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# Synthesis and microbial transformation of β-amino nitriles

Margit Winkler,[a] Ludmila Martínková,[b] Astrid C. Knall,[a] Stefan Krahulec[a] and Norbert Klempier[a,*]

[a]*Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria*
[b]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnska 1083, CZ 142 20 Prague 4, Czech Republic*

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**Abstract**—*Rhodococcus equi* A4, *Rhodococcus erythropolis* NCIMB 11540 and *Rhodococcus* sp. R312 were investigated towards their ability to produce β-amino amides and acids from β-amino nitriles. The microorganisms show comparable trends: five-membered alicyclic 2-amino nitriles were transformed significantly faster than the six-membered compounds and the products of *trans*-2-amino nitriles (amides and acids) were formed considerably faster than the *cis*-counterparts (amides). The *trans*-five membered nitriles gave the amides (**1b**, **5b**) in excellent enantiomeric excess (94–99%), the biotransformation of *trans*-six membered substrates resulted in the formation of the acid (**3c**, **7c**) in excellent ee (87–99%). The ee's of the *cis*-compounds were throughout lower. Fifteen new substances were synthesized and characterized in the course of this work.


## 1. Introduction

β-Amino acids are key structural components of a variety of natural products and drugs.[1,2] Recently, alicyclic β-amino acids, for example, *trans*-aminocyclopentane carboxylic acid, have been used for the synthesis of β-oligopeptides. The folding properties of such synthetic oligomers with unnatural backbones into defined three-dimensional structures have been explored.[3–5] On the other hand (1*R*,2*S*)-*cis*-aminocyclopentane carboxylic acid (cispentacin) itself is a strong antifungal antibiotic,[6] other alicyclic amino acids can be used in heterocyclic chemistry.[7] This interest in β-amino acids and their synthesis is reflected in several recent reviews.[1,2,8]

In recent years, enzymatic methods have been established as an alternative to the harsh reaction conditions of chemical hydrolysis of nitriles.[9] The respective biocatalysts—nitrilases,[10] nitrile hydratases[11] and amidases—are frequently used as whole cell systems or, less frequently, in purified form by several groups.

In this paper, we report the preparation of β-amino amides and carboxylic acids by whole cells of *Rhodococcus equi* A4, *Rhodococcus* sp. R312 and *Rhodococcus erythropolis* NCIMB 11540. These strains express nitrile hydratase and amidase activity.

## 2. Results and discussion

In previously published work, we have demonstrated that β-amino nitriles are readily converted to β-amino amides and acids by nitrile hydratase/amidase containing microorganisms.[12,13] In this work, we wish to report on the stereoselective transformation of *cis*- and *trans*-aminocyclopentane-/hexane nitriles (**1a**–**8a** depicted in Fig. 1) in dependence on ring size and relative configuration of the

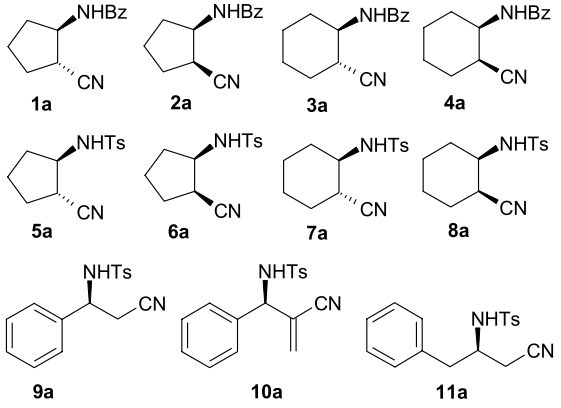


**Figure 1.** Structures of racemic β-amino nitriles for whole cell transformations (only one enantiomer is depicted).

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* Corresponding author. Tel.: +43 316 873 8245; fax: +43 316 873 8740; e-mail: klempier@orgc.tu-graz.ac.at

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**Table 1**. Biotransformations of racemic *N*-protected β-amino nitriles by whole cells—screening

| Entry | Substrate | *R. equi* A4[a] | | | *R. erythropolis* NCIMB 11540[b] | | | *R.* sp. R 312[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrile (%) | Amide (%) | Acid (%) | Nitrile (%) | Amide (%) | Acid (%) | Nitrile (%) | Amide (%) | Acid (%) |
| 1 | **1a** | 0 | 22 | 78 | 0 | 12 | 88 | 0 | 10 | 90 |
| 2 | **2a** | 97[d] | 3[d] | 0[d] | 93 | 4 | 3 | 93 | 3 | 3 |
| 3 | **3a** | 31 | 20 | 50 | 36 | 12 | 52 | 32 | 14 | 54 |
| 4 | **4a** | 99[d] | 1[d] | 0[d] | 91 | 9 | 0 | 94 | 6 | 0 |
| 5 | **5a** | 0 | 13 | 87 | 1 | 1 | 98 | 0 | 0 | 100 |
| 6 | **6a** | 0 | 76 | 24 | 1 | 70 | 29 | 17 | 46 | 37 |
| 7 | **7a** | 0 | 55 | 45 | 2 | 2 | 96 | 2 | 18 | 80 |
| 8 | **8a** | 3 | 96 | 1 | 2 | 92 | 7 | 1 | 90 | 8 |
| 9 | **9a** | 52 | 38 | 9 | 45 | 39 | 16 | 54 | 22 | 24 |
| 10 | **10a** | 6 | 94 | 0 | 2 | 98 | 0 | 6 | 94 | 0 |
| 11 | **11a** | 67 | 18 | 15 | 53 | 26 | 21 | 49 | 21 | 30 |

[a] Cell preparation OD 52.
[b] Cell preparation OD 68.
[c] Cell preparation OD 77.
[d] Cell preparation OD 12.

1,2-positions. The acyclic nitriles **9a**–**11a** serve as precursors for β-phenylalanine, α-methylene-β-amino acids and β-homo-phenylalanine, respectively, (Fig. 1).

## 2.1. Biotransformation

Initial screening experiments were performed using *Rhodococcus equi* A4, *Rhodococcus erythropolis* NCIMB 11540 and *Rhodococcus* sp. R312. Different from our previous work, the microorganisms were cultivated in an inductive medium yielding less biomass, yet with better activity. The results of these screening experiments are depicted in Table 1. In summary they suggest the following general trends: (i) the conversions by the microorganisms with respect to a single substrate structure are comparable. No significant differences in product formation were observed; (ii) the biotransformations of *trans*-compounds are in all cases faster than those of their *cis*-counterparts. The *cis*-benzoates **2a** and **4a** are not accepted as substrates, in contrast, *trans*-**1a** and *trans*-**3a** show rapid conversion; (iii) the conversion of tosylated substrate **6a** is resulting in a significant accumulation of the amide **6b**, while nitrile **5a** is converted to the acid **5c** without accumulating detectable amounts of amide; (iv) the transformation of five-membered alicycles is significantly faster than for the six-membered compounds.

Similar trends, that is, the faster conversion of *trans*-substrates compared to the *cis*-counterparts and the distinct influence of the ring-size on the transformation have been observed for the analogous β-hydroxy cyclopentane/hexane carbonitriles. This was previously reported for *R. equi* A4.[14]

As described later in this paper, the chemical route to the single *cis*-diastereoisomeric nitriles **2a**, **4a**, **6a** and **8a** is tedious and could be accomplished after all by preparative chromatographic separation of a mixture of *cis*/*trans*-diastereomers on silica gel. No synthetic approach to the *cis*-compounds is reported up to now and our efforts to synthesize the pure *cis*-isomers resulted in a 13:1 ratio at best for **2a** (Table 3) to nearly 1:1 for compound **6a** (Scheme 3). The rate difference found in the microbial transformation of *cis*- versus *trans*-isomers is, therefore, for the purpose of a diastereospecific preparation, of great practical use.

This diastereodiscrimination was found to be more distinct in case of the benzoates compared to the tosylates, thus nitriles **2a** and **4a** are not converted at all. Unexpectedly, the conversion rates in general were found to be faster for the tosylated substrates.

To demonstrate the preparative value, larger scale biotransformations were carried out with substrates chosen according to the best screening results in Table 1. The larger scale biotransformations were carried out with the tenfold substrate concentration in contrast to the screening experiments (10 mM versus 1 mM for the screening). In order to provide comparable conversion results, each reaction was

**Table 2**. Biotransformations of racemic *N*-protected β-amino nitriles by whole cells—isolated yields[a]

| Entry | Substrate[b] | *R. equi* A4 | | | *R. erythropolis* NCIMB 11540 | | | *R.* sp. R 312 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrile % (ee %)[c] | Amide % (ee %)[c] | Acid % (ee %)[c] | Nitrile % (ee %)[c] | Amide % (ee %)[c] | Acid % (ee %)[c] | Nitrile % (ee %)[c] | Amide % (ee %)[c] | Acid % (ee %)[c] |
| 1 | **1a** | 0 | 40 (94) | 55 (75) | 0 | 30 (>99) | 63 (48) | 0 | 7 (>99) | 87 (15) |
| 2 | **3a** | 38 (99) | 22 (56) | 36 (>95)[d] | 59 (44) | 16 (67) | 15 (>95)[d] | 61 (82) | 14 (38) | 7 (>95)[d] |
| 3 | **5a** | 40 (47) | 14 (>99) | 44 (2) | 0 | 13 (>99) | 86 (5) | 46 (30) | 10 (>99) | 34 (14) |
| 4 | **6a** | 71 (5) | 14 (51) | 0 | 50 (16) | 49 (15) | 0 | 11 (51) | 75 (7) | 0 |
| 5 | **7a** | 26 (78) | 54 (65) | 13 (>99) | 24 (98) | 56 (59) | 15 (97) | 33 (47) | 42 (77) | 16 (87) |
| 6 | **8a** | 47 (8) | 48 (6) | 0 | 50 (10) | 41 (8) | 0 | 44 (10) | 43 (4) | 0 |
| 7 | **10a** | 84 (0) | 10 (11) | 0 | 91 (1) | 2 (32) | 0 | 73 (0) | 25 (6) | 0 |

[a] Yields after chromatographic purification.
[b] Substrate amount given in the Section 4.
[c] Columns and chromatographic conditions for chiral separation are given in the Section 4.
[d] Since both enantiomers were not 100% baseline separated, the ee is given as >95%; the second enantiomer could not be actually detected.

stopped after a scheduled time (24 h), deliberately taking into account a mixture of nitrile, amide and acid. The yields of the remaining nitriles, product-amides and acids are given after isolation and chromatographic purification in Table 2.

Transformation of the benzoates **1a** and **3a** resulted, in consistency with the screening results, in a mixture of amide and acid. However, the purification of isolated amide **3b** turned out to be troublesome due to its poor solubility even in MeOH or DMSO. The tosylated nitriles **8a** and **10a** were transformed exclusively to the amides. Nitrile **10a**, prepared by aza-Baylis-Hillman reaction, is a precursor for α-methylene-β-amino acids. The related oxo-analogous Baylis–Hillman carbonitrile was recently reported to give exclusively the amide by incubation with cells of *Rhodococcus* sp. AJ270.[15] These results are in analogy to our aza-compound **10a**.

We have investigated the hydrolysis of *cis*-2-NHTs-cyclopentane nitrile (**6a**) over prolonged reaction time to find out, whether the transformation can be brought to completeness with regard to the acid (**6c**). These experiments were carried out on a 3 mM level in order to obtain sufficient material for the determination of the ee. In Figure 2, the conversion of **6a** using *Rhodococcus equi* A4 is plotted against the reaction time. On a 1 mM level, however, the acid could be detected as the sole product after prolonged reaction time. This is in agreement with results we obtained from experiments, whereby the extend of conversion of **6a** was determined in dependence on its concentration after a constant reaction time (20 h). In this case, the highest conversions were achieved within a concentration range of 0.9–1.5 mM of substrate. Rising the concentration turned out to be accompanied by a significant drop in conversion. As can be seen from Figure 2, 34% acid could be achieved after an incubation time of 206 h (3 mM). The application of extended reaction time to other substrates, for example nitrile **10a** resulted in 53% isolated yield of the respective amide (**10b**) as the only product after 120 h (70% conversion according to HPLC), though no acid was formed after this time.

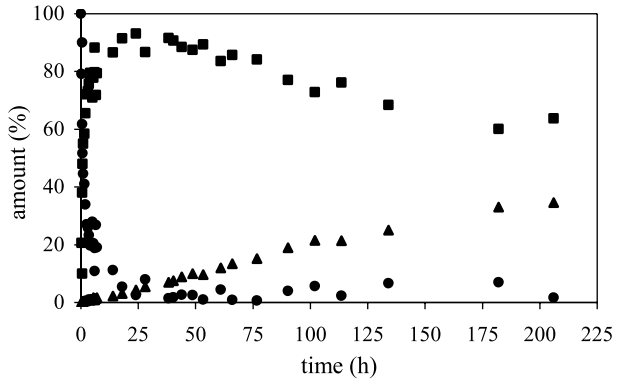



**Figure 2.** Biotransformation of **6a** by *R. equi* A4 in dependence on reaction time. (●) nitrile; (■) amide; (▲) acid.

Given the rather small structural variation within the *cis*- and *trans*-series of substrates **1**–**8**, the requirement of six different chiral HPLC columns (Chiralcel OD-H, Chiralpak AD-H, Chiralcel OJ, Chiral AGP, Chiral HSA and Chirobiotic R) for the determination of the enantiomeric excess of all products appears rather surprising. In the majority of cases a simultaneous separation of nitrile, amide and/or acid was not feasible. The respective results are included in Table 2. The detailed conditions are given in the Section 4.

Despite this, **3c** could not be resolved on any of the columns mentioned, instead, it was finally resolved by gas chromatographic separation of its methyl ester on Chirasil-Dex CB. Recently the liquid chromatographic separation of unprotected β-amino acids was reported employing a Chirobiotic T column[16] and a chiral crown ether (Crownpak CR(+)).[17] The latter one proved to be more efficient in separating *cis*- and *trans*-amino cyclohexane carboxylic acid, however, the formation of an ammonium ion is a prerequisite for successful separation. No separation of β-amino amides is reported up to now.

The enantioselectivity of the *Rhodococci* towards the *trans*-isomers (**1**, **3**, **5**, **7**) is significantly higher as compared to the *cis*-counterparts (**2**, **4**, **6**, **8**), providing the latter were accepted as substrates at all. Thus, the *trans*-amino cyclohexane carboxylic acids **3c** and **7c** could be prepared by all three microorganisms with very high ee (95–99%), instead, the intermediate amides were formed with moderate ee regardless of their protecting group. Differently, the five-membered *trans*-amides **1b** and **5b** were obtained throughout in very high enantiopurity, but not so their acids. The high enantiomeric purity of the remaining *trans*-nitriles **3a** and **7a** is remarkable, considering the extent of their conversions (38 and 24% resp.). At least for the transformation **3a** to **3b**, this can be attributed to the highly enantioselective nitrile hydratase reported for *Rhodococcus equi* A4.[18]

In Figure 3, the conversion of *cis*-2-NHTs-cyclopentane nitrile (**6a**), amide (**6b**) and acid (**6c**) is plotted versus their enantiomeric excess. Again, the experiments were carried out on a 3 mM level over a reaction time of 206 h. The curve in Figure 3 suggests the enantiopurity-conversion dependence expected from a kinetic resolution. Different from the entries in Table 2, where the acid did not form within the scheduled reaction time of 24 h, 33% acid could be obtained in 34% ee after 206 h. Even at low conversion, the ee of the acid did not exceed 56%. The reasons for that are unknown. One explanation is the presence of an additional amidase acting with opposite enantioselectivity.

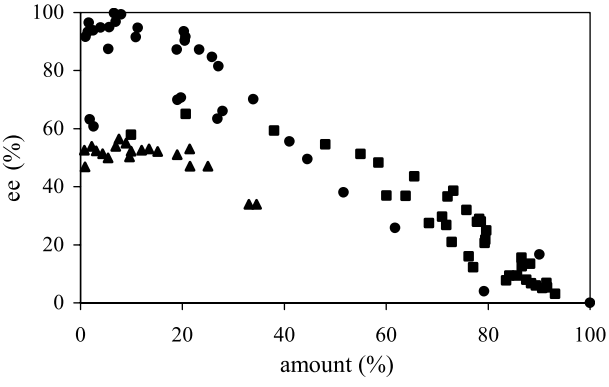
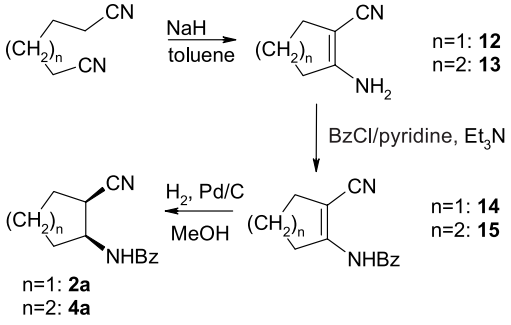



**Figure 3.** Enantiomeric excess of **6a**–**c** in dependence on conversion by *R. equi* A4. (●) nitrile; (■) amide; (▲) acid.

## 2.2. Synthesis of substrates

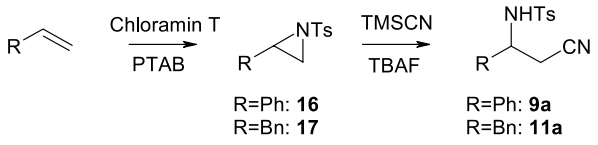
We have made several approaches to prepare **1a**–**4a**, the most preferred one for the *trans*-compounds being via the aziridine ring opening. Generally, the synthetic availability of benzoylated aziridines is limited.[13] However, the benzoylation of the corresponding tosylates with subsequent tosyl-deprotection turned out to be a practicable synthetic protocol for *trans*-compounds **1a** and **3a**, which has already been used in this laboratory for the preparation of Boc-protected amino nitriles.[13] To obtain the *cis*-compounds **2a** and **4a**, we developed a three step synthesis starting from commercially available adiponitrile and heptanedinitrile, respectively. Thorpe–Ziegler cyclization of the latter using NaH yielded unsaturated aminonitriles **12** and **13**.[19]

After benzoylation (BzCl/pyridine) to **14** and **15**, the double bond was catalytically hydrogenated using Pd/C in MeOH at 50 bar and ambient temperature (Scheme 1). The *cis*-selectivity in case of **2a** was 90% after 4 h and 97% conversion and could not be optimized further (Table 3). In the case of the six-membered ring the desired *cis*-compound **4a** could be isolated as a pure diastereoisomer after 3 days under the same conditions at 33% conversion.




**Scheme 1.**

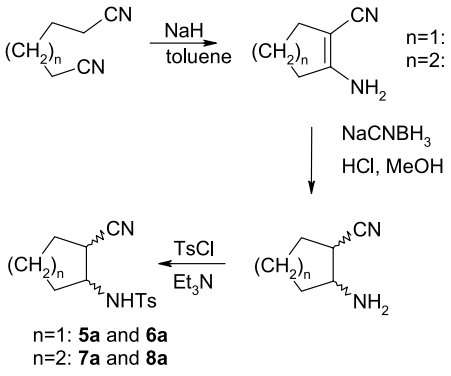
*N*-Ts-protected compounds **5a** and **7a** were prepared as described in our previous paper.[12] The same methodology was used for **9a** and **11a** via **16** and **17**, respectively,[20] (Scheme 2). The known structure **10a** was prepared by aza-Baylis-Hillman reaction, although the reaction conditions had to be optimized. We applied a number of modifications of the procedure described in the literature,[21] such as variation of the dehydrating agent (molecular sieve 4 Å, $PPh_3$, DCC, CDI), variation of the base (DABCO, quinuclidine) and the Lewis acid (Ti(i-OPr)$_4$, Sc–, Y–, Yb–, Gd–, La– and In-triflate). GC/MS evaluation revealed




**Scheme 2.**

that applying other dehydrating agents than molecular sieves partially results in formation of unwanted Baylis–Hillman product, that is the alcohol. Use of quinuclidine resulted in slightly better conversion rates versus DABCO. In-triflate was superior to all the other Lewis acids with respect to conversion and Baylis–Hillman product formation. Summarizing, the price of quinuclidine and In-triflate compared to DABCO and Ti(i-OPr)$_4$ rather suggests an application of the latter reagents.

*cis*-Tosylates **6a** and **8a** could not be prepared in an analogous way to Scheme 1, since the tosylation of **12** and **13** resulted in complex mixtures and the desired products could only be isolated in yields lower than 5%. Compounds **6a** and **8a** were available through the three step synthesis shown in Scheme 3, followed by silica gel separation of the diastereomers.[22]




**Scheme 3.**

Reference amides were prepared according to a standard procedure,[23] except **10b**, which was available only through biotransformation.

The chemical hydrolysis to reference acids was attempted by refluxing the corresponding nitriles in NaOH concd. Not surprisingly, the majority of the acids, that is, **1c**–**4c** and **6c**–**8c** could not be prepared by this way. Chemical hydrolysis of **7a** and **8a** resulted in mixtures of nitrile,

**Table 3**. Optimization of reaction conditions for catalytic hydrogenation of **14**

| Entry | Catalyst | Solvent | Pressure (bar) | Time | **14** (%) | **1a** (%) | **2a** (%) |
|---|---|---|---|---|---|---|---|
| 1 | $Rh/Al_2O_3$ | MeOH | 1 | 2d | 100 | 0 | 0 |
| 2 | 10% Pt/C | MeOH | 1 | 2d | 100 | 0 | 0 |
| 3 | 10% Pd/C | MeOH | 1 | 7d | 4 | 16 | 80 |
| 4 | 10% Pd/C | Ethyl acetate | 1 | 13d | <1 | 12 | 84 |
| 5 | 10% Pd/C | MeOH/$H_2O$ | 1 | 2d | <1 | 26 | 74 |
| 6 | Wilkinson | MeOH | 50 | 4 h | 100 | 0 | 0 |
| 7 | 5% Pd/C | Ethyl acetate | 50 | 3d | 9 | 17 | 74 |
| 8 | 5% Pd/C | MeOH | 50 | 20 h | 0 | 21 | 79 |
| 9 | 5% Pd/C | MeOH | 50 | 4 h | 3 | 7 | 90 |

amide and acid, even at reaction times longer than 14 days. *cis*-Nitrile **6a** epimerized under these drastic conditions to the *trans*-acid **5c**.

The benzoylated compounds were deprotected under these conditions. Thus, **2c–4c**, **7c** and **8c** were prepared by protecting the corresponding commercially available carboxylic acids using standard conditions. However, standard tosylation of (±)-cispentacin **18** to (±)-**6c** yielded compound **19**, which was confirmed by NOESY-NMR and single crystal structure (Fig. 4). A proposed mechanism for this reaction path is depicted in Scheme 4, where the amide **19** is generated by aminolysis of the intermediate β-lactam with another molecule of cispentacin **18**. The same side reaction did not occur during the benzoylation of (±)-cispentacin. Thus, the carboxylic acid **6c** was solely available by biotransformation.

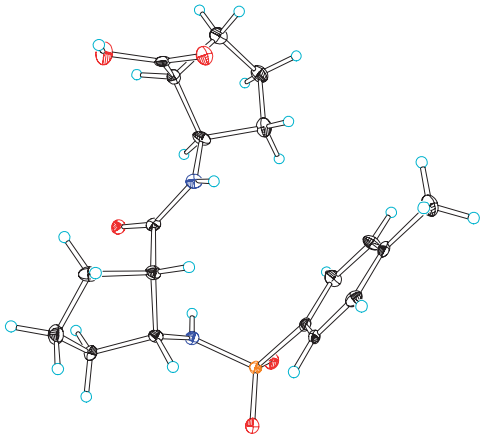


**Figure 4.** Molecular structure of **19** with thermal ellipsoids at the 30% probability level.

*trans*-2-Amino cyclopentane carboxylic acid **1c** was prepared by TFA-catalyzed deprotection of (±)-*trans*-2-*tert*-butoxycarbonylamino cyclopentane carboxylic acid[13] and subsequent benzoylation. (±)-2-[Phenyl-(toluene-4-sulfonylamino)-methyl]-acrylic acid **10c** could neither be prepared by chemical nor enzymatic hydrolysis under the above mentioned conditions.

To our knowledge, **1a–c**, **2a–c**, **4a**, **6a–c**, **8a–b**, **10b**, **15** and **19** have not been reported elsewhere up to now. Their spectroscopic data as well as other physical data are given in the Section 4.

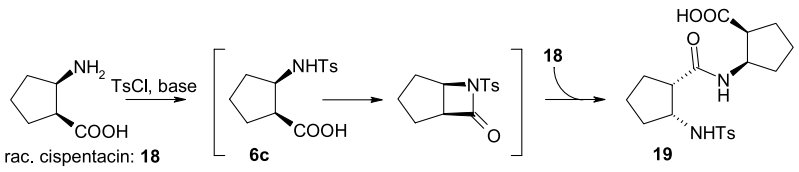
## 3. Conclusion

The biotransformation of five-membered alicyclic 2-amino nitriles proceeds significantly faster than in case of the six-membered compounds. More specific, the products of the *trans*-2-amino nitriles (amides and acids) are formed considerably faster than the products of the *cis*-counterparts (only amides). With exception of the α-methylene-β-amino carbonitrile structure, which is exclusively transformed to the amide, the other open chain nitriles show no clear trend concerning the formation of a preferred product. These product pattern mentioned in context with the alicyclic substrates (**1–8**) is similar for the three microorganisms investigated. In the same way, the enantioselectivities achieved for the alicyclic compounds are strongly dependent on the structure. Thus, the *trans*-five-membered nitriles give exclusively the amides in excellent enantiopurity (94–99% ee), in contrast, the biotransformation of *trans*-six-membered substrates result in the formation of the acid in excellent enantiopurity (87–99% ee). The ee's of the corresponding *cis*-compounds are throughout lower. The concentration level of the biotransformation reaction was found to exert some influence on the final distribution of the products. As a consequence of the kinetic resolution, improvement of product yield is gained at the expense of the enantiomeric purity.

In summary, the results suggest the application of the investigated *Rhodococci* to the enantioselective hydrolysis of five- and six-membered 2-amino substituted carbocyclic nitriles on a preparative scale.

## 4. Experimental

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 carried out with a Hewlett Packard Series 1100 HPLC using a G1315A diode array detector or MWD detector. For achiral analysis a LiChrospher 100 RP18e column (5 μm) was used. Chiral analysis was carried out with an Astec Chirobiotic R column, a Chromtech Chiral AGP 100.4 column (5 μm), a Chromtech Chiral HSA 100.4 column (5 μm), a Daicel Chiralpak AD-H (5 μm) and a Chiralcel OD-H column (5 μm). For preparative HPLC a Merck-Hitachi LC-6200 pump and L-4000 UV-detector was used. Separations were performed on a 21.2×250 mm Zorbax SB-C18 preparative HPLC column. CI-mass spectra were recorded with an Agilent 5973N MSD and Agilent 6890 Series II GC. Chiral gas chromatographic analyses were carried out on a Chrompack Chirasil-Dex CB (25 m× 0.32 mm; 0.25 μm film thickness; hydrogen carrier gas). EI-mass spectra were recorded with a Hewlett-Packard 5972 MSD and HP 6890 Series II GC equipped with a HP5 column. $^{1}$H NMR (199.98 MHz) and $^{13}$C NMR (50.29 MHz) spectra were recorded on a Varian GEMINI-200BB. $^{1}$H (499.82 MHz) and $^{13}$C NMR (125.69 MHz)




**Scheme 4.**

spectra were recorded on a Varian INOVA 500. 2D-techniques (HSQC, HMBC) as well as DEPT, NOESY, TOCSY and deuterium exchange were used to assist in structure elucidation. Melting points were determined on a Electrothermal MEL-TEMP apparatus and are uncorrected. The elemental analyses were performed on a Heraeus vario EL. X-ray crystal structures were measured on a Bruker AXS SMART APEX CCD diffractometer.

### 4.1. Microorganisms and cultivation

**4.1.1. Microorganisms.** *Rhodococcus equi* A4 was isolated in Prague by the group of V. Křen and L. Martínková and is deposited in the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic. *Rhodococcus* sp. R312 is commercially available (CBS 717.73). *Rhodococcus erythropolis* NCIMB 11540 was obtained from DSM Research, The Netherlands. *R. equi* A4 was maintained on MPA agarplates. Merck Standard I nutrient agar medium was used for maintainance of *R*. sp R312 and *R. erythropolis* NCIMB 11540 on agarplates.

**4.1.2. Cultivation.** All strains were cultivated on BSB medium[24] using acetonitrile as the only source of nitrogen. The microorganisms were cultured at 30 °C and 150 rpm in 250 ml shaking flasks, each containing 100 ml of the above described medium. During the exponential phase of growth, 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.

**4.1.3. Screening.** For screening experiments, 0.5 ml of cell suspension was put into Eppendorf vessels. The substrates were added as 200 mM solutions in DMSO (2.5 μl) to give a final concentration of 1 mM. The reactions were carried out at 32 °C in an Eppendorf Thermomixer at 850 rpm. After 20 h, 50 μl of HCl (1 N) were added. Unreacted nitriles and products were extracted twice with ethyl acetate. Conversions were determined by RP18 HPLC analysis. In the case of the benzoates **1**–**4** the ethyl acetate was removed and the remaining mixture diluted with methanol.

### 4.2. General procedure for large scale biotransformation

For preparative biotransformations, the cells were resuspended in the above given buffer, usually about 1 g wet cell weight per 10 ml of buffer. The optical density of the cell suspension was intended to be about 50, but as three different strains were used, the OD-value was not reproducible. The substrates were added as solutions in DMSO to give a final concentration of 10 mM. The DMSO portion was 2.5% (v/v) of the total volume. The reaction was performed in a rotary shaker at 150 rpm and 30 °C for 24 h. The biotransformations were stopped by addition of HCl (2 N). After centrifugation, unreacted nitriles as well as products were extracted from the aqueous phase using ethyl acetate. To prevent from losses of precipitated nitrile, amide and acid, the cells were also resuspended in ethyl acetate and again centrifuged. The combined organic layers were dried with $Na_2SO_4$ and concentrated in vacuo. Unreacted nitrile and products were purified by silica gel chromatography.

**4.2.1. (±)-2-[Phenyl-(toluene-4-sulfonylamino)-methyl]-acrylamide (10b).** *R. equi* A4 (13.8 g wet cells, 150 ml buffer, $OD_{610}=48$). Yield 79 mg (53%) at 70% conversion after 120 h from 141 mg (±)-**10a** (3 mM); *R. equi* A4 (6.0 g wet cells, 60 ml buffer, $OD_{610}=60$). Yield 20 mg (10%, ee=11%) from 187 mg (±)-**10a** (10 mM); *R. ery.* 11540 (0.8 g wet cells, 10 ml buffer, $OD_{610}=46$). Yield 3 mg (2%, ee=32%) from 125 mg (±)-**10a** (40 mM); *R*. sp R312 (5.0 g wet cells, 40 ml buffer, $OD_{610}=77$). Yield 33 mg (25%, ee=6%) from 124 mg (±)-**10a** (10 mM). White solid, mp 199–200 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.32 (s, 3H), 5.42 (d, 1H, $J=9.8$ Hz), 5.55 (s, 1H), 5.71 (s, 1H), 6.95 (s, 1H, N$H_2$), 7.04–7.06 (m, 2H), 7.14–7.19 (3H, m), 7.25 (d, 2H, $J=8.3$ Hz), 7.48 (s, 1H, N$H_2$), 7.54 (d, 2H, $J=8.3$ Hz), 8.32 (d, 1H, $J=9.8$ Hz, N*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.63, 57.44, 118.98, 127.12, 127.75, 127.93, 128.71, 129.94, 139.33, 140.59, 142.96, 144.56, 168.84; (CI, methane) *m*/*z* 331 $(M+1)^+$ (3), 313 (42), 260 (6), 172 (91), 160 (100), 155 (24). Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48. Found: C, 60.62; H, 5.48; N, 8.04. Chiral separation on Chiralcel OD-H, *n*-heptane/*i*-propanol 50:50, 0.38 ml/min, 15 °C.

**4.2.2. (±)-*cis*-2-(Toluene-4-sulfonylamino)-cyclopentane carboxylic acid (6c).** *R*. sp R312 (27.0 g wet cells, 250 ml buffer, $OD_{610}=61$). Yield 65 mg (92%) at 100% conversion after 76 h from 66 mg (±)-**6a** (1 mM). White solid, mp 139–141 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.48–1.56 (m, 1H), 1.65–1.82 (m, 3H), 1.88–2.00 (m, 2H), 2.43 (s, 3H), 2.91 (dt, 1H, $J=5.7$, 7.7 Hz, H-1), 3.78 (m, 1H, $J=8.1$ Hz, H-2), 6.16 (d, 1H, $J=9.3$ Hz, N*H*), 7.30 (d, 2H, $J=8.3$ Hz), 7.76 (d, 2H, $J=8.3$ Hz), 5.70 (s, br, 1H, COO*H*); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.78, 21.95, 28.06, 31.89, 46.55, 56.50, 127.31, 130.01, 137.77, 143.75, 178.46. Anal. Calcd for $C_{13}H_{17}NO_4S$: C, 55.12; H, 6.05; N, 4.94. Found: C, 55.11; H, 5.99; N, 4.97. Chiral separation on Chirobiotic R, polar organic mode (MeOH/$Et_3N$/AcOH 100:0.4:0.1), 0.80 ml/min, ambient temperature.

### 4.3. General procedure for benzoylation and detosylation

To a solution of **5a** or **7a** in anhydrous $CH_3CN$ 3.0 equiv of benzoylchloride and DMAP were added. After refluxing for 4 h 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 concentrated to give a crude oil, which was used for detosylation without further purification. Thus, the oil was dissolved in anhydrous MeOH. After addition of 5.0 equiv of magnesium turnings, the mixture was sonicated for 15 min. The mixture was allowed to react for another 45 min. Then, it was filtered through a plug of celite and washed with MeOH. The solvent was evaporated and the remaining oil diluted with $CH_2Cl_2$. The organic layer was washed with HCl (2 N), $NaHCO_3$ satd and brine. After drying with $Na_2SO_4$ and evaporation, the product was purified using silica gel chromatography.

**4.3.1. (±)-*trans*-*N*-(2-Cyano-cyclopentyl)-benzamide (1a).** White solid, mp 129–130 °C, $^1$H NMR ($CDCl_3$) $\delta$ 1.70–1.78 (m, 1H), 1.83–1.92 (m, 2H), 1.96–2.03 (m, 1H),

2.15–2.21 (m, 1H), 2.22–2.29 (m, 1H), 2.83 (dt, 1H, $J$=8.3, 7.1 Hz, H-1), 4.54 (m, 1H, $J$=7.3 Hz, H-2), 6.46 (d, 1H, $J$=6.3 Hz, N*H*), 7.42 (m, 2H), 7.51 (t, 1H, $J$=7.3 Hz), 7.75 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 23.20, 29.62, 31.77, 34.95, 56.40, 121.80, 127.23, 128.88, 132.10, 134.05, 167.80; *m/z* (EI) 213 $(M-1)^+$ (8), 161 (4), 105 (100), 77 (35). Anal. Calcd for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 71.19; H, 6.59; N, 12.43.

**4.3.2. (±)-*trans*-*N*-(2-Cyano-cyclohexyl)-benzamide (3a).** White solid, mp 167–168 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.31–1.39 (m, 1H), 1.47–1.55 (m, 2H), 1.72–1.84 (m, 3H), 2.13–2.16 (m, 2H), 2.82 (dt, 1H, $J$=3.4, 10.0 Hz, H-1), 4.20 (m, 1H, H-2), 6.23 (d, 1H, $J$=7.8 Hz, N*H*), 7.44 (m, 2H), 7.52 (m, 1H), 7.78 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 24.00, 24.04, 28.85, 31.66, 34.49, 50.27, 120.73, 127.23, 128.90, 132.02, 134.45, 167.47; *m/z* (EI) 228 $M^+$ (10), 160 (4), 123 (5), 105 (100), 77 (43). Anal. Calcd for $C_{14}H_{16}N_2O$: C, 73.66; H, 7.06; N, 12.27. Found: C, 74.16; H, 7.27; N, 12.08. Chiral separation on Chiralcel OD-H, *n*-heptane/*i*-propanol 50:50, 0.38 ml/min, 15 °C.

### 4.4. General procedure for benzoylation

Analogous to Section 4.3 but pyridine was used as the base.

**4.4.1. (±)-*trans*-2-Benzoylamino-cyclopentane carboxylic acid (1c).** *R. equi* A4 (7.1 g wet cells, 70 ml buffer, $OD_{610}$=40): Yield 90 mg (55%, ee=75%) from 150 mg (±)-**1a** (10 mM); *R. ery.* 11540 (8.2 g wet cells, 80 ml buffer, $OD_{610}$=32). Yield 118 mg (63%, ee=48%) from 171 mg (±)-**1a** (10 mM); *R.* sp R312 (7.9 g wet cells, 70 ml buffer, $OD_{610}$=46). Yield 192 mg (87%, ee=15%) from 150 mg (±)-**1a** (10 mM). White solid, mp 184–186 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.53–1.65 (m, 2H), 1.67–1.77 (m, 2H), 1.94–2.00 (m, 2H), 2.76 (dt, 1H, $J$=8.8, 7.3 Hz, H-1), 4.44 (m, 1H, $J$=7.4 Hz, H-2), 7.44 (m, 2H), 7.50 (m, 1H), 7.81–7.83 (m, 2H), 8.47 (d, 1H, $J$=7.8 Hz, N*H*), 12.20 (s, br, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 23.93, 29.35, 33.27, 49.98, 54.87, 127.96, 128.88, 131.80, 135.17, 166.63, 176.72. Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 65.86; H, 6.40; N, 5.96. Chiral separation on Chirobiotic R, polar organic mode (MeOH/$Et_3N$/AcOH 100:0.4:0.1), 0.80 ml/min, ambient temperature.

**4.4.2. (±)-*cis*-2-Benzoylamino-cyclopentane carboxylic acid (2c).** White solid, mp 187–189 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.47–1.55 (m, 1H), 1.73–1.86 (m, 3H), 1.87–2.00 (m, 2H), 2.93 (dt, 1H, $J$=7.3, 7.8 Hz, H-1), 4.55 (m, 1H, $J$=7.5 Hz, H-2), 7.42 (m, 2H), 7.49 (m, 1H), 7.76–7.78 (m, 2H), 8.17 (d, 1H, $J$=8.3 Hz, N*H*), 11.96 (s, br, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.90, 27.81, 31.50, 47.91, 52.98, 128.09, 128.74, 131.65, 135.58, 166.84, 175.30. Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.59; H, 6.47; N, 6.42.

**4.4.3. (±)-*trans*-2-Benzoylamino-cyclohexane carboxylic acid (3c).** *R. equi* A4 (4.1 g wet cells, 40 ml buffer, $OD_{610}$=40). Yield 36 mg (36%, ee>95%) from 93 mg (±)-**3a** (10 mM); *R. ery.* 11540 (9.0 g wet cells, 90 ml buffer, $OD_{610}$=38). Yield 33 mg (15%, ee>95%) from 205 mg (±)-**3a** (10 mM); *R.* sp. R312 (7.0 g wet cells, 60 ml buffer, $OD_{610}$=54). Yield 11 mg (7%, ee>95%) from 137 mg (±)-**3a** (10 mM). White solid, mp 217–219 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.12–1.22 (m, 1H), 1.24–1.35 (m, 2H), 1.42 (ddd, 1H, $J$=12.7, 12.7, 3.4 Hz), 1.69 (m, 2H), 1.79–1.82 (m, 1H), 1.90 (m, 1H), 2.43 (dt, 1H, $J$=3.3, 11.6 Hz, H-1), 3.98 (m, 1H, H-2), 7.42–7.44 (m, 2H), 7.47–7.51 (m, 1H), 7.76–7.78 (m, 2H), 8.29 (d, 1H, $J$=8.8 Hz, N*H*), 12.01 (s, br, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 25.08, 25.30, 29.55, 32.67, 48.94, 50.21, 127.91, 128.83, 131.68, 135.55, 166.01, 176.08. Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 66.78; H, 6.96; N, 5.62. Chirasil-Dex CB after derivatization with (trimethysilyl) diazomethane; 100 °C (2 min), 5°/min 200 °C ($H_2$, 1 bar).

**4.4.4. (±)-*cis*-2-Benzoylamino-cyclohexane carboxylic acid (4c).** White solid, mp 175–176 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.32–1.41 (m, 2H), 1.49–1.65 (m, 4H), 1.77–1.83 (m, 1H), 1.96–2.02 (m, 1H), 2.74 (m, 1H, H-1), 4.30 (m, 1H, H-2), 7.43 (m, 2H), 7.50 (m, 1H), 7.74–7.76 (m, 2H), 8.00 (d, 1H, $J$=8.3 Hz, N*H*), 12.22 (s, br, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.78, 23.55, 25.49, 29.95, 44.41, 48.19, 128.07, 128.83, 131.72, 135.60, 166.71, 175.52. Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 66.72; H, 6.86; N, 5.60.

**4.4.5. *N*-(2-Cyano-cyclopent-1-enyl)-benzamide (14).** White solid; mp 90–92 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 2.03 (m, 2H), 2.55 (t, 2H, $J$=6.8 Hz), 3.22 (t, 2H, $J$=7.7 Hz), 7.42–7.61 (m, 3H), 7.92 (m, 2H), 8.39 (s, 1H, N*H*); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 22.44, 30.33, 33.84, 89.90, 116.62, 127.72, 129.16, 133.03, 133.24, 156.67, 164.90; *m/z* (EI) 211 $(M-1)^+$ (31), 105 (100), 77 (50), 51 (11).

**4.4.6. *N*-(2-Cyano-cyclohex-1-enyl)-benzamide (15).** White solid; mp 102–104 °C; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.71–1.77 (m, 4H), 2.33 (m, 2H), 3.00 (m, 2H), 7.45–7.58 (m, 3H), 7.83 (m, 2H), 8.20 (s, 1H, N*H*); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 21.24, 21.72, 25.69, 27.96, 91.38, 118.30, 127.48, 129.23, 132.82, 133.93, 152.30, 165.25; *m/z* (EI) 226 $(M)^+$ (9), 206 (1), 105 (100), 77 (39), 51 (9).

### 4.5. General procedure for aziridine formation and ring opening

See Ref. 20.

**4.5.1. (±)-2-Phenyl-1-(toluene-4-sulfonyl)-aziridine (16).** White solid; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 2.39 (d, 1H, $J$=4.5 Hz), 2.43 (s, 3H), 2.98 (d, 1H, $J$=7.3 Hz), 3.78 (dd, 1H, $J$=7.3, 4.5 Hz), 7.20–7.37 (m, 7H), 7.87 (d, 2H, $J$=7.9 Hz); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 21.89, 36.18, 41.28, 126.81, 128.19, 128.55, 128.81, 130.14, 135.20, 135.29, 144.92; *m/z* (EI) 155 (1), 118 (32), 91 (100), 77 (5), 65 (44).

**4.5.2. (±)-2-Benzyl-1-(toluene-4-sulfonyl)-aziridine (17).** White solid; $^1$H NMR ($CDCl_3$) $\delta$ 2.18 (d, 1H, $J$=4.9 Hz), 2.43 (s, 3H), 2.69 (dd, 1H, $J$=14.5, 7.1 Hz), 2.72 (d, 1H, $J$=7.1 Hz), 2.82 (dd, 1H, $J$=14.5, 4.9 Hz), 2.96 (tt, 1H, $J$=7.1, 4.9 Hz), 7.03–7.06 (m, 2H), 7.15–7.18 (m, 3H), 7.22 (d, 2H, $J$=8.6 Hz), 7.69 (d, 2H, $J$=8.6 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.87, 33.07, 37.73, 41.43, 126.74, 128.11, 128.69,

128.96, 129.83, 135.06, 137.25, 144.56; *m/z* (EI) 287 $M^+$ (5), 172 (4), 155 (6), 132 (100), 105 (57), 91 (64).

**4.5.3. (±)-*trans*-*N*-(2-Cyano-cyclopentyl)-4-methyl benzene sulfonamide (5a).** White solid, mp 109–110 °C; $^1$H NMR ($CDCl_3$) $\delta$ 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, 6.2 Hz, H-1), 3.73 (m, 1H, $J$=6.6 Hz, H-2), 5.72 (d, 1H, $J$=7.2 Hz, N*H*), 7.35 (d, 2H, $J$=8.3 Hz), 7.81 (2H, d, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.84, 22.88, 29.22, 32.85, 35.89, 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). Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.63; H, 6.13; N, 10.63. Chiral separation on Chiralcel AD-H, *n*-heptane/*i*-propanol 50:50, 0.50 ml/min, 15 °C.

**4.5.4. (±)-*trans*-*N*-(2-Cyano-cyclohexyl)-4-methyl-benzene sulfonamide (7a).** White solid, mp 106–108 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.25–1.39 (m, 3H), 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 (ddt, 1H, $J$=4.1, 8.3, 8.3 Hz, H-2), 5.23 (d, 1H, $J$=8.3 Hz, N*H*), 7.34 (d, 2H, $J$=8.5 Hz), 7.82 (d, 2H, $J$=8.5 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 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). Anal. Calcd for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06. Found: C, 59.72; H, 6.42; N, 9.51. Chiral separation on Chiralcel OD-H, *n*-heptane/*i*-propanol 50:50, 0.38 ml/min, 15 °C.

**4.5.5. (±)-*N*-(2-Cyano-1-phenyl-ethyl)-4-methyl-benzene sulfonamide (9a).** White solid, mp 141–142 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.41 (s, 3H), 2.90 (dd, 1H, $J$=16.5, 7.0 Hz), 2.95 (dd, 1H, $J$=16.5, 5.5 Hz), 4.57 (dt, 1H, $J$=5.5, 7.0 Hz), 5.40 (d, 1H, $J$=7.0 Hz, N*H*), 7.11–7.13 (m, 2H), 7.24 (d, 2H, $J$=8.6 Hz), 7.27–7.29 (m, 3H), 7.67 (d, 2H, $J$=8.6 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.80, 26.55, 54.38, 116.69, 126.48, 127.38, 129.22, 129.44, 130.05, 136.70, 137.38, 144.29; *m/z* (EI) 260 (60), 155 (52), 145 (5), 91 (100), 77 (20). Anal. Calcd for $C_{16}H_{16}N_2O_2S$: C, 64.98; H, 5.37; N, 9.33. Found: C, 64.19; H, 5.33; N, 9.26.

**4.5.6. (±)-*N*-(1-Benzyl-2-cyano-ethyl)-4-methyl-benzene sulfonamide (11a).** White solid, mp 106–107 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.43 (s, 3H), 2.57 (dd, 1H, $J$=16.6, 3.9 Hz), 2.66 (dd, 1H, $J$=16.6, 6.3 Hz), 2.78 (dd, 1H, $J$=14.0, 7.6 Hz), 2.90 (dd, 1H, $J$=14.0, 6.8 Hz), 3.64 (m, 1H), 4.79 (d, 1H, $J$=7.3 Hz, N*H*), 6.98–7.00 (m, 2H), 7.19–7.23 (m, 5H), 7.55 (d, 2H, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.81, 24.39, 40.04, 51.49, 116.95, 127.19, 127.67, 129.19, 129.31, 130.11, 135.20, 136.50, 144.11; *m/z* (EI) 223 (57), 155 (100), 91 (100). Anal. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91. Found: C, 65.33; H, 5.79; N, 8.80.

### 4.6. General procedure for cyanoborohydride reduction and protection

To a solution of compound **12** or **13** in MeOH bromocresole green was added and the pH of the solution was adjusted with methanolic HCl till the indicator turned to yellow. $NaCNBH_3$ was added in portions while the pH was kept acidic using methanolic HCl. After stirring 30 min at room temperature, the solvent was removed under reduced pressure. The crude residue was diluted with NaOH (1 N). Solid NaCl was added to give a 10% solution. The aqueous phase was extracted with $CH_2Cl_2$. The product was removed from the organic phase by extraction with HCl (2 N) (CAUTION! Possible formation of HCN). Subsequently, the aqueous phase was made alkaline with NaOH concd and extracted four times with $CH_2Cl_2$. After drying with $Na_2SO_4$, the solvent was removed to give a brown oil which was used for standard tosylation of nitriles (see Section 4.7).

### 4.7. General procedure for tosylation of amino nitriles

The unprotected amino nitriles were suspended in $CH_2Cl_2$. 1.1 equiv of tosylchloride and 1.5 equiv of $Et_3N$ were added and the mixture was allowed to react at reflux. The reaction was monitored by TLC. After disappearance of starting material, the organic phase was washed with HCl (2 N), $NaHCO_3$ satd and NaCl satd. After drying with $Na_2SO_4$ the organic solvent was removed and the products were separated by silica gel chromatography.

**4.7.1. (±)-*cis*-*N*-(2-Cyano-cyclopentyl)-4-methyl-benzene sulfonamide (6a).** White solid, mp 117–119 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.57–1.65 (m, 2H), 1.82–1.96 (m, 3H), 1.98–2.04 (m, 1H), 2.44 (s, 3H), 2.83 (ddd, 1H, $J$=7.3, 6.8, 3.4 Hz, H-1), 3.77 (m, 1H, H-2), 5.29 (d, 1H, $J$=8.8 Hz, N*H*), 7.33 (d, 2H, $J$=8.3 Hz), 7.81 (d, 2H, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 20.85, 21.82, 28.28, 30.77, 35.23, 56.18, 119.91, 127.41, 130.15, 137.51, 144.15; *m/z* (EI) 264 $M^+$ (16), 210 (31), 155 (40), 109 (28), 91 (100). Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.30; H, 5.88; N, 10.75. Chiral separation on Chiralpak AD-H, EtOH, 0.55 ml/min, 40 °C.

**4.7.2. (±)-*cis*-*N*-(2-Cyano-cyclohexyl)-4-methyl-benzene sulfonamide (8a).** White solid, mp 133–134 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.12–1.21 (m, 1H), 1.35–1.52 (m, 3H), 1.53–1.61 (m, 2H), 1.68–1.72 (m, 1H), 1.88–1.91 (m, 1H), 2.37 (s, 3H), 3.01 (m, 1H, H-1), 3.24 (m, 1H, $J$=4.1 Hz, H-2), 4.91 (d, 1H, $J$=8.3 Hz, N*H*), 7.25 (d, 2H, $J$=8.3 Hz), 7.71 (d, 2H, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.13, 21.82, 24.80, 27.91, 29.89, 35.76, 52.72, 119.52, 127.11, 130.18, 138.10, 144.08; *m/z* (EI) 278 $M^+$ (0.1), 210 (1), 155 (2), 123 (4), 91 (100). Anal. Calcd for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06. Found: C, 60.60; H, 6.63; N, 10.01. Chiral separation on Chiralpak AD-H, *n*-heptane/*i*-propanol 50:50, 0.5 ml/min, 15 °C.

### 4.8. General procedure for tosylation of amino acids

The unprotected amino acids were suspended in $CH_3CN$. 1.1 equiv of tosylchloride and 1.5 equiv of $Et_3N$ were added and the mixture was allowed to react at reflux. The reaction was monitored by TLC. After disappearance of starting material, HCl (2 N) was added and the organic solvent was removed. The aqueous phase was extracted three times with $CH_2Cl_2$. The carboxylic acids were removed from the aqueous phase by extraction with NaOH (2 N) which was subsequently acidified with HCl (2 N). The product was extracted three times with $CH_2Cl_2$ and three times with ethyl acetate. After drying with $Na_2SO_4$, the solvent was

removed to give pure white solids (**7c** and **8c**) or a crude product (**19**), which was purified by silica gel chromatography.

**4.8.1. (±)-*trans*-2-(Toluene-4-sulfonylamino)-cyclohexane carboxylic acid (7c).** *R. equi* A4 (7 g wet cells, 80 ml buffer, $OD_{610}=53$). Yield 31 mg (13%, ee > 99%) from 223 mg (±)-**7a** (10 mM); *R. ery.* 11540 (7.5 g wet cells, 80 ml buffer, $OD_{610}=39$). Yield 35 mg (15%, ee = 97%) from 223 mg (±)-**7a** (10 mM); *R.* sp. R312 (8.1 g wet cells, 80 ml buffer, $OD_{610}=63$). Yield 39 mg (16%, ee = 87%) from 223 mg (±)-**7a** (10 mM). White solid, mp 175–176 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.16–1.30 (m, 3H), 1.47–1.55 (m, 1H), 1.67 (m, 2H), 1.96–1.99 (m, 2H), 2.32 (dt, 1H, $J$ = 3.6, 10.5 Hz, H-1), 2.42 (s, 3H), 3.36 (ddt, 1H, $J$ = 3.7, 7.6, 10.5 Hz, H-2), 5.31 (d, 1H, $J$ = 7.6 Hz, N*H*), 7.29 (d, 2H, $J$ = 8.3 Hz), 7.76 (d, 2H, $J$ = 8.3 Hz), 8.25 (s, br, 1H, COO*H*); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.83, 24.29, 24.52, 28.86, 33.38, 49.68, 54.03, 127.41, 129.86, 137.96, 143.63, 178.87. Anal. Calcd for $C_{14}H_{19}NO_4S$: C, 56.55; H, 6.44; N, 4.71. Found: C, 55.98; H, 6.44; N, 4.52. Chiral separation on Chiralpak AD-H *n*-heptane/ethanol 70:30, 0.80 ml/min, 15 °C.

**4.8.2. (±)-*cis*-2-(Toluene-4-sulfonylamino)-cyclohexane carboxylic acid (8c).** White solid, mp 155 158 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.25–1.29 (m, 2H), 1.45–1.49 (m, 2H), 1.54–1.60 (m, 1H), 1.66 (m, 1H), 1.76–1.84 (m, 1H), 2.07 (m, 1H), 2.43 (s, 3H), 2.78 (dt, 1H, $J$ = 4.4, 4.6 Hz, H-1), 3.42 (m, 1H, H-2), 6.01 (d, 1H, $J$ = 9.8 Hz, N*H*), 7.30 (d, 2H, $J$ = 8.3 Hz), 7.76 (d, 2H, $J$ = 8.3 Hz), 9.58 (s, br, 1H, COO*H*); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.80, 22.21, 24.42, 27.74, 29.89, 45.19, 52.68, 127.12, 129.97, 138.53, 143.60, 178.33. Anal. Calcd for $C_{14}H_{19}NO_4S$: C, 56.55; H, 6.44; N, 4.71. Found: C, 57.82; H, 6.99; N, 4.97.

**4.8.3. (±)-*cis*-2-{[*cis*-2-(Toluene-4-sulfonylamino) cyclopentanecarbonyl]-amino}-cyclopentane carboxylic acid (19).** White solid, mp 176–177 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.30–1.44 (m, 2H), 1.45–1.56 (m, 3H), 1.57–1.65 (m, 2H), 1.71–1.78 (m, 4H), 1.83–1.90 (m, 1H), 2.36 (s, 3H), 2.59 (q, 1H, $J$ = 7.4 Hz), 2.8 (q, 1H, $J$ = 7.6 Hz), 3.47 (m, 1H,), 4.27 (m, 1H), 7.32 (d, 1H, $J$ = 6.7 Hz, N*H*), 7.35 (d, 2H, $J$ = 8.0 Hz), 7.57 (d, 1H, $J$ = 8.7 Hz, N*H*), 7.64 (d, 2H, $J$ = 8.0 Hz), 11.95 (s, br, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.67, 22.11, 22.37, 27.20, 28.04, 31.98, 32.47, 47.22, 47.78, 52.42, 57.07, 127.31, 130.25, 138.68, 143.20, 172.95, 175.01. Crystal data[25] for $C_{19}H_{26}N_2O_5S$: orthorhombic, space group $P2_12_12_1(19)$, $a$ = 6.3805(13) Å, $b$ = 16.940(3) Å, $c$ = 17.430(4) Å, $\alpha=\beta=\gamma=90°$, $V$ = 1883.9(7) Å$^3$, $Z$ = 4, $d_c$ = 1.391 g cm$^{-1}$, $\mu$ = 0.206 mm$^{-1}$, (Mo K$\alpha$, $\lambda$ = 0.71073 Å) $T$ = 100 K, the structure was solved by direct methods and refined by full matrix least squares procedures (SHELXL97): $R_1$ = 0.0691 and 0.0756 (w$R_2$ = 0.1321 and 0.1345) for 3326 unique measured reflections. Goodness of fit: 1.277.

### 4.9. General procedure for Thorpe–Ziegler cyclization

1.05 equiv of NaH was stirred in dry toluene and a solution of adipodinitrile or heptanedinitrile in toluene was added dropwise. The mixture was then refluxed for 3 h. EtOH was added in order to destroy excess NaH. Subsequently, water and acetic acid were added slowly. The organic layer was separated and the aqueous phase extracted three times with ethyl acetate. The combined organic layers were dried with $Na_2SO_4$ and the solvent was evaporated. The product was purified by recrystallization.

**4.9.1. 2-Amino-cyclopent-1-ene carbonitrile (12).** Pale brown solid; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.90 (m, 2H), 2.47 (m, 4H), 4.50 (s, br, 2H, N$H_2$); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 22.19, 31.45, 34.48, 74.61, 119.26, 162.65; *m/z* (EI) 107 (M-1)$^+$ (100), 93 (1), 80 (20), 67 (3), 53 (10).

**4.9.2. 2-Amino-cyclohex-1-ene carbonitrile (13).** Pale brown solid; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 1.65 (m, 4H), 2.12–2.21 (m, 4H), 4.22 (s, br, 2H, N$H_2$); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 21.84, 22.21, 24.48, 28.41, 74.33, 121.17, 156.40; *m/z* (EI) 121 (M−1)$^+$ (61), 93 (100), 81 (7), 66 (25).

### 4.10. General procedure for catalytic hydrogenation

The catalyst (Pd/5% on charcoal) was added to a solution of the olefin **14** or **15** in MeOH. 1 bar (balloon) or 50 bar (autoclave) of hydrogen were applied after evaporating and purging the vessels three times. The reactions were carried out at room temperature and monitored by GC/MS.

**4.10.1. (±)-*cis*-*N*-(2-Cyano-cyclopentyl)-benzamide (2a).** White solid, mp 107–109 °C, $^1$H NMR ($CDCl_3$) $\delta$ 1.72–1.82 (m, 2H), 1.95–2.04 (m, 1H), 2.06–2.15 (m, 2H), 2.17–2.22 (m, 1H), 3.39 (dt, 1H, $J$ = 3.9, 7.3 Hz, H-1), 4.56–4.63 (m, 1H, H-2), 6.45 (d, 1H, $J$ = 6.5 Hz, N*H*), 7.44 (m, 2H), 7.52 (m, 1H), 7.80 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.85, 29.02, 30.50, 34.72, 52.63, 120.61, 127.30, 128.93, 132.14, 134.07, 168.03; *m/z* (EI) 214 M$^+$ (10), 161 (3), 105 (100), 77 (36). Anal. Calcd for $C_{13}H_{14}N_2O$: C, 72.87; H, 6.59; N, 13.07. Found: C, 72.56; H, 6.55; N, 12.75.

**4.10.2. (±)-*cis*-*N*-(2-Cyano-cyclohexyl)-benzamide (4a).** White solid, mp 177–178 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.43 (tq, 1H, $J$ = 3.7, 13.2 Hz), 1.53–1.63 (m, 1H), 1.67–1.75 (m, 3H), 1.92 (m, 2H), 2.06 (m, 1H), 3.48 (m, 1H, H-1), 4.14 (m, 1H, $J$ = 4.0 Hz, H-2), 6.38 (d, 1H, $J$ = 6.8 Hz, N*H*), 7.43–7.47 (m, 2H), 7.52 (m, 1H), 7.78 (d, 2H, $J$ = 7.8 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.48, 24.85, 27.68, 28.84, 34.21, 48.99, 120.30, 127.30, 128.91, 132.14, 134.08, 167.44; *m/z* (EI) 228 M$^+$ (16), 160 (7), 123 (6), 105 (100), 77 (38). Anal. Calcd for $C_{14}H_{16}N_2O$: C, 73.66; H, 7.06; N, 12.27. Found: C, 73.69; H, 7.13; N, 12.08.

### 4.11. General procedure for Aza-Baylis-Hillman reaction

Tosylamide was suspended in i-PrOH together with molecular sieve (4 Å) and 0.15 equiv of DABCO. 1.00 equiv of benzaldehyde, 1.10 equiv of acrylonitrile and 0.75 equiv of Ti(i-OPr)$_4$ were added subsequently. The reaction was stirred at room temperature and monitored by TLC. After 36 h, another 0.75 equiv of Ti(i-OPr)$_4$ were added and the reaction was stirred for another 24 h. Subsequently, the mixture was filtered over celite and washed with *i*-PrOH three times. The solvent was removed and the residue was treated with MeOH and $H_2SO_4$ (2 N) for

90 min. MeOH was removed under reduced pressure and the aqueous layer was extracted with $CH_2Cl_2$. The organic layer was washed with $NaHCO_3$ satd, water and brine. After drying with $Na_2SO_4$ the product was purified by silica gel chromatography.

**4.11.1. (±)-*N*-(2-Cyano-1-phenyl-2-propenyl)-4-methylbenzenesulfonamide (10a).** White solid, mp 126–127 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.44 (s, 3H), 5.06 (d, 1H, $J$=7.3 Hz), 5.23 (d, 1H, $J$=7.3 Hz, N*H*), 6.00 (d, 1H, $J$=1.0 Hz), 6.06 (d, 1H, $J$=1.0 Hz), 7.11–7.13 (m, 2H), 7.28 (d, 2H, $J$=8.5 Hz), 7.30–7.32 (m, 3H), 7.71 (d, 2H, $J$=8.5 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.82, 60.03, 116.77, 123.60, 127.08, 127.56, 129.35, 129.52, 130.02, 132.09, 136.35, 137.00, 144.33; *m*/*z* (EI) 260 (24), 157 (100), 155 (38), 91 (89), 77 (20). Anal. Calcd for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97. Found: C, 65.57; H, 5.05; N, 8.95. Chiral separation on AGP, 10 mM phosphatebuffer pH 7.02/acetonitrile 85:15, 0.80 ml/min, 15 °C.

## 4.12. General procedure for amide formation

100 mg of nitrile was dissolved in 5 ml of MeOH. 5.0 equiv of $K_2CO_3$ and 1 ml of aqueous $H_2O_2$ (35%) were added. The reaction was stirred at room temperature and monitored by TLC. After completion, the MeOH was removed under reduced pressure, the aqueous phase diluted with water and the product extracted three times with $CH_2Cl_2$. After drying with $Na_2SO_4$ and evaporation of the solvent, the product-amides crystallized as pure white substances.

**4.12.1. (±)-*trans*-*N*-(2-Carbamoyl-cyclopentyl)-benzamide (1b).** *R. equi* A4 (7.1 g wet cells, 70 ml buffer, $OD_{610}$=40): Yield 66 mg (40%, ee=94%) from 150 mg (±)-**1a** (10 mM); *R. ery.*11540 (8.2 g wet cells, 80 ml buffer, $OD_{610}$=32). Yield 55 mg (30%, ee > 99%) from 171 mg (±)-**1a** (10 mM); *R.* sp R312 (7.9 g wet cells, 70 ml buffer, $OD_{610}$=46). Yield 13 mg (7%, ee > 99%) from 150 mg (±)-**1a** (10 mM). White solid, mp 237–239 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.52–1.65 (m, 2H), 1.67–1.74 (m, 2H), 1.85–1.91 (m, 1H), 1.92–1.98 (m, 1H), 2.65 (dt, 1H, $J$=8.3, 7.6 Hz, H-1), 4.34 (m, 1H, $J$=7.3 Hz, H-2), 6.77 (s, br, 1H, N$H_2$), 7.27 (s, br, 1H, N$H_2$), 7.44 (m, 2H), 7.51 (m, 1H), 7.82–7.83 (m, 2H), 8.33 (d, 1H, $J$=7.8 Hz, N*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.24, 29.53, 33.41, 50.90, 55.08, 128.00, 128.84, 131.75, 135.33, 166.70, 176.41; *m*/*z* (EI) 232 $M^+$ (0.5), 188 (3), 187 (13), 127 (20), 105 (100), 77 (58). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 65.83; H, 6.62; N, 11.49. Chiral separation on AGP, 10 mM phosphate buffer pH 7.00 (with 1 mM dimethyloctylamine), 0.90 ml/min, ambient temperature.

**4.12.2. (±)-*cis*-*N*-(2-Carbamoyl-cyclopentyl)-benzamide (2b).** White solid, mp 198–199 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.45–1.55 (m, 1H), 1.73–1.86 (m, 1H), 1.88–1.97 (m, 4H), 2.85 (dt, 1H, $J$=7.3, 8.3 Hz, H-1), 4.43 (m, 1H, $J$=7.1 Hz, H-2), 6.87 (s, br, 1H, N$H_2$), 7.33 (s, br, 1H, N$H_2$), 7.44 (m, 2H), 7.48–7.51 (m, 1H), 7.74–7.76 (m, 2H), 8.12 (d, 1H, $J$=7.3 Hz, N*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 23.25, 28.66, 32.75, 47.24, 53.17, 127.77, 128.93, 131.73, 135.52, 166.47, 175.96; *m*/*z* (EI) 232 $M^+$ (0.1), 231 (2), 187 (6), 127 (10), 105 (100), 77 (55). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 66.47; H, 6.75; N, 11.53.

**4.12.3. (±)-*trans*-*N*-(2-Carbamoyl-cyclohexyl)-benzamide (3b).** *R. equi* A4 (4.1 g wet cells, 40 ml buffer, $OD_{610}$=40): Yield 22 mg (22%, ee=56%) from 93 mg (±)-**3a** (10 mM); *R. ery.* 11540 (9.0 g wet cells, 90 ml buffer, $OD_{610}$=38). Yield 35 mg (16%, ee=67%) from 205 mg (±)-**3a** (10 mM); *R.* sp. R312 (7.0 g wet cells, 60 ml buffer, $OD_{610}$=54). Yield 20 mg (14%, ee=38%) from 137 mg (±)-**3a** (10 mM). White solid, mp 290–291 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.87–1.12 (m, 3H), 1.24 (ddt, 1H, $J$=3.4, 12.9, 12.9 Hz), 1.47 (m, 2H), 1.56 (m, 1H), 1.66 (m, 1H), 2.15 (dt, 1H, $J$=3.4, 11.6 Hz, H-1), 3.69 (m, 1H, H-2), 6.51 (s, br, 1H, N$H_2$), 6.90 (s, br, 1H, N$H_2$), 7.20–7.23 (m, 2H), 7.28 (m, 1H), 7.58 (m, 2H), 7.94 (d, 1H, $J$=8.8 Hz, N*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 25.31, 25.41, 29.73, 33.18, 49.40, 50.46, 127.93, 128.82, 131.66, 135.58, 165.97, 176.13; *m*/*z* (EI) 246 $M^+$ (1), 201 (4), 141 (14), 105 (100), 77 (67). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 63.97; H, 7.13; N, 9.89. Chiral separation on HSA, 20 mM phosphate buffer (pH 7.00)/2-propanol 97:3, 0.90 ml/min, ambient temperature.

**4.12.4. (±)-*cis*-*N*-(2-Carbamoyl-cyclohexyl)-benzamide (4b).** White solid, mp 173–174 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29–1.38 (m, 2H), 1.44–1.62 (m, 4H), 1.92–1.99 (m, 2H), 2.57–2.60 (m, 1H, H-1), 4.19 (m, 1H, H-2), 6.86 (s, br, 1H, N$H_2$), 7.35 (s, br, 1H, N$H_2$), 7.44 (m, 2H), 7.50 (m, 1H), 7.73–7.76 (m, 2H), 7.91 (d, 1H, $J$=7.3 Hz, N*H*); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 22.50, 23.72, 25.88, 30.00, 44.51, 48.74, 127.84, 128.96, 131.78, 135.59, 166.56, 176.41; *m*/*z* (EI) 245 $(M-1)^+$ (7), 201 (11), 141 (28), 105 (100), 77 (52). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 64.00; H, 7.02; N, 10.28.

**4.12.5. (±)-*trans*-2-(Toluene-4-sulfonylamino)-cyclopentane carboxamide (5b).** *R. equi* A4 (7 g wet cells, 80 ml buffer, $OD_{610}$=56). Yield 32 mg (14%, ee > 99%) from 211 mg (±)-**5a** (10 mM); *R. ery.* 11540 (8.0 g wet cells, 80 ml buffer, $OD_{610}$=32). Yield 30 mg (13%, ee > 99%) from 211 mg (±)-**5a** (10 mM); *R.* sp. R312 (8.0 g wet cells, 80 ml buffer, $OD_{610}$=62). Yield 23 mg (10%, ee > 99%) from 211 mg (±)-**5a** (10 mM). White solid, mp 180–181 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.15–1.22 (m, 1H), 1.38–1.53 (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, N$H_2$), 7.14 (s, br, 1H, N$H_2$), 7.35 (d, 2H, $J$=8.1 Hz), 7.61 (d, 1H, $J$=7.8 Hz, N*H*), 7.65 (2H, d, $J$=8.1 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.68, 24.03, 29.97, 33.47, 51.60, 57.58, 127.23, 130.20, 139.36, 143.06, 176.09; *m*/*z* (EI) 281 $(M-1)^+$ (0.02), 238 (0.1), 155 (7), 127 (100), 110 (27), 91 (89). Anal. Calcd for $C_{13}H_{18}N_2O_3S$: C, 55.30; H, 6.43; N, 9.92. Found: C, 55.21; H, 6.12; N, 9.96. Chiral separation on Chiralcel OD-H, *n*-heptane/*i*-propanol 50:50, 0.38 ml/min, 15 °C.

**4.12.6. (±)-*cis*-2-(Toluene-4-sulfonylamino)-cyclopentane carboxamide (6b).** *R. equi* A4 (2.9 g wet cells, 30 ml buffer, $OD_{610}$=38). Yield 13 mg (14%, ee=51%) from 88 mg (±)-**6a** (10 mM); *R. ery.* 11540 (7.5 g wet cells, 70 ml buffer, $OD_{610}$=29). Yield 96 mg (49%, ee=15%) from 185 mg (±)-**6a** (10 mM); *R.* sp. R312 (5.3 g wet cells, 50 ml buffer, $OD_{610}$=50). Yield 106 mg (75%, ee=7%) from 132 mg (±)-**6a** (10 mM). White solid, mp 167–168 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.29–1.39 (m, 2H),

1.43–1.51 (m, 1H), 1.57–1.78 (m, 3H), 2.36 (s, 3H), 2.59 (dt, 1H, $J$=6.8, 8.1 Hz, H-1), 3.53 (m, 1H, $J$=6.3 Hz, H-2), 6.92 (s, br, 1H, N$H_2$), 7.26 (s, br, 1H, N$H_2$), 7.35 (d, 2H, $J$=8.1 Hz), 7.40 (d, 1H, $J$=6.8 Hz, N$H$), 7.67 (2H, d, $J$=8.1 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.66, 22.10, 27.72, 32.20, 47.56, 57.11, 127.33, 130.23, 138.92, 143.18, 175.55; *m/z* (EI) 281 $(M-1)^+$ (1), 238 (1), 155 (16), 127 (51), 110 (20), 91 (100). Anal. Calcd for $C_{13}H_{18}N_2O_3S$: C, 55.30; H, 6.43; N, 9.92. Found: C, 54.76; H, 6.42; N, 9.23. Chiral separation on Chiralpak AD-H, EtOH, 0.55 ml/min, 40 °C.

**4.12.7. (±)-*trans*-2-(Toluene-4-sulfonylamino)-cyclohexane carboxamide (7b).** *R. equi* A4 (7 g wet cells, 80 ml buffer, $OD_{610}$=53). Yield 129 mg (54%, ee=65%) from 223 mg (±)-**7a** (10 mM); *R. ery.* 11540 (7.5 g wet cells, 80 ml buffer, $OD_{610}$=39). Yield 133 mg (56%, ee=59%) from 223 mg (±)-**7a** (10 mM); *R.* sp. R312 (8.1 g wet cells, 80 ml buffer, $OD_{610}$=62). Yield 100 mg (42%, ee=77%) from 223 mg (±)-**7a** (10 mM). White solid, mp 212–213 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.93–1.09 (m, 3H), 1.32–1.39 (m, 1H), 1.44–1.51 (m, 3H), 1.69–1.71 (m, 1H), 2.03 (dt, 1H, $J$=3.9, 10.8 Hz, H-1), 2.35 (s, 3H), 3.26 (ddt, 1H, $J$=3.7, 9.3, 10.5 Hz, H-2), 6.70 (s, br, 1H, N$H_2$), 7.01 (s, br, 1H, N$H_2$), 7.32 (d, 2H, $J$=8.0 Hz), 7.42 (d, 1H, $J$=9.3 Hz, N$H$), 7.65 (d, 2H, $J$=8.0 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.66, 24.81, 24.84, 29.79, 33.12, 50.44, 53.92, 126.96, 130.04, 140.95, 142.69, 175.55; *m/z* (EI) 252 (1), 155 (10), 141 (100), 124 (55), 91 (83). Anal. Calcd for $C_{14}H_{20}N_2O_3S$: C, 56.74; H, 6.80; N, 9.45. Found: C, 56.77; H, 6.60; N, 9.46. Chiral separation on Chiralcel AD-H, *n*-heptane/ethanol 70:30, 0.80 ml/min, 15 °C.

**4.12.8. (±)-*cis*-2-(Toluene-4-sulfonylamino)-cyclohexane carboxamide (8b).** *R. equi* A4 (6.9 g wet cells, 60 ml buffer, $OD_{610}$=76): Yield 85 mg (48%, ee=6%) from 167 mg (±)-**8a** (10 mM); *R. ery.* 11540 (8.5 g wet cells, 80 ml buffer, $OD_{610}$=38). Yield 98 mg (41%, ee=8%) from 223 mg (±)-**8a** (10 mM); *R.* sp. R312 (8.3 g wet cells, 80 ml buffer, $OD_{610}$=52). Yield 103 mg (43%, ee=4%) from 223 mg (±)-**8a** (10 mM). White solid, mp 161–162 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.07–1.19 (m, 3H), 1.39–1.48 (m, 3H), 1.66–1.74 (m, 2H), 2.32–2.35 (m, 1H, H-1), 2.36 (s, 3H), 3.33 (m, 1H, H-2), 6.82 (s, br, 1H, N$H_2$), 7.24 (s, br, 1H, N$H_2$), 7.34 (m, 3H), 7.81 (d, 2H, $J$=7.8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.53, 21.66, 23.67, 25.64, 29.65, 45.44, 52.49, 127.22, 130.18, 139.25, 143.11, 176.08; *m/z* (EI) 252 (0.6), 155 (20), 141 (100), 124 (63), 91 (77). Anal. Calcd for $C_{14}H_{20}N_2O_3S$: C, 56.74; H, 6.80; N, 9.45. Found: C, 56.69; H, 6.87; N, 9.23. Chiral separation on Chiralcel OD-H, *n*-heptane/*i*-propanol 50:50, 0.38 ml/min, 15 °C.

**4.12.9. (±)-3-Phenyl-3-(toluene-4-sulfonylamino)-propionamide (9b).** White solid, mp 213–214 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.30 (s, 3H), 2.28–2.46 (m, 2H), 4.62 (dt, 1H, $J$=6.3, 8.8 Hz), 6.72 (s, br, 1H, N$H_2$), 7.08–7.14 (m, 5H), 7.18 (d, 2H, $J$=8.1 Hz), 7.23 (s, br, 1H, N$H_2$), 7.46 (d, 2H, $J$=8.1 Hz), 8.18 (d, 1H, $J$=8.8 Hz, N$H$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 21.60, 43.44, 55.17, 127.04, 127.43, 127.51, 128.56, 129.86, 139.26, 141.80, 142.77, 171.32; *m/z* (CI, methane) 319 $(M+1)^+$ (2), 260 (100), 172 (69), 155 (17), 148 (41). Anal. Calcd for $C_{16}H_{18}N_2O_3S$: C, 60.36; H, 5.70; N, 8.80. Found: C, 57.97; H, 5.48; N, 8.00.

**4.12.10. (±)-4-Phenyl-3-(toluene-4-sulfonylamino)-butyramide (11b).** White solid, mp 183–184 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.10 (d, 2H, $J$=6.8 Hz), 2.33 (s, 3H), 2.48 (dd, 1H, $J$=13.7, 7.8 Hz), 2.64 (dd, 1H, $J$=13.7, 5.4 Hz), 3.65 (m, 1H), 6.84 (s, br, 1H, N$H_2$), 7.02–7.03 (m, 2H), 7.12–7.18 (m, 3H), 7.22 (d, 2H, $J$=8.1 Hz), 7.30 (s, br, 1H, N$H_2$), 7.46 (d, 2H, $J$=8.1 Hz), 7.59 (d, 1H, $J$=8.3 Hz, N$H$); $^{13}$C NMR (acetone-$d_6$) $\delta$ 20.73, 38.55, 40.81, 53.05, 126.42, 127.06, 128.44, 129.60, 129.66, 138.36, 138.97, 142.89, 172.69. Anal. Calcd for $C_{17}H_{20}N_2O_3S$: C, 61.43; H, 6.06; N, 8.43. Found: C, 60.69; H, 5.94; N, 8.06.

## 4.13. General procedure for chemical hydratation of nitriles to carboxylic acids

100 mg of nitrile was suspended in 5 ml of NaOH concd and refluxed overnight. Except for the acids **7c** and **8c**, the saponification was complete. The acid was released by addition of HCl and dilution of the aqueous phase with water. Extraction with $CH_2Cl_2$ or/and ethyl acetate and drying with $Na_2SO_4$ generally yielded pure crystals. In some cases, the product had to be purified by recrystallization or silica gel chromatography.

**4.13.1. (±)-*trans*-2-(Toluene-4-sulfonylamino)-cyclopentane carboxylic acid (5c).** *R. equi* A4 (7 g wet cells, 80 ml buffer, $OD_{610}$=56). Yield 100 mg (44%, ee=2%) from 211 mg (±)-**5a** (10 mM); *R. ery.* 11540 (8.0 g wet cells, 80 ml buffer, $OD_{610}$=32). Yield 195 mg (86%, ee=5%) from 211 mg (±)-**5a** (10 mM); *R.* sp. R312 (8.0 g wet cells, 80 ml buffer, $OD_{610}$=62). Yield 76 mg (34%, ee=14%) from 211 mg (±)-**5a** (10 mM). White solid, mp 124–125 °C; $^1$H NMR ($CDCl_3$) $\delta$ 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, N$H$), 7.30 (d, 2H, $J$=8.2 Hz), 7.77 (d, 2H, $J$=8.2 Hz), 9.20 (s, br, 1H, COO$H$); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.80, 23.18, 28.47, 33.71, 50.87, 57.73, 127.53, 129.98, 137.13, 143.92, 179.74. Anal. Calcd for $C_{13}H_{17}NO_4S$: C, 55.11; H, 6.05; N, 4.94. Found: C, 55.27; H, 6.05; N, 4.92. Chiral separation on Chirobiotic R, polar organic mode (MeOH/$Et_3N$/AcOH 100:0.4:0.1), 0.8 ml/min, ambient temperature.

**4.13.2. (±)-3-Phenyl-3-(toluene-4-sulfonylamino)-propionic acid (9c).** White solid, mp 149–151 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.37 (s, 3H), 2.83 (dd, 1H, $J$=16.5, 6.3 Hz), 2.93 (dd, 1H, $J$=16.5, 6.1 Hz), 4.71–4.75 (m, 1H), 5.74 (d, 1H, $J$=7.8 Hz, N$H$), 7.10–7.12 (m, 2H), 7.17 (d, 2H, $J$=8.3 Hz), 7.19–7.21 (m, 3H), 7.60 (d, 2H, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.74, 40.89, 54.20, 126.68, 127.35, 128.16, 128.89, 129.75, 137.33, 139.15, 143.68, 174.90. Anal. Calcd for $C_{16}H_{17}NO_4S$: C, 60.17; H, 5.36; N, 4.39. Found: C, 59.72; H, 5.20; N, 4.42.

**4.13.3. (±)-4-Phenyl-3-(toluene-4-sulfonylamino)-butyric acid (11c).** White solid, mp 101–103 °C; $^1$H NMR ($CDCl_3$) $\delta$ 2.41 (s, 3H), 2.55 (d, 2H, $J$=4.9 Hz), 2.79 (dd, 1H, $J$=13.7, 6.9 Hz), 2.86 (dd, 1H, $J$=13.7, 7.8 Hz), 3.76 (m, 1H), 5.41 (d, 1H, $J$=8.3 Hz, N$H$), 7.01–7.03 (m, 2H), 7.20–7.23 (m, 5H), 9.72 (s, br, 1H, COO$H$), 7.62 (d, 2H, $J$=8.3 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.78, 37.92, 40.75, 51.91, 127.14, 127.22, 128.97, 129.46, 129.94, 136.87, 137.37,

143.69, 176.47. Anal. Calcd for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74; N, 4.20. Found: C, 62.47; H, 5.93; N, 4.16.

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25. CCDC 246741 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.