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Regio- and Stereoselective Lipase-Catalysed Acylation of Methyl α-D-Glycopyranosides with Fluorinated β-Lactams

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Burkholderia cepacia lipase (lipase PS-D) catalysed acylation with 3,3-difluoro-4-phenyl-, -thiophen-3-yl- and -4-pyridyl-azetidin-2-ones was examined for the formation of *N*-Boc-protected 6-*O*-acylated sugar–β-amino acid conjugates from methyl α-D-galacto-, -gluco- and mannopyranosides and Boc_2O. The 6-*O*-acylated glycopyranoside–β-amino acid conjugates were isolated and characterized. The low solubility of the gluco- and mannopyranosides and the high reactivity of the pyridylazetidinone restricted product formation. Activation of the β-lactam ring by the presence of fluorine substituents was shown to be necessary for the enzymatic acylation reaction. The (*S*)-enantiomers of the racemic β-lactam substrates reacted with the sugars.

Introduction

A four-membered β-lactam (azetidin-2-one) ring is a structural element of natural products and a precursor of β-amino acids, many of which are biologically and pharmacologically active compounds.[1,2] The ring-opening of β-lactam antibiotics by β-lactamase-catalysed mechanisms represents a threat to society in the form of growing bacterial resistance, similar ring-opening reactions in the hands of a chemist lead to intriguing synthetic possibilities.[2] Enantiomeric and diastereomeric purity is generally expected of pharmaceutically active ingredients. Enzymes are remarkably effective among all known catalysts in the degree of their acceleration of chemical reactions and their selectivity. Serine hydrolases such as lipases (EC 3.1.1.3) are valuable catalysts. Lipases accept a wide range of substrates in the different types of reactions they catalyse (e.g., hydrolysis, alcoholysis, aminolysis, interesterification and Michael addition),[1,3] and they also show a high stability and diversity. They have also been used as cheap regio- and enantioselective ring-opening catalysts of β-lactams in the preparation of β-amino acids[1] and their esters[4a] and amides, which could then be used, for example, in the synthesis of β-dipeptides.[4b,4c] Instances of the catalytic cleavage of amides by lipases are rare, and are mostly restricted to *Candida antarctica* lipases; a number of serine proteases readily fulfil this function.[5] The orientation of the nitrogen, which can stabilize the transition state of serine proteases, has been reported to be important for effective amide cleavages; lipases (and esterases) lack this stabilization.[6] That lipases cleave the amide bonds of β-lactams can be explained by the fact that the amide functional group lacks the resonance stabilization that typically stabilizes normal amide bonds.

A β-lactam serves as an irreversible acyl donor for lipase-catalysed acylation reactions. In mechanistic terms, the serine residue of the catalytic triad at the active site of the lipase enzyme first opens the β-lactam ring, leading to the formation of an ester intermediate (an acyl–enzyme intermediate). Typically, the formation of the intermediate should release the first reaction product (glycerol in the natural reaction). However, with a β-lactam substrate, no product is released in this mechanistic step. Reaction of the intermediate with a suitable nucleophile (NuH) then gives the β-amino acid or its derivative as the only reaction product (Scheme 1). For reactions in organic solvents, *Pseudomonas* sp. lipases (lipase AK and P-30) were first reported to catalyse the ring-opening of a *N*-benzoyl-activated β-lactam with methanol in *tert*-butyl methyl ether (TBME).[7a] Later, the value of lipase catalysis for the production of individual β-amino acid enantiomers from racemic β-lactams indicated that running the reaction in diisopropyl ether (DIPE) with just traces of added water could give effective ring-opening with *Candida antarctica* lipase B (CAL-B as a Novozym 435 preparation).[7b] Studies with mixtures of a 4-benzyl-β-lactam, methanol, and *Burkholderia cepacia* lipase (in the form of the lipase PS-D preparation) in dry organic solvents revealed potential side-reactions resulting from the presence of the so-called residual water in the enzyme preparation. These reactions led to the hydrolysis both of the β-lactam ring itself (Scheme 1, route B) and of the methyl ester (Scheme 1, route D) produced by ring-opening by methanol (Scheme 1, route A).[7c] Accordingly, studies where hydrolytic side-reactions are addressed and

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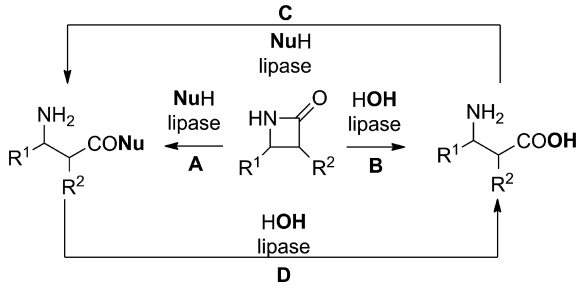
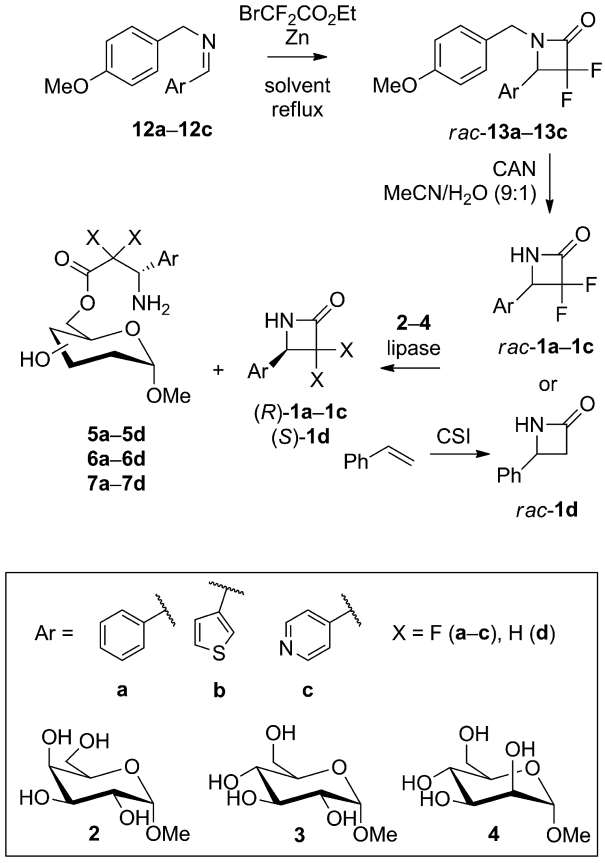
controlled are important for the enantioselective ring-opening of β-lactams by nucleophiles other than water.



Scheme 1. Possible reaction routes for the lipase-catalysed ring-opening of β-lactams: acylation with nucleophile (NuH) by ring-opening of the β-lactam (A), hydrolysis of the β-lactam (B), acylation with NuH by reaction with the free acid (C) and hydrolysis of formed sugar–β-amino acid conjugate (D).

As a continuation of our work towards the chemoenzymatic preparation of β-amino acids[1a] and sugar derivatives,[8] we aimed to combine our knowledge of these areas for the preparation of sugar–β-amino acid conjugates. Thus, we studied the regio- and stereoselective chemo-enzymatic formation by lipase catalysis of sugar esters **5**–**7**, starting from racemic β-lactams *rac*-**1a**–**1d** (for which the electron-withdrawing ability of the substituents increases in the order phenyl < thiophen-3-yl < pyridin-4-yl) and unprotected methyl α-D-galacto-, α-D-gluco- and α-D-mannopyranosides (**2**–**4**, respectively; Scheme 2). β-Lactam structures *rac*-**1a**–**1c** were chosen as starting materials as it had previously been shown that an electron-withdrawing fluorine substituent favoured the ring-opening reaction by methanol.[4a] Moreover, the presence of fluorine (an isostere of hydrogen) may introduce various positive effects to biologically active molecules, such as increased metabolic stability, target binding, and lipophilicity.[9] β-Lactam *rac*-**1d** was studied as a hydrogen-containing analogue of *rac*-**1a**. The lipase-catalysed ring-opening of *rac*-**1a**–**1c** and that of *rac*-**1d** with a primary alcohol group at the C-6 position of the sugars was planned to be the regio- and stereoselective step of the process. It was anticipated that the *S* (*R* for *rac*-**1d**) enantiomer of the β-lactam would react selectively in the ring-opening reaction, as observed in previous studies.[4,10] This selectivity was confirmed by testing enantiopure (*R*)- and (*S*)-**1b** in the enzymatic acylation of **2**. For reasons of cost, racemic β-lactams rather than pure enantiomers were used as acyl donors in the acylation of the unprotected methyl glycopyranosides. It is clear that the use of pure enantiomers for acylation would have the advantage of ensuring the diastereomeric purity of the sugar derivatives prepared.



Scheme 2. Chemoenzymatic route to sugar amino acid esters **5**–**7**.

Results and Discussion

Synthesis of β-Lactams

A slightly modified Reformatsky-type addition was used to prepare racemic 3,3-difluoro-4-arylazetidin-2-ones (*rac*-**1a**–**1c**).[4a,11] *N*-4-Methoxybenzyl-protected β-lactams *rac*-**13a**–**13c** were obtained by heating the corresponding 4-methoxybenzyl aldimines (i.e., **12a**–**12c**) with ethyl bromodifluoroacetate (2 equiv.) in the presence of Zn dust in refluxing 2-methyltetrahydrofuran (2-MeTHF) or tetrahydrofuran (THF). In the case of *rac*-**13c**, the isolated product was an inseparable mixture of the β-lactam and the corresponding β-amino ester (in a 1:3 ratio, according to ^{1}H NMR spectroscopy). *N*-Deprotection by treatment with ceric ammonium nitrate (CAN; 2 equiv.) gave *rac*-**1a**–**1c**. The synthesis of 4-phenylazetidin-2-one (*rac*-**1d**) was accomplished by the cycloaddition of chlorosulfonyl isocyanate (CSI) to styrene also by a known method.[4a,12] The synthetic details are given in the Supporting Information (Scheme S1).

For the preparation of the individual β-lactam enantiomers, *rac*-**1b** was first hydroxymethylated with paraformaldehyde under ultrasound conditions (Scheme S2, Supporting Information).[10] Thereafter, the lipase-catalysed enantioselective *O*-acylation of *N*-hydroxymethylated *rac*-**1b** with vinyl butanoate and lipase PS-D in toluene was performed followed by the separation of the unreacted (*R*)-alcohol (>99% *ee*) and the (*S*)-butanoate ester (74% *ee*). Subsequent enantiomeric purification of the butanoate product by enzymatic alcoholysis with 1-butanol in DIPE allowed the preparation of *N*-hydroxymethylated (*S*)-**1b**. Deprotection with $KMnO_4$ gave the (*R*)- and (*S*)-**1b** enantiomers with 99 and 98% *ee*, respectively. The same

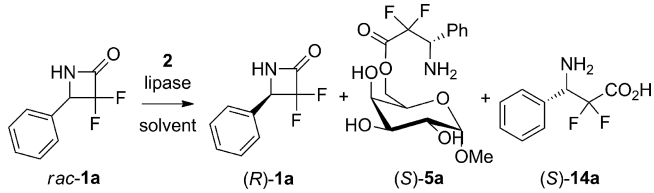
routine procedure could also be used for the preparation of other (*R*)- and (*S*)-lactams, but this was not done.

Optimization of Sugar–β-Amino Acid Conjugate Formation

The use of carbohydrates for the ring-opening of a β-lactam by lipase catalysis has potential for the preparation of intriguing sugar–β-amino acid conjugates (Scheme 2). Optimization was performed using model substrates, i.e., *rac*-**1a** as an acyl donor and methyl α-D-galactopyranoside (**2**) as a nucleophile. As the solubility of unprotected methyl glycopyranosides in the organic solvents that are typically used for lipase catalysed acylation might be limited, the solubility of **2** (50 mM) in 11 organic solvents was tested (Figure S2, Supporting Information), and three of them were selected for our studies on different grounds. *tert*-Amyl alcohol (*t*AmOH) as a water-soluble alcohol was chosen as it dissolves **2** well, and it was also used as a solvent in our previous lipase-catalysed acylations of **2**–**4**.[8c] TBME was included as it is an excellent solvent for enantioselective β-lactam ring cleavages with lipases.[1,4,7] 2-MeTHF was chosen as a more stable variant of THF. THF is a common solvent used for the enzymatic reactions of carbohydrates,[13] and 2-MeTHF has more recently been used as an alternative reaction solvent.[14] TBME and 2-MeTHF are both water insoluble, and although they dissolve **2** poorly, we hoped that the presence of other reagents and products would affect the solubility equilibrium in a positive way.[15]

For the lipase-screening, the model substrates [i.e., *rac*-**1a** (50 mM) and **2** (1 equiv.)] were dissolved as well as possible in the selected solvents, and then a catalyst [CAL-B, lipase PS-D, *Rhizomucor miehei* lipase (Lipozyme RM IM), or *Thermomyces lanuginosus* lipase (Lipozyme TL IM); 30 mg mL^{-1}] and molecular sieves (3 Å; 50 mg mL^{-1}) were added. To enhance the solubility of both the substrates and the products, the reactions were carried out at 47 °C. A chiral HPLC/UV method[4a,4b] was used to follow the diastereoselective ring-opening of *rac*-**1a** with **2**. However, products **5a** and (*S*)-**14a** were invisible to the chiral HPLC/UV detection. All of the lipases catalysed the ring-opening, and the reactivity was always highest in TBME (Table 1, entries 1–4). The Lipozyme RM IM and TL IM preparations were dropped from further studies as the conversions were discouraging, even after 48 h (Table 1, entries 3 and 4). The ring-opening reactions proceeded in a highly diastereoselective manner with both CAL-B and lipase PS-D, as indicated by the high enantiomeric excess (ee^{1a}) values of remaining **1a** at the given conversions in the 24 h samples (Table 1, entries 1 and 2). According to Scheme 1, ring-opening of *rac*-**1a** can occur by both of the competing processes of alcoholysis by the sugar to form the sugar–β-amino acid conjugate (i.e., **5a**; Scheme 1, route A) and the hydrolysis side-reaction leading to the corresponding β-amino acid [i.e., (*S*)-**14a**; Scheme 1, route B]. When *rac*-**1a** (50 mM) was subjected to the reaction conditions with lipase PS-D in the absence of the sugar, significant hydrolysis of *rac*-**1a** had occurred after 24 h (62% in TBME, 45% in 2-MeTHF, and 35% in *t*AmOH). This shows that there is the potential for substantial hydrolysis in all three solvents. The hydrolysis tendency with CAL-B (Novozym 435) was higher than with lipase PS-D, apparently because the hydrophobic methacrylate carrier of Novozym 435 gives up the bound water more readily than the Celite carrier of lipase PS-D (Figure S3, Supporting Information).[16] Accordingly, lipase PS-D was selected for further studies in dry *t*AmOH.

Table 1. Lipase screening for the diastereoselective ring opening of *rac*-3,3-difluoro-4-phenylazetidin-2-one (*rac*-**1a**; 50 mM) with methyl α-D-galactopyranoside (**2**; 1 equiv.) in the presence of a lipase preparation (30 mg mL^{-1}) and molecular sieves (3 Å; 50 mg mL^{-1}) in dried solvents; reaction time 24 h at 47 °C.



Entry	Lipase	TBME		2-MeTHF		*t*AmOH	
		Conv. [%][a]	ee^{1a} [%][b]	Conv. [%][a]	ee^{1a} [%][b]	Conv. [%][a]	ee^{1a} [%][b]
1	CAL-B	57/70	>99/>99	26/42	37/75	43/47	79/>99
2	PS-D	53/60	98/>99	50/53	93/>99	44/54	78/98
3	RM IM	33/40	50/70	10/14	12/17	7/9	5/7
4	TL IM	26/36	18/27	11/18	10/18	25/35	4[c]/8[c]

[a] Conversion as the disappearance of *rac*-**1a** against internal standard after 24 h/48 h. [b] ee^{1a} from the 24 h/48 h sample. [c] Opposite diastereoselectivity observed.

Another aspect of the reaction optimization concerns the amounts of the sugar and the β-lactam as well as their ratio in the reaction mixture. As the chiral HPLC/UV method did not allow the detection of sugars **2**–**4** or conjugate products **5**, an achiral RP-HPLC/UV-CAD method[8c] was developed to quantify the amount of unreacted β-lactam by UV detection, and the amounts of the unreacted sugar and conjugate products by CAD detection simultaneously from a single sample. The chiral HPLC-UV method was used only to determine the enantiomeric excess values ee^{1} of unreacted β-lactam **1**. Increasing the amount of **2** (1, 2, and 5 equiv.) relative to *rac*-**1a** did not affect the formation of **5a** (53% in every case after 24 h) in the presence of lipase PS-D (30 mg mL^{-1}) and molecular sieves in dry *t*AmOH. As expected, doubling the amount of *rac*-**1a** (100 mM) and keeping the amount of **2** unchanged (50 mM) allowed 75% of the sugar to be converted into **5a** in 24 h. We continued with our optimization using a 1:1 mixture of the reactants as using the commercially available methyl α-D-glycopyranosides to drive the progress of the reaction is economically more sensible than using in-house prepared β-lactams.

There was still an analytical problem. Sugar conjugate **5a** (Figure 1, dotted line) appeared as a broad peak with an indefinite shape, which made accurate quantification impossible. We concluded that the free amino group of the product should be protected for analysis. Di-*tert*-butyl di-

carbonate (Boc_2O) was selected as a protecting reagent as it reacts with amines (but not with alcohols) directly without catalysts or coupling reagents.[17] First, we tested whether or not the *N*-Boc-protected β-lactam could be formed under the ring-opening reaction conditions. Thus, *rac*-**1a** and **2** were allowed to react in the presence of lipase PS-D and molecular sieves in dry *t*AmOH for 48 h, and then the reaction mixture was split in two portions. The lipase PS-D was removed from one of the portions, and then Boc_2O was added to both portions. Subsequent analysis revealed that the unreacted starting material (i.e., **1a**) was unchanged, while the product (i.e., **5a**) was derivatized with a Boc group; the presence or absence of lipase PS-D did not affect this derivatization. Moreover, and as expected, the RP-HPLC/UV-CAD peak for the *N*-Boc-protected compound (i.e., **8a**) appeared sharp (Figure 2, solid line), unlike that of free **5a** (dotted line), and the retention times of the two compounds were different. We also found that the presence of Boc_2O in the enzymatic reaction mixture had no effect on the formation of **8a** in situ via **5a**. Thus, it is possible to choose either to prepare **5a** and derivatize a sample for analysis, or to prepare *N*-protected derivative **8a** directly.

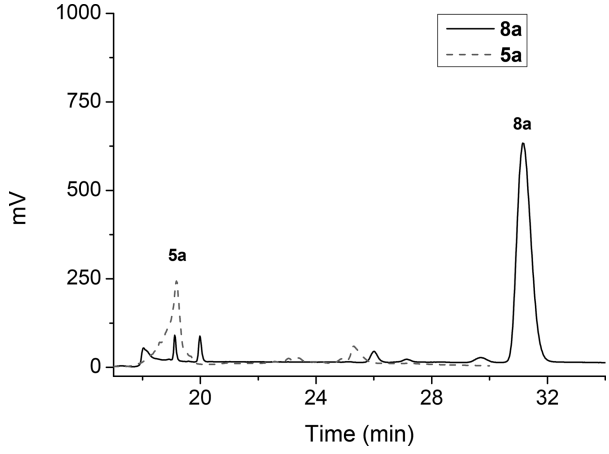


Figure 1. RP-HPLC/UV-CAD chromatograms: in the presence of Boc_2O (solid line) and without Boc_2O (dashed line).

Finally, the amount of lipase PS-D (5–50 mg mL^{-1}) was optimized for the acylation of *rac*-**1a** (50 mM) with **2** (1 equiv.) in the presence of Boc_2O (1 equiv.) and molecular sieves in dry *t*AmOH (Figure 2). The reactivity progressively increased with increasing amounts of catalyst, as determined by both the disappearance of *rac*-**1a** (solid lines with filled signs) and the formation of **8a** (dashed lines with open signs) in the 24 h sample. The difference between the solid curve and the corresponding dotted curve now more or less indicates the proportion of the hydrolysis side-reaction of the β-lactam ring under each of the acylation conditions. In the 24 h samples, the amount of hydrolysis was almost undetectable with low amounts of catalyst (5 and 10 mg mL^{-1}), but it slightly increased when the amount of enzyme was increased, and reached ca. 5% with 50 mg mL^{-1} of lipase PS-D. The fact that so little hydrolysis was seen in the formation of **8a** indicated that **2** effectively suppressed the potential of the residual water to act as a nucleophile.

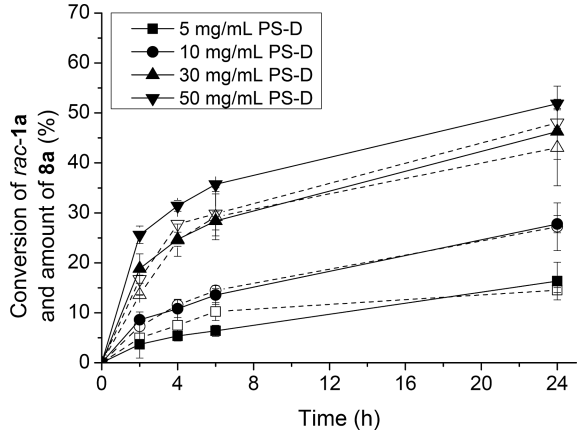


Figure 2. Effect of lipase PS-D (5–50 mg mL^{-1}) on the consumption of *rac*-**1a** (50 mM; filled signs) and the formation of **8a** (open signs) in the reaction with **2** (1 equiv.) and Boc_2O (1 equiv.) in the presence of molecular sieves (3 Å; 50 mg mL^{-1}) in *t*AmOH.

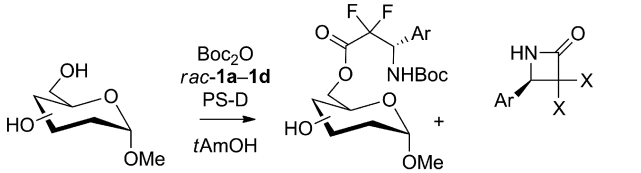
As already mentioned, 35% of *rac*-**1a** was hydrolysed in the absence of the sugar (and Boc_2O). A lipase PS-D content of 30 mg mL^{-1} was chosen to be optimal; as well as considerations about the hydrolysis side-reaction, the shaking of the reaction mixture was more effective when less solid material was present.

Substrate Scope Studies towards the Synthesis of Sugar–β-Amino Acid Conjugates

The optimized conditions were used in substrate-scope studies in which the structures of both the β-lactams and the methyl glycopyranosides were varied. Thus, the reactions between *rac*-**1a**–**1d** (50 mM) and **2**–**4** (1 equiv.) were carried out in the presence of lipase PS-D (30 mg mL^{-1}), Boc_2O (1 equiv.), and molecular sieves in dry *t*AmOH (Table 2). Each β-lactam except *rac*-**1d** (Table 2, entry 6) was reactive under the reaction conditions, as indicated by the disappearance of **1** after 24 h. The result with *rac*-**1d** was expected, as unfluorinated or otherwise unactivated compounds were also unreactive in previous ring-opening reactions.[4a] However, the yields of products **8**–**10** revealed that the product formation was clear only between *rac*-**1a** and sugars **2** and **3** and between *rac*-**1b** and **2** (Table 2, entries 1, 7 and 2). Experiments with the pure enantiomers revealed that it was the (*S*)-enantiomer of *rac*-**1b** that reacted with **2** (Table 2, entries 2–4). Secondly, the conversion of the β-lactam only gave a reasonable correlation with formation of the product (i.e., conjugates **8**–**10**) for the reactions of *rac*-**1a** with **2** and **3** (Table 2, entries 1 and 7). In other cases, the yields of the conjugate products (i.e., **8**–**10**) were very low, even though the β-lactam had been consumed to some extent, but also the amounts of recovered starting sugars **3** and **4** were low (Table 2, entries 8–12). These results could be due to the low solubilities of starting sugars **3** and **4** in these reaction mixtures. Thirdly, the conversion values for pyridine-substituted β-lactam *rac*-**1c** were around 80% for its reactions with sugars **2**–**4**, but no sugar-

conjugate formation was detected (Table 2, entries 5, 9, and 12). The high conversions might be due to the greater tendency of **1c** to undergo hydrolysis as a result of the strongly electron-withdrawing pyridine ring attached to the β-lactam ring. In addition, interactions between the basic pyridine nitrogen and other reagents and materials in the reaction mixture may reduce the effective β-lactam concentration under the reaction conditions. The appearance of several small unidentified peaks on the RP-HPLC/CAD chromatogram is consistent with this observation.

Table 2. Reaction between **1a**–**1d** (50 mM) and methyl α-D-glycopyranosides **2**–**4** (1 equiv.), and between *rac*-**1a** and 3,4-*O*-isopropylidene methyl α-D-galactopyranoside (**15**; 1 equiv.), in the presence of lipase PS-D (30 mg mL^{-1}), Boc_2O (1 equiv.), and molecular sieves (3 Å; 50 mg mL^{-1}) in *t*AmOH; reaction time 24 h at 47 °C.



Entry	Sugar	Lactam	Conversion [%][a]	*ee*[1] [%]	Yield [%][b] **2**–**4**	Yield [%][b] **8**–**10**
1	**2**	*rac*-**1a**	44	75	49	46
2	**2**	*rac*-**1b**	46	69	55	22
3	**2**	(*R*)-**1b**	13	99	100	–
4	**2**	(*S*)-**1b**	58	99	42	39
5	**2**	*rac*-**1c**	83[d]	16	64	–[e]
6	**2**	*rac*-**1d**	0	–	100	–
7	**3**	*rac*-**1a**	30	30	70	24[c]
8	**3**	*rac*-**1b**	26	33	47	<1[c]
9	**3**	*rac*-**1c**	83[d]	20	52	–[e]
10	**4**	*rac*-**1a**	15	15	22	<1[c]
11	**4**	*rac*-**1b**	22	16	20	<1[c]
12	**4**	*rac*-**1c**	76[d]	6	19	–[e]
13	**15**[f]	*rac*-**1a**	31	13	96[c]	4[c]

[a] Disappearance of *rac*-**1a**–**1d** against an internal standard. [b] [**2**–**4**]$_t$/[**2**–**4**]$_0$ and [**8**–**10**]$_t$/[**2**–**4**]$_0$. [c] Relative amount. [d] According to achiral analysis only. [e] Not determined due to the large number of peaks (see text). [f] Methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (**15**) was used instead of the unprotected glycopyranoside.

Next, preparative-scale reactions (with a 10 mL reaction volume) were carried out for the reactions of *rac*-**1a** with **2**–**4** and that of **1b** with **2**. The reactions between **1** (50 mM) and **2**–**4** (1 equiv.) were carried out in the presence of Boc_2O (1 equiv.), lipase PS-D (30 mg mL^{-1}), and molecular sieves in *t*AmOH, with the aim of isolating and characterizing the 6-*O*-acylated products (i.e., **8**–**10**; Table 3). For some reason, the yields of the products were much lower than in the small-scale reactions (Table 2), and the sugar–β-amino acid conjugates (i.e., **8a**–**10a** and **8b**) were isolated in 3–20% yield.

In addition to the unprotected methyl glycopyranosides, methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside (**15**), a more soluble sugar, was prepared from **2** by a known method.[18] In the previously reported lipase-catalysed acylation of carbohydrates with glyceric acid enantiomers, changing the substrate from **2** to **15** resulted in a significant increase in both the reactivity and the eventual product yield.[8c] Here, the results were less impressive (Table 2, entry 13). β-Lactam *rac*-**1a** reacted rather sluggishly with **15** in the presence of lipase PS-D, and the ring-opening proceeded with poor diastereoselectivity ($E < 10$ as estimated using the *ee*[1] value after the given conversion).

Product Diastereoisomerism

The ring-opening of a racemic β-lactam by a methyl glycopyranoside may lead to a mixture of two diastereomers, each of which is enantiomerically pure. As excellent enantioselectivity ($E > 200$) had previously been observed in the lipase-PS-D-catalysed ring-opening of *rac*-**1a** with methanol in TBME,[4a] a highly diastereoselective ring-opening leading to the formation of highly diastereomerically enriched sugar conjugates was expected also in this work. Unfortunately, chromatographic means failed to reveal whether more than one diastereomer was present for any of the isolated products (i.e., **8a**–**10a** and **8b**). The *ee*[1] value 75% at 44% conversion (giving *E* of the order of 100) for the unreacted (*R*)-**1a** indicates good diastereomeric purity for galactopyranoside derivative **8a**, while the *ee*[1] value 30% at 30% conversion (giving *E* of the order of 10) tends to propose low diastereomeric purity for glucoside derivative **9a** (Table 2, entries 1 and 7). However, the diastereoselective formation of the glucoconjugate **9a** might be better than approximated on the basis of the *E* value due to the low solubity of **3**. To further investigate the diastereoisomeric distribution of the products, we used enantiomerically pure β-lactams (*R*)- and (*S*)-**1b**. When the reaction between each of the individual enantiomers (*R*)- and (*S*)-**1b** (50 mM) and **2** (1 equiv.) was examined in the presence of lipase PS-D (30 mg mL^{-1}), Boc_2O (1 equiv.), and molecular sieves in *t*AmOH, (*R*)-**1b** failed to give any of the product (Table 2, entry 3) although some of the lactam was con-

Table 3. Preparative-scale synthesis of **8**–**10**.[a]

Entry	Lactam	Sugar	*ee*[1] [%]	**1a**–**1b** Yield [%][b]	**8**–**10** Yield [%][c]	**8**–**10** Yield [%][b]	**8**–**10** $[\alpha]_D^{25}$[d]
1	*rac*-**1a**	**2**	>99	–	25	20	+78.1
2	*rac*-**1b**	**2**	33	62	23	18	+82.2
3	*rac*-**1a**	**3**	26	37	18	10	+48.2
4	*rac*-**1a**	**4**	13	47	n.d.	3	n.d.

[a] **1** (50 mM), **2**–**4** (1 equiv.), Boc_2O (1 equiv.), lipase PS-D (30 mg mL^{-1}), molecular sieves (3 Å; 50 mg mL^{-1}), *t*AmOH, 47 °C. [b] Isolated yield. [c] Amount ([**8**–**10**]$_t$/[**2**–**4**]$_0$) in the reaction mixture. [d] (c = 1.0, CH_3CN).

sumed (31% conversion after 48 h; Figure 3). When the same experiment was conducted in the absence of the sugar, the conversion of (*R*)-**1b** still reached 29% after 48 h. Comparative experiments without lipase or sugar showed similar behaviour, with an 8% conversion, suggesting that non-enzymatic ring-opening of **1b** was occurring under the reaction conditions in addition to possible enzyme-catalysed hydrolysis. The (*S*)-enantiomer, on the other hand, gave a 58% conversion with respect to the lactam under identical reaction conditions, and diastereomerically and enantiomerically pure product **8b** was formed in 39% yield (Table 2, entry 4). It can be concluded that highly diastereomerically pure product **8a** was also obtained in the ring-opening reaction of *rac*-**1b** and **2** catalysed by lipase PS-D. The difference in the product yields [22% with *rac*-**1b** (50 mM) and 39% with (*S*)-**1b** (50 mM)] is consistent with the fact that only half of the racemic lactam was the reactive enantiomer.

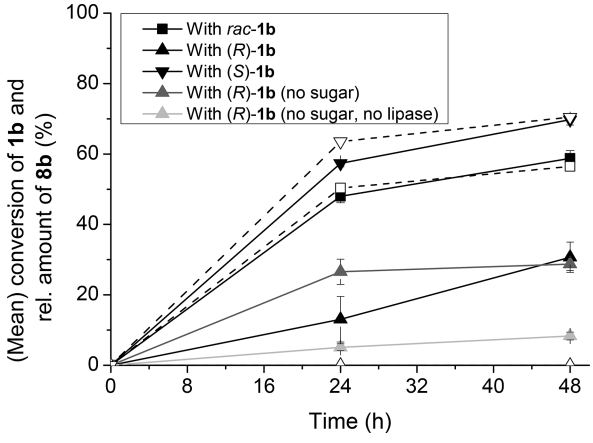


Figure 3. Progression curves for the lipase-PS-[d]-catalysed ring-opening of (*R*)-, (*S*)-, and *rac*-**1b** in *t*AmOH; consumption of **1b** (solid signs) and formation of **8b** (open signs). For reaction conditions, see text.

Conclusions

The regioselective formation of sugar–β-amino acid conjugates by lipase-PS-D-catalysed diastereoselective ring-opening of three racemic 3,3-difluoro-4-arylazetidin-2-ones (*rac*-**1a**–**1c**) and a non-fluorine-containing analogue (*rac*-**1d**) with methyl α-D-glycopyranosides (**2**–**4**) in the presence of Boc_2O and molecular sieves in *t*AmOH was examined. An achiral RP-HPLC/UV-CAD method was developed to quantify the amounts of the products formed, and a chiral HPLC/UV method was used to obtain the *ee* values of the unreacted β-lactams.

We have shown that the formation of sugar–β-amino acid conjugates by regio- and stereoselective lipase catalysis is possible. Conjugates **8a**–**10a** and **8b** were formed by the diastereoselective ring-opening of phenyl- (*rac*-**1a**) and thiophen-3-yl- (*rac*-**1b**) substituted 3,3-difluoro-4-arylazetidin-2-ones by sugars **2**–**4**, but the yield of **10a** (isolated yield 3%) was very low. The susceptibility of *rac*-**1a** to hydrolysis by the residual water in the lipase preparation was shown to be negligible in the presence of molecular sieves. By using pure enantiomers as acyl donors, it was shown that the (*S*)-enantiomer of *rac*-**1b** reacted with **2**, while (*R*)-**1b** was unreactive. β-Lactam *rac*-**1c**, with an electron-withdrawing and basic pyridine substitent on the lactam ring was consumed to a great extent in the reaction (ca. 80%), although conjugate formation was negligible or nil with sugars **2**–**4**. Unfluorinated β-lactam *rac*-**1d** did not react at all. Gluco- and mannopyranosides (**3** and **4**, respectively), with equatorial OH groups at C-4, were shown to be worse nucleophiles than the corresponding galactopyranoside, with the axial OH group at C-4, in the lipase-PS-D-catalysed reaction. In addition to any structural effect, the low solubility of **3** and **4** may restrict the formation of the desired sugar–β-amino acid conjugates, as enzymatic hydrolysis of the β-lactams by residual water is favoured at low sugar concentrations.

Experimental Section

General Remarks: All reagents and materials were used as received from commercial sources unless otherwise stated. Solvents were dried with molecular sieves (3 Å) before use. Powdered zinc was acid-washed before use.[19] Lipase preparations from Amano (*Burkholderia cepacia* lipase as Lipase PS-D and Lipase PS-C II) and Novozym (*Candida antarctica* lipase B as Novozym 435, *Rhizomucor miehei* lipase as Lipozyme RM IM, and *Thermomyces lanuginosus* lipase as Lipozyme TL IM) were used. HPLC analyses were performed with a HP 1090 HPLC/DAD (diode-array detector) equipped with a Daicel CHIRACEL-OD-H (4.6 mm × 250 mm × 5 μm) column or a Waters 2690 HPLC/DAD-CAD (charged-aerosol detector) equipped with an Agilent Technologies ZORBAX Eclipse XDB-C8 (4.6 mm × 150 mm × 5 mm) column. GC analyses were performed with a HP 6850 GC/FID equipped with a Chrompack CP-Chiralsil-DEX CB (25 m × 0.25 mm × 0.25 μm) column. Analytical thin-layer chromatography (TLC) was carried out on Merck Kieselgel $60F_{254}$ sheets, and the spots were visualized with UV light (254 nm) and by treatment with sulfuric acid (10% in ethanol) and heating. Chromatographic separations were carried out by column chromatography on Kieselgel 60 (0.063–0.200 μm). ^1H, ^{13}C, and ^{19}F NMR spectra were recorded with a Bruker Avance 500 spectrometer at 298 K in $CDCl_3$, [D_6]DMSO, or [D_4]methanol, and tetramethylsilane or 2,2,2-trifluoroethanol was used as an internal standard. Mass spectra were recorded with a Bruker Daltonics micrOTOF-Q (ESI-TOF) instrument in positive mode. Specific rotations were measured with a Perkin–Elmer 341 polarimeter using the sodium D line, and values are presented as deg cm^{-2} g^{-1}. Melting points were measured with a Gallenkamp device.

Small-Scale Enzymatic Experiments

Reactions between Methyl α-D-Glycopyranosides 2–4 and *rac*-1a–1d: In a typical small-scale reaction, a methyl α-D-glycopyranoside [*galacto* (**2**), *gluco* (**3**), or *manno* (**4**); 9.7 mg], a β-lactam (*rac*-**1a**–**1d**; 1–2 equiv.), Boc_2O (1 equiv.), a lipase preparation (30 mg mL^{-1}), molecular sieves (3 Å; 50 mg mL^{-1}), and a solvent (1 mL) were shaken (170 rpm) at +47 °C. Samples (50 μL) were taken at intervals, filtered, diluted with *n*-hexane (150 μL) or acetonitrile (100 μL), and analysed by the developed HPLC methods (see Supporting Information).

Preparative-Scale Reactions

Methyl 6-*O*-[(*S*)-2,2-Difluoro-3-phenyl-3-(*tert*-butoxycarbonyl)-aminopropanoyl]-α-D-galactopyranoside (8a): Methyl α-D-galactopyranoside (**2**; 97 mg, 0.5 mmol), *rac*-**1a** (91 mg, 1 equiv., 0.5 mmol), Boc_2O (109 mg, 1 equiv., 0.5 mmol), Lipase PS-D (30 mg mL^{-1}; 300 mg), molecular sieves (3 Å; 50 mg mL^{-1}; 500 mg), and *tert*-amyl alcohol (*t*AmOH; 10 mL) were shaken (170 rpm) at 47 °C for 24 h. Then the reaction mixture was filtered, and the solvents were evaporated. Column chromatography (ethyl acetate/ethanol, 95:5) gave (*R*)-**1a** as a mixture with Boc_2O (101 mg, >99% *ee*) and **8a** (47 mg, 0.1 mmol, 20%). Data for **8a**: $[\alpha]_D^{25}$ = +78.1 (*c* = 1.0, CH_3CN). ^{1}H NMR (500 MHz, 298 K, [D$_6$]DMSO): δ = 1.38 (s, 9 H, 3 CH_3), 3.29 (s, 3 H, OCH_3), 3.54 (ddd, *J* = 3.3, *J* = 5.6, *J* = 9.5 Hz, 1 H, 3-H), 3.60 (ddd, *J* = 3.6, *J* = 6.6, *J* = 10.1 Hz, 1 H, 2-H), 3.70 (t, *J* = 3.4 Hz, 1 H, 4-H), 3.82 (dd, *J* = 2.8, *J* = 8.4 Hz, 1 H, 5-H), 4.22 (dd, *J* = 3.0, *J* = 11.2 Hz, 1 H, 6a-H), 4.33 (dd, *J* = 8.7, *J* = 11.1 Hz, 1 H, 6b-H), 4.61 (d, *J* = 3.6 Hz, 1 H, 1-H), 4.63 (d, *J* = 6.6 Hz, 1 H, 2-OH), 4.69 (d, *J* = 5.7 Hz, 1 H, 3-OH), 4.74 (d, *J* = 4.4 Hz, 1 H, 4-OH), 5.32 (ddd, *J* = 11.3, *J* = 18.2, *J* = 21.6 Hz, 1 H, C*H*Ph), 7.37 (m, 3 H, arom.), 7.48 (m, 2 H, arom.), 8.22 (d, *J* = 10.2 Hz, 1 H, -NH) ppm. ^{13}C NMR (126 MHz, 298 K, [D$_6$]DMSO): δ = 27.87 (3 *C*H$_3$), 54.26 (O*C*H$_3$), 56.05 (*C*HPh), 66.80 (C-6), 67.81 (C-5), 67.92 (C-2), 68.87 (C-3), 68.94 (C-4), 78.93 [*C*(CH$_3$)$_3$], 99.93 (C-1), 128.25 (arom.), 128.52 (arom.), 128.63 (arom.), 133.30 (*C*F$_2$), 147.53 (arom.), 154.92 [*C*(O)O*t*Bu], 162.35 (*C*=O) ppm. ^{19}F NMR (471 MHz, 298 K, [D$_6$]-DMSO): δ = –113.0 (dd, *J* = 11.2, *J* = 250.2 Hz), –116.2 (dd, *J* = 17.9, *J* = 250.7 Hz) ppm. HRMS: calcd. for $C_{21}H_{29}F_2NO_9Na^+$ [M + Na]$^+$ 500.17026; found 500.17064.

Methyl 6-*O*-[(*S*)-2,2-Difluoro-3-(thiophen-3-yl)-3-(*tert*-butoxycarbonyl)aminopropanoyl]-α-D-galactopyranoside (8b): Methyl α-D-galactopyranoside (**2**; 97 mg, 0.5 mmol), *rac*-**1b** (95 mg, 1 equiv., 0.5 mmol), Boc_2O (109 mg, 1 equiv., 0.5 mmol), Lipase PS-D (30 mg mL^{-1}; 300 mg), molecular sieves (3 Å; 50 mg mL^{-1}; 500 mg), and *tert*-amyl alcohol (*t*AmOH; 10 mL) were shaken (170 rpm) at 47 °C for 24 h. Then the reaction mixture was filtered, and the solvents were evaporated. Column chromatography (ethyl acetate/petroleum ether, 1:9, and ethyl acetate/ethanol, 95:5) gave (*R*)-**1b** (57 mg, 0.3 mmol, 62%, 33% *ee*) as a colourless semisolid, and **8b** (43 mg, 91 μmol, 18%) as a colourless semisolid. Data for **8b**: $[\alpha]_D^{25}$ = +82.2 (*c* = 1.0, CH_3CN). ^{1}H NMR (500 MHz, 298 K, [D$_6$]-DMSO): δ = 1.39 (s, 9 H, 3 CH_3), 3.28 (s, 3 H, OCH_3), 3.54 (ddd, *J* = 3.2, *J* = 5.6, *J* = 9.5 Hz, 1 H, 3-H), 3.59 (ddd, *J* = 3.6, *J* = 6.5, *J* = 10.1 Hz, 1 H, 2-H), 3.70 (dd, *J* = 3.4, *J* = 3.6 Hz, 1 H, 4-H), 3.84 (dd, *J* = 2.8, *J* = 8.5 Hz, 1 H, 5-H), 4.21 (dd, *J* = 3.1, *J* = 11.2 Hz, 1 H, 6a-H), 4.33 (dd, *J* = 8.8, *J* = 11.0 Hz, 1 H, 6b-H), 4.60 (d, *J* = 3.5 Hz, 1 H, 1-H), 4.62 (d, *J* = 6.6 Hz, 1 H, 2-OH), 4.68 (d, *J* = 5.7 Hz, 1 H, 3-OH), 4.74 (d, *J* = 4.4 Hz, 1 H, 4-OH), 5.44 [ddd, *J* = 12.3, *J* = 15.8, *J* = 23.5 Hz, 1 H, C*H*(thiophenyl)], 7.25 (d, *J* = 4.9 Hz, 1 H, arom.), 7.55 (dd, *J* = 3.0, *J* = 4.9 Hz, 1 H, arom.), 7.65 (d, *J* = 2.2 Hz, 1 H, arom.), 8.09 (*J* = 10.1 Hz, 1 H, NH) ppm. ^{13}C NMR (126 MHz, 298 K, [D$_6$]DMSO): δ = 27.93 (3 *C*H$_3$), 54.32 (O*C*H$_3$), 55.72 [*C*H(thiophenyl)], 66.80 (C-6), 67.84 (C-5), 67.94 (C-2), 68.89 (C-3), 68.97 (C-4), 78.97 [*C*(CH$_3$)$_3$], 99.97 (C-1), 125.35 (arom.), 126.48 (arom.), 127.65 (arom.), 133.58 (arom.), 154.89 [*C*(O)O*t*Bu], 162.32 (*C*=O) ppm. ^{19}F NMR (471 MHz, 298 K, [D$_6$]DMSO): δ = –114.3 (dd, *J* = 11.6, *J* = 248.6 Hz), –116.6 (dd, *J* = 16.3, *J* = 248.6 Hz) ppm. HRMS: calcd. for $C_{19}H_{27}F_2NO_9SNa^+$ [M + Na]$^+$ 506.12668; found 506.12123.

Methyl 6-*O*-[(*S*)-2,2-Difluoro-3-phenyl-3-(*tert*-butoxycarbonyl)-aminopropanoyl]-α-D-glucopyranoside (9a): Methyl α-D-glucopyranoside (**3**; 97 mg, 0.5 mmol), *rac*-**1a** (91 mg, 1 equiv., 0.5 mmol), Boc_2O (109 mg, 1 equiv., 0.5 mmol), Lipase PS-D (30 mg mL^{-1}; 300 mg), molecular sieves (3 Å; 50 mg mL^{-1}; 500 mg), and *tert*-amyl alcohol (*t*AmOH; 10 mL) were shaken (170 rpm) at 47 °C for 48 h. Then the reaction mixture was filtered, and the solvents were evaporated. Column chromatography (ethyl acetate/petroleum ether, 1:9, and ethyl acetate/ethanol, 95:5) gave (*R*)-**1a** (34 mg, 0.2 mmol, 37%, 26% *ee*) and **9a** (34 mg, 51 μmol, 10%). Data for **9a**: $[\alpha]_D^{25}$ = +48.4 (*c* = 1.0, CH_3CN). ^{1}H NMR (500 MHz, 298 K, [D$_6$]DMSO): δ = 1.38 (s, 9 H, 3 CH_3), 3.07 (ddd, *J* = 6.3, *J* = 9.2, *J* = 15.4 Hz, 1 H, 4-H), 3.22 (ddd, *J* = 3.7, *J* = 6.5, *J* = 10.0 Hz, 1 H, 2-H), 3.28 (s, 3 H, OCH_3), 3.40 (ddd, *J* = 4.9, *J* = 9.2, *J* = 14.1 Hz, 1 H, 3-H), 3.60 (ddd, *J* = 1.5, *J* = 7.4, *J* = 9.3 Hz, 1 H, 5-H), 4.23 (dd, *J* = 7.5, *J* = 11.7 Hz, 1 H, 6a-H), 4.44 (dd, *J* = 1.4, *J* = 11.4 Hz, 1 H, 6b-H), 4.56 (d, *J* = 3.4 Hz, 1 H, 1-H), 4.83 (d, *J* = 6.5 Hz, 1 H, 2-OH), 4.95 (d, *J* = 4.9 Hz, 1 H, 3-OH), 5.24 (*J* = 5.7 Hz, 1 H, 4-OH), 5.31 (ddd, 1 H, *J* = 10.2 Hz, *J* = 17.1 Hz, *J* = 23.2 Hz, C*H*Ph), 7.37 (m, 3 H, arom.), 7.47 (m, 2 H, arom.), 8.20 (d, 1 H, *J* = 10.2 Hz, NH) ppm. ^{13}C NMR (126 MHz, 298 K, [D$_6$]DMSO): δ = 27.95 (3 *C*H$_3$), 54.25 (O*C*H$_3$), 56.06 (*C*HPh), 66.34 (C-6), 69.05 (C-5), 70.12 (C-4), 71.58 (C-2), 72.99 (C-3), 78.96 [*C*(CH$_3$)$_3$], 99.60 (C-1), 114.12 (*C*F$_2$), 128.30 (arom.), 128.55 (arom.), 128.64 (arom.), 133.41 (arom.), 154.93 [*C*(O)O*t*Bu], 162.35 (*C*=O) ppm. HRMS: calcd. for $C_{21}H_{29}F_2NO_9Na^+$ [M + Na]$^+$ 500.17026; found 500.17040.

Methyl 6-*O*-[(*S*)-2,2-Difluoro-3-phenyl-3-(*tert*-butoxycarbonyl)-aminopropanoyl]-α-D-mannopyranoside (10a): Methyl α-D-mannopyranoside (**4**; 97 mg, 0.5 mmol), *rac*-**1a** (91 mg, 1 equiv., 0.5 mmol), Boc_2O (109 mg, 1 equiv., 0.5 mmol), Lipase PS-D (30 mg mL^{-1}; 300 mg), molecular sieves (3 Å; 50 mg mL^{-1}; 500 mg), and *tert*-amyl alcohol (*t*AmOH, 10 mL) were shaken (170 rpm) at 47 °C for 48 h. Then the reaction mixture was filtered, and the solvents were evaporated. Column chromatography (ethyl acetate/petroleum ether, 1:9, and ethyl acetate/ethanol, 95:5) gave (*R*)-**1a** (43 mg, 0.2 mmol, 47%, 13% *ee*) and **10a** (8 mg, 17 μmol, 3%). Data for **10a**: ^{1}H NMR (500 MHz, 298 K, [D$_6$]DMSO): δ = 1.38 (s, 9 H, 3 CH_3), 3.26 (s, 3 H, OCH_3), 3.41 (m, 1 H, 4-H), 3.46 (m, 1 H, 3-H), 3.54 (m, 1 H, 5-H), 3.62 (ddd, *J* = 1.7, *J* = 3.2, *J* = 4.7 Hz, 1 H, 2-H), 4.24 (dd, *J* = 7.6, *J* = 11.6 Hz, 1 H, 6a-H), 4.46 (ddd, *J* = 1.7, *J* = 11.4, *J* = 13.1 Hz, 1 H, 6b-H), 4.53 (d, *J* = 1.3 Hz, 1 H, 1-H), 4.76 (d, *J* = 5.9 Hz, 1 H, 3-OH), 4.87 (d, *J* = 4.5 Hz, 1 H, 2-OH), 5.09 (*J* = 5.5 Hz, 1 H, 4-OH), 5.32 (ddd, *J* = 11.8, *J* = 17.5, *J* = 22.6 Hz, 1 H, C*H*Ph), 7.37 (m, 3 H, arom.), 7.47 (m, 2 H, arom.), 8.22 (d, *J* = 10.2 Hz, 1 H, NH) ppm. ^{13}C NMR (126 MHz, 298 K, [D$_6$]DMSO): δ = 27.95 (3 *C*H$_3$), 53.92 (O*C*H$_3$), 55.72 (*C*HPh), 66.60 (C-4, C-6), 69.96 (C-2), 70.30 (C-5), 70.59 (C-3), 78.97 [*C*(CH$_3$)$_3$], 100.97 (C-1), 128.30 (arom.), 128.55 (arom.), 128.65 (arom.), 133.43 (arom.), 154.94 [*C*OC(CH$_3$)$_3$], 162.14 (*C*=O) ppm. ^{19}F NMR (471 MHz, 298 K, [D$_6$]DMSO): δ = –113.3 (dd, *J* = 11.6, *J* = 250.7 Hz), –116.2 (dd, *J* = 17.3, *J* = 250.5 Hz) ppm. HRMS: calcd. for $C_{21}H_{29}F_2NO_9Na^+$ [M + Na]$^+$ 500.17026; found 500.17040.

Supporting Information (see footnote on the first page of this article): Synthesis of *rac*-**1a**–**1d**; retention times for compounds during HPLC analysis; solubility estimation for **2**; estimation of enzymatic hydrolysis by residual water; chemoenzymatic preparation of both enantiomers of **1b**; ^{1}H and ^{13}C NMR spectra for products and intermediates.

Acknowledgments

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[1] a) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854; b) E. Busto, V. Gotor-Fenández, V. Gotor, *Chem. Soc. Rev.* **2010**, *39*, 4504–4523; c) E. Busto, V. Gotor-Fernández, V. Gotor, *Chem. Rev.* **2011**, *111*, 3998–4035; d) E. Forro, F. Fülop, *Curr. Med. Chem.* **2012**, *19*, 6178–6187.
[2] a) C. Palomo, J. M. Aizpurua, I. Ganboa, M. Oiarbide, *Curr. Med. Chem.* **2004**, *11*, 1837–1872; b) B. Alcaide, P. Almendros, C. Aragoncillo, *Chem. Rev.* **2007**, *107*, 4437–4492; c) T. Heck, H.-P. E. Kohler, M. Limbach, O. Flogel, D. Seebach, B. Geueke, *Chem. Biodiversity* **2007**, *4*, 2016–2030.
[3] See, for instance: L. T. Kanerva, A. Liljeblad, *Transesterification – Biological*, in: *Encyclopedia of Catalysis*, Wiley Online Library, **2010**, DOI: 10.1002/0471227617.eoc197.
[4] a) X.-G. Li, M. Lähitie, M. Päiviö, L. T. Kanerva, *Tetrahedron: Asymmetry* **2007**, *18*, 1567–1573; b) X.-G. Li, M. Lähitie, L. T. Kanerva, *Tetrahedron: Asymmetry* **2008**, *19*, 1857–1861; c) P. D'Arrigo, L. T. Kanerva, X.-G. Li, C. Saraceno, S. Servi, D. Tessaro, *Tetrahedron: Asymmetry* **2009**, *20*, 1641–1645.
[5] a) H. Smidt, A. Fischer, P. Fischer, R. D. Schmid, *Biotechnol. Techniques.* **1996**, *10*, 335–338; b) T. Wagegg, M. M. Enzelberger, U. T. Bornscheuer, R. D. Schimd, *J. Biotechnol.* **1998**, *61*, 75–78; c) D. R. Duarte, E. L. Castillo, E. Bárzana, A. López-Munguía, *Biotechnol. Lett.* **2000**, *22*, 1811–1814; d) M. V. Sergeeva, V. V. Mozhaev, J. O. Rich, Y. L. Khmelnitsky, *Biotechnol. Lett.* **2000**, *22*, 1419–1422; e) A. Torres-Gavilán, J. Escalante, I. Regla, A. López-Munguía, E. Castillo, *Tetrahedron: Asymmetry* **2007**, *18*, 2621–2624; f) H. Ismail, R. M. Lau, F. van Rantwijk, R. A. Sheldon, *Adv. Synth. Catal.* **2008**, *350*, 1511–1516; g) A. Liljeblad, P. Kallio, M. Vainio, J. Niemi, L. T. Kanerva, *Org. Biomol. Chem.* **2010**, *8*, 886–895; h) J. Brem, L.-C. Nencze, A. Liljeblad, M. C. Turcu, C. Paizs, F.-D. Irimie, L. T. Kanerva, *Eur. J. Org. Chem.* **2012**, 3288–3294.
[6] a) P.-O. Syrén, P. Hendil-Forssell, L. Aumailley, W. Besenmatter, F. Gounine, A. Svendsen, M. Martinelle, K. Hult, *ChemCatChem* **2012**, *13*, 645–648; b) P.-O. Syrén, *FEBS J.* **2013**, *280*, 3069–3083.
[7] a) R. Brieva, J. Z. Crich, C. J. Sih, *J. Org. Chem.* **1993**, *58*, 1068–1075; b) E. Forró, F. Fülöp, *Org. Lett.* **2003**, *5*, 1209–1212; c) X.-G. Li, L. T. Kanerva, *Adv. Synth. Catal.* **2006**, *348*, 197–205.
[8] a) A. Hietanen, F. S. Ekholm, R. Leino, L. T. Kanerva, *Eur. J. Org. Chem.* **2010**, 6974–6980; b) A. Hietanen, L. T. Kanerva, *Eur. J. Org. Chem.* **2012**, 2729–2737; c) R. Sundell, L. T. Kanerva, *Eur. J. Org. Chem.* **2013**, 4971–4978.
[9] W. K. Hagmann, *J. Med. Chem.* **2008**, *51*, 4359–4369.
[10] a) P. Csomós, L. T. Kanerva, G. Bernáth, F. Fülöp, *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796; b) E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358; c) X.-G. Li, L. T. Kanerva, *Tetrahedron: Asymmetry* **2007**, *18*, 2468–2472.
[11] a) S. Marcotte, X. Pannecoucke, C. Feasson, J.-C. Quirion, *J. Org. Chem.* **1999**, *64*, 8461–8464; b) N. Boyer, P. Gloanec, G. De Nanteuil, P. Jubault, J.-C. Quirion, *Tetrahedron* **2007**, *63*, 12352–12366; c) N. Boyer, P. Gloanec, G. De Nanteuil, P. Jubault, J.-C. Quirion, *Eur. J. Org. Chem.* **2008**, 4277–4295.
[12] C. Palomo, M. Diarbide, S. Bindi, *J. Org. Chem.* **1998**, *63*, 2469–2474.
[13] B. Danieli, M. Luisetti, G. Sampognaro, G. Carrea, S. Riva, *J. Mol. Catal. B* **1997**, *3*, 193–201.
[14] a) Y. Simeó, J. V. Sinisterra, A. R. Alcántar, *Green Chem.* **2009**, *11*, 855–862; b) Z.-G. Chen, D.-N. Zhang, L. Cao, Y.-B. Han, *Bioresource Technol.* **2013**, *133*, 82–86.
[15] a) P. Tsavas, S. Polydorou, I. Faflia, E. C. Voutsas, D. Tassios, M. V. Flores, K. Naraghi, P. J. Halling, F. Chamouleau, M. Ghoul, J.-M. Engasser, M. Ferrer, F. J. Plou, *J. Chem. Eng. Data* **2002**, *47*, 807–810; b) E. C. Voutsas, P. Tsavas, K. Magoulas, D. Tassios, M. Ferrer, F. J. Plou, A. Ballesteros, *J. Chem. Eng. Data* **2002**, *47*, 1517–1520.
[16] L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer, U. Hanefeld, *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
[17] Y. Basel, A. Hassner, *J. Org. Chem.* **2000**, *65*, 6368–6380.
[18] A. Rashid, W. Mackie, I. J. Colquhoun, D. Lamba, *Can. J. Chem.* **1990**, *68*, 1122–1127.
[19] A. I. Vogel, B. S. Furniss, A. J. Hannaford, P. W. G. Smith, A. R. Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, 5th ed., Pearson Education Ltd., Harlow, UK, **1989**, p. 467.

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