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Solid-phase synthesis of peptoid-like oligomers containing diverse diketopiperazine units†

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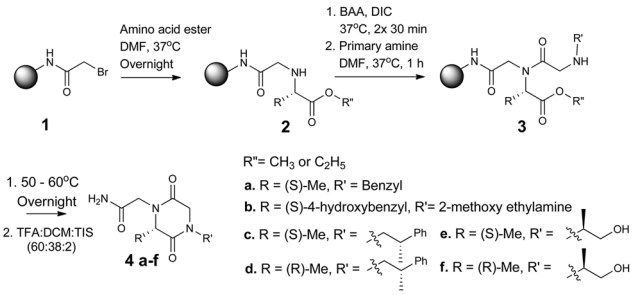
Diketopiperazine (DKP) units are found in many bioactive small molecules. Here we report facile chemistry for incorporating diverse DKP units within peptoid and peptoid-like libraries made by solid-phase split and pool synthesis.

Large combinatorial libraries of peptides, peptoids and other synthetic oligomers are a valuable source of protein ligands. There is continued interest in increasing the chemical diversity of the building blocks that can be included in such oligomer libraries by solid-phase synthesis. We have extended the utility of the "sub-monomer" synthesis of peptoids[1] by developing a variety of different units that can be used in this general process, which involves addition of the activated ester of bromoacetate to an amine followed by displacement of the bromide by a primary amine. In particular, we have been focused on building blocks that incorporate significant conformational constraints into the molecule with the idea that "stiffer" molecules will bind to proteins with higher affinity. For example, substitution of 2-bromoacetate with more complex γ-halo acids has allowed the incorporation of heterocycles,[2] chiral centers and unsaturation[3,4] into the oligomer main chain. We also demonstrated that 2-oxopiperazines can be assembled on-resin through a multi-step processes,[5] all of which proceed efficiently enough to be compatible with the creation of high-quality combinatorial libraries. Here we report another example of this type of multi-step on-resin construction of a cyclic unit, specifically a diketopiperazine (DKP),[6–8] a structural feature found in many bioactive molecules.[9]

The synthetic scheme that we imagined could be employed for the solid-phase synthesis of diverse DKPs is shown in Scheme 1. Addition of the activated ester of bromoacetic acid to a resin-bound amine would be followed by displacement of the bromide with an α-amino acid ester. Another round of peptoid synthesis on the N-terminal nitrogen would provide linear intermediate **3**, which we postulated should cyclize in high yield under basic conditions. A variety of substituents could be readily attached to the DKP through the use of diverse amino acid and amine building blocks.



Scheme 1

To test this scheme, compounds **4a–f** were synthesized using the conditions shown in Scheme 1 on Rink amide MBHA resin. The compounds were released from the resin and analyzed by NMR. DKPs **4a** and **4b** were formed cleanly with no significant side products or starting material apparent (ESI Fig. S1–S10†). To address possible racemization of the chiral center in the DKP, compounds **4c** and **4d** were synthesized, which differ in the absolute stereochemistry at this position and also have a chiral center external to the ring that renders the two compounds diastereomeric. Analysis of **4c** and **4d** showed that both were stereochemically pure (ESI Fig. S11–S15†). To further test the scope and limitation of our strategy, DKPs **4e** and **4f** were prepared, which have a chiral, branched center adjacent to the nitrogen undergoing cyclization. This might be expected to slow ring closure, possibly leading to some racemization in the ring. While the proton NMR of crude **4e** showed small amounts of other products (~10%) these were not the diastereomer **4f**, as evidenced by comparing their spectra (ESI Fig. S20†). Thus, even in this case of a difficult cyclization, the DKP-forming reaction proceeds with excellent stereochemical purity.

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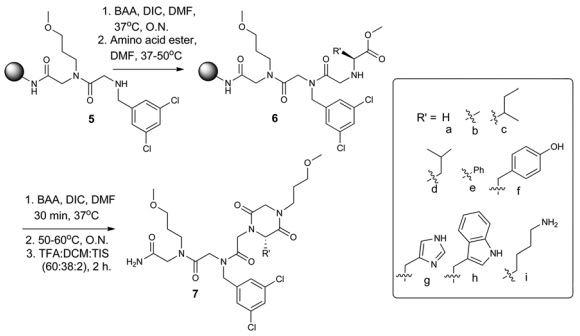

Scheme 2

To further test the scope of this process, DKPs **7a–i**, derived from nine different amino acid esters, were synthesized on-resin (Scheme 2). In each case, the DKP ring was assembled onto compound **5**. To establish rigorously the efficiency of the DKP-forming steps, further chain elongation was attempted, which should not proceed if the ring-forming reaction incorporates the nucleophilic nitrogen into an amide linkage. In the event, after cleavage of the compounds from the resin, HPLC analysis indicated that the expected DKP products were formed with purities ranging from 80–98%. No significant material from unwanted chain extension was observed by mass spectrometry in any of the nine cases (ESI Fig. S26–S34†).

The chemistry discussed above results in a DKP unit that cannot be extended further and thus represents a terminating modification for the synthesis of oligomers. We also examined the use of mono-protected diamines in this scheme since, after deprotection, this would afford the opportunity to further extend the molecule and allow the DKP unit to be flanked by diverse elements. As is shown the ESI,† this worked well. We synthesized two different compounds on Rink amide resin where the protected diamine was either incorporated as a sub-monomer prior to DKP formation or used as a part of the DKP ring. In each case, the alloc protecting group was removed after DKP formation and the chain was extended from this nitrogen, providing oligomers in which the DKP was an internal element in excellent yield and purity (see ESI S35–S38†) (Fig. 1).

Having validated the efficiency of the DKP-forming chemistry in the context of single compounds, we proceeded to construct a combinatorial library with the general structure shown in Fig. 2 using the split and pool strategy.[10,11] Following an invariant linker and one diversified peptoid unit, DKPs derived from nine different amino acid esters were incorporated into the second position of the main chain. A *N*-*t*Boc mono-protected ethylene diamine was employed as the primary amine in the formation of the DKP ring, allowing the addition of two additional residues following the DKPs. Both were constructed using the peptoid sub-monomer route. In the first of these positions, both (*S*)-2-bromopropionic acid and bromoacetic acid were employed as sub-monomers. In the last position, only bromoacetic acid was employed. 12 amines were

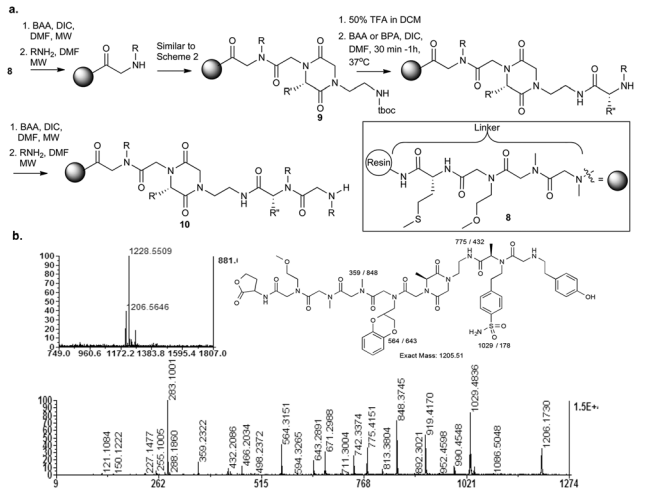

Fig. 1 Synthesis of a library containing a DKP unit inserted into the main chain. (a) General synthetic scheme for the construction of a combinatorial library containing internal DKP residues. The library was constructed on 150 μm TentaGel MB-NH2 resin. (b) Top: MALDI mass spectrum of a crude library member (from a single bead) after release form the bead with CNBr ([MH$^+$] = 1206.5) obtained after CNBr mediated single bead cleavage. Top-right: Structure of the peptoid identified after MALDI-TOF based sequencing. Bottom: MALDI-TOF MS/MS of the 1206.5 peak.

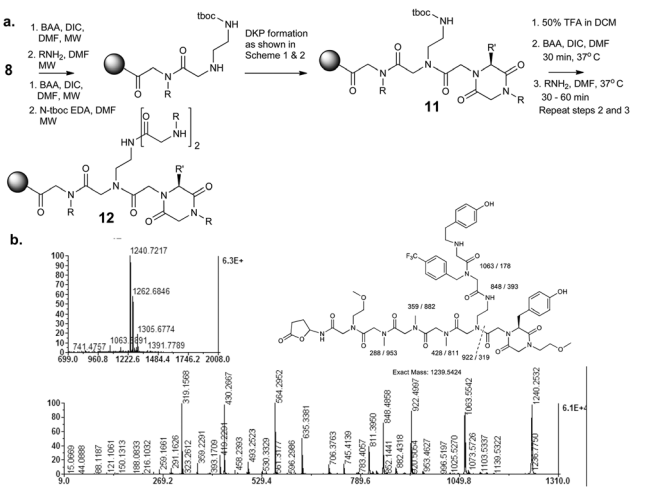

Fig. 2 Synthesis of a library of peptoids containing DKP side chains. (a) General synthetic scheme of DKP-SC peptoid library. (b) Top-left: MS of a peptoid ([MH$^+$] = 1240.7) obtained after CNBr mediated single bead cleavage. Top-right: Structure of the peptoid identified after MALDI-TOF based sequencing. Bottom: MS/MS of a peptoid obtained from MALDI-TOF with the precursor mass 1240.5.

used to construct the peptoid units, resulting in a library containing approximately 27 000 compounds. To test the quality of the library, 20 beads were chosen randomly from the population and cleaved from the resin at the invariant methionine using CNBr. In each case, a single, strong molecular ion was observed in the MALDI mass spectrum and the molecules could be sequenced unambiguously by tandem MALDI MS/MS, a result comparable to that obtained for most standard peptoid

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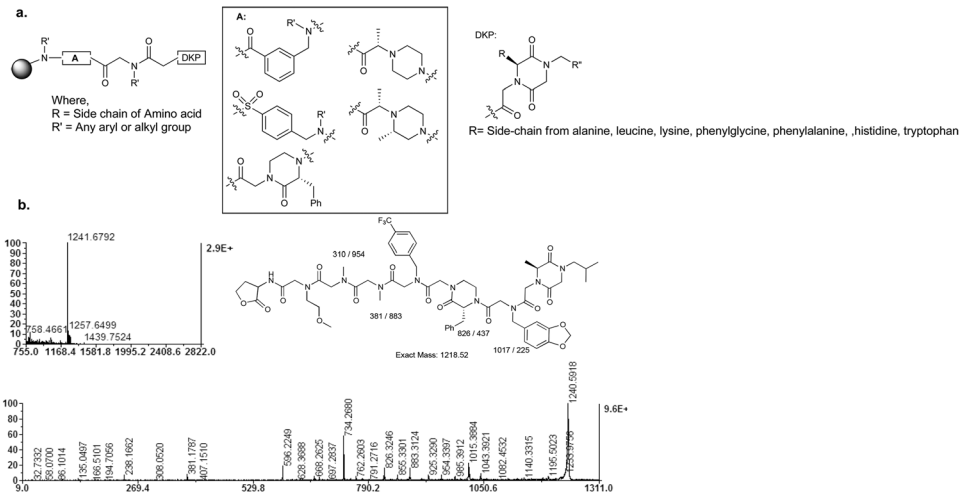

Fig. 3 Synthesis of a complex DKP-terminated library. (a) Left: General structure of the library. Middle: Structures of the five different main chain diversity elements used at second position of the library. Right: DKP units employed as a capping group in the library. (b) Top-left: MS of **11** ([MNa^+] = 1241.6) obtained after CNBr-mediated cleavage from a single bead picked from the library. Top-right: Structure of the peptoid identified after MALDI-TOF based sequencing. Bottom: MS/MS of **11** (from fragmentation of 1241.6 ion).

libraries (ESI S42–S57†). This demonstrates that high quality combinatorial libraries can be created with DKPs inserted into the main chain.

Another library was created in which diverse DKPs were included as a side chain in an otherwise standard peptoid library. To do this, a mono-*t*Boc-protected ethylene diamine was employed as the invariant sub-monomer at the second position in the library. After construction of the diverse DKP side chains using five amino acid esters and 12 amines, the other nitrogen of the ethylene diamine-derived unit was deprotected and an additional two peptoid units were added. This resulted in a library of approximately 100 000 compounds (Fig. 3). Again, the high quality of the library was established by MALDI MS and MS/MS analysis of the beads chosen randomly (ESI S58–S68†). This demonstrates that this chemistry is compatible with generating a high quality of library with DKP unit at a variety positions within the compounds.

With these encouraging results from the syntheses of relatively simple libraries, we constructed the more complex library shown in Fig. 3. The goal here was to begin to move towards more diverse main chain chemistry, then cap this library was diverse DKPs. A tetrameric hybrid library of approximately 135 000 compounds was created on Tentagel-MB-NH_2 resin. A detailed protocol for the incorporation of the various units at second position of the chain (A in Fig. 3) is provided in the ESI.† Briefly, for the piperazine units, after coupling 2-bromopropionic acid to the first peptoid residue, the bromide was either displaced with mono-*N*-*t*Boc-piperazine or mono-*N*-*t*-Boc-(*S*)-2-methyl piperazine, separately (see ESI†). The *t*-Boc group was deprotected before further chain extension with bromoacetic acid/DIC. (*S*)-3-Benzyl-2-oxopiperazine was introduced through *t*Boc-chemistry as reported previously.[5] 4-Bromomethyl benzenesulfonyl chloride or 3-chloromethyl benzoyl chloride were coupled with the nitrogen of the first peptoid residue under basic conditions. After coupling of the benzoyl and sulfonyl groups the beads were split and the halides were displaced with 12 different amines.

In total, 27 different structures were included in the library at this position (A in Fig. 3). Following a diversified unit **A**, conventional peptoid units were introduced at the third position of the library. Finally, 56 different 2,5-DKPs were incorporated into the terminal position by using seven different amino acid esters and eight primary amines. In the design of this library, peptoid units were placed between the **A** units and the terminating DKP in order to simplify interpretation of the MS/MS spectrum since peptoids fragment quite efficiently. The quality of the tetrameric complex peptoid library was confirmed by CNBr-mediated cleavage at methionine in the invariant linker region of 20 beads randomly selected from the pool of the library. More than 80% of the compounds were identified by MALDI-MS/MS (ESI S69–S84†). Further, HPLC analysis of several molecules synthesized on Rink amide resin with a variety of monomers at the second position indicated purities of 80–99%. (ESI S39–S41†). We conclude that this chemistry is efficient enough to create highly complex libraries of high quality.

Conclusions

In summary, we have demonstrated a facile approach for the on-resin synthesis of creating a highly substituted, enantiomerically pure 2,5-DKP units. High quality combinatorial libraries containing this unit can be created using the split and pool strategy. Depending on the precise nature of the reagents employed, the DKP can be incorporated as a terminating group, a side chain element, or inserted into the main chain of the oligomer and flanked by other units. We

anticipate that these libraries will serve as a useful source of bioactive compounds.

Acknowledgements

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