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TOTAL SYNTHESIS OF NEW 1,5-BISSUBSTITUTED *myo*-INOSITOL DERIVATIVES. SYNTHESIS OF D-*myo*-INOSITOL 1,5-BISPHOSPHATE, 3,5-BISPHOSPHATE AND OF *rac.* 1,5-BISSULPHATED AND 1,5-BISSULPHAMOYLATED ISOSTERIC ANALOGUES

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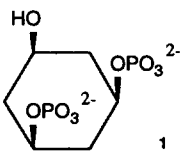
Abstract: *convenient synthesis of D-myo-inositol 1,5-bisphosphate, 3,5-bisphosphate and isosteric rac. 1,5-bissulphate and 1,5-bissulphonamide was accomplished from key intermediate 2,3,4,6-tetra-O-benzyl-myo-inositol.*

In many cell types, after binding of an extracellular signal molecule to a cellular receptor, D-myo-inositol 1,4,5-trisphosphate (IP_3) may be released intracellularly to effect a rise in cytosolic Ca^{++} concentration[1]. Subsequently, IP_3 and its congeners are substrates of several kinases and phosphatases in the so called phosphoinositide (PI) cycle[2]. The biological significance of the PI cycle as a signal transduction system makes it to an attractive target for pharmaceutical intervention[3] and accounts for the great synthetic efforts in the field of the inositol phospholipids and inositol (poly)phosphates[4].

Up to now all isomers of inositol (poly)phosphates detected in animal cells have been synthesized in order to study their possible function in biochemical processes related to the PI cycle.

Recently, the syntheses of two phosphorylated trihydroxycyclohexane derivatives, both interfering in the PI cycle, were reported. The 3,5,6-trisdeoxy derivative of myo-inositol 1-monophosphate was identified[5] as a very potent inhibitor of inositol monophosphatase, whereas *cis,cis*-cyclohexane 1,3,5triol bisphosphate



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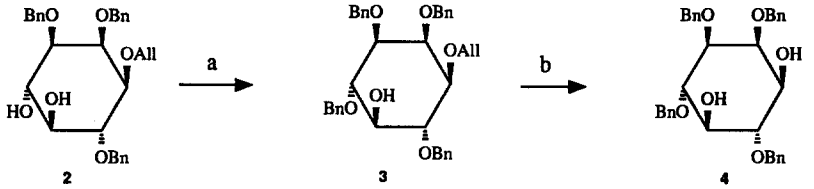
(**1**), a meso compound mimicking inositol 1,5-bisphosphate, turned out to be a full IP_3 agonist in releasing Ca^{++} from isolated vacuoles of *Neurospora crassa*[6]. To the best of our knowledge inositol 1,5-bisphosphate, an isomer not detected in mammalian cells[7], has been uninvestigated thus far. For this reason and taking into account the biological activity of compound **1** synthesis and biological evaluation of inositol 1,5-bisphosphate and analogues thereof would be attractive.

We now wish to report a short and convenient synthesis of D-myo-inositol 1,5-bisphosphate **9**, its enantiomer D-myo-inositol 3,5-bisphosphate **10** and of the racemic, isosteric analogs **11** and **12** containing sulphate and sulphonamido groups, respectively.

In order to prepare the suitably protected key intermediate **4**, two different routes were explored, i.e. the classical 'bis-ketal' route[8] and the 'orthoester' route[9]. The former route leads to intermediate **2**[10], but turned out to be rather tedious in the next step of the synthesis since no regioselectivity could be achieved in protecting the 4-OH of the 4,5-*vic*-diol of **2** (Scheme 1).

In order to circumvent the regioselective protection of the 4,5-*vic*-diol, we examined the use of inositol orthoformate **5**[11]. Perbenzylation of orthoformate **5** using sodium hydride/benzyl bromide in DMF



2 **3** **4**

Scheme 1

a: BaO/Ba(OH)$_2$/BnBr, 0°C, 20%; b: Ir[COD(PMePh$_2$)$_2$]PF$_6$/H$_2$ then HCl/dioxane/MeOH

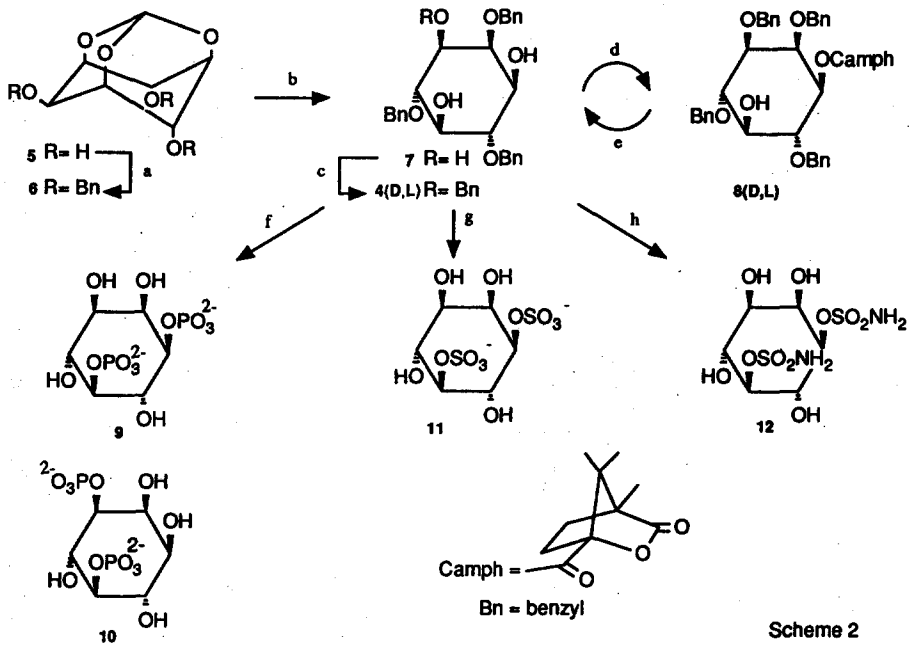
afforded the fully protected inositol **6**, which on removal of the orthoester furnished the meso 2,4,6-tri-O-benzyl derivative **7** (Scheme 2). Benzylation of triol **7** under phase transpher conditions[12] led predominantly to the desired 1,2,4,6-tetra-O-benzyl myo-inositol **4**[13] (71 % yield from **5**), the structure of which was readily deduced from its dissymmetric ^{1}H-NMR spectrum[12]. Key intermediate 1,5-diol **4** was subjected to optical resolution prior to phosphorylation experiments. The optical resolution of an 1,2-diol containing inositol derivative through a regioselective formation of diastereoisomeric 1-O-menthoxyacetates was reported previously[14]. After a number of experiments, we found that a diastereomeric mixture of the 1-O-camphanates **8**, prepared in a regioselective reaction of 1,5-diol **4** with 1.1 equivalent of (-)-camphanic acid chloride in pyridine at 0°C, could be separated efficiently by silica gel column chromatography. The optical purity of the separated diastereoisomers (**8D**, 47% yield, R_f 0.38, CH_2Cl_2/EtOAc, 97:3, v/v and **8L**, 44 % yield, R_f 0.55, CH_2Cl_2/EtOAc, 97:3, v/v) was checked by ^{1}H-NMR analysis since the diastereoisomeric camphanate methyl resonances exerted different chemical shifts. Alkaline hydrolysis (LiOH in 1:1 dioxane/methanol for 1.5 h at 20°C) of **8D** and **8L** afforded the enantiomerically pure diols **4D** ($[\alpha]_D$ 10.0, c=1, $CHCl_3$) and **4L** ($[\alpha]_D$ -9.1, c=1, $CHCl_3$), the absolute configurations of which were determined by comparison of the optical rotations with that of a sample synthesized in two steps from known enantiomeric **2**[10] (Scheme 1).

Phosphorylation of **4D** and **4L** was performed efficiently with a phosphoramidite reagent. Thus, to a stirred solution of the 1,5-diol (0.2 mmol) and bis(2-cyanoethyl)-*N,N*-diethylphosphoramidite[15] (0.6 mmol) in dichloromethane was added 1 ml of a 1M solution of 1*H*-tetrazole. After 1 hr, a solution of excess *tert*-butyl hydroperoxide and trietylamine in dichloromethane is added to the reaction mixture and after a further period of 4 hr, the mixture was concentrated and applied to a column of Sephadex LH-20. The appropriate fractions were treated with 0.2 N NaOH/MeOH/dioxane and neutralized (Dowex 50W, H^+-form). Subsequent hydrogenolysis (10% Pd/C) afforded the title compounds D-myo-inositol 1,5-bisphosphate **9** and 3,5-bisphosphate **10** in about 95% purity. Since biochemical experiments require highly purified preparations, the individual derivatives were separately subjected to DEAE column chromatography (1x30 cm^2, 0.2-0.8 M NH_4OAc). The appropriate fractions (detected by spot test using modified Jungnickel's reagent[16]) were lyophilized to furnish highly pure **9** (-D-, 83% yield from **4D**, $[\alpha]_D$ 6.0, c=0.5, H_2O) and **10** (-L-, 81% yield from **4L**, $[\alpha]_D$ -5.9, c=0.5, H_2O).

Upon considering the synthesis of isosteric analogs of myo-inositol 1,5-bisphosphate, we decided to replace the phosphate moieties by sulphate and by sulphonamide moieties to give a less negatively charged

(**11**) and a neutral (**12**) analogue, respectively.

Sulphation of diol **4** (0.2 mmol) was accomplished by reaction with excess triethylamine-sulphur trioxide complex[17] (2 mmol) in DMF at 50°C for 1 night. Removal of the excess reagent (Sephadex LH-20 in DMF containing 0.5% triethylamine) and neutralization (solid $NaHCO_3$) of the appropriate fractions turned out to be critical in the work-up procedure of the protected sulphated intermediate. Hydrogenolysis of the latter in 9:1 DMF/H_2O in the presence of 10% Pd on C furnished myo-inositol 1,5-bissulphate **11** in 88% yield from **4**.



Scheme 2

a, BnBr/NaH/DMF; b, CF_3COOH/H_2O 9:1, 0.5 h at 40°C and then NH_3/MeOH/H_2O for 4 h; c, BnBr/5% NaOH/$Bu_4N^+I^-/CH_2Cl_2$ 16h at 45°C; d, 1,1 eq. (-)-camphanic acid chloride in pyridine at 0°C; e, LiOH in dioxane/MeOH 1.5 h at 20°C; f, $(CNCH_2CH_2O)_2PN(CH_2CH_3)_2$/1-H-tetrazole in CH_2Cl_2/CH_3CN followed by tert-BuOOH/Et_3N and 0.2N NaOH/dioxane/MeOH; 10% Pd on C/H_2/DMF/H_2O; g, $(CH_3CH_2)_3N.SO_3$/DMF then 10% Pd on C/H_2/DMF/H_2O; h, NH_2SO_2Cl/NaH/DMF then 10%Pd on C/H_2/DMF/H_2O.

In order to obtain the sulphamoylated derivative **12**, diol **4** (0.2 mmol) was treated with sulphamoyl chloride (0.8 mmol) and NaH (0.8 mmol) in DMF at 0°C for 4 hr. Purification by silica gel column chromatography (eluent CH_2Cl_2/MeOH, 99:1, v/v) and subsequent hydrogenolysis (10% Pd on C) afforded myo-inositol 1,5-bissulphonamide **12** in a yield of 87% from **4**.

The identity and homogeneity of the title compounds were established by NMR spectroscopy and (FAB) mass spectrometry. The ^{1}H-NMR (360 MHz) spectral data are listed in the Table[18]. Noteworthy are the downfield shifts of the H-1 and H-5 resonances of the sulphated and sulphamoylated derivatives **11** and **12** with respect to the same resonances of the phosphorylated derivative **9**.

The biological data on the title compounds will be reported elsewhere in due time.

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12. A mixture of compound **7** (1 mmol), benzyl bromide (2.0 eq.), tetra-n-butylammonium iodide (0.25 eq.) and sodium hydroxide (3 ml, 5% solution) in CH_2Cl_2 (15 ml) is stirred vigorously for 16 h at 45°C. Work-up (standard) followed by silica gel column chromatography (eluent toluene/ethyl acetate, 9:1, v/v) afforded compound **4** as an oil in 71% yield. ^{1}H-NMR (200 MHz)δ (ppm): 3.35-3.50, c, 2H, H-1, H-5; 3.53, dd, 1H, H-3; 3.69, t, 1H, H-4; 3.88, t, 1H, H-6; 4.03, t, 1H, H-6; 4.65-5.05, c, 6H, 4xCH_2, 7.20-7.40, c, 20H, 4xPh.
13. 1,2,4,6-tetra-O-benzyl-myo-inositol (e.g. **4**) was isolated by Billington (ref. 9) as an undesired product in a synthesis towards myo-inositol 1,3-bisphosphate.
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18.

myo-inositol 1,5-	H_1	H_2	H_3	H_4	H_5	H_6
bisphosphate (**9**)	4.02 m	4.27 t	3.67 dd	3.79 t	3.90 c	3.90 c
bissulphate (**10**)	4.29 dd	4.42 t	3.68 dd	3.85 t	4.22 t	3.94 t
bissulphonamide(**11**)	4.52 dd	4.42 t	3.70 dd	3.90 t	4.40 t	4.08 t

Table, ^{1}H-NMR chemical shifts (360 MHz) of compounds **9**, **10** and **11**.

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