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A new approach to β-amino acids: biotransformation of *N*-protected β-amino nitriles

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Abstract—A number of novel *N*-protected β-amino nitriles were prepared as substrates for two nitrile-converting microorganisms, *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540. The respective biotransformation products, β-amino acids, are known to be pharmacological very potent compounds. © 2003 Published by Elsevier Science Ltd.

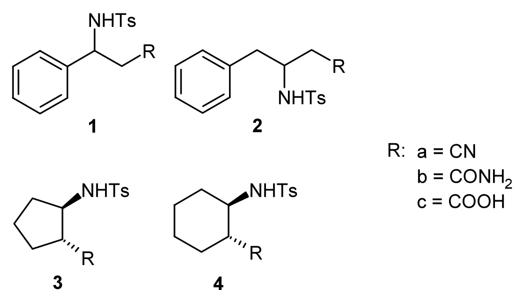
During the last years β-amino acids have gained considerable attention due to their antibiotic,[1] antifungal,[2] cytotoxic[3] and other important pharmacological properties.[4]

A known example is (2*R*,3*S*)-3-phenylisoserine, an essential constituent of the potent antitumor agent paclitaxel (Taxol®) and analogues thereof (e.g. Taxotere®).[4,5] β-Amino acids are key components of many naturally occurring peptides.[4,6] They also exhibit pharmacological properties per se, such as (1*R*,2*S*)-2-aminocyclopentane carboxylic acid (cispentacin), an antifungal antibiotic.[7] The replacement of α-amino acids in biologically active peptides by certain β-counterparts can have pronounced effects on their folding properties,[8] resulting in modified biological properties of the unnatural analogues.[9] Currently, the synthesis of oligopeptide chains of β-amino acids is attracting much interest because of their ability to fold into defined three-dimensional structures.[10] As a result, much effort has been made to develop efficient methods for the preparation of this compound class, as reflected by several recent reviews.[4,11]

The potential of both isolated enzymes and whole cell systems of several *Rhodococci* for the preparation of carboxylic acids and amides under very mild conditions has recently been demonstrated by others[12] and also by ourselves.[13]

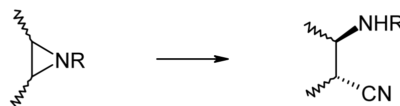
We now wish to report the preparation of β-amino acids using whole cells of *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540, both containing the nitrile hydratase/amidase enzyme system.[14]

In this work we have put emphasis on the preparation of aliphatic β-amino acids bearing a phenyl residue as well as on *trans*-configured alicyclic β-amino acids **1**–**4**.[15]



The corresponding *cis*-configured alicyclic β-amino nitriles are currently investigated and will be subject of a forthcoming paper.

The most feasible method to prepare β-amino nitriles is the (regioselective) ring-opening of aziridines (Scheme 1). Up to now, few studies have been dealing with the ring opening of aziridines using cyanides as nucle-



Scheme 1.

Keywords: β-amino nitriles; β-amino acids; aziridines; biotransformation; nitrile hydratase.

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Table 1. Screening of racemic *N*-protected β-amino nitriles

Entry	Substrate	*Rhodococcus* sp. R312			*Rhodococcus erythropolis* NCIMB 11540		
		Nitrile[a] (**a**)	Amide[a] (**b**)	Acid[a] (**c**)	Nitrile[a] (**a**)	Amide[a] (**b**)	Acid[a] (**c**)
1	**1a**	95	3	2	90	7	3
2	**2a**[16]	94	2	4	90	5	5
3	**3a**[17]	58	5	37	36	5	59
4	**4a**[18]	69	23	8	58	25	17

[a] % determined by HPLC; reference amides were prepared from the nitriles using aqueous H_2O_2 and K_2CO_3 in MeOH;[19] carboxylic acids were available by standard basic hydrolysis of the nitriles using NaOH, however, yields were poor and differed strongly from case to case.

Table 2. Biotransformations of racemic *N*-protected β-amino nitriles—isolated yields

Entry	Substrate	*Rhodococcus* sp. R312[28]			*Rhodococcus erythropolis* NCIMB 11540[28]		
		Nitrile (**a**)	Amide (**b**)	Acid (**c**)	Nitrile (**a**)	Amide (**b**)	Acid (**c**)
1	**3a**	43	8	29	63	3	19
2	**4a**	47	23	6	58	10	2

ophiles, except for lanthanoid tricyanides,[20] the tetrabutylammonium fluoride-catalyzed[21] reaction of TMSCN with *N*-tosylated aziridines and the reaction of NaCN with *N*-nosylaziridines.[22]

The aziridines for the preparation of **1a**–**4a** were easily accessible by bromine- or copper-catalyzed addition of Chloramine T[23] or *N*-(tolylsulfonyl)iminophenyliodinane[24] to the respective olefins. Subsequently, ring opening was carried out as described above.

The first experiments of biotransformation of **1a–4a** (Table 1) revealed that the tosyl group, despite its synthetic advantages, has some disadvantages for the biotransformation reaction, such as low substrate solubility[25] in aqueous media. Moreover, the removal of the tosyl group, following several published procedures,[26] can be troublesome, particularly in the presence of a cyano group.

Isolated yields[27] were determined for those substrates which showed promising screening results (Table 2). It can be seen from the results in Table 2 that the six-membered alicyclic substrate **4a** is mainly accumulated at the stage of the amide and, in contrast to the five-membered substrate **3a**, not further converted to the carboxylic acid.

In summary, we have developed a useful protocol for preparing β-amino acids starting from β-amino nitriles. Some of the products have so far not been reported in the literature, therefore their spectral data are included in the reference part.[16–18]

Future work in our laboratory will be directed towards more suitable *N*-protecting groups, preferably introduced at the beginning of the synthetic sequence, as well as towards the screening of other nitrile-converting microorganisms. The determination of the enantiomeric excess of all biotransformation products is planned since preliminary results found for product β-amino acid **3c**, even though not yet optimized, are encouraging.[29]

Acknowledgements

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14. For a typical experiment, cells of *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540 were freshly cultivated and harvested by centrifugation. After a washing step, the cells were resuspended in phosphate buffer. To 500 μL of resting cell suspension in Eppendorff vessels, 25 μL of the substrates were added as 5% solutions in DMSO. The bioconversions proceeded at 130 rpm and 30°C in a rotary shaker and were stopped after 24 h by addition of HCl (2N). Remaining starting material and products were extracted with ethylacetate and the conversion rates were determined by reversed-phase HPLC.
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16. Compound **2b**: ^{1}H NMR (acetone-d_6) δ 2.39 (3H, s), 2.74–2.87 (4H, m), 3.74 (1H, m), 6.38 (1H, s, br., N$\underline{H}_2$), 6.72 (1H, s, br., N$\underline{H}$), 6.90 (1H, s, br., N$\underline{H}_2$), 7.08–7.19 (5H, m), 7.28 (2H, d, J=8.2 Hz), 7.62 (2H, d, J=8.2 Hz); ^{13}C NMR δ 21.39, 39.27, 41.47, 53.70, 127.08, 127.71, 129.09, 130.26, 130.32, 139.02, 139.90, 143.55, 173.42.
17. Compound **3b**: ^{1}H NMR (acetone-d_6) δ 1.29–1.97 (6H, m), 2.41 (3H, s), 2.59–2.86 (1H, m), 3.78–3.89 (1H, m), 6.14 (1H, s, br., N$\underline{H}_2$), 5.54 (1H, s, br., N$\underline{H}$), 5.58 (1H, s, br., N$\underline{H}_2$), 7.37 (2H, d, J=7.3 Hz), 7.78 (2H, d, J=7.3 Hz); ^{13}C NMR (DMSO-d_6) δ 21.59, 23.77, 29.66, 33.22, 51.48, 57.65, 127.19, 130.30, 138.72, 143.59, 176.99. Compound **3c**: ^{1}H NMR ($CDCl_3$) δ 1.38–2.14 (6H, m), 2.42 (3H, s), 2.73 (1H, m, J=7.8 Hz), 3.81 (1H, m), 5.40 (1H, d, J=6.6 Hz, N$\underline{H}$), 7.30 (2H, d, J=8.2 Hz), 7.77 (2H, d, J=8.2 Hz), 8.79 (1H, s, br., COO$\underline{H}$); ^{13}C NMR δ 21.79, 23.20, 28.54, 33.66, 50.90, 57.73, 127.52, 129.98, 137.18, 143.90, 179.88.
18. Compound **4b**: ^{1}H NMR (DMSO-d_6) δ 1.04–1.75 (8H, m), 2.07 (1H, m), 2.38 (3H, s), 3.32 (1H, m), 6.71 (1H, s, br., N$\underline{H}_2$), 7.02 (1H, s, br., N$\underline{H}_2$), 7.34 (2H, d, J=7.9 Hz), 7.42 (1H, d, J=8.8 Hz), 7.69 (2H, d, J=7.5 Hz); ^{13}C NMR δ 21.52, 24.80, 29.76, 33.19, 50.41, 53.95, 126.97, 129.95, 140.98, 142.63, 175.53.
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27. *Typical procedure*: 2 mmol of nitrile were dissolved in 1.25 ml of DMSO and added to a suspension of resting cells (6 g wet weight suspended in 50 ml of phosphate buffer). The flask was shaken for 24 h at 30°C and 130 rpm in a rotary shaker. The reaction was stopped by adding 20 ml of HCl (2N). The cells were separated by centrifugation. The products were extracted twice from the aqueous phase with ethylacetate. To prevent from losses, the cells were also extracted twice with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and the solvent was removed under reduced pressure. Unreacted nitrile, product amide and acid were purified by chromatography and recrystallization.
28. HPLC analysis of the crude isolates gave for *R.* sp. R312: 48% **3a**, 13% **3b**, 39% **3c** and 47% **4a**, 45% **4b**, 8% **4c**; for *R. erythropolis* NCIMB 11540: 64% **3a**, 5% **3b**, 31% **3c** and 74% **4a**, 21% **4b**, 5% **4c**.
29. 27% e.e. for *R.* sp. R312 and 39% e.e. for *R. erythropolis* NCIMB 11540, obtained from HPLC analysis using a Chirobiotic R® column (MeOH/TEA/AcOH 100:0.4:0.1)