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A synthesis of L-α-phosphatidyl-D-*myo*-inositol 4,5-bisphosphate (4,5-PIP_2) and glyceryl lipid analogs

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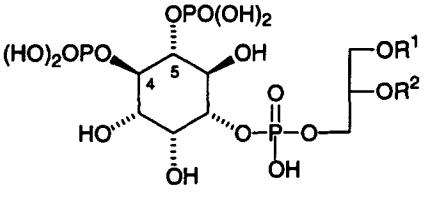
Abstract

The title bioactive phosphatidylinositide and short-chain glyceryl lipid analogs were prepared from deoxyinosose **2**, which was ultimately derived from 3-dehydroshikimic acid. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: cyclitols; hydroxylation; phospholipids; shikimic acid.

L-α-Phosphatidyl-D-*myo*-inositol 4,5-bisphosphate (4,5-PIP_2) (**1**: R^1, R^2=fatty esters) is a minor constituent of membrane phospholipids, yet plays a pivotal role in several essential intracellular functions.[1] Phospholipase C cleaves **1** into the second messengers inositol 1,4,5-P_3 (IP_3) and diacylglycerol (DAG).[2] Conversely, **1** is a substrate for phosphoinositide 3-kinases that convert it to phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate (3,4,5-PIP_3), a prominent member of the D-3 signal cascade.[3] More recent studies have established **1** itself as a bona fide second messenger,[4] capable of directly recruiting and/or modulating numerous regulatory proteins as spatially localized functional complexes via its strong affinity for pleckstrin homology domains.[5] In addition, **1** can regulate RNA transcription,[6] vesicle trafficking,[7] K_{ATP} channels[8] and cytoskeletal assembly.[9] The range of physiologic functions performed by **1** is still an area of intense scrutiny as are its interactions with cellular components. To help expedite these studies, we report herein an asymmetric synthesis of diacyl **1** and some useful glyceryl lipid analogs.



1: 4,5-PIP_2

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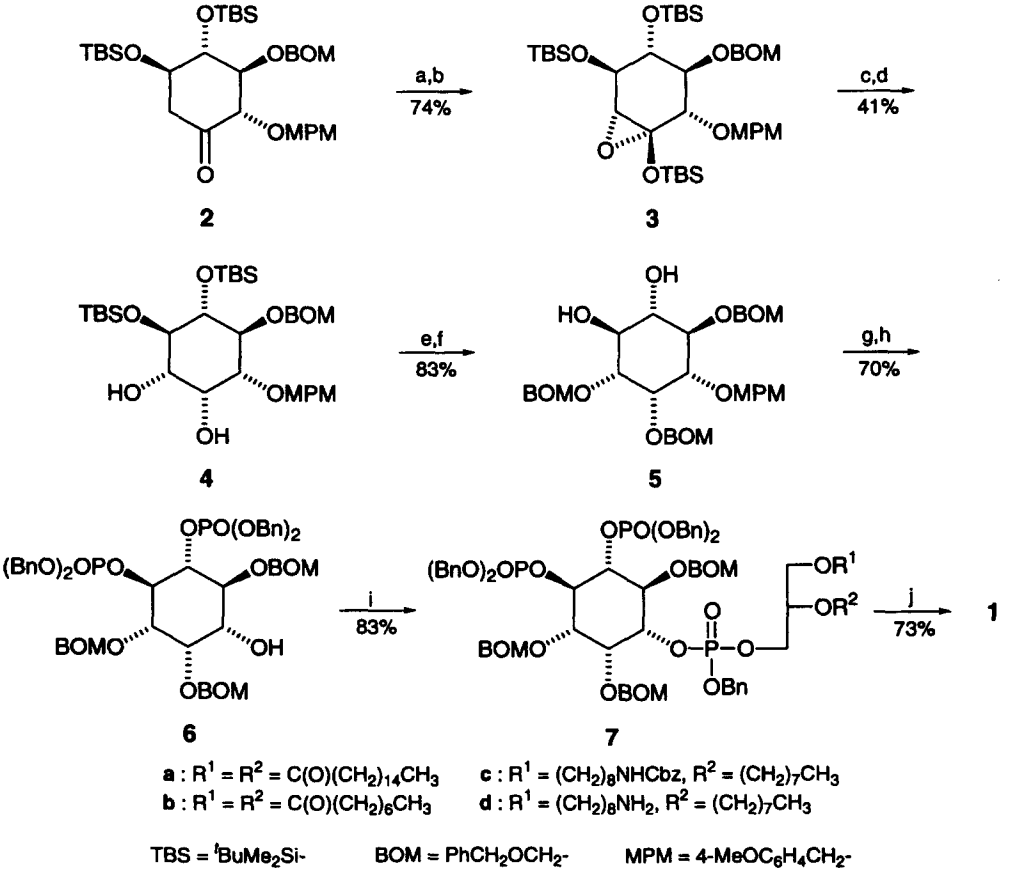
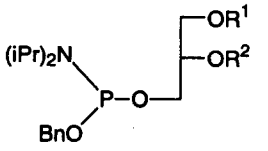
Most synthetic routes[10a,d,e] to **1** begin with inexpensive *myo*-inositol. These cases, however, necessitate a resolution if chiral material is desired and often incorporate unproductive protection–deprotection sequences to differentiate the six inositol hydroxyls. These limitations are largely avoided by a biomimetic approach[10b,c] that converts D-glucose to a mixture of inososes via an organomercurial intermediate from which a *myo*-inositol derivative can be secured by hydride reduction.[11]

Our synthetic strategy likewise exploited D-glucose, but eschewed heavy metals in favor of a biotechnology process that affords 3-dehydroshikimic acid.[12] This versatile reagent is easily transformed into deoxyinosose **2** by a short sequence as previously described.[13] Treatment of **2** with an excess of *tert*-butyldimethylsilyl trifluoromethanesulfonate as described by Corey[14] (Scheme 1) and epoxidation of the resultant $\Delta^{2,3}$-silyl enol ether using buffered *m*-chloroperbenzoic acid (*m*-CPBA) afforded the surprisingly robust α-(silyloxy)oxirane **3**[15] that could be isolated[16] and characterized [^{1}H NMR ($CDCl_3$): δ 3.07 (s)]. In practice, however, the crude oxidation product was hydrolyzed to the corresponding hydroxyketone by stirring overnight in THF/H_2O with camphorsulfonic acid (CSA). Preferential hydride addition from the less hindered β-face, assisted by coordination with the adjacent C(3)-alcohol, gave



Scheme 1. Reaction conditions: (a) TBS–OTf (2.5 equiv.), Et_3N (5 equiv.), CH_2Cl_2, 23°C, 6 h (87%); (b) *m*-CPBA (3 equiv.), Na_2HPO_4 (17 equiv.), CH_2Cl_2, –20°C to 0°C over 1 h, then 2 h at 0°C (85%); (c) CSA (0.5 equiv.), THF:H_2O (4:1), 23°C, 12 h (58%); (d) $NaBH_4$, MeOH, 0°C, 1 h (70%); (e) BOM-Cl, *i*Pr_2EtN, 65°C, 15 h (90%); (f) *n*Bu_4NF, THF, 23°C, 5 h (87%); (g) (*i*$Pr)_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (88%); (h) DDQ, CH_2Cl_2:H_2O (20:1), 23°C, 4 h (80%); (i) phosphoramidite **8**, 1*H*-tetrazole, CH_2Cl_2, 23°C, 2 h; *m*-CPBA, –40°C, 1 h (83%); (j) Pd black, H_2 (52 psi), $NaHCO_3$, *t*BuOH:H_2O (6:1), 23°C, 4 h (73%)

rise to diol **4**[17] with the desired *myo*-inositol configuration. The stereochemistry of the reduction was confirmed by inspection of the ^{1}H NMR of the diacetate derived from **4** which displayed a triplet at 5.42 ppm (J~3.0 Hz). Alkylation with BOM-Cl under standard conditions followed by desilylation with fluoride anion provided **5** that was bis-phosphorylated by the two-step phosphoramidite procedure of Tegge and Ballou.[18] Subsequent DDQ cleavage of the 4-methoxybenzyl (MPM) ether secured the key intermediate **6**. Phosphatidylation of the C(1)-alcohol in **6** with freshly prepared 1,2-di-*O*-hexadecanoyl-*sn*-glycerobenzyl (*N*,*N*-diisopropylamino)phosphoramidite[13] (**8a**) and peracid oxidation yielded **7a**.[19] Catalytic hydrogenolysis of **7a** in the presence of a stoichiometric $NaHCO_3$ afforded 4,5-PIP_2 (**1a**), isolated as the pentasodium salt.



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Repetition of the above condensation between **6** and **8b**[13] or **8c**[13] gave the dioctanoyl glyceryl analog **1b** and the ω-amino version **1c**, respectively. The former is more water soluble than the dihexadecanoyl form **1a** and has proven more tractable in some assays. The latter can be derivatized with fluorescent, radioactive, and affinity labels; its application in the isolation of several specific PIP binding proteins will be reported elsewhere.

Acknowledgements

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References

1. Review: Toker, A. *Curr. Opin. Cell Biol.* **1998**, *10*, 254–261.
2. Nishizuka, Y. *Science* **1992**, *258*, 607–614.
3. Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. *Cell* **1991**, *64*, 281–302.
4. Martin, T. F. J. *Ann. Rev. Cell Develop. Biol.* **1998**, *14*, 231–264.
5. Hsuan, J. J.; Minogue, S.; dos Santos, M. *Adv. Cancer Res.* **1998**, *74*, 167–216.
6. Yu, H. Y.; Fukami, K.; Watanabe, Y.; Ozaki, C.; Takenawa, T. *Euro. J. Biochem.* **1998**, *251*, 281–287.
7. Barylko, B.; Binns, D.; Lin, K. M.; Atkinson, M. A. L.; Jameson, D. M.; Yin, H. L.; Albanesi, J. P. *J. Biol. Chem.* **1998**, *273*, 3791–3797.
8. Ashcroft, F. *Science* **1998**, *282*, 1059–1060 and references cited therein.
9. Janmey, P. *Chem. Biol.* **1995**, *2*, 61–65.
10. Selected recent syntheses of 4,5-PIP_2: (a) Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **1988**, *29*, 6513–6516. (b) Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 6305–6312. (c) Gu, Q. M.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 8642–8647. (d) Toker, A.; Meyers, M.; Reddy, K. K.; Falck, J. R.; Aneja, R.; Aneja, S.; Parra, A.; Burns, D. J.; Ballas, L. M.; Cantley, L. C. *J. Biol. Chem.* **1994**, *269*, 32358–32367. (e) Watanabe, Y.; Nakamura, T.; Hiroyuki, M. *Tetrahedron Lett.* **1997**, *38*, 7407–7410.
11. Bender, S. L.; Budhu, R. J. *J. Am. Chem. Soc.* **1991**, *113*, 9883–9885. Estevez, V. A.; Prestwich, G. D. *J. Am. Chem. Soc.* **1991**, *113*, 9885–9887.
12. Li, K.; Mikola, M. R.; Draths, K. M.; Worden, R. M.; Frost, J. W. *Biotechnol. Bioeng.* **1999**, *64*, 61–73.
13. Reddy, K. K.; Saady, M.; Falck, J. R.; Whited, G. *J. Org. Chem.* **1995**, *60*, 3385–3390.

14. Corey, E. J.; Cho, H.; Rucker, C.; Hua, D. H. *Tetrahedron Lett.* **1981**, *22*, 3455–3458.
15. All new compounds were fully characterized by $^{1}H/^{13}C/^{31}P$ NMR, IR, and MS analysis using chromatographically homogeneous material.
16. Epoxy enol ethers are generally quite labile and are usually only detected as transient species: Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 295–400.
17. Spectral data for **4**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.11 (s, 6H), 0.13 (s, 6H), 0.86 (s, 9H), 0.89 (s, 9H), 3.04 (d, J=11.6 Hz, 1H, D_2O exchangeable), 3.17 (d, J=12 Hz, 1H, D_2O exchangeable), 3.73–3.76 (m, 1H), 3.77 (s, 3H), 3.83–3.88 (m, 2H), 4.08–4.16 (m, 3H), 4.39 (d, J=11.4 Hz, 1H), 4.60 (s, 2H), 4.64 (d, J=11.4 Hz, 1H), 4.71 (dd, J=7, 7 Hz, 2H), 6.84 (d, J=8.4 Hz, 2H), 7.20 (d, J=8.4 Hz, 2H), 7.28–7.39 (m, 5H). Intermediate **6**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.54 (ddd, J=2.6, 2.8, 9.4 Hz, 1H), 3.69 (dd, J=2.5, 9.4 Hz, 1H), 3.86 (apparent t, J=9.4 Hz, 1H), 4.22 (d, J=2.8 Hz, 1H, D_2O exchangeable), 4.27 (d, apparent t, J=2.6 Hz, 1H), 4.37–4.50 (m, 4H), 4.52–4.79 (m, 8H), 4.89–5.18 (m, 10H), 7.15–7.40 (m, 35H). Intermediate **7**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 0.85 (t, J=6.3 Hz, 3H), 0.87 (t, J=6.3 Hz, 3H), 1.12–1.38 (m, 16H), 1.49–1.58 (m, 4H). 2.18 (t, J=7.1 Hz, 2H), 2.20 (t, J=7.1 Hz, 2H), 3.68–3.74 (m, 1H), 3.91–3.98 (m, 1H), 4.12–4.32 (m, 4H), 4.38–4.56 (m, 7H), 4.58 (d, J=11.6 Hz, 1H), 4.64 (d, J=11.6 Hz, 1H), 4.72–5.18 (m, 18H), 7.05–7.39 (m, 40H). 4,5-PIP_2 (**1b**): ^{1}H NMR (D_2O, 250 MHz) δ 0.83 (apparent t, J=6.3 Hz, 6H), 1.12–1.38 (m, 16H), 1.44–1.62 (m, 4H), 2.32–2.41 (m, 4H), 3.66 (dd, J=2.4, 9.4 Hz, 1H), 3.70–4.36 (m, 8H), 4.38–4.47 (m, 1H), 5.28 (br s, 1H); ^{31}P NMR (D_2O, 121.4 MHz, 85% H_3PO_4 external reference) δ 0.35, 0.33, –2.10.
18. Tegge, W.; Ballou, C. E. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 94–98.
19. As a consequence of the newly created tetrahedral phosphorus, **7a** was obtained as a ~1:1.6 diastereomeric mixture by ^{31}P NMR analysis, but was typically used in the next step without separation.