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On the Synthesis of Conformationally Modified Peptides through Isonitrile Chemistry: Implications for Dealing with Polypeptide Aggregation

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Supporting Information

ABSTRACT: A method for introducing a dimethyleneoxy constraint joining the N atoms of two consecutive amino acids in the context of a polypeptide has been developed. This constraint can profoundly affect the tendency of a polypeptide to suffer aggregation and desolubilization, and it can be readily removed under mild conditions.

Peptides built from proteogenic L-amino acids often possess high affinity and selectivity in binding to various biotargets. However, the value of peptides bearing strictly natural motifs in clinical medicine tends to be compromised by poor in vivo pharmacoproperties, particularly bioavailability.[1] A widely considered strategy to upgrade the value of polypeptides envisions the incorporation of strategically placed conformational constraints into such molecules. Experience has shown that systems with designed motifs can have useful properties, including increased resistance to protease-induced degradation[2] and decreased flexibility relative to native linear peptides. Well-designed constraints in peptidic structures may result in enhanced affinity of the polypeptide to target receptors.[3] For instance, Kim and co-workers designed peptidomimetic inhibitors of HIV-1 infection that outperform analogous linear peptides lacking these conformational constraints.[4] Such successes have provided incentive for the development of improved methods for the synthesis of constrained peptides. In a particularly elegant example, Grubbs and co-workers[5] demonstrated that ring-closing metathesis can enable the construction of constrained peptide-like structures by exploiting enantiohomogeneous tertiary amides bearing appropriate unsaturation projecting from the amidic nitrogens.

In the research described below, we undertook the development of a logic wherein α-amino acids in a regular polypeptide motif would serve to anchor the constraint. In principle, our chemistry could encompass inclusion of either natural or "unnatural" amino acid building blocks. In the first instance, we chose to focus on strictly proteogenic amino acids *with the additional proviso that the restraining device should be detachable*, with restoration of the natural motif. The feature of "reversibility" could be of particular value in peptide synthesis. Thus, a molecular constraint might serve to attenuate the aggregation tendencies of prepolypeptide building blocks that are otherwise difficult to manage.[6−8] The constraint could be dismantled when a more stable (foldable) polypeptide is in hand.

For these purposes, we drew from the growing body of chemistry that emerged from our recent studies of isonitriles.[9] We revisited a key reaction that we discovered, namely, the coupling of a carboxylic acid (**a**) with an isonitrile (**b**) to afford an *N*-formylamide (**c**), presumably via the intermediacy of the formimidate carboxylate mixed anhydride (FCMA),[9a] as shown. This line of conjecture led to the proposal shown in Scheme 1. Thus, an acid (**1**) would combine with an amino acid-derived isonitrile (**2**).[9d] The coupling reaction described above and subsequent discharge of the protecting group (P) would give acid **3**. *Reiteration of this reaction with amino acid-derived isonitrile* **4** *would in principle give rise to* **5**, *which contains N-formyl groups on contiguous amidic nitrogens.* It was hoped that these proximal, imide-like formyl groups could be exploited to generate constrained tripeptide **6**. Elongation in both the N- and C-terminal directions would lead to site-specific constrained polypeptide **7**. Cleavage of the molecular constraint would then generate polypeptide **8** in a natural motif. While the concept is applicable in principle to many versions of "X," in our initial explorations described below, we examined the case where X = oxygen.

Our studies commenced with coupling of valine-related azide **9** with leucine-derived isonitrile **10**[9f] to afford **11** in 82% yield (Scheme 2). Cleavage of the ester generated acid **12**. We found it convenient to house the eventual N-terminii of our constructs in terminal azido linkages. Azides hold up well under the initial 1 + 1 acid + isonitrile coupling conditions, including the thermolytic O → N acyl migration step, and thereafter in the 2 + 1 coupling step, which sets the stage for building the constrained tripeptide motifs. As will be seen (see below), the reductive cleavage of the azido group serves as an effective means of exposing the N-terminus (NH_2) of the abbreviated constrained tripeptide to be elongated. In Table 1, we report three additional examples of this type of sequence.

The next phase of the plan involved building tripeptidyl constructs containing contiguous *N*-formyl groups. The key reaction for accomplishing this end is illustrated in the 2 + 1 coupling of acid **12** with isonitrile ester **16** (Scheme 3). This reaction was conducted under our conditions of hindered thiophenol mediation.[9g] In this case, a 50% yield of the target construct **17** was obtained. Curiously, this reaction also gave rise to a 5% yield of dipeptide **18**. An assortment of related

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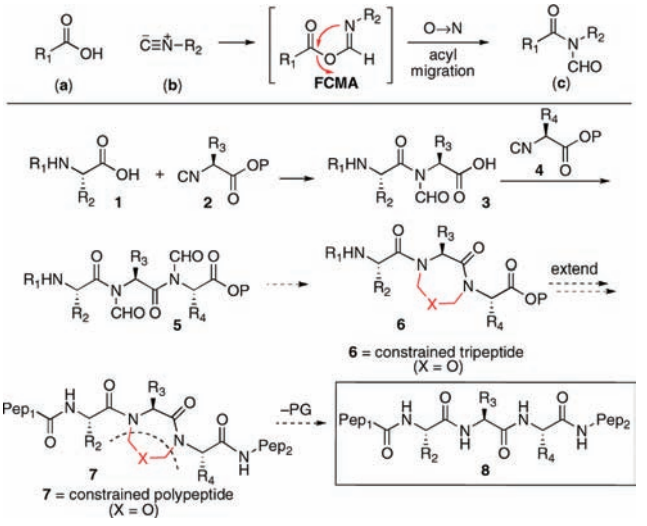
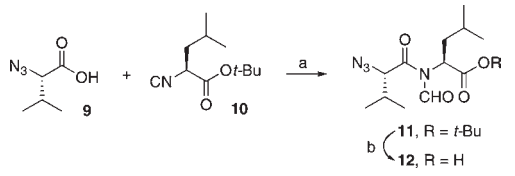
Scheme 1. Synthetic Strategy

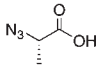
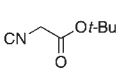
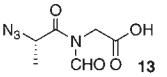
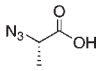
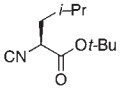
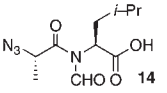
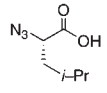
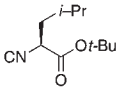
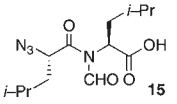


Scheme 2. Synthesis of Azido Precursors of Dipeptide Acids[a]



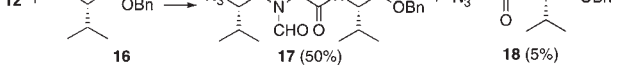
[a] Key: (a) microwave, 150 °C, $CHCl_3$, 82%; (b) HCO_2H, 100%.

Table 1. Reaction of Azido Acids with Isonitriles[a]

Entry	Amino acid	Isonitrile	Product	Yield
1			 13	85%
2			 14	80%
3			 15	87%

[a] Conditions: (a) microwave, 150 °C, $CHCl_3$; (b) HCO_2H.

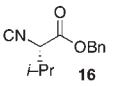
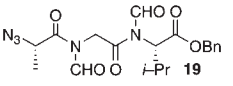
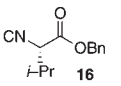
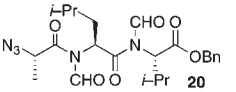
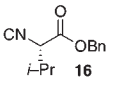
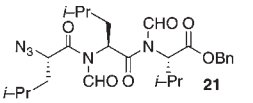
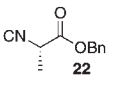
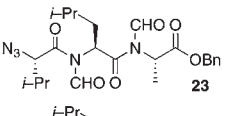
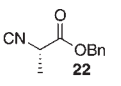
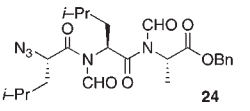
Scheme 3. ArSH-Mediated Synthesis of Bis(*N*-formyl) Tripeptides[a]



[a] Key: (a) microwave, 120 °C, $CHCl_3$, 2,6-dimethylthiophenol.

mechanisms to rationalize formation of this "deletion" product can be proposed, but the actual pathway has not been pinned down.[10] In this connection, we note that when the

Table 2. Synthesis of Tripeptides[a]

Entry	Dipeptide	Isonitrile	Product	Yield
1	13	 16	 19	65%
2	14	 16	 20	55%
3	15	 16	 21	55%
4	12	 22	 23	52%
5	15	 22	 24	53%

[a] Conditions: microwave, 120 °C, $CHCl_3$, 2,6-dimethylthiophenol.

reaction of **12** and **16** was conducted in the absence of hindered thiophenol (requiring thermolysis at 150 °C rather than 120 °C), the ratio of the unexpected product **18** to the target **17** was as high as 5:1.

The *N*-formyl group within **12** plays a key role in the critical second coupling reaction leading to bis(*N*-formyl) tripeptide **17** in that it stabilizes the intermediate FCMA arising from the reaction of **12** + **16** against oxazolone formation, thereby allowing the required O → N acyl tranfer to occur. In Table 2, we summarize five additional examples of this chemistry.[11]

With the bis(*N*-formyl) tripeptides in hand, we turned to the synthesis of the constrained systems. Initial studies centered on the reduction of the two *N*-formyl functions of the mixed imides, with the goal of generating the corresponding diol derivatives (cf. **26**). These efforts were complicated somewhat by the sensitivity of the imide-like *N*-formyl group to bases and nucleophiles. Fortunately, after considerable experimentation, it was found that treatment of bis(*N*-formyl) tripeptides **17**, **21**, **23**, and **24** with lithium borohydride in a mixed solvent system (20:1 CH_2Cl_2/*n*-PrOH) at −60 °C in the presence of acetic anhydride furnished the desired methylols (Table 3).[9l] The presence of acetic anhydride apparently served to suppress the formation of side products arising from deformylation, possibly by quenching the *n*-PrOLi generated during the course of the reaction.[12] Moreover, careful tuning of the workup conditions was crucial to ensure liberation of the diols from their presumed boronate esters [see the Supporting Information (SI)]. Happily, treatment of the crude diols with trifluoroacetic acid (TFA) in CH_2Cl_2 furnished the corresponding constrained tripeptides (**28**−**31**) in unoptimized but still manageable overall yields (Table 3).

Armed with a reliable method for obtaining these novel constrained tripeptides, we turned to the task of synthesizing larger polypeptides with site-specific constraining motifs. In this way, we hoped to probe the effect of the constraint on the peptide conformation and the resulting properties (Scheme 4). In a typical example, reductive cleavage of tripeptide **28** with triphenylphosphine (Ph_3P) followed by coupling with Boc−Lys−Gly−COOH employing HATU and DIPEA as condensing agents provided the desired pentapeptide in 75% yield over the two steps. Hydrogenolytic

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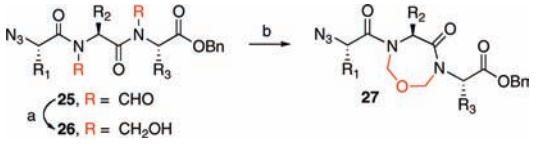
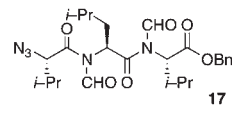
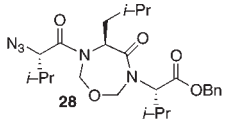
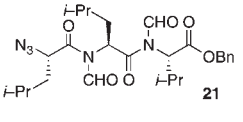
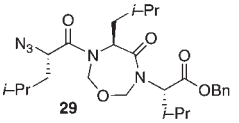
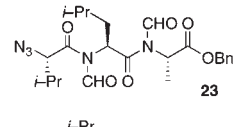
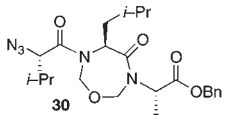
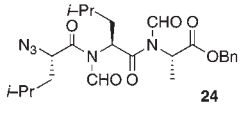
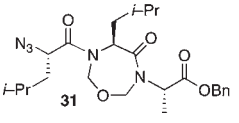
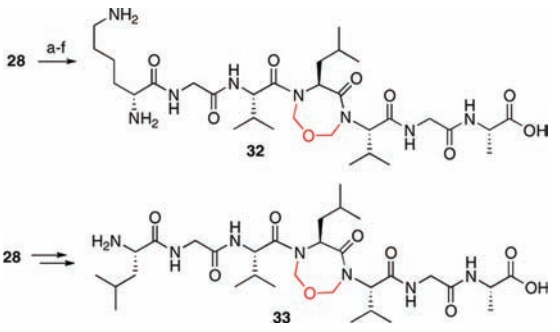
Table 3. Synthesis of Constrained Tripeptides[a]



Entry	Bis N–formyl tripeptide	Constrained peptide	Yield
1	 17	 28	60%
2	 21	 29	55%
3	 23	 30	53%
4	 24	 31	45%

[a] Key: (a) $LiBH_4$, CH_2Cl_2, *n*-PrOH, Ac_2O, −60 °C; (b) TFA, CH_2Cl_2.

Scheme 4. Synthesis of Large Constrained Peptides[a]

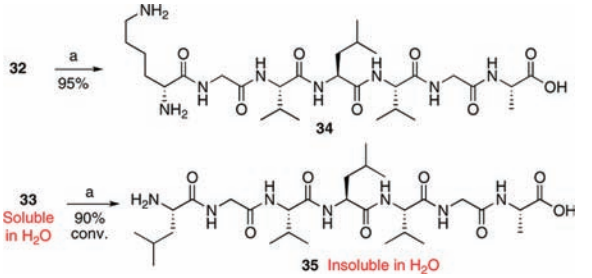


[a] Key: (a) PPh_3, THF, H_2O; (b) Boc−Lys−Gly−CO_2H, HATU, DIPEA, DMF; 75% over two steps; (c) Pd/C, MeOH, H_2; (d) HCl·H_2N−Gly−Ala−OBn, HATU, DIPEA, DMF; 80% over two steps; (e) 10% Pd/C, MeOH, H_2; (f) TFA, CH_2Cl_2; 80% over two steps.

cleavage of the derived benzyl ester followed by coupling of the resultant C-terminal acid with dipeptide H_2N−Gly−Ala−OBn afforded the heptapeptide in 80% yield over two steps. Finally, global deprotection furnished the desired heptapepeptide **32** in 80% yield over two steps. Following this general protocol, the same precursor **28** was converted to constrained system **33**.

In line with our original plan (see above), we hoped to remove the methylene bridging constraint to generate the native peptide. Indeed, treatment of **32** with 0.1 M HCl and 1,3-propanedithiol in trifluoroethanol at room temperature furnished the corresponding water-soluble linear heptapeptide **34** in 95% yield (Scheme 5).[13] When **33** was subjected to the same conditions, the reaction proceeded to ∼90% conversion (by LC−MS

Scheme 5. Deprotection of Constrained Peptides 34 and 35[a]



[a] Key: (a) 0.1 M HCl, CF_3CH_2OH, $HSCH_2CH_2CH_2SH$.

analysis), but most intriguingly, no product could be isolated because of the formation of an intractable precipitate, presumably as a result of aggregation of linearized **35**.[14] Efforts to solubilize the residue in H_2O, DMSO, DMF, CH_3CN, MeOH, and CH_2Cl_2 proved fruitless. The apparent insolubility of **35** in aqueous solution is especially striking considering that its constrained counterpart **33** is perfectly soluble in H_2O at concentrations of at least 10 mg/mL. This dramatic contrast suggests that the concept of using constraints to enforce intramolecular proximities, thereby attenuating proclivities to aggregation, may well have merit. *As noted above, such a capability could also be of advantage in synthesis. A particularly important application would be that of stabilizing aggregation-prone sequences of β-amyloids.*[15]

Extensive NMR studies provided deeper insights into the conformational consequences of our constrained heptamers **32** and **33**. Systematic inspection of the NOESY and ROESY data for each constrained peptide in pure D_2O and 9:1 H_2O/D_2O at 4 °C did not reveal suggestive through-space interactions between any of the backbone αH or amide NH protons. However, in both cases, an NOE correlation was observed between one of the α-protons of Gly-2 and a side-chain γ-methyl of Val-5 (shown for **32** in Figure 1a). In contrast, the deconstrained linear system **34** did not exhibit any discernible NOEs between protons of noncontiguous residues, as would be expected (Figure 1b). Taken together, these data indicate that the CH_2−O−CH_2 constraint induces a conformational bias wherein the peptide backbone is somehow folded such a way that residues 2 and 5 are brought into proximity. It could well be that this effect is responsible for the abatement of aggregation in **33**. The constraint may promote solubility by providing a scaffold that juxtaposes the hydrophobic surfaces of the molecule, enabling them to achieve energetically favorable noncovalent interactions at the intramolecular level.[16] Linear peptide **35**, lacking the constraint, achieves its noncovalent interactions by intermolecular means, namely, aggregation.

In summary, we have developed a method for assembling conformationally biased polypeptides that is enabled by our recently developed isonitrile coupling technology. Consecutive two-component coupling reactions of isonitriles exploiting a strategic N-terminal azide linkage in the first union afforded a novel tripeptide containing contiguous *N*-formyl amidic linkages. These *N*-formyl groups were used to establish a tripeptide bearing a CH_2−O−CH_2 bridge spanning contiguous amides. This system was further elaborated to construct heptameric peptides containing this bridge, which could then be dismantled under mild treatment. Studies of the constrained and linear systems so produced have underscored the possibility of modulating protein conformations that influence proclivities to aggregation. The broader implications of these findings are the subject of considerable current interest in our laboratory.

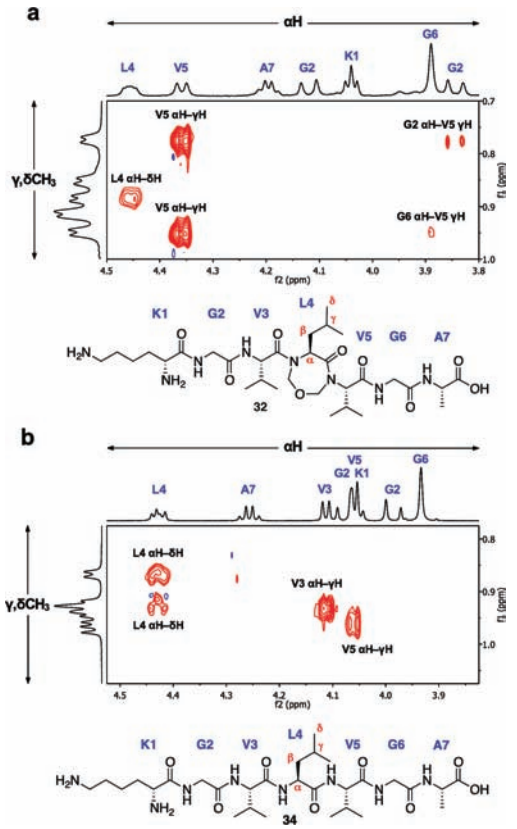


Figure 1. Selected 600 MHz NOESY data for (a) constrained peptide **32** and (b) linear peptide **34**. The data were obtained for 4.7 mM peptide samples at 4 °C in D_2O (pH 3.6−3.8, not corrected for deuterium) with a 250 ms mixing time.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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