Enzymatic Resolution of Sterically Demanding Bicyclo[3.2.0]heptanes: Evidence for a Novel Hydrolase in Crude Porcine Pancreatic Lipase and the Advantages of using Organic Media for Some of the Biotransformations

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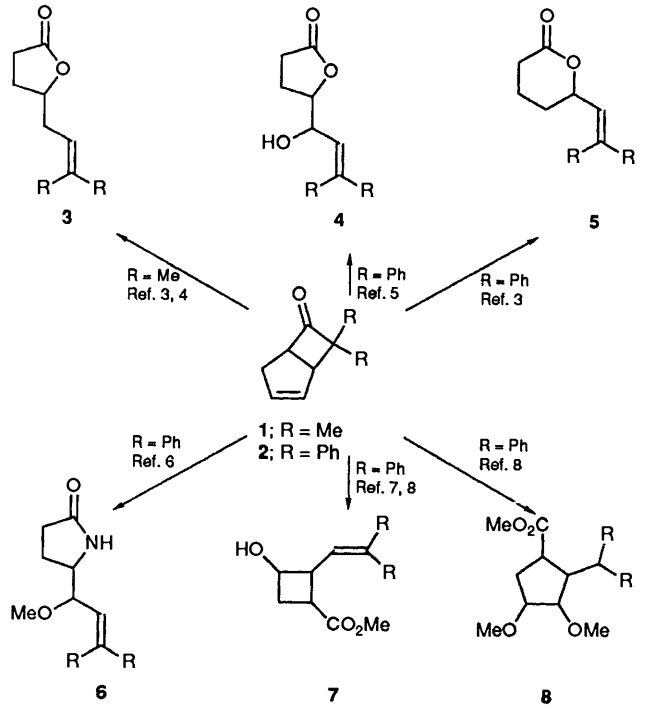
Sterically demanding 7,7-dimethylbicyclo[3.2.0]hept-2-en-6-one **1** was enzymatically resolved *via* the *exo*-acetate **11a** using crude porcine pancreatic lipase. By employing different fractions of hydrolases from the crude enzyme, evidence was obtained that an enzymatic 'impurity' was responsible for the highly selective reaction ($E > 300$). Alternatively, 7,7-dimethyl and 7,7-diphenyl derivatives **1** and **2** were equally well resolved, *via* bromohydrins **12b** and **13b**, by lipases from *Pseudomonas cepacia* and *Candida cylindracea* but only when acylations were conducted in organic media.

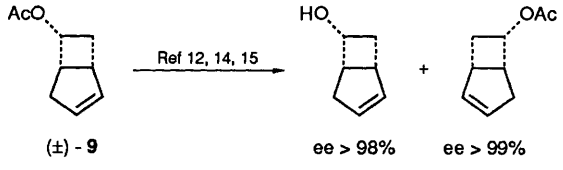
Bicyclo[3.2.0]heptan-6-one and 7,7-disubstituted derivatives thereof have been shown to be easily accessible intermediates for the synthesis of numerous bioactive compounds such as pheromones or leukotrienes.[1,2] Photochemically induced retro-[2 + 2]-addition of hydroxy- or amino-functionalized bicyclo-[3.2.0]heptan-6-ones led to 5- and 6-membered-ring lactones[3–5] **3–5** or lactams[6] **6**. The analogous photoinduced rearrangement of epoxy derivatives gave access to cyclobutane carboxylates **7** *via* bicyclic lactones.[7,8] On the other hand, cyclopentane derivatives **8** were synthesized by nucleophilic opening of highly strained tricycloheptane building blocks.[8] These reactions are summarized in Scheme 1.



Scheme 1 Transformation of bicyclo[3.2.0]heptenones **1** and **2**

In order to apply this wealth of synthetically useful reactions to the preparation of optically active compounds, easy access to sufficient quantities of both (7,7-disubstituted) bicyclo-[3.2.0]heptenone enantiomers is necessary. Hence, by using redox enzymes, ketone (±)-**1** was resolved either using whole fungal cells[9,10] or isolated dehydrogenase enzymes.[9–11]

Despite the high optical purity achieved, both methods were inapplicable to large-scale resolution. Use of whole cells of *Mortierella ramanniana* led to mixtures of diastereoisomeric alcohols which could not be separated easily by chromatography on a large scale[12] and the employment of isolated hydroxysteroid dehydrogenase made cofactor-recycling necessary. In contrast, enzymatic transformations using hydrolases do not have the above mentioned obstacles, and are generally amenable to scale-up.[13] Indeed, this approach has proved to be a useful method of resolution for 7-unsubstituted bicyclo-[3.2.0]heptane derivatives such as the acetate (±)-**9**,[12,14–16] but it failed when substituents such as chloro, methyl or phenyl were incorporated at the 7-position due to non-acceptance of the substrates by a variety of hydrolytic enzymes, this effect was probably caused by steric hindrance (Scheme 2).[12,15]



Scheme 2 Enzymatic hydrolysis of 7-unsubstituted ester (±)-**9**. *Reagents and conditions:* lipase, pH 7.

Results and Discussion

In order to extend the application of enzyme-catalysed enantioselective hydrolysis to sterically demanding 7,7-disubstituted derivatives **1** and **2** two independent approaches were chosen, both of which aimed at the relocation of the reaction site—*i.e.*, the highly shielded *endo*-ester moiety of compound **10a**—to a position that is more accessible for hydrolytic enzymes. In the first approach, the acetoxy moiety was inverted from the shielded *endo*- into the more accessible *exo*-position (substrate **11a**) and in the second approach the site of reaction was moved from the crowded 6-position to the less hindered 3-position (as in the bromohydrins **12b** and **13b**).

Synthesis of Substrates.—Ketones **1** and **2** were obtained by [2 + 2]-cycloaddition of cyclopentadiene to dimethyl- and diphenyl-ketene, respectively, generated *in situ* as previously described.[17,18] Sodium borohydride reduction of the dimethyl ketone **1** gave a readily separable mixture of the *endo*-**10b** and *exo*-alcohol **11b** in the ratio 78:22. Alternatively, pure *exo*-alcohol **11b** was obtained by using dichloroalumin-

Table 1 Enzymatic hydrolysis of *endo*-acetate (±)-**10a**[a]

Enzyme[b]	Time (*t*/h)	Conversion (%)	Alcohol product	Ee (%)	E^{23}
Rhizopus delemar lipase	96	~10	(1*S*,5*R*,6*R*)-**10b**	>95	>40
Lipozyme R	504	~15	(1*S*,5*R*,6*R*)-**10b**	>95	>46

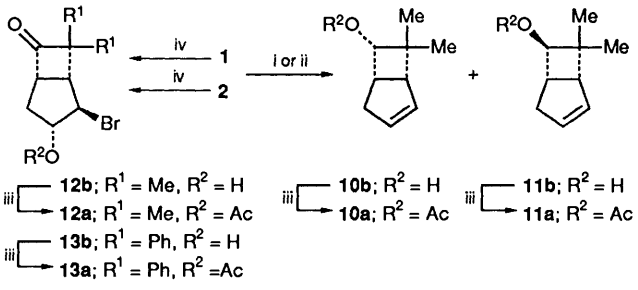
[a] No reaction was observed with either crude porcine pancreatic lipase or *Pseudomonas cepacia* lipase. [b] For details see Experimental section.

Table 2 Enzymatic hydrolysis of *exo*-acetate (±)-**11a**[a]

Enzyme[b]	Time (*t*/h)	Conversion (%)	Alcohol product	Ee (%)	Ester substrate recovered	Ee (%)	E^{23}
Candida cylindracea lipase	42	10	(1*R*,5*S*,6*R*)-**11b**	92	(1*S*,5*R*,6*S*)-**11a**	n.d.	27
Pseudomonas fluorescens lipase	90	8	(1*R*,5*S*,6*R*)-**11b**	96	(1*S*,5*R*,6*S*)-**11a**	n.d.	53
Steapsin	84	45	(1*R*,5*S*,6*R*)-**11b**	>98	(1*S*,5*R*,6*S*)-**11a**	78	>300
Steapsin	94	50	(1*R*,5*S*,6*R*)-**11b**	n.d.	(1*S*,5*R*,6*S*)-**11a**	87	
Cholesterol esterase	14	40	(1*R*,5*S*,6*R*)-**11b**	82	(1*S*,5*R*,6*S*)-**11a**	n.d.	17
Unknown hydrolase	120	35	(1*R*,5*S*,6*R*)-**11b**	>98	(1*S*,5*R*,6*S*)-**11a**	43	210

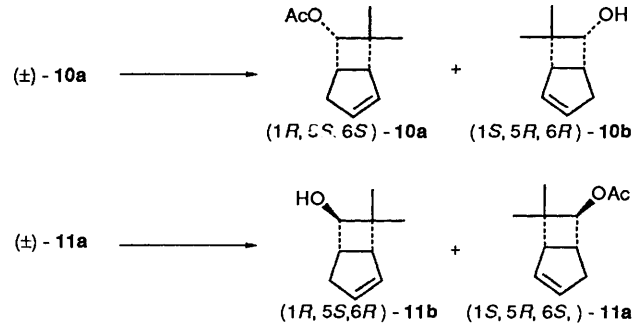
[a] No reaction was observed after 120 h with lipases from *Rhizopus delemar*, lipozyme R, pure porcine pancreas, or α-chymotrypsin. [b] For details see Experimental section.

ium hydride[19] as the reducing agent followed by thermal Oppenauer oxidation/Meerwein–Ponndorf–Verley reduction-mediated epimerization.[20] The bromohydrins **12b** and **13b** were synthesized by addition of hypobromous acid to the ketones **1** and **2** in a stereoselective fashion.[5,10,21] Acetylation of alcohols **10b–13b** by a standard procedure[22] led to the formation of the acetates **10a–13a** in excellent yield (Scheme 3).



Scheme 3 Synthesis of substrates. *Reagents and conditions:* i, $NaBH_4$, EtOH, 0 °C; **10b**:**11b** 78:22; ii, $LiAlH_4$, $AlCl_3$, Et_2O, room temp. then reflux; **11b**:**10b** > 98:2; iii, Ac_2O–pyridine, CH_2Cl_2, DMAP (cat.); iv, *N*-bromoacetamide, aq. acetone.

Enzymatic Resolution of endo-6-*Acetoxy*-7,7-*dimethylbicyclo*[3.2.0]*hept*-2-*ene* (±)-**10a**.—As expected from earlier findings,[12,15] the highly shielded *endo*-acetate (±)-**10a** was only marginally accepted by lipases, due to the location of the ester moiety on the inaccessible concave face of the rigid bicyclic framework. The enantioselectivity, however, was good but the slow reaction rates prevented this method from being of practical importance (see Scheme 4). Results of the hydrolysis are given in Table 1.



Scheme 4 Enzymatic hydrolysis of *endo*- and *exo*-acetates (±)-**10a** and (±)-**11a**. *Reagents and conditions:* hydrolase, pH 7.

Enzymatic Resolution of exo-6-*Acetoxy*-7,7-*dimethylbicyclo*[3.2.0]*hept*-2-*ene* (±)-**11a**.—As shown in Table 2, lipases from *Candida cylindracea* and *Pseudomonas cepacia* both accepted the *exo*-acetate (±)-**11a** at a low reaction rate and with moderate selectivity, but still did not provide a method of access to both enantiomers. In contrast, crude porcine pancreatic lipase (steapsin) proved to be an excellent biocatalyst for the required hydrolysis, with respect to both reaction rate and selectivity, thus making practical resolutions possible. From the observation that steapsin is known to be a crude enzyme preparation containing a number of other hydrolases[24] which might serve to diminish the observed selectivity, we investigated the above resolution by using pure porcine pancreatic lipase; to our surprise no reaction occurred. Similarly, α-chymotrypsin and cholesterol esterase—two known contaminant ester hydrolases present in steapsin—showed no activity or relatively low selectivity, respectively. Inspired by earlier observations[24] we then used a fraction of hitherto uncharacterized hydrolase purified from crude porcine pancreatic lipase which eventually exhibited a reaction rate and selectivity almost identical with that of steapsin. It is clear that authentic porcine pancreatic lipase is not the active enzyme; instead an enzyme 'impurity' present in the crude preparation is performing the stereoselective hydrolysis.

It is noteworthy that regardless of the configuration of the main framework of the substrate it was always the enantiomer possessing an (*R*)-configuration at the acetate-bearing carbon atom which preferentially reacted[25] in the case of both the diastereoisomeric substrates **10a** and **11a**.

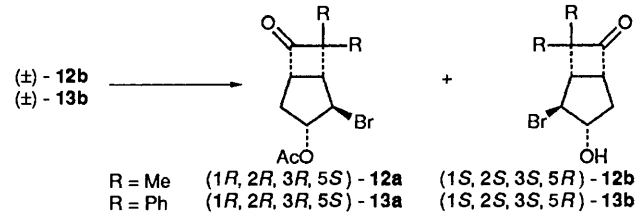
Enzymatic Resolution of Bromohydrins (±)-**12b** *and* (±)-**13b**.—Initial attempts at enzymatic hydrolysis of the bromohydrin acetates (±)-**12a** and (±)-**13a** in an aqueous system failed because, unlike esters (±)-**10a** and (±)-**11a**, the bromo acetates appeared to be completely insoluble or non-dispersible in water. Attempts to use water-miscible organic cosolvents such as isopropyl alcohol or acetone led only to marginal reaction rates, whereas their use at concentrations of >50% v/v caused rapid enzyme denaturation. Irreversible acyl transfer in an organic solvent,[26] however, occurred readily when using vinyl acetate both as the solvent and as the acyl donor (Scheme 5).[27] A related observation, where highly lipophilic substrates were successfully acylated using enzymes in an organic medium, was reported recently.[28] Results are given in Table 3.

Hence, as depicted in Table 3, both the bromohydrins (±)-

Table 3 Enzymatic resolution of bromohydrins (±)-**12b** and (±)-**13b**

Substrate	Enzyme[a]	Time (*t*/day)	Conversion (%)	Alcohol substrate recovered	Ee (%)	Ester product	Ee (%)	E^{23}
(±)-**12b**	*Pseudomonas cepacia* lipase	5	51	(1*S*,2*S*,3*S*,5*R*)-**12b**	>99	(1*R*,2*R*,3*R*,5*S*)-**12a**	96	>300
(±)-**13b**	*Candida cylindracea* lipase	9	39	(1*S*,2*S*,3*S*,5*R*)-**13b**	62	(1*R*,2*R*,3*R*,5*S*)-**13a**	>97	125

[a] For details see Experimental section.



Scheme 5 Enzymatic acylation of bromohydrins (±)-**12b** and (±)-**13b**. *Reagents:* lipase, vinyl acetate.

12b and (±)-**13b** were enzymatically resolved with very high selectivities but at a slow rate. In contrast to aqueous systems, where microbial contaminations can interfere with the desired transformation if reaction times exceed a period of 1–2 days, enzymatic reactions performed in an organic medium can be kept working over a period of several weeks if necessary.

The absolute configuration of the products was assigned as follows: Pyridinium dichromate (PDC) oxidation of the *endo*- and *exo*-alcohol, (1*S*,5*R*,6*R*)-**10b** and (1*R*,5*S*,6*R*)-**11b** gave optically active 7,7-dimethylbicyclo[3.2.0]hept-2-en-6-one **1** with known absolute configuration,[10] which in turn served as starting material for the synthesis of the optically active dimethylbromohydrin **12b**. The configuration of the diphenyl derivative **13b** was elucidated by ^{19}F NMR spectroscopy using Mosher's method:[29] note that this method gave the right answer for the dimethyl derivative **12b** as observed in a separate control experiment.

The studies show that sterically demanding bicyclo[3.2.0]-heptane derivatives can conveniently be resolved by hydrolytic enzymes if the centre of reaction is at a location on the bicyclic framework accessible by the enzyme. This technique allows the preparation of multigram amounts of building blocks in essentially optically pure state for leukotriene and pheromone synthesis.

Experimental

M.p.s were measured on a Büchi Tottoli apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 297 grating spectrophotometer. NMR spectra were obtained on a Bruker MSL 300 or WM 250 spectrometer. *J*-Values are given in Hz. Electron-impact mass spectra were determined on a Varian 311A machine. Optical-rotation values were measured on a Jasco DIP 200 or DIP 370 polarimeter. Gas chromatography was performed on a Dani 8500 chromatograph using a J & W capillary column DB 1701, 30 m × 0.25 mm, 0.25μ film, N_2 with flame ionization detection.

The following enzymes were used as received without further purification: Lipases from *Rhizopus delemar* (Biocatalysts Ltd.), *Mucor* sp. (lipozyme R, NOVO Industri), *Candida cylindracea* (Sigma type VII), *Pseudomonas cepacia* (Amano PS), *Pseudomonas fluorescens* (Biocatalysts Ltd.); pure porcine pancreatic lipase (Enzymatix), crude porcine pancreatic lipase (steapsin, Sigma type II), cholesterol esterase (Enzymatix Ltd.), α-chymotrypsin (Sigma type II), and an unknown ester hydrolase isolated from steapsin (Enzymatix).

Optical purities of alcohols were measured by ^{19}F NMR spectroscopy of their corresponding Mosher esters,[30] and for the bromohydrin **12b** GLC analysis of the corresponding (−)-menthyl carbonate was used.[31] Acetates were hydrolysed to the corresponding alcohols by standard procedures prior to derivatization.

The following compounds were synthesized according to known procedures: (±)-**1**,[17] (±)-**2**,[18] (±)-**12b**,[21] (±)-**13b**.[5]

(±)-7,7-*Dimethylbicyclo*[3.2.0]*hept*-2-*en*-6-endo-*ol* **10b** *and* (±)-7,7-*Dimethylbicyclo*[3.2.0]*hept*-2-*en*-6-exo-*ol* **11b**.—A solution of ketone **1** (4.0 g, 29.4 mmol) in anhydrous ethanol (7 cm^3) was added to a stirred solution of sodium borohydride (1.57 g, 42.5 mmol) in ethanol (20 cm^3) at 0 °C during 1 h. After the mixture had been stirred for 30 min the solvent was evaporated off and hydrochloric acid (2.0 mol dm^{-3}; 20 cm^3) was added to the residue. The mixture was extracted with diethyl ether (3 × 50 cm^3), the organic phase was washed successively with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3) and brine (2 × 50 cm^3). Drying ($MgSO_4$) and evaporation gave an oil, which was purified by silica gel chromatography (dichloromethane) to give the 6-*endo*-alcohol (±)-**10b** (2.55 g, 63%) as a clear oil, $\nu_{max}(CHCl_3)/cm^{-1}$ 3593 (OH, free), 3456 (OH, H-bonded) and 1604 (C=C); $\delta_H(CDCl_3)$ 5.82–5.60 (2 H, m, 2- and 3-H), 3.74 (1 H, d, $J_{6,5}$ 8, 6-H_{exo}), 3.60–2.94 (1 H, m, 1-H), 2.74–2.02 (4 H, m, 4-H_2, 5-H, and OH), 1.12 (3 H, s, 7-Me_{exo}) and 0.92 (3 H, s, 7-Me_{endo}); $\delta_C(CDCl_3)$ 133.7 and 132.8 (CH, C-2 and -3), 75.8 (CH, C-6), 54.2 (CH, C-1), 43.6 (C, C-7), 36.9 (CH, C-5), 30.8 (CH_2, C-4), 29.4 (Me_{exo}), and 17.1 (Me_{endo}); and the 6-*exo*-alcohol (±)-**11b** (0.72 g, 18%) as a solid; m.p. 36–38 °C; $\nu_{max}(CHCl_3)/cm^{-1}$ 3608 (OH, free) and 3429 (OH, H-bonded); $\delta_H(CDCl_3)$ 5.67–4.68 (2 H, m, 2- and 3-H), 3.47 (1 H, d, $J_{6,5}$ 5.5, 6-H_{endo}), 2.74–2.66 (1 H, m, 1-H), 2.64–2.54 (1 H, m, 5-H), 2.52–2.20 (2 H, m, 4-H_2), 2.06 (1 H, s, OH), 1.10 (3 H, s, 7-Me_{exo}), and 0.92 (3 H, s, 7-Me_{endo}); $\delta_C(CDCl_3)$ 132.2 (2 × CH, C-2 and -3), 80.8 (CH, C-6), 51.0 (CH, C-1), 43.4 (CH and C, C-5 and -7), 37.3 (CH_2, C-4), 22.6 (Me_{exo}) and 22.5 (Me_{endo}).

(±)-7,7-*Dimethylbicyclo*[3.2.0]*hept*-2-*en*-6-exo-*ol* **11b** (*Alternative Preparation*).—A solution of $LiAlH_4$ in diethyl ether (1.0 mol dm^{-3}; 27.5 cm^3, 27.5 mmol) was added dropwise to a stirred solution of anhydrous aluminium chloride (13.34 g, 100 mmol) in dry diethyl ether (100 cm^3) at 0 °C under nitrogen. The mixture was stirred at this temperature for 30 min and was then allowed to warm to room temperature. A solution of ketone (±)-**1** (14.3 g, 105 mmol) in dry diethyl ether (100 cm^3) was then added dropwise during 1 h. The reaction mixture was then refluxed for 1 h during which time a pink colour developed. Excess of hydride was destroyed at 0 °C by the dropwise addition of water and the precipitate thus formed was dissolved upon the addition of sulphuric acid (10% v/v; 50 cm^3). The organic phase was separated and the aq. phase was extracted with diethyl ether (100 cm^3); the combined organic phases were then dried and evaporated to give a brown oil. Chromatography (dichloromethane) gave the *exo*-alcohol (±)-**11b** (11.6 g, 80%) as a solid. No *endo*-alcohol (±)-**10b** was seen by TLC after the reflux period.

Table 4 Optical-rotation values

Compound	$[\alpha]_D^{20}$ (°)	c (g/100 cm³)	Solvent	Ee (%)
(1*S*,5*R*,6*R*)-**10b**	+115	0.40	$CHCl_3$	>95
(1*R*,5*S*,6*R*)-**11b**	−146	0.40	$CHCl_3$	>98
(1*R*,2*R*,3*R*,5*S*)-**12a**	+34.0	1.62	MeOH	96
(1*S*,2*S*,3*S*,5*R*)-**12b**	−92.1	1.17	MeOH	>99
(1*R*,2*R*,3*R*,5*S*)-**13a**	+6.3	0.95	MeOH	>97
(1*S*,2*S*,3*S*,5*R*)-**13b**	−28.3	1.14	MeOH	62

Esters (±)-**10a**–**13a** were prepared from the corresponding alcohols by a standard procedure.[22]

(±)-6-endo-*Acetoxy*-7,7-*dimethylbicyclo*[3.2.0]*hept*-2-*ene* **10a**. Yield 93%; b.p. 82 °C/13 mmHg; $\nu_{max}(CHCl_3)/cm^{-1}$ 1711 (C=O, ester) and 1608 (C=C); $\delta_H(CDCl_3)$ 5.82–5.52 (2 H, m, 2- and 3-H), 4.69 (1 H, dd, *J* 8 and 1.5, 6-H_{exo}), 3.14–3.02 (1 H, m, 1-H), 2.74 (1 H, br s, 5-H), 2.34–2.26 (2 H, m, 4-H), 1.98 (3 H, s, Ac), 1.12 (3 H, s, 7-Me_{exo}) and 0.82 (3 H, s, 7-Me_{endo}); $\delta_C(CDCl_3)$ 170.5 (C=O, ester), 133.4 (CH, C-3), 130.7 (CH, C-2), 76.6 (CH, C-6), 53.8 (CH, C-1), 43.1 (C, C-7), 35.3 (CH, C-5), 32.1 (CH_2, 4-C), 29.2 (CH_3, 7-Me_{exo}), 20.5 (CH_3, CO*Me*) and 17.7 (CH_3, 7-Me_{endo}) [Found: ($M^+ + NH_4^+$), 198.1494. $C_{11}H_{16}O_2$ requires (M + NH_4), 198.1494].

(±)-6-exo-*Acetoxy*-7,7-*dimethylbicyclo*[3.2.0]*hept*-2-*ene* **11a**. Yield 95%; b.p. 75 °C/12 mmHg; $\nu_{max}(CHCl_3)/cm^{-1}$ 1720 (C=O, ester); $\delta_H(CDCl_3)$ 5.81–5.69 (2 H, m, 2- and 3-H), 4.34–4.30 (1 H, m, 6-H_{endo}), 2.81–2.76 (2 H, m, 1- and 5-H), 2.49–2.40 (2 H, m, 4-H_2), 2.02 (3 H, s, Ac), 1.10 (3 H, s, 7-Me_{exo}) and 1.0 (3 H, s, 7-Me_{endo}); $\delta_C(CDCl_3)$ 170.6 (C=O, ester), 132.6 (CH, C-3), 131.6 (CH, C-2), 82.1 (CH, C-6), 51.6 (CH, C-1), 43.3 (C, C-7), 40.1 (CH, C-5), 37.2 (CH_2, C-4) and 23.4, 22.9 and 20.8 (Me) [Found: ($M^+ + NH_4^+$), 198.1494. $C_{11}H_{16}O_2$ requires (M + NH_4), 198.1494].

(±)-3-endo-*Acetoxy*-2-exo-*bromo*-7,7-*dimethylbicyclo*-[3.2.0]*heptan*-6-*one* **12a**. Yield 82%; m.p. 65–68 °C (lit.,[21] 68–70 °C); b.p. 90 °C/7 Pa (Kugelrohr); $\delta_H(CDCl_3)$ 5.37 (1 H, dd, *J* 5 and 1.5, 3-H), 4.34 (1 H, br s, 2-H), 3.94 (1 H, t, *J* 7, 5-H), 3.06 (1 H, d, *J* 7, 1-H), 2.51–2.40 (1 H, m, 4-H_{endo}), 2.22 (1 H, d, *J* 8, 4-H_{exo}), 1.98 (3 H, s, Me), 1.35 (3 H, s, Me_{exo}) and 1.17 (3 H, s, Me_{endo}); $\delta_C(CDCl_3)$ 212.4 (C, C-6), 170.6 (C, ester C=O), 83.4 (CH, C-3), 63.1 (C, C-7), 59.5 (CH, C-2), 53.5 (CH, C-5), 51.0 (CH, C-1), 32.0 (CH_2, C-4), 27.0 (CH_3, 7-Me_{exo}), 21.1 (CH_3, CO*Me*) and 17.9 (CH_3, 7-Me_{endo}).

(±)-3-endo-*Acetoxy*-2-exo-*bromo*-7,7-*diphenylbicyclo*-[3.2.0]*heptan*-6-*one* **13a**. Yield 85%; m.p. 63–65 °C; δ_H-($CHCl_3$) 7.53–7.12 (10 H, m, Ph), 5.28 (1 H, dd, *J* 5 and 1.2, 3-H), 4.46 (1 H, br s, 2-H), 4.19 (1 H, d, *J* 7, 1-H), 3.96 (1 H, t, *J* 7, 5-H), 2.59–2.48 (1 H, m, 4-H_{endo}), 2.29 (1 H, d, *J* 8, 4-H_{exo}) and 1.66 (3 H, s, Ac); $\delta_C(CDCl_3)$ 215.1 (C, C-6), 169.7 (C, ester C=O), 141.8 and 141.1 (c, arom.), 129.4, 128.9, 127.6, 127.1, 126.5 and 126.4 (CH, arom.), 83.1 (CH, C-3), 77.7 (C, C-7), 60.1 (CH, C-2), 52.2 and 51.7 (CH, C-1 and -5), 32.3 (CH_2, C-4) and 20.9 (CH_3, CO*Me*).

Enzymatic resolution by hydrolysis[13] and acyl transfer[28] was performed as previously described.

Acknowledgements

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