

Facile and Rapid Access to Inosine Puromycin Analogues through the Use of Adenylate Deaminase

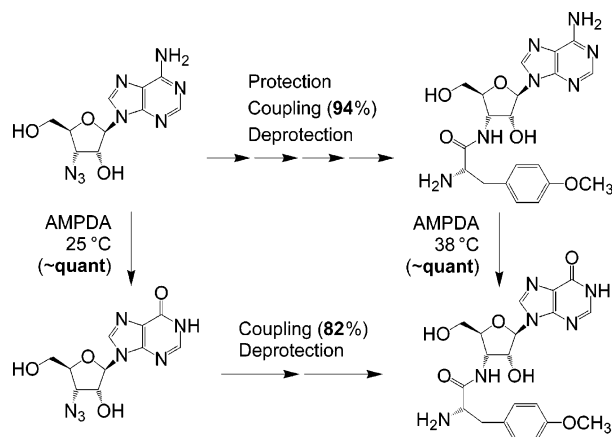
Adib Charafeddine, Hubert Chapuis, and Peter Strazewski*

Laboratoire de Synthèse de Biomolécules (UMR 5246, ICBMS), Bâtiment Eugène Chevreul (5ième étage), Université Claude Bernard Lyon 1, 43 boulevard du 11 novembre 1918, F-69622 Villeurbanne Cedex, France

strazewski@univ-lyon1.fr

Received April 5, 2007

ABSTRACT



To study the ribosomal peptidyl transfer, puromycin analogues are of interest in which adenine has been replaced by hypoxanthine. We synthesized inosine puromycin analogues from 3'-azidodeoxyadenosine derivatives using adenylate deaminase for the quantitative transformation of the N-heterocycle. The amino acid coupling was carried out under Staudinger–Villarsa conditions in 94% yield starting from the protected and in 82% using the unprotected azide, thus, in the presence of two hydroxyls and a lactam function.

Natural puromycin contains a dimethylamino group that replaces the usual N-heterocyclic 6-amino group with its adenine moiety. Whereas natural puromycin inhibits *in vivo* the growth of bacterial colonies by interrupting the bacterial ribosomal protein synthesis, which can be quantified *in vitro* in protein synthesis inhibition assays, the 6-amino puromycin analogue **3** is only active *in vitro* but completely inactive *in vivo*. Adenosine deaminase (adenosine aminohydrolase, ADA, EC 3.5.4.4) and adenylate deaminase (5'-adenylic acid deaminase, AMP deaminase, AMPDA, EC 3.5.4.6) are ubiquitously occurring metalloenzymes that belong to the class of hydrolases and catalyze the irreversible hydrolytic deamination of adenosine and its derivatives to the corre-

sponding inosines. The broad substrate specificity of these enzymes on nucleosides modified either on the purine base or in the ribose moiety has long been known.¹ The ADA reaction is thought to proceed via a tetrahedral intermediate at C-6 of the base. A similar mechanism may account for AMPDA as well.^{2–4} The use of enzymes in organic synthesis

(1) (a) Cory, J. G.; Suhadolnik, R. J. *Biochemistry* **1965**, *4*, 1729. (b) Wolfenden, R.; *J. Am. Chem. Soc.* **1966**, *88*, 3157. (c) Cory, J. G.; Suhadolnik, R. J. *Biochemistry* **1965**, *4*, 1733. (d) Minato, S.; Nakanishi, K. *J. Biochemistry* **1967**, *62*, 21. (e) Zielke, C. L.; Suelter, C. H. In *The Enzymes*; Boyer, P.D., Ed., Academic Press: New York, 1971; pp 47–78.

(2) Groziak, M. P.; Huan, Z. W.; Ding, H.; Meng, Z. Y.; Stevens, W. C.; Robinson, P. D. *J. Med. Chem.* **1997**, *40*, 3336.

(3) Merkle, D. J.; Kline, P. C.; Weiss, P.; Schramm, V. L. *Biochemistry* **1993**, *32*, 12993.

(4) Ciuffreda, P.; Buzzi, B.; Alessandrini, L.; Santaniello, E. *Eur. J. Org. Chem.* **2004**, 4405.

* To whom correspondence should be addressed. Phone: +334 72 44 82 34. Fax: +334 72 43 13 23.

is now widely accepted and will continue to gain momentum as more synthetic research utilizes enzymes as alternative and more desirable sources for specific biocatalysis. This is particularly true for the field of nucleoside chemistry.⁵ ADA and AMPDA are commercially available and have been used as biocatalysts for chemoenzymatic transformations to convert purine nucleoside derivatives into a variety of compounds⁶ that often show interesting pharmacological properties.⁷ Enzymes are also environmentally friendly resources that function best under aqueous conditions, thus decreasing the needed amount of toxic organic solvents.

Compared with ADA, the properties and the possible use of AMPDA as a biocatalyst are much less explored.^{5c,8} Information available from the literature seems to indicate that AMPDA is able to accept a wider range of substrates but is less stereoselective.⁹ It was gratifying for us to find that all tested compounds were substrates for the catalytic action of AMPDA and that this enzymatic deamination allowed a viable preparation of the hypoxanthine derivatives **1**, **2**, and **8** from the adenine derivatives **3**, **4**, and **7**, respectively (Figure 1 and Scheme 1).

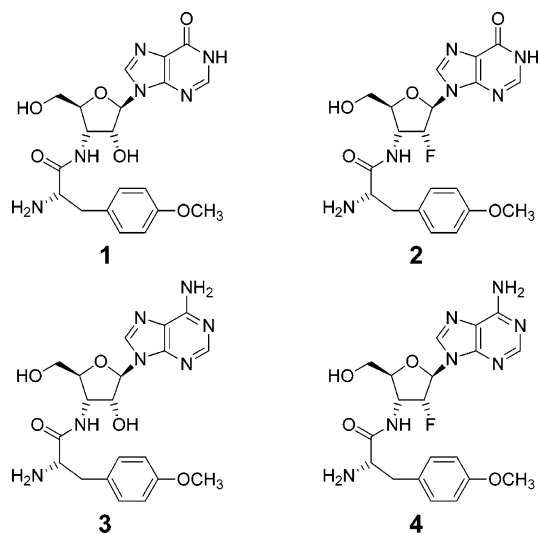


Figure 1. Puromycin analogues.

We previously reported the synthesis of compounds **3** and **4** from adenosine under optimized experimental conditions.¹⁰ Here, we present an alternative that gives direct and simple access to the corresponding unprotected inosine puromycin analogues. To obtain target compound **1** we proceeded via two synthetic routes (Scheme 1).

The first one, pathway A, started from fully protected 2'-azidodeoxynucleoside **5** that was first coupled with the 1-oxybenzotriazolyl ester of *N*-Fmoc-*O*-methyltyrosine to give **6** in 94% yield under the recently published Staudinger–Vilarrasa coupling conditions as developed for the synthesis of nonfluorinated^{10b} and fluorinated puromycin analogues.^{10c} Compound **6** was then deprotected in one pot with CH₃NH₂/

EtOH (33%) followed by 1 M TBAF/THF to give puromycin derivative **3** in 70% yield which was enzymatically deaminated in the presence of AMPDA in phosphate buffer at pH 6.5 and 38 °C to furnish after 5 h the target inosine puromycin analogue **1** in a quasi-quantitative isolated yield. Stirring the reaction at 25 instead of 38 °C resulted in a much prolonged deamination period of 48 h, yet still in quantitative yield. No deamination whatsoever of **3** (well soluble in water) was observed at 38 °C when AMPDA was replaced by ADA, most probably owing to the steric hindrance at the 3' position of the ribofuranose moiety (vide supra).

The second route, pathway B, began with the total deprotection of **5** in one pot using CH₃NH₂/EtOH (33%) followed by 1 M TBAF/THF to give the unprotected 3'-azido-deoxynucleoside **7** (Scheme 1). The enzymatic deamination of **7** could be carried out with both ADA or AMPDA in phosphate buffer (at pH 7.0 or 6.5, respectively). Both enzymes were equally capable of deaminating this substrate in a few minutes (~20 min) at 25 °C and gave the resulting 3'-azidodeoxyinosine derivative **8** in close to quantitative yield (>98% after chromatography).

The coupling of **8** with the 1-oxybenzotriazolyl ester of *N*-Boc-*O*-methyltyrosine under the same Staudinger–Vilarrasa conditions resulted in compound **9** in a remarkably good yield (82%) despite the presence of two hydroxyl groups and a lactam function. Compound **9** was then deprotected with CF₃COOH in water (3:7) to give the inosine puromycin analogue **1** in 80% yield after chromatography over silica gel and lyophilization.

Previously synthesized^{10c} compound **4** (Figure 1) was also subjected to an enzymatic deamination with AMPDA in phosphate buffer at pH 6.5 and 38 °C to give after 5 h the inosine 2'-deoxyfluoropuromycin analogue **2** in a quantitative yield. Like **3**, compound **4** was no substrate for ADA at 38 °C.

(6) (a) Secrist, J. A., III; Montgomery, J. A.; Shealy, Y. F.; O' Dell, C. A.; Clayton, S. J. *J. Med. Chem.* **1987**, *30*, 746. (b) Vince, R.; Brownell, J. *Biochem. Biophys. Res. Commun.* **1990**, *168*, 912. (c) Borthwick, A. D.; Biggadike, K.; Kirk, B. E.; Storer, R.; Weir, N. G.; Baxter, A. D.; Mo, C. L. *Eur. Pat. Appl.* 1991, 0 410 660. (d) Exall, A. M.; Jones, M. F.; Mo, C. L.; Myers, P. L.; Paternoster, I. L.; Singh, H.; Storer, R.; Weingarten, G. G.; Williamson, C.; Brodie, A. C.; Cook, J.; Lake, D. E.; Meerholz, C. A.; Turnbull, P. J.; Highcock, R. M. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2467. (e) Merlo, V.; Reece, F. J.; Roberts, S. M.; Gregson, M.; Storer, R. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1717. (f) Barai, V. N.; Zinchenko, A. I.; Eroshevskaya, L. A.; Zhernosek, E. V.; De Clercq, E.; Mikhailopulo, I. A. *Helv. Chim. Acta* **2002**, *85*, 1893. (g) Kakefuda, A.; Shuto, S.; Nagahata, T.; Seki, J.-I.; Sasaki, T.; Matsuda, A. *Tetrahedron* **1994**, *50*, 10167. (h) Seela, F.; Kaiser, K. *Chem. Pharm. Bull.* **1988**, *36*, 4153.

(7) (a) Ford, H., Jr.; Siddiqui, M. A.; Driscoll, J. S.; Marquez, V. E.; Kelley, J. A.; Mitsuya, H.; Shirasaka, T. *J. Med. Chem.* **1995**, *38*, 1189. (b) Zaitseva, G. V.; Zinchenko, A. I.; Barai, V. N.; Pavlova, N. I.; Boreko, E. I.; Mikhailopulo, I. A. *Nucleosides Nucleotides* **1999**, *18*, 687. (c) Tisdale, M.; Ellis, M.; Klumpp, K.; Court, S.; Ford, M. *Antimicrob. Agents Chemother.* **1995**, *39*, 2454. (d) Seela, F.; Heckel, M.; Rosemeyer, H. *Helv. Chim. Acta* **1996**, *79*, 1451. (e) Seela, F.; He, Y.; Wei, C. *Tetrahedron* **1999**, *55*, 9481. (f) Sheppard, T. L.; Rosenblatt, A. T.; Breslow, R. *J. Org. Chem.* **1994**, *59*, 7243.

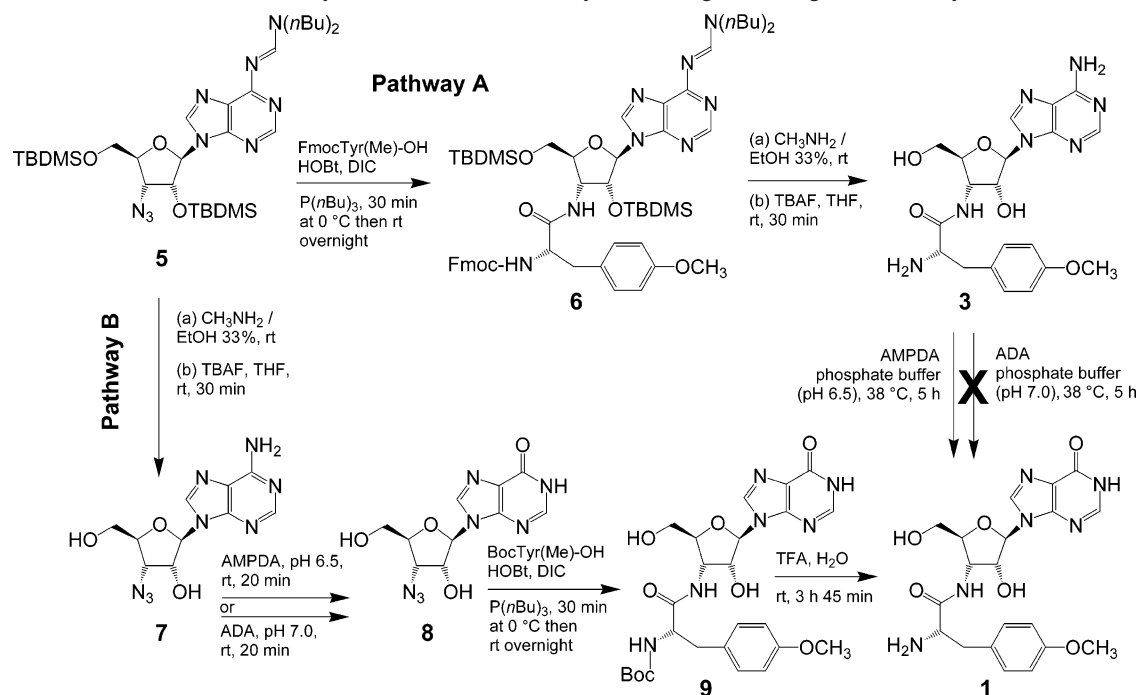
(8) (a) Samejima, H.; Kimura, I.-C.; Noguchi, S.; Shimura, G. In *Enzyme Engineering*; Pye, E. K., Weetall, H. H., Eds.; Plenum Press: New York, 1978; Vol. 3, pp 469–475. (b) Muzzarelli, R. A. A. *Enzyme Microb. Technol.* **1980**, *2*, 177.

(9) (a) Ciuffreda, P.; Loseto, A.; Alessandrini, L.; Terraneo, G.; Santaniello, E. *Eur. J. Org. Chem.* **2003**, 4748. (b) Margolin, A. L.; Borcherdig, D. R.; Wolf-Kugel, D.; Margolin, N. A. *J. Org. Chem.* **1994**, *59*, 7214.

(10) (a) Nguyen-Trung, N. Q.; Botta, O.; Terenzi, S.; Strazewski, P. *J. Org. Chem.* **2003**, *68*, 2038. (b) Chapuis, H.; Strazewski, P. *Tetrahedron* **2006**, *62*, 12108. (c) Charafeddine, A.; Dayoub, W.; Chapuis, H.; Strazewski, P. *Chem. Eur. J.* **2007**, *13*, 5566.

(5) (a) Ferrero, M.; Gotor, V. *Monatsh. Chem.* **2000**, *131*, 585. (b) Ferro, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319. (c) Santaniello, E.; Ciuffreda, P.; Alessandrini, L. *Synthesis* **2005**, 509. (d) Gupta, M.; Nair, V. *Collect. Czech. Chem. Commun.* **2006**, *71*, 769.

Scheme 1. Syntheses of Inosine Puromycin Analogue **1** Using Two Pathways



Earlier investigations already demonstrated that both enzymes, ADA and AMPDA, could tolerate some substitution at the 2',3'-positions. However, AMPDA seemed a more versatile biocatalyst, since it converted a larger number of adenosine into the corresponding inosine derivatives in a time well suitable for preparative purposes.⁹ Santaniello et al.'s studies showed that solubilizing the substrate with a few percent of a cosolvent like pyridine or DMSO reduced somewhat the deamination rate of ADA, and the recovery of the products often proved troublesome. However, the same reaction at 50–60 °C rendered many substrates soluble in water, and the ADA enzyme still showed good activity.¹¹ Generally, a quite high tolerance of ADA for changes in the N-heterocyclic positions 2 and 6 (to be hydrolyzed) is observed, yet steric hindrance at the 2'- and 3'-positions, or if bulky groups other than OH are present at the 5'-position, disfavors the complete fitting of the substrate within the active site of ADA, which leads to a slow reactivity or even total inhibition.^{12–14}

With this communication we wish to, first, add new evidence for AMPDA's tolerance toward higher temperature,

prolonged reactions times, replacement of the 2'-hydroxyl group by fluorine, and most interestingly, much bulkier substituents at the 3' position impossible to handle by ADA, all of which makes AMPDA a highly welcome alternative. AMPDA is commercially available as a practical lyophilate powder, and the cost per unit of reactivity may be some 30% lower than that of ADA. The experimental workup of large amounts of bulk enzyme poses absolutely no problems even for as highly polar molecules as our target compounds. A simple filtration of the reaction mixture over a short silica gel column using a step gradient mixture of ethyl acetate–methanol–water as the eluant provides, after lyophilization from water, solid product material in high yields and chemical purity (see the Experimental Part in the Supporting Information).

Second, the recently published optimized Staudinger–Vilarrasa coupling conditions,^{10b,c} which provide high yield access to amides directly from sterically demanding azides, proved much more chemoselective than observed under other modified Staudinger conditions.¹⁵ The activation of the Boc-amino acid by HOBt does not lead to significant side reactions when coupled to an iminophosphorane that contains at least two competing nucleophilic centers in addition to the one generated by the in situ addition of tributylphosphine to 2',5'-dihydroxyazide **8** (Scheme 1). The new synthetic intermediates and target compounds were characterized through MS and ¹H, ¹⁹F, and ¹³C NMR (Supporting Information) and will be subjected to biological assays. A more systematic investigation of this minimal protection version of the Staudinger–Vilarrasa coupling is currently underway (such as, for instance, the direct conversion of **7** into **3**).

(11) (a) Ciuffreda, P.; Casati, S.; Santaniello, E. *Tetrahedron* **2000**, *56*, 3239. (b) Ciuffreda, P.; Loseto, A.; Santaniello, E. *Tetrahedron* **2002**, *58*, 5767–5771.

(12) (a) Bloch, A.; Robins, M. J.; McCarthy, J. R., Jr. *J. Med. Chem.* **1967**, *10*, 908. (b) Ciuffreda, P.; Loseto, A.; Santaniello, E. *Tetrahedron: Asymmetry* **2002**, *13*, 239. (c) Ciuffreda, P.; Loseto, A.; Santaniello, E. *Tetrahedron: Asymmetry* **2004**, *15*, 203. (d) Ciuffreda, P.; Alessandrini, L.; Pavlovic, R.; Santaniello, E. *Nucleosides, Nucleotides, Nucleic Acids* **2007**, *26*, 121.

(13) (a) Maury, G.; Daiboun, T.; Elalaoui, A.; Genu-Dellac, C.; Périgaud, C.; Bergogne, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides, Nucleotides* **1991**, *10*, 1677–1692. (b) Nair, V.; Wiechert, R. *J. Bioorg. Chem.* **1980**, *9*, 423.

(14) (a) Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. *J. Med. Chem.* **1996**, *39*, 277. (b) Wilson, D. K.; Quiocho, F. A. *Biochemistry* **1993**, *32*, 1689.

(15) Kimmerlin, T.; Seebach, D. *J. Peptide Res.* **2005**, *65*, 229.

Supporting Information Available: Experimental protocols and chromatographic and spectroscopic characterization of all new synthetic intermediates and final products.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL070818Q