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Asymmetric synthesis of vinylogous β -amino acids and their incorporation into mixed backbone oligomers†

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Abstract

Chiral vinylogous β -amino acids (VBAA) were synthesized using enantioselective Mannich reactions of aldehydes with *in situ* generated *N*-carbamoyl imines followed by a Horner–Wadsworth–Emmons reaction. The efficiency with which these units could be incorporated into oligomers with different moieties on the C- and N-terminal sides was established, as was the feasibility of sequencing oligomers containing VBAA by tandem mass spectrometry. The data show that VBAA will be useful building blocks for the construction of combinatorial libraries of peptidomimetic compounds.

Introduction

To facilitate the rapid and low-cost discovery of bioactive compounds, several laboratories have focused on the development of large chemical libraries of oligomeric compounds displayed on a solid support.^{1–9} This technology was first developed for the construction of peptide libraries, but over the years has been adapted to other types of (mostly) amide oligomers with better serum stability and/or cell permeability than peptides. These bead-displayed libraries can be screened relatively easily and cheaply by simply incubating them with a tagged protein in the presence of a large excess of untagged competitor proteins and recovering beads that retain the tag.^{4,10} The structures of the “hits” can then be determined by tandem mass spectrometry (MS/MS) following cleavage from the bead.^{11–13} Alternatively, for molecules that cannot be sequenced easily by MS/MS, various encoding strategies¹⁴ have been developed.^{15–17} Until recently, this approach to protein ligand discovery was hampered by a variety of technical problems, but most of these have now been solved.^{18,19} Therefore, it is of great interest to begin to expand the type of chemical matter that can be utilized in the context of this inexpensive and powerful screening paradigm.

In particular, it is abundantly clear that a conformational constraint is desirable to reduce the entropic penalty a molecule must pay upon engaging its macromolecular target. Most compound collections used in traditional plate-based high throughput screening are

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dominated by molecules that achieve this “stiffness” by being comprised mostly of sp^2 centres, especially aromatic rings, to reduce the number of rotatable bonds. While this strategy can be replicated for encoded, bead displayed libraries, there is concern that this type of relatively flat, often hydrophobic molecular architecture can lead to off-target binding.^{20,21} Many investigators have suggested that it would instead be desirable to “escape flatland”²² and create screening collections of molecules that are stiff by virtue of conformational constraints due to interactions between sp^3 -hybridized chiral centres and other moieties.

Working toward this goal in the context of oligomeric molecules,²³ we have reported the construction of bead-displayed libraries of chiral oligomers of pentenoic amides (COPAs) (Fig. 1) and peptide tertiary amides (PTAs) (Fig. 1), in which chiral centres are placed adjacent to, or in between, substituted double bonds, resulting in strong $A_{1,3}$ strain effects that strictly limit the number of low energy conformers available to the molecule.²⁴ Lim and colleagues have created interesting smaller libraries of interesting helix mimetics containing chiral centers.¹⁶ Comparative screening experiments have demonstrated clearly the superiority of these libraries over collections of floppy molecules like peptoids (Fig. 1) for the discovery of high affinity and selectivity protein ligands.^{24–29} Thus, an attractive goal is to increase the number of available building blocks containing chiral sp^3 centres that display limited conformational flexibility.

β -Peptides (Fig. 1) were pioneered by Seebach and Gellman nearly two decades ago.^{30,31} β -Amino acids with substituents at both the alpha position and the beta position are conformationally constrained by *gauche* interactions between these centers³² and thus are potentially attractive as building blocks for the synthesis of libraries of conformationally constrained oligomers. However, in our library designs, which contain many peptoid (Fig. 1) or peptoid-inspired units,²³ coupling of an activated acid to a secondary amine is often required. Not surprisingly, when the acid partner is α -branched and lacks further activation, such as the presence of an electron-withdrawing unit at the α -carbon, amide bond formation is sluggish.^{25,33} This was anticipated to be a limitation when using β -amino acids in such libraries. Therefore, we focused on the creation of vinylogous β -amino acids (VBAAAs) in which the α -stereocenter is separated from the carboxylic acid by a sterically undemanding vinyl unit (Fig. 1). We report here an expedient synthesis of such building blocks and demonstrate that, as expected, they couple efficiently to most types of secondary amines that are present in our libraries.

Results and discussion

Asymmetric Mannich reaction of *in situ* generated *N*-carbamoyl aromatic imines

The organocatalyzed asymmetric Mannich reaction of imines and aldehydes provides rapid access to chiral β -amino aldehydes.^{34–41} We anticipated that these products would undergo facile Horner–Wadsworth–Emmons vinylation reactions to provide α,β -unsaturated VBAA esters that upon saponification, would provide the desired VBAAAs, such as **1a–d** shown in Fig. 2.

In the event, the chemistry proceeded smoothly, as anticipated. As model electron-rich and -poor benzaldehydes, piperonyl aldehyde and 4-nitrobenzaldehyde were converted to the Boc-protected amidosulfones shown in Fig. 2, then condensed with 3-methylbutanal in the presence of KF and an organocatalyst, providing Mannich products.³⁹ As expected, both *syn*- and *anti*-aldehydes were accessible *via* this route by utilizing the appropriate catalyst: the proline-derived tetrazole catalyst **A** or D-proline **B**, which are capable of forming specific hydrogen bonds⁴² for the *syn*-adducts and the TMS-diaryl prolinol- derived catalyst **C**, which enforces “steric-control approach”,^{43,44} for the *anti*-adduct, regardless of the substituents on the aryl aldehydes (Fig. 2). The Mannich products were >95% enantiomerically pure, as determined by reduction of the aldehyde and analysis of the Mosher esters by NMR (see the ESI[†]).

The Mannich products were then subjected to Horner–Wadsworth–Emmons reactions⁴⁵ (triethyl phosphonoacetate, LiCl and diisopropylethylamine (DIPEA)) to generate the expected α,β -unsaturated esters in 85–90% yields. The esters were hydrolyzed with LiOH to obtain the desired vinylogous β -amino acids (**1a–1d**, obtained in 28%, 12%, 11% and 11% overall yields, respectively, from the aromatic aldehyde starting materials).

The ¹³C-NMR spectrum of **1d** in CDCl₃ showed two sets of peaks (see Fig. S1 ESI[†]). This was also true when the compound was dissolved in DMSO-d₆, though the ratio between the two compounds was different ($\approx$ 60: 40 in CDCl₃ and 80: 20 in DMSO), suggesting that these are conformers. This was confirmed by heating the DMSO-d₆ solution to 100 °C, which resulted in a collapse of the two species into a single set of peaks. Similar results were obtained for all of the vinylogous β -amino acids.

The Mannich products were also converted to the corresponding β -amino acids (BAA) by Pinnick oxidation⁴⁶ in essentially quantitative yield. An example is shown in Fig. 3.

Oligomer synthesis using vinylogous β -amino acid building blocks

We are interested in the construction of peptoid-inspired combinatorial libraries of oligomers in which the backbone elements, in addition to the side chains, are treated as diversity elements.²³ In assessing the utility of β -amino acids and VBAAAs for this purpose, a critical issue is the efficiency with which various amines can be acylated with these units, as well as the efficiency with which various acid building blocks²³ can be added to their N-termini after removal of the Boc protecting group. Another important point is whether these units fragment in the mass spectrometer efficiently enough to allow their characterization. If not, they could be employed only in encoded libraries.^{15,17}

To address this issue, 90 μ m TentaGel beads were modified with a linker unit comprised of methionine, a peptoid unit with an ether-containing side chain (called Nmea), another peptoid with a bromobenzene-containing side chain and another Nmea unit (Fig. 4). The linker can be cleaved at the Met residue using cyanogen bromide⁴ and the bromine provides a useful isotope tag to facilitate identification of peaks in the mass spectrum.¹³ The Nmea residues simply provide additional mass to put the molecules in a mass range that is easy to

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analyze by MALDI MS/MS. Following the synthesis of this common linker, the beads were split into five flasks and the units shown in Fig. 4 (X_1 – X_5) were added. To each of these amino termini was coupled BAA **5b** or VBAA **1b**. The coupling efficiency as a function of time was determined by LC-MS (Table 1). In some cases, we could detect previously reported urea-type byproducts,³³ which originated from the parent compounds during the cleavage of the beads using cyanogen bromide (see ESI Fig. S2† for the plausible mechanism).

BAA **5b** did not add to any of the substrates rapidly and efficiently. Even for the case of acylating a primary amine of a preceding amino acid (X_1 and X_2), the yields were less than 50% at 24 h and the coupling was not complete even after four days under these conditions. For coupling of the BAA to a secondary amine (the peptoid X_3) only a trace amount of product could be detected at 24 h and the yield only reached 10% even after four days. Acylation of the PTA (X_4) and COPA (X_5) units with BAA was even more sluggish; it was impossible to detect even trace amounts of coupled product.

Clearly BAAs are not viable building blocks for oligoamide libraries containing tertiary amide bonds.

In contrast, VBAA **1b** was, as hoped, far more reactive. Primary amines (X_1 , X_2) and secondary amines (X_3 , X_5) were all acylated by activated **1b** efficiently. The coupling reactions were nearly complete in 8–24 h. The only exception was addition to the peptide tertiary amide (PTA; X_4) unit, a particularly hindered secondary amine adjacent to a branched α -substituent. This was not surprising and is in accordance with our previous experience that acylation of a PTA unit requires special, forcing conditions and even then is difficult.^{25,47}

With these results in hand, we next turned to the synthesis of oligomers containing a VBAA unit to confirm that acylation of their N-terminus would proceed efficiently and to determine if they would fragment in a fashion that would allow their facile characterization by MS/MS.

Using conditions published previously,^{24,47} several oligomers were constructed containing either VBAA **1a**, with *syn* stereochemistry and an electron-rich phenyl ring, or **1d**, with *anti* stereochemistry and an electron-deficient aromatic ring. Specifically, the five building blocks shown in Fig. 4 (X_1 – X_5) were appended to a short linker comprised of methionine and three peptoid units. This was then followed by VBAA **1a** or **1d**. After removal of the Boc protecting group, units X_1 – X_5 were then appended to the amine of the VBAA unit. Thus, we carried out 50 syntheses in all (25 with each VBAA). The yield of the desired product was determined by LC-MS (Table 2) after cleavage from the resin with CNBr (see Fig. 5 for an example).

Oligomers in which the amino acids or the peptoid unit (X_1 – X_3) preceded and followed the VBAA were produced in good yield and high purity. On the other hand, as discussed above, oligomers in which the VBAA followed a PTA unit could not be synthesized under these conditions. As an aside, we also found that the peptoid–PTA amide bond was not stable under the conditions employed to remove the Boc group, in line with the known acid sensitivity of this linkage.²⁵ On the other hand, PTA unit could be easily coupled to the N-

terminus of the VBAA (X₄-**1a**/**1d**-X_{1,2,3,5}-linker). As shown in Fig. 5, the major peak in the LC-MS represents the desired oligomer. This series of compounds was the only one in which the two VBAs behaved somewhat differently, with the oligomers containing **1a** being obtained in higher yield.

The peptidomimetic compounds that possess a COPA unit at the C-terminal position (X₂-**1d**-X₅-linker Fig. 6b, X₄-**1a**-X₅-linker Fig. 7b) exhibited slightly lower purities than the other compounds, in line with the modest reactivity of the activated ester of this building block, which has a branched centre adjacent to the carbonyl carbon and is not further activated electronically by an electron-withdrawing group at this position. However, even these oligomers were obtained in reasonable yield and purity.

We conclude that the VBAA building blocks are suitable for the synthesis of oligomer libraries as long as the design avoids placing a PTA residue C-terminal of the VBAA or involves sandwiching a VBAA between two COPA residues. Of course, more forcing conditions could be employed to presumably drive many of these yields up if desired.

Characterization of VBAA-containing oligomers using tandem mass spectrometry

Fig. 6 displays the LC trace of the compound X₂-**1d**-X₅-linker (Val-**1d**-COPA-linker). The MS data showed the anticipated pair of molecular ions (differing in the bromine isotopes). Tandem MALDI-TOF mass spectrometric analysis revealed several prominent fragments, including *b*-type ions resulting from fragmentation of the amide bonds on the N- and C-terminal sides of the VBAA unit (shown in blue in Fig. 6A) as well as two *a*-type ions (shown in red in Fig. 6A) resulting from fragmentation of bonds within this building block. The *a*-type ions can be explained by the formation of benzylic radical on 4-nitro benzyl position of **1d**, which led to further fragmentation of the building block. Compound X₄-**1a**-X₅-linker (PTA-**1a**-COPA-linker), possessing vinylogous β -amino acid with an electron donating moiety also showed the same pattern of *a*-type ions, which implies that the fragmentation of the vinylogous β -amino acid unit took place regardless of the electron density of the aromatic ring (Fig. 7). The presence of these readily detectable fragment ions in the MS/MS allows facile characterization of oligomers containing VBAs.

Elaboration of iodo-VBAAs by Suzuki coupling

The Mannich reaction employed to create the aldehyde precursor to the VBAA has been reported to proceed efficiently with a variety of aromatic aldehydes.^{36,37} This is an attractive feature for the purposes of library synthesis using VBAs. However, it would also entail maintaining stocks of a large number of VBAs as building blocks. As a compromise, we considered the creation of VBAs bearing functionalities on the aromatic ring that could be further elaborated by on bead synthesis to provide greater molecular diversity. Towards this goal, we processed 4-iodobenzaldehyde through the reaction sequence, providing VBAA **1e** (Fig. 8). We imagined that the aryl iodide would serve as an efficient partner in various organometallic coupling reactions.

To test this idea, **1e** was coupled to beads following a Met-Gly linker and the beads were treated with phenylboronic acid and palladium triphenylphosphine in the presence of K₃PO₄

in DMF at 37 °C overnight (Fig. 8).⁴⁸ Subsequent cleavage with CNBr and LC-MS analysis of the product showed that the expected Suzuki product **6a** was formed in 85% yield and excellent purity. A similar reaction employing 3-fluoro-4-methyl phenylboronic acid provided a 75% yield (Fig. 8). Thus, as anticipated from the literature precedent of successful Suzuki reactions in the solid phase,^{48,49} it will be possible to elaborate iodophenyl-containing VBAs in a combinatorial fashion for the creation of complex libraries.

Experimental

General procedure for the *syn*-Mannich reaction of isovaleraldehyde with *in situ* generated *N*-Boc imines

The reactions were carried out under air and moisture free conditions by using the proline-derived tetrazole catalyst **A** or D-proline **B**. Isovaleraldehyde (2 equiv.) was added to a solution of catalysts **A** or **B** (0.1 equiv.) in CHCl₃ at room temperature. After stirring for 30 min, α -amido sulfone **2** (1 equiv.) and KF (5 equiv.) were added successively. The reaction mixture was stirred for 24 h, then diluted with CH₂Cl₂ and flushed through a short plug of silica (1: 1 CH₂Cl₂/Et₂O). The solvent was removed *in vacuo* and the residue was purified by flash column chromatography with mixtures of ethyl acetate/hexane as the eluent. Since the Mannich products are prone to epimerization upon exposure to silica gel, the flash chromatography was performed quickly.

General procedure for the *anti*-Mannich reaction of isovaleraldehyde with *in situ* generated *N*-Boc imines

The reaction was performed by using 10% mol of TMS-diaryl prolinol-derived catalysts **C** following the same experimental procedure described above.

General procedure for Horner–Wadsworth–Emmons reaction with the Mannich product

The reactions were carried out under air and moisture free conditions. LiCl was weighed out in the glove box, and *N,N*-diisopropylethylamine (DIPEA) was distilled over calcium hydride. To a stirred suspension of LiCl (2.0 equiv.) in dry acetonitrile at room temperature, were added phosphonate (2.0 equiv.), DIPEA (1.8 equiv.) and the Mannich aldehyde (1.0 equiv.). The flash chromatography was performed to obtain a pure product. The HWE adducts **4a** and **4b** were not able to be purified by column chromatography, however, a small amount of the remaining aldehyde could be removed after hydrolysis of the ethyl ester.

Conclusion

We report here efficient methods for the synthesis of optically pure vinylogous beta amino acids (VBAs) and their incorporation into oligomers by solid-phase synthesis. We find that they are, in general much better coupling partners for the acylation of a variety of different primary and secondary amines than are the corresponding beta amino acids. The data (Tables 1 and 2) indicate that, in general, the VBA building blocks should be useful for the creation of a variety of diverse oligomer libraries using different backbone elements, as long as the coupling of a VBA to a PTA unit is avoided. Finally, the use of a phenyliodo-

containing VBAA provides a straight forward method for the further elaboration of the side chain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Structures of building blocks used previously for the construction of combinatorial libraries of oligomers. The asterisk indicates the presence of a chiral centre.

Fig. 2.

Synthesis of vinylogous β -amino acids. An electron-rich and an electron-deficient benzaldehyde were employed. Four compounds were made (1a–1d). (A) Scheme for the construction of 1a. (B) Structures of three other VBAs constructed. The organocatalyst employed in the asymmetric Mannich reaction is shown in the box.

Fig. 3.
Synthesis of β-amino acids.

Fig. 4.

Solid-phase synthesis of oligomers containing VBAA building blocks. (A) Overall reaction scheme starting from a common peptoid linker. (i) Coupling step for X_1 and X_2 , Fmoc-AA-OH, Oxyma, and DIC in DMF (3 equiv. each), then 20% piperidine in DMF 2 $\times$; for X_3 , bromoacetic acid and DIC (2.0 M each in DMF), and subsequent 2-methoxyethylamine (2.0 M in DMF); for X_4 , Fmoc-Ala-OH, Oxyma, and DIC in DMF (3 equiv. each), then 20% piperidine in DMF 2 $\times$, then benzaldehyde (10 equiv.), then excess NaBH₄ in MeOH/DCM; for X_5 , COPA monomer, DIC in DMF (3 equiv. each, 37 °C), then 3-methoxypropylamine (2.0 M in DMF). (ii) VBAA, Oxyma and DIC in DMF (3 equiv. each). (iii) 50% TFA in DCM, 2 $\times$. (iv) Repeat (i). (v) 200 mM CNBr in 200 mM HCl. (B) Structure of the VBAA building blocks (X_1 to X_5).

Fig. 5.

Analysis of PTA coupled after the VBAA position. (A) Crude LC-MS spectra of X₄-1a-X₃-linker. The LC trace was monitored at 254 nm. The major peak a was characterized as the desired product, and b was identified as a urea byproduct due to CNBr cleavage. (B) Mass spectra of the desired product a. (C) Structures of the desired product a and urea-type byproduct b.

Fig. 6. LC/MS and MS/MS data for the given compound X₂-1d-X₅-linker. (A) Structure of the compound with an indication of fragmentation ions being generated during MALDI analysis. (B) Crude LC-MS spectra of the given compound. The LC trace monitored at 254 nm. The insert showed MS data. (C) MALDI MS/MS fragments.

Fig. 7. LC/MS and MS/MS data for the given compound X_4-1a-X_5 -linker. (A) Structure of the compound with an indication of fragmentation ions being generated during MALDI analysis. (B) Crude LC-MS spectra of the given compound (peak a). The LC trace monitored at 254 nm. The insert showed MS data. Peak b corresponds to $[M + 43]^+$, urea byproduct previously reported³³ (C) MALDI MS/MS fragments.

Fig. 8. Suzuki coupling with iodo-containing VBAA 1e. (A) Synthetic scheme of VBAA 1e. (B) Solid phase Suzuki coupling with aryl boronic acids. Both unsubstituted and disubstituted aryl boronic acids were used. (C) Corresponding LC-MS spectra of compounds 6a and 6b synthesized in the solid phase.

Table 1

Coupling efficiency of BAA and VBAA with different types of Amines

Amine	Acid	1 h	2 h	4h	8 h	24 h	96 h
X ₁ -Linker	BAA 5b	—	6%	11%	19%	40%	84%
	VBAA 1b	35%	57%	75%	89%	100%	N.D.
X ₂ -Linker	BAA 5b	—	2%	6%	14%	36%	76%
	VBAA 1b	31%	53%	73%	94%	100%	N.D.
X ₃ -Linker	BAA 5b	—	—	—	—	2%	11%
	VBAA 1b	34%	54%	79%	96%	96%	N.D.
X ₄ -Linker	BAA 5b	—	—	—	—	—	—
	VBAA 1b	—	—	—	3%	8%	38%
X ₅ -Linker	BAA 5b	—	—	—	—	—	—
	VBAA 1b	37%	72%	84%	92%	94%	N.D.

The conversion yield was assessed by the peak area ratio of the product to that of the starting material (detected at 210 nm). In some cases, the starting material or product includes predictable byproducts during the cleavage of the beads using cyanogen bromide solution.³³ See the ESI for a detailed assessment. N.D. = not determined. — = product not detected.

Table 2

Yield of oligomers synthesized according to Fig. 4

		VBAA 1a					VBAA 1d				
		C-term.									
N-term.		X ₁	X ₂	X ₃	X ₄	X ₅	X ₁	X ₂	X ₃	X ₄	X ₅
X ₁		83	78	82	0	67	71	85	90	0	71
X ₂		81	71	80	0	75	70	86	90	0	82
X ₃		87*	70*	84*	0	87*	72*	72*	70*	0	65*
X ₄		83*	81*	80*	0	70*	62*	43*	57*	0	10*
X ₅		42	38	44	0	10	59	35	40	0	47

Yields are calculated from the peak area integrated from the LC traces (monitored at 254 nm; see the ESI). Asterisks mean that the product peak contains a known urea byproduct due to CNBr cleavage.³³