Chemistry of Natural Compounds and Bioorganic Chemistry

Glycoconjugates of amino acids. Preparation through *N*-alkylation of amino acids with *N*-chloroacetyl-β-glycopyranosylamines

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Monoalkylation of amino acids of different structural types with *N*-chloroacetylglycosylamines was shown to be applicable for the preparation of glycoconjugates containing β-D-galactose, *N*-acetyl-β-D-glucosamine, β-D-mannose, and lactose residues. The glycoconjugates were synthesized from amino acids with secondary (sarcosine, L-proline) or primary (L-2- and 4-aminobutyric acids, L-tryptophan) amino groups as well as from various amino dicarboxylic acids (*N*-methyl-DL-aspartic, DL-aspartic, L-glutamic, and DL-2-aminoadipic acids). The derivatives obtained may be of interest for glycotargeting of physiologically active compounds of this series.

Key words: glycoconjugates, amino acids, neurotransmitters, *N*-chloroacetylglycosylamines.

Earlier we have shown[1] that alkylation of primary and secondary amines with *N*-chloroacetylglycosylamines (*N*-glycosylated chloroacetamides) can be used as a simple and convenient method for synthesis of glycoconjugates of amines which contain fragments of *N*-glycylglycosylamines including derivatives of various physiologically active compounds. Now we report that this reaction can be used for the preparation of analogous derivatives of amino acids. Compounds of this type are of great interest as potential building blocks for the synthesis of biologically active peptides. On the other hand, some amino acids and their derivatives are important medicinal preparations themselves acting as analogs or antagonists of neurotransmitters of the mammalian nervous system.

A number of glycoconjugates of amino acids have been recently obtained by *N*-acylation of glycosylamines with the corresponding derivatives of amino acids, and the prospect of their use for glycotargeting of therapeutic drugs and oligo- and polynucleotides has been shown (see review[3]).

In the present paper, the previously reported[4] *N*-chloroacetyl-β-glycopyranosylamines **1a—d** containing the residues of β-D-galactose (as a monosaccharide or as a constituent of disaccharide lactose) and *N*-acetyl-β-D-glucosamine capable of specific binding with lectins of animal cell surface[5], as well as β-D-mannose derivatives, were used for the alkylation of amino acids.

The study of alkylation of amino acids containing secondary NH groups was carried out using sarcosine

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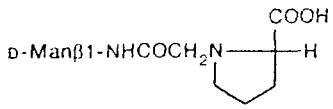
Sugβ1-NHCOCH$_2$Cl

1a–d

Sug = D-Gal (**a**), D-Man (**b**), D-Gal(β1-4)D-Glc (**c**), D-GlcNAc (**d**)

D-Galβ1-NHCOCH$_2$NCH$_2$COOH
Me

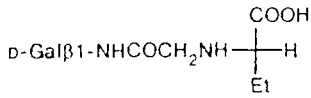
2



3

D-Gal(β1-4)D-Glcβ1-NHCOCH$_2$N—CHCOOH
Me CH$_2$COOH

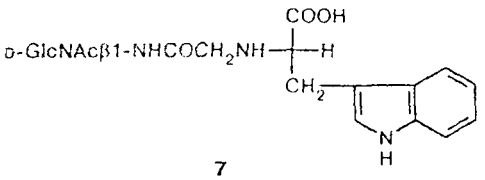
4



5

D-Manβ1-NHCOCH$_2$NH(CH$_2$)$_3$COOH

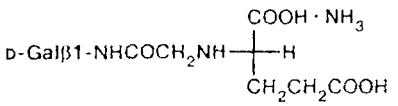
6



7

D-GlcNAcβ1-NHCOCH$_2$NHCHCOOH
CH$_2$COOH

8



9

D-Galβ1-NHCOCH$_2$NHCHCOOH · NH$_3$
CH$_2$(CH$_2$)$_2$COOH

10

(*N*-methylglycine) and L-proline; fragments of these amino acids are constituents of numerous drugs[2].

The reaction conditions used were similar to those described earlier[1] for the alkylation of secondary amines (a mixture of MeOH—water, 70 °C); however, Et_3N was added to the reaction mixture to neutralize COOH groups and as an acceptor of a hydrogen halide (molar ratio amino acid : *N*-glycosylchloroacetamide : tertiary amine, 2 : 1 : 3). Monitoring of the reaction by paper electrophoresis showed that the yield of the products reached 85—90% in 3 h after the beginning of the reaction. The treatment of the reaction mixture with Ac_2O for *N*-acetylation of the excess of amino acids facilitated the purification of the formed glycoconjugates **2** and **3** with the use of cation exchange resin. Additional purification by gel filtration afforded these compounds in 65 and 69% yield, respectively.

Alkylation of *N*-methyl-DL-aspartic acid, a racemate of the D-amino acid possessing neurostimulating activity[6,7], was studied as an example of the reaction with an amino dicarboxylic acid. In this case the reaction proceeded considerably more slowly and a satisfactory yield of glycoconjugate **4** was obtained only after heating of the mixture (molar ratio amino acid: *N*-glycosylchloroacetamide : tertiary amine 2 : 1 : 5) for 48 h. Attempts at using absorption on cation exchange resin for the purification of the product failed due to the formation of a by-product (probably a lactone) under the action of ion exchange resin in acidic form. Compound **4** was obtained as a mixture of diastereomers after two separations by gel chromatography: initially in alkaline medium (0.1 *M* NH_4OH) to separate it from the major amount of the starting amino acid and then in slightly acidic medium (0.05 *M* AcOH); the yield of the product was 59%.

Optimum conditions for mono-*N*-alkylation of amino acids containing a primary amino group with *N*-chloroacetylglycosylamines were developed using L-2- and 4-aminobutyric acids as examples. The first of these compounds belongs to the class of neuroactive amino acids[7,8], and the second is a natural inhibitory neurotransmitter and is used as a nootropic preparation[2,9].

In this case the alkylation proceeds smoothly when a MeOH—H_2O—DMSO mixture is used as solvent; the optimum molar ratio amino acid : *N*-glycosylchloroacetamide : tertiary amine to achieve monoalkylation is 3 : 1 : 4. The reaction was carried out at 70 °C; gel chromatography in an alkaline medium followed by gel chromatography in a slightly acidic medium as described above was used for the purification of the products. Glycoconjugates **5** and **6** were obtained in 70 and 55% yields, respectively.

Similar conditions were also found suitable for the monoalkylation of the NH_2 group of L-tryptophan exhibiting antidepressant activity[2]; reaction product **7** was isolated in 51% yield.

N-Chloroacetylglycosylamines can be also successfully used for the monoalkylation of amino dicarboxylic acids belonging to a group of neuromodulators[8,10,11]. The reaction with DL-aspartic acid proceeds smoothly in a mixture of DMSO—H_2O; the molar ratio amino acid : *N*-glycosylchloroacetamide : tertiary amine is 3 : 1 : 7. After gel chromatography glycoconjugate **8** was obtained in 53% yield.

It turned out, however, that these conditions were unsuitable for the similar reactions with L-glutamic and

DL-2-aminoadipic acids. The synthesis of glycoconjugates **9** and **10** should be performed in an aqueous solution since in the presence of DMSO the formation of a noticeable amount of five- or six-membered *N*-alkylated lactams was observed; the latter are easily formed from the glycoconjugates obtained. Moreover, partial formation of such lactams occurred when isolation of these compounds as derivatives of dicarboxylic acids was attempted; however, monoammonium salts of compounds **9** and **10** are quite stable.

Amorphous glycoconjugates **8** and **10** were obtained as mixtures of two diastereomers; their separation has not been undertaken.

The structures of compounds **2**—**10** were confirmed by elemental analysis and ^{1}H NMR spectroscopy data.

The results obtained have demonstrated that alkylation with *N*-chloroacetylglycosylamines can be used as a convenient method for introduction of carbohydrate residues into amino acids of different structural types. Glycoconjugates of biologically active compounds of this class, in particular of the analogs or antagonists of neuromediators, are of interest for their glycotargeting.

Experimental

^{1}H NMR spectra were recorded with a Bruker WM-250 (250 MHz) spectrometer for solutions in D_2O at 300 K. Optical rotations were measured with a Jasco DIP-360 polarimeter. Electrophoresis (12 V · cm^{-1}, 2 h) was performed on a Filtrak FN1 paper in 6% HCOOH. The compounds were visualized with ninhydrin or the following sequences of reagents: KIO_4—$AgNO_3$—KOH and Cl_2—KI—starch. Gel chromatography was monitored by UV detection at 206 nm. Water of crystallization was determined according to Fischer.

***N*-[*N*-Carboxymethyl-*N*-methylglycyl]-β-D-galactopyranosylamine (2).** A solution of *N*-chloroacetyl-β-D-galactopyranosylamine[4] (**1a**) (0.25 g, 1 mmol), sarcosine (0.18 g, 2 mmol), and Et_3N (0.42 mL, 3 mmol) in 2.8 mL of 60% aqueous MeOH was heated for 3 h at 70 °C. Methanol was evaporated, the residue was dissolved in H_2O (3 mL), and MeOH (0.15 mL) and Ac_2O (1 mL) were added to the solution. The mixture was stirred until the solution became homogeneous and then was kept for 16 h at 20 °C. The reaction mixture was diluted with MeOH (10 mL × 3) and concentrated to dryness (also three times). The residue was dissolved in 5 mL of H_2O, Dowex 50Wx8 (H^+) (15 mL) was added to the solution, and the mixture was stirred for 1 h. The resin was filtered off and washed with H_2O (150 mL) and then with 1.5 *M* NH_4OH (150 mL). Alkaline fractions were concentrated to dryness and the residue was subjected to gel chromatography (column 4×100 cm, Sephadex G-15, 0.05 *M* AcOH). Fractions containing the product were concentrated to dryness and the remaining AcOH was removed by addition and subsequent evaporation of a mixture of H_2O—MeOH—toluene (2 : 15 : 5). Yield 0.2 g (65%), m.p. 215—217 °C (MeOH—acetone, dec.), $[\alpha]_D^{20}$ +14.7° (*c* 1, H_2O). Found (%): C, 42.78; H, 6.52; N, 9.07. $C_{11}H_{20}N_2O_8$. Calculated (%): C, 42.86; H, 6.54; N, 9.08. ^{1}H NMR spectrum (ppm): 3.02 (s, 3 H, CH_3); 3.58—3.82 (m, 5 H, Gal); 3.83 (s, 2 H, CH_2COOH), 3.99 (br.d, 1 H, H(4) Gal, *J* = 2.5 Hz); 4.19 (br.s, 2 H, $COCH_3$); and 4.99 (d, 1 H, H (1) Gal, *J* = 9 Hz).

***N*-[(*S*)-2-Carboxypyrrolidinoacetyl]-β-D-mannopyranosylamine (3).** Compound **3** was prepared from *N*-chloroacetyl-β-D-mannopyranosylamine (**1b**) (0.25 g, 1 mmol), L-proline (0.23 g, 2 mmol), and Et_3N (0.42 mL, 3 mmol) as described for derivative **2**. Yield 0.24 g (68%, amorphous solid), $[\alpha]_D^{20}$ −54.4° (*c* 1, H_2O). Found (%): C, 44.59; H, 7.10; N, 7.91; H_2O, 5.70. $C_{13}H_{22}N_2O_8 \cdot H_2O$. Calculated (%): C, 44.31; H, 6.86; N, 7.95; H_2O, 5.11. ^{1}H NMR spectrum (ppm): 2.00—2.20 (m, 3 H, Pro); 2.46 (m, 1 H, Pro); 3.24 (m, 1 H, Pro); 3.49 (m, 1 H, H(5) Man); 3.61 (dd, 1 H, H(4) Man, *J* = 9.7 Hz and *J* = 9.7 Hz); 3.68—3.78 (m, 2 H, Man); 3.82—3.98 (m, 3 H); 4.07—4.31 (m, 3 H, NCH, $COCH_2$); and 5.29 (br.s, 1 H, H(1) Man).

***N*-[*N*-(DL-1,2-Dicarboxyethyl)-*N*-methylglycyl]-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (4).** A solution of *N*-chloroacetyl-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine monohydrate (**1c**) (215 mg, 0.05 mmol), *N*-methyl-DL-aspartic acid (147 mg, 1 mmol), and Et_3N (0.35 mL, 2.5 mmol) in 2 mL of 70% aqueous MeOH was heated for 48 h at 70 °C. Methanol was evaporated, and the residue was dissolved in 10 mL of 0.1 *M* NH_4OH and subjected to gel chromatography (column 5×90 cm, Sephadex G-25 (fine), 0.1 *M* NH_4OH). The fractions containing product **4** were concentrated to dryness, and the residue was dissolved in 10 mL of 0.05 *M* AcOH and subjected to gel chromatography (4×100 cm, Sephadex G-15, 0.05 *M* AcOH). The fractions containing product **4** were concentrated to dryness and the remaining AcOH was removed by addition and subsequent evaporation of a mixture of H_2O—MeOH—toluene (2 : 15 : 5). Yield 156 mg (59%, amorphous solid), $[\alpha]_D^{20}$ +4.5° (*c* 1, H_2O). Found (%): C, 42.81; H, 6.23; N, 5.48. $C_{19}H_{32}N_2O_{15}$. Calculated (%): C, 43.18; H, 6.10; N, 5.30. ^{1}H NMR spectrum (ppm): 2.97 (s, 3 H, CH_3); 3.00 (m, 2 H, $CH\underline{CH}_2$); 3.40—3.97 (m, 12 H, Glc, Gal); 4.18 (br.s, 2 H, $COCH_2$); 4.28 (m, 1 H, NCH); 4.44 (d, 1 H, H(1) Gal, *J* = 8 Hz); and 5.04 (d, 1 H, H(1) Glc, *J* = 9 Hz).

***N*-{*N*-[(*S*)-1-Carboxypropyl]glycyl}-β-D-galactopyranosylamine (5).** A solution of *N*-chloroacetyl-β-D-galactopyranosylamine (**1a**) (128 mg, 0.05 mmol), L-2-aminobutyric acid (154 mg, 1.5 mmol), and Et_3N (0.28 mL, 2 mmol) in a mixture of H_2O—DMSO—MeOH (1 : 1 : 2.5; 11.25 mL) was heated for 17 h at 70 °C. Water was removed from the reaction mixture by addition and subsequent evaporation of toluene (25 mL × 3); toluene (30 mL) was added to the residue and the precipitated product was washed with toluene and ether. The residue was subjected to gel chromatography on Sephadex G-25 and G-15 as described for compound **4**. Yield 120 mg (70%), m.p. 208—211 °C (H_2O— acetone), $[\alpha]_D^{20}$ +15.5° (*c* 1, H_2O). Found (%): C, 42.39; H, 7.14; N, 8.44; H_2O, 5.88. $C_{12}H_{22}N_2O_8 \cdot H_2O$. Calculated (%): C, 42.35; H, 7.11; N, 8.23; H_2O, 5.29. ^{1}H NMR spectrum (ppm): 0.96 (t, 3 H, CH_3, *J* = 8 Hz); 1.92 (m, 2 H, $\underline{CH}_2CH_3$); 3.57—3.81 (m, 6 H, Gal, NCH); 3.86—4.02 (m, 3 H, H(4) Gal, $COCH_2$); and 4.97 (d, 1 H, H(1) Gal, *J* = 9 Hz).

***N*-[*N*-(3-Carboxypropyl)glycyl]-β-D-mannopyranosylamine (6).** Compound **6** was prepared from *N*-chloroacetyl-β-D-mannopyranosylamine (**1b**) (128 mg, 0.05 mmol), 4-aminobutyric acid (154 mg, 1.5 mmol), and Et_3N (0.28 mL, 2 mmol) in a mixture of H_2O—DMSO—MeOH (1 : 2 : 3; 7.5 mL) after heating for 8 h at 70 °C as described for compound **5**. Yield 94 mg (55%), m.p. 141—143 °C (H_2O—MeOH—Et_2O), $[\alpha]_D^{20}$ −30.4° (*c* 1, H_2O). Found (%): C, 42.64; H, 7.34; N, 8.34; H_2O, 5.10. $C_{12}H_{22}N_2O_8 \cdot H_2O$. Calculated (%): C, 42.35; H, 7.11; N, 8.23; H_2O, 5.29. ^{1}H NMR spectrum (ppm): 1.92 (m, 2 H, $CH_2\underline{CH}_2CH_3$); 2.30 (t, 2 H, CH_2,

J = 7.1 Hz); 3.10 (t, 2 H, CH_2, J = 7.5 Hz); 3.46 (m, 1 H, H(5) Man); 3.58 (dd, 1 H, H(4) Man, J = 9.7 Hz and J = 9.7 Hz); 3.65—3.75 (m, 2 H, Man); 3.84—3.94 (m, 2 H, Man); 3.97 (br.s, 2 H, $COCH_2$); and 5.27 (br.s, 1 H, H(1) Man).

2-Acetamido-2-deoxy-1-*N*-{*N*-[(*S*)-1-carboxy-2-(3-indolyl)ethyl]glycyl}-β-D-glucopyranosylamine (7). Compound **7** was prepared from 2-acetamido-2-deoxy-1-*N*-chloroacetyl-β-D-glucopyranosylamine (**1d**) (148 mg, 0.05 mmol), L-tryptophan (306 mg, 1.5 mmol), and Et_3N (0.28 mL, 2 mmol) in a mixture of H_2O—DMSO—MeOH (1 : 2 : 6; 13.5 mL) after heating for 7 h at 70 °C as described for compound **5**. The reaction was performed in a screw-cap vial, which was closed after the air was displaced by methanol vapor. Yield 123 mg (51%), m.p. 180—182 °C (MeOH—EtOH—Et_2O), $[\alpha]_D^{20}$ +9.6° (*c* 1, H_2O). Found (%): C, 52.44; H, 6.36; N, 11.75; H_2O, 4.00. $C_{21}H_{28}N_4O_8 \cdot H_2O$. Calculated (%): C, 52.28; H, 6.26; N, 11.61; H_2O, 3.73. ^1H NMR spectrum (ppm): 2.03 (s, 3 H, CH_3); 3.40—3.68 (m, 5 H); 3.72—3.98 (m, 5 H); 4.11 (dd, NCH, J = 7.1 Hz and J = 5.9 Hz); 5.10 (d, 1 H, H(1) GlcN, J = 9.5 Hz); 7.21—7.40 (m, 2 H, Ar); 7.39 (s, 1 H, Ar); 7.61 (d, 1 H, Ar, J = 8 Hz); and 7.79 (d, 1 H, Ar, J = 8 Hz).

2-Acetamido-2-deoxy-1-*N*-[*N*-(DL-1,2-dicarboxyethyl)-glycyl]-β-D-glucopyranosylamine (8). Compound **8** was prepared from 2-acetamido-2-deoxy-1-*N*-chloroacetyl-β-D-glucopyranosylamine (**1d**) (148 mg, 0.05 mmol), DL-aspartic acid (200 mg, 1.5 mmol), and Et_3N (0.48 mL, 3.5 mmol) in a mixture of H_2O and DMSO (4 : 3; 3.5 mL) after heating for 48 h at 70 °C as described for compound **5**. Yield 104 mg (53%, amorphous solid), $[\alpha]_D^{20}$ +21.2° (*c* 1, H_2O). Found (%): C, 42.51; H, 6.29; N, 10.56. $C_{14}H_{23}N_3O_{10}$. Calculated (%): C, 42.75; H, 5.89; N, 10.68. ^1H NMR spectrum (ppm): 1.98 (s, 3 H, CH_3); 3.02 (m, 2 H, $CHC\underline{H}_2$); 3.42—3.54 (m, 2 H, GlcN); 3.60 (br.t, 1 H, H(3) GlcN, J = 8.8 Hz); 3.68—3.91 (m, 3 H, GlcN); 3.94—4.03 (m, 3 H, $COCH_2$, NCH); and 5.11 (d, 1 H, H(1) GlcN, J = 9.5 Hz).

***N*-{*N*-[(*S*)-1,3-Dicarboxypropyl]glycyl}-β-D-galactopyranosylamine, monoammonium salt (9) and *N*-[*N*-(DL-1,4-dicarboxybutyl)glycyl]-β-D-galactopyranosylamine, monoammonium salt (10).** Compounds **9** and **10** were prepared from *N*-chloroacetyl-β-D-galactopyranosylamine (**1a**) (128 mg, 0.05 mmol), L-glutamic (220 mg, 1.5 mmol), or DL-2-aminoadipic acid (241 mg, 1.5 mmol) and Et_3N (0.48 mL, 3.5 mmol) in 2.5 mL H_2O after heating for 24 h at 70 °C. The reaction mixture was concentrated to volume 0.5 mL, diluted with 7 mL of 0.1 *M* NH_4OH, and subjected to gel chromatography (column 5×90 cm, Sephadex G-25 (fine), 0.1 *M* NH_4OH).

Yield of compound **9** 121 mg (63%), m.p. 163—165 °C (H_2O—EtOH), $[\alpha]_D^{20}$ +3.2° (*c* 1, H_2O). Found (%): C, 40.63; H, 6.84; N, 10.84. $C_{13}H_{22}N_2O_{10} \cdot NH_3$. Calculated (%): C, 40.73; H, 6.57; N, 10.96. ^1H NMR spectrum (ppm): 2.10 (m, 2 H, CH_2); 2.40 (t, 2 H, CH_2, J = 7.7 Hz); 3.55—3.81 (m, 6 H); 3.94 (m, 3 H); and 4.95 (d, 1 H, H(1) Gal, J = 9 Hz).

Yield of compound **10** 119 mg (60%, amorphous solid), $[\alpha]_D^{20}$ +10.7° (*c* 1, H_2O). Found (%): C, 42.55; H, 6.81; N, 10.47. $C_{14}H_{24}N_2O_{10} \cdot NH_3$. Calculated (%): C, 42.31; H, 6.85; N, 10.57. ^1H NMR spectrum (ppm): 1.66 (m, 2 H, CH_2); 1.85 (m, 2 H, CH_2); 2.26 (t, 2 H, CH_2, J = 7.5 Hz); 3.57 (t, 1 H, NHCH, J = 6.2 Hz); 3.62—3.85 (m, 7 H); 4.00 (m, 1 H, H(4) Gal); and 4.99 (d, 1 H, H(1) Gal, J = 9 Hz).

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