# Organic & Biomolecular Chemistry

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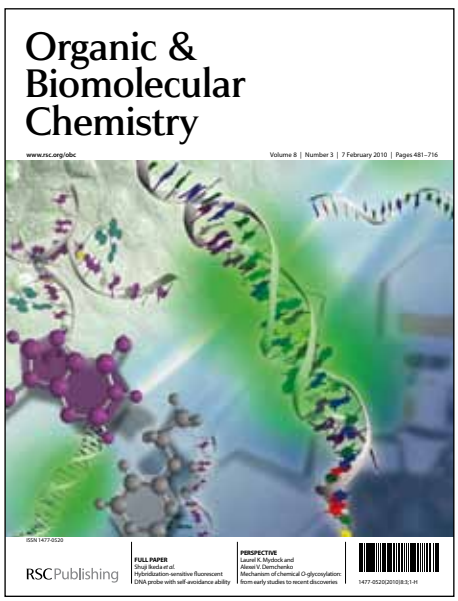


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# Synthesis of Libraries of Peptidomimetic Compounds Containing A 2-Oxopiperazine Unit In The Main Chain

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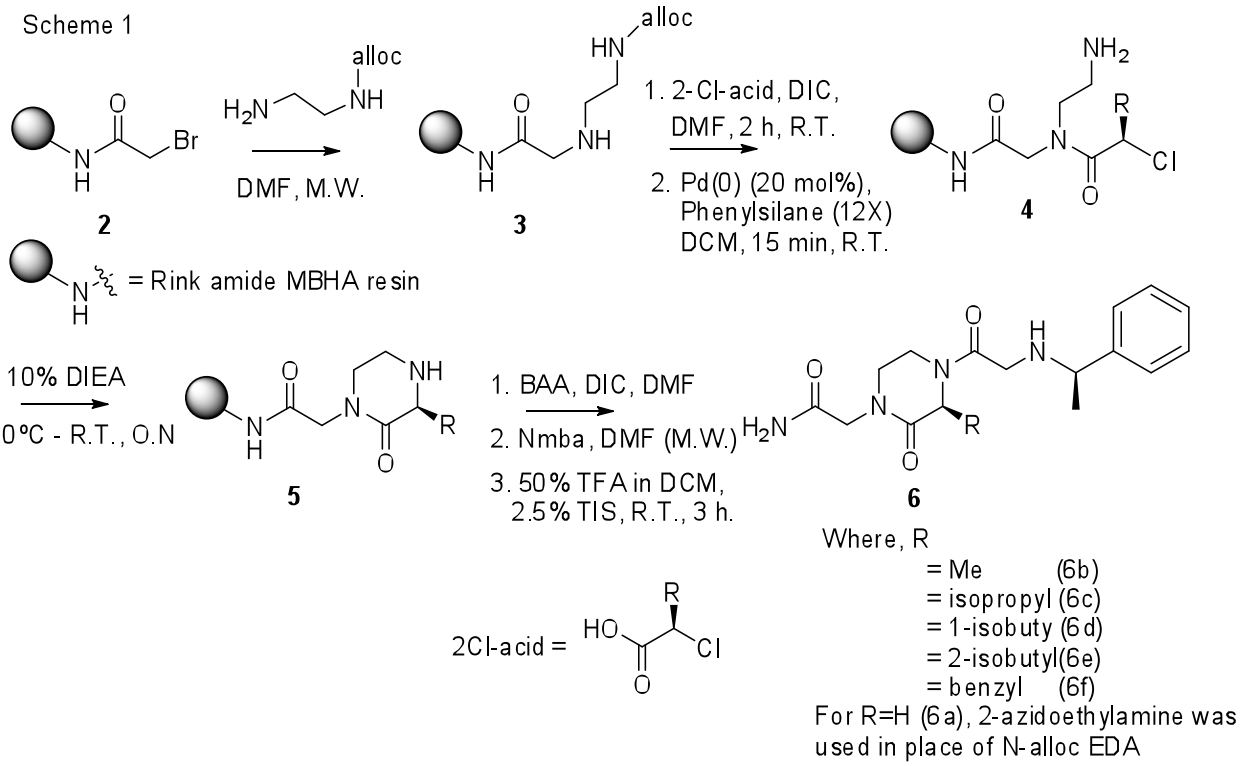
**Abstract**

**Peptoid libraries have been shown to be a useful source of protein-binding agents. However, simple linear peptoids lack conformational constraints, which may limit their binding affinity for proteins. Here we report facile chemistry for the assembly of 2-oxopiperazine rings into the main chain of peptoid-like oligomers, thus rigidifying the structure. This modified sub-monomer synthesis is suitable for the creation of high quality combinatorial libraries.**

Peptoids[1] (oligomers of N-substituted glycine) have several advantages over peptides and many other types of peptide-like oligomers as a potential source of bioactive compounds. Peptoids are more cell permeable than peptides [2, 3] and also are insensitive to proteases and peptidases[4]. Most importantly, large libraries of peptoids can be created easily using the solid-phase "sub-monomer" chemistry developed by Zuckermann and co-workers[5, 6] and the split and pool strategy[7], whereas most other types of oligomer libraries require far greater synthetic effort. The sub-monomer protocol involves two steps: acylation of an amine with 2-bromoacetic acid followed by displacement of the bromide with a primary amine. The large number of amines that are commercially available or synthesized readily allow libraries of tremendous diversity to be created rapidly without the need for synthesizing and maintaining extensive stocks of expensive precursors [8-10]. Several studies have shown that peptoid libraries can be mined to produce useful bioactive compounds[11-17]. However, with rare exceptions[11], primary screening hits that arise from peptoid libraries have not exhibited high affinity or potency. This may be due, in part, to the fact that common peptoids do not adopt well-defined conformations. Indeed, unlike peptides, both the *cis* and *trans* isomers of the amide bond are populated and there is little or conformational preference for the other two types of bonds in the molecule. Various strategies have been reported to address this limitation and create more conformationally constrained peptoids or peptoid analogues.[18-20] However, until recently[21], none of these solutions was based on chemistry that was efficient enough to support the creation of high quality combinatorial libraries. Recently, we have addressed this problem and have demonstrated the synthesis of libraries of peptoid-like oligomers with either main chain[22, 23] or side chain[24, 25] sub-monomer units that impose significant conformational restrictions. In this paper, we introduce another strategy for the creation of conformationally-restricted main chains via the insertion of 2-oxopiperazine units into the oligomer (Scheme 1). We demonstrate that this chemistry is efficient enough for the creation of high quality combinatorial libraries by solid-phase split and pool synthesis.

The synthesis of 2-oxopiperazine-containing peptoids was reported previously by workers at Chiron[26, 27]. However, the route employed resulted in a mixture of stereoisomers and did not allow facile extension of the oligomer following formation of the 2-oxopiperazine ring. Balasubramanian and colleagues published a diastereoselective synthesis that employed a chiral aldehyde in the key step [28] and Golebiowski, et al. developed a solid-pahse synthesis of 2-oxopiperazine-containing β-turn mimetics[29]. But neither scheme was adapted for embedding the molecules into oligomers. Our proposed approach (Scheme 1) involves addition of mono-protected 1,2-diaminoethane to the end of a growing peptoid chain. Another 2-halo acid is then added to the unprotected nitrogen, followed by deprotection and ring closure to create the 2-oxopiperazine unit. The oligomer chain can then be extended by acylation of the secondary amine in the ring (Scheme 1).

Scheme 1




**2** **3** **4** **5** **6**

To test this strategy, diisopropyl carbodiimide (DIC)-activated bromoacetic acid (BAA) was coupled to Rink amide MBHA resin (Scheme 1). The halide **2** was treated with mono-N-alloc-protected 1,2-diaminoethane and the resultant secondary amine **3** was coupled with DIC-activated 2-chloropropionic acid to obtain compound **4**. The alloc group was

then removed using palladium tetrakis triphenylphosphine in the presence of phenylsilane as a scavenger to afford the primary amine. Cyclization was effected under basic conditions (10% N, N' diisopropylethylamine, DIEA) to afford the 2-oxopiperazine ring **5**. Chain extension from the secondary amine in **5** was carried out by coupling with 2-bromo-acetic acid, followed by displacement of bromide with R-(+)-methyl benzyl amine (Nmba) to afford **6**, which was authenticated by MALDI-TOF mass spectrometry (MS). NMR and HPLC analysis of the product indicated high purity (see supplemental material).

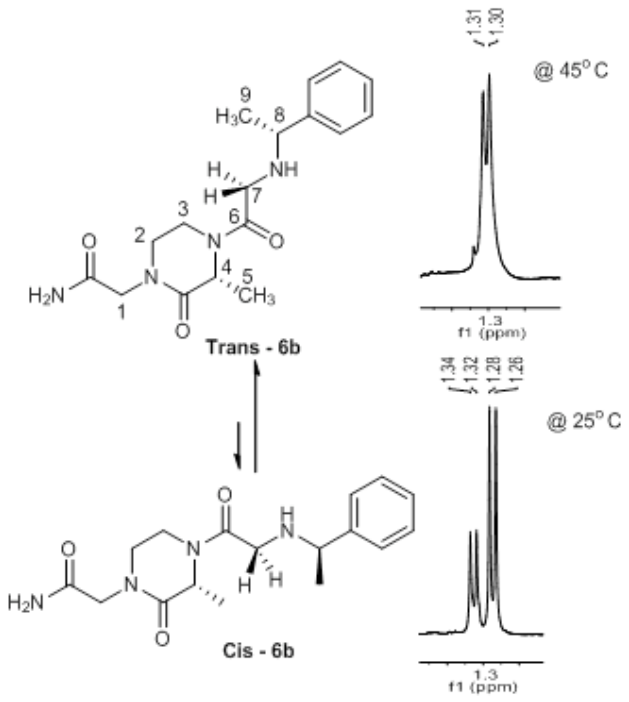



**Figure 1**: Left: Structure of the compound **6b**, showing cis and trans-amide bond geometries. Right: Portion of the $^{1}$H-NMR spectrum of **6b** taken at 25°C and 45°C, highlighting the resonance corresponding to the C5 methyl group.

Obviously, the 2-oxopiperazine ring in **6** enforces a relatively rigid main chain conformation for this part of the molecule. However, it was not clear if the amide bond on the N-terminal side of the ring would adopt either predominantly the cis or trans conformation due to the different substitution on C3 and C4 (Figure 1). To probe this point, NMR analysis was

carried out. Figure 1 displays part of the NMR spectrum of compound **6b**, highlighting the resonances of the C5 methyl group. Two doublets at δ 1.2 and 1.3 likely represent the signals from the cis and trans amide isomers of **6b**, which convert slowly on the NMR time scale at room temperature. This was confirmed by heating the sample to 45°C, which resulted in the merging of the signals. It is difficult to determine which isomer is predominant, but the approximately 2:1 ratio of the doublets in the spectrum show that both are well populated. The fact that only one doublet for the C5 methyl is observed at 45°C demonstrates that only a single enantiomer of compound **6b** is present and that the steps employed to create the 2-oxopiperazine ring proceeded without racemization (Supplemental information S2, S2.1).



Chiral 2-halocarboxylic acids can be made from α-amino acids in a single step with high stereochemical purity[30], so it should be possible to incorporate a number of different substituents at the chiral center of the 2-oxopiperazine. To test the scope of this process, five additional compounds (Scheme 1, **6a-f**) were synthesized in which the substituent at the chiral center in the 2-oxopiperazine ring was varied. The products were subsequently authenticated by MALDI-TOF and their purity tested by HPLC. The purity of the compounds ranged from 85-98% (supporting information Table T1 and Supplementary Figures **S11-S15**).

Therefore, we explored the use of this chemistry in the construction of a combinatorial library. A general structure of the one-bead-one-compound (OBOC) library is shown in Figure 2a. Following an invariant linker shown in red, a tetrameric 2-oxopiperazine-containing peptoid library containing 20,000 compounds was created using 15 different primary amines (linear or α-branched) and five 2-chloro acids. Since the library was created on tentagel-MB-$NH_2$, N-tboc protected ethylenediamine (EDA) was used rather than the N-alloc-protected EDA that was used with Rink amide resin (Scheme 1). The quality of the library was tested by picking 20 beads at random from the library. The compounds were released from the beads by cleavage at methionine in the invariant linker with CNBr. 80% of the compounds were sequenced successfully by tandem MALDI mass spectrometry (supplemental information, S30-S46), a

result comparable to that seen with standard peptoid libraries[31]. This demonstrates high quality combinatorial libraries with a 2-oxopiperazine unit inserted into a peptoid chain can be created.

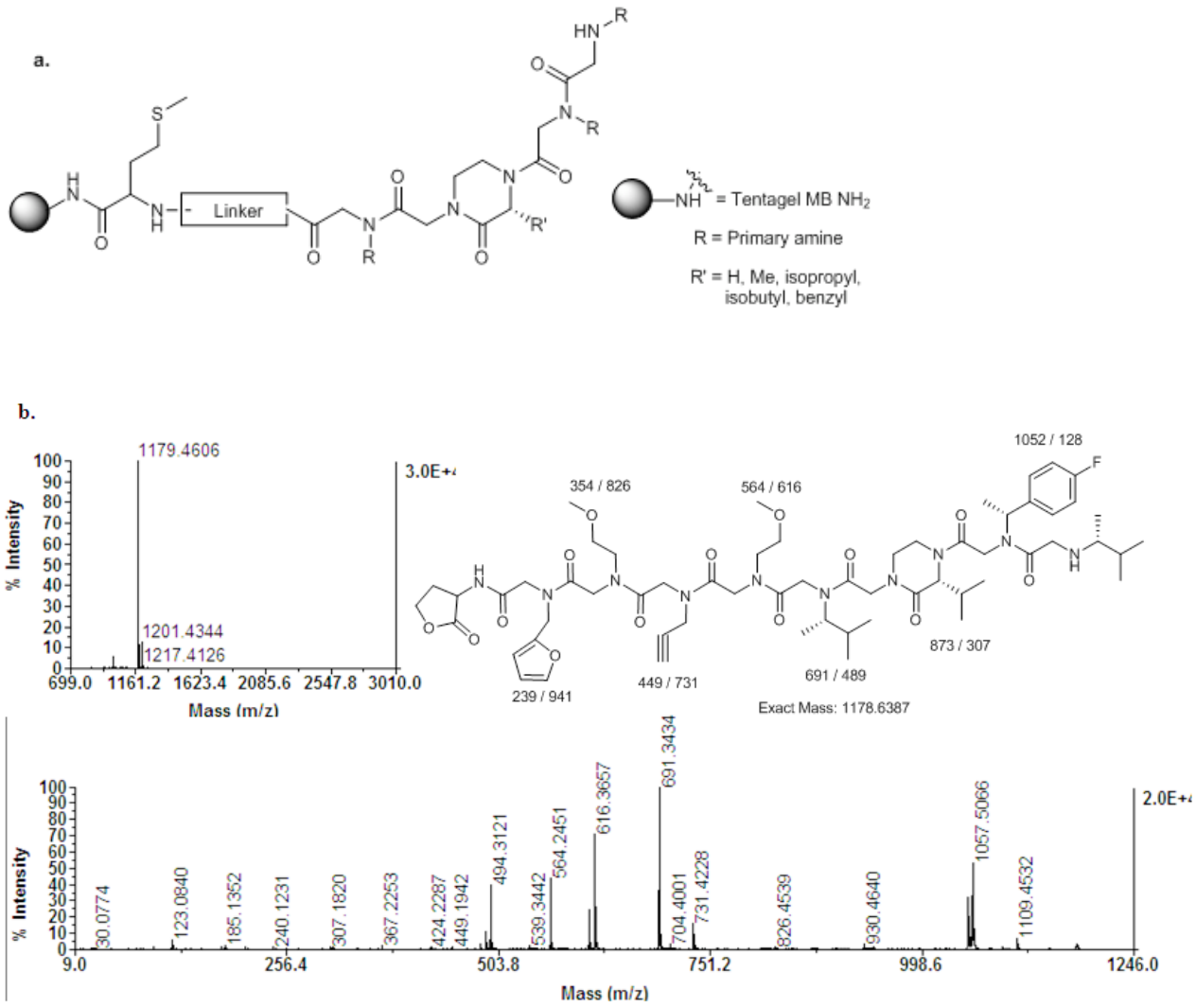



**Figure 2:** (a) General structure of a 2-oxopiperazine ring-containing peptoid library. (b) Top-left: MS of a peptoid ([$MH^+$]=1179.4) obtained after CNBr mediated single bead cleavage; Top-right: Structure of the peptoid identified after MALDI-TOF based sequencing; Bottom: MS/MS of a peptoid obtained from MALDI-TOF with the precursor mass 1179.4.

To study the scope of chain extension with respect to the unit N-terminal to the oxopiperazine ring, the addition of L-amino acids was attempted. We presumed that this would be a relatively difficult coupling given the crowding around the nitrogen, but worth assessing since it might bias the amide bond more towards the trans geometry. Thus, a series of compounds were made in which variously substituted 2-oxopiperazines were coupled with fmoc-protected amino acids, which were then acylated with activated chiral 2-halo acids followed by halide displacement with an amine

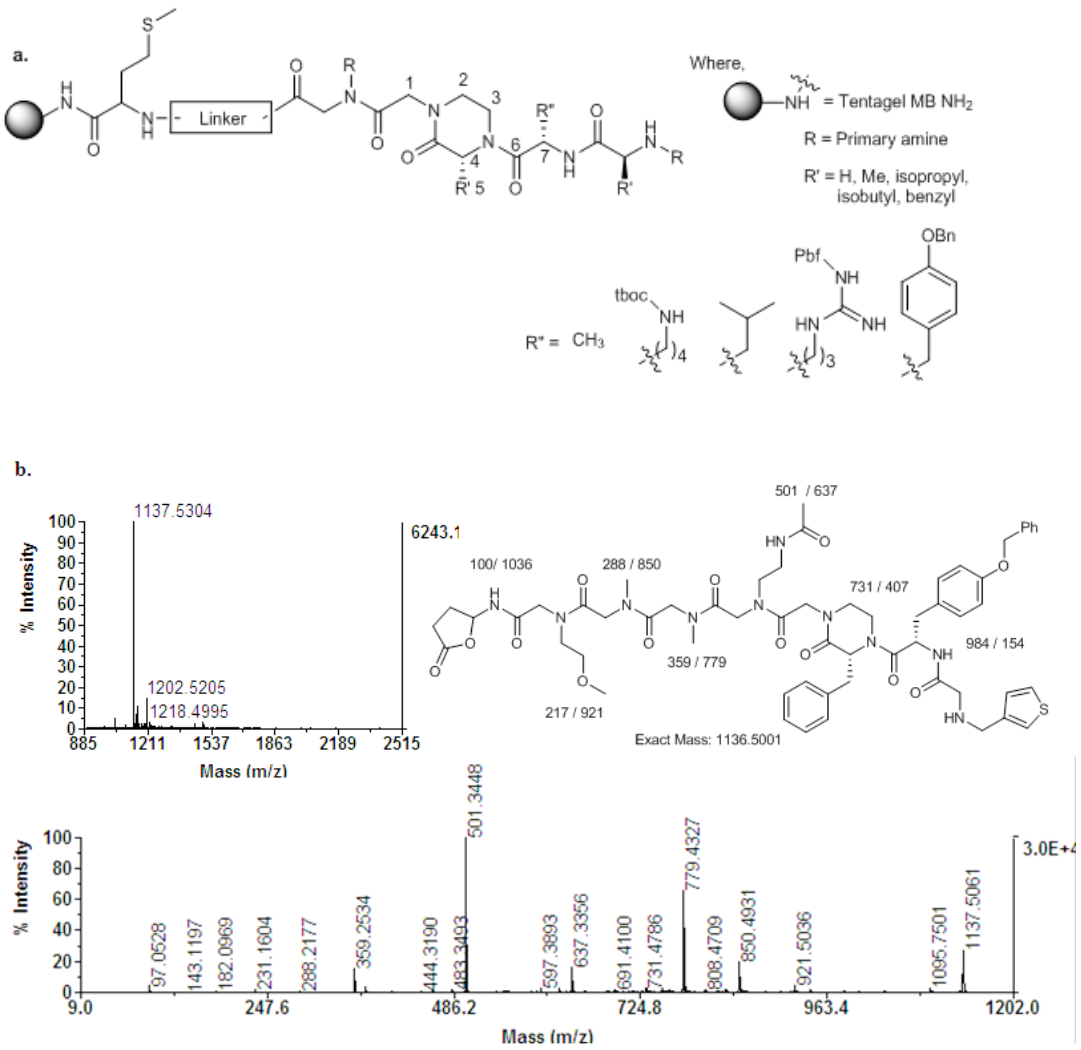



**Figure 3:** (a) General structure of a 2-oxopiperazine-containing hybrid library in which the ring is followed by an amino acid unit and then capped with a peptide tertiary amide. (b) Top-left: MS of a peptoid ([MH$^+$]=1137.5) obtained after CNBr mediated single bead cleavage; Top-right: Structure of the peptoid identified after MALDI-TOF based sequencing; Bottom: MS/MS of a peptoid obtained from MALDI-TOF with the precursor mass 1137.5.

(see Supplementary Scheme Sch1). Six different DIC/HOAt-activated Fmoc-amino acids were coupled to a 2-oxopiperazine moiety containing methyl and isobutyl substituents. The Fmoc group was removed using 20% piperidine in DMF and the resulting primary amine was coupled to 2-bromopropionic acid (2-chloro-4-methyl-pentanoic acid was also used successfully; see supplemental information, Scheme Sch1). The halides were then displaced by a primary amine. The compounds were cleaved from the Rink amide MBHA resin and analyzed by tandem MS and HPLC (Supplementary Figures S15-S29). The purity of the final products ranged from 80-91% (supplemental information, Table T2).

Given this encouraging result, we proceeded to construct a combinatorial library of compounds in which the 2-oxopiperazine unit is followed by an amino acid. After removal of Fmoc, a chiral $\alpha$-halo acid was then coupled to the amino acid and the bromide was then displaced with an amine. This library (Figure 3 and supplemental information Figure L2) was synthesized on tentagel resin utilizing 12 primary amines, 5 amino acids and 5 different types of α-halo acids to afford a library of approximately 18,000 compounds. The Fmoc-amino acids were coupled to the secondary amine of the 2-oxopiperazine ring using HOAt/DIC at elevated temperature (50°C). The quality of the tetramer library was verified by CNBr-mediated cleavage of 20 beads randomly selected from the pool. 80% of the compounds were identified by MALDI-MS/MS (supplemental information, S47-64), confirming the utility of this approach in generating the pure 2-oxopiperazine containing OBOC peptide tertiary amide library.

**Conclusions**

In summary, we have identified an effective solid-phase synthetic route to incorporate 2-oxopiperazine moieties into peptoids using α-haloacids and protected 1,2-diaminoethane. The ring can be substituted with a variety of side chains derived from chiral 2-haloacids that are themselves derived from $\alpha$-amino acids. When the following residue is a peptoid unit, both the cis- and trans-amide bond geometries on the N-terminal side of the ring are observed. Work focused on mining libraries of these compounds for protein ligands is underway.

**Acknowledgements**

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