Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1585–1595
Carbohydrate RESEARCH

Review

Fluorinated cyclitols as useful biological probes of phosphatidylinositol metabolism

David J. A. Schedler[a,*] and David C. Baker[b,*]

[a]*Department of Chemistry, Birmingham-Southern College, Birmingham, AL 35254, USA*
[b]*Department of Chemistry, The University of Tennessee, Knoxville, TN 37996-1600, USA*

Received 22 September 2003; accepted 31 March 2004
Available online 14 May 2004

Abstract—A number of deoxyfluoro cyclitols have been synthesized and evaluated as probes of the phosphatidylinositol pathway (PtdIns pathway), most notably 5-deoxy-5-fluoro-*myo*-inositol, which is incorporated into the pathway at about 25% the level of *myo*-inositol itself. Unfortunately, none of the cyclitols have proved effective in limiting cell proliferation, as the cells are able to 'synthesize around' the fraudulent cyclitols using natural *myo*-inositol as substrate. Inhibitors for 3-phosphatidylinositol kinase, which has importance in a number of pathological conditions, including cancer, have been intensively investigated. 3-Deoxy-3-fluoro-*myo*-inositol is incorporated into the PtdIns pathway; however, only related phosphatidyl derivatives, for example, a methyl ether derivative of the 3-deoxy inositol, showed significant antiproliferative activity. Synthesis of the deoxyfluoro analogues most often has been accomplished by DAST fluoro-de-hydroxylation of the appropriate cyclitol, generally leading to products of inversion.

Keywords: Deoxyfluoro cyclitols; Phosphatidylinositol; Phosphatidylinositol 3-kinase; Synthesis; DAST

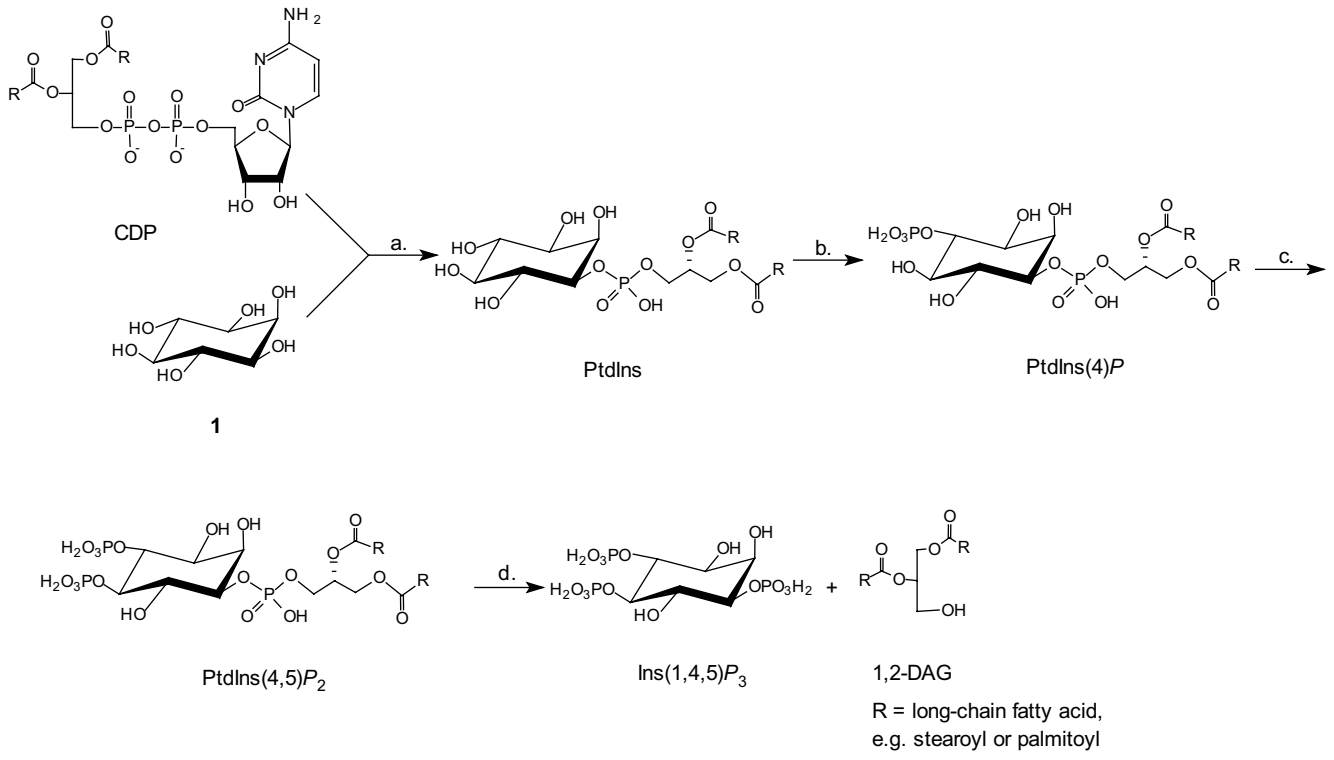
Contents

1. Introduction

Cyclitol is a generic term that is used to describe compounds that are cyclohexanehexitols, the most important of which is *myo*-inositol (**1**, Scheme 1), a compound that is ubiquitous in living systems.[1,2] *myo*-Inositol (**1**) and its derivatives {phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate [PtdIns(4)*P*], and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]} have been recognized for many years as important intermediates in the biosynthesis of the 'second messenger' compounds, inositol 1,4,5-trisphosphate [Ins (1,4,5)P_3] and diacylglycerol (1,2-DAG) that are

* Corresponding authors. Tel.: +1-423-974-3395; fax: +1-423-974-1536; e-mail addresses: dschedle@bsc.edu; dcbaker@utk.edu

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.03.030



Scheme 1.

associated with cell proliferation, cell growth, smooth muscle contraction, inflammatory cellular events, secretion, and other processes.[3,4] Certain malfunctions of these processes are implicated with a number of disease states.[5,6] In this minireview, we elaborate on the role of fluorinated cyclitols as biological probes for the reactions of the phosphatidyl inositol pathway as well as alternate pathways involving cyclitol biotransformations. Not covered in depth in this review are the roles played by various fluorinated inositol phosphates, that is, fluorinated analogues of the 'second messenger' compounds produced in the PtdIns pathway.

With a large body of chemistry having been developed on the cyclitols (e.g., all nine stereoisomers of the cyclohexanehexitols had been synthesized by about 1960),[†] it was natural that these compounds might become the focus of considerable exploration as the importance of PtdIns and its derivatives were discovered. The field has more recently developed into an intense area of research, beginning in the 1980s, with much of the activity directed toward probing the various pathways associated with cell proliferation and growth. Inasmuch as organofluorine compounds have been demonstrated to be effective replacements for their natural, nonfluorinated counterparts,[7,8] it is not surprising that fluorine-substituted analogues of *myo*-inositol (fluorinated cyclitols) have played an important role as biological probes.

2. The phosphatidylinositol pathway: points for pharmacological intervention

Central to these studies is probing the important pathway, termed the phosphatidylinositol pathway (Scheme 1), that serves to generate the second messengers, Ins(1,4,5)P_3 and 1,2-DAG. The first enzyme of the pathway, phosphatidylinositol synthase (EC 2.7.8.11), is responsible for the incorporation of a phosphatidyl group onto *myo*-inositol to give 1D-phosphatidyl *myo*-inositol, which in turn is phosphorylated by phosphatidylinositol 4-kinase [PtdIns(4) kinase, EC 2.7.1.67], then by phosphatidylinositol-4-phosphate 5-kinase [PtdIns-(4)*P* kinase, EC 2.7.1.68] to give phosphatidylinositol

[†] Posternak in his monograph[1] succinctly stated in the Foreword to the 1965 revised edition, "It is our personal opinion that the major problems in cyclitol chemistry are now solved. The structures of the naturally occurring cyclitols are now known, all theoretically possible inositols have been prepared, and the main stereochemical and structural points raised by them have been satisfactorily answered".

4,5-bisphosphate [PtdIns(4,5)P_2]. The latter intermediate is then cleaved by phosphatidylinositol-specific phospholipase C (PtdIns-PLC, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase, EC 3.1.4.11) to give Ins(1,4,5)P_3+1,2-DAG, the second messengers.[4]

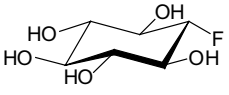
This pathway immediately presents two attractive points for pharmacological intervention: the two phosphorylation steps. By preventing either step—the phosphorylation at O-4 or the second phosphorylation at O-5—one should be able to effectively shut down the pathway by limiting the substrates for PtdIns-PLC, thus preventing the production of second messengers and, consequently, their effects on cellular function. Key to the success of any cyclitol being useful in this regard in intact cellular systems is that it must: (1) be lipophilic enough to cross cellular membranes and; (2) be a reasonably good substrate for the first enzyme, PtdIns synthase, thus facilitating its incorporation into the PtdIns cycle. Thus modifications on the cyclitol by replacement of either HO-4 or HO-5 with F could then, by effectively blocking phosphorylation at either the O-4 or O-5 position, serve to interrupt the pathway.

To this end, we in this laboratory began a program in collaboration with J.D. Dr. Moyer of the National Cancer Institute[9] in 1985 to selectively modify cyclitols and determine specific requirements for their incorporation into modified PtdIns analogues. A number of compounds were synthesized and evaluated for their PtdIns synthase activity.[10–12] High on our list of target compounds were the deoxyfluoro cyclitols, as it was hoped that these compounds, where F replaces OH or H, would serve as substrates for the enzyme. [The first reported deoxyfluoro cyclitol, 1-deoxy-1-fluoro-*scyllo*-inositol (**2**), was that of Yang and co-workers; however, the authors present no biological data for that compound.[13,14]] Among the compounds synthesized in our laboratory was 5-deoxy-5-fluoro-*myo*-inositol [(5d,5F)-Ins, **3**], which proved to be a good substrate for PtdIns synthase, with most determinations of competitive incorporation into the PtdIns pathway indicating that it was about 25% of the rate of *myo*-inositol. Compound **3** remains today as one of the most active, if not indeed the most active, of the modified cyclitols that act as substrates for PtdIns synthase.[12] (see Table 1) Further studies on the compound in intact L1210 leukemia cells showed that the rate of cellular uptake was similar to that of *myo*-inositol (**1**)[9] and that it achieved intracellular concentrations approximating those of **1**. More recently, detailed mass spectrometric studies of the lipid fraction produced by a crude microsomal preparation of PtdIns synthase from rat brain definitively showed that the compound is indeed incorporated into cellular lipid as the Ptd(5d,5F)Ins (**4**) compound.[15] Thus a simple modification, wherein the HO-5 group of *myo*-inositol is replaced by F, served to produce a compound that traverses cellular membranes, is a relatively good substrate for PtdIns synthase, is converted to Ptd(5d,5F)Ins (**4**), and is further phosphorylated to give what is indicated to be the 4-phosphate [Ptd(5d,5F)Ins(4)*P*, **5**]. Other salient features of the compound include the fact that it, like *myo*-inositol, is a meso compound and consequently does not require asymmetric synthesis.

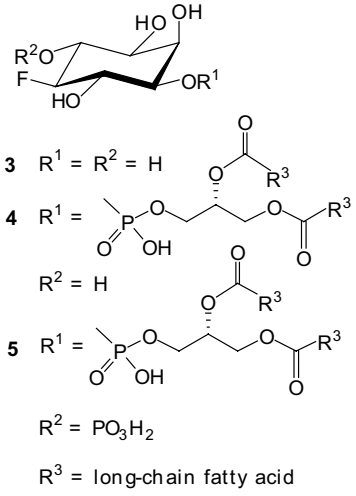
Table 1. *myo*-Inositol analogues as inhibitors and substrates for phosphatidylinositol synthase[a]

Compd no	Inhibition (%)[b]	Substrate activity (% of *myo*-inositol)[c]
1	68	100
3	30[d]	26 ± 4[e]
6	15	16 ± 2
7	<10	<5
8	75 ± 1	<5
9	37	7

[a] Compounds were evaluated at 5 mM.
[b] Inhibition was measured against incorporation of 0.04 mM *myo*-inositol. Average duplicate determinations agreed within ±10%. For details see Ref. 11.
[c] As a measure of CMP formation, where **1** = 100%. For details, see Ref. 11.
[d] A value of 22% is reported in Ref. 16 for a mixture that contained 0.5 mM *myo*-inositol (**1**) and 5 mM **3**.
[e] A value of 28% was reported under similar conditions in Ref. 16 for a mixture that contained 5 mM **3**.



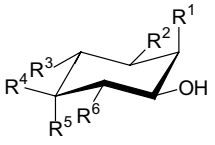
2



More disappointing, however, are the practical medicinal–chemical ramifications of these studies. It was hoped by achieving such enzymatic activity with a fraudulent cyclitol that an effective compound could be found that would halt cellular proliferation and growth. The ultimate objective was to find a new type of

antiproliferative (anticancer) drug that would act via the PtdIns pathway. Studies in whole cells, unfortunately, showed that cellular proliferation was not halted with administration of (5d,5F)Ins (**3**), and only in studies in a cell line that was deficient in *myo*-inositol (**1**) production was any antiproliferative effect noted.[12] In studies carried out with addition of an excess of *myo*-inositol (**1**) (up to a 200-fold excess), (5d,5F)Ins (**3**) showed uptake in a competitive manner. Thus the conclusions from these studies were that the production of *myo*-inositol (**1**) by indigenous means (i.e., by the normal pathway from D-glucose) was sufficient to overcome any effects that a fraudulent (5d,5F)Ins (**3**) might have. In other words, normal cells were able to 'synthesize around' the added fraudulent cyclitol, thereby limiting its cellular antiproliferative effects. By working through this pathway, the development of a fraudulent cyclitol as an antitumor compound was to no avail. Thus the use of an intact, synthetic phosphatidyl analogue (e.g., analogues of PtdIns such as **4** or **5**) would seem to be required; however, the latter type of compound has attendant problems of cell membrane permeability, instability, and problems with lengthier and more complex synthetic processes, all of which cast doubt on the potential for these phosphatidyl compounds as realistic drug candidates.

Other fluorinated analogues have also been examined, and rather strict structural requirements for PtdIns substrate became evident[12] as shown in Table 1. The 5-fluoro epimer of (5d,5F)Ins (**3**), 2-deoxy-2-fluoro-*neo*-inositol (**6**), showed $16 \pm 2\%$ incorporation; others, however, were shown to be much poorer substrates, all showing <5% incorporation as determined by measurement of CMP production from CDP-diglyceride: a 2-modified *myo*-inositol, 1-deoxy-1-fluoro-*scyllo*-inositol (**2**); a 4-modified derivative, 4-deoxy-4-fluoro-*myo*-inositol (**7**); and a 5,5-*gem*-difluoro analogue, 5-deoxy-5,5-difluoro-*myo*-inositol (**8**). In addition, **8** showed a fairly potent inhibition of PtdIns synthase. In a separate study,[16] the 3-deoxy-3-fluoro-*myo* derivative (**9**) was shown to have only a 7% incorporation.



6 $R^1 = R^2 = R^3 = R^6 = OH$; $R^4 = H$, $R^5 = F$

7 $R^1 = R^2 = R^3 = R^4 = OH$; $R^5 = H$, $R^6 = F$

8 $R^1 = R^2 = R^3 = R^6 = OH$; $R^4 = R^5 = F$

9 $R^1 = R^3 = R^4 = R^6 = OH$; $R^2 = F$, $R^5 = H$

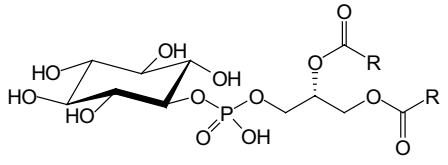
10 $R^2 = R^3 = R^4 = R^6 = OH$; $R^1 = F$, $R^5 = H$

In a related study that used turkey erythrocyte membranes, which served to avoid problems of cell penetration as with intact cells, 2-deoxy-2-fluoro-*myo*-inositol (**10**) and 1-deoxy-1-fluoro-*scyllo*-inositol (**2**) were examined, and it was confirmed that these were only very poorly converted into lipids.[17] When examined as the inhibitors of both the head-exchange reaction and PtdIns synthase, the compounds were found to effect an inhibition of $10.5 \pm 3.5\%$ (at 1 mM) and $93 \pm 3\%$ (at 10 mM), respectively, for the head-exchange process and an inhibition 23% (at 5 mM) and $61 \pm 5\%$ (at 10 mM), respectively, for the PtdIns synthase. These authors interestingly suggested further studies with the (5d,5F)Ins (**3**) analogue to determine the possible inhibitory effects of that compound. (These studies, to our knowledge, were never carried out.)

Largely because of the ability of intact cellular systems to synthesize around inhibition of the kinase enzymes of the PtdIns pathway, work has been aimed at directly inhibiting phosphatidylinositol-specific phosphlolipase C (PtdIns-PLC), another key enzyme (Scheme 1). PtdIns-PLC is a key enzyme in signal transduction and holds promise in rational drug design.[18] The enzyme has also been shown in many cases to be overexpressed in carcinomas and tumors;[19] therefore, inhibitors directed at regulating PtdIns-PLC activity could be attractive as agents to control the growth of these tumor cells. The mechanism of action of the enzyme is reminiscent of that for ribonuclease A[20] and has been shown to be a complex general acid–general base process.[21–23] To be an effective competitive inhibitor, the molecule must bind as a substrate for PtdIns-PLC, yet be nonhydrolyzable by the enzyme. The targets of modification have therefore been the phosphodiester linkage to the inositol ring and the adjacent axial hydroxy group at C-2. The axial HO-2 group is essential as an intramolecular nucleophile in the hydrolysis of PtdIns(4,5)P_2 to give the important second messengers Ins(1,4,5)P_3 and DAG.[24,25] However, studies of the active site have implied that the HO-2, although necessary for hydrolysis, is probably dispensable for substrate recognition and binding. Also, it appears that the phosphoryl moiety is necessary for both recognition and inhibition.[19,24,25] With this in mind, several PtdIns analogues discussed below have been investigated as nonhydrolyzable competitive inhibitors of PtdIns-PLC.

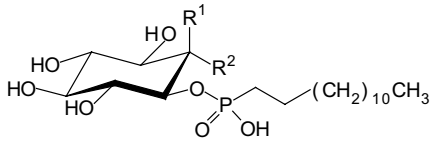
Modifications at the 2-position include deoxygenation,[24,26,27] inversion of the OH to the *scyllo* configuration, substitution with a fluoro group, or both. In work carried out by the groups of Shen and co-workers,[19,28,29] the *scyllo* analogue (**11**) of natural PtdIns has been identified as a potent inhibitor of PtdIns-PLC, and the same

principle has been extended to the fluorinated series. The most potent competitive inhibitor reported is the 2-deoxy-2-fluoro-*scyllo*-inositol-1-*O*-dodecylphosphonic acid (**12**). It inhibits GlyPtdIns-PLC to 85% activity at 1 mM. It was shown to be more potent than either the corresponding 2-deoxy-2-fluoro-*myo*-inositol (**13**) (47.9% at 5 mM) or the 2-deoxy-2,2-difluoro analogue (**14**) (78.7% at 1 mM). The efficacy of these inhibitors is dramatically influenced by the orientation of the fluoro substituent. The requirement that the fluoro substituent must be in the *scyllo* configuration is most likely due to its interaction with an active site residue. The addition of a fluoro group in the *scyllo* configuration to the 2-deoxy-2-fluoro-*myo*-inositol analogue (**13**) giving the 2-deoxy-2,2-difluoro analogue (**14**) is enough to nearly restore the efficacy. The alkylphosphonate side chain is necessary for inhibition as evidenced by the ineffectiveness of the fluorinated analogues lacking this group. Also, the methyl ester of the active phosphonate derivative, which lacks the negative charge, is ineffective as an inhibitor. In fact, the negative charge is also necessary for binding.[29] The best inhibitors are the result of two factors. The first is resistance to hydrolysis by PtdIns-PLC, and the second is the presence of a group at C-2 in the proper orientation to allow interaction with the enzyme active site.[19] The most potent inhibitors of PtdIns-PLC have both a dodecyl phosphonate at the 1-position of the 2-deoxyinositol and a fluoro group in the *scyllo* configuration at the 2-position.[28]



11

R = long-chain fatty acid



12 $R^1 = H, R^2 = F$

13 $R^1 = F, R^2 = H$

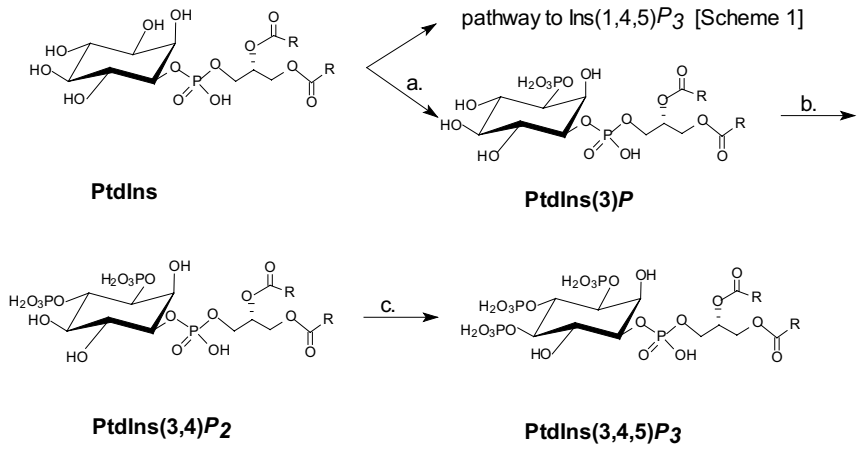
14 $R^1 = R^2 = F$

3. Inhibitors of the phosphatidylinositol 3-kinase pathway

In addition to the PI Pathway discussed in the foregoing section, a second pathway that involves phosphorylation at the 3-position of *myo*-inositol derivatives has been found to be important in cyclitol metabolism.[5,30,31] The enzyme, phosphatidylinositol 3-kinase (PtdIns 3-kinase, EC 2.7.1.137),[32] is responsible for the phosphorylation of PtdIns, PtdIns(4)*P*, or PtdIns(4,5)P_2 at the 3-position of the *myo*-inositol ring (see Scheme 2) that produces a set of multifunctional signaling molecules[33–35] that are involved in a plethora of cellular signaling events that impact insulin response,[36,37] inflammatory and immunological conditions,[38,39] and cellular proliferation, aspects of which are important in tumor pathogenesis, with implications of their potential use in cancer chemotherapy.[40] It is not surprising that these inositol 3-phosphates are targets for drug development, given the functional specialization of the various isoforms[35] of PtdIns 3-kinase that should allow for the design of specific inhibitors or antagonists with limited general toxicity.[41]

The resulting phosphorylated PtdIns's are, in general, poor substrates for hydrolysis by PtdIns-PLC,[42] which is associated with almost every growth factor, as well as oncogene transformation.[43,44] These 3-phosphates are modulators of protein interactions and enzyme activity via binding to the proteins, especially tyrosine kinases, at specific sites. However, details of the exact mechanism by which these 3-phosphorylated PtdIns's affects cell growth is not precisely known and is under intensive investigation.

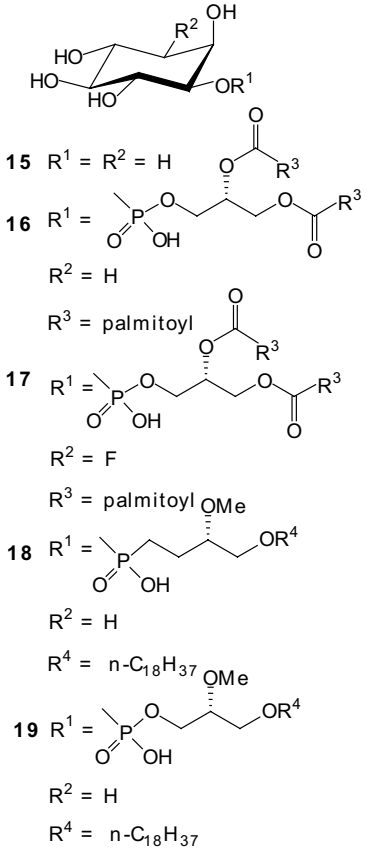
There is evidence that protein kinase C (PKC) is activated by PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, which leads to a cascade of events that culminate in DNA synthesis and cell proliferation.[44] These lipids have been found to occur in many cells, especially in transformed fibroblasts. Clear evidence exists that the ability of oncogenic polyoma virus to transform cells in culture and to induce tumors in animals depends on its ability to bind PtdIns 3-kinase through the middle T oncoprotein.[45,46] Because of this, inhibition of the formation of phosphorylated products by PtdIns 3-kinase could potentially be of value in controlling these cellular responses, with implications in the cancer area. Most of the work in this area has centered on selectively inhibiting the 3-kinase pathway without affecting the normal PtdIns pathway. Both cyclitols[16] and phosphatidyl analogues[44] have been studied. This approach is hoped to lead to pharmacological agents (i.e., anticancer drugs) that will selectively block the second messengers for cell proliferation and transformation without affecting normal cell growth. Although some early work centered on the ability of 3-modified inositols to inhibit cell growth,[47] their effect on specific enzymes in the PtdIns pathway was not reported. As with the earlier inhibitors previously discussed, it is important to design inhibitors of the 3-kinase that are substrates for PtdIns synthase, therein producing fraudulent PtdIns's that would block phosphorylation by the 3-kinase. As noted earlier, 3-deoxy-3-fluoro-*myo*-inositol (**9**) acts as a substrate for PtdIns synthase, although not to the extent of the



Scheme 2.

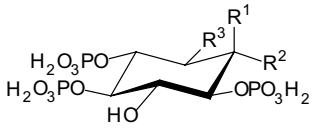
5-analogue. In the 5-analogue, the activity was attributed to the ability of the F atom to accept a hydrogen bond with the enzyme much the same as the OH in the natural substrate. In the 3-deoxyfluoro analogue **9** this explanation seems less likely as is evidenced by the nearly identical activity of 3-deoxy-*myo*-inositol (**15**) as a substrate.[16]

The phosphatidyl derivatives of these two inositols were synthesized, and their efficacy against cell growth with HT-29 human colon carcinoma cells was determined.[44] 1D-3-Deoxy-*myo*-PtdIns (**16**) and 1D-3-deoxy-3-fluoro-*myo*-PtdIns (**17**) showed IC_{50} values of 35 and 37 μM, respectively. The authors suggest that the observed low potency is probably due to their hydrolysis by PtdIns-PLC, producing DAG that would activate PKC and initiate tumor cell proliferation. An approach in the 3-deoxy case was to synthesize the phosphonate derivative **18** to prevent PtdIns-PLC hydrolysis; however, it showed only slightly improved inhibitory activity (IC_{50} 10 μM). Much greater improvement in activity came with the phosphatidyl derivative **19**, whereby ether lipid analogues were used to overcome the lipid hydrolysis problems, resulting in a compound with an IC_{50} of 2.1 μM, along with an inhibition of the 3-kinase with an IC_{50} of 2.5 μM. Furthermore, the compound was active against HT-29 human adenocarcinoma in SCID mice with a 67% reduction in tumor volume 10 days after inoculation at 150 mg/kg ip. These studies demonstrate that perhaps an active antitumor compound with limited toxicity can be derived from suitably ether-modified phosphatidyl derivatives of 3-deoxy cyclitols.[48]



There is another body of work involving fluorinated derivatives of Ins(1,4,5)P_3. This compound is a second messenger involved in the release of Ca^{2+} from intracellular stores.[49–51] In the search for an understanding of the molecular basis for the recognition of inositol polyphosphates by the IP_3 receptor and the metabolic enzymes, Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3 5-phosphatase, a number of analogues of these inositols have been synthesized and evaluated with the goal of

understanding the role of the three phosphates and hydroxy groups of Ins(1,4,5)P_3 in binding.[44,49,50,52–56] Because of the scope of this review, only the fluorinated analogues will be discussed. The four fluorinated analogues, 2-deoxy-2-fluoro-*scyllo*-inositol 1,4,5-trisphosphate {2-F-Ins(1,4,5)P_3 **20**}, 2-deoxy-2,2-difluoro-*myo*-inositol 1,4,5-trisphosphate {2,2-F_2-Ins(1,4,5)P_3 **21**}, 2-deoxy-2-fluoro-*myo*-inositol 1,4,5-trisphosphate {2d,2F-Ins(1,4,5)P_3 **22**}, and 3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate {3d,3F-Ins(1,4,5)P_3 **23**} are all full agonists for Ca^{2+} mobilization.[57] 3-Fluoro-D-*myo*-Ins-(1,4,5)P_3 is the most potent ligand and agonist of the analogues tested. The 3-F group therefore either appropriately mimics both the electronic environment and stereochemistry of the 3-OH of Ins(1,4,5)P_3 or is unimportant to binding. There is an established correlation between increasing molecular volume at the 3-position and respective decreases in both affinity and Ca^{2+}-mobilizing efficacy.[54] For example, Ins(1,3,4,5)P_4 binds with low affinity to the Ins(1,4,5)P_3 receptor. This could mean that the 3-OH may act as an anchor such that changes in its orientation or its replacement may adversely affect binding to the receptor. However, these four fluorinated analogues (**20**–**23**) are not as potent as Ins(1,4,5)P_3 indicating that the 2- and 3-positions may not be important for receptor binding. In regard to their interaction with the metabolic enzymes, as with the other 3-kinase, the 3-position is of particular interest because it is the site of phosphorylation by the enzyme. The environment around this position appears be crucial for 3-kinase activity and also 5-phosphatase-mediated hydrolysis.[54] As expected, the 3-deoxy-3-fluoro compound **23** is not a substrate for the Ins(1,4,5)P_3 3-kinase, while the 2-modified analogues are. Moreover, **23** is a substrate for the 5-phosphatase and inhibits dephosphorylation of Ins(1,4,5)P_3.[50,56] In contrast, the three 2-deoxy-2-fluoro analogues **20**–**22** are poor substrates for the 5-phoshatase; however, 2,2-F_2Ins(1,4,5)P_3 inhibits the 5-phosphatase with a high affinity ($K_i = 26 \pm 4.1\ \mu M$).[52,53] Position 2 does not appear then to be significantly involved in the recognition of, and the interaction with, either the Ins(1,4,5)P_3 receptor or Ins(1,4,5)P_3-3-kinase, but replacement of the 2-OH by fluorine produces Ins(1,4,5)P_3 analogues that are poor substrates for the 5-phosphatase.



20 R^1 = H, R^2 = F, R^3 = OH

21 R^1 = R^2 = F, R^3 = OH

22 R^1 = F, R^2 = H, R^3 = OH

23 R^1 = OH, R^2 = H, R^3 = F

4. Synthesis of deoxyfluoro cyclitols

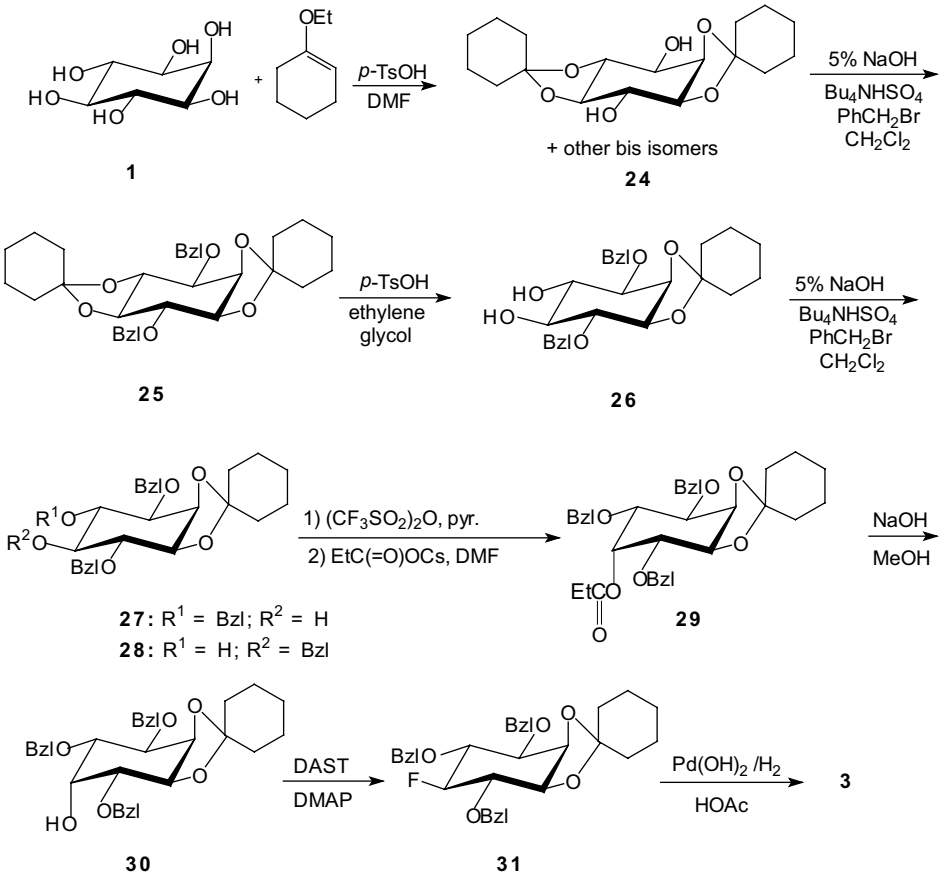
4.1. Chemical synthesis

Preparation of deoxyfluoro cyclitols has typically been carried out by fluoro-de-hydroxylation transformations that involve either displacement of a suitably activated sulfonate ester with fluoride ion, or directly in a one-step reaction by (diethylamino)sulfur trifluoride (DAST).[58] Protecting group chemistry often involves formation of acetal derivatives, benzyl ethers, and other protecting groups common in both the carbohydrate and cyclitol field. There is one report of a novel debenzylation–ring closure when using DAST.[59] *gem*-Difluoro analogues such as **8**[60] and others[61] are synthesized using DAST and the appropriately protected ketone. Attempts to use SF_4–anhydrous HF have resulted in formation of novel bicyclic compounds.[62]

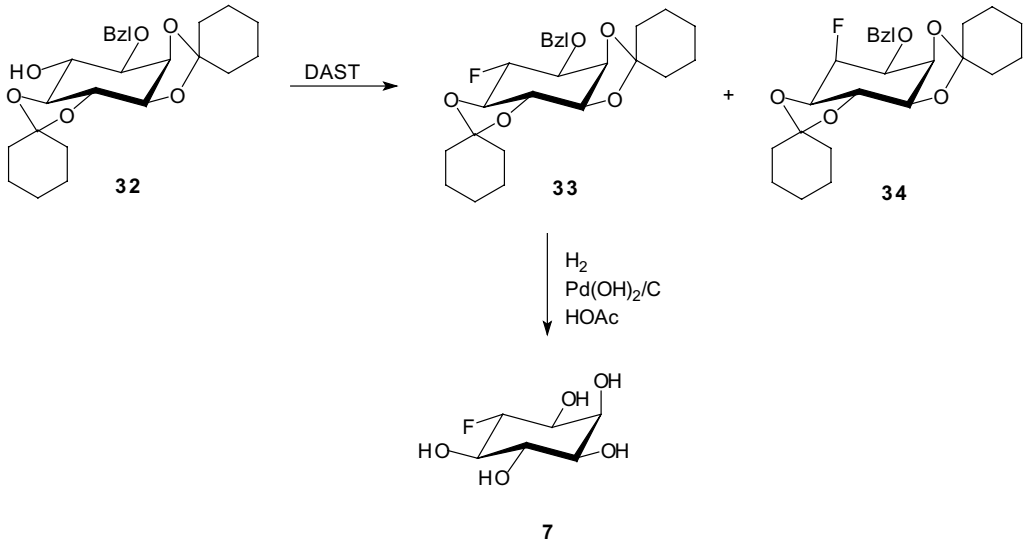
Several examples of selective fluoro-de-hydroxylations appear in the paper by Jiang et al.[10] For the most part, the reactions are predictable and proceed in respectable yields using DAST as the reagent, as was the case with the first deoxyfluoro cyclitols synthesized.[14] An example is shown by the synthesis of (5d,5F)Ins (**3**) (see Scheme 3).[60] 1:2,4:5-Di-*O*-cyclohexylidene-*myo*-inositol (note 1D-*myo* numbering) (**24**), prepared by an improved, acetal-exchange process,[63] was benzylated, then selectively mono-decyclohexylidenated to furnish the intermediate with the 4- and 5-positions open. Selective benzylation then gave a mixture of 3,4,6- (**27**) and 3,5,6-tri-*O*-benzyl-1,2-*O*-cyclohexylidene (**28**) derivatives that were separated by chromatography. Reaction of **27** with trifluoromethanesulfonic anhydride, then inversion of the C-5 center with cesium propionate, afforded the inverted *neo*-cyclitol derivative **29**. Hydrolysis of the ester group to **30**, followed by reaction with DAST, then gave the 5-deoxy-5-fluoro-*myo*-inositol derivative **31**. Deprotection by hydrogenolysis in acetic acid then afforded the target **3**.

In most cases DAST cleanly gave the product of inversion; however, in the reaction of DAST with 3-*O*-benzyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (**32**) (Scheme 4) a mixture of epimers **33** and **34** were obtained with a ratio of inversion to retention of ~1:4, giving the major product upon deptrotection, 4-deoxy-4-fluoro-*myo*-inositol (**7**).[10] Arguments can be made that the diethylaminodifluorosulfenyl intermediate formed from **32** with DAST is hindered from backside approach by the fluoride ion.

A clever use of DAST in a short synthesis of 3-deoxy-3-fluoro-*myo*-inositol (**9**) is shown in Scheme 5.[64]

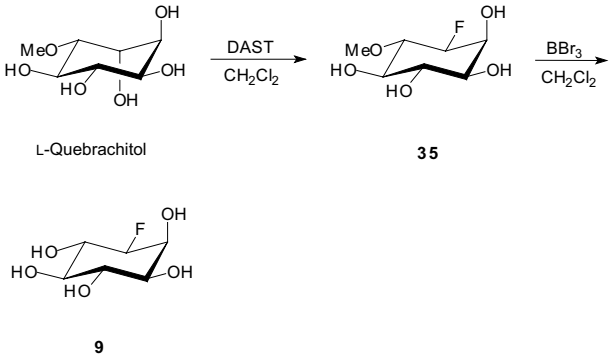


Scheme 3.



Scheme 4.

Powdered L-quebrachitol, cooled to −50 °C, is treated directly with DAST. Quenching the reaction with methanol, followed by demethylation using BBr_3, then affords optically active 3-deoxy-3-fluoro-*myo*-inositol (**9**). The selective fluoro-de-hydroxylation of an unprotected cyclitol is remarkable in this example.



Scheme 5.

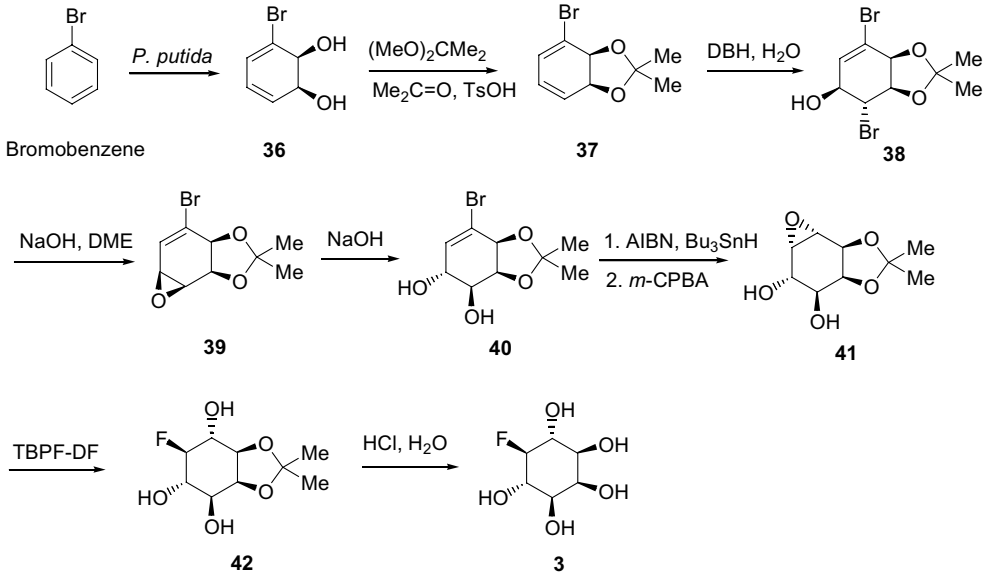
4.2. Chemoenzymatic synthesis

Using a strain of *Pseudomonas putida*, oxidation of substituted benzenes[65,66] gives cyclohexadiene diols that can be used in the synthesis of cyclitols.[67–70] For a process[68] (Scheme 6) to 5-deoxy-5-fluoro-*myo*-inositol (**3**), bromobenzene was so oxidized to give (5*S*,6*R*)-1-bromocyclohexa-1,3-diene-5,6-diol (**36**) that was acetal protected to give **37**, converted to the bromohydrin **38**, and treated with base to provide 4-bromo-3a,5a,6a,6b-tetrahydro-2,2-dimethyl-[3a*S*-(3aα,5aα,6aα,6bα)]-oxireno[*e*]-1,3-benzodioxole (**39**). Sodium hydroxide effected the oxirane opening to give the *trans*-diol **40**. Reaction with tributyltin hydride–AIBN resulted in debromination, and subsequent epoxidation with *m*-chloroperoxybenzoic acid gave the epoxide **41**. Ring opening of the epoxide with tetrabutylphosphonium fluoride dihydrofluoride (TBPF-DF) then furnished the fluoro derivative **42**, which was deprotected to give (5d,5F)Ins (**3**).

Similar strategies have been used to synthesize 3-deoxy-3-fluoro-L-*chiro*-inositol,[68] 6-deoxy-6-fluoro-*myo*-inositol,[67] and both (+)- and (−)-2-amino-1,2-dideoxy-1-fluoro-*allo*-inositols.[69] Aspects of this work have been reviewed.[70]

5. Conclusions

A number of deoxyfluoro cyclitols have proven their worth as probes of the phosphatidylinositol pathway (PtdIns pathway), most notably 5-deoxy-5-fluoro-*myo*-inositol (**3**), which is incorporated into the pathway at about 25% the level of *myo*-inositol itself. Unfortunately, none proved effective in limiting cell proliferation, as the cells were able to 'synthesize around' the fraudulent cyclitols using natural *myo*-inositol as substrate. Inhibitors for 3-phosphatidylinositol kinase, which has importance in a number of pathological conditions, including cancer, have been intensively investigated. 3-Deoxy-3-fluoro-*myo*-inositol (**9**) is incorporated into the PtdIns pathway; however, only related phosphatidyl derivatives, for example, **19**, showed significant antiproliferative activity. Synthesis of the deoxyfluoro analogues most often has been accomplished by DAST fluoro-de-hydroxylation of the appropriate cyclitol, generally leading to products of inversion.



DBH = 1,3-Dibromo-5,5-dimethylhydantoin
TBPF-DF = tetrabutylphosphoniumfluoride dihydrofluoride

Scheme 6.

References

1. Posternak, T. *The Cyclitols*; Holden-Day: San Francisco, 1962.
2. Anderson, L. The Cyclitols. In *The Carbohydrates*; Pigman, W., Horton, D., Eds.; Academic: San Diego, 1972; 2nd ed.; Vol. 1A, pp 519–579.
3. *Inositol and Phosphoinositides*; Bleasdale, J. E., Eichberg, J., Hauser, G., Eds.; Humana: Clifton, NJ, 1985.
4. *Inositol Lipids in Cell Signalling*; Michell, R. H., Drummond, A. H., Downes, C. P., Eds.; Academic: San Diego, 1989.
5. Powis, G.; Kozikowski, A. P. Inhibitors of *myo*-Inositol Signaling. In *New Molecular Targets for Cancer Chemotherapy*; Kerr, D. J., Workman, P., Eds.; CRC: Boca Raton, 1994; pp 81–95.
6. Kozikowski, A.; Fauq, A.; Malaska, M.; Tuchmantel, W.; Ognyanov, V.; Powis, G. *Curr. Med. Chem.* **1994**, *1*, 1–12.
7. Wakselman, C. *Ann. Pharm. Fr.* **1999**, *57*, 108–115.
8. Filler, R. *ACS Symp. Ser.* **2000**, *746*, 1–20.
9. Moyer, J. D.; Malinowski, N.; Napier, E. A.; Strong, J. *Biochem. J.* **1988**, *254*, 95–100.
10. Jiang, C.; Moyer, J. D.; Baker, D. C. *J. Carbohydr. Chem.* **1987**, *6*, 319–355.
11. Moyer, J. D.; Reizes, O.; Ahir, S.; Jiang, C.; Malinowski, N.; Baker, D. C. *Mol. Pharmacol.* **1988**, *33*, 683–689.
12. Moyer, J. D.; Reizes, O.; Malinowski, N.; Jiang, C.; Baker, D. C. *ACS Symp. Ser.* **1988**, *374*, 43–58.
13. Yang, S. S.; Beattie, T. R.; Shen, T. Y. *Tetrahedron Lett.* **1982**, *23*, 5517–5520.
14. Yang, S. S.; Beattie, T. R.; Durette, P. L.; Gallagher, T. F.; Shen, T. Y. U.S. Patent 4,515,722, 1985.
15. Baker, D. C.; Johnson, S. C.; Moyer, J. D.; Malinowski, N.; Tagliaferri, F.; Tuinman, A. *ACS Symp. Ser.* **1999**, *718*, 271–281.
16. Johnson, S. C.; Dahl, J.; Shih, T. L.; Schedler, D. J. A.; Anderson, L.; Benjamin, T. L.; Baker, D. C. *J. Med. Chem.* **1993**, *36*, 3628–3635.
17. McPhee, F.; Downes, C. P.; Lowe, G. *Biochem. J.* **1991**, *277*, 407–412.
18. Bruzik, K. S. In *Carbohydrate Drug Research*; Witczak, Z., Nieforth, K. A., Eds.; Dekker: New York, 1997; pp 385–431.
19. Morris, J. C.; Ping-Sheng, L.; Zhai, H.-X.; Shen, T.-Y.; Mensa-Wilmot, K. *J. Biol. Chem.* **1996**, *271*, 15468–15477.
20. Stryer, L. *Biochemistry*. 4th ed.; W.H. Freeman: New York, 1995; pp 216–218.
21. Bruzik, K. S.; Tsai, M.-D. *Bioorg. Med. Chem.* **1994**, *2*, 49–72.
22. Hondal, R. J.; Zhao, Z.; Riddle, S. R.; Suzette, R.; Kravchuk, A. V.; Liao, H.; Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1997**, *119*, 9933–9934.
23. Hondal, R. J.; Zhao, Z.; Kravchuk, A. V.; Liao, H.; Riddle, S. R.; Yue, X.; Bruzik, K. S.; Tsai, M.-D. *Biochemistry* **1998**, *37*, 4568–4580.
24. Martin, S. F.; Wagman, A. S. *J. Org. Chem.* **1996**, *61*, 8016–8023.
25. Aneja, S. G.; Ivanova, P. T.; Aneja, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1061–1064.
26. Schedler, D. J. A.; Baker, D. C. In *Abstr. Papers 201st ACS National Meeting*; American Chemical Society: Atlanta, GA, 1991, CARB-037.
27. Schedler, D. J. A. Ph.D. Dissertation, The University of Alabama, Tuscaloosa, AL, 1992.
28. Zhai, H.-X.; Lei, P.-S.; Morris, J. C.; Mensa-Wilmot, K.; Shen, T. Y. *Tetrahedron Lett.* **1995**, *36*, 7403–7406.
29. Morris, J. C.; Ping-Sheng, L.; Zhai, H.-X.; Shen, T.-Y.; Mensa-Wilmot, K. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 873–876.
30. Auger, K. R.; Carpenter, C. L.; Cantley, L. C.; Varticovski, L. *J. Biol. Chem.* **1989**, *264*, 20181–20184.
31. Carpenter, C. L.; Cantley, L. C. *Biochemistry* **1990**, *29*, 11147–11156.
32. Whitman, M.; Downes, C. P.; Keeler, M.; Keller, T.; Cantley, L. *Nature* **1988**, *332*, 644–646.
33. Fukui, Y.; Ihara, S.; Nagata, S. *J. Biochem. (Tokyo)* **1998**, *124*, 1–7.
34. Corvera, S.; Czech, M. P. *Trends Cell Biol.* **1998**, *8*, 442–446.
35. Vanhaesebroeck, B.; Waterfield, M. D. *Exp. Cell Res.* **1999**, *253*, 239–254.
36. Jones, D. R.; Varela-Nieto, I. *Mol. Med.* **1999**, *5*, 505–514.
37. Kido, Y.; Nakae, J.; Accili, D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 972–979.
38. Hanke, J. H.; Pollok, B. A.; Changelian, P. S. *Inflamm. Res.* **1995**, *44*, 357–371.
39. Myers, M. R.; He, W.; Hulme, C. *Curr. Pharm. Des.* **1997**, *3*, 473–502.
40. Roymans, D.; Slegers, H. *Eur. J. Biochem.* **2001**, *268*, 487–498.
41. Stein, R. C.; Waterfield, M. D. *Mol. Med. Today* **2000**, *6*, 347–357.
42. Serunian, L. A.; Haber, M. T.; Fukui, T.; Kim, J. W.; Rhee, S. G.; Lowenstein, J. M.; Cantley, L. C. *J. Biol. Chem.* **1989**, *264*, 17809–17815.
43. Toker, A.; Cantley, L. C. *Nature* **1997**, *387*, 673–676.
44. Qiao, L.; Nan, F.; Kunkel, M.; Gallegos, A.; Powis, G.; Kozikowski, A. P. *J. Med. Chem.* **1998**, *41*, 3303–3306.
45. Freund, R.; Dawe, C. J.; Benjamin, T. L. *Am. J. Pathol.* **1992**, *141*, 1409–1425.
46. Chen, Y.; Freund, R.; Listerud, M.; Wang, Z.; Talmage, D. A. *Oncogene* **1999**, *18*, 139–148.
47. Powis, G.; Aksoy, I. A.; Melder, D. C.; Aksoy, S.; Eichinger, H.; Fauq, A. H.; Kozikowski, A. P. *Cancer Chemother. Pharmacol.* **1991**, *29*, 95–104.
48. Gurwitz, D. *Drug Discov. Today* **1999**, *4*, 96–96.
49. Kozikowski, A. P.; Fauq, A. H.; Aksoy, I. A.; Seewald, M. J.; Powis, G. *J. Am. Chem. Soc.* **1990**, *112*, 7403–7404.
50. Safrany, S. T.; Wilcox, R. A.; Liu, C.; Potter, B. V. I.; Nahorski, S. R. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1992**, *226*, 265–272.
51. Stricker, R.; Chang, Y.-T.; Chung, S.-K.; Reiser, G. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 596–604.
52. Safrany, S. T.; Sawyer, D.; Wojcikiewicz, R. J. H.; Nahorski, S. R.; Potter, B. V. L. *FEBS Lett.* **1990**, *276*, 91–94.
53. Wilcox, R. A.; Nahorski, S. R.; Sawyer, D. A.; Liu, C.; Potter, B. V. L. *Carbohydr. Res.* **1992**, *234*, 237–246.
54. Wilcox, R. A.; Challiss, R. A. J.; Traynor, J. R.; Fauq, A. H.; Ognayanov, V. I.; Kozikowski, A. P.; Nahorski, S. R. *J. Biol. Chem.* **1994**, *269*, 26815–26821.
55. Guedat, P.; Poitras, M.; Spiess, B.; Guillemette, G.; Schlewer, G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1175–1178.
56. Wilcox, R. A.; Fauq, A.; Kozikowski, A. P.; Nahorski, S. R. *FEBS Lett.* **1997**, *402*, 241–245.
57. Sawyer, D. A.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1992**, 923–932.
58. Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574–578.
59. Chung, S.-K.; Kwon, Y.-U.; Ahn, Y.-H.; Jeong, T.-H.; Chang, Y.-T. *Bull. Korean Chem. Soc.* **2000**, *21*, 274–276.
60. Jiang, C.; Schedler, D. J. A.; Morris, P. E., Jr.; Zayed, A. H. A.; Baker, D. C. *Carbohydr. Res.* **1990**, *207*, 277–285.

61. da Silva, A. D.; Benicio, A. A. A.; Gero, S. D. *Tetrahedron Lett.* **1999**, *40*, 6531–6534.
62. Coe, P. L.; Maunder, P.; Frampton, C. S.; Martin, J. A.; Anthony, T. W., et al. *J. Fluorine Chem.* **2002**, *115*, 13–20.
63. Jiang, C.; Baker, D. C. *J. Carbohydr. Chem.* **1986**, *5*, 615–620.
64. Kozikowski, A. P.; Fauq, A. H.; Rusnak, J. M. *Tetrahedron Lett.* **1989**, *30*, 3365–3368.
65. Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653–2662.
66. Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626–1630.
67. Ley, S. V.; Parra, M.; Redgrave, A. J.; Sternfield, F. *Tetrahedron* **1990**, *46*, 4495–5026.
68. Nguyen, B. V.; York, C.; Hudlicky, T. *Tetrahedron* **1997**, *53*, 8807–8814.
69. Oppong, K. A.; Hudlicky, T.; Yan, F.; York, C.; Nguyen, B. V. *Tetrahedron* **1999**, *55*, 2875–2880.
70. Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195–1220.