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Baker's yeast reduction of cyclic δ-ketoesters: synthesis and chiroptical properties of condensed δ-lactones

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Abstract

Enantiomerically pure condensed δ-lactones have been prepared from the corresponding δ-ketoesters by the use of *Saccharomyces cerevisiae*. The reactions were not only highly enantioselective but also highly diastereoselective, provided the baker's yeast was preincubated at 50°C for 30 min. Interestingly, and contrary to what is usually found, the use of nutrients inhibited the bioreductions. The relative configurational assignments have been made by means of NMR, while the absolute configurations and conformations of the lactone rings were attributed by means of CD studies.

1. Introduction

The δ-lactone ring is present in a variety of natural products[1] isolated from insects, plants, fungi and marine organisms, normally as part of complex polycyclic skeletons. Examples can be found in quassinoid derivatives such as sergeolide[2] (isolated from *Picrolemma pseudocoffea*) and simalikalactone D[3] (isolated from the wood of *Simaba multiflora*) as well as in diterpenoids such as epinodosinol[4] (isolated from *Rabdosia angustifolia*) and longirabdolide G[5] (isolated from *Rabdosia longituba*). Many of them show biological activity; for instance, simalikalactone D is an antitumour while sergeolide is an antimalarial.

Therefore, a strategy giving access to optically active δ-lactones as chiral building blocks for the synthesis of more complex compounds seems highly interesting.

2. Results and discussion

In our previous papers[6,7] enantiomerically pure bicyclic γ-lactones were synthesized from the appropriate γ-nitroketones and γ-ketoesters, using raw baker's yeast in the enantiodifferentiating step.

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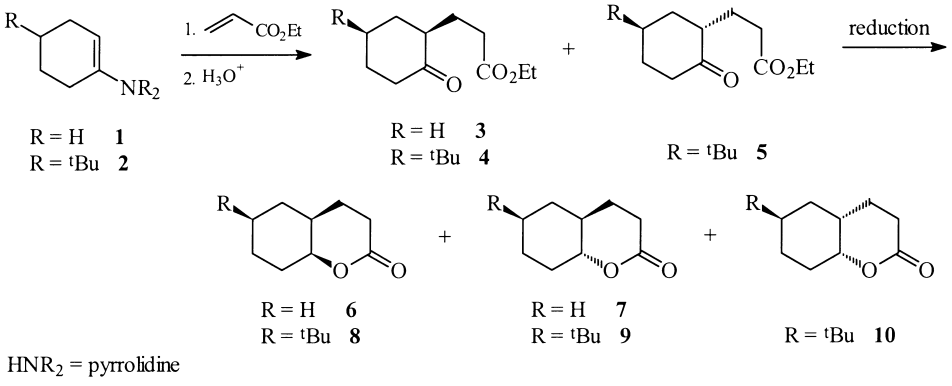
Since these bioreductions were not only highly enantioselective but also highly diastereoselective, it seemed interesting to verify whether the same strategy could also be useful for the synthesis of bicyclic δ-lactones, in particular starting from δ-ketoesters.

2.1. Synthesis of the substrates

Substrates used were the δ-ketoesters **3**, **4** and **5** (Scheme 1), derived from a Michael-type reaction between ethyl acrylate and the corresponding pyrrolidino cyclohexenes **1** and **2**, followed by hydrolysis under equilibrating acidic conditions.[8] The δ-ketoesters **4** and **5** could not be separated by column chromatography and, therefore, a mixture of thermodynamic formation (**4**:**5**, 9:1) was used in the subsequent steps.



Scheme 1.

2.2. Chemical reduction

Compound **3** and the mixture of **4** and **5** were reduced with both a chemical agent and baker's yeast. As reported in the literature,[9] among the reducing agents for δ-ketoesters, sodium cyanoborohydride is a better choice than sodium borohydride because it gives no by-products and also the diastereoselectivity is higher. Reduction of δ-ketoester **3** gave a mixture of *cis*- and *trans*-fused lactones **6**[10–12] and **7**[10] in the ratio 1:4, determined by HRGC. The δ-hydroxyester intermediates could not be detected, as they immediately cyclized to the corresponding δ-lactones in the reaction medium.

Similarly, when the 9:1 mixture of **4** and **5** was reduced under the same conditions, three lactones were identified, namely the *cis*-fused lactone **8**[9,13] (18%), the *trans*-fused lactone **9** (72%) both derived from **4**, and the *cis*-fused lactone **10** (10%) derived from **5**.

The geometry of the δ-lactones was established by analyzing their ^{1}H NMR spectra. In particular, in the *cis*-fused lactones **6** and **8** the respective oxygen atom was essentially axial, as indicated by the chemical shift and pattern of its geminal protons at C-8a (4.49 ppm, pseudo q, W_H 7.2 Hz and 4.48 ppm, pseudo q, W_H 5.2 Hz, respectively). In **7** and **9** the same protons H-8a were clearly axial as can be inferred from the values of the vicinal coupling constants as well as from the values of their respective chemical shifts (3.88 ppm, dt, J_1=J_2 10.4 Hz, J_3 4.4 Hz and 3.84 ppm, dt, J_1=J_2 10.5 Hz, J_3 4.4 Hz, respectively).

2.3. Baker's yeast reduction

Having isolated and characterized all the lactones formed by chemical reduction of the δ-ketoesters, the same substrates were reduced with baker's yeast in water. The conditions used for bioreduction did not allow for the isolation of the δ-hydroxyester intermediates and the corresponding δ-lactones were directly formed.

A few parameters were checked on ketoester **3** to optimize the reaction conditions, namely (a) use of raw[14] and dry yeast,[15] (b) preincubation of yeast,[16] (c) presence of glucose,[17] and (d) presence of ethanol.[18] The results are summarized in Table 1.

First, as anticipated, the presence of either glucose[17] as a nutrient or ethanol,[18] which is usually considered an inhibitor but in some cases was found to be an energy source,[18] greatly inhibited the bioreduction (in 18 days a conversion of only 8% was detected by HRGC). This was independent of the type of baker's yeast used. This result is quite unusual because this is the first time, as far as we know, that a nutrient (glucose) has acted as an inhibitor.

Pretreatment of yeast seemed to be an important factor for raw yeast, whereas it plays no role for dry yeast. In fact, preincubation of raw yeast at 50°C for 30 min increased the diastereoselectivity of the reduction (entry 2, Table 1), with the enantioselectivity remaining high. According to Nakamura et al.,[16] heat treatment denatures thermolabile dehydrogenases, thus increasing diastereoselectivity. It is evident, therefore, that these particular dehydrogenases are not present in dry yeast.

Actually, when the ketoester **3** was reduced with preincubated raw yeast, under a N_2 atmosphere,[†] the corresponding *cis*-fused lactone (−)-**6** was obtained in 98% diastereomeric as well as enantiomeric excess (entry 2). It is interesting to note, however, that reductions with dry baker's yeast were also satisfactory, provided the yeast was preincubated, as they furnished the lactone (−)-**6** with 92–94% d.e. and 99% e.e. Conversions ranged from 36% to 78%, the values exceeding 50% being due to the equilibration between the enantiomers of **3** occurring during the reaction.

Table 1
Reduction of the δ-ketoester (±)-**3** with baker's yeast

Entry	Type of b.y.	Method	B.y. (g/mmol)	Conc. of 3 (mol/l)	Reaction time (days)	Conv. (%)	(–)-6:7	(–)-6 e.e. (%)	7 e.e. (%)
1	Raw	A	10	0.05	14	54	60:40	>99	50
2	b.y.	B	10	0.05	16	78	99:1	98	-
3	Dry	A	4	0.05	11	36	96:4	>99	>99
4	b.y.	B	4	0.05	13	72	93:7	99	>99

Method A: without preincubation

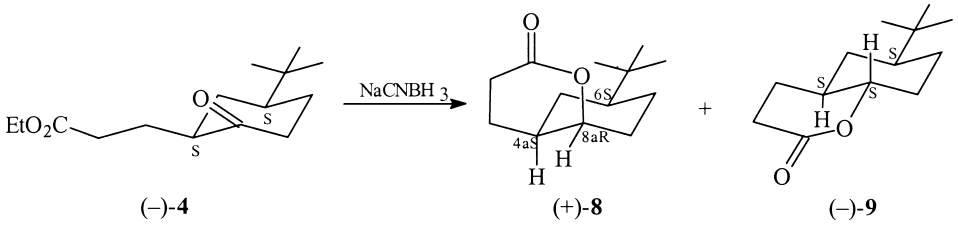
Method B: preincubated for 30min at 50°C

Therefore, reaction conditions, which gave the best result for the bioreduction of compound **3**, were used for reducing the thermodynamic mixture of δ-ketoesters **4** and **5**. This latter reaction was not only

[†] Since these bioreductions last more than 10 days, a nitrogen atmosphere is necessary to prevent yeast from rotting away; of course these can be done only if no nutrients are used.

highly enantioselective (99% e.e.) but also highly diastereoselective, leading to the lactone (−)-**8** as a single stereoisomer, after a 50% conversion. Therefore, of all the lactones shown in Scheme 1, it is possible to selectively obtain *cis*-fused lactones (−)-**6** and (−)-**8** directly and in high enantiomeric excess using preincubated raw baker's yeast.

From the bioreduction of the 9:1 mixture of **4** and **5**, the unreacted ketoester (−)-**4**, having 96% e.e., was recovered after chromatographic separation. It was reduced with sodium cyanoborohydride thus allowing for the isolation of the *trans*-fused lactone (−)-**9** in high enantiomeric excess. In fact, the reaction furnished a 4:1 mixture of the *cis*- and *trans*-fused lactones (+)-**8** and (−)-**9**, respectively, which were separated by flash chromatography (Scheme 2).



(–)-**4** (+)-**8** (–)-**9**

Scheme 2.

2.4. *Determination of the absolute configuration of compounds (−)-4, (−)-6, (−)-8 and (−)-9*

Absolute configuration of the enantiomerically pure compounds was established by means of CD spectroscopy. The CD spectrum of the unreacted ketoester (−)-**4** showed a positive Cotton effect at 289 nm ([Θ]=+2248) for the n → π* transition of the ketone carbonyl group. Using the simple octant rule[19] none of the substituents would make any contribution to the Cotton effect as both lie on symmetry planes. However, a large positive CE was observed for (+)-(2*S*,4*S*)-2-methyl-4-*t*-butylcyclohexanone and molecular mechanics calculations showed that the chair conformation is favoured.[19a] Geometry optimization up to a gradient of 10^{-4} kcal/molÅ performed with PM3[20] Hamiltonian afforded four possible conformations with enthalpy differences of almost 0.90 kcal/mol. In the present case the methyl group is substituted for a slightly longer chain that might jut into one of the front octant regions with concomitant large signed contribution. In any case, the large positive CE is consistent with the 2*S*,4*S*-configuration of the stereogenic centres of (−)-**4**. On the other hand, similar spectra were obtained for 2-(2-nitroethyl)-4-*t*-butylcyclohexanone[7a] having the same absolute configuration as (−)-**4**. As a consequence, absolute configurations of lactones (+)-**8** and (−)-**9**, both derived from (−)-**4**, are 4a*S*,6*S*,8a*R* and 4a*S*,6*S*,8a*S*, respectively, as shown in Scheme 2.

The absolute configuration of the enantiomeric lactone (−)-**8**, derived from the reduction of δ-ketoester (±)-**4** with baker's yeast, is 4a*R*,6*R*,8a*S*. The carbinol carbon atom in the hydroxyester intermediate, which was not isolated, was therefore *S*. Thus, once again the enantiopreference shown by the yeast is in accordance with Prelog's rule.[21] The *cis*-fused lactone (−)-**8** exhibited a negative Cotton effect at 212 nm as did (−)-**6** (Fig. 1). Therefore, C-4a and C-8a in (−)-**6** can be attributed the same absolute configuration as the same stereogenic centres in (−)-**8**, namely *R* and *S*, respectively.

An evaluation of the conformation of the lactone ring can be made from the CD curves.[22] In accordance with Korver,[22c] the boat conformation is responsible for a maximum appearing below 225 nm, while a maximum above 225 nm is indicative of a half-chair conformation for the lactone ring. Therefore, a boat conformation can be envisaged for both *cis*-fused δ-lactones (−)-**6** and (−)-**8** (Fig. 1 and Table 2), while for the *trans*-fused system (−)-**9** part of the half-chair conformation is surely present (Table 2).

In order to investigate the influence of steric factors on the equilibrium between chair and boat conformers, the *cis*-fused lactones (−)-**6** and (−)-**8** were α-methylated under the conditions used by

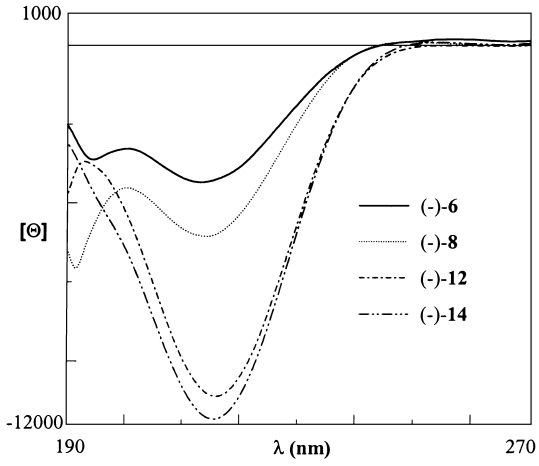


Figure 1. CD spectra of (−)-**6**, (−)-**8**, (−)-**12** and (−)-**14**

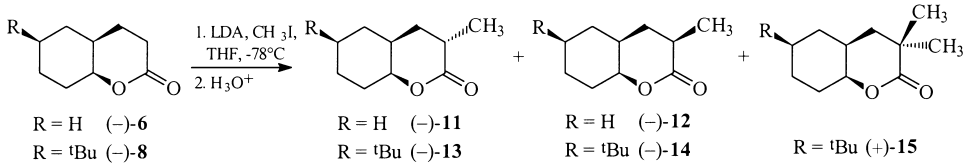
Table 2
Solvent effect

	CD								UV	
Compd.	Methanol		Acetonitrile		Dioxane		Cyclohexane		Methanol	
	λ	[Θ]	λ	[Θ]	λ	[Θ]	λ	[Θ]	λ	ε
(–)-**6**	213	-4345	218	-4009	219	-3832	221	-3348	210	85
(–)-**8**	214	-6067	216	-5097	218	-4433	219	-3826	212	69
(–)-**9**	235 208	+694 -1344	239 208	+918 -1068	240 213	+817 -395	240 209	+1429 -613	213	90
(–)-**11**	233 210	+1474 -884	234 218	+2291 +884	234	+2783	235 217	+3854 +1839	215	106
(–)-**12**	216	-11114	219	-10049	220	-9338	222	-8861	211	107

Grieco[23] for the stereoselective α-methylation of γ-lactones. In neither case was the stereoselectivity complete. Methylation of (−)-**6** afforded an 88:12 mixture of diastereoisomeric lactones (−)-**11** and (−)-**12**. Only lactone (−)-**11** was obtained as a pure compound, although contaminated by 3% of (−)-**12** (Scheme 3).

Analogous results were obtained for the methylation of (−)-**9**, which furnished (−)-**13** and (−)-**14**, besides the α-dimethylated lactone (−)-**15**. The ratio of (−)-**13**, (−)-**14** and (−)-**15** was 75:5:20. Separation of the mixture afforded (−)-**13** contaminated by 6% of its diastereoisomer (−)-**14**, while (−)-**15** was isolated as a pure compound.

It is interesting to underline that, although methylation of δ-lactones was not completely stereoselec-



Scheme 3.

tive, as is usual for γ-lactones, the preferred approach of methyl iodide to the enolate intermediate is still from the same side as that containing H-4a.

Inversion of configuration at C-3 of (−)-**11** and (−)-**13** furnished the corresponding isomers (−)-**12** and (−)-**14**. Inversion of configuration was accomplished by protonation of the corresponding enolates. Neither protonation was completely stereoselective. However, while (−)-**12** was obtained in admixture with (−)-**11** (13%), (−)-**14** could be isolated as a pure compound.

The ^{1}H NMR data for **11**–**14** of H-8a (**11**: 4.50 ppm, pseudo q, W_H 8.8 Hz; **12**: 4.50 ppm, pseudo q, W_H 11.3 Hz; **13**: 4.32 ppm, bq, W_H 8.9 Hz; **14**: 4.44 ppm, bq, W_H 8.0 Hz) can be compared with the values found for their non-methylated analogues **6** and **8**, whose data are reported above. From these data it can also be deduced that in the α-methylated systems the oxygen atom is essentially axial.

Orientation of the methyl group was established by means of DIFNOE measurements carried out on lactone (−)-**12** (Fig. 2). Of particular interest is the NOE effect found for H-3 when irradiating H-8a. It is clearly consistent with the boat conformation of the lactone ring which is confirmed by the position of the maximum in its CD curve (216 nm).[22c] Because of the boat conformation, the methyl group bisects the H–C_4–H angle, as proved by the same value of enhancement found for both H-4 (4%), when irradiating the methyl group. The Cotton effect exhibited by (−)-**12** is negative as it is for the analogous *t*-butylated derivative (−)-**14** (Fig. 2). Since (−)-**12** derives from (−)-**6**, the absolute configuration of (−)-**12** is 3*R*,4a*R*,8a*S* and that of (−)-**14** is 3*R*,4a*R*,6*R*,8a*S*.

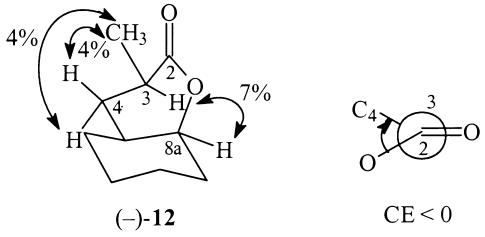


Figure 2. DIFNOE measurements on lactone (−)-**12**

3. Conclusions

All the configurational and conformational assignments made for the δ-lactones are in accordance with the Legrand and Bucourt rule[22e] that states that a negative Cotton effect is expected for a positive O–CO–Cα–Cβ torsional angle (Fig. 2). A negative Cotton effect was actually found for (−)-**12** as well as for (−)-**6**, (−)-**8** and (−)-**14**.

The *trans*-fused lactone (−)-**9** and the α-methylated lactones (−)-**11** and (−)-**13** exhibited bisignated CEs with a positive maximum around 235 nm, and a negative one around 210 nm (Fig. 3). On the basis of the previous considerations, an equilibrium between the boat and half-chair conformers can be envisaged for the lactone ring. Furthermore, since the band at around 210 nm shows a negative CE, the absolute configurations of (−)-**9**, (−)-**11** and (−)-**13** are 4a*S*,6*S*,8a*S*, 3*S*,4a*R*,8a*S* and 3*S*,4a*R*,6*R*,8a*S*, respectively.

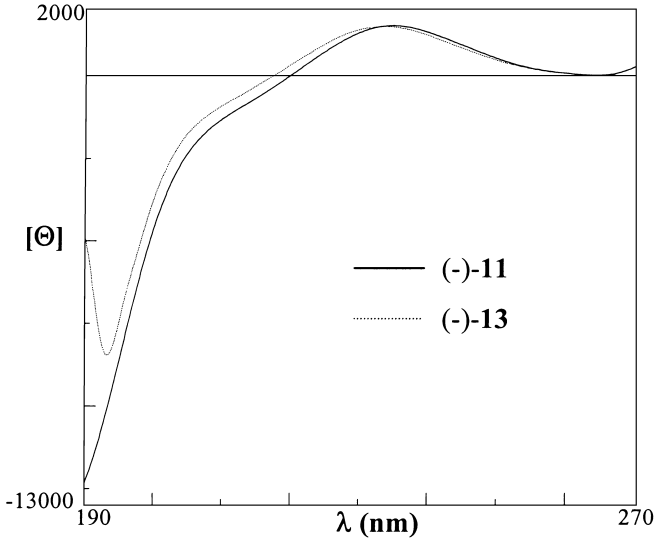


Figure 3. CD spectra of (−)-**11** and (−)-**13**

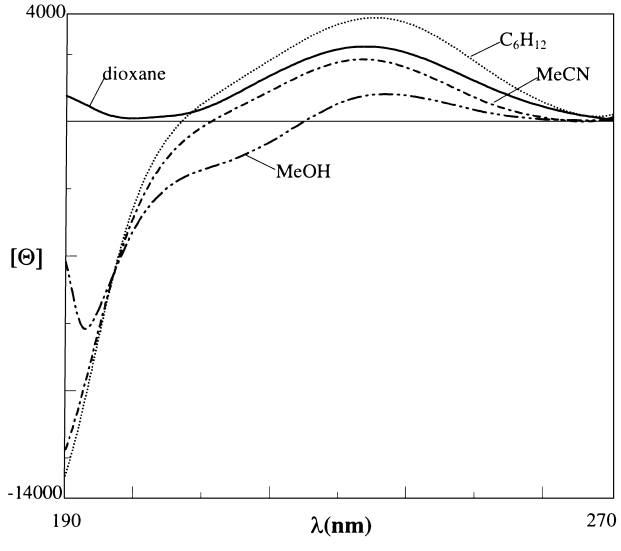


Figure 4. Solvent dependency of the CD spectra of (−)-**11**

The existence of this conformational equilibrium for the lactone ring is also confirmed by the solvent dependency of the CD spectra found for all lactones (Fig. 4 and Table 2).

4. Experimental

4.1. General

Melting points were determined with a Büchi apparatus and are uncorrected. IR spectra were recorded in $CHCl_3$, unless otherwise stated, on a Jasco FT/IR 200 spectrophotometer. ^{1}H NMR spectra were run on a Jeol EX-400 (400 MHz) spectrometer, using deuteriochloroform as the solvent and tetramethylsilane as the internal standard. ^{13}C NMR spectra were recorded on a Jeol EX-400 (100.5 MHz) instrument. Optical

rotations were determined on a Perkin–Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell); UV spectra were recorded on a Perkin–Elmer Lambda 2 and a Jasco V-550 spectrophotometers (1.0 cm cell) in methanol; GLC analyses were obtained on a Carlo Erba GC 8000 instrument, the capillary column being carbowax (30 m×0.32 mm; carrier gas He, 40 kPa) and a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (40 m×0.25 mm; carrier gas He, 180 kPa) or DMePe β-cyclodextrin (25 m×0.26 mm; carrier gas He, 110 kPa). Mass spectra were run by the electron-impact mode on a VG 7070 (70 eV) at the Central Facility for Mass Spectrometry of the University of Trieste and on a Hewlett–Packard 5971-A GC–MS instrument. TLCs were performed on Whatman K6F silica gel plates (eluant: light petroleum/ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with bp 40–70°C and ether to diethyl ether.

4.2. Synthesis of the substrates

Ethyl 3-(2-oxocyclohexyl)propionate **3** was prepared according to the literature.[8] Its IR, ^{1}H NMR and ^{13}C NMR were identical with the reported values.[24]

Ethyl 3-(5-(1,1-dimethylethyl)-2-oxo-cyclohexyl)propionate **4** was prepared by treatment of 1-*N*-pyrrolidinyl-4-(1,1-dimethylethyl)cyclohexene with ethyl acrylate in refluxing dioxane for 3 h. After addition of water and refluxing for 1 h, extraction with ether afforded a mixture of **4** and **5** in a 9:1 ratio, which was the thermodynamic composition. The two isomers could not be separated by flash chromatography. The spectroscopic data of compound (−)-**4** recovered after the bioreduction are given.

*4.2.1. (−)-*cis-*Ethyl 3-(5-(1,1-dimethylethyl)-2-oxo-cyclohexyl)propionate **4** (in admixture with 5% of the* trans-*isomer **5**)*

IR (film): 1730, 1710 cm^{-1} (C=O); ^{1}H NMR, δ, ppm: 4.12 (2H, q, *J* 7.3, COOC*H*$_2$CH$_3$), 2.36 (5H, m), 2.09 (3H, m), 1.52 (3H, m), 1.25 (3H, t, *J* 7.3, COOCH$_2$C*H*$_3$), 1.20 (1H, m), 0.91 [9H, s, C(CH$_3$)$_3$]; ^{13}C NMR, δ, ppm: 212.7 (s), 173.6 (s), 60.2 (t), 48.7 (d), 47.0 (d), 41.6 (t), 35.3 (t), 32.4 (s), 31.9 (t), 28.8 (t), 27.6 (3q), 24.8 (t), 14.2 (q); MS, m/z: 254 (M$^{+\cdot}$, 13), 209 (37), 208 (51), 193 (17), 180 (14), 167 (10), 151 (25), 124 (51), 96 (22), 83 (25), 69 (17), 57 (100), 55 (59); 96% e.e. (by chiral HRGC); $[\alpha]_D^{25}$=−18.1 (*c* 0.11, CH$_3$OH); $[\Theta]_{289}$=+2248 (CH$_3$OH); UV (CH$_3$OH), λ_{max} (ε, M^{-1} cm^{-1}): 282 (28), 229 (145).

*4.3. Chemical reduction of δ-ketoesters **3** and **4,5***

Reduction of **3** with NaCNBH$_3$ gave the corresponding lactones **6** and **7** in a 1:4 ratio. They were separated by flash chromatography.

Reduction of the 9:1 mixture of **4** and **5** with NaCNBH$_3$[9] afforded the corresponding lactones **8** and **9** in a 1:4 ratio. Lactone **10**, derived from the reductive cyclization of **5**, was also formed in 10%. Lactones **8**, **9** and **10** were partially separated by flash chromatography affording **9** as a pure compound together with a mixture of **8** and **10**.

4.3.1. cis-*6-(1,1-Dimethylethyl)-octahydro-2*H-*1-benzopyran-2-one **10***

Only a few signals could be identified in the mixture: ^{1}H NMR, δ, ppm: 4.45 (1H, dt, J_1=J_2 5.5, J_3 11.3, H-8a), 2.65 (1H, ddd, J_1 1.5, J_2 5.9, J_3 18.1, H-3), 2.47 (1H, dd, J_1 7.3, J_2 18.1 H-3), 2.30 (1H, m), 1.42 (1H, dt, J_1=J_2 13.2, J_3 4.9), 0.85 [9H, s, C(CH$_3$)$_3$], addition of a small amount of C$_6$D$_6$ shifted the H-8a signals of **10** at 4.45 from that of **8**, thus allowing the identification of the absorption; ^{13}C NMR,

δ, ppm: 170.6 (s), 81.0 (d), 40.7 (d), 32.7 (d), 32.0 [s, $C(CH_3)_3$], 30.4 (t), 30.1 (t), 29.1 (t), 27.4 [3q, $(CH_3)_3$], 25.4 (t), 20.8 (t).

4.4. Reduction with baker's yeast

4.4.1. Raw baker's yeast

Method A: 100 g of raw baker's yeast in 200 ml of water was added to 10 mmol of the δ-ketoester and the mixture was stirred at room temperature.

Method B: 100 g of raw baker's yeast in 200 ml of water was preincubated for 30 min at 50°C, added to 10 mmol of the δ-ketoester and the mixture was stirred at room temperature.

4.4.2. Dry baker's yeast

An amount of 4 g/mmol dry baker's yeast purchased from Sigma–Aldrich was used following the above procedures for methods A and B.

The course of the reduction was checked every 2 days by HRGC. At the end of the reaction, brine was added and the broth was continuously extracted with diethyl ether for 48 h. The organic phase was dried and evaporated.

4.4.3. (−)-(4*a*S,8*a*S)-cis-*Octahydro-2*H-*1-benzopyran-2-one* **6**

IR, ^{1}H NMR, ^{13}C NMR were identical with the reported values.[10a] MS, m/z: 154 ($M^{+\cdot}$, 10), 110 (29), 98 (49), 82 (88), 81 (100), 69 (85), 68 (95), 55 (88); 98% e.e. (β-cyclodextrin), $[\alpha]_D^{25}$=−45.7 (*c* 0.35, CH_3OH); $[\alpha]_D^{25}$=−53.5 (*c* 0.4, THF) [lit.[11] $[\alpha]_D$=−28 (*c* 2, THF)]; $[\Theta]_{213}$=−4345 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 209 (87).

4.4.4. trans-*Octahydro-2*H-*1-benzopyran-2-one* **7**

IR, ^{1}H NMR, ^{13}C NMR were identical with the reported values.[10a]

4.4.5. (−)-(4*a*R,6R,8*a*S)-cis-*6-(1,1-Dimethylethyl)-octahydro-2*H-*1-benzopyran-2-one* **8**

Mp 99–100°C (from light petroleum); elemental analysis: calculated: C, 74.24; H, 10.54%; found: C, 74.00; H, 10.75%; IR (Nujol): 1740 (–O–C=O); ^{1}H NMR, δ, ppm: 4.45 (1H, pseudo q, H-8a), 2.52 (2H, dd, J_1 6.6, J_2 8.3, H-3), 2.14 (2H, m, H-4 and H-8), 1.88 (1H, m, H-6), 1.56 (4H, m, H-4, H-5, H-7, H-8), 1.30 (1H, m, H-7), 1.23–1.08 (2H, m, H-5, H-6), 0.86 [9H, s, $C(CH_3)_3$]; ^{13}C NMR, δ, ppm: 172.8 (s, C-2), 77.3 (d, C-8a), 47.2 (d, C-6), 33.2 (d, C-4a), 32.4 [s, $C(CH_3)_3$], 30.9 (t, C-8), 27.4 [3q, $(CH_3)_3$], 27.3 (t, C-5), 26.4 (t, C-3), 25.1 (t, C-4), 20.4 (t, C-7); MS, m/z: 195 ($M-CH_3^+$, 5), 154 (83), 136 (42), 95 (37), 94 (100), 82 (38), 81 (32), 79 (21), 73 (18), 71 (20), 67 (25), 57 (53), 55 (35), 41 (56); >99% e.e. (β-cyclodextrin); $[\alpha]_D^{25}$=−11.6 (*c* 0.25, CH_3OH); $[\Theta]_{214}$=−6067 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 212 (69).

4.4.6. (−)-(4*a*S,6S,8*a*S)-trans-*6-(1,1-Dimethylethyl)-octahydro-2*H-*1-benzopyran-2-one* **9**

Oil; IR (film): 1740 (–O–C=O); ^{1}H NMR, δ, ppm: 3.84 (1H, dt, J_1=J_2 10.5, J_3 4.4, H-8a), 2.68 (1H, m, H-3), 2.54 (1H, m, H-3), 2.15 (1H, m, H-8), 1.87 (3H, m, H-4, H-5, H-7), 1.50 (3H, m, H-4, H-4a, H-8), 1.11 (2H, m, H-6, H-7), 0.87 [9H, s, $(CH_3)_3$], 0.86 (1H, m, H-5); ^{13}C NMR, δ, ppm: 171.9 (s, C-2), 83.6 (d, C-8a), 47.2 (d, C-6), 38.7 (d, C-4a), 32.4 [t, C-8 and s, $C(CH_3)_3$], 32.1 (t, C-5), 29.9 (t, C-3), 27.7 [3q, $(CH_3)_3$], 26.7 (t, C-4), 25.0 (t, C-7); MS, m/z: 195 ($M-CH_3^+$, 4), 167 (4), 155 (15), 136 (9), 94 (19), 57 (100), 41 (31); 96% e.e. (γ-cyclodextrin); $[\alpha]_D^{25}$=−33.5 (*c* 0.37, CH_3OH); $[\Theta]_{235}$=+694, $[\Theta]_{208}$=−1344, $[\Theta]_{191}$=−4480 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 213 (90).

4.5. α-Methylated lactones

4.5.1. (−)-(3S,4aS,8aS)-cis-3-Methyl-octahydro-2H-1-benzopyran-2-one **11** (in admixture with 3% of **12**)

IR (Nujol): 1735 (–O–C=O); ^{1}H NMR, δ, ppm: 4.50 (1H, pseudo q, W_H 8.8, H-8a), 2.62 (1H, ddq, W_H 28.8, H-3), 2.01 (1H, m, H-8), 1.90 (2H, m, H-4, H-4a), 1.74 (1H, m, H-6), 1.68 (1H, ddd, H-4), 1.51 (5H, m, 2 H-5, 2 H-7, H-8), 1.30 (4H, d and m, CH_3 and m); ^{13}C NMR, δ, ppm: 175.1 (s, C-2), 79.2 (d, C-8a), 34.2 (t, C-4), 33.3 (d, C-4a), 31.4 (d, C-3), 30.7 (t, C-8), 24.9 (t, C-5), 24.5 (t, C-6), 19.5 (t, C-7), 17.9 (q, CH_3); MS, m/z: 124 (8), 109 (10), 98 (14), 95 (24), 82 (96), 81 (59), 67 (100), 56 (43); 98% e.e.; $[\alpha]_D^{25}$=−30.0 (*c* 0.12, CH_3OH); $[\Theta]_{233}$=+1474, $[\Theta]_{210}$=−884, $[\Theta]_{193}$=−8463 (CH_3OH); UV, λ_{max} (ε, M^{-1} cm^{-1}): 215 (106).

4.5.2. (−)-(3R,4aS,8aS)-cis-3-Methyl-octahydro-2H-1-benzopyran-2-one **12** [in admixture with **11** (13%)]

IR (Nujol): 1740 (–O–C=O); ^{1}H NMR, δ, ppm: 4.50 (1H, pseudo q, W_H 11.3, H-8a), 2.60 (1H, ddq, H-3), 2.33 (1H, dt, J_1=J_2 8.7, J_3 13.7, H-4), 2.00 (2H, m, H-4a and H-8), 1.64 (3H, m, H-5, H-7 and H-8), 1.51 (2H, m, H-6 and H-7), 1.31 (2H, m, H-5 and H-6), 1.19 (3H, d, *J* 6.7, CH_3), 1.04 (1H, dt, J_1=J_2 13.7, J_3 4.0, H-4); ^{13}C NMR, δ, ppm: 176.6 (s, C-2), 75.8 (d, C-8a), 33.5 (t, C-4), 33.2 (d, C-4a), 32.5 (d, C-3), 29.7 (2t, C-6, C-8), 24.6 (t, C-5), 20.3 (t, C-7), 15.8 (q, CH_3); MS, m/z: 124 (7), 109 (10), 98 (12), 95 (22), 82 (89), 81 (55), 67 (100), 56 (51); 98% e.e.; $[\alpha]_D^{25}$=−67.7 (*c* 0.13, CH_3OH); $[\Theta]_{216}$=−11114 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 211 (107).

4.5.3. (−)-(3S,4aR,6R,8aS)-cis-6-(1,1-Dimethylethyl)-3-methyl-octahydro-2H-1-benzopyran-2-one **13**

Mp 147°C (from light petroleum); elemental analysis: calculated: C, 74.95%; H, 10.78%; found: C, 74.7%; H, 10.9%; IR (Nujol): 1730 (–O–C=O); ^{1}H NMR ($CDCl_3$+10% C_6D_6), δ, ppm: 4.32 (1H, bq, W_H 8.9, H-8a), 2.51 (1H, ddq, J_1 11.7, J_2 7.3, J_3 7.3, H-3), 2.06 (1H, dq, J_1=J_2=J_3 2.9, J_4 14.2, H-8), 1.73 (1H, ddd, J_1 2.4, J_2 7.3, J_3 13.7, H-4), 1.66 (1H, m, W_H 21.4, H-4a), 1.60 (1H, ddd, H-4), 1.54 (3H, m), 1.53 (1H, m, H-7), 1.38 (2H, m, H-5+H-8), 1.24 (3H, d, *J* 7.3, CH_3), 1.22 (1H, m, H-7), 1.12 (1H, m, H-5), 1.02 (1H, m, H-6), 0.83 [9H, s, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$+10% C_6D_6), δ, ppm: 175.0 (s, C-2), 78.6 (d, C-8a), 47.1 (d, C-6), 34.8 (t, C-4), 34.4 (d, C-4a), 32.4 [s, *C*$(CH_3)_3$], 31.3 (t+d, C-8+C-3), 27.3 [3q, $(CH_3)_3$], 25.8 (t, C-5), 20.3 (t, C-7), 17.7 (q, CH_3); MS, m/z: 209 ($M-CH_3^+$, 3), 168 ($M-C_4H_8^{+\cdot}$, 75), 123 (16), 95 (100), 87 (50), 82 (37), 81 (32), 69 (16), 67 (26), 57 ($C_4H_9^+$, 70), 55 (32), 43 (19), 41 (62); e.e. 99%; $[\alpha]_D^{25}$=−4.4 (*c* 0.04, CH_3OH); $[\Theta]_{234}$=+1543, $[\Theta]_{210}$=−1441 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 216 (89).

4.5.4. (−)-(3R,4aR,6R,8aS)-cis-6-(1,1-Dimethylethyl)-3-methyl-octahydro-2H-1-benzopyran-2-one **14**

Mp 87–88°C (from light petroleum); IR (Nujol): 1740 (–O–C=O); ^{1}H NMR, δ, ppm: 4.44 (1H, bq, W_H, 8.0, H-8a), 2.60 (1H, m, H-3), 2.42 (1H, dt, J_1=J_2 9.5, J_3 13.7, H-4), 2.15 (1H, m, H-8), 1.97 (1H, m, H-6), 1.54 (3H, m), 1.31 (1H, m), 1.18 (3H, d, *J* 6.8, CH_3), 1.00 (2H, m), 0.84 [9H, s, $C(CH_3)_3$]; ^{13}C NMR, δ, ppm: 176.8 (s, C-2), 74.6 (d, C-8a), 47.1 (d, C-6), 34.1 (t, C-4), 33.8 (d, C-4a), 32.3 (d, C-3), 32.0 [s, *C*$(CH_3)_3$], 30.9 (t, C-5), 30.1 (t, C-8), 27.3 [3q, $(CH_3)_3$], 20.7 (t, C-7), 15.5 (q, CH_3); MS, m/z: 209 ($M-CH_3^+$, 2), 168 (95), 150 (10), 123 (11), 95 (100), 87 (46), 81 (27), 67 (25), 57 (38), 55 (31); 99% e.e.; $[\alpha]_D^{25}$=−34.3 (*c* 0.14, CH_3OH); $[\Theta]_{216}$=−11909 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 214 (172).

*4.5.5. (+)-(4a*R*,6*R*,8a*S*)-*cis*-3,3-Dimethyl-6-(1,1-dimethylethyl)-octahydro-2*H*-1-benzopyran-2-one* ***15***

Mp 103°C (from light petroleum); elemental analysis: calculated: C, 75.58%; H, 10.99%; found: C, 74.4%; H, 11.0%; IR (Nujol): 1740 (–O–C=O); ^{1}H NMR, δ, ppm: 4.60 (1H, bq, H-8a), 2.13 (1H, m, H-8), 2.04 (1H, dd, J_1 7.3, J_2 14.2, H-4), 1.88 (1H, m, H-6), 1.66–1.34 (5H, m), 1.32 (3H, s, CH_3), 1.30 (3H, s, CH_3), 1.11 (2H, m), 0.85 [9H, s, $C(CH_3)_3$]; ^{13}C NMR, δ, ppm: 178.9 (s, C=O), 76.6 (d, C-8a), 47.8 (d, C-4a), 41.8 (t, C-4), 36.4 (s, C-3), 35.1 (d, C-6), 32.7 [s, $C(CH_3)_3$], 31.3 (t, C-8), 30.9 (q), 30.3 (q), 28.5 (t, C-5), 27.7 [q, $(CH_3)_3$], 20.6 (t, C-7); MS, m/z: 223 ($M-CH_3^+$, 2), 182 ($M-C_4H_8^{+\cdot}$, 15), 137 (20), 126 (37), 94 (65), 88 (23), 82 (21), 81 (37), 80 (21), 69 (22), 67 (21), 57 (100), 55 (29), 43 (20), 41 (56); 99% e.e.; $[\alpha]_D^{25}$=+4.9 (*c* 0.08, CH_3OH); $[\Theta]_{221}$=−4204 (CH_3OH); UV (CH_3OH), λ_{max} (ε, M^{-1} cm^{-1}): 212 (194).

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