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A New and Useful Method for the Macrocyclization of Linear Peptides

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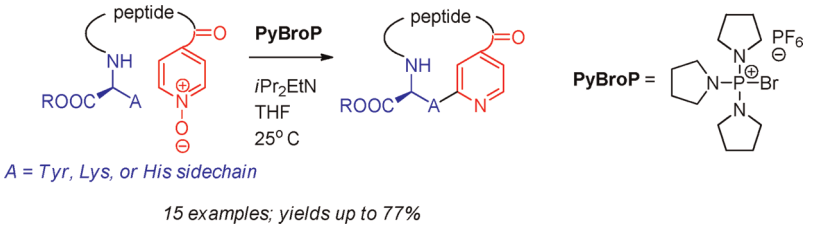
ABSTRACT



A new and useful procedure for the macrocyclization of linear peptides is described. The natural amino acid side chains of tyrosine (phenol), lysine (alkylamine), and histidine (imidazole) react in an intramolecular fashion with a pendent pyridine-*N*-oxide-carboxamide, which is selectively activated by the phosphonium salt, PyBroP. The reaction is mild, rapid, and efficient with a potentially large substrate scope. Multiple examples are provided with full characterization and analyses, including a novel aza-variant of the C–O–D ring system of vancomycin.

The discovery and development of peptide-based therapeutics are at the forefront of contemporary pharmaceutical research. Of particular interest are low molecular weight peptidomimetics that modulate protein function by competitive binding, allosteric regulation, or disruption of protein–protein interactions. Numerous linear peptide ligands have been discovered that affect these parameters, but very few have been developed into orally bioavailable chemotherapeutics.[1] This is due in large part to their poor gastrointestinal stability, poor permeability, and unpredictable secondary structure.[1,2] Macrocyclic peptides, however, can have markedly improved physicochemical properties when compared to their linear counterparts, with significant potential for development into novel oral therapies.[3] Consequently, there has been a resurgence of interest[4] in synthetic and naturally occurring cyclic peptides over the past few years as well as in new methods for their syntheses. Various procedures for the macrocyclization of small and medium sized rings are found in the literature, many of which have been applied to peptide-based substrates.[5] By far, the most challenging aspect of peptide macrocyclization is the ring-closure event, particularly when substrates contain seven amino acid residues or fewer.[6] In order for a short linear peptide to adopt the

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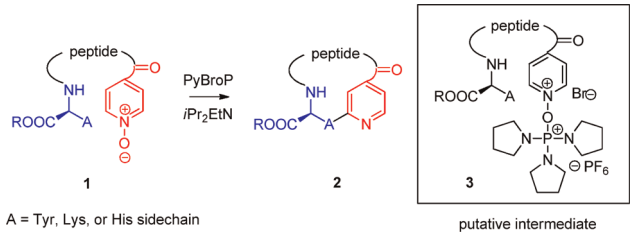
cyclic conformation necessary for intramolecular ring closure, it must overcome a significant entropic penalty. This is especially true for linear peptides that lack turn-inducing residues[7] or amphoteric termini.[6a,8] The cyclization event, therefore, usually requires forcing conditions and/or lengthy reaction times which may lead to the formation of oligimerized side products. High dilution[9] can prevent side reactions, but this, combined with extended reaction times, often leads to peptide epimerization. The strong scientific interest in macrocyclic peptidomimetics and the inherent limitations to their syntheses necessitates new and improved methods for their preparation. Our laboratory sought to develop such a procedure that would be mild, operationally simple, and broadly applicable to a wide class of peptide substrates.

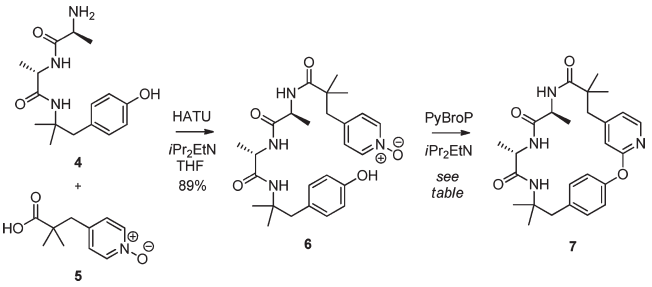
In previous communications,[10] we demonstrated that the phosphonium salt PyBroP[11] (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate) functioned as a general and mild pyridine-*N*-oxide activator for the intermolecular addition of varied N, O, S, and C nucleophiles, to afford the corresponding 2-substituted pyridines. We further described significant reaction optimization which resulted in a mild and operationally simple protocol. We rationalized that this procedure might prove effective for the macrocyclization of peptides (Scheme 1). In this case, the natural amino acid side chains of tyrosine (phenol), lysine (alkylamine), and histidine (imidazole) would function as tethered nucleophiles (blue) while a pendent pyridine-*N*-oxide-carboxamide (red) would act as the corresponding electrophile. From the onset, it was our goal to use standard iterative peptide syntheses for the facile production of cyclization substrates (**1**).

Scheme 1. New Method for Peptide Cyclization



We attempted our first peptide cyclization using substrate **6** (Table 1), which was readily synthesized via the amide coupling of **4** and **5**. Upon exposure of **6** to PyBroP and iPr$_2$EtN in THF at 25 °C, we were delighted to observe the formation of macrocycle **7** in 1 h. Minor modifications to our standard intermolecular protocol were required for successful intramolecular cyclization. Of particular importance were reagent addition order and reaction

Table 1. Reaction Optimization with Model System[a]



entry	solvent	concn (M)	% yield (**7**)
1	THF	0.001	60
2	THF	0.01	65
3	**THF**	**0.02**	**73**
4	THF	0.05	55[b]
5	THF	0.25	25[c]
6	ACN	0.02	40
7	TFE	0.02	n/r[d]
8	MeOH	0.02	n/r[d]
9	DMSO	0.02	<10[e]
10	DMF	0.02	<5[e]
11	HMPA	0.02	<5[e]
12	THF/DMSO[f]	0.02	<10[e]
13	THF/DMSO[g]	0.02	25

[a] Reaction conditions: A solution of **6** (1.00 equiv) was added in a dropwise fashion over 15 min to a stirred solution of PyBroP (1.30 equiv) and iPr$_2$EtN (3.75 equiv) in appropriate solvent at the indicated concentration. [b] 20% oligimerized mixture (HPLC). [c] 25% oligimerized mixture (HPLC). [d] No reaction. [e] Percent conversion (HPLC). [f] 1:1 THF/DMSO. [g] 10:1 THF/DMSO.

concentration. Owing to the rapid nature of this macrocyclization, we found it most effective to add substrate **6** over 15 min[12] to a THF solution of PyBroP and iPr$_2$EtN. A noncontrolled addition of substrate/reagents at the reaction onset led to the polymerization of substrate **6** in addition to the formation of macrocycle **7**. Higher reaction concentrations (≥0.05 M) also led to appreciable formation of polymerized side products. Interestingly, more dilute concentrations (<0.01 M), which are commonly used to prevent substrate oligimerization, were not necessary for selective monocyclization. Certain polar and protic solvents (entries 7−11) and corresponding mixtures with THF (entries 12−13) were not tolerated. Such media may preclude the formation of intermediate **3** and/or the critical charge association necessary for successful reactivity (*vide infra*). Under our newly optimized conditions, we were pleased to cleanly obtain a 73% yield of **7** (entry 3) in 1 h.

In an analogous fashion to our model system, we synthesized a series of cyclization substrates using iterative peptide coupling chemistry. The results of the subsequent macrocyclization events are shown in Figure 1. We varied three parameters: the peptide backbone (black),

(7) For example: L-proline, α-methyl/D-amino acids, L-glycine.
(8) Schmuck, C.; Wienand, W. *J. Am. Chem. Soc.* **2003**, *125*, 452–459.
(9) Often 10^{-4} or higher.
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(12) Substrates were added manually, in a dropwise fashion with a syringe. A syringe pump may be used, but no improvements in yields were noted.

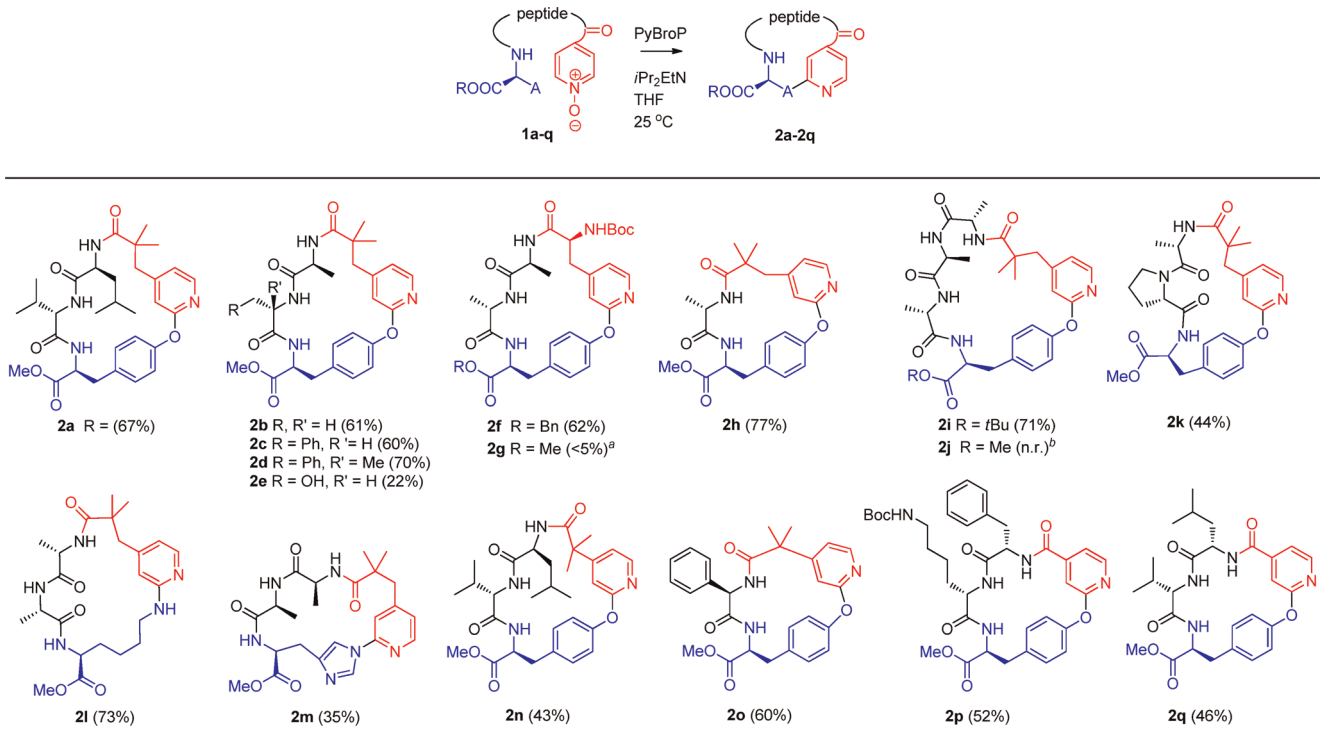

Figure 1. PyBroP-mediated macrocyclization of linear peptides. General conditions: **1a**−**1q** (1.00 equiv), PyBroP (1.30 equiv) and iPr$_2$EtN (3.75 equiv) in THF (0.02 M) at 25 °C for 15 h. See Supporting Information for detailed procedures. [a]Percent conversion (HPLC). [b]No reaction.

the nucleophilic amino acid (blue), and the electrophilic pyridine-*N*-oxide-carboxamide (red). Each cyclization reaction was carried out using the optimized conditions[13] mentioned above. In all examples, we were delighted to observe smooth macrocyclization, with, at most, trace amounts of oligimer. Upon completion,[14] the reactions were simply evacuated and subjected directly to reversed-phase HPLC purification. The desired products were isolated in modest to excellent yields with no observable epimerization.[15] All three substrate parameters were readily modified to afford macrocycles of varying residue and atom count. Due to the mild nature of this reaction, we were able to successfully integrate orthogonally protected side chains and termini (**2f** and **2p**). In certain instances (**2g** and **2j**), we found that the cyclization precursors were completely insoluble in THF and remained inert under the reaction conditions. By modifying the ester moieties of the respective C-termini (**2f** and **2i**), the reactant solubility was markedly improved, with the expected macrocyclization successful.

Of particular note is example **2o**, a novel aza-variant of the C−O−D ring system found in the antibiotic vancomycin. *De novo* structural modification of the macrocyclic core(s) found in glycoprotein antibacterials is an important strategy used to combat drug resistance.[16] New, diverse, and improved syntheses of the C−O−D ring from vancomycin are at the forefront of such efforts.[17]

We directly compared the physicochemical properties of macrocycles **2a** and **2q** to their corresponding open-chain variants (*des*-*N*-oxide-**1a** and -**1q**, respectively).[18] In these examples, we observed an average 7.5-fold improvement in passive cellular membrane permeability and a 0.6-log unit increase in lipophilicity, characteristics which may be favorable for improved peptide oral bioavailability.

In the instance of example **2i**, we obtained clear NMR evidence of peptide-like secondary structure.[19] Characteristic NOE interactions between NHi→NH(i+1) and CαHi→NH(i+1) combined with temperature-dependent ^{1}H NMR amide-NH resonances, and two unusually large $^3J_{\mathrm{NHC\alpha H}}$ coupling constants (>8 Hz) followed by two quite small $^3J_{\mathrm{NHC\alpha H}}$ coupling constants (<4 Hz), are all

(13) For macrocyclization substrates (**1**) with limited solubility in THF the following procedural modification was made: a mixture of **1** (1.00 equiv) in THF (0.02 M) was treated with iPr$_2$EtN (3.75 equiv) and PyBroP (1.30 equiv) sequentially. The substrate slowly enters into solution over the course of the reaction. See Supporting Information for details.

(14) Due to the limited solubility of certain macrocyclization substrates (**1**) in THF, all reactions were stirred for 15 h to ensure completion. In most cases, however, the reactions were complete (HPLC analysis) in 1 h.

(15) Determined by chiral HPLC and/or H NMR analysis.

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(18) See Supporting Information for data.

(19) See Supporting Information for detailed NMR data and analysis.

diagnostic of a β-turn mimetic.[20] Discrete secondary structure, such as a β-turn, may be advantageous for direct optimization of ligand/receptor interactions.

The lack of significant oligimerized side products during this macrocyclization event may be attributed to reactive intermediate **3** (Scheme 1). The electrostatic charge association[21] of the incoming nucleophile to the activated phosphonium complex can be considered enthalpically favorable and thus negate the entropic penalty associated with the linear peptide adopting a cyclic precyclization conformation. With the reactive counterparts in close proximity, facile cyclization ensues. Terminal ion pairing as a means to enforce cyclic conformation in linear peptides is known and has been shown to promote successful peptide macrocyclization by Yudin and co-workers.[6a]

We believe that the methodology described herein complements the growing interest in macrocyclic peptidomimetics. No specialized chemistry is necessary for the synthesis of the macrocyclization substrates. Both the electrophilic and nucleophilic reaction partners can be obtained from commercial sources or via short syntheses. The ability to choose from a pool of reactant components makes the potential diversity of this reaction very large. Normally challenging ring sizes are easily obtained without the need for traditional turn inducing residues, such as proline or α-methyl/D-amino acids, although each are tolerated. Most products are isolated in relatively high yields, especially when one considers the ubiquitous challenges associated with peptide cyclization. Finally, macrocyclic peptide mimetics obtained via this methodology can exhibit favorable physiochemical properties as well as defined secondary structure.

In conclusion, we have presented a novel macrocyclization procedure for the synthesis of varied cyclic peptides. A unique and diverse substrate scope combined with an operationally simple procedure makes this a useful reaction. Further secondary structural analyses of macrocyclic products obtained by this procedure will continue in our laboratory. We also hope to expand this methodology to incorporate other pyridine-*N*-oxide electrophiles as well as carbon and sulfur nucleophiles. The results of these studies will be published in due course.

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Supporting Information Available. Experimental details, procedures, supplementary data, and characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org. The authors declare no competing financial interest.

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(21) As described in our previous communications, the charge association of an incoming nucleophile to the activated phosphonium complex is critical for successful reactivity.

The authors declare no competing financial interest.