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A nitrilase-mediated entry to 4-carboxymethyl-β-lactams from chemically prepared 4-(cyanomethyl) azetidin-2-ones†

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(3*R*,4*S*)-3-Alkoxy/aryloxy-4-(cyanomethyl)azetidin-2-ones were efficiently prepared from readily available 1,2:5,6-di-*O*-isopropylidene-D-mannitol by means of a classical organic synthesis approach *via* 4-hydroxymethyl-β-lactams as key intermediates. The corresponding 4-carboxymethyl-β-lactams were subsequently obtained after selective hydrolysis of the nitrile functionality by means of a nitrilase enzyme without affecting the sensitive four-membered ring system, hence overcoming the difficulties associated with the chemical hydrolysis approach. Thus, the implementation of a biocatalytic step allows a convenient synthetic route to new 4-carboxymethyl-β-lactams as versatile building blocks for further elaboration.

Introduction

Since the accidental discovery of penicillin by A. Fleming in 1928,[1] β-lactam antibiotics belong to one of the most important classes of antimicrobial compounds.[2–5] Apart from their celebrated antibacterial activities and other medical applications, *e.g.* in the treatment of malaria, cancer and HIV,[6,7] β-lactams are also considered to be very interesting building blocks for further transformations due to their strained ring structure containing a reactive amide bond, a concept known as the 'β-lactam synthon method'.[8–11] In this way, these scaffolds can be deployed in the synthesis of a broad range of new nitrogen compounds with potential bioactivity, which has become a major incentive for the research and development of several new classes of β-lactams.[12–14]

One of those classes, the family of 4-(cyanomethyl)azetidin-2-ones, contains important synthons for the controlled synthesis of a variety of new compounds including functionalized γ-lactams, succinimide derivatives and diamino-β-lactams in optically pure form, all being promising compounds in terms of medicinal and synthetic applications.[15] Besides, the synthesis of multiple antibiotics, with the carbapenem antibiotic thienamycine as one of the most prominent examples, can be dedicated to the use of 4-(cyanomethyl)azetidin-2-one substrates.[16,17] Furthermore, functionalized polyalkoxypiperidines, or simply iminosugars, can be synthesized starting from 3-alkoxy-4-cyanomethyl-β-lactams.[18] These sugar analogues exhibit interesting biological properties, such as the selective inhibition of glycosidases and glycoprotein-processing enzymes, which makes them convenient for the treatment of diabetes, cancer and viral infections, including HIV.[19–22]

A second class of β-lactams attracting a lot of attention in both a chemical as well as a biological setting concerns the carboxyl-containing β-lactams, which are considered to be valuable building blocks for the preparation of antimicrobial agents and other biologically active compounds.[23–29] For example, 4-(1-carboxyethyl)-β-lactams, the synthesis of which has been performed by multiple methods to date,[30,31] are important intermediates in the preparation of carbapenem antibiotics.[32,33] 1-Benzyloxy-4-(2-carboxyethyl)azetidin-2-one on the other hand, has been reported to act as an intermediate in the synthesis of carbacephems, a class of broad-spectrum antibiotics, closely related to the cephalosporins.[34,35] Furthermore, optically pure (*R*)-1-benzyloxy-4-(2-carboxyethyl)azetidin-2-one can be used as a precursor for the synthesis of (1*S*,6*R*)-2-oxa-7-azabicyclo[4.2.0]octane-3,8-dione, a 3,4-fused bicyclic β-lactam, which has been employed as precursor in the synthetic route toward new β-lactam antibiotics or β-lactamase inhibitors.[36] Besides their use for further synthetic transformations, some carboxyl-containing azetidin-2-ones are also characterized by their intrinsic biological activities. This structural moiety can for example be found in macrocyclic β-lactam-fused enediynes.[37,38] Natural enediyne antibiotics are prodrugs, endowed with potent cytotoxic and anticancer activities, due to their capability of cleaving single- and double-stranded DNA.[37–39] Moreover, the di- and tri-organotin carboxylates

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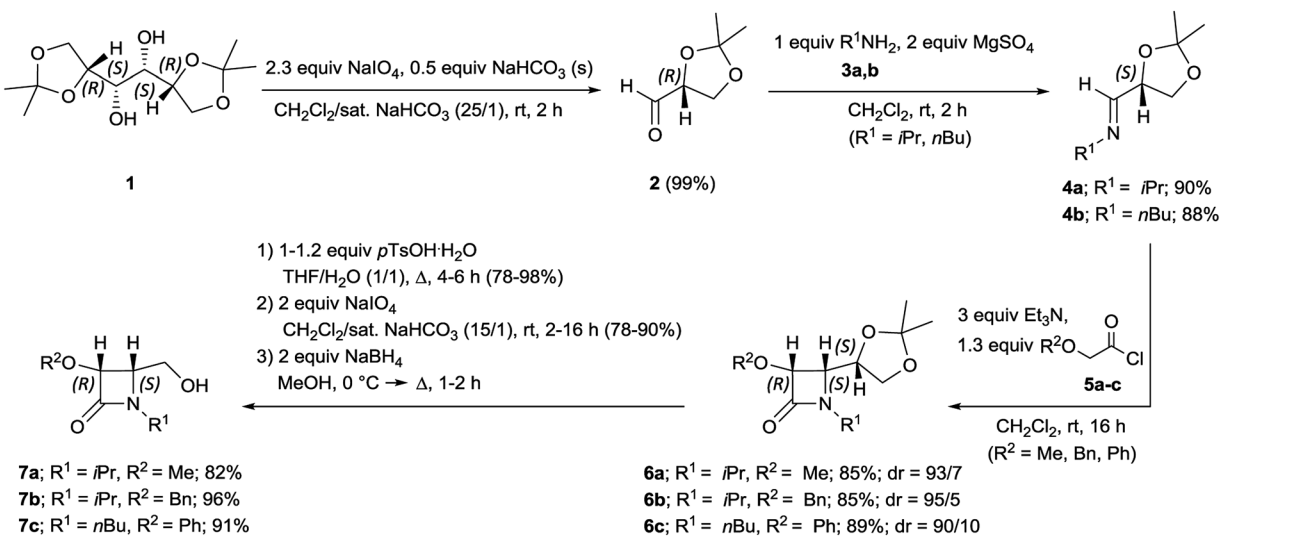
derived from silyl-protected 4-(1-carboxyethyl)-3-(1-hydroxyethyl) azetidin-2-one are known for their *in vitro* antitumor activity.[40]

In the framework of the biological relevance of both classes of compounds, as well as our synthetic interest in their straightforward development, optically pure 4-carboxymethyl-β-lactams are prepared *via* hydrolysis of the corresponding nitrile derivatives in this work. The latter are small scaffolds, consisting of a cyclic amide with a high ring tension in combination with a reactive cyano group, turning these compounds into attractive synthons from an organic synthesis point of view. The two main objectives of this work thus comprise (i) the design of an efficient synthetic route toward 4-cyanomethyl-β-lactams and (ii) their straightforward conversion into the corresponding 4-carboxymethyl-β-lactams without affecting the sensitive four-membered ring system.

Results and discussion

In a first section, the selective synthesis of *cis*-4-(cyanomethyl) azetidin-2-ones **11a–c** was performed. In that respect, (*E*)-*N*-[(2,2-dimethyl-1,3-dioxolan-4-yl)methylidene]amines **4a,b** were prepared in 88–90% yield *via* condensation of primary amines **3a,b** with (*R*)-glyceraldehyde acetonide **2**, obtained *via* periodate-induced oxidative cleavage of commercially available 1,2:5,6-di-*O*-isopropylidene-D-mannitol **1** in a quantitative way according to a literature procedure (Scheme 1).[41,42] The obtained imines **4a,b** were subsequently used as substrates in the Staudinger synthesis of (3*R*,4*S*)-3-alkoxy/aryloxy-4-(2,2-dimethyl-1,3-dioxolan-4-yl)-β-lactams **6a–c** by using 1.3 equiv. of methoxy–, benzyloxy– or phenoxyacetyl chloride **5a–c** in the presence of an excess of triethylamine as a base.[42–45] The optically pure β-lactams **6a–c** were prepared in 85–89% yield and with high diastereomeric ratios of (90/10)–(95/5), favoring the expected relative *cis*-configuration as confirmed by the ^{1}H NMR ($CDCl_3$) spectra. Indeed, the vicinal coupling constants between the protons on the 3- and 4-position of the β-lactam rings in compounds **6a–c** ranged between 5.0 and 5.5 Hz ($CDCl_3$), indicating a *cis*-relationship according to literature values.[44,46] Next, β-lactams **6a–c** were easily converted into the corresponding 4-hydroxymethyl-β-lactam key intermediates **7a–c** in 82–96% yield *via* a three-step approach, including (i) *p*-toluenesulphonic acid-mediated hydrolysis in a THF/water mixture (1/1) under reflux conditions for 4–6 hours,[42,44,45,47] (ii) subsequent oxidation using sodium periodate in the presence of sodium bicarbonate and stirring in dichloromethane for 2–16 hours at room temperature,[43–45,47] and (iii) selective reduction by means of two molar equiv. of sodium borohydride in methanol under reflux for 1–2 hours.[44,45]

In the following steps, modifications in the hydroxymethyl side chain attached to the 4-position of novel β-lactams **7a–c** were performed in order to install the nitrile functionality. At first, the hydroxyl group was converted into a better leaving group in order to create a more reactive compound, sensitive to nucleophilic substitution reactions (Scheme 2). Two options were thus explored. For the introduction of a mesyloxy group, β-lactams **7a,b** were treated with 1.5 equiv. of mesyl chloride and 4 equiv. of triethylamine, providing mesylated β-lactams **8a,b** in high yields.[48] In an analogous manner, stirring of 4-hydroxymethyl-β-lactams **7a–c** in the presence of tosyl chloride, triethylamine and DMAP under reflux conditions for nearly two days, afforded 4-tosyloxymethyl-β-lactams **9a–c** in 60–67% yield. The expected final step in this synthetic pathway consisted of the nucleophilic substitution of both sulfonyloxy leaving groups by a cyanide anion to give rise to 4-cyanomethyl-β-lactams **11a–c**. However, after a thorough optimization study, including variation of the number of equiv. of potassium cyanide, the solvent, the temperature (incl. microwave conditions), the reaction time…, no substitution of either the mesyloxy or the tosyloxy group by cyanide could be achieved. Therefore, inspired by similar cases concerning 4-(mesyloxymethyl)azetidin-2-ones described in the literature,[49–51] an alternative approach was studied consisting of the



Scheme 1

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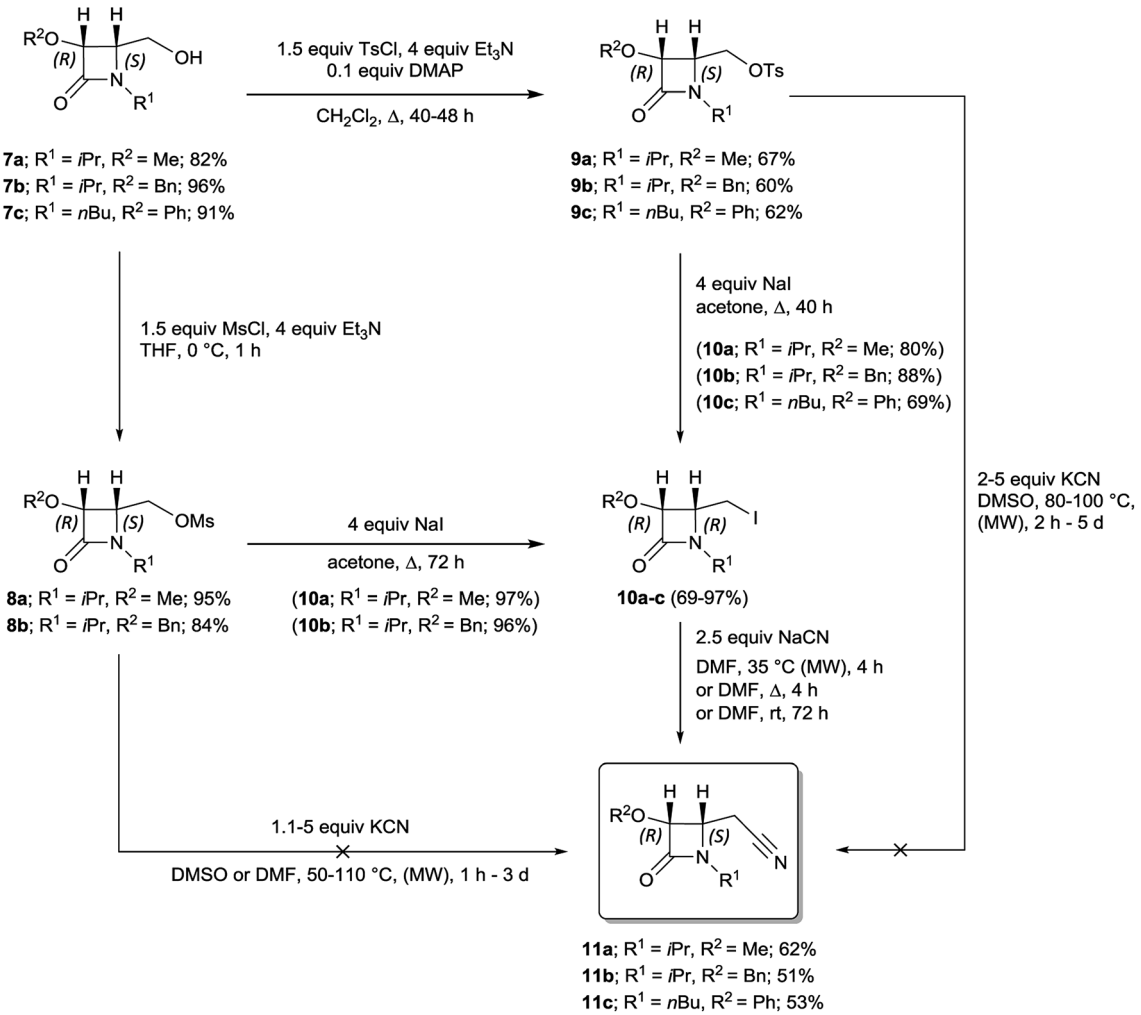

Scheme 2

preliminary conversion toward more reactive 4-iodomethyl-β-lactams **10a–c** as versatile intermediates,[52–54] as iodide is considered to be a very good leaving group combined with the expected decrease in steric hindrance during the substitution reactions as compared to sulfonyloxy leaving groups.[51] Both 4-mesyloxymethyl- and 4-tosyloxymethyl-β-lactams **8a,b** and **9a–c** were therefore converted into the corresponding iodides **10a–c** by means of an excess of sodium iodide in acetone under reflux (69–97%). Eventually, the premised new (3*R*,4*S*)-3-alkoxy/aryloxy-4-cyanomethyl-β-lactams **11a–c** could be obtained selectively in 51–62% yield after treatment with 2.5 equiv. of sodium cyanide in DMF.[51] It must be mentioned that the KCN-mediated direct displacement of 3-non-substituted 4-(tosyloxymethyl) azetidin-2-ones has been performed only once before, using 18-crown-6 as an additive, whereas sulfonyloxy displacements by cyanide in 3-substituted β-lactams have not been reported so far.[55]

In the next stage, the selective hydrolysis of the nitrile function within the synthesized (3*R*,4*S*)-3-alkoxy/aryloxy-4-cyanomethyl-β-lactams **11a–c** was studied. Whereas the hydrolysis of nitriles containing a β-lactam moiety toward the corresponding amides has been reported a few times, both in a chemical[49,56,57] and in an enzymatic way,[49,58] reports describing the conversion of the latter nitriles toward carboxylic acids are rather scarce. In one of these examples, 4-(cyanomethyl) azetidin-2-one was chemically converted to the corresponding methyl ester first, after which alkaline hydrolysis gave rise to the acid.[55] Similarly, a two-step chemical hydrolysis of silyl-protected 4-cyano-3-(1-hydroxyethyl)azetidin-2-one *via* the intermediate amide has been studied.[57] Otherwise, enzymatic hydrolysis of nitriles is typically performed *via* two different pathways, leading to either amides or carboxylic acids, depending on the application of nitrile hydratases or nitrilases (nitrilhydrolases), respectively.[59–62] If necessary, the amides formed by nitrile hydratases can be further hydrolyzed toward the acids using amidase enzymes.[59–62] Only two reports, however, describe the biocatalytic conversion of β-lactam-containing nitriles.[49,58] In these studies, catalyzed by *Rhodococcus erythropolis* AJ270 cells containing nitrile hydratases and amidases, the first set of enantiomers of racemic 3-non-substituted 4-cyano- and 4-cyanomethyl-β-lactams was converted toward the amides, while the others were fully hydrolyzed

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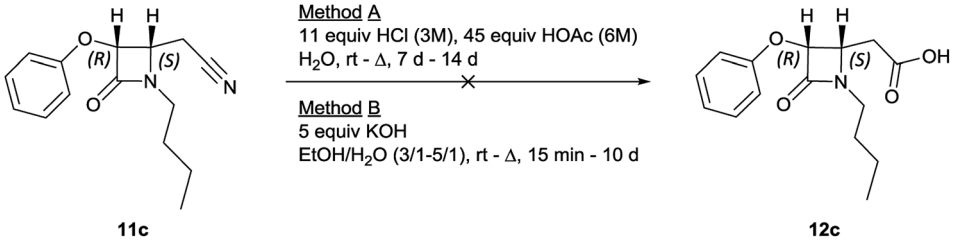
toward the carboxylic acids, which can be attributed to the high enantioselectivity exhibited by the amidases, in contrast to the lack of this stereocontrol in the case of nitrile hydratases.[63] In neither of these studies, however, the carboxylic acids were isolated. Instead, the corresponding methyl esters were formed by means of diazomethane immediately after the biocatalytic transformations. In other words, the selective and one-step hydrolysis of nitriles containing a β-lactam moiety toward the corresponding carboxylic acids, representing valuable building blocks in organic and medicinal chemistry, has not been described in the literature before and thus remains a challenge to be addressed.

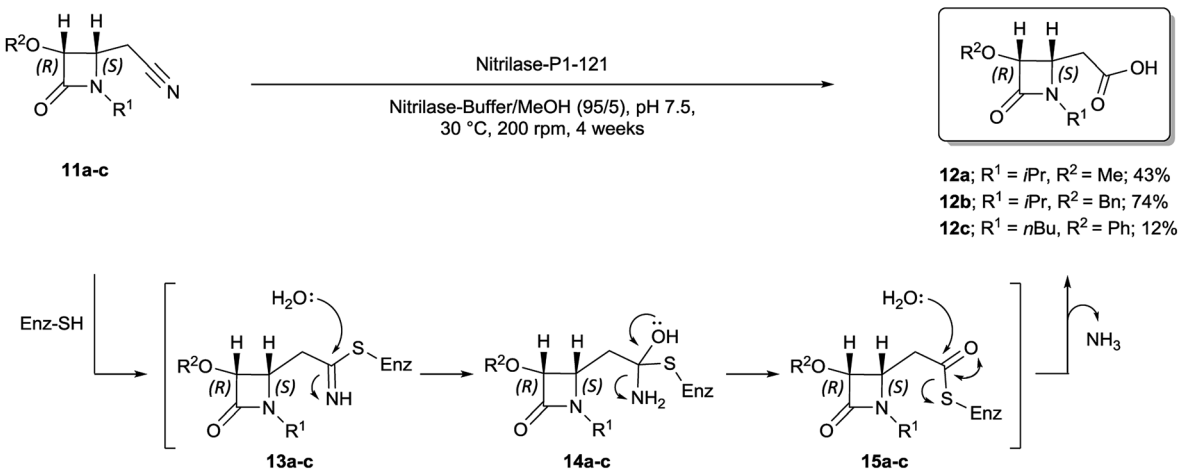
The chemical hydrolysis of 4-cyanomethyl-β-lactams **11a–c** was explored first (Scheme 3). To that end, (3*R*,4*S*)-1-butyl-4-cyanomethyl-3-phenoxyazetidin-2-one **11c** as a representative example was dissolved in water, after which HCl and HOAc were added (Method A). Unfortunately, no traces of the corresponding carboxylic acid **12c** could be observed after multiple attempts, as either no reaction occurred at low temperatures, or reflux conditions gave rise to complex mixtures resulting from decomposition of the reactants. Subsequently, KOH-induced alkaline hydrolysis was studied applying various reaction conditions (Method B), though leading to the same conclusions. These observations point to the precarious combination of a cyano group and a sensitive four-membered azaheterocycle, making a classical one-step acid- or base-catalyzed nitrile hydrolysis critical.

As the bottleneck of this reaction, *i.e.* the instability of the reactive four-membered ring under the above-mentioned conditions, required more mild – but still effective – procedures, we reverted to biocatalytic protocols for the hydrolysis of nitriles to carboxylic acids using isolated nitrilase enzymes as the hydrolyzing tools, as already applied successfully in previous work.[64,65] The repertoire of classical organic synthesis can indeed be significantly enriched by including biochemical methods, and the interface between organic chemistry and biocatalysis is offering new strategies and perspectives as valuable alternatives. This can be attributed to the enantio–, stereo– and regioselective, efficient and environmentally friendly catalysis applied by enzymes under mild reaction conditions, which fits perfectly in the framework of the integration of biocatalysis into the organic chemistry sector as an answer to the increasing demand for sustainable processes.[66,67]

To that end, 4-oxoazetidin-2-yl nitriles **11a–c** were dissolved in a mixture of methanol and a K_3PO_4–dithiothreitol–ethylenediaminetetra-acetic acid-based buffer (5/95), after which various nitrilases were added. The resulting reaction mixtures were incubated at 30 °C and 200 rpm (Scheme 4). After an extensive cluster screening, investigating 30 different nitrilases[68] at a small reaction scale using various solvents and enzyme and substrate concentrations, nitrilase-P1-121 seemed to be the only enzyme catalyzing the conversion of 4-cyanomethyl-β-lactams **11a–c** quantitatively toward novel (3*R*,4*S*)-3-alkoxy/aryloxy-4-carboxymethyl-β-lactams **12a–c**, as confirmed



Scheme 3



Scheme 4

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Table 1 Conversion, yields and purification efficiencies of the hydrolysis of 4-cyanomethyl-β-lactams **11a–c** toward 4-carboxymethyl-β-lactams **12a–c**

Compound	R^1	R^2	Conversion after 4 weeks of incubation[a] (%)	Yield Method A[b] (%)	Purification efficiency (%)	Yield Method B[c] (%)	Purification efficiency (%)
11a	iPr	Me	60	16	27	43	72
11b	iPr	Bn	93	—	—	74	80
11c	*n*Bu	Ph	70	12	17	—	—

[a] Conversion rate determined *via* LC-MS analysis. [b] Method A: removal of inactivated enzymes and buffer salts, followed by preparative HPLC of the crude reaction mixture. [c] Method B: removal of inactivated enzymes and buffer salts, followed by NaCl-mediated extraction, acidification of the aqueous phase and another extraction with ethyl acetate.

by ^{1}H NMR, IR and MS analysis. However, these reactions proceeded very slowly, probably due to the rigid structure of nitriles **11** which might impede an optimal positioning into the catalytic center of the enzyme. For this reason, relatively high enzyme concentrations (up till 4 mg mL^{-1}) were used when scaling up these reactions, with the nitrilase added gradually to the reaction mixture during the course of the weeks. The latter approach yielded reasonably constant reaction rates, leading to varying conversions of the substrates (60% for **11a**, 93% for **11b** and 70% for **11c** after 4 weeks, as determined *via* LC-MS analysis). It should however be mentioned that, when performing all reactions at a higher enzyme concentration and a longer incubation time, complete conversion of the substrate should be achievable, as was observed at a smaller reaction scale. In our case, however, we opted to make a compromise between acceptable substrate conversions and a time-efficient reaction protocol. Since no side products were formed during the hydrolysis, the conversion seemed completely selective toward the carboxylic acids **12a–c**. Finally, after inactivation and removal of the enzymes and filtration of the buffer salts, the isolation of the target compounds was performed using preparative HPLC (Method A). However, 4-carboxymethyl-β-lactams **12a** and **12c** could only be obtained in 12 and 16% yield respectively, implying a purification efficiency of only 17–27% taking the incomplete conversion rate into account (Table 1). Therefore, an alternative procedure was investigated (Method B). After extraction of the NaCl-saturated crude reaction mixtures with ethyl acetate, thus removing unreacted starting materials, the aqueous phases were acidified to pH 4 by means of HCl, turning the carboxylate ions into the organic solvent-soluble carboxylic acids **12a,b**, which could be recuperated in 43 and 74% yield, respectively. This elegant solution, based on a selective extraction method of the reaction mixture, is characterized by high purification efficiencies of 72–80% (Table 1).

Many efforts were devoted to the optimization of the enzymatic hydrolysis step in terms of reaction time and efficiency, and the reaction conditions presented here should thus be considered as an optimized procedure for this transformation.

Mechanistically, the formation of 4-carboxymethyl-β-lactams **12** can be explained as follows (Scheme 4). Nitrilase enzymes consist of an essential amino acid sequence in their active site: the Glu-Lys-Cys catalytic triade.[69,70] While glutamate and lysine are orienting the substrate in the correct position *via* non-covalent interactions, the catalytic cysteine thiol group ("Enz-SH") is capable of forming a covalent bond with the nitrile functional group in substrates **11**, thus giving rise to thioimidates **13**. Water then hydrolyzes these intermediates toward thioesters **15**, which are finally converted to the corresponding carboxylic acids **12** with concomitant expulsion of ammonia, after which the enzymes are released in their active form to enter a subsequent catalytic cycle.[59]

Conclusion

In this work, chiral *cis*-3-alkoxy/aryloxy-4-(2,2-dimethyl-1,3-dioxolan-4-yl)-β-lactams were employed successfully for the synthesis of *cis*-4-cyanomethyl-β-lactams in nine consecutive reaction steps *via* 4-hydroxymethyl-β-lactams as key intermediates, starting from the commercially available and enantiomerically pure 1,2:5,6-di-*O*-isopropylidene-D-mannitol. Next, the obtained *cis*-4-cyanomethyl-β-lactams were converted into the intended corresponding 4-carboxymethyl-β-lactams using nitrilase-P1-121 as the most active enzyme without affecting the sensitive four-membered ring, providing a useful substitute for the in this case inadequate classical chemical hydrolysis approach. In addition, a straightforward pH-based extraction protocol was designed for the efficient isolation of the carboxylic acids thus formed. In this way, the one-step selective hydrolysis of 3-alkoxy/aryloxy-4-cyanomethyl-β-lactams toward the corresponding carboxylic acids using isolated nitrilase enzymes, has been described for the first time, integrating biocatalytical processes into organic synthesis as a positive evolution fitting in the current trends of sustainability and responsible entrepreneurship. The thus obtained new 4-carboxymethyl-β-lactams can be considered as polyvalent and versatile building blocks for further deployment in organic synthesis.

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References and notes

1 A. Fleming, *Br. J. Exp. Pathol.*, 1929, **10**, 226–236.

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2 K. Bush, *Curr. Opin. Pharmacol.*, 2012, **12**, 527–534.
3 J. D. C. Yao and R. C. Moellering Jr, *Manual of Clinical Microbiology*, 9th edn, 2007, vol. 1, pp. 1077–1113.
4 R. Jimenez, J. Duarte, A. Zarzuelo and M. Galisteo, *Antibiotics*, 2005, 19–48.
5 M. A. Lopez-Ruz, M. Lopez-Gomez, J. Pasquau-Liano and J. Jimenez-Alonso, *Antibiotics*, 2005, 83–142.
6 G. Veinberg, I. Potorocina and M. Vorona, *Curr. Med. Chem.*, 2014, **21**, 393–416.
7 P. D. Mehta, N. P. S. Sengar and A. K. Pathak, *Eur. J. Med. Chem.*, 2010, **45**, 5541–5560.
8 I. Ojima and X. Qiu, *J. Am. Chem. Soc.*, 1987, **109**, 6537–6538.
9 I. Ojima, E. S. Zuniga and J. D. Seitz, *Top. Heterocycl. Chem.*, 2013, **30**, 1–64.
10 B. Alcaide and P. Almendros, *Curr. Med. Chem.*, 2004, **11**, 1921–1949.
11 M. D'hooghe, S. Dekeukeleire, E. Leemans and N. De Kimpe, *Pure Appl. Chem.*, 2010, **82**, 1749–1759.
12 N. A. A. El-Kanzi, *Heterocycl. Lett.*, 2013, **3**, 81–109.
13 C. R. Pitts and T. Lectka, *Chem. Rev.*, 2014, **114**, 7930–7953.
14 M. T. Aranda, P. Perez-Faginas and R. G. Muniz, *Adv. Org. Synth.*, 2013, **6**, 296–354.
15 B. Alcaide, P. Almendros, G. Cabrero and M. P. Ruiz, *Tetrahedron*, 2012, **68**, 10761–10768.
16 J. Fetter, K. Lempert, M. Kajtar-Peredy, G. Simig and G. Hornyak, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1453–1458.
17 M. Shiozaki, N. Ishida, T. Hiraoka and H. Maruyama, *Tetrahedron*, 1984, **40**, 1795–1802.
18 B. Alcaide, P. Almendros, G. Cabrero and M. P. Ruiz, *J. Org. Chem.*, 2007, **72**, 7980–7991.
19 R. J. Nash, A. Kato, C.-Y. Yu and G. W. J. Fleet, *Future Med. Chem.*, 2011, **3**, 1513–1521.
20 Y. Nishimura, *Curr. Top. Med. Chem.*, 2003, **3**, 575–591.
21 P. Greimel, J. Spreitz, A. E. Stutz and T. M. Wrodnigg, *Curr. Top. Med. Chem.*, 2003, **3**, 513–523.
22 L. Somsak, V. Nagy, Z. Hadady, T. Docsa and P. Gergely, *Curr. Pharm. Des.*, 2003, **9**, 1177–1189.
23 C. A. Townsend, M. J. Bodner, R. M. Phelan and M. F. Freeman, WO2011072287A2, 2011.
24 M. J. Bodner, R. Li, R. M. Phelan, M. F. Freeman, K. A. Moshos, E. P. Lloyd and C. A. Townsend, *ChemBioChem*, 2011, **12**, 2159–2165.
25 Y. Takahashi, H. Yamashita, S. Kobayashi and M. Ohno, *Chem. Pharm. Bull.*, 1986, **34**, 2732–2742.
26 T. Yoshioka, R. Miyata, T. Tsuchida, H. Tone and R. Okamoto, EP421440A2, 1991.
27 D. G. Melillo, T. Liu, K. Ryan, M. Sletzinger and I. Shinkai, *Tetrahedron Lett.*, 1981, **22**, 913–916.
28 P. Galletti, C. E. A. Cocuzza, M. Pori, A. Quintavalla, R. Musumeci and D. Giacomini, *ChemMedChem*, 2011, **6**, 1919–1927.
29 T. Hara, A. Yoshida and N. Matsui, JP10077263A, 1998.
30 A. H. Berks, *Tetrahedron*, 1996, **52**, 331–375.
31 T. Yamanaka, M. Seki, T. Kuroda, H. Ohmizu and T. Iwasaki, *Tetrahedron Lett.*, 1996, **37**, 4967–4968.
32 D. H. Shih, F. Baker, L. Cama and B. G. Christensen, *Heterocycles*, 1984, **21**, 29–40.
33 M. Sunagawa, A. Sasaki, H. Matsumura, K. Goda and K. Tamoto, *Chem. Pharm. Bull.*, 1994, **42**, 1381–1387.
34 C. M. Gasparski, A. Ghosh and M. J. Miller, *J. Org. Chem.*, 1992, **57**, 3546–3550.
35 M. A. Williams, M. J. Miller and N. P. Rath, *J. Org. Chem.*, 1991, **56**, 1293–1296.
36 P. R. Guzzo, M. Teng and M. J. Miller, *Tetrahedron*, 1994, **50**, 8275–8292.
37 A. Basak and S. Mandal, *Tetrahedron Lett.*, 2002, **43**, 4241–4243.
38 L. Banfi and G. Guanti, *Eur. J. Org. Chem.*, 2002, 3745–3755.
39 K. C. Nicolaou and W. M. Dai, *Angew. Chem.*, 1991, **103**, 1453–1481.
40 M. Gielen, H. Dalil, M. Biesemans, B. Mahieu, D. De Vos and R. Willem, *Appl. Organomet. Chem.*, 1999, **13**, 515–520.
41 C. H. Sugisaki, Y. Ruland and M. Baltas, *Eur. J. Org. Chem.*, 2003, 672–688.
42 K. Mollet, H. Goossens, N. Piens, S. Catak, M. Waroquier, K. W. Törnroos, V. Van Speybroeck, M. D'hooghe and N. De Kimpe, *Chem.–Eur. J.*, 2013, **19**, 3383–3396.
43 R. Grigg, M. Thornton-Pett, J. Xu and L.-H. Xu, *Tetrahedron*, 1999, **55**, 13841–13866.
44 W. Van Brabandt, M. Vanwalleghem, M. D'hooghe and N. De Kimpe, *J. Org. Chem.*, 2006, **71**, 7083–7086.
45 S. Dekeukeleire, M. D'hooghe, M. Vanwalleghem, W. Van Brabandt and N. De Kimpe, *Tetrahedron*, 2012, **68**, 10827–10834.
46 L. Jiao, Y. Liang and J. Xu, *J. Am. Chem. Soc.*, 2006, **128**, 6060–6069.
47 B. Alcaide, M. F. Aly, C. Rodriguez and A. Rodriguez-Vicente, *J. Org. Chem.*, 2000, **65**, 3453–3459.
48 F. Durrat, M. V. Sanchez, F. Couty, G. Evano and J. Marrot, *Eur. J. Org. Chem.*, 2008, 3286–3297.
49 Y.-F. Ao, D.-H. Leng, D.-X. Wang, L. Zhao and M.-X. Wang, *Tetrahedron*, 2014, **70**, 4309–4316.
50 J. Fetter, F. Bertha, T. Czuppon, M. Kajtar-Peredy and K. Lempert, *J. Chem. Res., Synop.*, 1995, 444–445.
51 G. Simig, G. Doleschall, G. Hornyak, J. Fetter, K. Lempert, J. Nyitrai, P. Huszthy, T. Gizur and M. Kajtar-Peredy, *Tetrahedron*, 1985, **41**, 479–484.
52 Y. Ito, Y. Kobayashi, T. Kawabata, M. Takase and S. Terashima, *Tetrahedron*, 1989, **45**, 5767–5790.
53 C. J. Ashcroft, J. Brennan, C. E. Newall and S. M. Roberts, *Tetrahedron Lett.*, 1984, **25**, 877–880.
54 S. Kishimoto, M. Sendai, M. Tomimoto, S. Hashiguchi, T. Matsuo and M. Ochiai, *Chem. Pharm. Bull.*, 1984, **32**, 2646–2659.
55 N. Ikota, H. Shibata and K. Koga, *Chem. Pharm. Bull.*, 1985, **33**, 3299–3306.
56 J. Fetter, K. Lempert, T. Gizur, J. Nyitrai, M. Kajtar-Peredy, G. Simig, G. Hornyak and G. Doleschall, *J. Chem. Soc., Perkin Trans. 1*, 1986, 221–227.
57 J. D. Buynak, WO2014070774A1, 2014.
58 D.-H. Leng, D.-X. Wang, Z.-T. Huang and M.-X. Wang, *Org. Biomol. Chem.*, 2010, **8**, 4736–4743.
59 A. Banerjee, R. Sharma and U. C. Banerjee, *Appl. Microbiol. Biotechnol.*, 2002, **60**, 33–44.

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60 T. Sugai, T. Yamazaki, M. Yokoyama and H. Ohta, *Biosci., Biotechnol., Biochem.*, 1997, **61**, 1419–1427.
61 M. Winkler, L. Martinkova, A. C. Knall, S. Krahulec and N. Klempier, *Tetrahedron*, 2005, **61**, 4249–4260.
62 V. K. Nigam, A. K. Khandelwal, A. Agarwal, A. S. Vidyarti, M. K. Mohan, R. K. Gothwal and P. Ghosh, *Recent Adv. Microbiol.*, 2013, **1**, 461–477.
63 M.-X. Wang, *Top. Organomet. Chem.*, 2011, **36**, 105–122.
64 K. Vervisch, M. D'hooghe, F. P. J. T. Rutjes and N. De Kimpe, *Org. Lett.*, 2012, **14**, 106–109.
65 K. Mollet, L. Decuyper, S. Vander Meeren, N. Piens, K. De Winter, T. Desmet and M. D'hooghe, *Org. Biomol. Chem.*, 2015, **13**, 2716–2725.
66 R. de Regil and G. Sandoval, *Biomolecules*, 2013, **3**, 812–847.
67 T. Schafer, T. W. Borchert, V. S. Nielsen, P. Skagerlind, K. Gibson, K. Wenger, F. Hatzack, L. D. Nilsson, S. Salmon, S. Pedersen, H. P. Heldt-Hansen, P. B. Poulsen, H. Lund, K. M. Oxenboell, G. F. H. Wu, H. Pedersen and H. Xu, *Adv. Biochem. Eng./Biotechnol.*, 2007, **105**, 59–131.
68 Two nitrilase screening kits were kindly donated by Codexis and Prozomix, from which nitrilase NIT-P1-121 (Codexis) was found to give the best results.
69 L. Martinkova and V. Kren, *Curr. Opin. Chem. Biol.*, 2010, **14**, 130–137.
70 C. Brenner, *Curr. Opin. Struct. Biol.*, 2002, **12**, 775–782.