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Preparation and conformational analysis of *C*-glycosyl β²- and β/β²-peptides

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ABSTRACT

Ten *C*-glycosyl β²- and β/β²-peptides with three to eight amino acid residues have been prepared. Solution and solid-phase peptide syntheses were employed to assemble β²-amino acids in which *C*-glycosylic substituents are attached to the C-2 position of β-amino acids. Conformational analysis of the *C*-glycosyl β²-peptides using NMR and CD spectra indicates that the tripeptide can form a helical secondary structure. Besides, helix directions of the *C*-glycosyl β/β²-peptides are governed by the configuration at the α-carbon of the peptide backbone that originates from the stereocenter of the *C*-glycosyl β²-amino acids.

1. Introduction

Carbohydrates are found ubiquitously in nature as natural products. They play key roles in numerous biological processes including modification of proteins, molecular recognition, and the immune response.[1–4] Glycoproteins or glycopeptides are involved in the central part of many processes mentioned above, adopting complex folded structures in order to perform their biological actions. Recently, mimetic compounds of bioactive polymers using non-natural amino acids[5–9] have attracted attention for the elucidation of structure–activity relationships. They are sometimes named as foldamers because their folding pattern can be predicted. They have been extensively studied to design new materials with functional properties or peptide-based drug candidates. One such example is a glycoprotein drug,[10,11] erythropoietin (EPO).[12] On the other hand, *C*-glycosylic compounds, in which the anomeric oxygen atoms in O-glycosides are replaced with carbon atoms, have been investigated as inhibitors of glycosidases or glycosyltransferases.[13–15] The C-glycoside analogue of KRN7000 was recently demonstrated to show outstanding biological activity in comparison with that of the corresponding O-glycoside.[16,17] *C*-glycosyl amino acids,[18–35] in which amino acid is connected to the sugar unit via carbon–carbon linkage, are attractive building blocks that constitute peptide mimetics because of their high stability toward chemical and enzymatic hydrolysis compared to naturally occurring *O*- or *N*-glycosyl amino acids.[36–39]

On the other hand, oligomers of β-amino acids, that is, β-peptides, are the most investigated peptidomimetic foldamers.[5–7,9] β-Peptides are more resistant toward proteolytic enzymes[40,41] than corresponding α-peptides and display many biological activities. For example, they show antibacterial properties[42–47] and act as β-peptidic somatostatin mimics.[48]

Structures of β-peptides are well studied. β-Peptides with as few as four amino acid residues have been shown to fold into helices, sheets, and turns, which are main structural elements of natural α-peptides.[5,9] The substitution pattern of individual residues determines the global conformation and the H-bond mode of β-peptides. Seebach and Gellman have demonstrated that β-peptides containing exclusively β-substituted β-amino acids (β³-amino acids) or exclusively α-substituted β-amino acids (β²-amino acids) tend to form the 14-, 12-helices, and that alternating β²- and β³-substituted peptides prefer the 10/12-helices.[49–51] Kessler and co-workers have reported that the 12/10/12-helix is adopted in mixed peptides containing furanoid β-sugar amino acids and β-alanine repeats.[52] Sharma et al. have reported that both 10/12 and 12/10 right-handed helices are formed by β-peptides derived from *C*-glycosyl β³-amino acids with alternating chirality at the β-carbon,[53] and right- and left-handed 10/12 and 12/10 helices are induced by mixed β-peptides with alternating *C*-glycosyl β³-amino acids and β-alanine repeats.[54]

The hybrid materials of β-peptides and *C*-glycosyl amino acids are expected to form predictable structures and are resistant to-

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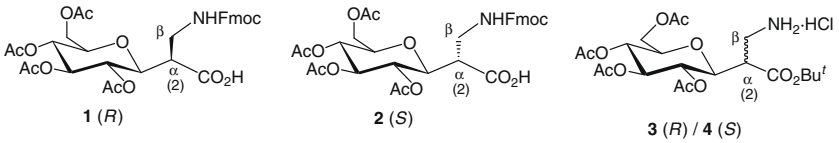


Chart 1.

ward proteases and glycosidase digestions. We have already reported the facile preparation of *C*-glycosyl β-alanine derivatives that have a β^2-amino acid skeleton (Chart 1).[35] In this paper, we describe the preparation of *C*-glycosyl β^2-tripeptides with homo or alternating chirality at the α-carbon by applying a solution-phase procedure and homochiral β/β^2-peptides containing β-alanine and *C*-glycosyl β^2-amino acid repeats in tetra-, hexa-, and octapeptides by applying Fmoc solid-phase peptide synthesis (SPPS) procedure. The solution conformations of these oligomers are also studied using both NMR spectroscopy and circular dichroism (CD). To the best of our knowledge, this is the first report for the synthesis and conformational study of β-peptides with *C*-glycosyl β^2-amino acid residues.

2. Results and discussion

2.1. Synthesis of tripeptides *RRR*, *RSR*, *SSS*, and *SRS*

Theoretical studies by Wu and co-workers demonstrated that (*S*)-β^2-peptides adopt the right-handed 10/12-helix and the left-handed 14-helix, and (*S*)-β^2/(*R*)-β^2-peptides would disrupt the formation of the 14-helix and might also form the 10/12-helix.[55,56] In this work, we prepared *C*-glycosyl β^2-tripeptides ***RRR*** (**5**), ***SSS*** (**6**), ***RSR*** (**7**), and ***SRS*** (**8**) (Fig. 1). Tripeptides ***RRR*** and ***SSS*** are oligomers consisting of the homo stereoisomer at the α-carbon, while tripeptides ***RSR*** and ***SRS*** are oligomers consisting of an alternating sequence. This difference in configuration at the α-carbon in the β-amino acid chain was expected to induce altered helix patterns in secondary structures.

Initially, diastereomerically pure carboxylic acid **1** (*R*) and a mixture of amines **3** and **4** (*R*:*S* = 65:35) were reacted by standard peptide coupling methods (EDC·HCl, HOBt·H_2O, and *N*-methylmolpholine) in solution phase. The products obtained were purified by silica gel column chromatography to afford a pair of dipeptides **9** and **10** in 49% and 17% yield, respectively. The absolute configurations of these dipeptides with respect to the α-carbon can be assessed as shown in Scheme 1 based on the isolated yields of the products, because these yields are based on the total amount of amines (**3** and **4**). The yield of the major product (**9** (*RR*), 49%) has exceeded the portion of minor amine (**4** (*S*), 35%). Similarly, the coupling reaction of the carboxylic acid counterpart **2** (*S*) with a mixture of amines **3** and **4** (*R*:*S* = 75:25) afforded dipeptides **11** and **12** in 41% and 15% yield, respectively. Subsequently, removal of the *tert*-butyl group with formic acid led to a set of dipeptide carboxylic acid **13**–**16** (Scheme 1).

The coupling reaction of carboxylic acid **13** with a mixture of amines **3** and **4** (*R*:*S* = 75:25) afforded tripeptides ***RRR*** and ***RRS*** (**17**) in 32% and 10% yield, respectively. Similarly, tripeptides ***RSR*** and ***RSS*** (**18**) were prepared from dipeptide carboxylic acid **14** and a mixture of amines **3** and **4** (*R*:*S* = 72:28) in 41% and 14% yield, respectively. Since diastereomerically pure amine **4** (*S*) can be obtained by a repeated recrystallization of a mixture of amines **3** and **4**, tripeptides ***SSS*** and ***SRS*** were prepared from the reaction of carboxylic acids **16** and **15** with amine **4** (*S*). The yields of ***SSS*** and ***SRS*** were 62% and 57%, respectively.

2.2. Conformational analysis of tripeptides

Structural studies on tripeptides **5**–**8** were carried out by using NMR spectroscopy and CD. The ^{1}H NMR spectra of the tripeptides in $CDCl_3$ showed well-resolved amide NH resonances (Tables 1–4). The low-field shifts for NH(3) compared with the other NH signals indicate the involvement of this NH proton in intramolecular hydrogen bonds. This hydrogen bonding was confirmed by the solvent titration studies (Fig. 2), in which the amide protons involved in hydrogen bonds show a small chemical shift change on DMSO addition, while those that are exposed to solvent tend to shift toward lower field.

Further studies of the solution-phase conformation of tripeptides were performed through nuclear *Overhauser* effect (NOE)

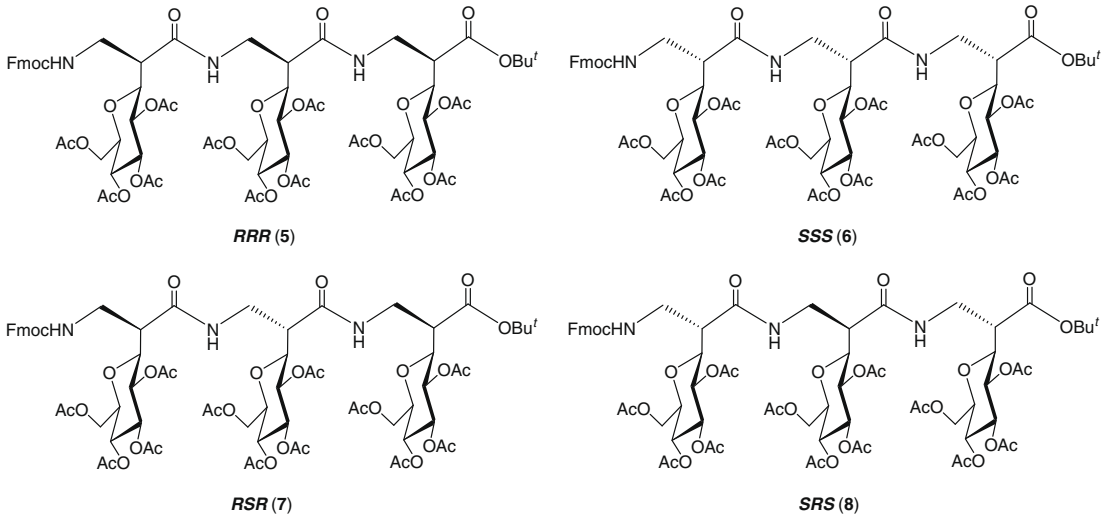
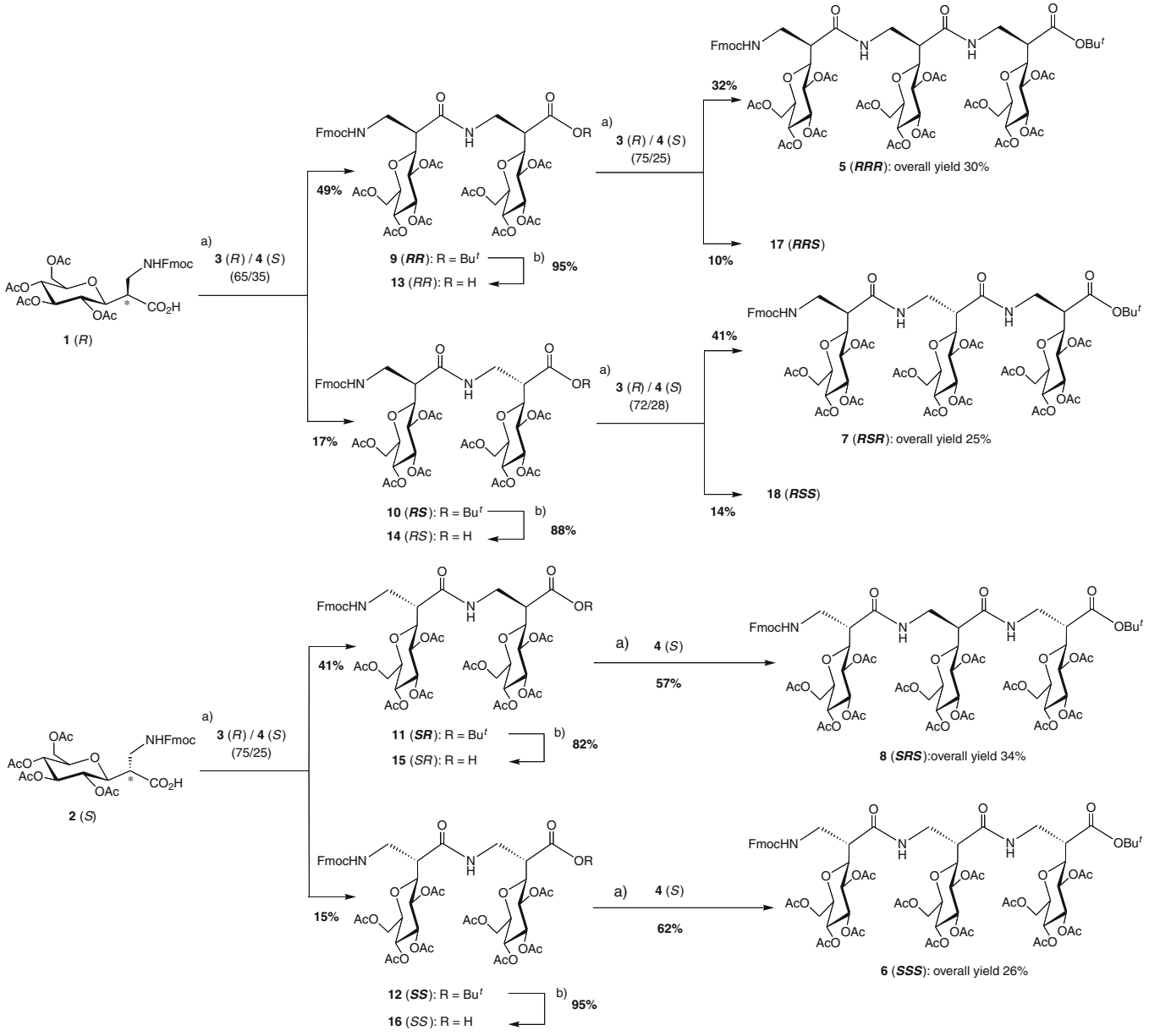


Figure 1. Structure of tripeptides ***RRR*** (**5**), ***SSS*** (**6**), ***RSR*** (**7**), and ***SRS*** (**8**).



Scheme 1. Synthesis of Tripeptide *RRR*, *SSS*, *RSR*, and *SRS*. Reagents and conditions: (a) (i) EDC·HCl, HOBt·H_2O, *N*-methylmorpholine, dry THF, rt, overnight, (ii) silica gel column chromatography; (b) HCOOH, rt, overnight.

measurements. The ROESY spectrum of ***RSR*** revealed the presence of long-range NOE correlations (CβHa(1)/NH(3) and CβHa(1)/C1H(3)). These NOEs were characteristic of a 12-membered ring that involves a hydrogen bond between Fmoc-CO and NH(3) (Fig. 3). However, the ROESY spectra of no other tripeptides showed such long-range NOE correlations. The CD spectra of the tripeptides measured in $CHCl_3$ (20 μM) solution showed no meaningful Cotton effects.

The ab initio molecular orbital energy of the tripeptide ***RSR*** involving 12-membered ring was optimized with the HF/6-31G method and the GAUSSIAN 03 program without solvation effects. The optimized structure involving a 12-membered ring is in good

Table 1
^1H and ^{13}C NMR chemical shifts of tripeptide ***RRR*** in $CDCl_3$

Residue	NH Cα	H-α Cβ	H-βa	H-βb C-1	H-1 C-2	H-2 C-3	H-3 C-4	H-4 C-5	H-5 C-6	H-6a	H-6b
1 (*R*)	5.74	2.67 49.99	3.45 43.63	3.52	3.58 78.96	5.00 72.58	5.17 76.85	5.02 71.19	3.61 79.12	4.15 64.94	4.24
2 (*R*)	6.89	2.71 49.21	3.41 42.21	3.70	3.64 78.88	5.04 72.50	5.17 76.85	5.04 71.18	3.69 79.22	4.15 64.99	4.29
3 (*R*)	7.19	2.78 48.64	3.50 40.99	3.62	3.69 79.22	5.32 72.66	5.12 77.47	5.06 70.72	3.68 78.48	4.09 64.55	4.20

Table 2
^{1}H and ^{13}C NMR chemical shifts of tripeptide ***SSS*** in $CDCl_3$

Residue	NH	H-α Cα	H-βa Cβ	H-βb	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6a C-6	H-6b
1 (*S*)	5.70	2.70 51.07	3.35 42.30	3.60	3.86 79.01	4.96 72.41	5.13 77.03	5.00 71.26	3.71 78.93	4.27 65.03	4.27
2 (*S*)	6.74	2.74 49.96	3.38 40.26	3.55	3.82 79.54	4.99 72.05	5.13 77.03	5.07 70.86	3.67 79.10	4.18 64.68	4.23
3 (*S*)	6.99	2.80 48.20	3.35 38.36	3.71	4.08 80.01	5.22 71.65	5.17 77.32	5.13 71.26	3.63 79.27	4.04 65.42	4.16

Table 3
^{1}H and ^{13}C NMR chemical shifts of tripeptide ***RSR*** in $CDCl_3$

Residue	NH	H-α Cα	H-βa Cβ	H-βb	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6a C-6	H-6b
1 (*R*)	5.83	2.66 50.10	3.40 43.50	3.57	3.58 78.84	5.07 72.36	5.18 76.68	5.10 71.22	3.62 79.11	4.05 64.94	4.39
2 (*S*)	6.92	2.54 50.36	3.50 39.95	3.73	3.92 79.54	5.04 72.36	5.19 76.68	5.06 70.82	3.71 79.27	4.14 64.52	4.31
3 (*R*)	7.34	2.73 47.59	3.48 41.44	3.58	3.67 79.15	5.32 72.49	5.03 77.44	5.01 70.47	3.64 78.13	3.97 64.37	4.21

Table 4
^{1}H and ^{13}C NMR chemical shifts of tripeptide ***SRS*** in $CDCl_3$

Residue	NH	H-α Cα	H-βa Cβ	H-βb	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6a C-6	H-6b
1 (*S*)	5.54	2.54 50.66	3.52 41.82	3.58	4.01 78.68	5.05 72.83	5.30 76.70	5.03 71.10	3.79 78.60	4.18 64.79	4.25
2 (*R*)	6.73	2.58 49.24	3.28 42.53	3.74	3.57 79.07	4.99 72.83	5.12 76.78	5.23 70.63	3.68 79.31	4.23 64.24	4.27
3 (*S*)	7.32	2.98 41.15	3.10 37.76	3.62	4.14 79.94	5.04 72.83	5.16 77.34	5.00 71.11	3.60 79.27	4.02 65.34	4.09

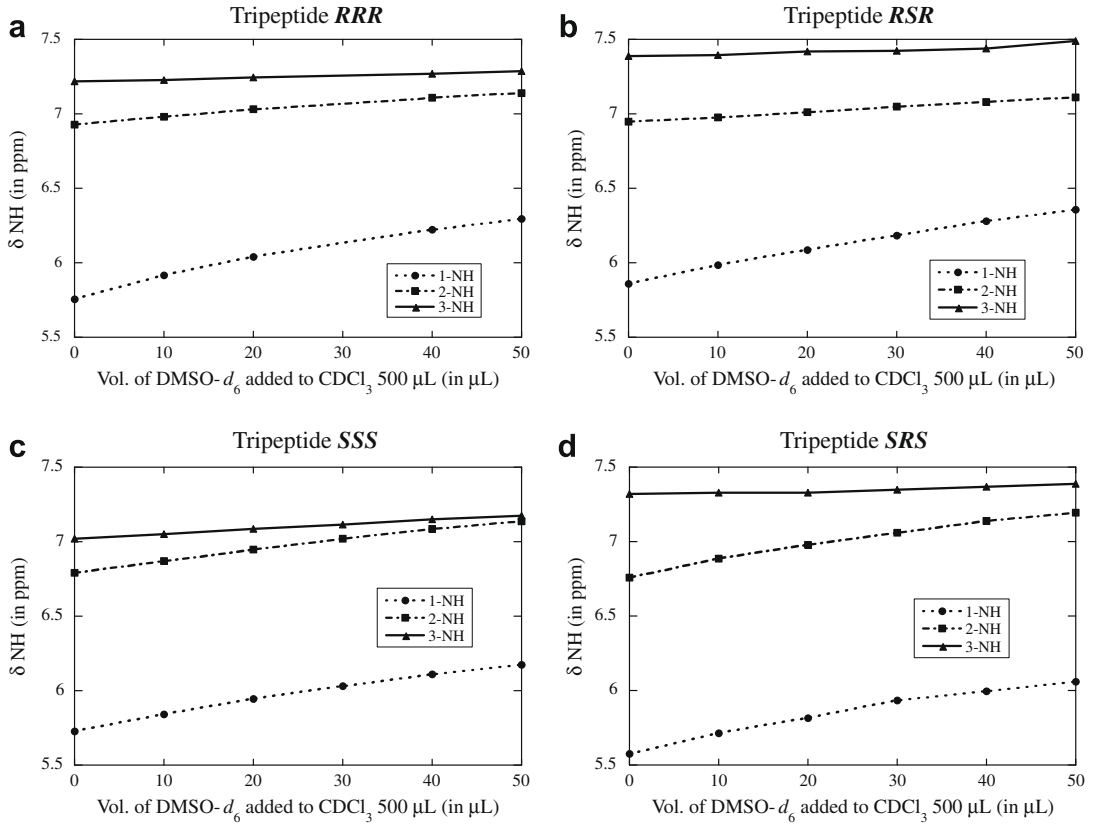


Figure 2. Plots showing the behavior of the amide NH chemical shifts on titration of DMSO-d_6 to a $CDCl_3$ (500 µL) solution of (a) ***RRR***, (b) ***RSR***, (c) ***SSS***, and (d) ***SRS***.

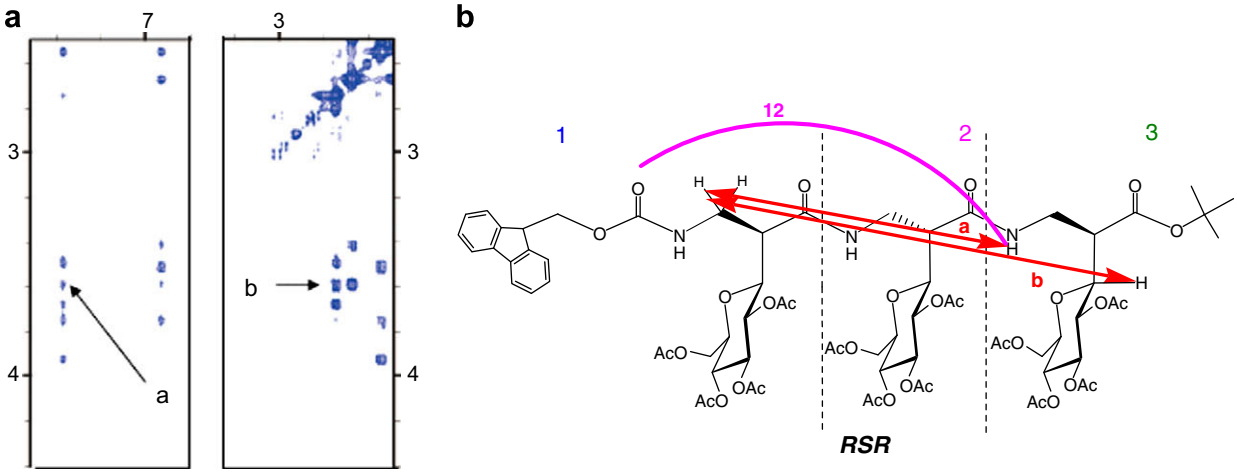


Figure 3. (a) ROESY spectrum of ***RSR*** in $CDCl_3$. The NOE NH(3)/CβHa (1) and C_1H(3)/CβHa (1) are marked as **a** and **b**, respectively. (b) Structure of tripeptide ***RSR*** (characteristic long-range interresidue NOE is shown by arrow, and with H-bonding indicated by curve).

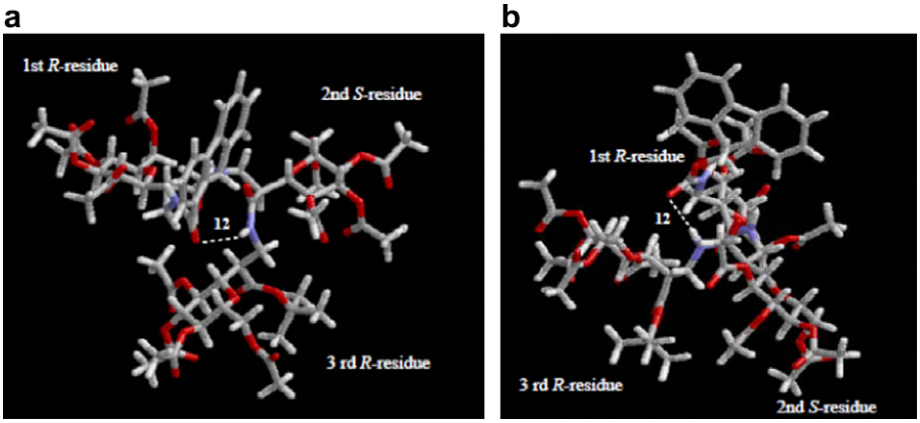
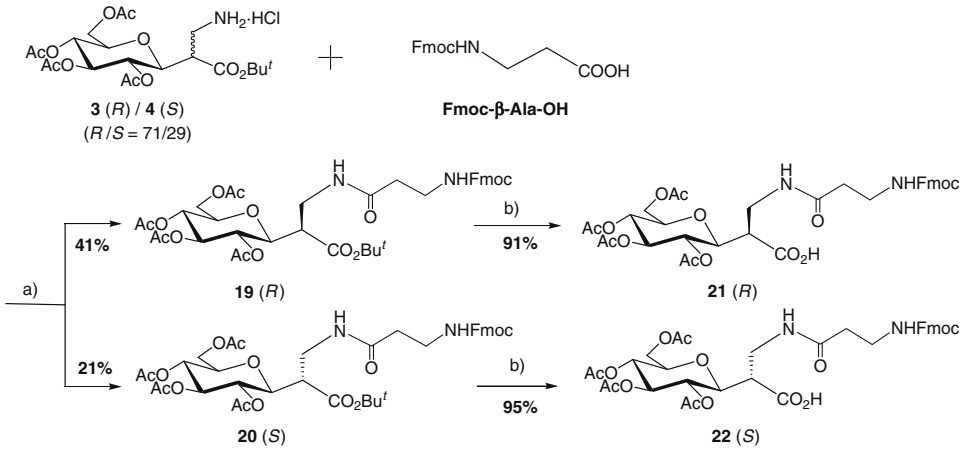


Figure 4. The HF/6-31G optimized structure of tripeptide ***RSR***. (a) Top view. (b) Side view.



Scheme 2. Synthesis of dipeptides **21** (*R*) and **22** (*S*). Reagents and conditions: (a) (i) EDC·HCl, HOBt·H_2O, *N*-methylmorpholine, dry THF, rt, overnight, (ii) silica gel column chromatography; (b) HCOOH, rt, overnight.

agreement with the structure discussed above (Fig. 4). The most stable structure of ***RSR*** involves a 12-membered hydrogen bond rather than its 14-membered counterpart. This result is in agreement with the previous theoretical study.[55]

2.3. Solid-phase synthesis of *C*-glycosyl β/β²-peptides

In general, β-peptides can be synthesized by common solid-phase methods without any alterations from the α-peptide synthesis.

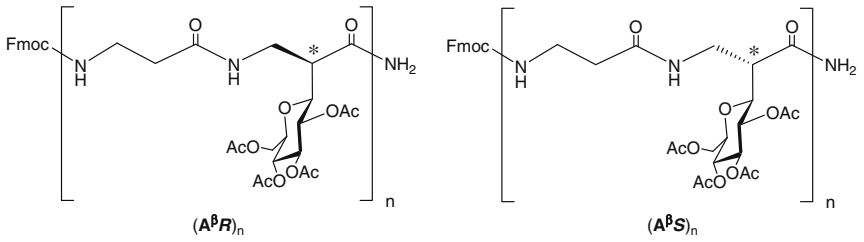
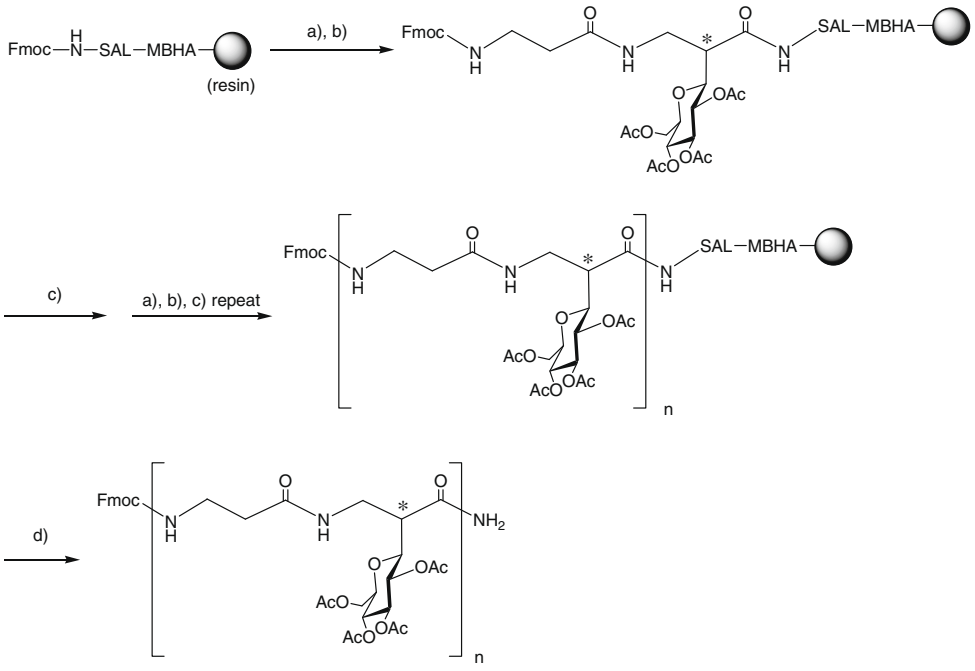


Figure 5. Structure of *C*-glycosyl β/β^2-peptides $(\mathbf{A^{\beta}R})_n$ or $(\mathbf{A^{\beta}S})_n$ (n = 2–4).



n = 2: **Tetra $\mathbf{A^{\beta}RA^{\beta}R}$ (23)**: 23%, **$\mathbf{A^{\beta}SA^{\beta}S}$ (24)**: 28%

n = 3: **Hexa $\mathbf{A^{\beta}RA^{\beta}RA^{\beta}R}$ (25)**: 11%, **$\mathbf{A^{\beta}SA^{\beta}SA^{\beta}S}$ (26)**: 20%

n = 4: **Octa $\mathbf{A^{\beta}RA^{\beta}RA^{\beta}RA^{\beta}R}$ (27)**: 25%, **$\mathbf{A^{\beta}SA^{\beta}SA^{\beta}SA^{\beta}S}$ (28)**: 37%

Scheme 3. Preparation of *C*-glycosyl β/β^2-peptides using Fmoc solid-phase peptide synthesis. Reagents and conditions: (a) 20% piperidine in NMP; (b) **21** (*R*) or **22** (*S*), HBTU, HOBt, and DIPEA in DMF; (c) 10% Ac_2O, 5% DIPEA in NMP; (d) 95:5 TFA–H_2O, RP-HPLC.

Table 5
^{1}H NMR chemical shifts of tetrapeptides $\mathbf{A^{\beta}RA^{\beta}R}$ in CD_3CN

Residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A^{β})	6.12	2.31		3.33								
2 (*R*)	6.86	2.64		3.32	3.52	3.76	5.01	5.15	5.01	3.72	4.11	4.20
3 (A^{β})	7.04	2.26		3.26								
4 (*R*)	6.84	2.70		3.44		3.81	5.03	5.17	5.00	3.76	4.08	4.20

Table 6
^{1}H NMR chemical shifts of tetrapeptides $\mathbf{A^{\beta}SA^{\beta}S}$ in CD_3CN

Residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A^{β})	6.22	2.32	2.35	3.34	3.37							
2 (*S*)	7.08		2.47	3.22	3.75	3.97	4.94	5.16	5.00	3.78	4.20	4.14
3 (A^{β})	7.02	2.12	2.26	3.10	3.53							
4 (*S*)	7.04	2.63		3.23	3.64	3.94	4.99	5.16	4.99	3.75	4.15	4.10

However, we found that the direct use of acetyl-protected Fmoc-*C*-glycosyl β^2-alanine **1** (*R*) as a monomer unit for solid-phase synthesis was unsuccessful because the acetyl rearrangement from the carbohydrate-protecting group to amino group generated after

Table 7
^{1}H NMR chemical shifts of hexapeptides **A$^\beta$*RA*$^\beta$*RA*$^\beta$*R*** in CD_3CN

residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A$^\beta$)	6.13	2.32		3.33								
2 (*R*)	6.93	2.64		3.31	3.55	3.78	4.99	5.15	5.01	3.73	4.10	4.20
3 (A$^\beta$)	7.13	2.25	2.32	3.28	3.45							
4 (*R*)	7.16	2.68		3.35	3.48	3.77	4.97	5.15	5.02	3.72	4.07	4.21
5 (A$^\beta$)	7.17	2.25	2.31	3.26	3.45							
6 (*R*)	6.87	2.70		3.41	3.46	3.82	5.03	5.18	5.01	4.77	4.08	4.21

Table 8
^{1}H NMR chemical shifts of hexapeptides **A$^\beta$*SA*$^\beta$*SA*$^\beta$*S*** in CD_3CN

Residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A$^\beta$)	6.26	2.35		3.36								
2 (*S*)	7.15	2.49		3.22	3.87	4.01	4.94	5.18	5.00	3.81	4.15	4.22
3 (A$^\beta$)	7.23	2.14	2.34	3.07	3.66							
4 (*S*)	7.63	2.48		3.10	3.84	3.97	4.90	5.14	4.97	3.77	4.10	4.18
5 (A$^\beta$)	7.52	2.16	2.31	3.09	3.66							
6 (*S*)	7.08	2.61		3.18	3.79	3.95	4.98	5.18	5.00	3.77	4.10	4.16

Table 9
^{1}H NMR chemical shifts of octapeptides **A$^\beta$*RA*$^\beta$*RA*$^\beta$*RA*$^\beta$*R*** in CD_3CN

Residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A$^\beta$)	6.17	2.34		3.34								
2 (*R*)	7.05	2.65		3.30	3.57	3.78	5.00	—	—	—	—	—
3 (A$^\beta$)	7.16	2.25	2.32	3.28	3.47							
4 (*R*)	7.26	2.66		3.31	3.53	3.80	5.01	5.17	5.16	—	—	—
5 (A$^\beta$)	7.32	2.29	2.37	3.29	3.49							
6 (*R*)	7.23	2.67		3.33	3.51	3.78	5.00	5.16	—	—	—	—
7 (A$^\beta$)	7.25	2.27	2.31	3.27	3.47							
8 (*R*)	6.96	2.72		3.40	3.49	3.83	5.04	5.19	5.04	3.78	—	—

Table 10
^{1}H NMR chemical shifts of octapeptides **A$^\beta$*SA*$^\beta$*SA*$^\beta$*SA*$^\beta$*S***r in CD_3CN

Residue	NH	H-αa	H-αb	H-βa	H-βb	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
1 (A$^\beta$)	6.30	2.33	2.40	3.36	3.41							
2 (*S*)	7.25	2.50		3.21	3.88	4.03	4.95	5.20	5.01	3.82	4.15	4.24
3 (A$^\beta$)	7.31	2.17	2.38	3.08	3.73							
4 (*S*)	7.75	2.51		3.10	3.92	4.01	4.90	5.17	4.98	3.81	4.11	4.20
5 (A$^\beta$)	7.86	2.21	2.41	3.10	3.80							
6 (*S*)	7.83	2.50		3.09	3.87	3.97	4.92	5.15	4.98	3.78	4.11	4.18
7 (A$^\beta$)	7.74	2.21	2.36	3.11	3.72							
8 (*S*)	7.14	2.62		3.17	3.70	3.95	4.99	5.18	5.00	3.78	4.11	4.18

deprotection of Fmoc group had occurred. In this context, β-alanine-capped dipeptides **21** and **22** (Scheme 2) were used as building units for the facile preparation of longer peptides using solid support. We successfully prepared tetra-, hexa-, and octapeptides with homochirality (Fig. 5). It is anticipated that the *C*-glycosyl β/β^2-peptides thus obtained, in which β-alanine (**A$^\beta$**) was introduced between *C*-glycosyl β^2-alanines, would provide enhanced conformational freedom.[56,57]

Starting from commercially available Fmoc-NH-SAL resin, dipeptide acids **21** (*R*) and **22** (*S*) were assembled, where N-deprotection was performed with 20% piperidine–1-methyl-2-pyrrolidinone (NMP). 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) were used to activate Fmoc-protected amino acids in *N*,*N*-dimethylformamide (DMF). After the peptide was cleaved from the resin with 95% trifluoroacetic acid (TFA)–H_2O, RP-HPLC purification yielded β-peptides **23**–**28** (11–37%, Scheme 3).

2.4. Conformational analysis of *C*-glycosyl β/β^2-peptides

Structural studies on β/β^2-peptides were next investigated by NMR spectroscopy and CD. The ^{1}H NMR spectra of β/β^2-peptides were measured in CD_3CN (Tables 5–10). For tetrapeptides **A$^\beta$*RA*$^\beta$*R*** and **A$^\beta$*SA*$^\beta$*S***, and hexapeptide **A$^\beta$*RA*$^\beta$*RA*$^\beta$*R***, no amide NH protons participated in hydrogen bonding, as judged from their chemical shifts (<7.20 ppm) and DMSO titration studies (Fig. 6). The low-field NH resonances (NH(4) and NH(5) for hexapeptide **A$^\beta$*SA*$^\beta$*SA*$^\beta$*S***, and NH(4)–NH(7) for octapeptide **A$^\beta$*SA*$^\beta$*SA*$^\beta$*SA*$^\beta$*S***) indicate the involvement of these NH groups in hydrogen bonding. However, the ROESY spectra of **A$^\beta$*SA*$^\beta$*SA*$^\beta$*S*** and **A$^\beta$*SA*$^\beta$*SA*$^\beta$*SA*$^\beta$*S*** exhibit only very weak NOE correlations probably due to large conformational freedom. The distinct differences in chemical shifts and sensitivities toward DMSO titration between **A$^\beta$*SA*$^\beta$*SA*$^\beta$*S*** and **A$^\beta$*RA*$^\beta$*RA*$^\beta$*R*** indicate that the *S*-peptide adopts a more hydrogen-bonded structure than the corresponding *R*-peptide in the present system.

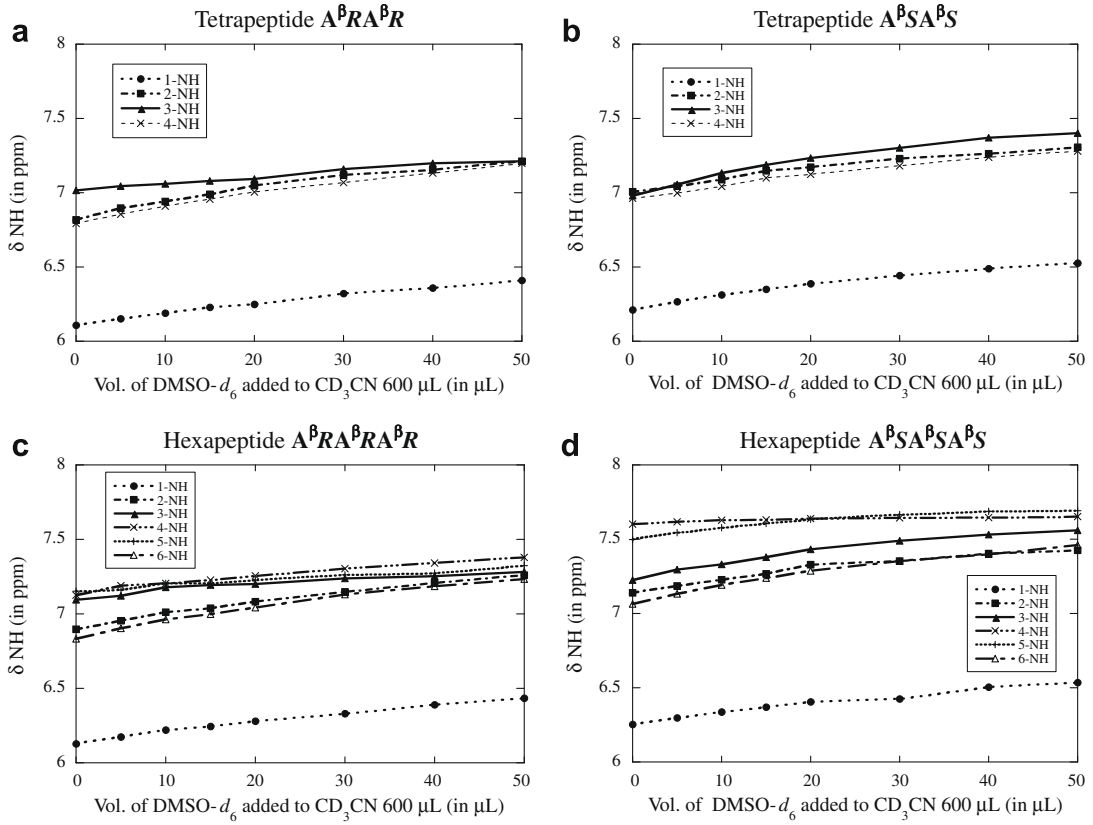


Figure 6. Plots showing the behavior of the amide NH chemical shifts on titration of DMSO-d_6 to a CD_3CN (600 μL) solution of (a) tetrapeptide **A$^{\beta}$RA$^{\beta}$R**, (b) tetrapeptide **A$^{\beta}$SA$^{\beta}$S**, (c) hexapeptide **A$^{\beta}$RA$^{\beta}$RA$^{\beta}$R**, and (d) hexapeptide **A$^{\beta}$SA$^{\beta}$SA$^{\beta}$S**.

Circular dichroism (CD) is frequently used to investigate secondary structures of α-peptides and proteins in solution. Although no simple correlation between the CD pattern and secondary structure for β-peptides is yet fully established, the Seebach and Gellman groups have recently indicated useful criteria for the secondary structure of β-peptides.[49,58–62] For the CD spectra of **A$^{\beta}$RA$^{\beta}$R**, **A$^{\beta}$RA$^{\beta}$RA$^{\beta}$R**, and **A$^{\beta}$RA$^{\beta}$RA$^{\beta}$RA$^{\beta}$R** composed of *R*-isomer recorded in CH_3CN (20 μM), a positive Cotton effect around 200 nm with the maximum wavelength and molar ellipticity θ increasing with peptide chain length was observed (Fig. 7). These CD spectra correspond to the previously observed CD curves of β-peptides,[50,54] indicating that the *R*-based β/β^2-peptides adopt a right-handed 12/10-helical conformation.

On the other hand, the CD spectra of β/β^2-peptides composed of the *S*-isomer showed a negative pattern with similar chain-length dependence. These CD features are in good agreement with the left-handed 12/10-helical pattern.[54] Molar ellipticity of about −26,000 deg cm^2 dmol^{-1} per residue in octapeptide **A$^{\beta}$SA$^{\beta}$SA$^{\beta}$SA$^{\beta}$S** is higher than the absolute values observed for **A$^{\beta}$RA$^{\beta}$RA$^{\beta}$RA$^{\beta}$R**. Since the monomeric amino acids exhibit negligible ellipticity around this resion, here again, the β/β^2-peptides composed of *S*-amino acids tend to show a more organized structure than that of *R*-isomer.

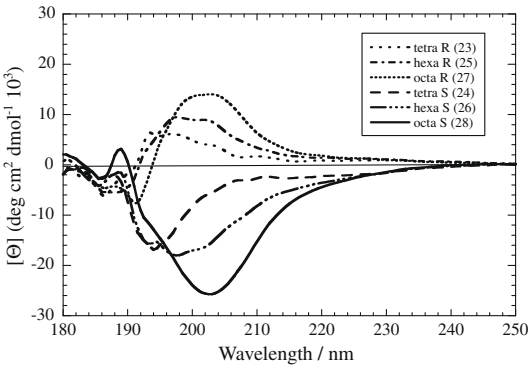


Figure 7. CD spectra of β/β^2-peptides **23**–**28** at 20 μM in CH_3CN.

The direction of the helical patterns was investigated by Newman projections with respect to the C_α–C_β bond (Fig. 8).[54] Dihedral angle θ is defined as a torsion angle about C_α–C_β bond (∠N–C_β–C_α–$C_{carbonyl}$). In an oligopeptide containing (*R*)-*C*-glycosyl β^2-alanine, the energetically favored arrangement is an *anti*-periplanar arrangement for the amide nitrogen atom and the sugar moiety ($\theta = 60°$, Fig. 8a). This arrangement leads to right-handed helical structure. In this right-handed helix, the opposite stereoisomer (*S*)-*C*-glycosyl β^2-alanine is in an energetically unfavored (−) *syn*-clinal arrangement with respect to the amide nitrogen atom and the sugar moiety (Fig. 8b). The stable *anti*-periplanar arrangement can be adopted in a left-handed helical structure ($\theta = -60$, Fig. 8c). The results of theoretical studies[55–57] are consistent with these observations. Chirality at the sugar moiety matches better with a left-handed helix in *S*-peptides than a right-handed helix with *R*-peptides.

3. Conclusion

Four β^2-tripeptides ***RRR***, ***SSS***, ***RSR***, and ***SRS*** were prepared by standard solution-phase peptide synthesis. Six β/β^2-peptides containing tetra-, hexa-, and octapeptides, (**A$^{\beta}$R**)$_n$ or (**A$^{\beta}$S**)$_n$ (n = 2–4), were prepared by Fmoc solid-phase synthesis using dipeptides

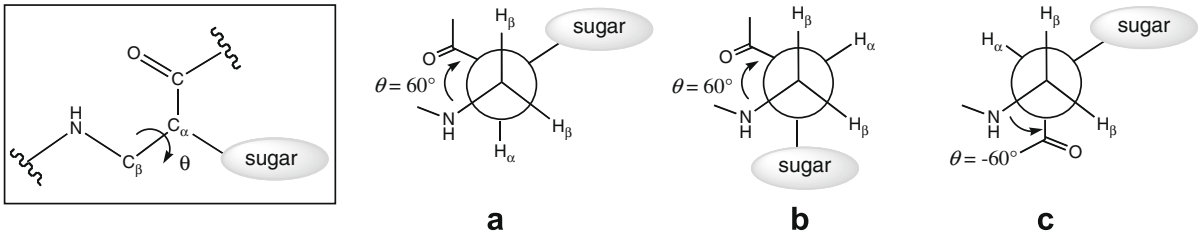


Figure 8. Newman projections illustrating the stereochemistries of (*R*)- and (*S*)-*C*-glycosyl β^2-alanines: (a) *R*-peptide in right-handed helix; (b) *S*-peptide in right-handed helix; (c) *S*-peptide in left-handed helix.

21 (*R*) or **22** (*S*). Rearrangement of the acetyl group from the sugar to the amino group was effectively surpressed by the introduction of a β-alanine residue in the building units. These β^2- and β/β^2-peptides are the first oligopeptide conjugates of *C*-glycosyl β-amino acids with a β^2-amino acid skeleton.

NOEs in ROESY spectra lead to the result that tripeptide ***RSR*** forms a stable secondary structure by a 12-membered ring H-bond. β/β^2-Peptides exhibit the helical conformation characteristic of the stereochemistry in the *C*-glycosyl β^2-amino acid confirmed by CD spectra. These *C*-glycosyl $(\beta/)\beta^2$-peptides with enzymatic and chemical tolerance are attractive substances for creating new materials and peptide-based drug candidates.

4. Experimental

4.1. General

All reagents and solvents used for synthesis were from commercial sources and used as received. *N,N*-Dimethylformamide (DMF, Wako) was dried over $CaSO_4$ and distilled under reduced pressure. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. All aqueous solutions were prepared using deionized and redistilled water. 4-(2,4-Dimethoxyphenyl-Fmoc-aminomethyl) phenoxy resin (Fmoc-NH-SAL resin) and 4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetoamide-norleucyl-*p*-methylbenzhydrylamine resin (Fmoc-NH-SAL-MBHA Resin) were purchased from Watanabe Chemical Ind., Ltd. TLC was performed on E. Merck Silica Gel 60 aluminum sheets. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) spectra were recorded on a Varian GEMINI 2000 spectrometer and referenced to internal TMS or solvent signals. Mass spectra were obtained by electrospray-ionization mass spectrometry (ESIMS) on a JEOL JMS-T 100LC. HPLC was carried out on a reversed-phase column (Cosmosil 5C18-AR-II (4.6 × 150 nm), or Cosmosil 5C18-AR-II (10 × 250 nm) (Nacalai Tesque) using an increasing linear gradient of acetonitrile in water. Peptides were detected by an absorbance measurement at 220 nm.

4.2. NMR spectroscopy

NMR spectra of tri-, tetra-, hexa-, and octapeptides were recorded at 298 K with Bruker DRX-600, DRX-500, and AV-400M spectrometers. For assignment of the ^{1}H and ^{13}C resonances, a series of two-dimensional (2D) experiments were performed: 2D ^{13}C–^{1}H heteronuclear single-quantum correlation (HSQC), ^{1}H–^{1}H total correlation spectroscopy (TOCSY) with mixing times of 40 and 70 ms, rotating frame *Overhauser* effect spectroscopy (ROESY) with a mixing time of 300 ms, and double-quantum filtered correlation spectroscopy (DQFCOSY).

For the TOCSY, ROESY, and DQFCOSY experiments on AV-400M (DRX-500, DRX-600), the direct dimensions were acquired with spectral widths of 22 (21, 20) ppm with 4096 (4096, 5120) complex points centered at 4.7 ppm, and the indirect dimensions were acquired using the TPPI-states method with spectral widths of 8.3 (11, 10) ppm with 900 complex points. The inter-scan delays were set at 2.0s, and 16–32 scans were accumulated for each free induction decay (FID).

For the ^{13}C–^{1}H HSQC experiment on AV-400M (DRX-500, DRX-600), the ^{1}H dimension was acquired with a spectral width of 16 (16, 20) ppm with 512 (1024, 1024) complex points centered at 4.7 ppm, and the ^{13}C dimension was acquired by the gradient-echo method[63] with a spectral width of 66 (80, 80) ppm with 300 (512, 400) complex points centered at 52.0 ppm. The inter-scan delay was set at 2.0s, and 32 scans were accumulated for each FID.

The NMR data were processed and analyzed using the NMRPIPE[63] and SPARKY[64] software packages, respectively. The chemical shift values were calibrated using tetramethylsilane (TMS) dissolved in deuterated chloroform ($CDCl_3$) or in deuterated acetonitrile (CD_3CN) solvent.

4.3. Circular dichroism spectroscopy

CD Spectra were acquired using JASCO-720 spectropolarimeter at room temperature in MeCN, using a 1-cm pathlength CD cell. Spectra represent the accumulation of 64 scans. The scans are carried out from 320 to 170 nm, at 20 μM concentration. Molecular ellipticities per residue are given as deg cm^2 dmol^{-1} per residue.

4.4. *tert*-Butyl (2*R*)-3-{(2*R*)-3-[(9-fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (*RR* (9)) and *tert*-butyl (2*S*)-3-{(2*R*)-3-[(9-fluorenylmethoxy)-carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (*RS* (10))

(2*R*)-3-[(9-Fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (**1**, (*R*), 545 mg, 0.85 mmol), *tert*-butyl (2*R*),(2*S*)-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-aminopropanoate monohydrochloride (**3** (*R*)/**4** (*S*), 434 mg, 0.85 mmol, **3** (*R*)/**4** (*S*) = 65:35), and HOBt·H_2O (130 mg, 0.85 mmol) were dissolved in dry THF (20 mL), and EDC·HCl (163 mg, 0.85 mmol) was added under Ar atmosphere. The mixture was stirred at room temperature, and *N*-methylmorpholine (0.46 mL, 4.25 mmol) was added dropwise. After 19 h, the reaction was monitored by TLC (EtOAc) to confirm the disappearance of the starting material, and a white precipitate was removed by filtration. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography three times (3:1 (1st), 2:1 (2nd), and 4:1 (3rd) EtOAc–hexane) to give ***RR*** (**9**) as a colorless solid (R_f 0.35; 4:1 EtOAc–hexane, 402 mg, 0.37 mmol, 43%) and ***RS*** (**10**) as a colorless solid (R_f 0.275; 4:1 EtOAc–hexane, 103 mg, 0.093 mmol, 11%), respectively.

For **9**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.76 (2H, d, *J* 7.6 Hz, Fmoc-Ar-4), 7.59 (2H, d, *J* 6.9 Hz, Fmoc-Ar-1), 7.41 (2H, dd, *J* 7.5, 7.6 Hz, Fmoc-Ar-3), 7.32 (2H, dd, *J* 6.9, 7.5 Hz, Fmoc-Ar-2), 7.07 (1H, br,

NHCO), 5.37–4.98 (7H, m, NHCOO, H-4, H-3, H-2), 4.41–4.08 (7H, m, Fmoc-CH_2O, Fmoc-CH, H-6), 4.06–3.44 (8H, m, H-5, H-1, H-β (R_1), H-β (R_2)), 2.80 (1H, m, H-α (R_2)), 2.61 (1H, m, H-α (R_1)), 2.07 (6H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.04 (6H, s, COCH_3), 1.99 (3H, s, COCH_3), 1.50 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.67 170.60, 170.31, 170.15, 170.07, 169.37, 169.23 (COCH$_3$, NHCO), 156.43 (NHCOO), 143.77, 143.73, 141.22 (Fmoc-Ar, C), 127.69 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 124.97 (Fmoc-Ar-1), 119.93 (Fmoc-Ar-4), 82.23 ((CH_3)$_3$C), 76.30, 76.08, 75.87 (C-1, C-5), 74.65, 74.09, 69.82, 69.31, 67.97 (C-3, C-2, C-4), 66.63 (Fmoc-CH_2O), 62.10, 61.87 (C-6), 47.13 (Fmoc-CH), 47.02 (C-α (R_2)), 45.90 (C-α (R_1)), 40.74, 38.23 (C-β), 28.01 ((CH_3)$_3$C), 20.63, 20.50 (COCH$_3$). ESIMS: Calcd for $C_{53}H_{66}N_2O_{23}Na$ ([M+Na]$^+$): 1121.39. Found: 1121.30. For **10**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.77 (2H, d, *J* 7.5 Hz, Fmoc-Ar-4), 7.61 (2H, d, *J* 7.2 Hz, Fmoc-Ar-1), 7.41 (2H, dd, *J* 7.5, 7.0 Hz, Fmoc-Ar-3), 7.33 (2H, dd, *J* 7.2, 7.0 Hz, Fmoc-Ar-2), 7.30 (1H, br, NHCO), 5.31 (1H, br, NHCOO), 5.26–4.98 (6H, m, H-4, H-3, H-2), 4.40 (2H, d, *J* 7.0 Hz, Fmoc-CH_2O), 4.35–4.03 (7H, m, Fmoc-CH, H-6, H-1), 3.66–3.58 (4H, m, H-5, H-β (R_1), H-β (S_2)), 3.42 (1H, m, H-β (R_1)), 3.19 (1H, m, H-β (S_2)), 2.95 (1H, m, H-α (S_2)), 2.54 (1H, m, H-α (R_1)), 2.10 (3H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.03 (3H, s, COCH_3), 2.01 (3H, s, COCH_3), 1.94 (3H, s, COCH_3), 1.52 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.04, 170.86, 170.58, 170.46, 170.05, 169.52, 169.36, 169.29, 169.19 (COCH$_3$, NHCO), 156.36 (NHCOO), 143.84, 141.23 (Fmoc-Ar, C), 127.66 (Fmoc-Ar-3), 127.03 (Fmoc-Ar-2), 125.05 (Fmoc-Ar-1), 119.91 (Fmoc-Ar-4), 81.53 ((CH_3)$_3$C), 77.00 (C-1), 76.58 (C-5), 76.01 (C-5), 74.57 (C-3), 74.14 (C-3), 68.60 (C-2), 67.96 (C-4), 66.65 (Fmoc-CH_2O), 62.49, 61.58 (C-6), 47.63 (C-α), 47.13 (Fmoc-CH), 44.58 (C-α (S_2)), 40.96 (C-β (R_1)), 35.11 (C-β (S_2)), 28.14 ((CH_3)$_3$C), 20.60, 20.53, 20.42 (COCH$_3$). ESIMS: Calcd for $C_{53}H_{66}N_2O_{23}Na$ ([M+Na]$^+$): 1121.39. Found: 1121.33.

4.5. *tert*-Butyl (2*R*)-3-{(2*S*)-3-[(9-fluorenylmethoxy) carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (*SR* (11)) and *tert*-butyl (2*S*)-3-{(2*S*)-3-[(9-fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoate (*SS* (12))

Compounds ***SR*** (**11**) and ***SS*** (**12**) were prepared by a method similar to the preparation of **9** and **10** using **2** (*S*) (641 mg, 1.00 mmol) and **3** (*R*)/**4** (*S*) (511 mg, 1.00 mmol, **3** (*R*)/**4** (*S*) 75:25) in place of **1** (*R*) and **3** (*R*)/**4** (*S*) (65:35). The residue was purified by silica gel column chromatography (7:3→1:0 EtOAc–hexane (1st), and 2:1 EtOAc–hexane (2nd)) to give ***SR*** (**11**) as a colorless solid (R_f 0.35; 2:1 EtOAc–hexane, 454 mg, 0.41 mmol) in 43% and ***SS*** (**12**) as a colorless solid (R_f 0.4; 2:1 EtOAc–hexane, 171 mg, 0.016 mmol) in 16% yield, respectively. For **11**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.77 (2H, d, *J* 7.5 Hz, Fmoc-Ar-4), 7.62 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.40 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-3), 7.32 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-2), 6.86 (1H, br, NHCO), 5.56 (1H, br, NHCOO), 5.35 (1H, dd, *J* 9.2, 9.8 Hz, H-2), 5.22–4.98 (5H, m, H-4, H-3, H-2), 4.38 (2H, m, Fmoc-CH_2O), 4.23–4.09 (5H, m, Fmoc-CH, H-6), 3.95 (1H, m, H-1 (S_1)), 3.70–3.66 (4H, m, H-5, H-1 (R_2), H-β (R_2)), 3.35 (2H, m, H-β (S_1)), 3.36 (1H, m, H-β (R_2)), 2.76 (1H, m, H-α (R_2)), 2.56 (1H, m, H-α (S_1)), 2.06 (3H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.02 (3H, s, COCH_3), 2.01 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.99 (3H, s, COCH_3), 1.98 (3H, s, COCH_3), 1.48 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5MHz): δ 170.76, 170.63, 170.36, 170.08, 169.73, 169.44, 169.32, 169.26, 169.06 (COCH$_3$, NHCO), 156.51 (NHCOO), 143.84, 141.20 (Fmoc-Ar, C), 127.66 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 125.09 (Fmoc-Ar-1), 119.91 (Fmoc-Ar-4), 82.37 ((CH_3)$_3$C), 76.84 (C-1 (S_1)), 76.40 (C-1 (R_2)), 76.21 (C-5), 75.90 (C-5), 74.65 (C-3), 74.06 (C-3), 70.33 (C-2), 69.64 (C-2), 68.13 (C-4), 67.91 (C-4), 66.81 (Fmoc-CH_2O), 61.74 (C-6), 48.41 (C-α (S_1)), 47.13 (Fmoc-CH), 45.51 (C-α (R_2)), 39.61 (C-β (S_1)), 38.40 (C-β (R_2)), 28.02 ((CH_3)$_3$C), 20.63, 20.53 (COCH$_3$). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M−Bu^t+H+Na]$^+$): 1065.33. Found: 1065.28. For **12**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.76 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.61 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.40 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-3), 7.31 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-2), 6.90 (1H, br, NHCO), 5.51 (1H, br, NHCOO), 5.21–4.99 (6H, m, H-4, H-3, H-2), 4.36 (2H, Fmoc-CH_2O), 4.23–4.06 (6H, Fmoc-CH, H-6, H-1 (S_1)), 3.89 (1H, m, H-1 (S_2)), 3.71–3.57 (4H, m, H-5, H-β (S_1), H-β (S_2)), 3.48–3.35 (2H, m, H-β (S_1), H-β (S_2)), 2.81 (1H, m, H-α (S_2)), 2.68 (1H, m, H-α (S_1)), 2.09 (3H, s, COCH_3), 2.07 (3H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.03 (3H, s, COCH_3), 2.02 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.98 (3H, s, COCH_3), 1.97 (3H, s, COCH_3), 1.48 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 170.83, 170.71, 170.58, 170.44, 170.13, 169.87, 169.57, 169.32, 169.36 (COCH$_3$, NHCO), 156.46 (NHCOO), 143.99, 143.89, 141.22 (Fmoc-Ar, C), 127.63 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 125.15 (Fmoc-Ar-1), 119.89 (Fmoc-Ar-4), 81.74 ((CH_3)$_3$C), 76.73, 76.58, 76.30 (C-1, C-5), 74.51, 74.23 (C-3), 69.19, 68.99 (C-2), 68.54, 68.10 (C-4), 66.89 (Fmoc-CH_2O), 62.49, 61.89 (C-6), 47.89, 47.13, 45.29 (Fmoc-CH, C-α), 39.25, 35.58 (C-β), 28.07 ((CH_3)$_3$C), 20.66, 20.55 (COCH$_3$). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M−Bu^t+H+Na]$^+$): 1065.33. Found: 1065.25.

4.6. (2*R*)-3-{(2*R*)-3-[(9-Fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (13 (*RR*))

RR (**9**) (273 mg, 0.248 mmol) was dissolved in formic acid (10 mL) and stirred at room temperature for 3 h. Water (30 mL) was added, and the organic material was extracted with $CHCl_3$ (2 × 30 mL), washed with brine (20 mL), and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was washed with MeOH to afford **13** (*RR*) as a colorless solid (219 mg, 0.21 mmol) in 85% yield. ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.76 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.59 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.40 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-3), 7.31 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-2), 7.18 (1H, br, NHCO), 5.51 (1H, br, NHCOO), 5.29–4.91 (6H, m, H-2, H-3, H-4), 4.39 (2H, d, *J* 7.0 Hz, Fmoc-CH_2O), 4.25–4.05 (5H, m, Fmoc-CH, H-6), 3.86–3.38 (8H, m, H-5, H-1, H-β (R_1), H-β (R_2)), 3.05 (1H, m, H-α (R_2)), 2.65 (1H, m, H-α (R_1)), 2.07 (6H, s, COCH_3), 2.04 (3H, s, COCH_3), 2.01 (12H, s, COCH_3), 1.98 (3H, s, COCH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 172.04, 171.05, 170.81, 170.47, 170.34, 170.21, 169.82, 169.57, 169.42 (COCH$_3$, COOH, NHCO), 156.57 (NHCOO), 143.73, 141.22 (Fmoc-Ar, C), 127.71 (Fmoc-Ar-3), 127.03 (Fmoc-Ar-2), 124.99 (Fmoc-Ar-1), 119.96 (Fmoc-Ar-4), 76.56 (C-1), 76.13 (C-1, C-5), 74.49 (C-3), 74.09 (C-3), 69.95 (C-2), 69.43 (C-2), 68.47 (C-4), 68.18 (C-4), 66.65 (Fmoc-CH_2O), 62.13 (C-6), 62.00 (C-6), 47.12 (Fmoc-CH), 46.70 (C-α (R_1)), 45.11 (C-α (R_2)), 40.53, 37.57 (C-β), 20.53 (COCH$_3$). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M+Na]$^+$): 1065.33. Found: 1065.25.

4.7. (2*S*)-3-{(2*R*)-3-[(9-Fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (14 (*RS*))

Compound **14** (*RS*) was prepared by a method similar to the preparation of **13** (*RR*) using ***RS*** (**10**) (79 mg, 0.072 mmol) in place of ***RR*** (**9**). The residue was purified by silica gel column chromatography (EtOAc) to give **14** (*RS*) as a colorless solid (R_f 0.025; EtOAc, 59 mg, 0.057 mmol) in 79% yield. ^{1}H NMR ($CDCl_3$, Me_4Si, 300

MHz): δ 7.77 (2H, d, *J* 7.2 Hz, Fmoc-Ar-4), 7.60 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.41 (2H, dd, *J* 7.2, 7.5 Hz, Fmoc-Ar-3), 7.33 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-2), 7.22 (1H, br, N*H*CO), 5.37 (1H, br, Fmoc-N*H*COO), 5.23–4.96 (6H, m, H-2, H-3, H-4), 4.40–4.03 (8H, m, Fmoc-CH_2O, Fmoc-C*H*, H-6, H-1 (S_2)), 3.75–3.39 (7H, m, H-5, H-1 (R_1), H-β), 2.98 (1H, m, H-α (S_2)), 2.60 (1H, m, H-α (R_1)), 2.09 (3H, s, COCH_3), 2.06 (9H, s, COCH_3), 2.02 (6H, s, COCH_3), 2.00 (6H, s, COCH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.48, 171.05, 170.78, 170.34, 170.25, 169.71, 169.44 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.47 (NH*C*OO), 143.79, 141.27 (Fmoc-Ar, C), 127.72 (Fmoc-Ar-3), 127.08 (Fmoc-Ar-2), 125.05 (Fmoc-Ar-1), 119.97 (Fmoc-Ar-4), 76.81 (C-1 (S_2)), 76.22 (C-1 (R_1), C-5), 74.17 (C-3), 69.25 (C-2), 68.41 (C-4), 66.71 (Fmoc-*C*H$_2$O), 61.95 (C-6), 47.63 (C-α (R_1)), 47.13 (Fmoc-*C*H), 45.30 (C-α (S_2)), 20.68 (COCH_3). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M+Na]$^+$): 1065.33. Found: 1065.26.

4.8. (2*R*)-3-{(2*S*)-3-[(9-Fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (15 (*SR*))

Compound **15** (*SR*) was prepared by a method similar to the preparation of **13** (*RR*) using ***SR*** (**11**) (419 mg, 0.38 mmol) in place of ***RR*** (**9**). Compound **15** (*SR*) was obtained as colorless solid (324 mg, 0.31 mmol) in 82% yield. ^{1}H NMR (CD_3CN, 300 MHz): δ 7.84 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.68 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.43 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-3), 7.35 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-2), 6.68 (1H, br, N*H*CO), 5.37 (1H, br, N*H*COO), 5.22–5.15 (3H, m, H-2, H-3), 5.04–4.98 (3H, m, H-2, H-4), 4.31 (2H, s, Fmoc-CH_2O), 4.29 (1H, m, Fmoc-C*H*), 4.13 (4H, m, H-6), 3.93 (2H, m, H-1), 3.76 (2H, m, H-5), 3.67 (2H, m, H-β (R_2)), 3.43 (2H, m, H-β (S_1)), 2.85 (1H, m, H-α (R_2)), 2.54 (1H, m, H-α (S_1)), 2.00, 1.99, 1.95, 1.94, 1.91 (24H, s, COCH_3). ^{13}C NMR(CD_3CN, 75.5 MHz): δ 171.97, 171.56, 171.45, 171.01, 170.62, 170.56, 170.36 (*C*OCH$_3$, *C*OOH, NH*C*O), 157.34 (NH*C*OO), 145.17, 142.08 (Fmoc-Ar, C), 128.68 (Fmoc-Ar-3), 128.15 (Fmoc-Ar-2), 126.27 (Fmoc-Ar-1), 121.03 (Fmoc-Ar-4), 76.83 (C-1), 76.76 (C-5), 75.05 (C-3), 71.70 (C-2), 70.37 (C-2), 69.43 (C-4), 67.23 (Fmoc-*C*H$_2$O), 62.90 (C-6), 48.72 (C-α (S_1)), 48.06 (Fmoc-*C*H), 46.28 (C-α (R_2)), 40.15 (C-β (S_1)), 37.73 (C-β (R_2)), 21.06, 20.88, 20.76 (COCH_3). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M+Na]$^+$): 1065.33. Found: 1065.22.

4.9. (2*S*)-3-{(2*S*)-3-[(9-Fluorenylmethoxy)carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (16 (*SS*))

Compound **16** (*SS*) was prepared by a method similar to the preparation of **13** (*RR*) using ***SS*** (**12**) (177 mg, 0.16 mmol) in place of ***RR*** (**9**). The residue was purified by silica gel column chromatography (7:1 EtOAc–hexane) to give **16** (*SS*) as a colorless solid (7:1 EtOAc–hexane, 128 mg, 0.12 mmol) in 76% yield. ^{1}H NMR (CD_3OD, 300 MHz): δ 7.83 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.71 (2H, d, *J* 7.0 Hz, Fmoc-Ar-1), 7.43 (2H, dd, *J* 7.3, 7.3 Hz, Fmoc-Ar-3), 7.36 (2H, dd, *J* 7.0, 7.3 Hz, Fmoc-Ar-2), 5.29–5.04 (6H, m, H-2, H-3, H-4), 4.39–4.10 (8H, m, Fmoc-CH_2O, Fmoc-C*H*, H-6, B-H-1), 3.95 (1H, dd, *J* 5.0, 10.1 Hz, H-1), 3.85–3.78 (2H, m, H-5), 3.67–3.43 (4H, m, H-β), 2.85 (1H, m, H-α), 2.77 (1H, m, H-α), 2.08 (6H, s, COCH_3), 2.05 (3H, s, COCH_3), 2.04 (6H, s, COCH_3), 2.03 (3H, s, COCH_3), 1.99 (3H, s, COCH_3), 1.97 (3H, s, COCH_3). ^{13}C NMR (CD_3OD, 75.5 MHz): δ 174.48, 173.00, 172.52, 172.45, 171.77, 171.34, 171.26, 171.21 (*C*OCH$_3$, *C*OOH, NH*C*O), 158.52 (NH*C*OO), 145.43, 145.34, 142.55 (Fmoc-Ar, C), 128.78 (Fmoc-Ar-3), 128.20 (Fmoc-Ar-2), 126.39 (Fmoc-Ar-1), 120.94 (Fmoc-Ar-4), 78.09 (C-1), 77.57 (C-1), 77.44 (C-5), 77.22 (C-5), 75.89 (C-3), 71.81 (C-2), 71.56 (C-2), 69.81 (C-4), 69.69 (C-4), 67.95 (Fmoc-*C*H$_2$O), 63.30 (C-6), 48.73 (C-α), 48.16 (Fmoc-*C*H), 47.54 (C-α), 39.79, 37.93 (C-β), 20.77, 20.59 (*C*OCH$_3$). ESIMS: Calcd for $C_{49}H_{58}N_2O_{23}Na$ ([M+Na]$^+$): 1065.33. Found: 1065.22.

4.10. *tert*-Butyl (2*R*)-3-{(2*R*)-3-{(2*R*)-3-[(9-fluorenylmethoxy)-carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (***RRR*** (**5**))

Compound **13** (*RR*) (156 mg, 0.15 mmol), **3** (*R*)/**4** (*S*) (78 mg, 0.15 mmol, **3** (*R*)/**4** (*S*) = 75:25) and HOBt·H_2O (23 mg, 0.15 mmol) were dissolved in dry THF (10 mL), and EDC·HCl (29 mg, 0.15 mmol) was added under an Ar atmosphere. The mixture was stirred at room temperature and *N*-methylmorpholine (0.16 mL, 1.5 mmol) was added dropwise. After 21 h, the reaction was monitored by TLC (silica, EtOAc) to confirm the disappearance of the starting material, and the white precipitate was removed by filtration. The solvent was removed in vacuo, and the residue was dissolved with EtOAc (30 mL). The organic material was washed with 4% aq $NaHCO_3$ (20 mL), 10% aq citric acid (20 mL), and water (20 mL), dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (10:1 EtOAc–hexane→10:1 EtOAc–$CHCl_3$) to give ***RRR*** (**5**) as a colorless solid (R_f 0.325; 10:1 EtOAc–$CHCl_3$, 60 mg, 0.04 mmol) in 27% yield. ^{13}C NMR ($CDCl_3$, 75.5 Hz): δ 170.81, 170.68, 170.36, 170. 23, 169.71, 169.63, 169.44, 169.36 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.62 (NH*C*OO), 143.87, 141.25 (Fmoc-Ar, C), 127.74, 127.13, 125.14, 119.97 (Fmoc-Ar-1, 2, 3, 4), 82.24 ((CH$_3$)$_3$*C*), 75.79, 74.77, 74.17, 69.93, 68.54, 68.02, 66.77, 62.28, 61.87, 47.20, 46.52, 45.98, 40.97, 39.50, 28.10, 28.04 ((*C*H$_3$)$_3$C), 20.60 (COCH_3). ESIMS: Calcd for $C_{70}H_{89}N_3O_{33}Na$ ([M+Na]$^+$): 1522.53. Found: 1522.45.

4.11. *tert*-Butyl (2*R*)-3-{(2*S*)-3-{(2*R*)-3-[(9-fluorenylmethoxy)-carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (***RSR*** (**7**))

Compound ***RSR*** (**7**) was prepared by a method similar to the preparation of ***RRR*** (**5**) using **14** (*RS*) (156 mg, 0.15 mmol) and **3** (*R*)/**4** (*S*) (77 mg, 0.15 mmol, **3** (*R*) /**4** (*S*) = 72:28) in place of **13** (*RS*) and **3** (*R*)/**4** (*S*) (75:25). The residue was purified by silica gel column chromatography (4:1 EtOAc–$CHCl_3$) to give ***RSR*** (**7**) as a colorless solid (R_f 0.35; 4:1 EtOAc–$CHCl_3$, 77 mg, 0.051 mmol) in 34% and ***RSS*** (**18**) (R_f 0.35; 4:1 EtOAc–$CHCl_3$, 32 mg, 0.021 mmol) in 14% yield, respectively. ESIMS: Calcd for $C_{70}H_{89}N_3O_{33}Na$ ([M+Na]$^+$): 1522.53. Found: 1522.47.

4.12. *tert*-Butyl (2*S*)-3-{(2*R*)-3-{(2*S*)-3-[(9-fluorenylmethoxy)-carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (***SRS*** (**8**))

Compound ***SRS*** (**8**) was prepared by a method similar to the preparation of ***RRR*** (**5**) using **15** (*SR*) (104 mg, 0.10 mmol) and **4** (*S*) (51 mg, 0.10 mmol) in place of **13** (*RR*) and **3** (*R*)/**4** (*S*) (75:25). The residue was purified by silica gel column chromatography (10:1 EtOAc–hexane) to give ***SRS*** (**8**) (R_f 0.20; 10:1 EtOAc–hexane, 85 mg, 0.057 mmol) in 57% yield. ^{13}C NMR ($CDCl_3$, 75.5 Hz): δ 171.26, 170.97, 170.84, 170.75, 17049. 170.39, 170.08, 169.94, 160.78, 169.52, 169.32, 169.29, 169.18, 169.11 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.54 (NH*C*OO), 143.86, 141.17 (Fmoc-Ar, C), 127.61, 127.03, 125.14, 119.89 (Fmoc-Ar-1, 2, 3, 4), 81.50 ((CH$_3$)$_3$*C*), 77.24, 76.26, 75.95, 75.84, 74.61, 73.99, 70.24, 68.60,

68.38, 67.86, 66.81, 62.63, 62.05, 61.52, 47.96, 47.10, 46.47, 44.32, 39.82, 39.20, 35.08, 28.14((CH_3)$_3$C), 20.52 (COCH$_3$). ESIMS: Calcd for $C_{70}H_{89}N_3O_{33}Na$ ([M+Na]$^+$): 1522.53. Found: 1522.45.

4.13. *tert*-Butyl (2*S*)-3-{(2*S*)-3-{(2*S*)-3-[(9-fluorenylmethoxy)-carbonylamino]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoate (*SSS* (**6**))

Compound ***SSS*** (**6**) was prepared by a method similar to the preparation of ***RRR*** (**5**) using **16** (*SS*) (83 mg, 0.080 mmol) and **4** (*S*) (41 mg, 0.080 mmol) in place of **13** (*RR*) and **3** (*R*)/**4** (*S*) (75:25). The residue was purified by silica gel column chromatography three times (4:1 EtOAc–hexane (1st), 3:1 EtOAc–hexane (2nd), 2:1 EtOAc–$CHCl_3$ (3rd)) to give ***SSS*** (**6**) as a white powder (R_f 0.25; 2:1 EtOAc–$CHCl_3$, 75 mg, 0.050 mmol) in 63% yield. ^{13}C NMR ($CDCl_3$, 75.5 Hz): δ 171.01, 170.89, 170.57, 170.47, 170.37, 170.18, 169.58, 169.45, 169.32 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.46 (NH*C*OO), 143.94, 141.20 (Fmoc-Ar, C), 127.63, 127.03, 125.20, 119.89 (Fmoc-Ar-1, 2, 3, 4), 81.63 ((CH_3)$_3$*C*), 76.40, 74.61, 74.40, 69.38, 68.91, 68.55, 68.18, 66.77, 62.73, 62.33, 62.00, 48.39, 47.29, 47.13, 45.43, 39.67, 37.60, 35.66, 28.09 ((CH_3)$_3$C), 20.55 (COCH$_3$). ESIMS: Calcd for $C_{70}H_{89}N_3O_{33}Na$ ([M+Na]$^+$): 1522.53. Found: 1522.45.

4.14. *tert*-Butyl (2*R*)-3-{3-[(9-fluorenylmethoxy) carbonylamino]propanolyamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoate (19 (*R*)) and *tert*-butyl (2*S*)-3-{3-[(9-fluorenylmethoxy)carbonylamino]propanolyamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoate (20 (*S*))

Compounds **19** (*R*) and **20** (*S*) were prepared by a method similar to the preparation of ***RRR*** (**5**) using 3-[(9-fluorenylmethoxy)carbonylamino]propanoic acid (**Fmoc-β-Ala-OH**) (609 mg, 1.96 mmol) and **3** (*R*)/**4** (*S*) (1.00 g, 1.96 mmol, **3** (*R*)/**4** (*S*) = 74:26) in place of **13** (*RR*) and **3** (*R*)/**4** (*S*) (75:25). The residue was purified by silica gel column chromatography (4:1 EtOAc–hexane) to give **19** (*R*) as a colorless solid (R_f 0.50; EtOAc, 620 mg, 0.81 mmol) in 41% and **20** (*S*) as a colorless solid (R_f 0.60; EtOAc, 374 mg, 0.48 mmol) in 25% yield. For **19**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.77 (2H, d, *J* 7.0 Hz, Fmoc-Ar-4), 7.60 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.40 (2H, dd, *J* 7.0, 7.3 Hz, Fmoc-Ar-3), 7.31 (2H, dd, *J* 7.0, 7.3 Hz, Fmoc-Ar-2), 6.18 (1H, br, N*H*CO), 5.53 (1H, br, N*H*COO), 5.37 (1H, dd, *J* 8.8, 10.4 Hz, H-2), 5.15–5.02 (2H, m, H-4, H-3), 4.37 (2H, d, *J* 7.6 Hz, Fmoc-CH_2O), 4.23–4.11 (3H, m, Fmoc-C*H*, H-6), 3.71–3.60 (3H, m, H-5, H-1, H-βa), 3.50 (3H, m, β-Ala CH_2, H-βb), 2.76 (1H, m, H-α), 2.42 (2H, m, β-Ala CH_2), 2.05 (3H, s, COCH_3), 2.03 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.49 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.51, 170.62, 170.36, 169.36, 169.27 (*C*OCH$_3$, NH*C*O), 156.43 (NH*C*OO), 143.90, 141.25 (Fmoc-Ar, C), 127.64 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 125.07 (Fmoc-Ar-1), 119.93 (Fmoc-Ar-4), 82.42 ((CH_3)$_3$*C*), 77.44 (C-1), 75.92 (C-5), 74.64 (C-3), 69.65 (C-2), 67.96 (C-4), 66.68 (Fmoc-CH_2O), 61.79 (C-6), 47.18 (Fmoc-*C*H), 45.43 (C-α), 38.56 (C-β), 37.02 (β-Ala CH_2), 35.82 (β-Ala CH_2), 28.10, 28.01 ((CH_3)$_3$C), 20.61 (COCH_3). HRESIMS: Calcd for $C_{35}H_{40}N_2O_{14}Na$ ([M-Bu^t+H+Na]$^+$): 735.2377. Found: 791.2392. For **20**: ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.76 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.60 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.40 (2H, dd, *J* 7.1, 7.3 Hz, Fmoc-Ar-3), 7.31 (2H, dd, *J* 7.2, 7.3 Hz, Fmoc-Ar-2), 6.23 (1H, br, N*H*CO), 5.66 (1H, br, N*H*COO), 5.21 (1H, dd, *J* 9.5, 9.8 Hz, H-3), 5.10 (1H, dd, *J* 9.2, 9.5 Hz, H-2), 5.02 (1H, dd, *J* 9.5, 9.8 Hz, H-4), 4.41–4.19 (4H, m, Fmoc-CH_2O, Fmoc-C*H*, H-6a), 4.14–4.05 (2H, m, H-6b, H-1), 3.86 (1H, m, H-βa), 3.65 (1H, m, H-5), 3.47 (2H, m, β-Ala CH_2), 3.37 (1H, m, H-βb), 2.64 (1H, m, H-α), 2.41 (2H, m, β-Ala CH_2), 2.06 (3H, s, COCH_3), 2.05 (3H, s, COCH_3), 2.04 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.45 (9H, s, (CH_3)$_3$C). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 171.38, 170.80, 170.33, 170.15, 160.55 (*C*OCH$_3$, NH*C*O), 156.43 (NH*C*OO), 143.95, 141.23 (Fmoc-Ar, C), 127.59 (Fmoc-Ar-3), 127.00 (Fmoc-Ar-2), 125.10 (Fmoc-Ar-1), 119.89 (Fmoc-Ar-4), 81.92 ((CH_3)$_3$*C*), 77.24 (C-1), 76.65 (C-5), 74.30 (C-3), 69.17 (C-2), 68.65 (C-4), 66.65 (Fmoc-CH_2O), 62.08 (C-6), 47.18 (Fmoc-*C*H), 45.34 (C-α), 37.04 (β-Ala *C*H$_2$), 35.64 (C-β, β-Ala CH_2), 27.99, 27.93 ((CH_3)$_3$C), 20.60 (COCH_3). HRESIMS: Calcd for $C_{35}H_{40}N_2O_{14}Na$ ([M-Bu^t+H+Na]$^+$): 735.2377. Found: 735.2386.

4.15. (2*R*)-3-{3-[(9-Fluorenylmethoxy)carbonylamino]-propanolyamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoic acid (21 (*R*))

Compound **21** (*R*) was prepared by a method similar to the preparation of **13** (*RR*) using **19** (*R*) (325 mg, 0.42 mmol) in place of ***RR*** (**9**). The residue was purified by silica gel column chromatography (4:1→1:0 EtOAc–$CHCl_3$) to give **21** (*R*) as a colorless solid (R_f 0; 4:1 EtOAc–$CHCl_3$, 300 mg, 0.42 mmol) in 100% yield. ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.74 (2H, d, *J* 7.2 Hz, Fmoc-Ar-4), 7.58 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.38 (2H, dd, *J* 7.2, 7.5 Hz, Fmoc-Ar-3), 7.29 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-2), 6.80 (1H, br, N*H*CO), 5.79 (1H, br, N*H*COO), 5.29 (1H, dd, *J* 9.3, 9.5 Hz, H-2), 5.15 (1H, dd, *J* 9.3, 9.3 Hz, H-3), 5.06 (1H, dd, *J* 9.3, 9.8 Hz, H-4), 4.34 (2H, d, *J* 7.0 Hz, Fmoc-CH_2O), 4.21–4.11 (3H, m, Fmoc-C*H*, H-6), 5.29 (1H, dd, *J* 9.5, 9.5 Hz, H-1), 3.74–3.63 (2H, m, H-5, H-βa), 3.45–3.43 (3H, m, β-Ala CH_2, H-βb), 2.94 (1H, m, H-α), 2.45 (2H, m, β-Ala CH_2), 2.04 (3H, s, COCH_3), 2.01 (3H, s, COCH_3), 1.98 (3H, s, COCH_3), 1.97 (3H, s, COCH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 172.46, 172.17, 170.86, 170.33, 169.55, 169.44 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.78 (NH*C*OO), 143.76, 141.19 (Fmoc-Ar, C), 127.66 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 125.04 (Fmoc-Ar-1), 119.93 (Fmoc-Ar-4), 77.00 (C-1), 76.18 (C-5), 74.46 (C-3), 69.28 (C-2), 68.09 (C-4), 66.86 (Fmoc-CH_2O), 61.94 (C-6), 47.04 (Fmoc-*C*H), 45.32 (C-α), 37.63 (C-β), 37.25 (β-Ala *C*H$_2$), 35.87 (β-Ala *C*H$_2$), 20.68, 20.58, 20.47 (COCH_3). ESIMS: Calcd for $C_{35}H_{40}N_2O_{14}Na$ ([M+Na]$^+$): 735.24. Found: 735.20. HRESIMS: Calcd for $C_{35}H_{39}N_2O_{14}Na_2$ ([M−H+2Na]$^+$): 757.21967. Found: 757.22350.

4.16. (2*S*)-3-{3-[(9-Fluorenylmethoxy)carbonylamino]-propanolyamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoic acid (22 (*S*))

Compound **22** (*S*) was prepared by a method similar to the preparation of **13** (*RR*) using **20** (*S*) (167 mg, 0.22 mmol) in place of ***RR*** (**9**). The residue was purified by silica gel column chromatography (4:1 EtOAc–$CHCl_3$) to give **22** (*S*) as a colorless solid (R_f 0; 4:1 EtOAc–$CHCl_3$, 154 mg, 0.21 mmol) in 95% yield. ^{1}H NMR ($CDCl_3$, Me_4Si, 300 MHz): δ 7.74 (2H, d, *J* 7.3 Hz, Fmoc-Ar-4), 7.58 (2H, d, *J* 7.3 Hz, Fmoc-Ar-1), 7.38 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-3), 7.29 (2H, dd, *J* 7.3, 7.5 Hz, Fmoc-Ar-2), 6.83 (1H, br, N*H*CO), 5.90 (1H, br, Fmoc N*H*COO), 5.18 (1H, dd, *J* 9.2, 9.5 Hz, H-3), 5.10–5.01 (2H, m, H-2, H-4), 4.34–4.32 (2H, m, Fmoc-CH_2O), 4.21–4.08 (3H, m,, Fmoc-C*H*, H-6), 4.03 (1H, dd, *J* 4.9, 10.0 Hz, H-1), 3.80 (1H, m, H-βa), 3.63 (1H, m, H-5), 3.50–3.43 (2H, m, β-Ala CH_2, H-βb), 2.74 (1H, dd, *J* 10.4, 5.0 Hz, H-α), 2.44 (2H, m, β-Ala CH_2), 2.03 (3H, s, COCH_3), 2.01 (3H, s, COCH_3), 2.00 (3H, s, COCH_3), 1.98 (3H, s, COCH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 173.34, 172.45, 170.99, 170.20, 169.71, 169.39 (*C*OCH$_3$, *C*OOH, NH*C*O), 156.78 (NH*C*OO), 143.79, 141.17 (Fmoc-Ar, C), 127.66 (Fmoc-Ar-3), 127.01 (Fmoc-Ar-2), 125.07 (Fmoc-Ar-1), 119.93 (Fmoc-Ar-4), 77.00 (C-1), 76.82 (C-5), 74.12 (C-3), 69.87 (C-2), 68.26 (C-4), 66.84 (Fmoc-CH_2O), 61.74 (C-6), 47.02 (Fmoc-*C*H), 45.76 (C-α), 37.30 (β-Ala *C*H$_2$), 35.52 (C-β), 35.90 (β-Ala *C*H$_2$), 20.53, 20.47 (COCH_3). ESIMS: Calcd for $C_{35}H_{40}N_2O_{14}Na$ ([M+Na$^+$]):

735.24. Found: 735.21. HRESIMS: Calcd for $C_{35}H_{39}N_2O_{14}Na_2$ ([M-H+2Na]$^+$): 757.21967. Found: 757.22779.

4.17. (2*R*)-3-{3-{(2*R*)-3-{3-[(9-Fluorenylmethoxy) carbonylamino]propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanamide (A$^{\beta}$*RA*$^{\beta}$*R* (23))

Starting from the Fmoc-NH-SAL-MBHA resin (0.64 mmol g^{-1} resin, 94 mg), **A$^{\beta}$*RA*$^{\beta}$*R***-SAL-MBHA resin was prepared manually following Fmoc SPPS. Each synthetic cycle consisted of removal of the Fmoc groups with a 20% piperidine solution of 1-methyl-2-pyrrolidinone (NMP), washing with NMP (×5), coupling of **21** (*R*) (64 mg, 0.090 mmol), which was preactivated by mixing for 3 min with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (32 mg, 0.085 mmol), 1-hydroxybenzotriazole (HOBt) (12 mg, 0.090 mmol), and *N*,*N*-diisopropylethylamine (DIEA) (23.5 μL, 0.135 mmol) in a mixture of DMF (1.2 mL), washing with NMP (×3), end capping with an NMP solution containing 10% Ac_2O and 5% DIEA, and washing with NMP (×3). After each coupling step, the completion of the coupling reaction was confirmed by using a ninhydrin color test. The resin was successively washed with NMP and MeOH, and dried in vacuo to afford **A$^{\beta}$*RA*$^{\beta}$*R***-SAL-MBHA resin (139 mg). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (89 mg). To the resin in a test tube was added 95% aq TFA (1.5 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. Then the volatile materials in the mixture were evaporated in a stream of Ar. The residue was added to aq acetonitrile. The resin-containing suspension was passed through a glass filter, and the filtrate was freeze-dried to give crude peptide (39 mg). This crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*RA*$^{\beta}$*R*** (**23**) (10.6 mg, 23% overall yield). Lyophilization provided the product as a white, fluffy material. HRESIMS: Calcd for $C_{55}H_{69}N_5O_{24}Na$ ([M+Na]$^+$): 1206.42302. Found: 1206.43117.

4.18. (2*S*)-3-{3-{(2*S*)-3-{3-[(9-Fluorenylmethoxy)-carbonylamino]propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanamide (A$^{\beta}$*SA*$^{\beta}$*S* (24))

Starting from the Fmoc-NH-SAL-MBHA resin (0.64 mmol g^{-1} resin, 156 mg), **A$^{\beta}$*SA*$^{\beta}$*S***-SAL-MBHA resin (252 mg) was prepared by a method similar to the preparation of **A$^{\beta}$*RA*$^{\beta}$*R*** (**23**) using **22** (*S*) (107 mg (0.15 mmol) for each cycle) in place of **21** (*R*). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (88 mg). To the resin in a test tube was added 95% aq TFA (1.5 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. Then the volatile materials in the mixture were evaporated in a stream of Ar. The residue was added to aq acetonitrile. The resin-containing suspension was passed through a glass filter, and the filtrate was freeze-dried to give crude peptide (36 mg). This crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*SA*$^{\beta}$*S*** (**24**) (12 mg, 28% overall yield). Lyophilization provided the product as a white, fluffy material. MALDI-TOFMS: Calcd for $C_{55}H_{69}N_5O_{24}Na$ ([M+Na]$^+$): 1206.42. Found 1206.39.

4.19. (2*R*)-3-{3-{(2*R*)-3-{3-{(2*R*)-3-{3-[(9-Fluorenylmethoxy)-carbonylamino]propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanamide (A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R* (25))

Starting from the Fmoc-NH-SAL-MBHA resin (0.64 mmol g^{-1} resin, 156 mg), **A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R***-SAL-MBHA resin (281 mg) was prepared by a method similar to the preparation of **A$^{\beta}$*RA*$^{\beta}$*R*** (**23**) using **21** (*R*) (107 mg (0.15 mmol) for each cycle). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (154 mg). To the resin in a test tube was added 95% aq TFA (1.5 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. Then the volatile materials in the mixture were evaporated in a stream of Ar. The residue was added to aq acetonitrile. The resin-containing suspension was passed through a glass filter, and the filtrate was freeze-dried to give crude peptide (78 mg). This crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R*** (**25**) (12 mg, 11% overall yield). Lyophilization provided the product as a white, fluffy material. HPLC (5C18-AR-II (10 × 250 mm); gradient MeCN 30–50%, 20 min) rt: 17.1 min. MALDI-TOFMS: Calcd for $C_{75}H_{97}N_7O_{35}K$ ([M+K]$^+$): 1694.56. Found 1694.07.

4.20. (2*S*)-3-{3-{(2*S*)-3-{3-{(2*S*)-3-{3-[(9-Fluorenylmethoxy)-carbonylamino]propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino} propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanamide (A$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*S* (26))

Starting from the Fmoc-NH-SAL-MBHA resin (0.64 mmol g^{-1} resin, 156 mg), **A$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*S***-SAL-MBHA resin (292 mg) was prepared by a method similar to the preparation of **A$^{\beta}$*RA*$^{\beta}$*R*** (**23**) using **22** (*S*) (107 mg (0.15 mmol) for each cycle) in place of **21** (*R*). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (99 mg). To the resin in a test tube was added 95% aq TFA (1.5 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. Then the volatile materials in the mixture were evaporated in a stream of Ar. The residue was added to aq acetonitrile. The resin-containing suspension was passed through glass filter, and the filtrate was freeze-dried to give crude peptide (49 mg). This crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*SA*$^{\beta}$-*SA*$^{\beta}$*S*** (**26**) (12 mg, 20% overall yield). ^{13}C NMR(CD_3CN): δ 174.29, 173.21, 173.11, 172.63, 172.19, 172.03, 171.61, 171.50, 170.99, 170.62 ($COCH_3$, *C*OOH, NHCO), 157.44 (NHCOO), 145.27, 142.18 (Fmoc-Ar, C), 128.72, 128.18, 126.19, 121.00 (Fmoc-Ar-4,3,2,1), 77.14, 76.89, 76.71, 76.47, 74.98, 72.44, 72.20, 71.81, 69.55, 69.45, 69.96, 63.03, 49.31, 49.14, 48.19, 47.98, 38.61, 37.19, 37.06, 21.14, 21.01, 20.91 ($COCH_3$). Lyophilization provided the product as a white, fluffy material. HPLC (5C18-AR-II (10 × 250 mm); gradient MeCN 30–50%, 20 min) rt: 20.0 min. MALDI-TOFMS: Calcd for $C_{75}H_{99}N_7O_{35}$ ([M+H]$^+$): 1656.61. Found 1656.00.

4.21. (2*R*)-3-{3-{(2*R*)-3-{3-{(2*R*)-3-{3-{(2*R*)-3-{3-[(9-Fluorenylmethoxy)carbonylamino]-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanamide (A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R* (27))

Starting from the Fmoc-NH-SAL resin (0.65 mmol g^{-1} resin, 92 mg), **A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R***-SAL resin (193 mg) was prepared by a method similar to the preparation of **A$^{\beta}$*RA*$^{\beta}$*R*** (**23**) using **21** (*R*) (64 mg (0.090 mmol) for each cycle). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (99 mg). To the resin in a test tube was added 95% aq TFA (1.2 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. Then the volatile materials in the mixture were evaporated in a stream of Ar. The residue was added to aq acetonitrile. The resin-containing suspension was passed through a glass filter, and the filtrate was freeze-dried to give crude peptide (73 mg). This

crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R*** (**27**) (16 mg, 25% overall yield). Lyophilization provided the product as a white, fluffy material. HPLC (5C18-AR-II (10 × 250 mm); gradient MeCN 30–50%, 20 min) rt: 15.1 min. MALDI-TOFMS: Calcd for $C_{95}H_{125}N_9O_{46}Na$ ([M+Na]$^+$): 2150.76. Found 2150.51.

4.22. (2*S*)-3-{3-{(2*S*)-3-{3-{(2*S*)-3-{3-{(2*S*)-3-{3-[(9-Fluorenylmethoxy)carbonylamino]-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-propanoylamino}-propanoylamino}-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)propanamide (A$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*S* (28))

Starting from the Fmoc-NH-SAL resin (0.65 mmol g^{-1} resin, 92 mg), **A$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*S***-SAL resin (204 mg) was prepared by a method similar to the preparation of **A$^{\beta}$*RA*$^{\beta}$*RA*$^{\beta}$*R*** (**23**) using **22** (*S*) (64 mg (0.090 mmol) for each cycle) in place of **21** (*R*). Experiments to isolate the synthetic glycopeptide were undertaken with a part of the resin (102 mg). To the resin in a test tube was added 95% aq TFA (1.2 mL), and the mixture was stirred for 2 h. Ether was added to the reaction mixture. The precipitate that formed was washed with ether three times and dissolved in aq acetonitrile. The resin-containing suspension was passed through a glass filter, and the filtrate was freeze-dried to give crude peptide (68 mg). This crude material was purified by RP-HPLC (column: Cosmosil 5C18-AR-II (10 × 250 mm) to give **A$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*SA*$^{\beta}$*S*** (**28**) (24 mg, 37% overall yield). ^{13}C NMR (CD_3CN): δ 174.18, 173.10, 172.66, 172.50, 172.39, 171.46, 170.99, 170.59 (*C*OCH$_3$, *C*OOH, NH*C*O), 157.45 (NH*C*OO), 145.27, 142.16 (Fmoc-Ar, C), 128.72, 128.15, 126.19, 121.00 (Fmoc-Ar-4,3,2,1), 76.91, 74.91, 69.43, 66.94, 62.99, 49.18, 48.16, 38.79, 37.28, 22.87, 20.91 (CO*C*H$_3$). Lyophilization provided the product as a white, fluffy material. HPLC (5C18-AR-II (10 × 250 mm); gradient MeCN 30 to 50%, 20 min) RT: 16.8 min. MALDI-TOFMS: Calcd for $C_{95}H_{125}N_9O_{46}Na$ ([M+Na]$^+$): 2150.76. Found 2150.73.

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