lected ($4° \le 2\theta \le 44°$; limited by weak diffraction), 3138 were unique, and 2238 were observed ($F_o = 4\sigma(F_o)$). For each molecule of **1**, the lattice contains a half-molecule of benzene. $R(F) = 0.0539$, $R(wf) = 0.0652$ for 292 variables. Further details of the crystal structure determination are obtainable from the director of the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge CB2 1EW (UK), on quoting the names of the authors and the journal citation.

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Reduction with Yeast Cells, the Key Step of an Efficient Synthesis of (3*S*, 4*S*)-4-Amino-3-hydroxypentanoic Acids**

By *Peter Raddatz, Hans-Eckart Radunz,* Günter Schneider,* and *Harry Schwartz**

Pepstatin,[1] a pentapeptide first isolated by *H. Umezawa,* contains the unusual γ-amino acid statine ((3*S*, 4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid). Statine and its analogues are central building blocks for many highly active, specific aspartyl protease inhibitors, including even renin inhibitors,[2] which are of interest as hypotensive drugs. Kinetic studies have shown that the hydroxy group in statines must be *S*-configurated, but the carbon chain at C-4 can be chemically modified.[3]

The current importance of such drugs warranted the search for a synthetic strategy enabling a flexible, diastereoselective synthesis of statine from readily accessible starting materials. At best, the methods of synthesis published so far[4] only partly fulfill these requirements, if at all.

In principle, in the case of the statine synthesis presented here any α-amino acid can be used as starting compound. This is allowed to react with a malonic acid building block, and the resulting γ-amino-β-ketoester can be diastereoselectively reduced to the β-hydroxy derivative with certain yeasts. The strategy will be described here for

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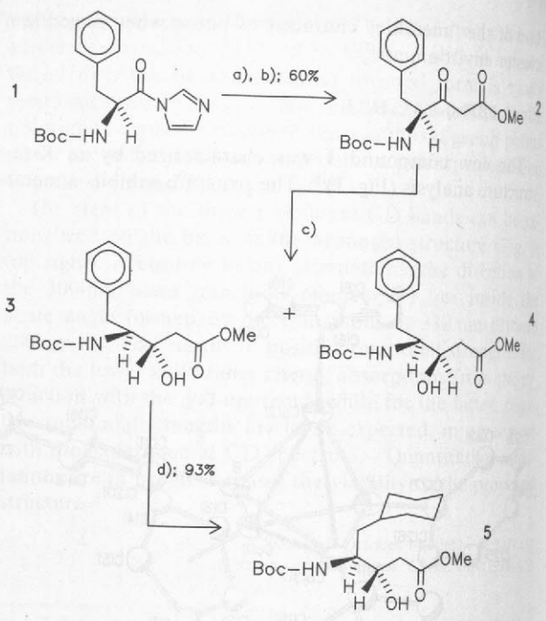
[**] We thank Dr. *M. Heinrich* for caryying out the fermentation and Herr *O. Kaser* for valuable assistance with the experiments.



Scheme 1. Synthesis of **5**. a) $HO_2C-CH_2-CO_2Me$, *i*PrMgBr; b) 15% HCl; c) reduction with yeast; d) Rh/Al_2O_3. Boc = *tert*-butoxycarbonyl. For physical and spectroscopic data of **2**–**5** see Table 1.

the synthesis of (3*S*, 4*S*)-*N*-Boc-4-amino-3-hydroxy-5-cyclohexylpentanoic acid methyl ester **5** (Scheme 1).

The crucial step is the stereoselective reduction of **2**, which is formed from *N*-Boc-L-phenylalanine-imidazolide **1** and the magnesium enolate of the malonic acid monomethyl ester,[4b] to the alcohol **3** with *S* configuration at C-3. The catalytic hydrogenation of **2** with Raney nickel affords a 1:1 mixture of the diastereomeric alcohols **3** and **4**. Neither of the diastereomers is preferentially formed, even with complex, sterically bulky hydrides. In order to alter the conditions in favor of one of the diastereomers, reduction of the β-ketoester with bakers' yeast was attempted.[5] The attempts with commercially available yeast (*Saccharomyces cerevisiae*) merely resulted in incomplete reaction (30–50%), but nevertheless the desired 3*S*,4*S*-diastereomer **3** was formed ($de = 60\%$).

Table 1. Selected physical and spectroscopic data of the compounds **2**–**5** [a]. $[\alpha]_D^{20}$ values with $c = 0.68$ to 1.26 g/100 mL methanol. 500-MHz ^1H-NMR spectra ([D$_6$]DMSO, coupling constants *J* in Hz).

2: m.p. 91 °C (petroleum ether/methyl *tert*-butyl ether): $[\alpha]_D^{20} = -64.8°$; ^1H-NMR: $\delta = 1.35$ (s, 9 H; $C(CH_3)_3$), 2.73 (dd, $J = 10.2$, 14, 1 H; CH_2Ph), 3.06 (dd, $J = 4.6$, 14, 1 H; CH_2Ph), 3.26 (br. s, 2 H; CH_2CO), 3.65 (s, 3 H; OCH_3), 4.25 (m, 1 H; CHN), 6.85 (br. m, 1 H, NH), 7.25 (m, 5 H; C_6H_5)

3: m.p. 99 °C (diisopropyl ether); $[\alpha]_D^{20} = -35.4°$; ^1H-NMR: $\delta = 1.32$ (s, 9 H; $C(CH_3)_3$), 2.30 (dd, $J = 9.2$, 15, 1 H; CH_2CO), 2.5 (dd, $J = 4$, 15, 1 H; CH_2CO), 2.60 (dd, $J = 9.5$, 13, 1 H; CH_2Ph), 2.84 (dd, $J = 5.3$, 13, 1 H; CH_2Ph), 3.55 (s, 3 H; OCH_3), 3.68 (m, 1 H; CHN), 3.9 (m, 1 H; *CH*OH), 4.9 (br. d, $J = 7$, 1 H; OH), 6.48 (br. d, $J = 7.5$, 1 H; NH), 7.23 (m, 5 H; C_6H_5)

4: m.p. 139 °C (methyl *tert*-butyl ether); $[\alpha]_D^{20} = -17.1°$; ^1H-NMR: $\delta = 1.25$ (s, 9 H; $C(CH_3)_3$), 2.30 (dd, $J = 9.3$, 15, 1 H; CH_2CO), 2.55 (m, 2 H; CH_2CO, CH_2Ph), 3.05 (dd, $J = 3.5$, 14, 1 H; CH_2Ph), 3.52 (m, 1 H; CHN), 3.6 (s, 3 H; OCH_3), 3.80 (m, 1 H; *CH*OH), 5.07 (br. d, $J = 7$, 1 H; OH), 6.61 (br. d, $J = 8$, 1 H; NH), 7.22 (m, 5 H; C_6H_5)

5: m.p. 69 °C (petroleum ether); $[\alpha]_D^{20} = -36.5°$; ^1H-NMR: $\delta = 0.7$–1.3 (m, 8 H), 1.42 (s, 9 H; $C(CH_3)_3$), 1.5–1.85 (m, 5 H), 2.25 (dd, $J = 9.2$, 15, 1 H; CH_2CO), 2.45 (dd, $J = 4$, 15, 1 H; CH_2CO), 3.55 (m, 1 H; CHN), 3.60 (s, 3 H; OCH_3), 3.85 (m, 1 H; *CH*OH), 4.75 (br. d, $J = 7$, 1 H; OH), 6.25 (d, $J = 7.5$, 1 H; NH)

[a] All the compounds gave correct elemental analyses.

Since baker's yeast behaves differently depending on its origin and strain we tried to improve the stereoselectivity by using pure cultures of selected yeast strains of the genera *Kloeckera, Hansenula, Candida,* and *Torulopsis*.[6] Only five of 74 yeast strains tested reduced the β-ketoester **2** completely. Four strains, including *Hansenula anomala,* formed the 3*S*, 4*S*-diastereomer **3** with $de = 92\%$, whereas predominantly the 3*R*, 4*S*-diastereomer **4** was formed (90% *de*) by the yeast *Candida boidinii*. Further optimization attempts with resting cells of the yeast *Hansenula anomala* during fermentation of glucose revealed that reduction of the β-ketoester **2** proceeds almost quantitatively if the glucose concentration is 10%, the yeast concentration 1%, and the substrate concentration 0.1%; this holds true for sample volumes of 100 mL to 1000 L. Irrespective of whether intact cells, cells immobilized in polyacrylamide gel[7] or cell-free crude extracts of the strain *Hansenula anomala* were used, **2** was reduced almost completely (90–95% conversion) on a laboratory scale (100 mg to 1 g). The procedure optimized for small amounts was then extended to larger substrate amounts (50 g to 1000 g). On using 50 g of **2**, 96% was reduced after 48 hours' incubation; the ratio of the alcohols **3** and **4** was 23:1. After work-up and recrystallization from diisopropyl ether we obtained 35 g of **3** with 98% *de*. Subsequent recrystallization from cyclohexane increased the diastereomeric excess to >99%.

Experimental

For the production of cell masses, yeasts of the genus *Hansenula* were incubated under sterile conditions in 100-L fermenters with continuous aeration at 28 °C in a medium consisting of 2% glucose, 0.3% malt extract, 0.3% yeast extract and 0.5% peptone from casein. After reaching the stationary growth phase the cells were separated from the medium and washed with cold 0.9% NaCl solution. The reduction was carried out in a 100-L fermenter at 28 °C. 500 g of yeast (wet weight) was suspended in 50 L of a 10% glucose solution. After commencement of fermentation, a solution of 50 g of β-ketoester **2** in 500 mL of ethanol was added and the mixture incubated for 48 h with continuous stirring (200 rpm) and gentle aeration.

For the extraction, the yeast suspension was treated twice with dichloromethane (a total of 35 L) and stirred for one hour. The separated organic phase was filtered over diatomaceous earth and the filtrate dried over Na_2SO_4. After removal of solvent the residue was recrystallized from 500 mL of diisopropyl ether.

The enantiomeric purity was determined HPL chromatographically and NMR spectroscopically via the MTPA amides[8] of **3** (MTPA = α-methoxy-α-trifluoromethylphenylacetic acid) and gave >98% *ee*. Subsequent hydrogenation of the benzene ring of **3** with Rh on Al_2O_3 in methanol afforded **5** in 93% yield.[9]

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Formation of Dioxirane from Carbonyl Oxide**

By *Dieter Cremer,* Thomas Schmidt, Jürgen Gauss,* and *T. P. Radhakrishnan*

Dedicated to Professor Emanuel Vogel on the occasion of his 60th birthday

Dioxirane **1** is an effective oxygen carrier for regio- and stereoselective epoxidations.[1] Spectroscopic investigations have provided evidence that **1** can be formed by thermal or photochemical isomerization of carbonyl oxide **2**, e.g. in the ozonolysis[2, 3] or the oxidation of carbenes.[4] According to ab initio calculations, the activation energy for the isomerization in the gas phase is 22.8 kcal mol^{-1}.[5] The transition state **3** (Fig. 1) of this reaction resembles **2** more closely than it does **1**, as is clear from the calculated geometries, in particular the large distance between C and the terminal O atom in **3** (Fig. 1).

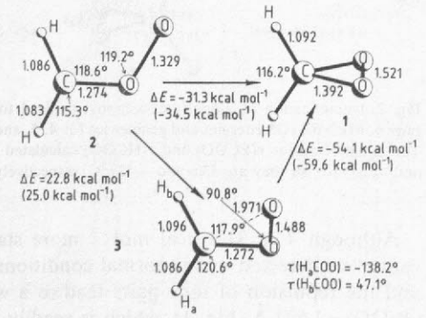

Fig. 1. Isomerization of carbonyl oxide **1** to dioxirane **2**. MP4(SDQ)/6-31G* energies and geometries for **1**, **2**, and **3**; distances in Å. The values in brackets are MP2/6-31G* results [5].

Further, the calculations show that the isomerization is not initiated by rotation about the CO bond but by pyramidalization at the central O atom. Negative charge is shifted from the C atom to the neighboring O atom, resulting in a weakening of the OO bonding due to enhanced charge repulsion (Fig. 1). The CO double-bond character, however, is essentially retained, since back-bonding between the orbital of a lone pair on the O atom and the depopulated 2pπ orbital on the C atom is possible (see **3a**).[6]

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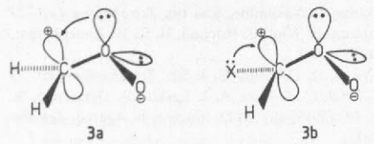


3a 3b

Accordingly, a substituent X with π-donor properties should stabilize the transition state (see **3b**), while π-acceptors should have a slight or noticeable destabilizing influence. These predictions are confirmed in the case of the monofluorocarbonyl oxides **4a** and **4b** (Fig. 2), whose isomerization to monofluorodioxirane **6** via **5a** and **5b** requires activation energies of 8 and 17 kcal mol^{-1}, respectively, according to ab initio calculations.[7] On the basis of these findings the formation of epoxides observed in the ozonolysis of fluoroalkenes[8] can be explained in terms of an enhanced formation of fluorodioxiranes, which epoxidize unchanged alkene.

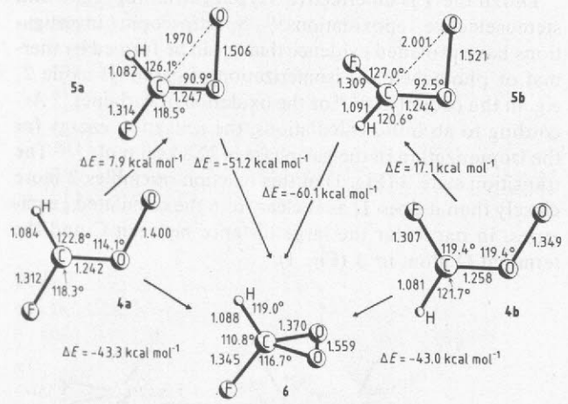


Fig. 2. Isomerization of monofluorocarbonyl oxide **4** to monofluorodioxirane **6**. MP2/6-31G* energies and geometries for **4**, **5**, and **6**; distances in Å. The dihedral angles τ(FCOO) and τ(HCOO) calculated for **5a** are −143.0 and 42.2°; for **5b** they are 53.6 and −137.3°, respectively.

Although **1** is 31.3 kcal mol^{-1} more stable than **2**, it cannot be detected under normal conditions.[2] Ring strain and the repulsion of lone pairs lead to a weak OO bond (R(OO) = 1.521 Å, Fig. 1), which is readily cleaved, either homolytically or, in the presence of Lewis acids, heterolytically.

The strain energy (SE) of **1** is estimated by ab initio calculations for the homodesmotic reaction (a) to be 24.7 kcal mol^{-1}.[9]

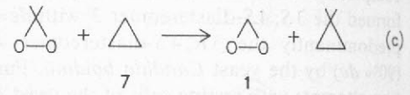
$$1 + H_2O_2 + 2CH_3OH \longrightarrow 2CH_3OOH + HOCH_2OH \quad (a)$$

For the comproportionation of **1** and cyclopropane **7** leading to **8** [Eq. (b)], a reaction energy ΔE of −38.2 kcal

$$1 + 7 \longrightarrow 2\,8 \quad (b)$$

mol^{-1} is calculated.[9] If one assumes that ΔE(b) is determined by the difference in the SE values of the three-membered rings (SE(**1**) = 24.7, SE(**7**) = 27.5,[10] SE(**8**) = 26.9 kcal mol^{-1}[10]) and the bond energies (BE) of C–C (80 kcal mol^{-1}), C–O (78 kcal mol^{-1}[11]), and O–O bonds, then BE(O–O) is estimated at 36 kcal mol^{-1}, a value which is close to the calculated dissociation energy of 31 kcal mol^{-1}.[12] In the isomerization, **2** is formed with an excess energy of 54 kcal mol^{-1}, which, if not reduced by collision deactivation, leads to cleavage of the O–O bond and subsequent decomposition of the molecule.

A thermodynamic stabilization of **1** is achieved by geminal methyl groups. It can be estimated via the reaction energy ΔE(c) to be 21 kcal mol^{-1}.[13] Geminal methyl groups



7 1 (c)

also increase the kinetic stability of **1**; they fix the CCC bond angle at a value of 117° by steric interaction and thereby render difficult a rehybridization at the C atom during cleavage of the OO bond. This explains the relatively high stability of dimethyldioxirane, which was recently prepared by oxidation of acetone with $KHSO_5$.[14]

Fluorine as substituent also increases the thermodynamic stability of **1**, but at the same time it reduces its kinetic stability, as manifested in the relatively large O–O distance (1.56 Å) and the reduction in the exocyclic angle to 110.8°. Intermediary fluorodioxiranes are therefore probably stronger epoxidizing agents than dialkyl dioxiranes.

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CAS Registry numbers:
1, 157-26-6; **2**, 78894-19-6; **4a**, 112897-49-1; **6**, 76694-11-6; dimethyldioxirane, 74087-85-7; dioxirane, 157-26-6.

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[5] 4th order Møller–Plesset calculations with the 6-31G* basis set (MP4(SDQ)/6-31G*) gave the following energies: −189.12016 (**1**), −189.07021(**2**), −189.03391 Hartree (**3**). The MP4 geometries were calculated using analytical gradients. J. Gauss, D. Cremer, *Chem. Phys. Lett. 138* (1987) 131.—For 2nd order Møller–Plesset calculations with the 6-31G* basis set (MP2/6-31G*) see D. Cremer, *J. Am. Chem. Soc. 101* (1979) 7199. Since MP2 calculations in the case of **2** favor diradical structures with a balanced π system, there results an isomerization barrier of 2.2 kcal mol^{-1} higher than with the more reliable MP4 calculations. This can be taken into account on correcting the MP2 calculations.
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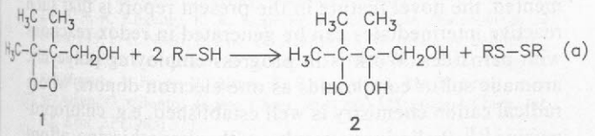
Facile Reduction of 1,2-Dioxetanes by Thiols as Potential Protective Measure against Photochemical Damage of Cellular DNA**

By *Waldemar Adam,* Bernd Epe, Dietmar Schiffmann, Franklin Vargas,* und *Dieter Wild*

In recent biological studies[1] it was demonstrated that 1,2-dioxetanes are genotoxic. Since these strained four-membered ring cyclic peroxides are known to be efficient chemical sources of n,π*-excited triplet carbonyl products,[2] we postulated that the observed DNA damage was of photochemical origin. However, in view of the quite moderate photo-genotoxicity displayed by the dioxetanes studied, we suspected that these labile peroxides were efficiently detoxified in the cell through chemical action. For example, the living cell guards itself against "oxidative stress"[3] by engaging glutathione, a tripeptide which deactivates reactive oxygen species including peroxides by reduction, itself being oxidized to its disulfide. We now report on the quantitative reduction of dioxetanes to the corresponding vicinal diols by glutathione [Eq. (a)], a reaction which also takes place with other thiols. The results are listed in Table 1.



1 2 (a)

Table 1. Reaction of **1** with thiols.

No.	Thiol [a] R–SH	Solvent	Reaction conditions T [°C]	t [h]	Product balance [%]	Product yields [mol] [b, c] Disulfide	**2**	Ketone [d]
1.	L-Glutathione	H_2O	5	0.16	97	0.97	0.96	—
2.	L-Cysteine	H_2O	20	0.16	95	0.98	0.92	—
3.	L-Penicillamine	H_2O	20	0.16	93	0.96	0.90	—
4.	*threo*-1,4-Dimercapto-2,3-butanediol	MeOH	10	1	99	0.99	0.99	—
5.	1,3-Propanedithiol	MeOh	−40	24	97	0.98	0.93	0.03
6.	Thiobenzyl alcohol	MeOH	−50	72	89	0.78	0.82	0.18
7.	Mercaptoacetic Acid	MeOH	−40	72	78	0.56	0.56	0.44
8.	Methyl Mercaptoacetate	MeOH	−40	72	80	0.59	0.58	0.42
9.	Thiophenol	MeOH	−100	22	50	0.49	0.49	0.51

[a] Stoichiometry 2:1 except entries 4 and 5, for which it was 1:1. [b] 100% conversion; normalized to 1.00 mol; ±2% error limits. [c] Determined ^{1}H-NMR spectroscopically and/or isolated. [d] Acetone and hydroxyacetone formed in equal amounts.

On mixing aqueous solutions of the hydroxydioxetane **1** with glutathione at 5°C in a 1:2 stoichiometry, a fast reaction (100% conversion in 10 min, monitored by ^{1}H-NMR) ensued, leading essentially quantitatively to the glutathione dimer [m.p. 178–180°C (decomp.)[4], m.p.$_{obs.}$ 178–182°C (decomp.)] and the triol **2**. Both products were isolated and identified by comparison with authentic materials. Similar results were obtained for cysteine, penicillamine, and *threo*-1,4-dimercapto-2,3-butanediol (Entries 2–4 in Table 1), except that in the latter case the cyclic disulfide 1,2-dithiane was isolated and small amounts (ca. 3%) of the dioxetane cleavage products (acetone and hydroxyacetone) were detected by ^{1}H-NMR. Such cleavage products gain greater importance for the simple thiols (Entries 6–9, Table 1), although the reactions were conducted at subambient temperatures, at which thermal decomposition of the dioxetane is negligible. For example, with thiophenol (Entry 9 in Table 1) the extent of dioxetane cleavage was as much as 50%, even at −100°C, thus seriously competing with reduction to the triol **2**. Similar results were obtained for tetramethyl-1,2-dioxetane, undergoing quantitative reduction by glutathione to pinacol, but appreciable cleavage into acetone with thiophenol.

That glutathione is an efficient reagent for the reduction of peroxides is well established,[3] but that the labile dioxetanes can be so cleanly transformed into vicinal diols is somewhat astonishing, since in the few reported studies with divalent sulfur compounds, oxygen transfer prevails. Thus, the dioxetane is converted into an epoxide and/or a ketone, while the sulfide is oxidized to the sulfoxide.[5] Furthermore, dialkyl sulfoxylates $S(OR)_2$ are transformed via intermediary tetraalkylorthosulfites $S(OR)_4$ into dialkyl sulfites.[6] These oxygen transfer reactions have been mechanistically interpreted in terms of either a nucleophilic attack by the sulfide[5] on the peroxide linkage or a biphilic insertion by the dialkylsulfoxylate.[6] Consequently, we likewise expected oxygenated products rather than disulfides in the reaction with thiols. Indeed, when only equimolar amounts of glutathione and dioxetane **1** were used, the glutathione was converted quantitatively in a fast reaction into its dimer, and in a subsequent slow reaction (hours) the disulfide was oxidized to its *S*-monoxide, as anticipated for an oxygen transfer reaction. With L-methionine (in H_2O, 20°C, 20 min, 1:1 stoichiometry), **1** was quantitatively converted into the epoxide 2,3-dimethyl-2,3-epoxy-1-butanol, while the methionine itself was converted into the sulfoxide. In the case of dimethyl sulfide (in $CHCl_3$, −5°C, 3 h, 1:1 stoichiometry) oxygen transfer was the major reaction (ca. 85%), affording 60% sulfoxide and 12% sulfone as sulfide derived products, and 44% 2,3-dimethyl-2,3-epoxy-1-butanol and 41% of the rearranged, ketone 1-hydroxy-3,3-dimethyl-2-butanone as dioxetane derived products. Dioxetane cleavage into equal amounts of acetone and hydroxyacetone was observed as secondary reaction (ca. 15%). A control experiment showed that in a much slower reaction dimethyl sulfoxide was oxidized by dioxetane **1** to its sulfone. Clearly, the sulfides behave differently than the thiols in their reaction with dioxetanes.

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