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Synthesis and regioselective deacylation studies on peracylated 2′-azido *arabino*- and *ribo*-thymine nucleosides: Towards 5′-*O*,2′-*N*-linked oligonucleotides

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Peracylated 1-(2′-azido-α-L-arabino-/β-D-ribofuranosyl)thymine has been chemoenzymatically synthesized and subjected to deacylation studies in the presence of CAL-B (*Candida antarctica* lipase-B immobilized on polyacrylate) in acetonitrile. It is observed that CAL-B mediates highly selective deacylation of the ester function involving C-5′ hydroxyl group of the nucleosides leading to the formation of 1-(2′-azido-3′-*O*-acyl-α-L-arabino / β-D-ribofuranosyl)thymine in very high yields, which are otherwise very difficult to prepare by classical chemical methods. The 3′-*O*-acylated azido-nucleosides may be used as key precursors for the preparation of 5′-*O*-2′-*N*-linked oligonucleotides of biological importance.

Keywords: Nucleosides, oligonucleotides, *Candida antarctica,* lipase-B, L-arabinose

The synthesis of amino nucleosides and oligo-nucleotides involving them is gaining importance due to their applications as antiviral agents and in nucleic acid based drug development[1,2]. It has been found that the C-2′ position in nucleosides is an attractive place for modifications as it interferes less with base pairing[3-6]. The use of 2′-amino-2′-deoxypyrimidine nucleosides as the building blocks greatly facilitates the attachment of the linkers. A number of recent reports have demonstrated that 2′,5′-linked oligonucleotides bind selectively to complementary single stranded RNA but not to DNA[7-9]. This unique property can be used for the development of potential antisense drugs and for other diagnostic applications. It was revealed by molecular modelling studies that a 2′,5′-linkage *via* ether, ester, or amides leads to structures that should be able to hybridise to the corresponding sense strand[10]. Silverman *et al.* developed a novel class of chimeric ONs composed of an activator moiety, 2′,5′-linked oligoadenylates (2-5A) attached to a standard antisense cassette[11-13]. The antisense portion of the chimera functioned to recruit RNase L to the targeted RNA molecule while the 2-5A moiety activated the latent enzyme RNase L. It is an endoribonuclease that is activated on binding with 2-5A, thereby initiating the decay of mRNA and rRNA[14]. Though RNase L target RNA, synthesis of 2-5A in the proximity of viral RNA promotes preferential degradation of viral transcripts, thereby inhibiting viral replication. Gish *et al*.[15] demonstrated that the various 5′-, 2′- and 3′-esters of ara-C anticancer drug showed high biological activity of a long duration compared to ara-C itself as these are not substrates for cytidine deaminase and slowly release ara-C following enzymatic hydrolysis. In the on going biotransformation studies on different nucleosides, we synthesised herein arabino- and ribo- configured azido thymine nucleosides and have studied selective deacylation of their peracylates in the presence of CAL-B (*Candida antarctica* lipase-B immobilized on polyacrylate) in acetonitrile.

Results and Discussion

The 1-(α-L-arabinofuranosyl)thymine **4** was synthesized from L-arabinose in four steps in good yields following literature procedure[16]. In the 1st step, C-5OH of L-arabinose **1** was selectively acetylated in the presence of CAL-B, followed by chemical

acetylation to afford the tetraacetate **2**, which was coupled with base to afford the acetylated nucleoside **3**, this on deacetylation afforded the compound **4**, which was converted to the anhydride **5** and then to azidonucleoside **6** in moderate yields. The azidonucleoside was acylated using acetic, propanoic and butanoic anhydrides to afford the acetate **7a**, propanoate **7b** and butanoate **7c**, which were subjected to selective lipase-mediated enzymatic deacylation studies (**Scheme I**).

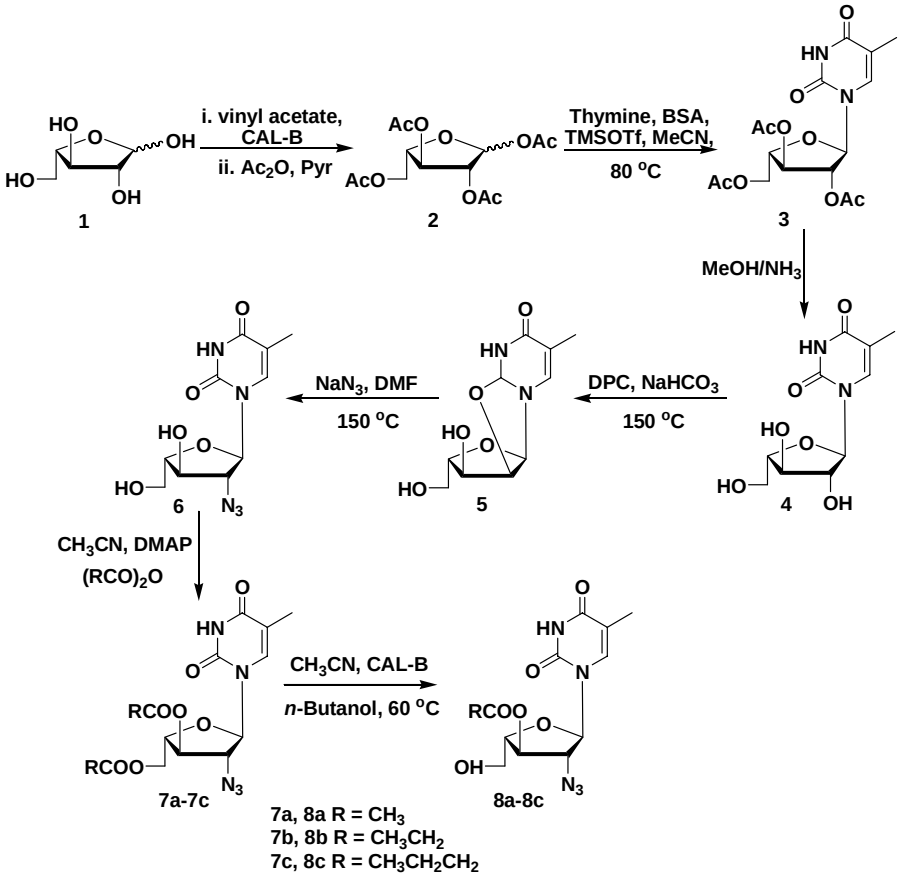
Different lipases, *e.g.* CAL-B (Novozyme 435), porcine pancreatic lipase (PPL), Amano PS, *Candida rugosa* lipase (CRL) and Lipozyme TL IM in various organic solvents, such as dioxane, tetrahydrofuran, diisopropyl ether (DIPE), acetonitrile, toluene and binary solvent systems such as toluene:DMF, toluene:DIPE, dioxane:acetonitrile, *etc*. in different ratio were screened for the selective deacylation of the peracylated compounds **7a-c**. It was observed that CAL-B in acetonitrile selectively catalyzed the deacylation in peracylates of both arabino- as well as ribo-configured 2′-azido thymidines. Other lipases screened for selective deacylation either did not catalyse the reactions or the reaction was too slow to be of any practical application. Further, it was found that acetonitrile was the solvent of choice in case of deacylation of peracylates of 2′-azido-α-L-arabinofuranosylthymine and the rate of the reaction was optimum at 60°C. While in case of deacylation of peracylates of 2′-azido-*β*-D-ribofuranosylthymine it was found that the reaction showed maximum selectivity in the binary mixture of toluene:DMF (3:1) at 50°C (**Scheme II**). The selectivity in case of 2′-azido-*β*-D-ribofuranosylthymine was not as good as in the case of 2′-azido-α-L-arabinofuranosylthymine; formation of another more polar completely deacylated product (may be completely deacylated 2′-azido-*β*-D-ribofuranosylthymine) was observed in minor amount.

In a typical reaction, to a solution of the peracylated nucleoside **7a**, **7b** or **7c** in acetonitrile, a drop of *n*-butanol, and CAL-B were added. The reaction mixture was stirred in an incubator shaker at 60°C and the progress of the reaction was monitored periodically by TLC. On completion, the reaction mixture was quenched by filtering off the enzyme and the residue was purified by column chromatography to afford 1-(3′-*O*-acetyl-2′-azido-*α*-L-arabinofuranosyl)thymine **8a**, 1-(2′-azido-3′-*O*-propanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine **8b** and 1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine **8c** in high yields: 93%, 91% and 88%, respectively (**Table I**). Similarly, selective deacylation of peracylated 2′-azido-*β*-D-ribofuranosylthymine derivatives **12a**, **12b** and **12c** was performed in the binary mixture of toluene:DMF (3:1) at 50°C (**Scheme II**). The reaction was quenched after completion and the products were isolated by silica gel column chromatography to yeield 1-(3′-*O*-acetyl-2′-azido-*β*-D-ribofuranosyl)thymine **13a**, 1-(2′-azido-2′-deoxy-3′-*O*-propanoyl-*β*-D-ribofuranosyl)thymine **13b** and 1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*β*-D-ribofuranosyl)-thymine **13c** in 65%, 72% and 78% yields, respectively.

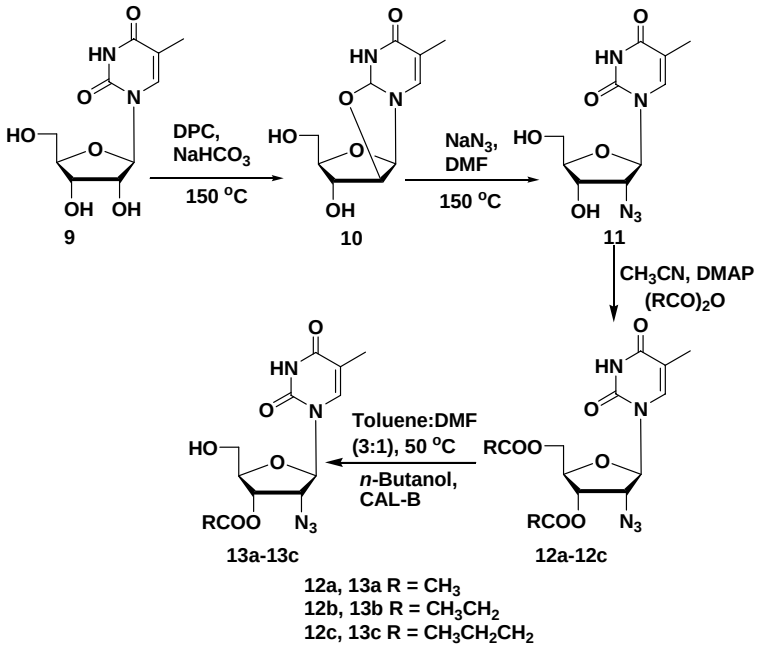
Thus, in all deacylation reactions, CAL-B selectively deacylated the ester function involving C-5′ hydroxyl group of the nucleosides leading to the formation of 1-(2′-azido-3′-*O*-acyl-α-L-arabino / β-D-ribofuranosyl)thymine in high to moderate yields, which is very difficult to prepare by classical chemical methods. The structures of all the peracylated 1-(2′-azido-α-L-arabino- /β-D-ribofuranosyl)-thymine derivatives **7a-c** and **12a-c** and enzymatically deacylated 1-(2′-azido-3′-*O*-acyl-α-L-arabino/β-D-ribofuranosyl)thymine derivatives **8a-c** and **13a-c** were unambiguously established on the basis of their spectral data (^{1}H and ^{13}C NMR, IR and HRMS) analysis. All these reactions did not yield any product when performed under similar conditions, but in the absence of CAL-B. The monoacylated nucleosides (**8a-c** and **13a-c**) were further reduced to afford 2′-amino-3′-acyl-α-L-arabinofuranosyl thymine (**14a-c**) and 2′-amino-3′-acyl-*β*-D-ribofuranosyl thymine (**15a-c**), respectively in the presence of 10% Pd-C/H_2 (**Scheme III**).

Experimental Section

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out using silica gel (100-200 mesh). Melting points were determined using H_2SO_4 bath and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 300 and at 75.5 MHz, respectively. The chemical shift values are reported as δ (ppm) relative to TMS used as internal standard and the coupling constants (*J*) are measured in Hz. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer. The FAB-HRMS spectra of all the compounds except **8b** and **8c** were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive mode using the matrix HEDS



Scheme I

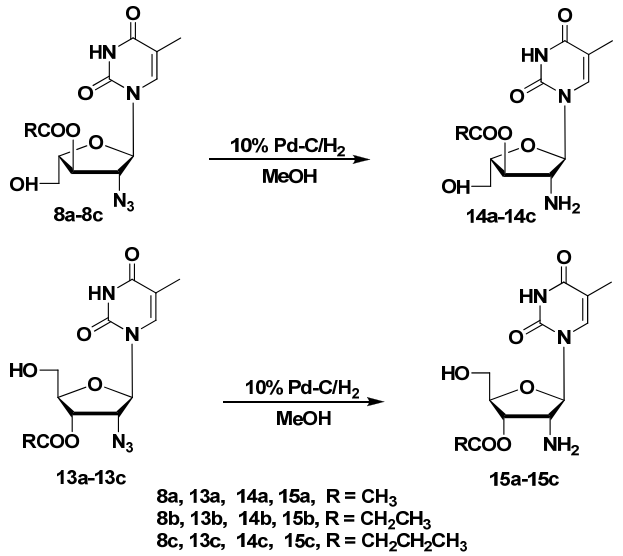


Scheme II

Table I — Novozyme-435 catalysed deacylation of preacylated nucleosides **7a-c** and **12a-c** in acetonitrile and toluene:DMF (3:1) at 60°C and 50°C, respectively in the presence of *n*-butanol as acyl scavenger.*

Reactant	Product	Reaction Time (hr)	Yield (%)
1-(3′,5′-Di-*O*-acetyl-2′-azido-2′-deoxy-*α*-L-arabinofuranosyl)thymine (**7a**)	1-(3′-*O*-Acetyl-2′-azido-2′-deoxy-*α*-L-arabinofuranosyl)thymine (**8a**)	18	93
1-(2′-Azido-2′-deoxy-3′,5′-di-*O*-propanoyl-*α*-L-arabinofuranosyl)thymine (**7b**)	1-(2′-Azido-2′-deoxy-3′-*O*-propanoyl-*α*-L-arabinofuranosyl)thymine (**8b**)	24	91
1-(2′-Azido-3′,5′-di-*O*-butanoyl-2′-deoxy-*α*-L-arabinofuranosyl)-thymine (**7c**)	1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine (**8c**)	30	88
1-(3′,5′-Di-*O*-acetyl-2′-azido-2′-deoxy-*β*-D-ribofuranosyl)thymine (**12a**)	1-(3′-*O*-Acetyl-2′-azido-2′-deoxy-*β*-D-ribofuranosyl)thymine (**13a**)	36	65
1-(2′-Azido-2′-deoxy-3′,5′-di-*O*-propanoyl-*β*-D-ribofuranosyl)thymine (**12b**)	1-(2′-Azido-2′-deoxy-3′-*O*-propanoyl-*β*-D-ribofuranosyl)thymine (**13b**)	28	72
1-(2′-Azido-3′,5′-di-*O*-butanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine (**12c**)	1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine (**13c**)	22	78

* All these reactions did not yield any product when performed under similar conditions, but in the absence of CAL-B



Scheme III

(*bis*hydroxyethyldisulphide) doped with sodium acetate. Mass spectra of compounds **8b** and **8c** were recorded on EM 6405 Transportable GC/ MS System. The *Candida antarctica* lipase and *Candida antarctica* lipolytic lipase (CAL, L on A, also known as Lipozyme TL IM), immobilized on accurel were a gift from Novozymes Inc. Company. The enzyme Amano PS was a gift from ISIS Pharmaceuticals. The enzymes *Candida rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (USA). The enzymes were dried over P_2O_5 under vacuum for 24 hr prior to use. All reactions were monitored with thin-layer chromatography (TLC) using silica gel coated plates with fluorescence indicator (SiO_2-60, F-254) and were visualized under UV light and by spraying with 5% conc. sulfuric acid in absolute ethanol (v/v), followed by heating.

General procedure for the esterification reaction

To a solution of 1-(2′-azido-2′-deoxy-*α*-L-arabinofuranosyl)thymine **(6**, 0.5 g) in acetonitrile and acid anhydride, *i.e.* acetic, propanoic or butanoic anhydride (2.2 equiv.) was added catalytic amount of DMAP (0.1 equiv.) and the reaction mixture was stirred for 4hr at RT and then acetonitrile was removed under vaccum. The residue was poured into ice-water. The mixture was extracted with ethyl acetate (3 × 25 mL), the combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution and dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The residue thus obtained was purified over silica gel column, eluting with 30-60% ethyl acetate-petroleum ether to afford the peracylated azido nucleosides **7a-c** in almost quantitave yields. Similarly, the peracylated azido nucleosides **12a-c** were obtained by treating the compound 1-(2′-azido-2′-deoxy-*β*-D-ribofuranosyl)-thymine (**11**, 0.5 g) with acid anhydrides, *i.e.* acetic, propanoic or butanoic anhydride (2.2 equiv.) in acetonitrile and catalytic amount of DMAP (0.1 equiv.). The work up was done in similar fashion as for **7a-c** and compounds **12a-c** were obtained after purification over silica gel, eluting with 30-40% ethyl acetate-petroleum ether.

1-(3′,5′-Di-*O*-acetyl-2′-azido-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 7a

It was obtained as an oil (0.602 g) in 93% yield. R_f = 0.45 (60% ethyl acetate-petroleum ether). IR (neat): 2115 (N_3), 1740 (CO), 1705, 1691 and 1463 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.96 (3H, s, C-5CH_3), 2.07 and 2.14 (6H, 2s, 2 × CH_3CO), 4.30 (2H, dd, *J* = 12 and 15.6 Hz, CH_2-OAc), 4.46 (1H, d, *J* = 2.7 Hz, C-2′H), 4.60 (1H, dd, *J* = 5.1 and 8.7 Hz, C-4′H), 5.12 (1H, t, *J* = 3 Hz, C-3′H), 5.84 (1H, d, *J* = 2.7 Hz, C-1′H), 7.15 (1H, s, C-6H) and 9.41 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.58 (C-5 CH_3), 20.06 and 20.77 (2 × CH_3), 63.02 (C-5′), 69.07 (C-3′), 76.62 (C-2′), 83.53 (C-4′), 91.35 (C-1′), 110.94 (C-5), 135.17 (C-6), 150.19 (C-4), 163.89 (C-2) and 169.55 and 170.58 (2 × CO); HRMS (ESI positive mode): *m/z* 390.1009 $[M+Na]^+$, Calcd for $C_{14}H_{17}N_5O_7$+Na 390.1020.

1-(2′-Azido-3′,5′-di-*O*-propanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 7b

It was obtained as an oil (0.628 g) in 90% yield. R_f = 0.60 (60% ethyl acetate-petroleum ether). IR (neat): 2120 (N_3), 1738 (CO), 1700, 1691 and 1460 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.10-1.20 (6H, m, 2 × C*H*$_3$CH$_2$CO), 1.95 (3H, s, C-5 CH_3), 2.28-2.46 (4H, m, 2 × CH_3C*H*$_2$CO), 4.25-4.36 (2H, m, CH_2-OPr), 4.43 (1H, d, *J* = 2.7 Hz, C-2′H), 4.59 (1H, dd, *J* = 5.1 and 8.1 Hz, C-4′H), 5.14 (1H, t, *J* = 2.7 Hz, C-3′H), 5.85 (1H, d, *J* = 2.7 Hz, C-1′H), 7.16 (1H, s, C-6H) and 9.47 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.75 and 8.96 (2 × *C*H_3CH$_2$CO), 12.56 (C-5CH_3), 27.33 (2 × CH_3*C*H$_2$CO), 62.93 (C-5′), 69.20 (C-3′), 76.61 (C-2′), 83.79 (C-4′), 91.28 (C-1′), 110.85 (C-5), 135.12 (C-6), 150.19 (C-4), 163.99 (C-2) and 172.96 and 174.02 (2 × CO); HRMS (ESI positive mode): *m/z* 418.1329 $[M+Na]^+$, Calcd for $C_{16}H_{21}N_5O_7$+Na 418.1333.

1-(2′-Azido-3′,5′-di-*O*-butanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 7c

It was obtained as an oil (0.709 g) in 95% yield. R_f = 0.60 (50% ethyl acetate-petroleum ether). IR (neat): 2118 (N_3), 1730 (CO), 1710, 1689 and 1464 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 and 0.96 (6H, 2t, *J* = 7.5 Hz, 2 × C*H*$_3$(CH$_2$)$_2$CO), 1.56-1.75 (4H, m, 2 × CH_3C*H*$_2$CH$_2$CO), 1.95 (3H, s, C-5 CH_3), 2.27 and 2.37 (4H, 2t, *J* = 7.2 and 7.5 Hz, 2 × CH_3CH_2C*H*$_2$CO), 4.24-4.36 (2H, m, CH_2-OBu), 4.43 (1H, t, *J* = 2.7 Hz, C-2′H), 4.58 (1H, dd, *J* = 5.1 and 8.4 Hz, C-4′H), 5.13 (1H, t, *J* = 2.7 Hz, C-3′H), 5.85 (1H, d, *J* = 3 Hz, C-1′H), 7.15 (1H, s, C-6H) and 9.29 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.57 (C-5CH_3), 13.54 and 13.62 (2 × *C*H_3(CH$_2$)$_2$CO), 18.20 and 18.29 (2 × CH_3*C*H$_2$CH$_2$CO), 35.79 and 35.86 (2 × CH_3CH_2*C*H$_2$CO), 62.81 (C-5′), 69.25 (C-3′), 76.49 (C-2′), 83.79 (C-4′), 91.29 (C-1′), 110.86 (C-5), 135.03 (C-6), 150.12 (C-4), 163.76 (C-2) and 172.16 and 173.15 (2 × CO); HRMS (ESI positive mode): *m/z* 446.1637 $[M+Na]^+$, Calcd for $C_{18}H_{25}N_5O_7$+Na 446.1646.

1-(3′,5′-Di-*O*-acetyl-2′-azido-2′-deoxy-*β*-D-ribofuranosyl)thymine, 12a

It was obtained as an oil (0.590 g) in 91% yield. R_f = 0.50 (60% ethyl acetate-petroleum ether). IR (neat): 2116 (N_3), 1740(CO), 1703, 1690 and 1450 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.95 (3H, s, C-5C*H*$_3$), 2.15 and 2.20 (6H, 2s, 2 × CH_3CO), 4.25 (1H, t, *J* = 5.4 Hz, C-2′H), 4.33-4.36 (3H, m, CH_2-OAc, C-4′H), 5.23 (1H, t, *J* = 5.4 Hz, C-3′H), 5.92 (1H, d, *J* = 5.1 Hz, C-1′H), 7.23 (1H, s, C-6H) and 9.41 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.72 (C-5CH_3), 20.48 and 20.83 (2 × CH_3), 62.70 (C-5′), 63.60 (C-3′), 71.49 (C-2′), 79.72 (C-4′), 88.10 (C-1′), 111.79 (C-5), 134.66 (C-6), 150.17 (C-4), 163.48 (C-2) and 170.05 and 170.11 (2 × CO); HRMS (ESI positive mode): *m/z* 390.1007 $[M+Na]^+$, Calcd for $C_{14}H_{17}N_5O_7$+Na 390.1020.

1-(2′-Azido-3′,5′-di-*O*-propanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine, 12b

It was obtained as an oil (0.656 g) in 94% yield. R_f = 0.65 (60% ethyl acetate-petroleum ether). IR (neat): 2110 (N_3), 1737 (CO), 1700, 1693 and 1455 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.16-1.25 (6H, m, 2 × C*H*$_3$CH$_2$CO), 1.95 (3H, s, C-5 CH_3), 2.37-2.52 (4H, m, 2 × CH_3C*H*$_2$CO), 4.19 (1H, t, *J* = 5.4 Hz, C-2′H), 4.31-4.35 (2H, m, CH_2-OPr), 4.38 (1H, d, *J* = 3 Hz, C-4′H), 5.24 (1H, t, *J* = 5.4 Hz, C-3′H), 5.94 (1H, d, *J* = 5.4 Hz, C-1′H), 7.26 (1H, s, C-6H) and 8.93 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.89 and 9.02 (2 × *C*H_3CH$_2$CO), 12.67 (C-5CH_3), 27.18 and 27.44 (2 × CH_3*C*H$_2$CO), 62.61 (C-5′), 63.70 (C-3′), 71.47 (C-2′), 79.95 (C-4′), 87.90 (C-1′), 111.83 (C-5), 134.54 (C-6), 150.07 (C-4), 163.23 (C-2) and 173.53 and 173.59 (2 × CO); HRMS (ESI positive mode): *m/z* 418.1325 $[M+Na]^+$, Calcd for $C_{16}H_{21}N_5O_7$ + Na 418.1333.

1-(2′-Azido-3′,5′-di-*O*-butanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine, 12c

It was obtained as an oil (0.717 g) in 96% yield. R_f = 0.50 (45% ethyl acetate-petroleum ether). IR (neat):

2117 (N_3), 1738 (CO), 1705, 1688 and 1464 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95-1.01 (6H, m, 2 × C*H*$_3$CH$_2$CH$_2$CO), 1.65-1.75 (4H, m, 2 × CH$_3$C*H*$_2$-CH$_2$CO), 1. 94 (3H, s, C-5 CH$_3$), 2.34-2.45 (4H, m, 2 × CH$_3$CH$_2$C*H*$_2$CO), 4.18 (1H, t, *J* = 4.8 Hz, C-2′H), 4.32-4.41 (3H, m, C-4′H and C*H*$_2$-OBu), 5.22 (1H, t, *J* = 5.1 Hz, C-3′H), 5.95 (1H, d, *J* = 5.1 Hz, C-1′H), 7.23 (1H, s, C-6H) and 8.99 (1H, s, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.66 (C-5CH$_3$), 13.56 and 13.65 (2 × *C*H$_3$CH$_2$CH$_2$CO), 18.23 and 18.33 (2 × CH$_3$*C*H$_2$CH$_2$CO), 35.59 and 35.97 (2 × CH$_3$CH$_2$-*C*H$_2$CO), 62.50 (C-5′), 63.71 (C-3′), 71.33 (C-2′), 79.95 (C-4′), 87.76 (C-1′), 111.81 (C-5), 134.49 (C-6), 150.11 (C-4), 163.33 (C-2) and 172.71 and 172.76 (2 × CO); HRMS (ESI positive mode): *m/z* 446.1643 $[M+Na]^+$, Calcd for $C_{18}H_{25}N_5O_7$+Na 446.1646.

General procedure for the selective biocatalytic desterification reaction: Generation of monoacylated azido nucleosides 8a-c and 13a-c

To a solution of the 1-(2′-azido-2′-deoxy-3′,5′-diacyl-*α*-L-arabinofuranosyl)thymine **7a-c** (1 mmole) in acetonitrile (15 mL) and *n*-butanol (1 equiv.) was added *Candida antarctica* lipase (CAL-B), also known as Novozyme 435 (0.150 g). The suspension was stirred at 60°C in a shaker incubator and the progress of the reaction was monitored periodically by TLC. Upon completion, the reaction was quenched by filtering off the enzyme and the solvent was removed under reduced pressure, and the residue thus obtained was purified by column chromatography over silica gel, eluting with 4-7% methanol in chloroform to afford the desired products 1-(2′-azido-2′-deoxy-3′-acyl-*α*-L-arabinofuranosyl)thymine **8a-c**. The azido nucleosides 1-(2′-azido-2′-deoxy-3′-acyl-*β*-D-ribofuranosyl)thymine **13a-c** were obtained by incubation of 1-(2′-azido-2′-deoxy-3′,5′-diacyl-*β*-D-ribofuranosyl)thymine **12a-c** (0.1 mmole) in a solution of toluene:DMF (3:1), *n*-butanol(1 equiv.) with the same enzyme *Candida antarctica* lipase (CAL-B, 0.150 g). The suspension was stirred at 50°C in an incubator shaker and the progress of the reaction was monitored periodically by TLC. Upon completion, the reaction was quenched by filtering off the enzyme and the solvent was removed under reduced pressure, and the residue thus obtained was purified by column chromatography over silica gel, eluting with 4-7% methanol in chloroform to afford the desired products **13a-c**.

1-(3′-*O*-Acetyl-2′-azido-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 8a

It was obtained as oil in (0.302 g) 93% yield. R_f = 0.45 (10% methanol-chloroform). IR (neat): 3388 (OH), 2113(N_3), 1713 (CO), 1664 and 1262 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (3H, s, C-5 CH$_3$), 2.05 (3H, s, CH$_3$CO), 3.54 (2H, dd, *J* = 2.4 and 5.2 Hz, C*H*$_2$-OH), 4.37 (1H, d, *J* = 4.2 Hz, C-4′H), 4.65 (1H, d, *J* = 4.8 Hz C-2′H), 5.15 (1H, d, *J* = 5.4 Hz, OH), 5.21 (1H, t, *J* = 4.5 Hz, C-3′H), 5.88 (1H, d, *J* = 5.1Hz, C-1′H), 7.56 (1H, s, C-6H) and δ 11.44 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 17.31 (C-5CH$_3$), 25.49 (CH$_3$), 66.33 (C-5′), 74.12 (C-3′), 81.28 (C-2′), 90.72 (C-4′), 95.06 (C-1′), 115.29 (C-5), 139.65 (C-6), 155.36 (C-4), 169.05 (C-2) and δ 174.48 (CO); HRMS (ESI positive mode): *m/z* 348.0904 $[M+Na]^+$, Calcd for $C_{12}H_{15}N_5O_6$+Na 348.0915.

1-(2′-Azido-3′-*O*-propanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 8b

It was obtained as an oil (0.308 g) in 91% yield. R_f = 0.5 (10% methanol-chloroform). IR (neat): 3410 (OH), 2115 (N_3), 1740 (CO), 1691 and 1260 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.01 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$CO), 1.78 (3H, s, C-5 CH$_3$), 2.33 (2H, d, *J* = 7.2 Hz, CH$_3$C*H*$_2$CO), 3.52 (2H, s, C*H*$_2$-OH), 4.37 (1H, d, *J* = 3 Hz, C-4′H), 4.61 (1H, s, C-2′H), 5.11 (1H, s, OH), 5.21 (1H, s, C-3′H), 5.86 (1H, d, *J* = 3.3 Hz, C-1′H), 7.53 (1H, s, C-6H) and 11.40 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 8.64 (*C*H$_3$CH$_2$CO), 12.04 (C-5CH$_3$), 26.73 (CH$_3$*C*H$_2$CO), 60.78 (C-5′), 67.69 (C-3′), 75.48 (C-2′), 83.91 (C-4′), 87.92 (C-1′), 109.54 (C-5), 135.84 (C-6), 150.36 (C-4), 163.75 (C-2) and 172.92 (CO); MS (ESI positive mode): *m/z* $[M]^+$ 339.

1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 8c

It was obtained as an oil (310 mg) in 88% yield. R_f = 0.65 (10% methanol-chloroform). IR (neat): 3387 (OH), 2110 (N_3), 1700 (CO), 1690 and 1258 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.84 (3H, t, *J* = 7.2 Hz, C*H*$_3$(CH$_2$)$_2$CO), 1.52 (2H, q, *J* = 7.5 Hz, CH$_3$C*H*$_2$CH$_2$CO), 1.79 (3H, s, C-5 CH$_3$), 2.29 (2H, t, *J* = 7.2 Hz), 3.52 (2H, s, C*H*$_2$-OH), 4.37 (1H, d, *J* = 3 Hz, C4′-H), 4.60 (1H, t, *J* = 4.8 Hz, C-2′H), 5.11 (1H, brs, OH), δ 5.22 (1H, t, *J* = 4.5 Hz, C-3′H), 5.85 (1H, d, *J* = 4.8 Hz, C-1′H), 7.54 (1H, s, C-6H) and 11.40

(1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.04 (C-5*C*H$_3$), 13.29 (*C*H$_3$(CH$_2$)$_2$CO), 17.69 (CH$_3$*C*H$_2$CH$_2$CO), 35.20 (CH$_3$CH$_2$*C*H$_2$CO), 60.76 (C-5′), 67.68 (C-3′), 75.33 (C-2′), 83.89 (C-4′), 87.91 (C-1′), 109.54 (C-5), 135.84 (C-6), 150.35 (C-4), 163.75 (C-2) and 170.07 (CO); MS (ESI positive mode): *m/z* [M]$^+$ 353.

1-(3′-*O*-Acetyl-2′-azido-2′-deoxy-*β*-D-ribofuranosyl)thymine, 13a

It was obtained as an oil (0.211 g) in 65% yield. R$_f$ = 0.5 (10% methanol-chloroform). IR (neat): 3390 (OH), 2112 (N$_3$), 1737 (CO), 1670 and 1255 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.87 (3H, s, C-5 CH$_3$), 2.01 (3H, s, CH$_3$CO), 3.62 (2H, s, C*H*$_2$-OH), 4.07 (1H, d, *J* = 2.7 Hz, C-4′H), 4.54 (1H, dd, *J* = 6.2 and 7.2 Hz, C-2′H), 5.36-5.38 (2H, m, OH, C-3′H), 5.91 (1H, d, *J* = 7.1 Hz, C-1′H), 7.84 (1H, s, C-6H) and 11.47 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.20 (C-5CH$_3$), 22.42 (*C*H$_3$CO), 60.63 (C-5′), 62.24 (C-3′), 72.35 (C-2′), 83.08 (C-4′), 85.12 (C-1′), 110.13 (C-5), 135.56 (C-6), 150.57 (C-4), 163.52 (C-2) and 171.89 (CO); HRMS (ESI positive mode): *m/z* 348.0902 [M+Na]$^+$, Calcd for C$_{12}$H$_{15}$N$_5$O$_6$+Na 348.0915.

1-(2′-Azido-3′-*O*-propanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine, 13b

It was obtained as an oil (0.244 g) in 72% yield. R$_f$ = 0.55 (10% methanol-chloroform). IR (neat): 3388 (OH), 2117 (N$_3$), 1738 (CO), 1660 and 1261 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.083 (3H, t, *J* = 7.5 Hz, C*H*$_3$CH$_2$CO), 1.79 (3H, s, C-5 CH$_3$), 2.40-2.50 (2H, m, CH$_3$C*H*$_2$CO), 3.64 (2H, d, *J* = 2.4 Hz, C*H*$_2$-OH), 4.08 (1H, d, *J* = 2.7 Hz, C-4′H), 4.53 (1H, dd, *J* = 6.3 Hz & 7.05 Hz, C-2′H), 5.35-5.39 (2H, m, C-3′H, OH), 5.90 (1H, d, *J* = 7.2 Hz, C-1′H), 7.71 (1H, s, C-6H) and 11.47 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 8.83 (*C*H$_3$CH$_2$CO), 12.26 (C-5*C*H$_3$), 26.61 (CH$_3$*C*H$_2$CO), 60.64 (C-5′), 62.20 (C-3′), 72.40 (C-2′), 83.08 (C-4′), 85.10 (C-1′), 110.13 (C-5), 135.57 (C-6), 150.56 (C-4), 163.53 (C-2) and 172.94 (CO); HRMS (ESI positive mode): *m/z* 362.1061 [M+Na]$^+$, Calcd for C$_{13}$H$_{17}$N$_5$O$_6$+Na 362.1071.

1-(2′-Azido-3′-*O*-butanoyl-2′-deoxy-*β*-D-ribofuranosyl)thymine, 13c

It was obtained as oil (274 mg) in 78% yield. R$_f$ = 0.65 (10% methanol-chloroform). IR (neat): 3397 (OH), 2120 (N$_3$), 1713 (CO), 1670 and 1212 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.924 (3H, t, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$CO), 1.54-1.64 (2H, m, CH$_3$C*H*$_2$CH$_2$CO), 1.79 (3H, s, C-5 C*H*$_3$), 2.41 (2H, t, *J* = 6.9 Hz, CH$_3$CH$_2$C*H*$_2$CO), 3.63 (2H, brs, C*H*$_2$-OH), 4.07(1H, d, *J* = 3 Hz, C4′-H), 4.55 (1H, dd, *J* = 6 and 7.2 Hz, C-2′H), 5.35-5.38 (2H, m, C-3′H & OH), 5.89 (1H, d, *J* = 7.2 Hz, C-1′H), 7.71 (1H, s, C-6H) and 11.47 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.26 (C-5*C*H$_3$), 13.27 (*C*H$_3$CH$_2$CH$_2$CO), 17.81 (CH$_3$*C*H$_2$CH$_2$CO), 35.31 (CH$_3$CH$_2$*C*H$_2$CO), 60.64 (C-5′), 62.24 (C-3′), 72.30 (C-2′), 83.08 (C-4′), 85.13 (C-1′), 110.12 (C-5), 135.57 (C-6), 150.56 (C-4), 163.53 (C-2) and 172.05 (CO); HRMS (ESI positive mode): *m/z* 376.1214 [M+Na]$^+$, Calcd for C$_{14}$H$_{19}$N$_5$O$_6$+Na 376.1228.

Procedure for the reduction of the azide group: Generation of monoacylated amino nucleosides 14a-c and 15a-c

To a solution of the monoacylated azido nucleoside **8a-8c** and **13a-13c** (0.5 mmole.) in 10 mL of dry methanol was added 15 mg of 10% Pd/C carefully, and the mixture was stirred under H$_2$ atmosphere for 5 hr. The catalyst was filtered off on a bed of *Celite* (in a scintered funnel) and the bed was washed with 20 mL of hot methanol. The washings were combined with the filterate and methanol removed under vaccum. The residue was purified by column chromatography over silica gel, eluting with 12-15% methanol in chloroform to afford the desired product **14a-14c** and **15a-15c** in 75-83% yields.

1-(3′-*O*-Acetyl-2′-amino-2′-deoxy-*α*-L-arabinofuranosyl)thymine, 14a

It was obtained as an oil (0.110 g) in 80% yield. R$_f$ = 0.45 (25% methanol-chloroform). IR (neat): 3404 (OH), 3321(NH$_2$), 1694 (CO), 1667 and 1120 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.78 (3H, s, CH$_3$CO), 1.92 (3H, s, C-5 CH$_3$), 3.58-3.61 (2H, m, C*H*$_2$-OH), 4.15 (1H, s, C-4′H), 4.12 (1H, t, *J* = 4.9 Hz, C2′-H), 4.47-4.52 (1H, m, OH), 5.19 (1H, t, *J* = 4.7 Hz, C-3′H), 5.75 (1H, d, *J* = 4.7 Hz, C-1′H), 5.89 (1H, d, *J* = 8.7 Hz, C2′-NH), 7.79 (1H, d, *J* = 8.4 Hz, C-2′NH), 7.80(1H, s, C-6′H) and 11.24 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.60 (C-5CH$_3$), 22.32 (CH$_3$), 55.18 (C-2′), 62.60 (C-5′), 72.34 (C-3′), 86.33 (C-4′), 88.45 (C-1′), 110.43 (C-5), 138.31 (C-6), 150.88 (C-4), 163.68 (C-2) and 171.80 (CO); HRMS (ESI positive mode): *m/z* 300.1186 [M+H]$^+$, Calcd for C$_{12}$H$_{17}$N$_3$O$_6$+H 300.1190.

1-(2′-Amino-3′-*O*-propanoyl-2′-deoxy-*α*-L-arabino-furanosyl)thymine, 14b

It was obtained as an oil (117 mg) in 75% yield. R_f = 0.50 (25% methanol-chloroform). IR (neat): 3415 (OH), 3320 (NH_2), 1667 (CO), 1650 and 1191 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$CO), 1.81 (3H, s, C-5 CH$_3$), 2.12 (2H, q, *J* = 7.5 Hz, CH$_3$C*H*$_2$CO), 3.61 (2H, dd, *J* = 4.3 and 7.8 Hz, C*H*$_2$-OH), 4.02 (1H, s, C-4′H), 4.13 (1H, t, *J* = 4.7 Hz, C-2′H), 4.46-4.52 (1H, m, OH), 5.19 (1H, t, *J* = 4.8 Hz, C-3′H), 5.71 (1H, d, *J* = 4.8 Hz, C-1′H), 5.87 (1H, d, *J* = 8.8 Hz, C-2′NH), 7.67 (1H, d, *J* = 9 Hz, C-2′NH), 7.73 (1H, s, C-6′H) and 11.27 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 9.89 (*C*H$_3$CH$_2$CO), 12.20 (C-5*C*H$_3$), 27.89 (CH$_3$*C*H$_2$CO), 56.21 (C-2′), 63.61 (C-5′), 71.37 (C-3′), 87.52 (C-4′), 88.50 (C-1′), 111.39 (C-5), 136.42 (C-6), 151.86 (C-4), 162.75 (C-2) and 172.34 (CO); HRMS (ESI positive mode): *m/z* 336.1154 $[M+Na]^+$, Calcd for $C_{13}H_{19}N_3O_6$+Na 336.1166.

1-(2′-Amino-3′-*O*-butanoyl-2′-deoxy-*α*-L-arabino-furanosyl)thymine, 14c

It was obtained as an oil (118 mg) in 78% yield. R_f = 0.50 (25% methanol-chloroform). IR (neat): 3382 (OH), 3330 (NH_2), 1680 (CO) and 1674 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.77 (3H, t, *J* = 7.5 Hz, C*H*$_3$(CH$_2$)$_2$CO), 1.43 (2H, q, *J* = 7.2 Hz, CH$_3$C*H*$_2$CH$_2$CO), 1.77 (3H, s, C-5 CH$_3$), 2.08 (2H, doublet of a triplet, *J* = 3 and 7.5 Hz, CH$_3$CH$_2$C*H*$_2$CO), 3.59 (2H, dd, *J* = 4.2 and 8.1 Hz, C*H*$_2$-OH), 3.90 (1H, s, C-4H), 4.05 (1H, t, *J* = 4.8 Hz, C-2H), 4.48-4.51 (1H, m, OH), 5.18 (1H, t, *J* = 4.8 Hz, C-3H), 5.65 (1H, d, *J* = 4.5 Hz, C-1H,) 5.88 (1H, d, *J* = 8.4 Hz, C-2′NH), 7.68 (1H, d, *J* = 9 Hz, C-2′NH), 7.74 (1H, s, C-6′H) and 11.24 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.28 (C-5*C*H$_3$), 13.32 (*C*H$_3$CH$_2$CH$_2$CO), 18.57 (CH$_3$*C*H$_2$CH$_2$CO), 36.79 (CH$_3$CH$_2$*C*H$_2$CO), 54.05 (C-2′), 61.61 (C-5′), 70.39 (C-3′), 85.48 (C-4′), 86.54 (C-1′), 109.33 (C-5), 136.34 (C-6), 150.86 (C-4), 163.69 (C-2), 172.57 (CO); HRMS (ESI positive mode): *m/z* 328.1498 $[M+H]^+$, Calcd for $C_{14}H_{21}N_3O_6$+H 328.1503.

1-(3′-*O*-Acetyl-2′-amino-2′-deoxy-*β*-D-ribofurano-syl)thymine, 15a

It was obtained as an oil (0.106 g) in 77% yield. R_f = 0.45 (25% methanol-chloroform). IR (neat): 3397 (OH), 3318 (NH_2), 1692 (CO), 1680 and 1103 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77 (3H, s, CH$_3$CO), 1.82 (3H, s, C-5 CH$_3$), 3.58 (2H, *J* = 3.6 Hz, C*H*$_2$-OH), 3.90 (1H, s, C-4′H), 4.05 (1H, s, C-2′H), 4.47-4.49 (1H, m, C-3′H), 5.19 (1H, brs, OH), 5.67 (1H, d, *J* = 3.6 Hz, C-1′H), 5.88 (1H, d, *J* = 8.4 Hz, C-2′NH), 7.74 (1H, s, C-6H), 7.83 (1H, d, *J* = 8.7 Hz, C-2′NH) and 11.25 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.26 (C-5CH$_3$), 22.42 (CH$_3$), 54.18 (C-2′), 61.60 (C-5′), 70.34 (C-3′), 85.33 (C-4′), 86.45 (C-1′), 109.43 (C-5), 136.31 (C-6), 150.88 (C-4), 163.68 (C-2) and 169.80 (CO); HRMS (ESI positive mode): *m/z* 322.1000 $[M+Na]^+$, Calcd for $C_{12}H_{17}N_3O_6$+Na 322.1010.

1-(2′-Amino-3′-*O*-propanoyl-2′-deoxy-*β*-D-ribofur-anosyl)thymine, 15b

It was obtained as an oil (0.123 g) oil in 79% yield. R_f = 0.55 (25% methanol-chloroform). IR (neat): 3400 (OH), 3325 (NH_2), 1670 (CO), 1668, 1100 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.92 (3H, t, *J* = 7.5 Hz, C*H*$_3$CH$_2$CO), 1.77 (3H, s, C-5 CH$_3$), 2.11 (2H, q, *J* = 7.5 Hz, CH$_3$C*H*$_2$CO), 3.59 (2H, d, *J* = 3.3 Hz, C*H*$_2$-OH), 3.90 (1H, s, C-4′H), 4.06 (1H, brs, C-2′H), 4.43-4.51 (1H, m, C-3′H), 5.19 (1H, brs, OH), 5.67 (1H, brs, C-1′H), 5.88 (1H, d, *J* = 8.7 Hz, C-2′NH), 7.67 (1H, d, *J* = 8.7 Hz, C-2′NH), 7.74 (1H, s, C-6H) and 11.22 (1H, s, NH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 9.75 (*C*H$_3$CH$_2$CO), 12.27 (C-5CH$_3$), 28.07 (CH$_3$*C*H$_2$CO), 54.21 (C-2′), 61.61 (C-5′), 70.37 (C-3′), 85.52 (C-4′), 86.50 (C-1′), 109.39 (C-5), 136.32 (C-6), 150.86 (C-4), 163.70 (C-2) and 173.53 (CO); HRMS (ESI positive mode): *m/z* 336.1154 $[M+Na]^+$, Calcd for $C_{13}H_{19}N_3O_6$+Na 336.1166.

1-(2′-Amino-3′-*O*-butanoyl-2′-deoxy-*β*-D-ribofura-nosyl)thymine, 15c

It was obtained as an oil (0.126 g) in 83% yield. R_f = 0.50 (25% methanol-chloroform). IR (neat): 3410 (OH), 3327 (NH_2), 1688 (CO), 1680 and 1220 cm^{-1}; ^{1}H NMR (300MHz, CD$_3$OD): δ 0.88 (3H, t, *J* = 7.5 Hz, C*H*$_3$CH$_2$CH$_2$CO), 1.58 (2H, q, *J* = 7.2 Hz, CH$_3$C*H*$_2$CH$_2$CO), 1.88 (3H, s, C-5 C*H*$_3$), 2.19 (2H, t, *J* = 7.5 Hz, CH$_3$CH$_2$C*H*$_2$CO), 3.30 (3H, s, C-2′NH & C*H*$_2$-OH), 3.77 (2H, brs, C-4′H & OH), 4.04 (1H, s, C-2′H), 4.22 (1H, d, *J* = 5.4 Hz, C-3′H), 5.71 (1H, brs, C-1′H), δ 6.03 (1H, d, *J* = 8.7 Hz, C-2′NH) and δ 7.83 (1H, s, C-6H); ^{13}C NMR (75.5 MHz, CD$_3$OD): δ 12.46 (C-5*C*H$_3$), 13.87 (*C*H$_3$CH$_2$CH$_2$CO), 20.20 (CH$_3$*C*H$_2$CH$_2$CO), 38.61 (CH$_3$CH$_2$*C*H$_2$CO), 56.58 (C-2′), 63.25 (C-5′), 72.13 (C-3′), 87.85 (C-4′), 88.57

(C-1′), 111.92 (C-5), 138.22 (C-6), 150.56 (C-4), 163.53 (C-2) and 172.05 (CO); HRMS (ESI positive mode): *m/z* 328.1487 $[M+Na]^+$, Calcd for $C_{14}H_{21}N_3O_6$+Na 328.1503.

Conclusions

We have successfully demonstrated the specificity and selectivity of CAL-B for the regioselective deacylation of acyl groups at primary position over that at secondary position in peracylated azido nucleosides. The biocatalytically deacylated nucleosides were further reduced to amino nucleosides which can be used for the synthesis of 5′-*O*-2′-*N*-linked oligonucleotides of biological importance.

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