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Regioselective Synthesis of Photolabile P(1,2)- and P(4,5)-(*o*-Nitrobenzyl) Esters of *myo*-Inositol 1,2,3,4,5,6-Hexakisphosphate

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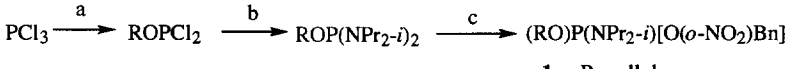
Summary. The regioselective synthesis of four caged $InsP_6$ derivatives is described. The synthesis employed allyl ester protecting groups, and Rh(I) could selectively deprotect all allyl phosphates in a single step without affecting the photolabile moieties.

Release of biochemical effectors by laser flash photolysis of biologically-inert but photosensitive precursors (commonly termed "caged" compounds) has proven to be a powerful method for introducing biological effector molecules into cells.[1] The *o*-nitrobenzyl phosphate group has been widely used as a photosensitive (but biologically-inert) moiety and has wide applications in biological chemistry.[2] Among the numerous inositol polyphosphates ($InsP_n$s) recognized as signaling molecules, only caged Ins(1,4,5)P_3 derivatives, such as the (*o*-nitrophenyl)ethyl esters, have been reported.[3] The synthesis employed diazo chemistry, leading to three isomers with the caging (*o*-nitrophenyl)ethyl group randomly distributed among the three phosphates.[3a] Thus, it appeared necessary to develop a general method to synthesize caged $InsP_n$s with regiochemical control over the number and location of caging moieties.

We have recently prepared a variety of affinity probes for proteins that mediate the biological activities of the major $InsP_n$s and phosphatidylinositol polyphosphates ($PtdInsP_n$).[4a] To determine the roles of specific phosphates of $InsP_6$ in cellular actions[4b] of $InsP_6$ in binding to assembly proteins[4c,d,e] and Golgi coatomer complexes[4a,f], and acting as a substrate for $InsP_6$ kinase[4g], two bis-caged and two tetrakis-caged derivatives were prepared. We report herein the synthesis of these P(1,2)- and P(4,5)- caged $InsP_6$ analogs in which specific masked phosphates were introduced regioselectively with two new *o*-nitrobenzyl-containing phosphoramidite reagents.

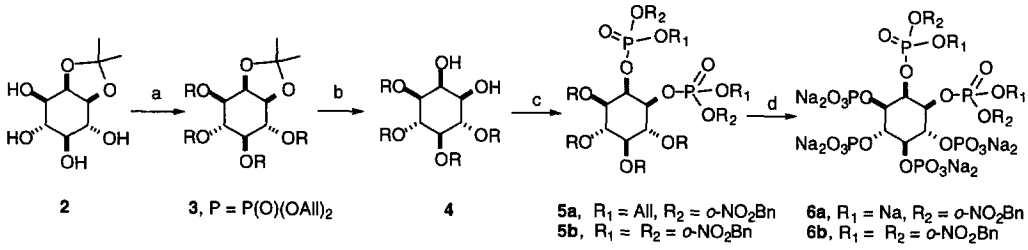
Phosphoramidites containing *o*-nitrobenzyl phosphites and allyl protecting groups were prepared as shown in **Scheme 1**. Allyl groups were employed because *o*-nitrobenzyl is labile to the hydrogenolytic conditions used in deprotection of benzyl groups. Thus, coupling reagents allyl *o*-nitrobenzyl **1a** and di-(*o*-nitrobenzyl)*N,N*-diisopropylphosphoramidite **1b** were synthesized from PCl_3.[5] Although labile phosphorus intermediates were not purified, reagents **1a** and **1b** were purified by SiO_2 chromatography using Et_2O with 5% Et_3N as eluent. Both reagents were stable for several months if kept at -20 °C and in the absence of oxygen.



1a, R = allyl
1b, R = *o*-nitrobenzyl

Scheme 1. (**a**) allyl alcohol or *o*-nitrobenzyl alcohol (1 equiv), Py, Et_2O, -78 °C to rt, overnight; (**b**) *i*-Pr_2NEt (10 equiv), Et_2O, -10 °C to rt overnight; (**c**) allyl alcohol or *o*-nitrobenzyl alcohol (1 equiv), diisopropylethylammonium 1-*H*-tetrazole (0.48 equiv), CH_2Cl_2, rt. 2-3 h.

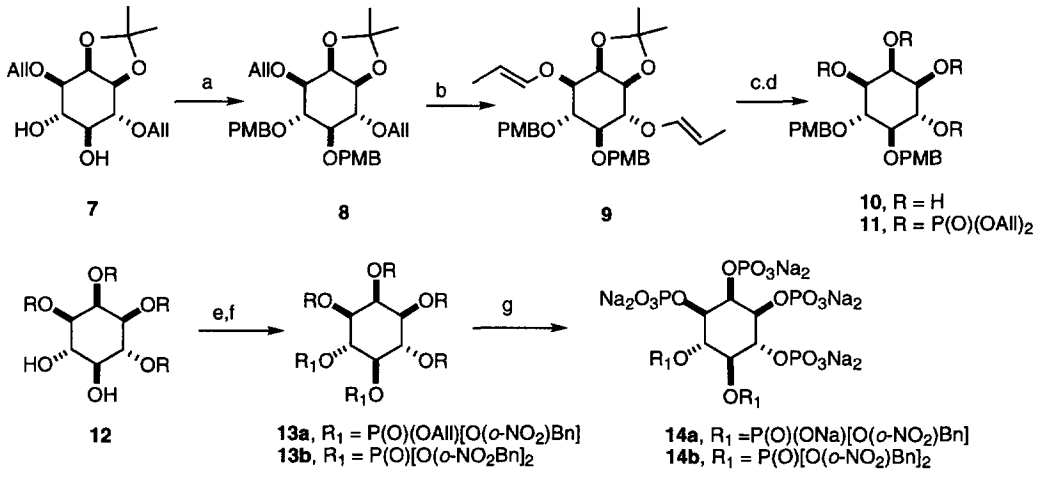
Coupling inositol **2**[6] with $(AllO)_2P(N\textit{i}\text{-}Pr_2)$[7] followed by *m*CPBA oxidation, gave tetrakisphosphate **3** in 84% yield. The allyl group was not affected during low-temperature oxidative conditions. Cleavage of the isopropylidene group was accomplished with CF_3CO_2H:CH_2Cl_2:CH_3OH (3:6:1) at 0 °C for 2.5 h to give compound **4** in 96% isolated yield.[8] Under these conditions, no phosphate migration could be detected by ^1H- or ^{31}P-NMR spectroscopy. Coupling of **4** with **1a** or **1b** gave the fully-protected $InsP_6$ **5a** or **5b** in 88% or 77% yield, respectively (**Scheme 2**).



Scheme 2. (**a**) $(AllO)_2PNPr_2$-*i* (2 equiv for each OH group), 1-*H*-tetrazole (4 equiv), CH_2Cl_2, rt, 1 h; then -40 °C, *m*CPBA (3 equiv), to rt, 1 h; (**b**) 1 mmol of **3** for 10 mL CF_3CO_2H:CH_2Cl_2:CH_3OH (3:6:1 v/v), 0 °C, 2.5 h; (**c**) **1a** or **1b** (2 equiv), similar procedure as (**a**); (**d**) $RhCl(PPh_3)_3$, 95% EtOH, *i*-Pr_2NEt, 1.5 h, 90 °C.

Attempts to remove allyl groups from **5a** using Pd^0 in THF failed.[9] Thus, reaction at rt for 24 h with $Pd(PPh_3)_4$ with butylamine and triphenylphosphine in THF gave only partial deprotection; increasing the reaction temperature led to extensive decomposition. However, the rhodium(I) Wilkinson's catalyst $RhCl(PPh_3)_3$[10] proved to be an efficient reagent for cleavage of all the allyl groups in a single high-yield process. Thus, a solution of **5a** in 95% EtOH containing $RhCl(PPh_3)_3$ (0.1 equiv per allyl group) and *i*-Pr_2NEt (0.3 equiv per allyl) was refluxed for 1.5 h; all the allyl groups were removed. After Chelex® ion exchange,[11] the P(1,2)-bis-caged $InsP_6$ **6a** was obtained in 83% yield as the sodium salt. The product mixture from the above Pd^0-catalyzed rt reaction was also subjected to this condition for 1.5 h, giving the same clean deallylated product **6a**. Similarly, the P(1,1,2,2,)-tetrakis-caged $InsP_6$ **6b** was obtained in 82% yield. Rh(I)-mediated cleavage of the allyl ether group generally involves a two-step reaction: isomerization of the double bond followed by acidic cleavage of the resulting 1-propenyl ether. For allyl phosphates, Rh(I) effects both the isomerization and cleavage.

This method was next used to synthesize P(4,5)-bis- and tetrakis-caged $InsP_6$ derivatives (**Scheme 3**). Thus, *p*-methoxybenzyl (PMB) protection of the two hydroxyls gave inositol **8**[12] in 87% yield. Isomerization of the allyl group to 1-propenyl ether **9** with *t*-BuOK and then acidic hydrolysis furnished the inositol **10** in 76% yield.[12] Coupling **10** with $(AllO)_2PNPr_2$-*i* followed by *m*CPBA oxidation, gave phosphate **11** in 75% yield. Removal[11] of PMB with DDQ gave compound **12** in 86% yield. Coupling of **12** with **1a** or **1b** gave the fully-protected $InsP_6$ derivatives **13a** (71%) or **13b** (67%). Rh(I)-catalyzed cleavage of the allyl groups gave P(4,5)-caged $InsP_6$ derivatives **14a** and **14b** in 70% and 56% yields, respectively, as their sodium salts.[13]



7 8 9 12

13a, R_1 = P(O)(OAll)[O(*o*-NO_2)Bn]
13b, R_1 = P(O)[O(*o*-NO_2Bn)]$_2$

14a, R_1 =P(O)(ONa)[O(*o*-NO_2)Bn]
14b, R_1 = P(O)[O(*o*-NO_2)Bn]$_2$

Scheme 3. (**a**) PMB-Cl, NaH, DMF, rt, 1h; (**b**) *t*-BuOK, DMSO, 60 °C, 4 h; (**c**) 5% *p*TsOH, MeOH, 1 h; (**d**) same procedure as (**a**) in **Scheme 2**; (**e**) 4 equiv DDQ, wet CH_2Cl_2, rt, 3 h; (**f**) **1a** or **1b**, same as (**c**) in **Scheme 2**. (**g**) $RhCl(PPh_3)_3$ (0.1 equiv per allyl), *i*-Pr_2NEt (0.3 equiv per allyl), 95% EtOH, reflux, 1.5 h; then Chelex ion exchange to obtain sodium form.

In conclusion, we have described herein the convenient synthesis of (*o*-nitrobenzyl) phosphoramidite reagents, a general method to synthesize the regiospecifically-caged derivatives of $InsP_6$, and a useful method to cleave all allyl groups in one step. This methodology should find wide application in the synthesis of many specifically-caged $InsP_n$ derivatives. The biological uses of these compounds will be reported in due course.

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13. Representative procedures and experimental data for reagents and caged products.

(a) *Synthesis of caging phosphoramidites.* To a mixture of PCl_3 (3 mmol) and pyridine (3 mmol) in dry ether (100 mL) cooled to -78 °C, was added dropwise a solution of *o*-nitrobenzyl alcohol (3 mmol) over 1 h. The mixture was stirred and warmed to rt overnight, and the solid was removed by filtration. The filtrate was cooled to -10 °C, 28 mL of *i*-Pr_2NH (30 mmol) was added dropwise over 1 h, the mixture was stirred overnight at rt, filtered and concentrated *in vacuo*. The resulting oil was dissolved in CH_2Cl_2 (100 mL); then *o*-nitrobenzyl alcohol (2.4 mmol) and *N,N*-diisopropylammonium 1-*H*-tetrazole (1.2 mmol) were added in one portion, and then stirred at rt for 2 h. Workup and purification on SiO_2 using ether with 5% Et_3N gave **1b** as yellow solid. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 7.8 Hz, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 7.8 Hz), 7.43 (t, *J* = 7.8 Hz, 1H), 4.86-4.65 (m, 4H, OCH_2), 3.80-3.60 (m, 2H, CHN), 1.24-1.21 (m, 12H, CH_3) ppm. ^{31}P-NMR (101 MHz, $CDCl_3$) δ: 150.4 ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 132.7, 129.3, 122.3, 121.7, 64.5, 64.2, 43.4, 43.2, 24.7, 24.6 ppm. FAB HRMS: $C_{20}H_{27}N_3O_6P$ (MH^+): Calcd. 436.1637. Found: 436.1626. MS: *m/z* 436, 420, 299, 283, 241, 136, 120.

(b) *Protected intermediate* ***5b***. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.20-8.05 (m, 8H), 7.80-7.40 (m, 8H), 6.00-5.80 (m, 8H), 5.55 (d, *J* = 9.1 Hz, 1H), 5.40-5.10 (m, 24H), 5.05-4.90 (m, 2H), 4.80-4.40 (m, 19H) ppm. ^{31}P-NMR (101 MHz, $CDCl_3$) δ: 0.63-1.7 (m) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 133.6-132.4, 129.7, 123.3-122.5 (m), 118.5, 87.3, 68.9, (m) ppm. FAB HRMS: $C_{58}H_{71}N_4O_{32}P_6$ (MH^+): Calcd. 1521.2477. Found: 1521.2539. MS: *m/z* 1521(MH^+), 1013, 861, 821, 473, 417, 337, 297, 219, 136.

(c) *P-1,1,2,2-Tetra-caged InsP$_6$ (**6b**).* A mixture of **5b** (1 mmol), $RhCl(PPh_3)_3$ (0.8 mmol), *i*-Pr_2NEt (2.4 mmol) in 20 mL of 95% EtOH was refluxed for 1.5 h, then concentrated to a brown solid and treated with acetone. The suspension was centrifuged and the acetone was decanted. This procedure was repeated several times until the acetone showed no further UV-absorbing material (TLC). The solid was dissolved in 1 mL of water and loaded onto a Chelex (Na^+ form) column, and eluted with water. The UV-active fractions were collected and lyophilized to give yellowish solid **6b**. ^{1}H-NMR (250 MHz, D_2O) δ: 8.30-7.40 (m, 16H), 5.20-4.00 (m, others) ppm. ^{31}P-NMR (101 MHz, $CDCl_3$) δ: 6.12-2.9 (m) ppm. ^{13}C-NMR (63 MHz, $CDCl_3$) δ: 137.2-136.1, 132.4 (m), 125.6-123.9 (m), 79.8, 79.0, 77.6, 76.3, 69.4, 68.7 ppm.

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