Tetrahedron Letters,Vol.28,No.20,pp 2309-2312,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

# SYNTHESIS OF D-*myo*-INOSITOL 1,4,5-TRISPHOSPHATE

Colin B. Reese* and John G. Ward

Department of Chemistry, King's College London, Strand, London WC2R 2LS, England

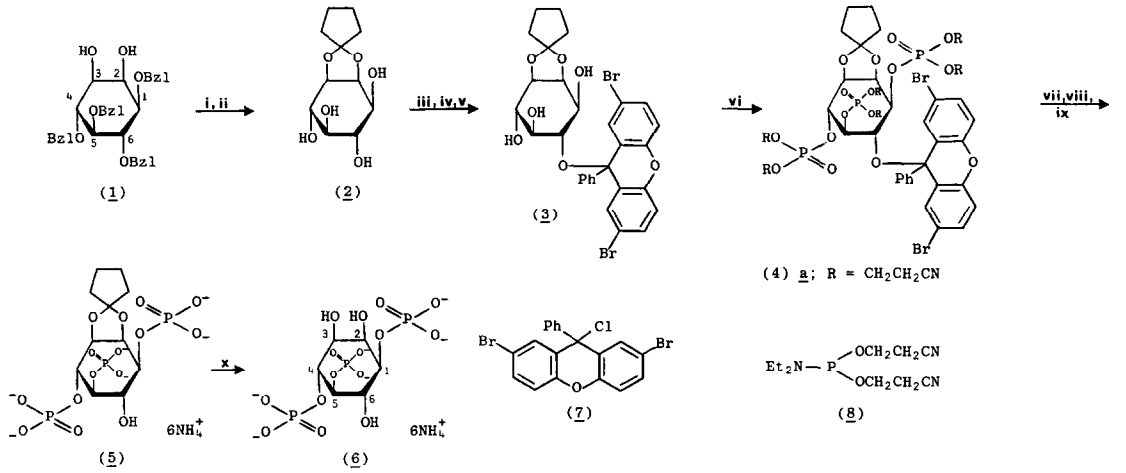
*Summary*: The conversion of *myo*-inositol into the ammonium salts both of racemic and enantiomerically pure D-*myo*-inositol 1,4,5-trisphosphate (6) is described. The n.m.r. spectroscopic properties and biological activity of synthetic and naturally-isolated (6) are virtually identical.

The recent discovery that D-*myo*-inositol 1,4,5-trisphosphate (6) acts as a second messenger[1] has, not unexpectedly, led to a renewed interest in synthetic studies[2,3] in the area of inositol phosphate chemistry. We now report a synthesis of (6) using an approach that should also be applicable to the preparation of analogues of (6) and other inositol phosphates.

We first undertook the synthesis of the racemic modification of *myo*-inositol 1,4,5-trisphosphate. Treatment of racemic 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol [(1), Scheme 1] prepared in three steps from *myo*-inositol by a modification of the published procedure[4], with an excess of 1,1-dimethoxypentane in the presence of toluene-*p*-sulphonic acid in acetonitrile solution gives its 2,3-cyclopentylidene derivative[5], m.p. 72-73°C, in quantitative yield. When this product is treated with sodium in liquid ammonia-tetrahydrofuran, racemic 2,3-cyclopentylidene-*myo*-inositol (2), m.p. 156-158°C, is obtained in 70% overall yield. The latter compound (2) is allowed to react first with t-butylchlorodimethylsilane [2.2 mol. equiv.] and imidazole [6.6 mol. equiv.] in acetonitrile solution[6] at room temperature for 5 hr, and then with 9-chloro-2,7-dibromo-9-phenylxanthene[7] [(7), 1.2 mol. equiv.] in pyridine-acetonitrile for 16 hr. Treatment of the products with an excess of tetraethylammonium fluoride in acetonitrile solution for 48 hr at room temperature, followed by chromatography on silica gel gives racemic 2,3-*O*-cyclopentylidene-6-*O*-(2,7-dibromo-9-phenylxanthen-9-yl)-*myo*-inositol (3)[9] as a crystalline solid [Found: C, 54.4; H, 4.2; Br, 24.4. $C_{30}H_{28}Br_2O_7$ requires: C, 54.57; H, 4.27; Br, 24.20%], m.p. 189-194°C, in 36% yield. Two, so far uncharacterized, more polar putative isomers of (3) are also obtained[11].

A solution of bis(2-cyanoethyl) diethylphosphoramidite[12] [(8); *ca.* 4.2 mmol] in dry dichloromethane is added (Scheme 1) to a stirred solution of the racemic protected *myo*-inositol derivative [(3); 0.32 mmol] and 1*H*-tetrazole [*ca.* 5.7 mmol] in dry dichloromethane at room temperature. After 1 hr, tetrahydrofuran-water (9:1 v/v) is added and, after a further period of 30 min, 2,6-lutidine and a large excess of t-butyl hydroperoxide[16] are added. The products are worked up after 16 hr and chromatographed on silica gel to give the racemic hexakis(2-cyanoethyl) ester of 2,3-*O*-cyclopentylidene-6-*O*-(2,7-dibromo-9-phenylxanthen-9-yl)-

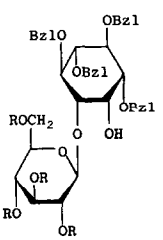
Scheme 1



Reagents: (i) 1,1-dimethoxycyclopentane, TsOH, MeCN; (ii) Na, liq. $NH_3$, tetrahydrofuran; (iii) $Bu^tSiMe_2Cl$, imidazole, acetonitrile; (iv) 9-chloro-2,7-dibromo-9-phenylxanthene (7), pyridine; (v) $Et_4NF$, acetonitrile; (vi) (a) $Et_2NP(OCH_2CH_2CN)_2$, tetrazole, $CH_2Cl_2$, (b) $Bu^tOOH$; (vii) $(Me_2N)_2C{=}NH$, EtOH-$H_2O$ (4:1 v/v); (viii) Dowex 50 ($H^+$ form); (ix) aqueous $NH_3$; (x) AcOH-$H_2O$ (2:1 v/v).

*myo*-inositol 1,4,5-trisphosphate (4a) as colourless crystals [Found: C, 47.3; H, 4.0; N, 6.9; Br, 13.4; P, 8.15. $C_{48}H_{49}N_6O_{16}P_3Br_2$ requires: C, 47.30; H, 4.05; N, 6.90; Br, 13.11; P, 7.62%], m.p. 133-135°C, in 50% yield.

The latter material [(4a); 0.07g] is readily unblocked (Scheme 1), under mild conditions, to give the ammonium salt of racemic *myo*-inositol 1,4,5-trisphosphate. When (4a) is treated with a large excess of $N^1,N^1,N^3,N^3$-tetramethylguanidine in ethanol-water (4:1 v/v) at 37°C for 24 hr and then at room temperature for 48 hr, the $N^1,N^1,N^3,N^3$-tetramethylguanidinium salt of (4; R = H) is obtained. When this product is dissolved in water (pH ~6) at room temperature, the 6-O-(2,7-dibromo-9-phenylxanthen-9-yl) protecting group is spontaneously removed[17] in *ca.* 1 hr. The aqueous products are passed through a Dowex 50 × 8 ($H^+$ form) cation exchange column, the eluate basified with aqueous ammonia and evaporated to dryness under reduced pressure. The residue, which is assumed to be (5) is then dissolved in acetic acid-water (2:1 v/v) at room temperature. After 2 hr, the products are evaporated to dryness under reduced pressure, re-evaporated with absolute ethanol, triturated with ether and then dried to give the putative hexa-ammonium salt of racemic *myo*-inositol 1,4,5-trisphosphate (6) as a colourless solid [0.014g, 33% yield, based on (4a)].



(9) a; R = Ac
b; R = H

Enantiomerically pure 1,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol (1) was obtained[18] from its 1-β-D-(2,3,4,6-tetra-*O*-acetyl)glucopyranosyl derivative (9a) using a slight modification of the procedure reported by Stepanov *et al.*[19]; this compound was converted into the putative hexa-ammonium salt of D-*myo*-inositol 1,4,5-trisphosphate (6) in the same way (see above, including Scheme 1) as the racemic modification of (1) was converted into the racemic modification of (6).

The n.m.r. spectra both of synthetic D-*myo*-inositol 1,4,5-trisphosphate[20] (6) and the racemic modification were identical to the spectra obtained[21] from natural material. Synthetic D-enantiomer (6) showed virtually identical activity to naturally-isolated (6)[1] in causing unfertilized sea urchin eggs to elevate fertilization envelopes[22], in the activation of transient hyperpolarisation following microinjection into N115-neuroblastoma cells[23] and in the release of calcium ions from permeabilised rat acinar cells[1]. In the last system, the racemic modification appeared to be significantly less active than the enantiomerically pure material.

Acknowledgements. We thank Professor Peter Baker of the Department of Physiology, King's College London for suggesting in the first place that we should undertake this study, and the Medical Research Council for generous financial support. We also thank Dr. John Lindon and his colleagues at the Wellcome Research Laboratories, Beckenham for measuring n.m.r. spectra of (6), Mr. Karl Swann (of the Department of Physiology, University College London), Dr. Irene Schulz and Mr. Sebastian Palm (of the Max Planck Institute for Biophysics, Frankfurt) and Dr. Ben Tilly (of the Netherlands Institute for Developmental Biology, Utrecht) for carrying out the biological tests, and Smith Kline & French Research Ltd., Welwyn for their help and cooperation. Finally, we thank Mr. Liu Changsheng for his assistance with the final stages of the synthetic work.

## REFERENCES AND FOOTNOTES

1 H. Streb, R.F. Irvine, M.J. Berridge, and I. Schulz, *Nature* 306, 67 (1983); M.J. Berridge and R.F. Irvine, *ibid.* 312, 315 (1984).

2 J. Gigg, R. Gigg, S. Payne, and R. Conant, *Carbohydr. Res.* 140, c1 (1985); *ibid.* 142, 132 (1985).

3 S. Ozaki, Y. Watanabe, T. Ogasawara, Y. Kondo, N. Shiotani, H. Nishii, and T. Matsuki, *Tetrahedron Lett.* 3157 (1986).

4 R. Gigg and C.D. Warren, *J. Chem. Soc. (C)* 2367 (1969).

5 A. Hampton, J.C. Fratantoni, P.M. Carroll, and S. Wang, *J. Am. Chem. Soc.*, 87, 5481 (1965).

6 Compound (2) has four equatorially-disposed secondary hydroxy functions that are difficult to distinguish between chemically. Preliminary studies, which need to be confirmed, have suggested that the 1- and 4-hydroxy functions of (2) are more susceptible to attack by t-butylchlorodimethylsilane than the 5- and 6-hydroxy functions.

7 Reaction between 2,7-dibromoxanthen-9-one[8] and phenylmagnesium bromide in ether solution gives 2,7-dibromo-9-phenylxanthen-9-ol as a crystalline solid, m.p. 149.5 - 150.5°C, in 69% yield. 9-Chloro-2,7-dibromo-9-phenylxanthene (7) is obtained as a pale yellow-orange solid by treating the latter alcohol with a large excess of acetyl chloride in anhydrous benzene solution at room temperature.

8 A. Lespagnol and J. Dupas, *Bull. Soc. Chim. France* 4, 541 (1937).

9 Treatment of (3) with a large excess of methyl iodide in the presence of barium oxide and barium hydroxide in anhydrous dimethylformamide[10] gives its 1,4,5-tri-*O*-methyl ether in nearly quantitative yield. When a solution of the latter material (0.04g) in dichloromethane (2.4 ml) and $d_4$-methanol (0.5 ml) is treated with trifluoroacetic acid (0.036 ml) at room temperature for 20 min, 2,3-cyclopentylidene-1,4,5-tri-*O*-methyl-*myo*-inositol is obtained. The structure of the latter compound follows clearly from its COSY spectrum (250 MHz, $d_6$-dimethyl sulphoxide), and this is the evidence on which the structure assigned to compound (3) is based. The latter compound (3) has a strong chromophore [$\lambda_{max}$ (95% EtOH) 303, 293, 253 (ε 3 600, 2 500, 23 000), $\lambda_{min}$ 297, 276 nm (ε 2 200, 1 200)] which greatly facilitates its detection, for example, on t.l.c. plates.

[10]R. Kuhn and H. Trischmann, *Chem. Ber.* 96, 284 (1963).

[11]The $R_F$'s (in ether) of these compounds, which are obtained in 13 and 12% yields, are 0.22 and 0.44, respectively; the $R_F$ (in ether) of compound (3) is 0.58.

[12]Bis(2-cyanoethyl) diethylphosphoramidite (8) is prepared by adding a solution of 2-cyanoethanol (1.57 ml, 23 mmol) and triethylamine (3.2 ml, 23 mmol) in anhydrous ether (15 ml) to a stirred solution of diethylphosphoramidous dichloride[13] (2.0g, 11.5 mmol) in ether (5 ml) at room temperature. After 1 hr, the products are filtered and the filtrate is evaporated under reduced pressure to give crude (8) as a gum. The latter material is always freshly prepared and is used without further purification. Other workers have very recently used bis(2-cyanoethyl) di-isopropylphosphoramidite[14] and bis-benzyl diethylphosphoramidite[15] as phosphitylating agents in a similar way.

[13]K. Issleib and W. Seidel, *Chem. Ber.* 92, 2681 (1959).

[14]E. Uhlmann and J. Engels, *Tetrahedron Lett.* 27, 1023 (1986).

[15]J.W. Perich and R.B. Johns, *Tetrahedron Lett.* 28, 101 (1987).

[16]A. Jäger and J. Engels, *Tetrahedron Lett.* 25, 1437 (1984).

[17]We had not anticipated that this protecting group would be so sensitive to hydrolytic cleavage. However, in (4; R = H) the equatorially-disposed 6-*O*-(2,7-dibromo-9-phenyl-9-xanthenoxy) group is flanked both by the equatorially-disposed 1- and 5-monophosphate groups. Presumably the latter functions provide considerable neighbouring group assistance to the hydrolytic process.

[18]Although the 1,4,5,6-tetra-*O*-benzyl-D-*myo*-inositol (1) was obtained by the acidic hydrolysis of what appeared to be its diastereoisomerically pure 1-β-D-glucopyranoside (9b), it had $[\alpha]_D^{20}$ = +18.8° (*c* 0.99, in $CHCl_3$) [lit.[19] + 25.1° (*c* 0.22, in $CHCl_3$)].

[19]A.E. Stepanov, B.A. Klyashchitskii, V.I. Shvets, and R.P. Evstigneeva, *Bioorg. Khim.* 2, 1627 (1976).

[20]$\delta_H$ [$D_2O$, pD 5.75, 360 MHz] 3.82 (1H, dd, *J* 2.8, 9.8 Hz), 4.01 (1H, t, *J* 9.5 Hz), 4.12 (2H, m), 4.37 (2H, m); $\delta_P$ [$D_2O$, pD 10.7, 145.8 MHz] 3.47 (d, *J* 3.7 Hz), 5.13 (d, *J* 7.0 Hz), 5.26 (d, *J* 7.6 Hz).

[21]J.C. Lindon, D.J. Baker, R.D. Farrant, and J.M. Williams, *Biochem. J.* 233, 275 (1986).

[22]M. Whitaker and R.F. Irvine, *Nature* 312, 5995 (1984).

[23]H. Higashida and D.A. Brown, *Nature* 323, 333 (1986).

(Received in UK 4 March 1987)