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Chemoenzymatic synthesis of the enantiomers of desoxymuscarine

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Abstract

Two different chemoenzymatic approaches allowed the preparation of the enantiomers of desoxymuscarine **5**, a muscarinic receptor agonist. Transesterification of racemic 5-hexen-2-ol **7** with vinyl butyrate under the catalysis of *Candida antarctica* B lipase was the key step for the preparation of (−)-**5** (2*R*,5*R*). On the other hand, lipase PS-catalyzed hydrolysis of iodo butyrate (±)-**14** was utilized to obtain (+)-**5** (2*S*,5*S*). Both enantiomers were prepared with enantiomeric excesses higher than 98%.

1. Introduction

Whenever target chiral compounds with high enantiomeric purity are needed, enzyme-catalyzed processes may represent an alternative (or complementary) approach to other strategies, i.e. asymmetric synthesis and conventional resolution methods. Among the different classes of enzymes, hydrolytic enzymes are by far the most versatile biocatalysts to afford kinetic resolutions of racemates and asymmetrizations of *meso* compounds, thus providing enantiomerically pure synthetic intermediates amenable to further functionalization.[1–4]

Stereochemistry is an essential feature of the molecular structure of biologically active chiral compounds, since opposite absolute configurations at pharmacophoric groups are usually responsible for differences in the biological response, mainly in terms of potency, toxicity and receptor subtype selectivity.[5] Therefore, the stereoisomeric composition of drug substances is currently receiving considerable attention owing to its pharmacological as well as industrial and regulatory implications.[6,7]

As a part of our investigation of the relantionship between structure and activity/selectivity of cholinergic ligands, we synthesized and tested a series of analogs of natural muscarine (+)-**1**, i.e.

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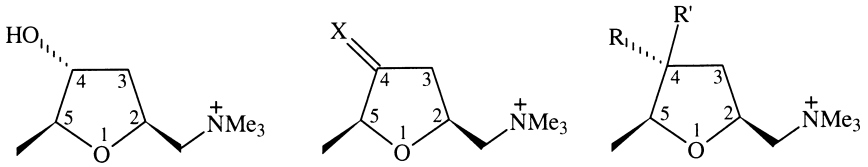


(+)-**1** (2*S*,4*R*,5*S*) (−)-**2** (2*S*,5*S*) : X = O
(−)-**3** (2*S*,5*S*) : X = CH_2
(+)-**4** (2*S*,5*S*) : R = R' = F
(+)-**5** (2*S*,5*S*) : R = R' = H

Fig. 1.

the four stereoisomers of muscarone,[8] methylenemuscarone,[9] and difluoromuscarine.[10] The absolute configurations of the most potent stereoisomers of muscarone (−)-**2**, methylenemuscarone (−)-**3**, and difluoromuscarine (+)-**4** are depicted in Fig. 1.

With the aim of studying further the nature of the interaction involving the binding of the hydroxy group of muscarine to the complementary receptor subsite ('muscarinic subsite'),[8–11] we planned the synthesis of the two enantiomers of desoxymuscarine **5**[12] (Fig. 1), a relatively potent muscarinic agonist with a tenfold selectivity for M_3 (ileal) versus M_2 (cardiac) muscarinic receptor subtype.[13]

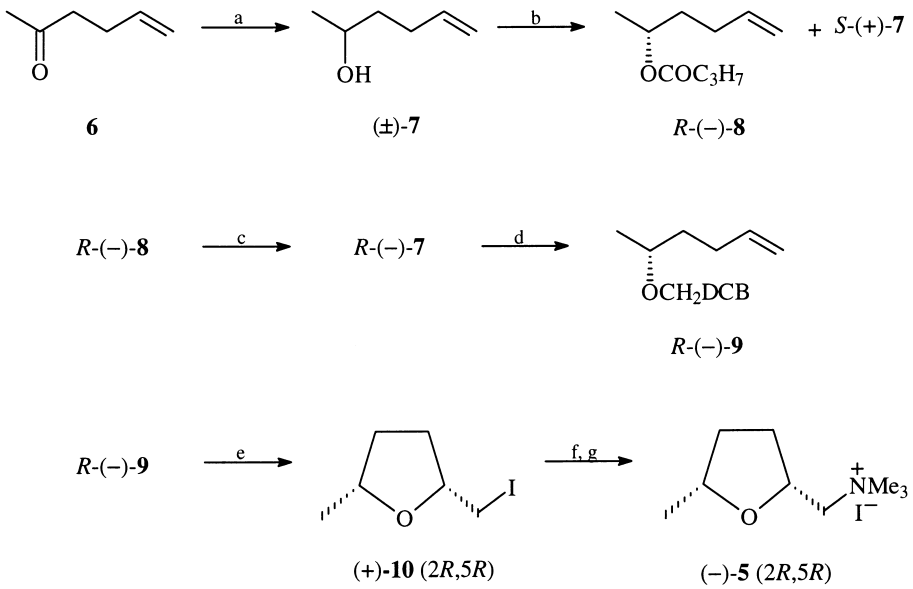
In previous papers we reported the synthesis of the enantiomers of compounds provided with a variety of biological activities, by taking advantage of enantioselective enzyme-catalyzed reactions.[14–18] As a part of an ongoing program, we describe the application of the chemoenzymatic strategy to the synthesis of both enantiomers of **5**. Furthermore, our approach allowed the assignment of the absolute configuration of (+)-**5** and (−)-**5**.

2. Results and discussion

We tackled the synthesis of the two enantiomers of desoxymuscarine by checking the feasibility of the two chemoenzymatic sequences reported in Schemes 1 and 2.

In the first approach, depicted in Scheme 1, we submitted racemic 5-hexen-2-ol **7** to a transesterification reaction with vinyl butyrate in petroleum ether under the catalysis of *Candida antarctica* B lipase. The substrate was in turn easily obtained by reducing an ether solution of commercially available 5-hexen-2-one (**6**) with lithium aluminum hydride. The enzymatic reaction was quite enantioselective (enantiomeric ratio,[1] E ≥300) with an enantiopreference for the *R* isomer, typical of *Candida antarctica* B lipase.[19] This result relies on the assignment of the absolute configuration to the enantiomers of **7**.[20,21] The degree of conversion and the enantiomeric excess (e.e.) of the produced butyrate were evaluated by chiral GLC analysis (see Experimental section). For preparative purposes, we stopped the reaction at 42% conversion and obtained *R*-(−)-**8**, after purification by column chromatography. The e.e. value of *R*-(−)-**8** was higher than 99%. Butyrate *R*-(−)-**8** was then hydrolyzed, in the presence of the same enzyme, to enantiomerically pure *R*-(−)-**7**. Treatment of *R*-(−)-**7** with sodium hydride and 2,6-dichlorobenzyl (DCB) bromide afforded the corresponding ether *R*-(−)-**9**, which was submitted to a iodocyclization reaction following the procedure described by Bartlett.[22] The electronic and steric properties of the substituent at oxygen control the stereochemical outcome of the cyclization,[22,23] to yield (2*R*,5*R*)-*cis*-2-iodomethyl-5-methyltetrahydrofuran (+)-**10** in a highly diastereoselective process. On the other hand, the corresponding benzyl ether gave rise to (+)-**10** contaminated by a substantial amount (35%) of the *trans*-(2*S*,5*R*)-isomer.

The synthetic sequence was then carried out to completion by reacting iodo derivative (+)-**10** with dimethylamine at 100°C followed by treatment with iodomethane, to give (2*R*,5*R*)-desoxymuscarine (−)-



a: $LiAlH_4$, ether; b: *Candida antarctica* B lipase, vinyl butyrate, petroleum ether; c: *Candida antarctica* B lipase, buffer, pH 7; d: NaH, 2,6-$Cl_2C_6H_3CH_2Br$; e: I_2, CH_3CN; f: $NHMe_2$; g: CH_3I.

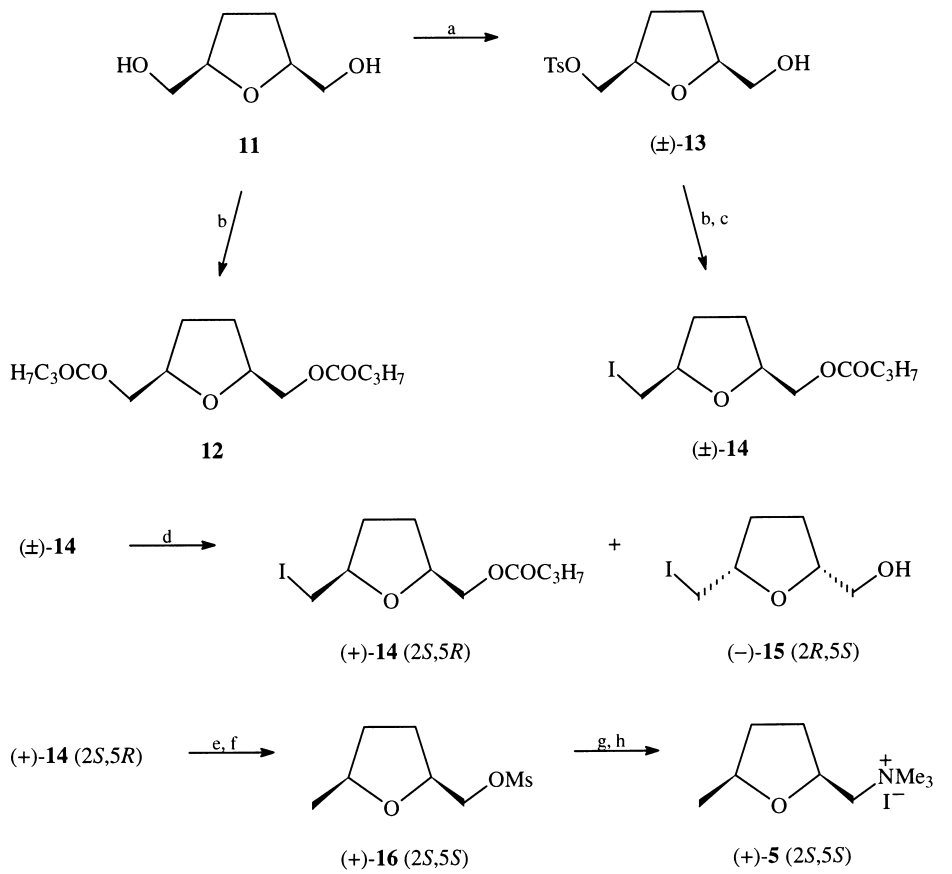
Scheme 1.

5. The absolute configuration at the two stereocenters was the result of both the *R* enantiopreference of the initial enzymatic transesterification and the stereoselective iodoetherification reaction. The relationship between the sign of specific rotation of (−)-**5** and absolute (2*R*,5*R*)-configurations is further confirmed by comparison to muscarine stereoisomers[24] and related analogs.[10]

It is worth noting that the reactions listed in Scheme 1 may be conveniently utilized also to synthesize the dextrorotatory enantiomer of desoxymuscarine. By extending transesterification slightly beyond 50% conversion, *S*-(+)-**7** was obtained with an e.e. value higher than 99%. Hence, the sequence illustrated above for the preparation of (−)-**5** was applied to accomplish the synthesis of enantiomerically pure (+)-**5**.

With the aim of reaching our synthetic goal in a more expeditious way, in a parallel study we investigated an alternative chemoenzymatic approach to the enantiomers of **5**, by selecting prochiral diester **12** as a substrate (Scheme 2). Derivative **12** already has the desired *cis* stereochemistry. Based on published results,[25] we tried at first pig liver esterase (PLE) induced asymmetrization of *meso* diester **12**, in turn prepared from *cis*-2,5-bis(hydroxymethyl)tetrahydrofuran **11**.[26] In our hands this attempt was unfruitful, owing to the poor selectivity of the PLE-catalyzed hydrolysis of **12**. Moreover, chiral GLC analysis did not allow separation of the enantiomers of the produced monoester, thus preventing any direct evaluation of the enantiomeric excess of the product. The e.e. value of such a compound was determined after transformation into iodo butyrate **14** (Scheme 2). The use of other biocatalysts, such as *Candida rugosa* lipase, was similarly unsuccessful.

Conversely, lipase from *Pseudomonas cepacia* (lipase PS) catalyzed with acceptable selectivity (E=14) the hydrolysis of butyrate (±)-**14**. The degree of conversion as well as the e.e. of the residual substrate and the produced alcohol were evaluated by chiral GLC (see Experimental section). By extending the hydrolysis at 65% conversion, iodo butyrate (+)-**14** (2*S*,5*R*) was isolated with an e.e. value higher than 98%. Lithium aluminum hydride reduction of both the iodomethyl group and the ester function gave



a: TsCl, Py; b: C_3H_7COCl, NEt_3; c: EtCOMe, NaI; d: Lipase PS, buffer pH 7; e: $LiAlH_4$; f: MsCl, NEt_3; f: $NHMe_2$; d: CH_3I

Scheme 2.

the volatile (2*S*,5*S*)-2-hydroxymethyl-5-methyltetrahydrofuran, which was readily converted into the corresponding mesylate (+)-**16**. The preparation of desoxymuscarine (+)-**5** was then accomplished by standard reactions. Absolute values of the specific rotation of the two desoxymuscarine enantiomers, obtained through the two different routes, were superimposable.

In summary, in this paper the results of two independent chemoenzymatic syntheses of the enantiomers of desoxymuscarine, a muscarinic receptor agonist, are reported and discussed. The key step of both the described sequences is an efficient enzyme-catalyzed kinetic resolution. The first strategy was more convenient, on account of the high enantioselectivity of its biocatalyzed transformation coupled to a remarkable diastereoselective iodocyclization process. Furthermore, this approach allowed the assignment of the absolute configuration to the enantiomers of (−)- and (+)-**5**. Based on the kind of reactions performed and the values of the polarimetric data on final compounds, we believe that each enantiomer has completely retained the enantiomeric purity induced by the enzyme-catalyzed transformations. The results of the pharmacological investigation of the two enantiomers of desoxymuscarine will be reported in due course.

3. Experimental section

Immobilized *Candida antarctica* B lipase (Novozym 435) was purchased from Novo and lipase from *Pseudomonas cepacia* (lipase PS) from Amano. Organic solvents were reagent grade. ^{1}H NMR spectra were recorded at 200 MHz in $CDCl_3$ (unless otherwise specified) solutions; chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. Chiral GLC analyses were conducted on a gas chromatograph equipped with a CP-Cyclodextrin-2,3,6-M-19 column (50 m, 0.25 mm). H_2 was used as the carrier gas at a flow rate of 1.2 mL/min; experimental conditions are reported in the appropriate paragraph. Boiling points of liquid compounds were determined either by conventional or Kugelrohr distillations. Rotary power determinations were carried out with a Perkin–Elmer 241 polarimeter coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microanalyses (C, H, N) of new compounds agreed with the theoretical value ±0.4%.

3.1. Synthesis of (RS)-5-hexen-2-ol (±)-7

To a suspension of lithium aluminum hydride (4.0 g) in anhydrous ether (300 mL) was added dropwise an ethereal solution (50 mL) of 5-hexen-2-one (14.0 g, 0.143 mol) at 0°C under stirring. The reaction mixture was stirred at room temperature for an additional 3 h, then water (25 mL) was cautiously added at 0°C and inorganic salts were separated by suction filtration. The remaining solution, dried over anhydrous sodium sulphate, was concentrated at atmospheric pressure, then distilled under vacuum to yield 12.73 g (89%) of the desired secondary alcohol. (*RS*)-5-Hexen-2-ol (±)-**7**: bp 83–84°C/125 mmHg (lit.[20] 138–140°C); ^{1}H NMR 1.19 (d, 3, CH_3; J=6.6); 1.47–1.58 (m, 3, H-3 and OH); 2.15 (m, 2, H-4); 3.82 (m, 1, H-2); 5.01 (m, 2, H-6); 5.83 (m, 1, H-5).

3.2. Enzymatic transesterification of (±)-7

A solution of 10.69 g (0.107 mol) of (±)-**7** in petroleum ether (300 mL) was treated with vinyl butyrate (15 mL, 0.118 mol) in the presence of *Candida antarctica* B lipase (70 mg). The reaction was stopped after about 3 h (42% conversion), the enzyme was filtered off and the solution was concentrated at atmospheric pressure. The residue was submitted to silica gel column chromatography (eluant: petroleum ether:diethyl ether=3:1) to afford 6.97 g (0.041 mol) of *R*-(−)-**8**[27] and 5.41 g (0.054 mol) of *S*-(+)-**7**.

(*R*)-5-Hexen-2-ol butyrate (−)-**8**: bp 100–110°C/22 mmHg; R_f 0.85 (petroleum ether:diethyl ether=3:1); $[\alpha]^{20}_D$ −9.11 (c 1.032, $CHCl_3$); e.e.>99%; ^{1}H NMR 0.94 (t, 3, CH_2CH_3; J=7.5); 1.20 (d, 3, CH_3; J=6.6); 1.42–1.80 (m, 4, H-3 and CH_2CH_3); 2.07 (m, 2, H-4); 2.25 (t, 2, $OCOCH_2$; J=7.2); 4.82–5.09 (m, 3, H-2 and H-6); 5.79 (m, 1, H-5). Anal. calcd for $C_{10}H_{18}O_2$: C, 70.53; H, 10.66. Found: C, 70.44; H, 10.87.

(*S*)-5-Hexen-2-ol (+)-**7**: R_f 0.21 (petroleum ether:diethyl ether=3:1); $[\alpha]^{25}_D$ +8.97 (c 1.026, Et_2O); e.e.=73% [after conversion into the corresponding *S*-(+)-**8**].

Capillary GLC analysis. Temperature program: 80°C (1 min.), then from 80°C to 95°C (0.5°C/min.). Injection volume: 10 μl. Relative retention times (min): *R*-(−)- and *S*-(+)-**7**, 6.85; *S*-(+)-**8**, 20.87, *R*-(−)-**8**, 21.72.

The same enzymatic transesterification was carried out on 2.14 g (21.4 mmol) of (±)-**7**. The reaction was stopped at 55% conversion and, after the above described work up, 0.79 g (37% yield) of *S*-(+)-**7** were obtained.

(*S*)-5-Hexen-2-ol (+)-**7**: $[\alpha]^{25}_D$ +17.28 (c 1.054, Et_2O) [lit.[20] $[\alpha]^{25}_D$ +21.8 (Et_2O)]; e.e.>99% [after conversion into the corresponding *S*-(+)-**8**]. *S*-(+)-**8**: $[\alpha]^{20}_D$ +9.52 (c 1.040, $CHCl_3$); e.e.>99%.

*3.3. Synthesis of (*R*)-5-hexen-2-ol (−)-7*

5.0 g (29.4 mmol) of (*R*)-(−)-**8** was dissolved in 150 mL of 0.1 M potassium phosphate buffer (pH 8) in the presence of *Candida antarctica* B lipase powder (100 mg). The hydrolysis was complete in about 4 h. The reaction mixture was extracted with ether (4×100 mL), the organic phase was dried over anhydrous sodium sulphate and the solvent was eliminated at atmospheric pressure. The residue was distilled at reduced pressure to give 2.23 g (76%) of (−)-**7**. (*R*)-5-Hexen-2-ol (−)-**7**: $[\alpha]^{25}_D$ −17.62 (c 1.016, Et_2O) [lit.[20] $[\alpha]^{25}_D$ −13.7 (Et_2O)].

*3.4. Synthesis of (*R*)-5-hexen-2-ol-2,6-dichlorobenzyl ether (−)-9*

To a cooled (−10°C) stirred suspension of sodium hydride (1.3 g of a 50% dispersion in mineral oil) in anhydrous THF (25 mL) was added 0.776 g (2.1 mmol) of tetra-n-butylammonium iodide under nitrogen. A solution of 2,6-dichlorobenzyl bromide (5.03 g, 21 mmol) in THF (20 mL) was then added dropwise. After 15 min, a solution of 2.10 g (21 mmol) of (*S*)-(−)-**7** in THF (5 mL) was added dropwise at −10°C. The reaction mixture was maintained under stirring at 0°C for about 5 h, then water (20 mL) was cautiously added and the aqueous phase was extracted with ether (4×20 mL). After the usual work up, the residue was column chromatographed (eluant: petroleum ether/5% diethyl ether) to yield 4.68 g (86%) of the desired compound. (*R*)-5-Hexen-2-ol-2,6-dichlorobenzyl ether (−)-**9**: bp 140–145°C/7 mmHg (see literature[22] for racemate); R_f 0.52 (petroleum ether/5% diethyl ether); $[\alpha]^{20}_D$ −35.69 (c 1.048, $CHCl_3$); ^{1}H NMR 1.25 (d, 3, CH_3; J=6.3); 1.50 (m, 1, H-3); 1.68 (m, 1, H-3′); 2.16 (m, 2, H-4); 3.57 (m, 1, H-2); 4.70 and 4.80 (d, 2, CH_2Ar; J=10.1); 4.95 (m, 2, H-6); 5.78 (m, 1, H-5); 7.09–7.35 (m, 3, arom.).

*3.5. Synthesis of (2*R*,5*R*)-2-iodomethyl-5-methyltetrahydrofuran (+)-10*

To a cooled (0°C) stirred solution of 4.50 g (17.37 mmol) of (−)-**9** in 30 mL anhydrous acetonitrile, a solution of iodine (6.0 g, 23.64 mmoL) in 80 mL dry acetonitrile was added dropwise under vigorous stirring. The progress of the reaction was monitored by TLC. After completion of the reaction (about 5 h at 0°C), 50 mL of a saturated solution of $Na_2S_2O_3$ was added at room temperature. Acetonitrile was evaporated under vacuum and the aqueous layer was extracted with ether (3×30 mL). After the usual work up, the residue was chromatographed on silica gel (eluant: petroleum ether:diethyl ether=9:1) to yield 2.55 g (65%) of iododerivative (+)-**10**. (2*R*,5*R*)-2-Iodomethyl-5-methyltetrahydrofuran (+)-**10**: bp 115–120°C/22 mmHg (see literature[22,23] for racemate); R_f 0.55 (petroleum ether:diethyl ether=9:1); $[\alpha]^{20}_D$ +11.24 (c 1.014, $CHCl_3$); ^{1}H NMR 1.26 (d, 3, CH_3; J=6.2); 1.42–1.82 (m, 2, H-4); 1.88–2.17 (m, 2, H-3); 3.17 and 3.24 (dd, 2, CH_2I; J=6.8, 9.8 and 4.8); 3.96 (m, 1, H-5); 4.07 (m, 1, H-2).

*3.6. Synthesis of (2*R*,5*R*)-2-[(dimethylamino)methyl]-5-methyltetrahydrofuran methiodide (−)-5*

A. A sealed metal container, filled with a solution of (+)-**10** (2.40 g, 10.62 mmol) in methanol (10 mL) and a tenfold excess of anhydrous dimethylamine, was heated at 100°C for 5 h. The container was cooled at 0°C, the solution was acified with 3 N HCl, and the volatiles were evaporated under vacuum. The residual aqueous phase was treated with ether (4×10 mL), made alkaline by portionwise addition

of solid K_2CO_3 and extracted with dichloromethane (4×10 mL). The pooled organic extracts were dried over anhydrous sodium sulphate, the solvent was evaporated under vacuum at 10°C, and the residue was Kugelrohr distilled to yield 0.94 g (62%) of tertiary base. (2*R*,5*R*)-2-[(Dimethylamino)methyl]-5-methyltetrahydrofuran: bp 130–135°C/140 mmHg (lit.[12] 86–89°C/55 mmHg for racemate); R_f 0.46 (chloroform:methanol=4:1); $[\alpha]^{20}_D$ −18.96 (c 1.092, $CHCl_3$); ^{1}H NMR 1.23 (d, 3, CH_3; J=6.0); 1.30–1.62 (m, 2, H-4); 1.83–2.08 (m, 2, H-3); 2.26 (s, 6, NMe_2); 2.29 and 2.44 (dd, 2, CH_2NMe_2; J=4.9, 12.5 and 7.5); 3.86–4.03 (m, 2, H-2 and H-5).

B. An ether solution of the tertiary amine was reacted with a fivefold excess of methyl iodide at room temperature. The salt precipited quantitatively and was crystallized from 2-propanol:ether (1:1). (2*R*,5*R*)-2-[(Dimethylamino)methyl]-5-methyltetrahydrofuran methiodide (−)-**5**: mp 141–142°C, dec., colorless needles from 2-propanol–ether (lit.[12] 154–155°C for racemate); $[\alpha]^{20}_D$ −28.16 (c 1.110, MeOH); ^{1}H NMR (D_2O) 1.24 (d, 3, CH_3; J=6.2); 1.42–1.78 (m, 2, H-4); 1.93–2.27 (m, 2, H-3); 3.18 (s, 9, NMe_3); 3.34–3.57 (m, 2, CH_2NMe_3); 4.16 (m, 1, H-5); 4.43 (m, 1, H-2). Anal. calcd for $C_9H_{20}INO$: C, 37.91; H, 7.07; N, 4.91. Found: C, 38.15; H, 7.19; N, 4.75.

3.7. Synthesis of cis*-2-(hydroxymethyl)-5-iodomethyltetrahydrofuran butyrate (±)-**14***

A. A 500 mL Erlenmeyer flask was charged with **11**[26] (8.0 g, 61 mmol), dichloromethane (250 mL), tetra-n-butylammonium hydrogen sulphate (2.55 g, 7.51 mmol) and 18 mL of a 15% aqueous solution of NaOH. To the suspension a solution of tosyl chloride (12.70 g, 67 mmol) in dichloromethane (75 mL) was added under vigorous stirring. The reaction mixture was stirred for 4 days at room temperature, then the organic phase was separated and sequentially washed with dilute HCl (100 mL) and a saturated solution of $NaHCO_3$ (100 mL). After the usual work up, the desired monotosylate (7.63 g, 44% yield) was obtained as a light yellow viscous oil by silica gel column chromatography (eluant: petroleum ether:ethyl acetate=1:4). *cis*-2-(Hydroxymethyl)-5-(tosyloxymethyl)tetrahydrofuran (±)-**13**: R_f 0.41 (cyclohexane:ethyl acetate=1:4); ^{1}H NMR 1.68–2.12 (m, 5, H-3, H-4 and OH); 2.44 (s, 3, CH_3); 3.42 and 3.65 (dd, 2, CH_2OH; J=5.7, 12.2 and 3.1); 3.93–4.21 (m, 4, H-2, H-5 and CH_2OSO_2); 7.35 and 7.80 (d, 4, arom.; J=8.2).

B. To a solution of (±)-**13** (7.63 g, 26.67 mmol) and triethylamine (12.20 mL, 84 mmol) in anhydrous dichloromethane (180 mL) was added dropwise a dichloromethane solution (40 mL) of butyryl chloride (8.40 mL, 80 mmol) at 0°C. The disappearance of the starting material was monitored by TLC (eluant: petroleum ether:ethyl acetate=1:4). The reaction mixture was acidified by addition of dilute HCl and the organic phase was dried and concentrated. The residue was purified by silica gel column chromatography (eluant: petroleum ether:ethyl acetate=7:3) to give 8.08 g (85%) of the expected diester as a light yellow viscous oil. *cis*-2-(Butyryloxymethyl)-5-(tosyloxymethyl)tetrahydrofuran: R_f 0.37 (cyclohexane:ethyl acetate=7:3); ^{1}H NMR 0.94 (t, 3, CH_2CH_3; J=7.5); 1.66 (m, 2, CH_2CH_3); 1.68–2.08 (m, 4, H-3 and H-4); 2.29 (t, 2, $OCOCH_2$; J=7.4); 2.45 (s, 3, CH_3); 3.83–4.18 (m, 6, H-2, H-5, CH_2OCO and CH_2OSO_2); 7.34 and 7.78 (d, 4, arom.; J=8.2).

C. A 250 mL Erlenmeyer flask, charged with *cis*-2-(butyryloxymethyl)-5-(tosyloxymethyl)tetrahydrofuran (7.95 g, 22.33 mmoL), 180 mL ethyl methyl ketone and NaI (7.0 g, 46.70 mmoL), was heated at reflux overnight under nitrogen. After cooling at room temperature and addition of 75 mL of a saturated solution of $Na_2S_2O_3$, the volatiles were evaporated under vacuum and the aqueous layer was extracted with dichloromethane (3×50 mL). After the usual work up, the desired compound (5,50 g, 79% yield) was purified by silica gel column chromatograpy (eluant: petroleum ether/15% ethyl acetate). *cis*-2-(Hydroxymethyl)-5-iodomethyltetrahydrofuran butyrate (±)-**14**: bp 155–160°C/2 mmHg; R_f 0.47 (cyclohexane:ethyl acetate=4:1); ^{1}H NMR 0.94 (t, 3, CH_2CH_3; J=7.5); 1.66 (m, 2, CH_2CH_3); 1.68–1.83

(m, 2, H-3); 1.90–2.21 (m, 2, H-4); 2.32 (t, 2, $OCOCH_2$; J=7.3); 3.17 and 3.26 (dd, 2, CH_2I; J=6.9, 9.7 and 4.7); 3.95–4.08 (m, 2, H-2 and H-5); 4.10–4.28 (m, 2, CH_2OCO). Anal. calcd for $C_{10}H_{17}IO_3$: C, 38.48; H, 5.49. Found: C, 38.65; H, 5.12.

*3.8. Enzymatic hydrolysis of (±)-**14***

A 500 mL Erlenmeyer flask was charged with (±)-**14** (8.80 g, 28.20 mmol), lipase PS (200 mg), 0.1 M potassium phosphate buffer, pH 7 (200 mL) and acetone (30 mL). The mixture was stirred at room temperature for about 6 h (65% conversion), then filtered and extracted with ethyl acetate (3×100 mL). The pooled organic phases were dried over anhydrous sodium sulphate and the solvent was evaporated at reduced pressure.The residue was submitted to silica gel column chromatography (eluant: petroleum ether:ethyl acetate=4:1) to afford 2.68 g (8.59 mmol) of (+)-**14** and 3.60 (14.88 mmol) of (−)-**15**.

(2*S*,5*R*)-(+)-**14**: $[\alpha]^{20}_D$ +32.61 (c 1.156, $CHCl_3$); e.e.>98%.

(2*R*,5*S*)-2-(Hydroxymethyl)-5-iodomethyltetrahydrofuran (−)-**15**: (see literature[28] for racemate) R_f 0.33 (cyclohexane:ethyl acetate 7:3); $[\alpha]^{20}_D$ −8.94 (c 1.044, $CHCl_3$); e.e.=53%; ^{1}H NMR 1.68–1.88 (m, 2, H-3); 1.90–2.18 (m, 3, H-4 and OH); 3.30 (d, 2, CH_2I); 3.53 and 3.73 (dd, 2, CH_2OH; J=5.1, 11.6 and 3.0); 3.90 (m, 1, H-5); 4.08 (m, 1, H-2).

Capillary GLC analysis. Temperature program: 150°C (80 min.). Injection volume: 10 μl. Relative retention times (min): (−)-**15**, 20.57; (+)-**15**, 20.90; (−)-**14**, 63.04; (−)-**14**, 63.65.

*3.9. Synthesis of (2S,5S)-2-[(dimethylamino)methyl]-5-methyltetrahydrofuran methiodide (+)-**5***

A. To a suspension of lithium aluminum hydride (1.80 g) in anhydrous ether (170 mL) was added dropwise an ethereal solution (20 mL) of (+)-**14** (2.16 g, 6.92 mmol) at 0°C under stirring. The reaction mixture was refluxed for an additional 2 h, then water (10 mL) was cautiously added at 0°C and inorganic salts were separated by suction filtration. To the remaining solution, dried over anhydrous sodium sulphate, was added triethylamine (4.82 mL, 34.6 mmol). A solution of methanesulfonyl chloride (2.42 mL, 31.14 mmol) in dry ether (10 mL) was then added dropwise at 0°C, and the reaction was stirred for about 20 h at room temperature. After addition of 3 N HCl (50 mL), the organic layer was separated and the aqueous phase was extracted with dichloromethane (3×30 mL). After the usual work up, the residue was submitted to silica gel column chromatography (eluant: petroleum ether:ethyl acetate=3:2) to afford 0.835 g (62% overall yield) of the expected mesylate. (2*S*,5*S*)-2-(Hydroxymethyl)-5-methyltetrahydrofuran mesylate (+)-**16**: bp 125–130°C/4 mmHg; R_f 0.37 (cyclohexane:ethyl acetate=3:2); $[\alpha]^{20}_D$ +21.90 (c 1.0, $CHCl_3$); ^{1}H NMR 1.25 (d, 3, CH_3; J=5.9); 1.38–1.59 (m, 1, H-3); 1.65–1.84 (m, 1, H-3′); 1.90–2.12 (m, 2, H-4); 3.07 (s, 3, OSO_2CH_3); 3.96–4.32 (m, 4, H-2, H-5 and CH_2OSO_2). Anal. calcd for $C_7H_{14}O_4S$: C, 43.28; H, 7.26. Found: C, 43.54; H, 7.11.

B. A sealed metal container, filled with a solution of (+)-**16** (0.80 g, 4.12 mmol) in methanol (5 mL) and a tenfold excess anhydrous dimethylamine, was heated at 100°C for 5 h. Following the experimental protocol described above for the preparation of the levorotatory enantiomer, 0.41 g (70% yield) of dextorotatory tertiary base were isolated. (2*S*,5*S*)-2-[(Dimethylamino)methyl]-5-methyltetrahydrofuran: $[\alpha]^{20}_D$ +18.52 (c 1.188, $CHCl_3$).

C. Quaternary ammonium salt (2*S*,5*S*)-(+)-**5** was prepared with the procedure described for (−)-**5**. (+)-**5**: mp 141–142°C, dec. $[\alpha]^{20}_D$ +28.06 (c 1.110, MeOH). Anal. calcd for $C_9H_{20}INO$: C, 37.91; H, 7.07; N, 4.91. Found: C, 38.09; H, 7.27; N, 4.88.

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