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Ritter-based glycoconjugation of amino acids and peptides—access to novel glycoconjugates displaying a β-amide linkage between amino acid and sugar moiety

Marlin Penner and Frank Schweizer*

Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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Abstract—β-Peptidic-D-*gluco*-, D-*galacto*-, and L-*fuco*-configured glycosyl amino acids can be prepared from the corresponding 2-deoxy-oct-3-ulopyranosonic acids via a one-pot intramolecular Ritter reaction. Initially, a ketopyranoside-based acid condenses under Lewis acid promoted conditions with nitriles (PhCN, MeCN) and a partially protected diamino ester (Boc-DAB-*O*-*t*-Bu, Boc-Orn-*O*-*t*-Bu) to form a β-peptidic glycosyl amino *t*-butylesters. The glycosyl amino *t*-butylesters can be converted into Fmoc-protected glycosyl amino acids that are suitably protected for solid-phase glycopeptide synthesis. Furthermore, replacement of the protected diamino ester by immobilized peptide amines permits post-synthetic N-terminal- and N(ε)-glycoconjugation of peptides on the solid phase.

Keywords: Glycoconjugates; C-Ketosides; Neoglycopeptides; Glycosyl amino acids; Glycomimetics

1. Introduction

Glycoconjugates involved in biological events such as tumor metastasis, inflammation, and immune response have a significant pharmaceutical potential.[1] Of particular interest are glycoproteins and glycopeptides containing modified glycosyl amino acids, thus exhibiting new properties. Artificial glycopeptide linkages such as *C*-glycosyl amino acids,[2] glycopeptoids,[3] retro amides,[4] and oxime-linked glycosyl amino acids[5] have been developed to overcome the inherent metabolic instability of glycoconjugates. Recently, it was reported that side-chain modified lysine glycoconjugates incorporated into the RGD tripeptide pharmacophore improved the receptor-subtype selectivity and pharmacokinetic properties of RGD peptides.[6] Similar approaches may also be applicable to other peptides but require access to unnatural glycosyl amino acids. In this paper, we report the synthesis of unnatural glycosyl amino acids in which the side-chain of DAB (diaminobutyric acid) or ornithine is conjugated via an β-amide linkage to a monosaccharide (glucose, galactose, fucose) using a one-pot Ritter-based condensation (Fig. 1). The building blocks can be converted into suitably protected glycosyl amino acids for use in solid-phase glycopeptide synthesis. In addition, we demonstrate that the Ritter condensation permits (a) incorporation of carbohydrate binding sites into the N-terminal position of resin-immobilized

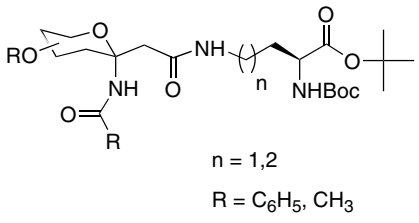

Figure 1. Glycosyl amino acids containing a β-amide linkage between carbohydrate and the side chain of diaminobutyric acid and ornithine.

* Corresponding author. Fax: +1 204 474 7608; e-mail addresses: schweize@cc.umanitoba.ca; schweize@ms.umanitoba.ca

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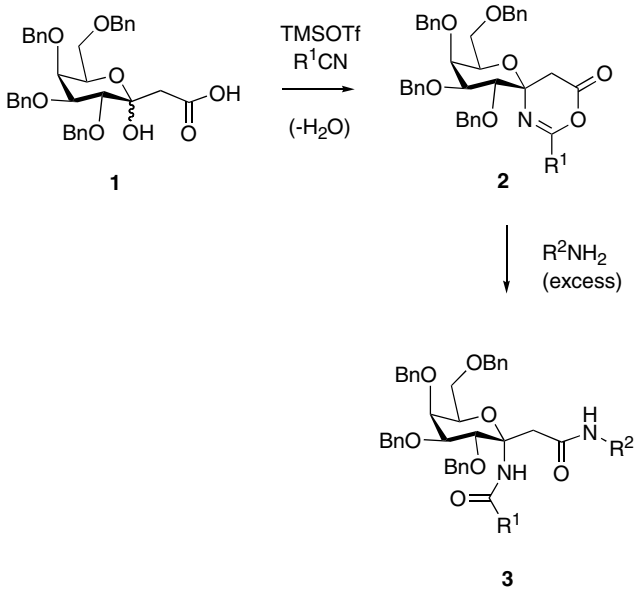


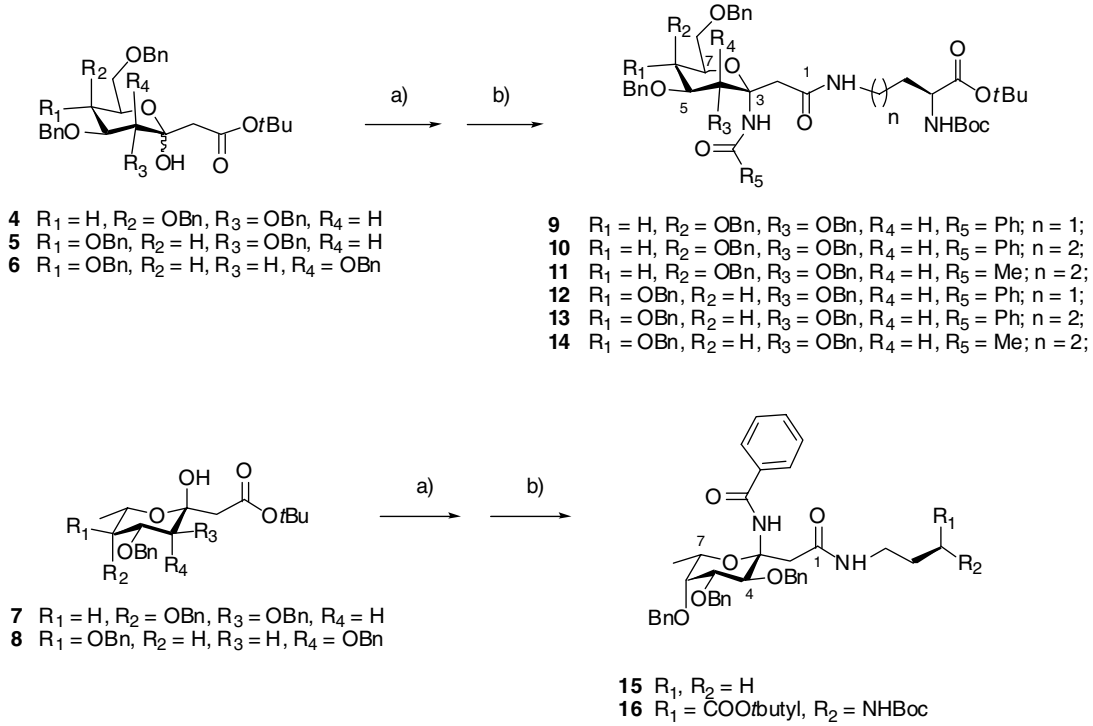
Scheme 1. Ritter-based one-pot three-component condensation that provides galactosyl-β-amides **3**. 2-Deoxy-α-D-*galacto*-oct-3-ulopyranosonic acid **1** condenses with a monofunctionalized nitrile under Lewis-acid promoted conditions to form imino anhydride **2**. Exposure of **2** to monofunctional amines yields galactosyl-β-amides **3**.

peptides and (b) post-synthetic internal N(ε)glycoconjugation of lysine-containing peptides on the solid phase. A preliminary account on the synthesis of D-*gluco*-, D-*galacto*-, and L-*fuco*-based β-peptidic glycosyl amino acids was recently communicated.[7]

Recently, we reported that α-D-*galacto*-2-deoxy-oct-3-ulopyranosonic acid **1** can be converted into galactose-configured-β-amides with high stereoselectivity using a Ritter-based three-component condensation. Initially, acid **1** was condensed with a monofunctionalized nitrile (aromatic and aliphatic) under Lewis-acid promoted conditions to form a cyclic imino anhydride **2** that undergoes a second condensation with excess of monofunctionalized amines to form galactosyl-β-amides **3** (Scheme 1).[8,9] We then became interested in expanding this three-component condensation to D-*gluco*-, D-*manno*, L-*fuco*-, and L-*rhamno*-based ulosonic acids, as well as to polyfunctionalized amino acids and immobilized peptidic amines.

2. Results and discussion

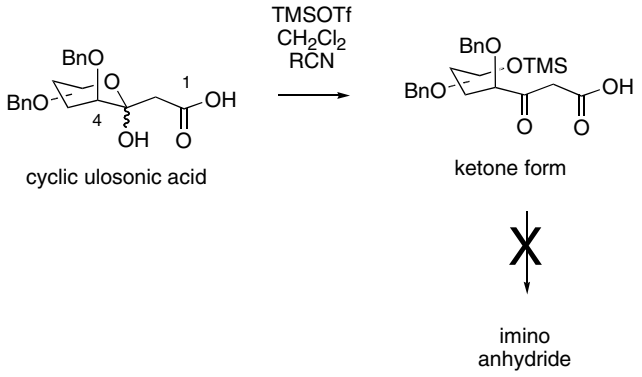
Ulosonic esters **4**–**8**[10] (Scheme 2) were prepared by the reaction of *t*-butylacetate enolate with the corresponding sugar lactone as previously described[8] and isolated in high yields averaging 90%. Initially, we were interested in studying whether polyfunctionalized amines such as side-chain deprotected α,γ-diaminobutyric *t*-butylester (Boc-DAB-*O*-*t*-Bu) and ornithine (Boc-Orn-*O*-*t*-Bu) are compatible with this Ritter-based



Scheme 2. Synthesis of glycosyl amino acids **9**–**16**. Reagents and conditions: (a) 50% TFA in CH_2Cl_2, 2 h; (b) sugar (4 equiv), TMSOTf (12 equiv), nitrile (MeCN or PhCN, 16 equiv), CH_2Cl_2, 2 h, 0 °C; quenching with DIPEA; then addition of Boc-DAB-*O*-*t*-Bu or Boc-Orn-*O*-*t*-Bu or $C_3H_7NH_2$, 12 h, 18–95%.

three-component condensation. Removal of the *t*-butyl group of the galactose-based ulosonic ester **4** with trifluoroacetic acid generated **1** in situ. Treatment of **1** with trimethylsilyl-trifluoromethanesulfonate (TMSOTf) and excess benzonitrile (PhCN) for 2 h, followed by quenching the reaction by the addition of excess diisopropylethylamine (DIPEA) and addition of Boc-DAB-*O*-*t*-Bu or Boc-Orn-*O*-*t*-Bu, produced the protected galactose-configured amino esters **9** and **10** as single stereoisomers in 75% and 64% yield, respectively (Scheme 2). Replacement of PhCN by acetonitrile (MeCN) and exposure to Boc-Orn-*O*-*t*-Bu provided the galactose-configured amino ester **11** in 61% yield.

After demonstrating the compatibility of polyfunctionalized amines in this three-component reaction, we explored the one-pot Ritter-based condensation using the glucose-based ulosonic acid derived from **5** by acidic ester hydrolysis. TMSOTf-promoted condensation of the acid with PhCN or MeCN and subsequent quenching with two amines (Boc-Dab-*O*-*t*-Bu or Boc-Orn-*O*-*t*-Bu) afforded the glucosyl amino esters **12**–**14** in 95%, 79%, and 55% yield, respectively (Scheme 2). By comparison, TMSOTf-promoted condensation of D-*manno*-based ulosonic acid, derived by acidic ester hydrolysis from ulosonic ester **5** with benzonitrile and Boc-Orn-*O*-*t*-Bu, did not afford the corresponding mannose-configured amino ester. Next we studied the use of the L-fucose- and L-*rhamno*-based ulosonic esters **7** and **8** in the three-component condensation (Scheme 2). The Ritter reaction of the L-fucose-based ulosonic acid with PhCN and trapping of the intermediate imino anhydride with propylamine and Boc-Dab-*O*-*t*-Bu provided the fucose-configured amide **15** and fucosyl amino acid **16** in 40% and 18% yield, respectively. This contrasts with the reaction of L-*rhamno*-based acid derived from ulosonic ester **8**, which did not provide the corresponding glycosyl amino ester. We speculate that the axial substituent at C-4 in the D-*manno*- and L-*rhamno*-based ulosonic acid destabilizes the cyclic form and favors the open ketone form, thereby preventing the formation of the corresponding β-peptidic glycosyl amino acids (Scheme 3).



Scheme 3. Destabilization of cyclic ulosonic acids bearing axial substituents at C-4. The axial substituent at C-4 in D-*manno*- and L-*rhamno*-based ulosonic acids destabilizes the cyclic form and leads to the formation of the acyclic ketone form, thereby preventing imino anhydride formation.

The absolute stereochemistry at C-3 of the protected glycosyl amino esters **9**–**16** was assigned based on inter-proton effects of H-7 (>0.6% NOE) measured relative to the singlet amide resonance signal. For instance, subjection of the singlet amide signal in **9** to a one-dimensional GOESY[11] experiment showed inter-proton effect to H-7 (0.8% NOE) measured relative to the singlet amide signal (Fig. 2). This is only compatible with an axial orientation of the anomeric amide group. Similar inter-proton NOE effects between H-7 and the singlet amide signal were observed for compounds **10**–**16** (Fig. 2).

To convert glycosyl amino ester **9** into a building block suitable for solid-phase glycopeptide synthesis, we applied a four-step deprotection/protection protocol (Scheme 4). First, the *O*-benzyl protecting groups on the galactose-moiety were removed by catalytic hydrogenation followed by acetylation of the free hydroxyl groups. Then the *t*-butylcarbamate and the *t*-butyl ester protecting groups were cleaved by treatment with TFA before selective deprotection of the amino function using 9-fluorenylmethyl pentafluorophenyl carbonate (Fmoc-OPfp), producing the Fmoc-protected galactose-based amino acid **19** in 76% overall yield (Scheme 4).

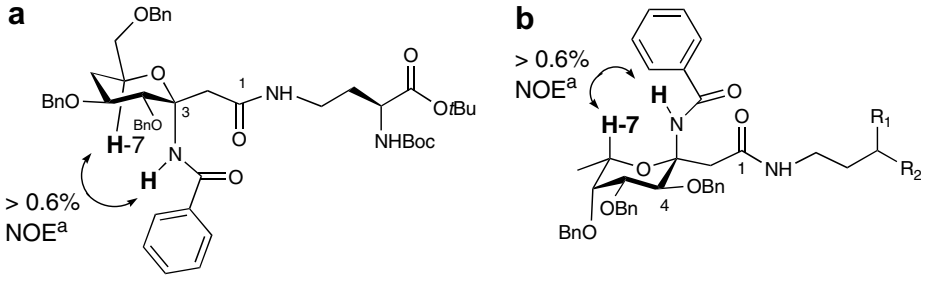
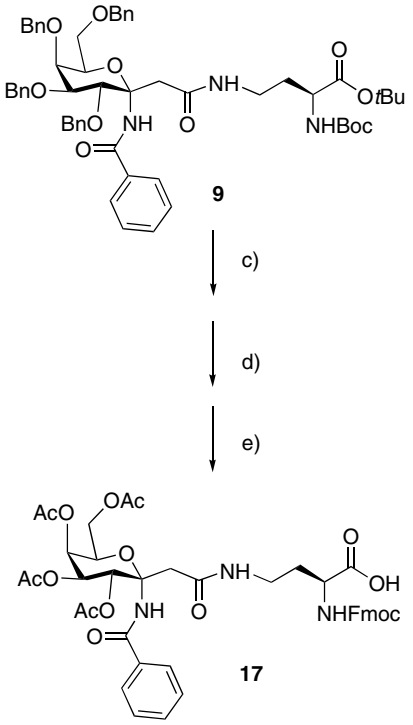


Figure 2. Conformationally relevant NOE interactions observed for compounds **9**–**14** (a) and compounds **15** and **16** (b); [a] in $CDCl_3$.

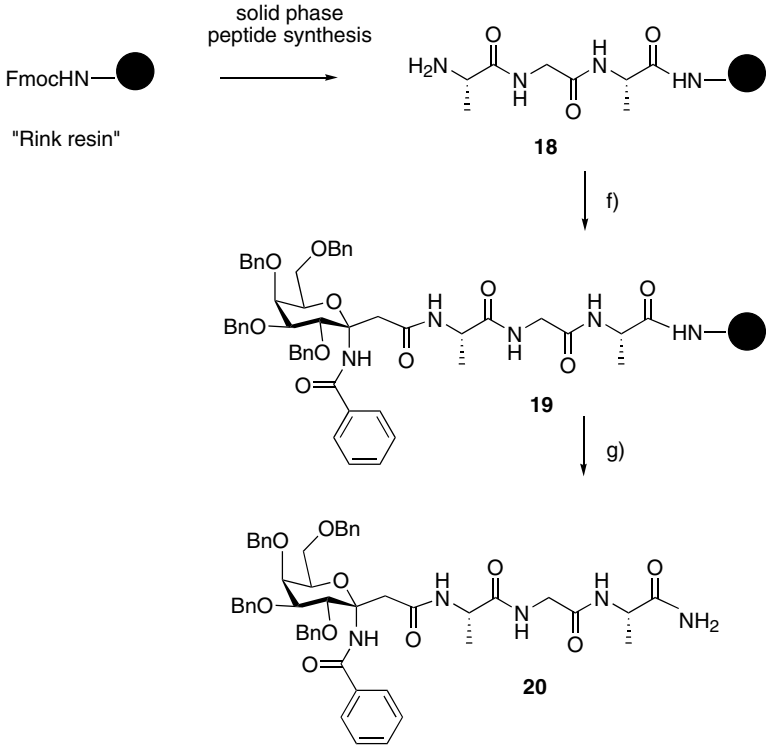


Scheme 4. Deprotection of glycosyl amino acid **9**. Reagents and conditions: (c) $Pd(OH)_2$, MeOH, H_2, then Ac_2O, C_5H_5N, 20 h; (d) 50% TFA in CH_2Cl_2, 0 °C to rt, 8 h; (e), THF–H_2O (2:1), Fmoc-OPfp (2 equiv), $NaHCO_3$, 12 h, 76% (over three steps).

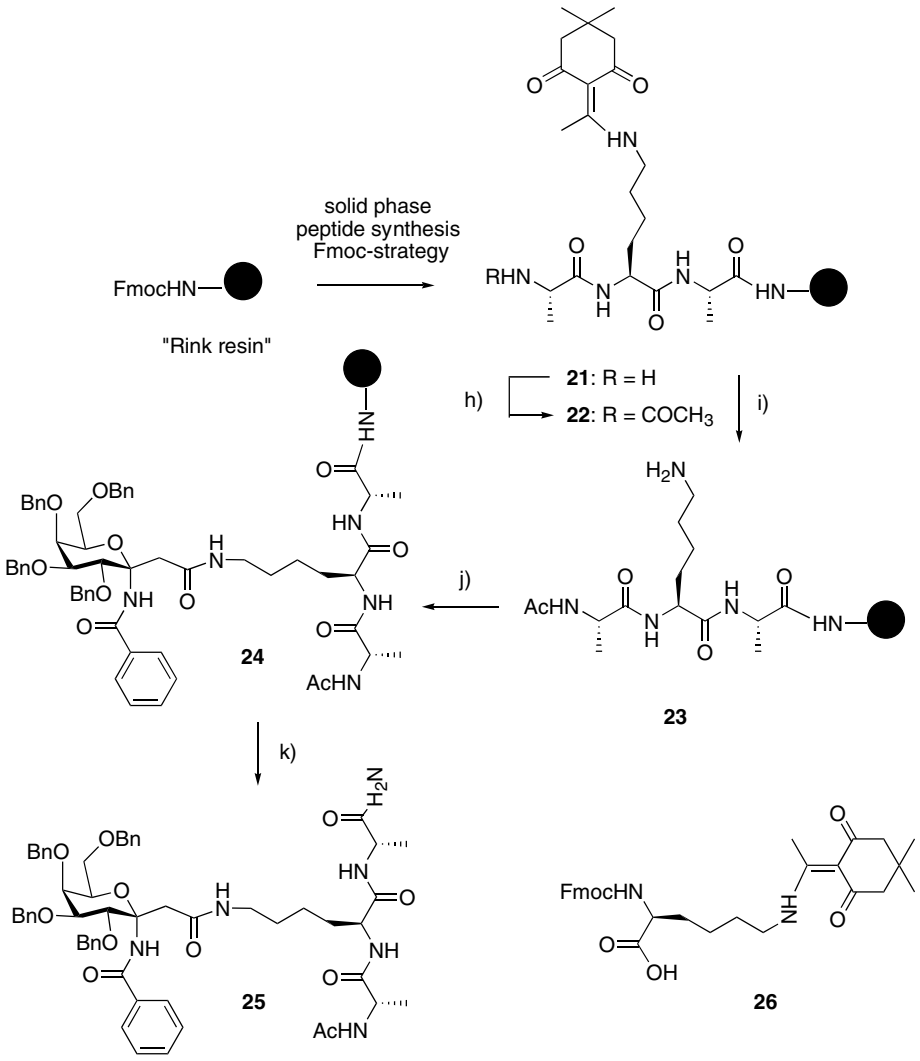
Compound **17** is suitably protected for solid-phase glycopeptide synthesis.

Next we studied the use of immobilized peptidic amines in this three-component reaction. At first we looked at N-terminal glycoconjugation of the immobilized tripeptide H-Ala-Gly-Ala-resin **18** synthesized by standard solid-phase peptide synthesis on the Rink-resin (Scheme 5). Exposure of tripeptide **18** to excess of galactose-based cyclic imino anhydride **2** provided immobilized glycopeptide **19** that was released from the resin by treatment with trifluoroacetic acid. Glycopeptide **20** was obtained in 35% isolated yield after chromatographic purification. Encouraged by these results, we explored Ritter-based side-chain glycoconjugation of the immobilized tripeptide Ac-Ala-Lys(H)-Ala-resin **23** synthesized by solid-phase peptide synthesis using orthogonally protected lysine building block **26** (Scheme 6). Exposure of immobilized tripeptide **23** to excess imino-anhydride **2** produced protected glycotripeptide **25** after resin cleavage (34% yield).

Our modified Ritter-based method allows fast access to glycosyl amino acids bearing a β-amide linkage between glucose, galactose, and fucose and the side chain of ornithine and diaminobutyric acid. The resulting glycosyl amino acids can be converted into Fmoc-protected glycosyl amino acid for use in solid-phase glycopeptide synthesis. Furthermore, our procedure allows



Scheme 5. Ritter-based N-terminal glycoconjugation of immobilized tripeptide H-Ala-Gly-Ala-resin **18**. Reagents and conditions: (f) (i) **1** (4 equiv), 50% TFA in CH_2Cl_2, 2 h, codistillation with toluene; (ii) TMSOTf (14 equiv), PhCN (40 equiv), CH_2Cl_2, 2 h, 0 °C, then addition of DIPEA (24 equiv), 2 min, then addition of **18** (1 equiv), 12 h; (g) 95% TFA in CH_2Cl_2, 20 min.



Scheme 6. Ritter-based side-chain glycoconjugation of immobilized tripeptide Ac-Ala-Lys(H)-Ala-resin **23**. Reagents and conditions: (h) CH_3COOH, TBTU, DIPEA; (i) 2% $N_2H_4 \times H_2O$ hydrazine in DMF; (j) (i) **1** (4 equiv), 50% TFA in CH_2Cl_2, 2 h, codistillation with toluene; (ii) TMSOTf (14 equiv), PhCN (40 equiv), CH_2Cl_2, 2 h, 0 °C, then addition of DIPEA (24 equiv), 2 min, then addition of **23**, 12 h; (k) 95% TFA in CH_2Cl_2, 20 min.

N-terminal glycoconjugation as well as internal N(ε)glycoconjugation of lysine-containing peptides immobilized on the Rink amide resin. The commercial availability of large numbers of nitriles, amino acids, and immobilized peptidic amines suggests that the methodology presented here should be attractive for the combinatorial synthesis of unnatural glycosyl amino acids and the preparation of neoglycopeptide libraries.[12]

3. Experimental

3.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 360 or 500 MHz for ^1H and at 75 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ (δ_H 7.26, δ_C (center of triplet) 77.0 ppm) or to CH_3OH (δ_H 73.35, δ_C (center of septet) 49.0 ppm) or to acetone as internal standard (D_2O). TLC was performed on E. Merck Silica Gel 60 F254 with detection by charring with 8% H_2SO_4 acid. Silica gel (0.040–0.063 mm) was used for column chromatography. Lactone **5** was purchased from Toronto Research Chemicals. Rink-amide MBHA resin was purchased from Novabiochem; compound **26** is commercially available from BACHEM.

3.2. General procedure for the synthesis of compounds 9–14, 15, and 16

The ketol (any of compounds **4**–**8**, 4.3 mmol) was dissolved in 1:1 mixture containing trifluoroacetic acid

and CH_2Cl_2 (30 mL) at 0 °C for 2 h. The solvent was removed under reduced pressure and codistilled with toluene (3 × 10 mL) to dryness. The oily residue was redissolved in CH_2Cl_2 (15 mL), PhCN or MeCN (17.2 mmol), and TMSOTf (12.9 mmol) was added at 0 °C. After 2 h, the reaction was quenched with DIPEA (16.12 mmol) and the amino acid (Boc-DAB-*O*-*t*-Bu × HCl or Boc-Orn-*O*-*t*-Bu × HCl; 1 mmol) dissolved in a 1:1 mixture of CH_2Cl_2 and DIPEA (4 mL) was added. Aqueous work-up after 12 h followed by chromatographic purification using hexane–EtOAc 1.5:1 (reaction using PhCN) or hexane–EtOAc 1:2.5 (reaction using MeCN) afforded protected glycosylamino ester in 18–95% yield.

3.3. (*S*)-*t*-Butyl 4-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)butanoate (9)

Compound **9** was obtained in 75% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.44 (s, 9H), 1.46 (s, 9H), 1.51–1.65 (m, 1H), 1.70–1.85 (m, 1H), 2.74 (d, 1H, $J = 15.3$ Hz), 2.84–3.01 (m, 1H), 3.03–3.24 (m, 1H), 3.64–3.81 (m, 4H), 3.86–3.94 (m, 1H), 3.96–4.07 (m, 1H), 4.11 (dd, $J = 2.0$, <1 Hz, 1H), 4.21 (d, 1H, $J = 9.6$ Hz), 4.41–5.09 (m, 8H + NH), 6.86 (s, 1H, NH), 7.01 (t, 1H, $J = 5.3$ Hz, NH), 7.22–7.66 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 28.0, 28.4, 31.2, 36.1, 43.0, 52.3, 67.8, 71.0, 72.1, 72.8, 73.6, 74.7, 76.1, 77.0, 79.6, 80.7, 81.8, 87.0, 126.9–128.7, 131.9, 134.6, 137.8 (×2), 138.3, 155.5, 166.7, 169.0, 171.5. ESIMS *m*/*z* Calcd for $C_{56}H_{68}N_3O_{11}$ $[M+H]^+$: 958.48539. Found: 958.48542. Anal. Calcd C, 70.20; H, 7.05; N, 4.39. Found: C, 70.44; H, 7.08; N, 4.35.

3.4. (*S*)-*t*-Butyl 5-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)pentanoate (10)

Compound **10** was obtained in 64% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.24–1.32 (m, 2H), 1.32–1.42 (m, 1H), 1.46 (s, 9H), 1.47 (s, 9H), 1.48–1.61 (m, 1H), 2.38–2.55 (m, 1H), 2.71 (d, 1H, $J = 15.6$ Hz), 3.09–3.23 (m, 1H), 3.65–3.85 (m, 4H), 3.88–3.96 (m, 1H), 3.99–4.10 (m, 1H), 4.13 (dd, 1H, $J = 2.2$, <1 Hz), 4.19 (d, 1H, $J = 9.6$ Hz), 4.49 (d, 1H, $J = 11.6$ Hz), 4.55 (d, 1H, $J = 10.6$ Hz), 4.57 (d, 1H, $J = 11.6$ Hz), 4.68 (d, 1H, $J = 11.7$ Hz), 4.80 (d, 1H, $J = 11.7$ Hz), 4.85–4.97 (m, 3H + NH), 6.78–6.92 (m, 2H, 2 × NH), 7.23–7.66 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 25.6, 28.0, 28.4, 30.2, 38.8, 43.1, 53.8, 67.7, 70.9, 72.1, 73.1, 73.6, 75.0, 76.2, 77.1, 79.5, 80.7, 81.7, 87.0, 126.9–128.7 (aromatic carbons), 131.9, 134.7, 137.7, 137.8, 138.4, 155.4, 166.7, 168.7, 171.8. ESIMS *m*/*z* Calcd for $C_{57}H_{70}N_3O_{11}$ $[M+H]^+$: 972.50104. Found: 972.50109.

3.5. (*S*)-*t*-Butyl 5-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-acetamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)pentanoate (11)

Compound **11** was obtained in 61% yield after chromatographic purification using hexane–EtOAc 1:2.5 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.18–1.30 (m, 2H), 1.30–1.38 (m, 1H), 1.45 (s, 9H), 1.46 (s, 9H), 1.47–1.46 (m, 1H), 1.95 (s, 3H), 2.32–2.51 (m, 1H), 2.63 (d, 1H, $J = 15.6$ Hz), 3.05–3.21 (m, 1H), 3.59–3.78 (m, 4H), 3.80–3.89 (m, 1H), 3.97–4.13 (m, 3H), 4.45–5.00 (m, 8H + 1 × NH), 6.06 (s, NH), 6.81 (dd, 1H, $J = 6.4$, 4.9 Hz, NH), 7.20–7.45 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 24.6, 25.5, 28.0, 28.4, 30.2, 38.7, 43.0, 53.8, 67.6, 70.7, 72.3, 73.1, 73.7, 75.0, 76.4, 77.0, 79.5, 80.9, 81.7, 86.7, 127.5–128.5 (aromatic carbons), 137.6, 137.7, 137.9, 138.4, 155.4, 168.7, 169.9, 171.8. ESIMS *m*/*z* Calcd for $C_{52}H_{68}N_3O_{11}$ $[M+H]^+$: 910.48539. Found: 910.48532.

3.6. (*S*)-*t*-Butyl 4-(2-((2*S*,3*R*,4*S*,5*S*,6*R*)-2-benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)butanoate (12)

Compound **12** was obtained in 95% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.45 (s, 9H), 1.46 (s, 9H), 1.58–1.76 (m, 1H), 1.88–2.06 (m, 1H), 2.75 (d, 1H, $J = 14.5$ Hz), 3.05–3.34 (m, 2H), 3.64–3.96 (m, 7H), 4.06–4.20 (m, 1H), 4.44–4.68 (m, 3H), 4.76–5.07 (m, 5H), 5.11 (d, 1H, $J = 8.0$ Hz, NH), 6.87 (s, 1H, NH), 6.97–7.73 (m, 25H + 1 × NH); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 28.0, 28.3, 32.6, 36.3, 42.8, 52.3, 68.0, 72.4, 73.4, 75.1, 75.7, 75.9, 77.4, 79.7, 80.4, 82.0, 83.4, 86.6, 126.9–128.7 (aromatic carbons), 132.0, 134.3, 137.7, 137.9, 138.2, 155.6, 166.7, 169.1, 171.6. ESIMS *m*/*z* Calcd for $C_{56}H_{68}N_3O_{11}$ $[M+H]^+$: 958.48539. Found: 958.48545. Anal. Calcd C, 70.20; H, 7.05; N, 4.39. Found: C, 70.38; H, 7.11; N, 4.44.

3.7. (*S*)-*t*-Butyl 5-(2-((2*S*,3*R*,4*S*,5*S*,6*R*)-2-benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)pentanoate (13)

Compound **13** was obtained in 79% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.37–1.50 (m, 3H), 1.42 (s, 9H), 1.46 (s, 9H), 1.55–1.73 (m,

1H), 2.73 (d, 1H, $J = 14.5$ Hz), 2.81–2.98 (m, 1H), 3.07–3.26 (m, 1H), 3.65–3.90 (m, 7H), 4.02–4.18 (m, 1H), 4.43–4.66 (m, 3H), 4.81–5.13 (m, 5H + 1 × NH), 6.83 (s, 1H, NH), 7.06 (t, 1H, $J = 5.8$ Hz, NH), 7.11–7.71 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 25.8, 28.0, 28.4, 30.3, 39.1, 42.9, 53.9, 68.1, 72.2, 73.5, 75.2, 75.8, 76.0, 77.3, 79.5, 80.5, 81.7, 83.3, 86.6, 126.9–128.7 (aromatic carbons), 132.0, 134.4, 137.8, 137.9, 138.1, 155.5, 166.6, 168.9, 171.8. ESIMS *m*/*z* Calcd for $C_{57}H_{70}N_3O_{11}$ $[M+H]^+$: 972.50104. Found: 972.50111.

3.8. (*S*)-*t*-Butyl 5-(2-((2*S*,3*R*,4*S*,5*S*,6*R*)-2-acetamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)pentanoate (14)

Compound **14** was obtained in 55% yield after chromatographic purification using hexane–EtOAc 1:2.5 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.34–1.50 (br m, 3H), 1.41 (s, 9H), 1.45 (s, 9H), 1.57–1.67 (m, 1H), 1.96 (s, 3H), 2.64 (d, 1H, $J = 14.7$ Hz), 2.77–2.94 (m, 1H), 3.05–3.21 (m, 1H), 3.58–3.87 (m, 7H), 4.01–4.16 (m, 1H), 4.43–4.96 (m, 8H), 5.03 (d, 1H, $J = 8.0$ Hz, NH), 6.02 (s, 1H, NH), 6.96 (t, 1H, $J = 5.8$ Hz, NH), 7.13–7.39 (m, 20H); ^{13}C NMR (75 MHz, $CDCl_3$, rt): δ 24.6, 25.8, 28.0, 28.3, 30.2, 39.1, 42.9, 53.9, 68.0, 72.0, 73.5, 75.2, 75.8, 76.2, 77.3, 79.5, 80.4, 81.7, 83.3, 86.2, 127.5–129.2 (aromatic carbons), 137.6, 137.9 (×2), 138.2, 155.5, 168.9, 169.8, 171.8. ESIMS *m*/*z* Calcd for $C_{52}H_{68}N_3O_{11}$ $[M+H]^+$: 910.48539. Found: 910.48532.

3.9. ((2*R*,3*S*,4*R*,5*S*,6*S*)-2-Benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)propane (15)

Compound **15** was obtained in 40% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 0.78 (t, 3H), 1.20–1.37 (m, 5H), 2.75 (d, 1H, $J = 15.1$ Hz), 2.76–2.85 (m, 1H), 3.03–3.18 (m, 1H), 3.75–3.85 (m, 3H), 3.85–3.90 (m, 1H), 4.20 (d, 1H, $J = 9.2$ Hz), 4.65 (d, 1H, $J = 10.9$ Hz), 4.70 (d, 1H, $J = 11.0$ Hz), 4.80 (d, 1H, $J = 11.0$ Hz), 4.90–5.05 (m, 3H), 6.85 (s, 1H, NH), 7.05 (t, 1H, $J = 7$ Hz, NH), 7.30–7.70 (m, 20H). ESIMS *m*/*z* Calcd for $C_{39}H_{45}N_2O_6$ $[M+H]^+$: 637.32776. Found: 637.32771. Anal. Calcd C, 73.56; H, 6.96; N, 4.40. Found: C, 73.84; H, 7.05; N, 4.48.

3.10. (*S*)-*t*-Butyl 4-(2-((2*R*,3*S*,4*R*,5*S*,6*S*)-2-benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(*t*-butoxycarbonyl-amino)butanoate (16)

Compound **16** was obtained in 18% yield after chromatographic purification using hexane–EtOAc 1.5:1 as eluent. ^{1}H NMR (300 MHz, $CDCl_3$, rt, TMS): δ 1.20–1.50 (m, 5H), 1.45–1.55 (m, 18H), 2.70–2.80 (m, 2H), 3.25–3.35 (m, 1H), 3.68–3.90 (m, 4H), 4.0–4.1 (m, 1H), 4.18 (d, 1H, $J = 9.3$ Hz), 4.65 (d, 1H, $J = 10.9$ Hz), 4.70 (d, 1H, $J = 11.0$ Hz), 4.80 (d, 1H, $J = 11.0$ Hz), 4.88–5.02 (m, 3H), 5.15 (d, 1H, $J = 7.5$ Hz), 6.81 (s, 1H, NH), 7.20 (t, 1H, NH), 7.23–7.65 (m, 20H). ESIMS *m*/*z* Calcd for $C_{49}H_{62}N_3O_{10}$ $[M+H]^+$: 852.44352. Found: 852.44344.

3.11. (*S*)-4-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-Benzamido-3,4,5-tris-(acetoxy)-6-acetoxymethyl-tetrahydro-2*H*-pyran-2-yl)acetamido)-2-(9-fluorenylmethoxycarbonylamino)butanoic acid (17)

Compound **9** (120 mg, 0.13 mmol) and Pearlman's catalyst (30 mg) were hydrogenated in MeOH (10 mL) at atmospheric pressure for 8 h. The solution was filtered and evaporated to dryness. The crude mixture was dissolved in a 1:1 mixture containing pyridine and acetic anhydride and left for 12 h, evaporated to dryness, and codistilled with toluene (3 × 10 mL) and purified by chromatography using EtOAc–hexane 3:1 as eluent to yield 70 mg of purified ester. The purified ester was dissolved in CH_2Cl_2 (2 mL) and trifluoroacetic acid (2 mL) and left for 90 min. The crude material was evaporated to dryness and codistilled with toluene (3 × 20 mL). The solid material was dissolved in tetrahydrofuran (2 mL), and 9-fluorenylmethyl pentafluorophenyl carbonate (74 mg, 0.26 mmol), and an aqueous solution containing sodium bicarbonate (46 mg, 0.55 mmol) were added. After 12 h, formic acid (0.2 mL) was added. The residue was concentrated under reduced pressure and water (10 mL) and CH_2Cl_2 (10 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatographic purification using 15% MeOH in EtOAc afforded compound 17 (76 mg, 84%). ^{1}H NMR (DMSO-d_6, 300 MHz, rt): δ 1.58–1.87 (m, 2H), 1.89 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.12 (s, 3H), 2.81 (d, 1H, $J = 14.6$ Hz), 2.87–3.20 (m, 3H), 3.59–3.72 (m, 2H), 3.97–4.10 (m, 4H), 4.14–4.29 (m, 1H), 4.34 (t, 1H, $J = 6.6$ Hz), 5.31 (d, 1H, $J = 10.3$ Hz), 5.34 (d, 1H, $J = 3.8$ Hz), 5.83 (dd, 1H, $J = 10.3$, 3.7 Hz), 6.58–6.77 (m, 1H, NH), 7.26–7.92 (m, 13 H), 7.92–8.06 (m, 1H, NH), 8.60 (s, 1H). ESIMS *m*/*z* Calcd for $C_{42}H_{46}N_3O_{15}$ $[M+H]^+$: 832.29290. Found: 832.29285.

3.12. General procedures for glycopeptide synthesis

The solid-phase synthesis of **18** and **22** was carried out manually using Fmoc chemistry. Building block **26** was used for the synthesis of tripeptide **23**. Starting with unprotected Rink-amide resin (loading level 0.22 mmol/g),

the next amino acid (4 equiv), activated with TBTU (4 equiv), and DIPEA (8 equiv) in DMF, was added. The Fmoc group was then removed using 20% piperidine in DMF. The coupling/deprotection cycles were repeated using the appropriate amino acids until the entire peptide sequence had been assembled. The side-chain protection of lysine in tripeptide **22** was removed by exposure to 2% hydrazine hydrate in DMF to produce unprotected tripeptide **23**. A Quest 210 (Agilent) manual synthesizer with 5 mL reaction vessels was used.

3.13. *N*-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-Benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetyl)-Ala-Gly-Ala-NH_2 (20)

Acid **1** (0.89 mmol, 0.58 g) was dissolved in CH_2Cl_2 (10 mL), and PhCN (8.88 mmol, 0.90 mL), and TMSOTf (3.11 mmol, 0.56 mL) were added at 0 °C. After 2 h, the reaction was quenched with DIPEA (16.12 mmol, 0.93 mL) and the immobilized peptide **18** (0.22 mmol) was added. After 12 h, the resin was filtered and washed with DMF (3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL) and dried. The resin was subjected to cleavage conditions (95% TFA, 2.5% thioanisole and 2.5% H_2O) for 20 min and the released glycopeptide was purified using 4% MeOH in EtOAc to afford **20** (69 mg, 35%). ^{1}H NMR (300 MHz, DMSO-d_6, rt): δ 1.11 (d, 3H, $J = 7.0$ Hz, Me(Ala)), 1.19 (d, 3H, $J = 7.2$ Hz, Me(Ala)), 2.80 (d, 1H, $J = 14.4$ Hz, H-2b), 3.48–3.71 (m, 5H, CH_2(Gly), H-8a, H-8b, H-2a), 3.90–3.98 (m, 1H, H-7), 4.11 (d, 1H, $J = 2.4$ Hz, H-6), 4.12–4.22 (m, 2H), 4.29 (dd, 1H, $J = 2.4$, 10.0 Hz, H-5), 4.38 (d, 1H, $J = 10.0$ Hz, H-4), 4.40 (d, 1H, $J = 11.8$ Hz, benzylic), 4.49–4.95 (m, 7H, benzylic), 7.02 (s, 1H, NH), 7.22–7.40 (m, 22H), 7.43–7.60 (m, 3H), 7.60–7.76 (m, 2H), 7.81 (d, 1H, $J = 7.4$, NH), 7.92 (t, 1H, $J = 5.8$ Hz, NH), 8.02 (d, 1H, $J = 6.6$ Hz, NH); ^{13}C NMR (75 MHz, CD_3OD, rt): δ 18.12, 18.15, 42.10, 48.01, 48.70, 54.91, 68.23, 70.80, 71.24, 72.26, 73.63, 73.80, 74.77, 77.43, 79.82, 86.92, 127.22–128.45 (aromatic), 131.54, 135.23, 138.15, 138.64, 138.76, 166.70, 168.26, 168.36, 172.31, 174.18. ESIMS *m*/*z* Calcd for $C_{51}H_{57}N_5NaO_{10}$ $[M+Na]^+$: 922.40031. Found: 922.40029.

3.14. NHAc-Ala-Lys(N^{ε}-(2-((2*S*,3*R*,4*S*,5*R*,6*R*)-2-Benzamido-3,4,5-tris-(benzyloxy)-6-benzyloxymethyl-tetrahydro-2*H*-pyran-2-yl)acetyl)-Ala-NH_2 (25)

Acid **1** (0.89 mmol, 0.58 g) was dissolved in CH_2Cl_2 (10 mL), and PhCN (8.88 mmol, 0.90 mL), and TMSOTf (3.11 mmol, 0.56 mL) were added at 0 °C. After 2 h, the reaction was quenched with DIPEA (16.12 mmol, 0.93 mL) and the immobilized peptide **23** (0.22 mmol) was added. After 12 h, the resin was filtered and washed with DMF (3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL) and dried. The resin was subjected to cleavage conditions (95% TFA, 2.5% thioanisole and 2.5% H_2O) for 20 min and the released glycopeptide was purified using 4% MeOH in EtOAc to afford **25** (75 mg, 34%). ^{1}H NMR (300 MHz, DMSO-d_6, rt): δ 0.94–1.34 (m, 10H), 1.35–1.64 (m, 2H, Hβ Lys), 1.83 (s, 3H), 2.62–2.75 (m, 1H, Hε Lys), 2.79 (d, 1H, $J = 14.1$ Hz, H-2b), 2.85–3.00 (m, 1H, Hε Lys), 3.28 (d, 1H, $J = 14.1$ Hz, H-2a), 3.46–3.70 (m, 2H, H-8a, H-8b), 3.89–3.99 (m, 1H, H-7), 4.02–4.33 (m, 6H, H(α)Ala, H(α)Ala, H(α)Lys, H-4, H-5, H-6), 4.99–4.39 (m, 8H, benzylic), 7.03 (s, 1H, NH), 7.22–7.41 (m, 24 H, Ar, NH_2), 7.64–7.45 (m, 3H, NH, Ar), 7.77–7.70 (m, 2H, NH, 1 × Ar), 7.92 (d, 1H, $J = 7.8$ Hz, NH), 8.08 (d, 1H, $J = 7.0$ Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6, rt): δ 17.96, 18.33, 22.49, 22.77, 28.54, 31.26, 31.28, 42.57, 47.92, 48.44, 52.69, 68.07, 70.46, 71.10, 72.27, 73.84, 74.06, 74.44, 77.43, 79.94, 86.72, 127.12–128.26 (aromatic), 131.49, 135.37, 138.17, 138.64, 138.85, 165.25, 166.66, 167.82, 169.32, 170.97, 172.61. ESIMS *m*/*z* Calcd for $C_{57}H_{68}N_6NaO_{11}$: 1035.48438 $[M+Na]^+$. Found: 1035.48447.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.11.006.

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