Tetrahedron Letters, Vol 32, No 42 pp 6021 6024, 1991
Printed in Great Britain

0040-4039/91 $3 00 + 00
Pergamon Press plc

Synthesis of 5-phosphonate analogues of *myo*-inositol 1,4,5-trisphosphate: possible intracellular calcium antagonists

C E Dreef[a], W Schiebler[b], G A van der Marel[a] and J H van Boom[a]

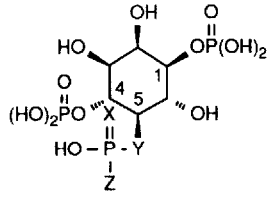
[a]*Gorlaeus Laboratories, P O Box 9502, 2300 RA Leiden, The Netherlands*
[b]*Hoechst AG, Pharma Forschung H 825, D-6230 Frankfurt am Main, FRG*

ABSTRACT The racemic 5-phosphonate analogues **IV** and **V** of *myo*-inositol 1,4,5-trisphosphate were readily accessible by bisphosphorylation of the common precursor **6**, removal of the *p*-methoxybenzyl group, phosphonylation and subsequent hydrogenolysis of the benzyl protecting groups The methylphosphonate analogue **IV** acted as a calcium antagonist in permeabilized human platelets, whereas the (difluoromethyl)phosphonate **V** exhibited only very little antagonistic activity

It is generally accepted now that hydrolysis of phosphatidylinositol [4,5]bisphosphate by receptor mediated activation of phospholipase C results in the formation of *myo*-inositol 1,4,5-trisphosphate (Ins[1,4,5]P_3)[1,2] and diacylglycerol[3] The intracellular second messenger Ins[1,4,5]P_3 is responsible for the release of calcium ions from intracellular stores located in the endoplasmic reticulum Deactivation of Ins[1,4,5]P_3 may proceed *via* two distinct pathways The major pathway for termination of the Ins[1,4,5]P_3 action is dephosphorylation by a specific 5-phosphatase[4] to give Ins[1,4]P_2, whereas the alternative pathway involves the phosphorylation of Ins[1,4,5]P_3 by a specific 3-kinase[5] to furnish the putative second messenger Ins[1,3,4,5]P_4[6]

The importance of Ins[1,4,5]P_3 as a calcium mobilizing intracellular second messenger has stimulated a considerable interest[7,8] in the synthesis of Ins[1,4,5]P_3 (**I**) and derivatives thereof Recently, the synthesis of the 5-phosphorothioate (*i e* **II**[9]) and 5-methylenephosphonate (*i e* **III**[10]) analogues of Ins[1,4,5]P_3, which both act as long-lived agonists, has been published

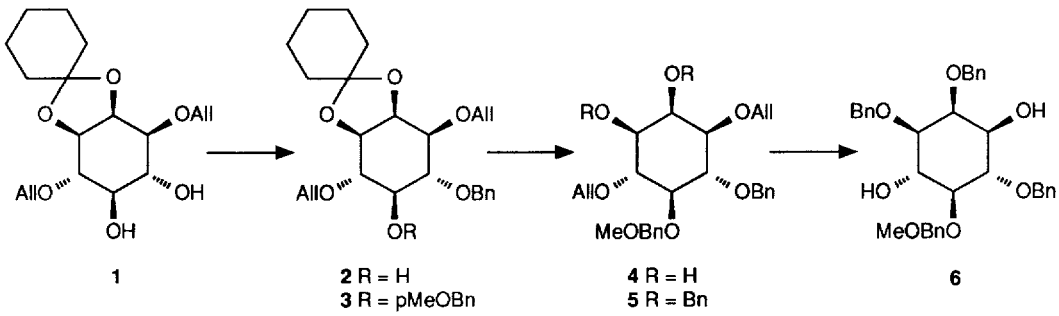
As part of a continuous programme directed towards the synthesis of *myo*-inositol phosphates and analogues thereof[11-13], we now report a convenient synthesis of the racemic *myo*-inositol 1,4,5-trisphosphate analogues **IV** and **V** the 5-phosphate of which is replaced by a phosphonate function



I X = Y = Z = O
II X = S, Y = Z = O
III X = Z = O, Y = CH_2
IV X = Y = O, Z = CH_3
V X = Y = O, X = CHF_2

The synthetic route we adopted for a successful conclusion of the racemic target compounds **IV** and **V** commences (see Scheme 1) with the preparation of the common precursor **6** the C-5 hydroxyl function of which is protected with a temporary *p*-methoxybenzyl protecting group Regioselective

Scheme 1



1 **2** R = H, **3** R = pMeOBn **4** R = H, **5** R = Bn **6**

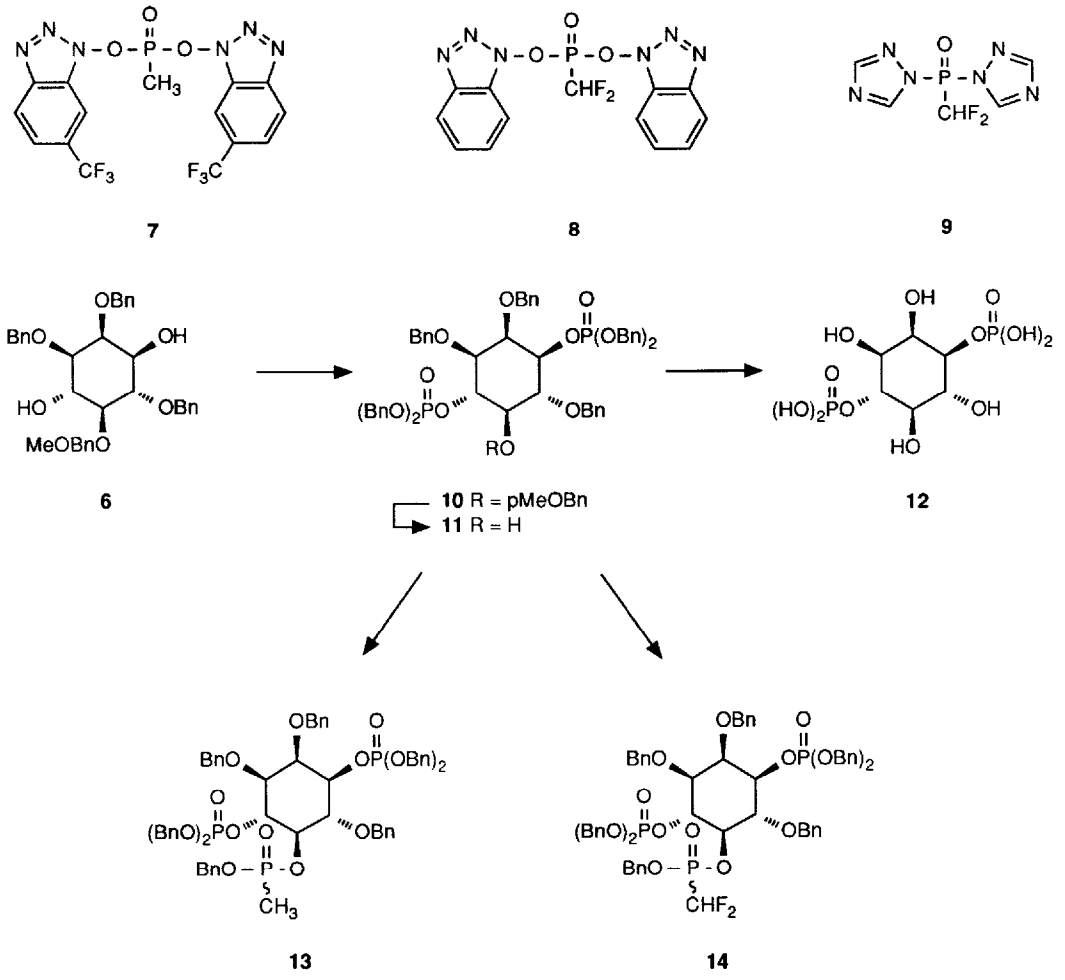
benzylation of the known 1,4-di-*O*-allyl-2,3-*O*-cyclohexylidene *myo*-inositol (**1**)[11 13] under phase transfer conditions[14] (BnBr/5% NaOH/Bu_4NHSO_4/CH_2Cl_2, 24 h at 45°C) afforded the mono-benzyl derivative **2**[15 16] in 53% yield Subsequent *p*-methoxybenzylation (*p*-MeOBnCl/NaH/DMF) of **2**, followed by acidic hydrolysis (0 05 N HCl in MeOH) of the 1,2-*cis*-cyclohexylidene group from **3** gave the crystalline diol **4** (mp 97-98°C) in 87% yield Benzylation (BnBr/NaH/DMF) of **4** furnished the fully protected *myo*-inositol derivative **5** (mp 69 5-70 5°C) in 97% yield Isomerization[17] of the allyl groups in **5** with 1,5-cyclooctadiene-bis[methyldiphenylphosphine]iridium hexafluorophosphate[18] (activated with H_2 for 2 min) in 1,2-dichloroethane, followed by mild acidic hydrolysis (0 1 N HCl in CH_2Cl_2/MeOH (1/1, v/v)) of the intermediate *trans*-prop-1-enyl groups, resulted in the isolation of the racemic 1,4-diol **6** (mp 97 5-98 5°C) in 92% yield

The next stage in the synthesis of the Ins[1,4,5]P_3 analogues **IV** and **V** entailed (see Scheme 2) phosphorylation of the properly protected *myo*-inositol derivative **6**, subsequent removal of the temporary protecting group and introduction of the respective phosphonate functions Thus, phosphitylation of diol **6** with *N,N*-diisopropyl dibenzyl phosphoramidite[12 19] in the presence of 1*H*-tetrazole, followed by oxidation of the intermediate phosphite-triesters with *tert*-butyl hydroperoxide[20] afforded the fully protected *myo*-inositol 1,4-bisphosphate **10** in 94% yield Cleavage of the *p*-methoxybenzyl group from **10** was effected by acidolysis (2 5% CF_3COOH in CH_2Cl_2) to afford the 5-OH-derivative **11** in 82% yield Hydrogenolysis of compound **11** resulted in the isolation of *myo*-inositol 1,4-bisphosphate (**12**), confirming that no phosphate migration had occurred during the removal of the *p*-methoxybenzyl group from **10**

The methylphosphonate function was introduced at the 5-position of **11** by a two-step one-pot phosphonylation procedure[21] Thus, phosphonylation of alcohol **11** with an excess of the bifunctional agent bis[6-(trifluoromethyl)benzotriazol-1-yl] methylphosphonate (**7**) gave, after 15 min at 20°C, the putative [6-(trifluoromethyl)benzotriazol-1-yl] methylphosphonate intermediate The latter was treated *in situ* with benzyl alcohol in the presence of *N*-methylimidazole to give, after 1 h at 20°C, the fully protected Ins[1,4,5]P_3 analogue **13** (diastereomeric mixture, ratio 1 3) in 75% overall yield Finally, hydrogenolysis (H_2/Pd(C)/(MeOH/H_2O, 4/1, v/v)) of compound **13** under pressure for 16 h gave the 5-methylphosphonate analogue of Ins[1,4,5]P_3 (**IV**), which was isolated as its sodium-salt[22]

On the other hand, the introduction of the (difluoromethyl)phosphonate function at the 5-position of **11** employing the reactive bifunctional agent bis(benzotriazol-1-yl) (difluoromethyl)phosphonate (**8**)[23] was unsuccessful The enhanced reactivity of **8** may be explained by the presence of the strongly electron withdrawing difluoromethyl group We reasoned that replacement of the benzotriazolyl- by triazolyl-groups would provide a less reactive phosphonylating agent Indeed, treatment of the alcohol **11** with an excess of the new bifunctional agent (difluoromethyl)phosphonic di(1,2,4-triazolide) (**9**)[24], followed by the addition of benzyl alcohol to the intermediate (difluoromethyl)phosphonic 1,2,4-triazolide in the presence

Scheme 2



of *N*-methylimidazole, afforded compound **14** (diastereomeric mixture, ratio 1 1) in 70% yield Finally, deprotection of compound **14** by hydrogenolysis (H_2/Pd(C)/(MeOH/H_2O, 4/1, v/v)) under pressure for 16 h yielded the sodium-salt of compound **V**[22]

Preliminary biological evaluation of the Ins[1,4,5]P_3 analogues **IV** and **V** indicated that the methylphosphonate analogue **IV** acted as a calcium antagonist in permeabilized human platelets, when Ins[1,4,5]P_3 was used to stimulate calcium release The calcium antagonistic effect of compound **IV** was not due to a chelation of calcium[25] The Ins[1,4,5]P_3 analogue **V** exhibited only very little antagonistic activity Full biological data of the compounds **IV** and **V** will be published elsewhere in due course

In conclusion, the results presented in this paper clearly demonstrate that the 5-phosphonate analogues **IV** and **V** are readily accessible from the common precursor **6** Furthermore, we believe that the *myo*-inositol derivative **11** may be of great value for the preparation of other 5-modified Ins[1,4,5]P_3 analogues At present, we are investigating the feasibility to prepare the corresponding 5-modified analogues of Ins[1,3,4,5]P_4, which may be of great value to study in detail the precise role of the putative second messenger Ins[1,3,4,5]P_4

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 Nishizuka, Y *Nature* **1984**, *308*, 693

4 Storey, D J , Shears, S B , Kirk, C J , Michell, R H *Nature* **1984**, *312*, 374

5 Irvine, R F , Letcher, A J , Heslop, J P , Berridge, M J *Nature* **1986**, *320*, 631

6 Batty, I R , Nahorski, S R , Irvine, R F *Biochem J* **1985**, *232*, 211

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 , Noble, N J , Payne, S , Gigg, R , Potter, B V L *J Chem Soc , Chem Commun* **1989**, 269

10 Falck, J R , Abdali, A , Wittenberger, S J *J Chem Soc , Chem Commun* **1990**, 953

11 Dreef, C E , Van der Marel, G A , Van Boom, J H *Recl Trav Chim Pays-Bas* **1987**, *106*, 161

12 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

13 Dreef, C E , Tuinman, R J , Lefeber, A W M , Elie, C J J , Van der Marel, G A , Van Boom, J H *Tetrahedron* **1991**, *47*, 4709

14 Jiang, C , Moyer, J D , Baker, D C *J Carbohydr Chem* **1987**, *6*, 319

15 Apart from the mono-benzylated derivative **2**, the corresponding positional isomer and the dibenzylated derivative were isolated in 36% and 5% yield, respectively

16 All new compounds were characterized by ^{1}H- and ^{13}C-NMR as well as combustion analysis

17 Oltvoort, J J , Van Boeckel, C A A , De Koning, J H , Van Boom, J H *Synthesis* **1981**, 305

18 Haines, L M , Singleton, E *J Chem Soc Dalton Trans* **1972**, 1891

19 Yu, K -L , Fraser-Reid, B *Tetrahedron Lett* **1988**, *29*, 979

20 Engels, J , Jager, A *Angew Chem Suppl* **1982**, 2010

21 Dreef, C E , Douwes, M , Elie, C J J , Van der Marel, G A , Van Boom, J H *Synthesis* **1991**, 443

22 Compound **IV** ^{31}P-NMR (D_2O, pH = 2 00) δ 0 29, 1 20 (P-1 and P-4) and 31 68 (P-5) ^{1}H-NMR (D_2O, pH = 2 00) δ 1 48 (d, 3H, CH_3, $J_{H,P}$ = 17 5 Hz), 3 75 (dd, 1H, H-3, $J_{3,4}$ = 9 5 Hz), 3 88 (dd, 1H, H-6, $J_{6,1}$ = 10 0 Hz), 4 03 (ddd, 1H, H-1, $J_{1,2}$ = 2 5 Hz, $J_{H,P}$ = 8 5 Hz), 4 12 (ddd, 1H, H-5, $J_{5,6}$ = 9 5 Hz, $J_{H,P}$ = 9 0 Hz), 4 28 (dd, 1H, H-2, $J_{2,3}$ = 3 0 Hz), 4 34 (ddd, 1H, H-4, $J_{4,5}$ = 9 0 Hz, $J_{H,P}$ = 9 0 Hz) Compound **V** ^{31}P-NMR (D_2O, pH = 2 00) δ 0 27, 1 16 (P-1 and P-4) and 4 68 (P-5, $J_{P,F}$ = 85 0 Hz) ^{1}H-NMR (D_2O, pH = 2 00) δ 3 78 (dd, 1H, H-3, $J_{3,4}$ = 9 5 Hz), 3 89 (dd, 1H, H-6, $J_{6,1}$ = 10 0 Hz), 4 03 (ddd, 1H, H-1, $J_{1,2}$ = 2 5 Hz, $J_{H,P}$ = 8 5 Hz), 4 13 (ddd, 1H, H-5, $J_{5,6}$ = 9 5 Hz, $J_{H,P}$ = 9 0 Hz), 4 27 (dd, 1H, H-2, $J_{2,3}$ = 3 0 Hz), 4 34 (ddd, 1H, H-4, $J_{4,5}$ = 9 0 Hz, $J_{H,P}$ = 9 0 Hz), 6 09 (ddd, 1H, CHF_2, $J_{H,F}$ = 49 0 Hz, $J_{H,P}$ = 24 0 Hz)

23 Bergstrom, D E , Shum, P W *J Org Chem* **1988**, *53*, 3953

24 (Difluoromethyl)phosphonic di(1,2,4-triazolide) (**9**) was prepared by dropwise addition of (difluoromethyl)phosphonic dichloride[23] (5 0 mmol) in dioxane (5 ml) to a cooled solution (0°C) of 1,2,4-triazole (12 5 mmol) and triethylamine (10 0 mmol) in dioxane (20 ml) After stirring for 1 h at 20°C, the salts were removed by filtration The thus obtained 0 2 M stock solution of **9** could be stored for several weeks at -20°C

25 Addition of compound **IV** to a control buffer solution containing only the fluorescent dye, used to measure the calcium concentration and calcium at low (≈ 100 nM) or high (100 μM) concentrations, did not show any detectable change in calcium induced fluorescence Furthermore, a first series of binding experiments of ^{3}H-Ins[1,4,5]P_3 in the presence of compound **IV** to membranes from bovine adrenocortical microsomes indicated that **IV** is a competitive receptor antagonist of the Ins[1,4,5]P_3-receptor

(Received in UK 14 August 1991)