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# Synthesis of Isosteric and Isopolar Phosphonate Substrate Analogues Designed as Inhibitors for Phosphatidylinositol-Specific Phospholipase C from *Bacillus Cereus*

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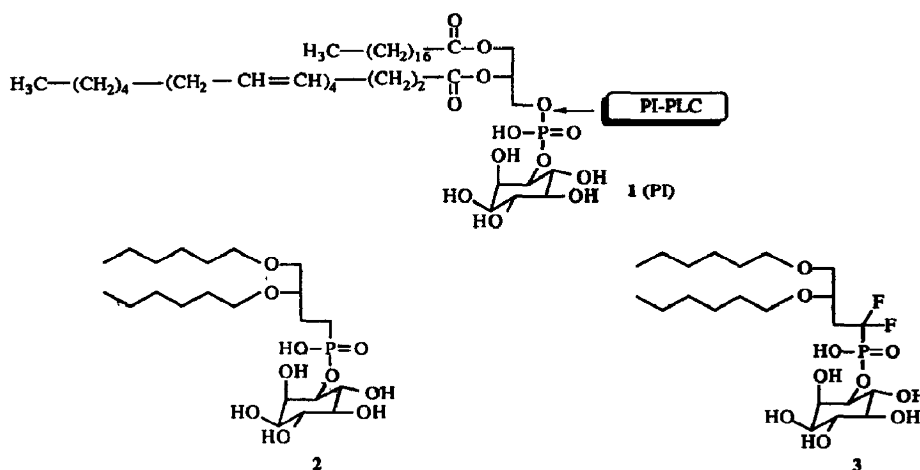
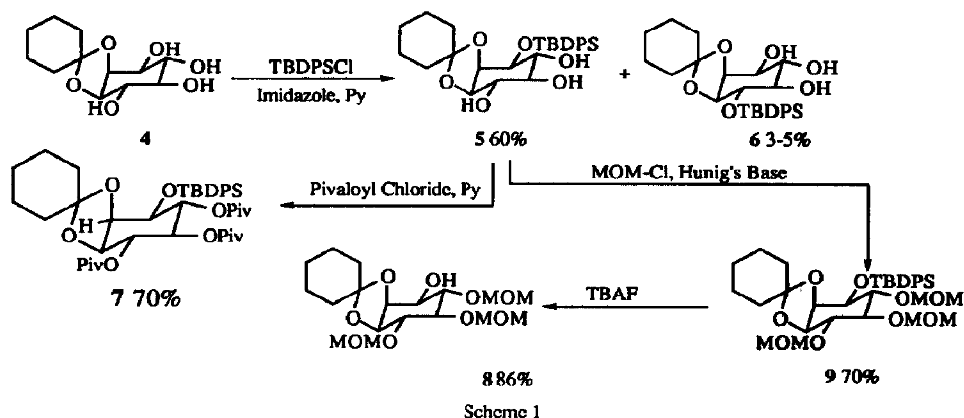
**Abstract:** The synthesis of the isosteric phosphonate substrate analogue inhibitor **2** and the isopolar difluoromethylenephosphonate inhibitor **3** for phosphatidyinositol-specific phospholipase C (PI-PLC) from *Bacillus cereus* is described. The key step involved a trichloroacetonitrile mediated condensation between the inositol derivative **8** and the corresponding phosphonic acids **16** and **18** to establish the central P-O bond in these inhibitors.

The phosphatidylinositol-specific phospholipase Cs (PI-PLCs) have been identified as a key family of enzymes involved in signal transduction in mammalian cells.[1] PI-PLCs cleave phosphorylated phosphoinositide components of cell membranes generating the second messengers, D-*myo*-inositol-1,4,5-trisphosphate ($IP_3$) and 1,2-diacylglycerol (DAG). Bacterial PI-PLCs, on the other hand, cleave non-phosphorylated versions of phosphatidylinositols (PIs), e.g., **1** and glycosyl-phosphatidylinositol (GPI) anchored proteins from mammalian cells, trypanosomes, and other organisms.[2] We have recently investigated the stereochemical requirement of the inositol head group in PI [3] and also examined the intermediates involved in the hydrolysis of PI when acted upon by PI-PLC from *Bacillus cereus*.[4] A collaborative effort to determine an X-ray structure of PI-PLC with and without substrate analogue inhibitors has been recently established[5] as part of an on-going effort to determine the structure of the enzyme and the mechanism of PI



hydrolysis.[6] Phosphonate analogues of PI are known to be PI-PLC inhibitors.[7] The isosteric phosphonate inhibitor **2** and the isopolar difluoromethylenephosphonate[8] inhibitor **3** were synthesized for this purpose. The availability of inhibitor **3** is expected to shed light on the effectiveness of the isopolar substitution, i.e. (P-O → P-$CF_2$) in designing potent inhibitors for PI-PLC. A comparison of structures of **2** and **3** with the natural substrate PI **1** also reveals that lengthy acyl chains on the *sn*-1 and *sn*-2 carbons of the glycerol unit are replaced by short chain ethers in our inhibitors. This particular structural variation is based on the need to increase monomeric water solubility[9] over that of **1**, the knowledge that the enzyme is tolerant to structural modifications on *sn*-1 and *sn*-2 carbons,[10] and that some of the natural GPI-anchored substrates contain an ether linkage at the *sn*-1 position.[2] The synthesis of the inhibitors **2** and **3** is reported herein.

The precursor employed for the inositol head group in **2** and **3** is the pentaprotected *myo*-inositol derivative **8**. The synthesis of **8** starts with readily prepared 2,3-*O*-cyclohexylidene-*myo*-inositol **4**.[11] Treatment of tetraol **4** with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) in the presence of imidazole provided 1-*O*-silyl protected derivative **5** (60%)[13] as the major product accompanied by the minor product **6** (3-5%), tentatively assigned as the 4-*O*-silyl derivative. The formation of **5** as the major product in this

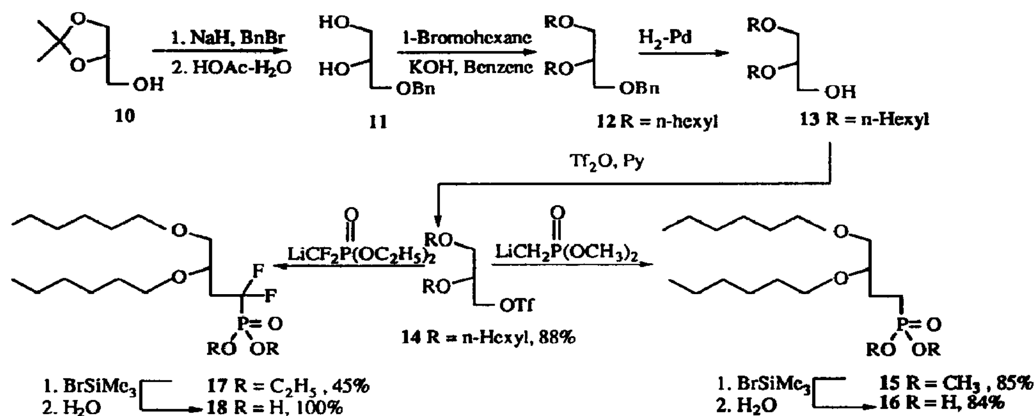



Scheme 1

reaction was reported earlier.[12] The structure of **5** was deduced by spectral comparison with structurally similar systems.[12] We therefore set out to ascertain unequivocally the regiochemistry of the silylation reaction leading to **5**. Towards this end, **5** was converted into the tripivaloyl derivative **7** (70%, mp 138-139 °C)[13] on treatment with excess pivaloyl chloride in pyridine. A $^1$H-$^1$H COSY spectrum of **7** indicated that the three downfield methine resonances (due to protons on carbons bearing ester groups) were contiguous in nature. The 2D-NMR also revealed that the single equatorial methine proton (C-2 H, shown on the structure) in the molecule appeared as a narrow triplet (J = 4.8 Hz) and did not exhibit a cross peak to any of the downfield resonances, confirming the assigned position of the silyl groups in **5**. Subsequently, the three remaining OH groups in **5** were exhaustively protected as their methoxymethyl ethers to give **9** (70%)[13] as an oil. Desilylation of **9** using tetra-*n*-butylammonium fluoride (TBAF) gave the necessary head group precursor **8** (70%, mp 76-77 °C).[13]

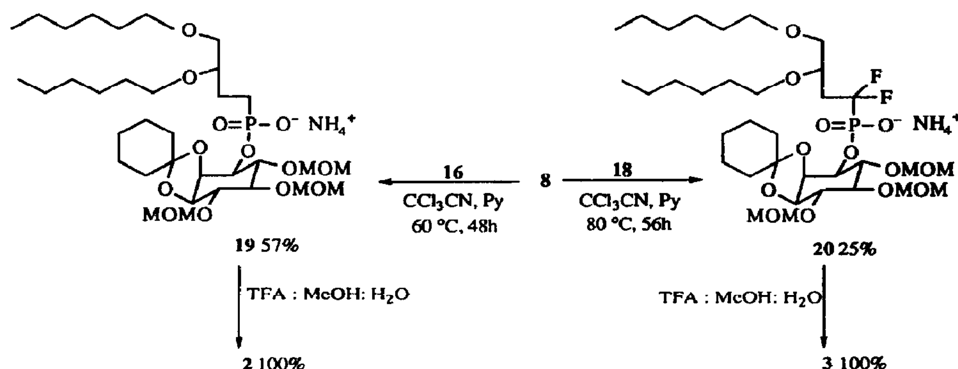
The synthesis of the double tail phosphonolipid unit in **2** and **3** started with commercially available racemic 1,3-dioxolane-4-methanol (solketal) **10**. Protection of the primary hydroxyl group in **10** as the benzyl ether followed by acid catalyzed cleavage of the ketal group gave the known diol **11** as a pale yellow oil.[14] Dialkylation of **11** with 1-bromohexane followed by hydrogenolysis of the benzyl group gave alcohol **13**[14] which was subsequently converted into triflate **14** (88%). Treatment of **14** with dimethyl (lithiomethyl)phosphonate gave dimethyl phosphonate ester **15** (85%)[13] which underwent facile dealkylation

to yield the phosphonic acid **16** (84%) using the protocol reported by McKenna.[15] The diethyl ester **17**[13] of the difluoromethylenephosphonic acid **18** was accessed by alkylating diethyl (lithiodifluoromethyl)-phosphonate[16] with triflate **14**, albeit in moderate yield (45%). Dealkylation was carried out with bromotrimethylsilane followed by water[14] providing difluoromethylenephosphonic acid **18**[13] in almost quantitative yield.




Scheme 2

Trichloroacetonitrile mediated condensation[17] of the pentaprotected-*myo*-inositol **8** and the phosphonic acid **16** gave **19** (57%) which was isolated as a homogenous ammonium salt. Removal of protecting groups from **19** using trifluoroacetic acid (TFA) gave **2**[13] in quantitative yield. The coupled product **20** (25%) was similarly prepared by condensation of **8** and **18**. Subsequent removal of the protecting groups gave **3**[13] in quantitative yield. A detailed enzymatic study of **2** and **3** with PI-PLC from *Bacillus cereus* will be reported in due course.




Scheme 3

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13. Compound **2** : $^1$H NMR ($CD_3OD$) δ 4.07 (m, 1 H), 3.74 (t, $J$ = 9.6 Hz, 1 H), 3.60 (m, 2 H), 3.43 (m, 7 H), 3.35 (m, 1 H), 3.16 (t, $J$ = 9.3 Hz, 1 H), 1.84 (m, 2 H), 1.53 (m, 6 H), 1.30 (m, 12 H), 0.88 (m, 6 H); $^{31}$P NMR ($CD_3OD$) δ 33.12; Anal. Calcd. for $C_{22}H_{45}O_{10}P$: C, 52.79; H, 9.06. Found: C, 52.90, H, 8.88. Compound **3**: $^1$H NMR ($CD_3OD$) δ 4.10 (m, 2 H), 3.90 (m, 1 H), 3.73 (m, 1 H), 3.61-3.48 (m, 9H), 2.38 (br.t, 2 H), 1.52 (m, 4 H), 1.30 (m, 12 H), 0.88 (m, 6 H); $^{31}$P NMR ($CDCl_3$:$CD_3OD$, 3:1 v/v) δ 2.50 (br.t). High resolution mass calcd. for $C_{22}H_{44}O_{10}F_2P$ ($MH^+$): 537.2660 Found: 537. 2640. The remaining new compounds reported in this paper, namely **5**, **7**, **8**, **9**, **15**, **16**, **17** and **18** were completely characterized by spectral methods and also gave satisfactory combustion (**5**, **7**, **8**, **9**, **15**, **16**) or high resolution mass spectral data (**17**).
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