Approaches to intramolecular sialylation
3.* Synthesis of 2,4-dimethoxybenzyl ester of per-*O*-acetylated *N*-acetylneuraminic acid thioglycoside and its attempted oxidation with DDQ in the presence of nucleophiles

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2,4-Dimethoxybenzyl ester of per-*O*-acetylated *N*-acetylneuraminic acid thioglycoside was synthesized and attempts at the oxidative (DDQ-induced) addition of O-nucleophiles (water and galactose derivatives with unprotected hydroxy group at C(6)) to the benzylic carbon atom of this ester were made.

Key words: sialic acids, *N*-acetylneuraminic acid, thioglycosides, substituted benzyl esters, oxidation, monosaccharides, NMR spectroscopy.

Development of new efficient methods for the synthesis of oligosaccharides containing sialic acid residues, in particular, *N*-acetylneuraminic acid (Neu5Ac), which are responsible for a series of immunological, neurobiological, oncological, and other biological processes,[2] is among the most challenging tasks of modern synthetic carbohydrate chemistry. In recent years, we have been developing[1,3] approaches to intramolecular sialylation (see review[4] on the use of intramolecular glycosylation to prepare the glycosides of other sugars and references cited therein). In this process, the target formation of the glycosidic bond is expected to compete more efficiently with the side elimination resulting in the Neu5Ac glycal, because in this case, glycosylation is a monomolecular reaction.

The first problem to be solved on the way to intramolecular sialylation is development of the synthesis of compounds containing glycosyl-donor (Neu5Ac derivative) and glycosyl-acceptor fragments chemically linked through a bridge by a nonglycosidic bond. In the case of Neu5Ac, it seemed most obvious to use the carboxy group for this purpose. Previously,[3] in a study of the synthesis of the disaccharide Neu5Ac-(α-2-3)-Gal containing a D-galactose residue sialylated at position 3, we demonstrated the possibility of efficient linking of the glycosyl donor (Neu5Ac), by a temporary ester bond, to the OH group at the O(2) atom of the glycosyl acceptor (Gal), which will not be further involved in the glycosidic bond formation.

However, a different approach based on the linking of the glycosyl donor, by a temporary bond, to the glycosyl-acceptor OH group that is to be involved in the glycosidic bond is also possible (Scheme 1). For example, the residue of a protected Neu5Ac thioglycoside can, apparently, be linked by its carboxy group through a methoxybenzylidene bridge (mixed acetal-acylal) to the OH group at the C(6) atom of the D-galactose residue subject to glycosylation. The activation of the anomeric position in the nonglycosidically linked Neu5Ac-thioglycoside disaccharide should give rise to the glycosidically linked disaccharide Neu5Ac-(2-6)-Gal (see Scheme 1). This approach is, in principle, similar to the use of mixed *p*-methoxybenzylidene acetal for binding the glycosyl donor and the glycosyl acceptor in the intramolecular synthesis of β-mannosides by a known method.[5]

The key problem along this line is how to create the required methoxybenzylidene bridge between the Neu5Ac and Gal residues. Both putative approaches (Scheme 2) to methoxybenzylidene derivative **1** are based on the addition of nucleophiles (carboxylic acid **2** (pathway *A*) or alcohol **5** (pathway *B*)) to the benzylic carbocation generated upon oxidation of methoxybenzyl ethers or esters (**3** or **4**, respectively) on treatment with DDQ. Here we describe the results of our attempted synthesis of methoxybenzylidene derivative **1** along pathway *B*.

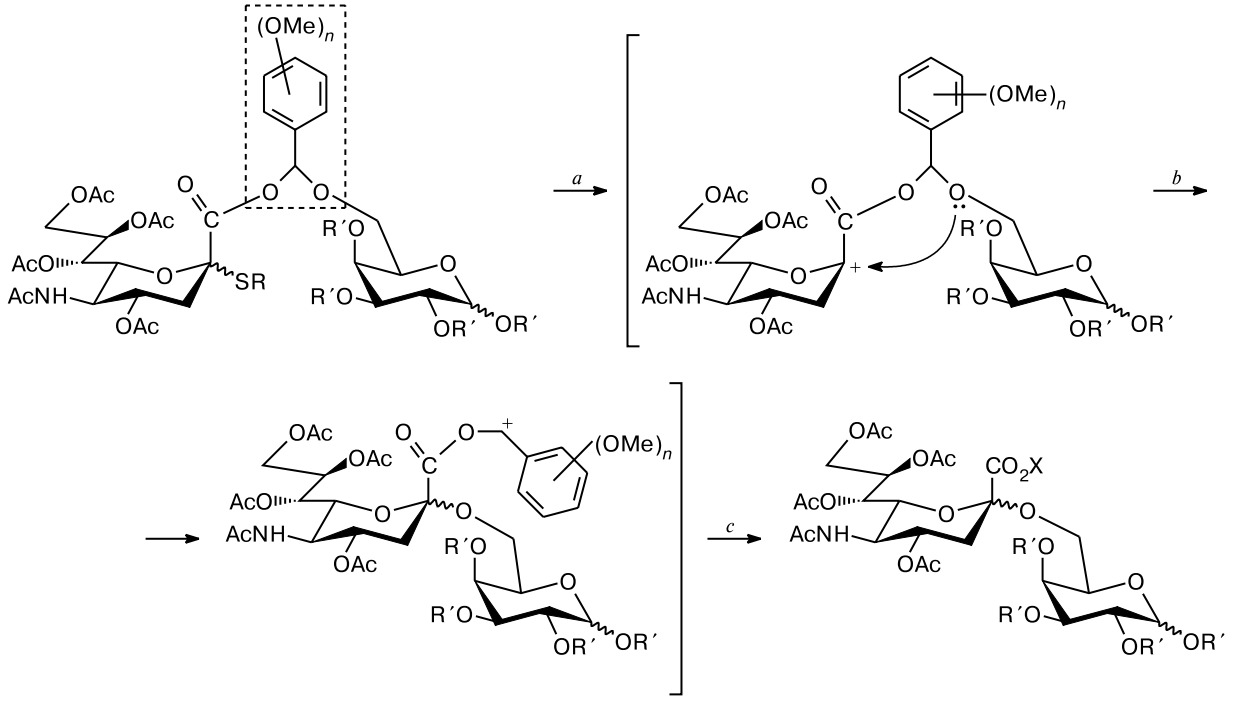
In order to implement this approach, we synthesized the 2,4-dimethoxybenzyl ester of acetylated Neu5Ac methyl thioglycoside (**7**) in 81% yield by the condensation of acid **6a** [1] with 2,4-dimethoxybenzyl alcohol in the presence of 1-mesitylsulfonyl-3-nitro-1,2,4-triazole

* For Part 2, see Ref. 1.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 244—248, January, 2004.

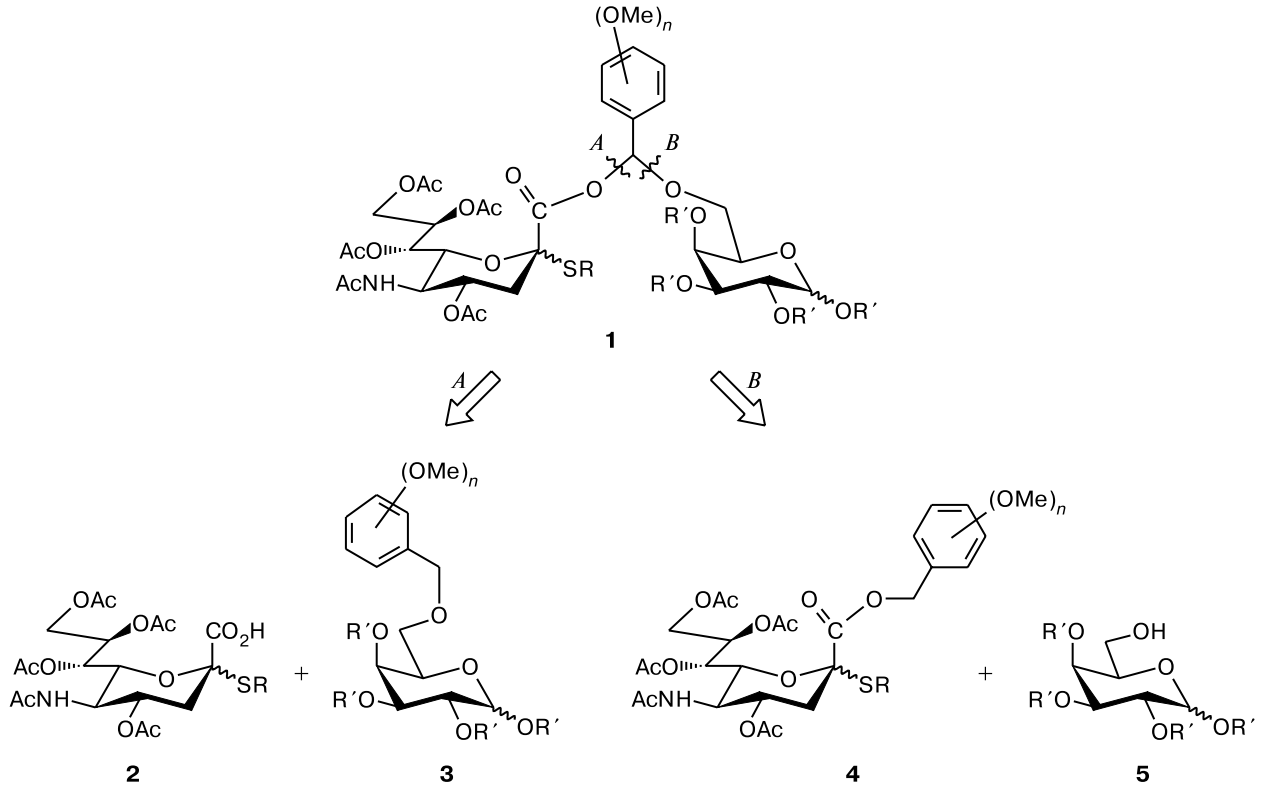
1066-5285/04/5301-0254

Scheme 1

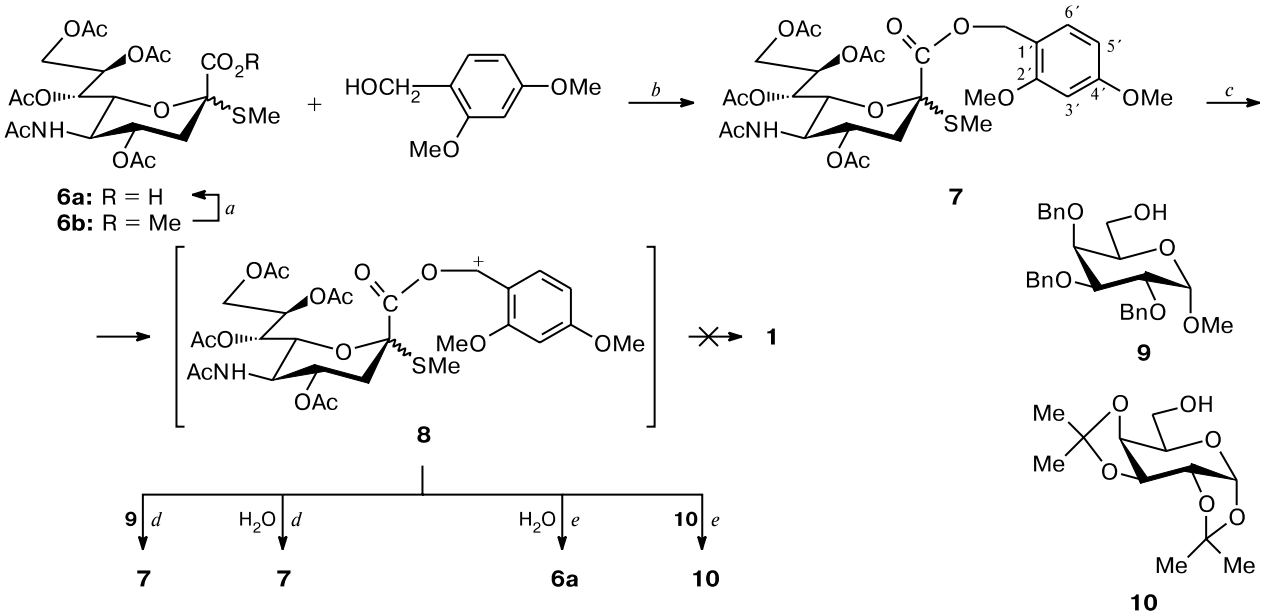


Conditions: *a*. Thioglycoside activation. *b*. Intramolecular nucleophilic attack. *c*. Termination of the reaction (addition of an external nucleophile).

Scheme 2



Scheme 3



Reagents and conditions: *a*. See Ref. 1. *b*. MSNT, MeIm, CH_2Cl_2. *c*. DDQ, 4 Å molecular sieves. *d*. CH_2Cl_2, 21—24 °C. *e*. CH_2ClCH_2Cl, 50—55 °C.

(MSNT)[6] and *N*-methylimidazole (MeIm) (Scheme 3). Ester **7** was purified by gel chromatography on a Bio-Beads S polystyrene sorbent (with toluene as the eluent). The use of adsorption chromatography on silica gel is problematic, as the acid-labile ester **7** partly decomposes during the process. In particular, this followed from the fact that the mass of ester **7** markedly decreased after preparative TLC on silica gel (see Experimental). Moreover, the attempts to isolate ester **7** by reversed-phase chromatography (C18, water—THF) resulted in its partial hydrolysis (probably, due to the presence of residual silanol groups on the sorbent surface). The structure of ester **7** followed from NMR and MS data. The ^1H and ^{13}C NMR chemical shifts observed for the Neu5Ac residue are similar to those of the starting acid **6a** [1] and the corresponding methyl ester **6b**.[7] The mass spectrum of the reaction product contains a molecular ion peak whose exact mass corresponds to the molecular formula of ester **7**.

The choice of dimethoxy derivative **7** for the attempted oxidative addition was due to the known[8] stability of monomethoxybenzyl esters against oxidative (DDQ-induced) deprotection. In addition, it is known[8] that dimethoxybenzyl esters of aliphatic acids are readily converted into free acids on treatment with DDQ (in the presence of water). Initially, it was assumed that in the case of dimethoxybenzyl ester **7**, nucleophiles would also add to the benzylic carbocation **8** generated from **7** under the action of the oxidant (DDQ). To verify this hypothesis, a mixture of ester **7** and methyl galactoside tribenzyl ether **9** [9] with the free OH group at the C(6) atom was treated with DDQ under the typical conditions (*cf.* Ref. 5) used to generate benzylic carbocations (CH_2Cl_2, 4 Å molecular sieves, 21 °C). After 24 h, alcohol **9** was completely consumed (TLC); however dimethoxybenzyl ester **7** was recovered unchanged from the reaction mixture (see Scheme 3). Apparently, the benzyl protective groups in ether **9** were oxidized by DDQ (this was indicated by the characteristic smell of benzaldehyde). When the same reaction was carried out in the presence of water, ester **7** was found to withstand oxidation. The oxidative hydrolysis of **7** could be accomplished by conducting the process at elevated temperature (50 °C, 2.5 h); acid **6a** was isolated from the reaction mixture (see Scheme 3). Apparently, generation of the benzylic carbocation **8** from ester **7** requires more drastic conditions than those reported[8] for similar esters of other acids. Therefore, the next attempt to prepare acetal **1** was made at the elevated temperature (1,2-dichloroethane, 55 °C). Since tribenzyl ether **9** proved to be unstable in the presence of DDQ even at ~20 °C (see above), the derivative **10** [10] with isopropylidene protective groups stable against oxidation was used under the chosen conditions. After 4 h, the reaction with DDQ was completed and the dimethoxybenzyl ester **7** has been completely consumed; however the starting alcohol **10** was the only isolable reaction product. Apparently, under the drastic conditions used, a nucleophilic attack by the thioglycoside S atom on the generated benzylic carbocation takes place; in the pres-

ence of excess oxidant, this results in degradation of the Neu5Ac derivative.

The arising problem could probably be solved, on the one hand, by the use of an equivalent amount of the oxidant and, on the other hand, by the use of an analogous Neu5Ac phenyl thioglycoside derivative in which the S atom is less nucleophilic, or by the preparation of trimethoxybenzyl ester of Neu5Ac thioglycoside, which should generate the benzylic carbocation under much milder conditions. The implementation of pathway *A* (see Scheme 2) might also allow one to avoid the difficulties arising along pathway *B*. Our further research will develop along these lines.

Experimental

All experiments were carried out in anhydrous solvents purified by standard procedures. Commercial reagents (Aldrich and Fluka) were used. TLC was performed on plates with silica gel 60 on aluminum foil (Merck) in a 1 : 1 toluene—acetone mixture. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol EX-270 instrument using Me_4Si as the internal standard in $CDCl_3$ or C_6D_6. The NMR signals were assigned using DEPT135 experiments and ^{1}H—^{1}H (COSY) and ^{13}C—^{1}H (HETCOR, LRHETCOR) 2D correlation experiments. Optical rotation was measured on a JASCO DIP-370 polarimeter at ~20—30 °C. Mass spectra were run on a Jeol JMS-HX-110 mass spectrometer (FAB, 3-nitrobenzyl alcohol as the matrix). The reactions with DDQ were terminated using the quenching solution (pH 7.0) obtained by dissolving ascorbic acid (1.75 g), citric acid (3.15 g), and NaOH (2.3 g) in 250 mL of water.

2,4-Dimethoxybenzyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate (7). *N*-Methylimidazole (46.5 μL, 0.585 mmol) was added to a solution of acid **6a** [1] (49.4 mg, 0.0973 mmol; α : β ≈ 10 : 1) in CH_2Cl_2 (1 mL) in an argon atmosphere. The resulting solution was transferred under argon into a flask containing MSNT (58.9 mg, 0.199 mmol) and then, under the same conditions, into the reaction flask with 2,4-dimethoxybenzyl alcohol (33.0 mg, 0.196 mmol). The first two flasks were additionally rinsed with CH_2Cl_2 (2×0.5 mL), and the obtained solutions were transferred to the reaction mixture, which was kept for 22 h at 20 °C (TLC showed the absence of the starting alcohol). The reaction mixture was diluted with AcOEt (50 mL), the organic phase was washed three times with a cold saturated solution of $NaHCO_3$ and brine and dried with $MgSO_4$, and the solvent was evaporated to dryness. The residue was dissolved in THF (1.5 mL) and purified by gel chromatography on a column (45×1.5 cm) with Bio-Beads SX-8 (200—400 mesh, Bio-Rad; THF as the eluent) to give the target ester **7** (51.8 mg, 81%, α : β ≈ 10 : 1), $[\alpha]_D^{29}$ −5.0 (*c* 1.0, AcOEt). MS (FAB, positive ion detection mode). Found: *m/z* 658.2177 [M + H]. $C_{29}H_{40}NO_{14}S$. Calculated: *m/z* 658.2170 [M + H]. No satisfactory results of elemental analysis could be obtained. ^{1}H NMR (C_6D_6), δ, α-**7**: 1.55 (s, 6 H, AcO, AcNH); 1.78 (s, 3 H, AcO); 1.89 (s, 3 H, AcO); 2.00 (dd, 1 H, H_{ax}(3)); 2.14 (s, 3 H, SMe); 2.20 (s, 3 H, AcO); 2.85 (dd, 1 H, H_{eq}(3), $J_{3eq,4}$ = 4.6 Hz, $J_{3eq,3ax}$ = 12.5 Hz); 3.28 (s, 3 H, OMe); 3.34 (s, 3 H, OMe); 3.85 (dd, 1 H, H(6), $J_{5,6}$ = 10.6 Hz, $J_{6,7}$ = 2.3 Hz); 4.31 (dd, 1 H, H_a(9), $J_{8,9a}$ = 5.6 Hz, $J_{9a,9b}$ = 12.5 Hz); 4.40 (ddd, 1 H, H(5), $J_{4,5}$ = 10.6 Hz); 4.68 (dd, 1 H, H_b(9), $J_{8,9b}$ = 3.0 Hz); 4.85 (ddd, 1 H, H(4), $J_{3ax,4}$ = 11.6 Hz); 5.25 (d, 1 H, C$\underline{H}_a$Ar, $J_{a,b}$ = 11.9 Hz); 5.42 (d, 1 H, C$\underline{H}_b$Ar); 5.47 (dd, 1 H, H(7), $J_{7,8}$ = 8.3 Hz); 5.78 (ddd, 1 H, H(8)); 6.34—6.41 (m, 2 H, H(3′), H(5′), Ar); 7.33 (m, 1 H, H(6′), Ar, *J* = 8.3 Hz). ^{1}H NMR (C_6D_6), δ, β-**7***: 2.58 (dd, 1 H, H_{eq}(3), $J_{3eq,4}$ = 4.6 Hz, $J_{3eq,3ax}$ = 12.5 Hz). ^{13}C NMR (C_6D_6), δ, α-**7**: 12.1 (SMe); 20.4, 20.5, 20.6, 21.3 (AcO); 23.0 (AcNH); 38.4 (C(3)); 49.2 (C(5)); 54.9, 55.0 (MeO); 62.7 (C(9)); 63.6 ($CO_2\underline{C}H_2Ar$); 67.5 (C(7)); 69.6 (C(8)); 70.0 (C(4)); 74.7 (C(6)); 83.6 (C(2)); 99.1 (C(5′), Ar); 104.4 (C(3′), Ar); 116.4 (C(1′), Ar); 131.9 (C(6′), Ar); 159.4, 162.2 (C(2′), C(4′), Ar); 169.2 (C(1)); 169.8 ($CH_3\underline{C}ONH$); 169.99, 170.02, 170.08 (CO).

Reactions of dimethoxybenzyl ester 7 with DDQ in the presence of nucleophiles

***A.* 2,4-Dimethoxybenzyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate (α-7).** Freshly activated 4 Å molecular sieves (0.2 g) were added to a solution containing ester **7** (25.5 mg, 0.0388 mol, α : β ≈ 10 : 1) and alcohol **9** [9] (18.1 mg, 0.038 mmol) in CH_2Cl_2 (1 mL) and the suspension was stirred for 15 min at 20 °C and cooled to 0 °C. A solution of DDQ (13.5 mg, 0.058 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred for 1 h at the same temperature (according to TLC, the composition of the reaction mixture was not changed) and then at 20 °C. After 24 h, the reaction was terminated by adding 1 mL of the quenching solution. The resulting suspension was stirred for 15 min, diluted with CH_2Cl_2 (20 mL), and filtered through Celite. The organic phase was washed twice with a cold saturated solution of $NaHCO_3$ and brine and dried with $MgSO_4$ and the solvent was evaporated to dryness. The residue was dissolved in THF (1.5 mL) and purified by gel chromatography on a column (45×1.5 cm) with Bio-Beads SX-8 (200—400 mesh; THF as the eluent) to give 28.5 mg of a product, which was mainly the initial ester **7**, according to NMR and TLC data. Additional purification by preparative TLC (a 20×20 cm plate, 0.5 mm-thick layer (Merck); successive development in acetone—toluene 10 : 90 (×2), 25 : 75 (×2), and 40 : 60 (×2) mixtures) gave a pure sample (7.4 mg, 29%) of ester α-**7**, $[\alpha]_D^{22}$ −3.0 (*c* 0.37, AcOEt). The NMR signals of α-**7** completely coincided with those of the major isomer present in the starting **7** (α : β ≈ 10 : 1).

B. Similarly to procedure ***A***, a mixture of ester **7** (2 mg), DDQ (5 mg), CH_2ClCH_2Cl (0.5 mL), and water (0.5 mL) was stirred for 4 h at 24 °C. No changes were detected by TLC. The reaction mixture was stirred for an additional 4 h at 50 °C. According to TLC, the starting ester **7** disappeared to give acid **6a**.

C. Similarly to procedure ***A***, DDQ (42.4 mg, 0.187 mmol) was added at 0 °C under argon to a mixture of ester **7** (24 mg, 0.0365 mol, α : β ≈ 10 : 1), alcohol **10** [10] (9.5 mg, 0.365 mmol), and 4 Å molecular sieves (0.2 g) in CH_2ClCH_2Cl (2 mL). The reaction mixture was stirred for 1 h at the same temperature, for 1.5 h at 20 °C (according to TLC, the composition of the reaction mixture was not changed) and for 4 h at 55 °C.

* A partial spectrum is given.

The reaction mixture was cooled to 0 °C and treated as described in procedure ***A***. The product was isolated by gel chromatography on a column (40×2.5 cm) with Bio-Beads SX-4 (200—400 mesh; toluene as the eluent) to give 9 mg of a product mainly containing the starting alcohol **10**, according to NMR and TLC data.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 02-03-32271), Russian Federation President (grant for the Support of Leading Scientific Schools NSh 1557.2003.3), and the Grant-in-Aid from the Ministry of Education, Science, and Culture of Japan.

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Received December 25, 2003