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Stereoselective synthesis of (*R*)- and (*S*)-1,2-diazetidine-3-carboxylic acid derivatives for peptidomimetics†

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The stereoselective synthesis of both enantiomers of *N*-protected 1,2-diazetidine-3-carboxylic acid (aAze) from homochiral glycidol is described. Orthogonal protection of this novel cyclic α-hydrazino acid allows for selective functionalisation at either N^{γ} or N^{δ}. This novel peptidomimetic building block was incorporated into the pseudotripeptides Gly-γaAze-Ala and Gly-δaAze-Ala.

Introduction

Cyclic α-hydrazino acids and their derivatives have been shown to possess a diverse set of biological activities.[1] They have been isolated from numerous natural sources, including plants, marine organisms and bacteria. Examples include the potent cytotoxins piperazimycin A–C from *Streptomyces* sp.[2] and the antimycobacterial cyclodepsipeptides lydiamycin A–D from *Streptomyces lydicus*.[3] As a class of modified amino acids, cyclic α-hydrazino acids mimic the structural constraints imposed by proline (Pro). Therefore, they have been widely applied in peptidomimetics where they can act as Pro surrogates to modulate the conformational flexibility, bioavailability, proteolytic stability and biological activity of target molecules.[4]

The six-membered cyclic α-hydrazino acid (n = 3), known as piperazic acid (Pip), is a non-proteinogenic amino acid and the only example of a naturally occurring member of this family (Fig. 1). Pip and its derivatives have been identified in over 140 natural products, many of which displaying potent biological activity, ranging from anti-malarial to anti-bacterial properties.[5] The five-membered derivative δ-azaproline (aPro, n = 2) has been used as proline mimic in peptidomimetics.[6] Detailed conformational studies of aPro containing di- and tripeptides have been performed, which revealed that aPro takes up a *cis* conformation in simple amides, whereas its carbamate-protected derivative adopts preferentially a *trans* conformation.[6a,7] Tripeptides containing aPro derivatives were found to be stable to proteolytic degradation, whereas native Pro analogues were readily digested.[8] Moreover, Sinha described the synthesis of the 5–9 membered cyclic α-hydrazino acids **1** (n = 2–6) using various synthetic strategies.[9]

L-Azetidine-2-carboxylic acid (Aze) was the first example of a naturally occurring azetidine,[10] and several natural products containing this non-proteogenic cyclic amino acid have been isolated.[11] Theoretical studies have shown that replacement of Pro by its lower homologue Aze, *i.e.* substitution of a pyrrolidine ring by an azetidine cycle, leads to a reduction of the conformational flexibility due to the quasi-planar geometry of the four-membered ring.[12] Similar to aPro-containing peptidomimetics, studies comparing Aze- and Pro-containing peptides have suggested a higher propensity of the amide bond involving the azetidine nitrogen to adopt a *cis* conformation.[13]

As part of our efforts in the synthesis of 3-substituted 1,2-diazetidines,[14] we were interested to explore the stereocontrolled synthesis of 1,2-diazetidine-3-carboxylic acid

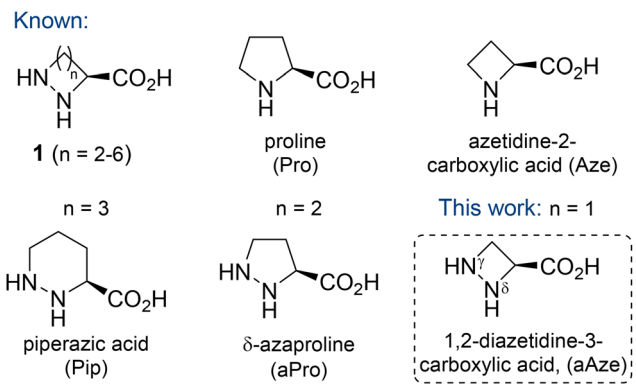

Fig. 1 Cyclic amino and α-hydrazino acids.

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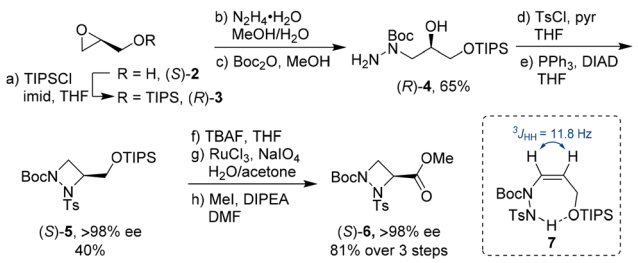
(γ-azaazetidine-2-carboxylic acid, aAze, $n = 1$), the lower homologue of aPro, and its potential application in peptidomimetics (Fig. 1). In particular, we were interested in exploring the structural consequences towards short peptide sequences after incorporation of this unnatural amino acid. Furthermore, we were intrigued if aAze could act as turn-inducing moiety for the cyclisation of short peptide sequences. Previously, we reported the synthesis of C3-functionalised diazetidines by ring opening of enantioenriched epoxides with hydrazine monohydrate, followed by iterative *N*-protection and Mitsunobu ring closure.[14a] Taking inspiration from this route, we explored possibilities to install a carboxylic acid in the C3 position of the 1,2-diazetidine ring to give access to the novel unnatural amino acid aAze. Here, we disclose the stereospecific synthesis of orthogonally protected (*R*)- and (*S*)-1,2-diazetidine-3-carboxylic acid, aAze derivatives, and their regioselective functionalisation as novel building blocks in peptidomimetics.

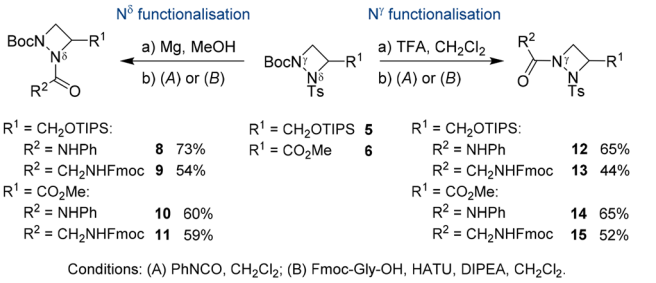
Results and discussion

With both glycidol enantiomers being commercially available and inexpensive, we commenced our synthesis with homochiral (*S*)-glycidol [(*S*)-**2**] as a source of chirality. Initially, similar to Williams's asymmetric synthesis of piperazic acid derivatives,[15] we masked the hydroxy group in glycidol as TBS (*tert*-butyldimethylsilyl) ether. However, ring opening of the silyl-protected glycidol with hydrazine hydrate gave inconsistent results with yields varying widely. Thus, we explored the sterically more hindered TIPS (triisopropylsilyl) protecting group. Stereocontrolled ring opening of homochiral epoxide (*R*)-**3**[16] with hydrazine hydrate at the unsubstituted carbon followed by selective protection of the secondary nitrogen gave Boc derivative (*R*)-**4** in 65% yield (Scheme 1).[17] This was followed by tosyl protection of the primary amine and azodicarboxylate-mediated Mitsunobu cyclisation to form the four-membered 1,2-diazetidine (*S*)-**5**. These steps were easily scalable and could be conducted on multi-gram scale.[18] The yield for the ring closure was only moderate as it was accompanied by the formation of elimination product **7** (up to 25%). Interestingly, **7** was selectively formed as the *cis* alkene as determined by ^{1}H NMR spectroscopy. We hypothesise that the selectivity in this elimination reaction is due to an intramolecular hydrogen bonding between the hydrogen on the tosyl protected amine and the TIPS protected oxygen. Removal of the primary TIPS group in (*S*)-**5** with TBAF followed by oxidation of the resulting primary alcohol with an excess of sodium periodate in the presence of catalytic amounts of ruthenium trichloride and esterification of the resulting carboxylic acid with iodomethane furnished orthogonally protected ester (*S*)-**6** as aAze derivative in 81% yield over the three step sequence.[19] Examination of the stereointegrity of (*S*)-**5** and (*S*)-**6** using chiral HPLC revealed that the formation of the 1,2-diazetidine from homochiral (*S*)-glycidol to (*S*)-**5** and its deprotection/oxidation/esterification to (*S*)-**6** occurred with virtually no racemisation (>98% ee).[18] The absolute stereochemical configuration of (*S*)-**5** was confirmed after TBAF-mediated removal of the TIPS group to the primary alcohol and comparison with the literature data.[14c] Alternatively, following the same reaction sequence starting from (*R*)-glycidol [(*R*)-**2**], (*R*)-**6** was prepared in comparable yield with >98% ee.[18]

Orthogonal protecting groups on N$^\gamma$ and N$^\delta$ allowed for selective functionalisation of the 1,2-diazetidine derivatives **5** and **6** (Scheme 2). The Boc group was selectively removed with trifluoroacetic acid, while the tosyl group was cleaved using magnesium turnings in methanol. The deprotected derivatives could be isolated after removal of either the Boc or tosyl group but were not stable upon storage. To avoid decomposition after deprotection, further functionalisation of the free N$^\gamma$ and N$^\delta$ was directly conducted on the crude material. Trapping of the free amine with phenyl isocyanate gave urea-containing compounds **8**, **10**, **12** and **14** in 60–73% yield. Alternatively, HATU-mediated coupling with Fmoc-Gly-OH provided pseudodipeptides **9**, **11**, **13** and **15** in 44–59% yield. These experiments demonstrate that either N$^\gamma$ or N$^\delta$ could be independently functionalised with similar reactivities observed for both nitrogen atoms. This observation stands in contrast to the reactivity of aPro derivatives, where the amide nitrogen was reported to be significantly less reactive.[7a] We attribute this increased reactivity of N$^\delta$ in aAze derivatives to its increased pyramidal character in 1,2-diazetidines, which moves the substituents out of the plane of the four-membered ring to avoid steric interactions.[14a] Moreover, both nitrogen atoms in the 1,2-diazetidine ring can successively engage in peptide couplings. Starting from **11**, removal of the Boc group from N$^\delta$ and



Scheme 1 Synthesis of (*S*)-1,2-diazetidine-3-carboxylic acid building block (*S*)-**6**.



Scheme 2 Orthogonal functionalisation of N$^\gamma$ and N$^\delta$ in **5** and **6**.

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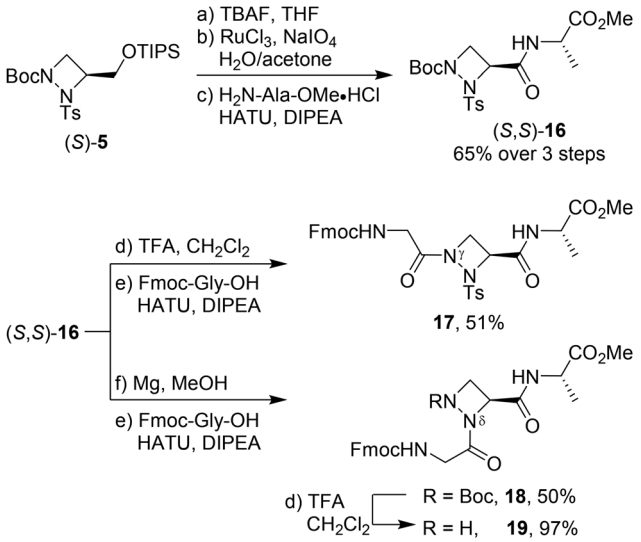

Scheme 3 Incorporation of 1,2-diazetidine building block (*S*)-**5** into pseudotripeptides.

coupling with Cbz-Ala-OH furnished an orthogonally protected pseudotripeptide.[18] In all cases, the C-terminus of silyl protected alcohol **5** and methyl ester **6** were fully compatible during *N*-functionalisation.

Having established conditions for the selective functionalisation of both nitrogen atoms, we explored the possibility to incorporate the aAza amino acid into pseudotripeptides. Starting from (*S*)-**5**, the silyl group was removed, and the free alcohol was oxidised to the carboxylic acid (Scheme 3). Without further purification, HATU-mediated coupling between the carboxylic acid and H-Ala-OMe furnished (*S*,*S*)-**16** as a single diastereomer in 65% yield over 3 steps. Alternatively, commencing from the enantiomer (*R*)-**5**, pseudodipeptide (*R*,*S*)-**16** was selectively formed proving that the subsequent deprotection, oxidation and amide coupling occurred without any racemisation at the C-terminus.[18]

With (*S*,*S*)-**16** in hand, removal of the Boc group from N^{γ} and coupling with Fmoc-Gly-OH gave pseudotripeptide **17** (Gly-γaAze-Ala) in 51% yield. On the other hand, tosyl deprotection from (*S*,*S*)-**16** and coupling with Fmoc-Gly-OH gave Gly-δaAze-Ala derivative **18** in 50% yield. While tosyl removal from **17** with magnesium in methanol caused decomposition, the Boc group from **18** was successfully removed with 25% TFA in dichloromethane leading to free amine **19** in nearly quantitative yield after washing with sodium bicarbonate. ^{1}H NMR analysis of pseudopeptides **18** and **19** at 298 K revealed a single set of signals, indicating the presence of only one conformer or a fast equilibrium between conformers in solution.

Conclusions

In summary, a practical synthesis of both enantiomers of orthogonally protected 1,2-diazetidine-3-carboxylic acid, derivates of the novel unnatural amino acid aAze, was developed using readily available homochiral glycidol as a source of chirality. The chemistry is operationally simple and can be performed on a preparative scale. Furthermore, orthogonal protection of 1,2-diazetidine building blocks **5** and **6** allowed for selective functionalisation on either N^{γ} or N^{δ} leading to different types of pseudopeptides. Due to the importance of 1,2-diazetidines, these cyclic unnatural amino acid derivatives have potential as novel scaffolds in peptidomimetics and as constrained analogues of natural amino acids. Studies of the structural consequences for the incorporation of aAze into short peptide sequences compared to the higher homologue aPro will be published in due course.

Author contributions

M. N., H. S. and S. R.: synthesis, spectral analysis, investigation, and methodology. M. S.: conceptualisation, resources, validation, and writing – review and editing. S. R.: conceptualisation, project administration, supervision, validation, writing – original draft, and writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

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References

1 (*a*) G. Le Goff and J. Ouazzani, *Bioorg. Med. Chem.*, 2014, **22**, 6529–6544; (*b*) C.-H. Küchenthal and W. Maison, *Synthesis*, 2010, 719–740.
2 E. D. Miller, C. A. Kauffman, P. R. Jensen and W. Fenical, *J. Org. Chem.*, 2007, **72**, 323–330.
3 X. Huang, E. Roemer, I. Sattler, U. Moellmann, A. Christner and S. Grabley, *Angew. Chem., Int. Ed.*, 2006, **45**, 3067–3072.
4 M. K. Singh and M. K. Lakshman, *Org. Biomol. Chem.*, 2022, **20**, 963–979.
5 (*a*) K. D. Morgan, R. J. Andersen and K. S. Ryan, *Nat. Prod. Rep.*, 2019, **36**, 1628–1653; (*b*) A. J. Oelke, D. J. France, T. Hofmann, G. Wuitschik and S. V. Ley, *Nat. Prod. Rep.*, 2011, **28**, 1445–1471; (*c*) M. A. Ciufolini and N. Xi, *Chem. Soc. Rev.*, 1998, **27**, 437–445.
6 (*a*) Y. M. Elbatrawi, K. P. Pedretty, N. Giddings, H. L. Woodcock and J. R. Del Valle, *J. Org. Chem.*, 2020, **85**, 4207–4219; (*b*) E. Voss, A. Arrault, J. Bodiguel and B. Jamart-Grégoire, *Tetrahedron: Asymmetry*, 2009, **20**, 1809–1812; (*c*) M. R. Mish, F. M. Guerra and E. M. Carreira, *J. Am. Chem. Soc.*, 1997, **119**, 8379–8380.

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7 (*a*) O. Ovdiichuk, L. Flauder, E. Humbert-Voss, B. Jamart-Grégoire and A. Arrault, *Tetrahedron Lett.*, 2019, **60**, 16–19; (*b*) I. Duttagupta, D. Misra, S. Bhunya, A. Paul and S. Sinha, *J. Org. Chem.*, 2015, **80**, 10585–10604; (*c*) E. Humbert-Voss, A. Arrault and B. Jamart-Grégoire, *Tetrahedron*, 2014, **70**, 363–370; (*d*) Y. Che and G. R. Marshall, *J. Org. Chem.*, 2004, **69**, 9030–9042; (*e*) W.-J. Zhang, A. Berglund, J. L.-F. Kao, J.-P. Couty, M. C. Gershengorn and G. R. Marshall, *J. Am. Chem. Soc.*, 2003, **125**, 1221–1235.

8 I. Duttagupta, J. Bhadra, S. K. Das and S. Sinha, *Tetrahedron Lett.*, 2016, **57**, 3858–3861.

9 (*a*) I. Duttagupta, K. Goswami, P. Chatla and S. Sinha, *Synth. Commun.*, 2014, **44**, 2510–2519; (*b*) I. Duttagupta, K. Goswami and S. Sinha, *Tetrahedron*, 2012, **68**, 8347–8357.

10 L. Fowden, *Nature*, 1955, **176**, 347–348.

11 For a review on Aze synthesis, see: F. Couty and G. Evano, *Org. Prep. Proced. Int.*, 2006, **38**, 427–465.

12 G. Revilla-López, J. G. Warren, J. Torras, A. I. Jiménez, C. Cativiela and C. Alemán, *Pept. Sci.*, 2012, **98**, 98–110.

13 (*a*) C. Melis, G. Bussi, S. C. R. Lummis and C. Molteni, *J. Phys. Chem. B*, 2009, **113**, 12148–12153; (*b*) D. Kern, M. Schutkowski and T. Drakenberg, *J. Am. Chem. Soc.*, 1997, **119**, 8403–8408.

14 (*a*) C. Dean, S. Roesner, S. Rajkumar, G. J. Clarkson, M. Jones and M. Shipman, *Tetrahedron*, 2021, **79**, 131836; (*b*) C. Dean, S. Rajkumar, S. Roesner, N. Carson, G. J. Clarkson, M. Wills, M. Jones and M. Shipman, *Chem. Sci.*, 2020, **11**, 1636–1642; (*c*) S. Rajkumar, G. J. Clarkson and M. Shipman, *Org. Lett.*, 2017, **19**, 2058–2061; (*d*) M. J. Brown, G. J. Clarkson, G. G. Inglis and M. Shipman, *Org. Lett.*, 2011, **13**, 1686–1689.

15 Y. Aoyagi, Y. Saitoh, T. Ueno, M. Horiguchi, K. Takeya and R. M. Williams, *J. Org. Chem.*, 2003, **68**, 6899–6904.

16 D. Lainé, M. Fujita and S. V. Ley, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1639–1645.

17 (*a*) A. Oliva, M. Ellis, L. Fiocchi, E. Menta and A. P. Krapcho, *J. Heterocycl. Chem.*, 2000, **37**, 47–55; (*b*) P. Cerea, C. Giannini, S. Dall'Angelo, E. Licandro, S. Maiorana and R. Marchelli, *Tetrahedron*, 2007, **63**, 4108–4119.

18 See the ESI† for a detailed description.

19 B. Long, S. Tang, L. Chen, S. Qu, B. Chen, J. Liu, A. R. Maguire, Z. Wang, Y. Liu, H. Zhang, Z. Xu and T. Ye, *Chem. Commun.*, 2013, **49**, 2977–2979.