Biosynthetic studies on the α-glucosidase inhibitor acarbose: the chemical synthesis of dTDP-4-amino-4,6-dideoxy-α-D-glucose

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Abstract

To study the biosynthesis of the pseudotetrasaccharide acarbose, dTDP-4-amino-4,6-dideoxy-α-D-glucose (**3**) was prepared from galactose in 16 steps. After initial protecting-group manipulations, the 6-position of galactose was deoxygenated by hydride displacement of a tosylate. Similarly a tosyl group at the 4-position was displaced upon reaction with sodium azide. Conversion of the resulting glycoside to a glycosyl phosphate was accomplished by reaction of a glycosyl trichloroacetimidate with dibenzyl phosphate. Subsequent removal of the benzyl protecting groups and reduction of the azide by hydrogenation and coupling with an activated nucleoside phosphate gave dTDP-4-amino-4,6-dideoxy-α-D-glucose. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Acarbose (**1**, Fig. 1) a pseudotetrasaccharide isolated from the fermentation broth of *Actinoplanes* sp., is an inhibitor of α-glucosidase and a clinically useful drug for the treatment of type II insulin-independent diabetes.[1] Structurally acarbose consists of an unsaturated cyclitol linked via a nitrogen bridge to a deoxyhexose, which is in turn linked to maltose. Previous studies on the biosynthesis of acarbose have demonstrated that the cyclitol moiety is derived from the pentose phosphate pathway.[2] In particular, through feeding experiments with various isotopically labeled cyclitols to *Actinoplanes* sp. SN223/29, it was found that only 2-*epi*-5-*epi*-[6-^{2}H$_2$]valiolone (**2**) was incorporated into acarbose, giving 15% specific incorporation.[3] 2-*epi*-5-*epi*-Valiolone in turn is formed from sedoheptulose 7-phosphate in a reaction catalyzed by a cyclase encoded by the *acbC* gene of the acarbose biosynthetic gene cluster of *Actinoplanes*, a homolog of dehydroquinate synthases.[4] Interestingly, neither the C-2 epimer (which has the same stereochemistry as acarbose) nor the C-5 epimer of **2**, nor their dehydrated forms, 2-*epi*-valienone and valienone, were incorporated.[3] The biosynthesis of the deoxyhexose unit involves the conversion of dTDP-D-glucose to dTDP-4-keto-6-deoxy-D-glucose by an intramolecular hydride transfer[5] catalyzed by dTDP-glucose 4,6-dehydratase, which is encoded by the *acbB* gene.[4] It is proposed that this keto-sugar is subsequently converted to dTDP-4-amino-4,6-dideoxy-D-glucose (**3**) by the action of a transaminase. The maltose moiety of **1** is derived directly from maltose in the fermentation medium.[2,6]

Two possible pathways for the biosynthesis of the pseudodisaccharide portion of acarbose are outlined in Scheme 1. The first proposes that condensation of the
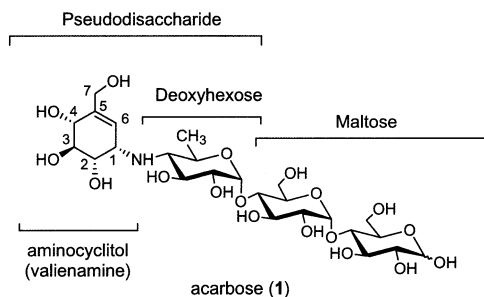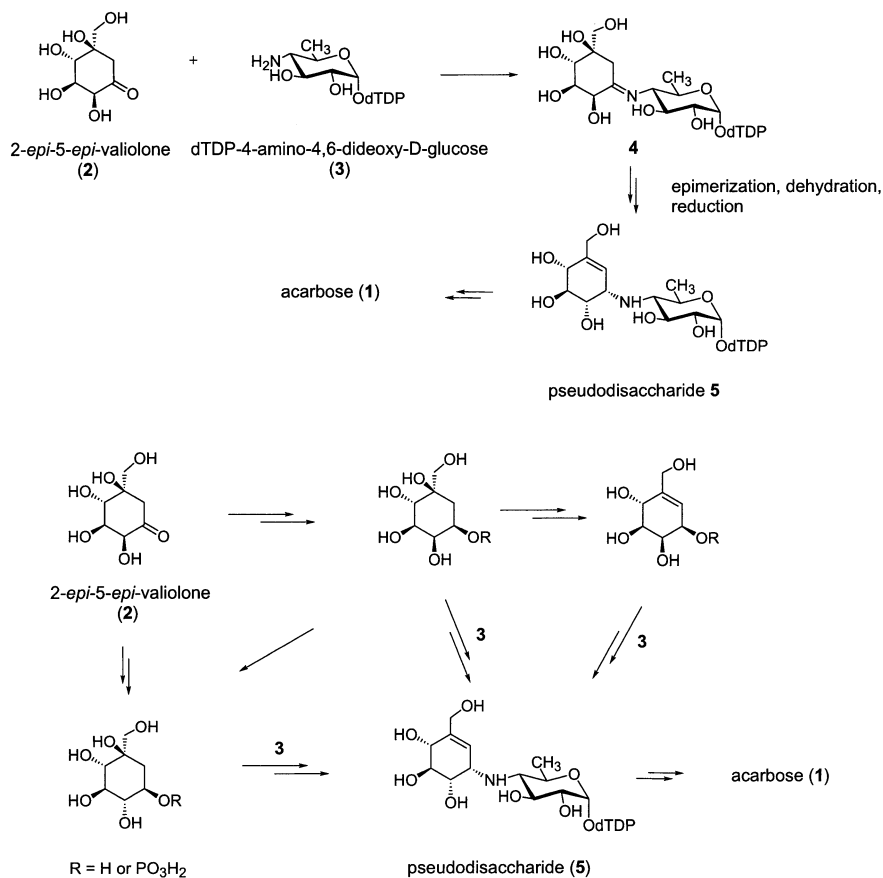
Fig. 1. Structure of acarbose.

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Scheme 1. Proposed pathways for the biosynthesis of acarbose.

keto-cyclitol **2** with amino sugar **3** gives the intermediate imine **4**. Subsequent epimerization at C-2 and dehydration between C-5 and C-6 followed by reduction of the imine to an amine, which all would have to occur on one enzyme to explain the absence of free intermediates, would afford the pseudodisaccharide nucleotide **5**.[3] an alternative hypothetical pathway involves the reduction of keto-sugar **2** to a 1-*epi*-valiolol derivative, followed by activation of the C-1 hydroxyl group as a phosphate and subsequent nucleophilic displacement by the nitrogen of amino sugar **3** to give pseudodisaccharide **5**. Such displacements are precedented in the biosynthesis of aristeromycin and neplanocin A.[7] In this pathway, the dehydration between C-5 and C-6 and the epimerization at C-2 of the cyclitol could occur either before or after the coupling with the amino sugar.

In order to determine whether either of the two proposed routes is operating in acarbose formation and to identify the actual biosynthetic pathway, it was necessary to have amino sugar **3** available as a substrate in the search for an enzyme coupling it to the cyclitol moiety and as a reference sample to aid in the determination whether the compound is generated enzymatically in acarbose-producing *Actinoplanes* sp. In this paper we describe the chemical synthesis of compound

3. An alternative synthesis of **3** has also recently been reported by Thorson and co-workers.[8]

2. Results and discussion

The chemical synthesis of amino sugar **3** started from fully protected galactoside **6** which is readily available from D-galactose in three steps.[9] The hydroxyl groups at the 2- and 3-positions were protected as benzyl ethers as follows (Scheme 2). Zemplén deacetylation of **6** was followed by protection of the 4,6-diol as a benzylidene acetal upon treatment with benzaldehyde dimethyl acetal and CSA to afford **7** in 79% yield. Subsequent benzylation of the 2- and 3-positions of **7** gave fully protected **8**, which was converted to diol **9** upon acid hydrolysis of the benzylidene acetal. Deoxygenation of the 6-position was accomplished by hydride displacement of a *p*-toluenesulfonyl leaving group. Chemoselective tosylation of the primary hydroxyl group of **9** was achieved using a slight excess of *p*-toluenesulfonyl chloride in combination with prolonged reaction times to afford tosylate **10** in 84% yield. Under these conditions no tosylation of the secondary hydroxyl was observed. Subsequent displacement of the primary tosylate with LiAlH$_4$ in THF afforded 6-deoxy derivative **11** in 83% yield.
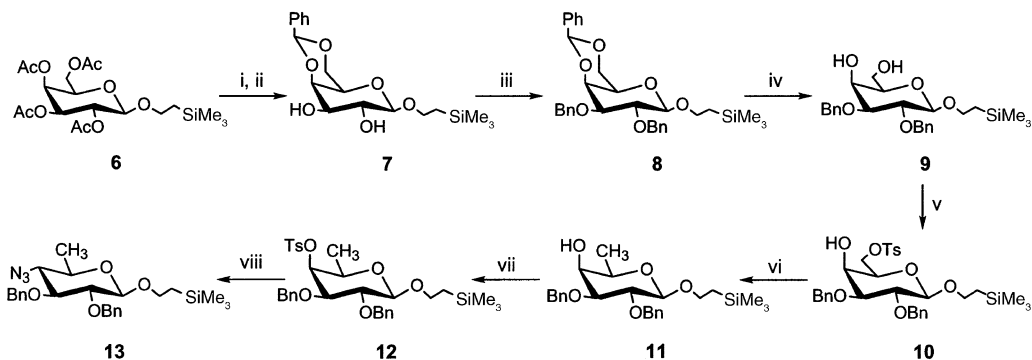
Scheme 2. Reagents and conditions: (i) NaOCH₃ (0.2 equiv), CH₃OH, 18 h; (ii) benzaldehyde dimethyl acetal (1.2 equiv), CSA, CH₃CN, 5 h, 79% (two steps); (iii) BnBr (3 equiv), NaH (4 equiv), DMF, 5 h, 89%; (iv) HOAc–water, 50 °C, 18 h, 93%; (v) TsCl (1.5 equiv), pyridine, 4 days, 84%; (vi) LiAlH₄ (2.2 equiv), THF, reflux, 18 h, 83%; (vii) TsCl (5 equiv), DMAP (0.5 equiv), pyridine, 50 °C, 18 h, 78%; (viii) NaN₃ (5 equiv), DMF, 70 °C, 3 days, 80%.

In a similar fashion nitrogen was introduced at the 4-position by displacement of a leaving group with azide. Since the tosylation of the 6-position required extended reaction times, it was expected that the secondary hydroxyl group of **11** would be much less reactive and possibly inert. Fortunately, when **11** reacted with a large excess of tosyl chloride and DMAP in pyridine at elevated temperatures, complete conversion to tosylate **12** was observed. Displacement of the tosyl group with sodium azide in DMF at 70 °C for 3 days afforded azide **13** in 80% yield. Furthermore, ¹H NMR spectroscopy indicated that the azido group of compound **13** was equatorial.

With compound **13** at hand, it remained to introduce an anomeric phosphate which would be coupled with an activated TMP derivative to give the final compound **3**. Various procedures exist for the preparation of glycosyl phosphates, including the reaction of dibenzyl phosphate with glycosyl donors such as thioglycosides,[10] glycosyl trichloroacetimidates,[11,12] and glycosyl bromides.[13] Alternatively it is possible to prepare an intermediate glycosyl phosphite from the corresponding

sugar hemi acetal, which is subsequently oxidized to the glycosyl phosphate.[13–15] The latter method was attempted first. The 2-(trimethylsilyl)ethyl anomeric protecting group was removed from fully protected glycoside **13** upon treatment with TFA in CH₂Cl₂ to give hemi acetal **14** in 89% yield (Scheme 3).[16] After treating **14** with the trivalent phosphitylating reagent, dibenzyl N,N-diisopropylphosphoramidite, and 1H-tetrazole, followed by addition of MCPBA and purification, the desired phosphate could not be isolated. After the failure of this approach, an alternative method for the preparation of the glycosyl phosphate was attempted using a glycosyl trichloroacetimidate as a glycosyl donor.[11,12] Hemi acetal **14** was treated with trichloroacetonitrile and potassium carbonate in CH₂Cl₂ and after purification, trichloroacetimidate **15** was obtained in 74% yield (1:5 α/β). Subsequent reaction of **15** with dibenzyl phosphate in CH₂Cl₂ afforded protected glycosyl phosphate **16** in 82% yield. ¹H NMR analysis of purified **16** indicated a mixture of anomers with an anomeric ratio of 5:1 α/β. All attempts to anomerize the mixture to the thermodynamically more
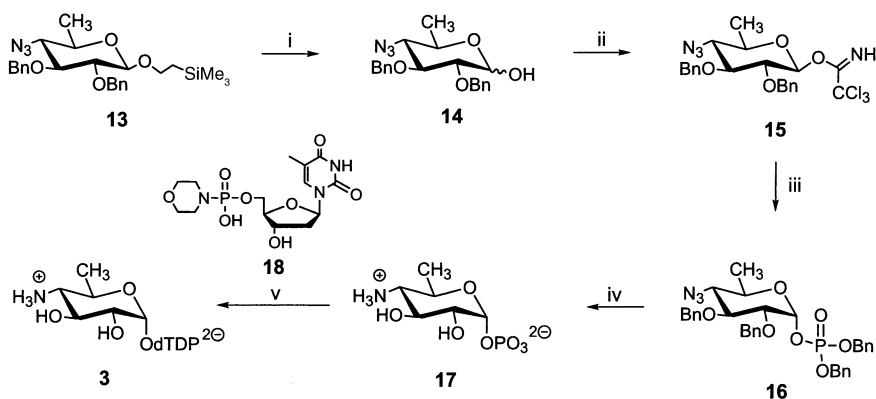
Scheme 3. Reagents and conditions: (i) 2:1 TFA–CH₂Cl₂ (v/v), 1 h, 89%; (ii) CCl₃CN (10 equiv), K₂CO₃ (5 equiv), CH₂Cl₂, 12 h, 74%; (iii) (BnO)₂P(O)OH (1.3 equiv), CH₂Cl₂, 1 h, 82%; (iv) H₂, Pd–C, 1:1 dioxane–water (v/v), 18 h; (v) 4-morpholine-N,N′-dicyclohexylcarboxamidinium thymidine 5′-monophosphomorpholidate (**18**) (1.2 equiv), 1H-tetrazole (3 equiv), pyridine, 5 days.

stable α anomer using p-toluenesulfonic acid[11] were unsuccessful and this material was used in the next step. Removal of the protecting groups and conversion of the azide to an amine was accomplished in one step via hydrogenation. Thus, compound **16** was stirred under a hydrogen atmosphere in the presence of Pd–C for 18 h, and the reaction mixture was maintained at pH 6 by the addition of 1 M NaOH. The product was purified on a Dowex 1X8 anion-exchange column (Cl⁻) which was eluted with an NaCl gradient. The resulting product was further purified on a BioGel P2 column (45 × 2 cm) to give **17** as a mixture of anomers (5:1 α/β) in 87% yield.

The final step involved the coupling of glycosyl phosphate **17** with the activated nucleoside phosphate, thymidine 5′-monophosphomorpholidate (**18**) (Scheme 3) using Wong's modified Moffat procedure.[17] A solution of **17**, 4-morpholine-N,N′-dicyclohexylcarboxamidinium thymidine 5′-monophosphomorpholidate and 1H-tetrazole in pyridine was stirred for 14 days.[17] Purification of the resulting products using Dowex 1X8 and size-exclusion chromatography on BioGel P2 resin gave **3** in 36% yield.

Currently, compound **3** is being utilized as a substrate for putative transaminases and transferases, which have been expressed from candidate genes in the acarbose biosynthetic gene cluster, in an attempt to identify the role, if any, of each enzyme in the formation of acarbose. The results will be reported separately in due course.

3. Experimental

General methods.—The ¹H and ¹³C NMR spectra were recorded on a Bruker AF-300 or AM-500 NMR spectrometer with MACNMR 5.5 PCI as the instrument controller and data processor. Low-resolution electrospray mass spectra were recorded on a Bruker–Esquire ion-trap mass spectrometer with electrospray, APCI, and nanospray ionization sources. A Fison VG Quattro II electrospray ionization-mass spectrometer was used to measure the high-resolution mass spectroscopy. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. All reactions were carried out under an atmosphere of dry argon in oven dried glassware unless noted otherwise. Reactions were monitored by TLC (Silica Gel 60 F₂₅₄, E. Merck) with detection by UV light and by charring with H_2SO_4–MeOH solution. All chemicals were purchased from Aldrich or Sigma and used without further purification unless otherwise noted. Column chromatography was performed on 230–400 mesh silica gel (Aldrich). Dowex 50 and Dowex 1 were from Aldrich. BioGel P-2 was purchased from Bio-Rad.

2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-β-D-galactopyranoside (**7**).—2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**6**) (41.9 g, 93.4 mmol) was dissolved in MeOH and NaOCH₃ (1 g, 18.7 mmol) was added. The reaction mixture was stirred for 18 h after which TLC (eluant 3:2 hexane–EtOAc) indicated complete conversion to a lower-running product. The solution was neutralized by the addition of Dowex 50 (H⁺), filtered and the filtrate concentrated under vacuum. The resulting oily residue was dissolved in CH₃CN (200 mL) and benzaldehyde dimethyl acetal (16.7 mL, 111.2 mmol) was added. Camphorsulfonic acid was added until the reaction mixture reached pH 3 and the resulting solution was stirred for 5 h, after which Et₃N (3 mL) was added dropwise and the solvents were removed under vacuum. The residue was redissolved in CH₂Cl₂ (200 mL) and washed with satd aq NaHCO₃ (3 × 200 mL), brine (2 × 200 mL), dried (MgSO₄), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 9:1–1:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **7** (26.9 g, 72.9 mmol, 79% over two steps) as a white foam; R_f 0.50 (EtOAc); $[\alpha]_D^{23}$ −43.6° (c 1, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ_H 7.53–7.32 (m, 5 H, ArH), 5.49 (s, 1 H, PhCH), 4.32 (dd, 1 H, $J_{5,6a}$ 1.2, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.28 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 4.11 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4), 4.10–4.04 (m, 2 H, H-6b, OCHHCH₂Si), 3.76 (dd, 1 H, $J_{1,2}$ 7.4, $J_{2,3}$ 9.9 Hz, H-2), 3.68 (dd, 1 H, $J_{3,4}$ 3.7, $J_{2,3}$ 9.9 Hz, H-3), 3.66–3.01 (m, 1 H, OCHHCH₂Si), 3.42 (bs, 1 H, H-5), 1.16–0.90 (m, 2 H, OCH₂CH₂Si), 0.00 (s, 9 H, (CH₃)₃Si); ¹³C NMR (125 MHz, CDCl₃) δ_C 137.4 (ArC_q), 128.9, 128.0, 126.3 (ArC), 102.1, 101.2 (C-1, CHPh), 75.3, 72.5, 71.5, 66.5 (C-2, C-3, C-4, C-5), 69.1, 67.2 (C-6, OCH₂CH₂Si), 18.2 (OCH₂CH₂Si), −1.3 (Si(CH₃)₃). Electrospray-MS: m/z 391.2 [M + Na]⁺. Electrospray-HRMS: m/z 391.1544 [M + Na]⁺, Calcd for $C_{18}H_{28}NaO_6Si$: 391.1553.

2-(Trimethylsilyl)ethyl 2,3-di-O-benzyl-4,6-di-O-benzylidene-β-D-galactopyranoside (**8**).—2-(Trimethylsilyl)ethyl 4,6-di-O-benzylidene-β-D-galactopyranoside (**7**) (25 g, 67.9 mmol) was dissolved in DMF (300 mL), and the solution was cooled to 0 °C. Sodium hydride (10.86 g, 271.6 mmol of 60% suspension) was added and the resulting suspension was stirred for 15 min. Benzyl bromide (24.2 mL, 203.6 mmol) was added dropwise and the reaction mixture was stirred for 5 h. Methanol (2 mL) was added and the resulting solution was extracted with ether (3 × 300 mL). The ether extracts were combined and washed with brine (2 × 700 mL), dried (MgSO₄), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 19:1–4:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **8** (33.2 g, 60.5 mmol, 89%) as a white

foam; R_f 0.44 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ 18.2° (c 1, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ_H 7.71–7.32 (m, 15 H, ArH), 5.51 (s, 1 H, PhC(H)O$_2$), 5.03, 4.92 (AB, 2 H, CH_2Ph), 4.90–4.84 (m, 2 H, CH_2Ph), 4.50 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 4.38 (d, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.21–4.18 (m, 2 H, H-4, OCHHCH$_2$Si), 4.10 (d, 1 H, $J_{6a,6b}$ 12.4 Hz, H-6b), 3.92 (dd, 1 H, $J_{1,2}$ 7.4, $J_{2,3}$ 9.3 Hz, H-2), 3.75–3.65 (m, 2 H, H-3, OCHHCH$_2$Si), 3.35 (s, 1 H, H-5), 1.20–1.14 (m, 2 H, OCH$_2$CH_2Si), 0.05 (s, 9 H, (CH_3)$_3$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 138.9, 138.4, 137.9, 128.7, 128.1, 127.9, 127.6, 127.4, 126.4, (ArC), 103.1, 101.0 (C-1, CHPh), 79.1, 78.5, 75.0, 73.6, 71.6, 69.1, 67.2, 66.1, 18.4, −1.3. Electrospray-MS: m/z 571.2 [M + Na]$^+$. Electrospray-HRMS: m/z 571.2474 [M + Na]$^+$, Calcd for C$_{32}$H$_{40}$NaO$_6$Si: 571.2492.

2-(Trimethylsilyl)ethyl 2,3-di-O-benzyl-β-D-galacto-pyranoside (**9**).—2-(Trimethylsilyl)ethyl 4,6-di-O-ben-zylidene-2,3-di-O-benzyl-β-D-galactopyranoside (**8**) (30.1 g, 54.8 mmol) was dissolved in 4:1 AcOH–water (300 mL) and heated to 50 °C. The reaction mixture was stirred for 18 h. The mixture was extracted with CH$_2$Cl$_2$ (5 × 100 mL) and the organic layers were combined and washed with satd aq NaHCO$_3$ (6 × 300 mL), brine (2 × 300 mL), dried (MgSO$_4$), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 4:1–1:1 hexane–EtOAc). Concentration of the appro-priate fractions afforded **9** (23.6 g, 51.2 mmol, 93%) as a white solid; R_f 0.26 (1:1 hexane–EtOAc); $[\alpha]_D^{23}$ 1.3° (c 0.44, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.50–7.12 (m, 10 H, ArH), 4.87, 4.69 (AB, 2 H, J_{AB} 10.9 Hz, CH_2Ph), 4.68–4.61 (m, 2 H, CH_2Ph), 4.32 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.03–3.95, 3.88–3.71, (2 × m, 4 H, H-4, H-6a, H-6b, OCHHCH$_2$Si), 3.68–3.50 (m, 2 H, H-2, OCHHCH$_2$Si) 3.42 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 3.1 Hz, H-3), 3.62 (dd, 1 H, $J_{5,6a}$ 5.2, $J_{5,6b}$ 6.2 Hz, H-5), 0.95 (dd, 2 H, J 8.8, 8.3 Hz, OCH$_2$CH_2Si); 0.01 (s, 9 H, (CH_3)$_3$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 139.5, 138.7 (ArC_q), 129.2–128.4 (ArC), 104.2 (C-1), 81.3, 79.9, 74.8, 67.8 (C-2, C-3, C-4, C-5), 75.9, 73.1, 68.3, 62.6 (C-6, 2 × CH$_2$Ph, OCH$_2$CH$_2$Si), 19.3 (OCH$_2$$CH_2$Si), −0.5 (Si(C$H_3$)$_3$). Electrospray-MS: m/z 483.2 [M + Na]$^+$. Electrospray-HRMS: m/z 483.2181 [M + Na]$^+$, Calcd for C$_{25}$H$_{36}$NaO$_6$Si: 483.2179.

2-(Trimethylsilyl)ethyl 2,3-di-O-benzyl-6-O-(p-tolyl-sulfonyl)-β-D-galactopyranoside (**10**).—2-(Trimethylsi-lyl)ethyl 2,3-di-O-benzyl-β-D-galactopyranoside (**9**) (20 g, 43.4 mmol) was dissolved in pyridine (200 mL) and p-toluenesulfonyl chloride (12.4 g, 65.1 mmol) was added. The reaction mixture was stirred for 4 days at rt after which TLC indicated complete conversion to a product of higher R_f. The solution was concentrated to 50 mL and subsequently diluted with CH$_2$Cl$_2$ (200 mL). The resulting solution was washed with satd aq NaHCO$_3$ (5 × 300 mL), brine (2 × 300 mL), dried (MgSO$_4$), filtered, and the filtrate was concentrated

under vacuum. The resulting residue was purified by column chromatography (eluant 9:1–7:3 hexane–EtOAc). Concentration of the appropriate fractions afforded **10** (22.4 g, 36.4 mmol, 84%) as a clear oil; R_f 0.41 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ − 5.3° (c 1.0, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.72 (d, 2 H, J 8.3 Hz, ArH), 7.32–7.20 (m, 12 H, ArH), 4.85 (1/2 AB, 1 H, J_{AB} 11.4 Hz, CHHPh), 4.66–4.58 (m, 3 H, CHHPh, CH_2Ph), 4.26 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.21 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 10.4 Hz, H-6a), 4.12 (dd, 1 H, $J_{5,6b}$ 6.8, $J_{6a,6b}$ 10.4 Hz, H-6b), 3.96–3.86 (m, 1 H, OCHHCH$_2$Si), 3.85 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 3.59 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{5,6b}$ 6.8 Hz, H-5), 3.55–3.45 (m, 2 H, H-2, OCHHCH$_2$Si), 3.40 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 3.1 Hz, H-3), 2.35 (s, 3 H, Ar-CH_3), 0.99 (dd, 2 H, J 8.8, 8.3 Hz, OCH$_2$CH_2Si), 0.01 (s, 9 H, (CH_3)$_3$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 145.7, 139.4, 138.4, 133.4 (4 × ArC_q), 130.7–128.4 (ArC), 103.8 (C-1), 80.9, 79.5, 72.4, 67.0 (C-2, C-3, C-4, C-5), 7.9, 73.3, 69.2, 68.3 (C-6, 2 × CH$_2$Ph, OCH$_2$CH$_2$Si), 22.4 (Ar-CH$_3$), 19.2 (OCH$_2$$CH_2$Si), − 0.6 (Si(C$H_3$)$_3$). Electrospray-MS: m/z 637.0 [M + Na]$^+$. Electrospray-HRMS: m/z 637.2263 [M + Na]$^+$, Calcd for C$_{32}$H$_{42}$NaO$_8$SSi: 637.2267.

2-(Trimethylsilyl)ethyl 2,3-di-O-benzyl-6-deoxy-β-D-galactopyranoside (**11**).—A solution of 2-(trimethyl-silyl)ethyl 2,3-di-O-benzyl-6-O-p-tolylsulfonyl-β-D-galactopyranoside (**10**) (16.3 g, 26.5 mmol) in THF (200 mL) was cooled to − 10 °C, and LiAlH$_4$ (2.21 g, 58.2 mmol) was added. The reaction mixture was stirred for 10 min and subsequently heated to reflux for 18 h. The resulting suspension was cooled to − 10 °C and quenched by the dropwise addition of EtOAc (50 mL), CH$_3$OH (50 mL) and satd aq potassium sodium tar-trate (50 mL). The mixture was extracted with ether (3 × 100 mL), the organic layers were combined, washed with satd aq potassium sodium tartrate (2 × 300 mL), brine (2 × 300 mL), dried (MgSO$_4$), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatogra-phy (eluant 4:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **11** (9.73 g, 21.9 mmol, 83%) as a clear oil; R_f 0.47 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ 6.4° (c 1, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.46–7.09 (m, 10 H, ArH), 4.86 (1/2 AB, 1 H, J_{AB} 10.9 Hz, CHHPh), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.68–4.64 (m, 3 H, CHHPh, CH_2Ph), 4.04–3.92 (m, 1 H, CHHCH$_2$Si), 3.66 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 3.58–3.37 (m, 4 H, H-2, H-3, H-5, CHHCH$_2$Si), 1.27 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 0.97 (dd, 2 H, J 8.8, 8.3 Hz, CH$_2$CH_2Si), 0.01 (s, 9 H, (CH_3)$_3$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 139.6, 138.8 (ArC_q), 129.2–128.3 (ArC), 103.9, (C-1), 81.7, 79.7, 70.7, 70.3 (C-2, C-3, C-4, C-5), 75.9, 73.1, 69.9 (2 × CH$_2$Ph, OCH$_2$CH$_2$Si), 19.3 (OCH$_2$$CH_2$Si), 17.2 (C-6), − 0.6 (Si(CH_3)$_3$). Electrospray-MS: m/z 467.24 [M + Na]$^+$. Electrospray-HRMS: m/z 467.2223 [M + Na]$^+$, Calcd for C$_{25}$H$_{36}$NaO$_5$Si: 467.2230.

2-(Trimethylsilyl)ethyl 2,3-di-O-benzyl-6-deoxy-4-O-(p-tolylsulfonyl)-β-D-galactopyranoside (**12**).—2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside (**11**) (9 g, 20.2 mmol), was dissolved in pyridine (200 mL) and *p*-toluenesulfonyl chloride (19.3 g, 101 mmol), and DMAP (1.24 g, 10.1 mmol) were added. The mixture was stirred for 18 h at 50 °C after which TLC indicated complete conversion to a product of higher R_f. The reaction mixture was concentrated to 50 mL and diluted with CH_2Cl_2 (300 mL). The resulting solution was washed with satd aq NaHCO$_3$ (5 × 300 mL), brine (2 × 300 mL), dried (MgSO$_4$), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 4:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **12** (9.48 g, 15.8 mmol, 78%) as a white solid; R_f 0.62 (7:3 hexane–EtOAc); $[\alpha]_D^{23}$ 71.6° (*c* 1, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.60 (d, 2 H, Ar*H*), 7.40–7.15 (m, 10 H, Ar*H*), 7.05 (d, 2 H, Ar*H*), 4.93 (d, 1 H, $J_{3,4}$ 2.1 Hz, H-4), 4.78, 4.60 (2 H, AB, *J* 11.4 Hz, C*H*$_2$Ph), 4.68, 4.48 (2 H, AB, *J* 11.9 Hz, C*H*$_2$Ph), 4.24 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.96–3.85 (m, 1 H, OC*H*HCH$_2$Si), 3.55 (q, 1 H, *J* 6.2 Hz, H-5), 3.48–3.36 (m, 3 H, H-2, H-3, OCH*H*CH$_2$Si), 2.28 (s, 3 H, ArC*H*$_3$), 1.22 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 0.94 (dd, 2 H, *J* 8.3, 8.8 Hz, OCH$_2$C*H*$_2$Si), −0.01 (s, 9 H, (C*H*$_3$)$_3$Si); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 144.8, 139.4, 138.6, 135.5 (Ar*C*$_q$), 130.0–128.3 (Ar*C*), 103.9 (C-1), 79.8, 79.6, 79.1, 69.7 (C-2, C-3, C-4, C-5), 75.8, 73.2, 68.4 (2 × *C*H$_2$Ph, O*C*H$_2$CH$_2$Si), 22.3, 17.9 (C-6, Ar*C*H$_3$), 19.3 (OCH$_2$*C*H$_2$Si), −0.6 (Si(*C*H$_3$)$_3$). Electrospray-MS: *m/z* 621.2 [M + Na]$^+$. Electrospray-HRMS: *m/z* 621.2304 [M + Na]$^+$, Calcd for $C_{32}H_{42}NaO_7SSi$: 621.2318.

2-(Trimethylsilyl)ethyl 4-azido-2,3-di-O-benzyl-4,6-dideoxy-β-D-glucopyranoside (**13**).—2-(Trimethylsilyl)-ethyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-(*p*-tolylsulfonyl)-β-D-galactopyranoside (**12**) (9 g, 15.0 mmol) was dissolved in DMF (150 mL) and NaN$_3$ (4.89 g, 75.2 mmol) was added. The reaction mixture was stirred at 70 °C for 3 days after which the solution was cooled to rt and extracted with ether (3 × 100 mL). The combined ether layers were washed with water (3 × 200 mL), dried (MgSO$_4$), filtered, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 19:1–4:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **13** (5.62 g, 12.0 mmol, 80%) as a clear oil; R_f 0.65 (9:1 hexane–EtOAc); $[\alpha]_D^{23}$ 87.7° (*c* 1, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.23–7.10 (m, 10 H, Ar*H*), 4.90, 4.83, 4.68, 4.65 (2 × AB, 4 H, 4 × CH*H*Ph), 4.27 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.93–3.82 and 3.56–3.43 (2 × m, 2 H, OC*H*HCH$_2$Si), 3.41–3.28 (m, 2 H, H-2, H-3), 3.17–3.00 (m, 2 H, H-4, H-5), 1.26 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 0.96 (dd, 2 H, *J* 8.3, 8.8 Hz, OCH$_2$C*H*$_2$Si), 0.01 (s, 9 H, (C*H*$_3$)$_3$Si); ^{13}C NMR (75

MHz, CDCl$_3$) δ_C 138.3, 137.8 (Ar*C*$_q$), 128.3–127.6 (Ar*C*), 102.8 (C-1), 82.6, 82.3, 70.3, 67.7 (C-2, C-3, C-4, C-5), 75.5, 74.7, 67.5 (2 × *C*H$_2$Ph, O*C*H$_2$CH$_2$Si), 18.5 (O*C*H$_2$CH$_2$Si), 18.4 (C-6), −1.4 (Si(*C*H$_3$)$_3$). Electrospray-MS: *m/z* 492.2 [M + Na]$^+$. Electrospray-HRMS: *m/z* 492.2280 [M + Na]$^+$, Calcd for $C_{25}H_{35}N_3NaO_4Si$: 492.2295.

4-Azido-2,3-di-O-benzyl-4,6-dideoxy-D-glucopyranose (**14**).—2-(Trimethylsilyl)ethyl 4-azido-2,3-di-*O*-benzyl-4,6-dideoxy-β-D-glucopyranoside (**13**) (2 g, 4.26 mmol) was dissolved in CH_2Cl_2 (15 mL), and TFA (30 mL) was added dropwise over 1 h. The reaction mixture was stirred for a further 2 h and subsequently diluted with EtOAc (10 mL) and toluene (20 mL). The solution was concentrated to 20 mL and toluene (30 mL) was added. This solution was evaporated to dryness and the residue taken up in toluene (3 × 20 mL) and evaporated. The resulting residue was purified by column chromatography (eluant 7:3–1:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **14** (1.38 g, 3.73 mmol, 89%, α:β 6:4) as a white solid; R_f 0.32 (7:3 hexane–EtOAc); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 7.37–7.06 (m, 10 H, Ar*H*), 5.05 (d, 0.6 H, $J_{1\alpha,2}$ 3.1 Hz, H-1α), 4.88–4.59 (m, 4 H, 2 × C*H*$_2$Ph), 4.57 (d, 0.4 H, $J_{1\beta,2}$ 7.3 Hz, H-1β), 3.80–3.69 (m, 1.2 H, H-3α, H-5α), 3.45 (dd, 0.6 H, $J_{1\alpha,2}$ 3.3, $J_{2,3}$ 9.5 Hz, H-2α), 3.43–3.38 (m, 0.4 H, H-3β), 3.30 (dd, 0.4 H, $J_{1\beta,2}$ 7.3, $J_{2,3}$ 9.3 Hz, H-2β), 3.19 (dd, 0.4 H, $J_{4,5\beta}$ 9.9, $J_{5\beta,6\beta}$ 5.7 Hz, H-5β), 3.15–2.98 (m, 1 H, H-4α, H-4β), 1.28 (d, 1.2 H, $J_{5\beta,6\beta}$ 5.7 Hz, H-6β), 1.20 (d, 1.8 H, $J_{5\alpha,6\alpha}$ 6.2 Hz, H-6α); ^{13}C NMR (125 MHz, CDCl$_3$) δ_C 138.7, 138.5, 138.4, 138.2 (Ar*C*$_q$), 129.2–128.6 (Ar*C*), 97.9, 91.8 (C-1), 83.9, 83.4, 80.8, 80.2, 71.4, 68.6, 68.3, 66.9 (C-2, C-3, C-4, C-5), 76.5, 76.4, 75.6, 73.9 (*C*H$_2$Ph), 19.3, 19.2 (C-6). Electrospray-MS: *m/z* 392.1 [M + Na]$^+$. Electrospray-HRMS: *m/z* 392.1598 [M + Na]$^+$, Calcd for $C_{20}H_{23}N_3NaO_4$: 392.1586.

4-Azido-2,3-di-O-benzyl-4,6-dideoxy-β-D-glucopyranosyl trichloroacetimidate (**15**).—4-Azido-2,3-di-*O*-benzyl-4,6-dideoxy-D-glucopyranose (**14**) (1.3 g, 3.52 mmol), was dissolved in CH_2Cl_2 (20 mL), and trichloroacetonitrile (3.53 mL, 35.2 mmol) was added followed by anhyd K$_2$CO$_3$ (2.43 g, 17.6 mmol). The reaction mixture was stirred for 12 h after which TLC (eluant 7:3 hexane–EtOAc) indicated that the reaction was complete. The suspension was filtered through a plug of Celite, and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (eluant 9:1–7:3 hexane–EtOAc, plus 0.5% Et$_3$N). Concentration of the appropriate fractions afforded **15** (1.33 g, 2.59 mmol, 74%) as a clear oil; R_f 0.58 (7:3 hexane–EtOAc); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H 8.75 (s, 1 H, N*H*), 7.45–7.30 (m, 10 H, Ar*H*), 5.79 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.03–4.67 (m, 4 H, 2 × C*H*$_2$Ph), 3.75 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 8.8 Hz, H-2), 3.64 (dd, 1 H, $J_{2,3}$ 8.8, $J_{3,4}$ 9.3 Hz, H-3), 3.45 (dq, 1 H, $J_{4,5}$

9.8, $J_{5,6}$ 6.2 Hz, H-5), 3.26 (dd, 1 H, $J_{3,4}$ 9.3, $J_{4,5}$ 9.8 Hz, H-4), 1.41 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C 161.1 (C(CCl$_3$)=NH), 137.7, 137.6 (ArC_q), 128.4–127.6 (ArC), 97.9 (C-1), 90.8 (C(CCl$_3$)=NH), 82.7, 81.0, 71.5, 67.3 (C-2, C-3, C-4, C-5), 75.7, 74.9 (CH$_2$Ph), 18.4 (C-6). Electrospray-MS: m/z 535.1 [M + Na]$^+$. Electrospray-HRMS: m/z 535.0683 [M + Na]$^+$, Calcd for $C_{22}H_{23}Cl_3NaN_4O_4$: 535.0683.

4-Azido-2,3-di-O-benzyl-4,6-dideoxy-α-D-glucopyranosyl dibenzyl phosphate (**16**).—4-Azido-2,3-di-*O*-benzyl-4,6-dideoxy-β-D-glucopyranosyl trichloroacetimidate (**15**) (1.2 g, 2.34 mmol) was dissolved in CH$_2$Cl$_2$ and dibenzyl phosphate (0.84 g, 3.04 mmol) was added. The reaction mixture was stirred for 1 h, after which TLC (eluant 7:3 hexane–EtOAc) indicated complete conversion to a product of lower R_f. The resulting solution was concentrated under vacuum, and the residue was purified by column chromatography (eluant 4:1 hexane–EtOAc). Concentration of the appropriate fractions afforded **16** (1.21 g, 1.92 mmol, 82%) as a clear oil; R_f 0.24 (7:3 hexane–EtOAc); ^1H NMR (500 MHz, CDCl$_3$) δ_H 7.51–7.25 (m, 20 H, ArH), 5.92 (dd, 1 H, $J_{1,2}$ 3.1, $J_{1,P}$ 6.8 Hz, H-1), 5.18–5.09 (m, 4 H, CH_2Ph), 4.98, 4.84 and 4.82, 4.72 (2 × AB, 4 H, 2 × CH_2Ph), 3.80, (t, 1 H, J 9.9 Hz, H-3), 3.73 (dq, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 6.2 Hz, H-5), 3.66 (dt, 1 H, J 9.9 Hz, 3.1, H-2), 3.18 (t, 1 H, J 9.9 Hz, H-4), 1.26 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6). Electrospray-MS: m/z 652.2 [M + Na]$^+$. Electrospray-HRMS: m/z 652.2167 [M + Na]$^+$, Calcd for $C_{34}H_{36}N_3NaO_7P$: 652.2189.

4-Amino-4,6-dideoxy-α-D-glucopyranosyl phosphate (**17**).—4-Azido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-glucopyranosyl dibenzyl phosphate (**16**) (206 mg, 327 μmol) was dissolved in 1:1 1,4-dioxane–water (6 mL), and Pd–C (100 mg, 50% wet) was added. The reaction mixture was stirred vigorously under an atmosphere of hydrogen for 18 h while maintaining pH 6 by the addition of 1 N NaOH. The resulting suspension was filtered through a plug of Celite and concentrated under vacuum. The residue was purified on a Dowex 1X8 anion-exchange column (Cl$^-$, 7 × 1.5 cm) and eluted with a NaCl gradient (0–700 mmol, 80 mL). The appropriate fractions were combined and lyophilized to dryness. The resulting white solid was redissolved in a minimum volume of water and purified on a Bio-Gel P2 column (45 × 2 cm) which was eluted with water to give **17** (69 mg, 284 μmol, 87%) as a white solid; R_f 0.13 (2:1 *i*PrOH–1 M NH$_4$HCO$_3$); ^1H NMR (500 MHz, CDCl$_3$) δ_H 5.22 (dd, 1 H, $J_{1,2}$ 3.6, $J_{1,P}$ 6.7 Hz, H-1), 3.98 (dq, 1 H, $J_{4,5}$ 10.4, $J_{5,6}$ 6.2 Hz, H-5), 3.64 (dd, 1 H, $J_{2,3} = J_{3,4}$ 9.9 Hz, H-3), 3.40–3.32 (m, 1 H, H-2), 2.75 (dd, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 10.4 Hz, H-4), 1.09 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (125 MHz, CDCl$_3$) δ_C 97.9 (C-1), 72.4, 69.2, 65.9, 57.7 (C-2, C-3, C-4, C-5), 17.7

(C-6). Electrospray-MS: negative ion m/z 242.2 [M − H]$^-$, positive ion m/z 266.0 [M + Na]$^+$. Electrospray-HRMS: m/z 266.0403 [M + Na]$^+$, Calcd for $C_6H_{14}NaNO_7$: 266.0406.

Thymidine 5′-diphospho-4-amino-4,6-dideoxy-α-D-glucopyranose (**3**).—4-Amino-4,6-dideoxy-α-D-glucopyranosyl phosphate (**17**) (33 mg, 136 μmol) and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium thymidine 5′-monophosphomorpholidate (186 mg, 272 μmol) were dissolved in pyridine (1.5 mL) and the resulting solution was concentrated to dryness. This process was repeated twice and the residue was dried at rt under vacuum for 48 h. To the dry residue was added pyridine (400 μL) and 1*H*-tetrazole (29 mg, 408 μmol), and the solution was stirred at rt for 14 days. Water (1 mL) was added and the solution was concentrated under vacuum. The resulting residue was purified by size-exclusion chromatography on Bio-Gel P2 resin, eluting with 250 mM NH$_4$HCO$_3$ to give **3** (26.5 mg, 48.4 μmol, 36%) as a white solid; R_f 0.39 (2:1 *i*PrOH–1 M NH$_4$HCO$_3$); ^1H NMR (300 MHz, CDCl$_3$) δ_H 7.50 (s, 1 H, H-6″), 6.11 (t, 1 H, J 6.7 Hz, H-1′), 5.35 (dd, 1 H, $J_{1,P}$ 6.8, $J_{1,2}$ 3.1 Hz, H-1), 4.42–4.38 (m, 1 H, H-3′), 4.00–3.87 (m, 3 H, H-4′, H-5a′, H-5b′), 3.80 (m, 1 H, H-5), 3.58 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 9.9 Hz, H-3), 3.40–3.31 (m, 1 H, H-2), 2.61 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 2.19–2.09 (m, 2 H, H-2a′, H-2b′), 1.70 (s, 3 H, H-5″-CH_3), 1.09 (d, 3 H, $J_{5,6}$ 5.7 Hz, H-6). Electrospray-MS: m/z 570.1 [M + Na]$^+$. Electrospray-HRMS: m/z 570.0845 [M + Na]$^+$, Calcd for $C_{16}H_{27}N_3NaO_{14}P_2$: 570.0866.

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