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**PAPER**

# Chemoselective cyclization of unprotected linear peptides by α-ketoacid–hydroxylamine amide-ligation†‡

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Cyclic peptides are important synthetic targets due to their constrained conformation, enhanced metabolic stability and improved bioavailability, which combine to make them promising lead compounds for drug candidates. They are typically synthesized by a multi-step sequence of carefully orchestrated protecting group manipulations and cyclization of side-chain protected linear precursors. In the present manuscript we disclose an alternative approach to the synthesis of peptide macrocycles by the α-ketoacid–hydroxylamine (KAHA) ligation. This reaction allows readily prepared linear peptides to be cyclized without reagents or side-chain protecting groups and delivers a native backbone amide bond at the ligation site. The precursors are prepared with Fmoc-based solid phase peptide synthesis using reagents that we have previously disclosed. No post-cyclization manipulations or deprotections other than purification are required. This protocol was applied to five different cyclic peptide natural products of varying ring sizes and side chain functionalities.

## Introduction

Cyclic peptides are widely recognized as powerful platforms for the development of biologically active molecules.[1] In addition to their widespread occurrence as natural products, cyclic peptides are now common motifs for the design of unnatural compounds as therapeutic agents. Their success is largely attributed to improved biological properties relative to their linear forms.[2] Confining a peptide into a cyclic structure results in increased resistance to proteolytic cleavage, enhanced stability and bio-availability, and reduced conformational freedom. For these reasons, cyclic peptides remain important synthetic targets for both chemists and biochemists.[3]

Current synthetic strategies towards cyclic peptides rely on the use of orthogonally protected precursors, which are selectively unmasked at specific functional groups.[4] Cyclization of these semi-protected linear peptides with excess amounts of coupling reagents can be performed either in solution or on solid support.[5] This is a successful method but requires several additional manipulations beyond standard solid-phase peptide synthesis including a late-stage global deprotection. The need to retain protecting groups on the side chains also induces steric demands than can hamper cyclizations. Furthermore, the use of coupling reagents limits, to some extent, the utility of high dilution methods that favour cyclic peptide formation. Native chemical ligation allows chemoselective intramolecular amide bond formation between C-terminal thioester and N-terminal cysteine to directly deliver unprotected cyclic peptides.[6] The primary constraints of NCL are the requirement for a cysteine residue at the cyclization site and the relative difficulties of obtaining the C-terminal thioesters. To address these limitations while maintaining the attractive properties of NCL, chemists have continually sought to apply novel ligation reactions to the preparation of cyclic peptides. Recent progress includes the use of traceless Staudinger ligation, which works most efficiently with glycine as the ligation residue,[7] and cyclizations of thioacids.[8] The synthesis of cyclic peptides with non-amide connections by azide–alkyne cyclization[9] or Ugi-type condensations[10] are also attractive routes to peptide-based macrocycles. Bioengineering approaches to the production of libraries of cyclic peptides are also of great contemporary interest.[11]

Our group has developed a chemoselective amide-forming ligation reaction between C-terminal peptide α-ketoacids and N-terminal hydroxylamine (KAHA ligation).[12] This reaction has been demonstrated to proceed in the presence of unprotected side chain functionalities,[13] which would allow the direct cyclization of unprotected linear oligopeptides. A final deprotection step is not necessary and cyclization can be performed on side-chain unprotected substrates. Importantly, the amide-formation proceeds over a wide range of concentrations (0.2 M–0.0001 M)

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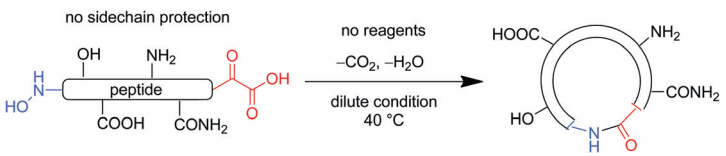
and does not require any added reagents or catalysts. In the context of peptide cyclization, reagent-less conditions would allow the use of high dilution without diminishing the cyclization rate, thereby favouring cyclization over oligomerization and potentially diminishing racemization. We envisioned that applying this strategy toward the cyclization of side-chain unprotected seco-peptides that could be prepared with standard Fmoc-based solid-phase peptide synthesis in combination with reagents and resins that we have previously reported. This article describes the successful implementation of this strategy and its application to several examples of cyclic peptide natural products (Scheme 1).[14]

## Results and discussion

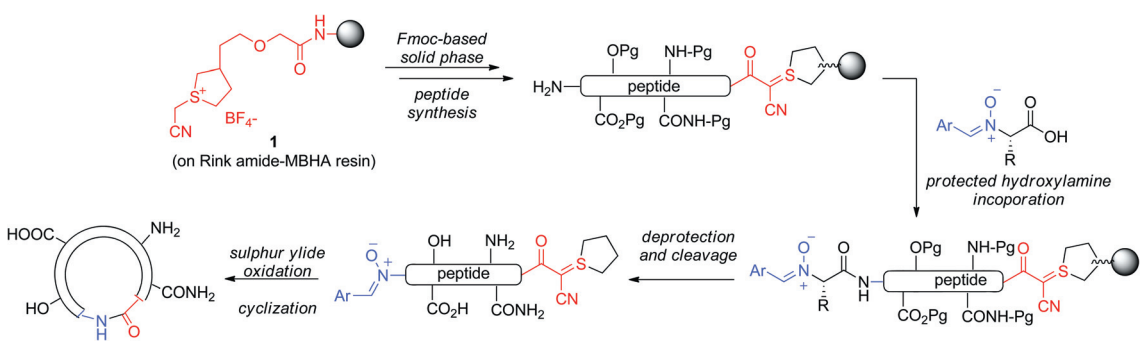
The greatest obstacle to implementing the KAHA ligation for the peptide cyclization is the installation of the requisite reactive partners into the linear peptide sequence prior to the cyclization step. The preparation of peptide-derived C-terminal α-ketoacids has been previously demonstrated in our laboratory with a solid-supported sulphur ylide linker that can be used with standard Fmoc-based solid phase peptide synthesis.[15] This process affords side-chain unprotected peptide sulphur ylides, which serve as the immediate precursors to unprotected peptide α-ketoacids.[16] Our group has also described general methods to synthesize N-terminal, *N*-hydroxyamino acids protected as *N*-benzylidene nitrones.[17] Deprotection of the nitrones can be performed to afford either side-chain protected or unprotected N-terminal peptide hydroxylamines and is suitable for the preparation of several enantiomerically pure *N*-hydroxylamino acid residues. The nitrone-protection also provides the flexibility of unmasking the *N*-hydroxyamino acid at different stages and enables us to carry the protected hydroxylamine through resin cleavage and side-chain deprotection. We envisioned that if a masked hydroxylamine was introduced at the end of a peptide bound to a resin *via* our sulphur ylide linker (Scheme 2), cleavage from the resin would afford a linear peptide sequence with an α-ketoacid–hydroxylamine precursor residing on each terminus. Provided that the nitrone-protected *N*-hydroxylamine survived the subsequent sulphur ylide oxidation, deprotection and cyclization would result in the desired cyclic peptide target.

We began our investigations with the synthesis of the prototypical cyclic peptide natural product Gramacidin S.[18] The preparation of linear peptide precursor **2** was accomplished using a MBHA resin-bound sulfonium salt using standard 9-fluorenylmethoxycarbonyl (Fmoc)-based solid phase peptide synthesis in analogy to our previously described protocols (Scheme 3).[15] The protected hydroxylamine **3** was prepared from the corresponding amino acid and introduced by standard peptide coupling protocols as previously described. Cleavage of the resin bound peptide (0.22–0.32 mmol $g^{-1}$) was performed with neat TFA at room temperature to give nitrone–sulphur ylide **4**. To avoid premature hydrolysis during the cleavage step, we found it essential to exclude moisture from the reaction by using only $CH_2Cl_2$ and TFA (no added $H_2O$). When 3% of water was added in the cleavage cocktail, rapid hydrolysis of the nitrone to the hydroxylamine occurred along with subsequent formation of an oxime side product. If desired, the cleaved peptide could be purified by reversed phase preparative HPLC prior to cyclization. Although subsequent cyclizations using purified precursors were somewhat cleaner, as assayed by HPLC, partial hydrolysis of the nitrone-protecting group during the purification was often observed and we therefore elected to perform the oxidation and cyclization of the unpurified peptides obtained directly from resin cleavage.

We have previously reported that oxidation of sulphur ylides to α-ketoacids occurs easily and cleanly with Oxone under aqueous solution.[16] When using *N*-benzylidene protected hydroxylamines, however, the acidic Oxone solution induced hydrolysis of the nitrone and led to the formation of hydroxylamine–α-ketoacids and the oxidized oxime–ketoacids as side products. Dimethoxyldioxirane (DMDO) was therefore

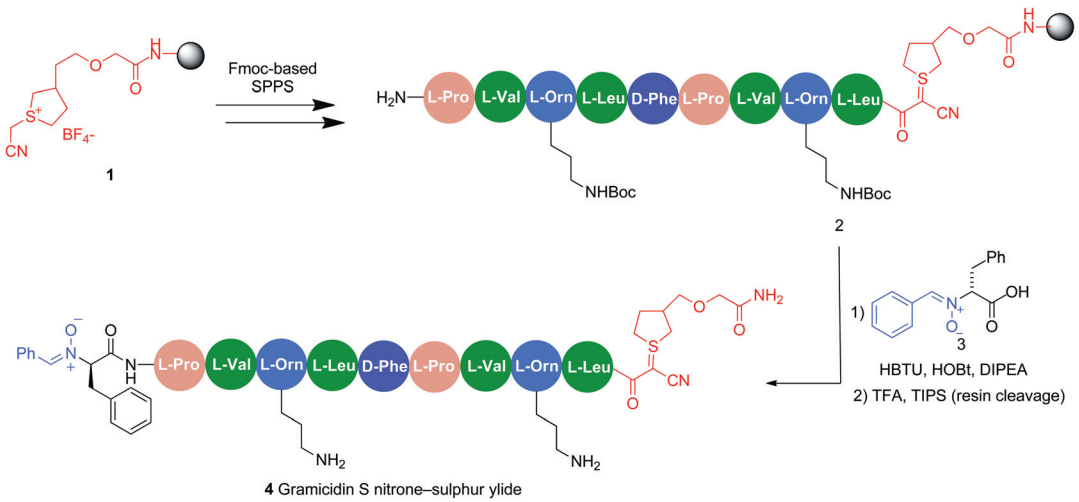



**Scheme 1** Cyclization of unprotected linear peptides by α-ketoacid–hydroxylamine amide ligation.

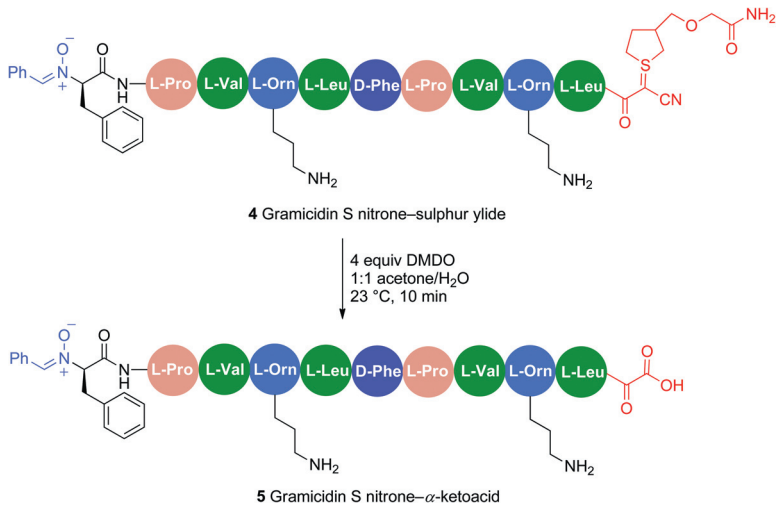



**Scheme 2** General strategy for preparation of unprotected linear peptides with N-terminal hydroxylamines and C-terminal α-ketoacids for direct, reagent-less cyclization.




**Scheme 3** Representative synthesis of N-terminal nitrone protected peptide α-ketoacids; the synthesis of *seco*-gramicidin S is shown. Other cyclic peptide precursors were prepared in an analogous fashion (see ESI‡ for details).

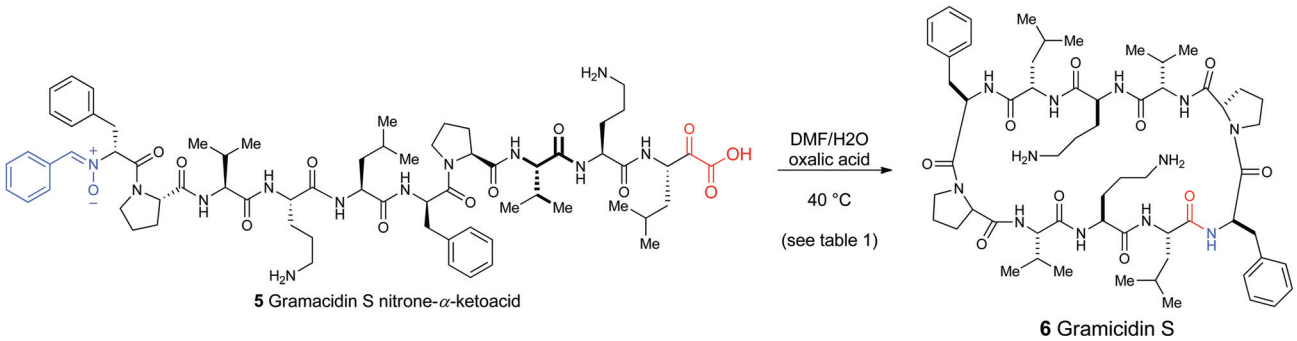



**Scheme 4** Chemoselective oxidation of sulphur ylide **4** without nitrone deprotection using aqueous DMDO.

chosen as an alternative oxidant that does not require acidic conditions.[19] DMDO was prepared as a solution in acetone and effected rapid oxidation of the sulphur ylide to the α-ketoacid. Dimethylsulphide was added at the end of the oxidation to quench any excess oxidant. The nitrone was found to be stable through the oxidation process. The Gramicidin S precursor is a representative example (Scheme 4): treatment of the crude nitrone–sulphur ylide **4** with DMDO in acetone and water mixture for 10 min yielded nitrone–ketoacid **5** with only small amounts of the nitrone–carboxylic acid and oxime–α-ketoacid as side products.

The crude peptide incorporating C-terminal α-ketoacid and N-terminal nitrone (**5**) was directly subjected to cyclization in degassed DMF and water mixture (Scheme 5). To suppress the formation of oxime, which arises from oxidation of the hydroxylamine,[17] and increase the ligation yield, we examined different solvents and acid additives. The presence of water is crucial; with only anhydrous DMF as solvent, very little or no ligation product was observed by LCMS analysis. This was attributed to the need for a small amount of water to hydrolyse the nitrone and reveal the hydroxylamine. The nitrone hydrolysis was aided by the addition of oxalic acid and we found that only a trace amount of the cyclized product was formed when no oxalic acid was used as an additive. The use of hydrochloric acid as an additive also produced the cyclized products, albeit in slightly diminished yields (Table 1).

The formation of dimerized products or oligomers by intermolecular cyclization is closely related to substrate concentration. As a model compound to examine the effect of concentration on the peptide cyclization, we selected the formation of semi-gramicidin S (**9**), which is well-known to prefer dimerization to cyclization under typical reaction conditions.[20] The semi-gramicidin S precursor **11** was subjected to three separate reactions under different concentrations (Scheme 6). Under dilute conditions (0.001 M), only trace amount of the dimer, gramicidin S was generated (47 : 1). As the concentration was increased to 0.01 M and 0.1 M, the amount of dimer formed also increased (17 : 1 and 2 : 1).




**5** Gramacidin S nitrone-$\alpha$-ketoacid

**6** Gramicidin S

**Scheme 5** Cyclization of unprotected seco-peptides by ketoacid–hydroxylamines.

**Table 1** Cyclization of linear peptides by KAHA ligation

| Entry | Cyclic peptide | Cyclization condition[a] | HPLC yield (%)[b] | Isolated yield (%)[c] | Average yield per step (%)[e] |
|---|---|---|---|---|---|
| 1 | **Gramicidin S** (**6**) *cyclo* (-$^{D}$FPVOL$^{D}$FPVOL-) | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (50 : 1) | 13 | 13 | 66 |
| 2 | | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (5 : 1) | 10 | nd[d] | 63 |
| 3 | | 0.001 M DMF : 1 M HCl : DMS (5 : 1 : 0.1) | 11 | nd[d] | 64 |
| 4 | **Tyrocidine A** (**7**) *cyclo*- (-$^{D}$FPF$^{D}$FNQYVOL-) | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (50 : 1) | 36 | 22 | 81 |
| 5 | | 0.001 M DMF : 1 M HCl : DMS (5 : 1 : 0.1) | 9 | 4 | 62 |
| 6 | **Hymenamide B** (**8**) cyclo(-FPPNFVE-) | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (50 : 1) | 15 | 8 | 68 |
| 7 | **semi Gramicidin S** (**9**) cyclo (-$^{D}$FPVOL-) | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (50 : 1) | 17 | 15 | 70 |
| 8 | **Stylostatin A**[f] (**10**) *cyclo*(-AISN$^{D}$FPL) | 0.1 M $(COOH)_2$, 0.001 M DMF : $H_2O$ (50 : 1) | 13 | 7 | |

[a] Reactions were carried out at 40 °C for 48 h. [b] The crude yield is determined from standard curve of purified cyclic peptide. [c] Isolated yield after purification on reversed phase preparative HPLC. [d] Not determined. [e] Average yield per step for hydroxylamine coupling, resin cleavage and deprotection, oxidation, nitrone hydrolysis, and cyclization. [f] A *p*-nitrobenzylidene nitrone was used instead of the benzylidene nitrone.

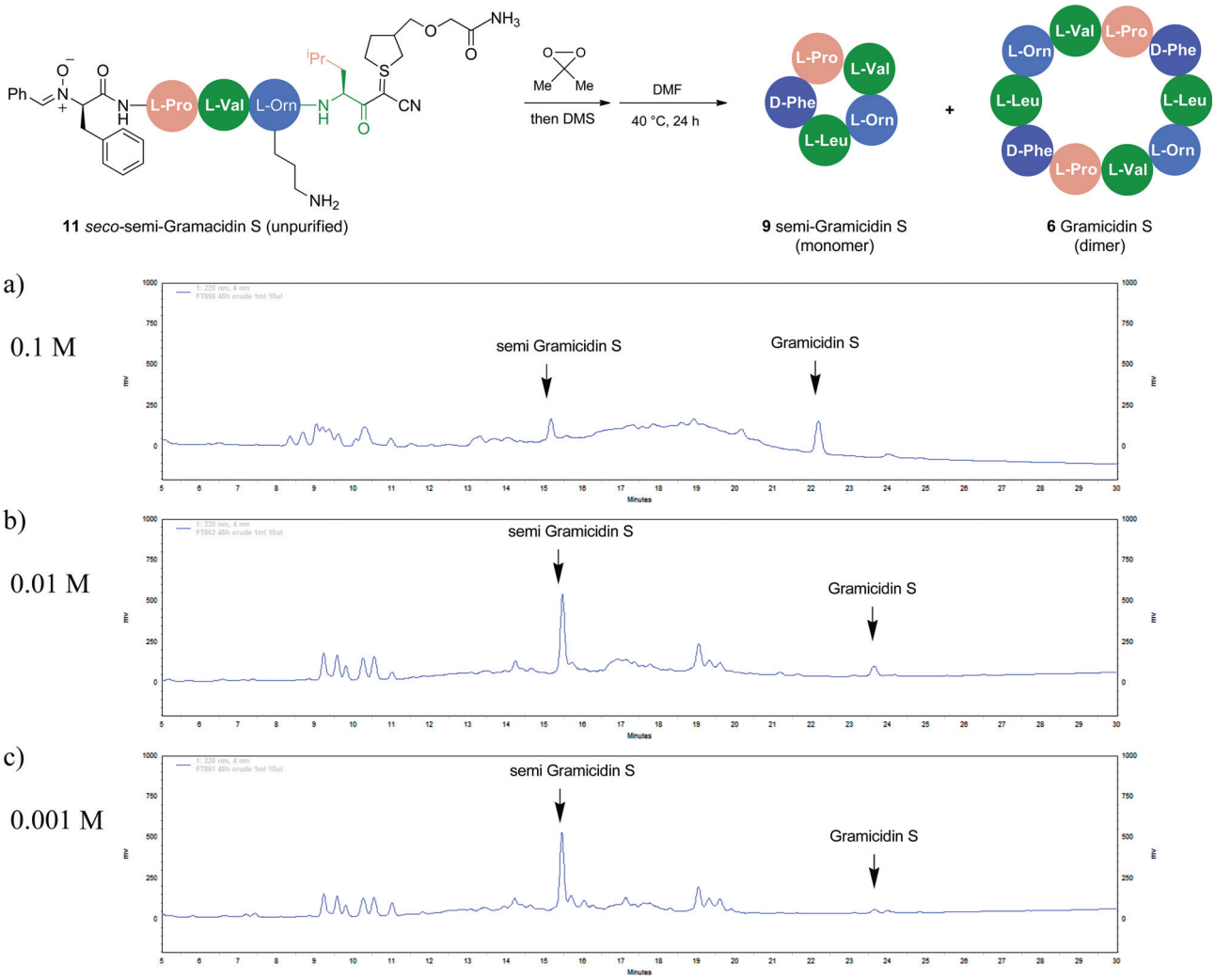


Epimerization is known to be one of the major concerns in peptide chemistry, particularly during the formation of cyclic peptides. In previous studies we have shown that some empimerization of the α-ketoacid can occur during oxidation of the sulphur ylide to the α-ketoacid. In our efforts to investigate the epimerization issue during intramolecular cyclization, cyclic peptide containing both epimeric amino acids at both the α-ketoacid as well as the nitrone/hydroxylamine sides of the cyclization point were prepared separately. Co-injection on HPLC demonstrated that a small amount (approx. 5%) of epimerization occurred at the α-ketoacid residue (see ESI‡). The nitrone–hydroxylamine residue was configurationally stable throughout the sequence. In all cases studied, the epimerized macrocyclic product had a significantly different retention time and the desired product was easily purified by preparative HPLC.

The established protocol was applied to formation of other cyclic peptides of several ring sizes and with various unprotected side chains (Fig. 1 and Table 1). The target peptides were chosen to demonstrate the compatibility of a variety of functional group to the method. The cyclic peptides selected include unprotected side chains with primary and secondary amines, hydroxyl moeities, and acid functionalities. The peptide sulphur ylides were prepared on a resin support with standard Fmoc-based SPPS. The nitrone-protected hydroxylamines were introduced during the final coupling step and cleavage of the Rink amide resin provided the side-chain unprotected nitrone–sulphur ylides. After oxidation and cyclization, the cyclic peptides were isolated as pure products in respectable yields by preparative HPLC. In all cases, the oxime formation was the most significant side product. In the case of longer peptides (>5 residues), dimer formation was not observed under the standard cyclization conditions (0.001 M).

We have previously noted that alanine and glycine derived nitrones are more resistant to hydrolysis.[17] To address this challenge, we chose Leu–Ala as the disconnection site during synthesis of stylostatin A. Under the standard cyclization conditions, the benzylidene nitrone of alanine was resistant to hydrolysis; no cyclized products were observed. Experiments performed using TFA instead of oxalic acid in different solvents, as well as with hydrochloric acid and *p*-toluenesolfonic acid provided only the starting nitrone and decomposition. Previously studies have suggested that nitrone hydrolysis is often kinetically feasible but thermodynamically unfavourable;[21] therefore, sequestering the




**Scheme 6** Effect of substrate concentration on cyclization *vs.* dimerization–cyclization of a seco-gramicidin S peptide. HPLC analysis was performed directly from the reaction mixtures without workup or prior purification.

benzaldehyde byproduct can facilitate the hydrolysis. We attempted to achieve deprotection by passing the protected α-ketoacid–nitrone peptide through a column of solid supported hydroxylamine and C-18 silica gel, a protocol we have previously reported,[17] but without success. When water was added during cleavage of the peptide from the resin, the alanine nitrone could be hydrolysed to yield the hydroxylamine–sulphur ylide. However, the unprotected hydroxylamine does not tolerate the subsequent oxidation and an alternative nitrone protecting group was sought.

A variety of derivatized benzylidene nitrones (Fig. 2) were prepared by condensing *O-tert*-butyl protected Ala-hydroxylamine with the corresponding aldehydes. After *tert*-butyl removal, the acids were loaded onto the end of the hexapeptide sulphur ylide bound to the resin. After cleavage, trace amounts of hydroxylamines were observed and the nitrone–sulphur ylides were obtained as the major products. The cleaved nitrone–sulphur ylide peptides were subjected to the standard DMDO oxidation to give the stable nitrone–α-ketoacids. We found that 4-methoxy, 2,4-dimethoxy, and 3-dimethylamino-substituted benzylidene nitrones were difficult to deprotect. Stirring in degassed hydrolysis conditions at 40–60 °C provided only the nitrone–α-ketoacids, nitrone-carboxylic acid and their oxime degradation products. Treating the cleaved sulphur ylide directly with acid and water revealed that these nitrones were robust enough to survive these conditions.

We found that 4-nitrobenzylidene nitrone **17**, which is an electron-deficient nitrone, was more prone to hydrolysis and release of the hydroxylamine. After peptide assembly and installation of the nitrone protected hydroxylamine as the last residue on solid phase, the peptide could be cleaved from the resin and purified on reversed phase preparative HPLC (Scheme 7). Although cyclization of the purified precursor was cleaner when monitored on HPLC, nitrone hydrolysis on the column was observed during the purification process; the crude peptide was therefore used directly. Using N-terminal *para*-nitrobenzylidene nitrone with a C-terminal α-ketoacid, the cyclized product stylostatin A (**10**) was isolated in 6% yield along with recovered nitrone–α-ketoacid and 13% of the oxime–ketoacid side product. These results suggest that modulation of the nitrone protecting group

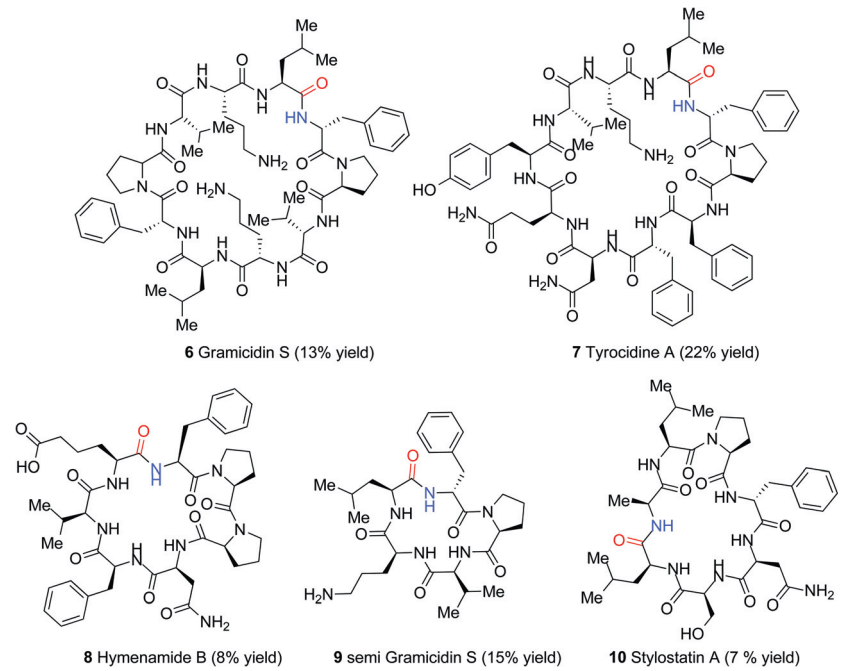



**Fig. 1** Cyclic peptides prepared by KAHA-ligation of unprotected linear peptides and isolated yields (preparative HPLC and based on resin loading) following Fmoc-solid phase peptide synthesis, resin cleavage and deprotection, sulphur ylide oxidation, and nitrone cleavage–cyclization. The ligation sites are indicated in color.

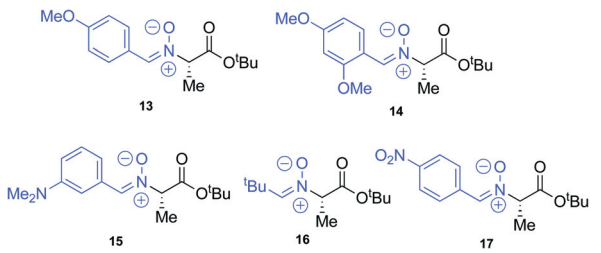
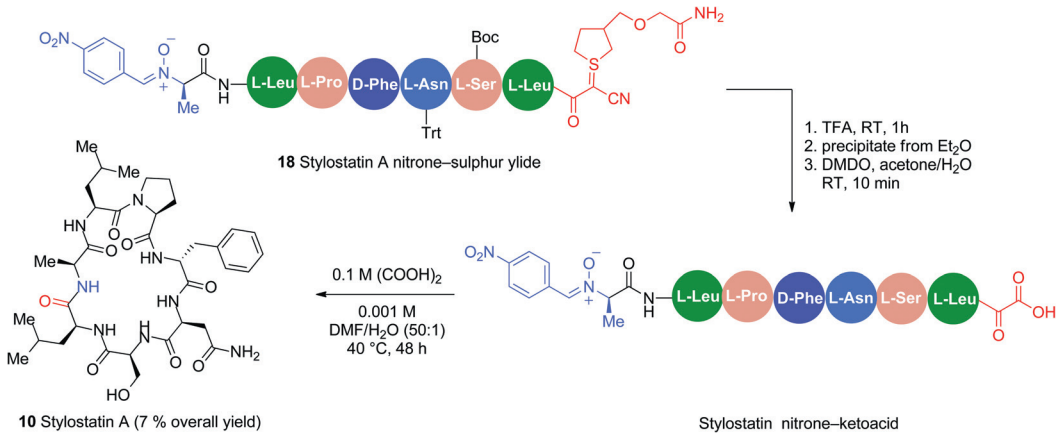



**Fig. 2** Various benzylidene nitrones investigated to facilitate hydrolysis of alanine hydroxylamines.

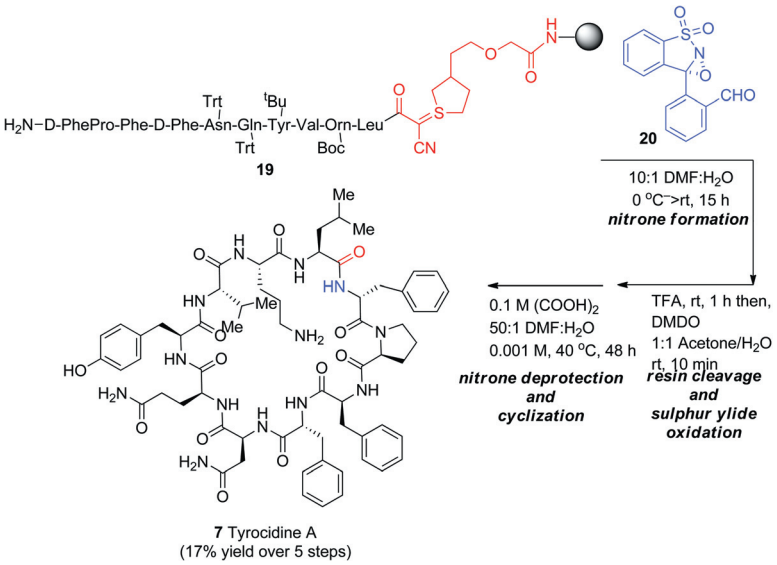
can be used to improve the cyclization conditions and adjust the rate at which the nitrone moiety undergoes deprotection. Increasing the amount of water present in the reaction mixture may facilitate nitrone hydrolysis, but our recent mechanistic studies into this amide-formation suggest that this may be detrimental to the amide-formation using these reaction partners.[22]

The method for the synthesis of cyclic peptides described above requires the cyanosulphur ylide linker and the appropriate nitrone-protected N-terminal amino acids. The latter must be prepared by a several step chemical synthesis from the corresponding protected α-amino acids. To avoid the need for the prior preparation of the protected *N*-hydroxyamino acid, we have reported the design and development of reagent **20** for the direct oxidation of solid-supported N-terminal peptide amines to nitrones, which can be hydrolysed to give the N-terminal peptide hydroxylamines.[23] The use of this reagent should allow for the preparation of the linear peptide precursors without the need for




**Scheme 7** Successful nitrone cleavage and cyclization of *para*-nitrobenzylidene protected N-terminal alanine hydroxylamine.




**Scheme 8** N-terminal hydroxylamine synthesis using oxaziridine reagent **20**. In this approach only the sulphur ylide linker and reagent **20** are needed to prepare cyclic peptides under standard Fmoc-SPPS conditions.

independent preparation of the hydroxylamine monomers. To test the suitability of this reagent for cyclic peptide synthesis, we performed the synthesis of tyrocidine A using **20** to introduce the N-terminal hydroxylamine from the linear peptide **19** with an N-terminal D-phenyl alanine residue (Scheme 8). Following resin cleavage, sulphur ylide oxidation, and cyclization we obtained tyrocidine A in 17% yield based on the resin loading. The yield is somewhat less than the route using the independently synthesized, benzylidene nitrone protected *N*-hydroxy-D-phenyl alanine. This route, however, utilizes a common resin for the α-ketoacid and a single reagent for hydroxylamine formation to be applicable to a larger variety of linear peptides as no separate synthesis of the hydroxylamine monomers is required.

## Conclusions

In summary, we have developed a new and convenient method for the synthesis of cyclic peptides by reagentless cyclization of peptides by α-ketoacid–hydroxylamine (KAHA) ligation. This strategy allows for the specific cyclization of linear peptides without the need for side chain protecting groups. Our present studies demonstrate that unprotected amines, carboxylic acids, phenols, and alcohols are tolerated. By using a generic sulphur ylide resin for introducing the α-ketoacid and oxaziridine reagent **20** for introducing the hydroxylamine, cyclic peptides can be prepared using standard Fmoc-based peptide synthesis. Isolation of the intermediate linear peptides is not necessary and no other regents, orthogonal protecting groups, or post-cyclization manipulations are needed. We anticipate that this mechanistically distinct amide-forming cyclization will find application for the ever-growing number of natural and unnatural cyclic peptide targets.

## Experimental

For experimental procedures, HPLC traces, compound characterization, and determination of resin loading please see the ESI.‡

### Synthesis of tyrocidine A with reagent 20

To resin bound peptide ($H_2N$–D-Phe-Pro-Phe-D-Phe-Asn-(Trt) Gln-Tyr-Val-(Boc)Orn-Leu-sulphur ylide-$NH_2$, 50.3 mg, approx. 0.20 mmol $g^{-1}$) in DMF–$H_2O$ (10 : 1, 0.5 ml) was added enantiopure (*R*)-**20** (14.3 mg, 0.0498 mmol, 5 equiv) in DMF–$H_2O$ (10 : 1, 0.5 ml) at 0 °C. The reaction mixture was gently stirred and slowly allowed to warm to rt over 15 h. The resin was filtered and washed with MeOH and $CH_2Cl_2$. The resin bound peptide was cleaved from the resin by TFA (1 ml) at rt for 1 h. The mixture was filtered through a small plug of cotton wool into a centrifuge tube and rinsed with a small volume of TFA. The filtrate was reduced to minimum volume by a nitrogen stream and $Et_2O$ was added. The white precipitate was collected by centrifugation and dried under reduced pressure to give the crude peptide. To a solution of the crude peptide in 1.0 mL acetone–$H_2O$ (1 : 1) at rt was added DMDO (0.175 M in acetone, 0.23 ml, 0.040 mmol, 4 equiv) and the mixture was stirred. After 10 min, the reaction was quenched by the addition of dimethylsulfide (4 drops). The solvent was removed under reduced pressure to give the crude peptide. The crude peptide was diluted to 0.001 M with degassed 10 mL 0.1 M $(COOH)_2$ DMF–$H_2O$ (50 : 1) and the temperature was raised to 40 °C for 48 h. The solvent was removed under reduced pressure and the crude material was diluted with MeOH (1 mL). Analysis of this sample against a standard curve showed that tyrocidine A (**7**) was formed in 17% yield for the overall process. For the isolation of tyrocidine A and other peptide, as well as characterization data and HPLC traces, see the ESI.‡

## Acknowledgements

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## References


1 (*a*) M. Zasloff, Antimicrobial peptides of multicellular organisms, *Nature*, 2002, **415**, 389–395; (*b*) M. Trabi and D. J. Craik, Circular proteins – no end in sight, *Trends Biochem. Sci.*, 2002, **27**, 132–138; (*c*) C. Jennings, J. West, C. Waine, D. Craik and M. Anderson, Biosynthesis and insecticidal properties of plant cyclotides: the cyclic knotted proteins from Oldenlandia affinis, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 10614–10619.

2 (*a*) D. J. Craik, Seamless proteins tie up their loose ends, *Science*, 2006, **331**, 1563–1564; (*b*) H. Kessler, Conformation and biological activity of cyclic peptides, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 512–523.

3 (*a*) P. Li, P. P. Roller and J. Xu, Current synthetic approaches to peptide and peptidomimetic cyclization, *Curr. Org. Chem.*, 2002, **6**, 411–440; (*b*) S. Jiang, Z. Li, K. Ding and P. P. Roller, Recent progress of synthetic studies to peptides and peptidomimetic cyclization, *Curr. Org. Chem.*, 2008, **12**, 1502–1542; (*c*) J. S. Davies, The cyclization of peptides and depsipeptides, *J. Pept. Sci.*, 2003, **9**, 471–501.

4 C. J. White and A. K. Yudin, Contemporary strategies for peptide macrocyclization, *Nat. Chem.*, 2011, **3**, 509–524.

5 (*a*) M. Katsara, Round and round we go: cyclic peptides in disease, *Curr. Med. Chem.*, 2006, **13**, 2221–2232; (*b*) W. F. DeGrado and E. T. Kaiser, Solid phase synthesis of protected peptides on a polymer-bound oxime, *J. Org. Chem.*, 1982, **47**, 3258–3261; (*c*) G. T. Bourne, W. D. Meutermans, P. F. Alewood, R. P. McGeary, M. Scanion, A. A. Watson and M. L. Smythe, A backbone linker for Boc-based peptide synthesis and on-resin cyclization: synthesis of stylostatin 1, *J. Org. Chem.*, 1999, **64**, 3095–3101.

6 (*a*) J. A. Camarero and T. W. Muir, Chemoselective backbone cyclization of unprotected peptides, *Chem. Commun.*, 1997, 1369–1370; (*b*) J. A. Camarero, J. Pavel and T. W. Muir, Chemical synthesis of a circular protein domain: evidence for folding-assisted cyclization, *Angew. Chem., Int. Ed.*, 1998, **37**, 347–349.

7 (*a*) O. David, W. J. N. Meester, H. Bieräugel, H. E. Schoemaker, H. Hiemstra and J. H. van Marrseveen, Intramolecular Staudinger ligation: a powerful ring-closure method to form medium-sized lactams, *Angew. Chem., Int. Ed.*, 2003, **42**, 4373–4375; (*b*) R. Kleineweischede and C. P. R. Hackenberger, Chemoselective peptide cyclization by traceless Staudinger ligation, *Angew. Chem., Int. Ed.*, 2008, **47**, 5984–5988.

8 K. Sasaki and D. Crich, Cyclic peptide synthesis with thioacids, *Org. Lett.*, 2010, **12**, 3254–3257.

9 (*a*) R. A. Turner, A. G. Oliver and S. R. Lokey, Click chemistry as a macrocyclization tool in the solid-phase synthesis of small cyclic peptides, *Org. Lett.*, 2007, **9**, 5011–5014; (*b*) V. D. Bock, D. Speijer, H. Hiemstra and J. H. van Maarseveen, 1,2,3-triazoles as peptide bond isosteres: synthesis and biological evaluation of cyclotetrapeptide mimics, *Org. Biomol. Chem.*, 2007, **5**, 971–975.

10 (*a*) R. Hili, V. Rai and A. K. Yudin, Macrocyclization of linear peptides enabled by amphoteric molecules, *J. Am. Chem. Soc.*, 2010, **132**, 2889–2891; (*b*) B. H. Rotstein, V. Rai, R. Hili and A. K. Yudin, Synthesis of peptide macrocycles using unprotected amino aldehydes, *Nat. Protoc.*, 2010, **5**, 1813–1822.

11 (*a*) J. W. Trauger, R. M. Kohli, H. D. Mootz, M. A. Marahiel and C. T. Walsh, Peptide cyclization catalysed by the thioesterase domain of tyrocidine synthetase, *Nature*, 2000, **407**, 215–218; (*b*) R. M. Kohli, M. D. Burke, J. Tao and C. T. Walsh, Chemoenzymatic route to macrocyclic hybrid peptide/polyketide-like molecules, *J. Am. Chem. Soc.*, 2003, **125**, 7160–7161.

12 J. W. Bode, R. M. Fox and K. D. Baucom, Decarboxylative condensations of α-ketoacids and *N*-alkylhydroxylamines: a new amide ligation reaction, *Angew. Chem., Int. Ed.*, 2006, **45**, 1248–1252.

13 J. Wu, J. Ruiz-Rodríguez, J. M. Comstock, J. Z. Dong and J. W. Bode, Synthesis of human GLP-1 (7–36) by chemoselective α-ketoacid–hydroxylamine peptide ligation of unprotected fragments, *Chem. Sci.*, 2011, **2**, 1976–1979.

14 We have recently reported the synthesis of epi-aza-Surfactin by cyclization of a linear peptide using the KAHA-amide formation. This work involved the use of an N-terminal beta-amino acid-derived hydroxylamine; Y. L. Huang, R. Frey, M. E. Juarez-Garcia and J. W. Bode, Synthesis of aza-surfactin and 3-epi-aza-surfactin, *Heterocycles*, 2012, **84**, 1179–1191.

15 L. Ju and J. W. Bode, A general strategy for the preparation of C-terminal peptide α-ketoacids by solid phase peptide synthesis, *Org. Biomol. Chem.*, 2009, **7**, 2259–2264.

16 L. Ju and A. R. Lippert, Stereoretentive synthesis and chemoselective amide-forming ligations of C-terminal peptide α-ketoacids, *J. Am. Chem. Soc.*, 2008, **130**, 4253–4255.

17 S. I. Medina, J. Wu and J. W. Bode, Nitrone protecting groups for enantiopure *N*-hydroxyamino acids: synthesis of *N*-terminal peptide hydroxylamines for chemoselective ligations, *Org. Biomol. Chem.*, 2010, **8**, 3405–3417.

18 G. F. Gause and M. G. Brazhnikova, Gramicidin S and its use in the treatment of infected wounds, *Nature*, 1944, **154**, 703.

19 M.-K. Wong, C.-W. Yu, W.-H. Yuen and D. Yang, *J. Org. Chem.*, 2001, **66**, 3606–3609.

20 M. Tamaki, S. Akabori and I. Muramatsu, CD spectra and cyclisation of linear pentapeptides as gramicidin S precursors with Orn(Z), *Bull. Chem. Soc. Jpn.*, 1993, **66**, 3113–3115.

21 S. M. Turega, C. Lorenz, J. W. Sadownik and D. Philip, Target-driven selection in a dynamic nitrone library, *Chem. Commun.*, 2008, 4076–4078.

22 I. Pusterla and J. W. Bode, The mechanism of the α-ketoacid-hydroxylamine (KAHA) amide-forming ligation, *Angew. Chem., Int. Ed.*, 2012, **51**, 513–516.

23 T. Fukuzumi and J. W. Bode, A reagent for the convenient, solid-phase synthesis of N-terminal peptide hydroxylamines for chemoselective ligations, *J. Am. Chem. Soc.*, 2009, **131**, 3864–3865.




 