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Catalyst-dependent syntheses of phosphatidylinositol-5-phosphate–DiC8 and its enantiomer

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ABSTRACT

Peptide-based catalysts have been applied to the enantioselective syntheses of the title compounds, with this being the first report of the synthesis of an *ent*-PI5P analogue. The key steps in the synthesis involve asymmetric phosphorylation catalysis. Additional maneuvers were developed with a protecting groups scheme that enabled efficient, streamlined syntheses of these important mediators of biochemical events.

1. Introduction

Phosphatidylinositol (**1**) and its variously phosphorylated analogues have been identified as tremendously important mediators of biochemical processes.[1] Among the monophosphates, phosphatidylinositol-5-phosphate (PtdIns-5P or 'PI5P', **2**) has gained significant attention (Fig. 1). Recent reports of cellular processes regulated by this agent are emerging, and its role in biological functions have only started to be appreciated. Among the examples, PI5P has been associated as a regulator of chromatin modification through its interaction with the tumor suppressor protein ING2.[2] It has also been implicated as a regulator of cell cycle progression,[3] as a modulator of gene expression,[4] and as an activator of signal transduction pathways.[1,5] Its role in living systems reaches beyond mammalian cells as it has been identified to play a role in the osmotic-stress response in plants.[6] Limited investigations of PI5P have identified it as a critical regulator of various cellular functions, however, further biochemical exploration is needed for our further understanding of these processes.

Synthetic chemistry has played a defining role in developing the chemical biology of PtdIns science.[7] The synthetic achievements in the field have yielded access to a number of important members of this family of targets.[8–11,14] The chiral pool has provided a common starting place for many of the synthetic approaches that deliver single-enantiomer compounds. The biomimetic approaches of Prestwich in particular have afforded many members of this class, as well as PI-based probes of PI-dependent biological events.[12] The dipalmitoyl-analogue of PI5P (PI5P–DiC16) has been previously synthesized by Prestwich[13] and Falck[14] as the natural enantiomer. Prestwich synthesized the inositol core via a Ferrier rearrangement of methyl α-D-glucopyranoside, while Falck relied on the diastereomeric separation of a camphor ketal intermediate. In addition, Watanabe[15] has synthesized the racemic form of PI5P–DiC16 directly from the *meso*-*myo*-inositol core.

Our own interest in these compounds stems from the synthetic challenge in developing rapid, streamlined syntheses of these molecules, and difficult-to-access analogues, such that high precision biochemical studies may be undertaken. In particular, we have reported enantioselective synthesis of phosphatidylinositol-3-phosphate,[16] phosphatidylinositol-3,5-bis(phosphate),[17] several deoxygenated analogues of these compounds, and in selected cases, enantiomeric versions that may serve as biological probes. In each of these syntheses, we capitalized on the power of desymmetrization catalysis to enter a chosen enantiomeric series (Scheme 1).

As shown in Scheme 1, catalysts **3** and **4** have emerged as workhorses in our laboratory for the rapid syntheses of compounds in the PtdIns family. Initially, we targeted enantioselective synthesis of inositol-1-phosphate, and its enantiomer inositol-3-phosphate. As shown in Scheme 1, rapid syntheses were indeed possible as a function of the two catalysts we discovered for these purposes.[18,19] Herein, we report the application of chiral catalysts **3** and **4** to the enantioselective syntheses of PI5P as well as *ent*-PI5P. Catalyst-dependent syntheses of these particular phosphatidylinositol-monophosphates have not yet been reported, and thus provide a new entry into each enantiomeric series. Efficient synthetic routes to these compounds could allow for further biochemical investigation of these important cellular messengers in an effort to elucidate their biological roles.

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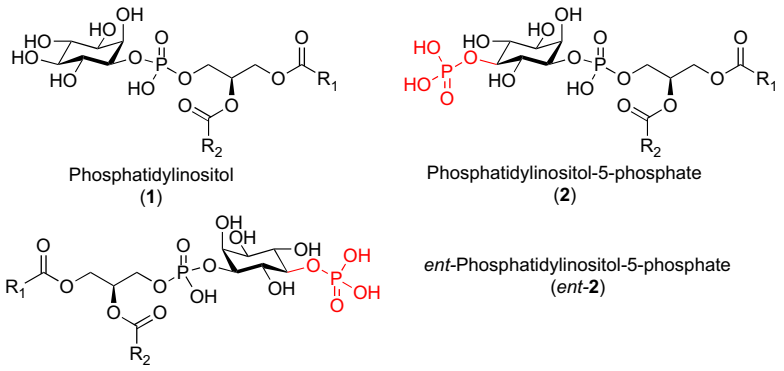
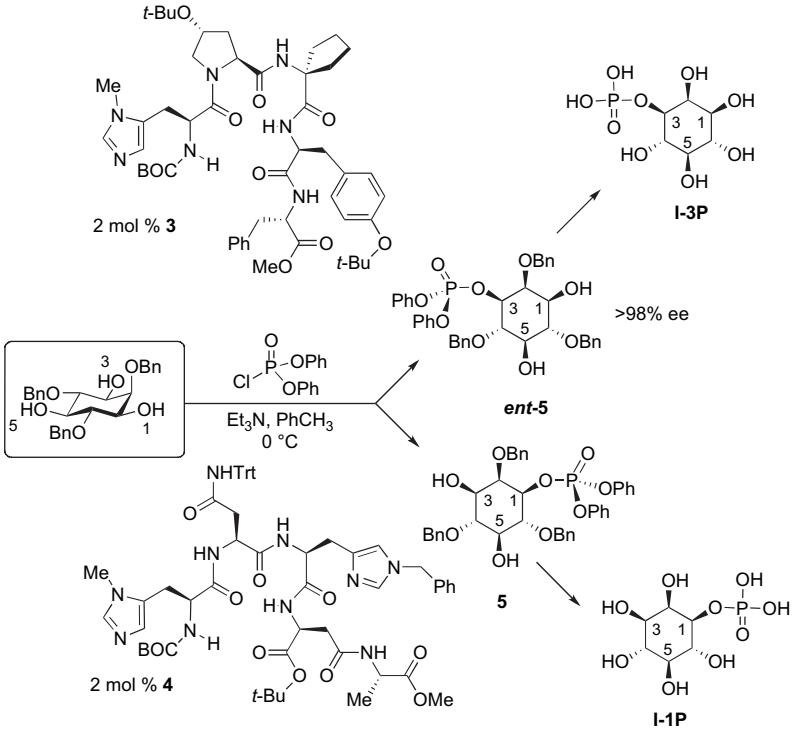


Figure 1. Phosphatidylinositol and its 5-phosphorylated variants.



Scheme 1.

2. Results and discussion

Peptide-based asymmetric catalysis provided direct routes to desymmetrized phosphoinositols **5** and *ent*-**5** and we envisioned manipulating these enantiomers to synthesize each PI5P and *ent*-PI5P, with dioctanoyl side chains, in an optically pure fashion (Fig. 2). The synthesis of these phosphatidylinositide derivatives bears inherent challenges due to the decreased reactivity toward phosphorylation of the C5 hydroxyl, in comparison to the C3 hydroxyl. Thus, retrosynthetically the C5 phosphate could be prepared from protected derivative **6**, which could be obtained through a selective protection (PG) of the C3 hydroxyl (Fig. 2). The glycerol fragment could be installed through the previously developed reaction for these systems of the corresponding phosphoric acid of **7** and alcohol **8** under Mitsunobu-type conditions.[16] Inositol derivative **7** can be readily derived from **5** through a previously developed three step sequence to convert the phenyl phosphate ester groups to benzyl phosphate ester groups.[16]

Large-scale production of both **5** and *ent*-**5**, utilizing selective catalysts **4** and **3**, respectively, provided a suitable starting point for the synthesis of each single-enantiomer PI5P compound (Scheme 1). As previously reported, it was necessary to exchange the phenyl phosphate ester groups to benzyl phosphate ester groups to provide an efficient final deprotection of the desired compounds. Thus the two remaining hydroxyl groups of **5** were protected as the silyl ethers followed by transesterification and subsequent deprotection to provide intermediate **7** (Scheme 2).

With late stage intermediate **7** in hand, our synthetic plan could either first install the glycerol functionality and then protect the C3 hydroxyl or vice versa. In the examination of the Mitsunobu reaction, it was intriguing to recount the reactions of **7** and deoxy-analogue **10**, using identical conditions.[16] Both analogues underwent monohydrolysis under basic conditions to deliver the corresponding phosphoric acids[20] followed by reaction with **8** employing Mitsunobu-type conditions to obtain phosphatidylinositol derivatives **9** and **11** (Scheme 3).[16] The yields for these two processes are remarkably different, with the deoxy-compound produced in 89% yield compared to 40% for **9**. Further, the purification of **9** was also difficult as a result of poor separation of the DEAD byproduct that is produced in the reaction. The requirement for multiple chromatographic

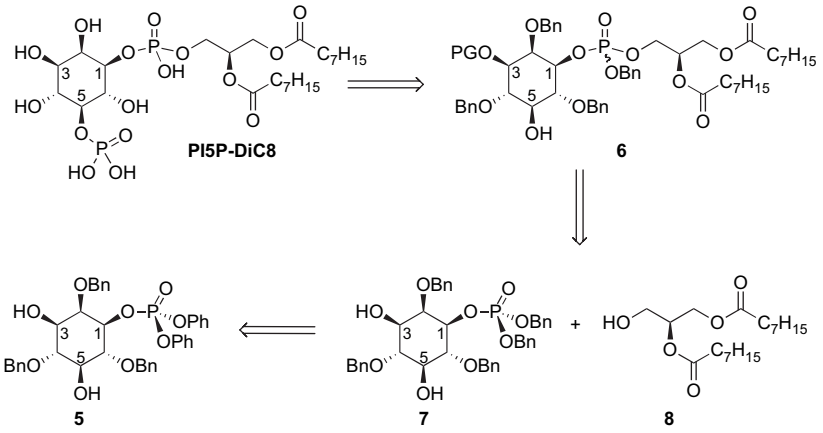
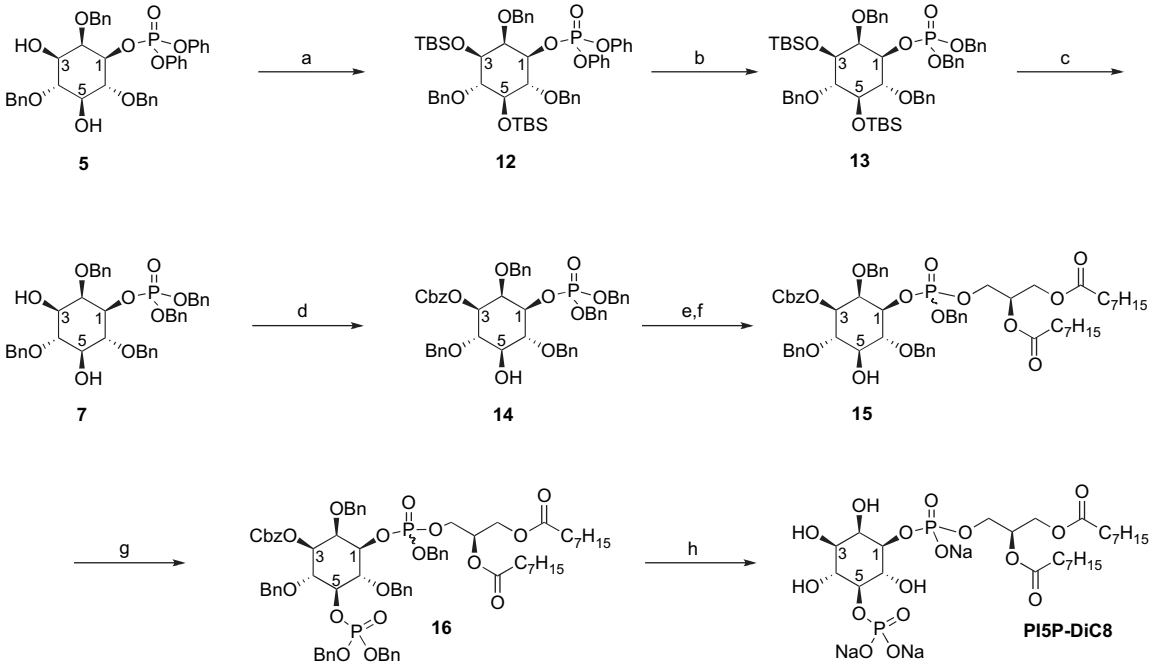


Figure 2. Retrosynthetic analysis for an enantiomeric series of PI5P–DiC8.

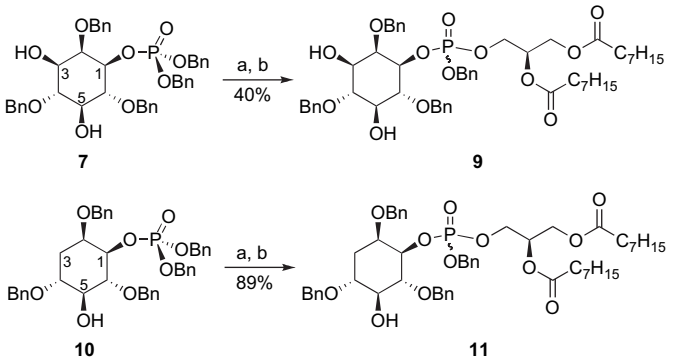
procedures could have contributed to the decreased isolated yield of the Mitsunobu product. Based on this evidence, we suspected that the relatively solvent exposed C3 hydroxyl may be impeding the reaction. Thus, masking this functionality could lead to increased yield of the desired product. As a result, our synthetic strategy evolved in the direction of protecting the C3 hydroxyl followed by subsequent installation of the glycerol fragment.

Two issues guided the choice of protecting group for the C3 hydroxyl. First, it was desirable to have one set of global deprotection conditions as the final synthetic step. Therefore the protecting group to be employed should be removable under previously developed hydrogenolysis conditions. Second, the protection needed to be selective for the C3 hydroxyl over the C5 hydroxyl. Initially, we attempted protection of the alcohol as the benzyl ether. We turned our attention toward Dudley's benzylation conditions, which uses MgO as a mild base.[21] These conditions were chosen with the thought that they should be sufficiently mild in order to preclude phosphate migration. The use of strong bases, such as NaH in the presence of benzyl bromide, was avoided as it was expected to cause racemization of **7** through a migration event. The reaction utilizing Dudley's reagent was sluggish with the use of toluene at 80 °C and provided only trace amounts of the desired product after 12 h. Dudley reports the reaction to be more efficient with the use of α,α,α-trifluorotoluene as a solvent, and thus we examined these conditions. These conditions proved to be more reactive, however, even at 80 °C for 3 days with 4 equiv of both the benzylating reagent and MgO, the desired product was obtained in only 18% yield in a 1:2:1 mixture of benzylation products (3,5-OBn/3-OBn/5-OBn). The poor selectivity and yield of the reaction was consistent with our previous experience of low site-selectivity for alkylation reactions when comparing the reactivity of the C1 or C3 hydroxyl with the more hindered C5 hydroxyl.

As previously mentioned, these two positions have shown remarkable differences in reactivity for the phosphorylation reaction.[18] Therefore, we envisioned protecting the C3 hydroxyl with a benzyloxycarbonyl, which should provide a more selective reaction as it proceeds through a tetrahedral intermediate, similar to the phosphorylation reaction. In addition, this group is removable under



Scheme 2. Reagents and conditions: (a) TBSCl, imidazole, DMF, 89%; (b) NaH, BnOH, THF, 84%; (c) HF–pyridine, THF, 86%; (d) CbzCl, Et_3N, cat. DMAP, CH_2Cl_2, 75%; (e) LiBr, acetone, reflux; then DOWEX 50×2-200; (f) DEAD, Ph_3P, **8**, THF, 74%, two steps; (g) 4,5-dicyanoimidazole, dibenzyl diisopropylphosphoramidite, CH_2Cl_2; then 30% H_2O_2/H_2O, 85%; (h) $Pd(OH)_2/C$, H_2, *t*-BuOH/H_2O (5:1), Chelex 100 (sodium form), 61%.



Scheme 3. Reagents and conditions: (a) LiBr, acetone, reflux; then DOWEX 50×2-200; (b) DEAD, Ph_3P, **8**, THF.

the global hydrogenolysis deprotection conditions. As predicted, the 3-OCbz-inositol derivative **14** was delivered in good yield with no detection of the 5-OCbz regioisomer. This reaction can be catalyzed by either *N*-methyl-imidazole (NMI), or 4-dimethylaminopyridine (DMAP), and shows a strong dependence on a 1.67:1 molar ratio between benzyl chloroformate and triethylamine. Protected derivative **14** was then subjected to monodebenzylation conditions and subsequent Mitsunobu reaction with glycerol fragment **8** to provide **15** as a mixture of diastereomers at the phosphorus atom. The Mitsunobu reaction with this derivative proceeded in 74% yield, which was a significant increase from the unprotected C3 hydroxyl derivative, which gave only 40% of the desired product under identical conditions. The Cbz protection may have further contributed to the yield of the reaction, as **15** was more easily separated from the many components of the reaction when compared to adduct **9**.

From **15**, the free hydroxyl at C5 of the *myo*-inositol core was subjected to coupling with dibenzyl diisopropylphosphoramidite under standard conditions to install the phosphate and provide fully protected PI5P–DiC8, **16** in excellent yield. Deprotection of **16** employing previously optimized hydrogenolysis conditions with Pearlman's catalyst (palladium hydroxide on carbon) and Chelex 100 sodium form resin under a hydrogen atmosphere in a water/*tert*-butanol mixture proceeded well to provide PI5P–DiC8 as the sodium salt. On occasion, an unidentified impurity was present in the sample, manifested by a large singlet at 1.91 ppm in the ^1H NMR. The presence of this contaminant at this stage has sporadically been a problem in previous synthesis of other phosphoinositide and phosphatidylinositide derivatives. However, exhaustive washing of Pearlman's catalyst prior to use, typically allows the global deprotection to proceed to deliver a pure product. Moreover, size exclusion chromatography proved effective for isolation of analytically pure PI5P–DiC8. In preparative runs, the deprotection sequence may be carried out, with passage of the product through a size exclusion column, to allow for the isolation of analytically pure PI5P–DiC8 in 61% yield, from the fully protected derivative. As a testimony to the robustness of this streamlined synthetic sequence, the entire synthetic procedure was repeated, starting from *ent*-**5**, to provide the unnatural enantiomer, *ent*-PI5P–DiC8, with comparable yields at every step.

3. Conclusion

In summary, we have completed the total syntheses of both the natural and unnatural enantiomers of PI5P with dioctanoyl side chains on the glycerol fragment. Following the catalytic desymmetrization of a central *myo*-inositol derivative, the key synthetic steps employed are the Mitsunobu coupling reaction and the use of a benzyloxycarbonyl protecting group. The use of the Cbz group provided selective protection of the C3 hydroxyl over the C5 hydroxyl of the inositol ring. The route employed in our synthesis was highly efficient and provided both enantiomers in their optically pure form, thus providing facile access to either a PI5P, or an *ent*-PI5P analogue (*ent*-PI5P–DiC8), from a common starting material. PI5P is a newly emerging member of the phospholipid family and its synthesis is essential toward the identification of its role in cellular processes. These syntheses provide rapid, efficient access to these molecules thus enabling the further exploration and identification of novel cellular functions of not only the natural enantiomer, but also the unnatural enantiomer, which could play alternate cellular roles.

4. Experimental

4.1. General

Proton NMR spectra were recorded on 400 or 500 MHz spectrometer. Proton chemical shifts are reported in parts per million (δ) relative to internal tetramethylsilane (TMS, δ 0.0 ppm) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26 ppm; CD_3OD, δ 3.31 ppm; D_2O, δ 4.79 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration). Carbon NMR spectra were recorded on 400 or 500 MHz spectrometer with complete proton decoupling. Carbon chemical shifts are reported in parts per million (δ) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ 77.0 ppm) except for D_2O in which case a drop of methanol was added as internal standard (CH_3OH, δ 49.5 ppm).[22] Phosphorous NMR spectra were recorded on 400 (162 MHz) or 500 (202 MHz) spectrometer with complete proton decoupling. Phosphorous chemical shifts are reported in parts per million (δ) relative to a 85% H_3PO_4 external standard. NMR data were collected at 25 °C, unless otherwise indicated. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F_{254} precoated plates (0.25 mm thickness). TLC R_f values are reported. Visualization was accomplished by irradiation with an UV lamp and/or staining with $KMnO_4$ or ceric ammonium molybdenate (CAM) solutions. Flash column chromatography was performed using Silica Gel 60 Å (32–63 μm).[23] Optical rotations were recorded at 20 °C at the sodium D line (path length 1.0 cm). High resolution mass spectra were obtained at institutional providers. The method of ionization is given in parentheses.

Analytical and preparative reverse phase and normal phase HPLC were performed employing a single wavelength UV detector (214 nm or 254 nm). Measurements of enantiomeric excess were carried out via analytical normal phase HPLC equipped with a diode array detector (214 nm and 254 nm) and employing a Chiralcel® OD column at a flow rate of 0.5 mL/min. All reactions were carried out under an argon or nitrogen atmosphere employing oven- and flame-dried glassware. All solvents were distilled from appropriate drying agents prior to use. Compounds **7** and *ent*-**7** were synthesized from peptide catalyzed phosphorylation and transesterification reactions.[16,17]

4.2. Specific procedures

*4.2.1. Compound **14***

To a stirred solution of **7** (190 mg, 0.267 mmol) in CH_2Cl_2 (0.534 mL) was added 4-(dimethylamino)pyridine (DMAP) (33 mg, 0.267 mmol) followed by triethylamine (0.112 mL, 0.801 mmol). Benzyl chloroformate (0.188 mL, 1.337 mmol) was then added quickly. The reaction solution was bubbled and a precipitate formed. The reaction mixture stirred at room temperature under a nitrogen atmosphere for 12 h. The reaction was then diluted with CH_2Cl_2 (50 mL) and the organic layer was washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried

(Na_2SO_4), filtered, and concentrated in vacuo to afford a yellow oil. This crude material was then purified by column chromatography (0–45% ethyl acetate/hexanes) to afford **14** as a clear thick oil (170 mg, 75%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.28–7.12 (m, 30H), 5.04 (ABq, J=12.1 Hz, 2H), 4.93–4.86 (m, 4H), 4.70 (ABq, J=11.2 Hz, 2H), 4.64–4.56 (m, 5H), 4.23–4.20 (m, 2H), 3.83 (td, J=9.5 and 2.3 Hz, 2H), 3.47 (td, J=9.2 and 2.3 Hz, 1H), 2.37 (d, J=2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 154.4, 138.3, 138.2, 138.1, 135.6, 135.6, 135.1, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.5, 79.6, 79.6, 78.9, 77.8, 77.8, 75.3, 75.2, 75.2, 74.4, 74.4, 69.8, 69.5, 69.5, 69.4, 69.3; ^{31}P NMR ($CDCl_3$, 202 Hz) δ −1.7; IR (film, cm^{-1}) 3387, 3089, 3064, 3032, 2946, 2880, 1744, 1495, 1450, 1262, 1013; TLC R_f 0.41 (40% ethyl acetate/hexanes); exact mass calcd for $[C_{49}H_{49}O_{11}P]^+$ requires m/z 845.3085, found 845.3064 (ESI$^+$); $[\alpha]_D$ +26.4 (c 1.0, $CHCl_3$).

4.2.2. Compound *15*

To a stirred solution of **14** (0.185 g, 0.219 mmol) in 14 mL of acetone (reagent grade) was added LiBr (0.035 g, 0.408 mmol), and the reaction mixture was refluxed for 10 h. It was then cooled to room temperature and concentrated. The residue was purified by silica gel chromatography eluting with 50% EtOAc/hexanes to 15% CH_3OH/CH_2Cl_2 to give the crude product as a lithium salt. The salt was dissolved in a minimal amount of CH_3OH and run through a H^+ DOWEX 50×2-200 ion exchange column. Fractions containing the desired product were combined and concentrated to give the crude phosphoric product. The crude residue was then dissolved in THF (1.1 mL), and diacylglycerol **8** (0.151 g, 0.437 mmol) and triphenylphosphine (0.115 g, 0.437 mmol) were added and the reaction was cooled to 0 °C. DEAD (68 μL, 0.437 mmol) was then added and the reaction mixture was stirred at 0 °C under N_2 for 48 h. The mixture was then concentrated under reduced pressure and purified by silica gel chromatography eluting with 0–40% EtOAc/hexanes to yield **15** as a colorless thick oil (0.165 g, 74%, over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29–7.15 (m, 25H), 5.07–4.88 (m, 5H), 4.76–4.57 (m, 7H), 4.28–3.81 (m, 8H), 3.50 (m, 1H), 2.48 (s, 1H), 2.17–2.10 (m, 4H), 1.48–1.46 (m, 4H), 1.18–1.15 (m, 16H), 0.79–0.77 (m, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 173.0, 173.0, 172.6, 154.3, 154.3, 138.2, 138.2, 138.1, 138.0, 137.9, 135.4, 135.4, 135.4, 135.3, 135.0, 135.0, 128.7, 128.5, 128.3, 128.2, 128.2, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 79.5, 79.4, 79.4, 78.9, 78.9, 77.9, 77.9, 77.8, 77.8, 77.8, 77.2, 76.8, 75.3, 75.2, 75.1, 74.4, 69.8, 69.7, 69.6, 69.5, 69.5, 69.2, 69.1, 65.7, 65.6, 65.4, 65.4, 61.5, 61.4, 34.0, 34.0, 33.9, 31.6, 29.0, 28.9, 28.8, 24.7, 24.7, 22.5, 14.0; ^{31}P NMR ($CDCl_3$, 162 Hz) δ −1.7, −1.8; IR (film, cm^{-1}) 3026, 2951, 2925, 2849, 1738, 1456, 1260, 1158, 1113, 1022; TLC R_f 0.21 (30% ethyl acetate/hexanes); exact mass calcd for $[C_{61}H_{78}O_{15}P]^+$ requires m/z 1081.5073, found 1081.5035 (ESI$^+$); $[\alpha]_D$ +11.6 (c 1.0, $CHCl_3$).

4.2.3. Protected PI5P–DiC8 (*16*)

To a stirred solution of **15** (0.100 mg, 0.092 mmol) in CH_2Cl_2 (18.5 mL) was added dibenzyl diisopropylphosphoramidite (311 μL, 0.92 mmol) followed by 4,5-dicyanoimidazole (0.131 g, 1.109 mmol). The reaction was stirred at room temperature under a nitrogen atmosphere for 15 h. The reaction was then cooled to 0 °C and 30% H_2O_2 (8.6 mL) was added. The reaction was stirred at 0 °C for 1 h at which time the reaction was quenched with saturated Na_2SO_3 (~70 mL) until no peroxides were detected via starch paper. The reaction was then extracted with DCM (3×50 mL) and the organic layers were combined, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting residue was then purified by silica gel column chromatography (0–38% ethyl acetate/hexanes) and a second column (70% diethyl ether/hexanes) to afford pure **16** as a thick oil (0.105 g, 85%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.315–7.32 (m, 31H), 6.98–6.94 (m, 4H), 4.98 (s, 2H), 4.94–4.61 (m, 14H), 4.45–4.15 (m, 3H), 4.04–3.72 (m, 6H), 2.17–2.09 (m, 4H), 1.51–1.44 (m, 4H), 1.23–1.15 (m, 16H), 0.82–0.77 (m, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 173.0, 172.7, 172.6, 154.2, 154.2, 138.0, 138.0, 137.9, 137.8, 135.9, 135.9, 135.8, 135.8, 135.4, 135.4, 135.3, 135.3, 134.9, 134.9, 128.6, 128.6, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 79.9, 79.8, 78.2, 78.2, 78.2, 78.1, 77.6, 77.5, 77.5, 77.5, 77.2, 76.6, 76.5, 76.3, 76.2, 75.4, 75.3, 74.7, 74.7, 69.9, 69.9, 69.8, 69.8, 69.6, 69.6, 69.3, 69.3, 69.2, 69.2, 69.2, 69.1, 65.8, 65.7, 65.6, 65.5, 61.4, 61.4, 34.0, 34.0, 33.9, 31.6, 29.0, 29.0, 28.9, 24.8, 24.7, 22.6, 14.0; ^{31}P NMR ($CDCl_3$, 202 Hz) δ −1.6, −1.9, −2.0; IR (film, cm^{-1}); TLC R_f 0.37 (30% ethyl acetate/hexanes); exact mass calcd for $[C_{75}H_{91}O_{18}P_2]^+$ requires m/z 1341.5675, found 1341.5661 (ESI$^+$); $[\alpha]_D$ +8.8 (c 1.0, $CHCl_3$).

4.2.4. PI5P–DiC8

Compound **16** (0.095 g, 0.071 mmol) was dissolved in t-BuOH/H_2O (5:1) (6 mL) and Chelex (Na form) resin was added till a thick slurry. Palladium hydroxide on carbon (0.190 g) was added and the chamber evacuated. The solution was placed under 1 atm of H_2 and stirred for 18 h. The reaction mixture was filtered over a Celite pad and the Celite was washed with ethanol (20 mL), ethanol/water (1:1) (15 mL), and H_2O (15 mL). The filtrate was filtered through a 0.22 μm filter and concentrated under reduced pressure (no heating). The resulting solid was dissolved in a minimal amount of water and run through a Sephadex G-10 size exclusion column eluting with water. Fractions containing pure product were combined and lyophilized. The solid was dissolved in a minimal amount of water and run through a Chelex (Na form) ion exchange column to fully form the desired sodium salt. Fractions containing the desired product were combined and lyophilized to yield the sodium salt of PI5P–DiC8 as a white fluffy solid (0.032 g, 61% yield). ^{1}H NMR (D_2O, 500 MHz) δ 5.32 (q, J=7.7 Hz, 1H), 4.43 (dd, J=12.2 and 1.5 Hz, 1H), 4.27 (dd, J=12.1 and 8.2 Hz, 1H), 4.22 (t, J=2.6 Hz, 1H), 4.08–4.03 (m, 3H), 3.93–3.85 (m, 2H), 3.80 (t, J=9.3 Hz, 1H), 3.62 (dd, J=10.0 and 2.4 Hz, 1H), 2.48–2.30 (m, 4H), 1.64–1.58 (m, 4H), 1.37–1.25 (m, 16H), 0.89–0.85 (m, 6H); ^{13}C NMR (D_2O, 500 MHz) δ 175.8, 175.7, 79.7, 79.7, 76.6, 76.5, 72.5, 71.7, 71.5, 71.5, 71.2, 63.9, 34.8, 34.8, 32.4, 29.7, 29.6, 29.6, 29.5, 25.5, 25.4, 23.2, 23.2, 14.5, 14.4; ^{31}P NMR (D_2O, 202 Hz) δ 4.3, −0.6; exact mass calcd for $[C_{25}H_{49}O_{16}P_2]^+$ requires m/z 667.2496, found 667.2476 (ESI$^+$); $[\alpha]_D$ +2.75 (c 2.0, H_2O, pH 9).

4.2.5. ent-PI5P–DiC8

Synthesis and spectral data were identical to PI5P–DiC8. $[\alpha]_D$ −8.75 (c 2.0, H_2O, pH 8).[24]

Acknowledgements

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