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SYNTHESES OF D- AND L-*MYO*-INOSITOL 1,2,4,5-TETRAKISPHOSPHATE AND STEREOSELECTIVITY OF THE I(1,4,5)P_3 RECEPTOR BINDING

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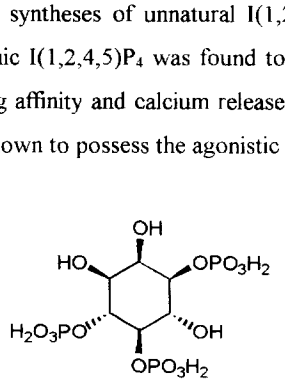
Abstract: D- and L-*myo*-Inositol 1,2,4,5-tetrakisphosphate [D- & L-I(1,2,4,5)P_4], which are analogues of D-*myo*-Inositol 1,4,5-trisphosphate [D-I(1,4,5)P_3], a calcium mobilizing second messenger, were synthesized via resolution of the camphanate ester of a *myo*-inositol derivative, and the binding affinities to I(1,4,5)P_3 receptor were measured.

Since the discovery that D-*myo*-inositol-1,4,5-trisphosphate [I(1,4,5)P_3, **1**] plays a pivotal role as a second messenger in the transmembrane signaling, thus mobilizing calcium ions from the intracellular storage, its interactions with the I(1,4,5)P_3 receptor and metabolic enzymes have been widely studied.[1] One of the major metabolic pathways involves a specific phosphorylation of I(1,4,5)P_3 to I(1,3,4,5)P_4 by I(1,4,5)P_3-3-kinase [IP3K].[2] It has been suggested that I(1,3,4,5)P_4 also acts as a second messenger mediating the entry of extracellular Ca^{2+} through a plasma membrane ion channel,[3] and to mobilize Ca^{2+} also from the intracellular calcium stores, albeit less potently than I(1,4,5)P_3.[4] A study with all possible regioisomers of IP_4s[5] for their ability to bind to the IP_3 receptor in bovine adrenal cortical membranes, and also for their ability to mobilize Ca^{2+} from IP_3-sensitive Ca^{2+} stores in permeabilized CHO cell, indicated that DL-I(1,2,4,5)P_4 had a binding affinity comparable to that of D-I(1,4,5)P_3.[6]

The syntheses of unnatural I(1,2,4,5)P_4 were reported both in the racemic form[7] and in chiral D-form.[8,9] Racemic I(1,2,4,5)P_4 was found to be 2-3 times less potent than the natural ligand, I(1,4,5)P_3 in terms of the binding affinity and calcium release effect from intracellular calcium store,[10] whereas chiral D-I(1,2,4,5)P_4 (**2D**) was shown to possess the agonistic property only 1.5-2 times less potent than I(1,4,5)P_3.[9]



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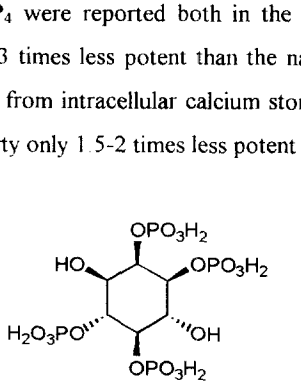
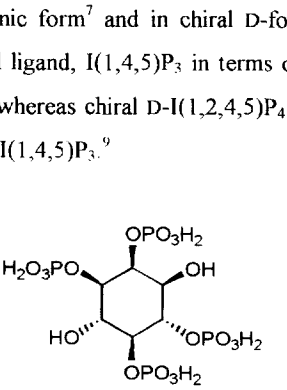


2D



2L

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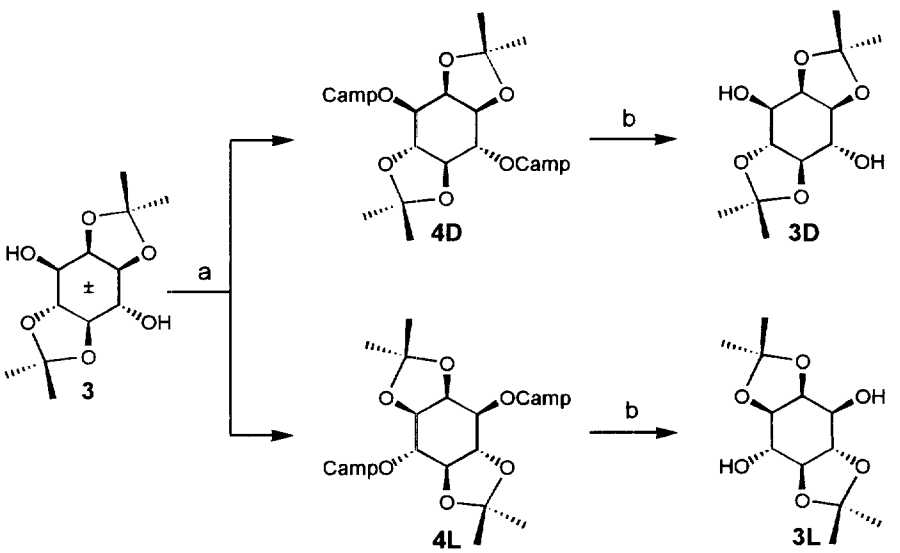
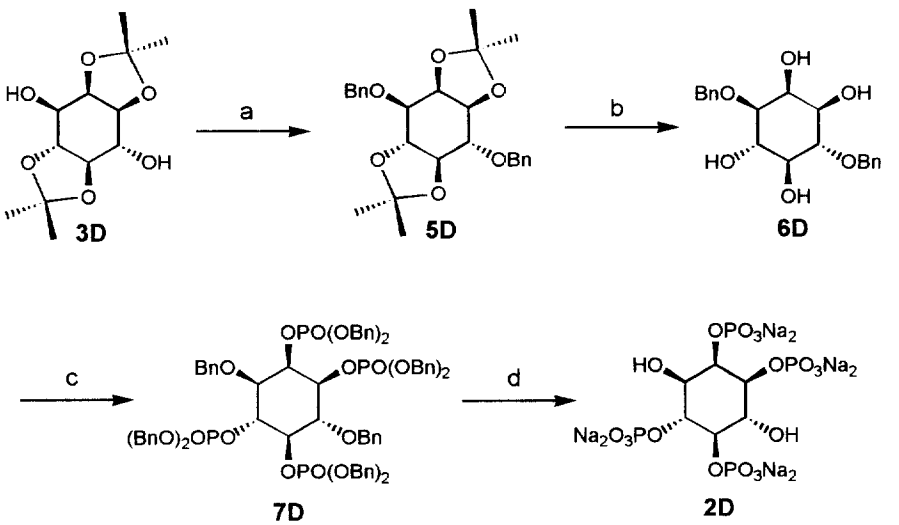
As L-I(1,4,5)P_3 is known to be essentially inactive in its binding to the IP_3 receptor or calcium releasing ability,[11] L-I(1,2,4,5)P_4 has also been assumed to be an inactive component in the binding study with the racemic IP_4 sample: an assumption never experimentally confirmed. Here we wish to report the first synthesis of L-I(1,2,4,5)P_4 (**2L**) and its binding property to IP_3 receptor.

Racemic diol **3**[12] was resolved via the diastereomers of its (-)-camphanate ester (Scheme 1), **4D** and **4L**.[13] After silica gel column chromatography, each diastereomer **4D** and **4L** was treated with NaOMe in MeOH to give the enantiomeric pair, **3D** (mp 169-170 °C, $[\alpha]_D$ - 41.7, c 1.58, CH_2Cl_2) and **3L** (mp 169-170 °C, $[\alpha]_D$ + 40.2, c 1.21, CH_2Cl_2; + 25.7, c 0.69, CH_3CN; lit.[14] mp 159-161 °C, $[\alpha]_D$ + 22.0, c 1.08, CH_3CN) (Scheme 1).



Scheme 1. a. (i) (1*S*)-(-)-camphanic chloride (Camp-Cl), pyridine. (ii) separation by column chromatography, **4D** (39%) is less polar than **4L** (35%). b. NaOMe, MeOH, Δ, 84%.

Diol **3D** was benzylated under the conventional conditions employing BnBr and NaH in DMF to give **5D**[15] Acid catalyzed hydrolysis of **5D** in aq. AcOH gave the tetraol, **6D**[16]. Compound **6D** was phosphorylated by successive treatments with dibenzyl *N,N*-diisopropylphosphoramidite and 1*H*-tetrazole, and then H_2O_2 to give the protected D-I(1,2,4,5)P_4, **7D**.[17] Hydrogenolysis of **7D** using Pd catalyst on activated charcol followed by an addition of NaOH to adjust pH 10 gave the sodium salt of D-I(1,2,4,5)P_4, **2D** (Scheme 2).[18] L-I(1,2,4,5)P_4, **2L** was synthesized according to the same procedure.



Scheme 2. a. BnBr, NaH, DMF, 87%. b. acetic acid - water (80 : 20), reflux, 80%. c. (i) dibenzyl *N,N*-diisopropylphosphoramidite, 1*H*-tetrazole, (ii) 30% H_2O_2, 79%. d. (i) H_2, Pd-C (10%). (ii) pH 10 (NaOH), quant.

The binding affinities of synthetic **2D** and **2L** were examined by the standard competition binding assay using 1.25 nM [^{3}H]-D-I(1,4,5)P_3 and I(1,4,5)P_3 binding protein, which was prepared from bovine adrenal cortex.[19] With D-I(1,4,5)P_3 (IC_{50} 15.3 nM) as the reference standard, **2D** showed a comparable binding affinity (IC_{50} 13.4 nM) to the natural ligand, while **2L** revealed a much lower affinity (IC_{50} 598 nM). It appears quite possible that even the low binding activity of **2L** (about 2% of **2D**) might be due to the contamination of **2D**, since the intermediate **4L** contained about 1.5% **4D**. Thus it is clear that the IP_3 receptor is quite stereospecific in its binding recognization.

In conclusion, we have prepared each enantiomer of I(1,2,4,5)P_4 and demonstrated that the D-form is indeed the active IP_3 receptor agonist as was previously assumed.

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13. **4D**: >99% de based on ^{1}H-NMR, $[\alpha]_D$ + 3.4 (c 1.04, CH_2Cl_2); **4L**: ca. 97% de. $[\alpha]_D$ - 18.6 (c 1.5, CH_2Cl_2). R_f values on silica gel TLC (ethyl acetate : CH_2Cl_2 = 1 : 7); **4D**: 0.52; **4L**: 0.44.
14. The assignments of **4D** and **4L** were based on the literature data for **3L**: Jones, M.; Rana, K. K.; Ward, J. G.; Young, R. C. *Tetrahedron Lett.* **1989**, *30*, 5353-5356.
15. **5D**: mp 155-156 °C, $[\alpha]_D$ - 45.2 (c 1.12, CH_2Cl_2); **5L**: mp 155-156 °C, $[\alpha]_D$ + 43.6 (c 1.43, CH_2Cl_2).
16. **6D**: mp 169-170 °C, $[\alpha]_D$ + 14.7 (c 1.03, CH_3OH); **6L**: mp 169-170 °C, $[\alpha]_D$ - 12.5 (c 0.95, CH_3OH).
17. **7D**: Oil, $[\alpha]_D$ - 3.3 (c 1.02, $CHCl_3$); **7L**: Oil, $[\alpha]_D$ + 2.8 (c 1.35, $CHCl_3$); ^{31}P-NMR ($CDCl_3$) δ 0.70, 1.05, 1.10, 1.61.
18. **2D**: $[\alpha]_D$ - 13.3 (c 1.0, H_2O, pH 10); **2L**: $[\alpha]_D$ + 12.1 (c 1.0, H_2O, pH 10). ^{31}P-NMR (D_2O, pH 10) δ 4.52, 5.06, 5.28, 5.37.
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