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AN EXPEDITIOUS SYNTHESIS OF BIOLOGICALLY IMPORTANT *MYO*-INOSITOL PHOSPHOROTHIOATES

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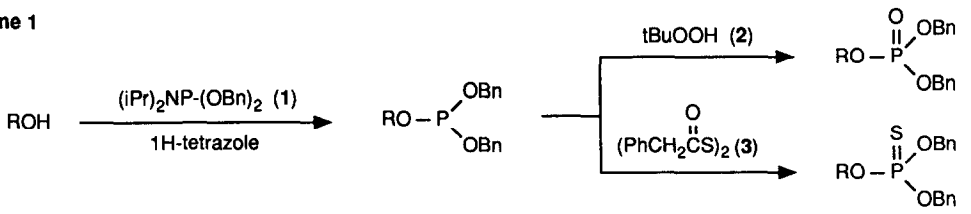
Abstract: The *myo*-inositol phosphorothioates **6**, **9**, **12** and **17** were readily accessible from properly protected precursors by phosphitylation with *N,N*-diisopropyl dibenzyl phosphoramidite (**1**), subsequent *in situ* sulfurization of the intermediate phosphite triesters with phenacetyl disulfide (**3**), and removal of all benzyl protecting groups.

It is well-known that *myo*-inositol 1,4,5-*tris*phosphate[1,2] is released upon phospholipase C catalyzed cleavage of phosphatidylinositol 4,5-*bis*phosphate after agonist stimulation of several cell surface receptors. The intracellular second messenger Ins[1,4,5]P_3 thus released is responsible for the mobilization of sequestered calcium ions from intracellular storage sites. The action of Ins[1,4,5]P_3 may be terminated *via* two distinct pathways. The major pathway for Ins[1,4,5]P_3 inactivation consists of dephosphorylation by a specific 5-phosphatase[3] to give Ins[1,4]P_2, which is further degraded by phosphatases to free *myo*-inositol. The alternate pathway entails phosphorylation of Ins[1,4,5]P_3 by a specific 3-kinase[4] to give the putative second messenger Ins[1,3,4,5]P_4[5]. Subsequent hydrolysis of the 5-phosphate affords Ins[1,3,4]P_3[6], which is metabolised to *myo*-inositol and higher *myo*-inositol phosphates.

The importance of Ins[1,4,5]P_3 as a calcium mobilizing second messenger has revived a considerable interest[7,8] in the chemical synthesis of *myo*-inositol phosphates and analogues thereof. The availability of Ins[1,4,5]P_3 analogues, having a modified phosphate function at a specific position, would be of great value to get a deeper insight into the metabolism of Ins[1,4,5]P_3. For example, Potter *et alia*[9-11] showed that Ins[1,4,5]P_3 phosphorothioate analogues, which are metabolically stable to degradation by phosphatases[12], are valuable tools to explore in detail the recognition of Ins[1,4,5]P_3 by enzymes and receptor sites[13].

As part of our continuous programme[14-17] directed towards the preparation of *myo*-inositol phosphates and analogues thereof, we now report an expeditious synthesis of some biologically important *myo*-inositol phosphorothioates.

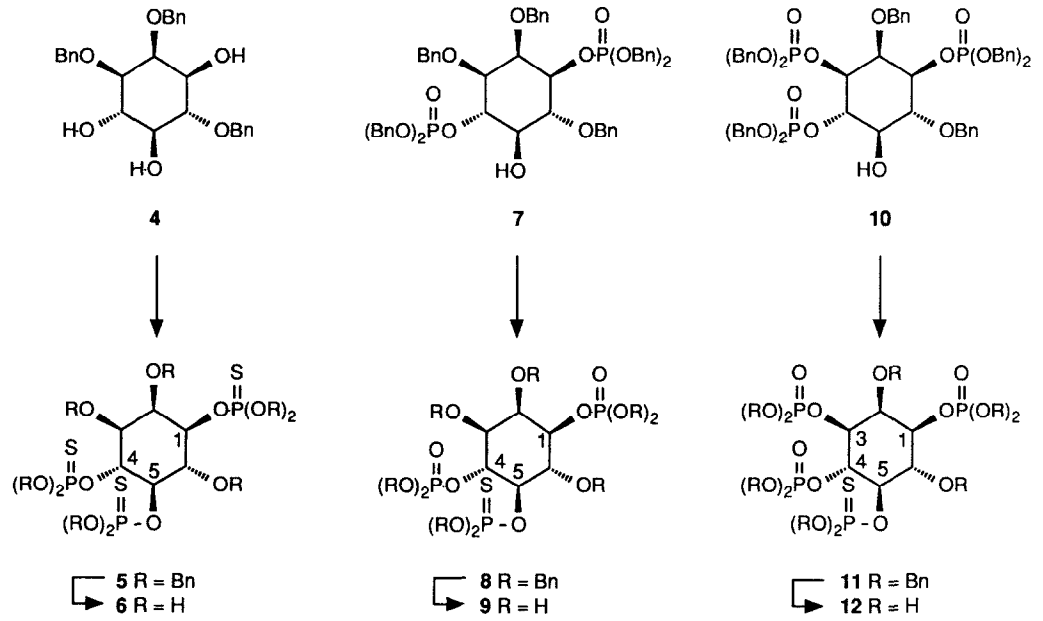
Scheme 1



Recent advances in the synthesis of *myo*-inositol phosphates demonstrated[16,18] that 1*H*-tetrazole-mediated phosphitylation (see Scheme 1) of properly protected *myo*-inositol derivatives with the easily accessible reagent *N,N*-diisopropyl dibenzyl phosphoramidite (**1**)[19], followed by oxidation of the intermediate phosphite triesters with *tert*-butyl hydroperoxide (**2**)[20], is a very efficient process. We earlier reported[21] that the sulfurizing reagent phenacetyl disulfide (**3**)[22] is an excellent alternative for the thioylation by elemental sulfur[23] of phosphite triester intermediates of nucleic acids. The efficacy of the thioylation reaction[24] urged us to employ the sulfurization reagent **3** for the preparation of *myo*-inositol phosphorothioates.

Phosphitylation (Scheme 2) of racemic 2,3,6-tri-*O*-benzyl-*myo*-inositol (**4**[16]; 0.33 mmol) with *N,N*-diisopropyl dibenzyl phosphoramidite (**1**; 1.50 mmol) in the presence of 1*H*-tetrazole (2.00 mmol) in a CH_2Cl_2 - CH_3CN mixture (10 mL, 1/1, v/v) afforded, within 15 min at 20°C, the intermediate *tris*phosphite triester (δ_p 141.49, 141.61 and 142.52 ppm). Monitoring of the *in situ* sulfurization of the latter phosphite triesters with reagent **3** (3.75 mmol) by ^{31}P-NMR spectroscopy revealed rapid formation (within 15 min) of the corresponding phosphorothioates (δ_p 68.00, 68.72 and 68.99 ppm). After work-up and purification by silica gel column chromatography, homogeneous 2,3,6-tri-*O*-benzyl-*myo*-inositol 1,4,5-*tris*(dibenzylphosphorothioate) (**5**)[25] was isolated in 88% yield. Removal of the benzyl protecting groups could be realized by reduction with sodium in liquid ammonia and tetrahydrofuran. The crude product was purified by gelfiltration over a Sephacryl column to give *myo*-inositol 1,4,5-trisphosphorothioate (**6**)[9], which was isolated as the sodium-salt in 51% yield. The ^{31}P-NMR spectrum of the Ins[1,4,5]P_3 analogue **6** showed the presence of only three equally intense phosphorothioate resonances at 49.46, 51.32 and 51.72 ppm.

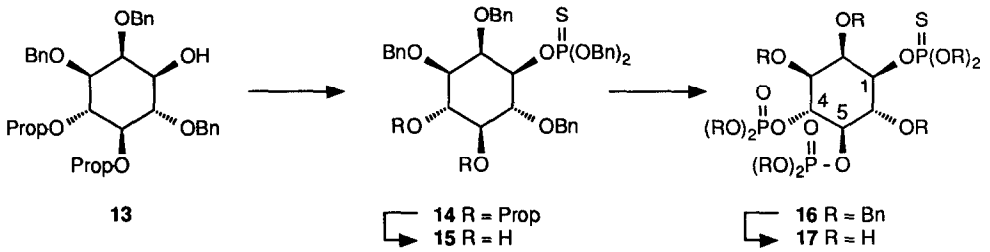
Scheme 2



The successful preparation of the *myo*-inositol 1,4,5-*tris*phosphorothioate (**6**) encouraged us to investigate whether the above described methodology was also amenable to the synthesis of the racemic *myo*-inositol 1,4,5-*tris*- and 1,3,4,5-*tetrakis*phosphate analogues **9**[10] and **12**, the 5-phosphate of which is replaced by a phosphatase-resistant phosphorothioate function. Thus, phosphitylation of the respective *myo*-inositol phosphate triesters **7**[26] and **10**[26] (Scheme 2), having a free hydroxyl function at the 5-position, with *N,N*-diisopropyl dibenzyl phosphoramidite (**1**) in the presence of 1*H*-tetrazole, followed by *in situ* sulfurization of the intermediate phosphite triesters with phenacetyl disulfide (**3**) for 15 min at 20°C afforded the fully protected *myo*-inositol 1,4,5-*tris*- and 1,3,4,5-*tetrakis*phosphate analogues **8** (δ_p -1.18, -0.97 and 69.26 ppm) and **11** (δ_p -1.09, -0.94, -0.48 and 69.90 ppm) in 92 and 87% yield, respectively. Removal of the benzyl protecting groups from **8** and **11** with sodium in liquid NH_3/THF gave the 5-phosphorothioate analogues **9** (δ_p 0.22, 1.44, 54.67 ppm) and **12** (δ_p 0.13, 0.31, 1.03 and 54.96 ppm) in 72 and 68% yield, respectively.

Finally, we turned our attention towards the synthesis of the 1-phosphorothioate analogue of Ins[1,4,5]P_3 (**17**)[11], which is a suitable precursor for the attachment of a fluorescent reporter group[11].

Scheme 3



Reaction of racemic 2,3,6-tri-*O*-benzyl-4,5-di-*O*-*trans*-prop-1-enyl-*myo*-inositol (**13**)[27] (Scheme 3) with *N,N*-diisopropyl dibenzyl phosphoramidite (**1**) and subsequent sulfurization of the intermediate phosphite triester with reagent **3** furnished, after mild acidolysis (0.1 N HCl in CH_2Cl_2/MeOH, 1/1, v/v) of the *trans*-prop-1-enyl groups at the 4- and 5-position from **14**, the 1-phosphorothioate **15** (δ_p 68.06 ppm) in 75% yield. Subsequent, phosphitylation of the phosphorothioate **15** with amidite **1** and oxidation of the intermediate phosphite triesters with *tert*-butyl hydroperoxide (**2**)[20] gave the fully protected *myo*-inositol derivative **16** (δ_p -1.42, -1.15 and 68.06 ppm) in 85% yield. Deprotection of **16** with sodium in liquid ammonia resulted in the isolation of *myo*-inositol 1-phosphorothioate 4,5-*bis*phosphate (**17**) (δ_p 1.21, 2.09 and 48.32 ppm) in 74% yield.

Preliminary biological studies indicated that the Ins[1,4,5]P_3 analogues **6**, **9** and **17** were substrates for the purified Ins[1,4,5]P_3 3-kinase from bovine brain. On the other hand, the Ins[1,3,4,5]P_4 analogue **12** acted as a competitive inhibitor of this enzyme. A detailed report on the biological properties of the *myo*-inositol phosphorothioates will be published in due course.

In conclusion, the results presented in this paper clearly demonstrate the usefulness of the phosphitylating reagent **1** in combination with the sulfurization reagent **3** for the preparation of *myo*-inositol phosphates, having phosphorothioate functions at predetermined positions.

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References and Notes

1. Streb, H.; Irvine, R.F.; Berridge, M.J.; Schulz, I. *Nature* **1983**, *306*, 67.
2. Berridge, M.J.; Irvine, R.F. *Nature* **1984**, *312*, 315.
3. Storey, D.J.; Shears, S.B.; Kirk, C.J.; Michell, R.H. *Nature* **1984**, *312*, 374.
4. Irvine, R.F.; Letcher, A.J.; Heslop, J.P.; Berridge, M.J. *Nature* **1986**, *320*, 631.
5. Batty, I.R.; Nahorski, S.R.; Irvine, R.F. *Biochem. J.* **1985**, *232*, 211.
6. Irvine, R.F.; Letcher, A.J.; Lander, D.J.; Downes, C.P. *Biochem. J.* **1984**, *223*, 237.
7. Billington, D.C. *Chem. Soc. Rev.* **1989**, *18*, 83.
8. Potter, B.V.L. *Nat. Prod. Rep.* **1990**, *7*, 1.
9. Cooke, A.M.; Gigg, R.; Potter, B.V.L. *J. Chem. Soc., Chem. Commun.* **1987**, 1525.
10. Cooke, A.M.; Noble, N.J.; Payne, S.; Gigg, R.; Potter, B.V.L. *J. Chem. Soc., Chem. Commun.* **1989**, 269.
11. Lampe, D.; Potter, B.V.L. *J. Chem. Soc., Chem. Commun.* **1990**, 1500.
12. Hamblin, M.R.; Flora, J.S.; Potter, B.V.L. *Biochem. J.* **1987**, *246*, 771.
13. Nahorski, S.R.; Potter, B.V.L. *Trends Pharmacol. Sci.* **1989**, *10*, 139.
14. Dreef, C.E.; Van der Marel, G.A.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 161.
15. Dreef, C.E.; Van der Marel, G.A.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 512.
16. Dreef, C.E.; Tuinman, R.J.; Elie, C.J.J.; Van der Marel, G.A.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1988**, *107*, 395.
17. Dreef, C.E.; Tuinman, R.J.; Lefeber, A.W.M.; Elie, C.J.J.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron* in press.
18. Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.
19. Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175.
20. Engels, J.; Jäger, A. *Angew. Chem. Suppl.* **1982**, 2010.
21. Kamer, P.C.J.; Roelen, H.C.P.F.; Van den Elst, H.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron Lett.* **1989**, *30*, 6757.
22. Frank, R.L.; Blegen, J.R., in: *Organic Syntheses*, Vol. 28, Snyder, H.R. (ed.), John Wiley and Sons, New York, 1948, p. 16.
23. Burgers, P.M.J.; Eckstein, F. *Tetrahedron Lett.* **1978**, 3835.
24. In our hands the sulfurization of dibenzyl phosphite triesters using elemental sulfur proceeded rather sluggishly.
25. All new compounds were fully characterized by ^{1}H- and ^{13}C-NMR as well as combustion analysis.
26. Dreef, C.E.; Jansze, J.-P.; Elie, C.J.J.; Van der Marel, G.A.; Van Boom, J.H. manuscript in preparation.
27. Dreef, C.E.; Elie, C.J.J.; Hoogerhout, P.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron Lett.* **1988**, *29*, 6513.