Tetrahedron Letters, Vol. 33, No. 24, pp. 3539-3542, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

THE SYNTHESIS OF CERAMIDE PHOSPHOINOSITOL

Alla Yu. Frantova, Alexander E. Stepanov, Anatoly S. Bushnev*,
Elena N. Zvonkova, Vitaly I. Shvets

Institute of Fine Chemical Technology, Department of Biotechnology,
prospekt Vernadskogo, 86, Moscow, 117571, Russia.

Abstract: *Total synthesis of the naturally occurring ceramide phosphoinositol has been accomplished via phosphite-triester method.*

Ceramide phosphoinositides are present in quite considerable quantities in higher plants, yeast, fungi[1,2], some bacterial cultures[3] and mutant yeasts[4]. Though their endogenic functions are not well understood, but they are believed to protect plant tissues from necrotic lesions and are supposed to be applicable as test for the diagnosis of histoplasmosis[2]. Recently studies of the lipopeptidophosphoglycan from *Trypanosoma cruzi* showed the presence of ceramide phosphoinositol as a constituent of this complex structure[5].

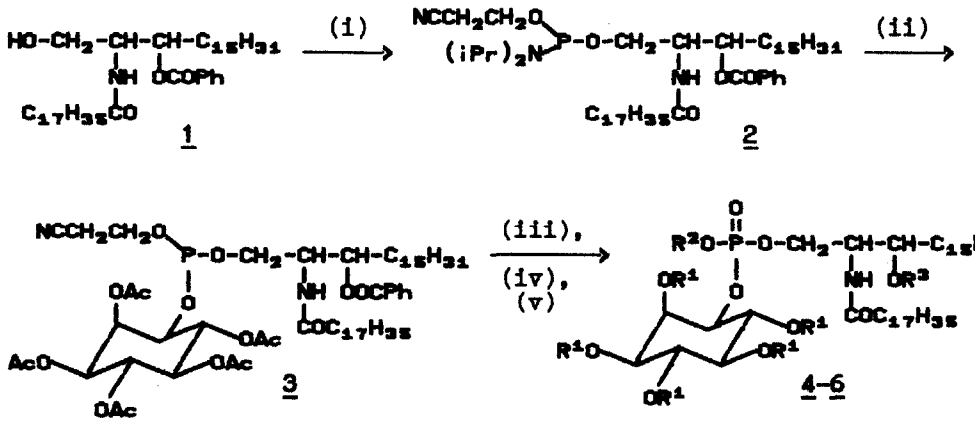
Inositol phosphosphingolipids isolated from natural sources contain ceramide and a *myo*-inositol part connected by a phosphodiester bond between 1-positioned hydroxyls from both moieties. The ceramide component as a rule has a 4-hydroxysphinganine structure N-acylated with hydroxy- or non-hydroxy fatty acids. The inositol moiety generally contains one or more monosaccharide residues attached at 2-OH or 6-OH of the *myo*-inositol cycle[1,2].

There has been quite a number of studies dealing with different synthetic approaches to major species inositol containing compounds such as phosphatidylinositols and their analogs[6,7]. Lately more reports appeared mentioning synthesis of *myo*-inositol phospholipid analogs[8,9], phosphothione derivatives of phosphatidylinositols included[10]. As to ceramide phosphoinositides, no communications appeared in the literature reporting the synthesis of these type *myo*-inositol lipids.

The aim of this work was to show that it is possible to synthesize compounds of this series beginning with the simplest one, ceramide

phosphoinositol. Our main purpose was to devise a synthetic route giving good yields of the target compounds and small losses of the reaction components, the latter being not easily available.

To build up the phosphodiester structure we chose the highly effective phosphoramidite approach, earlier used for phosphatidylinositols[8,10] and sphingophospholipids syntheses[11]. The present method was selected to minimize problems arising from side reactions, which have been observed in phosphorylation of 3-benzoylceramides by chlorophosphates[12].



	R¹	R²	R³
4	Ac	CH_2CH_2CN	PhCO
5	Ac	H	PhCO
6	H	H	H

Reagents:
(i) 7, diisopropylammonium tetrasolide;
(ii) 8, 1H-tetrazole; (iii) ^tBuOOH;
(iv) $^tBuNH_2$; (v) MeONa/MeOH

Thus the starting *rac*-3-benzoylceramide[13] 1 (0.75 mmol) was condensed with the bifunctional phosphitylating reagent 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite 7 (1.5 mmol) in the presence of diisopropylammonium tetrazolide (0.5 mmol) in CH_2Cl_2 (5 ml) under the argon atmosphere. After a work up with water and chromatographic purification (the eluting system buffered with triethylamine) we obtained phosphoramidite 2 (δ_p 148.10, 148.45 ppm[14]) with a 90-95% yield. Carefully dried (P_2O_5, high vacuo) phosphoramidite 2 (0.7 mmol) was coupled with 1(3),2,4(6),5,6(4)-pentaacetyl-*myo*-inositol[15] 8 (0.78 mmol) in a (1:2) mixture of CH_2Cl_2-acetonitrile (6 ml) in the presence of 1H-tetrazole (0.8 mmol) under dry argon. After 15 min TLC has shown

the amidite 2 to disappear with simultaneous appearance of a new diastereomeric products mixture of lower mobility. This was triester 3 (δ_p 142.63, 142.83, 143.22, 144.03 ppm) produced with a 80-90% yield as calculated from ^{31}P NMR data. The further oxydation of 3 *in situ* with tert-butylhydroperoxide afforded phosphotriester 4 (δ_p 0.49, 0.79, 1.10 ppm). The removal of 2-cyanoethyl group was performed by treating with tert.-butylamine for 30 min at 20°C. The standard extractive procedure and subsequent separation of the reaction mixture by chromatography gave phosphodiester 5 (δ_p 1.72, 1.89 ppm[16]) with a total yield of 75% as based on 2. Finally, deprotection of 2 with 0.1 M MeONa/MeOH followed by a washing with water and chromatographic purification on silica gel gave the target ceramide phospho-*myo*-inositol 6 (δ_p 1.44 ppm) as white crystals[17] with a yield of 91%.

References and Notes

1. R.A. Laine, *Chem. Phys. Lipids*, 42, 129 (1986); O. Lhomme, M. Bruneteau, C.E. Costello, P. Mas, P.M. Molot, A. Dell, P. Tiller and G. Michel, *Eur. J. Biochem.*, 191, 203 (1990).
2. K. Barr and R.L. Lester, *Biochemistry*, 23, 5581 (1984).
3. B.N. Singh, C.E. Costello, D.H. Beach and G.G. Holz, Jr., *Biochem. Biophys. Res. Commun.*, 157, 1239 (1988).
4. G.B. Weels and R.L. Lester, *J. Biol. Chem.*, 258, 10200 (1983).
5. R.M. Lederkremer, C. Lima, M.I. Ramirez and O.L. Casal, *Eur. J. Biochem.*, 192, 337 (1990).
6. A.E. Stepanov and V.I. Shvets, *Chem. Phys. Lipids*, 25, 247 (1979); V.I. Shvets, A.E. Stepanov, V.N. Krylova and P.V. Gulak, *myo-Inositol and phosphoinositides*, Moscow: Nauka Publishing House, 1987, 248 P. (in Russian).
7. D.C. Billington, *Chem. Soc. Rev.*, 18, 83 (1989).
8. C.J.J. Elie, C.E. Dreef, R. Verduyn, G.A. van der Marel and J.H. van Boom, *Tetrahedron*, 45, 3477 (1989); C.E. Dreef, C.J.J. Elie, P. Hoogerhout, G.A. van der Marel and J.H. van Boom, *Tetrahedron Lett.*, 29, 6513 (1988).
9. J.G. Ward and R.C. Young, *Tetrahedron Lett.*, 29, 6013 (1988); M. Jones, K.K. Rana and J.G. Ward, *ibid.*, 30, 5353 (1989).
10. G.M. Salamonczyk and K.S. Bruzik, *Tetrahedron Lett.*, 31, 2015 (1990); G. Lin, C.F. Bennett and M.D. Tsai, *Biochemistry*, 29, 2747 (1990).

11. K.S. Bruzik, *J. Chem. Soc. Chem. Commun.*, 329 (1986); K.S. Bruzik, *J. Chem. Soc. Perkin Trans 1*, 423 (1988); A.Yu. Frantova, A.S. Bushnev, E.N. Zvonkova and V.I. Shvets, *Bioorgan. Khim.*, 17, 1562 (1991).
12. E.N. Zvonkova, A.S. Bushnev, B.I. Mitsner and R.P. Evstigneeva, *Z. Org. Khim.*, 9, 31 (1973).
13. D. Shapiro, *"Chemistry of sphingolipids"*, Hermann, Paris, 1969. 111 P.
14. Compound 2 (a diastereomeric mixture due to the presence of a chiral phosphorus atom); ^{1}H NMR (200 MHz, $CDCl_3$), δ, ppm: 8.0–7.40 (m, 5H, C_6H_5), 6.12 (d, 1H, NH), 5.27 (dd, 1H, 3-CH), 4.45 (m, 1H, 2-CH), 3.83 (qrt, 2H, α-CH_2), 3.71 (qrt, 2H, 1-CH_2), 3.50 (m, 2H, α'-CH), 2.60 (t, 2H, β-CH_2), 2.22 (t, 2H, 3'-CH), 1.75 (m, 2H, 4-CH_2), 1.60 (m, 2H, 4'-CH_2), 1.25 [m, 54H, $(CH_2)_{13}$ and $(CH_2)_{14}$], 1.10 (m, 12H, CH_3), 0.90 (t, 6H, CH_3 chain). M.p. 40.5–41.5°C. Anal. Calc. for $C_{52}H_{94}N_3O_5P$: C 71.56, H 10.78, P 3.67; Found: C 71.51, H 10.65, P 3.64.
15. S.J. Angyal and M.E. Tate, *J. Chem. Soc.*, 6949 (1965).
16. Exact interpretation of the ^{1}H NMR spectra of compounds 5 and 6 is somewhat baffling, because many protons give overlapping multiplet signals, as the compounds synthesized are diastereomeric mixtures. Compound 5: ^{1}H NMR (200 MHz, $CDCl_3$–CD_3OD, 2:1), δ, ppm: 8.00–7.40 (m, 5H, C_6H_5), 5.70 (2t, J 15 Hz, 1H, 1-CH inositol), 3.90 (m, 2H, 1-CH_2), 5.60–2.70 (several overlapping multiplets), 1.80–2.05 (m, 15H, CH_3 acetyl), 1.50 (m, 4H, 4,4'-CH_2), 1.10 [m, 54H. $(CH_2)_{13}$ and $(CH_2)_{14}$], 0.80 (t, 6H, CH_3 chain). M.p. 44–45°C. Anal. Calc. for $C_{59}H_{98}O_{17}NP \cdot 0{,}5H_2O$: C 62.52, H 8.80, P 2.73; Found: C 62.54, H 8.94, P 2.46.
17. Compound 6: ^{1}H NMR (200 MHz, $CDCl_3$–CD_3OD–D_2O, 2:1:0.2), δ, ppm: the region of the proton signals from 3-CH of sphinganine (4.20–4.50) is overlapped by the broad signal from water molecules, 3.84 (m, 2H, 1-CH_2), 3.50–2.20 is a complex multiplet system, 12H, 2-CH and signals from inositol protons, 1.85 (t, 2H, 3'-CH_2), 1.20 (m, 4H, 4,4'-CH_2), 0.90 [m, 54H, $(CH_2)_{13}$ and $(CH_2)_{14}$], 0.50 (t, 6H, CH_3 chain). M.p. 155–160°C. Anal. Calc. for $C_{42}H_{84}O_{11}NP \cdot 3H_2O$: C 58.45, H 10.39, P 3.59; Found: C 58.86, H 10.20, P 3.20.

(Received in UK 29 January 1992)