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Note

Investigation into an efficient synthesis of 2,3-dehydro-*N*-acetyl neuraminic acid leads to three decarboxylated sialic acid dimers

Evan J. Horn, Jonel P. Saludes and Jacquelyn Gervay-Hague*

Department of Chemistry, University of California, Davis, One Shields Ave., Davis, CA 95616, USA

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Abstract—Sialic acid, an important carbohydrate found incorporated on the cell surface of many organisms, has been modified for use in a wide range of biological and pharmaceutical applications. We hypothesized that 4,7,8,9-tetra-*O*-acetyl-2-deoxy-2,3-dehydro-*N*-acetyl neuraminic acid methyl ester (**4**) could be efficiently synthesized in a one-pot reaction by heating peracetylated sialic acid (**2**) in pyridine and acetic anhydride to induce β-elimination. When reduced to practice, this reaction produced only modest yields of **4**. Six compounds, including three new decarboxylated sialic acid dimers, were also found to have been synthesized in the reaction. In an effort to better understand the chemistry and the mechanisms of this reaction, all of the side products were isolated and fully characterized.

Keywords: Sialic acid; Decarboxylation; Neu5Ac2en

Sialic acid (*N*-acetylneuraminic acid; Neu5Ac, **1**) has been modified for use in a wide range of biological and pharmaceutical applications. The 2,3-dehydro derivative of Neu5Ac (Neu5Ac2en, **4**) is a key intermediate in the preparation of the commercial anti-influenza drug, Zanamivir, which was found to be more effective than adamantane-based compounds.[1,2] Because of its synthetic importance, several methods have been reported for the synthesis of **4**.[3,4] These include the most widely used β-elimination of peracetylated Neu5Ac glycosyl chloride **3**[5] catalyzed by Et_3N or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Scheme 1),[6,7] TMSOTf-catalyzed elimination of peracetylated Neu5Ac methyl ester **2**,[8] and oxidation[9] or elimination[10] of Neu5Ac thioglycoside. A recent approach to the preparation of **4** from **3** utilized Na_2HPO_4 in refluxing acetonitrile for 3 h.[11] Although this method gave an almost quantitative yield, the preparation of **3** required several steps and careful handling of HCl gas.

Our laboratory has found derivatives of **1** and **4** to be useful building blocks for constructing amide-linked homooligomers with stable secondary structures,[12,13] and we typically employed DBU-catalyzed β-elimination to make **4**. However, it was our hypothesis that the C-2 acetate could be a sufficiently good leaving group under the basic conditions of peracetylation, and that by simply heating **2** in a one-pot technique, we could induce β-elimination to afford **4.** To test this hypothesis, **1** was peracetylated to yield nearly pure product as evidenced by ^{1}H NMR and MS. The solution was then heated to 100 °C for 4 h. The crude reaction mixture was carried forward and treated with ethereal diazomethane to methylate the carboxylic acid to facilitate purification (Scheme 2).

Initial ^{1}H NMR analysis showed that several products were formed in the reaction (Scheme 2), including compound **4** (confirmed via a doublet at δ 6.08, $J = 1.8$ Hz). The mixture was purified by a series of chromatographic procedures (Fig. S6, Supplementary data) to yield a total of seven compounds, with **4** isolated in only 21%. Two other products **5** (37%) and its anomer **6** (3%) were quite unexpected, with both having undergone

* Corresponding author. Tel.: +1 530 754 9577; fax: +1 530 752 8995; e-mail: gervay@chem.ucdavis.edu

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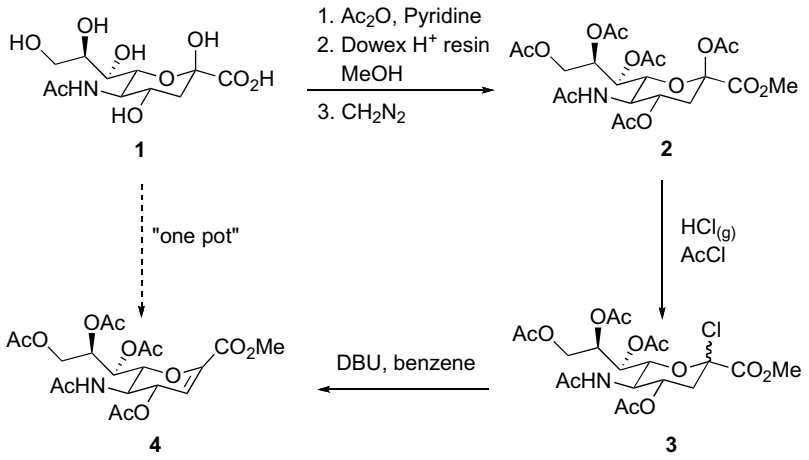
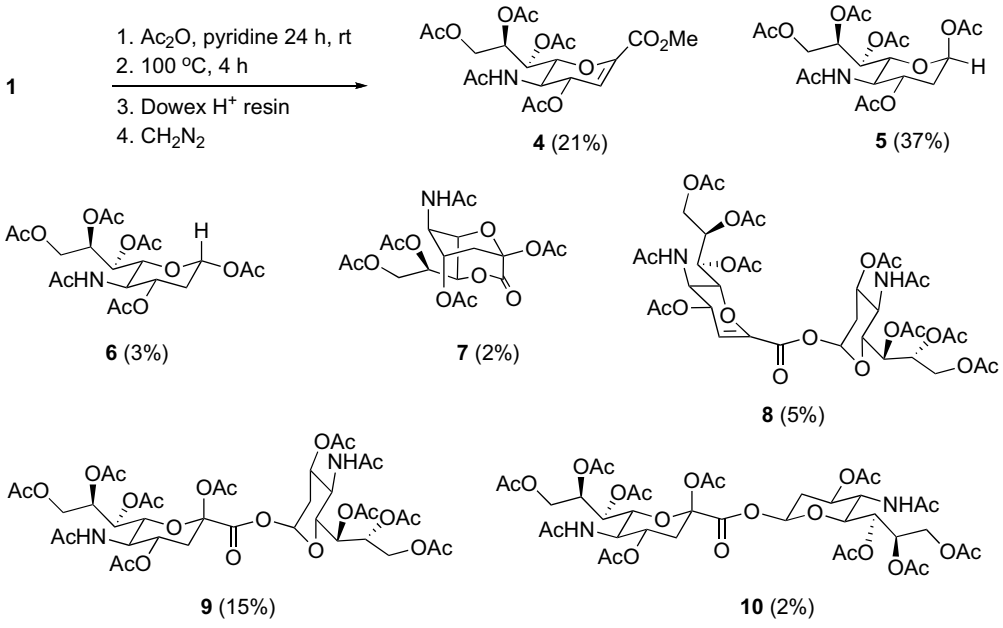


Scheme 1. The most widely used route to **4**, and our proposed one-pot synthesis.



Scheme 2. Products isolated from one-pot reaction.

decarboxylation. Both **5** and **6** were previously synthesized using 4:1 toluene–pyridine in the presence of lead(IV) acetate.[14] Formation of these compounds under standard peracetylation conditions was unprecedented. Compound **7**, a 1→7 lactone which is a common side product of peracetylation,[15] was also found in small amounts (2%).

Compounds **8**, **9**, and **10** are novel compounds. We first believed these to be dimers based on a few observations. Their mass spectra showed molecular ions close to twice that of the previously isolated monomers, and the number of acetate peaks in the ^{1}H NMR spectra corresponded to dimeric, peracetylated structures. In addition, their ^{1}H NMR spectra showed similarities to **2**, **4**, **5**, and **6**, leading us to believe that each of these dimers are composed of a combination of any two of these previously isolated compounds. Compound **9**, being the dimer isolated in highest yield, was characterized first. Its low-resolution mass of m/z 957.3 $[M+Na]^+$ suggest that it was composed of a combination of **2** and either **5** or **6**. Deuterium exchange and ^{1}H NMR studies revealed two exchangeable protons from NHAc, indicating that the dimer is connected via an ester linkage. The mass difference of 74 amu between **9** and the sum of **2** (m/z 533.2 $[M]^+$) and **5** (or **6**) (m/z 475.2 $[M]^+$) indicate the absence of an acetate and Me of the methyl ester. Heteronuclear correlation by HSQC revealed that the downfield ^{1}H NMR peak at δ 6.15 is anomeric (^{13}C NMR δ 93.5), and not due to an olefinic proton. The configuration of this anomeric center was determined

by applying *J*-based analysis using Karplus relationships to the anomeric proton resonance. We found that this proton is equatorial as shown by a doublet ($J = 1.8$ Hz) that is due to the coupling with axial methylene proton on C-2. If the anomeric H were axial, a distinct doublet of doublets would have been observed due to large trans diaxial and small equatorial–axial couplings ($J_{1,2ax} = \sim 10$ Hz; $J_{1,2eq} = \sim 2$ Hz). This led us to conclude that **9** is a β-linked dimer composed of **2** and **5**. The remaining ^{1}H and ^{13}C NMR resonances were assigned using a combination of COSY, HSQC, and HMBC (Table 2, Supplementary data). The key connectivity between **2** and **5** that completed the structural characterization of **9** was done by HMBC correlations ($J = 8$ Hz) between H-3′ and C-1′ and between C-1′ and H-1 (Fig. 1).

The structures of **8** and **10** were solved in the same manner as **9**. Compound **10** has the same mass and similar ^{1}H NMR spectra as **9**. The key difference is the resonance at δ 5.65 (dd, $J = 10.2$, 1.8 Hz) due to axial anomeric proton indicating that **10** is an α-linked dimer composed of **2** and **6**. The connectivity was confirmed by HMBC correlations ($J = 8$ Hz) as shown in Figure 1. The NMR spectra of **8** clearly indicate that it possesses the same olefinic system as **4** (^{1}H NMR δ 6.04; ^{13}C NMR δ 110.0) and the same equatorial anomeric proton as **9** (δ 6.38, d, $J = 1.8$ Hz). The low-resolution mass of m/z 897.2 $[M+Na]^{+}$ that is 74 amu less than the sum of **4** and **5** is equivalent to the absence of a Me and an acetate. Deuterium exchange studies also revealed two exchangeable protons from NHAc indicating an ester linkage. The remaining ^{1}H and ^{13}C NMR resonances were assigned (Table 3, Supplementary data), the β-linkage between **4** and **5** determined by *J*-based analysis on the anomeric proton (δ 6.38, d, $J = 1.8$ Hz) and their connectivity was confirmed by HMBC correlations ($J = 5$ Hz) as shown in Figure 1.

As an initial step in our investigation of the reaction mechanism, the methyl ester of **1** was subjected to the same peracetylation conditions shown in Scheme 1. We only recovered **2** and none of **4**–**10**. This suggests that the free acid plays a role in the formation of the observed products. In summary, our one-pot method generated seven compounds, including Neu5Ac2en (**4**) and three novel dimeric structures. Peracetylation of sialic acid using pyridine and acetic anhydride is common protecting group chemistry. It is surprising that relatively high amounts of **2** underwent decarboxylation under typical peracetylation conditions and heating. We are further investigating the mechanism of these reactions with the goal of directing the selectivity to the formation of **4**.

1. Experimental

1.1. General methods

NMR spectra were recorded on Varian 300 and 600 MHz NMR spectrometers. Chemical shifts were referenced to residual $CHCl_3$ ($\delta_H = 7.26$) and $CDCl_3$ ($\delta_C = 77.1$). Low-resolution mass spectra were obtained by electrospray ionization using Finnigan Mat LCQ-Deca mass spectrometer. High resolution mass spectra were obtained using Applied Biosystems 4700 MALDI-TOF with internal calibration. Reactions were monitored by TLC using Si Gel 60 F254 glass-backed plates (Merck). Reverse phase HPLC was performed using Waters 600 on a C_{18} column (250 × 10 mm, 7 μm particle size, Vydac) at 90 min elution time using a gradient of 5–50% MeOH in H_2O at 4 mL/min flow rate with detection set at 215 and 225 nm.

1.2. General synthesis

Pyridine (7.5 mL) and Ac_2O (7.5 mL) were added to sialic acid (500 mg, 1.6 mmol) under N_2 at rt. The suspension was stirred and the reaction was complete

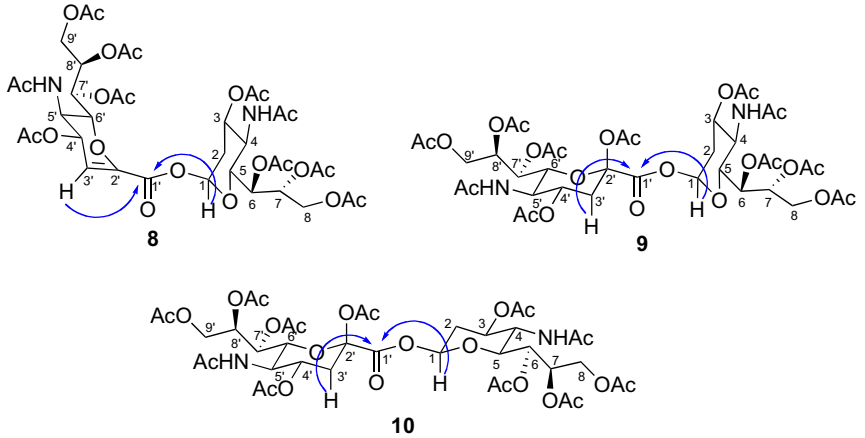


Figure 1. Key HMBC correlations in determining the connectivity for **8**, **9**, and **10**.

after 12 h as observed by TLC, resulting in full peracetylation ($R_f = 0.4$ in 65:15:8 $CHCl_3$–MeOH–H_2O). A small aliquot was removed from the solution, diluted with EtOAc, and washed with 1 N HCl. The organic layer was concentrated to dryness, the ^1H NMR spectrum recorded, and was found to be consistent with the literature[16] for **2**. The solution was heated to 100 °C for 4 h, at which time **2** had disappeared by TLC. The mixture was cooled to rt and concentrated. The crude mixture was dissolved in MeOH, treated with Dowex 50WX8-100 cationic resin for 30 min, filtered, and concentrated. The brown glassy oil was dissolved in 2:1 MeOH/Et_2O and followed by dropwise addition of freshly prepared CH_2N_2. A slight excess of CH_2N_2 was added to ensure complete methylation, and the reaction was quenched with HOAc. The mixture was concentrated to dryness in vacuo to yield a brownish glassy oil (710 mg). The crude reaction mixture was purified according to Figure S6, Supplementary data, to yield **4**, **5**, **6**, **7**, **8**, **9**, and **10**.

1.2.1. 4,7,8,9-Tetra-*O*-acetyl-2-deoxy-2,3-dehydro-*N*-acetyl neuraminic acid methyl ester (4). Isolated yield is 158 mg (23%) as a yellow oil. HPLC $t_R = 66.6$ min. ^1H and ^{13}C NMR are consistent with the literature.[7] LRMS *m*/*z*: calcd for $C_{20}H_{27}NO_{12}Na^+$, 496.1; found, 496.2.

1.2.2. 4-Acetamido-1,3,6,7,8-penta-*O*-acetyl-2-4-dideoxy-β-D-galacto-octopyranose (5). Isolated yield is 292 mg (37%) as a yellow glassy oil. HPLC $t_R = 53.8$ min. ^1H and ^{13}C NMR spectra are consistent with the literature.[14] LRMS *m*/*z*: calcd for $C_{20}H_{29}NO_{12}Na^+$, 498.2; found, 498.2.

1.2.3. 4-Acetamido-1,3,6,7,8-penta-*O*-acetyl-2-4-dideoxy-α-D-galacto-octopyranose (6). Isolated yield is 21 mg (3%) as a yellow glassy oil. HPLC $t_R = 61.6$ min. ^1H and ^{13}C NMR spectra are consistent with the literature.[14] LRMS *m*/*z*: calcd for $C_{20}H_{29}NO_{12}Na^+$, 498.2; found, 498.2.

1.2.4. 5-Acetamido-2,4,8,9-tetra-*O*-acetyl-β-D-glycero-D-galacto-2-nonulopyranosonic acid-1,7-lactone (7). Isolated yield is 18 mg (2%) as a yellow glassy oil. HPLC $t_R = 50.9$ min. ^1H and ^{13}C NMR spectra were found to be consistent with the literature.[15] LRMS *m*/*z*: calcd for $C_{19}H_{25}NO_{12}Na^+$, 482.1; found, 482.1.

1.2.5. 4-Acetamido-3,6,7,8-tetra-*O*-acetyl-1-[4,7,8,9-tetra-*O*-acetyl-2-deoxy-2,3-dehydro-*N*-acetyl neuraminyl]-2,4-dideoxy-β-D-galacto-octopyranose (8). Isolated yield is 32 mg (5%) as a colorless glassy oil. HPLC $t_R = 83.7$ min. ^1H NMR spectrum was also recorded in $CDCl_3$ with 3 drops of D_2O added. After shaking, the mixture was allowed to sit overnight, ^1H NMR spectrum was recorded and the disappearance of amide NH peaks at 5.50 and 6.71 ppm was observed. A coupling constant of 5 Hz was used to observe the HMBC correlations between C-1′ and H-1 and H-3′. All other HMBC correlations were observed at 8 Hz. ^1H NMR (600 MHz, $CDCl_3$): δ 6.72 (d, 1H, $J = 9.6$ Hz), 6.38 (d, 1H, $J = 1.8$ Hz), 6.04 (d, 1H, $J = 2.4$ Hz), 5.74 (dd, 1H, $J = 8.8$, 2.4 Hz), 5.50 (m, 3H), 5.37 (m, 2H), 5.09 (m, 1H), 4.47 (m, 2H), 4.37 (dd, 1H, $J = 12.6$, 2.4 Hz), 4.20 (m, 3H), 4.05 (dd, 1H, $J = 12.6$, 7.2 Hz), 3.97 (dd, 1H, $J = 10.2$, 1.8 Hz), 2.25 (dd, 1H, $J = 12.4$, 4.4 Hz), 2.18–1.89 (m, 32H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.3, 171.2, 171.1, 170.9, 170.7, 170.5, 170.4, 170.3, 170.0, 159.2, 145.1, 110.0, 92.2, 75.5, 71.8, 71.3, 69.5, 68.6, 68.3, 68.0, 66.5, 62.5, 61.5, 49.0, 47.4, 31.8, 23.3, 23.1, 21.2, 20.9, 20.8. HRMS *m*/*z*: calcd for $C_{37}H_{50}N_2NaO_{22}$, 897.2753; found, 897.2790.

1.2.6. 4-Acetamido-3,6,7,8-tetra-*O*-acetyl-1-[1,4,7,8,9-penta-*O*-acetyl-*N*-acetyl neuraminyl]-2,4-dideoxy-β-D-galacto-octopyranose (9). Isolated yield is 109 mg (15%) as a yellow glassy oil. HPLC $t_R = 84.4$ min. ^1H NMR spectrum was also recorded in $CDCl_3$ with 3 drops of D_2O added. After shaking, the mixture was allowed to sit overnight, ^1H NMR spectrum was recorded and the disappearance of amide NH peaks at 5.51 and 6.92 ppm was observed. The product was slightly unstable at room temperature at both dry and as $CDCl_3$ solution, and was found to decompose to the 2 monomer units. All HMBC correlations were recorded using a coupling constant of 8 Hz. ^1H NMR (600 MHz, $CDCl_3$): δ 6.92 (d, 1H, $J = 12.0$ Hz), 6.15 (d, 1H, $J = 1.8$ Hz), 5.51 (dd, 1H, $J = 7.8$, 2.4 Hz), 5.48 (t, 1H, 1.8 Hz), 5.44 (dt, 1H, $J = 4.2$, 2.4 Hz), 5.40 (d, 1H, $J = 4.8$ Hz), 5.36 (d, 1H, $J = 4.8$ Hz), 5.35 (d, 1H, $J = 10.2$ Hz), 5.17 (m, 1H), 4.48 (dd, 1H, $J = 12.6$, 2.4 Hz), 4.44 (dd, 1H, $J = 12.6$, 3.0 Hz), 4.30 (dd, 1H, $J = 10.8$, 1.2 Hz), 4.19 (m, 4H), 4.04 (dd, 1H, $J = 10.8$, 2.4 Hz), 2.51 (dd, 1H, $J = 13.2$, 4.8 Hz), 2.23 (ddd, 1H, $J = 13.2$, 4.2, 1.8 Hz), 2.15–1.89 (m, 35H). ^{13}C NMR (150 MHz, $CDCl_3$): δ171.6, 171.2, 171.1, 170.7, 170.6, 170.4, 170.3, 169.0, 165.2, 97.1, 93.5, 72.8, 72.5, 72.2, 70.7, 68.8, 68.3, 68.1, 66.5, 62.5, 61.7, 49.4, 49.1, 37.6, 36.7, 23.3, 21.3, 21.2, 21.1, 21.0, 20.9, 20.6, 20.1. HRMS *m*/*z*: calcd for $C_{39}H_{54}N_2NaO_{24}$, 957.2964; found, 957.2990.

1.2.7. 4-Acetamido-3,6,7,8-tetra-*O*-acetyl-1-[1,4,7,8,9-penta-*O*-acetyl-*N*-acetyl neuraminyl]-2,4-dideoxy-α-D-galacto-octopyranose (10). Isolated yield is 13 mg (1%) as a yellow glassy oil. HPLC $t_R = 73.7$ min. ^1H NMR spectrum was also recorded in $CDCl_3$ with 3 drops of D_2O added. After shaking, the mixture was allowed to sit overnight, ^1H NMR spectrum was recorded and the disappearance of amide NH peaks at 5.31 and 5.26 ppm was observed. The product was slightly unstable at room temperature at both dry and as $CDCl_3$ solution, and was found to decompose into

the 2 monomer units, as well as epimerize to **7** in $CDCl_3$. All HMBC correlations were recorded using a coupling constant of 8 Hz. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.65 (dd, 1H, $J = 10.2$, 1.8 Hz), 5.43 (dd, 1H, $J = 7.2$, 2.4 Hz), 5.37 (dd, 1H, $J = 4.2$, 2.4 Hz), 5.30 (m, 3H), 5.08 (m, 2H), 4.96 (m, 1H), 4.47 (dd, 1H, $J = 12.6$, 3.0 Hz), 4.30 (dd, 1H, $J = 12.6$, 2.4 Hz), 4.06 (m, 4H), 3.80 (dd, 1H, $J = 10.2$, 2.4 Hz), 2.48 (dd, 1H, $J = 13.8$, 4.8 Hz), 2.36 (ddd, 1H, $J = 12.6$, 4.8, 1.8 Hz), 2.14–1.83 (m, 35H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.9, 171.7, 171.3, 171.2, 170.9, 170.6, 170.5, 170.2, 168.6, 164.8, 96.7, 93.0, 74.0, 72.9, 71.9, 70.1, 69.9, 68.2, 68.0, 67.1, 62.2, 62.0, 49.7, 49.6, 36.1, 23.2, 21.0, 20.9, 20.8, 20.7, 20.6. HRMS m/z: calcd for $C_{39}H_{54}N_2NaO_{24}$, 957.2964; found, 957.2975.

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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.2008.01.033.

References

1. Dyason, J. C.; von Itzstein, M. *Aust. J. Chem.* **2001**, *54*, 663–670.
2. Thomson, R.; von Itzstein, M. In *Carbohydrate-based Drug Discovery*; Wong, C. H., Ed.; Wiley-VCH: Weinheim, 2003; Vol. 2, pp 831–862.
3. Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539–4566.
4. Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–469.
5. Meindl, P.; Tuppy, H. *Monatsh. Chem.* **1965**, *96*, 802–815.
6. Meindl, P.; Tuppy, H. *Monatsh. Chem.* **1969**, *100*, 1295–1306.
7. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631–636.
8. Ercegovic, T.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 3378–3384.
9. Kononov, L. O.; Komarova, B. S.; Nifantiev, N. E. *Russ. Chem. Bull.* **2002**, *51*, 698–702.
10. Ikeda, K.; Konishi, K.; Sano, K.; Tanaka, K. *Chem. Pharm. Bull.* **2000**, *48*, 163–165.
11. Kulikova, N. Y.; Shpirt, A. M.; Kononov, L. O. *Synthesis* **2006**, 4113–4114.
12. Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074–1078.
13. Gregar, T. Q.; Gervay-Hague, J. *J. Org. Chem.* **2004**, *69*, 1001–1009.
14. Potter, J. J.; vonItzstein, M. *Carbohydr. Res.* **1996**, *282*, 181–187.
15. Kirchner, E.; Thiem, F.; Dernick, R.; Heukeshoven, J.; Thiem, J. *J. Carbohydr. Chem.* **1988**, *7*, 453–486.
16. Marra, A.; Sinay, P. *Carbohydr. Res.* **1989**, *190*, 317–322.