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Efficient parallel synthesis of macrocyclic peptidomimetics

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ABSTRACT

A new method for solid phase parallel synthesis of chemically and conformationally diverse macrocyclic peptidomimetics is reported. A key feature of the method is access to broad chemical and conformational diversity. Synthesis and mechanistic studies on the macrocyclization step are reported.

Restricting the degrees of freedom of a molecule is a general paradigm in medicinal chemistry, aiming at not only decreasing the entropy of interaction between a synthetic compound and its target and increasing affinity, but also at improving the PK-ADME properties including oral bioavailability of the resulting molecule.[1] In the peptidomimetic field, the concept of conformational restriction has been explored in several directions, with some notable success using macrocyclization.[2] Indeed, whereas a linear peptide may have numerous conformations, its macrocyclic analogue is more restricted as a result of ring strain and transannular interactions. Unfortunately, general methods that can produce macrocyclic peptidomimetics in large numbers and with broad chemical diversity are scarce.[3]

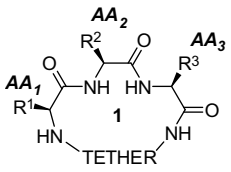

Figure 1. Representative structure of macrocyclic peptidomimetics (L stereochemistry exemplified).

We herein present a new class of macrocyclic peptidomimetics, exemplified by structure **1** (Fig. 1), empowered by an efficient solid phase parallel synthesis method for producing this target class. The discovery of potent modulators of gastrointestinal motility starting from high-throughput screening on a 10,000 membered library of these macrocycles was reported recently.[4,5] Specifically described is the discovery of an agonist of the ghrelin receptor presently in phase II clinical trials,[4] as well as the identification of selective antagonists of the motilin receptor.[5] The present account focuses on exemplifying the chemical diversity achievable with this synthetic approach, and reports general aspects of the method, with emphasis on the key macrocyclization step. Sequences presented in this Letter were chosen to illustrate the diversity that can be supported by this approach, in terms of chemical nature of amino acid side chains and tethers, stereochemistry of peptides, and ring size.

Structurally, macrocycle **1** is composed of a tripeptide cyclized backbone-to-backbone by a nonpeptidic 'tether'. Besides the stereochemical and chemical variations available from the commercial availability of numerous amino acids, macrocycle **1** also possesses an ionizable secondary amine and, most importantly,

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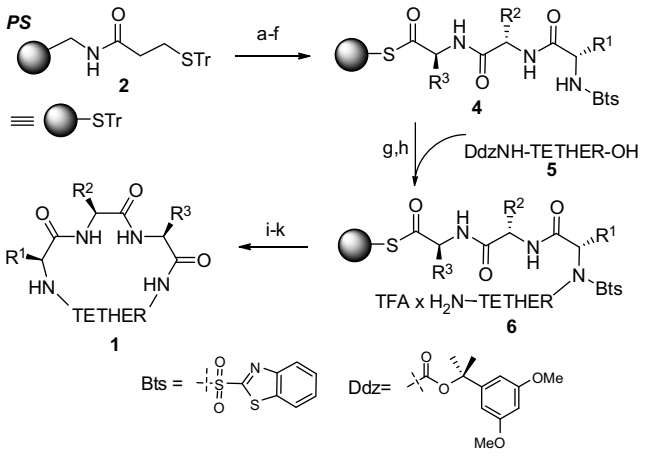


Scheme 1. Reagents and conditions: (a) 10%, TFA, Et_3SiH, DCM; (b) Ddz-AA_3-OH, PyBOP, DIPEA, NMP; (c) TFA, Et_3SiH, DCM; (d) Ddz-AA_2-OH, HBTU, DIPEA, NMP; (e) TFA, Et_3SiH, DCM; (f) Bts-AA_1-OH, HBTU, DIPEA, NMP; (g) **5**, PPh_3, DIAD, THF; (h) TFA, Et_3SiH, DCM; (i) $Ag(OCOCF_3)$, DIPEA, THF, MP-carbonate; (j) PS-thiophenol, KOTMS, THF/EtOH; (k) TFA, Et_3SiH, DCM.

the tether. The latter has multiple roles: firstly, in concert with the tripeptide moiety, it restricts the conformational space available to the molecule; secondly, it provides an additional molecular recognition sites and finally can serve as a handle to tune physico-chemical properties—and consequently overall pharmacological profile.

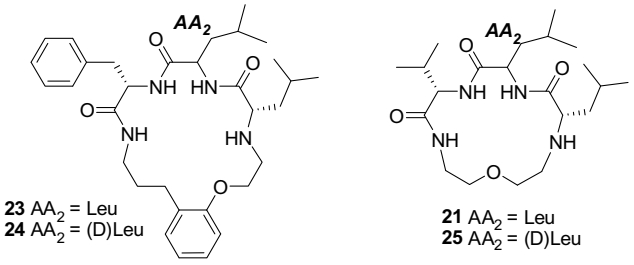
The method used to produce this macrocyclic class relies on solid phase parallel synthesis, some steps of which emanate from well-established peptide chemistry (Scheme 1). Synthesis was carried out on a polystyrene (PS) solid support, with the elongating peptide moiety anchored via a semi-labile thioester linker. Due to the electrophilicity of the thioester bond, a protection scheme was devised involving a highly acid labile protective group on the α-amino groups (the Ddz group,[6] cleaved with 1–2% TFA, Scheme 1), and more robust acid labile protective groups on the side chains (Boc carbamates, *t*-Bu esters and ethers, cleaved with 50–90% TFA post-macrocyclization), thus allowing selective removal. In order to give crude products of acceptable purity, macrocyclization was combined with cleavage from the resin in a cyclative release step,[7] allowing for easy removal of unreacted resin-bound materials by simple filtration.

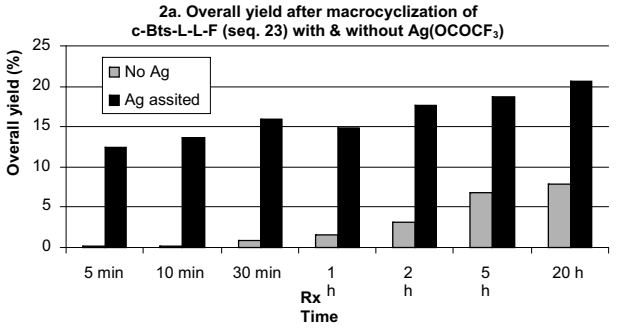
Specifically (Scheme 1), starting from commercially available trityl-protected mercaptopropionamide resin **2**, acidolysis followed by PyBOP-mediated formation of the thioester bond with the first amino acid (AA_3) gave Ddz-protected amino thioester resin. After Ddz cleavage, peptide bond formation with the second amino acid (AA_2) was effected using standard conditions. Deprotection of the second Ddz group and attachment of Bts-protected amino acid AA_1 as indicated gave resin-bound tripeptide **4**.

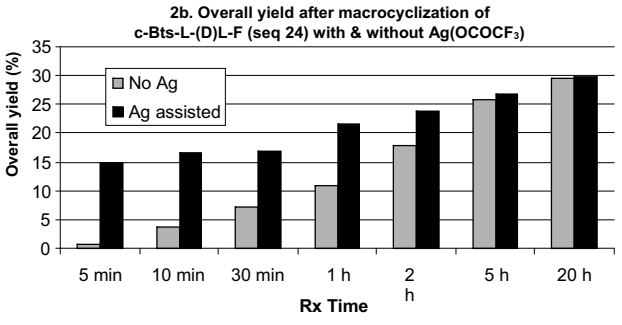
The Bts protective group[8] is crucial for attachment of protected tether alcohol **5** via Fukuyama–Mitsunobu alkylation,[9] although reductive amination was used effectively as well (vide infra). After attachment, subsequent cleavage of the tether Ddz group gave the desired linear precursor **6**. Cyclative release was performed in THF in the presence of Hünig's base, as well as a supported carbonate resin (MP-carbonate). During macrolactamization, the thioester undergoes intramolecular aminolysis, a process generally facilitated by the use of silver salts.[10] Removal of Bts protection from the cyclic product was performed in parallel solution phase, using a polymer-supported thiophenoxide.[11] Final side chain deprotections were performed in strongly acidic conditions (50–90% TFA) to deliver crude macrocycles.

At intermediate and final stages, macrocycles were quantified by RP-HPLC using the CLND detector.[12] Table 1 reports the results of macrocyclization for a series of representative macrocycles (yields based on quantitative CLND determination at the macrocyclization and final stages). Macrocyclization yields were determined with respect to effective loading of alkylated tripeptide 6, whereas overall yield was calculated with respect to the effective loading of the AA_3 moiety.[13] After final deprotection, macrocycles were purified by preparative HPLC for characterization.

In terms of scope (Table 1), the method supports the synthesis of macrocycles containing not only a broad side chain chemical diversity, but also all the possible combinations of (L), (D), α-, β-, γ-amino acids as well as N-methylated and α,α-disubstituted amino acids. Tether diversity encompasses 14- to 19-membered rings, including (but not limited to) moieties such as acetylene (**7**, **8**),







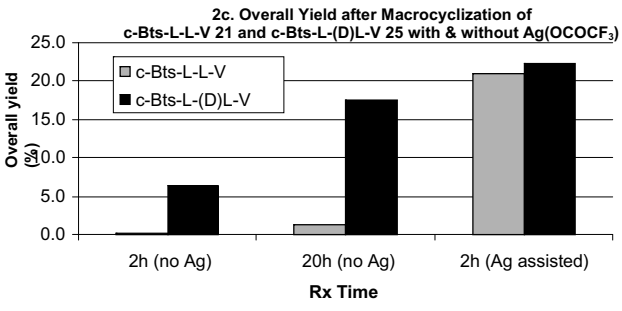
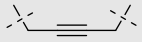
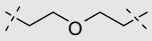
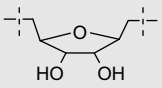
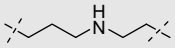
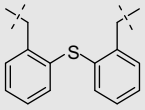
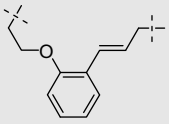
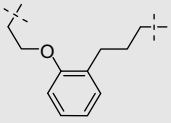
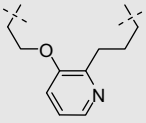
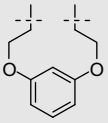
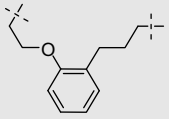
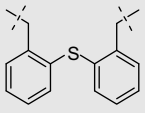
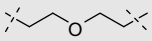
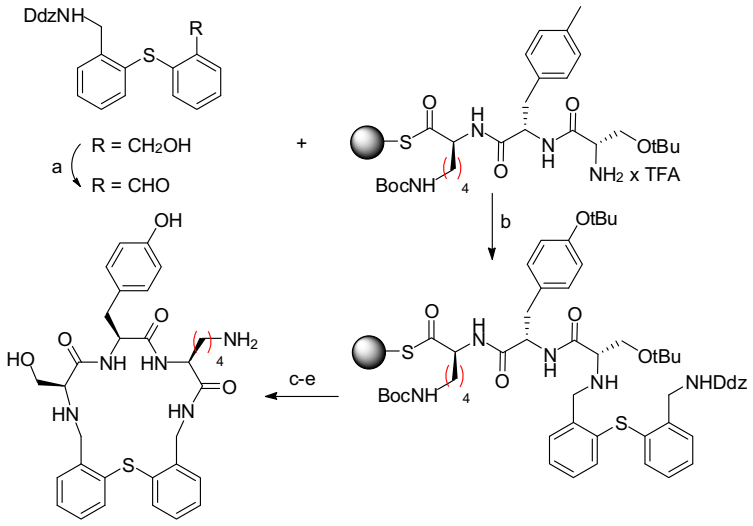


Figure 2. Influence of silver salts and sequence on macrocyclization.

Table 1
Representative sequences[14]

Compound	Sequence AA1-AA2-AA3	Tether	Ring size	Ag	Yield		Dimer (%)
					Overall (%)	Macro (%)	
7	Leu-(D)Leu-Phe		14	y	36.1[a]	67.2	nd
8	Leu-Leu-Phe			y	9.5	16.1	33
9	Orn-Ser-Glu		15	y	16.1	28.1	nd
10	Ser-(D)Ala-Orn		15	y	12.4	26	nd
11	Phe-Sar-Leu		16	y	14.1	30.6	nd
12	(D)Phe-(D)Lys-Met		17	y	15.8[b]	74.7	nd
13a	Ile-(D)Ala-Phe		18	y	22.5	35.6	6.7
13b				n	35.9	57	nd
14a	Ile-(D)Ala-(D)Phe			y	11.9	17.6	10.7
14b				n	13.2	19.4	5.3
15	Ile-Acp-3Thi[14]		18	y	5.6	33	5.1
16	(D)Nva-Asp-Phe		18	y	10.5[c]	25.5	
17	(D)Phe-(D)Ser-Tyr		19	y	13.7	30.8	
18	Leu-(D)Leu-Phe		18	y	16	26.3	7.4
19[e]				y	21	39	
20[e]	Ser-Tyr-(D)Lys		17	y	20	44	
21	Leu-Leu-Val		15	y	31[d]	56.7	nd
22				n	1.7	3.1	nd

[a] 7.4% of betsylated product.
[b] 5.5% of one diastereomer was observed.
[c] 2% betsylated materials detected.
[d] Including 9.8% betsylated product.
[e] Tether attachment performed by reductive amination.



Scheme 2. Reagents and conditions: (a) BH_3/C_5H_5N, $HC(OMe)_3$, MeOH; (b) 2% TFA, 3% Et_3SiH, DCM; (c) $Ag(OCOCF_3)$, DIPEA, THF, MP-carbonate; (d) 50% TFA, 3% Et_3SiH, DCM.

ether (**9**, **21**, **22**), sugar (**10**), amine (**11**), aromatic (**12**–**15**, **17**–**20**) and heteroaryl (**16**).

Whereas overall yields (11 steps) vary between 1% and 36%, several qualitative observations can be made regarding the macrocyclization step. Firstly, the yield of macrocyclization is greatly influenced by the stereochemistry and chemical nature of the building blocks. Generally, tripeptides of alternating stereochemistry give higher yields than their homochiral counterparts (see **7** vs **8** or **13** vs **14**). That difference is exacerbated in cases where macrocycles are more difficult to form (see **7** and **8**, see also Fig. 2). This effect is presumably due to unfavourable transannular interactions at the transition state in the homochiral series, making macrocyclization a more difficult process. Secondly, the presence of bulky amino acids in AA_3 (adjacent to the thioester moiety) is detrimental for macrocyclization when silver salts are absent, whereas the use of silver overcomes this issue (see **21** vs **22**, Fig. 2). More generally, the presence of silver salts seems to be beneficial for sequences reluctant to macrocyclize (see **21** vs **22**), yet does not seem to have a great impact on sequences that macrocyclize more readily (see **13a** vs **13b**). It is believed that silver has both an enthalpic effect (activation of the thioester bond) and an entropic templating effect (electrostatic interaction with electron-rich groups, thus helping folding of the linear precursor).[10c] Finally, macrocyclization yield is affected by ring size however, not in an apparently predictable way (14- to 19-membered rings). In terms of side products, the presence of dimer was observed in larger proportions with macrocycles that were difficult to form, accompanied by an overall lower yield and, occasionally, partial epimerization was observed at the AA_3 moiety (see **12**).

Altogether, the results presented in Table 1 testify to the breadth of chemical and conformational diversity that this approach can support.

Reductive amination was also used for tether attachment (Scheme 2).[15] Tethers were first oxidized to the corresponding aldehydes using IBX, then reacted with resin bound tripeptides under reductive amination conditions. Macrocyclization was performed using the previously described conditions. Comparison of entries **18** and **19** (Table 1) indicates that macrocyclization yield was superior when reductive amination was used, at least in this case (see also entry **20**).[16]

In order to gain a better preliminary understanding of the kinetics of macrocyclization, selected reactions were performed and stopped at set times on two similar sequences: Leu-Leu-Phe **23** and Leu-(D)Leu-Phe **24** (Fig. 2), giving some interesting observations. For example, on homochiral Leu-Leu-Phe sequence **23**, the use of silver almost tripled the yield compared to the same sequence macrocyclized without silver (Fig. 2a). Reaction was also more rapid and reached maximum yield within 2 h in the presence of silver salts. Conversely, on heterochiral Leu-(D)Leu-Phe sequence **24**, after 20 h there was no difference in yield with or without silver. However, the reaction was accelerated by silver salts (Fig. 2b).

Qualitatively, the use of silver trifluoroacetate facilitates difficult reactions (as in **23**), whereas it seems to have a marginal impact on easier reactions (as in **24**). It therefore appears to lower a high activation barrier, but has a limited impact when the activation barrier is low (as such, it truly acts as a catalyst). As a corollary, we believe that a certain degree of preorganization is associated with sequences of alternating stereochemistry (see **24**), which undergo macrocyclization easily in the absence or presence of silver salt. This may be due to favourable folding of the precursor chain at the transition state, facilitated either by steric or electrostatic factors or both. Further support for this notion is exemplified in Figure 2c, where a bulkier amino acid is used at AA_3 with the Leu-Leu-Val (**21**) and Leu-(D)Leu-Val (**25**) sequences, resulting in a more difficult macrocyclization. In the homochiral L-L-L series **21**, after 20 h there was almost no reaction without silver, whereas the presence of silver increased yield to 17%. In the L-D-L series, the yield was above 20% both with and without silver. Thus in this case, the presence of silver salts clearly allowed the more difficult reaction to occur, overcoming a larger energetic barrier.

In conclusion, the method reported herein allows for rapid solid phase parallel synthesis of chemically and conformationally diverse macrocyclic peptidomimetics. Application of this approach to drug discovery programs has already been reported and is continuing, based on high throughput screening of libraries of these macrocycles.[4,5] Preliminary results on the effects of silver on macrolactamization indicated that it appeared to be crucial in sequences difficult to macrocyclize, whereas it had less impact in sequences easier to macrocyclize.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.085.

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