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Tetrahedron Letters 44 (2003) 1157–1159

TETRAHEDRON LETTERS

Synthesis of caged *myo*-inositol 1,3,4,5-tetrakisphosphate

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Received 20 September 2002; revised 14 October 2002; accepted 14 December 2002

Abstract—The total synthesis of an enantiomerically pure Ins(1,3,4,5)P_4 derivative equipped with a photosensitive nitroveratryl group at the 3-*O*-phosphate is reported.

2-Nitrobenzyl esters of *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**], usually referred to as 'caged' Ins(1,4,5)P_3, are widely used tools for biochemical studies.[1–3] The cage group prevents its biological function, in particular the ability to release calcium from intracellular stores. Upon illumination with UV light the nitrobenzyl group is removed and active Ins(1,4,5)P_3 is released. In the typical synthesis, the nitrobenzyl ester is randomly attached to the 4-*O*- or 5-*O*-phosphate via a diazo reagent.[1] Alternatively, an essential hydroxy group could be masked by forming a photolabile veratroyl ether.[3] In cells, Ins(1,4,5)P_3 is phosphorylated at the 3-OH group to *myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4, **2** (Fig. 1)], thereby switching off the second messenger function of Ins(1,4,5)P_3 and generating another intracellular messenger, albeit controversially discussed in its function.

In order to generate larger synthetic versatility, it would be beneficial to regiospecifically introduce phosphate and cage in one synthetic step during a larger reaction sequence. The latter requires that the fully protected caged phosphate is stable under a variety of classical protecting group transformations. Only a few P(III) reagents, equipped with photolabile groups were prepared in the past.[4–6] Herein, we use the nitroveratryl phosphoramidite **3** with a fluorenylmethyl protecting (Fm) group to synthesize a 'caged' dimethoxynitrobenzyl-*myo*-inositol 1,3,4,5-tetrakisphosphate derivative [DMNBn-Ins(1,3,4,5)P_4, **4** (Fig. 1)]. The nitroveratryl group is specifically attached to the phosphate in position 3. The latter distinguishes Ins(1,3,4,5)P_4 from its biochemical precursor Ins(1,4,5)P_3 and should hence be essential for recognition by Ins(1,3,4,5)P_4-binding proteins. Furthermore, the interaction of phosphoinositide-binding protein domains with **2** is currently of particular interest, because **2** represents the headgroup of the second messenger phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3].[7]

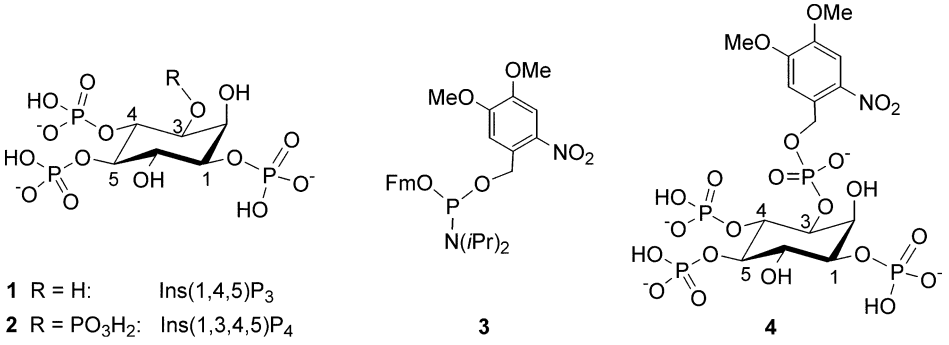

Figure 1. Structure of Ins(1,3,4,5)P_4 and its photoactivatable derivative.

Keywords: cyclitols; photoactivatable; caged compounds; phosphorylation; signal transduction.

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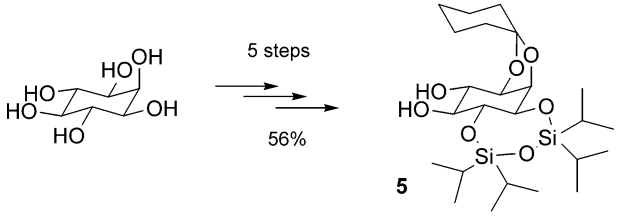
0040-4039/03/$ - see front matter
PII: S0040-4039(02)02834-4

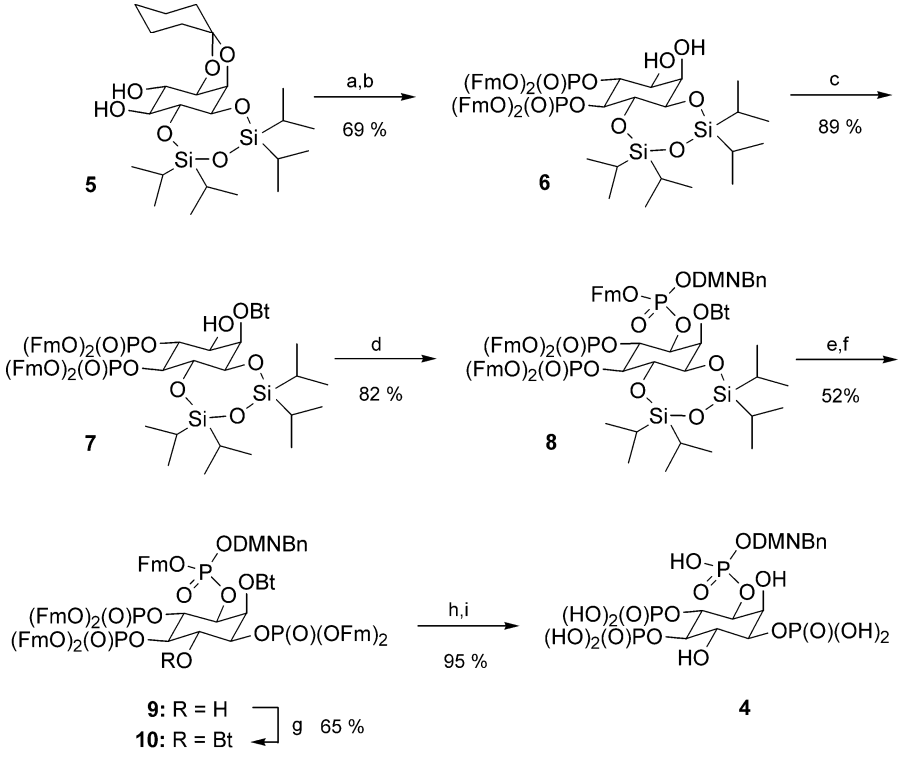
The phosphoramidite **3** was prepared as described in the preceeding paper.[8] How would the reagent perform within a more complex reaction sequence? The sequence (see Schemes 2–4 in Ref. 8) required various transformations after the cage was introduced to the orthogonally protected precursor, a good test for the feasibility of the method.

The enantiomerically pure precursor **5** was prepared as described before[9] (Scheme 1). In brief, *myo*-inositol was converted to the common racemic precursor 1,2-*O*-cyclohexylidene-*myo*-inositol that was regioselectively protected with the bidental tetraisopropyl disiloxane group.[10] The introduction of a mandeleic acid ester, followed by butyrylation, and separation of the diastereomers on silica, gave a fully protected compound in a one-pot procedure. Cleavage of all esters afforded the enantiomerically pure diol **5**. The latter was subsequently phosphorylated with bis(fluorenylmethyl) *N*,*N*-diisopropylphosphoramidite, followed by oxidation with *t*-BuOOH and removal of the ketale to give diol **6** (Scheme 2). The axial 2-OH-group was regioselectively esterified by treatment with trimethyl orthobutyrate and careful hydrolysis of the cyclic intermediate to give the butyric acid ester **7**. The remaining 3-OH-group was treated with **3** and the phosphite triester was oxidized with *t*-BuOOH to the fully protected compound **8**. To introduce the final phosphate, the bis(siloxyl) group was removed with aqueous HF in acetonitrile (70% yield). The final phosphitylation was accomplished with remarkable regioselectivity in favor of the 1-OH-group, probably due to the sterical hindrance of the fluorenylmethyl groups to give compound **9**. The hydroxy group of **9** was esterified with butyric acid anhydride in the presence of DIC, equimolar amounts of tetrazole, and traces of DMAP to give the fully protected derivative **10**. Structure elucidation of **10** by NMR was largely facilitated.



Scheme 1. The enantiomerically pure precursor **5** was prepared from *myo*-inositol in a five-step reaction sequence including the separation of distereomeric *O*-*tert*-butyldimethylsilyl (–)-mandeleic acid esters. The determination of the absolute configuration of **5** was previously described.[9]

To our knowledge, esterification of hydroxy groups vicinal to phosphate triesters was previously considered impossible, due to the formation of cyclic phosphate esters. However, fully esterified hydroxy groups of inositol phosphates are a pre-requisite for potential future manipulations of the phosphates. Therefore, the conditions described might be generally useful. The fluorenylmethyl groups were removed with pyrrolidine, followed



Scheme 2. *Reagents and conditions*: (a) $(FmO)_2P$-N*i*Pr_2, tetrazole, MeCN, rt, 5 h, then *t*BuOOH, rt, 30 min; (b) TFA, MeOH (wet), 0°C, 5 min; (c) $(MeO)_3CCH_2CH_2Me$, CSA, toluene, rt, 30 min, then MeOH (wet), rt, 2 h; (d) **3**, tetrazole, MeCN, rt, then *t*BuOOH, rt, 30 min; (e) HF (50% in water)/MeCN (1:10, v/v), rt, 4 h; (f) 1 equiv. $(FmO)_2P$-N*i*Pr_2, tetrazole, MeCN, rt, 5 h, then *t*BuOOH, rt, 30 min; (g) Bt_2O, DIC, tetrazole, DMAP, rt, 5 h; (h) pyrrolidine, DCM, rt, 5 min; (i) KOH (aq.), pH 13, rt, 6 h.

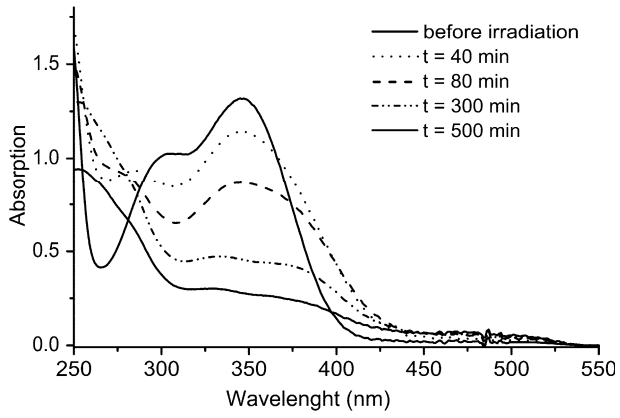


Figure 2. UV spectra of **4** (250 μM) after several intervals of illumination (360 nm, 6 W lamp, pH 7).

by purification on a RP-18 cartridge to remove 9-methylene-fluorene. Treatment with KOH gave the enantiomerically pure DMNBn-Ins(1,3,4,5)P_4 derivative **4** after elution from a Dowex WX-8 resin.[12] The compound exhibited the typical photospectral behavior of the nitroveratryl group when illuminated at 365 nm (Fig. 2).[11] The results showed also that the phosphotriester carrying the cage is surprisingly stable against nucleophiles (HF, tetrazole, or DMAP) and under basic conditions (pH 13).[8] In summary, it could be proved that reagent **3** was suitable in an elaborate synthesis.

The possibility to introduce phosphate and nitroveratryl group in a single step paves the way to site-specifically caged inositol polyphosphates. These compounds will be valuable tools for structure–activity studies of Ins(1,3,4,5)P_4 or other inositol polyphosphates to the respective binding proteins in the future. Biochemical experiments with the caged inositol 1,3,4,5-tetrakisphosphate are under way and will be published elsewhere in due time.

Acknowledgements

We thank SiChem GmbH, Germany, for chlorophosphoramidites, Dr. Marc Gentzel for MS analysis, and Ms. Nicole Heath for revising the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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12. Selected data of **4**: ^{1}H NMR (400 MHz, CD_3OD, pH 2): δ 7.79 (s, 1H), 7.43 (s, 1H), 5.51 (d, 6.3 Hz), 4.77 (ddd, 9.5 Hz, 9.4 Hz, 9.4 Hz, 1H, H-4), 4.53 (dd, 2.1 Hz, 2.1 Hz, 1H, H-2), 4.41 (ddd, 9.5 Hz, 8.8 Hz, 2.2 Hz, 1H, H-3), 4.27 (ddd, 9.2 Hz, 9.2 Hz, 9.2 Hz, 1H, H-5), 4.17 (ddd, 9.5 Hz, 9.2 Hz, 2.3 Hz, 1H, H-1), 4.03 (dd, 9.5 Hz, 9.5 Hz, 1H, H-6), 4.02 (s, 3H, OMe), 3.94 (s, 3H, OMe); ^{31}P NMR (162 MHz, CD_3OD, pH 2): δ 0.31 (s, 1P), 0.21 (s, 1P), −0.32 (s, 1P), −1.36 (s, 1P).