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Synthesis of novel *N*-protected β^3-amino nitriles: study of their hydrolysis involving a nitrilase-catalyzed step

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ABSTRACT

Several commercially available nitrilases were investigated with regard to their potential to hydrolyze N-protected β^3-amino nitriles into their corresponding N-protected β^3-amino acids.

The biotransformations were obtained in different proportions depending on the nitrilase involved. The best hydrolysis results were achieved for the *N*-Cbz-β^3-amino nitrile from L-alanine using the NIT-107, in a phosphate buffer at 0.05 M. However, no biotransformation into the corresponding acids was observed for the *N*-sulfonylamide β^3-amino nitriles. Two simple and efficient procedures to prepare the β^3-amino nitriles from their analogous α-amino acids are described. Thirty four new substances were synthesized and characterized over the course of this work.

1. Introduction

In recent years, there has been an increasing interest in the synthesis of β-amino acids due to their significant effects, such as antibiotic,[1] antifungal,[2] antitumor,[3] antihelminthic,[4] cytotoxic,[5] and other important pharmacological properties.[6] Many natural products with a β-amino acid moiety are potential lead structures for the development of new drugs.[7] The replacement of α-amino acids in biologically active peptides by certain β-counterparts can have pronounced effects on their folding properties. For instance, cyclic and linear oligomers of β-amino acids have revealed high biological activity as mimics for the peptide hormone somatostatin showing antiproliferative activities against human cancer cell lines.[6a]

Several routes to prepare β^3-amino acids have been developed,[6b,c,8] some from the corresponding α-amino acids.[9] The best known is the Arndt–Eistert methodology.[6b,10] Unfortunately, the Arndt–Eistert conditions for direct homologation are not suitable for large scale preparations and, besides, are not recommended for preserving the *N*-protected group necessary in peptidomimetic synthesis.[9b]

Several methods using β^3-amino nitriles as precursors of β^3-amino acids have been described.[11] Nitriles have been extensively used in the industry as precursors for the production of a wide variety of amides and carboxylic acids by chemical synthesis. However, the conventional chemical hydrolysis of nitriles suffers from several disadvantages, including the requirement for harsh acidic or basic conditions, high temperatures, the formation of undesirable by-products, racemization, low yields, and environmental problems due to the generation of waste salts.[12]

As a result, in recent years, considerable attention has been paid to the enzymatic hydrolysis of nitriles as an alternative route to the chemical synthesis of amides and carboxylic acids.[13] High conversion yields and selective hydrolysis of the –CN functionality of compounds containing labile groups as well as high chemo-, regio- and stereoselectivities can be obtained. On the other hand, biocatalysis frequently use a biodegradable catalyst and can be performed under mild reaction conditions (neutral pH, low temperature, and water as solvent).[14] Furthermore, the use of enzymes usually generates less waste and, hence, is both environmentally and economically more attractive than traditional organic syntheses.[14c]

The recent availability of 'ready to use' nitrilase preparations[15] which avoids the laborious handling of whole cell biotransformation systems has prompted us to search for an efficient and short synthesis of β^3-amino acids from the readily available corresponding nitriles.

In the drive toward Green, sustainable methodologies for chemicals manufacture, we herein report the synthesis of β^3-amino nitriles from their natural α-amino acids counterparts and their bioconversion into *N*-protected β^3-amino acids by purified nitrilases. These β^3-amino acids will be ready to use in peptidomimetic syntheses.

2. Results and discussion

2.1. Synthesis of β^3-amino nitriles

Initially, we were interested in the preparation of *N*-protected β^3-amino nitriles **7a–f** from their corresponding α-amino acids

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and then in their hydrolysis by a bioconversion step using nitrilases.

Aliphatic L-amino acids: alanine **1a**, valine **1b,** and isoleucine **1c**, and aromatic L-amino acids: phenylalanine **1d**, *O*-*tert*-butyl-tyrosine **1e,** and *O*-benzyl-tyrosine **1f** were employed. Either classical N-protecting groups such as Cbz and Boc were used in the peptide synthesis or uncommon protecting groups such as tosyl, nosyl, and diphenylphosphinyl (Dpp) were employed. We focused our attention on the study of the influence of these protecting groups in the biocatalysis step by nitrilases.

Taking into account that homologation of the parent chiral α-amino acids is one of the most powerful strategies to prepare the β-homologues, we designed two straightforward procedures, in which the key step of the whole conversion is represented by the synthesis of enantiomerically pure N-protected β^3-aminonitriles **7a**–**f** (Scheme 1).

Firstly the amino alcohols required are obtained in enantiomerically pure form via the reduction of the corresponding commercially available enantiopure α-amino acids. The reduction was achieved by sodium borohydride in tetrahydrofuran using $ZnCl_2$ as a Lewis acid catalyst.[16] The tendency of α-amino alcohols to form stable borate esters, and chelates with metal cations makes their isolation difficult.[17] Therefore, the key step of this protocol is the hydrolysis of the complex amino alcohol/borate by treatment of the crude reaction with 2 equiv of a solution of NaOH 50% (w/w) at 70 °C for 4 h. The amino alcohols **2a**–**f** were obtained in good yields (86–95%) and used in the next step without further purification.

The extent of racemization at the stereogenic center of the starting α-amino acids and/or β-amino alcohols was checked at various stages of the whole process by chiral HPLC analyses. Under our conditions, no trace amounts of racemized products could be detected.

The first strategy developed (method A) was employed in the preparation of the *N*-Cbz-β^3-amino nitriles **7a**–**f-Cbz**. This approach involved the formation of *N*–H aziridines, their activation by a benzyloxycarbonyl group, and their regioselective ring-opening using a cyanide ion as a nucleophile (Scheme 1).[11a,b]

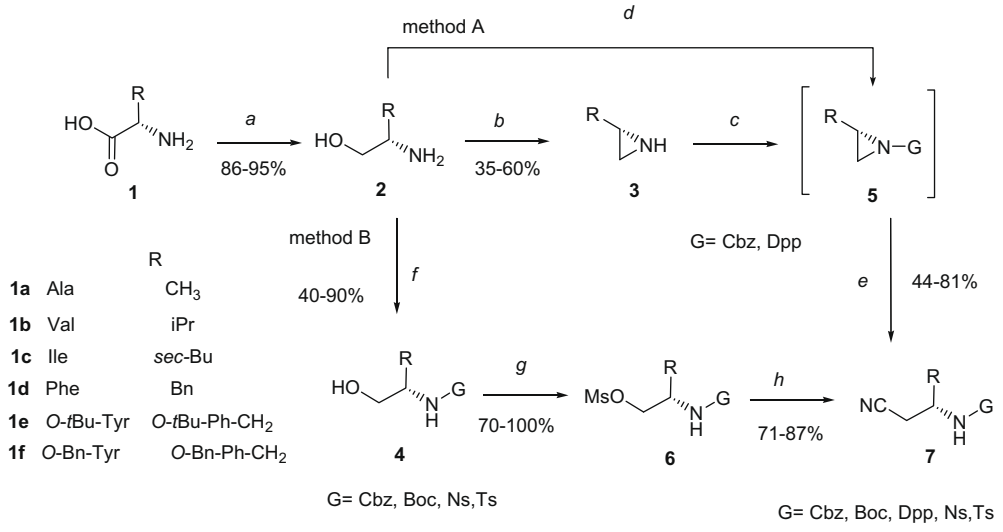
Chiral amino alcohols **2a**–**f** were converted into aziridines **3a**–**f** via a Mitsunobu reaction[18] using diethyl azodicarboxylate (DEAD) (1.05 equiv) and triphenylphosphine (Ph_3P) (1.05 equiv) in diethyl ether, toluene, or tetrahydrofuran. The modest yields obtained in this type of Mitsunobu reaction (35–60%) (Table 1) could be attributed to the low acidity of the amine moiety resulting in the slow formation of the oxyphosphonium intermediate and hence product formation.[19] Additionally, the instability due to the risk of polymerization, and in some cases, the volatile nature of N–H aziridines lead to a loss of more than 50% after work-up and during the purification step.

Next, our first attempt to prepare the *N*-Cbz-β^3-amino nitriles involved a one-pot procedure whereby the aziridines **3a**–**f** were directly transformed into the corresponding opening compounds **7a**–**f-Cbz** by the reaction with benzyl cyanoformate under phase transfer catalysis conditions. Recently Moss et al. have developed an efficient enantioselective alkylation of *N*-sulfonyl-protected aziridines using sophisticated phase transfer catalysts and a variety of base combinations.[20]

In our case, a most commonly used phase transfer agent, tetrabutylammonium bromide (TBABr), was employed in the presence of NaOH in stoichiometric quantities. As a result, the ring-opening reaction of intermediate aziridines **5a**–**f-Cbz** was carried out with NaCN in the presence of a catalytic amount of TBABr (10%, relative to the nucleophile) in a mixture of toluene/water (5:1) to obtain the corresponding *N*-Cbz-β^3-amino nitriles **7a**–**f-Cbz** in yields of 45–75%. It should be noted that significant amounts of polymeric materials were generated when this procedure was scaled up.

Subsequently, in order to improve the yields and make the work-up more easy, we decided to carry out the ring-opening reaction of aziridines **5a**–**f-Cbz** under mild conditions.[11b,21] First, aziridines **3a**–**f** were reacted with benzyl cyanoformate in acetonitrile to afford compounds **5a**–**f-Cbz**. The reaction was monitored by TLC and GC–MS and after removal of the solvent the activated aziridines were used in the next step without any purification. The crude *N*-Cbz aziridines **5a**–**f-Cbz** were reacted with additional NaCN (3 equiv) in a mixture of acetonitrile/water (9:1) at 80 °C and the reaction was completed in 16–21 h. The desired N-protected β-amino nitriles **7a**–**f-Cbz** were obtained with yields of 55–89% (Table 1). According to the NMR spectra, the aforementioned conditions produced a regioselective ring-opening of the heterocycle.

In order to improve the yields, the reaction time, and to avoid any intermediate work-up, a one-pot reaction was attempted. Unfortunately, even if the overall reaction time in the one-pot reaction decreased by half compared to the multi-step protocol (20 h vs



Scheme 1. Synthesis of β-amino nitriles. Reagents and conditions: (a) $NaBH_4$, $ZnCl_2$, THF; (b): PPh_3/DEAD, THF or toluene (c) CbzCN, CH_3CN; (d): DppCl, TEA, THF, 0 °C; (e) NaCN, CH_3CN/H_2O 9:1, reflux; (f) CbzCl, TEA CH_2Cl_2 (or THF) or Boc_2O, NaOH, dioxane or TsCl (or $_4$NsCl), $NaHCO_3$, THF (g) MsCl, TEA, THF; (h) NaCN, DMF.

Table 1
Mitsunobu reaction, ring-opening of aziridines in CH_3CN/H_2O, and overall yield of method A

2 **3** **5** **7**

2	R	**3-Cbz**		**7-Cbz**		Overall yield % (from **2**)
		Time (h)	Yield %	Time (h)	Yield %	
a	CH_3	4.5	25[a]	21	55	14
b	*i*Pr	40	35[b]	21	60	21
c	*sec*-Bu	18	60[b]	16	66	40
d	Bn	21	53[c]	21	81	43
e	O-*t*Bu-Ph-CH_2	19	43[c]	18	89	38
f	O-Bn-Ph-CH_2	22	30[c]	18	73	22

[a] Prepared in diethyl ether.
[b] Prepared in tetrahydrofurane.
[c] Prepared in toluene.

40 h), the overall yields obtained (17–35%) were not significantly better.

The *N*-Dpp β-amino nitriles **7a-Dpp**, **7c-Dpp**, **7d-Dpp**, and **7e-Dpp** were prepared following a modification of the procedure described above for method A. The use of a diphenylphosphinyl group as an alternative of protecting group in amino acid and peptide chemistry is well-known,[22] although, a few reports regarding *N*-Dpp aziridines synthesis are described.[22a,23] Firstly, the activated aziridines **5a-Dpp**, **5c-Dpp**, **5d-Dpp,** and **5e-Dpp** were synthesized from amino alcohols in a one-pot transformation by ring-closure of the corresponding *N*,*O*-diphenylphosphinylated intermediates.

Amino alcohols **2a**, **2c**, **2d**, and **2e** were reacted with diphenylphosphinyl chloride (DppCl) (2 equiv) and Et_3N (3 equiv) in THF at 25 °C for 20 h. Next, sodium hydride (5 equiv) was required to allow the cyclization to afford *N*-Dpp aziridines **5a-Dpp**, **5c-Dpp**, **5d-Dpp,** and **5e-Dpp** with yields of 55%, 76%, 67%, and 74%, respectively.

The ring-opening reaction of *N*-Dpp aziridines **5a-Dpp**, **5c-Dpp**, **5d-Dpp,** and **5e-Dpp** occurred smoothly using phase transfer conditions (TBABr 10 mol % in toluene/water 5:1 as described above for **5(a–f)-Cbz**) for 18 h and afforded the product as a single stereoisomer. The reaction gives the expected ring-opened product resulting from nucleophilic attack on the less substituted carbon atom. The *N*-Dpp amino nitriles were obtained regioselectively in good yields (74–86%, HPLC purity of 85–98%) (Table 2). To our knowledge, no ring-opening reaction of *N*-Dpp aziridines using such phase transfer conditions to afford *N*-Dpp β^3-aminonitriles is described. This method represents an interesting way to synthesize such *N*-Dpp β^3-aminonitriles.

As described above, the regioselective ring-opening reaction of aziridines (method A) is a synthetically powerful route to prepare N-protected-β^3-amino nitriles. Nevertheless, to circumvent the difficulties found during the synthesis of compounds **7a–f-Cbz** using method A, an alternative route (method B) was designed starting from amino alcohols **2a–f** to prepare the N-protected β-amino nitriles **7a–f-Cbz**. To extend the application of this method we wished to prepare other *N*-protected-β-amino nitriles **7a–c-Boc**, **7a–c-$_4$Ns,** and **7a–c-Ts**.

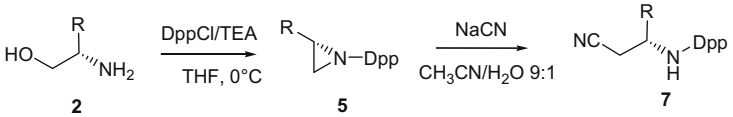
The new approach in method B involved amine group protection, activation of the alcohol function by mesylation, and S_N2 displacement by cyanide ion[13m,24] (Scheme 1). The synthesis of N-protected amino alcohols was carried out according to the literature procedures[25] and the results are reported in Table 3. To prepare the *N*-Cbz amino alcohols **4a–f-Cbz**, compounds **2a–f** were reacted with benzylchloroformate and triethylamine in THF or CH_2Cl_2 to afford the desired compounds **4a–f-Cbz** with yields ranging from 45% to 94%.

To obtain the *N*-Boc amino alcohols **4a–c-Boc**, the corresponding amino alcohols **2a–c** were reacted with $(Boc)_2O$ and NaOH 1 M in dioxane.[25] In the case of compounds **4a–c-$_4$Ns** and **4a–c-Ts**, β-amino alcohols **2a–c** were reacted with $_4$NsCl or TsCl (1.1 equiv) in the presence of $NaHCO_3$ (4 equiv) at room temperature in THF. The reaction times and the yields of the N-protection steps are summarized in Table 3.

In the next step, the *N*-protected amino alcohols **4a–f-Cbz**, **4a–c-Boc**, **4a–c-Ts,** and **4a–c-$_4$Ns** were treated with methanesulfonyl chloride in THF to afford the solid intermediates **6a–f-Cbz**, **6a–c-Boc**, **6a–c-Ts,** and **6a–c-$_4$Ns** with yields ranging from 70% to 100%. These compounds were unstable at room temperature and were used in the following reactions without further purification.

Finally, *N*-protected β-amino nitriles **7a–f-Cbz**, **7a–c-Boc**, **7a–c-$_4$Ns,** and **7a–c-Ts** were obtained by nucleophilic substitution of

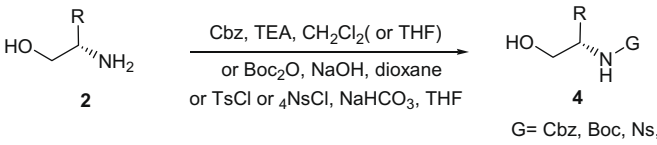
Table 2
Reaction times and yields of the *N*-Dpp aziridines **5a**,**c**,**d**,**e-Dpp** and **7a**,**c**,**d**,**e**-**Dpp**



2 **5** **7**

2	R	**5-Dpp**		**7-Dpp**		Overall yield % (from **2**)
		Time (h)	Yield %	Time (h)	Yield %	
a	CH_3	20	55	18	86	47
c	*sec*-Bu	20	76	18	74	56
d	Bn	20	67	18	75	50
e	O-*t*Bu-Ph-CH_2	20	74	18	74	74

Table 3
Yields of N-protected β-amino alcohols



2	R	4-Cbz		4-Boc		4-$_4$Ns		4-Ts	
		Time (h)	Yield %	Time (h)	Yield %	Time (h)	Yield %	Time (h)	Yield %
a	CH_3	12	60	3	85	5	95	28	71
b	*i*Pr	12	82	2.4	99	7	70	24	97
c	*sec*-Bu	3	55	2.6	65	5	78	24	83
d	Bn	10	92						
e	O-*t*Bu-Ph-CH_2	1.5	50[a]						
f	O-Bn-Ph-CH_2	3.5	94[a]						

[a] Prepared in CH_2Cl_2.

the corresponding β-amino mesylates by cyanide ion (NaCN, 1.5 equiv) in DMF at 70 °C without any deprotection. The reaction times and the yields obtained in the preparation of N-protected β-amino nitriles **7** are reported in Table 4. In the case of **7e-Cbz**, the low yield of 30% could be explained by the slow rate of the reaction (28 h) that induces the formation of polymers.

On the other hand, it was noticed that the required conditions for the preparation of *N*-Ts-β-amino nitriles **7b-Ts** and **7c-Ts** from their corresponding β-amino mesylates **6b-Ts** and **6c-Ts** afforded the corresponding *N*-tosyl aziridines in yields of 66% and 100%, respectively. Consequently, an additional step was necessary. The *N*-tosyl aziridines were opened using a cyanide ion as a nucleophile in a mixture of acetonitrile/water (9:1) at 80 °C. The desired *N*-Ts β-amino nitriles **7b-Ts** and **7c-Ts** were afforded in excellent yields of 92% and 94%, respectively. The reaction times and the yields of the *N*-protected-β-amino nitriles are summarized in Table 4.

In summary, the strategies described in this study (method A and method B) constitute as efficient approaches to prepare β-amino nitriles **7a–f-Cbz**, **7a–c-Boc**, **7a–c-$_4$Ns,** and **7a–c-Ts** from the corresponding amino alcohols. Moreover, a large number of compounds synthesized are reported here for the first time. The spectroscopic and physical data of these new compounds are given in Section 4.

Method A has shown that the regioselective ring-opening of aziridines is a potential tool to obtain β-amino nitriles in an easy way. The ring opening of aziridines by a cyanide ion requires an activating group and in our particular case, this reaction was particularly efficient with the diphenylphosphinyl group. However, our study shows that method B (applied to other protecting groups) is the best strategy for preparing *N*-protected-β-amino nitriles. This method used inexpensive reagents, and was cleaner and faster. On the other hand, the work-up was easier than that required for the method A and these operative conditions can be applicable on a large-scale. The reaction times and overall yields of the three steps for method B are reported in Table 5.

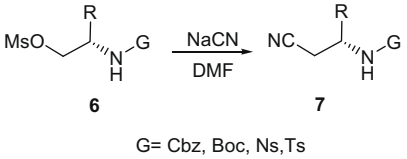
2.2. Biotransformation screening

The aim of the present study was to develop the hydrolysis of N-protected β-amino nitriles by a bioconversion step using nitrilases in order to avoid the harsh conditions required in the chemical hydrolysis of nitriles. We were also interested in studying the influence of the nature of *N*-protecting groups during the biotransformation process by nitrilases and in consequently evaluating their use for biocatalytic screening.

The recent availability of nitrilases 'ready for use' simplifies the reaction protocol considerably. To the best of our knowledge, very few publications showing the utilization of commercial nitrilases in biotransformation screening are available.[13m,o,r]

Most of the biotransformation reactions were monitored by reversed phase HPLC and UV-detection. For *N*-Boc-β-amino nitriles, the biotransformation is severely hindered by the problems arising in terms of reaction monitoring and product separation of strongly polar compounds. The detection of the aliphatic nitriles/amides is limited because of their poor UV-sensibility, especially on a screen-

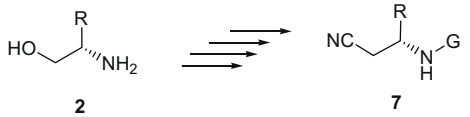
Table 4
Yields of N-protected β-amino nitriles



6	R	7-Cbz		7-Boc		7-Ns		7-Ts	
		Time (h)	Yield %	Time (h)	Yield %	Time (h)	Yield %	Time (h)	Yield %
a	CH_3	1	81	4	66	4	22	0.5	73
b	*i*Pr	4	85	1	43	4	81	2.5	92[a]
c	*sec*-Bu	4	87	5.5	47	48	40	4	94[a]
d	Bn	1	88						
e	O-*t*Bu-Ph-CH_2	28	30						
f	O-Bn-Ph-CH_2	1.5	89						

[a] Yields obtained from the *N*-tosyl aziridine intermediates.

Table 5
Reaction times and overall yields of method B in three steps



2 **7**

G= Cbz, Boc, Ns,Ts

Compound **7**	Time (h)	Overall yield % (from **2**)
7a-Cbz	15	46
7b-Cbz	7	54
7c-Cbz	8	47
7d-Cbz	11.5	57
7e-Cbz	29	14
7f-Cbz	7.5	74
7a-Boc	11	42
7b-Boc	5.4	40
7c-Boc	14	30
7a-$_4$Ns	19.5	19
7b-$_4$Ns	19	53
7c-$_4$Ns	58.5	30
7a-Ts	29.5	51
7b-Ts	29	88[a]
7c-Ts	26	74[a]

[a] Overall yield from **2** in four steps.

ing scale. When UV-detection was not possible, Micromass ZQ detection was used.

Initially, all the amino nitriles **7a**–**f-Cbz** (Scheme 2) were subjected to biotransformations on a screening level employing 12 nitrilases. The reactions were monitored by HPLC and for better comparability, all screening experiments were stopped at the time of the expected maximum conversion of the Cbz-β-amino nitriles **7a**–**f-Cbz** into the corresponding Cbz-β-amino acids **9a**–**f-Cbz** (after 18 h).

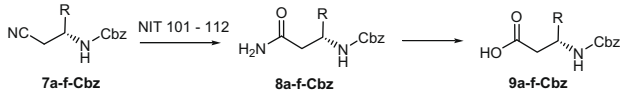
The initial biotransformation screening tests were achieved according to a modification of a protocol from the literature.[13m,o] In our study, the β-amino nitriles **7a**–**f-Cbz** (0.2 M) were subjected to a biotransformation on a screening level with twelve nitrilases (NIT-101–NIT-112)[15] in a phosphate buffer (pH 7) and DMSO or methanol as cosolvent when necessary at 30 °C.

On the basis of screening results, the reaction protocols were established for each β-amino nitrile. Therefore, new conditions were tested with each nitrilase (NIT-101–NIT-112) and different parameters were studied. The concentration of the substrate ranging of 0.2, 0.1, and 0.05 M was evaluated. On the other hand, the nature and concentration of the cosolvent (DMSO or methanol) at 3%, 4%, 5%, 10%, and 60% were also screened.

The first biocatalysis results dealing with the formation of *N*-Cbz-β-amino acids **9a**–**b-Cbz** from the corresponding *N*-Cbz-β-amino nitriles **7a**–**b-Cbz** with the different purified nitrilases are shown in Table 6.

The best results of the biotransformation screenings for the substrates **7a-Cbz** and **7b-Cbz** (0.2 M) were obtained for NIT-104, NIT-107, NIT-108, NIT-109, NIT-110, and NIT-112 without any cosolvent (Table 6, entries 1, 2, 5–8). It was noticed that the homogeneity of the media was of primary importance, rendering the use of a cosolvent unnecessary.

Table 6
Yields calculated by HPLC data during biotransformation screening of compounds **7a-Cbz** and **7b-Cbz**



7a-f-Cbz **8a-f-Cbz** **9a-f-Cbz**

Entry	Enzyme	Concn (M)	Nitrile %		Amide %		Acid %	
			7a	**7b**	**8a**	**8b**	**9a**	**9b**
1	NIT-104	0.2	54	96	—	2	46	2
2	NIT-107	0.2	48	59	—	26	52	15
3	NIT-107	0.1	28	52	—	31	72	17
4	NIT-107	0.05	**0**	14	—	43	**100**	43
5	NIT-108	0.2	80	96	—	2	20	2
6	NIT-109	0.2	54	96	—	3	46	1
7	NIT-110	0.2	55	95	—	5	45	0
8	NIT-112	0.2	55	95	—	3	45	2

Taking into account that the nitrilase NIT-107 showed the best results, we decided to limit the screening tests using only this enzyme at different concentrations of substrate (0.1 and 0.05 M). The results are depicted in Table 6 (entries 3 and 4). The best conditions for the biotransformation of **7a-Cbz** into the corresponding amino acid **9a-Cbz** were obtained with the nitrilase NIT-107, at a substrate concentration of 0.05 M, without any cosolvent (yield = 100%).

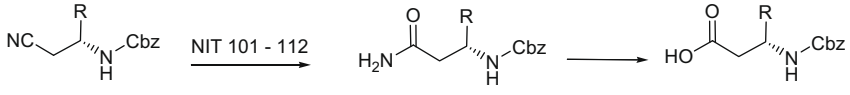
On the basis of these screening results, the optimized transformation protocols were established to synthesize **7a-Cbz** on a preparative scale with the appropriate nitrilase in high enantiomeric purity. The reactions were monitored by HPLC and stopped at the time established by the catalytic activity of enzyme. As expected, the biotransformation by the NIT-107 into the corresponding *N*-Cbz-β-amino acid **7a-Cbz** was complete without any formation of the amide. The yield of 98% obtained was determined after isolation by extraction and chromatographic purification (see Section 4).

An unexpected result was obtained for the *N*-Cbz-β-amino nitrile **7c-Cbz** derived from the L-isoleucine which showed a lower affinity for the purified nitrilases. The best rate of biotransformation of **7c-Cbz** into the corresponding acid **9c-Cbz** was observed using the NIT-107, methanol as cosolvent (5%) at a 0.1 M concentration of substrate (yield = 5.2%).

The biotransformation reactions of **7b-Cbz** were accompanied by the formation of the corresponding amide **8b-Cbz** (up to 43% for NIT-107). Such a nitrile hydratase activity has been noticed in the past and principally described for α-amino nitriles.[13j,m,26] Recently, Sheldon et al. have suggested a rational mechanism for amide formation.[27]

The specific rotations of **9a-Cbz** and **9b-Cbz** were measured and were in agreement with the data reported in the literature.[28] Hence, the absolute configuration of these compounds could be assigned as the (*S*)-isomer by comparison with the reported specific rotation value (see Section 4). These results clearly show that there is no racemization during the reaction sequences.

In the case of the *N*-Cbz-β-amino nitriles **7d**–**e-Cbz**, the results were very dissimilar. The first problem we had to face was the low solubility of the substrates. The insolubility of nitrile substrates in



7a-f-Cbz **8a-f-Cbz** **9a-f-Cbz**

Scheme 2. Biotransformation of β-amino nitriles **7a**–**f-Cbz** into amides and into carboxylic acids.

aqueous reaction media decreased the enzymatic reaction time. A lot of remarks dealing with the substrate solubility and the cosolvent compatibility in the biotransformation of nitriles is noticed in the literature.[29] However, this knowledge is hardly applicable in an unexplored area, such as the microbial transformation of β-amino nitriles.[13h] In our study, the nature and amount of the cosolvent had to be developed.

New conditions were tested for each nitrilase (NIT-101–NIT-112) requiring us to reestablish the concentration of the substrate and the cosolvent. Unfortunately, in spite of the high number of biotransformation reactions performed, no results were obtained for the Cbz-β-amino nitriles **7e-Cbz** and **7f-Cbz**. On the other hand, among more than forty screening tests carried out for **7d-Cbz**, only a poor biotransformation into the expected acid **9d-Cbz** was observed (2–5.5%) using the NIT-106.

At this stage in order to control the enantiospecificity of each nitrilases, we studied the same reaction using two racemic nitriles **7b-Cbz** and **7d-Cbz**. No biotransformation results were obtained with (±)-**7d-Cbz**. These first results of biotransformation suggested to us that Cbz-β-amino nitriles in the aromatic series were not suitable substrates for the purified nitrilases tested. The hydrolysis of (±)-**7b-Cbz** by the NIT-107 gave 23% of **9b-Cbz** which corresponds to the expected L-isomer.

Next, we studied the biotransformation of other *N*-protected-β-amino nitriles previously synthesized. As a result of the protocols previously established for *N*-Cbz-β-amino nitriles **7a-Cbz** and **7b-Cbz** (NIT-107, 0.05 M without cosolvent), the N-protected-β-amino nitriles **7a–c-Boc**, **7a–c-Ts**, **7a–c-$_4$Ns,** and **7a,c-Dpp** were also submitted to the biotransformation screenings.

Once more, the biocatalytic conditions had to be newly established. We were again confronted with the low solubility of the substrates; as a result DMSO and methanol were tested as cosolvents in 5%, 10%, and 20%. Under these new screening conditions, the nitrilases were used as cocktails: cocktail 1 (NIT-101–NIT-104), cocktail 2 (NIT-105–NIT-108), and cocktail 3 (NIT-109–NIT-112). Unfortunately, no biotransformation into the corresponding acids was observed under the newly explored conditions.

The nature of the *N*-protecting groups of β-amino nitriles on nitrilase activity has been investigated by a few authors.[13h] The protecting functional group could be a tool to modulate the substrate acceptance and selectivity of an enzyme. In terms of the requirements mentioned above, an alkoxycarbonyl N-protecting group, in particular the benzyloxycarbonyl group would be preferable. The β-amino nitriles *N*-protected by sulfonyl amide group did not undergo enzymatic hydrolysis with the nitrilases investigated. These results could be attributed to the inherent structural features of the substrates, especially the high substrate specificity of nitrilases regarding the nature of the protecting groups such as sulfonamides and aromatic groups.

Since there has not been an X-ray analysis of the nitrilases available, so that the actual structure of their active site remains unknown, such a difference in substrate reactivity cannot be easily rationalized. The same is true for the substrate specificity of NIT-107 toward β-amino nitriles **7a–c-Cbz** resulting in remarkably different biotransformation results.

3. Conclusion

The efficient preparation of β-amino nitriles was described by two procedures featuring a maximum of four steps from the corresponding commercially available α-amino acids. Both the efficiency and enantioselectivity of the commercial nitrilases used for the β-amino nitriles hydrolysis were strongly dependent upon the nature of the N-protecting group. The nitriles from the aliphatic α-amino acids with the protecting benzyloxycarbonyl group **7a–b-Cbz** have been successfully biotransformed. In spite of the β-amino nitriles with sulfonamide-like as well as diphenylphosphinyl-like N-protecting groups not being transformed by these nitrilases, their study contributes to a better understanding of nitrilase substrate specificities.

4. Experimental section

4.1. General

4.1.1. Chemicals

All reagents were obtained from commercial sources unless otherwise noted, and used as received. All reactions were monitored by thin layer chromatography (TLC) performed on precoated silica gel plates (60 F_{254}, Merck). TLC plates were viewed under UV (254 nm) and developed with ninhydrine or in an iodine chamber; frontal retention values R_f have been mentioned when necessary. Flash chromatography was performed on Silica Gel 60 (particle size 0.063–0.200 mm, Merck). The enzymes NIT-101–NIT-112 were purchased from a commercial supplier.[15] The enzymes used were delivered with the following specifications: NIT-101 (9 U/mg solid), NIT-102 (17 U/mg solid); NIT-103 (2.4 U/mg solid); NIT-104 (3.1 U/mg solid); NIT-105 (5.8 U/mg solid); NIT-106 (65 U/mg solid); NIT-107 (2.8 U/mg solid); NIT-108 (12 U/mg solid); NIT-109 (19 U/mg solid); NIT-110 (19 U/mg solid); NIT-111 (2.2 U/mg solid); and NIT-112 (11 U/mg solid).

4.1.2. Physical measurements

^{1}H and ^{13}C-NMR spectra were acquired on a Bruker BioSpin GmbH spectrometer 400 MHz, at room temperature. Chemical shifts δ are given in ppm and coupling constants J are measured in hertz. Coupling patterns are described by abbreviations: s (singlet), d (doublet), t (triplet), dd (doublet of a doublet), m (multiplet). GC analyses and EI-mass spectra were performed with an Agilent 6890N instrument equipped with a 15 m × 0.25 mm HP-5MS column and an Agilent 5973 N MS detector-column temperature gradient 50–300 °C for compounds **5c-Cbz**, **5a-Dpp**, **5e-Dpp**, and **7(a–c)-Cbz**; gradient 60–300 °C for compound **6a-Boc**; gradient 80–300 °C for compounds **3e**, **3f**, **4e-Cbz**, **4a-Ns**, **5(d-f)-Cbz**, **5d-Dpp**, **6c-Cbz**, **6f-Cbz**, **6b-Boc**, **6b-Ts**, **7(e–f)-Cbz**, **7c-Boc**, and **7c-Ts**, gradient 130–300 °C for compounds **4(b–c)-Ns,** and gradient 160–300 °C for compounds **6a-Ts** and **6c-Ts**. Infrared spectra were measured as KBr discs with a Nicolet FT-IR Avatar 320 spectrometer. High resolution mass spectra were performed with a Thermo Scientific LTQ Orbitrap mass spectrometer. The mass spectra were taken using electrospray (ESI) in the positive-ion mode. The *m/z* resulting from fragmentation processes were indicated, and sometimes assigned; the corresponding ionic abundances were reported in percentage relative to the more abundance. Specific rotations were determined on a Perkin Elmer 241. Melting points were determined on a Leica VMHB system Kofler apparatus. The HPLC analyses were performed using different columns. The chemical purity was determined using either a normal phase column Hypersil Si60 or Lichrosorb Si 60 or Krom Si 250, AME-OC 110205 (250 mm × 4.6 mm, 5 μm stationary phase) or with a reverse phase column (Hypersil ODS-C18, 5 μm stationary phase, 150 mm × 4.6 mm). The HPLC analyses for enantiomeric purity were performed with a chiral normal phase (Column AS chiralpak, 250 mm × 4.6 mm, 10 μm stationary phase). The HPLC analyses for the study were carried out with a reverse phase Column (YMC ODS AQ 2185, 250 mm, 5 μm) in an isocratic system of elution using a Photodiode Array Detector (PDA) Waters 996 (220–350 nm) or using a Waters Micromass ZQ detector (ESI/APCI source). The retention times t_R are expressed in minutes in the decimal system.

4.2. General procedure for the synthesis of chiral 2-substituted aziridines 3 (Mitsunobu reaction)

The amino alcohol was dissolved in toluene (1 mL per mmol) and added dropwise to a solution of triphenylphosphine (1 equiv) and DEAD (1 equiv) in toluene at 0 °C. After completion, the reaction mixture was poured into water and diluted with diethyl ether. The layers were separated, and the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was diluted with diethyl ether and kept in a freezer overnight. Precipitated triphenylphosphine oxide was filtered off. The precipitation step was repeated and the combined filtrates were concentrated in vacuo and purified by flash column chromatography on silica gel to afford the corresponding aziridines **3**. Yields: (*S*)-**3a**, 25%; (*S*)-**3b**, 35%; (*S*)-**3c**, 60%; (*S*)-**3d**, 53%; (*S*)-**3e**, 43%; (*S*)-**3f**, 30%.

4.2.1. (*S*)-2-(4-*tert*-Butoxybenzyl) aziridine 3e

Amino alcohol **2e** (1.13 g, 5.07 mmol) led to the expected aziridine **3e** after heating at reflux for 18 h according to the general procedure previously described. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 96:4) to afford the aziridine **3e** as a yellow powder. Yield: 440 mg (43%); R_f = 0.12 (CH_2Cl_2/MeOH 96:4); GC: t_R = 6.52; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.26 (s, 9H), 1.37 (d, 1H, J = 3.5 Hz), 1.74 (d, 1H, J = 6 Hz), 2.10–2.17 (m, 1H), 2.56 (dd, 1H, J = 14.6 Hz and J = 5.5 Hz), 2.68 (dd, 1H, J = 14.6 Hz and J = 6 Hz), 6.85 (d, 2H, J = 8.5 Hz), 7.07 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.8, 28.8, 30.9, 39.3, 78.2, 124.5, 129.1, 133.8, 153.7; *m/z* (EI): 205 (2), 149 (26), 107 (100), 91 (9), 77 (8), 65 (5); IR (KBr): ν 3030, 2975, 1505, 1233, 1159 1063 cm^{-1}; HRMS: calcd for $C_{13}H_{19}NO$ $[M+H]^+$ (206.15394); found (206.15384).

4.2.2. (*S*)-2-(4-(Benzyloxy)benzyl)aziridine 3f

Amino alcohol **2f** (1.31 g, 5 mmol) led to the expected aziridine **3f** after heating at reflux for 22 h according to the general procedure previously described. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 96:4) to afford the aziridine **3e** as a yellow oil. Yield: 365 mg (30%); R_f = 0.16 (CH_2Cl_2/MeOH 96:4); GC: t_R = 9.23; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.06 (s, 1H, N–H), 1.44 (d, 1H, J = 3.5 Hz), 1.80 (d, 1H, J = 6 Hz), 2.16–2.21 (m, 1H), 2.62 (dd, 1H, J = 14.6 Hz and J = 6.0 Hz), 2.75 (dd, 1H, J = 14.6 Hz and J = 6 Hz), 5.05 (s, 2H), 6.93 (d, 2H, J = 8.6 Hz), 7.18 (d, 2H, J = 9 Hz), 7.30–7.46 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.7, 31.1, 39.1, 70.1, 115.1, 127.5, 128.0, 128.6, 129.8, 131.3, 137.2, 157.5 ppm; *m/z* (EI): 239 (9), 197 (18), 91 (100), 77 (2), 65 (8); IR (KBr): ν 3031, 2991, 2906, 1509, 1233, 1175, 1016 cm^{-1}; HRMS: calcd for $C_{16}H_{17}NO$ $[M+H]^+$ (240.13101); found (240.13111).

4.3. General procedure for the synthesis of chiral 2-substituted *N*-Cbz aziridines 5-Cbz

Benzyl cyanoformate (1 equiv) was added to a solution of the appropriate 2-substituted aziridine **3** in acetonitrile (2 mL). The reaction mixture was stirred at room temperature and controlled by GC-MS. After 5 min of stirring, the solvent was removed in vacuo to give the crude **5-Cbz** which was used directly in the next step.

4.3.1. (*S*)-Benzyl-2-benzylaziridine-1-carboxylate 5d-Cbz

Aziridine **3d** (0.28 g, 2.1 mmol) led to the expected *N*-Cbz-aziridine **5d** according to the general procedure previously described. R_f = 0.16 (CH_2Cl_2/MeOH 96:4); GC: t_R = 9.10; *m/z* (EI): 267 (1); 176 (9), 132 (11), 91 (100), 77 (15); 65 (8).

4.3.2. (*S*)-Benzyl-2-(4-*tert*-butoxybenzyl)aziridine-1-carboxylate 5e-Cbz

The aziridine **3e** (0.29 g, 1.41 mmol) led to the expected *N*-Cbz-aziridine **5e** according to the general procedure previously described. R_f = 0.3 (CH_2Cl_2/MeOH 96:4); GC: t_R = 10.75; *m/z* (EI): 339 (M), 192 (48), 148 (14), 131 (45), 107 (22), 91 (100), 77 (8), 65 (7).

4.3.3. (*S*)-Benzyl 2-(4-(benzyloxy)benzyl)aziridine-1-carboxylate 5f-Cbz

Aziridine **3f** (0.27 g, 1.1 mmol) led to the expected *N*-Cbz-aziridine **5f** according to the general procedure previously described. R_f = 0.52 (CH_2Cl_2/MeOH 96:4); GC: t_R = 11.69; *m/z* (EI): 283 (5); 207 (3), 197 (16), 107(4), 91 (100), 77 (2), 65 (6).

4.4. General procedure for the synthesis of chiral 2-substituted *N*-Dpp aziridines 5-Dpp

To a solution of the corresponding amino alcohols **2** with Et_3N (3 equiv) in THF (3 mL per mmol) at 0 °C was added diphenylphosphinyl chloride (2 equiv). The resulting mixture was stirred for 20 h at 25 °C. Next, a suspension of NaH 60% in oil (5 equiv) was added and the solution was stirred for 20 h. Then 0.5 mL of water was added and the salts were filtered over Na_2SO_4. The crude residue was washed with EtOAc (3 × 25 mL) and filtered on silica gel. The organic layers were combined and concentrated to afford the corresponding *N*-Dpp aziridines **5-Dpp**. Yields: (*S*)-**5a-Dpp**, 55%; (*S*)-**5c-Dpp**, 76%; (S)-**5d-Dpp**, 67%; (S)-**5e-Dpp**, 74%.

4.4.1. (*S*)-2-(4-*tert*-Butoxybenzyl)-1-(diphenylphosphoryl)aziridine 5e-Dpp

The *O*-*t*Bu-tyrosinol **2e** (1.0 g, 4.5 mmol) led to the expected *N*-Dpp-aziridine **5e-Dpp** according to the general procedure previously described. Yield: 1.1 g (74%); mp 77–78 °C; $[\alpha]_D^{20} = -6.5$ (*c* 5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.29 (s, 9H), 1.99 (dd, 1H, J = 12.5 Hz and J = 3.5 Hz), 2.56 (dd, 1H, J = 12.5 Hz and J = 5.5 Hz); 2.77–2.81 (m, 2H), 2.95–3.00 (m, 2H), 6.80–6.79 (d, 2H, J = 8.6 Hz), 6.93–6.98 (d, 2H, J = 8.6 Hz), 7.33–7.55 (m, 6H), 7.89–7.95 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.9, 29.1 (d, J = 6.4 Hz), 36.2 (d, J = 6.39 Hz), 37.0 (d, J = 4.8 Hz), 78.3, 124.2, 129.1, 128.3, 128.4, 128.5, 131.5, 131.6, 131.7, 131.7, 131.8, 131.8, 132.1 (d, J = 22.1 Hz), 133.4 (d, J = 22.1 Hz), 132.5, 153.8 ppm; *m/z* (ESI+): 406 (M+H) (100); IR (KBr): ν 2963, 2930, 1152 (P=O) cm^{-1}; HRMS: calcd for $C_{25}H_{28}NO_2P$ $[M+H]^+$ (406.18577); found (406. 18586).

4.5. General procedure for the synthesis of chiral *N*-Cbz amino alcohols 4

To a solution of the corresponding amino alcohols **2** in CH_2Cl_2 (3.3 mL per mmol) at 0 °C was added dropwise benzyl chloroformate (1.2 equiv), followed by Et_3N (5 equiv). The resulting mixture was stirred at room temperature (monitoring by GC and TLC). After concentration, the crude residue was purified by flash column chromatography on silica gel to afford the corresponding *N*-Cbz amino alcohols **4-Cbz**. Yields: (*S*)-**4a-Cbz**, 60%; (*S*)-**4b-Cbz**, 82%, (*S*)-**4c-Cbz**, 55%; (S)-**4d-Cbz**, 92%; (S)-**4e-Cbz**, 50%, (S)-**4f-Cbz**, 94%.

4.5.1. (*S*)-Benzyl 1-(4-*tert*-butoxyphenyl)-3-hydroxypropan-2-ylcarbamate 4e-Cbz

The *O*-*t*Bu-tyrosinol **2e** (5.36 g, 15.03 mmol) led to the expected *N*-Cbz amino alcohol **4e-Cbz** after heating at reflux for 3 h according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 96:4) to afford **4e-Cbz** as a pale white solid. Yield: 2.7 g (50%); mp 121–123 °C; R_f = 0.32 (CH_2Cl_2/EtOAc 96:4); GC:

t_R = 13.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.27 (s, 9H), 2.79 (d, 2H, J = 4 Hz), 3.54 (m, 1H), 3.64 (m, 1H), 3.85–3.95 (m, 1H), 5.09 (s, 1H), 6.90 (d, 2H, J = 8 Hz), 7.05 (d, 2H, J = 8.1 Hz), 7.30–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.9, 36.7, 54.2, 63.9, 66.8, 78.4, 124.2, 128.0, 129.7, 136.4, 140.1, 154.0; *m/z* (EI): 357(M), 194 (23), 150 (29), 107(90), 91(100); *m/z* (ESI+): 380.1 [M+23] (100); IR (KBr): ν 3416, 3338 (N–H), 2929, 1695 (C=O), 1507, 1233, 1156 cm^{-1}; HRMS: calcd for $C_{21}H_{27}NO_4$ $[M+H]^+$ (358.194009); found (358.194209).

4.5.2. (S)-Benzyl 1-(4-(benzyloxy)phenyl)-3-hydroxypropan-2-ylcarbamate 4f-Cbz

The *O*-Bn-tyrosinol **2f** (0.7, 2.72 mmol) led to the expected *N*-Cbz amino alcohol **4e-Cbz** according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 96:4) to afford **4f-Cbz** as a white powder. Yield: 1 g (94%); R_f = 0.33 (CH_2Cl_2/MeOH 96:4); GC: t_R = 11.69; $[\alpha]_D^{24} = -13.3$ (*c* 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.52 (s, 1H, OH), 2.80 (d, 2H, J = 7 Hz), 3.56 (d, 1H, J = 9.5 Hz), 3.65 (d, 1H, J = 9.5 Hz), 3.91 (m, 1H), 5.04 (s, 2H), 5.08 (s, 2H), 6.90 (d, 2H, J = 8.5 Hz), 7.11 (d, 2H, J = 8 Hz), 7.31–7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 37.1, 54.0, 54.9, 64.5, 67.4, 115.6, 128.1, 128.5, 128.6, 128.7, 129.1, 129.2, 130.9, 137.0, 137.7, 157.1, 158.2; *m/z* (EI): 281 (5); 207 (33), 107 (6), 91 (100), 77 (2); 65 (6); IR (KBr): ν 3464, 3311, 3056, 3031, 2949, 2921, 1687, 1541, 1156, 1008 cm^{-1}; HRMS: calcd for $C_{24}H_{25}$ NO_4 $[M+H]^+$ (392.17835); found (392.17846).

4.6. General procedure for the synthesis of chiral *N*-$_4$Ns amino alcohols 4

A solution of *p*-nitrobenzenesulfonyl chloride (1.1 equiv) in THF (1.9 mL per mmol) was added dropwise to a solution of the corresponding amino alcohol and $NaHCO_3$ (4 equiv) in dry THF (1.5 mL per mmol) at 0 °C. The resulting mixture was stirred at room temperature and monitored by GC and TLC. The solution was concentrated and the yellow solid obtained was taken up in EtOAc/H_2O (7:3 v/v, 100 mL). The layers were separated, and the aqueous phase was back-extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by flash column chromatography on silica gel to afford the corresponding *N*-$_4$Ns amino alcohols **4(a–c)-$_4$Ns**. Yields: (*S*)-**4a-$_4$Ns**, 95%; (*S*)-**4b-$_4$Ns**, 70%, (*S*)-**4c-$_4$Ns**, 78%.

4.6.1. (*S*)-*N*-(1-Hydroxypropan-2-yl)-4-nitrobenzenesulfonamide 4a-$_4$Ns

Alaninol **2a** (843 mg, 11.22 mmol) led to the expected *N*-$_4$Ns amino alcohol **4a-$_4$Ns** after heating at reflux for 5 h according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc 3:7) to afford **4a-$_4$Ns** as a pale white solid. Yield: 2.78 g (95%); mp: 121–123 °C; R_f = 0.31 (cyclohexane/EtOAc 3:7); $[\alpha]_D^{20} = -8.4$ (*c* 1.0, MeOH); GC: t_R = 9.32; ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.20 (d, 3H, J = 6.4 Hz), 3.48–3.50 (m, 3H), 8.15 (m, 2H), 8.44 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 14.5, 52.8, 66.8, 125.3, 129.4, 149.4, 151.3; *m/z* (EI): 229 (100), 186 (36), 122 (38); IR (KBr): ν 3441, 3386 (N–H), 2941, 2877, 1542, 1351 ($\nu_{as}SO_2$), 1161 (ν_sSO_2) cm^{-1}; HRMS: calcd for $C_9H_{12}N_2O_5SNa$ $[M+Na]^+$ (283.03591); found (283.03587).

4.6.2. (*S*)-*N*-(1-Hydroxy-3-methylbutan-2-yl)-4-nitrobenzenesulfonamide 4b-$_4$Ns

The valinol **2b** (1.16 g, 11.22 mmol) led to the expected *N*-$_4$Ns amino alcohol **4b-$_4$Ns** after heating at reflux for 7 h according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc 1:1) to afford **4b-$_4$Ns** as a white solid. Yield: 2.27 g (70%); mp: 120–122 °C; R_f = 0.27 (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = +17.6$ (*c* 1.0, MeOH); GC: t_R = 10.6; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.82 (d, 3H, J = 6.8 Hz), 0.83 (d, 3H, J = 6.9 Hz), 1.77–1.90 (m, 1H), 3.11–3.19 (m, 1H), 3.58 (dd, 1H, J = 4.1 Hz and J = 11.3 Hz); 3.63 (dd, 1H, J = 5.4 Hz and J_{B-A} = 11.2 Hz), 8.08 (m, 2H), 8.36 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 18.7, 19.3, 29.8, 61.6, 62.9, 124.5, 128.5, 146.9, 150.1; *m/z* (EI): 257(100), 245 (19), 186 (14), 122 (25); IR (KBr): ν 3534, 3166 (N–H), 2959, 2926, 1524, 1355 ($\nu_{as}SO_2$), 1166 (ν_sSO_2) cm^{-1}; HRMS: calcd for $C_{11}H_{16}$ N_2O_5SNa $[M+Na]^+$ (311.06721); found (311.06728).

4.6.3. *N*-(2*S*)-1-Hydroxy-3-methylpentan-2-yl-4-nitrobenzenesulfonamide 4c-$_4$Ns

The isoleucinol **3c** (1.32 g, 11.22 mmol) led to the expected *N*-$_4$Ns amino alcohol **4c-$_4$Ns** after heating at reflux for 5 h according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc 1:1) to afford **4c-$_4$Ns** as a white powder. Yield: 2.71 mg (78%); mp: 125–127 °C; R_f = 0.25 (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = +9.7$ (*c* 1.0, MeOH); GC: t_R = 11.1; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.81 (m, 6H), 0.96–1.07 (m, 1H), 1.37–1.47 (m, 1H), 1.52–1.59 (m, 1H), 1.82 (s_b, 1H, OH), 3.20–3.26 (m, 1H), 3.58 (dd, 1H, J = 3.9 Hz and J = 11.2 Hz), 3.63 (dd, 1H, J = 5.6 Hz and J_{B-A} = 11.2 Hz), 5.12 (d, 1H, J = 8 Hz, NH), 8.09 (m, 2H); 8.36 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.6, 15.3, 25.5, 36.7, 60.3, 62.4, 124.4, 128.5, 146.9, 150.1. *m/z* (EI): 271 (100), 245 (58), 215 (65), 186 (40), 122 (38), 76 (22); *m/z* (ESI+): 325.1 [M+23] (100); IR (KBr): ν 3441, 3151 (N–H), 2961, 2915, 1530, 1350 ($\nu_{as}SO_2$), 1159 (ν_sSO_2) cm^{-1}; HRMS: calcd for $C_{12}H_{18}$ N_2O_5SNa $[M+Na]^+$ (325.08286); found (325.08279).

4.7. General procedure for the synthesis of N-protected amino alcohol methanesulfonates 6

To a stirred solution of the corresponding *N*-protected amino alcohols **4** and Et_3N (3 equiv) in THF (4 mL per mmol) at 0 °C was added dropwise methanesulfonyl chloride (1.1 equiv for **4a–e-Cbz** and **4a–b-Boc** or 2.2 equiv for **4a–c-$_4$Ns** and **4a-c-Ts**). The reaction mixture was stirred at room temperature and monitored by GC and TLC. The salts were filtered, the solution was concentrated, and the mixture was taken up in EtOAc/H_2O (2:1, v/v, 15 mL per mmol). The layers were separated and the aqueous phase was back-extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the corresponding N-protected amino alcohol methanesulfonates **6**, which were used in the next step without any further purification. Yields: (*S*)-**6a-Boc**, 74%; (*S*)-**6b-Boc**, 95%; (*S*)-**6c-Boc**, 99%; (*S*)-**6a-Cbz** 94%, (*S*)-**6b-Cbz** 77%; (*S*)-**6c-Cbz**, 98%; (*S*)-**6d-Cbz**, 70%; (*S*)-**6e-Cbz**, 94%; (*S*)-**6f-Cbz**, 88%; (*S*)-**6a-$_4$Ns**, 92%; (*S*)-**6b-$_4$Ns**, 94%; (*S*)-**6c-$_4$Ns**, 75%; (*S*)-**6a-Ts**, 99%; **(*S*)-6b-Ts**, 99%; (*S*)-**6c-Ts**, 95%.

4.7.1. (*S*)-2-(*tert*-Butoxycarbonylamino)propyl methanesulfonate 6a-Boc

The (*S*)-*tert*-butyl-1-hydroxypropan-2-ylcarbamate **4a-Boc** (0.86 g, 4.89 mmol) led to the expected compound **6a-Boc** after 4 h of stirring according to the general procedure previously described. Yellow powder, yield: 1.15 g (74%); R_f = 0.1 (cyclohexane/EtOAc 7:3); GC: t_R = 10.79; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.24 (d, 3H, J = 6.9 Hz), 1.46 (s, 9H), 3.05 (s, 1H), 3.94–4.05 (m, 1H), 4.16 (dd, 1H, J = 4.3 Hz and J = 10 Hz), 4.2 (dd, 1H, J = 3.3 Hz and J = 10 Hz, 4.6 (s_b, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 17.6, 29.2, 28.9, 38.0, 72.7, 80.2, 155.5; *m/z* (EI): 180 (4), 144

(37), 57 (100); IR (KBr): ν 3359 (N–H), 2975, 2937, 1687 (C=O), 1335 ($\nu_{as}SO_2$), 1161 (ν_sSO_2), 1003 cm^{-1}.

4.7.2. (*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylbutyl methanesulfonate 6b-Boc

The (*S*)-*tert*-butyl-1-hydroxy-3-methylbutan-2-ylcarbamate **4b-Boc** (1.0 g, 4.92 mmol) led to the expected compound **6b-Boc** after 1 h of stirring according to the general procedure previously described. Yellow powder, yield: 1.32 g (95%); R_f = 0.43 (cyclohexane/EtOAc 7:3); GC: t_R = 6.94; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.05 (d, 1H, J = 6.9 Hz), 1.10 (d, 1H, J = 6.8 Hz), 1.42 (s, 9H), 2.08–2.17 (m, 1H), 3.20 (s, 1H), 4.38–4.49 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.1, 29.7, 32.6, 37.4, 56.1, 66.6, 79.5, 160.3; *m/z* (EI): 281 (M), 172 (10), 57 (100); IR (KBr): ν 3395 (N–H), 2966, 2876, 1687 (C=O), 1356 ($\nu_{as}SO_2$), 1174 (ν_sSO_2) cm^{-1}.

4.7.3. (2*S*)-2-(*tert*-Butoxycarbonylamino)-3-methylpentyl meth- anesulfonate 6c-Boc

The *tert*-butyl-(2*S*)-1-hydroxy-3-methylpentan-2-ylcarbamate **4c-Boc** (0.9 g, 4.14 mmol) led to the expected compound **6c-Boc** after 5.5 h of stirring according to the general procedure previously described. Yellow powder, yield: 1.21 g (99%); R_f = 0.67 (cyclohexane/EtOAc 7:3); GC: t_R = 7.44; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.86–0.98 (m, 8H), 1.09–1.25 (m, 2H), 1.43 (s, 9H), 3.02 (s, 3H), 3.67–3.71 (m, 1H), 4.15–4.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.1, 15.6, 25.2, 28.2, 35.5, 37.4, 53.8, 69.7, 155.4; *m/z* (EI): 238 (2), 186 (5), 138 (43), 86 (49), 57 (100); IR (KBr): ν 3339 (N–H), 2965, 2875, 1677 (C=O), 1291 ($\nu_{as}SO_2$), 1174 (ν_sSO_2) cm^{-1}.

4.7.4. (2*S*)-2-(Benzyloxycarbonylamino)-3-methylpentyl methanesulfonate 6c-Cbz

The benzyl (2*S*)-1-hydroxy-3-methylpentan-2-ylcarbamate **4c-Cbz** (1 g, 3.9 mmol) led to the expected compound **6c-Cbz** after 1.5 h of stirring according to the general procedure previously described. Yellow oil, yield: 1.17 g (89%); R_f = 0.94 (CH_2Cl_2/MeOH 97:3); GC: t_R = 7.03; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.96 (d, 3H, J = 7.0 Hz), 0.98 (d, 3H, J = 7.1 Hz, 1.84–1.93 (m, 1H), 2.95 (s, 3H), 3.68–3.74 (m, 1H), 4.28 (s, 2H), 4.84 (s_b, 2H, NH), 5.08–5.15 (m, 2H), 7.28–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.1, 15.4, 25.2, 35.5, 37.3, 54.4, 66.9, 69.4, 128.8, 136.34, 156.1; *m/z* (EI): 233 (3), 176 (17), 91 (100), 77 (1); IR (KBr): ν 3327 (N–H), 3064, 3031, 2965, 2935, 2878, 1716 (C=O), 1355 ($\nu_{as}SO_2$), 1175 (ν_sSO_2) cm^{-1}.

4.7.5. (*S*)-2-(Ethoxycarbonylamino)-3-phenylpropyl methanesulfonate 6d-Cbz

(*S*)-Benzyl-1-hydroxy-3-phenylpropan-2-ylcarbamate **4d-Cbz** (0.84 g, 2.95 mmol) led to the expected compound **6d-Cbz** after 30 min of stirring according to the general procedure previously described. Yellow powder, yield: 750 mg (70%); R_f = 0.67 (CH_2Cl_2/MeOH 96:4); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.79 (d, 2H, J = 7.0 Hz), 2.87 (s, 3H), 4.01–4.15 (m, 2H), 4.16–4.25 (m, 1H), 4.85–5.95 (s_b, 1H), 7.05–7.35 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 37.1, 37.3, 51.4, 66.8, 69.5, 126.7, 128.1, 128.2, 128.6, 128.7, 129.4, 136.5, 137.8, 156.6; IR (KBr): ν 3347 (N–H), 3084, 3060, 2946, 1693 (C=O), 1536, 1349 ($\nu_{as}SO_2$), 1275 (ν_sSO_2), 1186, 1067 cm^{-1}.

4.7.6. (*S*)-3-(4-*tert*-Butoxyphenyl)-2-(ethoxycarbonylamino)-propyl methanesulfonate 6e-Cbz

(*S*)-Ethyl-1-(4-*tert*-butoxyphenyl)-3-hydroxypropan-2-ylcarbamate **4e-Cbz** (600 mg, 1.68 mmol) led to the expected compound **6e-Cbz** after 10 min of stirring according to the general procedure previously described. Yellow powder, yield: 638 mg (93%); R_f = 0.5 (cyclohexane/EtOAc 4:6); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.26 (s, 9H); 2.88 (s, 2H), 3.13 (s, 3H), 4.04–4.08 (m, 2H), 4.15–4.25 (m, 1H), 5.01 (s, 1H), 6.85 (d, 2H, J = 8.5 Hz), 7.02 (d, 2H, J = 8.5 Hz), 7.24–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.8, 31.6, 38.4, 46.2, 51.5, 71.6, 124.4, 128.2, 128.6, 129.7, 131.1, 136.3, 154.4, 155.7.

4.7.7. (*S*)-3-(4-(Benzyloxy)phenyl)-2-(ethoxycarbonylamino)propyl methanesulfonate 6f-Cbz

(*S*)-Benzyl-1-(4-(benzyloxy)phenyl)-3-hydroxypropan-2-ylcarbamate **4f-Cbz** (0.38 g, 0.97 mmol) led to the expected compound **6f-Cbz** after 2.5 h of stirring according to the general procedure previously described. White powder, yield: 0.4 g (88%); R_f = 0.75 (CH_2Cl_2/MeOH 96/4); GC: t_R = 11.68; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.83 (dd, 1H, J = 14.6 Hz and J = 7.5 Hz), 2.88 (dd, 1H, J = 14.6 Hz and J = 4.5 Hz), 2.95 (s, 3H), 4.12 (dd, 1H, J = 15.1 Hz and J = 7 Hz), 4.15 (dd, 1H, J = 15.1 Hz and J = 3.5 Hz), 4.24–4.26 (m, 1H), 4.92 (s_b, 1H, N–H), 5.04 (2H, s), 5.09 (s, 2H), 6.91 (d, 2H, J = 8.5 Hz), 7.10 (d, 2H, J = 8.6 Hz), 7.31–7.44 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 37.6, 52.1, 60.7, 67.3, 69.8, 70.4, 115.6, 127.8, 128.3, 128.5, 128.6, 128.9, 128,9, 130.6, 136.8, 137.3, 156.0, 158.3; *m/z* (EI): 283 (5), 197 (20), 107 (10), 91 (100), 77 (2); 65 (11); IR (KBr): ν 3384 (N–H), 3031, 2958, 2929, 2872, 1696 (C=O), 1344 ($\nu_{as}SO_2$), 1180 (ν_sSO_2) cm^{-1}.

4.7.8. (*S*)-2-(4-Nitrophenylsulfonamido)propyl methanesulfonate 6a-$_4$Ns

(*S*)-*N*-(1-Hydroxy propan-2-yl)-4-nitrobenzenesulfonamide **4a-$_4$Ns** (1.0 g, 3.84 mmol) led to the expected compound **6a-$_4$Ns** after 4.5 h of stirring according to the general procedure previously described. Yellow powder, yield: 1.2 g (92%); R_f = 0.7, Al_2O_3, (cyclohexane/EtOAc 1:1); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.97 (d, 3H, J = 6.7 Hz), 3.11 (s, 3H), 3.52–3.62 (m, 1H), 4.00 (dd, 1H, J = 5.9 Hz and J = 10.2 Hz), 4.04 (dd, 1H, J = 4.9 Hz and J = 10.3 Hz), 8.05–8.08 (m, 2H), 8.41 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 17.2, 36.6, 48.4, 72.2, 124.6, 127.9, 147.1, 149.5 ppm; *m/z* (ESI+): 361.1, M+23 (100), IR (KBr): ν 3062, 2083, 2894, 1360 ($\nu_{as}SO_2$), 1169 (ν_sSO_2) cm^{-1}.

4.7.9. (*S*)-3-Methyl-2-(4-nitrophenylsulfonamido)butyl methanesulfonate 6b-$_4$Ns

(*S*)-*N*-(1-Hydroxy-3-methylbutan-2-yl)-4-nitrobenzenesulfonamide **4b-$_4$Ns** (1.0 g, 3.47 mmol) led to the expected compound **6b-$_4$Ns** after 2.5 h of stirring according to the general procedure previously described. Yellow powder, yield: 1.2 g (94%); R_f = 0.78, Al_2O_3, (cyclohexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (d, 3H, J = 6.8 Hz), 0.88 (d, 3H, J = 6.8 Hz), 1.83–1.92 (m, 1H), 2.98 (s, 3H), 3.39 (m, 1H), 4.15 (dd, 1H, J = 10.7 Hz and J = 4.2 Hz); 4.18 (dd, 1H, J = 5.1 Hz and J = 10.6 Hz), 8.08 (m, 2H), 8.35 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 18.4, 19.1, 29.7, 37.4, 58.8, 69.2, 124.5, 128.4, 146.6, 150.2; *m/z* (ESI+): 361.1, M+23 (100).

4.7.10. (2*S*)-3-Methyl-2-(4-nitrophenylsulfonamido)pentyl methanesulfonate 6c-$_4$Ns

(*N*-((2*S*)-1-Hydroxy-3-methylpentan-2-yl)-4-nitrobenzenesulfonamide **4c-$_4$Ns** (570 mg, 1.89 mmol) led to the expected compound **6c-$_4$Ns** after 5 h of stirring according to the general procedure previously described. Yellow oil, yield: 541 mg (75%); R_f = 0.4, SiO_2, (CH_2Cl_2/MeOH 97/3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.81 (t, 3H, J = 7.3 Hz), 0.86 (d, 3H, J = 6.9 Hz), 0.98–1.08 (m, 1H), 1.36–1.48 (m, 1H), 1.58–1.68 (m, 1H), 2.97 (s, 3H), 3.40–3.47 (m, 1H), 4.09–4.20 (m, 2H), 8.08 (m, 2H), 8.35 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.2, 15.1, 25.2, 36.5, 37.4, 57.5, 68.9, 124.5, 128.4, 146.6, 150.2; IR (KBr): ν 3293 (N–H), 2968, 2937, 1530, 1351 ($\nu_{as}SO_2$), 1169 (ν_sSO_2) cm^{-1}.

4.7.11. (*S*)-2-(4-Methylphenylsulfonamido)propyl methanesulfonate 6a-Ts

(*S*)-*N*-(1-Hydroxy propan-2-yl)-4-methylbenzenesulfonamide **4a-Ts** (1.5 g, 6.55 mmol) led to the expected compound **6a-Ts** after

30 min of stirring according to the general procedure previously described. Yellow oil, yield: 2.01 g (100%); R_f = 0.5, SiO_2, (cyclohexane/EtOAc 4/6); GC: t_R = 7.40; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.1 (d, 3H, J = 6.9 Hz), 2.41 (s, 3H), 2.99 (s, 3H), 3.54–3.65 (m, 1H), 4.1(d, 2H, J = 4.6 Hz), 5.31 (d, 1H, NH), 7.27–7.32 (m, 2H), 7.73–7.78 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 20.4, 21.6, 26.3, 46.2, 116.8, 127.0, 129.9, 137.9, 144.0; m/z (EI): 307 [M] (1), 198 (100), 155 (71), 91 (69) 56(33); IR (KBr): ν 3290 (N–H), 2975, 2942, 1350 ($\nu_{as}SO_2$), 1168 (ν_sSO_2) cm^{-1}.

4.7.12. (*S*)-3-Methyl-2-(4-methylphenylsulfonamido)butyl methanesulfonate 6b-Ts

(*S*)-*N*-(1-Hydroxy-3-methylbutan-2-yl)-4-methylbenzenesulfonamide **4b-Ts** (1.5 g, 5.83 mmol) led to the expected compound **6b-Ts** after 30 min of stirring according to the general procedure previously described. Pale white powder, yield: 1.96 g (100%); R_f = 0.35, SiO_2, (cyclohexane/EtOAc 40/60); GC: t_R = 7.44; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.80 (d, 3H, J = 5.3 Hz), 0.82 (d, 3H, J = 5.4 Hz), 1.79–1.90 (m, 1H), 2.41 (s, 3H), 2.95 (s, 3H), 3.17–3.27 (m, 1H), 4.10 (dd, 1H, J = 4.6 Hz and J = 10.4 Hz), 4.18 (dd, 1H, J = 4.3 Hz and J = 10.4 Hz), 5.17 (d, 1H, NH), 7.29–7.31 (m, 2H), 7.75–7.77 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 18.2, 19.1, 21.6, 29.3, 37.2, 58.0, 69.1, 137.9, 127.2, 129.8, 137.6, 143.8; m/z (EI): 226 (1), 155 (10), 91 (32), 84 (100), 65 (12), 55 (23); IR (KBr): ν 3430 (N–H), 3254, 2964, 2876, 1353 ($\nu_{as}SO_2$), 1178 (ν_sSO_2) cm^{-1}.

4.7.13. (2*S*)-3-Methyl-2-(4-methylphenylsulfonamido)pentyl methanesulfonate 6c-Ts

N-((2*S*)-1-Hydroxy-3-methylpentan-2-yl)-4-methylbenzenesulfonamide **4c-Ts** (1.5 g, 5.53 mmol) led to the expected compound **6c-Ts** after 30 min of stirring according to the general procedure previously described. Pale white powder, yield: 1.83 g (95%); R_f = 0.48, SiO_2, (cyclohexane/EtOAc 4/6); GC: t_R = 8.91; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.78 (t, 3H; J = 7.4 Hz), 0.82 (d, 3H, J = 6.9 Hz), 0.95–1.05 (m, 1H), 1.38–1.50 (m, 1H), 1.55–1.65 (m, 1H), 2.42 (s, 3H), 2.94 (s, 3H), 3.27–3.37 (m, 1H), 4.13 (dd, 1H, J = 7.0 Hz, J = 10.4 Hz), 4.16 (dd, 1H, J = 5.7 Hz and J_{B-A} = 10.1 Hz), 5.07 (d, 1H, NH), 7.28–7.33 (m, 2H), 7.74–7.76 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.1, 15.1, 21.7, 24.97, 36.17, 37.3, 56.8, 68.8, 127.2, 129.9, 137.6, 143.8; m/z (EI): 292 (62), 240 (64), 155 (100), 91 (95) 65(20); IR (KBr): ν 3430 (N–H), 3276, 2970, 2876, 1359, 1172 ($\nu_{as}SO_2$), 1156 (ν_sSO_2) cm^{-1}.

4.8. General procedure for the synthesis of N-protected amino nitriles 7

Caution: Because of the high toxicity of sodium cyanide, all of the processes should be carried out taking into account the safety measures.

Method A: To a stirred solution of the corresponding N-protected aziridines **5** in a mixture of CH_3CN/H_2O 9:1 (3 mL per mmol) was added NaCN (1, 1.5, 2 or 3 equiv). The mixture was stirred at 80 °C. Upon completion of the reaction, the mixture was taken up in EtOAc/H_2O (1.5:1 v/v, 20 mL per mmol). The layers were separated, and the aqueous phase was back-extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash column chromatography on silica gel. Yields: (*S*)-**7a-Cbz**, 55%; (*S*)-**7b-Cbz**, 60%; (*S*)-**7c-Cbz**, 66%; (*S*)-**7d-Cbz**, 81%; (*S*)-**7e-Cbz**, 89%; (*S*)-**7f**-Cbz, 73%.

Method B: To a stirred solution of the corresponding N-protected amino alcohol methanesulfonates **6** in DMF (1.4 mL per mmol) was added NaCN (1 equiv for **7a**–**b-Cbz** and **7e**–**f-Cbz** or 1.5 equiv for **7c-Boc**, **7a-Ns**, and **7c-Ns**). The mixture was stirred at 70 °C and monitored by GC and TLC. The mixture was taken up in EtOAc/H_2O (4:1 v/v, 35 mL per mmol). The layers were separated, and the aqueous phase was back-extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash column chromatography on silica gel. Yields: (*S*)-**7a-Cbz**, 81%; (*S*)-**7b-Cbz**, 85%; (*S*)-**7c-Cbz**, 87%; (*S*)-**7d-Cbz**, 88%; (*S*)-**7e-Cbz**, 30%; (*S*)-**7f**-Cbz, 89%; (*S*)-**7a**-Boc, 66%, (*S*)-**7b**-Boc, 43%, (*S*)-**7c**-Boc, 47%; (*S*)-**7a-$_4$Ns**, 22%; (*S*)-**7b-$_4$Ns**, 81%; (*S*)-**7c-$_4$Ns**, 40%, (*S*)-**6a-Ts**, 73%; (*S*)-**6b-Ts**, 92%; (*S*)-**6c-Ts**, 94%.

4.8.1. (*R*)-Benzyl 1-cyano-3-methylbutan-2-yl carbamate 7b-Cbz

Method A: To a stirred solution of the (*S*)-benzyl 2-isopropylaziridine-1-carboxylate **5b-Cbz** (1.4 g, 6.4 mmol) in CH_3CN/H_2O 9:1 (35 mL) was added NaCN (0.43 g, 12.8 mmol). The expected compound **7b-Cbz** is obtained after 21 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

Method B: (*S*)-2-(Benzyloxycarbonylamino)-3-methylbutyl methanesulfonate **6b-Cbz** (0.9 g, 2.85 mmol) led to the expected compound **7b-Cbz** after 4 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

7b-Cbz was obtained as a yellow oil. Yield: 0.93 g (60%) method A, 0.6 g (85%) method B; R_f = 0.68 (CH_2Cl_2/MeOH 97:3); GC: t_R = 10.52; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.98 (d, 3H, J = 3.5 Hz), 1.00 (d, 3H, J = 3.5 Hz), 1.91 (m, 1H), 2.60 (dd, 1H, J = 12 Hz and J = 4.5 Hz), 2.70 (dd, 1H, J = 12 Hz and J = 5 Hz), 3.65–3.71(m, 1H), 4.82–4.92 (s_b, 1H, NH), 5.12 (s, 2H), 7.32–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 18.4, 19.4, 21.8, 31.0, 53.3, 60.4, 67.2, 128.4, 136.004, 155.8; *m/z* (EI): 246 (M), 207 (1), 162 (2), 108 (46), 91 (100), 79 (8); IR (KBr): ν 3326 (N–H), 3034, 2964, 2929, 2876, 2239 (C≡N) 1704, (C=O) cm^{-1}; HRMS: calcd for $C_{14}H_{18}N_2O_2Na$ [M+Na]$^+$ (269.12605); found (269.12585); HPLC purity: method A: 85%; method B: 90% on a Krom Si 250 column, *n*-heptane/dioxane 3:1, 1.0 mL min^{-1}, λ = 256 nm, t_R = 7,78 min.

4.8.2. Benzyl (2*R*)-1-cyano-3-methylpentan-2-ylcarbamate 7c-Cbz

Method A: To a stirred solution of the (*S*)-benzyl 2-*sec*-butylaziridine-1-carboxylate **5c-Cbz** (2.08 g, 14.5 mmol) in CH_3CN/H_2O 9:1 (43.5 mL) was added NaCN (0.87 g, 21.75 mmol). The expected compound **7c-Cbz** was obtained after 16 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

Method B: (2*S*)-2-(Benzyloxycarbonylamino)-3-methylpentyl methanesulfonate **6c-Cbz** (1.17 g, 3.54 mmol) led to the expected compound **7c-Cbz** after 4 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

7c-Cbz was obtained as a yellow powder. Yield: 2.5 g (66%) method A, 0.80 g (87%) method B; R_f = 0.32 (CH_2Cl_2/MeOH 97:3); $[\alpha]_D^{20} = -43.6$ (*c* 1.0, MeOH); GC: t_R = 11.01; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.92 (t, 3H, J = 7.5 Hz), 0.97 (d, 3H, J = 7 Hz), 1.14–1.23 (m, 1H), 1.50–1.74 (m, 2H), 2.60 (dd, 1H, J = 17 Hz and J = 5 Hz), 2.71 (dd, 1H, J = 17 Hz and J = 5 Hz), 3.71–3.78 (m, 1H), 4.91 (d, 1H, NH), 5.11 (s, 2H), 7.30–7.39 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): 10.1, 15.3, 21.7, 26.9, 30.9, 36.2, 44.5, 50.9, 66.1, 127.6–127.8, 135.0, 154.7; *m/z* (EI): 260 (M), 245 (1), 220 (1), 176 (2), 108 (42), 91 (100); IR (KBr): ν 3350 (N–H), 2930, 2885, 2240 (C≡N) 1706, (C=O) cm^{-1}; HRMS: calcd for $C_{15}H_{20}N_2O_2Na$ [M+Na]$^+$ (283.14170); found (283.14141); HPLC purity: method A = 77%; method B = 80% on a Krom Si 250 column, *n*-heptane/dioxane 3:1, 1.0 mL min^{-1}, λ = 256 nm, t_R = 7.08 min.

4.8.3. (*S*)-Benzyl 1-(4-*tert*-butoxyphenyl)-3-cyanopropan-2-ylcarbamate 7e-Cbz

Method A: To a stirred solution of the (*S*)-benzyl 2-(4-*tert*-butoxybenzyl)aziridine-1-carboxylate **5e-Cbz** (290 mg, 1.4 mmol) in CH_3CN/H_2O 9:1 (4.42 mL) was added NaCN (0.21 g, 4.23 mmol). The expected compound **7e-Cbz** was obtained after 18 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

Method B: (*S*)-3-(4-*tert*-Butoxyphenyl)-2-(ethoxycarbonylamino)propyl methanesulfonate **6e-Cbz** (683 mg, 1.48 mmol) led to the expected compound **7e-Cbz** after 2 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

7e-Cbz was obtained as a white solid. Yield: 455 mg (89%) method A, 60 mg (30%) method B; mp: 82–83 °C; R_f = 0.69 (CH_2Cl_2/MeOH 96/4); $[\alpha]_D^{21} = -3.1$ (*c* 1.0, EtOH); GC: t_R = 11.74; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.34 (s, 9H), 2.41 (dd, 1H, *J* = 16.6 Hz and *J* = 4 Hz), 2.67 (dd, 1H, *J* = 16.6 Hz and *J* = 4.5 Hz), 2.82 (dd, 1H, *J* = 13.6 Hz and *J* = 8.0 Hz), 2.93 (dd, 1H, *J* = 13.6 Hz and *J* = 6.8 Hz), 4.04–4.14 (m, 1H), 5.08 (s, 2H), 5.27 (d, 1H, *J* = 9.5 Hz, N–H), 6.94 (d, 2H, *J* = 8.5 Hz), 7.08 (d, 2H, *J* = 8.6 Hz), 7.28–7.38 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 22.5, 28.9, 38.6, 49.2, 67.0, 78.6, 117.3, 124.5, 128.1, 128.3, 128.6, 129.6, 130.7, 136.1, 154.7, 155.6; *m/z* (EI): 366 (M), 310 (20), 219 (25), 175 (23), 159 (45), 107 (100); 91 (90); 77 (15); IR (KBr): *v* 3351 (N–H), 3041, 2980, 2927, 2250 (C≡N), 1693 (C=O), 1526, 1526, 1256, 158 cm^{-1}; HRMS: calcd for $C_{22}H_{26}N_2O_3Na$ $[M+Na]^+$ (389.18356); found (389.18345); HPLC purity: 96.7% on a Lichrosorb Si 60 column, 250 mm 4.6 mm, 5 μm, Heptane/EtOAc 6:4, 1 mL min^{-1}, Detector PDA, t_R = 5.79 min, λ = 267 nm.

4.8.4. (*S*)-Benzyl 1-(4-(benzyloxy)phenyl)-3-cyanopropan-2-ylcarbamate 7f-Cbz

Method A: To a stirred solution of the (*S*)-benzyl-2-(4-(benzyloxy)benzyl)aziridine-1-carboxylate **5f-Cbz** (265 mg, 1.11 mmol) in CH_3CN/H_2O 9:1 (3.33 mL) was added NaCN (0.16 g, 3.33 mmol). The expected compound **7f-Cbz** was obtained after 18.5 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

Method B: (*S*)-3-(4-(Benzyloxy)phenyl)-2-(ethoxycarbonylamino)propyl methanesulfonate **6f-Cbz** (0.39 g, 0.83 mmol) led to the expected compound **7f-Cbz** after 1.5 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 7:3).

7f-Cbz was obtained as a white solid. Yield: 197 mg (73%) method A, 281 mg (89%) method B; mp = 117–118 °C; R_f = 0.82 (CH_2Cl_2/MeOH 96:4); $[\alpha]_D^{20} = -6.4$ (c 1.0, MeOH); GC: t_R = 10.44; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.46 (dd, 1H, *J* = 17 Hz and *J* = 4 Hz), 2.71 (dd, 1H, *J* = 16.6 Hz and *J* = 4.5 Hz), 2.84 (dd, 1H, *J* = 14 Hz and *J* = 8 Hz), 2.95 (dd, 1H, *J* = 14 Hz and *J* = 6.5 Hz), 4.04–4.15(m, 1H), 4.97 (d, 1H, *J* = 7 Hz, N–H), 5.05 (s, 2H), 5.10 (s, 2H), 6.94 (d, 2H, *J* = 8.5 Hz), 7.12 (d, 2H, *J* = 8 Hz), 7.30–7.45 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 22.5, 38.5, 49.2, 67.1, 70.1, 115.4, 117.1, 127.5, 128.0, 128.1, 128.3, 128.6, 128.6, 130.1, 136.0, 136.9, 155.5, 158.1; *m/z* (EI): 292 (10), 207 (1), 197 (2), 107 (3), 91 (100), 65(4); IR (KBr): *v* 3368 (N–H), 3062, 2928, 2243 (C≡N), 1697 (C=O), 1243, 1057 cm^{-1}; HRMS: calcd for $C_{25}H_{24}N_2O_3Na$ $[M+Na]^+$ (423.16791); found (423.16752); HPLC purity: 97.6% on a Lichrosorb Si 60 column, 250 mm 4.6 mm, 5 μm, Heptane/AcOEt 6:4, 1 mL min^{-1}, Detector PDA, t_R = 6.50 min, λ = 276 nm.

4.8.5. *tert*-Butyl (2*R*)-1-cyano-3-methylpentan-2-ylcarbamate 7c-Boc

(2*S*)-2-(*tert*-Butoxy carbonylamino)-3-methylpentyl methanesulfonate **6c-Boc** (1.0 g, 3.40 mmol) led to the expected compound **7c-Boc** after 5.5 h of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc 8:2) to give **7c-Boc** as a white solid. Yield: 362 mg (47%); mp: 103–104 °C; R_f = 0.53 (cyclohexane/EtOAc 8:2); $[\alpha]_D^{20} = -4.5$ (*c* 1.0, MeOH), GC: t_R = 5.52; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.81 (t, 3H, *J* = 7.4 Hz), 0.95 (d, 3H, *J* = 6.7 Hz), 1.05–1.3 (m, 2H), 1.43 (s, 9H), 2.54 (dd, 1H, *J* = 16.9 Hz and *J* = 4.8 Hz); 2.68 (dd, 1H, *J* = 5.0 Hz and *J* = 16.9 Hz), 3.62–3.69 (m, 1H), 4.69 (d_b, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.1, 15.6, 21.7, 25.3; 28.4, 37.4, 51.5, 80.2, 117.7, 155.3; *m/z* (EI): 211(2), 186 (5), 169 (7), 86 (14), 69 (46), 57 (100); IR (KBr): *v* 3367 (N–H), 2962, 2937, 2246 (C≡N), 1682 (C=O) cm^{-1}; HRMS: calcd for $C_{12}H_{22}N_2O_2Na$ $[M+Na]^+$ (249.15735); found (249.15720); HPLC purity: 95% on a YMC ODS AQ 2185 column, 250, 5 μm, CH_3CN/H_2O 3:1, 0.8 mL min^{-1}, Detector: PDA, λ = 210 nm t_R = 6.82 min.

4.8.6. *N*-((2*R*)-1-Cyano-3-methylpentan-2-yl)-4-nitrobenzenesulfonamide 7c-$_4$Ns

(2*S*)-3-Methyl-2-(4-nitrophenylsulfonamido)pentyl methanesulfonate **6c-$_4$Ns** (571 mg, 1.5 mmol) led to the expected compound **7c-$_4$Ns** after 3 days of stirring according to the general procedure previously described. The crude residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc 5:5) to give **7c-$_4$Ns** as a yellow oil. Yield: 190 mg (40%); R_f = 0.47 (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = -46.9$ (*c* 1.0, $CHCl_3$); GC: t_R = 10.43; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.80 (t, 3H, *J* = 7.4 Hz), 0.86 (d, 3H, *J* = 6.8 Hz), 0.97 (m, 1H), 1.4 (m, 1H), 1.67 (m, 1H), 2.59 (dd, 1H, *J* = 17.1 Hz and *J* = 5.18 Hz), 2.67 (dd, 1H, *J* = 5.4 Hz and *J* = 17.1 Hz), 3.42 (m, 1H), 8.09 (m, 2H), 8.38 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.0, 15.04, 22.5, 24.9, 38.0, 55.1, 117.0, 124.7, 128.4, 146.2, 150.3; *m/z* (EI): 271 (16), 254 (100), 186 (85), 122 (52), 76 (21); IR (KBr): *v* 3284 (N–H), 2968, 2932, 2251 (C≡N), 1530 ($v_{as}NO_2$arom), 1350 ($v_{as}SO_2$), 1167 (v_sSO_2) cm^{-1}; HRMS: calcd for $C_{13}H_{17}N_3O_4SNa$ $[M+Na]^+$ (334.08320); found (334.08306); HPLC purity: 99%; on a YMC ODS AQ 2185 column, 250, 5 μm, CH_3CN/H_2O 6:4, 0.7 mL min^{-1}, HCOOH 0.1%, Detector: PDA λ = 220 nm, t_R = 6.63 min.

4.9. General procedure for the synthesis of *N*-Dpp amino nitriles 7

To a stirred solution of the corresponding *N*-Dpp aziridines **5a-Dpp**, **5c-Dpp**, **5d-Dpp**, and **5e-Dpp** in toluene (5 mL per mmol) were added TBABr (0.1 equiv), NaCN (2 equiv), and water (1 mL per mmol). The reaction mixture was stirred at 85 °C and monitored by TLC. After cooling the mixture to room temperature, it was taken up in EtOAc/H_2O (6:1 v/v, 35 mL per mmol). The layers were separated and the organic layer was washed with water (5 mL per mmol), and then dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the corresponding *N*-Dpp amino nitriles **7 Dpp**. Yield: (*S*)-**7a-Dpp**, 86%; (*S*)-**7c-Dpp**, 74%, (*S*)-**7d-Dpp**, 75% (*S*)-**7e-Dpp**, 74%.

4.9.1. (S)-*N*-(1-Cyanopropan-2-yl)-*P*,*P*-diphenylphosphinic amide 7a-Dpp

(*S*)-1-(Diphenyl phosphoryl)-2-methylaziridine **5a-Dpp** (250 mg, 0.97 mmol) led to the expected compound **7a-Dpp** after 18 h of stirring according to the general procedure previously described. White solid. Yield: 238 mg (86%); mp: 178–180 °C, R_f = 0.32 (EtOAc); $[\alpha]_D^{23} = +28.9$ (*c* 3.6, CH_2Cl_2); GC: tr = 11.52; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.8 (d, 3H, *J* = 6.5 Hz), 2.55 (dd, 1H, *J* = 4.0 Hz and

J = 16.6 Hz), 2.70 (dd, 1H, J = 5.8 Hz and J = 16.6 Hz), 3.45–3.58 (m,1H), 7.35–7.56 (m, 6H), 7.79–7.93 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 22.8 (d, J = 6,4 Hz), 28.0 (d, J = 3.9 Hz), 44.1, 17.6, 128.6, 128.7, 128.7, 128.8, 131.8, 131.9, 132.1, 132.2; m/z (EI): 284 [M] (100), 269 (1), 244 (94), 201 (100), 77 (22); IR (KBr): ν 3146 (N–H), 2968, 2255 (C≡N), 1125 (P=O) cm^{-1}; HRMS: calcd for $C_{16}H_{17}N_2P$ [M+H]$^+$ (285.11570); found (285.1150).

4.9.2. *N*-((2*R*)-1-Cyano-3-methylpentan-2-yl)-P,P-diphenylphosphinic amide 7c-Dpp

(2*S*)-2-*sec*-Butyl-1-(diphenylphosphoryl)aziridine **5c-Dpp** (500 mg, 1.7 mmol) led to the expected compound **7c-Dpp** after 18 h of stirring according to the general procedure previously described as a white solid. Yield: 410 mg (74%); mp: 141.5–142.0 °C, R_f = 0.33 (EtOAc); $[\alpha]_D^{20} = -2.8$ (c 2.8, CH_2Cl_2); GC: tr = 12.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (t, 3H, J = 7.3 Hz), 0.92 (d, 3H, J = 6.8 Hz), 1.09–1.20 (m, H), 1.56–1.66 (m, 1H), 1.73–1.79 (m, 1H), 2.60 (dd, 1H, J = 4.3 Hz and J = 15.8 Hz), 2.75 (dd, 1H, J = 5.1 and J = 16.8 Hz), 3.14–3.25 (m, 2H), 7.42–7.52 (m, 6H), 7.87–7.92 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.3, 15.1, 23.4 (d, J = 3.2 Hz), 25.2, 39.4 (d, J = 5.6 Hz), 52.1(d, J = 1.6 Hz), 118.0, 128.6, 128.7, 128.7, 128.8, 131. 9, 131.9, 132.2, 132.2, 132.3, 131.3 (d, J = 30.4 Hz), 132.6 (d, J = 28.8 Hz); m/z (EI): 326 [M] (1), 286 (16), 269 (63), 201 (100), 77 (13); IR (KBr): ν 3140 (N–H), 2952, 2243 (C≡N), 1189 (P=O) cm^{-1}; HRMS: calcd for $C_{19}H_{23}N_2OP$ [M+H]$^+$ (327.15480); found (327.15420).

4.9.3. (S)-*N*-(1-(4-*tert*-Butoxyphenyl)-3-cyanopropan-2-yl)-*P*,*P*-diphenylphosphinic amide 7e-Dpp

(*S*)-2-(4-*tert*-Butoxybenzyl)-1-(diphenylphosphoryl)aziridine **5e-Dpp** (700 mg, 1.7 mmol) led to the expected compound **7e-Dpp** after 18 h of stirring according to the general procedure previously described as a white solid. The crude residue was purified by flash column chromatography on silica gel. Yield: 550 mg (74%); mp: 155–157 °C, $[\alpha]_D^{20} = -27.8$ (c 5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.34 (s, 9H), 2.55 (dd, 1H, J = 17.1 Hz and J = 4.0 Hz); 2.6 (dd, 1H, J = 17.1 Hz and J = 5.52 Hz); 2.99–3.00 (2H, m), 3.29–3.33 (m, 1H), 3.46–3.60 (m, 2H), 6.94–6.96 (d, 2H, J = 8.5 Hz), 7.04–7.06 (d, 2H, J = 8.5 Hz), 7.34–7.51 (m, 6H), 7.59–7.78 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.8, 25.7 (d, J = 2.4 Hz), 41.5 (d, J = 6.4 Hz), 49.7, 78.6, 117.6, 124.4, 129.9, 128.6, 128.64, 128.7, 131.5, 131.7, 131.8, 131.7, 131.8, 132.1, 132.2, 132.2, 132.3, 132.3, 131.2, 132.8 (d, J = 88 Hz), 131.3, 153.8; IR (KBr): ν 3140 (N–H), 2952, 2930, 2237 (C≡N), 1195 (P=O) cm^{-1}; HRMS: calcd for $C_{26}H_{30}O_2N_2P$ [M+H]$^+$ (433.20501); found (433.20540).

4.10. Screening

For screening experiments, the substrate (0.1 mmol) was dissolved in a phosphate buffer (125 μL, 50 mM potassium phosphate, 2 mM DTT, 1 mM EDTA, pH 7.5) and in case of low solubility of the substrate, MeOH or DMSO was used as a cosolvent. The enzyme (0.5 mg) was dissolved in a phosphate buffer (125 μL) and added to the substrate to afford a final concentration of 0.2 M. The reaction mixture was stirred with a magnetic bar and the temperature was adjusted at 30 °C. After 18 h, acetone (500 μL) was added. The reaction vessels were centrifuged for 15 min at room temperature at 4000 rpm to remove the precipitated proteins. The 300 μL of supernatant were concentrated and then analyzed by RP-18 HPLC using acetonitrile/water/formic acid (60:40: 0.1) using an isocratic mode.

4.11. Preparative scale biotransformations

Approximately 100 mg of substrate was dissolved in a phosphate buffer (50 mM potassium phosphate, 2 mM DTT, 1 mM EDTA, pH 7.5) in a round-bottomed flask. The commercial enzyme preparation was added as a solution in phosphate buffer to substrate to afford a final concentration of 0.2 M. Absolute amounts are added for the respective compounds listed below. The reaction mixture was stirred with a magnetic bar and the temperature was adjusted to 30–32 °C by use of an oil bath. The conversion was monitored by HPLC. After completion, the mixture was acidified by the addition of HCl and the protein was removed by $(NH_4)_2SO_4$ precipitation and filtration through a plug of Celite. The products were purified by silica gel chromatography (CH_2Cl_2/CH_3OH 95:5).

4.11.1. (*S*)-3-(Benzyloxycarbonylamino)butanoic acid 9a-Cbz

This compound was obtained as white solid mp: 107–110 °C, $[\alpha]_D^{24} = -21.5$ (c 0.4, $CHCl_3$) (lit.[28] $[\alpha]_D^{24} = -24.1$ (c 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): conform to data reported in the literature;[30] IR (KBr): ν 3432, 3311, 2952, 1686 (C=O) cm^{-1}; HRMS: calcd for $C_{12}H_{15}O_4N$ [M+Na]$^+$ (260.2500); found (260.01; HPLC purity: 98.6%; on a YMC ODS AQ 2185 column, 250, 5 μm, CH_3CN/H_2O 6:4, 1 mL min^{-1}, HCOOH 0.1%, Detector: PDA λ = 220 nm, t_R = 3.76 min.

4.11.2. (*S*)-3-(Benzyloxycarbonylamino)-4-methylpentanoic acid 9b-Cbz

This compound was obtained as white solid mp: 83–85 °C, $[\alpha]_D^{23} = -33.0$ (c 0.2, $CHCl_3$) (lit.[28] $[\alpha]_D^{23} = -33.6$ (c 0.2, $CHCl_3$) ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): conform to data reported in the literature.[31] IR (KBr): ν 3435, 2913, 2848, 1695 (C=O) cm^{-1}; HRMS: calcd for $C_{14}H_{19}O_4N$ [M+Na]$^+$ (288.300); found (288.3001); HPLC purity: 95%; on a YMC ODS AQ 2185 column, 250, 5 μm, CH_3CN/H_2O 6:4, 1 mL min^{-1}, HCOOH 0.1%, Detector: PDA λ = 220 nm, t_R = 4.71 min.

4.11.3. (*S*)-Benzyl 1-amino-4-methyl-1-oxopentan-3-ylcarbamate 8b-Cbz

This compound was not isolated, ESI-MS: calcd for $C_{14}H_{20}O_3N_2$ [M+Na]$^+$ (287.32); found (287.32); HPLC purity: 97%; on a YMC ODS AQ 2185 column, 250, 5 μm, CH_3CN/H_2O 6:4, 1 mL min^{-1}, HCOOH 0.1%, Detector: PDA λ = 220 nm, t_R = 3.72 min.

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