Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 997–999

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Stereoselective Synthesis of a *C*-Glycoside Analogue of *N*-Fmoc-Serine β-*N*-Acetylglucosaminide by Ramberg–Bäcklund Rearrangement

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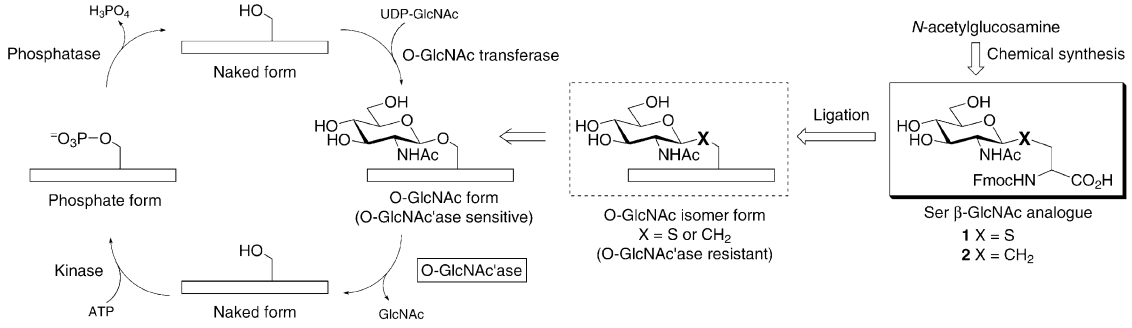
Received 20 November 2001; accepted 4 February 2002

Abstract—A *C*-glycoside analogue of *N*-Fmoc-serine β-*N*-acetylglucosaminide **1** was synthesized stereoselectively from a sulfone derivative of serinol thio-*N*-acetylglucosamide **8** using a Ramberg–Bäcklund rearrangement as a key step.

Post-translational modification with a β-*O*-linked *N*-acetylglucosamine (*O*-GlcNAc) residue has been found at Ser/Thr site of many nuclear and cytoplasmic proteins. It is thought to be one of the key factors regulating their functional roles in many diseases, including cancer, diabetes, and Alzheimer's disease.[1] These glycosylated Ser/Thr sites are often found to be phosphorylated, as is the case for c-*myc*, therefore, a site-directed mutagenesis of the Ser/Thr residues does not offer a promising approach to studying the actual role of *O*-GlcNAc. We envisioned that it would be very informative if a peptide with a nonhydrolyzable analogue replacing the naturally occurring Ser/Thr-linked *O*-GlcNAc can be constructed and used as a molecular probe.

We had previously prepared the *N*-Fmoc-S-GlcNAc **1**, a thio-glycoside analogue of GlcNAc, by a Mitsunobu reaction involving GlcNAc-SH and the hydroxyl group of a protected serine derivative.[2] This compound was



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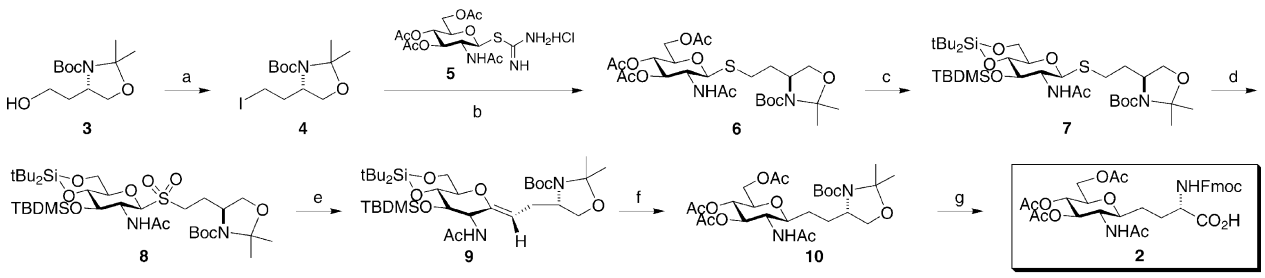
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00104-X



Scheme 1. Reagents and conditions: (a) imidazole, PPh_3, I_2/toluene, 77%; (b) K_2CO_3, $Na_2S_2O_5$/acetone–H_2O, 98%; (c) (i) Et_3N/MeOH–H_2O; (ii) tBu$_2$Si(OTf)$_2$, 2,6-lutidine/DMF, 88% (two steps); (iii) TBDMSCl, imidazole/DMF, 95%; (d) mCPBA, Na_2HPO_4/CH_2Cl_2, 77%; (e) KOH/Al_2O_3, $CBrF_2CBrF_2$/tBuOH, 50 °C, 38%; (f) (i) H_2, Pd(OH)$_2$/EtOAc, 78%; (ii) TBAF/THF; (iii) Ac_2O/pyridine, 68% (two steps); (g) (i) TFA/$CHCl_3$; (ii) FmocCl/iPr$_2$NEt/CH_2Cl_2–MeOH, 69% (two steps); (iii) Jones oxidation/acetone, 77%.

used as a building block for solid-phase peptide synthesis of a C-terminal sequence of CKII peptide, and the resulting glycopeptide analogue has been confirmed to be resistant to an enzymatic hydrolysis.[3] Since *C*-glycoside is a chemically more stable analogue of a naturally occurring *O*-glycoside than the corresponding *S*-glycoside, we turned our attention to the synthesis of a *C*-glycoside analogue of *O*-GlcNAc.

Although *C*-glycoside analogues of neutral sugar linked to amino acids have been prepared by a number of groups,[4] synthetic approaches to β-linked 2-*N*-acetamidoglycoside analogues are rather rare due to the incompatibility of the 2-amino function with common *C*-glycoside synthesis. Our attempt to synthesize a *C*-glycoside analogue of GlcNAc-Ser employing Strecker reaction[5] and Sharpless asymmetric aminohydroxylation[6] were unsuccessful. Strecker reaction gave cyclized products between terminal aldehyde and the *C*-2 nitrogen of the 2-*N*-acetamido groups. Sharpless asymmetric aminohydroxylation gave inseparable complex mixtures.[7]

Two synthetic approaches have been reported for *C*-glycoside analogues of GlcNAc-*N*-Boc-Ser. One involved a Wittig reaction of a *C*-GlcNAc aldehyde by Schmidt and Fuchs,[8] and the other employed a ring-opening reaction of a lactam derivative of serine by a dilithio derivative of GlcNAc by Westermann and co-workers.[9] However, these strategies required a separation of D- and L-serine moiety that was introduced at the later stage. Very recently, Franck and co-workers[10] published a synthesis of a *C*-glycoside analogue of glucosaminyl ceramide using Ramberg–Bäcklund (RB) rearrangement[11] as a key step. Inspired by this elegant synthesis, we anticipated that the L-configuration of the serine moiety would be easily introduced as a protected amino alcohol RB rearrangement, and it could be easily converted into serine derivatives. We report herein a convenient synthesis of *C*-glycoside analogue of β-GlcNAc-*N*-Fmoc-Ser derivative **2**, which is a suitable building block for a solid-phase synthesis of *C*-glycopeptide.

A known *N*-Boc amino alcohol **3**[12] was converted to iodide **4**[13] with I_2, Ph_3P, and imidazole (Scheme 1). A coupling reaction between **4** and isothiourea **5** was carried out under the *S*-alkylation condition[2] in the presence of K_2CO_3/$Na_2S_2O_5$ gave a thioglycoside **6**[14] in good yield. We replaced the base-sensitive *O*-acetyl groups to a stable di-*tert*-butylsilylene group for protecting the 4- and 6-hydroxyls and a *tert*-butyl-dimethylsilyl group for the 3-OH group for the upcoming RB reaction. *O*-Deacetylation of **6** with Et_3N/MeOH-H_2O followed by a sequential selective protection of the 4,6-hydroxyl group with di-*tert*-butylsilyl di-trifluoromethansulfonate and with TBDMSCl afforded a silyl-protected thioglycoside **7**.[14] Oxidation of **7** with *m*-chloroperbenzoic acid (mCPBA) and Na_2HPO_4 gave sulfone **8**.[14]

RB rearrangement of **8** was performed under the Frank and co-workers condition.[10,11b] Treatment of **8** with freshly prepared KOH/Al_2O_3 (KOH on Al_2O_3) in tBuOH/$(CBrF_2)_2$ at 50 °C afforded an *exo*-olefinic deoxyglucal **9**[14] in 38% yield. The chemical shift of an olefinic proton at δ 4.73 ppm indicated that the generated *exo*-deoxyglucal possessed a *Z*-olefinic configuration. Hydrogenation of **9** over Pearlman's catalyst gave a product as a single isomer. We confirmed its stereochemistry to be a β-configuration in **10**[14] after converting the *O*-silyl protective groups of **9** to *O*-acetyl groups by *O*-desilylation with TBAF and by acetylation. In its ^{1}H NMR spectrum in DMSO-d_6/D_2O, **10** revealed a large coupling constant for the *C*-2 proton ($J_{1,2}$ = 10 Hz) that supports a β-configuration at the *C*-1 stereochemistry.[15] The Boc group of **10** was replaced with an Fmoc group by treating with trifluoroacetic acid (TFA) and subsequently with Fmoc-Cl in the presence of Hoünig's base. The resulting Fmoc-amino alcohol was oxidized with Jones' reagent gave the *C*-GlcNAc-*N*-Fmoc-Ser **2**[14] in 77% yield.

In summary, we present a convenient synthetic route to β-GlcNAc-1-methylene-*N*-Fmoc-Ser **2** as a potentially useful molecular probe for studying a functional role of *O*-GlcNAc. This approach is highly stereoselective and avoids a potential epimerization step on amino acid moiety.

Acknowledgements

The NMR studies were performed in the Biochemistry NMR Facility at Johns Hopkins University, which was established by a grant from the National Institutes of Health (GM 27512) and a Biomedical

Shared Instrumentation Grant (1S10-RR06262-0). This research was partly supported by the National Institutes of Health (GM 52324). We thank Dr. Shirley Wacowich-Sgarbi (Optimer Pharmaceuticals, Inc.) for critical reading of this manuscript.

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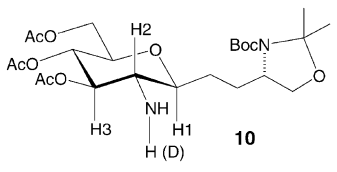
14. Analytical data for compound **6**: ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ 7.74 (1H, d, J = 8.4 Hz, –NH), 5.16 (1H, t, J = 9.6 Hz, H-3), 4.88 (1H, t, J = 9.6 Hz, H-4), 4.76 (1H, d, J = 10.4 Hz, H-1), 4.76 (1H, dd, J = 12.2, 5.0 Hz, H-6a), 4.17 (1H, dd, J = 12.2, 2.6 Hz, H-6b), 3.97–3.78 (5H, m), 2.66 (2H, m), 2.05 (3H, s, –OAc), 2.01 (3H, s, –OAc), 1.96 (3H, s, –OAc), 1.84 (3H, s, –NHAc), 1.53 (3H, s, Me), 1.48 (9H, s, *tert*-Bu), 1.46 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ 170.4, 170.1, 169.73, 169.69, 151.9, 93.5, 84.0, 79.7, 75.5, 74.5, 69.8, 67.2, 62.9, 56.9, 53.2, 34.9, 28.8, 28.7, 27.6, 26.3, 24.3, 23.1, 20.9, 20.84, 20.78; HRMS (FAB) m/z calcd for $C_{26}H_{43}N_2O_{11}SNa$ $(M+Na)^+$ 613.2407, found 613.2408.
For **7**: ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ 7.61 (1H, d, J = 9.2 Hz, –NH), 4.61 (1H, d, J = 10.0 Hz, H-1), 4.14 (1H, dd, J = 10.2, 5.0 Hz), 3.95–3.63 (7H, m), 3.45 (1H, m), 2.62 (2H, m), 1.90 (1H, m 1.84 (3H, s, –NAc), 1.75 (1H, m), 1.52 (3H, s, Me), 1.48 (9H, s, *tert*-Bu), 1.45 (3H, s, Me), 1.07 (9H, s, *tert*-Bu), 1.04 (9H, s, *tert*-Bu), 0.90 (9H, s, *tert*-Bu), 0.16 (3H, s, SiMe-a), 0.10 (3H, s, SiMe-b); ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ 169.3, 151.9, 93.4, 84.8, 79.6, 78.8, 77.1, 74.5, 67.2, 66.4, 57.1, 57.0, 34.4, 28.74, 28.68, 27.9, 27.8, 27.4, 26.3, 23.6, 22.8, 20.1, 18.3, −3.5, −4.0; HRMS (FAB) m/z calcd for $C_{34}H_{66}N_2O_8SSi_2Na$ $(M+Na)^+$ 741.4678, found 741.3999.
For **8**: ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ 7.81 (1H, d, J = 8.0 Hz, –NH), 4.80 (1H, d, J = 10.0 Hz, H-1), 4.20 (1H, dd, J = 10.2, 4.6 Hz), 3.98–3.62 (4H, m), 3.86–3.77 (2H, m), 3.69–3.66 (2H, m), 2.06 (2H, m), 1.93 (1H, m), 1.92 (3H, s, –NAc), 1.54 (3H, s, Me), 1.49 (9H, s, *tert*-Bu), 1.46 (3H, s, Me), 1.08 (9H, s, *tert*-Bu), 1.04 (9H, s, *tert*-Bu), 0.92 (9H, s, *tert*-Bu), 0.16 (3H, s, SiMe-a), 0.10 (3H, s, SiMe-b); ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ 170.0, 151.9, 93.7, 88.6, 80.0, 78.1, 76.1, 74.2, 67.0, 66.1, 56.3, 51.8, 47.0, 28.7, 28.6, 27.8, 27.4, 26.3, 25.4, 23.7, 22.7, 20.1, 18.3, 3.6, 4.0; HRMS (FAB) m/z calcd for $C_{34}H_{66}N_2O_{10}SSi_2Na$ $(M+Na)^+$ 773.3875, found 773.3901.
For **9**: ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ 7.79 (1H, d, J = 9.2 Hz,-NH), 4.79 (1H, dt, J = 7.4, 1.8 Hz, vinylic), 4.35 (1H, dt, J = 9.2, 1.2 Hz), 4.22 (1H, dd, J = 10.4, 5.2 Hz), 3.97–3.82 (4H, m), 3.64–3.56 (2H, m), 3.39 (1H, m), 2.38 (2H, m), 1.91 (3H, s, –NAc), 1.52 (3H, s, Me), 1.47 (9H, s, *tert*-Bu), 1.45 (3H, s, Me), 1.09 (9H, s, *tert*-Bu), 1.03 (9H, s, *tert*-Bu), 0.91 (9H, s, *tert*-Bu), 0.17 (3H, s, SiMe-a), 0.13 (3H, s, SiMe-b); ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ 169.2, 151.9, 151.6, 93.6, 79.6, 78.7, 77.2, 74.7, 66.8, 66.4, 56.8, 53.8, 28.8, 28.7, 27.9, 27.4, 26.3, 23.4, 22.8, 20.1, 18.3, −3.6, −4.0; HRMS (FAB) m/z calcd for $C_{34}H_{64}N_2O_8Si_2Na$ $(M+Na)^+$ 707.4099, found 707.4099.
For **10**: ^{1}H NMR (DMSO-d_6, 400 MHz, 80 °C) δ 7.63 (1H, d, J = 8.8 Hz, –NH), 5.06 (1H, dd, J = 10.0, 9.6 Hz, H-3′), 4.83 (1H, dd, J = 10.0, 9.6 Hz, H-4′), 4.17–4.06 (2H, m), 3.92 (1H, dd, J = 8.4, 6.0 Hz), 3.86–3.64 (4H, m), 3.47 (1H, m), 2.38 (2H, m), 2.03 (3H, s, –OAc), 2.01 (3H, s, –OAc), 1.95 (3H, s, –OAc), 1.82 (3H, s, –NAc), 1.51 (3H, s, Me), 1.47 (9H, s, *tert*-Bu), 1.44 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 100 MHz, 80 °C) δ 170.3, 170.2, 169.8, 169.7, 151.9, 93.3, 79.4, 78.4, 75.3, 70.2, 67.2, 63.2, 57.3, 53.6, 29.8, 29.4, 28.7, 28.6, 28.2, 27.3, 24.4, 23.0, 20.84, 20.82; HRMS (FAB) m/z calcd for $C_{26}H_{42}N_2O_{11}Na$ $(M+Na)^+$ 581.2687, found 581.2684.
For **2**: ^{1}H NMR (DMSO-d_6, 500 MHz, 80 °C) δ 7.85–7.30 (10H, m, –NH and aromatic), 5.01 (1H, dd, J = 10.0, 9.0 Hz, H-3), 4.77 (1H, dd, J = 10.0, 9.5 Hz, H-4′), 4.28–4.19 (3H, m), 4.13 (1H, dd, J = 12.0, 5.5 Hz, H-6′a), 3.99 (1H, dd, J = 12.0, 2.5 Hz, H-6′b), 3.73–3.62 (3H, m), 3.44 (1H, dt, J = 8.0, 3.0 Hz), 2.38 (2H, m), 1.98 (3H, s, –OAc), 1.95 (3H, s, –OAc), 1.89 (3H, s, –OAc), 1.74 (3H, s, –NAc); HRMS (FAB) m/z calcd for $C_{33}H_{38}N_2O_{12}Na$ $(M+H)^+$ 677.2323, found 677.2319.
15. The *C*-2 proton of **10** appeared as doublet of doublet of doublets in its ^{1}H NMR spectrum (DMSO-d_6). When D_2O was added into the NMR sample tube and vortexed, the *C*-2 amide proton disappeared from the spectrum and the coupling pattern of the *C*-2 proton (H_2) became a doublet of doublets. We concluded that, based on the large coupling constant (12.5, 10.0 Hz), both of the *C*-1 (H1) and *C*-3 (H3) protons are axial relationship to the *C*-2 proton, therefore the configuration at the *C*-1 alkyl substituent as β-stereochemistry.



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