JOC*Note*

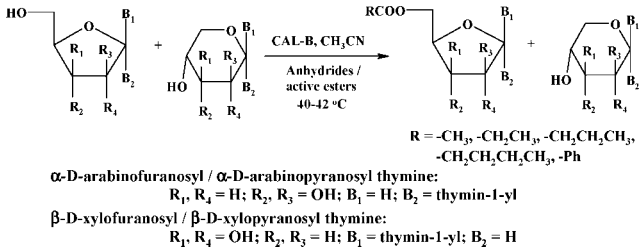
Efficient and Selective Enzymatic Acylation Reaction: Separation of Furanosyl and Pyranosyl Nucleosides

Jyotirmoy Maity,[†] Gaurav Shakya,[†] Sunil K. Singh,[†] Vasulinga T. Ravikumar,[‡] Virinder S. Parmar,[†] and Ashok K. Prasad*[,†]

Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi-110 007, India, and Isis Pharmaceuticals Inc., 2292 Faraday Avenue, Carlsbad, California 92008

ashokenzyme@yahoo.com

Received April 3, 2008



Candida antarctica lipase-B (CAL-B) immobilized on lewatite selectively acylated the primary hydroxyl group of the furanosyl nucleoside in a mixture of 1-(α-D-arabinofuranosyl)thymine and 1-(α-D-arabinopyranosyl)thymine. This selective biocatalytic acylation of furanosyl nucleoside has enabled us an easy separation of arabinofuranosyl thymine from an inseparable mixture with arabinopyranosyl thymine. The primary hydroxyl selective acylation methodology of arabinonucleoside has also been successfully used for the separation of 1-(β-D-xylofuranosyl)thymine and 1-(β-D-xylopyranosyl)thymine from a mixture of the two, which demonstrate the generality of the enzymatic methodology for separation of furanosyl and pyranosyl nucleosides.

Modified nucleosides of arabinofuranosyl moiety have attracted much attention as antiviral,[1] anticancer,[2] antimicrobial,[3] antitumor,[4] antihepatitis B virus,[5,6] antimycobacterial,[7] and cytostatic agents.[8]

Arabinonucleosides with pyranosyl configuration have been used as nucleoside monomers in the synthesis of oligonucleotides for the evaluation of their importance in etiology of nucleic acid structure.[9] In one of our research programs we aimed to synthesize α-D-arabinofuranosyl nucleosides but all our efforts of condensation of tetraacetylated arabinose with thymine led to the formation of an inseparable mixture of α-D-arabinofuranosyl and α-D-arabinopyranosyl nucleosides in varying proportions depending on the temperature of the reaction.

Imbach and co-workers[10] have generalized Guthrie−Smith[11] methodology for the synthesis of tetraacetate of five-membered aldofuranose sugars. They synthesized α- and β-anomers of tetra-*O*-acetyl-D-aldopentofuranosides starting from D-ribose, D-arabinose, D-xylose, and D-lyxose by their methanolysis, acetylation, and acetolysis protocol with an overall yield of ∼70%.

Contrary to this, Bristow and Lythgoe[12] pointed out that the preparative procedure for 1,2,3,5-tetra-*O*-acetylribofuranosyl starting from D-ribose cannot be applied in its entirety to other pentoses such as D-arabinose and D-xylose, possibly because of the existence of the sugar mainly in one cyclic form over the other at equilibrium in solution.

Further, during the synthesis of α- and β-D-xylofuranosyl nucleosides of the five naturally occurring bases, Imbach and co-workers[13] have also reported the existence of furanosyl sugar tetraacetate and the corresponding nucleosides with pyranose forms and found it very difficult to separate them on a preparative scale in contrast to their earlier report.[10] Thus far, no methodology exists for the efficient separation of mixtures of furanosyl and pyranosyl nucleosides.

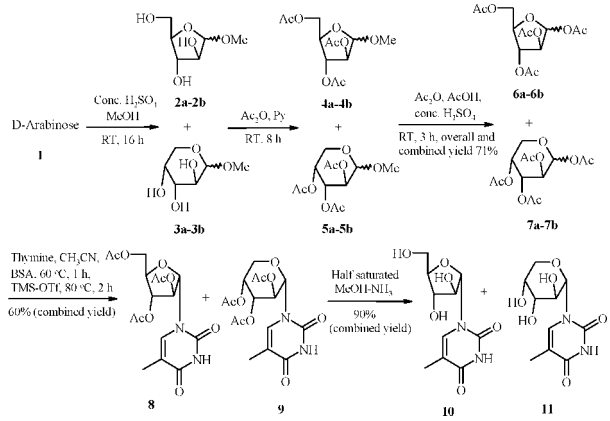
During the synthesis of α-D-arabinofuranosyl thymine from D-arabinose following literature procedure,[10] we obtained an anomeric mixture of 1,2,3,5-tetra-*O*-acetylarabinofuranoside (**6a**,**b**)[10] and 1,2,3,4-tetra-*O*-acetylarabinopyranoside (**7a**,**b**). The Vorbruggen's coupling[14] of thymine with the anomeric mixtures of furanosides **6a**,**b** and pyranosides **7a**,**b** afforded a mixture of 1-(2′,3′,5′-tri-*O*-acetyl-α-D-arabinofuranosyl)thymine (**8**)[15] and 1-(2′,3′,4′-tri-*O*-acetyl-α-D-arabinopyranosyl)thymine (**9**),

[†] University of Delhi.
[‡] Isis Pharmaceuticals Inc.

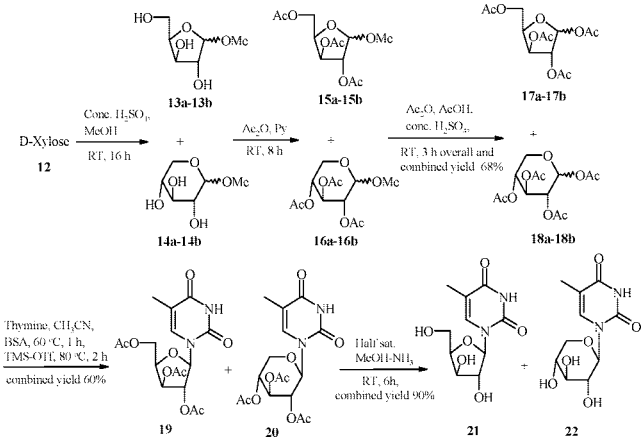
(1) Pankiewicz, K. W.; Nawrot, B.; Gadler, H.; Price, R. W.; Watanabe, K. A. *J. Med. Chem.* **1987**, *30*, 2314–2316.
(2) Miura, S.; Yoshimura, Y.; Endo, M.; Satoh, H.; Machida, H.; Sasaki, T. *Cancer Lett.* **1999**, *144*, 177–182.
(3) Bosco, M.; Bisseret, P.; Constant, P.; Eustache, J. *Tetrahedron Lett.* **2007**, *48*, 153–157.
(4) Ohtawa, M.; Ichikawa, S.; Teishikata, Y.; Fujimuro, M.; Yokosawa, H.; Matsuda, A. J. *Med. Chem.* **2007**, *50*, 2007–2010.
(5) Bhattacharya, B. K.; Ojwang, J. O.; Rando, R. F.; Huffman, J. H.; Revankar, G. R. *J. Med. Chem.* **1995**, *38*, 3957–3966.
(6) Ma, T.; Lin, J. S.; Newton, M. G.; Cheng, Y. C.; Chu, C. K. *J. Med. Chem.* **1997**, *40*, 2750–2754.
(7) Johar, M.; Manning, T.; Tse, C.; Desroches, N.; Agrawal, B.; Kunimoto, D. Y.; Kumar, R. *J. Med. Chem.* **2007**, *50*, 3696–3705.
(8) Jungmann, O.; Beier, M.; Luther, A.; Huynh, H. K.; Ebert, M. O.; Jaun, B.; Krishnamurthy, R.; Eschenmoser, A. *Helv. Chim. Acta* **2003**, *86*, 1259–1308.
(9) Cristofoli, W. A.; Wiebe, L. I.; De Clercq, E.; Andrei, G.; Snoeck, R.; Balzarini, J.; Knaus, E. E. *J. Med. Chem.* **2007**, *50*, 2851–2857.
(10) Kam, B. L.; Barascut, J. L.; Imbach, J. L. *Carbohydr. Res.* **1979**, *69*, 135–142.
(11) Guthrie, R. D.; Smith, S. C. *Chem. Ind.* **1968**, 547–548.
(12) (a) Bristow, N. W.; Lythgoe, B. *J. Chem. Soc.* **1949**, 2306–2309. (b) Chang, P.; Lythgoe, B. *J. Chem. Soc.* **1950**, 1992–1993.
(13) Gosselin, G.; Bergogne, M. C.; Rudder, J.; De Clercq, E.; Imbach, J. L. *J. Med. Chem.* **1986**, *29*, 203–213.
(14) Niedbella, U.; Vorbruggen, H. *J. Org. Chem.* **1974**, *39*, 3654–3660.
(15) Danishefsky, S.; Chow, K. *J. Org. Chem.* **1990**, *55*, 4211–4214.

10.1021/jo800731u CCC: $40.75
Published on Web 06/17/2008

SCHEME 1. Procedure for the Synthesis of 10 and 11



SCHEME 2. Procedure for the Synthesis of 21 and 22

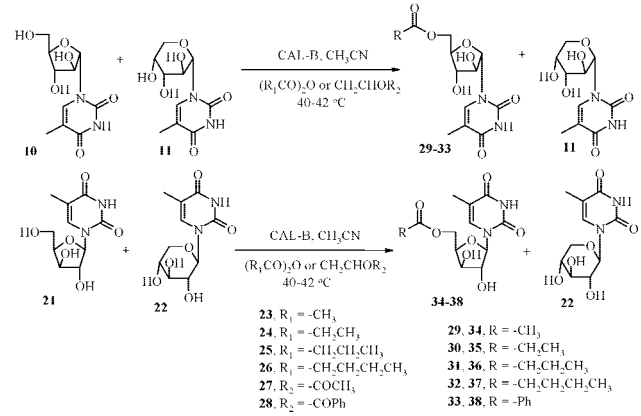


which on treatment with half-saturated methanolic ammonia gave a mixture of 1-(α-D-arabinofuranosyl)thymine (**10**)[16,17] and 1-(α-D-arabinopyranosyl)thymine (**11**)[8] in the ratio of 7.2:1 (Scheme 1).

The extension of study on D-xylose also led to the formation of 1,2,3,5-tetra-*O*-acetylxylofuranoside (**17a**,**b**)[10] and 1,2,3,4-tetra-*O*-acetylxylopyranoside (**18a**,**b**), which on Vorbruggen's coupling[14] with thymine followed by treatment of the resulting 1-(2′,3′,5′-tri-*O*-acetyl-β-D-xylofuranosyl)thymine (**19**)[15] and 1-(2′,3′,4′-tri-*O*-actyl-β-D-xylopyranosyl)thymine (**20**) with half-saturated methanolic ammonia afforded 1-(β-D-xylofuranosyl)thymine (**21**)[18,19] and 1-(β-D-xylopyranosyl)thymine (**22**)[19,20] in the ratio of 1.4:1 (Scheme 2).

The potential of enzymes in nucleoside chemistry[21] is well recognized for selective acylation of different functional groups of similar reactivity present in the molecule. Some of the lipases have been found to selectively acylate/deacylate primary hydroxyl over secondary hydroxyl group(s) of sugars[22] and nucleosides.[23] Earlier, lipases have been used for the separation

SCHEME 3. Separation of 10/ 11 and 21/ 22 by CAL-B-Catalyzed Selective Acylation Reaction



of nucleosides from their anomeric mixtures[24] and for the resolution of β-D/L-2′-deoxynucleosides.[25] Herein for the first time, a lipase-catalyzed selective acylation methodology has been developed for the separation of furanosyl and pyranosyl nucleosides from their mixture.

All our attempts to separate the mixture of arabinofuranosyl and arabinopyranosyl thymine **10** and **11**, and xylofuranosyl and xylopyranosyl thymine **21** and **22** by silica gel column chromatography failed. In the present study we have used *Candida antarctica* lipase-B (CAL-B)[26] for the separation of the furanosyl and the pyranosyl nucleosides of D-arabinose and D-xylose sugars. The CAL-B-catalyzed acylation of the furano and the pyrano nucleoside mixture of the two sugars was studied in different organic solvents, i.e., DCM, DIPE, THF, dioxane, and acetonitrile. The lipase in acetonitrile was found to be the best system for the efficient and selective acylation reaction of furano and pyrano nucleoside mixtures. CAL-B in acetonitrile selectively acylated the primary hydroxyl group in furano nucleosides of both sugars; since there is no primary hydroxyl group in pyrano nucleoside, it remained unreacted during the biocatalytic acylation reaction. There was an appreciable difference between the polarity of 5′-*O*-acylated furanonucleoside and pyranonucleoside, thus they were easily separated by column chromatography over silica gel.

In a typical reaction, the mixture of α-D-arabinofuranosyl and pyranosyl thymine **10** and **11**, or β-D-xylofuranosyl and pyranosyl thymine **21** and **22** and acetic anhydride **23** in acetonitrile were incubated with CAL-B (substrate−enzyme ratio, ∼1:0.5, w/w) at 40−42 °C and the progress of the reaction was monitored on TLC (Scheme 3). On completion of the reaction, the enzyme was filtered off and the solvent removed under reduced pressure. The residue thus obtained was purified by

(16) Adams, A. D.; Petrie, C. R.; Meyer, R. B. *Nucleic Acids Res.* **1991**, *19*, 3647–3651.

(17) Jorgensen, P. T.; Pedersen, E. B.; Nielsen, C. *Synthesis* **1992**, 1299–1306.

(18) (a) Gosselin, G.; Imbach, J. L. *Tetrahedron Lett.* **1981**, *22*, 4699–4702. (b) Gosselin, G.; Imbach, J. L. *J. Heterocycl. Chem.* **1982**, *19*, 597–602.

(19) Fox, J. J.; Yung, N.; Davoll, J.; Brown, G. B. *J. Am. Chem. Soc.* **1956**, *78*, 2117–2122.

(20) Wagner, T.; Huynh, H. K.; Krishnamurthy, R.; Eschenmoser, A. *Helv. Chim. Acta* **2002**, *85*, 399–416.

(21) (a) Prasad, A. K.; Trikha, S.; Parmar, V. S. *Bioorg. Chem.* **1999**, *27*, 135–154. (b) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319–4347.

(22) (a) Prasad, A. K.; Sorensen, M. D.; Parmar, V. S.; Wengel, J. *Tetrahedron Lett.* **1995**, *36*, 6163–6166. (b) Prasad, A. K.; Wengel, J. *Nucleosides, Nucleotides Nucleic Acids* **1996**, *15*, 1347–1359. (c) Prasad, A. K.; Kalra, N.; Yadav, Y.; Kumar, R.; Sharma, S. K.; Patkar, S.; Lange, L.; Wengel, J.; Parmar, V. S. *Chem. Commun.* **2007**, 2616–2617. (d) Prasad, A. K.; Kalra, N.; Yadav, Y.; Singh, S. K.; Sharma, S. K.; Patkar, S.; Lange, L.; Olsen, C. E.; Wengel, J.; Parmar, V. S. *Org. Biomol. Chem.* **2007**, *5*, 3524–3530.

(23) (a) Moris, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 10089–10098. (b) Ciuffreda, P.; Casati, P.; Santaniello, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1577–1582.

(24) (a) Damkjaer, D. L.; Petersen, M.; Wengel, J. *Nucleosides Nucleotides* **1994**, *13*, 1801–1807. (b) Garcia, J.; Diaz-Rodriguez, A.; Fernandez, S.; Sanghvi, Y.; Ferrero, M.; Gotor, V. *J. Org. Chem.* **2006**, *71*, 9765–9771.

(25) Garcia, J.; Fernandez, S.; Ferrero, M.; Sanghvi, Y.; Gotor, V. *Org. Lett.* **2004**, *6*, 3759–3762.

(26) The lipase CAL-B immobilized on lewatite has been obtained from Novozymes A/S (Copenhagen, Denmark).

TABLE 1. CAL-B-Catalyzed Regioselective Acylation of a Mixture of α-D-Arabinofuranosyl and Pyranosyl Thymine in Acetonitrile, Using Different Acylating Agents at 40−42 °C[a]

substrate	acylating agent	reaction time	product	yield[b] (%)
mixture of	**23**	6 days	**29**	40
furanoside **10** and	**24**	2.5 h	**30**	92
pyranoside **11**	**25**	45 min	**31**	97
	26	4 h	**32**	82
	27	1.5 h	**29**	95
	28	6 days	**33**	64

[a] All these reactions did not yield any product when performed in the absence of CAL-B. [b] The yields reported are based on the consideration of furanonucleosides as 100% in the mixture.

TABLE 2. CAL-B-Catalyzed Regioselective Acylation of a Mixture of β-D-Xylofuranosyl and Pyranosyl Thymine in Acetonitrile, Using Different Acylating Agents at 40−42 °C[a]

substrate	acylating agent	reaction time	product	yield[b] (%)
mixture of	**23**	6 days	**34**	25
furanoside **21** and	**24**	3 h	**35**	93
pyranoside **22**	**25**	1 h	**36**	96
	26	8 h	**37**	82
	27	2 h	**34**	92
	28	6 days	**38**	30

[a] All these reactions did not yield any product when performed in the absence of CAL-B. [b] The yields reported are based on the consideration of furanonucleosides as 100% in the mixture.

column chromatography to afford 5′-*O*-acetylated arabino and xylofuranosyl thymine **29** and **34** in 40% and 25% yields, respectively together with unreacted, recovered arabino and xylopyranosyl thymine **11** and **22** (Tables 1 and 2).

To find an efficient acylating agent, we carried out CAL-B-catalyzed acylation of a mixture of arabinofuranosyl/pyranosyl thymine **10/11** and xylofuranosyl/pyranosyl thymine **21/22** with different acid anhydrides **24**−**26** and two active esters, i.e., vinyl acetate **27** and vinyl benzoate **28**. The results compiled in Tables 1 and 2 clearly indicated that CAL-B most efficiently transfers the butanoyl group from butanoic anhydride to the primary hydroxyl group of α-D-arabinofuranosyl thymine **10** or β-D-xylofuranosyl thymine **21** when the mixtures of nucleosides **10/11** or **21/22** and butanoic anhydride were incubated with the lipase in acetonitrile at 40−42 °C. The efficiency of the acyl group transfer potential of the lipase decreases, both on descending or ascending the anhydrides from butanoic anhydride in the homologous series. Even the active ester, vinyl benzoate, was far from competing with the butanoic anhydride for the CAL-B-catalyzed benzoyl transfer reactions on nucleoside mixtures under study (Tables 1 and 2). In contrast to acetic anhydride, vinyl acetate was found to be a much better acetylating agent for the acetylation of both α-D-arabinofuranosyl thymine **10** and β-D-xylofuranosyl thymine **21** in the presence of CAL-B. Thus incubation of mixtures of nucleosides **10/11** or **21/22** with vinyl acetate leads to the formation of **29** and **34** in almost quantitative yields in 1.5 and 2.0 h, respectively; whereas the conversion was only 40% and 25% during the acetylation of the mixtures with acetic anhydride, that too in 6 days. However, butanoic anhydride was found to be a still better acylating agent than vinyl acetate. All the compounds prepared herein were unambiguously identified on the basis of their spectral (^{1}H, ^{13}C, ^{1}H−^{1}H COSY, NOE NMR, IR, and high-resolution mass spectral) data analysis. The structures of known compounds **6a**,**b**, **8**, **10**, **11**, **17a**,**b**, **19**, **21**, and **22** were further confirmed by the comparison of the physical and/or spectral data with those reported in the literature. All these reactions, when performed under identical conditions but without addition of CAL-B, did not yield any product.

In summary, it has been established that CAL-B selectively and efficiently catalyzes the butanoylation of the primary hydroxyl group in α-D-arabinofuranosyl thymine and β-D-xylofuranosyl thymine over the secondary hydroxyl groups in the molecule. It has also been demontrated for the first time that the primary hydroxyl group selectivity of CAL-B can efficiently be used for the separation of furanosyl and pyranosyl nucleoside mixtures of different pentoses, which is otherwise very difficult to achieve on preparative scale. This enzymatic methodology of separation of mixtures of arabinofuranosyl/pyranosyl thymine and xylofuranosyl/pyranosyl thymine may find applications in the preparation of pure derivatized furano nucleotides to be used for the synthesis of oligonucleotides on DNA synthesizer and for other useful applications.

Experimental Section

General Procedure for the Enzymatic Acylation Reaction on the Mixture of 10 and 11/21 and 22. To a mixture of 1-(α-D-arabinofuranosyl)thymine (**10**) and 1-(α-D-arabinopyranosyl) thymine (**11**)/1-(β-D-xylofuranosyl) thymine (**21**) and 1-(β-D-xylopyranosyl)thymine (**22**) (0.39 g, 1.5 mmol) and dry acetonitrile (25 mL), appropriate acid anhydride/vinyl acylate (1.05/0.7 equiv) was added, followed by CAL-B (200 mg). The reaction mixture was stirred in an incubator shaker at 40−42 °C and the progress of the reaction was monitored by TLC. Solubility of the reaction mixture increases with progress of the reaction. On completion, the reaction was stopped by filtering off the enzyme and the solvent was evaporated to dryness under reduced pressure. The crude product thus obtained was purified by column chromatography to afford 5′-*O*-acylated arabinofuranosyl thymine **29**−**33**/ 5′-*O*-acylated xylofuranosyl thymine **34**−**38** in 40−97%/25−96% yields (calculated by considering furanonucleoside as 100% in the mixture). The unreacted arabinopyranosyl thymine **11**/xylopyranosyl thymine **22** was recovered from the mixture in 60−85%/95−97% yields.

1-(5′-*O*-Acetyl-α-D-arabinofuranosyl)thymine (29). 29 was obtained as a colorless oil (0.16 g) in 40% yield (when acetic anhydride was used as acetylating agent)/(0.38 g) in 95% yield (when vinyl acetate was used as acetylating agent). R_f 0.45 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +43.6 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3416, 1720, 1698, 1474, 1372, 1265, 1046, and 783 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.64 (s, 3H), 1.88 (s, 3H), 3.75 (q, J = 4.5 Hz, 1H), 3.90−4.01 (m, 3H), 4.12−4.13 (m, 1H), 5.42 (d, J = 4.1 Hz, 1H), 5.57 (d, J = 4.7 Hz, 1H), 5.61 (d, J = 4.5 Hz, 1H), 7.39 (s, 1H), and 11.09 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + DMSO-d_6) δ 12.4, 20.9, 64.2, 75.7, 79.3, 83.0, 90.1, 109.5, 137.2, 150.9, 164.2, and 170.3; HRMS *m*/*e* calcd for $C_{12}H_{16}N_2O_7$ + Na (M + Na)$^+$ 323.0850, found 323.0837.

1-(5′-*O*-Propanoyl-α-D-arabinofuranosyl)thymine (30). 30 was obtained as a light yellow oil (0.38 g) in 92% yield. R_f 0.48 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +33.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3420, 1715, 1698, 1472, 1269, 1201, 1054 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (t, J = 7.4 Hz, 3H), 1.81 (s, 3H), 2.42 (q, J = 7.5 Hz, 2H), 4.25 (s, 1H), 4.29−4.41 (m, 2H), 4.51 (s, 2H), 5.03, 5.50 (2 × br s, 2H), 5.83 (s, 1H), 7.36 (s, 1H), and 10.91 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 9.4, 12.7, 27.8, 64.3, 76.3, 81.1, 85.5, 92.7, 110.2, 137.4, 151.9, 164.2, and 175.0; HRMS *m*/*e* calcd for $C_{13}H_{18}N_2O_7$ + Na (M + Na)$^+$ 337.1006, found 337.0997.

1-(5′-*O*-Butanoyl-α-D-arabinofuranosyl)thymine (31). 31 was obtained as a colorless oil (0.42 g) in 97% yield. R_f 0.5 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +31.1 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3442, 1703, 1477, 1270, 1099, 1059, 712 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, J = 7.3 Hz, 3H), 1.67 (hex, J = 7.3

Hz, 2H), 1.80 (s, 3H), 2.36 (t, J = 7.3 Hz, 2H), 4.22 (br s, 1H), 4.32−4.37 (m, 2H), 4.65 (br s, 1H), 5.34 (s, 1H), 5.85 (s, 1H), 5.90 (br s, 1H), 6.01 (br s, 1H), 7.37 (s, 1H), and 11.12 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.7, 14.0, 18.8, 36.4, 64.2, 76.3, 81.2, 85.4, 92.6, 110.2, 137.2, 152.0, 165.1, and 174.1; HRMS *m/e* calcd for $C_{14}H_{20}N_2O_7$ + Na $(M + Na)^+$ 351.1163, found 351.1161.

1-(5′-*O*-Pentanoyl-α-D-arabinofuranosyl)thymine (32). 32 was obtained as a white solid (0.37 g) in 82% yield. R_f 0.45 (10% methanol in chloroform, v/v); mp 186 °C; $[\alpha]^{32}_D$ +34.1 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3460, 1711, 1486, 1262, 1099, 989, and 706 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.5 Hz, 3H), 1.35 (sep, J = 7.5 Hz, 2H), 1.63 (p, J = 7.5 Hz, 2H), 1.78 (s, 3H), 2.38 (t, J = 7.5 Hz, 2H), 4.22 (br s, 1H), 4.30−4.34 (m, 3H), 4.51 (s, 2H), 5.23 (br s, 1H), 5.84 (s, 1H), 7.38 (s, 1H), and 11.10 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.7, 14.1, 22.6, 27.3, 34.2, 64.2, 76.4, 81.2, 85.9, 92.9, 110.0, 137.4, 151.9, 165.1, and 174.2; HRMS *m/e* calcd for $C_{15}H_{22}N_2O_7$ + Na $(M + Na)^+$ 365.1319, found 365.1308.

1-(5′-*O*-Benzoyl-α-D-arabinofuranosyl)thymine (33). 33 was obtained as a colorless oil (0.31 g) in 64% yield. R_f 0.53 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +16.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3437, 1712, 1694, 1271, 1096, 1061, and 708 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.76 (s, 3H), 4.31 (br s, 2H), 4.49−4.62 (m, 3H), 4.66 (s, 1H), 5.33 (br s, 1H), 5.90 (s, 1H), 7.38−7.45 (m, 3H), 7.53 (d, J = 7.4 Hz, 1H), 8.05 (d, J = 7.4 Hz, 2H), and 11.10 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.7, 64.7, 76.3, 81.2, 85.7, 92.8, 110.1, 128.9, 130.0, 130.2, 133.7, 137.3, 152.0, 165.1, and 166.9; HRMS *m/e* calcd for $C_{17}H_{18}N_2O_7$ + Na $(M + Na)^+$ 385.1006, found 385.0997.

1-(5′-*O*-Acetyl-β-D-xylofuranosyl)thymine (34). 34 was obtained as a sticky white solid (0.07 g) in 25% yield (when acetic anhydride was used as acetylating agent)/(0.24 g) in 92% yield (when vinyl acetate was used as acetylating agent). R_f 0.45 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +3.5 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3433, 1718, 1698, 1664, 1475, 1374, 1267, and 1054 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.85 (s, 3H), 2.03 (s, 3H), 3.45 (2 × br s, 2H), 4.06−4.08 (m, 2H), 4.33−4.40 (m, 3H), 5.82 (s, 1H), 7.64 (s, 1H), and 11.30 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + DMSO-d_6) δ 13.1, 21.3, 63.7, 76.0, 80.9, 81.6, 92.1, 109.8, 137.8, 151.3, 164.9, and 171.0; HRMS *m/e* calcd for $C_{12}H_{16}N_2O_7$ + Na $(M + Na)^+$ 323.0850, found 323.0842.

1-(5′-*O*-Propanoyl-β-D-xylofuranosyl)thymine (35). 35 was obtained as a sticky white solid (0.26 g) in 93% yield. R_f 0.48 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +7.3 (c 0.1, MeOH); IR (KBr) ν_{max} 3412, 1720, 1694, 1471, 1393, 1267, 1199, and 1086 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.51 (t, J = 7.5 Hz, 3H), 1.88 (s, 3H), 2.39 (q, J = 7.5 Hz, 2H), 4.11 (s, 1H), 4.22 (s, 1H), 4.33−4.42 (m, 2H), 4.50 (t, J = 7.3 Hz, 1H), 5.02, 5.32 (2 × br s, 2H), 5.78 (s, 1H), 7.62 (s, 1H), and 10.32 (br s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 9.7, 13.2, 27.6, 63.4, 75.9, 80.9, 81.4, 91.7, 109.5, 137.7, 151.4, 164.6, and 174.3; HRMS *m/e* calcd for $C_{13}H_{18}N_2O_7$ + Na $(M + Na)^+$ 337.1006, found 337.1000.

1-(5′-*O*-Butanoyl-β-D-xylofuranosyl)thymine (36). 36 was obtained as a white solid (0.28 g) in 96% yield. R_f 0.5 (10% methanol in chloroform, v/v); mp 69−70 °C; $[\alpha]^{32}_D$ +10.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3418, 1714, 1694, 1470, 1266, 1091, and 786 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 0.90 (t, J = 7.2 Hz, 3H), 1.56 (q, J = 7.2 Hz, 2H), 1.78 (s, 3H), 2.31 (t, J = 7.2 Hz, 2H), 3.98 (br s, 2H), 4.25−4.33 (m, 3H), 5.64 (br s, 1H), 5.71 (s, 1H), 5.80 (br s, 1H), 7.62 (s, 1H), and 11.40 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + DMSO-d_6) δ 11.6, 12.6, 17.1, 34.5, 61.7, 74.2, 79.3, 79.6, 90.0, 107.9, 136.1, 149.7, 163.0, and 171.9; HRMS *m/e* calcd for $C_{14}H_{20}N_2O_7$ + Na $(M + Na)^+$ 351.1163, found 351.1152.

1-(5′-*O*-Pentanoyl-β-D-xylofuranosyl)thymine (37). 37 was obtained as a sticky white solid (0.25 g) in 82% yield. R_f 0.45 (10% methanol in chloroform, v/v); $[\alpha]^{32}_D$ +24.9 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3412, 1708, 1695, 1470, 1266, 1178, and 1092 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.2 Hz, 3H), 1.26 (h, J = 7.2 Hz, 2H), 1.51 (p, J = 7.5 Hz, 2H), 1.76 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H), 3.97 (s, 2H), 4.22−4.24 (m, 1H), 4.30−4.32 (m, 2H), 5.65 (d, J = 3.0, 1H), 5.69 (s, 1H), 5.82 (d, J = 3.8 Hz, 1H), 7.61 (s, 1H), and 11.42 (br s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 11.6, 12.8, 20.8, 25.7, 32.4, 61.8, 74.1, 79.2, 79.5, 89.9, 107.9, 136.1, 149.8, 163.0, and 171.1; HRMS *m/e* calcd for $C_{15}H_{22}N_2O_7$ + Na $(M + Na)^+$ 365.1319, found 365.1311.

Acknowledgment. We thank the Department of Biotechnology (DBT, New Delhi) and ISIS Pharmaceuticals, USA for financial support to this work. J.M., G.S., and S.K.S. thank the Council of Scientific and Industrial Research (CSIR, New Delhi) for the award of senior research fellowships.

Supporting Information Available: Procedure for the preparation of **10/11** and **21/22**, spectral data of **11**, **22** and, **38**, and ^{1}H and ^{13}C spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800731U