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# A novel strategy toward the synthesis of *N*-(β-glycosyl)asparagines based on the alkylation of ethyl nitroacetate using *N*-(β-glycosyl)iodoacetamides

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ABSTRACT

A conceptually novel strategy has been developed for the synthesis of *N*-(β-glycosyl)asparagine precursors in good yield by the alkylation of ethyl nitroacetate using six per-O-acetylated *N*-(β-glycosyl)iodoacetamides derived from mono- and disaccharides. The use of a chiral organocatalyst, *N*-(9-anthracenylmethyl)cinchoninium chloride (10 mol %), resulted in diastereoselective alkylation up to 64% de. Single crystal structure analysis of the purified major diastereomer of the Glc derivative revealed an absolute configuration of S at the α-carbon of the monosubstituted ethyl nitroacetate which is a precursor of the L-asparagine conjugate.



Glycan components of glycoproteins play key roles in many biological processes.[1] In N-glycoproteins, the conserved pentasaccharide core, $Man_3GlcNAc_2$, is linked to the side chain amide nitrogen of Asn in the consensus sequence Asn-Xaa-Ser/Thr, where Xaa can be any amino acid except Pro.[2] Owing to the microheterogeneity[3] of the glycan chains that extend from the core, the expressed protein is most often a mixture of various glycoforms. As a result, the elucidation of structure–function correlations of glycoproteins has been a formidable challenge to overcome. Glycopeptides constitute structurally well-defined and homogeneous partial structures of glycoproteins and serve as valuable models for elucidating the functions of glycan chains of glycoproteins. Realizing the need for preparing structurally homogeneous glycopeptides in reasonable quantities to address the above-mentioned challenge, a number of chemical methods have been developed over the past several decades.[4] All these methods essentially belong to two types of synthetic strategies for the N-glycoamino acids and N-glycopeptides. The first one more closely resembles the amide bond formation (Scheme 1, Path A). The formation of the N-glycosidic bond has often been achieved by the coupling of a glycosylamine[5] with an activated Asp derivative that has been suitably protected to avoid side reactions. However, aspartic acid residue of peptides easily undergoes cyclization to afford the unwanted aspartimide as a side product. In addition, glycosylamines are relatively unstable resulting in not only anomerization but also hydrolysis to the hemiacetal during the coupling reaction.[6] These problems have been overcome to some extent by the use of glycosylamine equivalents such as glycosyl isothiocyanates[7] and glycosyl azides.[8] An alternative approach reported from the laboratory of Fraser-Reid[9] involved the trapping of a β-nitrilium ion, generated by the reaction of *n*-pentenyl 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside with acetonitrile using NBS as a promoter, with an aspartic acid derivative followed by selective N-deacetylation using piperidine. The second strategy, based on the biosynthesis of N-glycoproteins, involves N-glycosylation of protected asparagine and Asn containing di- and tripeptides using appropriately protected glycosyl trifluoroacetimidates as donors and TMSOTf as a catalyst (Scheme 1, Path B).[10] The β-D-glucosyl imidate with an acyl-protecting group at C2 position underwent conversion to the corresponding *N*-(β-D-glycosyl)asparagine with complete β-stereoselectivity in 98% yield, whereas the imidate derived from *N*-Troc glucosamine was obtained in moderate yield. The perbenzylated β-D-galactosyl trifluoroacetimidate, on the other hand, furnished the corresponding *N*-(β-D-galactosyl)asparagine in moderate yield and with poor β-selectivity (23%). Therefore, there is a need to develop general, versatile, and efficient methodologies for the synthesis of N-glycoamino acids and N-glycopeptides.

A conceptually novel strategy based on the retrosynthetic cleavage of $C_\alpha$–$C_\beta$ bond (Scheme 1, Path C) was planned to be developed

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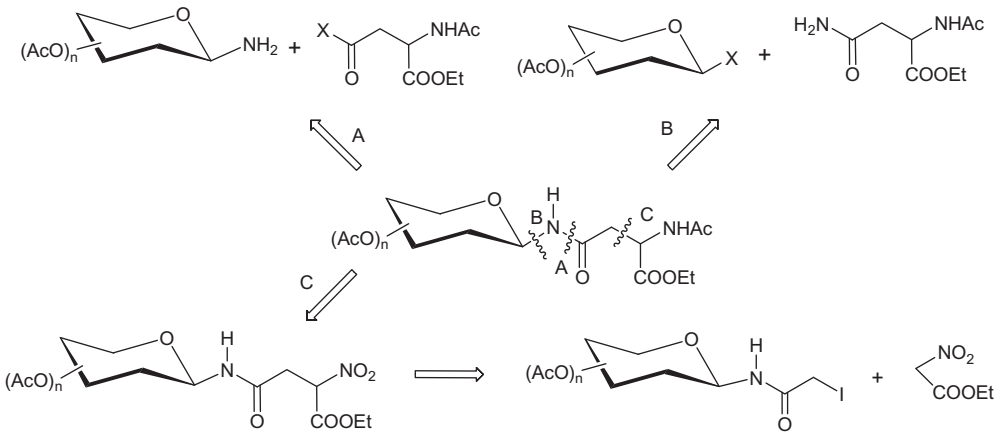
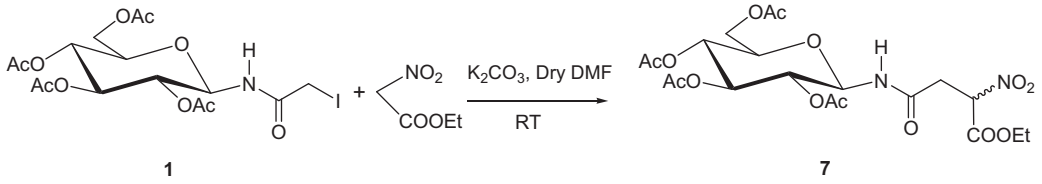


**Scheme 1.**



**Scheme 2.**

in the present work for the synthesis of N-glycoamino acids. To begin with, ethyl nitroacetate and fully protected *N*-(β-glycosyl)chloroacetamide were identified as synthons. The use of nitro group as a latent functionality for the amino group has been well-known in organic synthesis. However, this concept is yet to be exploited for the synthesis of N-glycoamino acids. We reasoned that the newer strategy would represent a general approach to glycoamino acid synthesis, as the key reaction is essentially alkylation of active methylene compounds. Furthermore, the newer strategy has the inherent potential to afford either L- or D-asparagine conjugate by the appropriate modulation of diastereoselectivity of alkylation reaction. The current strategy also gains an advantage by employing fully protected *N*-(β-glycosyl)chlorocetamides[11] as alkylating agents, which are readily prepared, stable, and easy to handle crystalline solids unlike glycosylamines used in the above-mentioned first strategy.

In order to facilitate the reaction at room temperature, fully acetylated *N*-(β-glycosyl)iodoacetamides (**1**–**6**) were actually chosen as facile-alkylating agents in the present work. These were prepared in excellent yield (91–95%) from the corresponding fully acetylated *N*-(β-glycosyl)chloroacetamides[11] by the displacement of chloride with iodide using KI in aqueous acetone at room temperature. The only known iodoacetamide (**4**) derived from GlcNAc was characterized based on the comparison of physical and spectral data with those reported in the literature,[12] whereas all the other five hitherto unknown compounds, **1**–**3** and **5**–**6**, were fully characterized based on physical and spectral methods. The signal assignable to the methylene carbon carrying the iodo group typically appears around −2.3 ppm in the $^{13}C$ NMR spectra of these iodoacetamido sugars, up-field shifted from that of the corresponding chloroacetamido derivatives (seen at 42.2 ppm), and this observation is consistent with the well-known heavy atom effect of iodine.

The initial alkylation was performed by reacting per-*O*-acetylated *N*-(β-D-glucopyranosyl)iodoacetamide (**1**) with ethyl nitroacetate using $K_2CO_3$ as the base in dry DMF at room temperature (Scheme 2).[13] After complete consumption of the starting material **1** in 6 h, the reaction mixture was worked-up and the crude product obtained was purified by column chromatography to afford the desired product **7** as a diastereomeric mixture in 62% yield. The $^1H$ NMR spectrum of **7** displayed a signal at 5.69 ppm, as a doublet of doublets with the coupling constants of 3.6 and 10.8 Hz, assignable to the α-hydrogen (CH proton) of one diastereomer of the monoalkylated ethyl nitroacetate. The CH proton signal of the other diastereomer was seen at 5.59 ppm as a triplet with a coupling constant of 6.8 Hz. The diastereomeric composition was determined to be 55:45 based on the integral intensities of these two signals. The signals of methylene protons adjacent to the above-mentioned CH protons of the two diastereomers were seen as multiplets in the range 3.26–3.12 and 3.09–2.97 ppm. This was confirmed by a pair of cross peaks with the chemical shift co-ordinates of 5.69 ppm and 3.19 ppm that established the spin connectivities between CH proton and the methylene protons of one diastereomer in the $^1H$–$^1H$ gradient COSY spectrum. Similarly, another pair of cross peaks with the chemical shift co-ordinates of 5.59 ppm and 3.04 ppm established the spin connectivities between CH proton and the methylene protons of the other diastereomer.

Prompted by the recent progress in asymmetric alkylation,[14] a diastereoselective synthesis of per-O-acetylated *N*-(β-glycosyl)asparagine precursors was then explored. Anthracenylmethyl ammonium salts derived from cinchonine and cinchonidine have been shown to be useful chiral organocatalysts in the enantioselec-

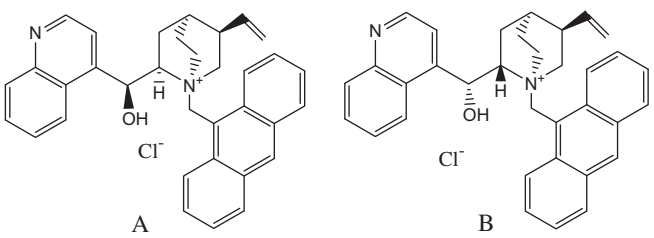
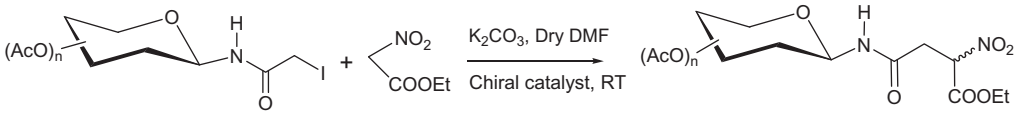


**Figure 1.** Chiral organocatalysts derived from cinchonine (A) and cinchonidine (B).

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**Scheme 3.**

**Table 1**
Reaction of various per-O-acetylated *N*-(β-glycosyl)iodoacetamides with ethyl nitroacetate in the presence of chiral catalyst A

| Entry | Glycosyliodoacetamide | Product | dr[a] | Yield[b] (%) |
|---|---|---|---|---|
| 1 | Glcβ(**1**) | **7** | 82:18 | 68 |
| 2 | Galβ(**2**) | **8** | 69:31 | 52 |
| 3 | Manβ(**3**) | **9** | 66:34 | 58 |
| 4 | GlcNAcβ(**4**) | **10** | 62:38 | 59 |
| 5 | L-Rhaβ(**5**) | **11** | 62:38 | 56 |
| 6 | Celloβ(**6**) | **12** | 66:34 | 55 |

[a] Determined based on $^1$H NMR.
[b] Yield of isolated pure product.

tive alkylation of benzophenone-imine derived from *tert*-butyl glycinate under phase-transfer conditions.[15] The feasibility of the diastereoselective alkylation was first examined by reacting *N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)iodoacetamide with ethyl nitroacetate in the presence of $K_2CO_3$ as a base and *N*-(9-anthracenylmethyl)cinchoninium chloride (Fig. 1A) as the chiral catalyst (10 mol %) at room temperature in dry DMF medium (Scheme 3).[16] After 6 h of stirring, when the starting material disappeared, the reaction mixture was worked-up. Column chromatography of the crude product over silica gel afforded the desired product, **7**, in 68 % yield. The diastereomeric ratio of the product (**7**) was determined to be 82:18 based on $^1$H NMR as described earlier.

The generality of the above-described diastereoselective alkylation was then examined by using several fully acetylated *N*-(β-glycosyl)iodoacetamides (Table 1). All the six fully acetylated *N*-(β-glycosyl)asparagine precursors (**7–12**) were obtained in fairly good yields and characterized based on physical and spectral methods including two-dimensional NMR and high resolution ESI mass spectrometry.[17a] Compound **10** is the precursor of the GlcNAc-Asn linkage conserved in all eukaryotic *N*-glycoproteins.[18a] Glc-Asn and L-Rha-Asn linkages are rare and known to occur in glycoproteins of *Halobacter halobium*[18b] and *Bacillus stearothermophillus*,[18c] respectively. Compounds **7** and **11** would serve as useful precursors for preparing such rare linkages. Besides these, the alkylation was successful with the iodoacetamides derived from other monosaccharides, Man, Gal and the disaccharide, cellobiose (affording the interesting analog, **12**, of chitobiosylasparagine), all in good yields and with diastereoselectivity ranging from 24% to 64%.

Efforts undertaken to elucidate the absolute configuration of the major diastereomer unambiguously based on X-ray diffractometry proved to be fruitful. Satisfyingly, crystallization of **7** from a mixture of ethyl acetate and hexane afforded single crystals. The crystal structure of the major isomer **7** was solved in the orthorhombic space group $P2_12_12_1$. The relevant details of data collection and refinement are given in Table 2. Analysis of the structure has shown that the major isomer is the one having an absolute configuration of S at the α-carbon of the monosubstituted ethyl nitroacetate moiety, which is a precursor of the naturally occurring L-asparagine conjugate (Fig. 2). The pyranose ring exists in $^4C_1$ conformation. The *N*-glycosidic torsion angle, $\varphi_N$, O5–C1–N1–C7, was found to be 104.9°. The side chain dihedral angle, $\chi_2$, N1–C7–C8–C9, turned out to be 158.5° revealing the *anti* conformation. A single hydrogen bond involving N1–H and O8 stabilizes the molecular packing in the crystal. The monoalkylated product **10** derived from *N*-(β-D-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-glucopyranosyl)iodoacetamide was also subjected to recrystallization. The diastereomeric ratio of the twice recrystallized product was estimated to be 94:6 based on $^1$H NMR.[17b] However, efforts made to get a single crystal of this twice recrystallized product for X-ray crystallographic analysis proved in vain.

The use of cinchonidine-derived ammonium salt ( Fig. 1B, 10 mol %), a pseudoenantiomer of the chiral catalyst A, in the reaction of fully acetylated *N*-(β-D-glucopyranosyl)iodoacetamide with ethyl nitroacetate also afforded **7** as a mixture of diastereomers in 66 % yield with a composition of 78:22, estimated based on its $^1$H NMR data. Crystallization of this mixture from a mixture of ethyl acetate and hexane afforded single crystals of the major isomer in pure form, which was confirmed by X-ray crystallographic analysis to be structurally identical with the same stereoisomer obtained from the earlier reaction catalyzed by *N*-(9-anthracenylmethyl)cinchoninium chloride (Fig. 1A).

**Table 2**
Data collection and refinement parameters for **7**

| Parameter | Compound **7** | Parameter | Compound **7** |
|---|---|---|---|
| Empirical formula | $C_{20}H_{28}N_2O_{14}$ | Crystal size (mm) | 0.3 × 0.2 × 0.2 |
| Formula weight | 520.44 | Theta range for data collection (°) | 2–18 |
| Wavelength (Å) | 0.71073 | Reflections collected/unique | 6288/6288 [*R*(int) = 0.0892] |
| Crystal system space group | $P2_12_12_1$, orthorhombic | Data restraint parameters | 6288/0/329 |
| Unit cell dimensions | *a* = 8.1416(3) Å | Index ranges | $-10 \leqslant h \leqslant 8$ |
| | *b* = 11.5681(6) Å | | $-15 \leqslant k \leqslant 15$ |
| | *c* = 27.0124(15) Å | | $-28 \leqslant l \leqslant 36$ |
| | α = 90 | | |
| | β = 90 | | |
| | γ = 90 | | |
| Volume (A$^3$) | 2544.1(2) | Final *R* indices [2<sigma> (*I*)] | $R_1$ = 0.0650 $wR_2$ = 0.1330 |
| Absorption coefficient (mm$^{-1}$) | 0.117 | *R* indices (all data) | $R_1$ = 0.2398 $wR_2$ = 0.1932 |
| *F* (0 0 0) | 1096 | Goodness-of-fit on $F^2$ | 0.902 |

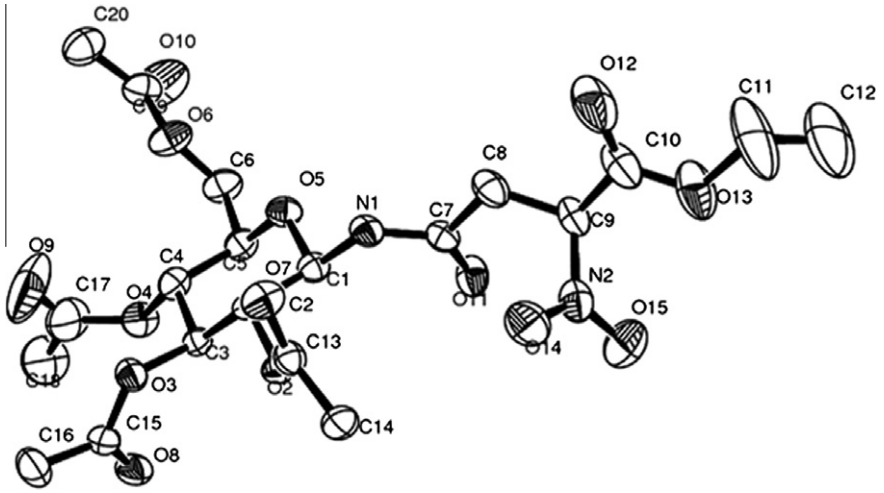


**Figure 2.** ORTEP with atom numbering of the major isomer of compound **7**.

To conclude, a newer and general synthetic strategy for *N*-(β-glycosyl)asparagine precursors has been developed based on the alkylation of ethyl nitroacetate using six different per-O-acetylated *N*-(β-glycosyl)iodoacetamides in good yield and with moderate diastereoselectivity. There is scope for improving the diastereoselectivity by screening a large number of newly emerging organocatalysts. Modulation of diastereoselectivity would enable the conjugation of the sugar to either L- or D-asparagine precursor thus facilitating the synthesis of natural as well as unnatural glycoaminoacids and glycopeptides.

X-ray crystallographic data: Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 780295. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.Uk).

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13. *Typical procedure for the synthesis of per-O-acetylated N-(β-D-glucopyranosyl)asparagine precursor* (**7**)*—uncatalyzed reaction*: To a stirred solution of anhydrous $K_2CO_3$ (207 mg, 1.5 mmol) in dry DMF (5 mL), ethyl nitroacetate (138 mg, 1 mmol) was added slowly at room temperature under nitrogen atmosphere. After stirring the reaction mixture for about 0.5 h, a solution of fully acetylated *N*-(β-D-glucopyranosyl)iodoacetamide (515 mg, 1 mmol) in dry DMF (3 mL) was added and stirring continued. The progress of the reaction was monitored by TLC analysis. Following completion of reaction in 6 h, the reaction mixture was diluted with ethyl acetate (50 mL). The resulting solution was washed with water (30 mL × 2), then with brine solution (30 mL), dried over anhydrous sodium sulfate and concentrated to dryness to get a sirup that was purified by column chromatography over silica gel (100–200 mesh) to obtain the product (**7**) as a mixture (55:45) of diastereomers.
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16. *General procedure for the synthesis of per-O-acetylated N-(β-glycosyl)asparagine precursors—catalyzed reaction*: To a stirred solution of anhydrous $K_2CO_3$ (207 mg, 1.5 mmol) and chiral catalyst A (10 mol %) in dry DMF (5 mL), ethyl nitroacetate (138 mg, 1 mmol) was added slowly at room temperature under nitrogen atmosphere. After stirring the reaction mixture for about 0.5 h at room temperature, a solution of *N*-(β-glycopyranosyl)iodoacetamide (1 mmol) in dry DMF (3 mL) was slowly added and stirring continued. The reaction and the work-up were performed as described above.[13] The crude product obtained was purified by column chromatography (100–200 mesh) to furnish analytically pure products, **7**–**12**, as diastereomeric mixtures.
17. (a) *N-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-3-nitro-4-oxobutan amide* (**7**): Sirup; $^1$H NMR ($CDCl_3$, 400 MHz): δ 6.86–6.78 (m, 1H, –N*H*), 5.69 (dd, 0.55H, *J* = 3.6 and 10.8 Hz, –C*H*$NO_2$–), 5.59 (t, 0.45H, *J* = 6.8 Hz, –C*H*$NO_2$–), 5.36–5.20 (m, 2H, H-1′ and H-3′), 5.11–5.02 (m, 1H, H-4′), 4.99–4.91 (m, 1H, H-2′), 4.36–4.24 (m, 3H, H-6a′ and –OC$H_2$–), 4.12–4.03 (m, 1H, H-6b′), 3.89–3.78 (m, 1H, H-5′), 3.26–3.12 (m, 1H, –NHCOC$H_2$–), 3.09–2.97 (m, 1H, –NHCOC$H_2$–), 2.11–2.01 (4s, 4 × –COC$H_3$), 1.31 (t, 3H, *J* = 7.2 Hz, –OCH$_2$C$H_3$); $^{13}$C NMR ($CDCl_3$, 100 MHz):

$\delta$ 171.5–169.6 (4 × –*C*OCH$_3$), 167.7, 167.5, 163.9 (–NHCOCH$_2$–), 163.7 (–NHCOCH$_2$–), 83.2 (–*C*HNO$_2$), 83.0 (–*C*HNO$_2$), 78.1 (C-1′), 73.7, 73.6, 72.6, 72.5, 70.5, 70.4, 68.1, 63.6, 63.5, 61.7, 61.6, 36.1 (–NHCOCH$_2$–), 20.7–20.5 (4s, 4 × –COCH$_3$), 13.8 (–OCH$_2$*C*H$_3$); ESI-MS: Calcd for $C_{20}H_{28}N_2O_{14}Na$: 543.1440 [M+Na]$^+$. Found: 543.1438.

(b) *N-(2′-Acetamido-2′-deoxy-3′,4′,6′-tri-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-3-nitro-4-oxobutanamide* (**10**): Sirup; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.44 (d, 1H, *J* = 8.0 Hz, –N*H*), 6.20 (d, 1H, *J* = 8.0 Hz, –N*H*′), 5.73–5.68 (m, 1H, –C*H*NO$_2$–), 5.17–5.00 (m, 3H, H-1′, H-3′ and H-4′), 4.38–4.22 (m, 3H, –OC*H*$_2$–, H-6a′), 4.20–4.03 (m, 2H, H-2′ and H-6b′), 3.76 (m, 1H, H-5′), 3.25–3.10 (m, 1H, –NHCOC*H*$_2$–), 3.06–2.94 (m, 1H, –NHCOC*H*$_2$–), 2.09, 2.08, 2.05, 2.03 (4 × –COC*H*$_3$), 1.31 (t, 3H, *J* = 7.1 Hz, –OCH$_2$C*H*$_3$); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 173.0, 171.9, 170.6, 169.2 (4 × –*C*OCH$_3$), 168.1, 163.9 (–NHCOCH$_2$–), 83.2 (–*C*HNO$_2$), 80.6 (C-1′), 73.6, 72.6, 67.6, 63.5, 61.7, 53.4, 36.1 (–NHCOCH$_2$–), 23.0, 20.7, 20.6, 20.5 (4 × –CO*C*H$_3$), 13.8 (–OCH$_2$CH$_3$); ESI-MS: Calcd for $C_{20}H_{29}N_3O_{13}Na$: 542.1597 [M+Na]$^+$. Found 542.1598.

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