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Streamlined Synthesis of Phosphatidylinositol (PI), PI3P, PI3,5P_2, and Deoxygenated Analogues as Potential Biological Probes

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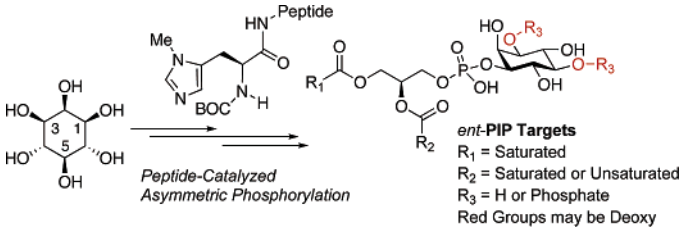

Highly direct total syntheses of phosphatidylinositol (PI), phosphatidylinositol-3-phosphate (PI3P), phosphatidylinositol-3,5-bisphosphate (PI3,5P_2), and a range of deoxygenated versions are reported. Each synthesis is carried out to deliver the target in optically pure form. The key step for each synthesis is a catalytic asymmetric phosphorylation reaction that affects desymmetrization of an appropriate *myo*-inositol precursor. Elaboration to each target compound is then carried out employing a diversity-oriented strategy from the common precursors. In addition to three natural products, several additional streamlined total syntheses of deoxygenated PI analogues are reported. These syntheses set the stage for high-precision biological investigations of polar headgroup/biological target interactions of these membrane-associated signaling molecules.

Introduction

Phosphatidylinositol (PI) and its various phosphorylated derivatives are now known to be central players in signal transduction and are ubiquitous in biochemistry (Figure 1).[1] Atomic resolution-level investigations of their structure−activity relationships would be greatly facilitated by streamlined syntheses in each enantiomeric series.[2] Furthermore, access to analogues where individual hydroxyl groups might be modified or deleted would create new opportunities for chemical biological studies.[3] Numerous synthetic approaches to these molecules have appeared.[4−7] Many strategies have capitalized on the chiral pool for both building blocks as well as chiral auxiliaries.

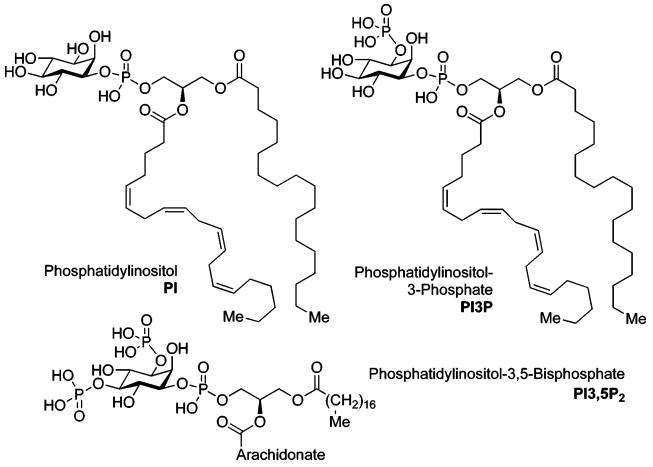

FIGURE 1. Phosphatidylinositol and representative derivative phosphates.

Biomimetic approaches have also inspired elegant approaches to many members of this class, as well as PI-based probes of PI-dependent biological events.[8]

We recently described an alternative synthetic approach based on asymmetric catalysis. In particular, we reported a new method

(1) Irvine, R. F.; Schell, M. J. *Nature Rev. Mol. Cell. Biol.* **2001**, *2*, 327−338.

(2) Prestwich, G. D. *Chem. Biol.* **2004**, *11*, 619−637.

(3) For examples of syntheses of deoxy-PI-type compounds with saturated side chains, see: (a) Wang, D. S.; Chen, C. S. *Bioorg. Med. Chem.* **2001**, *9*, 3165−3172. (b) Hu, Y.; Meuillet, E. J.; Qiao, L.; Berggren, M. M.; Powis, G.; Kozikowski, A. P. *Tetrahedron Lett.* **2000**, *41*, 7415−7418. (c) Andresen, T. L.; Skytte, D. M.; Madsen, R. *Org. Biomol. Chem.* **2004**, *2*, 2951−2957.

(4) For syntheses of PIP-compounds with unsaturated side chains, see: (a) Kubiak, R. J.; Bruziak, K. S. *J. Org. Chem.* **2003**, *68*, 960−968. (b) Gaffney, P. R. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **2001**, 192−205. (c) Watanabe, Y.; Nakatomi, M. *Tetrahedron* **1999**, *55*, 9743−9754.

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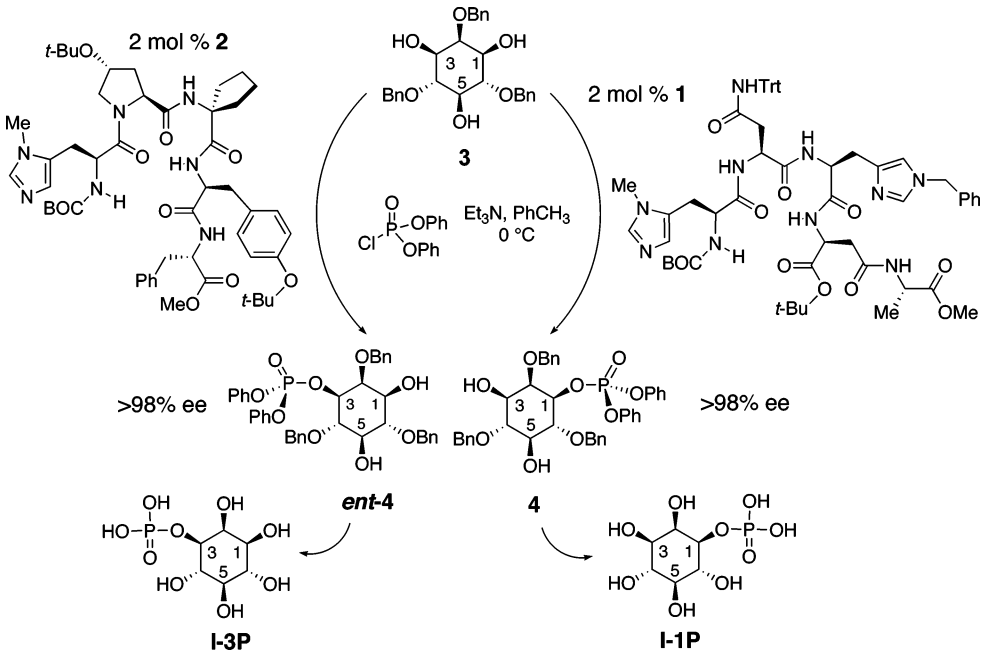
SCHEME 1



for catalytic asymmetric phosphorylation of *myo*-inositol derivatives that led to desymmetrization of starting material.[9] We then developed synthetic methodology such that the product of asymmetric phosphorylation could be elaborated to certain inositol phosphates and deoxygenated derivatives.[10] These compounds were utilized in chemical biological studies that probed the stereospecificity of several inositol monophosphatase enzymes. We also used the asymmetric phosphorylation as a springboard for the synthesis of each enantiomer of phosphatidylinositol-3-phosphate (PI3P), in each optically pure form.[11] We now report new total syntheses in this family of complex phospholipids, culminating in total syntheses of phosphatidylinositol (PI), phosphatidylinositol-3,5-bisphosphate ($PI3,5P_2$), and several key deoxygenated analogues. A key element of the present study is efficient Mitsunobu-type methodology for uniting the lipid portions of these molecules with appropriate polar headgroups. As in our previous studies, both the naturally occurring enantiomers of these phosphoinositides and the corresponding enantiomeric series are accessible in enantiopure form, with equivalent efficiency, based on the choice of catalyst used in the desymmetrization of a key common intermediate. The streamlined synthesis of these compounds sets the foundation for new chemical biological studies of these membrane-associated signaling molecules and their stereospecific interactions with their various biological targets.

Results and Discussion

The major hurdles associated with short, efficient synthesis of the PI-signaling molecules are the management of the six stereogenic hydroxyl groups of *myo*-inositol and the typically long step-counts associated with protecting group manipulations.[12] To address this challenge generally, we began with the development of catalytic, enantioselective phosphorylation catalysts. Specifically, we were able to find complementary pentapeptide catalysts **1** and **2** that enabled enantiospecific syntheses of both inositol-1-phosphate (**I-1P**) and the enantiomeric inositol-3-phosphate, **I-3P** (Scheme 1). Each peptide affected desymmetrization of *meso*-*myo*-inositol precursor **3** such that phosphates **4** and ***ent*-4** could be isolated with total enantiopurity. Each could then be subjected to one-step dissolving metal reductive cleavage of the protecting groups such that highly efficient syntheses of **I-1P** and **I-3P** were in hand.

To extend these studies to syntheses of naturally occurring PIs, we needed to develop a synthetic scheme that was compatible with the presence of the sensitive arachidonate side chain that is typical of the C2-ester of the glycerol moiety in the naturally occurring PI targets. As a result, the original desymmetrizations that we developed with benzyl groups on

(5) For representative syntheses of PI3P-compounds with saturated side chains, see: (a) Morisaki, N.; Morita, K.; Nishikawa, A.; Nakatsu, N.; Fukui, Y.; Hashimoto, Y.; Shirai, R. *Tetrahedron* **2000**, *56*, 2603−2614. (b) Falck, J. R.; Krishna, U. M.; Capdevila, J. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1711−1713. (c) Painter, G. F.; Grove, S. J. A.; Gilbert, I. H.; Holmes, A. B.; Raithby, P. R.; Hill, M. L.; Hawkins, P. T.; Stephens, L. R. *J. Chem. Soc., Perkins Trans. 1* **1999**, 923−936. (d) Chen, J.; Feng, L.; Prestwich, G. D. *J. Org. Chem.* **1998**, *63*, 6511−6522. (e) Wang, D. S.; Chen, C. S. *J. Org. Chem.* **1996**, *61*, 5905−5910. (f) Bruzik, K. S.; Kubiak, R. J. *Tetrahedron Lett.* **1995**, *36*, 2415−2418.

(6) For representative syntheses of $PI3,5P_2$-compounds with saturated side chains, see: (a) Falck, J. R.; Krishna, U. M.; Katipally, K. R.; Capderila, J. H.; Ulug, E. T. *Tetrahedron Lett.* **2000**, *41*, 4271−4275. (b) Han, F.; Hayashi, M.; Watanabe, Y. *Chem. Lett.* **2003**, *32*, 724−725. (c) Han, F.; Hayashi, M.; Watanabe, Y. *Eur. J. Org. Chem.* **2004**, 558−566. (d) Nishikawa, A.; Saito, S.; Hashimoto, Y.; Koga, K.; Shiri, R. *Tetrahedron Lett.* **2001**, *42*, 9195−9198.

(7) For representative syntheses of PI-compounds, see: Watanabe, Y.; Kiyosawa, Y.; Hyodo, S.; Hayashi, M. *Tetrahedron Lett.* **2005**, *46*, 281−284.

(8) Xu, Y.; Lee, S. A.; Kutateladze, T. G.; Sprissa, D.; Shisheva, A.; Prestwich, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 885−897.

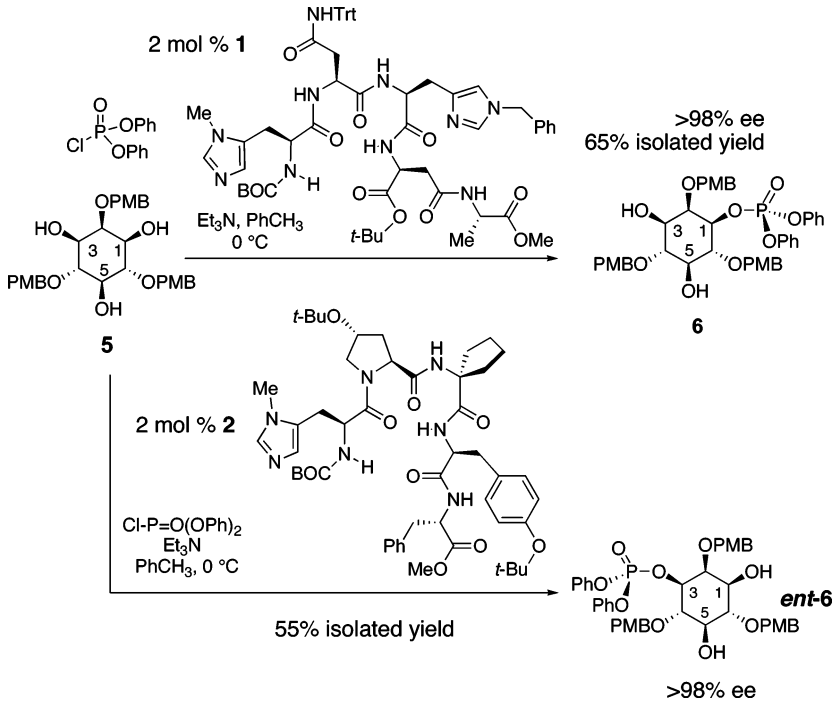
(9) (a) Sculimbrene, B. R.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10125−10126. (b) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 11653−11656. (c) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *Chem. Commun.* **2003**, 1781−1785.

(10) Morgan, A. J.; Wang, Y. K.; Roberts, M. F.; Miller, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 15370−15371.

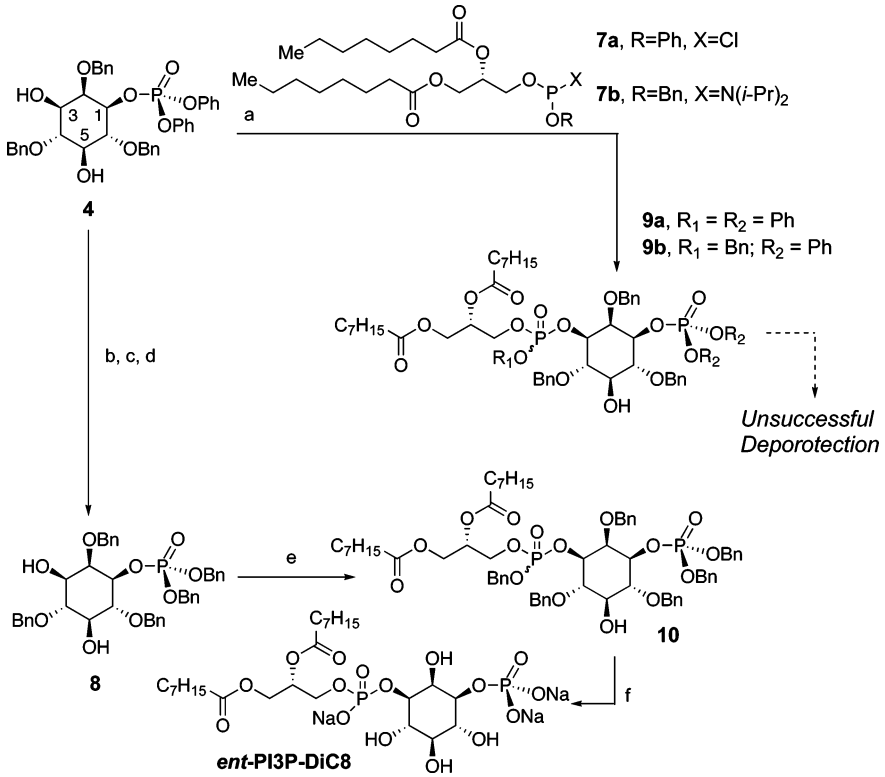
(11) Sculimbrene, B. R.; Xu, Y.; Miller, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 13182−13183.

(12) Gani, D. *Nature* **2001**, *414*, 703−705.

SCHEME 2



SCHEME 3 [a]

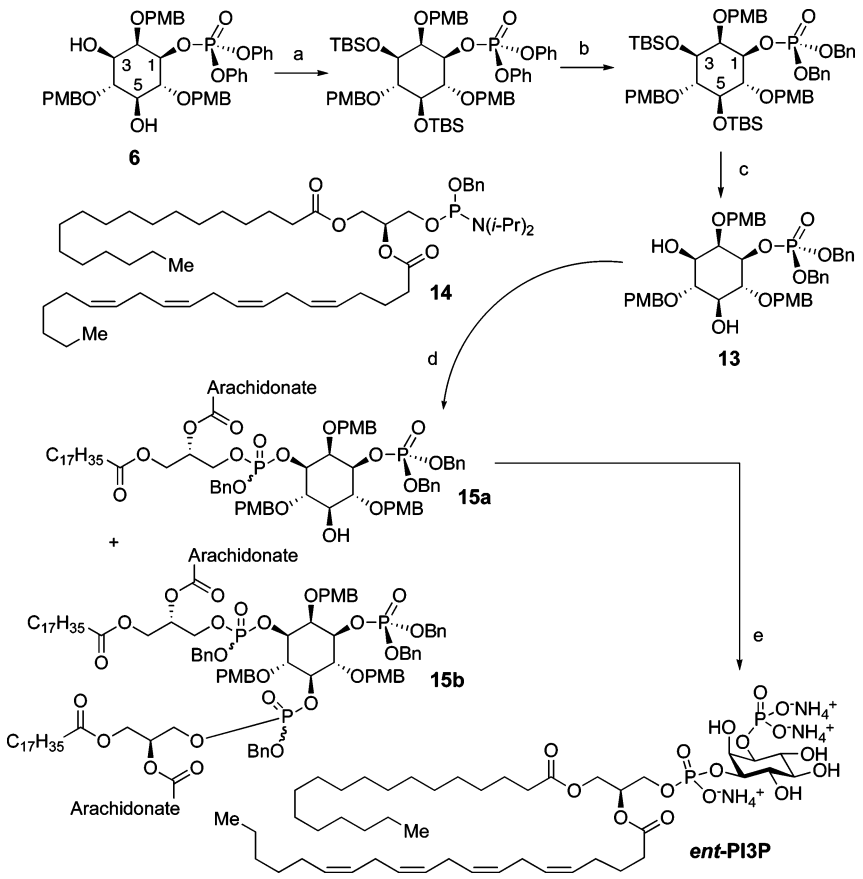


[a] Key: (a) **7a**, Hunig's base, THF, −78 °C; then 30% H_2O_2/H_2O, 31%; (b) TBSCl, imidazole, DMF, 89%; (c) NaH, BnOH, THF, 84%; (d) HF·pyridine, THF, 86%; (e) dicyanoimidazole, toluene/CH_2Cl_2 (1:1), **7b**; then 30% H_2O_2/H_2O; (f) H_2, $Pd(OH)_2/C$, *t*-BuOH/H_2O, $NaHCO_3$, 85%.

the 2-, 4-, and 6-positions of the inositol headgroups were not likely to be compatible with these more involved syntheses. That is, neither hydrogenolytic nor Birch-type cleavage of protecting groups would be compatible with arachidonoyl esters. As a result, we followed the precedent of Bruzik,[13] targeting a final, global deprotection that would be based on trimethylsilyl bromide dealkylation. The strategy requires that the desymmetrization of the *myo*-inositol headgroup be carried out on inositol-

(13) Kubiak, R. J.; Bruzik, K. S. *J. Org. Chem.* **2003**, *68*, 960−968.

SCHEME 4 [a]



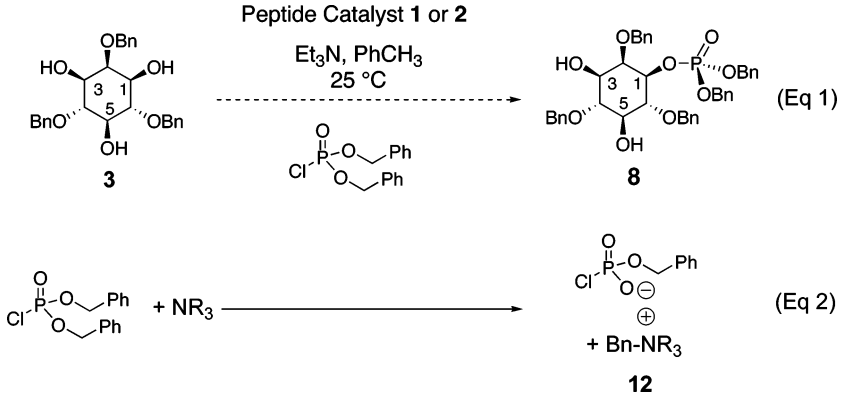
[a] Key: (a) TBSCl, imidazole, DMF, 89%; (b) NaH, BnOH, THF 99%; (c) HF·pyridine, THF, 77%; (d) 4,5-dicyanoimidazole, toluene/CH_2Cl_2 (1:1), **14**; then 30% H_2O_2/H_2O, 39%; (e) TMSBr (20 equiv)/$PhCH_3$, 70 °C; then NH_4OH, 61%.

derived triol **5** (Scheme 2), employing *p*-methoxybenzyl groups instead of benzyl protection. Indeed, the peptide-based phosphorylation catalysts are equivalently effective in the face of this minor change to the inositol structure, and each peptide enables the desymmetrization to be carried out with analogous efficiency.

PI3P and *ent*-PI3P Syntheses. In our initial studies to demonstrate the applicability of these approaches to the natural products and probes of relevance to phosphatidylinositol-dependent signaling, we targeted molecules with both the naturally occurring unsaturated side chains (e.g., arachidonoyl) and side chains that are saturated. We also wished to demonstrate syntheses in each enantiomeric series since the power of asymmetric catalysis translates into access to either optically pure series.[14] Our studies of application of catalytic asymmetric phosphorylation to phosphoinositide commenced with synthesis of PI3P, the product of phosphoinositide-3-kinase (PI3K), an enzyme known to be important in the biochemistry of cell cycle progression.[15]

For PI3P with saturated side chains, we were able to convert either compound **4** or ***ent*-4** to the corresponding target following a route that was compatible with 2,4,6-tribenzyl protection on the inositol. It was necessary, however, to convert the phenyl phosphate ester groups to benzyl phosphate ester groups following a high-yielding three-step sequence (Scheme 3). Compound **4** is, in principle, two steps from the target PI3P with saturated side chains. For example, we showed that we could convert **4** to compound **9a** or **9b** following standard chlorophosphite (**7a**) or phosphoramidite (**7b**) coupling methods.[16] However, we were not able to identify conditions that could efficiently remove the benzyl ether and phenyl phosphate ester protecting groups in one step, without competitive degradation of the material. Instead, we adopted a procedure wherein **4** was temporarily converted to a 3,5-disilylated analogue (TBSCl, imidazole; 89%). Sequential phosphate phenyl-to-benzyl transesterification/desilylation then afforded compound **8** in 72% yield (two steps). Diol **8** could then be subjected to a site-selective P(III)-coupling reaction to deliver protected PI3P analogue **10**.[17] Global hydrogenolysis then delivered *ent*-PI3P with the saturated side chains in 85% yield, after ion exchange. This synthetic plan, despite the phosphate ester exchange sequence, may represent the most efficient access to PI3P extant.

It is possible that an even further degree of streamlining may be achieved if the protecting group swap could be avoided. In this spirit, we have attempted to develop a catalytic asymmetric desymmetrization of **3** or **5** using di*benzyl* phosphochloridate, instead of our standard di*phenyl* phosphochloridate employed in Schemes 1 and 2 (eq 1). Such a transformation would allow conversion of **3** to **8** in a single step, rather than four steps. Unfortunately, when the catalytic P(V) chemistry is attempted under the peptide-catalyzed conditions, di*benzyl* phosphochloridate reacts as a carbon-based S_N2-type electrophile as shown in eq 2.[18] We are continuing to study alternatives to the ester swap through different strategies.



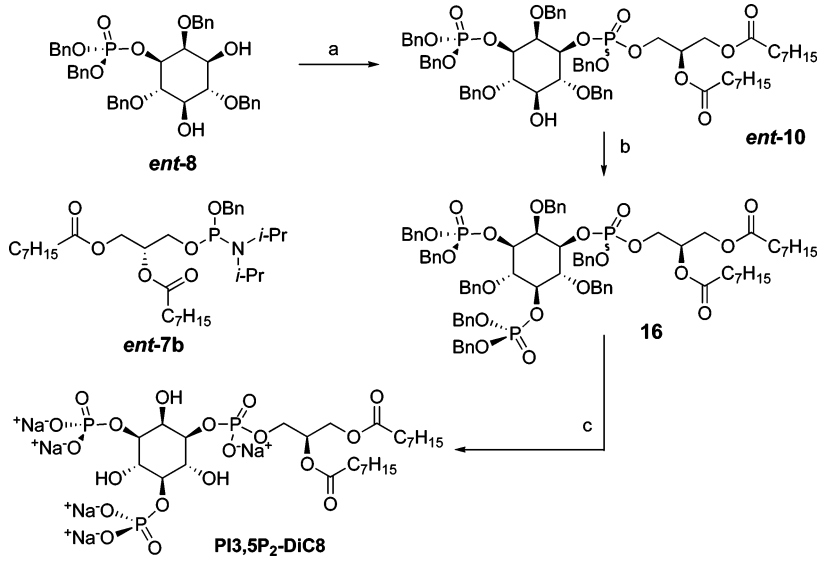
The desymmetrization of **5** also enables synthesis of PI3P with the naturally occurring side chains with unprecedented efficiency. Compound **6** may be converted to **13** through an analogous phosphate ester exchange (Scheme 4). Then, compound **13** may be subjected to a site-selective phosphoramidite coupling with reagent **14** to give **15a** in 39% yield. While the isolated yield is modest, it is a reflection of the difficulty in removing the minor product derived from phosphoramidite coupling at the C5-position of the inositol (**15b**). Since the next step is the final deprotection, and since final purification of phosphoinositides is challenging, it is essential that the penultimate precursor be exceptionally pure prior to deprotection. In fact, treatment of **15** with TMSBr delivers *ent*-PI3P in 61% isolated yield, after ion exchange, in extremely clean fashion.

PI3,5P$_2$ Synthesis. We next turned our attention to the PI3,5P$_2$ targets, once again with an eye to enantiospecific syntheses of targets with either saturated or unsaturated side chains. PI3,5P$_2$ is one of the most recently identified phosphoinositidyl bisphosphates, and it is implicated in a myriad of membrane-associated biochemical events.[19] As a result of our PI3P syntheses, we were able to extend these routes to streamlined syntheses of compounds in the PI3,5P$_2$ series. As shown in Scheme 5, ***ent*-8** was converted to ***ent*-10** as before (via ***ent*-7b)**. Then, the remaining hydroxyl group at C5 of the *myo*-inositol ring was subjected to coupling with dibenzyl diisopropylphosphoramidite using standard conditions to give **16** in 86% yield. Global hydrogenolysis delivers PI3,5P$_2$−DiC8 in the naturally occurring enantiomeric series in 97% isolated yield.

For the PI3,5P$_2$ analogues with naturally occurring unsaturated side chains, we also followed the route developed for PI3P. As shown in Scheme 6, ***ent*-13** could be converted to the C1-functionalized material ***ent*-15** in analogous yield to those observed in the PI3P studies. The second phosphorylation sequence delivered protected PI3,5P$_2$, **17**, in 88% isolated yield. In this case, the complete deprotection was achieved employing TMSI under carefully optimized conditions. PI3,5P$_2$ was thus obtained with excellent purity and in 41% isolated yield.

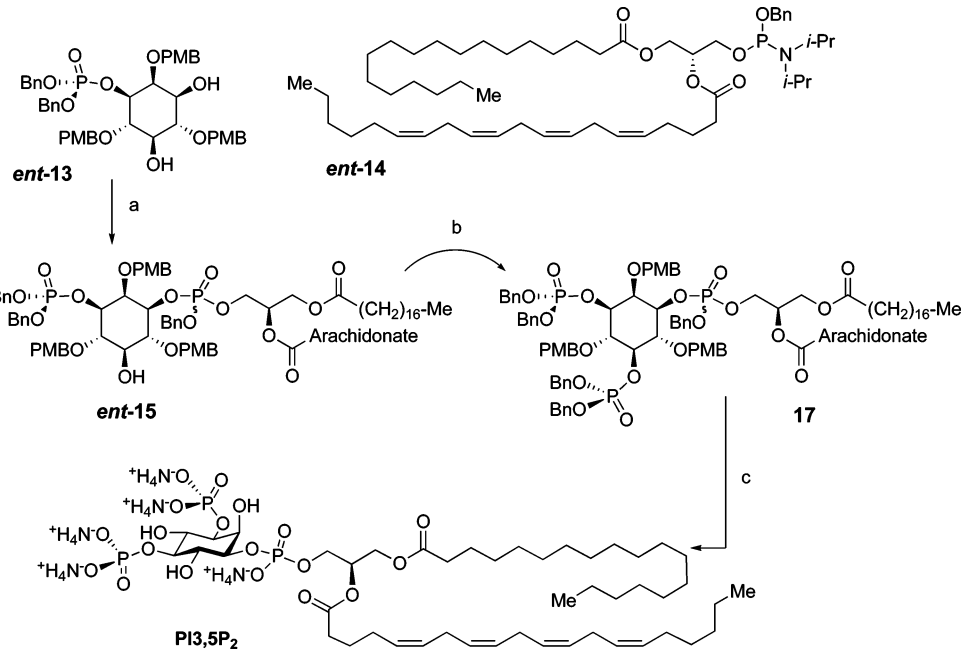
PI Synthesis. We next turned our attention to the synthesis of phosphatidylinositol in the optically pure form. Although this compound would seem the simplest of the PI-type natural products that we set out to synthesize, it is also the one that requires the development of a different method than those employed for PI3P or PI3,5P$_2$. Although we considered developing a catalytic asymmetric delivery of a glycerol-functionalized P(V) reagent, our experiences with di*benzyl* phosphochloridate diverted our attention to an approach that would utilize compounds such as **8** (Scheme 7), wherein the desymmetrization had already been performed. As such, we examined phosphotriester synthesis under Mitsunobu-type conditions.[20] Accordingly, monohydrolysis of **8** is achieved under basic

SCHEME 5 [a]



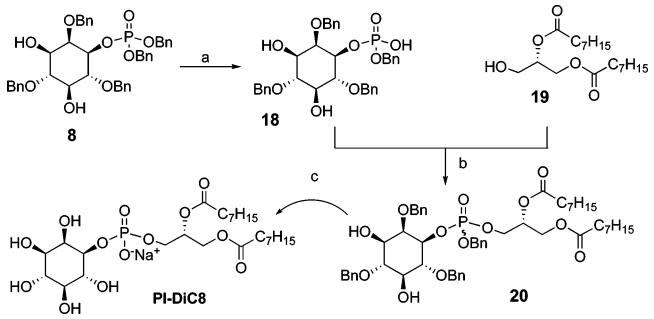
[a] Key: (a) 4,5-dicyanoimidazole, PhCH$_3$/CH$_2$Cl$_2$ (1:1), ***ent*-7b**; then 30% H$_2$O$_2$/H$_2$O, 40%; (b) 4,5-dicyanoimidazole CH$_2$Cl$_2$; dibenzyl diisopropylphosphoramidite; then 30% H$_2$O$_2$/H$_2$O, 86%;(c) Pd(OH)$_2$/C, H$_2$, *t*-BuOH/H$_2$O (5:1), Chelex 100 sodium form; 97%

SCHEME 6 [a]



[a] Key: (a) 4,5-dicyanoimidazole, toluene/CH_2Cl_2 (1:1), ***ent*-14**; then 30% H_2O_2/H_2O, 42%; (b) 4,5-dicyanoimidazole $PhCH_3/CH_2Cl_2$(1:1); dibenzyl diisopropylphosphoramidite; then 30% H_2O_2/H_2O, 88%; (c) TMSI, $CDCl_3$, 41%.

SCHEME 7[a]



[a] Key: (a) LiBr, acetone, reflux; then DOWEX 50 × 2-200; (b) DEAD, Ph_3P, **19**, THF 40% over two steps; (c) $Pd(OH)_2/C$, H_2, *t*-BuOH/H_2O (5:1), Chelex 100, sodium form; 98%.

conditions to deliver phosphoric acid **18**.[21] Coupling to **19** could then be achieved employing DEAD/PPh_3 conditions, delivering **20** in 40% yield from **8** (two steps). Hydrogenolysis of the protecting groups then allowed isolation of optically pure PI-DiC8 in 98% yield.

Synthesis of Deoxy-PI Analogues. We next wished to demonstrate the utility of the Mitsunobu chemistry for synthesizing optically pure analogues of the PI-derived compounds. For example, we have previously found deoxygenated inositol phosphate compounds to be valuable in studying the stereospecificity of IP interactions with the various enzymes that operate upon them.[10] We have therefore prepared analogous compounds in the PI series based on the combination of catalytic asymmetric phosphorylation and Mitsunobu-based phosphotriester synthesis. Such compounds, if available, could assist in elucidation of the key interactions between the various PI-compounds and their often membrane-associated protein targets.[22]

For 3-deoxygenated variants, we have previously demonstrated that precursor **4** could be monofunctionalized with phenylthionochloroformate at the 3-position (Scheme 8, 70%). The remaining free C5-hydroxyl group is then readily converted to TBS ether **21** (90%). Radical deoxygenation may then be induced under Barton-type conditions. Phosphate ester exchange and desilylation then afford compound **22**. Mono-deprotection of dibenzyl phosphate **22**, followed by Mitsunobu coupling to **19** results in compound **23** (89% over two steps.) Notably, compound **23** could be monophosphorylated in analogy to the other syntheses presented above, if a 5-phosphorylated analogue is desired. Nevertheless, hydrogenolysis then yields 3-deoxy-PI with, in this case, saturated C8-fatty acid side chains in 97% yield.

The 3,5-dideoxy variant of PI may also be achieved by a related route, shown in Scheme 9. In this case, exhaustive thiocarbonylation is carried out to give bis(thionocarbonate) **24**. Radical deoxygenation followed by transesterification then

(14) The sequences described below have indeed been executed equivalently in each enantiomeric series for a number of cases. Details of these syntheses, including characterization of enantiomers, are included in the Supporting Information where appropriate. For concise presentation, we describe in the text the preparation of only one enantiomer of each target.

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(16) Martin, S. F.; Josey, J. A.; Wong, Y. L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805−4820.

(17) Reddy, K. K.; Saady, M.; Falck, J. R. *J. Org. Chem.* **1995**, *60*, 3385−3390.

(18) This reactivity manifold is precedented. See: Atherton, F. R.; Howard, H. T.; Todd, A. R. *J. Chem. Soc.* **1948**, 1106−1111.

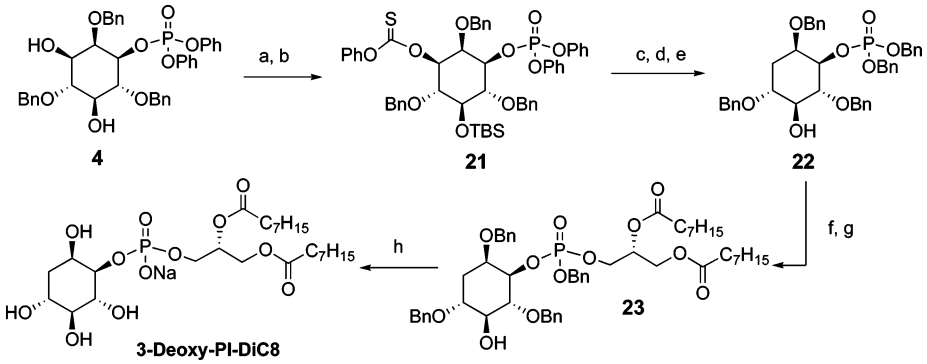
(19) Dove, S. K.; Piper, R. C.; McEwen, R. K.; Yu, J. W.; King, M. C.; Hughes, D. C.; Thuring, J.; Holmes, A. B.; Cooke, F. T.; Michell, R. H.; Parker, P. J.; Lemmon, M. A. *EMBO J.* **2004**, *23*, 1922−1933.

(20) (a) Mitsunobu, O. *Synthesis* **1981**, 1−28. (b) Hughes, D. L. *Org. React.* **1992**, *42*, 335−656. (c) Hughes, D. L. *Org. Prep. Proc. Int.* **1996**, *28*, 127−164. (d) Mitsunobu, O.; Kato, K.; Kimura, J. *J. Am. Chem. Soc.* **1969**, *91*, 6510−6511.

(21) Mahmoodi, N. O. *Phosphorus, Sulfur Silicon* **2002**, *177*, 2887−2893.

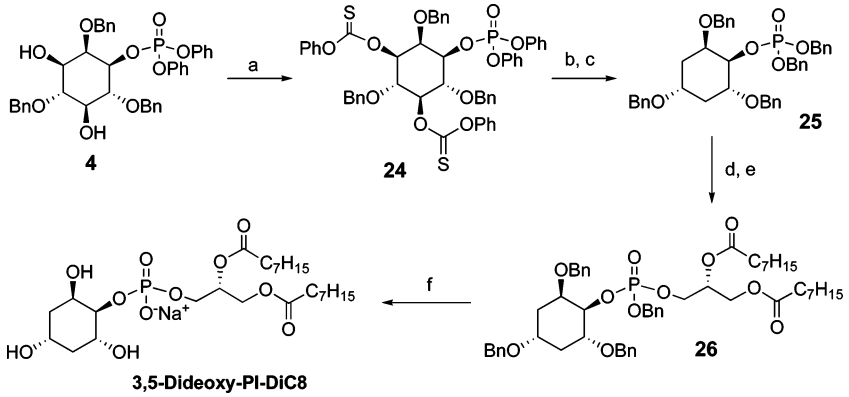
(22) Hurley, J. H.; Grobler, J. A. *Curr. Opin. Struct. Biol.* **1997**, *7*, 557−565.

SCHEME 8 [a]



[a] Key: (a) phenyl chlorothionoformate, DMAP, pyridine, CH_2Cl_2; 70%; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2; 90% (c) AIBN, Bu_3SnH, toluene; 70%; (d) BnOH, NaH, THF; 91%; (e) HF pyridine, THF; 75%; (f) LiBr, acetone, reflux; then DOWEX 50 × 2-200; (g) DEAD, Ph_3P, **19**, THF; 89% over two steps; (h) $Pd(OH)_2/C$, H_2, *t*-BuOH/H_2O (5:1), Chelex 100, sodium form; 97%.

SCHEME 9 [a]



[a] Key: (a) phenyl chlorothionoformate, DMAP, pyridine, CH_2Cl_2; 70%; (b) AIBN, Bu_3SnH, toluene; 60% (c) BnOH, NaH, THF; 67%; (d) LiBr, acetone, reflux; then DOWEX 50 × 2-200; (e) DEAD, Ph_3P, **19**, THF 55% over two steps; (f) $Pd(OH)_2/C$, H_2, *t*BuOH/H_2O (5:1), Chelex 100, sodium form; 93%.

delivers **25**, which may be carried through the hydrolysis−Mitsunobu sequence to afford **26**. As in the previous case, global hydrogenolysis delivers the final product, 3,5-dideoxy-PI, with the C8 fatty acid side chains installed in this case. Notably, the Mitsunobu sequences should be entirely compatible with either enantiomeric series and a wide variety of side chain functional types, including unsaturated versions, or variants that are functionalized with biologically interesting tethers as well.

Conclusions

In summary, we have completed a range of total syntheses of phosphatidylinositol derivatives. In either the PI3P, PI3,5P_2, or PI series, the sequences we have carried out are highly efficient and streamlined in comparison to prior approaches. The key step is the catalytic asymmetric phosphorylation reaction that effectively desymmetrizes the *myo*-inositol ring, such that either enantiomeric series may be synthesized. Efficient sequences that rely on a common approach to each target family allow unified reaction conditions to be applied throughout the series. For the PI compounds, a Mitsunobu coupling reaction was used that enables a wide variety of glycerol surrogates to be used. The schemes also enable the syntheses of a range of compounds wherein hydroxyls and/or phosphates at strategic positions in these PI-based targets may be deleted. These syntheses now set the stage for application of these probes to the high precision study of biological processes wherein the title compounds play significant roles.

Experimental Section

Protected PI3,5P_2-DiC8 (16). To a stirred solution of ***ent*-10** (0.023 g, 0.019 mmol) in CH_2Cl_2 (4.0 mL) was added dibenzyl diisopropylphosphoramidite (0.063 mL, 0.19 mmol) followed by 4,5-dicyanoimidazole (0.027 g, 0.23 mmol). The reaction was stirred at room temperature for 14 h and then cooled to 0 °C. 30% H_2O_2/H_2O (2 mL) was added, and the reaction was stirred at 0 °C for another 1 h. The reaction was then quenched with saturated Na_2SO_3 solution (15 mL) and the mixture extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried over sodium sulfate, and then concentrated under reduced pressure to afford an oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 0−50% ethyl acetate/hexanes to afford pure product as a clear oil (0.024 g, 86% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−7.12 (m, 36H), 7.00 (m, 4H), 5.06 (m, 1H), 5.00−4.66 (m, 16H), 4.53 (m, 1H), 4.46 (m, 1H), 4.37 (m, 1H), 4.26 (m, 1H), 4.13−3.93 (m, 4H), 3.86 (m, 1H), 3.78 (m, 1H), 2.14 (m, 4H), 1.54 (m, 4H), 1.25 (m, 16H), 0.88 (m 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 173.0, 172.9, 140.0, 138.5, 138.1, 138.0, 135.8, 135.7, 135.6, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 80.2, 78.3, 78.1, 76.1, 76.0, 75.0, 74.9, 70.2, 70.0, 69.9, 69.8, 69.7, 69.5, 69.4, 65.9, 61.7, 34.3, 34.2, 31.9, 31.2, 29.3, 29.2, 29.1, 25.0, 24.9, 22.8, 14.3; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −0.73, −0.79, −0.84, −0.88; IR (film, cm^{-1}) 2927, 2855, 1742, 1497, 1455, 1271, 1158, 1013, 880,

735, 696; TLC R_f 0.24 (50% ethyl acetate/hexanes); exact mass calcd for $[C_{81}H_{97}O_{19}P_3 + H]^+$ requires *m/z* 1467.5915, found 1467.5924 (ESI+); $[\alpha]_D = +2.0$ (4.0, $CHCl_3$).

PI3,5P$_2$-DiC8. To a stirred solution of **16** (0.023 g, 0.016 mmol) in *t*-BuOH/H_2O (5:1, 3 mL) was added sodium ion-exchange resin (Chelex 100 sodium form, 50−100 dry mesh, washed by H_2O) followed by $Pd(OH)_2$/C (40 mg, washed by H_2O). The reaction was then stirred at 1 atm of H_2 for 32 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure and lyophilized to afford a white solid (0.013 g, 97% yield): ^{1}H NMR (D_2O, 300 MHz) δ 5.16 (m, 1H), 4.30 (m, 2H), 4.15 (m, 1H), 3.90−3.88 (m, 4H), 3.74 (m, 3H), 2.29 (t, J = 7.2 Hz, 2H), 2.25 (t, J = 7.8 Hz, 2H),1.45 (m, 4H), 1.14 (m, 16H), 0.72 (m, 6H); ^{31}P NMR (D_2O, 121 MHz) δ 3.8, 2.3, 0.2; exact mass calcd for $[C_{25}H_{49}O_{19}P_3 - H]^-$ requires *m/z* 745.2003, found 745.2016 (ESI−); $[\alpha]_D = +5.2$ (1.0, H_2O at pH = 9).

Protected PI3,5P$_2$ (17). To a stirred solution of ***ent*-15** (0.0500 g, 0.0310 mmol) in CH_2Cl_2/toluene (1.5 mL/ 1.5 mL) was added dibenzyl diisopropylphosphoramidite (0.216 g, 0.626 mmol) followed by 4,5-dicyanoimidazole (0.0920 g, 0.782 mmol). The reaction mixture was stirred at room temperature for 12 h and then cooled to 0 °C. 30% H_2O_2/H_2O (3 mL) was added, and the reaction was stirred at 0 °C for another 1 h. The reaction was then quenched with saturated Na_2SO_3 solution (20 mL) and the mixture extracted with $CHCl_3$ (3 × 75 mL). The organic layers were combined, dried over sodium sulfate, and then concentrated under reduced pressure to afford an oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 10−50% ethyl acetate/hexanes to afford pure product as a clear oil (0.050 g, 88% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32−7.18 (m, 25H), 7.06 (m, 5H), 6.84 (m, 3H), 6.76−6.66 (m, 4H), 5.38−5.30 (m, 8H), 5.10 (m, 1H), 5.00−4.85 (m, 8H), 4.76 (t, J = 10.0 Hz, 4H), 4.70−4.63 (m, 4H), 4.50 (m, 1H), 4.36 (m, 2H), 4.26 (m, 2H), 4.12 (m, 1H), 4.01 (t, J = 9.6 Hz, 3H), 4.00−3.83 (m, 1H), 3.82 (s, 3H), 3.73−3.70 (s, 6H), 2.80 (m, 6H), 2.21 (m, 4H), 2.04 (m, 4H), 1.60 (m, 4H), 1.29 (m, 34H), 0.88 (m 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.2, 159.0, 130.7, 130.3, 129.5, 129.4, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.7, 113.9, 113.7, 74.6, 69.7, 60.7, 55.6, 55.5, 34.3, 33.9, 32.3, 31.9, 30.1, 30.0, 29.9, 29.8, 29.7, 29.6, 27.6, 26.9, 26.1, 26.0, 25.2, 25.1, 23.1, 23.0, 21.5, 14.7, 14.6, 14.5; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −0.48, −0.51, −0.58, −0.61, −0.64, −0.70; IR (film, cm^{-1}) 2924, 2853, 1743, 1613, 1514, 1456, 1379, 1249, 1015, 881, 823, 737, 697; TLC R_f 0.69 (50% ethyl acetate/hexanes); exact mass calcd for $[C_{106}H_{139}O_{22}P_3 + Na]^+$ requires *m/z* 1879.8869, found 1879.8850 (ESI+); $[\alpha]_D = +0.6$ (6.0, $CHCl_3$).

PI3,5P$_2$. Compound **17** (0.012 g, 0.0064 mmol) was dissolved in 1.0 mL of $CDCl_3$, and iodotrimethylsilane (0.028 mL, 0.19 mmol) was added. The reaction mixture was stirred at room temperature for 45 min and then cooled to 0 °C and concentrated via vacuum transfer. The residue was dissolved in 1.5 mL of toluene and then azeotroped at 0 °C via vacuum transfer. This procedure was repeated twice. The residue was then dissolved in 1.0 mL of methanol and stirred at 0 °C for 1 h. This solution was then concentrated under reduced pressure. The residue was dissolved in a minimal amount of CH_3OH/CH_2Cl_2 (1:1) and purified by silica gel chromatography eluting with $CHCl_3/CH_3OH$/2.2 M NH_4OH (9:7:2) to yield 3 mg (41% yield) of **PI3,5P$_2$** as the ammonium salt: ^{1}H NMR ($CD_3OD/CDCl_3/D_2O$ (4:3:1), 400 MHz) δ 5.39 (m, 8H), 5.30 (m, 1H), 4.23 (m, 3H), 4.09 (m, 5H), 3.93 (m, 3H), 2.84 (m, 5H), 2.40 (t, J = 6.8 Hz, 2H), 2.33 (t, J = 6.8 Hz, 2H), 2.10 (m, 4H), 1.73 (m, 2H), 1.61 (m, 2H), 1.30 (m, 34H), 0.92 (m, 6H); ^{31}P NMR ($CD_3OD/CDCl_3/D_2O$ (4:3:1), 121 MHz) δ 3.0, 1.9, 0.7; TLC R_f 0.10 (9:7:2 $CHCl_3/CH_3OH$/2.2 M NH_4OH); exact mass calcd for $[C_{47}H_{85}O_{19}P_3 + H]^+$ requires *m/z* 1047.4976, found 1047.5001 (ESI+).

Protected PI-DiC8 (20). To a stirred solution of **8** (0.058 g, 0.082 mmol) in 5 mL of acetone (reagent grade) was added LiBr (0.011 g, 0.12 mmol), and the reaction mixture was refluxed for 10 h. It was then cooled to rt and concentrated. The residue was purified by silica gel chromatography eluting with EtOAc/hexanes (1:1) to CH_2Cl_2/MeOH (1:1) to give the crude product as a lithium salt. The salt was dissolved in MeOH, and H^+ ion-exchange resin was added. The mixture was then filtered through cotton. The filtrate was concentrated to give the crude phosphoric diester product. The crude residue was then dissolved in 5 mL of THF, and diacylglycerol **19** (0.076 g, 0.12 mmol) and triphenylphosphine (0.032 g, 0.12 mmol) were added followed by DEAD (19 μL, 0.12 mmol). The reaction mixture was stirred at rt under N_2 for 12 h. The mixture was then concentrated under reduced pressure and purified by silica gel chromatography eluting with 10−50% EtOAc/hexanes to yield 32 mg of **20** (yield = 40% over two steps): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36−7.20 (m, 20H), 5.10 (m, 1H), 5.03 (m, 2H), 4.88−4.71 (m, 6H), 4.31−4.09 (m, 4H), 4.07−3.93 (m, 2H), 3.88 (m, 1H), 3.67 (t, J = 9.6 Hz, 1H), 3.54 (m, 2H), 2.42 (m, 1H), 2.21 (m, 5H), 1.55 (m, 4H), 1.23 (m, 16H), 0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 172.7, 138.5, 138.3, 128.8, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 81.4, 81.3, 80.2, 80.1, 79.1, 79.0, 78.9, 75.7, 75.6, 75.5, 75.4, 75.2, 75.0, 74.9, 72.0, 70.0, 69.9, 69.8, 69.7, 69.5, 69.4, 65.9, 65.8, 65.7, 65.6, 61.8, 61.7, 34.4, 34.2, 31.9, 30.0, 29.4, 29.3, 29.2, 25.1, 22.9, 14.4; ^{31}P NMR ($CDCl_3$, 121 MHz): δ −0.4, −0.5; IR (film, cm^{-1}): 3550, 3461, 3064, 3031, 2926, 2856, 2360, 1743, 1497, 1455, 1259, 1109, 1020, 895, 737, 697; **TLC**: Rf = 0.54 (1:1EtOAc/Hexanes); exact mass calcd for $[C_{53}H_{71}O_{13}P + H]^+$ requires *m/z* 947.4711, found 947.4708 (ESI+); $[\alpha]_D = +2.4$ (1.0, $CHCl_3$).

PI-DiC8. To a stirred solution of **20** (0.028 g, 0.030 mmol) in *t*-BuOH/H_2O (5:1, 3 mL) was added sodium ion-exchange resin (Chelex 100 sodium form, 50−100 dry mesh; washed by H_2O) followed by $Pd(OH)_2$/C (washed by H_2O). The reaction was then stirred at 1 atm of H_2 for 24 h. The reaction was filtered through Celite, and the filtrate was concentrated under reduced pressure and lyophilized to afford a white solid (0.018 g, 98% yield): ^{1}H NMR (D_2O, 300 MHz) δ 5.13 (m, 1H), 4.27 (m, 1H), 4.06 (m, 2H), 3.90 (m, 2H), 3.77 (t, J = 7.8 Hz, 1H), 3.58 (t, J = 9.6 Hz, 1H), 3.45 (t, J = 9.6 Hz, 1H), 3.38 (m, 1H), 3.17 (t, J = 9.3 Hz, 1H), 2.26−2.05 (m, 4H), 1.43 (m, 4H), 1.11 (m, 16H), 0.69 (m, 6H); ^{31}P NMR (D_2O, 121 MHz) δ 0.4; exact mass calcd for $[C_{25}H_{46}NaO_{13}P + H]^+$ requires *m/z* 609.2652, found 609.2669 (ESI+); $[\alpha]_D = +2.8$ (1.0, H_2O at pH = 9).

2,4,6-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-*myo*-inositol-1-diphenylphosphate-3-*O*-thiocarbonic Acid Phenyl Ester (21). To a stirred solution of 2,4,6-tri-*O*-benzyl-D-*myo*-inositol-1-diphenyl phosphate-3-*O*-thiocarbonic acid phenyl ester (0.572 g, 0.698 mmol) in dichloromethane (20 mL) was added 2,6-lutidine (326 μL, 2.79 mmol). TBSOTf (481 μL, 2.10 mmol) was then added and the reaction mixture stirred for 3 h at which time saturated NH_4Cl solution (25 mL) was added and the mixture was extracted with dichloromethane (3 × 75 mL). The organic layers were combined, dried over sodium sulfate, and then concentrated under reduced pressure to afford a yellow, oily solid. The crude product was purified using silica gel flash chromatography eluting with a gradient of 0−20% ethyl acetate/hexanes to afford pure product as a clear oil (580 mg, 90% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43−7.13 (m, 28H), 6.89 (m, 2H), 5.41 (dd, J = 2.4, 10.0 Hz, 1H), 4.92−4.69 (m, 8H), 4.14 (t, J = 10.0 Hz, 1H), 4.04 (t, J = 9.2 Hz, 1H), 3.74 (t, J = 9.2 Hz, 1H), 0.97 (s, 9H), 0.06 (s, 3H), −0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 194.5, 153.5, 150.7, 138.7, 138.5, 138.3, 130.0, 129.9, 129.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.6, 127.5, 127.4, 127.2, 126.8, 125.6, 125.5, 122.0, 120.3, 120.1, 83.6, 80.0, 79.7, 79.6, 79.5, 76.0, 75.7, 75.6, 74.8, 26.2, 18.2, 0.2, −3.8, −3.9; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −11.6; IR (film, cm^{-1}) 2927, 2855, 2360, 1590, 1490, 1288, 1217, 1190, 1039, 1025, 960, 837, 777, 689; TLC R_f 0.50 (20% ethyl acetate/hexanes); exact mass calcd for $[C_{52}H_{57}O_{10}PSSi + H]^+$ requires *m/z* 933.3258, found 933.3232 (ESI+); $[\alpha]_D = +22$ (1.0, $CHCl_3$).

2,4,6-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-*myo*-inositol-3-deoxy-1-diphenyl Phosphate. To a stirred solution of **21** (0.180

g, 0.193 mmol) in toluene (5 mL) was added tributyltin hydride (105 μL, 0.386 mmol) followed by AIBN (0.010 g, 0.058 mmol). The reaction mixture was stirred at reflux for 4 h. The mixture was cooled to room temperature and concentrated under reduced pressure to afford an oil. The crude product was then purified using silica gel flash chromatography eluting with a gradient of 0−20% ethyl acetate/hexanes to afford pure product as a clear oil (106 mg, 70% yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38−7.09 (m, 25H), 4.82 (m, 2H), 4.62 (m, 2H), 4.49−4.34 (m, 3H), 4.14 (m, 1H), 3.90 (t, J = 9.0 Hz, 1H), 3.64 (m, 2H), 2.16 (dt, J = 4.0, 14.0 Hz, 1H), 1.30 (m, 1H), 0.92 (s, 9H), 0.08 (s, 3H), −0.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.9, 150.8, 138.9, 138.8, 138.6, 130.0, 129.9, 129.8, 129.6, 128.5, 128.3, 128.1, 128.0, 127.8, 127.5, 127.1, 125.5, 125.3, 120.4, 120.3, 120.2, 120.1, 82.4, 82.3, 80.7, 80.6, 77.0, 75.5, 74.8, 72.9, 72.2, 30.4, 26.3, 18.4, −3.8, −3.9; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −11.4; IR (film, cm^{-1}) 2927, 2855, 1590, 1490, 1288, 1191, 1088, 1070, 1024, 955, 835, 777; TLC R_f 0.29 (20% ethyl acetate/hexanes); exact mass calcd for $[C_{45}H_{53}O_8PSi + H]^+$ requires m/z 781.3326, found 781.3340 (ESI+); $[\alpha]_D$ = −24 (1.0, $CHCl_3$).

2,4,6-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-*myo*-inositol-3-deoxy-1-dibenzyl Phosphate. To a stirred solution of 2,4,6-tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-*myo*-inositol-3-deoxy-1-diphenyl phosphate (0.116 g, 0.148 mmol) in THF (5 mL) was added benzyl alcohol (34.0 μL, 0.327 mmol) followed by sodium hydride (0.0150 g, 0.594 mmol). The reaction mixture was stirred and monitored by thin-layer chromatography. Starting material was consumed after 3 h, and the reaction was quenched with 0.5 M citric acid until pH = 7. The reaction mixture was then concentrated under reduced pressure and extracted with diethyl ether (3 × 75 mL) in 25 mL of brine. The organic layers were combined, dried over sodium sulfate, and then concentrated under reduced pressure to afford an oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 0−20% ethyl acetate/hexanes to afford pure product as a clear oil (110 mg, 91% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37−7.07 (m, 25H), 4.87-(m, 3H), 4.77 (m, 3H), 4.59−4.31 (m, 4H), 4.27 (m, 1H), 4.02 (m, 1H), 3.77 (t, J = 9.0 Hz, 1H), 3.63−3.49 (m, 2H), 2.05 (dt, J = 4.2, 14.1 Hz, 1H), 1.16 (m, 1H), 1.10 (s, 9H), 0.03 (s, 3H), −0.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0, 138.7, 138.6, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.3, 127.2, 81.3, 81.2, 81.0, 75.5, 74.8, 72.9, 72.5, 69.5, 69.4, 69.3, 30.7, 26.5; ^{31}P NMR ($CDCl_3$, 121 MHz) δ −0.5; IR (film, cm^{-1}) 2927, 2889, 2855, 1497, 1455, 1259, 1144, 1088, 1071, 1015, 1001, 835, 735, 696; TLC R_f 0.12 (25% ethyl acetate/hexanes); exact mass calcd for $[C_{47}H_{57}O_8PSi + H]^+$ requires m/z 809.3639, found 809.3624 (ESI+); $[\alpha]_D$ = −21 (1.0, $CHCl_3$).

2,4,6-Tri-*O*-benzyl-D-*myo*-inositol-3-deoxy-1-dibenzyl Phosphate (22). To a stirred solution of 2,4,6-tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-*myo*-inositol-3-deoxy-1-dibenyl phosphate (0.060 g, 0.074 mmol) in THF (5 mL) (Teflon container) was added HF/pyridine (0.5 mL). After 48 h, the reaction was neutralized with saturated sodium bicarbonate solution (50 mL) until basic and extracted with chloroform (3 × 75 mL). The organic layers were combined and concentrated under reduced pressure to afford a viscous oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 20−50% ethyl acetate/hexanes to afford pure product as a clear oil (38 mg, 75% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.19 (m, 25H), 5.01−4.91 (m, 4H), 4.80 (m, 2H), 4.60−4.45 (m, 4H), 4.30 (m, 1H), 4.08 (m, 1H), 3.87 (t, J = 9.2 Hz, 1H), 3.68 (m, 1H), 3.57 (t, J = 9.2 Hz, 1H), 2.61 (m, 1H), 2.18 (dt, J = 4.0, 14.0 Hz, 1H), 1.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.6, 138.4, 135.9, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 80.7, 80.6, 80.3, 80.2, 76.9, 76.2, 75.4, 75.1, 72.7, 72.5, 69.6, 69.5, 69.4, 69.3, 30.6; ^{31}P NMR ($CDCl_3$, 121 MHz) δ −0.5; IR (film, cm^{-1}) 3411, 3063, 3031, 2929, 2874, 1497, 1455, 1267, 1213, 1069, 1017, 911, 735, 697; TLC R_f 0.33 (50% ethyl acetate/hexanes); exact mass calcd for $[C_{41}H_{43}O_8P + H]^+$ requires m/z 695.2774, found 695.2759 (ESI+); $[\alpha]_D$ = −15 (1.0, $CHCl_3$).

Protected 3-Deoxy-PI-DiC8 (23). To a stirred solution of **22** (0.060 g, 0.086 mmol) in reagent grade acetone (5 mL) was added lithium bromide (0.011 g, 0.12 mmol). The reaction mixture was stirred at reflux for 12 h. The mixture was then cooled to room temperature and concentrated under reduced pressure to afford a white solid. The crude product was then purified using silica gel flash chromatography eluting with $CHCl_3$/MeOH/2.2 M NH_4OH (9:7:1) to afford the product as a lithium salt. The lithium salt was then dissolved in a minimum amount of methanol and loaded onto a proton ion-exchange resin column. The acidic fractions were combined and concentrated under reduced pressure (no heat) to afford a clear oil (acid form). The oil was then dissolved in THF (5 mL). Triphenylphosphine (0.034 g, 0.13 mmol) was added followed by diacylglycerol **19** (0.081 g, 0.13 mmol). DEAD (21 μL, 0.13 mmol) was added, and the reaction was stirred for 12 h and then concentrated under reduced pressure to afford an orange oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 5−50% ethyl acetate/hexanes to afford pure product as a clear oil (0.073 g, 89% yield, two steps): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39−7.22 (m, 20H), 5.11 (m, 1H), 4.97 (m, 2H), 4.81 (m, 2H), 4.65−4.46 (m, 4H), 4.27−3.92 (m, 6H), 3.86 (t, J = 9.6 Hz, 1H), 3.69 (m, 1H), 3.60 (q, J = 7.5 Hz, 1H), 2.60 (m, 1H), 2.20 (m, 5H), 1.55 (m, 5H), 1.26 (m, 16H), 0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 173.0, 172.7, 138.6, 138.4, 138.3, 128.7, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 80.8, 80.7, 80.6, 80.2, 80.1, 76.2, 75.5, 75.4, 75.2, 75.1, 72.7, 72.6, 72.5, 72.4, 69.8, 69.7, 69.6, 69.5, 65.9, 65.8, 65.6, 65.5, 61.9, 61.8, 34.4, 34.3, 31.9, 30.5, 30.4, 29.4, 29.3, 29.2, 25.2, 22.9, 14.1; ^{31}P NMR ($CDCl_3$, 121 MHz) δ −0.6, −0.7; IR (film, cm^{-1}) 3447, 3031, 2928, 2857, 1742, 1497, 1455, 1360, 1265, 1155, 1102, 1069, 1023, 736, 697; TLC R_f 0.48 (50% ethyl acetate/hexanes); exact mass calcd for $[C_{53}H_{71}O_{12}P + H]^+$ requires m/z 931.4761, found 931.4771 (ESI+); $[\alpha]_D$ = −8.6 (1.0, $CHCl_3$).

3-Deoxy-PI-DiC8. To a stirred solution of **23** (0.026 g, 0.028 mmol) in *t*-BuOH/H_2O (5:1, 3 mL) was added sodium ion-exchange resin (Chelex 100 sodium form, 50−100 dry mesh; washed by H_2O) followed by $Pd(OH)_2$/C (washed by H_2O). The reaction mixture was then stirred at 1 atm of H_2 for 24 h. The mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure and lyophilized to afford a white solid (0.016 g, 97% yield): ^{1}H NMR (D_2O, 300 MHz) δ 5.17 (m, 1H), 4.28 (m, 1H), 4.11 (m, 2H), 3.93 (m, 2H), 3.80 (t, J = 7.8 Hz, 1H), 3.62 (m, 1H), 3.57 (t, J = 9.6 Hz, 1H), 3.18 (t, J = 9.3 Hz, 1H), 2.24 (m, 4H), 1.97 (dt, J = 3.9, 13.5 Hz, 1H), 1.44 (m, 5H), 1.14 (m, 16H), 0.73 (m, 6H); ^{31}P NMR (D_2O, 121 MHz) δ 0.7; exact mass calcd for $[C_{25}H_{47}O_{12}P + H]^+$ requires m/z 593.2703, found 593.2687 (ESI+); $[\alpha]_D$ = +3.2 (1.0, H_2O at pH = 9).

2,4,6-Tri-*O*-benzyl-D-*myo*-inositol-3,5-dideoxy-1-dibenzyl Phosphate (25). To a stirred solution of 2,4,6-tri-*O*-benzyl-D-*myo*-inositol-3,5-dideoxy-1-diphenyl phosphate (0.172 g, 0.264 mmol) in THF (10 mL) was added benzyl alcohol (68.0 μL, 0.661 mmol) followed by sodium hydride (0.0200 g, 0.793 mmol). The reaction mixture was stirred and monitored by thin-layer chromatography. Starting material was consumed after 3 h, and the reaction was quenched with 0.5 M citric acid until pH = 7. The reaction was then concentrated under reduced pressure and extracted with diethyl ether (3 × 75 mL) in 20 mL of brine. The organic layers were combined, dried over sodium sulfate, and then concentrated under reduced pressure to afford an oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 0−25% ethyl acetate/hexanes to afford pure product as a clear oil (0.120 g, 67% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.18 (m, 25H), 4.99 (m, 4H), 4.65−4.43 (m, 6H), 4.39 (dt, J = 2.8, 8.4 Hz, 1H), 4.12 (m, 1H), 3.91 (m, 1H), 3.77 (m, 1H), 2.45 (m, 1H), 2.24 (m, 1H), 1.53−1.40 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.6, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.5, 75.4, 75.0, 74.9, 72.6, 72.2, 71.5, 70.9, 69.4, 69.3, 69.2; ^{31}P NMR

($CDCl_3$, 162 MHz) δ −0.5; IR (film, cm^{-1}) 3062, 3031, 2942, 2866, 1497, 1454, 1359, 1278, 1212, 1071, 1016, 736, 696; TLC R_f 0.22 (30% ethyl acetate/hexanes); exact mass calcd for $[C_{41}H_{43}O_7P + H]^+$ requires *m*/*z* 679.2825, found 679.2797 (ESI+); $[\alpha]_D = -16$ (4.8, $CHCl_3$).

Protected 3,5-Dideoxy-PI-DiC8 (26). To a stirred solution of **25** (0.095 g, 0.14 mmol) in reagent grade acetone (8 mL) was added lithium bromide (0.016 g, 0.18 mmol). The reaction mixture was stirred at reflux for 12 h. The mixture was then cooled to room temperature and concentrated under reduced pressure to afford a white solid. The crude product was then purified using silica gel flash chromatography eluting with $CHCl_3$/MeOH/2.2 M NH_4OH (9:7:1) to afford the product as a lithium salt. The lithium salt was then dissolved in a minimum amount of methanol and loaded onto a proton ion-exchange resin column. The acidic fractions were combined and concentrated under reduced pressure (no heat) to afford a clear oil (acid form). The oil was then dissolved THF (5 mL). Triphenylphosphine (0.040 g, 0.15 mmol) was added followed by diacylglycerol **19** (0.096 g, 0.15 mmol). DEAD (24 μL, 0.15 mmol) was added, and the reaction was stirred for 12 h and then concentrated under reduced pressure to afford an orange oil. The crude product was purified using silica gel flash chromatography eluting with a gradient of 5−50% ethyl acetate/hexanes to afford pure product as a clear oil (0.070 g, 55% yield, two steps): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (m, 20H), 5.10 (m, 1H), 4.97 (m, 2H), 4.65−4.43 (m, 6H), 4.36 (m, 1H), 4.19−3.98 (m, 5H), 3.90 (m, 1H), 3.76 (m, 1H), 2.46 (m, 1H), 2.20 (m, 5H), 1.55 (m, 4H), 1.47 (m, 2H), 1.23 (m, 16H), 0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 172.7, 138.5, 138.4, 136.0, 135.9, 128.6, 128.5, 128.4, 128.3, 127.8, 127.7, 127.6, 81.7, 74.9, 72.6, 72.1, 72.0, 71.4, 71.3, 70.9, 69.6, 69.5, 69.4, 65.7, 65.5, 62.0, 34.4, 34.3, 34.2, 32.0, 29.4, 29.3, 29.2, 25.2, 25.2, 23.0, 14.4; ^{31}P NMR ($CDCl_3$, 162 MHz) δ −0.6, −0.7; IR (film, cm^{-1}) 3063, 3031, 2928, 2857, 1742, 1497, 1455, 1360, 1282, 1158, 1097, 1070, 1023, 737, 697; TLC R_f 0.64 (50% ethyl acetate/hexanes); exact mass calcd for $[C_{53}H_{71}O_{11}P + H]^+$ requires *m*/*z* 915.4812, found 915.4796 (ESI+); $[\alpha]_D = -10$ (1.0, $CHCl_3$).

3,5-Dideoxy-PI-DiC8. To a stirred solution of **26** (0.063 g, 0.069 mmol) in *t*-BuOH/H_2O (5:1, 3 mL) was added sodium ion-exchange resin (Chelex 100 sodium form, 50−100 dry mesh; washed by H_2O) followed by $Pd(OH)_2$/C (washed by H_2O). The reaction was then stirred at 1 atm of H_2 for 24 h. The reaction was filtered through Celite, and the filtrate was concentrated under reduced pressure and lyophilized to afford a white solid (0.037 g, 93% yield): ^{1}H NMR (D_2O, 300 MHz) δ 5.11 (m, 1H), 4.25 (m, 1H), 4.07 (m, 2H), 3.88 (m, 3H); 3.74 (m, 2H), 2.22 (t, J = 6.6 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 2.06 (m, 1H), 1.94 (m, 1H), 1.42 (m, 5H), 1.26 (m, 1H), 1.10 (m, 16H), 0.69 (m, 6H); ^{31}P NMR (D_2O, 121 MHz) δ 0.8; exact mass calcd for $[C_{25}H_{47}O_{11}P + H]^+$ requires *m*/*z* 577.2754, found 577.2738 (ESI+); $[\alpha]_D = -4.0$ (1.0, H_2O at pH = 9).

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Supporting Information Available: Experimental procedures and product characterization for additional intermediates discussed in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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