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A Study of Baker's Yeast Reduction of Piperidone-carboxylates

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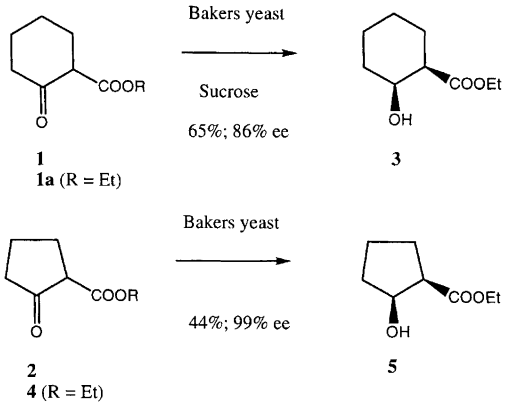
The stereoselective baker's yeast reduction of various *N*-protected piperidone-carboxylic acids have been studied, and the enantioselectivity was found to be widely dependent on whether fermenting or non-fermenting conditions were employed. Thus reaction of *N-tert*-butoxycarbonyl-4-oxopiperidine-3-carboxylic acid ethyl ester (**6**) with fermenting baker's yeast gave almost racemic *N-tert*-butoxycarbonyl-4-hydroxypiperidine-3-carboxylic acid ethyl ester (**7**), however, with complete diastereoselectivity. Reduction of **6** with non-fermenting yeast gave **7** with a 24–41% enantiomeric excess. Similarly, reduction of *N-tert*-butoxycarbonyl-3-oxopiperidine-4-carboxylic acid ethyl ester (**17**) with fermenting baker's yeast gave racemic *N-tert*-butoxycarbonyl-3-hydroxypiperidine-4-carboxylic acid ethyl ester [(±)-**18**] diastereoselectively. A convenient method for determining the enantiomeric excess of the hydroxypiperidine carboxylic acids derivatives was found in the reaction with Sanger's reagent followed by HPLC on a chiral column.

In a project aimed at obtaining hydroxypiperidine-carboxylic acids for peptide synthesis we became intrigued by the possibility of obtaining these compounds in a simple manner through baker's yeast reduction of piperidone-carboxylates.

Baker's yeast can be used for the stereoselective reduction of ketones, and reduction of β-keto esters is a particularly favorable case. Furthermore the baker's yeast reduction of cyclic β-keto esters such as alkyl cyclohexanone-2-carboxylates (**1**) and alkyl cyclopentanone-2-carboxylates (**2**) have been found to be both highly diastereoselective and enantioselective.[1] Thus 2-ethoxycarbonylcyclohexanone (**1a**) is reduced by baker's yeast to give almost only one of four possible stereoisomers **3** (Scheme 1).[2,3] The high degree of selectivity is caused, first by the high enantioselectivity of the reduction of the ketone by the yeast dehydrogenases, second, by a preference for reduction of one enantiomer of the ketone, and third, by *in situ* equilibration of the non-preferred enantiomer of the ketone into the preferred one. Similarly 2-ethoxycarbonylcyclopentanone (**4**) is reduced to (1*S*,2*R*)-2-ethoxycarbonylcyclopentanol (**5**) with an ee of >99%.[3]

This reaction seemed highly useful for enantioselective synthesis of chiral hydroxypiperidinecarboxylic acids, because 4-oxopiperidine-3-carboxylates or 3-oxo-piperidine-4-carboxylates are readily available. Thus



Scheme 1. Baker's yeast reduction of some keto esters.

4-hydroxypiperidine-3-carboxylic acid or 3-hydroxy-piperidine-4-carboxylic acid might be obtained optically pure with baker's yeast.

Indeed we found in the literature that the former reaction had recently been investigated.[4] Thus *N-tert*-butoxycarbonyl-3-ethoxycarbonylpiperidin-4-one (**6**) had been reported to be reduced by fermenting baker's yeast to give ethyl (3*R*,4*S*)-*N-tert*-butoxycarbonyl-3-ethoxy-carbonylpiperidin-4-ol (**7**) with more than 93% ee. Our initial aim was therefore to repeat this reaction and deprotect the product to a new amino acid as well as studying the reduction of other piperidones. In this paper we report the results of these studies and the surprising

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finding that baker's yeast reduction of these piperidones proceeds with low enantioselectivity but complete diastereoselectivity.

Results and discussion

To investigate thoroughly the baker's yeast reduction of **6**, we prepared a number of protected derivatives of the commercially available 3-ethoxycarbonylpiperidin-4-one (**8**). Compound **8** was reacted with 9-fluorenylmethyl chloroformate, acetic anhydride and di-*tert*-butyl dicarbonate to give **9**, **10** and **6**, respectively, in 67–98% yield. Notably, NMR spectroscopy revealed that keto ester **6** was exclusively on the enol form (Scheme 2).

Baker's yeast reduction of all three compounds was attempted under fermenting conditions.[3] However only **6** was reduced satisfactorily. Compound **9** was not reduced, perhaps because it was too insoluble in water. Compound **10** might have been reduced, but the polar products were extremely difficult to extract from the sugar-rich aqueous medium. Reduction of **6** under fermenting conditions gave, in our hands, only one diastereoisomer, the *cis*-alcohol **7**, together with some unreduced **6** (Scheme 3). However, compound **7** was obtained with a rotation of $+0.2°$, in sharp contrast with the previously published $+25°$.[4] This result suggested that the **7** obtained was almost racemic. This was verified by removal of the BOC-group from (±)-**7** with TFA and reaction with Sanger's reagent (2,4-dinitrofluorobenzene), which gave yellow arylpiperidine (±)-**11** in 87% yield. HPLC of (±)-**11** on a chiral column measuring at 390 nm showed both enantiomers to be present in identical amounts (Fig. 1).

Variation in reaction time or source of baker's yeast did not change the course of reaction or give the anticipated result. Prolonging the reaction time up to 5 days even led to negative rotation ($[\alpha]_D - 4° \approx 7\%$ ee), i.e. the reverse selectivity compared with the literature. Common baker's yeast and yeast obtained from Sigma gave identical results (Table 1). These findings were disturbing because (1) they directly conflicted with the previous specific reports on baker's yeast reduction of **6** and (2) Seebach and coworkers have reduced many similar compounds with great succes. For example, baker's yeast reduction of **12** was highly enantioselective (98% ee).[3] To check our yeast on a known system we reduced 2-ethoxycarbonylcyclohexanone **1a** and obtained **3** with a rotation identical with that reported by Seebach *et al.*[2] (Table 1).

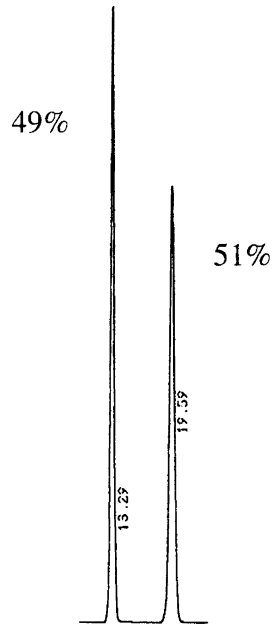
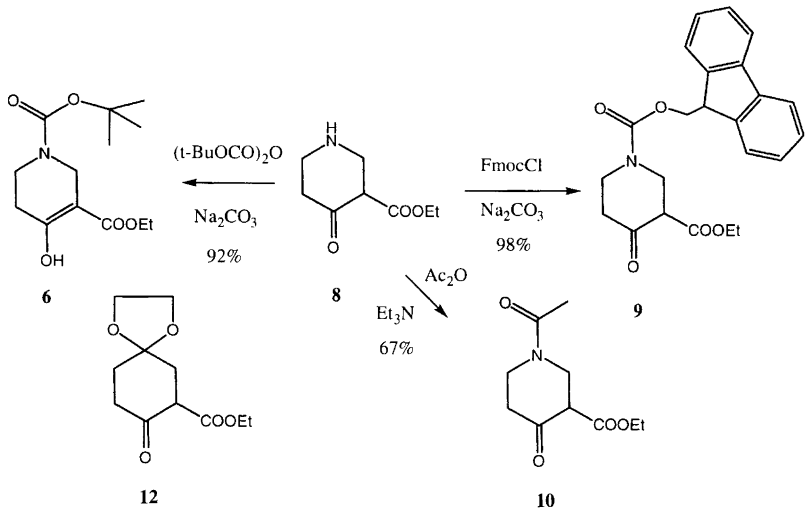
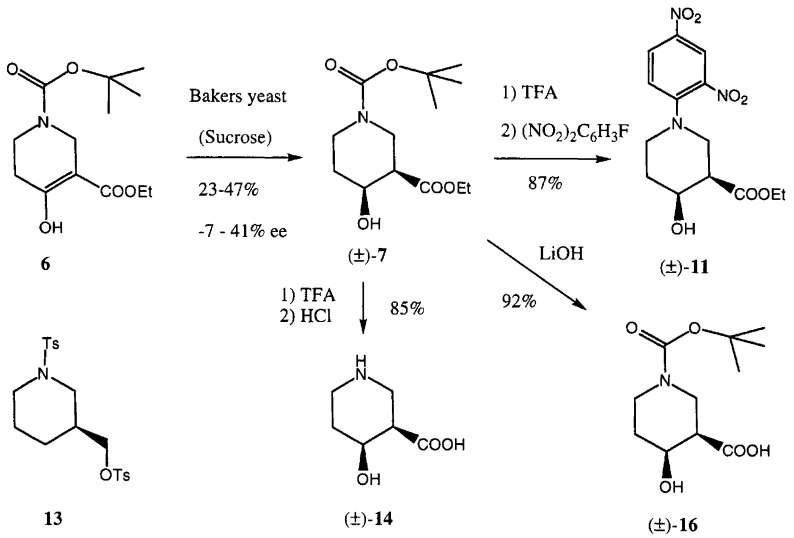


Fig. 1. Chiral HPLC of compound **11** made with fermenting yeast.

Changing to non-fermenting conditions has been reported by Seebach and coworkers to improve stereoselectivity. This was found to increase the enantioselectivity of the reduction of **6** significantly. Thus, without sugar added, **7** was obtained with a rotation of $+15°$ to



Scheme 2. N-Protection of **8**.



Scheme 3. Synthesis and transformations of **7**.

Table 1. Baker's yeast reduction of **1a**, **6** and **17**.

Substrate	Product	g yeast/ g substrate	g sugar/ g yeast	*t*/day	Yield (%)	Recovered (%)	Rotation/°	ee (%)
6	**7**	7.1	1.5	1	39	28	+0.2	0
6	**7**	2.8	4	1	47	27	+0.4	—
6	**7**	5.9	1.5	3	34	55	−2	—
6	**7**	6.9	1.5	5	23	43	−4	—
6	**7**	7.4[a]	1[b]	1	46	24	+3	—
6	**7**	18	0	1	39	7	+15	24
6	**7**	2.0	0	1	37	33	+23	41
6	**7**	7.4[a]	0[c]	1	6	57	+12.6	—
17	**18**	13.3	1.5	3	40	10	0	—
17	**18**	17	0	1	11	0	+2.8	12
1a	**3**	6[a]	0	1	21	20	+23.6	—

[a]Yeast from Sigma used. [b]Allyl alcohol added. [c]Ethyl chloroacetate added.

+23°, which was close to the +25° reported for fermenting conditions. However unlike that report we did not find that our material had high optical purity. The +15° material was found, by conversion into **11**, to have an ee of 24% (Fig. 2). The +23° compound was found to have an ee of 41%. Thus non-fermenting conditions improved the stereoselectivity and almost reversed the selectivity.

Knight *et al.* found that **7** with a rotation of +25° was at least 93% enantiomerically pure, while these results suggest an ee of 40–45%. The discrepancy between this and our results is not clear, but unless an error in measurement of rotation has occurred the following can be argued. Knight *et al.*'s conclusion is based on a seven step conversion of **7** into the known ditosylate **13**, which was found by comparison of rotation and chiral shift NMR spectra to be almost optically pure. Though column fractions and mother liquors were checked to see whether enantiomeric enrichment was occurring during the synthesis, given the relative length of the synthesis, enantiomeric enrichment may have occurred unnoticed.

It is however not clear why we did not get any

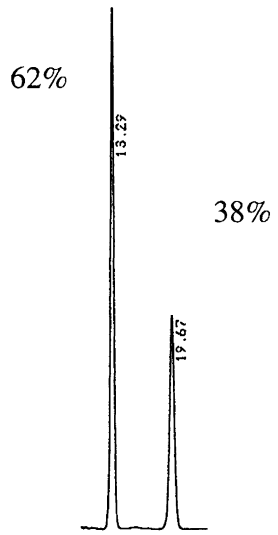


Fig. 2. Chiral HPLC of compound **11** made with non-fermenting yeast.

enantioselectivity with fermenting baker's yeast, and had to carry out a non-fermenting reaction to get a rotation similar to Knight *et al.* Apparently those authors did not carry out the non-fermenting reaction. Since they did not publish a detailed experimental procedure for the transformation there might be differences in experimental procedures.

To obtain some amino acid building blocks a racemic batch of **7** was deprotected using TFA to give piperidinecarboxylic acid (±)-**14**. (±)-**14** was then reacted with 9-fluorenylmethyl chloroformate to give the Fmoc amino acid (±)-**15**. Alternatively the ethyl ester of (±)-**7** was saponified with LiOH in THF to give the acid (±)-**16** in 92% yield.

We also carried out baker's yeast reduction of ketone (±)-**17** (Scheme 4). Known *N*-benzyl-4-ethoxycarbonylpiperidin-3-one was first hydrogenolysed to the amine using palladium-on-carbon as the catalyst, and then BOC-protected by reaction with di-*tert*-butyl dicarbonate which gave (±)-**17** in 99% yield. Like **6**, the keto ester **17** was also exclusively in enol form. Reduction of **17** with fermenting baker's yeast was diastereoselective giving a 37% yield of *cis* reduction product **18** together with 10% of unchanged **17**.

The reduction gave no enantioselectivity however ($[\alpha]_D$ 0°). In another experiment (Table 1) shorter reaction time gave a material with a small rotation. When the BOC group was removed, and the resulting piperidine reacted with Sanger's reagent to give **19**, then chiral HPLC of **19** revealed it to be an almost racemic mixture having only 12% e.e (Fig. 3). The ethyl ester of (±)-**18** was hydrolysed with LiOH to give acid (±)-**20** in 63% yield.

It may seem puzzling that these reactions lack the enantioselectivity usually observed in baker's yeast reductions of cyclic β-keto esters, but have maintained the diastereoselectivity; especially because the diastereoselectivity has often been explained as a preferred enantioselective reduction of one stereoisomer of the ketone and rapid racemisation of the wrong isomer.[5] It may seem unlikely that one stereoisomer of **6** was reduced from one side by the yeast, while the other stereoisomer was reduced from the opposite site. NMR spectroscopy of **6** and **17** revealed that both compounds were mostly in the enol form. It may therefore be argued that the baker's yeast reduction of **6** and **17** occurred by conjugate addition to the enone catalysed by enoate reductases. However, as such reductions are known to give *anti* addition of hydrogen to the double bond,[6] which would give *trans* isomers in this case, involvement of enoate reductases is unlikely. Thus one stereoisomer of the ketone must be reduced from one side, and the enantiomer from the opposite side (Fig. 4).

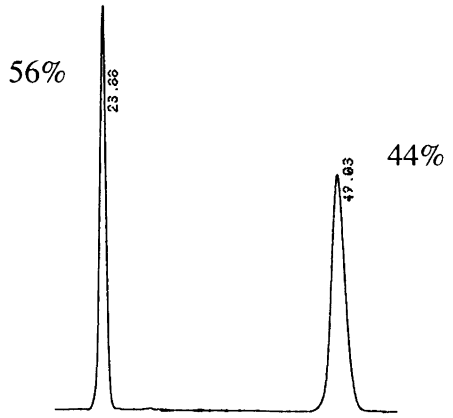
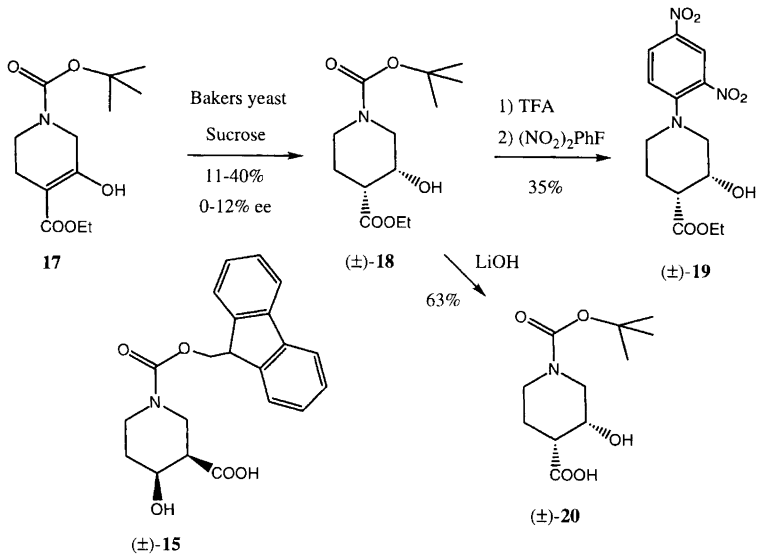


Fig. 3. Chiral HPLC of compound **19**.

It is likely that these reactions are carried out by at least two different dehydrogenases, as evidence from β-keto ester reductions suggests that several enzymes with different selectivities play a role in baker's yeast reductions.[7] It has been found that adding compounds



Scheme 4. Synthesis and transformations of **18**.

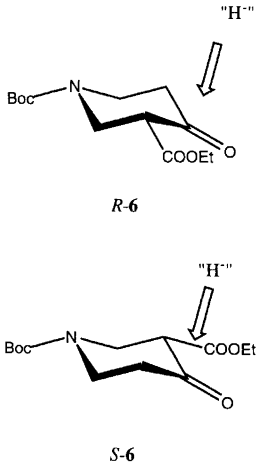


Fig. 4. Preferred reduction face of the two enantiomers of **6**.

that can inhibit one of these enzymes can dramatically change the stereochemical outcome of the reaction.[8,9] For example addition of ethyl chloroacetate inhibited the socalled 'D-enzyme', while allyl alcohol inhibited the 'L-enzyme'.

In our case the L-enzyme might be responsible for formation of the (3*R*,4*S*)-isomer, while the D-enzyme might be responsible for formation of the (3*S*,4*R*)-isomer. Thus we tried to improve the selectivity by adding allyl alcohol to the fermenting yeast reduction of **6** and ethyl chloroacetate to the non-fermenting reduction of **6** expecting the former reaction to give more (3*S*,4*R*)-isomer and the latter more (3*R*,4*S*)-isomer. Exactly the opposite happened; in both cases the selectivity decreased slightly (Table 1).

This work has shown that baker's yeast reduction of piperidines containing the β-keto ester moiety proceeds with low enantioselectivity. Thus the piperidones **6** and **17** had structures very close to compounds that were previously known to be reduced with very high stereoselectivity. Nevertheless **6** and **17** were reduced with no or very low stereoselectivity. Therefore these transformations, in our opinion, must be regarded as unpredictable even for closely related substrates, and the optical purity of the products should be regarded with the highest degree of suspicion. These results contrast the general impression obtained in the literature regarding baker's yeast reduction of cyclic β-keto esters[1,2] and also the specific report on reduction of **6**.[4] It is unlikely that the discrepancies are a result of the use of different strains of yeast because (a) we tried yeast from different sources with identical results and (b) our yeast reduced **1a** to **3** with the same high stereoselectivity as was obtained by Seebach *et al.*[4] The conflict of our results with those of Knight *et al.* seems to partly have been caused by an erroneous measurement of either the enantiomeric excess or the rotation on their part. In the former case subtle differences in yeast or experimental set-up apparently gave those authors a better enantiomeric excess.

Experimental

General. ^{13}C NMR and ^{1}H NMR spectra were recorded on a Varian Instruments Gemini 200. When $CDCl_3$ was used as the solvent Me_4Si and $CDCl_3$ (^{13}C NMR: δ 76.93) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Concentrations were performed on a rotary evaporator at a temperature below 40 °C. HPLC was performed on a Chiralcel AD column from Daicel Chemical Industries Ltd.

N-tert-*Butoxycarbonyl-3-ethoxycarbonyl-4-piperidone* (**6**). 3-Ethoxycarbonyl-4-piperidone hydrochloride (**8**, 2.08 g, 10 mmol) was dissolved in 50% aqueous THF (30 ml), and Na_2CO_3 (1.09 g, 10.2 mmol) was added. At 0 °C a solution of di-*tert*-butyl dicarbonate (2.48 g, 11.4 mmol) and Na_2CO_3 (1.07 g, 12 mmol) in 50% aqueous THF (20 ml) was added. The solution was kept for 30 min at 0 °C and then allowed to reach room temperature over 2 h. The mixture was acidified with conc. HCl, and water (20 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 20 ml), dried ($MgSO_4$) and concentrated to give a crystalline product (2.5 g, 92%). M.p. 52–56 °C. TLC: (EtOAc–pentane 10:1) R_f 0.5. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.1 (s, 1 H, OH), 4.21 (q, 2 H, *J* 7 Hz, CH_2CH_3), 4.04 (s, 2 H, H-2), 3.53 (t, *J* 6 Hz, H-6), 2.35 (t, 2 H, *J* 6 Hz, H-5), 1.45 [s, 9 H, $(CH_3)_3$], 1.23 [t, 3 H, *J* 7 Hz, CH_2CH_3]. ^{13}C NMR ($CDCl_3$, 200 MHz): δ 171.2 (*C*OOEt), 170.3 (C-4), 155.0 (N*C*OO), 96.7 (C-3), 80.6 [*C*$(CH_3)_3$], 61.0 (*C*H_2CH_3), 40.7, 40.1, 29.4 (C-2, C-5, C-6), 28.9 [C(*C*$H_3)_3$], 14.7 (CH_2CH_3). MS (EI): *m/z* 271 (M^+), 171 ($M-C_5H_8O_2$).

3-Ethoxycarbonyl-N-(9-fluorenylmethoxycarbonyl)-4-piperidone (**9**). 3-Ethoxycarbonyl-4-piperidone hydrochloride (**8**, 0.87 g, 4.2 mmol) was dissolved in 50% aqueous THF (15 ml) and Na_2CO_3 (0.56 g, 5.3 mmol) was added. At 0 °C a solution of 9-fluorenylmethoxycarbonyl chloride (1.40 g, 5.4 mmol) and Na_2CO_3 (0.60 g, 5.7 mmol) in 50% aqueous THF (10 ml) was added. The solution was kept for 30 min at 0 °C, and then allowed to reach room temperature overnight. The mixture was acidified with conc. HCl, and water (20 ml) was added. The mixture was extracted with EtOAc (3 × 20 ml), dried ($MgSO_4$) and concentrated to give a crystalline product (1.6 g, 98%). M.p. 92–94 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.85–7.33 (m, 8 H, Ar), 4.53 (m, 2 H, NCOOCH_2), 4.33–4.20 (m, q, 3 H, *J* 7 Hz, COOCH_2C*H*, CH_2CH_3), 4.08 (m, 2 H, H-2), 3.62 (m, 2 H, H-6), 2.35 (m, 2 H, H-5), 1.37 (t, 3 H, *J* 7 Hz, CH_2CH_3). MS (EI): *m/z* 393 (M^+).

N-*Acetyl-3-ethoxycarbonyl-4-piperidone* (**10**). 3-Ethoxycarbonyl-4-piperidone hydrochloride (**8**, 2.00 g, 9.6 mmol) was dissolved in EtOH (12.5 ml) and Et_3N (2.42 g, 23.9 mmol), and Ac_2O (2.40 g, 22.4 mmol) was added. The solution was kept for 2 h at room temperature and then concentrated. Water (10 ml) was added, and

the mixture was extracted with EtOAc (3 × 20 ml), dried ($MgSO_4$) and concentrated to give a crystalline product (1.37 g, 67%). M.p. 50–52 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.30–4.21 (q, 2 H, *J* 7 Hz, CH_2CH_3), 4.19–4.09 (m, 1 H, H-3), 3.75–3.70 (m, 2 H, H-2), 3.69–3.58 (m, 2 H, H-6), 2.42–2.31 (m, 2 H, H-5), 2.17 (s, 3 H, Ac), 1.33 (t, 3 H, *J* 7 Hz, CH_2CH_3). MS(EI): *m/z* 213 (M^+), 184 ($M-CH_2CH_3$), 170 ($M-COCH_3$), 168 ($M-OCH_2CH_3$), 140 (M-$COOCH_2CH_3$), 43 ($COCH_3$).

Baker's yeast reduction under fermenting conditions. General procedure. Baker's yeast (10 g) was dissolved in tap water (80 ml) at 30 °C, and sucrose (15 g) was added. [Optionally, inhibitor (allyl alcohol, 6.3 mmol) was added at this stage and the mixture was incubated for 30 min at this temperature.] After 1 h at 30 °C, compound **6**, **9**, **10** or **17** (6.3 mmol) was added, and the reaction was kept at 30 °C for 18 h. Further sucrose (10 g) in H_2O (25 ml) at 40 °C was added, and the mixture was kept for 2 days at 30 °C. Celite (1 g) was added, and the mixture was filtered. The filter was washed with $CHCl_3$ (3 × 20 ml), which was subsequently used to extract the filtrate. The combined $CHCl_3$ phase was dried ($MgSO_4$), concentrated and subjected to flash chromatography. Eluting with the appropriate EtOAc–pentane mixture gave first unchanged ketone, then product.

*Specific details. Ethyl 3,4-*cis-N-tert-*butoxycarbonyl-4-hydroxypiperidine-3-carboxylate* (**7**). Elution with EtOAc–pentane 1:15 gave unchanged **6** (0.93 g, 54%), while elution with EtOAc gave (±)-**7** (0.59 g, 34%, m.p. 56–60 °C, $[\alpha]_D^{25}$ −2 (*c* 0.75, CH_2Cl_2)).

*Ethyl 3,4-*cis-N-tert-*butoxycarbonyl-3-hydroxypiperidine-4-carboxylate* (**18**). From **17** (0.400 g, 1.48 mmol) was obtained on elution with EtOAc–pentane 1:10 unchanged **17** (0.039 g, 10%). Elution with EtOAc gave **18** (0.150 g, 37%). $[\alpha]_D^{25}$ +1.6 (*c* 1.61; $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.19 (q, 3 H, *J* 7 Hz, CH_2CH_3, H-3), 4.07–3.96 (m, 2 H, H-2), 3.01–2.77 (m, 2 H, H-6), 2.59–2.50 (m, 2 H, H-4, OH), 2.16–1.77 (m, 2 H, H-5), 1.46 [s, 9 H, $(CH_3)_3$], 1.27 (t, 3 H, *J* 7 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 174.2 (*C*OOEt), 156.1 (N*C*OO), 80.4 [*C*$(CH_3)_3$], 65.9, 61.4 (C-3, *C*H_2CH$_3$), 49.5, 45.8, 43.3, 23.1 (C-2, C-4, C-5, C-6), 28.8 [C(*C*H_3)$_3$], 14.6 (CH_2CH_3).

Non-fermenting conditions. General procedure. Baker's yeast (20 g) was dissolved in tap water (150 ml) at 30 °C. [Optionally inhibitor (ethyl chloroacetate, 4.1 mmol) was added at this stage and the mixture was incubated for 30 min at this temperature.] After 15 min **6** or **17** was added (4.1 mmol). The reaction was kept at 30 °C for 24 h. Celite (1 g) was added, and the mixture was filtered. The filter was washed with EtOAc (3 × 100 ml), which was subsequently used to extract the filtrate. The combined EtOAc phase was dried ($MgSO_4$), concentrated and subjected to flash chromatography. Elution with the appropriate EtOAc–pentane mixture gave first unchanged ketone then product.

*Specific details. Ethyl 3,4-*cis-N-tert-*butoxycarbonyl-4-hydroxypiperidine-3-carboxylate* (**7**). Elution with EtOAc–pentane 1:10 gave unchanged **6** (78 mg, 7%), while elution with EtOAc gave **7** (0.44 g, 39%), $[\alpha]_D^{25}$ +23.2 (*c* 3.85, CH_2Cl_2), ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.28 (ddd, 1 H, J_{4eq5ax} 3 Hz, J_{4eq5eq} 3 Hz, J_{4eq3ax} 5 Hz, H-4eq), 4.19 (q, 2 H, *J* 7 Hz, CH_2CH_3), 3.99 (br dd, 1 H, J_{2eq3ax} 3 Hz, J_{2eq2ax} 13 Hz, $J_{2eq6eq} \approx 0$, H-2eq), 3.72 (dt, 1 H, J_{6eq5eq} 4 Hz, J_{6eq5ax} 4 Hz, J_{6eq6ax} 13 Hz, H-6eq), 3.40 (dd, 1 H, J_{2ax3ax} 10 Hz, H-2ax), 3.27 (ddd, 1 H, J_{6ax5ax} 11 Hz, H-6ax), 2.62 (ddd, 1 H, H-3ax), 1.82 (dddd, 1 H, J_{5eq5ax} 14 Hz, H-5eq), 1.63 (dddd, 1 H, H-5ax), 1.45 [s, 9 H, $(CH_3)_3$], 1.27 (t, 3 H, *J* 7 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 173.8 (*C*OOEt), 155.2 (N*C*OO), 80.3 [*C*$(CH_3)_3$], 65.5, 61.5 (C-4, *C*H_2CH$_3$), 46.4, 41.2, 39.2, 31.9 (C-2, C-3, C-5, C-6), 28.9 [C(*C*H_3)$_3$], 14.6 (CH_2CH_3). MS (EI): *m/z* 273 (M^+), 200 ($M-COOEt$), 172 ($M-COOCMe_3$).

*3,4-*cis-*4-hydroxypiperidine-3-carboxylic acid, hydrochloride* (**14**). Hydroxy ester (±)-**7** (0.203 g, 0.75 mmol) was dissolved in CH_2Cl_2 (10 ml). CF_3COOH (10 ml) was added. The solution was stirred for 15 min, after which it was concentrated. TLC: (butanol–acetic acid–water 4:1:1) R_f 0.25. ^{1}H NMR (D_2O, 200 MHz): δ 4.48–4.45 (m, 1 H, H-4), 4.22–4.11 (q, 2 H, *J* 7 Hz, CH_2CH_3), 3.43–3.18 (m, 4 H, H-2, H-6), 3.06–2.97 (m, 1 H, H-3), 2.0–1.92 (m, 2 H, H-5), 1.20 [t, 3 H, *J* 7 Hz, CH_3 (Et)]. ^{13}C NMR (D_2O, 200 MHz): 174.9 (COOEt), 65.8, 65.3 (C-4, *C*H_2CH$_3$), 46.1, 42.2, 41.5, 31.2 (C-2, C-3, C-5, C-6), 16.1 (CH_2CH_3). 3,4-*cis*-3-ethoxycarbonyl-4-hydroxypiperidine was dissolved in 1 M HCl (20 ml) and refluxed at 100 °C for 3 h. The mixture was concentrated to give a crystalline product (0.114 g, 85%). M.p. 191–194 °C. TLC: (butanol–acetic acid–water 4:1:1) R_f 0. ^{1}H NMR (D_2O, 200 MHz): δ 4.47–4.42 (m, 1 H, H-4), 3.43–3.16 (m, 4 H, H-2, H-6), 3.04–2.95 (m, 1 H, H-3), 2.0–1.92 (m, 2 H, H-5). ^{13}C NMR (D_2O, 200 MHz): δ 176.4 (COOH), 65.7 (C-4), 45.7, 42.2, 41.5, 31.0 (C-2, C-3, C-5, C-6).

*3,4-*cis-N-*(9-fluorenylmethoxycarbonyl)-4-hydroxypiperidine-3-carboxylic acid*, (±)-**15**. Amino acid (±)-**14** (114 mg, 0.63 mmol, ee 1.5%) was dissolved in 50% aqueous THF (5 ml) and Na_2CO_3 (83 mg, 0.78 mmol) was added. At 0 °C, a mixture of 9-fluorenylmethoxycarbonyl chloride (224 mg, 0.87 mmol) and Na_2CO_3 (92 mg, 0.87 mmol) in 50% aqueous THF (6 ml) was added and the mixture was allowed to reach room temperature overnight, after which it was acidified with conc. HCl, and water (20 ml) was added. The mixture was extracted with EtOAc (3 × 20 ml), dried ($MgSO_4$) and concentrated. The product was subjected to flash chromatography (EtOAc–pentane 1:4–4:1, 1% HCOOH) to give (±)-**15** (51 mg, 22%). M.p. 68–71 °C. $[\alpha]_D^{25}$ −2 (*c* 0.59, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz):

δ 7.76–7.30 (m, 8 H, Ar), 4.45 (d, 2 H, *J* 5.5 Hz, $NCOOCH_2$), 4.34 (m, 1 H, H-4), 4.23 (t, 1 H, *J* 5.5 Hz, $COOCH_2CH$), 4.16–3.25 (m, 4 H, H-2, H-6), 2.75–2.41 (m, 1 H, H-3), 1.85–1.5 (m, 2 H, H-5). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 176.8 (*C*OOH), 155.9 (N*C*OO), 144.3, 141.8 (4 C, Ar), 128.2, 127.6, 125.5, 120.5 (8 C, Ar), 68.0, 65.4 (C-4, $NCOOCH_2$), 47.8, 46.1, 41.1, 39.4, 31.9 (C-2, C-3, C-5, C-6, $NCOOCH_2CH$).

3,4-cis-N-tert-*Butoxycarbonyl-4-hydroxypiperidine-3-carboxylic acid* (**16**). Ester **7** (0.216 g, 0.79 mmol, ee 24%) was dissolved in THF (2 ml). 1 M LiOH (1.6 ml) was added. The solution was stirred at 25 °C for 30 min, after which the solution was acidified with 1 M HCl, and water (10 ml) was added. The mixture was extracted with EtOAc (3 × 15 ml), dried ($MgSO_4$) and concentrated to give a crystalline product (0.178 g, 92%). M.p. 140–144 °C. $[\alpha]_D^{25}$ +14.4 (*c* 4, $CHCl_3$). ^1H NMR ($CDCl_3$, 200 MHz): δ 4.33 (ddd, 1 H, J_{4eq5ax} 3 Hz, J_{4eq5eq} 3 Hz, J_{4eq3ax} 4 Hz, H-4eq), 3.97 (br dd, 1 H, J_{2eq3ax} 3 Hz, J_{2eq2ax} 13 Hz, J_{2eq6eq} ≈0 Hz, H-2eq), 3.7 (dt, 1 H, J_{6eq5eq} 4 Hz, J_{6eq5ax} 4 Hz, J_{6eq6ax} 14 Hz, H-6eq), 3.43 (dd, 1 H, J_{2ax3ax} 10 Hz, H-2ax), 3.27 (ddd, 1 H, J_{6ax5ax} 11 Hz, H-6ax), 2.66 (ddd, 1 H, H-3ax), 1.83 (dddd, 1 H, J_{5eq5ax} 14 Hz, H-5eq), 1.66 (dddd, 1 H, H-5ax), 1.45 [s, 9 H, $(CH_3)_3$)]. ^{13}C NMR ($CDCl_3$, 200 MHz): δ 176.9 (*C*OOH), 155.5 (N*C*OO), 80.9 [$C(CH_3)_3$], 65.7 (C-4), 46.2, 41.0, 39.3, 31.9 (C-2, C-3, C-5, C-6), 28.9 [$C(CH_3)_3$]. MS (EI): *m/z* 246 (M+1), 146 ($M-C_5H_7O_2$).

Ethyl N-tert-*butoxycarbonyl-3-oxopiperidine-4-carboxylate* (**17**). Ethyl *N*-benzyl-3-oxopiperidine-4-carboxylate (0.503 g, 1.69 mmol) was dissolved in a solution of 50% aqueous EtOH (10 ml) and 3 M HCl (1 ml) and hydrogenated at 6 atm. for 48 h using 10% Pd–C (50 mg) catalyst. The mixture was filtred and concentrated to give an oil (0.305 g, 87%). ^1H NMR (D_2O, 200 MHz): δ 4.15 (q, 2 H, *J* 6 Hz, CH_2CH_3), 3.42–3.26 (m, 2 H, H-2), 3.16–3.0 (m, 2 H, H-6), 2.1–1.99 (m, 2 H, H-5), 1.2 (t, 3 H, *J* 6 Hz). ^{13}C NMR (D_2O, 200 MHz): δ 181.4, 178.7, 176.0 [COOEt, C-3′ (enol), C-3 (ketone)], 91.9 (C-4′), 64.9 (CH_2CH_3), 53.1, 52.0, 46.0, 44.1, 25.0, 21.6, 16.0 (C-2, C-2′, C-4, C-5, C-5′, C-6, C-6′), 11.9 (CH_2CH_3). Ethyl 3-oxopiperidine-4-carboxylate hydrochloride (0.305 g, 1.47 mmol) was BOC-protected using the same procedure as for **6** using aqueous THF (20 ml), Na_2CO_3 (0.176 g, 1.66 mmol) and a mixture of di-*tert*-butyl dicarbonate (0.374 g, 1.72 mmol) and Na_2CO_3 (0.181 g, 1.71 mmol) in aqueous THF (10 ml). The solution was acidified with conc. HCl and water (20 ml) was added. The mixture was extracted with CH_2Cl_2 (3 × 30 ml), dried ($MgSO_4$) and concentrated to give an oil (0.395 g, 99%). TLC: (EtOAc–pentane 10:1) R_f 0.5. ^1H NMR ($CDCl_3$, 200 MHz): δ 12.08 (s, 1 H, OH), 4.28 (q, 2 H, *J* 7 Hz, CH_2CH_3), 4.02 (s, 2 H, H-2), 3.48 (t, 2 H, *J* 6 Hz, H-6), 2.31 (t, 2 H, *J* 6 Hz, H-5), 1.46 [s, 9 H, $(CH_3)_3$], 1.30 (t, 3 H, *J* 7 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 172.3 (COOEt), 168.0 (C-3), 154.9 (NCOO), 97.4 (C-4), 80.7 [$C(CH_3)_3$], 61.1 (CH_2CH_3), 45.5, 40.7, 40.4 (C-2, C-5, C-6), 28.9 [$C(CH_3)_3$], 14.7 (CH_2CH_3).

3,4-cis-N-tert-*Butoxycarbonyl-3-hydroxypiperidine-4-carboxylic acid* [(±)-**20**]. Ester (±)-**18** (88 mg, 0.32 mmol) was dissolved in THF (0.7 ml). 1 M LiOH (0.7 ml) was added. The solution was stirred at 25 °C for 30 min. The solution was acidified with 1 M HCl and water (10 ml) was added. The mixture was extracted with EtOAc (3 × 10 ml), dried ($MgSO_4$) and concentrated to give an oil (50 mg, 63%). ^1H NMR ($CDCl_3$, 200 MHz): δ 4.28–4.00 (m, 3 H, H-3, H-2), 3.02–2.77 (m, 2 H, H-6), 2.62–2.53 (m, 1 H, H-4), 2.15–1.68 (m, 2 H, H-5), 1.45 [s, 9 H, $(CH_3)_3$]. ^{13}C NMR ($CDCl_3$, 200 MHz): δ 177.6 (*C*OOH), 156.4 (N*C*OO), 81.0 [$C(CH_3)_3$], 65.9 (C-3), 49.5, 45.6, 30.2, 22.8 (C-2, C-4, C-5, C-6), 28.9 [$C(CH_3)_3$].

Reaction of hydroxypiperidines with Sanger's reagent. Piperidide **7** or **18** (0.282 g, 1.03 mmol) was dissolved in CH_2Cl_2 (10 ml). CF_3COOH (10 ml) was added. The solution was stirred at 25 °C for 15 min and then concentrated. First saturated $NaHCO_3$ (5 ml) was added and then a mixture of 2,4-dinitrofluorobenzene (0.4 ml) in EtOH (4 ml, 99.9%) was added. The solution was stirred at 25 °C for 1 h. H_2O (10 ml) was then added and the mixture was extracted with ether (5 × 20 ml). The concentrated ether solution was dried ($MgSO_4$) and subjected to flash chromatography.

Specific details. Ethyl N-*(2,4-dinitrophenyl)-4-hydroxypiperidine-3-carboxylate* (**11**). Eluting with ether–pentane 1:4 gave **11** (0.306 g, 87%). ^1H NMR ($CDCl_3$, 200 MHz): δ 8.7 (d, 1 H, J_{meta} 3 Hz, H-3′), 8.25 (dd, 1 H, J_{ortho} 10 Hz, J_{meta} 3 Hz, H-5′), 7.18 (d, 1 H, J_{ortho} 10 Hz, H-6′), 4.44 (m, 1 H, H-4eq), 4.29 (q, 2 H, *J* 7 Hz, CH_2CH_3), 3.68–3.17 (m, 4 H, H-2ax, H-6ax, H-2eq, H-6eq), 2.92 (m, 1 H, H-3ax), 2.74 (br s, 1 H, OH), 1.99–1.91 (m, 2 H, H-5eq, H-5ax), 1.27 (t, 3 H, *J* 7 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 172.5 (COOEt), 150.2, 139.0, 138.8 (Ar), 128.8, 124.2, 120.3 (Ar), 64.6, 61.9 (C-4, CH_2CH_3), 48.2, 46.6, 46.4, 31.9 (C-2, C-3, C-5, C-6), 14.6 (CH_2CH_3). MS (EI): *m/z* 339 (M^+).

Ethyl N-*(2,4-dinitrophenyl)-3-hydroxypiperidine-4-carboxylate* (**19**). Procedure above using **18** (28 mg, 0.103 mmol), CH_2Cl_2 (1.4 ml), CF_3COOH (1.4 ml), $NaHCO_3$ (0.49 ml, sat.), 2,4-dinitrofluorobenzene (42 ml) and EtOH (0.42 ml, 99.9%). Purification by preparative TLC in ether–pentane 1:4 gave **19** (12 mg, 35%). ^1H NMR ($CDCl_3$, 200 MHz): δ 8.7 (d, 1 H, J_{meta} 3 Hz, H-3′), 8.24 (dd, 1 H, J_{ortho} 10 Hz, J_{meta} 3 Hz, H-5′), 7.30 (d, 1 H, J_{ortho} 10 Hz, H-6′), 4.35–4.3 (m, 1 H, H-3), 4.22 [q, 2 H, *J* 7 Hz, CH_2 (Et)], 3.74–3.13 (m, 4 H, H-2, H-6), 2.74–2.65 (m, 1 H, H-4), 2.34–1.84 (m, 2 H, H-5), 1.3 [t, *J* 7 Hz, CH_3 (Et)]. ^{13}C NMR ($CDCl_3$, 200 MHz): δ 174.0 (*C*OOEt), 150.7, 138.8, 138.5 (Ar), 128.7, 124.2, 121.5 (Ar), 65.9, 61.9 (C-3, CH_2CH_3), 55.9, 51.1, 45.0, 23.3 (C-2, C-4, C-5, C-6), 14.6 (CH_2CH_3). MS (EI): *m/z* 339 (M^+).

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