Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2981–2984

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Three Enantiomeric Pairs of *scyllo*-Inositol Phosphate and Molecular Interactions Between All Possible Regioisomers of *scyllo*-Inositol Phosphate and Inositol 1,4,5-Trisphosphate 3-Kinase

Yong-Uk Kwon,[a] Jungkyun Im,[a] Gildon Choi,[b] Young-Soo Kim,[a] Kwan Yong Choi[b] and Sung-Kee Chung[a,*]

[a]*Department of Chemistry, Division of Molecular and Life Sciences, Pohang University of Science & Technology, Pohang 790-784, South Korea*

[b]*National Research Laboratory of Protein Folding and Engineering, Division of Molecular and Life Sciences, Pohang University of Science & Technology, Pohang 790-784, South Korea*

Received 6 February 2003; accepted 21 March 2003

Abstract—*scyllo*-Inositol phosphates, which are among the stereoisomers of *myo*-inositol phosphate, can have 15 possible regioisomers including three enantiomeric pairs: *scyllo*-I(1,2)P_2, *scyllo*-I(1,2,4)P_3, *scyllo*-I(1,2,3,4)P_4. We herein describe the facile synthetic routes to the three enantiomeric pairs of *scyllo*-inositol phosphate and the molecular interactions between 15 regioisomers of *scyllo*-inositol phosphate and inositol 1,4,5-trisphosphate 3-kinase. Geometry of the enzyme binding site is discussed.

D-*myo*-Inositol 1,4,5-trisphosphate [I(1,4,5)P_3], which is generated from the phospholipase C catalyzed cleavage of membrane bound phosphatidylinositol bisphosphate (PIP_2), plays a pivotal role as a second messenger by inducing Ca^{2+} release from the intracellular storage.[1] In addition, nuclear inositol phosphates were also reported to control mRNA export and transcription, thus suggesting that activation of IP_n signaling might regulate gene expression as well.[2] I(1,4,5)P_3 is metabolized by two major pathways which yield I(1,3,4,5)P_4 by I(1,4,5)P_3 3-kinase [IP3K] and I(1,4)P_2 by I(1,4,5)P_3 5-phosphatase. I(1,3,4,5)P_4 has recently attracted a lot of attention because of its roles in relation with I(1,4,5)P_3 as well as in its own right as second messenger and regulator.[3] IP3K, a member of inositol polyphosphate kinase family, shows a remarkable stereo- and regioselectivity toward the recognition of I(1,4,5)P_3; in some cases the binding selectivity appears to be higher than that of either I(1,4,5)P_3 receptor or I(1,4,5)P_3 5-phosphatase.[1b,4] At the level of cDNAs, several isoforms (A, B, and C) of IP3K have been cloned: A-C from human, A and B from rat, and A from chicken.[5] The catalytic domain of the enzyme has been mapped to be in the C-terminus with the last 275 amino acid residues being indispensable for the enzyme activity. It has been proposed that in rat isoform A Lys-197 and Asp-414 are involved in the ATP binding, whereas Lys-262 is important for the I(1,4,5)P_3 binding.[6]

For the molecular design of selective and potent inhibitors of the enzyme as opposed to random screening, it would be highly desirable to have some detailed geometry information on the binding domain of the enzyme.[7] We previously proposed a binding site model for IP3K on the basis of the correlations between the distinct stereochemical disposition of all 38 regioisomers of *myo*-inositol phosphates [IP_n, where $n = 1$–6] and the enzyme.[8] As an extension of this investigation, we now have carried out the syntheses of all possible optically active regioisomers of *scyllo*-inositol phosphates from enzymatically resolved conduritol B derivatives, and utilized them together with the previously synthesized achiral *scyllo*-inositol phosphates as molecular probes in order to obtain a refined picture of the binding model of IP3K.

Although *scyllo*-inositol and *scyllo*-inositol phosphates have been found in nature, the understanding of their

*Corresponding author. Tel.: +82-54-279-2103; fax: +82-54-279-3399; e-mail: skchung@postech.ac.kr

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00629-2

biological activities leaves a lot to be desired.[9] *scyllo*-Inositol phosphates, which are among the stereoisomers of *myo*-inositol phosphate, can have 15 possible regioisomers, including three enantiomeric pairs: *scyllo*-I(1,2)P_2, *scyllo*-I(1,2,4)P_3, *scyllo*-I(1,2,3,4)P_4 (Fig. 1). We previously synthesized for the first time all possible 12 regioisomers of *scyllo*-inositol phosphate in meso or racemic forms.[10] To our knowledge, only L-*scyllo*-I(1,2,4)P_3 has been synthesized in optically pure form.[11]

Our synthetic approaches to homochiral regioisomers of *scyllo*-IP_n are based on the lipase-catalyzed resolution of racemic conduritol B diacetate which we previously utilized in the syntheses of optically active stereoisomers of conduritol and inositol.[12] Enantiomerically enriched *scyllo*-inositol derivative [(−)-**4**] was obtained by the Mitsunobu reaction of *myo*-inositol diol [(−)-**3**], which was derived from enzymatically enriched conduritol B diol [(+)-**1**] (Scheme 1). Compound (−)-**4** might be considered as a suitably protected intermediate for the synthesis of chiral *scyllo*-IP_n. The vicinal diol [(−)-**5**] was easily prepared by debenzoylation of compound (−)-**4** with a catalytic amount of NaOMe in MeOH at reflux (Scheme 2). Acid-catalyzed hydrolysis of compound (−)-**4** afforded the triol (−)-**6**, which could be converted to L-*scyllo*-I(1,2,4)P_3. Benzoylation of compound (−)-**4** under conventional conditions, followed by hydrogenolysis in the presence of a small amount of AcOH provided the vicinal dibenzoate (+)-**8**.

The precursors (−)-**5**, (−)-**6** and (+)-**8** were phosphorylated by successive treatments with the phosphoramidite reagent and 1*H*-tetrazole, and then *m*CPBA to give compounds (−)-**9**, (−)-**10** and (+)-**11** all in good yields. In the final steps, all protecting groups of compound (−)-**9** were removed by hydrogenolysis in the presence of a small amount of AcOH, thus giving the Na salt of D-*scyllo*-I(1,2)P_2 (**IID**) after pH adjustment with NaOH. Hydrogenolysis of compounds (−)-**10** and (+)-**11**, followed by treatment of LiOH removed all protecting groups. The target compounds, the optically

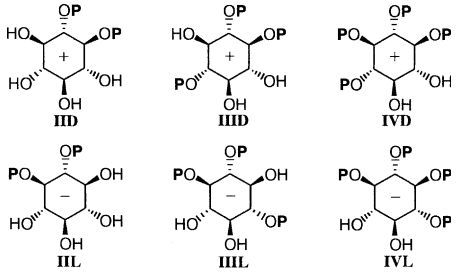
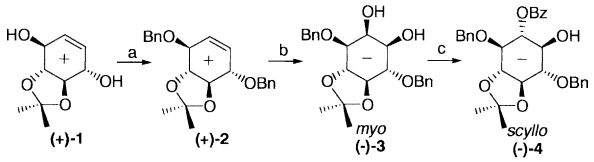
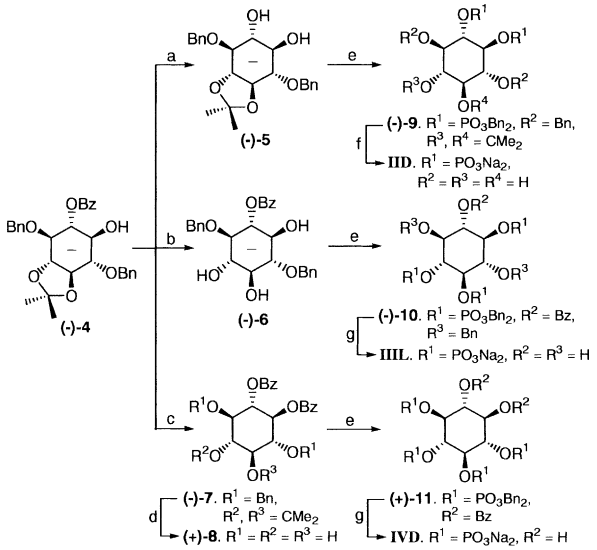


Figure 1. Three enantiomeric pairs of *scyllo*-inositol phosphate.



Scheme 1. Reagents and conditions: (a) BnBr, NaH, DMF, 96%; (b) OsO_4, NMO, aq acetone, 94.4%; (c) BzOH, Ph_3P, DEAD, toluene, 80 °C, 79%.



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, reflux, 96%; (b) 80% aq AcOH, 100 °C, quant; (c) BzCl, pyridine, 99%; (d) H_2 (50 psi), $Pd(OH)_2$/C, AcOH, EtOAc/MeOH, 96%; (e) (i) $iPr_2NP(OBn)_2$, 1*H*-tetrazole, $CHCl_3$; (ii) *m*CPBA; 75% for (−)-**9**, 96% for (−)-**10**, 77% for (+)-**11**; (f) (i) H_2 (50 psi), Pd/C, AcOH, EtOH/MeOH; (ii) pH 10 (NaOH), 94%; (g) (i) H_2 (50 psi), Pd/C, EtOH/MeOH; (ii) 1 N LiOH; (iii) Dowex 50WX8-100 (H^+); (iv) pH 10 (NaOH), 91% for **IIIL**, 89% for **IVD**.

active *scyllo*-I(1,2,4)P_3 (**IIIL**) and *scyllo*-I(1,2,3,4)P_4 (**IVD**) were obtained after chromatography on Dowex 50WX8-100 (H^+), pH adjustment to 10 with NaOH, and then lyophilization. Identical series of reactions were also carried out with (+)-**4** to afford compounds **IIL**, **IIID** and **IVL**. All products were fully characterized with NMR spectroscopy, and their optical rotations are listed in Table 1.

Table 1. Optical rotations of three enantiomeric pairs of *scyllo*-IP_n

scyllo-IP_n [P = P(O)(ONa)$_2$]	$[\alpha]_D^{25}$ (in H_2O)	
	D-form	L-form
scyllo-I(1,2)P_2	+24.8 (*c* 0.34, pH 9.5)	−20.2 (*c* 0.45, pH 9.2)
scyllo-I(1,2,4)P_3	+25.9 (*c* 0.91, pH 9.5)	−20.6 (*c* 0.57, pH 9.2)[a]
scyllo-I(1,2,3,4)P_4	+16.9 (*c* 2.23, pH 9.2)	−15.1 (*c* 1.79, pH 9.2)

[a]Lit.[11] $[\alpha]_D^{21}$ −24.1 (*c* 0.4, $CHCl_3$).

Bioassays with IP3K

IP3K that catalyzes the ATP-dependent phosphorylation of I(1,4,5)P_3 to generate I(1,3,4,5)P_4, is one of the key metabolic enzymes in the phosphoinositide signal transduction system. Though we could get much useful information on the binding mode of IP3K from our previous results using *myo*-IP_ns,[8] all 15 synthetic *scyllo*-IP_n regioisomers were tested for their binding affinity toward IP3K to obtain more detailed information. We have constructed recombinant plasmids for the rat brain IP3K, overexpressed them in *Escherichia coli*, and purified the enzyme to near homogeneity.[13] Assays were performed with 1 μM [^{3}H]-D-*myo*-I(1,4,5)P_3 by increasing the concentration of each *scyllo*-IP_n regioisomer in the absence of Ca^{2+}.[8] The IC_{50} values of 15 *scyllo*-IP_n

regioisomers have been determined by inhibition assays at various concentrations and are listed in Table 2.

Table 2. Inhibition of IP3K by 15 *scyllo*-IP_n regioisomers

scyllo-Inositol phosphates	IC_{50} (μM)
D-*myo*-I(1,4,5)P_3	2.6±0.3
scyllo-IP_1	N.D.[a]
4 *scyllo*-IP_2s	N.D.[a]
D-*scyllo*-I(1,2,4)P_3	43.7±7.2
L-*scyllo*-I(1,2,4)P_3	6.3±0.7
scyllo-I(1,2,3)P_3	27.4±2.0
scyllo-I(1,3,5)P_3	90±16
D-*scyllo*-I(1,2,3,4)P_4	>100
L-*scyllo*-I(1,2,3,4)P_4	>100
scyllo-I(1,2,3,5)P_4	27.8±0.1
scyllo-I(1,2,4,5)P_4	39.4±2.1
scyllo-IP_5	N.D.[a]
scyllo-IP_6	N.D.[a]

[a]IC_{50} values were not determined for the compounds that showed very weak or negligible inhibition effect even at 200 μM.

All regioisomers of *scyllo*-inositol monophosphate, *scyllo*-inositol bisphosphate, *scyllo*-inositol pentakisphosphate, and *scyllo*-inositol hexakisphosphate were found to be very weak or negligible in inhibiting the IP3K activity even at 200 μM. DL-*scyllo*-I(1,2,4)P_3 was previously reported to be a good substrate for IP3K.[11,14] As expected, L-*scyllo*-I(1,2,4)P_3 which may be visualized as having the geometry of D-*myo*-I(1,4,5)P_3 with inverted 2-OH, showed comparable inhibitory activity to that of D-*myo*-I(1,4,5)P_3, the natural substrate of IP3K. D-*scyllo*-I(1,2,4)P_3 was also found to be a good inhibitor although it was about 7 times less potent than L-*scyllo*-I(1,2,4)P_3. However, it is surprising to find that *scyllo*-I(1,2,3)P_3 shows a potent inhibitory activity against radiolabeled D-*myo*-I(1,4,5)P_3 even though it does not have the usual structural requirement of the three phosphate groups, that is the D-1,4,5-trisphosphate motif, and also in view of the prior observations that its positional analogues, DL-*myo*-I(3,4,5)P_3 and *myo*-I(4,5,6)P_3 did not have any meaningful inhibitory activities.[8] *scyllo*-I(1,2,3,5)P_4 and *scyllo*-I(1,2,4,5)P_4 also significantly inhibited the phosphorylation of D-*myo*-[^{3}H]-I(1,4,5)P_3 by IP3K. But *scyllo*-I(1,3,5)P_3, D-*scyllo*-I(1,2,3,4)P_4 and L-*scyllo*-I(1,2,3,4)P_4 displayed less potent inhibitory activities than the other regioisomers of *scyllo*-IP_3 and *scyllo*-IP_4.

These observations imply that all three phosphate groups of D-*myo*-I(1,4,5)P_3 play important roles for the efficient binding to IP3K, but the 1-phosphate group appears less critical than the other two. It is apparent that IP3K has a vacant space in the C-2 axial direction of D-*myo*-I(1,4,5)P_3 and negative charges near the vacant space surrounding the C-2 position.[8,15] In addition, inhibitory activities of L-*scyllo*-I(1,2,4)P_3 and *scyllo*-I(1,2,4,5)P_4 indicate that not only IP3K does have a vacant space in the C-2 equatorial direction but also the negative charges in the C-2 equatorial direction are well-tolerated. D-*myo*-I(1,4,5)P_3 derivatives with a bulky substituent such as 3-benzoyl or 3-methylbenzoyl group are known to be ineffective in inhibiting the IP3K activity, implying that IP3K does not tolerate a bulky

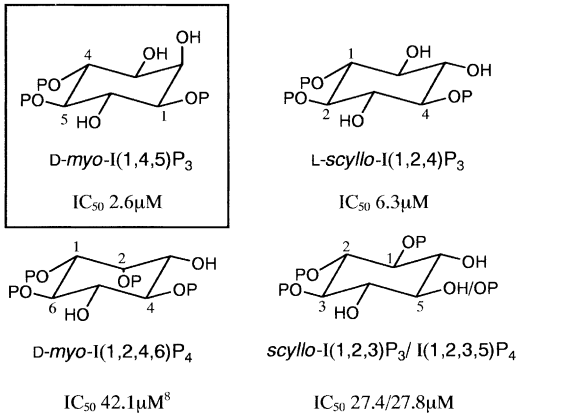


Figure 2. Geometry comparison between D-*myo*-I(1,4,5)P_3 and compounds with inverted C-2 configuration.

substituent at that position.[14] It is intriguing that the equatorially oriented, negatively-charged phosphate group at C-1 of *scyllo*-I(1,2,3)P_3 and *scyllo*-I(1,2,3,5)P_4 is well tolerated, even though D-*myo*-I(1,3,4,5)P_4, the product of IP3K, is known to be a very weak inhibitor presumably due to the repulsive collision between the C-3 phosphate and the negative charge and/or steric tightness on the enzyme.[1b,8] This observation is consistent with the prior observation that D-*myo*-I(1,2,4,6)P_4 showed a good inhibitory activity, and these results suggest that the inverted 2-OH causes somewhat twisted or tilted binding of the *scyllo*-IP_3 and-IP_4 to IP3K, and the C-1 phosphate group is now directed away from the usual orientation of C-3 phosphate of D-*myo*-I(1,3,4,5)P_4. This in turn suggests that IP3K has a pocket in the C-3 axial direction of D-*myo*-I(1,4,5)P_3 (Fig. 2). It has been previously speculated that the empty space in the C-3 axial direction is to be occupied by ATP.[8] *scyllo*-I(1,2,3,4)P_4 with the bulky phosphate group at the C-6 position of D-*myo*-I(1,4,5)P_3 showed a very weak inhibitory activity, strongly indicating that the enzyme binding pocket does not have as much leeway in the direction of the equatorial 6-OH of D-*myo*-I(1,4,5)P_3 as compared to 2-OH and 3-OH.

In sum, the binding interaction data between the 15 regioisomers of *scyllo*-IP_n and IP3K are in good accord with the binding site model previously proposed, and we are now proceeding to design effective inhibitors of IP3K as well as attempting to crystallize the enzyme.

Acknowledgements

This work was financially supported by the Ministry of Education/Basic Science Research Institute fund, POSTECH/POSCO, and the KISTEP/NRL program.

References and Notes

1. (a) Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933 and references therein. (b) Berridge, M. J. *Nature* **1993**, *361*, 315.
2. (a) Chi, T. H.; Crabtree, G. R. *Science* **2000**, *287*, 1937.

(b) Odom, A. R.; Stahlberg, A.; Wente, S. R.; York, J. D. *Science* **2000**, *287*, 2026.
3. (a) Irvine, R. *Cur. Biol.* **2001**, *11*, R172. (b) Schell, M. J.; Erneux, C.; Irvine, R. F. *J. Biol. Chem.* **2001**, *276*, 37537. (c) Shen, X.; Xiao, H.; Ranllo, R.; Wu, W. H.; Wu, C. *Science* **2003**, *299*, 112. (d) Steger, D. J.; Haswell, E. S.; Miller, A.; Wente, S. R.; O'Shea, E. K. *Science* **2003**, *299*, 114.
4. Narhorski, S. R.; Potter, B. V. L. *Trends Pharmacol. Sci.* **1989**, *10*, 139.
5. (a) Bertsch, U.; Deschermeier, C.; Fanick, W.; Girkontaite, I.; Hillemeier, K.; Johnen, H.; Weglohner, W.; Emmrich, F.; Mayr, G. W. *J. Biol. Chem.* **2000**, *275*, 1557. (b) Dewaste, V.; Roymans, D.; Moreau, C.; Erneux, C. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 400.
6. (a) Communi, D.; Takazawa, K.; Erneux, C. *Biochem. J.* **1993**, *291*, 811. (b) Togashi, S.; Takazawa, K.; Endo, T.; Erneux, C.; Onaya, T. *Biochem. J.* **1997**, *326*, 221.
7. (a) Chang, Y. T.; Choi, G.; Bae, Y. S.; Burdett, M.; Moon, H. S.; Lee, J. W.; Gray, N. S.; Schultz, P. G.; Meijer, L.; Chung, S. K.; Choi, K. Y.; Suh, P. G.; Ryu, S. H. *ChemBioChem.* **2002**, *3*, 897. (b) Ahn, Y. H.; Chung, S. K. *Bull. Kor. Chem. Soc.* **2002**, *23*, 515.
8. (a) Choi, G.; Chang, Y. T.; Chung, S. K.; Choi, K. Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2709. (b) Choi, G.; Chang, Y. T.; Chung, S. K.; Choi, K. Y. *Kor. J. Med. Chem.* **1997**, *7*, 106.
9. (a) Seaquist, E. R.; Gruetter, R. *Magn. Reson. Med.* **1998**, *39*, 313. (b) Narasimhan, B.; PliskaMatyshak, G.; Kinnard, R.; Carstensen, S.; Ritter, M. A.; von Weymarn, L.; Murthy, P. P. N. *Plant Physiol.* **1997**, *113*, 1385. (c) Lien, Y. H. H.; Michaelis, T.; Moats, R. A.; Ross, B. D. *Life Sci.* **1994**, *54*, 1507.
10. Chung, S. K.; Kwon, Y. U.; Chang, Y. T.; Sohn, K. H.; Shin, J. H.; Park, K. H.; Hong, B. J.; Chung, I. H. *Bioorg. Med. Chem.* **1999**, *7*, 2577.
11. Lampe, D.; Liu, C. S.; Mahon, M. F.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1717.
12. (a) Kwon, Y. U.; Lee, C.; Chung, S. K. *J. Org. Chem.* **2002**, *67*, 3327. (b) Kwon, Y. U.; Chung, S. K. *Org. Lett.* **2001**, *3*, 3013.
13. Choi, K. Y.; Kim, H. Y.; Lee, S. Y.; Moon, K. H.; Sim, S. S.; Kim, J. W.; Chung, H. K.; Rhee, S. G. *Science* **1990**, *248*, 64.
14. Wilcox, R. A.; Safrany, S. T.; Lampe, D.; Mills, S. J.; Nahorski, S. R.; Potter, B. V. L. *Eur. J. Biochem.* **1994**, *223*, 115.
15. Hirata, M.; Watanabe, Y.; Kanematsu, T.; Ozaki, S.; Koga, T. *Biochem. Biophys. Acta* **1995**, *1244*, 404.