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New prototypical O-linked-glycopeptidomimetics corresponding to the linkage region of proteoglycans

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Abstract

A new class of glycopeptidomimetic composed of N-substituted oligoglycine (peptoid) mimicking the β-D-Xyl-(1 → 3)-*O*-L-Ser linkage region of proteoglycans was synthesized using a convergent approach. Reiterative N-alkylation–N-bromoacetylation reactions were used starting from *tert*-butyl bromoacetate (**1**) and isopropylamine. Perbenzoylated 2-aminoethyl β-D-xylopyranoside **10** constituted the key glycan L-Ser mimic. © 1997 Elsevier Science Ltd. All rights reserved.

Keywords: D-Xylose; Oligoglycine; Peptoid; Peptidomimetic; Proteoglycan

1. Introduction

Mammalian tissues are made of proteoglycans (PGs) composed of glycosaminoglycan repeating units generally anchored to core polypeptide backbones through a tetrasaccharide ending with β-D-Xyl-(1 → 3)-*O*-L-Ser/Thr sequences [1,2]. In initial biosynthetic studies, it was thought that specific amino acid recognition patterns of the peptide backbones were necessary for the attachment of Ser/Thr to the connecting β-D-xylose residues. It was later found that many different amino acid sequences could be recognized by the xylosyltransferases. Consequently, conformational arguments were raised to explain the regioselectivity of the glycosylation. In light of these discrepancies, it is of interest to construct glycopeptide libraries. One such strategy, which also includes the possibility of glycopeptidomimetic libraries of potential therapeutic value, is to synthesize conformationally flexible mimetics based on recently described N-substituted oligoglycine modules (peptoids) [3–5]. A schematic representation showing the structural analogies between glycopeptides and glycopeptoids is illustrated in Fig. 1.

2. Results and discussion

As an extension of ongoing activities on the syntheses of conformationally flexible N- [6–8] and O-linked [9] glycopeptidomimetics, we describe herein the synthesis of a glyco pentapeptoid **22** corresponding to part of the linkage region of human bone and cartilage proteoglycans [2]. To this end, we chose the sequence Val–Phe–Ser-(β-D-Xyl)–Glu–Ala as a prototype glycopeptidomimetic.

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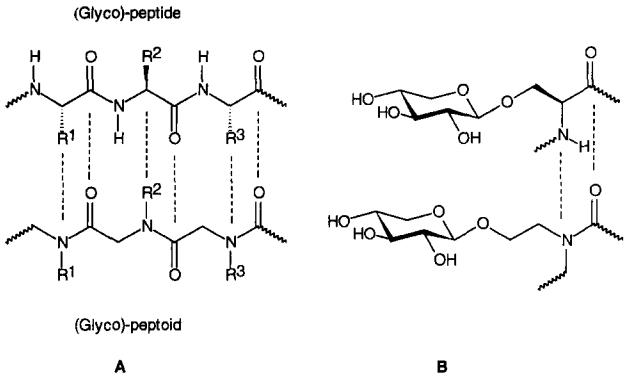
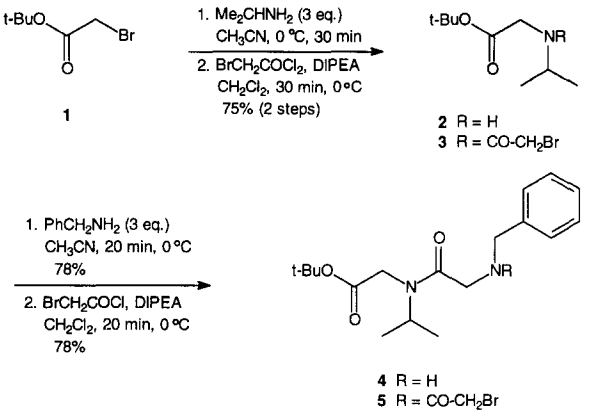


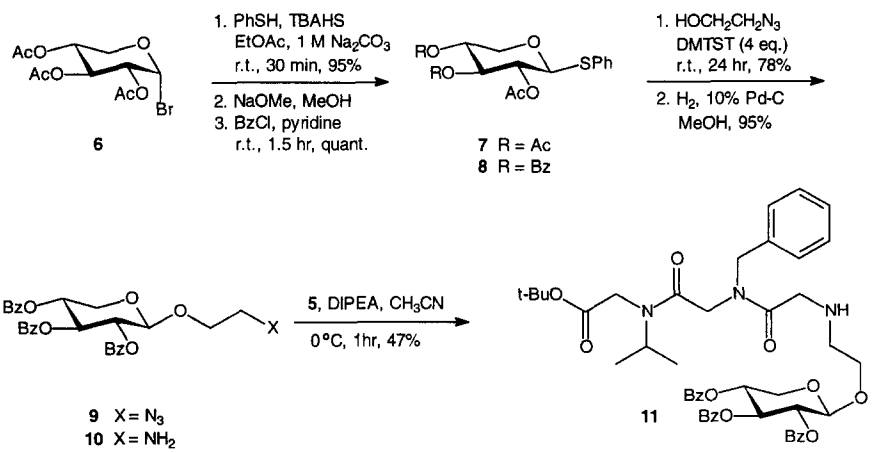
Fig. 1. (A) Comparison of (glyco)-peptide and N-substituted oligoglycine (NSG) sequences. (B) Typical β-D-Xylose-(1 → 3)-L-Ser linkage and its O-linked homoserine 'glycopeptidomimetic'.



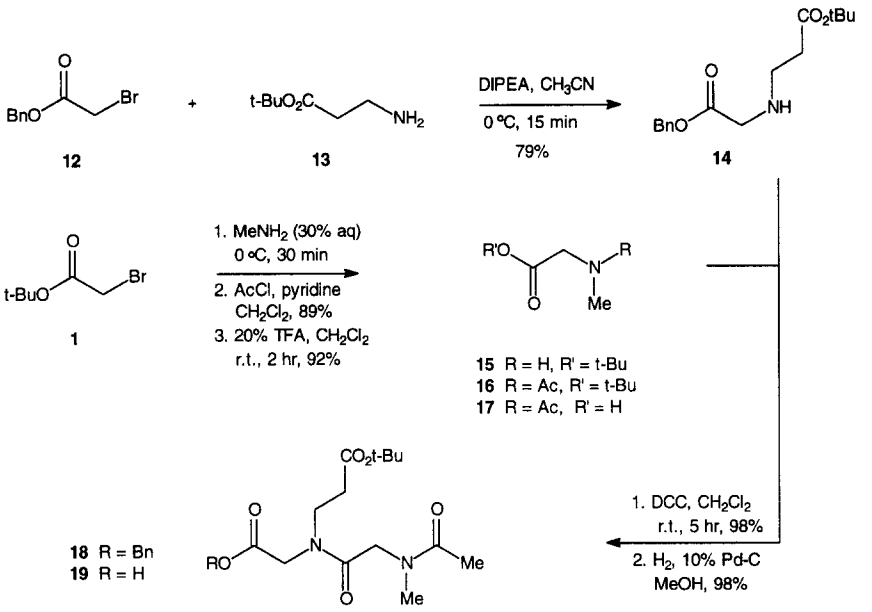
Scheme 1.

The left-hand N-terminus of the N-substituted glycylglycine portion of the peptidomimetic corresponding to Val–Phe was prepared from *tert*-butyl bromoacetate (**1**) by a series of reiterative N-alkylation (Me_2CHNH_2, then $PhCH_2NH_2$) and bromoacetylation ($BrCH_2COCl$, DIPEA, CH_2Cl_2, 20–30 min, 0 °C) sequences as described in Scheme 1. The resulting secondary amine **4** was obtained in 59% yield for the three steps. It was shown to exist as slow equilibrating rotamers in a ratio of 1:1.6 as measured from the relative integration of the two *tert*-butyl signals shown at 1.39 and at 1.43 ppm in its ^1H NMR spectrum. (Note: For dipeptoids and higher homologues, 2^n slow equilibrating rotamers, where n represents the number of secondary amide bonds, are possible.) These early observations allowed us to speculate that peptoid resulting from such a strategy would offer distinct advantages to probe large conformational spaces in potential receptors. N-Bromoacetylation of **4** provided **5** in 78% yield, which was shown to exist as four rotamers in a ratio of 1.3:1.7:1.1:1.0 (Scheme 1).

The synthesis of the key glycan portion, 2-azidoethyl β-D-xylopyranoside homoserine mimic **9**, together with its attachment to N-bromoacetylglycylglycinate derivative **5** to give **11**, are illustrated in Scheme 2. Due to difficulties encountered in the direct Koenigs–Knorr glycosylation of tri-*O*-acetyl-α-D-xylopyranosyl bromide (**6**) [10] with 2-azidoethanol [11], we chose to synthesize 2-azidoethyl β-D-xylopyranoside **9** using thioglycoside chemistry. To this end, bromide **6** was stereospecifically transformed into the known [12] phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (**7**) in 95% yield using phase-transfer catalyzed glycosylation developed in this group (PhSH, TBAHS, EtOAc, M Na_2CO_3, r.t., 30 min) [13–15]. As peracetylated phenyl-1-thio-xylopyranoside **7** was prone to orthoester formation, it was further transformed into its perbenzoylated derivative **8** in a two-step sequence involving Zemplén O-deacetylation and O-benzoylation in pyridine



Scheme 2.

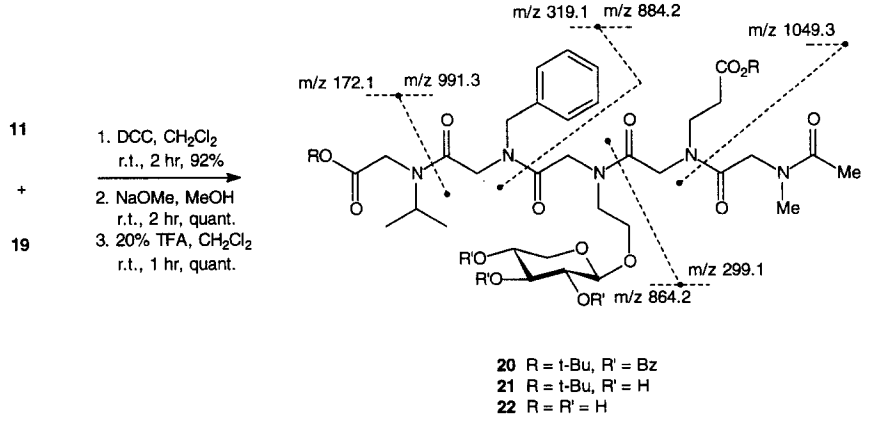


Scheme 3.

(quant). Glycosidation of **8** with azidoethanol using dimethyl(methylthio)-sulfonium triflate (DMTST) in dichloromethane occurred in 78% yield. Hydrogenation of azide **9** into amine **10** (H_2, 10% Pd–C, MeOH, 95%) and coupling to fragment **5** (DIPEA, CH_3CN) afforded peptoid-block **11** in 47% yield (Scheme 2).

The synthesis of the right-hand side of the molecule corresponding to the C-terminal Glu–Ala mimetic is illustrated in Scheme 3. Benzyl bromoacetate (**12**) was used to N-alkylate β-alanine *tert*-butyl ester (**13**) to provide glutamic acid mimic **14** in 79% yield. The *N*-acetyl-Ala mimic **16** was obtained by treating **1** with methylamine (30% aq) to give *tert*-butyl *N*-methylglycinate **15** which was transformed (AcCl, pyridine, CH_2Cl_2) into **16** in 89% yield for the two steps. The ^1H NMR spectrum of **16** showed it as a mixture of rotamers in a 1:2.4 ratio, as judged from the relative integration of the two *tert*-butyl signals at 1.34 and 1.36 ppm, respectively. Hydrolysis of the *tert*-butyl ester of **16** using 20% trifluoroacetic acid (TFA) in CH_2Cl_2 provided **17** in 92% yield. Coupling of secondary amine **14** with acid **17** using DCC afforded fragment **18** in 98% yield. Hydrogenolysis (H_2, 10% Pd–C, MeOH) of the benzyl ester furnished acid **19** in 98% yield (Scheme 3).

Final coupling of amine **11** and acid **19** was also accomplished with DCC (CH_2Cl_2, r.t., 2 h) to provide protected xylopeptoid **20** in 92% yield (Scheme 4). Mild deprotection of the benzoate group of the xylose moiety using Zemplén conditions (NaOMe, MeOH) gave derivative **21**, which, after treatment



Scheme 4. Xylopeptoid **20** and its positive-ion FABMS fragmentation pattern.

with 20% TFA in CH_2Cl_2, afforded final compound **22** in quantitative yield for the two steps.

In conclusion, an efficient and convergent approach toward a new prototype glycopeptidomimetic was illustrated by the synthesis of an N-substituted oligoglycine (peptoid)-bearing a Val–Phe–Ser-(β-D-Xyl)–Glu–Ala mimic unit corresponding to part of the linkage region of human proteoglycans. Work is now in progress to evaluate the biological and conformational properties of this new class of compounds (Scheme 4).

3. Experimental

General methods.—Melting points were determined on a Gallenkamp apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 500 instrument or a Varian Gemini 200 MHz. IR spectra were run on a Bomen Michelson FT-IR instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and were run at 23 °C for 1% solutions in chloroform unless stated otherwise. Elemental analyses were performed by the analytical services of the Department of Chemistry of the University of Ottawa. Mass spectra were recorded on a VG 7070-E spectrometer (CI, ether) and a Kratos Concept IIH for FABMS. Thin layer chromatography (TLC) was performed on pre-coated Silica Gel 60 F_{254} plates and column chromatography on Silica Gel 60 (231–400 mesh, E. Merck No. 9385). All solvents and reagents were reagent grade and were used without further purification.

tert-*Butyl* N-*isopropylglycinate* (**2**).—*tert*-Butyl bromoacetate (1.0 g, 5.13 mmol) in CH_3CN (10 mL) was added dropwise to a solution of diisopropylethylamine (DIPEA) (0.91 g, 15.4 mmol) in CH_3CN (10 mL) at 0 °C. The solution was stirred at that temperature for 30 min. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2. The solution was washed with water (30 mL × 2) and the organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum to give crude compound **2**, which was used for the next step without further purification: ^1H NMR ($CDCl_3$): δ 0.90 (d, 6 H, *J* 6.2 Hz, CMe_2), 1.31 (s, 9 H, CMe_3), 1.45 (bs, 1 H, NH), 2.62 (m, 1 H, C*H*Me_2), 3.14 (s, 2 H, $COCH_2$).

tert-*Butyl* N-*bromoacetyl*-N-*isopropylglycinate* (**3**).—To a solution of **2** and diisopropylethylamine (5.13 mmol) in CH_2Cl_2 (20 mL) was added dropwise bromoacetyl chloride (0.81 g, 5.13 mmol). The solution was stirred at 0 °C for 30 min, after which time it was washed with water (15 mL), 5% aq HCl and 5% aq $NaHCO_3$. The organic phase was dried over anhydrous Na_2SO_4, concentrated, and purified by silica gel column chromatography using 4:1 hexane–ethyl acetate (R_f 0.28) as eluant to give pure compound **3** as a colorless oil (1.13 g, 3.85 mmol) in 75% yield for the two steps; CIMS: *m/z* 294.0 (M + 1, 73.3%); ^1H NMR ($CDCl_3$): δ 1.05, 1.21 (2d, 6 H, *J* 6.9 Hz, CHC*Me*$_2$), 1.43, 1.45 (2s, 9 H, CMe_3), 3.77–4.00 (m, 4 H, 2 × CH_2), 4.12, 4.77 (2m, 1 H, C*H*Me_3); ratio of rotamers = 1:1.6.

tert-*Butyl* (N-*phenylglycyl*)-N-*isopropylglycinate* (**4**).—Benzylamine (368 mg, 3.43 mmol) was added to a solution of **3** (336 mg, 1.14 mmol) in CH_3CN (15 mL) at 0 °C, and the solution was stirred for 20 min. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2, washed with water (15 mL), satd $NaHCO_3$ (15 mL), and dried over anhydrous Na_2SO_4. Silica gel column chromatography of the residue using 96:4 CH_2Cl_2–MeOH (R_f 0.44) afforded **4** (658 mg, 2.06 mmol) as a colorless oil in 78% yield: ^1H NMR ($CDCl_3$): δ 1.05, 1.11 (2d, 6 H, *J* 6.9 Hz, CH*Me*$_2$), 1.39, 1.43 (2s, 9 H, CMe_3), 2.32 (bs, 1 H, NH), 3.26–3.82 (m, 6 H, 3 × CH_2), 3.94, 4.86 (2m, 1 H, C*H*Me_2), 7.16–7.41 (m, 5 H, Ph).

tert-*Butyl* (N-*bromoacetyl*-N-*phenylglycyl*)-N-*isopropylglycinate* (**5**).—Diisopropylethylamine (121 mg, 936 μmol) was added to a solution of **4** (250 mg, 780 μmol) in CH_2Cl_2 (10 mL). Then, bromoacetyl chloride (147 mg, 936 μmol) was added dropwise at 0 °C and the resulting solution was stirred at that temperature for 20 min. The reaction mixture was washed with 5% HCl (10 mL), satd $NaHCO_3$ (10 mL), and water (10 mL). The dried organic solution (anhydrous Na_2SO_4) was concentrated under vacuum and the residue was purified by silica gel column chromatography using 2:3 ethyl acetate–hexane (R_f 0.36) as eluant to give **5** (267 mg, 605 μmol) as a colorless oil in 78% yield: CIMS gave *m/z* (ion, relative intensity) for $C_{20}H_{29}BrN_2O_4$: 441 (M + 1, 10.0%), 385 (M + 1 − *t*-Bu, 37.4%), 268 (M + 1 − *t*-$BuO_2CCH_2NCHMe_2$, 100%); ^1H NMR ($CDCl_3$): δ 1.02, 1.03, 1.11 (3d, 6 H, *J* 6.9 Hz, CH*Me*$_2$), 1.31, 1.42, 1.43 (3s, 9 H, CMe_3), 3.63–4.90 (m, 9 H, 4 × CH_2, C*H*Me_2), 7.20–7.37 (m, 5 H, Ph).

*Phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-*D*-xylopyranoside* (**7**).—To a solution of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (**6**) (0.50 g, 1.47 mmol) and tetrabutylammonium hydrogen sulfate (TBAHS) (0.50

g, 1.47 mmol) in ethyl acetate (5 mL) was added thiophenol (0.49 g, 4.42 mmol) in M sodium carbonate (5 mL). The reaction mixture was vigorously stirred at room temperature for 40 min. Ethyl acetate (15 mL) was added, and the organic phase was separated from the aq phase. The organic solution was washed with M NaOH (20 mL), water (20 mL × 2), and brine (15 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The crude compound was purified by silica gel column chromatography to give the title compound **7** (0.53 g, 1.43 mmol) in 95% yield: mp 77.6–77.9 °C; $[\alpha]_D$ −54.9° (*c* 1.0 $CHCl_3$); Lit. [12] mp 79–80 °C, $[\alpha]_D$ −55° (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.02 (s, 6 H, 2 × OAc), 2.07 (s, 3 H, OAc), 3.40 (dd, 1 H, $J_{5a,4}$ 8.8 Hz, H-5a), 4.26 (dd, 1 H, $J_{5e,4}$ 4.9 Hz, $J_{5e,5a}$ 11.8 Hz, H-5e), 4.78 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.90 (m, 1 H, H-4), 4.92 (t, 1 H, $J_{2,3}$ 8.2 Hz, H-2), 5.16 (t, 1 H, $J_{3,4}$ 8.2 Hz, H-3), 7.28–7.30 (m, 3 H, *o,p*-Ar), 7.44–7.45 (m, 2 H, *m*-Ar); ^{13}C NMR ($CDCl_3$): δ 20.7 (3 × COMe), 65.2 (C-5), 68.4 (C-4), 69.8 (C-2), 72.0 (C-3), 86.3 (C-1), 128.2 (*o*-C_{Ar}), 129.0 (*p*-C_{Ar}), 132.2 (*ipso*-C_{Ar}), 132.7 (*m*-C_{Ar}); Anal. Calcd for $C_{17}H_{20}O_7S$: C, 55.42; H, 5.48. Found: C, 55.55; H, 5.43.

*Phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-*D*-xylopyranoside* (**8**).—Phenyl 1-thio-β-D-xylopyranoside was obtained from phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranoside (**7**) under the usual Zemplén conditions (NaOMe, MeOH, r.t., 4 h). To a solution of phenyl 1-thio-β-D-xylopyranoside (1.97 g, 8.15 mmol) in pyridine (10 mL) at 0 °C was added dropwise benzoyl chloride (5.16 g, 36.7 mmol). The solution was then stirred for 1.5 h at room temperature. The reaction mixture was then treated with ice-water (15 mL) for 1 h to decompose the excess benzoyl chloride. The organic phase was separated and washed with satd $NaHCO_3$ (20 mL × 2), water (20 mL × 2), and brine (20 mL). The organic phase was dried over anhydrous Na_2SO_4, concentrated under vacuum, and the residue was purified by silica gel column chromatography using 4:1 hexane–ethyl acetate as eluant. Compound **8** (3.96 g, 7.16 mmol) was obtained as a white solid in 88% yield: mp 60.5–61.6 °C; $[\alpha]_D$ −29.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.81 (dd, 1 H, $J_{5a,5e}$ 12.2 Hz, $J_{5a,4}$ 6.6 Hz, H-5a), 4.70 (dd, 1 H, $J_{5e,4}$ 3.9 Hz, H-5e), 5.27 (d, 1 H, $J_{1,2}$ 6.2 Hz, H-1), 5.26–5.30 (m, 1 H, H-4), 5.45 (t, 1 H, $J_{2,3}$ 6.3 Hz, H-2), 5.77 (t, 1 H, $J_{3,4}$ 6.6 Hz, H-3), 7.18–7.57 (m, 6 H, *o*-Ar), 7.95–8.09 (m, 14 H, Ar); ^{13}C NMR ($CDCl_3$): δ 64.2 (C-5), 69.3 (C-4), 70.6 (C-2), 71.0 (C-3), 87.0 (C-1), 128.7, 129.0, 129.5, 129.7, 129.8, 130.5, 130.6, 130.8, 133.2, 133.7, 134.0, 134.1 (Ar), 165.7, 165.8, 166.1 (CO). The product was used directly in the next step.

*2-Azidoethyl 2,3,4-tri-O-benzoyl-β-*D*-xylopyranoside* (**9**).—To the solution of phenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-xylopyranoside (**8**) (1.0 g, 1.80 mmol) and 2-azidoethanol (0.47 g, 5.40 mmol) in CH_2Cl_2 containing 4 Å molecular sieves (3.0 g) was added DMTST (1.86 g, 7.22 mmol) at 0 °C under nitrogen. The reaction mixture was stirred for 7 h at 0 °C and for another 18 h at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then filtered through a Celite pad and the filtrate was concentrated under vacuum. The crude residue was purified by silica gel column chromatography using 1:4 ethyl acetate–hexane as eluant to give **9** (0.74 g, 1.40 mmol) as a white solid in 78% yield: mp 115.0–115.5 °C; $[\alpha]_D$ −38.8° (*c* 0.34, $CHCl_3$); CIMS gave *m/z* for $C_{28}H_{25}N_3O_8$: 532 ($[M + 1]^+$, 0.9%), 504 ($[M + 1 - N_2]^+$, 7.7%), 445 ($[M + 1 - OCH_2CH_2N_3]^+$, 55.3%); FTIR ($CHCl_3$) ν 2105 cm^{-1} (N_2); ^{1}H NMR ($CDCl_3$): δ 3.36–3.41, 3.44–3.49 (m, 2 H, CH_2N_3), 3.69–3.73, 3.99–4.03 (m, 2 H, OCH_2), 3.73 (dd, 1 H, $J_{5a,5e}$ 12.2 Hz, $J_{5a,4}$ 6.7 Hz, H-5a), 4.46 (dd, 1 H, $J_{5e,4}$ 4.2 Hz, H-5e), 4.89 (d, 1 H, $J_{1,2}$ 5.2 Hz, H-1), 5.28–5.31 (m, 1 H, H-4), 5.38 (dd, 1 H, $J_{2,3}$ 7.1 Hz, H-2), 5.75 (t, 1 H, $J_{3,4}$ 7.0 Hz, H-3), 7.30–7.38 (m, 6 H, *m*-Ar), 7.47–7.54 (m, 3 H, *p*-Ar), 7.94–8.00 (m, 6 H, *o*-Ar); ^{13}C NMR ($CDCl_3$): δ 50.7 (CH_2N_3), 61.2 (C-5), 67.6 (OCH_2), 69.0 (C-4), 70.0 (C-2, C-3), 100.0 (C-1), 128.33, 128.40, 128.43 (3 × *m*-C_{Ar}), 129.12, 129.24, 129.29 (3 × *ipso*-C_{Ar}), 129.89, 129.91 (3 × *o*-C_{Ar}), 133.27, 133.37, 133.40 (3 × *p*-C_{Ar}), 165.17, 165.36, 165.56 (3 × CO).

*2-Aminoethyl 2,3,4-tri-*O*-benzoyl-β-*D*-xylopyranoside* (**10**).—A solution of 2-azidoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside (**9**) (0.53 g, 1.00 mmol) in MeOH (5 mL) was added to a suspension of 10% Pd–C (0.10 g) in methanol (15 mL). Nitrogen was bubbled into the solution for 5 min and then hydrogen overnight. The suspension was filtered through a Celite pad, and the filtrate was concentrated under vacuum to provide **10** (0.48 g, 0.95 mmol) in 95% yield: FABMS gave *m/z* for $C_{28}H_{27}NO_8$: 506 ($[M + 1]^+$, 1.1%), 446 ($[M + 1 - OCH_2CH_2NH_2]^+$, 1.1%); ^{1}H NMR ($CDCl_3$): δ 1.56 (bs, 2 H, NH_2), 2.81–2.90 (m, 2 H, CH_2N), 3.54–3.58 (m, 1 H, OCH_2), 3.69 (dd, 1 H, $J_{5a,5e}$ 12.1 Hz, $J_{5a,4}$ 7.4 Hz, H-5a), 3.87–3.91 (m, 1 H, OCH_2), 4.42 (dd, 1 H, $J_{5e,4}$ 4.4 Hz, H-5e), 4.83 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 5.28–5.32 (m, 1 H, H-4), 5.38 (dd, 1 H, $J_{2,3}$ 7.6 Hz, H-2), 5.76 (t, 1

H, $J_{3,4}$ 7.5 Hz, H-3), 7.32–7.40 (m, 6 H, *m*-Ar), 7.45–7.53 (m, 3 H, *p*-Ar), 7.94–8.03 (m, 6 H, *o*-Ar); ^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2N), 61.5 (C-5), 69.3 (C-4), 70.5 (C-2, C-3), 71.8 (OCH_2), 100.5 (C-1), 128.4 (*m*-C_{Ar}), 129.1 (*ipso*-C_{Ar}), 129.8 (*o*-C_{Ar}), 133.4 (*p*-C_{Ar}), 165.2, 165.4, 165.6 (3 × CO).

N-*Alkylation of* **10** with **5** to give **11**.—To a solution of dipeptoid unit **5** (154 mg, 350 μmol) and diisopropylethylamine (54 mg, 420 μmol) in CH_3CN (15 mL) was added 2-aminoethyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside (**10**) (212 mg, 420 μmol). The solution was stirred for 1 h at 0 °C. The reaction mixture was then concentrated, and the residue was purified by silica gel column chromatography using a mixture of 18:1:1 $CHCl_3$–MeOH–MeCN (R_f 0.40) as eluant to give **11** (152 mg, 175 μmol) as a white foam in 47% yield: positive-ion FABMS gave *m/z* for $C_{48}H_{55}N_3O_{12}$: 866 ([M + 1]$^+$, 19.7%), 445 (M$^+$ − aglycon, 2.0%); ^{1}H NMR ($CDCl_3$): δ 1.04, 1.12, 1.13 (3d, 6 H, *J* 6.9 Hz, CH*Me*$_2$), 1.34, 1.39, 1.44 (3s, 9 H, CMe_3), 2.08 (bs, 1 H, NH), 2.83, 2.91 (2dd, 2 H, *J* 5.5 Hz, 2.0 Hz, NCH_2CH_2OXyl), 3.38–4.21 (m, 10 H), 4.41 (dd, 1 H, $J_{5a,5e}$ 12.1 Hz, $J_{5e,4}$ 4.2 Hz, H-5e), 4.58–4.61 (m, 2 H), 4.85 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1), 5.26 (m, 1 H, H-4), 5.32 (t, 1 H, $J_{2,3}$ 7.1 Hz, H-2), 5.72 (t, 1 H, $J_{3,4}$ 7.3 Hz, H-3), 7.17–7.40 (m, 11 H, *m*-Ar, Ph), 7.44–7.53 (m, 3 H, *p*-Ar), 7.93–8.00 (m, 6 H, *o*-Ar).

Benzyl N-*[2-(*tert-*butyloxycarbonyl)ethyl]glycinate* (**14**).—To a solution of β-alanine *tert*-butyl ester hydrochloride (**13**) (825 mg, 4.54 mmol) in CH_3CN was added diisopropylethylamine (1.47 g, 11.35 mmol). The resulting solution was stirred for 10 min, and then benzyl bromoacetate (**12**) (800 mg, 3.49 mmol) was added. The reaction mixture was then stirred for 15 min at 0 °C. The concentrated solution was purified by chromatography as above using 3:2 hexane–ethyl acetate (R_f 0.28) as eluant. Compound **14** (813 mg, 2.77 mmol) was obtained as a colorless oil in 79% yield: ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9 H, CMe_3), 1.84 (bs, 1 H, NH), 2.40 (t, 2 H, *J* 6.5 Hz, CH_2CO), 2.83 (t, 2 H, *J* 6.5 Hz, NCH_2), 3.44 (s, 2 H, $COCH_2N$), 5.15 (s, 2 H, $PhCH_2O$), 7.33–7.35 (m, 5 H, Ph).

tert-*Butyl* N-*acetyl*-N-*methylglycinate* (**16**).—*tert*-Butyl bromoacetate (**1**) (1.0 g, 5.13 mmol) in CH_3CN (10 mL) was added dropwise to a solution of methylamine (30% w/w in water, 2.4 g, 15.4 mmol) in CH_3CN. The reaction was allowed to proceed at 0 °C for 30 min. The solution was concentrated, and the residue was diluted with CH_2Cl_2. The organic solution was washed with water, dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give crude intermediate **15**, which was used for the next step without further purification. To a solution of **15** and diisopropylethylamine in CH_2Cl_2 (10 mL) was added acetyl chloride (0.40 g, 5.13 mmol) in CH_2Cl_2 (5 mL). The solution was then stirred for 30 min at 0 °C. The reaction mixture was washed with water, 5% aq HCl, and satd $NaHCO_3$. The organic solution was dried over Na_2SO_4 and concentrated. Purification of the residue by silica gel column chromatography gave **16** as a white solid in 89% yield: ^{1}H NMR ($CDCl_3$): δ 1.34, 1.36 (2s, 9 H, CMe_3), 1.91, 2.01 (2s, 3 H, $COCH_3$), 2.83, 2.95 (2s, 3 H, NCH_3), 3.81, 3.89 (2s, 2 H, CH_2); ratio of two rotamers = 1:2.4.

N-*Acetyl*-N-*methylglycine* (**17**).—Compound **16** (1.69 g, 9.04 mmol) was treated with a 20% solution of TFA in CH_2Cl_2 (120 mL) for 2 h at room temperature. The reaction mixture was then concentrated and coevaporated a few times with toluene to give **17** (1.09 g, 8.34 mmol) in 92% yield): ^{1}H NMR ($CDCl_3$): δ 2.15, 2.23 (2s, 3 H, $COCH_3$), 3.03, 3.14 (2s, 3 H, NCH_3), 4.13, 4.20 (2s, 2 H, CH_2), 11.73 (bs, 1 H, CO_2H); ratio of two rotamers = 1:4.4.

Coupling of **14** *to* **17** *to give* **18**.—To a mixture of **14** (500 mg, 1.71 mmol) and **17** (224 mg, 1.71 mmol) in CH_2Cl_2 (20 mL) was added 1,3-dicyclohexylcarbodiimide (DCC, 528 mg, 2.56 mmol). The solution was stirred for 5 h at room temperature. The white solid dicyclohexylurea formed during the reaction was filtered through a cotton wool, and the filtrate was concentrated. Column chromatography with 98:2 CH_2Cl_2–MeOH (R_f 0.30) as eluant afforded **18** (679 mg, 1.67 mmol) as a colorless oil in 98% yield): ^{1}H NMR ($CDCl_3$): δ 1.39, 1.41 (2s, 9 H, CMe_3), 1.88, 1.97, 2.04, 2.11 (4s, 3 H, $COCH_3$), 2.52, 2.55 (2t, 2 H, *J* 6.8 Hz, CH_2CO_2), 2.85, 2.91, 2.99, 3.05 (4s, 3 H, CH_3N), 3.56, 3.61 (2t, 2 H, *J* 6.8 Hz, NCH_2), 4.03–4.30 (m, 4 H, 2 (CH_2), 5.12–5.15 (m, 2 H, $PhCH_2O$), 7.32–7.34 (m, 5 H, Ph); rotamer ratio = 5:3:1.5:1.

Compound **19**.—Nitrogen was passed for 5 min in a solution of **18** (55.6 mg, 137 μmol) in MeOH. Pd–C (10% w/w, 6.0 mg) was added to the solution, and hydrogen was then passed through the solution for 3 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated to afford **19** (42.5 mg, 134 μmol) as a colorless oil in 98% yield: CIMS gave *m/z* for $C_{14}H_{24}N_2O_6$: 317 ([M + 1]$^+$ 2.6%); ^{1}H NMR ($CDCl_3$): δ 1.39, 1.40, 2 × 1.41 (4s, 9 H, CMe_3), 1.97, 1.99, 2.10, 2.13 (4s, 3 H, $COCH_3$), 2.51, 2.54 (2t, 2 H, *J* 6.8 Hz, CH_2CO_2), 2.90, 2.91, 3.05, 3.06 (4s, 3 H, NCH_3), 3.57, 3.60

(2t, 2 H, *J* 6.8 Hz, NCH_2), 4.00–4.40 (m, 4 H, $2 \times CH_2$), 7.90 (bs, 1 H, CO_2H); rotamer ratio = 6.8:6.5:1.5:1.

Compound **20**.—To a mixture of **11** (119 mg, 137 μmol) and **19** (43.4 mg, 137 μmol) in CH_2Cl_2 (10 mL) was added 1,3-dicyclohexylcarbodiimide (28.4 mg, 137 μmol). The reaction mixture was stirred for 2 h at room temperature. The white precipitate that formed was filtered, and the solution was concentrated. The residue was then purified by column chromatography using 18:1:1 $CHCl_3$–MeOH–MeCN (R_f 0.31) as eluant to give **20** (147 mg, 126 μmol) as a white solid in 92% yield: mp 85.0–86.5 °C; $[\alpha]_D$ −9.22° (*c* 1.8, $CHCl_3$); FABMS gave *m/z* for $C_{62}H_{77}N_5O_{17}$: 1164.3 ($[M + 1]^+$ 0.5%); Anal. Calcd for $C_{62}H_{76}N_5O_{17}$: C, 64.01; H, 6.58; N, 6.02. Found: C, 63.97; H, 6.64; N, 5.93.

Compound **22**.—A solution of **20** (40 mg, 34.4 μmol) in methanol (5 mL) was treated with a M $NaOCH_3$ solution in MeOH until pH 9. The solution was then stirred for 2 h at room temperature. The reaction mixture was treated with H^+ resin for 30 min, filtered, and then concentrated to give **21** as a white solid in quantitative yield. Compound **21** was used for the next step without further purification. It was dissolved in CH_2Cl_2 (2 mL) containing 20% TFA, and the resulting solution was stirred for 1 h at room temperature to give the fully deprotected pentapeptoid **22** in essentially quantitative yield; positive-ion FABMS gave *m/z* for $C_{33}H_{49}N_5O_{14}$: 740 ($[M + 1]^+$, 1.1%).

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