

# Synthesis and conformational studies of peptides from new C-linked carbo- $\beta$ -amino acids ( $\beta$ -Caas) with anomeric methylamino- and difluorophenyl moieties†

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New C-linked carbo- $\beta$ -amino acids ( $\beta$ -Caas), Cbz-(*S*)- $\beta$ -Caa-(NH<sub>2</sub>Boc)-OMe (**1**) and Cbz-(*R*)- $\beta$ -Caa-(NH<sub>2</sub>Boc)-OMe (**2**), with an additional amine group (methylamino group of NH<sub>2</sub>Boc) at the C-1 position of the lyxofuranoside side chain and Boc-(*S*)- $\beta$ -Caa-(diFP)-OMe (**3**) and Boc-(*R*)- $\beta$ -Caa-(diFP)-OMe (**4**), with a C-difluorophenyl (diFP) moiety at the anomeric position of the lyxofuranoside side chain were prepared from D-mannose.  $\beta$ -Peptides [tetra- and hexapeptides] were synthesized from these  $\beta$ -Caas, 'epimeric' [at the amine stereocentre (C <sub>$\beta$</sub> )], using the concept of 'alternating chirality' to carry out their conformational studies [NMR (CDCl<sub>3</sub>), CD and MD]. In the monomer design, it was envisaged that the presence of an additional amine group in **1** or **2** would help in solubilizing the peptides in water, while, the C-difluorophenyl (diFP) moiety of **3** and **4** is expected to enhance the biological activity. The peptides having **1** and **2**, though could not retain their 12–10-mixed helices in water, have shown moderate activity against Gram positive and Gram negative bacterial strains. The peptides prepared from **3** and **4**, much against our expectations, did not display any biological activity.

## Introduction

Designing non-biological polymers, which fold into predictable secondary and tertiary structures, referred to as 'foldamers',<sup>1</sup> has been an area of intense research activity.<sup>2</sup>  $\beta$ -Peptides, obtained from  $\beta$ -amino acids,<sup>3</sup> the higher homologues of  $\alpha$ -amino acids, display a wide variety of secondary structures,<sup>4,6</sup> thereby providing an unparalleled opportunity to understand the factors governing protein structure and folding.<sup>7</sup> The wide variety of biological activities<sup>8</sup> of  $\beta$ -peptides, besides their resistance to enzyme degradation<sup>9</sup> and enhancing protein binding ability,<sup>10</sup> has made them attractive targets in the area of peptidomimetics and drug discovery. From extensive theoretical and molecular dynamics simulation studies<sup>11</sup> it was found that there is an intrinsic preference for the formation of 10–12-mixed helices in these  $\beta$ -peptides. The mixed 10–12-helices ( $\beta$ -helices), containing intertwined 10- and 12-membered (mr) H-bond rings, unique to  $\beta$ -peptides, were first reported by Seebach *et al.*<sup>12</sup> by using alternating  $\beta^2$ – $\beta^3$ -amino acids as a motif, while, Kessler *et al.*<sup>13</sup> realized them using furanose amino acid (FAA)– $\beta$ -hGly ( $\beta$ -homo glycine). In

our earlier studies, we designed 'new motifs' using C-linked carbo- $\beta$ -amino acids<sup>14</sup> ( $\beta$ -Caa; 'epimeric' at the amine stereocentre), having carbohydrate moieties as side chains,<sup>15–17</sup> and demonstrated that peptides derived with 'alternating chirality' generate very robust and stable right handed 10–12-mixed helices.<sup>18a</sup> Similarly, peptides made from the dipeptide repeats of  $\beta$ -Caa– $\beta$ -hGly ( $\beta$ -homo glycine), resulted in both right- and left-handed mixed helices,<sup>18b</sup> where the 'switch' in the handedness was governed by the stereochemistry at the C <sub>$\beta$</sub>  of the  $\beta$ -Caa used in the design. Our further designs with dipeptide repeats to enhance the skeletal diversity resulted in  $\alpha$ – $\gamma$ -hybrid peptides,<sup>19a</sup> which generated a 10–12-helix devoid of  $\beta$ -amino acid. Yet another new motif for the realization of 10–12- $\beta$ -helices from peptides with  $\alpha$ -aminoxy acid (Ama) and (*R*)- $\beta$ -Caa alternating was recently reported.<sup>19b</sup>

Therapeutic applications of cationic  $\beta$ -peptides or their biomimetic analogues, as antibacterial agents are promising.<sup>20,21</sup> Their direct action on the bacterial cell membrane is likely to prevent rapid development of bacterial resistance. The designs used by different research groups<sup>22</sup> for imparting antibacterial activity in  $\beta$ -peptides had helical structures with charged hydrophilic residues on one face of the helix and hydrophobic residue on its other face, providing amphiphilicity to the helix.

In our studies, the design of  $\beta$ -Caas was based on the C-linked ribosyl glycine moiety that is present in nikkomycins,<sup>23</sup> the dipeptide antibiotics. The  $\beta$ -peptides investigated by us however could not be explored for therapeutic applications due to their inherent limitation of insolubility in water. To address the problems associated with water solubility and biological activity, we designed new  $\beta$ -Caa monomers. In the first simple design on bifunctional  $\beta$ -Caas, an additional amine group (NH<sub>2</sub>Boc) was introduced at the C-1 position of a carbohydrate side chain, which

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is expected to help enhancing solubility of derived peptides in water.

In yet another novel design, we envisaged  $\beta$ -Caas having C-difluorophenyl (diFP) glycoside as side chain, to promote biological activity. The rationale behind such a design was two fold: firstly, C-aryl glycoside moieties<sup>24</sup> are present in several bio-active compounds. The difluorophenyl moiety is part of the structure of the drugs such as fluconazole and diflunisal. Secondly, the highly electronegative character of fluorine imparts very different properties to the molecule,<sup>25</sup> allowing exploration by replacing hydrogen with fluorine in drug design and discovery.<sup>26</sup> Further, it is well documented that fluorinated amino acids enhance protein stability and support in other applications.<sup>27</sup> Based on the above observations, the preparation of a new class of  $\beta$ -Caas, having difluorophenyl (diFP) C-glycofuranoside as side chain, was considered to improve the biological activity of the  $\beta$ -peptides thus derived. The present work reports the synthesis of 'epimeric' (at the amine stereocentre, C <sub>$\beta$</sub> ), bifunctional  $\beta$ -Caas **1** and **2** and  $\beta$ -Caa (diFP) **3** and **4** (Fig. 1), with NHBoc and C-difluorophenyl (diFP) groups respectively at the anomeric position (C-1) of lyxofuranoside side chain from D-mannose, conversion of these monomers into the corresponding tetra- and hexapeptides **7–16** (Fig. 2 and 3) and their structural studies by extensive NMR, MD and CD analysis. Thus, the structural features incorporated in the newly designed  $\beta$ -Caas **1–4** in the present study: a) would help in solubilizing the peptides in water due to the additional amine group (NHBoc) and b) the C-difluorophenyl (diFP) glycoside side chain might provide desirable biological activity to the  $\beta$ -peptides **13–16**.

## Results and discussions

### 1. Synthesis of amino acids **1** and **2**

The main strategy in the synthesis of **1** and **2**, from D-mannose lies in the conversion of a) the anomeric (C-1) carbon into a Boc protected aminomethyl group and b) to install the amino acid side chain at the C-4 centre using the C-5 carbon. Accordingly, the known alcohol **17**<sup>28</sup> was treated with *p*-TsCl, Et<sub>3</sub>N and DMAP (catalytic) in CH<sub>2</sub>Cl<sub>2</sub> at room temperature for 3 h to give **18** in 83% yield (Scheme 1). Tosylate **18** was further reacted with NaN<sub>3</sub> in DMF at 70 °C for 6 h to afford azide **19** (65%), which on

reduction with Ph<sub>3</sub>P in methanol for 1 h, followed by protection with (Boc)<sub>2</sub>O for 5 h furnished **20** (75%).

Further, hydrolysis of the 5,6-acetonide in **20**, on reaction with PTSA in aq. MeOH at room temperature for 8 h gave the diol **21** in 87% yield. Oxidative cleavage of the diol in **21** with NaIO<sub>4</sub> in a solution of MeOH–H<sub>2</sub>O at room temperature for 2 h afforded aldehyde **22**, which on subsequent Wittig olefination with (methoxycarbonylmethylene)triphenylphosphorane in benzene at reflux for 5 h furnished  $\alpha,\beta$ -unsaturated ester **23** as a *cis–trans* mixture of isomers (81%). Michael addition on **23** with benzylamine<sup>29</sup> at room temperature for 12 h gave an 'epimeric' (at the amine stereocentre) mixture of esters **24** (43%) and **25** (25%) separable by column chromatography. Hydrogenolysis of esters **24** and **25** in the presence of 10% Pd–C in methanol under hydrogen atmosphere afforded the respective amines **26** and **27**, which were treated with DIPEA and Cbz-Cl in CH<sub>2</sub>Cl<sub>2</sub> at room temperature for 2 h to furnish **1** (87%) and **2** (90%) respectively. Base hydrolysis (aq. NaOH in methanol) of **1** and **2** gave the corresponding acids **28** (83%) and **29** (86%) respectively. The overall yield was 14%.

### 2. Synthesis of amino acids **5** and **6**

Reaction of the ester **30** with benzylamine<sup>19,29</sup> (Scheme 2) at room temperature for 12 h gave a separable mixture of esters **31** (38%) and **32** (26%), which on reaction with 10% Pd–C in methanol and subsequent treatment of the resulting amines **33** and **34** with Et<sub>3</sub>N and (Boc)<sub>2</sub>O in THF furnished **5** (92%) and **6** (88%) respectively.

### 3. Synthesis of peptides **7–12**

The synthesis of the peptides **7–12** is outlined in Schemes 3 and 4. Accordingly, Boc-(*R*)- $\beta$ -Caa-OMe **6** (Scheme 3) was saponified with aq. 4 N NaOH to give acid **35** (90%), which on coupling with **33** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> afforded the dipeptide **36** (83%). Ester **36** on exposure to CF<sub>3</sub>COOH in CH<sub>2</sub>Cl<sub>2</sub> was converted into the salt **37**, which on condensation with acid **28** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> furnished the tripeptide **7** in 60% yield.

Base hydrolysis of tripeptide **7** gave the corresponding acid **38**, which on coupling with **27** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> afforded the tetrapeptide **8** in 49% yield. Peptide **8** on sequential treatment with base, followed by Cbz deprotection with 10% Pd–C in MeOH at room temperature under hydrogen

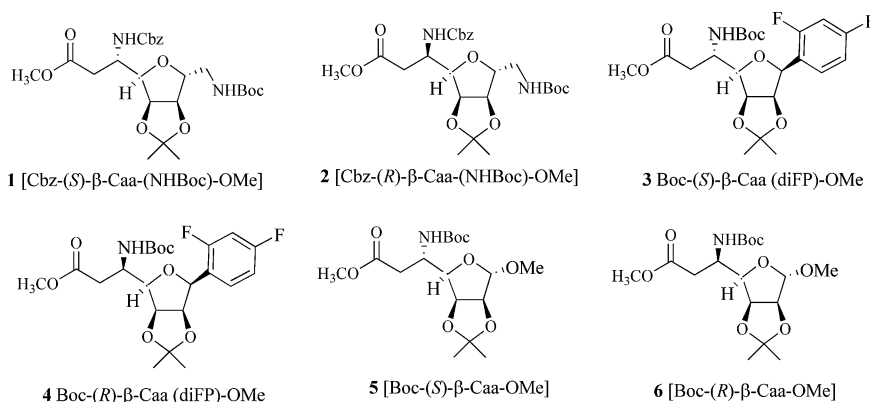
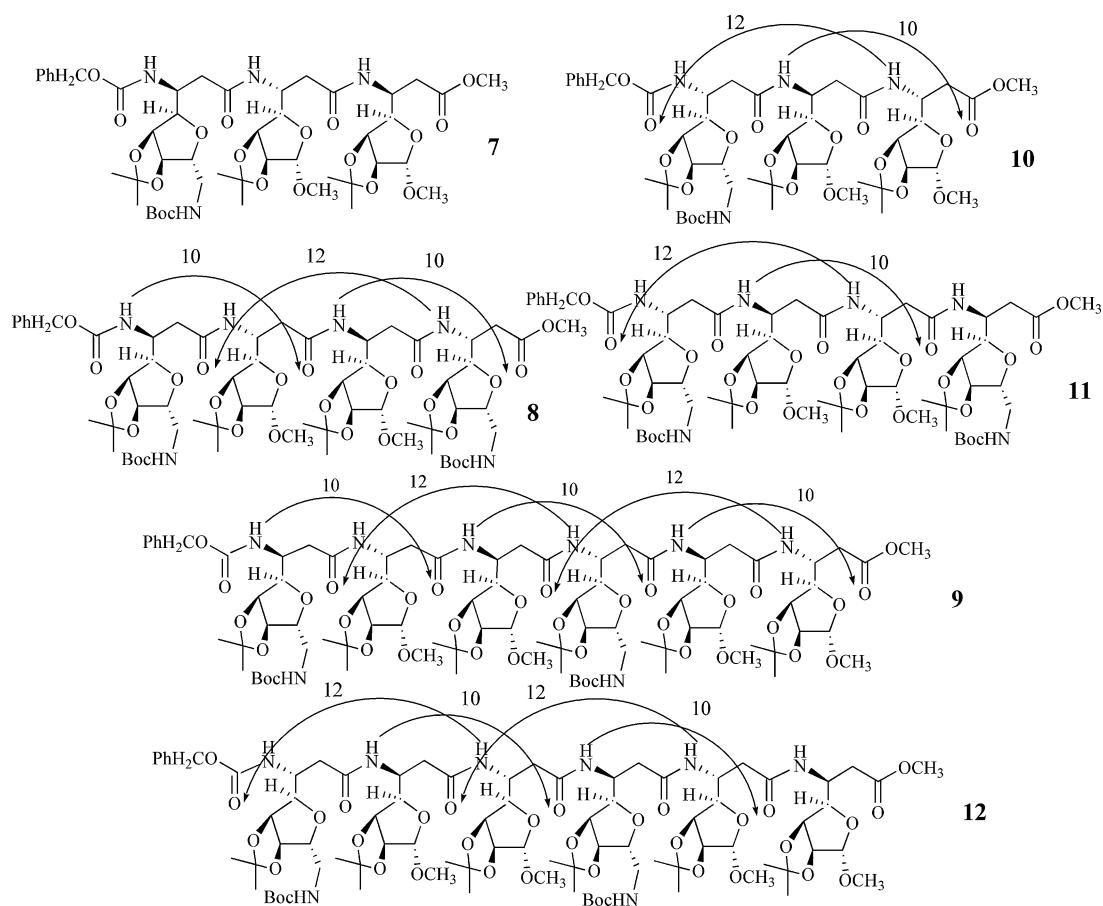
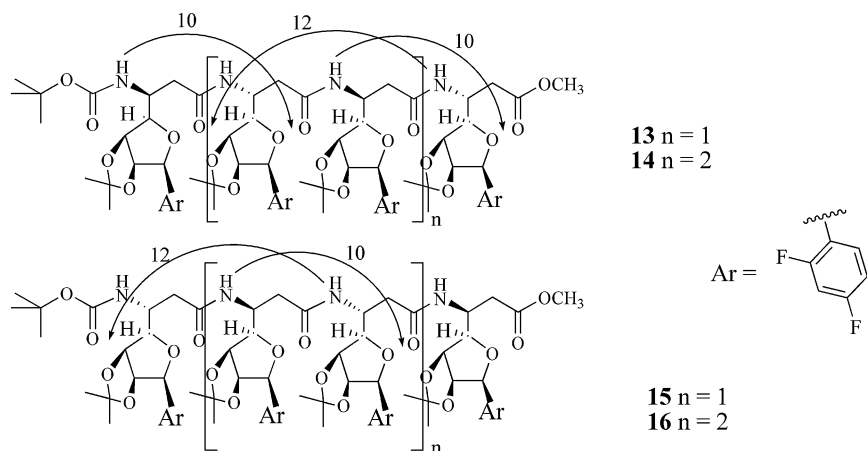


Fig. 1 Structures of C-linked carbo  $\beta$ -amino acids **1–6**.



**Fig. 2** Structures of peptides **7–12** containing  $\beta$ -Caas **1**, **2**, **5** and **6**.



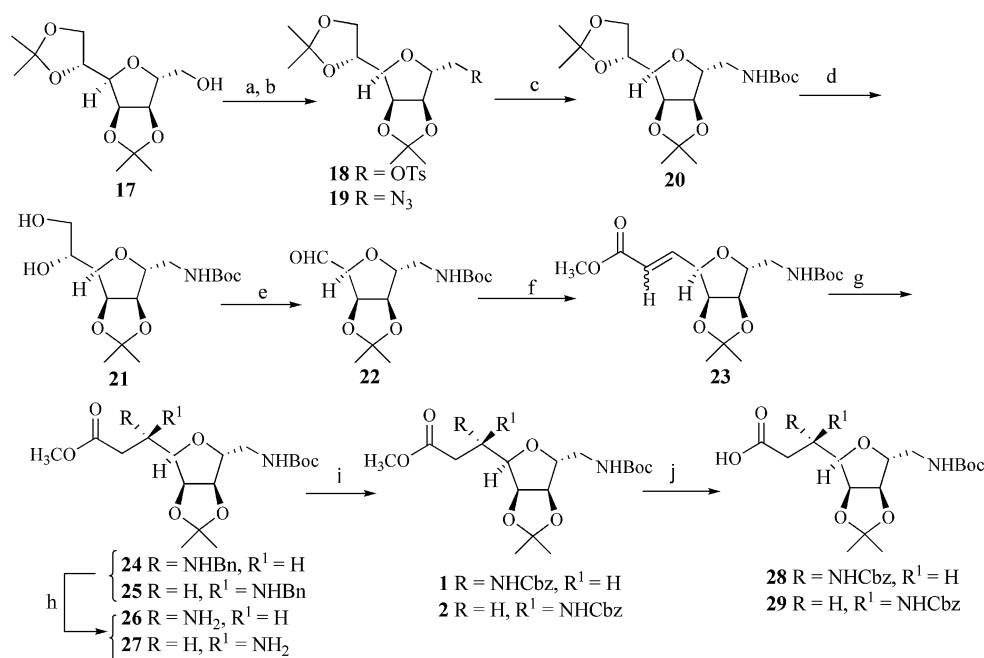
**Fig. 3** Structures of peptides **13–16** derived from  $\beta$ -Caas **3** and **4**.

atmosphere and subsequent exposure to  $\text{CF}_3\text{COOH}$  gave the acid salt **8a**. Similarly, peptide **8** on exposure to  $\text{CF}_3\text{COOH}$  underwent Boc deprotection to furnish the salt **8b**.

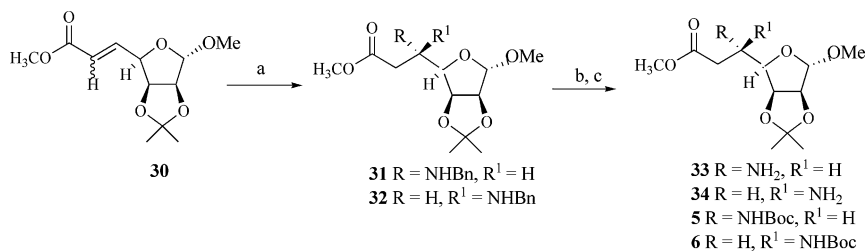
Likewise, Boc-(*S*)- $\beta$ -Caa-OMe **5** (Scheme 3) was saponified with aq. 4 N NaOH to give acid **40**, which on coupling with **34** in the presence of EDCI, HOBt and DIPEA in  $\text{CH}_2\text{Cl}_2$  afforded the dipeptide **41** (84%). Coupling of the salt **42** (obtained from **41** by the exposure to  $\text{CF}_3\text{COOH}$ ) with acid **29** gave peptide **10** (67%). Hydrolysis of **10** with base afforded the corresponding acid **43**,

while, exposure to  $\text{CF}_3\text{COOH}$  furnished the salt **44**. Peptide **43** on further Cbz and Boc deprotection with 10% Pd-C and  $\text{CF}_3\text{COOH}$  respectively gave the salt **10a**.

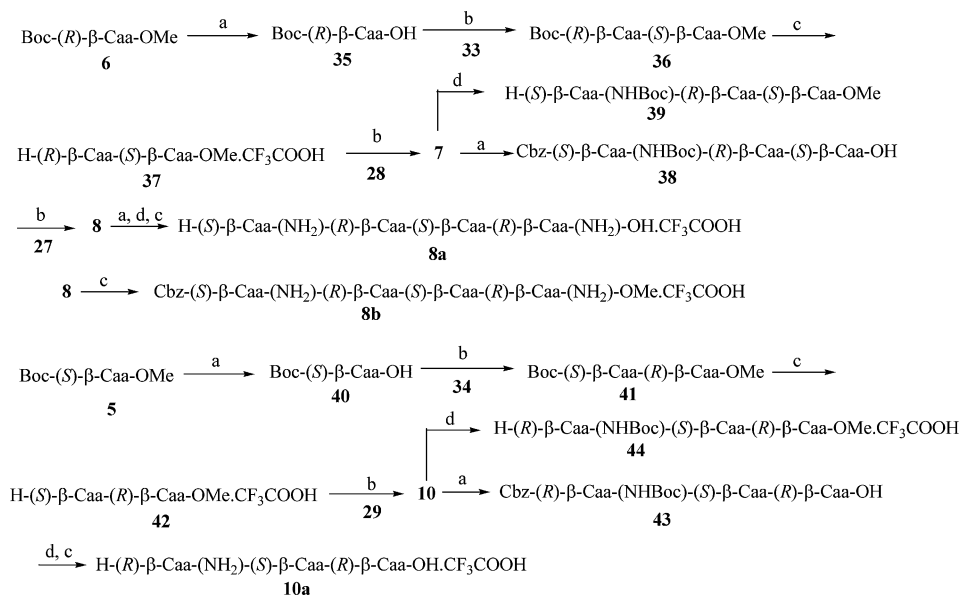
In a further study, acid **38** on coupling with **44** (Scheme 4) in the presence of EDCI, HOBt and DIPEA in  $\text{CH}_2\text{Cl}_2$  gave the peptide **9** (54%) yield. Base hydrolysis of **9** and subsequent Cbz and Boc deprotections on the resulting acid, using 10% Pd-C and  $\text{CF}_3\text{COOH}$  in  $\text{CH}_2\text{Cl}_2$ , respectively afforded the salt **9a**. Similarly, peptide **9** on reaction with  $\text{CF}_3\text{COOH}$  furnished the salt **9b**.



**Scheme 1** Synthesis of amino acids **1** and **2**. *Reagents and conditions*: a) *p*-TsCl, Et<sub>3</sub>N, cat. DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT, 3 h; b) NaN<sub>3</sub>, DMF, 70 °C, 6 h; c) Ph<sub>3</sub>P, CH<sub>3</sub>OH, 1 h, then (Boc)<sub>2</sub>O, RT, 5 h; d) PTSA, CH<sub>3</sub>OH–H<sub>2</sub>O, 5 : 1, RT, 8 h; e) NaIO<sub>4</sub>, MeOH–H<sub>2</sub>O (5 : 1), RT, 2 h; f) Ph<sub>3</sub>P=CHCOOCH<sub>3</sub>, benzene, reflux, 5 h; g) BnNH<sub>2</sub>, RT, 12 h; h) H<sub>2</sub>, 10% Pd–C, CH<sub>3</sub>OH, RT, 12 h; i) Cbz–Cl, DIPEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT, 2 h; j) aq. 4 N NaOH, CH<sub>3</sub>OH, 0 °C–RT, 2 h.



**Scheme 2** Synthesis of amino acids **5** and **6**. *Reagents and conditions*: a) BnNH<sub>2</sub>, RT, 12 h; b) H<sub>2</sub>, 10% Pd–C, CH<sub>3</sub>OH, RT, 12 h; c) (Boc)<sub>2</sub>O, Et<sub>3</sub>N, THF, 0 °C–RT, 2 h.



**Scheme 3** Synthesis of peptides **7**, **8** and **10**. *Reagents and conditions*: a) aq. 4 N NaOH, CH<sub>3</sub>OH, 0 °C–RT; b) HOBt (1.2 equiv.), EDCI (1.2 equiv.), DIPEA (2 equiv.), dry CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT; c) CF<sub>3</sub>COOH, dry CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT; d) H<sub>2</sub>, 10% Pd–C, CH<sub>3</sub>OH, RT.



of **47** (NaIO<sub>4</sub>, aq. NaHCO<sub>3</sub>) in CH<sub>2</sub>Cl<sub>2</sub> furnished aldehyde **48**, which on subsequent Wittig olefination in MeOH gave ester **49** in 78% yield as a *cis*-isomer, as adjudged from the NMR data. Ester **49** on reductive deoxygenation with triethylsilane<sup>31</sup> and BF<sub>3</sub>·OEt<sub>2</sub> in dry CH<sub>3</sub>CN furnished β-C-aryl glycoside **50** (86%) as an exclusive product. The exclusive formation of β-glycoside **50** may be attributed to the presence of 2,3-acetonide group at the β-face, which thereby directs the nucleophile attack from the α-face.

Michael addition on ester **50** (Scheme 5) with benzylamine<sup>29</sup> at room temperature gave a mixture of esters **51** (41%) and **52** (20%) as epimers at the amine stereocentre. Hydrogenolysis of **51** and **52** with 10% Pd–C in MeOH and subsequent reaction of **53** and **54** with (Boc)<sub>2</sub>O and Et<sub>3</sub>N in CH<sub>2</sub>Cl<sub>2</sub> furnished **3** (89%) and **4** (94%), respectively. Esters **3** and **4** on hydrolysis with aq. NaOH solution gave the respective acids **55** and **56**, while exposure to CF<sub>3</sub>COOH afforded the TFA salts **57** and **58** respectively. The overall yield was 24%.

The absolute stereochemistry of **3** was unambiguously obtained from the spectral analysis (Fig. 4). From the <sup>1</sup>H NMR spectrum (500 MHz) of **3** at 303 K, the observed couplings, <sup>3</sup>J<sub>C<sub>1</sub>H–C<sub>2</sub>H</sub> = 3.7 Hz, <sup>3</sup>J<sub>C<sub>2</sub>H–C<sub>3</sub>H</sub> = 6.2 Hz, <sup>3</sup>J<sub>C<sub>3</sub>H–C<sub>4</sub>H</sub> = 3.5 Hz and NOEs such as: C<sub>1</sub>H–C<sub>4</sub>H and C<sub>1</sub>H–C<sub>3</sub>H in the NOESY spectrum provide ample proof, that the difluorophenyl ring at C<sub>1</sub> is in the β-position and the sugar pucker is °E. Very strong NOEs, CH<sub>3</sub>(*pro-R*)–C<sub>2</sub>H and CH<sub>3</sub>(*pro-R*)–C<sub>3</sub>H, suggest an envelop conformation for isopropylidene ring, where C<sub>2</sub>, C<sub>3</sub> and both oxygens are in one plane.

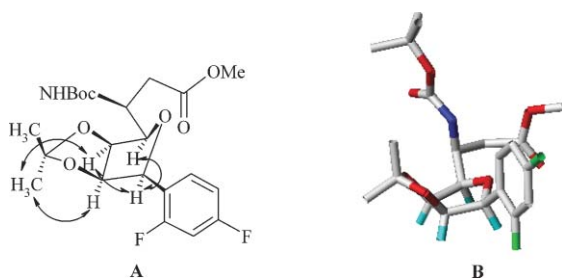
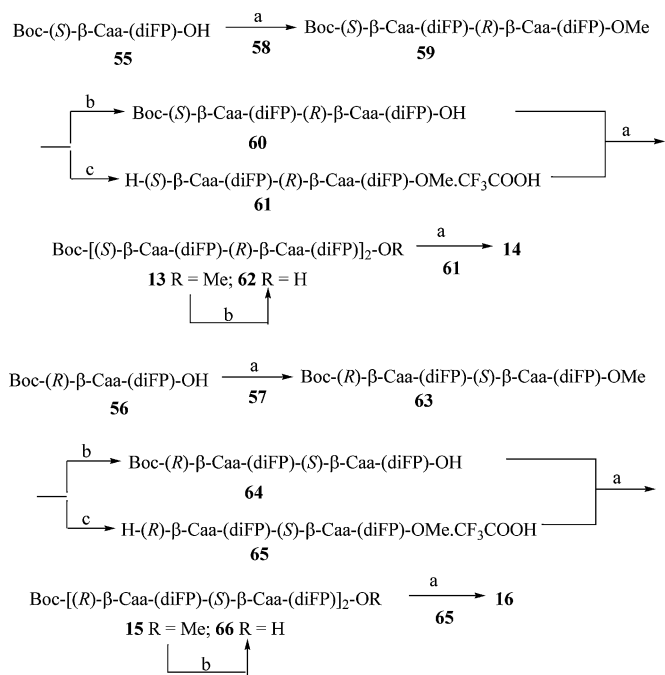


Fig. 4 Ester **3** (A) characteristic NOE correlations; (B) energy minimized structure.

## 5. Synthesis of peptides 13–16

Condensation of acid **55** with the salt **58** (Scheme 6) in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> afforded dipeptide **59** (73%), which on base (aq. 4 N NaOH) hydrolysis gave **60**, while exposure to CF<sub>3</sub>COOH afforded salt **61**. Condensation of acid **60** with **61** gave the tetrapeptide **13** (45%). Hydrolysis of **13** with base furnished the acid **62**, which on reaction with **61** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> afforded the hexapeptide **14** (34%).

Likewise, condensation of acid **56** with salt **57** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> furnished the dipeptide **63** (64%) yield. Ester **63** on base hydrolysis with aq. 4 N NaOH gave the corresponding acid **64**, which on coupling with the salt **65** (obtained from **63** by the exposure to CF<sub>3</sub>COOH) afforded tetrapeptide **15** (48%). Base hydrolysis of **15** and coupling of the corresponding acid **66** with **65** in the presence of EDCI, HOBt and DIPEA in CH<sub>2</sub>Cl<sub>2</sub> resulted in the hexapeptide **16** (41%).



Scheme 6 Synthesis of peptides **13–16**. Reagents and conditions: a) HOBt (1.2 equiv.), EDCI (1.2 equiv.), DIPEA (2 equiv.), dry CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT, 4 h; b) 4 N NaOH, CH<sub>3</sub>OH, 0 °C–RT, 2 h; c) CF<sub>3</sub>COOH, dry CH<sub>2</sub>Cl<sub>2</sub>, 0 °C–RT, 2 h.

## 6. Conformational analysis of peptides 7–16

Having obtained the first family of water soluble peptides derived from bifunctional β-Caas, it was important to learn as to whether the design sustains a stable helix in water. As a first step, therefore, NMR studies on 5–10 mM solution of peptides **8b**, **9b** and **11a** were conducted in water and DMSO-*d*<sub>6</sub> at 303 K.<sup>32</sup> The spectra in water (90% H<sub>2</sub>O + 10% D<sub>2</sub>O) did not display any distinct signatures of a secondary structure. Most of the NH resonances were grouped in the region of 7.70–8.40 ppm. The large value of the temperature coefficients of the amide proton chemical shifts (Δδ/ΔT > 7.6 ppb °C<sup>-1</sup>) imply that they do not participate in H-bonding. The absence of medium range NOEs further ruled out well defined folds. The strong propensity to form intermolecular H-bonds with water molecules appears to destabilize formation of the secondary structure. The spectra of these peptides in DMSO-*d*<sub>6</sub> were, however, broad and lacked the desired resolution, leading us to abandon our efforts for a detailed NMR study. The NMR studies of **9b** were also carried out in CD<sub>3</sub>OH. Limited dispersion of amide chemical shift and resonance overlap of C<sub>β</sub>H and C<sub>α</sub>H region did not permit the analysis. Though the destabilization of helices in water is observed for most of the β-peptides,<sup>21b,33</sup> it has been demonstrated that these peptides may reorganize into helices upon docking to their receptors.<sup>34</sup> Since, a preorganized structure is not a prerequisite for antimicrobial activity,<sup>21b</sup> it does not preclude these peptides from displaying biological activity.

The lack of secondary structures in peptides **8b**, **9b** and **11a**, obtained by using the concept of ‘alternating chirality’, compelled us to check the validity of the design principle. Therefore the NMR studies of 5–10 mM solutions of peptides **7–16** in CDCl<sub>3</sub> solutions were undertaken in order to find the presence of 10–12-helical structures.<sup>32</sup>

The tripeptide **7** does not seem to have a folded structure. For tetrapeptide **8**, a highly dispersed spectrum in the amide and  $C_\alpha$ H region, with chemical shift ( $\delta$ ) dispersion of 1.68 and 0.64 ppm respectively, indicate the presence of a well defined structure. The amide protons, NH(1)–NH(4) resonated at 6.73, 7.23, 7.33 and 8.31 ppm respectively, suggesting involvement of several of them in H-bonds. To confirm this observation, solvent titration studies<sup>35</sup> were carried out by adding up to 50% v/v DMSO- $d_6$  and small variation in their  $\delta$  values ( $\Delta\delta_{\text{NH}}$ ) confirmed, that except NH(2), all amide protons participate in intramolecular H-bonding. The  $^3J_{\text{NH-C}\beta\text{H}} = 8.6\text{--}9.3$  Hz for all the four residues suggest that NH and  $C_\beta$ H protons are in *antiperiplanar* (*ap*) arrangement corresponding to  $C(\text{O})\text{--N--C}_\beta\text{--C}_\alpha(\phi) \sim \pm 120^\circ$  (Fig. 5). Values of  $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}} > 10$  Hz and  $< 5$  Hz imply predominance of a single conformation about  $\text{N--C}_\beta\text{--C}_\alpha\text{--C}(\text{O})(\theta)$ .

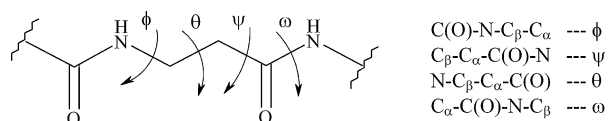


Fig. 5 Schematic representation of backbone torsion angles in  $\beta$ -peptides.

The NOEs such as: NH(2)– $C_\alpha$ H(*pro-R*)(1), NH(3)– $C_\alpha$ H(*pro-S*)(2), NH(4)– $C_\alpha$ H(*pro-R*)(3) support a value of  $\theta \sim 60^\circ$ . These observations along with the presence of medium range NOEs,  $C_\beta$ H(2)–NH(4) and  $C_\beta$ H(2)– $C_\alpha$ H(*pro-R*)(4) and weak NOEs, NH(1)–NH(2) and NH(3)–NH(4), in the ROESY experiments (Fig. 6) confirm the 10–12–10-H-bonded arrangement in tetrapeptide **8**. The structure is stabilized by one 12-mr H-bond NH(4)–CO(1) and two 10-mr H-bonds [NH(1)–CO(2) and NH(3)–CO(4)]. The exchange peaks in the ROESY spectrum indicate the presence of a very small amount of other isomer.

Hexapeptide **9**, an extension of **8** at the C-terminal, again displayed all the signatures of a well defined 10–12-mixed helix. All the amide protons, excluding NH(2) participate in intramolecular H-bonding, which was confirmed by the solvent titration studies.<sup>35</sup> The distinctive NOEs,  $C_\beta$ H(2)–NH(4),  $C_\beta$ H(2)– $C_\alpha$ H(*pro-R*)(4),  $C_\beta$ H(4)–NH(6) and  $C_\beta$ H(4)– $C_\alpha$ H(*pro-R*)(6), confirm the 12-mr H-bonds between NH(4)–CO(1) and NH(6)–CO(3), whereas 10-mr H-bonds between NH(1)–CO(2), NH(3)–CO(4) and NH(5)–CO(6) were confirmed by the NOEs, NH(1)–NH(2), NH(3)–NH(4) and NH(5)–NH(6) respectively. The above data along with the coupling constants and the H-bonding information,

provide compelling evidence for the presence of extended 10–12-helix for the peptide **9**.

The  $\text{CDCl}_3$  spectrum of **10** shows the appearance of two amide signals with  $\delta > 7$  ppm, which hints at their participation in intramolecular H-bonds. Solvent titration studies<sup>35</sup> confirmed that NH(2) and NH(3) participate in H-bonds because of small  $\Delta\delta_{\text{NH}}$  ( $< 0.51$  ppm). The observation of the  $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}}$  with values  $> 9$  Hz and  $< 5$  Hz, suggest predominantly a single conformation about  $C_\alpha\text{--C}_\beta$ . The  $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}}$  values, along with various  $C_\alpha\text{H--C}_\beta\text{H}$  and strong sequential NOEs,  $C_\alpha\text{H}(i-1)\text{--NH}(i)$  confirm that the  $\theta$  for the predominant conformation is  $\sim 60^\circ$ , which is found to be consistent with a right handed helix. Additionally, NOEs,  $C_\beta\text{H}(1)\text{--NH}(3)$  and  $C_\beta\text{H}(1)\text{--C}_\alpha\text{H}(\textit{pro-R})(3)$  demonstrate the presence of a 12–10-mixed helical structure. However, larger deviations from extreme values of the couplings as well as weaker NOEs compared to **8** and **9** indicate the presence of a sizeable fraction of disordered structures in **10**. In the present study **10** is the smallest peptide that generated a well defined structure.

Peptide **11**, a tetrapeptide, made by an elongation of **10** with monomer **1** at the C-terminal end, again shows the features very similar to those of **10**. The participation of the NH(2) and NH(3) in H-bonding was confirmed from their large  $\delta$  as well as small  $\Delta\delta_{\text{NH}}$  shifts in the solvent titration studies.<sup>35</sup> The indirect couplings as well as the medium range NOEs,  $C_\beta\text{H}(1)\text{--NH}(3)$  and  $C_\beta\text{H}(1)\text{--C}_\alpha\text{H}(\textit{pro-R})(3)$ , are consistent with a 12–10-H-bonded arrangement.

For peptide **12**, the extended structure of **11**, all but the first and the sixth amide protons are not involved in H-bonds.<sup>35</sup> The  $^3J_{\text{NH-C}\beta\text{H}} = 8.0\text{--}9.8$  Hz, for all the residues corresponds to  $\phi \sim \pm 120^\circ$ , which is in accordance with the value for mixed helices.  $^3J_{\text{C}\alpha\text{H-C}\beta\text{H}}$  values of  $> 10$  Hz and  $< 5$  Hz for all the residues suggest predominance of a single conformation about  $C_\alpha\text{--C}_\beta$ , which along with some sequential NH– $C_\alpha$ H NOEs confirm a value of  $\sim 60^\circ$  for  $\theta$ . The distinct signatures for the 12–10–12–10-H-bonds in the ROESY spectrum are very clearly noticeable. The medium range NOEs like  $C_\beta\text{H}(1)\text{--NH}(3)$ ,  $C_\beta\text{H}(1)\text{--C}_\alpha\text{H}(\textit{pro-R})(3)$ ,  $C_\beta\text{H}(3)\text{--NH}(5)$  and  $C_\beta\text{H}(3)\text{--C}_\alpha\text{H}(\textit{pro-R})(5)$  strongly support the presence of 12-mr H-bonds between NH(3)–CO(Boc) and NH(5)–CO(2) and medium NOEs NH(2)–NH(3) and NH(4)–NH(5) show the presence of 10-mr H bonds exist between NH(2)–CO(3) and NH(4)–CO(5).

$^1\text{H}$  NMR study on tetrapeptide **13**, in  $\text{CDCl}_3$  showed a well dispersed spectrum in both amide and alpha regions. Most of the amide protons appear at low field. Solvent titration studies<sup>35</sup> confirm that except NH(2) all of them participate in intramolecular H-bonding. The  $^3J_{\text{NH-C}\beta\text{H}} = 8.1\text{--}9.5$  Hz for all

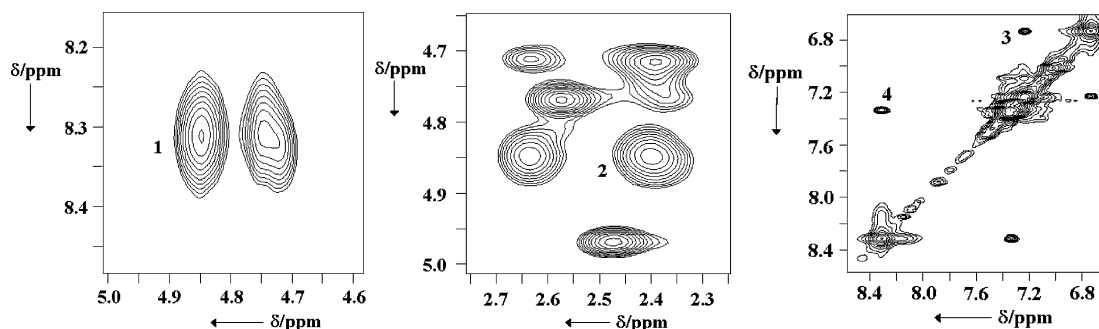
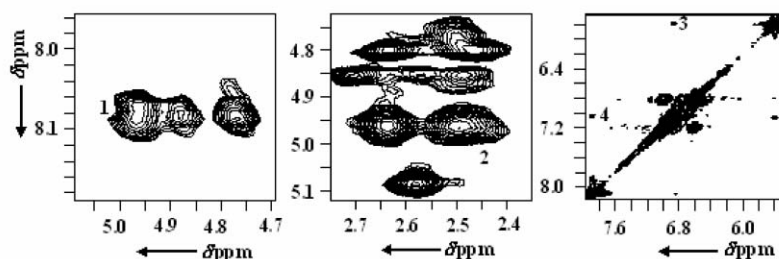


Fig. 6 ROESY spectrum of **8**: The characteristic NOE interactions  $C_\beta\text{H}(2)\text{--NH}(4)$ ,  $C_\beta\text{H}(2)\text{--C}_\alpha\text{H}(\textit{pro-R})(4)$ , NH(1)–NH(2) and NH(3)–NH(4) are marked as 1, 2, 3 and 4 respectively.



**Fig. 7** ROESY spectrum of **13**: The characteristic NOE interactions  $C_{\beta}H(2)$ – $NH(4)$ ,  $C_{\beta}H(2)$ – $C_{\alpha}H_{(pro-R)}(4)$ ,  $NH(1)$ – $NH(2)$  and  $NH(3)$ – $NH(4)$  are marked as **1**, **2**, **3** and **4** respectively.

the four residues, suggest that  $NH$  and  $C_{\beta}H$  protons are in *ap* arrangement, corresponding to  $C(O)$ – $N$ – $C_{\beta}$ – $C_{\alpha}(\phi) \sim \pm 120^{\circ}$ . Observation of  ${}^3J_{C_{\alpha}H-C_{\beta}H} > 10$  Hz and  $< 5$  Hz, clearly demonstrates the predominance of a single conformation around  $C_{\alpha}$ – $C_{\beta}$ , while the characteristic NOEs (Fig. 7) like,  $C_{\beta}H(2)$ – $NH(4)$ ,  $C_{\beta}H(2)$ – $C_{\alpha}H_{(pro-R)}(4)$ , qualify 12-mr H-bond,  $NH(4)$ – $CO(1)$ , while 10-mr H-bonds,  $NH(1)$ – $CO(2)$  and  $NH(3)$ – $CO(4)$ , are confirmed by the NOEs,  $NH(1)$ – $NH(2)$  and  $NH(3)$ – $NH(4)$ . These observations fully support a 10–12–10-H-bonded arrangement in peptide **13**.

For hexapeptide **14**, the participation of all amide protons, excluding  $NH(2)$ , was confirmed by their low field  $\delta_s$  as well as solvent titration studies ( $\Delta\delta_{NH} < 0.34$  ppm).<sup>35</sup> A single conformation around  $C_{\alpha}$ – $C_{\beta}$  is indicated by the values of  $J_{C_{\alpha}H-C_{\beta}H} (> 10$  Hz and  $< 5$  Hz), while, the characteristic NOEs, such as:  $C_{\beta}H(2)$ – $NH(4)$ ,  $C_{\beta}H(2)$ – $C_{\alpha}H_{(pro-R)}(4)$ ,  $C_{\beta}H(4)$ – $NH(6)$ ,  $C_{\beta}H(4)$ – $C_{\alpha}H_{(pro-R)}(6)$ ,  $NH(1)$ – $NH(2)$ ,  $NH(3)$ – $NH(4)$  and  $NH(5)$ – $NH(6)$  qualified the extended 10–12-mixed helix.

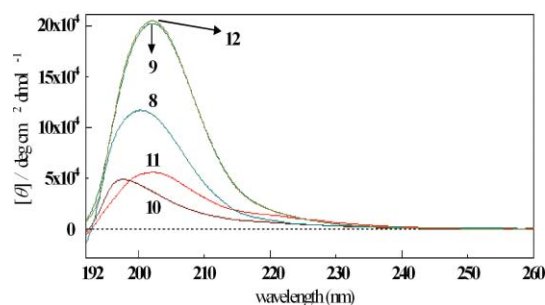
${}^1H$  NMR spectra of tetrapeptide **15** and hexapeptide **16** distinctly show the presence of a 12–10-mixed helix, as confirmed by the low field amide resonances, solvent titration studies, coupling constants and characteristic NOEs.<sup>35</sup> In tetrapeptide **15**, the presence of a very small amount of the other rotamer is indicated by the exchange peaks in the ROESY spectrum.

Except for the second residue in **8**, **9**, **13** and **14**, and the first residues in **10–12**, **15** and **16**, for all  $\beta$ -Caa residues  ${}^3J_{C_{\beta}H-C_{\alpha}H} > 9$  Hz implies  $\chi_1(C_{\beta}H-C_{\alpha}H-C_{\gamma}-C_{\delta}H) \sim 180^{\circ}$ . However, like earlier observations,<sup>15</sup> the second residue in **8**, **9**, **13** and **14** and the first residue in **10–12**, **15** and **16** have  ${}^3J_{C_{\beta}H-C_{\alpha}H} \sim 6$  Hz, suggesting predominance of structures with  $|\chi_1| \sim 60^{\circ}$ . The sugar ring couplings of  ${}^3J_{C_{1H}-C_{2H}} \sim 0$  Hz,  ${}^3J_{C_{2H}-C_{3H}} \sim 5.8$  Hz and  ${}^3J_{C_{3H}-C_{4H}} \sim 3.2$  Hz are in conformity with the  ${}^2T_3$  sugar pucker for the furanose rings.<sup>36</sup>

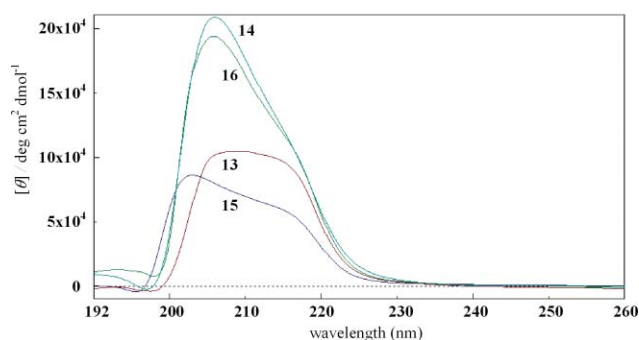
The CD spectra (100  $\mu$ M solution in methanol) of the peptides **8**, **9**, **11** and **12** (Fig. 8) show diagnostic signatures of a right handed 12–10-mixed helix with a maxima at about 203 nm, with very little excursion in the negative molar ellipticity. For **10** the maximum has shifted to 197 nm, partly due to fraying at the termini and contributions from other disordered structures.

On the other hand, **13–16** (Fig. 9) show distinctly different signatures. Though the maxima around 203–208 nm are noticeable, a shoulder at higher wavelength around 218 nm is unmistakably present. For **15**, the maximum has shifted to a lower wavelength compared to **13**, **14** and **16**, possibly due to fraying and contributions from other disordered structures.

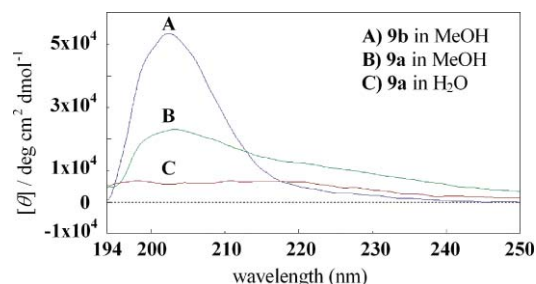
The peptides **9a** and **9b** display rather unusual CD spectra (Fig. 10).



**Fig. 8** CD spectra of **8–12**.



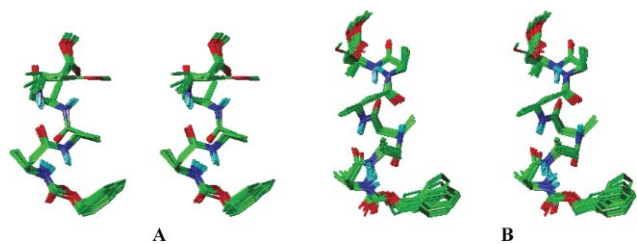
**Fig. 9** CD spectra of **13–16**.



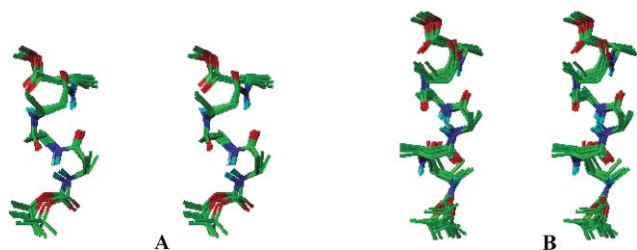
**Fig. 10** CD spectra of **9b** and **9a**.

For the restraint molecular dynamics (MD) studies (Fig. 11 and 12), constraints were derived from the volume integrals obtained from the ROESY spectra using a two-spin-approximation. Fig. 11A and B show the 20 lowest energy superimposed structures of **8** and **9** respectively. The MD structures depict the features already alluded to the heavy atom and the backbone RMSDs respectively of 1.56 Å and 0.74 Å for **8** and 1.67 Å and 1.10 Å for **9**. These values are significantly larger than those observed for the corresponding peptides studied earlier, which resulted in very





**Fig. 11** Stereoviews of the superimposition of the 20 lowest energy structures of (A): peptide **8** and (B): peptide **9** (sugars are replaced with methyl groups after calculations).



**Fig. 12** Stereoviews of the superimposition of the 20 lowest energy structures of (A): peptide **13** and (B): peptide **14** (sugars are replaced with methyl groups after calculations).

robust mixed helices. Fig. 12A and B show the 20 lowest energy superimposed structures of **13** and **14** respectively. The heavy atom and the backbone RMSDs respectively are 1.86 Å and 0.51 Å for **13** and 1.71 Å and 0.62 Å for **14**.

## 6. Evaluation of antibacterial activity

The  $\beta$ -peptides **9a**, **10a**, **11b** and **12a** were evaluated for their antibacterial activity. The standard measurement of antibacterial potency of a compound is the minimum inhibitory concentration (MIC), required for complete inhibition of growth, were obtained by the broth dilution method.<sup>37</sup> The above peptides showed antibacterial activity against a variety of bacterial strains whose MIC values towards the six selected bacterial strains are shown in Table 1. The MIC required for complete inhibition of growth was 6.3  $\mu\text{g mL}^{-1}$  for **9a** against Gram positive *Bacillus sphaericus* and Gram negative *Chromobacterium violaceum* (standard: melittin). The MIC values of peptide **12a** (6.3  $\mu\text{g mL}^{-1}$ ) also showed

good antibacterial activity against *Chromobacterium violaceum* compared to all other bacterial strains. The MIC values for peptide **11b** were better in Gram negative *Pseudomonas oleovorans* and *Chromobacterium violaceum* bacteria when compared to Gram positive bacteria. The activity of all these peptides however is very much inferior to that observed for melittin.

Similarly, the peptides **13–16** were tested for their antimicrobial activity against *S. aureus*, *E. faecalis*, *E. faecium* and *E. coli* and found to show no activity.<sup>32</sup>

## 8. Conclusions

New C-linked carbo- $\beta$ -amino acids ( $\beta$ -Caas) have been prepared, with a methylamino (NH<sub>2</sub>Boc) group and a difluorophenyl (diFP) moiety at the anomeric (C-1) position of the lyxofuranoside side chain. The new ‘epimeric’ (at C <sub>$\beta$</sub> ) monomers were utilized for the synthesis of  $\beta$ -peptides, using the design principle of ‘alternating chirality’ to realize the 12–10-mixed helical patterns in them. The helical structures in the new peptides were ascertained from the NMR, CD and MD studies. No helical patterns were observed in aqueous solutions for the peptides having bifunctional  $\beta$ -Caas, after the removal of protecting groups. Though, some of the water soluble peptides have shown moderate antibacterial activity, the peptides derived from  $\beta$ -Caas (diFP) have shown no activity, much against our expectations. Further, no effect of the bulky aromatic ring, with fluorines, was observed on the helix formation and stability. The present study thus creates new  $\beta$ -Caas with anomeric substitution in the sugar side chain. Expanding the conformational space by using new  $\beta$ -amino acids opens up novel options and enhances the diversity in synthetic peptides and proteins.

## Experimental section

### (3aR,4R,6R,6aS)-6-[(4R)-2,2-Dimethyl-1,3-dioxolan-4-yl]-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl-methyl 4-methyl-1-benzenesulfonate (**18**)

A solution of **17** (5 g, 18.2 mmol) and Et<sub>3</sub>N (4.9 mL, 36.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) containing DMAP (0.1 eq.) at 0 °C was treated with *p*-TsCl (3.82 g, 20 mmol) and stirred at ambient temperature for 3 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL), washed with water (30 mL), brine (30 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of solvent and purification of the residue by column chromatography (silica gel, 20% EtOAc in petroleum

**Table 1** Antibacterial activities of water soluble carbo- $\beta$ -peptides<sup>a</sup>

Peptide	Microorganism					
	Gram positive			Gram negative		
	<i>Bacillus subtilis</i>	<i>Bacillus sphaericus</i>	<i>Serratia marcescens</i>	<i>Pseudomonas oleovorans</i>	<i>Klebsiella aerogenes</i>	<i>Chromobacterium violaceum</i>
<b>9a</b>	25	6.3	12.5	12.5	12.5	6.3
<b>10a</b>	50	25	25	25	50	25
<b>11b</b>	25	25	25	12.5	25	12.5
<b>12a</b>	25	12.5	12.5	12.5	12.5	6.3
Melittin	0.8	0.4	0.4	6.3	6.3	3.1

<sup>a</sup> Activities are expressed as minimum inhibitory concentration (MIC,  $\mu\text{g mL}^{-1}$ ) defined as lowest concentration required for inhibition of growth of bacterial strain.

ether) afforded **18** (6.48 g, 83%) as a colorless syrup;  $[\alpha]_D = +9.7$  (*c* 1.0, CHCl<sub>3</sub>); IR (neat): 2950, 1580, 1210, 1175, 1005, 825 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  7.79 (d, 2H, *J* = 8.3 Hz, Ar-H), 7.37 (d, 2H, *J* = 8.3 Hz, Ar-H), 4.77 (dd, 1H, *J* = 3.7, 6.0 Hz, C<sub>3</sub>H), 4.72 (dd, 1H, *J* = 1.3, 6.0 Hz, C<sub>2</sub>H), 4.30 (ddd, 1H, *J* = 4.5, 6.3, 7.4 Hz, C<sub>3</sub>H), 4.19 (ddd, 1H, *J* = 1.3, 4.6, 5.8 Hz, C<sub>1</sub>H), 4.08 (dd, 1H, *J* = 4.5, 10.5 Hz, C<sub>6</sub>H), 4.02 (dd, 1H, *J* = 5.8, 8.6 Hz, CH<sub>2</sub>a), 4.01 (dd, 1H, *J* = 4.5, 10.5 Hz, C<sub>6</sub>H), 3.90 (dd, 1H, *J* = 4.6, 8.6 Hz, CH<sub>2</sub>b), 3.86 (dd, 1H, *J* = 3.7, 7.4 Hz, C<sub>4</sub>H), 2.46 (s, 3H, Ar-CH<sub>3</sub>), 1.47 (s, 3H, Me), 1.41 (s, 3H, Me), 1.36 (s, 3H, Me), 1.32 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  130.0, 127.9, 112.9, 109.1, 82.5, 82.4, 81.7, 80.9, 73.3, 69.6, 66.5, 26.8, 26.0, 25.0, 24.5, 21.6; HRMS (ESI): *m/z* calculated for C<sub>20</sub>H<sub>28</sub>O<sub>8</sub>S (M<sup>+</sup>+H) 429.1583, found 429.1578.

**(3aR,4R,6R,6aS)-6-[(4R)-2,2-Dimethyl-1,3-dioxolan-4-yl]-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl-methyl azide (19)**

To a solution of **18** (7.03 g, 16.42 mmol) in dry DMF (15 mL), NaN<sub>3</sub> (3.18 g, 49.0 mmol) was added and stirred at 70 °C for 6 h. The reaction mixture was diluted with EtOAc (20 mL), washed with water (20 mL), brine (20 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of solvent and purification of the residue by column chromatography (silica gel, 10% EtOAc in petroleum ether) afforded **19** (3.19 g, 65%) as a white semi solid; mp 65–67 °C;  $[\alpha]_D = -4.9$  (*c* 0.5, CHCl<sub>3</sub>); IR (neat): 2988, 2951, 2168, 2092, 1455, 1248, 1207, 1163, 1088, 887 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  4.81 (dd, 1H, *J* = 3.8, 6.0 Hz, C<sub>3</sub>H), 4.63 (dd, 1H, *J* = 1.5, 6.0 Hz, C<sub>2</sub>H), 4.38 (ddd, 1H, *J* = 4.7, 6.3, 7.4 Hz, C<sub>3</sub>H), 4.22 (ddd, 1H, *J* = 1.5, 4.7, 6.6 Hz, C<sub>1</sub>H), 4.10 (dd, *J* = 6.3, 8.8 Hz, C<sub>6</sub>H), 4.05 (dd, 1H, *J* = 4.7, 8.8 Hz, C<sub>6</sub>H), 3.94 (dd, 1H, *J* = 3.8, 7.4 Hz, C<sub>4</sub>H), 3.41 (dd, 1H, *J* = 6.6, 13.0 Hz, CH<sub>2</sub>a), 3.24 (dd, 1H, *J* = 4.7, 13.0 Hz, CH<sub>2</sub>b), 1.50 (s, 3H, Me), 1.45 (s, 3H, Me), 1.38 (s, 3H, Me), 1.35 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  113.0, 109.2, 83.5, 83.1, 81.6, 80.9, 73.3, 66.7, 51.3, 26.8, 26.1, 25.0, 24.6; HRMS (ESI): *m/z* calculated for C<sub>13</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub> (M<sup>+</sup>+Na) 322.1378, found 322.1386.

**tert-Butyl N-((3aR,4R,6R,6aS)-6-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-ylmethyl)carbamate (20)**

A solution of **19** (2.5 g, 8.36 mmol) and Ph<sub>3</sub>P (4.37 g, 16.7 mmol) in methanol (10 mL) was stirred below 20 °C for 1 h and then treated with (Boc)<sub>2</sub>O (2.22 mL, 8.36 mmol) and stirred at ambient temperature for 5 h. Methanol was evaporated and the residue purified by column chromatography (silica gel, 25% EtOAc in petroleum ether) to give **20** (2.33 g, 75%) as a pale yellow syrup,  $[\alpha]_D = +4.8$  (*c* 0.5, CHCl<sub>3</sub>); IR (neat): 3365, 2981, 2936, 1701, 1522, 1455, 1369, 1253, 1210, 1118, 850 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  4.78 (dd, 1H, *J* = 3.7, 6.0 Hz, C<sub>3</sub>H), 4.67 (br s, 1H, NH), 4.57 (d, 1H, *J* = 6.0 Hz, C<sub>2</sub>H), 4.39 (ddd, 1H, *J* = 4.7, 6.4, 7.4 Hz, C<sub>3</sub>H), 4.09 (dd, *J* = 6.4, 8.7 Hz, C<sub>6</sub>H), 4.07 (m, 1H, C<sub>1</sub>H), 4.03 (dd, 1H, *J* = 4.7, 8.7 Hz, C<sub>6</sub>H), 3.85 (dd, 1H, *J* = 3.7, 7.4 Hz, C<sub>4</sub>H), 3.26 (m, 1H, CH<sub>2</sub>a), 3.07 (ddd, 1H, *J* = 4.6, 8.9, 14.0 Hz, CH<sub>2</sub>b), 3.24 (dd, 1H, *J* = 4.7, 13.0 Hz, CH<sub>2</sub>b), 1.49 (s, 3H, Me), 1.45 (s, 9H, Boc), 1.44 (s, 3H, Me), 1.37 (s, 3H, Me), 1.33 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  155.8, 112.8, 109.1, 83.6, 83.1, 80.7, 80.5, 73.3, 66.8, 40.0, 28.3, 26.9, 26.1, 25.1, 24.6; HRMS

(ESI): *m/z* calculated for C<sub>18</sub>H<sub>31</sub>NO<sub>7</sub> (M<sup>+</sup> + Na) 396.1998, found 396.2007.

**Methyl (E-Z)-3-((3aS,4R,6R,6aR)-6-[(tert-butoxycarbonyl)amino]methyl-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl)-2-propenoate (23)**

To a solution of **21** (2.66 g, 8.0 mmol) in MeOH–H<sub>2</sub>O (30 mL; 5 : 1), NaIO<sub>4</sub> (1.71 g, 8.0 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. MeOH was removed, the residue extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 30 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give aldehyde **22** as a yellow liquid, which was used as such for the next reaction.

A solution of **22** (1.90 g, 6.31 mmol) in benzene (10 mL) was added to a stirred solution of (methoxycarbonylmethylene)triphenylphosphorane (2.52 g, 7.54 mmol) in benzene (10 mL) and heated at reflux for 5 h. Benzene was evaporated and residue purified by flash column chromatography (silica gel, 15% EtOAc in petroleum ether) to give a *cis*–*trans*-mixture of **23** (1.82 g, 81%) as a pale yellow syrup.

**trans 23.**  $[\alpha]_D = -19.0$  (*c* 2.0, CHCl<sub>3</sub>); IR (neat): 3370, 2979, 1791, 1521, 1438, 1368, 1270, 1252, 1165, 1098, 860 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  6.96 (dd, 1H, *J* = 5.2, 15.7 Hz, C<sub>5</sub>H), 6.12 (dd, 1H, *J* = 1.6, 15.7 Hz, C<sub>6</sub>H), 4.78 (dd, 1H, *J* = 4.3, 5.9 Hz, C<sub>3</sub>H), 4.73 (br s, 1H, NH), 4.61 (dd, 1H, *J* = 1.3, 5.9 Hz, C<sub>2</sub>H), 4.51 (ddd, 1H, *J* = 1.6, 4.3, 5.2 Hz, C<sub>4</sub>H), 4.16 (m, 1H, C<sub>1</sub>H), 3.75 (s, 3H, COOMe), 3.33 (m, 1H, CH<sub>2</sub>a), 3.11 (ddd, 1H, *J* = 4.8, 8.7, 13.9 Hz, CH<sub>2</sub>b), 1.45 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.31 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  166.3, 155.9, 141.8, 122.6, 113.4, 83.3, 83.2, 82.2, 79.4, 51.6, 40.2, 29.6, 28.3, 26.2, 25.1; HRMS (ESI): *m/z* calculated for C<sub>17</sub>H<sub>27</sub>NO<sub>7</sub> (M<sup>+</sup> + Na) 380.1685, found 380.1680.

**cis 23.**  $[\alpha]_D = -126.3$  (*c* 0.3, CHCl<sub>3</sub>); IR (neat): 3374, 2967, 1799, 1535, 1429, 1373, 1265, 1241, 1171, 1092, 845 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  6.33 (dd, 1H, *J* = 6.7, 11.7 Hz, C<sub>5</sub>H), 5.97 (dd, 1H, *J* = 1.6, 11.7 Hz, C<sub>6</sub>H), 5.33 (ddd, 1H, *J* = 1.6, 4.0, 6.7 Hz, C<sub>4</sub>H), 5.01 (dd, 1H, *J* = 4.0, 5.9 Hz, C<sub>3</sub>H), 4.77 (br s, 1H, NH), 4.59 (d, 1H, *J* = 5.9 Hz, C<sub>2</sub>H), 4.18 (dd, 1H, *J* = 5.4, 9.2 Hz, C<sub>1</sub>H), 3.73 (s, 3H, COOMe), 3.35 (m, 1H, CH<sub>2</sub>a), 3.04 (ddd, 1H, *J* = 3.8, 9.2, 14.0 Hz, CH<sub>2</sub>b), 1.47 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.30 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  165.9, 155.6, 145.3, 120.5, 112.6, 83.3, 83.1, 82.5, 79.6, 51.5, 40.1, 29.7, 28.4, 26.2, 24.8; HRMS (ESI): *m/z* calculated for C<sub>17</sub>H<sub>27</sub>NO<sub>7</sub> (M<sup>+</sup> + Na) 380.1685, found 380.1672.

**Methyl (3S)-3-((3aS,4R,6R,6aR)-6-[(tert-butoxycarbonyl)amino]methyl-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl)-3-(benzylamino)propanoate (24) and methyl (3R)-3-((3aS,4R,6R,6aR)-6-[(tert-butoxycarbonyl)amino]methyl-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl)-3-(benzylamino)propanoate (25)**

A mixture of **23** (1.84 g, 5.15 mmol) and benzylamine (1.38 mL, 12.8 mmol) was stirred at room temperature. After 12 h, the reaction mixture was directly purified by column chromatography. First eluted (silica gel 25% EtOAc in petroleum ether) was **25** (0.59 g, 25%) as a pale yellow syrup;  $[\alpha]_D = +37.25$  (*c* 1.3, CHCl<sub>3</sub>); IR (neat): 3366, 2980, 2939, 2397, 1714, 1561, 1446, 1264, 1168, 1103, 754 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.36–7.20 (m, 5H, Ar-H), 4.79 (dd, 1H, *J* = 3.8, 6.1 Hz, C<sub>3</sub>H), 4.76 (br s,

1H, NH), 4.51 (d, 1H,  $J = 6.1$  Hz, C<sub>2</sub>H), 4.04 (dd, 1H,  $J = 5.3, 9.7$  Hz, C<sub>1</sub>H), 3.88 (d, 1H,  $J = 13.1$  Hz, BnCH<sub>2</sub>a), 3.85 (d, 1H,  $J = 13.1$  Hz, BnCH<sub>2</sub>b), 3.78 (dd, 1H,  $J = 3.6, 8.4$  Hz, C<sub>4</sub>H), 3.68 (s, 3H, COOMe), 3.47 (ddd, 1H,  $J = 4.7, 6.5, 8.4$  Hz, C<sub>β</sub>H), 3.29 (m, 1H, CH<sub>2</sub>a), 2.99 (ddd, 1H,  $J = 3.9, 9.7, 13.6$  Hz, CH<sub>2</sub>b), 2.72 (dd, 1H,  $J = 4.7, 15.2$  Hz, C<sub>α</sub>H), 2.60 (dd, 1H,  $J = 6.5, 15.2$  Hz, C<sub>α</sub>H), 1.45 (s, 3H, Me), 1.44 (s, 9H, Boc), 1.32 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz):  $\delta$  172.8, 155.8, 140.3, 128.9, 128.8, 128.2, 128.1, 126.8, 112.5, 83.0, 82.4, 81.2, 80.7, 79.4, 79.1, 53.2, 51.4, 51.0, 39.6, 36.0, 28.3, 26.2, 24.9; HRMS (ESI):  $m/z$  calculated for C<sub>24</sub>H<sub>37</sub>N<sub>2</sub>O<sub>7</sub> (M<sup>+</sup> + H) 465.2600, found 465.2598.

Second eluted was (30% EtOAc in petroleum ether) **24** (1.02 g, 42.6%) yield as a yellow solid; mp 84–86 °C;  $[\alpha]_D^{25} = +21.5$  ( $c$  0.5, CHCl<sub>3</sub>); IR (neat): 3392, 2976, 2871, 2358, 1739, 1685, 1531, 1274, 1164, 1040, 862, 701 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  7.35–7.21 (m, 5H, Ar-H), 4.75 (dd, 1H,  $J = 3.7, 6.0$  Hz, C<sub>3</sub>H), 4.73 (br s, 1H, BocNH), 4.55 (d, 1H,  $J = 6.0$  Hz, C<sub>2</sub>H), 4.08 (dd, 1H,  $J = 5.4, 9.2$  Hz, C<sub>1</sub>H), 3.97 (dd, 1H,  $J = 3.7, 8.4$  Hz, C<sub>4</sub>H), 3.91 (d, 1H,  $J = 12.9$  Hz, PhCH<sub>2</sub>a), 3.82 (d, 1H,  $J = 12.9$  Hz, PhCH<sub>2</sub>b), 3.69 (s, 3H, COOMe), 3.44 (dt, 1H,  $J = 10.8, 5.4$  Hz, C<sub>β</sub>H), 3.27 (m, 1H, CH<sub>2</sub>a), 3.02 (ddd, 1H,  $J = 4.3, 9.2, 13.8$  Hz, CH<sub>2</sub>b), 2.74 (dd, 1H,  $J = 5.1, 15.1$  Hz, C<sub>α</sub>H), 2.57 (dd, 1H,  $J = 5.7, 15.1$  Hz, C<sub>α</sub>H), 1.44 (s, 3H, Me), 1.42 (s, 9H, Boc), 1.30 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz):  $\delta$  172.4, 158.8, 140.0, 128.4, 128.3, 127.0, 112.5, 83.1, 82.8, 81.2, 80.6, 79.5, 54.3, 51.7, 51.2, 39.7, 35.3, 28.2, 26.1, 24.8; HRMS (ESI):  $m/z$  calculated for C<sub>24</sub>H<sub>37</sub>N<sub>2</sub>O<sub>7</sub> (M<sup>+</sup> + H) 465.2600, found 465.2607.

### Cbz-(S)-β-Caa(NHBoc)-OCH<sub>3</sub> (1)

A solution of **24** (0.162 g, 3.49 mmol) in methanol (20 mL) was treated with 10% Pd–C (cat.) and stirred at room temperature under hydrogen atmosphere for 12 h. The reaction mixture was filtered and filtrate evaporated to give methyl (3S)-3-((3aS,4R,6R,6aR)-6-[(*tert*-butoxycarbonyl)amino]methyl)-2,2-dimethylperhydrofuro[3,4-*d*][1,3]dioxol-4-yl)-3-aminopropanoate (**26**) as a yellow liquid, which was used as such for the next reaction.

A solution of **26** (1.50 g, 4.01 mmol) and DIPEA (1.40 mL, 8.02 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) at 0 °C was treated with Cbz-Cl (0.82 g, 4.82 mmol) and stirred at room temperature for 2 h. Solvent was evaporated and purified the residue by column chromatography (silica gel, 30% EtOAc in petroleum ether) to give **1** (1.76 g, 86%) as a pale yellow syrup;  $[\alpha]_D^{25} = +16.9$  ( $c$  0.5, CHCl<sub>3</sub>); IR (neat): 3354, 2980, 2941, 2284, 1669, 1523, 1226, 1168, 753 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  7.37–7.28 (m, 5H, Ar-H), 5.40 (d, 1H,  $J = 8.6$  Hz, NH), 5.12 (d, 1H,  $J = 12.4$  Hz, PhCH<sub>2</sub>a), 5.10 (d, 1H,  $J = 12.4$  Hz, PhCH<sub>2</sub>b), 4.68 (br m, 1H, NH), 4.68 (dd, 1H,  $J = 3.9, 6.0$  Hz, C<sub>3</sub>H), 4.54 (d, 1H,  $J = 6.0$  Hz, C<sub>2</sub>H), 4.37 (m, 1H, C<sub>β</sub>H), 4.09 (dd, 1H,  $J = 3.9, 9.5$  Hz, C<sub>4</sub>H), 4.08 (dd, 1H,  $J = 5.3, 9.3$  Hz, C<sub>1</sub>H), 3.67 (s, 3H, COOMe), 3.21 (ddd, 1H,  $J = 5.3, 7.1, 14.1$  Hz, CH<sub>2</sub>a), 3.05 (ddd, 1H,  $J = 4.9, 9.3, 14.1$  Hz, CH<sub>2</sub>b), 2.80 (dd, 1H,  $J = 5.4, 16.2$  Hz, C<sub>α</sub>H), 2.73 (dd, 1H,  $J = 6.0, 16.2$  Hz, 1H, C<sub>α</sub>H), 1.45 (s, 3H, Me), 1.42 (s, 9H, Boc), 1.29 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  171.9, 155.9, 155.8, 136.4, 128.4, 128.1, 128.0, 113.0, 83.0, 80.6, 79.6, 79.1, 66.6, 51.7, 47.9, 39.7, 36.3, 28.3, 26.0, 24.7; HRMS (ESI):  $m/z$  calculated for C<sub>25</sub>H<sub>36</sub>N<sub>2</sub>O<sub>9</sub> (M<sup>+</sup> + H) 509.2499, found 509.2486.

### Boc-(R)-β-Caa-(S)-β-Caa-OCH<sub>3</sub> (36)

A solution of **35** (0.8 g, 2.2 mmol), HOBt (0.35 g, 2.65 mmol), EDCI (0.50 g, 2.6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was stirred at 0 °C under N<sub>2</sub> atmosphere for 15 min and treated sequentially with the amine **33**, DIPEA (0.7 mL, 2.24 mmol) and stirred for 8 h. The reaction mixture was quenched at 0 °C with sat. NH<sub>4</sub>Cl (10 mL) solution. After 10 min, the reaction mixture was diluted with CHCl<sub>3</sub> (10 mL), washed with 1 N HCl (10 mL), water (10 mL), aq. sat. NaHCO<sub>3</sub> (10 mL) solution and brine (10 mL) solution. The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give the residue, which was purified by column chromatography (silica gel, 55% EtOAc in petroleum ether) to give **36** (1.12 g, 83%) as a white solid; mp 143–145 °C;  $[\alpha]_D^{25} = +63.4$  ( $c$  0.25, CHCl<sub>3</sub>); IR (KBr): 3431, 3343, 2927, 1744, 1689, 1508, 1375, 1170, 1093, 1021, 996, 872 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  6.55 (br s, 1H, NH-2), 5.68 (br s, 1H, NH-1), 4.88 (s, 2H, C<sub>1</sub>H-1, 2), 4.77 (dd, 1H,  $J = 3.4, 5.9$  Hz, C<sub>3</sub>H-2), 4.77 (dd, 1H,  $J = 3.4, 5.9$  Hz, C<sub>3</sub>H-1), 4.59 (m, 1H, C<sub>β</sub>H-2), 4.54 (d, 1H,  $J = 5.9$  Hz, C<sub>2</sub>H-2), 4.52 (d, 1H,  $J = 5.9$  Hz, C<sub>2</sub>H-1), 4.31 (m, 1H, C<sub>β</sub>H-1), 4.18 (dd, 1H,  $J = 3.3, 8.0$  Hz, C<sub>4</sub>H-2), 4.13 (dd, 1H,  $J = 3.4, 6.7$  Hz, C<sub>4</sub>H-1), 3.68 (s, 3H, COOMe), 3.31 (s, 3H, OMe), 3.30 (s, 3H, OMe), 2.79 (dd, 1H,  $J = 5.3, 15.9$  Hz, C<sub>α</sub>H(*pro-R*)-1), 2.77 (dd, 1H,  $J = 5.6, 15.9$  Hz, C<sub>α</sub>H(*pro-S*)-1), 2.72 (m, 1H, C<sub>α</sub>H(*pro-R*)-2), 2.56 (dd, 1H,  $J = 5.6, 15.0$  Hz, C<sub>α</sub>H(*pro-S*)-2), 1.48 (s, 3H, Me), 1.46 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.30 (s, 3H, Me), 1.29 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  172.0, 170.2, 155.8, 112.8, 112.6, 106.9, 106.8, 85.2, 84.9, 79.9, 79.5, 78.9(2), 54.7, 54.6, 51.7, 47.6, 46.2, 38.6, 35.6, 28.3, 26.0, 25.9, 24.7, 24.5; FABMS:  $m/z$  calculated for C<sub>28</sub>H<sub>46</sub>N<sub>2</sub>O<sub>13</sub> 619.9 [52, (M + H)<sup>+</sup>], 519.9 [100, (M + H – Boc)<sup>+</sup>], 345.9 (6), 276.8 (14), 115.5 (18), 57.3 (36); HRMS (ESI):  $m/z$  calculated for C<sub>28</sub>H<sub>46</sub>N<sub>2</sub>O<sub>13</sub> (M<sup>+</sup> + Na) 641.2897, found 641.2897.

### Cbz-(S)-β-Caa(NHBoc)-(R)-β-Caa-(S)-β-Caa-OCH<sub>3</sub> (7)

A solution of **36** (0.87 g, 1.41 mmol) and TFA (0.8 mL) in CH<sub>2</sub>Cl<sub>2</sub> was stirred at 0 °C to room temperature for 2 h. The solvent was evaporated under reduced pressure, resulting salt **37** was dried under high vacuum and used as such for further reaction.

A mixture of acid **28** (0.70 g, 1.41 mmol), HOBt (0.22 g, 1.70 mmol), EDCI (0.32 g, 1.70 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was stirred at 0 °C under N<sub>2</sub> atmosphere for 15 min and treated sequentially with the amine **37** and DIPEA (0.5 mL, 2.11 mmol) and stirred for 8 h. Workup as described for **36** and purification by column chromatography (silica gel, 70% EtOAc in petroleum ether) gave **7** (0.84 g, 60%) as a white solid; mp 100–102 °C;  $[\alpha]_D^{25} = +58.0$  ( $c$  0.25, CHCl<sub>3</sub>); IR (KBr): 3370, 2986, 2942, 1717, 1666, 1520, 1337, 1100, 972, 864 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  7.35–7.28 (m, 5H, Ar-H), 7.28 (br s, 1H, NH-3), 7.03 (d, 1H,  $J = 9.2$  Hz, NH-2), 6.12 (br s, 1H, NH-1), 5.11 (d, 1H,  $J = 12.4$  Hz, PhCH<sub>2</sub>a), 5.00 (d, 1H,  $J = 12.4$  Hz, PhCH<sub>2</sub>b), 5.05 (t, 1H,  $J = 12.4$  Hz, NHBoc), 4.89 (s, 1H, C<sub>1</sub>H-3), 4.88 (s, 1H, C<sub>1</sub>H-1), 4.82 (dd, 1H,  $J = 3.6, 6.1$  Hz, C<sub>3</sub>H-3), 4.79 (dd, 1H,  $J = 3.6, 6.1$  Hz, C<sub>3</sub>H-2), 4.77 (dd, 1H,  $J = 3.5, 6.1$  Hz, C<sub>3</sub>H-1), 4.56 (d, 1H,  $J = 6.1$  Hz, C<sub>2</sub>H-3), 4.53 (d, 1H,  $J = 6.1$  Hz, C<sub>2</sub>H-2), 4.51 (d, 1H,  $J = 6.1$  Hz, C<sub>2</sub>H-1), 4.67 (m, 1H, C<sub>β</sub>H-2), 4.43 (m, 1H, C<sub>β</sub>H-3), 4.27 (dd, 1H,  $J = 3.6, 8.8$  Hz, C<sub>4</sub>H-3), 4.24 (m, 1H, C<sub>β</sub>H-1), 4.05 (dd, 1H,  $J = 3.5, 6.3$  Hz, C<sub>4</sub>H-2), 3.95 (dd, 1H,  $J = 3.7, 6.0$  Hz, C<sub>4</sub>H-1), 3.61 (s, 3H, COOMe), 3.36 (s, 3H, OMe), 3.30 (s, 3H, OMe),

3.29–2.97 (m, 1H, CH<sub>2</sub>a), 3.01 (dd, 1H, *J* = 5.0, 16.8 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-3), 2.97 (m, 1H, CH<sub>2</sub>b), 2.76 (dd, 1H, *J* = 5.3, 14.7, Hz C<sub>α</sub>H<sub>(pro-S)</sub>-1), 2.73 (dd, 1H, *J* = 5.4, 16.8 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-3), 2.61 (dd, 1H, *J* = 7.6, 15.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-2), 2.58 (dd, 1H, *J* = 4.0, 14.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-1), 2.50 (dd, 1H, *J* = 4.7, 15.7 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-2), 1.50 (s, 3H, Me), 1.48 (s, 3H, Me), 1.46 (s, 3H, Me), 1.40 (s, 9H, Boc), 1.29 (s, 6H, Me), 1.27 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz): δ 173.2, 171.3, 169.9, 156.1, 155.9, 136.5, 128.5, 128.1, 128.0, 112.8, 107.2, 106.7, 96.1, 85.1, 84.8, 83.1, 82.9, 80.5, 79.9, 79.4, 79.3, 79.0, 78.9, 78.1, 66.5, 55.0, 54.7, 51.9, 48.9, 47.2, 46.0, 39.5, 39.2, 38.1, 34.5, 29.7, 28.3, 26.2, 26.1, 25.9, 24.9, 24.8, 24.1; FABMS: *m/z* calculated for C<sub>47</sub>H<sub>70</sub>N<sub>4</sub>O<sub>19</sub> 1017 [14, (M + Na)<sup>+</sup>], 995 [40, (M + H)<sup>+</sup>], 895 [16, (M + H – Boc)<sup>+</sup>], 519 (30), 470 (29), 377 (28), 276 (100), 227 (30); HRMS (ESI): *m/z* calculated for C<sub>47</sub>H<sub>70</sub>N<sub>4</sub>O<sub>19</sub> (M<sup>+</sup> + Na) 1017.4531, found 1017.4497.

### **Cbz-(S)-β-Caa(NHBoc)-(R)-β-Caa-(S)-β-Caa-(R)-β-Caa(NHBoc)-OCH<sub>3</sub> (8)**

A mixture of **38** (0.18 g, 0.18 mmol), HOBt (0.03 g, 0.22 mmol), EDCI (0.04 g, 0.22 mmol) and DIPEA (0.04 mL, 0.28 mmol) in CH<sub>2</sub>Cl<sub>2</sub> was stirred at 0 °C for 15 min and treated with amine **27** (0.07 g, 0.12 mmol) under nitrogen atmosphere for 8 h. Workup as described for **36** and purification by column chromatography (silica gel, 1.8% methanol in CHCl<sub>3</sub>) afforded **8** (0.12 g, 49%) as a white solid; mp 117–120 °C; [α]<sub>D</sub> = +64.5 (*c* 0.25, CHCl<sub>3</sub>); IR (KBr): 3412, 2939, 1720, 1657, 1546, 1378, 1209, 1101, 1026, 966, 879 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.39–7.27 (m, 5H, Ar-H), 8.31 (d, 1H, *J* = 9.2 Hz, NH-4), 7.33 (d, 1H, *J* = 9.2 Hz, NH-3), 7.23 (d, 1H, *J* = 9.7 Hz, NH-2), 6.73 (d, 1H, *J* = 8.5 Hz, NH-1), 5.16 (br s, 1H, BocNH-1), 5.12 (d, 1H, *J* = 12.5 Hz, PhCH<sub>2</sub>a), 5.10 (d, 1H, *J* = 12.5 Hz, PhCH<sub>2</sub>b), 5.05 (br s, 1H, BocNH-4), 4.97 (dd, 1H, *J* = 3.6, 6.0 Hz, C<sub>3</sub>H-3), 4.89 (s, 1H, C<sub>1</sub>H-2), 4.85 (s, 1H, C<sub>1</sub>H-3), 4.85 (m, 1H, C<sub>β</sub>H-2), 4.77 (dd, 1H, *J* = 3.6, 6.0 Hz, 1H, C<sub>3</sub>H-1), 4.74 (dd, 1H, *J* = 3.7, 6.0 Hz, C<sub>3</sub>H-4), 4.73 (m, 1H, C<sub>β</sub>H-4), 4.71 (dd, 1H, *J* = 3.5, 6.0 Hz, C<sub>3</sub>H-2), 4.56 (d, 1H, *J* = 6.0 Hz, C<sub>2</sub>H-4), 4.54 (d, 1H, *J* = 6.0 Hz, C<sub>2</sub>H-1), 4.53 (d, 1H, *J* = 6.0 Hz, C<sub>2</sub>H-2), 4.52 (d, 1H, *J* = 6.0 Hz, C<sub>2</sub>H-3), 4.39 (m, 1H, C<sub>β</sub>H-3), 4.33 (m, 1H, C<sub>β</sub>H-1), 4.16 (dd, 1H, *J* = 4.9, 9.9 Hz, C<sub>1</sub>H-1), 4.13 (dd, 1H, *J* = 5.5, 9.8 Hz, C<sub>1</sub>H-4), 4.07 (dd, 1H, *J* = 3.6, 9.5 Hz, C<sub>4</sub>H-3), 4.04 (dd, 1H, *J* = 3.6, 7.8 Hz, C<sub>4</sub>H-1), 3.98 (m, 1H, C<sub>4</sub>H-2), 3.82 (dd, 1H, *J* = 3.7, 7.8 Hz, C<sub>4</sub>H-4), 3.67 (s, 3H, COOMe), 3.30 (m, 1H, CH<sub>2</sub>a), 3.29 (m, 1H, CH<sub>2</sub>c), 3.28 (s, 3H, OMe), 3.27 (s, 3H, OMe), 2.96 (m, 1H, CH<sub>2</sub>d), 2.95 (m, 1H, CH<sub>2</sub>b), 2.94 (dd, 1H, *J* = 3.6, 12.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-4), 2.64 (dd, 1H, *J* = 2.7, 12.5 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-2), 2.58 (m, 1H, C<sub>α</sub>H<sub>(pro-S)</sub>-1, C<sub>α</sub>H<sub>(pro-R)</sub>-1), 2.48 (dd, 1H, *J* = 4.0, 13.0 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-3), 2.41 (dd, 1H, *J* = 10.1, 12.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-4), 2.39 (t, 1H, *J* = 12.5 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-2), 2.37 (dd, 1H, *J* = 4.6, 13.0 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-3), 1.49 (s, 6H, Me), 1.45 (s, 3H, Me), 1.44 (s, 3H, Me), 1.43 (s, 9H, Boc), 1.40 (s, 9H, Boc), 1.31 (s, 3H, Me), 1.30 (s, 3H, Me), 1.26 (s, 3H, Me), 1.25 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz): δ 174.4, 171.1, 170.0, 169.4, 156.1, 155.9, 155.8, 136.6, 128.4, 127.8, 127.7, 113.1, 112.9, 112.7, 112.4, 106.9, 106.8, 85.0, 84.9, 83.4, 83.2, 83.1, 83.0, 80.7, 80.6, 80.5, 79.9, 79.7, 79.4, 79.3, 79.2, 78.4, 78.3, 66.3, 54.6, 54.2, 52.2, 48.6, 48.3, 46.6, 46.3, 46.2, 40.4, 40.1, 39.4, 37.9, 37.6, 28.3, 26.2, 25.9, 25.8, 25.1, 24.9, 24.8, 24.0; FABMS: *m/z* calculated for C<sub>63</sub>H<sub>96</sub>N<sub>6</sub>O<sub>25</sub> 1360 [10, (M + Na)<sup>+</sup>], 1338 [39, (M + H)<sup>+</sup>], 1238 [14, (M + H – Boc)<sup>+</sup>], 619 (23), 562 (44), 377 (46),

319 (82), 212 (100); HRMS (ESI): (M<sup>+</sup> + Na) 1359.6322, found 1359.6333.

### **(3aS,4R,6R,6aS)-4-(2,4-Difluorophenyl)-6-[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-ol (46)**

A stirred solution of 1-bromo-2,4-difluorobenzene (14.96 g, 77.5 mmol) in dry THF (100 mL) at –78 °C was treated with *n*-BuLi (48.4 mL, 77.5 mmol) dropwise over a period of 15 min. A solution of lactone **45** (10 g, 38.75 mmol) in dry THF (100 mL) was added dropwise at the same temperature and the reaction mixture allowed to reach room temperature and stirred for 5 h. The reaction mixture was quenched with NH<sub>4</sub>Cl solution (10 mL), water (100 mL) was added and the mixture was extracted with EtOAc (2 × 150 mL). The organic layer was washed with water (100 mL), brine (100 mL) and dried (Na<sub>2</sub>SO<sub>4</sub>). It was evaporated and purified by column chromatography (silica gel, 15% EtOAc in petroleum ether) to afford **46** (12.26 g, 85%) as a white solid; mp 145–147 °C; IR (KBr): 3325, 3087, 2987, 2928, 1625, 1584, 1469, 1373, 1270, 1055, 846 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.49 (m, 1H, Ar-H), 6.89–6.78 (m, 2H, Ar-H), 4.96 (m, 1H, C<sub>2</sub>H), 4.87 (m, 1H, C<sub>3</sub>H), 4.45 (m, 1H, C<sub>6</sub>H), 4.31–4.06 (m, 3H, C<sub>6</sub>H, C<sub>4</sub>H, C<sub>5</sub>H), 1.30 (s, 3H, Me), 1.29 (s, 3H, Me), 1.28 (s, 3H, Me), 1.24 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 162.3, 158.9, 133.2–130.0 (4C), 112.9, 112.2, 111.9, 86.9, 79.3, 79.2, 73.4, 66.4, 26.5, 25.6, 25.3, 24.8; HRMS (ESI): *m/z* calculated for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>F<sub>2</sub> (M<sup>+</sup> + Na) 395.1282, found 395.1286.

### **(1R)-1-[(3aS,4R,6R,6aS)-6-(2,4-Difluorophenyl)-6-hydroxy-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl]ethane-1,2-diol (47)**

A mixture of **46** (12.26 g, 32.9 mmol) and 60% aq. AcOH (85 mL) was stirred at room temperature for 6 h. The reaction mixture was neutralized with solid NaHCO<sub>3</sub> and sat. aq. NaHCO<sub>3</sub> solution (pH = 7) and extracted with EtOAc (3 × 300 mL). Organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), evaporated and the residue purified by column chromatography (silica gel, 50% EtOAc in petroleum ether) to give **47** (8.16 g, 75%) as a colorless syrup; IR (neat): 3390, 2986, 2939, 1618, 1466, 1378, 1212, 1035, 893 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.49 (m, 1H, Ar-H), 6.91–6.79 (m, 2H, Ar-H), 4.96 (m, 2H, C<sub>2</sub>H, C<sub>3</sub>H), 4.30 (m, 1H, C<sub>6</sub>H), 3.90–3.87 (m, 3H, C<sub>6</sub>H, C<sub>4</sub>H, C<sub>5</sub>H), 1.29 (s, 3H, Me), 1.24 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 162.2, 161.3, 133.3 (4C), 113.1, 86.8, 79.6, 79.5, 78.8, 69.5, 63.9, 25.8, 25.0; HRMS (ESI): *m/z* calculated for C<sub>15</sub>H<sub>18</sub>O<sub>6</sub>F<sub>2</sub> (M<sup>+</sup> + Na) 355.0969, found 355.0976.

### **Methyl (Z)-3-[(3aS,4R,6aS)-6-(2,4-difluorophenyl)-6-hydroxy-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxol-4-yl]-2-propenoate (49)**

A stirred solution of **47** (8.16 g, 24.57 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was treated with NaIO<sub>4</sub> (5.25 g, 24.57 mmol), sat. aq. NaHCO<sub>3</sub> solution (4.9 mL) at 0 °C and allowed to stir at room temperature for 5 h. The reaction mixture was filtered, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure to afford (3aS,4S,6aR)-6-(2,4-difluorophenyl)-2,2-dimethylperhydrofuro[3,4-d][1,3]dioxole-4-carbaldehyde (**48**) as a colorless syrup, which was used as such for the next reaction.

A solution of **48** (6.70 g, 22.3 mmol) and (methoxycarbonylmethylene)triphenylphosphorane (8.20 g, 24.56 mmol) in MeOH

(70 mL) was stirred at 0 °C and allowed to stir at room temperature for 5 h. Methanol was evaporated and the residue purified by column chromatography (silica gel, 15% EtOAc in petroleum ether) to give **49** (6.2 g, 78%) as a pale yellow syrup; IR (neat): 3434, 2988, 1719, 1617, 1467, 1209, 1033, 820 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 7.51 (m, 1H, Ar-H), 6.92–6.80 (m, 2H, Ar-H), 6.45 (dd, 1H, *J* = 6.7, 11.7 Hz, C<sub>5</sub>H), 6.03 (dd, 1H, *J* = 1.7, 11.7 Hz, C<sub>6</sub>H), 5.70 (ddd, 1H, *J* = 1.7, 4.0, 6.7 Hz, C<sub>4</sub>H), 5.20 (dd, 1H, *J* = 4.0, 5.8 Hz, C<sub>3</sub>H), 5.01 (m, 1H, C<sub>2</sub>H), 3.75 (s, 3H, OMe), 1.27 (s, 3H, Me), 1.25 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 166.2, 161.3, 158.3, 144.9, 133.3 (4C), 120.7, 113.4, 113.0, 86.9, 81.2, 77.3, 51.6, 25.9, 25.1; HRMS (ESI): *m/z* calculated for C<sub>17</sub>H<sub>18</sub>O<sub>6</sub>F<sub>2</sub> (M<sup>+</sup> + Na) 379.0969, found 379.0976.

#### Methyl (*Z*)-3-[(3*aS*,4*R*,6*S*,6*aR*)-6-(2,4-difluorophenyl)-2,2-dimethylperhydrofuro[3,4-*d*][1,3]dioxol-4-yl]-2-propenoate (**50**)

A stirred solution of **49** (7.5 g, 21.0 mmol) and Et<sub>3</sub>SiH (6.70 mL, 42.1 mmol) in dry CH<sub>3</sub>CN (80 mL) was treated with BF<sub>3</sub>·OEt<sub>2</sub> (2.64 mL, 21.0 mmol) dropwise at -10 °C and stirred for 1 h. The reaction mixture was quenched with sat. aq. NaHCO<sub>3</sub> solution (10 mL), water (100 mL) and extracted with EtOAc (2 × 150 mL). The organic layers were washed with water (100 mL), brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, 8% EtOAc in petroleum ether) to afford **50** (6.18 g, 86%) as a yellow syrup; [α]<sub>D</sub> = +197.9 (*c* 0.75, CHCl<sub>3</sub>); IR (KBr): 2985, 1720, 1618, 1471, 1205, 1103, 996 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 303K, 500 MHz): δ 7.47 (m, 1H, Ar-H), 6.91–6.78 (m, 2H, Ar-H), 6.52 (dd, 1H, *J* = 6.3, 11.6 Hz, C<sub>5</sub>H), 6.03 (dd, 1H, *J* = 1.6, 11.6 Hz, C<sub>6</sub>H), 5.16 (m, 2H, C<sub>3</sub>H, C<sub>4</sub>H), 5.0 (d, 1H, *J* = 4.0 Hz, C<sub>1</sub>H), 4.86 (dd, 1H, *J* = 4.0, 5.7 Hz, C<sub>2</sub>H), 3.75 (s, 3H, OMe), 1.48 (s, 3H, Me), 1.27 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ 166.2, 162.0, 159.0, 145.2, 132.7 (4C), 120.6, 112.9, 82.5, 81.6, 78.8, 77.1, 51.5, 25.2, 24.3; HRMS (ESI): *m/z* calculated for C<sub>17</sub>H<sub>18</sub>O<sub>5</sub>F<sub>2</sub> (M<sup>+</sup> + Na) 363.1020 found 363.1037.

#### Boc-(*S*)-β-Caa(diFP)-(R)-β-Caa(diFP)-OMe (**59**)

A mixture of **55** (0.55 g, 1.24 mmol), HOBt (0.20 g, 1.48 mmol), EDCI (0.28 g, 1.48 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was stirred at 0 °C under N<sub>2</sub> atmosphere for 15 min and treated with **58** (0.56 g, 1.24 mmol; obtained from **4** on exposure to TFA and DIPEA (0.43 mL, 2.48 mmol) under nitrogen atmosphere for 8 h. Workup as described for **36** and purification by column chromatography (silica gel, 60% EtOAc in petroleum ether) afforded **59** (0.71 g, 73%) as a white solid; mp 95–97 °C; [α]<sub>D</sub> = +128.1 (*c* 0.28, CHCl<sub>3</sub>); IR (KBr): 3440, 2929, 1736, 1629, 1471, 1207, 1108, 994 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 303K, 500 MHz): δ 7.26–7.20 (m, 2H, Ar-H), 6.87–6.84 (m, 4H, Ar-H), 6.76 (d, 1H, *J* = 8.6 Hz, NH-2), 5.37 (m, 1H, NH-1), 4.96 (d, 1H, *J* = 3.7 Hz, C<sub>1</sub>H-1), 4.95 (m, 1H, C<sub>1</sub>H-2), 4.88 (m, 1H, C<sub>3</sub>H-1), 4.87 (m, 1H, C<sub>3</sub>H, C<sub>2</sub>H-2), 4.83 (m, 1H, C<sub>3</sub>H-2), 4.80 (m, 1H, C<sub>2</sub>H-2), 4.79 (m, 1H, C<sub>2</sub>H, C<sub>2</sub>H-1), 4.39 (m, 1H, C<sub>6</sub>H-1), 3.94 (dd, 1H, C<sub>4</sub>H-1), 3.87 (dd, 1H, *J* = 3.6, 5.6 Hz, C<sub>4</sub>H-2), 3.69 (s, 3H, COOMe), 2.86 (dd, 1H, *J* = 7.1, 16.1 Hz, C<sub>α</sub>H-2), 2.81 (dd, 1H, *J* = 6.4, 16.1 Hz, C<sub>α</sub>H-2), 2.70 (m, 2H, C<sub>α</sub>H, C<sub>α</sub>H-1), 1.50 (s, 3H, Me), 1.48 (s, 3H, Me), 1.45 (s, 9H, Boc), 1.27 (s, 3H, Me), 1.26 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz): δ 172.0, 170.5, 162.1 (2C), 160.5 (2C), 155.8, 129.6 (8C), 113.0, 112.8, 111.6–

111.5 (4C), 81.7, 81.4, 80.9 (2C), 80.7, 80.3, 79.1, 76.6, 47.8, 45.8, 28.4 (3C), 25.2, 25.1, 24.2, 23.9; HRMS (ESI): *m/z* calculated for C<sub>38</sub>H<sub>46</sub>N<sub>2</sub>O<sub>11</sub>F<sub>4</sub> (M<sup>+</sup> + Na) 805.2935, found 805.2926.

#### Boc-[(*S*)-β-Caa(diFP)-(R)-β-Caa(diFP)]<sub>2</sub>-OMe (**13**)

A mixture of **60** (0.24 g, 0.31 mmol), HOBt (0.05 g, 0.37 mmol) and EDCI (0.07 g, 0.37 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was stirred at 0 °C for 15 min and treated with **61** (0.24 g, 0.31 mmol; obtained from **59** on exposure to TFA) and DIPEA (0.10 mL, 0.62 mmol) under nitrogen atmosphere for 8 h. Workup as described for **36** and purification by column chromatography (silica gel 1.4% MeOH in CHCl<sub>3</sub>) afforded **13** (0.201 g, 45%) as a white solid; mp 156–158 °C; [α]<sub>D</sub> = +162.7 (*c* 0.27, CHCl<sub>3</sub>); IR (KBr): 3435, 3300, 2983, 1723, 1655, 1470, 1272, 1108, 995 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 303 K, 500 MHz): δ 8.08 (d, 1H, *J* = 8.8 Hz, NH-4), 7.06 (d, 1H, *J* = 9.5 Hz, NH-2), 7.03 (d, 1H, *J* = 8.4 Hz, NH-3), 7.22–7.15 (m, 4H, Ar-H), 6.85–6.76 (m, 8H, Ar-H), 5.78 (d, 1H, *J* = 8.1 Hz, NH-1), 5.09 (d, 1H, *J* = 4.4 Hz, C<sub>1</sub>H-3), 5.04 (d, 1H, *J* = 4.4 Hz, C<sub>1</sub>H-1), 4.97 (m, 1H, C<sub>6</sub>H-2), 4.87 (d, 2H, *J* = 4.4 Hz, C<sub>1</sub>H-2, C<sub>1</sub>H-4), 4.86 (m, 1H, C<sub>3</sub>H-1), 4.85 (m, 1H, C<sub>3</sub>H-3), 4.80 (m, 2H, C<sub>2</sub>H-1, C<sub>3</sub>H-2), 4.85 (m, 1H, C<sub>3</sub>H-3), 4.77 (m, 2H, C<sub>1</sub>H-3, C<sub>6</sub>H-4), 4.76 (m, 2H, C<sub>2</sub>H-4, C<sub>3</sub>H-4), 4.60 (m, 1H, C<sub>6</sub>H-3), 4.46 (m, 1H, C<sub>6</sub>H-1), 3.93 (m, 1H, C<sub>4</sub>H-1), 3.79 (dd, 1H, *J* = 3.8, 9.5 Hz, C<sub>4</sub>H-3), 3.70 (dd, 1H, *J* = 3.7, 9.5 Hz, C<sub>4</sub>H-4), 3.68 (s, 3H, COOMe), 3.67 (m, 1H, C<sub>4</sub>H-2), 2.93 (dd, 1H, *J* = 4.4, 13.7 Hz, C<sub>α</sub>H(*pro-S*)-4), 2.70 (m, 1H, C<sub>α</sub>H(*pro-S*)-1), 2.64 (dd, 1H, *J* = 3.8, 13.0 Hz, C<sub>α</sub>H(*pro-S*)-2), 2.58 (m, 1H, C<sub>α</sub>H(*pro-S*)-3), 2.57 (dd, 1H, *J* = 4.5, 12.3 Hz, C<sub>α</sub>H(*pro-R*)-1), 2.51 (m, 1H, C<sub>α</sub>H(*pro-R*)-4), 2.49 (dd, 1H, *J* = 5.1, 13.1 Hz, C<sub>α</sub>H(*pro-R*)-3), 2.46 (dd, 1H, *J* = 11.0, 13.0 Hz, C<sub>α</sub>H(*pro-R*)-2), 1.52 (s, 3H, Me), 1.47 (s, 3H, Me), 1.46 (s, 9H, Boc), 1.44 (s, 3H, Me), 1.43 (s, 3H, Me), 1.26 (s, 3H, Me), 1.25 (s, 3H, Me), 1.25 (s, 3H, Me), 1.21 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz): δ 173.4, 170.8, 170.1, 169.9, 162.1 (4C), 160.4 (4C), 155.7, 129.6–129.4 (16C), 112.9–112.7 (4C), 111.6–111.4 (8C), 81.8, 81.7, 81.5, 81.3, 81.2, 81.2, 80.9, 80.7, 80.5, 80.2, 80.0, 78.8, 76.5, 76.4, 47.3, 47.2, 46.5, 46.0, 28.2 (3C), 25.3 (3C), 25.1, 24.4, 24.3, 24.1, 23.8; HRMS (ESI): *m/z* calculated for C<sub>70</sub>H<sub>80</sub>N<sub>4</sub>O<sub>19</sub>F<sub>8</sub> (M<sup>+</sup> + Na) 1455.5186, found 1455.5186.

#### Boc-[(*S*)-β-Caa(diFP)-(R)-β-Caa(diFP)]<sub>3</sub>-OMe (**14**)

A mixture of **62** (0.1 g, 0.07 mmol), HOBt (0.01 g, 0.08 mmol) and EDCI (0.016 g, 0.08 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was stirred at 0 °C for 15 min and treated with **61** (0.055 g, 0.070 mmol), and DIPEA (0.02 mL, 0.14 mmol) under nitrogen atmosphere for 8 h. Workup as described for **36** and purification by column chromatography (silica gel 1.9% MeOH in CHCl<sub>3</sub>) afforded **14** (0.05 g, 34%) as a white solid; mp 162–165 °C; [α]<sub>D</sub> = +122.9 (*c* 0.15, CHCl<sub>3</sub>); IR (KBr): 3434, 2932, 1722, 1653, 1471, 1378, 1272, 1109, 997 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 303 K, 500 MHz): δ 9.07 (d, 1H, *J* = 9.0 Hz, NH-4), 8.69 (d, 1H, *J* = 9.4 Hz, NH-6), 8.45 (d, 1H, *J* = 8.5 Hz, NH-3), 7.76 (d, 1H, *J* = 9.0 Hz, NH-5), 7.26–7.13 (m, 6H, Ar-H), 6.89–6.79 (m, 12H, Ar-H), 7.20 (d, 1H, *J* = 9.7 Hz, NH-2), 5.88 (d, 1H, *J* = 9.1 Hz, NH-1), 5.25 (dd, 1H, *J* = 3.7, 6.0 Hz, C<sub>3</sub>H-5), 5.18 (dd, 1H, *J* = 3.7, 6.0 Hz, C<sub>3</sub>H-3), 5.14 (d, 1H, *J* = 4.5 Hz, C<sub>1</sub>H-1), 5.09 (m, 1H, C<sub>6</sub>H-2), 5.02 (d, 1H, *J* = 4.5 Hz, C<sub>1</sub>H-2), 4.92 (m, 3H, C<sub>1</sub>H-3, C<sub>1</sub>H-6, C<sub>2</sub>H-6), 4.91 (m, 4H, C<sub>3</sub>H-1, C<sub>1</sub>H-4, C<sub>3</sub>H-4, C<sub>6</sub>H-6), 4.84 (m, 3H, C<sub>2</sub>H-1, C<sub>3</sub>H-2, C<sub>1</sub>H-5), 4.82

(m, 1H, C<sub>2</sub>H-3), 4.79 (m, 1H, C<sub>2</sub>H-4), 4.76 (m, 1H, C<sub>2</sub>H-2), 4.75 (dd, 1H, *J* = 4.5, 6.0 Hz, C<sub>2</sub>H-5), 4.75 (m, 1H, C<sub>β</sub>H-3), 4.74 (m, 2H, C<sub>β</sub>H-4, C<sub>2</sub>H-5), 4.59 (m, 2H, C<sub>β</sub>H-1, C<sub>β</sub>H-5), 4.03 (m, 1H, C<sub>4</sub>H-1), 3.91 (dd, 1H, *J* = 3.4, 9.7 Hz, C<sub>4</sub>H-3), 3.80 (m, 1H, C<sub>4</sub>H-5), 3.77 (m, 1H, C<sub>4</sub>H-2), 3.74 (m, 1H, C<sub>4</sub>H-6), 3.72 (s, 3H, COOMe), 3.63 (m, 1H, C<sub>4</sub>H-4), 3.06 (dd, 1H, *J* = 3.3, 12.5 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-6), 2.93 (dd, 1H, *J* = 3.4, 12.1 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-4), 2.86 (dd, 1H, *J* = 2.7, 12.1 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-2), 2.76 (dd, 1H, *J* = 5.4, 13.8 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-1), 2.74 (dd, 1H, *J* = 3.4, 12.7 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-3), 2.66 (dd, 1H, *J* = 3.2, 12.7 Hz, C<sub>α</sub>H<sub>(pro-S)</sub>-5), 2.65 (dd, 1H, *J* = 5.1, 13.8 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-1), 2.61 (m, 1H, C<sub>α</sub>H<sub>(pro-R)</sub>-6), 2.55 (dd, 2H, *J* = 5.1, 12.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-3, C<sub>α</sub>H<sub>(pro-R)</sub>-5), 2.42 (dd, 1H, *J* = 12.1, 12.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-2), 2.20 (dd, 1H, *J* = 12.1, 12.7 Hz, C<sub>α</sub>H<sub>(pro-R)</sub>-4), 1.52 (s, 3H, Me), 1.51 (s, 3H, Me), 1.49 (s, 3H, Me), 1.45 (s, 9H, Boc), 1.44 (s, 3H, Me), 1.41 (s, 3H, Me), 1.41 (s, 3H, Me), 1.31 (s, 3H, Me), 1.28–1.26 (s, 12H, Me), 1.21 (s, 3H, Me); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz): δ 173.9, 171.2, 170.9, 170.8, 169.9, 169.6, 162.2 (6C), 160.5 (6C), 155.7, 129.5 (24C), 113.0–112.4 (6C), 112.1–111.3 (12C), 84.4–76.4 (20C), 48.3, 47.3, 47.2, 46.8, 46.6, 46.2, 28.4 (3C), 25.4–24.9 (6C), 24.4–23.6 (6C); HRMS (ESI): *m/z* calculated for C<sub>102</sub>H<sub>114</sub>N<sub>6</sub>O<sub>27</sub>F<sub>12</sub> (M<sup>+</sup> + Na) 2105.7432, found 2105.7433.

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