128.55, 128.53, 128.3, 128.10, 128.06, 127.97, 127.95, 95.1, 79.6, 77.8, 73.3, 72.9, 72.8, 68.3, 67.2, 60.0, 58.9, 57.7, 40.8, 38.5, 37.1, 35.2, 34.0, 30.9, 29.8, 27.1, 26.6, 25.8; $[\alpha]_D$ +26.1 (c 1.0, $CHCl_3$); HRMS for $C_{49}H_{59}N_5O_{10}$ calculated ($M+H^+$) 878.43347, found 878.43746.



Compound 3.57a: To a solution of **3.55** (11 mg, 0.026 mmol) and **3.56a** (21 mg, 0.024 mmol) in DCM (2 mL) at 0 °C were added fresh *recrystallized* DEPBT (11 mg, 0.036 mmol) and 2,6-lutidine (8 μL, 0.072 mmol). After addition, the reaction mixture was allowed to warm to R.T. slowly, and stirred for 20 h, then cooled to 0 °C. To the mixture, **3.55** (6 mg, 0.013 mmol) in DCM (0.3 mL), fresh *recrystallized* DEPBT (11 mg, 0.036 mmol) and 2,6-lutidine (8 μL, 0.072 mmol) were added at 0 °C. After addition, the reaction mixture was allowed to warm to R.T. slowly, and stirred for 24 h, then diluted with DCM (10 mL), washed with 0.5 M HCl aqueous (15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/DCM 7:13) to give crude

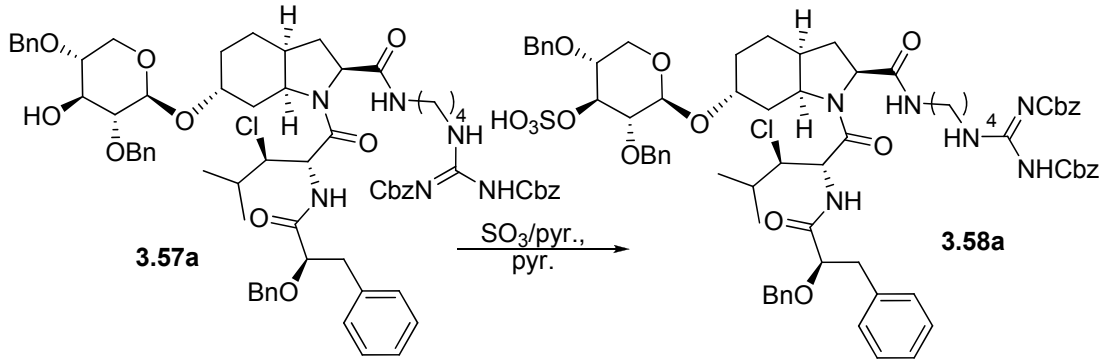
product, which was purified by RP-LC-MS (C4 column, 26 min., isocratic of 80% CH_3CN in 0.1% aqueous FA) to afford pure **3.57a** (21 mg, 69%), as a colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.73 (s, 1H), 8.30 (t, 1H, *J*=5.2Hz), 7.41-7.22 (m, 30H), 7.12 (d, 1H, *J*=9.3 Hz), 6.83 (t, 1H, *J*=5.6 Hz), 5.15 (d, 4H, *J*=14.2 Hz), 4.99 (dd, 2H, *J*=10.8 Hz, *J*=20.8 Hz), 4.77-4.69 (m, 3H), 4.54-4.47 (m, 3H), 4.44-4.36 (m, 2H), 4.20 (dd, 1H, *J*=5.0 Hz, *J*=11.7 Hz), 4.11-4.08 (m, 2H), 3.86 (d, 1H, *J*=10.4 Hz), 3.71 (t, 1H, *J*=9.0Hz), 3.49 (dt, 1H, *J*=5.1 Hz, *J*=9.4 Hz, *J*=9.1 Hz), 3.39-3.21 (m, 4H), 3.16 (dd, 1H, *J*=3.7 Hz, *J*=14.4 Hz), 3.06 (dt, 1H, *J*=6.1 Hz, *J*=12.5 Hz, *J*=12.2 Hz), 2.96 (dd, 1H, *J*=6.9 Hz, *J*=14.1 Hz), 2.54-2.46 (br, m, 2H), 2.35 (t, 2H, *J*=6.6Hz), 2.14-2.00 (m, 2H), 1.79-1.68 (m, 2H), 1.60-1.39 (m, 7H), 1.31 (d, 1H, *J*=21.0 Hz), 0.89 (d, 6H, *J*=6.5 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.3, 170.9, 169.8, 164.0, 156.2, 154.0, 138.6, 138.5, 137.07, 137.00, 136.98, 134.8, 130.0, 129.0, 128.9, 128.73, 128.72, 128.69, 128.64, 128.62, 128.35, 128.31, 128.29, 128.24, 128.13, 128.06, 128.04, 128.00, 126.9, 102.7, 82.0, 80.6, 76.0, 74.9, 74.2, 73.03, 72.99, 68.5, 68.3, 67.3, 63.8, 60.2, 55.9, 52.4, 40.7, 39.2, 38.6, 36.1, 32.2, 29.8, 28.5, 26.6, 26.5, 24.0, 21.0, 20.0, 15.2; $[\alpha]_D$ +1.5 (c 1.0, $CHCl_3$); HRMS for $C_{71}H_{83}ClN_6O_{13}$ calculated ($M+H^+$) 1263.57794, found 1263.57212.



Compound 3.57b: To a solution of **3.55** (10 mg, 0.024 mmol) and **3.56b** (20 mg, 0.022 mmol) in DCM (2 mL) at 0 ◦C were added fresh *recrystallized*

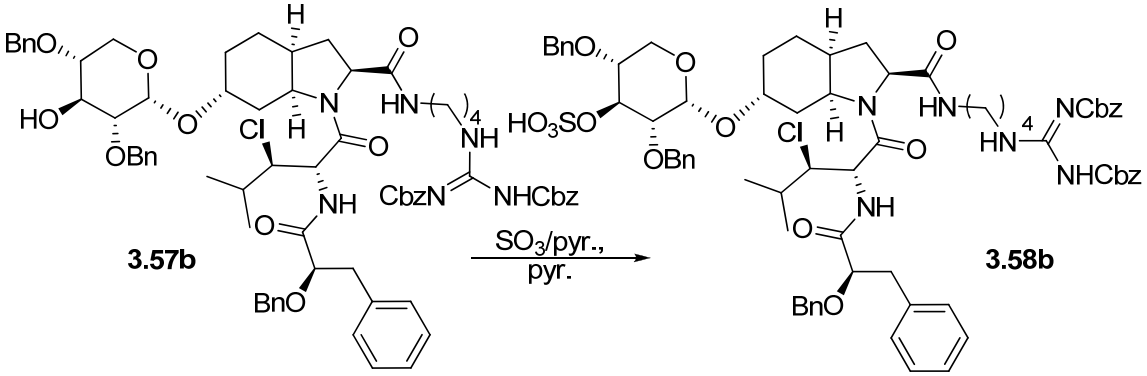
DEPBT (10 mg, 0.033 mmol) and 2,6-lutidine (8 μL, 0.066 mmol). After addition, the solution was allowed to warm to R.T. slowly, and stirred for 20 h, then cooled to 0 °C, and then **3.55** (4 mg, 0.011 mmol) in DCM (0.3 mL), fresh *recrystallized* DEPBT (10 mg, 0.033 mmol), and 2,6-lutidine (5 μL, 0.044 mmol) were added. After addition, the mixture was allowed to warm to R.T. slowly, and stirred for 24 h, then cooled to 0 °C, and then **3.55** (4 mg, 0.011 mmol) in DCM (0.3 mL), fresh *recrystallized* DEPBT (10 mg, 0.033 mmol) and 2,6-lutidine (5.2 μL, 0.044 mmol) were added at 0 °C. After addition, the reaction mixture was allowed to warm to R.T. slowly, and stirred for 24 h, then diluted with DCM (10 mL), washed with 0.5 M HCl aqueous (15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (MeOH/DCM 1:49) to give crude product, which was purified by RP-LC-MS (C4 column, 26 min., isocratic of 80% CH_3CN in 0.1% aqueous FA) to afford **3.57b** (17 mg, 60%), as a colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ 11.74 (s, 1H), 8.33 (t, 1H, *J*=5.5 Hz), 7.47-7.22 (m, 30H), 7.07 (d, 1H, *J*=9.9 Hz), 6.68 (t, 1H, *J*=5.0 Hz), 5.22 (d, 1H, *J*=2.5 Hz), 5.16 (d, 4H, *J*=8.7 Hz), 5.02 (t, 1H, *J*=10.3 Hz), 4.93 (d, 1H, *J*=12.4 Hz), 4.78 (dd, 2H, *J*=12.1 Hz, *J*=18.8 Hz), 4.65 (d, 1H, *J*=11.8 Hz), 4.55 (d, 1H, *J*=11.4 Hz), 4.44-4.34 (m, 3H), 4.11 (dd, 1H, *J*=3.2 Hz, *J*=6.5 Hz), 4.06 (d, 1H, *J*=8.1 Hz), 4.00-3.95 (m, 2H), 3.74-3.61 (m, 1H), 3.54-3.46 (m, 2H), 3.43-3.31 (m, 3H), 3.24 (dd, 1H, *J*=6.9 Hz, *J*=13.6 Hz), 3.19-3.09 (m, 2H), 2.94 (dd, 1H, *J*=6.8 Hz, *J*=14.1 Hz), 2.57 (s, 1H), 2.48-2.44 (br, m, 1H), 2.42-2.34 (m, 1H), 2.32-2.18 (m, 2H), 2.04-1.97 (m, 1H), 1.81-1.46 (m, 10H), 1.35-1.27 (m, 1H), 0.89 (d, 1H, *J*=6.3 Hz), 0.82 (dd, 5H, *J*=6.5 Hz, *J*=14.4 Hz); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 171.5, 171.0, 169.3, 163.8, 156.2, 154.0, 138.5, 138.4, 137.04, 136.98, 136.93, 134.7, 130.0, 128.99, 128.89, 128.64, 128.61, 128.33, 128.29, 128.22, 128.19, 128.13, 128.03, 128.00, 126.8, 94.2, 80.5,

78.5, 73.5, 72.9, 72.8, 71.8, 70.7, 68.3, 68.2, 67.3, 60.7, 60.3, 55.6, 52.5, 40.7, 39.2, 38.7, 36.5, 30.0, 29.9, 28.3, 26.5, 26.4, 25.1, 20.8, 20.1, 15.5; $[\alpha]_D$ +45.0 (c 1.0, $CHCl_3$); HRMS for $C_{71}H_{83}ClN_6O_{13}$ calculated ($M+H^+$) 1263.57794, found 1263.58015.



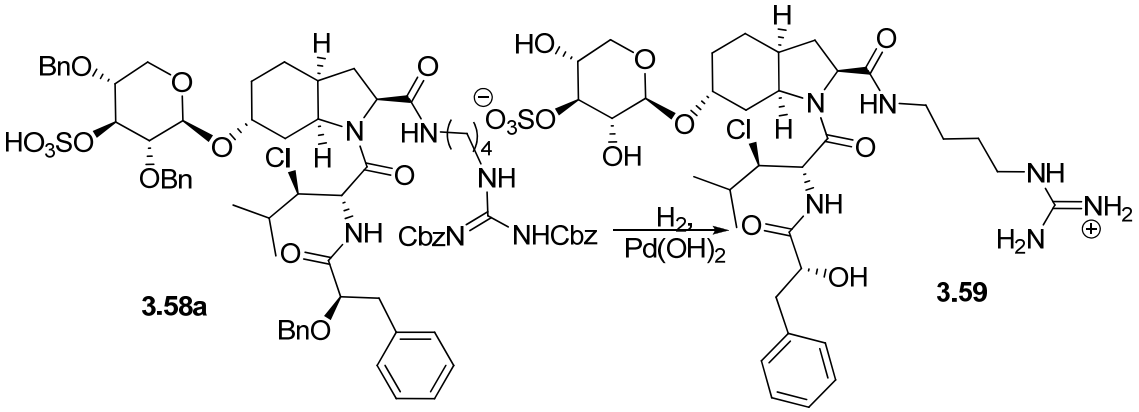
Compound 3.58a: To a solution of **3.57a** (10 mg, 0.008 mmol) in dry pyridine (3 mL) at R.T., was added sulfur trioxide pyridine complex (64 mg, 0.4 mmol). After addition, the mixture was heated to 50 °C and stirred for 48 h, TLC (DCM/MeOH 19:1) showed the conversion of **3.57a** into **3.58a**, then cooled to R.T. and pyridine was evaporated. The crude residue was suspended in 15mL H_2O and extracted with 15 mL×4 DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:24 to MeOH/ DCM 2:23) to give **3.58a** (10 mg, 88%), as a colorless film: ^{1}H NMR, (700 MHz, MeOH-d_4) δ (major rotamer) 8.01 (dd, 1H, *J*=4.8 Hz, *J*=6.5 Hz), 7.72 (d, 1H, *J*=9.5 Hz), 7.50 (d, 2H, *J*=7.1 Hz), 7.46 (d, 2H, *J*=7.0 Hz), 7.41-7.15 (m, 26H), 5.11 (d, 2H, *J*=6.9 Hz), 5.10 (d, 2H, *J*=2.1 Hz), 5.00 (d, 1H, *J*=10.5 Hz), 4.76 (d, 1H, *J*=10.5 Hz), 4.69 (d, 1H, J=11.9 Hz), 4.57 (dd, 2H, *J*= 8.6 Hz, *J*=9.9 Hz), 4.51 (d, 2H, *J*=7.2 Hz), 4.41 (d, 1H, *J*=11.6 Hz), 4.32 (dd, 1H, *J*=8.0 Hz, *J*=10.0 Hz), 4.11 (dd, 1H, *J*=3.9 Hz, *J*=6.5 Hz), 4.05 (s, 1H), 4.03 (dd, 1H,

J=5.3 Hz, *J*=12.0 Hz), 4.00 (dd, 1H, *J*=1.7 Hz, *J*=10.8 Hz), 3.64-3.60 (m, 1H), 3.39 (dd, 1H, *J*=7.3 Hz, *J*=8.7 Hz), 3.37 (d, 1H, *J*=4.8 Hz), 3.35-3.31 (m, 2H), 3.13 (dd, 1H, *J*=3.8 Hz, *J*=14.1 Hz), 3.07 (qd, 1H, *J*=5.4 Hz, *J*=10.7 Hz), 2.96 (dd, 1H, J=6.6 Hz, *J*=14.2 Hz), 2.62 (d, 1H, *J*=14.0 Hz), 2.31 (ddd, 1H, *J*=8.1 Hz, *J*=14.7 Hz, *J*=23.0 Hz), 2.12–2.07 (m, 1H), 2.06–2.02 (m, 1H), 1.99 (td, 1H, *J*=10.7 Hz, *J*=12.9 Hz), 1.81 (t, 1H, *J*=12.1 Hz), 1.71 (d, 1H, *J*=13.4 Hz), 1.59- 1.44 (m, 8H), 1.34-1.30 (br, d, 2H), 0.86 (dd, 6H, *J*=6.6 Hz, *J*=11.5Hz); ^{13}C NMR, (175 MHz, MeOH-*d*$_4$) δ (major rotamer) 172.40, 171.83, 168.82, 163.4, 155.7, 153.2, 138.59, 138.56, 137.17, 136.90, 136.83, 134.9, 129.7, 128.4, 128.37, 128.19, 128.11, 128.10, 128.03, 128.0, 127.9, 127.8, 127.7, 127.69, 127.66, 127.5, 127.3, 127.2, 126.3, 101.64, 82.2, 80.6, 80.2, 76.0, 74.7, 73.5, 72.9, 72.3, 67.9, 67.0, 63.3, 60.7, 55.5, 53.8, 52.1, 40.4, 38.2, 37.8, 36.4, 31.8, 30.8, 28.1, 25.6, 23.0, 19.8, 19.2, 14.3; $[\alpha]_D$ +4.2 (c 0.95, MeOH); HRMS for $C_{71}H_{83}ClN_6O_{16}S$ calculated ($M+Na^+$) 1365.5167, found 1365.51264.



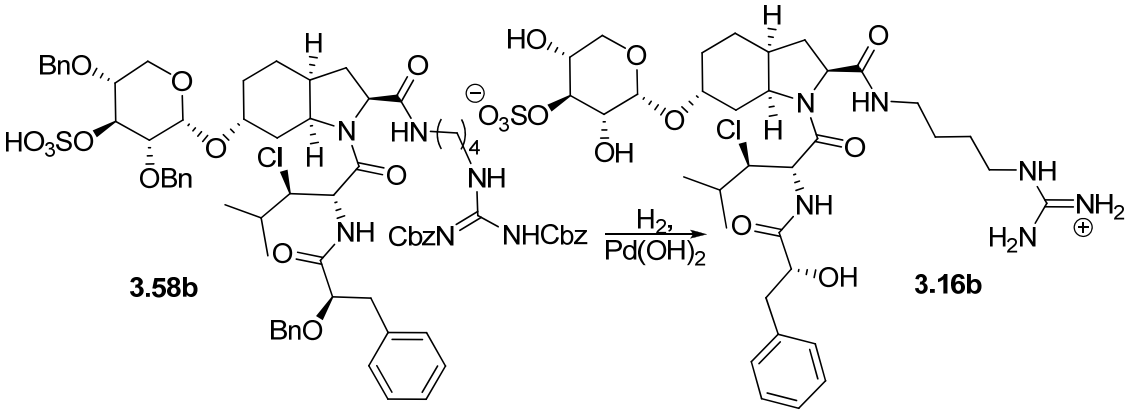
Compound 3.58b: To a solution of **3.57b** (9 mg, 0.007 mmol) in dry pyridine (2 mL) at R.T., was added sulfur trioxide pyridine complex (59 mg, 0.37 mmol). After addition, the mixture was heated to 50 °C and stirred for 48 h, TLC (DCM/MeOH 19:1) showed the conversion of **3.57b** into **3.58b**, then cooled to R.T. and pyridine was evaporated. The crude residue was suspended

in 15 mL H_2O and extracted with 15 mL×4 DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:24 to MeOH/DCM 2:23) to give **3.53b** (9 mg, 85%), as a colorless film: ^{1}H NMR, (700 MHz, MeOH-d_4) δ (major rotamer) 7.55 (d, 2H, *J*=7.1Hz), 7.42-7.29 (m, 18H), 7.26-7.20 (m, 9H), 7.16-7.11 (m, 4H), 5.17 (d, 1H, *J*=2.8 Hz), 5.14-5.09 (m, 3H), 5.04 (s, 2H), 4.89-4.87 (m, 2H), 4.67 (s, 1H), 4.60 (dd, 2H, *J*=11.6 Hz, *J*=20.0 Hz), 4.44 (dd, 1H, *J*=9.6 Hz, *J*=15.0 Hz), 4.42 (d, 1H, *J*=11.6 Hz), 4.32 (dd, 1H, *J*=8.1 Hz, *J*=10.1 Hz), 4,17 (dd, 1H, *J*=3.9 Hz, *J*=6.6 Hz), 4.08 (dd, 1H, *J*=1.9 Hz, *J*=10.9 Hz), 4.03 (br, m, 1H), 3.68-3.63 (m, 3H), 3.61-3.58 (m, 1H), 3.45-3.41 (1H, m), 3.35-3.33 (dr, m, 1H), 3.17 (dd, 1H, *J*=3.7 Hz, *J*=14.2 Hz), 3.06 (dt, 1H, *J*=5.2Hz, *J*=13.1Hz), 3.00 (dd, 1H, *J*=6.6 Hz, *J*=14.1 Hz), 2.66-2.63 (br, m, 0.4H), 2.42-2.37 (m, 0.6H), 2.31-2.26 (m, 1H), 2.14-2.10 (m, 1H), 2.05-1.99 (m, 1H), 1.86-1.83 (m, 1H), 1.76-1.74 (d, 1H, *J*=14.3 Hz), 1.67-1.50 (m, 8H), 1.30 (br, m, 2H), 0.81 (dd, 6H, *J*=1.8 Hz, *J*=6.6 Hz); ^{13}C NMR, (175 MHz, MeOH-d_4) δ (major rotamer) 173.9, 173.2, 170.2, 165.0, 157.3, 154.6, 140.2, 140.1, 138.8, 138.5, 138.4, 136.4, 131.2, 129.91, 129.89, 129.63, 129.59, 129.51, 129.47, 129.36, 129.33, 129.31, 129.24, 129.17, 129.14, 129.02, 128.7, 128.5, 127.8, 97.2, 81.8, 79.4, 77.8, 76.9, 74.13, 74.01, 73.94, 72.0, 69.4, 69.3, 68.5, 63.3, 62.4, 56.9, 53.6, 41.9, 49.5, 39.3, 38.2, 32.3, 31.0, 29.4, 27.0, 26.0, 21.2, 21.0, 16.3; $[\alpha]_D$ +23.3 (c 0.8, MeOH); HRMS for $C_{71}H_{83}ClN_6O_{16}S$ calculated ($M+H^+$) 1343.53476, found 1343.53163.



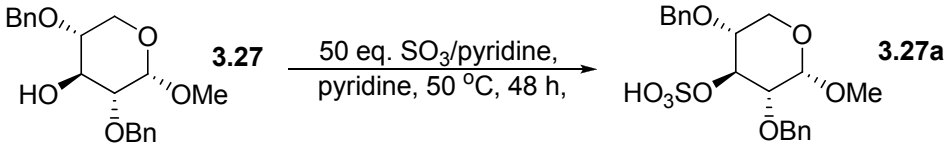
Compound 3.59: To a solution of **3.58a** (9 mg, 0.007 mmol) in MeOH (8 mL) was added $Pd(OH)_2/C$ (20 wt. %, max. 50% water)(65 mg) and the suspension was stirred under H_2 at R.T. for 6 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to obtain crude product, which was purified by RP-LC-MS (Column: Synergi Polar-RP, 12 min., gradient of 60-80% MeOH in H_2O) to afford pure final **3.59** (5 mg, 85%), as a white powder. ^{1}H NMR (700 MHz, DMSO-d_6) δ (major rotamer) 8.43 (s, 1H), 7.97 (t, 2H, *J*=5.5 Hz), 7.52 (d, 1H, *J*=9.5 Hz), 7.37 (s, 1H), 7.25 (dd, 2H, *J*=10.0 Hz, *J*=17.2 Hz), 7.22 (d, 2H, *J*=6.9 Hz), 7.20–7.17 (m, 1H), 6.69 (s, 1H), 5.97 (d, 1H, *J*=5.8 Hz), 5.40 (d, 1H, *J*=3.0 Hz), 5.26 (d, 1H, *J*=3.4 Hz), 4.86 (t, 1H, *J*=10.0 Hz), 4.28–4.23 (m, 2H), 4.20–4.15 (m, 2H), 3.92 (t, 2H, *J*=8.9 Hz), 3.87 (ddd, 2H, *J*=3.6 Hz, *J*=5.7 Hz, *J*=10.4 Hz), 3.45–3.39 (m, 1H), 3.34 (s, 1H), 3.16 (t, 1H, *J*=11.0 Hz), 3.10 (dd, 1H, *J*=6.4 Hz, *J*=13.2 Hz), 3.08–3.03 (m, 3H), 2.99 (dd, 1H, *J*=6.3 Hz, *J*=12.1 Hz), 2.96 (dd, 1H, *J*=3.7 Hz, *J*=13.9 Hz), 2.82 (dd, 1H, *J*=7.0 Hz, *J*=13.8 Hz), 2.27 (d, 1H, *J*=13.3 Hz), 2.20 (td, 1H, *J*=6.1 Hz, *J*=12.6 Hz), 2.06–1.96 (m, 2H), 1.81 (dd, 1H, *J*=12.2 Hz, *J*=22.7 Hz), 1.70–1.64 (m, 2H), 1.63 (dd, 1H, *J*=7.0 Hz, *J*=14.3 Hz), 1.49–1.36 (m, 6H), 0.87 (d, 3H, *J*=6.5 Hz), 0.84 (d, 3H, *J*= 6.7 Hz); ^{13}C NMR (175 MHz, DMSO-d_6) δ (major rotamer) 172.11, 171.19, 167.60, 156.91, 137.86, 129.80, 127.90, 126.14, 102.20, 82.98, 72.95, 72.32, 71.70, 69.28, 68.53, 65.32, 59.86,

54.66, 51.05, 40.38, 40.01, 37.97, 35.74, 31.71, 30.91, 27.79, 26.29, 25.86, 23.12, 20.79, 19.16, 15.02; $[\alpha]_D$ +13.0 (c 0.1, MeOH); HRMS for $C_{34}H_{53}ClN_6O_{12}S$ calculated ($M+H^+$) 805.32035, found 805.32158.



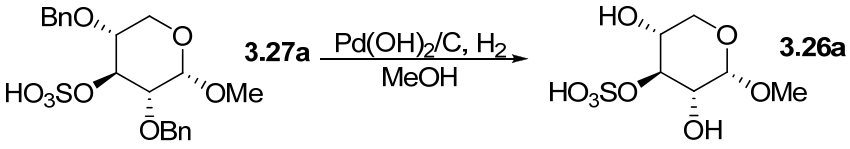
Compound 3.16b: To a solution of **3.58b** (10 mg, 0.008 mmol) in MeOH (10 mL) was added $Pd(OH)_2/C$ (20 wt. %, max. 50% water)(70 mg) and the suspension was stirred under H_2 at R.T. for 5.5 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to obtain crude product, which was purified by RP-LC-MS (Column: Synergi Polar-RP, 12 min., gradient of 60-80% MeOH in H_2O) to afford pure final **3.16b** (5 mg, 84%), as a white powder. ^{1}H NMR (700 MHz, DMSO-d_6) δ (major rotamer) 8.83 (s, 1H), 8.05 (t, 1H, *J*=5.5 Hz), 7.83 (s, 2H), 7.67 (d, 1H, *J*=9.5 Hz), 7.24 (dd, 2H, *J*=8.0 Hz, *J*=15.0 Hz), 7.22 (d, 2H, *J*=6.7 Hz), 7.17 (t, 1H, *J*=7.7 Hz), 6.69 (s, 1H), 6.01 (s, 1H), 5.32 (d, 1H, *J*=2.4 Hz), 5.09 (d, 1H, *J*=3.8 Hz), 4.93 (t, 1H, *J*=9.7 Hz), 4.82 (d, 1H, *J*=4.2 Hz), 4.35 (dt, 1H, *J*=6.1 Hz, *J*=12.0 Hz), 4.22–4.13 (m, 3H), 3.97 (dd, 1H, *J*=1.6 Hz, *J*=10.7 Hz), 3.86 (s, 1H), 3.45 (ddd, 2H, *J*=3.6 Hz, *J*=8.4 Hz, *J*=12.2 Hz), 3.39 (br, m, 1H), 3.35 (br, m, 1H), 3.16 (s, 1H), 3.07 (dd, 1H, *J*=6.7 Hz, *J*=13.5 Hz), 3.03 (s, 2H), 3.00 (dd, 1H, *J*=6.4 Hz, *J*=13.9 Hz), 2.94 (dd, 1H, *J*=3.7 Hz, *J*=14.0 Hz), 2.78 (dd, 1H, *J*=7.5 Hz, *J*=13.8 Hz), 2.32 (d, 1H, *J*=11.8 Hz), 2.26 (dt, 1H, *J*=6.9 Hz, *J*=13.1 Hz),

2.13 (ddd, 1H, *J*=6.4 Hz, *J*=11.2 Hz, *J*=19.8 Hz), 2.01 (dt, 1H, *J*=6.8 Hz, *J*=13.3 Hz), 1.85 (dd, 1H, *J*=13.1 Hz, *J*=23.5 Hz), 1.66 (dt, 1H, *J*=6.9 Hz, *J*=13.3 Hz), 1.58 (d, 1H, *J*=14.4 Hz), 1.54 (d, 2H, *J*=12.8 Hz), 1.49 (d, 1H, *J*=13.6 Hz), 1.46–1.43 (m, 2H), 1.43–1.38 (m, 2H), 0.85 (d, 3H, *J*=6.6 Hz), 0.84 (d, 3H, *J*=6.6 Hz); ^{13}C NMR (175 MHz, DMSO-d_6) δ (major rotamer) 172.37, 171.16, 167.45, 157.47, 138.05, 129.75, 127.91, 126.08, 94.20, 80.45, 71.77, 70.71, 68.96, 68.58, 67.33, 61.43, 59.81, 54.03, 51.10, 40.25, 40.03, 38.04, 35.92, 30.84, 28.78, 27.52, 26.25, 25.89, 24.89, 20.85, 19.51, 15.37; $[\alpha]_D$ +39.4 (c 0.5, MeOH); HRMS for $C_{34}H_{53}ClN_6O_{12}S$ calculated (M-H^+) 803.30579, found 803.30339.

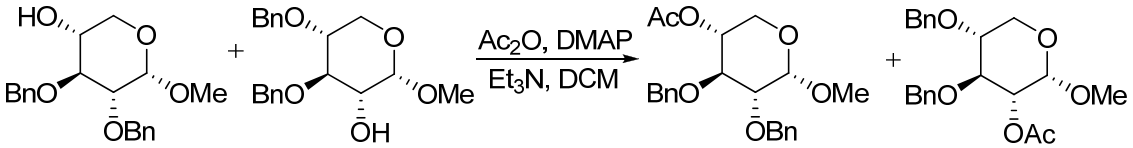


Methyl 3-*O*-sulfated-2,4-di-*O*-benzyl-α-D-xylopyranoside 3.27a: To a solution of methyl 2,4-di-*O*-benzyl-α-D-xylopyranoside **3.27** (172 mg, 0.5 mmol) in dry pyridine (10 mL) at R.T. were added sulfur trioxide pyridine complex (3.98 g, 25 mmol). After addition, the reaction mixture was heated to 50 °C slowly, and stirred for 24 h, when TLC (EtOAc/hexane 3:7) showed the conversion of **3.27** into **3.27a**, then cooled to R.T. and pyridine was evaporated. The crude residue was suspended in 15 mL H_2O and extracted with 15 mL×4 DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM to MeOH/DCM 2:23) to give **3.27a** (220 mg, 87%), as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.40 (m, 4H), 7.36–7.23 (m, 6H), 4.91 (dd, 2H, *J*=2.0 Hz, *J*=11.9 Hz), 4.75 (td, 1H, *J*=3.8 Hz, *J*=8.4 Hz), 4.65 (dd, 2H, *J*=11.9 Hz, *J*=17.5 Hz), 4.54 (d, 1H, *J*=3.3 Hz), 3.60 (dd, 1H, *J*=4.0 Hz, *J*=8.6 Hz), 3.57 (dd, 1H, *J*=3.3 Hz, *J*=7.9 Hz), 3.53 (dd, 1H, *J*=3.3 Hz,

J=5.9 Hz), 3.50 (td, 1H, *J*=3.5 Hz, *J*=9.2 Hz), 3.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.8, 139.7, 129.5, 129.42, 129.41, 129.35, 128.9, 128.8, 100.3, 80.6, 79.0, 77.3, 74.9, 74.5, 61.8, 55.8; Chemical Formula: $C_{20}H_{24}O_8S$.



Methyl 3-*O*-sulfated-α-D-xylopyranoside 3.26a: To a solution of methyl 3-*O*-sulfated-2,4-di-*O*-benzyl-α-D-xylopyranoside **3.27a** (55 mg, 0.13 mmol) in dry MeOH (10 mL) was added $Pd(OH)_2$/C 20 w.t.% (2 mg) and the suspension was stirred under H_2 (1 atm) at R.T. for 4 h. The $Pd(OH)_2$/C catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **3.26a** (31 mg, 96%), as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (d, 1H, *J*=3.5 Hz), 4.40(t, 1H, *J*=8.8 Hz), 3.70 (ddd, 1H, *J*=5.5 Hz, *J*=8.3 Hz, *J*=9.9 Hz), 3.65 (dd, 1H, *J*=5.5 Hz, *J*=10.9 Hz), 3.60 (dd, 1H, *J*=3.5 Hz, *J*=9.2 Hz), 3.52 (t, 1H, *J*=10.5 Hz), 3.42 (s, 3H), 3.35 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.2, 82.8, 71.9, 70.2, 62.8, 55.8; HRMS for $C_6H_{12}O_8S$ calculated $(M-H)^-$ 243.01801, found 243.01846.

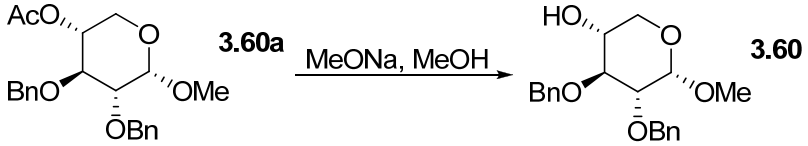


Compounds 3.60a and 3.61a: The mixture of **3.60** and **3.61** (43 mg, 0.125 mmol) was dissolved in dry DCM (5 mL), and cooled at 0 °C. To the solution at 0 °C, Ac_2O (17 μL, 0.18 mmol), DMAP (1 mg, 0.01 mmol), and Et_3N (35 μL, 0.25 mmol) were added, then warmed to R.T.. After stirring for 3 h, the reaction mixture was diluted with DCM (10 mL), washed with 1 M HCl aqueous (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The

crude residue was purified by flash column chromatography (hexane to EtOAc/hexane 1:5) to afford the mixture of **3.60a** and **3.61a** (45mg, 93%), as a colorless oil, which was separated by RP-LC-MS (Column: Synergi Polar-RP, 30 min., gradient of 70-73% MeOH in H_2O with 0.1% FA) to give **3.60a** (17 mg, 35%), as a colorless oil and **3.61a** (23 mg, 48%), as a colorless oil.

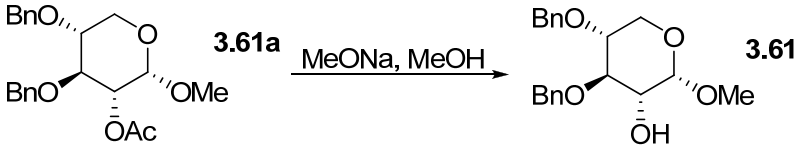
Methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-xylopyranoside 3.60a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.24 (m, 10H), 4.86 (ddd, 2H, *J*=4.0 Hz, *J*=8.9 Hz, *J*=15.2 Hz), 4.78 (d, 1H, *J*=12.1 Hz), 4.66 (dd, 2H, *J*=11.8 Hz, *J*=15.7 Hz), 4.54 (d, 1H, *J*=3.5 Hz), 3.89 (t, 1H, *J*=9.4 Hz), 3.67 (dd, 1H, *J*=5.9 Hz, *J*=10.7 Hz), 3.50 (dd, 1H, *J*=3.5 Hz, *J*=9.5 Hz), 3.42 (t, 1H, *J*=10.7 Hz), 3.36 (s, 3H), 1.93 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.3, 138.8, 138.2, 128.7, 128.5, 128.3, 128.2, 128.0, 127.8, 98.5, 79.6, 79.0, 75.6, 73.8, 71.4, 58.7, 55.6, 21.1; ESI/MS for $C_{22}H_{26}O_6$ calculated ($M+Na^+$) 409, found 409.

Methyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-xylopyranoside 3.61a: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.37–7.24 (m, 10H), 4.85 (d, 1H, *J*=11.4 Hz), 4.80 (s, 1H), 4.77 (d, 1H, *J*=3.6 Hz), 4.72 (dd, 2H, *J*=2.4 Hz, *J*=11.3 Hz), 4.62 (d, 1H, *J*=11.6 Hz), 3.89 (t, 1H, *J*=8.8 Hz), 3.65 (dd, 1H, *J*=3.9 Hz, *J*=7.5 Hz), 3.64 (dq, 2H, *J*=5.4 Hz, *J*=8.6 Hz), 3.57–3.46 (m, 1H), 3.35 (s, 3H), 2.04 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 170.6 138.8, 138.3, 128.7, 128.6, 128.1, 128.0, 127.8, 97.3, 79.5, 78.3, 75.6, 73.7, 73.3, 60.0, 55.3, 21.2; ESI/MS for $C_{22}H_{26}O_6$ calculated ($M+Na^+$) 409, found 409.



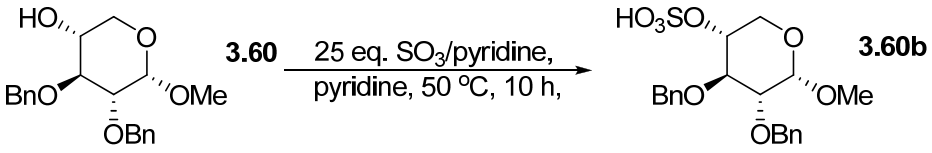
Methyl 2,3-di-*O*-benzyl-α-D-xylopyranoside 3.60: A solution of methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-xylopyranoside **3.60a** (15 mg, 0.04 mmol) in dry fresh MeOH (3 mL) was treated with 0.5 M MeONa in MeOH (0.16 mL, 0.08

mmol) carefully. After stirring at R.T. for 2 h, the reaction mixture was diluted with solid NH_4Cl, filtered and concentrated under reduced pressure. The crude residue was redissolved in DCM (10 mL), filtered and concentration under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 1:4) to afford **3.60** (13 mg, 96%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.40–7.25 (m, 10H), 4.95 (d, 1H, *J*=11.4 Hz), 4.73 (d, 1H *J*=12.0 Hz), 4.68 (d, 1H, *J*=1.9 Hz), 4.66 (d, 1H, *J*=11.4 Hz), 4.56 (d, 1H, *J*=3.1 Hz), 3.71 (t, 1H, *J*=8.3 Hz), 3.68–3.57 (m, 2H), 3.54 (dd, 1H, *J*=7.0 Hz, *J*=10.6 Hz), 3.48 (dd, 1H, *J*=3.1 Hz, *J*=8.7 Hz), 3.38 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 138.7, 138.1, 128.8, 128.7, 128.3, 128.2, 128.1, 98.8, 81.2, 79.4, 75.3, 73.4, 69.7, 62.0, 55.6; ESI/MS for $C_{20}H_{25}O_5$ calculated ($M+H^+$) 345, found 345.

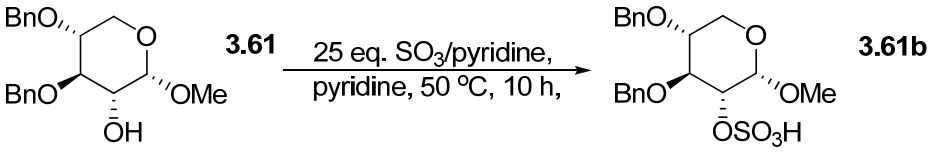


Methyl 3,4-di-*O*-benzyl-α-D-xylopyranoside 3.61: A solution of methyl 2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-xylopyranoside **3.61a** (19 mg, 0.05 mmol) in dry fresh MeOH (4 mL) was treated with 0.5 M MeONa in MeOH (0.2 mL, 0.1 mmol) carefully. After stirring at R.T. for 2 h, the reaction mixture was diluted with solid NH_4Cl, filtered and concentrated under reduced pressure. The crude residue was redissolved in DCM (10 mL), filtered and concentration under reduced pressure. The residue was purified by flash column chromatography (EtOAc/hexane 1:4) to afford **3.61** (17 mg, 96%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.38–7.24 (m, 10H), 4.88 (d, 1H, *J*=11.3 Hz), 4.81 (d, 1H, *J*=11.3 Hz), 4.70 (d, 1H, *J*=11.9 Hz), 4.68 (d, 1H, *J*=3.9 Hz), 4.61 (d, 1H, *J*=11.6 Hz), 3.68 (dd, 2H, *J*=7.2 Hz, *J*=16.0 Hz), 3.60 (ddd, 1H, *J*=3.3 Hz, *J*=6.6 Hz, *J*=9.8 Hz), 3.55 (d, 2H, *J*=6.4 Hz), 3.41 (s, 3H); ^{13}C NMR, (100 MHz,

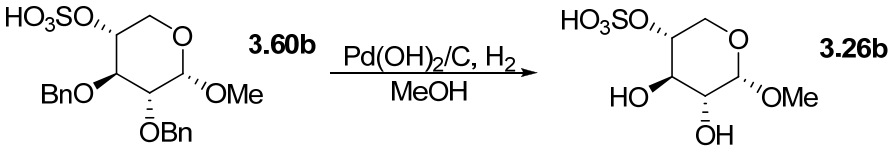
$CDCl_3$) δ 138.8, 138.3, 128.7, 128.6, 128.12, 128.05, 127.96, 127.93, 99.6, 81.7, 77.7, 75.3, 73.3, 72.3, 62.5, 55.5; ESI/MS for $C_{20}H_{25}O_5$ calculated ($M+H^+$) 345, found 345.



Methyl 4-*O*-sulfated-2,3-di-*O*-benzyl-α-D-xylopyranoside 3.60b: To a solution of methyl 2,3-di-*O*-benzyl-α-D-xylopyranoside **3.60** (13 mg, 0.036 mmol) in dry pyridine (2 mL) at R.T. were added sulfur trioxide pyridine complex (143 mg, 0.9 mmol). After addition, the reaction mixture was heated to 50 °C slowly, and stirred for 10 h, when TLC (EtOAc/hexane 3:7) showed the conversion of **3.60** into **3.60b**, then cooled to R.T. and pyridine was evaporated. The crude residue was suspended in 15 mL H_2O and extracted with 15 mL×4 DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM to MeOH/DCM 2:23) to give **3.60b** (14 mg, 92%), as a colorless oil: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.47–7.40 (m, 2H), 7.37–7.20 (m, 8H), 5.01 (d, 1H, *J*=10.9 Hz), 4.72 (d, 1H, *J*=4.7 Hz), 4.69 (d, 1H, *J*=3.8 Hz), 4.66 (d, 1H, *J*=3.6 Hz), 4.63 (d, 1H, *J*=11.8 Hz), 4.38 (ddd, 1H, *J*=5.9 Hz, *J*=9.0 Hz, *J*=10.7 Hz), 4.00 (dd, 1H, *J*=5.9 Hz, *J*=11.1 Hz), 3.78 (t, 1H, *J*=9.2 Hz), 3.56 (t, 1H, *J*=11.0 Hz), 3.48 (dd, 1H, *J*=3.6 Hz, *J*=9.5 Hz), 3.36 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 140.3, 139.8, 129.6, 129.5, 129.31, 129.27, 129.0, 128.6, 99.5, 80.6, 77.8, 76.3, 74.5, 60.7, 55.7; Chemical Formula: $C_{20}H_{24}O_8S$.

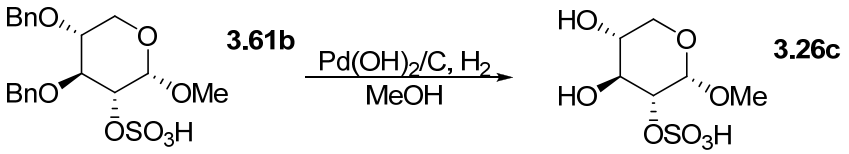


Methyl 2-*O*-sulfated-3,4-di-*O*-benzyl-α-D-xylopyranoside 3.61b: To a solution of methyl 3,4-di-*O*-benzyl-α-D-xylopyranoside **3.61** (16 mg, 0.046 mmol) in dry pyridine (2 mL) at R.T. were added sulfur trioxide pyridine complex (183 mg, 1.15 mmol). After addition, the reaction mixture was heated to 50 °C slowly, and stirred for 10 h, when TLC (EtOAc/hexane 3:7) showed the conversion of **3.61** into **3.61b**, then cooled to R.T. and pyridine was evaporated. The crude residue was suspended in 15 mL H_2O and extracted with 15 mL×4 DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (DCM to MeOH/DCM 2:23) to give **3.61b** (18 mg, 91%), as a colorless oil: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.45–7.40 (m, 2H), 7.34–7.20 (m, 8H), 5.03 (d, 1H, *J*=3.5 Hz), 4.99 (d, 1H, *J*=10.9 Hz), 4.68 (dd, 2H, *J*=2.3 Hz, *J*=11.2 Hz), 4.62 (d, 1H, *J*=11.6 Hz), 4.27 (dd, 1H, *J*=3.6 Hz, *J*=9.7 Hz), 3.80 (d, 1H, *J*=8.8 Hz), 3.82–3.75 (m, 1H), 3.78 (d, 1H, *J*=9.5 Hz), 3.66 (dd, 1H, *J*=5.4 Hz, *J*=10.2 Hz), 3.56 (ddd, 1H, *J*=5.4 Hz, *J*=8.6 Hz, *J*=10.4 Hz), 3.47 (t, 1H, *J*=10.4 Hz), 3.39 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 140.3, 140.0, 129.6, 129.5, 129.3, 129.1, 128.8, 128.6, 99.7, 80.8, 79.3, 78.9, 76.3, 74.6, 60.8, 55.9; Chemical Formula: $C_{20}H_{24}O_8S$.

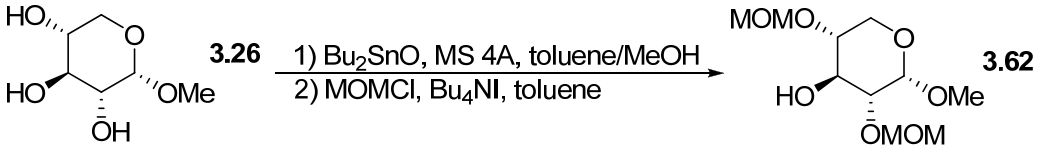


Methyl 4-*O*-sulfated-α-D-xylopyranoside 3.26b: To a solution of methyl 4-*O*-sulfated-2,3-di-*O*-benzyl-α-D-xylopyranoside **3.60b** (14 mg, 0.033 mmol) in dry MeOH (5 mL) was added $Pd(OH)_2$/C 20 w.t.% (2 mg) and the suspension was stirred under H_2 (1 atm) at R.T. for 4 h. The $Pd(OH)_2$/C catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **3.26b** (8 mg, 96%), as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6)

δ 5.01 (d, 1H, *J*=3.0 Hz), 4.88 (d, 1H, *J*=6.6 Hz), 4.48 (d, 1H, *J*=3.6 Hz), 3.90 (ddd, 1H, *J*=5.8 Hz, *J*=8.9 Hz, *J*=10.7 Hz), 3.66 (dd, 1H, *J*=5.8 Hz, *J*=10.9 Hz), 3.46 (td, 1H, *J*=3.0 Hz, *J*=9.2 Hz), 3.32–3.27 (m, 1H), 3.25 (s, 3H), 3.30–3.20 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 99.8, 74.8, 71.9, 71.7, 59.3, 54.7; HRMS for $C_6H_{12}O_8S$ calculated $(M-H)^-$ 243.01801, found 243.01851.

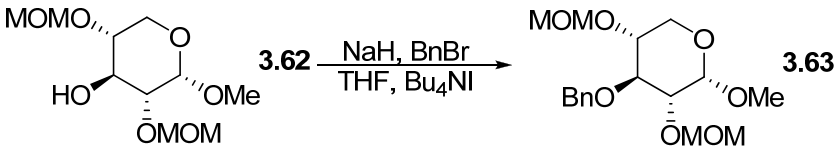


Methyl 2-*O*-sulfated-α-D-xylopyranoside 3.26c: To a solution of methyl 2-*O*-sulfated-3,4-di-*O*-benzyl-α-D-xylopyranoside **3.61b** (17 mg, 0.13 mmol) in dry MeOH (10 mL) was added $Pd(OH)_2$/C 20 w.t.% (2 mg) and the suspension was stirred under H_2 (1 atm) at R.T. for 4 h. The $Pd(OH)_2$/C catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **3.26c** (9 mg, 97%), as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.07 (d, 1H, *J*=4.9 Hz), 4.92 (d, 1H, *J*=2.2 Hz), 4.72 (d, 1H, *J*=3.3 Hz), 3.81 (dd, 1H, *J*=3.2 Hz, *J*=9.4 Hz), 3.49–3.37 (m, 2H), 3.36–3.28 (m, 1H), 3.23 (s, 3H), 3.22 (dd, 1H, *J*=5.5 Hz, *J*=13.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 98.3, 76.2, 71.8, 70.3, 61.2, 54.7; HRMS for $C_6H_{12}O_8S$ calculated $(M-H)^-$ 243.01801, found 243.01820.



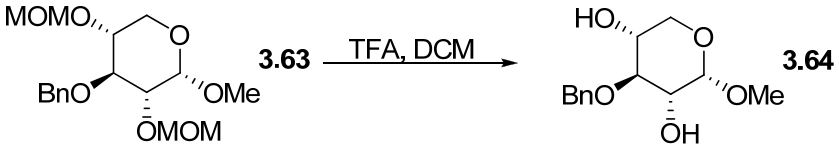
Methyl 2,4-di-*O*-MOM-α-D-xylopyranoside 3.62: To a solution of Methyl α-D-xylopyranoside **3.26** (82 mg, 0.5 mmol) in dry toluene (12 mL) and dry MeOH (2 mL) at R.T. was added bis(tributyltin) oxide (274 mg, 1.1 mmol) and activated 4Å molecular sieves. After heating at 70 °C for 10 h, solvent was evaporated. The crude residue was redissolved in dry dry toluene (5 mL), then

treated with MOMCl (84 µL, 1.1 mmol) and Bu_4NI (185 mg, 0.5mmol) at R.T. for 10 h. The suspension was filtered over celite and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexane 3:7) to afford major product **3.62** (59 mg, 47%), as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.83 (d, 1H, *J*=6.9 Hz), 4.77 (d, 2H, J= 6.9 Hz), 4.76 (dd, 2H, *J*=2.6 Hz, *J*=3.6 Hz), 4.71 (d, 1H, *J*=7.0 Hz), 3.87 (t, 1H, *J*=8.8 Hz), 3.71 (d, 2H, *J*=4.9 Hz), 3.52 (d, 2H, *J*=6.4 Hz), 3.43 (s, 3H), 3.42 (s, 3H), 3.41 (s, 3H),; ^{13}C NMR (75 MHz, $CDCl_3$) δ 98.9, 98.0, 97.5, 80.0, 78.7, 71.8, 59.8, 55.86, 55.85, 55.4; $[\alpha]_D$ +76.8 (c 1.0, $CHCl_3$), ESI/MS for $C_{10}H_{20}O_7$ calculated ($M+H^+$) 253, found 253.

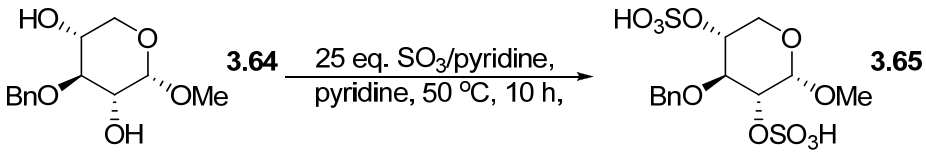


Methyl 3-*O*-benzyl-2,4-di-*O*-MOM-α-D-xylopyranoside 3.63 : A solution of methyl 2,4-di-*O*-MOM-α-D-xylopyranoside **3.62** (20 mg, 0.08 mmol) in THF (5 mL) was cooled to 0°C, and treated with NaH (5 mg, 60 dispersion in mineral oil) under argon atmosphere, and warmed up to R.T.. After stirring for 1 h, BnBr (14 µL, 0.12 mmol) and Bu_4NI (30 mg, 0.08 mmol) were added, and stirred for 10 h. The mixture was quenched by H_2O (0.5 mL) carefully and concentrated under reduced pressure. Then the residue was redissolved in a mixture of DCM (15 mL) and H_2O (15 mL). The phases were separated, and the organic phase was washed with H_2O (15 mL) and brine (15 mL), then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane to EtOAc/hexane 3:17) to give **3.63** (25 mg, 89%), as a colorless oil: ^{1}H NMR, (400 MHz, $CDCl_3$) δ 7.29 (d, 4H, *J*=4.4 Hz), 7.28–7.21 (m, 1H), 4.79 (d, 1H, *J*=6.8 Hz),4.75 (dt, 3H, *J*=7.4 Hz, *J*=10.0 Hz), 4.68 (d, 1H, *J*= 6.7 Hz), 4.60 (d, 1H, *J*=6.7 Hz), 3.76 (t, 1H, *J*= 9.3 Hz),

3.70 (dd, 1H, *J*=5.5 Hz, *J*=10.3 Hz), 3.61 (ddd, 1H, *J*=5.5 Hz, *J*=8.8 Hz, *J*=10.4 Hz), 3.53 (dd, 1H, *J*=3.6 Hz, *J*=9.6 Hz), 3.50 (t, 1H, *J*=10.4 Hz),3.41 (s, 3H), 3.35 (s, 3H), 3.31 (s, 3H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ 138.9, 128.5, 127.9, 127.8, 99.4, 97.9, 97.4, 80.7, 78.8, 77.0, 75.9, 60.5, 55.7, 55.6, 55.4; ESI/MS for $C_{17}H_{26}O_7$ calculated ($M+H^+$) 343, found 343.

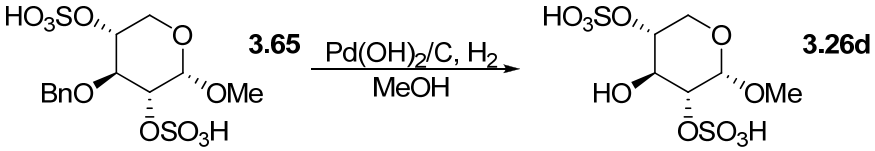


Methyl 3-*O*-benzyl-α-D-xylopyranoside 3.64: Methyl 3-*O*-benzyl-2,4-di-*O*-MOM-α-D-xylopyranoside **3.58** (24 mg, 0.07 mmol) was treated with >99.9% TFA/DCM (1:2, 3 mL) at R.T.. After stirring for 3 h, the reaction mixture was diluted with EtOAc (20 mL) and concentrated under reduced pressure. The crude residue was suspended in saturated aqueous Na_2CO_3 (20 mL) and extracted with DCM (3×30 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:49) to afford **3.64** (15 mg, 82%), as a colorless oil: ^{1}H NMR, (400 MHz, MeOH-d_4) δ 7.43 (d, 2H, *J*=7.3 Hz), 7.31 (t, 2H, *J*=7.4 Hz), 7.25 (t, 1H, *J*= 7.2 Hz), 4.86 (d, 2H, *J*=4.0 Hz), 4.61 (d, 1H, *J*=3.3 Hz), 3.65–3.56 (m, 1H), 3.55 (dd, 1H, *J*=7.2 Hz, *J*=9.3 Hz), 3.58–3.51 (m, 1H), 3.48 (dd, 2H, *J*=9.5 Hz, *J*=19.7 Hz), 3.40 (s, 3H); ^{13}C NMR, (100 MHz, MeOH-d_4) δ 140.6, 129.3, 129.2, 128.6, 101.9, 83.8, 76.3, 73.7, 71.5, 63.1, 55.7; ESI/MS for $C_{13}H_{18}O_5$ calculated ($M+H^+$) 255, found 255.

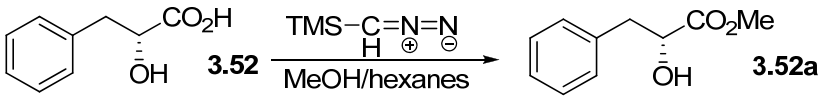


Methyl 3-*O*-benzyl-2,4-di-*O*-sulfated-α-D-xylopyranoside 3.65: To a solution of methyl 3-*O*-benzyl-α-D-xylopyranoside **3.64** (12 mg, 0.04 mmol) in

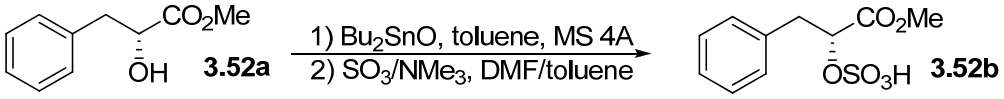
dry pyridine (2 mL) at R.T. were added sulfur trioxide pyridine complex (159 mg, 1.0 mmol). After addition, the reaction mixture was heated to 50 °C slowly and stirred for 10 h, when TLC (MeOH/DCM 1:19) showed the conversion of **3.64** into **3.65**, then cooled to R.T.. The reaction mixture was filtered and pyridine was evaporated. The residue was purified by flash column chromatography (MeOH/DCM 1:9 to MeOH/DCM 1:4) to give **3.65** (14 mg, 87%), as a colorless oil: ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.52 (d, 2H, *J*=7.2 Hz), 7.29 (t, 2H, *J*=7.3 Hz), 7.24–7.18 (m, 1H), 5.05 (d, 1H, *J*=3.4 Hz), 4.88 (d, 2H, *J*=19.3 Hz), 4.40 (ddd, 1H, *J*=6.1 Hz, *J*=8.9 Hz, *J*=10.6 Hz), 4.29 (dd, *J*=3.5 Hz, *J*=9.7 Hz), 4.02 (dd, 1H, *J*=6.0 Hz, *J*=11.1 Hz), 3.77 (t, 1H, *J*=9.4 Hz), 3.60 (t, 1H, *J*=10.1 Hz), 3.40 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 139.9, 129.7, 129.2, 128.5, 99.6, 78.5, 78.4, 77.1, 75.9, 60.7, 56.0; Chemical Formula: $C_{13}H_{18}O_{11}S_2$.



Methyl 2,4-di-*O*-sulfated-α-D-xylopyranoside 3.26d: To a solution of methyl 3-*O*-benzyl-2,4-di-*O*-sulfated-α-D-xylopyranoside **3.65** (10 mg, 0.024 mmol) in dry MeOH (5 mL) was added $Pd(OH)_2$/C 20 w.t.% (2 mg) and the suspension was stirred under H_2 (1 atm) at R.T. for 3 h. The $Pd(OH)_2$/C catalyst was removed by filtration and filtrate was concentrated under reduced pressure to give **3.26d** (7 mg, 96%), as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.97 (d, 1H, J=2.6 Hz), 4.79 (d, 1H, *J*=3.5 Hz), 3.93 (ddd, 1H, *J*=5.9 Hz, *J*=9.0 Hz, *J*=10.8 Hz), 3.83 (dd, 1H, J=3.6 Hz, *J*=9.8 Hz), 3.76 (dd, *J*=5.9 Hz, *J*=11.0 Hz), 3.53 (td, 1H, *J*=2.5 Hz, *J*=9.5 Hz), 3.26 (dd, 1H, J=12.6 Hz, *J*=23.7 Hz), 3.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 97.9, 76.0, 74.9, 69.4, 59.0, 54.8; HRMS for $C_6H_{12}O_{11}S_2$ calculated $(M-H)^-$ 322.97483, found 322.97594.



Compound 3.52a: To a solution of D-(+)-3-phenyllactic acid **3.52** (50 mg, 0.3 mmol) in MeOH (0.5 mL) and hexanes (1.0 mL) was added 2 M trimethylsilyldiazomethane in hexanes (0.6 mmol, 0.3 mL) at R.T., and stirred for 1 h (TLC monitoring, EtOAc/hexanes 7:13), then solvents were evaporated. The crude residue was purified by flash column chromatography (EtOAc/hexanes 1:4) to give **3.52a** (51 mg, 94%), as a colorless needle: ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.29–7.25 (m, 4H), 7.24–7.18 (m, 1H), 4.38 (dd, 1H, *J*=5.2 Hz, *J*=6.5 Hz), 4.29 (s, 1H), 3.67 (s, 3H), 3.06 (dd, 1H, *J*=4.6 Hz, *J*=13.8 Hz), 2.91 (dd, 1H, *J*=7.6 Hz, *J*=13.8 Hz); ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.9, 138.6, 130.4, 129.0, 127.3, 72.6, 52.1, 41.4; ESI/MS for $C_{10}H_{12}O_3$ calculated ($M+H^+$) 181, found 181.



Compound 3.52b: To a solution of **3.52a** (25 mg, 0.14 mmol) in dry toluene (10 mL) was added activated 4Å molecular sieves and bis(tributyltin) oxide (69 mg, 0.28 mmol) at R.T.. After heating at 80 °C for 10 h, solvent was evaporated. The crude residue was redissolved in dry DMF (3 mL) and dry toluene (3 mL) at R.T., and treated with sulfur trioxide trimethylamine complex (38 mg, 0.28 mmol) for 10 h. The suspension was filtered over celite and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM to MeOH/DCM 1:24 to MeOH/DCM 1:12) to afford **3.52b** (23 mg, 65%), as a colorless oil: ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.26–7.24 (m, 4H), 7.23–7.16 (m, 1H), 5.07 (t, 1H, *J*=6.3 Hz), 3.59 (s, 3H), 3.17 (d, 1H, *J*=4.4 Hz), 3.15 (d, 1H, *J*=5.1 Hz); ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.3, 137.1, 130.6, 129.1, 127.5, 77.2, 52.4, 39.2; HRMS for $C_{10}H_{12}O_6S$ calculated $(M-H)^-$

259.02818, found 259.02827.

3-5 References:

1. M. Murakami, Y. Okita, H. Matsuda, T. Okino, and K. Yamaguchi, *Tetrahedron Lett.*, **1994**, *35*, 3129.
2. J. L. Rios Steiner, M. Murakami, and A. Tulinsky, *J. Am. Chem. Soc.,* **1998**, *120*, 597.
3. N. Valls, M. LSpez-Canet, M. Vallribera, and J. Bonjoch, *J. Am. Chem. Soc.,* **2000**, *122*, 11248.
4. N. Valls, M. LSpez-Canet, M. Vallribera, and J. Bonjoch, *Chem. Eur. J.,* **2001**, *7*, 3446.
5. P.Wipf, J.-L. Methot, *Org. Lett.*, **2000**, *2*, 4213.
6. S. Konetschny-Rapp, H.-W. Krell, and U. Martin, *PCT WO96/11941*, 1996 [*Chem. Abst.,* **1996**, *124*, 315175].
7. R. Engh, S. Konetschny-Rapp, H.-W. Krell, U. Martin, and C. Tsaklakidis, *PCT WO97/21725*, 1997 [*Chem. Abst.*, **1997**, *127*, 122002].
8. S. Hanessian, M. Tremblay, and J. F. W. Petersen, *J. Am. Chem. Soc.*, **2004**, *126*, 6064.
9. A. R. Carroll, M. S. Buchanan, A. Edser, E. Hyde, M. Simpson, and R. J. Quinn, *J. Nat. Prod.,* **2004**, *67*, 1291.
10. A. R. Carroll, G. K. Pierens, G. Fechner, P. de Almeida Leone, A. Ngo, M. Simpson, E. Hyde, J. N. A. Hooper, S.-L. Bostrom, D. Musil, and R. J. Quinn, *J. Am. Chem. Soc.,* **2002**, *124*, 13340.
11. S. Hanessian, R. Margarita, A. Hall, S. Johnstone, M. Tremblay, and L. Parlanti, *J. Am. Chem. Soc.*, **2002**, *124*, 13342.
12. G. H. Goetz, G. G. Harrigan, J. J. Likos, and T. P. Kasten, *PCT WO03/ 051831*, 2003 [*Chem. Abst.,* **2003**, *139*, 47155].

13. S. Hanessian, J. R. Del Valle, Y. Xue, and N. Blomberg, *J. Am. Chem. Soc.,* **2006**, *128*,10491.
14. K. Ersmark, J. R. Del Valle, and S. Hanessian, *Angew. Chem. Int. Ed.,* **2008**, *47*, 1202.
15. J. H. Shin, H. Matsuda, M. Murakami, and K. Yamaguchi, *J. Org. Chem.* **1997***, 62*, 1810.
16. M. Murakami, K. Ishida, T. Okino, Y. Okita, and K. Yamaguchi, *Tetrahedron Lett.,* **1995**, *36*, 2785.
17. A. M. Burja, B. Banaigs, E. Abou-Mansour, J. G. Burgess, and P. C. Wright, *Tetrahedron,* **2001**, *57*, 9347
18. W. J. Gerwick, L. T. Tan, and N. Sitachitta, Vol. 57 (Ed.: G. A. Cordell), Academic Press, San Diego, 2002, p. 75
19. J. B. Stewart, V. Bornemann, J. L. Chen, R. E. Moore, F. R. Caplan, H. Karuso, L. K. Larsen, and G. M. L. Patterson, *J. Antibiot.,* **1998**, *41*, 1048.
20. J. L. Chen, R. E. Moore, and G. M. L. Patterson, *J. Org. Chem.,***1991**, *56*, 4360
21. K. Fujii, K. Sivonen, K. Adachi, K. Noguchi, Y. Shimizu, H. Sano, K. Hirayama, M. Suzuki, and K. Harada, *Tetrahedron Lett.*, **1997**, *38*, 5529
22. A. Pluotno and S. Carmeli, *Tetrahedron Lett.,* **2005**, *61*, 575
23. N. Toyooka, A. Makazawa, T. Himiyama, and H. Nemoto, *Heterocycles,* **2003**, *59*, 75.
24. N. Valls, M. Vallribera, M. Font-Bardia, X. Solans, and J. Bonjoch, *Tetrahedron: Asymmetry,* **2003**, *14*, 1241.
25. N. Valls, M. Borregán, and J. Bonjoch, *Tetrahedron Lett.,* **2006**, *47*, 3701.
26. S. Hanessian, K. Ersmark, X. Wang, J. R. Del Valle, N. Blomberg, Y. Xue, and O. Fjellstrom, *Bioorg. & Medicinal Chem. Lett.,* **2007**, *17*, 3480.
27. R. R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, **1984**, 1343.

28. S. Hanessian, V. Mascitti and O. Rogel, *J. Org. Chem.,* **2002**, *67*, 3346.
29. O. N. Nadein, and A. Kornienko, *Org. Lett.,* **2004**, *6*, 831.
30. I. G. lvarez, G. Corrales, E. D. Perez, A. Munoz, M. N. Sampedro, and A. F. Mayoralas, *J. Med. Chem.,* **2007**, *50*, 364.
31. P. Greimel, S. Jabs, S. Storch, S. Cherif, K. Honke, T. Braulkeb, and J. Thiem, *Carbohydrate Research*, **2006**, *341*, 918.
32. A. G. Goncalves, M. D. Noseda, M. E. R. Duarteb, and T. B. Grindley *Carbohydrate Research*, **2006**, *341*, 1753.
33. A. Rosch and H. Kunz, *Carbohydrate Research*, **2006**, *341*, 1597.
34. N. Morishima, S. Koto, C. Kusuhara, and S. Zen, *Bull. Chem. Soc. Jpn.,* **1982**, *55*, 631.
35. A. D. Proud, J. C. Prodger, and S. L. Flitsch, *Tetrahedron Lett.,* **1997**, *38*, 7243.
36. W. A. Slusarchyk, J. Gougoutas, W. H. Koster, D. R. Kronenthal, M. Malley, M. G. Perri, F. L. Routh, J. E. Sundeen, E. R. Weaver, and R. Zahler, *Tetrahedron Lett.,* **1986**, *27*, 2789.
37. K. Feichtinger, C. Zapf, H. L. Sings, and M. Goodman, *J. Org. Chem.,* **1998**, *63*, 3804.

Université de Montréal

CRYSTAL AND MOLECULAR STRUCTURE OF

C15 H25 N O5 COMPOUND (benit94)

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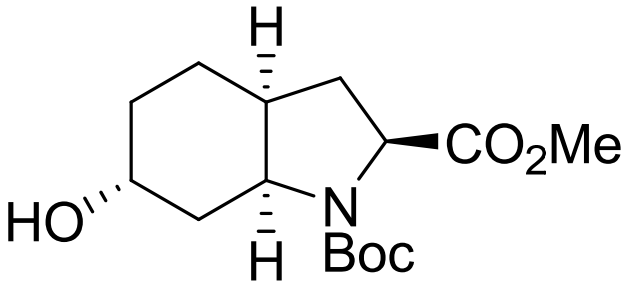


Structure solved and refined in the laboratory of X-ray diffraction Université de Montréal by Benoît Deschênes Simard.

Table 1. Crystal data and structure refinement for C15 H25 N O5.

Identification code	benit94
Empirical formula	C15 H25 N O5
Formula weight	299.36
Temperature	150K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	a = 8.8510(2) Å α = 90°
	b = 11.2574(3) Å β = 115.248(1)°
	c = 9.0523(2) Å γ = 90°
Volume	815.80(3)Å^3
Z	2
Density (calculated)	1.219 g/cm^3
Absorption coefficient	0.750 mm^{-1}
F(000)	324
Crystal size	0.21 x 0.20 x 0.18 mm
Theta range for data collection	5.40 to 72.02°
Index ranges	$-10 \leq h \leq 10$, $-13 \leq k \leq 13$, $-11 \leq \ell \leq 10$
Reflections collected	10191
Independent reflections	3035 [R_{int} = 0.034]
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8737 and 0.8000
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3035 / 1 / 196
Goodness-of-fit on F^2	1.101
Final R indices [I>2sigma(I)]	R_1 = 0.0403, wR_2 = 0.0957
R indices (all data)	R_1 = 0.0406, wR_2 = 0.0961
Absolute structure parameter	0.02(14)
Extinction coefficient	0.119(4)
Largest diff. peak and hole	0.433 and -0.304 e/Å^3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for C15 H25 N O5.
U_{eq} is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U_{eq}
O(1)	2928(1)	3923(1)	10370(1)	42(1)
O(2)	-1309(2)	3994(1)	3001(2)	42(1)
O(3)	-1813(1)	2213(1)	1776(1)	38(1)
O(4)	2378(1)	3659(1)	3177(1)	30(1)
O(5)	3819(1)	4457(1)	5719(1)	32(1)
N(1)	1836(1)	3125(1)	5305(1)	25(1)
C(1)	2189(2)	3101(1)	7056(2)	24(1)
C(2)	1347(2)	4144(1)	7502(2)	30(1)
C(3)	1248(2)	3944(2)	9124(2)	36(1)
C(4)	337(2)	2786(2)	9092(2)	39(1)
C(5)	1184(2)	1718(2)	8723(2)	38(1)
C(6)	1479(2)	1882(1)	7184(2)	28(1)
C(7)	-79(2)	1787(1)	5562(2)	29(1)
C(8)	485(2)	2328(1)	4313(2)	24(1)
C(9)	-945(2)	2978(1)	2972(2)	26(1)
C(10)	-3306(2)	2675(2)	461(2)	55(1)
C(11)	2672(2)	3751(1)	4617(2)	23(1)
C(12)	5014(2)	5158(1)	5331(2)	29(1)
C(13)	4111(2)	6024(1)	3945(2)	38(1)
C(14)	6137(2)	4328(2)	4927(2)	39(1)
C(15)	5981(3)	5801(2)	6931(2)	51(1)

Table 3. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for C15 H25 N O5.

	x	y	z	U_{eq}
H(1)	2917	3814	11283	63
H(1A)	3422	3119	7745	29
H(2A)	1984	4880	7570	35
H(2B)	205	4251	6629	35
H(3)	634	4621	9334	43
H(4A)	-830	2842	8249	47
H(4B)	307	2669	10163	47
H(5A)	482	1006	8586	46
H(5B)	2271	1577	9668	46
H(6)	2292	1261	7193	34
H(7A)	-1020	2242	5597	34
H(7B)	-423	948	5293	34
H(8)	922	1690	3831	28
H(10A)	-4079	2958	902	82
H(10B)	-3843	2045	-340	82
H(10C)	-3008	3335	-69	82
H(13A)	3497	5581	2931	57
H(13B)	4928	6548	3810	57
H(13C)	3327	6501	4199	57
H(14A)	6658	3755	5817	59
H(14B)	7007	4791	4791	59
H(14C)	5470	3902	3911	59
H(15A)	5223	6321	7167	77
H(15B)	6867	6279	6848	77
H(15C)	6478	5219	7813	77

Table 4. Anisotropic parameters ($Å^2$ x 10^3) for C15 H25 N O5.

The anisotropic displacement factor exponent takes the form:

$$-2\pi^2 [h^2 a^{*2} U_{11} + ... + 2 h k a^* b^* U_{12}]$$

	U11	U22	U33	U23	U13	U12
O(1)	38(1)	68(1)	23(1)	-6(1)	14(1)	-12(1)
O(2)	41(1)	33(1)	49(1)	1(1)	16(1)	8(1)
O(3)	35(1)	41(1)	27(1)	-4(1)	4(1)	-3(1)
O(4)	35(1)	36(1)	23(1)	-3(1)	17(1)	-8(1)
O(5)	35(1)	40(1)	25(1)	-7(1)	17(1)	-17(1)
N(1)	26(1)	30(1)	20(1)	-4(1)	12(1)	-7(1)
C(1)	24(1)	30(1)	19(1)	1(1)	10(1)	-4(1)
C(2)	35(1)	32(1)	25(1)	-2(1)	16(1)	-3(1)
C(3)	36(1)	50(1)	26(1)	-8(1)	18(1)	-4(1)
C(4)	38(1)	61(1)	26(1)	-1(1)	21(1)	-10(1)
C(5)	43(1)	46(1)	27(1)	8(1)	15(1)	-10(1)
C(6)	33(1)	27(1)	25(1)	3(1)	14(1)	-3(1)
C(7)	32(1)	28(1)	28(1)	-1(1)	14(1)	-8(1)
C(8)	24(1)	25(1)	23(1)	-3(1)	11(1)	-3(1)
C(9)	25(1)	30(1)	26(1)	0(1)	14(1)	-2(1)
C(10)	36(1)	81(2)	32(1)	2(1)	-1(1)	3(1)
C(11)	23(1)	26(1)	23(1)	-1(1)	12(1)	-2(1)
C(12)	27(1)	30(1)	34(1)	-1(1)	18(1)	-8(1)
C(13)	36(1)	29(1)	53(1)	6(1)	22(1)	1(1)
C(14)	30(1)	34(1)	59(1)	2(1)	23(1)	1(1)
C(15)	55(1)	60(1)	43(1)	-17(1)	25(1)	-35(1)

Table 5. Bond lengths [Å] and angles [°] for C15 H25 N O5

		N(1)-C(1)-C(6)	101.02(10)
O(1)-C(3)	1.4325(19)	C(2)-C(1)-C(6)	113.82(11)
O(2)-C(9)	1.1919(19)	C(3)-C(2)-C(1)	112.02(13)
O(3)-C(9)	1.3398(17)	O(1)-C(3)-C(2)	107.11(12)
O(3)-C(10)	1.4459(19)	O(1)-C(3)-C(4)	111.29(14)
O(4)-C(11)	1.2194(17)	C(2)-C(3)-C(4)	110.73(12)
O(5)-C(11)	1.3395(16)	C(3)-C(4)-C(5)	111.81(12)
O(5)-C(12)	1.4766(16)	C(4)-C(5)-C(6)	113.20(13)
N(1)-C(11)	1.3512(16)	C(7)-C(6)-C(5)	115.50(12)
N(1)-C(8)	1.4580(16)	C(7)-C(6)-C(1)	102.70(11)
N(1)-C(1)	1.4791(16)	C(5)-C(6)-C(1)	114.18(12)
C(1)-C(2)	1.534(2)	C(6)-C(7)-C(8)	103.60(11)
C(1)-C(6)	1.5349(19)	N(1)-C(8)-C(9)	112.39(11)
C(2)-C(3)	1.5243(19)	N(1)-C(8)-C(7)	103.21(10)
C(3)-C(4)	1.527(2)	C(9)-C(8)-C(7)	110.89(11)
C(4)-C(5)	1.527(3)	O(2)-C(9)-O(3)	124.57(13)
C(5)-C(6)	1.534(2)	O(2)-C(9)-C(8)	125.94(13)
C(6)-C(7)	1.5306(19)	O(3)-C(9)-C(8)	109.38(12)
C(7)-C(8)	1.5419(18)	O(4)-C(11)-O(5)	126.51(11)
C(8)-C(9)	1.5171(17)	O(4)-C(11)-N(1)	122.64(12)
C(12)-C(15)	1.516(2)	O(5)-C(11)-N(1)	110.84(11)
C(12)-C(14)	1.518(2)	O(5)-C(12)-C(15)	101.41(11)
C(12)-C(13)	1.520(2)	O(5)-C(12)-C(14)	109.75(12)
		C(15)-C(12)-C(14)	111.32(15)
C(9)-O(3)-C(10)	116.20(14)	O(5)-C(12)-C(13)	111.19(12)
C(11)-O(5)-C(12)	122.14(10)	C(15)-C(12)-C(13)	111.49(15)
C(11)-N(1)-C(8)	120.23(10)	C(14)-C(12)-C(13)	111.30(13)
C(11)-N(1)-C(1)	126.64(11)		
C(8)-N(1)-C(1)	113.05(10)		
N(1)-C(1)-C(2)	111.36(11)		

Table 6. Torsion angles [°] for C15 H25 N O5.

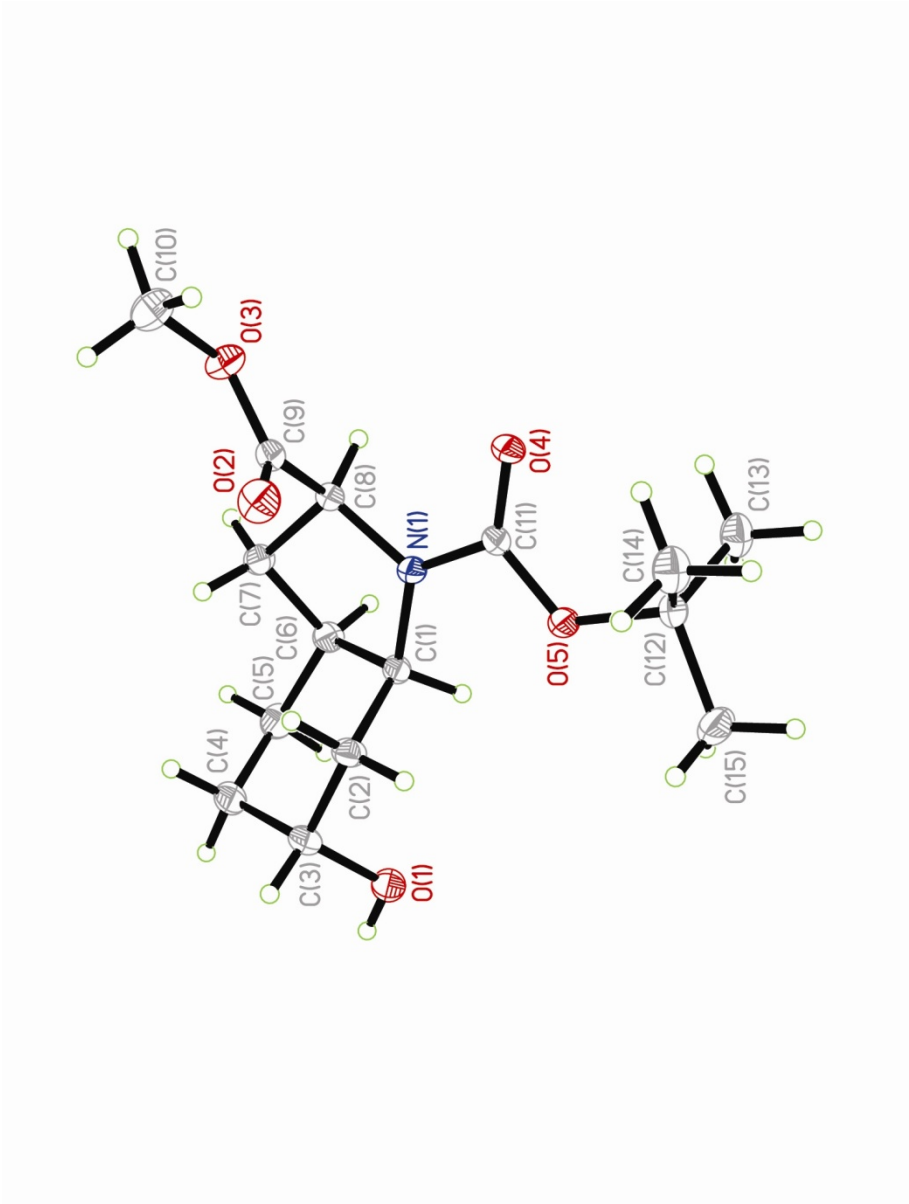
C(11)-N(1)-C(1)-C(2)	84.54(16)	C(11)-N(1)-C(8)-C(7)	178.71(12)
C(8)-N(1)-C(1)-C(2)	-98.56(12)	C(1)-N(1)-C(8)-C(7)	1.59(14)
C(11)-N(1)-C(1)-C(6)	-154.26(12)	C(6)-C(7)-C(8)-N(1)	-25.49(14)
C(8)-N(1)-C(1)-C(6)	22.64(13)	C(6)-C(7)-C(8)-C(9)	-146.05(12)
N(1)-C(1)-C(2)-C(3)	163.00(12)	C(10)-O(3)-C(9)-O(2)	-1.2(2)
C(6)-C(1)-C(2)-C(3)	49.60(15)	C(10)-O(3)-C(9)-C(8)	175.12(14)
C(1)-C(2)-C(3)-O(1)	65.05(16)	N(1)-C(8)-C(9)-O(2)	-26.55(19)
C(1)-C(2)-C(3)-C(4)	-56.47(16)	C(7)-C(8)-C(9)-O(2)	88.40(17)
O(1)-C(3)-C(4)-C(5)	-61.12(16)	N(1)-C(8)-C(9)-O(3)	157.19(11)
C(2)-C(3)-C(4)-C(5)	57.91(17)	C(7)-C(8)-C(9)-O(3)	-87.85(14)
C(3)-C(4)-C(5)-C(6)	-52.09(17)	C(12)-O(5)-C(11)-O(4)	-5.9(2)
C(4)-C(5)-C(6)-C(7)	-73.87(17)	C(12)-O(5)-C(11)-N(1)	174.33(11)
C(4)-C(5)-C(6)-C(1)	44.89(17)	C(8)-N(1)-C(11)-O(4)	-1.98(19)
N(1)-C(1)-C(6)-C(7)	-37.35(13)	C(1)-N(1)-C(11)-O(4)	174.71(13)
C(2)-C(1)-C(6)-C(7)	82.09(14)	C(8)-N(1)-C(11)-O(5)	177.82(11)
N(1)-C(1)-C(6)-C(5)	-163.15(12)	C(1)-N(1)-C(11)-O(5)	-5.49(18)
C(2)-C(1)-C(6)-C(5)	-43.71(16)	C(11)-O(5)-C(12)-C(15)	178.98(15)
C(5)-C(6)-C(7)-C(8)	164.47(13)	C(11)-O(5)-C(12)-C(14)	-63.22(17)
C(1)-C(6)-C(7)-C(8)	39.53(14)	C(11)-O(5)-C(12)-C(13)	60.35(18)
C(11)-N(1)-C(8)-C(9)	-61.76(15)		
C(1)-N(1)-C(8)-C(9)	121.13(12)		

Table 7. Bond lengths [Å] and angles [°] related to the hydrogen bonding for C15 H25 N O5.

D-H	..A	d(D-H)	d(H..A)	d(D..A)	<DHA
O(1)-H(1)	O(4)#1	0.84	1.97	2.7994(14)	167.6

Symmetry transformations used to generate equivalent atoms:

#1 x,y,z+1



ORTEP view of the C15 H25 N O5 compound with the numbering scheme adopted. Ellipsoids drawn at 30% probability level. Hydrogen atoms are represented by sphere of arbitrary size.

REFERENCES

Flack, H.D. (1983). Acta Cryst. A39, 876-881.

Flack, H.D. and Schwarzenbach, D. (1988). Acta Cryst. A44, 499-506.

SAINT (2006) Release 7.34A; Integration Software for Single Crystal Data. Bruker AXS Inc., Madison, WI 53719-1173.

Sheldrick, G.M. (2007). SADABS, Bruker Area Detector Absorption Corrections. Bruker AXS Inc., Madison, WI 53719-1173.

Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.

SHELXTL (2001) version 6.12; Bruker Analytical X-ray Systems Inc., Madison, WI 53719-1173.

APEX2 (2008) ; Bruker Molecular Analysis Research Tool. Bruker AXS Inc., Madison, WI 53719-1173.

Spek, A.L. (2008). PLATON, A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands.

Maris, T. (2004). UdMX, University of Montréal, Montréal, QC, Canada.

XPREP (2008) Version 2008/2; X-ray data Preparation and Reciprocal space Exploration Program. Bruker AXS Inc., Madison, WI 53719-1173.

Université de Montréal

CRYSTAL AND MOLECULAR STRUCTURE OF

C33 H43 N O9 COMPOUND (bent112)

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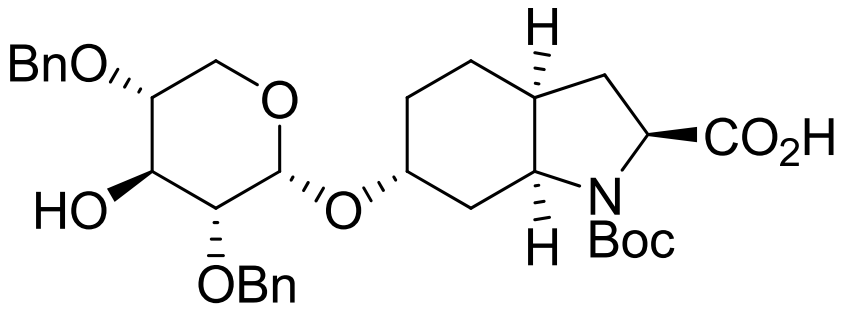


Structure solved and refined in the laboratory of X-ray diffraction Université de Montréal by Benoît Deschênes Simard.

Table 1. Crystal data and structure refinement for C33 H43 N O9.

Identification code	bent112
Empirical formula	C33 H43 N O9
Formula weight	597.68
Temperature	150K
Wavelength	1.54178 Å
Crystal system	Orthorhombic
Space group	P212121
Unit cell dimensions	a = 10.4333(5) Å α = 90°
	b = 12.4407(5) Å β = 90°
	c = 23.9905(10) Å γ = 90°
Volume	3113.9(2)Å^3
Z	4
Density (calculated)	1.275 g/cm^3
Absorption coefficient	0.758 mm^{-1}
F(000)	1280
Crystal size	0.22 x 0.15 x 0.14 mm
Theta range for data collection	3.68 to 72.64°
Index ranges	$-12 \leq h \leq 11$, $-15 \leq k \leq 15$, $-29 \leq \ell \leq 29$
Reflections collected	40744
Independent reflections	6091 [R_{int} = 0.044]
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.87 and 0.85
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6091 / 4 / 449
Goodness-of-fit on F^2	0.967
Final R indices [I>2sigma(I)]	R_1 = 0.0352, wR_2 = 0.0868
R indices (all data)	R_1 = 0.0404, wR_2 = 0.0890
Absolute structure parameter	-0.10(12)
Extinction coefficient	0.00326(19)
Largest diff. peak and hole	0.136 and -0.186 e/Å^3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for C33 H43 N O9.
U_{eq} is defined as one third of the trace of the orthogonalized Uij tensor.

	Occ.	x	y	z	U_{eq}
O(1)	1	4977(1)	-82(1)	566(1)	37(1)
O(2)	1	4361(1)	-5982(1)	210(1)	43(1)
O(3)	1	3168(1)	-4514(1)	54(1)	35(1)
O(4)	1	5309(1)	-2561(1)	-946(1)	48(1)
O(5)	1	5496(1)	-4379(1)	-822(1)	38(1)
O(6)	1	6259(1)	946(1)	-263(1)	36(1)
O(7)	1	7752(1)	2389(1)	412(1)	46(1)
O(9)	1	4052(1)	1522(1)	882(1)	50(1)
N(1)	1	5406(1)	-3312(1)	-84(1)	33(1)
C(1)	1	5414(2)	-2288(1)	222(1)	33(1)
C(2)	1	4093(2)	-1734(1)	206(1)	37(1)
C(3)	1	3978(2)	-876(1)	653(1)	39(1)
C(4)	1	4168(2)	-1370(1)	1223(1)	44(1)
C(5)	1	5504(2)	-1865(1)	1276(1)	43(1)
C(6)	1	5841(2)	-2646(1)	808(1)	37(1)
C(7)	1	5235(2)	-3771(1)	862(1)	38(1)
C(8)	1	5370(2)	-4258(1)	279(1)	34(1)
C(9)	1	4260(2)	-5015(1)	167(1)	33(1)
C(10)	1	5387(2)	-3347(1)	-648(1)	36(1)
C(11)	1	5086(2)	-4682(1)	-1391(1)	41(1)
C(12)	1	3677(2)	-4385(2)	-1454(1)	52(1)
C(13)	1	5259(2)	-5890(2)	-1384(1)	56(1)
C(14)	1	5901(2)	-4169(2)	-1836(1)	61(1)
C(15)	1	4562(2)	948(1)	419(1)	40(1)
C(16)	1	5709(2)	1556(1)	190(1)	37(1)
C(17)	1	6739(2)	1745(1)	631(1)	38(1)
C(20)	1	6369(2)	1546(1)	-772(1)	43(1)
C(21)	1	6816(2)	824(1)	-1238(1)	40(1)
C(22)	1	6604(2)	-275(1)	-1246(1)	40(1)
C(23)	1	6997(2)	-890(2)	-1696(1)	46(1)
C(24)	1	7604(2)	-423(2)	-2143(1)	55(1)
C(25)	1	7810(2)	681(2)	-2140(1)	65(1)
C(26)	1	7417(2)	1297(2)	-1693(1)	55(1)
O(8A)	0.807(4)	6993(5)	2280(4)	1596(3)	50(1)
C(18A)	0.807(4)	6131(9)	2298(11)	1131(3)	44(1)
C(19A)	0.807(4)	4972(13)	1664(17)	1319(5)	52(1)
C(27A)	0.807(4)	7977(2)	3074(2)	1625(1)	59(1)
C(28A)	0.807(4)	7533(2)	4171(1)	1812(1)	41(1)
C(29A)	0.807(4)	8306(2)	5051(2)	1691(1)	61(1)
C(30A)	0.807(4)	8005(3)	6057(2)	1907(2)	74(2)
C(31A)	0.807(4)	6931(4)	6183(2)	2243(2)	72(2)
C(32A)	0.807(4)	6158(3)	5303(2)	2364(1)	57(1)
C(33A)	0.807(4)	6459(2)	4298(2)	2148(1)	44(1)
O(8B)	0.193(4)	6850(20)	2249(18)	1669(11)	67(7)
C(18B)	0.193(4)	6180(40)	2290(50)	1146(10)	44(1)
C(19B)	0.193(4)	4990(50)	1680(70)	1310(20)	52(1)
C(27B)	0.193(4)	6446(13)	3039(8)	2032(5)	60(4)
C(28B)	0.193(4)	7000(9)	4160(6)	1925(5)	34(3)
C(29B)	0.193(4)	7897(8)	4325(7)	1505(5)	57(3)
C(30B)	0.193(4)	8438(9)	5335(9)	1430(5)	80(6)
C(31B)	0.193(4)	8082(14)	6182(6)	1775(7)	104(9)
C(32B)	0.193(4)	7186(16)	6018(7)	2195(6)	65(6)
C(33B)	0.193(4)	6645(12)	5007(9)	2270(4)	59(4)

Table 3. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for C33 H43 N O9.

	Occ.	x	y	z	U_{eq}
H(3)	1	2559	-4955	70	53
H(7)	1	8211	2014	201	68
H(1)	1	6075	-1799	58	39
H(2A)	1	3964	-1402	-165	44
H(2B)	1	3413	-2280	261	44
H(3A)	1	3116	-526	632	46
H(4A)	1	3512	-1932	1285	53
H(4B)	1	4056	-810	1512	53
H(5A)	1	6147	-1279	1282	52
H(5B)	1	5560	-2249	1637	52
H(6)	1	6793	-2735	803	44
H(7A)	1	5697	-4209	1142	46
H(7B)	1	4322	-3718	971	46
H(8)	1	6198	-4658	251	41
H(12A)	1	3189	-4684	-1142	78
H(12B)	1	3348	-4678	-1805	78
H(12C)	1	3589	-3601	-1456	78
H(13A)	1	6161	-6062	-1313	84
H(13B)	1	5003	-6188	-1746	84
H(13C)	1	4725	-6202	-1090	84
H(14A)	1	5789	-3387	-1824	91
H(14B)	1	5642	-4440	-2202	91
H(14C)	1	6804	-4346	-1770	91
H(15)	1	3891	890	123	48
H(16)	1	5413	2267	44	45
H(17)	1	7096	1036	752	46
H(20A)	1	6987	2141	-721	51
H(20B)	1	5527	1861	-870	51
H(22)	1	6186	-610	-940	48
H(23)	1	6845	-1643	-1696	55
H(24)	1	7880	-848	-2449	66
H(25)	1	8224	1014	-2448	77
H(26)	1	7557	2052	-1697	67
H(18A)	0.807(4)	5886	3053	1037	53
H(19A)	0.807(4)	5253	950	1453	63
H(19B)	0.807(4)	4559	2043	1634	63
H(27A)	0.807(4)	8375	3143	1252	70
H(27B)	0.807(4)	8647	2820	1885	70
H(29A)	0.807(4)	9041	4965	1461	73
H(30A)	0.807(4)	8534	6658	1824	89
H(31A)	0.807(4)	6725	6870	2390	87
H(32A)	0.807(4)	5423	5390	2593	68
H(33A)	0.807(4)	5930	3696	2230	52
H(18B)	0.193(4)	5956	3051	1056	53
H(19C)	0.193(4)	5246	968	1455	63
H(19D)	0.193(4)	4571	2072	1622	63
H(27C)	0.193(4)	6676	2818	2415	73
H(27D)	0.193(4)	5500	3084	2012	73
H(29B)	0.193(4)	8140	3746	1269	69
H(30B)	0.193(4)	9051	5448	1143	96
H(31B)	0.193(4)	8452	6873	1724	124
H(32B)	0.193(4)	6943	6596	2431	78
H(33B)	0.193(4)	6032	4895	2557	71

Table 4. Anisotropic parameters ($Å^2$ x 10^3) for C33 H43 N O9.
The anisotropic displacement factor exponent takes the form:

$$-2\pi^2 [h^2 a^{*2} U_{11} + ... + 2 h k a^* b^* U_{12}]$$

	U11	U22	U33	U23	U13	U12
O(1)	33(1)	27(1)	50(1)	0(1)	4(1)	2(1)
O(2)	44(1)	27(1)	58(1)	3(1)	-3(1)	2(1)
O(3)	32(1)	26(1)	47(1)	2(1)	1(1)	-2(1)
O(4)	71(1)	32(1)	40(1)	3(1)	8(1)	-7(1)
O(5)	42(1)	32(1)	40(1)	-5(1)	1(1)	2(1)
O(6)	40(1)	29(1)	39(1)	5(1)	0(1)	6(1)
O(7)	45(1)	33(1)	59(1)	-6(1)	3(1)	-5(1)
O(9)	43(1)	40(1)	67(1)	-8(1)	13(1)	10(1)
N(1)	38(1)	27(1)	35(1)	0(1)	5(1)	-4(1)
C(1)	33(1)	27(1)	38(1)	-2(1)	4(1)	-6(1)
C(2)	35(1)	31(1)	44(1)	0(1)	-2(1)	-2(1)
C(3)	33(1)	32(1)	51(1)	-4(1)	7(1)	0(1)
C(4)	50(1)	36(1)	46(1)	-7(1)	13(1)	-9(1)
C(5)	55(1)	36(1)	39(1)	0(1)	-3(1)	-10(1)
C(6)	35(1)	35(1)	41(1)	0(1)	-3(1)	-3(1)
C(7)	42(1)	35(1)	37(1)	3(1)	-3(1)	-2(1)
C(8)	31(1)	30(1)	42(1)	2(1)	0(1)	1(1)
C(9)	38(1)	28(1)	34(1)	1(1)	3(1)	2(1)
C(10)	35(1)	32(1)	41(1)	-2(1)	6(1)	-3(1)
C(11)	45(1)	37(1)	40(1)	-7(1)	3(1)	-5(1)
C(12)	52(1)	48(1)	56(1)	2(1)	-7(1)	-5(1)
C(13)	71(1)	41(1)	56(1)	-13(1)	-2(1)	4(1)
C(14)	70(1)	65(1)	47(1)	-14(1)	18(1)	-18(1)
C(15)	37(1)	32(1)	51(1)	-3(1)	2(1)	8(1)
C(16)	41(1)	24(1)	47(1)	1(1)	2(1)	8(1)
C(17)	41(1)	25(1)	48(1)	-2(1)	-1(1)	3(1)
C(20)	51(1)	33(1)	45(1)	10(1)	-5(1)	-1(1)
C(21)	39(1)	39(1)	41(1)	7(1)	-9(1)	-5(1)
C(22)	42(1)	38(1)	41(1)	8(1)	-6(1)	-3(1)
C(23)	51(1)	39(1)	47(1)	1(1)	-10(1)	-5(1)
C(24)	65(1)	56(1)	42(1)	-4(1)	-2(1)	-8(1)
C(25)	89(2)	59(1)	46(1)	2(1)	10(1)	-24(1)
C(26)	73(1)	43(1)	50(1)	5(1)	-1(1)	-20(1)
O(8A)	66(2)	38(2)	47(2)	-9(1)	-4(1)	16(2)
C(18A)	52(1)	28(1)	53(1)	-5(1)	-2(1)	9(1)
C(19A)	63(1)	43(1)	51(1)	-11(1)	11(1)	9(1)
C(27A)	47(2)	64(2)	65(2)	-29(1)	-4(1)	7(1)
C(28A)	40(2)	47(2)	35(2)	-4(1)	-3(1)	-4(1)
C(29A)	54(2)	67(2)	60(2)	13(2)	-13(2)	-10(2)
C(30A)	84(4)	45(2)	94(3)	16(2)	-46(2)	-21(2)
C(31A)	111(4)	39(2)	67(3)	-8(2)	-41(2)	15(2)
C(32A)	74(3)	50(2)	45(2)	-11(1)	-12(2)	19(2)
C(33A)	52(2)	40(1)	38(1)	-6(1)	1(1)	3(1)
O(8B)	93(12)	64(10)	43(8)	-5(6)	-14(8)	-43(8)
C(18B)	52(1)	28(1)	53(1)	-5(1)	-2(1)	9(1)
C(19B)	63(1)	43(1)	51(1)	-11(1)	11(1)	9(1)
C(27B)	87(9)	39(6)	56(7)	4(5)	17(6)	-21(5)
C(28B)	26(7)	29(5)	47(7)	1(4)	5(5)	-12(4)
C(29B)	32(6)	66(8)	75(9)	14(7)	-4(6)	3(5)
C(30B)	59(9)	61(9)	119(15)	31(10)	-51(9)	-3(6)
C(31B)	110(20)	71(14)	126(19)	29(14)	-57(14)	4(12)
C(32B)	68(10)	30(7)	97(15)	2(8)	-48(10)	1(8)
C(33B)	59(10)	83(13)	35(6)	7(7)	-3(6)	2(8)

Table 5. Bond lengths [Å] and angles [°] for C33 H43 N O9

		C(29b)-C(30b)	1.39
O(1)-C(15)	1.3973(18)	C(30b)-C(31b)	1.39
O(1)-C(3)	1.4517(19)	C(31b)-C(32b)	1.39
O(2)-C(9)	1.2116(18)	C(32b)-C(33b)	1.39
O(3)-C(9)	1.3279(19)		
O(4)-C(10)	1.2139(19)	C(15)-O(1)-C(3)	115.98(12)
O(5)-C(10)	1.3559(18)	C(10)-O(5)-C(11)	120.18(13)
O(5)-C(11)	1.479(2)	C(20)-O(6)-C(16)	113.51(11)
O(6)-C(20)	1.4355(19)	C(15)-O(9)-C(19A)	112.5(9)
O(6)-C(16)	1.4436(19)	C(15)-O(9)-C(19B)	112(4)
O(7)-C(17)	1.426(2)	C(19A)-O(9)-C(19B)	1(3)
O(9)-C(15)	1.423(2)	C(10)-N(1)-C(8)	124.59(13)
O(9)-C(19a)	1.432(5)	C(10)-N(1)-C(1)	121.76(13)
O(9)-C(19b)	1.432(17)	C(8)-N(1)-C(1)	113.61(12)
N(1)-C(10)	1.353(2)	N(1)-C(1)-C(6)	101.94(12)
N(1)-C(8)	1.4639(18)	N(1)-C(1)-C(2)	111.72(12)
N(1)-C(1)	1.4692(18)	C(6)-C(1)-C(2)	114.22(13)
C(1)-C(6)	1.540(2)	C(3)-C(2)-C(1)	111.56(13)
C(1)-C(2)	1.542(2)	O(1)-C(3)-C(4)	108.23(14)
C(2)-C(3)	1.519(2)	O(1)-C(3)-C(2)	108.60(13)
C(3)-C(4)	1.511(2)	C(4)-C(3)-C(2)	110.09(13)
C(4)-C(5)	1.529(3)	C(3)-C(4)-C(5)	111.03(14)
C(5)-C(6)	1.526(2)	C(6)-C(5)-C(4)	113.90(15)
C(6)-C(7)	1.541(2)	C(5)-C(6)-C(1)	114.94(13)
C(7)-C(8)	1.531(2)	C(5)-C(6)-C(7)	114.95(14)
C(8)-C(9)	1.516(2)	C(1)-C(6)-C(7)	102.71(12)
C(11)-C(14)	1.506(3)	C(8)-C(7)-C(6)	104.19(13)
C(11)-C(13)	1.513(2)	N(1)-C(8)-C(9)	114.49(13)
C(11)-C(12)	1.523(3)	N(1)-C(8)-C(7)	103.12(12)
C(15)-C(16)	1.519(2)	C(9)-C(8)-C(7)	109.71(13)
C(16)-C(17)	1.528(2)	O(2)-C(9)-O(3)	123.92(15)
C(17)-C(18a)	1.521(5)	O(2)-C(9)-C(8)	122.39(15)
C(17)-C(18b)	1.524(16)	O(3)-C(9)-C(8)	113.56(12)
C(20)-C(21)	1.507(2)	O(4)-C(10)-N(1)	124.36(14)
C(21)-C(22)	1.385(2)	O(4)-C(10)-O(5)	125.87(15)
C(21)-C(26)	1.391(2)	N(1)-C(10)-O(5)	109.75(14)
C(22)-C(23)	1.385(3)	O(5)-C(11)-C(14)	112.45(14)
C(23)-C(24)	1.375(3)	O(5)-C(11)-C(13)	102.06(14)
C(24)-C(25)	1.390(3)	C(14)-C(11)-C(13)	111.18(17)
C(25)-C(26)	1.380(3)	O(5)-C(11)-C(12)	107.99(14)
O(8a)-C(27a)	1.426(6)	C(14)-C(11)-C(12)	111.80(17)
O(8a)-C(18a)	1.432(5)	C(13)-C(11)-C(12)	110.93(16)
C(18a)-C(19a)	1.512(5)	O(1)-C(15)-O(9)	112.33(14)
C(27a)-C(28a)	1.510(3)	O(1)-C(15)-C(16)	107.68(12)
C(28a)-C(29a)	1.39	O(9)-C(15)-C(16)	109.09(13)
C(28a)-C(33a)	1.39	O(6)-C(16)-C(15)	108.92(12)
C(29a)-C(30a)	1.39	O(6)-C(16)-C(17)	108.89(12)
C(30a)-C(31a)	1.39	C(15)-C(16)-C(17)	112.30(14)
C(31a)-C(32a)	1.39	O(7)-C(17)-C(18A)	110.2(6)
C(32a)-C(33a)	1.39	O(7)-C(17)-C(18B)	109(2)
O(8b)-C(27b)	1.38(3)	C(18A)-C(17)-C(18B)	2.4(15)
O(8b)-C(18b)	1.436(17)	O(7)-C(17)-C(16)	110.61(14)
C(18b)-C(19b)	1.512(17)	C(18A)-C(17)-C(16)	108.9(3)
C(27b)-C(28b)	1.532(11)	C(18B)-C(17)-C(16)	111.2(13)
C(28b)-C(29b)	1.39	O(6)-C(20)-C(21)	110.19(13)
C(28b)-C(33b)	1.39	C(22)-C(21)-C(26)	118.61(17)

C(22)-C(21)-C(20)	123.32(16)	C(32A)-C(31A)-C(30A)	120
C(26)-C(21)-C(20)	118.00(16)	C(31A)-C(32A)-C(33A)	120
C(21)-C(22)-C(23)	120.61(17)	C(32A)-C(33A)-C(28A)	120
C(24)-C(23)-C(22)	120.68(18)	C(27B)-O(8B)-C(18B)	112.2(19)
C(23)-C(24)-C(25)	119.05(19)	O(8B)-C(18B)-C(19B)	99(2)
		O(8B)-C(18B)-C(17)	120(2)
C(26)-C(25)-C(24)	120.43(19)	C(19B)-C(18B)-C(17)	108(5)
C(25)-C(26)-C(21)	120.61(18)	O(9)-C(19B)-C(18B)	116(4)
C(27A)-O(8A)-C(18A)	118.6(7)	O(8B)-C(27B)-C(28B)	115.4(14)
O(8A)-C(18A)-C(19A)	105.3(6)	C(29B)-C(28B)-C(33B)	120
O(8A)-C(18A)-C(17)	110.2(5)	C(29B)-C(28B)-C(27B)	120.6(9)
C(19A)-C(18A)-C(17)	109.4(11)	C(33B)-C(28B)-C(27B)	119.3(9)
O(9)-C(19A)-C(18A)	112.5(9)	C(28B)-C(29B)-C(30B)	120
O(8A)-C(27A)-C(28A)	114.9(3)	C(31B)-C(30B)-C(29B)	120
C(29A)-C(28A)-C(33A)	120	C(30B)-C(31B)-C(32B)	120
C(29A)-C(28A)-C(27A)	118.2(2)	C(33B)-C(32B)-C(31B)	120
C(33A)-C(28A)-C(27A)	121.4(2)	C(32B)-C(33B)-C(28B)	120
C(30A)-C(29A)-C(28A)	120		
C(29A)-C(30A)-C(31A)	120		

Table 6. Torsion angles [°] for C33 H43 N O9.

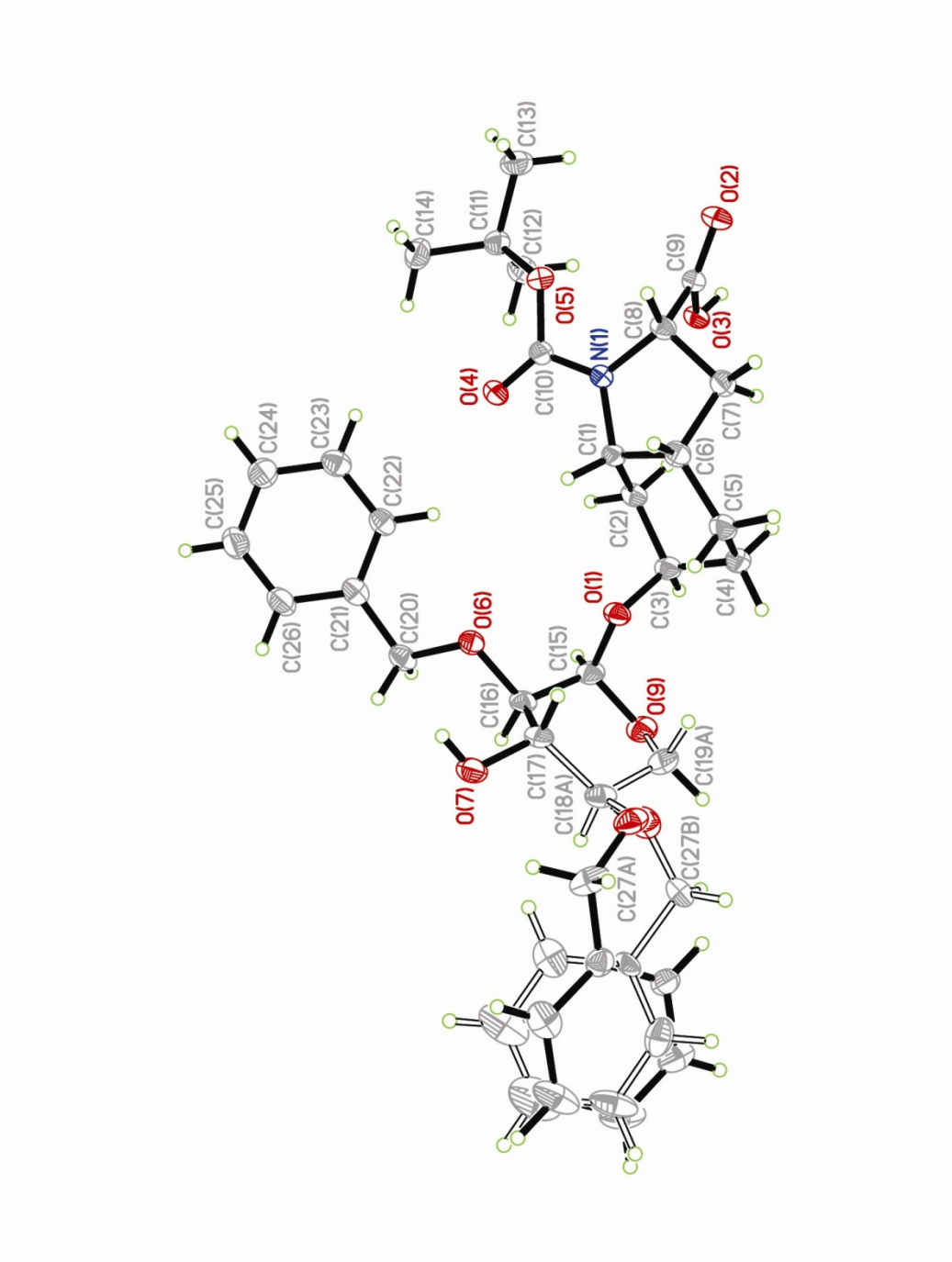
C(10)-N(1)-C(1)-C(6)	-163.69(14)	C(16)-O(6)-C(20)-C(21)	-173.99(13)
C(8)-N(1)-C(1)-C(6)	18.60(16)	O(6)-C(20)-C(21)-C(22)	26.2(2)
C(10)-N(1)-C(1)-C(2)	73.94(18)	O(6)-C(20)-C(21)-C(26)	-156.94(16)
C(8)-N(1)-C(1)-C(2)	-103.78(15)	C(26)-C(21)-C(22)-C(23)	0.7(3)
N(1)-C(1)-C(2)-C(3)	162.80(12)	C(20)-C(21)-C(22)-C(23)	177.55(16)
C(6)-C(1)-C(2)-C(3)	47.76(17)	C(21)-C(22)-C(23)-C(24)	0.1(3)
C(15)-O(1)-C(3)-C(4)	-126.13(14)	C(22)-C(23)-C(24)-C(25)	-0.6(3)
C(15)-O(1)-C(3)-C(2)	114.36(14)	C(23)-C(24)-C(25)-C(26)	0.4(4)
C(1)-C(2)-C(3)-O(1)	59.19(16)	C(24)-C(25)-C(26)-C(21)	0.4(4)
C(1)-C(2)-C(3)-C(4)	-59.15(17)	C(22)-C(21)-C(26)-C(25)	-0.9(3)
O(1)-C(3)-C(4)-C(5)	-57.19(17)	C(20)-C(21)-C(26)-C(25)	-177.9(2)
C(2)-C(3)-C(4)-C(5)	61.38(17)	C(27A)-O(8A)-C(18A)-C(19A)	-160.1(1)
C(3)-C(4)-C(5)-C(6)	-52.25(18)	C(27A)-O(8A)-C(18A)-C(17)	82(1)
C(4)-C(5)-C(6)-C(1)	40.90(19)	O(7)-C(17)-C(18A)-O(8A)	-70.9(1)
C(4)-C(5)-C(6)-C(7)	-78.04(18)	C(18B)-C(17)-C(18A)-O(8A)	-1(71)
N(1)-C(1)-C(6)-C(5)	-159.38(14)	C(16)-C(17)-C(18A)-O(8A)	167.6(7)
C(2)-C(1)-C(6)-C(5)	-38.73(18)	O(7)-C(17)-C(18A)-C(19A)	173.9(6)
N(1)-C(1)-C(6)-C(7)	-33.81(15)	C(18B)-C(17)-C(18A)-C(19A)	-116(72)
C(2)-C(1)-C(6)-C(7)	86.84(15)	C(16)-C(17)-C(18A)-C(19A)	52.4(8)
C(5)-C(6)-C(7)-C(8)	163.38(14)	C(15)-O(9)-C(19A)-C(18A)	60.9(15)
C(1)-C(6)-C(7)-C(8)	37.82(16)	C(19B)-O(9)-C(19A)-C(18A)	-13(10)
C(10)-N(1)-C(8)-C(9)	-53.7(2)	O(8A)-C(18A)-C(19A)-O(9)	-174.9(12)
C(1)-N(1)-C(8)-C(9)	123.89(14)	C(17)-C(18A)-C(19A)-O(9)	-56.5(16)
C(10)-N(1)-C(8)-C(7)	-172.87(15)	C(18A)-O(8A)-C(27A)-C(28A)	77.2(6)
C(1)-N(1)-C(8)-C(7)	4.77(17)	O(8A)-C(27A)-C(28A)-C(29A)	-160.6(3)
C(6)-C(7)-C(8)-N(1)	-26.33(16)	O(8A)-C(27A)-C(28A)-C(33A)	26.5(4)
C(6)-C(7)-C(8)-C(9)	-148.72(13)	C(33A)-C(28A)-C(29A)-C(30A)	0
N(1)-C(8)-C(9)-O(2)	144.37(15)	C(27A)-C(28A)-C(29A)-C(30A)	-173.1(2)
C(7)-C(8)-C(9)-O(2)	-100.29(18)	C(28A)-C(29A)-C(30A)-C(31A)	0
N(1)-C(8)-C(9)-O(3)	-39.60(19)	C(29A)-C(30A)-C(31A)-C(32A)	0
C(7)-C(8)-C(9)-O(3)	75.74(16)	C(30A)-C(31A)-C(32A)-C(33A)	0
C(8)-N(1)-C(10)-O(4)	173.53(16)	C(31A)-C(32A)-C(33A)-C(28A)	0
C(1)-N(1)-C(10)-O(4)	-3.9(3)	C(29A)-C(28A)-C(33A)-C(32A)	0
C(8)-N(1)-C(10)-O(5)	-7.8(2)	C(27A)-C(28A)-C(33A)-C(32A)	172.8(2)
C(1)-N(1)-C(10)-O(5)	174.75(13)	C(27B)-O(8B)-C(18B)-C(19B)	-83(5)
C(11)-O(5)-C(10)-O(4)	-21.3(2)	C(27B)-O(8B)-C(18B)-C(17)	161(4)
C(11)-O(5)-C(10)-N(1)	160.02(14)	O(7)-C(17)-C(18B)-O(8B)	-77(5)
C(10)-O(5)-C(11)-C(14)	65.8(2)	C(18A)-C(17)-C(18B)-O(8B)	172(76)
C(10)-O(5)-C(11)-C(13)	-174.99(15)	C(16)-C(17)-C(18B)-O(8B)	161(4)
C(10)-O(5)-C(11)-C(12)	-58.03(18)	O(7)-C(17)-C(18B)-C(19B)	171(3)
C(3)-O(1)-C(15)-O(9)	75.66(17)	C(18A)-C(17)-C(18B)-C(19B)	60(71)
C(3)-O(1)-C(15)-C(16)	-164.21(13)	C(16)-C(17)-C(18B)-C(19B)	49(3)
C(19A)-O(9)-C(15)-O(1)	60.3(8)	C(15)-O(9)-C(19B)-C(18B)	59(7)
C(19B)-O(9)-C(15)-O(1)	62(3)	C(19A)-O(9)-C(19B)-C(18B)	166(10)
C(19A)-O(9)-C(15)-C(16)	-59.0(8)	O(8B)-C(18B)-C(19B)-O(9)	-179(6)
C(19B)-O(9)-C(15)-C(16)	-58(3)	C(17)-C(18B)-C(19B)-O(9)	-52(7)
C(20)-O(6)-C(16)-C(15)	125.27(14)	C(18B)-O(8B)-C(27B)-C(28B	-79(3)
C(20)-O(6)-C(16)-C(17)	-111.96(15)	O(8B)-C(27B)-C(28B)-C(29B)	-3.2(17)
O(1)-C(15)-C(16)-O(6)	55.25(16)	O(8B)-C(27B)-C(28B)-C(33B)	179.9(13)
O(9)-C(15)-C(16)-O(6)	177.41(13)	C(33B)-C(28B)-C(29B)-C(30B)	0
O(1)-C(15)-C(16)-C(17)	-65.45(16)	C(27B)-C(28B)-C(29B)-C(30B)	-176.9(1)
O(9)-C(15)-C(16)-C(17)	56.71(16)	C(28B)-C(29B)-C(30B)-C(31B)	0
O(6)-C(16)-C(17)-O(7)	63.83(16)	C(29B)-C(30B)-C(31B)-C(32B)	0
C(15)-C(16)-C(17)-O(7)	-175.46(12)	C(30B)-C(31B)-C(32B)-C(33B)	0
O(6)-C(16)-C(17)-C(18A)	-174.9(6)	C(31B)-C(32B)-C(33B)-C(28B)	0
C(15)-C(16)-C(17)-C(18A)	-54.2(6)	C(29B)-C(28B)-C(33B)-C(32B)	0
O(6)-C(16)-C(17)-C(18B)	-174(3)	C(27B)-C(28B)-C(33B)-C(32B)	176.9(1)
C(15)-C(16)-C(17)-C(18B)	-54(3)		

Table 7. Bond lengths [Å] and angles [°] related to the hydrogen bonding for C33 H43 N O9.

D-H	..A	d(D-H)	d(H..A)	d(D..A)	<DHA
O(7)-H(7)	O(2)#1	0.84	2.01	2.8488(16)	171.8
O(3)-H(3)	O(6)#2	0.84	1.89	2.7195(15)	168.4

Symmetry transformations used to generate equivalent atoms:

#1 x+1/2,-y-1/2,-z #2 x-1/2,-y-1/2,-z



ORTEP view of the C33 H43 N O9 compound with the numbering scheme adopted. Ellipsoids drawn at 30% probability level. Hydrogen atoms are represented by sphere of arbitrary size.

REFERENCES

Flack, H.D. (1983). Acta Cryst. A39, 876-881.

Flack, H.D. and Schwarzenbach, D. (1988). Acta Cryst. A44, 499-506.

SAINT (2006) Release 7.34A; Integration Software for Single Crystal Data. Bruker AXS Inc., Madison, WI 53719-1173.

Sheldrick, G.M. (1996). SADABS, Bruker Area Detector Absorption Corrections. Bruker AXS Inc., Madison, WI 53719-1173.

Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122.

SHELXTL (2001) version 6.12; Bruker Analytical X-ray Systems Inc., Madison, WI 53719-1173.

APEX2 (2008) ; Bruker Molecular Analysis Research Tool. Bruker AXS Inc., Madison, WI 53719-1173.

Spek, A.L. (2008). PLATON, A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands.

Maris, T. (2004). UdMX, University of Montréal, Montréal, QC, Canada.

XPREP (2008) Version 2008/2; X-ray data Preparation and Reciprocal space Exploration Program. Bruker AXS Inc., Madison, WI 53719-1173.