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Enzymatic resolution of *syn*-2-azido-1,3,4-trihydroxybutane catalysed by lipases in the transesterification mode

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Abstract

The enzymatic resolution of both *syn*-2-azido-1,3,4-trihydroxybutane **1** and *syn*-2-azido-1,4-diacetoxy-3-hydroxybutane **2** have been undertaken with different lipases as catalysts and vinyl acetate as acylating agent. Lipases Amano PS and Amano AK proved to be the superior catalysts for this resolution. Indeed, both enantiomers of **1** are easily available in good yields and very good e.e.s (up to >99%). The use of chiral HPLC with a Chiralcel OD-H column allowed the determination of e.e.s of both diacetate **2** and triacetate **3** (*syn*-2-azido-1,3,4-triacetoxybutane) in a single analysis and thus facilitated the precise control of the reaction.

1. Introduction

Until recently, the 'chiral pool' was the main and simplest source of non-racemic materials available to chemists to perform asymmetric synthesis or asymmetric catalysis. In recent years the extraordinary development of both asymmetric catalysis[1] and bioconversion[2] has given chemists the opportunity to access new chiral synthons out of the 'chiral pool'. In this connection we were interested in the resolution of *syn*-2-azido-1,3,4-trihydroxybutane **1** by bioconversion, using lipases in the transesterification mode. Indeed, **1** is a multifunctional small molecule, readily available in racemic form through simple chemistry, starting from commercial *cis*-2,3-butene-1,4-diol (Scheme 1). Enantiomerically enriched derivatives of **1** have already been described: they have been obtained, for example, from transformations of ascorbic acid[3] or monoprotected optically active *cis*-1,4-dihydroxy-2,3-butene oxide.[4] The latter could be generated through Sharpless asymmetric epoxidation[4] or by bioconversion.[5,6] In the first case the transformation of ascorbic acid is tedious and, in the second case, a mixture of isomers is formed when opening the epoxide

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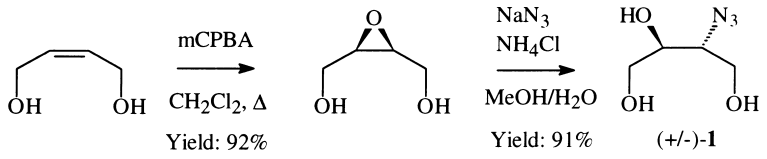


Scheme 1. Synthesis of *syn*-2-azido-1,3,4-trihydroxy-butane **1**

functionality with NaN_3 unless some regiocontrol could be exerted in the azidation process. It should also be noted that the enzymatic resolution of related 2,3-azido alcohols has been reported previously.[7] We believed that if a method could be developed to access both enantiomers of **1**, such a molecule would be a valuable chiral building block in the synthesis, for example, of sphingosines and aza-sugars.

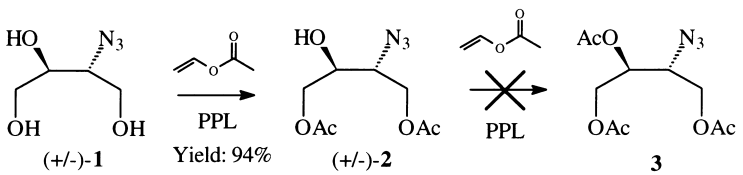
2. Results and discussion

2.1. Synthesis of *1*

As mentioned in the introduction, **1** is easily available from *cis*-2,3-butene-1,4-diol through simple chemistry and in good yield (84%), as depicted in Scheme 1.

2.2. Enzymatic access to racemic syn-*2-azido-1,4-diacetoxy-3-hydroxy-butane 2*

In a first attempt to realise the enzymatic resolution of **1**, we made the observation that porcine pancreatic lipase (PPL) was able to convert (+/–)-**1** to the corresponding racemic 1,4-diacetate **2**, with vinyl acetate as both solvent and acyl donor, in very good yield (Scheme 2). This reaction proved to be superior to its chemical counterpart in terms of regioselectivity since in our hands the chemical acetylation of (+/–)-**1** led to a mixture of mono-, di- and triacetates of **1**.



Scheme 2. Enzymatic synthesis of (+/–)-**2** with PPL

No triacetate **3** could be detected, indicating that **2** is not a substrate of PPL. This result prompted us to investigate the resolution of both (+/–)-**1** and enzymatically formed (+/–)-**2**.

2.3. Enzymatic resolution of diacetate *2*

Three lipases were tested, i.e. lipase Amano AK, lipase Amano PS and lipase Amano AY, respectively, from bacteria *Pseudomonas* sp. (both AK and PS) and from yeast *Candida rugosa* (formerly *C. cylindracea*). In the three cases vinyl acetate was used both as solvent and acyl donor and the influence of added co-solvents such as benzene and THF was also investigated. The

reactions were followed by chiral HPLC using a Chiralcel OD-H column. Both diacetate **2** and triacetate **3** are simultaneously resolved by this column using a mixture of hexane/isopropanol (95/5, vol./vol.) as eluent, delivered at a flow rate of 0.8 mL/min.[8] The results obtained are grouped in Table 1 (entries 1 to 5).

Table 1
Enzymatic resolution of diacetate **2** and triol **1**

Entry	Substrate	Lipase/cosolvent	Time (h)	Conversion	e.e. of **2***	e.e. of **3***	E
1	(+/-)-**2**	PS/none	43	34%	38%	74%	10
2	(+/-)-**2**	AY/none	43	8%	2%	23%	2
3	(+/-)-**2**	AK/none	43	58%	>99%	71%	26
4	(+/-)-**2**	AK/THF	24	46%	66%	77%	15
5	(+/-)-**2**	AK/benzene	24	56%	>99%	76%	29
6	(+/-)-**1**	AK/none	94	100%	95%	95%	n.a.
7	(+/-)-**1**	AK/benzene	90	100%	94%	87%	n.a.
8	(+/-)-**1**	PS/none**	94	100%	50%	95%	n.a.
9	(+/-)-**1**	PS/benzene**	114	100%	>99%	91%	n.a.

Enzymatic resolution of diacetate **2** and triol **1** using various lipases with vinyl acetate as acylating agent. In these reactions 50 mg of **2** or **1** were added in 10 mL of vinyl acetate or in a mixture of 5 mL of vinyl acetate and 5 mL of the co-solvent. Then 50 mg of lipase were added. * The e.e.'s were determined using HPLC with a Chiralcel OD-H column and a mixture of hexane-isopropanol (95/5) as eluent, delivered at 0.8 mL/min. UV detection was set at 214 nm. ** A further 50 mg portion of lipase PS was added at 48h. n.a. : not applicable.

It can be seen from Table 1 (entries 1 to 3) that lipase Amano AK is the most successful enzyme in the resolution of (+/–)-**2** (E = 26). In order to enhance the selectivity of lipase Amano AK, we have investigated the possible role of benzene and THF as co-solvent[3] in the reaction. From Table 2 (entries 4 and 5) it could be concluded that the use of THF as co-solvent has a negative impact on the selectivity of lipase Amano AK (E = 15). With benzene a slightly better enantiomeric factor is obtained (E = 29) and the reaction is twice as fast.

2.4. *Enzymatic resolution of triol **1***

Following the resolution of diacetate **2**, we are interested in that of the parent compound, triol **1**. For this study, only lipases Amano PS and AK were selected and the reactions run either with only vinyl acetate as solvent or in a 1/1 mixture with benzene. The results are grouped in Table 1 (entries 6 to 9). It is worth mentioning that in all cases, at the end of the reaction, only diacetate **2** and triacetate **3** are detected either by TLC or chiral HPLC. Also, the reaction times are higher

Table 2
Enzymatic preparative resolution of triol **1** and diacetate **2**

Lipase/substrate	Time	E.e. of (2*S*,3*R*)-**2***/$[\alpha]^{20}_D$	Yield	E.e. of (2*R*,3*S*)-**3***/$[\alpha]^{20}_D$	Yield
AK/**2**	22 h	>99%/+13.6(c=3.28,$CHCl_3$)	33%	70%/+7.5(c=5.62,$CHCl_3$)	48%
AK/**1**	45 h	57%/+7.8(c=1.50,$CHCl_3$)	48%	94%/+10.4(c=1.38,$CHCl_3$)	19%
AK** /**1**	42 h	96%/+13.0(c=6.58,$CHCl_3$)	42%	91%/+10.0(c=8.53,$CHCl_3$)	46%
PS***/**1**	168 h	98%/+13.2(c=6.46,$CHCl_3$)	41%	80%/+8.7(c=9.09,$CHCl_3$)	48%

Enzymatic resolution of triol **1** and diacetate **2** using various lipases with vinyl acetate as acylating agent. In these reactions 1g of substrate was added to a 1/1 mixture of vinyl acetate and benzene and 1 g of lipase was then added. * The e.e.'s were determined using HPLC with a Chiralcel OD-H column and a mixture of hexane-isopropanol (95/5) as eluent, delivered at 0.8 mL/min. UV detection was set at 214 nm. ** A further 1 g portion of lipase was added at 24h. *** Three 1 g portions of lipase PS were further added at 24, 48 and 96h.

when **1** is used as substrate (probably due to lower solubility); improved selectivities are obtained for reactions involving lipase Amano AK without co-solvent and for lipase Amano PS with added benzene (entries 6 and 9).

2.5. *Enzymatic preparative resolutions of **2** and **1***

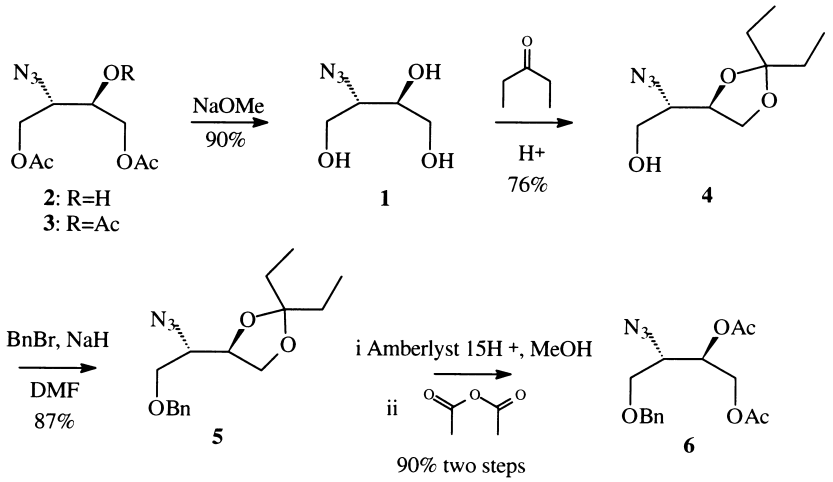
Following the results described in Table 1, we have undertaken the enzymatic preparative resolution of diacetate **2** and triol **1**, according to the best procedures developed previously. The reactions have been done on a 1 gram scale, using lipases Amano PS or AK in a 1/1 mixture of vinyl acetate and benzene and followed by chiral HPLC chromatography. When the desired e.e.s were reached, purification of both **2** and **3** has been undertaken and specific rotations determined, as well as the absolute configurations of enantiomerically enriched **2** and **3**. The results are collected in Table 2.

The results depicted in Table 2 indicate that (2*S*,3*R*)-**2** is easily available in high e.e.s (up to >99%) and satisfactory yields, using either Amano AK or PS lipases as catalysts and triol **1** or diacetate **2** as substrates. On the other hand, if (2*R*,3*S*)-**3** is to be obtained with good e.e. and good yield, lipase Amano AK is the catalyst of choice with triol **1** as substrate.

2.6. *Determination of absolute configurations*

In 1994, Fuji et al.[9] have described, in a work devoted to the synthesis of enantiomerically pure 2-aziridinemethanols, the preparation and absolute configuration of (2*S*,3*R*)-2-azido-1-benzyloxy-3,4-diacetoxybutane **6** (Scheme 3). Compound **6** could easily be obtained from **2** or **3** by the reaction sequence depicted in Scheme 3.

In order to determine the absolute configurations of enzymatically formed **2** and **3**, we have first transformed (+)-**2** (e.e. 57%, $[\alpha]^{20}_D$ +7.8 (c = 1.50, $CHCl_3$)) into (–)-**3** (e.e. 57%, $[\alpha]^{20}_D$ –7.2 (c = 2.89, $CHCl_3$)) by acetylation with acetic anhydride and then, following the sequence depicted



Scheme 3. Conversion of diacetate **2** or triacetate **3** to compound **6**

in Scheme 3, into (+)-**6** (e.e. 59%,[10] $[\alpha]_D^{20}$ +6.7 (c = 2.81, $CHCl_3$)). The positive sign of the specific rotation thus obtained for compound **6** allowed the assignment of its absolute configuration as (2*S*,3*R*)-2-azido-1-benzyloxy-3,4-diacetoxybutane[9] and hence that of starting (+)-**2**, that is also (2S,3*R*). This means that this enantiomer of **2** is the first eluted one on Chiralcel OD-H column.[8] By the way, the first eluted enantiomer of **3**,[8] having a negative optical rotation sign, has the same (2S,3*R*) absolute configuration.

3. Conclusion

By using very simple chemistry followed by lipase resolution we were able to access, on a preparative scale, both enantiomers of triol **1**, with very high e.e.s (up to > 99%) and good yields. We believe that such a small multifunctional chiral synthon could have a lot of synthetic applications, some of which have already been described.[3–5] Indeed, as shown in Scheme 3, the *vic*-diol functionality is easily differentiated from the third alcohol functionality using 3-pentanone. After suitable protection of the remaining alcohol function, the acetal could be released, leaving one primary alcohol and one secondary alcohol, that are thus easily differentiated chemically.

4. Experimental

4.1. General methods

Porcine pancreatic lipase was from Sigma. Lipases AK, AY and PS were a gift from Amano. *cis*-2-Buten-1,4-diol, *m*-CPBA and Amberlyst 15 were from Fluka. Vinyl acetate, *p*-toluenesulfonic acid, 3-pentanone, sodium methoxide (0.5 M in methanol), sodium hydride, benzyl bromide and acetic anhydride were from Aldrich. NMR spectra were run on a Bruker AC 200 spectrometer with TMS as internal standard. Chemical shifts are given in ppm downfield of TMS (s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quadruplet,

m = multiplet). Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Chiral HPLC analyses were run with a Chiralcel OD-H column, using a 95/5 (hexane/2-propanol) mixture as eluent, delivered at 0.8 mL/min. UV detection occurred at $\lambda = 214$ nm. Enzymatic reactions were carried out in screw cap tubes. The tubes were incubated at 30°C, on a reciprocal shaker, working at 60 oscillations per minute.

4.2. *Synthesis of 1,4-dihydroxy-*cis*-2,3-butene oxide*

In a 0.5 L round-bottomed flask, 2.2 g (25 mmol) of 1,4-dihydroxy-*cis*-2,3-butene were dissolved in 50 mL of CH_2Cl_2. Then 9.5 g (27.5 mmol, 1.1 equiv.) of *m*-CPBA dissolved in 100 mL of CH_2Cl_2 were added slowly. At the end of the addition, the reaction mixture was vigorously stirred and brought to reflux. The progress of the reaction was followed by TLC with a 7/3 CH_2Cl_2/MeOH eluent. Consumption of 1,4-dihydroxy-*cis*-2,3-butene was complete after 4 h. The reaction mixture was then poured on a silica column equilibrated with Et_2O. Unreacted *m*-CPBA and formed *m*-CBA were first eluted with 2% acetic acid in Et_2O. The product was then eluted with an 8/2 mixture of CH_2Cl_2/MeOH affording 2.33 g (23 mmol) of 1,4-dihydroxy-*cis*-2,3-butene oxide as a white amorphous solid in 92% yield, mp 38–40°C. ^{1}H NMR (200 MHz, CD_3OD) δ 3.11–3.19 (2H, m), 3.56–3.80 (4H, m), 4.73 (2H, br. s). ^{13}C NMR (50 MHz, CD_3OD) δ 57.7 (CH), 61.1 (CH_2).

4.3. *Synthesis of* syn-*2-azido-1,3,4-trihydroxy-butane* ***1***

In a 0.25 L round-bottomed flask, 2.25 g (22 mmol) of 1,4-dihydroxy-*cis*-2,3-butene oxide were dissolved in 90 mL of an 8/1 methanol/water mixture. Then 2.8 g (44 mmol, 2 equiv.) of NaN_3 and 5.8 g (110 mmol, 5 equiv.) of NH_4Cl were added and the reaction brought to reflux. The progress of the reaction was followed by TLC (CH_2Cl_2/MeOH, 7/3). After 24 h, the starting epoxide was totally consumed. The salts were then filtered and the product first eluted on a silica column using methanol as eluent. The fractions containing **1** were collected, evaporated and taken to dryness with P_2O_5 under vacuum. This crude mixture was then purified by flash chromatography on silica gel with a CH_2Cl_2/MeOH mixture (7/3) affording 2.9 g (20 mmol) of **1** as a colourless oil (91% yield). ^{1}H NMR (200 MHz, CD_3OD) δ 3.45 (1H, br. s), 3.52 (1H, m), 3.62 (1H, m), 3.70 (2H, m), 4.36 (2H, br. s). ^{13}C NMR (50 MHz, CD_3OD) δ 62.8 (CH), 64.0 (CH), 65.4 (CH_2), 72.4 (CH_2).

4.4. *Enzymatic synthesis of racemic diacetate* ***2***

In a screw cap tube were placed 1 g of **1** (6.8 mmol), 10 mL of dry THF and 20 mL of vinyl acetate. Then 1 g of PPL was added and the tube was incubated at 30°C, on a reciprocal shaker, working at 60 oscillations per minute. The reaction progress was followed by TLC (CH_2Cl_2/MeOH, 95/5). After two days of reaction, 500 mg of PPL were further added. After one week of reaction the lipase was filtered, the solvents evaporated and the product finally purified over silica (CH_2Cl_2/Et_2O, 80/20) affording 1.48 g (6.4 mmol) of (+/–)-**2** (94% yield) as a white solid, mp 44–45°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.12 (6H, s), 2.83 (1H, br. s), 3.73 (1H, ddd, $J = 7.8$, 4.9, 3.6 Hz), 3.97 (1H, m), 4.20 (2H, m), 4.30 (1H, dd, $J = 11.6$, 7.8 Hz), 4.40 (1H, dd, $J = 11.6$, 4.9 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.7 (CH_3), 20.8 (CH_3), 61.4 (CH), 63.8 (CH_2), 65.3 (CH_2), 69.1 (CH), 170.8 (CO), 171.1 (CO).

4.5. Preparative enzymatic resolution of (+/–)-1 and (+/–)-2

In a screw cap tube were placed 1 g (6.8 mmol) of **1** or 1 g (4.3 mmol) of **2**, 20 mL of vinyl acetate and 20 mL of benzene (**1**) or 40 ml of vinyl acetate (**2**). One gram of lipase was then added and the tube incubated at 30°C, on a reciprocal shaker, working at 60 oscillations per minute. The reaction progress was followed by chiral HPLC. When the desired e.e.s were obtained, the enzyme was filtered and diacetate **2** and triacetate **3** were purified by flash column chromatography over silica, using a 9/1 to 3/7 gradient of petroleum ether (low bp) and diethyl ether. See Table 2 for more details upon yields, e.e.s and specific rotations. Compound **3** (colourless oil): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.08 (3H, s), 2.11 (3H, s), 2.13 (3H, s), 3.87 (1H, ddd, $J=7.2$, 4.7, 4.7 Hz), 4.15 (2H, m), 4.31 (1H, dd, $J=7.2$, 4.7 Hz), 4.34 (1H, dd, $J=7.2$, 4.7 Hz), 5.22 (1H, ddd, $J=6.0$, 4.7, 4.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.7 (CH_3), 59.6 (CH), 62.3 (CH_2), 63.0 (CH_2), 70.0 (CH), 169.9 (CO), 170.4 (CO).

4.6. Chemical acetylation of diacetate (+)-2

In a 50 mL two necked round-bottomed flask equipped with a reflux condenser were placed 494 mg (2 mmol) of enzymatically generated (+)-**2** (e.e. 57%, $[\alpha]_D^{20}$ +7.8 (c = 1.50, $CHCl_3$)). Then 224 mg of acetic anhydride (2.2 mmol, 1.1 equiv.), 1 mL of triethylamine and 20 mL of anhydrous dichloromethane were added and the reaction mixture was brought to reflux under magnetic agitation. After overnight refluxing the solvent and triethylamine were first evaporated under reduced pressure and the crude residual mixture purified over silica using a 7/3 mixture of petroleum ether (low bp)/diethyl ether as eluent. 519 mg of triacetate **3** were thus recovered (1.9 mmol, 95% yield, $[\alpha]_D^{20}$ –7.2 (c = 2.89, $CHCl_3$)) as a colourless oil which partially solidified upon standing. Same spectral characteristics as reported above.

4.7. Chemical deacetylation of triacetate (–)-3

In a two necked 50 mL round-bottomed flask equipped with a magnetic stirrer were placed 450 mg (1.65 mmol) of triacetate (–)-**3**. Dry methanol (20 mL) was added and the flask placed under inert atmosphere (N_2). Then 3.6 mL of a 0.5 M NaOMe solution in methanol (1.8 mmol) were added and the reaction placed under magnetic agitation at 20°C. After 1 h of reaction, methanol was removed under reduced pressure and the crude residual mixture purified over silica using a 7/3 mixture of dichloromethane/methanol as eluent affording 219 mg of (+)-**1** (1.49 mmol, 90% yield, $[\alpha]_D^{20}$ +12.4 (c = 1.31, MeOH)) as a colourless oil. Same spectral characteristics as reported above.

4.8. Synthesis of syn-2-azido-1-hydroxy-3,4-(pentane-3,3-diyloxy)-butane 4

In a 50 mL round-bottomed flask were placed 200 mg (1.36 mmol) of (+)-**1**, 20 mL of freshly distillated 3-pentanone, 10 mg of *p*-toluenesulfonic acid and 1 g of molecular sieves. The reaction was then warmed at 80°C under magnetic agitation for 3 days. The reaction mixture was then filtered, 3-pentanone evaporated under reduced pressure and the crude residue purified over silica using a 9/1 to 7/3 CH_2Cl_2/MeOH gradient of elution affording 216 mg of acetal (+)-**4** (1.0 mmol, 76% yield, $[\alpha]_D^{20}$ +9.1 (c = 1.05, $CHCl_3$)) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (3H, t, $J=7.4$ Hz), 0.93 (3H, t, $J=7.3$ Hz), 1.64 (2H, q, $J=7.3$ Hz), 1.70 (2H, q, $J=7.4$ Hz), 2.86 (1H, br. s), 3.47 (1H, dd, $J=11.0$, 5.8 Hz), 3.6–3.8 (3H, m), 4.07 (1H, dd, $J=8.1$, 6.4 Hz),

4.22 (1H, ddd, $J=8.3$, 6.3, 6.3 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 8.1 (CH_3), 28.9 (CH_2), 29.5 (CH_2), 32.5 (CH_2), 64.6 (CH_2), 63.0 (CH), 66.7 (CH_2), 76.4 (CH), 113.9 (C).

4.9. *Synthesis of* syn-*2-azido-1-benzyloxy-3,4-(pentane-3,3-diyloxy)-butane* **5**

In a two necked 50 ml round-bottomed flask were placed under nitrogen 190 mg (0.88 mmol) of (+)-**4**, 10 ml of dry DMF, 23 mg of NaH (0.97 mmol, 1.1 equiv.) and 165 mg of benzyl bromide (0.97 mmol, 1.1 equiv.). The reaction mixture was left overnight under magnetic agitation at 20°C. The reaction was then diluted with 100 mL of water and extracted three times with 100 mL of diethyl ether. The mixed organic phases were washed twice with water (100 mL), dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude mixture thus obtained was purified over silica using a 9/1 to 7/3 gradient of petroleum ether (low bp)/diethyl ether affording 235 mg of (+)-**5** (0.77 mmol, 87% yield, $[\alpha]_D^{20}$ +9.7 (c = 0.85, $CHCl_3$)) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, $J=7.4$ Hz), 0.94 (3H, t, $J=7.3$ Hz), 1.64 (2H, q, $J=7.4$ Hz), 1.69 (2H, q, $J=7.3$ Hz), 3.50–3.61 (3H, m), 3.75 (1H, dd, $J=7.9$, 7.9 Hz), 4.01 (1H, dd, $J=8.1$, 6.4 Hz), 4.16 (1H, ddd, $J=7.5$, 6.0, 6.0 Hz), 4.54 (2H, s), 7.33 (5H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ 8.1 (CH_3), 29.1 (CH_2), 29.6 (CH_2), 62.5 (CH), 66.8 (CH_2), 69.8 (CH_2), 73.6 (CH_2), 76.3 (CH), 113.6 (C), 127.7 (CH), 127.9 (CH), 128.5 (CH), 137.5 (C).

4.10. *Synthesis of* syn-*2-azido-1-benzyloxy-3,4-diacetoxy-butane* **6**

In a 50 mL round-bottomed flask equipped with a reflux condenser were placed 200 mg of (+)-**5** (0.85 mmol), 20 mL of MeOH and 200 mg of Amberlyst 15. The reaction was then brought to reflux for 3 days. After total deprotection of the acetal (TLC analysis) the reaction mixture was filtered and evaporated to dryness under reduced pressure. The crude residue thus obtained was then acetylated overnight with acetic anhydride (191 mg, 1.87 mmol, 2.2 equiv.) in 10 mL of dry dichloromethane containing 0.5 mL of triethylamine under refluxing conditions. Following completion of the reaction, the mixture was evaporated to dryness under reduced pressure and the crude residue purified over silica using a 1/1 mixture of petroleum ether (low bp)/diethyl ether affording 247 mg of (+)-**6** (0.77 mmol, 90% yield, two steps, $[\alpha]_D^{20}$ +6.7 (c = 2.81, $CHCl_3$)) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.03 (3H, s), 2.05 (3H, s), 3.60 (2H, m), 3.70 (1H, ddd, $J=6.8$, 4.4, 4.4 Hz), 4.12 (1H, dd, $J=11.9$, 6.2 Hz), 4.32 (1H, dd, $J=11.9$, 4.4 Hz), 4.54 (2H, s), 5.24 (1H, ddd, $J=6.1$, 4.5, 4.5 Hz), 7.27–7.36 (5H, m). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.7 (CH_3), 60.5 (CH), 62.6 (CH_2), 69.3 (CH_2), 70.3 (CH), 73.6 (CH_2), 127.8 (CH), 128.0 (CH), 128.5 (CH), 137.3 (C), 169.9 (CO), 170.4 (CO).

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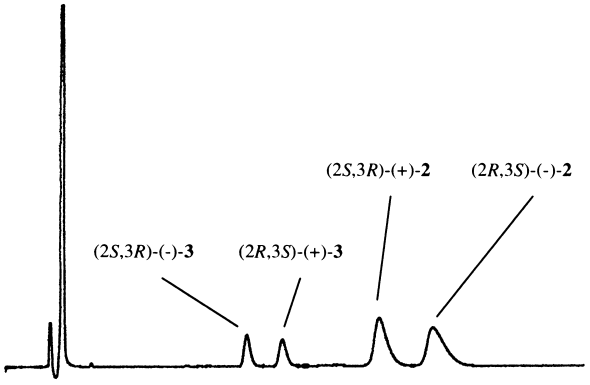
8. Chiral HPLC chromatogram trace of (+/–)-**2** and (+/–)-**3**. Column Chiralcel OD-H, eluent 95/5 hexane/isopropanol delivered at 0.8 mL/min, UV detection at 214 nm. Retention times: (2*S*,3*R*)-(–)-**3** 20.4 min, (2*R*,3*S*)-(+)-**3** 23.4 min, (2*S*,3*R*)-(+)-**2** 31.5 min, (2*R*,3*S*)-(–)-**2** 36.0 min.



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10. The e.e. of the chemically synthesised **6** was determined based on the comparison of its specific rotation with that of the same compound having an e.e. of 88%, $[\alpha]_D^{20}$ +9.73 (c = 0.781, $CHCl_3$).[4]