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Facile synthesis of *N*-glycosyl amides using a *N*-glycosyl-2,4-dinitrobenzenesulfonamide and thioacids

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ABSTRACT

N-Glucosyl-2,4-dinitrobenzenesulfonamide was prepared from *N*-acetyl-D-glucosamine and 2,4-dinitrobenzenesulfonyl chloride. Amidation of several thioacids using the *N*-glucosylsulfonamide donor proceeded smoothly to give the desired *N*-glucosylamides in good to high yields. The amidation reactions were carried out at room temperature, under mild conditions, and were completed in a very short time.
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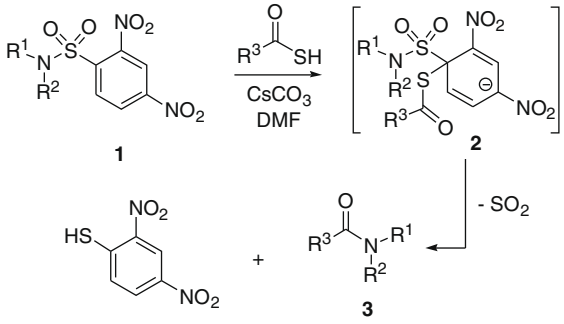
Initially introduced as a protecting group for the controlled alkylation of primary amines to secondary amines,[1] the 2,4-dinitrobenzenesulfonyl group as a sulfonamide derivative **1** (Scheme 1) was adapted to facilitate the formation of amides **3** with thioacids.[2] The reaction proceeds with the *ipso* attack of the thioacid to the sulfonamide to give a Meisenheimer-type complex **2**. The sulfonated amine traps the nearby thioacid upon decomposition of the Meisenheimer-type complex. This chemistry quickly proved to be applicable to the coupling of amino thioacids, peptide fragments, and neoglycoconjugates.[3,4] In these studies, we sought to determine whether or not an *N*-glycosylsulfonamide, has the potential to react with thioacids to provide β-configured glycosyl amides. The *N*-glycosylsulfonamide depicted in Figure 1 contains the 2-acetamido-2-deoxy-β-D-glucopyranosyl unit that in N-linked glycopeptides/proteins is frequently found attached to an asparagine residue.[5] *N*-Glucosylsulfonamides of this type may lend themselves to the convergent assembly of N-linked glycopeptides/proteins[6] considering that the 2,4-dinitrobenzenesulfonyl-thioacid ligation is relatively unaffected by the steric bulk of the reacting fragments.[3] In this paper, we wish to communicate the use of a *N*-glucosylsulfonamide (Fig. 1) as glycosyl donor to access *N*-glycosyl amides.

The 2-acetamido-3,4,6-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine **6** was obtained after a series of functional group manipulations at the anomeric carbon following literature procedures (Scheme 2).[7–9] Sulfonation of the glycosyl amine with 2,4-dinitrobenzenesulfonyl chloride provided the glycosyl donor **6**. Condensation of the glycosyl donor with a simple thioacid, thioacetic acid, in the presence of cesium carbonate and anhydrous DMF afforded the *N*-β-glucosylacetamide **9** in respectable yield of 65%. Employing the same reaction conditions with thiobenzoic acid



Scheme 1. Mechanism of amide formation.

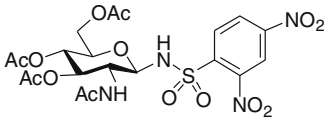


Figure 1. *N*-glucosylsulfonamide.

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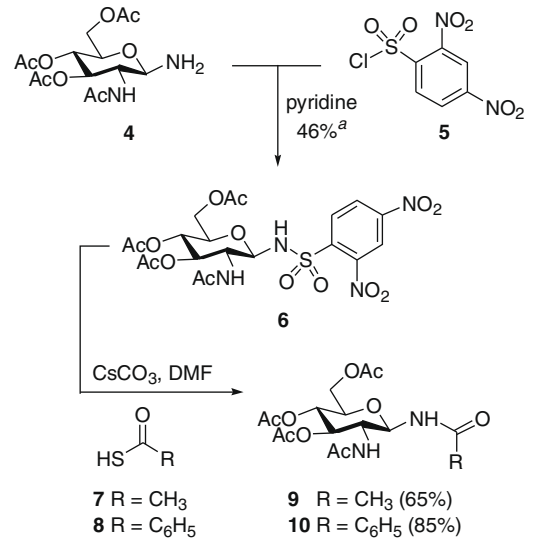
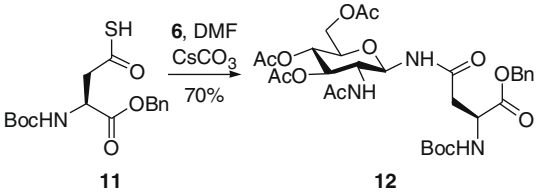


Scheme 2. *N*-Glucosyl amides from sulfonamide **6** (yield over two steps from glucosylazide to glucosyl amine to glucosylsulfonamide).



Scheme 3. *N*-Glycosylation of aspartic acid.

gave a high yield of 85% of the corresponding *N*-β-glucosylbenzamide **10**. In both examples, the reaction was performed at room temperature and was complete in 30 min, indicating the relative ease with which this reaction could be carried out.

Encouraged by these results, we immediately turned our attention to the synthesis of *N*-(β-D-glucosyl)asparagine derivative **12** (Scheme 3). The requisite aspartic acid **11** with a thioacid-modified side chain was prepared following a five-step protocol reported in the literature.[3,10] Reaction of the *N*-glucosylsulfonamide with thioacid **11** under similar conditions as the previous examples gave an isolated yield of 69% of the desired *N*-(β-D-glucosyl)asparagine derivative **12**. The observed yield was comparable with other methods such as the Staudinger ligation reaction involving glycosyl azides.[11,12,6b] The preservation of the β-configuration in the product is a valuable feature of this method in light of the fact that it is a crucial aspect of convergent N-linked glycopeptide synthesis.

In summary, we have demonstrated that *N*-glucosylsulfonamide **12** is an efficient and convenient glycosylating agent of thioacids that is able to introduce the desired β-glycosidic linkage as shown by the preparation of several *N*-glucosylamides. The reaction proceeds at room temperature under mild conditions in relatively short time. This method for forming the *N*-(β-D-glycosyl)asparagine linkage is expected to have applications for the convergent synthesis of homogeneous N-linked glycopeptides/proteins.

1. Experimental

1.1. General

Aspartic acid and other fine chemicals were purchased from commercial suppliers and were used without further purification. Pyridine and DMF were dried over 3 Å and 4 Å molecular sieves, respectively. Crude products were purified by flash column chromatography on silica gel (230–400 mesh) using commercial solvents as received. Analytical thin layer chromatography (TLC, Silica Gel 60, F_{254}) was performed in the specified developing solvents and visualized under UV or charring in the presence of 5% H_2SO_4/MeOH or cerium ammonium molybdate. ^{1}H spectra were taken on either a 400 or a 600 MHz spectrometer and ^{13}C NMR spectra were obtained on either 100 or 150 MHz spectrometer in $CDCl_3$ with the residual $CHCl_3$ signal as internal reference ($CDCl_3$: ^{1}H NMR at δ 7.27, ^{13}C NMR at δ 77.23). ^{1}H–^{1}H gCOSY was performed on a 600 MHz spectrometer. Low resolution mass spectra were obtained on an electrospray ionization mass spectrometer operated in the positive ion mode and high resolution mass spectra were determined on a TOF mass spectrometer.

1.2. *N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4-dinitrobenzenesulfonamide (6)

The crude glucosylamine **4,** obtained from glucosylazide (0.11 g, 0.29 mmol) following literature procedure,[9] was reacted with 2,4-dinitrobenzenesulfonyl chloride (0.12 g, 0.44 mmol) in anhydrous pyridine (4 mL) at room temperature under N_2 atmosphere. The reaction was monitored by TLC and appeared complete within 3 h. After completion of the reaction, EtOAc (50 mL) was added and the diluted mixture was washed with water, saturated aqueous sodium bicarbonate, and brine. The organic phase was dried (anhydrous Na_2SO_4) and filtered, and the filtrate was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (7.5 × 3.0 cm) using EtOAc–hexanes (3:2) to generate *N*-glucosylsulfonamide **6** as a yellow fluffy solid: yield 46% (0.078 g, in two steps); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.99 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 3.67 (m, 1H, H-5), 3.83 (dd, 1H, *J* = 4.2, 12.6 Hz, H-6), 4.07 (dd, 1H, *J* = 1.8, 12.6 Hz, H-6′), 4.16 (dd, 1H, *J* = 9.6, 19.2 Hz, H-2), 4.88 (t, 1H, *J* = 9.0 Hz, H-1), 5.00 (t, 1H, *J* = 9.6 Hz, H-4), 5.13 (t, 1H, *J* = 9.6 Hz, H-3), 6.51 (d, 1H, *J* = 7.8 Hz, N*H*Ac), 7.74 (d, 1H, *J* = 7.2 Hz, N*H*SO_2Ar), 8.36 (d, 1H, *J* = 9.0 Hz, aromatic), 8.51 (dd, 1H, *J* = 1.8, 8.4 Hz, aromatic), 8.65 (d, 1H, *J* = 1.8 Hz, aromatic); ^{13}C NMR (100.56 MHz, $CDCl_3$): δ 20.8 (*C*H_3CO), 20.9 (*C*H_3CO), 20.93 (*C*H_3CO), 23.3 (*C*H_3CO), 53.4 (C-2), 61.4 (C-6), 68.0, 72.5, 73.7, 84.9 (C-1), 120.6, 127.1, 132.5, 140.8, 148.0, 149.9 (six aromatic carbons), 169.5 (C═O), 170.3 (C═O), 171.9 (C═O), 173.1 (C═O); mass spectrum (HRMS): *m/z* = 577.1098 $[M+H]^+$ ($C_{20}H_{25}N_4O_{14}S$ requires 577.1088).

1.3. General procedure for *N*-glycopeptide synthesis

To a suspension of cesium carbonate (2 equiv) in anhydrous DMF in a 50-mL round-bottomed flask was added appropriate thioacid (2 equiv). The mixture was stirred for 10 min at room temperature under N_2 atmosphere before addition of sulfonamide starting material (1 equiv). The resulting solution (approximately 0.2 M, red in color) was stirred further for a given time until judged complete by TLC. After completion of the reaction, the reaction mixture was diluted with EtOAc (60 mL), and washed with saturated aqueous NH_4Cl (2 × 30 mL), followed by brine. The organic phase was dried (anhydrous Na_2SO_4), filtered, and the filtrate concentrated under reduced pressure to afford a crude material. The crude material was purified by silica gel flash column chromatography to furnish the desired product.

1.3.1. *N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)acetamide (9)

Glucosylsulfonamide **6** (0.039 g, 0.068 mmol) was converted to **9** (0.017 g, 0.044 mmol) as a glassy yellow solid following the

general procedure described earlier with a reaction time of 30 min. The crude product was purified by silica gel flash column chromatography (6 × 3 cm) using MeOH–$CHCl_3$ (1:19): yield 65%; R_f = 0.5 (1:9 MeOH–$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.96 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 3.77 (m, 1H, H-5), 4.08 (dd, 1H, J = 1.8, 12.6 Hz, H-6), 4.13 (dd, 1H, J = 10.2, 18.6 Hz, H-2), 4.29 (dd, 1H, J = 4.8, 12.6 Hz, H-6′), 5.04–5.13 (m, 3H, H-1, H-3 and H-4), 6.16 (d, 1H, J = 8.4 Hz, N*H*Ac$_{C\text{-}2}$), 7.02 (d, 1H, J = 8.4 Hz, N*H*Ac$_{C\text{-}1}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.8 (*C*H_3CO), 20.9 (*C*H_3CO), 21.0 (*C*H_3CO), 23.4 (*C*H_3CO), 23.6 (*C*H_3CO), 53.6, 61.9, 67.9, 73.2, 73.6, 80.5 (C-1), 169.5 (C═O), 171.0 (C═O), 171.2 (C═O), 172.2 (C═O), 172.3 (C═O). This compound was reported in the literature.[12]

1.3.2. *N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)benzamide (10)

Glucosylsulfonamide **6** (0.030 g, 0.052 mmol) was converted to **10** (0.020 g, 0.044 mmol) as an amorphous off white cotton-like solid, following the general procedure described earlier with a reaction time of 30 min. The crude product was purified by silica gel flash column chromatography (6 × 3 cm) using MeOH–$CHCl_3$ (1:19): yield 85%; R_f = 0.62 (1:9 MeOH/$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.92 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 3.86 (m, 1H, H-5), 4.12 (dd, 1H, J = 1.8, 12.0 Hz, H-6), 4.28 (dd, 1H, J = 10.2, 18.6 Hz, H-2), 4.35 (dd, 1H, J = 4.2, 12.6 Hz, H-6′), 5.14 (t, 1H, J = 9.6 Hz, H-3), 5.20 (t, 1H, J = 9.6 Hz, H-4), 5.28 (dd, 1H, J = 8.4, 9.6 Hz, H-1), 6.18 (d, 1H, J = 7.8 Hz, N*H*Ac), 7.44 (t, 2H, J = 7.2 Hz, aromatic), 7.52 (t, 1H, J = 7.2 Hz, aromatic), 7.82 (d, 2H, J = 7.8 Hz, aromatic), 7.84 (d, 1 H, J = 8.4 Hz, N*H*COPh); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (*C*H_3CO), 21.0 (*C*H_3CO), 23.4 (*C*H_3CO), 29.9 (*C*H_3CO), 53.8 (C-2), 61.9 (C-6), 67.8, 73.2, 73.7, 81.4 (C-1), 127.6, 128.9, 132.5, 132.9, 167.7 (C═O), 169.5 (C═O), 170.9 (C═O), 172.4 (C═O), 172.6 (C═O); mass spectrum (HRMS): m/z = 473.1545 $[M+Na]^+$ ($C_{21}H_{26}N_2NaO_9$ requires 473.1536). This compound was reported in the literature.[12]

1.3.3. Benzyl N^{α}-*tert*-butoxycarbonyl-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginate (12)

Glucosylsulfonamide **6** (0.031 g, 0.054 mmol) was converted to **12** (0.024 g, 0.037 mmol) as an amorphous light green solid, following the general procedure described earlier with a reaction time of 60 min. The crude product was purified by silica gel flash column chromatography (10 × 2.5 cm) using hexane–$CHCl_3$–MeOH–acetone (60:30:5:5): yield 69%; R_f = 0.09 (60:30:5:5, hexane–$CHCl_3$–MeOH–acetone); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.42 (s, 9H, C(CH_3)$_3$), 1.95 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.67 (dd, 1H, J = 4.2, 16.2 Hz, Asp-β-CH), 2.94 (dd, 1H, J = 4.2, 16.2 Hz, Asp-β-CH), 3.72–3.75 (m, 1H, H-5), 4.06 (dd, 1H, J = 1.8, 12.0 Hz, H-6′), 4.08–4.13 (m, 1H, H-2), 4.31 (dd, 1H, J = 4.2, 12.0 Hz, H-6), 4.56–4.57 (m, 1H, Asp-α-CH), 5.01–5.04 (t, 2H, J = 8.4 Hz, H-1, H-4), 5.12–5.20 (m, 3H, H-3, OCH_2Ph), 5.55 (d, 1H, J = 8.4 Hz, Asp-α-NH), 5.96 (d, 1H, J = 7.8 Hz, N*H*Ac$_{C\text{-}2}$), 7.04 (d, 1H, J = 8.4 Hz, N*H*Ac$_{C\text{-}1}$), 7.33–7.37 (m, 5H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): 20.8 (*C*H_3CO), 20.9 (*C*H_3CO), 20.9 (*C*H_3CO), 23.3 (*C*H_3CO), 28.5 (C(*C*H_3)$_3$), 38.4 (Asp-β-CH_2), 50.3 (Asp-α-CH), 53.6, 61.9, 67.6, 67.7, 73.0, 73.8, 80.3, 80.5 (C-1), 128.4, 128.5, 128.7, 135.5, 155.7 (C═O), 169.5 (C═O), 170.9 (C═O), 170.9 (C═O), 171.3 (C═O), 172.2 (C═O), 172.4 (C═O); mass spectrum (ESI-MS): m/z = 674.3 $[M+Na]^+$ ($C_{30}H_{41}N_3Na\ O_{13}$ requires 674.3). The ^{1}H NMR and ^{13}C NMR were identical to the reported compound.[12]

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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.2009.07.002.

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