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Synthesis of 2-Deoxy-2-Fluorinated Inositol-1-O-Dodecylphosphonates as Inhibitors of Glycosyl Phosphatidylinositol Phospholipase C

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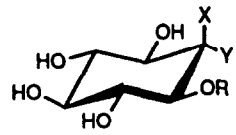
Abstract: Three 2-deoxy-2-fluorinated inositols and their 1-O-dodecylphosphonate derivatives have been synthesized as non-cleavable inhibitors of glycosyl phosphatidylinositol phospholipase C. Their structure-activity relationship is discussed.

Phosphatidylinositol phospholipase C (PI-PLC) is a key enzyme involved in the metabolism of membrane phosphatidylinositol (PI) in signal transduction pathway. The distinct glycosyl phosphatidylinositol phospholipase C (GPI-PLC) plays an active role in the cleavage of GPI membrane anchors of surface proteins. There has been considerable interest in the chemistry and biological activity of the fluorinated inositols. Such analogs could be useful as inhibitors of PI-PLC and GPI-PLC, and for the characterization of their enzymatic activities. The synthesis of fluorinated inositols, with mono- or di-fluoro substituents at positions 2,3 and 5, in the conformation of *myo* or *scyllo*, have been reported previously.[1,2] Herein we describe the synthesis of a group of 2-deoxy-2-fluorinated inositols (**1-3**), and their new 1-O-dodecylphosphonic acid derivatives (**4-6**) for biological investigations.

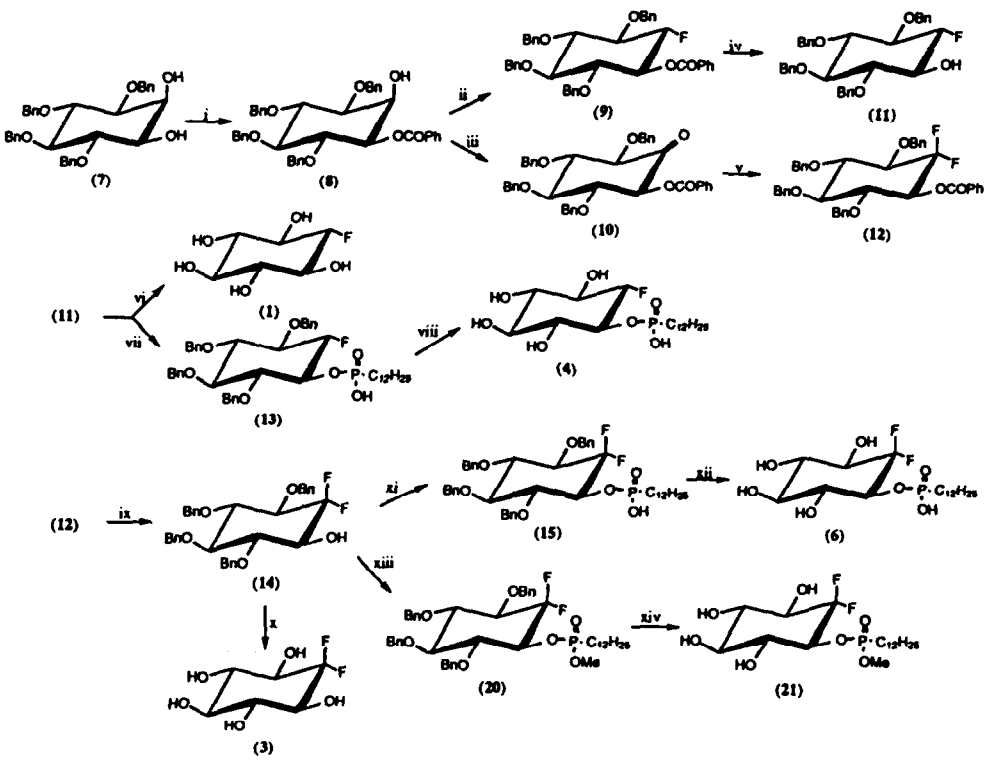


	X	Y	R
1	H	F	H
2	F	H	H
3	F	F	H
4	H	F	*P*
5	F	H	*P*
6	F	F	*P*

P = dodecylphosphonic acid

As the starting material 1,4,5,6-tetra-O-benzyl-*myo*-inositol (**7**) was prepared by modifications of a published procedure.[2,3] Compound **7** was then regiospecifically benzoylated at the 3-position to generate 1-O-benzoyl-3,4,5,6-tetra-O-benzyl-*myo*-inositol (**8**). This compound was used in two ways. First, following a published method[1a], it was treated with the diethylaminosulfur trifluoride (DAST) and then debenzoylated to give, with inversion of the C-2 configuration, 2-deoxy-2-fluoro-1,4,5,6-tetra-O-benzyl-*scyllo*-inositol(**11**), which yielded 2-deoxy-2-fluoro-*scyllo*-inositol (**1**) after debenzylation. Compound **11** was phosphorylated with dodecylphosphonic dichloride in the presence of a base to yield 3,4,5,6-tetra-O-benzyl-2-deoxy-2-fluoro-*scyllo*-inositol-1-O-dodecylphosphonic acid (**13**), which was debenzylated to 2-deoxy-2-fluoro-*scyllo*-inositol-1-O-dodecylphosphonic acid (**4**) by catalytic hydrogenation with 10% Pd/C. In another route, oxidation of **8** afforded 1-O-benzoyl-3,4,5,6-tetra-O-benzyl-inososc-2 (**10**), which was converted to 1-O-benzoyl-3,4,5,6-

tetra-O-benzyl-2-deoxy-2,2-difluoro-*myo*-inositol (12) by treatment with DAST. Using the same procedure as in the preparation of compound 1 and 4, 2-deoxy-2,2-difluoro-*myo*-inositol (3) and 2-deoxy-2,2-difluoro-*myo*-inositol-1-O-dodecylphosphonic acid (6) were obtained respectively. The methyl ester of 6 compound (21) was also prepared. (**Scheme 1**)

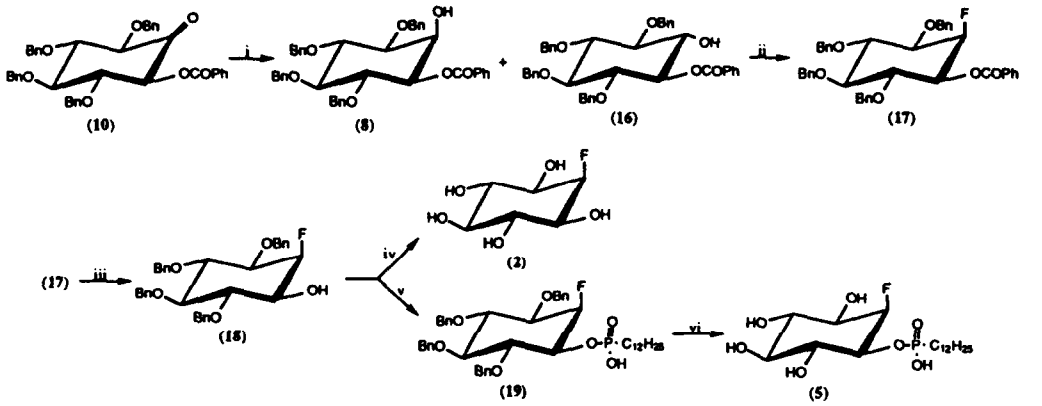


Reagents and conditions: i: PhCOCl, base,82%; ii: DAST,CH_2Cl_2,0°C; iii: Ac_2O,DMSO,88%; iv: aq. NaOH,90%; v: DAST,CH_2Cl_2,0°C,62%;vi: 10% Pd/C,EtOH,45%; vii: $C_{12}H_{25}P(O)Cl_2$,base,0°C,75%; viii: 10% Pd/C,EtOH,r.t.,70%; ix: aq.NaOH,r.t.,91%; x: 10% Pd/C,MeOH,r.t.,75%; xi: $C_{12}H_{25}P(O)Cl_2$,base,r.t.,63%; xii: 10% Pd/C,EtOH,r.t.,51%. xiii: $C_{12}H_{25}P(O)Cl_2$,base,0°C, MeOH,65%; xiv: 10% Pd/C,EtOH,48%.

Scheme 1

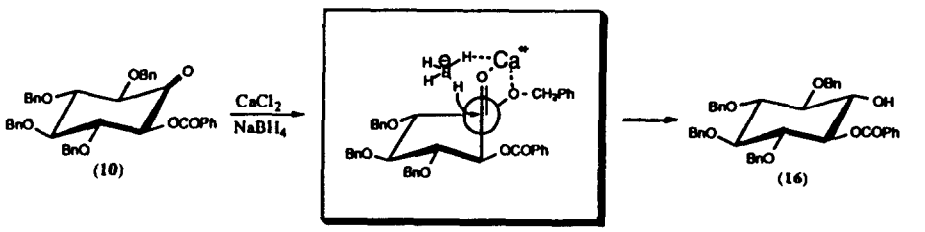
As shown in scheme 2, 10 was further used as the starting material for the successful synthesis for the first time of 2-deoxy-2-fluoro-*myo*-inositol (2). Its 1-O-dodecylphosphonic acid derivative (5) was also prepared. In scheme 2, stereoselective reduction of 10 with sodium borohydride in the presence of calcium chloride[4] afforded a mixture of *scyllo*- & *myo*-inositol derivatives in the ratio of 3/1, whereas a 1/1 ratio was observed in the absence of calcium chloride. The stereoselective formation of the *scyllo*- product is attributed to the attack of the carbonyl carbon from the less hindered side by the hydride (**Scheme 3**). ^{1}H-NMR investigation showed that upon addition of calcium chloride, the signal of the H-3 proton of the complex of inososе-2 and calcium chloride was moved downfield. The *scyllo*-derivative, 1-O-benzoyl-3,4,5,6-tetra-O-benzyl

scyllo-inositol (16), was treated with DAST, with partial inversion of the *scyllo*-configuration of the C-2 hydroxyl group, to yield a mixture of the *myo*-2-deoxy-2-fluoro derivative, 1-O-benzoyl-3,4,5,6-tetra-O-benzyl-2-deoxy-2-fluoro-*myo*-inositol (17) and the *scyllo* isomer **9**. Compound **17** was then debenzoylated by hydrolysis with aq. sodium hydroxide to yield 1,4,5,6-tetra-O-benzyl-2-deoxy-2-fluoro-*myo*-inositol (18),



Reagents and conditions: i: MeOH,$CaCl_2$,$NaBH_4$,0°C,1h,93%(total,ratio of (8)/(16)=1/3; ii: DAST,CH_2Cl_2,0°C, 32%; iii: aq.NaOH,r.t.; iv: 10% Pd/C,EtOH,r.t.,80%; v: $C_{12}H_{25}P(O)Cl_2$, base,83%; vi: 10% Pd/C,EtOH,r.t.,61%.

Scheme 2



Scheme 3

from which the desired compounds 2-deoxy-2-fluoro-*myo*-inositol (2) and 2-deoxy-2-fluoro-*myo*-inositol-1-O-dodecylphosphonic acid (5) were obtained by the same procedure as in the preparation of compounds **1**, **3**, **4**, **6**.

Glycosyl phosphatidylinositol phospholipase C (GPI-PLC) from *Trypanosoma brucei* and phosphatidylinositol phospholipase C (PI-PLC) from *Bacillus spp.* both cleave glycosylphosphatidylinositols (GPIs). Substrate requirements of both enzymes have been examined with glycosylinositol analogs of GPI core glycan components as potential inhibitors.[5] Using a similar approach (Table), we show that GPI-PLC is inhibited potently and competitively (Morris *et. al.*, in preparation) by compound **4** (85.1% at 1 mM), while compound

5 is less effective, inhibiting GPI-PLC 47.9% at 5 mM. This observation suggests that the configuration of the fluoro group has a dramatic effect on the potency of these inhibitors; the *scyllo* is preferable to the *myo* configuration, possibly due to interaction with an active site residue. Addition of a second fluoro group in the *scyllo* conformation to compound **5**, to yield **6**, partially restored the inhibition originally observed with compound **4**. However, the degree of effectiveness of **6** was less than that of **4**. These results suggest that an additional fluoro group in the *myo* configuration at the 2-position of 2-deoxyinositol(**6**) reduces the effectiveness of compound **4**. An alkylphosphonate side chain is essential for inhibition as the fluorinated inositols (**2** and **3**) are both inactive. The methyl ester (**21**) is also much less active than the free acid (**6**). The most potent inhibitors of GPI-PLC tested to date have both a fluoro group in the *scyllo* configuration at the 2-position and a dodecylphosphonate at the 1-position of 2-deoxyinositol (**4** and **6**). These inhibitors are at least 5-fold more inhibitory than *myo*-inositol-1-O-dodecylphosphonic acid.[5] Lastly, although **4** and **6** were equally effective against *T. brucei* GPI-PLC, **4** was 3-fold more effective than **6** against *B. cereus* PI-PLC (Table), confirming the notion that the two enzymes represent mechanistic subclasses of GPI phospholipases C.[5]

Table: Selective inhibition of phospholipases C by fluoro-Ins analogs[a]

Compound	*T. brucei* GPI-PLC % Inhibition at 1 mM	*B. cereus* PI-PLC % Inhibition at 1 mM
4	85.1	92.6
5	(47.9)[b]	(6.2)[b]
6	78.7	26.7
21	22.9	0.0

[a] Enzyme activities were determined as described, using [^{3}H]-labeled variant surface glycoprotein of *T. brucei* as substrate.

[b] Inhibition at 5 mM.

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