Stereoselective Access to Azetidine-Based α-Aminoacids and Applications to Small Peptides Synthesis

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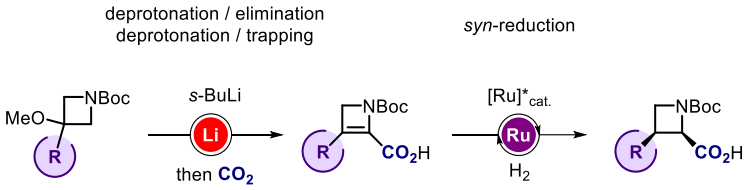
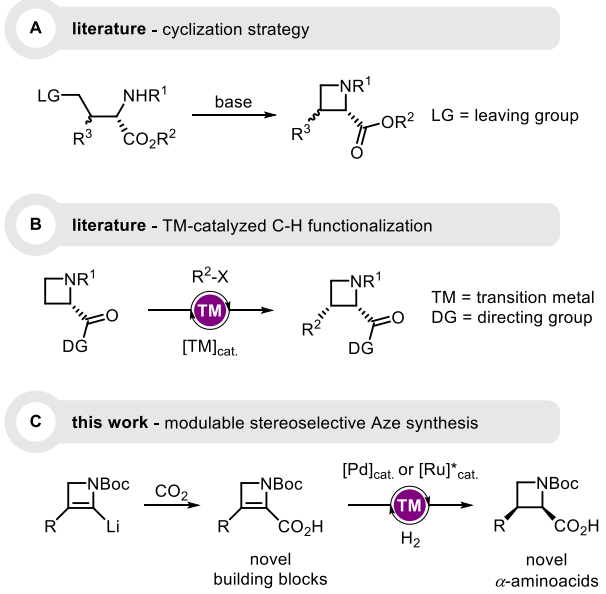
Supporting Information Placeholder



ABSTRACT: Recent progress on four-membered ring building blocks has led us to investigate the formation of non-natural azetidine-based amino acids (*Aze*). A simple organometallic route was developed to access unsaturated carboxylic acids, which were further engaged in metal catalyzed asymmetric reduction. Functionalized *Aze* derivatives were finally employed in the formation of small peptide chains.

α-Amino acids are essential building blocks that constitute the backbone of every protein. The potential behind the development of new non-natural amino acids is broad since it unlocks new features in protein engineering. The introduction of cyclic amino acid scaffolds causes substantial changes in the secondary structure of peptide chains, justifying therefore the interest in azetidine 2-carboxylic acid compounds (*Aze*) as versatile foldamer elements.[1]

Scheme 1. Synthesis of Aze derivatives



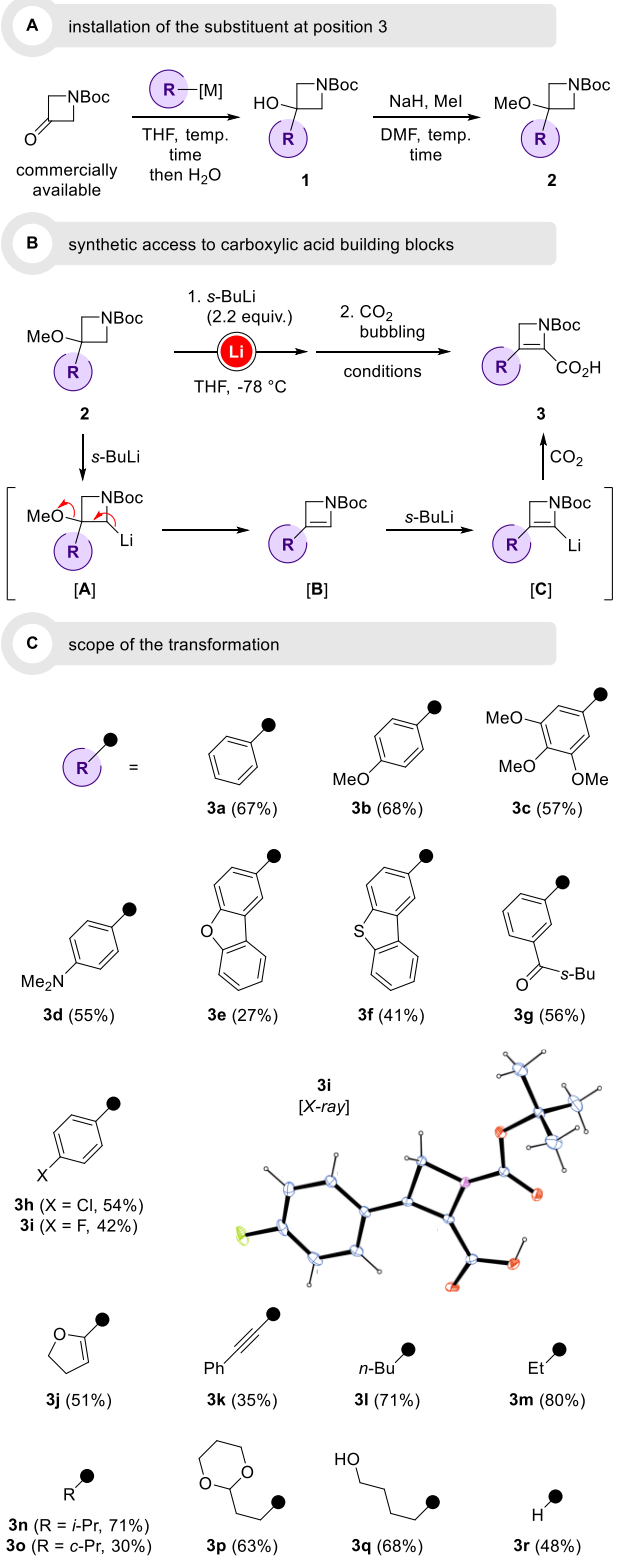
For instance, the groups of Martín-Martínez[2] and Toniolo[3] reported that γ-turns can be induced by the presence of 2-azetidinylcarboxylic acids within peptide chains.

Aze are traditionally synthesized by cyclization of an adequately protected α-amino acid possessing a leaving group in the γ-position, in the presence of a base (Scheme 1A).[4] Recently, the groups of Schreiber and Baran have showcased the use of directing groups at position 2 of stereodefined *Aze* derivatives to promote a *cis*-selective functionalization at position 3 through C-H activation strategies (Scheme 1B).[5]

Following our recent advances on the synthesis of four-membered carbo- and heterocycles,[6-8] we set out to investigate the formation of non-natural substituted *Aze* through a simple stereocontrolled sequence involving the intermediate formation of unsaturated prochiral azetinyl-carboxylic acids. We envisioned that further asymmetric hydrogenation would furnish the desired functionalized *Aze* compounds diastereo- and enantioselectively (Scheme 1C). As previously established by our group,[7a] the addition of organometallic nucleophiles to commercial sources of 3-azetidiones (Scheme 2) allows for an efficient access to tertiary alcohols **1**, which is then transformed into the corresponding methyl ether **2**.[9] The addition of *s*-BuLi promotes a sequence of α-lithiation/β-elimination, leading to an azetinyllithium species [**C**]. Corresponding carboxylic acids **3** are simply obtained after bubbling CO_2 in the reaction mixture. A model substrate possessing a phenyl group at position 3 led to the corresponding *Aze*-carboxylic acid 3a in 67% yield. Functional group tolerance was examined next by introducing various substituents on the aryl moiety of the substrate. Electron-donor groups such as methoxy- and polymethoxy-substituents

gave satisfactory yields (**3b-c**, up to 68%), and the dimethylanilin derivative furnished **3d** in 55% yield. A decrease in efficiency was noted with dibenzofurans and thiophenes (**3e-f**), only isolated in up to 41% yield. The presence of a nitrile group resulted in the formation of the corresponding ketone (**3i**) via 1,2-addition of *s*-BuLi.

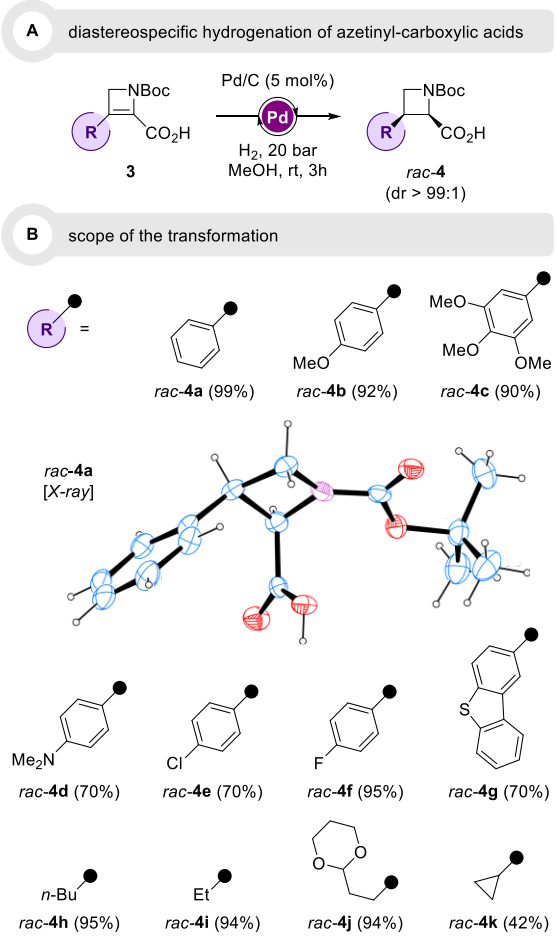
Scheme 2. Synthetic sequence towards azetinyl-carboxylic acids 3



Halogen-substituted structures were also tolerated, furnishing **3e-f** in moderate yields. It is important to note that these strained unsaturated carboxylic acids proved to be stable towards air and moisture. We were therefore able to crystallize compound **3i** (X-ray given in Scheme 2C),[10] which showed hydrogen bonding between the proton of the carboxylic acid and the *N*-Boc protecting group, partially accounting for the overall stability of these structures. Alkenyl and alkynyl groups were also introduced (**3j-k**), although lower yields were generally observed in comparison with alkyl groups (**3l-q**), except for cyclopropyl which proved relatively unstable under these conditions. A moderate yield was obtained for the unsubstituted derivative **3r**, isolated in 48%.

Having established a new library of unsaturated *Aze* derivatives, we started investigating the diastereospecific synthesis of functionalized 2-azetidinecarboxilic acids **4** through palladium-catalyzed *cis*-hydrogenation (Scheme 3).[11]

Scheme 3. Diastereospecific hydrogenation towards functionalized *cis*-*Aze* compounds 4

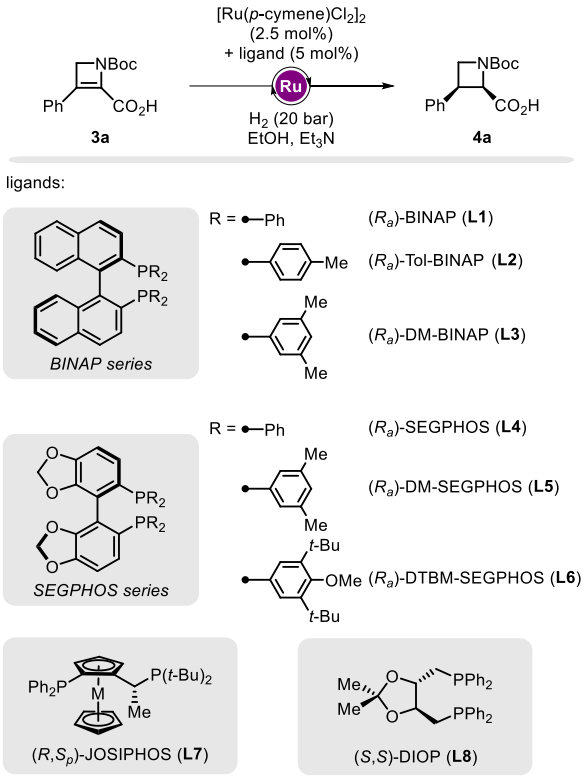


Selected examples were hydrogenated using Pd/C (5 mol%) under H_2 atmosphere (20 bar) in methanol, furnishing *cis*-isomers *rac*-**4a-j** in excellent yields (up to 98%), independently from the nature of the substituent at position 3. The relative stereochemistry of these derivatives was assessed by analogy with X-ray measurements performed on *rac*-**4a**. Although substrates possessing primary alkyl groups gave the desired compounds *rac*-**4h-j** in high yields, the presence of a cyclopropyl only yielded 42% of compound *rac*-**4k**. It is worth noting that the classical lithiation of saturated cyclic systems usually leads to

trans-isomers due to the sterical hindrance engendered by surrounding substituents. Our method allows for the selective formation of *cis*-isomers, offering therefore an efficient stereodivergent alternative to existing strategies.[12]

Optimizations of the asymmetric hydrogenation were logically carried out next on model compound **3a** in order to identify the best ligand system to be used in the formation of enantioenriched *Aze* compound **4a**. Results employing [Ru(*p*-cymene)Cl_2]$_2$ (2.5 mol%) are given in Table 1, as transition metal complex of rhodium[13] or iridium (Crabtree's precatalyst)[14] did not give satisfactory results.

Table 1. Optimizations on enantioselective hydrogenation



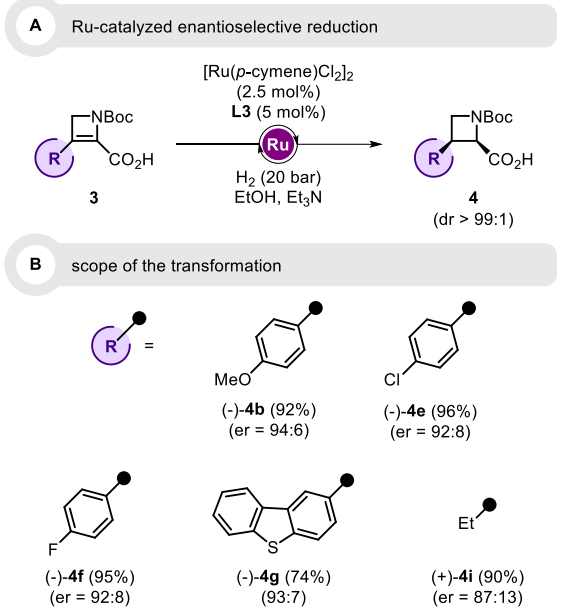
Ligand	Variation to the conditions	Yield	er
L1	none	90%	88:12
L2	none	91%	94.5:5.5
L3	none	98%	95:5
L3	MeOH instead of EtOH	20%	nd
L3	pyridine instead of Et_3N	< 5%	nd
L3	DIPEA instead of Et_3N	98%	95:5
L3	10 bar instead of 20 bar H_2	95%	95:5
L4	none	90%	90:10
L5	none	< 5%	nd
L6	none	< 5%	nd
L7	none	94%	75:25
L8	none	< 5%	nd

Inspired by the pioneering work of Noyori on asymmetric hydrogenation,[15] BINAP-based ligands **L1**-**3** were evaluated first and gave both best yields and enantioselectivities (up to 98% and er = 95:5 for **L3**). Other usually efficient ligand systems such as SEGPHOS (**L4**-**6**),[16] JOSIPHOS (**L7**)[17] and DIOP (**L8**)[18] proved less efficient, with enantiomeric ratios ranging from 75:25 to 90:10.

Using methanol as solvent only gave 20% of the desired product **4a**. This study also showed that the nature of the tertiary amine (Et_3N or DIPEA) did not influence the reaction, while pyridine only led to traces of **4a**. Decreasing the pressure of hydrogen to 10 bar gave similar results, although the reaction had to be stirred for extended time.

With optimized conditions in hands, the scope of asymmetric hydrogenation was evaluated on selected aryl, heteroaryl and alkyl derivatives (Scheme 4). Electron-donating and electron-deficient substituents in *para* position of phenyl groups furnished (-)-**4b**, (-)-**4e**-**f** in high yields (92 to 96%) and good enantiomeric ratios up to 94:6. With a dibenzothiophenyl moiety, product **4h** was isolated in 74% yield and 93:7 er. However, the presence of an alkyl group (ethyl, (+)-**4i**) diminished the enantiomeric ratio to 87:13.

Scheme 4. Ru-catalyzed enantioselective reduction of azentinylcarboxylic acids

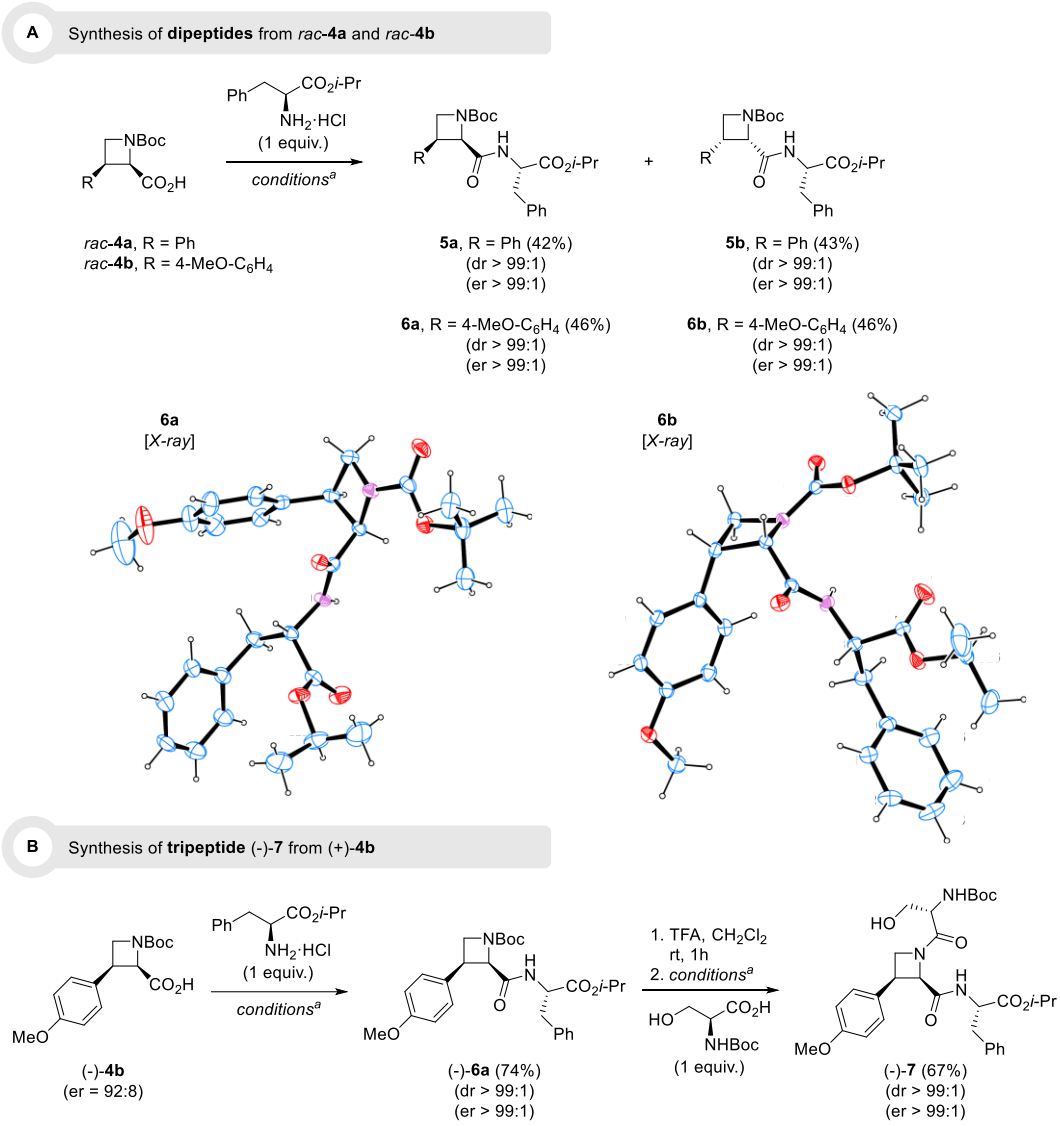


With a novel library of racemic and enantioenriched *Aze* building blocks at our disposal, we lastly aimed at their incorporation into small peptidic chains. *Rac*-**4a** was chosen to undergo amidification with stereodefined L-phenylalanine isopropyl ester (L-Phe-*O*-*i*-Pr) in the presence of HTBU as peptide coupling agent.[19] The two enantiopure diastereoisomers **5a** and **5b** could be separated via chromatography and isolated with high yields (42 and 43%, respectively). Similarly, *p*-methoxyphenyl substituted substrate *rac*-**4b** gave two separable isomers **6a** and **6b** in 92% overall yield. The absolute configuration of both isomers was ascertained by X-ray crystallography of **6a** and **6b**, as shown in Scheme 5A. These results were also used to determine the absolute configurations of molecules presented in Scheme 4B, by analogy.

Enantioenriched amino acid (-)-**4b** (92:8 er) was easily resolved through peptide coupling with L-Phe-*O*-*i*-Pr under previous conditions, yielding the enantiopure dipeptide (-)-**6a** in 74%. The azetidinyl moiety was further deprotected with TFA

and engaged with a L-serine derivative (L-Ser-*N*-Boc) towards the formation of tripeptide (-)-**7** which was isolated in its enantiopure form in 67% yield.

Scheme 5. Di- and tripeptide synthesis



[a] HTBU, DIPEA, CH_2Cl_2, rt, 6h

In summary, the synthesis of enantioenriched four-membered amino acids was achieved using a simple and practical two-step procedure through intermediate formation of isolable 2-azetinylcarboxylic acids. Their reduction was performed either with palladium or chiral ruthenium complexes and the resulting saturated amino acids were efficiently resolved after peptide coupling. Di- and tripeptides were obtained in good yields and excellent enantiopurity. We believe that such unusual architectures will be of high interest in future protein engineering and in the study of *Aze*-containing secondary structures.

ASSOCIATED CONTENT

Supporting Information

Contains all experimental procedures and characterization (IR, HRMS, and ^1H and ^{13}C NMR data) for all new compounds.
The SI is available free of charge on the ACS Publications website.

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[9] See supporting information

[10] CCDC 2031218 (**3i**), 2031215 (*rac*-**4a**), 20131216 (**6a**) and 2031217 (**6b**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the Cambridge Crystallographic Data Centre.

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