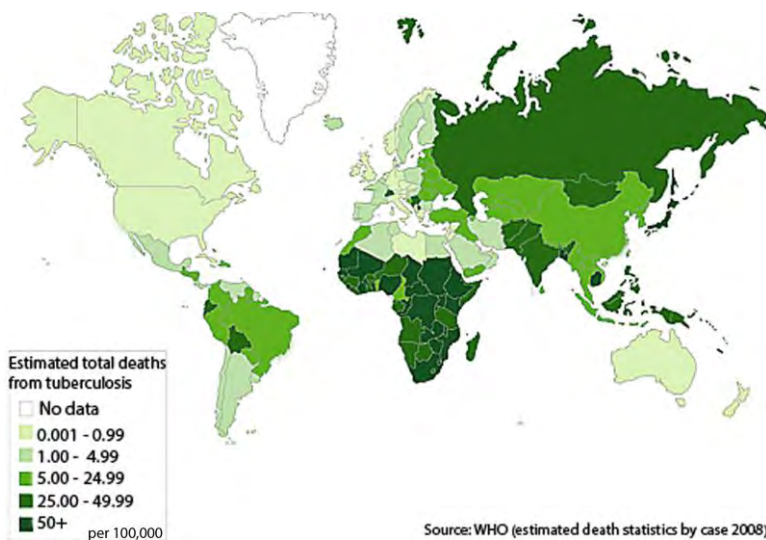


Synthesis of a novel class of antitubercular peptidomimetics based on β -aminoboronates

Alexey Gorovoy

Dissertation for the degree of Philosophiae Doctor

November 2012



Synthesis of a novel class of antitubercular peptidomimetics based on β -aminoboronates.

ACKNOWLEDGEMENTS

This thesis would not have been possible without several people who inspired, guided and supported me during the whole study.

First and foremost, I am grateful to my supervisor Professor Tore Lejon for his wisdom, sense of humor, optimism and firm belief in our ability to overtake any problem we met on the course of the investigations. He has always given me great freedom to follow independent work remaining available at any time I needed an advice.

I am deeply grateful to my friend, lab mate and co-worker Olga Gozhina for her impact to the project and lively and productive lab life.

I want to express my gratitude to Fred Leeson and Truls Ingebrigtsen for the proofreading of the thesis and helpful suggestions.

Magnus Engqvist has been a true friend. I can't overestimate his advices, his help in the lab and I am really grateful to Magnus for the introduction to the world of fishing.

I would like to show my gratitude to Professor John Sigurd Svendsen for useful discussions and support throughout the whole project.

It is an honor for me to thank Valentina Burkow Vollan for her expertise, wish and ability to help in almost any situation.

I am grateful to Annette Bayer, Rolf Carlson, Laima Grineviciute, David Guyader, Elizaveta Igumnova, Johan Isaksson, Jostein Johansen, Rasmus Karstad, Evaldas Kasiulis, Arnfinn Kvarsnes, Kinga Leszczak, Marianne Paulsen, Britt Paulsen, Wenche Stensen, Ivar Thomassen for making me feel that I was a part of the departmental team.

I owe my deepest gratitude to those introduced me to the organic chemistry: Konstantin A. Krasnov, Marina N. Yurova, and to all people I collaborated with Anton Liaimer, Victor Tetz, Steffi Munack, Victor Khrustalev, Jim Werngren, Sven Hoffner.

I want to thank the good friends that I have made in Tromso: Oleg and Natalia Sidorenkovy, Dmitrii Shcherbin, Inna Tolskaya, Roman and Natalia Kopusovy.

I also gratefully acknowledge Professor Kenneth Ruud and the Department of Chemistry for the financial support that I have received while working on this project as well as the support from The Norwegian Science Council (FORNY program).

Last but not least I would like to thank Nadya and Anya, as well as my parents, for their patience and love.

ABSTRACT

The unique properties of antimicrobial peptides have attracted the attention of chemists since the 1970's. This class of compounds provides additional tools in the treatment of infections and the work described in this thesis was therefore focused on the synthesis of short peptidomimetics on the basis of modified amino acids and evaluation of their antimicrobial activity.

The compounds of choice were β -aminoboronic acid derivatives with different substitution patterns and this work describes the development of a synthetic approach to the synthesis of this class of compounds. The skeleton of a β -amino acid was built using a step-wise approach utilizing the Matteson homologation. New chiral centers were obtained with high levels of diastereoselectivity provided by the use of chiral directors with particular configurations.

The variety of β -aminoboronates was provided not only by different substituents in positions α - and β - to boron, but also by the opposite configurations of these chiral centers. As their configuration was dependent on the chiral directors used, several attempts to obtain all possible stereoisomers were undertaken.

Derivatives of β -aminoboronic acid were coupled with different amino acids (both natural and non-natural) and the library of (~70) short peptidoids was characterized and tested on the set of microorganisms (*Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Candida albicans*). High activity of the synthesized compounds against *Mycobacterium tuberculosis* (H37Rv) induced additional investigations on a set of resistant strains of this pathogen. Lead compounds have shown activity against all resistant strains tested.

Investigation of the affinity of our compounds to secreted and internal chorismate mutase showed the absence of binding and led to the exclusion of this mechanism of inhibition from consideration.

Antifungal activity (including resistant strains) was detected in one of the molecules synthesized. The low levels of toxicity of the lead compounds are very encouraging with regard to the prospects for developing drugs based on these compounds.

LIST OF ABBREVIATIONS

^{13}C	carbon NMR
^{19}F	fluorine NMR
^1H	proton NMR
AMP	antimicrobial peptides
Boc	tert-butoxycarbonyl
COSY	correlation spectroscopy
de	diastereomeric excess
DICHD	1,2-dicyclohexyl-1,2-ethanediol
DMSO	dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
HOBt	hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
MDR	multidrug-resistant
MIC	minimum inhibitory concentration
MW	microwave
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
ppm	parts per million (NMR)
QSAR	quantitative structure–activity relationship
ROESY	rotating-frame nuclear Overhauser effect
SMI	Smittskyddsinstitutet (Swedish Institute for Infectious Disease Control)
THF	tetrahydrofuran
TLC	thin layer chromatography
TB	tuberculosis
WHO	World Health Organization
XDR	extremely drug-resistant

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	3
ABSTRACT.....	5
LIST OF ABBREVIATIONS.....	6
TABLE OF CONTENTS.....	7
1. INTRODUCTION.....	9
1.1 General considerations.....	9
1.1.1. Treatment of infections.....	9
1.1.2. Antimicrobial peptides.....	9
1.1.3. β -Amino acids.....	11
1.1.4. β -Aminoboronic acids.....	14
1.2. Chemical background.....	15
1.2.1. Structural features of boronic acids.....	15
1.2.2. Matteson homologation-alkylation.....	17
1.2.3. Synthesis of (+)- or (-)-pinanediol.....	21
AIMS OF THE PROJECT.....	23
References.....	24
2. RESULTS AND DISCUSSION.....	26
2.1. Strategy and methods.....	26
2.2. Synthesis of α - alkyl (or aryl-) substituted β -amino boronates.....	28
2.3. Synthesis of β -alkyl (or aryl-) substituted β -amino boronates.....	32
2.3.1. Synthesis of β -phenyl- β -aminoboronates.....	34
2.4. Synthesis of α,β -disubstituted β -amino boronates.....	39
2.5. Synthesis α,β -diamino- β -alkylsubstituted boronates.....	43
2.5.1. Towards the cyclisation of α,β -diaminoderivatives of boronic acid.....	49
2.6. Investigations of the synthesis of all possible configurations of α,β -dialkyl substituted β -aminoboronates.....	52
2.7. Measurement of diastereomeric ratio.....	56
2.8. Towards the synthesis of β -amino acids.....	61

2.9. Reduction of the azido group using the Staudinger reaction	66
References	71
3. MICROBIOLOGICAL INVESTIGATIONS.....	72
3.1. General outline.....	72
3.2. Results and discussions.....	73
3.2.1. Testing of α -substituted- β -aminoboronates.....	73
3.2.2. Testing of β -substituted- β -aminoboronates.....	77
3.2.3. Testing of α,β -disubstituted- β -aminoboronates	79
3.2.4. Testing of α,β -diaminosubstituted derivatives	81
3.2.5. Inhibition of resistant strains of <i>Mycobacteria tuberculosis</i>	82
3.2.6. Inhibition of resistant strains of <i>Candida albicans</i> (<i>Paper III</i>)	86
3.3. Experimental part.....	88
References	89
4. MECHANISTIC INVESTIGATION AND TOXICITY TESTING.....	91
4.1. Evaluation of chorismate mutase binding	91
4.2. Initial toxicity testing.....	91
4.3. Experimental part.....	93
References	95
5. CONCLUSIONS.....	96
6. SUMMARY OF SYNTHESIZED PEPTIDOMIMETICS	97
7. EXPERIMENTAL SECTION.....	98
Patent.....	175
Paper I.....	177
Paper II	189
Paper III.....	201
Paper IV	207

1. INTRODUCTION

1.1 General considerations

1.1.1. Treatment of infections

Humanity is in eternal confrontation with pathogenic microorganisms and most of the time an average person can control their influence and remain healthy. Sometimes external factors like hypothermia, wounds, poisoning etc. break the relatively stable internal equilibrium and the person becomes infected. At this point the immune system starts to work in emergency mode and sometimes is in need of help. In the course of centuries this help evolved from ritual tambourine dancing around a sick person to the immunomodulation and pharmacotherapy. As a result, average life expectancy has grown from 30-40 years at the beginning of the 20th century to 67 at present. The discovery in 1928 and mass production in 1940`s of penicillin ushered in the era of antibiotics. Unfortunately, initial optimism was very soon reduced significantly by appearance of drug-resistant bacteria and workers in the field of drug discovery were presented with a new challenge – the search for new types and classes of antibiotics able to withstand the natural mutability of pathogenic microorganisms.

1.1.2. Antimicrobial peptides

Today several classes of antibiotics are on the active list of practicing physicians, namely: β -lactams, tetracyclines, macrolides, aminoglycosides, laevomycetins, glycopeptides, lincosamides,

quinolones and antifungal antibiotics. Antimicrobial peptides (AMP) were discovered in the '70s¹ and their role in various types of immune response such as inflammation, wound repair and regulation of the adaptive immune system is now well established.²⁻⁵

Antimicrobial peptides (AMP) have become an important class of antibacterial drugs and approximately 1000 active peptides have been described to date.⁶

The therapeutic potential of this class of compounds seems to be great, though there is disagreement over the extent of this potential, with estimates ranging from highly optimistic^{7,8} to moderate.⁹

The broad spectrum of antipathogenic activity, absence of the toxicity to the host cells, speed of action and absence of the mechanisms (or very rare examples) for the development of resistant bacterial strains¹⁰ are the significant advantages of AMP. At the same time, the natural origins of these compounds result in such drawbacks as the propensity to enzymatic hydrolysis.¹¹ This problem can be solved by chemical modification of the active compound making it less sensitive to the natural mechanisms of degradation.

One of the approaches investigated which pointed towards the development of less toxic, more stable, active and selective drugs was bioisosteric replacement of pharmacophoric groups.¹² *Bioisoster* is the compound where one atom (or a group of atoms) is replaced by another atom (or a group of atoms) with retention of biological activity.¹² It is necessary to distinguish between the terms *isoster* and *bioisoster*. The history of these concepts started in 1919 with the work of I. Langmuir.¹³ Langmuir proposed that compounds with the same number of atoms and the same total number of electrons have a similar distribution of electrons and similar physical properties.

Such compounds were named *isosteres*. The theory was developed by H.G. Grimm¹⁴, and later by H. Erlenmeyer¹⁵ who considered not only physical but also physiological properties of isosteres.

The term *bioisosteres* was introduced by H. Friedman in 1951.¹⁶ According to Friedman bioisosteres must satisfy the definition of isosteres, have the same type of biological activity and attack the same target in the biological system. This theory transformed later to the QSAR methodology.

This project was planned and conducted in the light of earlier investigations in the field of antimicrobial peptides started in 1995 at the University of Tromsø. Taking into account antimicrobial properties of bovine lactoferrin peptide derivatives¹⁷, a new concept was developed and some important conclusions were drawn concerning the active site of this peptide and the importance of certain substituents for keeping the high level of antimicrobial activity and selectivity. Synthetic peptides were then investigated in order to improve activity as well as to find a means to improve uptake and to avoid side effects e.g. enzymatic degradation. From these results it was clear that the smallest peptidomimetics still exhibiting desired effect were tripeptides with certain features, i.e. two bulky side-groups and two charged side-chains.¹⁸⁻²¹

In the present work it was decided to extend the range of short peptides and produce a library of derivatives with β -amino analogs of amino acids.

1.1.3. β -Amino acids

Amino acids can be considered as carboxylic acids in which one or several hydrogen atoms are substituted by amino-groups. They can be classified depending on the number of carboxyl- and

amino-groups, side-chain type or position of amino-group relative to the carboxyl moiety (Figure 1.1).

Living organisms use α -amino acids for the synthesis of compounds with both peptide and non-peptide nature (like nucleotides, thyroxin, choline, amines etc.). Modified α -amino acids (or unnatural amino acids) are extensively utilized in peptide research and in many drug compositions.²²

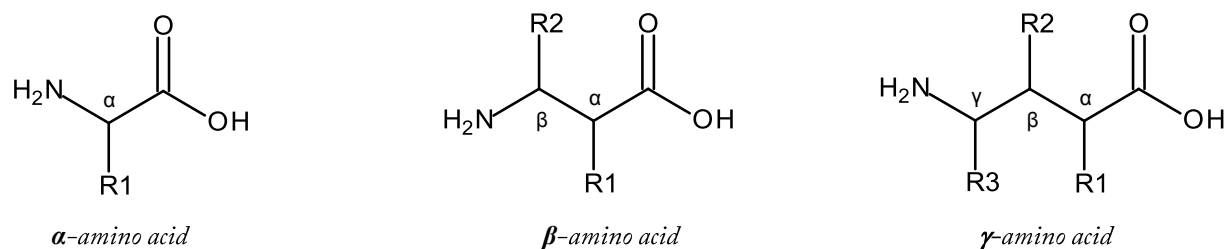


Figure 1.1 Classification of amino acids depending on amino-group position.

β -Amino acids and their analogs also attract the attention of organic, bioorganic and pharmaceutical chemists.²²

Some β -amino acids are considered as biologically and physiologically active compounds both in free form and as fragments in distinct natural products such as peptides, alkaloids and terpenoids.^{23,24} E. Juaristi in his preface to the first edition of *Enantioselective Synthesis of β -Amino Acids* (John Wiley & Sons: New York, 1997) writes: “ β -Amino acids, although less abundant than their α -analogs, are also present in peptides and in other natural products, and in free form they show interesting pharmacological effects. Furthermore, β -amino acids are

synthetic precursors of β -lactams, which are potentially biologically active and of current interest”.

The additional carbon atom in β -amino acids provides an opportunity for the synthesis of mono- (α - or β -), di-, tri- and even tetrasubstituted derivatives. These compounds can be synthesized in high yield and with excellent diastereoselectivity. The extra carbon in β -amino acids leads to the appearance of one more dihedral angle and hence higher flexibility (Figure 1.2).

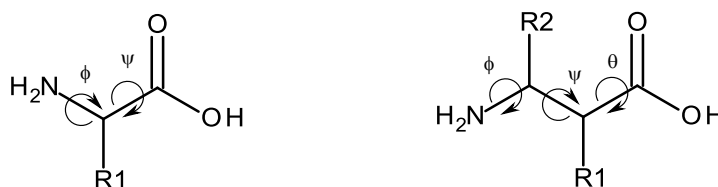


Figure 1.2 Dihedral angles in α - and β -amino acids.

This feature can result in less structured oligopeptides. However, there is a great deal of evidence that β -peptides can form various types of secondary or tertiary structure²⁵ (such as, β -turns and β -sheets), quaternary structures²⁶ and simulate properties of natural peptides showing more stability towards enzymatic hydrolysis.²³

β -Amino acids are important constituents of natural²⁴ and synthetic materials. There are a number of methods for the stereoselective synthesis of β -amino acids. These approaches include transition metal catalysis, organocatalysis and biocatalytic routes. Most of them are summarized and described in the book by E. Juaristi and V. Soloshonok²⁷ and several reviews.²⁸⁻³⁰

However, even though a number of different strategies have already been developed, there is still a considerable need for new stereoselective approaches to the synthesis of this class of compounds.

1.1.4. β -Aminoboronic acids

As referred to earlier, β -amino acids possess a greater ability to resist enzymatic hydrolysis and it is believed that this stability could be amplified by using isosteric substitution of the carboxy-group. There are other amino acids that can be considered as isosteres of natural amino acids. Amongst these are aminophosphonic acids³¹, aminosulphonic acids (taurin or aminoethanesulphonic acid is an example of naturally occurring β -aminosulphonic acid), aminoarsenic acids and aminoboronic acids. All of them find application in medicine and the latter class of compounds is actually the core of the present work. Boron-containing compounds do not have any significant toxicity³² (although some toxicity was reported for fat-soluble derivatives of boronic acids³³). They are air-stable and can be stored on shelf, though inert atmosphere is preferable.

There are few examples of naturally occurring boron containing compounds exhibiting biological activity, but boromycin and aplasmomycin are examples of naturally occurring macrocycles which do in fact exhibit antibiotic activity.³⁴ In addition, synthetic boron containing compounds have been shown to act as enzyme inhibitors³⁵, and bortezomib, an amino boronic acid containing compound, is used in cancer therapy.³⁶ However, to the best of our knowledge, peptidomimetics with amino boronic moieties have not been used before as antimicrobials.

1.2. Chemical background

1.2.1. Structural features of boronic acids

Boronic acids have a set of unique properties that mark them out from other types of boron-containing compounds. Due to the structural similarity of boron to carbon it is reasonable to consider β -aminoboronic acids as potentially useful chemical and biological analogs of β -amino acids.

Examining the structure and bonding in boronic acids one can see that the CBO_2 part is planar and similar to the carbonyl group whereas tetracoordinated boron becomes tetrahedral with angles close to conventional tetrahedral sp^3 -hybridized carbon atom (Figure 1.3). The C-B- and B-O-bonds lengths of are also quite close to the lengths of the C-C and C-O-bonds.

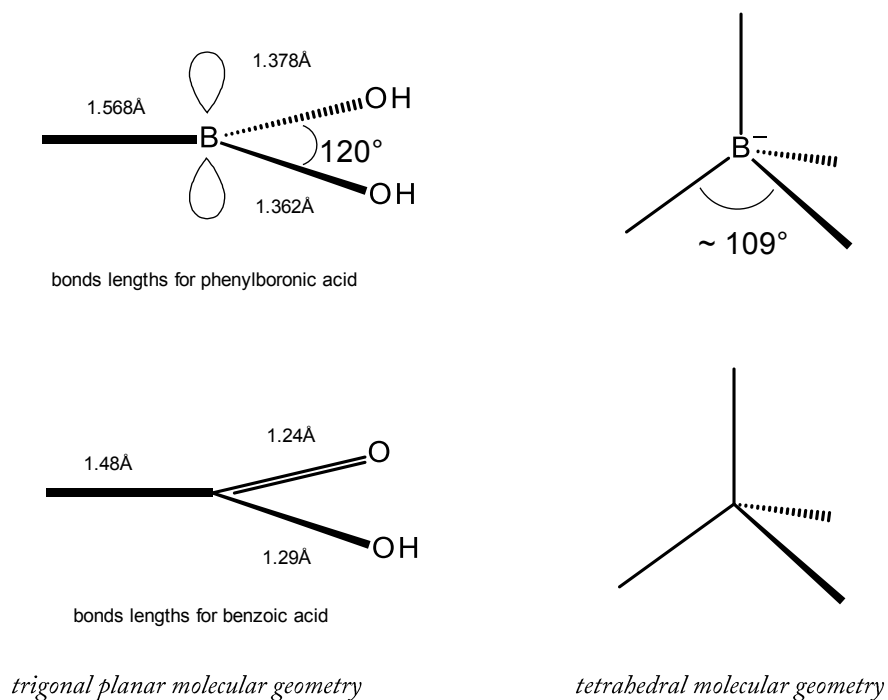
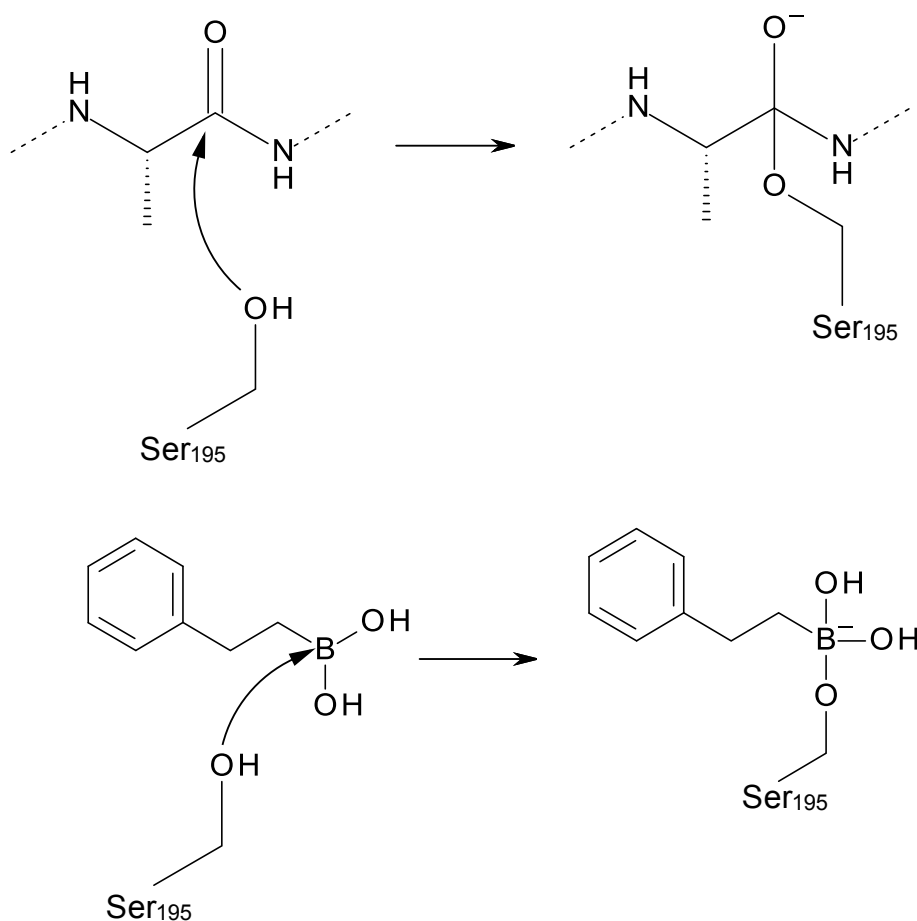


Figure 1.3 Comparison of molecular geometry of boron and carbon atom.

Boronic acids are very similar to the classic acids. Due to the weak Lewis acidity they remain uncharged and tricoordinated at physiological pH. Having a vacant orbital, they can react with various bases to complete the octet, giving rise to a tetrahedral product (Scheme 1.1).



Scheme 1.1 Comparison serine protease binding to amino acids and boronic acids.³⁸

This feature explains the fact that boronic acids act as potent inhibitors of serine proteases by mimicking the tetrahedral transition state of peptide-bond hydrolysis.^{37, 38}

It is essential that boronic acids can form cyclic anhydrides which are usually trimers (Figure1.4). Boroxines have the same chemical properties as boronic acids, but also influence

some physical constants and the stoichiometry of reactions. There is substantial evidence that substances currently used in therapy exist initially as cyclic structures.³⁶

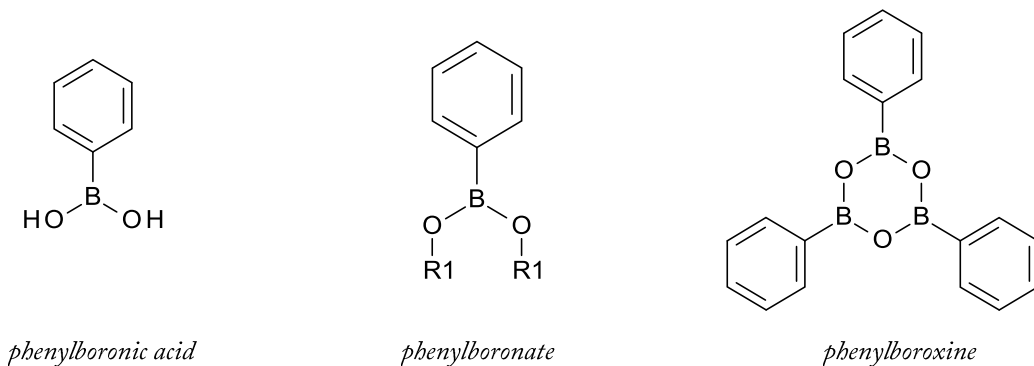


Figure 1. 4 Phenylboronic acid, phenylboronate and phenylboroxine.

1.2.2. Matteson homologation-alkylation.*

The strategy chosen (see Results and Discussion) for the stereoselective synthesis of target compounds was based mainly on the Matteson homologation. The reaction can be applied to boronic acids protected in a variety of ways, but in our case the use of pinanediol (Figure 1.5) as a protective group was chosen for several reasons. In theory, there are many variants of diol-protection of the boronic acid, but only a few of them give derivatives stable enough to work up and use in succeeding reactions. Pinanediol not only gives products that are stable towards acidic or basic hydrolysis, but also acts as chiral director, that drives the reaction to the *a priori* defined configuration of final compound.

* *Homologation or homologisation is generally accepted term for certain type of reactions that lead to transformation of the substrate to the next or previous member of homologous series.*

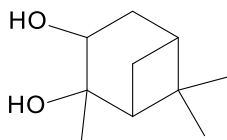


Figure 1.5 Pinanediol.

Matteson homologation is an efficient procedure for the synthesis of enantiomerically pure α -chloroalkylboronates.^{39,40} Subsequent nucleophilic substitution of the chlorine atom by C-, N-, S- or O-nucleophiles also proceeds with stereocontrol⁴¹ and there are a number of publications devoted to applications of chiral α -chloroalkylboronates in asymmetric synthesis.³⁹ Combining Matteson homologation and nucleophilic substitution thus appeared interesting as a method for the synthesis of variously substituted chiral derivatives of β -amino acids or β -amino boronic acid. Matteson homologation gives not only complete and fast transformation of substrate to the desired substituted α -haloboronate, but also proceeds with high stereoselectivity.

It is necessary to mention that different groups tolerate the conditions of Matteson homologation and the reaction was utilized in numerous stereoselective synthetic pathways leading to different classes of organic compounds such as: chiral alcohols, aminoalcohols, polyols, α -amino acids, amides, azidoderivatives etc.

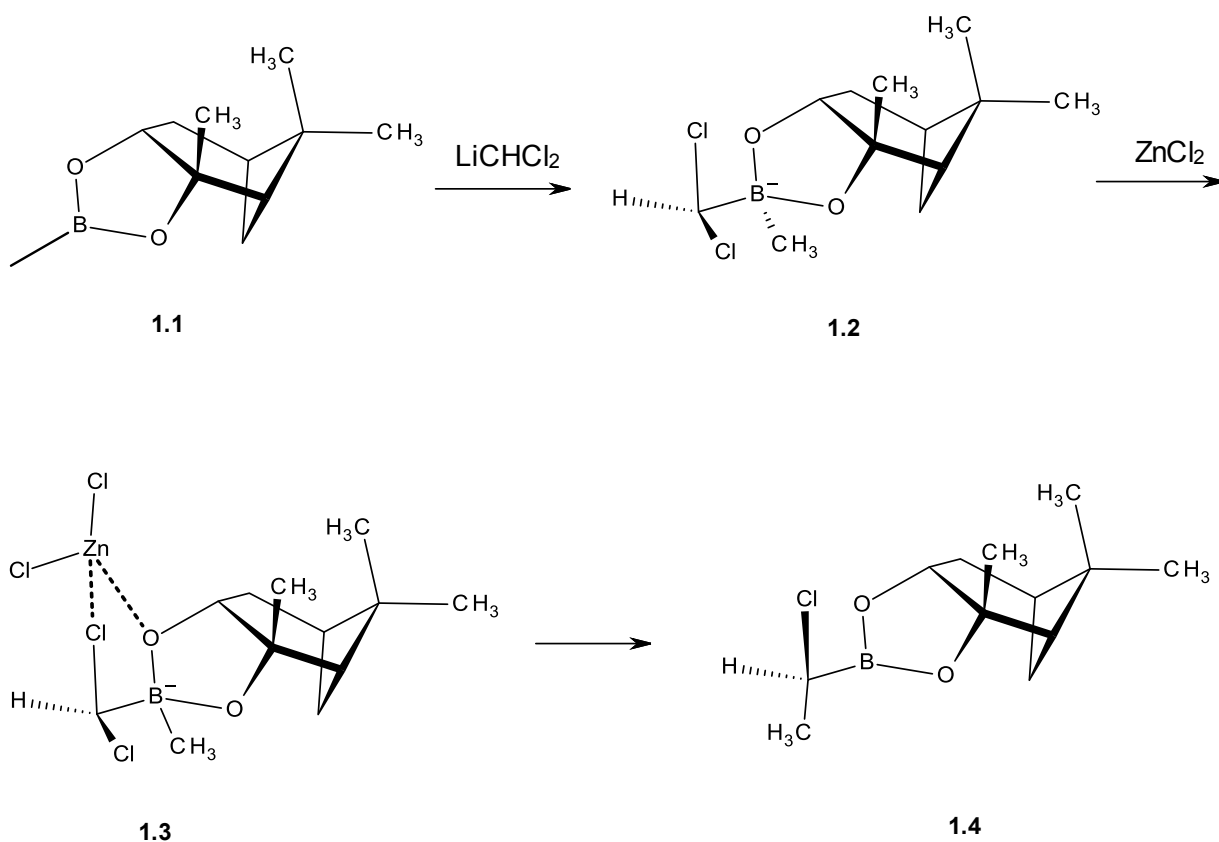
The typical procedure includes generation of dichloromethyl lithium which forms a chiral intermediate **1.2** (Scheme 1.2) that determines the configuration of the final product. The structure of the intermediate complex depends on the spatial structure of the chiral director. The chiral diols most commonly used in this reaction are (-)- or (+)-pinanediol⁴², 1,2-dicyclohexylethane-1,2-diol⁴³, 1,2-diisopropyl-1,2-diol^{44,45} and 2,3-butanediol.⁴⁶ Due to the influence of chiral, bulky diols, high diastereoselectivity (up to 1000:1) was achieved.⁴⁴ The

transformation (as a consequence of addition of ZnCl_2) of the highly unstable (at temperatures above $-100\text{ }^\circ\text{C}$) intermediate complex **1.2** to the more stable and sterically established compound **1.3** is an important feature of this reaction.

The role played by ZnCl_2 is crucial in:

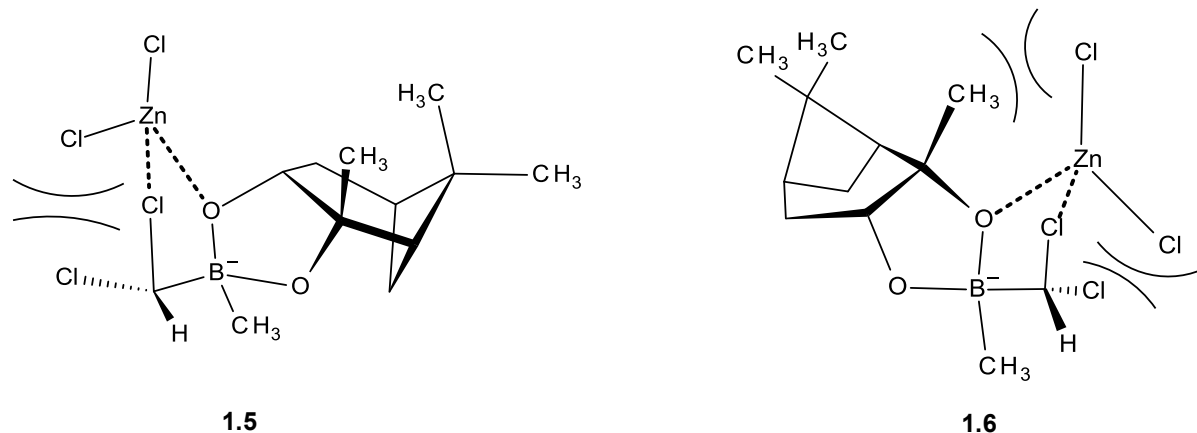
- promoting the intermediate complex rearrangement
- providing stereoselection
- inhibiting epimerization by capturing Cl^- as ZnCl_3^- and/or ZnCl_4^{2-}

This stereoselectivity can be explained by considering the intermediate complex **1.3** compared to two possible alternatives (**1.5** and **1.6**) (Scheme 1.3).⁴⁷



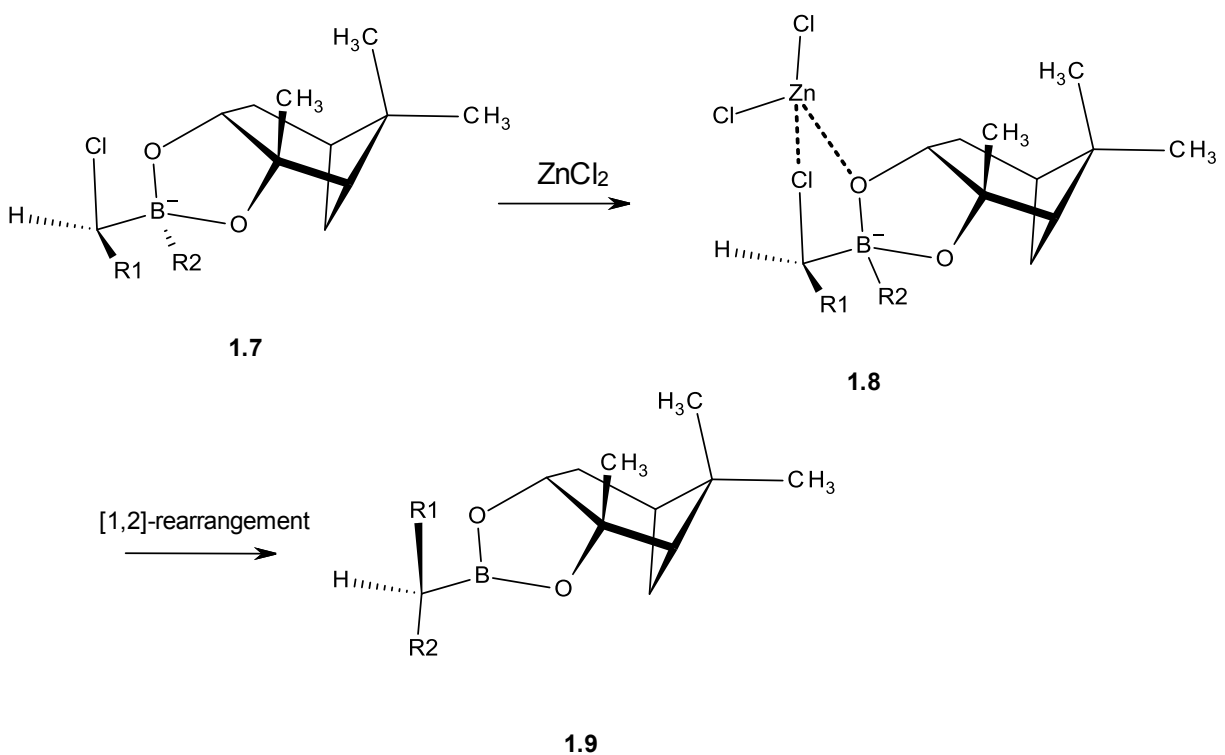
Scheme 1.2 Generally accepted homologation mechanism (exemplified by the methyl boronate).

Transformation of complex **1.5** to the final product results in the wrong stereoisomer, but fortunately formation of **1.5** is much less favorable due to steric reasons. Complex **1.6** can convert to the product with correct stereochemistry but steric hindrance is even larger in this case.



Scheme 1.3 Alternative configurations of intermediate complex **1.3**.

In the last step, the complex with favorable configuration **1.3** undergoes 1,2-rearrangement releasing the final product **1.4**.



Scheme 1.4 Alkylation mechanism.

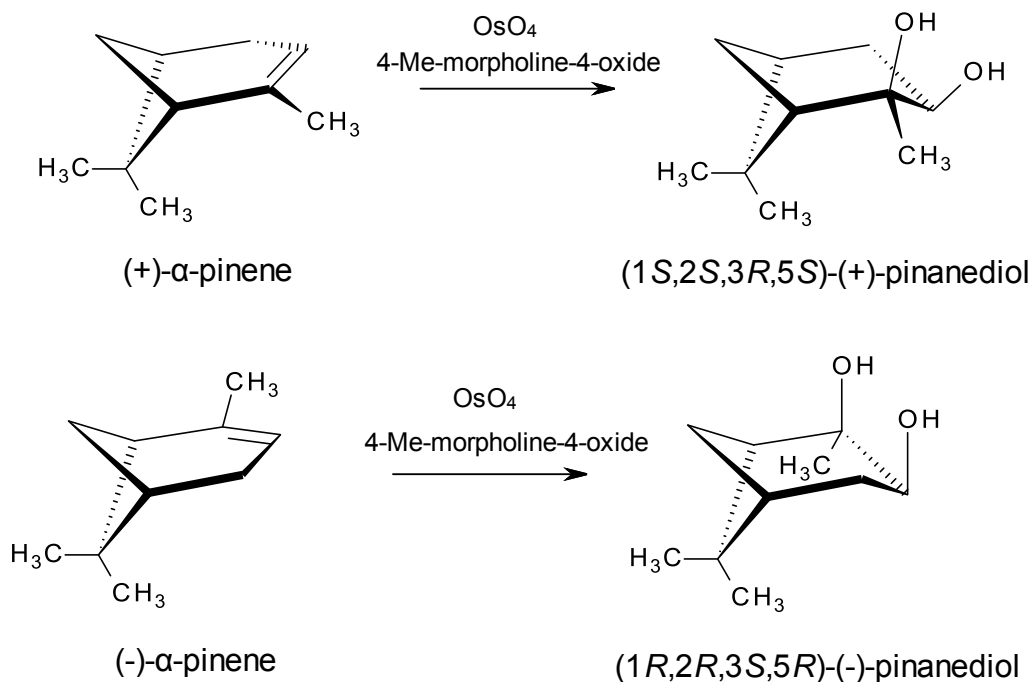
The reaction of alkylation of α -chloro derivative **1.4** with C-, N-, S- or O-nucleophiles goes via an intermediate complex which is very similar to **1.3** (Scheme 1.4).

High stereoselectivity and the stability of the different functional groups present in the molecule make this reaction a flexible tool for asymmetric synthesis.

1.2.3. Synthesis of (+)- or (-)-pinanediol

The choice of a chiral diol was defined by several reasons such as the level of provided stereoselectivity, ease of synthesis and commercial availability. There are several diols in the arsenal of the chemist using the homologation reaction.⁴⁸ Symmetric, non-chiral diols assist Matteson homologation but give racemic products that were not the goal of this investigation. The use of the best C₂-symmetric one (according to the diastereoselection level) – 1,2-dicyclohexyl-1,2-ethanediol (DICHEd) is limited because of its high cost. Due to the relatively low production costs and high level of stereocontrol it was decided to use pinanediol as a chiral director in the homologation reaction. Both (1*R*,2*R*,3*S*,5*R*)-(-)-pinanediol and (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol are commercially available but it is also possible to synthesize them according to the published procedure (Scheme 1.5).^{49, 50}

One of the advantages of pinanediol esters was the remarkable stability towards hydrolysis allowing chromatography on silica and the clear splitting pattern in NMR spectra.



Scheme 1.5 Synthesis of (+)- or (-)-pinanediol.

For reasons of simplicity the pinanediol derived from (+)- α -pinene was named (+)-pinanediol (1.10) and the pinanediol derived from (-)- α -pinene was named (-)-pinanediol (1.11) (Figure 1.6).

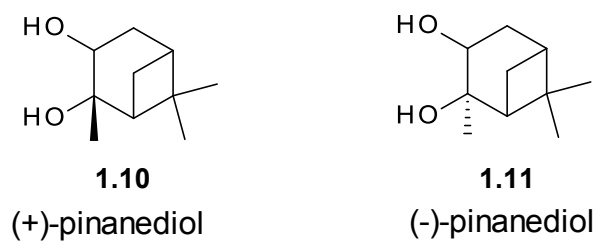


Figure 1.6 Two isomers of pinanediol.

AIMS OF THE PROJECT

The synthesis of potentially biologically active bioisosteres of natural amino acids was the overall goal of this work.

The specific aims of the investigation were:

- to develop a general strategy for the stereoselective synthesis of modified β -amino acids
- to obtain a set of variously substituted β -aminoboronic acids (or their esters)
- to obtain a set of stereopure β -aminoboronic acids (or their esters) with different configurations of chiral carbon atoms in the α - and/or β -position to boron
- to assess the applicability of the approach used in this work to future syntheses of different chiral substrates and their further derivatisation
- to create a library of short peptidomimetics containing modified amino acid moieties
- to measure antimicrobial activity of oligopeptides obtained, on a selected set of microorganisms
- to evaluate structure-activity relationships (if any)

References

1. Boman H.G., Nilsson I., and Rasmuson B., (1972), *Nature*, 237, 232-235.
2. Zasloff M., (2002), *Nature*, 415, 389-395.
3. Auvynet C. and Rosenstein Y., (2009), *FEBS Journal*, 276, 6497-6508.
4. Holzl M.A, Hofer J., Steinberger P., Pfistershammer K. and Zlabinger G.J., (2008), *Immunol Lett.*, 119, 4-11.
5. Lai Y. and Gallo R.L., (2009), *Trends Immunol.*, 30, 131-141.
6. Wang G., Li X., Wang Z. (2009) APD2: the updated antimicrobial peptide database and its application in peptide design. *Nucleic acids Res*; 37:D933-D937
7. Andres E., Dimarcq J.L., (2004), *J. Intern. Med.*, 255, 519-520.
8. Peters B.M., Shirliff M.E., Jabra-Rizk M.A., (2010), *Plos pathogens*, 6, 10, 1-4.
9. Gordon Y.J. and Romanowski E.G., (2005), *Curr. Eye Res.*, 30(7), 505-515.
10. Boman H.G., (2003), *J. Intern. Med.*, 254, 197-215.
11. Ryge T.S., Frimodt-Møller N., Hansen P.R., (2008), *Chemotherapy*, 54, 152-156.
12. Zefirova O.N., Zefirov N.S., (2002), *Vestnik Moskovskogo Universiteta, Ser. 2, Khimiya*, vol. 43, iss. 4, page 251.
13. Langmuir I., (1919), *J. Am. Chem. Soc.*, 41, 1543.
14. Grimm H.Z., (1934), *Electrochem.*, 47, 594.
15. Erlenmeyer H., Willi E., (1935), *Helv.Chim. Acta.*, 18, 740.
16. Friedman H. L., Influence of Isosteric Replacements upon Biological Activity. NAS-NRS Publication No. 206; NAS-NRS: Washington, DC, (1951); 206, 295-358.
17. Tomita M., Takase M., Bellamy W. and Shimamura S., (1994), *Acta. Paed. Jpn.*, 36(5), 585-91.
18. Haug B.E., Svendsen J.S., (2001), *J. Pept. Sci.*, 7(4), 190-196.
19. Haug B.E., Skar M.L. and Svendsen J. S., (2001), *J. Pept. Sci.*, 7(8), 425-432.
20. Strom M.B., Haug B.E., Rekdal O., Skar M.L., Stensen W. and Svendsen J.S., (2002), *Biochemistry and Cell Biology*, 80(1), 65-74.
21. Haug B.E., Stensen W., Stiberg T. and Svendsen J.S., (2004), *J. Med. Chem.*, 47(17), 4159-62.
22. Ma J.S., (2003), *Chimica Oggi Chemistry today*, June, 65-68.
23. Steer D.L., Lew R.A., Perlmutter P., Smith A.I., Aguilar M.-I., (2002), *Curr. Med. Chem.*, 811-822.
24. Patočka J., (2011), *Mil. Med. Sci. Lett. (Voj. Zdrav. Listy)*, 80, p. 2-11.
25. Cheng R.P., DeGrado W. F., (2001), *J. Am. Chem. Soc.*, 123, 5162-5163.
26. Daniels D.S., Petersson E.J., Qiu J.X., Schepartz A., (2007), *J. Am. Chem. Soc.*, 129, 1532-1533.
27. Juaristi E., Soloshonok V., `Enantioselective Synthesis of β -Amino Acids` (John Wiley& Sons: New York, 2005)
28. Seebach D., Beck A.K., Capone S., Deniau G., Groselj U., Zass E., (2009), *Synthesis*, (1), 1-32.

29. Liu M., Sibi M.P., (2002), *Tetrahedron*, 58, 7991–8035.
30. Weiner B., Szymanski W., Janssen D.B., Minnaard A.J., Feringa B.L., (2010), *Chem. Soc. Rev.*, 39, 5, 1656-1691.
31. Orsini F., Sello G., Sisti M., (2010), *Curr. Med. Chem.*, 17, 264-89.
32. Baker S.J., Ding C.Z., Akama T., Zhang Y-K., Hernandez V., Xia Y., (2009) *Future Med. Chem.*, 1(7), 1275-1288.
33. Matteson D.S., Soloway A.H., Tomlinson D.W., Campbell J.D., Nixon G.A., (1964), *J. Med. Chem.*, 7(5), 640-3.
34. Rezanka, T., Sigler K., (2008), *Phytochemistry*, 69(3), 585-606.
35. Jabbour A., Smoum R., Takroui K., Shalom E., Zaks B., Steinberg D., Rubinstein A., Goldberg I., Katzhendler J., Srebnik M., (2006), *Pure Appl. Chem.* 78(7), 1425-1453.
36. Ivanov A.S., Zhalnina A.A., Shishkov S.V., (2009), *Tetrahedron*, 65(34), 7105-7108.
37. LeBeau A.M., Singh P., Isaacs J.T., Denmeade S.R., (2008), *Chemistry & Biology*, 15, 665–674.
38. Walker B., Lynas J.F., (2001), *Cell. Mol. Life Sci.*, 58, 596–624.
39. Matteson D.S., (1989), *Chem.Rev.*, 89, (7), 1535-51.
40. Matteson D.S. *Boronic Acids*. Wiley-VCH Verlag GmbH & Co. (2005) vol.305 iss.342, 305-342.
41. Thomas S.P., French R.M. et al. (2009), *Chem. Rec.*, 9(1), 24-39.
42. Matteson D.S., Sadhu K.M., (1983), *J. Am. Chem. Soc.*, 105, 2077.
43. Ditrich K., Bube T., Sturmer R., Hoffmann R. W., (1986), *Angew. Chem., Int. Ed.*, 25, 1028.
44. Tripathy P.B., Matteson D.S., (1990), *Synthesis*, 200-206.
45. Matteson D.S., Ray R., Rocks R.R., Tsai D.J.S., (1983), *Organometallics*, 2, 1536-1543.
46. Sadhu K.M., Matteson D.S., Hurst G.D., Kurosky J.M., (1984), *Organometallics*, 3, 804.
47. Corey E.J., Barnes-Seeman D., Lee T.W., (1997), *Tetrahedron: Asymmetry*, 8, 22, 3711-3713.
48. Matteson D.S., (1999), *J. Organomet. Chem.*, 581, 51 - 65.
49. Ray R., Matteson D.S., (1980), *Tetrahedron Lett*, 21, 449-450.
50. Wityak J., Earl R.A., Abelman M.M., Bethel Y.B., Fisher B.N., Kauffman G.S., Kettner Ch.A., Ma Ph., McMillan J.L., (1995), *J. Org. Chem.*, 60, 12, 3717-3722.

2. RESULTS AND DISCUSSION

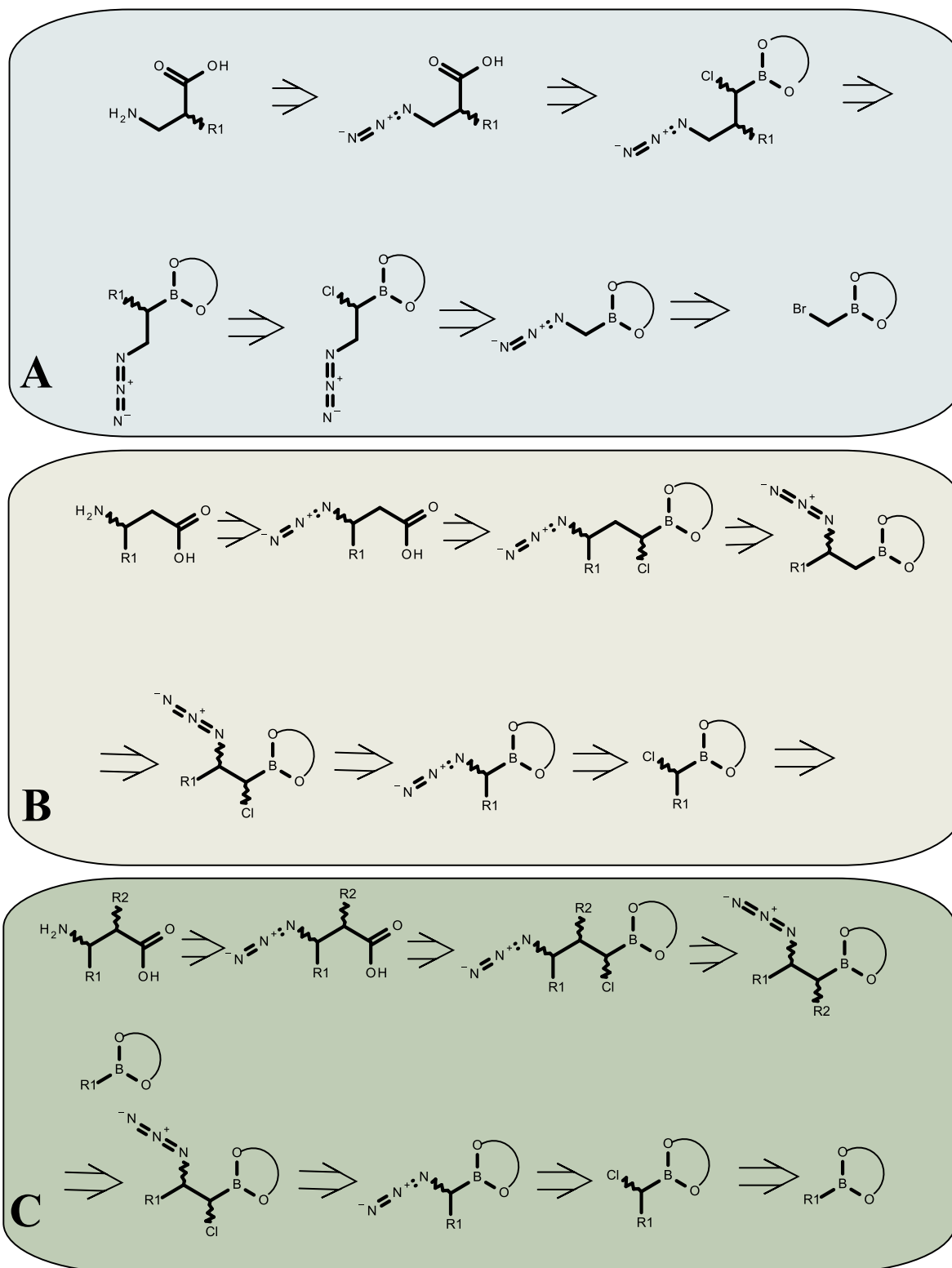
2.1. Strategy and methods

A new application of Matteson homologation that has not been described before in the literature has been employed. A step-by-step approach was used in the construction of the molecular skeleton of β -aminoboronates and β -amino acids. Strategies vary with the desired substitution pattern (Scheme 2.1 A, B, C). It is essential that configuration of new chiral centers appearing during the synthesis can be set *a priori*, on the planning stage, according to the needs of the researcher.

Even though numerous literature examples of the application of the methodology used confirm the high level of stereoselectivity of these transformations, we made several attempts to confirm the level of stereopurity of our target compounds (Chapter 2.7).

Compounds obtained on the way from starting compounds to product, provided the opportunity to investigate the reactivity of these intermediates (which was especially interesting for compounds not synthesized before). There appeared to be a number of approaches to the different types of synthetic transformations such as click-reactions, couplings, cyclizations etc. However, it was impractical to evaluate every possibility that arose during the course of work. Unexpectedly high biological activity of derivatives of β -aminoboronic acids obliged us to focus on the synthesis of short peptides containing a boronic acid moiety and set aside (or only touch on) the final part of the synthesis that generates the carboxylic group. It is necessary to specify that optimization of the procedures and yields was not a goal of this study and that sometimes efficiency had to be sacrificed at certain steps in order to arrive at the final product within a rational time frame. In spite of this, we estimate the general procedure as efficient enough for most of the examined cases.

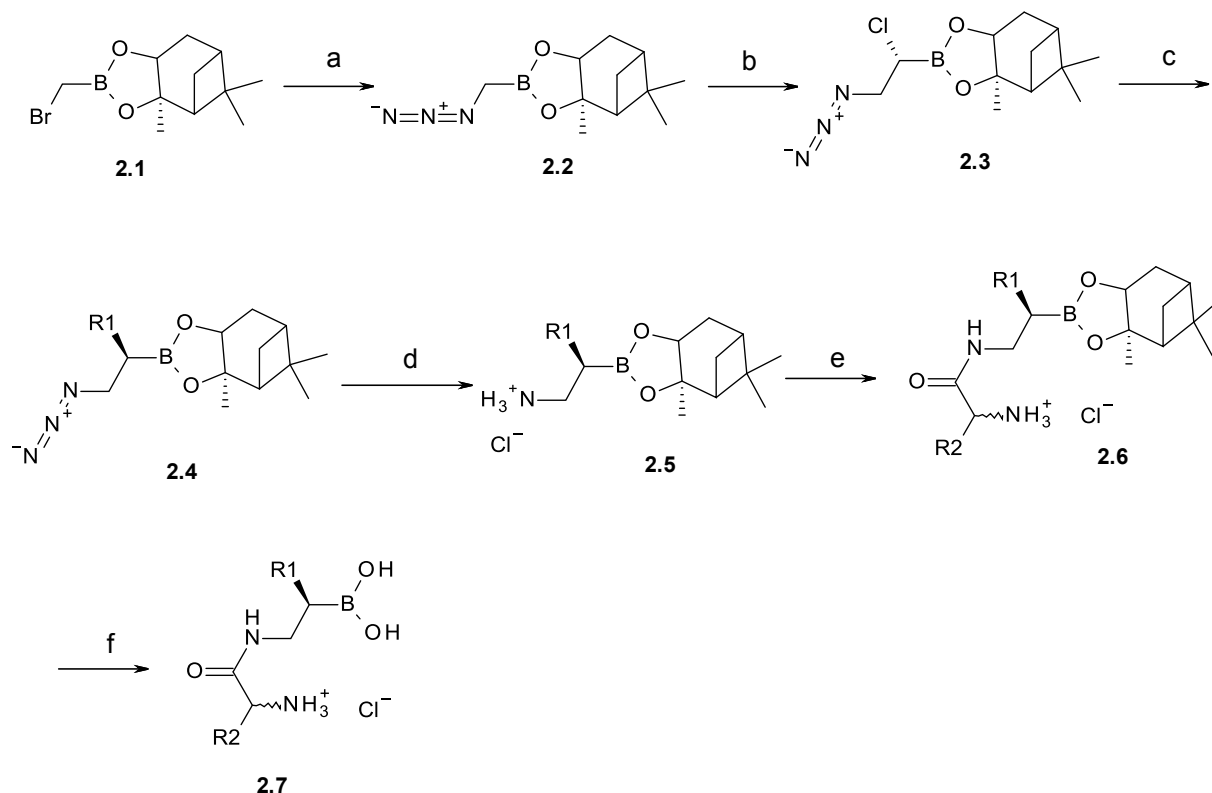
The detailed synthesis can be found in the following chapters.



Scheme 2.1 Retrosynthetic analysis of the synthesis of α -alkyl (or aryl-) substituted- β -amino acids **A**, β -alkyl (or aryl-) substituted- β -amino acids **B** and α,β -disubstituted- β -amino acids **C**.

2.2. Synthesis of α -alkyl (or aryl-) substituted β -amino boronates

Compounds were synthesized according to the route shown in Scheme 2.2



Scheme 2.2 General outline for synthesis of α -alkyl (or aryl-) substituted- β -aminoboronic acids and boronates exemplified using (-)-pinanediol.

a: NaN_3 , $(\text{Bu})_4\text{N}^+\text{Br}^-$, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ **b:** 1. CH_2Cl_2 , $n\text{-BuLi}$, Ar, THF, $-100\text{ }^\circ\text{C}$, 2. ZnCl_2 , $-78\text{ }^\circ\text{C}$ **c:** R1-MgCl , ZnCl_2 , $-78\text{ }^\circ\text{C}$, Ar, THF. For **2.4a**: lithium triethylborohydride, $-78\text{ }^\circ\text{C}$, Ar, THF **d:** 1. $\text{LiAlH}_4/\text{THF}$ 2. HCl/MeOH **e:** 1. N-Boc-amino acid, 1-HOBT, EDC, N-methylmorpholine, CH_2Cl_2 2. HCl/MeOH **f:** A: 3M $\text{HCl}/\text{H}_2\text{O}$, $90\text{ }^\circ\text{C}$ B: phenylboronic acid, $\text{Et}_2\text{O}/\text{H}_2\text{O}$ r.t.

The (-)-pinanediol-(bromomethyl)boronate **2.1** was prepared from 1,3-propandiol-(bromomethyl)boronate by a transesterification reaction which gave quantitative yield, in contrast to direct synthesis of **2.1** from trimethoxyborate, dibromomethane and butyllithium followed by addition of pinanediol. The approach used is one step longer but it avoided loss of valuable pinanediol. The starting material, 1,3-propandiol-(bromomethyl)boronate, was obtained by the

procedure analogous to the published one¹ with a yield of 57% and transesterification by (-)-pinanediol gave **2.1** in 93% yield. In the next step bromine was substituted by azide and the resulting (azidomethyl)boronate **2.2** was homologated to the corresponding (1-chloro -2-azidoethyl)boronate **2.3**. Compound **2.3** was then substituted by different Grignard reagents to obtain (1-substituted-2-azidoethyl)boronate **2.4** with (in most cases) high yields (Table 2.1). Between experiments some loss of stereoselectivity, which varied from 0% to 25%, was observed depending on the workup. It was found that even gentle heating during the concentration of the reaction mixture in the homologation step led to significant epimerization of product. To avoid this problem the procedure for work up was modified. After the completion of the reaction, the mixture was diluted by diethyl ether and the resulting mixture was washed with saturated ammonium chloride solution in order to remove any lithium chloride present (*vide infra*). The organic layer could then be concentrated at elevated temperature without any loss of diastereoselectivity.

Unsubstituted β -azidoboronate **2.4a** was obtained by reducing the C-Cl bond by super-hydride (lithium triethylborohydride) in THF at -78 °C followed by overnight stirring at room temperature. The reaction gave reasonably high yields (87%, Table 2.1) with over-reduction to the corresponding aminoboronic ester as a probable reason for such losses as occurred.

One approach to the reduction of an azido-group is the Staudinger reaction^{2,3} which gives good yield and provides a mild method that tolerates (in theory) a wide number of different functional groups. Unfortunately, this method was not suitable for our goals, as it unexpectedly resulted in a product that, whilst interesting, was outside of the scope of this study (see Chapter 2.9).

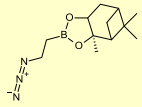
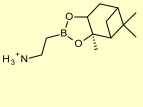
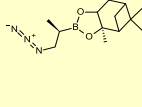
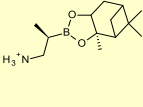
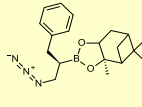
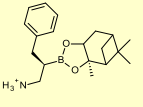
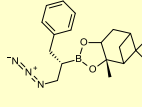
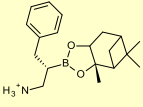
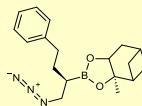
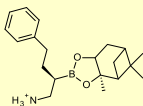
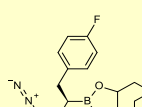
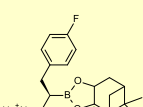
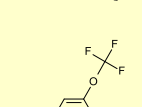
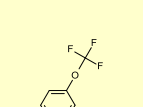
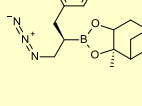
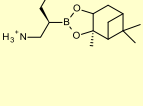
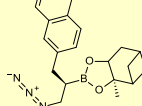
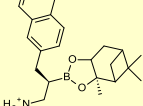
This unexpected complication obliged us to turn to the method utilizing LiAlH_4 as reducing agent. By means of this reaction, we managed to reduce β -azides to the corresponding amines and then convert them to their hydrochloric salts **2.5** with moderate to high yields (Table 2.1). It is necessary to be careful not to reduce the boronate moiety with LiAlH_4 ⁴ since that significantly decreases the overall yield. We suspect that this was the cause of the low yields in some reactions (entries **2.5c** and **2.5i**, Table 2.1).

The amines obtained **2.5a-i** were coupled with a set of diverse α -amino acids according to a general procedure for amino acid coupling⁵ and provided a library of Boc-protected derivatives of **2.6** (Table 3.1) in good yield (Experimental section*). Deprotection of the amine by means of HCl in methanol at 0 °C gave the corresponding hydrochloric salt **2.6** in quantitative yields (Experimental section).

Release of the boronic acid from its pinanediol ester was not a trivial task. Pinanediol esters possess a remarkable stability toward hydrolysis. This feature helps to avoid unnecessary side-reactions and provides significant flexibility in the choice of reaction conditions, but complicates conversion back to the boronic acid. There are very few methods for recovery of the acidic group. Transesterification with different chiral diols gave only moderate conversion and the esters obtained are also stable under hydrolytic conditions.⁶ Reacting these compounds with BCl_3 is not compatible with the labile functional groups and destroys the pinanediol moiety. In certain cases reaction with KHF_2 can give corresponding BF_3K -salt that can be transformed to the boronic acid. A few attempts to perform this reaction gave the desired product in low yield (*vide infra*).

* Yields of Boc-protected derivatives are given after the corresponding deprotected product **2.6**

Table 2.1 A set of synthesized α -substituted β -azido- and aminoboronates.

N	structure	Yield, %	N	structure	Yield, %
2.4a		89	2.5a		64
2.4b		86	2.5b		74
2.4c		97	2.5c		37
2.4d		36*	2.5d		58
2.4e		81	2.5e		82***
2.4f		30	2.5f		87
2.4g		78	2.5g		78
2.4h		98**(50)	2.5h		74
2.4i		92**(50)	2.5i		17

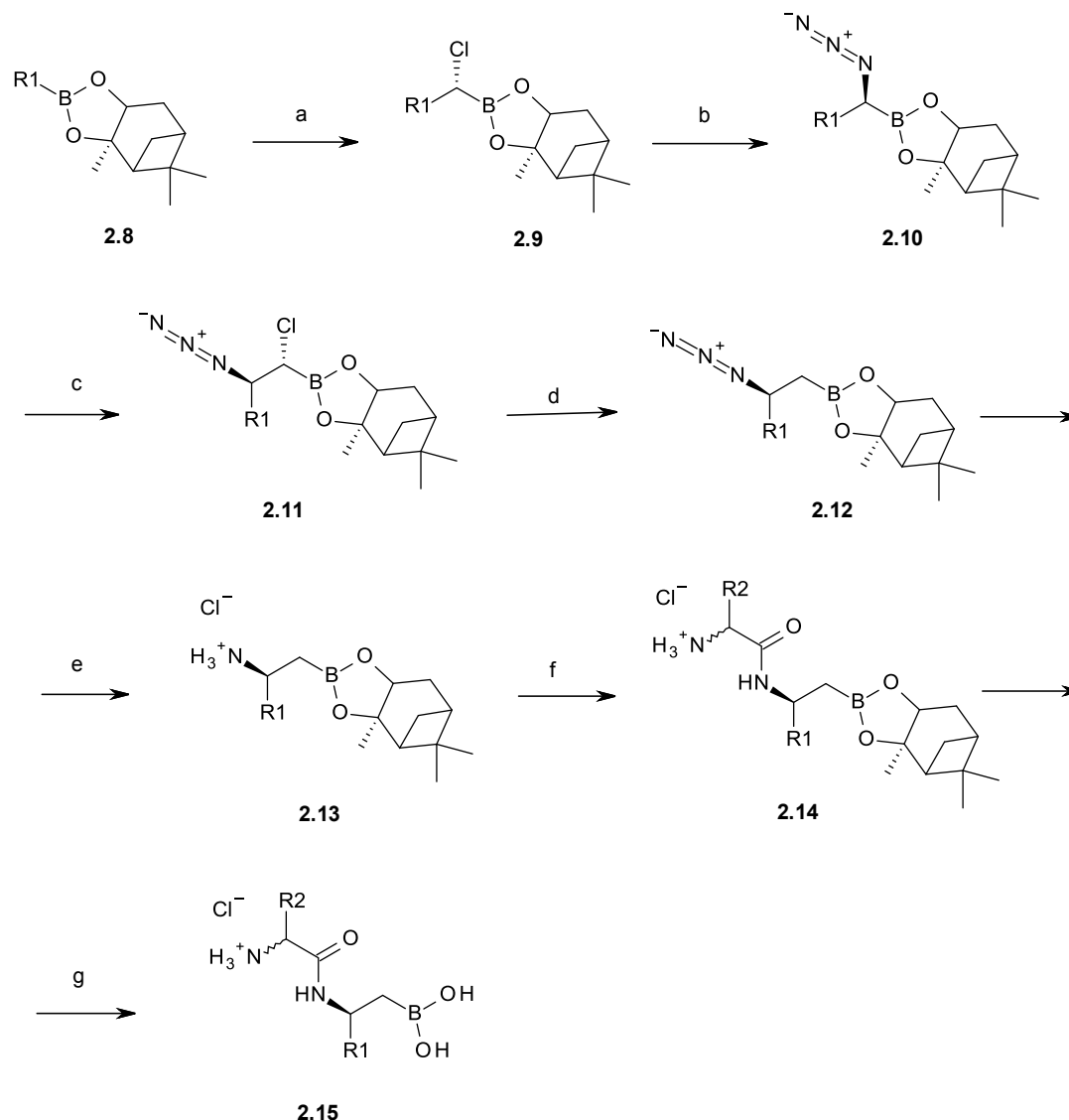
*yield after distillation **crude yield (estimated yield product) ***doubled signals in NMR (see Experimental Section)

Given the significant inefficiency of these approaches it was decided to employ either hydrolysis by means of 3M aqueous HCl or a two-phase transesterification method.⁷ The latter method was

especially convenient for the deprotection of compounds **2.6** and gave pure aminoboronic acids **2.7** with high yields (see Experimental section).

2.3. Synthesis of β -alkyl (or aryl-) substituted β -amino boronates

Compounds were synthesized according to the route shown in Scheme 2.3



Scheme 2.3 General outline for synthesis of β -alkyl (or aryl-) substituted β -aminoboronic acids and boronates exemplified using (-)-pinanediol. a: CH_2Cl_2 , n-BuLi, Ar, THF, $-100\text{ }^\circ\text{C}$, ZnCl_2 , $-78\text{ }^\circ\text{C}$ b: NaN_3 , $(\text{Bu})_4\text{N}^+\text{Br}^-$, $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, c: CH_2Cl_2 , n-BuLi, Ar, THF, $-100\text{ }^\circ\text{C}$, ZnCl_2 , d: $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$, THF, Ar, $0\text{ }^\circ\text{C}$, e: 1. $\text{LiAlH}_4/\text{THF}$ 2. HCl/MeOH , f: 1. N-Boc-amino acid, 1-HOBT, EDC, N-methylmorpholine, CH_2Cl_2 2. HCl/MeOH , g: A: 3M $\text{HCl}/\text{H}_2\text{O}$, $90\text{ }^\circ\text{C}$ B: phenylboronic acid, $\text{Et}_2\text{O}/\text{H}_2\text{O}$ r.t.

Another strategy was used for the synthesis of β -alkyl (or aryl-) substituted β -azidoboronates. Alkylboronic esters **2.8b-e** were synthesized according to the published procedure⁸ and homologated under standard conditions. Compounds **2.9b-d** were then substituted by sodium azide to obtain **2.10b-e** and homologated one more time to obtain α -chloro- β -azidoboronates **2.11b-d**. Compounds **2.11b-d** were reduced to β -substituted β -azidoboronates **2.12b-d** (Table 2.2) with super-hydride ($\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$).

Table 2.2 A set of synthesized β -alkyl (or aryl-) substituted β -azido- and aminoboronates.

N	structure	Yield, %	N	Structure	Yield, %
2.4a		89	2.5a		64
2.12b		71	2.13b		31
2.12c		85	2.13c		56
2.12d		98*	2.13d		45

* crude yield

Another possibility to obtain compounds **2.12** was a direct one-carbon homologation using CH_2Cl_2 and lithium diisopropylamine⁹ or iodochloromethane and *n*-butyllithium.¹⁰ Although

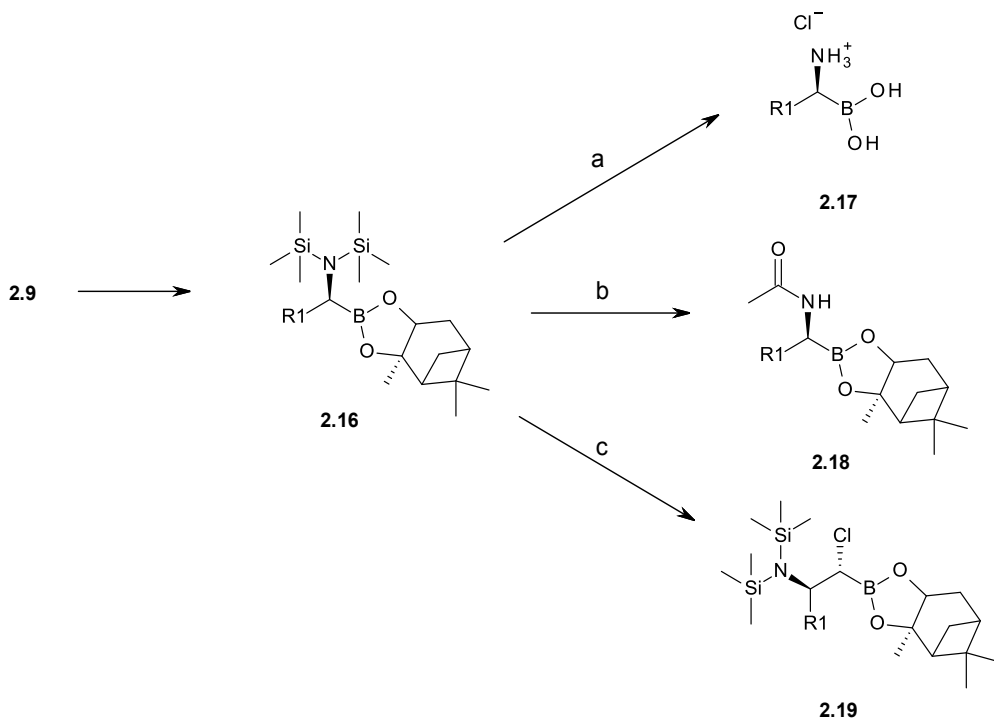
one step longer, the first procedure gave higher yields than the direct homologation introducing a methylene moiety.

Reduction of the azido group was performed with LiAlH_4 in a way similar to the reduction of the α -substituted analogs **2.4**. Coupling and deprotection reactions were also universal for either α - and β -alkyl (or aryl-) substituted β -aminoboronates (see Experimental section).

2.3.1. Synthesis of β -phenyl- β -aminoboronates

Significant difficulties were observed during synthesis of the β -phenylsubstituted derivative **2.10e** ($\text{R1} = \text{phenyl}$). Substitution of the α -chloro atom by azide resulted in a mixture of the desired product **2.10e** and benzaldehyde ($\approx 20\%$). This by-product brought additional difficulties into the next steps and resulted in a complicated mixture making spectra impossible to interpret. Taking into account the behavior of the azidoboronate in this particular case, we decided to use hexamethyldisilazane derivative **2.16** to overcome these problems.

In general, the hexamethyldisilazane group provided precursors to the amines which were quite stable to air and moisture and could be easily deprotected providing hydrochloride salts of α -amino boronic acids **2.17** or α -amido boronates **2.18** (Scheme 2.4).¹¹⁻¹³ However, although the hexamethyldisilazane group was an extremely convenient precursor for an amino group, the β -silylamino- α -chloroboronic acid derivatives (**2.19** $\text{R1} = \text{benzyl}$) synthesized by homologation of (**2.16** $\text{R1} = \text{benzyl}$) were highly moisture sensitive and could not be purified using chromatography or distillation.^{14,15} In addition, conversion of **2.16** to **2.19** was not effective, probably due to



Scheme 2.4 Application of α -hexamethyldisilazyl derivatives in the synthesis of α -aminoboronates.

steric reasons as evidenced from X-ray analysis of the naphthylmethyl analog (Figure 2.1). Shielding of the boron atom by bulky substituents in the α -position reduces yield and provides complicated mixtures making analysis rather difficult.

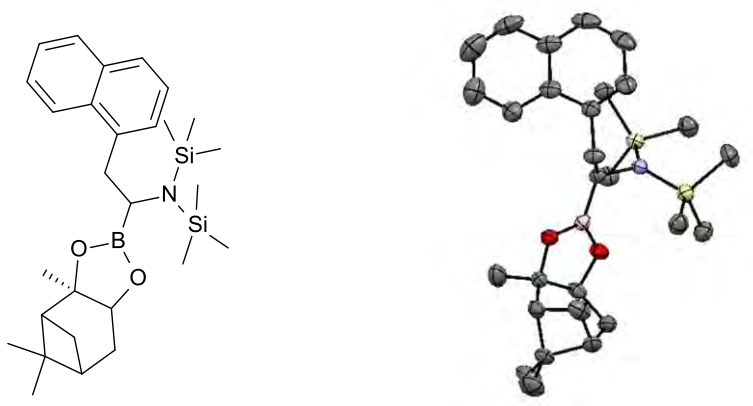
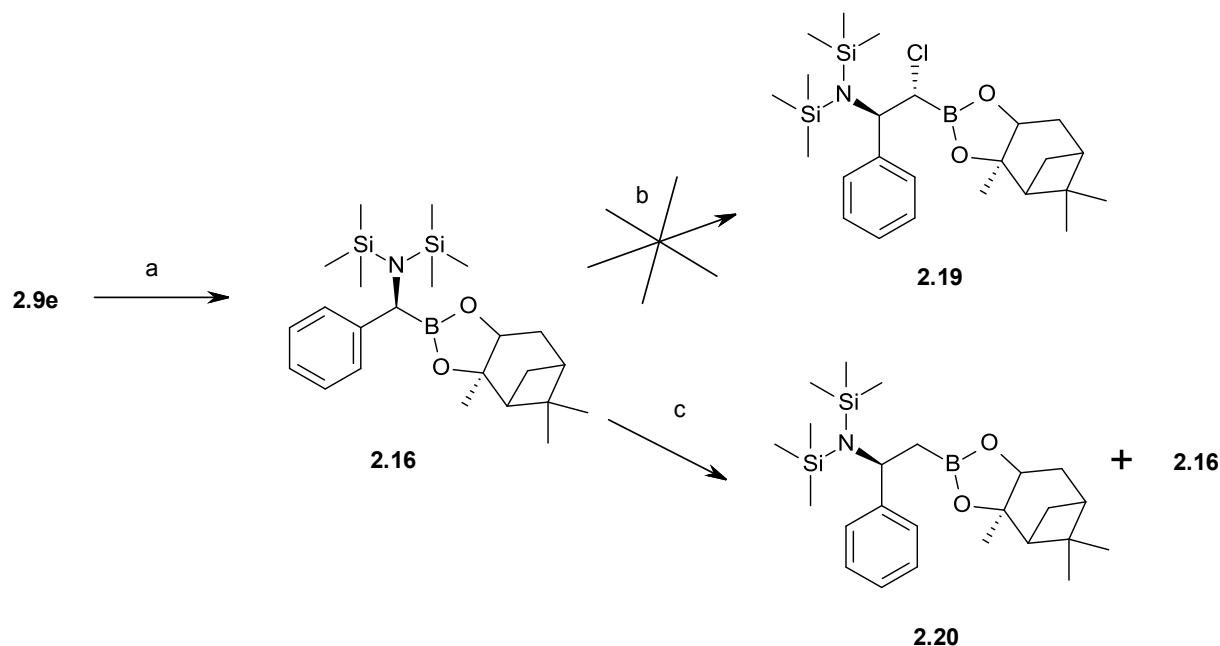


Figure 2.1 ORTEP drawing (right) and skeletal formula (left) of the sterically hindered α -hexamethyldisilazane derivative 2.16 (R1= α -naphthylmethyl). Boron and α -carbon atom are surrounded by bulky substituents, making the formation of the intermediate complex extremely complicated and rearrangement not effective.

Moreover, the homologation of the phenyl-substituted derivative **2.16** (R1=phenyl) failed completely (Scheme 2.5). Starting compound was recovered.

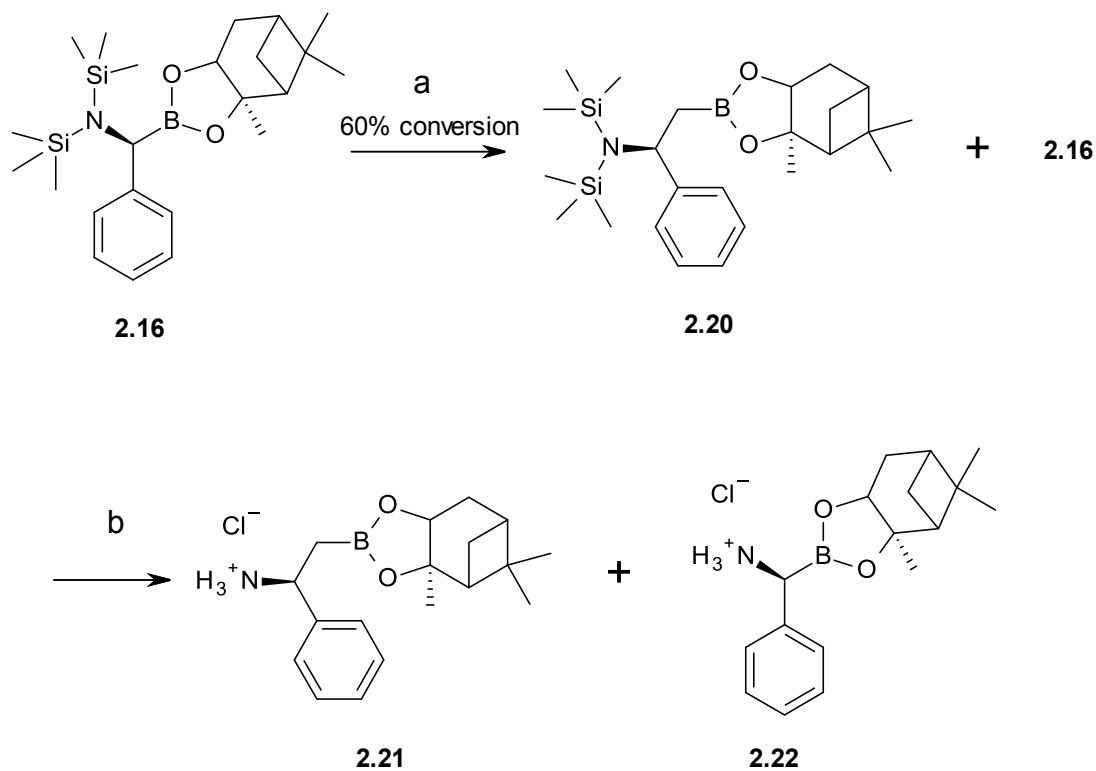


Scheme 2.5 Homologation of phenyl-substituted boronate. **a**: HMDS, -78 °C, Ar, THF **b**: 1. CH₂Cl₂, n-BuLi, Ar, THF, -100 °C, 2. ZnCl₂, -78 °C **c**: 1. ICH₂Cl, n-BuLi, Ar, THF, -100 °C, 2. ZnCl₂, -78 °C.

It is known, that direct insertion of a methylene group can be achieved by adding *n*-butyllithium to the complex of iodochloromethane and the corresponding boronate.¹⁰ This reaction provided a 60% conversion to the product **2.20** contaminated with unreacted starting compound **2.16**.

In this case we were compelled to work with mixtures (which were not very complicated for interpretation by NMR) in the hope that the last step would allow washing out all starting compounds and impurities. Despite this not very effective approach, it was possible to get the final amine hydrochloride **2.21** (Scheme 2.6) though as a mixture with α -aminoboronate **2.22**.

The mixture obtained (2.21+2.22) was coupled with Boc-protected amino acid and expectedly gave us a mixture of two dipeptides which were separated by means of preparative TLC resulting in two pure homologs (Figure 2.2) of the same dipeptide (Experimental section).



Scheme 2.6 Synthesis of β -phenyl- β -aminoboronate. **a:** 1. ICH_2Cl , $n\text{-BuLi}$, Ar, THF, $-100\text{ }^\circ\text{C}$, 2. ZnCl_2 , $-78\text{ }^\circ\text{C}$ **b:** HCl/MeOH , $-20\text{ }^\circ\text{C}$.

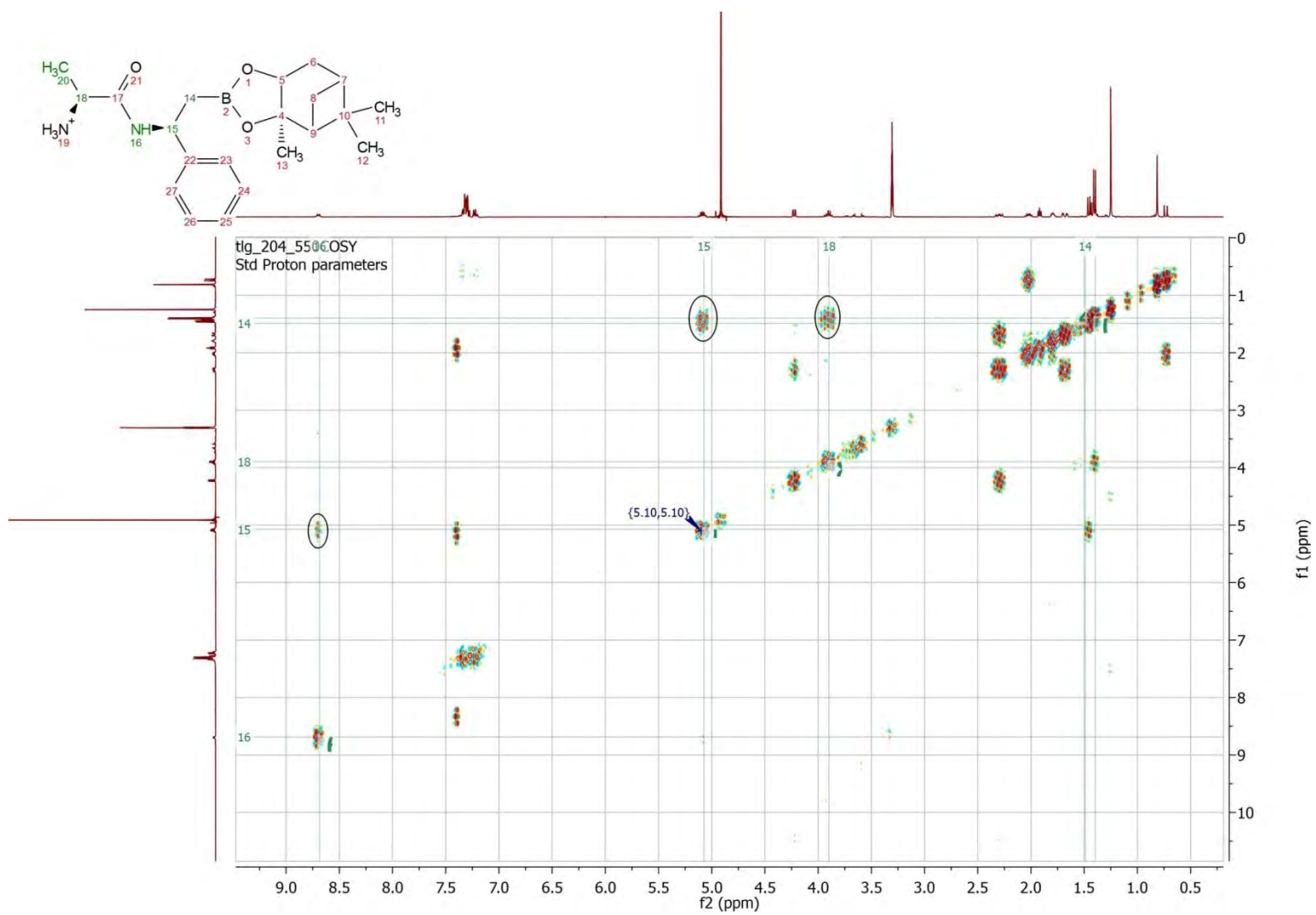
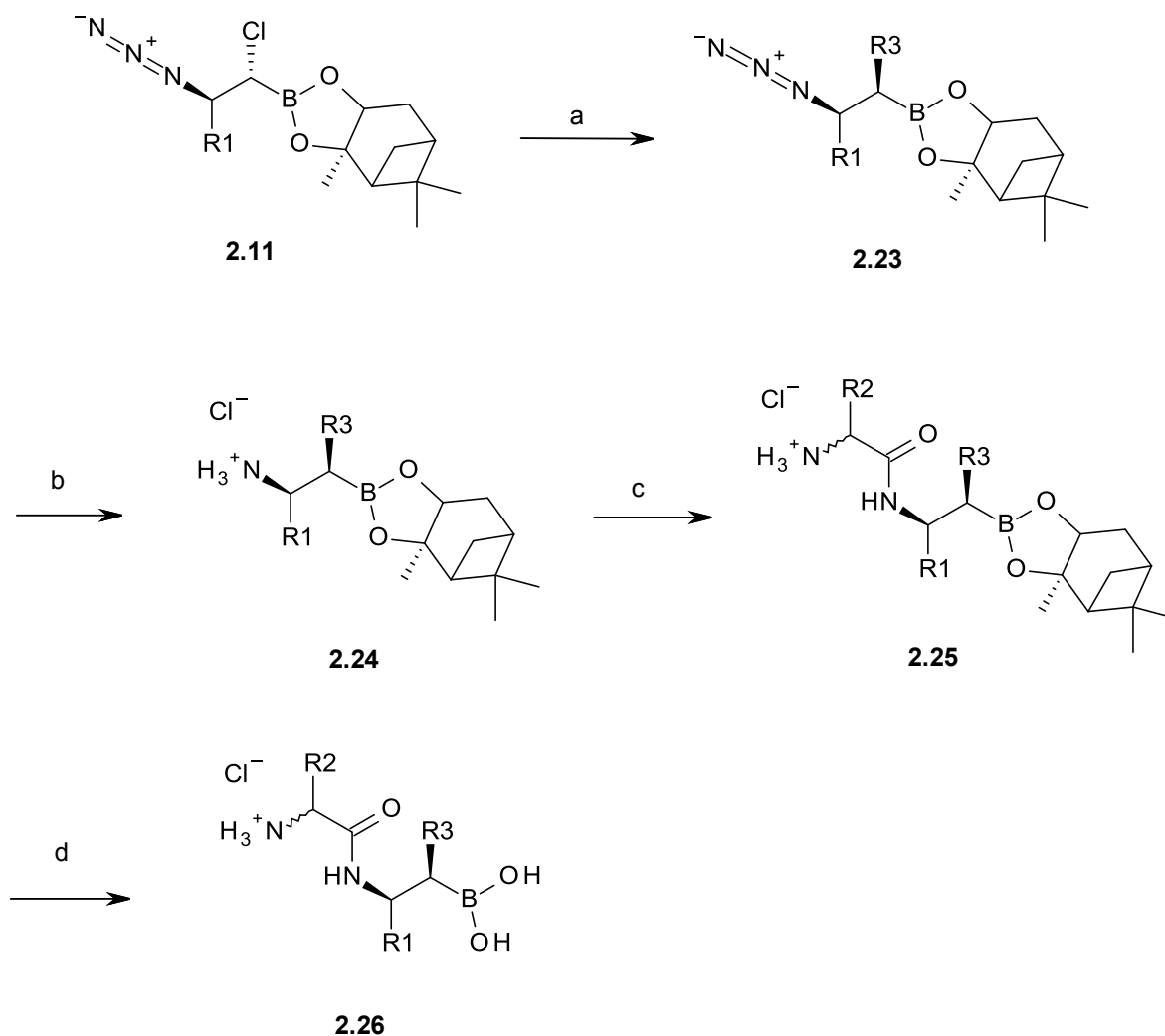


Figure 2.2 COSY spectrum (400 MHz, CDCl₃) of 2.21-1 (contaminated by 2.22-N-Ala) showing the coupling between H14 and H15, H15 and H16.

2.4. Synthesis of α,β -disubstituted β -amino boronates

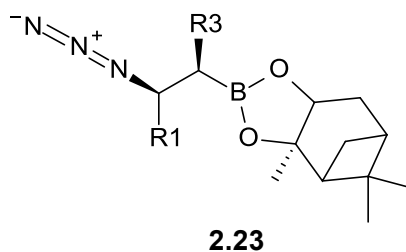
Compounds were synthesized according to the route shown in Scheme 2.7



Scheme 2.7 General outline for synthesis of α,β -disubstituted β -aminoboronic acids and boronates exemplified using (-)-pinanediol. a: R3-MgCl, -78 °C, ZnCl₂, THF b: 1. LiAlH₄/THF 2. HCl/MeOH, c: 1. N-Boc-amino acid, 1-HOBt, EDC, N-methylmorpholine, CH₂Cl₂ 2. HCl/MeOH, d: A: 3M HCl, 90 °C B: phenylboronic acid, Et₂O/H₂O r.t.

Using compound **2.11** it was possible to introduce the second alkyl group into the molecule and obtain various α,β -disubstituted β -azido boronates **2.23**. Unlike the substitution of (1-chloro -2-azidoethyl)boronate **2.3** which gave complete conversion to the 1-alkyl-2-azidoboronates **2.4** the analogous reaction of **2.11** never gave 100% transformation to the product **2.23** providing instead mixtures with starting compounds. While substitution of the α -chlorine atom proceeded without difficulties in the case of MeMgCl certain problems were observed with more bulky Grignard reagents. The conversion of starting compound fell from the 90% to 0% depending on the R3 group on Grignard reagent (Table 2.3). On the basis of these results it seems very likely that steric factors play a very important role in the accessibility of reactive sites and the process of intermediate complex formation.

Table 2.3 Conversion of **2.11** to **2.23** when using different Grignard reagents (R3MgCl).



<i>R3</i>	<i>Me</i>	<i>Bz</i>	<i>Ph</i>	<i>2-methyl-2-phenylpropyl</i>
% of conversion* <i>R1</i> = <i>Benzyl</i>	80-90(2.23b)	55-60(2.23d)	48(2.23f)	0(2.23g)
% of conversion <i>R1</i> = <i>Methyl</i>	78-85(2.23a)	55-65(2.23c)	32-36(2.23e)	-
% of conversion <i>R1</i> = <i>H</i>	100(2.4b)	100(2.4c)	50(2.4i)	-

* Reactions were performed at 0.5 g scale. None of the compounds were isolated in the model experiment. The conversion to product was estimated by ¹H NMR. Samples were taken after 24 hrs of stirring.

Reduction of the azido-group was achieved by means of lithium aluminum hydride using

standard procedures. Coupling and deprotection reactions were also identical for all types of β -aminoboronates.

It was not always possible to get analytical sample of the amines. HRMS data unequivocally indicated the presence of the correct product, but referencing NMR signals was complicated due to broadening. Thus, in the case of **2.24a** it was decided to analyze it in its Boc-protected form.

The corresponding derivative was obtained as white crystals, suitable for X-ray analysis (Figure 2.3).

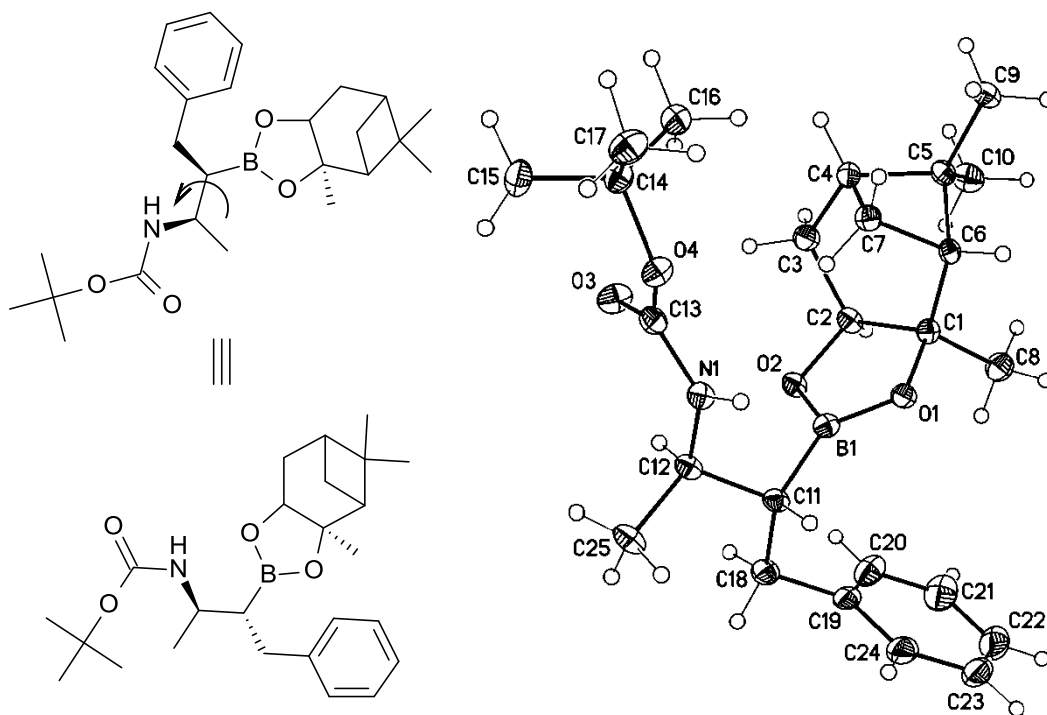


Figure 2.3 Skeletal formula and ORTEP drawing of boc-protected amine **2.24**.

It is known from the literature¹⁶ that analogous α -amidoderivatives form a 5-membered ring with a bond between the amide oxygen and the boron atom (bond length 1.638Å) (Figure 2.4).

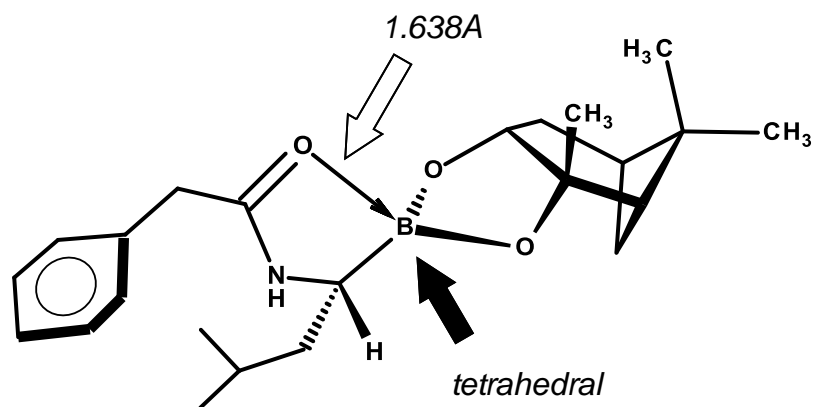


Figure 2.4 Established spatial structure for α -amidoderivatives.¹⁶

In the case of β -amidoderivatives the bond between the amido-oxygen and the boron did not exist and the interatomic distance was 4.010 Å and hence there was no 6-membered ring. It was also clear that the CBO₂ fragment of the molecule **2.24a** remained planar (Figure 2.3) while α -amidoderivative showed tetrahedral molecular geometry (Figure 2.4).

In compound **2.24a** the relative positions of the benzyl and boronate moieties in relation to the rest of the molecule is open to dispute. Theoretically, both the benzyl and boronate substituents at the α -carbon could end up in the *anti*-position to the amide at the β -carbon. In this respect, comparison of the conformations in both crystal form and solution would be useful in any further investigations. A set of NMR experiments were performed on compound **2.24a** (see Experimental section for the detailed spectra).

The ROESY spectra of **2.24a** reveal two stable rotamers at room temperature on the NMR time scale in approximately 3:1 ratio (Figure S1 in the Experimental section). The separation of chemical shifts between the rotamers indicates that rotation around the C11-C12 (Figure 2.3)

bond is the origin of the rotamers, a conclusion which is further supported by the significantly different $^3J_{\text{HH}}$ coupling constants between H11 and H12 for the two rotamers and analysis of ROESY spectra (Figure S1 and S2 in the Experimental section).

The data shows that there is a conformational difference in the solid state and in solution (Figure 2.4) and the lack of coupling between the hydrogens in the minor rotamer suggests that these are perpendicular in relation to each other.

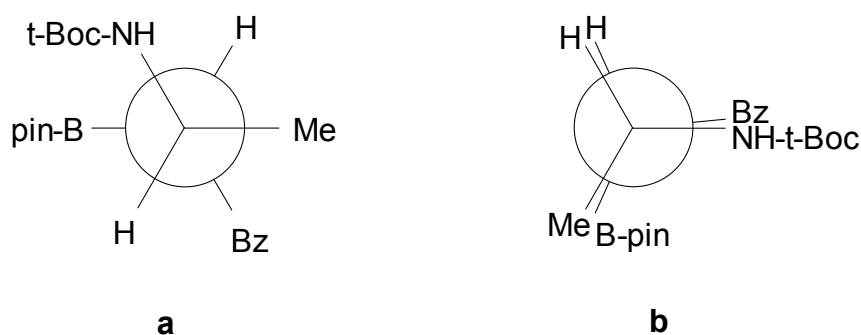


Figure 2.4 Newman projections of rotamer in crystal (a) and major rotamer in solution (b).

2.5. Synthesis α,β -diamino- β -alkylsubstituted boronates

Diamino acids are an extremely important motif in nature. Recently simple derivatives of various diamino acids (along with ordinary amino acids) were found in meteorites, providing important information concerning the origins of life on Earth.¹⁷

These types of amino acids are a part of such complex natural compounds as bleomycin¹⁸, capremycin¹⁹, penicillin and some other types of antibiotics. They can also be considered as stabilizers of the secondary structure of peptides.

Therefore, it was interesting to develop and apply a novel approach to the synthesis of this class of molecules and obtain synthetically interesting α,β -diamino- β -alkyl analogs of the target β -aminoboronates (Figure 2.5).

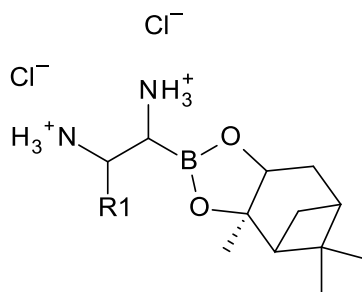
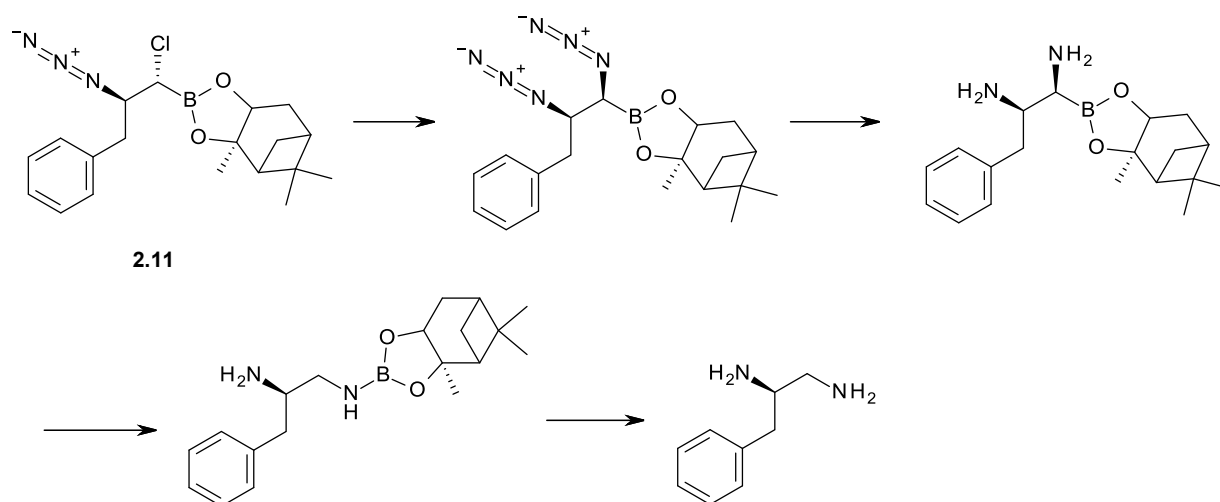


Figure 2.5 General structure of the α,β -diamino- β -alkyl aminoboronates.

We pursued the idea that two adjacent amino groups could be collinear and proved access to interesting synthetic opportunities (see Chapter 2.5.1.). The mutual arrangement of the amido-groups in the molecule was important as we could deduce the presence of a turn in the molecule under investigation and identify the possibility of intramolecular cyclisation.

At this point, the way of introducing an α -amino-group played a very important role. The original intention to use an azido-group as a source of α -nitrogen was found to be unworkable as it was impossible to release free amine in the position α - to the boron atom. The reason for this was illustrated by D. Matteson during the early stages of exploration of the limitations of his reaction.²⁰

If not immediately trapped as an amide or salt, the molecule rearranges, releasing the free amine (Schemes 2.8 and 2.20). Reduction of the azides in our substrates, with help of lithium aluminum hydride, hypothetically gives two free amino groups simultaneously, leaving no chance for α,β -diaminoboronate to survive (Scheme 2.8).



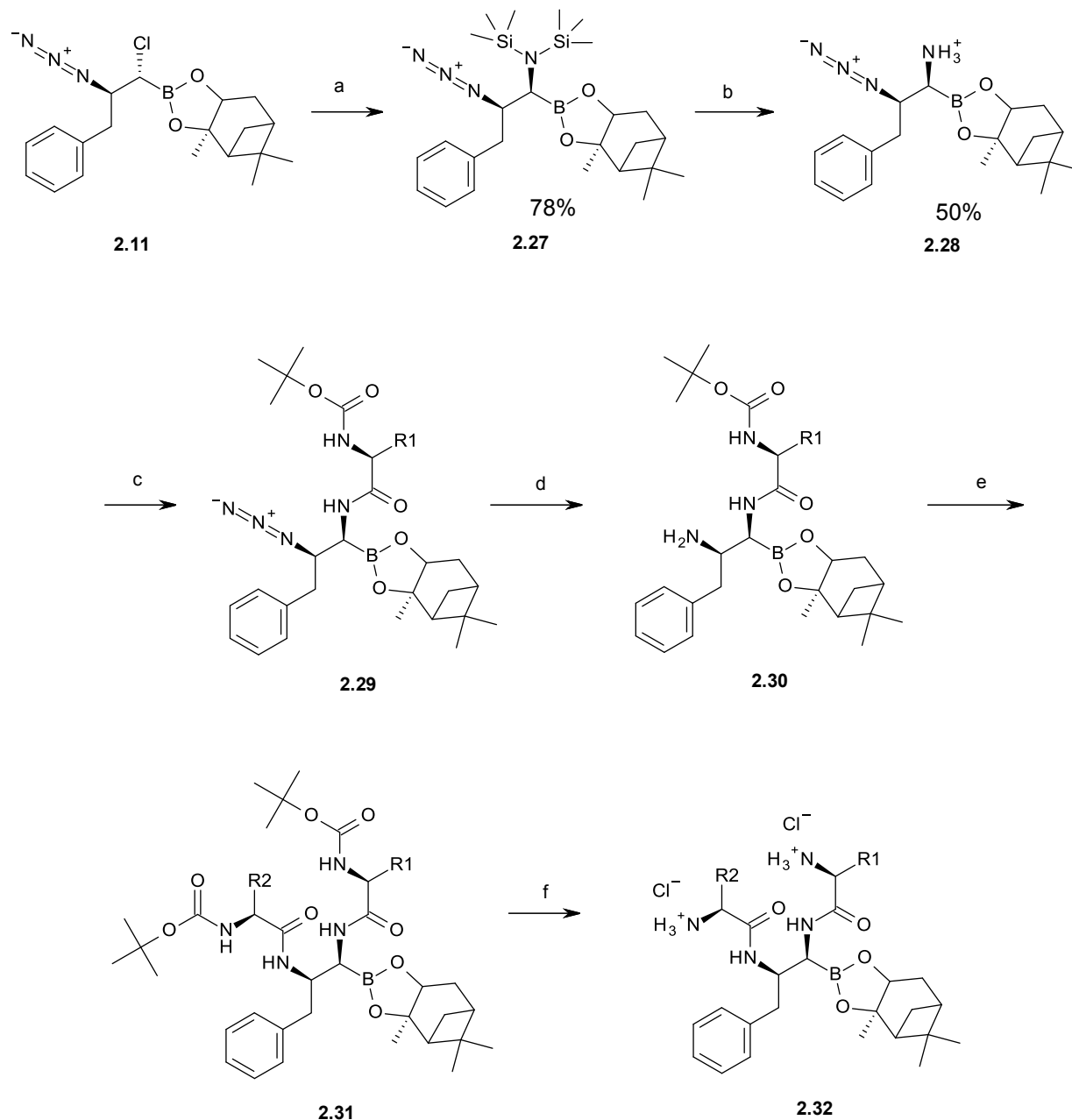
Scheme 2.8 Rearrangement of free α -aminoboronate to primary amine.

Due to this reason it was decided to apply another approach that included substitution of an α -chloro atom in compound **2.11** with a hexamethyldisilazane group by means of a well-established procedure.⁸ It was also important that the method developed, provided the opportunity to release the amino-groups on separate steps in any suitable order.

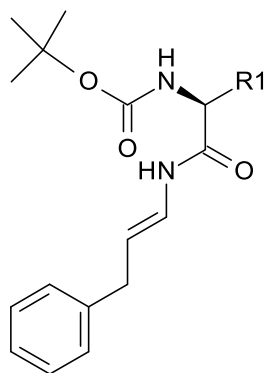
The next step, generating an α -amino group, has been demonstrated by workers in the field in recent decades^{21,22} and applying this approach functioned as anticipated, the target compound **2.28** being obtained with satisfactory yield (Scheme 2.9).

Coupling these derivatives with amino acids gave the corresponding dipeptides (**2.29** R1=benzyl) in high yield.

Reduction of the azide by means of lithium aluminum hydride gave a mixture of the target molecule **2.30** (R1=benzyl) and an unexpected by-product **2.33** (Figure 2.6).



Scheme 2.9 General outline for synthesis of α,β -diamino- β -benzyl β -aminoboronic acids and boronates exemplified using (-)-pinanediol. a: LiHMDS, -78°C , THF b: HCl/MeOH, c: 1. N-Boc-amino acid, 1-HOBt, EDC, N-methylmorpholine, CH_2Cl_2 d: $\text{LiAlH}_4/\text{THF}$ e: 1. N-Boc-amino acid, 1-HOBt, EDC, N-methylmorpholine, CH_2Cl_2 f: HCl/MeOH.

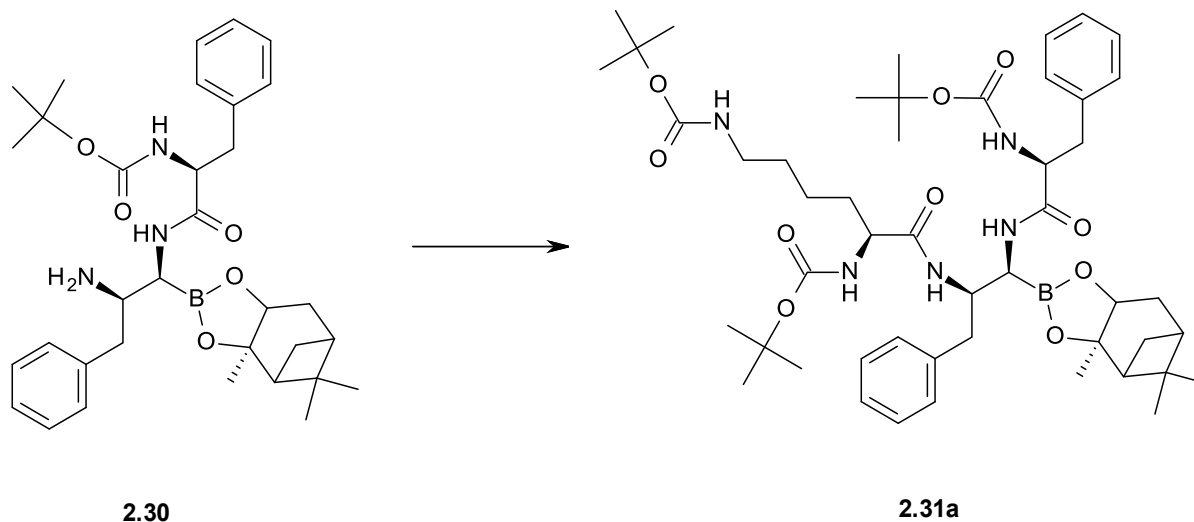


2.33

Figure 2.6 By-product (R1=benzyl) detected and isolated from the reaction mixture.

We suggest that the formation of compound **2.33** was a result of an elimination process similar to one described in the literature for free α -aminoboronates (Schemes 2.8 and 2.20). The main difference was in the final product of this (in most cases) undesirable reaction. Matteson had described²⁰ the decomposition product as an amine, but in our case (according to HRMS and NMR) the amino group was not found in the leaving fragment, probably staying attached to the boronic ester moiety. The leaving group we in fact detected contained a double bond. An analytical sample of this compound was obtained by means of straight phase LC (pentane/diethyl ether, 100% to 70% pentane gradient). Proton and COSY spectra were consistent with the proposed structure; though the Boc-group was not detected in carbon NMR. Coupling of compound **2.30** (R1=benzyl) with an amino acid expectedly gave us a tripeptide **2.31a** (see Scheme 2.10). N-Debocylation of the fully protected product went smoothly and the corresponding hydrochloride **2.32a** was obtained in good yield. The analysis of the spatial structure of diamidoboronates left a lot of space for discussions. In the previous chapter we considered formation of an intramolecular bond between carbonyl oxygen and boron atom

separately for the α - and β -amidoderivatives. Now we united them in one molecule that produced new structural alternatives.



Scheme 2.10 Synthesis of the model tripeptide on the basis of α,β -diamino- β -benzylboronate.

As discussed earlier (Chapter 2.4), formation of the 5-membered ring was more favorable than the 6-membered one. This fact gave us the opportunity to consider the presence of a 5-membered ring in the molecule **2.31a** exclusively, without any competition from the carbonyl oxygen of β -amido moiety. The two most obvious consequences of the presence of the 5-membered ring: were distortion of the planarity of CBO_2 part of the molecule and increasing of the rotation barrier around the $\text{C}_\alpha\text{-C}_\beta$ bond.

It is necessary to mention, that even if the mutual arrangement of amino-groups does not meet our expectations, there is still a possibility that the rotation barrier around $\text{C}_\alpha\text{-C}_\beta$ bond is low enough to facilitate generation of the favorable configuration *in situ*.

Taking into account configuration of chiral centers in the similar molecule **2.24a** and the presence

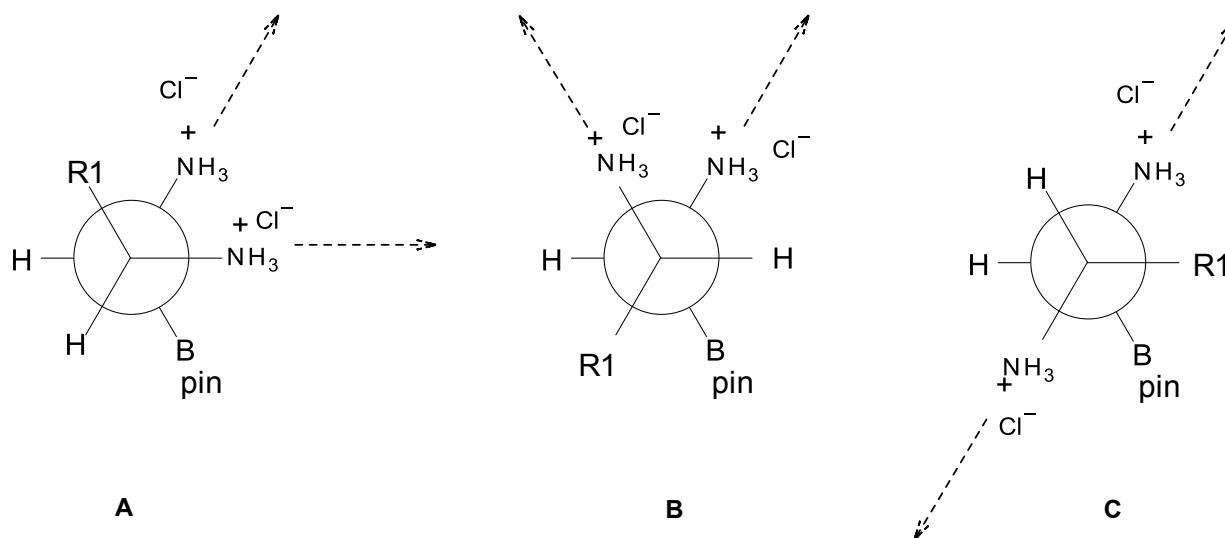


Figure 2.7 Three possible rotamers of α,β -diamino- β -alkylboronate.

of bulky substituent in the position β to boron, we can consider three possible rotamers (Figure 2.7). Two of them (**A** and **B**) generate nearly collinear vectors and provide the possibility for the γ -turn and facilitate cyclization *vide infra*.

2.5.1. Towards the cyclisation of α,β -diaminoderivatives of boronic acid

It is impossible to overemphasise the importance of cyclic peptides in contemporary medicine. Starting from the discovery of the decapeptide gramicidin S by G. Gause and M. Brazhnikova²³ this class of compounds has developed into a powerful branch of therapeutic agents. Their ability to resist proteases is widely used in drug development.

The synthesis of cyclic peptides is associated with certain difficulties. It has to be planned well and requires relatively advanced laboratory techniques, high dilutions (to avoid *intermolecular* side reactions as far as is possible) or solid support methods as an alternative to dilution.

Cyclization of large peptides (in this context ≥ 7 amino acid residues) is, usually, not a problem.

However, shorter derivatives are strongly dependent upon colinearity of the two reactive sites.

There are numerous reactions facilitating formation of intramolecular bonds e.g.²⁴:

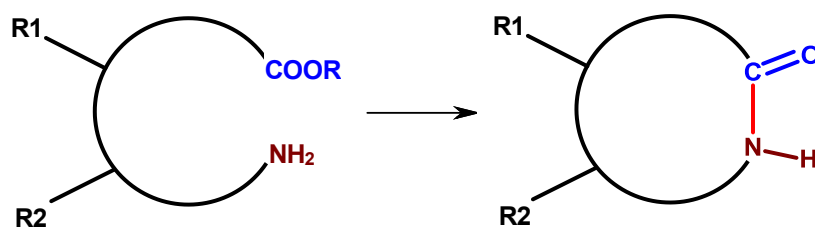
- azide-alkyne cycloadditions,
- formation of esters
- formation of peptide bond
- formation of sulfur bridges

Some of these methods can be applied to our model compounds.

It is essential to have the two reactive centers close to each other to avoid steric constraints and thereby promote *intramolecular* interaction.

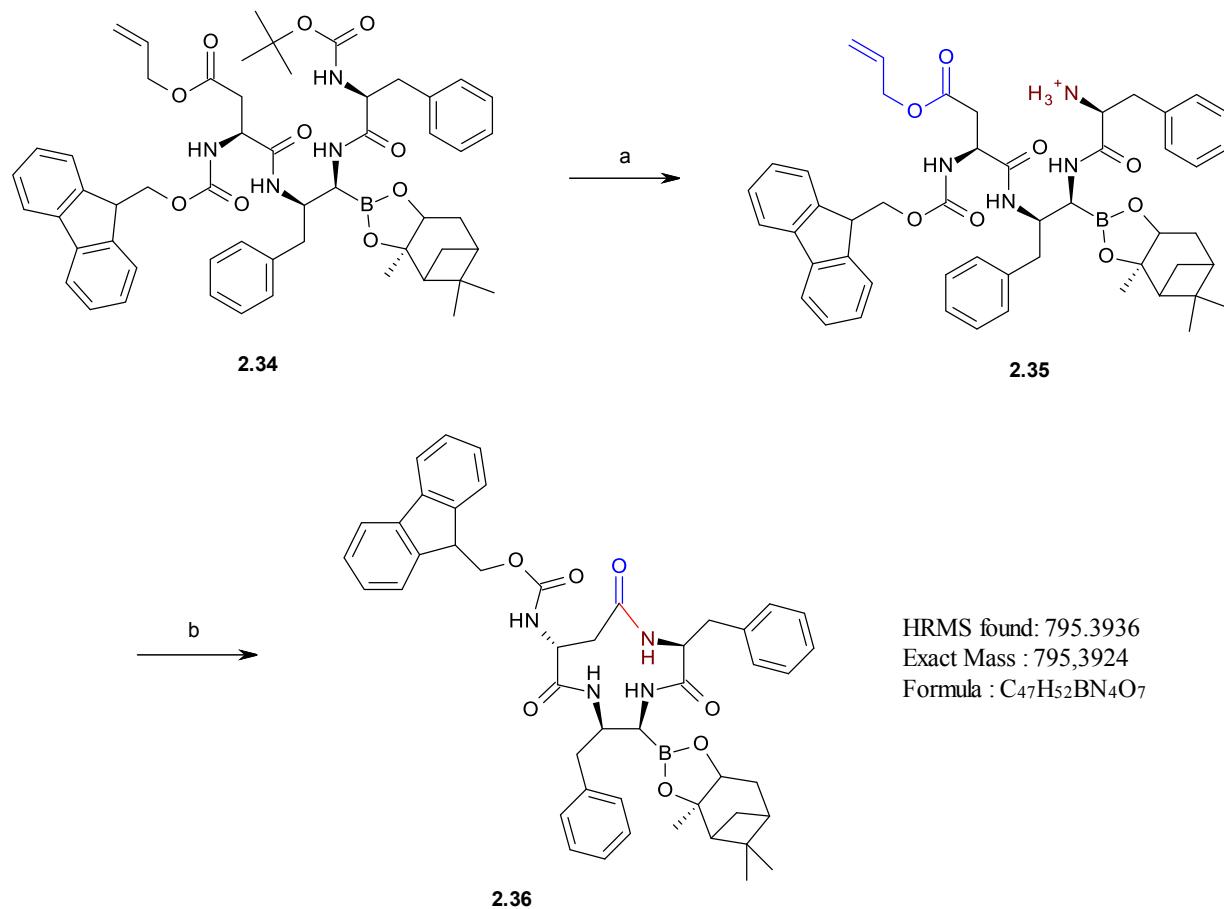
When considering the configuration of adjacent chiral centers in the positions α - and β - to the boron atom, it becomes clear that the amino groups α - and β - to boron can be oriented in a way that favors the formation of cyclic peptides (if suitable amino acids are chosen for the coupling).

The model, chosen for the first cyclization attempt, was a protected tripeptide-mimetic synthesized according to Scheme 2.9. The initial idea being to create a peptide bond in a “head-to-tail manner” (Scheme 2.11).



Scheme 2.11 “Head-to-tail” cyclization.

For this purpose we synthesized a compound that combined a protected aspartic acid residue in the β -position and a protected phenylalanine moiety in the α -position (**2.34**) (see Scheme 2.12).



Scheme 2.12 11-membered ring formation. a: HCl/MeOH b: MW, t°, H₂O/tBuOH.

Making a careful choice of protecting groups provided the opportunity to deprotect and modify various positions separately. At the next step we deprotected the amino-group of phenylalanine releasing **2.35** which could be cyclized directly. The method of choice was a microwave assisted cyclisation similar to the one utilized in the cyclisation of diketopiperazines.²⁵ This method seemed convenient for the test reactions, as it did not require a lot of starting compound, so we could start immediately with the allyl-protected substrate **2.35**. In the event that this approach

failed, we would still be able to deprotect the aspartic acid and work with other coupling procedures.

In the model reaction, the hydrochloric salt of **2.35** was heated to 100 °C under microwave irradiation in a water/*tert*-butanol solution in the presence of a base for 10 or 20 minutes and the reaction was repeated at 140 °C. The reaction mixture was analyzed and HRMS spectra of the crude reaction mixture showed the presence of the target molecule **2.36** even when the lowest temperature and shortest reaction time were used. Some by-products were detected, but there were no products of *intermolecular* condensation. Prolonged reaction time and higher temperatures resulted in decomposition of the starting compound. It was not clear if starting compound reacted and then decomposed or starting compound decomposed at higher temperature. An attempt to purify **2.36** by means of HPLC was unfortunately unsuccessful, but the principle for the synthesis of cyclic derivatives of boronic acids was successfully demonstrated. It will of course be necessary to optimize the procedure for different substrates for cyclization. However, these preliminary results are most promising and are an excellent foundation for further work with these syntheses.

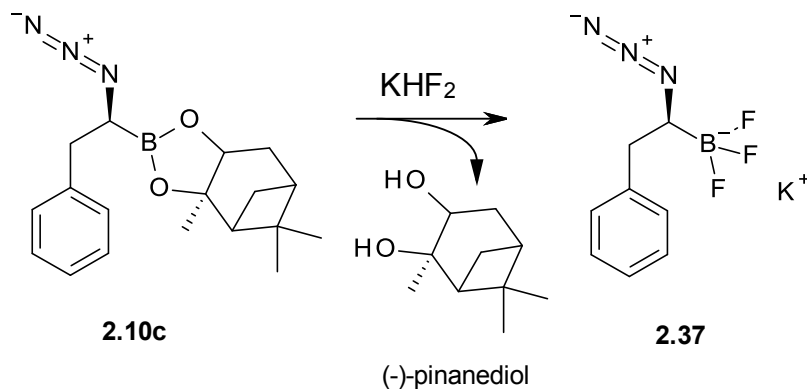
2.6. Investigations of the synthesis of all possible configurations of α,β -dialkyl substituted β -aminoboronates

The characteristic features of Matteson homologation made it possible to generate new chiral centers in consecutive order with configuration dependent on the configuration of the chiral director (specifically pinanediol).

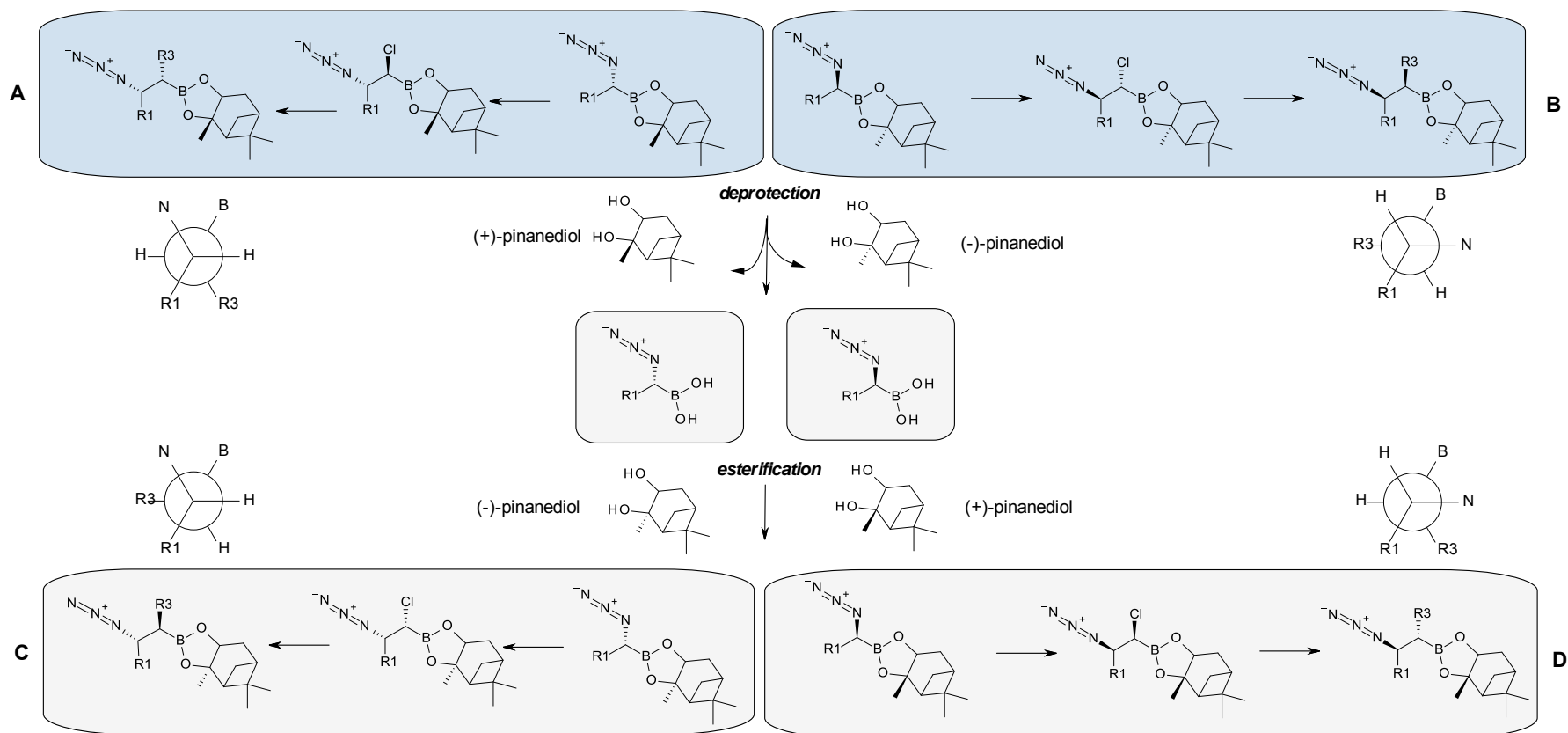
The two adjacent chiral centers present in our molecules will in general produce 4 stereoisomers. However, continuous use of a chiral director of the same optical orientation from the first to the last step gave only two stereoisomers (**A** or **B**, Scheme 2.13) and the other two isomers (**C** or **D**) required changing the pinanediol used (from (-) to (+)) in the middle of the synthetic sequence. For this reason we needed a procedure for the removing pinanediol from the ester because this manipulation would make it possible to produce the missing diastereomers.

As described earlier, the recovery of boronic acid from its pinanediol ester is not a trivial task. Available methods are limited and include cleavage by direct hydrolysis, transborylation with boron trichloride, reduction with lithium aluminum hydride, transesterification in two-phase system using phenylboronic acid and conversion to BF_3K salts.

Unfortunately all attempts to employ these methods gave unsatisfactory results. An azide in the α -position to the boron simply will not tolerate hydrolysis or transborylation conditions. The transesterification method failed because of the low water solubility of the product and the only approach that worked was the reaction with potassium bifluoride (Scheme 2.14) which gave **2.37** that can then be hydrolyzed to the corresponding boronic acid.



Scheme 2.14 Scheme of the synthesis of trifluoroborate salts.



Scheme 2.13 Synthesis of 4 possible diastereomers.

Potassium and cesium bifluorides have been successfully used for the deprotection of boronic esters.^{26,27} Reaction of **2.10c** with potassium bifluoride in methanol/water gave the target compound **2.37** in satisfactory yield. The crystals obtained were suitable for X-ray analysis (Figure 2.8).²⁸

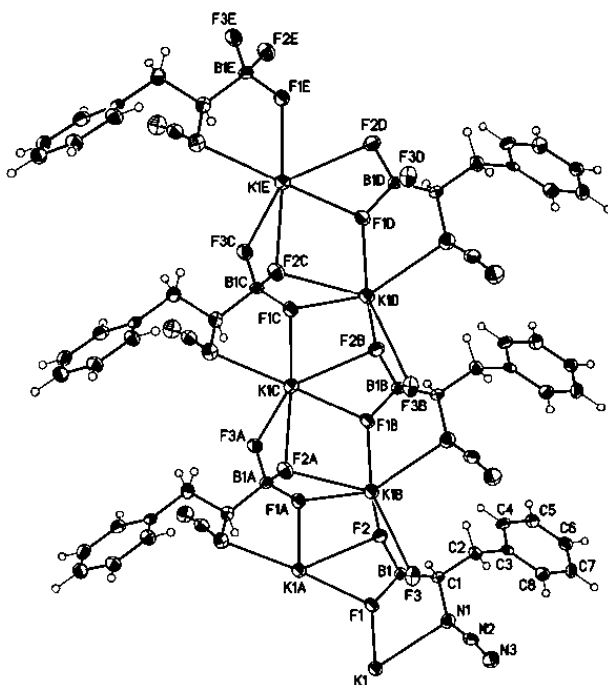


Figure 2.8 ORTEP drawing of the structure of **2.37**.

Trifluoroborates are considered to be stable precursors of dihaloboranes which, in turn, can be transformed to boronic acids and esters. Methods available for the recovery of boronic ester moiety include:

- Treatment of BF_3K -salts with SiCl_4 or Me_3SiCl providing dichloroborane followed by hydrolysis to boronic acids or esterification with alcohols to give boronic esters.
- Reaction with an excess of aqueous LiOH and subsequent esterification.

Unfortunately, application of these protocols to our substrate led to complicated mixtures and unsatisfactory yields.

Despite the ease of synthesis **2.37**, the practical application of these approaches to the deprotection of these boronic acids was limited by low yield or complete loss of product during hydrolysis and consequently, this aspect of the project was temporarily set aside but is now under renewed investigation.

2.7. Measurement of diastereomeric ratio

Matteson homologation is a well-established reaction which provides a remarkable level of stereoselectivity. Careful compliance with the methodology makes it possible to avoid any significant losses of stereopurity. Despite this, the synthesis contains some weak points (described earlier) which can lead to the partial epimerization. There are several methods for the measurement of the enantiomeric or diastereomeric excess (*ee* or *de*)²⁹ and the choice of method depends on the properties of the molecule under investigation and availability and usability of the equipment.

According to literature data³⁰ diastereomeric excess of chiral compounds containing pinanediol moiety can be estimated by NMR, measuring the characteristic doublets at 1.0 – 1.2 ppm.

Two main problems were encountered during the analysis of NMR spectra:

- Difficulties in distinguishing between signals from the product, starting compounds and diastereomers (signals resolved).
- Overlapping of signals in aliphatic region.

In order to detect products of epimerization and separate them from the set of similar peaks it was decided to provoke epimerization of α -chloroalkylboronates³⁰ by stirring a solution of the

product in THF in the presence of LiCl for 1 hour. A small sample of an α -chloroderivative was treated and a new proton NMR spectrum of the mixture was recorded. Spectrum obtained clearly showed the striking change in the integral ratio from 100:0 to 50:50 (Figure 2.9). It was, therefore, concluded that there were only traces of the second diastereomer in the initial mixture. This approach was extended to several similar compounds with the same observable result.

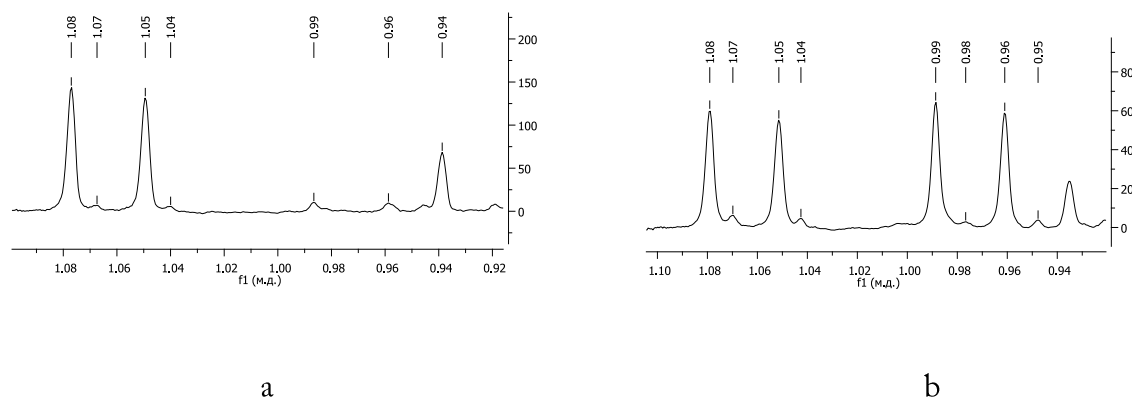
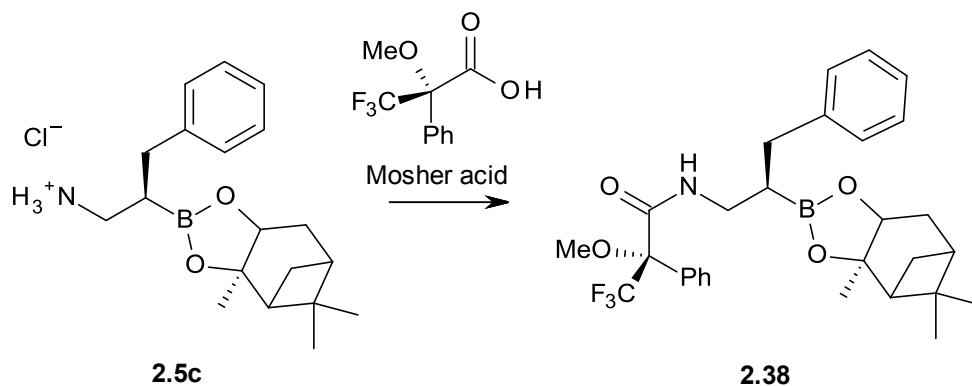


Figure 2.9. *a*: fragment of the typical ^1H NMR spectrum of the model compound before treatment by LiCl. *b*: fragment of the typical ^1H NMR spectrum of the model compound after treatment by LiCl.

Another method of determination of *de*, consisting of the irreversible conversion of target molecules to reference compounds with known configurations and optical properties is also described in the literature.³¹

We found it convenient to convert our β -aminoboronates to dipeptide-like derivatives of Mosher acid – a chiral compound containing fluorine atoms (Scheme 2.15). Even though these types of peptidoids are not described in the literature, it would be possible to prove the presence of the second diastereomer in the mixture (and determine the amount of it) by simply checking the ratio of the signals in ^{19}F NMR spectrum.

For the model experiment, a partly epimerized compound **2.5c** was obtained and coupled with Mosher acid in the usual manner.



Scheme 2.15 Synthesis of Mosher acid derivatives.

As has been mentioned earlier, the proton spectra of the synthesized compounds were usually crowded in the aliphatic region (see Figure 2.10, 0.8– 1.0 ppm as an example), and analysis of the ^{19}F spectra was a good alternative for the estimation of diastereomeric ratio.

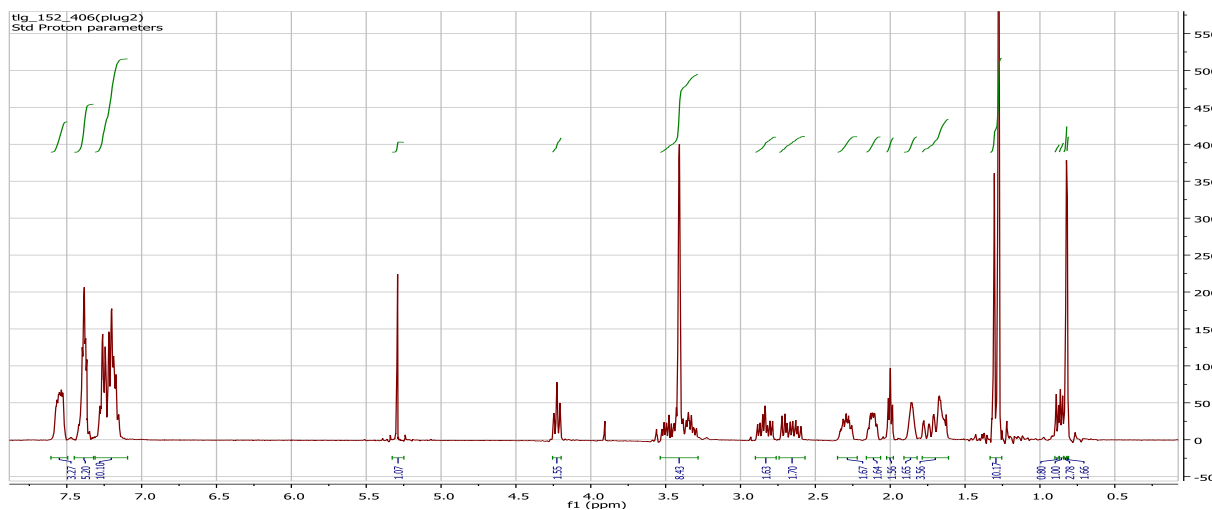


Figure 2.10 ^1H NMR spectrum (CDCl_3) of **2.38**.

Fortunately, the ^1H spectrum was not very complicated this time, thus enabling a direct comparison with the ^{19}F spectrum (Figure 2.11). As is shown in Figure 2.11 intensities of peaks were in full accord with each other.

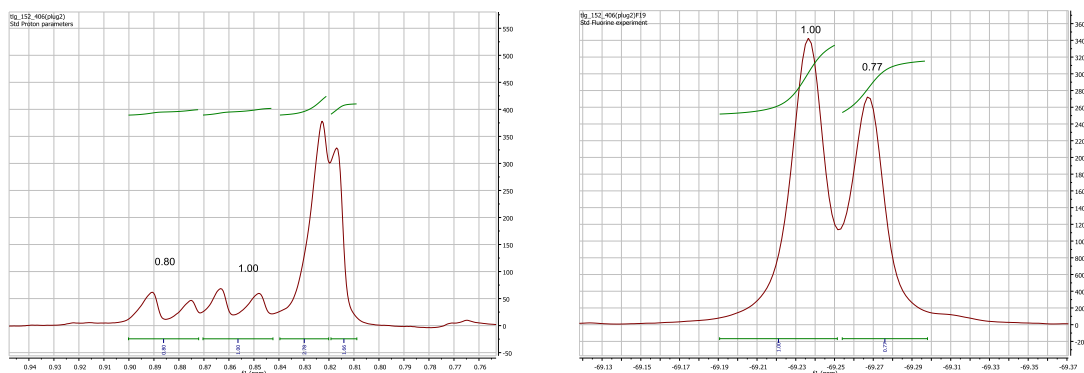
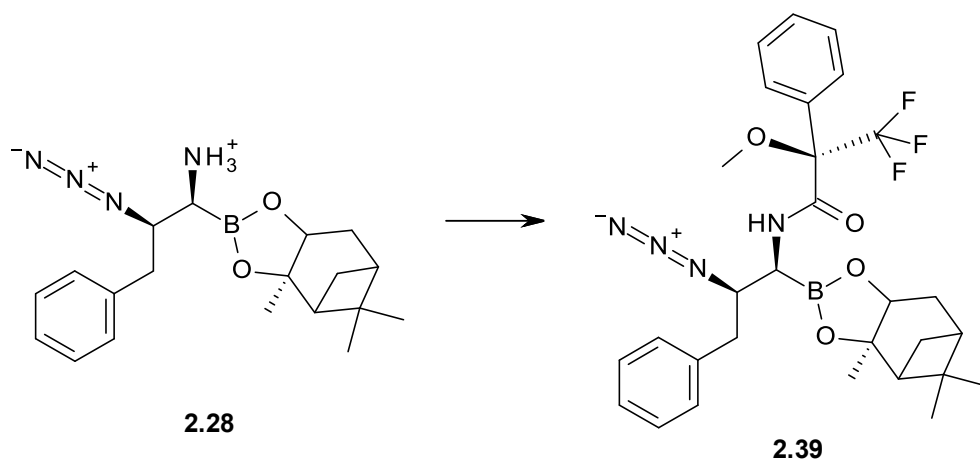


Figure 2.11 Relevant fragments of ^1H (left) and ^{19}F (right) spectra for **2.38**.

By means of this method we decided to check a compound with two adjacent chiral centers in the aminoboronate part in order to estimate losses of stereopurity during multiple rearrangements.

The coupling reaction of a model α -amino- β -azido derivative **2.28** with the Mosher acid resulted in the corresponding dipeptide **2.39** (Scheme 2.16) which was analyzed in the same manner as **2.38** (Figures 2.12 and 2.13).



Scheme 2.16 Synthesis of Mosher acid derivative **2.39**.

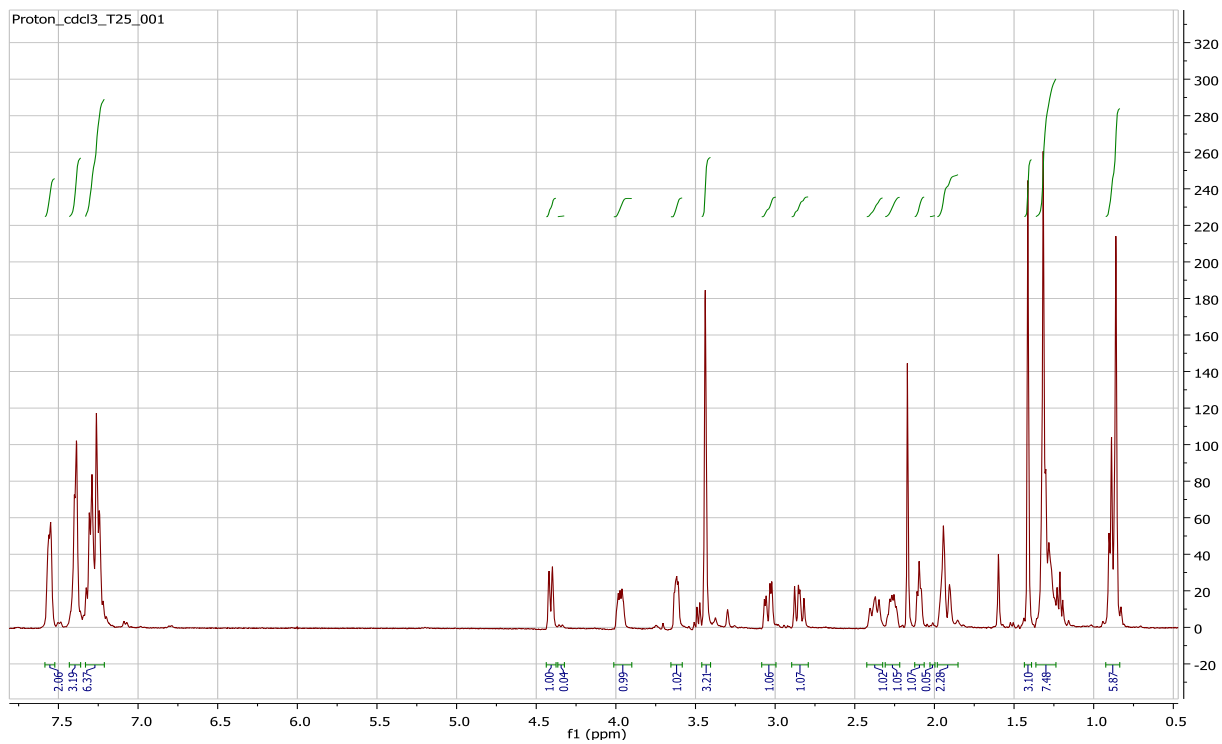


Figure 2.12 ^1H NMR spectrum (CDCl_3) of 2.39.

Analysis of the ^1H NMR spectrum revealed a trace signal at $\sim 4.3\text{ppm}$ (Figure 2.12). The signal could either belong to one of the starting compounds or to the second diastereomer. Analysis of the ^{19}F spectrum (Figure 2.13) showed the minor peak of the same intensity as in the ^1H NMR spectrum, providing evidence of the presence of another diastereomer.

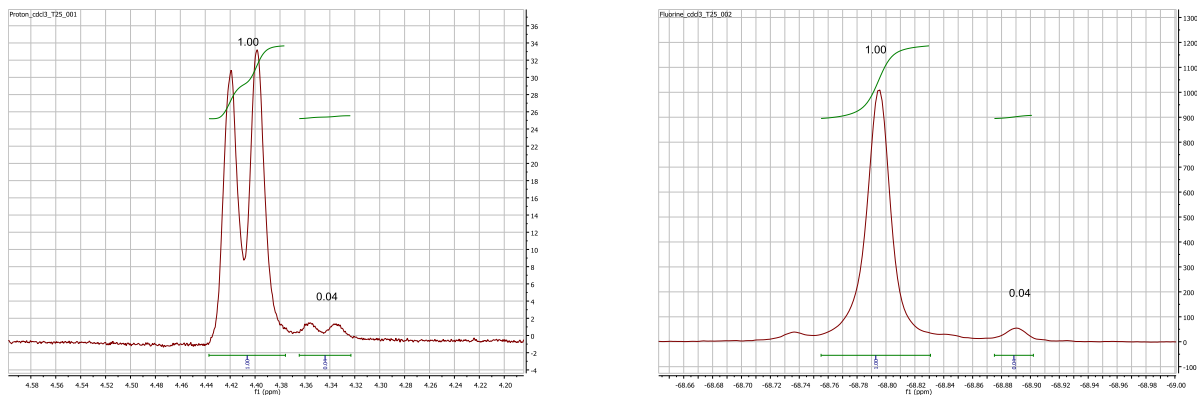


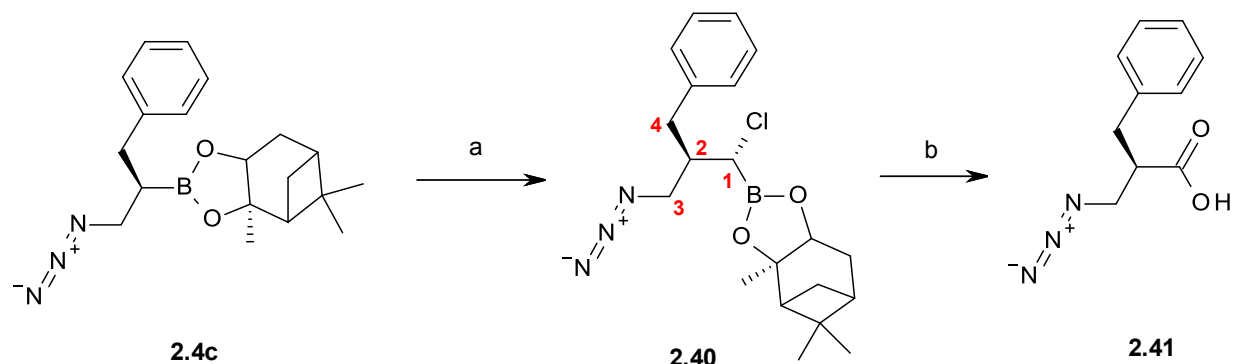
Figure 2.13 Relevant fragments of ^1H (left) and ^{19}F (right) spectra for 2.39 providing signals intensities of different diastereomers.

The experiment showed that losses of stereopurity during four consequent transformations which either created or affected chiral centers were only ~4%.

Results obtained during this investigation confirm high stereoselectivity of the homologation reaction and provide us with a convenient method for the estimation of diastereomeric excess for α - and β -aminoboronates.

2.8. Towards the synthesis of β -amino acids

Once the method for the synthesis of β -aminoboronic acids had been established, the need arose to determine whether the synthesis of β -amino acids (Schemes 2.1, 2.2, 2.3) was possible. In order to prove the concept and obtain these products, two subsequent steps were run (Scheme 2.17).



Scheme 2.17 a: 1. CH_2Cl_2 , $n\text{-BuLi}$, Ar, THF, $-100\text{ }^\circ\text{C}$, 2. ZnCl_2 , $-78\text{ }^\circ\text{C}$ b: NaClO_2 , 1-methylcyclohexene, $\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$, $t\text{BuOH}$, r.t., 16hrs.

Starting compound **2.4c** was homologated under standard conditions to obtain **2.40** with 80% conversion (see Figures 2.14-2.17 additionally supported by IR spectrum with characteristic azide asymmetric stretch: 2101 cm^{-1}).

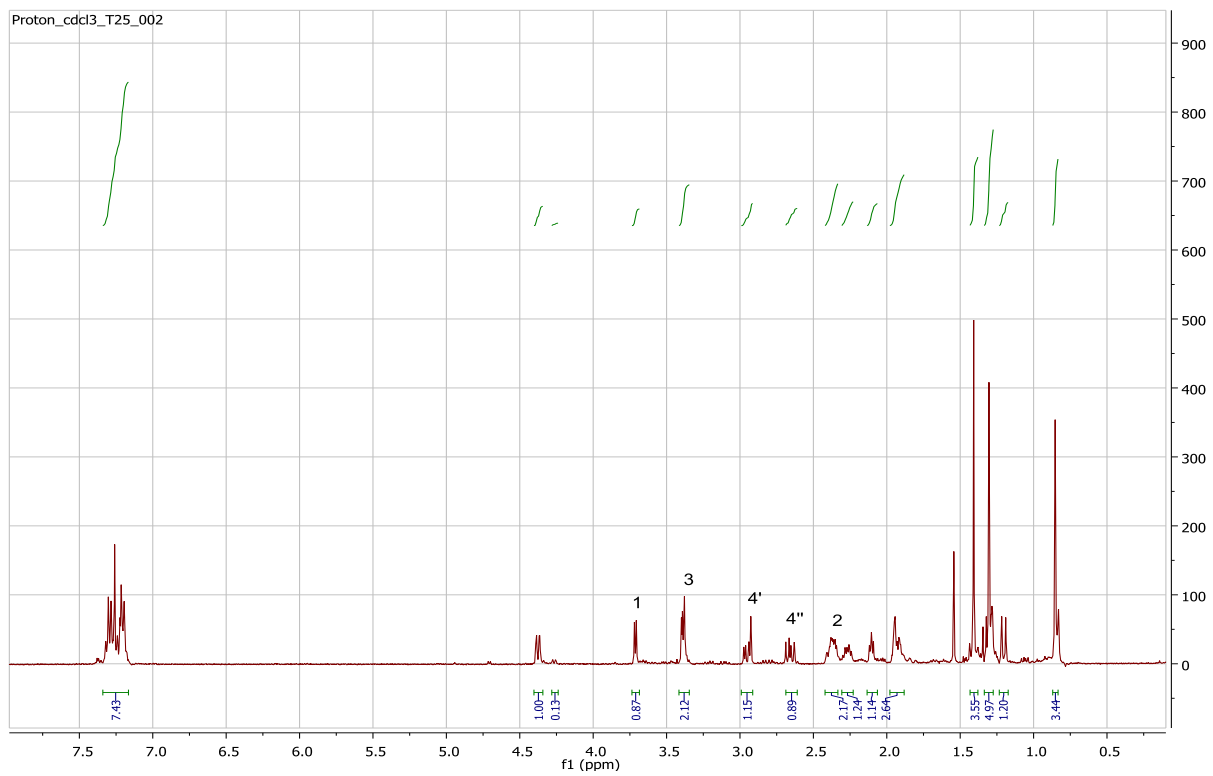


Figure 2.14 ^1H NMR spectrum (CDCl_3) of 2.40.

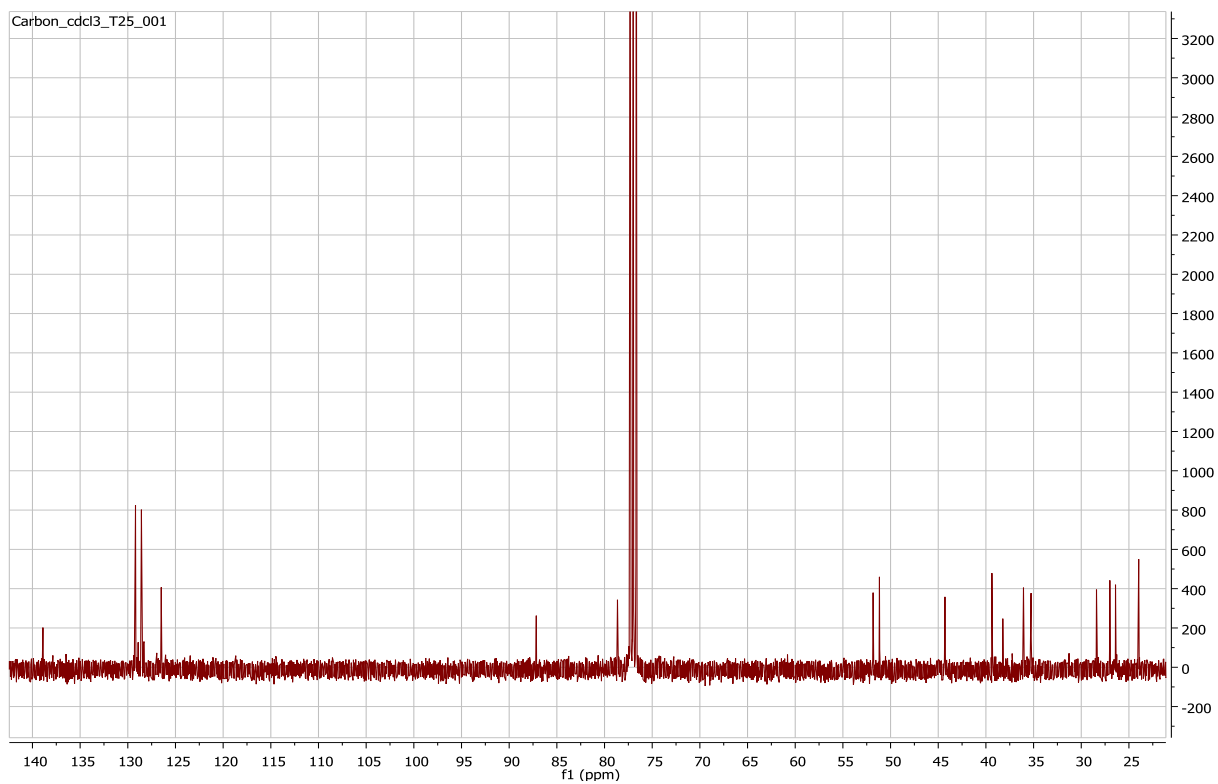


Figure 2.15 ^{13}C NMR spectrum (CDCl_3) of 2.40.

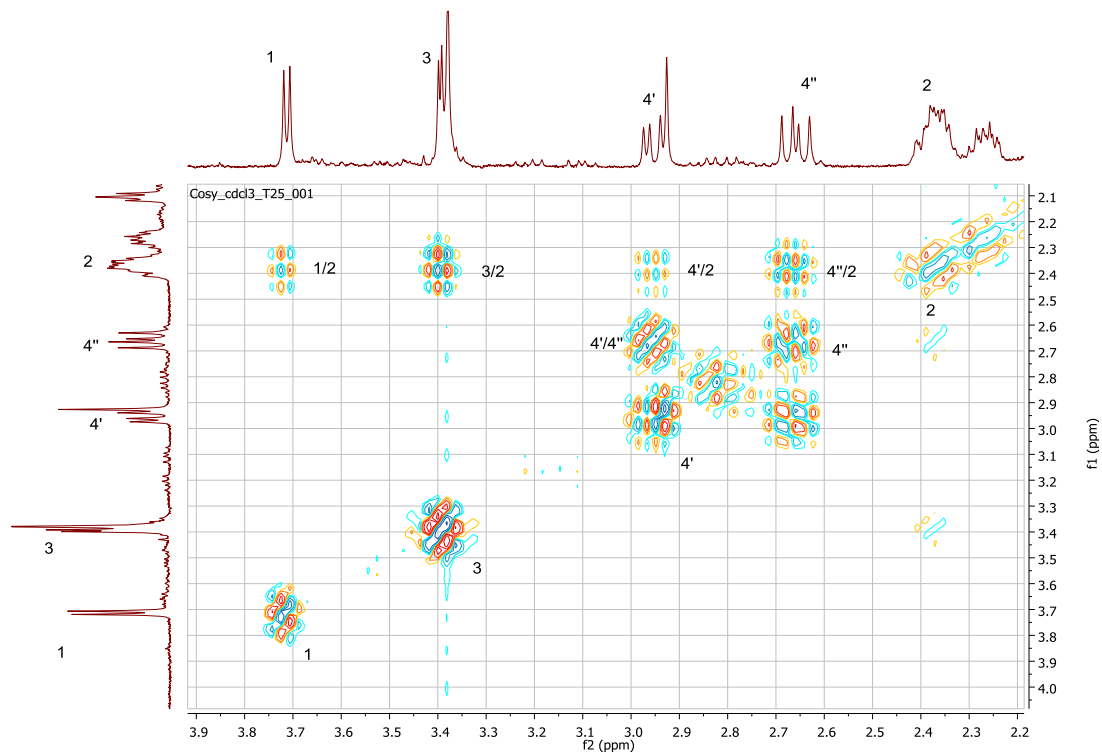


Figure 2.16 COSY spectrum (CDCl_3) of 2.40.

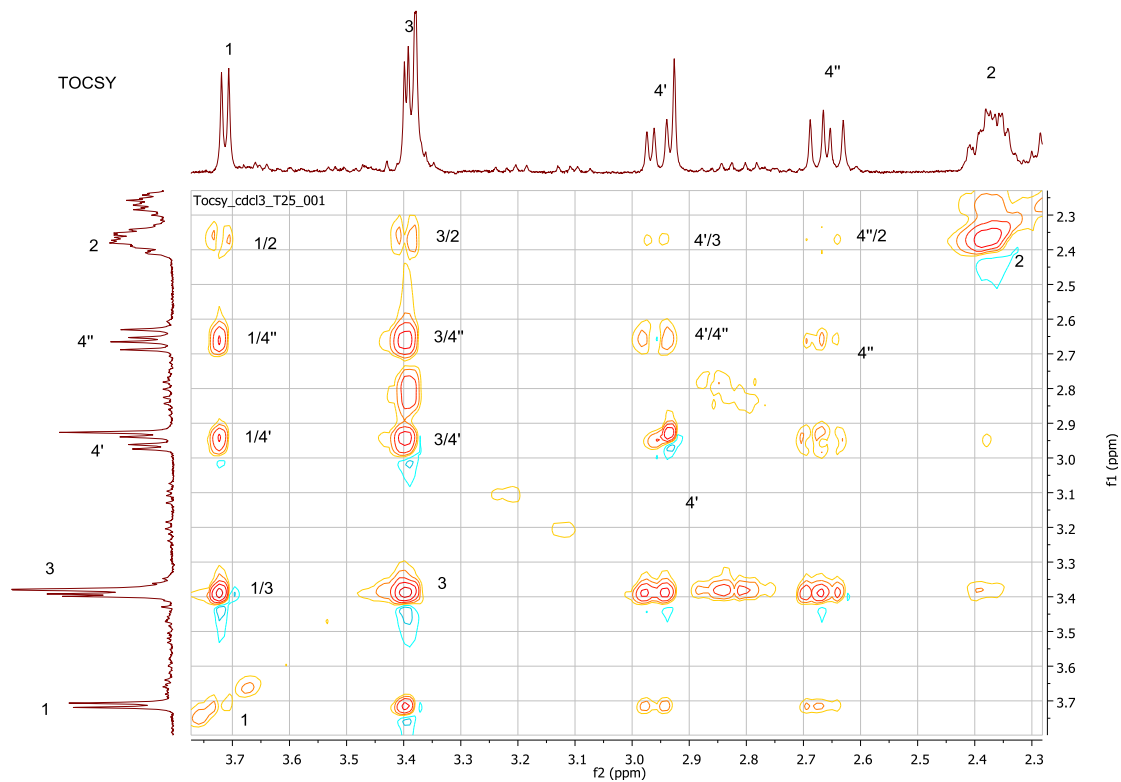


Figure 2.17 TOCSY spectrum (CDCl_3) of 2.40.

The α -chloro- β -azido derivative **2.40** was oxidized under mild conditions in accordance with literature procedures^{13,14,32} to provide a direct precursor of the desired β -amino acid **2.41** (see Figures 2.18-2.20, additionally supported by IR spectrum (cm^{-1}): broad O-H stretch (3400-2600), carboxylic C=O stretch (1706), azide asymmetric stretch (2101)).

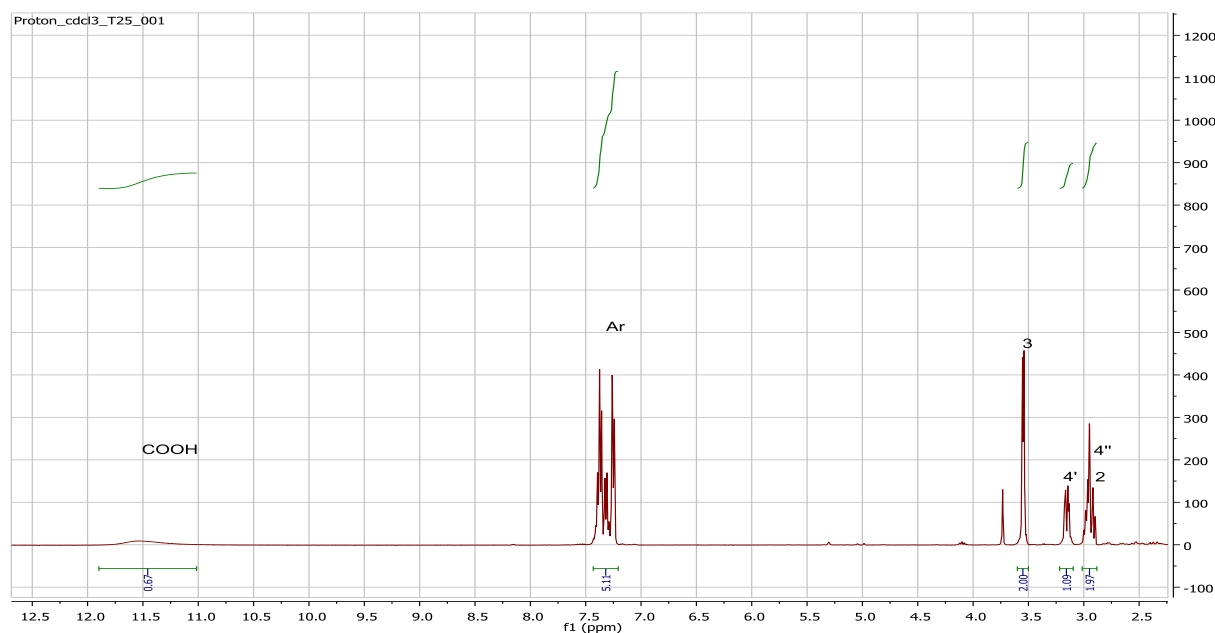


Figure 2. ^1H NMR spectrum (CDCl_3) of **2.41**.

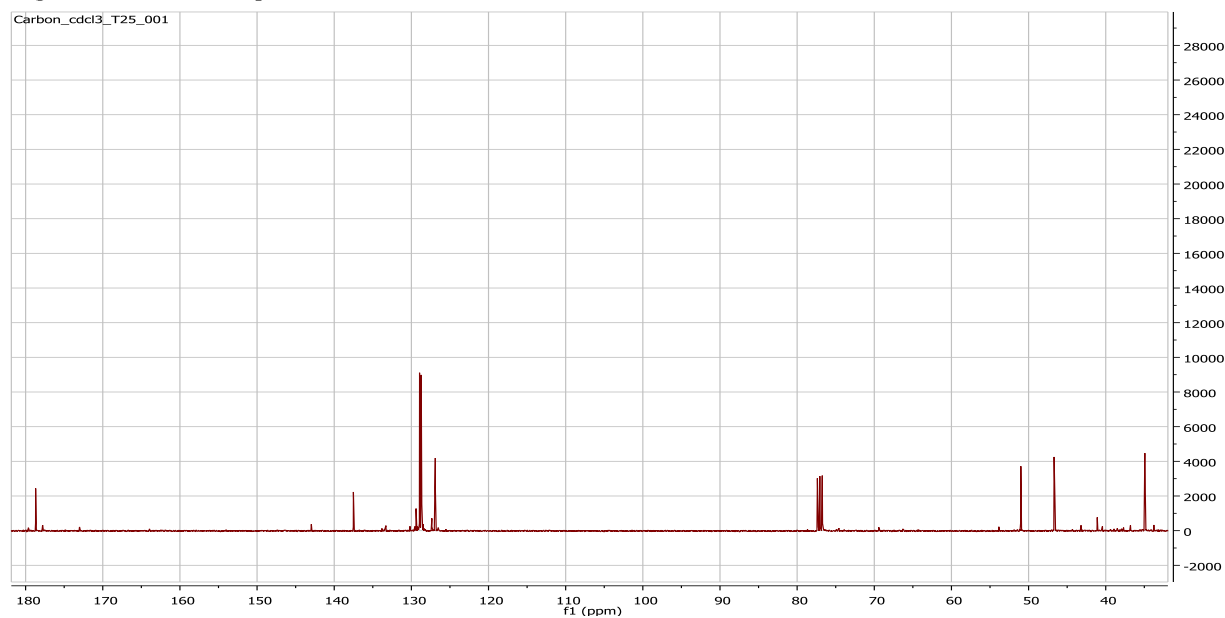


Figure 2. ^{13}C NMR spectrum (CDCl_3) of **2.41**.

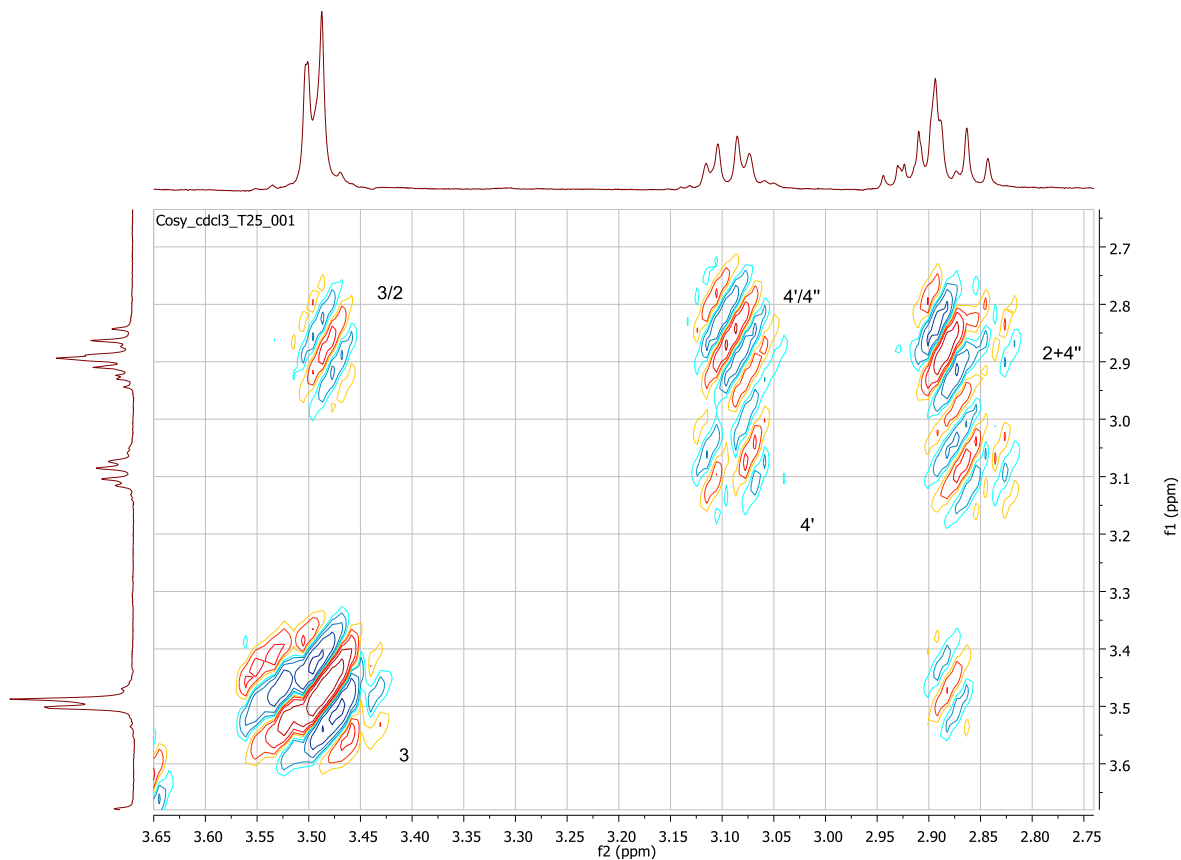


Figure 2.20 COSY spectrum (CDCl_3) of 2.41.

The spectral data was consistent with the literature data for the analogous compound.³³ The reduction of the azide is well-described in the literature and presented no difficulties.³³

The only unresolved question analytically is to positively demonstrate the stereopurity of the azide obtained, work which remains to be carried out. However, results from other syntheses that have been discussed earlier and the synthetic approach outlined in the retrosynthetic analysis indicate strongly that stereo-integrity is preserved (as no new stereocenters appeared and no reactions which touched existing stereocenters were carried out) the choice of configuration being entirely under the control of the chemist carrying out the work. With the caveat with regard to completing the analytical work taken into account we believe that this method may be regarded

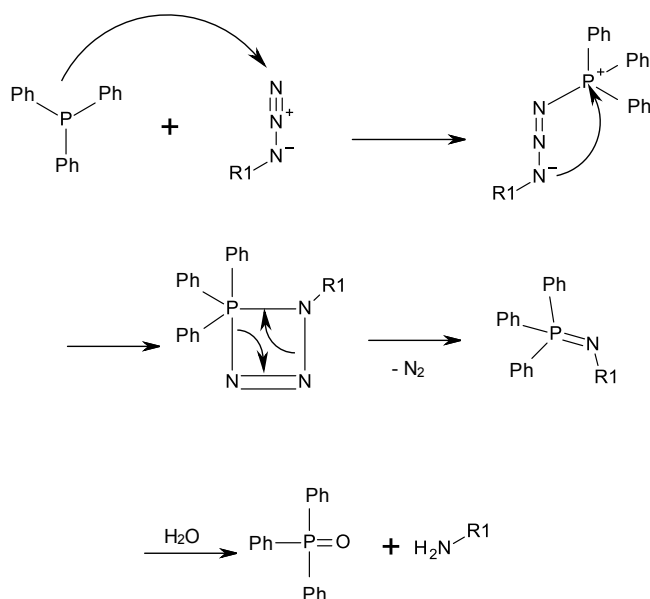
as a new stereoselective approach to the synthesis of β -substituted β -amino acids and can be warmly recommended as a method of choice for constructing libraries based on this class of non-natural amino acids.

2.9. Reduction of the azido group using the Staudinger reaction

"Marge, I agree with you - in theory. In theory, communism works. In theory."

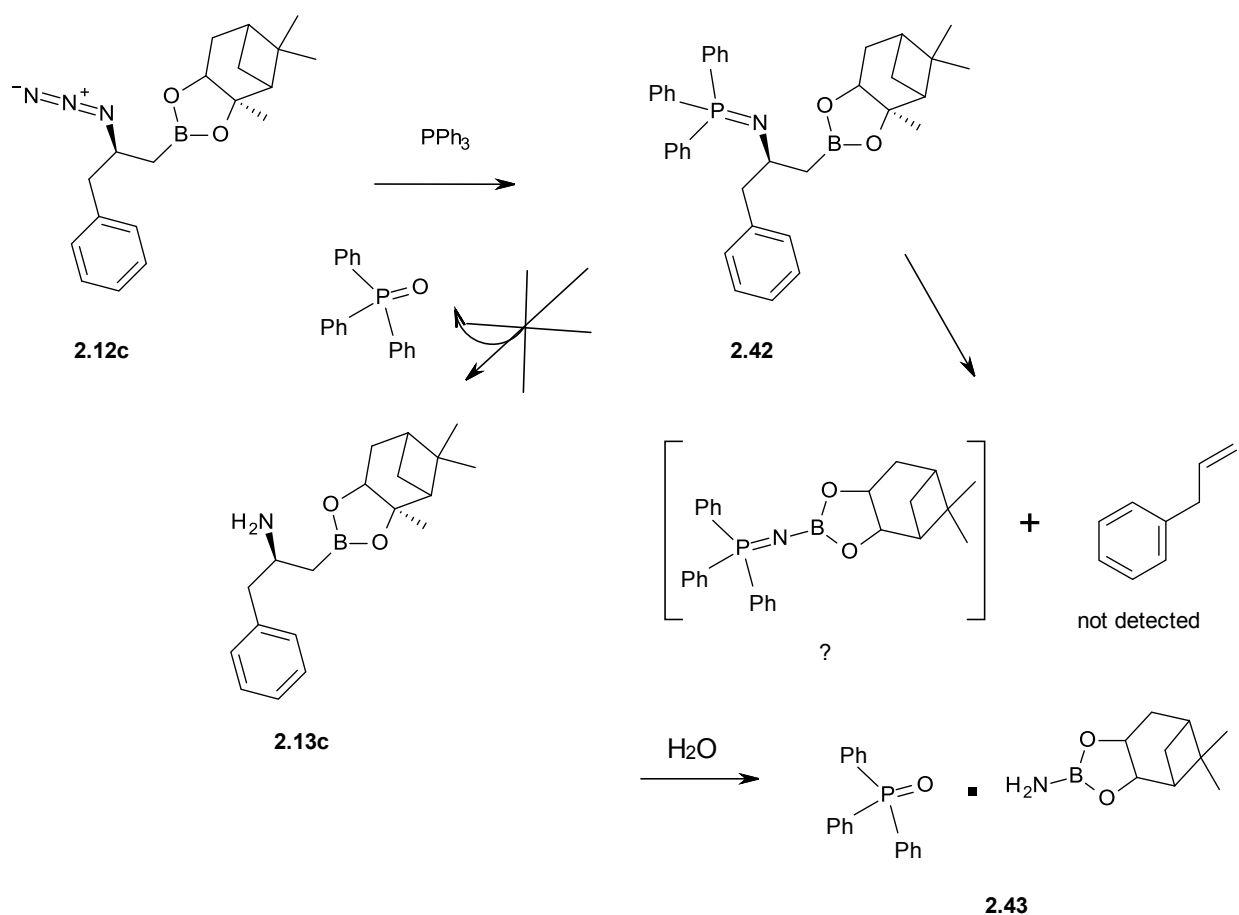
Homer Simpson.

As previously mentioned (chapter 2.2), it was attempted to obtain amino-group by reducing the azide under Staudinger reaction conditions. It is well established, that the reaction of organic azides with triphenylphosphine in absence of water provides phosphazide followed by release of nitrogen and iminophosphorane formation. After reaction with water, this intermediate results in the phosphine oxide and a free amine (Scheme 2.18).



Scheme 2.18 Mechanism of the Staudinger reaction.

In our experiments the general procedure for the Staudinger reaction was followed (see Experimental section). Unexpectedly, in the case of compound **2.12c**, instead of the β -amino- β -benzylboronate **2.13c** the compound obtained corresponded to a complex of aminoboronic ester and triphenylphosphine oxide **2.43** (Scheme 2.19). The structure of this complex was established with the help of X-ray analysis (Figure 2.21, Experimental Part) and NMR. To the best of our knowledge this was the first stable compound, containing a free amino-group directly connected to the boronic ester moiety.



Scheme 2.19 Staudinger reaction for β -azidoboronates.

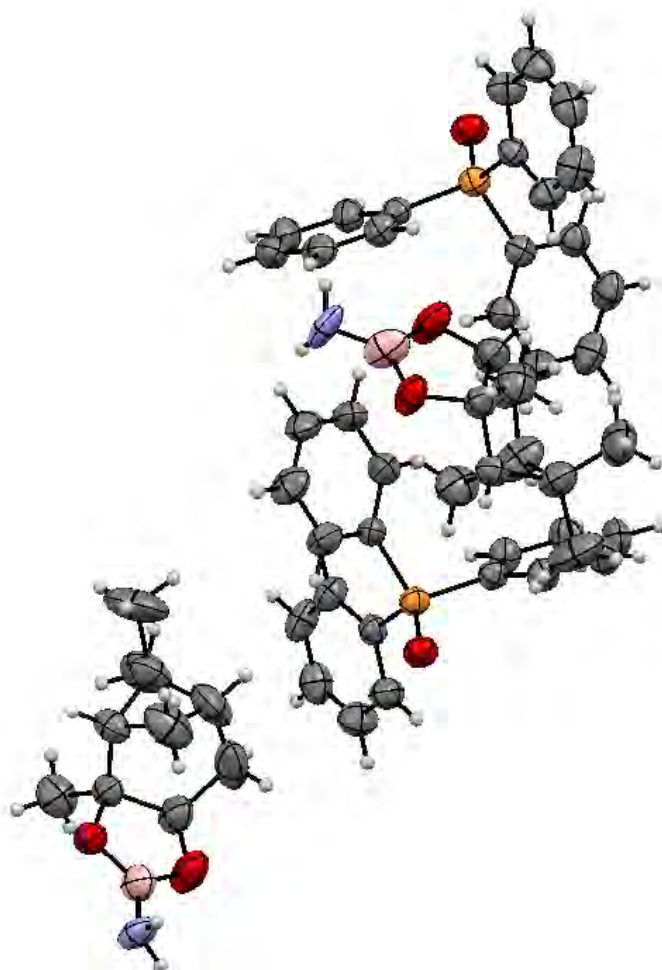


Figure 2.21. ORTEP drawing of the complex 2.43.

Compound **2.12c** was used as a substrate for a set of model reactions which were performed in order to find a plausible mechanism of this unusual transformation. In a typical experiment, triphenylphosphine and the azide **2.12c** (in 1:1 ratio) were dissolved in CDCl_3 in the NMR tube and proton spectra were recorded periodically (Figure 2.22).

After 24 hrs the reaction mixture was exposed to air or treated with water and product was extracted with pentane. The pentane solution was concentrated under vacuum and analyzed.

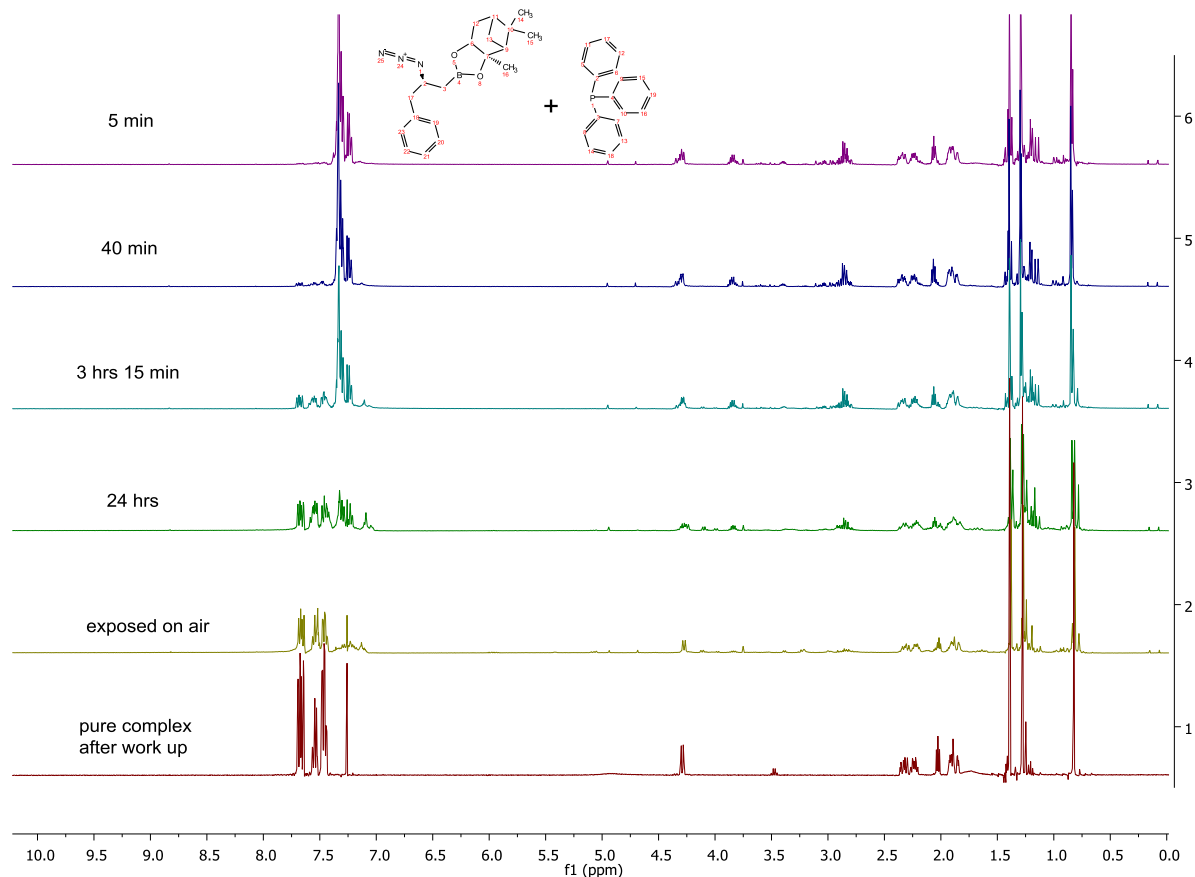
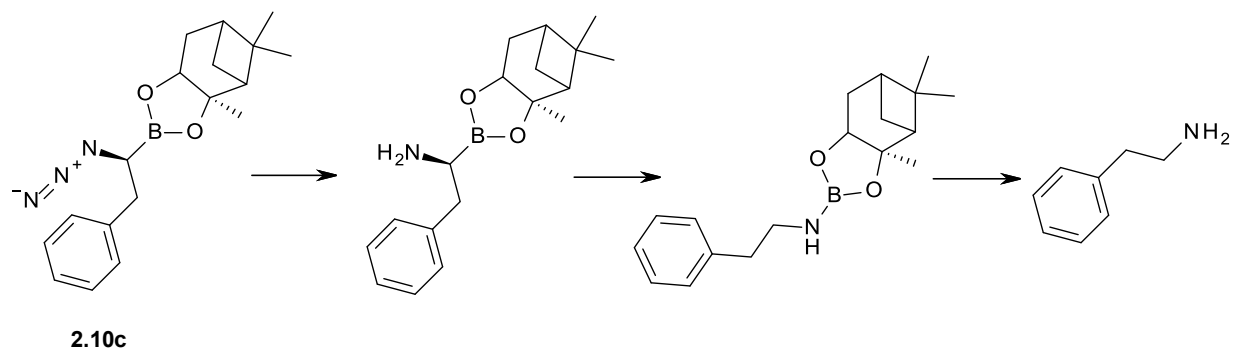


Figure 2.22 Stacked NMR spectra (CDCl₃) of the crude mixture of (2.12c) and triphenylphosphine recorded at certain time intervals.

It was quite easy to follow the formation of the product due to the appearance of new set of signals above 7.5 ppm. Unfortunately, no intermediate or product containing a benzyl moiety of the starting molecule was detected. Establishing the structure of this intermediate would assist in determining the mechanism of the reaction and testing other available azides would in all likelihood provide additional information about this transformation.

We hypothesized that α -azidoderivative **2.10c** would behave differently in this reaction. In theory, the substrate **2.10c** after reduction would result in the corresponding α -aminoboronate and rearrange to the primary amine (Scheme 2.20).²⁰



Scheme 2.20 Rearrangement of free α -aminoboronate to the primary amine.²⁰

The reaction was performed under standard conditions, but transformation resulted in the same complex of triphenylphosphine oxide and aminoboronate **2.43** (Figure 2.21). We concluded that the reaction, in all probability, went *via* the same transition state as analogous reaction for the β -azido-derivative **2.12c** (see Scheme 2.19, complex in brackets).

The next model experiment was performed with α -chloro- β -azidoderivative **2.11c**. This time, NMR spectra of the reaction mixture clearly showed the presence of double bond (see Figure 2.23) together with the same complex of aminoboronate and triphenylphosphine oxide **2.43**.

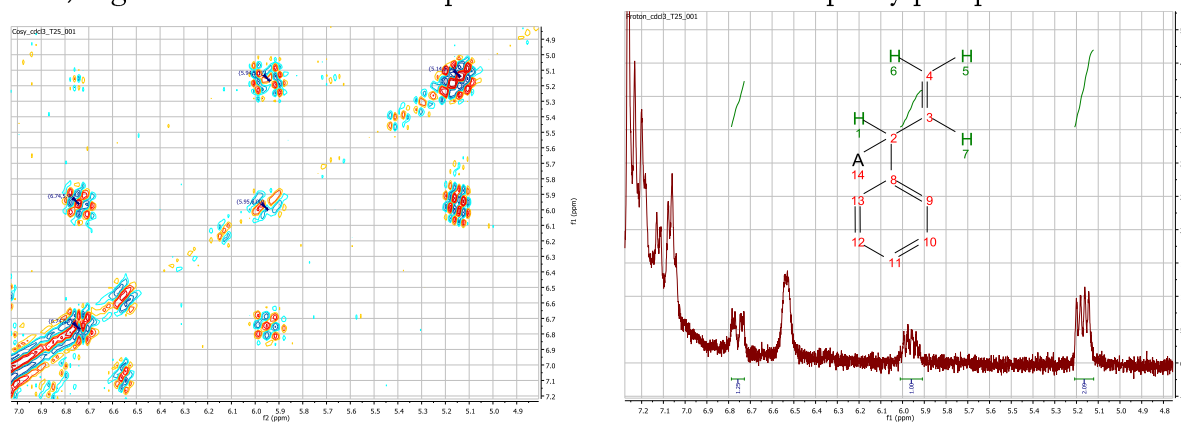


Figure 2.23 COSY and proton spectra of the reaction mixture after work up.

Even though we were able to detect a derivative that contained a double bond which could be the product of a β -elimination, the final structure and mechanism of this reaction has yet to be determined and is currently under extended investigation.

References

1. Davoli P., Fava R. et al., (2005), *Tetrahedron*, 61(18), 4427-4436.
2. Staudinger H., Meyer J., (1919), *J. Helv. Chim. Acta*, 2, 635.
3. Tian W.Q., Wang Y.A., (2004), *J. Org. Chem.*, 69, 4299-4308.
4. Brown H.C., Rangaishenvi M.V., (1988), *J. Organomet. Chem.*, 358, 15-30.
5. Snow R.J. et al., (1994), *J. Am. Chem.Soc.*, 116, 10860-10869.
6. Roy C.D., Brown H.C., (2007), *Monatsh. Chem.*, 138, 747-753.
7. Wityak J., Earl R. A., Abelman M. M., Bethel Y. B., Fisher B. N., Kauffman G. S., Kettner C. A., Ma P., McMillan J. L., (1995), *J. Org. Chem.*, 60, 3717-3722.
8. Martichonok V., Jones J. B., (1996), *J. Am. Chem. Soc.*, 118, 950-958.
9. Brown H.C., Singh S.M., Rangaishenvi M.V., (1986), *J. Org. Chem.*, 51, 3150-3155.
10. Sadhu K.M., Matteson D.S., (1985), *Organometallics*, 4, 1687-1689.
11. Kettner C.A., Shenvi A.B., (1984), *J.Biol.Chem.*, 24, 15106-15114.
12. Kettner C.A., Mersinger L., Knabb R. J., (1990), *Biol.Chem.*, 30, 18289-18297.
13. Matteson D.S., (2008), *Med. Res. Reviews*, 28, 2, 233-246.
14. Matteson D.S., Beedle E.C., Christenson E., Dewey M.A., Peterson M.L., (1987), *J. Labelled Compd. Radiopharm.*, 25, (6), 675-683.
15. Matteson D.S., Beedle E.C., (1987), *Tetrahedron Lett.* 28, 4499-4502.
16. Inglis S.R., Woon E.C.Y., Thompson A.L., Schofield C.J., (2010), *J. Org. Chem.*, 75, 468-471.
17. Meierhenrich U.J., Munoz Caro G.M., Bredehoff J.H., Jessberger E.K., Thiemann W.H.-P., (2004), *Proc Natl Acad Sci USA.*, 101(25), 9182-9186.
18. Umenzawa H., Maeda K, Takeuchi T., (1966), *J. Antibiot.*, (Tokyo), Ser. A, 19, 200.
19. Zierski M., (1969), *Tubercle.*, 50, supplement:37-39.
20. Matteson D.S., Sadhu K.M., (1984), *Organometallics*, 10, 614-618.
21. Mantri P., Duffi E.D., Kettner C.A., (1996), *J. Org. Chem.*, 61, 5690-5692.
22. Nakamura H., Watanabe M., Ban H. S., Asai A., (2009), *Bioorg. Med. Chem. Lett.*, 19, 3220-3224.
23. Gause G.F., Brazhnikova M.G., (1944), *Nature*, 154, 703-703.
24. White C.J., Yudin A.K., (2011), *Nature Chemistry*, 3, 509-524.
25. Tullberg M., Grøtli M., Luthman K., (2006), *Tetrahedron*, 62, 7484-7491.
26. Matteson D.S., Maliakal D., Pharazyn P.S., Kim B., (2006), *Synlett.*, 3501-3503.
27. Matteson D.S., Kim G.Y., (2002), *Org. Lett.*, 4, 2153-215.
28. Lejon T., Gorovoy A.S., Khrustalev V.N., (2012), *Acta Cryst.*, E68, m1048. (Paper III)
29. Eliel E.L., Wilen S.H., Doyle M.P., (2001), *Basic Organic Stereochemistry*. Wiley-Inter-Science, New York.
30. Matteson D.S., Erdik E., (1983), *Organometallics*, 2, 1083-1088.
31. Matteson D.S., (1991) *Pure & Appl. Chem.*, 63, 3, 339-344.
32. Lindgren B.O., Nilsson T., (1973), *Acta Chem. Scand.*, 27, 888-890.
33. Ma, Da-You Wang, De-Xian, Zheng, Qi-Yu, Wang, Mei-Xiang, (2006), *Tetrahedron: Asymmetry*, 17, 16, 2366-2376.

3. MICROBIOLOGICAL INVESTIGATIONS

3.1. General outline.

The literature database search gives 27 hits on the publications containing information about synthesis and application of β -aminoboronates. Application of this class of compounds was limited by inhibition¹⁻³ and stabilization⁴ of enzymes with the single work devoted to their virucide and antihypertensive properties⁵; therefore it was decided to screen the activity of this class of compounds against a selected set of microorganism. The set included gram-positive and gram-negative bacteria, fungi and *Mycobacterium tuberculosis*, *i.e.* covering the main types of microorganisms (see Experimental part 3.3 (A) for the details).

We tried to design the set of tested compounds in such a way as to cover a wide range of substitution patterns in the newly synthesized β -aminoboronates (red) and ordinary amino acids (blue) (Figure 3.1). It should also be mentioned that data from this microbiological testing was fed back into the criteria for the choice of substituents in both parts of the molecule as the synthetic work progressed.

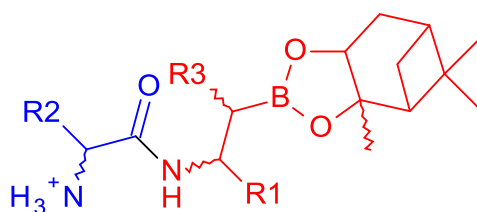


Figure 3.1 Scaffold for the tested compounds.

3.2. Results and discussions

3.2.1. Testing of α -substituted- β -aminoboronates

The investigation was initially focused on α -substituted- β -aminoboronates due to relative simplicity of synthesis. The variety of starting compounds was the largest in this case and as a result around 35 new peptidomimetics were synthesized according to certain parameters.

Parameters to screen included (see Table 3.1):

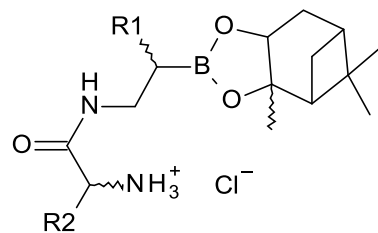
- The substituent α to the boron (R3)
- Incorporated amino acid (R2) (natural/non-natural; polar/non-polar; cationic/anionic)
- Boronic acids/esters
- Stereochemistry on either side of the amide bond

These parameters were based on prior knowledge⁶⁻⁹ discussed in the Introduction (1.1.3). The substituent α to the boron varied from hydrogen to methyl, benzyl, naphthylmethyl and phenyl in order to increase the steric bulk.

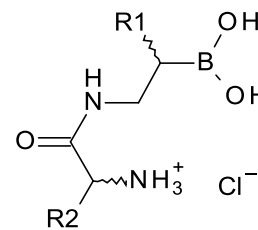
Interestingly, all tested derivatives of β -aminoboronic acid exhibited antitubercular activity to some degree (Table 3.1). The preliminary analysis of structure-activity relationships showed that the substitution pattern influenced the activity of the tested compounds.

We must conclude that there was no clear trend in activity of the boronic acids/esters, as there were compounds for which the acid was more active while the opposite was true for other compounds. Also, the larger groups incorporated into the α -position were more active than the smaller groups as seen from the low activity for unsubstituted boronates and methyl boronate.

Table 3.1 Antimicrobial activity of α -substituted- β -amino boronic acid derivatives 2.6 and 2.7.



2.6



2.7

N	R1	R2	Acid/Ester	MIC values, mg/l												
				<i>M. tub.</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>S. pyogenes</i>		<i>C. albicans</i>		
				liq.	solid	liq.	solid	liq.	solid	liq.	solid	liq.	solid	Liq.	solid	
2.6-1	H	L-Lys	(-)-pinanediol	≤500	≤500	≤500	-	-	-	-	-	-	≤500	-	≤500	≤50
2.6-2	Me	L-Phe	(-)-pinanediol	≤500	≤500	-	-	≤500	-	-	-	-	-	-	≤500	≤500
2.6-3	Me	L-Lys	(-)-pinanediol	≤50	≤500	-	-	-	-	-	-	-	-	-	≤500	-
2.6-4	phenethyl	D-Lys	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	-	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.6-5	phenethyl	L-Phe	(-)-pinanediol	≤50	≤50	-	-	≤50	≤50	-	-	≤500	≤500	-	-	-
2.7-5	phenethyl	L-Phe	acid	≤500	≤500	≤500	≤500	-	-	-	-	-	-	-	-	-
2.6-6	phenethyl	L-Lys	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	-	≤500	-	≤500	≤500	≤500	≤500	≤500
2.7-6	phenethyl	L-Lys	acid	≤500	≤500	-	-	-	-	-	-	-	-	-	≤500	≤500
2.6-7	benzyl	L-Arg	(-)-pinanediol	≤5	≤50	≤500	-	≤500	≤500	≤500	-	≤500	≤500	≤500	≤500	≤500
2.6-8	benzyl	D-Lys	(-)-pinanediol	≤50	≤500	≤500	≤500	≤500	≤500	≤500	-	≤500	≤500	≤500	≤500	≤500
2.7-8	benzyl	D-Lys	acid	≤5	≤500	-	-	-	-	-	-	-	-	-	-	-
2.6-9	benzyl	L-Phe	(-)-pinanediol	≤5	≤50	-	-	≤500	-	≤500	≤500	≤500	≤500	-	-	-
2.6-10	benzyl	L-Phe	(+)-pinanediol	≤50	≤50	-	-	-	-	-	-	≤500	≤500	≤50	≤50	-
2.6-11	benzyl	L-Lys	(-)-pinanediol	≤5	≤5	-	-	≤500	≤500	≤500	≤500	≤500	≤500	≤500	-	-
2.7-11	benzyl	L-Lys	acid	≤5	≤5	≤50	≤500	≤500	-	-	-	≤500	-	≤500	≤500	≤500

2.6-12	benzyl	L-Lys	(+)pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	-	≤500	≤500	≤500	≤50
2.6-13	benzyl	L-Ala	(+)pinanediol	≤50	≤500	≤500	≤500	≤500	≤500	-	-	-	-	≤500	≤500
2.6-14	benzyl	L-Asp	(-)pinanediol	≤50	≤500	-	-	-	-	-	-	-	-	-	-
2.6-15	benzyl	L-Asp(OMe)	(-)pinanediol	≤500	limited	-	-	-	-	-	-	-	-	-	-
2.6-16	benzyl	L-Lys-L-Gly- L-Phe-	(-)pinanediol	≤50	≤50	≤500	≤500	-	-	-	-	-	-	≤500	≤500
2.6-17	4-(F)-benzyl	L-Lys	(-)pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.6-18	4-(F)-benzyl	L-Lys	pinacole	≤50	≤50	-	-	-	-	-	-	-	-	-	-
2.7-17	4-(F)-benzyl	L-Lys	acid	≤5	≤50	-	-	-	-	-	-	-	-	≤500	-
2.6-19	4-(F)-benzyl	L-Phe	(-)pinanediol	≤50	≤50	-	≤500	-	≤500	-	-	-	≤500	-	≤500
2.7-19	4-(F)-benzyl	L-Phe	acid	≤50	≤50	≤500	≤500	≤500	≤500	≤500	-	≤500	≤500	-	-
2.6-20	4-(CF ₃ O)-benzyl	D-Lys	(-)pinanediol	≤50	≤50	-	≤500	-	≤500	≤500	≤500	≤500	≤50	≤500	≤500
2.7-20	4-(CF ₃ O)-benzyl	L-Lys	acid	≤500	≤500	≤500	≤500	≤500	≤500	≤500	-	≤500	≤500	≤500	-
2.6-21	4-(CF ₃ O)-benzyl	L-Lys	(-)pinanediol	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.6-22	4-(CF ₃ O)-benzyl	L-Ala	(-)pinanediol	≤50	≤50	-	≤500	≤50	≤500	-	≤500	≤50	≤50	≤50	≤500
2.6-23	4-(CF ₃ O)-benzyl	L-Phe	(-)pinanediol	≤5	≤50	-	-	≤50	≤50	-	-	≤50	≤50	≤500	≤500
2.7-23	4-(CF ₃ O)-benzyl	L-Phe	acid	≤50	≤500	≤500	≤500	-	≤500	≤500	-	≤500	≤500	≤500	≤500
2.6-24	2-naphthylmethyl	L-Lys	(-)pinanediol	≤5	≤50	-	≤500	≤50	≤500	≤500	≤500	≤500	≤50	≤500	≤500
2.6-25	2-naphthylmethyl	L-Phe	(-)pinanediol	≤5	≤5	-	-	-	-	-	-	≤50	≤50	≤5	≤5
2.6-26	phenyl	L-Phe	(-)pinanediol	≤500	≤500	-	-	≤500	-	-	-	-	-	≤500	≤500
2.6-27	phenyl	L-Lys	(-)pinanediol	≤500	≤500	-	-	-	-	-	-	-	-	-	-

This suggests that, as for our earlier results, a certain bulk was necessary for activity. If this was purely an effect of size or, if it was related to lipophilicity is not clear at this stage. The activity of compounds with the phenyl directly attached to the α -carbon atom (2.6-26 and 2.6-27) was also low.

That there was a weak or no discrimination between compounds of opposite stereochemistry at the α -position was also an interesting observation and it may be argued that the mechanism did not involve enzymes.

At the N-terminal end arginine and lysine exhibited high activity; this was also in accordance with earlier observations on anti-bacterial peptides in which charge was essential for activity.²⁻⁵ On the other hand, charge was not necessary for anti-tubercular activity, as seen from compounds with phenylalanine that were also highly active.

It is necessary to mention the absence of discrimination between D- and L-forms of the amino acids. Non-natural amino acids exhibited the same level of activity as their natural analogues (2.7-8 and 2.7-11) and this fact can serve as additional proof of non-enzymatic mechanism of action of these compounds.

The increase of the length of the amino acid chain was not important for the activity either, as the compound 2.6-16 showed only moderate antimicrobial activity in the standard experiment.

3.2.2. Testing of β -substituted- β -aminoboronates

The influence of alkyl- (or aryl-) substitution at β -carbon atom was also checked on the same set of microorganisms though the number of tested compounds was smaller. The set of derivatives included almost the same types of substituents except naphthylmethyl- and p-substituted benzyl group (see Table 3.2).

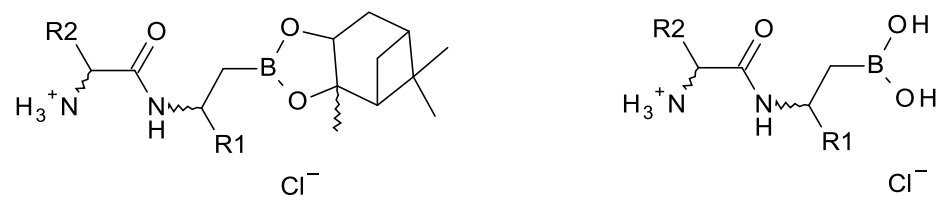
The results were similar to the α -analogs in several respects.

- Almost all compounds were efficient against *M.tuberculosis*.
- Less bulky substituents had no or very little activity.
- Phenyl derivative (2.21-1) had poor or no effect on any type of microorganisms.
- There was no trend in acid/ester activity.
- Several compounds were moderately active (≤ 50 mg/L).
- At the N-terminal end, L-lysine and L-phenylalanine showed the highest activity.

The comparison of similar derivatives with D-lysine (2.14-4) and L-lysine (2.14-3) showed a difference by a factor of hundred.

In conclusion, almost all structure-activity observations are valid for both types of mono-substituted β -aminoboronates (α and β)

Table 3.2 Antimicrobial activity of β -substituted- β -amino boronic acid derivatives 2.14 and 2.15.



2.14

2.15

N	R2	R1	acid/ester	MIC values, mg/l												
				<i>M. tub.</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>P. aeruginosa</i>		<i>S. pyogenes</i>		
				Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	
2.15-1	L-lys	methyl	acid	-	-	-	-	-	-	-	-	-	-	-	-	≤500
2.14-1	L-lys	methyl	(-)-pinanediol	≤500	-	-	-	-	-	-	-	-	-	-	-	-
2.15-2	L-lys	phenethyl	acid	-	-	-	-	-	-	-	-	-	-	-	-	-
2.14-2	L-lys	phenethyl	(-)-pinanediol	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.14-3	L-lys	benzyl	(-)-pinanediol	≤5	≤5	≤500	≤500	≤500	≤500	n/a	n/a	-	-	n/a	n/a	
2.15-3	L-lys	benzyl	acid	≤50	≤50	≤500	≤500	≤500	≤500	n/a	n/a	≤500	≤500	n/a	n/a	
2.14-4	D-lys	benzyl	(-)-pinanediol	≤500	≤500	≤500	≤500	≤500	-	≤500	-	≤50	-	≤500	≤500	
2.14-5	L-phe-L-phe	benzyl	(-)-pinanediol	≤50	≤500	-	-	≤50	≤500	≤500	≤500	-	-	-	-	
2.14-6	L-phe	benzyl	(-)-pinanediol	≤50	≤500	-	-	0	≤500	≤500	≤500	-	-	-	≤500	
2.15-6	L-phe	benzyl	acid	≤5	≤500	-	-	-	-	-	-	-	-	-	-	
2.14-7	L-trp	benzyl	(-)-pinanediol	≤50	≤500	-	-	≤500	≤500	-	-	-	-	-	-	
2.14-8*	L-arg	benzyl	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	≤500	-	-	≤500	≤500	
2.15-9	L-ala	benzyl	acid	≤500	≤500	-	-	-	-	-	-	-	-	-	-	
2.14-9	L-ala	benzyl	(-)-pinanediol	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	
2.21-1	L-ala	phenyl	(-)-pinanediol	≤500	≤500	-	-	≤500	≤500	-	-	-	-	≤500	≤500	

* Spectral data were not possible to use for the conclusions about purity.

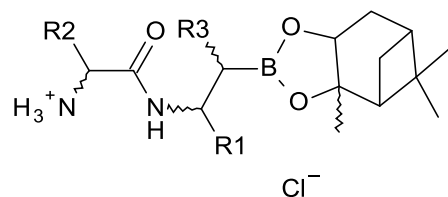
3.2.3. Testing of α,β -disubstituted- β -aminoboronates

α,β -Disubstituted derivatives possess higher conformational stability due to a relatively high rotation barrier. Taking into account the biological activity (see Table 3.3) and obviously higher conformational stability (see Results and Discussion) of these compounds they represent a convenient model for future structural investigations and structure-activity correlations. Using data from the biological investigations of α - and β -mono substituted β -aminoboronates, benzyl substituent which was presented in the active compounds was chosen and α,β -disubstituted analogs were synthesized.

Though the set of synthesized compounds included only 7 derivatives, the activity pattern was very similar to the activity of the mono-substituted analogs. The most active compound 2.25-7 (with the benzyl group in α -position) showed antitubercular activity at the level of 5mg/L. The rest of the set was also active against *Mycobacteria tuberculosis* with MIC values from 50 to 500 mg/L (in the single case 2.25-3) (Table 3.3).

Directed synthesis of the compounds with certain substitution pattern serves as a confirmation of the observations on the influence of specific substituents on the activity of the peptidomimetics.

Table 3.3 Antimicrobial activity of α,β -disubstituted- β -amino boronic acid derivatives **2.25**.



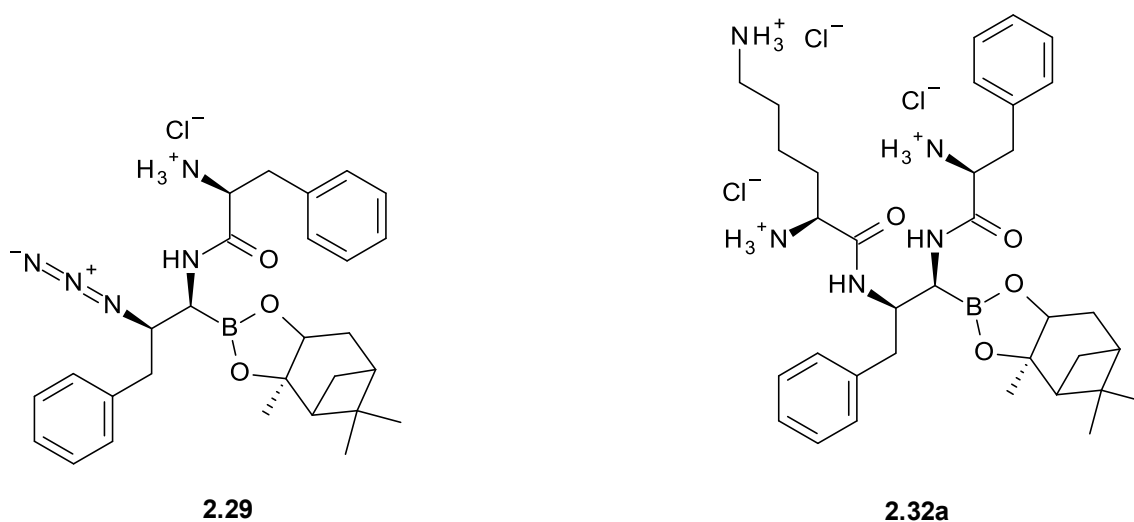
N	R3	R2	R1	acid/ester	MIC values, mg/l											
					<i>M. tub.</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>P. aeruginosa</i>		<i>S. pyogenes</i>	
					Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar
2.25-1	methyl	L-lys	benzyl	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	-	≤500	≤500
2.25-2	benzyl	L-lys	benzyl	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.25-3	methyl	L-pro	benzyl	(-)-pinanediol	≤500	≤500	-	-	≤500	-	≤500	≤500	≤500	-	≤500	-
2.25-4	methyl	L-phe	benzyl	(-)-pinanediol	≤50	≤50	-	-	≤500	≤500	≤500	≤500	-	-	≤500	≤500
2.25-5	methyl	L-lys-L-phe	benzyl	(-)-pinanediol	≤50	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500
2.25-6	benzyl	L-phe	methyl	(-)-pinanediol	≤50	≤50	-	-	-	-	≤500	≤500	-	-	≤500	≤500
2.25-7	benzyl	L-lys	methyl	(-)-pinanediol	≤5	≤50	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500	≤500

3.2.4. Testing of α,β -diaminosubstituted derivatives

Two derivatives of α,β -diaminoboronic acid (2.29) and (2.32a) were synthesized according to the Scheme 2.12 and tested.

The antimicrobial activity of these compounds was low or not detected at the level tested, for all types of selected microorganisms (see Table 3.4).

Table 3.4 Antimicrobial activity of α,β -diaminosubstituted derivatives 2.29 and 2.32a.



		MIC values, mg/l											
N	<i>M. tub.</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>P. aeruginosa</i>		<i>S. pyogenes</i>		
	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	Sol.	Agar	
2.29	≤500	≤500	-	-	-	-	≤500	≤500	-	-	-	-	
2.32a	≤500	≤500	-	-	-	-	≤500	≤500	-	-	≤500	≤500	

The number of the tested samples was too small to make any general conclusions about the influence of the structure on activity, but the presence of an α -amido oxygen atom resulted in decreasing of the antimicrobial activity of the tested compounds (compare 2.32a and 2.14-3).

The reasons for this effect are not clear but we suggested that tetrahedral configuration of boron atom (described in previous chapter) had changed the spatial arrangement of the whole molecule, reduced the Lewis acidity of boron atom and, hence affected the biological properties.

3.2.5. Inhibition of resistant strains of *Mycobacteria tuberculosis*

According to the WHO reports¹⁰, in 2010 about 9 million new cases and 1.5million deaths due to tuberculosis (TB) were detected on a global basis. Treatment of this disease is time consuming and complicated by the appearance of multidrug-resistant (MDR) and extensively drug-resistant (XDR) forms of TB.^{11,12} In this respect, several of our hit-candidates (Table 3.5) were tested on a set of 25 strains of *M.tuberculosis*, covering all possible resistance patterns. The testing was performed at the Karolinska Institutet in Stockholm, according to procedure B (see experimental details in chapter 3.3). Unfortunately, the cultivation of the *M.tuberculosis* and standardization of the biomass is not an easy task and the investigations on the resistant strains are even more resource-demanding, so some deviations are present in the results. The gap in the MIC values (when using methods A or B) can be explained by the difference in the cultivation procedures and methods of quantification of the mycobacterial biomass, used in different laboratories. According to the estimations, the bacterial load achieved in the method A was 10 times lower than in the case of method B. We considered this problem as a minor issue and concentrated on the effect that of our compounds exhibited on the resistant TB strains.

The results confirmed the inhibiting activity of our compounds, though in a lower degree (Table 3.5). The best of our compounds, **2.6-25**, was a (-)-pinanediol ester of boronic acid and

had a naphthylmethyl group in the α -position and a phenylalanine moiety thus corresponding to a bulky and hydrophobic compound.

The most important result of the investigation was the total inhibition of all types of resistant strains, something that may be an indication that a new mechanism of action is involved.

These results can give additional hints about SAR and make the search for the correct direction for the further modification of our derivatives easier.

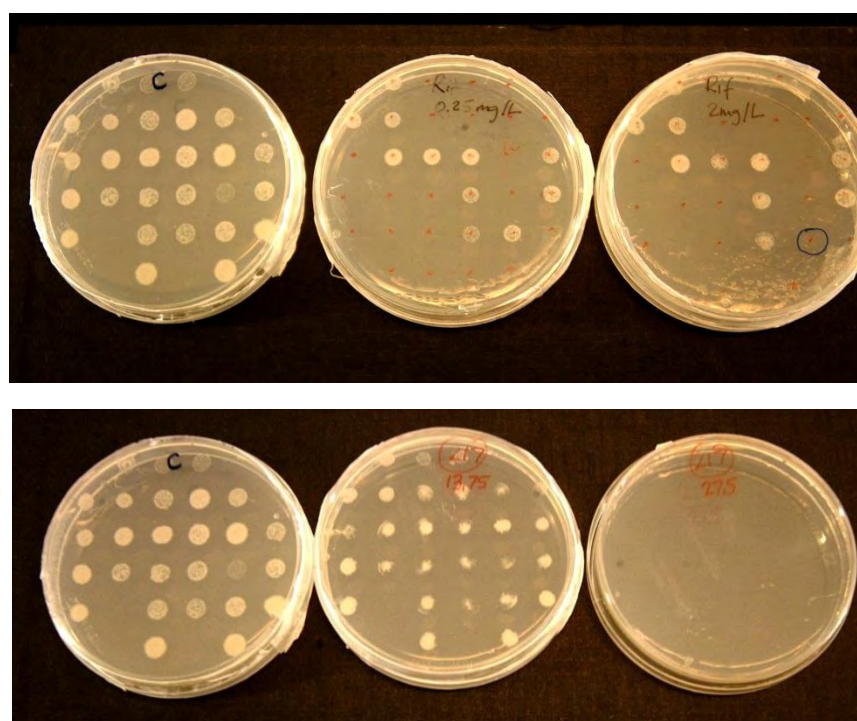


Figure 3.2 Colonies of the resistant *M. tuberculosis* strains in the petri dishes. Upper picture: Control / Rifampicin 0.25mg/L / Rifampicin 2mg/L. Lower picture: Control / 2.6-25 (13.75mg/L) / 2.6-25 (27.5mg/L).

Table 3.5 Activity of the selected lead-compounds against drug-resistant *Mycobacterium tuberculosis* (Rifampicin was used as internal control for strains and methodology technique: agar dilution, method B (Experimental part 3.3)).

SMI ID	INH	SM	RIF	EMB	OFX	CAP	AMI	KM	Rif range: 0.25-2 mg/L	2.7-11	2.14-3	2.7-17	2.6-23	2.6-8	2.6-12	2.6-25 range: 6.875-55 mg/L
XTB10-215	R	R	R	R	R	R	R	R	>2	>128	>128	>128	64	128	128	27.5
XTB10-213	R	R	R	S	S	S	S	S	>2	>128	>128	>128	64	128	128	27.5
XTB10-212	R	R	R	R	S	R	R	R	>2	>128	>128	>128	64	128	128	27.5
XTB10-210	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	128	128	27.5
XTB10-208	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	128	128	27.5
XTB10-207	S	S	S	S	S	S	S	S	≤0,25	128	128	>128	64	128	128	27.5
XTB10-206	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	128	128	27.5
XTB11-176	R	R	R	R	R	S	S	S	>2	>128	>128	>128	64	128	128	27.5
XTB11-177	R	R	R	S	S	S	S	S	>2	>128	>128	>128	64	>128	128	27.5
XTB11-180	R	R	R	S	S	S	S	R	>2	>128	>128	>128	64	128	128	27.5
XTB11-181	S	S	S	S	S	S	S	S	≤0,25	128	>128	>128	64	128	128	27.5
XTB11-182	R	R	R	R	S	S	S	S	>2	>128	>128	>128	64	128	128	27.5
XTB11-183	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	128	128	27.5
XTB11-184	R	R	R	S	S	S	S	R	>2	>128	>128	>128	64	128	128	27.5
XTB11-189	S	S	S	S	S	S	S	S	≤0,25	128	>128	>128	64	128	128	27.5
XTB11-190	S	S	S	S	S	S	S	S	≤0,25	128	>128	>128	64	128	128	27.5
XTB11-191	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	128	128	27.5
XTB11-192	S	S	S	S	S	S	S	S	≤0,25	>128	64	>128	64	64	128	27.5

XTB11-193	R	R	R	R	S	S	S	S	2	>128	>128	>128	64	>128	128	27.5
XTB11-194	R	R	R	S	S	S	S	S	>2	>128	>128	>128	32	128	128	27.5
XTB11-195	R	R	R	S	S	S	S	S		>128	>128	>128	64	>128	128	27.5
BTB 11-299	R	R	R	R	R	S	S	S	>2	>128	>128	>128	64	128	128	27.5
BTB 10-092	R	R	R	R	R	S	S	S	no growth	no growth	no growth	no growth	no growth	no growth	no growth	no growth
BTB 10-344	R	R	S	R	R	S	R	R	≤0,25	>128	>128	>128	64	>128	128	27.5
BTB 10-349	R	R	S	R	R	S	S	S	≤0,25	>128	>128	>128	64	>128	128	27.5
H37Rv	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	>128	128	27.5
H37Rv	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	>128	128	27.5
H37Rv	S	S	S	S	S	S	S	S	≤0,25	>128	>128	>128	64	>128	128	27.5

AMI, amikacin; CAP, capreomycin; EMB, ethambutol; INH, isoniazid; KM, kanamycin; OFX, ofloxacin; RIF, rifampin; SM, streptomycin.

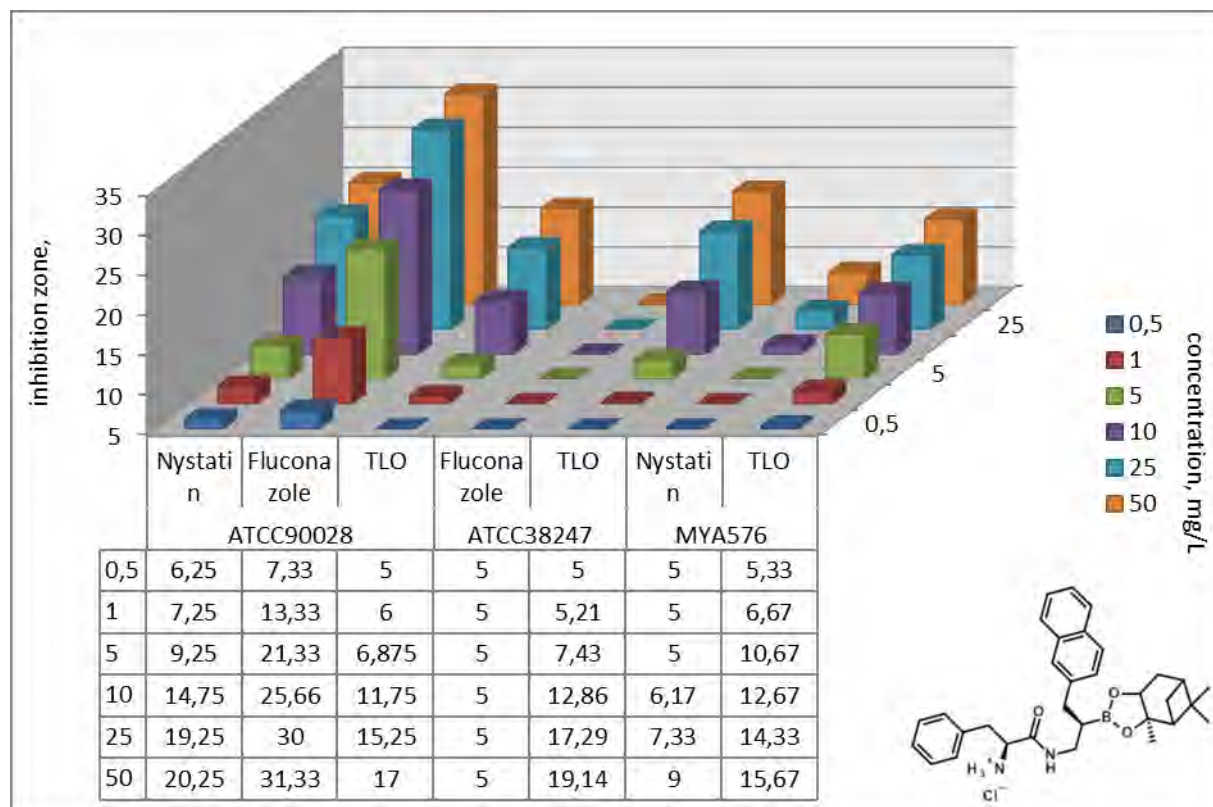
3.2.6. Inhibition of resistant strains of *Candida albicans* (Paper III)

In the course of antimicrobial screening, the exceptional antifungal activity of **2.6-25** against the common human fungal pathogen *Candida albicans* was detected. According to the data obtained (Table 3.1) the compound inhibited fungal growth at concentrations less than 5 mg/l. Further investigations were undertaken in order to determine limits of the antifungal activity of the **2.6-25**.

In addition to *C. albicans* ATCC90028, two other strains *C. albicans* ATCC38247 and *C. albicans* ATCC MYA576 possessing resistance to the two medicines (fluconazole and nystatin respectively) widely used for topical applications in treatments of mycosis, were tested. Investigations were performed by paper disk sensitivity test as described by Jennison and Stenton.¹³ The summary of the sensitivity test are presented in the Table 3.6.

Even though the compound under investigation exhibited lower activity against the sensitive strain ATCC90028 when compared with nystatin and fluconazole, the overall level of activity was considered as high (~1 mg/L). In addition, compound **2.6-25** remained active against strains resistant to the reference substances (Table 3.6) which suggests that the cellular target of this compound and the mechanism of action is different than those of nystatin and fluconazole, which effect structure or biosynthesis of fungal ergosterol containing cell membranes.^{14,15}

Table 3.6 Antifungal activity of 2.6-25. The evaluation was done by measurement of inhibition zones diameter, where value of 5 mm corresponds to the disk diameter and no inhibitory effect (see paper III for the experimental details).



3.3. Experimental part

A: Experiments were performed at the Department of Microbiology, Virology, and Immunology, St Petersburg State Pavlov Medical University.

In the screening experiments *Mycobacterium tuberculosis* (H37Rv), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* (ATCC 19615) and *Pseudomonas aeruginosa* (ATCC 27853) were included.

The liquid media used for growth were Luria-Bertani (Becton Dickinson, Sparks, MD) and Mueller-Hinton (bio-Merieux, Paris, France) broths and Middlebrook 7H9 medium (Difco) for mycobacteria.

The strains were grown at 37 °C and after suitable cell concentrations had been reached, 100 µL of each cell suspension was added to a tube with growth media and test compound. Cultivation of the bacteria was then carried out in the presence of each test compound at 37 °C and the tubes examined for visible growth. The compounds were tested at concentrations of 500 mg/l, 50 mg/l or 5 mg/l. This assay was repeated twice.

M. tuberculosis (strain H37Rv) was cultivated in Middlebrook 7H9 medium (Difco) or on Middlebrook 7H10 agar plates at 37 °C. For testing *M. tuberculosis* was cultivated in 4 mL of broth until the culture reached a concentration of approximately 1×10^8 cfu/mL and then diluted 10 times in PBS to yield a suspension with minimal viscosity and a concentration of bacteria of approximately 1×10^8 cfu/mL and 100 µL of this suspension was added to a tube with media with tested agent in concentrations 500.0, 50.0 or 5.0 mg/l. After cultivation at 37 °C from the tube where growth was determined the aliquots were plated on Middlebrook 7H12 agar to determine the presence of bacterial growth and identification of bactericidal or bacteriostatic effect of tested peptide.

In a further experiment, a sample of cells from the test tubes in which the above broth activity assays were plated on agar to determine the presence of bacterial or fungal growth. The CFUs were counted.

B: Experiments were performed at Karolinska Institutet, Stockholm, Sweden.

M. tuberculosis strains

Twenty five clinical isolates of *M. tuberculosis* were selected from the strain collection at SMI. All strains had earlier been tested for resistance to the anti-tuberculosis drugs isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin, capreomycin and ofloxacin in the Bactec MGIT 960 system (Becton Dickinson Biosciences, Sparks, MD). Of these 13 exhibited multidrug resistance (MDR), i.e. resistance to at least isoniazid and rifampicin, one was classified extensively drug resistant (XDR), i.e. MDR plus resistance to a fluoroquinolone and one of the second line injectables and 10 was fully drug susceptible. The *M. tuberculosis* reference strain (ATCC 25618) was included as a drug susceptible control.

MIC determinations using agar dilution method

The DST (drug susceptibility test) inoculums, to be used for MIC (minimum inhibitory concentration) determinations, was prepared from bacterial growth on Löwenstein-Jensen egg medium in 37 °C, not older than three weeks. Briefly, two 1µL-loops of bacteria were suspended in 3 ml of phosphate buffered saline (PBS) in a small glass tube with glass beads. Homogenization of the bacterial suspension was made using an ultrasound water bath to disperse clumps. Thereafter the suspension was left to sediment for 20 min and the upper 2 mL were transferred to a new tube and let to sediment for another 15 min. The suspensions were then adjusted to a

turbidity equivalent to that of a 0.5 McFarland standard with additional PBS. Subsequently, 200 μ L of each adjusted suspension was transferred to a 32-well plate. One well was filled with PBS to serve as a negative control and the fully drug susceptible *M. tuberculosis* reference strain was tested in triplicates as a control of reproducibility. To define the MIC of each strain, stock solutions of 80-1280 mg/L of each of the seven drugs (for rifampicin: 2.5-20 mg/L) were prepared by dissolving the compound in DMSO (Sigma-Aldrich, Germany) and diluting in sterile water. Middlebrook 7H10 agar (BD AB, Stockholm, Sweden) enriched with 10% oleic acid/albumin/dextrose/catalase (BD AB) and 5% glycerol was prepared in 9 cm Petri dishes each containing 25 mL of agar. Serial two-step dilutions of the stock solutions were incorporated (2.5 mL drug solution + 22.5 mL agar) in the plates reaching final antibiotic concentrations of 8 to 128 mg/L. For rifampicin the test concentration range was 0.25 to 2 mg/L, which includes the critical test concentration 1 mg/L recommended for susceptibility testing using 7H10 agar.

By using a 32-stick replicator each *M. tuberculosis* isolate suspension was transferred to the Middlebrook plates with the two-step dilution series (8, 16, 32, 64 and 128 mg/L) of the respective antibiotic, beginning with the lowest antibiotic concentration. Drug-free control plates were replicated before and after each antibiotic plate series. The plates were sealed with parafilm and placed in plastic bags and then incubated at 37 °C for 3 weeks. The MIC was defined as the lowest antibiotic concentration fully inhibiting bacterial growth. For this study MIC determination of rifampicin (0.25-2 mg/L) (Sigma-Aldrich, Germany) was performed on all strains as a control methodological reproducibility.

References

1. Li X., Zhang Yong-Kang Liu, Yang D., Charles Z., Li Q., Zhou Y., Plattner J.J., Baker S.J., Qian X., Fan D., et al., (2010), *Bioorg. Med. Chem. Lett.*, 20(12), 3550-3556.
2. Lee J.T., Chen D.Y., Yang Z., Ramos A.D., Hsieh J.J.-D., Bogyo M., (2009), *Bioorg. Med. Chem. Lett.*, 19(17), 5086-5090.
3. Ndubaku C., Varfolomeev E., Wang L., Zobel K., Lau K., Elliott L.O., Maurer B., Fedorova A.V., Dynek J.N., Koehler M., et al., (2009), *ACS Chemical Biology*, 4(7), 557-566.
4. Elliott R.Ph., Weisgerber D.J., Saunders Ch.W., (2003), *PCT Int. Appl.*, WO 2003041680 A1 20030522.
5. Kleemann H.W., Urbach H., Koenig W., Ruppert D., Winkler I., (1990), *Ger. Offen. DE 3840452 A1* 19900607.
6. Haug B.E., Svendsen, J.S., (2001), *J. Pept. Sci.*, 7(4), 190-196.
7. Haug, B.E., Skar M.L. and Svendsen J.S., (2001), *J. Pept. Sci.*, 7(8), 425-432.
8. Haug B.E., Stensen W., Stiberg T. and Svendsen J.S., (2004), *J. Med. Chem.*, 47(17), 4159-62.
9. Strom M.B., Haug B. E., Rekdal O., Skar M.L., Stensen W. and Svendsen J.S., (2002), *Biochem. Cell Biol.*, 80(1), 65-74.
10. WHO reports. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 Global Report on Surveillance and Response. ISBN 978 92 4 159919 1
11. Dormandy J., Somoskovi A., Kreiswirth B.N., Driscoll J.R., Ashkin D., Salfinger M., (2007), *Chest*, 131, 497-501.

12. Caminero J.A., Sotgiu G., Zumla A., Migliori G.B., (2010), *The Lancet Infectious Diseases*, 10, 9, 621-629.
13. Jennison R.F., Stenton P., (1957), *J. Clin. Pathol.*, 10(3), 219-221.
14. Lampen J.O., (1966), *Sym. Soc. Gen. Microbiol.*, 16, 111-130.
15. Pasko M.T., Piscitelli S.C., Van Slooten A.D., (1990), *DICP*. 24(9), 860-7.

4. MECHANISTIC INVESTIGATION AND TOXICITY TESTING

4.1. Evaluation of chorismate mutase binding

Chorismate mutase¹ is an enzyme existing only in bacteria, fungi and higher plants and is responsible for the synthesis of aromatic amino acids *via* the shikimate pathway², thus acting as an attractive target for inhibitors of different kinds.

Thermofluor and enzyme assays were run on a set of 4 compounds (2.15-6, 2.6-9, 2.7-17, 2.14-3) in order to estimate the level of their binding to the secreted chorismate mutase (*MtCM) and the internal chorismate mutase (MtCM) of *Mycobacterium tuberculosis* (see Experimental part for the details).

According to the test results (Experimental part 4.3.) compounds under investigation did not exhibit any affinity for the enzyme and this mechanism of inhibition of *M. tuberculosis* can be excluded from consideration.

4.2. Initial toxicity testing

Determination of cytotoxicity is an important step in drug development process. A set of tests was performed in order to find possible perspectives and limitations for the use of this class of compounds on biological models. Results for the selected compounds (2.15-6, 2.6-25, 2.7-20, 2.6-17) which were chosen in a way to represent different substitution patterns, has shown the high potential of this class of peptidomimetics as drug candidates.

According to the data obtained, **2.7-20** with p-OCF₃ substituted α -benzyl group, exhibited no toxicity in tests up to 100 μ M, while β -benzyl substituted compound **2.15-6** and α -naphthylmethyl derivative **2.6-25** both showed some indications of cytochrome c release increasing, which implicates the activation of a signaling cascade leading to apoptosis. Compound **2.6-17** and **2.6-25** showed cell loss at 100 μ M indicating some toxicity at these concentrations.

Also, **2.15-6** showed moderate permeability in the Epi-Airway tissues whilst all other compounds show low permeability here.

The data obtained provided us with additional information about the effect of substituents in the boronic acid part, their position and amino acid used for coupling. The most toxic compound **2.15-6** contained a benzyl substituent in the position β - to boron and phenylalanine as an amino acid residue. In contrast, p-(OCF₃)-benzyl group in the α -position and free boronic acid, **2.7-20**, had the desired properties, i.e. low toxicity, high solubility and low permeability in EpiAirway.

Kinase profiling was also interesting for the β -aminoboronates providing information on protein kinase affinity. 5 Compounds were selected (**2.7-19**, **2.6-9**, **2.14-3**, **2.25-1**, **2.5g**) and tested on a set of 136 kinases (tests were performed in the International Center for Kinase Profiling, University of Dundee, UK). The results showed low affinity of tested β -aminoboronates to all kinases used in the assay (with 2 exceptions for CAMK1 and SmMLCK) supporting the conclusion about low toxicity of this class of compounds.

4.3. Experimental part*

1. Chorismate mutase binding:

Thermofluor assays

(Experiments were performed by S. Munack, Department of Chemistry, University of Oslo, Norway)

In order to evaluate the stabilization effects of the compounds (2.15-6, 2.6-9, 2.14-3 and 2.7-17) they were subjected to a thermal shift assay, determining the shift of the melting points T_m of the secreted chorismate mutase (*MtCM) and the internal chorismate mutase (MtCM) of *Mycobacterium tuberculosis*. The final protein concentration in the assay is 0,5 mg/ml (*MtCM = 27 μ M, MtCM = 49,6 μ M). The compounds were dissolved in DMSO to a concentration of 200 mM and used in the assay at a final concentration of 8 mM. SYPRO® Orange (Sigma Aldrich) was used as fluorescent dye for the readout. The experiments were performed on a LightCycler® 480 real time PCR machine from Roche in a volume of 25 μ l in 384 well plates heating up the plate from 20 to 95 °C. The melting curve determinations were undertaken in triplicates. Furthermore, interactions of the compound with the dye or the protein alone were investigated in order to rule out false positives or negatives. For *MtCM the transition state analogue (TSA) of the reaction could be used as reference compound.

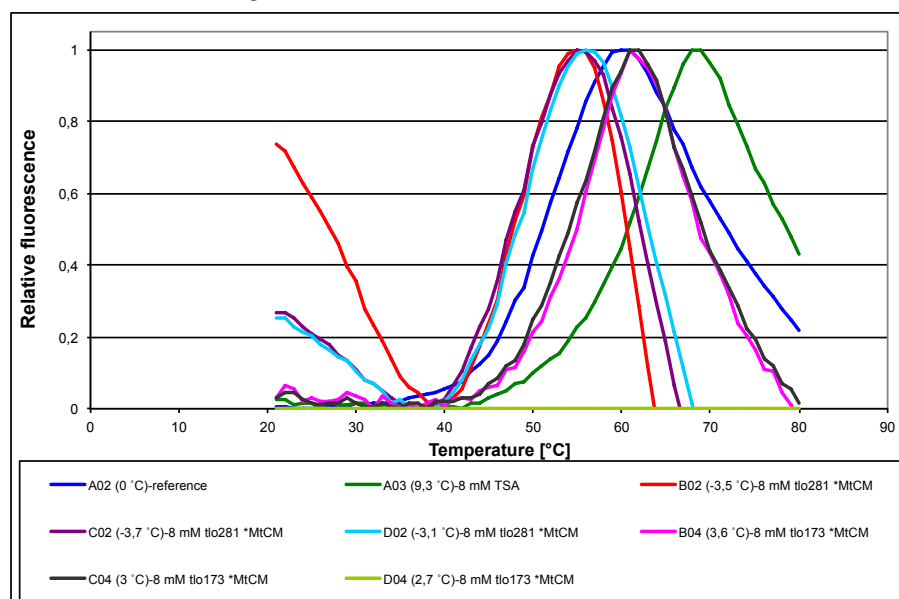
Reaction conditions

*MtCM: 0,5 mg/ml C-terminally His-tagged *MtCM, Sypro Orange 1:1000, 100 mM Potassium Phosphate buffer pH 7.5, 150 mM NaCl, 8 mM compound.

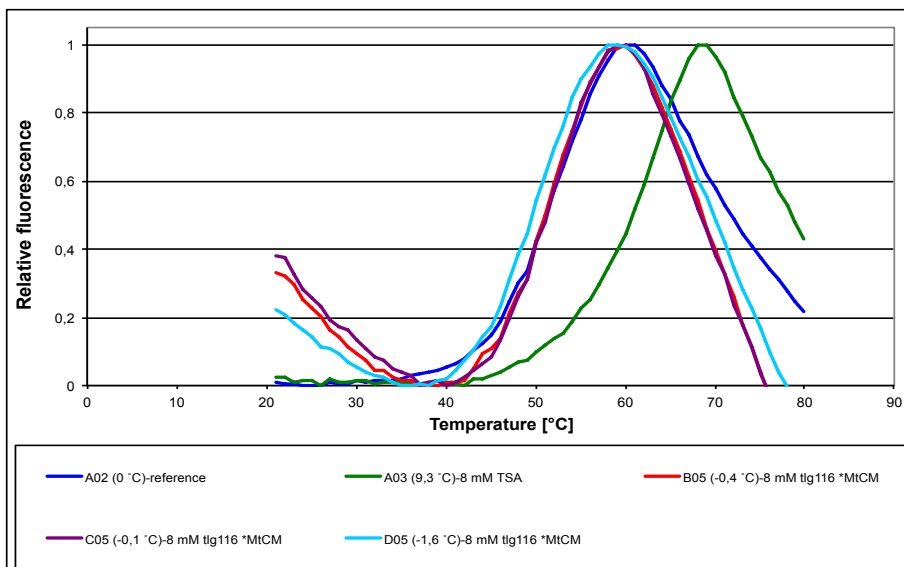
MtCM: 0,5 mg/ml MtCM, Sypro Orange 1:1000, 100 mM bicine buffer pH 9.0, 150 mM NaCl, 8 mM compound.

Results *MtCM

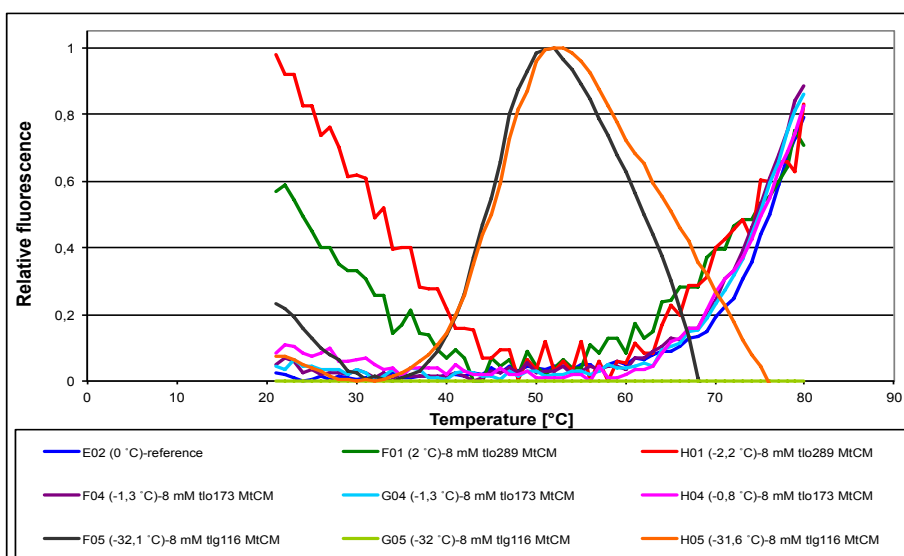
For (2.6-9), no melting curve was obtained.



* Compound numbering is not consistent with the numbering in the thesis. Read tlg-116 as 2.15-6, tlo258 as 2.6-9, tlo281 as 2.14-3, tlo289 as 2.7-17. tlo173 is not belonging to the class of β -aminoboronates.



Results MtCM



Enzyme assay

(Experiments were performed by S. Munack, Department of Chemistry, University of Oslo, Norway)

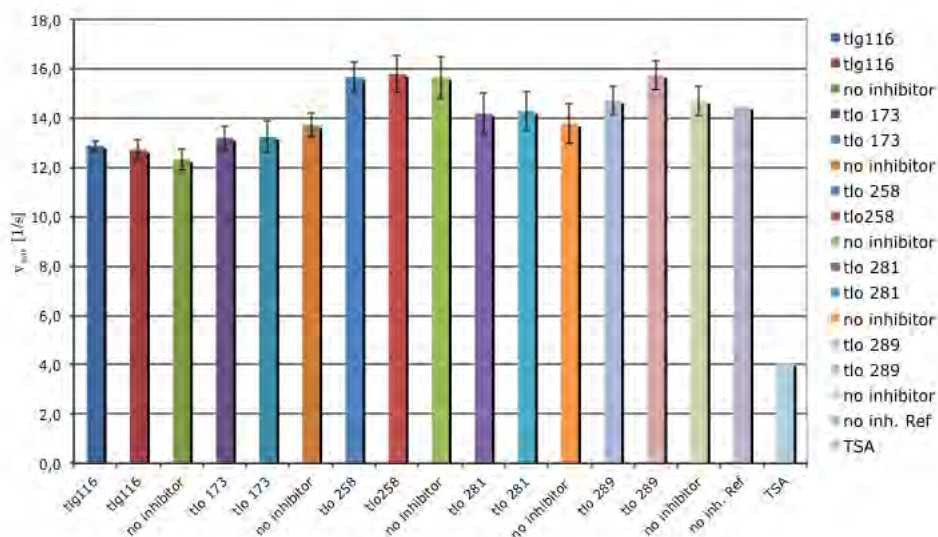
*MtCM stop assay

Initial rates of the enzyme-catalyzed conversion of chorismate to prephenate were determined. Reactions were carried out in a volume of 500 μl in 50 mM Potassium phosphate buffer, pH 7.5, 100 μM chorismate, 3 nM *MtCM and stabilization additives. The compounds 2.15-6, 2.6-9, 2.14-3 and 2.7-17 were dissolved in DMSO to 12 mM, added to the assay solutions (120 μM final concentration in the assay) and incubated at 30 °C in a *Eppendorf thermomixer comfort* at 550 rpm. The reaction was stopped after 3.5, 6.5 and 10 min, respectively, by addition of 50 μl 5 M HCl and incubation was continued at 30 °C in order to convert prephenate to phenylpyruvic acid. Thereafter, 50 μl 10 M NaOH were added to deprotonate the acid to phenylpyruvate. With that method, the formation of prephenate could be recorded by absorption measurements at 320 nm. Blank absorbance from samples at 0 min incubation was prepared for subtraction from the absorbance measured for the enzyme activity determination. The initial rates were determined from the slope of the resulting graph ($A_{320\text{nm}}$ against time) and they were always corrected for the spontaneous background reaction at 30 °C. The

resulting initial rates (v_{init} , [s⁻¹]), were compared to the reaction without compounds present (individually for each assay run with one compound) and the reaction performed in presence of the transition state analogue of the enzymatic reaction, the best inhibitor known to date for *MtCM (final concentration in assay: 11.1 μM, K_i = 3.7 μM [1], v_{init} = 4.07 ± 0.27 s⁻¹, $31 \pm 1,2$ % residual activity). The results show that none of the tested compounds have any inhibitory effect against *MtCM, the slight enhanced residual activity that occurs in some cases might be due to systematic errors.

(Sasso S., Ramakrishnan C., Gamper M., Hilvert D., Kast P., (2005), FEBS Journal, 272, 375.)

Chart of all measurements combined



Compound	v_{init} [s ⁻¹]	Residual activity (%) (based on respective control)
2.15-6	12,88 ± 0,19	104,5 ± 0,8
2.15-6	12,72 ± 0,40	103,2 ± 1,7
Control	12,33 ± 0,43	100 ± 1,8
2.6-9	15,66 ± 0,60	100 ± 2,5
2.6-9	15,79 ± 0,76	100,8 ± 3,2
Control	15,66 ± 0,85	100 ± 3,6
2.14-3	14,19 ± 0,85	103,0 ± 3,6
2.14-3	14,29 ± 0,81	103,8 ± 3,4
Control	13,77 ± 0,80	100 ± 3,4
2.7-17	14,71 ± 0,57	100,1 ± 2,4
2.7-17	15,75 ± 0,60	107,2 ± 2,5
Control	14,69 ± 0,61	100 ± 2,6

References

1. Qamra R., Prakash P., Aruna B., Hasnain S.E., Mande S.C., (2006), Biochemistry, 45 (23), 6997–7005.
2. Goerisch H., (1978), Biochemistry, 17 (18), 3700.

5. CONCLUSIONS

In order to obtain bioisosteric modifications of natural amino acids, a new approach for the stereoselective synthesis of:

- α -alkyl(or aryl)substituted- β -aminoboronates
- β -alkyl(or aryl)substituted- β -aminoboronates
- α,β -disubstituted- β -aminoboronates

was developed. It has also been demonstrated that the approach is suitable for the synthesis of substituted β -amino acids.

Possibilities for the further synthetic modifications were investigated and new ways towards the synthesis of prospective derivatives of aminoboronates, such as cyclic peptidomimetics and α,β -diaminoboronates, were found.

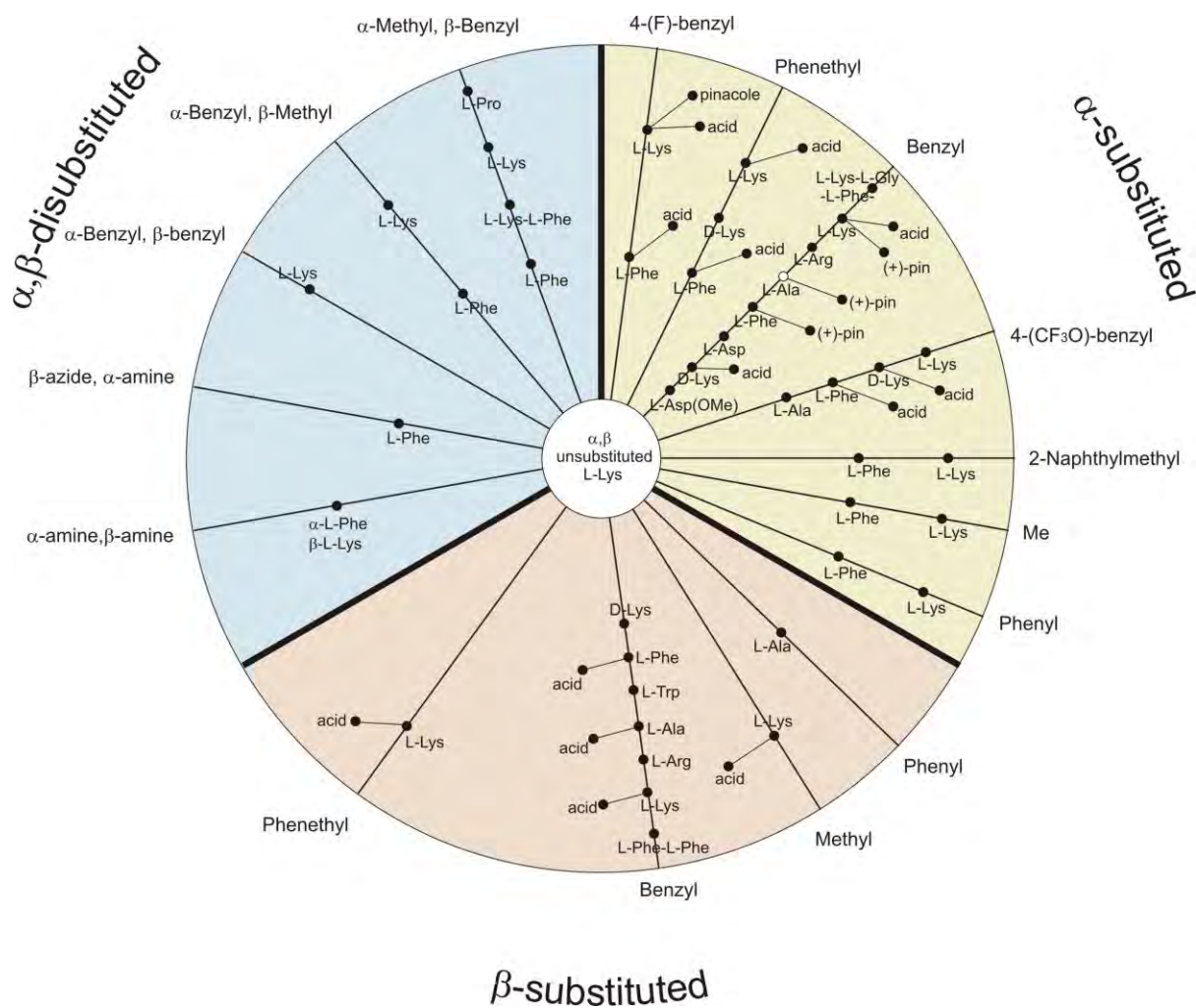
A library of approximately 70 oligopeptides was synthesized (Chapter 6) using the aforementioned substrates. The library was tested on a set of microorganisms which included *Mycobacterium tuberculosis* (H37Rv), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* (ATCC 19615) *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 90028). High activity and selectivity of the compounds under investigation against *M.tuberculosis* (including 25 resistant strains) was detected.

One compound (2.6-25) with high activity against different strains (including 2 resistant) of *Candida albicans* was found.

Several important conclusions concerning structure-activity relationships were reached. The study revealed a correlation between the size of the substituent in the position α - or β - to boron atom and antitubercular activity. A benzyl group (with various modifications) in the α - and/or β -position to boron, was necessary for high levels of antitubercular activity. Replacement of this group by methyl, phenyl, or phenethyl groups dramatically decreased the antibacterial activity. No discrimination was found between activities of boronic acids or esters. Lysine and phenylalanine gave better chances for the keeping high activity level. The high activity of peptidomimetics, containing non-natural amino acids could serve as indirect evidence of a possible non-enzymatic mechanism for inhibition in *M. tuberculosis*. This conclusion is supported by the poor affinity of lead-compounds for bacterial chorismate mutase. The absence of strains resistant to boron-containing peptidomimetics (both *M.tuberculosis* and *C.albicans*) may be evidence of a completely new mechanism of action.

The low levels of toxicity found are an advantage in the future development of these compounds into drug-candidates.

6. SUMMARY OF SYNTHESIZED PEPTIDOMIMETICS



7. EXPERIMENTAL SECTION

THF was freshly distilled from sodium benzophenone ketyl. All reactions were performed under an atmosphere of argon in oven-dried glassware. *n*-Butyllithium 2.7 M in heptane, ZnCl₂ 1 M in diethyl ether and all Grignard reagents were purchased from Aldrich Chemical Co. Inc. Protected amino acids, EDC and HOBT were purchased from Sigma-Aldrich Co., Chem-Impex International Inc. and Shanghai Mocell Biotech Co.,Ltd. and used as received. Routine NMR spectra were recorded in CDCl₃ on a Varian Mercury 400 plus (399.65/100.54 MHz). ¹³C NMR spectra were obtained with broadband proton decoupling. Signals from carbons α -to boron were not detected. The residual signal from CHCl₃ in CDCl₃ was used as internal standard and set to 7.26 ppm for ¹H and to 77.16 for ¹³C. The residual signal from DMSO in DMSO-*d*₆ was used as internal standard and set to 2.50 ppm for ¹H and to 39.50 for ¹³C. The residual signal from CH₃OH in CD₃OD was used as internal standard and set to 3.31 ppm for ¹H and to 49.00 for ¹³C. The residual signal from H₂O in D₂O was used as internal standard and set to 4.79 ppm for ¹H.

NMR data for **2.24a-Boc** were acquired on a Varian/Agilent Inova spectrometer operating at 599.934 and 150.863 MHz for ¹H and ¹³C respectively, using a cryogenically cooled inverse detection HCN probe with enhanced proton channel (2nd generation). 2D correlation experiments, COSY, HSQC and HMBC were acquired for assignment using gradient selection and adiabatic pulses for the heteronuclei experiments. ROESY, using adiabatic pulses and 300 ms spinlock duration at 36 dB, and NOESY, using 200, 500 and 800 ms mixing times, were acquired to study the rotamers. All spectra were acquired at 298 K in CDCl₃ (Sigma-Aldrich).

IR spectra were recorded on a Varian 7000e FT-IR spectrometer. Optical rotation was measured on an AA-10R polarimeter (Optical activity Ltd.). Mass spectra were measured on a Thermo electron LTQ Orbitrap XL + Electrospray ion source (ION-MAX). Samples were dissolved in pure methanol and infused by syringe pump at a flow rate of 5 μ l/min. No molecular ion was detected for compounds containing boronic acid. No molecular ion was detected for the compounds containing azido group.

Preparative TLC was made on Silica gel 60 F₂₅₄ 2mm (Merck). Multiple development technique was used to improve separation, if R_f is not reported. Silica 60A 6 μ m-35 μ m (Davisil, Grace Davison) was used for the LC.

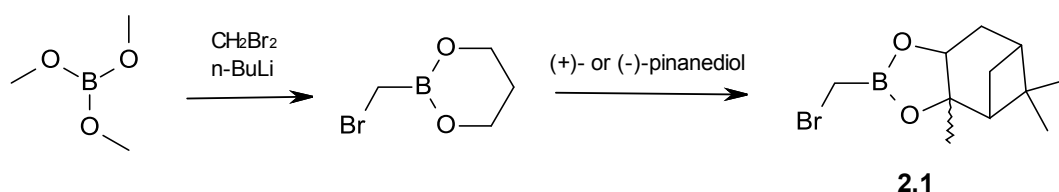
[1R-(1 α ,2 α ,3 α ,5 α)]-2,6,6-Trimethylbicyclo[3.1.1]heptane-2,3-diol or (-)-pinanediol

2-liter three-necked flask equipped with mechanical stirrer, heating mantle and condenser was loaded with (-)- α -pinene (34.00 g, 1 eq.), N-methylmorpholine N-oxide 50 % solution in water (62.1 mL, 1.2 eq.), water (22.5 mL), acetone (250 mL), pyridine (0.25 mL) and OsO₄ (0.25 g). The mixture was stirred vigorously under reflux. The color of the reaction mixture turned deep purple in 3-4 days that was an indicator of the end of the reaction. The reaction mixture was cooled on the ice bath and mixed with sodium metabisulfite (10.00 g), Magnesol (5.00 g) and Sodium sulphate (20.00 g). The mixture was stirred at room temperature for 2 hrs and filtered through Celite. The solution was concentrated under vacuum; the residue was diluted with water and extracted with diethyl ether. Organic layer was washed with saturated Na₂S₂O₃, 2N HCl, water, saturated NHCO₃ solution and brine. The solution was dried with MgSO₄ and concentrated to give oil that was crystallized from heptane to give 37.00 g (87 % yield) of pure (-)-pinanediol.

All spectroscopic data were in accordance with earlier published data:

Ray R., Matteson D. S., (1980), Tetrahedron letters, 21, 449-450.

(3a*R*,4*R*,6*R*,7a*S*)-2-bromomethylhexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.1) (or enantiomer)



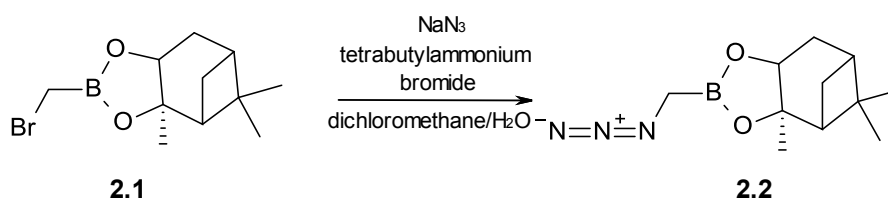
To a solution of dibromomethane (19.4 g, 0.11 mol) and trimethylborate (10.3 g, 0.1 mol) in dry THF (50 mL) *n*-butyllithium (67.7 mL as 1.6M solution in THF, 0.11 mol) was added drop-wise at $-78\text{ }^{\circ}\text{C}$. The reaction mixture was stirred for 1 hr and then quenched with chloro trimethylsilane (13.1 g, 0.12 mol). The resulting mixture was allowed to warm to ambient temperature and stirred for 14 hrs. during which time a white precipitate formed. After treating the suspension with 1,3-propanediol (7.6 g, 0.1 mol) and stirring for 14 hrs., the solvent was evaporated under vacuum. The oily residue was extracted by pentane, dried over magnesium sulfate, concentrated and distilled under high vacuum. (bp: $42\text{--}44\text{ }^{\circ}\text{C}$, 0.043 mbar) to leave a colorless oil (10.2 g, 57 %).

To a solution of 1,3-propanediol bromomethylboronate (10.0 g, 0.056 mol) in diethyl ether (100 mL), (-)-pinanediol (9.5 g, 0.056 mol) was added and the resulting mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the resulting oil was dissolved in pentane and filtered through silica. The pentane solution was dried over magnesium sulfate and the solvent was removed to obtain pure product as colorless oil (14.1 g, 93 %).

All spectroscopic data were in accordance with earlier published data:

Davoli P., Fava R., Morandi S., Spaggiari A. and Prati F., (2005), *Tetrahedron*, 61(18), 4427-4436

(3a*R*,4*R*,6*R*,7a*S*)-2-azidomethylhexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.2)

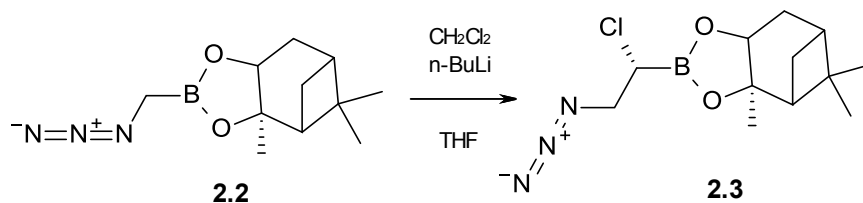


A solution of 2.1 (3.00 g, 0.011 mol) in dichloromethane (30 mL) was placed in a dropping funnel and added drop wise to a vigorously stirred mixture of sodium azide (7.00 g, 0.1 mol) and tetrabutylammonium bromide (0.18 g, 0.00056 mol) in a mixture of dichloromethane (200 mL) and water (45 mL). The reaction mixture was stirred overnight at room temperature. The organic phase was separated and the water phase was extracted twice by additional portions of dichloromethane. The extracts were combined and concentrated *in vacuo*. The resulting oily residue was dissolved in dichloromethane and filtered through a pad of silica to remove traces of the phase-transfer catalyst. The final dichloromethane solution was dried over magnesium sulfate and concentrated *in vacuo* to obtain the product as colorless oil (2.50 g, 97 %).

All spectroscopic data were in accordance with earlier published data:

Matteson D. S., Maliakal D. and Fabry-Asztalos L., (2008), *J. Organomet. Chem.*, 693(13), 2258-2262

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-chloroethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.3) (or diastereomer)

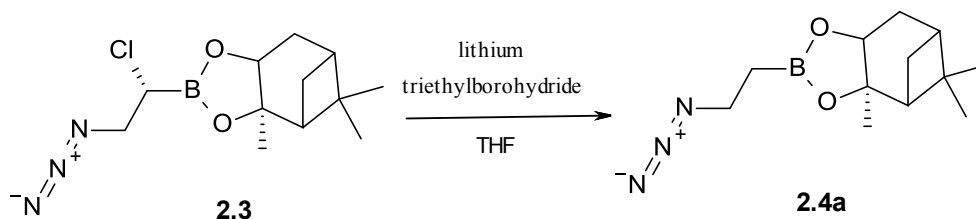


Dichloromethyl lithium was prepared by the addition of *n*-butyllithium (4.75 mL as 2.5M solution in THF, 0.012 mol) to dichloromethane (1.66 g, 0.02 mol) in anhydrous THF at -100 °C under argon. A solution of azidomethylboronic ester **2.2** (2.30 g, 0.01 mol) was slowly added to the vigorously stirred slurry of dichloromethyl lithium. After 10 min the reaction temperature was raised to -78 °C and the mixture was stirred for 30 min. Anhydrous zinc chloride (9.6 mL as 1M solution in diethyl ether, 0.01 mol) was then added and the mixture was allowed to warm to room temperature. After 2 hrs diethyl ether was added to the reaction mixture and the suspension obtained was washed with saturated ammonium chloride. The solvent was evaporated and the oily residue was dissolved in diethyl ether, washed with brine and dried over magnesium sulfate. Diethyl ether was removed under vacuum to obtain the product as slightly yellow oil (1.90 g, 68 %).

All spectroscopic data are in accordance with earlier published data:

Matteson D. S., Maliakal D. and Fabry-Asztalos L., (2008), *J. Organomet. Chem.*, 693(13), 2258-2262

(3*aR*,4*R*,6*R*,7*aS*)-2-[2-azidoethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4a)



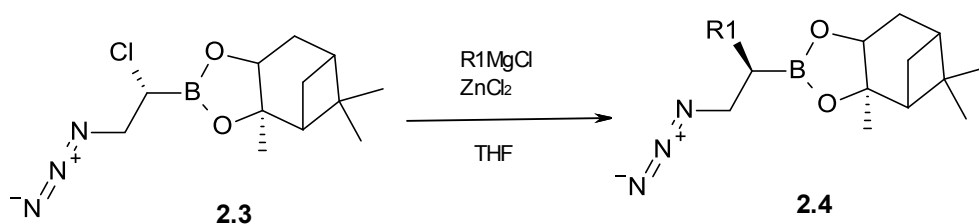
A solution of the α -chloro derivative **2.3** (5.00 g, 0.0133 mol, 1 eq) in dry THF (50 mL) was cooled to -78 °C and a solution of lithium triethylborohydride (1.84 g, 0.0173 mol, 17.3 mL of 1M solution in THF, 1.3 eq) was added drop wise to the vigorously stirred solution. The reaction mixture was stirred overnight, concentrated under vacuum and the residue was dissolved in diethyl ether, washed with saturated ammonium chloride and dried over magnesium sulfate. The dried solution was concentrated under vacuum to give product contaminated with the compound **5a** resulting from reduction of the azido-group. m(crude)= 4.00 g (88.9 % yield)

¹H NMR (400 MHz, DMSO) δ 4.32 (d, J = 7.3 Hz, 1H), 2.88 – 2.75 (m, 2H), 2.34 – 2.23 (m, 1H), 2.22 – 2.11 (m, 1H), 1.95 (t, J = 5.4 Hz, 1H), 1.86 (s, 1H), 1.71 (d, J = 14.7 Hz, 1H), 1.32 (s, 3H), 1.24 (s, 3H), 1.16 – 1.06 (m, 2H), 0.99 (d, J = 10.7 Hz, 1H), 0.80 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 77.93, 51.19, 47.71, 39.46, 38.11, 35.33, 28.57, 27.03, 26.41, 23.96, 9.43 (contained impurities)

IR (cm⁻¹) 2923.1, 2869.5, 2093.5

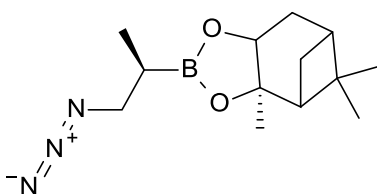
(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-alkylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4) exemplified by 2.4b.



Grignard reagent (for methylmagnesium chloride (2.4b)): 0.96 g, 4.3 mL 3 M solution in THF, 0.0128 mol, 1.2 eq) was added drop wise to the solution cooled to $-78\text{ }^{\circ}\text{C}$ of α -chloroalkylboronate (3.00 g, 0.0106 mol, 1eq.) in THF. The reaction mixture was stirred for 30 min. A zinc chloride solution (5.80 g, 42.6 mL 1 M solution in diethyl ether, 0.0426 mol, 4 eq.) was added drop wise to the reaction mixture; the solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was concentrated under vacuum, dissolved in pentane and washed with saturated ammonium chloride solution. The organic layer was dried over magnesium sulfate and concentrated under vacuum to obtain the product as colorless oil. $m = 2.4\text{ g}$, (86 % yield)

Some Grignard reagents did not give full conversion. If conversion was not complete, the crude mixture was used in the subsequent step without further purification.

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-methylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4b)



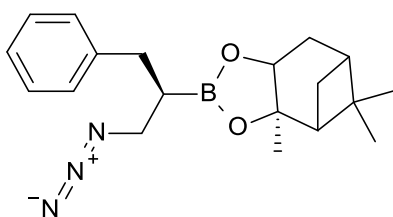
$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.28 (dd, $J = 8.8, 1.7\text{ Hz}$, 1H), 3.37 (ddd, $J = 19.8, 11.9, 7.1\text{ Hz}$, 2H), 2.40 – 2.29 (m, 1H), 2.22 (ddd, $J = 8.2, 6.1, 3.0\text{ Hz}$, 1H), 2.05 (t, $J = 5.5\text{ Hz}$, 1H), 1.95 – 1.80 (m, 2H), 1.42 (m, 1H) 1.38 (s, 3H), 1.29 (s, 3H), 1.11 (d, 1H), 1.07 (d, $J = 7.5\text{ Hz}$, 3H), 0.88 (s, 1H), 0.84 (s, 3H).

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 85.92, 77.95, 55.24, 51.22, 39.48, 38.14, 35.43, 28.60, 27.05, 26.35, 23.97, 13.40

IR (cm^{-1}) 2920.6, 2872.4, 2092.9, 1367.9, 1280.9

$[\alpha]_{\text{D}} = -30$ ($c = 0.332$, toluene, $18\text{ }^{\circ}\text{C}$)

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-benzylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4c)



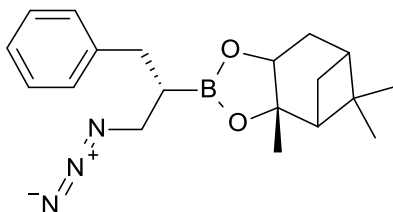
^1H NMR (400 MHz, CDCl_3) δ 7.34 – 7.19 (m, 5H), 4.31 (dd, J = 8.8, 2.0 Hz, 1H), 3.42 (dd, J = 6.7, 2.0 Hz, 2H), 2.94 – 2.77 (m, 2H), 2.37 (ddt, J = 14.1, 8.8, 2.4 Hz, 1H), 2.22 (dtd, J = 8.3, 6.1, 2.2 Hz, 1H), 2.10 – 2.05 (m, 1H), 1.93 (td, J = 5.7, 2.9 Hz, 1H), 1.90 – 1.83 (m, 1H), 1.77 – 1.68 (m, 1H), 1.37 (s, 3H), 1.32 (s, 3H), 1.10 (d, J = 11.0 Hz, 1H), 0.87 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 140.94, 129.15, 128.54, 126.27, 86.31, 78.24, 52.65, 51.36, 39.67, 38.35, 35.54, 34.34, 28.76, 27.27, 26.48, 24.21

IR (cm^{-1}) 2920.8, 2870.1, 2092.9, 1386.1, 1280.3

97 % yield (can contain 10-15 % impurity that varies from run to run)

(3a*S*,4*S*,6*S*,7a*R*)-2-[(1*S*)-2-azido-1-benzylethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4d)



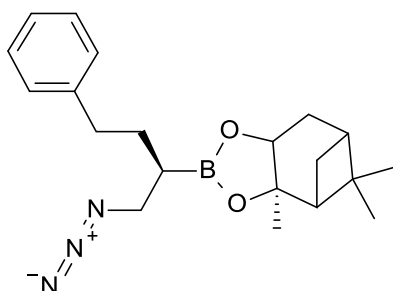
^1H NMR (400 MHz, CDCl_3) δ 7.31 – 7.15 (m, 1H), 4.27 (dd, J = 8.8, 2.0 Hz, 1H), 3.38 (dd, J = 6.7, 1.6 Hz, 1H), 2.81 (qd, J = 13.8, 7.6 Hz, 1H), 2.37 – 2.27 (m, 1H), 2.21 – 2.13 (m, 1H), 2.05 – 2.00 (m, 1H), 1.92 – 1.86 (m, 1H), 1.82 (ddd, J = 14.6, 3.3, 2.1 Hz, 1H), 1.73 – 1.63 (m, 1H), 1.32 (s, 1H), 1.28 (s, 1H), 1.05 (d, J = 11.0 Hz, 1H), 0.83 (d, J = 2.4 Hz, 1H).

^{13}C NMR (101 MHz, CDCl_3) δ 140.64, 128.92, 128.32, 126.04, 86.08, 77.99, 52.38, 51.10, 39.42, 38.11, 35.33, 34.08, 28.52, 27.03, 26.27, 23.99

b.p. 110C ($5 \cdot 10^{-4}$ torr) partly decomposes

36 % yield

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*)-2-azido-1-phenylethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4e)

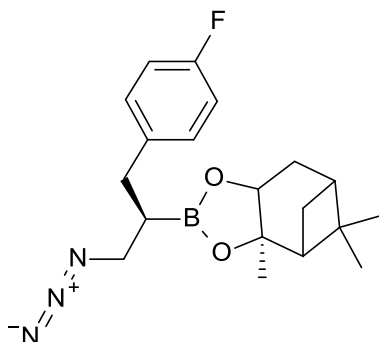


^1H NMR (400 MHz, CDCl_3) δ 7.27 – 7.07 (m, 5H), 4.24 (dd, J = 8.8, 1.9 Hz, 1H), 3.37 (ddd, J = 37.3, 12.0, 7.1 Hz, 1H), 2.68 – 2.49 (m, 2H), 2.33 – 2.23 (m, 1H), 2.21 – 2.12 (m, 1H), 2.00 (t, J = 5.5 Hz, 1H), 1.87 – 1.68 (m, 5H), 1.33 (s, 3H), 1.23 (s, 3H), 1.09 (d, J = 10.9 Hz, 1H), 0.78 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 141.18, 127.38, 127.31, 124.78, 84.99, 76.95, 52.31, 50.18, 38.49, 37.14, 34.49, 33.88, 29.51, 27.69, 26.04, 25.46, 22.99

81 % yield

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-(4-fluoro)benzylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4f)



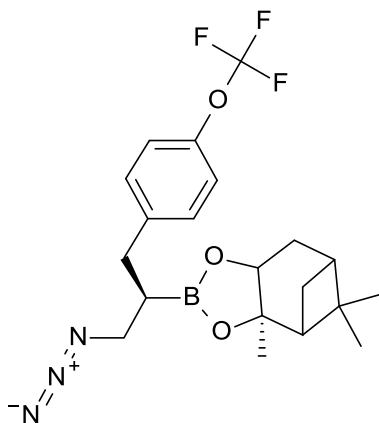
^1H NMR (400 MHz, CDCl_3) δ 7.09 - 6.88 (m, 4H), 4.19 (dd, $J = 8.8, 2.1$ Hz, 1H), 3.38 - 3.21 (m, 2H), 2.71 (t, $J = 8.2$ Hz, 2H), 2.32 - 2.18 (m, 1H), 2.14 - 2.03 (m, 1H), 1.95 (m, 1H), 1.85 - 1.68 (m, 2H), 1.63 - 1.51 (m, 1H), 1.25 (s, 3H), 1.21 (s, 3H), 0.94 (d, $J = 11.0$ Hz, 1H), 0.76 (s, 3H).

^{19}F NMR (376 MHz, CDCl_3) δ -117.42 (ddd, $J = 14.2, 8.9, 5.5$ Hz).

IR (cm^{-1}) 2923.8, 2870.8, 2094.8, 1509.1

30 % yield

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-(4-trifluoromethoxy)benzylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4g)



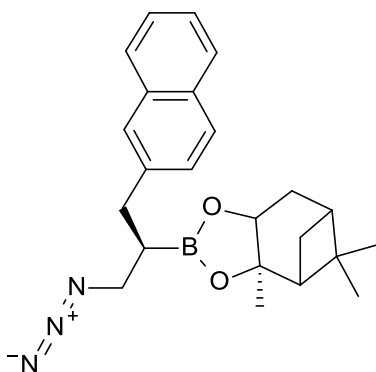
^1H NMR (400 MHz, CDCl_3) δ 7.20 - 6.96 (m, 4H), 4.18 (dd, $J = 8.8, 2.0$ Hz, 1H), 3.38 - 3.25 (m, 2H), 2.74 (dd, $J = 7.7, 1.8$ Hz, 2H), 2.29 - 2.19 (m, 1H), 2.08 (m, 1H), 1.94 (t, $J = 5.6$ Hz, 1H), 1.85 - 1.68 (m, 2H), 1.66 - 1.53 (m, 1H), 1.23 (s, 3H), 1.20 (s, 3H), 0.90 (d, $J = 11.0$ Hz, 1H), 0.75 (s, 3H).

^{19}F NMR (376 MHz, CDCl_3) δ -57.97 (s)

IR (cm^{-1}) 2926.2, 2872.4, 2096.7, 1254.6

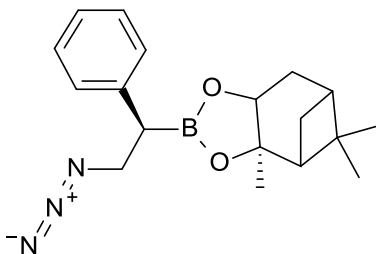
78 % yield

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-(2-methyl-naphthyl)ethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4h)



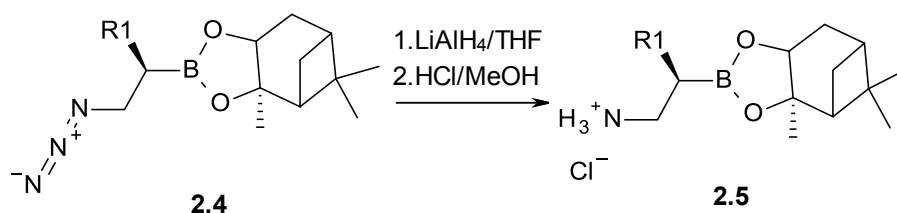
¹H NMR (400 MHz, CDCl₃) δ 7.40 – 7.22 (m, 10H), 4.19 (dd, *J*= 8.8, 2.0 Hz, 1H), 3.38 – 3.27 (m, 2H), 2.99 – 2.81 (m, 2H), 2.30 – 2.24 (m, 1H), 2.21 – 2.16 (m, 1H), 1.95 (dd, *J*= 6.1, 5.0 Hz, 1H), 1.88 – 1.84 (m, 1H), 1.83 – 1.75 (m, 1H), 1.73 – 1.67 (m, 1H), 1.24 (s, 3H), 1.19 (s, 3H), 0.98 (d, *J*= 11.0 Hz, 1H), 0.74 (s, 3H).
50 % Conversion according to NMR. The product was used in the next step without further purification.
m (crude)= 5.1g (from 3.8g of starting compound) 98 % yield.

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*)-2-azido-1-phenylethyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.4i)



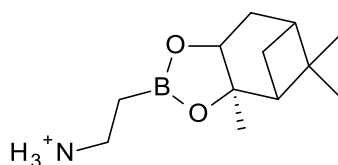
¹H NMR (400 MHz, CDCl₃) δ 7.31 – 7.08 (m, 5H), 4.24 (dd, *J*= 8.8, 2.0 Hz, 1H), 3.56 (m, 2H), 2.64 (t, *J*= 8.0 Hz, 1H), 2.32 – 2.18 (m, 1H), 2.15 – 2.05 (m, 1H), 2.00 – 1.93 (m, 1H), 1.89 – 1.79 (m, 1H), 1.73 (m, 1H), 1.29 (s, 3H), 1.21 (s, 3H), 0.98 (d, *J*= 11.0 Hz, 1H), 0.76 (s, 3H).
IR (cm⁻¹) 2922.2, 2871, 2095, 1376.9
30-50 % Conversion according to NMR. The product was used in the next step without further purification.
m (crude)= 4.5g (from 4.3g of st comp) 92 % crude yield.

General procedure for synthesis of amines 2.5



β -azidoboronate **2.4** (1eq) was dissolved in dry THF and cooled to $-78\text{ }^\circ\text{C}$. To the solution lithium aluminum in THF (1.2 eq) was added drop wise and the resulting mixture was allowed to warm to room temperature and stirred overnight. Water was added slowly to the reaction mixture to decompose unreacted lithium aluminum hydride. The white precipitate was filtered off and washed several times with diethyl ether. Organic layers were combined, washed with saturated ammonium chloride solution and dried over magnesium sulfate. The solution was concentrated under vacuum and dissolved in pentane. The precipitate (if present) was filtered off. To the solution of amine an excess of hydrochloric acid (1.25 M solution in methanol) was added at $0\text{ }^\circ\text{C}$ and the resulting mixture was stirred overnight at room temperature. Solvents were evaporated and the oily residue was washed with pentane. The solvent was decanted leaving pure product.

2-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)ethylammonium chloride (2.5a)



HRMS found: 224.1817, Exact Mass: 224.1816, Calculated for: $\text{C}_{12}\text{H}_{23}\text{BNO}_2[\text{M}+\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 4.38 (dd, $J = 8.8, 2.0$ Hz, 1H), 3.06 (m, 2H), 2.48 – 2.32 (m, 1H), 2.25 (m, 1H), 2.02 (t, $J = 5.5$ Hz, 1H), 1.91 (d, $J = 5.2$ Hz, 1H), 1.82 (ddd, $J = 14.5, 3.4, 1.9$ Hz, 1H), 1.40 (s, 3H), 1.29 (s, 3H), 1.24 (m, 2H), 1.06 (d, $J = 10.9$ Hz, 1H), 0.85 (s, 3H).

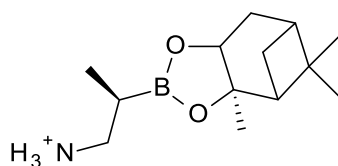
^{13}C NMR (101 MHz, CD_3OD) δ 86.22, 78.03, 51.16, 39.41, 37.84, 34.83, 27.46, 26.04, 25.81, 22.85, 11.74

IR (cm^{-1}) 2918.7, 1367.6, 1281.0, 1207.1, 1029.8

$[\alpha]_{\text{D}} = -18.2^\circ$ ($c = 0.33$, MeOH, $18\text{ }^\circ\text{C}$)

64 % yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)propylammonium chloride (2.5b)]



HRMS found: 238.1972 Exact Mass: 238.1973 Calculated for: $\text{C}_{13}\text{H}_{25}\text{BNO}_2[\text{M}+\text{H}]^+$

^1H NMR (400 MHz, DMSO) δ 7.96 (s, 1H), 4.32 (d, J = 7.3 Hz, 1H), 2.92 (dt, J = 11.0, 5.5 Hz, 1H), 2.71 – 2.55 (m, 1H), 2.28 (dd, J = 14.4, 8.9 Hz, 1H), 2.16 (dd, J = 9.9, 5.5 Hz, 1H), 1.94 (t, J = 5.5 Hz, 1H), 1.84 (s, 1H), 1.71 (d, J = 14.4 Hz, 1H), 1.41 – 1.34 (m, 1H), 1.32 (s, 1H), 1.23 (s, 1H), 0.97 (t, J = 9.0 Hz, 1H), 0.79 (s, 2H).

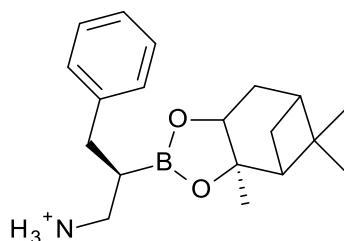
^{13}C NMR (101 MHz, DMSO) δ 94.38, 85.99, 77.47, 51.22, 42.01, 38.21, 35.30, 28.62, 27.27, 26.29, 24.04, 22.50, 13.36

IR (cm^{-1}) 3348.4, 2917.4, 2871.7, 1456.4, 1367.8

$[\alpha]_{\text{D}} = -17.9^\circ$ (c = 0.39, MeOH, 18 °C)

74 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-phenyl]propylammonium chloride (2.5c)



HRMS found: 314.2287 Exact Mass: 314.2286 Calculated for: $\text{C}_{19}\text{H}_{29}\text{BNO}_2$ $[\text{M}+\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.52 – 7.03 (m, 5H), 4.35 (dd, J = 8.8, 2.1 Hz, 1H), 3.07 – 2.89 (m, 2H), 2.83 (m, 2H), 2.35 (m, 1H), 2.16 (m, 1H), 2.00 (t, J = 5.6 Hz, 1H), 1.90 – 1.68 (m, 3H), 1.35 (s, 3H), 1.28 (s, 3H), 0.94 (d, J = 11.0 Hz, 1H), 0.85 (s, 3H).

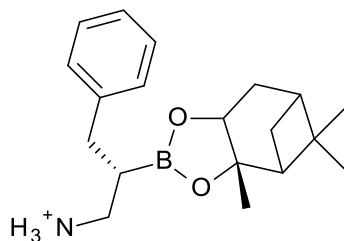
^{13}C NMR (101 MHz, CD_3OD) δ 139.73, 128.60, 128.11, 126.10, 86.41, 78.12, 51.05, 40.06, 39.35, 37.80, 34.64, 33.59, 27.43, 26.03, 25.79, 22.86

IR (cm^{-1}) 3379.3, 3025.9, 2979.4, 2915.2, 2869.8, 1375.4

$[\alpha]_{\text{D}} = -14.7^\circ$ (c = 0.408, MeOH, 18 °C)

37 % yield

[2*S*-((3*aS*,4*S*,6*S*,7*aR*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-phenyl]propylammonium chloride (2.5d)



HRMS found: 314.2286 Exact Mass: 314.2286 Calculated for: $\text{C}_{19}\text{H}_{29}\text{BNO}_2$ $[\text{M}+\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.38 – 7.08 (m, 5H), 4.35 (dd, J = 8.8, 2.0 Hz, 1H), 3.08 – 2.89 (m, 2H), 2.89 – 2.77 (m, 2H), 2.44 – 2.26 (m, 1H), 2.21 – 2.11 (m, 1H), 2.00 (t, J = 5.5 Hz, 1H), 1.93 – 1.70 (m, 3H), 1.35 (s, 3H), 1.28 (s, 3H), 0.95 (d, J = 11.0 Hz, 1H), 0.85 (s, 3H).

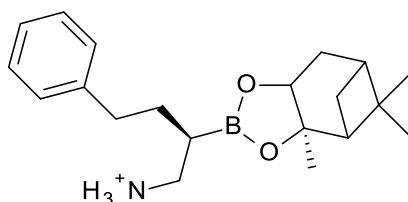
^{13}C NMR (101 MHz, CD_3OD) δ 139.75, 128.62, 128.12, 126.11, 86.41, 78.12, 51.05, 40.08, 39.36, 37.81, 34.64, 33.60, 27.44, 26.04, 25.79, 22.88

IR (cm^{-1}) 3423.7, 3025.8, 2968.8, 2915.5, 2869.3, 1603.2

$[\alpha]_{\text{D}} = -14.4^\circ$ ($c = 0.694$, MeOH, 18°C)

58 % yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-4-phenyl]butylammonium chloride (2.5e)



HRMS found: 328.2445 Exact Mass: 328.2442 Calculated for: $\text{C}_{20}\text{H}_{31}\text{BNO}_2$ $[\text{M}+\text{H}]^+$

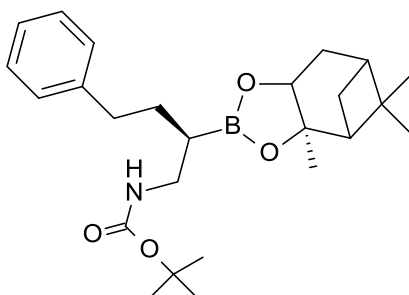
^1H NMR (400 MHz, CDCl_3) δ 7.36 – 7.08 (m, 5H), 4.31 (, 1H), 3.00 – 2.83 (m, 2H), 2.81 – 2.57 (m, 2H), 2.35 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.86 (m, 3H), 1.79 – 1.57 (m, 1H), 1.41 (s+s, 3H), 1.37 (m, 1H), 1.30 (s+s, 6H), 1.12 (m, 1H), 0.85 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 142.38, 142.23, 128.57, 128.51, 128.42, 128.31, 128.29, 125.77, 125.73, 86.28, 86.06, 77.94, 77.81, 65.83, 51.24, 51.16, 49.71, 42.64, 39.48, 39.21, 38.18, 38.12, 35.49, 35.39, 34.99, 34.82, 30.87, 30.72, 28.71, 28.58, 27.02, 26.55, 26.47, 23.97, 23.95, 15.26.

m(crude) 4.0g (from 5.6g of st.comp) 81.6 % crude yield

Resonances are doubled due to some coordination effects. This feature disappeared after Boc-protection of the amino group.

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-4-phenyl]butyl-N-Boc-amine (2.5e-Boc)



^1H NMR (400 MHz, CDCl_3) δ 7.45 – 7.05 (m, 5H), 4.81 (b.s, 1H), 4.29 (dd, $J = 8.8, 2.0$ Hz, 1H), 3.28 (t, $J = 6.6$ Hz, 2H), 2.68 (t, $J = 8.1$ Hz, 2H), 2.36 (m, 1H), 2.28 – 2.17 (m, 1H), 2.07 (t, $J = 5.5$ Hz, 1H), 1.92 (dd, $J = 5.4, 2.8$ Hz, 1H), 1.89 – 1.62 (m, 3H), 1.44 (s, 9H), 1.39 (s, 3H), 1.30 (s+m, 3+1H), 1.11 (d, $J = 10.9$ Hz, 1H), 0.85 (s, 3H).

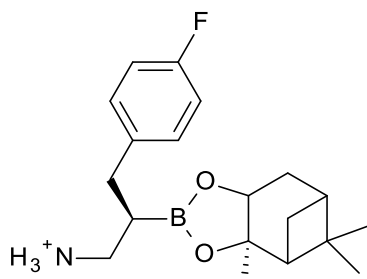
^{13}C NMR (101 MHz, CDCl_3) δ 155.96, 142.58, 128.44, 128.25, 125.66, 85.77, 77.77, 51.19, 41.43, 39.51, 38.12, 35.56, 35.06, 30.73, 28.70, 28.45, 27.06, 26.53, 24.00

IR (cm^{-1}) 3443.3, 3376.8, 2974.0, 2925.1, 2869.5, 1704.2, 1506.5, 1365.8

$[\alpha]_{\text{D}} = +1.6^\circ$ ($c = 0.63$, MeOH, 18°C)

30 % yield after preparative TLC (pentane/diethyl ether)

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-(4-fluorophenyl)]propylammonium chloride (2.5f)



HRMS found: 332.2191 Exact Mass: 332.2192 Calculated for: C₁₉H₂₈BFNO₂ [M+H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.26 (dd, *J* = 8.5, 5.7 Hz, 2H), 7.01 (t, *J* = 8.8 Hz, 2H), 4.35 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.08 – 2.89 (m, 2H), 2.83 (dd, *J* = 7.4, 2.0 Hz, 2H), 2.36 (m, 1H), 2.16 (m, 1H), 2.00 (t, *J* = 5.6 Hz, 1H), 1.91 – 1.77 (m, 2H), 1.74 (t, *J* = 7.5 Hz, 1H), 1.35 (s, 3H), 1.29 (s, 3H), 0.92 (d, *J* = 10.9 Hz, 1H), 0.85 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 161.63 (d, *J* = 243.3 Hz), 135.70 (d, *J* = 3.4 Hz), 130.30 (d, *J* = 7.9 Hz), 114.64 (d, *J* = 21.4 Hz), 86.44, 78.12, 51.03, 40.05, 39.34, 37.80, 34.63, 32.76, 27.41, 26.00, 25.77, 22.84.

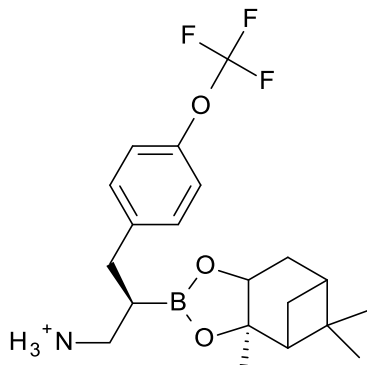
¹⁹F NMR (376 MHz, CD₃OD) δ -118.94 (dt, *J* = 8.7, 3.8 Hz).

IR (cm⁻¹) 2968.5, 2917, 2871, 1600.7, 1508.9

[α]_D = -11.76° (c = 0.51, MeOH, 18 °C)

87.4 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-(4-trifluoromethoxyphenyl)]propylammonium chloride (2.5g)



HRMS found: 398.2113 Exact Mass: 398.2109 Calculated for: C₂₀H₂₈BF₃NO₃ [M+H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.42 – 7.10 (m, 4H), 4.39 – 4.31 (m, 1H), 3.00 (ddd, *J* = 39.8, 12.8, 7.7 Hz, 2H), 2.87 (d, *J* = 7.5 Hz, 2H), 2.41 – 2.29 (m, 1H), 2.19 – 2.08 (m, 1H), 1.99 (t, *J* = 5.6 Hz, 1H), 1.88 – 1.73 (m, 3H), 1.33 (m, 3+1H), 1.28 (s, 3H), 0.85 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 147.7, 139.18, 130.27, 120.71, 86.47, 78.15, 51.01, 40.10, 39.32, 37.79, 34.59, 32.91, 27.37, 25.98, 25.73, 22.82 (OCF₃-signal intensity was lower than the noise level due to splitting to quartet)

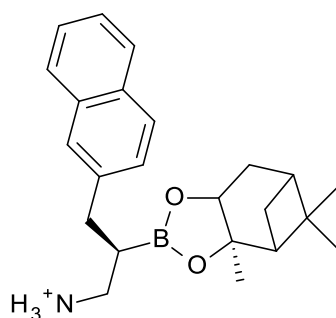
¹⁹F NMR (376 MHz, CD₃OD) δ -59.62 (s)

IR (cm⁻¹) 2918.4, 1508.2, 1259.9

[α]_D = -6.7° (c = 0.33, MeOH, 18 °C)

78 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-(2-naphthyl)]propylammonium chloride (2.5h)



HRMS found: 364.2446 Exact Mass: 364.2442 Calculated for: C₂₃H₃₁BNO₂ [M+H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.92 – 7.65 (m, 4H), 7.53 – 7.31 (m, 3H), 4.33 (dd, *J*= 8.5, 1.9 Hz, 1H), 3.10 – 2.90 (m, 4H), 2.32 (ddd, *J*= 11.5, 8.5, 2.5 Hz, 1H), 2.05 (dd, *J*= 11.0, 6.3 Hz, 1H), 1.97 (t, *J*= 5.4 Hz, 1H), 1.88 (q, *J*= 7.6 Hz, 1H), 1.84 – 1.72 (m, 2H), 1.32 (s, 3H), 1.24 (s, 3H), 0.89 (d, *J*= 10.9 Hz, 1H), 0.82 (s, 3H).

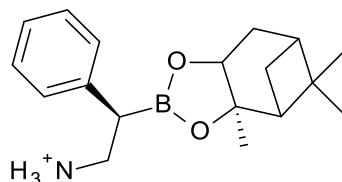
¹³C NMR (101 MHz, CD₃OD) δ 137.25, 133.54, 132.35, 127.78, 127.22, 127.06, 126.99, 125.73, 125.14, 86.43, 78.09, 50.97, 40.10, 39.28, 37.77, 34.59, 33.74, 27.43, 26.00, 25.70, 22.86

IR (cm⁻¹) 3049.8, 2970.8, 2911.3, 2867.7, 1393.1

[α]_D= -10.3° (c= 0.29, MeOH, 18 °C)

74 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-2-phenyl]ethylammonium chloride (2.5i)



HRMS found: 300.2134 Exact Mass: 300.2129 Calculated for: C₁₈H₂₇BNO₂ [M+H]⁺

¹H NMR (400 MHz, DMSO) δ 7.78 (s, 3H), 7.24 (m, 5H), 4.33 (d, *J*= 8.3 Hz, 1H), 3.16 (m, 1H), 2.73 (m, 1H), 2.32 – 2.15 (m, 1H), 2.06 (m, 1H), 1.93 (t, *J*= 5.4 Hz, 1H), 1.77 (m, 1H), 1.57 (m, 1H), 1.28 (s, 3H), 1.20 (s, 3H), 0.83 (d, *J*= 10.8 Hz, 1H), 0.77 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 137.45, 128.78, 128.32, 126.61, 86.52, 78.16, 51.16, 41.32, 39.24, 37.87, 34.69, 27.33, 25.99, 25.76, 22.82

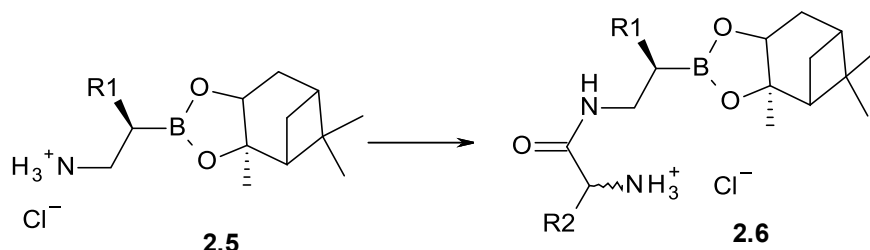
IR (cm⁻¹) 2910.6, 2868.3, 2705.3, 2611.2, 1600.4, 1388.5

[α]_D= +21.7° (c= 0.23, MeOH, 18 °C)

17 % yield

General procedure for synthesis of dipeptides (2.6)

Snow R. J., Bachovchin W. W., Barton R. W., Campbell S. J., Coutts S. J., Freeman D. M., Gutheil W. G., Kelly T. A., Kennedy C. A., Krolkowski D. A., Leonard S. F., Pargellis C. A., Tong L. and Adams J., (1994), J. Am. Chem. Soc., 116(24), 10860-9.



To a stirred, ice-cold solution of Boc-protected amino acid (1 eq) in dichloromethane was added 1-hydroxybenzotriazole hydrate (1 eq) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (1.3 eq). After 30 min a solution of aminoboronate hydrochloride (1 eq) was added followed by *N*-methylmorpholine (2 eq). The mixture was allowed to warm slowly to room temperature and was stirred for 8 hrs. The solution was washed with water, 1 M potassium hydrogen sulfate and saturated sodium bicarbonate consecutively. The organic solution was filtered through a pad of silica gel with ethyl acetate as eluent. The resulting solution was evaporated to give pure product.

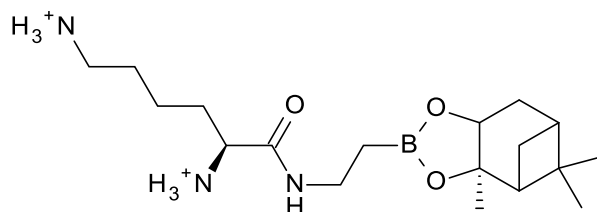
Yields of Boc-protected compounds are reported after the name of the compound.

Amine deprotection:

Boc-protected dipeptide (1 eq) was dissolved in MeOH and treated with a 5-fold excess of 1.25 M solution of hydrochloric acid in methanol at 0 °C for 1hr. Solvents were removed under vacuum, the residue was washed with diethyl ether and the solids were dried under vacuum to give pure product.

[2-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]ethylamine-*N*-(*L*)-lysine dichloride (2.6-1)

diBoc-protected analog: 91 % yield



HRMS found: 352.2770 Exact mass: 352.2766 Calculated for: C₁₈H₃₄BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 4.37 – 4.25 (m, 1H), 3.88 (m, 1H), 3.31 (m, 2H), 2.97 (m, 2H), 2.43 – 2.31 (m, 1H), 2.23 (m, 1H), 2.00 (t, *J* = 5.5 Hz, 1H), 1.95 – 1.67 (m, 6H), 1.51 (m, 2H), 1.38 (s, 3H), 1.30 (s, 3H), 1.09 (m, 3H), 0.86 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 168.22, 85.63, 77.58, 52.76, 51.16, 39.42, 38.91, 37.80, 35.47, 34.99, 30.72, 27.72, 26.62, 26.18, 25.94, 23.00, 21.62

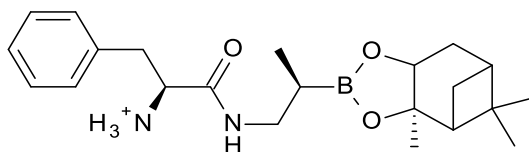
IR (cm⁻¹) 3220.6, 2919.5, 1667.7, 1384.7

[α]_D = n/a

56 % yield.

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]propylamine-*N*-(*L*)-phenylalanine chloride (2.6-2)

Boc-protected analog: yield 80 %



HRMS found: 385.2659 Exact mass: 385.2657 Calculated for: C₂₂H₃₄BN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.53 – 7.19 (m, 5H), 4.30 (dd, *J* = 8.7, 1.9 Hz, 1H), 4.11 – 3.95 (m, 1H), 3.27 – 2.99 (m, 4H), 2.36 (m, 1H), 2.22 (m, 1H), 2.04 – 1.96 (m, 1H), 1.88 (m, 1H), 1.79 (ddd, *J* = 14.7, 3.4, 1.9 Hz, 1H), 1.36 (s, 3H), 1.29 (s, 3H), 1.26 – 1.19 (m, 1H), 1.06 (d, *J* = 10.9 Hz, 1H), 0.89 (d, *J* = 7.4 Hz, 3H), 0.86 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 168.10, 134.29, 129.09, 128.69, 127.42, 85.60, 77.63, 54.43, 51.16, 42.26, 39.40, 37.80, 37.37, 34.98, 27.61, 26.06, 25.84, 22.88, 12.32

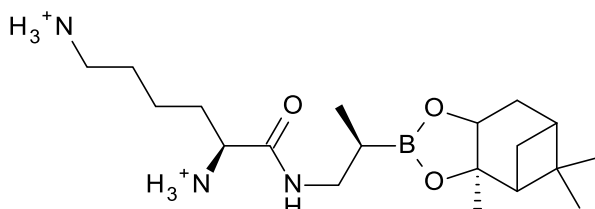
IR (cm⁻¹) 3208.35, 3062.27, 3030.45, 2917.99, 2871.55, 1665.16, 1386.18, 1029.65, 698.53

[α]_D = +25° (c = 0.4, MeOH, 18 °C)

Quantitative yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]propylamine-*N*-(*L*)-lysine dichloride (2.6-3)

diBoc-protected analog: yield 38 %



HRMS found: 366.2926 Exact mass: 366.2922 Calculated for: C₁₉H₃₇BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 4.32 (dd, *J* = 8.8, 1.9 Hz, 1H), 3.86 (m, 1H), 3.39 – 3.32 (m, 1H), 3.22 (dd, *J* = 13.4, 9.1 Hz, 1H), 2.98 – 2.83 (m, 2H), 2.38 (m, 1H), 2.30 – 2.16 (m, 1H), 2.01 (t, *J* = 5.5 Hz, 1H), 1.96 – 1.77 (m, 3H), 1.77 – 1.64 (m, 2H), 1.48 (dd, *J* = 17.6, 9.7 Hz, 2H), 1.38 (s, 3H), 1.30 (s, 1+3H), 1.08 (d, *J* = 10.9 Hz, 1H), 1.01 (d, *J* = 7.4 Hz, 3H), 0.87 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 161.94, 85.63, 77.68, 52.81, 51.22, 42.28, 39.44, 38.88, 37.82, 35.01, 30.80, 27.62, 26.74, 26.05, 25.85, 22.86, 21.61, 12.36

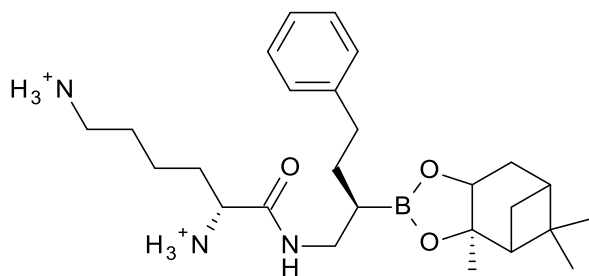
IR (cm⁻¹) 3207.9, 2920.4, 2872.4, 1670.1, 1375.9

[α]_D = +5.7° (c = 0.35, MeOH, 18 °C)

Quantitative yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-4-phenyl]butylamine-N-(*D*)-lysine dichloride (2.6-4)

diBoc-protected analog: yield 57 %



HRMS found: 456.3399 Exact mass: 456.3392 Calculated for: C₂₆H₄₃BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.31 – 7.06 (m, 5H), 4.34 (d, *J* = 8.7 Hz, 1H), 3.91 (m, 1H), 3.54 – 3.45 (m, 1H), 3.27 – 3.16 (m, 2H), 2.94 (m, 2H), 2.73 (m, 1H), 2.65 – 2.53 (m, 1H), 2.45 – 2.34 (m, 1H), 2.25 (m, 1H), 2.07 – 2.03 (m, 1H), 1.95 – 1.81 (m, 4H), 1.77 – 1.69 (m, 4H), 1.51 (m, 2H), 1.41 (s, 3H), 1.35 (m, 1H) 1.30 s, 3H), 1.24 – 1.11 (m+d, 2H), 0.87 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 168.52, 142.34, 128.06, 127.95, 125.41, 85.72, 77.67, 52.77, 51.24, 40.35, 39.50, 38.89, 37.86, 35.12, 34.56, 30.87, 30.63, 27.82, 26.65, 26.17, 26.09, 23.01, 21.67.

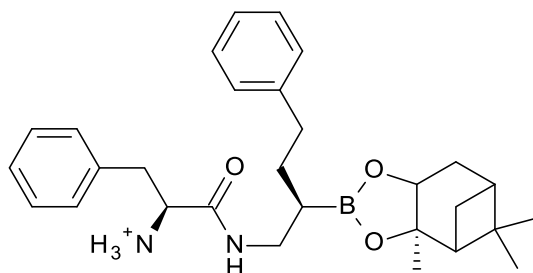
IR (cm⁻¹) 3805.1, 2922.37, 1669.81, 1495.78, 1387.27, 1280.12, 1029.61

[α]_D = -1.43° (c = 1.4, MeOH, 18 °C)

Quantitative yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-4-phenyl]butylamine-N-(*L*)-phenylalanine chloride (2.6-5)

Boc-protected analog: yield 57 %



HRMS found: 475.3137 Exact mass: 475.3127 Calculated for: C₂₉H₄₀BN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.39 – 7.06 (m, 10H), 4.34 (dd, *J* = 8.8, 2.0 Hz, 1H), 4.04 (t, *J* = 7.5 Hz, 1H), 3.38 – 3.21 (m, 2H), 3.10 (ddd, *J* = 51.5, 13.8, 7.1 Hz, 2H), 2.74 – 2.64 (m, 1H), 2.53 (m, 1H), 2.44 – 2.33 (m, 1H), 2.26 (m, 1H), 2.04 (t, *J* = 5.6 Hz, 1H), 1.92 (m, 1H), 1.83 (m, 1H), 1.62 (m, 2H), 1.39 (s, 3H), 1.30 (s, 3H), 1.28 – 1.21 (m, 1H), 1.13 (d, *J* = 10.8 Hz, 1H), 0.87 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 168.04, 142.27, 134.21, 129.01, 128.70, 128.06, 127.90, 127.45, 125.39, 85.72, 77.68, 54.43, 51.19, 40.30, 39.47, 37.83, 37.38, 35.07, 34.40, 30.33, 27.74, 26.07, 26.04, 22.91.

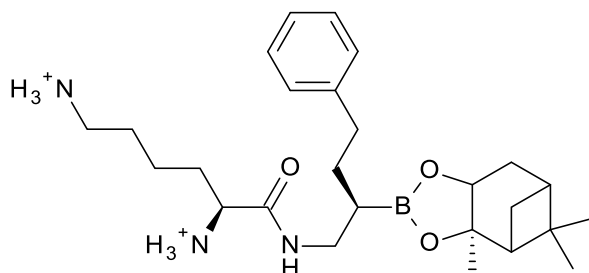
IR (cm⁻¹) 3212.74, 3026.4, 2910.18, 2868.3, 1666.56, 1378.06, 1029.25

[α]_D = +31.88° (c = 1.6, MeOH, 18 °C)

Quantitative yield

[(2*R*)-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-4-phenyl]butylamine-*N*-(*L*)-lysine dichloride (2.6-6)

diBoc-protected analog: 49 % yield after preparative TLC (EtAc/pentane 1:1, $R_f = 0.5$)



HRMS found: 456.3393 Exact mass: 456.3392 Calculated for: $C_{26}H_{43}BN_3O_3 [M-H]^+$

1H NMR (400 MHz, CD_3OD) δ 7.37 – 7.05 (m, 5H), 4.37 (dd, $J = 8.7, 1.9$ Hz, 1H), 3.85 (m, 1H), 3.44 (dd, $J = 13.6, 8.9$ Hz, 1H), 3.01 – 2.91 (m, 1H), 2.89 – 2.81 (m, 2H), 2.79 – 2.50 (m, 2H), 2.41 (ddd, $J = 14.1, 8.8, 2.4$ Hz, 1H), 2.33 – 2.22 (m, 1H), 2.06 (t, $J = 5.6$ Hz, 1H), 1.96 – 1.80 (m, 4H), 1.78 – 1.62 (m, 4H), 1.54 – 1.35 (m, 3+3H), 1.32 (s, 3H), 1.16 (d, $J = 10.8$ Hz, 1H), 0.89 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.60, 142.40, 127.99, 127.96, 125.42, 85.73, 77.67, 52.81, 51.22, 40.15, 39.50, 38.87, 37.86, 35.12, 34.39, 30.84, 30.51, 29.56, 27.80, 26.68, 26.11, 22.96, 21.64.

Signals were doubled due to some coordination effects.

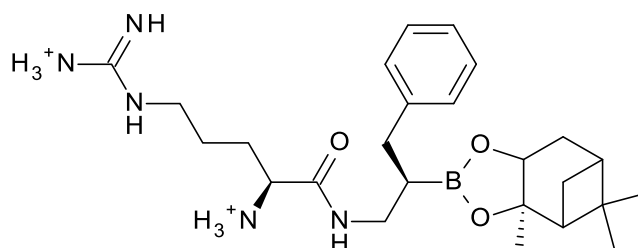
IR (cm^{-1}) 3366.05, 2921.1, 2845.5, 1747.74, 1672.6, 1496.19, 1387.7, 1278.68, 1237.68, 1029.35

$[\alpha]_D = +9.16^\circ$ ($c = 4.8$, MeOH, 18 °C)

85 % yield

[2*R*]-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-*N*-(*L*)-arginine dichloride (2.6-7)

Boc-protected analog: 55 % yield



HRMS found: 470.3290 Exact mass: 470.3297 Calculated for: $C_{25}H_{41}BN_5O_3 [M-H]^+$

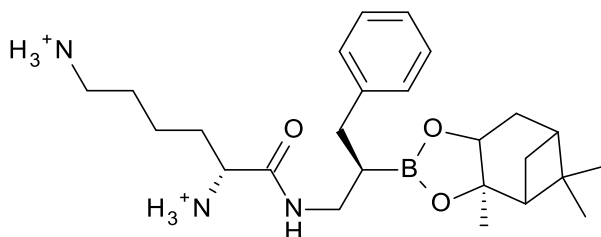
1H NMR (400 MHz, CD_3OD) δ 8.51 (s, 0H), 7.88 (d, $J = 8.3$ Hz, 1H), 7.74 (d, $J = 8.2$ Hz, 1H), 7.62 – 7.44 (m, 2H), 7.30 – 7.07 (m, 5H), 4.29 – 4.20 (m, 1H), 3.95 (t, $J = 6.7$ Hz, 1H), 3.38 (dd, $J = 13.4, 8.1$ Hz, 1H), 3.25 (d, $J = 6.2$ Hz, 3H), 2.88 – 2.62 (m, 2H), 2.39 – 2.24 (m, 1H), 2.07 (td, $J = 6.7, 3.6$ Hz, 1H), 1.92 (q, $J = 9.5, 7.7$ Hz, 3H), 1.85 – 1.79 (m, 1H), 1.78 – 1.64 (m, 5H), 1.27 (s, 4H), 1.26 (s, 3H), 0.86 (d, $J = 11.2$ Hz, 2H), 0.82 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.45, 157.17, 140.86, 128.65, 127.85, 127.00, 125.93, 125.64, 117.15, 110.07, 85.73, 77.63, 52.64, 51.05, 40.54, 40.46, 39.36, 37.75, 34.79, 34.37, 28.46, 27.58, 26.06, 25.82, 24.15, 22.91.

IR (cm⁻¹) 3323.25, 3251.82, 3154.41, 3062.44, 2922.47, 2869.96, 1663.41, 1387.18, 1375.58, 1238.7, 1029.27
[α]_D = -6.9° (c = 0.29, MeOH, 18 °C)
Quantitative yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(D)-lysine dichloride (2.6-8)

diBoc-protected analog: 86 % yield



HRMS found: 442.3235 Exact mass: 442.3236 Calculated for: C₂₅H₄₁BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.32 – 7.05 (m, 5H), 4.24 (dd, *J* = 8.8, 1.8 Hz, 1H), 3.96 – 3.87 (m, 1H), 3.45 (m, 1H), 3.27 – 3.17 (m, 1H), 2.95 (t, *J* = 7.8 Hz, 2H), 2.84 – 2.65 (m, 2H), 2.38 – 2.20 (m, 1H), 2.14 – 2.01 (m, 1H), 1.97 – 1.79 (m, 4H), 1.77 – 1.63 (m, 4H), 1.59 – 1.44 (m, 2H), 1.29 (s, 3H), 1.26 (s, 3H), 0.86 (d, *J* = 10.8 Hz, 1H), 0.83 (s, 3H)

¹³C NMR (101 MHz, CD₃OD) δ 168.52, 141.00, 128.66, 127.89, 125.66, 85.75, 77.69, 52.83, 51.14, 40.51, 39.40, 38.91, 37.79, 34.83, 34.43, 30.85, 27.59, 26.71, 26.11, 25.82, 22.94, 21.70

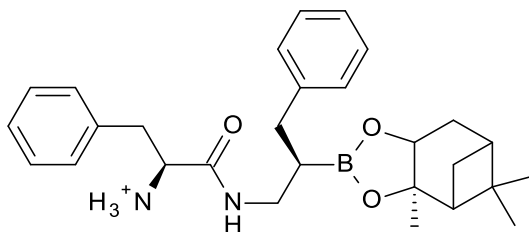
IR (cm⁻¹) 3202, 2917, 1747, 1672, 1495, 1387, 1238, 1029

[α]_D = -9.6° (c = 1.04, MeOH, 18 °C)

65 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-phenylalanine chloride (2.6-9)

Boc-protected analog: 86 % yield



HRMS found: 461.2977 Exact mass: 461.2970 Calculated for: C₂₈H₃₈BN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.38 – 7.12 (m, 10H), 4.23 (dd, *J* = 8.7, 2.1 Hz, 1H), 4.10 (t, *J* = 7.3 Hz, 1H), 3.29 – 2.98 (m, 4H), 2.61 (m, 2H), 2.39 – 2.24 (m, 1H), 2.11 – 2.01 (m, 1H), 1.93 (t, *J* = 5.5 Hz, 1H), 1.81 (dt, *J* = 5.5, 3.0 Hz, 1H), 1.77 – 1.66 (m, 1H), 1.65 – 1.52 (m, 1H), 1.25 (s+s, 6H), 0.86 (d, *J* = 11.0 Hz, 1H), 0.81 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.09, 140.86, 134.28, 129.09, 128.72, 128.66, 127.80, 127.45, 125.59, 85.70, 77.65, 54.43, 51.07, 40.37, 39.36, 37.74, 37.33, 34.79, 34.15, 27.57, 26.06, 25.80, 22.88.

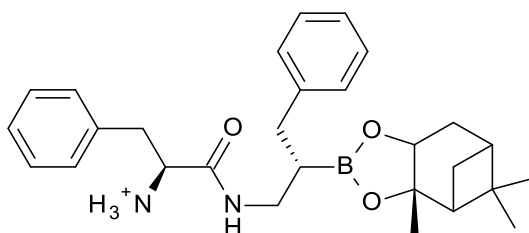
IR (cm^{-1}) 3337.15, 3212.81, 3060.66, 3028.16, 2921.3, 1671.26, 1560.22, 1496.12, 1387.75, 1029.41

$[\alpha]_{\text{D}} = +21.69^\circ$ ($c = 0.876$, MeOH, 18°C)

81 % yield

[2*S*-((3*aS*,4*S*,6*S*,7*aR*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-phenylalanine chloride (2.6-10)

Boc-protected analog: quantitative yield



HRMS found: 461.2967 Exact mass: 461.2970 Calculated for: $\text{C}_{28}\text{H}_{38}\text{BN}_2\text{O}_3$ $[\text{M}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.46 – 7.04 (m, 10H), 4.24 (d, $J = 8.3$ Hz, 1H), 4.09 – 3.95 (m, 1H), 3.41 (dd, $J = 13.4, 6.5$ Hz, 0.5H), 3.24 – 2.97 (m, 3.5H), 2.63 (qd, $J = 14.0, 8.0$ Hz, 2H), 2.31 (m, 1H), 2.08 (m, 1H), 1.97 – 1.90 (m, 1H), 1.82 (m, 1H), 1.77 – 1.69 (m, 1H), 1.59 (m, 1H), 1.27 (m, 6H), 0.90 – 0.74 (m, 1+3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.05, 140.92, 134.33, 129.14, 128.73, 128.67, 127.82, 127.45, 125.61, 85.71, 77.65, 54.40, 51.09, 40.32, 39.36, 37.75, 37.35, 34.81, 34.18, 27.58, 26.09, 25.81, 22.92.

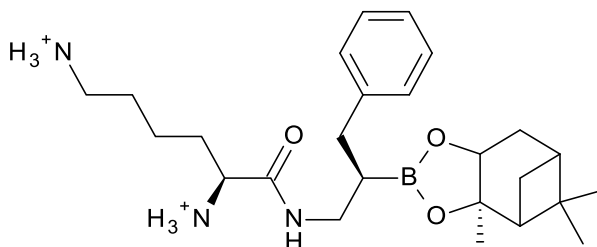
IR (cm^{-1}) 3400.3, 3027.69, 2918.41, 2901.5, 1664.04, 1496.06, 1454.7, 1386.95, 1029.54

$[\alpha]_{\text{D}} = +38.4^\circ$ ($c = 1.12$, MeOH, 18°C)

80 % yield.

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-lysine dichloride (2.6-11)

diBoc-protected analog: 74 % yield after preparative TLC (EtAc/pentane 7:3, $R_f = 0.2$)



HRMS found: 442.3245 Exact mass: 442.3236 Calculated for: $\text{C}_{25}\text{H}_{41}\text{BN}_3\text{O}_3$ $[\text{M}-\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.45 – 7.04 (m, 5H), 4.26 (dd, $J = 8.8, 1.9$ Hz, 1H), 3.87 (m, 1H), 3.39 (m, 1H), 3.29 – 3.24 (m, 1H), 2.93 (t, $J = 8.1$ Hz, 2H), 2.84 – 2.63 (m, 2H), 2.38 – 2.26 (m, 1H), 2.12 – 2.01 (m, 1H), 1.94 (t, $J = 5.6$ Hz, 1H), 1.92 – 1.80 (m, 3H), 1.78 – 1.65 (m, 4H), 1.54 – 1.45 (m, 2H), 1.27 (s+s, 6H), 0.86 (d, $J = 10.9$ Hz, 1H), 0.83 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.58, 140.90, 128.63, 127.85, 125.64, 85.71, 77.62, 52.77, 51.07, 40.51, 39.36, 38.89, 37.76, 34.81, 34.39, 30.81, 27.59, 26.72, 26.06, 25.81, 22.91, 21.65

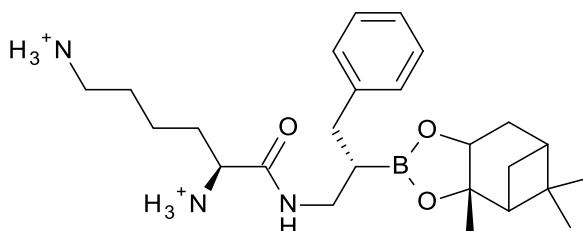
IR (cm^{-1}) 2917.78, 1669.37, 1387.03, 1238.38, 1029.05

$[\alpha]_{\text{D}} = -0.83^\circ$ ($c = 2.4$, MeOH, 18°C)

Quantitative yield

[2*S*-((3*aS*,4*S*,6*S*,7*aR*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-lysine dichloride (2.6-12)

diBoc-protected analog: 75 % yield



HRMS found: 442.3244 Exact mass: 442.3235 Calculated for: $\text{C}_{25}\text{H}_{41}\text{BN}_3\text{O}_3$ $[\text{M}-\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.62 – 6.92 (m, 5H), 4.24 (dd, $J = 8.7, 2.0$ Hz, 1H), 3.89 (t, $J = 6.7$ Hz, 1H), 3.45 (dd, $J = 13.4, 7.1$ Hz, 1H), 3.22 (dd, $J = 13.4, 8.1$ Hz, 1H), 2.94 (t, $J = 7.9$ Hz, 2H), 2.86 – 2.61 (m, 2H), 2.35 – 2.24 (m, 1H), 2.07 (m, 1H), 1.98 – 1.79 (m, 4H), 1.77 – 1.63 (m, 4H), 1.59 – 1.40 (m, 2H), 1.28 (s+s, 6H), 0.83 (s+d, 3+1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.49, 140.95, 128.62, 127.86, 125.64, 85.72, 77.64, 52.78, 51.08, 40.45, 39.35, 38.87, 37.76, 34.80, 34.39, 30.83, 27.54, 26.72, 26.06, 25.79, 22.90, 21.67

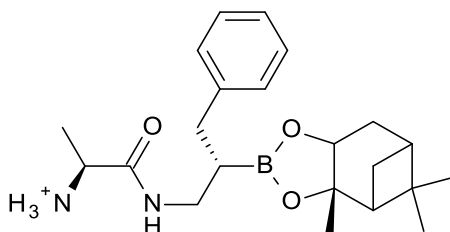
IR (cm^{-1}) 3205.21, 3024.41, 2917.41, 2869.77, 1670.09, 1387.13, 1238.53, 1029.09, 698.8

$[\alpha]_{\text{D}} = +8.6^\circ$ ($c = 1.05$, MeOH, 18°C)

73 % yield.

[2*S*-((3*aS*,4*S*,6*S*,7*aR*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-alanine chloride (2.6-13)

Boc-protected analog: quantitative yield.



HRMS found: 385.2651 Exact mass: 385.2657 Calculated for: $\text{C}_{22}\text{H}_{34}\text{BN}_2\text{O}_3$ $[\text{M}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.42 – 7.03 (m, 1H), 4.24 (dd, $J = 8.7, 2.1$ Hz, 0H), 3.90 (q, $J = 7.0$ Hz, 0H), 3.44 (dd, $J = 13.3, 7.6$ Hz, 0H), 3.19 (dd, $J = 13.4, 7.8$ Hz, 0H), 2.80 – 2.65 (m, 0H), 2.37 – 2.24 (m, 0H), 2.13

- 2.02 (m, 0H), 1.94 (t, $J = 5.5$ Hz, 0H), 1.82 (dt, $J = 5.3, 3.0$ Hz, 0H), 1.78 – 1.60 (m, 0H), 1.49 (d, $J = 7.0$ Hz, 1H), 1.28 (s, 1H), 1.26 (s, 1H), 0.86 (d, $J = 11.4$ Hz, 0H), 0.82 (s, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 169.38, 141.00, 128.58, 127.86, 125.62, 85.71, 77.62, 51.06, 48.83, 40.40, 39.34, 37.74, 34.80, 34.40, 27.54, 26.07, 25.83, 22.90, 16.44

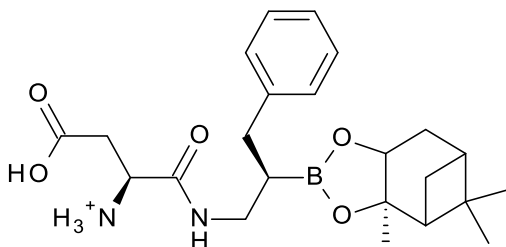
IR (cm^{-1}) 3211.02, 3061.15, 3026.19, 2918.23, 2870.27, 2595.79, 2510.31, 1667.84, 1386.87, 698.47

$[\alpha]_{\text{D}} = +1.4^\circ$ ($c = 1.08$, MeOH, 18°C)

Quantitative yield.

[2R-((3aR,4R,6R,7aS)hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-Aspartic acid chloride (2.6-14)

To a solution of 2.6-15 in THF, a solution of potassium hydroxide (4eq.) was added. The mixture was stirred at room temperature for 1hr. To the reaction mixture HCl in methanol was added (*quantum satis* until pH= 7). Solvents were evaporated, the residue was dissolved in methanol. The insoluble part was filtered off and the methanol solution was concentrated to provide pure product (2.6-14)



HRMS found: 451.2379 Exact mass: 451.2375 Calculated for: $\text{C}_{23}\text{H}_{33}\text{BN}_2\text{NaO}_5$ $[\text{M}+\text{Na}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.32 – 7.05 (m, 5H), 4.24 (dd, $J = 8.7, 2.2$ Hz, 1H), 4.08 (dd, $J = 9.3, 4.9$ Hz + 3.83 dd, $J = 8.7, 3.7$ Hz, 1H), 3.41 – 3.17 (m, 2H), 2.96 – 2.54 (m, 4H), 2.36 – 2.24 (m, 1H), 2.08 (qd, $J = 6.7, 3.2$ Hz, 1H), 1.93 (t, $J = 5.4$ Hz, 1H), 1.85 – 1.60 (m, 3H), 1.33 – 1.18 (m, 6H), 0.89 (d, $J = 10.7$ Hz, 1H), 0.82 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 171.02, 168.99, 141.14, 128.63, 127.83, 125.56, 85.57, 77.56, 51.68, 51.08, 40.29, 39.37, 37.75, 34.89, 34.41, 34.21, 27.61, 26.12, 25.81, 22.95

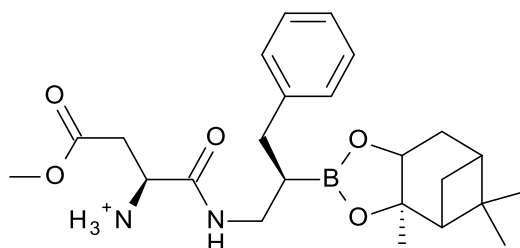
IR (cm^{-1}) 3026.5, 2920.6, 2869.5, 1619.6

$[\alpha]_{\text{D}}$ n/a

73 % yield.

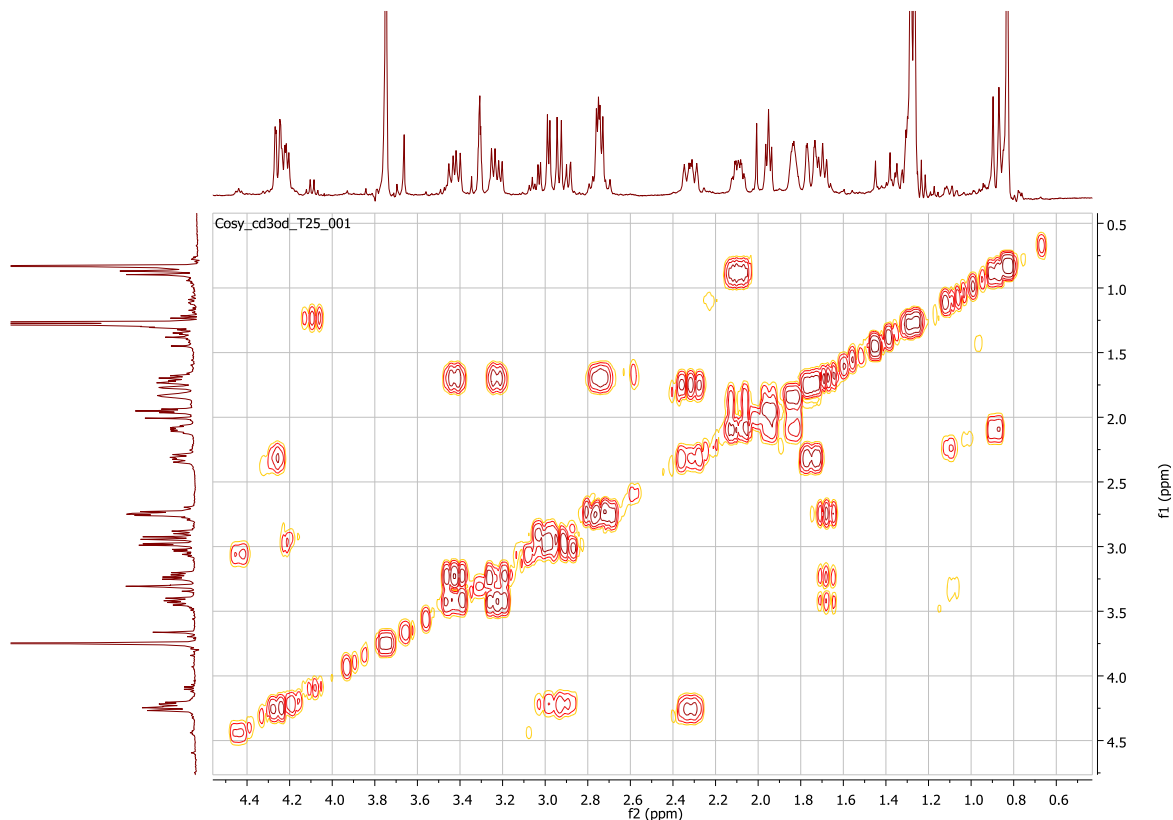
[2R-((3aR,4R,6R,7aS)hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-N-(L)-Aspartic acid methyl ester chloride (2.6-15)

Boc-protected analog: 59 % yield



HRMS found: 443.2718 Exact mass: 443.2712 Calculated for: $C_{24}H_{36}BN_2O_5 [M]^+$

1H NMR (400 MHz, CD_3OD) δ 8.30 (d, $J = 5.9$ Hz, 0H), 7.31 – 7.10 (m, 2H), 4.24 (ddd, $J = 15.2, 8.2, 3.4$ Hz, 1H), 3.75 (s, 1H), 3.43 (dd, $J = 13.2, 8.0$ Hz, 0H), 3.31 (p, $J = 1.7$ Hz, 0H), 3.23 (dd, $J = 13.4, 6.6$ Hz, 0H), 3.04 – 2.84 (m, 1H), 2.80 – 2.67 (m, 1H), 2.38 – 2.25 (m, 0H), 2.13 – 2.05 (m, 0H), 1.95 (t, $J = 5.6$ Hz, 0H), 1.84 (dd, $J = 5.6, 2.8$ Hz, 0H), 1.80 – 1.66 (m, 1H), 1.27 (d, $J = 6.5$ Hz, 3H), 0.88 (d, $J = 11.0$ Hz, 1H), 0.83 (s, 1H).
COSY:

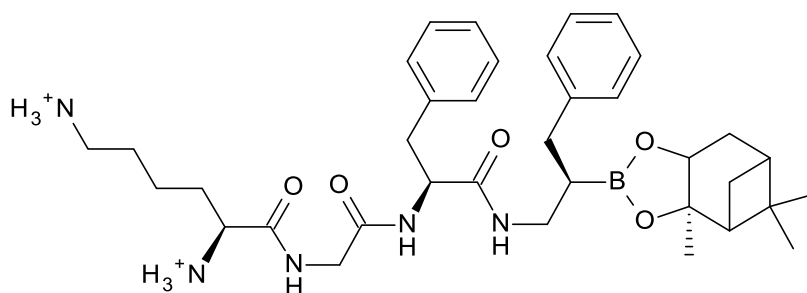


^{13}C NMR (101 MHz, CD_3OD) δ 170.05, 167.32, 140.91, 128.62, 127.85, 125.64, 85.73, 77.67, 51.62, 51.09, 49.42, 40.43, 39.38, 37.75, 34.86, 34.79, 34.24, 27.58, 26.09, 25.83, 22.91

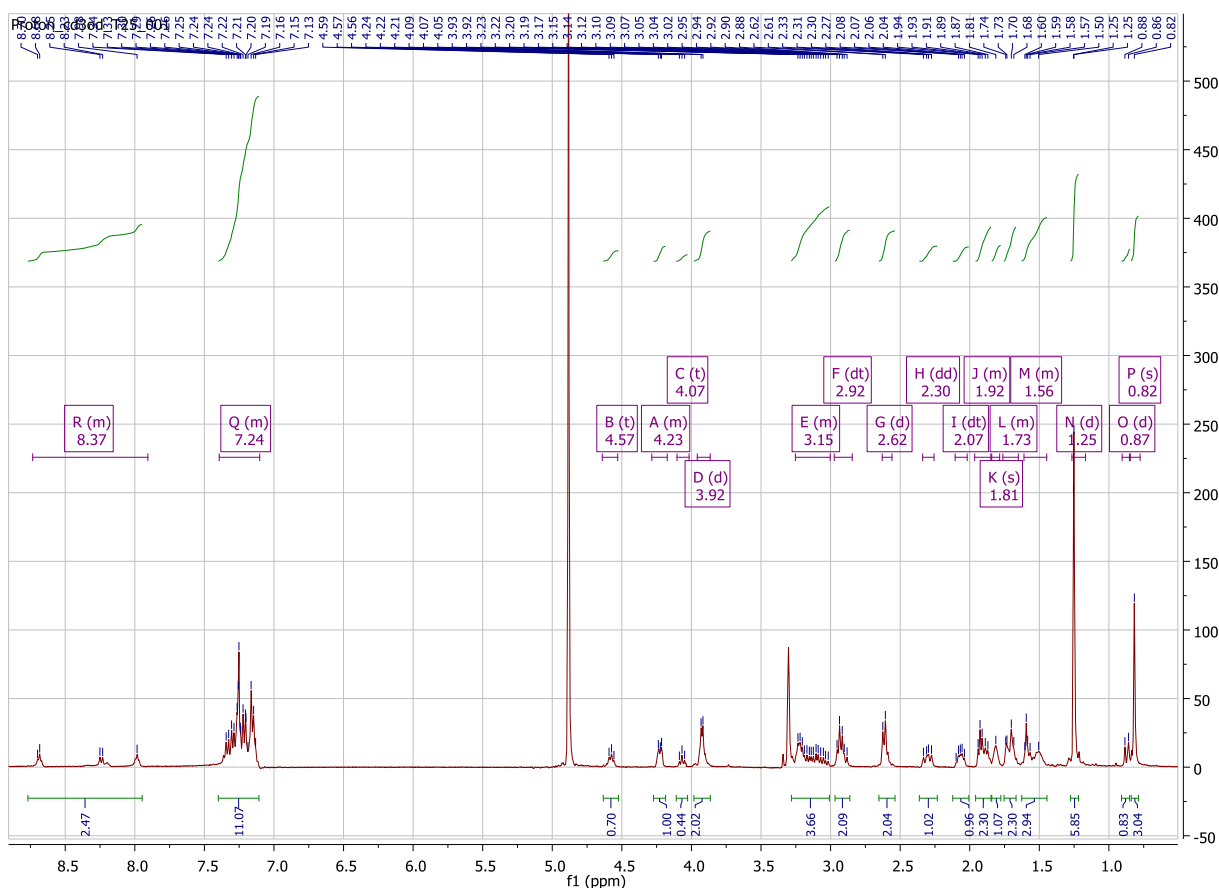
96 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl]propylamine-*N*-(*L*)-phenylalanine-glycine-(*L*)-lysine dichloride (2.6-16)

Spectra are difficult to interpret. Probably contains some impurities or rotamers.

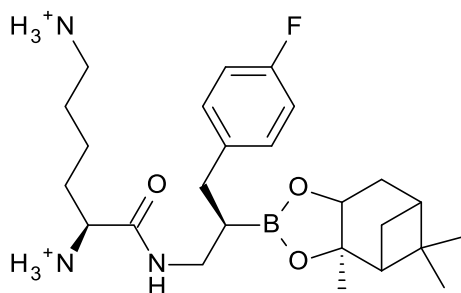


HRMS found: 646.4138 Exact mass: 646.4134 Calculated for: $C_{36}H_{53}BN_5O_5 [M-H]^+$



[2R-((3aR,4R,6R,7aS)hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-fluorophenyl)]propylamine-N-(L)-lysine dichloride (2.6-17)

Boc-protected analog: quantitative yield



HRMS found: 460.3139 Exact mass: 460.3141 Calculated for: $C_{25}H_{40}BFN_3O_3$ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.24 (dd, *J* = 8.5, 5.6 Hz, 2H), 6.96 (t, *J* = 8.8 Hz, 2H), 4.26 (dd, *J* = 8.8, 1.9 Hz, 1H), 3.92 (t, *J* = 6.5 Hz, 1H), 3.44 – 3.24 (m, 4H), 2.94 (t, *J* = 7.9 Hz, 2H), 2.84 – 2.64 (m, 2H), 2.40 – 2.26 (m, 1H), 2.13 – 2.05 (m, 1H), 1.95 (m, 1H), 1.85 (m, 2H), 1.77 – 1.64 (m, 5H), 1.53 (m, 2H), 1.28 (s+s, 6H), 0.86 – 0.82 (s+d, 4H).

¹³C NMR (101 MHz, CD₃OD) δ 168.59, 161.40 (d, *J* = 243.1 Hz), 136.84 (d, *J* = 3.0 Hz), 130.28 (d, *J* = 7.7 Hz), 114.35 (d, *J* = 21.3 Hz), 85.76, 77.67, 52.80, 51.09, 40.44, 39.37, 38.89, 37.77, 34.81, 33.51, 30.80, 27.59, 26.72, 26.05, 25.81, 22.88, 21.65.

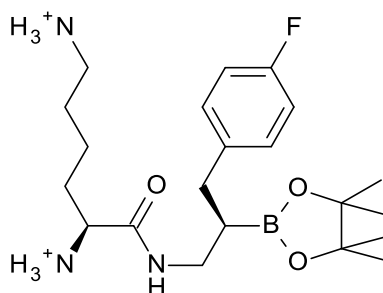
IR (cm⁻¹) 3390.7, 3205.4, 2929.1, 1674.1, 1509.0, 1388.3, 1220.8, 667.8

[α]_D = +0.5° (c = 2.0, MeOH, 18 °C)

Quantitative yield

(2*R*)-3-(4-fluorophenyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propan-1-amine-N-(L)-lysine dichloride (2.6-18)

The mixture of 2.7-17 (1 eq), pinacole (1 eq) and THF was stirred for 10 hrs at room temperature. Solvent was evaporated to give white precipitate of 2.6-18 with quantitative yield.



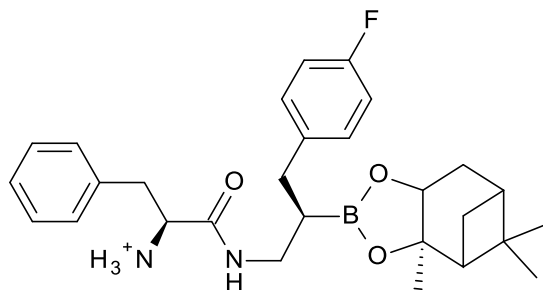
HRMS found: 408.2834 Exact mass: 408.2828 Calculated for: C₂₁H₃₆BFN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.35 – 7.14 (m, 2H), 6.98 (m, 2H), 3.97 – 3.87 (m, 1H), 3.41 – 3.12 (m, 4H), 2.95 (m, 2H), 2.83 – 2.56 (m, 2H), 1.88 (s, 2H), 1.72 (m, 2H), 1.52 (m, 3H), 1.25 – 1.10 (s+s, 12H).

¹³C NMR (101 MHz, CD₃OD) δ 168.59, 161.40 (d, *J* = 242.3 Hz), 136.85 (d, *J* = 3.2 Hz), 130.29 (d, *J* = 7.8 Hz), 114.32 (d, *J* = 21.3 Hz), 83.42, 74.41, 52.78, 40.98, 40.25, 38.88, 34.40, 33.39, 30.85, 26.72, 23.83, 23.61, 21.68

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-fluorophenyl)]propylamine-N-(L)-phenylalanine chloride (2.6-19)

Boc-protected analog: 96 % yield



HRMS found: 479.2870 Exact mass: 479.2875 Calculated for: C₂₈H₃₇BFN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.41 – 7.22 (m, 5H), 7.16 (dd, *J* = 8.5, 5.6 Hz, 2H), 6.95 (dd, *J* = 9.5, 7.9 Hz, 2H), 4.24 (dd, *J* = 8.8, 1.9 Hz, 1H), 4.08 (t, *J* = 7.4 Hz, 1H), 3.24 – 2.97 (m, 4H), 2.57 (m, 2H), 2.31 (m, 1H), 2.15 – 2.03 (m, 1H), 1.93 (t, *J* = 5.5 Hz, 1H), 1.83 (m, 1H), 1.72 (m, 1H), 1.26 (s, 3H), 1.25 (s, 3H), 0.84-0.82 (m, 1H+3H).

¹³C NMR (101 MHz, CD₃OD) δ 168.09 (s), 161.38 (d, *J* = 242.3 Hz), 136.76 (d, *J* = 3.2 Hz), 134.26 (s), 130.28 (d, *J* = 7.7 Hz), 129.05 (s), 128.73 (s), 127.46 (s), 114.28 (d, *J* = 21.2 Hz), 85.74 (s), 77.67 (s), 54.43 (s), 51.06 (s), 40.25 (s), 39.35 (s), 37.74 (s), 37.33 (s), 34.77 (s), 33.23 (s), 27.55 (s), 26.02 (s), 25.78 (s), 22.85 (s).

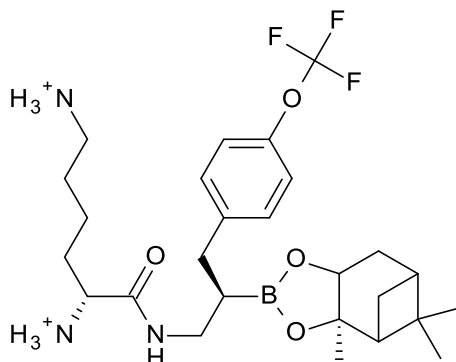
IR (cm⁻¹) 3219, 2919, 1668, 1508, 1387, 1220, 699

[α]_D = +18.3° (c = 1.8, MeOH, 18 °C)

Quantitative yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-trifluoromethoxyphenyl)]propylamine-*N*-(*D*)-lysine dichloride (2.6-20)

Boc-protected analog: 59 % isolated yield



HRMS found: 526.3058 Exact mass: 526.3058 Calculated for: C₂₆H₄₀BF₃N₃O₄ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 8.49 (s, 0.5H), 7.33 (d, *J* = 8.9 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 4.24 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.91 (m, 1H), 3.44 (dd, *J* = 13.3, 7.1 Hz, 1H), 3.25 (dd, *J* = 13.7, 8.4 Hz, 1H), 2.95 (t, *J* = 7.9 Hz, 2H), 2.89 – 2.65 (m, 2H), 2.35 – 2.25 (m, 1H), 2.09 – 1.99 (m, 1H), 1.92 (m, 1H), 1.84 (m, 2H), 1.76 – 1.65 (m, 5H), 1.54 (m, 2H), 1.27 (s+s, 6H), 0.82 (s, 3H), 0.75 (d, *J* = 10.9 Hz, 1H).

¹³C NMR (101 MHz, CD₃OD) δ 168.54, 147.37, 140.38, 130.28, 120.48, 120.52 (q, *J* = 254.9 Hz OCF₃), 85.78, 77.68, 52.79, 51.04, 40.35, 39.32, 38.87, 37.74, 34.74, 33.61, 30.83, 27.48, 26.72, 26.02, 25.71, 22.87, 21.67.

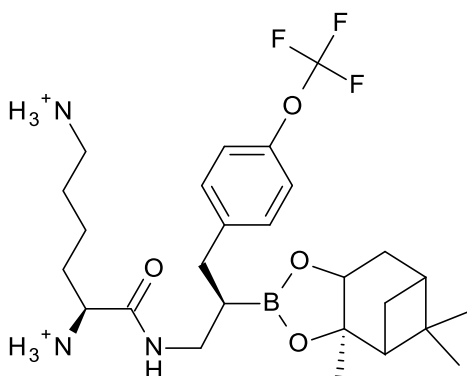
IR (cm⁻¹) 3379.44, 3210.89, 2932.97, 1673.43, 1508.27, 1388.98, 1260.96, 1222.57, 1161.57, 667.9

[α]_D = -10° (c = 1.0, MeOH, 18 °C)

Quantitative yield.

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-trifluoromethoxyphenyl)]propylamine-*N*-(*L*)-lysine dichloride (26-21)

diBoc-protected analog: 76 % yield



HRMS found: 526.3057 Exact mass: 526.3058 Calculated for: C₂₆H₄₀BF₃N₃O₄ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 8.54 (b.s, 1H), 7.37 – 7.30 (m, 2H), 7.15 (m, 2H), 4.26 (dd, *J* = 8.8, 2.0 Hz, 4H), 3.93 (t, *J* = 6.5 Hz, 4H), 3.38 (m, 2H), 2.94 (t, *J* = 7.8 Hz, 2H), 2.88 – 2.67 (m, 2H), 2.35 – 2.26 (m, 1H), 2.08 – 2.01 (m, 1H), 1.96 – 1.78 (m, 5H), 1.71 (d, *J* = 9.4 Hz, 2H), 1.52 (p, *J* = 8.0 Hz, 2H), 1.26 (s+s, 6H), 0.82 (s, 3H), 0.76 (d, *J* = 10.7 Hz, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.64, 147.39, 140.35, 130.28, 120.50, 120.52 (q, J = 255.0 Hz (OCF_3), 85.77, 77.65, 52.77, 51.02, 40.44, 39.32, 38.87, 37.74, 34.75, 33.64, 30.80, 27.53, 26.72, 26.02, 25.73, 22.87, 21.65.

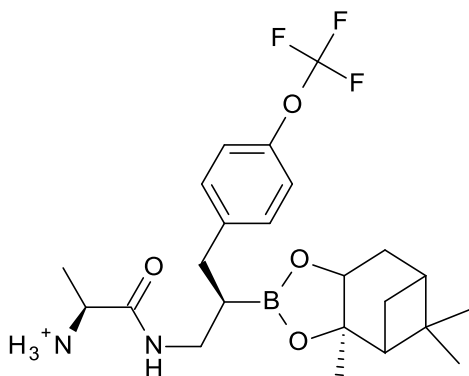
IR (cm^{-1}) 3382, 3206, 2926, 1673, 1508, 1260, 1222, 1161

$[\alpha]_{\text{D}} = +2.35^\circ$ (c = 1.7, MeOH, 18 $^\circ\text{C}$)

Quantitative yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-trifluoromethoxyphenyl)]propylamine-*N*-(*L*)-alanine chloride (2.6-22)

Boc-protected analog: 75 % yield



HRMS found: 469.2484 Exact mass: 469.2480 Calculated for: $\text{C}_{23}\text{H}_{33}\text{BF}_3\text{N}_2\text{O}_4$ [M] $^+$

^1H NMR (400 MHz, CD_3OD) δ 7.32 (d, J = 8.6 Hz, 2H), 7.14 (d, J = 8.2 Hz, 2H), 4.26 (dd, J = 8.8, 2.0 Hz, 1H), 3.95 (q, J = 7.0 Hz, 1H), 3.43 – 3.14 (m, 2H), 2.88 – 2.67 (m, 2H), 2.36 – 2.24 (m, 1H), 2.06 (ddd, J = 10.8, 6.2, 2.1 Hz, 1H), 1.93 (t, J = 5.6 Hz, 1H), 1.86 – 1.78 (m, 1H), 1.79 – 1.67 (m, 2H), 1.50 (d, J = 7.1 Hz, 3H), 1.26 (s+s, 3H+3H), 0.82 (s, 3H), 0.79 (d, J = 10.8 Hz, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 169.54, 147.39, 147.37, 140.38, 130.22, 120.45, 120.53 (q, J = 254.8 Hz), 85.76, 77.69, 51.05, 48.83, 40.33, 39.35, 37.73, 34.76, 33.57, 27.53, 26.04, 25.76, 22.86, 16.44.

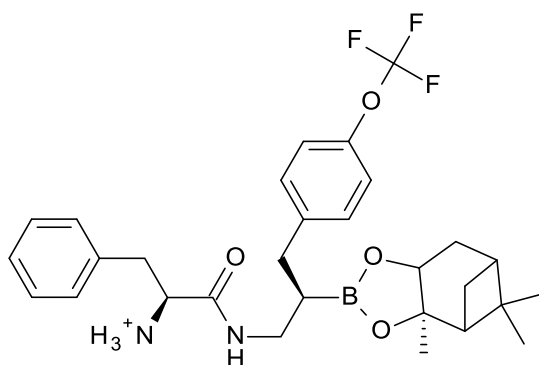
IR (cm^{-1}) 3211, 3063, 2983, 2931, 1670, 1508, 1389, 1262, 1222, 1162

$[\alpha]_{\text{D}} = -8.16^\circ$ (c = 0.49, MeOH, 18 $^\circ\text{C}$)

Quantitative yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(4-trifluoromethoxyphenyl)]propylamine-*N*-(*L*)-phenylalanine chloride (2.6-23)

Boc-protected analog: 67 % yield



HRMS found: 545.2793 Exact mass: 545.2793 Calculated for: $C_{29}H_{37}BF_3N_2O_4[M]^+$

1H NMR (400 MHz, CD_3OD) δ 7.36 – 7.06 (m, 10H), 4.23 (dd, J = 8.8, 2.1 Hz, 1H), 4.14 (t, J = 7.3 Hz, 1H), 3.27 – 3.04 (m, 4H), 2.60 (qd, J = 13.8, 7.6 Hz, 2H), 2.34 – 2.22 (m, 1H), 2.03 (m, 1H), 1.91 (t, J = 5.5 Hz, 1H), 1.80 (dt, J = 5.5, 2.9 Hz, 1H), 1.74 – 1.66 (m, 1H), 1.64 – 1.55 (m, 1H), 1.24 (s+s, 6H), 0.81 (s, 3H), 0.76 (d, J = 10.9 Hz, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.17, 147.36, 140.31, 134.32, 130.31, 129.14, 128.70, 127.42, 120.38, δ 120.54 (q, J = 255.0 Hz (- OCF_3)), 85.75, 77.68, 54.44, 51.04, 40.26, 39.33, 37.72, 37.31, 34.74, 33.36, 27.53, 26.05, 25.73, 22.87.

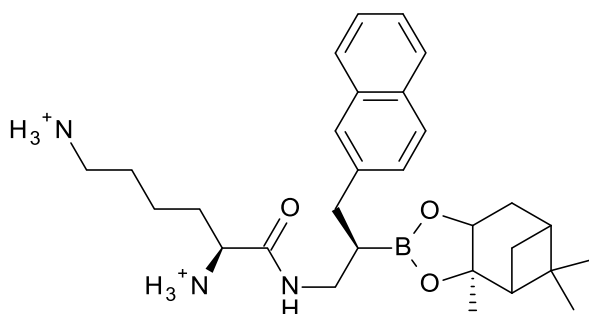
IR (cm^{-1}) 3208, 2929, 1669, 1508, 1388, 1262, 1223, 1162

$[\alpha]_D = +26.4^\circ$ (c = 0.53, MeOH, 18 °C)

Quantitative yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-(2-naphthyl)]propylamine-*N*-(*L*)-lysine dichloride (2.6-24)

Boc-protected analog: 32 % yield after preparative TLC (EtOAc/pentane 3:7, R_f = 0.2)



HRMS found: 492.3390 Exact mass: 492.3392 Calculated for: $C_{29}H_{43}BN_3O_3[M-H]^+$

1H NMR (400 MHz, CD_3OD) δ 7.82 – 7.67 (m, 4H), 7.46 – 7.35 (m, 3H), 4.24 (dd, J = 8.7, 1.9 Hz, 1H), 3.94 (t, J = 6.6 Hz, 1H), 3.51 – 3.32 (m, 2H), 2.94 (m, 4H), 2.28 (dd, J = 14.3, 8.8 Hz, 1H), 1.99 – 1.78 (m, 5H), 1.72 (m, 4H), 1.53 (m, 2H), 1.24 (s, 3H), 1.22 (s, 3H), 0.80 (s, 3H), 0.77 (d, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.61, 138.46, 133.58, 132.21, 127.41, 127.32, 127.15, 127.01, 126.82, 125.52, 124.86, 85.75, 77.67, 52.82, 51.06, 40.61, 39.31, 38.90, 37.73, 34.76, 34.57, 30.80, 27.58, 26.71, 26.03, 25.70, 22.87, 21.67.

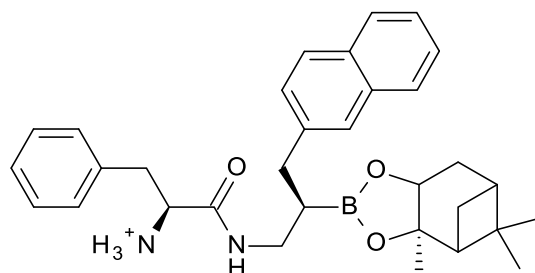
IR (cm^{-1}) 3413.96, 3212.61, 3049.19, 2972.11, 2870.32, 1671.67, 1388.83

$[\alpha]_D = 0^\circ$ (c = 1.31, MeOH, 18 °C)

81 % yield

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)]-3-(2-naphthyl)propylamine-*N*-(*L*)-phenylalanine chloride (2.6-25)

Boc-protected analog: 49 % yield after preparative TLC (EtOAc/pentane 4:10, $R_f = 0.2$)



HRMS found: 511.3123 Exact mass: 511.3127 Calculated for: $C_{32}H_{40}BN_2O_3 [M]^+$

1H NMR (400 MHz, CD_3OD) δ 8.37 (s, 0.5H), 7.84 – 7.55 (m, 4H), 7.47 – 7.20 (m, 8H), 4.23 – 4.07 (m, 2H), 3.49 – 3.02 (m, 4H), 2.87 – 2.68 (m, 2H), 2.21 (m, 1H), 1.95 – 1.77 (m, 2H), 1.76 – 1.59 (m, 3H), 1.29 – 1.06 (m, 6H), 0.83 – 0.65 (m, 1+3H). Signals are broadened.

^{13}C NMR (101 MHz, CD_3OD) δ 168.20, 138.49, 134.36, 133.55, 132.18, 129.23, 128.73, 127.44, 127.38, 127.22, 127.07, 126.91, 125.53, 124.87, 85.70, 77.62, 54.46, 50.99, 40.42, 39.26, 37.69, 37.35, 34.75, 34.51, 34.32, 27.63, 26.10, 25.73, 22.95.

Signals were doubled due to some coordination effects.

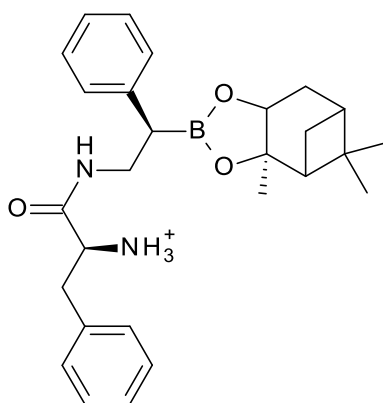
IR (cm^{-1}) 3227.0, 2968.01, 2919.48, 2868.74, 1668.37, 1387.6, 745.99

$[\alpha]_D = +23^\circ$ ($c = 0.35$, MeOH, $18^\circ C$)

91 % yield.

[2*R*-((3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-2-phenyl]ethylamine-*N*-(*L*)-phenylalanine chloride (2.6-26)

Boc-protected analog contained app. 20 % of impurities. Was used without further purification.



HRMS found: 447.2810 Exact mass: 447.2813 Calculated for: $C_{27}H_{36}BN_2O_3 [M]^+$

app. 15-20 % impurity of starting boronate.

1H NMR (400 MHz, CD_3OD) δ 7.47 – 7.06 (m, 10H), 4.33 (dd, $J = 8.9, 2.0$ Hz, 1H), 3.95 (dd, $J = 8.4, 5.5$ Hz, 1H), 3.77 – 3.49 (m, 2H), 2.97 – 2.70 (m, 2H), 2.67 (dd, $J = 10.8, 5.7$ Hz, 1H), 2.40 – 2.28 (m, 1H), 2.15 (m,

1H), 2.01 (t, $J= 5.5$ Hz, 1H), 1.83 (m, 1H), 1.71 (m, 1H), 1.34 (s, 3H), 1.27 (s, 3H), 0.97 (d, $J= 11.0$ Hz, 1H), 0.84 (s, 3H)

^{13}C NMR (101 MHz, CD_3OD) δ 168.15, 139.91, 134.06, 128.96, 128.67, 128.38, 128.17, 127.38, 125.66, 85.99, 77.84, 54.16, 51.17, 41.16, 39.26, 37.84, 37.26, 34.84, 27.50, 26.03, 25.80, 22.87

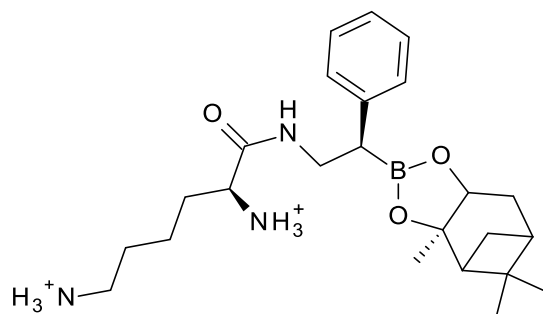
IR (cm^{-1}) 3062.7, 3028.9, 2922.3, 2872.3, 1669.1

$[\alpha]_{\text{D}} = +30^\circ$ ($c= 0.5$, MeOH, 18°C)

33 % yield.

[2R-((3aR,4R,6R,7aS)hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-2-phenyl]ethylamine-N-(L)-lysine dichloride (2.6-27):

Boc-protected analog: 76 % yield



HRMS found: 428.3079 Exact mass: 428.3070 Calculated for: $\text{C}_{24}\text{H}_{40}\text{BN}_3\text{O}_3$ $[\text{M}-\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.38 – 7.06 (m, 4H), 4.33 (dd, $J= 8.8, 2.1$ Hz, 1H), 3.95 (dd, $J= 13.9, 11.7$ Hz, 1H), 3.71 (t, $J= 6.5$ Hz, 1H), 3.56 – 3.40 (m, 1H), 2.82 – 2.60 (m, 3H), 2.38 – 2.27 (m, 1H), 2.22 – 2.09 (m, 1H), 2.00 (t, $J= 5.5$ Hz, 1H), 1.84 (dd, $J= 5.4, 2.6$ Hz, 1H), 1.70 (ddd, $J= 14.7, 3.3, 1.9$ Hz, 1H), 1.62 – 1.43 (m, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 1.12 – 0.99 (m, 2H), 0.95 (d, $J= 10.9$ Hz, 1H), 0.84 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.25, 139.97, 128.43, 128.10, 125.59, 86.00, 77.88, 52.69, 51.20, 40.66, 39.28, 38.77, 37.84, 34.84, 30.56, 27.48, 26.65, 26.02, 25.77, 22.83, 21.04

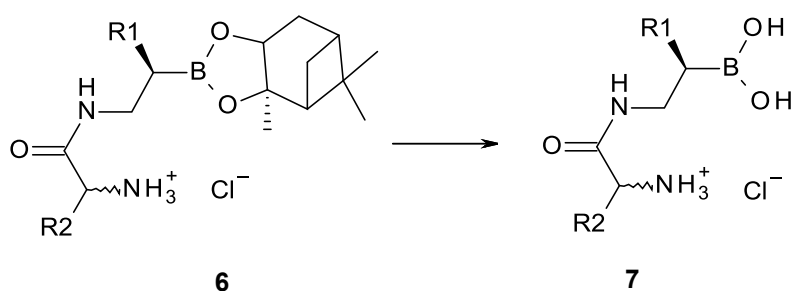
IR (cm^{-1}) n/a

$[\alpha]_{\text{D}}$ n/a

83 % yield.

General procedures for synthesis of amino boronic acids 2.7

Wityak J., Earl R. A., Abelman M. M., Bethel Y. B., Fisher B. N., Kauffman G. S., Kettner C. A., Ma P. and McMillan J. L., (1995), *J. Org. Chem.*, 60(12), 3717-22



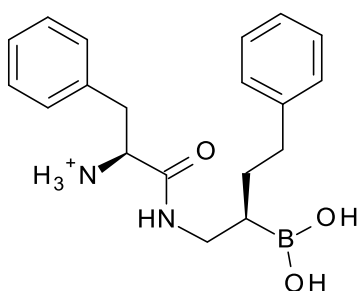
The diol was removed by one of the two following procedures:

A: Dipeptide (1 eq) was stirred at 90 °C in hydrochloric acid (3M solution, 10-fold excess) for 1hr. The reaction mixture was cooled to room temperature extracted with dichloromethane and the water layer was concentrated under vacuum to give the product as a white precipitate.

B: To a solution of dipeptide (1 eq) in (6 mL per 100 mg of peptide) diethyl ether/water (1:1) phenylboronic acid (4 eq) was added. The reaction mixture was stirred for 10 hrs. The aqueous layer was separated, washed with diethyl ether (3 x 3 mL) and concentrated under vacuum to give pure product in quantitative yield.

[(1S)-1-benzyl-2-[(2R)-2-borono-4-phenyl-butyl]amino]-2-oxo-ethyl]ammonium chloride (2.7-5)

Deprotection by method A.



^1H NMR (400 MHz, CD_3OD) δ 7.38 – 7.02 (m, 10H), 4.09 (t, J = 7.4 Hz, 1H), 3.34 – 3.24 (m, 1H), 3.21 – 3.00 (m, 3H), 2.58 – 2.46 (m, 2H), 1.68 – 1.47 (m, 2H), 1.27 (b.s, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.08, 142.19, 134.32, 129.08, 128.64, 128.10, 127.88, 127.37, 125.39, 54.41, 40.65, 37.35, 34.56, 30.45

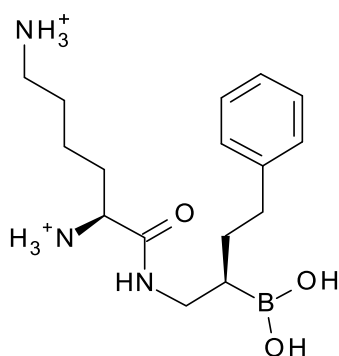
IR (cm^{-1}) 3235.22, 3026.6, 2924.84, 2861.06, 1665.29, 1496.15, 1371.51

$[\alpha]_{\text{D}} = +43.18^\circ$ (c = 0.88, MeOH, 18 °C)

70 % yield

[(5S)-5-azaniumyl-6-[(2R)-2-borono-4-phenyl-butyl]amino]-6-oxo-hexyl]ammonium dichloride (2.7-6)

Deprotection by method A



^1H NMR (400 MHz, CD_3OD) δ 7.32 – 7.08 (m, 5H), 3.88 (t, J = 6.6 Hz, 1H), 3.42 (dd, J = 13.3, 7.4 Hz, 1H), 3.20 (dd, J = 13.3, 6.5 Hz, 1H), 2.99 – 2.84 (m, 2H), 2.65 – 2.53 (m, 2H), 1.94 – 1.78 (m, 2H), 1.77 – 1.65 (m, 4H), 1.49 (m, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.51, 142.18, 128.02, 127.92, 125.44, 52.74, 40.63, 38.83, 34.57, 30.87, 30.69, 26.71, 21.64

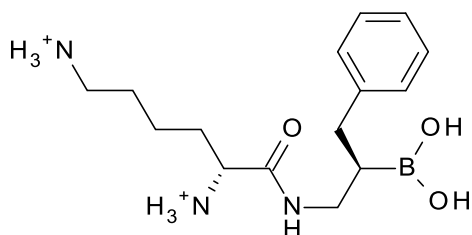
IR (cm^{-1}) 3350.47, 3226.68, 2924.47, 1666.63, 1495.48, 1378.18, 1073.18

$[\alpha]_{\text{D}} = +12.5^\circ$ ($c = 2.8$, MeOH, 18°C)

52 % isolated yield after preparative TLC (MeOH/ Et_2O , 7:3, $R_{\text{f}} = 0.2$)

[(5*R*)-5-azaniumyl-6-[[*(2R)*-2-borono-3-phenyl-propyl]amino]-6-oxo-hexyl]ammonium dichloride (2.7-8):

Deprotection by method A.



^1H NMR (400 MHz, CD_3OD) δ 7.32 – 7.09 (m, 5H), 3.93 (t, $J = 6.6$ Hz, 1H), 3.39 (dd, $J = 13.2, 7.7$ Hz, 1H), 3.18 (m, 1H), 3.00 – 2.91 (m, 2H), 2.80 – 2.57 (m, 2H), 1.97 – 1.69 (m, 4+1H), 1.60 – 1.45 (m, 2H)

^{13}C NMR (101 MHz, CD_3OD) δ 168.49, 141.37, 128.26, 128.02, 125.66, 52.77, 41.13, 38.88, 35.32, 30.83, 26.67, 21.57

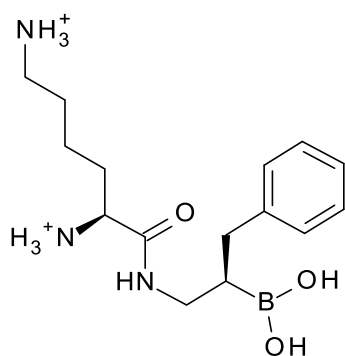
IR (cm^{-1}) 3224, 3025, 2932, 1670, 1567, 1495, 1386

$[\alpha]_{\text{D}} = -7.14$ ($c = 0.98$, MeOH, 18°C)

39 % yield after LC ($\text{Et}_2\text{O}/\text{MeOH}$, 25 % to 75 % of MeOH)

[(5*S*)-5-azaniumyl-6-[[*(2R)*-2-borono-3-phenyl-propyl]amino]-6-oxo-hexyl]ammonium dichloride (2.7-11)

Deprotection by method A.



^1H NMR (400 MHz, CD_3OD) δ 7.37 – 7.03 (m, 5H), 3.91 (m, 1H), 3.31 – 3.14 (m, 2H), 3.00 – 2.87 (m, 2H), 2.81 – 2.54 (m, 2H), 1.95 – 1.67 (m, 5H), 1.52 (m, 2H)

^{13}C NMR (101 MHz, CD_3OD) δ 168.62, 141.37, 128.28, 128.00, 125.64, 52.75, 41.16, 38.86, 35.37, 30.86, 26.68, 21.66

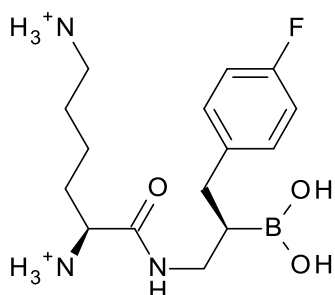
IR (cm^{-1}) 3223.6, 3023.98, 2927.83, 1668.26, 1495.16, 1381.29, 1070.83

$[\alpha]_{\text{D}} = -5.59^\circ$ ($c = 1.79$, MeOH, 18°C)

50 % yield after LC ($\text{Et}_2\text{O}/\text{MeOH}$, 30 %-100 % MeOH)

[(5*S*)-5-azaniumyl-6-[[*(2R)*-2-borono-3-(4-fluorophenyl)propyl]amino]-6-oxo-hexyl]ammonium dichloride (2.7-17)

Deprotection by method B.



^1H NMR (400 MHz, CD_3OD) δ 8.70 – 7.84 (group of broad singlets, 1H), 7.19 (m, 2H), 6.96 (m, 2H), 3.98 (b.m., 1H), 3.40 – 3.10 (m, 2H), 2.96 (m, 2H), 2.78 – 2.52 (m, 2H), 1.90 (b.m., 2H), 1.81 – 1.69 (m, 3H), 1.60 – 1.48 (m, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.80, 168.71, 162.54, 162.43, 160.13, 160.03, 137.50, 137.39, 137.35, 130.08, 130.05, 130.00, 129.97, 114.65, 114.47, 114.44, 114.26, 52.78, 40.99, 40.75, 39.01, 38.92, 34.42, 34.01, 30.86, 26.63, 21.73, 21.71, 21.64 resonances were doubled

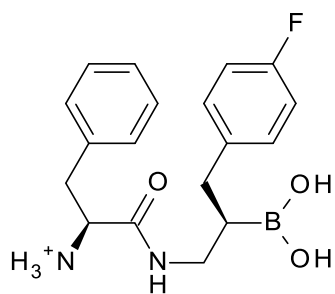
IR (cm^{-1}) 3229.32, 2931.22, 1670.99, 1508.72, 1382.99, 1220.28, 667.81

$[\alpha]_{\text{D}} = +6.96^\circ$ ($c = 1.58$, MeOH, 18°C)

84 % yield.

[(1*S*)-1-benzyl-2-[[*(2R)*-2-borono-3-(4-fluorophenyl)propyl]amino]-2-oxo-ethyl]ammonium chloride (2.7-19)

Deprotection by method B.



^1H NMR (400 MHz, CD_3OD) δ 8.37 (b.s, 0.26H)+7.71 (d, $J = 7.4$ Hz, 0.4H) amide proton, 7.43 – 7.20 (m, 5+1H), 7.11 (m, 2H), 6.94 (m, 2H), 4.17 (b.s, 1H), 3.15 (m, 4H), 2.60 – 2.42 (b.s, 2H), 1.60 (b.s, 1H). Resonances were broadened

^{13}C NMR (101 MHz, CD_3OD) δ 168.22, 161.28 (d, $J = 242.0$ Hz), 137.32 (b.s), 134.38, 133.42, 130.03 (d, $J = 7.7$ Hz), 129.18, 128.69, 127.40, 127.16, 114.42 (d, $J = 21.0$ Hz), 54.43, 37.35

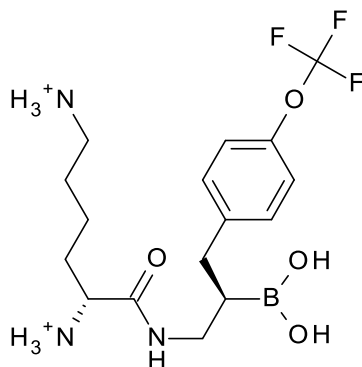
IR (cm^{-1}) 3245.4, 3065.5, 2932.2, 1669.0, 1508.4, 1373.2, 1221.0, 667.8

$[\alpha]_{\text{D}} = +29.46^\circ$ ($c = 1.12$, MeOH, 18°C)

82 % yield.

[(5*R*)-5-azaniumyl-6-[[*(2R)*-2-borono-3-[4-(trifluoromethoxy)phenyl]propyl]amino]-6-oxo-hexyl]ammonium dichloride (2.7-20)

Deprotection by method B.



^1H NMR (400 MHz, CD_3OD) δ 8.5-7.5 (broad signals, 0.2H), 7.29 (d, J = 8.1 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 3.95 (t, J = 6.4 Hz, 1H), 3.35 (dd, J = 13.6, 7.6 Hz, 2H), 3.22 (dd, J = 13.5, 6.8 Hz, 2H), 2.96 (m, 2H), 2.81 – 2.55 (m, 2H), 1.89 (m, 3H), 1.75 (b.s, 2H), 1.60 – 1.47 (b.m, 2H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.73, 147.38, 140.84, 129.94, 120.57, 120.52 (q, J = 255.0 Hz (OCF_3)), 52.76, 40.92, 38.89, 34.48, 30.86, 26.65, 21.68.

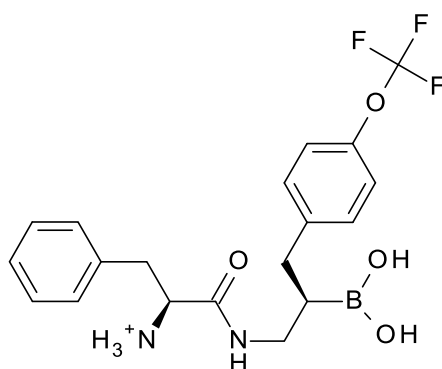
IR (cm^{-1}) 3337.73, 2931.48, 1663.48, 1508.13, 1374.67, 1258.87, 1160.11, 668.01

$[\alpha]_{\text{D}} = +9.43^\circ$ (c = 0.53, MeOH, 18 °C)

41 % isolated yield.

[(1*S*)-1-benzyl-2-[[*(2R)*-2-borono-3-[4-(trifluoromethoxy)phenyl]propyl]amino]-2-oxo-ethyl]ammonium chloride (2.7-23)

Deprotection by method A. Contained approximately 13 % of impurity



^1H NMR (400 MHz, CD_3OD) δ 7.39 – 7.06 (m, 1H), 4.14 (t, J = 7.1 Hz, 1H), 3.28 – 3.01 (m, 1H), 2.55 (d, J = 7.1 Hz, 1H), 1.60 (s, 1H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.21, 147.33, 140.79, 134.36, 129.94, 129.11, 128.67, 127.39, 120.46, 120.53 (q, J = 255.0 Hz (OCF_3)) 54.42, 40.68, 37.33, 35.88, 34.11.

IR (cm^{-1}) 3233, 2928, 1668, 1508, 1263, 1222, 1164

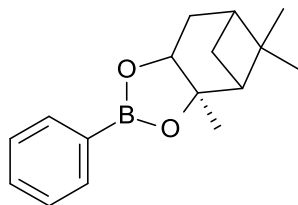
$[\alpha]_{\text{D}} = \text{n/a}$

31 % yield.

General procedure for the synthesis of compounds 2.8, exemplified by 2.8e.

(3*aR*,4*R*,6*R*,7*aS*)-2-phenylhexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.8e, R1=phenyl, Scheme 2.3)

To a stirred solution of trimethoxyborate (5.20 g, 0.05 mol) kept at -78 °C in dry THF (50mL) phenylmagnesium chloride (6.85 g, 0.05 mol) was added drop wise. White precipitate formed after 2 hrs of stirring and the mixture was allowed to warm to room temperature with continuing stirring. To the reaction mixture water (70 mL) was added followed by 10 % H₂SO₄ (40ml). The resulting mixture was extracted with diethyl ether (3 x 70 mL) and the organic extracts were washed with water and extracted with 2M KOH. The aqueous extract was saturated with sodium chloride and acidified by 10 % H₂SO₄ to the pH~1. The solution was extracted with diethyl ether (3 x 50 mL) and the organic phase was dried over magnesium sulfate, filtered, concentrated to a volume of 100 mL. To this solution pinanediol (5.00 g, 0.03 mol) was added at room temperature and stirred overnight. The reaction mixture was concentrated under vacuum to obtain an oily residue that was dissolved in pentane, filtered through a plug of silica gel, dried over magnesium sulfate and concentrated under vacuum to give final product as colorless crystals. (7.00 g, 54.6 % yield)



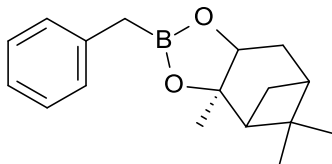
¹H NMR (400 MHz, CDCl₃) δ 7.82 (dt, *J*= 8.1, 1.4 Hz, 2H), 7.52 – 7.43 (m, 1H), 7.41 – 7.33 (m, 2H), 4.50 – 4.42 (m, 1H), 2.48 – 2.34 (m, 1H), 2.30 – 2.20 (m, 1H), 2.16 (t, *J*= 5.2 Hz, 1H), 2.03 – 1.90 (m, 2H), 1.49 (s, 3H), 1.32 (s, 3H), 1.25 – 1.20 (m, 1H), 0.90 (s, 3H).

All spectroscopic data were in accordance with earlier published data:

Matteson D. S., Ray R., Rocks R. R., Tsai D. J., *Organometallics*, (1983), 2, 11, 1536-1543.

The structure was also confirmed by X-ray analysis.

(3*aR*,4*R*,6*R*,7*aS*)-2-benzylhexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12c)



¹H NMR (400 MHz, CDCl₃) δ 7.33 – 7.15 (m, 5H), 4.32 (dd, *J*= 8.7, 2.0 Hz, 1H), 2.41 – 2.32 (m, 3H), 2.27 – 2.19 (m, 1H), 2.10 (t, *J*= 5.6 Hz, 1H), 1.93 (ddd, *J*= 8.5, 5.9, 2.9 Hz, 1H), 1.87 (ddd, *J*= 14.5, 3.3, 2.0 Hz, 1H), 1.43 (s, 3H), 1.33 (s, 3H), 1.11 (d, *J*= 10.9 Hz, 1H), 0.88 (s, 3H).

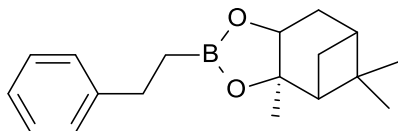
¹³C NMR (101 MHz, CDCl₃) δ 139.00, 129.16, 128.50, 125.07, 86.04, 78.15, 51.52, 39.69, 38.37, 35.68, 28.83, 27.29, 26.63, 24.21

All spectroscopic data were in accordance with earlier published data:

Martichonok V., Jones J. B., (1996), *J. Am. Chem. Soc.*, 118, 950-958.

2-phenethyl-1,3,2-dioxaborolane (2.12d)

The product was obtained by stirring phenethyl boronic acid with pinanediol (1:1 ratio) in ethyl acetate at room temperature, overnight. The solvent was evaporated and the oily residue was dissolved in pentane, washed with brine and dried over magnesium sulfate. Pentane was removed under vacuum to obtain the product as slightly yellow oil (95.2 % yield)

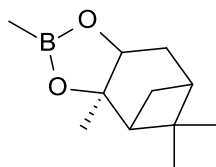


^1H NMR (400 MHz, CDCl_3) δ 7.36 – 7.12 (m, 5H), 4.29 – 4.21 (m, 1H), 2.84 – 2.71 (m, 2H), 2.39 – 2.26 (m, 1H), 2.17 (m, 1H), 2.04 (t, J = 5.5 Hz, 1H), 1.86 (m, 2H), 1.37 (s, 3H), 1.28 (s, 3H), 1.23 – 1.14 (m, 2H), 1.01 (d, J = 10.9 Hz, 1H), 0.84 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 144.39, 128.18, 127.98, 125.49, 85.52, 77.67, 51.25, 39.49, 38.10, 35.44, 30.09, 28.65, 27.07, 26.36, 23.98

(3a*R*,4*R*,6*R*,7a*S*)-2-methyl hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12b)

A mixture of trimethylboroxine (0.5 g, 0.004 mol), pinanediol (2.0 g, 0.012 mol) and diethyl ether (10 mL) was stirred under argon atmosphere at room temperature for 2 hrs and left without a stopper under a stream of argon, followed by pumping under high vacuum for 30 minutes to remove residual solvent. The resulting oil was pure product (1.8 g, 76.8 % yield).



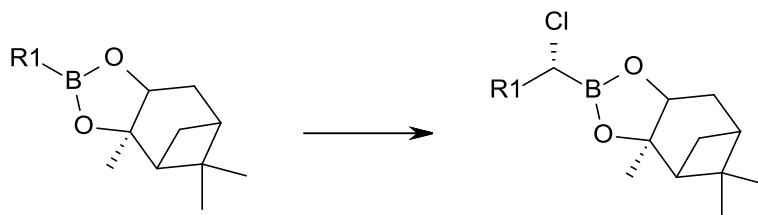
^1H NMR (400 MHz, CDCl_3) δ 4.24 (dd, J = 8.7, 1.8 Hz, 1H), 2.38 – 2.26 (m, 1H), 2.25 – 2.14 (m, 1H), 2.02 (t, J = 5.6 Hz, 1H), 1.96 – 1.76 (m, 2H), 1.37 (s, 3H), 1.27 (s, 3H), 1.11 (d, J = 10.9 Hz, 1H), 0.83 (s, 3H), 0.27 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 85.39, 77.62, 51.29, 39.51, 38.10, 35.45, 28.65, 27.07, 26.42, 23.97

All spectroscopic data were in accordance with earlier published data:

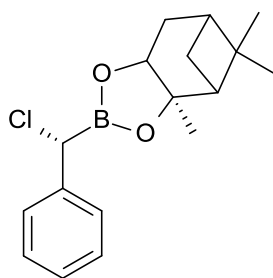
Matteson D. S., Ray R., Rocks R. R., Tsai D. J., *Organometallics*, (1983), 2, 11, 1536-1543.

General procedure for the synthesis of compounds 2.9, exemplified by 2.9e.



(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*)-chloro(phenyl)methyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.9e)

Dichloromethylithium was prepared by the addition of n-butyllithium (5.5 mL, 2.7 M solution in THF, 0.015 mol) to dichloromethane (2.0 g, 0.024 mol) in anhydrous THF at -100 °C under argon. A solution of phenylboronic ester (3.0 g, 0.011 mol) was slowly added to the vigorously stirred slurry of dichloromethylithium. After 10 min the reaction temperature was raised to -78 °C and the mixture was stirred for 30 min. Anhydrous zinc chloride (2.7g, 1M solution in diethyl ether, 0.02 mol) was then added and the mixture was allowed to warm to room temperature. After 2 hrs diethyl ether was added to the reaction mixture and the suspension obtained was washed with a saturated ammonium chloride solution. The solvent was evaporated under vacuum and the oily residue was dissolved in diethyl ether, washed with brine and dried over magnesium sulfate. Diethyl ether was removed under vacuum to obtain the product as slightly yellow oil (3.0 g, 84 %).



¹H NMR (400 MHz, CDCl₃) δ 7.46 – 7.20 (m, 5H), 4.50 (s, 1H), 4.35 (dd, *J*= 8.8, 1.8 Hz, 1H), 2.36 – 2.26 (m, 1H), 2.23 – 2.14 (m, 1H), 2.07 (dd, *J*= 6.0, 4.8 Hz, 1H), 1.90 – 1.78 (m, 3H), 1.37 (s, 3H), 1.25 (s, 3H), 1.11 (d, *J*= 11.0 Hz, 1H), 0.80 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 139.02, 128.87, 128.72, 128.04, 87.31, 79.05, 51.41, 39.48, 38.51, 35.45, 28.59, 27.22, 26.49, 24.21

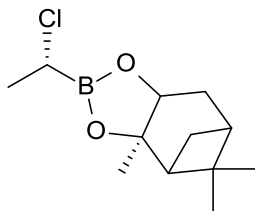
[α]_D = +15.6° (c= 0.26, toluene)

All spectroscopic data were in accordance with earlier published data:

Matteson D. S., Beedle E. C., Christenson E., Dewey M. A., Peterson M. L., (1987), Journal of Labelled Compounds and Radiopharmaceuticals, vol 25, (6), 675-683.

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*)-chloroethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.9b)

The reaction was performed according to the general procedure (83.7 % yield).



¹H NMR (400 MHz, CDCl₃) δ 4.36 (dd, *J*= 8.8, 1.8 Hz, 1H), 3.57 (q, *J*= 7.5 Hz, 1H), 2.40 – 2.30 (m, 1H), 2.24 (m, 1H), 2.11 – 2.05 (m, 1H), 1.95 – 1.85 (m, 2H), 1.57 (d, *J*= 7.5 Hz, 3H), 1.42 (s, 3H), 1.29 (s, 3H), 1.16 (d, *J*= 11.1 Hz, 1H), 0.84 (s, 3H).

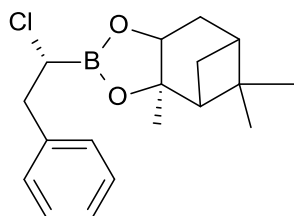
¹³C NMR (101 MHz, CDCl₃) δ 86.74, 78.57, 51.18, 39.32, 38.22, 35.24, 28.39, 26.99, 26.27, 23.94, 20.57

IR (cm⁻¹) 2969.3, 2921.2, 2871.7, 1378.3, 1029.5

All spectroscopic data were in accordance with earlier published data:
Martichonok V., Jones J. B., (1996), J. Am. Chem. Soc., 118, 950-958.

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*)-1-chloro-2-phenyl-ethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.9c)

The reaction was performed according to the general procedure (96 % yield).



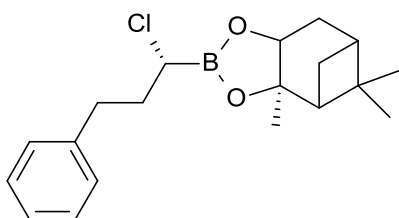
^1H NMR (400 MHz, CDCl_3) δ 7.33 – 7.14 (m, 5H), 4.34 (dd, $J = 8.8, 1.9$ Hz, 1H), 3.70 – 3.60 (m, 1H), 3.15 (ddd, $J = 22.4, 13.9, 8.0$ Hz, 2H), 2.39 – 2.27 (m, 1H), 2.17 (ddd, $J = 8.3, 6.1, 2.2$ Hz, 1H), 2.06 (t, $J = 5.5$ Hz, 1H), 1.93 – 1.79 (m, 2H), 1.34 (s, 3H), 1.28 (s, 3H), 1.06 (d, $J = 11.0$ Hz, 1H), 0.83 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 138.66, 129.42, 128.59, 126.96, 87.03, 78.76, 51.38, 40.61, 39.57, 38.45, 35.38, 28.58, 27.24, 26.47, 24.19

All spectroscopic data were in accordance with earlier published data:
Martichonok V., Jones J. B., (1996), J. Am. Chem. Soc., 118, 950-958.

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*)-1-chloro-3-phenyl-propyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.9d)

The reaction was performed according to the general procedure (82.3 % yield)



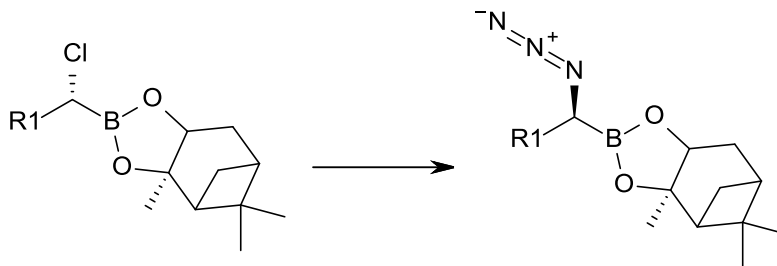
^1H NMR (400 MHz, CDCl_3) δ 7.33 – 7.17 (m, 5H), 4.36 (dd, $J = 8.8, 1.8$ Hz, 1H), 3.48 (dd, $J = 8.1, 6.6$ Hz, 1H), 2.83 (m, 2H), 2.40 – 2.32 (m, 1H), 2.28 – 2.21 (m, 1H), 2.15 (m, 2H), 2.09 (t, $J = 5.5$ Hz, 1H), 1.91 (m, 2H), 1.41 (s, 3H), 1.30 (s, 3H), 1.18 (d, $J = 11.0$ Hz, 1H), 0.85 (s, 3H).

Contains 15 % of impurity.

^{13}C NMR (101 MHz, CDCl_3) δ 141.08, 128.65, 128.41, 126.00, 86.79, 78.57, 51.16, 39.35, 38.22, 35.83, 35.26, 33.30, 28.44, 27.01, 26.37, 23.96

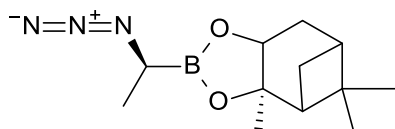
Material was used in the subsequent step without further purification.

General procedure for the synthesis of compounds 2.10, exemplified by 2.10b.



3a*R*,4*R*,6*R*,7a*S*)-2-[(1*S*)-azidoethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.10b)

A solution of **2.9b** (3.70 g, 0.015 mol) in dichloromethane (30 mL) was placed in a dropping funnel and added drop wise to a vigorously stirred mixture of sodium azide (10.00 g, 0.15 mol) and tetrabutylammonium bromide (0.25 g, 0.00078 mol) in a mixture of dichloromethane (200 mL) and water (45 mL). The reaction mixture was stirred overnight at room temperature. The organic phase was separated and the water phase was extracted by additional portions of dichloromethane (3 x 20 mL). The extracts were combined and concentrated under vacuum. The resulting oily residue was dissolved in dichloromethane and filtered through a pad of silica to remove traces of the phase-transfer catalyst. The final dichloromethane solution was dried over magnesium sulfate and concentrated under vacuum to obtain colorless oil that was distilled (b.p. 94-96 °C (3.0*10⁻³mbar)) to give pure product (3.20 g, 84.2 %).



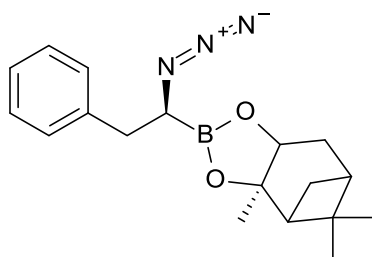
¹H NMR (400 MHz, CDCl₃) δ 4.35 (dd, *J* = 8.7, 1.7 Hz, 1H), 3.23 (q, *J* = 7.5 Hz, 1H), 2.41 – 2.30 (m, 1H), 2.26 (ddd, *J* = 10.9, 6.1, 3.1 Hz, 1H), 2.08 (t, *J* = 5.4 Hz, 1H), 1.96 – 1.84 (m, 2H), 1.41 (s, 3H), 1.37 (d, *J* = 7.7 Hz, 3H), 1.29 (s, 4H), 1.08 (d, *J* = 11.0 Hz, 1H), 0.84 (s, 4H).

¹³C NMR (101 MHz, CDCl₃) δ 104.99, 86.80, 78.51, 77.31, 76.99, 76.67, 51.08, 39.38, 38.10, 35.20, 28.45, 26.98, 26.39, 23.91

IR (cm⁻¹) 2921.9, 2089.8, 1378.2, 1204.2

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*S*)-1-azido-2-phenylethyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.10c)

The reaction was performed according to the general procedure (94.0 % yield).



^1H NMR (400 MHz, CDCl_3) δ 4.31 (dd, $J = 8.8, 1.9$ Hz, 1H), 3.36 (dd, $J = 8.9, 5.8$ Hz, 1H), 2.96 (ddd, $J = 23.0, 14.0, 7.4$ Hz, 2H), 2.35 – 2.27 (m, 1H), 2.21 – 2.12 (m, 1H), 2.04 (t, $J = 5.5$ Hz, 1H), 1.91 – 1.81 (m, 2H), 1.35 (s, 3H), 1.26 (s, 3H), 0.96 (d, $J = 11.0$ Hz, 1H), 0.81 (s, 3H).

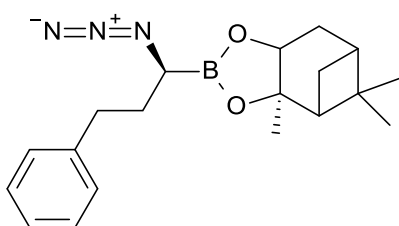
^{13}C NMR (101 MHz, CDCl_3) δ 138.86, 129.33, 128.69, 126.90, 87.19, 78.77, 77.57, 77.25, 76.93, 51.26, 39.63, 38.34, 37.03, 35.40, 28.69, 27.23, 26.59, 24.18

IR (cm^{-1}) 3028.7, 2920.7, 2870.9, 2089.9, 1376.3

$[\alpha]_{\text{D}} = -12.6^\circ$ ($c = 0.476$, toluene, 18°C)

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*S*)-1-azido-3-phenyl-propyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.10d)

The reaction was performed according to the general procedure (89.8 % yield).

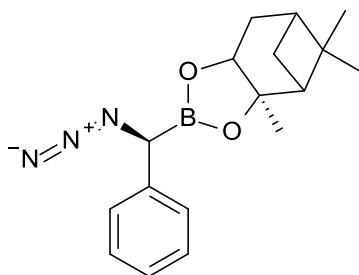


^1H NMR (400 MHz, CDCl_3) δ 7.19 (m, 5H), 4.31 – 4.24 (m, 1H), 3.06 (t, $J = 7.1$ Hz, 1H), 2.80 – 2.56 (m, 2H), 2.35 – 2.23 (m, 1H), 2.19 (m, 1H), 2.01 (t, $J = 5.5$ Hz, 1H), 1.98 – 1.76 (m, 4H), 1.34 (s, 3H), 1.22 (s, 3H), 1.03 (d, $J = 11.0$ Hz, 1H), 0.77 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 141.24, 128.55, 128.42, 126.00, 86.88, 78.51, 51.07, 39.43, 38.11, 35.24, 33.35, 32.50, 30.91, 28.53, 26.99, 26.50, 23.94

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*S*)-azido(phenyl)methyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.10e)

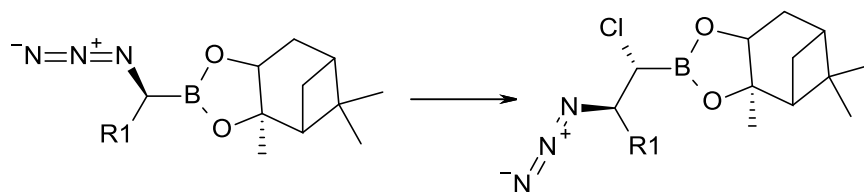
The reaction was performed according to the general procedure (68.6 % yield).



^1H NMR (400 MHz, CDCl_3) δ 7.50 – 7.12 (m, 5H), 4.36 – 4.17 (m, 1H), 2.30 – 2.17 (m, 1H), 2.04 (dtd, $J = 8.2, 6.1, 2.5$ Hz, 1H), 1.96 (t, $J = 5.4$ Hz, 1H), 1.76 (ddd, $J = 14.0, 10.1, 3.1$ Hz, 2H), 1.31 (s, 3H), 1.16 (s, 3H), 0.84 (d, $J = 11.0$ Hz, 1H), 0.72 (s, 3H).

The method was discontinued for this compound as a fair amount of benzaldehyde was formed.

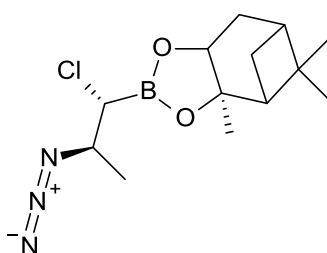
General procedure for the synthesis of compounds 2.11, exemplified by 2.11b.



The reaction was performed according to the general homologation procedure.

(89.2 % yield).

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*,2*R*)-2-azido-1-chloro-propyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.11b)

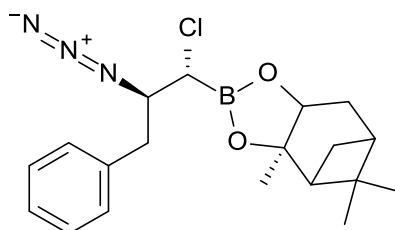


¹H NMR (400 MHz, CDCl₃) δ 4.39 (dd, *J* = 9.0, 1.6 Hz, 1H), 3.96 – 3.77 (m, 1H), 3.44 (t, *J* = 6.3 Hz, 1H), 2.42 – 2.31 (m, 1H), 2.26 (m, 1H), 2.09 (t, *J* = 5.5 Hz, 1H), 1.98 – 1.83 (m, 2H), 1.43 (d, *J* = 6.6 Hz, 1H), 1.42 (s, 3H), 1.40 (d, *J* = 6.6 Hz, 2H), 1.29 (s, 3H), 1.19 (d, *J* = 11.0 Hz, 1H), 0.84 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) δ 87.27, 78.80, 59.61, 51.04, 39.29, 38.23, 35.15, 28.39, 26.97, 26.33, 23.97, 17.50, 17.08

IR (cm⁻¹) 2973.2, 2925.3, 2872.4, 2103.3, 1377.2

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*,2*R*)-2-azido-1-chloro-3-phenyl-propyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.11c)



¹H NMR (400 MHz, CDCl₃) δ 7.37 – 7.11 (m, 5H), 4.36 (dd, *J* = 8.8, 1.7 Hz, 1H), 3.90 (td, *J* = 7.1, 4.8 Hz, 1H), 3.47 (d, *J* = 4.7 Hz, 1H), 3.14 – 2.90 (m, 2H), 2.39 – 2.28 (m, 1H), 2.23 (dt, *J* = 11.1, 6.0 Hz, 1H), 2.09 – 2.05 (m, 1H), 1.93 – 1.85 (m, 2H), 1.40 (s, 3H), 1.27 (s, 3H), 1.18 (d, *J* = 11.0 Hz, 1H), 0.81 (s, 3H).

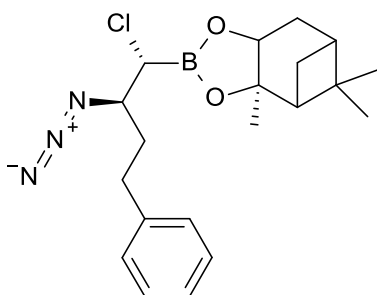
¹³C NMR (101 MHz, CDCl₃) δ 137.01, 129.59, 128.98, 127.29, 87.72, 79.22, 65.59, 51.32, 39.56, 38.45, 38.25, 35.42, 28.64, 27.22, 26.61, 24.20

IR (cm⁻¹) 2922.9, 2102.4, 1394.3

[α]_D = -22.4° (toluene, *c* = 0.4204, 18 °C)

81.6 % yield

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*,2*R*)-2-azido-1-chloro-4-phenyl-butyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12d)



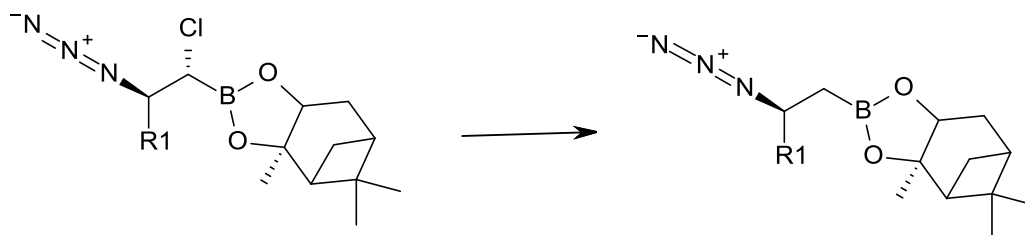
^1H NMR (400 MHz, CDCl_3) δ 7.33 – 7.17 (m, 5H), 4.39 (dd, J = 8.8, 2.0 Hz, 1H), 3.72 – 3.65 (m, 1H), 3.58 (d, J = 6.2 Hz, 1H), 2.77 (m, 2H), 2.40 – 2.32 (m, 1H), 2.30 – 2.22 (m, 1H), 2.09 (t, J = 5.4 Hz, 1H), 2.03 – 1.86 (m, 4H), 1.42 (s, 3H), 1.30 (s, 3H), 1.18 (d, J = 11.0 Hz, 1H), 0.84 (s, 3H).

Contained approximately 10 % of starting compound.

^{13}C NMR (101 MHz, CDCl_3) δ 140.57, 128.57, 128.40, 126.23, 87.36, 78.87, 63.84, 51.04, 39.29, 38.23, 35.14, 33.97, 32.42, 28.40, 26.98, 26.37, 23.98

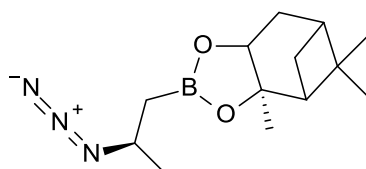
83.2 % crude yield

General procedure for the synthesis of compounds 2.12 exemplified by 2.12b.



(3*aR*,4*R*,6*R*,7*aS*)-2-[(2*R*)-2-azidopropyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12b)

A solution of the α -chloro derivative of the boronate (4.00 g, 0.013 mol) in dry THF (30 mL) was cooled to -78°C and a solution of lithium triethylborohydride (1.84 g, 0.0173 mol, 17.3 mL of 1M sol. in THF) was added drop wise to the vigorously stirred solution. The reaction mixture was stirred overnight, concentrated under vacuum and the residue was dissolved in diethyl ether, washed with saturated ammonium chloride and dried over magnesium sulfate. The dried solution was concentrated under vacuum to give pure product as slightly yellow oil (2.50 g, 71.4 % yield)



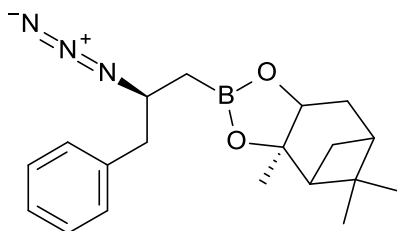
^1H NMR (400 MHz, CDCl_3) δ 4.28 (dd, J = 8.7, 2.0 Hz, 1H), 3.80 – 3.69 (m, 1H), 2.38 – 2.30 (m, 1H), 2.27 – 2.19 (m, 1H), 2.06 – 2.02 (m, 1H), 1.95 – 1.89 (m, 1H), 1.85 (m, 1H), 1.39 (s, 3H), 1.29 (s+d, 6H), 1.11 (d, J = 11.0 Hz, 1H), 0.84 (s, 6H).

^{13}C NMR (101 MHz, CDCl_3) δ 85.91, 77.86, 55.36, 51.14, 39.45, 38.11, 35.37, 28.60, 27.03, 26.44, 23.99, 21.87

IR (cm^{-1}) 2970.2, 2920.4, 2872.7, 2097.6, 1375.4

(3a*R*,4*R*,6*R*,7a*S*)-2-[(2*S*)-2-azido-3-phenyl-propyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12c)

The reaction was performed according to the general procedure (85.4 % yield).



^1H NMR (400 MHz, CDCl_3) δ 7.35 – 7.19 (m, 5H), 4.28 (dd, J = 8.8, 2.0 Hz, 1H), 3.88 – 3.79 (m, 1H), 2.92 – 2.78 (m, 2H), 2.34 (dddd, J = 10.4, 8.7, 4.3, 2.0 Hz, 1H), 2.28 – 2.19 (m, 1H), 2.08 – 2.04 (m, 1H), 1.96 – 1.81 (m, 3H), 1.39 (d, J = 1.7 Hz, 4H), 1.29 (d, J = 1.6 Hz, 4H), 1.19 (d, J = 7.1 Hz, 2H), 1.15 (d, J = 11.1 Hz, 1H), 0.85 (s, 4H).

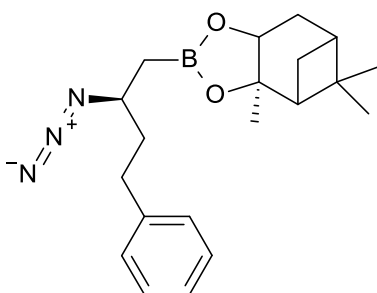
^{13}C NMR (101 MHz, CDCl_3) δ 138.29, 129.66, 128.67, 126.86, 86.25, 78.19, 61.56, 51.46, 43.23, 39.74, 38.38, 35.63, 28.84, 27.29, 26.71, 24.23.

IR (cm^{-1}) 2919.3, 2100.3, 1377.1

$[\alpha]_{\text{D}} = -13.46^\circ$ (c = 0.52, methanol, 18 °C)

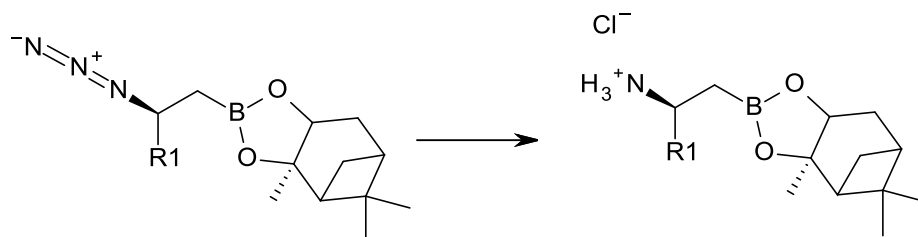
(3a*R*,4*R*,6*R*,7a*S*)-2-[(2*S*)-2-azido-4-phenyl-butyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.12d)

The reaction was performed according to the general procedure (98 % yield).



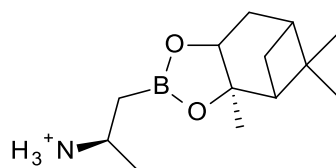
Spectra were difficult to interpret but the product contained azide (IR (cm^{-1}): 2098.9) and amine (IR (cm^{-1}): 3400) and no starting material could be detected. The compound was used in the subsequent step without further purification.

General procedure for the synthesis of compounds (2.13) exemplified by 2.13b.



[(1*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-methyl-ethyl]ammonium chloride (2.13b)

β -azidoboronate (2.50 g, 1eq) was dissolved in dry THF (15 mL) and cooled to $-78\text{ }^{\circ}\text{C}$. To the solution lithium aluminum hydride in THF (0.52 g, 6.9 mL 2M solution in THF, 1.3 eq) was added drop wise and the resulting mixture was allowed to warm to room temperature and was stirred overnight. Water was added slowly to the reaction mixture to decompose unreacted lithium aluminum hydride. The white precipitate was filtered off and washed several times with diethyl ether. Organic layers were combined, washed with saturated ammonium chloride solution and dried over magnesium sulfate. The solution was concentrated under vacuum and dissolved in pentane. The precipitate (if present) was filtered off. To the solution of amine, an excess of hydrochloric acid (1.25 M solution in methanol) was added at $0\text{ }^{\circ}\text{C}$ and the resulting mixture was stirred overnight at room temperature. Solvents were evaporated and the oily residue was washed with pentane. The solvent was decanted leaving pure product as oil (0.9 g, 31 % yield).



HRMS found: 238.1968 Exact mass: 238.1973 Calculated for: $\text{C}_{13}\text{H}_{25}\text{BNO}_2[\text{M}]^+$

^1H NMR (400 MHz, CD_3OD) δ 4.37 (dd, $J= 8.8, 1.9$ Hz, 1H), 3.53 – 3.41 (m, 1H), 2.40 (m, 1H), 2.32 – 2.20 (m, 1H), 2.06 – 2.01 (m, 1H), 1.93 (m, 1H), 1.84 (m, 1H), 1.40 (s, 3H), 1.33 (d, $J= 6.5$ Hz, 3H), 1.31 (s, 3H), 1.10 (d, $J= 10.9$ Hz, 1H), 0.88 (s, 3H).

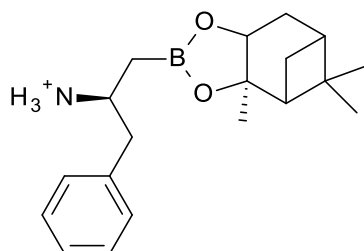
^{13}C NMR (101 MHz, CD_3OD) δ 86.17, 77.91, 51.13, 45.21, 39.43, 37.81, 34.83, 27.51, 26.02, 25.89, 22.85, 19.42

IR (cm^{-1}) 2918.2, 2871.8, 1376.8

$[\alpha]_{\text{D}} = -15.6^{\circ}$ ($c= 0.64$, methanol, $18\text{ }^{\circ}\text{C}$)

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethyl]ammonium chloride (2.13c)

The reaction was performed according to the general procedure (56 % yield).



HRMS found: 314.2287 Exact Mass: 314.2286 Calculated for: C₁₉H₂₉BNO₂ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.39 – 7.22 (m, 5H), 4.30 (dd, *J* = 8.6, 2.0 Hz, 1H), 3.65 (m, 2H), 2.95 (m, 2H), 2.42 – 2.33 (m, 1H), 2.29 – 2.21 (m, 1H), 2.03 (t, *J* = 5.5 Hz, 1H), 1.95 – 1.80 (m, 2H), 1.37 (s, 3H), 1.30 (s, 3H), 1.22 (m, 2H), 1.07 (d, *J* = 10.9 Hz, 1H), 0.86 (s, 3H).

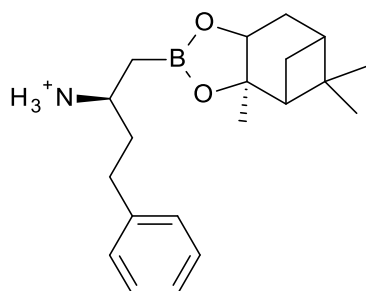
¹³C NMR (101 MHz, CD₃OD) δ 135.66, 129.12, 128.63, 127.10, 86.28, 77.97, 51.14, 50.30, 40.37, 39.40, 37.81, 34.75, 27.48, 26.01, 25.87, 22.84

IR (cm⁻¹) 3026.8, 2982.9, 2915.5, 2870.2, 1375.8

[α]_D = -20.7° (c = 0.15, methanol, 18 °C)

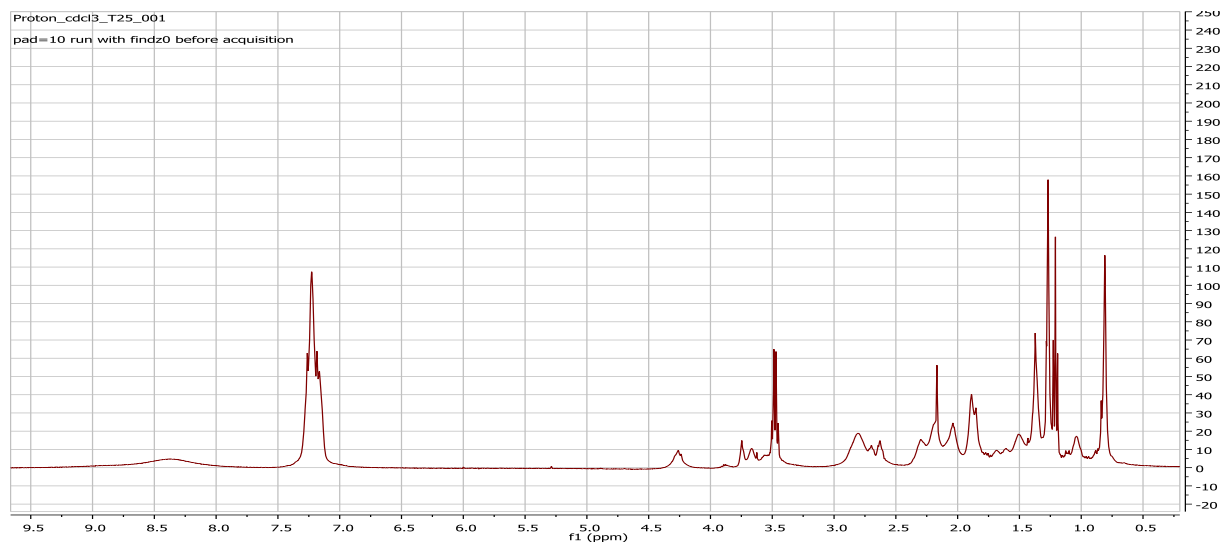
[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-phenethyl]ammonium chloride (2.13d)

The reaction was performed according to the general procedure (48.8 % crude yield).



HRMS found: 328.2443 Exact Mass: 328.2442 Calculated for: C₂₀H₃₁BNO₂ [M]⁺

NMR spectra are complicated and difficult to interpret. HRMS shows correct mass one compound.

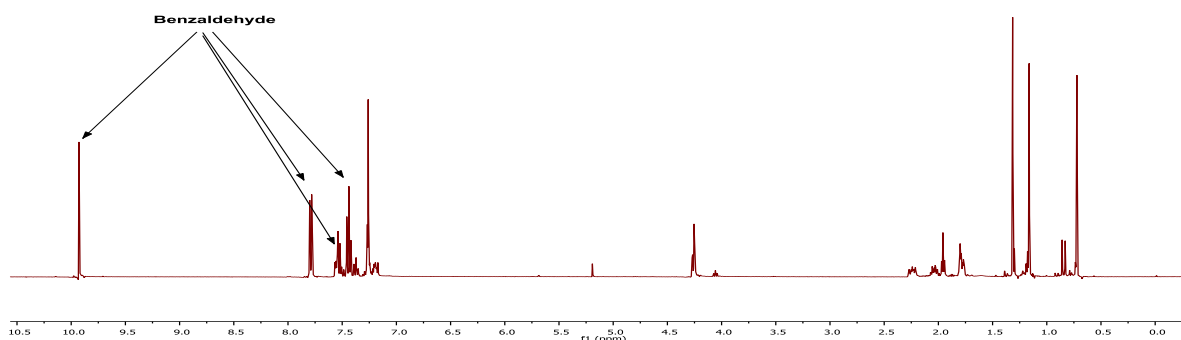
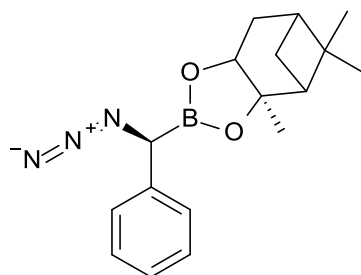


IR (cm⁻¹) 3061.9, 3026.1, 2921.8, 2868.9, 1375.8

Synthesis of β-phenyl-β-aminoboronates.

Substitution of the α-chloro atom by azide resulted in the mixture of desired product (2.10e) and benzaldehyde (≈20 %). These brought additional difficulties into the next steps and resulted in complicated mixture that was impossible to interpret.

(3*aR*,4*R*,6*R*,7*aS*)-2-[(*S*)-azido(phenyl)methyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.10e)

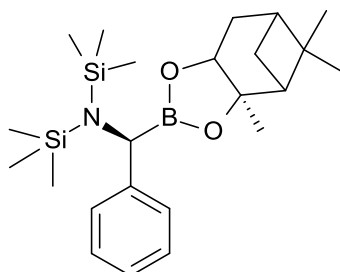


^1H NMR (400 MHz, CDCl_3) δ 7.50 – 7.12 (m, 5H), 4.36 – 4.17 (m, 1H), 2.30 – 2.17 (m, 1H), 2.04 (dtd, J = 8.2, 6.1, 2.5 Hz, 1H), 1.96 (t, J = 5.4 Hz, 1H), 1.76 (ddd, J = 14.0, 10.1, 3.1 Hz, 2H), 1.31 (s, 3H), 1.16 (s, 3H), 0.84 (d, J = 11.0 Hz, 1H), 0.72 (s, 3H).

68.6 % crude yield

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*S*)-1-phenyl-*N,N*-bis(trimethylsilyl)methanamine]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.16e)

To a stirred solution of **2.9e** (9.50 g, 0.031 mol) in dry THF, lithium hexamethyldisylazane (6.25 g, 37.7 mL 1M solution in THF, 0.037 mol) was added drop wise at -78°C . The resulting mixture was stirred overnight at room temperature. Solvents were evaporated under vacuum and the residue was dissolved in pentane. Insoluble part was filtered and the pentane solution was concentrated under vacuum to give target compound containing 20 % of impurity. No starting compound was observed. (14.7 g, 109 % crude yield)

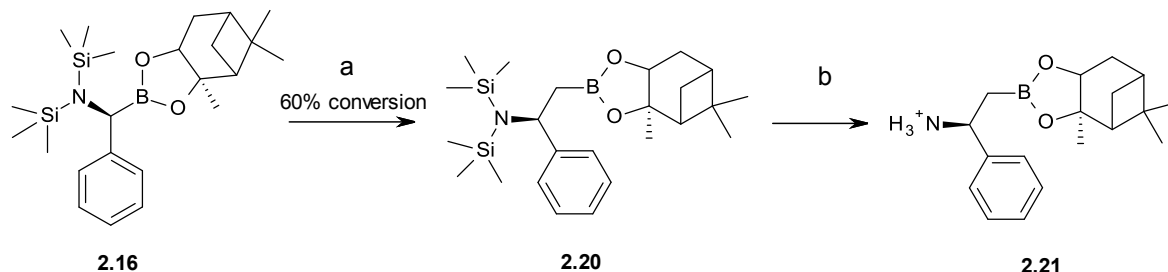


^1H NMR (400 MHz, CDCl_3) δ 7.47 (dt, J = 8.5, 1.2 Hz, 2H), 7.34 – 7.20 (m, 2H), 7.17 – 7.05 (m, 1H), 4.36 (dd, J = 8.8, 1.8 Hz, 1H), 4.08 (s, 1H), 2.40 – 2.23 (m, 1H), 2.11 (dd, J = 6.0, 4.8 Hz, 1H), 1.99 – 1.90 (m, 2H), 1.42 (s, 3H), 1.31 (s, 3H), 0.9 (1H), 0.86 (s, 3H), 0.17 (s, 3H), 0.09 (s, 15H).

^{13}C NMR (101 MHz, CDCl_3) δ 142.46, 125.02, 124.14, 122.60, 83.50, 75.99, 48.95, 36.97, 35.71, 32.94, 25.85, 24.57, 24.03, 21.54, 19.85, 11.59, 0.90, 0.00

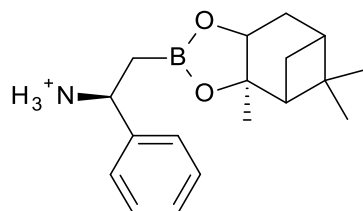
Product contains app. 20 % of imp. 109.7 % crude yield

Target compound (**2.21**) was obtained in two steps from (**2.20**).



To a mixture of **2.16** (1 eq) and iodochloromethane (1.2 eq) in dry THF, n-butyllithium was added drop wise at $-78\text{ }^\circ\text{C}$. The reaction mixture was warmed to room temperature and stirred overnight. Solvents were evaporated under vacuum, the residue was dissolved in the pentane, precipitate was filtered off and pentane solution was concentrated to give **2.20** as a mixture with starting compound. (52.8 % crude yield, 60 % conversion to the product)

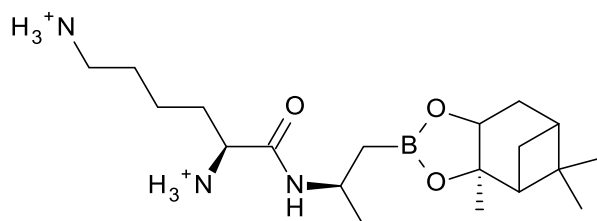
Treatment of **2.20** with the solution of HCl in methanol at $-78\text{ }^\circ\text{C}$ provided the target compound **2.21** as a mixture with the hydrochloric salt of α -amino- α -phenylboronate. The separation was done during purification of corresponding dipeptides. The mixture was used without further purification.



HRMS found: 300.2134 Exact mass: 300.2129 Calculated for: $\text{C}_{26}\text{H}_{43}\text{BN}_3\text{O}_3$ [M] $^+$

[(1*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-methyl-ethylamino-*N*-(*L*)-lysine dihydrochloride (**2.15-1**)

diBoc-protected analog: quantitative yield



HRMS found: 366.2929 Exact mass: 366.2922 Calculated for: $\text{C}_{19}\text{H}_{38}\text{BN}_3\text{O}_3$ [M-H] $^+$

^1H NMR (400 MHz, CD_3OD) δ 8.58 – 7.47 (m, 1H), 4.31 (dd, J = 8.6, 1.9 Hz, 1H), 4.11 – 3.98 (m, 1H), 3.91 (m, 1H), 3.00 (d, J = 7.4 Hz, 2H), 2.38 – 2.32 (m, 1H), 2.27 – 2.17 (m, 1H), 2.00 (t, J = 5.5 Hz, 1H), 1.95 – 1.85 (m, 3H), 1.86 – 1.70 (m, 3H), 1.58 – 1.47 (m, 2H), 1.37 (s, 3H), 1.29 (s, 3H), 1.24 – 1.09 (m+d, J = 10.8 Hz, 1+3H), 1.05 (m+d, J = 16.9 Hz, 2H), 0.85 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.36, 85.54, 77.54, 52.80, 51.21, 43.17, 39.48, 38.98, 37.85, 35.10, 30.75, 27.90, 26.54, 26.34, 26.04, 23.16, 21.64, 21.46

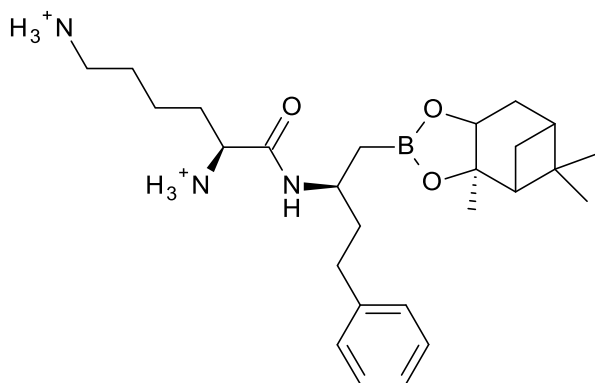
IR (cm⁻¹) 3386.96, 3230.1, 2921.47, 1665.18, 1376.02, 1029.14

[α]_D = +4.15° (c = 6.5, MeOH, 18 °C)

96 % yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-phenethylamino-*N*-(*L*)-lysine dihydrochloride (2.14-2)

diBoc-protected analog: quantitative yield



HRMS found: 456.3399 Exact Mass: 456.3392 Calculated for: C₂₆H₄₃BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.36 – 7.05 (m, 5H), 4.30 (dd, *J* = 8.8, 1.9 Hz, 1H), 4.04 (m, 1H), 3.92 (t, *J* = 6.6 Hz, 1H), 2.94 (m, 2H), 2.76 – 2.51 (m, 2H), 2.43 – 2.30 (m, 1H), 2.26 – 2.15 (m, 1H), 2.04 – 1.70 (m, 9H), 1.54 (t, *J* = 7.9 Hz, 2H), 1.36 (s, 3H), 1.29 (s, 3H), 1.17 (m, 2H), 1.07 (d, *J* = 10.7 Hz, 1H), 0.85 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 167.77, 141.55, 128.03, 127.97, 125.54, 85.58, 77.61, 52.92, 51.19, 46.89, 39.46, 38.91, 37.79, 37.64, 35.00, 32.20, 30.93, 27.68, 26.65, 26.12, 26.01, 22.94, 21.75.

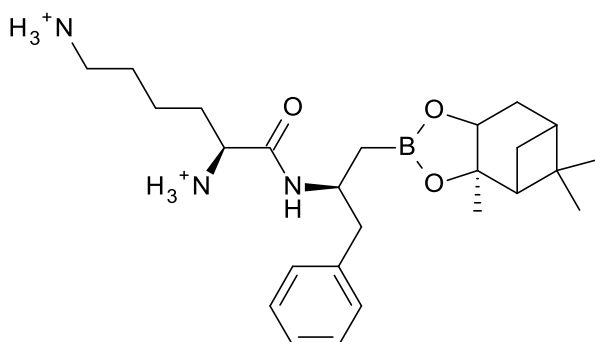
IR (cm⁻¹) 3414.2, 3024.9, 2927.5, 1667.2

[α]_D = +3.17° (c = 0.63, MeOH, 18 °C)

quantitative yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-*N*-(*L*)-lysine dihydrochloride (2.14-3)

Boc-protected analog: 60 % yield



HRMS found: 442.3231 Exact Mass: 442.3235 Calculated for: C₂₅H₄₂BN₃O₃ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.34 – 7.15 (m, 5H), 4.34 (m, 2H), 3.76 (m, 1H), 3.08 – 2.92 (m, 2H), 2.79 (m, 2H), 2.69 (dd, *J* = 13.7, 10.2 Hz, 1H), 2.39 (m, 1H), 2.25 (m, 1H), 2.04 (m, 1H), 1.96 – 1.81 (m, 2H), 1.73 (m, 1H), 1.63 – 1.49 (m, 2H), 1.42 – 1.38 (m, 2H), 1.30 (d, *J* = 2.0 Hz, 2H), 1.16 (d, *J* = 29.0 Hz, 1H), 1.05 (dt, *J* = 11.5, 5.7 Hz, 1H), 0.87 (d, *J* = 1.8 Hz, 2H). Contains ~40 % of amino acid

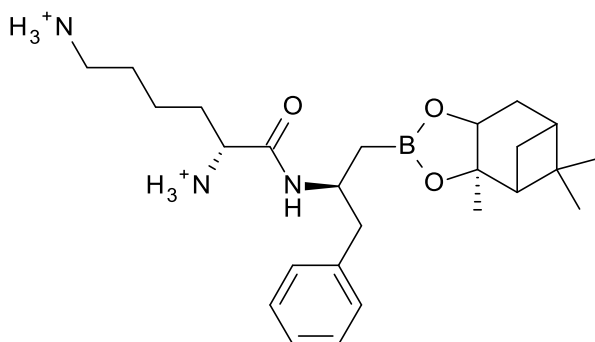
^{13}C NMR (101 MHz, CD_3OD) δ 167.33, 138.91, 129.00, 127.99, 126.08, 85.70, 77.68, 52.70, 52.34, 51.23, 48.36, 41.76, 39.49, 38.81, 37.83, 35.02, 30.61, 29.57, 27.69, 26.61, 26.11, 25.98, 22.94, 21.72, 20.97.

$[\alpha]_{\text{D}} = -3.7^\circ$ ($c = 1.9$, MeOH , 18°C)

68 % yield.

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-N-(*D*)-lysine dihydrochloride

diBoc-protected analog: 92 % isolated yield



HRMS found: 442.3230 Exact Mass: 442.3235 Calculated for: $\text{C}_{25}\text{H}_{42}\text{BN}_3\text{O}_3$ $[\text{M}-\text{H}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.25 (m, 5H), 4.26 (m, 2H), 3.81 (t, $J = 6.3$ Hz, 1H), 2.99 – 2.80 (m, 4H), 2.42 – 2.34 (m, 1H), 2.28 – 2.20 (m, 1H), 2.01 – 1.66 (m, 7H), 1.51 (m, 2H), 1.37 (s, 3H), 1.30 (s, 3H), 1.18 – 0.99 (m, 3H), 0.86 (s, 3H). Contains ~15 % of amino acid

^{13}C NMR (101 MHz, CD_3OD) δ 167.56, 138.42, 129.10, 127.99, 126.05, 85.65, 77.62, 52.75, 51.22, 48.62, 41.80, 39.44, 38.88, 37.81, 34.99, 30.96, 27.73, 26.71, 26.09, 26.00, 22.92, 21.67

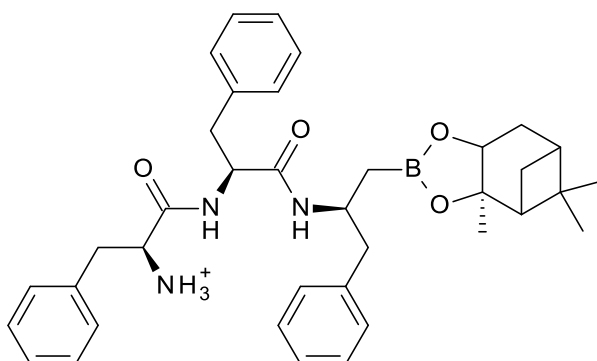
IR (cm^{-1}) 3379.4, 3205.4, 2917.12, 1747.34, 1671.16, 1496.39, 1379.55, 1030.06, 700.76

$[\alpha]_{\text{D}} = -17.3^\circ$ ($c = 2.2$, MeOH , 18°C)

65 % yield.

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-N-(*L*)-penylalanine-(*L*)-phenylalanine hydrochloride (2.14-5)

Boc-protected analog: 93 % yield



HRMS found: 608.3673 Exact Mass: 608.3654 Calculated for: $\text{C}_{37}\text{H}_{47}\text{BN}_3\text{O}_4$ $[\text{M}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.45 – 6.96 (m, 15H), 4.57 (t, $J = 7.2$ Hz, 1H), 4.30 – 4.20 (m, 1+1H), 4.13 (dd, $J = 8.3, 4.9$ Hz, 1H), 3.31 – 3.23 (m, 1H), 3.05 – 2.76 (m, 3H), 2.75 – 2.63 (m, 2H), 2.31 (dd, $J = 14.5, 8.7$

Hz, 1H), 2.21 (q, $J = 6.8, 5.6$ Hz, 1H), 1.99 (t, $J = 5.5$ Hz, 1H), 1.91 – 1.75 (m, 2H), 1.35 (s, 3H), 1.26 (s, 3H), 1.13 – 0.98 (m, 1+2H), 0.81 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 170.21, 167.84, 138.43, 136.75, 134.05, 129.28, 129.18, 129.03, 128.73, 128.14, 127.96, 127.43, 126.47, 126.02, 85.58, 77.56, 55.01, 53.98, 51.18, 41.78, 39.44, 37.95, 37.78, 37.23, 35.04, 29.57, 27.77, 26.15, 26.04, 22.98.

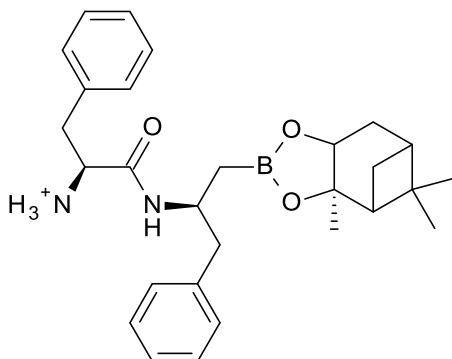
IR (cm^{-1}) 3286.9, 2927.1, 1641.3

$[\alpha]_{\text{D}} = 0^\circ$ ($c = 0.21$, methanol, 18°C)

Quantitative yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-*N*-(*L*)-penylalanine hydrochloride (2.14-6)

Boc-protected analog: 62.5 % yield



HRMS found: 461.2966 Exact Mass: 461.2970 Calculated for: $\text{C}_{28}\text{H}_{38}\text{BN}_2\text{O}_3$ $[\text{M}]^+$

^1H NMR (600 MHz, DMSO) δ 8.40 (d, $J = 8.1$ Hz, 1H), 8.03 (d, $J = 5.6$ Hz, 3H), 7.32 – 6.97 (m, 10H), 4.27 (dd, $J = 8.9, 1.8$ Hz, 1H), 4.08 (td, $J = 7.7, 5.2$ Hz, 1H), 3.84 (d, $J = 7.3$ Hz, 1H), 2.86 (dd, $J = 14.0, 5.3$ Hz, 1H), 2.74 – 2.65 (m, 2H), 2.63 – 2.56 (m, 1H), 2.32 – 2.23 (m, 1H), 2.19 – 2.12 (m, 1H), 1.95 (t, $J = 5.5$ Hz, 1H), 1.84 (dt, $J = 5.6, 2.7$ Hz, 1H), 1.70 (dt, $J = 14.5, 2.7$ Hz, 1H), 1.29 (s, 3H), 1.22 (s, 3H), 1.01 – 0.93 (m, 3H), 0.79 (s, 3H).

^1H NMR (400 MHz, CD_3OD) δ 8.20 (b.s, 0.5H), 7.35 – 6.93 (m, 10H), 4.37 – 4.24 (m, 1H), 3.99 (b.m, 1H), 2.98 (dd, $J = 14.4, 5.6$ Hz, 1H), 2.90 – 2.73 (m, 2H), 2.68 (dd, $J = 13.8, 8.5$ Hz, 1H), 2.39 (m, 1H), 2.29 – 2.20 (m, 1H), 2.02 (m, 1H), 1.96 – 1.76 (m, 2H), 1.38 (s, 3H), 1.30 (s, 3H), 1.17 – 1.03 (m, 3H), 0.87 (m, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.01, 138.45, 134.02, 129.17, 129.09, 128.67, 128.02, 127.39, 126.16, 85.71, 77.67, 54.21, 51.21, 41.67, 39.47, 39.39, 37.82, 37.24, 34.99, 27.66, 26.10, 25.97, 22.92

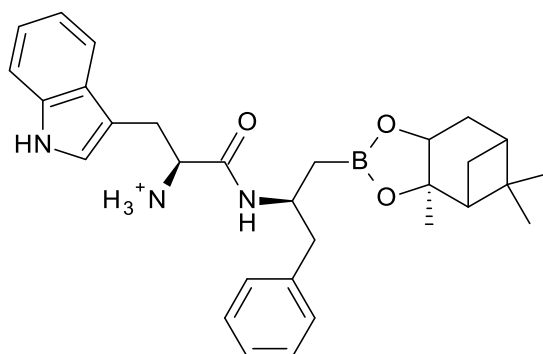
IR (cm^{-1}) 3204.3, 3027.59, 2917.49, 1660.145, 1453.87, 1376.26, 1029.67, 699.29

$[\alpha]_{\text{D}} = -4.2^\circ$ ($c = 1.2$, methanol, 18°C)

92 % yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-*N*-(*L*)-tryptophane hydrochloride (2.14-7)

Boc-protected analog: 86.9 % yield



HRMS found: 500.3074 Exact Mass: 500,3079 Calculated for: C₃₀H₃₉BN₃O₃ [M]⁺

¹H NMR (600 MHz, DMSO) δ 10.95 (d, *J* = 2.6 Hz, 1H), 8.41 (d, *J* = 8.2 Hz, 1H), 7.93 (m, 3H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 2H), 7.13 (d, *J* = 7.3 Hz, 1H), 7.10 – 7.02 (m, 3H), 6.98 (t, *J* = 7.4 Hz, 1H), 4.28 (dd, *J* = 8.8, 1.9 Hz, 1H), 4.17 – 4.07 (m, 1H), 3.79 (dd, *J* = 9.4, 5.0 Hz, 1H), 2.98 (dd, *J* = 14.8, 5.0 Hz, 1H), 2.82 – 2.68 (m, 2H), 2.64 – 2.56 (m, 1H), 2.32 – 2.24 (m, 1H), 2.20 – 2.12 (m, 1H), 1.95 (t, *J* = 5.5 Hz, 1H), 1.84 (m, 1H), 1.70 (m, 1H), 1.29 (s, 3H), 1.22 (s, 3H), 1.02 – 0.96 (m, 3H), 0.79 (s, 3H).

¹H NMR (400 MHz, CD₃OD) δ 8.08 (d, *J* = 8.4 Hz, 0.3H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.28 – 6.92 (m, 9H), 4.36 – 4.20 (m, 2H), 4.00 (dd, *J* = 8.2, 6.1 Hz, 1H), 3.14 (dd, *J* = 14.9, 6.2 Hz, 1H), 2.95 (dd, *J* = 14.8, 8.3 Hz, 1H), 2.78 (dd, *J* = 13.7, 5.7 Hz, 1H), 2.61 (dd, *J* = 13.6, 7.9 Hz, 1H), 2.41 – 2.31 (m, 1H), 2.23 (m, 1H), 1.99 (t, *J* = 5.6 Hz, 1H), 1.90 (m, 1H), 1.81 (m, 1H), 1.36 (s, 3H), 1.29 (s, 3H), 1.13 – 1.05 (m, 3H), 0.86 (s, 3H).

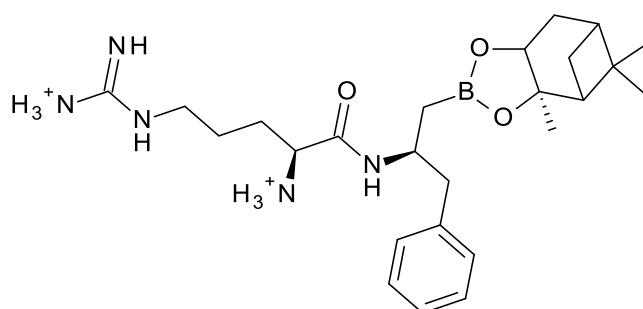
¹³C NMR (101 MHz, CD₃OD) δ 167.48, 138.30, 136.87, 129.13, 127.97, 126.81, 126.09, 124.08, 121.50, 118.87, 117.69, 111.24, 106.58, 85.68, 77.60, 56.84, 53.54, 51.12, 48.13, 41.44, 39.43, 37.79, 34.98, 27.62, 27.57, 26.07, 25.95, 22.91.

IR (cm⁻¹) 3343.8, 3211.6, 3058.8, 3027.5, 2917.4, 2868.9, 1667.7, 1378.0, 741.1, 699.8

[α]_D = +15° (c = 0.4, MeOH, 18 °C)

80 % yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzylethylamino-*N*-(*L*)-arginine hydrochloride (2.14-8)

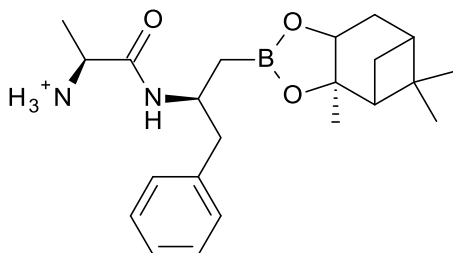


HRMS found: 470.3292 Exact Mass: 470.3297 Calculated for: C₂₅H₄₂BN₅O₃ [M-H]⁺

NMR signals were overlapping in the NMR spectra making assignment impossible.

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-1-benzylethylamino-N-(L)-alanine hydrochloride (2.14-9)

Boc-protected analog: 73.8 % yield



HRMS found: 385.2661 Exact Mass: 385.2657 Calculated for: C₂₂H₃₄BN₂O₃ [M]⁺

¹H NMR (400 MHz, CDCl₃) δ 7.51 – 7.38 (m, 1H), 7.30 – 7.11 (m, 5H), 4.39 – 4.28 (m, 1H), 4.24 (td, *J*= 8.3, 7.9, 1.9 Hz, 1H), 3.98 (s, 3H), 3.75 – 3.58 (m, 1H), 2.94 – 2.68 (m, 2H), 2.31 (ddd, *J*= 14.5, 8.9, 2.4 Hz, 1H), 2.19 (ddd, *J*= 10.5, 5.9, 1.7 Hz, 1H), 2.02 (td, *J*= 6.3, 5.7, 1.9 Hz, 1H), 1.90 (m, 1H), 1.85 – 1.75 (m, 1H), 1.35 (s+s, 3H), 1.33 – 1.28 (d, 1H) + 1.27 (d+s, 5H), 1.09 (m, 3H), 0.82 (s, 3H).

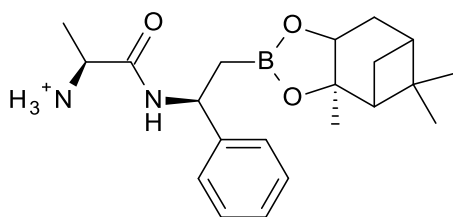
¹³C NMR (101 MHz, CDCl₃) δ 172.52, 138.66, 129.78, 128.47, 126.51, 86.07, 77.90, 51.38, 50.51, 47.75, 42.78, 39.68, 38.33, 35.61, 28.83, 27.27, 26.80, 24.22, 20.27

IR (cm⁻¹) 3349.1, 3060.7, 3026.8, 2919.7, 1654.6

92 % yield

[(1*S*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-1-phenylethylamino-N-(L)-alanine hydrochloride (2.21-1)

The Boc-protected dipeptide was obtained in a mixture with the α-aminoderivative. The pure product was obtained after preparative TLC (diethyl ether/pentane, 60:40. Multiple developments were needed as higher concentration of diethyl ether gave bad resolution and higher concentration of pentane gave too small R_f. The system was left for several hours with the half-opened lead to provide constant evaporation of the eluent from the top of the TLC plate.)



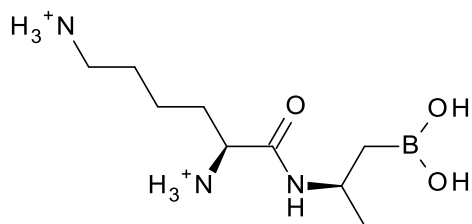
HRMS found: 371.2505 Exact mass: 371.2500 Calculated for: C₂₁H₃₂BN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 8.70 (d, *J*= 7.8 Hz, 1H), 7.38 – 7.18 (m, 5H), 5.09 (qd, *J*= 7.8, 5.8 Hz, 1H), 4.23 (dd, *J*= 8.8, 1.9 Hz, 1H), 3.91 (m, 1H), 2.30 (ddt, *J*= 14.5, 8.8, 2.4 Hz, 1H), 2.07 – 1.97 (m, 1H), 1.92 (dd, *J*= 6.0, 5.1 Hz, 1H), 1.79 (tt, *J*= 6.0, 3.1 Hz, 1H), 1.68 (ddd, *J*= 14.5, 3.3, 1.9 Hz, 1H), 1.46 (d, *J*= 8.0 Hz, 2H), 1.40 (d, *J*= 7.1 Hz, 3H), 1.25 (s, 6H), 0.81 (s, 3H), 0.73 (d, *J*= 10.9 Hz, 1H).

¹³C NMR (101 MHz, CD₃OD) δ 168.35, 143.61, 128.31, 127.18, 126.42, 85.90, 77.83, 51.24, 50.91, 48.94, 39.53, 37.94, 35.07, 27.77, 26.25, 25.92, 23.09, 16.35

(1*R*)-2-borono-1-methyl-ethylamino-*N*-(*L*)-lysine dihydrochloride (2.15-1)

Deprotection by method A.



¹H NMR (400 MHz, CD₃OD) δ 4.14 – 4.02 (m, 1H), 3.94 – 3.80 (m, 2H), 3.08 – 2.78 (m, 5H), 1.89 (s, 1H), 1.75 (d, *J*= 7.8 Hz, 3H), 1.53 (d, *J*= 9.3 Hz, 3H), 1.37 – 1.00 (m, 10H). Resonances were broadened

¹³C NMR (101 MHz, CD₃OD) δ 167.32, 52.79, 43.26, 38.94, 30.80, 26.62, 21.59, 21.33, 21.31

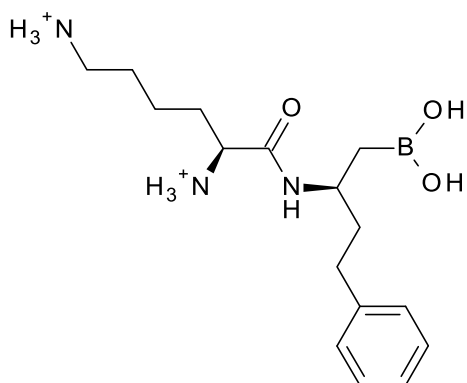
IR n/a

[α]_D = +28.9 (c= 0.64, MeOH, 18 °C)

53 % yield

(1*S*)-2-borono-1-phenethylamino-*N*-(*L*)-lysine dihydrochloride (2.12-2)

Deprotection by method B.



¹H NMR (400 MHz, CD₃OD) δ 7.33 – 7.06 (m, 5H), 4.03 (m, 1H), 3.93 (t, *J*= 6.5 Hz, 1H), 2.94 (m, 2H), 2.74 – 2.49 (m, 2H), 2.03 – 1.68 (m, 6H), 1.59 – 1.49 (m, 2H), 1.19 – 1.06 (m, 2H).

¹³C NMR (101 MHz, CD₃OD) δ 167.78, 141.69, 128.00, 127.95, 125.48, 52.89, 47.14, 38.91, 37.84, 32.31, 30.95, 26.65, 21.74

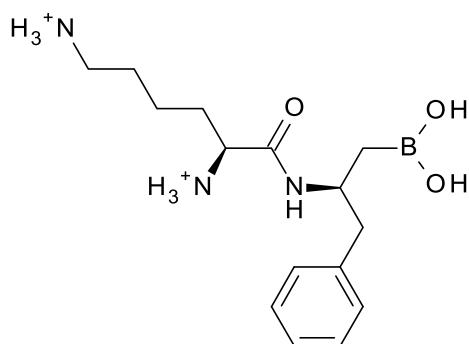
IR (cm⁻¹) 3213.6, 3025.0, 2943.5, 1666.1

[α]_D = +16.1° (c= 1.12, MeOH, 18 °C)

Quantitative yield

(1S)-2-borono-1-benzyl-ethylamino-N-(L)-lysine dihydrochloride (2.15-3)

Deprotection by method A.



^1H NMR (400 MHz, D₂O) δ 7.38 – 7.17 (m, 5H), 4.06 (t, J = 6.4 Hz, 1H), 3.69 (t, J = 7.2 Hz, 1H), 3.05 – 2.80 (m, 4H), 1.96 (m, 2H), 1.69 (m, 2H), 1.48 (m, 2H), 1.24 – 1.03 (m, 2H).

^{13}C NMR (101 MHz, D₂O) δ 172.17, 136.01, 129.81, 129.17, 127.70, 52.88, 51.07, 40.64, 39.22, 29.44, 26.46, 21.60

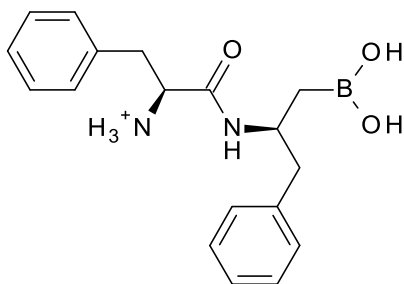
IR (cm⁻¹) 3204.6, 2926.2, 1735.2

$[\alpha]_{\text{D}} = +5.2^\circ$ (c= 1.945, methanol, 18 °C)

62 % yield

(1S)-2-borono-1-benzyl-ethylamino-N-(L)-phenylalanine hydrochloride (2.15-6)

Deprotection by method B.



^1H NMR (400 MHz, CD₃OD) δ 7.40 – 7.05 (m, 10H), 4.36 – 4.17 (m, 1H), 4.03 (t, J = 6.2 Hz, 1H), 2.89 (m, 2H), 2.76 – 2.57 (m, 2H), 1.05 (m, 2H).

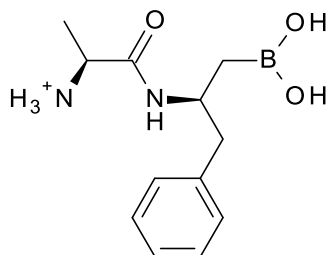
^{13}C NMR (101 MHz, CD₃OD) δ 167.05, 138.73, 134.07, 129.14, 129.07, 128.65, 127.96, 127.36, 126.06, 54.21, 48.58, 41.85, 37.27

IR (cm⁻¹) 3249.3, 3061.8, 2927.8, 1653.2

85.7 % yield

(1*S*)-2-borono-1-benzyl-ethylamino-*N*-(*L*)-alanine hydrochloride (2.15-9)

Deprotection by method A.



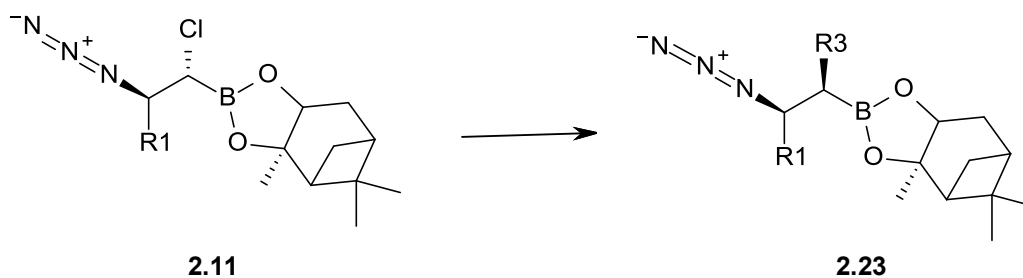
¹H NMR (400 MHz, D₂O) δ 7.40 – 7.19 (m, 5H), 4.05 (q, *J* = 7.3 Hz, 1H), 3.74 – 3.60 (m, 1H), 2.90 (tdd, *J* = 15.7, 12.8, 7.3 Hz, 2H), 1.50 (dd, *J* = 7.3, 1.2 Hz, 3H), 1.23 – 1.00 (m, 2H).

¹³C NMR (101 MHz, D₂O) δ 173.14, 135.88, 129.71, 129.07, 127.62, 50.97, 49.02, 40.55, 15.42

IR (cm⁻¹) 3181.3, 3062.1, 3027.9, 2969.8, 1713.7

58.8 % yield

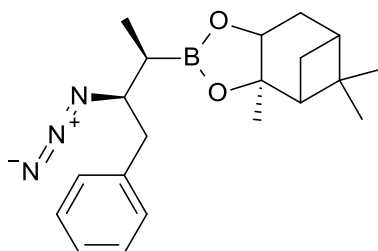
General procedure for the synthesis of compounds 2.23 exemplified by 2.23a.



Some Grignard reagents did not give full conversion. If conversion was not complete, the crude mixture was used in the subsequent step without further purification. Pure compounds were obtained after peptide coupling.

(3*aR*,4*R*,6*R*,7*aS*)-2-[(1*R*,2*R*)-2-azido-1-methyl-3-phenyl-propyl]hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.23a)

Methyl magnesium chloride (0.73 g, 3.2 mL 3 M solution in THF, 0.01 mol, 1.3 eq) was added drop wise to solution of α -chloroalkylboronate (2.80 g, 0.0075 mol, 1eq) in THF (10 mL) at -78 °C . The reaction mixture was stirred for 30 min. A zinc chloride solution (4.00 g, 29.4 mL 1 M solution in diethyl ether, 0.03 mol, 4 eq) was added drop wise to the reaction mixture; the solution was allowed to warm to room temperature and was stirred overnight. The reaction mixture was concentrated under vacuum, residues were dissolved in pentane and washed with saturated ammonium chloride solution. The organic layer was dried over magnesium sulfate and concentrated under vacuum to obtain the product as colorless oil. (85.7 % yield)

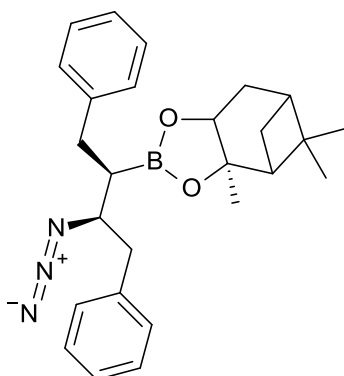


^1H NMR (400 MHz, CDCl_3) δ 7.48 – 7.01 (m, 5H), 4.31 (dd, J = 8.8, 2.0 Hz, 1H), 3.69 (ddd, J = 9.6, 5.6, 4.6 Hz, 1H), 3.01 – 2.74 (m, 2H), 2.36 (m, 1H), 2.25 (m, 1H), 2.09 (t, J = 5.5 Hz, 1H), 1.96 – 1.85 (m, 2H), 1.47 – 1.42 (m, 1H), 1.40 (s, 3H), 1.30 (s, 3H), 1.19 (d, 1H), 1.12 (d, J = 7.3 Hz, 3H), 0.85 (s, 3H).

^{13}C NMR (101 MHz, CDCl_3) δ 138.86, 129.59, 128.71, 126.79, 86.11, 78.10, 68.55, 51.46, 39.88, 39.74, 38.41, 35.78, 28.93, 27.30, 26.69, 24.27, 12.72

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*,2*R*)-2-azido-1-benzyl-3-phenyl-propyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.23b)

The product was obtained as a mixture with starting compound. 85 % crude yield. 50 % conversion to product. An analytical sample was obtained using preparative TLC (pentane/diethyl ether, 95:5, R_f = 0.9 for product and 0.7 for starting compound)



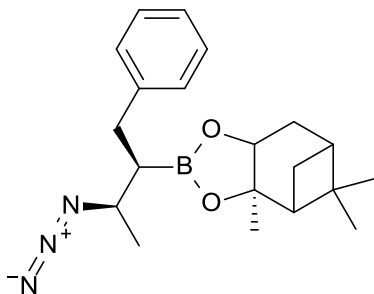
^1H NMR (400 MHz, CDCl_3) δ 7.36 – 7.11 (m, 12H), 4.31 (dd, J = 8.7, 2.0 Hz, 1H), 3.67 (dt, J = 8.7, 5.1 Hz, 1H), 3.11 – 2.74 (m, 5H), 2.43 – 2.28 (m, 1H), 2.20 (ddt, J = 8.3, 6.0, 3.0 Hz, 1H), 2.07 (t, J = 5.5 Hz, 1H), 1.96 – 1.81 (m, 3H), 1.72 (td, J = 8.3, 4.8 Hz, 1H), 1.31 (s, 6H), 1.15 (d, J = 10.9 Hz, 1H), 0.86 (s, 4H).

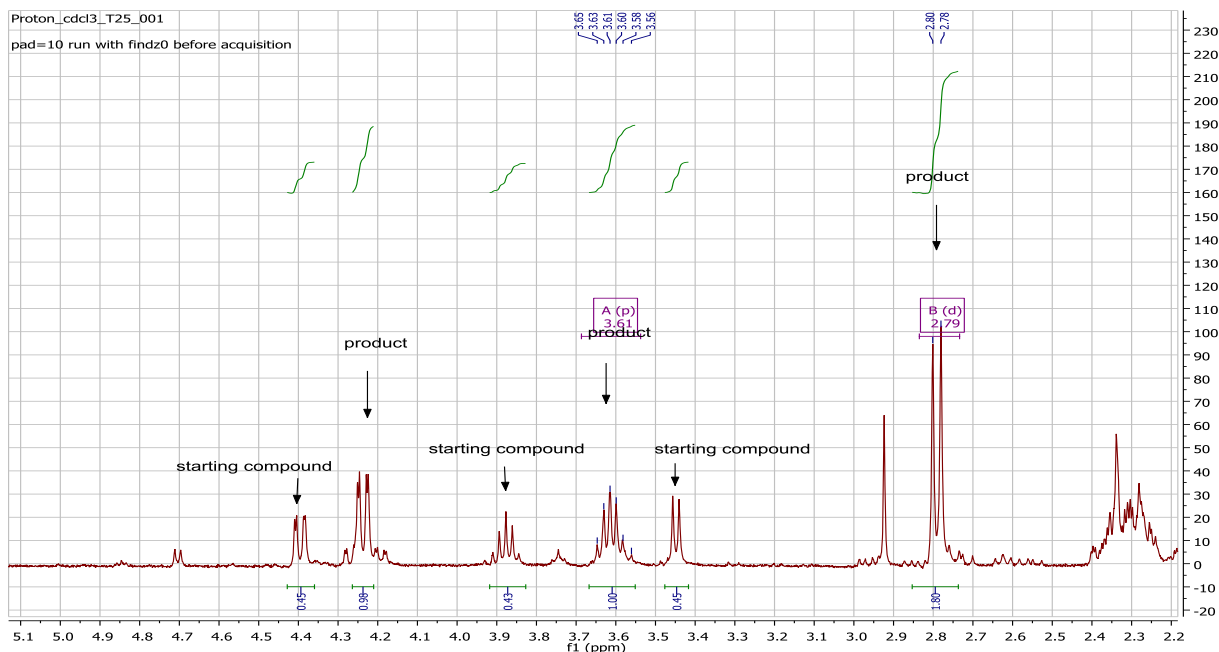
^{13}C NMR (101 MHz, CDCl_3) δ 140.81, 138.21, 129.32, 128.85, 128.51, 128.33, 126.62, 126.00, 85.96, 77.89, 65.93, 51.17, 40.37, 39.52, 38.15, 35.50, 34.44, 28.60, 27.08, 26.38, 24.04

IR (cm^{-1}) 3062.5, 3027.3, 2970.1, 2918.4, 2870.0, 2096.6

$[\alpha]_D = -15.6^\circ$ (c = 0.64, MeOH, 18°C)

(3a*R*,4*R*,6*R*,7a*S*)-2-[(1*R*,2*R*)-2-azido-1-benzyl-propyl]hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole (2.23e)





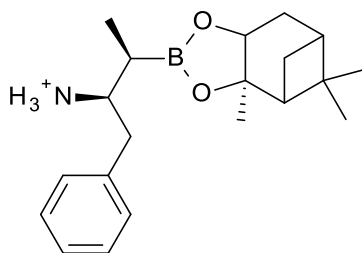
The product was obtained as a mixture with starting compound. New signals belonging to the product are ^1H NMR (400 MHz, CDCl_3) δ 7.33 – 7.11 (m, 5H), 3.61 (m, 1H), 2.79 (d, J = 8.3 Hz, 2H). The rest is difficult to interpret because of overlapping in aliphatic region.

48.5 % crude yield. 55 % conversion to product.

Material was used in subsequent step without further purification.

Synthesis of compounds (2.24):

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-1-benzyl-2-methyl-ethyl]ammonium chloride (2.24b)



HRMS found: 328.2442 Exact mass: 328.2442 Calculated for: $\text{C}_{20}\text{H}_{31}\text{BNO}_2$ [M] $^+$

^1H NMR (400 MHz, CDCl_3) δ 8.33 (b.s, 3H), 7.32 – 7.09 (m, 5H), 4.21 (d, J = 8.6 Hz, 1H), 3.69 (m, 1H), 3.27 (dd, J = 13.7, 6.4 Hz, 1H), 2.87 (dd, J = 13.7, 8.1 Hz, 1H), 2.39 – 2.14 (m, 2H), 2.04 (t, J = 5.4 Hz, 1H), 1.95 – 1.80 (m, 2H), 1.73 – 1.58 (m, 1H), 1.31 (s, 3H), 1.28 (s, 3H), 1.25 (d, J = 9.5 Hz, 3H), 1.05 (d, J = 10.9 Hz, 1H), 0.81 (s, 3H).

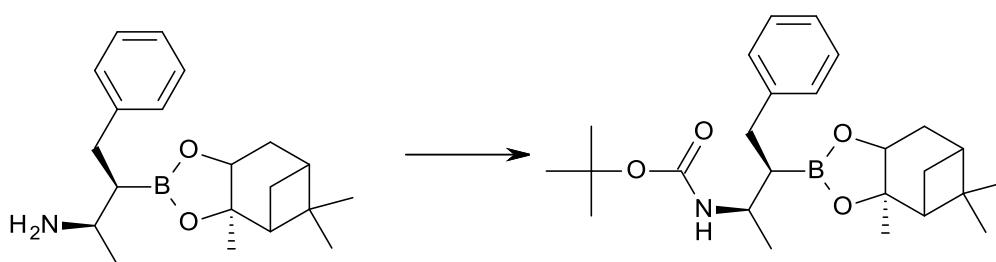
^{13}C NMR (101 MHz, CDCl_3) δ 136.32, 129.58, 128.75, 127.05, 86.35, 78.01, 56.66, 51.10, 39.42, 38.32, 38.11, 35.19, 28.47, 26.99, 26.46, 23.91, 11.53

IR (cm^{-1}) 3197.5, 3027.5, 2916.7, 2870.9

$[\alpha]_{\text{D}} = -18.4^\circ$ (c = 0.38, MeOH, 18 $^\circ\text{C}$)

46.5 % yield

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-methyl-2-benzyl-ethyl-*N*-Boc-amine (2.24a-Boc)



To the solution of the amine **2.24a** (3.3 g, 0.009 mol) in THF (20 mL) triethylamine (0.0099 mol) was added. White precipitate formed immediately. To the mixture Boc-anhydride (3.0g, 0.137 mol) was added and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under vacuum and the oily residue was recrystallized from pentane to give product as long needles. m.p.= 127-129C (0.7g, 17 % yield) HRMS found: 450.2791 Exact mass: 450.2786 Calculated for: C₂₅H₃₈BNNaO₄ [M+Na]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.33 – 7.00 (m, 1H), 6.08 (d, *J*= 9.2 Hz, 0H), 4.24 (d, *J*= 8.9 Hz, 0H), 3.71 (q, *J*= 7.9, 7.3 Hz, 0H), 2.82 – 2.54 (m, 0H), 2.32 (t, *J*= 11.7 Hz, 0H), 2.10 (t, *J*= 8.6 Hz, 0H), 1.92 (t, *J*= 5.5 Hz, 0H), 1.81 (s, 0H), 1.70 (d, *J*= 14.7 Hz, 0H), 1.53 (dt, *J*= 9.6, 6.8 Hz, 0H), 1.43 (s, 2H), 1.26 (s, 1H), 1.19 – 1.05 (m, 1H), 1.00 (d, *J*= 10.9 Hz, 0H), 0.81 (s, 1H).

¹³C NMR (101 MHz, CDCl₃) δ 155.50, 141.42, 128.90, 128.19, 125.83, 104.99, 85.73, 78.65, 77.70, 50.99, 39.43, 38.08, 35.52, 35.01, 28.48, 27.41, 27.02, 26.52, 23.98, 22.26

IR (cm⁻¹) 3361.3, 2997.2, 2976.3, 2924.6, 2870.4, 1705.0

[α]_D= +22.5° (c= 0.31, MeOH, 18 °C)

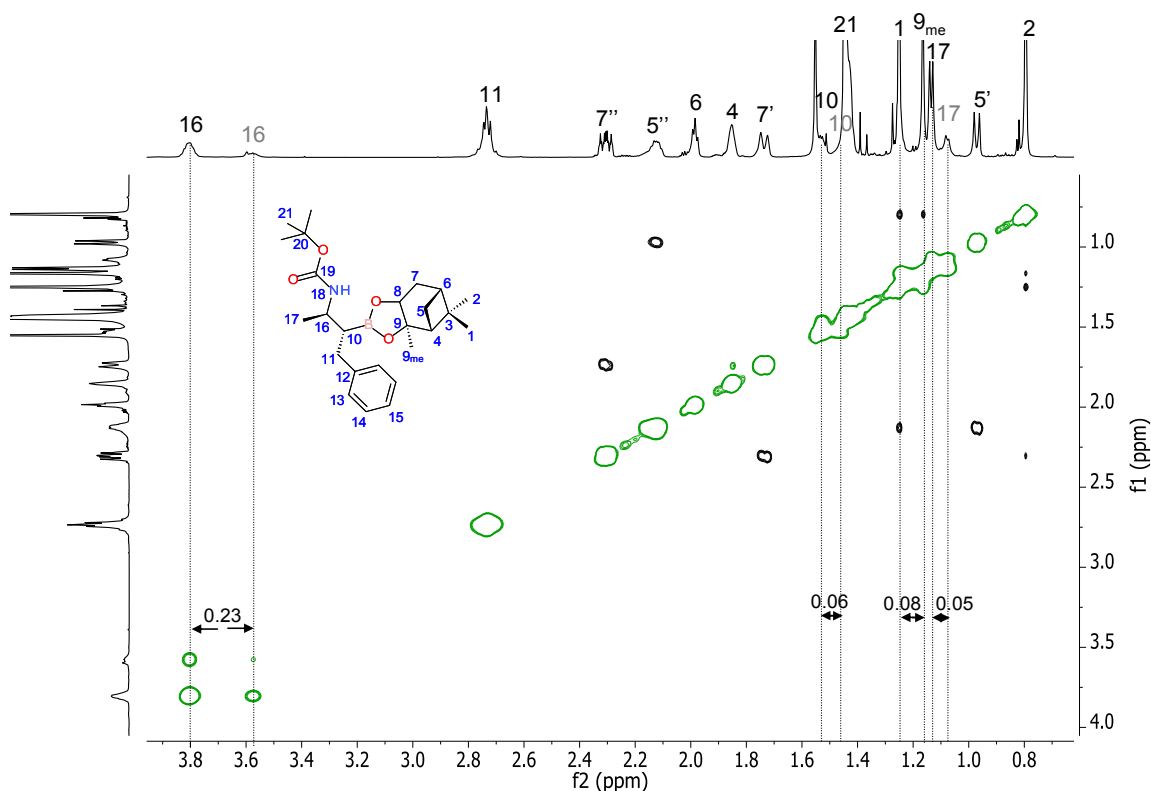


Figure S1. 300ms ROESY spectra of **1** at 298 K. Crosspeaks with the same sign as the diagonal (green) reflect conformational exchange between two rotamers in approximately 3:1 ratio (major: black, minor: grey). The $\Delta\delta$ (ppm) for each proton pair is given in the figure, marked by arrows, indicating that the rotation is around the C10-C16 bond.

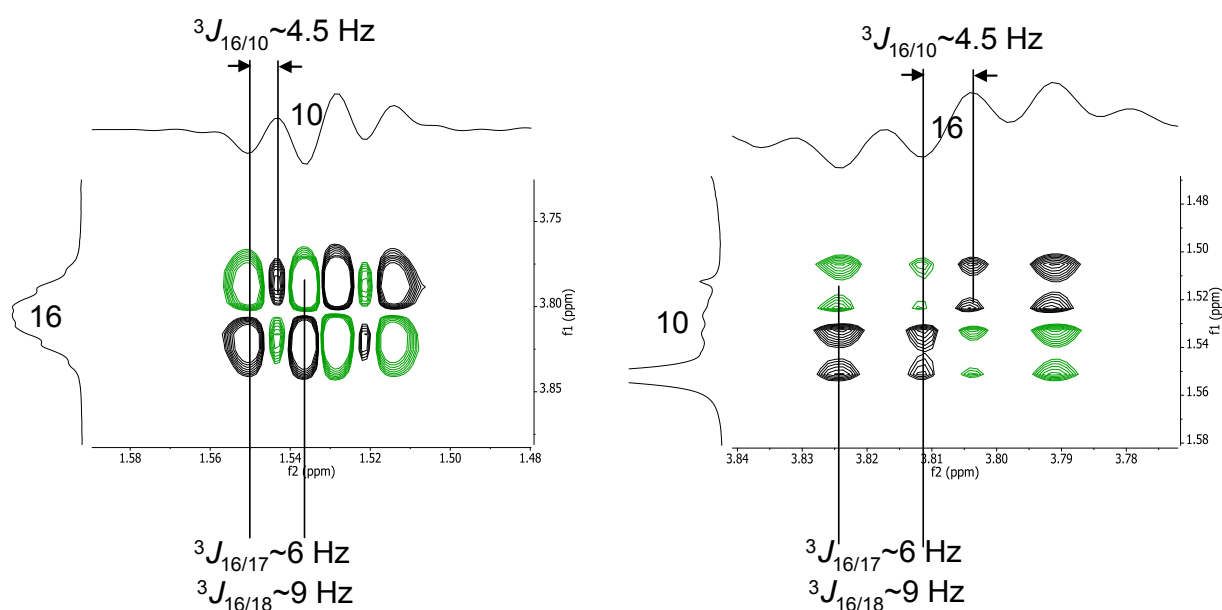


Figure S2. DQF-COSY showing the coupling between H16 and H10 of the major rotamer (estimated to ~ 4.5 Hz). The corresponding coupling is not detectable for the minor rotamer.

Crystal data and structure refinement for (2.24a-Boc).

Identification code	2.24a-Boc	
Empirical formula	C ₂₅ H ₃₈ B N O ₄	
Formula weight	427.37	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Trigonal	
Space group	<i>P</i> ₃ ₁	
Unit cell dimensions	a= 12.3865(6) Å	a= 90°.
	b= 12.3865(6) Å	b= 90°.
	c= 13.9385(7) Å	g= 120°.
Volume	1852.0(2) Å ³	
Z	3	
Density (calculated)	1.150 Mg/m ³	
Absorption coefficient	0.076 mm ⁻¹	
F(000)	696	
Crystal size	0.20 x 0.20 x 0.15 mm ³	
Theta range for data collection	1.46 to 32.59°.	
Index ranges	-18 ≤ h ≤ 18, -18 ≤ k ≤ 18, -21 ≤ l ≤ 21	
Reflections collected	28682	
Independent reflections	8998 [R(int)= 0.0414]	
Completeness to theta= 32.59°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.989 and 0.985	

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	8998 / 1 / 288
Goodness-of-fit on F^2	1.001
Final R indices [for 8313 rflns with $I > 2\sigma(I)$]	R1= 0.0386, wR2= 0.0853
R indices (all data)	R1= 0.0446, wR2= 0.0885
Largest diff. peak and hole	0.200 and -0.178 e.Å ⁻³

Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{Å}^2 \times 10^3$)

for (2.24a-Boc) U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Atom	x	y	z	U(eq)
O(1)	7570(1)	5818(1)	7517(1)	20(1)
O(2)	7321(1)	5358(1)	5913(1)	21(1)
O(3)	3954(1)	2710(1)	5823(1)	29(1)
O(4)	3097(1)	2346(1)	7327(1)	23(1)
N(1)	5023(1)	2706(1)	7154(1)	20(1)
B(1)	7403(1)	4974(2)	6824(1)	19(1)
C(1)	7672(1)	6933(1)	7066(1)	19(1)
C(2)	7381(1)	6557(1)	5979(1)	21(1)
C(3)	6159(2)	6435(2)	5588(1)	27(1)
C(4)	5456(1)	6754(1)	6338(1)	22(1)
C(5)	6350(1)	7999(1)	6864(1)	21(1)
C(6)	6694(1)	7172(1)	7516(1)	18(1)
C(7)	5384(1)	6059(1)	7281(1)	21(1)
C(8)	9006(1)	7970(2)	7241(1)	30(1)
C(9)	5623(2)	8483(1)	7440(1)	26(1)
C(10)	7326(2)	9078(2)	6266(1)	30(1)
C(11)	7295(2)	3669(1)	7000(1)	20(1)
C(12)	6025(1)	2633(1)	6643(1)	20(1)
C(13)	4020(1)	2595(1)	6685(1)	22(1)
C(14)	1951(1)	2335(1)	7018(1)	23(1)
C(15)	1303(2)	1405(2)	6205(1)	31(1)
C(16)	2276(2)	3657(2)	6752(1)	27(1)
C(17)	1153(2)	1898(2)	7920(1)	32(1)
C(18)	8383(2)	3608(2)	6507(1)	27(1)
C(19)	9633(2)	4680(2)	6811(1)	27(1)
C(20)	10306(2)	5720(2)	6226(1)	35(1)
C(21)	11446(2)	6708(2)	6513(2)	44(1)

C(22)	11931(2)	6679(2)	7403(2)	44(1)
C(23)	11270(2)	5669(2)	8001(1)	44(1)
C(24)	10129(2)	4672(2)	7710(1)	35(1)
C(25)	5848(2)	1326(2)	6756(1)	29(1)

Bond lengths [Å] and angles [°] for (2.24a-Boc)

O(1)-B(1)	1.3624(18)	C(9)-H(9B)	0.9800
O(1)-C(1)	1.4639(17)	C(9)-H(9C)	0.9800
O(2)-B(1)	1.3773(18)	C(10)-H(10A)	0.9800
O(2)-C(2)	1.4523(17)	C(10)-H(10B)	0.9800
O(3)-C(13)	1.2172(16)	C(10)-H(10C)	0.9800
O(4)-C(13)	1.3598(17)	C(11)-C(12)	1.533(2)
O(4)-C(14)	1.4772(18)	C(11)-C(18)	1.548(2)
N(1)-C(13)	1.3494(19)	C(11)-H(11)	1.0000
N(1)-C(12)	1.4723(18)	C(12)-C(25)	1.529(2)
N(1)-H(1N)	0.8800	C(12)-H(12)	1.0000
B(1)-C(11)	1.572(2)	C(14)-C(17)	1.521(2)
C(1)-C(6)	1.5188(19)	C(14)-C(16)	1.524(2)
C(1)-C(8)	1.523(2)	C(14)-C(15)	1.526(2)
C(1)-C(2)	1.5731(19)	C(15)-H(15A)	0.9800
C(2)-C(3)	1.545(2)	C(15)-H(15B)	0.9800
C(2)-H(2)	1.0000	C(15)-H(15C)	0.9800
C(3)-C(4)	1.534(2)	C(16)-H(16A)	0.9800
C(3)-H(3A)	0.9900	C(16)-H(16B)	0.9800
C(3)-H(3B)	0.9900	C(16)-H(16C)	0.9800
C(4)-C(7)	1.549(2)	C(17)-H(17A)	0.9800
C(4)-C(5)	1.560(2)	C(17)-H(17B)	0.9800
C(4)-H(4)	1.0000	C(17)-H(17C)	0.9800
C(5)-C(10)	1.525(2)	C(18)-C(19)	1.511(2)
C(5)-C(9)	1.535(2)	C(18)-H(18A)	0.9900
C(5)-C(6)	1.5795(19)	C(18)-H(18B)	0.9900
C(6)-C(7)	1.5509(19)	C(19)-C(20)	1.395(3)
C(6)-H(6)	1.0000	C(19)-C(24)	1.398(2)
C(7)-H(7A)	0.9900	C(20)-C(21)	1.387(3)
C(7)-H(7B)	0.9900	C(20)-H(20)	0.9500
C(8)-H(8A)	0.9800	C(21)-C(22)	1.386(3)
C(8)-H(8B)	0.9800	C(21)-H(21)	0.9500
C(8)-H(8C)	0.9800	C(22)-C(23)	1.380(3)
C(9)-H(9A)	0.9800	C(22)-H(22)	0.9500

C(23)-C(24)	1.393(3)	C(25)-H(25A)	0.9800
C(23)-H(23)	0.9500	C(25)-H(25B)	0.9800
C(24)-H(24)	0.9500	C(25)-H(25C)	0.9800
B(1)-O(1)-C(1)	109.17(10)	C(10)-C(5)-C(6)	122.04(13)
B(1)-O(2)-C(2)	108.66(10)	C(9)-C(5)-C(6)	110.67(11)
C(13)-O(4)-C(14)	120.31(10)	C(4)-C(5)-C(6)	85.29(10)
C(13)-N(1)-C(12)	121.42(11)	C(1)-C(6)-C(7)	108.69(11)
C(13)-N(1)-H(1N)	119.3	C(1)-C(6)-C(5)	112.86(11)
C(12)-N(1)-H(1N)	119.3	C(7)-C(6)-C(5)	87.33(10)
O(1)-B(1)-O(2)	113.42(13)	C(1)-C(6)-H(6)	114.9
O(1)-B(1)-C(11)	125.52(12)	C(7)-C(6)-H(6)	114.9
O(2)-B(1)-C(11)	121.06(12)	C(5)-C(6)-H(6)	114.9
O(1)-C(1)-C(6)	107.77(11)	C(4)-C(7)-C(6)	86.65(10)
O(1)-C(1)-C(8)	105.80(11)	C(4)-C(7)-H(7A)	114.2
C(6)-C(1)-C(8)	113.84(12)	C(6)-C(7)-H(7A)	114.2
O(1)-C(1)-C(2)	103.60(10)	C(4)-C(7)-H(7B)	114.2
C(6)-C(1)-C(2)	111.68(11)	C(6)-C(7)-H(7B)	114.2
C(8)-C(1)-C(2)	113.29(12)	H(7A)-C(7)-H(7B)	111.4
O(2)-C(2)-C(3)	109.36(12)	C(1)-C(8)-H(8A)	109.5
O(2)-C(2)-C(1)	104.55(10)	C(1)-C(8)-H(8B)	109.5
C(3)-C(2)-C(1)	115.90(11)	H(8A)-C(8)-H(8B)	109.5
O(2)-C(2)-H(2)	108.9	C(1)-C(8)-H(8C)	109.5
C(3)-C(2)-H(2)	108.9	H(8A)-C(8)-H(8C)	109.5
C(1)-C(2)-H(2)	108.9	H(8B)-C(8)-H(8C)	109.5
C(4)-C(3)-C(2)	112.91(11)	C(5)-C(9)-H(9A)	109.5
C(4)-C(3)-H(3A)	109.0	C(5)-C(9)-H(9B)	109.5
C(2)-C(3)-H(3A)	109.0	H(9A)-C(9)-H(9B)	109.5
C(4)-C(3)-H(3B)	109.0	C(5)-C(9)-H(9C)	109.5
C(2)-C(3)-H(3B)	109.0	H(9A)-C(9)-H(9C)	109.5
H(3A)-C(3)-H(3B)	107.8	H(9B)-C(9)-H(9C)	109.5
C(3)-C(4)-C(7)	108.48(12)	C(5)-C(10)-H(10A)	109.5
C(3)-C(4)-C(5)	111.13(12)	C(5)-C(10)-H(10B)	109.5
C(7)-C(4)-C(5)	88.09(10)	H(10A)-C(10)-H(10B)	109.5
C(3)-C(4)-H(4)	115.3	C(5)-C(10)-H(10C)	109.5
C(7)-C(4)-H(4)	115.3	H(10A)-C(10)-H(10C)	109.5
C(5)-C(4)-H(4)	115.3	H(10B)-C(10)-H(10C)	109.5
C(10)-C(5)-C(9)	107.85(12)	C(12)-C(11)-C(18)	111.69(11)
C(10)-C(5)-C(4)	118.03(12)	C(12)-C(11)-B(1)	109.52(11)
C(9)-C(5)-C(4)	111.47(12)	C(18)-C(11)-B(1)	110.63(12)

C(12)-C(11)-H(11)	108.3	C(11)-C(18)-H(18B)	109.3
C(18)-C(11)-H(11)	108.3	H(18A)-C(18)-H(18B)	108.0
B(1)-C(11)-H(11)	108.3	C(20)-C(19)-C(24)	117.98(17)
N(1)-C(12)-C(25)	109.64(12)	C(20)-C(19)-C(18)	121.61(15)
N(1)-C(12)-C(11)	109.61(10)	C(24)-C(19)-C(18)	120.37(16)
C(25)-C(12)-C(11)	113.41(13)	C(21)-C(20)-C(19)	121.39(18)
N(1)-C(12)-H(12)	108.0	C(21)-C(20)-H(20)	119.3
C(25)-C(12)-H(12)	108.0	C(19)-C(20)-H(20)	119.3
C(11)-C(12)-H(12)	108.0	C(22)-C(21)-C(20)	119.9(2)
O(3)-C(13)-N(1)	125.31(13)	C(22)-C(21)-H(21)	120.1
O(3)-C(13)-O(4)	125.35(14)	C(20)-C(21)-H(21)	120.1
N(1)-C(13)-O(4)	109.34(11)	C(23)-C(22)-C(21)	119.71(19)
O(4)-C(14)-C(17)	102.24(11)	C(23)-C(22)-H(22)	120.1
O(4)-C(14)-C(16)	108.95(13)	C(21)-C(22)-H(22)	120.1
C(17)-C(14)-C(16)	111.05(13)	C(22)-C(23)-C(24)	120.48(17)
O(4)-C(14)-C(15)	111.34(13)	C(22)-C(23)-H(23)	119.8
C(17)-C(14)-C(15)	109.97(13)	C(24)-C(23)-H(23)	119.8
C(16)-C(14)-C(15)	112.80(13)	C(23)-C(24)-C(19)	120.55(19)
C(14)-C(15)-H(15A)	109.5	C(23)-C(24)-H(24)	119.7
C(14)-C(15)-H(15B)	109.5	C(19)-C(24)-H(24)	119.7
H(15A)-C(15)-H(15B)	109.5	C(12)-C(25)-H(25A)	109.5
C(14)-C(15)-H(15C)	109.5	C(12)-C(25)-H(25B)	109.5
H(15A)-C(15)-H(15C)	109.5	H(25A)-C(25)-H(25B)	109.5
H(15B)-C(15)-H(15C)	109.5	C(12)-C(25)-H(25C)	109.5
C(14)-C(16)-H(16A)	109.5	H(25A)-C(25)-H(25C)	109.5
C(14)-C(16)-H(16B)	109.5	H(25B)-C(25)-H(25C)	109.5
H(16A)-C(16)-H(16B)	109.5		
C(14)-C(16)-H(16C)	109.5		
H(16A)-C(16)-H(16C)	109.5		
H(16B)-C(16)-H(16C)	109.5		
C(14)-C(17)-H(17A)	109.5		
C(14)-C(17)-H(17B)	109.5		
H(17A)-C(17)-H(17B)	109.5		
C(14)-C(17)-H(17C)	109.5		
H(17A)-C(17)-H(17C)	109.5		
H(17B)-C(17)-H(17C)	109.5		
C(19)-C(18)-C(11)	111.53(12)		
C(19)-C(18)-H(18A)	109.3		
C(11)-C(18)-H(18A)	109.3		
C(19)-C(18)-H(18B)	109.3		

Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for (2.24a-Boc). The anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^*2U^{11} + \dots + 2 h k a^* b^* U^{12}]$

Atom	U ¹¹	U ²²	U ³³	U ²³	U ¹³	U ¹²
O(1)	25(1)	23(1)	16(1)	0(1)	-1(1)	15(1)
O(2)	29(1)	23(1)	14(1)	1(1)	3(1)	16(1)
O(3)	33(1)	42(1)	17(1)	5(1)	2(1)	22(1)
O(4)	22(1)	31(1)	17(1)	3(1)	1(1)	13(1)
N(1)	24(1)	24(1)	13(1)	1(1)	2(1)	14(1)
B(1)	19(1)	24(1)	17(1)	2(1)	3(1)	12(1)
C(1)	20(1)	19(1)	16(1)	0(1)	1(1)	9(1)
C(2)	26(1)	21(1)	16(1)	2(1)	4(1)	13(1)
C(3)	37(1)	34(1)	19(1)	-4(1)	-6(1)	24(1)
C(4)	23(1)	20(1)	22(1)	-3(1)	-4(1)	11(1)
C(5)	24(1)	17(1)	23(1)	2(1)	2(1)	10(1)
C(6)	20(1)	18(1)	15(1)	-1(1)	1(1)	9(1)
C(7)	20(1)	18(1)	24(1)	3(1)	2(1)	8(1)
C(8)	20(1)	26(1)	38(1)	-3(1)	-1(1)	8(1)
C(9)	30(1)	24(1)	28(1)	-1(1)	1(1)	17(1)
C(10)	32(1)	23(1)	35(1)	9(1)	6(1)	14(1)
C(11)	26(1)	25(1)	15(1)	-1(1)	1(1)	18(1)
C(12)	27(1)	21(1)	15(1)	2(1)	4(1)	15(1)
C(13)	27(1)	20(1)	20(1)	2(1)	3(1)	13(1)
C(14)	21(1)	26(1)	20(1)	0(1)	-1(1)	10(1)
C(15)	30(1)	26(1)	30(1)	-4(1)	-7(1)	9(1)
C(16)	28(1)	27(1)	29(1)	-2(1)	-3(1)	14(1)
C(17)	24(1)	41(1)	29(1)	5(1)	4(1)	15(1)
C(18)	30(1)	36(1)	24(1)	-8(1)	-1(1)	23(1)
C(19)	26(1)	41(1)	23(1)	-9(1)	-2(1)	25(1)
C(20)	26(1)	56(1)	27(1)	-5(1)	1(1)	22(1)
C(21)	27(1)	55(1)	44(1)	-5(1)	6(1)	17(1)
C(22)	29(1)	58(1)	50(1)	-22(1)	-8(1)	25(1)
C(23)	47(1)	67(1)	36(1)	-22(1)	-18(1)	42(1)
C(24)	40(1)	49(1)	30(1)	-10(1)	-7(1)	33(1)

C(25) 39(1) 22(1) 31(1) 1(1) 4(1) 20(1)

Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for (2.24a-Boc).

Atom	x	y	z	U(iso)
H(1N)	5080	2824	7779	24
H(2)	8094	7177	5580	25
H(3A)	6354	6997	5030	32
H(3B)	5611	5570	5362	32
H(4)	4649	6669	6116	26
H(6)	6836	7418	8208	22
H(7A)	5365	5257	7186	26
H(7B)	4720	5964	7726	26
H(8A)	9166	8081	7933	44
H(8B)	9127	8749	6962	44
H(8C)	9586	7749	6942	44
H(9A)	6203	9193	7834	38
H(9B)	5016	7817	7856	38
H(9C)	5184	8747	6998	38
H(10A)	7861	9774	6690	45
H(10B)	6908	9348	5807	45
H(10C)	7834	8805	5917	45
H(11)	7347	3560	7706	24
H(12)	5956	2777	5946	24
H(15A)	1758	1751	5605	46
H(15B)	447	1243	6137	46
H(15C)	1288	624	6352	46
H(16A)	2887	3962	6229	41
H(16B)	2630	4202	7312	41
H(16C)	1520	3658	6544	41
H(17A)	957	1047	8075	48
H(17B)	378	1910	7811	48
H(17C)	1611	2454	8454	48
H(18A)	8341	2811	6675	32
H(18B)	8298	3630	5802	32

H(20)	9977	5751	5618	42
H(21)	11894	7404	6101	53
H(22)	12714	7351	7600	53
H(23)	11596	5654	8614	53
H(24)	9684	3980	8127	42
H(25A)	4975	703	6629	43
H(25B)	6067	1221	7411	43
H(25C)	6387	1216	6299	43

Torsion angles [°] for (2.24a-Boc)

C(1)-O(1)-B(1)-O(2)	-2.69(16)
C(1)-O(1)-B(1)-C(11)	177.87(13)
C(2)-O(2)-B(1)-O(1)	-2.70(16)
C(2)-O(2)-B(1)-C(11)	176.77(13)
B(1)-O(1)-C(1)-C(6)	124.83(12)
B(1)-O(1)-C(1)-C(8)	-113.05(13)
B(1)-O(1)-C(1)-C(2)	6.37(14)
B(1)-O(2)-C(2)-C(3)	-118.30(12)
B(1)-O(2)-C(2)-C(1)	6.41(14)
O(1)-C(1)-C(2)-O(2)	-7.65(13)
C(6)-C(1)-C(2)-O(2)	-123.37(12)
C(8)-C(1)-C(2)-O(2)	106.50(13)
O(1)-C(1)-C(2)-C(3)	112.80(13)
C(6)-C(1)-C(2)-C(3)	-2.92(17)
C(8)-C(1)-C(2)-C(3)	-133.05(14)
O(2)-C(2)-C(3)-C(4)	119.81(13)
C(1)-C(2)-C(3)-C(4)	2.00(18)
C(2)-C(3)-C(4)-C(7)	-47.67(16)
C(2)-C(3)-C(4)-C(5)	47.57(16)
C(3)-C(4)-C(5)-C(10)	41.20(18)
C(7)-C(4)-C(5)-C(10)	150.29(14)
C(3)-C(4)-C(5)-C(9)	166.82(12)
C(7)-C(4)-C(5)-C(9)	-84.08(13)
C(3)-C(4)-C(5)-C(6)	-82.81(12)
C(7)-C(4)-C(5)-C(6)	26.29(10)
O(1)-C(1)-C(6)-C(7)	-63.36(13)

C(8)-C(1)-C(6)-C(7)	179.63(12)
C(2)-C(1)-C(6)-C(7)	49.79(14)
O(1)-C(1)-C(6)-C(5)	-158.47(11)
C(8)-C(1)-C(6)-C(5)	84.51(14)
C(2)-C(1)-C(6)-C(5)	-45.33(15)
C(10)-C(5)-C(6)-C(1)	-37.41(18)
C(9)-C(5)-C(6)-C(1)	-165.91(12)
C(4)-C(5)-C(6)-C(1)	82.90(12)
C(10)-C(5)-C(6)-C(7)	-146.58(14)
C(9)-C(5)-C(6)-C(7)	84.92(13)
C(4)-C(5)-C(6)-C(7)	-26.27(10)
C(3)-C(4)-C(7)-C(6)	84.90(12)
C(5)-C(4)-C(7)-C(6)	-26.77(10)
C(1)-C(6)-C(7)-C(4)	-86.82(11)
C(5)-C(6)-C(7)-C(4)	26.43(10)
O(1)-B(1)-C(11)-C(12)	119.40(15)
O(2)-B(1)-C(11)-C(12)	-60.00(16)
O(1)-B(1)-C(11)-C(18)	-117.07(15)
O(2)-B(1)-C(11)-C(18)	63.53(18)
C(13)-N(1)-C(12)-C(25)	-99.25(15)
C(13)-N(1)-C(12)-C(11)	135.65(13)
C(18)-C(11)-C(12)-N(1)	178.19(12)
B(1)-C(11)-C(12)-N(1)	-58.91(13)
C(18)-C(11)-C(12)-C(25)	55.29(15)
B(1)-C(11)-C(12)-C(25)	178.19(12)
C(12)-N(1)-C(13)-O(3)	-17.5(2)
C(12)-N(1)-C(13)-O(4)	163.06(12)
C(14)-O(4)-C(13)-O(3)	-7.0(2)
C(14)-O(4)-C(13)-N(1)	172.39(12)
C(13)-O(4)-C(14)-C(17)	174.58(13)
C(13)-O(4)-C(14)-C(16)	-67.83(16)
C(13)-O(4)-C(14)-C(15)	57.20(17)
C(12)-C(11)-C(18)-C(19)	176.24(12)
B(1)-C(11)-C(18)-C(19)	53.97(17)
C(11)-C(18)-C(19)-C(20)	-101.30(17)
C(11)-C(18)-C(19)-C(24)	76.59(18)

C(24)-C(19)-C(20)-C(21)	1.3(2)
C(18)-C(19)-C(20)-C(21)	179.24(16)
C(19)-C(20)-C(21)-C(22)	-0.7(3)
C(20)-C(21)-C(22)-C(23)	-0.5(3)
C(21)-C(22)-C(23)-C(24)	1.0(3)
C(22)-C(23)-C(24)-C(19)	-0.3(3)
C(20)-C(19)-C(24)-C(23)	-0.8(2)
C(18)-C(19)-C(24)-C(23)	-178.79(15)

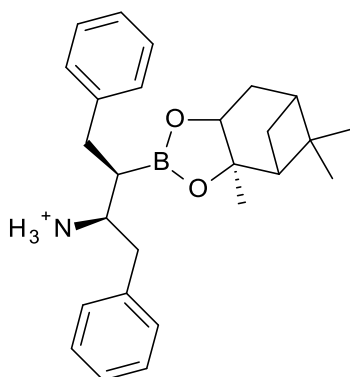
Hydrogen bonds for (2.24a-Boc) [\AA and $^\circ$].

D-H...A	d(D-H)	d(H...A)	d(D...A)	\angle (DHA)
N(1)-H(1N)...O(2)#1	0.88	2.24	3.0227(14)	147

Symmetry transformations used to generate equivalent atoms:

#1 $-y+1, x-y, z+1/3$

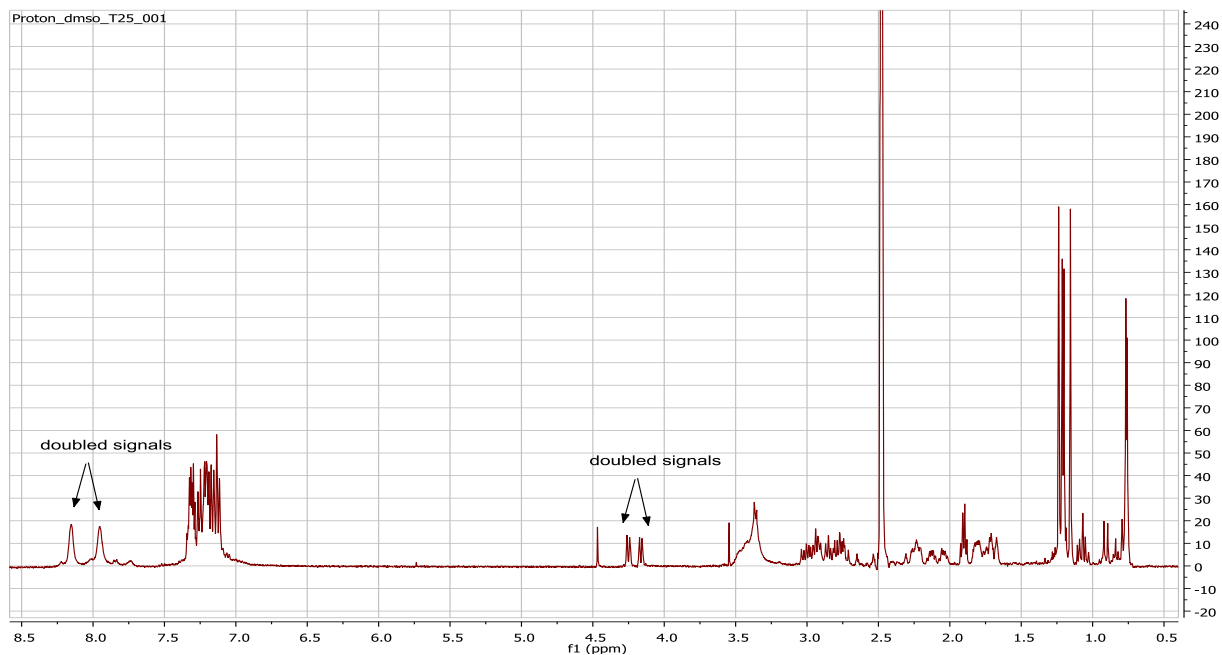
[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-1-benzyl-2-benzyl-ethylammonium chloride (2.24d)



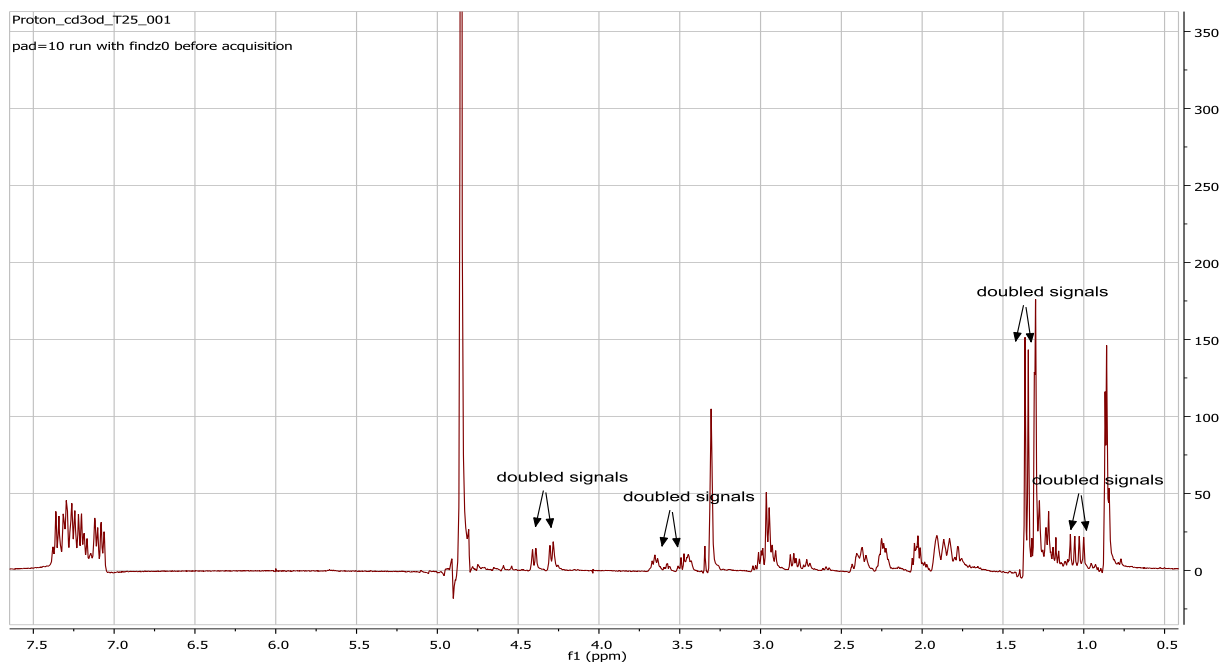
HRMS found: 404.2762 Exact mass: 404.2755 Calculated for: $\text{C}_{26}\text{H}_{35}\text{BNO}_2$ $[\text{M}]^+$

Resonances are doubled in NMR spectra probably due to slow rotation or aggregation effects. Epimerization was excluded from consideration as such an effect was not observed neither in previous steps nor in the subsequent one.

DMSO

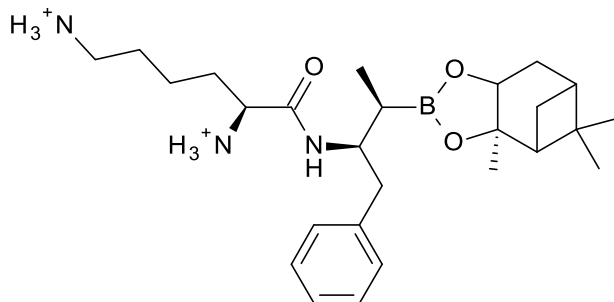


CD3OD



[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzyl-2-methyl-ethylamino-*N*-(*L*)-lysine dihydrochloride (2.25-1)

diBoc-protected analog: 58.1 % yield.



HRMS found: 456.3390 Exact mass: 456.3392 Calculated for: $C_{26}H_{43}BN_3O_3$ $[M-H]^+$

1H NMR (400 MHz, CD_3OD) δ 7.25 (m, 5H), 4.40 (m, 2H), 3.79 (t, J = 6.1 Hz, 1H), 2.99 – 2.84 (m, 1H), 2.73 (m, 3H), 2.47 – 2.36 (m, 1H), 2.28 (m, 1H), 2.07 (t, J = 5.4 Hz, 1H), 1.97 – 1.81 (m, 2H), 1.71 (m, 1H), 1.58 – 1.43 (m, 6H), 1.42 (s, 3H), 1.32 (s, 3H), 1.16 (d, J = 10.6 Hz, 1H), 1.09 (d, J = 7.5 Hz, 3H), 0.89 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.73, 139.38, 128.85, 127.98, 126.00, 85.71, 77.77, 53.38, 52.67, 51.26, 39.50, 38.79, 37.87, 37.77, 35.09, 30.62, 27.71, 26.67, 26.07, 26.00, 22.91, 20.83, 9.20.

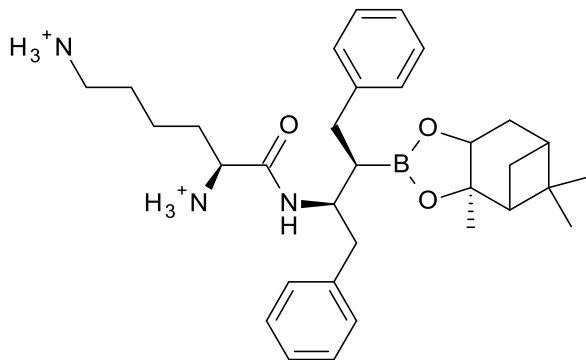
IR (cm^{-1}) 3429.2, 3208.1, 2918.1, 2872.2, 1665.9

$[\alpha]_D = n/a$

58.8 % yield

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzyl-2-benzyl-ethylamino-*N*-(*L*)-lysine dihydrochloride (2.25-2)

Boc-protected analog: 78.8 % isolated yield.



HRMS found: 532.3701 Exact mass: 532.3704 Calculated for: $C_{32}H_{47}BN_3O_3$ $[M-H]^+$

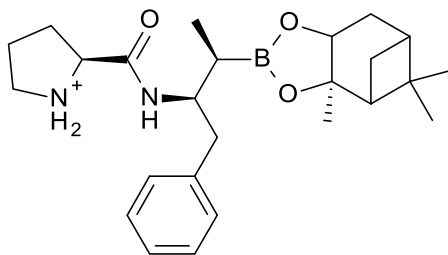
1H NMR (400 MHz, CD_3OD) δ 7.32 – 7.10 (m, 10H), 4.42 (dt, J = 10.3, 4.2 Hz, 1H), 4.34 (d, J = 8.3 Hz, 1H), 3.86 (m, 1H), 3.07 – 2.67 (m, 6H), 2.37 (dd, J = 13.9, 8.7 Hz, 1H), 2.13 (dt, J = 11.2, 6.0 Hz, 1H), 1.99 (t, J = 5.4 Hz, 1H), 1.88 – 1.68 (m, 3H), 1.62 – 1.44 (m, 4H), 1.27 (s+s, 6H), 1.12 – 0.89 (m+d, 3H), 0.84 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.78, 141.44, 138.95, 128.87, 128.59, 128.07, 127.8, 126.11, 125.52, 85.77, 77.76, 52.75, 52.52, 51.12, 39.42, 39.01, 38.82, 37.79, 35.00, 32.55, 30.72, 27.66, 26.74, 26.05, 26.02, 22.9, 20.9

IR (cm^{-1}) 3364.7, 3025.9, 2975.1, 2929.5, 2867.3, 1685.9

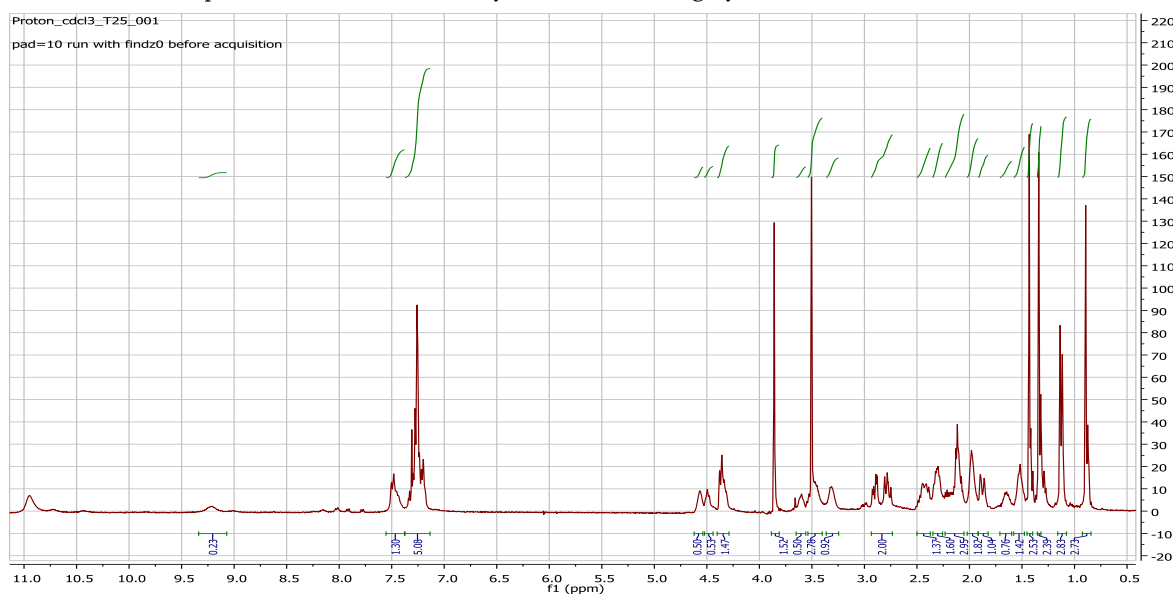
(19.2 % yield)

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzyl-2-methyl-ethylamino-*N*-(*L*)-proline hydrochloride (2.25-3)

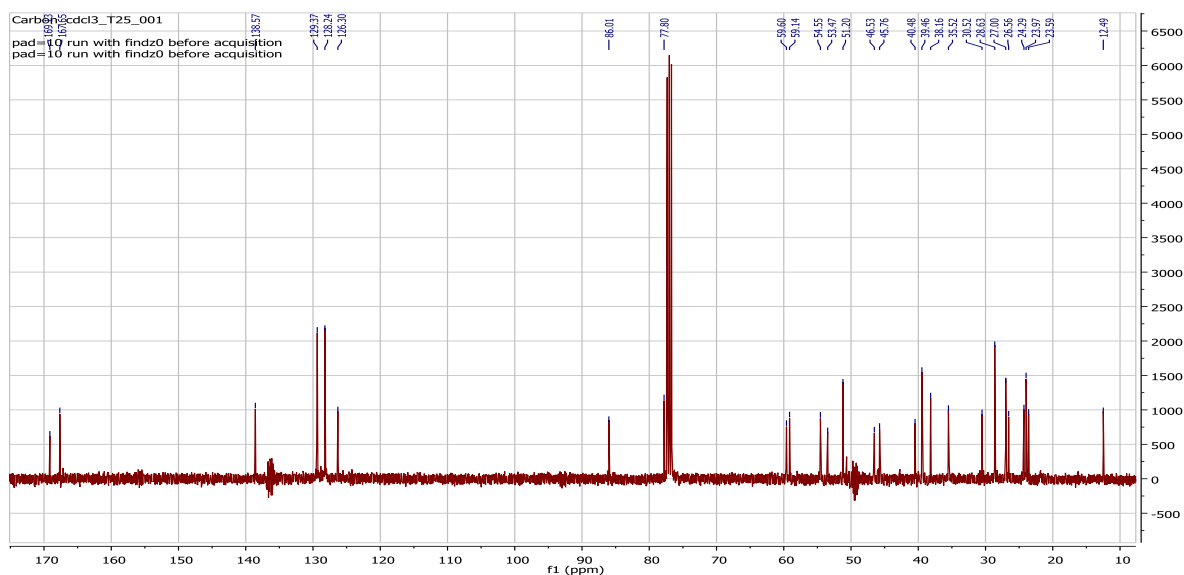


HRMS found: 425.2962 Exact mass: 425.2970 Calculated for: C₂₅H₃₈BN₂O₃ [MH]⁺

¹H NMR (400 MHz, CDCl₃): signals were doubled due to some aggregation effect. The amount of 2.25-3 was too small and the compound was unstable to analyze it more thoroughly.

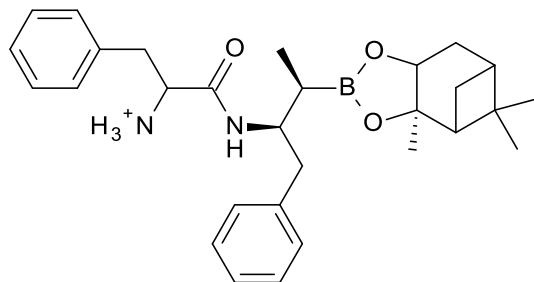


¹³C NMR (101 MHz, CDCl₃)



[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzyl-2-methyl-ethylamino-*N*-(*L*)-phenylalanine hydrochloride (2.25-4)

Boc-protected analog: 74.3 % isolated yield.



HRMS found: 475.3131 Exact mass: 475.3127 Calculated for: C₂₉H₄₀BN₂O₃ [M]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.26 (m, 10H), 4.33 (m, 2H), 4.07 (t, *J* = 6.7 Hz, 1H), 3.02 – 2.60 (m, 4H), 2.41 (m, 1H), 2.26 (m, 1H), 2.05 (t, *J* = 5.4 Hz, 1H), 1.96 – 1.78 (m, 2H), 1.48 (m, 1H), 1.38 (s, 3H), 1.31 (s, 3H), 1.13 (d, *J* = 10.6 Hz, 1H), 1.06 (d, *J* = 7.4 Hz, 3H), 0.88 (s, 3H).

¹³C NMR (101 MHz, CD₃OD) δ 167.47, 139.09, 133.89, 129.10, 128.87, 128.65, 128.09, 127.35, 126.15, 85.70, 77.75, 54.13, 53.83, 51.25, 39.50, 38.48, 37.86, 37.23, 35.11, 27.74, 26.11, 26.03, 22.95, 9.90

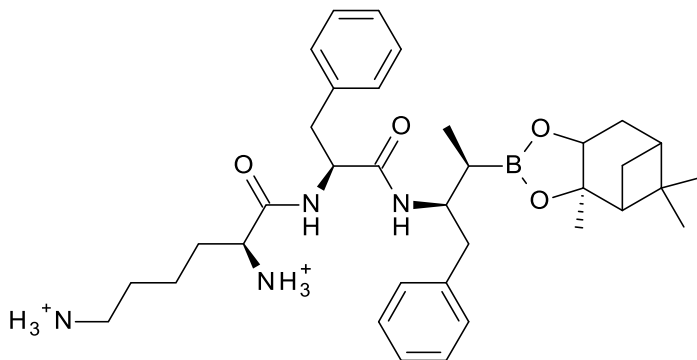
IR (cm⁻¹) 3060.2, 3028.1, 2917.2, 2870.5, 1747.7, 1668.4

[α]_D = -3.1° (c = 0.32, MeOH, 18 °C)

65.2 % yield

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-benzyl-2-methyl-ethylamino-*N*'-(*L*)-lysine dihydrochloride (2.25-5)

diBoc-protected analog: 87.8 % yield.



HRMS found: 603.4121 Exact mass: 603.4076 Calculated for: C₃₅H₅₂BN₄O₄ [M-H]⁺

¹H NMR (400 MHz, CD₃OD) δ 7.36 – 7.06 (m, 10H), 4.54 (t, *J* = 7.0 Hz, 1H), 4.40 – 4.30 (m, 1H), 4.18 (m, 1H), 3.87 (s, 1H), 2.95 (s, 3H), 2.70 (m, 3H), 2.41 (m, 1H), 2.31 – 2.21 (m, 1H), 2.05 (t, *J* = 5.4 Hz, 1H), 1.96 – 1.80 (m, 4H), 1.70 (s, 3H), 1.50 (s, 3H), 1.38 (s, 3H), 1.32 (s, 3H), 1.15 (d, *J* = 10.7 Hz, 1H), 1.02 (d, *J* = 7.2 Hz, 3H), 0.88 (s, 3H)

^{13}C NMR (101 MHz, CD_3OD) δ 171.16, 168.27, 138.96, 136.75, 128.86, 128.80, 128.16, 128.00, 126.47, 126.00, 85.63, 77.62, 55.24, 53.75, 52.42, 51.27, 39.52, 39.08, 38.86, 37.86, 37.43, 35.13, 30.64, 27.81, 26.53, 26.11, 26.06, 22.96, 21.05, 10.85

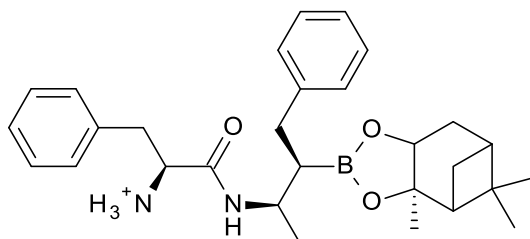
IR (cm^{-1}) 3207.4, 3027.2, 2925.2, 1649.2

$[\alpha]_{\text{D}} = +15.6^\circ$ ($c = 0.64$, MeOH, 18°C)

Quantitative yield.

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-methyl-2-benzyl-ethylamino-*N*-(*L*)-phenylalanine hydrochloride (2.25-6)

Boc-protected analog: 75.5 % yield.



HRMS found: 475.3124 Exact mass: 475.3127 Calculated for: $\text{C}_{29}\text{H}_{40}\text{BN}_2\text{O}_3$ $[\text{M}]^+$

^1H NMR (400 MHz, CD_3OD) δ 7.50 – 6.94 (m, 10H), 4.22 (d, $J = 8.3$ Hz, 1H), 4.16 – 3.94 (m, 2H), 3.21 – 2.98 (m, 2H), 2.74 – 2.59 (m, 2H), 2.30 (dd, $J = 14.5, 8.3$ Hz, 1H), 2.14 – 2.02 (m, 1H), 1.92 (t, $J = 5.3$ Hz, 1H), 1.82 (b.s, 1H), 1.62 (m, 2H), 1.26 (s, 3H), 1.16 (s, 3H), 0.96 (d, $J = 6.5$ Hz, 3H), 0.79 (s+d, 3+1H).

^{13}C NMR (101 MHz, CD_3OD) δ 167.04, 141.28, 134.38, 129.24, 128.75, 128.61, 127.84, 127.44, 125.54, 85.66, 77.62, 54.58, 50.96, 46.54, 39.34, 37.73, 37.47, 34.97, 32.91, 27.62, 26.07, 25.95, 22.94, 18.70.

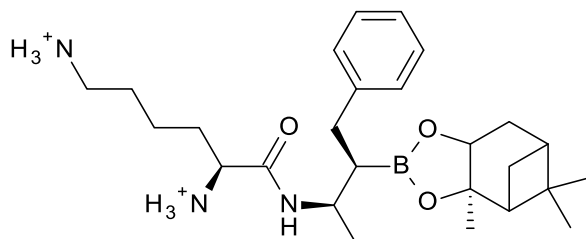
IR (cm^{-1}) 3387.7, 3198.55, 3027.81, 2967.05, 2922.47, 2869.8, 1675.29, 1386.67, 1029.05, 744.37, 698.57

$[\alpha]_{\text{D}} = +33.3^\circ$ ($c = 0.36$, MeOH, 18°C)

11.0 % yield

[(1*R*,2*R*)-2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl]-1-methyl-2-benzyl-ethylamino-*N*-(*L*)-lysine dihydrochloride (2.25-7)

diBoc-protected analog: 47.5 % yield



HRMS found: 456.3389 Exact mass: 456.3392 Calculated for: $\text{C}_{26}\text{H}_{43}\text{BN}_3\text{O}_3$ $[\text{M}-\text{H}]^+$

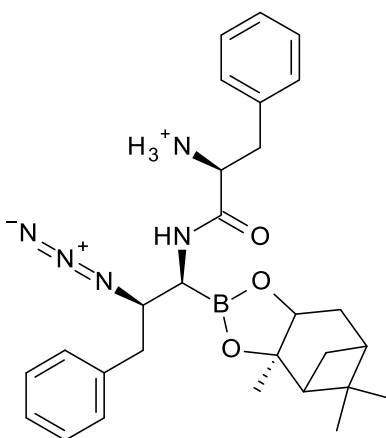
^1H NMR (400 MHz, CD_3OD) δ 7.24 (m, 3H), 7.13 (m, 2H), 4.28 (dd, $J = 9.0, 2.0$ Hz, 1H), 4.18 – 4.09 (m, 1H), 3.93 (m, 1H), 2.96 (t, $J = 7.9$ Hz, 2H), 2.88 – 2.64 (m, 2H), 2.33 (m, 1H), 2.16 – 2.05 (m, 1H), 2.01 – 1.80 (m, 5H), 1.78 – 1.64 (m, 4H), 1.52 (d, $J = 7.7$ Hz, 3H), 1.26 (s, 3H), 1.23 (s, 2H), 0.86 (d, 1H), 0.83 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 157.16, 141.11, 128.61, 127.85, 125.55, 79.61, 78.40, 77.59, 50.96, 45.82, 39.57, 39.37, 37.77, 35.37, 35.11, 34.13, 31.50, 29.19, 27.39, 26.04, 25.73, 22.92, 20.06

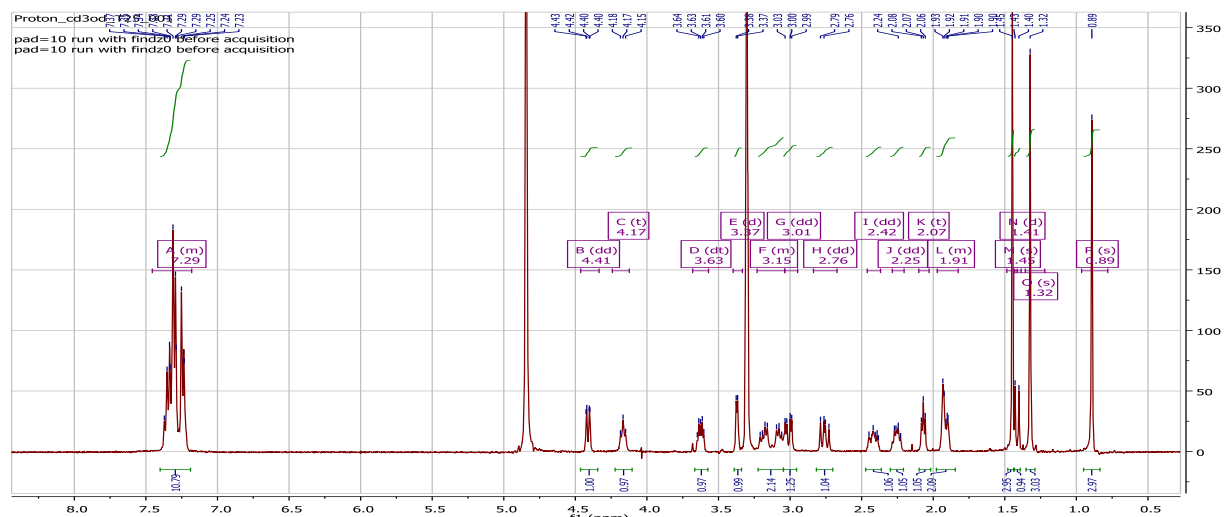
10.0 % yield.

[(1*S*,2*R*)-2-azido-3-phenyl-1-(2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)propylamino-N-(L)-phenylalanine (2.29)

Boc-protected analog: 81.2 % yield



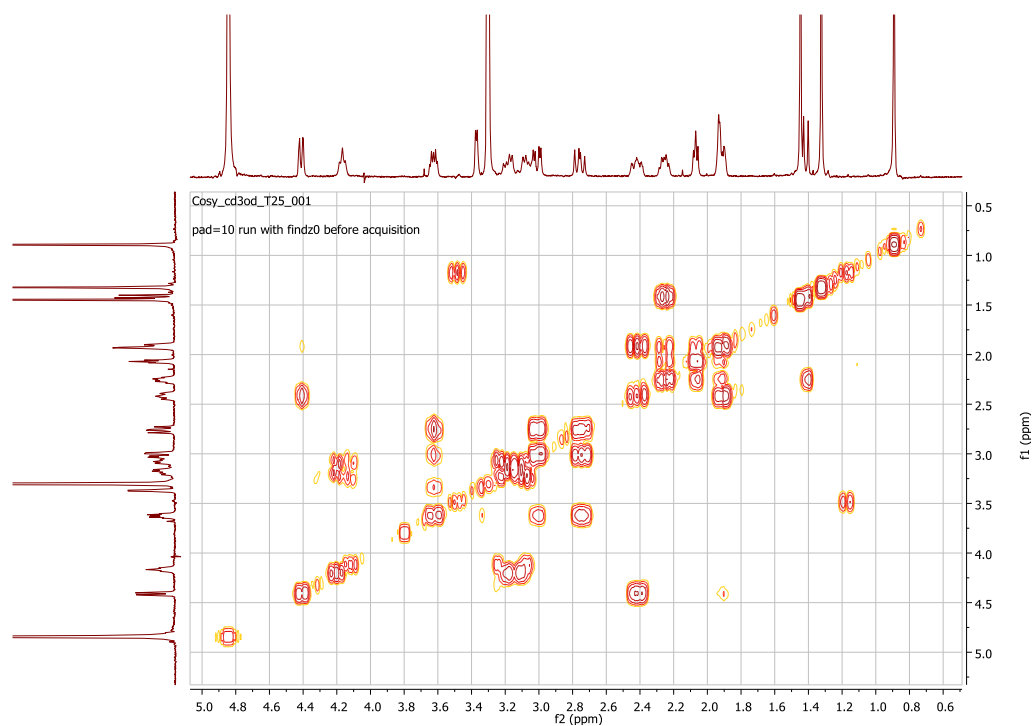
HRMS found: 502.2998 Exact mass: 502,2984 Calculated for: $\text{C}_{28}\text{H}_{37}\text{BN}_5\text{O}_3$ $[\text{M}]^+$



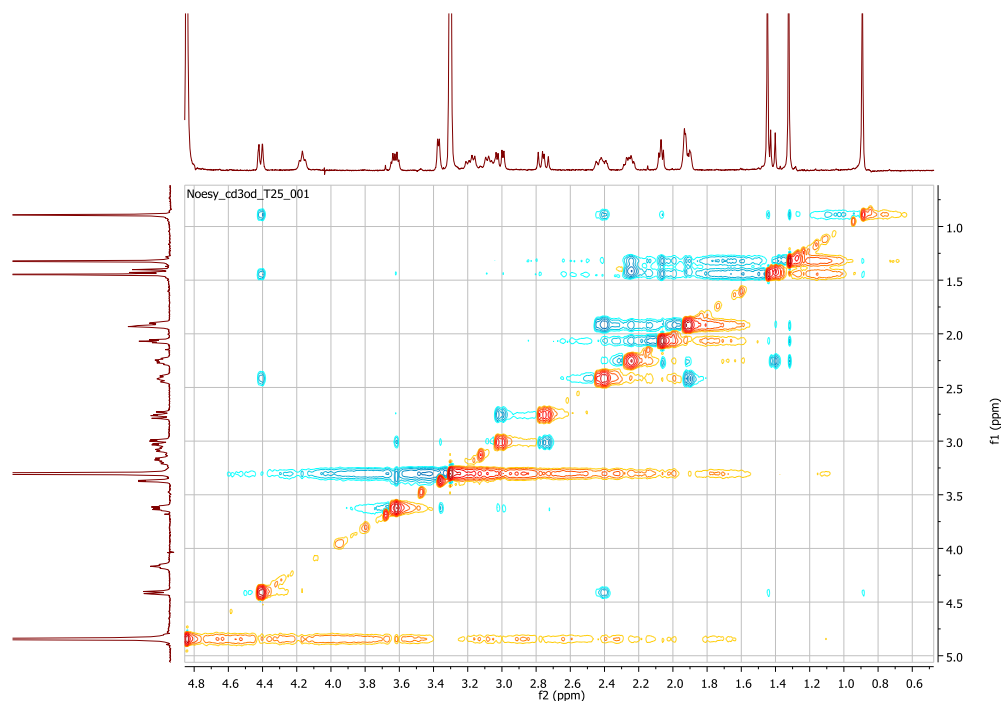
^1H NMR (400 MHz, CD_3OD) δ 7.46 – 7.18 (m, 10H), 4.41 (dd, $J = 8.8, 2.0$ Hz, 1H), 4.17 (t, $J = 7.1$ Hz, 1H), 3.63 (dt, $J = 9.4, 4.3$ Hz, 1H), 3.37 (d, $J = 4.0$ Hz, 1H), 3.23 – 3.04 (m, 1H), 3.01 (m, 1H), 2.76 (m, 1H), 2.42 (m, 1H), 2.25 (m, 1H), 2.07 (t, $J = 5.4$ Hz, 1H), 1.97 – 1.83 (m, 2H), 1.45 (s, 3H), 1.41 (d, $J = 10.8$ Hz, 1H), 1.32 (s, 3H), 0.89 (s, 3H).

^{13}C NMR (101 MHz, CD_3OD) δ 168.86, 137.94, 134.04, 129.21, 128.99, 128.70, 128.17, 127.52, 126.40, 86.42, 78.01, 66.71, 53.65, 51.35, 39.50, 38.02, 37.89, 37.28, 35.02, 27.71, 26.10, 25.82, 22.96.

COSY:

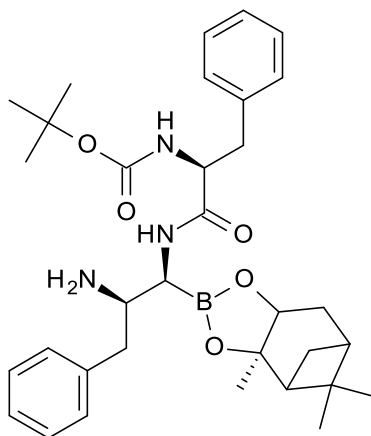


NOESY:



Quantitative yield.

[(1*S*,2*R*)-2-amino-3-phenyl-1-(2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)propylamino-1-*N*-(Boc-(*L*)-phenylalanine) (2.30)

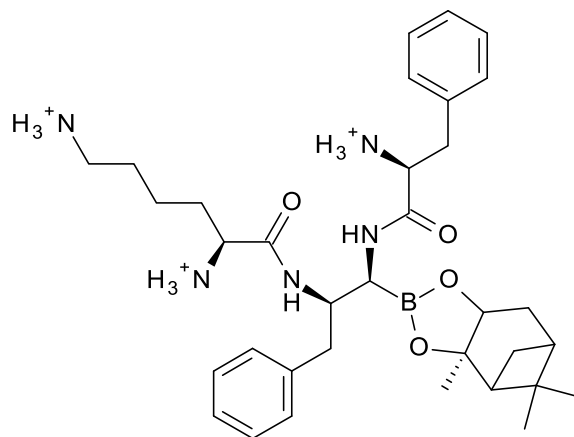


HRMS found: 576.3603 Exact Mass : 576,3603 Calculated for $C_{33}H_{47}BN_3O_5$ $[M+H]^+$

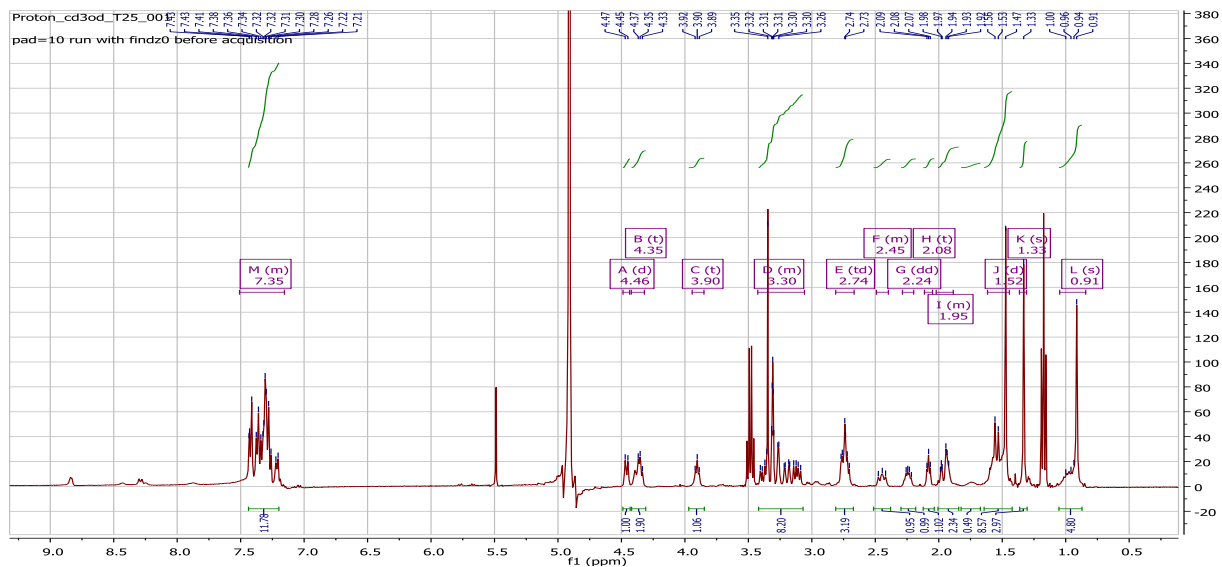
Compound was used in the next step without purification. Crude yield 64.5 %

(1*S*,2*R*)-1-(2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3-phenyl-propane-1,2-diamine-1-*N*-(*L*)-phenylalanine-2-*N*-(*L*)-lysine trihydrochloride (2.32a)

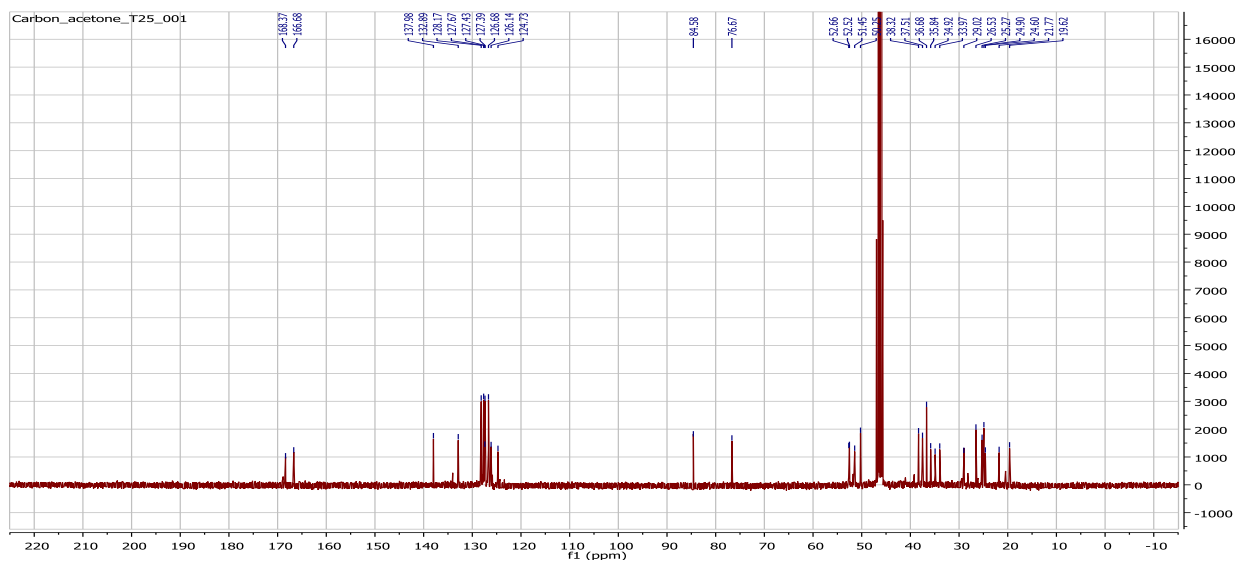
triBoc-protected analog (2.31a): 38.9 % isolated yield



HRMS found: 604.4044 Exact Mass : 604,4029 Calculated for $C_{34}H_{51}BN_5O_4$ $[M-2H]^+$

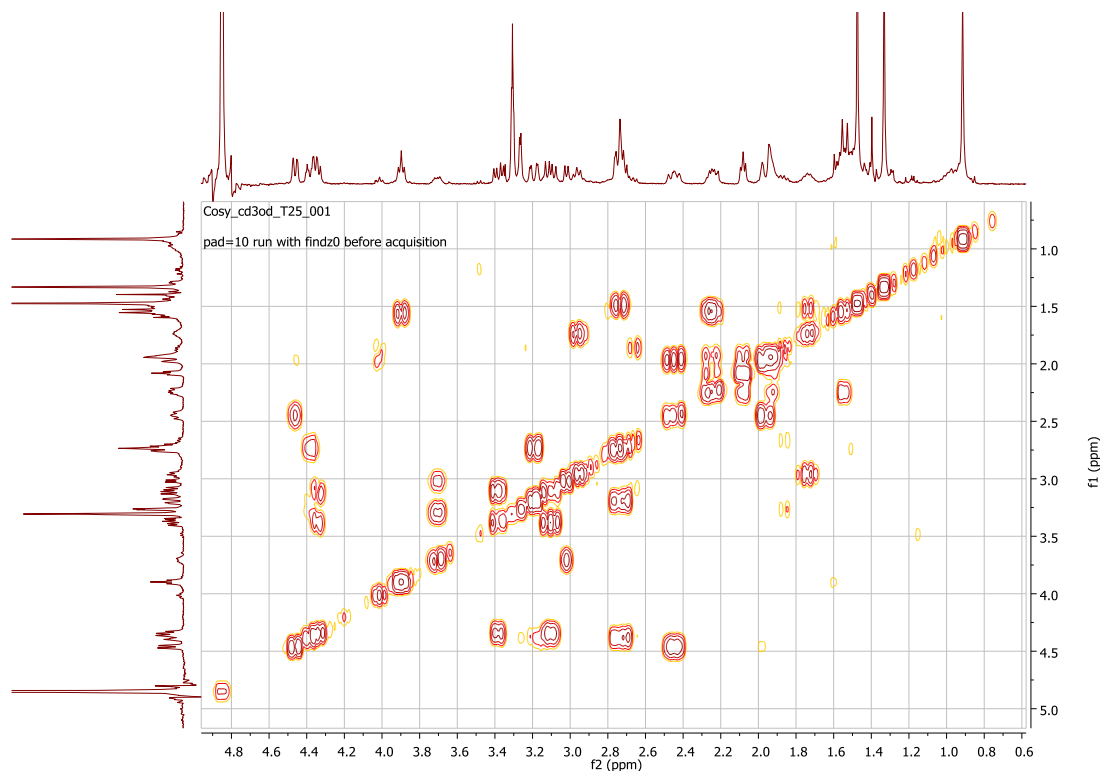


^1H NMR (400 MHz, CD_3OD) δ 7.51 – 7.16 (m, 10H+1H), 4.46 (d, J = 8.5 Hz, 1H), 4.35 (t, J = 6.6 Hz, 2H), 3.90 (t, J = 6.4 Hz, 1H), 3.43 – 3.06 (m, 4H), 2.74 (m, 2H+1H), 2.49 – 2.40 (m, 1H), 2.24 (dd, J = 10.8, 5.7 Hz, 1H), 2.08 (t, J = 5.3 Hz, 1H), 2.02 – 1.89 (m, 2H), 1.52 (m, 6H+3H+1H), 1.33 (s, 3H), 0.91 (s, 3H).

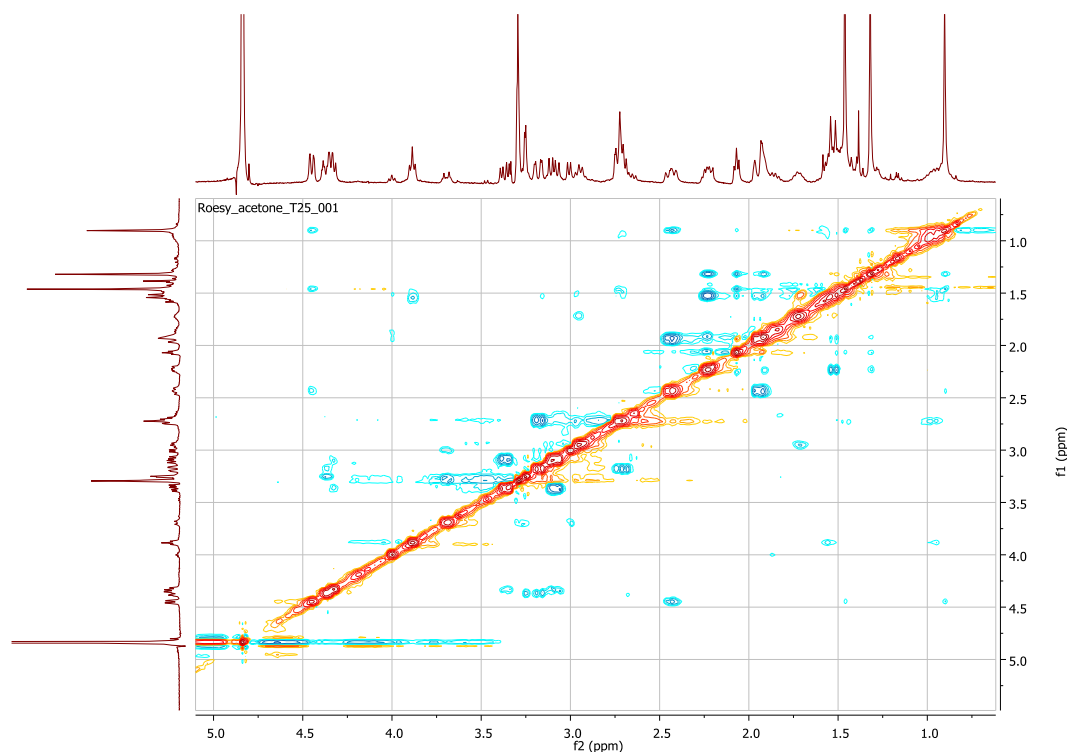


^{13}C NMR (101 MHz, $(\text{CD}_3)_2\text{CO}$) δ 168.37, 166.68, 137.98, 132.89, 128.17, 127.67, 127.39, 126.68, 126.14, 124.73, 84.58, 76.67, 52.66, 52.52, 51.45, 50.25, 38.32, 37.51, 36.68, 35.84, 34.92, 33.97, 29.02, 26.53, 25.27, 24.90, 24.60, 21.77, 19.62.

COSY



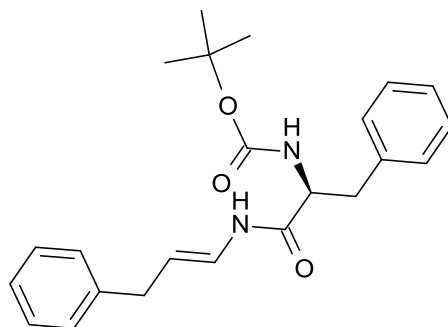
NOESY:



IR (cm⁻¹) 3384.5, 2928.1, 2867.6, 1661.1

Quantitative yield

tert-butyl N-[(1*S*)-1-benzyl-2-oxo-2-[[*(E)*-3-phenylprop-1-enyl]amino]ethyl]carbamate (2.33)



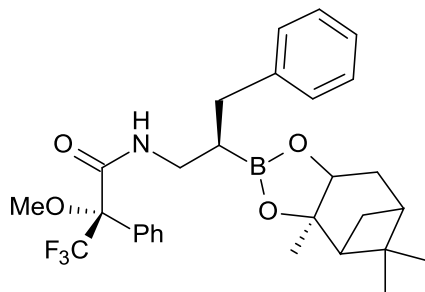
HRMS found: 403.1999 Exact Mass : 403.1992 Calculated for $C_{23}H_{28}N_2NaO_3$ [M+Na]⁺

24 % yield

Potassium [(1*S*)-1-azido-2-phenylethyl]trifluoroborate (2.37)

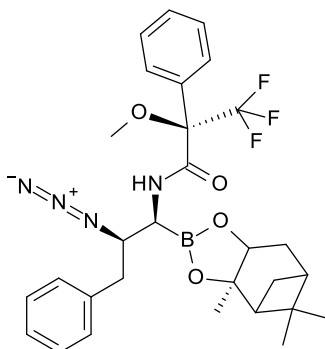
See paper I for the experimental details: Lejon T., Gorovoy A.S., Khrustalev V.N., (2012), Acta Cryst., E68, m1048

(2*S*)-3,3,3-trifluoro-2-methoxy-2-phenyl-N-[(2*R*)-3-phenyl-2-((1*S*,2*R*)-1-(2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl) propyl]propanamide (2.38)



(see Chapter 2.7 for the spectral data)

(2*R*)-N-[(1*S*,2*R*)-2-azido-3-phenyl-1-(2-(3*aR*,4*R*,6*R*,7*aS*)hexahydro-3*a*,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborolan-2-yl)-3,3,3-trifluoro-2-methoxy-2-phenyl]propanamide (2.39)



(see Chapter 2.6 for the spectral data); $[\alpha]_D = +88.2$ ($c = 0.34$, MeOH, 18 °C); Quantitative yield.

Patent

GB 1200338.0 (9 January 2012).

Gorovoy AS, Gozhina OV, Svendsen JS, Lejon T.

Paper I

Potassium [(1S)-1-azido-2-phenylethyl]trifluoridoborate

Tore Lejon, Alexey S. Gorovoy and Victor N. Khrustalev

Acta Cryst. (2012). E68, m1048

Paper II

Boron containing peptidomimetics – a novel class of selective anti-tubercular drugs

Alexey S. Gorovoy, Olga V. Gozhina, John Sigurd Svendsen, Anna A. Domorad, George V. Tetz, Victor V. Tetz and Tore Lejon

“Chemical Biology and Drug Design”

Accepted for publication

Paper III

β -Aminoboronate based peptidomimetic as potential antifungal agent

Alexey S. Gorovoy, Tore Lejon, Anton Liaimer

Manuscript. Submitted to “Antimicrobial agents and Chemotherapy”

Paper IV

Syntheses and anti-tubercular activity of β -substituted and α,β -disubstituted β -aminoboronates and boronic acids

Alexey S. Gorovoy, Olga Gozhina, John-Sigurd Svendsen, George V. Tetz, Anna Domorad, Victor V. Tetz and Tore Lejon

Manuscript.



ISBN xxx-xx-xxxx-xxx-x