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Iterative, Aqueous Synthesis of β^3-Oligopeptides without Coupling Reagents

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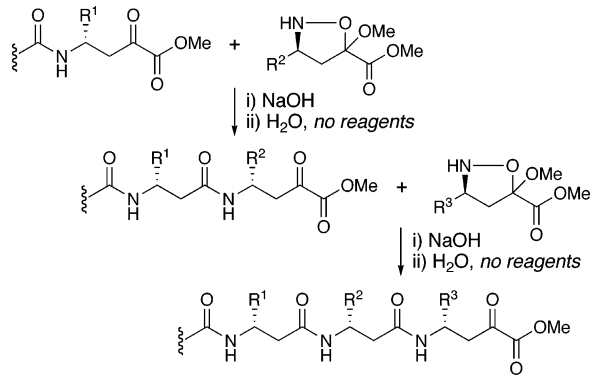
The discovery that oligomers of β-amino acids fold into discrete, stable secondary structures has fuelled an explosion in the synthesis, study, and application of these peptidomimetics.[1] Reported properties, including membrane translocation,[2] antimicrobial activity,[3] somastatin antagonism,[4] and disruption of protein−protein interactions,[5] attest to their diverse therapeutic potential. Further advances, however, are limited by the relative difficulty of preparing β-oligopeptides.[6,7] The constituent monomers, β-amino acids, often require multiple steps for their preparation, and the known routes are not readily scaled.[8] Furthermore, the assembly of β-oligopeptides by standard deprotection/activation peptide coupling approaches is plagued by sluggish reactivity, necessitating the use of several equivalents of the precious protected β-amino acids. These synthetic challenges conspire to make the preparation of biologically interesting poly-β-peptides a time-consuming and expensive endeavor, thereby limiting the further advancement of these highly promising peptidomimetics.

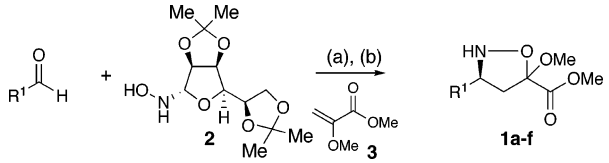
We recently developed a novel route to the construction of amide bonds by the decarboxylative condensation of α-ketoacids and *N*-alkylhydroxylamines.[9] This powerful, mechanistically unique amidation requires no reagents and produces no byproducts. However, in order for it to be applied to the stepwise synthesis of peptide oligomers, challenges in the design, synthesis, and reactivity of suitable monomers must be addressed. We selected the preparation of β^3-oligopeptides as an initial target and now disclose an approach for their synthesis by iterative couplings of isoxazolidines under aqueous conditions (Scheme 1).

After an initial screen of monomer classes suitable for the iterative synthesis of β-oligopeptides, we were pleased to find that isoxazolidine acetals **1a**−**f** could be readily prepared in enantiomerically pure form and were exceptional substrates in peptide forming reactions with α-ketoacids. Their synthesis takes advantage of Vasella's diastereoselective nitrone cycloaddition with carbohydrate-derived chiral auxiliaries,[10,11] a protocol that was easily extended to cycloaddition reactions with methyl 2-methoxyacrylate (**3**).[12] Cycloadditions occurred with high regio- and diastereoselectivity simply by heating a mixture of the D-mannose-derived hydroxylamine **2**, acrylate **3**, and the appropriate aldehyde. The isoxazolidines were isolated with >99% enantiomeric purity by chromatography or crystallization.[13] Auxiliary removal with acid or hydroxylamine proceeded in good yield with a small, but inconsequential, amount of epimerization at the acetal center. This practical approach was applied to the synthesis of monomers for common β-peptides on multigram scales (Chart 1).[14]

Scheme 1. Iterative Synthesis of β-Oligopeptides by Ketoacid−Isoxazolidine Peptide Formations in Water

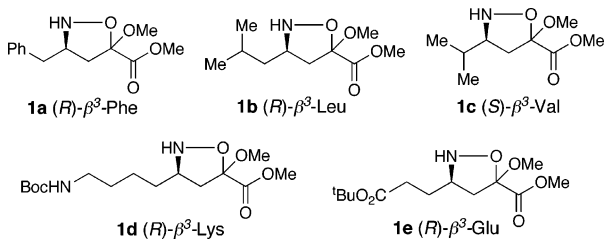


Scheme 2[a]



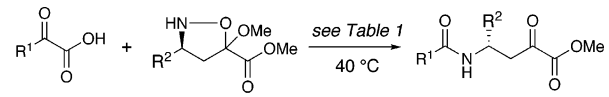
[a] Conditions: (a) C_6H_6, 80 °C, cat. Bu_2SnO, 12−36 h; (b) $HClO_4$, MeOH or $NH_2OH{\cdot}HCl$, MeOH.

Chart 1



Isoxazolidines **1a**−**f** undergo facile decarboxylative peptide couplings with α-ketoacids (Table 1). Our initial efforts with isoxazolidine **1f** revealed an interesting solvent effect on the amide formation. Surprisingly, DMF, which was the ideal solvent for the coupling of O-unsubstituted hydroxylamines with α-ketoacids,[9] was unproductive. In contrast, nonpolar solvents, including CH_2Cl_2 and toluene, gave the desired amides in good yield. Protic solvents were also effective, and water was particularly beneficial for the reactions.

Table 1.



4 R^1 = Bn **5** R^1 = Me **1f** R^2 = *p*-Tol **1a** R^2 = Bn **6** R^1 = Bn, R^2 = *p*-Tol **7** R^1 = Me, R^2 = Bn

entry	R^1	Isox.	conditions	time (h)	yield[a] (%)
1	Bn	**1f**	DMF (0.1 M)	21	nr
2	Bn	**1f**	toluene (0.1 M)	21	75
3	Bn	**1f**	CH_2Cl_2 (0.1 M)	21	82
4	Bn	**1f**	pH 5 Ac buffer (0.1 M)[b]	21	73
5	Bn	**1f**	1:1 ^{t}BuOH/H_2O (0.1 M)	21	92
6	Me	**1a**	1:1 ^{t}BuOH/H_2O (0.2 M)	8	92
7	Me	**1a**	1:1 ^{t}BuOH/H_2O (0.2 M), rt	8	72
8	Me	**1a**	1:1 ^{t}BuOH/H_2O (0.5 M)	1	93

[a] Yield of pure product following chromatography. [b] Heterogeneous reaction mixture.

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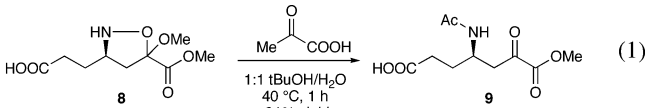
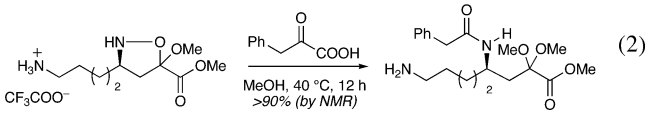
From these studies, we selected a 1:1 mixture of *tert*-BuOH/H_2O for reasons of substrate solubility and reactivity. At higher concentrations (0.5 M), the reactions were done within minutes and proceeded with visible loss of carbon dioxide. The isoxazolidines also coupled with α-ketoacids at room temperature with synthetically useful reaction rates (entry 7).

The amide formation is chemoselective. Unprotected glutamic acid substrate **8** reacted cleanly with pyruvic acid to give amino acid derivative **9** in excellent yield (eq 1). In the context of solution phase synthesis, reactions with unprotected amines were most conveniently performed in MeOH, which upon removal of the solvent afforded the corresponding amide−acetals (eq 2).



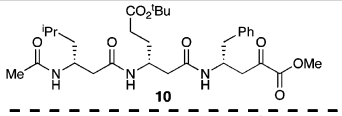
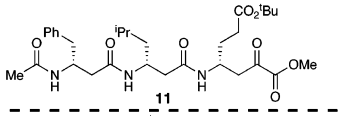
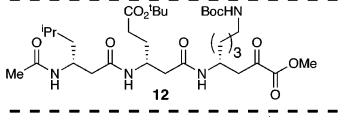
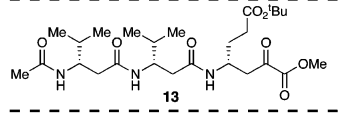
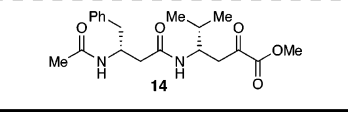
(1)



(2)

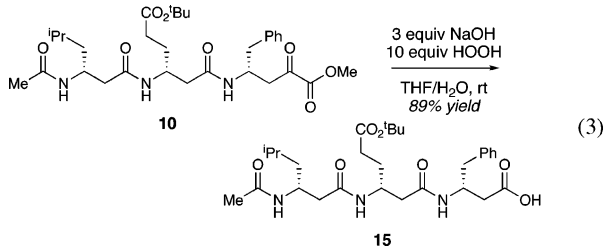
The products of the reaction of isoxazolidines with ketoacids were methyl α-ketoesters, which were easily saponified to α-ketoacids, and the peptide chain was extended by reaction with another isoxazolidine (Scheme 1). This two-step iteration could be carried out without purification or isolation of the intermediate acid. Using this approach, we have prepared a number of di- and tripeptides (Table 2). While there are some decreases in coupling rates as larger molecules and lower concentrations are used, in all cases, the peptide couplings proceed cleanly. For preliminary studies, we employed side-chain-protected substrates and isolated the intermediate peptides. Less than optimal yields for some substrates, especially those containing *β*h-valine residues, reflect the insolubility of the *β*-oligopeptides during workup and silica gel chromatography and not limitations of the amide formation step. Further studies employing solid phase synthesis and optimization of protocols for multiple, iterative couplings without isolating intermediates are in progress.

Table 2. Selected *β*-Oligopeptides Prepared by Isoxazolidine Couplings (see Scheme 1)

entry	product	iterat.	yield (%)[a]
1	 **10**	1st	90
		2nd	87
		3rd	90
2	 **11**	1st	93
		2nd	75
		3rd	46
3	 **12**	1st	90
		2nd	87
		3rd	44
4	 **13**	1st	90
		2nd	56
		3rd	45
5	 **14**	1st	93
		2nd	63

[a] Overall yield of pure, isolated product for (i) ketoester hydrolysis, (ii) isoxazolidine coupling, and (iii) purification. See Supporting Information for details.

The ketoester products could be directly converted to the corresponding carboxylic acids by oxidative decarboxylation in the presence of basic hydrogen peroxide (eq 3).



(3)

The ketoacid−hydroxylamine peptide ligation uses a unique set of functional groups to achieve chemoselective amide bond formation in the presence of unprotected functionalities and without reagents. Further applications, including the identification of new monomers suitable for other amide-based targets, will contribute to new methods for the preparation of peptidic structures and materials.

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

References

(1) (a) Cheng, P. R.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiv.* **2004**, *1*, 1111−1239.
(2) (a) Rueping, M.; Mahajan, Y.; Sauer, M.; Seebach, D. *ChemBioChem* **2002**, 257−259. (b) Umezawa, N.; Gelman, M. A.; Haigis, M. C.; Raines, R. T.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 368−369.
(3) (a) Porter, E. A.; Wang, X.; Lee, D.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *123*, 565−565. (b) Arvidsson, P. I.; Ryder, N. S.; Weiss, H. M.; Hook, D. F.; Escalante, J.; Seebach, D. *Chem. Biodiv.* **2005**, *2*, 401−420.
(4) Seebach, D.; Rueping, M.; Arvidsson, P. I.; Kimmerlin, T.; Micuch, P.; Noti, C.; Langenegger, D.; Hoyer, D. *Helv. Chim. Acta* **2001**, *84*, 3503−3510.
(5) (a) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468−9469. (b) Sadowsky, J. D.; Schmitt, M. A.; Lee, H.-S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11966−11968. (c) Werder, M.; Hauser, H.; Abele, S. Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774−1783.
(6) Murray, J. K.; Gellman, S. H. *Org. Lett.* **2005**, *7*, 1517−1520.
(7) Arvidsson, P. I.; Frackenpohl, J.; Seebach, D. *Helv. Chim. Acta* **2003**, *86*, 1522−1553.
(8) Steer, D. L.; Lew, R. A.; Perlmutter, P.; Smith, A. I.; Aguilar, M. I. *Curr. Med. Chem.* **2002**, *9*, 811−822.
(9) Bode, J. W.; Fox, R. M.; Baucom, K. D. *Angew. Chem., Int. Ed*. in press. dx.doi.org/10.1002/anie.200503991.
(10) Vasella, A. *Helv. Chim. Acta* **1977**, *60*, 1273−1295.
(11) For other recent applications of carbohydrate-derived chiral auxiliaries for the synthesis of hydroxylamines and isoxazolidines, see: (a) Fässler, R.; Frantz, D. E.; Oetiker, J.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 3054−3056. (b) Kasahara, K. Iida, H.; Kibayashi, C. *J. Org. Chem.* **1989**, *54*, 2225−2233.
(12) For other practical approaches to enantioenriched isoxazolidines, see: (a) Fuller, A. A.; Chen, B.; Miner, A. R.; Mapp, A. K. *J. Am. Chem. Soc.* **2005**, *127*, 5376−5383. (b) Sibi, M. P.; Prabagaran, N.; Ghorpade, S. G.; Jasperse, C. P. *J. Am. Chem. Soc.* **2003**, *125*, 11796−11797.
(13) Enantiomeric purities and absolute configurations were confirmed by conversion to known compounds and by chiral HPLC studies.
(14) For this study, we utilized a D-mannose-derived chiral auxiliary that afforded the "unnatural" peptide enantiomers. We have also prepared the "natural" enantiomers by using a chiral auxiliary derived from D-gulose. Further studies on the syntheses of these and other isoxazolidine monomers will be reported separately.

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