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β-Amino Acid Facilitates Macrocyclic Ring Closure in a Combinatorial Library

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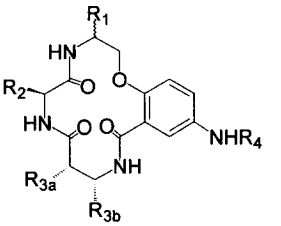
Abstract: A solid phase synthesis of 14-membered macrocycles, resulting from resin-bound protected amino alcohols is described. A nucleophilic aromatic substitution strategy was used to effect the macrocyclization.

Since RNA molecules play key roles in vital biological processes, they are emerging as important targets in drug discovery.[1] Using a combinatorial chemistry approach to these targets,[2] we have enabled a solid phase synthesis of a 14-membered macrocyclic library (Figure 1). The macrocyclic scaffold is attractive as it can be assembled with a diverse array of building blocks including α-amino alcohols, α-amino acids and β-amino acids. The use of β-amino building blocks in drug discovery is of current interest as a β-peptide-containing oligomer has recently been found to exhibit biological activity.[3] To effect macrocyclization, the nucelophilic aromatic substitution (S_NAr) linker,[4] 2-fluoro-5-nitrobenzoic acid, was employed to react with the hydroxyl group of the various amino alcohol moieties. Unlike other macrocyclization strategies, a fixed amino acid side chain is not required in the S_NAr reaction[4] and therefore another diversity site is made available. The aryl nitro group of the linker is a useful latent combinatorial diveristy site as it can be reduced to the amine and subsequently functionalized with a collection of RNA-binding carboxylic acids. The high purity of 14-membered aryl ether macrocycles is another attractive feature of this library as no purification is required prior to biological testing.

Figure 1. Macrocyclic scaffold



In order to explore the feasibility of a combinatorial macrocyclic synthesis, model compound **7a** was prepared (Scheme 1, Table 1). To begin the solid-phase synthesis, ethanolamine was loaded onto ArgoGel™–MB-CHO resin[5] *via* a BH_3·pyridine mediated reductive amination procedure.[6] The free hydroxyl group was then *tert*-butyl dimethylsilyl (TBDMS)-protected to give resin **2a**. The protected amino acid Fmoc-L-Ala-OH was then coupled to resin **2a** using bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP)[7] activation. Following Fmoc removal with piperidine, the β-amino acid Fmoc-β-Ala-OH was reacted. It was important to incorporate the β-amino acid, instead of an α-amino acid to allow for the formation of a favorable 14-membered macrocycle. The attempted macrocyclization of a linear analog of **5a**, where both Fmoc-(L)-Gly-OH and Fmoc-(L)-Phe-OH were incorporated instead of Fmoc-β-Ala-OH, gave only 26-membered dimeric structures, according to MS analysis. After Fmoc-deprotection, the S_NAr cyclization linker, 2-fluoro-5-nitrobenzoic acid, was reacted with the N-terminus. The TBDMS protection was then removed using triethylamine trihydrofluoride (TREAT-HF) and the cyclization step was carried out in a 0.2 M solution of DBU in DMF.

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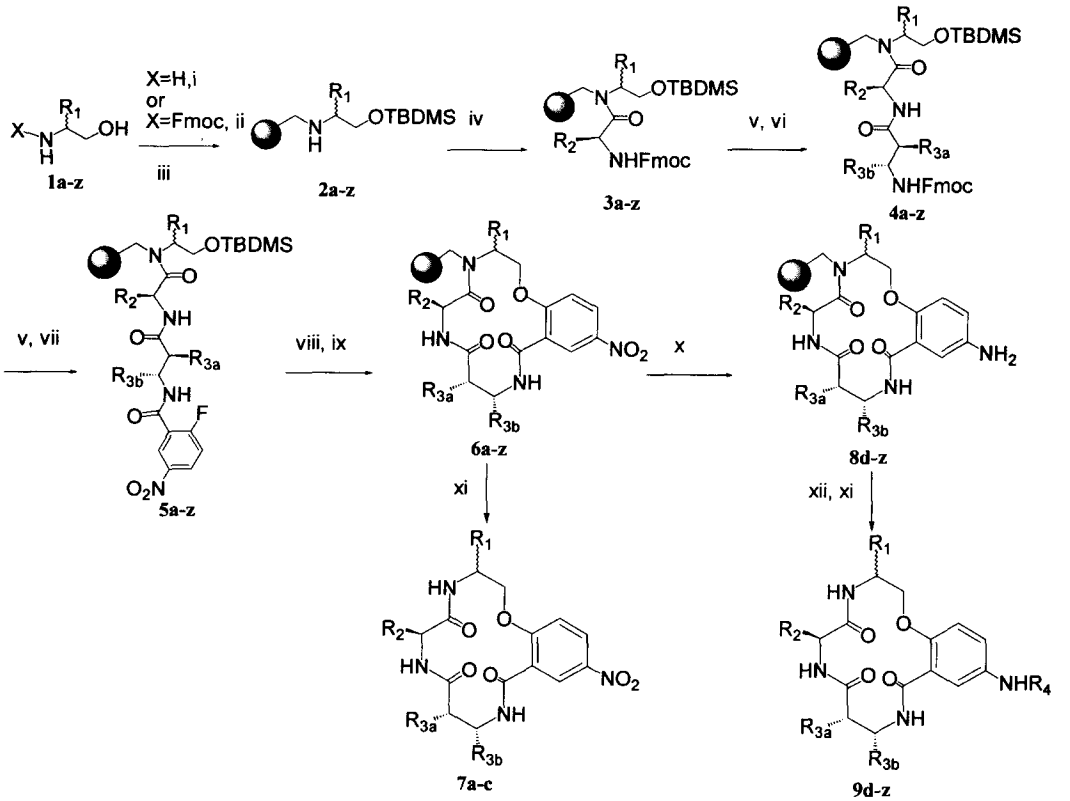
Following cleavage of resin **6a** with TFA, macrocycle **7a** was obtained in >95% purity according to HPLC and MS analysis.[8] The quality of the synthesis was verified at several steps by TFA cleaving portions of resins **3a**, **4a** and **5a** and analyzing the products by HPLC and MS techniques.

The synthesis is flexible since several amino alcohols can be loaded onto the solid support and many Fmoc-amino acids and β-amino acids[9] are commercially available or easily obtained. In addition, the aryl nitro group can be reduced with tin(II)chloride[10,4a] and subsequently functionalized, thus creating a fourth diversity site.

Scheme 1. Synthesis of 14-membered macrocycles.



Reagents and Conditions: i) 3.0 equiv. **1**, 1.0 equiv. ArgoGel™-MB-CHO, 4:1 MeOH/$CH(OMe)_3$, RT, 24 h ; 2 equiv. BH_3·pyridine, 2 equiv. AcOH, RT, 24 h; ii) 1.3 equiv. **1**, 2.0 equiv. NaOH, MeOH, 4 h; 2.2 equiv. AcOH, 30 min; 1.0 equiv. ArgoGel™-MB-CHO, $CH(OMe)_3$, RT, 24 h; 2 equiv. BH_3.pyridine, 2 equiv. AcOH, RT, 24 h; (iii) 3.0 equiv. TBDMS-Cl, 3.0 equiv. TEA, 0.1 equiv. DMAP, DCM, RT, 24 h; iv) 0.25 M PyBroP, 0.5 M DIEA, 0.25 M Fmoc-α-amino acid, DCM, RT, 48 h; v) 20% piperidine, DMF, RT, 30 min; vi) 0.11 M Fmoc-β-amino acid, 0.11 M HATU, 0.22 M collidine, DMF, RT, 24 h; vii) 0.11 M 2-fluoro-5-nitrobenzoic acid, 0. 11 M HATU, 0.22 M collidine , DMF/DCM (1/1; v/v), RT, 24 h; viii) 0.2 M TREAT-HF, THF, RT, 24 h; ix) 0.2 M DBU, DMF, RT, 48 h; x)1.5 M $SnCl_2$, DMF/EtOH (10/1; v/v), RT, 24 h; xi) TFA/triisopropylsilane (95/5;v/v), RT, 4 h; xii) 0.11 M carboxylic acid, 0.11 M HATU, DMF, RT, 24 h or 0.22 M isocyanate, DMF, RT, 24 h.

A library of 5x6x3x4 members was synthesized using the IRORI™ directed sorting technology.[11] Racemic D,L-amino alcohols [Fmoc-Tyr(*t*Bu)-ol, Fmoc-Ala-ol, Fmoc-Arg(Pmc)-ol, Fmoc-Lys(*t*Bu)-ol and Fmoc-Ser(*t*Bu)-ol] were first immobilized onto solid support to provide starting resins, according to Scheme 1. A total of six Fmoc-L-amino acids [Fmoc-Phe-OH, Fmoc-Leu-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Glu(*t*Bu)-OH, Fmoc-Arg(Pmc)-OH, and Fmoc-Asn(Trt)-OH] and three Fmoc-β-amino acids [Fmoc-β-Ala-OH, N-α-Boc-N-β-Fmoc-(S)-2,3-diaminopropionic acid (Boc-L-Dpr(Fmoc)-OH) and Fmoc-L-Asp(*t*Bu)-OH] were used in the chain elongation. Following DBU-induced S_NAr macrocyclization, the nitro groups of the resin-bound structures were reduced with tin (II) chloride and subsequently functionalized with either 2-pyrazine carboxylic acid, thymine-1-acetic acid, Boc-isonipecotic acid or 4-methoxybenzyl isocyanate. The macrocycles were cleaved from the support to provide structures **9d-z** (Scheme 1). The MS and purity data for a representative set of compounds is summarized in Table 1. NMR data was also obtained on non-diastereomeric samples **7b** and **7c**, whose synthesis was carried out on a larger scale.[12] Analysis of the data in Table 1 reveals that a variety of functionality and steric constraints are well tolerated in the macrocyclization.

Table 1. Individual 14-membered macrocycles.

	R_1	R_2	R_{3a}	R_{3b}	R_4	MS-EI, (M+H)	HPLC Purity[a] (%)
7a	H	methyl	H	H	-	351	>95
7b	methyl (up)	hydroxymethyl	amino	H	-	396	>95
7c	methyl (up)	isobutyl	amino	H	-	422	>95
9d	3-(amidinoamino)propyl	carbamoylmethyl	H	H	2-pyrazinecarboxyl	569	84
9e	3-(amidinoamino)propyl	isobutyl	H	H	carbamoyl	505	85
9f	methyl	2-carboxyethyl	amino	H	isonipecotyl	519	82
9g	hydroxymethyl	benzyl	H	carboxy	isonipecotyl	582	92
9h	hydroxymethyl	isobutyl	amino	H	2-pyrazinecarboxyl	514	93
9i	4-hydroxybenzyl	benzyl	H	H	isonipecotyl	614	>95
9j	hydroxymethyl	isobutyl	amino	H	thymine-1-acetyl	574	78
9k	4-aminobutyl	carbamoylmethyl	H	H	2-pyrazinecarboxyl	541	77
9l	3-(amidinoamino)propyl	isobutyl	amino	H	2-pyrazinecarboxyl	583	89
9m	4-aminobutyl	isobutyl	amino	H	thymine-1-acetyl	615	91
9n	4-hydroxybenzyl	carbamoylmethyl	amino	H	thymine-1-acetyl	651	82
9o	methyl	3-(amidinoamino)propyl	amino	H	thymine-1-acetyl	601	75
9p	4-aminobutyl	benzyl	H	H	thymine-1-acetyl	634	93
9q	methyl	isobutyl	H	carboxy	isonipecotyl	532	79
9r	4-aminobutyl	2-carboxyethyl	H	H	2-pyrazinecarboxyl	556	73
9s	4-hydroxybenzyl	benzyl	H	carboxy	isonipecotyl	658	89
9t	methyl	isobutyl	amino	H	carbamoyl	435	>95
9u	4-aminobutyl	isobutyl	H	H	carbamoyl	477	76
9v	4-aminobutyl	hydroxymethyl	H	H	carbamoyl	451	93
9w	4-aminobutyl	hydroxymethyl	H	H	thymine-1-acetyl	574	80
9x	4-hydroxybenzyl	benzyl	H	carboxy	2-pyrazinecarboxyl	653	81
9y	hydroxymethyl	isobutyl	H	carboxy	isonipecotyl	548	72
9z	3-(amidinoamino)propyl	hydroxymethyl	amino	H	thymine-1-acetyl	617	91

[a] Reversed-phase HPLC employed an evaporative light-scattering detector (SEDEX). In some cases the macrocyclic diastereomers were not HPLC-resolved and purities reflect the sum of two peaks.

In summary we have developed a versatile route to high purity 14-membered macrocycles starting from resin-bound amino alcohols. We are currently applying this synthesis scheme to a mixture-based combinatorial library of > 10,000 macrocycles.

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12. Syntheses were carried out on a 100 mmol scale. **7b:** ^{13}C NMR (100 MHz, dmso-d_6) 169.74, 166.92, 164.00, 160.93, 140.96, 128.32, 126.90, 122.35, 114.83, 70.74, 69.78, 60.56, 58.29, 52.64, 40.13, 16.66; ^1H NMR (400 MHz, dmso-d_6) 8.78 (d, 1 H, *J*=6.0 Hz), 8.70 (s, 1 H), 8.30-8.39 (m, 3 H), 8.18 (br s, 2 H), 7.82 (d, 1 H, *J*=6.0 Hz), 7.53 (d, 1 H, *J*=9.6 Hz), 5.10 (br s, 1H), 4.33-4.40 (m, 3 H), 4.01-4.03 (m, 1 H), 4.08-4.13 (m, 2 H), 3.95-3.98 (m, 2 H), 3.64-3.66 (m, 2 H), 1.18 (d, 3 H, *J*=7 Hz). **7c:** ^{13}C NMR (100 MHz, dmso-d_6) 171.83, 166.64, 163.79, 160.99, 140.94, 128.34, 126.97, 122.22, 114.74, 70.71, 69.77, 53.65, 52.42, 44.47, 24.11, 22.72, 21.45, 16.42; ^1H NMR (400 MHz, dmso-d_6) 8.71 (s, 1 H), 8.52 (d, 1 H, *J*=6.0 Hz), 8.38 (d, 1H, *J*=9.2 Hz), 8.23-8.31 (m, 3 H), 7.86 (d, 1H, *J*=6 Hz), 7.53 (d, 1 H, *J*=9.6 Hz), 4.40-4.45 (m, 1H), 4.35-4.31 (m, 1H), 4.09-4.12 (m, 2H), 3.98 (m, 1 H), 3.90-3.94 (m, 2 H), 1.43-1.67 (m, 3 H), 1.18 (d, *J*=6.8 Hz), 8.75 (dd, 6 H).