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A chemoenzymatic synthesis of deoxy sugar esters involving stereoselective acetylation of hemiacetals catalyzed by CALB

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ABSTRACT

An extension of the scope of the chemoenzymatic strategy for the synthesis of stereochemically pure pyranose deoxy sugar esters of different carboxylic acids has been achieved. The objective of the work was to extend the strategy to the synthesis of furanose deoxy sugar derivatives and additionally, to *N*-Boc-protected amino acid esters. With all used carboxylic acids (deoxycholic acid, α-methoxyphenylacetic acid, *N*-Boc-L-phenylalanine and *N*-Boc-L-tyrosine) the lipase-catalyzed stereoselective acetylation of furanose or pyranose hemiacetal moiety as a key step afforded one desired stereochemically pure acetylated hemiacetal deoxy sugar ester in high *de*.

1. Introduction

The synthesis of (deoxy) sugars and their derivatives of both, pyranose and furanose form continues to be one of the focus points of organic synthesis as well as drug development [1]. Also, a number of functionalized tetrahydrofurans and tetrahyropyrans, which represent parent ring systems for deoxy furanosides and pyranosides, respectively, are embedded in many biologically important natural products [2,3]. Several carbohydrates and their derivatives have proven to be efficient and versatile tools in stereoselective synthesis. The compounds of this type are also used as organocatalysts, chiral auxiliaries, ligands, *etc.* [4]. The carbohydrate esters of amino acids have found application as sweetening agents and surfactants, as well as in the delivery of physiologically active agents [5]. Recently, some of the esters have shown antitumor, plant growth inhibiting and antibiotic activities [6,7]. The sulfur-containing glucosamine esters are used for the synthesis of glycopeptides [8]. Therefore, different approaches are needed to achieve variable structures of carbohydrate derivatives.

For the synthesis of deoxy sugars many methods have been developed [9]. Several chemical methods are available for the synthesis of sugar esters [7]. However, in last decades these compounds have been prepared predominantly by the enzymatic acylation [6,10]. A lot of work has been done with hydrolases, among which mainly lipases can be found. Lipases, called the "workhorses" of biocatalysis, are widely used to catalyze stereoselective reactions in both laboratory scale as well as in an industrial scale fine organic synthesis [11]. These enzymes are applicable in the acyl transfer reactions, for example, in the reactions involving esters, carboxylic acids, alcohols, *etc.*, playing an important role in the kinetic resolution of racemic mixtures. Lipases have been used for separation of α- and β-glucopyranosides [12], but also for derivatization of carbohydrates in synthesis of oligosaccharides [13] as well as in a chemoenzymatic approach for the synthesis of several regioprotected fructose derivatives [14], *etc.* Amino acid esters of carbohydrates have been prepared by Lohith and Divakar who used lipases to obtain mixtures of mono- and diesters [15].

The sugars as well as deoxy sugars form two types of thermodynamically favored cyclic hemiacetals, *i.e.* furanoses and pyranoses. These rings of carbohydrates, five- and six-membered, respectively, greatly differ in their molecular geometry and, therefore, in the accessibility of the corresponding hydroxyl groups to the enzyme. From this has arisen the main purpose of the current study – to investigate whether the chemoenzymatic synthetic strategy developed initially for the synthesis of pyranose deoxy sugar esters [16] is applicable for the synthesis of their furanose counterparts. The basic concept of the approach is as follows (Scheme 1):

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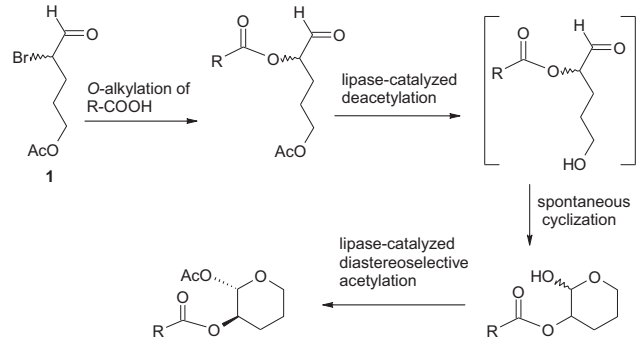


Scheme 1. A chemoenzymatic approach for the synthesis of deoxy sugar esters.

1. A carboxylic acid is *O*-alkylated by a deoxy sugar precursor (α-bromo-ω-acetoxy aldehyde (**1**)).
2. The following lipase-catalyzed hydrolysis cleaves the acetate with high regioselectivity.
3. A spontaneous cyclization takes place affording a mixture of hemiacetal deoxy sugar esters.
4. Only one of the hemiacetal deoxy sugar ester diastereomers is acetylated by the lipase. A kinetic dynamic resolution of hemiacetal stereoisomers takes place. The stereopreference of the CALB was in accordance with the Kazlauskas' rule for secondary alcohols.

α-Bromo-ω-acetoxy pentanal (**1**) was used as a precursor of the deoxy sugar (3,4-dideoxy-D-ribose and 3,4-dideoxy-D-arabinose) moiety in the synthesis of pyranose deoxy sugar esters [16]. For the synthesis of furanose deoxy sugar esters racemic α-bromo-ω-acetoxy butanal (**2**), as a deoxy sugar precursor (affording 3-deoxy D-erythrose and 3-deoxy D-threose), has to be used.[1] Thus, one of the goals of the current work was to investigate the applicability of the above approach to the synthesis and especially resolving of the stereoisomers of furanose deoxy sugar esters. An additional important step to be made in this study in the development of the synthetic strategy was the synthesis of deoxy sugar esters of *N*-Boc-protected α-amino acids (involving deoxy sugar in both, furanose as well as pyranose form)

In this work all starting compounds and targets are mere examples representing corresponding type of organic compounds.

In order to obtain furanose deoxy sugar esters the reaction sequence in the Scheme 1 was followed using α-bromobutanal **2** for the *O*-alkylation of carboxylic acids. Four carboxylic acids were chosen for the deoxy sugar ester synthesis. First, deoxycholic acid (DCA[2]; structures in Table 1) as a sterically demanding dihydroxy carboxylic acid bearing unprotected hydroxyl groups was chosen. (The glycoconjugates of bile acids itself have long been a subject of interest to researchers from different fields [18].) α-Methoxyphenylacetic acid (MPA) was involved because of its role of the chiral derivatizing agent allowing to determine the absolute stereochemistry of the ester diastereomers by measuring of differential shielding effects in the NMR spectra of diastereomers [19,20]. As an extension of the synthetic approach two *N*-Boc-protected α-amino acids, *viz.* *N*-Boc-L-phenylalanine and *N*-Boc-L-tyrosine were introduced in the synthesis of stereochemically pure 3-deoxy D-erythrose (furanose) esters as well as 3,4-dideoxy D-ribose (pyranose) esters. A comparative study of the stereoselectivity and yield of the products of CALB-catalyzed acetylation was performed.

2. Experimental section

2.1. General methods and materials

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker 400, 500 or 800 MHz spectrometers. All signals were referenced relatively to solvent signal (7.27 ppm for ^{1}H and 77.00 ppm for ^{13}C). 2D FT methods were used for the full assignment of NMR spectra. TLC was performed using DC-Alufolien Kieselgel 60 F_{254} (Merck) silica gel plates and the compounds were visualized by staining upon heating with anisaldehyde solution. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). Commercial reagents were used without purification. The *Candida antarctica* lipase B (CALB) preparation (Novozym® 435) was obtained from Novozymes A/S.

The *O*-alkylation of carboxylic acids as well as the lipase-catalyzed acetylation and deacetylation reactions (General Procedures A, B and C) were performed according to the standard procedures (only incubation times were varied in accordance with reaction velocity) [16].

2.1.1. General Procedure A: the O-alkylation of a carboxylic acid followed by the lipase-catalyzed deacetylation

Carboxylic acid (1 mmol) was dissolved in acetonitrile (8 ml), 4 eq. of DIPEA was added, followed by 0.7 mmol of bromoaldehyde (1 or 2) dissolved in 2 ml of acetonitrile. The reaction was monitored by TLC. After disappearance of the starting material (**1** or **2**) Novozym® 435 (600 mg) and H_2O (5 mmol) were added, the mixture was shaken at rt and the reaction was monitored by TLC. The solution was then diluted with Et_2O, the enzyme was filtered off, and the solution was washed with water, 1 M $NaHSO_4$, water and brine, and dried with Na_2SO_4. The product was evaporated and purified by column chromatography over silica.

2.1.2. General Procedure B: the lipase-catalyzed acetylation of hemiacetal compounds (**9**–**14**)

To the solution of 0.2 mmol of hemiacetal in 4 ml of chloroform, 1 ml of vinyl acetate and 200 mg of Novozym® 435 were added. The reaction mixture was shaken at rt and the reaction was monitored by TLC. After the process had been completed, the enzyme was filtered off and the solution evaporated. The products were separated by column chromatography over silica.

2.1.3. General Procedure C: the lipase catalyzed deacetylation of compounds **15**–**20**

To the solution of 0.5 mmol of an acetylated compound in 8 ml of acetonitrile (containing 2% of water) 350 mg of Novozym® 435 was added. The reaction mixture was shaken at rt and the process was monitored by TLC. The enzyme was filtered off, the reaction mixture was evaporated and the products were purified by column chromatography over silica.

2.2. Compound characterization

2.2.1. Deoxycholic acid 4′-acetoxy-1′-formyl-propyl ester (**3**)

TLC: R_f = 0.4 (EtOAc). Reaction time 28 h.

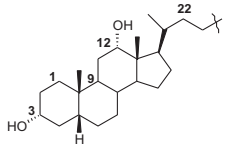
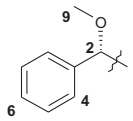
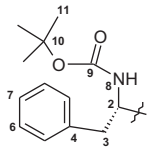
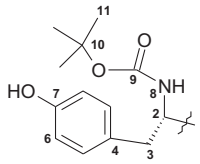
2.2.2. (S)-(+)-α-Methoxyphenylacetic acid 4′-acetoxy-1′-formyl-propyl ester (**4**)

TLC: R_f = 0.14 (30% EtOAc/hexane). Reaction time 3 h.

[1] The synthesis and characterization of ω-acetylated aldehydes **1** and **2** (Scheme 2) has been described in detail [17].

[2] *Abbreviations*: DCA – deoxycholic acid, MPA – α-methoxyphenylacetic acid, *N*-Boc-Phe – *N*-Boc-L-phenylalanine, *N*-Boc-Tyr – *N*-Boc-L-tyrosine, Boc – *tert*-butoxycarbonyl group, CALB – *Candida antarctica* lipase B.

Table 1
The yield and stereoisomeric purity of the products.

Structure of acid residueR-COOH	Abbr.	Comp. no	Yield (%)	de
	DCA	**9**	35[a]	–
	3-Ac-DCA	**15**	27[b]	>98
	DCA	**9a**	54[c]	>98[d]
	MPA	**10**	44[a]	–
		16	33[b]	>98
		10a	42	>98[d]
	*N*-Boc-Phe	**11**	62[a]	–
		13	72[a]	–
		17	41[b]	>98
		19	44[b]	>98
		11a	68	>98[d]
		13a	89	>98[d]
	*N*-Boc-Tyr	**12**	32[a]	–
		14	53[a]	–
		18	27[b]	>98
		20	24[b]	>98
		12a	80	>98[d]

[a] Overall yield for the "one-pot synthesis".
[b] Theoretically, maximum yield of the reaction can be 50%.
[c] Conversion rate was 80%.
[d] Two anomers are accounted as one diastereomer.

*2.2.3. N-(tert-Butoxycarbonyl)-L-phenylalanine 4′-acetoxy-1′-formyl-propyl ester (**5**)*

TLC: R_f = 0.15 (30% EtOAc/hexane). Reaction time 6 h.

*2.2.4. N-(tert-Butoxycarbonyl)-L-tyrosine 4′-acetoxy-1′-formyl-propyl ester (**6**)*

TLC: R_f = 0.25 (50% EtOAc/hexane). Reaction time 6 h.

*2.2.5. N-(tert-Butoxycarbonyl)-L-phenylalanine 4′-acetoxy-1′-formyl-butyl ester (**7**)*

TLC: R_f = 0.2 (30% EtOAc/hexane). Reaction time 24 h.

*2.2.6. N-(tert-Butoxycarbonyl)-L-tyrosine 4′-acetoxy-1′-formyl-butyl ester (**8**)*

TLC: R_f = 0.25 (40% EtOAc/hexane). Reaction time 24 h.

*2.2.7. Deoxycholic acid 2′-hydroxy-tetrahydrofuran-3′-yl ester (**9**)*

The reaction was carried out following the General Procedure A starting with 1 mmol of DCA to yield 172 mg (35%, overall yield for "one-pot synthesis") of 9 in 120 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ *trans*: 5.36, 5.06, 4.13/4.04, 3.97, 3.59, 2.39/1.93, 2.37/2.23, 1.84/1.25, 1.82/1.25, 1.79, 1.78/1.51, 1.77/1.33, 1.72/0.96, 1.69, 1.66/1.36, 1.59/1.06, 1.53, 1.50, 1.41/1.12, 1.40, 1.38, 0.95, 0.89, 0.66 ppm; *cis*: 5.47, 5.00, 4.10/3.85, 3.97, 3.59, 2.27/2.08, 2.42/2.32, 1.84/1.25, 1.82/1.25, 1.79, 1.78/1.51, 1.77/1.33, 1.72/0.96, 1.69, 1.66/1.36, 1.59/1.06, 1.53, 1.50, 1.41/1.12, 1.40, 1.38, 0.95, 0.89, 0.66 ppm. ^{13}C NMR[3] (125 MHz, $CDCl_3$) δ *trans*: 173.87 < 173.85 (C24), 100.31 > 100.28 (C2′), 73.16 < 73.14 (C12), 71.7 (C3), 78.24 < 78.23 (C3′), 66.73 < 66.72 (C5′), 48.2 (C14), 47.15 < 47.11 (C17), 46.45 < 46.44 (C13), 42.0 (C5), 36.2 (C4), 36.0 (C20), 35.2 (C1), 35.14 < 35.11 (C8), 34.1 (C10), 33.5 (C9), 31.3 < 31.2 (C23), 30.73 < 30.72 (C22), 30.3 (C2), 28.5 (C11), 29.27 > 29.25 (C4′), 27.50 < 27.49 (C16), 27.1 (C6), 26.1 (C7), 23.7 (C15), 23.1 (C19), 17.22 < 17.21 (C21), 12.7 (C18) ppm; *cis*: 173.85 < 173.83 (C24), 95.1 < 95.0 (C2′), 73.16 < 73.15 (C12), 71.7 (C3), 78.26 < 78.23 (C3′), 64.4 < 64.3 (C5′), 48.14 < 48.13 (C14), 47.1 < 47.0 (C17), 46.5 < 46.4 (C13), 42.0 (C5), 36.3 (C4), 36.0 (C20), 35.2 (C1), 35.1 (C8), 34.1 (C10), 33.5 (C9), 31.01 < 30.97 (C23), 30.69 < 30.68 (C22), 30.3 (C2), 28.51 < 28.46 (C11), 28.37 (C4′), 27.50 < 27.46 (C16), 27.1 (C6), 26.1 (C7), 23.7 (C15), 23.1 (C19), 17.3 < 17.2 (C21), 12.7 (C18) ppm. MS (*m*/*z*): 460.15, 391.10, 373.15, 355.15, 273.10, 255.10. IR (neat, cm^{-1}): 1045, 1244, 1374, 1740, 2866–2938, 3426. Anal. Calcd for $C_{28}H_{46}O_6$ (478.74): C, 70.24; H, 9.71. Found: C, 70.17; H, 9.68. TLC: R_f = 0.2 (EtOAc). Flash chromatography eluent: 30% → 100% EtOAc/hexane.

*2.2.8. (S)-(+)-α-Methoxyphenylacetic acid 2′-hydroxy-tetrahydrofuran-3′-yl ester (**10**)*

The reaction was carried out following the General Procedure A starting with 1 mmol of MPA to yield 111 mg (44%, overall yield for "one-pot synthesis") of **10** in 25 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42, 7.36, 7.34, 5.50, 5.39, 5.35, 5.18, 5.12, 4.86, 4.84, 4.77, 4.76, 4.10, 3.83, 2.41, 2.28, 1.95, 1.68 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ *trans*: 170.14 < 170.09 (C1), 135.71 < 135.68 (C3), 128.89 (C6), 128.7 (C7), 128.7 (C5), 127.19 > 127.17 (C4), 127.19 (C8), 100.01 < 99.98 (C2′), 82.3 < 82.2 (C2), 78.8 > 78.7 (C3′), 66.71 > 66.67 (C5′), 57.33 > 57.29 (C9), 29.4 > 29.0 (C4′) ppm; *cis*: 170.01 < 169.94 (C1),135.6 < 135.5 (C3), 128.85 (C6), 128.6 (C7), 128.7 < 128.64(C5), 127.1 > 127.0 (C4), 127.1 (C8), 95.4 < 95.1 (C2′), 82.1 < 81.9 (C2), 74.0 > 73.8 (C3′), 64.4 > 64.3 (C5′), 57.25 > 57.24 (C9), 28.7 > 28.3 (C4′) ppm. MS (*m*/*z*):251.95, 164.95, 121.05, 105.00, 91.05, 77.00. IR (neat, cm^{-1}): 699, 1032–1105, 1178–1247, 1494, 1739, 2831–2942,

[3] Carbon atoms of deoxy sugar moiety are indicated with prime symbol.

3441. Anal. Calcd for $C_{13}H_{16}O_5$ (252.29): C, 61.89; H, 6.41. Found: C, 61.81; H, 6.38. TLC: R_f = 0.35 (50% EtOAc/hexane). Flash chromatography eluent: 30% → 50% EtOAc/hexane.

*2.2.9. N-(tert-Butoxycarbonyl)-L-phenylalanine 2′-hydroxy-tetrahydrofuran-3′-yl ester (**11**)*[4]

The reaction was carried out following the General Procedure A starting with 1 mmol of Boc-Phe to yield 217 mg (62%, overall yield for "one-pot synthesis") of 11 in 24 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22, 7.21, 7.08, 7.07, 5.25, 5.15, 5.00, 4.47, 3.95, 2.99, 2.98, 2.45, 2.28, 1.99, 1.82, 1.34 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (C1), 155.2 (C9), 135.89/135.83 (C4), 129.5/129.4 (C5), 128.6/128.5 (C6), 127.1/126.9 (C7), 99.9 (C2′), 80.2 (C10), 79.3/79.1 (C3′), 64.5 (C5′), 54.5 (C2), 38.4/38.3 (C3), 28.3 (C4′), 25.2 (C11) ppm. MS (*m*/*z*): 332.90, 259.90, 247.95, 233.90, 85.95, 70.00, 57.00. IR (neat, cm^{-1}):702, 1048, 1168–1248, 1369, 1605, 1738–1739, 2980, 3365. Anal. Calcd for $C_{18}H_{25}NO_6$ (351.44): C, 61.51; H, 7.18; N, 3.99. Found: C, 61.69; H, 7.20; N, 4.01. TLC: R_f = 0.6 (20% isopropanol/hexane). Flash chromatography eluent: 10% isopropanol/hexane.

*2.2.10. N-(tert-Butoxycarbonyl)-L-tyrosine 2′-hydroxy-tetrahydrofuran-3′-yl ester (**12**)*

The reaction was carried out following the General Procedure A starting with 1 mmol of Boc-Tyr to yield 117 mg (32%, overall yield for "one-pot synthesis") of **12** in 24 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88, 6.86, 6.7, 6.6, 5.2, 5.0, 4.95, 4.4, 4.04, 4.02, 2.9, 2.8, 2.3, 2.0, 1.3 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 171.8, 171.7, 155.8, 155.5, 155.4, 130.5, 127.3, 127.0, 115.7, 100.1, 100.0, 80.7, 80.6, 79.3, 79.1, 66.7, 66.6, 60.7, 37.6, 37.1, 29.2, 29.1, 28.4, 25.1, 21.1 ppm. MS (*m*/*z*): 366.90, 348.90, 263.95, 249.90, 219.90, 146.90, 107.00, 87.00, 57.05. IR (neat, cm^{-1}): 830, 1047, 1170–1247, 1517, 1616, 1739, 2937–2981, 3366. Anal. Calcd for $C_{18}H_{25}NO_7$ (367.44): C, 58.83; H, 6.87; N, 3.81. Found: C, 58.95; H, 6.88; N, 3.80. TLC: R_f = 0.4 (10% isopropanol/toluene). Flash chromatography eluent: 10% isopropanol/toluene.

*2.2.11. N-(tert-Butoxycarbonyl)-L-phenylalanine 2′-hydroxy-tetrahydropyran-3′-yl ester (**13**)*

The reaction was carried out following the General Procedure A starting with 1.3 mmol of Boc-Phe to yield 339 mg (72%, overall yield for "one-pot synthesis") of 13 in 24 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32, 7.30, 7.27, 7.25, 7.21, 4.98, 4.88, 4.71, 4.76, 4.60, 4.55, 4.01, 3.93, 3.53, 3.12, 2.09, 1.97, 1.76, 1.67, 1.64, 1.58, 1.54, 1.43 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 > 171.4 (C1), 155.5 < 155.2 (C9), 135.94 > 135.86 (C4), 129.5 > 129.3 (C5), 128.7 > 128.5 (C6), 127.2 > 127.0 (C7), 94.4 > 91.7 (C2′), 80.3 < 80.0 (C10), 72.2 > 71.3 (C3′), 63.2 > 62.0 (C6′), 54.8 < 54.4 (C2), 38.1 (C3), 28.31 < 28.28 (C11), 25.7 > 24.6 (C4′), 22.7 > 22.3 (C5′) ppm. MS (*m*/*z*): 308.95, 290.95, 274.00, 248.05, 164.00, 147.90, 120.05, 100.00, 90.90, 84.00, 57.05. IR (neat, cm^{-1}): 702, 1047, 1168–1245, 1371, 1498, 1718–1740, 2937–2979, 3365. Anal. Calcd for $C_{19}H_{27}NO_6$ (365.47): C, 62.44; H, 7.46; N, 3.83. Found: C, 62.32; H, 7.44; N, 3.82. TLC: R_f = 0.3 (5% isopropanol/toluene). Flash chromatography eluent: 10% → 25% isopropanol/hexane.

*2.2.12. N-(tert-Butoxycarbonyl)-L-tyrosine 2′-hydroxy-tetrahydropyran-3′-yl ester (**14**)*

The reaction was carried out following the General Procedure A starting with 1 mmol of Boc-Tyr to yield 240 mg (53%, overall yield for "one-pot synthesis") of **14** in 24 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01, 6.98, 6.75, 5.24, 5.19, 5.14, 5.12, 4.91, 4.88, 4.83, 4.67, 4.64, 4.51, 4.43, 3.51, 2.89, 2.08, 2.02, 1.75, 1.67, 1.54 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 171.8, 171.72, 171.66 (C1), 155.7, 155.5 (C9), 155.46, 155.43, 155.38, 155.32 (C7), 130.5, 130.3 (C5), 126.93, 126.87, 126.80 (C4), 115.6, 115.52, 115.45 (C6), 91.5, 91.2 (C2′), 80.4, 80.3, 80.2, 80.1 (C10), 72.0, 71.8, 71.3, 71.1 (C3′), 62.7, 62.6, 61.9, 61.3 (C6′), 55.1, 54.82, 54.79, 54.56 (C2), 37.3, 37.2, 36.9, 36.6 (C3), 28.23, 28.20 (C11), 24.6, 24.5, 24.1 (C4′), 22.6, 22.4, 22.3 (C5′) ppm. MS (*m*/*z*): 306.90, 263.90, 245.90, 200.85, 178.90, 146.95, 136.00, 107.00, 84.00, 57.05. IR (neat, cm^{-1}): 734, 1046, 1169–1247, 1370–1517, 1739, 2978, 3374. Anal. Calcd for $C_{19}H_{27}NO_7$ (381.47): C, 59.82; H, 7.15; N, 3.67. Found: C, 59.96; H, 7.16; N, 3.66. TLC: R_f = 0.5 (20% isopropanol/hexane). Flash chromatography eluent: 8% → 20% isopropanol/hexane.

*2.2.13. 3-Acetyl deoxycholic acid (2′S,3′R)-2′-acetoxy-tetrahydrofuran-3′-yl ester (**15**)*

The reaction was carried out following the General Procedure B to yield 210 mg (27%) of **15** in 240 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15, 5.20, 4.71, 4.14, 3.99, 2.40/2.27, 2.09, 2.01, 2.06/1.78, 1.87/1.27, 1.85/1.53, 1.83/1.24, 1.81/1.37, 1.81, 1.77, 1.76/1.03, 1.69, 1.61/1.05, 1.56, 1.51, 1.44, 1.43, 1.41/1.10, 1.40, 0.98, 0.92, 0.68 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3 (C24), 170.8 (C25), 169.7 (C27), 99.5 (C2′), 76.8 (C3′), 74.2 (C3), 73.1 (C12), 68.2 (C5′), 48.2 (C14), 47.2 (C17), 46.4 (C13), 41.7 (C5), 35.9 (C20), 35.0 (C8), 34.8 (C1), 34.1 (C10), 33.5 (C9), 32.1 (C4), 31.0 (C23), 30.6 (C22), 29.4 (C4′), 28.6 (C11), 27.4 (C16), 26.9 (C6), 26.4 (C2), 25.9 (C7), 23.5 (C15), 23.1 (C19), 21.5 (C26), 21.2 (C28), 17.2 (C21), 12.7 (C18) ppm. $[\alpha]^{25}_{546}$ = 23.1 (c 2.96, MeOH). MS (*m*/*z*): 523, 495, 484, 410, 368, 341, 256, 129. IR (neat, cm^{-1}): 1044, 1244, 1374, 1448, 1739, 2940, 3535. Anal. Calcd for $C_{32}H_{50}O_8$ (562.82): C, 68.28; H, 8.97. Found: C, 68.09; H, 8.95. TLC: R_f = 0.25 (30% EtOAc/hexane). Flash chromatography eluent: 25% EtOAc/hexane.

*2.2.14. (S)-(+)-α-Methoxyphenylacetic acid (2′S,3′R)-2′-acetoxy-tetrahydrofuran-3′-yl ester (**16**)*

The reaction was carried out following the General Procedure B to yield 49 mg (33%) of **16** in 160 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42, 7.37, 7.34, 6.18, 5.22, 4.78, 4.03/3.68, 3.42, 2.05, 1.73/2.25 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) 169.7 (C1), 169.5 (C10), 135.7 (C3), 128.9 (C6), 128.7 (C5,7), 127.1 (C4,8), 99.3 (C2′), 82.1 (C2), 77.6 (C3′), 68.0 (C5′), 57.4 (C9), 29.3 (C4′), 21.1 (C11) ppm. $[\alpha]^{25}_{546}$ = +0.4 (c 1.38, MeOH). MS (*m*/*z*): 293.90, 234.90, 164.90, 129.00, 121.00, 105.00, 91.00, 77.00. IR (neat, cm^{-1}): 699, 1014, 1116, 1173–1231, 1367, 1494, 1748, 2907–2939. Anal. Calcd for $C_{15}H_{18}O_6$ (294.33): C, 61.21; H, 6.18. Found: C, 61.16; H, 6.19. TLC: R_f = 0.6 (50% EtOAc/hexane). Flash chromatography eluent: 20% EtOAc/hexane

*2.2.15. N-(tert-Butoxycarbonyl)-L-phenylalanine (2′S,3′R)-2′-acetoxy-tetrahydrofuran-3′-yl ester (**17**)*

The reaction was carried out following the General Procedure B to yield 99 mg (41%) of 17 in 260 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31, 7.28, 7.17, 5.18, 4.99, 4.58, 4.08, 3.98, 3.09, 2.30, 2.07, 1.44 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2 (C1), 169.5 (C12), 155.1 (C9), 135.8 (C4), 129.3 (C5), 128.7 (C6), 127.2 (C7), 99.4 (C2′), 80.2 (C10), 77.9 (C3′), 68.1 (C5′), 54.5 (C2), 38.5 (C3), 29.4 (C4′), 28.3 (C11), 21.1 (C13) ppm. MS (*m*/*z*): 333.00, 278.00, 276.95, 259.95, 164.00, 120.00, 70.00, 57.05. IR (neat, cm^{-1}):702, 756, 1167–1233, 1368, 1498, 1605, 1715–1747, 2932–2979, 3373. $[\alpha]^{25}_{546}$ = −58.6 (c 1.41, MeOH). Anal. Calcd for $C_{20}H_{27}NO_7$ (393.48): C, 61.05; H, 6.93; N, 3.56. Found: C, 61.12; H, 6.95; N, 3.55. TLC: R_f = 0.2 (20% EtOAc/hexane). Flash chromatography eluent: 15% EtOAc/hexane.

*2.2.16. N-(tert-Butoxycarbonyl)-L-tyrosine (2′S,3′R)-2′-acetoxy-tetrahydrofuran-3′-yl ester (**18**)*

The reaction was carried out following the General Procedure B to yield 22 mg (27%) of **18** in 182 h. ^{1}H NMR (500 MHz,

[4] In the case of *N*-Boc-protected amino acids E- and Z-isomers (caused by urethane bond in Boc-group) complicate the spectra by exchange broadened peaks.

$CDCl_3$) δ 6.99, 6.76, 6, 32, 6.09, 5.16, 5.03, 4.51, 4.07/3.98, 2.98, 2.29/1.85, 2.11 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3 (C1), 169.7 (C12), 155.22 (C7), 155.16 (C9), 130.3 (C5), 127.1 (C4), 115.5 (C6), 99.3 (C2′), 80.3 (C10), 77.7 (C3′), 68.0 (C5′), 54.6 (C2), 37.5 (C3), 29.3 (C4′), 28.2 (C11), 21.1 (C13) ppm. MS (*m*/*z*): 365.95, 306.90, 200.90, 178.90, 107.00, 84.00, 57.05. IR (neat, cm^{-1}): 757, 1012–1108, 1165–1236, 1369–1517, 1615, 1744, 2935–3020, 3377. $[\alpha]^{25}_{546} = -45{,}4$ (c 0.57, MeOH). Anal. Calcd for $C_{20}H_{27}NO_8$ (409.48): C, 58.66; H, 6.66; N, 3.42. Found: C, 58.73; H, 6.64; N, 3.40. TLC: $R_f = 0.3$ (40% EtOAc/hexane). Flash chromatography eluent: 30% EtOAc/hexane.

*2.2.17. N-(tert-Butoxycarbonyl)-L-phenylalanine (2′S,3′R)-2′-acetoxy-tetrahydropyran-3′-yl ester (**19**)*

The reaction was carried out following the General Procedure B to yield 121 mg (44%) of 19 in 200 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30, 7.25, 7.16, 5.76, 4.99, 4.74, 4.60, 3.87/3.66, 3.09, 2.11, 2.00/1.65, 1.78/1.47 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0 (C1), 169.2 (C12), 154.9 (C9), 135.7 (C4), 129.2 (C5), 128.5 (C6), 127.0 (C7), 90.9 (C2′), 79.9 (C10), 68.4 (C3′), 62.7 (C_6′), 54.4 (C2), 38.3 (C3), 28.2 (C11), 24.1 (C4′), 20.6 (C5′), 21.0 (C13) ppm. MS (*m*/*z*): 347.00, 291.00, 274.00, 164.00, 128.05, 120.10, 100.05, 84.00, 57.05, 43.05. IR (neat, cm^{-1}): 702, 1012–1075, 1166–1233, 1369, 1498, 1605, 1716–1748, 2935–2976, 3374. $[\alpha]^{25}_{546} = -34.9$ (c 1.69, MeOH). Anal. Calcd for $C_{21}H_{29}NO_7$ (407.51): C, 61.89; H, 7.19; N, 3.44. Found: C, 61.74; H, 7.17; N, 3.45. TLC: $R_f = 0.5$ (10% isopropanol/toluene). Flash chromatography eluent: 15% EtOAc/hexane.

*2.2.18. N-(tert-Butoxycarbonyl)-L-tyrosine (2′S,3′R)-2′-acetoxy-tetrahydropyran-3′-yl ester (**20**)*

The reaction was carried out following the General Procedure B to yield 56 mg (24%) of **20** in 214 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98, 6.96, 6.75, 6.73, 5.77, 5.10, 4.75, 4.53, 4.39, 3.86, 3.66, 2.99, 2.88, 2.16, 2.01, 1.79, 1.68, 1.48, 1.41 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2 (C1), 169.4 (C12), 155.3 (C7), 155.2 (C9), 130.2 (C5), 126.9 (C4), 115.4 (C6), 90.9 (C2′), 80.2 (C10), 68.4 (C3′), 62.6 (C_6′), 54.6 (C2), 37.4 (C3), 28.2 (C11), 24.0 (C4′), 21.0 (C13), 20.5 (C5′) ppm. MS (*m*/*z*): 423.00, 363.05, 348.95, 307.00, 201.00, 179.05, 106.90, 107.00, 84.00, 57.05. IR (neat, cm^{-1}): 757, 1012–1108, 1165–1236, 1369–1517, 1615, 1744, 2935–3020, 3377. $[\alpha]^{25}_{546} = -38.8$ (c 1.07, MeOH). Anal. Calcd for $C_{12}H_{29}NO_8$ (423.51): C, 59.55; H, 6.92; N, 3.31. Found: C, 59.38; H, 6.90; N, 3.29. TLC: $R_f = 0.4$ (10% isopropanol/toluene). Flash chromatography eluent: 30% EtOAc/hexane

*2.2.19. Deoxycholic acid (3′R)-2′-hydroxy-tetrahydrofuran-3′-yl ester (**9a**)*

The reaction was carried out following the General Procedure C to yield 32 mg (54%, conversion 80% in 21 days) of 9a. ^{1}H NMR (800 MHz, $CDCl_3$) 5.35 s > 5.47 d 4.2 Hz (H2′), 5.06 dd 5.6 and 0.9 Hz > 4.99 ddd 7.8, 7.2 and 4.2 Hz (H3′), 4.14–4.02 m (H5′), 3.98 t 2 × 1.9 Hz (H12), 3.58 tt 2 × 4.8 and 2 × 10.6 Hz (H3), 2.42–2.21 m (H23, H4′), 1.94–0.95 m from ring and side chain protons of isomers, 0.95 d 6.4 Hz (H21), 0.89 s (H19), 0.661 < 0.659 s (H18). δ ^{13}C NMR (200 MHz, $CDCl_3$) δ 173.92 < 173.91 (C24), 100.18 > 94.86 (C2′), 78.21 < 73.19 (C3′), 73.15 < 73.14 (C12), 71.64 (C3), 66.65 > 65.32 (C5′), 48.06 > 48.03 (C14), 47.06 > 46.99 (C17), 46.40 < 46.39 (C13), 41.95 < 41.94 (C5), 36.15 (C4), 35.89 < 35.88 (C8), 35.2 (C1), 35.17 (C1 and C20), 34.05 < 34.04 (C10), 33.43 (C9), 31.22 > 30.94 (C23), 30.66 > 30.60 (C22), 30.22 < 30.20 (C2), 29.17 > 28.27 (C4′), 28.42 > 28.40 (C11), 27.50 < 27.49 (C16), 27.04 > 27.03 (C6), 26.08 < 26.07 (C7), 23.64 < 23.62 (C15), 23.05 (C19), 17.15 < 17.13 (C21), 12.64 (C18). $[\alpha]^{25}_{546} = 36.3$ (c 1.2, MeOH). TLC: $R_f = 0.2$ (10% isopropanol/toluene). Flash chromatography eluent: 70% EtOAc/hexane.

*2.2.20. (S)-(+)-α-Methoxyphenylacetic acid (3′R)-2′-hydroxy-tetrahydrofuran-3′-yl ester (**10a**)*

The reaction was carried out following the General Procedure C to yield 11 mg (42%) of **10a** in 72 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ *trans*: 7.3–7.5, 5.41, 5.13, 4.78, 4.08/3.87, 3.41, 2.31/1.71 ppm; *cis*: 7.3–7.5, 5.51, 5.04, 4.86, 4.01/3.76, 3.42, 2.21/1.96 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ *trans*: 170.0 (C1), 135.9 (C3), 128.83 (C6), 128.63 (C5,7), 127.07 (C4,8), 100.0 (C2′), 82.0 (C2), 78.8 (C3′), 66.8 (C5′), 57.2 (C9), 29.0 (C4′) ppm; *cis*: 170.1 (C1), 135.9 (C3), 128.89 (C6), 128.69 (C5,7), 127.14 (C4,8), 95.0 (C2′), 82.0 (C2), 73.9 (C3′), 64.2 (C5′), 57.4 (C9), 29.0 (C4′) ppm. $[\alpha]^{25}_{546} = 59.5$ (c 0.3, MeOH). Flash chromatography eluent: 20% EtOAc/hexane.

*2.2.21. N-(tert-Butoxycarbonyl)-L-phenylalanine (3′R)-2′-hydroxy-tetrahydrofuran-3′-yl ester (**11a**)*

The reaction was carried out following the General Procedure C to yield 24 mg (68%) of 11a in 260 h. ^{1}H NMR (125 MHz, $CDCl_3$) δ 7.23, 7.21, 7.19, 7.09, 7.07, 5.25, 5.01, 4.99, 4.96, 4.94, 4.5, 4.48, 4.04, 3.99, 3.98, 3.85, 3.83, 3.10, 2.99, 2.98, 2.27, 2.25, 1.97, 1.67, 1.34 ppm. ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.5, 155.1, 135.9, 135.9, 129.3, 128.7, 128.6, 127.2, 127.2, 100.2, 80.1, 79.1, 74.2, 66.8, 54.5, 38.5, 29.2, 28.3, 23.9, 14.2 ppm. $[\alpha]^{25}_{546} = -20.9$ (c 0.77, MeOH). TLC: $R_f = 0.43$ (10% isopropanol/toluene). Flash chromatography eluent: 25% EtOAc/hexane.

*2.2.22. N-(tert-Butoxycarbonyl)-L-tyrosine (3′R)-2′-hydroxy-tetrahydrofuran-3′-yl ester (**12a**)*

The reaction was carried out following the General Procedure C to yield 12 mg (80%) of **12a** in 216 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99, 6.76, 5.38, 5.23, 5.14, 5.06, 4.53, 4.37, 4.13, 4.07, 3.99, 3.85, 3.03, 2.98, 2.33, 2.19, 1.93, 1.75, 1.43 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 (C1), 155.4 (C7), 155.2 (C9), 130.4 (C5), 127.1 (C4), 115.5 (C6), 99.9 > 95.5 (C2′), 80.5 (C10), 78.9 > 74.0 (C3′), 66.6 > 64.4 (C5′), 54.6 (C2), 37.6 (C3), 29.6 (C4′), 28.3 (C11) ppm. $[\alpha]^{25}_{546} = -18.9$ (c 0.4, MeOH). TLC: $R_f = 0.3$ (10% isopropanol/toluene). Flash chromatography eluent: 40% EtOAc/hexane.

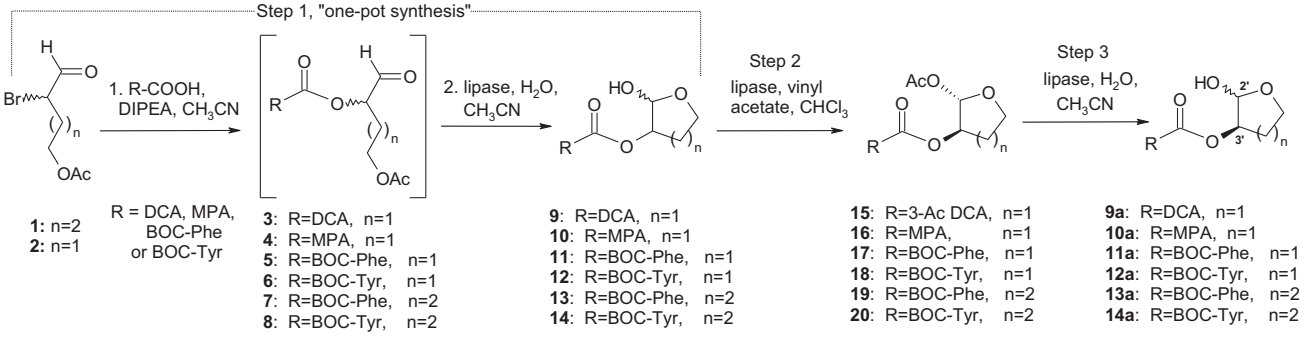
*2.2.23. N-(tert-Butoxycarbonyl)-L-phenylalanine (3′R)-2′-hydroxy-tetrahydropyran-3′-yl ester (**13a**)*

The reaction was carried out following the General Procedure C to yield 39 mg (89%) of 13a in 72 h. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23, 7.20, 7.10, 5.02, 4.85, 4.66, 4.59, 3.90, 3.67, 3.45, 3.02, 2.04, 2.01, 1.62, 1.46, 1.34 ppm. ^{13}C NMR (125 MHz, $CDCl_3$) 171.8, 171.7 (C1), 155.5, 155.2 (C9), 136.0, 135.9 (C4), 129.4, 129.3 (C5), 128.6, 128.6 (C6), 127.2, 127.1 (C7), 94.2, 91.8 (C2′), 80.5, 80.1 (C10), 71.9, 71.1 (C3′), 62.8, 62.4 (C6′), 55.1, 54.7 (C2), 38.4, 37.8 (C3), 28.3 (C11), 25.4, 24.8 (C4′), 22.5, 22.2 (C5′) ppm. $[\alpha]^{25}_{546} = -11.1$ (c 1.56, MeOH). TLC: $R_f = 0.45$ (10% isopropanol/toluene). Flash chromatography eluent: 30% EtOAc/hexane.

3. Results and discussion

The detailed synthetic scheme of deoxy sugar esters consists of following steps (Scheme 2):

Step 1. The first reaction of this step is the *O*-alkylation of carboxylic acid with bromoaldehyde (**1** or **2**). The *O*-alkylation is followed by the lipase-catalyzed deacetylation of the ω-hydroxyl group, the hydroxy aldehyde formed undergoes a spontaneous cyclization affording a mixture of diastereomers of deoxy sugar ester in hemiacetal form. These two reactions were carried out as a "one-pot synthesis". This increased the yield of the products as the separation of the aldehyde intermediates **3**–**8** over silica appeared to be quite complicated and the base used for alkylation did not interfere with the enzymatic reaction. The diastereomeric



Scheme 2. The chemoenzymatic synthesis of deoxy sugar esters of different carboxylic acids.

esters of hemiacetal deoxy sugar formed (**9**–**14**) were inseparable by column chromatography and were only purified as a mixture from side products.

Step 2. The diastereomeric mixture of each of the hemiacetal esters (**9**–**14**) was further enzymatically acetylated in stereoselective manner affording one diastereomer of *trans*-hemiacetal acetate (**15**–**20**), which was easily separated by column chromatography over silica.

Step 3. The deacetylation of individual hemiacetal acetates by lipase gave desired stereochemically pure deoxy sugar esters, albeit as a mixture of two anomers under the conditions used (**9a**–**14a**).

It should be emphasized that the lipase-catalyzed acetylation (Step 2) is diastereoselective for the compounds investigated in the current work; in this synthetic step the configuration of the stereogenic center at C_2 of deoxy sugar skeleton controls the stereochemical result of the enzymatic reaction. The hemiacetal acetate that is formed is of *trans* geometry and at the same time the stereochemistry of this acetate is in accordance with the Kazlauskas' rule for secondary alcohols [16].

3.1. The synthesis of furanose deoxy sugar esters of DCA

The *O*-alkylation of the DCA carboxyl group with bromobutanal (**2**) followed by the lipase-catalyzed deacetylation of the terminal hydroxyl group of deoxy sugar provided a mixture of hemiacetal deoxy sugar ester diastereomers (**9**) with 35% yield (Table 1). This could be considered as a satisfactory result taking into account two issues: 1) that DCA as an acyl group on C_2-OH of furanose deoxy sugar ester is accessible to the lipase and 2) adding methanol to the reaction medium was necessary. The first point has been proven by identification of some (modest) amount of parent deoxy sugar (tetrahydrofuran-2,3-diol) in the crude product of the deacetylation. The solubility of DCA in acetonitrile medium is low; therefore, methanol was added to improve the homogenization of the reaction mixture of *O*-alkylation. (Unfortunately, methanol is not inert towards an α-bromo-aldehyde under basic conditions used. As a result – the yield of *O*-alkylation was somewhat diminished.) Due to the presence of methanol in the reaction mixture, the deacetylation step necessitated no additional nucleophile (water has been added in other cases) but only enzyme preparation. Along with the target compound **9** also two byproducts were separated and identified as DCA methyl ester (7%) and parent deoxy sugar (10%). The mixture of diastereomers purified over column chromatography was subjected to the enzymatic acetylation, which appeared to be slower as compared to corresponding pyranose deoxy sugar esters. The acetylation gave target compound as individual diastereomer – acetylated cyclic *trans* hemiacetal deoxy sugar ester (**15**), which was separated by flash chromatography. Another product obtained was a mixture of stereoisomers of the open-chain (aldehyde) deoxy sugar ester. In the lipase-catalyzed acetylation also the hydroxyl group attached to the C_3 atom of the steroid skeleton was reactive, but the subsequent lipase-catalyzed deacetylation cleaved both of the acetates. The latter reaction appeared to be a rather time-consuming process (conditions were not optimized). The deacetylation resulted during incubation with lipase for 21 days at room temperature in the formation of the stereochemically pure furanose deoxy sugar (3-deoxy D-erythrose) ester of DCA (**9a**), which exists as a mixture of two anomers in solution.

3.2. The synthesis of furanose deoxy sugar esters of (S)-MPA

The "one-pot synthesis" of MPA hemiacetal deoxy sugar esters (**10**) gave this stereoisomeric mixture with reasonable yield and time. The resolution of diastereomers by lipase-catalyzed acetylation gave two products of which the more polar one was separated over silica and identified by NMR to be the desired acetylated furanose hemiacetal as an individual diastereomer (**16**) while the less polar product decomposed in contact with silica gel during column chromatography and thus remaining unidentified. The deacetylation of ester **16** proceeded smoothly (during 72 h) and selectively – no byproducts were detected. The 3-deoxy D-erythrose ester **10a** was obtained in the form of a mixture of two anomers.

An important feature of the synthesis of MPA esters regarding the whole strategy towards the stereochemically pure furanose deoxy sugar esters was the successful experimental determination of the absolute configuration of deoxy sugar ester **10a**, which is preferably acetylated by CALB. This was easy to achieve by determination of the differential shielding effect for the C'_4 atom of the furanose ring from the ^{13}C NMR spectrum of the diastereomeric mixture **10**. The corresponding value of chemical shift difference (differential shielding), $\Delta\delta$, was found to be as large as 0.350 ppm (29.35–29.00) for the pair of the ester diastereomers **10** (determined for the *trans* anomers). The individual deoxy sugar ester **10a** was identified by observed significant shielding of C'_4 of furan ring by the phenyl group. As a result, it was proven that CALB catalyzes acetylation of furanose deoxy sugar ester hemiacetals preferring the stereoisomer in accordance with the Kazlauskas' rule for secondary alcohols. The same has been shown for pyranose deoxy sugar hemiacetals [16].

The absolute configuration of **10a** and of other resolved deoxy sugar ester stereoisomers correspond to what has been depicted in the Scheme 2.

3.3. The synthesis of furanose and pyranose deoxy sugar esters of N-Boc-phenylalanine

The first two steps of the synthetic sequence integrated into a "one-pot synthesis" of the esterification of *N*-Boc-L-phenylalanine

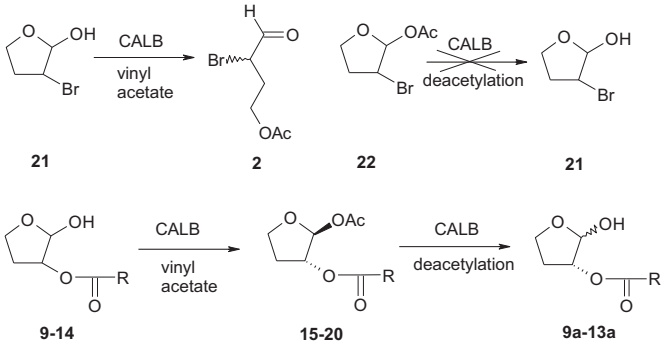
using either aldehyde **2** or **1** as a deoxy sugar precursor proceeded smoothly and gave hemiacetal deoxy sugar esters **11** and **13**, respectively, both of them in good yield. Again, the parent deoxy sugars – tetrahydrofuran-2,3-diol for the former and tetrahydropyran-2,3-diol for the latter, were determined as byproducts, evidently due to the breaking of the ester group formed by the carboxylic acid *N*-Boc-Phe and 2-hydroxyl group of deoxy sugar moiety. The latter evidence observed indicates not very high functional group selectivity of the process in some cases. Nevertheless, in both cases, the desired deoxy sugar esters were obtained in good yield and the reaction time all together did not exceed 48 h under standard conditions. On the contrary, the lipase-catalyzed acetylation used for the separation of diastereomers occurred slowly. The incubation of deoxy furanose ester (**11**) with CALB during 260 h afforded only acetylated hemiacetal **17** that was successfully separated and identified. In the case of the pyranose deoxy sugar ester (**13**), again, only an acetylated desired product **19** was obtained, while the other starting diastereomer afforded only traces of several byproducts that were discarded. The yields (41% and 44%) of the hemiacetal acetates (**17**) and (**19**), respectively, were high considering the fact that only one half of the substance should be converted into the acetylated product. Finally, the CALB-catalyzed deacetylation of the hemiacetal acetates (**17**) and (**19**) resulted in the formation of the desired stereochemically pure deoxy sugar esters (**11a**) and (**13a**) with good (68%) to excellent (89%) yields, respectively. The reaction proceeded more easily for the pyranose hemiacetal while the furanose hemiacetal acetate required 3.5 times longer incubation time under the same conditions.

3.4. The synthesis of furanose and pyranose deoxy sugar esters of *N*-Boc-tyrosine

In the case of *N*-Boc-L-tyrosine, the *O*-alkylation was smooth for both starting bromoaldehydes and also occurred similarly to the above synthesis of analogous *N*-Boc-Phe derivatives. The *O*-alkylation and the subsequent enzymatic hydrolysis step produced, together with the desired deoxy sugar esters (**12**) and (**14**), respectively, also the aforementioned parent deoxy sugars, as a major byproduct. For the "one-pot synthesis" the overall yield of the pyranose deoxy sugar ester of *N*-Boc-Tyr (**14**) was higher than that of corresponding furanose deoxy sugar ester (**12**) but notably lower than that of corresponding *N*-Boc-Phe esters (**11**) and (**13**). In the lipase-catalyzed kinetic resolution of diastereomers the yield of *N*-Boc-Tyr esters (**18**) and (**20**) was lower than that observed for *N*-Boc-Phe esters (**17**) and (**19**). At the same time, there was no great difference between the yield of acetylated furanose and pyranose deoxy sugar esters in case of both of the *N*-Boc protected amino acid esters.

The deacetylation of *N*-Boc-Tyr esters (**18**) and (**20**) were performed by CALB in accordance with standard procedure. The deacetylation of **18** led to the formation of the desired deoxy furanose ester (**12a**) in good yield. On the other hand, lipase-catalyzed deacetylation of pyranose deoxy sugar ester (**20**) remained, even after several attempts, unsuccessful. Currently, it is hard to explain this dramatic difference in the behavior of the pyranose *vs*. furanose deoxy sugar esters. Further experimental investigation of this synthesis supported by molecular modeling studies is needed to clarify the reasons for such different results compared to the other pyranose deoxy sugar esters.

In summary, the main difference between furanose and pyranose deoxy sugar esters is that in most cases the lipase-catalyzed acetylation of furanose hemiacetals produces only acetylated cyclic form. On the contrary, when starting from pyranose deoxy sugar esters, also an open-chain aldehyde form has often been gained besides the stereochemically pure cyclic hemiacetal acetate [16].



Scheme 3. The chemoselectivity (and feasibility) of the reactions depends on the substituent at the 3rd position of the furan cycle.

However, this does not apply for *N*-Boc-L-amino acid esters as, apparently, the amino group has crucial influence on the decyclization of hemiacetal in the active site of the enzyme or causes (makes possible) the occurrence of further uncontrolled reactions [21].

It is important to note that the CALB-catalyzed acetylation of the α-bromo-ω-hydroxy butanal (**21**, Scheme 3), which mainly exists in a furanose hemiacetal form, results almost solely in the formation of an extended-chain α-bromo-ω-acetoxy butanal (**2**) [17]. The current work has shown that the lipase-catalyzed acetylation of furanose hemiacetal deoxy sugar esters **9**–**14** (the analogues of **21**) affords, prevalently, acetylated cyclic hemiacetals **15**–**20** in high *de*. There is still no convincing rationalization available for this important difference.

It has also been observed that the CALB-catalyzed deacetylation of the acetylated [17] stereoisomeric bromotetrahydrofuranols **22** (Scheme 3) failed in our hands. In this work, on the contrary, the deacetylation of the related compounds **15**–**20** catalyzed by the same enzyme was successful with most of the esters under investigation producing hemiacetals **9a**–**13a**.

In summary, we have to conclude that the substituents at the 3rd position of the 2-furanols greatly influence the selectivities of the lipase-catalyzed acetylation; they may affect the feasibility of the acetylation and deacetylation of this type of compounds in general, independently of the stereochemistry of the substrate in some cases.

4. Conclusions

The synthetic strategy developed previously for the synthesis and stereochemical resolution of the pyranose dideoxy sugar esters is also applicable to the most of the furanose deoxy sugar esters investigated.

In the case of all four carboxylic acids tested – deoxycholic acid, α-methoxyphenylacetic acid, *N*-Boc-phenylalanine and *N*-Boc-tyrosine – the corresponding stereochemically pure 3-deoxy D-erythrose ester was obtained in satisfactory to good yield.

In addition, 3,4-dideoxy D-ribose esters of *N*-Boc-phenylalanine and *N*-Boc-tyrosine were synthesized in a diastereoselective manner.

By evaluating yields of the products and incubation times needed for the enzymatic resolution of stereoisomers, the overall tendency is that pyranose deoxy sugar derivatives are, in general (still, with one exception – 3,4-dideoxy D-ribose of *N*-Boc-Tyr ester), slightly more proper target compounds for the current synthetic strategy than furanose deoxy sugar derivatives.

Finally, it should be emphasized that for the synthesis of deoxy sugar esters within the frames of the current synthetic approach – the remote hydroxyl groups of carboxylic acids (for instance, DCA and *N*-Boc-Tyr) need no protection.

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