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Total Synthesis of Cyclosporine: Access to N-Methylated Peptides via Isonitrile Coupling Reactions

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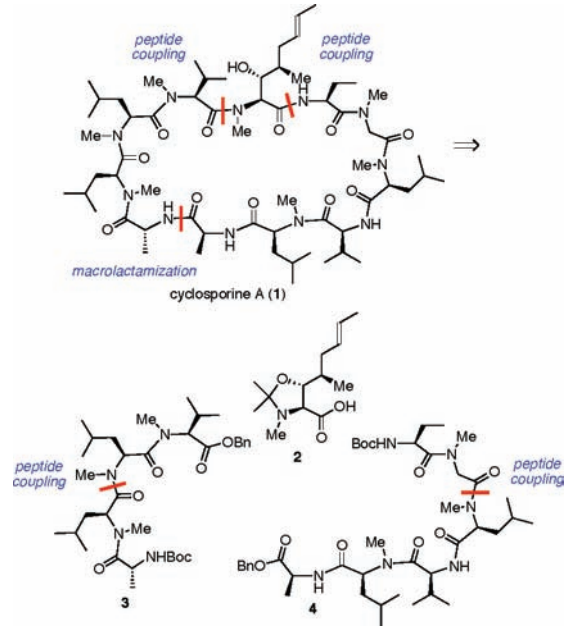
Recently, we described a series of findings using isonitriles in the formation of amide bonds.[1−4] These early works suggested that isonitrile-mediated bond constructions might be applicable to the synthesis of tertiary amides. There is currently a great deal of interest in such systems, particularly with respect to improving the pharmacoavailability of biologically active polypeptides.[5]

We thought that our findings in this area were sufficiently promising that it would be appropriate to explore various ways to generate tertiary amides in the context of a total synthesis of a challenging target system. In view of these considerations, it was only natural to turn to cyclosporine A (**1**) as a worthy goal. Cyclosporine A is a reversible inhibitor of cytokines in T helper cells[6] that was isolated from the fungus *Tolypocladium inflatum gams*.[7] Its structure was confirmed by chemical degradation, NMR, and X-ray crystallographic studies,[8] and its total synthesis was reported by Wenger in 1984.[9] The immunosuppressive properties of cyclosporine A, which enable otherwise nonsustainable transplantations, are very well established.[10] In addition, cyclosporine A has been applied to the treatment of Beçhet's syndrome, endogenous uveitis, psoriasis, atopic dermatitis, rheumatoid arthritis, active Crohn's disease, and nephrotic syndrome.[10] Indeed, its bioavailability with respect to proteolysis is highly dependent on the pattern of N-methylation present in 7 of the 11 amino acid residues of the cyclic polypeptide. As established by Wenger, Rich, and Galpin through analogue studies,[11] the unusual amino acid (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-methylamino-6-octenoic acid (MeBmt) is of particular importance to the biological activity of **1** as well as the sequence MeBmt, Abu, Sar, MeLeu. Herein, we demonstrate the emerging versatility and power of isonitrile chemistry in the context of a total synthesis of cyclosporine A.

Retrosynthetic planning began by disconnecting the macrocycle at the Ala-(D-Ala) junction and on either side of the MeBmt residue, thus revealing MeBmt acetonide **2**, tetrapeptide **3**, and hexapeptide **4** (Scheme 1). The protected MeBmt residue **2** is a known compound that is obtainable via the route charted by Evans and co-workers.[12] Tetrapeptide **3** was to be assembled from its component dipeptides, each of which could be reached via isonitrile-mediated coupling methods. Hexapeptide **4** was seen as arising from dipeptide and tetrapeptide fragments.

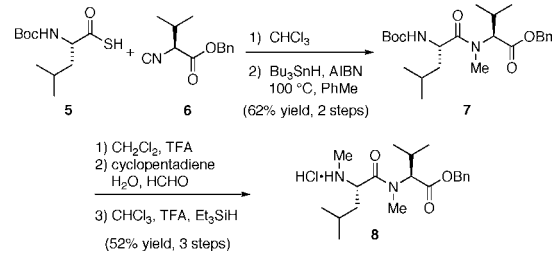
We began by targeting dipeptide **8** en route to tetrapeptide **3**. Leucine-derived thioacid **5**[13] was smoothly coupled with valine isonitrile **6**[14] to afford the corresponding *N*-thioformyl amide (Scheme 2). Subsequent conversion to the *N*-methyl amide was challenging. Treatment with Raney Ni as reported by Chupp and co-workers[15] was unsuccessful, presumably because of the base-

Scheme 1



sensitive nature of the thioformyl amide. Ultimately, it was found that the reduction proceeded well under free radical conditions. Thus, treatment of the crude *N*-thioformyl amide with Bu_3SnH and azobis(isobutyronitrile) (AIBN) at 100 °C in toluene afforded the desired *N*-methyl dipeptide **7** in 62% yield over two steps. Following cleavage of the carbamate, the elegant retro-aza-Diels−Alder methylation approach described by Greico et al. provided access to the corresponding N-methylated derivative **8**, without epimerization, in 52% yield over three steps.[16]

Scheme 2



A similar strategy was applied for the second key dipeptide fragment. Thus, D-alanine thioacid **9**[13] underwent coupling with leucine isonitrile **10**[14] in $CHCl_3$ at ambient temperature, followed by radical reduction, to afford the desired N-methylated dipeptide

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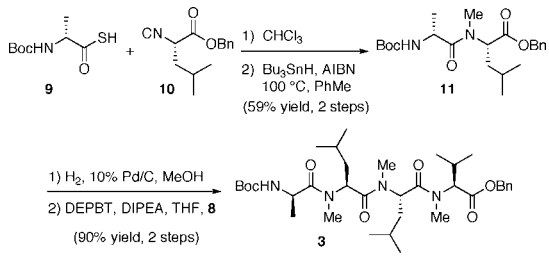
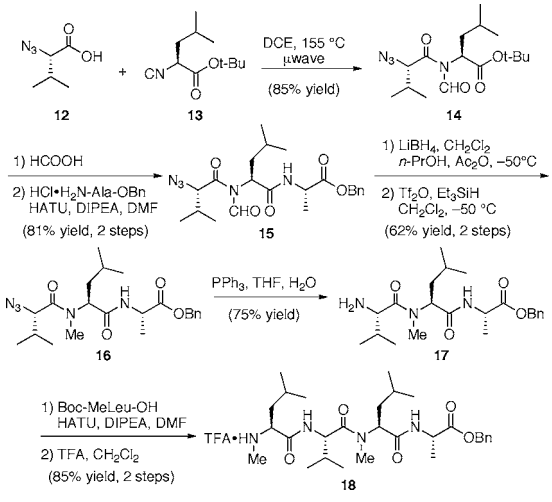
Scheme 3



11 in 59% yield over two steps (Scheme 3). At this point, we were poised to join the two dipeptide fragments. Hydrogenolysis of the benzyl ester afforded the corresponding acid, and subsequent coupling with fragment **8** was accomplished upon treatment with DEPBT[17] and Hünig's base in THF. Using these optimized conditions,[18] we were able to synthesize the desired tetrapeptide **3** in 90% yield over the two steps with no observable epimerization.

With tetrapeptide fragment **3** in hand, we turned our attention to the synthesis of hexapeptide **4**. Microwave irradiation of azido acid **12**[19] and leucine isonitrile **13**[14] afforded *N*-formyl amide **14** in 85% yield (Scheme 4). We reasoned that the presence of the *N*-formyl group could be exploited to decrease the extent of oxazolone formation, which might otherwise lead to epimerization during the C-terminal extension with a suitably protected alanine.[1c,d] Maintenance of the *N*-formyl group during acidic removal of the *tert*-butyl ester and during coupling with alanine benzyl ester was possible, thereby providing tripeptide **15** in 81% yield and >20:1 dr. The reduction of the *N*-formyl amide was very challenging because of its propensity to undergo either nonselective reduction, resulting in destruction of the peptide, or deformylation, resulting in formation of the native amide bond. Substantial optimization was required to identify conditions under which the required transformation could be performed chemoselectively. Ultimately, a combination of lithium borohydride and 0.5 equiv of acetic anhydride in a mixture of CH_2Cl_2 and *n*-propanol at −50 °C was used to provide the relatively unstable *N*-hydroxymethyl intermediate. Immediate reduction of the crude product with triflic anhydride and triethylsilane in CH_2Cl_2 afforded the desired N-methylated tripeptide **16** in 62% yield over two steps. Selective reduction of the azide functionality was readily accomplished by treatment of **16** with triphenylphosphine in the presence of water, forming **17**. The final

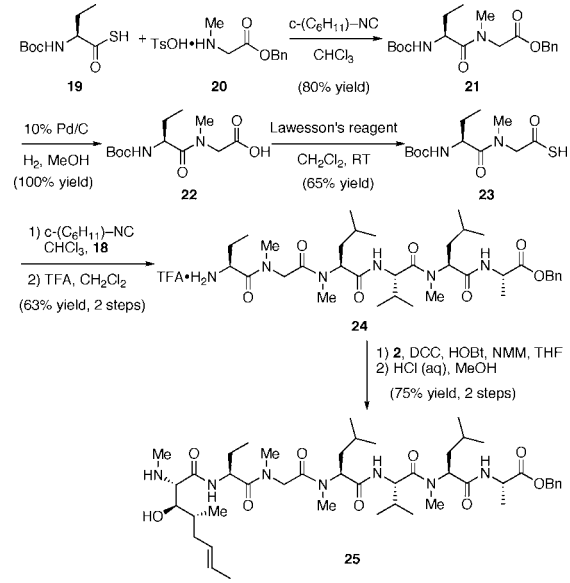
Scheme 4



methyl-leucine residue was attached via a HATU coupling. Acidic removal of the Boc protecting group afforded trifluoroacetic acid (TFA) salt **18**, which bears the secondary amide bond at the valine residue, in 85% yield over two steps.

We were now well-positioned to test additional isonitrile-mediated amide bond formations.[4] Thus, thioacid **19** was treated with a "sacrificial isonitrile"[4] to provide a putative formimidate (thio)carboxylate mixed anhydride (thio-FCMA) that was intercepted by amine **20**, thereby producing dipeptide **21** in 80% yield (Scheme 5). Hydrogenolysis of the benzyl ester was achieved quantitatively, providing known dipeptide **22**. We recently disclosed a method by which carboxylic acids could be treated with Lawesson's reagent under microwave conditions or at room temperature to afford the corresponding thioacids.[20] With this approach, **22** was converted to thioacid **23** (65% yield), which was then coupled with secondary amine **18**. Following cleavage of the Boc group, hexapeptide **24** was obtained in 63% yield.

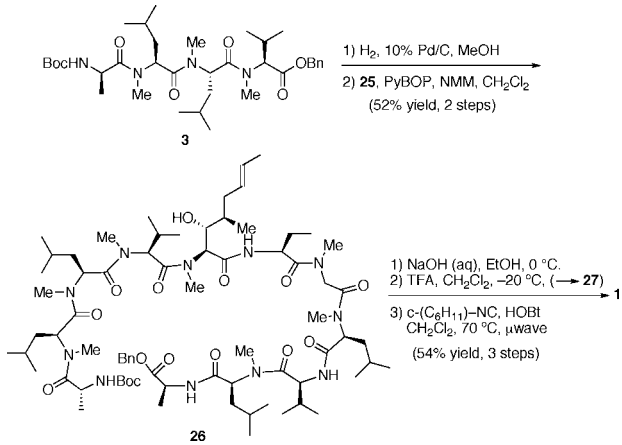
Scheme 5



We next began the final series of fragment couplings of the seco system. The protected MeBmt residue, **2**, was coupled to hexapeptide **24** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt). Subsequent treatment with aqueous HCl provided heptapeptide **25** in 75% yield over two steps. At this point, we sought to couple the final two fragments: tetrapeptide **3** and heptapeptide **25**. Hydrogenolysis of the C-terminus of **3** followed by smooth coupling with fragment **25** afforded undecapeptide **26** in 52% yield (Scheme 6). Basic hydrolysis of **26** exposed the C-terminus, and subsequent acidic treatment cleaved the *tert*-butoxy carbamate from the N-terminus.

The stage was now set for applying the logic of our recently developed[4] coupling method to a macrolactamization. This was no minor extension of our earlier work because, in the case at hand, we would be attempting to apply the logic to a C-terminal acid rather than to a thioacid.[4] In the event, exposure of the unprotected undecapeptide **27** to 10 equiv of cyclohexyl isonitrile at 70 °C under microwave radiation resulted in isolation of cyclosporine A (**1**) in 30% yield over three steps. We note that ordinarily with carboxylic acids (as opposed to thioacids), amide bond formation by 1,3-O→N acyl transfer does not occur below ∼130 °C under microwave mediation. We had conjectured that the FCMA actually forms at lower temperature but that the usual 1,3-O→N acyl transfer requires

Scheme 6



higher temperature for efficient rearrangement. In the case at hand, the substrate was likely preorganized as a result of intrastrand hydrogen bonding.[21] Thus, the FCMA of the seco acid, once formed, had an increased proclivity for cyclization, and macrolactamization was achieved at 70 °C, with only a trace amount of 1,3-*O*→*N* acyl transfer visible by LC−MS of the crude reaction mixture.

While this finding was encouraging, the yield was nonetheless disappointing. Fortunately, the yield could be significantly increased through the addition of HOBt (1.5 equiv) to the cyclization medium. Actually, the reaction seemed very clean and appeared to proceed with high conversion. However, the isolated yield of cyclosporine A is at this time 54%. It is tempting to suppose, *but is certainly not proven*, that an initial FCMA formed from **27** and cyclohexylisonitrile is intercepted with HOBt, thereby generating an active HOBt ester. The latter could well be the actual intermediate for macrolactamization, thereby attenuating the tendency for either 1,3-O→N acyl transfer or FCMA hydrolysis. Importantly, no dimerization was observed in either the HOBt or HOBt-free cyclization reactions.

In summary, we have shown how the chemistry of isonitriles can be applied to the construction of a variety of tertiary amides. These findings made possible a total synthesis of cyclosporine A in a fashion that allows for a more detailed mapping of its SAR.[1a] Further applications of isonitrile logic in peptide, cyclic peptide, and glycopeptide settings will be disclosed in due course.

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Supporting Information Available: Experimental procedures, copies of spectral data, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Standard coupling conditions, including HATU/DIPEA/DMF, PyBOP/NMM/CH_2Cl_2, and DCC/HOBt/NMM/THF, led to low yields (40−45%) and high levels of epimerization (1:1 to 2:1 ratios of diastereomers).
(19) Prepared according to the following procedure: Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797–3800.
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(21) This preorganization was originally proposed by Wenger in ref 9a. Our ^{1}H NMR data support this hypothesis. Resolution of the seven *N*-Me groups demonstrated that **26** exists largely as one rotamer. The acetonide-protected precursor to heptapeptide **25**, which would possess only two potential intrastrand hydrogen bonds, exists as a mixture of rotamers. See the Supporting Information for images of these spectra.

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