ELSEVIER

Carbohydrate Research 266 (1995) 285–292

CARBOHYDRATE RESEARCH

Note

Synthesis of GDP-3-acetamido-3-deoxy-α-D-mannose and GDP-3-azido-3-deoxy-α-D-mannose

Werner Klaffke [1]

Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg 13, Germany

Received 16 February 1994; accepted in revised form 21 July 1994

Keywords: GDP-3-acetamido-3-deoxy-α-D-mannose; GDP-3-azido-3-deoxy-α-D-mannose; Morpholidate procedure; Brewers' yeast extract; Cofactor regeneration

In complex glycosylated compounds, such as macrolides or anthracyclines, aminodeoxy sugars are found to influence markedly the pharmacokinetical properties of the respective drug. The carbohydrate moieties not only govern solubility, resorption, and bioavailability of the antibiotic[1], but they may also determine the site of interaction with other biomolecules, such as DNA [2,3]. By changing the glycosylation patterns of drugs based on secondary metabolites of bacteria, improvements in performance and therapeutic range may be achieved [4,5].

Numerous chemical approaches toward glycosides of antibiotics have been described [6,7]. However, they often suffer from poor stereo- and regio-selectivity due to multiple acceptor sites in the aglycon or lower anchimeric control in glycosylation reactions with deoxygenated glycosyl donors. Enzymatic glycosylation reactions would therefore present a route to more efficient syntheses since they are stereo- and regio-selective. Unfortunately, these techniques are still hampered by the low availability of both nucleoside diphosphate-activated glycosyl donors and the corresponding bacterial sugar transferases.

Having available a synthesis of 3-azido-3-deoxy-D-mannose [8], we therefore sought routes to activated 3-amino-3-deoxymannose derivatives. Common chemical syntheses of glycosyl phosphates in which a hydroxy group is replaced by an azido group would fail

[1] Present address: Unilever Research Laboratorium, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands.

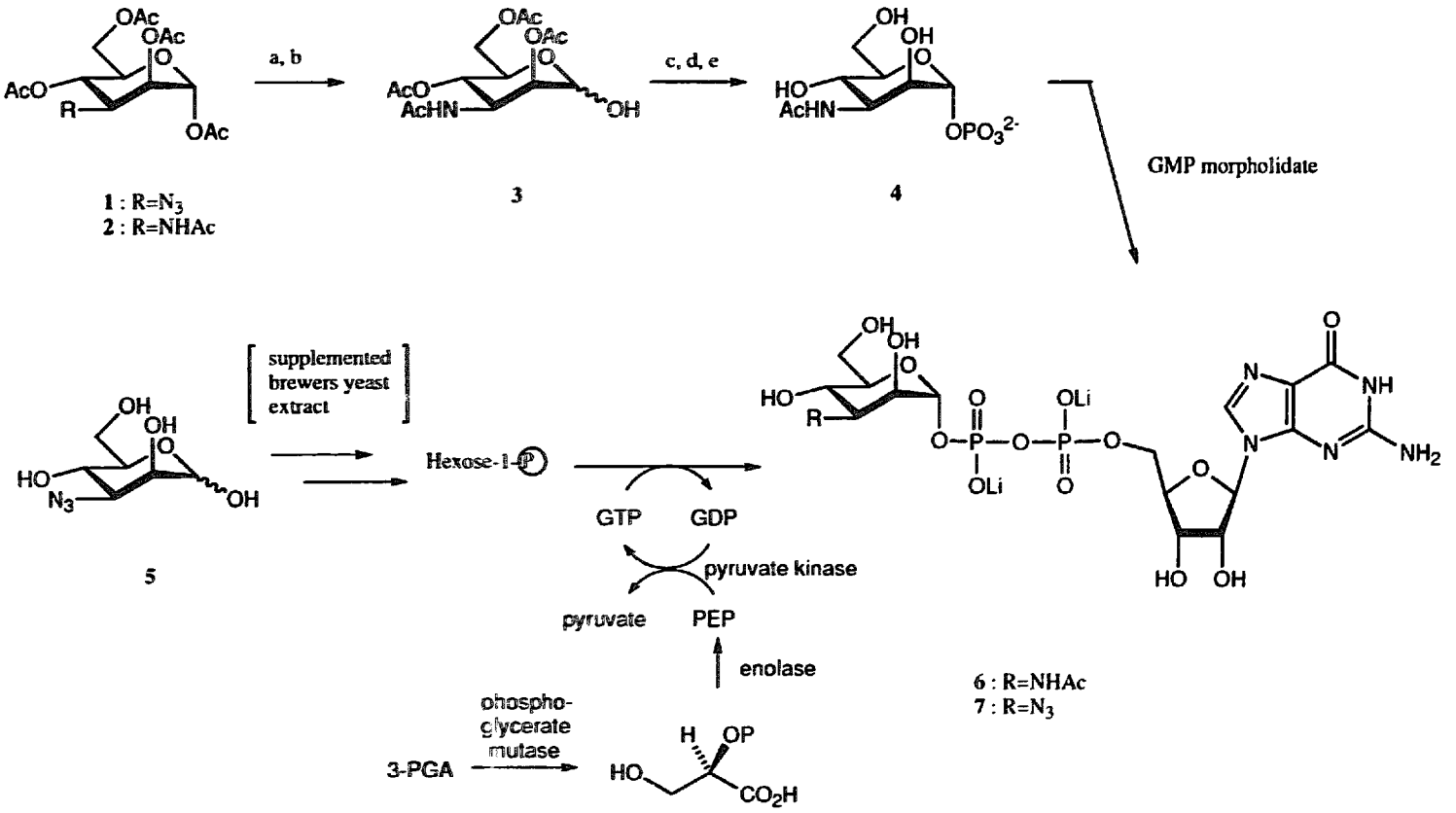
SSDI 0008-6215(94)00261-4

because of obvious incompatibilities. Hydrogenolysis of diphenyl or dibenzyl phosphates would also reduce the azide to yield the amine; a synthesis via the phosphoramidite route [9] would result in an unwanted Staudinger reaction of the P^{III}-species and the azide [10,11].

Therefore, two individual routes for the synthesis of the target compounds are presented, the first by chemical synthesis of the diphosphate sugar after azide reduction to give the corresponding acetamido compound **6**, the other by following a biochemical pathway to give the GDP-azidomannose **7**.

For the synthesis of the GDP-acetamidomannose **6** from the previously synthesized tetra-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranose (**1**) [8], reduction of the azide group by hydrogenation was attempted. However, hydrogenolysis was not successful and gave rise to a range of products with various degrees of acetylation. Re-acetylation of this mixture did not result in the formation of **2**, and reduction of the azido function was therefore carried out with triphenylphosphine in dichloromethane–water [12,13]. By this procedure, compound **1** was reduced in high yield via its readily hydrolyzed phosphinimine intermediate. After evaporation, acetylation gave **2**. It should be added that similar experiments with the analogous 3- and 4-azidoglucose derivatives were less successful. In those cases, the phosphinimine intermediate withstood hydrolysis, which under acidic conditions gave a stable amino phosphonium salt [14]. The mannopyranose **3** could easily be obtained from the acetamido derivative **2** by conversion into the glycosyl bromide and hydrolysis in acetone–water in the presence of silver carbonate. This anomerically deblocked hexose was then subjected to phosphitylation in the presence of base employing 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, following the procedure described by van Boom and co-workers [9]. After completion of the reaction, the solvent was removed in a stream of nitrogen; silica gel chromatography then yielded a crude product, which could be identified by ^1H NMR data. Surprisingly, only the diastereomeric phosphoramidites of α configuration were formed. This difference in stereoselectivity to the *gluco* series [14,15] may certainly be rationalized by the marked steric effect of the axially oriented acetoxy group at C-2 in the *manno* series. The phosphoramidites were transformed in situ into the bis(cyanoethyl)phosphite triester, which then was oxidized by *tert*-butyl hydroperoxide to yield the corresponding labile phosphate triester. Deprotection of this by ammonia in methanol, followed by ion-exchange chromatography with a linear gradient of NH_4HCO_3, yielded the phosphate as a crude product. This could be coupled to commercially available GMP-morpholidate [16] in 36% yield to give the desired GDP-sugar **6**, isolated after ion-exchange and gel permeation chromatography (Scheme 1).

In contrast to the chemical routes, enzymatic methods should leave the azido function intact and thus allow the synthesis of the GDP-sugar **7**. Experiments on the substrate specificity of yeast hexokinase, the enzyme which initiates the phosphorylation procedure by converting the sugar into a 6-phosphate, were promising. The epimeric 3-azido-3-deoxyglucose has been shown to serve as a substrate in the mM-range, which resulted in a chemo-enzymatic approach toward dTDP-3-azido-3-deoxy-D-glucose [17]. With these results in hand, the enzymatic synthesis of GDP-mannose employing fresh brewers' yeast supplemented with hexokinase and D-glucose-1,6-bisphosphate [18–21] has been applied to derivative **5** [8]. To guarantee low stationary levels of phosphoenol pyruvate, which is



Scheme 1. a: HBr, HOAc; b: Ag_2CO_3, water–acetone; c: 2-cyanoethyl *N,N*-diisopropylchlorophosphoamidite, *N,N*-diisopropylethylamine, CH_2Cl_2; d: 3-hydroxypropionitrile, 1*H*-tetrazole, then *tert*-butyl hydroperoxide; e: NH_3 in EtOH, 0°C → room temperature. 3-PGA: 3-phospho-D-glyceric acid; PEP: phosphoenol pyruvate; GTP/GDP: guanosine tri(di)-phosphate.

used to satisfy the GTP demand of the phosphorylation steps, 3-phosphoglyceric acid was added to the mixture, along with PGA-mutase, enolase, and pyruvate kinase. Various enzyme sources were investigated, and in our hands none of the commercial products catalyzed the synthesis of GDP-sugars. Fresh preparations of the raw enzyme fraction from brewers' yeast obtained directly from a local brewery (Holsten Brauerei, Hamburg), however, brought about the synthesis. After 18 h at room temperature, formation of the GDP-mannose derivative **7** could be detected by TLC (7:3:1 isopropyl alcohol–water–satd ammonia in water). The mixture was filtered through a 10-kDa membrane, treated with alkaline phosphatase, denatured, and centrifuged. The supernatant solution was applied to a Dowex 2 X8 (Cl^-) ion-exchange column and the product was separated by gradient elution (0 → 0.8 M LiCl). After a double desalting step on Biogel P2, product **7** was obtained as a white powder (53 mg, 8%, dilithium salt) and could be identified by both ^1H and ^{13}C NMR spectroscopy.

Both diphosphate sugars **6** and **7** showed small $^3J_{1'',2''} < 1$ Hz and $^3J_{1'',P}$ 7.5 Hz couplings, which correspond to a 1,2-*trans*-diequatorial arrangement of H-1″ and H-2″. A ^4J-hetero coupling between the anomeric phosphorus atom and H-2″, which is usually observed in the *gluco* series [11] (O-1–H-2″ dihedral angle 180°) is not detected for **6** and **7** and thus confirms the α configuration (O-1–H-2″ dihedral angle 60°). Furthermore, the dd-signal observed at 3.80 ppm in the spectrum of compound **7**, with $J_{2'',3''}$ 3.5 and $J_{3'',4''}$ 10.0 Hz, could be assigned to H-3″, and thus C-3″ was still bearing the azido group in an equatorial position. This was also underlined by the presence of a characteristic IR absorption at 2100 cm^{-1} [22,23].

The presence of the acetamido group in derivative **6** was indicated by a singlet at 2.10 ppm (for $COCH_3$) in the ^1H NMR spectrum; in the carbon spectrum, the signals at 23.2 and 176.5 ppm could be assigned to $COCH_3$ and $COCH_3$, respectively. For both acetamido compound **6** and azide **7**, C-1″ and C-2″ were split by carbon-hetero couplings of 6.0 and 10.1 Hz, respectively, which were indicative of an α-mannosyl phosphate [24].

The chemical and the chemo-enzymatic route [5] comprise facile and complementary methods for the preparation of structural variants of nucleoside diphosphate mannose. GDP-3-azido-3-deoxy-D-mannose is to our knowledge not obtainable by current chemical procedures. Further research is now directed to the question of substrate specificities of various enzymes of the deoxy sugar pathway and therefore to achieve integration of the enzymatic routes described above.

1. Experimental

General procedures.—^1H NMR spectra were recorded with a Bruker AM-300 or AM-400 spectrometer at the frequencies indicated, employing standard pulse angles. If necessary, coupled protons were assigned by $^1H,^1H$-COSY. All reactions were monitored by TLC on silica gel plates (GF_{254}, Merck) and detected by either UV absorption or charring with 5% H_2SO_4 in EtOH and subsequent heating to 500°C. Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck) with solvents listed below. GTP (sodium salt), D-glucose 1,6-bisphosphate (potassium salt), ATP (disodium salt, $3H_2O$), 3-phosphogly-

ceric acid (tricyclohexylammonium salt), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40), phosphoglycerate mutase (EC 2.7.5.3), yeast hexokinase (EC 2.7.1.1), calf intestine alkaline phosphatase (EC 3.1.3.1), and guanosine 5′-phosphoromorpholidate (*N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt) were purchased from Sigma Chemicals. Brewers' yeast was obtained fresh at 4°C from Holsten Brauerei, Hamburg.

3-Acetamido-1,2,4,6-tetra-O-*acetyl-3-deoxy-α*-D-*mannose* (**2**).—To a solution of 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranose (**1**) [8] (100 mg, 0.27 mmol) in CH_2Cl_2 (10 mL) were added Ph_3P (83.5 mg, 0.64 mmol) and water (0.5 mL). The mixture was stirred at room temperature until TLC (1:1 toluene–acetone) showed complete conversion. The solvent was evaporated and the residue was re-suspended in anhyd pyridine (10 mL) and Ac_2O (5 mL). After 3 h, the mixture was evaporated, co-distilled with toluene (3×50 mL) to remove traces of acid and anhydride, and the residue was subjected to chromatography on silica gel (4:1 toluene–acetone) to yield **2** (86 mg, 82%) as colourless oil; $[\alpha]_D^{20}$ +34° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.14 (d, H-1), 5.74 (br d, N-H), 5.11 (dd, H-4), 5.00 (dd, H-2), 4.70 (ddd, H-5), 4.30 (dd, H-6a), 4.05 (m_c, 2 H, H-3,6b), and 2.18, 2.10, 2.08, 2.05, 1.98 (5 s, 15 H, 4 OAc, 1 NH*Ac*); $J_{1,2}$ 1.5, $J_{2,3}$ 3.0, $J_{3,4} = J_{4,5} = 10.5$, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 12.5, $J_{3,NH}$ 8.5 Hz; ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.9, 170.6, 169.9, 169.7, 169.4 (5 s, 5 C=O), 90.0 (C-1), 70.3 (C-2), 69.8 (C-3), 66.1 (C-4), 62.5 (C-6), 48.3 (C-5), and 23.2, 20.8, 20.7, 18.9, 18.8 [5 s, 5 C(O)CH_3]. Anal. Calcd for $C_{16}H_{23}NO_{10}$ (389.4): C, 49.36; H, 5.95; N, 3.60. Found: C, 50.02; H, 5.91; N, 3.57.

3-Acetamido-2,4,6-tri-O-*acetyl-3-deoxy*-D-*mannopyranose* (**3**).—Compound **2** (100 mg, 0.26 mmol) was dissolved in anhyd CH_2Cl_2 (5 mL) and at 0°C HBr–HOAc (30%, 30 mL) was added. After 3 h the mixture was poured onto ice and extracted 3 times with small portions of CH_2Cl_2, and the organic phase was co-evaporated with toluene. The residue was redissolved in 5:1 acetone–water (10 mL) and Ag_2CO_3 (50 mg) was added. After 3–4 h at room temperature, TLC showed the formation of a more polar product and the reaction was worked up by filtration and evaporation of the solvents. The residue was subjected to silica gel chromatography (2:1 toluene–acetone) to yield **3** (85 mg, 92%) as a colourless syrup, which was processed further. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.64 (br d, N*H*), 5.20 (br s, H-1), 5.05 (dd, H-4), 5.05 (dd, H-2), 4.75 (ddd, H-5), 4.26 (m_c, 2 H, H-3,6b), 4.14 (m ≈ dd, H-6a), 2.08 (m ≈ 3 s), and 1.92 (s) (12 H, OAc and NH*Ac*); $J_{2,3}$ 5.0, $J_{3,4} = J_{4,5} = 10.5$, $J_{5,6a}$ 3.0, $J_{5,6b}$ 9.0, $J_{6a,6b}$ 10.0, $J_{3,NH}$ 8.0 Hz.

GDP-3-acetamido-3-deoxy-α-D-*mannopyranose [guanosine 5′-(3-acetamido-3-deoxy-α*-D-*mannopyranosyl diphosphate] dilithium salt* (**6**).—Anomerically deblocked **3** (70 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (2 mL) and equilibrated for 1 h over 4A molecular sieves under Ar. To the mixture were added *N,N*-diisopropylethylamine (87 μL, 0.5 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (58.1 μL, 0.26 mmol). In order to drive the reaction to completion, a further 0.5 equiv of reagent was added after 2 h. The mixture was allowed to attain room temperature and was monitored by TLC (3:1 toluene–acetone). After completion, the solvent was removed by a stream of N_2 and the residue was quickly passed through a column of thoroughly dried silica gel. Elution with 3:1 toluene–acetone (containing 0.1% of Et_3N) yielded a mixture of diastereomeric phosphoramidites of α configuration, as was proven by ^{1}H NMR of the crude material. This was dried in

vacuo and dissolved in MeCN, to which was added subsequently, and with strict exclusion of moisture, 3-hydroxypropionitrile (36 μL, 0.44 mmol) and 1*H*-tetrazole (0.4 mmol, dissolved in anhyd MeCN). After stirring for 3 h at room temperature, aq 70% *tert*-butyl hydroperoxide (95 μL, 3.5 mol equiv) was added and stirred for 2–3 h. At 0°C, a solution of NH_3 in MeOH (7 M, 1.6 mL) was added, and the mixture was allowed to reach room temperature and stirred further for 6 h. Excess of NH_3 was removed by a stream of N_2 through the mixture and the solvent was evaporated in vacuo. A solution of the gummy residue in water (15 mL) was passed through a bed of Dowex 50W resin (pyridinium form) and the eluate was applied to a column of Dowex 1 X8 resin (HCO_3^-). After a washing step with water–10% MeOH (30 mL), the phosphate was eluted with a linear gradient of 0 → 0.4 M NH_4HCO_3. Fractions were pooled around concentrations of 0.15 M NH_4HCO_3, after being individually checked by TLC (7:3:1 isopropyl alcohol–water–NH_3), and lyophilized to yield a white solid. This was dissolved in pyridine (20 mL) and tributylamine (47.6 μL, 0.2 mmol) was added. The mixture was concentrated by evaporation and dried in vacuo, the residue (with thorough exclusion of moisture) was dissolved in pyridine (5 mL), and previously dried guanosine 5′-phosphoromorpholidate (*N,N′*-dicyclohexyl-4-morpholinecarboxamidine salt, 435 mg, 0.26 mmol) was added. After 15 days at room temperature, water (20 mL) was added and the resulting solution was applied to a Dowex 2 X8 (Cl^-) column (10×2.5 cm) and eluted with a linear gradient (0 → 0.8 M, 250 mL). Diphosphate was detected by UV absorption and appropriate fractions were pooled, carefully lyophilized to ca. 2–3 mL of solvent, and subjected twice to Sephadex G-10 chromatography (160×2 cm), to give **6** (46 mg, 35%); ^{1}H NMR (400 MHz, D_2O): δ 7.84 (s, H-8), 5.70 (d, H-1′), 5.31 (d, H-1″), 4.54 (dd ≈ t, H-2′), 4.38 (dd ≈ t, H-3′), 4.18–4.10 (m_c, 4 H, H-4′,5a′,5b′,3″), 3.90 (br m, H-2″), 3.75–3.52 (m, 4 H, H-4″,5″,6a″,6b″), and 2.10 (s, 3 H, NHC(O)CH_3); $J_{1',2'} \approx J_{2',3'} \approx J_{3',4'} \approx 5.5$, $J_{1'',2''} < 1.0$, $^3J_{1'',P}$ 7.7 Hz; ^{13}C NMR (100.6 MHz, D_2O): δ 157.8 (C-4), 154.3 (C-2), 138.1 (C-8), 116.0 (C-5), 94.0 (d, C-1″), 86.5 (C-1′), 83.1 (C-4′), 76.2 (C-4″), 75.3 (C-2′), 71.7 (C-3′), 69.8 (C-2″), 65.9 (C-5′), 62.8 and 62.4 (each s, C-5″,6″), 61.3 (C-3″); $^2J_{C-1'',P}$ 5.8, $^3J_{C-2'',P}$ 10.2 Hz. Anal. Calcd for $C_{18}H_{26}Li_2N_6O_{16}P_2$ (658.3): C, 32.84; H, 3.98; N, 12.77. Found C, 33.10; H, 4.07; N, 13.95.

*GDP-3-azido-3-deoxy-α-*D*-mannose dilithium salt* (**7**).—After fast vacuum-filtration of fresh brewers' yeast at 4°C, raw cell mass (ca. 400 g) was crumbled into liquid N_2. The cells were further treated as described previously [18], to yield an extract which was resuspended in 0.1 M Tris-HCl buffer (40 mL, pH 7.5). To this were added NaF (350 mmol), EDTA (3.15 mmol), $MgCl_2 \cdot 6H_2O$ (3.15 mmol), 3-azido-3-deoxy-D-mannose [8] (**5**, 0.7 mmol, 140 mg), GTP (980 mg, 0.24 mmol), D-glucose 1,6-bisphosphate (20 μmol), ATP (4 μmol), and 3-phosphoglyceric acid (1.4 mmol, previously transformed into its sodium salt by passing through a Dowex 50W-Na^+ ion-exchanger column). The solution was degassed by a stream of N_2 (15 min) and enolase (4 U, EC 4.2.1.11), pyruvate kinase (20 U, EC 2.7.1.40), phosphoglycerate mutase (20 U, EC 2.7.5.3), and yeast hexokinase (200 U, EC 2.7.1.1) were added. The mixture was shaken at 25°C for 18 h, protein was filtered by passing through an Amicon cell (10 kDa), and the filtrate was incubated at 37°C for 60 min with alkaline phosphatase (CIAP, 300 U, EC 3.1.3.1). After denaturing (100°C, 2 min) and centrifugation, the supernatant solution was concentrated to ca. 10 mL final volume

by lyophilization, then applied to a strongly basic ion-exchanger column (Dowex 2 X8, Cl^- form, 20×2 cm) and eluted by a linear LiCl gradient (500 mL water, 500 mL 0.8 M LiCl). The appropriate UV-active, diphosphate-containing fractions were pooled and, after concentration by lyophilization, desalted by passing them twice through a Biogel P2 column (160×2 cm) to give **7** as its dilithium salt (53 mg, 84 μmol, 8%); ν_{max} 2100 cm^{-1} (azide); ^1H NMR (400 MHz, D_2O): δ 7.98 (s, H-8), 5.78 (d, H-1′), 5.25 (d, H-1″), 4.60 (dd ≈ t, H-2′), 4.39 (dd ≈ t, H-3′), 4.23 (mc, H-4′), 4.10 (m, 2 H, H-5a′,5b′), 3.88 (mc, H-2″), 3.80 (dd, H-3″), and 3.56–3.76 (m, 4 H, H-4″,5″,6a″,6b″); $J_{1',2'}=J_{2',3'}=J_{3',4'}=5.5$, $J_{1'',2''}<1.0$, $^3J_{1'',P}$ 7.5, $J_{2'',3''}$ 3.5, $J_{3'',4''}$ 10.0 Hz; ^{13}C NMR (100.6 MHz, D_2O): δ 159.0 (C-4), 154.0 (C-2), 137.6 (C-8), 116.3 (C-5), 95.3 (d, C-1″), 87.2 (C-1′), 83.8 (C-4′), 74.7 (C-4″), 74.1 (C-2′), 70.5 (C-3′), 67.8 (C-2″), 65.5 (C-5′), 61.3 and 61.2 (each s, C-5″,6″), 53.4 (C-3″); $^2J_{C-1'',P}$ 6.0, $^3J_{C-2'',P}$ 10.1 Hz.

Acknowledgements

The author thanks Brigitte Leon for skillful technical assistance. Generous support of this project given by Professor Joachim Thiem is gratefully acknowledged. This research enjoyed financial support by the Verband der Chemischen Industrie (Liebig-Habilitationsstipendium) and the Deutsche Forschungsgemeinschaft (grant Kl 461/4-1).

References

[1] S. Neidle and M.J. Waring (Eds.), *Molecular Aspects of Anti-Cancer Drug Action*, VCH, Weinheim, 1983.
[2] K.C. Nicolaou, S.-C. Tsay, T. Suzuki, and G.F. Joyce, *J. Am. Chem. Soc.*, 114 (1992) 7555–7557.
[3] S.L. Walker, L.L. Kiessling, and S.L. Schreiber, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 1105–1109.
[4] D.W. Henry, *ACS Symp. Ser.*, 30 (1976).
[5] D. Füllenbach and G.A. Nagel, in S. Seeber (Ed.), *Adriamycin Symposium 1981*, Karger Verlag, Basel, 1981.
[6] I.F. Pelyvás, C. Monneret, and P. Herczegh, *Synthetic Aspects of Aminodeoxy Sugars of Antibiotics*, Springer Verlag, Berlin, 1988.
[7] J. Thiem and W. Klaffke, *Top. Curr. Chem.*, 154 (1990) 285–334.
[8] E.W. Holla, V. Sinnwell, and W. Klaffke, *Synlett*, (1992) 413–415.
[9] P. Westerduin, G.H. Veeneman, J.E. Marugg, G.A.A. van der Marel, and J.H. van Boom, *Tetrahedron Lett.*, 27 (1986) 1211–1214.
[10] B. Leon and W. Klaffke, *XVIth Int. Carbohydr. Symp.*, Paris, 1992, *Abstr.*, A231.
[11] B. Leon, S. Liemann, and W. Klaffke, *J. Carbohydr. Chem.*, 12 (1993) 597–610.
[12] H. Staudinger and J. Meyer, *Helv. Chim. Acta*, 2 (1919) 635–646.
[13] S. Friedrich-Bochnitschek, H. Waldmann, and H. Kunz, *J. Org. Chem.*, 54 (1989) 751–656.
[14] W. Klaffke and S. Liemann, unpublished results.
[15] A. Naundorf, S. Liemann, and W. Klaffke, *Eurocarb VII*, Cracow, Poland, 1993, *Abstr.*, C004.
[16] J.G. Moffatt, *Methods Enzymol.*, 8 (1966) 136–142.
[17] W. Klaffke, in H. Kirst, K. Krohn, and H. Maas (Eds.), *Antibiotics and Antiviral Compounds*, VCH, Weinheim, 1993, pp 389–402.
[18] T.J. Grier and J.R. Rasmussen, *Anal. Biochem.*, 127 (1982) 100–104.
[19] W.A. Braell, M.A. Tyo, S.S. Krag, and P.W. Robbins, *Anal. Biochem.*, 74 (1976) 484–487.
[20] S. Chang, K.O. Broschat, and G.S. Serif, *Anal. Biochem.*, 144 (1985) 253–257.
[21] E.S. Simon, S. Grabowski, and G.M. Whitesides, *J. Org. Chem.*, 55 (1990) 1834–1841.

[22] A. Treinin, *The Chemistry of the Azido Group*, Interscience, New York, 1971.
[23] R.K. Evans and B.E. Haley, *Biochemistry*, 26 (1987) 269–276.
[24] J. Niggemann and J. Thiem, *Liebigs Ann. Chem.*, (1992) 535–538.