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Synthesis of D-1,2-dideoxy-1,2-difluoro-*myo*-inositol 3,4,5,6-tetrakisphosphate and its enantiomer as analogues of *myo*-inositol 3,4,5,6-tetrakisphosphate

Kevin R.H. Solomons[a], Sally Freeman[a,*], Carl H. Schwalbe[b], Stephen B. Shears[c], Deborah J. Nelson[d], Weiwen Xie[d], Karol S. Bruzik[e], Marcia A. Kaetzel[f]

[a] *School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

[b] *Department of Pharmaceutical and Biological Sciences, Aston University, Birmingham B4 7ET, UK*

[c] *Inositide Signaling Section, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA*

[d] *Department of Neurology, University of Chicago, 5841 S Maryland Avenue, Chicago, IL 60637, USA*

[e] *Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago IL 60612-7231, USA*

[f] *Department of Molecular and Cellular Physiology, College of Medicine, University of Cincinnati, OH 45267-0576, USA*

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Abstract

DL-3,4,5,6-Tetra-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol was resolved using (−)-(*1S*, *4R*)-camphanyl chloride. The diastereoisomers formed were separated and the structure of D-3,4,5,6-tetra-*O*-benzyl-2-(*1S,4R*)-camphanyl-1-deoxy-1-fluoro-*scyllo*-inositol was solved by X-ray crystallography to an R-factor of 4.2%. A series of manipulations led to the preparation of D-1,2-dideoxy-1,2-difluoro-*myo*-inositol 3,4,5,6-tetrakisphosphate and its enantiomer. The D-1,2-difluoro enantiomer stereospecifically inhibited CaMK II-activated Cl^- current, but with low potency; however, efficacy of this compound was greatly enhanced by *myo*-inositol 3,4,5,6-tetrakisphosphate itself.

Keywords: Inositol polyphosphates; Chloride current; Myo-inositol 3,4,5,6-tetrakisphosphate; Crystal structure

* Corresponding author. Fax: 0044 (0)161 275 2396; e-mail: sfreeman@fsl.pa.man.ac.uk

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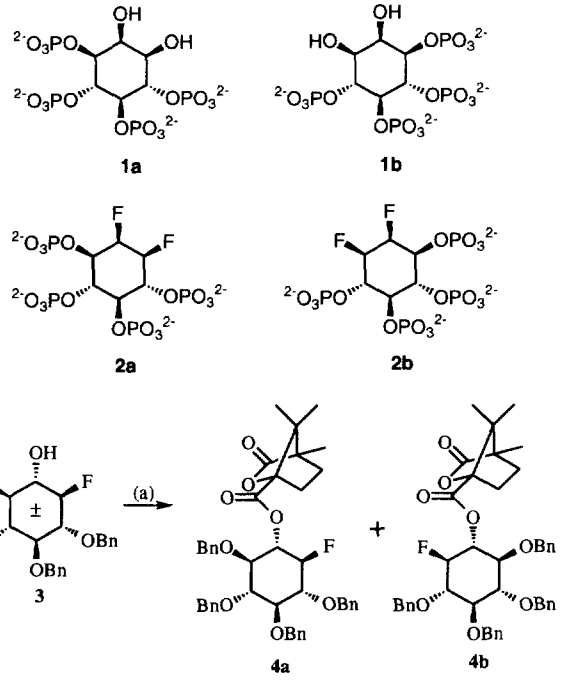
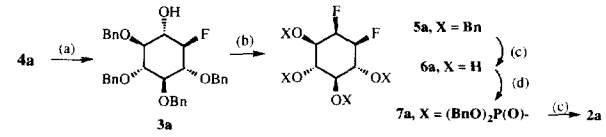
1. Introduction

Since the finding that *myo*-inositol 1,4,5-trisphosphate is the second messenger linking receptor activation to the mobilisation of calcium from intracellular stores [1], there has been widespread investigation into the metabolism and effects of inositol phosphates as mediators of cell signalling transduction pathways [2,3]. D-*myo*-Inositol 3,4,5,6-tetrakisphosphate (Ins[3,4,5,6]P_4, **1a**) has been shown to be involved in the long-term uncoupling of chloride secretion from intracellular calcium levels [4]. More recently it has been demonstrated that **1a** inhibits calcium-dependent chloride conductance, which is important in the cellular control of salt and fluid secretion, and may have implications in the treatment of cystic fibrosis [5]. Tetrakisphosphate **1a** has also been shown to be a potent inhibitor of *myo*-inositol 1,3,4-trisphosphate 5/6-kinase activity [6]. In addition, levels of its enantiomer, D-*myo*-inositol 1,4,5,6-tetrakisphosphate (Ins[1,4,5,6]P_4, **1b**), may increase after cell transformation with the *src* oncogene [7], and also after treatment with phosphoinositidase (PIC)-linked agonists [8].

Fluorine is a sterically conservative replacement for a hydroxy group: the significant difference is that fluorine can only accept hydrogen bonds, whereas hydroxy can both accept and donate [9]. We have previously reported the synthesis of racemic DL-1,2-dideoxy-1,2-difluoro-*myo*-inositol 3,4,5,6-tetrakisphosphate (**2**) [10], and here the enantiomers **2a** and **2b** have been synthesised. Enantiomer **2a** represents the first biologically-active analogue of **1a**. Schultz and coworkers have recently reported the synthesis of the corresponding 1,2-dichloro and 1,2-dimethoxy analogues [11,12].



Scheme 1. Resolution of DL-3,4,5,6-tetra-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol. Reagents: (a) (−)-(*1S,4R*)-camphanyl chloride, DMAP, Et_3N, CH_2Cl_2.



Scheme 2. Synthesis of D-1,2-dideoxy-1,2-difluoro-*myo*-inositol 3,4,5,6-tetrakisphosphate (**2a**). Reagents: (a) aq NaOH, MeOH; (b) DAST, CH_2Cl_2, Et_3N; (c) H_2, 10% Pd-C, THF–H_2O (1:1); (d) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, THF, then MCPBA.

2. Results and discussion

The synthesis of **2a** is shown in Schemes 1 and 2. The enantiomer **2b** was prepared analogously from **4b**. DL-3,4,5,6-Tetra-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3** was prepared in three steps from DL-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol [10]. Camphanyl esters have been useful in determining the absolute configuration of inositol derivatives [13–15] and here the resolution of **3** was achieved by treatment with (−)-(*1S, 4R*)-camphanyl chloride (Scheme 1). The diastereoisomeric (*1S,4R*)-camphanyl esters **4a** and **4b** were readily separated by flash chromatography and were fully characterised, with data including ^1H and ^{13}C NMR spectra, elemental analysis and specific optical rotation measurements. The second eluted diastereoisomer crystallised from diethyl ether–hexane to give X-ray quality crystals. The structure was refined to $R = 4.2\%$.[1] The atomic coordinates are given in Table 1 and Fig. 1 gives the molecular geometry. Correct stereochemistry was confirmed by the known camphanyl geometry and the structure was determined as diastereoisomer **4a**. The five inositol oxygens and the fluoro substituent are all equatorial, as required for the *scyllo* isomer. The inositol ring adopts a distorted, locally flattened chair

[1] Tables of atomic coordinates, bond lengths, and bond angles have been deposited with the Cambridge Crystallographic Data Centre. These tables may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 union Road, Cambridge, UK, CB2 1EZ.

conformation: the torsion angles around the ring vary in magnitude from a maximum for C1–C2–C3–C4 [55.9(4)°] to a minimum for C4–C5–C6–C1 [−49.3(4)°], compared with the consensus value of 56° in cyclohexane [17]. The best-preserved symmetry elements of a cyclohexane chair are the mirror plane through C2 with asymmetry parameter [18] $\Delta C_S = 1.7°$ and the two-fold axis through the midpoint of C2 and C3 with $\Delta C_2 = 1.5°$. The C–C inositol ring bond lengths (range 1.499–1.529 Å) are comparable with those of *myo*-inositol [19]. The C–C–C inositol ring bond angles (range 109.9–113.0°) are close to those of a perfect chair [17].

Table 1
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for **4a**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor

	x	y	z	U(eq)
C(2)	7126(2)	5608(4)	4786(2)	59(1)
C(1)	6239(2)	5898(3)	4122(2)	54(1)
C(6)	5218(2)	5188(3)	4318(2)	55(1)
C(5)	4930(2)	5449(4)	5228(2)	55(1)
C(4)	5855(2)	5212(4)	5897(2)	56(1)
C(3)	6852(2)	5949(4)	5690(2)	59(1)
F(8)	8016(2)	6326(3)	4575(2)	88(1)
O(7)	6529(2)	5401(2)	3304(1)	61(1)
O(12)	4414(2)	5694(3)	3717(1)	65(1)
O(11)	4087(2)	4554(3)	5390(2)	65(1)
O(10)	5538(2)	5649(2)	6710(1)	64(1)
O(9)	7691(2)	5541(2)	6284(1)	67(1)
C(13)	6821(3)	6305(4)	2721(2)	64(1)
O(14)	6844(3)	7484(3)	2844(2)	103(1)
C(15)	7122(3)	5634(4)	1925(2)	60(1)
O(16)	7327(2)	6693(3)	1313(2)	76(1)
C(17)	7959(4)	6154(5)	734(2)	79(1)
C(18)	8123(4)	4719(5)	968(2)	85(1)
C(19)	8179(3)	4844(4)	1962(2)	76(1)
C(20)	6333(4)	4692(6)	1444(3)	101(2)
C(21)	7032(5)	4093(6)	763(3)	117(2)
O(22)	8246(3)	6803(4)	150(2)	112(1)
C(23)	9044(5)	4048(7)	560(3)	134(2)
C(24)	9114(3)	5712(8)	2321(3)	111(2)
C(25)	8172(6)	3515(6)	2429(3)	135(3)
C(26)	8361(4)	6584(5)	6628(3)	100(2)
C(27)	9230(3)	5957(4)	7196(2)	65(1)
C(28)	10031(3)	5261(5)	6846(3)	82(1)
C(29)	10822(3)	4679(6)	7371(4)	108(2)
C(30)	10821(5)	4818(7)	8240(5)	118(2)
C(31)	10056(5)	5519(8)	8587(3)	113(2)
C(32)	9262(3)	6090(5)	8073(3)	87(1)
C(33)	5679(4)	4670(5)	7381(2)	84(1)
C(34)	5296(3)	5246(4)	8190(2)	72(1)
C(35)	5845(4)	6217(6)	8648(3)	102(2)
C(36)	5499(5)	6738(7)	9386(3)	119(2)
C(37)	4575(5)	6285(8)	9679(3)	122(2)
C(38)	4010(4)	5313(8)	9244(4)	122(2)
C(39)	4361(4)	4799(7)	8498(3)	100(1)
C(40)	3274(3)	5077(4)	5906(2)	71(1)
C(41)	2353(2)	5636(4)	5375(2)	58(1)
C(42)	2120(3)	6966(4)	5373(3)	80(1)
C(43)	1252(4)	7476(5)	4902(4)	102(2)
C(44)	599(3)	6659(5)	4420(3)	90(1)
C(45)	804(4)	5305(5)	4426(3)	94(1)
C(46)	1666(3)	4794(4)	4897(3)	83(1)
C(47)	3829(3)	4724(5)	3237(3)	85(1)
C(48)	3085(3)	5399(5)	2574(2)	72(1)
C(49)	2555(3)	6564(5)	2763(3)	94(1)
C(50)	1851(4)	7152(7)	2165(5)	132(2)
C(51)	1627(5)	6563(11)	1388(5)	142(3)
C(52)	2135(5)	5391(12)	1192(3)	144(3)
C(53)	2850(4)	4796(8)	1792(3)	112(2)

Alkaline hydrolysis of esters **4a** and **4b** yielded the enantiomeric alcohols **3a** and **3b** in quantitative yield, which were converted to the dideoxy-difluoro-*myo*-inositol tetrakisphosphates, **2a** and **2b**, by methods previously described for the racemate of **2** (Scheme 2, shown for **a**) [10]. This required fluorination of **3a** and **3b** with DAST to give the difluoro compounds **5a** and **5b**, debenzylation by hydrogenolysis to give the tetraols **6a** and **6b**, phosphorylation with dibenzyl *N,N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole, followed by MCPBA to give the protected tetrakisphosphates **7a** and **7b**, and subsequent removal of the P-OBn groups by hydrogenolysis to give the tetrakisphosphates **2a** and **2b**.

An alternative preparation of **4a** and **4b** is outlined in Scheme 3. This requires the reaction of DL-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**8**) with (−)-(*1S,4R*)-camphanyl chloride. Selectivity was observed at the 1-/3-positions to give a mixture of diastereoisomers **9a** and **9b**. Separation was achieved by crystallisation, however it was more

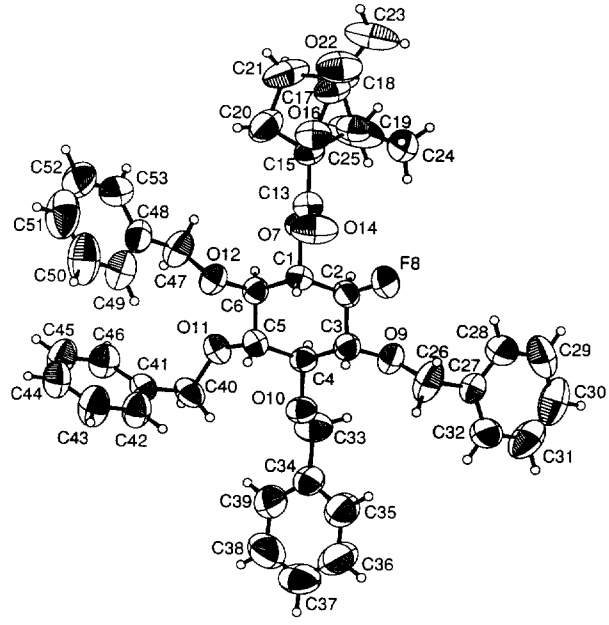
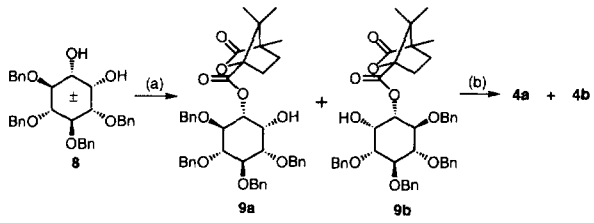


Fig. 1. ORTEP drawing [16] of **4a**, showing the labelling scheme for non-H atoms. Thermal ellipsoids are drawn at the 50% probability level.



Scheme 3. Alternative syntheses of camphanyl esters **4a** and **4b**. Reagents: (a) (−)-(*1S,4R*)-camphanyl chloride, DMAP, Et_3N, CH_2Cl_2; (b) DAST, toluene.

convenient to fluorinate the diastereoisomeric mixture with DAST to give **4a** and **4b** in excellent yield, which could be separated by flash chromatography.

Most of the biological studies conducted with **2a** and **2b** have been reported [20]. Briefly *myo*-inositol 1,4,5-trisphosphate causes the release of cytosolic calcium which binds to calmodulin and activates calmodulin dependant protein kinase II (CaMK II). Through phosphorylation this activates one type of chloride conductance (gCl_{CaMK}) (Fig. 2, bar A). This chloride current is down-regulated by the tetrakisphosphate **1a** (Fig. 2, bar B) with an IC_{50} of 6 μM [5], which shows non-cooperative binding at low concentration and cooperative binding at high concentration [20]. The 1,2-difluoro analogue **2a** inhibited the chloride current to provide the first agonist of **1a**, albeit with lower affinity (IC_{50} 100 μM) [20]. In contrast, **2a** showed non-cooperative binding only, suggesting that one or both of the hydroxy groups in **1a** contributes to the cooperative binding through hydrogen-bond donation [20]. The inhibition was stereospecific as the enantiomer **2b** had no effect on the CaMK II-activated Cl^- current [20]. In addition, here it is reported that **1a** increases the potency of inhibition of compound **2a**: a 10 μM concentration of **2a** had little effect on the CaMK II-activated Cl^- current (Fig. 2, bar C), whereas there was a synergistic decrease in the current when 6 μM **2a** was combined with 6 μM **1a** (Fig. 2, bar D). This **1a**-mediated increase in the potency of **2a** is consistent with inhibition of Cl^- current being highly cooperative in vivo [5]. Thus, compound **2a** has contributed to defining the importance of hydrogen-bonding in the cooperative inhibition of **1a**, which should assist in the design of agonists and antagonists for potential therapeutic applications. The major route of metabolism of **1a** in vivo is by phosphorylation by a 1-OH kinase [21]. Therefore compound **2a** also has a potential application as a metabolically stable analogue.

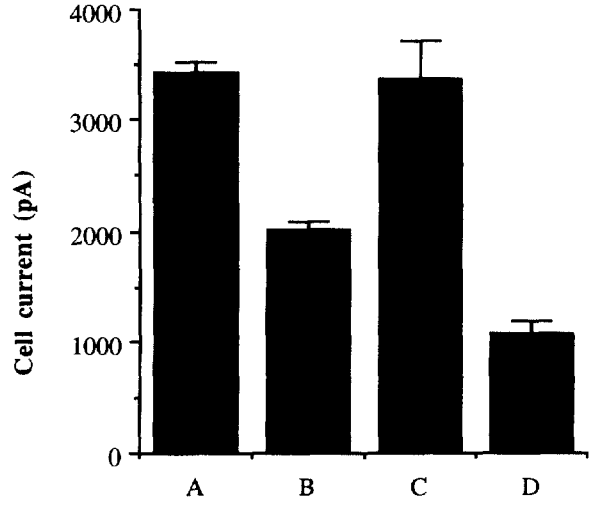


Fig. 2. The effect of **2a** upon the chloride current ($I_{Cl,CaMK}$) when added together with **1a**. The mean ($n=3$) peak current amplitudes at 110 mV are given after 12–15 min treatment with CaMK II plus the following concentrations of **1a** and **2a**: bar A-CaMK II activated (control); bar B-6 μM **1a**; bar C-10 μM **2a**; bar D-6 μM **2a** + 6 μM **1a**.

3. Experimental

General.—Instrumentation, techniques and reagent suppliers were as previously described [10] with the following addition: optical rotations were measured on an Optical Activity AA-100 polarimeter using a 0.25 dm cell at 25 °C. For compounds **3a/b, 5a/b, 7a/b** and **2a/b** only brief experimental information and ^1H NMR data, melting points and specific optical rotations are given: the procedures and other spectral data (^{13}C and ^{19}F NMR) were identical to those reported for the racemic materials [10].

Crystal structure determination of (**4a**).—$C_{44}H_{47}FO_8$, M = 722.8. Monoclinic, a = 12.646(2), b = 9.939(1), c = 15.611(2) Å, β = 94.107(9)°, V = 1957.0(4) Å^3 (by least squares analysis of setting angles of 25 reflections, $23.0 \leq \theta \leq 27.0°$, λ = 1.54178 Å), space group $P2_1$, Z = 2, D_c = 1.227 Mg m^{-3}. Needle with dimensions: 0.4×0.15×0.05 mm, μ = 0.71 mm^{-1}.

Enraf-Nonius CAD4 diffractometer, ω-2θ scan technique, graphite-monochromated Cu radiation: 4262 reflections measured ($2 \leq \theta \leq 67°$, for $-1 \leq h \leq 15$, $0 \leq k \leq 11$, $-18 \leq l \leq 18$), 3689 unique, giving 2895 with $F > 4\sigma$. The structure was solved by direct methods [22] and refined by full-matrix least-squares [23] with anisotropic displacement parameters for non-H atoms, and H atoms in calculated

positions. Final discrepancy indices are $R = 4.2\%$ for observed data and 6.4% for all data. Correct stereochemistry is confirmed by the known camphanyl geometry and an absolute structure parameter [23] value of 0.2(2).

D-*3,4,5,6-Tetra*-O-*benzyl-2-(*1′S,4′R*)-camphanyl-1-deoxy-1-fluoro*-scyllo-*inositol* (**4a**) *and* D-*1,4,5,6-tetra*-O-*benzyl-2-(*1′S,4′R*)-camphanyl-3-deoxy-3-fluoro*-scyllo-*inositol* (**4b**).—A solution of (−)-(*1S,4R*)-camphanyl chloride (212 mg, 0.978 mmol) in dichloromethane (1.2 mL) was added dropwise to a stirred solution of DL-3,4,5,6-tetra-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3** [10] (265 mg, 0.489 mmol), DMAP (10 mg, 0.082 mmol) and triethylamine (0.125 mL, 0.897 mmol) in anhydrous dichloromethane (6 mL) at 0 °C under an atmosphere of nitrogen. The mixture was allowed to warm to room temp. and then stirred for 24 h. The reaction mixture was washed with saturated aq. $NaHCO_3$ (2×10 mL) and water (2×10 mL), dried (Na_2SO_4) and the solvent was evaporated off in vacuo. Flash chromatography on silica eluting with diethyl ether–dichloromethane (2:98) gave **4a** (R_f 0.30) and **4b** (R_f 0.41).

Diastereoisomer **4a** was recrystallised from diethyl ether–hexane to give needles which were suitable for X-ray crystallography (125 mg, 36%); mp 112–113 °C; $[\alpha]_D$ +1.1 ± 0.6° (*c* 0.0143 g/mL, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 0.89 (3 H, s, CH_3), 1.05 (3 H, s, CH_3), 1.10 (3 H, s, CH_3), 1.6–1.75 (1 H, m, Camph-CH_2), 1.85–2.0 (2 H, m, Camph-CH_2), 2.3–2.4 (1 H, m, Camph-CH_2), 3.5–3.8 (4 H, m, 3-, 4-, 5- and 6-H), 4.51 (1 H, dt, $^2J_{HF}$ 51.5, $J_{1/2}$~$J_{1/6}$~9.3, 1-H), 4.65–4.95 (8 H, m, 4×CH_2Ph), 5.42 (1 H, br dt, $^3J_{HF}$ 11.2, $J_{2/1}$~$J_{2/3}$ ~9.9, 2-H), 7.2–7.35 (20 H, m, Ph); ^{13}C NMR data ($CDCl_3$): δ 9.6 (CH_3), 16.3 (CH_3), 16.7 (CH_3), 28.95 (CH_2), 30.7 (CH_2), 54.2 (quat.), 54.7 (quat.), 73.0 (d, $^2J_{CF}$ 18.3, C-2 or C-6), 75.4, 75.5, 76.0, 76.1 [4×CH_2Ph], 78.7 (d, $^3J_{CF}$ 11.0, C-3 or C-5), 80.4 (d, $^2J_{CF}$ 15.8, C-6 or C-2), 81.1 (d, $^3J_{CF}$ 12.2, C-5 or C-3), 82.2 (s, C-4), 90.8 (quat.), 92.95 (d, $^1J_{CF}$ 186.7, C-1), 127.1–128.4 (20×arom CH), 137.6 (arom C), 137.7 (arom C), 137.8 (arom C), 138.0 (arom C), 166.4 (C=O), 177.9 (C=O); MS data (CI): 740 ($M + NH_4^+$, 14%), 216 (40), 108 (51), 91(100). Anal. Calcd for $C_{44}H_{47}FO_8$: C, 73.1; H, 6.55. Found: C, 72.9; H, 6.6.

Diastereoisomer **4b** was recrystallised from ethanol (118 mg, 34%); mp 139–140 °C; $[\alpha]_D$ −3.6 ± 0.5° (*c* 0.0135 g/mL, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 0.99 (3 H, s, CH_3), 1.04 (3 H, s, CH_3), 1.12 (3 H, s, CH_3), 1.55–1.7 (1 H, m, Camph-CH_2), 1.75–1.95 (2 H, m, Camph-CH_2), 2.35–2.5 (1 H, m, Camph-CH_2), 3.5–3.8 (4 H, m, 3-, 4-,5- and 6-H), 4.54 (1 H, dt, $^2J_{HF}$ 51.8, $J_{1/2}$~$J_{1/6}$~9.6, 1-H), 4.7–4.95 (8 H, m, 4×CH_2Ph), 5.40 (1 H, br dt, $^3J_{HF}$ 11.9, $J_{2/1}$~$J_{2/3}$~9.9, 2-H), 7.2–7.35 (20 H, m, Ph); ^{13}C NMR data ($CDCl_3$): δ 9.7 (CH_3), 16.4 (2×CH_3), 28.8 (CH_2), 30.5 (CH_2), 54.7 (quat.), 54.8 (quat.), 73.3 (d, $^2J_{CF}$ 17.1, C-2 or C-6), 75.4, 75.7, 75.95, 76.1 [4×CH_2Ph], 78.9 (d, $^3J_{CF}$ 9.8, C-3 or C-5), 80.5 (d, $^2J_{CF}$ 15.9, C-6 or C-2), 81.1 (d, $^3J_{CF}$ 11.0, C-5 or C-3), 82.1 (s, C-4), 91.0 (quat.), 92.7 (d, $^1J_{CF}$ 185.5, C-1), 127.3–128.4 (20×arom CH), 137.75 (2×arom C), 137.9 (arom C), 138.0 (arom C), 166.8 (C=O), 178.3 (C=O); MS data (CI): 740 ($M + NH_4^+$, 40%), 108 (35), 91(100). Anal. Calcd for $C_{44}H_{47}FO_8.0.5\ H_2O$: C, 72.2; H, 6.6. Found C, 72.3; H, 6.4.

Alternatively, **4a** and **4b** were prepared directly by heating a diastereoisomeric mixture of 3,4,5,6-tetra-*O*-benzyl-1-(*1′S,4′R*)-camphanyl-*myo*-inositol **9a** and 1,4,5,6-tetra-*O*-benzyl-3-(*1′S,4′R*)-camphanyl-*myo*-inositol **9b** with DAST (16 eq) in toluene at 70–80 °C for 2 h. Aqueous work-up, flash chromatography and recrystallisation as described above gave **4a** and **4b** in 48 and 25% yields, respectively.

D-*3,4,5,6-Tetra*-O-*benzyl-1-deoxy-1-fluoro*-scyllo-*inositol* (**3a**) *and* D-*1,4,5,6-tetra*-O-*benzyl-3-deoxy-3-fluoro*-scyllo-*inositol* (**3b**).—Aqueous sodium hydroxide (3 M, 2 mL) was added to a stirred solution of each camphanyl ester, **4a** or **4b** (160 mg, 0.29 mmol) in warm methanol (15 mL). After 18 h at room temp., methanol was removed by evaporation in vacuo and the residue was partitioned between dichloromethane (2×30 mL) and water (30 mL). The combined organic layers were washed with water (30 mL), dried (Na_2SO_4), and the solvent evaporated to give the alcohols **3a** or **3b** in quantitative yields. Recrystallisation from ethyl acetate–hexane gave crystals of **3a**; mp 121–122 °C; $[\alpha]_D$ −7.75 ± 0.3° (*c* 0.0258 g/mL, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.49 (1 H, s, OH), 3.40 (1 H, t, $J_{4/3}$ ~$J_{4/5}$~9.0, 4-H), 3.5–3.8 (4 H, m, 2-, 3-, 5- and 6-H), 4.42 (1 H, dt, J_{HF} 52.4, $J_{1/2}$~$J_{1/6}$~9.0, 1-H), 4.75–4.95 (8 H, m, 4×CH_2Ph), 7.25–7.35 (20 H, m, Ph). **3b**; mp 122–123 °C; $[\alpha]_D$ +6.1 ± 1.0 (*c* 0.0085 g/mL, $CHCl_3$).

D-*3,4,5,6-Tetra*-O-*benzyl-1,2-dideoxy-1,2-difluoro*-myo-*inositol* (**5a**) *and* D- *1,4,5,6-tetra*-O-*benzyl-2,3-dideoxy-2,3-difluoro*-myo-*inositol* (**5b**).—Recrystallisation from ethanol gave needles of **5a**; mp

87–88 °C; $[\alpha]_D$ −20.0 ± 0.7 (*c* 0.0108 g/mL, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.45 (1 H, br dd, $^3J_{3/F\text{-}2}$ 28.0, $J_{3/4}$ 9.5, 3-H), 3.47 (1 H, t, $J_{5/4}\sim J_{5/6}\sim$9.4, 5-H), 3.97 (1 H, td, $J_{4/3}\sim J_{4/5}\sim$9.6, $^4J_{4/F\text{-}2}$ 1.3, 4-H), 4.04 (1 H, dtd, $^3J_{6/F\text{-}1}$ 9.9, $J_{6/1}\sim J_{6/5}\sim$9.6, $^4J_{6/F\text{-}2}$ 1.3, 6-H), 4.42 (1 H, dddd, $^2J_{HF}$ 46.5, $^3J_{HF}$ 27.7, $J_{1/6}$ 9.7, $J_{1/2}$ 1.8, 1-H), 4.7–4.95 (8 H, m, 4×CH_2Ph), 5.12 (1 H, ddt, $^2J_{HF}$ 53.1, $^3J_{HF}$ 9.6, $J_{2/1}\sim J_{2/3}\sim$1.6, 2-H), 7.25–7.35 (20 H, m, Ph). **5b**; mp 92–93 °C; $[\alpha]_D$ +17.7 ± 0.4 (*c* 0.0162 g/mL, $CHCl_3$).

D-*1,2-Dideoxy-1,2-difluoro*-myo-*inositol 3,4,5,6-tetrakis(dibenzyl phosphate)* (**7a**) *and* D-*2,3-dideoxy-2,3-difluoro*-myo-*inositol 1,4,5,6-tetrakis(dibenzyl phosphate)* (**7b**).—Compounds **5a** (85 mg, 0.156 mmol) or **5b** (69 mg, 0.127 mmol) were hydrogenated to give tetraols **6a** or **6b**. Without purification these were phosphorylated using dibenzyl *N,N*-diisopropylphosphoramidite (5 eq) to give the protected phosphates as oils, **7a** (141 mg, 74%) or **7b** (79 mg, 51%). Enantiomer **7a**; $[\alpha]_D$ +2.4 ± 0.6 (*c* 0.0139 g/mL, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 4.15–4.35 (1 H, m, inositol H), 4.4–4.65 (1 H, m, inositol H), 4.85–5.1 (19 H, m, 8×CH_2Ph and 3×inositol H), 5.35 (1 H, br dd, $^2J_{HF}$ 51.1, $^3J_{HF}$ 8.9, 2-H), 7.1–7.4 (40 H, m, Ph); ^{31}P NMR data ($CDCl_3$, referenced to 85% phosphoric acid, ^1H decoupled): δ −1.74 (s), −1.63 (s), −0.88 (s), −0.72 (s); MS data (+ve electrospray): 1225 ($M+H^+$, 5%), 1242 ($M+H_2O^+$, 100), 1247 ($M+Na^+$, 27). Enantiomer **7b**; $[\alpha]_D$ −2.8 ± 0.5 (*c* 0.0145 g/mL, $CHCl_3$).

Sodium salts of D-*1,2-dideoxy-1,2-difluoro*-myo-*inositol 3,4,5,6-tetrakisphosphate* (**2a**) *and* D-*2,3-dideoxy-2,3-difluoro*-myo-*inositol 1,4,5,6-tetrakisphosphate* (**2b**).—Enantiomer **2a**; mp > 300 °C; $[\alpha]_D$ −2.7 ± 0.4 (*c* 0.0125 g/mL, H_2O); ^1H NMR data (D_2O, referenced to benzene at 7.44 ppm) 4.2–4.3 (2 H, m, inositol-H), 4.35–4.65 (2 H, m, inositol-H), 4.59 (1 H, ddd, $^2J_{HF}$ 46.8, $^3J_{HF}$ 29.0, $J_{1/6}$ 9.4, 1-H), 5.45 (1 H, dd, $^2J_{HF}$ 52.1, $^3J_{HF}$ 8.9, 2-H). Enantiomer **2b**; mp > 300 °C; $[\alpha]_D$ +2.8 ± 0.3 (*c* 0.0160 g/mL, H_2O); ^{31}P NMR data (D_2O, pH 13, referenced to 85% phosphoric acid, ^1H decoupled): δ 4.00 (s), 4.32 (s), 4.44 (s), 4.64 (s); MS data (−ve electrospray): 502.9 ($[M^{8-}+7H^+]^-$, 100%), 524.8 ($[M^{8-}+6H^++Na^+]^-$, 12), 546.9 ($[M^{8-}+5H^++2Na^+]^-$, 11), 568.8 ($[M^{8-}+4H^++3Na^+]^-$, 23), 590.8 ($[M^{8-}+3H^++4Na^+]^-$, 5).

D-*3,4,5,6-Tetra*-O-*benzyl-1-(1'S,4'R)-camphanyl*-myo-*inositol* (**9a**) *and* D-*1,4,5,6-tetra*-O-*benzyl-3-(1'S, 4'R)-camphanyl*-myo-*inositol* (**9b**).—A mixture of **9a** and **9b** was prepared by the reaction of (−)-(*1S,4R*)-camphanyl chloride and DL-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol **8** (2.37 g, 4.39 mmol) using a procedure similar to that described for **4a/b**. Crystallisation from ethanol gave a mixture of **9a** and **9b** (1.54 g, 49%); ^1H NMR data ($CDCl_3$): δ 0.89 (3 H, s, CH_3a), 0.97 (3 H, s, CH_3b), 1.01 (3 H, s, CH_3b), 1.08 (3 H, s, CH_3a), 1.10 (3 H, s, CH_3a), 1.11 (3 H, s, CH_3b), 1.55–1.75 (1 H, m, Camph-CH_2), 1.8–2.0 (2 H, m, Camph-CH_2), 2.25–2.4 (1 H, m, Camph-CH_2), 3.54 (1 H, t, $J_{HH}\sim J_{HH}\sim$8.9, inositol H), 3.57 (1 H, d, $J_{3/4}$ 9.2, 3-H), 3.95 (1 H, t, $J_{HH}\sim J_{HH}\sim$9.6, inositol Ha), 3.97 (1 H, t, $J_{HH}\sim J_{HH}\sim$9.6, inositol Hb), 4.12 (1 H, t, $J_{HH}\sim J_{HH}\sim$9.6, inositol Ha), 4.14 (1 H, t, $J_{HH}\sim J_{HH}\sim$9.6, inositol Hb), 4.29 (1 H, t, $J_{2/1}\sim J_{2/3}\sim$2.3, 2-Hb), 4.32 (1 H, t, $J_{2/1}\sim J_{2/3}\sim$2.3, 2-Ha), 4.65–4.95 (9 H, m, 4×CH_2Ph and 1-H), 7.2-7.4 (20 H, m, Ph).

Biological methods.—The electrophysiological methods evaluating the synergistic inhibition of CaMK II-activated chloride current in T84 colonic epithelial cells by **2a** in the presence of **1a** was as previously described [20].

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