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Peptide Macrocycles Featuring a Backbone Secondary Amine: A Convenient Strategy for the Synthesis of Lipidated Cyclic and Bicyclic Peptides on Solid Support

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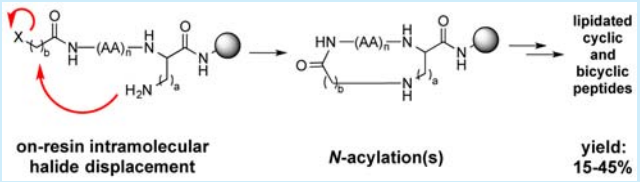
Supporting Information

ABSTRACT: A convenient strategy for the on-resin synthesis of macrocyclic peptides (3- to 13-mers) via intramolecular halide substitution by a diamino acid is described. The method is compatible with standard Fmoc/tBu SPPS and affords a tail-to-side-chain macrocyclic peptide featuring an endocyclic secondary amine. This functional group is still reactive toward acylation, allowing for the continuation of the synthesis. An application to the synthesis of lipidated cyclic and bicyclic antimicrobial peptides is presented.



Cyclic peptides are a scaffold of high pharmaceutical interest[1] and much work has been dedicated to the improvement of the critical ring-closure reaction, as described in many excellent reviews.[2]

Hereby we present the successful on-resin synthesis of modified peptide macrocycles consisting of 3 to 13 residues and featuring a backbone secondary amine. This was accomplished by combining two previously described methods. The first is a strategy originally presented by Robey and Fields and further developed by Roberts et al., which achieves ring closure by intramolecular nucleophilic substitution of an aliphatic bromide by a thiol group (e.g., from a cysteine residue), thus affording a macrocyclic peptide-thioether.[3] The second is the solid-phase synthesis of *N*-substituted glycines (i.e., NSG or peptoids) via the so-called submonomer approach, in which bromoacetic acid is coupled to the resin-bound peptide and the bromide is then substituted by a primary amine.[4]

The combination of these two strategies thus affords macrocycles featuring a backbone secondary amine. The advantages offered by this combination are considerable, as the resulting secondary amines are more chemically stable than the corresponding thioethers and they still have one proton that can be substituted, opening avenues to interesting synthetic applications.

Indeed, the possibility to continue the synthesis on the backbone after the macrocyclization step proved very convenient for the synthesis of cyclic lipopeptides, a scaffold of critical antimicrobial importance,[5] as well as bicyclic peptides of various descriptions. The advantages over existing synthetic protocols were considerable in terms of both yields (15−45%, Table 1) and crude purity (Figure 1). For a comparison, Li et al. achieved 8−13% yields for the solid-supported synthesis of similar lipidated cyclic γAA-peptides involving allyl-based protecting groups to access a third level of orthogonality.[6] A recent investigation of novel polymyxin analogs obtained with a combination of solid-phase synthesis and solution-phase cyclization resulted in similar or lower yields.[7] Karskela et al. reported ≤5% yields for their solid-supported synthesis of fused bicyclic peptides,[8] whereas **12** (Figure 2) was obtained in 19.6% yield.

Table 1. Summary of the 12 Macropeptoids Synthesized in This Study, Including Yields and Biological Activities[a]

						MIC		
no.	a	b	n	R[b]	yield	sa[c]	ec[c]	pa[c]
1	1	1	1	Dec	15%	>16	>16	>16
2	4	1	2	Dec	15%	>32	>32	>32
3	1	1	3	Dec	21%	>32	>32	>32
4	4	1	3	Dec	37%	>32	>32	>32
5	4	1	5	Pal	21%	4	4	4
6	4	1	7	Pal	24%	2	4	4
7	4	2	7	Pal	37%	>32	>32	>32
8	4	3	7	Pal	22%[d]	>32	>32	>32
9	4	4	7	Pal	45%	4	16	8
10[e]	4	1	9	Dec	25%	>32	32	>32
11[e]	4	1	11	Dec	24%	16	16	16
12	N/A[f]			Pal	20%	2	4	16

[a]For a description of a, b, n, and R, see Scheme 1. All ring closure reactions via halide displacements occurred overnight in the presence of 0.25 M DIEA. [b]Decanoyl or palmitoyl. [c]*Staphylococcus aureus* ATCC 29213; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; MIC in μM concentrations. [d]Calculated from a 27% yield of an 82% pure product. [e]Compounds **10** and **11** feature the D-form of phenylalanine. [f]The lipidated bicycle **12** is displayed in Figure 2.

As illustrated in Scheme 1, the key elements in the linear precursor are the ω-halocarboxylic acid and the diamino acid, which are involved in an intramolecular reaction resulting in

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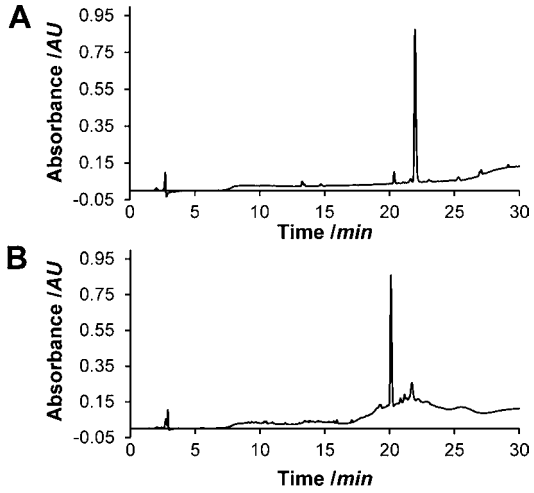


Figure 1. Crude RP-HPLC chromatograms (λ = 220 nm) of **5** (A) and **12** (B). Absorbance is expressed in arbitrary units (AU).

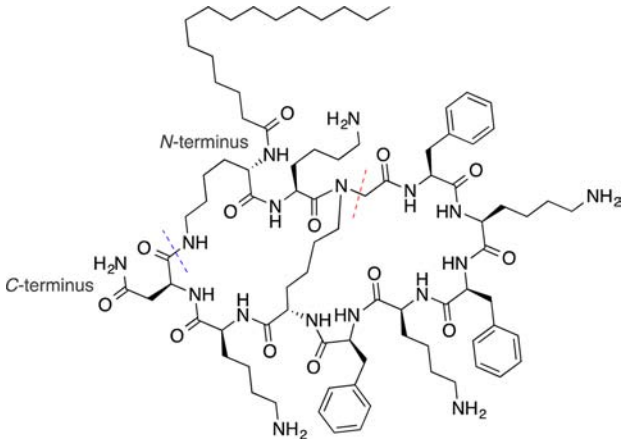


Figure 2. Structure of **12**. A red and a blue dashed line highlight the first and the second cyclization points, respectively.

ring closure. Due to the analogy with peptoids, these macrocyclic peptidomimetics could be called "*macropeptoids*".

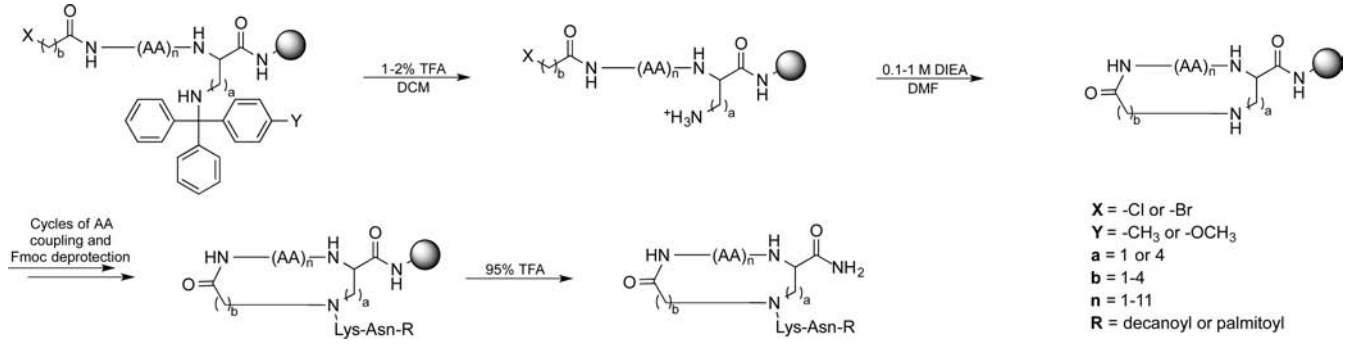
The diamino acid can occupy the *C*-terminal position or can be located within the sequence; its ω-amino functionality should be protected with an acid-labile group such as methoxytrityl (Mmt) or methyltrityl (Mtt), typically removed with <2% trifluoroacetic acid (TFA) in dichloromethane (DCM). The resin linker should instead release the peptide by treatment with >50% TFA (e.g., Wang or Rink-Amide linkers). In this way it is possible to remove Mmt/Mtt groups selectively, as the difference in acid sensitivity with Boc/*t*Bu groups and Rink/Wang linkers is wide enough to be *quasi*-orthogonal. Chain elongation can proceed according to the standard Fmoc/tBu strategy, with alternating cycles of amino acid coupling and deprotection. Finally, an ω-halocarboxylic acid preactivated with diisopropylcarbodiimide (DIC) is coupled to the *N*-terminus. After removing the Mmt- or Mtt-group, the deprotonation of the primary amine activates it toward substitution. The resulting macrocycle features a backbone secondary amine and a variable degree of backbone flexibility depending on the diamino acid and the halocarboxylic acid employed. The secondary amine provided the anchoring point for the (lipidated) exocyclic chain.

Crucial to our investigation was the assessment of whether lipidated macropeptoids would display the expected biological activity. For a proof of concept we therefore synthesized several peptides consisting of repeated aromatic and cationic amino acids, since this scaffold has proven robust antimicrobial activity in a variety of backbone motifs, in both linear and cyclic arrangements.[6,9] As a lipidated exocyclic chain we likewise adopted the previously reported Palmitoyl-Asn-Lys sequence,[6] but due to solubility issues, for some compounds decanoic acid was preferred. We report that the antimicrobial activity was maintained at similar or identical levels as previously reported for analogous compounds (Table 1).[6]

The general ring-core structure *cyclo*(AA_n-Xda-NH_2) is displayed in Scheme 1, where AA is either Phe or Lys (alternated after the first Phe) and Xda is the N^{ω}-(carboxyalkyl)-α,ω-diamino acid (e.g., N^6-(carboxymethyl)-lysine in **2** and **4**−**11**). For **10** and **11**, D-Phe has been preferred to facilitate handling in aqueous solutions (reduce foaming). The resulting N^{ω}-(carboxyalkyl)-diaminoacyl moieties resemble the lysinoalanyl residue observed in some naturally occurring antimicrobial compounds.[10] The COMU/Oxyma[11] combination was chosen for couplings on primary α-amino groups, but HATU/HOAt[12] were preferred for the couplings on the backbone secondary amine. The completion of the latter was assessed via the chloranil test.[13]

The lipidated, fused bicycle **12** (Figure 2) was obtained from the linear precursor bromoacetyl-(Phe-Lys(Boc))$_3$-Lys(Mmt)-Phe-Lys-Asp-ODmab. The synthesis started by loading Fmoc-Asp-ODmab on the solid-supported Rink amide linker (RAM). After ring closure, Fmoc-Lys(Boc)-OH was coupled to the backbone secondary amine, followed by Fmoc-Lys(Dde)-OH. After removal of the Fmoc-group, palmitic acid was coupled. Finally, after the simultaneous removal of the Dmab- and Dde-groups with a 2% hydrazine/DMF solution, the second ring

Scheme 1. Pathway for the Solid-Supported Synthesis of Lipidated Macropeptoids



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closed via amide bond formation using HOAt/PyBOP[14] as coupling reagents.

Besides its similarity with the synthesis of peptoids and cyclic thioethers, our approach also displays a few core differences: no excess of nucleophile can be added to drive the reaction to completion, and while thiols are weakly acidic, the amino-nucleophile also competes in protonation equilibria and can undergo substitution more than once. On the other hand, intermolecular reactivity is a shortcoming of all cyclization methods. Overall, in the studied conditions dimerization was not found to substantially affect the yields of the desired compounds, provided that a low-loading support is employed. Medium-loading supports (≈0.6 mmol/g) were found to affect crude purity even for very small macrocycles, such as the one featured in **1**. Although we only employed TentaGel resins, we foresee no complication in using low-loading Wang supports. In that case, the liberation of a peptide acid might prove to be a highly convenient strategy for the synthesis of anionic, Ca-dependent antimicrobial lipopeptides such as daptomycin.[5a]

In order to react as a nucleophile, the ω-amino group of the diamino acid must not be protonated. The substitution reaction produces HX as a byproduct, where X^- is the halide; ideally, this proton would be accepted by the secondary amine of a closed ring, as this would inhibit further reactions. Realistically, from previous literature[15] we expected it to have a lower pK_a than e.g. lysine's primary ε-amino group. Additional base is thus required to drive the reaction to completion. Non-nucleophilic bases such as diisopropylethylamine (DIEA) can be safely employed at high concentration. However, it could be assumed that a stronger (yet still hindered) base would accelerate the cyclization reaction by deprotonating lysine's ε-amino group more effectively. Bicyclic amidine bases fit this description. In particular, 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) has been employed as a non-nucleophilic alternative to piperidine for the removal of Fmoc-groups.[16] Unfortunately, whenever DBU was used to promote the ring closure reaction, mass spectra showed signals from presumable peptide-DBU adducts (see Supporting Information). Therefore, DIEA still appears to be the base of choice.

Cyclization rates were not rigorously studied, as they arguably depend on the composition of individual sequences. We report however that, for the studied sequences, acceptable cyclization times (maximum overnight) were observed with base concentrations between 0.1 and 1 M and a total volume corresponding to 20 equiv. For medium-sized rings (e.g., **5**−**9**), the cyclization time was found to be ≤90 min; such peptides have also been obtained in an encouraging 21−45% yield. Although the reaction course could be conveniently monitored with the TNBS test,[17] we observed a high rate of *pseudo*-false positives presumably deriving from negligible amounts of unreacted primary amine; cyclization completion was therefore assessed by cleaving the peptide from a small amount of resin.

Both acylation and substitution steps proceed satisfactorily for bromoacetic acid, but a substantial amount of impurities were observed in the crude HPLC chromatogram for longer ω-bromocarboxylic acids. A C-activated ω-halocarboxylic acid presents two electrophilic sites, the carbonyl- and the ω-carbon, both bearing good leaving groups that can be substituted by the resin-bound α-amino group. The relative reactivity of the leaving groups determines the product composition. Moving the halide away from the α-carbon decreases the acylating efficiency of the DIC-activated carboxyl function and encourages side reactions involving the ω-bromide. As previously described, replacing the ω-bromides with ω-chlorides can effectively hinder such undesired reactions by decreasing the reactivity of the ω-leaving group.[18] The reactivity toward substitution, necessary for the ring closure reaction, was later reestablished *in situ* by adding KI (1 M) to the DIEA/DMF solution (see Scheme 1).

The high purity of the crude products lead us to expect even higher yields for the purified compounds. We presume that in some cases the yields were affected by the low water solubility of small lipidated compounds and/or the surfactant-like behavior (foaming) of some others, which made them difficult to handle when in aqueous solutions (e.g., during and after purification). Indeed, we failed to obtain all-L versions of compounds **10** and **11**. However, the replacemement of L-Phe with its D-form allowed us to obtain the compounds easily and in good yields. Unfortunately, our equipment proved unable to fully separate all impurities from **8**.

In conclusion, a promising method for the solid-supported preparation of peptide macrocycles featuring a backbone secondary amine has been described. The strategy appears very convenient for the synthesis of modified cyclic and bicyclic peptides in a useful size range (3- to 13-membered rings). Its intrinsic suitability to the synthesis of cyclic antimicrobial lipopeptides makes it a useful tool in the fight against multidrug-resistant pathogens, a worldwide emergency exacerbated by the lack of novel therapeutics.[19] Moreover, we envisage that the possibility to easily obtain very small, bifunctional macrocycles could find a wide range of applications in the design of conformationally controlled peptides as high-affinity receptor ligands.[20]

■ ASSOCIATED CONTENT

Supporting Information

List of materials, experimental methods, MALDI spectra and analytical HPLC chromatograms are included as Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01026.

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Notes

The authors declare no competing financial interest.

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