ChemPubSoc Europe

DOI: 10.1002/ejoc.201501412

EurJOC European Journal of Organic Chemistry

Communication

Acyclic Nucleoside Synthesis

A Chemoenzymatic Route To Prepare Acyclic Nucleoside Analogues

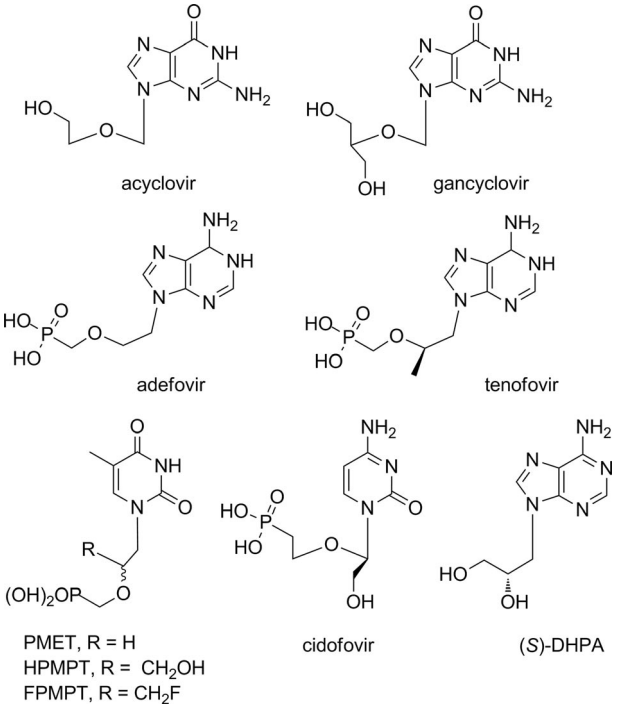
Martín A. Palazzolo,[a,b] Mariano J. Nigro,[a] Adolfo M. Iribarren,[a,c] and Elizabeth Sandra Lewkowicz*[a]

Abstract: Acyclic nucleosides are potential antiviral and antitumor agents. In this work, their preparation through a novel chemoenzymatic procedure involving the *N*-alkylation of a nucleobase and subsequent aldol condensation catalyzed by a dihydroxyacetone phosphate-dependant aldolase (Rabbit Muscle Aldolase, RAMA) is described.

Introduction

Nucleoside analogues have are a valuable source of antiviral and antitumor agents.[1] The discovery of the antiviral activity of two acyclic analogues of guanosine and adenosine in 1978, 9-(2-hydroxyethoxymethyl)guanine (acyclovir)[2] and *S*-9-(2,3-dihydroxypropyl)adenine (*S*-DHPA),[3] encouraged the study on this family of nucleosides (Scheme 1). Acyclic nucleoside (AN) analogues are used as antiviral agents due to the ability of the corresponding triphosphate derivatives to function as chain terminators during DNA or RNA biosynthesis and because of their enhanced chemical and metabolic stability.[4] Among them, acyclovir and ganciclovir are potent antiherpetic drugs, whereas thymine (phosphonomethoxy) alkyl derivatives such as 1-[2-(phosphonomethoxy)ethyl]thymine (PMET), 1-[3-hydroxy-2-(phosphonomethoxy)propyl]thymine (HPMPT) and 1-[3-fluoro-2-(phosphonomethoxy)propyl]thymine (FPMPT) were found as inhibitors of thymidine phosphorylase from SD-lymphoma.[5]

AN phosphonates also exhibit antiviral and cytostatic activity. Adefovir and tenofovir are useful compounds against HBV and HIV infections[6] while cidofovir has been approved for treatment of CMV retinitis in AIDS patients and in other viral diseases like papilloma virus-induced warts.[7] As a general statement, ANs possess a branched or linear alkyl chain, substituted with at least one hydroxy group, attached to the N atom of the nucleobase that mimics the pentofuranose ring in natural nucleosides. Moreover, the flexibility of the alkyl chain enables the acyclic analogues to adopt a suitable conformation to fit in the active site of the enzymes involved in their metabolism.[8] These characteristics, combined with the need of new active compounds against drug resistant viruses, have led to the constant development of new AN structures and consequently, methodologies for their synthesis.



Scheme 1. Acyclic nucleoside analogues.

Efforts have also been focused on the preparation of different derivatives of ANs with the aim of tuning characteristics like hydrophilicity, solubility, electronic properties and specific binding, as in the case of phosphonates, that provide improved phosphatase resistance,[9,10] or L-valine esters prodrugs with increased bioavailability.[11] The stereochemistry of the ANs often plays an important role in determining their biological activity

[a] *Laboratorio de Biocatálisis y Biotransformaciones, Departamento de Ciencia y Tecnología*
Universidad Nacional de Quilmes
Roque Sáenz Peña 352, 1876 Bernal, Buenos Aires, Argentina
E-mail: elewko@unq.edu.ar
[b] *Dr. M. Palazzolo. Present address*
Area de Química Orgánica, Facultad de Química, Bioquímica y Farmacia
Universidad Nacional de San Luis, INTEQUI-CONICET
San Luis 5700, Argentina
[c] *Laboratorio de Química de Ácidos Nucleicos*
INGEBI-CONICET
Vuelta de Obligado 2490, 1428 C.A.B.A, Argentina
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201501412.

and selectivity. For instance, the (*R*)-enantiomer of tenofovir, is about 50 times more effective against HIV than its (*S*)-counterpart.[9]

A number of synthetic routes to prepare acyclic nucleoside analogues in enantiomerically pure form have been reported, starting from optically active compounds like carbohydrates, diethyl L-tartrate, L-ascorbic acid, D-lactose or nucleosides.[12] Acyclic pyrimidine nucleoside analogues were successfully synthesized by coupling a commercially available acyclic moiety with 6-azapyrimidines using the Vorbrüggen procedure.[13] Special efforts have been directed towards the synthesis of acyclic purine analogues, since the preparation of such derivatives comprises low-yield multistep procedures.[6] Recently, we reported an efficient organocatalysed strategy for the synthesis of novel ANs, though racemic mixtures were obtained.[14]

One of the most explored biocatalyzed routes that provided a wide platform of nucleoside analogues involves the use of nucleoside phosphorylases as biocatalysts.[15] Nevertheless, the synthesis of acyclic derivatives of nucleosides by means of biocatalysis has not been explored so far.

Aldolases catalyze the reversible formation of C–C bonds by the aldol addition of a nucleophilic donor, usually a ketone enolate, onto an electrophilic aldehyde acceptor. These enzymes can usually accept a wide variety of aldehydes as acceptors, but the donor compound is often structurally invariable.[16] For this reason, aldolases can be classified into different classes according to their donor specificity. The most studied group utilizes dihydroxyacetone phosphate (DHAP) as donor, being its best known member fructose 1,6-bisphosphate (FDP) aldolase. The rabbit muscle aldolase (RAMA), the most versatile and useful enzyme among the available FDP aldolases, displays a broad synthetic scope, which includes the preparation of sugars, deoxysugars, fluorosugars, iminocyclitols, macrocycles, macrolide antibiotics, pheromones, terpenoids, and alkaloids.[17] Wong and co-workers explored the use of RAMA in the synthesis of adenosine analogues carrying modified sugar moieties, describing the production of 6-adenyl-6-deoxy-D-fructose and 6-adenyl-6-deoxy-L-sorbose.[18] Apart from RAMA, the utility of a glycine-dependent aldolase was also demonstrated in the synthesis of the precursors for the preparation of a short-chain RNA mimic reported by Miura et al.[19]

The production of monophosphorylated nucleoside derivatives as prodrugs is highly desired.[20] The presence of a phosphate group increases the solubility of the parent drug by several orders of magnitude[21] and furthermore, phosphate esters are readily cleaved by endogenous phosphatases releasing rapidly the pharmacologically active component. To this group of derivatives belongs fludarabine monophosphate (9-β-D-arabinofuranosyl-2-fluoroadenine-5′-monophosphate), a hydrophilic prodrug widely used in the treatment of chronic lymphocytic leukaemia therapies.[22]

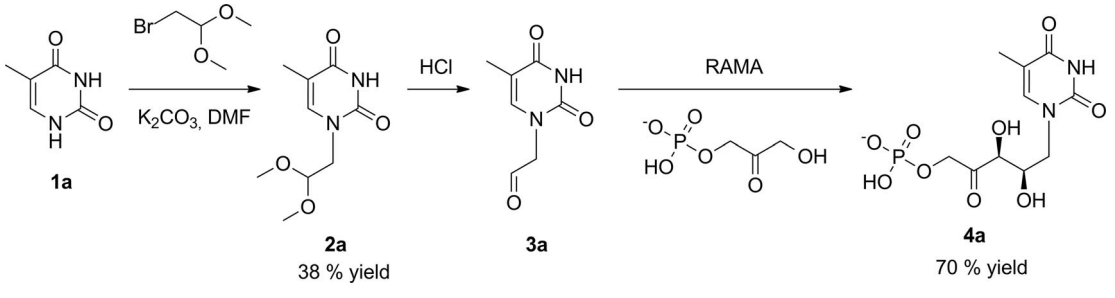
In the present communication, we report the production of AN monophosphates by means of a novel chemoenzymatic route that comprises the stereoselective formation of a specific C–C bond catalyzed by RAMA.

Results and Discussion

The traditional strategy for the synthesis of ANs consists of the cumbersome stereoselective preparation of the acyclic chain followed by regioselective coupling to the nucleobase. On the contrary, the chemoenzymatic route herein proposed takes advantage of the benefits provided by biocatalyzed reactions like simplicity, chemo-, regio-, and stereoselectivity and sustainability[23] allowing the easy and stereoselective building of the acyclic chain. However, enzyme specificity is still a concern and only particular kinds of acyclic moieties can be generated.

As a first attempt, we explored the synthesis of an acyclic derivative of thymine (Scheme 2). A key intermediate, the protected aldehyde derivative of the nucleobase, was prepared by adapting the procedure reported by Tanaka et al.[24] for the synthesis of CMP-sialic acid analogues (CMP = cytidine monophosphate). In this way, a mixture of thymine (**1a**) and K_2CO_3 in DMF was stirred in the presence of a suitable alkylating agent, such as 2-bromo-1,1-dimethoxyethane. After 24 h, the crude mixture was filtered and the solvent removed under reduced pressure to obtain compound **2a** in 38 % yield after purification by silica gel column chromatography.

Once quantitative acid hydrolysis of the acetal **2a** was achieved, the resulting reaction mixture containing the aldehyde **3a** was neutralized and incorporated into a buffered solution comprising DHAP and RAMA, and then stirred at room temperature. The consumption of **3a** was monitored by silica gel TLC whereas the formation of 5-(1-thymidyl)-3,4-dihydroxy-2-oxopentyl phosphate **4a** was quantified by HPLC-UV (86 % conversion) and its molecular weight confirmed by HPLC-MS. Finally, the product **4a** was isolated by reverse phase chromatography (70 % yield) and its structure analyzed by NMR spectroscopy. The overall yield of the reaction was 26.6 %, but it



Scheme 2. Schematic representation of the chemoenzymatic synthesis of an acyclic nucleoside analogue.

could be improved since the first chemical step was not optimized.

It is well established that aldolases have a high level of stereocontrol of the configuration at the formed chiral centers.[25] Therefore, the stereochemistry of the reaction is virtually independent of the structure of the acceptor substrate and thus the stereochemical outcome is highly predictable.[26]

In particular, in the active site of RAMA, the nucleophilic attack of the enamine intermediate formed between DHAP and the enzyme occurs on the *si*-face of the acceptor aldehyde, thus affording the product with (3*S*, 4*R*) stereochemistry with respect to the carbonyl group[16] as was previously stated by Schoewaart et al.[27] The coupling constant between H9 and H10 (*J*:1.89 Hz) confirms the *syn* configuration of the hydroxy groups in the stereogenic centers of **4a**.

The structural arrangement of compound **4a** yields an AN that mimics arabinothymidine monophosphate. Arabinonucleosides display antiviral and antitumoral activity and in particular, vidarabine – the corresponding adenine analogue – is an active nucleoside analogue currently used against viral infections caused by herpes simplex and varicella zoster viruses.[28]

After preparing the respective aldehydes, other nucleobases, such as adenine (**3b**), uracil (**3c**), and cytosine (**3d**), were evaluated as acceptors in RAMA-catalyzed reactions employing the developed chemoenzymatic strategy. HPLC analysis of the unpurified mixtures revealed similar conversions compared to the thymine-based molecule (**4b**: 88 %, **4c**: 83 %, **4d**: 88 %).

Conclusions

In this communication we have explored the stereoselective RAMA-catalyzed synthesis of novel AN analogues with potential pharmacological applications through a simple, clean and effective procedure. Ongoing research is devoted to the production of novel families of ANs by extending the chemoenzymatic route described herein employing other nucleobases, alkylating agents, and aldolases. The search for biological activities is also in progress.

Experimental Section

General Procedure for the Preparation of Aldehydes 3a–d

Mixtures of **1a**–**1d** (1 equiv.) and K_2CO_3 (2 equiv.) in DMF (10 mL) were stirred at 90 °C in the presence of 2-bromo-1,1-dimethoxyethane (2 equiv.). After 24 h, the reactions were filtered and the solvent was removed under reduced pressure. The crude mixtures were purified by column chromatography and characterized by 1D and 2D NMR spectroscopy. Aldehydes **3a**–**3d** were obtained after hydrolysis of the corresponding acetals **2a**–**2d** in HCl (1 N) at 90 °C for 1 h and neutralization with NaOH (10 N).

Biocatalyzed Aldol Condensation

The neutral reaction mixture containing **3a**–**3d** (0.08 mmol), DHAP (0.04 mmol) and commercially available rabbit muscle aldolase (RAMA, EC 4.1.2.13, 2 U) in phosphate buffer pH 7.5 (50 mM, 2 mL) was stirred at 200 rpm and 25 °C. After 3 h an additional portion of RAMA (2 U) was added. The crude biotransformation mixtures were filtered through a celite pad to remove the enzyme and the filtrates purified by C18 reverse-phase silica column chromatography. The purity was determined by HPLC and the identity was confirmed by NMR spectroscopy.

Acknowledgments

This work was supported by Universidad Nacional de Quilmes, CONICET and Secretaría de Ciencia y Técnica de la Nación, Argentina. E. S. L. and A. M. I. are research members of CONICET; M. A. P. and M. J. N. are CONICET and ANPCyT fellows respectively.

Keywords: Nucleosides · Enzyme catalysis · Lyases · Aldol reactions · Antiviral agents

[1] a) E. De Clercq, *J. Med. Chem.* **2010**, *53*, 1438–1450; b) L. P. Jordheim, D. Durantel, F. Zoulim, C. Dumontet, *Nature Rev. Drug Discov.* **2013**, *12*, 447–464.
[2] a) G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, H. J. Schaeffer, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5716–5720; b) H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, P. Collins, *Nature* **1978**, *272*, 583–585.
[3] E. De Clercq, J. Descamps, P. De Somer, A. Holý, *Science* **1978**, *200*, 563–565.
[4] S. Zhang, C. Switzer, J. C. Chaput, *Chem. Biodiversity* **2010**, *7*, 245–258.
[5] I. Votruba, K. Pomeisl, E. Tloustova, A. Holy, B. Otova, *Biochem. Pharmacol.* **2005**, *69*, 1517–1521.
[6] B. A. Trofimova, A. G. Mal'kina, V. V. Nosyreva, O. A. Shemyakina, A. P. Borisova, L. I. Larina, O. N. Kazheva, G. G. Alexandrov, O. A. Dyachenko, *Tetrahedron* **2010**, *66*, 1699–1705.
[7] M. M. Kaiser, P. Jansa, M. Dracínsky, Z. Janeba, *Tetrahedron* **2012**, *68*, 4003–4012.
[8] D. D'Alonzo, A. Guaragna, G. Palumbo, *Chem. Biodiversity* **2011**, *8*, 373–413.
[9] A. Paju, M. Päri, A. Selyutina, E. Žusinaite, A. Merits, T. Pehk, K. Siirde, A.-M. Müürisepp, T. Kailas, M. Lopp, *Nucleosides Nucleotides Nucleic Acids* **2010**, *29*, 707–720.
[10] a) K. Panigrahi, M. J. Eggen, J. Maeng, Q. Shen, D. B. Berkowitz, *Chem. Biol.* **2009**, *16*, 928–936; b) K. Panigrahi, D. L. Nelson, D. B. Berkowitz, *Chem. Biol.* **2012**, *19*, 666–667.
[11] a) E. De Clercq, *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 1–24; b) L. Iglesias, E. Lewkowicz, R. Medici, P. Bianchi, A. Iribarren, *Biotechnol. Adv.* **2015**, *33*, 412–434.
[12] a) A. Hadj-Bouazza, R. Zerrouki, P. Krausz, G. Laumond, A. M. Aubertin, Y. Champavier, *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 1249–1263; b) M.-S. Xie, H.-Y. Niu, G.-R. Qu, H.-M. Guo, *Tetrahedron Lett.* **2014**, *55*, 7156–7166.
[13] M. Jasamai, C. Simons, J. Balzarini, *Nucleosides Nucleotides Nucleic Acids* **2010**, *29*, 535–541.
[14] M. A. Palazzolo, M. Pérez-Sánchez, A. M. Iribarren, E. S. Lewkowicz, P. Domínguez de María, *Tetrahedron Lett.* **2012**, *53*, 6797–6800.
[15] E. S. Lewkowicz, A. M. Iribarren, *Curr. Org. Chem.* **2006**, *10*, 1197–1215.
[16] a) E. García-Junceda, in *Multi-Step Enzyme Catalysis: Biotransformations and Chemoenzymatic Synthesis* (Ed.: E. García-Junceda), Wiley-VCH, Weinheim, **2008**, pp. 61–81; b) P. Clapés, W.-D. Fessner, G. A. Sprenger, A. K. Samland, *Curr. Opin. Chem. Biol.* **2010**, *14*, 154–167.
[17] a) M. D. Bednarski, E. S. Simon, N. Bischofberger, W.-D. Fessner, M.-J. Kim, W. Lees, T. Saito, H. Waldmann, G. M. Whitesides, *J. Am. Chem. Soc.* **1989**, *111*, 627–635; b) S. M. Dean, W. A. Greenberg, C.-H. Wong, *Adv. Synth. Catal.* **2007**, *349*, 1308–1320; c) M. Brovetto, D. Gamenara, P. Sáenz Méndez, G. A. Seoane, *Chem. Rev.* **2011**, *111*, 4346–4403.
[18] K. K.-C. Liu, C.-H. Wong, *J. Org. Chem.* **1992**, *57*, 4798–91.
[19] T. Miura, F. Masayuki, S. Kazushi, K. Ikuo, J. Naganoma, T. Kajimoto, I. Yoshiteru, *Tetrahedron Lett.* **1998**, *39*, 7313–7316.
[20] R. Médici, J. I. Garaycoechea, A. L. Valino, C. A. Pereira, E. S. Lewkowicz, A. M. Iribarren, *Appl. Microbiol. Biotechnol.* **2014**, *98*, 3013–22.

[21] T. Heimbach, D. Fleischer, A. Kaddoumi, in *Prodrugs: challenges and rewards* (Eds.: S. V. Borchart, M. Hageman, R. Oliya, H. Maag, J. W. Tilley), Springer, New York, **2007**, pp. 160–99.
[22] T. Robak, A. Korycka, E. Lech-Maranda, P. Robak, *Molecules* **2009**, *14*, 1183–1226.
[23] N. J. Turner, E. O'Reilly, *Nat. Chem. Biol.* **2013**, *9*, 285–288.
[24] T. Tanaka, M. Ozawa, T. Miura, T. Inazu, S. Tsuji, T. Kajimoto, *Synlett* **2002**, *9*, 1487–1490.
[25] a) M. G. Silvestri, G. Desantis, M. Mitchell, C.-H. Wong, in *Topics in Stereochemistry* (Ed.: S. E. Denmark), John Wiley & Sons Inc., Hoboken, NJ, **2003**, pp. 267–342; b) H. J. M. Gijsen, L. Qiao, W. Fitz, C.-H. Wong, *Chem. Rev.* **1996**, *96*, 443–474.
[26] J. Sukumaran, U. Hanefeld, *Chem. Soc. Rev.* **2005**, *34*, 530–542.
[27] R. Schoewaart, F. van Rantwijk, R. A. Sheldon, *Tetrahedron: Asymmetry* **1999**, *10*, 705–711.
[28] a) N. Miwa, K. Kurosaki, Y. Yoshida, M. Kurokawa, S. Saito, K. Shiraki, *Antiviral Res.* **2005**, *65*, 49–55; b) A. Shokar, A. Au, S. Hwan, E. Tong, G. Garza, J. Zayas, S. F. Wnuk, K. M. Land, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4203–4205.

Received: November 6, 2015
Published Online: January 29, 2016