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Revisiting N-to-O Acyl Shift for Synthesis of Natural Product-like Cyclic Depsipeptides

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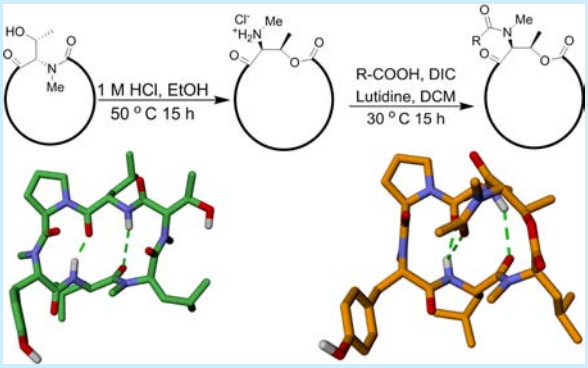
Supporting Information

ABSTRACT: Despite the prevalence of head-to-side chain threonine linkages in natural products, their incorporation has been underexplored in synthetic cyclic peptides. Herein we investigate a cyclic peptide scaffold able to undergo an *N*−*O* acyl rearrangement. Upon acylation of the amine with diverse carboxylic acids, the resulting cyclic depsipeptides displayed favorable cellular permeability and a conformation similar to the parent peptide. The rearrangement was found to be scaffold and conformation dependent as evidenced by molecular dynamics experiments.



Cyclic depsipeptides are a diverse class of natural products that have shown a wide variety of biological activities.[1−5] Many cyclic depsipeptides are defined by a single C-terminal-to-side-chain ester (i.e., "depsi") linkage through either a threonine or serine residue. These linkages result in lariat-type structures in which the amino termini are further elongated and/or capped with diverse lipid tails. It has been shown in structure−activity studies with the kahalalides, for example, that both the depsi-linkage and tail are required for biological activity.[6−9]

The *N*-acyl-*O*-acylthreonine linkages found in many cyclic depsipeptides[9] involve a significant backbone rearrangement of the parent homodetic peptides and allow for further diversification, by decoration of the exocyclic amine with any of a variety of peptide extensions or capping groups. Cyclic peptide backbone elements, e.g., ring size, stereochemistry, and amide *N*-methylation, are known to have an impact not only on pharmacological activity, but also on ADME properties such as cell permeability and metabolic stability.[10−13] However, despite the ubiquitous presence of backbone-to-side chain linkages among cyclic peptide natural products, their effect on physicochemical and pharmacokinetic properties has not been investigated in detail.

Previously our laboratory undertook an investigation into the influence of side chain polarity on the permeability and metabolic stability of the cell permeable and orally bioavailable *N*-methylated cyclic hexapeptide cyclo[Leu1-D(NMe)Leu2-(NMe)Leu3-Leu4-DPro5-(NMe)Tyr6] (**1NMe3**).[13,14] The (NMe)Leu3 residue was substituted with a variety of different amino acids, and the ADME properties of the derivatives were compared with those of the parent compound. We found that the derivative in which (NMe)Leu3 was replaced with (NMe)Thr (**1**, Scheme 1) had good oral bioavailability in rat (F = 24%), nearly equal to that of the parent compound. However, in the course of analysis via reversed phase HPLC, we found that **1** had partially converted to a more polar and less permeable isomer.

NMR analysis of this isomer revealed its structure to be compound **2** (Scheme 1), the product of an *N*-to-*O* acyl migration from the main chain to the Thr side chain. Since its discovery in 1922,[15] this rearrangement has been used as a prodrug strategy to enhance the solubility of lipophilic peptides, and has been observed as a side reaction during the acidic deprotection of synthetic peptides that contain Thr and Ser residues.[16,17] In addition, this reaction has been explored as a degradation pathway of the clinically relevant drug cyclosporine A.[18] In light of the recent surge of interest in macrocycles as "beyond-rule-of-5" (bRo5) scaffolds in drug discovery[19] and the prevalence of depsipeptides among cyclic peptide natural products, we were compelled to investigate the potential of

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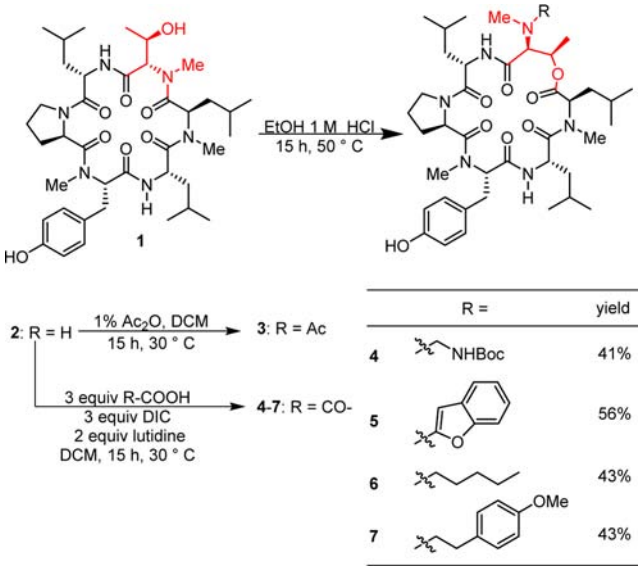
Scheme 1. Acyl Rearrangement and Subsequent Acylation



this unanticipated side reaction as entry into lariat peptides as a subgenre of synthetic, cell-permeable macrocycles.

Although the isoacyl depsipeptide **2** was stable as its HCl salt, it quickly rearranged back to the amide upon neutralization in aqueous buffer.[18] To stabilize the ester and allow for a more direct comparison between the uncharged lactam **1** and the corresponding depsipeptide, the free amine **2** was acetylated with acetic anhydride to generate cyclic depsipeptide **3** in good yield (62% after HPLC purification) and purity. Compound **3** contains the same *N*-acetyl-*O*-acylthreonine moiety commonly found in many bioactive cyclic peptide natural products such as the micropeptins, aspergillicins, xenobactin, and some members of the kahalahide series.[6−9]

Inspired by cyclic depsipeptides containing chemically diverse "tails", we sought to explore functionalization of the exocyclic amine. To that end we synthesized a small set of compounds from the HCl salt of **2**, using standard carbodiimide coupling chemistry. The resulting cyclic depsipeptides were isolated in reasonable yield over two steps starting with **1** and in good purity after HPLC purification. The addition of Boc-glycine was used as a possible entry point into solution phase peptide synthesis that would allow for access to peptidic tails such as those observed in a majority of head-to-side chain cyclic depsipeptides. The addition of the lipidic hexanoic acid yielded a macrocycle similar to the lipopeptide antibiotics[20] and demonstrates the potential of this route for late stage diversification.

To explore the generality of this acyl rearrangement within different macrocycles, a series of cyclic peptides were synthesized and subjected to the acidic conditions employed in the acyl shift of **1**. These peptides were varied with respect to ring size, *N*-methylation pattern, and amino acid constitution. Since many cyclic depsipeptide natural products contain primarily hydrophobic residues, another series was designed that incorporated *N*-methyl Phe and Ala in addition to Leu, Tyr, and Pro. The peptides in Table 1 were synthesized using standard Fmoc-SPPS and subjected to either global selective *N*-methylation[13] or in-sequence *N*-methylation using NBS- or TFA- protecting group strategies.[21,22]

Purified peptides were then subjected to 1 M ethanolic HCl at 50 °C overnight. In some scaffolds these conditions were found to cause degradation, in which case gentler conditions (0.1 M ethanolic HCl at 30 °C overnight) were used. The depsipeptide-to-peptide ratio was quantified by HPLC. The sequences and rearrangement efficiencies are outlined in Table 1. Notably, compounds **14** and **15** rearranged quantitatively under the mild conditions. Although all compounds that quantitatively rearrange in the conditions tested contain an *N*-methyl Thr, *N*-methylation does not appear to be a requirement. Of the three compounds not containing an *N*-methyl Thr tested, **8**, **10**, and **11** all rearrange to some extent, with **8** achieving nearly 50% conversion. Since *N*-methylation impacts both conformation and electronics, it is difficult to draw any strong conclusions about the direct effect of *N*-methylation on the rearrangement efficiency.

Indeed, the dramatic difference in rearrangement efficiency between **1** and **9** (100% and 0% conversion, respectively), which have the same backbone geometry and differ only in the location (and stereochemistry) of the Thr side chain, suggested that the position of the Thr residue in the β-turn ($i+1$ for **9** vs $i+2$ for **1**) was the source of their differential reactivity. The optimal attack angle for nucleophilic attack at an sp^2 center is ∼105°, known as the Burgi−Dunitz angle.[23] We hypothesized that access to the productive conformer required for nucleophilic attack of the Thr-OH onto the $i-1$ carbonyl carbon, defined by both the Burgi−Dunitz angle and the distance between the reacting groups, was related to the side chain's conformational preference with respect to the conformation of the backbone of the $i-1$ residue.

We ran molecular dynamics (MD) simulations on **1** and **9**, monitoring the distance and angle between the reacting atoms. Analysis of 2000 snapshots from each simulation (Figure 1) showed that reactive conformations for **1** were sampled twice as

Table 1. Sequence and Rearrangement Efficiencies for Cyclic Peptides

compd	cond	1	2	3	4	5	6	7	conv %[c]
8	*a*	Thr	**dLeu**	**Leu**	Leu	dPro	**Tyr**		45.8
9	*a*	Leu	**dThr**	**Leu**	Leu	dPro	**Tyr**		0
1	*a*	Leu	**dLeu**	**Thr**	Leu	dPro	**Tyr**		100
10	*b*	Leu	**dLeu**	**Leu**	Thr	dPro	**Tyr**		6.4
11	*b*	Leu	dLeu	dThr	Leu	dPro	Tyr		19.2
12	*b*	Leu	**Leu**	**Thr**	Leu	dPro	**Tyr**		0.9
13	*a*	Leu	**Thr**	Ala	**Phe**	dPro			41.3
14	*b*	Leu	**Thr**	Ala	**Phe**	Leu	dPro		100
15	*b*	Leu	**Thr**	Ala	**Phe**	Leu	Leu	dPro	100

[a]1 M ethanolic HCl, 50 °C, 15 h. [b]0.1 M ethanolic HCl, 30 °C, 15 h. Bold indicates *N*-methylated amino acids. [c]As quantified by UV (220 nm) integration of HPLC spectra.

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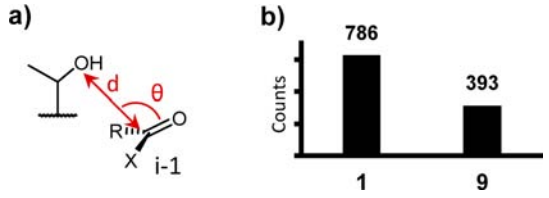

Figure 1. (a) Distance and angle measured between Thr-OH and $i-1$ carbonyl carbon; (b) graph of snapshots containing an −OH to C═O distance of <4 Å and O−C−O angle within ±10° of the Burgi−Dunitz angle.

frequently as they were for **9** (SI Figure 2a,b; shaded boxes). Thus, while the stereochemistry and/or *N*-Me status of the Thr and its neighboring residues may not, in themselves, be predictive of rearrangement efficiency, they may allow or disallow access to reactive conformations by virtue of their impact (along with other factors) on the global conformation of the macrocycle. Combined with the fact that under highly acidic conditions the reverse acyl shift is unlikely, the MD results point toward a kinetic rather than thermodynamic effect driving the observed differences in % conversion.

In an attempt to gain a deeper understanding, the biochemical properties imparted by the amide-to-ester transformation, the ADME properties of all depsipeptides, and the conformations of **1** and **3** were investigated in detail. Despite its increased size and flexibility, the cell permeability of **3** was comparable to that of **1** (Table 2). The depsipeptides containing more complex tails, **4**−**7**, exhibited favorable cellular permeability as well, at least as high as that of the orally bioavailable cyclic peptide natural product **CSA**. In an attempt to explain the cell permeabilities of **1** and **3** we sought to solve their three-dimensional solution conformations.

Table 2. *In Vitro* Cell Permeability for Selected Compounds

compound	MDCK-LE (10^{-6} cm/s)
1	2.7
3	3.3
4	2.2
5	1.1
6	2.9
7	2.0
CSA	1.1
1NMe3	4.0

Compound **1** and its acetylated depsipeptide analog **3** provide a model system with which to explore the effect of the depsipeptide linkage on backbone conformation. Peak assignments were made using COSY, TOCSY, HSQC, and HMBC experiments, and NOESY-derived interproton distances were calculated based on crosspeak volumes. ^3J H−H couplings were also used to provide dihedral information. Conformers were generated using molecular dynamics (MD) simulations, and ensembles were fit to the distance and dihedral restraints using the "NMR analysis of molecular flexibility in solution" (NAMFIS)[24] protocol in the program DISCON.[25,26] This algorithm fits NMR data to ensembles of conformers rather than *a priori* assuming the presence of a single, low energy conformation in solution.

Compound **1** showed a single, dominant conformer (98% of the ensemble) when analyzed with DISCON. In contrast, compound **3** showed a mixture of four different conformations with the major one constituting 54% of the ensemble. Minor conformations are shown in the Supporting Information (SI Figure 1). Interestingly, the major solution conformation of **3** showed a backbone conformation with transannular hydrogen bonds flanked by β-turns, very similar to those of **1** and **1NMe3** despite the amide-to-ester ring expansion (Figure 2d).

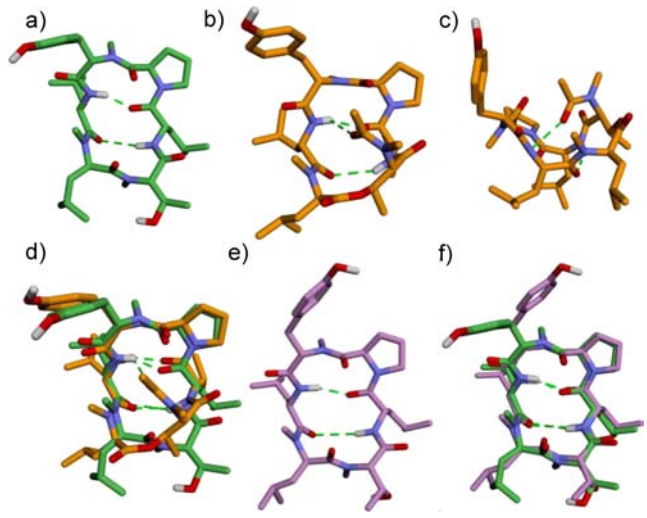

Figure 2. (a, b) Major conformation of the DISCON ensemble for **1** and **3**, respectively. (c) Conformation of **3**, highlighting tail-to-backbone hydrogen bond. (d) Overlay of **1**, green, and **3**, orange. (e, f) X-ray structrure of **1** and an overlay of the X-ray structure, purple, and solution conformation, green.

In addition to the DISCON structure, crystallization of **1** by vapor diffusion between benzene and cyclohexane yielded X-ray diffraction-quality crystals. The X-ray structure of **1** was similar to that of the solution conformation (backbone and Cβ RMSD = 0.321 Å). In addition, both the X-ray and solution structure adopted a conformation similar to that of the solution structure previously determined for **1NMe3** in $CDCl_3$.[13] This conformation is defined by two transannular hydrogen bonds flanked on either side by opposing β-turns (Figure 2a). The similarity in backbone conformation between **1** and **1NMe3** lends further support to the observation that stereochemistry and *N*-methylation are major determinants of backbone geometry in cyclic peptides in this size range.

The presence of intramolecular hydrogen bonds has been shown to be a significant determinant of cell permeability in cyclic peptides[27,28] and are present in the major conformations of both **1** and **3**. Variable temperature experiments were conducted to independently assess the solvent exposure of the backbone amides. Both amide protons in **1** and **3** exhibited temperature coefficients consistent with their engagement in hydrogen bonds (Supporting Information Table 4). The solution structure of **3** also shows a lower solvent exposed polar surface area compared to that of **1** (130 Å^2 vs 162 Å^2) and a slightly decreased radius of gyration (5.1 Å vs 5.4 Å), both parameters which have recently been linked to passive membrane permeation.[29] Furthermore, the acetyl carbonyl of **3** reaches back over the ring and forms another hydrogen bond with an amide proton of Leu1, decreasing its solvent accessibility. Scaffolds other than **1NMe3**, which do not contain a transannular hydrogen bond in their homodedic form, may benefit more from the ring expansion and added flexibility afforded by the depsi-bond in accessing lipophilic conformations.

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By exploiting the *N*−*O* acyl shift, often considered an unwanted side reaction in peptide synthesis, we have synthesized a diversifiable cyclic depsipeptide bearing a striking resemblance to natural products aspergillicin and xenobactin, while maintaining the cellular permeability of the parent scaffold. In some cases the acid catalyzed acyl rearrangement may represent an effective synthetic strategy for either diversity oriented synthesis or total synthesis of natural products given the ability of about half of scaffolds presented here to undergo this rearrangement. Although this rearrangement was previously used in the synthesis of actinomycin precursors, it has not been explored in other scaffolds.[30] From the library of compounds presented here it appears that this rearrangement may be used in some macrocycles, though there is a strong dependence of conversion efficiency on both conformation and ring size. Further studies will be directed toward more fully exploring the scope of this chemistry in other scaffolds.

■ ASSOCIATED CONTENT

Supporting Information

These data include synthetic and computational details as well as thorough characterization of all compounds synthesized. 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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