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Cyclic Carbo-Isosteric Depsipeptides and Peptides as a Novel Class of Peptidomimetics

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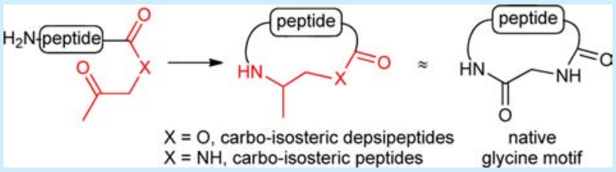
Ⓢ *Supporting Information*

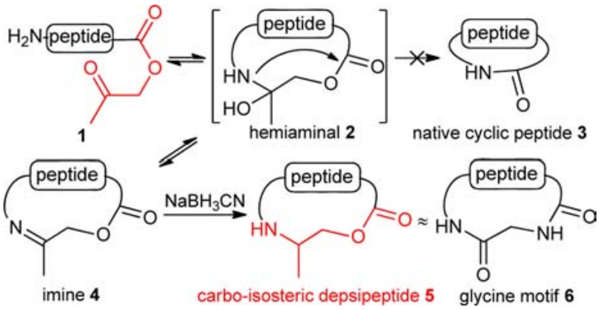
ABSTRACT: A novel and highly efficient cyclization method has been developed to access a new class of cyclic carbo-isosteric depsipeptides and carbo-isosteric peptides. Our strategy requires easily accessible C-terminal methyl ketone ester or amide functionalized linear precursors as starting materials. The well-known reductive amination has then been used to afford cyclic tetra- to octa-pseudopeptides *via* a selective intramolecular formation of a glycine peptidomimetic unit under moderate dilution.



Over the years, both academic groups and the pharmaceutical industry have extended their research toward peptide chemistry.[1] Peptides with their high modularity and potential diversity give rise to a high likelihood for strong affinity for a broad range of biological targets. Consequently, they have become interesting candidates for the development of novel drug-like molecules.[2] However, only a few of them have reached the market due to their poor bioavailability and lack of permeability and metabolic stability. Multiple factors are known to improve the pharmacological properties of peptidic drugs such as *N*-methylation,[3,4] glycosylation,[5] and the use of D-amino-acids and CPP transporters.[6] Head-to-tail cyclization of peptides[7−9] has additionally been performed to increase their *in vivo* stability and proteolysis resistance.[10,11] Another major strategy is the use of pseudopeptidic motifs[12,13] which mimic the secondary structure of peptides and can modulate their bioavailability.[14,15] As a state of the art option, cyclic peptidomimetics[16] represent a very interesting and powerful combination to access highly potent compounds with improved pharmacokinetic properties.[17]

With the aim of developing new methods to access macrocyclic molecules, we focused on the possible modification of the Tam ligation.[18,19] Using a linear peptide functionalized with a C-terminal methyl ketone ester **1**, we first expected to perform a head-to-tail cyclization *via* a reversible hemiaminal **2** formation which could then undergo a ring contraction to afford, after spontaneous elimination, a native cyclic peptide **3** (Scheme 1). A wide range of conditions were studied. Unfortunately, the ring contracted product **3** was never observed and only the starting material **1** was detected. To understand the lack of detectable product resulting from the hemiaminal/ring contraction strategy, sodium cyanoborohydride was added to the reaction mixture leading to the formation of a cyclic amine in 100% conversion. Surprisingly, only the monomeric product **5** was formed without oligomerization. Even though the reductive amination is a well-known transformation, it has, to the best of our knowledge, never been reported in the literature to afford cyclic pseudopeptides. Compared to the native cyclic peptide scaffold **6**, the obtained motif **5** displays the same number of ring atoms with the methyl being a replacement for the carbonyl unit and would presumably mimic the glycine amino acid structure. Additionally, the resulting cyclic lactone **5** resembles the depsipeptide family consisting of a cyclic peptidic structure containing a lactone unit. We therefore propose herein to name our new peptidomimetic family, "carbo-isosteric" depsipeptides (Scheme 1).

Scheme 1. Attempted Prior-Capture/Ring Contraction Strategy Giving Rise to Cyclic Pseudopeptide *via* a Novel Glycine Peptidomimetic Motif



It is important to state that interesting syntheses of depsipeptides have been reported in the literature.[20,21] This compound class exhibits remarkable biological activity (e.g., anticancer[22,23] and antimicrobial[24]) and is widely investigated for therapeutic applications. In general, the cyclic depsipeptides have mainly been found in natural environments. For example, Jaspamidine[25] isolated from the indo-pacific sponge displays cytotoxic, antifungal, and insecticidal activities. Petriellin A

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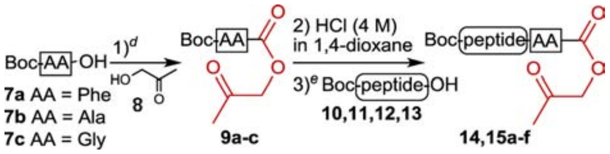
isolated from the organic extracts of a fungus, *Petriella sordida*, also shows antifungal activity.[26] In accordance, our first disclosed carbo-isosteric depsipeptide family could represent a valuable starting point for the development of new drug-like molecules.

Encouraged by the above-mentioned observations and the interest in the cyclic depsipeptide class of peptidomimetics, we decided to apply this novel methodology to the synthesis of various ring size carbo-isosteric depsipeptides. First, the methyl ketone motif was introduced in linear peptides (Table 1).

Table 1. Synthesis of Methyl Ketone Ester Linear Peptidic Precursors 14 and 15a–f



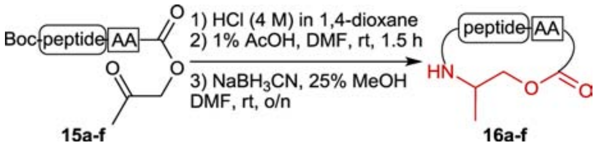
entry	AA[a]	step 1 yield (%)	peptide	step 3 yield (%)[b]	product
1	n.a.	n.a.	**10**, GGFL	54[c,d] 0[e]	**14**, Boc-GGFL-$CO_2CH_2COCH_3$
2	**7a**	**9a**, 91	**10**	35[e]	**15a**, Boc-GGFLF-$CO_2CH_2COCH_3$
3	**7b**	**9b**, 89	**10**	44[e]	**15b**, Boc-GGFLA-$CO_2CH_2COCH_3$
4	**7c**	**9c**, 89	**10**	44[e]	**15c**, Boc-GGFLG-$CO_2CH_2COCH_3$
5	**7b**	**9b**, 89	**11**, LFPG K(Me_2)A	69[e]	**15d**, Boc-LFPG K(Me_2)AA-$CO_2CH_2COCH_3$
6	**7a**	**9a**, 91	**12**, F^{D}AG	65[e]	**15e**, Boc-F^{D}AGF-$CO_2CH_2COCH_3$
7	**7a**	**9a**, 91	**13**, A^{D}E(Bzl)	17[e]	**15f**, Boc-A^{D}E(Bzl)F-$CO_2CH_2COCH_3$

[a] SM used in step 1 unless otherwise stated. [b] Isolated yield over 2 steps after preparative RP-HPLC. [c] Complete racemization observed (dr 50:50 determined by RP-UPLC-MS analysis). [d] Hydroxyacetone (3 equiv), EDC·HCl (1.2 equiv), DMAP (0.3 equiv), DCM, 0 °C to rt, o/n. [e] Optimized conditions: **8** or **9a–c** (2.5 equiv), HCTU (1.45 equiv), 2,6-lutidine (2.5 equiv), DCM/DMF 1:1, 0 °C to rt, o/n; racemization was not observed using RP-UPLC-MS analysis.

Commercially available hydroxyacetone **8** was directly reacted with linear peptide **10** using EDC·HCl and DMAP to give product **14** as a 50:50 mixture of epimers at the C-terminal leucine (entry 1). Using other coupling conditions did not allow the formation of the desired product. Alternatively, *N*-Boc-protected phenylalanine **7a** was esterified without racemization using alcohol **8** and EDC·HCl and DMAP (entry 2). The resulting phenylalanine **9a** was then treated with hydrogen chloride and coupled to peptide **10** using HCTU to afford precursor **15a** without observable epimerization. In a similar fashion, compound **8** was reacted with *N*-Boc-protected alanine **7b** and glycine **7c** and introduced into tetrapeptide **10** to give compounds **15b** and **15c** (entries 3 and 4).

To enhanced the ring closure study, linear peptides **11–13** were reacted with methyl ketone ester amino-acids **9a** and **9b** to afford functionalized hepta-, tetra- and tripeptides **15d–f** (entries 5–7).

Table 2. Cyclic Carbo-Isosteric Depsipeptides 16a–f Using Optimized Reductive Amination Conditions[27]



entry	SM	c[a]	yield (%)[b]	dr[c]	product[d]
1	Boc-GGFLF-$CO_2CH_2COCH_3$	8	74	52:48	**16a**
2	Boc-GGFLF-$CO_2CH_2COCH_3$	50	51	52:48	**16a**
3	Boc-GGFLA-$CO_2CH_2COCH_3$	8	59	54:46	**16b**
4	Boc-GGFLG-$CO_2CH_2COCH_3$	8	69	n.a.	**16c**
5	Boc-LFPGK(Me_2)AA-$CO_2CH_2COCH_3$	8	63	68:32	**16d**
6	Boc-F^{D}AGF-$CO_2CH_2COCH_3$	8	85	81:19	**16e**
7	Boc-A^{D}E(Bzl)F-$CO_2CH_2COCH_3$	8	40	78:22	**16f**

[a] Final concentration in mM at step 3. [b] Isolated yield over 3 steps after preparative RP-HPLC. [c] dr determined by RP-UPLC-MS or chiral-HPLC analysis. [d] For structures see Figure 1.

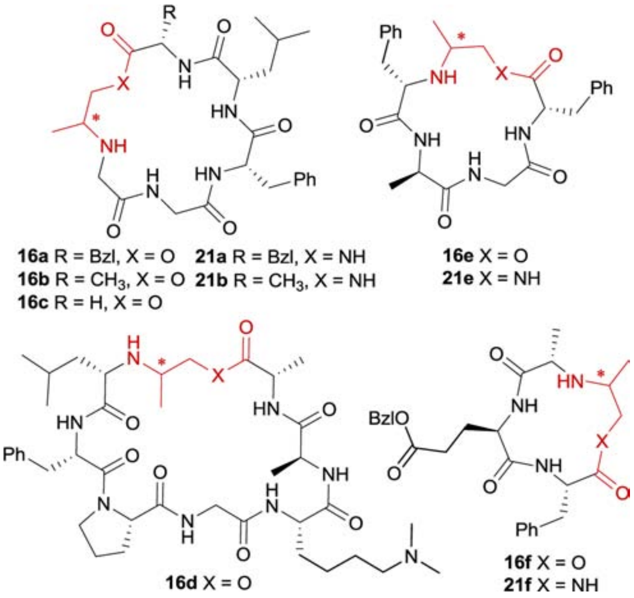


Figure 1. Structure of the obtained cyclic carbo-isosteric depsipeptides and carbo-isosteric peptides.

Our attention next focused on the use of our cyclization method to afford various ring size carbo-isosteric depsipeptides (Table 2 and Figure 1). After extensive experimentation, we found the optimal conditions for the ring closing reductive amination to be 1% acetic acid in DMF followed by addition of sodium cyanoborohydride and 25% MeOH onto the imine intermediate. The desired monomeric cyclic hexapeptidomimetic **16a** was successfully isolated in 74% yield (entry 1). It is noteworthy that the cyclization was performed at relatively high concentration (*c* = 8 mM) with respect to reported peptide cyclizations. Even when the concentration was increased to 50 mM, the cyclic dimer was detected in only 21% conversion and the desired cyclic monomer **16a** was observed with 79% conversion and isolated in 51% yield after purification by preparative RP-HPLC (entry 2). Variation of the C-terminal amino acid from phenylalanine to alanine or glycine did not noticeably influence the yield of the cyclization (entries 1, 3, and 4).

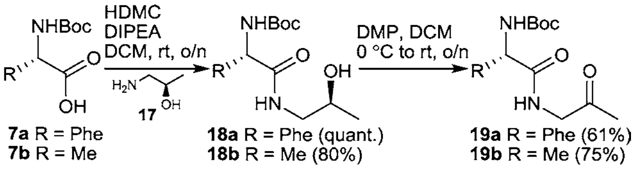
By increasing the linear precursor length to eight units, the intramolecular reductive amination allowed the selective formation of cyclic carbo-isosteric octa-depsipeptide **16d** in good yield (entry 5). However, the lysine backbone amino acid was protected to avoid side chain backbone cyclization. Alternatively, the azide protecting group for lysine residue would be compatible with our system.

By decreasing the ring size, constrained cyclic penta-pseudopeptide **16e** was successfully isolated in 85% yield. From a more challenging aspect and knowing the difficulty in accessing cyclic tetra-peptides using conventional amide bond formation methods, we studied the feasibility of our concept to access cyclic tetra-depsipeptide carbo-isosteres with the expectation that the reversible prior-capture step of imine formation could ease such difficult cyclizations. To our delight, cycle **16f** was obtained in an acceptable isolated yield of 40% without observable oligomerization.

As expected when the N-terminal amino acid differs from a glycine unit, a slight induction toward one diastereoisomer was achieved during the reduction of the imine intermediate (entries 5 and 7). Remarkably for cyclic carbo-isosteric penta-depsipeptide **16e** containing a N-terminal phenylalanine, a 81:19 diastereoisomeric ratio was observed (entry 6). In the case of cyclic pseudopeptides **16b**, **16d**, and **16e**, each diastereoisomer was successfully separated by preparative chiral-HPLC or RP-HPLC (see Supporting Information). However the absolute configuration could not be established due to the lack of relevant NOE correlations.

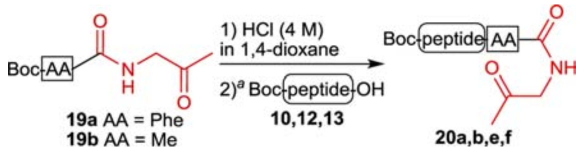
The last part of our study aimed to enrich our cyclic pseudopeptidic family by expanding the lactone unit to a lactam group to increase the pool of peptidomimetic scaffolds. We focused our feasibility study on four selected lactone analogues. Starting with the synthesis of the methyl ketone amide motif, *N*-Boc-protected phenylalanine **7a** and alanine **7b** were coupled with commercially available amino-alcohol **17** to give alcohols **18a** and **18b**, respectively. Subsequent Dess-Martin periodinane oxidation afforded the amide methyl ketones **19a**[28] and **19b**[29] in good yield (Scheme 2).

Scheme 2. Synthesis of the Methyl Ketone Amide Functionalized Amino Acids 19a and 19b



After Boc deprotection of precursors **19a** and **19b** and using the optimal coupling conditions (see Table 1), the free amine unfortunately self-reacted with the ketone to form as the major product the corresponding methylpyrazinone after rearrangement. After extensive experimentation, HATU and 2,6-lutidine with a 1:1 acid/amine ratio gave the desired methyl ketone amide linear peptides **20a,b,e,f** in good yields without epimerization and only traces of the unwanted side product (Table 3, entries 1−4).

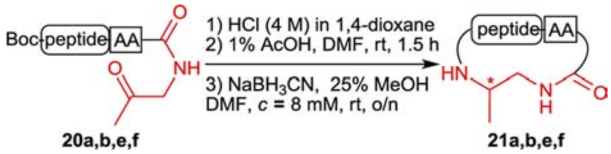
Table 3. Synthesis of the Methyl Ketone Amide Linear Peptidic Precursors 20a,b,e,f



entry	SM	peptide	yield (%)[b]	product
1	**19a**	**6**	53	**20a**, Boc-GGFLF-CONHCH$_2$COCH$_3$
2	**19b**	**6**	60	**20b**, Boc-GGFLA-CONHCH$_2$COCH$_3$
3	**19a**	**8**	52	**20e**, Boc-F^{D}AGF-CONHCH$_2$COCH$_3$
4	**19a**	**9**	59	**20f**, Boc-A^{D}E(Bzl)F-CONHCH$_2$COCH$_3$

[a] Optimized conditions: peptide (1 equiv), **19a** or **19b** (1 equiv), HATU (1.45 equiv), 2,6-lutidine (1.22 equiv), DCM/DMF (3:1), 0 °C to rt, o/n. [b] Isolated yield over 2 steps after preparative RP-HPLC; racemization was not detected using RP-UPLC-MS analysis.

Table 4. Extension of Our Method to the Synthesis of Cyclic Carbo-Isosteric Peptides 21a,b,e,f[27]



entry	SM	yield (%)[a]	dr[b]	product[c]
1	Boc-GGFLF-CONHCH$_2$COCH$_3$	70	n.a.	**21a**
2	Boc-GGFLA-CONHCH$_2$COCH$_3$	54	70:30	**21b**
3	Boc-F^{D}AGF-CONHCH$_2$COCH$_3$	72	88:12	**21e**
4	Boc-A^{D}E(Bzl)F-CONHCH$_2$COCH$_3$	dimer[d]	n.a.	**21f**[d]

[a] Isolated yield over 3 steps after preparative RP-HPLC. [b] dr determined by chiral-HPLC analysis. [c] See Figure 1 for structures. [d] Desired monomeric product was not observed; cyclic dimer was isolated in 16% yield as a complex mixture of four diastereoisomers.

Finally, the ring closing reductive amination was performed with the methyl ketone amide analogues using the previously optimized conditions. Cyclic carbo-isosteric hexa-peptides containing either phenylalanine **21a** or alanine **21b** as C-terminal amino acids were isolated in 70% and 54% yield respectively (Table 4, entries 1 and 2). The constrained cyclic penta-lactam **21e** was also successfully obtained in excellent yield with a 88:12 diastereoisomeric ratio, and the two diastereoisomers were isolated by preparative chiral-HPLC (entry 3). In analogy to its carbo-isosteric depsipeptide analogue **16e**, a similar yield and diastereoisomeric ratio were obtained.

Unfortunately, highly challenging cyclic tetra-pseudopeptide **21f** could not be obtained and only the dimer (cyclic octa-peptidomimetic) was isolated in 16% yield (entry 4). Alternative methods, conditions, and different sequences for the ring closing reductive amination of cyclic carbo-isosteric tetra-peptides will have to be studied in the future. However, as a preliminary remark, the replacement of the lactone by a lactam decreases the ring flexibility which in the case of a cyclic tetramer is extremely critical and does not give rise to the desired cyclic monomer.

To further demonstrate the impact of our cyclization method, the synthesis of a native cyclic hexapeptide *cyclo*[-GGFLAG-], an analogue of the obtained pseudopeptide **21b,** was attempted by cyclization of a linear peptide Boc-GGFLAG-OH using standard coupling reagents with a concentration of 1 mM. By using PyOxP,[30] the ring closure was achieved in only 4% yield. The maximum yield was obtained using the HATU/HOAt system, but in that case, even at 1 mM the cyclic peptide could only be isolated in 35% yield. In contrast, its carbo-isosteric peptide analogue **21b** was successfully isolated in 54% yield at 8 mM (entry 2).

To conclude, we have developed a powerful method to access novel cyclic peptidomimetics. Our strategy affords cyclic carbo-isosteric penta- to octa-depsipeptides and peptides in high yields under moderate dilution. Importantly, a constrained cyclic lactone tetra-pseudopeptide was successfully isolated in good yield. In our study, *N*-Boc-protected linear peptides were arbitrarily chosen. However, the precursors could be replaced by Fmoc protected linear peptides without affecting the efficacy of our reductive amination cyclization strategy. From a structural aspect, the resulting α-methyl secondary amine provides an interesting chemical handle to diversify the obtained cyclic peptidomimetic structure. Future work will investigate the scope and limitation of our strategy. Computational and NMR conformational studies will be performed to understand the rules that govern the intramolecular selectivity and diastereoselectivity of our novel methodology. Their potential biological applications will additionally be investigated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization data for all compounds. Analytical RP-UPLC-MS profiles for **14** and reaction mixtures of **16a** at *c* = 8 and 50 mM. Complete analytical RP-UPLC-MS profiles for the synthesis of cyclic carbo-isosteric depsipeptide **16e**. Analytical RP-UPLC-MS or analytical chiral-HPLC profiles for the final cyclic peptidomimetic products (**16a,b,d,e,f** and **21b,e**). Copies of ^{1}H and ^{13}C NMR spectra for the precursors and the cyclic peptidomimetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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