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The C-Glycosyl Analog of an N-Linked Glycoamino Acid

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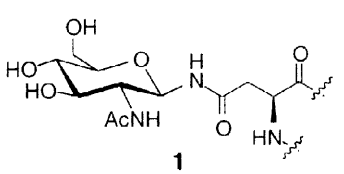
Abstract: The synthesis of a new glycoamino acid derivative, a direct C-analog of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-Asn is described. The C-glycoside is prepared by a tandem Horner-Emmons-Wadsworth olefination-Michael addition between an aspartyl β-keto phosphonate and a 4,6-O-benzylidene GlcNAc sugar.

Keywords: C-Glycosides; glycopeptides; phosphonates; Wittig reactions.

In N-linked glycoproteins, N-acetyl-glucosamine (GlcNAc) is attached via a β-1-N glycosidic bond to the Asn side chain carboxamide (**1**), while in O-linked glycoproteins carbohydrates are attached to the Ser/Thr side chain.[1] Carbon analogs of these glycoamino acids can be produced by replacing the C-N or C-O glycosidic bond with a C-C bond. C-glycoside analogs should be resistant to chemical and enzymatic hydrolysis and may serve as enzyme inhibitors,[2] or as ligands for molecular recognition.[3] Replacement of O by a methylene group has provided C-glycosyl analogs of O-linked glycoamino acids.[4,5] While the syntheses of retroamide **2**,[6] glycosyl methylamide **3**,[7] and C-glycoside **4**[8] have been described, direct replacement of the Asn NH by CH_2 to give a C-linked glycoamino acid such as **5** has not yet been reported. To obtain specific glycoamidase inhibitors we seek to prepare glycopeptides containing the C-glyco amino acid **5**. Herein, we describe the synthesis of a new glycoamino acid derivative **9**, a direct C-analog of N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-Asn **1**.



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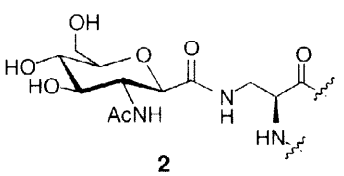

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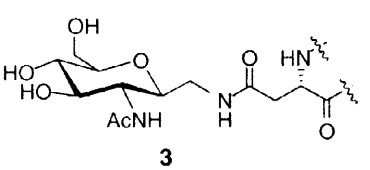

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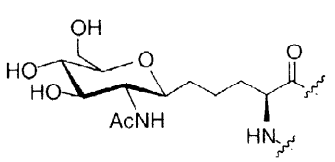

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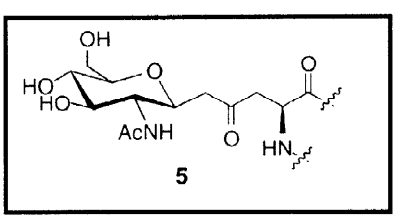

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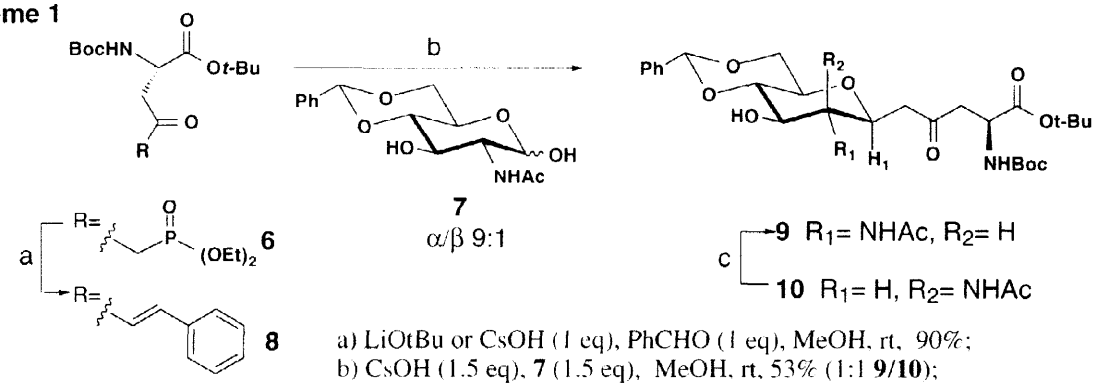
PII: S0040-4039(98)02065-6

The crucial step in the synthesis of C-glycoside **9** involves a tandem Horner-Emmons-Wadsworth (HWE) olefination-Michael addition between aspartyl β-ketophosphonate **6**[9] and 4,6-O-benzylidene GlcNAc **7**[10] (Scheme 1). While HWE strategies exist to make C-linked glycosides from 2-amino[11-13] and 2-oxo sugars,[14,15] the phosphonate reagents used were of limited structural complexity. Amino acid (S)-phosphonate **6** {$[\alpha]_D$= +16.7 (c=1.0, $CHCl_3$); lit. $[\alpha]_D$= +16.2 (c=0.5, $CHCl_3$)[9]} was prepared in 78% overall yield from t-BOC-Asp-α-*t*-butyl ester by esterification with diazomethane, followed by condensation with $LiCH_2PO(OEt)_2$.[16] Two problems were encountered during HWE olefination of GlcNAc **7**: 1) reduced reactivity of the hemi-acetal form of **7**, and 2) epimerization of the GlcNAc C2 position. Outlined below, is our approach to solving these problems in the preparation of C-glycosyl amino acid **9**.

Scheme 1



a) LiOtBu or CsOH (1 eq), PhCHO (1 eq), MeOH, rt, 90%;
b) CsOH (1.5 eq), **7** (1.5 eq), MeOH, rt, 53% (1:1 **9**/**10**);
c) LiOtBu (4 eq), MeOH, rt, 100%.

Anticipating reactivity problems due to the hemi-acetal form of **7**, we first optimized the nucleophilicity of the phosphonate anion of **6** by varying the solvent and base. Deprotonation of β-keto phosphonates gives two possible enolates: the Z-enolate, with the cation chelated to the oxygens, or the E-enolate with the cation loosely coordinated to the anion (Table 1).[17,18] We found that the structure and reactivity of the anion derived from phosphonate **6** were influenced by the solvent and cation. Addition of LiOtBu (1 equiv) to β-keto phosphonate **6** in either CD_3CN, d_6-DMSO or CD_3OD, caused diagnostic changes in the NMR spectra. In CD_3CN and d_6-DMSO, the ^{31}P NMR signal moved from ~20 ppm for **6** to a single resonance at higher frequency for the anion (δ=31.5 ppm in CD_3CN and δ=34.2 ppm in d_6-DMSO). Based on β–keto-phosphonate literature, these NMR chemical shifts are consistent with formation of the Li^+-chelated Z-enolate.[17,18] Addition of LiOtBu to **6** in CD_3OD gave a 2:1 ratio of Z-enolate (δ=34.3 ppm) and E-enolate (δ=35.6 ppm). Changing the cation from Li^+ to Cs^+ in CD_3OD further increased the amount of E-enolate (Z/E ≈ 1/2.5). To correlate structure with reactivity, the HWE reaction of phosphonate anions of **6** and benzaldehyde (1 equiv) to give α,β-unsaturated ketone **8** and diethylphosphate was monitored by ^{31}P NMR. The HWE reaction of benzaldehyde and the Li^+ enolate of **6** was faster in CD_3OD than in either d_6-DMSO or CD_3CN (Table 1). The HWE reaction rate was further accelerated in CD_3OD when using the Cs^+ enolate of **6**. These relative rates are consistent with the phosphonate's E-enolate being more nucleophilic than the Z-enolate. Indeed, the E-enolate is more reactive than the Z-enolate for the related β-diketones.[19]

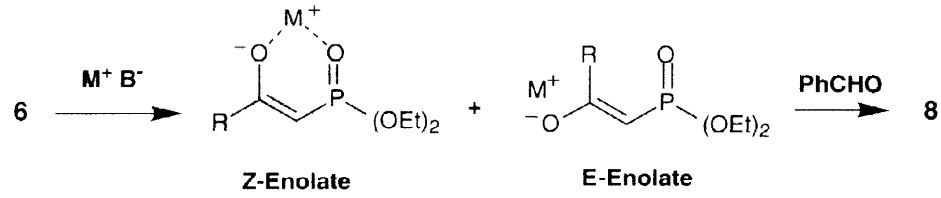


Table 1. The Effect of Solvent and Counter-Cation on the HWE Reaction of **6** with Benzaldehyde.

Entry	Solvent	Base[a]	Z/E Ratio[b]	Time	% Conversion[c]
1	CD_3CN	LiOtBu	>95/5	7 h	5
2	DMSO-d_6	LiOtBu	>95/5	7 h	35
3	CD_3OD	LiOtBu	≈ 2/1	1 h	75
4	CD_3OD	CsOH	≈ 1/2.5	0.5 h	85

a. The phosphonate anion of **6** was generated by addition of 1 equiv of base.

b. Determined by ^{31}P NMR. Z and E-enolates were assigned by analogy to diethyl (2-oxo-propyl) phosphonate.[17]

c. Monitored by ^{31}P NMR by following formation of diethyl phosphate (δ≈0.0 ppm) and disappearance of the phosphonate anion of **6**. Monitoring the appearance of **8** using ^{1}H NMR showed similar kinetics.

Having optimized reaction of phosphonate **6** with benzaldehyde the HWE reaction was performed with GlcNAc **7**. This reaction was slower than with benzaldehyde, due to predominance of the hemi-acetal of **7**. Nonetheless, reaction of the Cs^+-enolate of **6** with 1.5 equivalents of **7** in CH_3OH at 20 °C for 96 h produced a 1:1 mixture of two diastereomers in 53 % purified yield (Scheme 1). The isomers were separated by chromatography. Analysis of 2D COSY and NOESY NMR data identified the two diastereomers to be the desired C-linked glycoside, GlcNAc **9**, and its C2-epimer ManNAc **10**. In particular, the chemical shift difference for the H2 resonance of **9** (δ=3.67 ppm) and **10** (δ=4.30 ppm) suggested that C2 epimerization occurred during the HWE reaction. Both GlcNAc **9** and ManNAc **10** had strong H1-H3 and H1-H5 NOEs, indicating that these C-glycosides had a C1 β–configuration. The stereochemistry at C2 was deduced from coupling constants. GlcNAc **9** had $^{3}J_{1,2}$ = 9.9 Hz, while the coupling constant for **10** ($^{3}J_{1,2}$=1.6 Hz) was consistent with its manno configuration. NOE data also supported the C2 configuration for GlcNac **9** and ManNAc **10**. Thus, the NH of ManNAc **10** (NH2) had a strong NOE to H4, but no NH2-H1 or NH2-H3 NOEs. As expected for a gluco isomer, GlcNAc **9** had NH2-H1, NH2-H2, and NH2-H3 NOEs, but no NH2-H4 NOE.

Initial attempts to suppress GlcNAc C2 epimerization during the HWE reaction failed. The Masamune-Roush HWE modification,[20] or HWE olefination of an N2-t-Boc GlcNAc derivative both gave 1:1 mixtures of gluco and manno isomers. Molecular mechanics calculations (321-G*) show GlcNac **9** to be lower in energy than ManNAc **10** by 2.8 kcal/mol, indicating that ManNAc **10** should be equilibrated to **9** by a base-catalyzed retro-Michael reaction. Initial attempts to epimerize ManNAc **10** to GlcNAc **9** are promising. In one set of experiments purified **9** and **10** were treated separately with 4 equivalents of LiOtBu in MeOH. Under these conditions, ManNAc **10** was converted to GlcNAc **9**, while GlcNAc **9** was recovered unchanged. If we are unable to find HWE conditions that give only GlcNAc **9** then equilibration of ManNAc **10** to **9** promises to solve the C2 epimerization problem.

The preparation of C-glycosyl amino acid derivative **9** represents the first synthesis of a direct C-isostere of N^4-(2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-L-Asn **1**. Our preliminary work shows that HWE-Michael reaction of aspartate-derived phosphonates and reducing sugars in protic solvents is a straightforward method for the synthesis of C-glycosyl amino acids. Using both stepwise and convergent approaches we plan to incorporate C-glycosyl amino acids into peptides. Development of C-glycopeptides as specific enzyme inhibitors, and study of C-glycopeptide solution conformation, are ongoing.

Analysis of C-Linked GlcNAc 9: ^{1}H NMR (500 MHz, CD_3CN) δ 7.47-7.35 (m, 5H), 6.41 (d, 1H, NHAc, J= 8.7 Hz), 5.55 (s, 1H), 5.50 (d, 1H, BOC NH, J= 7.9 Hz), 4.29 (m, 1H, α-H), 4.15 (dd, 1H, $H6_{eq}$, J= 4.7, 10.3 Hz), 3.84 (ddd, 1H, H1, J= 3.2, 8.8, 9.9 Hz), 3.67 (m, 1H, H2), 3.61 (m, 2H, H3, $H6_{ax}$), 3.46 (dd, 1H, H4, J= 9.5, 9.5 Hz), 3.38 (ddd, 1H, H5, J= 4.7, 9.5, 10.3 Hz), 2.91 (dd, 1H, βH_a, J= 6.4, 17.5 Hz), 2.83 (dd, 1H, βH_b, J= 4.8, 17.5 Hz), 2.62 (dd, 1H, $H7_a$, J= 3.2, 16.7 Hz), 2.54 (dd, 1H, $H7_b$, J= 8.8, 16.7 Hz), 1.88 (s, 3H, CH_3), 1.39 (s, 18H); ^{13}C NMR (125 MHz, CD_3CN) δ 206.7, 171.7, 171.6, 156.4, 139.0, 129.9, 129.1, 127.2, 102.2, 82.6, 82.3, 76.4, 73.3, 71.2, 69.3, 56.7, 55.2, 51.1, 46.6, 45.5, 28.5, 28.1, 23.2; IR: ($CHCl_3$, cm^{-1}): 3450, 1725, 1711, 1640, 1630; FAB-MS(%) (M^++1) 579 (25.1); HRMS-FAB calc. for (M^++1) $C_{29}H_{43}O_{10}N_2$ 579.2918, found 579.2959.

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