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Lipase-catalyzed regio- and stereoselective deacylation: Separation of anomers of peracylated α,β-D-ribofuranosides

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Efficient regio- and stereoselective deacylation of acyloxy function involving C-5′ hydroxyl group of α-anomer over the other similar C-5′ acyl group of β-anomer and acyl groups involving secondary hydroxyls in anomeric mixture of peracylates of D-ribose has been achieved during deacylation reaction mediated by Lipozyme® TL IM (*Thermomyces lanuginosus* lipase immobilized on silica). This enzymatic methodology has been efficiently used for the separation of anomeric mixtures of peracylated α,β-D-ribofuranosides.

Keywords: Anomeric mixture, Lipozyme® TL IM, peracylated α,β-D-ribofuranosides, regio- and stereoselective deacylation

Acylated and partially acylated carbohydrates are key intermediates in the preparation of oligosaccharides, glycopeptides and modified nucleosides[1-4]; in particular, sugar monoesters are important as biodegradeable surfactants[5]. Due to the polyhydroxylated structure of sugars, the preparation of partially acylated derivatives has been traditionally carried out using protection-deprotection procedures which yield mixtures of products. Enzyme-catalyzed deacetylation reactions have been successfully utilized in selective deacetylation of peracetylated sugars. At present, hydrolases are well recognized biocatalysts for the regioselective transformation of polyhydroxylated compounds and therefore, they provide a useful access to partially acylated carbohydrates[6-8]. Even when the literature describes many examples of the regioselective modification of carbohydrates through enzymatic acylation and deacylation of peracylated derivatives, most of them involve hexopyranose rings; in contrast, the enzymatic regioselective transformation of pentofuranoses has been studied to a lesser extent[7-13].

Among the enzymatic deacetylations related to pentofuranosides, Fernandez-Lorente *et al.*[12] have used *Candida rugosa* lipase (CRL) immobilised on octyl agarose to catalyse regioselective deacetylation at the C-5 position of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose to afford 1,2,3-tri-*O*-acetyl-β-D-ribofuranose in 80% and 47% yields in phosphate buffer-DMF and phosphate buffer, respectively. Whereas, Chien and Chern[13] have carried out native CRL (Sigma) catalysed hydrolysis of α-anomer of 1,2,3,5-tetra-*O*-acetylribofuranose to afford the corresponding 5-hydroxyl derivative, Inigo *et al.*[14] have studied *Candida antarctica* lipase B catalysed deacetylation of 1-*O*-methyl-2,3,5-tri-*O*-acetyl-α-D-ribofuranoside and 1-*O*-methyl-2,3,5-tri-*O*-acetyl-β-D-ribofuranoside, separately and observed regioselective deacetylation in the former compound to afford 1-*O*-methyl-2,3-di-*O*-acetyl-α-D-ribofuranoside in 81% yield; selectivity was not observed in β-diastereoisomer. This stimulating background and our own interest in the lipase-mediated chemical transformations[15-19] prompted us to explore the possibility of lipase-mediated selective deacylation studies on anomeric mixtures of peracylated α,β-D-ribofuranosides with an aim to develop efficient and simple methodology for the separation of α- and β-anomers.

Results and Discussion

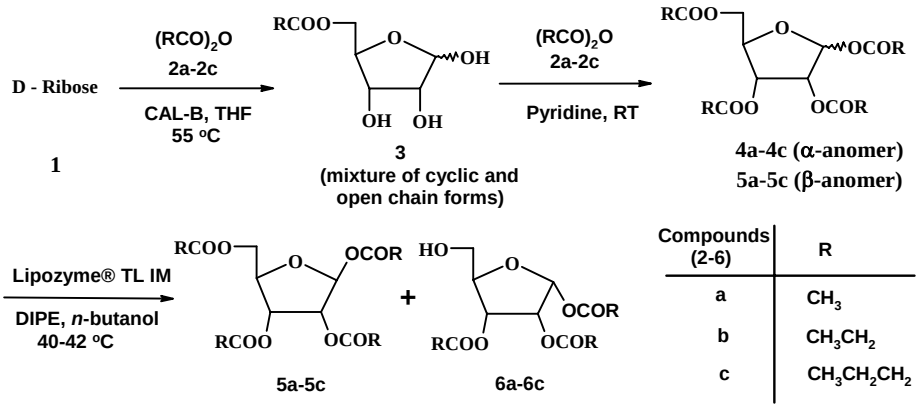
Tetra *O*-acylated α,β-D-ribofuranosides **4a-4c** and **5a-5c** were chemoenzymatically synthesized in two

steps, *i.e.* by selective acylation of lone primary hydroxyl group of D-ribose with acetic-, propanoic- and butanoic anhydride catalyzed by CAL-B in THF at 55°C followed by classical peracylation of the other three secondary hydroxyl groups with same acylating agents in pyridine at RT in quantitative yields[15] (**Scheme I**). The ratio of the α- and β-anomer in the anomeric mixtures **4a-5a**, **4b-5b** and **4c-5c** were determined on the basis of comparison of intensities of anomeric protons of the two anomers in the ^{1}H NMR spectrum of the anomeric mixtures. The anomeric proton of β-anomer appeared as a singlet, whereas the anomeric proton of α-anomer appeared as a doublet or as a broad singlet at higher δ value with respect to the corresponding proton of the β-anomer[13,20,21].

Four different lipases, *i.e. Candida rugosa* lipase (CRL), *Candida antarctica* lipase B immobilized on accurel [CAL-L(A)], *Candida antarctica* lipase B immobilized on polyacrylate (lewatit, CAL-B) and *Thermomyces lanuginosus* lipase immobilised on silica (Lipozyme® TL IM) were screened for regioselective deacetylation of acetoxy function of one anomer over the other acetoxy functions of the same anomer and acetoxy functions of the other anomer in different organic solvents, *i.e.* tetrahydrofuran, dioxane, acetonitrile, toluene, ethanol and diisopropyl ether (DIPE) at different temperature (35°C to 60°C). It was observed that Lipozyme® TL IM in DIPE at 40-42°C selectively and most efficiently deacetylates the acetoxy function involving C-5′-hydroxyl group of the α-anomer **4a** over the other acetoxy functions present in tetra-*O*-acetylated α,β-D-ribofuranosides **4a-5a**. Although CAL-B initially showed selectivity for the deacetylation of C-5′-acetoxy group of α-anomer, it started deacetylation of acetoxy functions of the β-anomer as well with time and led to a mixture of compounds. CRL and CAL-L(A) did not show any appreciable selectivity in the deacetylation studies on anomeric mixtures of tetra-*O*-acetylated α,β-D-ribofuranosides.

In a typical reaction, a solution of the anomeric mixture of peracetylated α,β-D-ribofuranosides **4a-5a** in diisopropyl ether containing a small amount of *n*-butanol was agitated with Lipozyme® TL IM in an incubator shaker at 40-42°C. On completion of the reaction, as indicated by TLC examination, the enzyme was filtered off and the solvent removed under reduced pressure. The crude product thus obtained was passed through a small silica gel column to afford the pure deacetylated compound **6a**, with lower R_f value than the starting anomeric mixture **4a-5a** in 92% yield and the unreacted, recovered 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranoside **5a** in 91% yield (**Scheme I**, **Table I**). The structure of the triacetylated compound **6a** was established as 1,2,3-tri-*O*-acetyl-α-D-ribofuranose by a detailed study of its IR, ^{1}H and ^{13}C NMR and HRMS, and the comparison of its ^{1}H NMR spectrum with that of the unreacted, recovered 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranosides **5a**.

Further, to explore enzyme selectivity and reaction rate for the different acyl groups, anomeric mixtures of tetra-*O*-propanoylated α,β-D-ribofuranosides **4b-5b** and tetra-*O*-butanoylated α,β-D-ribofuranosides **5c-6c** were synthesized and incubated with Lipozyme® TL IM in DIPE at 40-42°C. It was observed that in both the cases, Lipozyme® TL IM selectively catalyzed the deacylation of acyloxy functions involving C-5′-hydroxyl group of α-anomer over the other acyloxy function of the α- and β-anomers in the mixture, as in case of deacetylation of tetra-*O*-acetylated α,β-D-ribofuranosides **4a-5a**. However, the rate of deacyla-



Scheme I — Synthesis and lipase-catalyzed selective deacylation of 1,2,3,5-tetra-*O*-acyl-α,β-D-ribofuranosides

Table I — Lipozyme® TL IM-catalyzed regio- and stereo-selective deacylation study on mixture of α,β-anomers of peracylated ribofuranosides in DIPE at 40-42°C

Compd[a]	Ratio of α:β anomers	Reaction time (hr)	Deacylated and recovered, unreacted ribofuranosides	Yield[b] (%)
4a,**5a**	1.0:2.5	12.0	**6a**	92
			5a	91
4b,**5b**	1.0:1.4	6.0	**6b**	87
			5b	93
4c,**5c**	1.0:3.3	3.0	**6c**	91
			5c	95

[a]All these reactions did not yield any product when performed in the absence of Lipozyme® TL IM. [b]The yields reported are calculated by taking the amounts of α- and β-anomers in the mixture as 100%.

tion of acyloxy function involving C-5′-hydroxyl of α-anomers **4a**, **4b** and **4c** was different. The Lipozyme® TL IM catalyzed the debutanoylation of α-anomer **5c** four times faster than the deacetylation of the α-anomer **4a** and twice as fast as the depropanoylation of the α-anomer **4b**. This indicates that increase in lipophilicity of the peracylates make them better substrates for the enzyme. The structures of all the pure compounds **5a**, **5b** and **5c** and **6a**, **6b** and **6c** synthesized during enzymatic deacylation studies were established on the basis of their spectral data (^{1}H and ^{13}C NMR, IR spectra and HRMS) analysis. The structures of the known compound **5a** was further confirmed by the comparison of its reported data in the literature[13].

Selective deacylation of the ester function invoving the C-5′ hydroxyl group of α-anomers **4a**, **4b** and **4c** over the other ester functions in the anomeric mixtures **4a**-**5a**, **4b**-**5b** and **4c**-**5c** results in the sufficient difference in the polarity of the monohydroxy α-D-ribofuranosides and their corresponding tetraacylated β-anomers. This selective lipase mediated deacylation methodology enabled us to achieve the separation of α- and β-anomers of peracylates of D-ribose, which is otherwise almost impossible to achieve by simple chromatographic methods. All these reactions did not yield any product when performed in the absence of Lipozyme® TL IM. The yields reported for partially deacylated α-anomers **6a**-**6c** and unreacted, recovered peracylated β-anomers **5a**-**5c** are calculated by taking the amounts of α- and β-anomers in the starting mixtures as 100%.

Experimental Section

Melting points were determined on a Mettler FP 62 instrument or in a sulfuric acid bath and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer by preparing KBr disc for solid samples and thin film for oils. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300 Avance spectrometer at 300 and 75.5 MHz, respectively using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz. The peak for OH group in the ^{1}H NMR spectra is ascertained on the basis of comparison of the spectrum of the compound in the neat deuterated solvent and in the deuterated solvent plus D_2O. The HRMS analysis was carried out on a microTOF-Q instrument from Bruker Daltonics, Bremen. They were run in ESI positive mode. *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), *Candida antarctica* lipase B immobilized on accurel [CAL-L(A)] and *Candida antarctica* lipase B immobilized on polyacrylate (lewatite, CAL-B) were gifted by Novozymes Inc. (Copenhagen, Denmark), whereas *Candida rugosa* lipase (CRL) was purchased from Sigma Chemical Co. (USA). All enzymes were used after storing *in vacuum* over P_2O_5 for more than 24 hr. Acetonitrile and diisopropyl ether was distilled and kept over molecular sieves (4 Å) prior to use. Analytical TLCs were run on precoated Merck silica-gel $60F_{254}$ plates; the spots were detected either under UV light or by charring with 4% alcoholic H_2SO_4. Silica gel (100-200 mesh) was used for column chromatography.

General procedure for Lipozyme® TL IM-catalyzed selective deacylation of peracylated α,β-D-ribofuranosides 4a-5a, 4b-5b and 4c-5c

The anomeric mixture of peracylated α,β-D-ribofuranosides **4a-5a, 4b-5b** and **4c-5c** (1.5 mmol) was dissolved in diisopropyl ether (25 mL) and incubated with Lipozyme® TL IM (0.24-0.32 g, substrate-enzyme ratio, approximately 1:0.5, w/w) and *n*-butanol (0.05 mL) at 40-42°C. The progress of the reaction was monitored by TLC. 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 the unreacted, recovered β-anomers **5a**-**5c** and the 5′-*O*-deacylated compounds **6a**-**6c** in high yields. All the unreacted, recovered peracylated β-D-ribofuranosides

5a-5c and enzymatically deacylated 1,2,3-tri-*O*-acyl-α-D-ribofuranoside **6a-6c** were unambiguously identified on the basis of their spectral data. The structures of the known compound **5a** was further confirmed by the comparison of its data with that reported in the literature[13].

1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranoside, 5a

It was obtained as colourless oil (0.31 g) in 91% yield. R_f = 0.35 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = -0.6° (*c* 0.1, MeOH); IR (Nujol): 1756, 1446, 1230, 1098, 1059, 971 and 884 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.08, 2.09, 2.10 and 2.13 (12H, 4s, 3H each, 4 × $COCH_3$), 4.16 (m, 1H, C-5H$_\alpha$), 4.31-4.36 (m, 2H, C-4H and C-5H$_\beta$), 5.34-5.36 (m, 2H, C-2H and C-3H) and 6.16 (s, 1H, C-1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 19.17, 19.41, 19.71 (4×CO*C*H$_3$), 62.35 (C-5), 69.26 (C-3), 72.86 (C-2), 78.04 (C-4), 96.90 (C-1), and 167.67, 168.11, 168.37 and 169.11 (4 × CO); HRMS: *m/z* Calcd for ($C_{13}H_{18}O_9$+Na) 341.0843. Found 341.0839.

1,2,3,5-Tetra-*O*-propanoyl-β-D-ribofuranoside, 5b

It was obtained as colourless oil (0.30 g) in 93% yield. R_f = 0.50 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = -0.5° (*c* 0.1, MeOH); IR (Nujol): 1748, 1452, 1240, 1088, 1034 and 883 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.98-1.07 (m, 12H, 4 × $COCH_2CH_3$), 2.26-2.45 (m, 8H, 4 × $COCH_2CH_3$), 4.10 (dd, *J* = 12.0 and 5.1 Hz, 1H, C-5H$_\alpha$), 4.27-4.36 (m, 2H, C-4H and C-5H$_\beta$), 5.26-5.30 (m, 2H, C-2H and C-3H) and 6.05 (s, 1H, C-1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.50, 8.70, 8.81 and 8.82 (4 × $COCH_2CH_3$), 26.47, 26.51, 26.76 and 26.89 (4 × $COCH_2CH_3$), 63.00 (C-5), 70.05 (C-3), 73.51 (C-2), 79.12 (C-4), 97.71 (C-1), and 172.09, 172.49, 172.64 and 173.17 (4×CO); HRMS: *m/z* Calcd for ($C_{17}H_{26}O_9$+Na) 397.1469. Found 397.1463.

1,2,3,5-Tetra-*O*-butanoyl-β-D-ribofuranoside, 5c

It was obtained as colourless oil (0.47 g) in 95% yield. R_f = 0.65 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = -0.3° (*c* 0.1, MeOH); IR (Nujol): 1751, 1436, 1220, 1078 and 876 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 0.85-0.92 (m, 12H, 4×$COCH_2CH_2CH_3$), 1.47-1.61 (m, 8H, 4 × $COCH_2CH_2CH_3$), 2.23-2.41 (m, 8H, 4 × $COCH_2CH_2$), 4.10 (dd, *J* = 12.0 and 5.0 Hz, 1H, C-5H$_\alpha$), 4.24-4.35 (m, 2H, C-4H and C-5H$_\beta$), 5.24-5.30 (m, 2H, C-2H and C-3H) and 6.05 (s, 1H, C-1H); ^{13}C NMR (75.5 MHz, DMSO-*d*$_6$): δ 13.20, 13.23, 13.31 and 13.48 (4 × $COCH_2CH_2CH_3$), 17.56, 17.66, 17.77 and 17.89 (4 × $COCH_2CH_2CH_3$), 34.87, 34.93, 35.22 and 35.56 (4 × $COCH_2CH_2CH_3$), 62.94 (C-5), 70.01 (C-3), 73.41 (C-2), 79.06 (C-4), 97.63 (C-1), and 171.18, 171.57, 171.78 and 172.32 (4 × CO); HRMS: *m/z* Calcd for ($C_{21}H_{34}O_9$+Na) 453.2095. Found 453.2095.

1,2,3-Tri-*O*-acetyl-α-D-ribofuranose, 6a

It was obtained as colourless oil (0.11 g) in 92% yield. R_f = 0.20 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = +3.9° (*c* 0.1, MeOH); IR (Nujol): 3480, 1747, 1434, 1231, 1102, 1047 and 899 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 2.01, 2.06 and 2.08 (9H, 3s, 3H each, 3×$COCH_3$), 3.53 (m, 2H, C-5H$_{\alpha+\beta}$), 4.21 (m, 1H, C-4H), 5.10 (t, *J* = 5.4 Hz, 1H, -OH), 5.17-5.24 (m, 2H, C-2H and C-3H) and 6.28 (d, *J* = 4.2 Hz, 1H, C-1H); ^{13}C NMR (75.5 MHz, DMSO-*d*$_6$): δ 20.08, 20.45 and 20.84 (3 × CO*C*H$_3$), 60.65 (C-5), 69.64 (C-3), 69.84 (C-2), 80.40 (C-4), 93.67 (C-1), and 169.12, 169.57 and 169.87 (3 × CO); HRMS: *m/z* Calcd for ($C_{11}H_{16}O_8$+Na) 299.0737. Found 299.0726.

1,2,3-Tri-*O*-propanoyl-α-D-ribofuranoside, 6b

It was obtained as colourless oil (0.17 g) in 87% yield. R_f = 0.25 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = +2.4° (*c* 0.1, MeOH); IR (Nujol): 3470, 1742, 1440, 1248, 1091 and 903 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 1.05-1.10 (m, 9H, 3 × $COCH_2CH_3$), 2.17-2.40 (m, 6H, 3 × $COCH_2CH_3$), 3.53 (m, 2H, C-5H$_{\alpha+\beta}$), 4.22-4.23 (m, 1H, C-4H), 5.10 (brs, 1H, -OH), 5.21 (dd, *J* = 6.3 and 4.5 Hz, 1H, C-2H), 5.27 (dd, *J* = 6.3 and 2.1 Hz, 1H, C-3H) and 6.31 (d, *J* = 4.5 Hz, 1H, C-1H); ^{13}C NMR (75.5 MHz, DMSO-*d*$_6$): δ 8.71, 8.77 and 8.93 (3 × $COCH_2CH_3$), 26.51, 26.85 and 27.13 (3 × $COCH_2CH_3$), 60.73 (C-5), 69.62 (C-3), 70.00 (C-2), 84.56 (C-4), 93.69 (C-1), and 172.09, 172.61 and 172.91 (3 × CO); HRMS: *m/z* Calcd for ($C_{14}H_{22}O_8$+Na) 341.1207. Found 341.1191.

1,2,3-Tri-*O*-butyl-α-D-ribofuranoside, 6c

It was obtained as colourless oil (0.11 g) in 91% yield. R_f = 0.30 (20% ethyl acetate in pet. ether, v/v); $[\alpha]_D^{24}$ = +1.8° (*c* 0.1, MeOH); IR (Nujol): 3440, 1753, 1436, 1230, 1083 and 881 cm^{-1}; ^1H NMR (300 MHz, DMSO-*d*$_6$): δ 0.84-0.95 (m, 9H, 3 × $COCH_2CH_2CH_3$), 1.47-1.63 (m, 6H, 3 × $COCH_2CH_2CH_3$), 2.21-2.34 (m, 6H, 3 × $COCH_2CH_2CH3$), 3.53 (m, 2H, C-5H$_{\alpha+\beta}$),

4.20-4.21 (m, 1H, C-4H), 5.10 (brs, 1H, -OH), 5.19 (dd, J = 6.5 and 4.5 Hz, 1H, C-2H), 5.28 (dd, J = 6.5 and 2.1 Hz, 1H, C-3H) and 6.32-6.34 (d, J = 4.5 Hz, 1H, C-1H); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.24, 13.36 and 13.48 (3 × $COCH_2CH_2CH_3$), 17.65, 17.76 and 17.89 (3 × $COCH_2CH_2CH_3$), 34.89, 35.36 and 35.63 (3 × $COCH_2CH_2CH_3$), 60.74 (C-5), 69.59 (C-3), 70.07 (C-2), 84.75 (C-4), 93.51 (C-1), and 171.34, 171.74 and 172.03 (3 × CO); HRMS: *m/z* Calcd for ($C_{17}H_{28}O_8$+Na) 383.1676. Found 383.1674.

Conclusion

The present study reveals that Lipozyme® TL IM is very selective for the deacylation of acyloxy function involving primary hydroxyl group over the acyloxy function involving secondary hydroxyl group in peracylates of α-anomer of D-ribose. At the same time, the lipase exhibited extreme diastereoselectivity and did not deacylate any of the acyloxy function present in the peracetates of β-anomer of D-ribose, when anomeric mixture of peracylates of α,β-D-ribofuranoside is incubated with lipase. Further, it was discovered during the study that the enzyme deacylates the acyloxy function involving primary hydroxyl group in α-anomer of the anomeric mixture which is in less quantity in the mixture. The enzymatic methodology developed herein may find application in separation of anomeric mixtures of sugars or nucleosides.

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References

1 Newman R H & Zhang J, *Nature Chem Bio*, 4, **2008**, 382.
2 Walsh G & Jefferis R, *Nature Biotech*, 24, **2006**, 1241.
3 Davies B G, *Chem Rev*, 102, **2002**, 579.
4 Gruner S A W, Locardi E, Lohof E & Kessler H, *Chem Rev*, 102, **2002**, 491.
5 Park O J, Kim D Y & Dordick J S, *Biotechnol Bioeng*, 70, **2000**, 208.
6 Bornsheuer U & Kazlauskas R, *Hydrolases in Organic Chemistry* (Wiley-VCH, Weinheim), **1999**.
7 Kadereit D & Waldmann H, *Chem Rev*, 101, **2001**, 3367.
8 Ferla B La, *Monatsh Chem*, 133, **2002**, 351.
9 Prasad A K, Roy S, Kumar R, Kalra N, Wengel J, Olsen C E, Cholli A L, Samuelson L I, Kumar J, Watterson A C, Gross R A & Parmar V S, *Tetrahedron*, 59, **2003**, 1333.
10 Mastihubova M, Szemesova J & Biely P, *Tetrahedron Lett*, 44, **2003**, 1671.
11 Hennen W J, Sweers H M, Wang Y F & Wong C H, *J Org Chem*, 53, **1988**, 4939.
12 Fernandez-Lorente G, Palomo J M, Cocca J, Mateo J C, Moro P, Terreni M, Fernandez-Lafuente R & Guisan J M, *Tetrahedron*, 59, **2003**, 5705.
13 Chien T C & Chern J W, *Carbohydr Res*, 339, **2004**, 1215.
14 Inigo S, Porro M T, Montserrat J M, Iglesias L E & Iribarren A M, *J Mol Catal B: Enzymatic*, 35, **2005**, 70.
15 Prasad A K, Serensen M D, Parmar V S & Wengel J, *Tetrahedron Lett*, 36, **1995**, 6163.
16 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.
17 Prasad A K, Kalra N, Yadav Y, Singh S K, Kumar R, Sharma S K, Patkar S, Lange L, Wengel J & Parmar V S, *Org Biomol Chem*, 5, **2007**, 3524.
18 Singh S K, Sharma R K, Maity J, Wengel J, Parmar V S & Prasad A K, *Nucleic Acids Symposium Series No* 52, **2008**, 273.
19 Maity J, Shakya G, Singh S K, Ravikumar V T, Parmar V S & Prasad A K, *J Org Chem*, 73, **2008**, 5629.
20 Boryski J & Grynkiewicz G, *Synthesis*, 14, **2001**, 2170.
21 Stevens J D & Fletcher H G Jr, *J Org Chem*, 33, **1968**, 1799.