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INFLUENCE OF THE ABSOLUTE CONFIGURATION AT C-4 IN THE BINDING OF D-MYO INOSITOL 1,4,5 TRISPHOSPHATE ANALOGUES TO IP3 RECEPTOR

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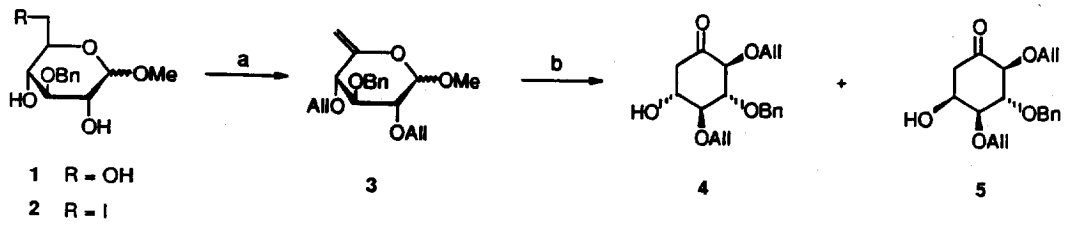
Abstract : Biomimetic syntheses of enantiomerically pure 3-deoxy-D-*muco*- and D-*myo*-inositol-1,4,5 trisphosphate from D-glucose are described. Preliminary biological studies show a dramatic influence of the stereochemistry at C-4 on the binding to IP_3 receptor.

Since the discovery of the role of D-*myo*-inositol 1,4,5-trisphosphate (IP_3) as a second intracellular messenger,[1,2,3] the synthesis and biological evaluation of analogues has received much interest.[4,5] Among them, several analogues have been used to determine the functions required for the biological activity. It has been shown in particular that 3-deoxy and 3-substituted IP_3 analogues are agonists of the natural messenger.[6,7,8,9] Due to the lack of hydroxyl substituent, these analogues do not undergo phosphorylation by 3-kinase in a metabolic pathway of IP_3 which forms inositol 1,3,4,5 tetrakisphosphate, whose biological role remains a matter of debate.[10] Consequently the use of 3-deoxy IP_3 as a pharmacological tool is of interest. Provided it should be accessible, it could be also a good starting material for the synthesis of more lipophilic derivatives which could cross the cellular membrane and could be of interest for biological studies on intact cells. We have developed a new approach to 3-deoxy IP_3 and its C-4 epimer 3-deoxy-*muco*-inositol 1,4,5-trisphosphate and we report in this manuscript the synthesis and preliminary biological evaluation of these compounds.

Previous synthesis of 3-deoxy IP_3 makes use of L quebrachitol[6,8] as a starting compound. We choose an approach in which the carbocyclic ring is constructed from a suitably protected carbohydrate precursor. Given the availability of the Ferrier carbocyclisation[11], we started from D-glucose.[12] Biosynthesis of IP_3 occurs from glucose the C-4 of which becomes the C-1 of inositol.[13,14] Thus a permanent protecting group must be introduced at C-3 (future C-6). The methyl glucoside derivative **1** was prepared by standard chemistry. Selective tosylation of **1** followed by iodine substitution gave the iodo derivative **2** which was converted into the protected olefin **3** in one step according to our previously reported procedure.[15] Ferrier carbocyclisation of **3** using a catalytic procedure [16] gave a 1/6 mixture of epimers at C-4 (inositol numbering) **4** and **5** in 57% yield.To the best of our knowledge, this is the first example of a Ferrier carbocyclisation in the presence of allyl ethers as protecting groups; no interaction of Hg^{2+} with the allyl ether was observed.

After separation of **4** ($[\alpha]_D$ = - 29.9°, c 1.1, $CHCl_3$) and **5** ($[\alpha]_D$ = - 23.7°, c 1, $CHCl_3$), protection of the hydroxyl group as a tetrahydropyranyl ether[17] gave **6** or **7** in 70% yield . For the synthesis of 3-deoxy IP_3, compound **6** was reduced using $NaBH_4$ to provide alcohol **8** which was benzylated under standard conditions to give **10** in 63 % yield.



1 R = OH
2 R = I

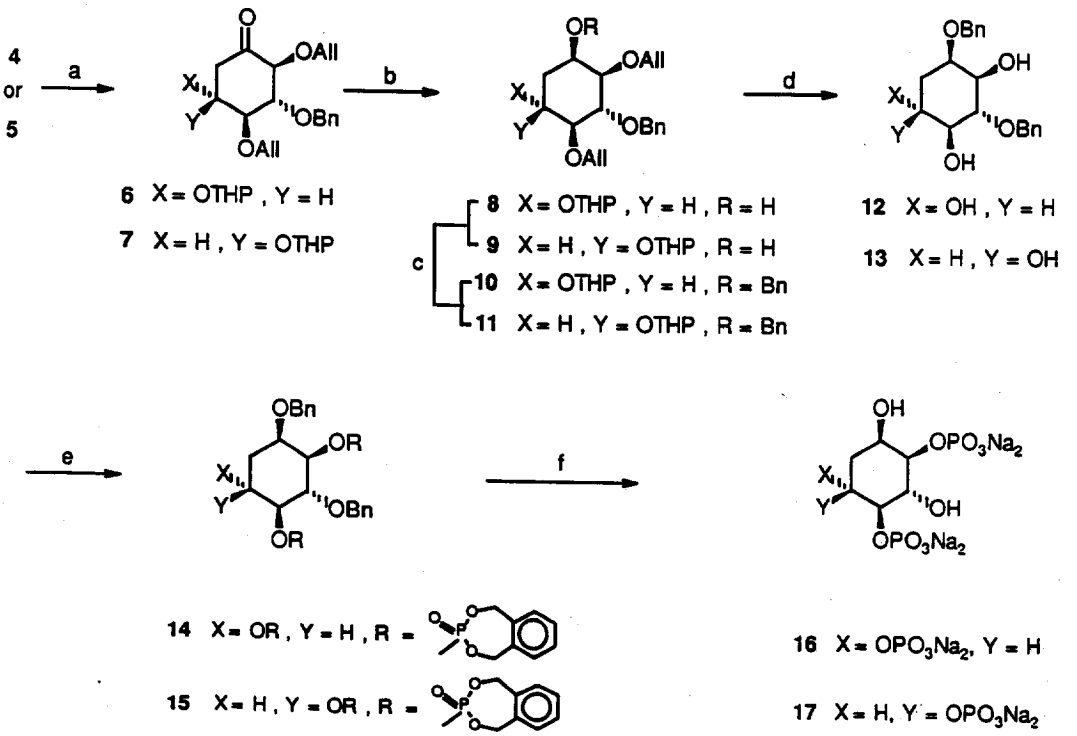
3

4

5

Reagents: a) NaH, AllBr, DMF, 60 %; b) $HgSO_4$, H_2SO_4 (5mM), 1,4-dioxane, 60° C, 57 %.

Conventional removal of allyl groups using potassium *t*-butoxide in DMSO[18] followed by acid treatment gave the triol **12** in 60 % yield. Treatment of this triol with 2-(*N*,*N*-diisopropylamino)-5,6-benzo-1,3,2 dioxaphosphepane[19] in the presence of tetrazole gave the expected trisphosphite which was immediately oxidized with *t*-butyl hydroperoxide into the trisphosphate **14**. Extensive purification by column chromatography of this material was then performed. Pure **14** was obtained as shown by the presence of a set of three ^{31}P signals. Deprotection was cleanly effected by hydrogenolysis under 10 atm. in the presence of palladium (10 %) on charcoal. After removal of the catalyst, neutralisation with sodium hydroxide gave 3-deoxy IP_3 hexasodium salt **16** in 85 % yield.



Reagents: a) DHP, APTS, CH_2Cl_2, 70 %; b) $NaBH_4$, MeOH; c) NaH, BnBr, DMF; d) tBuOK, DMSO, 50° C then HCl, MeOH reflux, 60 %; e) $(iPr)_2NP(OCH_2)_2C_6H_4$, CH_2N_4, then $tBuO_2H$, CH_2Cl_2, 47 %; f) H_2, 10 % Pd/C, 10 atm, MeOH then NaOH, 85 %.

Accordingly the 3-deoxy-*muco*-inositol derivative (mIP_3)[20] was prepared from **7**. In this case the reduction of the carbonyl group of **7** proceeded in a highly stereoselective fashion using sodium borohydride giving **9** in 68 % yield. Benzylation of the resulting hydroxyl group gave **11** which upon treatment with potassium *t*-butoxide in DMSO followed by acid hydrolysis gave **13**. The phosphorylation sequence already described for **12** gave pure **15** in 47 % overall yield. Once again the purity of **15** was checked by ^{31}P nmr. Deprotection of **15** gave 3-deoxy-D-*muco* IP_3 **17** in 82 % yield.

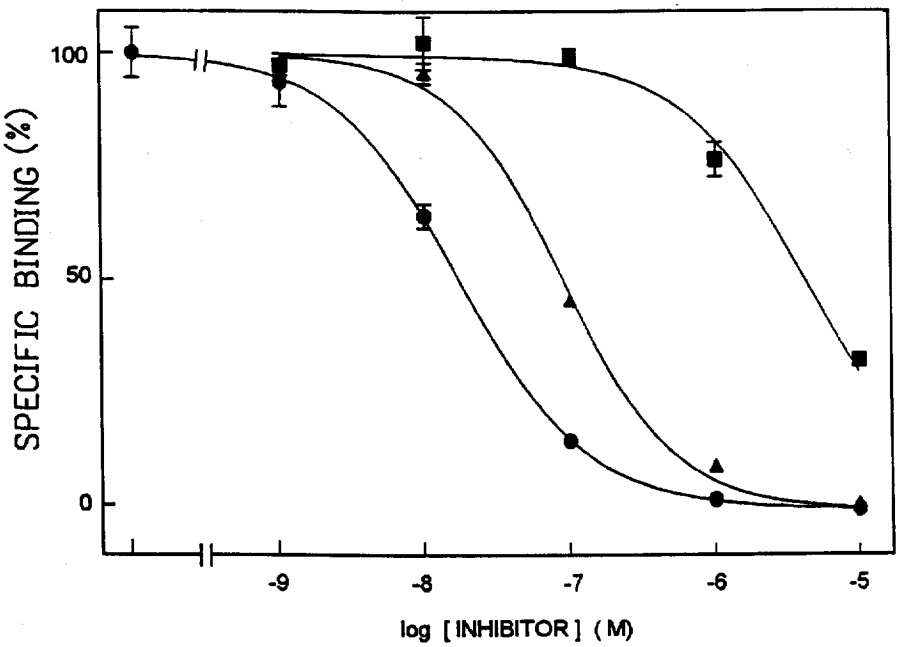


Fig 1: Inhibition of [^3H] IP_3 binding to rat cerebellar membranes[21] by IP_3 (circles), 3-deoxy-IP_3 (triangles) and 3-deoxy-*muco*-IP_3 (squares)

Both compounds have been tested for their inhibitory effects on the binding of [^3H] $InsP_3$ to rat cerebellar membranes which have been shown to contain a high density of IP_3 receptors.[22] Fig 1 shows that both 3-deoxy IP_3 and 3-deoxy-*muco*-IP_3 inhibit [^3H]$InsP_3$ binding to the membranes, indicating that they recognise $InsP_3$ receptors. The 3-deoxy-*myo* derivative is about ten fold less potent than IP_3 (see also ref. 6) and the affinity of the 3-deoxy-*muco*-IP_3 is almost 3 order of magnitude lower than affinity of IP_3. This shows that in the 3-deoxy series, ***the 4,5-trans relationship of the phosphate groups should be a primary requirement for the binding to the receptor of IP_3.*** We have tested the ability of these compounds to release Ca^{2+} from permeabilized hepatocytes by using an experimental procedure already described.[23] The two compounds **16** and **17** released Ca^{2+} from the intracellular compartment as IP_3 did, with the following order of potency : IP_3 > 3-deoxy-IP_3 (**16**) > 3-deoxy-*muco*-IP_3 (**17**), in agreement with the order found for the inhibition of [^3H] IP_3 binding.

Further comparison of these two compounds in terms of biological properties, release of calcium, and metabolism will be reported in due course.

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References and Notes :

1. Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197.
2. Berridge, M. J.; Irvine, R. F. *Nature* **1984**, *312*, 315.
3. Berridge, M. J.; *Nature* **1993**, *361*, 315.
4. Potter, B. V. L. *Nat. Prod. Reports* **1990**, 1.
5. Billington, D. C. VCH, Weinheim, **1993**, 1-153.
6. Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 4429.
7. Liu, C.; Thomas, N. F.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1993**, 1687.
8. Seewald, M. J.; Aksoy, I. A.; Powis, G.; Fauq, A. H.; Kozikowski, A. P. *J. Chem. Soc., Chem. Commun.* **1990**, 1638.
9. Kozikowski, A. P.; Fauq, A. H.; Wilcox, R. A.; Challiss, R. A. J.; Nahorski, SR *J. Med. Chem.* **1994**, *37*, 868.
10. Berridge, M. J. *Annu. Rev. Biochem.* **1987**, *56*, 159.
11. Ferrier, R. J.; Middleton, S. *Chem. Rev.* **1993**, *93*, 2779.
12. For relative biomimetic syntheses starting from D-glucose see: Estevez, V. A.; Prestwich, G. D. *Tetrahedron Lett.* **1991**, *32*, 1623. Bender, S. L.; Budhu, R. J. *J. Am. Chem. Soc.* **1991**, *113*, 9883.
13. Loewus, F. A. *Recent Adv. Phytochem.* **1974**, *8*, 179.
14. Loewus, F. A.; Loewus, M. W. *Annu. Rev. Plant. Physiol.* **1983**, *34*, 137.
15. Chrétien, F. *Synth. Commun.* **1989**, *19*, 1015.
16. Machado, A. S.; Olesker, A.; Lukacs, G. *Carbohydr. Res.* **1985**, *135*, 231.
17. Blattner, R.; Ferrier, R. J. *Carbohydr. Res.* **1986**, *150*, 151
18. Lampe, D.; Mills, S. J.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2899.
19. Uhlmann, E.; Engel, J. *Tetrahedron Lett* **1986**, *41*, 1023.
20. The correct nomenclature of compound **17** should be either 3-deoxy-D-*epi*-inositol 1,4,5-trisphosphate or 3-deoxy-D-*muco*-inositol 1,4,5-trisphosphate. Indeed D-*epi* and D-*muco* inositol differ only by the stereochemistry at C-3.
21. Hilly, M.; Piétri-Rouxel, F.; Coquil, J.-F.; Guy, M.; Mauger, J.-P. *J. Biol. Chem.* **1993**, *268*, 16488.
22. Ferris, C. D.; Snyder, S. H. *Annu. Rev. Physiol.* **1992**, *54*, 469.
23. Combettes, I.; Dumont, M.; Berthon, B.; Erlinger, S.; Claret, M. *J. Biol. Chem.* **1988**, *263*, 2299.

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