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Difficult Macrocyclizations: New Strategies for Synthesizing Highly Strained Cyclic Tetrapeptides

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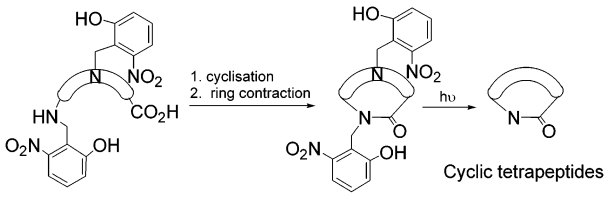
ABSTRACT



Cyclic tetrapeptides are an intriguing class of natural products. To synthesize highly strained cyclic tetrapeptides we developed a macrocyclization strategy that involves the inclusion of 2-hydroxy-6-nitrobenzyl (HnB) group at the N-terminus and in the "middle" of the sequence. The N-terminal auxiliary performs a ring closure/ring contraction role, and the backbone auxiliary promotes *cis* amide bonds to facilitate the otherwise difficult ring contraction. Following this route, the all-L cyclic tetrapeptide *cyclo*-[Tyr-Arg-Phe-Ala] was successfully prepared.

Historically natural products have played an important role in drug development, as either chemical tools to validate targets or as a rich source of drug or lead compounds.[1] Cyclic tetrapeptides[2−11] are a class of natural products that have been characterized as potent and highly selective molecules in a diverse range of therapeutic areas (i.e., a privileged structure).[12] Typical examples are the cytotoxic and antimitogenic agents HC toxin,[2,3] chlamydocin,[2−4] the antitumor agent

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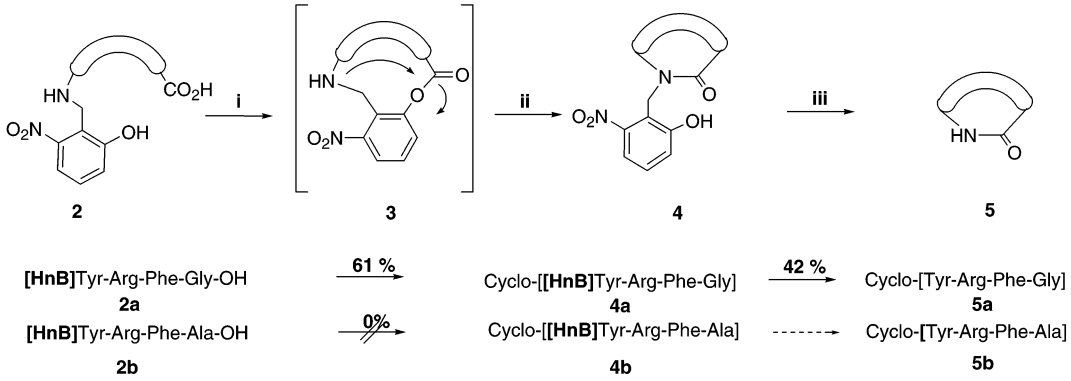
Scheme 1. Synthesis of Difficult Sequences through a Ring Contraction Strategy[a]



[a] Reagents and conditions: (i) 1 equiv of BOP, 1 mM in DMF, 2 equiv of DIEA, 3 h/rt. (ii) 10 equiv of DIEA, 20 h/rt. (iii) *hν*, 1% HOAc in DMSO, 1:1, 2 h.

trapoxin,[3,5] the tyrosinase inhibitor *cyclo*-[(L)Pro-(L)Tyr-(L)-Pro-(L)Val], and the antimalarial apicidins.[6] One compound is reported to show in vivo activity by both parenteral and oral administration in mice.[7]

Cyclic tetrapeptides are very rigid 12-membered-ring structures. Different conformers of these molecules have been isolated, and these have been shown to display differing biological activities.[8] Although isolation and structure determination of these compounds has taken place over the past three decades, there has been little success in synthesizing representative compounds. This is surprising, given that cyclic tetrapeptides may be considered a rich source of drug like molecules due to their wide-ranging biological activities, low molecular weight, favorable pharmacokinetic characteristics, and unique 12-atom cyclic backbone, providing a rigid framework that can support a wide range of functional groups.

The ring strain inherent in this framework makes the synthesis of these molecules very difficult.[13] The primary reason for ineffective cyclization originates from a sequence-related inefficiency to bring the termini together for cyclization.[14] Because peptide bonds contain strong π-character and preferentially adopt a trans conformation, linear peptides prefer a more extended conformation. Incorporation of turn-inducing elements such as Gly, Pro, or D-amino acids are known to enhance cyclization yields.[15] As a result, the few cyclic tetrapeptides that have been synthesized contain either D-residues or at least one tertiary amide in the sequence.[2−11]

Small head-to-tail cyclic peptides that do not contain turn-inducing elements are known to be very difficult to synthesize,[14−16] as macrocyclization of the linear sequences produce linear and cyclic oligomers in preference to the monocyclic target, even when cyclization is performed at high dilution or the sequence is rotated for optimal cyclization yield.[14] The problem becomes more prominent for shorter medium-sized ring peptides.[14]

In a previous report, the development of a novel ring-contraction auxiliary that could be used to facilitate the room temperature cyclization of difficult cyclic pentapeptide sequences was described.[17] It was anticipated that this approach could also be applied to yield 12-membered cyclic tetrapeptides. Cyclization of a 2-hydroxy-6-nitrobenzyl (HnB) N-terminally substituted tetrapeptide **2** would initially generate a more accessible but reactive cyclic nitrophenylester intermediate **3** (Scheme 1), which would ring contract through an O-to-N acyl transfer to generate the desired, substituted, target compound **4**. Photolytic removal of the HnB auxiliary would provide the target cyclic product **5**.

When this strategy was applied to the tetrapeptide [HnB]-Tyr-Arg-Phe-Gly-OH **2a** none of the desired cyclic product **4a** was obtained when cyclization was conducted at room temperature. Fortunately, cyclization to **4a** could be achieved through formation of the nitrophenyl ester intermediate **3a**, followed by further addition of DIEA and heating at 70 °C overnight. This cyclic product is not accessible from the unsubstituted H-Tyr-Arg-Phe-Gly-OH **1** sequence. The extra thermal energy must be required to overcome various ring strain elements in the formation of the 12-membered-ring products, despite the higher effective concentration of the C- and N-termini and the entropic advantages when using HnB for cyclization. This strategy has subsequently proven to be useful in the synthesis of a variety of glycine-containing cyclic tetrapeptides. However, glycine is a Cα-unsubstituted amino acid, and its presence in a cyclic peptide should greatly

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Table 1. Cyclization of Linear Tetrapeptides **8** and Corresponding Yields of Cyclic Product **9** and Photolysis to Give Product **5**

		cyclic product (**9**)[b]			
entry	linear peptide (**8**)[a]	% yield by HPLC[c]	% isolated material[c]	% desired isomer[d]	photolysis[e] (**5**)
a	[HnB]-Tyr-Arg-[HnB]-Phe-Gly-OH	75	33		50%
b	[HnB]-Tyr-Arg-[HnB]-Phe-Ala-OH	78	59	26	0 (20%)[f]
c	[HnB]-Tyr-Arg-[HnB]-Phe-D-Ala-OH	68	51	22	42%
d	[HnB]-Tyr(Bn)-Arg-[HnB]-Phe-Ala-OH	71	48	23	25%[h]

[a] All naturally occurring amino acids are the L-isomer unless otherwise stated. [b] Cyclization methods: (i) 1 equiv of BOP or HATU, 2 equiv of DIEA, 1 mM in DMSO, 3 h/rt; (ii) 10 equiv of DIEA, 20 h/rt. [c] Combined yield of L- and D-product. [d] % isolated yield of the correct isomer. [e] Photolysis method: *hν*, CH_3CN/H_2O or 1% HOAc in DMSO. [f] Photolysis conducted upon purified all-L MnB-isomer **10b**. [h] Photolysis conducted upon crude all-L MnB-isomer **10d**.

facilitate cyclization as a result of its flexibility. More constrained cyclic peptides that do not contain glycine are therefore far more challenging synthetic targets. As a result, the next target was *cyclo*-[Tyr-Arg-Phe-Ala]. Unfortunately, cyclization of the HnB-substituted linear peptide **2b** at 70 °C overnight yielded none of the desired cyclic tetrapeptide **4b**, even when the temperature, activating conditions, and time were varied.

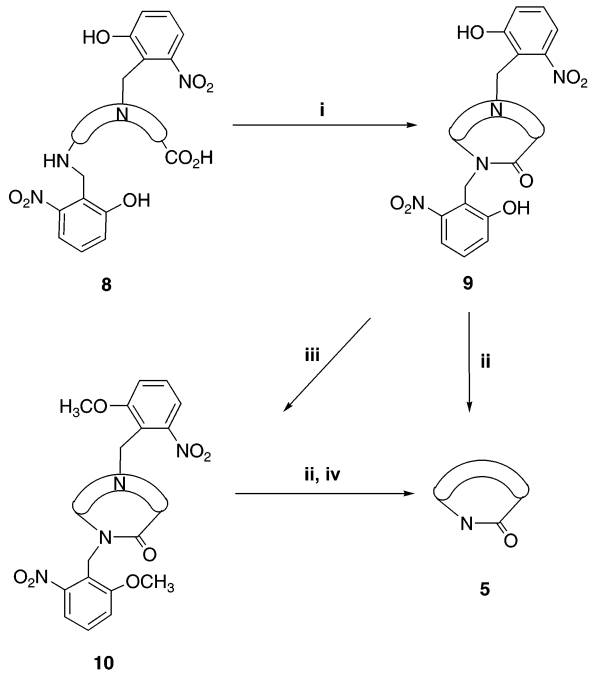
These results suggest that for non-glycine-containing all-L tetrapeptides a significant barrier for ring contraction exists. This barrier could be lowered by increasing the propensity for *cis*-amide bonds in the linear precursor, an idea especially attractive given that crystal and NMR structures of cyclic tetrapeptides have at least one *cis* amide bond.[10] As N-alkylation of amide bonds have been previously reported to lower the *trans*-*cis* amide bond barrier,[11,16] this general strategy was a promising route for cyclic tetrapeptide synthesis.

It was reasoned that HnB should be an ideal backbone substituent to aid cyclization as it is a bulky *N*-alkyl substituent (increasing the propensity for *cis* amide bonds[16]), the secondary amine substituted with HnB can be readily acylated[17] (even with bulky amino acids), and the substituent is removed by photolysis. To this end, H-Tyr-Arg-[HnB]-Phe-Gly-OH **6** was synthesized, which contained HnB in the "middle" of the sequence. Cyclization of **6** yielded the desired cyclic product *cyclo*-[Tyr-Arg-[HnB]-Phe-Gly] **7** in 15% isolated yield and significant dimer (10% isolated yield).

Previous observations indicated that an advantage of including HnB at the N-terminus, followed by cyclization, is the significant reduction of dimer formation.[16,17] A combination of HnB at the N-terminus (to increase the effective concentration of the C- and N-termini, entropically favoring cyclization and reducing oligomerization) and a HnB substituent at the *N*2 position (to promote *cis* amide conformations and facilitate ring contraction) should therefore provide a suitable strategy for the synthesis of highly strained macrocycles (Scheme 2). This strategy proved to be successful. Synthesis and cyclization of the bis-HnB substituted tetrapeptide [HnB]-Tyr-Arg-[HnB]-Phe-Gly-OH **8a** yielded the desired HnB-substitued cyclic product in 33% isolated yield at room temperature, with no dimer formation (Table 1). This *room temperature* ring contraction confirmed the previous hypothesis on the value of the HnB combination approach in the synthesis of such difficult targets. On the basis of this successful result, this combination approach was applied to the synthesis of *cyclo*-[Tyr-Arg-Phe-Ala] **5b**, which had previously proven to be synthetically inaccessible. Because of the increased ring strain, as a result of four Cα-alkyl amino acids, ring contraction was expected to proceed at a slower rate than for glycine-containing analogues.

Therefore cyclization of [HnB]-Tyr-Arg-[HnB]-Phe-Ala-OH **8b** was examined at room temperature for 20 h, at 70 °C for 1 h, and at 70 °C for 20 h. Surprisingly, reasonable yields of cyclic products (59%) were generated at *room temperature* (Table 1). Chiral amino acid analysis, MS, and NMR were used to characterize the isolated reaction

Scheme 2. Synthesis of Difficult Sequences via Combination Strategy[a]



[a] Reagents and conditions: **(**i) 1 equiv of BOP or HATU, 1 mM in DMSO, 2 equiv of DIEA, 3 h/rt then 10 equiv of DIEA, 20 h/rt. (ii) *hν*, CH_3CN/H_2O or 1% HOAc in DMSO, 1:1, 2 h. (iii) CH_2N_2 EtOH, 30 min. (iv) H_2/Pd, MeOH, 1 h.

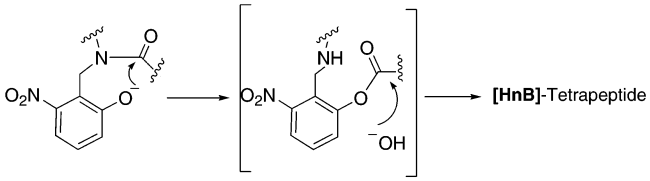
products. The constrained *cyclo*-([HnB]-Tyr-Arg-[HnB]-Phe-Ala) **9b** product was isolated in 26% yield.

The only competing side reaction during cyclization was racemization of Ala to yield *cyclo*-([HnB]-Tyr-Arg-[HnB]-Phe-D-Ala) in 33% yield, which was not significantly different when using other activating agents or bases. Cyclization of both the bis-HnB L-Ala **8b** and D-Ala **8c** linear peptides yielded similar ratios of cyclic L- and D-products, suggesting that racemization is significantly faster than cyclization and that cyclization rates for L-Ala **8b** and D-Ala **8c** are similar (Table 1).

Previously, it was demonstrated that the HnB group could be removed from the amide backbone by photolysis.[17] Photolysis of the glycine-containing cyclic tetrapeptides **4a** and **9a** proceeded in reasonable yield and purity (Scheme 1), and products were isolated and characterized by MS, chiral amino acid analysis, and NMR.

In contrast, whereas photolysis of the D-Ala cyclic product **9c** yielded **5c** cleanly, the more strained all-L-cyclic tetrapeptide **9b** yielded only hydrolyzed product. It may be expected that the amide bonds of the highly strained all-L analogues to be out of plane and more susceptible to hydrolysis. This may occur via a ring-expansion reaction. (Scheme 3).

Scheme 3. Proposed Reaction Pathway during Photolysis of **9b**



Alkylation of the 2-hydroxy functionality on HnB should prevent these decomposition reactions. This was attempted by treatment with excess diazomethane in ethanol, but methylation of the HnB groups was slower than expected and partial methylation of the tyrosine side chain was inevitable. A 10 min diazomethane treatment provided the best yield of the desired bis(2-methoxy-6-nitrobenzyl) (bis-MnB) peptide cyclo-[[MnB]-Tyr-Arg-[MnB]-Phe-Ala] **10b** (10% yield after HPLC purification). Photolysis of **10b** generated the desired all-L-cyclic tetrapeptide *cyclo*-[Tyr-Arg-Phe-Ala] **5b** in 20% yield (after HPLC purification), confirming our proposed hydrolysis pathway.

To avoid the need for selective methylation a benzyl-protected tyrosine was subsequently employed. Thus the all-L monocyclic peptide **9d** was isolated in 25% yield (the D-Ala analogue was also isolated in 23% yield) from **8d**. A 30 min diazomethane treatment of **9d** generated the bis-MnB-substituted analogue **10d**. Following photolysis and purification, *cyclo*-[Tyr(Bn)-Arg-Phe-Ala] **5d** was isolated in 25% overall yield. Hydrogenolysis generated the target *cyclo*-[Tyr-Arg-Phe-Ala] **5b**.

In conclusion, despite three decades of discovery of biologically active cyclic tetrapeptides and their reported oral activity, they have been largely unexplored in the pharmaceutical industry. Primarily this is due to the difficulties in preparing these rigid 12-membered-ring compounds. This paper reports the development of a synthetic strategy that uses a combination of two reversible auxiliaries, one at the N-terminus and one at the middle backbone amide nitrogen atom. The first auxiliary performs a ring closure/ring contraction role, which increases the effective concentration of the C- and N-termini, entropically favoring cyclization and effectively preventing oligomerization from occurring, and the latter serves as a *cis*-amide bond promotor to facilitate the otherwise difficult ring contraction. HnB is suitable for both auxiliary functions owing to its superior acyl transfer properties, its ready accessibility, and its photolability.

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Supporting Information Available: Full experimental procedures including TOCSY data for *cyclo*-[Tyr-Arg-Phe-Gly] showing the amide NH region. This material is available free of charge via the Internet at http://pubs.acs.org.

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