# *N*-Bromoacetyl-β-D-glycopyranosylamines in the synthesis of glycoconjugates of nitrogen-containing physiologically active compounds*

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A series of nitrogen-containing physiologically active compounds underwent smooth *N*-monoalkylation with *N*-bromoacetyl-β-glycopyranosylamines derived from *N*-acetyl-D-glucosamine and lactose. This reaction was demonstrated to be promising for the introduction of carbohydrate residues into heterocyclic compounds, *viz.*, pyridine, imidazole, pyrimidinetrione, carboline, and piperazine derivatives, and into an amino acid, 5-hydroxy-L-tryptophan, which is unstable in alkaline media.

**Key words:** *N*-bromoacetyl-β-glycopyranosylamines, *N*-monoalkylation, glycoconjugates.

Glycosylamines and their derivatives are finding ever-increasing application in the synthesis of glycoconjugates used in various biological studies.[1,2] A possible procedure for the preparation of glycoconjugates involves the use of *N*-haloacetylglycosylamines for the attachment of carbohydrate residues. Earlier,[3,4] we have demonstrated that *N*-monoalkylation of compounds containing primary or secondary amino groups with *N*-chloroacetyl-β-glycopyranosylamines[5] can serve as a convenient approach to the synthesis of glycoconjugates of amines[3] and amino acids.[4] Recently,[6,7] we have described a new efficient procedure for the synthesis of glycosylamines derived from mono- and disaccharides with the use of ammonium carbamate. Starting from these glycosylamines, we prepared *N*-bromoacetyl-β-glycopyranosylamines by an improved method.[8] In the present study, we used two compounds of this series, *viz.*, *N*-acetyl-D-glucosamine and lactose derivatives (**1a** and **1b**, respectively), for the synthesis of glycoconjugates of nitrogen-containing physiologically active compounds. We chose these carbohydrate derivatives because it is well known that the animal cell surface contains proteins (lectins) that specifically bind *N*-acetyl-β-D-glucosamine and β-D-galactose, a lactose component.

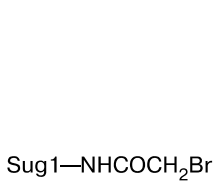
The use of *N*-bromoacetylglycopyranosylamines in the synthesis of glycopeptide analogs by *S*-alkylation of the cysteine residues in peptides[9] or the *N*-acetyl-3-thio-D-galactosamine residue in a glycopeptide[10] was documented. *N*-Bromoacetylglycopyranosylamines were also used for the introduction of an affinity label into the active site of glycosidases[11—17] by alkylation of methionine residues[12,13] or carboxy groups.[14—17] However, the possibility of using these compounds for alkylation at the nitrogen atom has not been examined so far. In the present study, we investigated this reaction. We synthesized glycoconjugates of selected physiologically active compounds of the following nitrogen-containing heterocyclic systems: pyridine (nicotinic acid (**2**)), imidazole (2-benzyl-1*H*-benzimidazole (**3**)), pyrimidinetrione (5,5-diethylbarbituric acid (**4**)), and carboline (9-benzyl-3-methyl-1,2,3,4-tetrahydro-3-carboline (**5**)). It was demonstrated that the use of *N*-bromoacetyl-β-glycopyranosylamines makes possible monoalkylation of the primary amino group in alkali-unstable 5-hydroxyl-L-tryptophan (**6**) and the secondary amino group in 1-(3-chlorophenyl)piperazine (**7**).

The acidic groups in compounds **2**, **4**, and **6** were neutralized and hydrochloride **7** was converted into the free base using sterically hindered amine $EtNPr^{i}_{2}$, which did not react with bromoacetamides **1a,b** under the conditions used for alkylation. Alkylation was carried out in MeOH (compound **1a**) or in 85% aqueous MeOH (compound **1b**) in the presence of an excess (up to 2 equiv.) of the amino component. The reaction was monitored and subsequent purification of the glycoconjugates (**8—13**) was carried out by paper chromatography and/or paper electrophoresis. Compounds **2** and **5—7** reacted with bromoacetamide **1a** or **1b** at room temperature, whereas it was necessary to increase the reaction time and temperature in the synthesis of glycoconjugate **10** from diethylbarbituric acid **4**. In the synthesis of glycoconjugate **9** from benzimidazole **3**, the best results were obtained when the reaction was carried out in *N*-methyl-2-pyrrolidone at 36 °C. Under the conditions used, monoalkylation of compounds **2—5** occurred smoothly, only in the case of

* Dedicated to Academician N. K. Kochetkov on the occasion of his 90th birthday.

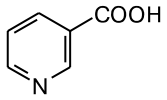
Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 1256—1259, May, 2005.

1066-5285/05/5405-1294 

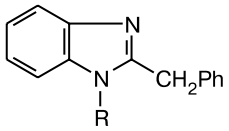


**1a,b**

Sug = β-D-GlcNAc (**a**),
β-D-Gal(1—4)-β-D-Glc (**b**)

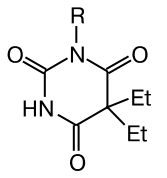


**2**



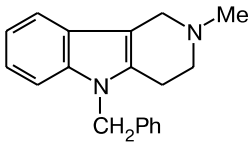
**3, 9**

R = H (**3**),
β-D-GlcNAc1—NHCOCH$_2$ (**9**)

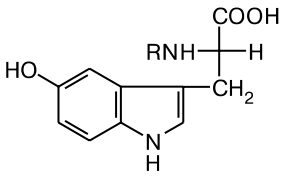


**4, 10**

R = H (**4**),
β-D-Gal(1—4)-β-D-Glc1—NHCOCH$_2$ (**10**)

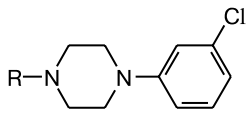


**5**



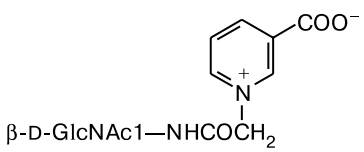
**6, 12**

R = H (**6**),
β-D-GlcNAc1—NHCOCH$_2$ (**12**)

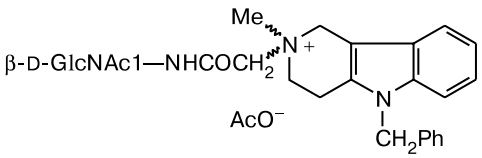


**7, 13**

R = H · HCl (**7**),
β-D-Gal(1—4)-β-D-Glc1—NHCOCH$_2$ (**13**)



**8**



**11**

compounds **6** and **7** this was accompanied by dialkylation. To decrease the yield of the dialkylation products, alkylation of compounds **6** and **7** was carried out in dilute solutions (0.1 *M* solutions of bromoacetamides **1a,b**).

Glycoconjugates **8**, **9**, and **13** precipitated from the reaction mixtures (51—63% yields) and did not contain bromide ions. Glycoconjugates **10—12** were isolated by anion-exchange chromatography and/or gel chromatography on Sephadex G-15 (50—66% yields).

The structures of glycoconjugates **8—13** were confirmed by data from elemental analysis and $^1$H NMR spectroscopy. The absence of the bromide ion in glycoconjugate **13** indicates that alkylation occurs at the secondary rather than tertiary amino group. Glycoconjugate **11** was prepared from carboline derivative **5** as a 1 : 1 mixture of two diastereomers, as evidenced by the $^1$H NMR spectrum, which shows two signals for the MeN group (δ 3.28 and 3.31) and two signals for the Ac group (δ 1.79 and 2.00) of equal intensities. We did not make special attempts to separate the diastereomers.

To summarize, the use of *N*-bromoacetyl-β-glycopyranosylamines, which are more reactive than *N*-chloroacetyl-β-glycopyranosylamines,[3,4] makes it possibile to perform *N*-monoalkylation of new types of amino compounds and prepare glycoconjugates from compounds containing a primary or secondary amino group under milder conditions.

## Experimental

The $^1$H NMR spectra were recorded in $D_2O$ at 24 °C on a Bruker WM-250 spectrometer (250 MHz) relative to acetone as the external standard (δ 2.225). The optical rotation was determined on a PU-07 polarimeter (Russia). Electrophoresis (12 V cm$^{-1}$, 1 h) was carried out on a Filtrak FN1 paper in 4% HCOOH or in a pyridinium acetate buffer (0.05 *M* with respect to Py, pH 4.5). Ascending chromatography was performed on a Filtrak FN15 paper in a BuOH—AcOH—$H_2O$ solvent system (4 : 1.4 : 2.5). Compounds were visualized with ninhydrin and a $KIO_4$—$AgNO_3$—KOH reagent sequence.[18] The presence of crystallization water was determined by the Fischer method.[19] Gel chromatography on a column (5×100 cm) with Sephadex G-15 was monitored by following UV absorption at 206 nm.

**2-Acetamido-*N*-[(3-carboxylato-1-pyridinium)acetyl]-2-deoxy-β-D-glucopyranosylamine (8).** *N*,*N*-Diisopropylethylamine (0.34 mL, 2 mmol) was added with stirring to a heterogeneous mixture of 2-acetamido-*N*-bromoacetyl-2-deoxy-β-D-glucopyranosylamine[8] (341 mg, 1 mmol) (**1a**) and nicotinic acid (**2**) (246 mg, 2 mmol) in MeOH (7 mL). The resulting solution was kept at 20 °C for 48 h. The precipitate that formed was filtered

off, washed with MeOH and diethyl ether, recrystallized from 80% aqueous MeOH, and dried. Compound **8** was obtained in a yield of 250 mg (60%), m.p. 256—257 °C, $[\alpha]_D^{20}$ +36.7 (*c* 1, $H_2O$). Found (%): C, 48.85; H, 5.62; N, 10.60; $H_2O$, 2.46. $C_{16}H_{21}N_3O_8 \cdot 0.5\ H_2O$. Calculated (%): C, 48.98; H, 5.65; N, 10.70; $H_2O$, 2.30. $^1$H NMR, δ: 2.01 (s, 3 H, Me); 3.47—3.58 (m, 2 H, H(4), H(5)); 3.64 (t, 1 H, H(3), $J_{2,3} = J_{3,4} = 9.0$ Hz); 3.78 (dd, 1 H, H(6a), $J_{5,6a} = 5.0$ Hz, $J_{6a,6b} = 12.5$ Hz); 3.84—3.96 (m, 2 H, H(2), H(6b)); 5.12 (d, 1 H, H(1), $J = 9.5$ Hz); 5.58 (s, 2 H, $CH_2CO$); 8.18 (m, 1 H, Ar); 8.81 (d, 1 H, Ar, $J = 6.0$ Hz); 8.96 (d, 1 H, Ar, $J = 8.0$ Hz); 9.11 (s, 1 H, Ar).

**2-Acetamido-*N*-[(2-benzyl-1*H*-benzimidazol-1-yl)acetyl]-2-deoxy-β-D-glucopyranosylamine (9).** A mixture of compound **1a** (341 mg, 1 mmol) and 2-benzyl-1*H*-benzimidazole (**3**) (416 mg, 2 mmol) in *N*-methyl-2-pyrrolidone (2.5 mL) was kept at 36 °C for 40 h. The precipitate that formed was filtered off, washed with MeOH and diethyl ether, and dried. Product **9** was obtained in a yield of 218 mg. The filtrate and washings were combined, concentrated to 2.5 mL, and diluted with diethyl ether (30 mL). The precipitate that formed was separated, washed with toluene (3×5 mL), and suspended in $H_2O$ (20 mL). Then $Et_3N$ was added to pH ~11, the suspension was washed with toluene (4×4 mL), the suspension was concentrated to dryness, and the residue was suspended in $H_2O$ (5 mL). The precipitate was filtered off, washed with $H_2O$ (2×2 mL) and MeOH (2×1.5 mL), and dried. An additional amount of product **9** was obtained (200 mg). The products were combined and dissolved in $H_2O$ (50 mL) at 80 °C. The solution was concentrated to 10 mL. The precipitate that formed was filtered off, washed with MeOH and diethyl ether, and dried. Compound **9** was obtained in a yield of 296 mg (63%), m.p. 278—279 °C, $[\alpha]_D^{20}$ +35.3 (*c* 1, DMSO). Found (%): C, 61.52; H, 6.10; N, 12.08. $C_{24}H_{28}N_4O_6$. Calculated (%): C, 61.52; H, 6.02; N, 11.96. $^1$H NMR (0.01 *M* DCl), δ: 2.01 (s, 3 H, Me); 3.53—3.62 (m, 2 H, H(4), H(5)); 3.70 (m, 1 H, H(3)); 3.79—4.02 (m, 3 H); 4.70 (s, 2 H, $CH_2Ph$); 5.11 (d, 1 H, H(1), $J = 9.5$ Hz); 5.41 (s, 2 H, $CH_2CO$); 7.46 (m, 2 H, Ar); 7.57 and 7.73 (both m, 3 H each, Ar); 7.85 (m, 1 H, Ar).

***N*-[(5,5-Diethyl-2,4,6(1*H*,3*H*,5*H*)-trioxopyrimidin-1-yl)acetyl]-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (10).** Water (1 mL) and $EtNPr^i_2$ (0.17 mL, 1 mmol) were added with stirring to a heterogeneous mixture of *N*-bromoacetyl-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine[8] (**1b**) (231 mg, 0.5 mmol) and 5,5-diethylbarbituric acid (**4**) (185 mg, 1 mmol) in MeOH (5 mL). The reaction mixture was kept at 25 °C for 72 h and filtered. The filtrate was diluted with $H_2O$ (3 mL). The solution was concentrated to 2 mL and kept at 5 °C for 16 h. The precipitate that formed was filtered off and washed with cold $H_2O$ (2×0.3 mL). The filtrate was diluted with 0.2 *M* AcOH (1.3 mL) and chromatographed on Sephadex in 0.05 *M* AcOH. The fractions containing the product were concentrated to dryness, and traces of AcOH from the residue were removed by evaporation with a 2 : 15 : 5 $H_2O$—MeOH—toluene mixture to dryness. The residue was dried. Amorphous compound **10** was obtained in a yield of 160 mg (50%), $[\alpha]_D^{20}$ +2.5 (*c* 1, $H_2O$). Found (%): C, 41.35; H, 6.68; N, 6.35; $H_2O$, 11.06. $C_{22}H_{35}N_3O_{14} \cdot 4\ H_2O$. Calculated (%): C, 41.44; H, 6.70; N, 6.59; $H_2O$, 11.30. $^1$H NMR, δ: 0.86 (t, 6 H, 2 Me, $J$ = 7.5 Hz); 1.95—2.09 (m, 4 H, 2 C$\underline{H}_2$Me); 3.41—3.58 (m, 2 H); 3.60—3.86 (m, 8 H); 3.88—3.97 (m, 2 H); 4.45 (d, 1 H, H(1), Gal, $J = 7.5$ Hz); 4.63 and 4.73 (AB system, 2 H, $CH_2CO$, $J$ = 16.1 Hz); 5.01 (d, 1 H, H(1), Glc, $J = 9.0$ Hz).

**2-Acetamido-*N*-[(DL-9-benzyl-3-methyl-1,2,3,4-tetrahydro-3-carbolinium)acetyl]-2-deoxy-β-D-glucopyranosylamine acetate (11).** A solution of compound **1a** (170 mg, 0.5 mmol) and 9-benzyl-3-methyl-1,2,3,4-tetrahydro-3-carboline (**5**) (207 mg, 0.75 mmol) in MeOH (1.5 mL) was kept at 20 °C for 16 h. The reaction mixture was dried to dryness and the residue was suspended in $H_2O$ (6 mL). The suspension was washed with $CHCl_3$ (3×1.7 mL), and the aqueous layer was centrifuged and concentrated to 2 mL. Dowex 1wx8 anion-exchange resin ($AcO^-$) (0.5 mL) was added to the solution, the mixture was stirred for 30 min, and the resin was filtered off and washed with $H_2O$ (3×1.5 mL). The filtrate and washings were concentrated to 3 mL and chromatographed on Sephadex in 0.05 *M* AcOH containing 10% MeOH. The fractions containing product **11** were concentrated to dryness, and traces of AcOH from the residue were removed by evaporation with a 2 : 15 : 5 $H_2O$—MeOH—toluene mixture to dryness. The residue was dissolved in $H_2O$ (3 mL), lyophilized, and dried. Amorphous compound **11** was obtained in a yield of 208 mg (66%), $[\alpha]_D^{20}$ +10.5 (*c* 1, $H_2O$). Found (%): C, 59.00; H, 7.04; N, 9.15; $H_2O$, 5.73. $C_{29}H_{37}N_4O_6^+ \cdot AcO^- \cdot 2\ H_2O$. Calculated (%): C, 58.85; H, 7.01; N, 8.85; $H_2O$, 5.69. $^1$H NMR, δ: 1.79 (s, 1.5 H, Ac); 1.90 (s, 3 H, $AcO^-$); 2.00 (s, 1.5 H, Ac); 3.05 (br.s, 2 H, C$\underline{H}_2$Ph); 3.28 and 3.31 (both s, 1.5 H each, MeN); 3.45—4.00 (m, 8 H); 4.11 (m, 2 H); 4.57, 4.89 (both m, 1 H each); 5.12 (d, 1 H, H(1), $J$ = 9.5 Hz); 5.19 (br.s, 2 H, $CH_2CO$); 6.99 and 7.16 (both m, 2 H each, Ar); 7.28 (m, 4 H, Ar); 7.44 (m, 1 H, Ar).

**2-Acetamido-1-*N*-{*N*-[(*S*)-1-carboxy-2-(5-hydroxy-3-indolyl)ethyl]glycyl}-2-deoxy-β-D-glucopyranosylamine (12).** 5-Hydroxyl-L-tryptophan (**6**) (220 mg, 1 mmol) and $EtNPr^i_2$ (0.17 mL, 1 mmol) were added with stirring to a solution of compound **1a** (170 mg, 0.5 mmol) in MeOH (5 mL). The solution was kept at 18 °C for 24 h. The reaction mixture was concentrated to 0.5 mL, 1 *M* AcOH (5 mL) was added, and the mixture was chromatographed on Sephadex in 0.05 *M* AcOH. The fractions containing product **12** were lyophilized, the residue was dissolved in $H_2O$ (5 mL), the solution was lyophilized, and the residue was dried *in vacuo* at 60 °C. Amorphous compound **12** was obtained in a yield of 160 mg (62.5%), $[\alpha]_D^{20}$ +2.8 (*c* 1, $H_2O$). Found (%): C, 49.24; H, 6.37; N, 10.48; $H_2O$, 6.64. $C_{21}H_{28}N_4O_9 \cdot 2\ H_2O$. Calculated (%): C, 48.83; H, 6.24; N, 10.85; $H_2O$, 6.98. $^1$H NMR, δ: 2.02 (s, 3 H, Me); 3.35 (dd, 1 H, $J$ = 8.0 Hz, $J$ = 15.5 Hz); 3.48—3.60 (m, 3 H); 3.66 (t, 1 H, H(3), $J_{2,3} = J_{3,4}$ = 9.0 Hz); 3.74—3.84 (m, 3 H); 3.85—4.01 (m, 2 H); 4.07 (dd, 1 H, CHCO, $J = 2.0$ Hz, $J = 8.0$ Hz); 5.10 (d, 1 H, H(1), $J$ = 9.5 Hz); 6.94 (dd, 1 H, Ar, $J = 1.5$ Hz, $J = 8.5$ Hz); 7.20 (d, 1 H, Ar, $J = 1.5$ Hz); 7.38 (s, 1 H, Ar); 7.48 (d, 1 H, Ar, $J = 8.5$ Hz).

***N*-{[1-(3-Chlorophenyl)piperazin-4-yl]acetyl}-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (13).** Methanol (4.25 mL) and $EtNPr^i_2$ (0.17 mL, 1 mmol) were added with stirring to a solution of compound **1b** (230 mg, 0.5 mmol) and 1-(3-chlorophenyl)piperazine hydrochloride (**7**) (233 mg, 1 mmol) in $H_2O$ (0.75 mL). The reaction mixture was kept at 18 °C for 16 h and then at 5 °C for 5 h. The precipitate that formed was filtered off, washed with MeOH and diethyl ether, crystallized from MeOH, and dried. Compound **13** was obtained in a yield of 150 mg (51%), m.p. 140—141 °C, $[\alpha]_D^{20}$ +4.1 (*c* 1, $H_2O$). Found (%): C, 47.30; H, 6.76; Cl, 5 69; N, 7.16;

$H_2O$, 6.12. $C_{24}H_{36}ClN_3O_{11} \cdot 2\ H_2O$. Calculated (%): C, 46.94; H, 6.57; Cl, 5.77; N, 6.84; $H_2O$, 5.87. $^1H$ NMR, δ: 2.70 and 3.17 (both br.s, 4 H each, $CH_2NCH_2$); 3.25 (br.s, 2 H, $CH_2CO$); 3.40—3.59 (m, 2 H); 3.60—3.86 (m, 8 H); 3.87—3.97 (m, 2 H); 4.45 (d, 1 H, H(1), Gal, $J$ = 7.5 Hz); 5.02 (d, 1 H, H(1), Glc, $J$ = 9.0 Hz); 6.92—7.00 (m, 2 H, Ar); 7.09 (s, 1 H, Ar); 7.27 (t, 1 H, Ar, $J$ = 8.0 Hz).

This study was financially supported by the Russian Foundation for Basic Research (Project No. 01-03-33267a) and the Council on Grants of the President of the Russian Federation (Program for State Support of Leading Scientific Schools of the Russian Federation, Grant NSh-1557.2003.3).

## References

1. M. J. Grogan, M. R. Pratt, L. A. Marcaurelle, and C. R. Bertozzi, *Ann. Rev. Biochem.*, 2002, **71**, 593.
2. M. Monsigny, C. Quetard, S. Bourgerie, D. Delay, C. Pichon, P. Midoux, R. Mayer, and A.-C. Roche, *Biochimie*, 1998, **80**, 99.
3. L. M. Likhosherstov, O. S. Novikova, and V. N. Shibaev, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 1244 [*Russ. Chem. Bull.*, 1998, **47**, 1214 (Engl. Transl.)].
4. L. M. Likhosherstov, O. S. Novikova, and V. N. Shibaev, *Izv. Akad. Nauk, Ser. Khim.*, 1999, 1377 [*Russ. Chem. Bull.*, 1999, **48**, 1365 (Engl. Transl.)].
5. L. M. Likhosherstov, O. S. Novikova, V. N. Shibaev, and N. K. Kochetkov, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1848 [*Russ. Chem. Bull.*, 1996, **45**, 1760 (Engl. Transl.)].
6. L. M. Likhosherstov, O. S. Novikova, and V. N. Shibaev, *Dokl. Akad. Nauk*, 2002, **383**, 500 [*Dokl. Chem.*, 2002, **383**, 89 (Engl. Transl.)].
7. L. M. Likhosherstov, O. S. Novikova, and V. N. Shibaev, *Dokl. Akad. Nauk*, 2003, **389**, 482 [*Dokl. Chem.*, 2003, **389**, 73 (Engl. Transl.)].
8. L. M. Likhosherstov, O. S. Novikova, A. O. Zheltova, and V. N. Shibaev, *Izv. Akad. Nauk, Ser. Khim.*, 2004, 676 [*Russ. Chem. Bull., Int. Ed.*, 2004, **53**, 709].
9. D. Macmillan, A. M. Daines, M. Bayrhuber, and S. L. Flitsch, *Org. Lett.*, 2002, **4**, 1467.
10. L. A. Marcaurelle and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2001, **123**, 1587.
11. E. W. Thomas, *Methods Enzymol.*, 1977, **46**, 362.
12. T. S. Black, L. Kiss, D. Tull, and S. G. Withers, *Carbohydr. Res.*, 1993, **250**, 195.
13. T. Kiss, A. Erdel, and L. Kiss, *Arch. Biochem. Biophys.*, 2002, **399**, 188.
14. D. J. Vocaldo, J. Wicki, K. Rupitz, and S. G. Withers, *Biochemistry*, 2002, **41**, 9736.
15. J. Chir, S. G. Withers, C. F. Wan, and Y. K. Li, *Biochem. J.*, 2002, **365**, 857.
16. Z. Keresztessy, L. Kiss, and M. A. Hughes, *Arch. Biochem. Biophys.*, 1994, **315**, 323.
17. D. Tull, D. L. Burgoyne, D. T. Chow, S. G. Withers, and R. Aebersold, *Anal. Biochem.*, 1996, **234**, 119.
18. A. I. Usov and M. A. Rekhter, *Zh. Obshch. Khim.*, 1969, **39**, 912 [*J. Gen. Chem. USSR*, 1969, **39** (Engl. Transl.)].
19. K. Fischer, *Angew. Chem.*, 1935, **48**, 394.

*Received February 2, 2005;*
*in revised form March 24, 2005*