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Facile synthesis of chiral *N*-glycosylated amino acids

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Abstract Five designed chiral glycosylated amino acids have been synthesized for the first time by coupling of 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine sulfate (**2**), previously prepared by direct acetylation of D-glucosamine hydrochloride with acetic anhydride, with chiral Fmoc-protected amino acids and DIC, HOBt, and DIEA under mild conditions. The structures of these new compounds were characterized by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy and ESI MS.

Keywords *N*-Glycosylated amino acid · Fmoc-protected amino acid · Glucosamine · Chiral

Introduction

Most of the proteins occurring in nature are glycosylated with *O* and *N*-linked oligosaccharides [1]. Carbohydrate moieties in glycoproteins play a crucial role in a number of biological processes including cell recognition, cell adhesion, infection, and tumor metastasis [2]. Glycosylation of peptides has also been shown to increase proteolytic stability and to promote blood brain barrier (BBB) permeability [3, 4]. Glycosylation-enhanced crossing of the blood–brain barrier has been observed with nonnatural glycosylation of enkephalin peptides [5, 6]. However, despite the biological relevance of glycopeptide and, more generally, glycoconjugate structures, the preparation of such compounds by chemical synthesis is still a challenging and laborious task.

Glucosamine, a natural component of glycoproteins found in connective tissues and gastrointestinal mucosal membranes, has therapeutic potential for the treatment of a variety of diseases, including arthritis, inflammatory bowel disease, and general

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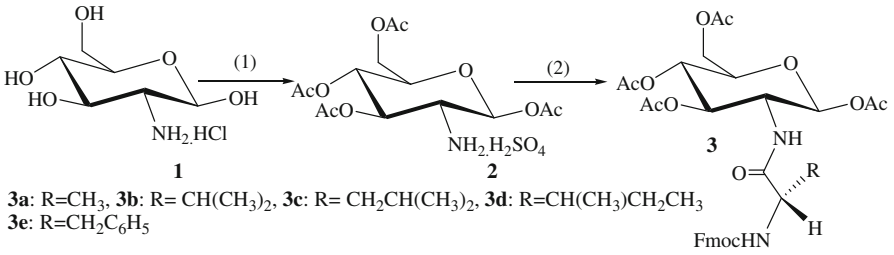


Scheme 1 The synthetic route to the target compounds. Reagents (1) Ac_2O, H_2SO_4, (2) Fmoc-protected amino acid, Et_3N, DIC, HOBt, DIEA

inflammatory damage [7, 8]. 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine, a key intermediate in the synthesis of derivatives of D-glucosamine, is obtained by selective protection of 1,3,4,6-hydroxyl groups of D-glucosamine. Various methods have been reported for synthesis of the intermediate:

(1) all the groups of D-glucosamine were protected with acetic anhydride, and the amino protection group was then removed selectively [9];
(2) different protecting groups were used to protect the amino group and four hydroxyl group, and then the amino-protecting group was removed [10–13]; and
(3) one-pot synthesis of 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine sulfate with acetic anhydride under acidic conditions[14, 15].

Of these three methods, the conditions used for the first method are harsh. The second method is inconvenient because of relatively long reaction steps. Comparatively, the third method has convenient operation, low cost, and good yield.

In our study the third method was further improved to obtain **2**; this was then coupled with protected amino acids to produce the designed new target derivatives **3**, which will be used as synthetic building blocks of glycopeptides. The synthetic route is shown in Scheme 1.

Experimental

*Synthesis of 1,3,4,6-tetraacetyl-β-D-glucosamine sulfate (**2**)*

To a stirred solution of acetic anhydride (11.3 mL), cooled in an ice-bath, was added D-glucosamine hydrochloride (2.6 g, 12 mmol). After stirring for 10 min, sulfuric acid (1 mL) was added dropwise to the ice-cold reaction mixture and stirring at room temperature was continued for 1 day. Much white solid appeared after dropwise addition of ethanol (5 mL) to the reaction mixture at 0 °C. The reaction mixture was filtered, then the residue was washed with ethyl acetate until the smell of acetic anhydride disappeared. The white product obtained was dried in vacuo to yield 74.6%. The compound was characterized by determination of melting point, IR spectroscopy, and MS. 1,3,4,6-Tetra-*O*-acetyl-*β*-D-glucosamine sulfate (II): IR (cm^{-1}): 3459, 3108 (ν_{N-H}), 2992, 2942 (ν_{C-H}), 1754 ($\nu_{C=O}$), 1622 (δ_{N-H}),

1238 (s, br, ν_{C-O-C}), 1036 (s, ν_{C-O-C}); ESI MS: 370.2 ($M^+ + 23$); 371.2 ($M^+ + 23 + 1$).

A general method for synthesis of ***3***

A solution of protected amino acid (1.3 mmol), DIC (204 mg, 1.62 mmol), HOBt (220 mg, 1.62 mmol), and DIEA (168 mg, 1.3 mmol) in THF (5 mL) was stirred in a round-bottomed flask at 0 °C for 1 h. A solution of D-glucosamine sulfate (579 mg, 1.3 mmol in 5 mL THF) containing triethylamine (263 mg, 2.6 mmol) was then added. After stirring for 12 h, the solvent was removed under reduced pressure. The solid residue was dissolved in 20 mL ethyl acetate and washed with 0.1 M hydrochloric acid (2 × 10 mL) and 5% potassium bicarbonate solution (2 × 15 mL). The organic layer was dried over Na_2SO_4 and the volume was reduced to 4 mL and purified via flash chromatography (SiO_2) with ethyl acetate and petroleum ether as eluent. The chemical structures of these products were identified by IR and NMR spectroscopy and MS.

Results and discussion

Considering the esterification of all the hydroxyl groups of D-glucosamine and protection of amino group, our initial efforts focused on the amount of acetic anhydride (Table 1) and sulfuric acid used (Table 2). The experimental results showed that the optimum molar ratio of **1**/Ac_2O/H_2SO_4 was 1:10:1.6. It was found that when the molar ratio of Ac_2O/**1** was 6:1 a lower yield was obtained. This was because the least amount of acetic anhydride was unfavorable to dissolution of D-glucosamine hydrochloride, and less white product was precipitated when ethanol was added dropwise to the reaction mixture. When the ratio was 12:1, not only was more ethyl acetate needed to wash the residue, but the yield also decreased. The effect of temperature on the reaction was then investigated (Table 3). Although the yield was almost same at 25 or 40 °C, the reaction at 40 °C was faster than that at 25 °C. In a series of exploratory experiments the highest yield was 91.5%, validating the optimum reaction condition.

A comparative study of the coupling reaction between glucosamine and protected amino acids was performed using different solvents (Table 4) and use of different coupling reagents for amide bond formation, for example TBTU, HATU, DIC, and

Table 1 Effect on experimental results of the amount of acetic anhydride used

Entry	**1**/Ac_2O (mol/mol)	Yield (%)	m.p. (°C)
1	1:6	38	168.0–170.1
2	1:8	64	168.2–169.6
3	1:10	75	167.8–169.5
4	1:12	71	167.6–170.0

r.t., **1**/H_2SO_4 = 1:1.6 (molar ratio)

Table 2 Effect on experimental results of the amount of sulfuric acid used

Entry	**1**/H_2SO_4 (mol/mol)	Yield (%)	m.p. (°C)
1	1:1.1	64.1	168.2–171.2
2	1:1.3	68.4	167.5–170.9
3	1:1.6	74.6	167.6–169.3
4	1:1.8	73.3	167.5–172.1

r.t., **1**/Ac_2O = 1:10 (molar ratio)

Table 3 Effect on experimental results of reaction temperature

Entry	*T* (°C)	Yield (%)	m.p. (°C)
1	10	71.1	167.6–169.7
2	25	75.4	167.9–170.1
3	40	76.3	168.2–170.3
4	60	72.1	161.2–169.4

1/Ac_2O/H_2SO_4 = 1:10:1.6 (molar ratio)

Table 4 The effect of different solvents on the coupling reaction of glucosamine with Fmoc-Ala-OH

Entry	Solvent	Yield (%)
1	DCM	46
2	THF	61
3	CH_3CN	54
4	DMF	32

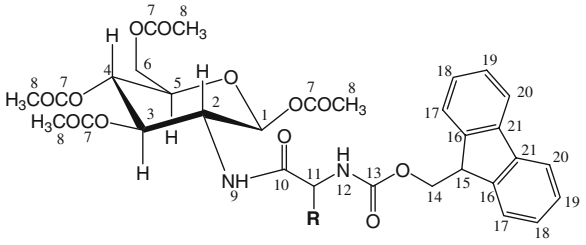
Reagents: Fmoc-Ala-OH (0.5–1.5 mmol), GlcAc4-$NH_2 \cdot H_2SO_4$ (1 equiv), TBTU/HOBt (1 equiv), Et_3N (2 equiv), DIEA (1 equiv)

Table 5 The effect of different coupling reagents on the reaction of glucosamine with Fmoc-Ala-OH

Entry	Coupling reagent	Yield (%)
1	DCC	31
2	DIC	74
3	TBTU	59
4	PyBOP	47

Reagents: Fmoc-Ala-OH (0.5–1.5 mmol), GlcAc4-$NH_2 \cdot H_2SO_4$ (1 equiv), THF (5 mL), HOBt (1 equiv), Et_3N (2 equiv), DIEA (1 equiv)

PyBOP (Table 5). The experimental results showed that the coupling reaction of Fmoc-Ala-OH with tetraacetyl-protected glucosamine worked well using HOBt and DIC as coupling reagents in THF. During selection of the solvents it was found experimentally that for fewer by-product solvents with low dielectric constant should be used as far as possible to dissolve the reactants. Using the same reaction condition, four other *N*-glycosylated amino acids target compounds were obtained



3a, R= CH_3;
3b, R= $CH(CH_3)_2$;
3c, R= $CH_2CH(CH_3)_2$;
3d, R= $CH(CH_3)CH_2CH_3$;
3e, R=$CH_2C_6H_5$;

Scheme 2 The structures of the target compounds

by coupling **2** with four other protected amino acids (Fmoc-Leu-OH, Fmoc-IIe-OH, Fmoc-Val-OH, and Fmoc-Phe-OH) (Scheme 2); the yields were 71, 78, 58, and 83% respectively.

***3a**: Fmoc-Ala-NH-Glucose (Ac)*: m.p. 198.6–202.3 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 7.94 (d, 1H, J = 8.3 Hz, H-9), 7.89 (d, 2H, J = 7.4 Hz, H-20), 7.72 (d, 2H, J = 7.3 Hz, H-17), 7.48 (d, 1H, J = 8.0 Hz, H-12), 7.42 (t, 2H, J = 7.4 Hz, H-19), 7.33 (t, 2H, J = 7.3 Hz, H-18), 5.92 (d, 1H, J = 3.0 Hz, H-1), 5.20 (t, 1H, J = 10.1 Hz, H-3), 5.02 (t, 1H, J = 9.5 Hz, H-4), 4.26–4.01 (m, 8H, H-2,5,6,11,14,15), 2.19, 2.02, 1.99, 1.93 (s, each, 3H, H-8), 1.5 (d, 3H, J = 6.8 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 173.4 (C-10), 170.1, 169.9, 169.2, 169.1 (C-7), 155.6 (C-13), 144.0, 143.8 (C-16), 140.7 (C-21), 127.7 (C-17), 127.1 (C-20), 125.3 (C-18), 120.1 (C-19), 89.7 (C-1), 70.1 (C-3), 69.1 (C-5), 67.9 (C-4), 65.6 (C-14), 61.4 (C-6), 49.9 (C-2), 49.7 (C-11), 46.7 (C-15), 20.9, 20.5, 20.4 (C-8), 18.3 (CH_3); IR (cm^{-1}): 3341 (ν_{N-H}), 3068 ($\nu_{=C-H}$), 2986, 2908 (ν_{C-H}), 1754 ($\nu_{C=O}$), 1676 ($\nu_{O=C-N}$), 1604, 1451 ($\nu_{C=C}$), 1236 (s, br, ν_{C-O-C}), 1044 (s, ν_{C-O-C}); ESI MS: 663.3 ([M + Na]$^+$), 679.2 ([M + K]$^+$).

***3b**: Fmoc-Val-NH-Glucose (Ac)*: m.p. 199.6–204.8 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 7.96 (d, 1H, J = 8.2 Hz, H-9), 7.88 (d, 2H, J = 7.4 Hz, H-20), 7.73 (d, 2H, J = 7.3 Hz, H-17), 7.50 (d, 1H, J = 8.0 Hz, H-12), 7.42 (t, 2H, J = 7.4 Hz, H-19), 7.33 (t, 2H, J = 7.3 Hz, H-18), 6.01 (d, 1H, J = 3.1 Hz, H-1), 5.17 (t, 1H, J = 10.1 Hz, H-3), 4.97 (t, 1H, J = 9.5 Hz, H-4), 4.37–4.03 (m, 8H, H-2,5,6,11,14,15), 2.21–2.32 (m, 1H, C<u>H</u> $(CH_3)_2$), 2.16, 2.01, 1.96, 1.94 (s, each, 3H, H-8), 0.92 (d, 6H, J = 5.93 Hz, CH $(C\underline{H}_3)_2$); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 173.3 (C-10), 170.0, 169.8, 169.2, 169.1 (C-7), 155.7 (C-13), 144.0, 143.7 (C-16), 140.8 (C-21), 127.7 (C-17), 127.1 (C-20), 125.3 (C-18), 120.1 (C-19), 89.5 (C-1), 70.0 (C-3), 69.1 (C-5), 68.0 (C-4), 65.6 (C-14), 61.4 (C-6), 51.4 (C-2), 51.1 (C-11), 46.7 (C-15), 31.6 (CH), 20.9, 20.5, 20.4 (C-8), 19.0, 17.8 (CH_3); IR (cm^{-1}): 3334 (ν_{N-H}), 3072 ($\nu_{=C-H}$), 2989, 2904 (ν_{C-H}), 1753 ($\nu_{C=O}$), 1674 ($\nu_{O=C-N}$), 1601, 1454 ($\nu_{C=C}$), 1237 (s, br, ν_{C-O-C}), 1036 (s, ν_{C-O-C}); ESI MS: 691.3 ([M + Na]$^+$), 707.3 ([M + K]$^+$).

***3c**: Fmoc-Leu-NH-Glucose (Ac)*: m.p. 211.5–215.2 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.01 (d, 1H, J = 8.3 Hz, H-9), 7.89 (d, 2H, J = 7.4 Hz, H-20), 7.72 (d, 2H, J = 7.3 Hz, H-17), 7.48 (d, 1H, J = 8.0 Hz, H-12), 7.42 (t, 2H, J = 7.3 Hz, H-19), 7.33 (t, 2H, J = 7.2 Hz, H-18), 5.94 (d, 1H, J = 3.3 Hz, H-1),

5.19 (t, 1H, $J = 10.2$ Hz, H-3), 5.02 (t, 1H, $J = 9.6$ Hz, H-4), 4.28–4.02 (m, 8H, H-2,5,6,11,14,15), 2.19, 2.03, 1.98, 1.91 (s, each, 3H, H-8), 1.39–1.57 (m, 3H, CH_2CH), 0.87–0.92 (m, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm):173.2 (C-10), 170.0, 169.7, 169.2, 169.0 (C-7), 155.8 (C-13), 144.1, 143.7 (C-16), 140.7 (C-21), 127.6 (C-17), 127.1 (C-20), 125.3 (C-18), 120.1 (C-19), 89.5 (C-1), 69.8 (C-3), 69.1 (C-5), 68.1 (C-4), 65.5 (C-14), 61.4 (C-6), 52.2 (C-2), 50.1 (C-11), 46.7 (C-15), 40.6 (CH_2), 22.8, 21.6 (CH_3), 21.13 (CH), 20.9, 20.5, 20.4 (C-8); IR (cm^{-1}): 3322 (ν_{N-H}), 2960, 2901 (ν_{C-H}), 1754 ($\nu_{C=O}$), 1602, 1468 ($\nu_{C=C}$), 1236 (s, br, ν_{C-O-C}), 1032 (s, ν_{C-O-C}); ESI MS: 705.3 ($[M + Na]^+$), 721.2 ($[M + K]^+$).

***3d**: Fmoc-IIe-NH-Glucose (Ac)*: m.p. 205.4–208.3 °C; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 7.98 (d, 1H, $J = 8.2$ Hz, H-9), 7.88 (d, 2H, $J = 7.4$ Hz, H-20), 7.72 (d, 2H, $J = 7.3$ Hz, H-17), 7.49 (d, 1H, $J = 8.0$ Hz, H-12), 7.42 (t, 2H, $J = 7.3$ Hz, H-19), 7.33 (t, 2H, $J = 7.3$ Hz, H-18), 5.96 (d, 1H, $J = 3.2$ Hz, H-1), 5.18 (t, 1H, $J = 10.1$ Hz, H-3), 5.01 (t, 1H, $J = 9.5$ Hz, H-4), 4.35–4.03 (m, 8H, H-2,5,6,11,14,15), 2.18, 2.10 (s, each, 3H, H-8), 1.97–2.01 (m, 7H, H-8 and CH), 1.18–1.46 (m, 2H, CH_2), 0.89–0.95 (m, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm):173.3 (C-10), 170.0, 169.8, 169.2, 169.1 (C-7), 155.7 (C-13), 144.0, 143.7 (C-16), 140.7 (C-21), 127.7 (C-17), 127.1 (C-20), 125.2 (C-18), 120.1 (C-19), 89.5 (C-1), 69.9 (C-3), 69.1 (C-5), 68.1 (C-4), 65.6 (C-14), 61.4 (C-6), 53.5 (C-2), 50.0 (C-11), 46.7 (C-19), 38.5 (CH), 23.6 ($\underline{C}H_2CH_3$), 20.8, 20.5, 20.4 (C-8), 15.7 ($CH\underline{C}H_3$), 11.7 ($CH_2\underline{C}H_3$); IR (cm^{-1}): 3342 (ν_{N-H}), 2962, 2925 (ν_{C-H}), 1756 ($\nu_{C=O}$), 1608, 1475 ($\nu_{C=C}$), 1234 (s, br, ν_{C-O-C}), 1038 (s, ν_{C-O-C}); ESI MS: 705.3 ($[M + Na]^+$).

***3e**: Fmoc-phe-NH-Glucose (Ac)*: m.p. 207.5–209.1 °C; ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 8.17 (d, 1H, $J = 8.7$ Hz, H-9), 7.88 (d, 2H, $J = 7.50$ Hz, H-20), 7.65–7.61 (m, 2H, ArH), 7.43–7.19 (m, 10H, H-12 and ArH), 5.98 (d, 1H, $J = 3.3$ Hz, H-1), 5.24 (t, 1H, $J = 10.1$ Hz, H-3), 5.03 (t, 1H, $J = 9.8$ Hz, H-4), 4.29–3.99 (m, 8H, H-2,5,6,11,14,15), 2.89–2.84 (m, 1H, CH_2), 2.78–2.51 (m, 1H, CH_2), 2.21, 2.03, 1.99, 1.91 (s, each, 3H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 173.4 (C-10), 170.0, 169.8, 169.2, 169.1 (C-7), 155.7 (C-13), 143.8, 143.6 (C-16), 140.6 (C-21), 137.7, 129.1, 128.0, 126.3 ($\underline{C}_6H_5$), 127.6 (C-17), 127.0 (C-20), 125.2 (C-18), 120.0 (C-19), 89.5 (C-1), 69.9 (C-3), 69.1 (C-5), 68.0 (C-4), 65.6 (C-14), 61.3 (C-6), 55.6 (C-11), 50.1 (C-2), 46.5 (C-15), 20.8, 20.5, 20.4 (C-8); IR (cm^{-1}): 3341 (ν_{N-H}), 3071 ($\nu_{=C-H}$), 2984, 2914 (ν_{C-H}), 1756 ($\nu_{C=O}$), 1676 ($\nu_{O=C-N}$), 1608, 1457 ($\nu_{C=C}$), 1238 (s, br, ν_{C-O-C}), 1045 (s, ν_{C-O-C}); ESI MS: 739.3 ($[M + Na]^+$), 755.3 ($[M + K]^+$).

Conclusion

Five designed chiral glycosylated amino acids were firstly synthesized in the yield of 58–83% under mild conditions. One practical synthesis of 2 was developed with promising application industrially for its convenient operation, low cost and stable high yield. All of these will lay foundation to a certain extent for the scale synthesis of glycoproteins.

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