OL Organic Letters

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Stereoselective Access to Azetidine-Based α-Amino Acids and Applications to Small Peptide Synthesis

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Cite This: https://dx.doi.org/10.1021/acs.orglett.0c03131 Read Online

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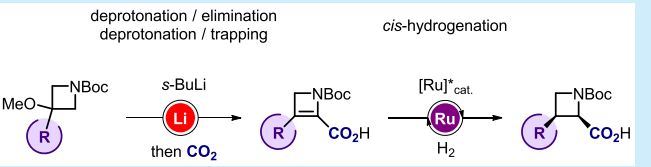
ABSTRACT: Non-natural azetidine-based amino acids (Aze) present interesting features in protein engineering. A simple organometallic route toward unsaturated carboxylic acid precursors is presented. Subsequent metal-catalyzed asymmetric reduction allowed for the synthesis of a new library of 2-azetidinylcarboxylic acids, which were finally employed in the formation of small peptide chains.



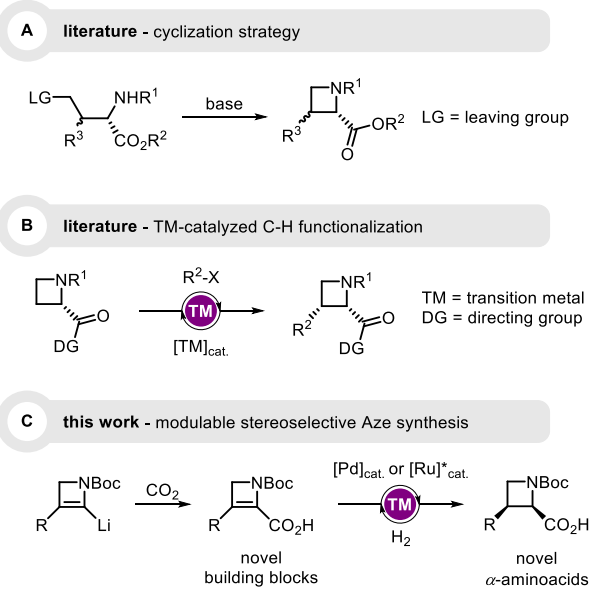
Constituting the backbone of every protein, α-amino acids are essential building blocks. The potential behind the development of new non-natural amino acids is broad since it unlocks new features in protein engineering. The introduction of cyclic amino acid scaffolds causes substantial changes in the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

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.

2-Azetidinylcarboxylic acids are traditionally synthesized by cyclization of an adequately protected α-amino acid possessing a leaving group in the γ-position under basic conditions (Scheme 1A).[4] Recently, the groups of Schreiber and Baran have showcased the use of directing groups at position 2 of stereodefined derivatives to promote a *cis*-selective functionalization at position 3 through C−H activation strategies (Scheme 1B).[5] In addition, efforts have recently been made toward the synthesis of 3-azetidinylcarboxylic acids.[6]

Following our recent advances on the synthesis of four-membered carbo- and heterocycles,[7−9] we set out to investigate the formation of non-natural substituted amino acids through a simple stereocontrolled sequence involving the intermediate formation of unsaturated prochiral azetinylcarboxylic acids. We envisioned that further asymmetric hydrogenation would furnish the desired functionalized azetidine carboxylic acid compounds diastereo- and enantioselectively (Scheme 1C).[10] The addition of organometallic nucleophiles to commercial sources of 3-azetidinones (Scheme 2) allows for an efficient access to tertiary alcohol **1**, which is then transformed into the corresponding methyl ether **2**.[11] As previously established by our group,[8a] the formation of lithiated species [**C**] is accomplished through an α-lithiation/β-elimination/α-lithiation sequence via addition of *s*-BuLi. Corresponding carboxylic acids **3** are simply obtained after bubbling CO_2 in the reaction mixture. A model substrate

Scheme 1. Synthesis of Aze Derivatives



possessing a phenyl group at position 3 led to the corresponding 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

Received: September 17, 2020

https://dx.doi.org/10.1021/acs.orglett.0c03131
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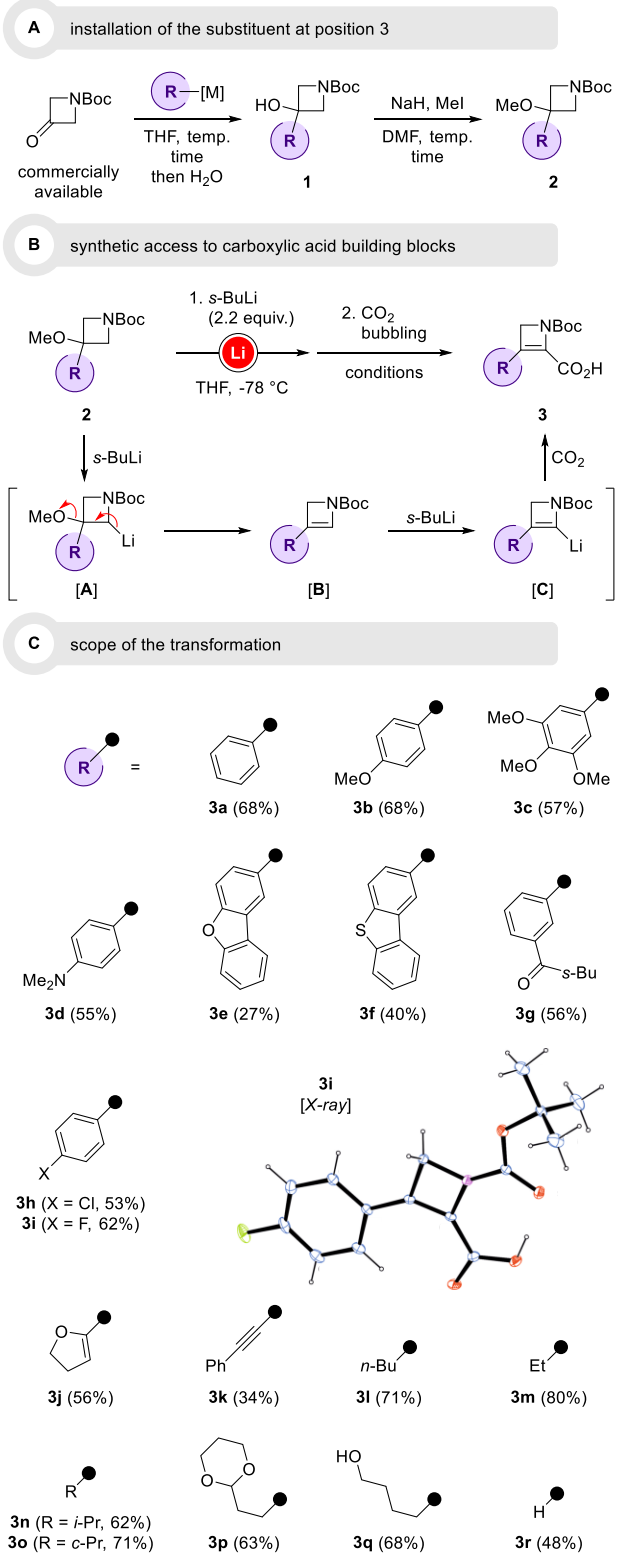
Scheme 2. Synthetic Sequence Towards Azetinyl-Carboxylic Acids 3

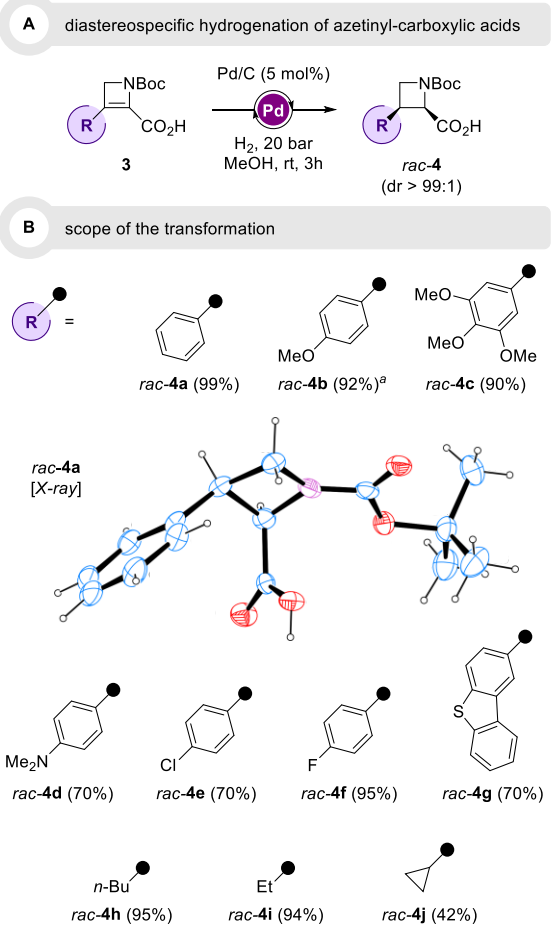


gave satisfactory yields (**3b** and **3c**, 68%), and the *N*,*N*-dimethylanilin derivative furnished **3d** in 55% yield.

A decrease in efficiency was noted with dibenzofurans and thiophenes (**3e**, **3f**), which were only isolated in up to 40% yield. The presence of a nitrile group resulted in the formation of the corresponding ketone (**3g**) via 1,2-addition of *s*-BuLi. Halogen-substituted structures were also tolerated, furnishing **3h** and **3i** in moderate yields up to 62%. It is important to note that these strained unsaturated carboxylic acids proved to be stable toward air and moisture. We were therefore able to crystallize compound **3i** (X-ray given in Scheme 2C), 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**, **3k**), although lower yields were generally observed in comparison with alkyl groups (**3l**−**3q**, 62−80%). A moderate yield was obtained for the unsubstituted derivative **3r**, isolated in 48%.

Having established a new library of unsaturated derivatives, we started investigating the diastereospecific synthesis of functionalized 2-azetidinecarboxylic acids **4** through palladium-catalyzed *cis*-hydrogenation (Scheme 3).[12]

Scheme 3. Diastereospecific Hydrogenation Towards Functionalized *cis*-Aze Compounds 4



[a]The reaction was also performed on 3.5 mmol of **3b**, giving 952 mg (89%) of compound *rac*-**4b**.

Selected examples were hydrogenated using Pd/C (5 mol %) under H_2 atmosphere (20 bar) in methanol, furnishing *cis*-isomers *rac*-**4a**−**4j** 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**−**4j** in high yields, the

https://dx.doi.org/10.1021/acs.orglett.0c03131
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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,[5] offering therefore an efficient stereodivergent complementary alternative to existing strategies.[13]

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 2-azetidinylcarboxylic acid **4a**.

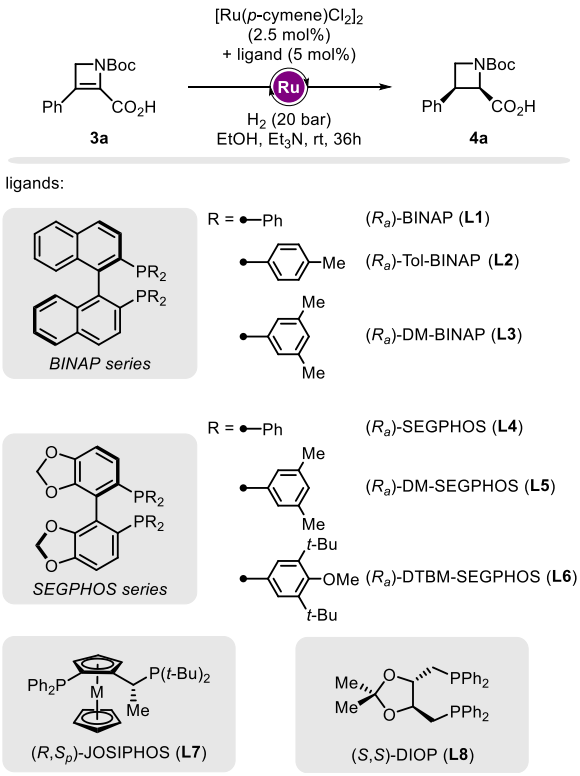
Results employing $[Ru(p\text{-cymene})Cl_2]_2$ (2.5 mol %) are given in Table 1, as transition metal complexes of rhodium[14] or iridium (Crabtree's precatalyst)[15] did not give satisfactory results. Inspired by the pioneering work of Noyori on asymmetric hydrogenation,[16] BINAP-based ligands **L1**−**L3** 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**−**L6**),[17] JOSIPHOS (**L7**)[18] and DIOP (**L8**)[19] proved less efficient, with enantiomeric ratios ranging from 75:25 to 90:10.

Using methanol as a 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 an extended time.

With optimized conditions in hand, the scope of asymmetric hydrogenation was evaluated on selected aryl, heteroaryl, and alkyl derivatives (Scheme 4). Electron-donating and electron-deficient substituents in the *para* position of phenyl groups furnished (−)-**4b** and (−)-**4e**−**4f** in high yields (91−96%) 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.

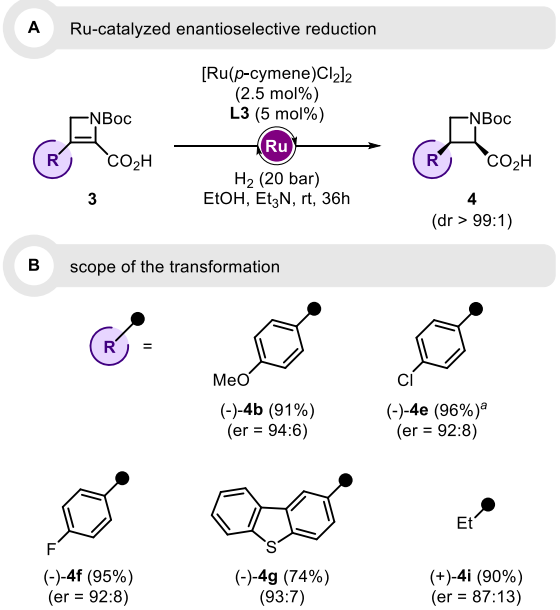
With a novel library of racemic and enantioenriched amino acid building blocks at our disposal, we last aimed at their incorporation into small peptidic chains. *Rac*-**4a** was chosen to undergo amidification with stereodefined L-phenylalanine isopropyl ester (L-Phe-*O*-*i*-Pr) in the presence of HBTU as a peptide coupling agent.[20] The two enantiopure diastereoisomers **5a** and **5b** could be separated via chromatography and isolated with high yields (42% and 43%, respectively, 85% overall). 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

Table 1. Optimizations on Enantioselective Hydrogenation



ligand	deviation from 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

Scheme 4. Ru-Catalyzed Enantioselective Reduction of Azetinylcarboxylic Acids



[a]The reaction was performed on 1.5 mmol of **3h**, giving 447 mg of compound (−)-**4e**.

With a novel library of racemic and enantioenriched amino acid building blocks at our disposal, we last 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 HBTU as a peptide coupling agent.[20] The two enantiopure diastereoisomers **5a** and **5b** could be separated via chromatography and isolated with high yields (42% and 43%, respectively, 85% overall). 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

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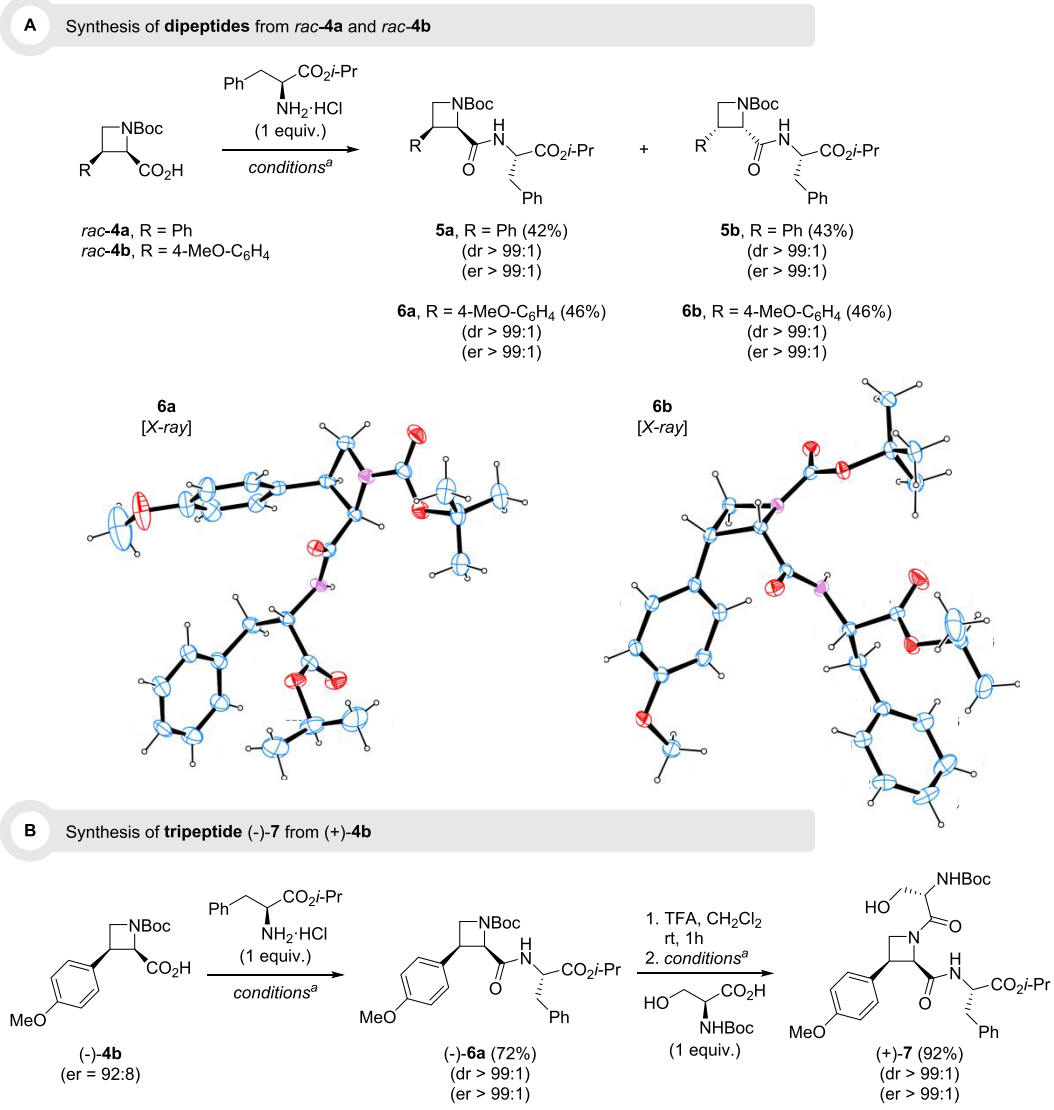
Scheme 5. Di- and Tripeptide Synthesis



[a]HBTU (1.1 equiv), DIPEA (2.2 equiv), CH_2Cl_2, rt, 6 h.

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 72%. The azetidinyl moiety was further deprotected with TFA and engaged with an L-serine derivative (L-Ser-*N*-Boc) toward the formation of tripeptide (+)-**7** which was isolated in its enantiopure form in 92% yield (Scheme 5B).

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 with either 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 azetidine-containing secondary structures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c03131.

Representative example for the asymmetric reduction of azetines (PDF)

Accession Codes

CCDC 2031215−2031218 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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https://dx.doi.org/10.1021/acs.orglett.0c03131
Org. Lett. XXXX, XXX, XXX−XXX

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

D.D. and F.R. are grateful to the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft (DFG grant: DI 2227/2-1 and Heisenberg fellowship: DI 2227/4-1), the SFB749, and the Ludwig-Maximilians University for PhD funding and financial support. Dr. Peter Mayer, Prof. Konstantin Karaghiosoff, and Dr. Andreas N. Baumann (LMU, Munich) are kindly acknowledged for X-ray and NMR measurements, and preliminary experiments.

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