Institute of Pharmaceutical Chemistry
University of Szeged

Syntheses of mono- and dihydroxy-substituted cyclopentane-, cyclohexane- and cyclooctane-β-amino acids

PhD Thesis

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CONTENTS

ABBREVIATIONS AND SYMBOLS ..... **ii**
PUBLICATIONS ..... **iii**
1. INTRODUCTION AND AIMS **1**
2. LITERATURE **3**
2.1. Hydroxy-β-amino acids 3
2.2. Synthesis of cyclic hydroxy-β-amino acids 4
2.2.1. Reactions of the olefinic bond 4
2.2.1.1. Opening of the oxirane ring 4
2.2.1.2. Oxazoline, oxazine or iodolactone intermediates 9
2.2.1.3. Osmium-catalysed or $KMnO_4$-mediated dihydroxylation 14
2.2.2. Conversion of other functional groups 15
3. RESULTS AND DISCUSSION **19**
3.1. Novel cyclic mono- and dihydroxy-substituted alicyclic β-amino acids 19
3.1.1. Syntheses of the starting compounds 19
3.1.2. Monohydroxy derivatives via epoxide rings 24
3.1.3. Monohydroxy-substituted cyclopentane- and cyclooctane-β-amino acids via oxazoline or iodolactone intermediates 26
3.1.4. Dihydroxy-substituted cyclopentane-, cyclohexane- and cyclooctane-β-amino acids 30
3.2. High-performance liquid chromatography of 2-aminomono- and dihydroxy-cyclopentanecarboxylic acids and 2-aminodihydroxycyclohexanecarboxylic acids 37
3.3. Methods 38
4. SUMMARY **41**
5. REFERENCES **44**
6. ACKNOWLEDGEMENTS **49**
ANNEX **50**
TABLE OF THE SYNTHESIZED COMPOUNDS **51**

ABBREVIATIONS AND SYMBOLS

Ac	acetyl
AcCl	acetyl chloride
ACHC	2-aminocyclohexanecarboxylic acid
ACPC	2-aminocyclopentanecarboxylic acid
AIBN	azobis(isobutyronitrile)
Boc	*tert*-butoxycarbonyl
Bz	benzoyl
CSA	(1*R*)-(–)-10-camphorsulfonic acid
CSI	chlorosulfonyl isocyanate
CSPs	chiral stationary phases
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
ee	enantiomeric excess
Fmoc	9-fluorenylmethoxycarbonyl
Ipc_2BH	diisopinocamphenyl borane
KHMDS	potassium hexamethyldisilylazide dimer
Lipolase	lipase B from *Candida antartica*
mCPBA	*m*-chloroperbenzoic acid
Ms	mesyl
MsCl	methane sulfonyl chloride
MW	microwave
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
ORTEP	Oak Ridge thermal ellipsoid plot program
PLE	pig liver esterase
TBAF	tetrabutylammonium fluoride
TfO_2	trifluoromethanesulfonyl anhydride
Z	carboxybenzyloxy
ZCl	benzyl chloroformate

PUBLICATIONS

Papers related to the thesis

I. **Gabriella Benedek**, Márta Palkó, Edit Wéber, Tamás A. Martinek, Enikő Forró, Ferenc Fülöp:
Efficient synthesis of hydroxy-substituted cispentacin derivatives
Eur. J. Org. Chem. **2008**, 3724–3730.

II. **Gabriella Benedek**, Márta Palkó, Edit Wéber, Tamás A. Martinek, Enikő Forró, Ferenc Fülöp:
Efficient synthesis of 3,4- and 4,5-dihydroxy-2-aminocyclohexanecarboxylic acid enantiomers
Tetrahedron: Asymmetry **2009**, *20*, 2220-2225.

III. Márta Palkó, **Gabriella Benedek**, Enikő Forró, Edit Wéber, Mikko Hänninen, Reijo Sillanpää, Ferenc Fülöp:
Synthesis of mono- and dihydroxy-substituted 2-aminocyclooctanecarboxylic acid enantiomers
Tetrahedron: Asymmetry **2010**, *21*, 957-961.

IV. Róbert Berkecz, István, Ilisz, **Gabriella Benedek**, Ferenc Fülöp, Daniel W. Armstrong, Antal Péter:
High-performance liquid chromatographic enantioseparation of 2-aminomono- and dihydroxycyclopentanecarboxylic and 2-aminodihydroxy-cyclohexanecarboxylic acids on macrocyclic glycopeptide-based phases
J. Chromatogr. A. **2009**, *1216*, 927-932.

Conference lectures

V. **Benedek Gabriella:**
Ciklopenténvázas β-aminosav-származékok diasztereo- és régioszelektív hidroxilálása
„A Szegedi Ifjú Szerves Kémikusok Támogatásáért" Alapítvány tudományos előadóülése,
Szeged, 2007. január 17.

VI. **Gabriella Benedek**, Márta Palkó, Loránd Kiss, Tamás A. Martinek, Ferenc Fülöp:
Efficient syntheses of hydroxy-substituted β-aminocyclopentanecarboxylic acids
Blue Danube Symposia on Heterocyclic Chemistry (BDSCH) 12,
June 10-13, 2007, Tihany, Hungary, Abstr.: P-06.

VII. **Benedek Gabriella:**
Ciklopenténvázas β-aminosav-származékok diasztereo- és régioszelektív hidroxilálása
Magyar Tudomány ünnepe – PhD hallgatóink eredményei
Szeged, 2007. november 6.

VIII. **Benedek Gabriella**, Palkó Márta, Wéber Edit, Martinek A. Tamás, Forró Enikő, Fülöp Ferenc:
Hidroxilált ciszpentacin származékok előállítása
MKE Vegyészkonferencia
Hajdúszoboszló, 2008. június 19-21., Abstr.: P-02.

IX. **Gabriella Benedek**, Márta Palkó, Edit Wéber, Tamás A. Martinek, Enikő Forró, Ferenc Fülöp:
Efficient syntheses of hydroxy-substituted cispentacin derivatives
XXIIIrd European Colloquium on Heterocyclic Chemistry
September 9-13, 2008, Antwerp, Belgium, Abstr.: P-22.

X. **Gabriella Benedek**, Márta Palkó, Edit Wéber, Tamás A. Martinek, Enikő Forró, Ferenc Fülöp:
Efficient syntheses of hydroxy-substituted β-aminocyclohexanecarboxylic acids
COST Action CM0803, Foldamers: building blocks, structure and function
September 24-26, 2009, Szeged, Hungary, Abstr.: P-05.

1. INTRODUCTION AND AIMS

Although the β-amino acids[1-3] derived from β-lactams[4,5] are less abundant than their α-counterparts, they have captured the interest of a number of synthetic research groups as a consequence of their useful biological effects and occurrence in many pharmacologically relevant compounds. These compounds are to be found in natural products, *e.g.* cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, an antifungal antibiotic, and amipurimycin, which also contains the *cis*-2-aminocyclopentane-carboxylic acid moiety, isolated from *Streptomyces novoguineensis*, which is strongly active both *in vitro* and *in vivo* against *Pyricularia oryzae*, responsible for rice blast disease.[6,7-10] The synthetic 4-methylene derivative of cispentacin (Icofungipen, PLD-118, BAY 10-8888) is active *in vitro* against *Candida species* and is currently advancing through clinical development for the treatment of yeast infections (Figure 1).[11-16] These molecules are also useful as starting materials, *e.g.* anatoxin-a,[17-20] a nicotinic acetylcholine receptor agonist, was efficiently synthesized from β-lactam.[21]

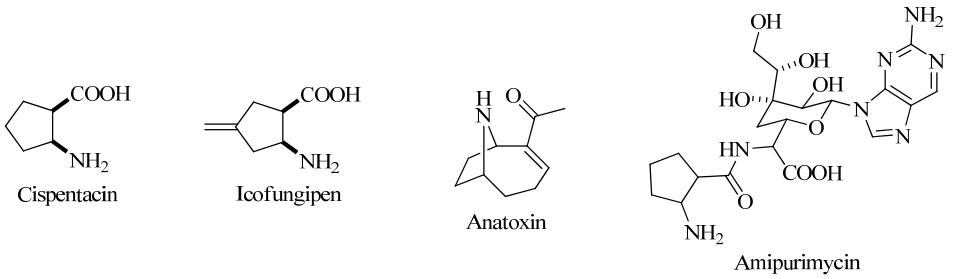
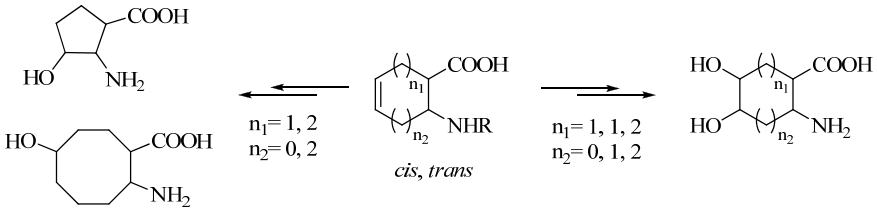


Figure 1

In view of the growing importance of β-amino acid derivatives, my PhD work had the major aim of developing simple and efficient routes for the preparation of new hydroxy-functionalized alicyclic β-amino acids.

The preparative work was focused on preparing new mono- and dihydroxy-substituted cyclopentane, cyclohexane and cyclooctane β-amino acid derivatives, starting from β-lactam via amino acids and aminocarboxylates, exploiting the reactivity of the ring double bond [I, II, III, IV] (Scheme 1).



Scheme 1

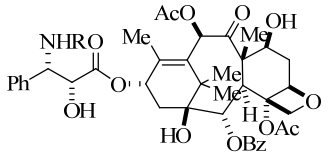
A further aim was to study the stereo- and regioselectivity of the different approaches followed, based on the formation of bromooxazoline or iodolactonization and diastereoselective OsO_4-catalysed dihydroxylation of esters.

The publications on which the thesis is based are referred to in square brackets, while other references are given as superscripts.

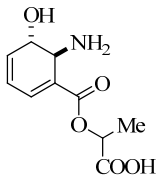
2. LITERATURE

2.1. Hydroxy-β-amino acids

Hydroxylated β-amino acids have captured interest as a consequence of their possible exploitation in many fields of medicinal and pharmaceutical chemistry. The importance of these molecules resides in their biologically interesting properties. They are integral parts of many naturally occurring, pharmaceutically active compounds.[22] In this connection, paclitaxel (Taxol®) and docetaxel (Taxotere®) are effective chemotherapeutic agents against breast and ovarian cancers.[22-24] Illustrative examples are the aminopeptidase inhibitors amastatin[25] and bestatin,[26-28] the renin inhibitor KRI-1314,[29] the angiotensin-converting enzyme inhibitory migroginins[30] and the antibacterial agent dideoxy-kanamycin A.[31] Although of less biological importance than their open-chain analogues, some cyclic hydroxylated β-amino acid derivatives have antibiotic (oryzoxymycin)[32-35] or antifungal[36] activities and are building blocks for pharmaceutically important natural substances (Figure 2).[37]



R=Bz; Taxol
R=CO_2tBu; Taxotere



Oryzoxymycin

Figure 2

In recent years, the importance and preparation of β-amino acids, in either racemic or enantiopure form, has come into the foreground of interest, because of their widespread use in peptides, heterocyclic and combinatorial chemistry and drug research.[38-43] β-Amino acids and especially their cyclic counterparts, can serve as useful building blocks for peptides which may have modified, and even increased biological activities. Their resistance to enzymatic degradation can lead to peptide-based synthetic targets.[44] Woll *et al*. studied the structures of β-peptides built up from *trans*-2-aminocyclopentanecarboxylic acid monomers hydroxy- or amino-substituted at position 3. These structures assumed a robust 12-helix, like the β-peptides formed from monomers without functional groups at position 3 of the ring.[45] This allows the

conclusion that functionalized β-peptides built up from substituted β-amino acid derivatives are appropriate non-natural polymers which are able to form well-defined secondary structures for biological applications.

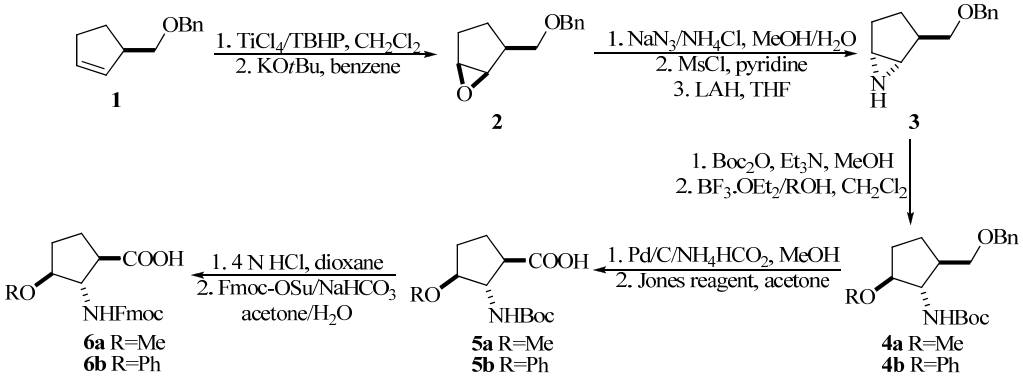
2.2. Synthesis of cyclic hydroxy-β-amino acids

The extended use of cyclic hydroxy-β-amino acid derivatives as building blocks for peptidomimetics has led to the conclusion that there is a need for efficient, new synthetic protocols for the preparation of racemic and enantiopure starting materials.[37]

2.2.1. Reactions of the olefinic bond

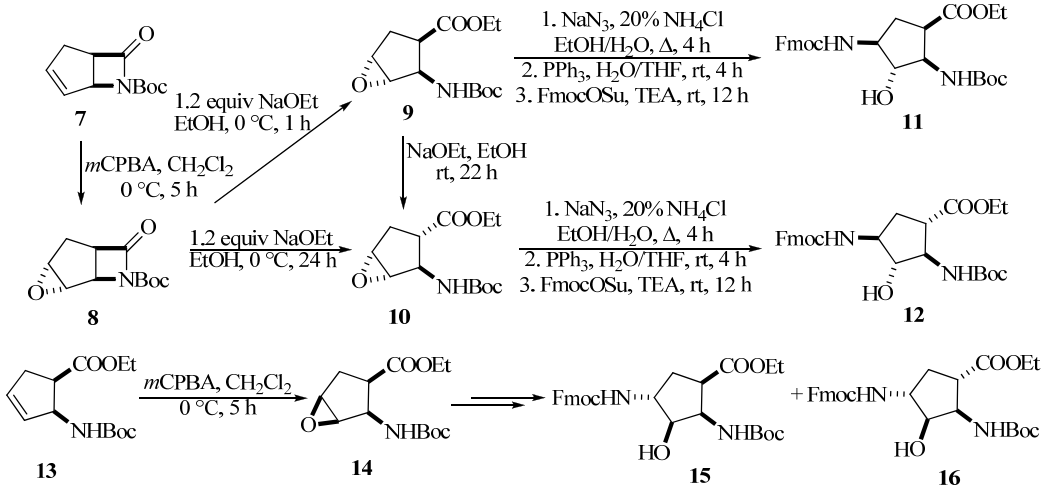
2.2.1.1. Opening of the oxirane ring

Woll *et al.* prepared *trans*-2-aminocyclopentanecarboxylic acid (*trans*-ACPC) enantiomers hydroxy-functionalized at the position 3 in order to introduce them into peptides.[45] Transformation of alkene **1** to *cis* epoxide **2** by Sharpless chloro-hydroxylation and reaction with NaN_3, followed by alcohol activation with MsCl, yielded a 2:3 regioisomeric mixture of azido-mesylates, which was converted to **3** by azide reduction and ring closure. Intermediates **4a** and **4b** were obtained by ring opening with BF_3 and an alcohol. Removal of the benzyl protecting group, followed by oxidation and change of the Boc protecting group to Fmoc, afforded β-amino acid derivatives **6a** and **6b** for solid-phase synthesis (Scheme 2).



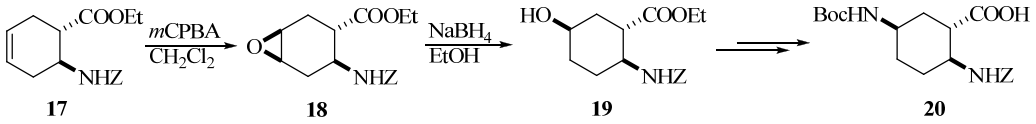
Scheme 2

An epoxidation protocol[46] was used by Kiss *et al.* to prepare hydroxy-functionalized, orthogonally protected 2,4-diaminocyclopentanecarboxylate diastereomers containing both the β- and the γ-amino ester unit on a cyclopentane moiety (Scheme 3).[47] Epoxidation and ring opening of the *N*-protected azetidinone **7** with *m*CPBA in CH_2Cl_2 resulted in a *trans* oxirane ring (**9**, **10**) relative to the 2-amino group, while in the case of *N*-protected ester **13**, where the *cis*-stereodirecting effect predominates, it led to the *cis*-epoxy amino ester **14**, which can be interpreted in terms of hydrogen bonds between the amide and the *m*CPBA in the transition state.[48] Epoxides **9**, **10** and **14** were refluxed in EtOH/H_2O with NaN_3 in the presence of a catalytic amount of NH_4Cl. The attack of the azido group occurred at position 4 from the less hindered face of the cyclopentane ring. Following reduction of the azido function with PPh_3 and protection of the amino group, the hydroxy-substituted *N*-protected diaminocarboxylates **11**, **12**, **15** and **16** were obtained.



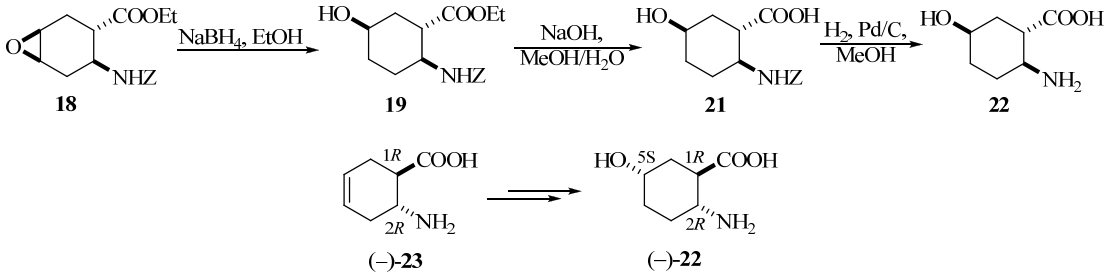
Scheme 3

The epoxidation protocol was also applied with a slight modification to prepare the protected 2,5-diaminocyclohexanecarboxylic acid **20** via 2-amino-5-hydroxy-cyclohexanecarboxylic acid derivative **19** (Scheme 4).[49] The starting epoxide **18** was prepared by *m*CPBA-mediated epoxidation, where the attack on the olefinic bond occurred from the side of the protected amino group.[46] The selectivity can be interpreted by the stereodirecting effect of the Z-protected amino group. Reductive opening of the oxirane ring with $NaBH_4$/EtOH at room temperature regioselectively afforded only **19**, in which the hydroxy group is on position 5 of the cyclohexane ring.



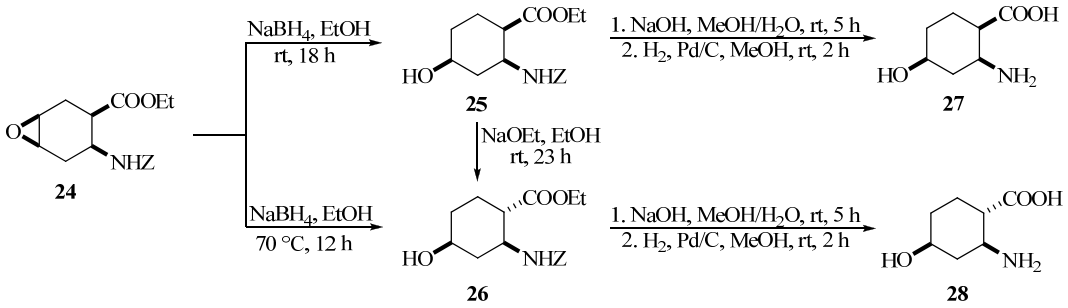
Scheme 4

The same research group reported a procedure for the synthesis of hydroxy-functionalized cyclohexane-β-amino acids, both in racemic and in optically pure form, using the previously mentioned synthetic routes (Scheme 5).[50]



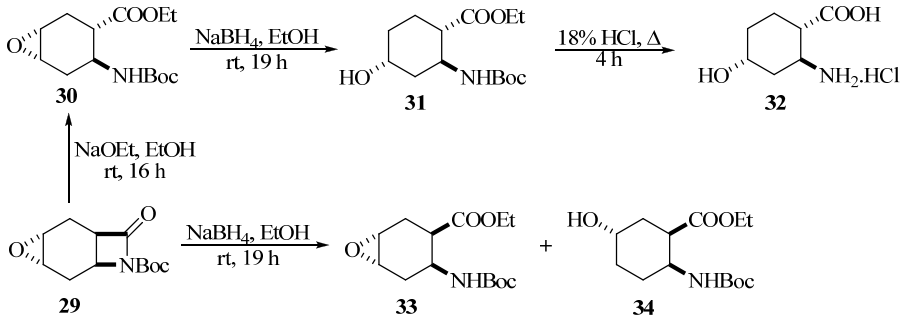
Scheme 5

A simple method for the preparation of hydroxy-functionalized β-amino-cyclohexane-carboxylic acid derivatives was investigated by Kiss *et al*.[51] By means of the epoxidation protocol, the epoxides **24** and **29** were prepared (Schemes 6 and 7). Reductive opening of the oxirane ring at room temperature gave the *all-cis*-hydroxy derivative **25**, whereas at 70 °C isomerization at C-1 was observed, resulting in hydroxy derivative **26**. The formation of 4-hydroxy-substituted *cis*-amino ester **25** from **24** probably involves hydride attack from the sterically less hindered side of the cyclohexane skeleton. Since the oxirane ring opening reaction by hydride is faster than the isomerization at C-1, the same explanation is valid for the formation of hydroxy derivative **26**. Cleavage of the protecting groups gave 2-amino-4-hydroxy-cyclohexanecarboxylic acid derivatives **27** and **28** (Scheme 6).



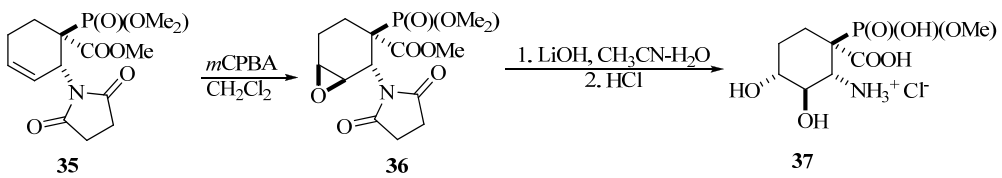
Scheme 6

Starting from *N*-Boc-protected lactam **29**, using the above methods, the hydroxy group was introduced *trans* relative to the amino group on position 4 or 5 (**32** and **34)** (Scheme 7).



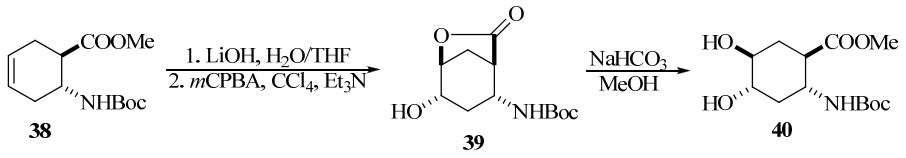
Scheme 7

In 2001, Defacqz *et al.* reported a method for the preparation of new hydroxy-functionalized aminophosphonic acid derivatives.[52] Compound **37** was obtained by treatment of **35** with *m*CPBA in CH_2Cl_2. Epoxide **36** was transformed to 1-methoxyphosphoryl-2-amino-3,4-dihydroxycyclohexane-1-carboxylic acid hydrochloride (**37**) by LiOH-mediated opening of the oxirane ring, followed by acidic hydrolysis of the protecting groups (Scheme 8). The final product **37** exhibits *trans* orientation between the OH groups on positions 3 and 4 due to the favourable hydrogen-bonding interactions between OH(C-4) and NH_3(C-2), and OH(C-3) and P=O(C-1).



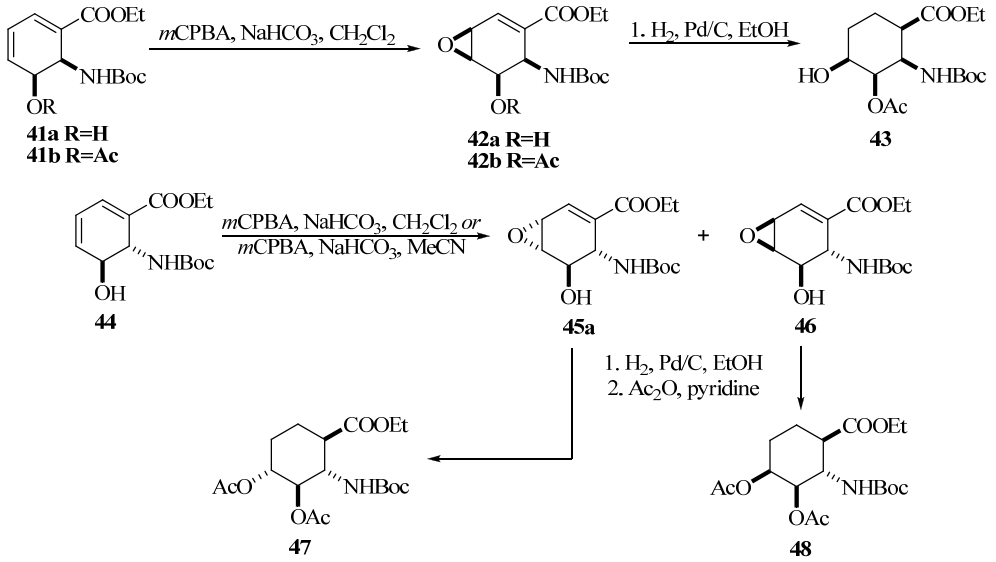
Scheme 8

Methyl (1*R*,2*R*,4*S*,5*S*)-2-*tert*-butoxycarbonylamino-4,5-dihydroxycyclohexane-carboxylate (**40**) was prepared by Wipf and Wang.[46] Epoxidation of *trans*-2-amino-cyclohex-4-enecarboxylate **38** with *m*CPBA and *in situ* cyclization of the epoxy carboxylate resulted in hydroxy lactone **39**. This was methanolysed to obtain the tetrasubstituted cyclohexane derivative **40**, in which the OH groups are *trans* to one another (Scheme 9).



Scheme 9

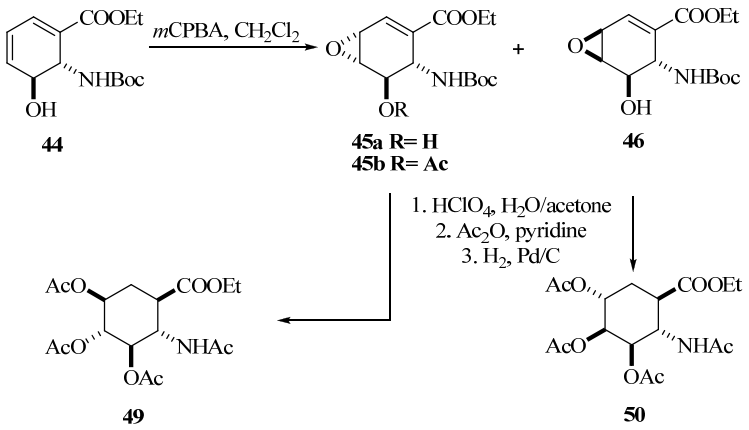
3,4-Dihydroxy ACHC derivatives were prepared from protected esters **41a**,**b** and **44** through simple substrate-stereocontrolled operations by epoxidation.[53] For **41a** and **41b**, the reaction with *m*CPBA was highly stereoselective and afforded the epoxide on the same side as the carbamate and the hydroxy groups. Reduction furnished the *all-cis* isomer **43** as the only final product. In the case of **44**, under the same conditions the reaction resulted in two isomeric epoxides, **45a** and **46**, in a ratio of 9:1, with the major isomer arising from attack from the same face as the Boc carbamate. When the solvent was changed from CH_2Cl_2 to MeCN, the yields changed to a ratio of 1:2 (Scheme 10).



Scheme 10

Reductive opening of the epoxides and addition of the hydride in the presence of Pd-C under a hydrogen atmosphere gave dihydroxy derivatives **47** and **48** (Scheme 10). In the case of **42b**, the addition occurs on the less hindered diastereo face of the alkene, and in the second reaction (**45a** and **46**) on the more hindered side.[53]

The same research group prepared 4,5-*trans* diols, starting from diene **44**. Reaction with *m*CPBA in CH_2Cl_2 afforded a 9:1 mixture of epoxides **45a**:**46**.[53,54] Acetylation of the hydroxy group raised the selectivity and resulted in epoxide **45b** as a single isomer. Treatment of epoxides **45a** and **46** with aqueous $HClO_4$ resulted in trihydroxylated cyclohexyl-β-amino acid derivatives **49** and **50** (Scheme 11).

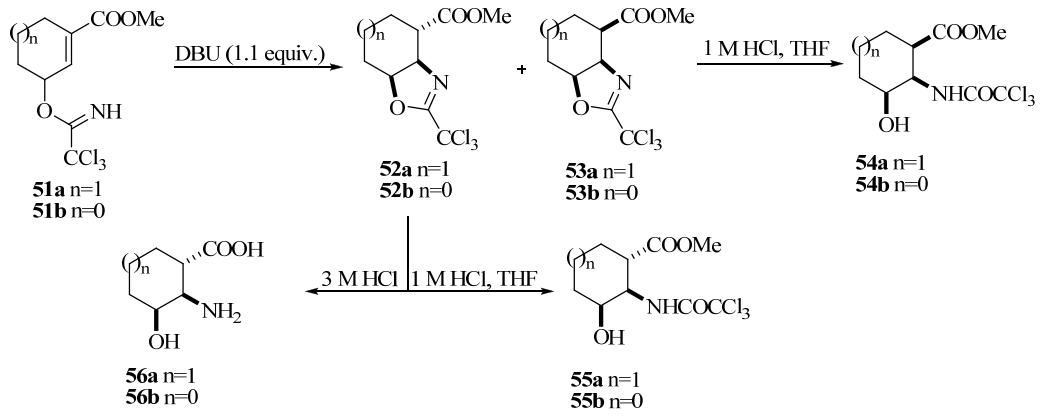


Scheme 11

2.2.1.2. Oxazoline, oxazine or iodolactone intermediates

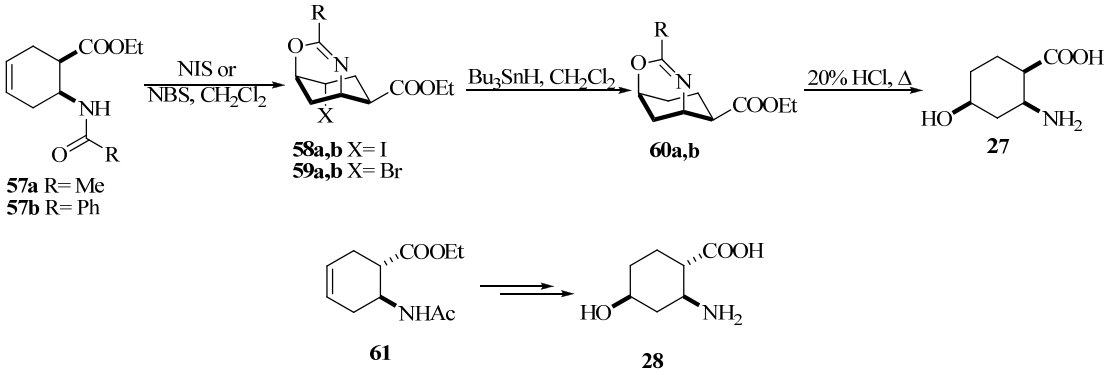
Matsushima and Kino recently developed a novel route for the preparation of 3-hydroxy-substituted ACHC and ACPC and their derivatives.[55] The subsequent conjugate addition of trichloroacetimidates **51a** and **51b** (Scheme 12) with the stoichiometric amount of DBU in MeCN afforded the cyclized oxazoline products **52a,b** and **53a,b** in moderate yields. The stereochemistry was confirmed by NOE analysis.

Treatment with 1 M HCl in THF cleaved the oxazoline ring and resulted in methyl 2-trichloroacetamido-hydroxycycloalkanecarboxylates **54a,b** and **55a,b**. The methyl ester groups and oxazoline rings of **52a** and **52b** were hydrolysed with 3 M HCl to furnish the free hydroxy-substituted amino acid derivatives **56a,b**.



Scheme 12

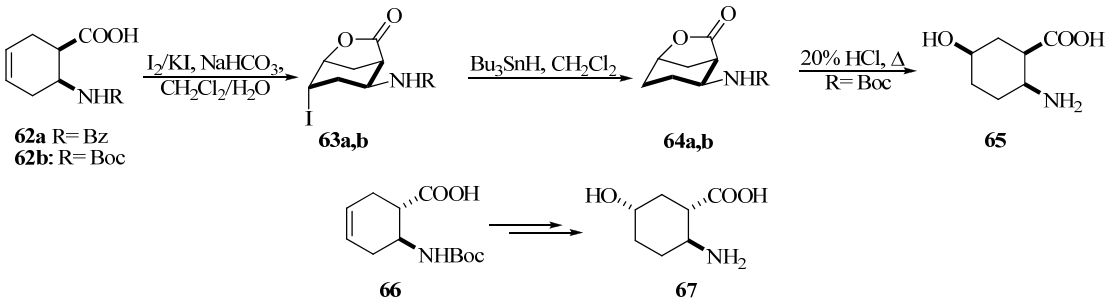
In 2005, a synthetic route was reported for the preparation of hydroxylated *cis*- and *trans*-2-amino-4-cyclohexanecarboxylic acid derivatives via 1,3-oxazine or γ-lactone intermediates.[56,57] The halofunctionalization[58] of double bonds in a cyclic system is a simple strategy that proceeds under high regio- and stereocontrol, as shown in Scheme 13. The reaction of **57a,b** with NIS or NBS furnished oxazines **58a,b** and **59a,b**. Subsequent dehalogenation with Bu_3SnH under Ar and further acidic hydrolysis with 20% aqueous HCl resulted in (1*R**,2*S**,4*S**)-2-amino-hydroxycyclohexane-carboxylic acid (**27**) (Scheme 13). Via the same synthetic route, starting from *trans*-2-acetylamino-cyclohex-4-enecarboxylic acid (**61**), (1*S**,2*S**,4*S**)-2-amino-4-hydroxycyclohexanecarboxylic acid (**28**) was prepared.



Scheme 13

2-Amino-5-hydroxycarboxylic acids **62** and **67** were prepared by stereoselective iodolactonization. Iodolactones **63a,b** were obtained with excellent regio- and diastereoselectivity from **62a,b** in a two-phase reaction with I_2/KI/$NaHCO_3$ in CH_2Cl_2/H_2O. After dehalogenation and hydrolysis by the above-mentioned method,

2-amino-5-hydroxycyclohexanecarboxylic acid **65** was obtained. Isomer **67** was also prepared by the same reaction route (Scheme 14).

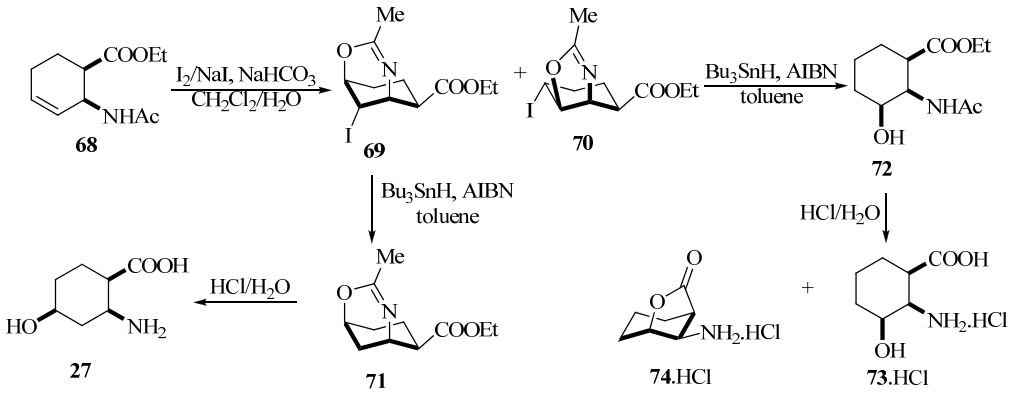


Scheme 14

The given stereochemistry and the relative configurations of the compounds were proved by using vicinal couplings and characteristic NOEs (Scheme 14).

Novel pathways were developed for the synthesis of 4-hydroxy- (**27**) and 3-hydroxy-β-amino acids (**73**.HCl, **73**.HBr) via iodooxazine, iodooxazoline or iodolactone intermediates (Schemes 15 and 16) by Szakonyi *et al.*[59] The method was applied for the enantiopure compounds too.

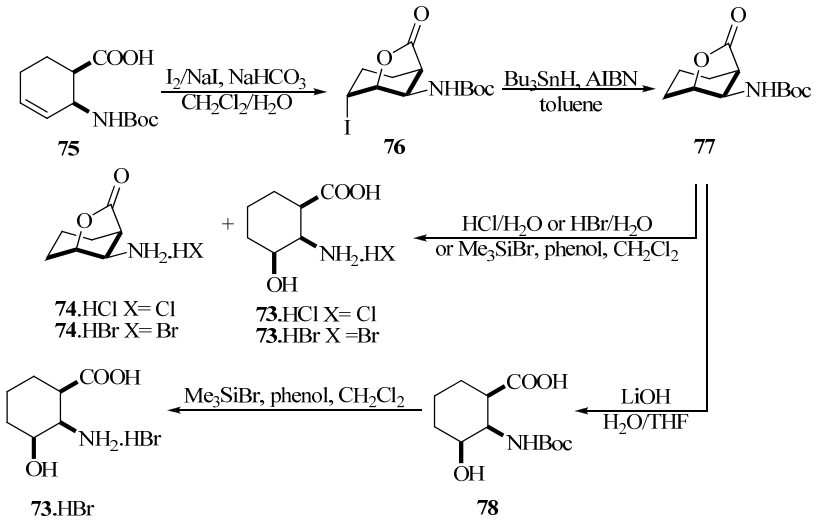
The iodocyclization of *N*-acetylamino ester **68** was accomplished with NaI, I_2 and $NaHCO_3$ in a two-phase solvent system. The reaction was not selective and resulted in 30:70 mixtures of iodooxazine **69** and iodooxazoline **70**. They were separated and deiodinated with Bu_3SnH in the presence of a catalytic amount of AIBN under N_2. Hydrolysis of oxazine **71** under acidic conditions resulted in 4-hydroxyamino acid **27** (Scheme 15).



Scheme 15

Deiodination of iodooxazoline **70** gave only the ring-opened product **75**, which, after hydrolysis, yielded a mixture of 3-hydroxyamino acid hydrochloride **73**.HCl and amino lactone hydrochloride **74**.HCl (Scheme 15).

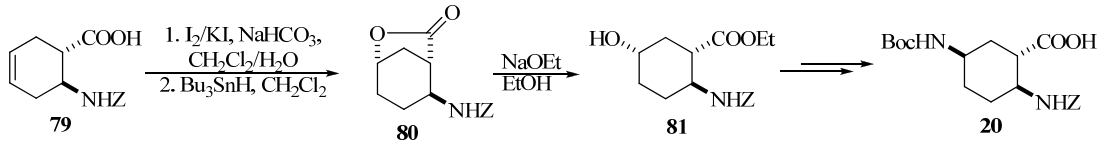
Focus was next placed on the iodolactonization protocol (Scheme 16). Iodolactonization of *N*-Boc amino acid **75** was achieved under the same reaction conditions as for iodooxazine **69** and iodooxazoline **70**. In this case, only the five-membered iodolactone was obtained, although the chances of a five- or six-membered lactone ring being formed are the same. Dehalogenation followed by ring opening with different acidic reagents resulted in hydroxyamino acid salts **73**.HCl and **73**.HBr, together with amino lactone salts **74**.HCl and **74**.HBr.



Scheme 16

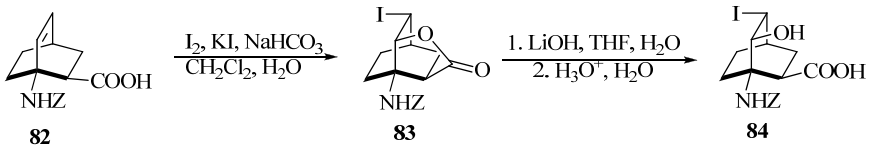
When the hydrolysis was carried out with aqueous LiOH in THF, the *N*-Boc-hydroxyamino acid was obtained selectively (Scheme 16). After deprotection with Me_3SiBr and phenol, *all-cis*-2-amino-3-hydroxycyclohexanecarboxylic acid hydrobromide (**73**.HBr) was obtained.

The iodolactonization[37] protocol was applied with a slight modification to prepare protected 2,5-diaminocyclohexanecarboxylic acid **20** via 2-amino-5-hydroxy-cyclohexanecarboxylic acid derivative **81** (Scheme 17).[49]



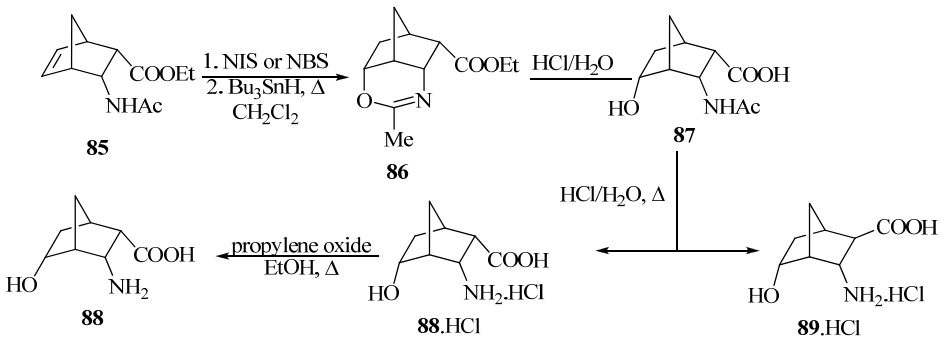
Scheme 17

Songis *et al.* reported a method for the preparation of enantiopure iodo hydroxy-β-amino acid derivative via the iodolactone.[60] The starting material was prepared by asymmetric Diels-Alder cycloaddition[61] and reacted in a biphasic mixture of I_2/KI under alkaline conditions to result in the iodolactone **83**, which was treated with LiOH in a THF/H_2O mixture to give the enantiopure iodo-hydroxy-β-amino acid **84** in good yield (Scheme 18).



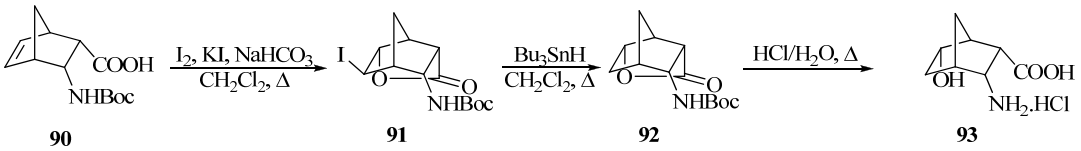
Scheme 18

Using NIS- and NBS-mediated cyclization, Palkó *et al.* prepared 3-amino-6-hydroxybicyclo[2.2.1]heptane-2-carboxylic acid epimers **88**.HCl and **89**.HCl (Scheme 19).[62] The structures of the hydroxy-functionalized compounds were proved by NMR after chemical transformations.



Scheme 19

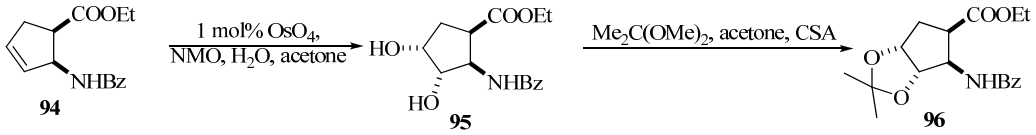
The *all-endo*-3-amino-6-hydroxybicyclo[2.2.1]heptane-2-carboxylic acid (**93**) was also prepared stereoselectively by iodolactonization (Scheme 20).[62]



Scheme 20

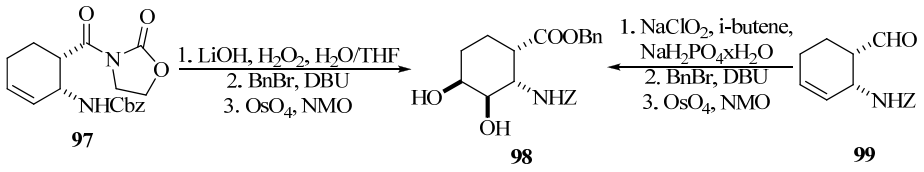
2.2.1.3. Osmium-catalysed or $KMnO_4$-mediated dihydroxylation

In 2003, Coldham *et al.* reported a method for the preparation of dihydroxy-cyclopentanecarboxylates.[63] *N*-Benzoyl derivative **94** was reacted with a catalytic amount of OsO_4 and NMO to give a single diol product **95**, which was transformed to the acetonide **96**. The relative stereochemistry of the acetonide was confirmed by NOESY studies (Scheme 21).



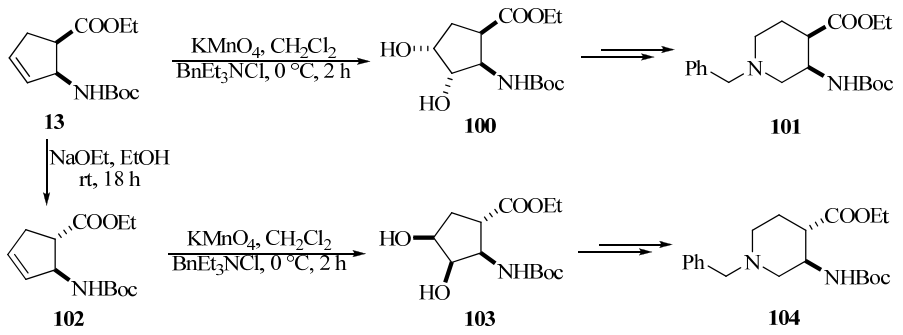
Scheme 21

Wipf and Wang reported an efficient approach towards dihydroxylated *cis*-2-aminocyclohexanecarboxylate derivative **98**.[46] Compound **97** was prepared by Kobayashi's chiral scandium-catalysed, Lewis acid-mediated Diels-Alder reaction.[64] Saponification of **97** and transesterification, followed by dihydroxylation with OsO_4, gave the desired diol **98**. An alternative route was the nucleophile-catalysed Diels-Alder reaction with phenylalanine-derived imidazolidinone.[65] From the intermediate **99**, after oxidation, esterification and dihydroxylation, diol **98** was obtained (Scheme 22).



Scheme 22

Kiss *et al.* reported a method for $KMnO_4$-induced dihydroxylation of the cyclopentene skeleton in the presence of $BnEt_3NCl$ as phase-transfer catalyst (Scheme 23).[66-68] The two hydroxy groups are formed on the side opposite to the carboxylate on C-1 (**100** and **103**). The reactions were carried out on both racemic and enantiopure starting materials.

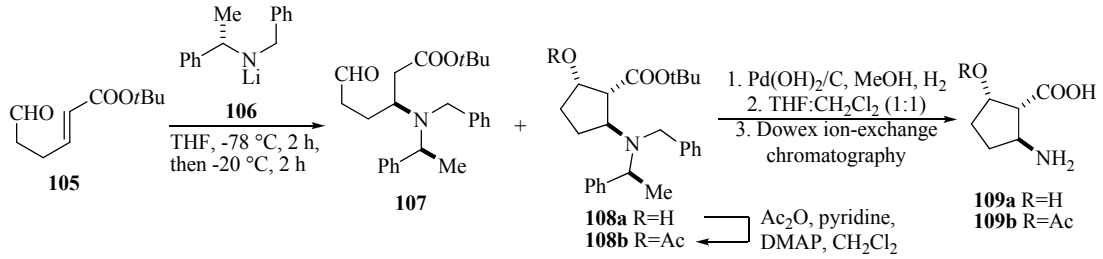


Scheme 23

2.2.2. Conversion of other functional groups

Davies *et al.* reported a method for the synthesis of hydroxy-substituted transpentacin and transhexacin and their derivaties via lithium amide-promoted tandem asymmetric conjugate addition-cyclization reactions.[69]

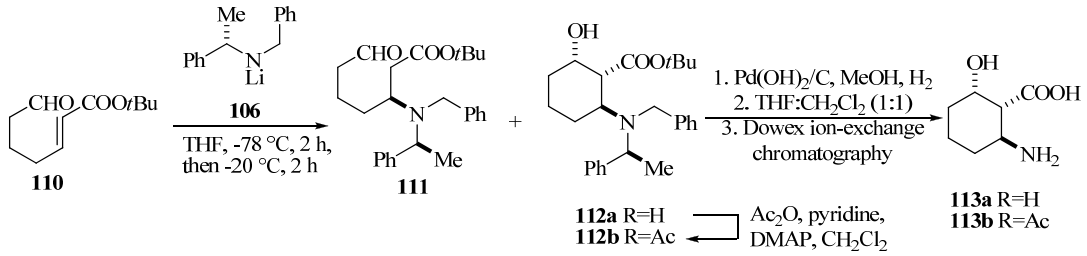
Addition of lithium amide **106** to *tert*-butyl-(*E*)-6-oxohex-2-enoate (**105**) gave a 9:91 mixture of β-amino-ε-aldehyde ester **107** and 1,2-*syn*-1,5-*anti*-cyclic β-amino ester **108a** (Scheme 24).



Scheme 24

The same cyclization method was extended to lithium amide addition to *tert*-butyl-(*E*)-7-oxohept-2-enoate (**110**) at low temperature to result in a 27:73 mixture of β-amino ester **111** and cyclic 1,2-*syn*-1,6-*anti*-β-amino ester **112a**, in > 98% de (Scheme 25).

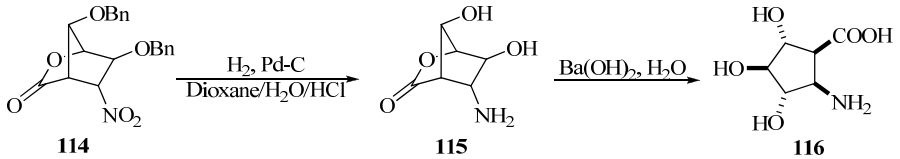
The cyclic products were presumably prepared through chair (six-membered ring) or envelope (five-membered ring) transition states.



Scheme 25

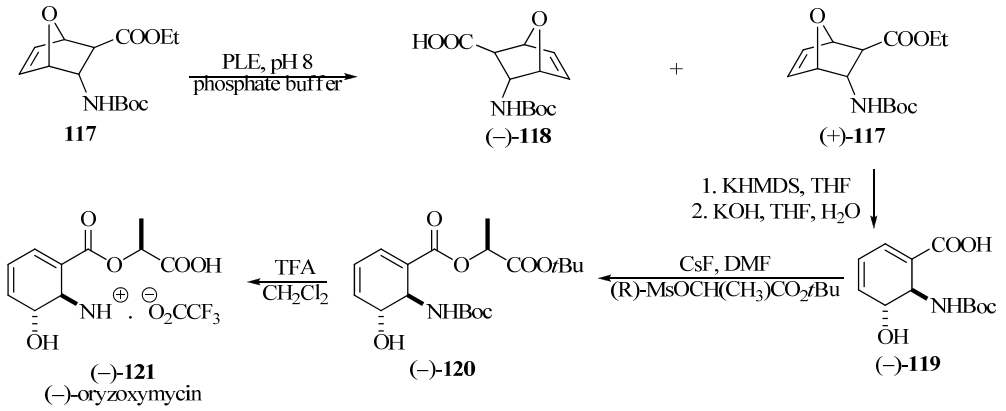
Finally, deprotection of cyclic β-amino esters **108a,b** and **112a,b** by hydrogenolysis, followed by ester hydrolysis and purification by Dowex ion-exchange chromatography, resulted in 2-hydroxy- and 2-acetoxy-transpentacin **109a,b** and 2-hydroxy- and 2-acetoxy-transhexacin **113a,b** (Schemes 24 and 25).

Soengas *et al.* reported a method for the synthesis of (1*S*,2*R*,3*S*,4*S*,5*R*)-3,4,5-trihydroxy-2-aminocyclopentanecarboxylic acid (**116**) (Scheme 26).[70] Nitrolactone **114** was obtained from L-idose in nine steps and was then transformed to aminolactone **115** by hydrogenation to yield finally the desired amino acid **122** after ring opening.[71]



Scheme 26

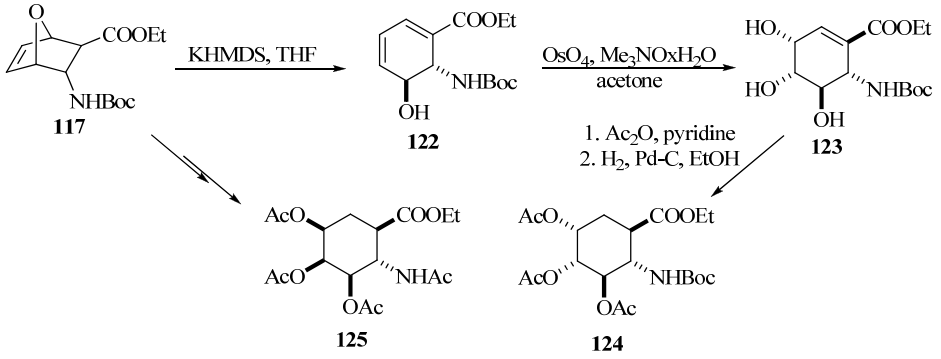
In 2003, Bunnage *et al.* described a short route for the asymmetric synthesis of the enantiomer of natural oryzoxymycin ((+)-oryzoxymycin), the structure of which was determined by spectroscopic, analytical and degradation studies (Scheme 27).[32-34,35] Kinetic resolution of protected amino ester **117** with PLE in pH 8 phosphate buffer/Et_2O afforded chiral ester (+)-**117**.[72,73] Hydrolysis and reaction with mesylate, followed by deprotection with TFA, provided the enantiomer of natural oryzoxymycin as a TFA salt (-)-**121**. Comparison of the IR, NMR and optical rotation data of the natural and synthetic oryzoxymycins revealed significant differences. The interpretation of these findings is still in progress.



Scheme 27

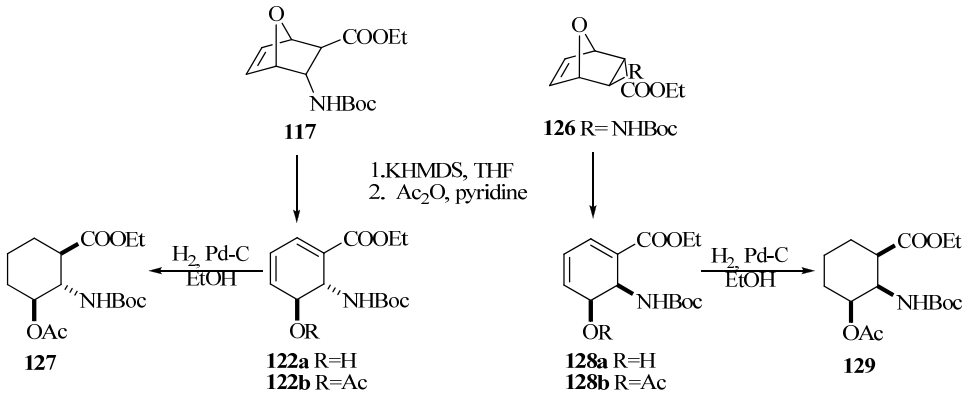
Routes for the stereoselective synthesis of tetraacetoxylated 2-aminocyclohexanecarboxylate were reported by Masesane and Steel.[54] Starting from oxabicyclic ester **117**,[35] treatment with KHMDS provided the hydroxy-substituted dihydroanthranilate ester **122a**. Dihydroxylation with a catalytic amount of OsO_4, peracylation and reduction resulted in tetraacetoxylated derivative **124** (Scheme 28). The new hydroxy groups were formed on the same face as the carbamate and on the face opposite the free allylic hydroxy group.[74]

To obtain the isomer with all the hydroxy groups on the same face (**125**), oxanorbornenyl carbamate **117** was subjected to OsO_4-mediated dihydroxylation.



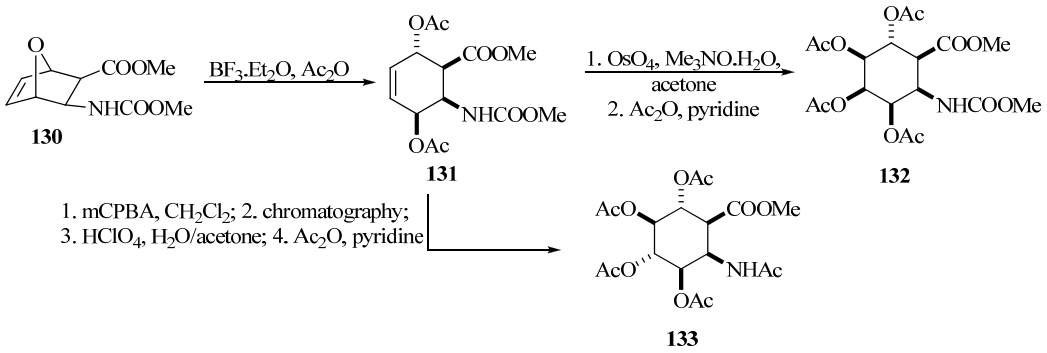
Scheme 28

A new route for the preparation of 3-hydroxy derivatives of 2-aminocyclohexanecarboxylic acids was reported by Masesane and Steel (Scheme 29).[53] First, β-elimination of the oxygen bridge gave dihydroanthranilates **122a** and **128a**. Acylation and reduction of acetates **122b** and **128b** with H_2 over Pd-C afforded 3-hydroxy ACHC derivatives **127** and **129**.



Scheme 29

In 2008, Chola and Masesane described a stereoselective synthesis of racemic 3,4,5,6-tetraacetoxycyclohexyl-β-amino acid derivatives.[75] Opening of oxabicyclic adduct **130** with the Lewis-acid $BF_3.Et_2O$ in the presence of nucleophilic acetic anhydride and *syn*-selective OsO_4-mediated dihydroxylation[74] followed by acylation afforded **132**. An alternative oxygenation route[53] involved epoxidation of the prepared dihydroxy derivative with *m*CPBA, followed by acid-catalysed opening of the epoxide to give 2-amino-3,4,5,6-tetraacetoxycyclohexanecarboxylic acid derivative **133** (Scheme 30).



Scheme 30

3. RESULTS AND DISCUSSION

3.1. Novel cyclic mono- and dihydroxy-substituted alicyclic β-amino acids

Methods have recently been published for the introduction of a monohydroxy functionality onto the cyclohexane ring, *e.g.* via dihydrooxazine or oxazoline derivatives or by iodolactonization of *cis*- and *trans*-2-aminocyclohex-3-enecarboxylic acids and *cis*- and *trans*-2-aminocyclohex-4-enecarboxylic acids.[56,59,60] Another method involves hydroxylation of 2-aminocyclohex-4-enecarboxylic acid by functionalization of the double bond through epoxidation or by applying the same method for the preparation of 2,4-diaminocyclopentanecarboxylate diastereomers with a hydroxy moiety on position 3 of the cyclopentane ring.[47-50] The osmium-catalysed dihydroxylation of olefins is one of the most efficient and useful methods for the preparation of 1,2-diols.[67,76-78]

Despite their promising role in peptide chemistry and in drug research, there are only few examples in the literature on mono- and dihydroxylated 2-aminocyclopentane-carboxylic acid derivatives or dihydroxycyclohexanecarboxylic acids, and no example of mono- or dihydroxycyclooctane β-amino acids.[15,67]

In the frame of the work described in this thesis, we investigated epoxidation, halolactonization, halocyclofunctionalization and dihydroxylation as appropriate mono- and dihydroxylation synthetic protocols.

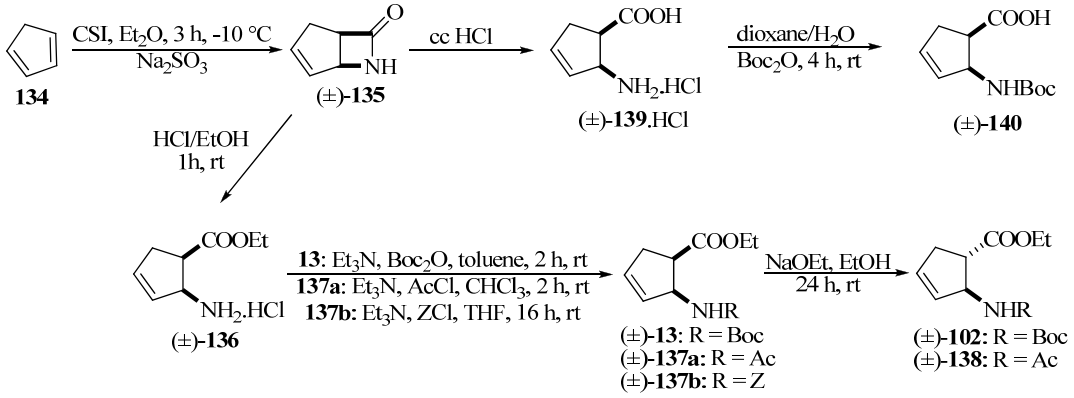
3.1.1. Syntheses of the starting compounds

The starting materials were prepared in both racemic and enantiomeric form by using literature procedures with efficient modifications designed to optimize the synthetic methods.

In the case of cyclopentene starting compounds, the well-known 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate and cyclopentadiene (**134**) was used to prepare the diastereomerically pure β-lactam (±)-**135** by a literature method with some modifications. In contrast with the known method, CSI in dry Et_2O was added to a solution of freshly distilled cyclopentadiene (**134**) and dry Et_2O. Hydrolysis of the sulfonyl chloride group of the cycloadduct with Na_2SO_3 in the presence of KOH gave

β-lactam (±)-**135**, which was purified by column chromatography (Scheme 31).[79] Ring opening of the β-lactam (±)-**135** with ethanolic HCl resulted in amino ester hydrochloride (±)-**136**, the amino group of which was protected by reaction with AcCl, Boc_2O or ZCl in various solvents to give *N*-acylated esters (±)-**13** and (±)-**137a**,**b**.

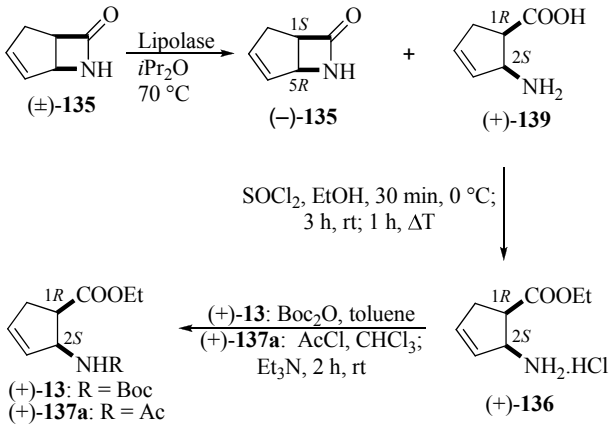
The base-mediated isomerization of amino esters with NaOEt in EtOH is a widely used method for isomerization of the carboxyl ester group on position 1 of the ring.[80] When *cis N*-protected amino esters (±)-**13** and (±)-**137a** were reacted at room temperature, a slow transformation was observed, and after 24 h *trans* (±)-**102** and (±)-**138** were isolated in yields of 37-51% (Scheme 31) [I]. This can be interpreted as the hydrolysis of the ester group, with the amino acid remaining in the aqueous phase in the course of the work-up.



Scheme 31

The optically pure starting materials were prepared by Lipolase (lipase B from *C. antarctica*)-catalysed enantioselective ring cleavage of *rac*-β-lactam ((±)-**135**) in *i*Pr_2O at 70 °C (Scheme 32).[81] At this temperature, the enzyme did not lose its activity and these conditions proved best for preparation of the optically pure compounds in 48% yield and in *ee* > 99%.

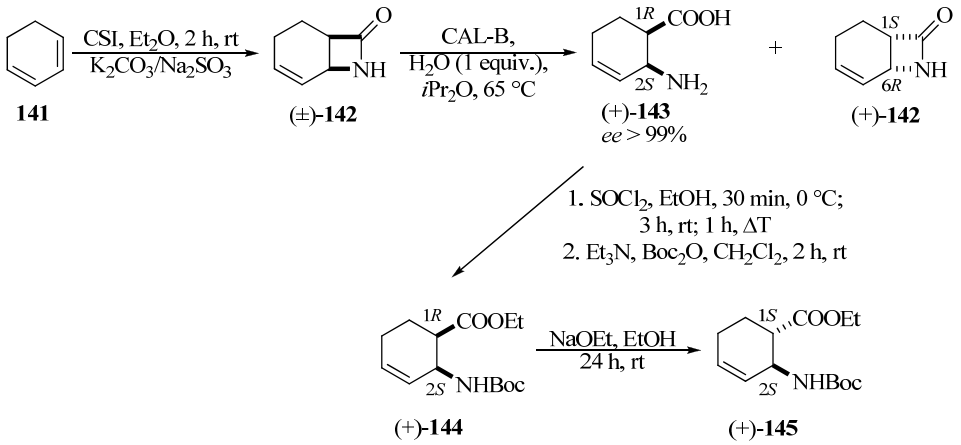
An alternative synthetic route was used in the cases of (+)-**13** and (+)-**137a**. The starting amino acid (+)-**139** was esterified with $SOCl_2$ to ethyl (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylate hydrochloride ((+)-**136**), subsequent protection of the free amino group with Boc_2O or AcCl leading to the desired *N*-acylated ester enantiomers (Scheme 32).



Scheme 32

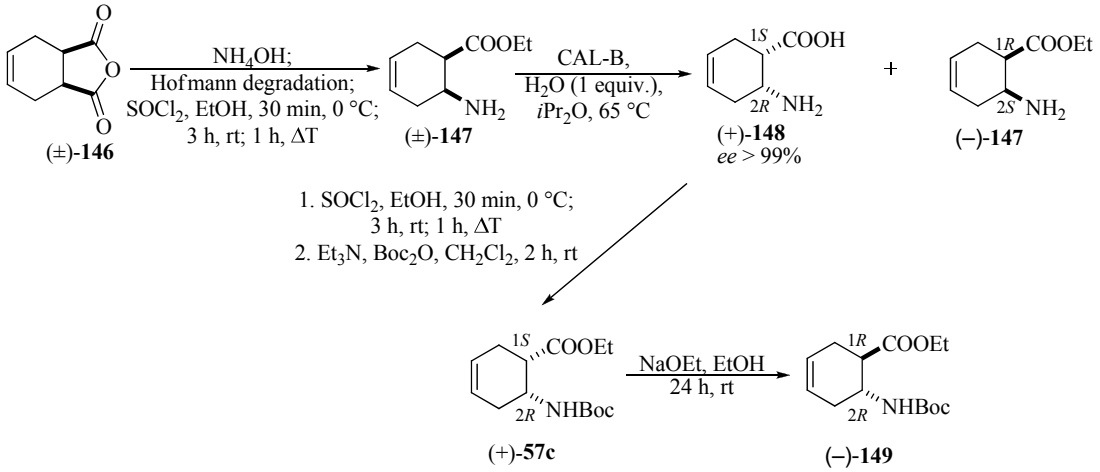
The *ee* value for the starting (+)-**139** (> 99%) was determined by gas chromatography on a Chromopak Chiralsil-Dex CB column after double derivatization. The carboxyl group of (+)-**139** was first esterified with CH_2N_2, which was followed by acylation with Ac_2O in the presence of 4-dimethylaminopyridine and pyridine.[82] In the case of ester (+)-**136** (> 99%), the *ee* was determined under the same conditions, but the sample was treated only with Ac_2O in the presence of 4-dimethylaminopyridine and pyridine.

Next, utilizing the highly enantioselective CAL-B-catalysed hydrolysis method[81,83] with one equivalent of H_2O in iPr_2O at 65 °C, (1*R*,2*S*)-2-aminocyclohex-3-enecarboxylic acid ((+)-**143**) and (1*S*,2*R*)-2-amino-cyclohex-4-enecarboxylic acid ((+)-**148**) were synthesized from β-lactam (±)-**142**[59] and β-amino ester (±)-**147**.[57] The 1,2-dipolar cycloaddition of CSI to 1,3-cyclohexadiene **141** takes place regio- and stereoselectively, resulting in *rac*-7-azabicyclo[4.2.0]oct-4-en-8-one ((±)-**142**). Ammonolysis of *cis*-1,2,3,6-tetrahydrophthalic anhydride **146**, followed by hypochlorite-mediated Hofmann degradation and esterification, was used for the preparation of ethyl *cis*-2-aminocyclohex-4-enecarboxylate ((±)-**147**). The reactions of (+)-**143** and (+)-**148** (*ee* > 99%) with $SOCl_2$ in EtOH gave amino ester hydrochlorides, which were reacted with Boc_2O to afford *N*-Boc-protected amino esters (+)-**144** and (+)-**57c** in yields of 82-89% (Schemes 33 and 34).



Scheme 33

C-1 Epimerization of (+)-**144** and (+)-**57** with NaOEt at room temperature in EtOH resulted in *trans*-*N*-Boc amino esters (+)-**145** and (–)-**149** in yields of 43-49%**.** The yield of the base-mediated isomerization of *N*-Boc-protected cyclopentene amino ester (±)-**13** was similar (51%), which indicated that this system is also disposed to hydrolysis under the isomerization conditions (Schemes 33 and 34) [II].

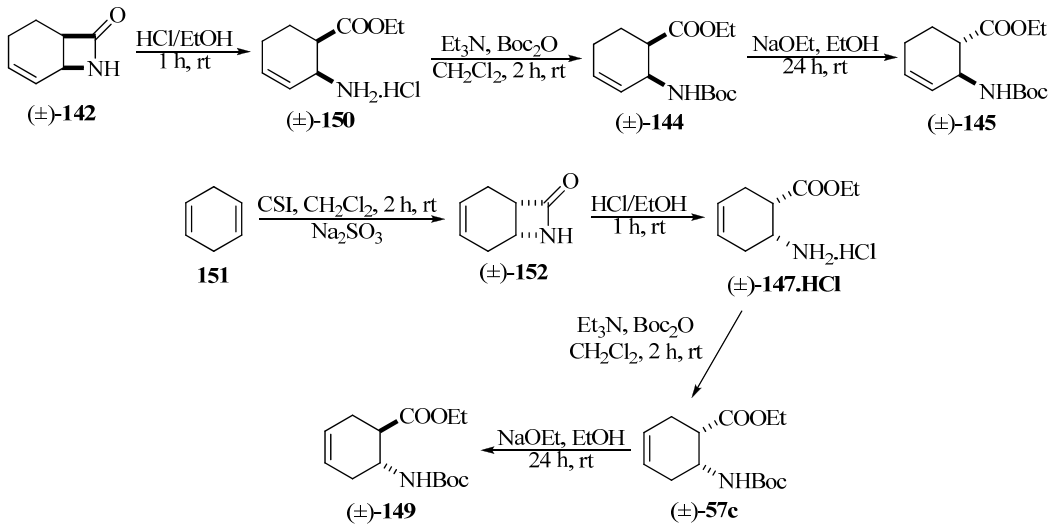


Scheme 34

The *ee* values for the starting (1*S*,2*R*)-2-aminocyclohex-3-enecarboxylic acid ((+)-**143**) and (1*R*,2*S*)-2-aminocyclohex-4-enecarboxylic acid ((+)-**148**) (> 99%) were determined by gas chromatography on a Chromopak Chiralsil-Dex CB column after reaction with CH_2N_2 and Ac_2O in the presence of 4-dimethylaminopyridine and pyridine.[82]

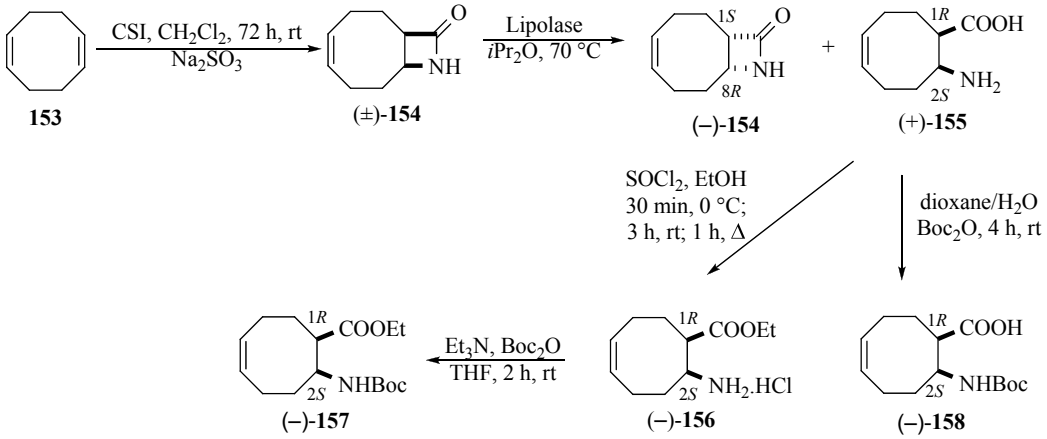
The synthetic protocol specified above was also applied for the preparation of racemic starting materials with some relevant modifications. β-Lactams (±)-**142** and

(±)-**152** were prepared by CSI addition to 1,3- and 1,4-cyclohexadiene at room temperature (Scheme 35). β-Lactams (±)-**142** and (±)-**152** were next opened to the expected *cis* amino ester hydrochloride salts (±)-**150**.HCl and (±)-**147**.HCl with EtOH containing dry HCl, and the amino groups were acylated with Boc_2O. The final step was the base-mediated isomerization of protected amino esters (±)-**144** and (±)-**57c** with NaOEt in EtOH.



Scheme 35

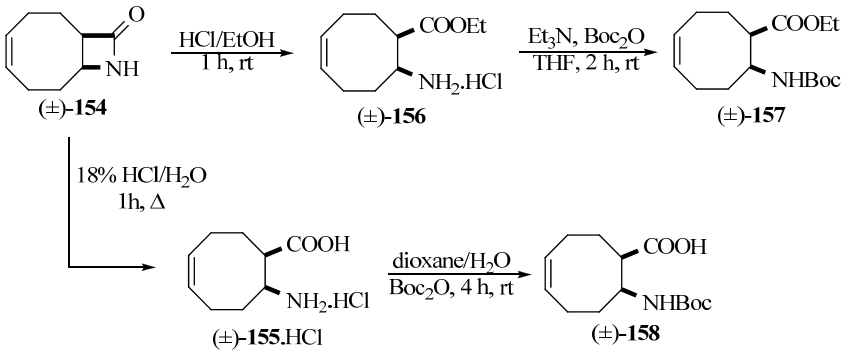
For the preparation of *rac*-9-azabicyclo[6.2.0]dec-4-en-10-one ((±)-**154**), CSI was reacted with 1,5-cyclooctadiene (**153**) in dry CH_2Cl_2 at room temperature. Treatment of the resulting *N*-chlorosulfonyl-β-lactam with Na_2SO_3 in slightly alkaline solution gave a yellow solid, which was recrystallized from *i*Pr_2O, resulting in diastereomerically pure β-lactam (±)-**154** in a yield of 42%.[20] The highly enantio-selective Lipolase-catalysed ring cleavage of azetidinone (±)-**154** in *i*Pr_2O at 70 °C resulted in (1*R*,2*S*)-2-aminocyclooct-5-enecarboxylic acid ((+)-**155**) (Scheme 36). This compound was transformed to the amino ester hydrochloride (–)-**156** with $SOCl_2$ in EtOH, which was then acylated with Boc_2O in THF to give *N*-protected ester (–)-**157** in good yield. *N*-Boc protection of (+)-**155** in dioxane/H_2O led to *N*-Boc-amino acid (–)-**158** [III].



Scheme 36

The *ee* (> 99%) value for the starting compound was determined by gas chromatography after double derivatization: esterification with CH_2N_2 and then acylation with Ac_2O in the presence of 4-dimethylaminopyridine and pyridine.[82]

The racemic starting compounds of the enantiopure cyclooctane-β-amino acids were achieved by using an alternative synthetic protocol. The selective ring opening of β-lactam (±)-**154** with EtOH containing HCl, followed by protection of the amino group with Boc_2O, gave *N*-Boc ester (±)-**157**. In order to observe the *N*-Boc-protected amino acid (±)-**158**, β-lactam (±)-**154** was submitted to heating in 18% HCl for 1 h. The following Boc protection of the amino group resulted in the desired product (Scheme 37).



Scheme 37

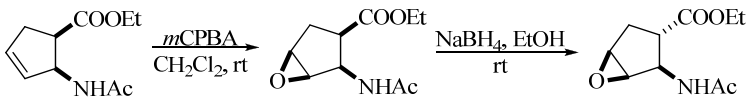
3.1.2. Monohydroxy derivatives via epoxide rings

Functionalization of the olefinic bond of an alkene, utilizing *m*CPBA or other reagents, such as DMDO, as oxidant, is a well-known and widely-used synthetic

protocol for the preparation of the 3-membered oxirane ring.[84,85] The epoxides play a crucial role in synthetic chemistry because they are capable of assisting in nucleophilic ring opening and hence, depending on the nucleophilic reagent, the preparation of various kinds of substituted compounds.[86] This advantage is exploited in the functionalization of unsaturated cyclic β-amino acids, where the newly-forming oxirane ring is oriented from the less hindered side of the starting β-amino acid. In our case, opening of the epoxide[47] with reducing agent such as $NaBH_4$ is one possible way to introduce a hydroxy group onto the ring. A significant body of work has been devoted to the preparation of hydroxy β-amino acids through the epoxidation protocol (see Introduction).

Epoxidation of ethyl *cis*-2-acetylamino-cyclohex-3-enecarboxylate ((±)-**137a**) in the presence of *m*CPBA in CH_2Cl_2 under stirring at room temperature gave the corresponding *cis*-epoxide (±)-**159** as the only diastereomer.[49-51] The epoxidation occurred on the same face as the carbamate and the ester group, which is consistent with the reported finding.[47] The orientation of the oxirane ring was deduced from the large NOE signals of H-2, H-3 and H-4, which suggest an epoxide ring *cis* to the amino group. The absence of couplings between H-1, H-3 and H-4 confirms the *trans* orientation relative to the ester moiety.

All attempts to open the oxirane ring and prepare a hydroxy compound using $NaBH_4$ in EtOH failed: the epoxide moiety remained unchanged, but isomerization occurred at position 1, which can be ascribed to the basic conditions under which the reaction was carried out (Scheme 38).



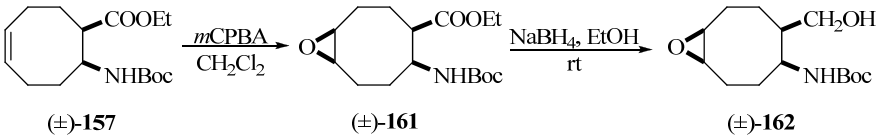
(±)-**137a** (±)-**159** (±)-**160**

Scheme 38

The same synthesis methods were applied for the preparation of ethyl *cis*-5-*tert*-butoxycarbonylamino-9-oxabicyclo[6.1.0]nonane-4-carboxylate ((±)-**161**). The epoxide group is located on the same side of the cyclooctane ring as the other functional groups, as indicated by the NOE cross-peaks between H-1, H-5 and H-6, and between H-2, H-5 and H-6. The opening of the oxirane ring proceeds in an analogous fashion with $NaBH_4$, in contrast with the previously experienced isomerization to *tert*-butyl

(1*R**,4*S**,5*R**,8*S**)-5-(hydroxymethyl)-9-oxabicyclo[6.1.0]nonan-4-ylcarbamate ((±)-**162**). This transformation can be rationalized in terms of the reductive conditions of $NaBH_4$. The NOE cross-peaks between H-1 and H-5, H-1 and H-6, H-2 and H-5, and H-2 and H-6 suggest the *all-cis* stereochemistry of the compound and point to the conformational flexibility of (±)-**161** (Scheme 39).

X-ray crystallography unequivocally confirmed the structure of (±)-**161**. Figure 3 depicts the boat-chair conformation of the cyclooctane ring,[87] with the hydroxymethyl and the bulky *tert*-butoxycarbonylamino groups in equatorial positions.



Scheme 39

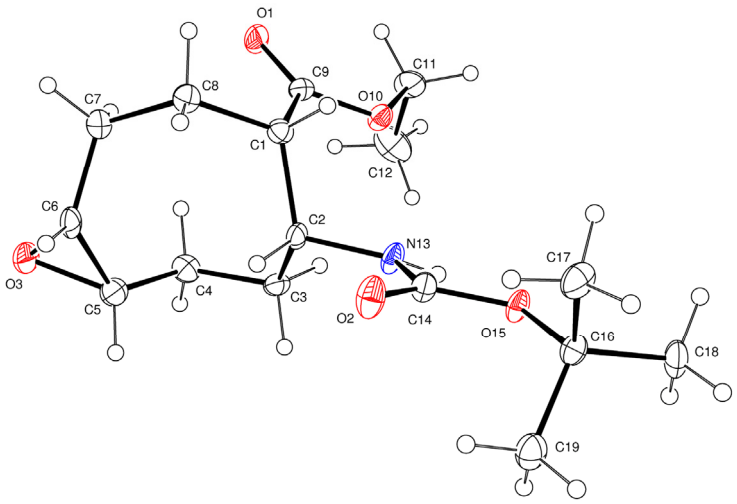


Figure 3 ORTEP plot of the X-ray structure of (±)-161

3.1.3. Monohydroxy-substituted cyclopentane- and cyclooctane-β-amino acids via oxazoline or iodolactone intermediates

Use of the halolactonization strategy in the stereocontrolled synthesis of complex organic molecules has significantly increased recently, as shown by the frequent publications relating to this topic.[88] This cyclofunctionalization proceeds by a common mechanistic pathway in which the transition state generally reflects an intramolecular opening of the 3-membered ring intermediate, which arises from acceptance of the halogen-derived electrophiles, by a heteroatom oxygen, which could

be part of a carboxylic acid, an ester or an amide. The substrates for cyclization that allow complete stereocontrol include those that contain the double bond within a ring. The resulting fused lactones strongly preserve *cis* stereochemistry at the ring junction.

The diastereoselective electrophile-induced bromocyclocarbamation of different homoallylic carbamates has been reported regularly in organic synthesis. In the course of halocyclofunctionalization of the double bond in a cyclic system via *O,N*-heterocycles, a hydroxy group can be introduced *cis* to the already existing amino group under high regio- and stereocontrol.[89-92]

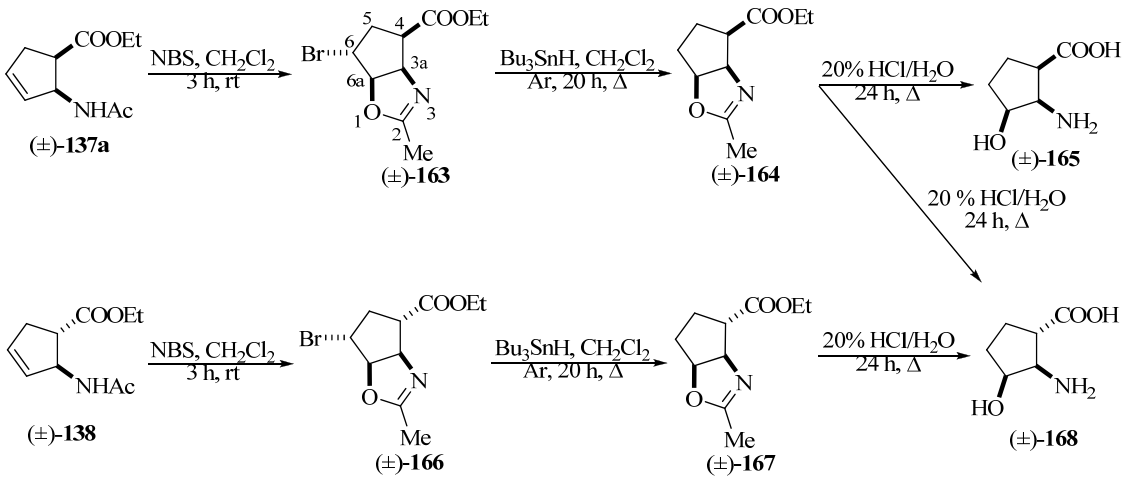
The reaction of *cis*- and *trans*-*N*-acetylcyclopentene derivatives (±)-**137a** and (±)-**138** with NBS in CH_2Cl_2 yielded bicyclic bromooxazoline derivatives (±)-**163** and (±)-**166** regio- and diastereoselectively (Scheme 40).[69] Reduction of the bromo group with Bu_3SnH under an Ar atmosphere resulted in oxazolines (±)-**164** and (±)-**167**. Ring opening and deprotection of (±)-**164** under acidic conditions under reflux, followed by ion-exchange chromatography and finally fractional crystallization, led to the stereoisomer (1*S**,2*R**,3*S**)-2-amino-3-hydroxycyclopentanecarboxylic acid ((±)-**168**), which was in contrast with expectations. In the last step of the reaction, partial *cis* → *trans* isomerization occurred at position 1.

Although acid-mediated isomerization is less frequent than the base-catalysed methods, it is possible under the conditions we applied. Protonation of the heteroatom at the α-position enhances the hydrogen abstraction at C-1, and it can be deprotonated even with a weak base such as water.[80]

From the mother liquor *all-cis*-2-amino-3-hydroxycyclopentanecarboxylic acid ((±)-**165**) was also isolated in a low yield by crystallization (Scheme 40).

Hydrolysis of oxazoline (±)-**167** produced hydroxy-amino acid (±)-**169** as the only product as a white crystalline solid (Scheme 40).

The novel compounds were characterized by NMR measurements. For (±)-**168**, the small NOE signal between H-1 and H-2 suggested a *trans* orientation, whereas the large NOE between H-2 and H-3 pointed to *cis* C-2 and C-3 substituents. The large NOE signal between H-1 and H-2 for (±)-**165** suggested a *cis* carboxyl group relative to the amino group, and the signals between H-1 and H-3 showed *cis* orientation between C-1 and C-3.



Scheme 40

The synthesis of (1*R*,2*R*,3*S*)-3-hydroxyamino acid derivative (+)-**165** was carried out similarly as for the *rac* compound, starting from enantiopure ethyl (1*R*,2*S*)-2-acetylaminocyclopent-3-enecarboxylate ((+)-**137a**) (Schemes 32 and 40).

The selective iodolactonization of *cis*- and *trans*-2-aminocyclohex–4–enecarboxylic acids and *cis*-2-amino-cyclohex-3-enecarboxylic acid was previously reported.[56,59] Cyclization of *cis*-2-*tert*-butoxycarbonylamino-cyclopent-3-enecarboxylic acid[47] ((±)-**140**) with I_2/KI in aqueous $NaHCO_3$ and CH_2Cl_2 was carried out according to the literature procedure, but not even traces of iodolactone product were observed.[56] The starting compound remained unchanged, most probably because of the unstable bicyclic lactone ring system.

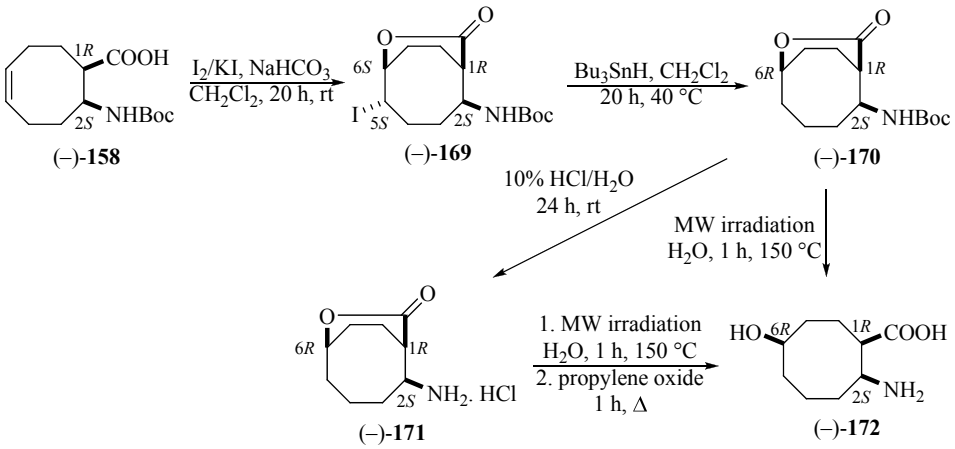
The iodolactonization of cyclooctane derivatives was first optimized on racemic *cis*-2-*tert*-butoxycarbonylaminocyclooct-5-enecarboxylic acid ((±)-**158**) and applied for the optically pure compounds.

The reaction of (1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclooct-5-ene-carboxylic acid ((–)-**158**) in a two-phase solvent system with I_2/KI/aqueous $NaHCO_3$ in CH_2Cl_2 regio- and diastereoselectively yielded the six-membered iodolactone (–)-**169** as a white crystalline product, in a yield of 71%. The other possible product would be the seven-membered lactone, but only (–)-**169** was obtained.

Reduction of the iodo group with Bu_3SnH in CH_2Cl_2 yielded the lactone (–)-**170**, aqueous hydrolysis and deprotection with MW irradiation[93,94] gave (1*R*,2*S*,6*R*)-2-amino-6-hydroxycyclooctanecarboxylic acid ((–)-**172**) in good yield. When ring opening of Boc-lactone (–)-**170** was attempted with aqueous HCl, deprotected lactone (–)-**171** was formed, which was transformed to amino acid (–)-**172**

upon MW irradiation followed by heating in propylene oxide (Scheme 41). The presence of the lactone ring in (–)-**169** and (–)-**171** was confirmed by the cross-peak between H-6 and the carbonyl carbon in the HMBC spectra. The structures of iodo-lactones (–)-**169** and (–)-**171** were unambiguously confirmed by the X-ray analyses (Figures 4 and 5). Figures 4 and 5 illustrate the twist-boat conformation of the cyclooctane ring[87], and the equatorial position of the amino functional group in both lactones.

In the case of the final hydroxy amino acid (–)-**172**, the NOE cross-peak between H-1 and H-6 suggests that the hydroxy group should be *cis* relative to the carboxylic group. This result is in accordance with the literature examples of the analogous reactions of 2-aminocyclohex-3- and 2-aminocyclohex-4-enecarboxylic acids.[56,59]



Scheme 41

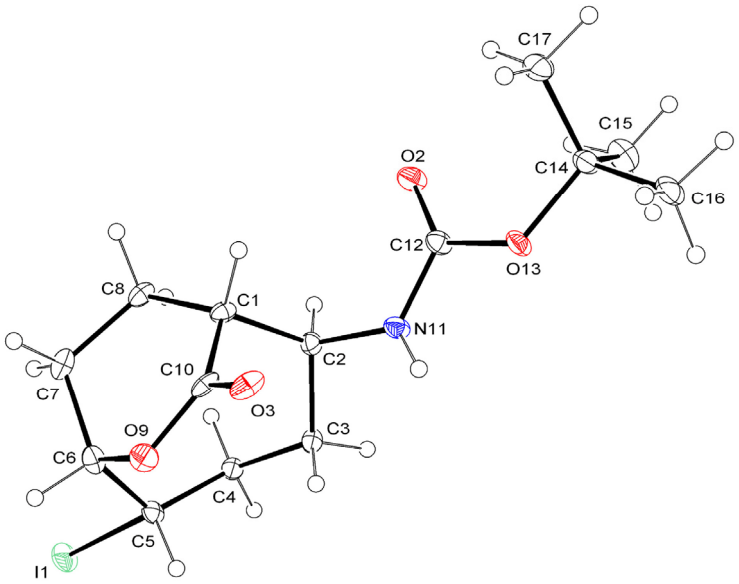


Figure 4 ORTEP plot of the X-ray structure of Boc-protected lactone (±)-169

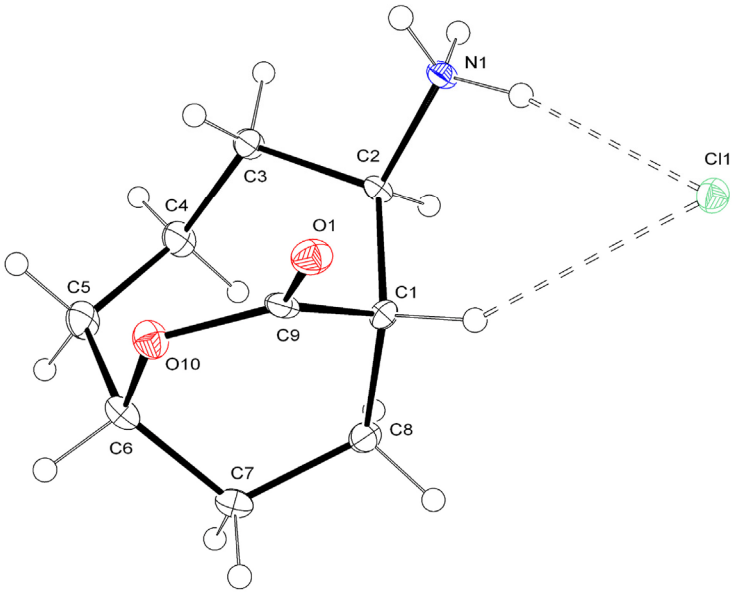
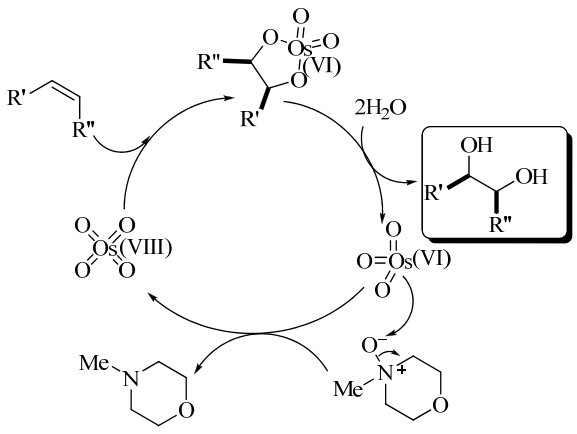


Figure 5 ORTEP plot of the X-ray structure of lactone (±)-171

The *ee* (> 99%) value for the monohydroxy-substituted 2-aminocyclo-octanecarboxylic acid (–)-**172** was determined by HPLC.

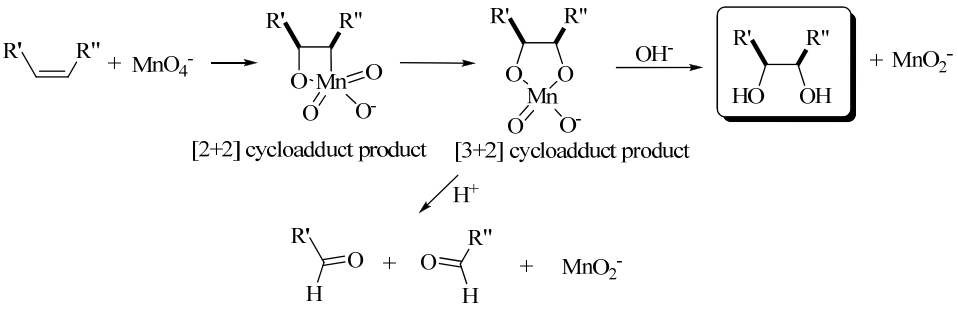
3.1.4. Dihydroxy-substituted cyclopentane-, cyclohexane- and cyclooctane-β-amino acids

Osmium-catalysed oxidation is one of the most useful methods for dihydroxylation of olefins to provide the corresponding vicinal diols. This oxidation proceeds in the presence of a catalytic amount of OsO_4 with a co-oxidant such as NMO (Upjohn procedure, Scheme 42),[95] potassium ferricyanide, NaOCl, H_2O_2, O_2 or H_2O_2-flavine base.[96-98] The Sharpless process[99] combining the use of a co-oxidant and a chiral ligand (cinchona alkaloid) is now used to carry out the catalytic asymmetric dihydroxylation of alkenes. Although these reactions have widespread applications in organic synthesis, there have been few large-scale industrial applications, due to the toxicity, high cost and volatility of the reagent.[100]



Scheme 42

The oxidation of carbon–carbon double bonds by permanganate ion (MnO_4^-) is an important and well-known reaction in organic chemistry. Under alkaline conditions, olefins are converted into the corresponding diols, while in neutral or slightly acidic solutions α-hydroxy ketones or carbonyl compounds are produced. It has been widely accepted that MnO_4^- reacts with alkenes in a cycloaddition to form the cyclic Mn(V) ester intermediate, which subsequently decomposes into a 1,2-diol or carbonyl compounds, depending upon the reaction conditions (Scheme 43).[101]



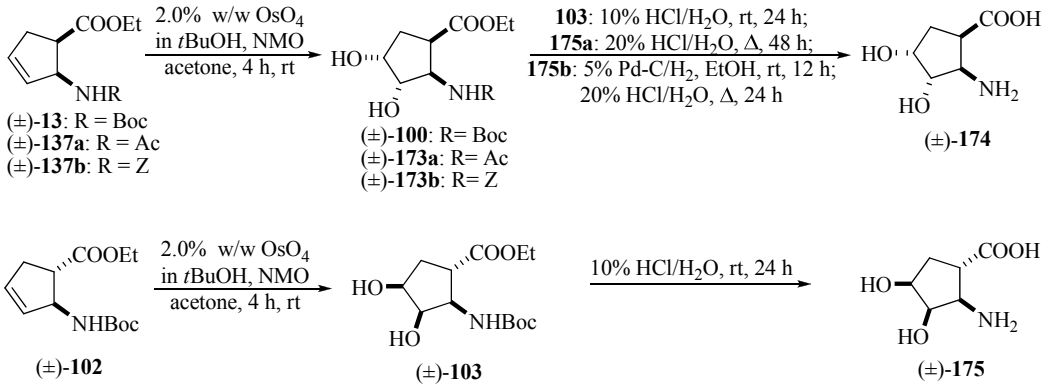
Scheme 43

Since aqueous $KMnO_4$-mediated dihydroxylation results in the same diastereoselectivity as with osmylation, but with lower yields, we carried out the former method. The best reaction conditions included the use of OsO_4 (2 w/w%) with NMO as the stoichiometric co-oxidant at room temperature for 4 h, with *t*BuOH and acetone as the solvent mixture. The yield of the isolated products lay in the range 71-91%.

The dihydroxylation of olefins (±)-**13** and (±)-**137a**,**b**, like that of the corresponding *trans N*-Boc-ester (±)-**102** with catalytic OsO_4 and NMO, resulted in the products (±)-**100**, (±)-**173a**,**b** and (±)-**103** as single diastereomers, as determined by ^{1}H

NMR spectroscopy (Scheme 44). The ester and the *N*-protecting groups were then cleaved off under acidic conditions to obtain the expected (1*R**,2*R**,3*S**,4*R**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid ((±)-**174**) and (1*S**,2*R**,3*R**,4*S**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid ((±)-**175**).

The double bonds in (±)-**13** and (±)-**137a**,**b** undergo oxidation on the sterically less hindered side of the cyclopentane ring. The diastereoselectivity of the dihydroxylation of (±)-**103** is not likely to be determined by simple steric repulsion, because both the ester and the protected amino group are in equatorial positions. In this case, the newly formed hydroxy groups have the same steric orientation as the protected amino group in the final product. This can be rationalized by the probability of an electrostatically advantageous interaction in the intermediate complex of OsO_4 and (±)-**103** between the partially positive amide hydrogen atom and the partially negative oxygen atom attacking the sp^2 carbon atom vicinal to the protected amine substituent. This interaction is possible only if OsO_4 is in juxtaposition with the NHBoc moiety.

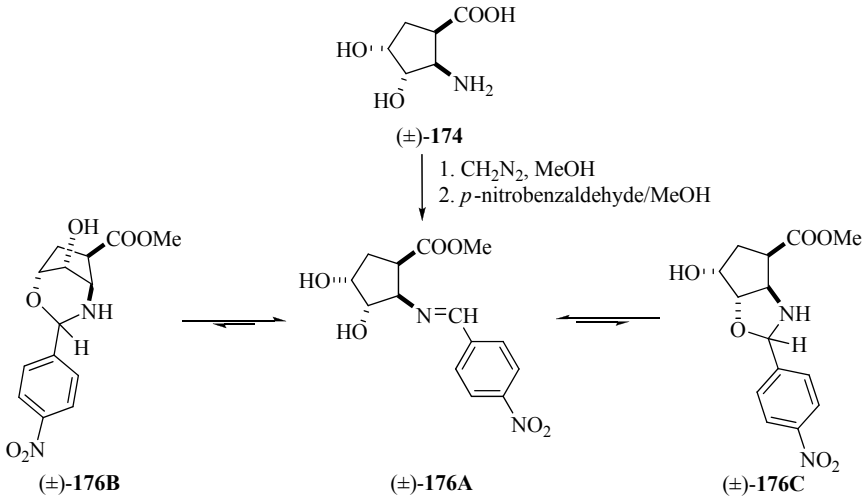


Scheme 44

The interesting differences observed in the ring-closure reactions of 1,2-disubstituted 1,2- and 1,3-difunctionalized cycloalkanes[102,103] helped us prove the relative configurations of dihydroxy amino acids (±)-**174** and (±)-**175** not merely by means of NMR, but also by chemical transformations. If the 3- and 4-hydroxy substituents of ester derivatives of (±)-**174** are in the *cis* position relative to the 2-amino substituent, the ester derivatives should undergo ring closure; otherwise, if they are *trans* to the amino substituent, the ester derivatives will not undergo ring closure.

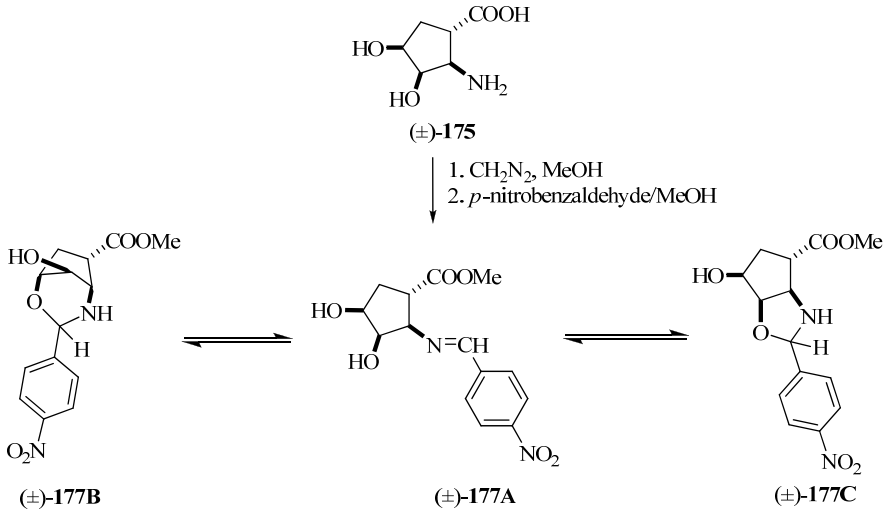
Using the above theory, we attempted to prove the relative configurations of (±)-**174** and (±)-**175**. (1*R**,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclopentanecarboxylic acid ((±)-**174**) was reacted with CH_2N_2, followed by treatment with 1 equivalent of

p-nitrobenzaldehyde in MeOH. After evaporation and purification, a well-defined product was obtained. For tautomeric equilibrium to be reached, the substance was allowed to stand for 24 h in $CDCl_3$ (Scheme 45). The ^1H NMR spectrum of the product (±)-**176** indicated a well-defined signal at δ = 8.4 ppm ((±)-**176A** chain form N=C*H* singlet) and no peaks were detected at δ = 5–6 ppm ((±)-**176B** and (±)-**176C** ring form NH-C*H*Ar-O singlet), which proves the *trans* orientation of 2-NH_2 and 3-OH. This means that this compound exists only as the open Schiff base form (±)-**176A**.[103]



Scheme 45

In contrast, for the similar transformation of (1*S**,2*R**,3*R**,4*S**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid ((±)-**175**), both the open Schiff base form (±)-**177A** and the ring-closure products (±)-**177B** and (±)-**177C** were observed in the ^1H NMR spectrum, which underlines the *cis* orientation of the 2-NH_2 and 3-OH substituents (Scheme 46).



Scheme 46

The optically pure dihydroxy compound (–)-**174** was prepared by following the analogous synthetic procedure as for the racemic counterpart. The ^{1}H NMR and ^{13}C NMR data and elemental analyses for the product and intermediates were in accordance with those for racemic derivatives. Since the *ee* of ethyl (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylate hydrochloride ((+)-**136**) was higher than 99%, and not even traces of other diastereomers were detected in the prepared compounds by ^{1}H NMR, the *ee* values for the products were undoubtedly higher than 99%.[81]

Dihydroxylation of the cyclohexane derivatives was first optimized on the racemic starting materials and then applied for the optically pure compounds. The racemic dihydroxy compounds ((±)-**179**, (±)-**181**, (±)-**183** and (±)-**185**) were prepared as free amino acids and also as hydrochloride salts.

Dihydroxylation of regioisomeric ethyl (1*R*,2*S*)- and (1*S*,2*S*)-2-aminocyclohex-3-enecarboxylate ((+)-**144** and (+)-**145**) and ethyl (1*S*,2*R*)- and (1*R*,2*R*)-2-aminocyclohex-4-enecarboxylate ((+)-**57c** and (–)-**149**) yielded the desired products (–)-**178** and (–)-**180**, and (+)-**182** and (–)-**184** as single diastereomers in yields of 72-85% (Schemes 47 and 48).

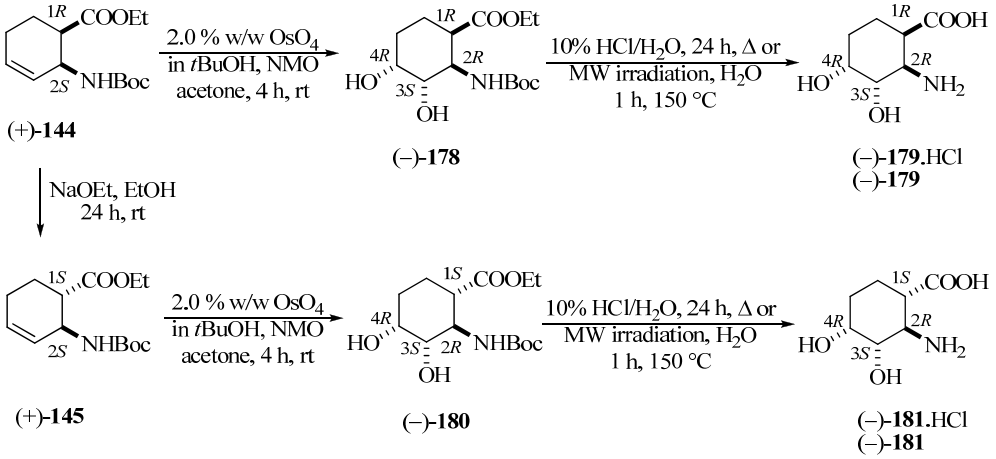
In accord with expectations, osmylation of (+)-**144** and (+)-**57c** proceeded with *anti* selectivity with regard to the ester and the amino groups, on the sterically less-hindered side of the ring. The orientation of the hydroxy groups was deduced from the couplings and NOEs of their vicinal hydrogens.

Osmylation of the double bond in (+)-**145** and (–)-**149**, where the ester and amino groups are on opposite sides of the ring, led to hydroxy groups on the ester side, *anti* to the amino group. This selectivity can be explained by the steric bias of the substituents. The bulkier *N*-Boc protecting group interacts unfavourably with the forming hydroxy groups, and osmylation occurs from the sterically less-hindered face.[78]

The acidic hydrolysis of (–)-**178** and (–)-**180** resulted in the corresponding dihydroxyamino acid hydrochlorides (–)-**179**.HCl and (–)-**181**.HCl in yields of 45-47%. For (+)-**182** and (–)-**184**, a different deprotection method was used in the last step of the synthesis: reaction first with LiOH in THF to deprotect the carboxylic group, followed by hydrolysis with HCl/H_2O.[59] The yields were obviously the same.

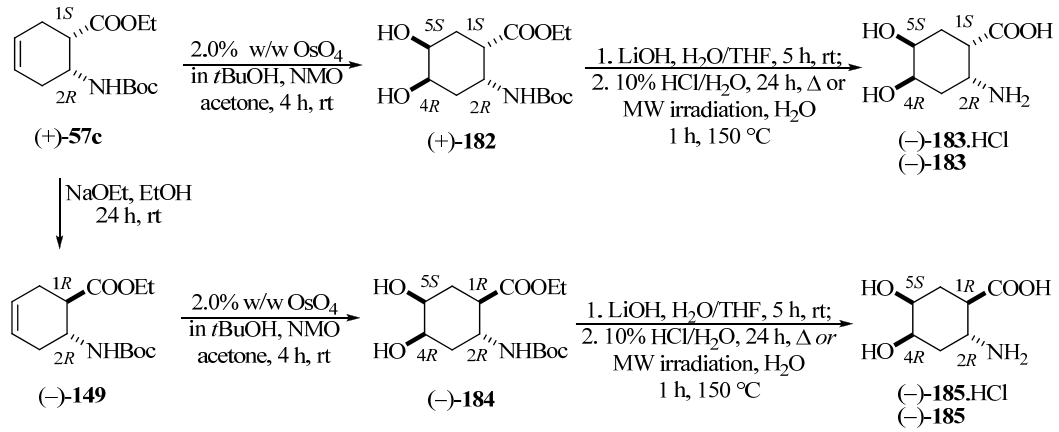
In order to improve the yields of the final products, the simple and efficient catalyst-free water-mediated novel deprotection protocol introduced by Wang *et al.* was applied with a MW reactor.[94] The importance of this synthetic method is that the MW conditions used are similar to subcritical water conditions, which replace the catalyst in

this acid-catalysed reaction. The dihydroxy compounds (–)-**178**, (–)-**180**, (+)-**182** and (–)-**184** were placed into a MW tube, subjected to MW irradiation in H_2O at 150 °C for 1 h, and crystallized from acetone[93,94] (Schemes 47 and 48).



Scheme 47

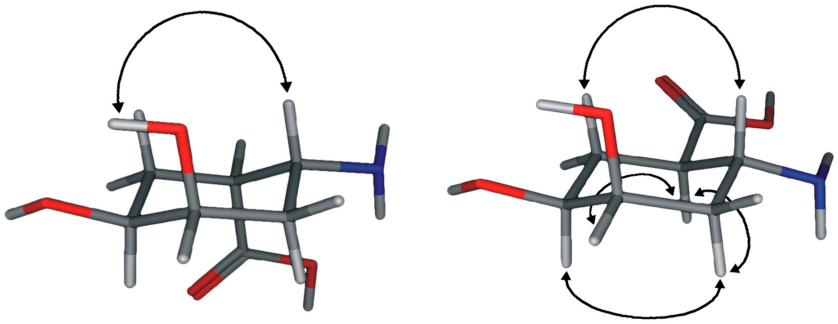
The *ee* values (> 99%) for the final products (–)-**179**, (–)-**181**, (–)-**183** and (–)-**185** and their HCl salts were determined by HPLC on a Chirobiotic TAG and a Chirobiotic T column, using MeOH containing 0.1% TEA and 0.1% AcOH and 0.1% aqueous triethylammonium acetate/EtOH = 20/80 as the mobile phase.



Scheme 48

(–)-**183** Gave the NOE cross-peak between H-2 and the 4-hydroxy group, which points to the *trans* orientation of the amino and the hydroxy groups. For (–)-**185**, NOE signals are observed between H-1 and H-5 and between H-3ax and H-1 and H-5, which points to the *cis* orientation of the carboxyl and hydroxy groups (Figure 6). These structures were built and energy-minimized by using the Molecular Operating

Environment software (MOE 2008.10). Structures were drawn with the builder module of MOE, and energy-minimized by using MMFF94 force-field and distance/dihedral constraints based on NMR data.



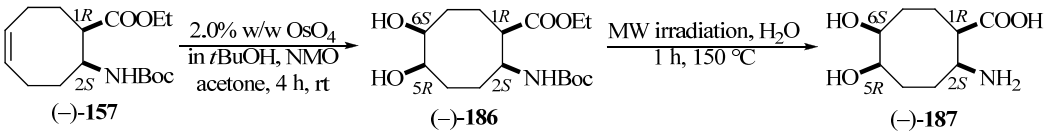
(–)-**183** (–)-**185**

Figure 6

(1*R*,2*S*,5*R*,6*S*)-2-Amino-5,6-dihydroxycyclooctanecarboxylic acid (–)-**187** was prepared by osmium-catalysed dihydroxylation followed by MW-assisted deprotection. Oxidation of the double bond of *N*-Boc ester (–)-**157** afforded the expected product (–)-**186** as a single diastereomer in a yield of 91%. After deprotection of (–)-**186** with MW irradiation in H_2O at 150 °C, the corresponding dihydroxy-amino acid (–)-**187** was obtained (Scheme 49).[93,94] The orientation of the hydroxy groups was deduced from the NOE pattern of the compound, while the coupling constants had only a limited role in the analysis because of the conformational flexibility of the compound. The *all-cis* stereochemistry of (–)-**186** was proved by the NOE cross-peaks between H-2 and H-5 and between H-1 and H-6. For (–)-**187**, a similar NOE pattern was observed, which indicates the *all-cis* orientation of the functional groups and underlines that the MW irradiation did not affect the stereochemistry.

The stereochemistry of (–)-**187** was checked by X-ray diffraction (Figure 7). From a racemic mixture, a single enantiomer crystallized out in a zwitterionic form in the crystal investigated. However, the data did not permit confirmation of which enantiomer this was.

The *ee* values of > 99% for the final product (-)-**187** was determined by HPLC on a Chirobiotic TAG column, using MeOH containing 0.1% TEA and 0.1% AcOH as the mobile phase.



Scheme 49

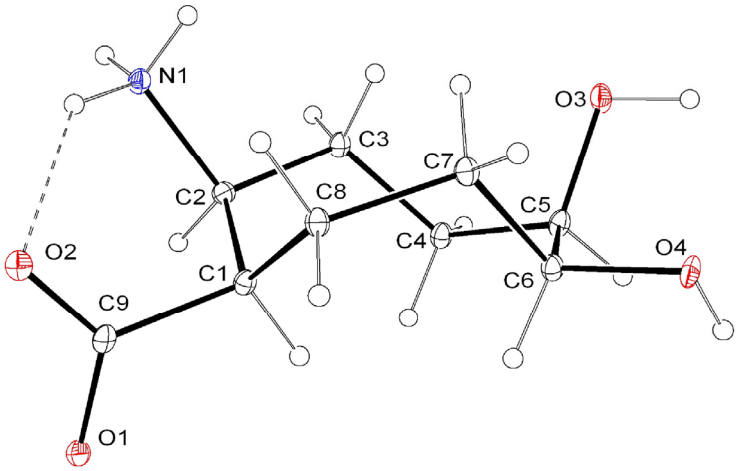


Figure 7 ORTEP plot of the X-ray structure of zwitterionic dihydroxy-substituted amino acid (±)-187

3.2. High-performance liquid chromatography of 2-aminomono- and dihydroxy-cyclopentanecarboxylic acids and 2-aminodihydroxycyclohexanecarboxylic acids

The direct separation of the enantiomers of hydroxy amino acids (±)-**165**, (±)-**168**, (±)-**174**, (±)-**175**, (±)-**179**, (±)-**181**, (±)-**183** and (±)-**185** was performed by HPLC on CSPs containing different macrocyclic glycopeptide antibiotics as chiral selectors (Chirobiotic T, T2, TAG and R columns) [IV].

The influence of the pH (6.52-3.00), the mobile phase composition, the nature of the alcoholic modifier (MeOH, EtOH, PrOH and IPA) was investigated. Besides carboxyl and primary amino groups, analogues (±)-**165** and (±)-**168** bear one hydroxy group, while (±)-**174**, (±)-**175**, (±)-**179**, (±)-**181**, (±)-**183** and (±)-**185** possess two hydroxy groups. This distinction results in different steric effects and hydrogen bonding, and influences the hydrophobicity, bulkiness and rigidity of the molecules. On the same column, the values of the separation factor were generally lower for amino acids (±)-**165** and (±)-**168**, which possess a single hydroxy group, but it seems that the position of the hydroxy groups on the cycloalkane skeleton (positions 3, 4 or 4, 5) resulted in only a small effect on the selectivity factor. The hydroxy groups attached to

the cycloalkane skeleton contribute significantly to the chiral recognition, and therefore Chirobiotic phases are much more suitable for the enantioseparation of hydroxy-cycloalkane β-amino acids, than unhydroxylated cycloalkane β-amino acids.

Of the four Chirobiotic columns, Chirobiotic T and TAG appeared most suitable for enantioseparation of the 2-aminomono- or dihydroxycycloalkanecarboxylic acids (reverse-phase mode using 0.1% TEAA, pH 4.1, and an alcoholic modifier). The elution sequence was determined in most cases (1*S* < 1*R* or 2*S* < 2*R*), but no general rule could be established as concerns the relation of the elution sequence to the absolute configuration.

3.3. Methods

^{1}H NMR spectra were recorded at 400, 500 or 600 MHz, and the ^{13}C NMR spectra at 100, 125 or 150 MHz in $CDCl_3$, CD_3OD, *d6*-DMSO or D_2O with a Bruker AM 400, a Bruker DRX 500 or a Bruker AV 600 spectrometer, usually at ambient temperature. Elemental analyses were performed with a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer. Melting points were measured with a Kofler melting point apparatus, and optical rotations with a Perkin-Elmer 341 polarimeter. MW reactions were performed in a CEM Discover MW reactor, using a 10-mL pressurized reaction vial. The enantiopurities of the starting materials and the final products were determined by GC and by HPLC.

Details of synthesis, physical and analytical data on the new compounds described in the thesis can be found in the Experimental part of the enclosed publications, except for compounds (±)-**159**, (±)-**160**, (±)-**161** and (±)-**162**, which are described in the following.

General procedure for epoxidation of (±)-137a and (±)-157 to (±)-**159 and** (±)-**161**: To a solution of *N*-protected esters (5 mmol) in CH_2Cl_2 (50 mL), *m*CPBA (6 mmol) was added at 0 °C. After stirring for 1 day, CH_2Cl_2 (50 mL) was added and the mixture was washed with saturated $NaHCO_3$ solution in H_2O (3x20 mL). The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was crystallized from *n*-hexane, and recrystallized from *i*Pr_2O.

Ethyl (1*R,2*R**,3*R**,5*S**)-2-acetamido-6-oxabicyclo[3.1.0]hexane-3-carboxy-late ((±)-159)**: a white crystalline solid (0.95 g, 89%), mp 119-122 °C. ^{1}H NMR (400 MHz, DMSO, 24 °C): δ = 1.15 (t, *J* = 7.1 Hz, 3 H, C*H*$_3$CH$_2$), 1.86 (s, 3 H, COC*H*$_3$), 1.94

(ddd, *J* = 14.5, 9.1, 1.2 Hz, 1 H, H-5ax), 2.42 (d, *J* = 14.5 Hz, 1 H, H-5eq), 2.91 (dt, *J* = 9.1, 9.1, 1.4 Hz, 1 H, H-1), 3.43 (dd, *J* = 2.5, 1.1 Hz, 1 H, H-3), 3.48 (d, *J* = 2.5 Hz, 1 H, H-4), 3.95-4.02 (m, 2 H, CH_3CH_2), 4.59 (dt, *J* = 9.1, 9-1, 1.1 Hz, 1 H, H-2), 8.06 (d, *J* = 8.7 Hz, 1 H, N*H*) ppm. ^{13}C NMR (100 MHz, DMSO, 24 °C): δ = 14.8, 23.3, 29.8, 39.8, 52.9, 54.5, 58.3, 60.7, 170.2, 173.1 ppm. Anal. Calcd for $C_{10}H_{15}NO_4$ (213.23): C, 56.33; H, 7.09; N, 6.57. Found: C, 56.52; H, 7.01; N, 6.62.

Ethyl (1*S,4*R**,5*S**,8*R**)-5-*tert*-butoxycarbonylamino-9-oxabicyclo[6.1.0]nonane-4-carboxylate ((±)-161)**: a white crystalline solid (1.3 g, 83%), mp 83-87 °C. ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.19 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.37 (s, 9H, *t*Bu), 1.35-1.45 (m, 1H, H-4), 1.49-1.59 (m, 1H, H-7), 1.61-1.70 (m, 2H, H-3, H-8), 1.73-1.80 (m, 1H, H-3), 1.81-1.88 (m, 1H, H-8), 1.88-1.94 (m, 1H, H-4), 1.96-2.02 (m, 1H, H-7) 2.71 (dd, *J* = 8.5, 3.4 Hz, 1H, H-1), 2.86-2.92 (m, 2H, H-5, H-6), 3.94-4.09 (m, 3H, H-2, CH_2CH_3), 6.77 (d, *J* = 6.6 Hz, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 13.9, 22.8, 23.0, 23.7, 28.1, 28.1, 45.9, 50.5, 54.0, 54.1, 59.6, 77.7, 154.9, 173.2 ppm. Anal. Calcd for $C_{16}H_{27}NO_5$ (313.39): C, 61.32; H, 8.68; N, 4.47. Found: C, 61.07; H, 8.85; N, 4.44.

General procedure for opening of the epoxide ring of (±)-159 and (±)-161: To a solution of epoxide (2.5 mmol) in EtOH (20 mL), $NaBH_4$ (236 mg, 6.25 mmol) was added in portions. The mixture was stirred at room temperature for 1 day, and the solvent was then evaporated off. The residue was taken up in EtOAc (50 mL), washed with H_2O (20 mL), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (EtOAc/*n*-hexane 1:1).

Ethyl (1*R,2*R**,3*S**,5*S**)-2-acetamido-6-oxabicyclo[3.1.0]hexane-3-carboxylate ((±)-160)**: a white crystalline solid (187 mg, 35%), mp 127-130 °C. ^{1}H NMR (400 MHz, DMSO, 24 °C): δ = 1.14 (t, *J* = 7.1 Hz, 3 H, CH_3CH_2), 1.79-1.90 (m, 4 H, $COCH_3$, H-5eq), 2.23-2.33 (m, 2 H, H-1, H-5ax), 3.49 (dd, *J* = 2.6, 1.3 Hz, 1 H, H-3), 3.53 (d, *J* = 2.0 Hz, 1 H, H-4), 4.02 (q, *J* = 7.1 Hz, 2 H, CH_3CH_2), 4.38 (t, *J* = 8.1 Hz 1 H, H-2), 8.19 (d, *J* = 7.6 Hz, 1 H, N*H*) ppm. ^{13}C NMR (100 MHz, DMSO, 24 °C): δ = 14.8, 23.3, 31.0, 42.9, 54.4, 54.8, 57.9, 61.1, 170.2, 173.9 ppm. Anal. Calcd for $C_{10}H_{15}NO_4$ (213.23): C, 56.33; H, 7.09; N, 6.57. Found: C, 56.78; H, 7.25; N, 6.46.

***tert*-Butyl (1*S**,2*R**,5*R**)-5-hydroxy-2-hydroxymethyl-cyclooctylcarbamate ((±)-162)**: a colourless oil (450 mg, 58%). ^{1}H NMR (500 MHz, DMSO, 25 °C): δ = 1.38 (s, 9H, *t*Bu), 1.39-1.48 (m, 3H, H-4, H-7, H-8), 1.47-1.56 (m, 2H, H-3ax, H-8), 1.61-1.67 (m, 1H, H-3eq), 1.73-1.78 (m, 1H, H-1), 1.87-1.93 (m, 2H, H-4, H-6), 2.82-2.91

(m, 2H, H-5, H-6), 3.19-3.25 (m, 1H, C*H*$_2$OH), 3.33-3.39 (m, 1H, C*H*$_2$OH), 3.81-3.86 (m, 1H, H-2), 4.43 (t, *J* = 4.8 Hz, 1H, CH$_2$O*H*), 6.49 (m, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 25 °C): δ = 23.2, 23.5, 23.7, 28.3, 28.8, 43.3, 49.1, 54.5, 54.8, 62.7, 77.7, 155.6 ppm. Anal. Calcd for $C_{14}H_{27}NO_4$ (273.37): C, 61.51; H, 9.96; N, 5.12. Found: C, 61.59; H, 9.88; N, 5.07.

4. SUMMARY

Simple and efficient regio- and stereospecific routes have been developed for the preparation of mono- and dihydroxy-functionalized cyclopentane-, cyclohexane- and cyclooctane-β-amino acids.

The racemic starting substances were prepared via the 1,2-cycloaddition of CSI and cyclopentadiene, which resulted in *cis*-6-azabicyclo[3.2.0]hept-3-en-7-one ((±)-**135**). This was reacted with ethanolic HCl to obtain ethyl *cis*-2-aminocyclopent-3-enecarboxylate hydrochloride ((±)-**136**.HCl), while treatment with aqueous HCl led to 2-aminocyclopent-3-enecarboxylic acid hydrochloride ((±)-**139**.HCl) in a yield of 74%. Protection of the amino group of ester hydrochloride (±)-**136**.HCl resulted in *N*-acetyl- ((±)-**137a**), *N*-Boc- ((±)-**13**) or *N*-Z-protected esters ((±)-**137b**), which was followed by NaOEt isomerization of (±)-**137a** and (±)-**13** to give the *trans N*-protected amino esters (±)-**102** and (±)-**138** in a yield of 37-51%. *N*-Protection of (±)-**139**.HCl with Boc_2O furnished *N*-Boc-protected amino acid (±)-**140**.

The reactions of ethyl *cis*- and *trans*-2-acetylaminocyclopent-3-enecarboxylate ((±)-**137a**, (±)-**138)** with NBS at room temperature afforded the bicyclic ethyl (3a*R**,4*R**,6*R**,6a*R**)- ((±)-**163**) and ethyl (3a*R**,4*S**,6*R**,6a*R**)-6-bromo-2-methyl-4,5,6,6a-tetrahydro-3aH-cyclopentaoxazole-4-carboxylate ((±)-**166**) in yields of 71-86%. On reduction of the bromo group with Bu_3SnH, followed by opening of the oxazoline ring by refluxing in 20% aqueous HCl, (1*S**,2*R**,3*S**)-2-amino-3-hydroxy-cyclopentanecarboxylic acid ((±)-**168**) was obtained. (1*R**,2*R**,3*S*)-2-Amino-3-hydroxycyclopentanecarboxylic acid ((±)-**165**) could be isolated from the mother liquor only by fractional crystallization.

Dihydroxylation of ethyl *cis*-2-acetylaminocyclopent-3-enecarboxylate ((±)-**13)**, ethyl *cis*- and *trans*-2-*tert*-butoxycarbonylaminocyclopent-3-enecarboxylate ((±)-**137a,b)** and ethyl *cis*-2-benzyloxycarbonylaminocyclopent-3-enecarboxylate ((±)-**102**) was carried out with a catalytic amount of OsO_4 and NMO as a stochiometric co-oxidant in acetone. The synthesized ethyl (1*R**,2*S**,3*S**,4*R**)-2-acetylamino-3,4-dihydroxycyclopentanecarboxylate ((±)-**173a**) and its *N*-Boc ((±)-**100**) and *N*-Z-protected ((±)-**173b**) counterparts, just like ethyl (1*R**,2*R**,3*S**,4*R**)-2-*tert*-butoxycarbonylamino-3,4-dihydroxycyclopentanecarboxylate ((±)-**103**), were deprotected under acidic conditions to result in (1*R**,2*R**,3*S**,4*R**)-2-amino-3,4-

dihydroxycyclopentanecarboxylic acid ((±)-**174**) and (1*S**,2*R**,3*S**,4*R**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid ((±)-**175**).

The optically pure mono- and dihydroxycyclopentane-β-amino acids ((+)-**165** and (–)-**174**) were also prepared by the synthetic methods mentioned above, but with a slight modification. The starting amino acid (+)-**139** was synthesized from racemic β-lactam (±)-**135** by Lipolase-catalysed ring opening, followed by esterification with $SOCl_2$ in EtOH.

Osmylation was accomplished from (1*S*,2*R*)-2-aminocyclohex-3-enecarboxylic acid ((+)-**143**) and (1*R*,2*S*)-2-aminocyclohex-4-enecarboxylic acid ((+)-**148**), which were prepared by CAL-B-catalysed hydrolysis of racemic β-lactam (±)-**142** or amino ester (±)-**147**. After esterification and *N*-protection, the compounds were isomerized to esters (+)-**145** and (–)-**149** with NaOEt. In the acidic hydrolysis of the protecting groups, a newly published deprotection reaction was also applied in order to improve the yields of the final products. MW irradiation in water at 150 °C for 1 h resulted in the expected (1*R*,2*R*,3*S*,4*R*)- and (1*S*,2*R*,3*S*,4*R*)-2-amino-3,4-dihydroxycyclohexane-carboxylic acids ((–)-**179** and (–)-**181**) and (1*S*,2*R*,4*R*,5*S*)- and (1*R*,2*R*,4*R*,5*S*)-2-amino-4,5-dihydroxycyclohexanecarboxylic acids ((–)-**183** and (–)-**185**) in yields of 70-77%.

The racemic dihydroxy compounds were prepared via ring opening of β-lactams (±)-**142** and (±)-**152** with EtOH/HCl**,** followed by *N*-protection of the amino group. Azetidinones (±)-**142** and (±)-**152** were prepared by CSI addition to 1,3- or 1,4-cyclohexadiene (**141** and **151**)**.**

Iodolactonization was applied for the preparation of (1*R*,2*S*,6*R*)-2-amino-6-hydroxycyclooctanecarboxylic acid (–)-**172**. The starting chiral (1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclooct-5-enecarboxylic acid ((–)-**158**) was prepared by the Lipolase-catalysed reaction of (1*R**,2*S**)-9-azabicyclo[6.2.0]dec-4-en-10-one ((±)-**154**), followed by esterification with $SOCl_2$ in EtOH and *N*-protection of the amino group with Boc_2O. Reaction of the enantiopure *N*-Boc-amino acid (–)-**158** with I_2/KI in a two-phase solvent system resulted in iodolactone (–)-**169**. Reduction of the iodo group with Bu_3SnH and hydrolysis of the lactone ring by MW irradiation gave the (1*R*,2*S*,6*R*)-2-amino-6-hydroxycyclooctanecarboxylic acid ((–)-**172**) in *ee* > 99%. The same final product was observed when amino group was deprotected first with aqueous HCl and subsequent opening of the lactone ring with MW irradiation in H_2O.

The investigations of OsO_4 dihydroxylation were extended to the cyclooctane skeleton. (1*R*,2*S*,5*R*,6*S*)-2-Amino-5,6-dihydroxy-cyclooctane-carboxylic acid ((–)-**187**

was prepared by using a catalytic amount of OsO_4 and NMO. In the last step, deprotection was performed with MW irradiation.

The racemic mono- and dihydroxycyclooctane-β-amino acids (±)-**172** and (±)-**187** were synthesized from β-lactam (±)-**154**, which was prepared by 1,2-cycloaddition of CSI and 1,5-cyclooctadiene (**153**), and transformed to the amino ester hydrochloride by ring opening with EtOH/HCl.

The enantiopurities of the starting materials and final products were proved by GC and HPLC. The stereochemistry of the novel materials was determined by NMR spectroscopy and X-ray crystallography.

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ANNEX

TABLE OF THE SYNTHESIZED COMPOUNDS

Compound number in the thesis	Article number/ Compound number in the article
13	I/4b
57c	II/4
100	I/13b
102	I/15
103	I/16
134	I/1
135	I/2
136	I/3
137a,b	I/4a,c
138	I/9
139.HCl	I/5
142	II/10
143	II/11
144	II/13
145	II/16
147	II/1
148	II/2
149	II/7
154	III/1
155	III/2
156	III/4
157	III/5
158	III/6

Compound number in the thesis	Article number/ Compound number in the article
163	I/6
164	I/7
165	I/8
166	I/10
167	I/11
168	I/12
169	III/7
170	III/8
171	III/9
172	III/10
173 a,b	I/13a,c
174	I/14
175	I/17
178	II/14
179	II/15
180	II/17
181	II/18
182	II/5
183	II/6
184	II/8
185	II/9
186	III/11
187	III/12

I

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Efficient Synthesis of Hydroxy-Substituted Cispentacin Derivatives

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Keywords: Amino acids / Hydroxycispentacin / Hydroxylation / Diastereoselectivity / Regioselectivity

Starting from *N*-protected *cis*- and *trans*-2-aminocyclopent-3-enecarboxylic acid derivatives, isomers of 2-amino-3-hydroxycyclopentanecarboxylic acid (**8** and **12**) were prepared via oxazoline intermediates, whereas the stereoisomeric 2-amino-3,4-dihydroxycyclopentanecarboxylic acids **14** and **17** were synthesized by OsO_4-catalyzed oxidation. The enantiomers of **8** and **14** were also prepared by the same pathway. The structures, stereochemistry and relative configurations of the synthesized compounds were proved by NMR spectroscopy.

Introduction

The importance of alicyclic β-amino acids,[1,2] derived from β-lactams,[3,4] has recently increased because of their occurrence in many pharmacologically important compounds.[5] They can also be introduced into peptides to increase and modify their biological activity.[6] These compounds are found in a large number of natural products, some of which, for example, cispentacin [(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid], exhibit antifungal activity.[7–9] The 4-methylene analogue of cispentacin[10–12] (Icofungipen, PLD-118) is a representative of a novel class of antifungals that are active in vitro against *Candida* species.[13] This β-amino acid actively accumulates in yeast, competitively inhibiting isoleucyl-*t*RNA synthetase and consequently disrupting protein biosynthesis.[14,15]

Hydroxy-functionalized β-amino acids play an important role in medicinal chemistry because they also occur in many important and essential products such as Paclitaxel (Taxol) and Docetaxel (Taxotere), which have chemotherapeutic effects.[16–18] Although of less biological importance than their open-chain analogues, some cyclic hydroxylated β-amino acid derivatives have antibiotic (oryzoxymycin)[19–22] or antifungal activities and are building blocks for pharmaceutically important natural substances.[23]

In our earlier work we described several methods for the synthesis of hydroxy-substituted cyclohexane β-amino acids. The introduction of a hydroxy group into the cyclohexane ring was accomplished stereoselectively starting from *cis*- and *trans*-2-aminocyclohexenecarboxylic acids by iodolactonization or via the corresponding oxazine derivatives.[24,25] Another method involves the hydroxylation of the 2-aminocyclohexenecarboxylic acid by functionalization of the olefinic bond by epoxidation.[26–28]

Only a few syntheses of hydroxylated 2-aminocyclopentanecarboxylic acid derivatives have been reported.[29–32] For example, 2-amino-4-hydroxycyclopentanecarboxylic acid was synthesized from the 4-methylene analogue of cispentacin. After esterification and protection, ozonolysis of the olefinic bond, reduction of the carbonyl group, hydrolysis and deprotection resulted in a diastereomeric (3:1) mixture of the 4-hydroxylated amino acid.[10,31] The all-*cis*-2-amino-4-hydroxycyclopentanecarboxylic acid was accessible by a Curtius reaction of the half acid derived from the *meso*-diester of the appropriate 4-oxocyclopentane and subsequent removal of the oxo group by Clemensen reduction.[30] (1*R*,2*S*,5*S*)-5-Amino-2-hydroxycyclopentanecarboxylic acid can be obtained by lithium amide conjugate addition to ε-oxo α,β-unsaturated esters and subsequent intramolecular cyclization.[29] The polyhydroxylated *trans*-2-aminocyclopentanecarboxylic acid was formed from a D-glucose derivative via a bicyclic sugar nitrolactone.[32]

The aim of this work was to functionalize the olefinic bond of *cis*-2-amino-3-cyclopentenecarboxylic acid and to synthesize and structurally analyze new mono- or dihydroxy-substituted derivatives.

Results and Discussion

Diastereomerically pure β-lactam **2** was prepared by 1,2-cycloaddition of chlorosulfonyl isocyanate (CSI) in Et_2O at –10 °C by a modification of a literature procedure.[33] Ring-opening of the β-lactam with ethanolic HCl resulted in the amino ester hydrochloride **3**, which was protected with AcCl, di-*tert*-butyl dicarbonate or benzyl chloroformate to give *N*-acylated esters **4a**–**c** (Scheme 1). An alternative syn-

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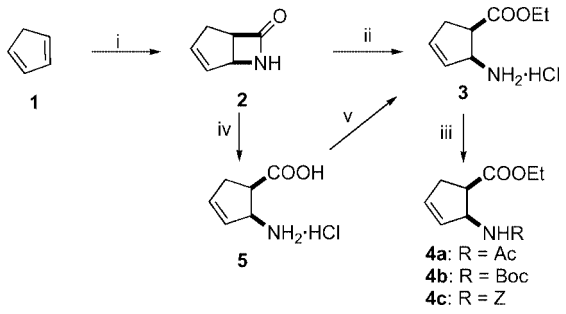
thesis of **3** comprised hydrolysis of β-lactam **2** with concentrated aqueous HCl at room temperature to amino acid hydrochloride **5**, which was esterified in the presence of EtOH and $SOCl_2$ to give amino ester hydrochloride **3**, which was then acylated by the above methods.



Scheme 1. Reagents and conditions: (i) CSI, Et_2O, 3 h, –10 °C, Na_2SO_3, 61%; (ii) HCl/EtOH, room temp., 1 h, 64%; (iii) R = Ac: Et_3N, AcCl, $CHCl_3$, 2 h, room temp., 73%; R = Boc: Et_3N, Boc_2O, toluene, 2 h, room temp., 69%; R = Z: Et_3N, ZCl, THF, 16 h, room temp., 65%; (iv) concd. HCl, 74%; (v) $SOCl_2$, EtOH, 30 min, 0 °C; 3 h room temp.; 1 h, Δ*T*, 86%.

The selective iodolactonization of *cis*- and *trans*-2-amino-4-cyclohexenecarboxylic acids and *cis*-2-amino-3-cyclohexenecarboxylic acid has previously been reported.[24,25] The iodolactonization of *cis*-2-*tert*-butoxycarbonylaminocyclopent-3-enecarboxylic acid with I_2/KI/$NaHCO_3$ was attempted, but not even traces of the iodolactone product were observed. The starting compound remained unchanged, most probably because of the unstable lactone ring.

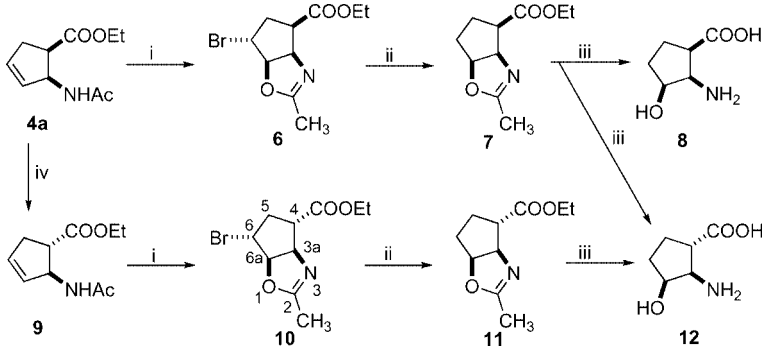
Another possible way to introduce a hydroxy group is the epoxidation of the double bond and then opening of the oxirane ring.[28] Epoxidation of ethyl *cis*-2-acetylamino- (**4a**) and ethyl *cis*-2-benzyloxycarbonylaminocyclopent-3-enecarboxylate (**4c**) in the presence of *m*-chloroperbenzoic acid in CH_2Cl_2 gave the corresponding *cis*-epoxides.[28] In the presence of $NaBH_4$ in EtOH, the oxirane ring remained unchanged.

When *N*-acetyl derivative **4a** was treated with *N*-bromosuccinimide (NBS), bicyclic bromooxazoline derivative **6** was obtained regio- and diastereoselectively (Scheme 2). Not even traces of other regio- or diastereomers were observed in the crude product, according to Markovnikov's rule.[34] Bromooxazoline **6** was then converted into oxazoline **7** by reduction of the bromo group with Bu_3SnH under argon. When **7** was heated to reflux in a 20% aqueous solution of HCl, partial *cis* → *trans* isomerization took place, and ion-exchange chromatography followed by fractional crystallization resulted in (1*S**,2*R**,3*S**)-2-amino-3-hydroxycyclopentanecarboxylic acid (**12**). From the mother liquor the all-*cis*-2-amino-3-hydroxycyclopentanecarboxylic acid (**8**) was also isolated by crystallization (Scheme 2).

The isomerization of **4a** with NaOEt gave the *trans*-*N*-acetyl amino ester **9**. It is known from the literature that such reactions are generally not quantitative and the yields are therefore low.[28] Hydrolysis of **7** followed by isomerization resulted in the corresponding (1*S**,2*R**,3*S**)-2-amino-3-hydroxycyclopentanecarboxylic acid (**12**).

Compounds **6**, **7**, **10** and **11** were characterized by NMR measurements. The values of ^3J(3a-H,6a-H) were in the range of 7.2–7.8 Hz, and large NOE signals were observed between 3a-H and 6a-H, which supports the expected *cis* ring anellation. The fact that no coupling was observed between 6-H and 6a-H for **6** and **10** suggests a *trans*-diequatorial orientation. Consequently, the 6-bromo group should be *trans* relative to the oxazoline ring. In **6** and **7**, ^3J(3a-H,4-H) = 7.2 Hz, and the large NOE signal between 3a-H and 4-H indicates a *cis* (synperiplanar) orientation for 3a-H and 4-H, and consequently a *cis* orientation for the C-4 substituent relative to the oxazoline ring. For **10** and **11**, no coupling was observed between 4-H and 3a-H, which indicates that 4-H and 3a-H are perpendicular to each other. This, together with the small NOE signal between 4-H and 3a-H, suggests a *trans*-diequatorial orientation for the hydrogen atoms and a *trans*-diaxial orientation for the C-3a and C-4 substituents.

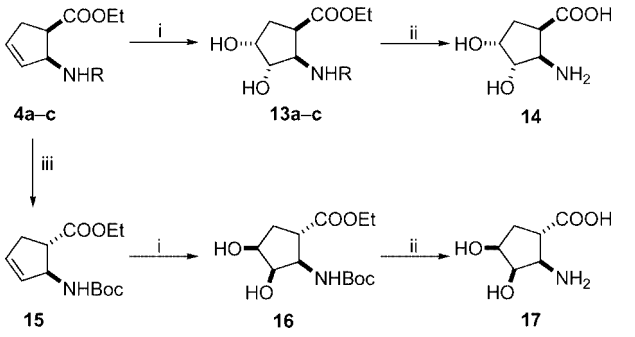
For **12**, the small NOE signal between 1-H and 2-H and the value of ^3J(1-H,2-H) = 9.5 Hz suggest a *trans*-diaxial orientation. Thus, the C-1 and C-2 substituents are *trans*. ^3J(2-H,3-H) = 5.0 Hz indicates an equatorial position for 3-H, which, together with the large NOE between 2-H and 3-H, points to *cis*-oriented C-2 and C-3 substituents. The large NOE signal between 1-H and 2-H and the value ^3J(1-H,2-H) = 6.5 Hz for **8** suggest a *cis*-oriented C-1 carboxy group relative to the C-2 amino group. This is sup-



Scheme 2. Reagents and conditions: (i) NBS, CH_2Cl_2, 3 h, room temp., 71–86%; (ii) Bu_3SnH, CH_2Cl_2, Ar, 20 h, Δ*T*, 63–65%; (iii) 20% HCl/H_2O, 24 h, Δ*T*, 22–67%; (iv) NaOEt, EtOH, 24 h, room temp., 37%.

ported by the fact that an NOE signal was observed between 1-H and 3-H in **8**, whereas no signal was observed between these hydrogen atoms in **12**.

The osmium-catalyzed dihydroxylation of olefins is one of the most efficient methods for the preparation of vicinal diols.[35,36] Ethyl 2-benzoylaminocyclopent-3-enecarboxylate has been successfully dihydroxylated with OsO_4 and 4-methylmorpholine *N*-oxide (NMO).[36] The facile dihydroxylation of olefins **4a**–**c** by oxidation with catalytic OsO_4 and NMO as the stoichiometric co-oxidant afforded the desired products **13a**–**c** as single diastereomers in good yields (Scheme 3). Not even traces of other diastereomers were observed in the crude product, as determined by ^{1}H NMR spectroscopy. The ester functions of **13a**–**c** were then hydrolyzed and the amino group then deprotected to furnish the desired (1*R**,2*R**,3*S**,4*R**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid (**14**).



Scheme 3. Reagents and conditions: (i) 2.0 wt.-% solution of OsO_4 in *t*BuOH, NMO, acetone, 4 h, room temp.; R = Ac: 47%; R = Boc: 56%; R = Z: 58%; (ii) R = Ac: 20% HCl/H_2O, Δ*T*, 48 h; R = Boc: 10% HCl/H_2O, 24 h, room temp.; R = Z: 5% Pd/C, H_2, EtOH, 12 h, room temp.; 20% HCl/H_2O, Δ*T*, 24 h, 35–37%; (iii) NaOEt, EtOH, 24 h, room temp., 51%.

The isomerization of **4b** with NaOEt resulted in *trans*-*N*-Boc amino ester **15**. By a transformation similar to that of the *cis* isomer **4a**, *trans*-*N*-Boc amino ester **15** reacted via the dihydroxy ester intermediate **16** to yield the corresponding (1*S**,2*R**,3*R**,4*R**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid (**17**) (Scheme 3).

For **13c** the value ^3J(1-H,2-H) = 8.1 Hz and the very large NOE signal between 1-H and 2-H indicate a *cis* (synperiplanar) orientation of the C-1 and C-2 substituents. The 3-H and 4-H atoms and consequently the C-3 and C-4 hydroxy groups should also be *cis* relative to each other because of the large NOE signal between 3-H and 4-H and the value ^3J(3-H,4-H) = 3.5 Hz. Moreover, the hydroxy groups are located on opposite sides of the cyclopentane ring to the C-1 and C-2 substituents; this can be concluded from the small NOE signal between 2-H and 3-H, the large signal between 3-H and the NH group, and the absence of an NOE signal between 3-H and 1-H or between 4-H and 1-H. The same pattern was detected for **13a**,**b**.

The double bonds in **4a**–**c** undergo oxidation on the sterically less hindered side of the ring. The diastereoselectivity of the dihydroxylation of **15** is not likely to be determined by simple steric repulsion because both the ester and the protected amino group are in equatorial positions. In this case, the hydroxy groups have the same steric orientation as the protected amino group in the final product. This can be rationalized by the probability of an electrostatically advantageous interaction in the intermediate complex of OsO_4 and **15** between the partially positive amide hydrogen atom and the partially negative oxygen atom attacking the sp^2 carbon atom vicinal to the protected amine substituent. This interaction is possible only if OsO_4 is in juxtaposition with the NHBoc moiety.

In the ring closures of the 1,2-disubstituted 1,2- and 1,3-difunctionalized cycloalkanes, striking differences were observed in the cyclization reaction: although the *cis* isomers reacted readily, their *trans* counterparts did not undergo ring closure in most cases.[37,38]

On this basis, we also attempted to prove the relative configurations of **14** and **17** chemically. Compound **14** was esterified with CH_2N_2 and then allowed to react in MeOH with 1 equiv. of *p*-nitrobenzaldehyde. A well-defined product was obtained. ^{1}H NMR spectroscopy indicated that in $CDCl_3$ this compound exists solely as the open Schiff base form.[38] A well-defined signal was observed at δ = 8.4 ppm (s, 1 H, N=CH). No peaks were detected in the interval δ = 5–6 ppm (ring closure product: s, 1 H, NCHO), which underlines the *trans* orientation of the 2-NH_2 and 3-OH substituents. In contrast, for the similar transformation of **17**, both the open Schiff base form and the ring-closure product were observed in the ^{1}H NMR spectra, which points to a *cis* orientation of the 2-NH_2 and 3-OH substituents.

For **15**, the value ^3J(1-H,2-H) = 8.6 Hz and the small NOE signal between 1-H and 2-H suggest a *trans* orientation for 1-H and 2-H. Consequently, the C-1 and C-2 substituents are *trans*. Compound **9** exhibits the same coupling pattern and stereochemistry. For **16** and **17**, the values ^3J(1-H,2-H) = 6–7 Hz and the small NOE signals between 1-H and 2-H point to a *trans* orientation for 1-H and 2-H. Consequently, the carboxy and amino groups should again be *trans*. The relative orientation of the hydroxy groups is *cis* because of the large NOE and the small coupling constant between 3-H and 4-H, and they are located on the same side of the cyclopentane ring as the C-2 substituent. This is proved by the large NOE between 2-H and 3-H. Moreover, in **16**, which was dissolved in [D_6]DMSO for the NMR experiments, NOE signals were observed between the NH and OH groups. The positions of the hydroxy groups are supported by the fact that no NOE signal was detected between 1-H and 3-H or between 1-H and 4-H, whereas a small NOE was found between 2-H and 4-H.

All the above reactions were also performed starting from the enantiomeric (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylic acid hydrochloride [(+)-**5**]. Compound (+)-**5** was prepared by Lipolase (lipase B from *Candida antarctica*) catalyzed enantioselective ring cleavage of *rac*-6-azabicyclo[3.2.0]hept-3-en-7-one (**2**) in iPr_2O at 70 °C.[39] This enantiopure amino acid was transformed with $SOCl_2$ into ethyl (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylate hydrochloride [(+)-**3**]. The 3-hydroxy- and 3,4-dihydroxyamino

acid derivatives (+)-**8** and (–)-**14**, respectively, were synthesized similarly to the corresponding *rac* compounds (Schemes 2 and 3).

Conclusion

Effective and stereoselective routes to mono- and dihydroxylated *cis*- and *trans*-2-aminocaclopentanecarboxylic acid has been developed via oxazoline intermediates and OsO_4-catalyzed oxidation, respectively. Ongoing work uses these amino acids to create foldameric structures.

Experimental Section

General Procedures: ^{1}H NMR spectra were recorded at 400.13 MHz and ^{13}C NMR spectra at 100.62 MHz in D_2O or in [D_6]DMSO at ambient temperature with a Bruker AM 400 spectrometer. Some spectra were recorded with a Bruker AV 600 spectrometer at 600.20 and 150.94 MHz for the ^{1}H and ^{13}C NMR spectra, respectively. Chemical shifts are given in δ (ppm) relative to TMS as the internal standard. Elemental analyses were performed with a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer. Melting points were measured with a Kofler melting point apparatus and are uncorrected. The *ee* for (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylic acid hydrochloride [(+)-**5**] (>99%) was determined by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m) after double derivatization with (i) CH_2N_2 and (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine [120 °C for 5 min → 190 °C (rate of temperature rise 10 °C/min, 140 kPa), retention time: 11.51 min], whereas the *ee* for the corresponding ethyl (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylate hydrochloride [(+)-**3**] (>99%) was determined under the same conditions, but the sample was derivatized only with Ac_2O (retention time: 12.28 min). As the *ee* of ethyl (1*R*,2*S*)-2-aminocyclopent-3-enecarboxylate hydrochloride [(+)-**3**] is >99% and not even traces of other diastereomers were detected in the prepared compounds by ^{1}H NMR spectroscopy, the *ee* values for the products are undoubtedly >99%.

***cis*-6-Azabicyclo[3.2.0]hept-3-en-7-one (2):** A solution of CSI (10.5 g, 74.19 mmol) in dry Et_2O (50 mL) was added dropwise to a solution of freshly distilled 1,3-cyclopentadiene (7.00 g, 105.90 mmol) dissolved in dry Et_2O (100 mL) at –10 °C. After the addition was completed, the resulting colourless solution was stirred for 40 min. The reaction mixture was then poured into a stirred solution of Na_2SO_3 (7.6 g, 60.8 mmol) in water (100 mL) and the pH was adjusted to 8–9 with 15% KOH. After stirring at 0 °C for 3 h, the organic layer was separated and the aqueous layer washed with Et_2O (2 × 250 mL) and then with EtOAc (2 × 250 mL). The combined organic layers were dried with Na_2SO_4, and the solution was concentrated to dryness under reduced pressure. The residue was purified by column chromatography (*n*-hexane/EtOAc, 1:3) to afford white crystals (7.04 g, 61%), m.p. 30–32 °C (ref.[33] oil). ^{1}H NMR (400 MHz, $CDCl_3$, 30 °C): δ = 2.42–2.49 (m, 1 H, 2-H), 2.69–2.76 (m, 1 H, 2-H), 3.82–3.84 (m, 1 H, 1-H), 4.50–4.51 (m, 1 H, 5-H), 5.93–5.95 (m, 1 H, 4-H), 6.01–6.03 (m, 1 H, 4-H), 6.01–6.03 (m, 1 H, 3-H), 6.48 (br. s, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 30 °C): δ = 31.5, 54.0, 59.9, 131.3 137.7, 173.0 ppm. C_6H_7NO (109.13): calcd. C 66.04, H 6.47, N 12.84; found C 66.22, H 6.55, N 12.61.

Ethyl *cis*-2-Aminocyclopent-3-enecarboxylate Hydrochloride (3): A solution of β-lactam **2** (5 g, 45.82 mmol) in EtOH containing 22% HCl (50 mL) was stirred at room temperature for 1 h. After removal of the solvent, amino ester hydrochloride **3** was obtained, which was recrystallized from EtOH/Et_2O. Colourless crystals (5.62 g, 64%), m.p. 190–193 °C (ref.[28] 198–200 °C). ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.24 (t, *J* = 7.1 Hz, 3 H, CH_3CH$_2$), 2.60 (dd, *J* = 8.8, 16.8 Hz, 1 H, 5-H$_{eq}$), 2.76 (dddd, *J* = 2.0, 4.5, 8.1, 16.8 Hz, 1 H, 5-H$_{ax}$), 3.42 (ddd, *J* = 7.7, 8.1, 8.8 Hz, 1 H, 1-H), 4.09–4.18 (m, 2 H, CH$_3$CH_2), 4.28 (d, *J* = 7.7 Hz, 1 H, 2-H), 5.78–5.82 (m, 1 H, 3-H), 6.12–6.17 (m, 1 H, 4-H), 8.19 (s, 3 H, NH_2) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.8, 34.9, 44.6, 56.1, 61.7, 128.2, 137.7, 171.8 ppm. $C_8H_{14}ClNO_2$ (191.70): calcd. C 50.08, H 7.30, Cl 18.52, N 7.30; found C 50.12, H 7.45, N 7.19.

Ethyl *cis*-2-Aminocyclopent-3-enecarboxylate Hydrochloride (3): Thionyl chloride (4.42 g, 37.15 mmol) was added dropwise with stirring to dry EtOH (31 mL) at –15 °C. Compound **5** (5.55 g, 30.92 mmol) was added in one portion to this mixture, which was then stirred at 0 °C for 30 min. After stirring at room temperature for 3 h, the mixture was refluxed for a further 1 h and then concentrated. The residue was recrystallized from EtOH/Et_2O to give colourless crystals. Yield (5.59 g, 86%); m.p. 190–193 °C (ref.[28] 198–200 °C). ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.24 (t, *J* = 7.1 Hz, 3 H, CH_3CH$_2$), 2.60 (dd, *J* = 8.8, 16.8 Hz, 1 H, 5-H^{eq}), 2.76 (dddd, *J* = 2.0, 4.5, 8.1, 16.8 Hz, 1 H, 5-H^{ax}), 3.42 (ddd, *J* = 7.7, 8.1, 8.8 Hz, 1 H, 1-H), 4.09–4.18 (m, 2 H, CH$_3$CH_2), 4.28 (d, *J* = 7.7 Hz, 1 H, 2-H), 5.78–5.82 (m, 1 H, 3-H), 6.12–6.17 (m, 1 H, 4-H), 8.19 (s, 3 H, NH_2) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.8, 34.9, 44.6, 56.1, 61.7, 128.2, 137.7, 171.8 ppm. $C_8H_{14}ClNO_2$ (191.70): calcd. C 50.08, H 7.30, Cl 18.52, N 7.30; found C 50.12, H 7.45, N 7.19.

Ethyl *cis*-2-Acetylaminocyclopent-3-enecarboxylate (4a): Et_3N (4.05 g, 40 mmol) and AcCl (1.88 g, 24 mmol) were added to a suspension of **3**·HCl (3.83 g, 20 mmol) in $CHCl_3$ (50 mL), and the mixture was stirred at room temperature for 2 h and then washed with water (2 × 20 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined organic phases were dried (Na_2SO_4) and the solvents evaporated. The residue was purified by column chromatography (*n*-hexane/EtOAc, 1:3) to afford white crystals (2.88 g, 73%), m.p. 84–86 °C. ^{1}H NMR (400 MHz, [D_6]-DMSO, 30 °C): δ = 1.14 (t, *J* = 7.1 Hz, 3 H, CH_3CH$_2$), 1.74 (s, 3 H, COCH$_3$), 2.41 (dd, *J* = 8.7, 16.7 Hz, 1 H, 5-H$_{eq}$), 2.73 (dddd, *J* = 2.3, 4.9, 7.2, 16.7 Hz, 1 H, 5-H$_{ax}$), 3.27 (ddd, *J* = 7.2, 8.6, 8.7 Hz, 1 H, 1-H), 3.93–4.05 (m, 2 H, CH$_3$CH_2), 5.17 (dd, *J* = 8.6, 9.2 Hz, 1 H, 2-H), 5.51–5.54 (m, 1 H, 3-H), 5.90–5.94 (m, 1 H, 4-H), 7.75 (d, *J* = 9.2 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.0, 22.2, 33.3, 45.8, 55.0, 59.7, 129.5, 132.8, 168.7, 172.3 ppm. $C_{10}H_{15}NO_3$ (197.24): calcd. C 60.90, H 7.67, N 7.10; found C 60.67, H 8.05, N 7.47.

Ethyl *cis*-2-*tert*-Butoxycarbonylaminocyclopent-3-enecarboxylate (4b): Et_3N (4.05 g, 40 mmol) and di-*tert*-butyl dicarbonate (5.24 g, 24 mmol) were added to a suspension of **3**·HCl (3.83 g, 20 mmol) in toluene (50 mL) at 0 °C. Stirring was continued at room temperature for 2 h, after which the organic layer was washed with H_2O (2 × 20 mL) and the aqueous layer extracted with EtOAc. The combined organic phases were dried (Na_2SO_4) and the solvents evaporated. The residue was recrystallized from *n*-hexane to give a white solid (3.52 g, 69%), m.p. 97–99 °C (ref.[28] 89–91 °C). ^{1}H NMR (600 MHz, [D_6]DMSO, 25 °C): δ = 1.17 (t, *J* = 7.1 Hz, 3 H, CH_3CH$_2$), 1.35 (s, 9 H, *t*Bu), 2.32 (dd, *J* = 8.5, 16.5 Hz, 1 H, 5-H$_{eq}$), 2.72 (dddd, *J* = 2.5, 4.5, 7.4, 16.5 Hz, 1 H, 5-H$_{ax}$), 3.21 (ddd, *J* = 7.4, 8.5, 8.6 Hz, 1 H, 1-H), 3.97–4.04 (m, 2 H, CH$_3$CH_2), 4.85 (dd, *J* = 8.6, 9.4 Hz, 1 H, 2-H), 5.51–5.54 (m, 1 H, 3-H), 5.86 (dd,

J = 2.1, 5.1 Hz, 1 H, 4-H), 6.70 (d, J = 9.4 Hz, 1 H, NH) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C): δ = 14.0, 28.2, 33.0, 46.2, 56.9, 59.7, 77.6, 129.3, 132.7, 154.6, 171.8 ppm. $C_{13}H_{21}NO_4$ (255.32): calcd. C 61.16, H 8.29, N 5.49; found C 61.18, H 8.63, N 5.83.

Ethyl *cis*-2-Benzyloxycarbonylaminocyclopent-3-enecarboxylate (4c): Benzyl chloroformate (5.12 g, 30 mmol) was added to a solution of **3**·HCl (3.83 g, 20 mmol) and Et_3N (4.05 g, 40 mmol) in THF (160 mL) at 0 °C. After stirring at room temperature for 16 h, the mixture was taken up in EtOAc (300 mL), washed with H_2O, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was recrystallized from *n*-hexane to give a white solid (4.2 g, 65%), m.p. 55–57 °C (ref.[28] 60–65 °C). ^{1}H NMR (400 MHz, [D$_6$]-DMSO, 30 °C): δ = 1.08 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 2.36 (dd, J = 8.6, 16.7 Hz, 1 H, 5-H$_{eq}$), 2.74 (dddd, J = 2.3, 4.8, 8.2, 16.7 Hz, 1 H, 5-H$_{ax}$), 3.28 (ddd, J = 8.2, 8.6, 9.0 Hz, 1 H, 1-H), 3.95 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2), 4.89–5.00 (m, 3 H, 2-H, OCH_2Ph), 5.53–5.57 (m, 1 H, 3-H), 5.89 (d, J = 4 Hz, 1 H, 4-H), 7.26 (d, J = 9.5 Hz, 1 H, NH), 7.28–7.39 (m, 5 H, Ph) ppm. ^{13}C NMR (100 MHz, [D$_6$]-DMSO, 30 °C): δ = 14.8, 33.9, 47.1, 58.4, 60.6, 66.0, 128.6, 129.1, 130.1, 133.9, 138.0, 156.2, 172.5 ppm. $C_{16}H_{19}NO_4$ (289.33): calcd. C 66.42, H 6.62, N 4.84; found C 66.54, H 6.92, N 5.11.

***cis*-2-Aminocyclopent-3-enecarboxylic Acid Hydrochloride (5):** A solution of β-lactam **2** (5 g, 45.82 mmol) in concentrated HCl (50 mL) was stirred at room temperature for 1 h. After removal of the solvent, the resulting amino acid hydrochloride **5** was recrystallized from EtOH/Et_2O to give a white crystalline solid (5.55 g, 74%), m.p. 185–188 °C (ref.[28] 178–180 °C). ^{1}H NMR (400 MHz, D_2O, 30 °C): δ = 2.58–2.64 (m, 2 H, 5-H), 3.26 (q, J = 8.2 Hz, 1 H, 1-H), 4.24 (d, J = 7.6 Hz, 1 H, 2-H), 5.79–5.83 (m, 1 H, 3-H), 6.18 (d, J = 4.4 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, D_2O, 30 °C): δ = 34.9, 47.8, 57.0, 128.5, 137.7, 180.1 ppm. $C_6H_{10}ClNO_2$ (163.6): calcd. C 44.05, H 6.16, Cl 21.67, N 8.56; found C 43.85, H 6.32, N 8.45.

Ethyl *trans*-2-Acetylaminocyclopent-3-enecarboxylate (9): Freshly prepared NaOEt (1.46 g, 21.54 mmol) was added to a solution of ethyl *cis*-2-acetylaminocyclopent-3-enecarboxylate (**4a**; 5.5 g, 21.54 mmol) in anhydrous EtOH (40 mL), and the mixture was stirred at room temperature for 24 h. It was then concentrated under reduced pressure and taken up in EtOAc, washed with H_2O (2 × 20 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/EtOAc, 1:3) to afford white crystals (2.05 g, 37%), m.p. 56–58 °C. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30 °C): δ = 1.18 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.79 (s, 3 H, COCH$_3$), 2.43 (dddd, J = 2.0, 4.2, 5.8, 15.8 Hz, 1 H, 5-H$_{eq}$), 2.66–2.79 (m, 2 H, 1-H, 5-H$_{ax}$), 4.07 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2), 4.97–5.03 (m, 1 H, 2-H), 5.54–5.58 (m, 1 H, 3-H), 5.80–5.84 (m, 1 H, 4-H), 8.06 (d, J = 7.4 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, 30 °C): δ = 14.9, 23.4, 36.3, 50.0, 59.5, 61.0, 131.9, 131.9, 169.5, 175.0 ppm. $C_{10}H_{15}NO_3$ (197.24): calcd. C 60.90, H 7.67, N 7.10; found C 60.78, H 7.56, N 7.02.

General Procedure for the Synthesis of Bromooxazolines 6 and 10: A solution of *N*-acetylamino ester **4a** or **9** (2 g, 10.14 mmol) in CH_2Cl_2 (80 mL) was treated with 1.1 equiv. of NBS, and the reaction mixture was stirred at room temperature for 3 h. When the reaction was complete (monitored by TLC), the mixture was treated with aqueous NaOH solution (10%, 3 × 20 mL). The aqueous solution was next extracted with CH_2Cl_2 (3 × 40 mL), the combined organic layers were dried (Na_2SO_4) and the solvents evaporated. The residue was purified by column chromatography (CH_2Cl_2/EtOAc, 10:1) to give a yellow oil.

Ethyl (3a*R,4*R**,6*R**,6a*R**)-6-Bromo-2-methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (6):** Yield: 2.41 g, 86%. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30 °C): δ = 1.21 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.87 (s, 3 H, CH$_3$), 2.07 (dd, J = 5.8, 14.9 Hz, 1 H, 5-H$_{eq}$), 2.17 (ddd, J = 4.8, 12.5, 14.9 Hz, 1 H, 5-H$_{ax}$), 3.40 (ddd, J = 6.2, 7.2, 12.5 Hz, 1 H, 4-H), 4.04–4.15 (m, 2 H, CH$_3$CH_2), 4.55 (d, J = 4.8 Hz, 1 H, 6-H), 4.88 (dd, J = 7.2, 7.3 Hz, 1 H, 3-H), 5.05 (d, J = 7.3 Hz, 1 H, 7-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, 30 °C): δ = 13.9, 15.0, 34.7, 48.2, 53.5, 60.9, 72.4, 89.4, 164.6, 170.9 ppm. $C_{10}H_{14}BrNO_3$ (276.13): calcd. C 43.50, H 5.11, N 5.07; found C 43.39, H 5.13, N 5.20.

Ethyl (3a*R,4*S**,6*R**,6a*R**)-6-Bromo-2-methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (10):** Yield: 1.99 g, 71%. ^{1}H NMR (600 MHz, [D$_6$]DMSO, 25 °C): δ = 1.21 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.89 (s, 3 H, CH$_3$), 2.19–2.27 (m, 1 H, 5-H$_{eq}$), 2.40 (ddd, J = 4.6, 5.0, 14.5 Hz, 1 H, 5-H$_{ax}$), 2.78 (ddd, J = 2.8, 5.1, 7.8 Hz, 1 H, 4-H), 4.12–4.22 (m, 2 H, CH$_3$CH_2), 4.35 (d, J = 4.8 Hz, 1 H, 6-H), 4.81 (d, J = 7.8 Hz, 1 H, 3a-H), 5.01 (d, J = 7.8 Hz, 1 H, 6a-H) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO, 25 °C): δ = 13.3, 13.9, 35.8, 49.7, 52.1, 60.5, 72.4, 88.9, 162.8, 171.9 ppm. $C_{10}H_{14}BrNO_3$ (276.13): calcd. C 43.50, H 5.11, N 5.07; found C 43.42, H 5.17, N 4.95.

General Procedure for the Dehalogenation of Bromooxazolines 6 and 10 to 7 and 11: Bu_3SnH (4.07 g, 14 mmol) was added to a solution of the bromooxazoline (1.93 g, 7 mmol) in CH_2Cl_2 (120 mL) under Ar, and the reaction mixture was stirred at 40 °C for 20 h. The solvent was then evaporated and the residue purified by column chromatography on silica gel (*n*-hexane/EtOAc, 9:1) to afford the oxazoline as an oil.

Ethyl (3a*R,4*R**,6a*S**)-2-Methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (7):** Yield: 0.89 g, 65%. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30 °C): δ = 1.19 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.54–1.69 (m, 3 H, 5-H$_{ax}$, 5-H$_{eq}$, 6-H), 1.81–1.86 (m, 4 H, 6-H, CH$_3$), 2.88 (ddd, J = 6.2, 7.2, 12.5 Hz, 1 H, 4-H), 3.99–4.12 (m, 2 H, CH$_3$CH_2), 4.60 (dd, J = 7.2, 7.3 Hz, 1 H, 3a-H), 4.89 (dd, J = 4.8, 7.3 Hz, 1 H, 6a-H) ppm. ^{13}C NMR (100 MHz, [D$_6$] DMSO, 30 °C): δ = 14.1, 15.0, 24.4, 33.4, 50.1, 60.4, 73.4, 84.2, 165.6, 171.8 ppm. $C_{10}H_{15}NO_3$ (197.24): calcd. C 60.90, H 7.67, N 7.10; found C 60.97, H 7.38, N 7.35.

Ethyl (3a*R,4*S**,6a*S**)-2-Methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (11):** Yield: 0.87 g, 63%. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30 °C): δ = 1.19 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.65–1.87 (m, 4 H, 5-H, 6-H), 1.86 (s, 1 H, CH$_3$), 2.73 (d, J = 7.1 Hz, 1 H, 4-H), 4.07 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2), 4.56 (d, J = 7.7 Hz, 1 H, 3a-H), 4.92 (dd, J = 5.8, 7.7 Hz, 1 H, 6a-H) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO, 30 °C): δ = 14.2, 14.9, 26.5, 33.4, 51.5, 61.1, 74.9, 84.4, 164.6, 173.6 ppm. $C_{10}H_{15}NO_3$ (197.24): calcd. C 60.90, H 7.67, N 7.10; found C 60.51, H 7.49, N 7.42.

Synthesis of Stereoisomeric 2-Amino-3-hydroxycyclopentanecarboxylic Acids 8 and 12: A solution of oxazoline derivative **7** (0.6 g, 3.04 mmol) was dissolved in aqueous HCl (20%, 20 mL) and heated at reflux for 24 h. The solvent was then evaporated to afford the crude amino acid hydrochloride. The free amino acid base was liberated by ion-exchange chromatography with Dowex 50. After concentration, the residue was dissolved in water (5 mL) and diluted with acetone (25 mL). After filtration, the solution was left to stand in a refrigerator for 1 h, which resulted in crystalline (1*S**,2*R**,3*S**)-2-amino-3-hydroxycyclopentanecarboxylic acid (**12**) as the main product. From the mother liquor, all-*cis*-2-amino-3-hydroxycyclopentanecarboxylic acid (**8**) was isolated as a diastereomerically enriched (9:1) mixture, which was separated from

12 by crystallization (water/acetone). Compound **12** was synthesized in the same way starting from **11** (0.6 g, 3.04 mmol).

(1*R,2*R**,3*S**)-2-Amino-3-hydroxycyclopentanecarboxylic Acid (8):** Compound **8** was prepared as a white crystalline solid (0.11 g, 25%), m.p. 250–255 °C (dec.). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 1.63–1.70 (m, 1 H, 4-H), 1.89–2.07 (m, 3 H, 4-H, 5-H_{ax}, 5-H_{eq}), 2.92 (ddd, J = 6.5, 7.0, 8.7 Hz, 1 H, 1-H), 3.60 (dd, J = 5.4, 6.5 Hz, 1 H, 2-H), 4.31 (ddd, J = 5.4, 5.9, 6.7 Hz, 1 H, 3-H) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 25.3, 30.0, 44.9, 55.0, 71.1, 180.4 ppm. $C_6H_{11}NO_3$ (145.16): calcd. C 49.65, H 7.64, N 9.65; found C 49.41, H 7.43, N 9.58.

(1*S,2*R**,3*S**)-2-Amino-3-hydroxycyclopentanecarboxylic Acid (12):** Compound **12** was prepared as a white crystalline solid (0.097 g, 22% from **7**; 0.20 g, 45% from **11**), m.p. 262–266 °C (dec.). ^{1}H NMR (400 MHz, D_2O, 30 °C): δ = 1.65–1.78 (m, 2 H, 4-H, 5-H), 2.01–2.10 (m, 1 H, 4-H), 2.15–2.25 (m, 1 H, 5-H), 2.80 (q, J = 9.2 Hz, 1 H, 1-H), 3.61 (dd, J = 5.1, 9.3 Hz, 1 H, 2-H), 4.33 (ddd, J = 2.6, 4.9, 5.1 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100 MHz, D_2O, 30 °C): δ = 25.9, 31.5, 48.3, 57.6, 71.3, 181.4 ppm. $C_6H_{11}NO_3$ (145.16): calcd. C 49.65, H 7.64, N 9.65; found C 49.41, H 7.43, N 9.58.

Ethyl *trans*-2-*tert*-Butoxycarbonylaminocyclopent-3-enecarboxylate (15): Freshly prepared NaOEt (0.79 g, 11.75 mmol) was added to a solution of ethyl *cis*-2-*tert*-butoxycarbonylaminocyclopent-3-enecarboxylate (**4b**) (3 g, 11.75 mmol) in anhydrous EtOH (35 mL), and the mixture was stirred at room temperature for 24 h. It was then concentrated under reduced pressure, taken up in EtOAc and washed with H_2O (2 × 20 mL). The combined organic phases were dried (Na_2SO_4) and the solvents evaporated. The residue was recrystallized from *n*-hexane to give a white solid (1.53 g, 51%), m.p. 65–67 °C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.18 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.38 (s, 9 H, *t*Bu), 2.40 (dd, J = 6.9, 16.0 Hz, 1 H, 5-H_{eq}), 2.65 (dd, J = 9.6, 16.0 Hz, 1 H, 5-H_{ax}), 2.81 (ddd, J = 6.9, 8.6, 9.6 Hz, 1 H, 1-H), 4.08 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2), 4.72–4.76 (m, 1 H, 2-H), 5.52–5.55 (m, 1 H, 3-H), 5.75–5.77 (m, 1 H, 4-H), 7.08 (d, J = 8.0 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.9, 29.1, 36.0, 50.1, 60.9, 61.2, 78.1, 131.4, 132.1, 156.2, 174.7 ppm. $C_{13}H_{21}NO_4$ (255.32): calcd. C 61.16, H 8.29, N 5.49; found C 60.99, H 8.21, N 5.37.

General Procedure for the Dihydroxylation of *N*-Acylamino Esters 4a–c and 15: OsO_4 (3.2 mL, 0.25 mmol; a 2.0 wt.-% solution in *t*BuOH) was added to a stirred solution of *N*-methylmorpholine *N*-oxide (1.73 g, 14.81 mmol) and **4a–c** or **15** (5 mmol) in acetone (35 mL), and the mixture was stirred for 4 h. When the reaction was complete (monitored by TLC), the mixture was treated with aqueous Na_2SO_3 (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL), the combined organic layers were dried (Na_2SO_4), and the solvent was removed by evaporation under reduced pressure to afford **13a–c** and **16**, which were recrystallized from EtOAc.

Ethyl (1*R,2*R**,3*S**,4*R**)-2-Acetylamino-3,4-dihydroxycyclopentanecarboxylate (13a):** Compound **13a** was prepared as a white crystalline solid (0.54 g, 47%), m.p. 160–162 °C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.14 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.69 (ddd, J = 2.3, 9.2, 13.7 Hz, 1 H, 5-H_{ax}), 1.77 (s, 3 H, COCH$_3$), 2.09 (ddd, J = 6.1, 7.0, 13.7 Hz, 1 H, 5-H_{eq}), 3.11 (ddd, J = 7.0, 8.9, 9.2 Hz, 1 H, 1-H), 3.73–3.78 (m, 1 H, 3-H), 3.91–4.07 (m, 3 H, 4-H, CH$_3$CH_2), 4.24 (ddd, J = 7.8, 8.4, 8.9 Hz, 1 H, 2-H), 4.57 (d, J = 3.9 Hz, 1 H, OH), 4.63 (d, J = 6.4 Hz, 1 H, O*H*), 7.80 (d, J = 8.4 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.9, 23.3, 33.4, 43.5, 56.0, 60.6, 70.4, 76.6, 170.1, 174.2 ppm. $C_{10}H_{17}NO_5$ (231.25): calcd. C 51.94, H 7.41, N 6.06; found C 52.15, H 7.71, N 6.42.

Ethyl (1*R,2*R**,3*S**,4*R**)-2-*tert*-Butoxycarbonylamino-3,4-dihydroxycyclopentanecarboxylate (13b):** Compound **13b** was prepared as a white crystalline solid (0.81 g, 56%), m.p. 122–124 °C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.16 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.37 (s, 9 H, *t*Bu), 1.64 (ddd, J = 2.3, 8.7, 14.0 Hz, 1 H, 5-H_{ax}), 2.07 (ddd, J = 6.1, 7.0, 14.0 Hz, 1 H, 5-H_{eq}), 3.11 (ddd, J = 7.0, 8.7, 9.0 Hz, 1 H, 1-H), 3.72–3.77 (m, 1 H, 3-H), 3.89–4.06 (m, 4 H, 2-H, 4-H, CH$_3$CH_2), 4.51 (d, J = 3.5 Hz, 1 H, OH), 4.60 (d, J = 6.2 Hz, 1 H, OH), 6.72 (d, J = 8.4 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.9, 29.1, 33.3, 43.9, 57.8, 60.6, 70.5, 76.7, 78.4, 156.2, 174.0 ppm. $C_{13}H_{28}NO_6$ (289.33): calcd. C 53.97, H 8.01, N 4.84; found C 54.21, H 8.35, N 5.20.

Ethyl (1*R,2*R**,3*S**,4*R**)-2-Benzyloxycarbonylamino-3,4-dihydroxycyclopentanecarboxylate (13c):** Compound **13c** was prepared as a white crystalline solid (0.93 g, 58%), m.p. 118–121 °C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30 °C): δ = 1.08 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.66 (ddd, J = 2.3, 9.2, 13.6 Hz, 1 H, 5-H_{ax}), 2.10 (ddd, J = 6.0, 7.3, 13.6 Hz, 1 H, 5-H_{eq}), 3.14 (ddd, J = 7.3, 8.9, 9.2 Hz, 1 H, 1-H), 3.72–3.78 (m, 1 H, 3-H), 3.90–3.99 (m, 3 H, 4-H, CH$_3$CH_2), 4.06 (ddd, J = 7.7, 8.8, 8.9 Hz, 1 H, 2-H), 4.56 (d, J = 3.9 Hz, 1 H, OH), 4.67 (d, J = 6.1 Hz, 1 H, OH), 5.01 (d, J = 2.2 Hz, 2 H, OCH_2Ph), 7.27 (d, J = 8.8 Hz, 1 H, NH), 7.30–7.39 (m, 5 H, Ph) ppm. ^{13}C NMR (100 MHz, [D_6]DMSO, 30 °C): δ = 14.8, 33.2, 43.8, 58.1, 60.6, 66.1, 70.4, 76.6, 128.6, 129.2, 138.0, 156.9, 173.8 ppm. $C_{16}H_{21}NO_6$ (323.35): calcd. C 59.23, H 6.55, N 4.33; found C 59.01, H 6.39, N 4.52.

Ethyl (1*S,2*R**,3*R**,4*R**)-2-*tert*-Butoxycarbonylamino-3,4-dihydroxycyclopentanecarboxylate (16):** Compound **16** was prepared as a white crystalline solid (0.78 g, 54%), m.p. 150–153 °C. ^{1}H NMR (600 MHz, [D_6]DMSO, 25 °C): δ = 1.16 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$), 1.37 (s, 9 H, *t*Bu), 1.77–1.88 (m, 2 H, 5-H), 2.74–2.79 (m, 1 H, 1-H), 3.72–3.76 (m, 1 H, 3-H), 3.89 (ddd, J = 4.7, 8.4, 8.7 Hz, 1 H, 2-H), 3.93–3.97 (m, 1 H, 4-H), 4.03 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2), 4.68 (d, J = 6.0 Hz, 1 H, OH), 4.83 (d, J = 4.2 Hz, 1 H, OH), 6.28 (d, J = 8.7 Hz, 1 H, NH) ppm. ^{13}C NMR (150 MHz, [D_6]DMSO, 25 °C): δ = 14.0, 28.1, 33.4, 46.0, 55.5, 59.9, 71.0, 72.7, 77.8, 154.9, 174.7 ppm. $C_{13}H_{28}NO_6$ (289.33): calcd. C 53.97, H 8.01, N 4.84; found C 54.08, H 7.79, N 5.03.

General Synthesis of the Stereoisomeric 2-Amino-3,4-dihydroxycyclopentanecarboxylic Acids 14 and 17: A solution of dihydroxy ester **13b** or **16** (2.3 mmol) was dissolved in aqueous HCl (20%, 20 mL), and the mixture was stirred at room temperature for 24 h. In the case of **13a**, the mixture was refluxed for 48 h. The solvent was then evaporated to afford the crude amino ester hydrochloride. The free amino acid base was liberated by ion-exchange chromatography with Dowex 50. An exception was for **13c**: the protected dihydroxy amino acid was first stirred with 10% Pd/C (80 mg) in EtOH (30 mL) under H_2 for 2 h. The catalyst was then filtered off, and the filtrate was concentrated under reduced pressure and subsequently treated with aqueous HCl by the above method.

(1*R,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclopentanecarboxylic Acid (14):** Compound **14** was prepared as a white crystalline solid (0.13 g, 35%), m.p. 230–232 °C. ^{1}H NMR (400 MHz, D_2O, 30 °C): δ = 2.14 (ddd, J = 2.3, 9.3, 14.8 Hz, 1 H, 5-H_{ax}), 2.23 (ddd, J = 5.9, 6.8, 14.8 Hz, 1 H, 5-H_{eq}), 3.11 (ddd, J = 6.8, 8.9, 9.3 Hz, 1 H, 1-H), 3.60 (dd, J = 8.4, 8.9 Hz, 1 H, 2-H), 4.15–4.23 (m, 2 H, 3-H, 4-H) ppm. ^{13}C NMR (100 MHz, D_2O, 30 °C): δ = 35.2, 41.0, 55.5, 69.9, 75.5, 180.6 ppm. $C_6H_{11}NO_4$ (161.16): calcd. C 44.72, H 6.88, N 8.69; found C 44.89, H 8.25, N 9.01.

(1*S,2*R**,3*R**,4*S**)-2-Amino-3,4-dihydroxycyclopentanecarboxylic Acid (17):** Compound **17** was prepared as a white crystalline solid (0.12 g, 37%), m.p. 175–179 °C. ^{1}H NMR (400 MHz, D_2O, 30 °C): δ = 2.06–2.23 (m, 2 H, 5-H), 3.26 (ddd, J = 7.5, 9.2, 9.6 Hz, 1 H, 1-H), 3.92 (dd, J = 6.1, 6.3 Hz, 1 H, 3-H), 4.21–4.29 (m, 2 H, 3-H, 4-H) ppm. ^{13}C NMR (100 MHz, D_2O, 30 °C): δ = 32.9, 44.9, 54.1, 71.9, 72.0, 177.2 ppm. $C_6H_{11}NO_4$ (161.16): calcd. C 44.72, H 6.88, N 8.69; found C 44.95, H 6.71, N 8.89.

(1*R*,2*R*,3*S*)-2-Amino-3-hydroxycyclopentanecarboxylic Acid (+)-(8) and (1*R*,2*R*,3*S*,4*R*)-2-Amino-3,4-dihydroxycyclopentanecarboxylic Acid (–)-(14): The same synthetic route as used for the racemic compounds **8** and **14** was applied, starting from *cis*-2-aminocyclopentenecarboxylic acid hydrochloride [(+)-(**5**)],[39] via intermediate (+)-**6** or (–)-**13b**. The ^{1}H NMR spectroscopic data for the intermediates and products are similar to those for the racemates.

Ethyl (1*R*,2*S*)-2-Aminocyclopent-3-enecarboxylate Hydrochloride (+)-(3): White crystals, m.p. 89–91 °C, $[\alpha]_D^{20}$ = +85.4 (c = 0.5, EtOH).

Ethyl (1*R*,2*S*)-2-Acetylaminocyclopent-3-enecarboxylate (+)-(4a): White crystals, m.p. 109–111 °C, $[\alpha]_D^{20}$ = +33.5 (c = 0.5, EtOH).

Ethyl (3a*R*,4*R*,6*R*,6a*R*)-6-Bromo-2-methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (+)-(6): Yellow oil, $[\alpha]_D^{20}$ = +71.1 (c = 0.5, CH_2Cl_2).

Ethyl (3a*R*,4*R*,6a*S*)-2-Methyl-4,5,6,6a-tetrahydro-3a*H*-cyclopentaoxazole-4-carboxylate (+)-(7): Yellow oil, $[\alpha]_D^{20}$ = +96.8 (c = 0.5, EtOH).

(1*R*,2*R*,3*S*)-2-Amino-3-hydroxycyclopentanecarboxylic Acid (+)-(8): White crystals, m.p. 228 °C (dec.), $[\alpha]_D^{20}$ = +28.4 (c = 0.584, H_2O).

Ethyl (1*R*,2*S*)-2-*tert*-Butoxycarbonylaminocyclopent-3-enecarboxylate (+)-(4b): Colourless crystals, m.p. 89–90 °C, $[\alpha]_D^{20}$ = +45.1 (c = 0.5, EtOH).

Ethyl (1*R*,2*R*,3*S*,4*R*)-2-*tert*-Butoxycarbonylamino-3,4-dihydroxycyclopentanecarboxylate (–)-(13b): White crystals, m.p. 118–120 °C, $[\alpha]_D^{20}$ = –117.2 (c = 0.5, EtOH).

(1*R*,2*R*,3*S*,4R)-2-Amino-3,4-dihydroxycyclopentanecarboxylic Acid (–)-(14): White crystals, m.p. 223 °C (dec.), $[\alpha]_D^{20}$ = –112.6 (c = 0.5, H_2O).

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II

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Efficient synthesis of 3,4- and 4,5-dihydroxy-2-amino-cyclohexanecarboxylic acid enantiomers

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ABSTRACT

An efficient method for the synthesis of (1*S*,2*R*,4*R*,5*S*)- and (1*R*,2*R*,4*R*,5*S*)-2-amino-4,5-dihydroxycyclohexanecarboxylic acids (−)-**6** and (−)-**9** and (1*R*,2*R*,3*S*,4*R*)- and (1*S*,2*R*,3*S*,4*R*)-2-amino-3,4-dihydroxycyclohexanecarboxylic acids (−)-**15** and (−)-**18** was developed by using the OsO_4-catalyzed oxidation of Boc-protected (1*S*,2*R*)-2-aminocyclohex-4-enecarboxylic acid (+)-**2** and (1*R*,2*S*)-2-aminocyclohex-3-enecarboxylic acid (+)-**11**. Good yields were obtained. The stereochemistry of the synthesized compounds was proven by NMR spectroscopy.

1. Introduction

Alicyclic β-amino acids[1,2] derived from β-lactams[3,4] have attracted the interest of a number of synthesis research groups as a consequence of their useful biological effects and occurrence in many pharmacologically relevant compounds.[5] Peptides containing β-amino acids can increase and modify biological activities and are not degradable by proteases, which can lead to peptide-based synthetic targets.[6] These compounds can be found in natural products, for example, cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, an antifungal antibiotic, as is amipurimycin, which was isolated from *Streptomyces novoguineensis*.[5,7–9] The synthetic 4-methylene derivative of cispentacin (Icofungipen, PLD-118) is active in vitro against *Candida* species.[10,11] In recent years, the preparation of enantiopure β-amino acids has come into the foreground of interest, because of their widespread use in peptide, heterocyclic and combinatorial chemistries and drug research.[12–15]

Among the β-amino acids, the hydroxy-functionalized derivatives are of considerable importance in medicinal chemistry, because they occur in many important products, such as paclitaxel (Taxol) and docetaxel (Taxotere), which have chemotherapeutic effects.[16–18] Some cyclic hydroxylated β-amino acid derivatives have antibiotic (oryzoxymycin)[19–22] or antifungal activities, and are used as building blocks for pharmaceutically significant natural substances.[23]

A number of methods have recently been published for the stereoselective introduction of a mono-hydroxy functionality onto the cyclohexane or cyclopentane ring, for example, by iodolactonization of *cis*- and *trans*-2-aminocyclohexenecarboxylic acids or *cis*- and *trans*-2-aminocyclopentenecarboxylic acids, or via the corresponding dihydrooxazine or oxazoline derivatives.[24–29] Another method involves the hydroxylation of the 2-aminocyclohexenecarboxylic acid by functionalization of the olefinic bond through epoxidation.[29–31]

The OsO_4-catalyzed dihydroxylation of olefins provides one of the most efficient methods for the preparation of vicinal diols.[32–38] The $KMnO_4$-induced oxidation of the double bond is another well-known route to dihydroxy derivatives.[39]

Our present aim was the dihydroxylation of the olefinic bond of enantiopure and racemic, *cis*- and *trans*-2-amino-4-cyclohexenecarboxylic acids and *cis*- and *trans*-2-amino-3-cyclohexenecarboxylic acids, and the structural analysis of the new dihydroxy-substituted derivatives.

2. Results and discussion

The starting (1*S*,2*R*)-2-aminocyclohex-4-enecarboxylic acid (+)-**2** and (1*R*,2*S*)-2-aminocyclohex-3-enecarboxylic acid (+)-**11** were synthesized from β-amino ester (±)-**1** and β-lactam (±)-**10** by highly enantioselective CAL-B-catalyzed hydrolysis with one equivalent of H_2O in *i*-Pr_2O at 65 °C.[40,41] The enantiopure amino acids (+)-**2** and (+)-**11** (ee >99%) were esterified in the presence of EtOH and $SOCl_2$ to give amino ester hydrochlorides, which were reacted with *tert*-butoxy pyrocarbonate to afford the *N*-Boc-protected amino esters (+)-**4** and (+)-**13**, respectively (Schemes 1 and 2).

The isomerization of (+)-**4** and (+)-**13** with NaOEt at room temperature resulted in *trans*-*N*-Boc amino esters (−)-**7** and (+)-**16**. The *trans*-configuration was confirmed by the NOE signal of relatively low intensity between H-1 and H-2 and the large ^3J(H-1, H-2) coupling at around 9–10 Hz.

Dihydroxylation of protected esters (+)-**4**, (−)-**7** and (+)-**13**, (+)-**16** with a catalytic amount of OsO_4 and 4-methylmorpholine

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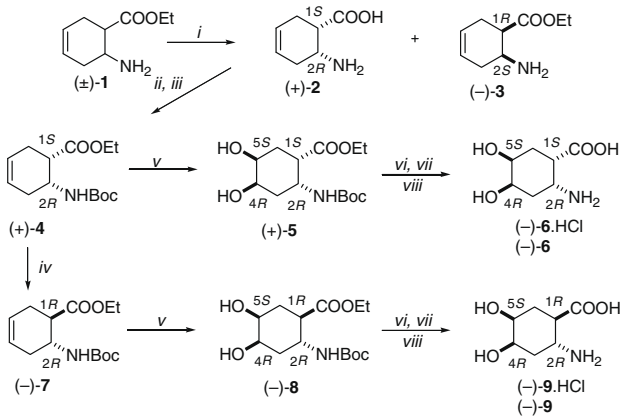
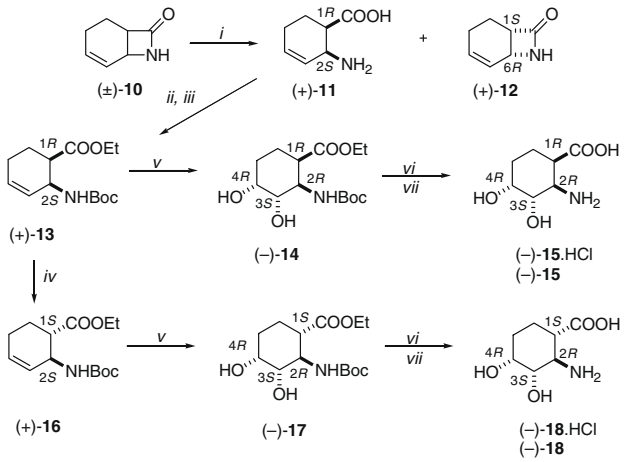


Scheme 1. Reagents and conditions: (i) CAL-B, H_2O (1 equiv), *i*-Pr_2O, 65 °C; (ii) $SOCl_2$, EtOH, 0 °C–Δ, 97%; (iii) Et_3N, Boc_2O, CH_2Cl_2, 2 h, rt, 89%; (iv) NaOEt, EtOH, 24 h, rt, 43%; (v) 2.0 w/w% OsO_4 solution in *t*-BuOH, NMO, acetone, 4 h, rt, 78–85%; (−)-**6**·HCl, (−)-**9**·HCl: (vi) LiOH, H_2O/THF, rt, 5 h, 92–96%; (vii) 10% HCl/H_2O, 24 h, Δ, 45–47%; (−)-**6**, (−)-**9**; (viii) microwave irradiation, H_2O, 150 °C, 1 h, 70–77%.



Scheme 2. Reagents and conditions: (i) CAL-B, H_2O (1 equiv), *i*-Pr_2O, 65 °C; (ii) $SOCl_2$, EtOH, 0 °C–Δ, 90%; (iii) Et_3N, Boc_2O, CH_2Cl_2, 2 h, rt, 82%; (iv) NaOEt, EtOH, 24 h, rt, 65%; (v) 2.0 w/w% OsO_4 solution in *t*-BuOH, NMO, acetone, 4 h, rt, 72–74%; (−)-**15**·HCl, (−)-**18**·HCl: (vi) 10% HCl/H_2O, 24 h, Δ, 43–49%; (−)-**15**, (−)-**18**; (vii) microwave irradiation, H_2O, 150 °C, 1 h, 72–74%.

N-oxide (NMO) as the stoichiometric co-oxidant afforded the desired products (+)-**5**, (−)-**8** and (−)-**14**, (−)-**17** as single diastereomers in good yields.

The dihydroxylations of (+)-**4** and (+)-**13** exhibit *anti* selectivity with regard to the ester and protected amino groups, on the sterically less-hindered side of the ring. The orientation of the hydroxy groups was deduced from the couplings and NOEs of their vicinal hydrogens. For (+)-**5**, H-4 and H-1 display large couplings (^3J = 9–10 Hz), indicating their axial positions. The singlet of H-5 suggests its equatorial position. NOE signals were observed between the axial 5-OH and the axial H-1 and H-3ax. Moreover, the signal between H-4 and the amide hydrogen confirms the *trans* orientation of the hydroxy groups relative to the ester and amide groups. For (−)-**14**, the coupling constants suggest equatorial H-3 and axial H-4 and H-1, and the NOEs prove the stereochemistry: the signal between 3-OH and H-1, H-4 and H-6ax and between the amide hydrogen and H-4 and H-6x.

Following the osmylation of the double bond in (−)-**7** or (+)-**16**, where the ester and amino groups are on opposite sides of the ring, the hydroxy groups project on the ester side, that is, *anti* relative to the amino group. In this case, H-1 and H-2 are in a *trans-diaxial* position, and the NOE signals between H-1 and the axial H-5 and between H-1 and the axial H-3 indicate the orientation of the hydroxy groups for (−)-**8** and (−)-**17**, respectively. This selectivity can be interpreted in terms of the steric bias of the substituents. The bulkier *N*-Boc-protecting group interacts unfavourably with the forming hydroxy groups, and hence osmylation will occur from the sterically less-hindered face.[32]

It is relevant that dihydroxylation by $KMnO_4$ results in the same diastereoselectivity as for osmylation, but the yields are not so good.[42]

The acidic hydrolysis of (−)-**14** and (−)-**17** resulted in the corresponding dihydroxy-amino acid hydrochlorides (−)-**15**·HCl and (−)-**18**·HCl in moderate yields.

For (+)-**5**, (−)-**8**, (±)-**5** and (±)-**8**, a different deprotection method was used in the last step of the synthesis: reaction first with LiOH in THF to deprotect the carboxylic group, followed by hydrolysis with HCl/H_2O. The yields were obviously the same.

In order to improve the yields of the final products, a new deprotection protocol was applied: dihydroxy compounds (+)-**5**, (−)-**8**, (−)-**14** and (−)-**17** and their racemic counterparts were subjected to microwave irradiation in H_2O at 150 °C for 1 h.[43,44]

Due to the possible isomerization after hydrolysis, we analyzed the structures of the deprotected dihydroxy-amino acids (−)-**6**, (−)-**9**, (−)-**15** and (−)-**18** as well. Because of the higher conformational flexibility of the compounds, some of the NMR signals were broadened; consequently the smaller coupling constants could not be determined exactly. For (−)-**9** and (−)-**18**, the small NOE signal between H-1 and H-2 and the large coupling ^3J(H-1, H-2) = 11–12 Hz suggest a *trans*-orientation for the carboxyl and amino groups, while for (−)-**6** and (−)-**15**, the small NOE couplings and the large NOE signals between H-1 and H-2 indicate *cis*-substituents. The orientations of the hydroxy groups can be deduced from the couplings and the NOE patterns of their vicinal hydrogens.

For (−)-**6**, whose conformational flexibility was pronounced, the stereochemistry was proved unequivocally by measurements in CD_3OD and in DMSO. In this case, the coupling constants suggest axial H-2 and H-5 and equatorial H-4. This would involve hydroxy groups on the opposite side of the ring from the amino group, which is supported by the absence of NOE signals between H-2 and H-4 and by the NOE cross peak between H-2 and one of the hydroxyl groups (Fig. 1).

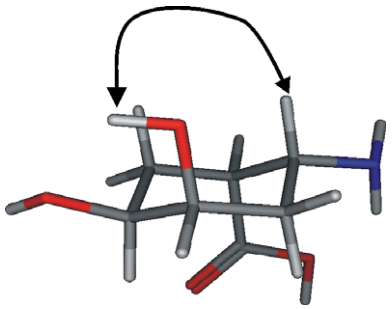


Figure 1. Molecular structure of (−)-**6**.

For (−)-**9**, H-1 and H-2 are in a *trans-diaxial* position (concluded from ^3J(H-1, H-2) = 12 Hz) and H-5 should also be axial, while H-4 is equatorial. NOE signals can be observed between H-1 and H-5 and between H-3ax and H-1 and H-5, which suggest that the hydroxy groups are *cis* relative to the carboxyl group (Fig. 2).

For (−)-**15**, whose spectra were measured in D_2O because of the overlapping signals in DMSO, the coupling constants indicate axial H-2 and H-3, and equatorial H-4, which requires *trans*-hydroxy groups relative to the amino and carboxyl groups. The NOE signal

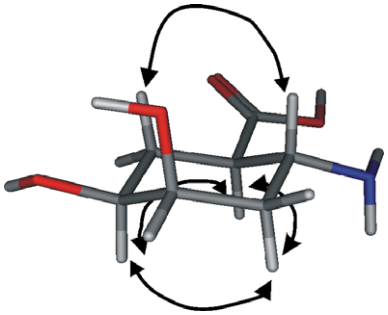


Figure 2. Molecular structure of (−)-**9**.

between H-3 and H-5ax is in accordance with this structure. For (−)-**18**, similar couplings and NOE signal patterns were observed for H-2, H-3 and H-4, which indicates that the hydroxy groups are on the opposite side of the cyclohexane ring from the amino group.

3. Conclusions

An effective route has been devised for the preparation of enantiopure 2-amino-4,5-dihydroxycyclohexanecarboxylic acids and 2-amino-3,4-dihydroxycyclohexanecarboxylic acids. Catalytic osmylation with OsO_4 and NMO as co-oxidants was used to introduce the dihydroxy functionality on the cyclohexene ring. After microwave irradiation of the protected amino acids, the appropriate products were obtained. These new β-amino acid derivatives can be used as enantiopure building blocks to produce peptides or heterocycles. The synthesis of further substances is also to be expected.

4. Experimental

4.1. General

The ^{1}H NMR spectra were recorded at 500 MHz or at 600 MHz while the ^{13}C NMR spectra at 125 MHz or at 150 MHz in DMSO-d_6, except for (−)-**6** (CD_3OD) and (−)-**15** (D_2O), which were recorded at ambient temperature, with Bruker DRX 500 and AV 600 spectrometers, respectively. Chemical shifts are given in δ (ppm) relative to TMS as the internal standard. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II Elemental Analyzer. Melting points were measured with a Kofler melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. Microwave reactions were performed in a CEM Discover MW reactor. Ester (±)-**1** was prepared by hypochlorite-mediated Hoffman degradation of the carboxamide obtained by the ammonolysis of *cis*-1,2,3,6-tetrahydrophthalic anhydride,[47] while β-lactam (±)-**10** was formed by the addition of chlorosulfonyl isocyanate to 1,3-cyclohexadiene.[25] Amino acid (+)-**2** was esterified in the presence of EtOH and $SOCl_2$, and the amino group was then protected with di-*tert*-butyl dicarbonate to give Boc-protected ester (+)-**4**.[26] The ee values for the starting (1*S*,2*R*)-2-aminocyclohex-4-enecarboxylic acid (+)-**2** and (1*R*,2*S*)-2-aminocyclohex-3-enecarboxylic acid (+)-**11** (>99%) were determined after a simple and rapid double derivatization by using GC instrumentation equipped with CP-Chirasil L-Val columns.[45]

The ee values for the final products were determined by HPLC. For (−)-**9**·HCl, (−)-**15**·HCl and (−)-**18**·HCl, a Chirobiotic TAG 5μ column (0.46 cm × 25 cm) was used at room temperature; the mobile phase was MeOH containing 0.1% TEA and 0.1% AcOH; flow rate 1 mL/min; detection at 205 nm; retention times (min): (−)-**9**·HCl, 11.37 (antipode: 18.77); (−)-**15**·HCl, 16.11 (antipode: 14.43); (−)-**18**·HCl, 12.16 (antipode: 13.41). For (−)-**6**·HCl, a Chirobiotic T 5μ column (0.46 cm × 25 cm) was used at room temperature; the mobile phase was 0.1% aqueous triethylammonium acetate (TEEA)/EtOH = 20/80; flow rate 0.5 mL/min; detection at 205 nm; retention time (min): 22.77 (antipode: 21.08).[46] The ee values for compounds (−)-**6**, (−)-**9**, (−)-**15** and (−)-**18** were determined by the above-mentioned methods; the samples were derivatized with concentrated HCl.

4.2. Ethyl (1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclohex-3-enecarboxylate, (+)-13

At first, $SOCl_2$ (1.47 g, 12.4 mmol) was added dropwise with stirring to dry EtOH (11 mL) at −15 °C. To this mixture, (+)-**11** (2.00 g, 14.17 mmol) was added in one portion, followed by stirring for 30 min at 0 °C. After further stirring for 3 h at room temperature, the mixture was refluxed for an additional 1 h and then evaporated. The residue was recrystallized from EtOH/Et_2O to give a colourless crystalline product.

To the product (2.00 g, 9.7 mmol) in CH_2Cl_2 (50 mL), Et_3N (1.97 g, 19.4 mmol) and di-*tert*-butyl dicarbonate (2.33 g, 10.7 mmol) were added at 0 °C. The mixture was stirred at room temperature for 2 h, and then washed with water (2 × 20 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic phase was dried (Na_2SO_4) and the solvents were evaporated off. The residue was recrystallized from *n*-hexane to give a white solid. Yield: 2.25 g (74%), mp 82–84 °C, $[\alpha]_D^{20}$ = +165.2 (*c* 0.55, EtOH). ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.16 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.36 (s, 9H, *t*-Bu), 1.61–1.68 (m, 1H, H-6eq), 1.76–1.84 (m, 1H, H6-ax), 1.88–1.96 (m, 1H, H-5ax), 2.02 (dt, *J* = 17.8, 5.4, 5.2 Hz, 1H, H-5eq), 2.62 (ddd, *J* = 12.4, 4.6, 3.0 Hz, 1H, H-1), 3.96–4.02 (m, 2H, CH_2CH_3), 4.35–4.39 (m, 1H, H-2), 5.53–5.59 (m, 1H, H-3), 5.73–5.78 (m, 1H, H-4), 6.73 (d, *J* = 9.4 Hz, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 13.5, 18.4, 23.4, 27.8, 43.2, 44.6, 59.1, 77.5, 126.0, 128.8, 154.6, 172.5 ppm. Anal. Calcd for $C_{14}H_{23}NO_4$ (269.34): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.34; H, 8.59; N, 5.27.

4.3. General procedure for isomerization of Boc-protected *cis* amino esters, (+)-4 and (+)-13

Freshly prepared NaOEt (0.25 g, 3.7 mmol) was added to a solution of (+)-**4** or (+)-**13** (1.00 g, 3.7 mmol) in dry EtOH (12 mL), and the mixture was stirred for 24 h at room temperature. It was then concentrated under reduced pressure, taken up in EtOAc and washed with H_2O (2 × 20 mL). The combined organic phase was dried (Na_2SO_4) and the solvent was evaporated off. The residue was purified by column chromatography (*n*-hexane/EtOAc, 9:1) to give a white solid.

4.3.1. Ethyl (1*R*,2*R*)-2-*tert*-butoxycarbonylaminocyclohex-4-enecarboxylate, (−)-7

Yield: 0.43 g (43%), mp 45–47 °C, $[\alpha]_D^{20}$ = −23.7 (*c* 0.5, EtOH). ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.17 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.36 (s, 9H, *t*-Bu), 1.95–2.02 (m, 1H, H-3ax), 2.17 (dt, *J* = 17.3, 4.5, 4.5 Hz, 1H, H-3eq), 2.23–2.27 (m, 2H, H-6), 2.53 (dt, *J* = 10.7, 8.0, 8.0 Hz, 1H, H-1), 3.64–3.74 (m, 1H, H-2), 4.03 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 5.56–5.59 (m, 2H, H-4, H-5), 6.79 (d, *J* = 8.7 Hz, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 13.5, 27.1, 27.4, 31.1, 44.8, 46.5, 59.1, 77.9, 124.4, 124.8, 154.8, 174.3 ppm. Anal. Calcd for $C_{14}H_{23}NO_4$ (269.34): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.27; H, 8.71; N, 5.17.

4.3.2. Ethyl (1*S*,2*S*)-2-*tert*-butoxycarbonylaminocyclohex-3-enecarboxylate, (+)-16

Yield: 0.65 g (65%), mp 75–78 °C, $[\alpha]_D^{20}$ = +103.6 (*c* 0.53, EtOH). ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.17 (t, *J* = 7.1 Hz, 3H,

CH_2CH_3), 1.63–1.69 (m, 1H, H-6ax), 1.82–1.89 (m, 1H, H-6eq), 1.36 (s, 9H, *t*-Bu), 1.95–2.02 (m, 2H, H-5), 2.42–2.47 (m, 1H, H-1), 3.97–4.11 (m, 2H, CH_2CH_3), 4.21 (d, J = 8.4 Hz, 1H, H-2), 5.42 (d, J = 9.8 Hz, 1H, H-3), 5.67–5.72 (m, 1H, H-4), 6.96 (d, J = 8.7 Hz, 1H, NH) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 14.0, 23.3, 24.2, 28.2, 45.1, 48.0, 59.9, 77.7, 127.7, 128.7, 155.0, 173.8 ppm. Anal. Calcd for $C_{14}H_{23}NO_4$ (269.34): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.45; H, 8.67; N, 5.22.

4.4. General procedure for dihydroxylation of *N*-Boc-protected esters, (+)-4, (−)-7, (+)-13 and (+)-16

At first, OsO_4 (1.02 mL 0.08 mmol; a 2.0% w/w solution in *t*-BuOH) was added to a stirred solution of *N*-methylmorpholine *N*-oxide (0.55 g, 4.7 mmol) and (+)-**4**, (−)-**7**, (+)-**13** or (+)-**16** (0.43 g, 1.6 mmol) in acetone (15 mL), and stirring was continued for a further 4 h. When the reaction was completed (monitored by TLC), the mixture was treated with aqueous Na_2SO_3 (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL), and the combined organic layer was dried (Na_2SO_4). The solvent was removed by evaporation under reduced pressure to afford crystalline products (+)-**5**, (−)-**8** and (−)-**17**, which were recrystallized from *n*-hexane/EtOAc to give white crystalline solids. The oily compound (−)-**14** was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1:1) to afford white crystals.

4.4.1. Ethyl (1*S*,2*R*,4*R*,5*S*)-2-*tert*-butoxycarbonylamino-4,5-dihydroxycyclohexanecarboxylate, (+)-5

Yield: 379 mg (78%), mp 136–137 °C, $[\alpha]_D^{20} = +27.3$ (c 0.49, EtOH). ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.14 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.36 (s, 9H, *t*-Bu), 1.51 (d, J = 11.8 Hz, 1H, H-3eq), 1.66 (ddd, J = 12.8, 5.0, 4.5 Hz, 1H, H-6eq), 1.73 (dd, J = 11.8, 9.4 Hz, 1H, H-3ax), 1.91 (dd, J = 12.8, 11.8 Hz, 1H, H-6ax), 2.75–2.82 (ddd, J = 10.5, 5.0, 4.2 Hz, 1H, H-1), 3.69 (d, J = 9.4 Hz, 1H, H-4), 3.72 (s, 1H, H-5), 3.93-4.04 (m, 2H, CH_2CH_3), 4.13 (s, 1H, H-2), 4.28 (s, 1H, OH), 4.35 (d, J = 2.5 Hz, 1H, OH), 6.79 (d, J = 5.9 Hz, 1H, NH) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 13.9, 28.1, 28.3, 33.7, 39.9, 47.2, 59.5, 66.4, 67.2, 77.5, 154.9, 172.8 ppm. Anal. Calcd for $C_{14}H_{25}NO_6$ (303.35): C, 55.43; H, 8.31; N, 4.62. Found: C, 55.35; H, 8.29; N, 4.53.

4.4.2. Ethyl (1*R*,2*R*,4*R*,5*S*)-2-*tert*-butoxycarbonylamino-4,5-dihydroxycyclohexanecarboxylate, (−)-8

Yield: 413 mg (85%), mp 114–117 °C, $[\alpha]_D^{20} = -25.2$ (c 0.51, EtOH). ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.15 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.34 (s, 9H, *t*-Bu), 1.40 (dd, J = 12.2, 13.0 Hz, 1H, H-3ax), 1.55 (ddd, J = 12.4, 3.9, 3.6 Hz, 1H, H-6eq), 1.73 (ddd, J = 13.0, 4.0, 3.8 Hz, 1H, H-3eq), 1.83 (q, J = 12.4 Hz, 1H, H-6ax), 2.31 (ddd, J = 12.4, 12.3, 3.4 Hz, 1H, H-1), 3.36 (ddd, J = 11.0, 6.5, 5.5 Hz, 1H, H-5), 3.73 (s, 1H, H-4), 3.81–3.88 (m, 1H, H-2), 3.93–4.05 (m, 2H, CH_2CH_3), 4.42 (s, 1H, OH), 4.49 (d, J = 5.5 Hz, 1H, OH), 6.63 (d, J = 9.3 Hz, 1H, NH) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 14.5, 28.6, 30.9, 37.8, 45.8, 47.8, 60.0, 68.5, 69.7, 77.5, 155.2, 172.6 ppm. Anal. Calcd for $C_{14}H_{25}NO_6$ (303.35): C, 55.43; H, 8.31; N, 4.62. Found: C, 55.60; H, 8.35; N, 4.54.

4.4.3. Ethyl (1*R*,2*R*,3*S*,4*R*)-2-*tert*-butoxycarbonylamino-3,4-dihydroxycyclohexanecarboxylate, (−)-14

Yield: 359 mg (74%), mp 49–51 °C $[\alpha]_D^{20} = -43.2$ (c 0.54, EtOH). ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.13 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.36 (s, 9H, *t*-Bu), 1.39–1.48 (m, 2H, H-5), 1.49–1.56 (m, 1H, H-6eq), 1.76 (td, J = 12.4, 6.2, 6.2 Hz, 1H, H-6ax), 2.73 (td, J = 12.2, 4.1, 4.1 Hz, 1H, H-1), 3.48 (s, 1H, H-3), 3.63 (dt, 1H, J = 10, 5.6, 5.6 Hz, H-4), 3.90–4.05 (m, 2H, CH_2CH_3), 4.10 (dt, J = 9.8, 4.5, 4.5 Hz, 1H, H-2), 4.28 (d, J = 5.7 Hz, 1H, OH), 4.72 (d, J = 3.4 Hz, 1H, OH), 6.73 (d, J = 9.8 Hz, 1H, NH) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 13.7, 20.2, 26.3, 27.8, 39.4, 52.6, 59.2, 65.8, 70.9, 78.1, 155.2, 174.0 ppm. Anal. Calcd for $C_{14}H_{25}NO_6$ (303.35): C, 55.43; H, 8.31; N, 4.62. Found: C, 55.49; H, 8.37; N, 4.65.

4.4.4. Ethyl (1*S*,2*R*,3*S*,4*R*)-2-*tert*-butoxycarbonylamino-3,4-dihydroxycyclohexanecarboxylate, (−)-17

Yield: 349 mg (72%), mp 153–156 °C, $[\alpha]_D^{20} = -12$ (c 0.28, EtOH). ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.16 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.32 (d, J = 13.3 Hz, 1H, H-5ax), 1.35 (s, 9H, *t*-Bu), 1.42–1.47 (m, 1H, H-6eq), 1.67 (ddd, J = 13.5, 6.9, 3.5 Hz, 1H, H-5eq), 1.81 (dq, J = 13.1, 12.9, 12.9, 3.3 Hz, 1H, H-6ax), 2.28 (dt, J = 12.3, 12.3, 3.7 Hz, 1H, H-1), 3.18–3.23 (m, 1H, H-3), 3.73 (q, J = 10.20 Hz, 1H, H-2), 3.80 (s, 1H, H-4), 3.93–4.04 (m, 2H, CH_2CH_3), 4.20 (d, J = 7.0 Hz, 1H, OH), 4.45 (s, 1H, OH), 6.54 (d, J = 9.4 Hz, 1H, NH) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 14.5, 22.6, 28.7, 30.0, 48.5, 52.2, 60.1, 69.0, 73.6, 77.5, 155.7, 173.6 ppm. Anal. Calcd for $C_{14}H_{25}NO_6$ (303.35): C, 55.43; H, 8.31; N, 4.62. Found: C, 55.47; H, 8.40; N, 4.69.

4.5. General synthesis of stereoisomeric 2-amino-4,5-dihydroxycyclohexanecarboxylic acid hydrochlorides, (−)-6·HCl and (−)-9·HCl, and 2-amino-3,4-dihydroxycyclohexanecarboxylic acid hydrochlorides, (−)-15·HCl and (−)-18·HCl

Dihydroxy ester (−)-**14** or (−)-**17** (364 mg, 1.2 mmol) was dissolved in aqueous HCl (10%; 20 mL) and the mixture was refluxed for 24 h. The solvent was then evaporated off to afford the crude amino acid hydrochloride, which was recrystallized from EtOH/Et_2O to give a pale-yellow crystalline solid. Compounds (+)-**5** and (−)-**8** were first hydrolyzed with LiOH in H_2O/THF at room temperature for 5 h and subsequently treated with aqueous HCl by the above-mentioned method.

4.5.1. (1*S*,2*R*,4*R*,5*S*)-2-Amino-4,5-dihydroxycyclohexanecarboxylic acid hydrochloride, (−)-6·HCl

Yield: 119 mg (47%), mp 240–243 °C (dec.), $[\alpha]_D^{20} = -7.3$ (c = 0.324, H_2O); ee >99%. ^{1}H NMR (600 MHz, CD_3OD, 40 °C): δ = 2.03–2.12 (m, 3H, H-3, H-3, H-6ax), 2.17 (dt, J = 13.6, 4.7, 4.7 Hz, H-6eq), 3.12 (q, J = 4.8 Hz, 1H, H-1), 3.74 (dt, J = 9.8, 4.7, 4.7 Hz 1H, H-2), 3.77 (d, J = 10.0 Hz, 1H, H-5), 3.99 (dt, J = 5.0, 2.9, 2.9 Hz, 1H, H-4) ppm. ^{13}C NMR (150 MHz, MeOD, 40 °C): δ = 28.5, 31.7, 39.6, 46.0, 67.0, 67.6, 174.2 ppm. Anal. Calcd for $C_7H_{14}ClNO_4$ (211.64): C, 39.72; H, 6.67; N, 6.62. Found: C, 39.79; H, 6.57; N, 6.65.

4.5.2. (1*R*,2*R*,4*R*,5*S*)-2-Amino-4,5-dihydroxycyclohexanecarboxylic acid hydrochloride, (−)-9·HCl

Yield: 114 mg (45%), mp 222–225 °C (dec.), $[\alpha]_D^{20} = -48.8$ (c = 0.46, H_2O); ee >99%. ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.58 (t, J = 12.4 Hz, 1H, H-3ax), 1.67 (q, J = 12.2 Hz, 1H, H-6ax), 1.79–1.84 (m, 1H, H-6eq), 2.06 (dt, J = 12.6, 4.0, 4.0 Hz, 1H, H-3eq), 2.55 (t, J = 12.4 Hz, 1H, H-1), 3.36–3.47 (m, 2H, H-2, H-5), 3.79 (s, 1H, H-4), 4.69–4.86 (m, 2H, OH), 8.06 (s, 3H, NH) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 30.3, 33.7, 43.9, 45.3, 66.9, 69.0, 173.8 ppm. Anal. Calcd for $C_7H_{14}ClNO_4$ (211.64): C, 39.72; H, 6.67; N, 6.62. Found: C, 39.77; H, 6.67; N, 6.58.

4.5.3. (1*R*,2*R*,3*S*,4*R*)-2-Amino-3,4-dihydroxycyclohexanecarboxylic acid hydrochloride, (−)-15·HCl

Yield: 109 mg (43%), mp 224 °C (dec.), $[\alpha]_D^{20} = -84.8$ (c = 0.55, H_2O); ee >99%. ^{1}H NMR (600 MHz, D_2O, 27 °C): δ = 1.51–1.60 (dd, J = 14.8, 12.0 Hz 1H, H-5ax), 1.74–1.79 (m, 1H, H-5eq), 1.87 (tt, J = 14.2, 14.2, 4.2, 4.2 Hz, 1H, H-6ax), 1.93–2.01 (m, 1H, H6-eq), 3.11 (q, J = 4.2 Hz, 1H, H-1), 3.50 (dd, J = 10.7, 4.6 Hz, 1H, H-2), 3.99 (dd, J = 10.7, 3.20 Hz, 1H, H-3), 4.04 (q, J = 3.1 Hz, 1H, H-4) ppm. ^{13}C NMR (150 MHz, D_2O, 27 °C): δ = 20.6, 26.8, 41.3, 50.7,

68.5, 68.5, 173.8 ppm. Anal. Calcd for $C_7H_{14}ClNO_4$ (211.64): C, 39.72; H, 6.67; N, 6.62. Found: C, 39.65; H, 6.54; N, 6.71.

4.5.4. (1*S*,2*R*,3*S*,4*R*)-2-Amino-3,4-dihydroxycyclohexanecarboxylic acid hydrochloride, (−)-18·HCl

Yield: 124 mg (49%), mp 214 °C (dec.), $[\alpha]_D^{20} = -61.5$ (c = 0.5, H_2O); ee >99%. ^{1}H NMR (600 MHz, DMSO, 27 °C): δ = 1.47 (s, 1H, H-5ax), 1.62–1.69 (m, 1H, H-6), 1.69–1.74 (m, 2H, H-5eq, H-6), 2.47–2.51 (m, 1H, H-1), 3.23–3.29 (m, 1H, H-2), 3.45 (dd, J = 12.9, 4.2 Hz, 1H, H-3), 3.85 (s, 1H, H-4), 4.89 (s, 1H, O*H*), 5.42 (br s, 1H, O*H*), 7.90 (br s, 3H, N*H*), 12.70 (br s, 1H, COO*H*) ppm. ^{13}C NMR (150 MHz, DMSO, 27 °C): δ = 22.5, 29.6, 44.6, 51.4, 67.4, 71.4, 173.7 ppm. Anal. Calcd for $C_7H_{14}ClNO_4$ (211.64): C, 39.72; H, 6.67; N, 6.62. Found: C, 39.55; H, 6.83; N, 6.64.

4.6. General synthesis of stereoisomeric 2-amino-4,5-dihydroxycyclohexanecarboxylic acids (−)-6, (−)-9 and 2-amino-3,4-dihydroxycyclohexanecarboxylic acids, (−)-15 and (−)-18

Dihydroxy esters (+)-**5**, (−)-**8**, (−)-**14** or (−)-**17** (0.2 g, 0.66 mmol) were dissolved in water (4 mL) in a CEM-Discover microwave pressure tube. The reaction mixture was then stirred at 150 °C for 60 min under a maximum microwave irradiation of 150 W. After cooling, the mixtures were diluted with acetone (6 mL) and the products crystallized out. The crude amino acids were recrystallized from H_2O/acetone to afford pale-yellow crystalline solids.

4.6.1. (1*S*,2*R*,4*R*,5*S*)-2-Amino-4,5-dihydroxycyclohexanecarboxylic acid, (−)-6

Yield: 81 mg (70%), mp 248–250 °C (dec.), $[\alpha]_D^{20} = -11.7$ (c = 0.35, H_2O); ee >99%. ^{1}H NMR (500 MHz, D_2O, 27 °C): δ = 1.86–1.93 (m, 1H, H-6), 1.96–2.08 (m, 3H, H-3, H-3, H-6), 2.78 (q, J = 4.7 Hz, 1H, H-1), 3.61 (dt, J = 9.8, 4.7, 4.7 Hz 1H, H-2), 3.70 (d, J = 8.7 Hz, 1H, H-5), 3.98–4.01 (m, 1H, H-4) ppm. ^{13}C NMR (125 MHz, D_2O, 27 °C): δ = 29.3, 31.8, 41.1, 46.7, 67.9, 67.9, 179.1 ppm. Anal. Calcd for $C_7H_{13}NO_4$ (175.18): C, 47.99; H, 7.48; N, 8.00. Found: C, 48.07; H, 7.62; N, 8.15.

4.6.2. (1*R*,2*R*,4*R*,5*S*)-2-Amino-4,5-dihydroxycyclohexanecarboxylic acid, (−)-9

Yield: 89 mg (77%), mp 236–240 °C (dec.), $[\alpha]_D^{20} = -56$ (c = 0.37, H_2O); ee >99%. ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.40 (t, J = 12.4 Hz, 1H, H-3ax), 1.47 (q, J = 12.4 Hz, 1H, H-6ax), 1.77 (dt, J = 3.0, 12.4, 12.4 Hz, 1H, H-1), 1.88–1.95 (m, 2H, H-6eq, H-3eq), 2.98 (dt, J = 3.0, 11.7, 11.7 Hz,1H, H-2), 3.35 (dt, J = 11.6, 4.0. 3.4 Hz, 1H, H-5), 3.76 (s, 1H, H-4), 4.25–4.54 (m, 2H, O*H*), 8.51 (s, 2H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 30.3, 36.6, 43.9, 47.0, 68.2, 70.5, 176.5 ppm. Anal. Calcd for $C_7H_{13}NO_4$ (175.18): C, 47.99; H, 7.48; N, 8.00. Found: C, 47.55; H, 7.58; N, 8.05.

4.6.3. (1*R*,2*R*,3*S*,4*R*)-2-Amino-3,4-dihydroxycyclohexanecarboxylic acid, (−)-15

Yield: 86 mg (74%), mp 227–230 °C (dec.), $[\alpha]_D^{20} = -81.4$ (c = 0.35, H_2O); ee >99%. ^{1}H NMR (500 MHz, D_2O, 47 °C): δ = 1.98 (dd, J = 14.8, 12.0 Hz 1H, H-5ax), 2.15–2.28 (m, 2H, H-5eq, H-6ax), 2.34–2.43 (m, 1H, H-6eq), 3.27 (s, 1H, H-1), 3.92 (d, J = 10.3, 4.7 Hz, 1H, H-2), 4.39 (d, J = 10.3 Hz, 1H, H-3), 4.50 (s, 1H, H-4) ppm. ^{13}C NMR (125 MHz, D_2O, 47 °C): δ = 21.6, 27.8, 42.6, 52.2, 69.3, 69.4, 174.9 ppm. Anal. Calcd for $C_7H_{13}NO_4$ (175.18): C, 47.99; H, 7.48; N, 8.00. Found: C, 48.12; H, 7.51; N, 7.89.

4.6.4. (1*S*,2*R*,3*S*,4*R*)-2-Amino-3,4-dihydroxycyclohexanecarboxylic acid, (−)-18

Yield: 83 mg (72%), mp 258 °C (dec.), $[\alpha]_D^{20} = -5.2$ (c = 0.38, H_2O); ee >99%. ^{1}H NMR (500 MHz, D_2O, 27 °C): δ = 1.66–1.76 (m, 2H, H-5, H-6), 1.96–2,02 (m, 2H, H-5, H-6), 2.38–2.44 (m, 1H, H-1), 3.49 (t, 11.0 Hz, 1H, H-2), 3.74 (d, J = 10.5 Hz, 1H, H-3), 4.16 (s, 1H, H-4) ppm. ^{13}C NMR (125 MHz, D_2O, 27 °C): δ = 22.6, 29.3, 46.8, 53.0, 68.8, 71.8, 174.7 ppm. Anal. Calcd for $C_7H_{13}NO_4$ (175.18): C, 47.99; H, 7.48; N, 8.00. Found: C, 48.04; H, 7.35; N, 7.93.

4.7. Racemic compounds

All the reactions for the racemic compounds were first optimized. The ^{1}H and ^{13}C NMR spectroscopic data and elemental analyses on the racemic derivatives are in accordance with those for the enantiomers. Representative data on the racemates.

4.7.1. Ethyl (1*S**,2*R**,4*R**,5*S**)-2-*tert*-butoxycarbonylamino-4,5-dihydroxycyclohexane-carboxylate, (±)-5

White crystals, mp 76–78 °C.

4.7.2. (1*S**,2*R**,4*R**,5*S**)-2-Amino-4,5-dihydroxycyclohexane-carboxylic acid hydrochloride, (±)-6·HCl

Pale-yellow crystals, mp 227 °C (dec.).

4.7.3. (1*S**,2*R**,4*R**,5*S**)-2-Amino-4,5-dihydroxycyclohexane-carboxylic acid, (±)-6

Pale-yellow crystals, mp 231 °C (dec.).

4.7.4. Ethyl (1*R**,2*R**,4*R**,5*S**)-2-*tert*-butoxycarbonylamino-4,5-dihydroxycyclohexane-carboxylate, (±)-8

White crystals, mp 99–101 °C.

4.7.5. (1*R**,2*R**,4*R**,5*S**)-2-Amino-4,5-dihydroxycyclohexane-carboxylic acid hydrochloride, (±)-9·HCl

Pale-yellow crystals, mp 220 °C (dec.).

4.7.6. (1*R**,2*R**,4*R**,5*S**)-2-Amino-4,5-dihydroxycyclohexane-carboxylic acid, (±)-9

Pale-yellow crystals, mp 251 °C (dec.).

4.7.7. Ethyl (1*R**,2*S**)-2-*tert*-butoxycarbonylaminocyclohex-3-enecarboxylate, (±)-13

White crystals, mp 67–69 °C.

4.7.8. Ethyl (1*R**,2*R**,3*S**,4*R**)-2-*tert*-butoxycarbonylamino-3,4-dihydroxycyclohexane-carboxylate, (±)-14

White crystals, mp 123–125 °C.

4.7.9. (1*R**,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclohexane-carboxylic acid hydrochloride, (±)-15·HCl

Pale-yellow crystals, mp 213 °C (dec.).

4.7.10. (1*R**,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclohexane-carboxylic acid, (±)-15

Pale-yellow crystals, mp 233 °C (dec.).

4.7.11. Ethyl (1*S**,2*S**)-2-*tert*-butoxycarbonylaminocyclohex-3-enecarboxylate, (±)-16

White solid, mp 71–74 °C.

4.7.12. Ethyl (1*S**,2*R**,3*S**,4*R**)-2-*tert*-butoxycarbonylamino-3,4-dihydroxycyclohexane-carboxylate, (±)-17

White crystals, mp 165–168 °C.

4.7.13. (1*S**,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclohexane-carboxylic acid hydrochloride, (±)-18·HCl

Pale-yellow crystals, mp 225 °C (dec.).

4.7.14. (1*S**,2*R**,3*S**,4*R**)-2-Amino-3,4-dihydroxycyclohexanecarboxylic acid, (±)-18

Pale-yellow crystals, mp 275 °C (dec.).

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Synthesis of mono- and dihydroxy-substituted 2-aminocyclooctanecarboxylic acid enantiomers

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ABSTRACT

(1*R*,2*S*,6*R*)-2-Amino-6-hydroxycyclooctanecarboxylic acid (−)-**10** was synthesized from (1*R*,2*S*)-2-aminocyclooct-5-enecarboxylic acid (+)-**2** via an iodolactone intermediate, while (1*R*,2*S*,3*R*,4*S*)-2-amino-5,6-dihydroxycyclooctanecarboxylic acid (−)-**12** was prepared by using the OsO_4-catalysed oxidation of Boc-protected amino ester (−)-**5**. The stereochemistry and relative configurations of the synthesized compounds were determined by 1D and 2D NMR spectroscopy (based on 2D NOE cross-peaks and ^3J(H,H) coupling constants) and X-ray crystallography.

1. Introduction

The stereoselective synthesis of alicyclic β-amino acids,[1,2] derived from β-lactams,[3,4] has attracted attention because of their occurrence in many pharmacologically relevant compounds.[5] They can become important components for peptides with the aim of modifying their biological activities.[6] These compounds are to be found in various natural and synthetic biologically active molecules, for example, natural cispentacin and its synthetic 4-methylene analogue (Icofungipen, PLD-118) exhibit antifungal activity.[7–11] These molecules are also useful starting materials; as an example anatoxin-*a*,[12–14] a nicotinic acetylcholine receptor agonist, was efficiently synthesized from the β-lactam.[15]

Among the β-amino acids, hydroxy-functionalized counterparts also play an important role in medicinal chemistry because of their presence in many essential products, such as Paclitaxel (Taxol®) and its synthetic derivative Docetaxel (Taxotere®), which exert significant chemotherapeutic effects.[16–18] Some cyclic derivatives have antibiotic (oryzoxymycin) and antifungal activities.[19–22]

We earlier reported several methods for the mono- or dihydroxylation of the cyclopentene and cyclohexene rings, but there is no example involving mono- or dihydroxycyclooctane β-amino acids in the literature. By iodolactonization[23] and epoxidation[24,25] of the double bond or via dihydrooxazine[26] and oxazoline[27] derivatives, the corresponding monohydroxy β-amino acids can be efficiently synthesized. For dihydroxylation, well-known routes involve oxidation of the double bond with $KMnO_4$ or with catalytic OsO_4.[26,28]

Our present work focuses on functionalization of the double bond of N-protected *cis*-2-aminocyclooct-5-enecarboxylic acid derivatives and analysis of the structures of the newly prepared mono- and dihydroxy-substituted enantiopure and racemic molecules.

2. Results and discussion

The racemic β-lactam (±)-**1** was prepared by 1,2-cycloaddition of chlorosulfonyl isocyanate (CSI) in dry CH_2Cl_2 at room temperature by modification of a literature process.[29] The starting (1*R*,2*S*)-2-aminocyclooct-5-enecarboxylic acid (+)-**2** was synthesized from (±)-**1** by highly enantioselective Lipolase-catalysed ring opening with 1 equiv of H_2O in iPr_2O at 70 °C.[30]

The enantiopure amino acid (+)-**2** (ee >99%) was esterified in the presence of EtOH and $SOCl_2$ to furnish amino ester hydrochloride (−)-**4**, which was then reacted with *tert*-butoxy pyrocarbonate, affording the *N*-Boc-protected amino ester (−)-**5**. An alternative synthesis of (±)-**5**, which was used in the case of racemic compounds, comprised hydrolysis of (±)-**1** with 22% ethanolic HCl at room temperature to give (±)-**4**, which was then acylated by the above method (Scheme 1).

The starting material in the iodolactonization reaction was *cis*-2-*tert*-butoxycarbonylaminocyclooct-5-enecarboxylic acid (−)-**6**. Enantiopure (−)-**6** was prepared from (+)-**2** with Boc_2O, while (±)-**6** was synthesized by the ring opening of (±)-**1** with 18% aqueous HCl and after acylation with di-*tert*-butyl dicarbonate. The N-protected acid (−)-**6** was reacted with I_2/KI/aqueous

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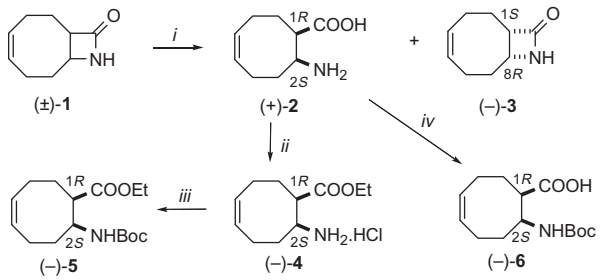
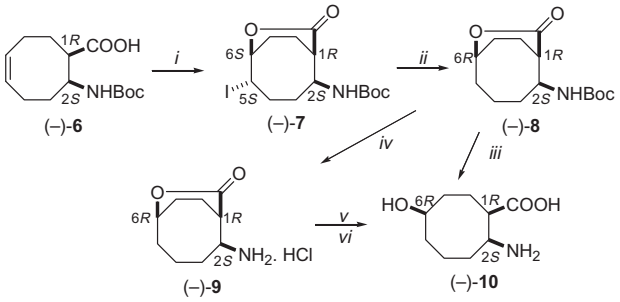


Scheme 1. Reagents and conditions: (i) Lipolase, iPr_2O, 70 °C; (ii) $SOCl_2$, EtOH, 30 min, 0 °C, 3 h rt, 1 h, △, 88%; (iii) Et_3N, Boc_2O, THF, 2 h, rt, 91%; (iv) dioxane/H_2O, Boc_2O, 4 h, rt, 76%.

$NaHCO_3$ in CH_2Cl_2 to give iodolactone (−)-**7** regio- and diastereoselectively as a white crystalline product, in good yield. Reduction of the iodo group with Bu_3SnH in CH_2Cl_2 yielded lactone (−)-**8** which, after hydrolysis with microwave irradiation, gave (1*R*,2*S*,6*R*)-2-amino-6-hydroxycyclooctanecarboxylic acid ((−)-**10**) in good yield. When ring opening of the Boc–lactone (−)-**8** was attempted with hydrochloric acid, deprotected lactone (−)-**9** was observed, which was transformed to hydroxy-amino acid (−)-**10** upon microwave irradiation followed by heating in propylene oxide (Scheme 2). The presence of the lactone ring in (−)**7**-(−)-**9** was confirmed by the cross-peak between H-6 and the carbonyl carbon in the HMBC spectra. In the case of (−)-**10**, the NOE cross-peak between H-1 and H-6 suggests that the hydroxyl group should have a *cis* configuration relative to the carboxyl group. A small NOE cross-peak was also observed between H-2 and H-6, which indicates the conformational flexibility of the compound and also proves the orientation of the amino group. The stereochemistry of (±)-**7** and (±)-**9** was confirmed by X-ray diffraction (Figs. 1 and 2).



Scheme 2. Reagents and conditions: (i) I_2/KI, $NaHCO_3$, CH_2Cl_2, 20 h, rt, 71%; (ii) Bu_3SnH, CH_2Cl_2, 20 h, 40 °C, 76%; (iii) microwave irradiation, H_2O, 1 h, 150 °C, 67%; (iv) 10% HCl/H_2O, 24 h, 82%; (v) microwave irradiation, H_2O, 1 h, 150 °C, 65%; (vi) propylene oxide, 1 h, △, 62%.

(1*R*,2*S*,5*R*,6*S*)-2-Amino-5,6-dihydroxycyclooctane-carboxylic acid (−)-**12** was obtained by OsO_4-catalysed dihydroxylation. The oxidation of *N*-Boc ester (−)-**5** with catalytic OsO_4 and *N*-methylmorpholine *N*-oxide (NMO) as the stoichiometric co-oxidant afforded the desired product (−)-**11** as a single diastereomer in good yield. After deprotection of the compound, microwave irradiation in water resulted in the corresponding dihydroxy-aminoacid (−)-**12** in good yield (Scheme 3). In the case of (−)-**11**, the all-*cis* stereochemistry of the substituents can be proved by the NOE cross-peaks between H-2 and H-5 and between H-1 and H-6. The presence of these NOE signals not only confirms the *cis* orientation of the functional groups, but also indicates the conformational flexibility of (−)-**11**. For (−)-**12**, a similar NOE pattern was observed which indicates the all-*cis* orientation of the functional groups and reveals that the microwave irradiation did not affect the stereochemistry. The stereochemistry of **12** was also confirmed by X-ray diffraction (Fig. 3). From a racemic mixture, a single enantiomer crystallized out in a zwitterionic form in the crystal investigated. However, the data did not permit confirmation of which enantiomer this was.

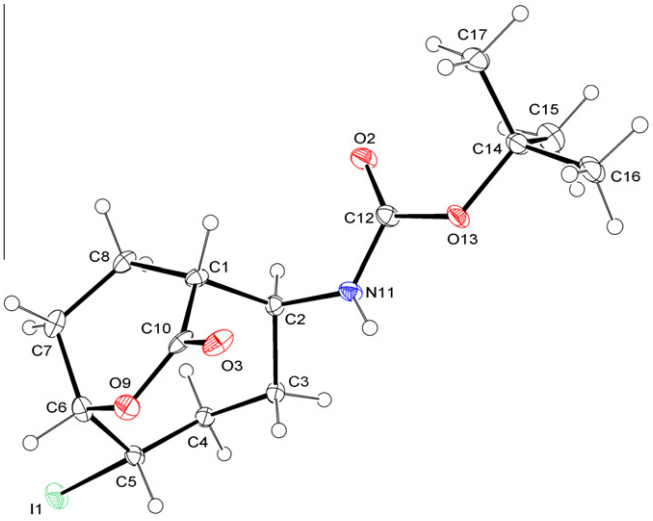


Figure 1. ORTEP plot of the X-ray structure of iodolactone (±)-**7**.

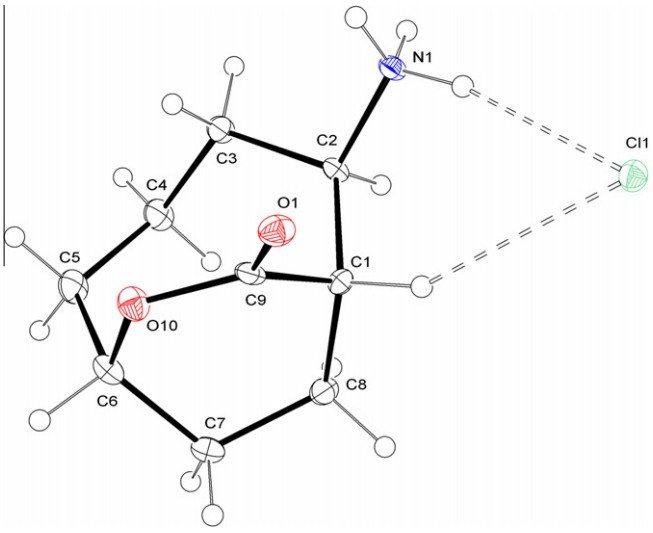
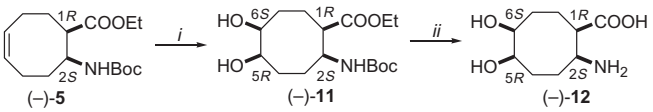


Figure 2. ORTEP plot of the X-ray structure of lactone (±)-**9**.



Scheme 3. Reagents and conditions: (i) 2.0% w/w solution of OsO_4 in *t*-BuOH, NMO, acetone, 4 h, rt, 91%; (ii) microwave irradiation, H_2O, 1 h, 150 °C, 69%.

3. Conclusions

In summary, we have successfully synthesized either racemic and enantiomeric 6-hydroxy- and 5,6-dihydroxy-2-aminocyclooctanecarboxylic acid derivatives by using iodolactonization and OsO_4-catalysed dihydroxylation. All the racemic and enantiopure derivatives produced can be used for further valuable transforma-

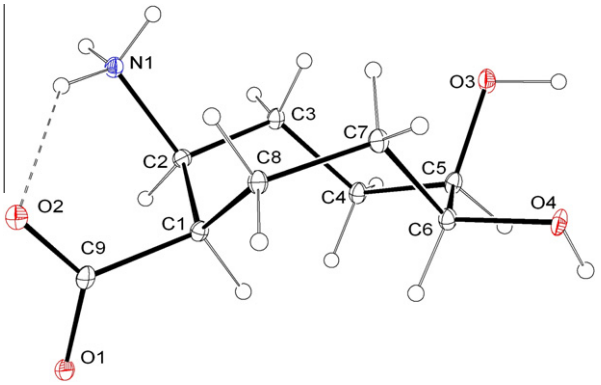


Figure 3. ORTEP plot of the X-ray structure of zwitterionic dihydroxy-substituted amino acid **12**.

tions, and they are good starting materials for the synthesis of peptides and different heterocycles with potential biological activity.

4. Experimental

4.1. General

The NMR spectra were recorded at ambient temperature in $CDCl_3$, DMSO-d_6 or D_2O with a Bruker AV 500 spectrometer at 500 MHz and at 125 MHz for ^1H and ^{13}C, respectively. Chemical shifts are given in δ (ppm) relative to TMS as an internal standard. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II Elemental Analyzer. Melting points were measured with a Kofler melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin Elmer 341 polarimeter. Microwave reactions were performed in a CEM Discover LabMate MW reactor.

The ee value for the starting (1*R*,2*S*)-2-aminocyclooct-5-enecarboxylic acid (+)-**2** was determined by using GC after double derivatization.[31]

The ee values for the HCl salts of the final products were determined by HPLC. A Chirobiotic TAG 5 μ column (0.46 cm × 25 cm) was used at room temperature; the mobile phase was MeOH containing 0.1% TEA and 0.1% AcOH; flow rate 1 mL/min; detection at 216 nm; retention times (min): (−)-**10**, 28.08 (antipode: 30.92) and (−)-**12**, 25.49 (antipode: 27.36).

4.2. Ethyl (1*R*,2*S*)-2-aminocyclooct-5-enecarboxylate hydrochloride (−)-4

Thionyl chloride (0.86 g, 7.27 mmol) was added dropwise with stirring to dry EtOH (5 mL) at −15 °C. Compound (+)-**2** (1 g, 6.61 mmol) was added in one portion to this mixture, which was then stirred at 0 °C for 30 min. After subsequent stirring at room temperature for a further 3 h, the mixture was refluxed for 1 h and then concentrated to give colourless crystals (1.5 g, 88%) mp 108–110 °C, lit. mp 112–117 °C,[29] $[\alpha]_D^{20} = -1.5$ (*c* 1, EtOH). The ^1H NMR data are in accordance with those reported in the literature.[29] ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 14.3, 23.2, 23.7, 27.0, 30.3, 44.5, 52.0, 61.4, 128.8, 130.2, 172.9 ppm.

4.3. Ethyl (1*R*,2*S*)-2-*tert*-butoxycarbonylaminocyclooct-5-enecarboxylate (−)-5

To a suspension of (−)-**4** (1.5 g, 6.42 mmol) in THF (50 mL) were added Et_3N (1.29 g, 12.84 mmol) and di-*tert*-butyl dicarbonate (1.54 g, 7.06 mmol) at 0 °C. Stirring was continued for 3 h at room temperature, after which the organic layer was diluted with EtOAc and washed with H_2O (2 × 20 mL). The aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic phase was dried (Na_2SO_4) and the solvents were evaporated off. The residue was purified by column chromatography (*n*-hexane/EtOAc 5:1) to afford a colourless oil (1.74 g, 91%), $[\alpha]_D^{20} = -53.4$ (*c* 1, EtOH). ^1H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.28 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.43 (s, 9H, *t*Bu), 1.72–1.82 (m, 2H, H-3, H-8), 1.86–1.94 (m, 1H, H-3), 1.96–2.02 (m, 1H, H-4), 2.07–2.21 (m, 2H, H-7, H-8), 2.26–2.34 (m, 1H, H-4), 2.44–2.51 (m, 1H, H-7), 2.81–2.85 (m, 1H, H-1), 4.11–4.22 (m, 3H, H-2, CH_2CH_3), 4.99–5.05 (m, 1H, N*H*), 5.61–5.72 (m, 2H, H-5, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 14.2, 23.3, 24.6, 27.3, 28.4, 32.9, 48.0, 50.2, 60.4, 79.2, 129.2, 130.4, 155.0, 174.2 ppm. Anal. Calcd for $C_{16}H_{27}NO_4$ (297.39): C, 64.62; H, 9.15; N, 4.71. Found: C, 64.57; H, 9.11; N, 4.84.

4.4. (1*R*,2*S*)-2-*tert*-Butoxycarbonylaminocyclooct-5-enecarboxylic acid (−)-6

Amino acid (+)-**2** (0.98 g, 4.76 mmol) was dissolved in a mixture of dioxane (10 mL) and water (5 mL), and 1 M NaOH (5 mL) and *tert*-butoxypyrocarbonate (1.14 g, 5.24 mmol) were added to the solution at 0 °C. The pH was adjusted to 8.5 with 1 M NaOH and the mixture was stirred at room temperature for 5 h. The solvent was then evaporated down to one-third volume, and the mixture was diluted with EtOAc (30 mL) and acidified with 10% H_2SO_4 (pH 2.5). The mixture was extracted with EtOAc (3 × 15 mL), the combined organic phase was dried ($NaSO_4$), and the solvents were evaporated off. The residue was recrystallized from iPr_2O to give a white crystalline solid (0.97 g, 76%), mp 125–127 °C, $[\alpha]_D^{20} = -59.0$ (*c* 1, EtOH). ^1H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.45 (s, 9H, *t*Bu), 1.79–1.88 (m, 2H, H-3, H-8), 1.93–2.00 (m, 1H, H-3), 2.00–2.21 (m, 3H, H-4eq, H-7eq, H-8), 2.27–2.36 (m, 1H, H-4ax), 2.44–2.52 (m, 1H, H-7ax), 2.97 (s, 1H, H-1), 4.16–4.22 (m, 1H, H-2), 5.09 (s, 1H, N*H*), 5.68–5.74 (m, 2H, H-5, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 22.9, 24.6, 27.6, 28.4, 33.0, 48.2, 50.0, 80.1, 129.6, 130.7, 155.8, 177.9 ppm.. Anal. Calcd for $C_{14}H_{23}NO_4$ (269.34): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.36; H, 8.52; N, 5.14.

4.5. (1*R*,2*S*,5*S*,6*S*)-2-*tert*-Butoxycarbonylamino-5-iodo-7-oxabicyclo[4.2.2]decan-8-one (−)-7

To a solution of carboxylic acid derivative (−)-**6** (1 g, 3.71 mmol) in CH_2Cl_2 (25 mL) were added $NaHCO_3$ (0.5 M, 22 mL), KI (3.69 g, 22.26 mmol) and I_2 (1.88 g, 7.42 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 20 h, and saturated $Na_2S_2O_3$ (25 mL) was then added. The mixture was extracted with CH_2Cl_2 (3 × 15 mL), in the extract was dried (Na_2SO_4), and the solvents were evaporated off. The residue was recrystallized from iPr_2O to give iodolactone (−)-**7** (1.04 g, 71%), mp 105–107 °C, $[\alpha]_D^{20} = -76.2$ (*c* 1, EtOH). ^1H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.31–1.40 (m, 1H, H-3ax), 1.43 (s, 9H, *t*Bu), 2.00–2.14 (m, 3H, H-3eq, H-7, H-8), 2.26–2.37 (m, 2H, H-4ax, H-8), 2.47–2.54 (m, 1H, H-4eq), 2.53–2.56 (m, 1H, H-7), 3.04 (dt, *J* = 11.3, 2.5, 2.5 Hz, 1H, H-1), 3.82–3.89 (m, 1H, H-2), 4.55 (dt, *J* = 12.0, 4.0, 4.0 Hz, 1H, H-5), 5.02–5.06 (m, 2H, N*H*, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 18.9, 22.5, 28.4, 31.7, 33.3, 34.2, 43.5, 55.4, 79.8, 82.7, 155.3, 172.0 ppm. Anal. Calcd for $C_{14}H_{22}INO_4$ (395.23): C, 42.54; H, 5.61; N, 3.54. Found: C, 42.46; H, 5.52; N, 3.56.

4.6. (1*R*,2*S*,6*R*)-2-*tert*-Butoxycarbonylamino-7-oxabicyclo[4.2.2]decan-8-one (−)-8

At first, Bu_3SnH (1.36 mL, 5.06 mmol) was added to a solution of iodolactone (−)-**7** (1 g, 2.53 mmol) in dry CH_2Cl_2 (35 mL) under argon. After stirring for 20 h at 40 °C, the solvent was evaporated off,

and the crude lactone was crystallized from *n*-hexane and recrystallized from iPr_2O (0.52 g, 76%), mp 102–105 °C, $[\alpha]_D^{20} = -93.1$ (*c* 0.5, EtOH). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.34–1.47 (m, 10H, *t*Bu, H-3ax), 1.64–1.74 (m, 1H, H-4ax), 1.75–1.87 (m, 3H, H-4eq, H-5, H-7), 1.98–2.06 (m, 1H, H-8), 2.07–2.20 (m, 3H, H-3eq, H-5, H-7), 2.27 (ddd, *J* = 13.1, 11.4, 10.6 Hz, 1H, H-8), 2.99 (dt, *J* = 11.2, 2.7, 2.7 Hz, 1H, H-1), 3.81–3.87 (m, 1H, H-2), 4.84 (dt, *J* = 6.0, 3.0, 3.0 Hz, 1H, H-6), 5.14 (d, *J* = 7.6 Hz, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 20.7, 22.3, 22.7, 28.2, 31.8, 35.3, 43.2, 55.8, 78.6, 79.5, 154.9, 173.1 ppm. Anal. Calcd for $C_{14}H_{23}NO_4$ (269.34): C, 62.43; H, 8.61; N, 5.20. Found: C, 62.35; H, 8.66; N, 5.15.

4.7. (1*R*,2*S*,6*R*)-2-Amino-7-oxabicyclo[4.2.2]decan-8-one hydrochloride (−)-9

A solution of lactone (−)-**8** (0.6 g, 2.23 mmol) was dissolved in aqueous HCl (10%; 20 mL) and the mixture was stirred for 24 h at rt. The solvent was then evaporated off to afford the crude amino lactone hydrochloride which was recrystallized from EtOH/Et_2O (0.33 g, 68%), mp 250–252 °C, $[\alpha]_D^{20} = -23.9$ (*c* 0.4, H_2O). ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.37 (ddd, *J* = 14.0, 11.5, 9.8 Hz, 1H, H-3ax), 1.67–1.87 (m, 4H, H-4, H-4, H-5, H-7), 1.89–2.08 (m, 3H, H-5, H-7, H-8), 2.09–2.16 (m, 1H, H-3eq), 2.20 (ddd, *J* = 12.7, 11.3, 8.9 Hz, 1H, H-8), 3.09 (dt, *J* = 11.1, 2.5, 2.5 Hz, 1H, H-1), 3.45 (ddd, *J* = 10.8, 4.6, 2.8 Hz, 1H, H-2), 4.81–4.84 (m, 1H, H-6), 8.08 (s, 3H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 19.0, 21.0, 21.6, 29.1, 34.5, 40.5, 55.0, 78.3, 170.9 ppm. Anal. Calcd for $C_9H_{16}ClNO_2$ (205.09): C, 52.56; H, 7.84; Cl, 17,24; N, 6.81. Found: C, 52.35; H, 7.66; Cl, 17,43; N, 7.05.

4.8. (1*R*,2*S*,6*R*)-2-Amino-6-hydroxycyclooctanecarboxylic acid (−)-10

4.8.1. Method A

The lactone (−)-**8** (178 mg, 0.66 mmol) was dissolved in water (4 mL) in a 10-mL pressurized reaction vial, and the mixture was then stirred at 150 °C for 60 min at max. One hundred and fifty watts microwave irradiation. After cooling, the mixture was diluted with acetone (6 mL) and the product crystallized out from the solvent. The crude amino acid was recrystallized from H_2O/acetone to afford a pale-yellow crystalline solid (83 mg, 67%), mp 211–213 °C (dec).

4.8.2. Method B

Lactone (−)-**9** (205 mg, 1 mmol) was dissolved in water (4 mL) in a 10-mL pressurized reaction vial, and the reaction mixture was then stirred at 150 °C for 60 min at max. One hundred and fifty watts microwave irradiation. The solvent was evaporated off, the residue was dissolved in propylene oxide (10 mL) and the mixture was refluxed for 1 h. The product crystallized out from the solvent. The crude amino acid was recrystallized from H_2O/acetone to afford a pale-yellow crystalline product (116 mg, 62%), mp 210–212 °C (dec), $[\alpha]_D^{20} = -9.0$ (*c* 0.4, H_2O), ee >99%. ^{1}H NMR (500 MHz, D_2O, 27 °C): δ = 1.36–1.44 (m, 1H, H-4), 1.51–1.58 (m, 1H, H-5), 1.67–1.98 (m, 8H, H-3, H-3, H-4, H-5, H-7, H-7, H-8, H-8), 2.62 (dt, *J* = 10.1, 3.7, 3.7 Hz, 1H, H-1), 3.46 (dt, *J* = 10.0, 4.0, 4.0 Hz, 1H, H-2), 3.82–3.87 (m, 1H, H-6) ppm. ^{13}C NMR (125 MHz, D_2O, 27 °C): δ = 19.1, 22.6, 28.9, 32.3, 34.3, 44.5, 51.2, 70.3, 180.9 ppm. Anal. Calcd for $C_9H_{17}NO_3$ (187.24): C, 57.73; H, 9.15; N, 7.48. Found: C, 57.68; H, 9.22; N, 7.45.

4.9. Ethyl (1*R*,2*S*,5*R*,6*S*)-2-*tert*-butoxycarbonylamino-5,6-dihydroxycyclooctanecarboxylate (−)-11

OsO_4 (1 mL, 0.08 mmol; a 2.0% w/w solution in *t*BuOH) was added to a stirred solution of *N*-methylmorpholine *N*-oxide (1.18 g, 10.13 mmol) and (−)-**5** (0.5 g, 1.68 mmol) in acetone (20 mL) and the mixture was stirred at room temperature for a further 4 h. When the reaction was complete (monitored by TLC), the mixture was treated with saturated aqueous Na_2SO_3 (20 mL). The aqueous layer was next extracted with EtOAc (3 × 20 mL), the combined organic layer was dried (Na_2SO_4) and the solvent was removed by evaporation under reduced pressure. Compound (−)-**11** was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1:1) to afford a colourless oil (0.5 g, 91%), $[\alpha]_D^{20} = -27.2$ (*c* 1, EtOH). ^{1}H NMR (500 MHz, DMSO, 27 °C): δ = 1.16 (t, *J* = 7.1 Hz, 3H, CH_2CH_3), 1.30–1.40 (m, 11H, *t*Bu, H-4, H-7), 1.42–1.48 (m, 1H, H-3), 1.53–1.60 (m, 1H, H-8), 1.80–1.96 (m, 3H, H-3, H-4, H-8), 1.98–2.06 (m, 1H, H-7), 2.62 (dt, *J* = 11.0, 3.5, 3.5 Hz, 1H, H-1), 3.60 (t, *J* = 6 Hz, 1H, H-6), 3.65 (br s, 1H, H-5), 3.91–4.04 (m, 3H, CH_2CH_3, H-2), 4.22 (d, *J* = 3.3 Hz, 1H, O*H*), 4.30 (d, *J* = 4.6 Hz, 1H, O*H*), 6.61 (d, *J* = 9.2 Hz, 1H, N*H*) ppm. ^{13}C NMR (125 MHz, DMSO, 27 °C): δ = 14.0, 20.3, 26.1, 27.7, 28.1, 28.2, 44.5, 50.1, 59.8, 71.5, 71.5, 77.5, 154.8, 173.9 ppm. Anal. Calcd for $C_{16}H_{29}NO_6$ (331.40): C, 57.99; H, 8.82; N, 4.23. Found: C, 57.81; H, 8.89; N, 4.12.

4.10. (1*R*,2*S*,5*R*,6*S*)-2-Amino-5,6-dihydroxycyclooctanecarboxylic acid (−)-12

Dihydroxy ester (−)-**11** (0.2 g, 0.9 mmol) was dissolved in water (4 mL) in a 10-mL pressurized reaction vial and the reaction mixture was stirred at 150 °C for 60 min at max. One hundred and fifty watts microwave irradiation. After cooling, the mixture was diluted with acetone (5 mL) and the product crystallized out from the solvent. The crude amino acid was recrystallized from H_2O/acetone to afford a pale-yellow crystalline solid (126 mg, 69%), mp 207–210 °C (dec), $[\alpha]_D^{20} = -7.6$ (*c* 0.5, H_2O), ee >99%. ^{1}H NMR (500 MHz, D_2O, 27 °C): δ = 1.57–1.66 (m, 2H, H-4, H-7), 1.76–1.87 (m, 2H, H-3, H-8), 1.89–2.06 (m, 4H, H-3, H-4, H-7, H-8), 2.62 (dt, *J* = 10.1, 3.5, 3.5 Hz, 1H, H-1), 3.54 (ddd, *J* = 10.3, 5.0, 3.5 Hz, 1H, H-2), 3.84 (dt, *J* = 8.6, 2.2, 2.2 Hz, 1H, H-6), 3.92 (dt, *J* = 7.4, 2.3, 2.6 Hz, 1H, H-5) ppm. ^{13}C NMR (125 MHz, D_2O, 27 °C): δ = 22.3, 23.5, 25.9, 27.1, 43.8, 50.9, 71.1, 72.1, 181.0 ppm. Anal. Calcd for $C_9H_{17}NO_4$ (203.24): C, 53.19; H, 8.43; N, 6.89. Found: C, 53.01; H, 8.57; N, 6.78.

4.11. Racemic compounds

All the reactions were first optimized for the racemic compounds. The ^{1}H and ^{13}C NMR spectroscopic data and elemental analyses on the racemic derivatives are in accordance with those for the enantiomers.

4.11.1. (1*R**,2*S**)-9-Azabicyclo[6.2.0]dec-4-en-10-one (±)-1

To a solution of 1,5-cyclooctadiene (30 g, 0.28 mol) in dry CH_2Cl_2 (250 mL) was added dropwise a solution of CSI (39.63 g, 0.28 mol) in dry CH_2Cl_2 (150 mL) at room temperature. After stirring for a further 72 h, the resulting liquid was poured into a stirred solution of Na_2SO_3 (54.5 g, 0.43 mol) in water (148 mL), and the pH was adjusted to 8–9 with 20% KOH solution. The mixture was stirred at room temperature for 3 h, after which the organic layer was separated off and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The resulting yellow solid was recrystallized from iPr_2O to give (17.78 g, 42%) of pure (±)-**1**, mp 110–113 °C, lit. 112–113 °C.[29] The ^{1}H NMR, ^{13}C NMR and elemental analysis data are in accordance with those reported in the literature.[30]

4.11.2. (1*R**,2*S**)-2-Aminocyclooct-5-enecarboxylic acid hydrochloride (±)-2·HCl

A solution of β-lactam (±)-**1** (1.5 g, 9.92 mmol) in H_2O containing 18% of HCl (15 mL) was refluxed for 1 h. After removal of the

solvent, amino acid hydrochloride (±)-**2**·HCl was obtained, which was recrystallized from EtOH to Et_2O. Colourless crystals (1.67 g, 82%), mp 229–231 °C, lit. mp 218–220 °C.[29] The ^1H NMR, ^{13}C NMR and elemental analysis data are in accordance with those reported in the literature.[30]

4.11.3. Ethyl (1*R**,2*S**)-2-aminocyclooct-5-enecarboxylate hydrochloride (±)-4

A solution of β-lactam (±)-**1** (3 g, 19.84 mmol) in EtOH containing 22% HCl (30 mL) was stirred for 2 h at room temperature. After removal of the solvent, amino ester hydrochloride **3** was obtained, which was recrystallized from EtOH to Et_2O. Colourless crystals (4.13 g, 89%), mp 108–110 °C, lit. mp 112–117 °C.[29] The ^1H NMR, ^{13}C NMR and elemental analysis data are in accordance with those reported in the literature.[30]

4.11.4. Representative data on the racemates (±)-5-(±)-12

4.11.4.1. Ethyl (1*R,2*S**)-2-*tert*-Butoxycarbonylaminocyclooct-5-enecarboxylate (±)-5.** Colourless oil.

4.11.4.2. (1*R,2*S**)-2-*tert*-Butoxycarbonylaminocyclooct-5-enecarboxylic acid (±)-6.** White crystals, mp 119–123 °C.

4.11.4.3. (1*R,2*S**,5*S**,6*S**)-2-(*tert*-Butoxycarbonylamino)-5-iodo-7-oxabicyclo-[4.2.2]decan-8-one (±)-7.** White crystals mp, 144–146 °C.

4.11.4.4. (1*R,2*S**,6*R**)-2-(*tert*-Butoxycarbonylamino)-7-oxabicyclo-[4.2.2]decan-8-one (±)-8.** Pale-yellow crystals, mp 86–88 °C.

4.11.4.5. (1*R,2*S**,6*R**)-2-Amino-6-hydroxycyclooctanecarboxylic acid (±)-9.** White crystals, mp 255–258 °C.

4.11.4.6. (1*R,2*S**,6*R**)-2-Amino-6-hydroxycyclooctanecarboxylic acid (±)-10.** White crystals, mp 220–222 °C (dec).

4.11.4.7. Ethyl (1*R,2*S**,5*R**,6*S**)-2-*tert*-butoxycarbonylamino-5,6-dihydroxycyclooctanecarboxylate (±)-11.** Colourless crystals, mp 90–93 °C.

4.11.4.8. (1*R,2*S**,5*R**,6*S**)-2-Amino-5,6-dihydroxycyclooctanecarboxylic acid (±)-12.** White crystals, mp 262–264 °C (dec).

5. X-ray crystallographic studies

All single-crystals for **7**, **9**, and **12** were obtained from racemic mixtures. Compound **12** crystallized as a single enantiomer. Crystallographic data were collected at 123 K with a Nonius-Kappa CCD area detector diffractometer, using graphite-monochromatized MoK radiation ($\lambda = 0.71073$ Å) as reported earlier,[32] except that the absorption corrections were carried out with SADABS[33] The structures were solved by direct methods, and full-matrix, least-squares refinements on F^2 were performed with the SHELXL-97 program.[34] The CH hydrogen atoms were included at fixed distances with fixed displacement parameters from their host atoms. The NH hydrogen atoms were refined isotropically with fixed displacement parameters.

The deposition numbers CCDC 769713–769715 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

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IV

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High-performance liquid chromatographic enantioseparation of 2-aminomono- and dihydroxycyclopentanecarboxylic and 2-aminodihydroxycyclohexanecarboxylic acids on macrocyclic glycopeptide-based phases

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ABSTRACT

The direct separation of the enantiomers of four 2-aminomono- or dihydroxycyclopentanecarboxylic acids and four 2-aminodihydroxycyclohexanecarboxylic acids was performed on chiral stationary phases containing macrocyclic glycopeptide antibiotics such as teicoplanin (Astec Chirobiotic T and T2), teicoplanin aglycone (Chirobiotic TAG) or ristocetin A (Chirobiotic R) as chiral selectors. The effects of the nature of organic modifiers, the pH, the mobile phase composition and the structures of the analytes on the separation were investigated. Chirobiotic TAG, and in some cases Chirobiotic T, proved to be the most useful of these columns. The elution sequence was determined in most cases.

1. Introduction

β-Amino acids are key building blocks of numerous bioactive molecules [1]. These compounds are to be found in a large number of natural products, β-lactams and antibiotics, some of which, *e.g.* cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, exhibit antifungal activity [2–4]. They can be introduced into peptides in order to modify and increase their biological activities [5]. The hydroxy-functionalized β-amino acids play important roles in medicinal chemistry because of their occurrence in many biologically relevant compounds, such as paclitaxel (Taxol, Bristol Myers Squibb, New York, USA) and docetaxel (Taxotere, Sanofi-aventis, Paris, France), which have chemotherapeutic effects [6–8]. Some cyclic hydroxylated β-amino acid derivatives, *e.g.* oryzoxymycin have antibiotic or antifungal activities [9–12].

The enantioselective syntheses [13,14] requires analytical methods as a check on the enantiopurity of the final products. Few papers deal with the chromatographic enantioseparation of alicyclic β-amino acids. In the past decade, our group has examined the high-performance liquid chromatographic (HPLC) enantioseparations of alicyclic-β-amino acids by using either chiral derivatizing agents [15–17] or chiral stationary phases (CSPs) [18,19].

The aim of the present work was to investigate the effectiveness of different macrocyclic glycopeptide-based CSPs for the separation of 2-aminomono- or dihydroxycycloalkanecarboxylic acids. The influence of the pH, the mobile phase composition, the nature of the alcoholic modifier and the specific structural features of the

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analytes and selectors on the retention are discussed. The elution sequence was determined in most cases.

2. Experimental

2.1. Materials and methods

Racemic *cis*- and *trans*-2-amino-3-hydroxycyclopentanecarboxylic acid (**1** and **2**; Fig. 1) were prepared from ethyl *cis*- and *trans*-2-acetylaminocyclopent-3-enecarboxylate, which were transformed to ethyl (3a*R**,4*R**,6*R**,6a*R**)-6-bromo-2-methyl-4,5,6,6a-tetrahydro-3aH-cyclopentaoxazole-4-carboxylate and ethyl (3a*R**,4*S**,6a*S**)-2-methyl-4,5,6,6a-tetrahydro-3aH-cyclopentaoxazole-4-carboxylate with *N*-bromosuccinimide, and then converted by selective reduction of the halogen group and subsequent hydrolysis to the desired product [20].

Racemic (1*R**,2*R**,3*S**,4*R**)-2-amino-3,4-dihydroxycyclopentanecarboxylic acid (**3**) was synthetized by the oxidation of ethyl-

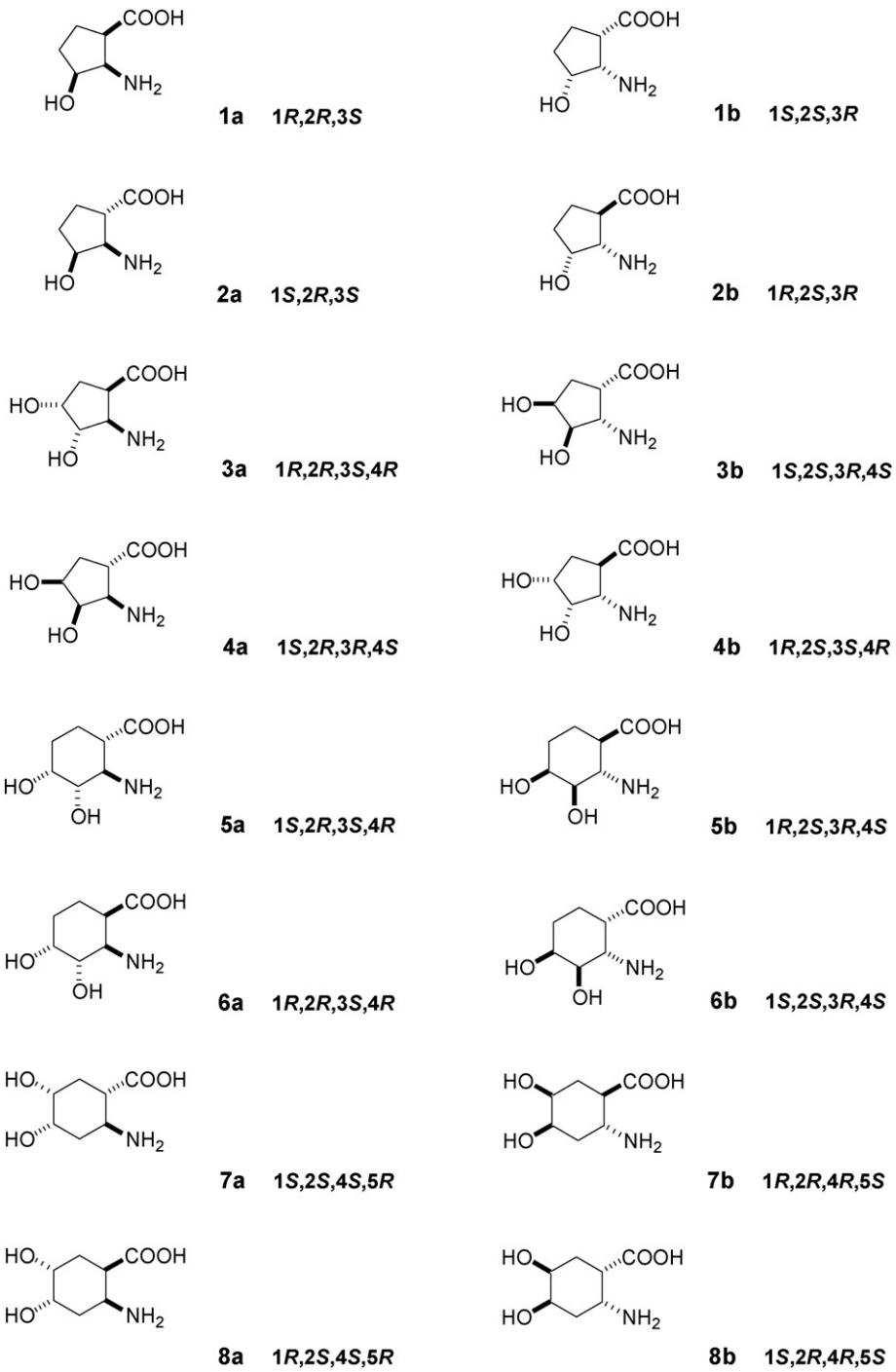

Fig. 1. Structures of analytes: **1a** (1*R*,2*R*,3*S*)- and **1b** (1*S*,2*S*,3*R*)-2-amino-3-hydroxycyclopentane carboxylic acid; **2a** (1*S*,2*R*,3*S*)- and **2b** (1*R*,2*S*,3*R*)-2-amino-3-hydroxycyclopentane carboxylic acid; **3a** (1*R*,2*R*,3*S*,4*R*)- and **3b** (1*S*,2*S*,3*R*,4*S*)-2-amino-3,4-dihydroxycyclopentane carboxylic acid; **4a** (1*S*,2*R*,3*R*,4*S*)- and **4b** (1*R*,2*S*,3*S*,4*R*)-2-amino-3,4-dihydroxycyclopentane carboxylic acid; **5a** (1*S*,2*R*,3*S*,4*R*)- and **5b** (1*R*,2*S*,3*R*,4*S*)-2-amino-3,4-dihydroxycyclohexane carboxylic acid; **6a** (1*R*,2*R*,3*S*,4*R*)- and **6b** (1*S*,2*S*,3*R*,4*S*)-2-amino-3,4-dihydroxycyclohexane carboxylic acid; **7a**, (1*S*,2*S*,4*S*,5*R*)- and **7b** (1*R*,2*R*,4*R*,5*S*)-2-amino-4,5-dihydroxycyclohexane carboxylic acid; **8a** (1*R*,2*S*,4*S*,5*R*)- and **8b** (1*R*,2*R*,4*R*,5*S*)-2-amino-4,5-dihydroxycyclohexane carboxylic acid.

cis-2-*tert*-butoxycarbonylaminocyclopent-3-enecarboxylate with a catalytic amount of OsO_4 and 4-methylmorpholine *N*-oxide as the stoichiometric co-oxidant, which selectively afforded the expected product. The other dihydroxy compounds, *e.g.* cyclopentane **4** and cyclohexane **5**–**8** derivatives, were prepared according to the method described for **3** [20]. For the enantiomers, the same synthesis route was followed as for the racemic compounds [20].

Methanol (MeOH) of HPLC grade was purchased from Scharlau (Sentmenat, Spain). Triethylamine (TEA), glacial acetic acid (AcOH), ethanol (EtOH), *n*-propanol (PrOH), 2-propanol (IPA) and other reagents of analytical reagent grade were from Sigma–Aldrich (St. Louis, MO, USA). The Milli-Q water was further purified by filtration on a 0.45-μm filter, type HV, Millipore (Molsheim, France).

Most of the separations were performed with mobile phases of 0.1% aqueous triethylammonium acetate (TEAA, pH 4.1)/MeOH = 20/80 (v/v) and 0.1% TEAA (pH 4.1)/EtOH = 20/80 (v/v) on the four different Chirobiotic columns at 25 °C using Spark Mistral column thermostat (Spark Holland, Emmen, The Netherlands).

2.2. Apparatus

The HPLC separations were carried out on a Waters HPLC system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium^{32} Chromatography Manager data system (Waters Chromatography, Milford, MA, USA) equipped with Rheodyne Model 7125 injector (Cotati, CA, USA) with 20 μL loop.

The macrocyclic glycopeptide-based stationary phases used for analytical separation were teicoplanin-containing Chirobiotic T and T2, teicoplanin aglycone-containing Chirobiotic TAG or ristocetin A-containing Chirobiotic R columns, 250 mm × 4.6 mm I.D., 5-μm (for each column) (Astec, Whippany, NJ, USA). Chirobiotic T and T2 are both based on silica gel with a 5 μm, but the Chirobiotic T material has a 120-Å pore size and the Chirobiotic T2 material has a 200-Å pore size. Moreover, the linkage chain in Chirobiotic T2 is approximately twice as long as that in Chirobiotic T. Hence, the coverage and spacing will be different for the two. This will manifest itself mainly in the form of steric interaction differences between the two columns.

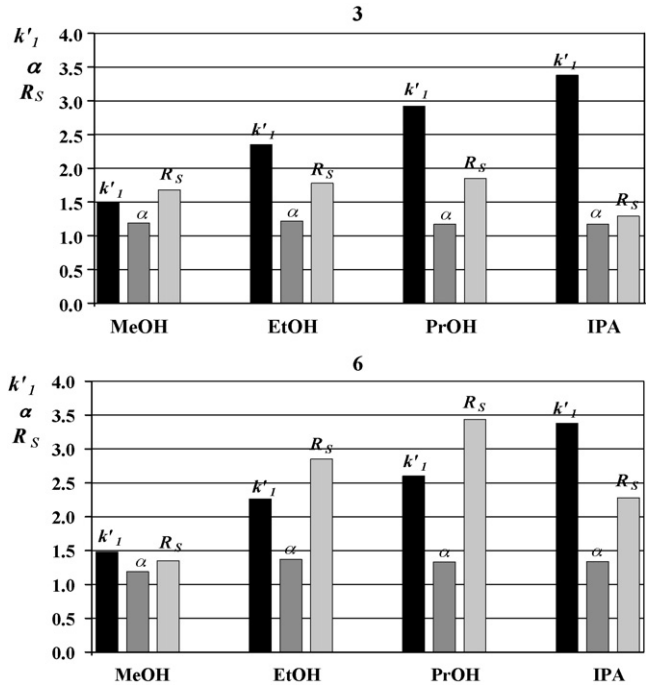

Fig. 2. Effects of the nature of the alcoholic modifier on the retention factor of the first-eluting enantiomer (k'_1), the separation factor (α) and the resolution (R_S) for analytes **3** and **6** on the Chirobiotic T2 column. Chromatographic conditions: mobile phase, 0.1% TEAA (pH 4.1)/alcoholic modifier = 20/80 (v/v); alcoholic modifiers, MeOH, EtOH, PrOH and IPA; flow rate, 0.5 mL min^{-1}; detection, 205 nm.

3. Results and discussion

The experimental conditions, including the pH of the mobile phase, the buffer type and concentration, and the nature of the organic modifier, were investigated in the course of the separation process. The analytes in this study (Fig. 1) possess either a cyclopentane or a cyclohexane skeleton. Besides carboxy and primary amino groups, analogs **1** and **2** bear one hydroxy group, while analytes **3**–**8** possess two hydroxy groups This distinction results in different steric effects and hydrogen bonding, and influences the hydrophobicity, bulkiness and rigidity of the molecules.

3.1. Effect of pH

A decrease in the pH of the 0.1% aqueous TEAA (pH 4.1)/EtOH = 20/80 (v/v) eluent system from 6.52 to 3.00 considerably increased the retention factors of analytes **3** and **6** on Chirobiotic T2, while the selectivity and resolution decreased. Similar results were obtained by Armstrong et al. [21] on a teicoplanin CSP for analytes with free carboxylic acid groups. The pH that produced the highest α also yielded the best resolution. The discontinuities in k', α and R_S at low pH are most probably due to the protonation of the teicoplanin CSP and analyte. Protonation of teicoplanin either directly affects the charge–charge or dipolar interactions between the analyte and CSP, or indirectly

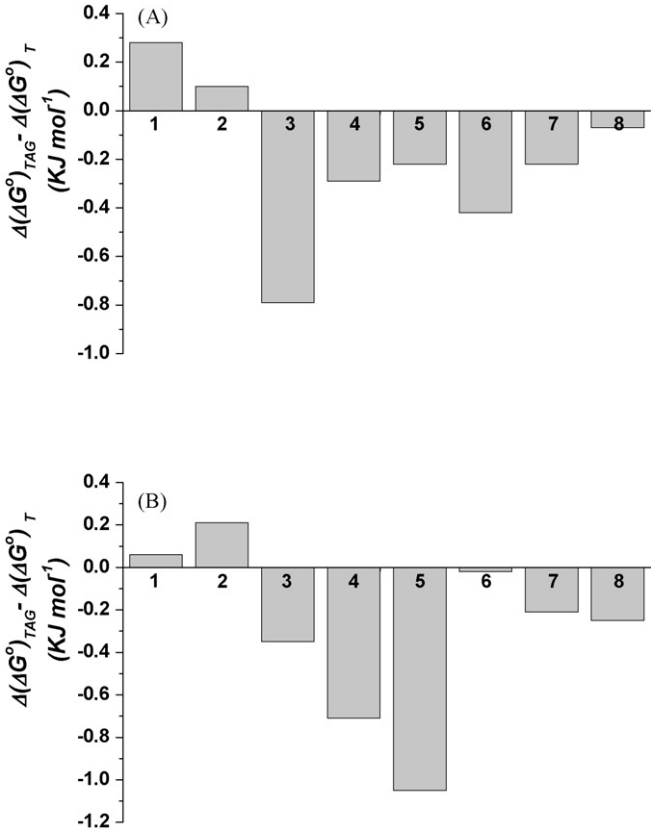

Fig. 3. Enantioselectivity free energy differences, $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, between aglycone and native teicoplanin CSPs. Chromatographic conditions: mobile phase, **A**, 0.1% TEAA (pH 4.1)/MeOH = 20/80 (v/v), **B**, 0.1% TEAA (pH 4.1)/EtOH = 20/80 (v/v); flow rate, 0.5 mL min^{-1}; detection, 205 nm.

Table 1
Chromatographic data, retention factor (k'), separation factor (α) and resolution (R_S) for alicyclic mono- and dihydroxyamino acids on macrocyclic glycopeptide-based CSPs with variation of the type of the organic modifier.

Compound	Column	Mobile phase	k'_1	k'_2	α	R_S
1	T	**a**	2.73	3.21	1.18	1.65
	T	**b**	4.22	5.20	1.23	2.10
	T2	**a**	2.39	2.75	1.15	1.60
	T2	**b**	4.00	4.68	1.17	1.35
	TAG	**a**	5.11	5.38	1.05	0.40
	TAG	**b**	7.61	9.17	1.20	0.80
	R	**a**	1.92	2.11	1.10	1.40
	R	**b**	n.d.	n.d.	n.d.	n.d.
2	T	**a**	3.56	3.70	1.04	0.50
	T	**b**	3.72	4.05	1.09	1.15
	T2	**a**	2.61	2.74	1.05	0.50
	T2	**b**	3.59	3.87	1.08	0.60
	TAG	**a**	5.09	5.09	1.00	0.00
	TAG	**b**	6.95	6.95	1.00	0.00
	R	**a**	1.97	1.97	1.00	0.00
	R	**b**	3.14	3.21	1.02	0.40
3	T	**a**	2.43	2.74	1.13	1.70
	T	**b**	3.47	3.88	1.12	1.30
	T2	**a**	1.76	2.14	1.22	1.80
	T2	**b**	2.90	3.57	1.23	1.20
	TAG	**a**	3.26	5.04	1.55	3.95
	TAG	**b**	4.55	5.87	1.29	2.45
	R	**a**	1.81	1.93	1.06	0.65
	R	**b**	2.99	3.26	1.09	0.80
4	T	**a**	2.96	3.36	1.14	1.75
	T	**b**	3.89	4.81	1.24	2.50
	T2	**a**	2.76	2.98	1.08	1.05
	T2	**b**	3.77	4.53	1.20	1.70
	TAG	**a**	4.66	5.96	1.28	2.90
	TAG	**b**	5.67	9.33	1.65	2.95
	R	**a**	2.11	2.11	1.00	0.00
	R	**b**	3.60	3.60	1.00	0.00
5	T	**a**	2.13	2.48	1.17	1.90
	T	**b**	2.97	4.07	1.37	3.20
	T2	**a**	1.61	1.86	1.15	1.40
	T2	**b**	2.43	3.35	1.38	2.90
	TAG	**a**	3.02	3.86	1.28	2.75
	TAG	**b**	3.79	7.96	2.10	4.95
	R	**a**	1.75	1.75	1.00	0.00
	R	**b**	2.80	2.94	1.05	0.40
6	T	**a**	2.27	2.49	1.10	1.00
	T	**b**	3.33	4.09	1.23	2.30
	T2	**a**	1.67	2.03	1.22	1.50
	T2	**b**	2.58	3.73	1.44	2.75
	TAG	**a**	3.34	4.35	1.30	2.50
	TAG	**b**	5.66	7.03	1.24	0.80
	R	**a**	1.59	1.66	1.04	0.40
	R	**b**	2.65	2.82	1.06	0.80
7	T	**a**	2.20	2.59	1.18	2.10
	T	**b**	2.96	3.79	1.28	2.80
	T2	**a**	1.57	1.79	1.14	1.30
	T2	**b**	2.19	2.78	1.27	2.00
	TAG	**a**	2.93	3.77	1.29	2.60
	TAG	**b**	4.18	5.81	1.39	2.40
	R	**a**	1.79	2.22	1.24	2.10
	R	**b**	2.93	3.84	1.31	2.90
8	T	**a**	2.73	2.94	1.08	1.05
	T	**b**	3.90	4.41	1.13	1.55
	T2	**a**	1.80	2.18	1.21	1.83
	T2	**b**	2.92	3.78	1.29	2.15
	TAG	**a**	4.01	4.45	1.11	1.20
	TAG	**b**	7.29	9.12	1.25	1.50
	R	**a**	1.84	2.03	1.10	1.10
	R	**b**	3.07	3.48	1.13	1.40

Chromatographic condition: mobile phase, **a**, 0.1% aqueous triethylammonium acetate (TEAA, pH 4.1)/MeOH = 20/80 (v/v), **b**, 0.1% TEAA (pH 4.1)/EtOH = 20/80 (v/v); flow rate, 0.5 mL min^{-1}; detection, 205 nm; n.d., no data available.

influences the separation by changing the conformation of the selector.

3.2. Effect of organic modifier nature and content

The nature of the alcoholic modifier exerted considerable effects. Fig. 2 reveals that at constant organic modifier content the retention factors increased with increasing chain length of the alcohol, especially for alcohols with branched and bulky side-chains. The apolar character of the mobile phase increased in the sequence MeOH, EtOH, PrOH, IPA, while at a constant percentage of alcohol modifier, the molar concentrations of the longer-chain alcohols were less because of their higher molar mass. Increasing carbon number is disadvantageous for polar interactions between the mobile phase and analytes: the retention factor may increase. The lower molar concentration of propanols relative to MeOH may decrease the apolar character of the mobile phase. The overall resultant of these two effects is that the retention factor increases with increasing chain length of the alcohol (Fig. 2). This behavior was more pronounced for IPA. In this case, the steric effect probably contributes to the decreased interactions between the mobile phase and the analytes. As concerns resolution, the highest R_S values were obtained on application of EtOH or PrOH.

The effects of the organic modifier content on the separation were also investigated. On variation of the ratio of 0.1% TEAA (pH 4.1) and EtOH in the range 80/20 to 0/100 (v/v), as the EtOH content of the mobile phase was increased, the retention factor increased; this was due to the reduced solubility of the polar analytes in the EtOH-rich, more apolar mobile phase. This behavior was observed on all four CSPs. In all cases, the α values varied slightly, while the R_S values progressively increased with increasing EtOH content in the mobile phase.

Relevant separation data for all compounds are given in Table 1. As expected, EtOH exhibited larger k' values than MeOH. When the same mobile phases were used, the retention factors were smallest on ristocetin A and largest on the teicoplanin aglycone chiral selector. The native teicoplanin phases (Chirobiotic T and T2) exhibited intermediate k' values. Similar trends, with higher k' values on Chirobiotic TAG than on a Chirobiotic T column, were observed by Berthod et al. [22], D'Acquarica et al. [23] and Péter et al. [18,19,24–26] for unusual α-amino acids and cyclic β-amino acids. Comparison of the Chirobiotic T and T2 columns revealed that the retention factors on Chirobiotic T were somewhat larger (Table 1). Slightly higher k' values on Chirobiotic T2 than on Chirobiotic T were observed by Péter et al. [26] for β^3-homoamino acids.

Another obvious trend indicated by the data in Table 1 was that α and R_S were smallest on the ristocetin A selector (with the exception of analyte **7**), while larger α and R_S values were obtained on both teicoplanin and teicoplanin aglycone selectors.

3.3. Comparison of separation performances and effect of sugar units of Chirobiotic columns

The different separation ability of the Chirobiotic TAG column relative to Chirobiotic T for hydroxyamino acids with cycloalkane skeletons indicates a possible difference in the separation mechanism on the teicoplanin versus the teicoplanin aglycone CSPs. From the aspect of enantiomeric separations, the sugar moieties of the native teicoplanin may intervene in the chiral recognition process

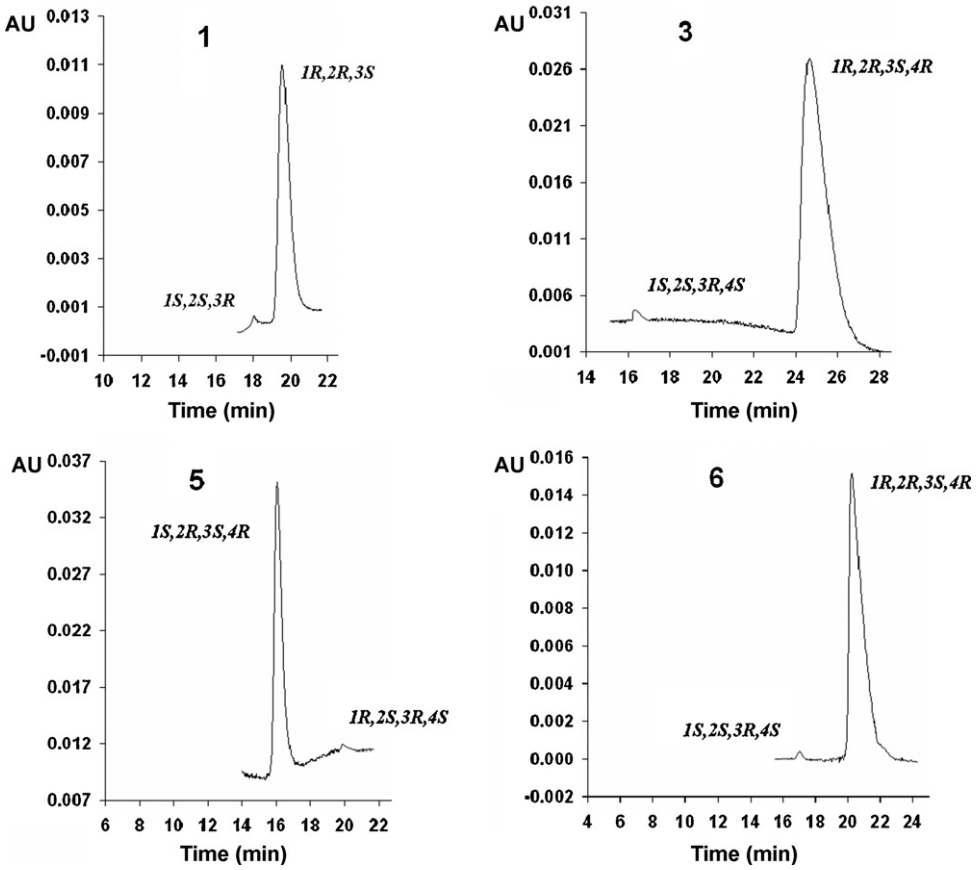

Fig. 4. Separation of minor enantiomers of **1**, **3**, **5** and **6** when it is present in an excess of the major isomer. Chromatographic conditions: column, Chirobiotic T2 for **1**, Chirobiotic TAG for **3**, and Chirobiotic T for **5** and **6**; mobile phase for **1** 0.1% TEAA (pH 6.5)/MeOH = 20/80 (v/v); for **3**, **5** and **6** 0.1% TEAA (pH 4.1)/EtOH = 20/80 (v/v), 0.5 mL min^{-1}; detection, 205 nm.

in at least three ways [22]: (i) sugar units occupy the space inside the "basket"; (ii) they block the possible interaction sites on the aglycone (phenolic hydroxy groups and an alcohol moiety); (iii) they offer competing interaction sites, since the three sugars are themselves chiral and have hydroxy, ether and amido functional groups.

To quantify the effects of the sugar units, the differences in enantioselective free energies between the two CSPs, $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, were used. $\Delta(\Delta G^\circ)$ values were taken from Table 1 [$-\Delta(\Delta G^\circ) = RT\ln\alpha$]. The $\Delta(\Delta G^\circ)_{TAG} - \Delta(\Delta G^\circ)_T$, values were plotted as shown in Fig. 3. A negative number means that the stereoisomers were better separated on the aglycone CSP. A positive number means that the stereoisomers are better separated on the native teicoplanin CSP. As can be seen in Fig. 3, hydroxyamino acid enantiomers were much better separated by the aglycone CSP (exceptions were analytes **1** and **2**). The negative energy difference means that the absence of sugar units increases the amino acid enantiorecognition. It also indicates that the aglycone basket of the teicoplanin molecule is solely responsible for the enantiorecognition.

3.4. Effect of the structures of the analytes

The structures of the analytes influenced the chiral recognition. On the same column, the α values were generally lower for analytes **1** and **2**, which possess a single hydroxy group. As indicated by the positive free energy differences for analytes **1** and **2**, the contribution of the interaction of the sole hydroxy group with the basket resulted in decreased enantioselectivity (Fig. 3). However, the additional hydroxy groups in analytes **3**–**8** contributed considerably to chiral recognition by the aglycone basket (*i.e.* Chirobiotic TAG). Interestingly, it seems that the positions of the hydroxy groups on the cycloalkane skeleton (positions 3, 4 or 4.5) produced only a small effect on the selectivity factor.

β-Amino acids possessing cycloalkane skeletons exhibited much lower enantioselectivity on Chirobiotic columns [18,19]. The hydroxyl groups attached to the cycloalkane skeleton significantly contribute to the chiral recognition therefore Chirobiotic phases are much more suitable for the enantioseparation of hydroxycycloalkane β-amino acids.

The limit of detection (LOD) was determined as a peak whose area was three times the baseline noise. For the monohydroxy derivatives 5.12 nmol, while for the dihydroxy derivatives 4.23 nmol were obtained as LOD. The relative standard deviation for the LOD was 3%. Further, the determination limit for the minor isomer is less than 0.1% when it is present in an excess of the major isomer (Fig. 4).

3.5. Elution sequences of investigated analytes

On the Chirobiotic T, T2 and TAG columns for analytes **1, 3** and **6**, the elution sequence was **b** < **a** (Fig. 1), while for analytes **5** and **8** it was **a** < **b**; these retention sequences were independent of the organic modifier used (*e.g.* MeOH or EtOH). On the Chirobiotic R column for analyte **1**, **b** < **a**, while for analytes **3, 6** and **8**, the elution sequence **a** < **b** was found. Neither the configuration of the carbon atom attached to the carboxyl group nor the configuration of the carbon atom attached to the amino group determined the elution sequence, in most cases the elution sequence 1*S* < 1*R* or 2*S* < 2*R* was observed.

4. Conclusions

The enantioseparations of hydroxycycloalkane amino acid analogs were investigated by using macrocyclic glycopeptide-based CSPs, *i.e.* Chirobiotic T, T2, TAG and R columns. The separations could be accomplished in reversed-phase mode by using 0.1% TEAA (pH 4.1)/alcoholic modifier mobile phases in different compositions. Of the four Chirobiotic columns, Chirobiotic T and TAG appeared most suitable for the enantioseparation of 2-aminomono- or dihydroxycycloalkanecarboxylic acids. The elution sequence was determined in most cases, but no general rule could be established relating the elution sequence to the absolute configuration.

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