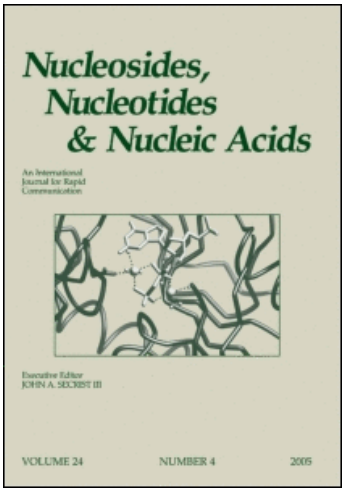


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

NOVEL REVERSION REACTION OF D-*ARABINO*-HEXOSE PHENYLOSOTRIAZOLE. A USEFUL MODEL IN NATURAL GLYCOSIDE AND POLYSACCHARIDE ANALYSIS

Hammed H. A. M. Hassan[a]; Amel H. F. El-Husseiny[a]
[a] Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

Online publication date: 30 October 2001

To cite this Article Hassan, Hammed H. A. M. and El-Husseiny, Amel H. F.(2001) 'NOVEL REVERSION REACTION OF D-*ARABINO*-HEXOSE PHENYLOSOTRIAZOLE. A USEFUL MODEL IN NATURAL GLYCOSIDE AND POLYSACCHARIDE ANALYSIS', Nucleosides, Nucleotides and Nucleic Acids, 20: 9, 1683 — 1690
To link to this Article: DOI: 10.1081/NCN-100105904
URL: http://dx.doi.org/10.1081/NCN-100105904

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(9), 1683–1690 (2001)

NOVEL REVERSION REACTION OF D-*ARABINO*-HEXOSE PHENYLOSOTRIAZOLE. A USEFUL MODEL IN NATURAL GLYCOSIDE AND POLYSACCHARIDE ANALYSIS

Hammed H. A. M. Hassan* and Amel H. F. El-Husseiny

Chemistry Department, Faculty of Science,
Alexandria University, P.O. Box 426 Ibrahimia,
21321-Alexandria, Egypt

ABSTRACT

Treatment of D-*arabino*-hexose phenylosotriazole with conc. hydrochloric acid afforded a new type of α- and β-glycosides of D-erythrose formed by reaction of the 3,6-anhydro derivative with the *in situ* formed 2-phenyl-4-(formylmethyl)-1,2,3-triazole.

INTRODUCTION

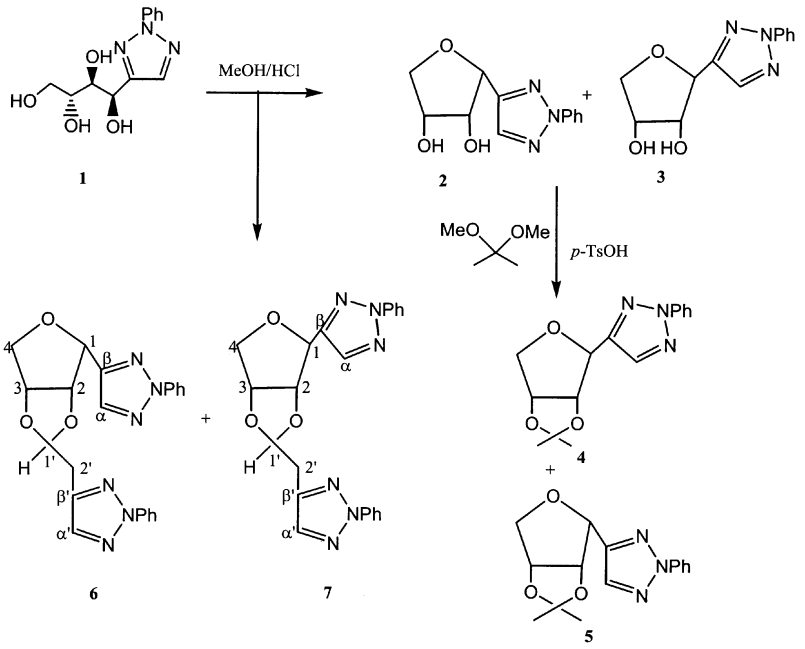
The last decade has witnessed a remarkable growth in the field of glycoconjugates syntheses, purification and structural analyses[1]. The analysis progresses through the identification of any monosaccharides present, characterization of oligosaccharide size and monosaccharide sequence and determination of the linkages between the monosaccharides and the protein[2]. To carry out this analysis, the glycoprotein under investigation is digested by chemical degradation (usually highly acidic conditions) to release the oligosaccharides followed by structural and conformational analysis by

*Corresponding author. E-mail: hassan@safwa.com.eg

spectroscopic methods. Nevertheless, the problem arises from the unusual changes that glycosides may undergo in such drastic acidic conditions[3] and the typical question arises, is such unusual modification present in the protein or it is an artificial product of analysis. In continuation of our work in the chemistry of natural C-glycosides[4] and their analogues[5–7], we recently reported our effort towards the synthesis of C-mannosylated tryptophan[8], an unusual C-glycoside linkage to protein[9]. This research led us to re-address the problem of the unusual modifications that may occur for simple C-glycosides when treated with concentrated mineral acids[3]. In particular, reversion products can be troublesome in natural glycoside and polysaccharide analysis. In this paper we wish to report the isolation of a new sterically less favored reversion product upon heating the well known D-*arabino*-hexose phenylosotriazole[10] with conc. HCl. We selected the C-glycoside 1,2,3-triazole as a model compound because it resembles, to some extent, the C(1)sugar-C(2)-amino acid[9] bond and also due to the fact that so many kinds of Cephalosporins bearing 1,2,3-triazole or its heterocyclic analogue as a partial structure are still under active investigations in the search of more active and safer compounds[11].

RESULTS AND DISCUSSIONS

Since the first preparation of sugar osazones[12] and their osotriazole derivatives[10] a long time ago, their chemistry still has renewed interest[13]. Osazones are usually prepared by the action of three molecular proportions of the unsubstituted hydrazine (or its hydrochloride salt) on one molecular proportion of the saccharide in aqueous acetic acid (or sodium acetate). They can be easily converted into their osotriazole derivatives **1** by oxidation-reduction process mediated by ($Cu^{(II)} \rightarrow Cu^{(0)}$) through a dimeric complex[14]. Boiling a solution of sugar phenylosotriazole in CH_3OH/H_2SO_4 (10%) or MeOH/HCl (15%) causes the elimination of water and formation of a 3,6-anhydro ring with partial epimerization at C-3[15] of the hexose, leading to a mixture of D-*arabino* and D-*ribo* cyclized products **2** and **3**, which can be considered as α- and β-C-glycosides of D-erythrose, Scheme 1. To the best of our knowledge, the ratio of α- and β-C-glycosides **2** and **3** has not been reported previously. To obtain some information, the mixture was masked as acetonide derivatives **4** and **5** by treatment with 2,2-dimethoxypropane/*p*-TsOH mixture. Spectral data (H-H COSY experiment) proved that the product contains a 90:10 mixture of β- and α-isomers, respectively. The protection of the anhydro derivatives as acetonides **4** and **5** not only improve the limited solubility of the mixture in organic solvents, but also made their separation on a cation-exchange resin column possible using a 10% mixture of MeOH in H_2O as eluent.



Scheme 1.

Upon treatment of **1** with methanol/mineral acid mixture, we observed the formation of a dark brown oil layer after 10–15 min reaction. Increasing the concentration of acid (conc. HCl) was found to strongly affect the yield of **2** and **3** and increase to some extent the amount of the oil layer. Longer reaction time did not show any influence on the ratio of the two phases, even after heating for 24 h. Heating of **1** with conc. HCl in the absence of methanol for several hours followed by separation of the oil layer and standard work-up purification by successive cation-exchange resin column chromatography yielded a colorless crystalline product (15% yield, m.p. 91°C). Spectral data proved that this product consists of a mixture of two stereoisomers **6** and **7** (90:10 ratio). Separation of these two stereoisomers using cation-exchange resin column chromatography was quite difficult owing to their relatively long separation time using a 10%, 20% and 30% ethanol-water gradient as eluent.

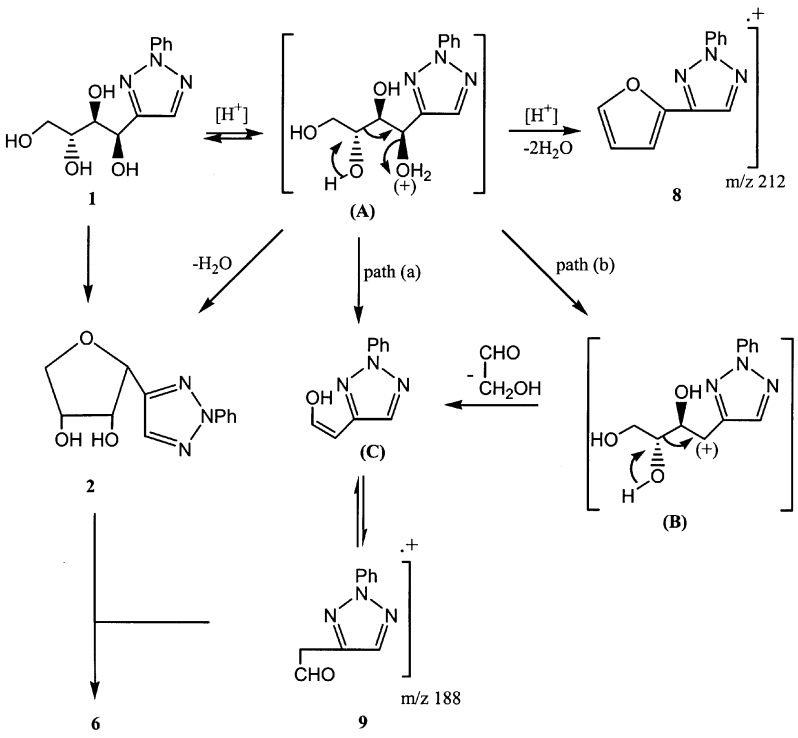
The structure of the major and minor isomers **6** and **7** were determined by NMR techniques (H-H-COSY and H-C HETCOR experiments), accurate mass spectrometry and elemental analysis. The ^{1}H-NMR of **6** shows H-1, H-2, H-3 and H-1′ of the stereogenic centers at δ 5.351 ppm, δ 5.225 ppm ($J_{2,1} = 0.9$ Hz, $J_{2,3} = 6.3$ Hz), δ 4.890 ppm ($J_{3,4} = 3.9$ Hz), δ 5.325 ppm ($J_{1',2'} = 4.6$ Hz), respectively, whereas the ^{13}C NMR spectrum

shows these carbons at δ 78.61, 85.23, 82.03 and 105.27 ppm, respectively. On the other hand, the ^{1}H NMR shows these chiral centers for the minor isomers **7** at δ 5.351 (hidden 1H (COSY)), δ 5.252 ppm ($J_{2,1} = 1.2$ Hz, $J_{2,3} = 5.6$ Hz), δ 4.945 ppm, δ 5.620 ppm ($J_{1',2'} = 4.4$ Hz) and the ^{13}C NMR shows signals of these carbons at δ 79.12 (C-1), 84.31 (C-2), 81.11 (C-3), and 105.07 ppm (C-1′), respectively. The $J_{1,2}$ values (0.9 and 1.2 Hz) accord with the *eq*,*eq* relationship and thus confirm the given stereochemistry at the anomeric carbons atoms. Furthermore, the structure **6** was confirmed by accurate mass spectrometry (calculated for $C_{22}H_{20}O_3N_6$ 416.190; Found 416.1550). The FAB-MS technique shows the base peak at m/z 417 (M^++1, 100%) whereas the EI-MS technique clearly showed two characteristic fragments at m/z 212 (100%) and m/z 188 (29%) which are probably due to the cleavage of the C-O bonds at C-2 and C-3 to give the corresponding 2-phenyl-4-(furan-2-yl)-1,2,3-triazole **8** and 2-phenyl-4-(formylmethyl)-1,2,3-triazole **9**, Scheme 2. The molecular ion in the EI-MS spectrum is found at m/z 416 (5%).

As demonstrated in Scheme 2, a plausible pathway of this reaction is related to the typical reactions of free sugars and acyclic C-glycosides with concentrated mineral acids[16]. Formation of the 3,6-anhydro phenylosotriazole **2** from **1** involves initial protonation of the OH group on the α-carbon of **1**, intermediate (**A**), followed by either an S_{N^1} or S_{N^2} intramolecular ring closure to yield **2**. Furthermore, dehydration/cyclization of (**A**) gives the dehydro non-carbohydrate analogue **8**. A further competing reaction with ring closure occurs under heating conditions causing fragmentation of (**A**) to produce aldehyde **9**. The formation of **9** could proceed by a concerted hydration-fragmentation of (**A**) to give the enol form of aldehyde (**C**) which could then react with **2** to give **6** (path a). Alternatively, **9** could form by a step-wise loss of H_2O from (**A**) followed by fragmentation of the intermediate carbocation (**B**) (path b).

It is worth mentioning that further addition of **2** to the separated aqueous layer, without heating, increased the yield of **6** up to 70% yield. Additionally, placing a mixture of anhydro derivatives **2** and **3** under the conditions where **1** produces **6** and **7** yielded only an inseparable mixture of dehydrated products. This observation serves not only as further evidence for the fragmentation of **1** to produce **9** and thus confirms the present reaction mechanism, but also affords a simple method to obtain new types of C-glycosides which may have a synthetic value.

In conclusion, a new type of reversion products are isolated from the reaction of D-*arabino*-hexose phenylosotriazole **1** with conc. HCl. To our knowledge, such types of C-glycosides have not been reported previously. In addition, according to the present reaction mechanism, the formation of aldehyde **9** gives an additional angle to the well established reactions of furan derivatives with conc. HCl[14]. These findings could find immediate use in natural glycoside and polysaccharide analyses, since some disaccharides



Scheme 2.

isolated from the hydrolysates in low yield may be reversion products of this type.

EXPERIMENTAL

General: Melting points were determined on a *Reichert-Jung* thermometer apparatus and were uncorrected. Spectra were recorded as follows: ^{1}H-NMR, *Varian Gemini* 300 spectrometer at 300 MHz and *Varian VXR* spectrometer at 200 MHz; ^{13}C-NMR, *Varian Gemini* at 75 MHz and *Varian VXR* spectrometer at 50 MHz in deuterated chloroform solutions; mass spectra (electron impact), *VG Micromass* 16F spectrometer, operating at 70 eV with an accelerating voltage of 4 kV and a variable source temperature. High resolution mass spectra (FAB) were determined on a KRATOS limited MS 9/50 spectrometer. Microanalyses were performed at the Physical and Chemical Research (Riken), Wako-shi, Saitama, Japan.

Preparation of 4-(2,3-*O*-isopropylidene-β-D-erythrofuranosyl)-2-phenyl-1,2,3-triazole (4) and 4-(2,3-*O*-isopropylidene-α-D-erythrofuranosyl)-2-phenyl-

1,2,3-triazole (5). To a mixture of anhydroosotriazole derivatives **2** and **3** (1 g, 4.0 mmol) dissolved in 10 ml of MeOH, 20 mg of *p*-TsOH and 10 ml of 2,2-dimethoxypropane were added. The reaction mixture was stirred at r.t. for 12 h and then the solvent was evaporated to dryness to give 1 g (86% yield) of compounds **4** and **5** (9:1 mixture, respectively). The following spectral data were recorded: **^{1}H-NMR** (**4**) (200 MHz): δ 8.02–7.98 (m, 2H, arom), 7.85 (s, 1H, H-5′), 7.50–7.30 (m, 3H, arom), 5.24 (d, $J_{1,2} = 7.6$ Hz, 1H, H-1), 4.34 (dd, $J_{2,3} = 7.8$, $J_{2,1} = 7.6$ Hz, 1H, H-2), 4.14–4.04 (m, 1H, H-3), 3.79 (m, 2H, H-4), 1.50 (s, 3H, CH_3), 1.48 (s, 3H, CH_3). **^{13}C-NMR** (**4**) (50 MHz): δ 134.05 (1C), 129.49 (2C), 127.95 (1C), 118.94 (2C), 110.34 (1C), 90.68 ($-O_2\underline{C}(CH_3)_2$), 81.94 (C-1), 72.30 (C-2), 71.51 (C-3), 63.28 (C-4), 26.57 (CH_3), 26.95 (CH_3). **^{1}H-NMR** (**5**) (200 MHz): δ 7.82 (s, 1H, H-5′), 5.18 (d, $J_{1,2} = 7.0$ Hz, 1H, H-1), 4.36–4.04 (2H, hidden (COSY), H-2, H-3), 3.85–3.74 (m, 1H, H-4), 1.31 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). **^{13}C-NMR** (**5**) (50 MHz): δ 134.35 (1C), 129.40 (2C), 128.04 (1C), 121.36 (2C), 112.70 (1C), 92.03 ($-O_2\underline{C}(CH_3)_2$), 81.32 (C-1), 73.00 (C-2), 71.40 (C-3), 63.45 (C-4), 25.53 (CH_3), 25.26 (CH_3).

Preparation of 2-Phenyl-4-[2,3-*O*-(2-(2-phenyl-1,2,3-triazol-4-yl)ethylidene)-α-D-erythrofuranosyl]-1,2,3-triazole (6) and 2-phenyl-4-[2,3-*O*-(2-(2-phenyl-1,2,3-triazol-4-yl)ethylidene)-β-D-erythrofuranosyl]-1,2,3-triazole (7). A solution of D-*arabino*-hexose phenylosotriazole (**1**) (5 g) in 25 ml of conc. HCl was heated at reflux for 3 h. The resulting oil layer was separated, dissolved in 20 ml of $CHCl_3$, neutralized with solid Na_2CO_3 (3 g), filtered and the filtrate was evaporated to dryness. The remaining dark brown residue was dissolved in EtOH (10 ml) and passed through an Amberlite ® IR-120 (plus) column (500 g) using pre-boiled distilled water as eluent. The solvent was evaporated under diminished pressure (bath temperature 30°C). The residue was once again passed through an Amberlite ® IRA 410 column (500 g) using pre-boiled water as eluent followed by removal of the solvent. The remaining brown residue was purified over an Amberlyst ® A-21 column (500 g) using a 10%, 20% and 30% EtOH-H_2O gradient as eluent. The collected colorless fractions were left to stand several days (2–3 days) after which compounds **6** and **7** had crystallized out as a colorless needles. Yield 1.2 g (15%), m.p. 91°C. Accurate mass for $C_{22}H_{20}O_3N_6$ is 416.1550 (require 416.190). **FAB-MS**: 417 (M^++1) (100%), 357 (14), 341 (3), 315 (3), 272 (6), 258 (34), 230 (19), 212 (58), 200 (9), 188 (27), 172 (14), 158 (54), 136 (25), 107 (10), 91 (18), 77 (44). **EI-MS**-: 416 (M^+)(5%), 258 (95), 240 (4), 229 (56), 212 (100), 200 (5), 188 (9), 184 (29), 172 (41), 158 (53), 103 (31), 91 (51), 77 (51). **^{1}H-NMR** (**6**) (300 MHz): δ 8.075–8.00 (m, 4H, *o*-H, arom), δ 7.80 (s, 1H, H-α), δ 7.757 (s, 1H, H-α′), δ 7.494–7.429 (m, 4H, *m*-H, arom), δ 7.373–7.289 (m, 2H, *p*-H, arom), δ 5.351 (br s, 1H, H-1), δ 5.325 (t, 1H, $J_{1',2'} = 4.6$ Hz, H-1′), δ 5.225 (dd, 1H, $J_{2,3} = 6.3$, $J_{2,1} = 0.9$ Hz, H-2),

δ 4.890 (dd, 1H, $J_{3,2} = 6.3$, $J_{3,4b} = 3.9$ Hz, H-3), δ 4.156 (d, 1H, $J_{4a,4b} = 10.8$ Hz, H-4_a), δ 3.822 (ddd, 1H, $J_{4b,4a} = 10.8$, $J_{4b,3} = 3.9$ Hz, $J_{4b,1} = 0.6$ Hz H-4_b), δ 3.310 (d, 2H, $J_{2',1'} = 4.6$ Hz, H-2′). **^{13}C-NMR** (75 MHz): δ 146.75 (s, C-β), δ 144.16 (s, C-β′), δ 139.92 (N-C), δ 135.70 (d, C-α), δ 134.29 (d, C-α′), δ 129.32 (d, *m*-C), δ 129.23 (d, *m*-C), δ 127.72 (d, *p*-C), δ 127.22 (d, *p*-C), δ 118.86 (d, *o*-C), δ 118.78 (d, *o*-C), δ 105.27 (d, C-1′), δ 85.23 (d, C-2), δ 82.03 (d, C-3), δ 78.61 (d, C-1), δ 72.26 (t, C-4), δ 30.70 (t, C-2′). **^{1}H-NMR** (**7**) (300 MHz): δ 5.620 (t, 1H, $J_{1',2'} = 4.4$ Hz, H-1′), δ 5.351 (hidden 1H (COSY), H-1), δ 5.252 (dd, 1H, $J_{2,3} = 5.6$, $J_{2,1} = 1.2$ Hz, H-2), δ 4.945 (m, 1H, H-3), δ 4.128 (d, 1H, $J_{4a,4b} = 11.1$ Hz, H-4_a), δ 3.874 (ddd, 1H, $J_{4b,4a} = 11.1$, $J_{4b,3} = 4.4$, $J_{4b,1} = 0.7$ Hz, H-4_b), δ 3.216 (d, 2H, $J_{2',1'} = 4.4$ Hz, H-2′). **^{13}C-NMR** (**7**) (75 MHz): δ 146.85 (s, C-β), δ 144.04 (s, C-β′), δ 139.66 (N-C), δ 135.59 (d, C-α), δ 134.08 (d, C-α′), δ 104.07 (d, C-1′), δ 84.31 (d, C-2), δ 81.11 (d, C-3), δ 79.12 (d, C-1), δ 72.94 (t, C-4), δ 30.78 (t, C-2′). Anal. Calc. For $C_{22}H_{20}O_3N_6$: C, 63.4; H, 4.8; N, 20.1. Found: C, 63.1; H, 4.8; N, 19.7.

REFERENCES

1. Gabius, H.J.; Gabius, S. (Eds). *Glycosciences, Status and Perspectives*; Chapmann & Hall: Weinheim, New York, 1997.
2. a) Hounsell, E.F. Adv. Carbohydr. Chem. Biochem. **1994**, *50*, 311. b) Hounsell, E.F. Mol. Biotech. **1994** *2*, 45.
3. Goffey, S. (Ed). *Rodd's Chemistry of Carbon Compounds*; Elsevier: Amsterdam, 1967; Vol. 1F, Chaps. 22, 23.
4. Hassan, H.H.A.M.; Bredenkamp, M.W. Abstracts Book, 19th International Carbohydrate Symposium, San Diego, California, **1998**, BP 295.
5. Hassan, H.H.A.M.; Abdel-Rahman, M.M. Synth. Comm. **2000**, *30*, 201.
6. Hassan, H.H.A.M. Heterocycles, **2000**, *53*, 397.
7. Hassan, H.H.A.M.; El-Husseiny, A.F.H. Polish J. Chem. accepted for publication.
8. Hassan, H.H.A.M.; Spies, H.S.; Bredenkamp, M.W. Polish J. Chem. **2000**, *74*, *in press.*
9. Hofsteenge, J.; Muller, D.R.; De Beer, T.; Loffler, A.; Richter, W.J.; Vliegenthart, J.F.G. Biochemistry **1994**, *33*, 13524.
10. Hann, R.M.; Hudson, C.S. J. Am. Chem. Soc. **1944**, *66*, 735.
11. a) Micetich, R.G.; Maiti, S.N.; Spevak, P.; Hall, T.W.; Yambe, S.; Ishida, M.; Tanaka, T.; Yamazaki, T.; Nakai, A.; Ogawa, K. J. Med. Chem. **1987**, *30*, 1469. b) Harada, K.; Odo, M.; Matsushite, A.; Shirai, M. Syn. Lett. **1998**, 431.
12. Richtmyer, N.K. Methods Carbohydr. Chem. **1963**, *2*, 127.
13. Oikawa, N.; Muller, C.; Kunz, M.; Lichtenthaler, F.W. Carbohydr. Res. **1998**, *309*, 269.
14. El Khadem, H.S. Carbohydr. Res. **1998**, *313*, 255.

15. a) Hardegger, E.; Schrier, E. Helv. Chim. Acta **1952**, *35*, 232, 623. b) Bayer, S. J. Chem. Soc. **1952**, 4993. c) Schrier, E.; Stohr, E.; Hardegger, E. Helv. Chim. Acta **1954**, *37*, 34, 574.
16. Speck, J.C. Jr, Adv. Carbohydr. Chem. **1958**, *13*, 63.

Received June 29, 2000
Accepted February 20, 2001