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

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Dedicated to the memory of Professor Herbert Holland[‡]

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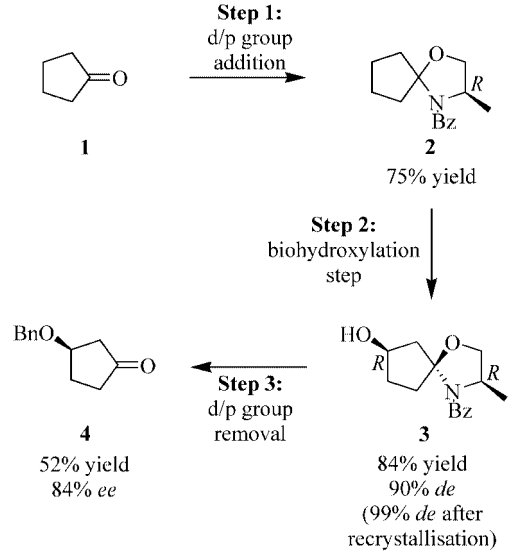
An enantiopure *tert*-butyl-substituted derivative of cyclopentanone, which is a vital member of the chiral docking/protecting group series, is employed, for the first time, to stereoselectively (90 % *de*) introduce an (*S*)-configured hydroxyl group onto an unactivated carbon atom present in the cyclopentane ring using the fungus *Beauveria bassiana* ATCC 7159.

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 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.



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, K_2CO_3, CH_2Cl_2, 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), CH_3CN, 20 °C

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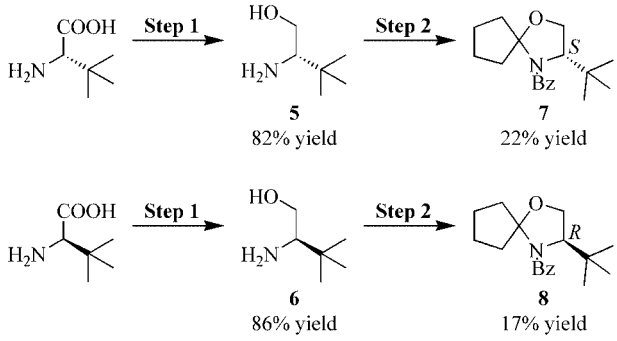
Despite the fact that a wide range of chiral d/p groups has been investigated, while the (*R*)-configured product (**3**) could be prepared in 90% *de* − recrystallisation increased this value to over 99% − the (*S*)-configured counterpart could not be obtained with more than 20% *de* from other substrates.

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*.

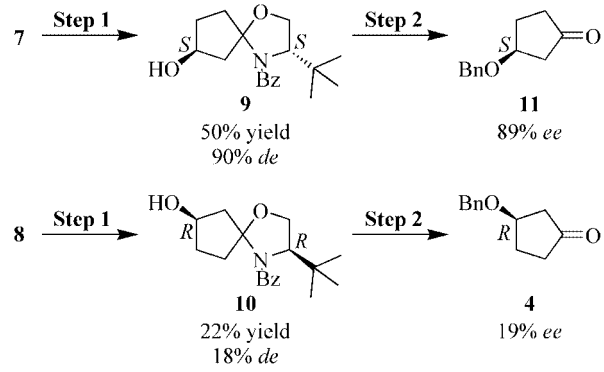
Results and Discussion

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: $NaBH_4$, I_2, THF; step 2: cyclopentanone, K_2CO_3, CH_2Cl_2, 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), CH_3CN, 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 ^{13}C 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).

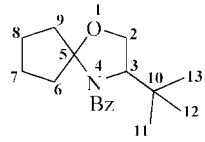


Figure 1. Numbering scheme for the spirooxazolidine derivatives

Amino Alcohols 5 and 6: ^1H and ^{13}C 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–

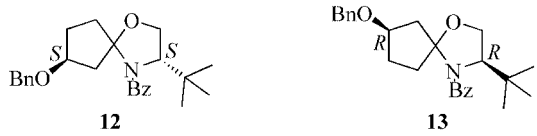
97.5 °C. $[\alpha]_D^{20}$ = +65.3 (*c* = 1.11 in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ = 0.72 (br. s, 9 H, 11-H, 12-H, 13-H), 1.40–2.10 (br. m, 6 H, 6^a-H, 7-H, 8-H, 9^a-H), 2.28, 2.73 (2× br. m, 2×1 H, 6^b-H, 9^b-H), 3.95 (m, 3 H, 2-H, 3-H), 7.38 (s, 5 H, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 24.7, 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 (CO*Ph*), 170.2 (*C*OPh) ppm. MS (70 eV): *m*/*z* (%) = 287 (15) $[M]^+$, 258 (13) $[M - C_2H_5]^+$, 230 (43) $[M - C_4H_9]^+$, 105 (100) $[Bz]^+$, 77 (16) $[Ph]^+$. 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). ^{1}H NMR ($CDCl_3$): δ = 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, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 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 (CO*Ph*) ppm. MS (70 eV): *m*/*z* (%) = 287 (15) $[M]^+$, 258 (12) $[M - C_2H_5]^+$, 230 (43) $[M - C_4H_9]^+$, 105 (100) $[Bz]^+$, 77 (17) $[Ph]^+$. 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*,5Ξ,7*R*)-**9** = 17.6 min, retention time of (3*S*,5Ξ,7*S*)-**9** = 20.8 min. ^{1}H NMR ($CDCl_3$): δ = 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, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 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 (CO*Ph*) ppm. MS (70 eV): *m*/*z* (%) = 303 (12) $[M]^+$, 286 (2) $[M - OH]^+$, 274 (10) $[M - C_2H_5]^+$, 258 (16), 246 (28) $[M - C_4H_9]^+$, 124 (42), 105 (100) $[Bz]^+$, 77 (27) $[Ph]^+$. 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*,5Ξ,7*S*)-**9** = 16.0 min, retention time of (3*R*,5Ξ,7*R*)-**9** = 22.1 min. ^{1}H NMR ($CDCl_3$): δ = 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, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 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 (CO*Ph*) ppm. MS (70 eV): *m*/*z* (%) = 303 (11) $[M]^+$, 286 (2) $[M - OH]^+$, 274 (10) $[M - C_2H_5]^+$, 258 (13), 246 (27) $[M - C_4H_9]^+$, 124 (40), 105 (100) $[Bz]^+$, 77 (26) $[Ph]^+$. 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). ^{1}H NMR ($CDCl_3$): δ = 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, C*H*$_2$Ph), 7.24 (m, 10 H, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 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 (*C*H$_2$Ph), 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 (CO*Ph*) ppm. MS (70 eV): *m*/*z* (%) = 393 (3) $[M]^+$, 364 (1) $[M - C_2H_5]^+$, 336 (3) $[M - C_4H_9]^+$, 287 (5), 258 (9), 245 (7), 214 (5), 182 (4), 146 (4), 124 (5), 105 (100) $[Bz]^+$, 91 (37) $[Bn]^+$, 77 (28) $[Ph]^+$. HRMS: calcd. 393.23039; found 393.23040.



12 **13**

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. ^{1}H NMR ($CDCl_3$): δ = 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, C*H*$_2$Ph), 7.24 (m, 10 H, CO*Ph*) ppm. ^{13}C NMR ($CDCl_3$): δ = 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* (*C*H$_2$Ph), *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 (CO*Ph*) ppm. MS (70 eV): *m*/*z* (%) = 393 (2) $[M]^+$, 364 (1) $[M - C_2H_5]^+$, 336 (2) $[M - C_4H_9]^+$, 287 (3), 258 (5), 245 (4), 214 (3), 182 (2), 146 (5), 124 (2), 105 (100) $[Bz]^+$, 91 (49) $[Bn]^+$, 77 (25) $[Ph]^+$. 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 benzylated derivatives **13** and **12**, employing published conditions.[3] These compounds were then compared with reference substances available from previous studies.

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[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.
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