Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 10, pp. 1231-1234, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00196-0

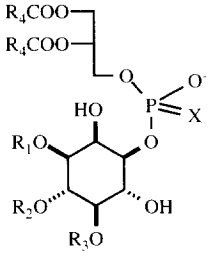
SYNTHESIS OF PHOSPHOROTHIOATE ANALOGS OF PHOSPHATIDYLINOSITOL 3,4,5-TRISPHOSPHATE

Robert J. Kubiak and Karol S. Bruzik*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Abstract: Phosphorothioate analogs of phosphatidylinositol 3,4,5-trisphosphate have been synthesized using Me_3N-Me_3SiCl reagent in a critical exhaustive cleavage of *O*-benzyl phosphorothioate triester intermediates.

Phosphatidylinositol phosphates (PIP) are important precursors of inositol phosphate second messengers, or are the cellular signals themselves.[1] In particular, the 3-phosphorylated phosphatidylinositols **1-3** have been implicated in interactions with various cellular targets to mediate diverse physiological events ranging from vesicular trafficking to insulin response.[2] The primary metabolic pathways of the 3-phosphorylated PIs involve a series of specific phosphatases rapidly removing phosphates from the 5-, 4- and 3-positions to finally form phosphatidylinositol.[2,3] One approach to study the function of these phospholipids would be to increase their biological lifetime, by making them resistant to specific phosphatases or phosphodiesterases. Such resistance can be conferred by substituting the phosphate groups with the corresponding phosphorothioates. Phosphorothioate analogs of phosphate monoesters and diesters have been long known to undergo slower enzymatic hydrolysis than their phosphate counterparts,[4] and the phosphatase-resistant phosphorothioate analogs of inositol 1,4,5-trisphosphate (IP_3) have been previously used to investigate the role of IP_3 in calcium release.[5] This work describes the first synthesis of phosphorothioate analogs of phosphatidylinositol phosphates **4** and **5**.[6]

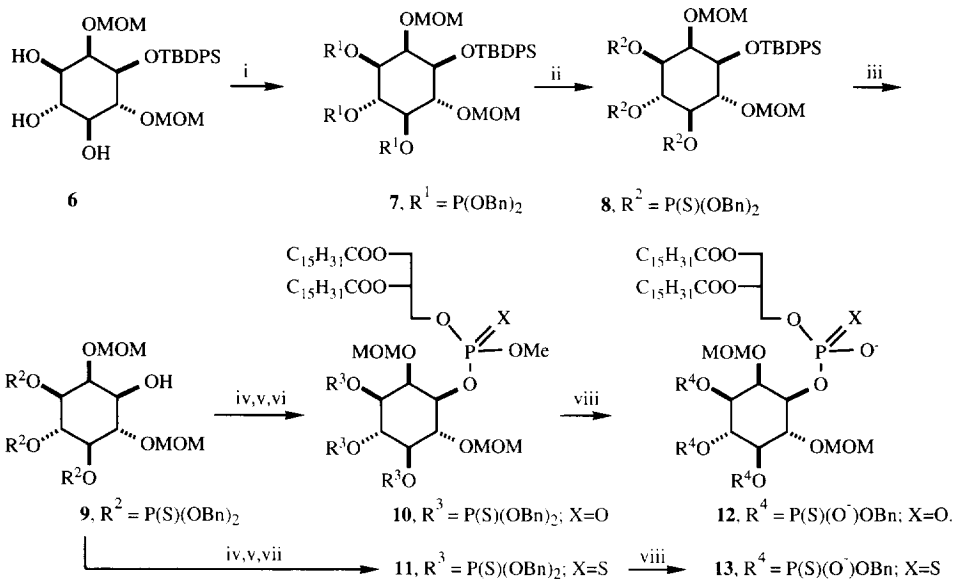


$R_1 = PO_3^{2-}$, $R_2 = R_3 = H$, X = O, PI-3-P, **1**
$R_1 = R_2 = PO_3^{2-}$, $R_3 = H$, X = O, PI-3,4-P_2, **2**
$R_1 = R_2 = R_3 = PO_3^{2-}$, X = O, PI-3,4,5-$P_3$, **3**
R_4 = alkenyl, alkyl
$R_1 = R_2 = R_3 = PO_2S^{2-}$, X = O, PI-3,4,5-$PS_3$, **4**
$R_1 = R_2 = R_3 = PO_2S^{2-}$, X = S, PsI-3,4,5-$PS_3$, **5**
$R_4 = C_{15}H_{31}$

Despite the close structural analogy between phosphates and phosphorothioates, synthesis of the latter is more difficult. For example, a convenient hydrogenolytic deprotection of phosphate groups used by all methods reported to date[6] is not applicable in the presence of sulfur, and the instability of the P-S bond makes also a number of other deprotection methods unsuitable. Below we present a deprotection scheme that enables synthesis of phosphorothioate monoesters in the polyphosphate environment, in the presence of the labile acyl ester groups.

The previously synthesized triol **6**[6d,7] was treated with *O,O*-dibenzyl-*N,N*-diisopropylphosphoramidite to give the corresponding 3,4,5-trisphosphite **7**. This compound was added with elemental sulfur to produce the trisphosphorothioate **8**, which was consecutively treated with tetra-n-butylammonium fluoride to afford the

alcohol **9**. The reaction of this alcohol with *N,N*-diisopropyl-*O*-methyl phosphoroamidochloridite, followed by condensation of the resulting phosphoramidite with 1,2-dipalmitoyl-*sn*-glycerol (DPG) and oxidation with dinitrogen tetroxide or sulfurization gave the fully protected PI-3,4,5-Ps_3 **10** and the fully protected PsI-3,4,5-Ps_3 **11**, respectively.

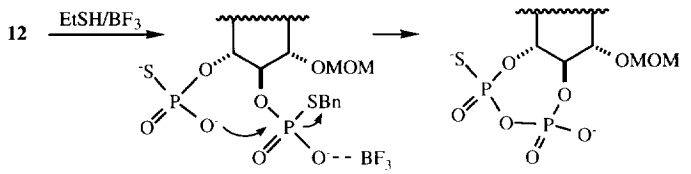


(i) iPr_2N-$P(OBn)_2$, tetrazole; (ii) S_8; (iii) $Bu_4N^+F^-$; (iv) Cl-P(OMe)($NiPr_2$), $EtiPr_2N$; (v) DPG, tetrazole; (vi) N_2O_4; vii: S_8; (viii) Me_3N

The demethylation of the phosphate group in the 1-position was performed using trimethylamine to give the corresponding phosphodiester group. This reagent was found to also simultaneously cleave benzyl groups from the *O,O*-dibenzylphosphorothioate residues at the 3, 4- and 5-positions to give the corresponding 3,4,5-tris-*O*-benzylphosphorothioates **12** and **13**. Due to the presence of chiral centers at each phosphorothioate group these products were mixtures of stereoisomers giving rise to complex ^{31}P NMR spectra. Further dealkylation of a phosphodiester to a phosphomonoester does not occur with this (and other nucleophilic reagents) due to a poor leaving group character of a phosphorothioate (phosphate) monoester dianion; a further conversion to a monoester requires some protic or Lewis acid catalysis.

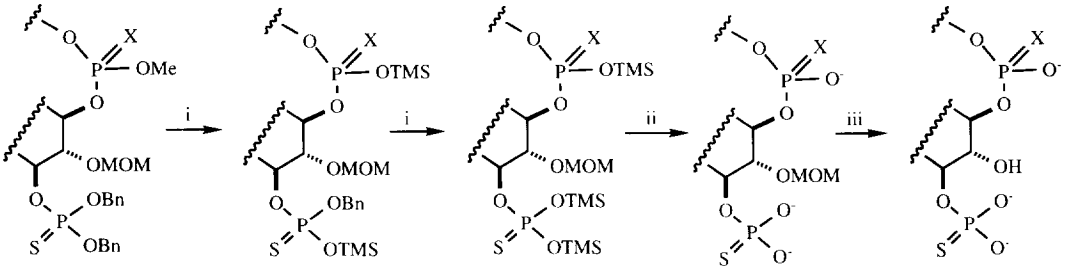
The typical approaches to cleave the mono-*O*-benzyl phosphorothioate group proved problematic. First, the debenzylation of the phosphodiester groups in **12** with ethanethiol-BF_3 was unsuccessful. The reaction with this reagent yielded large amounts of by-products (50%) giving rise to the ^{31}P NMR signals at 20-30 ppm, and additional by-products at ca. -10 ppm. The range of the observed chemical shifts suggested that in addition to the *O*-benzyl ester cleavage, a partial thiono-thiolo rearrangement[8] took place to give the corresponding *S*-benzyl derivatives **14** (δ ca. 20 ppm). These thiolesters could then undergo a nucleophilic displacement of the *S*-benzyl group by the adjacent phosphorothioate nucleophile to give a cyclic pyrophosphate **15** (δ ca. -10 ppm, see the scheme below). The attempted solvolysis of benzyl esters in TFA/$CHCl_3$ produced only the rearrangement and cyclization products **14** and **15**. The reaction of the phosphorothioate **12** with TMS iodide resulted in a partial

cleavage of the phosphodiester bond at the 1-position, rendering this potential method of deprotection unsuitable, as well. It has to be stressed, that due to the fact that all three phosphorothioate groups have to be deprotected to give the desired product, but that isomerization at any single phosphorothioate function produces an unusable side-product, a relatively low rate of isomerization translates into large losses of yield. Furthermore, the very polar nature of the final products and their low solubility in organic media essentially preclude product purification by typical chromatographic methods.



14 15

In contrast, the treatment of both esters **10** and **11** with the mixture of trimethylamine-TMS chloride afforded exclusively the desired persilylated derivatives of tris-monophosphorothioates **16** and **17**, respectively.[9] The rationale for using this reagent is to persilylate the initially formed tris-*O*-benzyl diester **12** to form the neutral tris-*O*-benzyl-*O*-trimethylsilylphosphorothioate **18**. This triester underwent further debenzylation, albeit at a slower rate than the original dibenzyl phosphate. The reaction was followed by ^{31}P NMR and the progress of dealkylations at the 3,4,5-positions and at the 1-position could be precisely evaluated due to the 10-13 ppm upfield shift upon replacement of each alkyl group by the silyl group, and due to the fact that only silylation at the oxygen atom (not sulfur) was observed. Thus, the removal of the first benzyl group resulted in the shift from ca. 70 ppm (*O*,*O*-dibenzyl phosphorothioate group in **10**) to 57 ppm (*O*-TMS-*O*-benzyl phosphorothioate group in **18**), and the removal of the second group gave a signal at 44 ppm (*O*,*O*-bisTMSphosphorothioate group in **16**). Analogously, the removal of the methyl group from the phosphate at the 1-position in the derivative **10** resulted in the shift from 2 ppm to -8 ppm. Since these dealkylations were neither regio- nor stereo-selective, a large number of isomeric compounds was formed at the intermediate reaction times, which eventually converged into two diastereomeric species of the product, due to a random silylation of the pro-*R* and pro-*S* oxygen atoms of the phosphate group at the 1-position in the product **16**.



10, X = O
11, X = S

18, X = O
19, X = S

16, X = O
17, X = S

20, X = O
21, X = S

4, X = O
5, X = S

i: TMS-Cl/Me_3N (1:4 w/w, used as solvent), 23°C, 10 days ; ii: acetate buffer; iii: EtSH/BF_3

The cleavage of the *O*-silyl derivatives **16** and **17** was achieved by aqueous hydrolysis at neutral pH to give exclusively MOM-ethers **20** and **21**. It is important that the neutral pH be maintained during the hydrolysis, as

the hydrolysis under unbuffered conditions lead to desulfurization and formation of phosphoanhydrides, apparently by the displacement of the sulfhydryl group by the adjacent phosphorothioate group, analogously as shown above. The MOM-derivatives **20** and **21** were finally deprotected by ethanethiol-BF_3 at room temperature to give the final products **4** and **5**, respectively. The obtained compounds **4** and **5** were fully characterized by ^1H and ^{31}P NMR and ES MS.[10,11] Their evaluation as potential inhibitors of inositol-related enzymes and metabolically stable analogs of PIP_3 is currently underway. We believe that in view of the mild character of the deprotection conditions described here and the lability of the synthesized analogs of PIP_3, this scheme should be well applicable to synthesis of other phosphorothioate monoester analogs of complex natural phosphates.

Acknowledgments: This work was supported in parts by The Department of Medicinal Chemistry and Pharmacognosy of UIC and the NIH grant GM30327.

REFERENCES and NOTES

1. Berridge, M. J. *Nature (London)* **1993**, *361*, 315.
2. Stephens, L. R.; Jackson, T. R.; Hawkins, P. T. *Biochim. Biophys. Acta* **1993**, *1179*, 27.
3. Woscholski, R.; Waterfield, M. D.; Parker, P. J. *J. Biol. Chem.* **1995**, *270*, 31001.
4. Herschlag, D.; Piccirilli, J. A.; Cech, T. R. *Biochemistry* **1991**, *30*, 4844.
5. Taylor, C. W.; Berridge, M. J.; Cooke, A. M.; Potter, B. V. L. *Biochem. J.* **1989**, *259*, 645.
6. Synthesis of the natural 3-phosphorylated phosphatidylinositols: (a) Falck, J. R.; Abdali, A. in *Inositol Phosphates and Derivatives. Synthesis, Biochemistry, and Therapeutic Potential*, Reitz, A. B. Ed.; *ACS Symp. Ser.* **1991**, *463*, 145; (b) Gou, D.-M.; Chen, C.-S. *J. Chem. Soc. Chem. Commun.* **1994**, 2125; (c) Watanabe, Y.; Hirofuji, H.; Ozaki, S. *Tetrahedron Lett.* **1994**, *35*, 123; (d) Bruzik, K. S.; Kubiak, R. J. *Tetrahedron Lett.* **1995**, 36, 2415; (e) Watanabe, Y.; Tomioka, M.; Ozaki, S. *Tetrahedron* **1995**, *33*, 8969; (f) Reddy, K. K.; Saady, M.; Falck, J. R.; Whited, G. *J. Org. Chem.* **1995**, *60*, 3385. (g) Desai, T.; Gigg, J.; Gigg, R.; Martin-Zamora, E. in *Synthesis in Lipid Chemistry*, Tyman, J. H. P., Ed.; Royal Society of Chemistry, London, 1995; (h) Prestwich, G. D. *Acc. Chem. Res.* **1996**, *29*, 503; (i) Wang, D.-S.; Chen, C.-S. *J. Org. Chem.* **1996**, *61*, 5905; (j) Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996**, *91*, 6305.
7. Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361.
8. The ^{31}P NMR spectra are simplified by the debenzylation reaction yielding the persilylated derivatives **16** and **17**, where only two diastereomers are present due to chirality at the silylated phosphate group at the 1-position.
9. Bruzik, K. S.; Stec, W. J. *J. Org. Chem.* **1981**, *46*, 1618; *ibid.* **1981**, *46*, 1625.
10. An analogous method employing DBU/TMS-Cl was used recently for the cleavage of the 2-cyanoethyl phosphate; Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Org. Chem.* **1992**, *57*, 1964.
11. The 1;1 ratio of diastereomers of the product **5** is indicated by the ^1H NMR spectrum of the derivative **11**.
12. **4** (NH_4^+): ^1H NMR ($CDCl_3$:CD_3OD:D_2O, 1:1:0.3) δ 0.89 (t, J = 6.7 Hz, 6 H), 1.22-1.38 (m, 48 H), 1.52-1.68 (m, 4 H), 2.29-2.37 (m, 4 H), 3.94-3.99 (m, 1 H), 4.04-4.11 (m, 3 H), 4.18-4.24 (m, 1 H), 4.32-4.37 (m, 1 H), 4.43 (m, 1 H), 4.52 (m, 1 H), 4.82 (m, 1 H), 5.27 (m, 1 H) (the two missing inositol protons are most likely overlapped by the broad OH signal); ^{31}P NMR ($CDCl_3$:CD_3OD:D_2O, 1:1:0.3) δ -0.61 (1P), 52.4 (2P), 53.2 (1P); ES MS $[M-H]^-$=1097. **5** (NH_4^+): ^1H NMR ($CDCl_3$:CD_3OD:D_2O, 1:1:0.3) δ 0.89 (t, J = 6,6 Hz, 6H), 1.27 (m, 48 H), 1.51-1.68 (m, 4 H), 2.29-2.36 (m, 4 H), 3.93-4.01 (m, 1 H), 4.03-4.38 (m, 6 H), 4.41-4.46 (m, 1 H), 4.79 (m, 1 H), 5.22-5.34 (m, 1 H); the missing inositol proton is most likely overlapped by a broad OH resonance; ^{31}P NMR ($CDCl_3$:CD_3OD:D_2O, 1:1:0.3) δ 52.3, (1P), 52.6 (1P), 53.2 (1P), 56.7 (1P); ES MS $[M-H]^-$=1113.

(*Received in USA* 26 *February* 1997; *accepted* 8 *April* 1997)