Biotransformation of β-amino nitriles: The role of the *N*-protecting group

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Abstract—*N*-Tolylsulfonyl- and *N*-butyloxycarbonyl-protected β-amino nitriles were prepared to study the effect of the *N*-protecting group on the biotransformation of the β-amino nitriles to the corresponding β-amino amides and acids. The bioconversions were carried out by using whole cells of *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540. The bioconversion products of five-membered carbocyclic nitriles were mainly the respective acids whereas the carbocyclic six-membered nitriles were accumulated at the stage of the amide.

Keywords: β-amino nitriles, β-amino acids, protecting groups, biotransformation, nitrile hydratase

1. Introduction

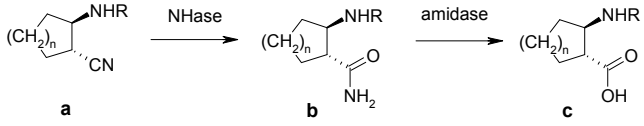
In recent years, β-amino acids have been subject of considerable attention due to their significant effects, such as antibiotic [1-3], antifungal [4,5] cytotoxic [6] and other important pharmacological properties [7].

β-Amino acids occur as key components in many peptidic natural products [7,8]. In functionalized mode, they occur in a variety of bioactive molecules, a known example is paclitaxel (Taxol®). They also exhibit pharmacological properties *per se*, such as cispentacin, (*1R,2S*)-2-aminocyclopentane carboxylic acid, an antifungal antibiotic [9,10]. The replacement of α-amino acids in biologically active peptides by certain β-counterparts can have pronounced effects on their folding properties [11], resulting in modified biological properties [12,13]. Therefore, considerable efforts have been made to develop synthetic methods [7,14-16].

Nitriles are versatile precursors to carboxylic acids. Their chemical conversion requires harsh conditions whereas enzymatic reactions occur under mild conditions. As a result, the synthetic potential of many nitrile converting microbial strains has been subject of intense investigation as reflected by several reviews [17,18].

We have demonstrated that *Rhodococcus equi* A4 is an efficient biocatalytic system for diastereo-selective [19] and chemoselective [20] *trans*-formations.

In this laboratory, we have recently reported on the microbial transformation of *N*-tosylated β-amino nitriles to β-amino amides/acids using whole cells of *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540, both containing the nitrile hydratase/ amidase enzyme system [21].



Scheme 1. Biotransformation of alicyclic nitriles to amides and carboxylic acids

Although the literature seems not to be short on subjects dealing with substrate solubility and co-solvent compatibility in biotransformations [22,23],

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this knowledge is hardly applicable in an unexplored area, such as the microbial transformation of β-amino nitriles. Frequently, nature and amount of the cosolvent have to be newly developed.

2. Experimental

2.1. Materials and methods

Analytical thin layer chromatography was carried out on Merck Silica gel 60 F_{254} plates. Flash chromatography was performed on Merck Silica gel 60, 230-400 mesh. Analytical HPLC was conducted with a Hewlett Packard Series 1100 HPLC using a LiChrospher®100 RP18e column, 5μm particle size and a G1315A diode array detector. For preparative HPLC a Merck-Hitachi LC-6200 pump and L-4000 UV-detector was used. Separations were performed on a 21.2 x 250 mm Zorbax SB-C18 preparative HPLC column. EI-mass spectra were recorded with a Hewlett-Packard 5972 MSD and HP 6890 Series II GC. ^{1}H-NMR (199.98 MHz) and ^{13}C-NMR (50.29) spectra were recorded on a Varian GEMINI-200BB. ^{1}H (499.82 MHz) and ^{13}C-NMR (125.69 MHz) spectra were recorded on a Varian INOVA 500. 2D-techniques (HSQC, HMBC) as well as DEPT and deuterium exchange were used to assist in structure elucidation. Melting points were determined on a Electrothermal MEL-TEMP apparatus.

2.2. Microorganisms and cultivation

2.2.1. Medium

Rhodococcus sp. R312 is commercially available (CBS 717.73). *Rhodococcus erythropolis* NCIMB 11540 was obtained from DSM Research, The Netherlands. The medium used for maintainance on agarplates (15.0 g/L agar) and cultivation was sterilized in five separate groups: Group I: 4.97 g/L Na_2HPO_4 and 2.04 g/L KH_2PO_4, Group II: 0.20 g/L $MgSO_4*7H_2O$, Group III: 0.02 g/L $CaCl_2*2H_2O$, ammonium ferric (III) citrate and 1.00 ml/L trace element solution (100 mg/L $ZnSO_4*7H_2O$, 300 mg/L H_3BO_3, 200 mg/L $CoCl_3*6H_2O$, 6 mg/L $CuSO_4$, 20 mg/L $NiCl_2*6H_2O$, 30 mg/L $NaMoO_4*2H_2O$, 25 mg/L $MnCl_2*2H_2O$), Group IV: 1.00 g/L yeast extract and 10.0 g/L peptone from meat, Group V: 10.0 g/L glucose.

2.2.2. Cultivation

Both strains were subcultured at 30 °C and 150 rpm in 250 ml shaking flasks, each containing 100 ml of the above described medium. After 24 hours, 5 ml of the subcultures were inoculated into 1000 ml shaking flasks containing 250 ml of medium. After 20 hours of incubation at 30 °C and 150 rpm in a rotary shaker, the cells were harvested by centrifugation (5500 rpm, 20 min, 4 °C). The cells were washed with phosphate buffer (4.98 g/L Na_2HPO_4, 2.04 g/L KH_2PO_4, pH 7.5) and again centrifuged.

2.3. Synthesis of substrates

2.3.1. *N*-Ts-protected compounds **1a** and **2a**

Substrates **1a** and **2a** were prepared as previously described [21].

2.3.2. *N*-Boc-protected compounds **3a** and **4a**

To a solution of **1a** or **2a** in anhydrous CH_3CN 2.2 equiv. of Boc_2O and 0.1 equiv. of DMAP were added. After stirring for 24 h at 40 °C, the solvent was removed under reduced pressure. The remaining oil was diluted with CH_2Cl_2 and washed with saturated NH_4Cl. The aqueous phase was extracted with CH_2Cl_2 three times. The combined organic layers were dried with Na_2SO_4 and evaporated to give a crude oil which was used for the detosylation step without further purification. Thus, the oil was dissolved in anhydrous MeOH. After addition of 5 equiv. of Mg turnings ultrasound was applied for 15 min. The reaction mixture was diluted with CH_2Cl_2 and washed with HCl (2N), $NaHCO_3$ and brine. After drying with Na_2SO_4 and evaporation, the products were purified using silica gel chromatography.

2.4. Biotransformation using whole cells

2.4.1. *N*-Ts-protected compounds **1** and **2**

To a suspension of 6 g washed wet cells in 50 ml of the above mentioned phosphate buffer, 2 mmol of the substrates were added as powder in case of rather water soluble cyclopentane derivative **1a**, alternatively, for compound **2a** as solution in DMSO (1.25 ml). The baffeled Erlenmeyer flasks were shaken for 24 h at 30 °C and 150 rpm. The reaction was stopped by adding 20 ml of HCl (2N). After centrifugation (10000 rpm, 20 min, 4 °C) the supernatant was extracted three times with 50 ml of CH_2Cl_2. Therefore, the aqueous phase was cooled to 4 °C before extraction, otherwise phase separation was

unsatisfying. To prevent from losses with respect to the unreacted nitriles, the cells were also extracted twice with ethyl acetate. The combined organic layers were dried with Na_2SO_4 and the solvent was removed under reduced pressure. Unreacted nitrile and the products were purified by either silica gel chromatography and recrystallization or preparative HPLC on a Zorbax SB-C18 column using an acetonitrile / 0.1% H_3PO_4 gradient elution system.

2.4.2. *N*-Boc-protected compounds **3a** and **4a**

To a suspension of 6g washed wet cells in 50 ml of the above mentioned phosphate buffer, 2 mmol of the substrates were added as powder in case of the strongly watersoluble cyclopentane derivative **3a** or as a solution in DMSO (1.25 ml) for compound **4a**. The baffeled Erlenmeyer flasks were shaken for 24 h at 30 °C and 150 rpm. The reaction was stopped by centrifugation (10000 rpm, 20 min, 4 °C). The supernatant was extracted three times with 50 ml of CH_2Cl_2. The cells were also extracted twice with ethyl acetate. The combined organic layers were dried with Na_2SO_4 and the solvent was removed under reduced pressure. Since the respective *N*-Boc-cyclopentane carboxylic acids and amides are highly water soluble, their extraction was not possible. Thus, CELITE was added to the supernatant and the water was removed under reduced pressure. After that, unreacted nitrile and products were purified by silica gel chromatography.

2.4.3. (±)-trans-N-(2-Cyanocyclopentyl)-4-methyl benzene sulfonamide ***1a***

White solid, mp 109-110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44-1.51 (m, 1H), 1.65-1.80 (m, 2H), 1.83-1.90 (m, 1H), 1.93-2.00 (m, 1H), 2.06-2.13 (m, 1H), 2.44 (s, 3H), 2.83 (dt, 1H, J = 8.6, 6.0 Hz, H-1), 3.73 (m, 1H, J = 6.7 Hz, H-2), 5.72 (d, 1H, J = 7.2 Hz, NH), 7.35 (2H, d, J = 8.3 Hz), 7.81 (2H, d, J = 8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.84, 22.88, 29.22, 32.84, 35.88, 58.92, 121.41, 127.49, 130.26, 136.76, 144.36; m/z (EI) 264 M^+ (10), 210 (27), 155 (38), 109 (26), 91 (100), 65 (22).

2.4.4. (±)-trans-2-(Toluene-4-sulfonylamino) cyclo-pentane carboxamide ***1b***

White solid, mp 166-169 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.15-1.22 (m, 1H), 1.38-1.52 (m, 4H), 1.78-1.82 (m, 1H), 2.37 (s, 3H), 2.44 (dt, 1H, J = 8.8, 6.8 Hz, H-1), 3.66 (m, 1H, J = 7.1 Hz, H-2), 6.72 (s, br., 1H, NH_2), 7.14 (s, br., 1H, NH_2), 7.35 (d, 2H, J = 8.1 Hz), 7.61 (d, 1H, J = 7.8 Hz, NH), 7.65 (2H, d, J = 8.1 Hz); ^{13}C NMR (125 Hz, DMSO-d_6) δ 21.68, 24.03, 29.97, 33.47, 51.60, 57.58, 127.23, 130.20, 139.36, 143.05, 176.09; Mass spectrum can not be given due to decomposition.

2.4.5. (±)-trans-2-(Toluene-4-sulfonylamino) cyclo-pentane carboxylic acid ***1c***

White solid, mp 124-125 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44-1.52 (m, 1H), 1.60-1.76 (m, 2H), 1.78-1.85 (m, 1H), 1.95-2.09 (m, 2H), 2.43 (s, 3H), 2.73 (dt, 1H, J = 8.8, 7.5 Hz, H-1), 3.80 (m, 1H, J = 7.0 Hz, H-2), 5.19 (d, 1H, J = 6.4 Hz, NH), 7.30 (d, 2H, J = 8.2 Hz), 7.77 (d, 2H, J = 8.2 Hz), 9.20 (s, br., 1H, COOH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.79, 23.18, 28.47, 33.71, 50.87, 57.73, 127.53, 129.98, 137.13, 143.91, 179.73; m/z (EI) partial decomposition: 254 (1), 210 (7), 172 (2), 155 (27), 128 (98), 110 (20), 91 (100), 82 (33), 65 (31), 56 (22).

2.4.6. (±)-trans-N-(2-Cyanocyclohexyl)-4-methyl-benzene sulfonamide ***2a***

White solid, mp 106-108 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25-1.39 (m, 6H), 1.58-1.68 (m, 3H), 1.93-1.97 (m, 1H), 2.01-2.06 (m, 1H), 2.44 (s, 3H), 2.62-2.68 (m, 1H, H-1), 3.35-3.41 (dq, 1H, J = 4.1, 8.3 Hz, H-2), 5.23 (d, 1H, J = 8.3 Hz, NH), 7.34 (d, 2H, J = 8.5 Hz), 7.82 (d, 2H, J = 8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.83, 22.78, 23.14, 27.43, 31.66, 34.69, 52.91, 120.46, 127.46, 130.11, 137.31, 144.19; m/z (EI) 278 M^+ (4), 210 (33), 155 (32), 123 (16), 91 (100), 65 (31).

2.4.7. (±)-trans-2-(Toluene-4-sulfonylamino) cyclo-hexane carboxamide ***2b***

White solid, mp 212-213 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95-1.06 (m, 3H), 1.32-1.38 (m, 1H), 1.43-1.47 (m, 3H), 1.68-1.71 (m, 1H), 2.03 (dt, 1H, J = 3.7, 10.8 Hz, H-1), 2.35 (s, 3H), 3.26 (dq, 1H, J = 3.7, 9.8 Hz, H-2), 6.70 (s, br., 1H, NH_2), 7.01 (s, br., 1H, NH_2), 7.32 (d, 2H, J = 8.0 Hz), 7.42 (d, 1H, J = 9.8 Hz, NH), 7.65 (d, 2H, J = 8.0 Hz); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 21.65, 24.81, 24.84, 29.79, 33.13, 50.43, 53.92, 126.96, 130.04, 140.95, 142.69, 175.55; Mass spectrum can not be given due to decomposition.

2.4.8. (±)-trans-2-(Toluene-4-sulfonylamino) cyclo-hexane carboxylic acid ***2c***

White solid, mp 175-176 °C ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13-1.25 (m, 3H), 1.45-1.54 (m, 1H), 1.64

(2H, m), 1.96-1.99 (m, 2H), 2.34 (dt, 1H, J = 3.6, 10.7 Hz, H-1), 2.40 (s, 3H), 3.36 (dq, 1H, J = 3.7, 8.9 Hz, H-2), 5.31 (d, 1H, J = 8.9 Hz, NH), 7.29 (d, 2H, J = 8.3 Hz), 7.76 (d, 2H, J = 8.3 Hz), 8.25 (s, br., 1H, COOH); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 21.83, 24.29, 24.52, 28.86, 33.37, 49.67, 54.03, 127.41, 129.85, 137.96, 143.63, 178.83; Mass spectrum can not be given due to decomposition.

2.4.9. (±)-(2-Cyano-cyclopentyl) carbamic acid tert-butyl ester ***3a***

White solid, mp 97-98 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.55 (m, 1H), 1.77-1.86 (m, 2H), 1.92-1.99 (m, 1H), 2.09-2.18 (m, 2H), 2.84 (s-like, br., 1H, H-1), 4.13 (m, 1H, J = 6.6 Hz, H-2), 4.66 (s, br., 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.93, 28.55, 29.29, 31.80, 35.23, 56.95, 80.23, 121.87, 155.25; m/z (EI) 209 $(M-1)^+$ (1), 153 (5), 109 (14), 82 (9), 59 (38), 57 (100), 56 (58), 41 (28).

2.4.10. (±)-(2-Carbamoyl-cyclopentyl) carbamic acid tert-butyl ester ***3b***

White solid, mp 186-188 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32-1.39 (m, 1H), 1.36 (9H, s), 1.48-1.62 (m, 3H), 1.76-1.87 (m, 2H), 2.39-2.43 (q, 1H, J = 7.8 Hz, H-1), 3.86 (m, 1H, J = 7.2 Hz, H-2), 6.74 (s, br., 1H, NH_2), 6.82 (d, 1H, J = 7.2 Hz, NH), 7.17 (s, br., 1H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.86, 28.95, 29.32, 33.69, 50.91, 55.74, 78.27, 155.82, 176.59; m/z (EI) 172 (10), 155 (6), 127 (27), 111 (8), 83 (18), 72 (42), 57 (100).

2.4.11. (±)-2-tert-Butoxycarbonylamino cyclo-pentane carboxylic acid ***3c***

White solid, mp 137-138 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.47 (m, 1H), 1.68-1.73 (2H, m), 1.88-1.92 (m, 1H), 2.06-2.12 (m, 2H), 2.73 (s-like, br., 1H, H-1), 4.04 (s-like, br., 1H, H-2), 4.94 (s, br., 1H, NH), 11.43 (s, br., 1H, COOH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.64, 28.52, 28.54, 33.85, 52.73, 56.04, 81.23, 157.31, 176.79; Mass spectrum can not be given due to decomposition.

2.4.12. (±)-(2-Cyano-cyclohexyl) carbamic acid tert-butyl ester ***4a***

White solid, mp 122-124 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24-1.10 (m, 3H), 1.46 (s, 9H), 1.63-1.76 (m, 3H), 2.01-2.10 (m, 2H), 2.59 (s-like, br., 1H, H-1), 3.68 (m, 1H, H-2), 4.69 (s, br., 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.92, 28.54, 28.60, 31.83, 35.02, 50.83, 80.25, 120.87, 155.13; m/z (EI) 223 $(M-1)^+$ (1), 167 (7), 150 (4), 124 (20), 108 (8), 81 (8), 59 (42), 57 (100), 56 (98).

2.4.13. (±)-(2-Carbamoyl-cyclohexyl) carbamic acid tert-butyl ester ***4b***

White solid, mp 214-215 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.01-1.19 (m, 3H), 1.29-1.37 (m, 1H), 1.32 (s, 9H), 1.57-1.62 (m, 2H), 1.66-1.69 (m, 1H), 1.76-1.78 (m, 1H), 2.05 (dt, 1H, J = 3.1, 11.5 Hz, H-1), 3.28-3.35 (m, 1H, H-2), 6.46 (d, 1H, J = 8.8 Hz, NH), 6.71 (s, br., 1H, NH_2), 6.91 (s, br., 1H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.21, 25.28, 28.95, 29.82, 33.55, 49.47, 51.15, 78.07, 155.37, 176.19; m/z (EI) 242 M^+ (1), 185 (19), 168 (16), 141 (60), 124 (32), 97 (25), 83 (30), 72 (18), 57 (100), 56 (64), 55 (18).

2.4.14. (±)-N-(Toluene-4-sulfonyl)-6-azabicyclo [3.1.0] hexane ***5***

White solid; mp 87-88 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43-1.64 (m, 4H), 1.81-1.93 (m, 2H), 2.37 (s, 3H), 3.26 (s, 2H), 7.26 (d, 2H, J = 8.4 Hz), 7.75 (d, 2H, J = 8.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.68, 21.69, 27.05, 46.85, 127.68, 129.78, 136.04, 144.29; m/z (EI) 237 M^+ (1), 173 (9), 118 (3), 91 (23), 82 (84), 65 (21), 55 (100).

2.4.15. (±)-N-(Toluene-4-sulfonyl)-7-azabicyclo [4.1.0] heptane ***6***

White solid; mp 58-59 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19-1.49 (m, 4H), 1.75-1.78 (m, 4H), 2.44 (s, 3H), 2.97 (t, 2H, J = 1.3 Hz), 7.32 (d, J = 8.2 Hz), 7.81 (d, 2H, J = 8.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.64, 21.84, 23.01, 40.04, 127.84, 129.82, 136.20, 144.25; m/z (EI) 251 M^+ (1), 154 (3), 96 (100), 91 (21), 69 (27), 67 (11).

2.4.16. (±)-6-Aza-bicyclo[3.1.0]hexane-N-carboxylic acid tert-butyl ester ***7***

Colourless oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15-1.29 (m, 2H), 1.44 (s, 9H), 1.58-1.65 (m, 4H), 2.02-2.12 (m, 2H), 2.91 (s-like, br., 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.70, 26.69, 27.62, 43.22, 85.39, 146.97; m/z (EI) 168 (1), 127 (3), 83 (24), 82 (17), 68 (28), 67 (18), 57 (100), 41 (42).

3. Results and discussion

With regard to water solubility, the transformation of the unprotected amino nitriles would appear to be the method of choice. However, the biotransformation is severely hindered by the difficulties arising in terms of reaction monitoring and product isolation of strongly polar compounds. The high solubility of 2-amino cyclopentane/hexane carboxylic acid/amide in aqueous media makes their isolation by extraction into an organic solvent difficult. TLC, in general a powerful tool for screening of biotransformation reactions [24], frequently fails in its resolution power of carboxylic acids versus carboxamides. Most of the biotransformation reactions of polar organic compounds can be monitored by reversed phase-HPLC and UV-detection. However, the detection of aliphatic and alicyclic nitriles/amides/acids is restricted because of their poor UV-sensitivity, in particular on a screening scale.

A major part of these drawbacks can be circumvented by protecting the amino group.

The effect of protecting groups on substrate solubility of α-amino acids has been investigated in the context of a new approach to enzymatic peptide synthesis [25]. Another important effect is functional group protection as a tool to modulate the substrate acceptance and selectivity of an enzyme [26].

The aim of the present work is the evaluation of *N*-protecting groups with respect to their applicability for biotransformations. In our case the choice of the proper protecting group is governed by the following considerations: 1) introduction of the protecting group 2) sufficient substrate solubility in aqueous buffer 3) improved reaction monitoring by UV for TLC and HPLC and 4) ease of removal.

It is obvious that the combined requirements can hardly be met in a single protecting group.

In terms of the abovementioned requirements, an amide-like protecting group, in particular the benzoyl-group would be preferable. Our experiments, however, have revealed that the benzoic acid amides were subject of prompt enzymatic amide bond hydrolysis.

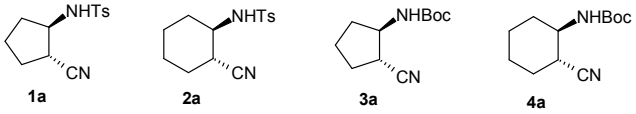
With respect to preparation, we found that *N*-sulfonylamides are excellent to handle. More important, they are not prone to enzymatic hydrolysis by the strains investigated.

Generally, the *N*-benzyl-derivatives of the respective nitriles are of oily consistence and therefore easier available for the enzyme. However, as secondary amines, they cannot be considered as amino-protecting groups.

The carbamoyl-group on the other hand, fits the requirements in particular with regard to 2) and 4), since it is sufficiently water soluble for biotransformation and its deprotection is conveniently achieved.

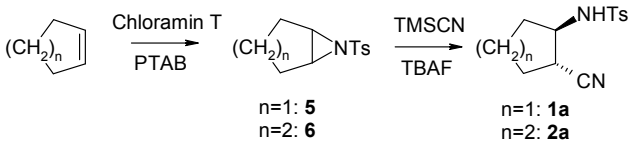
3.1. Introduction of the protecting group

The application of the aforementioned protecting groups for β-amino nitriles is dependent on requirements associated with their synthesis.



Scheme 2. *N*-protected β-amino nitriles

We have found the catalytic aziridination of olefinic precursors and subsequent ring opening of the aziridine ring by cyanide to be the best protocol for preparing the alicyclic *trans*-configured β-amino nitriles **1a** and **2a** [27,28] (Scheme 3). The cyanide driven ring opening requires an activating group and is accomplished best with the tolylsulfonyl-group [29].

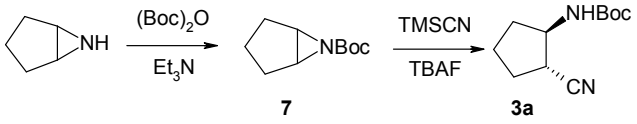


Scheme 3. Preparation of *trans*-configured alicyclic β-amino nitriles

However, when the nitrogen atom is bearing a protecting group different from tosyl, considerable problems concerning this step arise (Scheme 4). There is virtually no equivalent to Chloramine T and tosyliodinane for the analogous preparation of, for example, *N*-acyl- or *N*-carbamoyl protected aziridines. Thus, these *N*-protected azirdinies are solely available *via* the free aziridines.

Substrate **3a** was initially prepared by Iodazide addition to the olefinic bond of cyclopentene,

following reduction with $LiAlH_4$ to the corresponding bicyclic heterocompound 6-azabicyclo[3.1.0]hexane [30,31]. Unfortunately, the extreme volatility of this compound lead to losses in excess of 50% after workup. The aziridine-nitrogen was then masked with the *tert*-butyloxycarbonyl-protecting group. However, the analogue ring opening reaction, successfully applied to **1a** and **2a,** only yielded in 38% of **3a** (given 40% conversion), even though an excess of Boc_2O and elevated temperatures were applied.

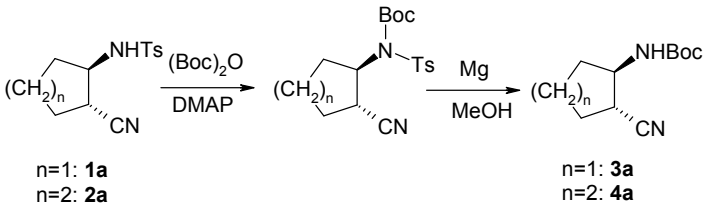


Scheme 4. Preparation of **3a** via the free aziridine

Although the cyanide mediated ring opening of *N*-protected aziridines other than tosyl has been mentioned in the literature [29,32,33], our own investigations on this subject have revealed that the *N*-Boc, *N*-benzoyl and *N*-benzyl derivatives undergo ring opening only sluggishly. The cyanide mediated nucleophilic ring opening reaction of a *N*-Boc-protected aziridine has not been reported in the literature.

In summary, the overall yield following this procedure was too low for a preparative purpose.

The unsatisfying results in the preparation of **3a** and **4a** prompted us to investigate alternative protection/deprotection sequences. Recently, the preparation of *N*-Boc protected amines from *N*-tosylates has been published [34].



Scheme 5. Preparation of Boc-protected β-amino nitriles

Thus, in maintaining the smooth ring opening of *N*-Ts-aziridine **1a** and **2a**, an exhaustive protection of the amino group was effected by using Boc-anhydride. In this case, the following deprotection step of the tosyl-group could be achieved in excellent yields using Mg and ultrasonification within minutes [35] (Scheme 5).

3.2. *Sufficient substrate solubility in aqueous buffer*

Table 1 shows the results of the biotransformations of the *N*-tosylated nitriles. Due to the low water solubility of compounds **1a** and **2a**, the addition of a cosolvent was necessary. After testing several cosolvents such as toluene, ethyl acetate, ethanol, DMF and DMSO in different amounts, 5 Vol % of DMSO turned out to be the best choice for the screenings.

Therefore we concentrated on the carbamate-type protecting groups. We anticipated that, in terms of water-solubility and ease of deprotection, the butyloxycarbonyl-group meets our requirements for a microbial transformation at best, although at the expense of good UV-sensitivity.

3.3. Improved reaction monitoring by UV for TLC and HPLC

High UV-sensitivity is crucial for screening experiments. For alicyclic substrates without any chromophor, sufficient UV-activity should be introduced by the protecting group.

The tosylated substrates **1** and **2** have an important property: very good UV-activity. In contrast, the bocylated derivatives **3** and **4** can hardly be detected by HPLC. Neither UV nor RI activity is high enough for the low concentrations used for screening experiments. GC screening methods also fail due to decomposition of the product acids. However, TLC monitoring is possible.

3.4. Removal of the N-protecting group

The Boc-group in the biotransformation products can be easily cleaved under well established conditions [36]. Contrary to that, the standard conditions (30% HBr/acetic acid, reflux or Na/NH_3 liq.) for the cleavage of the *N*-tosyl-group are very harsh. In this case we applied an alternative recently described deprotection protocol [37,38], although with moderate success.

Table 1.

Biotransformations of racemic *N*-protected β-amino nitriles – isolated yields

Entry	Substrate	*Rhodococcus* sp. R312			*Rhodococcus erythropolis* NCIMB 11540		
		nitrile **a** (%)	amide **b** (%)	acid **c** (%)	nitrile **a** (%)	amide **b** (%)	acid **c** (%)
1	**1a**	43	8	29	63	3	19
2	**2a**	47	23	6	58	10	2
3	**3a**	<1	0	85	1	0	85
4	**4a**	17	59	0	17	80	0

3.5. Biotransformation results

The results of the biotransformation of substrates **1a - 4a** are given in Table 1. All *N*-protected nitriles could be readily transformed, although their final products differ considerably. No consecutive hydrolyses of the cyclohexane amides **2b** and **4b** occurred with respect to both *rhodococcus* strains.

These results can be attributed to the inherent structural features of the substrates rather than to the nature of the protecting group, nevertheless the extent of the transformation to the amide could be improved applying the Boc-protected substrate **4a**. In contrast, the influence of the protecting group can be deduced from the results of transformations of the five-membered alicyclic substrates **1a** and **3a**. In this case, the yields of acids **1c** and **3c**, both representing the major transformation products of the two investigated strains, differ considerably. The Boc-protected acid **3c** could be isolated in much higher yield. An effect due to product isolation can be excluded, since product stability as well as extraction properties into organic solvents are both superior for the *N*-tosyl-protected compounds. Even though, we cannot exclude any inhibiting influence of polar product acids nor of the tolylsulfonamide moiety *per se*.

4. Conclusions

Sulfonamide-like as well as carbamate-like *N*-protecting groups were investigated regarding their effect on substrate acceptance and product specificity in the biotransformation of alicylic β-amino nitriles. Whereas the nature of the product appears to be a result of the inherent structure (ring size) of the substrate, the amount of the respective product formed within the same incubation time is higher in case of the carbamate-protected derivatives.

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References

[1] P. Garner, S. Ramakanth, J. Org. Chem. 51 (1986) 2609.
[2] F. Czernecki, S. Franco, J.-M. Valery, J. Org. Chem. 62 (1997) 4845.
[3] S. G. Davies, G. D. Smyth, A. M. Chippindale, J. Chem. Soc. Perkin Trans. 1 (1999) 3089.
[4] S. G. Davies, O. Ichihara, I. Lenoir, I. A. S. Walters, J. Chem. Soc. Perkin Trans. 1 (1994) 1411.
[5] F. Theil, S. Ballschuh, Tetrahedron Asymmetry 7 (1996) 3565.
[6] H. Sone, T. Nemoto, H. Ishiwata, M. Ojika, K. Yamada, Tetrahedron Lett. 34 (1993) 8449.
[7] E. Juaristi, (Ed.), Enantioselective Synthesis of β-Amino Acids, Wiley-VCH, 1997.
[8] G. Cardillo, C. Tomasini, Chem. Soc. Rev. 25 (1996) 117.
[9] M. Konishi, M. Nishio, K. Saitoh, T. Miyaki, T. Oki, H. Kawaguchi, J. Antibiotics 42 (1989) 1749.
[10] T. Iwamoto, E. Tsujii, M. Ezaki, A. Fujie, S. Hashimoto, M. Okuhara, M. Kohsaka, H. Imanaka, K. Kawabata, Y. Inamoto, K. Sakane, J. Antibiotics 43 (1990) 1.
[11] S. H. Gellman, Acc. Chem. Res. 31 (1998) 173.
[12] A. Giannis, T. Kolter, Angew. Chem. 105 (1993) 1303.
[13] J. A. Robl, M. P. Cimarusti, L. M. Simpkins, H. N. Weller, Y. Y. Pan, M. Malley, J. D. DiMarco, J. Am. Chem. Soc. 116 (1994) 2348.

[14] E. Juaristi, D. Quintana, J. Escalante, Aldrichim. Acta 27 (1994) 3.
[15] D. C. Cole, Tetrahedron 50 (1994) 9517.
[16] M. Liu, M. P. Sibi, Tetrahedron 58 (2002) 7991.
[17] L. Martinková V. Kren, Biocatal. Biotrans. 20 (2002) 73.
[18] T. Sugai, T. Yamazaki, M. Yokoyama, H. Ohta, Biosci. Biotech. Biochem. 6 (1997) 1419.
[19] L. Martínková, N. Klempier, M. Preiml, M. Ovesná, M. Kuzma, V. Mylerova, V. Kren, Can. J. Chem. 80 (2002) 724.
[20] L. Martínková, N. Klempier, J. Bardakji, A. Kandelbauer, M. Ovesná, T. Podarilová, M. Kuzma, I. Prepechalová, H. Griengl, V. Kren, J. Mol. Catal. B: Enzymatic 14 (2001) 95.
[21] M. Preiml, K. Hillmayer, N. Klempier, Tetrahedron Lett. 44 (2003) 5057.
[22] S. G. Cull, J. M. Woodley, G. J. Lye, Biocatalysis and Biotransformation 19 (2001) 131.
[23] R. Leon, P. Fernandez, H. M. Pinheiro, J. M. S. Cabral, Enzyme Microb. Technol. 23 (1988) 483.
[24] M. Pressnig, N. Klempier, H. Schwab, J. Biotechnol., submitted for publication.
[25] A. Fischer, A. S. Bommarius, K. Drauz, C. Wandrey, Biocatalysis 8 (1994) 289.
[26] K. Faber, Biotransformation in Organic Chemistry: a Textbook, 4th ed., Springer, Berlin, 2000.
[27] D. A. Evans, M. M. Faul, M. T. Bilodeau, J. Am. Chem. Soc. 116 (1994) 2742.
[28] J. U. Jeong, B. Tao, H. Hennings, K. B. Sharpless, J. Am. Chem. Soc. 20 (1998) 6844.
[29] J. Wu, X.-L. Hou, L.-X. Dai, J. Org. Chem. 65 (2000) 1344.
[30] Z. da Zang, R. Scheffold, Helv. Chim. Acta 76 (1993) 2602.
[31] A. Hassner, G. J. Matthews, F. W. Fowler, J. Am. Chem. Soc. 91 (1969) 5046.
[32] T. Katagiri, M. Takahashi, Y. Fujiwara, H. Ihara, K. Uneyama, J. Org. Chem. 64 (1999) 7323.
[33] J. Farras, X. Ginesta, P. W. Sutton, J. Taltavull, F. Egeler, P. Romea, F. Urpí, J. Vilarrasa, Tetrahedron 57 (2001) 7665.
[34] J. L. Matthews, D. R. McArthur, K. W. Muir, Tetrahedron Lett. 43 (2002) 5401.
[35] B. Nyasse, L. Grehn, U. Ragnarsson, Chem. Commun. (1997) 1017.
[36] T. W. Greene, P. G. M. Wuts, Protective Groups in Organic Synthesis, 3rd ed., John Wiley & Sons, New York, 1999.
[37] S. C. Bergmeier, P. P. Seth, Tetrahedron Lett. 40 (1999) 6181.
[38] D. A. Alonso, P. G. Andersson, J. Org. Chem. 63 (199) 9455.