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A divergent approach toward synthesis of *myo*-inositol phosphates: Acyl migrations and regioselectivity

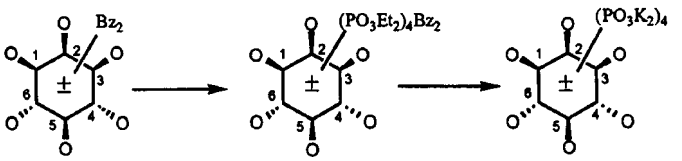
Sung-Kee Chung,* Young-Tae Chang and Youngha Ryu

Department of Chemistry, Pohang University of Science & Technology, Pohang 790-784, Korea

Abstract:
Syntheses of all possible 9 and 12 regioisomers of *myo*-inositol tetrakis- and tris-phosphates(IP_4 and IP_3), respectively were accomplished from *myo*-inositol via its di- and tri-benzoate derivatives. The requisite sets of *myo*-inositol benzoate intermediates were most conveniently produced by base-catalyzed migration of the benzoate group followed by suitable separation methods.
Kinetics of the acyl migration showed that generally *cis* migrations are faster than *trans* migrations but not to the extent to give a high selectivity. However, a reasonable degree of *cis/trans* selectivity could be observed in compounds with rigid conformations. The acyl migration rate increases with increasing solvent polarity indicating that the intermediate or transition state involved is more polar than the stating material.
1,2:4,5-Di-O-isopropylidene-*myo*-inositol shows a high degree of regioselectivity in reactions such as alkylation, acylation and silylation at 3-OH in preference to 6-OH. The origin of the regioselectivity was found to be the enhanced nucleophilicity of the 3-alkoxide due to its interaction with the *cis*-vicinal oxygen of the isopropylidene group possibly via "through-space α effect".

The phospholipase C catalyzed cleavage of membrane bound phosphatidylinositol bisphosphate (PIP_2) into *myo*-inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol is a crucially important means of cellular signalling processes. A large fraction of receptors identified to date are linked to the increase of PIP_2 turnover following the receptor activation (ref. 1). More recently, a number of *myo*-inositol phosphates including IP_4 and IP_5 have also been implicated as either second messengers or key metabolic intermediates in the intracellular signal transduction pathways (ref. 2 and 3).

Scheme 1



1

Dibenzoate

a. I(1,2)Bz_2 = I(2,3)Bz_2
b. I(1,3)Bz_2
c. I(1,4)Bz_2 = I(3,6)Bz_2
d. I(1,5)Bz_2 = I(3,5)Bz_2
e. I(1,6)Bz_2 = I(3,4)Bz_2
f. I(2,4)Bz_2 = I(2,6)Bz_2
g. I(2,5)Bz_2
h. I(4,5)Bz_2 = I(5,6)Bz_2
i. I(4,6)Bz_2

⟹

Inositol-tetrakisphosphate

a. I(3,4,5,6)P_4 = I(1,4,5,6)P_4
b. I(2,4,5,6)P_4
c. I(2,3,5,6)P_4 = I(1,2,4,5)P_4
d. I(2,3,4,6)P_4 = I(1,2,4,6)P_4
e. I(2,3,4,5)P_4 = I(1,2,5,6)P_4
f. I(1,3,5,6)P_4 = I(1,3,4,5)P_4
g. I(1,3,4,6)P_4
h. I(1,2,3,6)P_4 = I(1,2,3,4)P_4
i. I(1,2,3,5)P_4

As a part of our efforts to understand the molecular recognition aspect of the *myo*-inositol phosphate dependent signal transduction, we set out to synthesize all possible regioisomers of IP_n. There are 39 racemic or meso regioisomers (enantiomerically 63) available for IP_n: IP_1, 4(6); IP_2, 9(15); IP_3, 12(20); IP_4, 9(15); IP_5, 4(6); IP_6, 1. One of the key problems in the synthesis of inositol phosphates is to prepare suitable, selectively protected inositol intermediates. We have envisioned that the synthetic challenge may be reduced to the facile generation of all possible regioisomers of *myo*-inositol benzoate(IBz_n) intermediates, which are expected to be readily phosphorylated to provide the target structures(IP_n) (Scheme 1). Indeed, the requisite sets of *myo*-inositol benzoate intermediates have been conveniently prepared by base-catalyzed migrations of the benzoate group followed by suitable separation methods, e.g. HPLC, column chromatography, and/or crystallization(ref. 4 and 5).

The acyl migrations among vicinally related diol systems are well precedented in the literature. For example, the initial observation that the acyl groups in mono- and di-acylglycerols migrate from the oxygen to which they are attached to an adjacent free hydroxyl group by a transesterification process catalyzed by acid, base or heat goes back to E. Fischer. In fact, the facile acyl group migration presents a persistant major difficulty in the synthesis of pure acylglycerols (ref. 6). The acyl group migrations in the glucopyranoside and glucofuranoside systems have been frequently observed under the conditions employing silver oxide or a mild base (ref. 7). In the case of aminoacyl-tRNA, the critical component in the biosynthesis of proteins, the aminoacylation occurs on the 2' or 3'-OH group of the adenosine at the 3' end of tRNA, and the ester formed initially equilibrates very rapidly ($t_{1/2}$ of ca. 0.2 ms) between the two vicinal hydroxyl groups (ref. 8). The acetyl group migrations in partially acetylated derivatives of *myo*-inositols are also known, and some *cis/trans*-selectivity has been reported in the methylation of 1,4,5,6-tetra-O-acetyl-*myo*-inositol by employing reaction conditions of neutral silver oxide and a limited amount of methyl iodide in a solvent of low polarity at a low temperature (ref. 9).

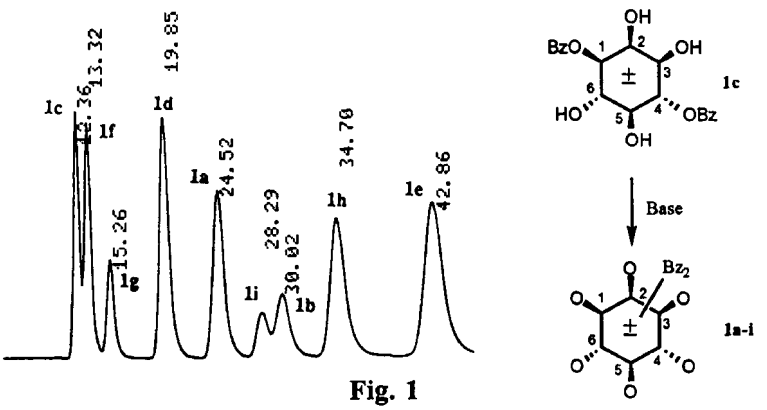


Fig. 1

We investigated the acyl migration in partially benzoylated *myo*-inositols as a quick way of generating all of the requisite regioisomers of IBz_2 and IBz_3. We chose the readily available **1 c**, and its mono acetal derivatives **2**, **3** as the starting materals for our study. The 9 regioisomers of IBz_2(**1 a**-**i**) generated from **1 c** by treatment with aq. pyridine could be nicely analyzed by HPLC. The increasing order of the HPLC retention time of these isomers has been found to be I-1,4(**1 c**), 2,4(**1 f**), 2,5(**1 g**), 1,5(**1 d**), 1,2(**1 a**), 4,6(**1 i**),1,3(**1 b**), 4,5(**1 h**) and 1,6(**1 e**)Bz_2 (Fig.1). When partially protected derivatives of I(1,4) Bz_2, **2** and **3** were subjected to the 60 % aq. pyridine conditions and then 80 % aqueous acetic acid at reflux, two sets of 5 isomers of IBz_2 were obtained from the limited benzoyl group migrations (Fig. 2).

From the kinetic profiles of **1 c**, **2**, **3** at various temperatures, it is quite apparent that the *cis*-1,2-benzoyl migration is generally faster than the *trans* migration as expected, and that the product distributions are largely due to initial one migrations and then slowly followed up by two migrations to reach the equilibrium. Studies at variable temperature showed substantial temperature effects on the overall migration rate to the equilibrium, but not much on the *cis/trans* selectivity. With compound **2**, no discernible selectivity was observed under all conditions examined, suggesting that the energy barriers in the available *trans* migrations in **2** are similar.

In order to understand the nature of the migratory behavior we have also subjected compounds **1 c** and **3** to various other reaction conditions and the results are shown in Table 1. Several trends are clearly discernible. From the data in entries 1-3, the *cis*-migration rate in **3** increases with the increasing solvent polarity (ref. 10). The entries 4-6 show that an increasing amount of water facilitates the migration, but the

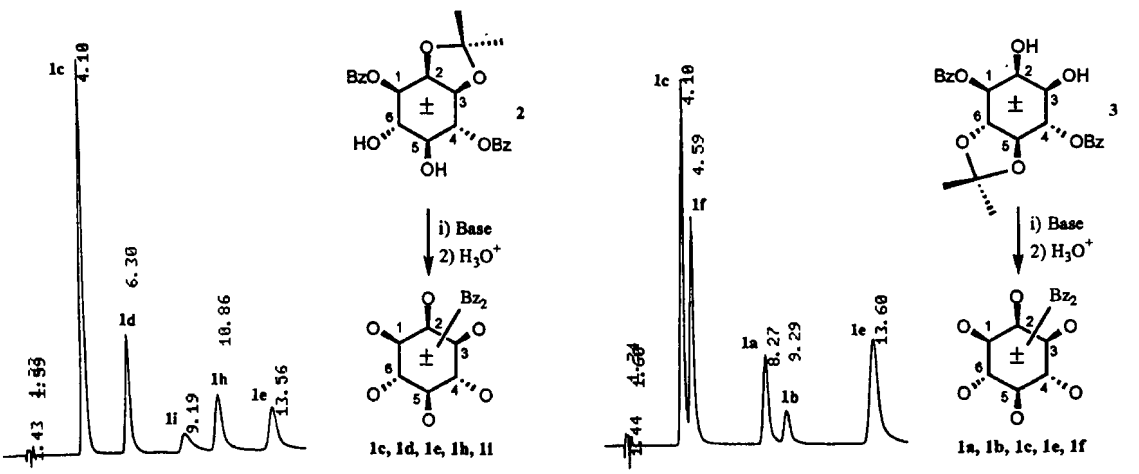


Fig. 2

initially observed *cis/trans* selectivity slowly disappears with the increasing reaction time under these conditions. The product distribution in entry 6 was found to represent essentially the equilibrium mixture. The same solvent polarity effect on the migration rate and the loss of the *cis/trans* selectivity could be seen with sodium carbonate base at room temperature (entries 7-9). The benzoyl migration in **1c** showed similar trends (entries 10-13). With sodium carbonate base in DMF, the migration in **1c** took place more slowly than in **3**(entries 8-9 vs. 11-12). The *cis/trans* selectivity of the migration in **1c** in aq. pyridine could not be improved by lowering the reaction temperature (entry 10).

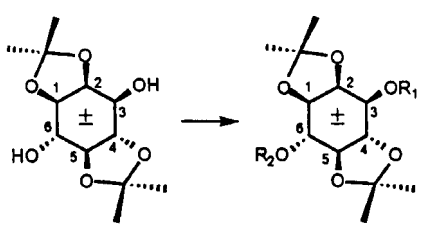
These results might be explained on the basis of the following. 1) The *cis*-migrations generally involve lower activation energies than the competing *trans*-migrations because of the apparent geometric advantages (ref. 12). 2) The benzoyl migration presumably involves a tetrahedral intermediate or transition state, which is more polar than the starting material and thus better stabilized by more polar solvent environment, and procceds at a faster rate in polar solvents such as aq. pyridine and DMF. The effect of water on the reaction rate appears to be primarily due to its polarity rather than its protic nature. 3) The reasonably high *cis/trans* selectivity observed in **3** as opposed to **1c** is most likely due to its rigid conformation which is more conducive to the *cis*-migration. Therefore, in order to obtain a good *cis/trans* selectivity in compounds such as **1c** with flexible conformations, one needs to find conditions which allow reasonably fast migrations while conformational rigidity is maintained (ref. 13).

Table 1

Run	Substrate	Conditions	Product Distribution								
			1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**
1	**3**	pyridine-toluene(1:1) 100 °C, 1h	-	-	89	-	-	11	-	-	-
2	**3**	pyridine 100 °C, 1h	-	-	80	-	-	20	-	-	-
3	**3**	pyridine-DMF(1:1) 100 °C, 1h	-	-	74	-	-	26	-	-	-
4	**3**	pyridine-water(9:1) 100 °C, 1h	-	2	64	-	7	27	-	-	-
5	**3**	pyridine-water(8:2) 80 °C, 1h	1	-	48	-	15	36	-	-	-
6	**3**	pyridine-water(8:2) 100 °C, 10 min	36	12	24	-	17	11	-	-	-
7	**3**	THF(Na_2CO_3) R.T., 1day	-	-	74	-	9	17	-	-	-
8	**3**	DMF(Na_2CO_3) R.T., 2 h	-	-	29	-	24	47	-	-	-
9	**3**	DMF(Na_2CO_3) R.T., 4 h	6	2	27	-	20	45	-	-	-
10	**1c**	pyridine-water(9:1) 60 °C, 1h	12	4	33	12	8	16	7	3	5
11	**1c**	DMF(Na_2CO_3) R.T., 2 h	-	-	92	-	-	8	-	-	-
12	**1c**	DMF(Na_2CO_3) R.T., 40 h	2	4	48	5	6	26	3	-	6

In the course of our synthetic studies of *myo*-inositol phosphates, we have had an occasion to examine the regioselectivity problems in the isopropylidene protected *myo*-inositols (ref. 14). For example, 1,2:4,5-Di-O-isopropylidene-*myo*-inositol, **4** could be selectively functionalized in reactions including alkylation, acylation and silylation at 3-OH in preference to 6-OH (Scheme 2 and Table 2).

Scheme 2



4

5a. R_1= Bn, R_2= H
b. R_1= H, R_2= Bn
c. R_1, R_2= Bn

6a. R_1= pMeOBn, R_2= H
b. R_1= H, R_2= pMeOBn
c. R_1, R_2= pMeOBn

7a. R_1= $(CH_3)_3CC(O)$, R_2= H
b. R_1= H, R_2= $(CH_3)_3CC(O)$
c. R_1, R_2= $(CH_3)_3CC(O)$

8a. R_1= CF_3SO_2, R_2= H
b. R_1= H, R_2= CF_3SO_2
c. R_1, R_2= CF_3SO_2

9a. R_1= $tBuMe_2Si$, R_2= H
b. R_1= H, R_2= $tBuMe_2Si$
c. R_1, R_2= $tBuMe_2Si$

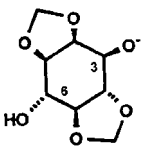
10a. R_1= $(PhO)_2P(O)$, R_2= H
b. R_1= H, R_2= $(PhO)_2P(O)$
c. R_1, R_2= $(PhO)_2P(O)$

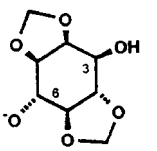
Table 2. Regioselective functionalizations of diol **4**

entry	reaction conditions	products (isolated yield, %)[a]			starting material recovered, %
1	BnBr(1.2eq), NaH(1.2eq) DMF, RT, 11hrs	5a(46)[b]	5b(0)	5c(15)	ND
2	BnBr(1.2eq), NaH(1.2eq) toluene, reflux, 12hrs	5a(48.1)	5b(11.5)	5c(19.0)	8.5
3	p-(CH_3O)BnCl(1.0eq), NaH(5.0eq), DMF, RT, 5hrs	6a(20)	6b(0)	6c(24)	ND
4	p-(CH_3O)BnCl(1.2eq), NaH(1.2eq), DMF, RT, 3hrs	6a(30.5)	6b(0)	6c(29.2)	ND
5	$(CH_3)_3CC(O)Cl$(1.8eq), pyridine, RT, 28hrs	7a(42.3)	7b(9.1)	7c(15.0)	7.9
6	$(CF_3SO_2)_2O$(1.15eq), pyridine(2.0eq), CH_2Cl_2 -20°C, 3hrs	8a(52.0)	8b(0)	8c(24.9)	0
7	$tBuMe_2SiCl$(1.1eq), imidazole(3.3eq), DMF, RT, 12hrs	9a(55.4)	9b(0)	9c(6.8)	25.3
8	$(PhO)_2P(O)Cl$(1.2eq), TEA(3.0eq), cat.DMAP, CH_2Cl_2, RT, 4hrs	10a(30)	10b(trace)	10c(8)	ND

a: isolated by column chromatography unless indicated otherwise
b: isolated by recrystallization
ND: not determined

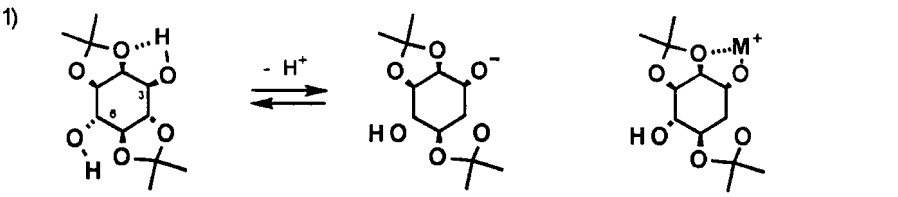
Table 3. The *ab initio* results of **Ions 3** and **6**.





Species	Relative Energy	HOMO Energy	Polarizability
ion 3	377.45 kcal/mol	-0.1532 eV	102.99
ion 6	376.36 Kcal/mol	-0.1541 eV	102.29

An explanation for the reactivity difference based on steric hindrance appears unlikely since the more reactive 3-OH group is sterically more hindered than the 6-OH group. We have envisaged the possible origins of the regioselectivity in terms of kinetic acidity, intrinsic thermodynamic acidity, relative stabilization of chelated species, and enhanced nucleophilicity (Scheme 3). First, a stronger hydrogen bonding involving with the 6-OH group over the 3-OH may impart a higher kinetic acidity to the 3-OH group. Second, intrinsically higher thermodynamic acidity of the 3-OH group can account for the observed regioselectivity. Alternatively, the selective chelation stabilization of sodium alkoxide at C-3 by the vicinal, *cis* ether oxygen may provided a possible explanation for the observed regioselectivity. In fact, such a cation chelation effect had been invoked to explain a hydroxyl regioselectivity in the alkylation of the mono-orthoformate of *myo*-inositol (ref. 15). The last possibilitiy is that the through-space interaction between the alkoxide anion at C-3 and the vicinal, *cis* ether oxygen may enhance the nucleophilicity of the alkoxide (ref. 16).



Scheme 3. Possible Origins of the Regioselectivity Observed

The IR spectrum of **4** in a highly diluted(<0.01M) CH_2Cl_2 solution revealed the presence of the intramolecularly hydrogen bonded hydroxyl group(3585 cm^{-1}) and free hydroxy group(3683 cm^{-1}). ^{1}H-NMR spectra of **4** at 300MHz in deuteriochloroform displayed two hydroxyl peaks each at δ 2.36(d, J=8.8, C3-OH) and 2.45(d, J=2.9, C6-OH). The *ab initio* generated geometry of **4** has indicated that the dihedral angles between the hydroxyl proton and the ring methine proton at C-3 and C-6 are 167.3° and 59.7°, respectively, and these angles are in good agreement with the observed vicinal coupling constants.

Furthermore, these dihedral angles suggest that these hydroxyl protons are hydrogen bonded to nearby *cis* and *trans* vicinal ether oxygens of the isopropylidene groups, respectively.

The geometry obtained from the single crystal X-ray diffraction of **4** showed a chair conformation. Although the hydrogen positions of **4** could be determined from the diffraction(not calculation) data, no direct evidence for the intramolecular hydrogen bond could be found in the crystal structure, for intermolecular H-bonding was predominant in its unit cell. However, the interatomic distance between the 3-oxygen and its nearest oxygen in the *cis*-isopropylidene moiety was found as 2.7456 A, whereas the distance between the 6-oxygen and its nearest oxygen in the *trans*-isopropylidene group as 2.9382 A, suggesting that the former case could provide a better intramolecular hydrogen bonding distance(ref. 17).

In order to deduce conformations in the solution phase, vicinal coupling constants were determined from the ^{1}H-NMR of **4** in both $CDCl_3$ and DMSO-d_6. Since observed coupling constants are virtually identical in both solvent systems, it might be assumed that conformation (or conformational equilibrium) of **4** stays the same. Comparisons of the coupling constants derived from both the crystal and MM-2 generated structures by a generalized Karplus equation show that all of them are in good agreement with those from ^{1}H-NMR. It is likely that the solution conformation of **4** is similar to the conformation in the crystalline phase. These findings together argue for a lower rather than higher kinetic acidity for the 3-OH group than the 6-OH group, because the 3-OH is held more tightly by the hydrogen bonding with the oxygen atom in the *cis*-isopropylidene group.

The other possibilities have been evaluated by *ab initio* calculations for the reactions employing strong bases such as sodium hydride. The *ab initio* calculations have been performed on **ion 3** and **ion 6** by using various basis sets including 6-31+G* with the Hartree-Fock and MP2 methods. The calculated relative energies, HOMO energy levels and the average polarizability are summarized in Table 3 (ref. 18). The relative energies by Hartree-Fock method (shown in the table 3) indicate that the C-6 alkoxide species is more stable than the C-3 alkoxide by ca. 1.09 kcal/mol, i.e. the intrinsic thermodynamic acidity of the 6-OH is higher than the 3-OH. An identical energy difference was observed with MP2 method. The nucleophilicity of a species is often correlated with the HOMO energy level and polarizability. The corresponding values shown in Table 3 unequivocally indicate that the C-3 alkoxide is more nucleophilic than the C-6 alkoxide. The *ab initio* calculations of the model reactions between **Ions 3** and **6** with NaH and MeCl using the basis set of STO-3G also showed that the transition state energy with **Ion 3** is lower than that with **ion 6** by ca. 2 kcal/mol in accord with the nucleophilicity arguments. Therefore, we suggest that the origin of the regioselectivity is due to the enhanced nucleophilicity of the 3-alkoxide, and that the nucleophilicity enhancement is most likely due to its electronic interaction with the *cis*-vicinal oxygen of the isopropylidene group via "through-space α effect".

Acknowledgments

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10. Solvent polarity is defined as polarity index, P', which is a measure of the ability of the solvent to interact with various polar test solutes. P' for Pyridine = 5.3, Pyridine-toluene(1:1) = 3.9, Pyridine-DMF(1:1) = 5.9, Pyridine-water(9:1) = 5.8, Pyridine-water(8:2) = 6.3, THF = 4.0, DMF = 6.4 (see ref. 11).
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