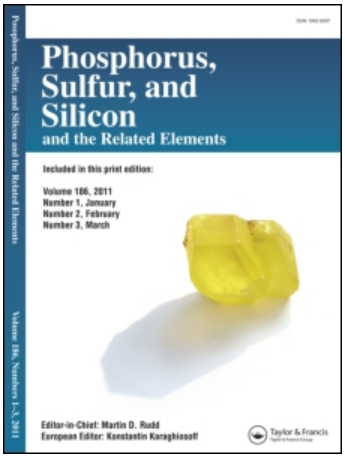

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Synthesis and Characterization of N-(O,O-Dialkyl Phosphoryl)-D-glucosamine

Jing Guo[a]; Li Ma[a]; Pu Liu[a]
[a] Department of Chemistry, Zhengzhou University, Zhengzhou, P. R. China

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SYNTHESIS AND CHARACTERIZATION OF N-(O,O-DIALKYL PHOSPHORYL)-D-GLUCOSAMINE

Jing Guo, Li Ma, and Pu Liu
Department of Chemistry, Zhengzhou University, Zhengzhou, P. R. China

Using D-glucosamine hydrochloride as a starting material and alkylphosphite as the phosphorylating agent, a new analogue of glycosyl phosphate, namely N-(O, O-dialkyl phosphoryl)-D-glucosamine, was synthesized without hydroxyl protection by Atherton–Todd reaction. The structure of N-(O, O-dialkyl phosphoryl)-D-glucosamine was determined by FT-IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and HR MS. This concise and convenient synthetic pathway provides a new approach for the preparation of N-phosphoryl glucosamine derivatives.

Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.

Keywords Characterization; N-(O,O-dialkyl phosphoryl)-D-glucosamine; glucosamine

INTRODUCTION

Chitin, a poly-β-(1,4)-N-acetyl-D-glucosamine, is the second most abundant natural biopolymer presented in the exoskeleton of crustaceans and in cell walls of fungi, insects, and yeast. A series of oligomers and monosaccharides, such as D-glucosamine and N-acetyl-D-glucosamine, can be obtained by either chemical or enzymatic hydrolysis of chitosan and chitin.[1] The reactive hydroxyl and amino groups offer a wide possibility for obtaining new properties that are known to have a range of biological activities.[2–5]

Glycosyl phosphates play an important role in carbohydrate metabolism.[6] It is known that they are glycosyl donors in the biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates, and they also have a range of other biological activities. For example, tetrasaccharide phosphate is a protected form of the linkage region of the arabinogalactan-peptidoglycan complex in the mycobacterial cell wall.[7] Phosphorylated glucosamine inhibits adipogenesis in 3T3-L1 adipocytes.[8] α-D-Glucose-1-phosphate is formed by phosphorylase catalytic splitting of glycogen.[9] The synthesis of analogues is a worthy endeavor for a better understanding of the enzymatic pathways involving such glycosyl phosphates. More importantly, the synthesis of analogues that could regulate metabolism would lead to the rational development of carbohydrate-based therapeutics.

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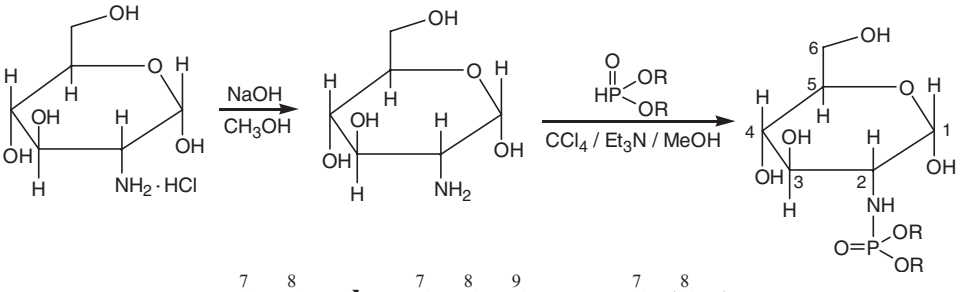
Address correspondence to Pu Liu, Department of Chemistry, Zhengzhou University, Zhengzhou 450001, P. R. China. E-mail: liupu@zzu.edu.cn

A literature survey on the synthesis of analogues of glycosyl phosphates reveals several reports on the synthesis of derivatives that contain O—P bond or C—P bond.[10–17] But the synthesis of N-phosphorylated glucosamine has received much less coverage. Jayakumar et al. synthesized phosphorylated chitosan/chitin by using $H_3PO_4/P_2O_5/Et_3PO_4$/hexanol method with high yields and degree of substitution.[18–20] However, these research efforts were limited in the derivatives of phosphoric acid.[18–20] Kannan et al. described a two-step synthesis of glycosyl phosphoramidates starting from the corresponding per-O-acetylated glycosyl azides by the Staudinger reaction.[21]

In this article, we describe a concise, effective, and one-step synthetic method of N-phosphorylated glucosamine starting from D-glucosamine hydrochloride without protection of hydroxyl by the Atherton–Todd reaction.[22–26]



a R=$\overset{7}{C}H_2\overset{8}{C}H_3$ **b** R=$\overset{7}{C}H_2\overset{8}{C}H_2\overset{9}{C}H_3$ **c** R=$\overset{7}{C}H(\overset{8}{C}H_3)_2$

d R=$\overset{7}{C}H_2\overset{8}{C}H_2\overset{9}{C}H_2\overset{10}{C}H_3$ **e** R=$\overset{7}{C}H_2\overset{8}{C}H(\overset{9}{C}H_3)_2$

Scheme 1

RESULTS AND DISCUSSION

The structures of compounds **a–e** were established on the basis of their analytical spectral data. The ^1H and ^{13}C NMR spectra of compounds **a–e** are similar except for the signals resulting from the alky groups in alkylphosphite. Figures 1 and 2 (and Figures S1–S5, Supplemental Materials, available online) are the spectra of N-(O, O-dibutyl phosphite)-D-glucosamine (compound **d**) as a typical example.

Furthermore, ESI-MS/MS was performed in order to corroborate the structure. Compounds **a–e** showed similar fragmentation pathways. The stepwise fragmentations of their $[M+Na]^+$ ions showed that they all underwent the loss of H_2O molecules, alkyl groups, and methylene groups. The structures also transformed from six-membered rings into five-membered rings. However, the sequence of these transformations varies from case to case. In typical ESI-MS/MS research regarding *N-(O,O*-dibutyl phosphite*)-D*-glucosamine (compound **d**), the molecule ion is 394 $[M+Na]^+$, the characteristic fragment ions 376$[M-H_2O+Na]^+$, 358$[M-2H_2O+Na]^+$, 320$[M-C_4H_8-H_2O+Na]^+$, 302$[M-C_4H_8-2H_2O+Na]^+$, 284$[M-C_4H_8-3H_2O+Na]^+$, 264$[M-2C_4H_8-H_2O+Na]^+$, 246$[M-2C_4H_8-2H_2O+Na]^+$, 254$[M-C_4H_8-3H_2O-CH_2O+Na]^+$, 216$[M-2C_4H_8-2H_2O-CH_2O+Na]^+$, 198$[M-2C_4H_8-3H_2O-CH_2O+Na]^+$, and 184$[M-2C_4H_8-3H_2O-CH_2O-CH_2+Na]^+$ appeared. Based on these fragment ions, the fragmentation pathway of compound **d** is shown in Figure 3.

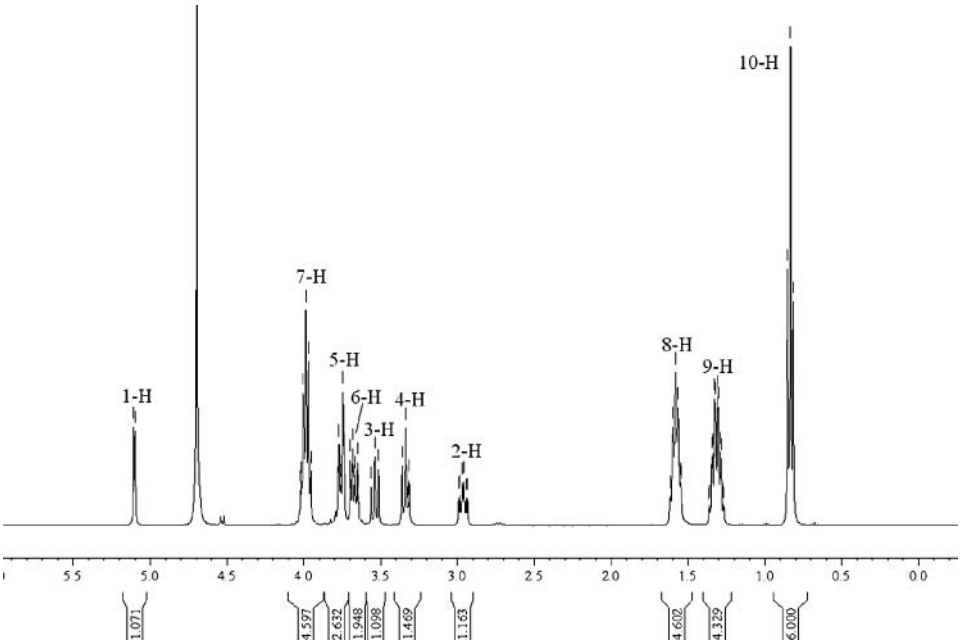


Figure 1 ^{1}H NMR spectra of compound **d**.

EXPERIMENTAL

Melting points were determined on an XT4A melting point apparatus, and were uncorrected. FT-IR spectra were recorded in a Perkin-Elmer FT-IR 1750 series spectrophotometer at room temperature with KBr pellet method in the range of 400–4000 cm^{-1}. Mass

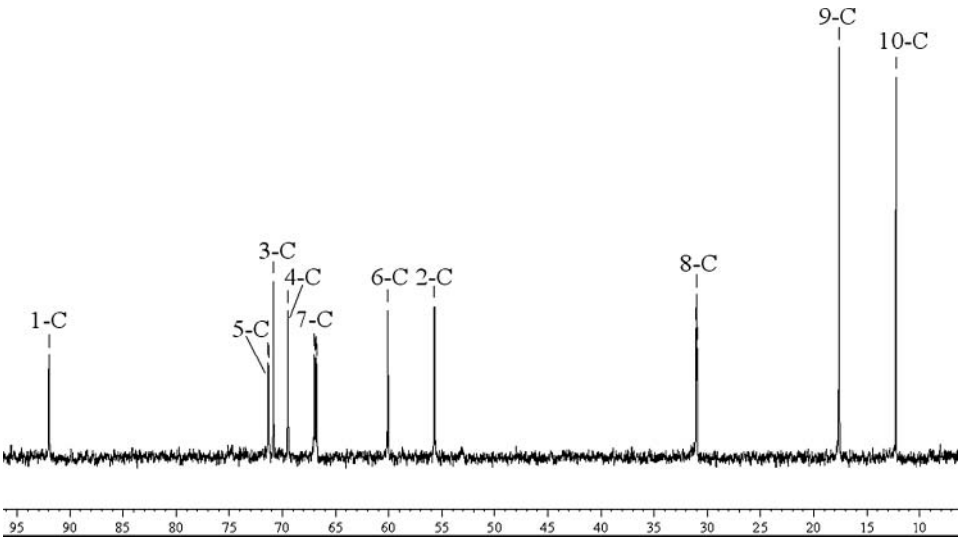


Figure 2 ^{13}C NMR spectra of compound **d**.

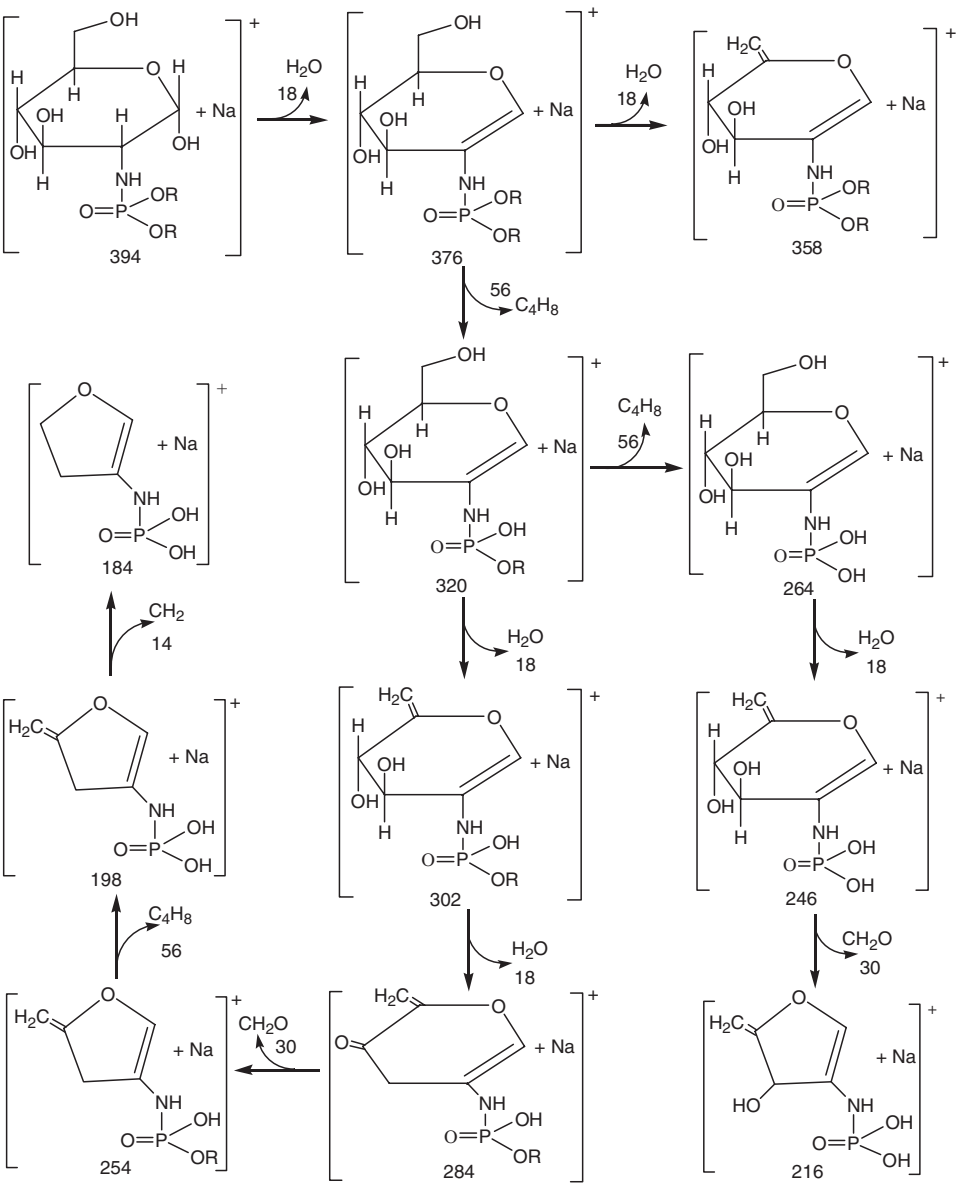


Figure 3 ESI-MS/MS fragmentation pathway of compound **d** (R=$CH_2CH_2CH_2CH_3$).

spectra were obtained on a Bruker Esquire 3000 mass spectrometer by electrospray ionization (ESI). HR MS were obtained on a Q-TOF Micro by electrospray ionization. Optical rotations were measured in solution of H_2O in 1 dm cells at 20°C on a Perkin Elmer 341 automatic spectropolarimeter. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR were recorded in D_2O on a Bruker-DTX-400 using internal TMS (^{1}H, ^{13}C) and external 85% H_3PO_4 (^{31}P) as standard. Chemical shifts were expressed in parts per million (ppm). Coupling constants (*J*) are given in Hertz (Hz). UV-vis was measured on a TU-1901 double-beam UV-vis

Spectrophotometer. Thin layer chromatography (TLC) was performed on thick plates of silica gel. Chromatography was performed with chloroform:methanol (1:3) and visualized by spraying the plates with 1,2,3-triketohydrindene ethanol solution and heating in an oven at 110°C for 10 min until the color developed.

General Synthesis Procedure: N-(O,O-Dialkylphosphoryl)-D-glucosamine (a–e)

Glucosamine hydrochloride (0.01 mol) and NaOH (0.01 mol) were added to methanol (30 mL), and the reaction mixture was stirred for 5 min. Then the solution was filtered to remove the salt. Triethylamine (10 mL) was added into the filtrate, then a solution of alkylphosphite (0.02 mol) and CCl_4 (10 mL) was added dropwise with vigorous stirring in an ice-salt bath. The reaction was allowed to stir for 3 h at about –5°C, then proceeded for 21 h at room temperature. The mixture was filtered to remove the salt. The filtrate was concentrated by a rotary evaporator under reduced pressure below 40°C. A white viscous mixture was obtained. Ethyl acetate was added into the mixture in order to precipitate the product. By filtration and washing with ethyl acetate, trichloromethane and, NaOH saturated solution completely, the white powder N-(O,O-dialkyl phosphoryl)-D-glucosamine was obtained (Scheme 1).

N-(O,O-Diethyl phosphite)-D-glucosamine (a)

Yield: 42.9%. Mp 99–100°C. HR MS calcd for $C_{10}H_{21}NO_8P$ 338.0983[M+Na]$^+$, found 338.0981[M+Na]$^+$. $[\alpha]_D^{20}$+ 45.2 (c 0.50, H_2O). ^{1}H NMR (400 MHz, D_2O), δ(ppm): 5.19–5.18(d, J = 3.6 Hz, 1H, 1-H), 4.15–4.07(m, 4H, 7-H), 3.90–3.02(m, 1H, 5-H), 3.78–3.71(m, 1H, 6-H), 3.64–3.59(t, J = 10.2 Hz, 1H, 3-H), 3.44–3.39(t, J = 10.2 Hz, 1H, 4-H), 3.08–3.02 (m, 1H, 2-H), 1.33–1.30(m, 6H, 8-H). ^{13}C NMR (400 MHz, D_2O), δ(ppm): 92.50 (1-C), 71.8 (3-C), 71.41 (5-C), 70.05 (4-C), 63.90 (7-C), 60.61 (6-C), 56.2 (2-C), 15.30 (8-C). ^{31}P NMR (400 MHz, D_2O), δ (ppm): 10.13. FT-IR: ν_{NH}: 3346 cm^{-1}, $\nu_{P=O}$: 1207cm^{-1}, ν_{P-O-C}: 1030 cm^{-1}, ν_{-CH3}: 2984 cm^{-1}. UV: λ_{max} = 273 nm.

N-(O,O-Dipropyl phosphite)-D-glucosamine (b)

Yield: 31.1%. Mp 139–140°C. HR MS calcd for $C_{12}H_{26}NO_8P$ 366.1295[M+Na]$^+$, found 366.1294[M+Na]$^+$. $[\alpha]_D^{20}$+ 44.6(c 0.50, H_2O). ^{1}H NMR (400 MHz, D_2O), δ(ppm): 5.08–5.07 (d, 1H, J = 3.6 Hz, 1-H), 3.94–3.87 (m, 4H, 7-H), 3.79–3.71(m, 1H, 5-H), 3.66–3.58 (m, 2H, 6-H), 3.53–3.48 (t, 1H, J = 9.6 Hz, 3-H), 3.33–3.28 (m, 1H, 4-H), 2.97–2.91 (m, 1H, 2-H), 1.60–1.54 (m, 4H, 8-H), 0.84–0.80 (m, 6H, 9-H). ^{13}C NMR (400 MHz, D_2O), δ(ppm): 92.87(1-C), 72.24(5-C), 71.76(3-C), 70.39(4-C), 69.58(7-C), 60.97(6-C), 56.63(2-C), 23.41(8-C), 9.69(9-C). ^{31}P NMR (400 MHz, D_2O), δ(ppm): 10.75. FT-IR: ν_{NH}: 3355 cm^{-1}, $\nu_{P=O}$: 1207cm^{-1}, ν_{P-O-C}: 1013 cm^{-1}, ν_{-CH3}: 2968 cm^{-1}, δ_{-CH3}: 1375 cm^{-1}, $\nu_{\text{pyranose ring}}$: 921 cm^{-1}, 769 cm^{-1}. UV: λ_{max} = 267 nm.

N-(O,O-Diisopropy phosphite)-D-glucosamine (c)

Yield: 36.8%. Mp 125–126°C. HR MS calcd for $C_{12}H_{26}NO_8P$ 366.1292[M+Na]$^+$, found 366.1294[M+Na]$^+$. $[\alpha]_D^{20}$+ 46.5(c 0.50, H_2O). ^{1}H NMR (400 MHz, D_2O), δ(ppm): 5.09–5.08 (d, 1H, J = 3.6 Hz, 1-H), 4.59–4.45 (m, 2H, 7-H), 3.75–3.70 (m, 1H, 5-H),

3.66–3.51 (m, 2H, 6-H), 3.49–3.31 (t, 1H, $J = 9.6$ Hz, 3-H), 3.29–3.20 (t, 1H, $J = 9.2$ Hz, 4-H), 2.95–2.92 (m, 1H, 2-H), 1.21–1.19 (t, 12H, $J = 2.8$ Hz, 8-H). ^{13}C NMR (400 MHz, D_2O), δ(ppm): 92.88(1-C), 73.44(7-C), 72.31(3-C), 71.72(5-C), 70.45(4-C), 60.91(6-C), 56.78(2-C), 23.19(8-C). ^{31}P NMR (400 MHz, D_2O), δ (ppm): 9.02. FT-IR: ν_{NH}: 3362 cm^{-1}, $\nu_{P=O}$: 1219cm^{-1}, ν_{P-O-C}: 1024 cm^{-1}, ν_{-CH3}: 2979 cm^{-1}, δ_{-CH3}: 1386 cm^{-1}, $\nu_{\text{pyranose ring}}$: 940 cm^{-1}, 777 cm^{-1}. UV: $\lambda_{max} = 274$ nm.

N-(O,O-Dibutyl phosphite)-D-glucosamine (d)

Yield: 33.2%. Mp 131–132°C. HR MS calcd for $C_{14}H_{30}NO_8P$ 394. 1597[M+Na]$^+$, found 394.1607[M+Na]$^+$. $[\alpha]_D^{20}+$ 43.0(c 0.50, H_2O). ^{1}H NMR (400 MHz D_2O), δ (ppm): 5.07–5.06 (d, 1H, $J = 3.6$ Hz, 1-H), 3.98–3.91 (m, 4H, 7-H), 3.74–3.70 (m, 1H, 5-H), 3.66–3.60 (m, 2H, 6-H), 3.52–3.47 (t, 1H, $J = 9.2$ Hz, $J = 10.4$ Hz, 3-H), 3.32–3.27 (t, 1H, $J = 9.2$ Hz, $J = 9.6$ Hz, 4-H), 2.95–2.90 (m, 1H, 2-H), 1.56–1.53 (t, 4H, $J = 6.8$ Hz, $J = 6.4$ Hz, 8-H), 1.33–1.23 (m, 4H, 9-H), 0.81–0.78 (t, 6H, $J = 7.2$ Hz, $J = 7.6$ Hz, 10-H). ^{13}C NMR (400 MHz, D_2O), δ (ppm): 91.94(1-C), 71.31(5-C), 70.84(3-C), 69.44(4-C), 66.86(7-C), 60.13(6-C), 55.70 (2-C), 30.98(8-C), 17.60(9-C), 12.42(10-C). ^{31}P NMR (400 MHz, D_2O), δ (ppm): 10.72. FT-IR: ν_{NH}: 3359 cm^{-1}, $\nu_{P=O}$: 1206 cm^{-1}, ν_{P-O-C}: 1024 cm^{-1}, ν_{-CH3}: 2959 cm^{-1}, δ_{-CH3}: 1377 cm^{-1}, $\nu_{\text{pyranose ring}}$: 922 cm^{-1}, 773 cm^{-1}. UV: $\lambda_{max} =$ 274 nm.

N-(O,O-Diisobutyl phosphite)-D-glucosamine (e)

Yield: 36.4%. Mp 153–154°C. HR MS calcd for $C_{14}H_{30}NO_8P$ 394.1605[M+Na]$^+$, found 394.1607[M+Na]$^+$. $[\alpha]_D^{20}+$ 44.8(c 0.50, H_2O). ^{1}H NMR (400 MHz, D_2O), δ (ppm): 5.08–5.07 (d, 1H, $J = 3.6$ Hz, 1-H), 3.78–3.69 (m, 4H, 7-H), 3.78–3.69 (m, 1H, 5-H), 3.66–3.61 (m, 2H, 6-H), 3.53–3.48 (t, 1H, $J = 9.6$ Hz, $J = 10.0$ Hz, 3-H), 3.33–3.28 (t, 1H, $J = 9.6$ Hz, $J = 9.2$ Hz, 4-H), 2.97–2.91 (m, 1H, 2-H), 1.87–1.78 (m, 2H, 8-H), 0.83–0.81(d, 12H, $J = 6.4$ Hz, 9-H), ^{13}C NMR (400 MHz, D_2O), δ (ppm): 92.53(1-C), 73.39(5-C), 71.89(3-C), 71.40(4-C), 70.03(7-C), 60.63(6-C), 56.30(2-C), 28.46(8-C), 17.84(9-C). ^{31}P NMR (400 MHz, D_2O), δ(ppm): 10.17. FT-IR: ν_{NH}: 3330 cm^{-1}, $\nu_{P=O}$: 1217cm^{-1}, ν_{P-O-C}: 1048 cm^{-1}, ν_{-CH3}: 2960 cm^{-1}, δ_{-CH3}: 1368 cm^{-1}, $\nu_{\text{pyranose ring}}$: 916 cm^{-1}, 779 cm^{-1}. UV: $\lambda_{max} = 273$ nm.

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