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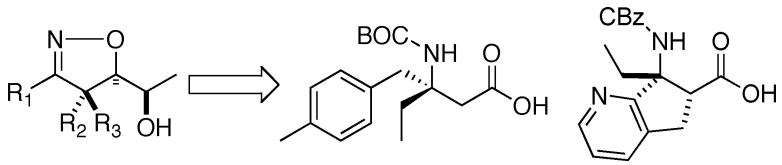

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Succinct Synthesis of β-Amino Acids via Chiral Isoxazolines

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Abstract: β-Amino acids are important synthetic targets due to their presence in a wide variety of natural products, pharmaceutical agents, and mimics of protein structural motifs. While β-amino acids containing geminal substitution patterns have enormous potential for application in these contexts, synthetic challenges to the stereoselective preparation of this class of compound have thus far limited more complete studies. We present here a straightforward method employing chiral isoxazolines as key intermediates to access five different β-amino acid structural types with excellent selectivity. Of particular note is the use of this approach to prepare highly substituted *cis*-β-proline analogues. The ready access to these diversely substituted compounds is expected to facilitate future studies of the structure and function of this important class of molecules.

Introduction

β-Amino acids are important constituents of biologically active natural products (e.g., taxol, dolastatin) and pharmaceutical agents as well as valuable precursors to such structures as β-lactams (Figure 1a).[1,2] In addition, oligomers of β-amino acids (β-peptides) have attracted attention as useful peptidomimetics because of their propensity to adopt stable secondary structures (e.g., helices, β-turns)[3] and their proteolytic stability relative to natural peptides.[4] These features make β-peptides attractive targets for use in a number of biological applications. There are several recent reports, for example, detailing β-peptides with positively charged residues that exhibit potent antimicrobial activities, presumably through disruption of bacterial cell membranes.[5] In all of the β-amino acid applications, the substitution pattern and the stereochemistry at the C2 and/or C3 positions strongly influence both structural characteristics and stability. Thus, effective stereoselective synthetic approaches for β-amino acids remain highly sought after.[2,6−8]

Two particularly intriguing classes of β-amino acids are also among the most challenging synthetic targets. The first of these contains geminal disubstitution at C2 and/or C3 such as the $\beta^{3,3}$- and $\beta^{2,3,3}$-amino acids depicted in Figure 1b.[8] Given the dense substitution and their resistance to proteolytic degradation, this molecular class provides excellent building blocks for bioactive molecules. Further, preliminary studies and predictive models indicate that the secondary structures adopted by β-peptides incorporating these amino acids will be unique and potentially quite stable.[8] However, due to the relatively small number of such structures available in stereoisomerically pure form, more complete studies have not been forthcoming.[9,10] A second class of β-amino acids of particular interest includes the cyclic proline

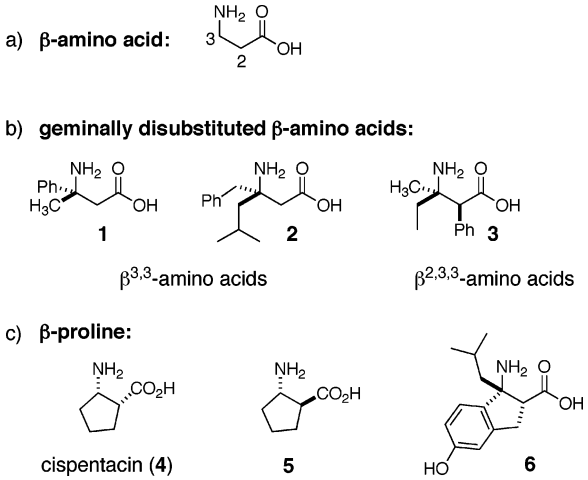

Figure 1. Structural classes of β-amino acids. (a) The basic structure of a β-amino acid. The nomenclature of Seebach in which the superscript after "β" describes the substitution pattern of a β-amino acid is used throughout.[8] According to this system, for example, a $\beta^{2,3,3}$-amino acid has a single substituent at the C2 position and two substituents at the C3 position. (b) Examples of geminally disubstituted β-amino acids, a particularly challenging class to prepare in stereoisomerically pure form. (c) β-Proline containing either a cis relationship between the amine group and the carboxylic acid (as in **4**) or a trans relationship (**5**) produces predictable secondary structures upon oligomerization.

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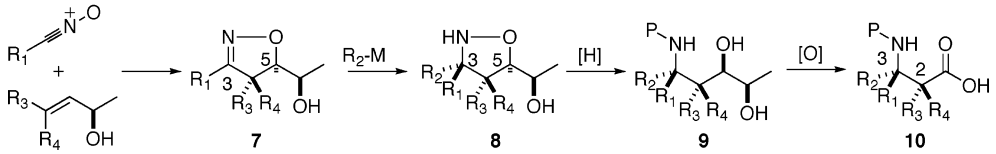

Figure 2. Isoxazoline strategy for the synthesis of β-amino acids. If R^1 and R^4 or R^2 and R^3 are covalently linked, access to cyclic β-amino acid analogues such as the β-prolines can be envisioned.

analogues ("β-proline"), *cis*-2-aminocyclopentanoic acid (cispentacin, **4**) and *trans*-2-aminocyclopentanoic acid (**5**) (Figure 1c).[11,12] Illustrative of the relationship between stereochemistry and structure, oligomers of **4** form strands,[13] whereas oligomers of **5** form helices.[14] Due to their predictable and well-defined structural characteristics, this class of β-amino acids has enormous potential for the formation of higher order structures, transitioning to protein-like structure and function for catalyst development and pharmaceutical applications.[15] As with their α-amino acid counterparts, the formation of higher order assemblies (discrete helical bundles or β-barrels, for example) will be dependent upon interactions between side-chain functional groups such as hydrophobic packing, salt bridges, and hydrogen bonding.[16] Efficient synthetic approaches to single stereoisomers of both **4** and **5** have been reported,[11,12] but the introduction of additional substituents (e.g., **6**) remains a substantial synthetic challenge. Thus, the further development of both classes of β-amino acids is contingent upon synthetic strategies that will provide access to single stereoisomers of these compounds.

The essential synthetic challenge for the β-amino acid classes described above is one shared by many synthetic targets, including a diverse group of natural products and other biologically active agents: stereocenters bearing an amine substituent, in particular tertiary and quaternary stereocenters.[17] Thus, synthetic advances in highly substituted β-amino acid preparation also impact a variety of fields. The traditional method of β-amino acid synthesis, the Arndt−Eistert homologation,[1] is a powerful approach for the preparation of β^3-amino acids but is relatively ineffective for more substituted versions because the yields and selectivities of the reaction suffer significantly with increased substitution.[18] In addition, cyclic β-amino acids cannot be prepared using this approach. Alternatively, β-amino acids can be accessed via a conjugate addition of an amine into an acrylate;[6] this transformation has been carried out asymmetrically by employing a chiral amine, a chiral ester, or a chiral Lewis acid.[19] However, extending this method to the synthesis of highly substituted targets is problematic as it involves overcoming both the steric hindrance and the reduced reactivity of the corresponding acrylates. Asymmetric catalytic reduction of β-amino acrylates has provided a highly efficient entry to various β-amino acids in high enantiomeric excesses,[20] although this method is limited by its intrinsic inability to access geminally disubstituted β-amino acids such as **2**, **3**, or **6**. Perhaps the most versatile addition to the repertoire of synthetic approaches is the addition of enolates to chiral imines,[21,22] and this has provided a method for the facile preparation of a range of β-amino acids including a recent report of the parent *trans*-β-proline (**5**) prepared via a chiral sulfinyl imine.[23] Nonetheless, this approach does not provide ready entry into certain classes of highly substituted β-amino acids, particularly those with sterically similar substituents at C3 (e.g., **2** and **3**), for example, or members of the *cis*-substituted β-proline class (e.g., **4** and **6**). Thus, access to the highly substituted and/or conformationally constrained β-amino acids remains a significant obstacle to the study of β-peptides incorporating these building blocks.

To address this synthetic challenge, we developed an orthogonal approach employing chiral isoxazolines as key intermediates for the preparation of a diverse array of β-amino acids, including the particularly challenging cyclic and highly substituted variants.[24] Isoxazolines are readily accessible as single stereoisomers via a 1,3-cycloaddition reaction between a nitrile oxide and a chiral allylic alcohol employing the conditions originally described by Kanemasa et al.[25] and further expanded by Carreira and co-workers[26] (Figure 2). We reasoned that nucleophilic addition to the C3 C═N bond of the isoxazo-

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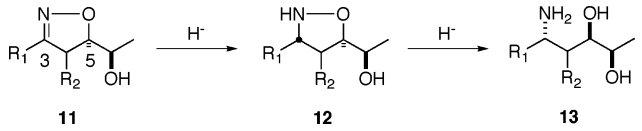


Figure 3. Directed hydride addition to isoxazolines.

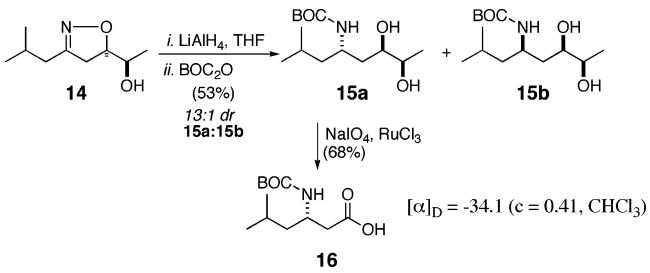
line (**7**) would be strongly influenced by the nearby C5 substituent, enabling us to establish the C3 stereochemistry in a controlled manner. Addition of hydride at this stage would provide key intermediates for the synthesis of β^3- and $\beta^{2,3}$-amino acids, whereas the inter- or intramolecular addition of carbon nucleophiles would create the nitrogen-bearing stereogenic centers present in more substituted acyclic and cyclic variants. As there existed few examples of the addition of nucleophiles other than hydride to isoxazolines,[27,28] this was the primary point of investigation throughout the development of the approach. The product of the nucleophilic addition, an isoxazolidine (**8**), contains all of the functionality and stereochemical relationships present in the final β-amino acid product (**10**). Conversion to the β-amino acid could be accomplished through reductive cleavage of the N−O bond followed by protection of the resulting amine and subsequent cleavage of the diol intermediate. We provide here a full account of this approach and its application to the synthesis of a diverse array of β^3-, $\beta^{2,3}$-, $\beta^{3,3}$-, and β-^{2,3,3}-amino acids, significantly expanding the diversity of β-amino acids available for study and application. In addition, we describe the further development of the strategy to include the synthesis of a series of densely substituted *cis*-β-proline analogues previously inaccessible by conventional means.

Results and Discussion

Synthesis of β^3- and $\beta^{2,3}$-Amino Acids. To develop the approach outlined above, our initial efforts were directed toward less substituted β-amino acid targets, the β^3- and the $\beta^{2,3}$-amino acids that contain either one or two tertiary stereogenic centers and are commonly used in β-peptide applications. Addition of hydride to the C═N bond would provide the key stereocenter in the final β-amino acid product (Figure 3). We identified one example in a report by Jäger in which a proximal hydroxymethyl substituent apparently served to direct the reduction of an isoxazoline to provide a β-amino alcohol with modest diastereoselectivity (1.7:1).[29] In addition, there are several examples of oximes (stereoelectronically similar to isoxazolines) undergoing reductions directed by a nearby hydroxyl group with agents such as $Me_4NBH(OAc)_3$.[30] Given this precedent and the presence of the hydroxyl on the C5 substituent of the isoxazoline, we anticipated that hydride attack directed by this group would be the most effective means to set the C3 stereocenter.

To investigate this, isoxazoline **14**, the precursor to β-leucine (**16**), was prepared as a single stereoisomer under standard conditions and subjected to a variety of reduction conditions (Scheme 1). $NaCNBH_4$, $Me_4NBH(OAc)_3$, and DIBAL-H were ineffective in this reaction, with no conversion to product observed under several different conditions. The more reactive agent $Zr(BH_4)_2$, previously shown to effect directed reduction,[31] did produce the desired product but in low yield and poor diastereoselectivity. The conditions of Jäger ($LiAlH_4/Et_2O$) provided good conversion of the isoxazoline to the product amine diols, but the selectivity was modest (1.5:1).[29,32] It was noted during this study that, regardless of the reaction conditions, the intermediate isoxazolidine (e.g., **12**) was never observed.

Scheme 1. Reduction of the Isoxazoline



This was particularly surprising in the case of $Zr(BH_4)_2$ because this reagent has been used to reduce the C═N bond selectively in the context of an oxime.[31] We reasoned that in our system the C5′ hydroxyl, deprotonated under the reaction conditions, and the ring oxygen might be forming a chelate with the reducing agent, thus activating the N−O bond for cleavage as well as attenuating the selectivity of the hydride delivery. Identification of conditions that would disrupt such a chelate could then lead to enhanced selectivity for the reduction.

Following this rationale, the use of $LiAlH_4$ in THF to reduce the isoxazoline followed by immediate protection of the amine provided the desired balance of reactivity and selectivity with the formation of a 13:1 ratio of diastereomers (Scheme 1). To verify the facial selectivity of the hydride addition, the protected amine diol was converted to β-leucine by oxidative cleavage of the diol moiety and the optical rotation of the final product was compared to that reported in the literature.[33] This provided confirmation that the hydride approach was from the same face of the isoxazoline ring as the C5 hydroxyethyl substituent. The same series of reactions was then carried out on isoxazolines bearing different substituents at C3 and C4 to explore the scope of the approach (Table 1). Increasing the steric bulk at C3 (**20** and **26**) has little effect on the key reaction. Further, less robust functional groups such as the alkyne in **29** are also well tolerated to provide a β^3-amino acid typically more difficult to obtain. Substituents at C4 of the isoxazoline can be incorporated by employing a disubstituted olefin in the cycloaddition, as in **32**.[25,26] Conversion of all of the amine diols to the final β^3- and $\beta^{2,3}$-amino acid targets also proceeded without incident. Overall, this approach provides a rapid, selective entry into these commonly employed classes of β-amino acids.

Synthesis of $\beta^{3,3}$- and $\beta^{2,3,3}$-Amino Acids. As noted in the Introduction, $\beta^{3,3}$- and $\beta^{2,3,3}$-amino acids are targets of particular interest for the preparation of structurally defined oligomers but are also among the most difficult to prepare stereoselectively due to the C3 quaternary stereocenter and, in the case of $\beta^{2,3,3}$-amino acids, the vicinal substitution.[8] To access the C3 geminal disubstitution present in this interesting and synthetically challenging class of compounds, we sought conditions for the selective addition of carbon nucleophiles to C3 of appropriately

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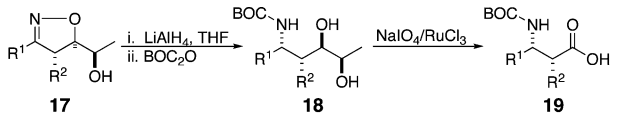
Table 1. Reduction of Isoxazolines and Conversion to β-Amino Acids



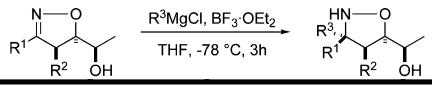
17 **18** **19**

Entry	Isoxazoline	Amine diol	dr[a] (3-step yield)[b]	β-Amino acid (yield)
1	**20**	**21**	7:1[c] (64%)	**22** (76%)
2	**23**	**24**	7:1 (44%)	**25** (70%)
3	**26**	**27**	15:1 (54%)	**28** (72%)
4	**29**	**30**	10:1[c] (40%)	**31** (59%)[d]
5	**32**	**33**	12:1[c] (63%)	**34** (67%)

[a] Diastereomeric ratios determined by ^{1}H NMR spectral integration prior to separation. [b] Combined yield of diastereomers; only a single diastereomer was carried on to the acid. [c] Due to poorly dispersed resonances in the ^{1}H NMR spectra, dr was determined by mass comparisons of isolated compounds. [d] Treatment with $NaIO_4$ followed by $NaClO_2$ provided **31**.

functionalized isoxazolines. In contrast to the hydride addition described in the previous section, it was anticipated that the facial selectivity of these nucleophiles would be sterically driven, with approach opposite the C5 ring substituent being more favorable. However, while numerous examples of the selective addition of organometallic nucleophiles to imines have been documented,[34] only a few examples of carbon nucleophiles added to analogous acyclic ketoxime ethers can be found in the literature.[35] References documenting carbon nucleophile addition to isoxazolines are even more rare,[27,28] making this a largely unexplored reaction.

Table 2. Addition of Stabilized Grignard Reagents to Isoxazolines



Entry	Substrate	R^3	Product	dr[a] (yield)
1	**23**		**35**	10:1 (95%)
2	**14**		**36**	9:1 (90%)
3	**26**		**37**	16:1 (74%)
4	**14**		**38**	18:1 (90%)[b]
5	**23**		**39**	Single diast. (96%)
6	**23**		**40**	24:1 (83%)
7	**41**		**42**	Single diast. (81%)[b]
8	**43a**: R^1 = Et **43b**: R^1 = *i*-Bu		**44a**: R^1 = Et **44b**: R^1 = *i*-Bu	**44a:** 1:1 (72%) **44b:** Single diast. (70%)

[a] Diastereomer ratio determined by ^{1}H NMR analysis of the crude reaction mixture. [b] Isolated as the HCl salt.

Organometallic reagents commonly used for additions to imines (organocerium, organozinc, and cuprate reagents, for example) provided poor conversion to product (0−15% yield) under a variety of conditions. In contrast, the more reactive Grignard reagent allylmagnesium chloride was an effective nucleophile, providing the desired adduct in good to excellent yields (entries 1−3, Table 2). Optimal diastereoselectivity was obtained using THF as solvent and upon complexation of the isoxazoline with $BF_3{\cdot}OEt_2$ to activate the C=N bond prior to addition of the Grignard reagent. Less polar solvents such as diethyl ether provided comparable product yields but reduced diastereoselectivity (2−3:1). The facial selectivity of the addition was verified by NOE difference experiments on a derivative of **36** in which the allyl group was oxidatively transformed into a primary alcohol to maximize signal dispersion (Figure 4). As indicated, the signals for the protons on the primary alcohol chain were enhanced upon irradation of the H4 proton syn to H5, whereas the isobutyl methylene was enhanced upon

(33) Elmarini, A.; Roumestant, M. L.; Viallefont, P.; Razafindramboa, D.; Bonato, M.; Follet, M. *Synthesis* **1992**, 1104−1108.
(34) Bloch, R. *Chem. Rev.* **1998**, *98*, 1407−1438. Enders, D.; Reinhold, U. *Tetrahedron: Asymmetry* **1997**, *8*, 1895−1946. Kobayashi, S.; Ishitani, H. *Chem. Rev.* **1999**, *99*, 1069−1094.
(35) Moody, C. J.; Gallagher, P. T.; Lightfoot, A. P.; Slawin, A. M. Z. *J. Org. Chem.* **1999**, *64*, 4419−4425. Hanessian, S.; Yang, R. Y. *Tetrahedron Lett.* **1996**, *37*, 8997−9000. Greven, R.; Jütten, P.; Scharf, H. D. *Carbohydr. Res.* **1995**, *275*, 83−93. Marco, J. A.; Carda, M.; Murga, J.; González, F.; Falomir, E. *Tetrahedron Lett.* **1997**, *38*, 1841−1844. Marco, J. A.; Carda, M.; Murga, J.; Rodríguez, S.; Falomir, E.; Oliva, M. *Tetrahedron: Asymmetry* **1998**, *9*, 1679−1701. Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Bando, T.; Hughes, R.; Winssinger, N.; Natarajan, S.; Koumbis, A. E. *Chem.-Eur. J.* **1999**, *5*, 2648−2667.

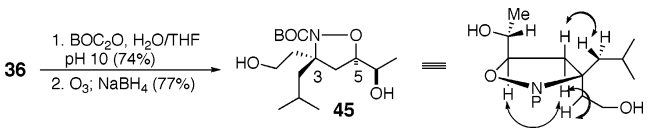


Figure 4. NOE difference experiment to confirm facial selectivity of addition.

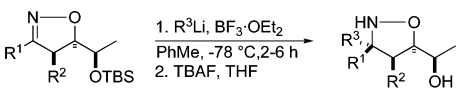
irradiation of H4 syn to the C5 hydroxyethyl substituent, consistent with the stereochemical assignment indicated.

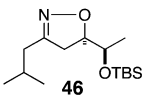
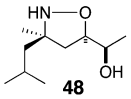
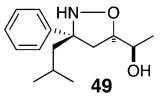
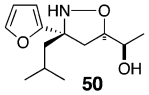
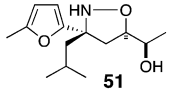
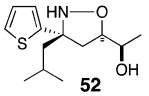
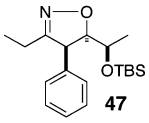
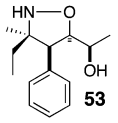
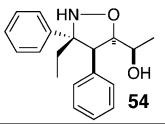
Similar reaction conditions were successfully employed with several different stabilized Grignard reagents (entries 4−6, Table 2) to provide isoxazolidines containing the requisite quaternary center at C3 in good to excellent yields (83−96% yield). Predictably, increasing the steric bulk of the nucleophile corresponded to an increase in diastereoselectivity observed (up to >20:1 dr). To obtain $\beta^{2,3,3}$-amino acid precursors, an additional substituent at C4 of the isoxazoline can readily be incorporated, as in entries 7 and 8. When the C4 group is syn to the C5 substituent, only a single diastereomer is observed in the nucleophilic addition reaction (entry 7). When the substituents are anti, however, the diastereoselectivity is further influenced by the C3 substituent (entry 8). In the case of **43a** in which both C3 and C4 bear an ethyl group, a 1:1 mixture of diastereomeric isoxazolidines is isolated. In contrast, an increase in the size of C3 as in **43b** leads to a completely diastereoselective reaction; an NOE difference study of an acetylated version of **44b** confirmed exclusive approach of the nucleophile from the *si* face (see Supporting Information for details). Analogous selectivity was observed with larger C4 substituents such as benzyl (C3 = Et, 1.2:1 dr; C3 = *i*-Bu, single diastereomer). This suggests that the steric interplay between the C3 and C4 substituents selectively shields the *re* face of the isoxazoline. These findings indicate that, through judicious choice of substituents at these positions, the stereochemistry at C3 can be set independently of C4, enabling access to an even wider range of $\beta^{2,3,3}$-amino acid precursors.

Initial attempts to expand this reaction beyond stabilized Grignard reagents were unsuccessful as the use of aryl or alkyl organometallic agents provided only recovered starting material. Given the sites available for deprotonation within the isoxazoline, competitive deprotonation appeared a likely culprit. [28,36] In support of this, a deuterium quench (D_2O) of a reaction with phenyllithium/BF_3·OEt_2 and **23** produced an isoxazoline labeled with deuterium at the C4 position. Because intramolecular proton transfer from the C5 hydroxyethyl group was a possible contributor, we explored protection of this group. This would also have the added advantage of increasing the steric bulk at this position and potentially further increasing the diastereoselectivity of addition. Of the various protecting groups screened to serve this purpose, the TBS ether was most effective because of both the stability of this protecting group under the Lewis acidic reaction conditions and the ease of its removal in subsequent steps.

With the side-chain hydroxyl masked, organolithium reagents could be employed as nucleophiles to provide the isoxazolidine adducts in excellent diastereoselectivity (Table 3), although even with this modification, nonstabilized Grignard reagents remained ineffective. Again employing BF_3·OEt_2 as a Lewis acid, methyllithium and phenyllithium as well as heteroaryllithium reagents were suitable nucleophiles for this transformation. In contrast to the reactions with Grignard reagents in Table 2, the use of toluene as solvent provided the best combination of yield and selectivity. As shown in entries 6 and 7, additional substitution could be incorporated into the isoxazoline to prepare vicinally substituted isoxazolidines in excellent diastereoselectivity; in the case of entry 7, however, the yield was lower despite prolonged reaction times, likely due to additional steric hindrance about the reactive center. An NOE difference study on a peracetylated derivative of **48** provided evidence that the approach of the nucleophile is opposite the sterically bulky C5 substituent (see Supporting Information for details).

Table 3. Addition of Organolithium Reagents to Isoxazolines



Entry	Substrate	R^3	Product	dr[a] (2-step yield)
1	 **46**	Me	 **48**	20:1 (80%)
2	**46**		 **49**	20:1 (60%)
3	**46**		 **50**	13:1 (80%)
4	**46**		 **51**	10:1 (80%)
5	**46**		 **52**	15:1 (82%)
6	 **47**	Me	 **53**	Single diast. (70%)
7	**47**		 **54**	Single diast. (49%)

[a] Determined by ^{1}H NMR spectral integration of crude product mixture.

It remained for us to convert these diverse isoxazolidines, with all of the requisite substitution and stereochemical relationships installed, to the target β-amino acids (Figure 5). The synthetic plan entailed reduction of the N−O bond followed by protection of the nitrogen with a carbamate protecting group. In several of the substrates, the nitrogen is benzylic; thus we sought conditions that were mild enough such that these compounds would not be over-reduced. Use of 10% Pd/C with NH_4O_2CH, H_2−$Pd(OH)_2$ in HCl−MeOH,[37] or SmI_2 conditions[38] gave incomplete conversions and/or inconsistent yields. How-

(36) Annunziata, R.; Cinquini, M.; Cozzi, F.; Raimondi, L. *Tetrahedron* **1986**, *42*, 2129−2134. Shatzmiller, S.; Shalom, E.; Lidor, R.; Tartkovski, E. *Liebigs Ann. Chem.* **1983**, 906−912. Curran, D. P.; Chao, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 3036−3040. Kozikowski, A. P.; Ghosh, A. K. *J. Org. Chem.* **1984**, *49*, 2762−2772. Grund, H.; Jäger, V. *Liebigs Ann. Chem.* **1980**, 80−100. Jäger, V.; Schwab, W. *Tetrahedron Lett.* **1978**, 3129−3132.

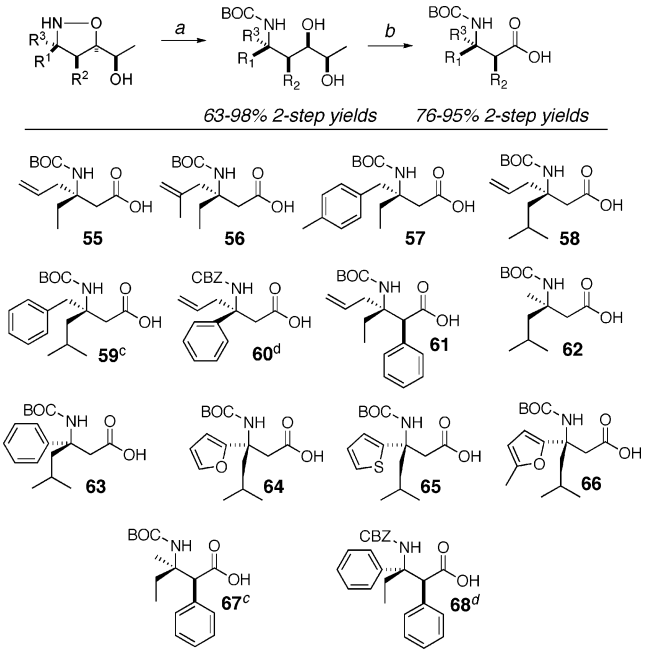


Figure 5. Conversion of isoxazolidines to $\beta^{3,3}$- and $\beta^{2,3,3}$-amino acids. Reaction conditions: (a) (i) $LiAlH_4$, Et_2O, 0 °C to room temperature, 3 h; (ii) BOC_2O, THF/H_2O, pH 10, 12 h. (b) (i) $NaIO_4$, CH_3CN/H_2O (1:1), room temperature, 0.5 h; (ii) $NaClO_2$, 2-methyl-2-butene, KH_2PO_4, *t*-BuOH, H_2O, room temperature, 2 h. (c) N−O bond cleavage effected by 10% Pd/C, NH_4O_2CH. (d) Amine protected as benzyl carbamate following N−O bond reduction: CbzCl, Na_2CO_3, THF/H_2O (5:1), 12 h.

ever, treatment of the isoxazolidines with $LiAlH_4$ in Et_2O cleanly provided the target amine diols that were immediately protected to facilitate isolation. In most cases, a BOC group was used, but in the case of **37** and **54**, the amine diol proved resistant to protection, probably because of the significant steric encumbrance of the nitrogen. For these examples, the sterically less demanding benzyl carbamate (CBZ) protecting group proved successful. In contrast to the conditions employed earlier for the β^3- and $\beta^{2,3}$-amino acids, we found that a two-step procedure employing $NaIO_4$ to cleave the diol followed by standard $NaClO_2$ conditions to oxidize the intermediate aldehyde proceeded with shorter reaction times and greater overall yields of the suitably protected β-amino acids, perhaps due to the shorter exposure of potentially sensitive side chains to the oxidizing conditions.[39]

As illustrated by the $\beta^{3,3}$- and $\beta^{2,3,3}$-amino acids in Figure 5, the isoxazoline-based synthetic strategy is remarkably effective for the preparation of a wide range of structures. One of the most notable features of this approach is that the C3 substituents in the β-amino acid are not required to be sterically dissimilar for stereoselective synthesis to be achieved,[40] as is most often the case with other synthetic strategies.[21,41] An excellent example of this is **59** with a benzyl and isobutyl group at C3; in the key stereocenter-forming step, the diastereoselectivity achieved is 18:1. Many of the side chains present in natural α-amino acids are sterically similar, and thus this class of targets is particularly

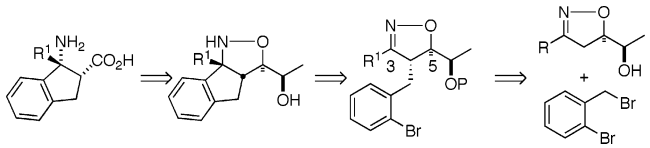


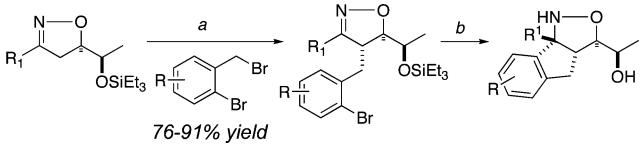
Figure 6. Retrosynthetic analysis of a *cis*-β-proline analogue. In this example, the latent nucleophile is an aryl bromide. By choosing a different electrophile in the alkylation reaction, the synthesis of *cis*-β-proline variants containing a range of substitution patterns can be envisioned.

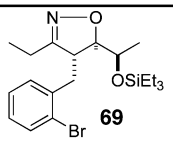
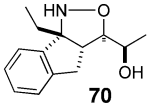
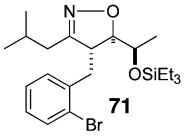
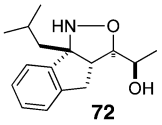
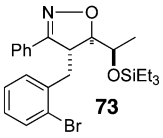
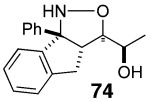
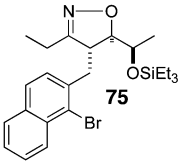
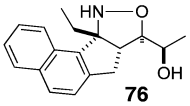
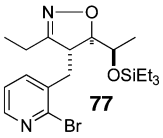
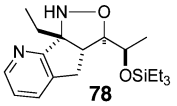
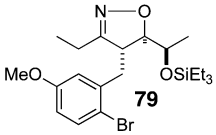
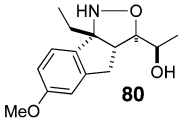
desirable. Also noteworthy among the structures we have synthesized are the substituents that can readily be transformed into a variety of other functional groups using straightforward means. The allyl and methylallyl groups, for example, are readily oxidized to hydroxyl and carbonyl groups (for example, see Figure 4), themselves excellent precursors to amines and guanidinium functionality. The methylfuryl group is also readily converted to a carboxylic acid under mild oxidative conditions.[42] Thus, a full range of substituents and substitution patterns can be accessed using this synthetic strategy.

Synthesis of *cis*-β-Prolines: Conformationally Constrained $\beta^{2,3,3}$-Amino Acids. As outlined in the Introduction, oligomers of cispentacin (*cis*-2-aminocyclopentanoic acid, **4**) form β-strands,[13] a common protein fold. Cispentacin itself is a natural product, originally isolated independently from *Bacillus cereus*[43] and *Streptomyces setonii*[43,44] and possessing antifungal activity.[45] From both a structural and a biological activity standpoint, cispentacin analogues are thus synthetic targets of great interest.[46] The most common synthetic approach employs a [2+2]-cycloaddition to form a β-lactam that is then hydrolyzed to the desired β-amino acid.[11,47] However, the stereoselective installation of additional substituents within the β-proline backbone, particularly at the β-position, is difficult by this or other methods.[48] To directly address these points, we envisioned that isoxazolines bearing a substituent at C4 containing a nascent nucleophilic group would be versatile intermediates for the preparation of this structural class as an intramolecular nucleophilic attack would generate the requisite *cis*-substituted cyclopentane with a nitrogen-bearing quaternary center at C3. A retrosynthetic analysis of a benzofused cispentacin in accordance with this strategy is depicted in Figure 6. Isoxazolines bearing C4 substituents are readily available by alkylation of the core ring structure as shown,[28,36] with substitution along the alkylating

(37) Beccalli, E. M.; Broggini, G.; Farina, A.; Malpezzi, L.; Terraneo, A.; Zecchi, G. *Eur. J. Org. Chem.* **2002**, 2080−2086.
(38) Bode, J. W.; Carreira, E. M. *Org. Lett.* **2001**, *3*, 1587−1590.
(39) Additional confirmation of the stereochemical assignments was obtained through X-ray crystallographic analysis of acid **67**. See ref 24 for details.
(40) For rare examples, see ref 10 and: Espino, C. G.; Wehn, P. M.; Chow, J.; Du Bois, J. *J. Am. Chem. Soc.* **2001**, *123*, 6935−6936. Satoh, T.; Fukuda, Y. *Tetrahedron* **2003**, *59*, 9803−9810. Davis, F. A.; Deng, J. H.; Zhang, Y. L.; Haltiwanger, R. C. *Tetrahedron* **2002**, *58*, 7135−7143.
(41) For elegant approaches to β-amino acids in which substituents are sterically dissimilar, see: Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 12−13. Gais, H.-J.; Loo, R.; Roder, D.; Das, P.; Raabe, G. *Eur. J. Org. Chem.* **2003**, 1500−1526. Kobayashi, K.; Matoba, T.; Irisawa, S.; Takanohashi, A.; Tanmatsu, M.; Morikawa, O.; Konishi, H. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 2805−2809. Ishitani, H.; Ueno, M.; Kobayashi, S. *J. Am. Chem. Soc.* **2000**, *122*, 8180−8186.
(42) Borg, G.; Chino, M.; Ellman, J. A. *Tetrahedron Lett.* **2001**, *42*, 1433−1436.
(43) Konishi, M.; Nishio, M.; Saitoh, K.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1989**, *42*, 1749−1755.
(44) Iwamoto, T.; Tsujii, E.; Ezaki, M.; Fujie, A.; Hashimoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H.; Kawabata, K.; Inamoto, Y.; Sakane, K. *J. Antibiot.* **1990**, *43*, 1−7.
(45) Oki, T.; Hirano, M.; Tomatsu, K.; Numata, K. I.; Kamei, H. *J. Antibiot.* **1989**, *42*, 1756−1762.
(46) Ohki, H.; Inamoto, Y.; Kawabata, K.; Kamimura, T.; Sakane, K. *J. Antibiot.* **1991**, *44*, 546−549.
(47) Fülöp, F.; Palkó, M.; Kámán, J.; Lázár, L.; Sillanpää, R. *Tetrahedron: Asymmetry* **2000**, *11*, 4179−4187. Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593−1600. Szakonyi, Z.; Fülöp, F.; Bernáth, G.; Evanics, F.; Riddell, F. G. *Tetrahedron* **1998**, *54*, 1013−1020.
(48) Gees, T.; Schweizer, W. B.; Seebach, D. *Helv. Chim. Acta* **1993**, *76*, 2640−2653. Hanselmann, R.; Zhou, J. C.; Ma, P.; Confalone, P. N. *J. Org. Chem.* **2003**, *68*, 8739−8741. Aggarwal, V. K.; Roseblade, S. J.; Barrell, J. K.; Alexander, R. *Org. Lett.* **2002**, *4*, 1227−1229. Konosu, T.; Oida, S. *Chem. Pharm. Bull.* **1993**, *41*, 1012−1018.

Table 4. Intramolecular Nucleophilic Addition



Entry	Substrate	Product	Yield
1	 **69**	 **70**	87%
2	 **71**	 **72**	69%
3	 **73**	 **74**	84%
4	 **75**	 **76**	86%
5	 **77**	 **78**	73%[c]
6	 **79**	 **80**	93%

[a] (i) LDA, THF, −78 °C, 1.5 h; (ii) RCH_2Br, 2 h. [b] (i) *t*-BuLi, THF, −78 °C; (ii) H_3O^+, 0 °C to room temperature. [c] To facilitate isolation of this very polar isoxazolidine, the TES group was not removed after the nucleophilic addition reaction.

agent backbone providing additional functional groups within the β-proline product.[49] As with the acyclic β-amino acid variants, the absolute stereochemistry of the final β-amino acid product is dictated by the initial choice of allylic alcohol absolute stereochemistry, enabling ready access to either enantiomer.

The *cis*-β-proline variants selected as synthetic targets contain both a quaternary center as well as a fused aryl or heteroaryl group, lending further conformational rigidity to the final product in addition to added positions for functionalization. To initiate the synthesis, isoxazolines bearing various C3 substituents were treated with LDA to form an internal azaenolate followed by addition of an electrophile such as 2-bromobenzyl bromide (Table 4). The alkylation proceeds with excellent regio- and diastereoselectivity with only a single isomer observed in the crude product mixture by either GC or ^{1}H NMR analysis. An NOE study carried out on isoxazoline **71** confirmed that, as expected, the alkylation event takes place on the face of the isoxazoline opposite the sterically bulky C5 subsitutent, resulting in a C4,C5-anti relationship in the product. We anticipated that the aryl bromide would be converted upon treatment with *tert*-butyllithium into a potent nucleophile poised for intramolecular addition to the adjacent C=N bond. Indeed, this reaction was found to proceed smoothly, and the triethylsilyl protecting groups in the product were removed conveniently by quenching the reaction with acid to provide tricyclic isoxazolidines (Table 4). In contrast to the intermolecular nucleophilic additions described in previous sections, addition of an intramolecular nucleophile does not require a Lewis acid, and the yields are nearly identical with or without added $BF_3{\cdot}Et_2O$. The addition is similarly tolerant of R^1 side chains of varying steric and electronic characteristics. Further, the nucleophile itself can be electron-deficient (entry 5) or bear electron-donating substituents (entry 6) without deleterious effects on the yield. The example in entry 5 is particularly noteworthy as the pyridine ring serves to increase water solubility of the product, useful for later biological applications, and also provides a hydrogen-bond acceptor within the backbone for assembly purposes.

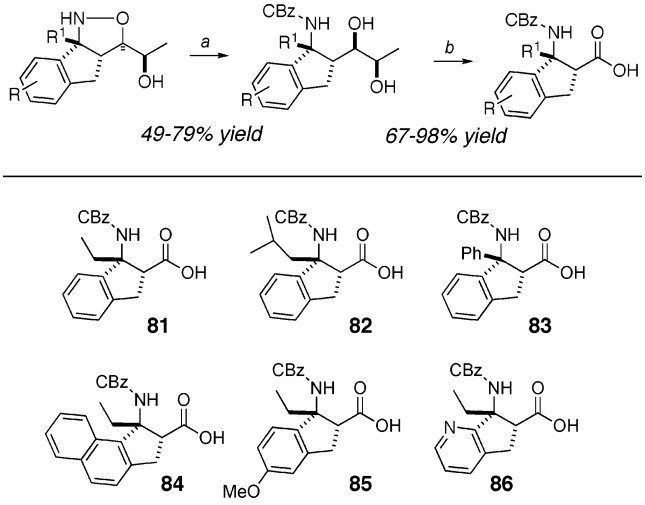


Figure 7. Conversion of isoxazolidines to conformationally constrained $\beta^{2,3,3}$-amino acids.

Using the conditions previously identified, it was straightforward to convert the highly functionalized, tricyclic isoxazolidines to the corresponding β-amino acids. The isoxazolidine N−O bond was reduced with $LiAlH_4$, and, due to the steric hindrance about the resulting amine, protection of this functional group was best accomplished using benzyl chloroformate to provide a benzyl carbamate moiety. A two-step oxidation procedure ($NaIO_4$ then $NaClO_2$) was likewise effective for oxidative cleavage of the diol moiety, providing the β-amino acid products in good to excellent overall yields (Figure 7). The ready synthesis of these β-proline examples illustrates the versatility of the isoxazoline-based synthetic approach for this functionally dense compound class. With the additional substitution, these structures are promising candidates for developing β-peptides that form higher order structures.

(49) Alternatively, employing a 1,2-disubstituted olefin in the initial 1,3-dipolar cycloaddition to form the isoxazoline can be used to install a C4 substituent. This approach is generally less desirable for producing a diversity of isoxazolines, however, because the yields of the cycloaddition often decrease as olefin substitution increases, and, further, the allylic alcohols are less readily available.

Conclusions

We have described here a versatile and efficient approach for the synthesis of a diverse array of β-amino acids. The approach employs chiral isoxazolines as the centerpiece, translating the high diastereoselectivity available from a 1,3-dipolar cycloaddition reaction into the formation of structures with up to four contiguous stereocenters. The initial implementation provided access to β^3- and $\beta^{2,3}$-amino acids, classes of β-amino acids that have been widely employed in pharmaceutical targets and peptidomimetics. When applied to the synthesis of highly substituted β-amino acids, targets that demand the installation of an amine-bearing quaternary stereocenter, the method has proven to be especially valuable; the novel targets described here significantly expand the set of C3-disubstituted β-amino acids available for structure/function studies of β-peptide oligomers. Elaborating on the same general strategy, we have demonstrated that appropriately substituted isoxazolines can be used to efficiently generate a high degree of molecular complexity toward the preparation of *cis*-β-proline variants. Such densely functionalized structures should find immediate use in both structural and biological studies. In addition to the valuable β-amino targets addressed here, we also anticipate that the general strategy will find even broader use. In particular, we have shown that isoxazolines are useful precursors for targets containing tertiary and quaternary amine-bearing stereocenters; thus one can envision using isoxazolines en route to other synthetic targets including various nitrogen-containing pharmaceuticals and alkaloid natural products.

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

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