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First synthesis of natural phosphatidyl-β-D-glucoside

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Abstract

Herein, we report the chemical synthesis of naturally occurring mammalian phosphatidyl-β-D-glucoside (PtdGlc), in order to confirm the proposed structure and to clarify its stereochemistry. We designed a convergent synthetic strategy, suitable to prepare sensitive PtdGlc derivatives. As an initial demonstration of our strategy, we successfully prepared both PtdGlc diastereomers as well as its sensitive arachidonyl analogue. The presence of both diastereomers in the natural sample was confirmed.

Keywords: Phosphatidylglucoside; *t*-Butyl orthoester; H-phosphonate; Selective deprotection; Phospholipids

1. Introduction

Glucose containing phospholipids are rare in nature. In 1970, Short and White[1] identified phosphatidylglucoside (**1**, PtdGlc) in the lipid fraction of *Staphylococcus aureus*. About 30 years later, the first isolation from mammalian sources was reported by Nagatsuka et al.[2,3] Recently, PtdGlc was isolated from rat embryonic brain[4] tissue, comprising a single molecular species containing exclusively 18:0/20:0 fatty acyl chains, rarely occurring in known mammalian lipids. It was speculated, that this unusual fatty acyl chain pattern might be responsible for its presence within distinct membrane microdomains on astroglial cells. Furthermore, it was suggested that PtdGlc plays an important role in glial cell development and differentiation. However, the biological roles of PtdGlc in glial cells remain largely unknown.

A variety of approaches toward chemical syntheses of PtdGlc and its derivatives, which predominantly bear palmityl (16:0) or stearyl (18:0) residues, have been explored. Typically, the free acid or metal salt of the corresponding phosphatidic acid derivative was condensed with an activated carbohydrate moiety, such as α-D-glycopyranosyl bromide,[5] trichloroacetimidate,[6,7] or 1,2-(*tert*-Butyl orthoacetate).[7,8] On the other hand, Ramirez et al.[9] took a unique approach by first condensing benzylated glucose with bis-(1,2-dimethylethenylene) pyrophosphate. Thus, the resultant cyclic phosphate was linked with 1,2-di-*O*-palmityl-*sn*-glycerol. However, the configuration of the anomeric center was not determined and deprotection of the final compound was not successful.

Enzymatic approaches have also been studied, using phospholipase D from various vegetable[10] or actinobacteria.[11,12] With vegetable enzyme, carbohydrates did not act as acceptors. In contrast, phospholipase DA from *Actinomadura* sp.[11] catalysed transphosphorylation to various carbohydrates, including glucose. Although the structural analyses of the resulting carbohydrates have not been reported, secondary evidence[12] point toward primary hydroxyl groups as phosphatidyl moiety acceptors.

Our synthetic plan toward PtdGlc and its derivatives is depicted in Scheme 1. In contrast to previous[5–8] approaches, we decided to separately furnish the stereochemically defined components, a β-glycopyranosyl H-phosphonate unit and an enantiomerically pure glycerol unit. The condensation of the glycerol and carbohydrate units was expected to provide the desired phosphatidyl glycerol. This approach was considered to be more suitable to prepare sensitive PtdGlc derivatives. Since mammalian

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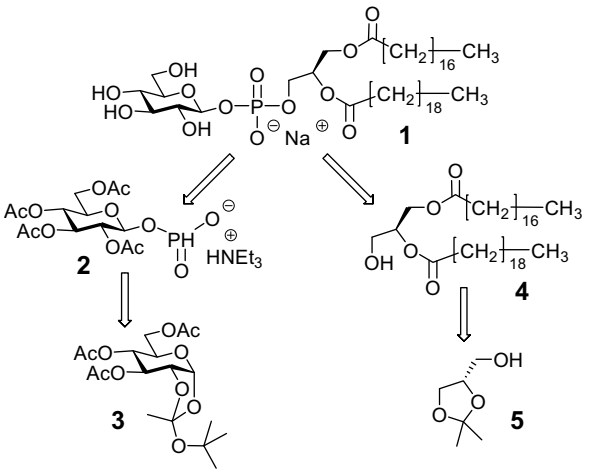


Scheme 1. Synthetic strategy towards PtdGlc (**1**) and its derivatives.

PtdGlc was initially proposed to have a multiple unsaturated arachidonic acid substituent, we selected an acetyl (Ac) group for the protection of the hydroxyl groups.

Herein, we report the first unambiguous syntheses as well as the structural confirmation of naturally occurring mammalian PtdGlc.[2–4]

2. Syntheses

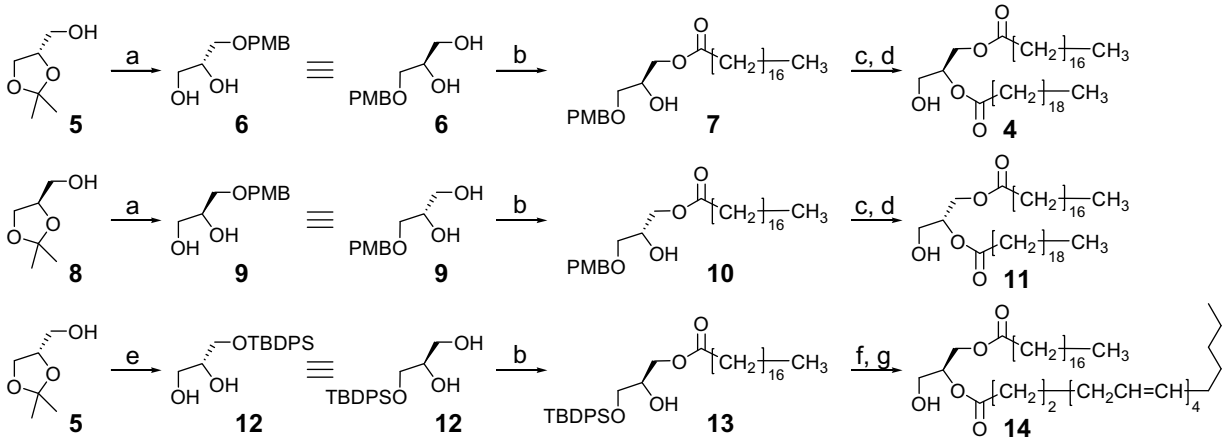
To begin with, glycerol moiety **4** was synthesized from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (**5**; Scheme 2). Thus, protection with *p*-methoxybenzyl (PMB) group was followed by the removal of isopropylidene group to give **6**.[13] Compound **6** was esterified with stearic acid using DCC to give monostearate **7** (63%) together with its regioisomer (8%) and distearate (10%). Further esterification with arachidic acid, followed by DDQ-mediated removal of the PMB protecting group, under carefully controlled conditions, gave glycerol moiety **4**. Its enantiomer **11** was prepared in an identical manner from **8** via **9** and **10**. Diacylglycerol derivatives are prone to ester migration during prolonged reaction as well as during slow chromatographic purification.[14] Thus, short reaction times and rapid flash chromatography over neutral silica gel were essential to successfully suppress the acyl migration.

Initially, preparation of arachidonate carrying glycerol **14** was attempted starting from **7**. However, this approach was abandoned, because conjugated double bonds were not tolerant to DDQ treatment and TBDPS-protected **12** was employed as the starting material. After sequential esterification, the TBDPS group was removed at 0 °C within 1 h to give **14** (46% yield), with 50% of the starting material recovered. More forcing conditions increase the risk of the above-described ester migration considerably.

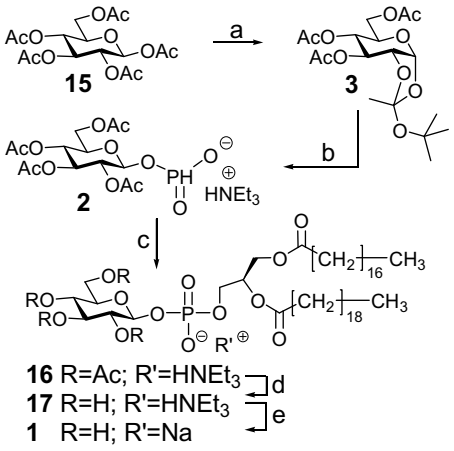
The glucose moiety **2** was prepared via a two-step sequence from the corresponding peracetate **15** (Scheme 3). Firstly, **15** was converted to *t*-butyl orthoester **3** under Lewis acid mediated conditions.[15] The use of DMAP instead of 2,6-lutidine gave superior yields of **3**. Subsequently, autocatalytic reaction with phosphonic acid gave the desired β-phosphonate **2**.[16]

Linkage of the carbohydrate and glycerol moieties was furnished in a unified manner with a 'one-pot' procedure through pivalyl chloride-mediated H-phosphonate activation and subsequent oxidation.[17] Iodine was used as an oxidant, which was shown to be well-compatible with the conjugated double bonds of the arachidonic acid residue, giving **21** in a reasonable yield.

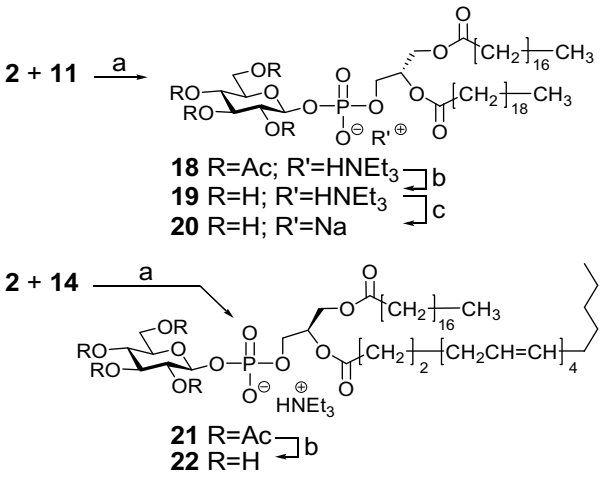
The final deprotection to yield compounds **17**, **19**, and **22** required the selective removal of acetyl groups under conservation of the fatty acid esters. This seemingly challenging strategy was chosen in order to approach **22**, which corresponds to the initially proposed structure of PtdGlc, carrying an arachidonic acid residue.[2] In addition, β-phosphate diesters are poorly tolerant to acidic conditions. Initial attempts to employ a catalytic amount of sodium



Scheme 2. Syntheses of asymmetric glycerol units **4**, **11** and **14**. Reagents and conditions: (a) (1) PMBCl, NaH, TBAI, DMF; (2) Amberlite H^+, $CHCl_3$/MeOH = 2:1 (60% for **6**, 54% for **9**); (b) DCC, DMAP, stearic acid, DCM (63% for **7**, 67% for **10**, 61% for **13**); (c) DCC, DMAP, arachidic acid, DCM (82% on route to **4**, 93% on route to **11**); (d) DDQ, DCM/H_2O = 18:1 (88% for **4**, 83% for **11**); (e) (1) TBDPSCl, imidazole, DCM; (2) Amberlite H^+, $CHCl_3$/MeOH = 2:1 (60%); (f) DCC, DMAP, arachidonic acid, DCM (94%); and (g) TBAF, AcOH, THF, 0 °C (46%).



Scheme 3. Syntheses of carbohydrate unit **2**, linkage and selective deprotection to yield PtdGlc (**1**). Reagents and conditions: (a) $AlCl_3$, DMAP, *t*-BuOH, $CHCl_3$ (89%); (b) (i) H_3PO_3, THF, (ii) NEt_3, 4 °C (55%); (c) (i) **4**, PivCl, pyridine, THF, 0 °C→rt, (ii) I_2, pyridine/H_2O (76%); (d) N_2H_4/HOAc = 4:1, $CHCl_3$/MeOH = 1:2.5 (42%); and (e) Dowex 50WX8, $CHCl_3$/MeOH = 2:1 (94%).



Scheme 4. Linkage and selective deprotection to yield PtdGlc derivatives **20** and **22**. Reagents and conditions: (a) (i) PivCl, pyridine, THF, 0 °C→rt, (ii) I_2, pyridine/H_2O (96% for **18**, 51% for **21**); (b) N_2H_4/HOAc = 4:1, $CHCl_3$/MeOH = 1:2.5 (67% for **19**, 67% for **22**); and (c) Dowex 50WX8, $CHCl_3$/MeOH = 2:1 (94%).

methoxide were futile, showing poor selectivity, while guanidine/guanidinium nitrate[18] exhibited somewhat better selectivity. Most satisfactory was a modified hydrazine mediated deacetylation protocol[5a] yielding the desired products within 5 min in 42–67% yield. The exchange of counter ion from triethylammonium to sodium via Dowex 50WX8 Na^+ form column gave the desired **1** and its stereoisomer **20** in good yields. PtdGlc (**1**[19]) and its derivatives (**17**, **19**, **20**,[20] and **22**[21]) exhibited poor solubility in any organic solvents, for example, chloroform, methanol, DMSO, or ether, as well as in water, thus enabling NMR measurements only in very dilute solutions (Scheme 4).

3. Structure confirmation

The presence of an arachidonyl moiety (**22**) in the natural sample, as initially proposed,[2] could be ruled out, due to the absence of vinylic proton signals around 5.4 ppm. In the case of PtdGlc (**1**) and its diastereomer **20**, proton

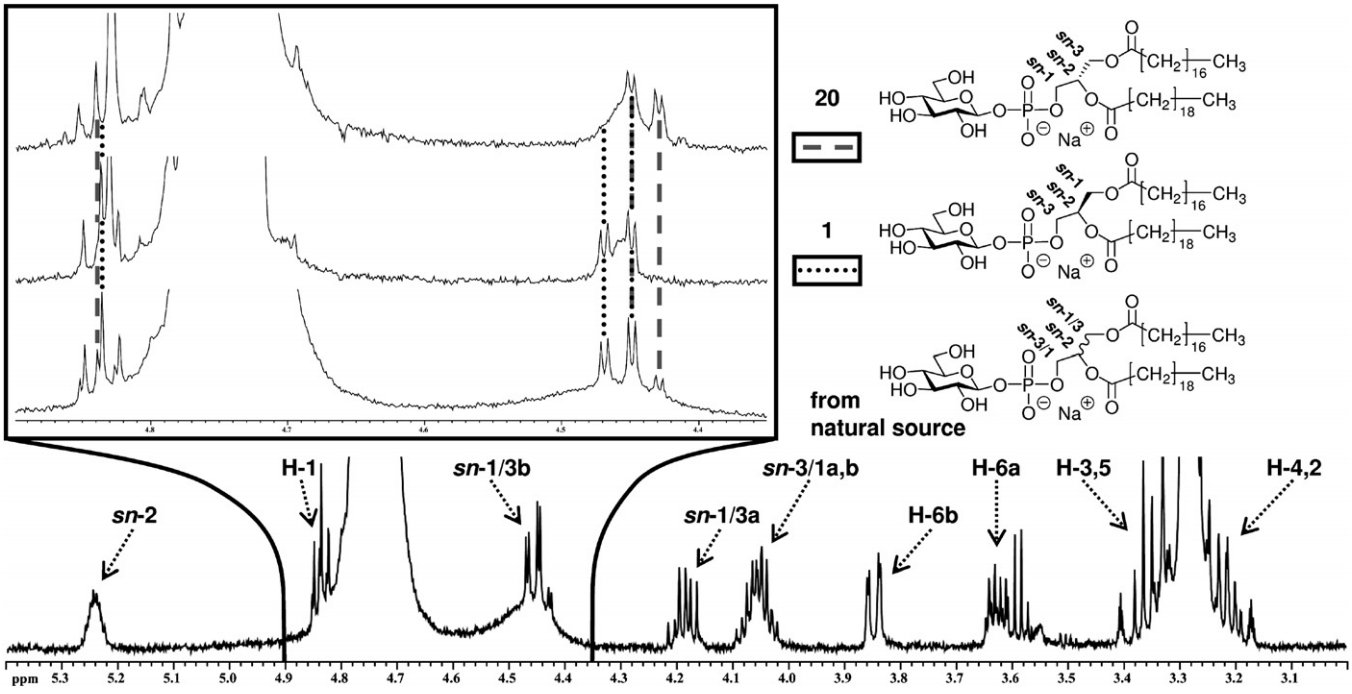


Figure 1. Comparison of NMR data from synthetic PtdGlc diastereomers **1** and **20**, as well as from natural source[4] (spectrum at the bottom) in methanol-d_4.

NMR spectra are virtually identical. The methylene group bearing the primary fatty acid residue, designated *sn*-1/3b and *sn*-1/3a, exhibited the most prominent difference in the chemical shift of about 0.02 ppm, while the difference between the anomeric signals was less substantial. Upon comparison (see Fig. 1) with the previously published natural PtdGlc,[4] similarity of the major constituent from the natural sample with the synthetic compound **1** was readily observed, confirming its stereochemistry. Intriguingly, the minor constituent of the natural sample, about 15%, coincides well with synthetic diastereomer **20**, supporting the hypothesis[4] that natural PtdGlc is a mixture of both diastereomers.

4. Conclusion

Diastereomerically pure PtdGlc and its derivatives were prepared by linkage of β-configured H-phosphonate and asymmetric diacyl glycerol via a longest linear sequence of 7 steps. The preparation of β-H-phosphonate was conducted by the direct conversion of the corresponding orthoester. Further investigations to diversify the carbohydrate as well as the glycerol moiety are currently underway and will be reported in due course.

Comparison of ^{1}H NMR data of PtdGlc (**1**) and its diastereomer **20** suggested the presence of both diastereomers in the natural sample, obtained from rodent brain.[4] As expected, *sn*-1,2-di-*O*-acyl PtdGlc (**1**) represents the major compound in the natural sample.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.036.

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19. *Spectroscopic properties of PtdGlc* (**1**): ^{1}H NMR (600 MHz, MeOH-d_4, 35 °C): $\delta = 5.25$–5.22 (m, 1H; *sn*-2), 4.84 (dd, ^3J (H,P) = 7.6 Hz, $J = 7.6$ Hz, 1H; H-1), 4.46 (dd, $J = 3.0$ Hz, $J = 12.1$ Hz, 1H; *sn*-1b), 4.18 (dd, $J = 6.6$ Hz, $J = 12.1$ Hz, 1H; *sn*-1a), 4.08 (ddd, $J = 5.7$ Hz, ^3J (H,P) = 5.7 Hz, $J = 11.3$ Hz, 1H; *sn*-3b), 4.04 (ddd, $J = 5.5$ Hz, ^3J (H,P) = 5.5 Hz, $J = 11.1$ Hz, 1H; *sn*-3a), 3.84 (dd, $J = 2.0$ Hz, $J = 12.1$ Hz, 1H; H-6b), 3.64 (dd, $J = 6.0$ Hz, $J = 12.1$ Hz, 1H; H-6a), 3.37 (dd, $J = 9.1$ Hz, $J = 9.1$ Hz, 1H; H-3), 3.35–3.34 (m, 1H; H-5), 3.26 (dd, $J = 9.6$ Hz, $J = 9.6$ Hz, 1H; H-4), 3.22 (dd, $J = 8.1$ Hz, $J = 9.1$ Hz, 1H; H-2), 2.32 (t, $J = 7.3$ Hz, 2H; Stea-2), 2.30 (t, $J = 7.6$ Hz, 2H; Ara-2), 1.63–1.56 (n.r., 4H; Stea-3, Ara-3), 1.28 (s, 60H; Stea-4-17, Ara-4-19), 0.89 (t, $J = 7.1$ Hz, 6H; Stea-18, Ara-20); ^{31}P NMR (240 MHz, MeOH-d_4, 35 °C): $\delta = -0.70$ (1P; P); HRMS (ESI-TOF, neg) calcd for $C_{47}H_{90}O_{13}NaP$ $[M-Na]^-$: 893.6119, found: 893.6075.
20. *Spectroscopic properties of* **20**: ^{1}H NMR (600 MHz, MeOH-d_4, 35 °C): $\delta = 5.25$–5.22 (m, 1H; *sn*-2), 4.84 (dd, ^3J (H,P) = 7.1 Hz, $J = 7.6$ Hz, 1H; H-1), 4.44 (dd, $J = 3.0$ Hz, $J = 12.1$ Hz, 1H; *sn*-3b), 4.20 (dd, $J = 7.1$ Hz, $J = 12.1$ Hz, 1H; *sn*-3a), 4.07 (ddd, $J = 5.7$ Hz, ^3J (H,P) = 5.7 Hz, $J = 11.6$ Hz, 1H; *sn*-1b), 4.04 (ddd, $J = 5.7$ Hz, ^3J (H,P) = 5.7 Hz, $J = 11.3$ Hz, 1H; *sn*-1a), 3.84 (dd, $J = 2.0$ Hz, $J = 12.1$ Hz, 1H; H-6b), 3.65 (dd, $J = 5.5$ Hz, $J = 12.1$ Hz, 1H; H-6a), 3.37 (dd, $J = 9.1$ Hz, $J = 9.1$ Hz, 1H; H-3), 3.34–3.34 (m, 1H; H-5), 3.25 (dd, $J = 9.6$ Hz, $J = 9.8$ Hz, 1H; H-4), 3.22 (dd, $J = 8.1$ Hz, $J = 9.1$ Hz, 1H; H-2), 2.32 (t, $J = 7.1$ Hz, $J = 7.6$ Hz, 2H; Stea-2), 2.30 (t, $J = 7.1$ Hz, 2H; Ara-2), 1.62–1.57 (n.r., 4H; Stea-3, Ara-3), 1.28 (s, 60H; Stea-4-17, Ara-4-19), 0.89 (t, $J = 6.8$ Hz, 6H; Stea-18, Ara-20); ^{31}P NMR (240 MHz, MeOH-d_4, 35 °C): $\delta = -0.70$ (1P; P); HRMS (ESI-TOF, neg) calcd for $C_{47}H_{90}O_{13}NaP$ $[M-Na]^-$: 893.6119, found: 893.6117.
21. *Spectroscopic properties of* **22**: ^{1}H NMR (600 MHz, $CDCl_3$/MeOH-d_4 = 2:1, 25 °C): $\delta = 5.42$–5.32 (n.r., 8H; Ara-DB), 5.28–5.25 (n.r., 1H; *sn*-2), 4.85 (dd, $J = 7.6$ Hz, ^3J (H,P) = 7.6 Hz, 1H; H-1), 4.46 (dd, $J = 3.0$ Hz, $J = 12.1$ Hz, 1H; *sn*-1b), 4.20 (dd, $J = 6.6$ Hz, $J = 12.1$ Hz, 1H; *sn*-1a), 4.09–4.04 (m, 2H; *sn*-3a/b), 3.88 (dd, $J = 2.5$ Hz, $J = 12.1$ Hz, 1 H; H-6b), 3.67 (dd, $J = 6.0$ Hz, $J = 12.1$ Hz, 1H; H-6a), 3.43 (dd, $J = 9.1$ Hz, $J = 9.1$ Hz, 1H; H-3),

3.39 (ddd, $J = 2.5$ Hz, $J = 6.6$ Hz, $J = 9.4$ Hz, 1H; H-5), 3.31 (dd, $J = 9.1$ Hz, $J = 9.6$ Hz, 1H; H-4), 3.30 (dd, $J = 8.1$ Hz, $J = 9.1$ Hz, 1H; H-2), 3.14-3.13 (n.r., 6H; NEt_3), 2.86–2.81 (m, 6H; Ara-7,Ara-10,Ara-13), 2.36 (t, $J = 7.6$ Hz, 2H; Ara-2), 2.31 (t, $J = 7.6$ Hz, 2H; Stea-2), 2.13 (dt, $J = 6.6$ Hz, $J = 7.1$ Hz, 2H; Ara-4), 2.07 (dt, $J = 7.1$ Hz, $J = 7.6$ Hz, 2H; Ara-16), 1.74–1.67 (m, 2H; Ara-3), 1.62–1.58 (m, $J = 7.1$ Hz, 2H; Stea-3), 1.40–1.35 (m, $J = 7.3$ Hz, 2H; Ara-17), 1.33 (t, $J = 7.6$ Hz, 9H; NEt_3), 1.27 (n.r., 32H; Stea-4-17, Ara-18-19), 0.90 (t, $J = 7.1$ Hz, 3H; Ara-20), 0.89 (t, $J = 7.1$ Hz, 3H; Stea-18); ^{31}P NMR (240 MHz, $CDCl_3$/MeOH-d_4 = 2:1, 25 °C): $\delta = -0.66$ (1P; P); HRMS (ESI-TOF, neg) calcd for $C_{53}H_{98}NO_{13}P$ $[M-HNEt_3]^-$: 885.5493, found: 885.5463.