Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5602–5604
Bioorganic & Medicinal Chemistry Letters

Preparation of a protected phosphoramidon precursor via an *H*-Phosphonate coupling strategy

Matthew G. Donahue and Jeffrey N. Johnston*

Department of Chemistry, Vanderbilt University, 7330 Stevenson Center, Station B 351822, Nashville, TN 37235, USA

Received 28 July 2006; accepted 2 August 2006
Available online 28 August 2006

Abstract—The preparation of a phosphoramidon precursor is described using a phosphorus(III) coupling protocol.

Phosphoramidon (**1**) was isolated from *Streptomyces tanashiensis* in 1972 by a Japanese group at Keio University.[1] It consists of a leucyl-tryptophan dipeptide coupled to α-L-rhamnose via a phosphoramidate linkage (Fig. 1). Phosphoramidon possesses an array of biological activity, including inhibition of the bacterial zinc protease thermolysin,[2] the mammalian plasma membrane zinc peptidase,[3] and a metalloprotease endothelin converting enzyme.[4] Its ability to inhibit endothelin converting enzyme has rendered it a valuable tool in the study of hypertension,[5] stroke,[6] and diseases of the kidney.[7,8] Phosphoramidon has been particularly scrutinized as a potent and specific inhibitor of a zinc metalloproteinase identified as an endothelin converting endopeptidase. As a result, it has been a model for the development of numerous novel inhibitors developed over the last decade.[9] Phosphoramidon has also been used in the study of the upstream inhibition of endothelin conversion.

To support studies of this type, phosphoramidon is sold commercially. In practice however, supplies of phosphoramidon are subject to both high cost (ca. $45/mg), and inconsistent availability.[10] This report details our effort to produce gram quantities of phosphoramidon, which led ultimately to an improvement of the convergent coupling of the monosaccharide, phosphate ester, and dipeptide fragments of the natural product.

Our initial synthetic route to phosphoramidon followed the first report of its synthesis by De Nanteuil and coworkers in 1995 (Fig. 2),[11] in which three fragments served as key intermediates: triacylated L-rhamnose (**2**), phenyl dichlorophosphate (**3**), and the ethyl ester of leucyl-tryptophan (**4**). In their report, the three components were linked in a one-pot, double condensation reaction to afford the protected intermediate (**11**), which was saponified to afford **1** in 7.5% yield over two steps.

Toward that end, commercially available L-rhamnose **5** was peracylated under Steglich conditions to afford **6** in 99% yield (Scheme 1).[12] Chemoselective deacylation of **6** at the anomeric position gave α-anomer **2** as a white solid in 77% yield.[13] The carbobenzyloxy protected

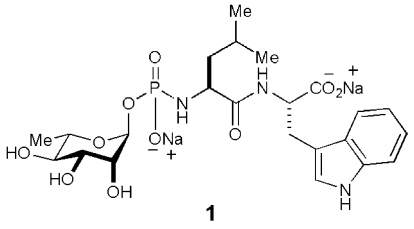


Figure 1. Phosphoramidon.

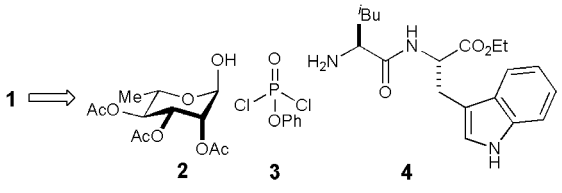
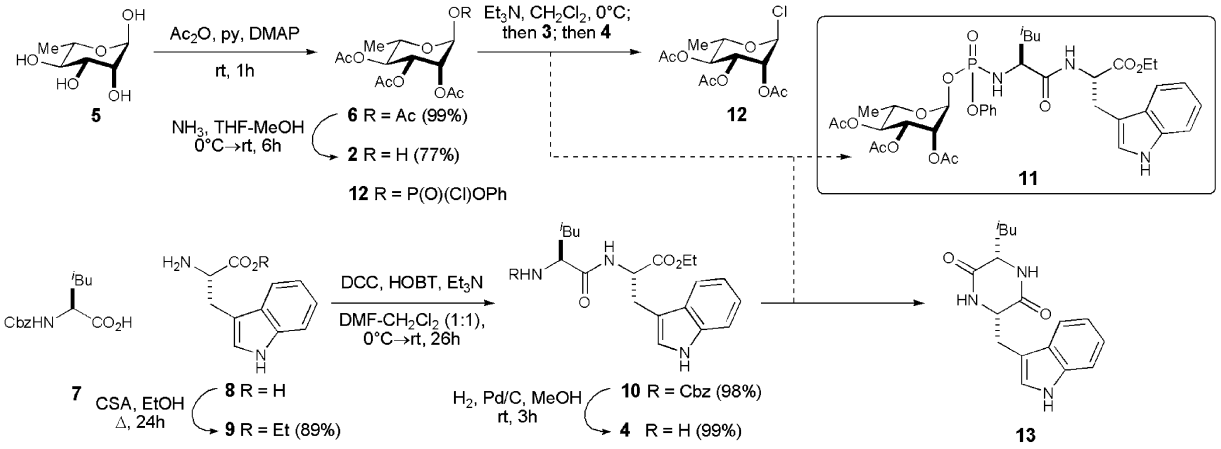


Figure 2. Key fragments for the synthesis of phosphoramidon (De Nanteuil).

Keywords: Phosphoramidon; *H*-Phosphonate; Synthesis.
* Corresponding author. Tel.: +1 615 322 7376; fax: +1 615 343 1234; e-mail: jeffrey.n.johnston@vanderbilt.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.024



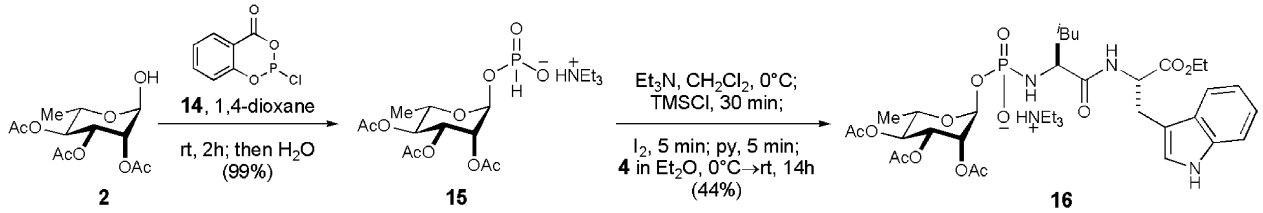
Scheme 1. Unsuccessful coupling based on literature protocol.

dipeptide **10** was prepared in 98% yield via union of commercially available *N*-carbobenzyloxy-L-leucine (**7**) with the ethyl ester of L-tryptophan (**9**) (prepared in 89% yield by esterification of L-tryptophan **8** in refluxing ethanol with two equivalents of DL-camphorsulfonic acid) using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT).[14] Palladium catalyzed hydrogenolysis of the Cbz group of **10** gave dipeptide **4** in 99% yield.

With ample quantites of **2** and **4** in hand, the literature coupling conditions involving phenyl dichlorophosphate **3** failed to provide any product **11**. As the result of several attempts to effect the coupling, we successfully isolated glycosyl chloride **12** (anomeric proton, δ 5.87 (d, $J = 2$ Hz)). Furthermore, we noted the propensity for dipeptide **4** to transform slowly to cyclic dipeptide **13**, even upon standing in purified form at low temperature. Through various modifications to the coupling protocol, we surmised that formation of the glycosyl chloride was both evidence that phosphorylation of the monosaccharide was occurring (leading to **12**), as well as an indication that chloride displacement of the phosphate is competitive with dipeptide coupling. Therefore, we turned to a more effective phosphorus lynchpin for these couplings.

To circumvent the failings of the doubly activated phosphorus reagent, the coupling route was redesigned to a step-wise approach. We hypothesized that acceleration of the dipeptide-glycosyl phosphate coupling might be achieved through the use of more electrophilic phosphorus(III) *H*-phosphonates, for which several bench stable options can be found in the literature.[15] Phosphitylation of the anomeric alcohol of **2** with **14**[16] was followed by a hydrolysis protocol to afford *H*-phosphonate **15** in quantitative yield (Scheme 2).[17] This step required chromatographic separation over silica gel to remove the salicylic acid. The subsequent coupling involves four distinct steps: (1) conversion of the tetracoordinate P(III) *H*-phosphonate to the tri-coordinate P(III) bis(silyl)phosphite, (2) oxidation of phosphorus with molecular iodine, (3) formation of the presumed phosphoryl pyridinium ion and (4) trapping with dipeptide **4** to afford **16**. We found that this sequence furnished the desired aminophosphonate in 44% yield after silica gel chromatography. The anomeric center was assigned as α by comparison of $^{3}J_{C(1)H\text{-}P}$ (7.5 Hz) to known α linked phosphoramidates of rhamnose.[18] The ^{31}P NMR (carbon and proton decoupled) showed a singlet at 3.83 ppm. As a practical note, the isolable compounds contain achiral phosphorus, thereby simplifying the spectral data. A total of 18.8 g of **16** was produced by this method, and the final saponification to phosphoramidon has been reported by De Nanteuil.[11]

In summary, a two step coupling protocol utilizing salicylate phosphorus(III) reagent **14** allowed the efficient, multigram scale synthesis of phosphoramidon precursor **16**. This synthesis should result in a more consistent and inexpensive supply of this highly employed, pharmacologically important agent as a tool in chemical biology.



Scheme 2. Phosphorus(III) coupling protocol.

Acknowledgment

We are grateful to Procter and Gamble for financial support of this work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.024.

References and notes

1. Umezawa, S.; Tatsuta, K.; Izawa, O.; Tsuchiya, T. *Tetrahedron Lett.* **1972**, *1*, 97.
2. Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 621.
3. Kenny, A. J. In *Proteinases in Mammalian Cells and Tissues*; Barrett, A. J., Ed.; Elsevier: Amsterdam, 1977; p 393.
4. Review of the pharmacology of endothelin converting enzymes: Turner, A. J.; Murphy, L. *J. Biochem. Pharmacol.* **1996**, *51*, 91.
5. Matsumura, Y.; Hisaki, K.; Takaoka, M.; Morimoto, S. *Eur. J. Pharmacol.* **1990**, *185*, 103.
6. Simonson, M. S.; Dunn, M. J. *J. Lab. Clin. Med.* **1992**, *119*, 622.
7. Kohan, D. E. *Am. J. Kidney Dis.* **1993**, *22*, 493.
8. Review: Warner, T. D.; Battistini, D.; Doherty, A. M.; Corder, R. *Biochem. Pharmacol.* **1994**, *48*, 625.
9. (a) Lloyd, J.; Schmidt, J. B.; Hunt, J. T.; Barrish, J. C.; Little, D. K.; Tymiak, A. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1323; (b) Hanessian, S.; Rogel, O. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2441.
10. (a) http://www.agscientific.com/Item/P1101.html.; (b) This worked commenced due to an indefinite backorder for phosphoramidon.
11. De Nanteuil, G.; Benoist, A.; Rémond, G.; Descombes, J.-J.; Barou, V.; Verbeuren, T. J. *Tetrahedron Lett.* **1995**, *36*, 1435.
12. Steglich, W.; Hoefle, G. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 981.
13. Fiandor, J.; Garcìa-López, M. T.; De Las Heras, F. G.; Méndez-Castrillón, P. P. *Synthesis* **1985**, 1121.
14. Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067.
15. Stawinski, J.; Kraszewski, A. *Acc. Chem. Res.* **2002**, *35*, 952.
16. Young, R. W. *J. Am. Chem. Soc.* **1952**, *74*, 1672.
17. Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661.
18. Sun, Q.; Xiao, Q.; Ju, Y.; Zhao, Y. F. *Chin. Chem. Lett.* **2003**, *14*, 685.