Pergamon

Tetrahedron Letters, Vol. 36, No. 48, pp. 8719-8722, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01893-X

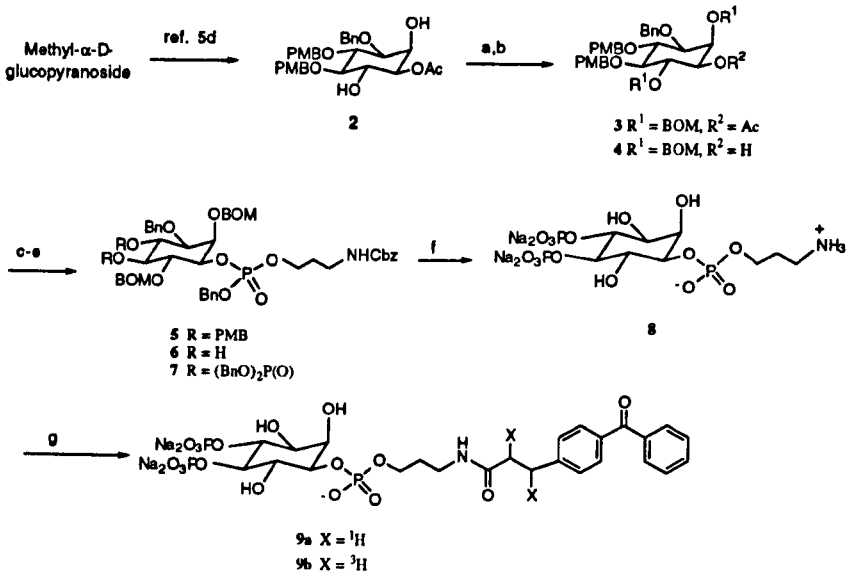
Synthesis of D-*myo*-P-1-(*O*-Aminopropyl)-Inositol-1,4,5-Trisphosphate Affinity Probes from α-D-Glucose

György Dormán, Jian Chen, and Glenn D. Prestwich*
Department of Chemistry
University at Stony Brook
Stony Brook, New York 11794-3400

Abstract: D-*myo*-P-1-(*O*-3-Aminopropyl)-Ins(1,4,5)P_3 has been synthesized from methyl α-D-glucopyranoside. This optically-pure tethered IP_3 derivative has been converted to a selective photoaffinity label for modification of the ligand binding site of IP_3 receptor proteins.

D-*myo*-Inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3 or IP_3) is a second messenger in a vast number of important signal transduction processes.[1] IP_3 interacts stereospecifically with membrane receptors to promote the release of Ca^{2+} from intracellular stores.[2] Numerous methods have been developed to synthesize D-*myo*-1,4,5-IP_3 and other inositol polyphosphates and their analogues in racemic or optically-active forms.[3-5] The 1-*O*-aminoalkyl-phospho-*myo*-inositol-4,5-bisphosphates, a family of aminoalkyl-tethered analogues of *myo*-inositol-1,4,5-trisphosphate, have been used to prepare IP_3-affinity probes for receptor purification and for photoaffinity labeling of the active sites of IP_3 receptors.[6] The 1-*O*-aminoethyl[7] and 1-*O*-aminopropyl[8] tethered IP_3 materials showed significantly reduced receptor affinity relative to IP_3 itself. To date, these IP_3 analogues have only been available in racemic form. Thus, to reduce nonselective binding and to improve the efficiency of active site modifications, affinity reagents based on optically-active D-*myo*-inositol-1,4,5-trisphosphate derivatives were required. We report herein the synthesis of enantiomerically-pure 1-tethered Ins(1,4,5)P_3 *via* the Ferrier rearrangement[9] of a suitably protected α-D-glucose derivative. Photoaffinity probes containing the 4-benzoyldihydrocinnamide group were prepared in both radioactive and radioinert forms.

The synthesis utilized a modified version of the Ferrier rearrangement route[4] initially developed for 1-tethered Ins(1,3,4,5)P_4,[5b] and subsequently applied to the preparation of P-5-tethered Ins(1,2,5,6)P_4[5c] and P-2-tethered Ins(1,2,4,5)P_4.[5d] The intermediate **2** was obtained in seven steps from methyl α-D-glucopyranoside **1**.[5d] Introduction of two benzyloxymethyl (BOM) groups onto the two remaining hydroxyl groups required judicious selection of conditions[5b] to avoid the migration of the acetyl group. This was achieved by stepwise etherification with BOM-Cl, Proton Sponge® and *n*-Bu_4NBr in CH_3CN (25 °C, 10 h; 35 °C, 10 h; and 55 °C, 10 h)[10] giving the fully protected inositol **3** in 61% yield. Basic methanolysis of the acetate **3** gave the C-1 alcohol **4** in 90% yield, which served as the pivotal intermediate to the enantiomerically-pure P-1-modified IP_3 analogue.



Scheme 1. Reagents and Conditions: (a) BOM-Cl, Bu_4NBr, H^+ sponge, CH_3CN, rt to 55 °C; (b) NaOH, MeOH, reflux, 2 h; (c) (1) (*i*-Pr_2N)(OBn)P($OCH_2CH_2CH_2NHCbz$), 1-*H* tetrazole, CH_2Cl_2, rt; (2) *m*CPBA, -45 °C to 0 °C, 30 min; (d) DDQ, wet, CH_2Cl_2, rt, 6 h; (e) (1) $(BnO)_2P(NPr_2\text{-}i)$, 1-*H* tetrazole, CH_2Cl_2, rt; (2) *m*CPBA, -45 °C to 0 °C, 2 h; (f) Pd-C, H_2, 95% EtOH, 5 atm, rt, 10 h; (g) BZDC-NHS ester, DMF-0.25 M TEAB buffer, rt, overnight, or Et_3N, DMF, rt, overnight, or [^{3}H]BZDC-NHS ester, DMF-0.25 M TEAB buffer, rt, overnight.

Phosphitylation of 1-OH with (benzyloxy)[(3-*N*-carboxyamino)propyl] (diisopropylamino)phosphine[6a] in the presence of 1-*H*-tetrazole, followed by oxidation, gave the protected aminopropyl-tethered inositol **5** in 75% yield as a mixture of two diastereoisomers due to the chiral phosphotriester. Two ^{31}P resonances were clearly observed for P-1. Removal of the two *p*-methoxybenzyl (PMB) groups with DDQ in wet methylene chloride gave the diol **6** in 78% yield after silica gel chromatography. Condensation of the diol **6** with di(benzyloxy) (diisopropylamino) phosphine (as for **5**) gave the fully protected IP_3 derivative **7**[11] (75% yield). The ^{31}P-NMR resonances for the diastereomeric P-1 were not resolved for **6** or **7**. Hydrogenolysis with 10% Pd/C removed all protecting groups to provide the optically-active, P-1-(3-aminopropyl)-tethered D-*myo*-Ins(1,4,5)P_3 **7** (sodium salt) in nearly quantitative yield after ion-exchange chromatography on Chelex (sodium form)[6a] (Scheme 1).

Reaction of the P-1-(3-aminopropyl)-tethered D-*myo*-Ins(1,4,5)P_3 (**7**) with the N-hydroxysuccinimido ester of 4-benzoyldihydrocinnamic acid (BZDC-NHS ester) in DMF-0.25 M triethylammonium bicarbonate (TEAB) buffer[8] or in pure DMF suspension with Et_3N[12] at rt for overnight gave, after purification on DEAE-cellulose, the BZDC derivative **8a** in 70% yield. The radiolabeled [^{3}H]BZDC probe **8b** was prepared in 30% radiochemical yield using the DMF-TEAB method.[8] Studies of inositol polyphosphate and phosphoinositide binding proteins using these probes will be described in due course.

Acknowledgment: We thank the NIH (NS 29632) for financial support, Dr. J.F. Marecek and Ms. A. Chaudhary for advice, and Dr. J.D. Olszewski, in conjunction with Dr. D.G. Ahern (Du Pont-New England Nuclear), for providing the BZDC-NHS reagents.

References

1. (a) Berridge, M.J.; Irvine, R.F. *Nature* **1984**, *312*, 315-321. (b) Willcocks, A.L.; Cooke, A.M.; Potter, B.V.L.; Nahorski, S.R. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 1071-1078.

2. (a) Berridge, M.J. *Annu. Rev. Biochem.* **1987**, *56*, 159-193. (b) Berridge, M.J. *Molec. Cell. Endocrinol.* **1994**, *98*, 119-124. (c) Downes, C.P.; Macphee, C.H. *Eur. J. Biochem.* **1990**, *193*, 1-18.

3. (a) *The Inositol Phosphates: Chemical Synthesis and Biological Significance,* (Billington, D.C. Ed.), VCH, **1993**. (b) Potter, B.V.L. *Natural Product Reports,* **1990**, 1-24. (c) Beaucage, S.L.; Iyer, R.P. *Tetrahedron*, **1993**, *49*, 10441-10488. (d) Falck, J.R.; Yadagiri, P. *J. Org. Chem.* **1989**, *54*, 5851-5852. (e) Ling, L.; Ozaki, S. *Carbohydr. Res.*, **1994**, *256*, 49-58. (f) Gou, D.-M.; Liu, Y.-C. *Carbohydr. Res.*, **1992**, *234*, 51-64.

4. Bender, S.L.; Budhu, R.J. *J. Am. Chem. Soc.* **1991**, *113*, 9883-9885.

5. (a) Prestwich, G.D.; Marecek, J.F. in *Inositol Phosphates and Derivatives: Synthesis, Biochemistry, and Therapeutic Potential* (Reitz, A.B. Ed.) ACS Symposium Series, No. 463, American Chemical Society; Washington D.C. **1991** 122-131. (b) Estevez, V.A.; Prestwich, G.D. *J. Am. Chem. Soc.* **1991**, *113*, 9885-9887. (c) Chaudhary, A.; Dormán, G.; Prestwich, G.D. *Tetrahedron Lett.* **1994**, *35*, 7521-7524. (d) Chen, J.; Dormán, G.; Prestwich, G.D. *J. Org. Chem.*, submitted (1995).

6. (a) Prestwich, G.D.; Marecek, J.F.; Mourey, R.J.; Theibert, A.B.; Ferris, C.D.; Danoff, S.K.; Snyder, S.H. *J. Am. Chem. Soc.* **1991**, *113*, 1822-1825. (b) Kanemastu, T.; Takeya, H.; Watanabe, Y.; Ozaki, S.; Yoshida, M.; Koga, T.; Iwanaga, S.; Hirada, M. *J. Biol. Chem.*, **1992**, *267*, 6518-6525.

7. Schäfer, R.; Nehls-Sahabandu, M.; Grabowsky, B.; Dehlinger-Kremer, M.; Schulz, I.; Mayr, G. *Biochem. J.* **1990**, *272*, 817-825.

8. Mourey, R.J.; Estevez, V.A.; Marecek, J.F.; Barrow, R.K.; Prestwich, G.D.; Snyder, S.H. *Biochemistry* **1993**, *32*, 1719-1726.

9. Ferrier, R.J.; Middleton, S. *Chem. Rev.* **1993**, *93*, 2779-2831.

10. Evans, D.A.; Bender, S.L. *Tetrahedron Lett.* **1986**, *27*, 799-802.

11. Satisfactory spectroscopic and analytical data were obtained for all the compounds. ^{31}P shifts are reported in ppm from 85% phosphoric acid as an external standard.

Compound **3** (an oil): ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 19H), 6.83, 6.78 (2 x d, 4H), 4.97-4.50 (m, 14H), 4.43(bs, 1H), 4.20 (t, 1H), 3.99 (t, 1H), 3.80-3.75 (m, 1H), 3.77 (s, 6H), 3.50-3.45 (m, 2H), 1.81 (s, 3H) ppm. FAB HRMS: $C_{47}H_{52}O_{11}$ + Na^+ ($M+Na^+$). (Note: Compounds acquired Na^+ from the FAB matrix.) Anal. Calcd for 815.3407. Found: 815.3426.

Compound **4**: mp 83-85 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ: 7.29-7.22 (m, 19H), 6.86-6.81 (m, 4H), 5.00-4.55 (m, 14H), 4.20 (bs, 1H), 3.97 (t, 1H), 3.8 (t, 1H), 3.79 (s, 6H), 3.72 (d, 1H), 3.50-3.45 (m, 3H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 159.7, 138.4, 138.1, 130.4, 129.6, 129.3, 128.5, 128.1, 127.8, 127.7, 113.8, 96.6, 95.8, 82.7, 81.5, 80.0, 75.5, 72.6, 71.2, 70.1, 69.7, 55.3 ppm. FAB *m/z*: 750 (M^+), 749 (M^+ - 1). Anal. Calcd for $C_{45}H_{50}O_{10}$: C, 71.99; H, 6.71. Found: C, 72.16; H, 6.78.

Compound **5** (viscous colorless oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31-7.11 (m, 29H, phenyl), 6.81-6.72 (m, 4H, PMB), 5.2-4.99 (m, 31H), 3.78 (s, 6H, OMe), 3.41 (m, 2H), 3.17 (m, 2H), 1.66 (m, 2H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 159.0, 157.0, 138.0, 130.8, 130.7, 129.6, 129.2, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7, 127.6, 113.8, 96.2, 95.4, 82.6, 81.2, 79.7, 77.1, 75.5, 75.4, 74.0, 72.6, 70.2, 70.1, 69.7, 66.6, 55.3, 37.0 ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.75, 0.48 corresponding to two diastereoisomers. FAB HRMS: $C_{63}H_{70}NO_{15}P$ + Na^+ ($M+Na^+$). Anal. Calcd for 1134.4381. Found: 1134.4440.

Compound **6** (an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.32-7.26 (m, 25H, phenyl), 5.20-3.80 (m, 17H), 3.30-3.00 (m, 6H), 1.73 (m, 2H) ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.53 ppm. FAB HRMS: $C_{47}H_{54}NO_{13}P$ + Na^+ ($M+Na^+$). Anal. Calcd for 894.3231. Found: 894.3216.

Compound **7** (a syrup): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22-9.19 (m, 45H, phenyl), 5.2-3.8(m, 30H), 3.52(m, 1H), 3.15(m, 2H), 1.57 (m, 2H) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 159.0, 138.0, 130.8, 130.6, 129.7, 129.3, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.5, 127.4, 113.8, 96.2, 95.4, 82.6, 81.2, 79.7, 77.1, 75.5, 75.4, 74.0, 72.6, 70.2, 70.1, 69.7, 66.6, 55.3, 37.0 ppm. ^{31}P NMR (101 MHz, $CDCl_3$) δ: 0.33, 0.10, -0.27 ppm. FAB HRMS: $C_{75}H_{80}NO_{19}P_3$ + Na^+ ($M+Na^+$). Anal. Calcd for 1414.4435. Found: 1414.4360.

Compound **8** (a colorless glass): ^{1}H NMR (300 MHz, D_2O) δ: 4.15-3.65 (m, 7H), 3.58 (d, J = 9.6 Hz, 1H), 3.02 (t, J = 6.9 Hz, 2H), 1.90-1.86 (m, 2H) ppm. ^{13}C NMR (63 MHz, D_2O) δ: 79.5, 79.0, 77.5, 74.4, 73.4, 66.5, 66.3, 40.1, 30.4 ppm. ^{31}P NMR (101 MHz, D_2O) δ: 8.57, 8.44, 3.44 (1:1:1) ppm. FAB *m/z:* 563 M^+ -2), 541 (M^+ - Na -1), 519 (M^+ - 2Na).

Compound **9a** (a glass): ^{1}H NMR (250 MHz, D_2O) δ: 7.70-7.55 (m, 5H), 7.40 (t, 2H), 7.26 (t, 2H), 4.8-3.4 (m, 8H), 3.06 (t, J = 6.5 Hz, 2H), 2.90 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H), 1.65 (m, 2H) ppm. ^{31}P NMR δ: 7.93, 7.32, 3.6 ppm.

12. Olszewski, J.D.; Dormán, G.; Elliott, J.T.; Hong, Y.; Ahern, D.G.; and Prestwich, G.D. *Bioconjugate Chem.*, **1995**, *6*, 395-400.

(*Received in USA* 31 *August* 1995; *revised* 28 *September* 1995; *accepted* 30 *September* 1995)