Synthesis and Metabolism of the *myo*-Inositol Pentakisphosphates

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All six isomeric *myo*-inositol pentakisphosphates ($InsP_5$), consisting of the two *meso* compounds *myo*-inositol 1,3,4,5,6-pentakisphosphate [Ins(1,3,4,5,6)P_5] (**18**) and *myo*-inositol 1,2,3,4,6-pentakisphosphate [Ins(1,2,3,4,6)P_5] (**22**) and two pairs of enantiomers *myo*-inositol 1,2,4,5,6-pentakisphosphate [Ins(1,2,4,5,6)P_5] (**15**) *myo*-inositol 2,3,4,5,6-pentakisphosphate [Ins(2,3,4,5,6)P_5] (*ent*-**15**) and *myo*-inositol 1,2,3,5,6-pentakisphosphate [Ins(1,2,3,5,6)P_5] (**20**) *myo*-inositol 1,2,3,4,5-pentakisphosphate [Ins(1,2,3,4,5)P_5] (*ent*-**20**), respectively, were synthesized. These compounds have been found in tissue, and although not resolved as pure enantiomers, their primary metabolism in a cytosolic extract from fetal calf thymus was therefore investigated by analytical HPLC. Four isomers were dephosphorylated to singly defined inositol tetrakisphosphates, while Ins(1,2,4,5,6)P_5 was phosphorylated to *myo*-inositol hexakisphosphate ($InsP_6$). Interestingly, Ins(2,3,4,5,6)P_5 was the only isomer which was not metabolized. These data demonstrate that chemically synthesized, enantiomerically pure inositol pentakisphosphate isomers are valuable tools for the unravelling of the metabolic pathways of $InsP_5$ turnover in living cells.

Introduction

Since the second messenger function of *myo*-inositol 1,4,5-trisphosphate[1] [Ins(1,4,5)P_3] was discovered in 1983 the biochemistry[2] and consequently the field of preparative organic chemistry of inositol phosphates has experienced enormous growth[3]. Of the 63 possible *myo*-inositol phosphates the higher phosphorylated inositol polyphosphates have only very recently drawn more attention. This interest has arisen from the reporting of the first suggestions for their physiological functions inside[4][5] and outside cells[6]. It was shown that Ins(1,3,4,5,6)P_5 is hydrolyzed receptor-mediated to the newly discovered intracellular messenger Ins(3,4,5,6)P_4[4][7]. Other functions of inositol pentakisphosphates discussed include the intracellular regulation of surface receptors and ion channels[8]. Microinjection of Ins(1,3,4,5,6)P_5 blocked the transmission in the preterminal giant synapse of squid[9]. For future investigations of their biological functions and their metabolism it would be desirable to consider all six isomeric *myo*-inositol pentakisphosphates, since most of them are abundant in cells. However, only Ins(1,3,4,5,6)P_5 is currently commercially available. The preparative isolation of the other isomers from natural material is laborious and enantiomerically pure isomers cannot be obtained by the methods presented so far[10]. We therefore aimed to prepare all six inositol pentakisphosphates. It should be noted, however, that some of them have been synthesized before in their racemic form[11].

Apart from the biological functions the metabolism of most of the pentakisphosphate isomers is currently unknown. We here report (i) the enantioselective chemical synthesis of all six $InsP_5$ isomers, and (ii) the usage of these synthetic compounds as substrates for a cytosolic extract from fetal calf thymus.

Results and Discussion

Synthesis of Regioselectively Protected Precursors: The enantiomers Ins(1,2,4,5,6)P_5 and Ins(2,3,4,5,6)P_5 were prepared starting from the common precursor *rac*-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*rac*-**1**)[12]. As we have shown before, *rac*-**1** may be easily transformed to the diastereomerically pure 1-*O*- and 3-*O*-camphanate, respectively (Scheme 1)[13]. The benzyl groups were removed by catalytic hydrogenolysis (10% Pd on C) in glacial acetic acid to give the monocamphanates **2** and *dia*-**2**. The regioselective protection of the axial 2-OH group for the synthesis of the *meso* compound Ins(1,3,4,5,6)P_5 also started from *rac*-**1**. The only equatorial hydroxyl group was selectively alkylated with the help of a cyclic dibutyltin intermediate as described before[13][14]. The remaining 2-OH group was esterified with butyric anhydride in pyridine and subsequently the benzylic groups were removed by catalytic hydrogenolysis to give the desired 2-*O*-butyryl-*myo*-inositol (**5**) in 65% yield from *rac*-**1**.

For the synthesis of the remaining pentakisphosphates different starting materials were required. The preparation

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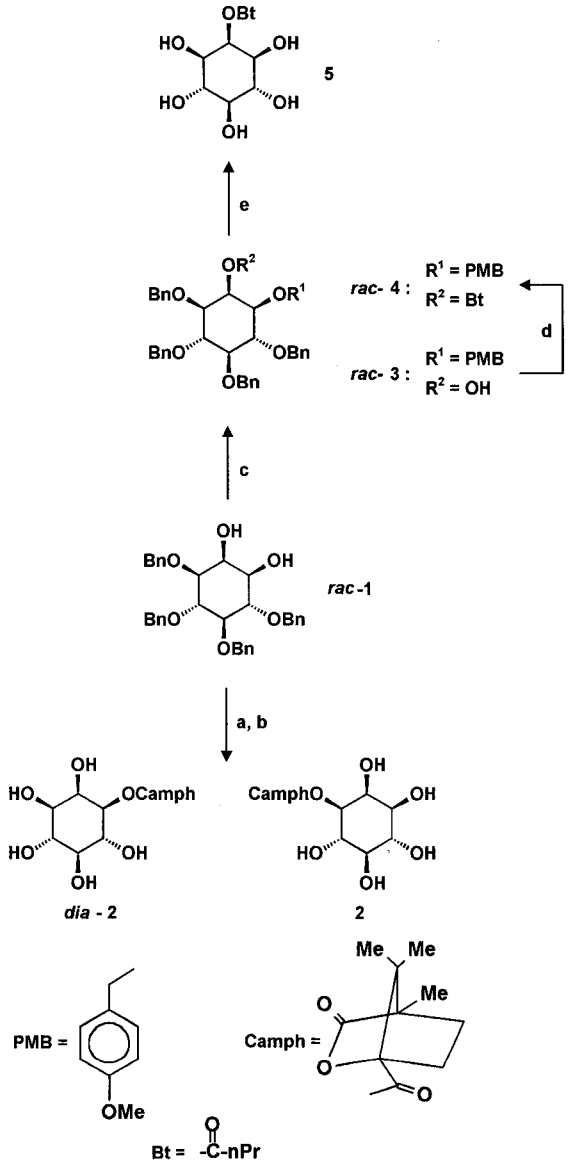
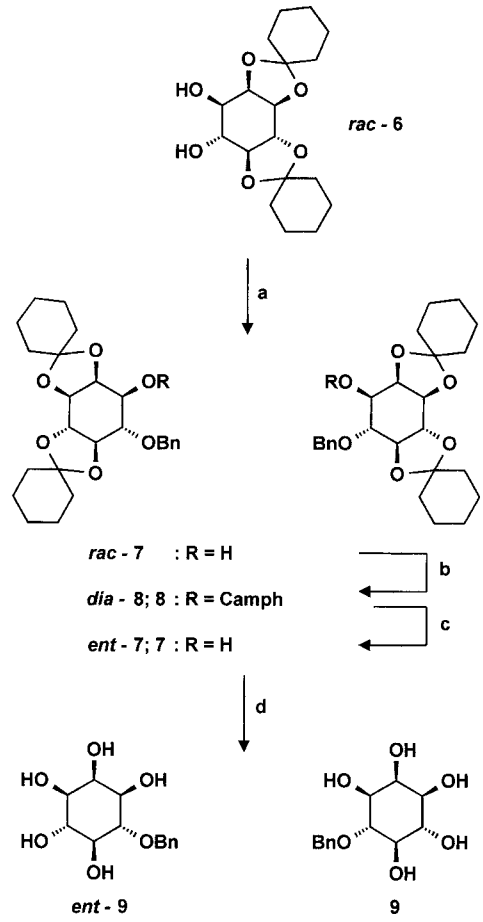
Scheme 1. Preparation of the regioselectively protected precursors 3-*O*-camphanoyl-*myo*-inositol (**2**), 1-*O*-camphanoyl-*myo*-inositol (*dia*-**2**) and 2-*O*-butyryl-*myo*-inositol (**5**)



Reagents and conditions: a: (−)-Camphanoyl chloride, pyridine. − b: H_2, Pd/C (10%), MeCOOH. − c: (i) Bu_2SnO, toluene, Soxhlet with molecular sieves, 3 Å, (ii) PMB-Cl, CsF, DMF. − d: Bt_2O, DMAP, pyridine. − e: H_2, Pd/C (10%), MeCOOH.

of the regioselectively benzylated enantiomers 4-*O*-benzyl- and 6-*O*-benzyl-*myo*-inositol (**9** and *ent*-**9**, respectively) began with the regioselective benzylation of the 1,2:5,6-diketal *rac*-**6** (Scheme 2) again employing a cyclic dibutyltin intermediate. Replacement of the latter with benzyl bromide gave predominantly (3:1) the desired 4-*O*-benzyl ether (*rac*-**7**), which could be separated from the 3-*O*-benzyl regioisomer by preparative HPLC on a 50 × 250 mm reversed phase column. The remaining hydroxyl group was used to prepare the diastereomeric (−)-camphanates **8** and *dia*-**8**, which were separated by flash chromatography (Si 60; CH_2Cl_2:diisopropyl ether, 98:2). Removal of the camphanates and subsequently the ketals yielded the monobenzyl ethers **9** and *ent*-**9**, respectively, in excellent yield.

Scheme 2. Preparation of the regioselectively protected precursors 4-*O*-benzyl-*myo*-inositol (**9**) and its enantiomer 6-*O*-benzyl-*myo*-inositol (*ent*-**9**)

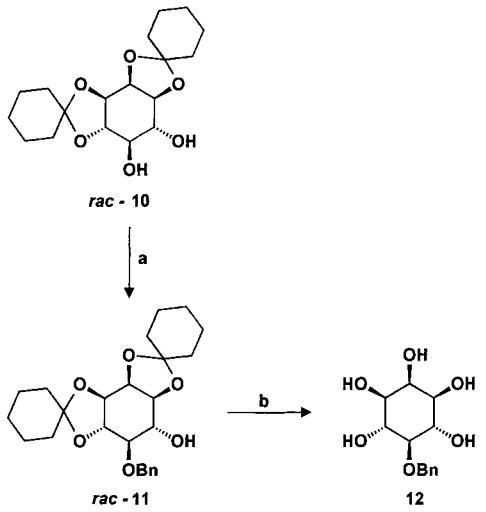


Reagents and conditions. a: (i) Bu_2SnO, toluene, Soxhlet with molecular sieves, 3 Å; (ii) BnCl, CsF, DMF. − b: (−)-Camphanoyl chloride, pyridine. − c: KOH, MeOH. − d: H_2, Pd/C (10%), MeCOOH.

To selectively protect the hydroxyl group in the 5-position *rac*-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (**10**) was alkylated with benzyl bromide again via dibutyl tin activation. The selectivity was 3:1 in favor of the desired 5-*O*-benzyl ether *rac*-**11** which was purified by preparative HPLC. The ketals were hydrolyzed by treatment with aqueous TFA in acetonitrile to give the pentol **12**. ^{1}H-NMR analysis of **12** clearly indicated the symmetric structure of the *meso* compound.

Phosphorylation and Deprotection: All pentols were phosphorylated by reaction with the phosphite reagent dibenzyl *N*,*N*-diisopropylphosphoramidite and subsequent oxidation to the fully protected phosphates **13**, *dia*-**13**, **16**, **19**, *ent*-**19**, and **21** with peracetic acid (Scheme 4)[15]. Yields were about 70% after purification by preparative HPLC (50 × 250 mm, RP-18, 10 μm) with 90% methanol as the eluent. At this stage the enantio and diastereopure compounds were checked for enantiomeric excess or diastereomeric purity, respectively, on a chiral HPLC-column (4 × 250 mm, Chiradex, 10 μm, Merck AG, Germany) with 100% acetonitrile as the eluent. No contamination with the corresponding

Scheme 3. Preparation of the regioselectively protected precursor 5-*O*-benzyl-*myo*-inositol (**12**)



Reagents and conditions: a: (i) Bu_2SnO, toluene, Soxhlet with molecular sieves, 3 A; (ii) BnCl, CsF, DMF. – b: H_2, Pd/C (10%), MeCOOH.

stereoisomer was detected. The fully protected pentakis(dibenzyl)phosphates were deprotected by catalytic hydrogenolysis on Pd/C (10%) to **14**, *ent*-**14**, **17**, **20**, *ent*-**20**, and **22**. Where necessary butyrates or camphanates were hydrolyzed by treatment with aqueous KOH at pH 12.8 to give the *myo*-inositol pentakisphosphates **15**, *ent*-**15**, **18**, respectively. To provide comparable samples for NMR analysis (Table 1) the potassium salts of **15**, *ent*-**15**, and **18** were eluted from Dowex 50 WX 8 to give the free acids.

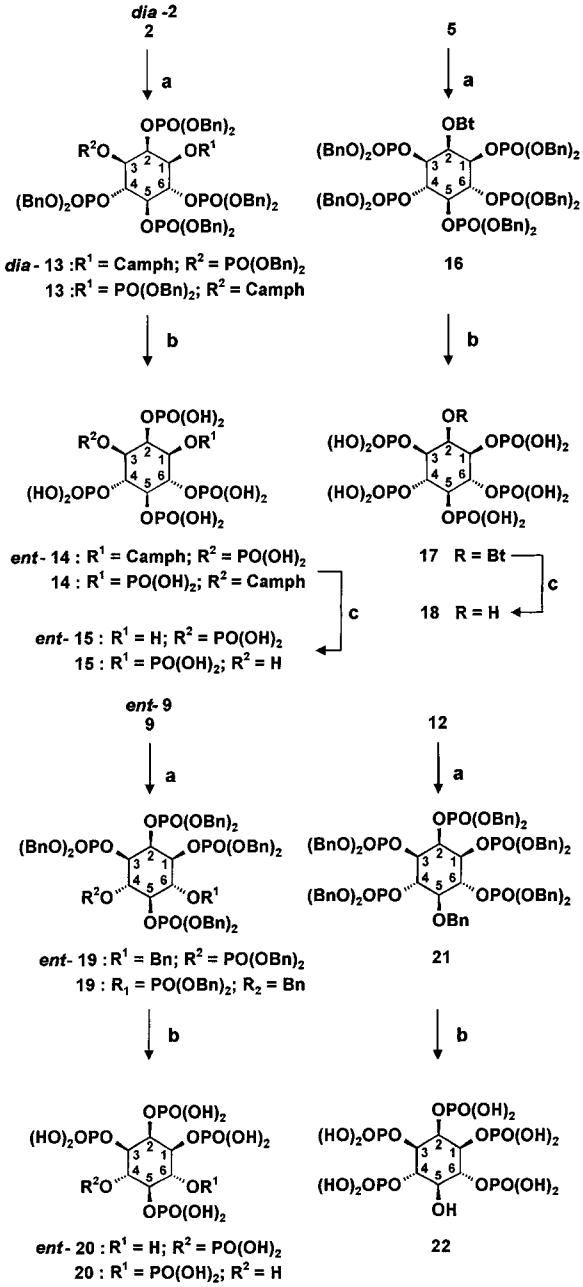
Biochemistry

To investigate the metabolism of the *myo*-inositol pentakisphosphates all isomers were incubated with a crude cytosolic extract from fetal calf thymus for different periods of time. Microbore-HPLC analysis of the products using co-injection with known *myo*-inositol polyphosphate standards[21] revealed the presence of the different enzyme activities as shown in Table 2. Five of the six isomers were metabolized (Table 2). Interestingly, each isomer yielded a single distinct product under these conditions. Four of the $InsP_5$-isomers were dephosphorylated to $InsP_4$-isomers, while in the presence of ATP Ins(1,2,4,5,6)P_5 was phosphorylated to $InsP_6$ by a 3-kinase enzyme activity. Ins(2,3,4,5,6)P_5 did not appear to be a substrate, at least under the conditions (time scale, buffer etc.) used.

At least three $InsP_5$-metabolizing enzymes were present in the thymic extract, since chromatographic purification of the inositol pentakisphosphate 5-phosphatase removed the Ins(1,2,4,5,6)P_5 3-kinase and the Ins(1,3,4,5,6)P_5 1/3-phosphatase activity (data not shown). This partially purified 5-phosphatase accepted only pentakisphosphates with phosphates in positions 1, 2 and 3. Whether both enzymatic activities are combined in a single protein remains to be investigated.

The results decribed here indicate that in fetal calf thymus Ins(1,2,4,5,6)P_5 is the metabolic precursor of $InsP_6$.

Scheme 4. Synthesis of the six *myo*-inositol pentakisphosphates starting from the regioselectively protected precusors



Reagents and conditions: a: (i) $(BnO)_2PN(iPr)_2$, 1*H*-tetrazole, MeCN, (ii) MeCOOOH, −40 °C. – b: H_2, Pd/C (10%), MeCOOH. – c: KOH, pH = 12.8.

Similar results have been obtained in cytosolic extracts from Jurkat T cells[22]. However, in the latter study the racemic Ins(1,2,4,5,6)P_5 was used as a substrate. In our study, we were able to show that the 3-kinase used only D-Ins(1,2,4,5,6)P_5 as a substrate. In contrast, the 5-phosphatase appeared to accept both Ins(1,2,3,4,5)P_5 and its enantiomer Ins(1,2,3,5,6)P_5.

Our data hint towards a somewhat different scenario for the metabolism of the highly phosphorylated inositol phosphates as compared to liver. For that tissue Shears and coworkers postulated the formation of $InsP_6$ from

Table 1. ^{1}H-NMR data of the six *myo*-inositol pentakisphosphates

Protons[a]		Products[b]			
		Ins(1,2,3,4,5)P_5[c]	Ins(1,2,4,5,6)P_5[d]	Ins(1,3,4,5,6)P_5[e]	Ins(1,2,3,4,6)P_5
	δ	4.14	3.81	4.24	4.29
1-H	m[f]	dddd	d	ddd	dddd
	J [Hz]	9.4, 9.4, 2.6, 1.9	9.8	9.8, 9.4, 2.8	9.5, 9.5, 2.0, 2.0
	δ	4.88	4.68	4.40	4.88
2-H	m	ddd	d	dd	ddd
	J [Hz]	9.8, 2.6, 2.2	9.8	2.8, 2.5	9.8, 2.0, 2.0
	δ	4.27	4.27		
3-H	m	dddd	ddd	see 1-H	see 1-H
	J [Hz]	9.8, 9.4, 2.2, 1.9	9.8, 9.4, 9.4		
	δ	4.43	4.47	4.49	4.37
4-H	m	ddd	ddd	ddd	ddd
	J [Hz]	9.4, 9.4, 9.4	9.4, 9.4, 9.1	9.8, 9.4, 9.4	9.5, 9.2, 9.2
	δ	4.12	4.24	4.28	3.68
5-H	m	ddd	ddd	ddd	dd
	J [Hz]	9.8, 9.4, 9.0	9.4, 9.1, 0.1	9.4, 9.4, 9.4	9.2, 9.2
	δ	3.86	4.35		
6-H	m	dd	ddd	see 4-H	see 4-H
	J [Hz]	9.8, 9.4	9.8, 9.4, 9.1		

[a] ^{1}H-NMR spectra were recorded on a 360 MHz spectrometer, Bruker AM 360. Chemical shifts were measured in ppm relative to tetramethyl silane (TMS) as internal standard. The solvent was D_2O. – [b] All products were prepared as free acids and were measured at pH = 1.6. – [c] An identical ^{1}H-NMR spectra was obtained for the enantiomeric Ins(1,2,3,5,6)P_5. – [d] ^{1}H-NMR data correspond to Stephens et al.[10b]. An identical ^{1}H-NMR spectrum was obtained for the enantiomeric Ins(2,3,4,5,6)P_5. – [e] ^{1}H-NMR data correspond to Lu et al.[25]. – [f] Multiplicity.

Table 2. Metabolism of synthetic inositol pentakisphosphates by a crude cytosolic extract from fetal calf thymus

Substrate[a]	Product[b]	Enzyme activity[c]
Ins(1,2,3,4,6)P_5	D/L-Ins(1,2,3,4)P_4	4/6-phosphatase
Ins(1,2,3,4,5)P_5	Ins(1,2,3,4)P_4	5-phosphatase
Ins(1,2,3,5,6)P_5	Ins(1,2,3,6)P_4	5-phosphatase
Ins(1,2,4,5,6)P_5	InsP_6	3-kinase
Ins(2,3,4,5,6)P_5	no product	no reaction
Ins(1,3,4,5,6)P_5	D/L-Ins(1,4,5,6)P_4	1/3-phosphatase

[a] Substrate concentrations were 22 μM. All other conditions are given in the Experimental Section. – [b] The analysis of the products was performed using microbore metal-dye detection HPLC as described previously[21]. – [c] Enzyme assays were carried out as described in the Experimental Section.

Ins(1,3,4,5,6)P_5, the most abundant inositol pentakisphosphate[5]. Although this finding is not supported by our results with bovine thymus, the well-documented hydrolysis of Ins(1,3,4,5,6)P_5 to Ins(3,4,5,6)P_4 and/or its enantiomer Ins(1,4,5,6)P_4 was confirmed by our experiments[5][22]. The chromatographic properties of the Ins(1,3,4,5,6)P_5 1/3-phosphatase activity from thymus indicate that it is the same enzyme as that purified from rat liver[23].

Future investigations with purified enzymes should allow a more detailed description of the metabolism of the inositol pentakisphosphates. The full set of synthetic pentakisphosphates described here appears to be crucial in order to perform these experiments. The enantiomerically pure compounds in particular have already proved to be very useful in defining the substrate-specificity of the novel Ins(1,2,4,5,6)P_5 3-kinase and the D/L-Ins(1,2,3,4,5)P_5 5-phosphatase.

We thank *J. Stelten* for recording the NMR spectra, Dr. *P. Schulze* for providing FAB-MS data and *Miller-Pharma* (Hamburg) for fetal calf thymus. We are indebted to the *Deutsche Forschungsgemeinschaft* (Schu 943/1-4, 1-5 u. 1-6; Gu 360/1-1 u. 1-2) for financial support.

Experimental Section

General: Melting points: Büchi B-540 apparatus (uncorrected). – ^{1}H-NMR spectra: 360 MHz, Bruker AM 360, TMS as internal standard. – ^{31}P-NMR spectra: 145.8 MHz, Bruker AM 360, 85% H_3PO_4 as external standard. Chemical shifts are reported as usual in ppm units. – Mass spectra: Finnigan Mat 8222 mass spectrometer with fast atom bombardment (FAB) ionization. High-resolution masses were determined relative to known compounds with a mass not differing by more than 10%. – Optical rotations were measured at the sodium *D*-line in a 10 cm cell with a Perkin-Elmer 1231 polarimeter. – Ultrafiltration of the palladium/carbon catalyst was performed with a Sartorius filtration apparatus SM 162 01 using filters from regenerated cellulose (Sartorius, SM 116 04). – Elemental analysis were performed by Mikroanalytisches Labor Beller, Göttingen, FRG.

All reagents were obtained in the highest purity available. Where necessary, solvents were dried and/or distilled before use. Acetonitrile was distilled from phosphorus(V) oxide and stored over 3 Å molecular sieves, as was DMF. Pyridine and toluene were stored over 4 Å molecular sieves. Palladium on carbon (10%) was from Acros Chemie. (1*S*)-(−)-Camphanoyl chloride, dibenzyl *N,N*-diisopropylphosphoramidite and tetrazole were from Aldrich. Butyric anhydride was from Merck. Trifluoroacetic acid, benzyl bromide, 4-(dimethylamino)pyridine (DMAP), cesium fluoride and 4-methoxybenzyl chloride were from Fluka. The ion-exchange resin Dowex 50 WX 8, H^+-form, was from Serva, Heidelberg. All other reagents were from Riedel-de Haën. *rac*-1,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (*rac*-**1**), D-3,4,5,6-tetra-*O*-benzyl-1-*O*-camphanoyl-*myo*-inositol and D-1,4,5,6-tetra-*O*-benzyl-3-*O*-camphanoyl-*myo*-inositol were synthesized according to the procedures described previously[13]. The method of Angyal and Tate[12] was used to synthesise *rac*-1,2-5,6-Di-*O*-cyclohexylidene-*myo*-inositol (*rac*-**6**) and *rac*-1,2-3,4-di-*O*-cyclohexylidene-*myo*-inositol (**10**).

General Procedure for Phosphorylations: The selectively protected *myo*-inositol derivative and tetrazole were dissolved under argon in dry acetonitrile before dibenzyl *N,N*-diisopropylphosphoramidite was added. After stirring the mixture at room temp. for 4 h to 2 d, HPLC analysis showed no further reaction. The reaction mixture was cooled to −40°C and peracetic acid (32% v/w; 1 mol equiv. for each mol equiv. of phosphoramidite) was added with vigorous stirring. The mixture was allowed to warm to room temp. The solvent was removed under reduced pressure and the residual oil was purified by preparative HPLC to give the fully protected inositol pentakisphosphate derivative.

General Procedure for Deprotection of Benzyl Groups by Hydrogenolysis: A solution of the fully protected *myo*-inositol pentakisphosphate or the tetrabenzyl-inositol, respectively, in acetic acid was vigorously stirred with palladium on carbon (10%; 0.1 mol palladium for each mol of benzyl groups) under hydrogen in a self-built hydrogenation apparatus for 4−15 h. The catalyst was removed by ultrafiltration and the filtrate was freeze dried to give the respective product.

1-O-Camphanoyl-myo-inositol (*dia*-**2**): 3,4,5,6-Tetra-*O*-benzyl-1-*O*-camphanoyl-*myo*-inositol (150 mg, 208 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give pentol *dia*-**2** (74.5 mg, 99%) as a white powder after freeze drying, m.p. 210−211°C (EtOH). − $[\alpha]^{24}$ = +26.0 (*c* = 0.55, MeOH). − ^{1}H NMR ([D$_6$]DMSO): δ = 4.99 (d, *J* = 3.8 Hz, 1 H, OH), 4.93 (d, *J* = 5.6 Hz, 1 H, OH), 4.84 (d, *J* = 4.5 Hz, 1 H, OH), 4.73 (d, *J* = 4.9 Hz, 1 H, OH), 4.69 (d, *J* = 4.9 Hz, 1 H, OH), 4.62 (dd, *J* = 9.9, 2.4 Hz, 1 H, 1-H), 3.84 (ddd, *J* = 3.8, 2.4, 2.4 Hz, 1 H, 2-H), 3.60 (ddd, *J* = 9.7, 9.4, 5.6 Hz, 1 H, 4-H), 3.38 (ddd, *J* = 9.9, 9.3, 4.5 Hz, 1 H, 6-H), 3.22 (ddd, *J* = 9.7, 4.9, 2.4 Hz, 1 H, 3-H), 3.01 (ddd, *J* = 9.3, 9.3, 4.9 Hz, 1 H, 5-H), 2.43 (m, 1 H, camph), 1.93 (m, 2 H, camph), 1.55 (m, 1 H, camph), 1.06 (s, 3 H, CH_3), 1.01 (s, 3 H, CH_3), 0.86 (s, 3 H, CH_3). − MS (FAB); *m/z* (%): 361 (15) [M + H$^+$], 195 (35) [camphO$^+$], 359 (12) [M − H$^+$], 197 (100) [camphO$^-$]. − $C_{16}H_{24}O_9$: calcd. C 53.33, H 6.71; found C 53.14, H 6.86.

3-O-Camphanoyl-myo-inositol (**2**): 1,4,5,6-Tetra-*O*-benzyl-3-*O*-camphanoyl-*myo*-inositol (150 mg, 208 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give pentol **2** (74 mg, 98%) as a white powder after freeze drying, m.p. 226−227°C (EtOH). − $[\alpha]^{24}$ = -33.6 (*c* = 0.39, MeOH). − ^{1}H NMR ([D$_6$]DMSO): δ = 4.99 (d, *J* = 3.8 Hz, 1 H, OH), 4.93 (d, *J* = 5.6 Hz, 1 H, OH), 4.84 (d, *J* = 4.5 Hz, 1 H, OH), 4.73 (d, *J* = 4.9 Hz, 1 H, OH), 4.66 (d, *J* = 4.9 Hz, 1 H, OH), 4.56 (dd, *J* = 9.9, 2.4 Hz, 1 H, 3-H), 3.85 (ddd, *J* = 3.8, 2.4, 2.4 Hz, 1 H, 2-H), 3.61 (ddd, *J* = 9.7, 9.4, 5.6 Hz, 1 H, 6-H), 3.39 (ddd, *J* = 9.9, 9.3, 4.5 Hz, 1 H, 4-H), 3.24 (ddd, *J* = 9.7, 4.9, 2.4 Hz, 1 H, 1-H), 3.01 (ddd, *J* = 9.3, 9.3, 4.9 Hz, 1 H, 5-H), 2.43 (m, 1 H, camph), 1.93 (m, 2 H, camph), 1.55 (m, 1 H, camph), 1.05 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3). − MS (FAB); *m/z* (%): 361 (21) [M + H$^+$], 195 (45) [camphO$^+$], 359 (10) [M − H$^+$], 197 (100) [camphO$^-$]. − $C_{16}H_{24}O_9$: calcd. C 53.33, H 6.71; found C 53.25, H 6.79.

1-O-Camphanoyl-myo-inositol 2,3,4,5,6-Pentakis(dibenzyl)-phosphate (*dia*-**13**): A solution of pentol *dia*-**2** (62.3 mg, 173 μmol) and tetrazole (245 mg, 3.36 mmol) in acetonitrile (3 ml) was treated with dibenzyl *N,N*-diisopropylphosphoramidite (1.17 ml, 3.46 mmol) for 16 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC (93% MeOH; 40 ml/min; t_R = 29.10 min) gave compound *dia*-**13** (177 mg, 60%) as an oil. − $[\alpha]^{24}$ = +0.82 (*c* = 3.7, $CHCl_3$). − ^{1}H NMR (360 MHz, $CDCl_3$), δ = 7.10−7.45 (m, 50 H, ArH), 5.26−5.36 (m, 3 H, 2-H, 4-H, 6-H), 4.85−5.22 (m, 21 H, 5 × CH_2 ph, 1-H), 4.60 (ddd, *J* = 9.0, 9.0, 9.0 Hz, 1 H, 5-H), 4.46 (dddd, *J* = 9.8, 9.5, 2.3, 2.3 Hz, 1 H, 3-H), 2.26 (m, 1 H, camph), 1.92 (m, 1 H, camph), 1.72 (m, 1 H, camph), 1.40 (m, 1 H, camph), 1.07 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3). − ^{31}P NMR (145.8 MHz, ^{1}H-decoupled, $CDCl_3$): δ = −0.31(1 P, s), −0.42 (1 P, s), −0.94 (1 P, s), −0.96 (1 P, s), −2.01 (1 P, s). − MS (FAB); *m/z* (%): 1661 (5) [M + H$^+$], 91 (100) [Bn$^+$], 1659 (12) [M − H$^+$], 277 (100) [OPO(OBn)$_2^-$]. − $C_{79}H_{82}O_{24}P_5$: calcd. [M − Bn$^+$] 1569.3884; found 1569.3889 (MS).

3-O-Camphanoyl-myo-inositol 1,2,4,5,6-Pentakis(dibenzyl)-phosphate (**13**): A solution of pentol **2** (53 mg, 148 μmol) and tetrazole (208 mg, 2.96 mmol) in acetonitrile (3 ml) was treated with dibenzyl *N,N*-diisopropylphosphoramidite (1.00 ml, 2.96 mmol) for 17 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC (93% MeOH; 40 ml/min; t_R = 27.15 min) gave compound **13** (171 mg, 69%) as an oil. − $[\alpha]^{24}$ = −0.10 (*c* = 3.4, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 7.10−7.46 (m, 50 H, ArH), 5.36 (ddd, *J* = 9.1, 2.4, 2.4 Hz, 1 H, 2-H), 5.20−5.25 (m, 2 H, 4-H, 6-H), 4.90−5.18 (m, 21 H, 5 × CH_2 ph, 3-H), 4.59 (ddd, *J* = 9.0, 9.0, 9.0 Hz, 1 H, 5-H), 4.49 (dddd, *J* = 9.8, 9.5, 2.4, 2.3 Hz, 1 H, 1-H), 2.40 (m, 1 H, camph), 2.12 (m, 1 H, camph), 1.62 (m, 1 H, camph), 1.43 (m, 1 H, camph), 1.06 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 092 (s, 3 H, CH_3). − ^{31}P NMR (^{1}H decoupled, $CDCl_3$): δ = −0.40 (1 P, s), −0.46 (1 P, s), −0.74 (1 P, s), −1.07 (1 P, s), −1.70 (1 P, s). − MS (FAB); *m/z* (%): 1661 (< 1) [M + H$^+$], 91 (100) [Bn$^+$], 1570 (19) [M − Bn$^+$], 277 (100) [OPO(OBn)$_2^-$]. − $C_{79}H_{82}O_{24}P_5$: calcd. [M − Bn$^+$] 1569.3884; found 1569.3844 (MS).

1-O-Camphanoyl-myo-inositol 2,3,4,5,6-Pentakisphosphate (*dia*-**14**): Compound *dia*-**13** (170 mg, 102 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give title compound *dia*-**14** (71.5 mg, 89%) as a solid after freeze drying. − $[\alpha]^{24}$ = +15.4 (*c* = 0.48, MeOH). − ^{1}H NMR (D_2O): δ = 5.28 (dd, *J* = 9.9, 2.2 Hz, 1 H, 1-H), 4.97 (ddd, *J* = 9.3, 2.5, 2.2 Hz, 1 H, 2-H), 4.66 (ddd, *J* = 9.9, 9.9, 9.6 Hz, 1 H, 4-H), 4.55 (ddd, *J* = 9.6, 9.6, 9.0 Hz, 1 H, 6-H), 4.40 (m, 2 H, 3-H, 5-H), 2.57 (m, 1 H, camph), 2.00 (m, 2 H, camph), 1.61 (m, 1 H, camph), 1.05 (s, 3 H, CH_3), 1.03 (s, 3 H, CH_3), 0.91 (s, 3 H, CH_3). − ^{31}P NMR (^{1}H decoupled, D_2O): δ = 1.30 (1 P, s), 0.80 (1 P, s), 0.23 (1 P, s), −0.25 (1 P, s), −0.89 (1 P, s). − MS (FAB); *m/z* (%): 761 (9) [M + H$^+$], 82 (100) [OP(OH)$_2^+$], 759 (100) [M − H$^+$]. − $C_{16}H_{28}O_{24}P_5$: calcd. [M − H$^+$] 758.9659; found 758.9689 (MS).

3-O-Camphanoyl-myo-inositol 1,2,4,5,6-Pentakisphosphate (**14**): The fully protected phosphate **13** (157 mg, 95 μmol) was hydrogenated as described for the other diastereomer to give compound **14** (67 mg, 90%). − $[\alpha]^{24}$ = −19.1 (*c* = 0.35, MeOH). − ^{1}H NMR (D_2O): δ = 5.30 (dd, *J* = 9.9, 2.2 Hz, 1 H, 3-H), 4.98 (ddd, *J* = 9.3, 2.5, 2.2 Hz, 1 H, 2-H), 4.67 (ddd, *J* = 9.9, 9.9, 9.6 Hz, 1 H, 6-H), 4.56 (ddd, *J* = 9.6, 9.6, 9.0 Hz, 1 H, 4-H), 4.41 (m, 2 H, 1-H, 5-H), 2.62 (m, 1 H, camph), 2.04 (m, 2 H, camph), 1.63 (m, 1 H, camph), 1.10 (s, 3 H, CH_3), 1.05 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3). − ^{31}P NMR (^{1}H decoupled, D_2O): δ = 1.24 (1 P, s), 0.78 (1 P, s), 0.25 (1 P, s), −0.02 (1 P, s), −1.30 (1 P, s). MS (FAB); *m/z* (%): 761 (< 1) [M + H$^+$], 82 (100) [OP(OH)$_2^+$], 759 (100) [M − H$^+$]. − $C_{16}H_{28}O_{24}P_5$: calcd. [M − H$^+$] 758.9659; found 758.9679 (MS).

myo-Inositol 2,3,4,5,6-Pentakisphosphate (*ent*-**15**): Compound *dia*-**14** (58 mg, 73 μmol) was treated with 0.5 mol KOH (1.61 ml) to adjust the pH = 12.8. The solution was stirred at room temp. for 2 d. The reaction mixture was directly poured onto an ion-exchange column (Dowex 50 WX 8, H$^+$) for purification. The

aqueous phase was washed twice with CH_2Cl_2 (15 ml) and twice with ethyl acetate (15 ml). Lyophilization gave the title compound *ent*-**15** (34 mg, 80%). – $[\alpha]^{24} = -6.2$ ($c = 0.96$ in water, pH = 1.6). – ^{31}P NMR (^{1}H decoupled, D_2O, free acid): δ = 0.72 (1 P, s), 0.49 (2 P, s), −0.03 (1 P, s), −0.29 (1 P, s), −1.30 (1 P, s). – MS (FAB); *m/z* (%): 581 (40) [M + H$^+$], 579 (100) [M − H$^+$]. – $C_6H_{16}O_{21}P_5$: calcd. [M − H$^+$] 578.8872; found 578.8862 (MS).

myo-Inositol 1,2,4,5,6-Pentakisphosphate (**15**): A similar reaction and workup of compound **14** afforded the pentakisphosphate **5**. – $[\alpha]^{24} = -7.1$ ($c = 0.83$ in water, pH 1.6). – Spectral data were identical to those of enantiomer *ent*-**5**. – $C_6H_{16}O_{21}P_5$: calcd. [M − H$^+$] 578.8872; found 578.8861 (MS).

rac-3,4,5,6-Tetra-O-benzyl-1-O-(4-methoxybenzyl)-myo-inositol (*rac*-**3**): Dry *rac*-**1** (550 mg, 1 mmol) and dry dibutyl tin oxide (248 mg, 1.01 mmol) were heated to reflux in dry toluene (100 ml) in a Soxhlet apparatus filled with activated molecular sieve (3 Å) for 4 h. The reaction mixture was cooled to room temp. and evaporated to dryness under diminished pressure. CsF (303 mg, 2 mmol) was added to the residual oil, and the mixture was kept under high vacuum for 2 h. The residual syrup was dissolved in dry DMF (5 ml) under argon and 4-methoxybenzyl chloride (283 µl, 2.8 mmol) was added. After stirring the solution for 5 h at 60°C, HPLC analysis (90% MeOH; 1.5 ml/min; t_R = 5.55 min) showed no further reaction. Excess of 4-methoxybenzyl chloride and DMF were removed in high vacuum. The crude product was chromatographed by preparative HPLC (90% MeOH; 40 ml/min; t_R = 31 min) to give compound *rac*-**3** (0.45g, 70%) as a solid, m.p. 129−130°C (MeOH) [ref.[13] m.p. 128−129°C (MeOH)]. – ^{1}H NMR ($CDCl_3$): δ = 7.25−7.40 (m, 22 H, ArH), 6.65 (d, 2 H, PMB ArH), 4.75−4.96 (m, 8 H, 4 × CH_2 Ph), 4.66 (s, 2 H, CH_2 of PMB), 4.20 (dd, J = 2.7, 2.7 Hz, 1 H, 2-H), 4.01 (dd, J = 9.5, 9.5 Hz, 4-H), 3.98 (dd, J = 9.5, 9.5 Hz, 6-H), 3.81 (s, 3 H, OCH_3), 3.46 (dd, J = 9.5, 9.5 Hz, 1 H, 5-H), 3.39 (dd, J = 9.5, 2.7 Hz, 1 H, 3-H), 3.36 (dd, J = 9.5, 2.7 Hz, 1 H, 1-H). – MS (FAB); m/z (%): 659 (100) [M − H$^+$], 569 (20) [M − Bn$^+$].

rac-3,4,5,6-Tetra-O-benzyl-2-O-butyryl-1-O-(4-methoxybenzyl)-myo-inositol (*rac*-**4**): A solution of *rac*-**3** (297 mg, 450 µmol), butyric anhydride (326 ml, 900 µmol) and DMAP (56 mg, 45 µmol) in dry pyridine (4 ml) was stirred at room temp. for 3 h. The solvents were evaporated under high vacuum to give an oil. Residual pyridine was removed by evaporing three times with octane. The residue was dissolved in *tert*-butyl methyl ether (15 ml) and washed once with phosphate buffer (10 ml), sodium hydrogen carbonate (10 ml), sodium hydrogen sulfate (10 ml) and then with brine (10 ml) The organic layer was dried with Na_2SO_4 and filtered. Evaporation of the solvent gave pure *rac*-**4** (310 mg, 94%) as an oil. – ^{1}H NMR ($CDCl_3$): δ = 7.25−7.35 (m, 20 H, ArH), 6.85 (m, 2 H, PMB ArH), 5.89 (dd, J = 2.7, 2.7 Hz, 1 H, 2-H), 4.45−4.91 (m, 10 H, 4 × CH_2 ph and CH_2 in PMB), 3.89 (dd, J = 9.7, 9.6 Hz, 1 H, 4-H), 3.86 (dd, J = 9.6, 9.4 Hz, 1 H, 6-H), 3.80 (s, 3 H, OCH_3), 3.50 (dd, J = 9.7, 9.6 Hz, 1 H, 5-H), 3.48 (dd, J = 9.6, 2.7 Hz, 1 H, 3-H), 3.46 (dd, J = 9.4, 2.7 Hz, 1 H, 1-H), 2.49 (t, J = 7.4 Hz, 2 H, CH_2CO), 1.69 (m, 2 H, CH_2), 0.94 (t, J = 7.4 Hz, 2 H, CH_3). – MS (FAB); *m/z* (%): 731 (4) [M + H$^+$], 121 (100) [PMB$^+$]. – $C_{42}H_{43}O_7$: calcd. [M − Bt$^+$] 659.3009; found 659.2994 (MS).

2-O-Butyryl-myo-inositol (**5**): Compound *rac*-**4** (195 mg, 267 µmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give pentol **5** (65 mg, 99%) as a white powder, m.p. 160−161°C (EtOH). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.20 (dd, J = 2.8, 2.8 Hz, 1 H, 2-H), 4.78 (s, 2 H, OH), 4.74 (s, 2 H, OH), 4.70 (s, 1 H, OH), 3.29 (dd, J = 9.8, 9.8 Hz, 2 H, 4-H and 6-H), 3.25 (dd, J = 9.8, 2.8 Hz, 2 H, 1-H and 3-H), 2.96 (dd, J = 9.8, 9.8 Hz, 1 H, 5-H), 2.25 (t, J = 7.3 Hz, 2 H, CH_2CO), 1.55 (m, 2 H, CH_2), 0.90 (t, J = 7.4 Hz, 3 H, CH_3). – MS (FAB); *m/z* (%): 251 (100) [M + H$^+$], 70 (100) [Bt$^+$], 249 (28) [M − H$^+$]. – $C_{10}H_{18}O_7$: calcd. C 48.00, H 7.25; found C 48.07, H 7.20.

2-O-Butyryl-myo-inositol 1,3,4,5,6-Pentakis(dibenzyl)phosphate (**16**): A solution of alcohol **5** (48 mg, 193 µmol) and tetrazole (274 mg, 3.86 mmol) in acetonitrile (4 ml) was treated with dibenzyl *N,N*-diisopropylphosphoramidite (1.31 ml, 3.86 mmol) for 17 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC (91% MeOH; 40 ml/min; t_R = 37.00 min) gave compound **16** (177 mg, 60%) as an oil. – ^{1}H NMR ($CDCl_3$): δ = 7.11−7.36 (m, 50 H, ArH), 6.07 (dd, J = 2.7, 2.3 Hz, 1 H, 2-H), 4.89−5.06 (m, 22 H, CH_2 ph, 4-H and 6-H), 4.43−4.54 (m, 3 H, 5-H, 1-H and 3-H), 2.27 (t, J = 7.3 Hz, 2 H, CH_2CO), 1.58 (m, 2 H, CH_2), 0.90 (t, 3 H, CH_3). – ^{31}P NMR (^{1}H decoupled, $CDCl_3$): δ = −1.18 (2 P, s), −0.74 (2 P, s), −0.48 (1 P, s). – MS (FAB); *m/z* (%): 1551 (3) [M + H$^+$], 91 (100) [Bn$^+$], 1459 (12) [M − Bn$^+$], 277 (100) [OPO(OBn)$_2^-$]. – $C_{73}H_{76}O_{22}P_5$: calcd. [M − Bu$^+$] 1459.3517; found 1459.3509 (MS).

2-O-Butyryl-myo-inositol 1,3,4,5,6-Pentakisphosphate (**17**): The fully protected pentakisphosphate **16** (170 mg, 110 µmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give the pentakisphosphate **17** (70 mg, 98%) as a solid after freeze drying. – ^{1}H NMR (D_2O): δ = 5.66 (dd, J = 2.9, 2.9 Hz, 1 H, 2-H), 4.42 (ddd, J = 9.8, 9.4, 9.4 Hz, 2 H, 4-H and 6-H), 4.31 (dddd, J = 9.8, 9.4, 2.9, 1.8 Hz, 2 H, 1-H and 3-H), 4.23 (ddd, J = 9.4, 9.4, 9.4 Hz, 1 H, 5-H), 2.31 (t, J = 7.4 Hz, 2 H, CH_2CO), 1.50 (m, 2 H, CH_2), 0.78 (t, J = 7.4 Hz, 3 H, CH_3). – ^{31}P NMR (^{1}H decoupled, D_2O): δ = 0.60 (1 P, s), 0.30 (2 P, s), −0.49 (2 P, s). – MS (FAB); *m/z* (%): 651 (10) [M + H$^+$], 72 (100) [Bt$^+$], 649 (100) [M − H$^+$]. – $C_{10}H_{22}O_{22}P_5$: calcd. [M − H$^+$] 648.9291; found 648.9263 (MS).

myo-Inositol 1,3,4,5,6-Pentakisphosphate (**18**): Compound **17** (28 mg, 43 µmol) was treated with 0.5 M KOH (963 µl) to adjust the pH = 12.8. The solution was stirred at room temp. for 2 d. The reaction mixture was directly poured onto an ion-exchange column (Dowex 50 WX 8, H$^+$) for purification. Lyophilization gave the title compound **18** (24 mg, 95%). – ^{31}P NMR (^{1}H decoupled, D_2O, free acid): δ = 0.88 (1 P, s), 0.50 (2 P, s), −0.11 (2 P, s). – MS (FAB); *m/z* (%): 579 (65) [M − H$^+$]. – $C_6H_{16}O_{21}P_5$: calcd. [M − H$^+$] 578.8872; found 578.8868 (MS).

rac-4-O-Benzyl-1,2:5,6-di-O-cyclohexylidene-myo-inositol (*rac*-**7**): Dry *rac*-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (900 mg, 2.65 mmol) and dry dibutyl tin oxide (667 mg, 2.67 mmol) were heated under reflux in dry toluene (100 ml) in a Soxhlet apparatus filled with activated molecular sieve (3 Å) for 4 h. The reaction mixture was cooled to room temp. and evaporated to dryness under diminished pressure. CsF (797 mg, 5.24 mmol) was added to the residual oil, and the mixture was left for 2 h under high vacuum. The syrup was dissolved in dry DMF (7.5 ml) under argon and benzyl bromide (882 ml, 7.42 mmol) was added. After stirring the solution for 36 h at room temp., HPLC analysis (80% MeOH; 1.5 ml/min; t_R = 9.45 min) showed no further reaction. Excess benzyl bromide and DMF were removed in high vacuum. The crude product was separated by preparative HPLC (80% MeOH; 40 ml/min; t_R = 50.30 min) to give compound *rac*-**7** (0.65g, 60%) as a solid. For spectroscopic data see the enantiomeric pure compound **7**.

Preparation of Enantiomerically Pure 4-O-Benzyl-1,2:5,6-di-O-cyclohexylidene-myo-inositol (**7**) *and 6-O-Benzyl-2,3;4,5-di-O-cyclohexylidene-myo-inositol* (*ent*-**7**): (−)-Camphanoyl chloride (653 mg,

3.02 mmol) was added to a solution of *rac*-4-*O*-benzyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (*rac*-**7**) (650 mg, 1.51 mmol) and DMAP (18.3 mg, 14.3 μmol) in dry pyridine (5 ml). The reaction mixture was stirred at room temp. for 18 h. The solvent was removed under diminshed pressure to give an oil. Residual pyridine was removed by evaporation three times with octane. The oil was dissolved in *tert*-butyl methyl ether (40 ml) and was washed once with phosphate buffer (20 ml), sodium hydrogen carbonate solution (20 ml), aqueous sodium hydrogen sulfate (20 ml) and finally with brine (20 ml). The organic layer was dried with Na_2SO_4 and filtered. The mixture was chromatographed (Si 60, 200 g, CH_2Cl_2/diisopropyl ether, 97.5:2.5) to afford the two diastereomers 4-*O*-benzyl-3-*O*-camphanoyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**8**) and 6-*O*-benzyl-1-*O*-camphanoyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (*dia*-**8**). **8**: M.p. 155°C (MeOH) (ref.[16]: m.p. 152–153°C) – $[\alpha]^{24} = -39.6$ (c = 0.96, $CHCl_3$) {ref.[16]: [α] = −37 (c = 1, $CHCl_3$)}. – ^{1}H NMR ($CDCl_3$): δ = 7.27–7.38 (m, 5 H, ArH), 5.25 (dd, J = 4.9, 4.4 Hz, 1 H, 3-H), 4.75 (AB, J = 10.1 Hz, 2 H, CH_2 ph), 4.62 (dd, J = 6.3, 4.4 Hz, 1 H, 2-H), 4.33 (dd, J = 8.2, 6.3 Hz, 1 H, 3-H), 3.87 (dd, J = 8.2, 4.9 Hz, 1 H, 4-H), 3.85 (dd, J = 10.2, 8.2 Hz, 1 H, 6-H), 3.50 (dd, J = 10.2, 8.2 Hz, 1 H, 5-H), 2.38 (m, 1 H, camph), 2.00 (m, 1 H, camph), 1.89 (m, 1 H, camph), 1.28–1.78 (m, 21 H, 2 × cyclohexylidene, camph), 1.10 (s, CH_3), 1.05 (s, 3 H, CH_3), 090 (s, 3 H, CH_3). – MS (FAB); *m/z* (%): 611 (19) [M + H$^+$], 91 (100) [Bn$^+$], 610 (< 1) [M − H$^+$], 197 (100) [camphO−]. *dia*-**8**: M.p. 120°C (MeOH) {ref.[16]: m.p. 121–123°C}. – $[\alpha]^{24} = +26$ (c = 0.58, $CHCl_3$) {ref.[16]: [α] = +23 (c = 1, $CHCl_3$)}. – ^{1}H NMR ($CDCl_3$): δ = 7.27–7.39 (m, 5 H, ArH), 5.27 (dd, J = 4.9, 4.4 Hz, 1 H, 3-H), 4.74 (AB, J = 10 Hz, 2 H, CH_2 ph), 4.59 (dd, J = 6.3, 4.4 Hz, 1 H, 2-H), 4.32 (dd, J = 8.3, 6.3 Hz, 1 H, 1-H), 3.86 (dd, J = 8.3, 4.9 Hz, 1 H, 6-H), 3.84 (dd, J = 10.3, 8.3 Hz, 1 H, 4-H), 3.53 (dd, J = 10.3, 8.3 Hz, 1 H, 5-H), 2.42 (m, 1 H, camph), 2.01 (m, 1 H, camph), 1.91 (m, 1 H, camph), 1.26–1.72 (m, 21 H, 2 × cyclohexylidene, camph), 1.11 (s, CH_3), 1.00 (s, 3 H, CH_3), 091 (s, 3 H, CH_3). – MS (FAB); *m/z* (%): 611 (12) [M + H$^+$], 91 (100) [Bn$^+$], 610 (2) [M − H$^+$], 197 (100) [camphO$^-$]. To generate the enantiomerically pure **7** and *ent*-**7** each ester was dissolved in MeOH, 1 M KOH (to pH ≈ 13) was added, and the solution was stirred at 40°C for 16 h. The reaction mixture was evaporated under reduced pressure and the product was extracted with *tert*-butyl methyl ether. The organic layer was washed with a sodium hydrogene sulfate solution (5%), phosphate buffer and brine, dried with Na_2SO_4, filtered and evaporated. Crystallization from MeOH gave pure the enantiomers **7** (226 mg, 75%) and *ent*-**7** (230 mg, 76%). **7**: M.p. 87°C (MeOH) (ref.[16]: m.p. 87–89°C). – $[\alpha]^{24} = +3.9$ (c = 1.1, $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 7.25–7.40 (m, 5 H, ArH), 4.73 (AB, J = 10 Hz, 2 H, CH_2 ph), 4.44 (dd, J = 7.5, 3.8 Hz, 1 H, 2-H), 4.35 (dd, J = 7.5, 7.5 Hz, 1 H, 3-H), 4.19 (dd, J = 10.9, 7.5 Hz, 1 H, 4-H), 4.04 (dd, J = 3.8, 2.1 Hz, 1 H, 1-H), 3.90 (dd, J = 7.9, 2.1 Hz, 1 H, 6-H), 3.56 (dd, J = 10. 9, 7.9 Hz, 1 H, 5-H) 2.60 [s (br), 1 H, OH], 1.53–1.76 (m, 20 H, 2 × cyclohexylidene). – MS (FAB); *m/z* (%): 429 (8) [M − H$^+$], 431 (20) [M + H$^+$], 91 (100) [Bn$^+$]. *ent*-**7**: $[\alpha]^{24} = -4.1$ (c = 1.2, $CHCl_3$) {ref.[17]: [α] = −4.0 (c = 1, $CHCl_3$)}. All other spectroscopic data were in accordance with those of **7**.

4-O-Benzyl-myo-inositol (**9**): A solution of **7** (114 mg, 265 μmol) in CH_3CN/H_2O (100:1, 10 ml) was stirred with trifluoroacetic acid (2 ml) at room temp. for 2 h. The solvent was evaporated under reduced pressure. Crystallisation from EtOH gave the title compound **9** (64 mg, 90%), m.p. 173°C (EtOH) {ref.[18]: m.p. *175–177°C (MeOH/propanol)*}. – $[\alpha]^{24} = +6.1$ (c = 0.5, MeOH) {ref.[18]: [α] = +6.0 (c = 1, MeOH)}. – ^{1}H NMR ($CDCl_3$): δ = 7.20–7.40 (m, 5 H, ArH), 4.75 (AB, J = 9.9 Hz, 2 H, CH_2 ph), 4.36 [s (br), 5 H, 5 × OH], 3.70 (dd, J = 2.7, 2.3 Hz, 1 H, 2-H), 3.32 (dd, J = 9.6, 2.7 Hz, 1 H, 3-H), 3.37–3.42 (m, 2 H, 4-H, 6-H), 3.13 (dd, J = 9.6, 2.3 Hz, 1 H, 1-H), 3.10 (dd, J = 9.3, 8.9 Hz, 1 H, 5-H). – MS (FAB); *m/z* (%): 269 (100) [M − H$^+$], 271 (10) [M + H$^+$], 91 (100) [Bn$^+$].

6-O-Benzyl-myo-inositol (*ent*-**9**): A similar reaction and workup procedure to that of compound *ent*-**8**, described above, afforded the pentol *ent*-**9**. – $[\alpha]^{24} = -5.9$ (c = 1.1, MeOH) {ref.[18]: [α] = −6.0 (c = 1, MeOH)}. All other spectral data were in accordance with those obtained for enantiomer **9**.

4-O-Benzyl-myo-inositol 1,2,3,5,6-Pentakis(dibenzyl)phosphate (**19**): A solution of alcohol **9** (81 mg, 300 μmol) and tetrazole (422 mg, 6.00 mmol) in acetonitrile (3 ml) was treated with dibenzyl *N,N*-diisopropylphosphoramidite (2.00 ml, 6.00 mmol) for 17 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC (95% MeOH; 35 ml/min; t_R = 25.00 min) gave compound **19** (350 mg, 74%) as an oil. – $[\alpha]^{24} = +4.50$ (c = 1.1, $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 6.90–7.30 (m, 55 H, ArH), 5.54 (ddd, J = 8.9, 2.2, 2.2 Hz, 1 H, 2-H), 4.55–5.24 (m, 23 H, 11 × CH_2 ph, 6-H), 4.49 (ddd, J = 9.4, 9.4, 9.4 Hz, 1 H, 5-H), 4.42 (dddd, J = 10.2, 9.8, 2.2, 1.8 Hz, 1 H, 1-H), 4.30 (dddd, J = 9.8, 9.4, 2.2, 1.8 Hz, 1 H, 3-H), 3.92 (dd, J = 9.4, 9.4 Hz, 1 H, 4-H). – ^{31}P NMR (^{1}H-decoupled, $CDCl_3$): δ = −0.37 (1 P, s), −0.62 (1 P, s), −0.95 (1 P, s), −1.14 (1 P, s), −1.48 (1 P, s). – MS (FAB); *m/z* (%): 1571 (10) [M + H$^+$], 91 (100) [Bn$^+$], 1480 (8) [M − Bn$^+$], 277 (100) [$OPO(OBn)_2^-$]. – $C_{69}H_{70}O_{21}P_5$: calcd. [M − 2 × Bu$^+$ + H$^+$] 1389.3098; found 1389.3081 (MS).

6-O-Benzyl-myo-inositol 1,2,3,4,5-Pentakis(dibenzyl)phosphate (*ent*-**19**): Compound *ent*-**9** was phosphorylated as described above to give compound *ent*-**19**. – $[\alpha]^{24} = -4.2$ (c = 1.1, $CHCl_3$). All other spectral data were in accordance with those obtained for enantiomer **19**. – $C_{69}H_{70}O_{21}P_5$: calcd. [M − 2 × Bu$^+$ + H$^+$] 1389.3098; found 1389.3093 (MS).

myo-Inositol 1,2,3,5,6-Pentakisphosphate (**20**): The fully protected inositol phosphate **19** (54mg, 34.5 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give compound **20** (15.4 mg, 77%) as a solid after freeze drying. – $[\alpha]^{24} = +4.30$ (c = 0.43, H_2O, pH = 1.6). – ^{31}P NMR (^{1}H decoupled, D_2O, pH 1.6): δ = 0.85 (1 P, s), 0.35 (1 P, s), 0.12 (1 P, s), 0.00 (1 P, s), −0.61 (1 P, s). – MS (FAB); *m/z* (%): 581 (21) [M + H$^+$], 579 (100) [M − H$^+$]. – $C_6H_{16}O_{21}P_5$: calcd. [M − H$^+$] 578.8872; found 578.8868 (MS).

myo-Inositol 1,2,3,4,5-Pentakisphosphate (*ent*-**20**): In a similar reaction compound *ent*-**19** was hydrogenated to give the pentakisphosphate *ent*-**20**. – $[\alpha]^{24} = -4.00$ (c = 0.23, H_2O, pH = 1.6). All other spectral data were in accordance with those obtained for enantiomer **20**. – $C_6H_{16}O_{21}P_5$: calcd. [M − H$^+$] 578.8872; found 578.8857 (MS).

rac-5-O-Benzyl-1,2:3,4-di-O-cyclohexylidene-myo-inositol (*rac*-**11**): Dry *rac*-1,2:3,4-di-*O*-cyclohexylidene-*myo*-inositol (145 mg, 426 μmol) and dry dibutyltin oxide (107 mg, 431 μmol) were heated to reflux in dry toluene (100 ml) in a Soxhlet apparatus filled with activated molecular sieve (3 Å) for 4 h. The reaction mixture was cooled to room temp. and evaporated to dryness under reduced pressure. CsF (130 mg, 852 μmol) was added to the residual oil, and the mixture was left for 2 h under high vacuum. The residual syrup was dissolved in dry DMF (3 ml) under argon and benzyl bromide (142 ml, 1.19 mmol) was added. After stirring the solution for 16 h at room temp., HPLC analysis (80% MeOH; 1.5 ml/min; t_R = 7.51 min) showed no further reaction. Excess of benzyl bro-

mide and DMF were removed in high vacuum. The crude product was chromatographed by preparative HPLC (79% MeOH; 35 ml/min; t_R = 50.25 min) to give compound *rac*-**11** (107 mg, 60%) as a solid, m.p. 85–86°C (MeOH) (ref.[19]: m.p. 87–89°C). – ^{1}H NMR ($CDCl_3$): δ = 7.27–7.40 (m, 5 H, ArH), 4.80 (AB, *J* = 9.8 Hz, 2 H, CH_2 Ph), 4.62 (dd, *J* = 5.5, 3.0 Hz, 1 H, 2-H), 4.20 (dd, *J* = 6.0, 5.5 Hz, 1 H, 1-H), 4.06 (dd, *J* = 9.8, 9.5 Hz, 1 H, 4-H), 3.86 (dd, *J* = 6.5, 5.5 Hz, 1 H, 6-H), 3.71 (dd, *J* = 9.8, 3.0 Hz, 1 H, 3-H), 3.53 (dd, *J* = 9.8, 6.5 Hz, 1 H, 5-H) 2.62 [s (br), 1 H, OH], 1.32–1.70 (m, 20 H, 2 × cyclohexylidene). – MS (FAB); *m/z* (%): 431 (5) [M + H$^+$], 91 (100) [Bn$^+$].

5-O-Benzyl-myo-inositol (**12**): A solution of *rac*-**12** (95 mg, 221 μmol) in CH_3CN/H_2O (100:1.5 ml) was stirred with trifluoroacetic acid (1 ml) at room temp. for 2 h. The solvent was evaporated under reduced pressure. Crystallization from EtOH gave the title compound **12** (53 mg, 90%), m.p. 286°C (EtOH) {ref.[20]: m.p. 281–283°C (EtOH)}. – ^{1}H NMR ([D$_6$]DMSO): δ = 7.20–7.42 (m, 5 H, ArH), 4.79 (AB, *J* = 10 Hz, 2 H, CH_2 Ph), 4.64 [s (br), 1 H, OH], 4.52 [s (br), 2 H, OH]; 4.45 [s (br), 2 H, OH], 3.71 (dd, *J* = 2.8, 2.8 Hz, 1 H, 2-H), 3.53 (dd, *J* = 9.8, 9.8 Hz, 2 H, 4-H and 6-H), 3.17 (dd, *J* = 9.8, 2.8 Hz, 1 H, 1-H and 3-H), 2.98 (dd, *J* = 9.8, 9.8 Hz, 1 H, 5-H). – (FAB); *m/z* (%): 269 (10) [M – H$^+$], 271 (< 1) [M + H$^+$], 91 (100) [Bn$^+$].

5-O-Benzyl-myo-inositol 1,2,3,4,6-Pentakis(dibenzyl)phosphate (**21**) A solution of alcohol **12** (30 mg, 111 μmol) and tetrazole (156 mg, 2.22 mmol) in acetonitrile (3 ml) was treated with dibenzyl *N,N*-diisopropylphosphoramidite (746 ml, 2.22 mmol) for 17 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC (92% MeOH; 40 ml/min; t_R = 34.45 min) gave compound **21** (136 mg, 79%) as an oil. – ^{1}H NMR ($CDCl_3$): δ = 6.90–7.45 (m, 55 H, ArH), 5.63 (ddd, *J* = 9.3, 2.8, 2.7 Hz, 1 H, 2-H), 4.81–5.23 (m, 22 H, 11 × CH_2 Ph), 4.58 (ddd, *J* = 9.8, 9.8, 9.4 2 H, 4-H and 6-H), 4.38 (dddd, J = 9.2, 2.8, 2.7, 1.8 Hz, 2 H, 1-H and 3-H), 3.53 (dd, *J* = 9.8, 9.4 Hz, 1 H, 5-H). – ^{31}P NMR (^{1}H decoupled, $CDCl_3$): δ = –2.18 (1 P, s), –0.89 (2 P, s), –0.14 (1 P, s). – MS (FAB); *m/z* (%): 1571 (< 1) [M + H$^+$], 91 (100) [Bn$^+$], 1478 (8) [M – Bn$^+$], 277 (100) [OPO(OBn)$_2^+$]. – $C_{69}H_{70}O_{21}P_5$: calcd. [M – 2 × Bu$^+$ + H$^+$] 1389.3098; found 1389.3098.

myo-Inositol 1,2,3,4,6-Pentakisphosphate (**22**): The fully protected phosphate **21** (106 mg, 67 μmol) was hydrogenated as described in the general procedure to give compound **22** (24 mg, 60%). – ^{31}P NMR (^{1}H decoupled, D_2O): δ = 0.57 (2 P, s), 0.47 (2 P, s), –0.54 (1 P, s). – MS (FAB); *m/z* (%): 581 (100) [M + H$^+$], 579 (45) [M – H$^+$]. – $C_6H_{16}O_{21}P_5$: calcd. [M – H$^+$] 578.8872; found 578.8862 (MS).

Biochemistry - General: Fetal calf thymus were obtained from the local slaughterhouse. The tissue was prepared quickly after the death of the cow and the material was immediately used to prepare a crude cytosoiv extract (see below) which was stored at -80°C. Hydroxyapatite resin, ATP, phosphocreatine and creatine phosphokinase were obtained from Sigma, Deisenhofen, Germany. Leupeptin, pepstatin, AEBSF and antipain were from Biomol, Hamburg, Germany. Sephadex G25-medium and Q-Sepharose HP were obtained from Pharmacia Biotech, Freiburg, Germany. Throughout all experiments we used bidistilled and MilliQ-filtered water (Millipore-Waters, Eschborn, Germany).

Preparation of the Thymic Cytosolic Extract: 1–3 g of frozen fetal calf thymus were cut into small pieces and mixed with ice-cold extraction buffer (110 mM KCl, 10 mM NaCl, 1 mM KH_2PO_4, 10 mM $MgCl_2$, 20 mM HEPES, 2 mM EGTA, 1 mM DTT, pH = 7.5 adjusted with KOH) with a mixture of protease inhibitors (final concentrations: 50 μM leupeptin, 1 μM pepstatin, 50 μM antipain and 1 mM AEBSF) in a ratio of 3 ml buffer per gram tissue wet-weight. The tissue was mixed with a turrax-mixer 10 times for 10 seconds on ice. The homogenate was centrifuged at 35 000 × g (Beckman centrifuge J-21 C, Rotor type: JA 21) for 30 min at 4°C. The supernatant liquid was harvested and aliquots were stored for enzyme assays or protein purification at –80°C. If it was necessary to remove small soluble contaminations the cytosolic extract was separated by chromatography using HiTrap desalting columns (Pharmacia, Freiburg, Germany) equilibrated with extraction buffer. An aliquot (5 μl) of the extract was assayed for protein content using the BioRad protein assay.

Partial Purification of InsP_5-phosphatases: Cytosolic extract (50 ml) from 50 g fetal calf thymus was desalted on G-25 medium using a buffer containing 25 mM Hepes, 1 mM DTT, pH = 7.5. The desalted protein (600 mg) was diluted twice with double-distilled water to about 1000 ml and loaded onto a column containing Q-Sepharose FF (Pharmacia Biotech) at a flow rate of 5.5 ml/min. Protein was eluted at 5.5 ml/min applying a linear gradient from 25 mM Hepes, 1 mM DTT, pH = 7.5 to 25 mM HEPES, 1 mM DTT, 1 M NaCl, pH = 7.5 in 126 min. The Ins(1,2,3,4,5)P_5 5-phosphatase did not bind to Q-Sepharose under these conditions whereas Ins(1,2,4,5,6)P_5 3-kinase and Ins(1,3,4,5,6)P_5 1/3-phosphatse did. Ins(1,2,3,4,5)P_5 5-phosphatase was then further purified on a hydroxyapatite-column using a gradient to 500 mM KH_2PO_4 for elution.

For enzyme assays from Q-Sepharose or hydroxyapatite, chromatography fractions were desalted by passing over disposable HiTrap desalting columns. Enzyme assays were carried out with these partially purified enzyme preparations as described below.

Metabolism of the Inositol Pentakisphosphates: The standard incubation mixture contained 100 μg protein and 3 nmol InsP_5 isomer in a volume of 100 μl extraction buffer (for composition see above). For a typical time course 440 μg protein and 4.4 nmol InsP_5 in a volume of 440 μl were incubated at 37°C. When samples were assayed for InsP_5-kinase activity, an ATP generating system (1 mM ATP, 10 mM creatine phosphate and 20 U/ml creatine phosphokinase) was added. Also, a mixture of protease inhibitors (final concentrations: 50 μM leupeptin, 1 μM pepstatin, 50 μM antipain and 1 mM AEBSF) was included. At 0, 30, 60 and 90 min. 100 μl of the mixture were removed and the reaction was stopped by mixing with 150 μl of ice-cold 10% (v/v) perchloric acid and 50 μl of 0.2 M EDTA solution.

Microbore and Standard Metal-dye Detection HPLC: Samples were prepared for HPLC as detailed recently[21][24]. The separations were performed essentially as described either on a standard HPLC using a MonoQ (10/5) column (Pharmacia) or on a microbore HPLC (SMART-system, Pharmacia) using a MiniQ PC3.2/3 column[21][24].

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