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PAPER

An expedient biocatalytic procedure for abasic site precursors useful in oligonucleotide synthesis†

Saúl Martínez-Montero,[a] Susana Fernández,[a] Yogesh S. Sanghvi,[b] Vicente Gotor*[a] and Miguel Ferrero*[a]

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Preparation of abasic site precursors through a divergent chemoenzymatic synthesis has been accomplished. Several biocatalysts and acylating agents were studied furnishing a practical and scalable green method useful for industrial applications. Highly regioselective acylation and deacylation reactions with 1,2-dideoxy-D-ribose are described resulting in excellent yield. A fast, atom-efficient and convenient synthesis of 3-, and 5-*O*-DMTr-1,2-dideoxyribose **17** and **19** has been achieved. These compounds are useful precursors for the preparation of phosphoramidites required for the assembly of oligonucleotides containing the tetrahydrofuran abasic lesions.

Introduction

Abasic sites are one of the most common cellular DNA damage in the genome. They result from the cleavage of the glycosidic bond, which can occur spontaneously under physiological conditions, or during the intermediate steps of base excision repair when DNA is exposed to various endogenous and exogenous damaging agents.[1] For example, antitumor antibiotic bleomycin is reported to cause cleavage of thymine-deoxyribose glycosidic bond in DNA.[2] A variety of bifunctional agents, such as mitomycin C and cisplatin can react with bases in DNA leading to the generation of abasic sites.[3] This process of cellular damage is also responsible for interstrand cross-links in duplex DNA leading to cytotoxic effects.[4,5] Therefore, studies of repair of DNA is important for maintaining the integrity of the genome.[6] *In vivo*, the abasic sites are believed to be repaired by the action of cellular endonucleases.[7]

Several oligonucleotide analogues containing abasic sites have been synthesized to determine the structural basis of these lesions that are responsible for their distinct biochemical effects.[8] Noteworthy is the unoxidized abasic site from the hydrolysis of the glycosidic bond (**1**, AP; Chart 1) and the oxidized abasic lesions C4-AP (**2**, C4-oxidized abasic site), 2-deoxyribonolactone (**3**, L), or 5-(2-phosphoryl-1,4-dioxobutane) (**4**, DOB), which inhibits repair by DNA polymerase β.[9] Other abasic lesions such as the tetrahydrofuran (**5**, F), the hydrocarbon derivatives CPA (**6**) and CPE (**7**), the lactam (**8**, Lm), the ketone (**9**, K) and the methylene cyclopentane (**10**, MCP) analogues have already been reported as probes of replication in cells. It has been shown that oligodeoxynucleotides containing the tetrahydrofuran group (**5**) act as substrates for AP endonucleases repair in *Escherichia coli*, and also serve as effective templates for AuV reverse transcriptase and other DNA polymerases of eukaryotic and prokaryotic origin.[8c,10] In addition, abasic sugars were introduced into therapeutic siRNAs to inhibit degradation by nucleases.[11]

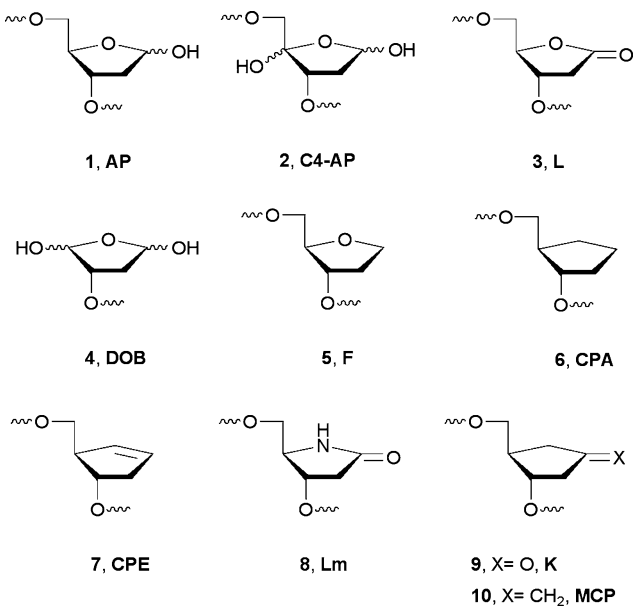

Chart 1

The widespread use of furanoid glycals as key intermediates for the synthesis of interesting biological compounds[12] has aroused considerable interest in scalable and cost-efficient procedures for their preparation. Most of the synthetic methods employed 2-deoxy-D-ribose as starting material.[8c,13] In 1984, Millican *et al.*[10b] reported the synthesis of 1,2-dideoxy-D-ribose starting from 2′-deoxyadenosine *via* destructive protocol (loss of atoms) where

[a]*Departamento de Química Orgánica e Inorgánica and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, 33006, Oviedo, (Asturias), Spain. E-mail: vgs@uniovi.es, mferrero@uniovi.es; Fax: +34 985 103 448; Tel: +34 985 103 448*

[b]*Rasayan Inc., 2802 Crystal Ridge Road, Encinitas, CA, 92024-6615, USA*

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purine portion of the molecule was cleaved off to give the cyclic enol ether. In 1994, Pedersen *et al.*[14] and subsequently in 2002 Beigelman *et al.*[15] employed thymidine as starting material in the preparation of the 1,2-dideoxy-D-ribose. Clearly, these protocols are not atom efficient where a good portion of the molecule is sacrificed to obtain 1,2-dideoxy-D-ribose.

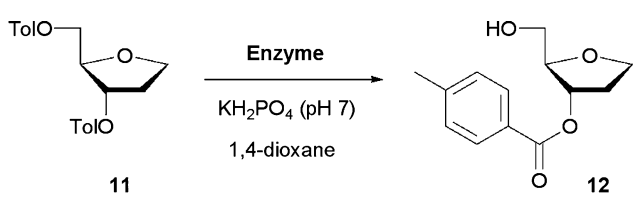
Biocatalytic methods have been recognized as practical procedures in the nucleoside area.[16] For manipulation of protecting groups, application of biocatalysts in organic synthesis has become an attractive alternative to conventional chemical methods. Thus, enzyme–catalyzed chemical transformations, which satisfy increasingly stringent environmental constraints, are in great demand by the pharmaceutical and chemical industries.

Herein we describe an efficient, scalable and atom economical (non-destructive) synthesis of 1,2-dideoxy-D-ribose from simple starting materials that are currently synthesized on metric ton scale for the synthesis of 2-deoxynucleosides.[17] Herein, we explored the potential of the enzymatic catalysis for the manipulation of 1,2-dideoxy-D-ribose with two orthogonal protecting groups at any site that is desired. The orthogonally protected 1,2-dideoxy-D-ribose could serve as very useful building-block in the synthesis of carbohydrate analogues and in the generation of building-blocks required for inserting abasic sites in oligonucleotides with greater ease.

Results and discussion

We have previously reported that *Candida antarctica* lipase B (CAL-B) catalyzes the hydrolysis at the 5′-*O*-acyl group of 2′-deoxynucleosides selectively, whereas *Pseudomonas cepacia* lipase (PSL) shows unusual regioselectivity towards the acyl group at 3′-position.[18] As an extension of our earlier studies and further exploration of enzyme substrate specificity and selectivity, we elected di-*O*-toluoyl-1,2-dideoxy-D-ribose (**11**) as the starting material.

The di-*O*-toluoyl-protected **11** was conveniently prepared from the commercial 1-α-chloro-3,5-di-*O*-*p*-toluoyl-2-deoxy-ribofuranose following literature procedure.[17] The regioselective hydrolysis of **11** is shown in Scheme 1 and the experimental data are summarized in Table 1. For an initial screening of suitable lipases, enzymatic hydrolysis of **11** was carried out with CAL-B, PSL, and *Cromobacterium viscosum* lipase (CVL). Treatment of **11** with 0.15 M KH_2PO_4 (pH 7) containing 1,4-dioxane as cosolvent in the presence of CAL-B at 55 °C afforded after 48 h (monitored by TLC) the 5-hydroxyl derivative **12** as the only product (entry 1, Table 1). As expected, CAL-B exhibited complete regioselectivity in the hydrolysis of the acyl group at the 5-position of compound **11**.



Scheme 1

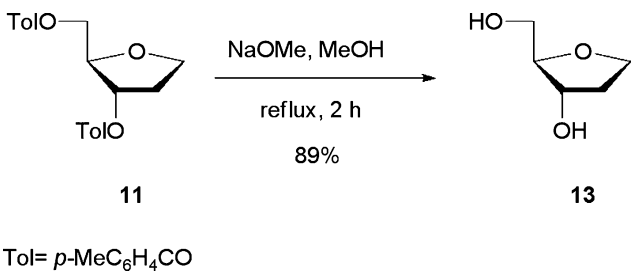
Table 1 Enzymatic hydrolysis of di-*O*-toluoyl-1,2-dideoxyribose (**11**)

Entry	Enzyme	**11** : Enzyme[a]	*T*/°C	t (h)	Conv. (%)[b]	**12** (%)[b,c]
1	CAL-B[d]	1 : 1	55	48	>97	>97
2	PSL-C[e]	1 : 2	50	2	>97	>97 (95)
3	PSL-SD[f]	1 : 2	50	15	>97	>97
4	CVL[g]	1 : 0.25	50	1.5	>97	>97

[a] Ratio **11** : enzyme (w/w). [b] Calculated by ^{1}H NMR. [c] Percentage of isolated yields are given in parentheses. [d] Novozym 435. [e] Lipase PS "Amano" immobilized on ceramic. [f] Crude lipase PS "Amano" SD. [g] *Cromobacterium viscosum* lipase.

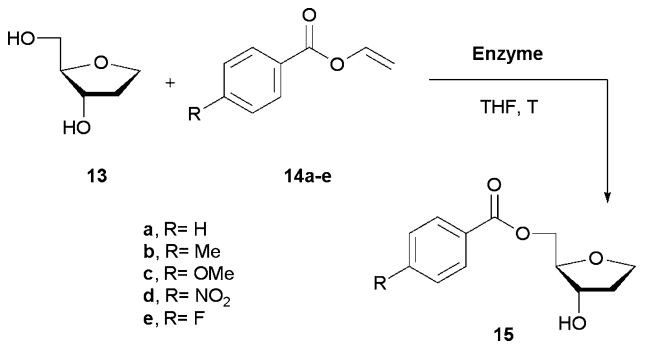
Enzymatic hydrolysis of **11** with PSL revealed that this lipase showed the same 5-*O*-acyl group selectivity as CAL-B (entries 2 and 3, Table 1). The rate of hydrolysis with PSL was significantly faster compared to CAL-B furnishing exclusively **12** in excellent yield (95%). Interestingly, PSL displayed opposite selectivity during hydrolysis of di-*O*-acyl-β-D-2′-deoxynucleosides suggesting that the presence of the nucleosidic base is pivotal in how the substrate fits in the active site of the enzyme.[19] Similar results were also obtained with the enzyme CVL furnishing the 3-*O*-acyl derivative in < 2 h (entry 4, Table 1). Nevertheless, PSL-C was the enzyme of choice for the transformation of **11** into **12** on large-scale. This is because PSL-C is immobilized and provides several advantages such as enhanced stability, reusability, convenient separation by filtration from the reaction mixture, preventing protein contamination in the product, and consistent performance of the commercial preparations. Additionally, easy isolation of **12** has been accomplished *via* extraction avoiding expensive column chromatography. Furthermore, the toluoyl protecting group in **11** appears to be an ideal substrate for recognition by lipase. It also offers easy detection by UV and more importantly, facilitates the extraction of **12** in organic solvent.

Next, we decided to prepare the 5-*O*-acyl derivative through the enzymatic acylation of 1,2-dideoxy-D-ribose expecting that the complementary behaviour of these lipases in hydrolysis and acylation reactions may enable the synthesis of the desired regioisomer. 1,2-Dideoxy-D-ribose was conveniently prepared by the treatment of **11** with sodium methoxide in MeOH at reflux to afford **13** in 89% yield (Scheme 2).



Scheme 2

Evaluation of the selective acylation of **13** in the presence of immobilized CAL-B and PSL as catalysts was undertaken. We employed commercially available vinyl benzoate (**14a**) as the acylating agent for this study. Therefore, 1,2-dideoxy-D-ribose (**13**) was dissolved in anhydrous THF under nitrogen and treated with 2 equiv of vinyl benzoate and CAL-B or PSL (Scheme 3). Regioselective acylation was observed with both enzymes furnishing



Scheme 3

exclusively 5-*O*-benzoyl-1,2-dideoxyribose (**15a**). Shorter reaction times were achieved with PSL-C, which affords **15a** in almost quantitative yield after 3 h at 50 °C (entries 1, 2, and 3, Table 2). Importantly, pure **15a** was isolated *via* precipitation without a need for column chromatographic purification. It is noteworthy that we are using less equiv of vinyl benzoate compared to an earlier protocol reported by us for the preparation of 5′-*O*-benzoyl-2′-deoxynucleoside derivatives.[20] During the transformation of **13** to **15a**, it is not necessary to use an excess of vinyl benzoate to drive the reaction to completion. Compared to the earlier nucleoside studies, in case of carbohydrate **13**, the overall reaction time is shorter without loss of selectivity.

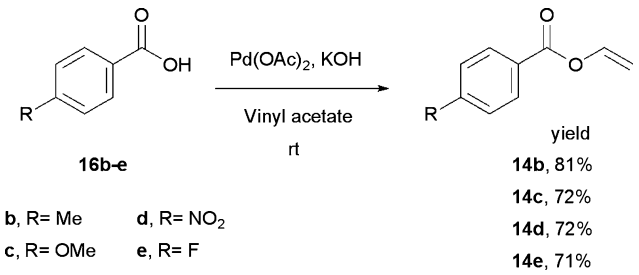
To demonstrate the general application of acylation protocol with other acyl derivatives and to study the influence of the electronic nature of the acyl moiety, we carried out the enzymatic acylation with four *p*-substituted vinyl benzoates as acylating agents. Thus, vinyl esters **14b–e** were synthesized in good yields through *trans*-vinylation reaction of the corresponding carboxylic acid in the presence of palladium(II) acetate as catalyst (Scheme 4).[21]

When *p*-methyl vinylbenzoate (**14b**) was used for the acylation of **13**, total selectivity at the primary hydroxyl was observed with both PSL-C and CAL-B. The rate of conversion was higher with PSL-C, which afford **15b** after 3 h in 97% yield (entry 4, Table 2). To optimize conditions for CAL-B, longer reaction times were examined. However, a maximum 85% conversion was reached after 168 h at 55 °C (entries 5, 6, and 7, Table 2).

Table 2 Enzymatic acylation of 1,2-dideoxy-D-ribose (**13**)

Entry	*R*	Enzyme	**13** : Enzyme[a]	*T*/°C	t (h)	Conv. (%)[b]	**15** (%)[b,c]
1	H	CAL-B[d]	1 : 1	55	62	>97	>97
2	H	PSL-C[e]	1 : 2	50	3	>97	>97 (98)
3	H	PSL-IM[f]	1 : 2	50	5	>97	>97
4	Me	PSL-C[e]	1 : 2	50	3	>97	>97 (97)
5	Me	CAL-B[d]	1 : 1	55	65	42	>97
6	Me	CAL-B[d]	1 : 1	55	96	59	>97
7	Me	CAL-B[d]	1 : 1	55	168	85	>97
8	OMe	CAL-B[d]	1 : 1	55	96	32	>97
9	OMe	PSL-C[e]	1 : 2	50	26	>97	>97 (86)
10	NO_2	CAL-B[d]	1 : 1	55	57	>97	>97
11	NO_2	PSL-C[e]	1 : 2	50	0.5	>97	>97 (90)
12	F	CAL-B[d]	1 : 1	55	96	90	>97
13	F	PSL-C[e]	1 : 2	50	2.5	>97	>97 (86)

[a] Ratio **13** : enzyme (w/w). [b] Calculated by ^{1}H NMR. [c] Percentage of isolated yields are given in parentheses. [d] Novozym 435. [e] Lipase PS "Amano" immobilized on ceramic. [f] Lipase PS "Amano" SD immobilized on diatomaceous earth.



Scheme 4

Although excellent selectivity was also achieved with *p*-methoxy vinylbenzoate (**14c**), CAL-B-catalyzed acylation of **13** gave lower conversion (entry 8, Table 2). The acylating agent **14c** contains an electron donating group lowering the formation of acylated **15c** catalyzed by CAL-B compared to the vinylbenzoate and *p*-methyl vinylbenzoate discussed above. Similar behaviour was observed with PSL-C. However, this enzyme drives the reaction to completion after 26 h at 50 °C (entry 9, Table 2). Interestingly, the acylation proceeded faster with *p*-nitro vinylbenzoate (**14d**), which possesses an electron withdrawing group. 1,2-Dideoxy-D-ribose was converted selectively to the 5-*O*-*p*-nitrobenzoyl derivative **15d** with both CAL-B and PSL-C (entries 10 and 11, Table 2). The later furnished **15d** in 90% yield within 0.5 h at 50 °C. To further enhance our understanding of the influence of *p*-substituent during enzymatic acylation, the reaction was conducted with *p*-fluoro vinylbenzoate (**14e**). The presence of a weakly deactivating group in the *para* position of the benzoyl group provided longer reaction times relative to the nitro derivative (entries 12 and 13 *vs.* entries 10 and 11, Table 2), but significantly shorter than electron donating methyl or methoxy substituents. Overall, the regioselective 5-*O*-acylation was observed with all of the enol esters tested, providing shorter reaction times with PSL-C than CAL-B.

It is noteworthy that conventional acylation of **13** with an acid chloride was non-selective furnishing mixtures of acylated products. As an example, the reaction of **13** with 1.5 equiv of 4-methylbenzoyl chloride in pyridine as solvent and DMAP as catalyst offered 50% of the 5-*O*-esterified product, 2% of the 3-*O*-acylated product, and 11% of the diacyled derivative and 37% of starting material despite of extended reaction (5 days).

To make this enzymatic process attractive for industrial applications, we use the vinyl ester in 1 : 1.1 ratio with respect to the starting material and tested the efficacy of enzyme recycle on a 5 mmol scale (Table 3). The reaction of **13** with 1.1 equiv of vinyl benzoate catalyzed by PSL-C was completed in 7.5 h and

Table 3 Recycling study of PSL-C in the enzymatic acylation of **13**[a]

Run	t (h)	Conv. (%)[b]	**15a** (% isolated yield)
1	7.5	>97	96
2	8.5	>97	96
3	26	>97	94
4	32	91	86
5	54	89	86

[a] The reaction was carried out on 5 mmol scale at 50 °C, in 1 M concentration with 1.1 equiv of vinyl benzoate. [b] Calculated by ^{1}H NMR.

work-up of the reaction provided crude 5-*O*-benzoyl-1,2-dideoxy-D-ribose, which was easily isolated in pure state *via* extraction and drying under vacuum (96%). Next, recycling experiments using the recovered lipase were carried out. To improve the activity, the immobilized enzyme was reactivated by hydration. For that, the enzyme was poured into water, filtered, and dried under vacuum. Five consecutive catalytic cycles were performed and excellent yields have been achieved. With the exception of longer reaction times in subsequent runs similar behaviour of the PSL-C was observed. A conversion of ~90% was reached in the fourth and fifth catalytic cycle. Despite lower efficiencies in the 4/5 cycles, the product is easily isolated following the standard extraction work-up which removes the starting material in aq. wash. Overall, the isolation of **15a** was accomplished without column chromatography.

Ionic liquids have proven to be environmentally green and attractive reaction media for biocatalysis.[22] Therefore, we tested [BMIM]-[PF_6] (1-butyl-3-methylimidazolium) hexafluorophosphate for lipase catalyzed acylation of 1,2-dideoxy-D-ribose and compared it to the THF as solvent. The acylation of **13** with vinyl benzoate (1.1 equiv) in [BMIM]-[PF_6] at 50 °C catalyzed by PSL-C proceeded with high regioselectivity in 10 h, only trace amount of diacyl derivative (<2%) was detected compared to the reaction in THF.

The enzymatic catalysis provides useful synthetic intermediates that can be employed in the assembly of other complex molecules. For example, dimethoxytrityl (DMTr) protected **17** and **19** are key precursors for the synthesis of corresponding amidites that are used for incorporation into therapeutic oligonucleotides.[11] Therefore, synthesis of **17** and **19** was envisioned from a common starting material **11** in a straightforward manner (Scheme 5). The tritylation of **12** and **15** with dimethoxytrityl chloride furnished **16** and **18** in 87% and 83% yield, respectively. The synthetic ease and accessibility of these orthogonally protected 1,2-dideoxy-D-ribose **16** and **18** may further enhance the value of these molecules in carbohydrate chemistry. Next, the toluoyl or benzoyl groups are selectively cleaved with sodium methoxide in MeOH, leading to the 5- and 3-*O*-DMTr monomers **17** and **19**, useful building blocks in carbohydrate and oligonucleotide synthesis.

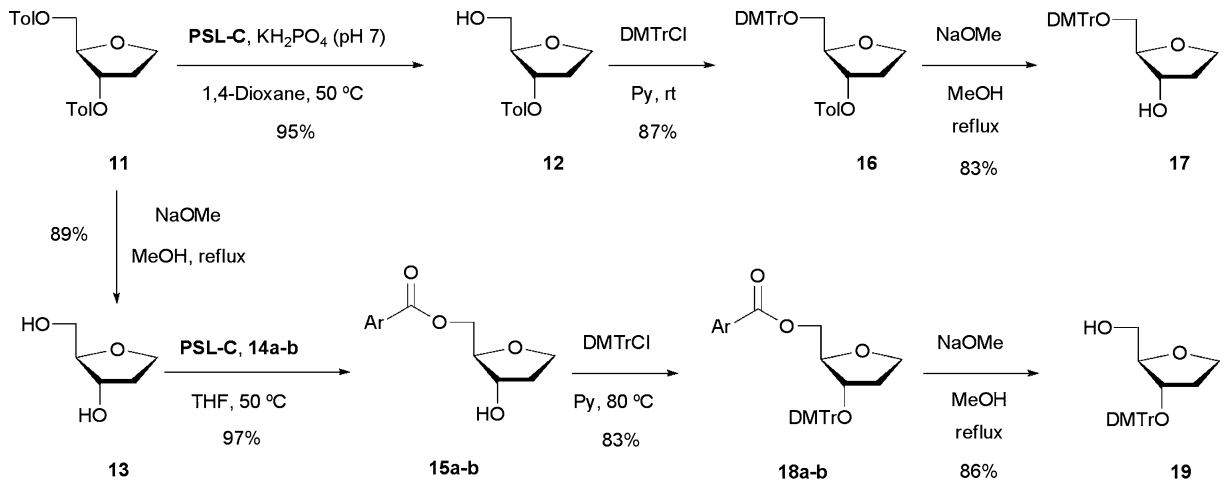
Conclusions

We have developed a green protocol for the preparation of 3-, and 5-*O*-DMTr-1,2-dideoxy-D-ribose, which are of interest due to their incorporation into therapeutic oligonucleotides as abasic site precursors. *Candida antarctica* lipase B and *Pseudomonas cepacia* lipase catalyze the regioselective hydrolysis of di-*O*-toluoyl-1,2-dideoxy-D-ribose furnishing the 3-*O*-toluoyl derivative in quantitative yield. Meanwhile, both lipases exhibited complementary behaviour in the acylation reaction of 1,2-dideoxy-D-ribose, providing exclusively the opposite regioisomer. The presence of electron donating substituents in the *para*-position of the benzoate vinyl ester decreased the rate of enzymatic acylation. However, the reaction with electron withdrawing group in the acylating agent proceeded at a faster rate. The protocol described herein offers a fast, reliable and scalable route to orthogonally protected carbohydrate building-blocks in excellent overall yields. Specially, the 3-*O*-DMTr precursor is very useful when reverse amidite is required for therapeutic oligonucleotides synthesis. Moreover, enzyme catalyzed reactions are less hazardous, less polluting, and less energy-consuming than the conventional chemistry-base methods. In addition, the enzyme has been reused to make the process further economical for industrial applications. In summary, we have developed atom efficient and low environmental impact protocols without sacrificing the stereo- and regiocontrol requirements of the modern day carbohydrate chemistry.

Experimental section

Enzyme activities

Pseudomonas cepacia lipase (PSL-C, Amano PS-C Type II, 1950 U/g) was purchased from Aldrich. *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300 PLU/g) was a gift from Novo Nordisk Co. *Chromobacterium viscosum* lipase (CVL, 4100 U/mg) was a gift from Genzyme Co. *Pseudomonas cepacia* lipase powder immobilized in diatomaceous earth (PSL-IM, 943 U/g) and *Pseudomonas cepacia* powder as a crude enzyme preparation containing dextrin as diluents (PSL-SD, 24700 U/g) were generously provided by Amano Europe.



Scheme 5

Enzymatic hydrolysis of di-*O*-toluoyl-1,2-dideoxyribose. Synthesis of 3-*O*-toluoyl-1,2-dideoxy-D-ribose (12)

To a solution of **11** (400 mg, 1.13 mmol) in 1,4-dioxane (3.7 mL) was added 0.15 M phosphate buffer pH 7 (7.4 mL) and the corresponding lipase [ratio of **11**:CAL-B was 1 : 1 (w/w); ratio of **11**:PSL was 1 : 2 (w/w); ratio of **11**:CVL was 1 : 0.25 (w/w)]. The mixture was allowed to react at 250 rpm for the time and at the temperature indicated in Table 1. The reactions were monitored by TLC (10% MeOH/CH_2Cl_2). The enzyme was filtered off and washed with CH_2Cl_2, the solvents were distilled under vacuum, and the residue was taken up in CH_2Cl_2 and washed with $NaHCO_3$ (aq). The combined organic layers were dried over Na_2SO_4 and evaporated to give pure **12**. R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 88–90 °C. $[\alpha]_D^{20}$ = +26 (c 1.0, CH_2Cl_2). IR (KBr): υ 3460, 3055, 2987, 1713, 1612 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 2.23 (m, 2H, H-2), 2.40 (s, 3H, CH_3), 4.15 (m, 3H, $2H_i$+H_4), 5.33 (dt, 1H, $^3J_{HH}$ 2.4, $^3J_{HH}$ 6.4 Hz), 7.23 (d, 2H, H_m, $^3J_{HH}$ 8.3 Hz), 7.91 (d, 2H, H_o, $^3J_{HH}$ 8.3 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 22.3 (CH_3), 33.8 (C_2), 63.6 (C_5), 68.3 (C_1), 77.2 (C_3), 85.6 (C_4), 127.7 (C_i), 129.8 (C_m), 130,3 (C_o), 144.7 (C_p), 167.2 (C═O). MS (ESI^+, *m/z*): 237 [$(M+H)^+$, 10%], 259 [$(M+Na)^+$, 100]. HRMS (ESI^+) calc for $C_{13}H_{16}NaO_4$ $[M+Na]^+$: 259.0941, found: 259.0938.

Synthesis of 1,2-dideoxy-D-ribose (13)

NaOMe (1.9 g, 35.25 mmol) was added to a solution of **11** (5 g, 14.1 mmol) in anhydrous MeOH (28 mL) at room temperature. Reaction was stirred at reflux and monitored by TLC (10% MeOH/CH_2Cl_2) indicating the total disappearance of the starting material in 2 h. Once the reaction was concluded, solvents were evaporated under vacuum and the residue was purified by flash chromatography (gradient elution with 2–10% MeOH/CH_2Cl_2) to obtain **13** as a colorless oil (1.48 g, 89% yield). R_f (10% MeOH/CH_2Cl_2): 0.20. IR: υ 3370, 2945, 2882, 1441 cm^{-1}. ^1H NMR (MeOH-d_4, 300.13 MHz): δ 1.95 (m. 1H, H-2), 2.09 (m, 1H, H-2), 3.51 (m, 2H, H-5), 3.75 (m, 1H, H_4), 3.91 (m, 2H, H-1), 4.21 (dt, 1H, $^3J_{HH}$ 3.1 Hz, $^3J_{HH}$ 6.1 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 36.2 (C-2), 63.9 (C-5), 68.2 (C-1), 74.0 (C-3), 88.4 (C-4). HRMS (ESI^+) calc for $C_5H_{10}NaO_3$ $[M+Na]^+$: 141.0522, found: 141.0524.

Synthesis of vinyl esters 14b–e

General Procedure. A mixture of carboxylic acid (**16b–e**) (5 mmol), vinyl acetate (50 mL), $Pd(OAc)_2$ (0.8 mmol; 1.6 mmol for **16d**), and KOH (55 mg, 1 mmol) under nitrogen was stirred overnight at room temperature. After filtration over Celite, solvent was evaporated and the residue was purified by flash chromatography on silica gel (20% EtOAc/hexane) to afford **14** (81% yield for **14b**; 72% yield for **14c**; 72% yield for **14d**; 71% yield for **14e**).

Vinyl 4-methylbenzoate (14b)

Colorless liquid. R_f (20% EtOAc/hexane): 0.57. IR: υ 3055, 2987, 1730, 1645, 1613 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 2.40 (s, 3H, CH_3), 4.67 (d, 1H, H-2, $^3J_{HH}$ 6.4 Hz), 5.06 (d, 1H, H-2, $^3J_{HH}$ 14.0 Hz), 7.24 (d, 2H, H_o, $^3J_{HH}$ 8.3 Hz), 7.51 (dd, 1H, $^3J_{HH}$ 6.4 Hz, $^3J_{HH}$ 14.0 Hz), 7.98 (d, 1H, $^3J_{HH}$ 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 22.3 (CH_3), 98.5 (C-2), 126.8 (C_i), 129.8 (C_m), 130.6 (C_o), 142.0 (C-1), 145.0 (C_p), 164.3 (C═O).

Vinyl 4-methoxybenzoate (14c)

White solid. R_f (20% EtOAc/hexane): 0.57. mp: 52–54 °C. IR (KBr): υ 3055, 2971, 2842, 1726, 1645, 1607 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 3.86 (s, 3H, OCH_3), 4.67 (d, 1H, H-2, $^3J_{HH}$ 6.3 Hz), 5.04 (d, 1H, H-2, $^3J_{HH}$ 14.2 Hz), 6.95 (d, 2H, H_m, $^3J_{HH}$ 9.0 Hz), 7.52 (dd, 1H, H_1 $^3J_{HH}$ 6.3 Hz, $^3J_{HH}$ 14.2 Hz), 8.06 (d, 1H, H_o, $^3J_{HH}$ 8.4 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 55.4 (CH_3), 97.6 (C-2), 113.8 (C_m), 121.2 (C_i), 132.1 (C_o), 141.5 (C-1), 163.3-163.9 (C═O + C_p). HRMS (ESI^+) calc for $C_{10}H_{10}NaO_3$ $[M+Na]^+$: 201.0522, found: 201.0510.

Vinyl 4-nitrobenzoate (14d)

Pale yellow solid. R_f (20% EtOAc/hexane): 0.57. mp: 74–76 °C. IR (KBr): υ 3056, 2987, 1737, 1647, 1609, 1530 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 4.80 (dd, 1H, H-2, $^2J_{HH}$ 2.0 Hz, $^3J_{HH}$ 6.0 Hz), 5.14 (dd, 1H, H-2, $^3J_{HH}$ 2.0 Hz, $^3J_{HH}$ 14.0 Hz), 6.95 (d, 2H, H_m, $^3J_{HH}$ 9.0 Hz), 7.52 (dd, 1H, H-1 $^3J_{HH}$ 6.3 Hz, $^3J_{HH}$ 13.9 Hz), 8.28 (m, 4H, H_o+H_m). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 99.5 (C-2), 123.7 (C_m), 131.1 (C_o), 134.3 (C_i), 141.1 (C-1), 161.8 (C_P and C═O).

Vinyl 4-fluorobenzoate (14e)

Colorless liquid. R_f (20% EtOAc/hexane): 0.57. mp: 52–54 °C. IR: υ 3093, 2958, 1737, 1646, 1604 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 4.70 (dd, 1H, H-2, $^2J_{HH}$ 2.0 Hz, $^3J_{HH}$ 6.3 Hz), 5.06 (d, 1H, H-2, $^3J_{HH}$ 13.9 Hz), 7.14 (m, 2H, H_m), 7.48 (dd, 1H, H_1, $^3J_{HH}$ 6.3 Hz, $^3J_{HH}$ 13.9 Hz), 8.14 (m, 2H, H_o). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 98.3 (C-2), 115.7 (d, C_m, $^2J_{CF}$ 22.1 Hz), 125.2 (C_i), 132.6 (d, C_o, $^3J_{CF}$ 9.3 Hz), 141.3 (C-1), 162.6 (C═O), 254.9 (d, C_p, $^1J_{CF}$ 64.1 Hz). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 104.4

Enzymatic acylation of 1,2-dideoxy-D-ribose

General procedure for the synthesis of 15a–e. A suspension of **13** (59 mg, 0.5 mmol), the corresponding vinyl ester **14** (1 mmol), and the lipase [ratio of **13**:CAL-B was 1 : 1 (w/w); ratio of **13**:PSL was 1 : 2 (w/w)] in anhydrous THF (2.5 mL) under nitrogen was stirred at 250 rpm for the time and at the temperature indicated in Table 2. The reactions were monitored by TLC (10% MeOH/CH_2Cl_2). The enzyme was filtered off and washed with CH_2Cl_2. The combined organic layers were washed with $NaHCO_3$ (aq),dried over Na_2SO_4 and evaporated. The volatile acylation agents (**14a**, **14b**, **14e**) were removed by drying under vacuum at 60 °C to give pure **15a**, **15b** and **15e**. With acylating agents **14c–d**, the residue obtained after extraction was subjected to flash chromatography (2% MeOH/CH_2Cl_2) to afford **15c–d** as pure white solids.

5-*O*-Benzoyl-1,2-dideoxy-D-ribose (15a)

98% yield. R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 57–59 °C. $[\alpha]_D^{20}$ = +29 (c 1.0, CH_2Cl_2). IR (KBr): υ 3445, 2986, 2953, 1721, 1602 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.95 (m, 1H, H-2), 2.18 (m, 1H, H-2), 2.78 (br s, 1H, OH), 4.05 (m, 3H, H-1 + H-4), 4.36 (m, 3H, H-3 + H-5), 7.42 (t, 2H, H_m $^3J_{HH}$ 7.6 Hz), 7.55 (tt, 2H, H_p

$^3J_{HH}$ 1.3 Hz, $^3J_{HH}$ 6.7 Hz), 8.03 (m, 2H, H_o). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 34.9 (C-2), 64.9 (C-5), 67.1 (C-1), 73.5 (C-3), 83.8 (C-4), 128.4–129.6 (5C, C_i+C_o+C_m), 133.2 (C_p), 166.6 (C═O). MS (ESI$^+$, *m/z*): 259 [(M+Na)$^+$, 100]. HRMS (ESI$^+$) calc for $C_{12}H_{15}O_4$ [M+H]$^+$: 223.0965, found: 223.0959.

5-*O*-Toluoyl-1,2-dideoxy-D-ribose (15b)

R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 45–47 °C. $[\alpha]_D^{20}$ = +26 (c 1.0, CH_2Cl_2). IR (KBr): υ 3429. 3037, 2949, 1717, 1612 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300.13 MHz), δ 1.92 (m, 1H, H-2), 2.14 (m, 1H, H-2), 2.37 (s, 3H, CH_3), 3.03 (br s, 1H, OH), 4.01 (m, 3H, H-1 + H-4), 4.31 (m, 3H, H-3 + H-5), 7.19 (d, 2H, H_m, $^3J_{HH}$ 8.1 Hz), 7.89 (d, 2H, H_o, $^3J_{HH}$ 8.1 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 21.5 (CH_3), 34.9 (C-2), 64.7 (C-5), 67.1 (C-1), 73.3 (C-3), 83.8 (C-4), 126.8 (C_i), 129.0 (C_m), 129.6 (C_o), 143.8 (C_p), 166.7 (C═O). MS (ESI$^+$, *m/z*): 237 [(M+H)$^+$, 20%], 259 [(M+Na)$^+$, 100]. HRMS (ESI$^+$) calc for $C_{13}H_{16}NaO_4$ [M+Na]$^+$: 259.0941, found: 259.922.

5-*O*-*p*-Methoxybenzoyl-1,2-dideoxy-D-ribose (15c)

R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 67–69 °C. $[\alpha]_D^{20}$ = +21 (c 0.72, CH_2Cl_2). IR (KBr): 3455, 3054, 2985, 1712, 1606 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.96 (m, 1H, H_2), 2.19 (m, 1H, H_2), 3.85 (s, 3H, OCH_3), 4.02 (m, 2H, H-1), 4.08 (c, 1H, H-4, $^3J_{HH}$ 4.9 Hz), 4.35 (m, 3H, H-5 and H-3), 6.90 (d, 2H, H_m, $^3J_{HH}$ 8.9 Hz), 7.89 (d, 2H, $^3J_{HH}$ 8.9 Hz). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 35.0 (C-2), 55.4 (OCH_3), 64.7 (C-5), 67.2 (C-1), 73.5 (C-3), 84.0 (C-4), 113.7 (C_m), 122.1 (C_i), 131.8 (C_o), 163.7 and 166.4 (C_p and C═O). MS (ESI$^+$, *m/z*): 275.0 [(M+Na)$^+$, 100%]. HRMS (ESI$^+$) calc for $C_{13}H_{16}NaO_5$ [M+Na]$^+$: 275.0890, found: 275.0880.

5-*O*-*p*-Nitrobenzoyl-1,2-dideoxy-D-ribose (15d)

R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 90–92 °C. $[\alpha]_D^{20}$ = +26 (c 1.0, CH_2Cl_2). IR (KBr): 3352, 3074, 1716, 1606, 1527 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.96 (m, 1H, H-2), 2.21 (m, 1H, H-2), 4.00–4.12 (m, 3H, H-1 and H-4), 4.32–4.50 (m, 3H, H-3, and H-5), 8.26 (m, 4H, H_o and H_m). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 35.0 (C-2), 65.7 (C-5), 67.2 (C-1), 73.6 (C-3), 83.6 (C-4), 123.6 (C_m), 130.8 (C_o), 135.2 (C_i), 150.7 (C_p), 164.7 (C═O). MS (ESI$^+$, *m/z*): 290.0 [(M+Na)$^+$, 100%]. HRMS (ESI$^+$) calc for $C_{12}H_{13}NaO_6$ [M+Na]$^+$: 290.0635, found: 290.0639.

5-*O*-*p*-Fluorobenzoyl-1,2-dideoxy-D-ribose (15e)

R_f (10% MeOH/CH_2Cl_2): 0.52. mp: 59–61 °C. $[\alpha]_D^{20}$ = +41 (c 1.0, CH_2Cl_2). IR (KBr): 3372, 3070, 1712, 1507, 1512 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.96 (m, 1H, H-2), 2.20 (h, 1H, H-2, $^3J_{HH}$ 8.0 Hz), 3.99–4.10 (m, 3H, H-1 and H-4), 4.32–4.50 (m, 3H, H-3, and H-5), 7.11 (m, 2H, H_m), 8.06 (m, 2H, H_o). ^{13}C NMR ($CDCl_3$, 100.61 MHz): δ 35.1 (C-2), 65.0 (C-5), 67.2 (C-1), 73.6 (C-3), 83.8 (C-4), 115.6 (d, C_m, $^2J_{CF}$ 22.0 Hz), 130.8 (C_o), 126.0 (C_i), 132.3 (C_o), 165.7 (C═O), 165.9 (d, C_p, $^2J_{CF}$ 254.5 Hz). ^{19}F NMR ($CDCl_3$, 282 MHz): δ 105.2. MS (ESI$^+$, *m/z*): 263.0 [(M+Na)$^+$, 100%]. HRMS (ESI$^+$) calc for $C_{12}H_{13}FNaO_6$ [M+Na]$^+$: 263.0690, found: 263.0690.

Synthesis of 5-*O*-dimethoxytrityl-3-*O*-toluoyl-1,2-dideoxy-D-ribose (16)

To a solution of **12** (80 mg, 0.34 mmol), in anhydrous pyridine (3.4 mL) was added 4,4′-dimethoxytrityl chloride (127 mg, 0.37 mmol), and the mixture was stirred at room temperature. The progress of the reaction was monitored by TLC (30% hexane/CH_2Cl_2), which indicates the total disappearance of starting material in 3 h. The mixture was concentrated under reduced pressure and the crude product was purified by column chromatography using neutral silica gel 60 Å (32–63 μm) pH 7 (20% hexane/CH_2Cl_2) to obtain **16** (158 mg, 87% yield). mp: 43–45 °C. R_f (30% hexane/CH_2Cl_2): 0.37. $[\alpha]_D^{20}$ = 39 (c 1.0, CH_2Cl_2). IR (KBr): υ 3055, 2966, 1714, 1609 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300.13 MHz), δ 2.09 (m, 1H, H-2), 2.33 (m, 1H, H-2), 2.39 (s, 3H, CH_3), 3.25 (m, 2H, 2*H*-5), 3.73 (s, 6H, OCH_3), 4.09 (m, 3H, H_1 and H_4), 5.39 (dt, 1H, H_3, $^3J_{HH}$ 2.2 Hz, $^3J_{HH}$ 6.4 Hz), 6.82 (d, 4H, DMT, $^3J_{HH}$ 8.8 Hz), 7.25 (m, 9H, DMT), 7.43 (d, 2H, H_m, $^3J_{HH}$ 8.3 Hz), 7.88 (d, 2H, H_o $^3J_{HH}$ 8.3 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 21.7 (CH_3), 33.7 (C-2), 55.6 (2C, OCH_3), 65.3 (C-5), 68.6 (C-1), 78.3 (C-3), 84.7 (C-4), 87.5 (C-DMT), 114.1 (4C), 127.8–131.3 (14C), 137.3 (2C, DMT), 145.5–146.4 (2C), 160.1 (2C), 167.6 (C═O). MS (ESI$^+$, *m/z*): 561 [(M+Na)$^+$, 100]. HRMS (EI$^+$) calc for $C_{34}H_{34}O_6$ [M$^+$]: 538.2355, found: 538.2351.

Synthesis of 5-*O*-dimethoxytrityl-1,2-dideoxy-D-ribose (17)

Compound **16** (100 mg, 0.19 mmol) was dissolved in anhydrous MeOH (2.0 mL) and NaOMe (21 mg, 0.38 mmol) was added at room temperature under continuous stirring. The reaction is stirred at reflux for 2 h. The solvent was then evaporated and the residue subjected to flash chromatography on silica gel (40% EtOAc/hexane) to give **17** (64 mg, 83% yield). R_f (40% EtOAc/hexane): 0.26. mp: 42–44 °C. $[\alpha]_D^{20}$ = +14 (c 1.0, CH_2Cl_2). IR (KBr): υ 3433, 3055, 2987, 1609 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.86 (m, 1H, H_2), 2.06 (m, 1H, H-2), 3.08 (m, 2H, H-5), 3.75 (s, 6H, OCH_3), 3.94 (m, 3H, H-1+H-4), 4.22 (dt,1H, H-3, $^3J_{HH}$ 2.6 Hz, $^3J_{HH}$ 5.7 Hz), 6.83 (d, 4H, DMT, $^3J_{HH}$ 8.8 Hz), 7.26 (m, 7H, DMT), 7.44 (d, 2H, DMT, $^3J_{HH}$ 7.7 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 36.1 (C-2), 56.0 (2C, OMe), 65.9 (C-5), 68.4 (C-1), 74.6 (C-3), 87.2 (C-4) and 87.7 (C-DMT), 114.3 (4C), 128.0–131.6 (9C), 137.6 (2C), 146.8 (1C), 160.4 (2C). MS (ESI$^+$, *m/z*): 443 [(M+Na)$^+$, 10]. HRMS (EI$^+$) calc for $C_{26}H_{28}O_5$ [M$^+$]: 420.1937, found: 420.1936.

Synthesis of 5-benzoyl-3-dimethoxytrityl-1,2-dideoxy-D-ribose (18a) or 3-dimethoxytrityl-5-toluoyl-1,2-dideoxy-D-ribose (18b)

To a solution of **15a** or **15b**(0.34 mmol) in anhydrous pyridine (3.4 mL) was added 4,4′-dimethoxytrityl chloride (127 mg, 0.37 mmol), and the mixture was stirred at 70 °C. The progress of the reaction was monitored by TLC (30% hexane/CH_2Cl_2) which indicates the total disappearance of the starting material in 3 h. The mixture was concentrated under reduced pressure and the crude product was purified by column chromatography using neutral silica gel 60 Å (32–63 μm) pH 7 (20% hexane/CH_2Cl_2) to obtain **18**.

18a. 83% yield. mp: 47–49 °C. R_f (30% hexane/CH_2Cl_2): 0.27. IR (KBr): υ 3055, 2986, 2957, 1720, 1608 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.58 (m, 1H, H-2), 1.74 (m, 1H, H-2), 3.74

(s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.84–4.05 (m, 5H, H-1, H-4, H-5), 4.23 (dt, 1H, H-3, $^3J_{HH}$ 2.2 Hz, $^3J_{HH}$ 5.9 Hz), 6.85 (m, 4H, DMT), 7.10–7.69 (m, 11H), 7.61 (t, 1H, H_p, $^3J_{HH}$ 1.3 Hz, $^3J_{HH}$ 6.8 Hz), 7.88 (m, 2H, H_o). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 35.0 (C-2), 55.6 (2C, OCH3), 65.7 (C-5), 68.5 (C-1), 76.8 (C-4), 84.4 and 88.3 (C-3+C-DMT), 114.1 (4C), 127.8–134.2 (15C), 137.6 and 137.8 (2C), 147.0 (1C), 160.2 (2C), 167.5 (C═O). MS (ESI$^+$, m/z): 547 [(M+Na)$^+$, 100%], 1099 [(2 M+Na)$^+$, 40]. HRMS (EI$^+$) calc for $C_{33}H_{32}O_6$ [M$^+$]: 524.2199, found: 524.2204.

18b. 83% yield. mp: 48–50 °C. R_f (30% hexane/CH_2Cl_2): 0.27. $[\alpha]_D^{20}$ = +13 (c 1.0, CH_2Cl_2). IR (KBr): υ 3055, 2966, 1716, 1609 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.53 (m, 1H, H-2), 1.70 (m, 1H, H-2), 2.38 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.80–4.10 (m, 5H, H-1, H-4, H-5), 4.23 (dt, 1H, H-3, $^3J_{HH}$ 2.2 Hz, $^3J_{HH}$ 5.9 Hz), 6.82 (m, 4H, DMT), 7.09–7.39 (m, 9H), 7.46 (d, 2H, H_m, $^3J_{HH}$ 8.3 Hz), 7.88 (d, 2H, H_o, $^3J_{HH}$ 8.1 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 21.9 (CH_3), 35.4 (C_2), 56.0 (2C, O-Me), 65.9 (C-5), 68.9 (C-1), 77.1 (C-4), 84.8–84.6 (C-3+C-DMT), 114.1 (4C) 128.2–131.9 (14C), 137.9–138.2 (2C), 145.6 and 147.4 (2C), 160.6 (2C), 168.0 (C═O). MS (ESI$^+$, m/z): 561 [(M+Na)$^+$, 100%], 1099 [(2M+Na)$^+$, 60]. HRMS (EI$^+$) calc for $C_{34}H_{34}O_6$ [M$^+$]: 538.2355, found: 538.2347.

Synthesis of 3-dimethoxytrityl-1,2-dideoxy-D-ribose (19)

Same procedure as described above for the synthesis of **17**. 86% yield. R_f (40% EtOAc/hexane): 0.24. mp: 54–56 °C. $[\alpha]_D^{20}$ = +36 (c 1.0, CH_2Cl_2). IR (KBr): υ 3406, 3055, 2986, 1609 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.34 (m, 1H, H-2), 1.56 (m, 1H, H-2), 3.15 (dd, 1H, H-5, $^3J_{HH}$ 5.3 Hz, $^2J_{HH}$ 11.9 Hz), 3.30 (dd, 1H, H-5, $^3J_{HH}$ 3.5 Hz, $^2J_{HH}$ 12.7 Hz), 3.75 (s, 6H, 2OMe), 3.83 (m, 3H, $2H_1$+H_4), 4.14 (dt, 1H, H_3, $^3J_{HH}$ 1.7 Hz, $^3J_{HH}$ 5.7 Hz), 6.84 (m, 4H, DMT), 7.27 (m, 7H, DMT), 7.46 (m, 2H, DMT). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 35.8 (C_2), 56.0 (2C, OMe), 63.7 (C_5), 68.8 (C_1), 77.2 (C_3), 88.0–88.4 (C_t+C_4), 87.7 (C_4), 114.4 (4C), 128.2–131.9 (9C), 138.4 (2C), 147.4 (1C), 160.5 (2C). MS (ESI$^+$, m/z): 443 [(M+Na)$^+$, 100%], 863 [(2M+Na)$^+$, 20]. HMRS (EI$^+$) calc for $C_{26}H_{28}O_5$ [M$^+$]: 420.1937, found: 420.1942.

Procedure for the recycling of PSL-C

A stirred suspension of **13** (590 mg, 5 mmol), vinyl benzoate (814 mg, 5.5 mmol), and PSL-C (1.18 g) in anhydrous THF (5 mL) under nitrogen was heated at 50 °C and 250 rpm for 7.5 h. Once the reaction is finished, the enzyme was filtered off and washed with CH_2Cl_2. The combined filtrate were washed with $NaHCO_3$ (aq), dried over Na_2SO_4 and evaporated. The small excess of vinyl benzoate was removed under vacuum at 60 °C to afford **15a** in 96% yield. This process was repeated four times employing the same PSL-C (see Table 3). To reactivate the enzyme, PSL-C was poured into water, filtered, and dried under vacuum after each cycle.

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