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SYNTHESIS OF *myo*-INOSITOL-1-PHOSPHATASE INHIBITORS IN WHICH THE PHOSPHATE GROUP IS REPLACED BY LESS POLAR GROUPS

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Abstract: The phosphate group in the inositol-1-phosphatase inhibitor (**1**) is replaced by less polar phosphorus containing groups (substituted phosphate and phosphonates), by sulphur containing substituents (sulphate, sulphonamides) and by a carboxylate and carbamate. The effect of the replacement is studied on the enzyme *myo*-inositol-1-phosphatase.

In drug design the introduction of highly polar groups is generally undesirable because it reduces the transfer of compounds across biological membranes. We are interested in reducing the polarity of phosphate groups to facilitate transport of inhibitors of the enzymes *myo*-inositol-monophosphatase (IMPase) through the Blood Brain Barrier (BBB). In the work presented here, we replaced the phosphate group of a known IMPase inhibitor by a less polar group and studied the effect on the target enzyme.

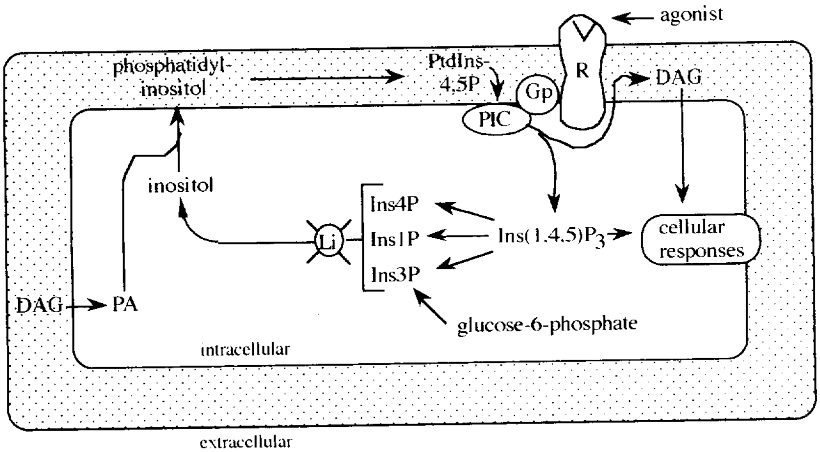
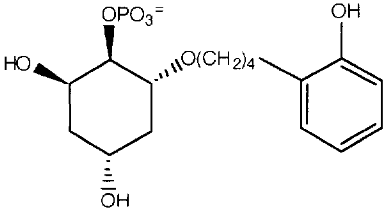


Figure 1: The inositol phosphate system with arrows indicating enzymic conversions. The dephosphorylation of the inositol monophosphates are blocked by lithium salts. In addition, the *de novo* synthesis starting from glucose-6-phosphate is also inhibited. (Ptd)Ins(1,3,4,5)P indicates (phosphatidyl)inositol(1,3,4,5)phosphate; DAG, diacylglycerol; R, receptor; Gp, a coupling G-protein and PIC, phosphoinositidase C; PA, phosphatidic acid. For a more detailed description of this system and the intermediate reaction steps, see reference 1.

Since 1949 lithium has been used in treatment of manic-depressive illness. A major hypothesis of its mechanism of action is that lithium inhibits several enzymes in the inositol-phosphate cycle (see figure 1). Because peripheral inositol does not penetrate the BBB, the required inositol supply in neuronal cells in the central nervous system is regulated by the inositol-phosphatases. Inhibition of the crucial inositol-monophosphatases by lithium salts reduces the supply of inositol, thereby quenching the (over)activation of inositol-phosphate cycle, which could be the case in bipolar-mood disorders. In this respect it has been postulated that inhibitors of inositol-monophosphatases (IMPase-inhibitors) may mimic the action of lithium in the treatment of bipolar-mood disorders[1].



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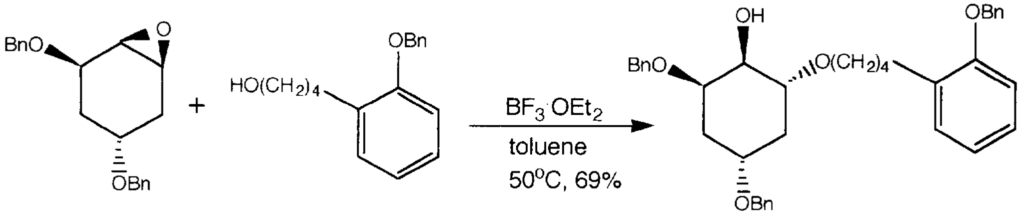
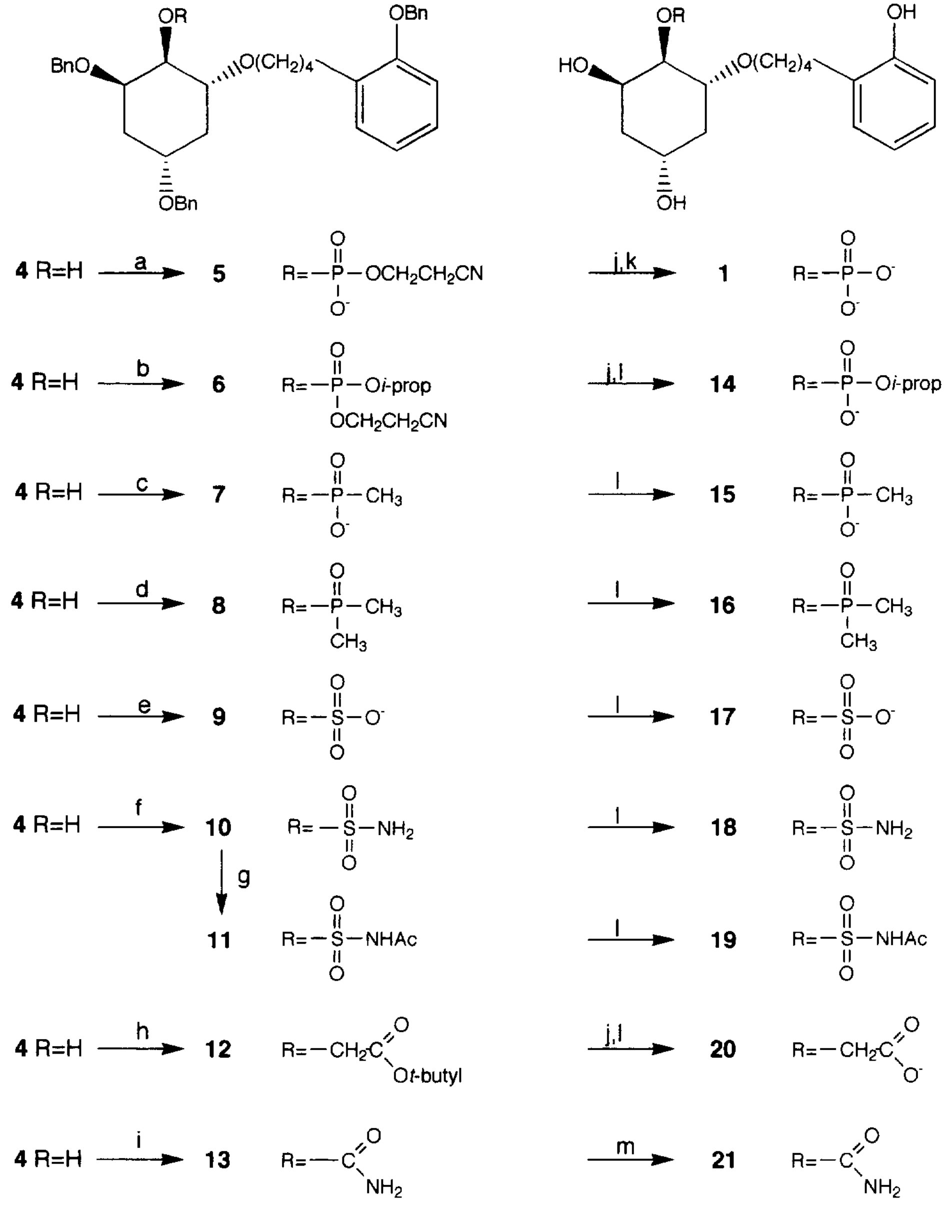
Scheme 1

The most active inhibitor of *myo*-inositol-1-phosphatase found so far, the 6α-substituted 2β,4α-dihydroxy-cyclohexane derivative (**1**), was chosen as a tool in studying the replacement of the phosphate group[2]. During the synthesis of **1** we followed the route as described in literature[2], except for the epoxide opening with the spacer alcohol (*scheme 2*). In our hands, the literature procedure in which aluminium oxide in toluene was used to open the epoxide ring, gave only low yields (~20%), whereas the use of borontrifluoride etherate in toluene at 50°C yielded compound **4** in 69% yield. It should be noted that all synthesized deoxyinositol compounds derived from **4** were tested as pair of enantiomers.



2 **3** **4**

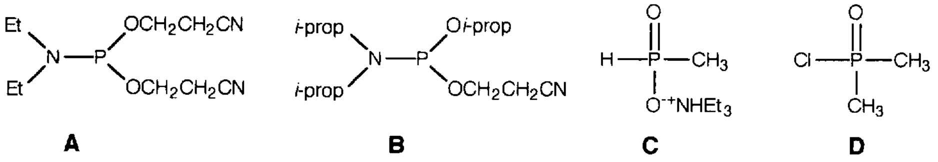
Scheme 2



Scheme 3

a) reagent **A**, 1H-tetrazole, CH_3CN, CH_2Cl_2, than Et_3N, *t*-BuOOH; 75%. **b**) reagent **B**, 1H-tetrazole,CH_3CN than *t*-BuOOH;100%. **c**) reagent **C**, pyr, pivCl than I_2/pyr/H_2O; 86%. **d**) reagent **D**, pyr, CH_2Cl_2; 46%. **e**) Et_3NSO_3, DMF; 80%. **f**) NaH, sulphamoyl chloride, DMF; 82%. **g**) Ac_2O, pyr; 85%. **h**) $BrCH_2COO$*t*-Bu, NaH, DMF; 98%. **i**) NaOCN (2 eq.), TFA (2.1 eq.), CH_2Cl_2; 79%. **j**) 4N NaOH, dioxane, MeOH. **k**) H_2, 10% Pd/C, DMF/H_2O; 100%. **l**) H_2, 10% Pd/C, *t*-BuOH/H_2O; ~95%. **m**) H_2, 10% Pd/C, EtOH/H_2O; 85%.

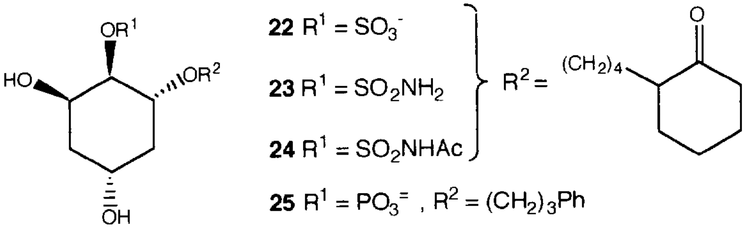
Phosphorylation of **4** (see *scheme 3*) with the phosphoramidite reagent **A**[3,4] (*scheme 4*) activated by 1H-tetrazole, followed by oxidation using *t*-butylhydroperoxide in the presence of triethylamine gave the phosphate diester **5**. Subsequent deprotection of the remaining cyanoethyl group and benzyl functions afforded **1**, which was used as a reference in our inositol-1-phosphatase assay. After synthesizing the reference compound **1**, we prepared some less polar phosphorus containing derivatives, *viz*. the *i*-propylphosphate ester **14**, the methylphosphonate **15** and the dimethylphosphinate derivative **16** (see also *scheme 3*). The *i*-propylphosphate ester was synthesized using the phosphorylating agent **B**[5] (*scheme 4*) with 1H-tetrazole as a promoter. In situ oxidation of the intermediate phosphite with *t*-butylhydroperoxide (→**6**, quantitative) and removal of the cyanoethyl group with sodium hydroxide followed by hydrogenolysis gave **14**. The preparation of the methylphosphonate **15** was performed via the H-phosphonate procedure which we reported previously[3,6]. Phosphonate reagent **C** was condensed with **4**, applying pivaloyl chloride as a coupling agent. In this case, oxidation of the H-phosphonate was done using iodine in a pyridine/water system to give **7**. Hydrogenolysis of **7** in *t*-butanol/water in the presence of 10% palladium on charcoal gave **15**. After condensation of dimethylphosphic chloride (**D**) with **4** in pyridine/dichloromethane, compound **8** was isolated in a modest yield (46%). Deprotection gave **16** (*scheme 3*). In addition a phosphate containing compound (**25**), with a phenylpropyl substituent instead of the hydroxylated aromatic moiety was prepared as described for **1** (*scheme 5*).



Scheme 4

To test whether sulphate derivatives can mimic the phosphate group, three sulphur containing compounds were synthesized: the sulphate derivative **17**, the sulphonamide **18** and the N-acetylsulphonamide **19** (*scheme 3*). The synthesis of the intermediate compounds **9**, **10** and **11** was quite straightforward. Sulphation of **4** using the $SO_3.NEt_3$ complex in dimethylformamide, followed by purification on LH-20 (dimethylformamide) gave **9** (80%)[3,7]. The sulphonamide[3,7] and N-acetyl sulphonamide were prepared using sulphamoyl chloride/sodium hydride in dimethylformamide (→**10**, 82%). Acetylation of **10** afforded the N-acetyl-sulphonamide **11** (85%). However, during hydrogenolysis of the sulfur containing intermediates in the presence of 10% palladium on charcoal in dimethylformamide/water a side-product was observed. Separation by preparative TLC (*n*-butanol : water : acetic acid 50:1:1) showed that a side-product was present in a ratio of (side-product : compound) 1:2 for **17**, 1:4 for **18** and 1:3 for **19**. NMR spectroscopy of the side-product showed that the aromatic ring was hydrogenated. Because in all cases the molecular weight, as determined by FAB-mass spectrometry, was 4 mass units higher than the target compound, the side-product was identified as a derivative carrying a cyclohexanone ring (**22**, **23** and **24**, see *scheme 5*).

Finally, two compounds, containing neither sulphur nor phosphorus, were synthesized: the carboxymethylene derivative **20** and the carbamate **21**. Condensation of *t*-butyl bromoacetate with **4** gave **12** in excellent yield[3]. Saponification and hydrogenolysis gave the methylcarboxylate **20**. The carbamate was prepared using sodium cyanate and trifluoroacetic acid (→**13**, 79%)[8]. Attempts to acetylate **13** failed. Hydrogenolysis of the carbamate in ethanol/water gave **21**.



Scheme 5

The method of Pachter[9] was used for the isolation and purification of bovine brain *myo*-inositol-1-phosphatase. The inhibitory constants (IC_{50}) of the synthesized derivatives were determined by incubating the purified inositol-1-phosphatase (15 min, 37°C) with the radioactive ^{3}H-D-inositol-1-phosphate and the non labeled D-inositol-1-phosphate and/or the synthesized compounds. The substrate ^{3}H-D-inositol-1-phosphate was separated from the formed ^{3}H-D-inositol by anion-exchange chromatography and the amount of ^{3}H-D-inositol formed was measured by liquid scintillation counting. The results of the enzyme assays are presented in Table 1.

Table 1: The inhibitory constants of the synthesized compounds for *myo*-inositol-1-phosphatase. The values given are the average of 2-3 experiments, performed in triplicate. n.a.: not active at 300μM.

compound	IC_{50}(μM)	compound	IC_{50}(μM)
1	0.3	**19**	>300
25	0.8	**20**	>300
14	50	**21**	n.a.
15	87	**22**	100
16	>300	**23**	200
17	90	**24**	>300
18	>300		

The replacement of the phosphate (compound **1**) by less polar groups (**14-24**) has a dramatic effect on the inhibitory constant. Substitution of the phosphate group by derivatives containing only one negative charge (**14, 15, 17, 22**) resulted in a 200- to 300-fold decrease of the IC_{50}, whereas neutral mimics (**16, 18, 19, 21, 24**) are not active. On the other hand, no significant difference in inhibition was observed whether either a sulphate (**17**), a di-substituted phosphate (**14**), or phosphonate group (**15**) was present in the inhibitor. In contrast, the carboxylate derivative (**20**) was found to be a very weak inhibitor. Substitution of the spacer with a non-hydroxylated aromatic ring (**25**) had only little influence on the IC_{50}.

In summary it can be concluded that the presence of a phosphate group, bearing two negative charges, is required for inhibition of *myo*-inositol-1-phosphatase. Substitution by a less polar sulphate or phosphate group results in a reduction of IMPase inhibition. On the other hand these derivatives are considerably less polar and may have better prospects in penetrating the blood brain barrier.

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