

Chiral Auxiliaries as Docking/Protecting Groups in Biohydroxylation: (S)-Specific Hydroxylation of Enantiopure *tert*-Butyl-Substituted Spirooxazolidines Derived From Cyclopentanone

Dieter F. Münzer,^[a] Herfried Griengl,^[a] Alexandra Mouttzi,^[a] Robert Saf,^[b] Tullio Terzani,^[a] and Anna de Raadt^{*[a]}

Dedicated to the memory of Professor Herbert Holland^[‡]

Keywords: Biohydroxylation / Biotransformations / Enantioselectivity / Substrate engineering

An enantiopure *tert*-butyl-substituted derivative of cyclopentanone, which is a vital member of the chiral docking/protecting group series, is employed, for the first time, to stereoselectively (90 % *de*) introduce an (*S*)-configured hydroxyl group onto an unactivated carbon atom present in the cyclo-

pentane ring using the fungus *Beauveria bassiana* ATCC 7159.

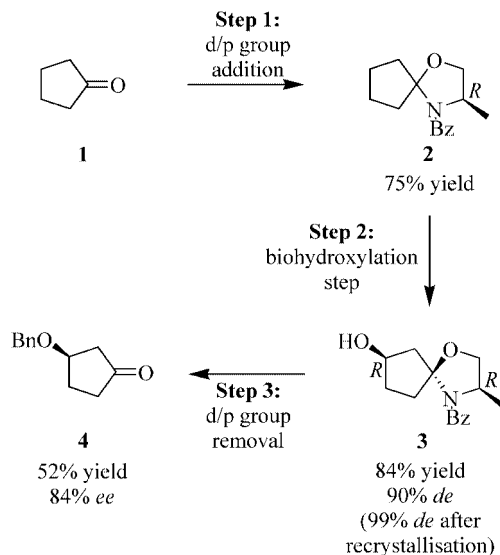
(© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2005)

Introduction

The stereoselective introduction of hydroxyl groups onto unactivated carbon atoms present in organic compounds is, generally, still a very daunting task in modern synthetic chemistry.^[1,2] For this reason, we have been investigating the docking/protecting (d/p) group concept as a means to easily employ biohydroxylation in preparative chemistry.^[3,4] We have found that a range of organic compounds, such as carboxylic acids, alcohols, aldehydes and ketones, can be easily hydroxylated following this concept.

Chiral d/p groups have also been investigated^[5,6] and employed for the hydroxylation of ketones. In this manner, the stereoselective functionalisation of these compounds was achieved. Indeed, depending on the nature of the chiral d/p group used, the configuration of the introduced hydroxyl moiety could be readily determined.

The d/p concept is a three-step process (Scheme 1). Using parent ketone **1** and a chiral d/p group as an example, derivatisation gives the enantiopure biohydroxylation substrate **2** in the first step. Subsequent exposure of this spirooxazolidine-



Scheme 1. The d/p concept as exemplified by a chiral d/p group and model ketone **1**: step 1: (2*R*)-2-amino-1-propanol, K₂CO₃, CH₂Cl₂, 20 °C, 24 h, followed by BzCl, 20 °C, 24 h; step 2: *Beauveria bassiana* ATCC 7159; step 3: BnBr, NaH, THF/DMF, 20 °C followed by IR 120 (H⁺, cat), CH₃CN, 20 °C

ine derivative to a suitable microorganism, for example *Beauveria bassiana* ATCC 7159, yields hydroxylated product **3**. The third and final step of this approach is to remove the d/p group to furnish the desired hydroxylated product **4**. In this particular example, an atypical protection step (benzylation) was also needed to prevent elimination of the introduced group.

^[a] Institut für Organische Chemie der Technischen Universität Graz, Stremayrgasse 16, 8010 Graz, Austria
Fax: +43-316-873-8740
E-mail: deraadt@orgc.tu-graz.ac.at

^[b] Institut für Chemische Technologie Organische Stoffe der Technischen Universität Graz, Stremayrgasse 16, 8010 Graz, Austria
Fax: +43-316-873-8959
E-mail: robert.saf@TUGraz.at

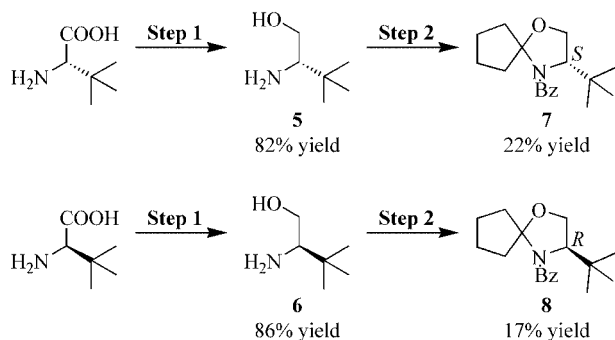
^[‡] Not only a leader in the field of biohydroxylation but a most valued friend and colleague

Despite the fact that a wide range of chiral d/p groups has been investigated, while the (*R*)-configured product (**3**) could be prepared in 90% *de* – recrystallisation increased this value to over 99% – the (*S*)-configured counterpart could not be obtained with more than 20% *de* from other substrates.

In this account we wish to disclose results obtained from the last member of this chiral d/p group series, namely compounds containing *tert*-butyl substituents. In this manner, the synthesis of the (*S*)-configured biohydroxylation product could be accomplished with 90% *de*.

Results and Discussion

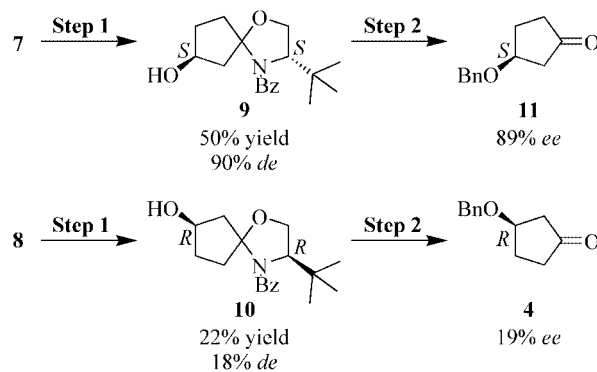
The commercially available, but relatively expensive, *tert*-butyl amino alcohols **5** and **6** required for substrate synthesis could be easily prepared from the corresponding cheaper amino acids in high yield (over 80%) by the reduction method reported by Drauz and coworkers.^[7] Subsequent reaction with cyclopentanone afforded the desired, enantiopure substrates **7** and **8**, respectively (Scheme 2). In contrast to previous observations, these compounds were obtained in only modest yields under standard, unoptimized conditions^[5,8] (22% and 17%, respectively).



Scheme 2. Preparation of enantiopure *tert*-butyl biohydroxylation substrates **7** and **8**: step 1: NaBH₄, I₂, THF; step 2: cyclopentanone, K₂CO₃, CH₂Cl₂, 20 °C, 24 h followed by BzCl, 20 °C, 24 h

For the biohydroxylation step, the fungus *Beauveria bassiana* ATCC 7159 was then employed in the usual manner to afford crystalline products **9** and **10** in modest yields (50% and 22%, respectively; Scheme 3). We believe that this outcome could be improved by optimising the fermentation parameters if required.^[9] More importantly, however, the *de* and configuration of the introduced alcohol were deemed to be very interesting. While the *de* of product **10** was found to be rather modest (18%) by HPLC, compound **9** exhibited a high *de* of 90%. After subsequent benzylation and d/p group removal, the configuration of the newly introduced chiral center could be determined by GC comparison of the resulting ketones **4** and **11** with model compounds. Satisfyingly, although ketone **4** was found to be (*R*)-configured (19% *ee*), ketone **11** was found to be (*S*)-configured and with a high *ee* (89%). It should be mentioned at this

point that, based on published experiments^[5] with product **3**, this *ee* value should increase after simple recrystallisation of compound **9**. This result nicely complements those obtained from the (*R*)-methyl d/p used in previous studies (Scheme 1) where product **3** could be obtained in 90% *de* in a single biohydroxylation step.



Scheme 3. Biohydroxylation of *tert*-butyl substrates **7** and **8**, subsequent benzylation and d/p removal: step 1: *Beauveria bassiana* ATCC 7159; step 2: BnBr, NaH, THF/DMF, 20 °C followed by IR 120 (H⁺, cat), CH₃CN, 20 °C

Conclusions

In conclusion, it has been shown that, for the biohydroxylation step, the appropriate choice of chiral d/p group can be exploited to obtain either configuration of the newly introduced alcohol in 90% *de*. Simple recrystallisation of this biohydroxylation product could improve this value to over 99% *de* if desired. Simple removal of the chiral d/p group yields the corresponding ketone derivative.

Experimental Section

General Remarks: Please refer to ref.^[3] for all general methods unless stated otherwise. NMR: signals from the minor isomer are given in *italics*. The ¹³C NMR spectra for spirooxazolidine derivatives **7** to **13** suggested that a number of different conformations were present in the NMR sample (see Figure 1 for numbering scheme).

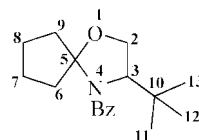


Figure 1. Numbering scheme for the spirooxazolidine derivatives

Amino Alcohols **5 and **6**:** ¹H and ¹³C NMR spectroscopic data, optical rotations and melting points were in agreement with published values.^[10]

Spirooxazolidine Derivative **7:** Employing the standard procedure for preparing spirooxazolidine derivatives,^[5] compound **7** was prepared in 22% yield as a pale-yellow, crystalline solid. M.p. 97.0–

97.5 °C. $[\alpha]_D^{20} = +65.3$ ($c = 1.11$ in CH_2Cl_2). ^1H NMR (CDCl_3): $\delta = 0.72$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.40–2.10 (br. m, 6 H, 6^a-H, 7-H, 8-H, 9^a-H), 2.28, 2.73 (2×br. m, 2×1 H, 6^b-H, 9^b-H), 3.95 (m, 3 H, 2-H, 3-H), 7.38 (s, 5 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 24.7, 25.2$ (2×br. s, C-7, C-8), 27.4 (C-11, C-12, C-13), 35.2, 37.5 (2×br. s, C-6, C-9), 35.7 (C-10), 65.5, 66.2 (2×br. s, C-2, C-3), 105.7 (C-5), 127.7, 128.4, 129.9, 138.8 (*COPh*), 170.2 (*COPh*) ppm. MS (70 eV): m/z (%) = 287 (15) $[\text{M}]^+$, 258 (13) $[\text{M} - \text{C}_2\text{H}_5]^+$, 230 (43) $[\text{M} - \text{C}_4\text{H}_9]^+$, 105 (100) $[\text{Bz}]^+$, 77 (16) $[\text{Ph}]^+$. HRMS: calcd. 287.1885; found 287.1887.

Spirooxazolidine Derivative 8: Employing the standard procedure for preparing spirooxazolidine derivatives,^[5] compound **8** was prepared in 17% yield as a pale-yellow, crystalline solid. M.p. 92.0–95.0 °C. $[\alpha]_D^{20} = -57.8$ ($c = 1.51$ in CH_2Cl_2). ^1H NMR (CDCl_3): $\delta = 0.72$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.40–2.10 (br. m, 6 H, 6^a-H, 7-H, 8-H, 9^a-H), 2.28, 2.73 (2×br. m, 2×1 H, 6^b-H, 9^b-H), 3.95 (m, 3 H, 2-H, 3-H), 7.38 (s, 5 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 24.7, 25.2$ (2×br. s, C-7, C-8), 27.4 (C-11, C-12, C-13), 35.2, 37.5 (2×br. s, C-6, C-9), 35.7 (C-10), 65.5, 66.2 (2×br. s, C-2, C-3), 127.7, 128.4, 129.9, 138.8 (*COPh*) ppm. MS (70 eV): m/z (%) = 287 (15) $[\text{M}]^+$, 258 (12) $[\text{M} - \text{C}_2\text{H}_5]^+$, 230 (43) $[\text{M} - \text{C}_4\text{H}_9]^+$, 105 (100) $[\text{Bz}]^+$, 77 (17) $[\text{Ph}]^+$. HRMS: calcd. 287.1885; found 287.1875.

Biohydroxylation Product 9: Employing published methods,^[5] treatment of compound **7** (1.277 g) with *Beauveria bassiana* ATCC 7159 gave the title compound (586.6 mg), together with unreacted starting material (97.1 mg), as a white solid (50% yield, taking into account unreacted starting material). M.p. 89.0–104.0 °C. $[\alpha]_D^{20} = +79.4$ ($c = 1.06$ in CH_2Cl_2); 90% *de* (HPLC: CHIRALCEL AD, $T = 10$ °C, 0.5 mL min⁻¹, *n*-heptane/IPA = 4:1, measured at 238 nm), retention time of (3*S*,5*E*,7*R*)-**9** = 17.6 min, retention time of (3*S*,5*E*,7*S*)-**9** = 20.8 min. ^1H NMR (CDCl_3): $\delta = 0.72$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.65–2.90 (3×br. m, 7 H, 6-H, 8-H, 9-H, OH), 4.00 (br. m, 3 H, 2-H, 3-H), 4.39 (br. s, 1 H, 7-H), 7.39 (s, 5 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 27.4$ (C-11, C-12, C-13), 34.3, 35.3 (2×br. s, C-6, C-9), 35.6 (C-10), 42.7 (br. s, C-8), 65.8 (br. s, C-2, C-3), 73.4 (br. s, C-7), 105.0 (br. s, C-5), 127.6, 128.4, 130.0, 138.4 (*COPh*) ppm. MS (70 eV): m/z (%) = 303 (12) $[\text{M}]^+$, 286 (2) $[\text{M} - \text{OH}]^+$, 274 (10) $[\text{M} - \text{C}_2\text{H}_5]^+$, 258 (16), 246 (28) $[\text{M} - \text{C}_4\text{H}_9]^+$, 124 (42), 105 (100) $[\text{Bz}]^+$, 77 (27) $[\text{Ph}]^+$. HRMS: calcd. 303.1834; found 303.1831.

Biohydroxylation Product 10: Employing published methods,^[5] treatment of compound **8** (759.6 mg) with *Beauveria bassiana* ATCC 7159 gave the title compound (160.6 mg), together with unreacted starting material (65.8 mg), as a white solid (22% yield, taking into account unreacted starting material). M.p. 85.0–105.0 °C; 18% *de* (HPLC, CHIRALCEL OD-H, $T = 10$ °C, 0.5 mL/min, *n*-heptane/IPA = 7:2, measured at 238 nm), retention time of (3*R*,5*E*,7*S*)-**9** = 16.0 min, retention time of (3*R*,5*E*,7*R*)-**9** = 22.1 min. ^1H NMR (CDCl_3): $\delta = 0.72$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.65–2.90, 3.12 (4×m, 7 H, 6-H, 8-H, 9-H, OH), 4.00 (m, 3 H, 2-H, 3-H), 4.39 (br. m, 1 H, 7-H), 7.39 (s, 5 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 27.4$ (C-11, C-12, C-13), 34.4, 34.8, 35.3 (3×br. s, C-6, C-9), 35.7 (C-10), 42.7, 46.0 (2×br. s, C-8), 66.0 (br. s, C-2, C-3), 73.5 (br. s, C-7), 127.6, 128.5, 130.1, 138.4 (*COPh*) ppm. MS (70 eV): m/z (%) = 303 (11) $[\text{M}]^+$, 286 (2) $[\text{M} - \text{OH}]^+$, 274 (10) $[\text{M} - \text{C}_2\text{H}_5]^+$, 258 (13), 246 (27) $[\text{M} - \text{C}_4\text{H}_9]^+$, 124 (40), 105 (100) $[\text{Bz}]^+$, 77 (26) $[\text{Ph}]^+$. HRMS: calcd. 303.1834; found 303.1841.

Compound 12: Standard benzylation^[5] of **9** (135.0 mg) furnished derivative **12** (146.2 mg; Figure 2) in 83% yield as a pale-yellow syrup. $[\alpha]_D^{20} = +65.5$ ($c = 1.90$ in CH_2Cl_2). ^1H NMR (CDCl_3): $\delta = 0.65$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.72, 2.13, 2.55 (3×m, 6 H, 6-H, 8-H, 9-H, OH), 3.60, 3.95 (2×m, 3 H, 2-H, 3-H), 4.18 (m, 1 H,

7-H), 4.45 (br. s, 2 H, CH_2Ph), 7.24 (m, 10 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 27.4$ (C-11, C-12, C-13), 31.3, 36.0 (2×br. s, C-6, C-9), 35.6 (C-10), 42.4 (br. s, C-8) 65.8, 66.3 (2×br. s, C-2, C-3), 70.7 (CH_2Ph), 79.8 (br. s, C-7) 104.6 (br. s, C-5), 127.0, 127.5, 127.6, 127.8, 128.4, 128.7 129.9, 131.4, 138.6, 139.0 (*COPh*) ppm. MS (70 eV): m/z (%) = 393 (3) $[\text{M}]^+$, 364 (1) $[\text{M} - \text{C}_2\text{H}_5]^+$, 336 (3) $[\text{M} - \text{C}_4\text{H}_9]^+$, 287 (5), 258 (9), 245 (7), 214 (5), 182 (4), 146 (4), 124 (5), 105 (100) $[\text{Bz}]^+$, 91 (37) $[\text{Bn}]^+$, 77 (28) $[\text{Ph}]^+$. HRMS: calcd. 393.23039; found 393.23040.

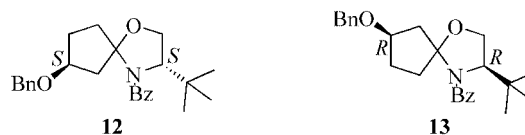


Figure 2. Compounds **12** and **13**

Compound 13: Standard benzylation^[5] of **10** (73 mg) furnished derivative **13** (83.9 mg; Figure 2) in 89% yield as a pale-yellow syrup. ^1H NMR (CDCl_3): $\delta = 0.65$ (br. s, 9 H, 11-H, 12-H, 13-H), 1.72, 2.13, 2.55, 3.08 (4×m, 6 H, 6-H, 8-H, 9-H, OH), 3.60, 3.95 (2×m, 3 H, 2-H, 3-H), 4.18 (m, 1 H, 7-H), 4.45 (br. s, 2 H, CH_2Ph), 7.24 (m, 10 H, *COPh*) ppm. ^{13}C NMR (CDCl_3): $\delta = 27.4$ (C-11, C-12, C-13), 31.3, 36.0 (2×br. s, C-6, C-9), 35.6 (C-10), 42.5, 43.9 (2×br. s, C-8) 65.8, 66.3 (2×br. s, C-2, C-3), 70.6, 71.3 (CH_2Ph), 79.3, 79.8 (2×br. s, C-7) 104.6 (br. s, C-5), 127.0, 127.5, 127.8, 127.8, 128.4, 128.7 130.0, 131.5, 138.6, 139.0 (*COPh*) ppm. MS (70 eV): m/z (%) = 393 (2) $[\text{M}]^+$, 364 (1) $[\text{M} - \text{C}_2\text{H}_5]^+$, 336 (2) $[\text{M} - \text{C}_4\text{H}_9]^+$, 287 (3), 258 (5), 245 (4), 214 (3), 182 (2), 146 (5), 124 (2), 105 (100) $[\text{Bz}]^+$, 91 (49) $[\text{Bn}]^+$, 77 (25) $[\text{Ph}]^+$. HRMS: calcd. 393.23039; found 393.22975.

Ketones 4 and 11: The known^[5] title compounds were obtained for chiral GC experiments from the corresponding benzyolated derivatives **13** and **12**, employing published conditions.^[3] These compounds were then compared with reference substances available from previous studies.

Acknowledgments

Financial support from Spezialforschungsbereich F001 Biokatalyse is gratefully acknowledged. The authors also wish to express their thanks to B. Fetz, H.-J. Weber and C. Illaszewicz for their technical assistance.

- [1] D. H. R. Barton, *Tetrahedron* **1998**, *54*, 5805–5817.
- [2] G. Asensio, R. Mello, M. E. González-Núñez, G. Castellano, J. Corral, *Angew. Chem.* **1996**, *108*, 196–198; *Angew. Chem. Int. Ed.* **1996**, *35*, 217–218.
- [3] G. Braunnegg, A. de Raadt, S. Feichtenhofer, H. Griengl, I. Kopper, A. Lehmann, H. Weber, *Angew. Chem. Int. Ed.* **1999**, *38*, 2763–2766.
- [4] A. de Raadt, H. Griengl, H. Weber, *Chem. Eur. J.* **2001**, *7*, 27–31.
- [5] A. de Raadt, B. Fetz, H. Griengl, M. F. Klingler, I. Kopper, B. Krenn, D. F. Münzer, R. G. Ott, P. Plachota, H. Weber, G. Braunnegg, W. Mosler, R. Saf, *Eur. J. Org. Chem.* **2000**, 3835–3847.
- [6] A. de Raadt, B. Fetz, H. Griengl, M. F. Klingler, B. Krenn, K. Mereiter, D. F. Münzer, P. Plachota, H. Weber, R. Saf, *Tetrahedron* **2001**, *57*, 8151–8157.
- [7] M. J. McKennon, A. I. Meyers, K. Drauz, M. Schwarm, *J. Org. Chem.* **1993**, *58*, 3568–3571.

- [8] A number of reaction variables, such as reaction temperature, reaction solvent and order of reagent addition, were examined in an effort to improve the yields of compounds **7** and **8**.
- [9] An example of how fermentation conditions can influence a product yield and optical purity can be found in the following reference: A. Kraemer-Schafhalter, S. Domenek, H. Boehling, S. Feichtenhofer, H. Griengl, H. Voss, *Appl. Microbiol. Biotech.* **2000**, 53, 266–271.
- [10] *Aldrich Handbook and Fine Chemicals and Laboratory Equipment (2003–2004)*, Sigma–Aldrich Co.

Received: September 7, 2004