Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2273–2278

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

COMBINATORIAL CHEMISTRY: FROM PEPTIDES AND PEPTIDOMIMETICS TO SMALL ORGANIC AND HETEROCYCLIC COMPOUNDS

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Received 1 April 1998; accepted 1 June 1998

Abstract: Modified dipeptides have been used successfully for the generation of a variety of small organic and heterocyclic combinatorial libraries, including linear urea, polyamine, hydantoin, thiohydantoin, cyclic urea, cyclic thiourea and bicyclic guanidine. The synthesis and screening results for a number of these libraries are described. The solid phase synthesis of heterocyclic compounds such as diazepine and thiomorpholinone are also described.
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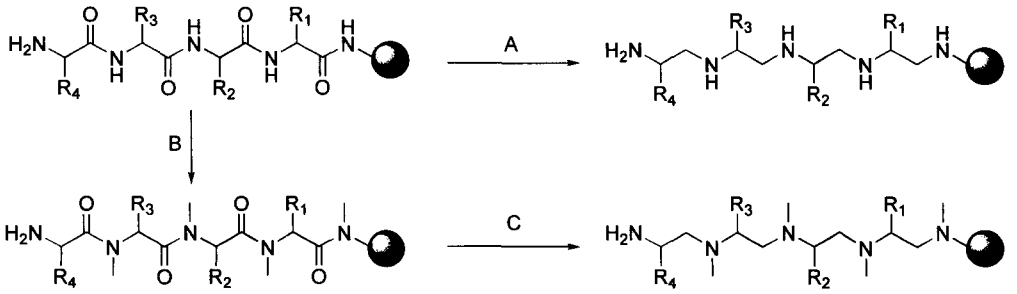
The concept of combinatorial chemistry is based on Merrifield's solid-phase methods for the synthesis of peptides.[1] Large scale solid-phase combinatorial parallel peptide synthesis was first carried out on pins[2] and standard resin packets[3] in 1984 and 1985, respectively, and on glass surfaces in 1990.[4] Early work from this laboratory has shown the broad utility of mixture-based synthetic combinatorial libraries (SCLs) composed of peptides[5,6] for the identification of potent analgesics,[7] highly active antimicrobials,[8] enzyme inhibitors,[9] highly specific antigenic determinants,[10] and inhibitors of melittin's hemolytic activity.[11] In addition to linear peptide sequences, this laboratory has synthesized a cyclic peptide template SCL and identified highly active chymotrypsin inhibitors.[12]

Since peptides often have limitations as pharmaceuticals due to their poor bioavailability and rapid enzymatic degradation, we have developed an efficient method for the generation of peptidomimetic libraries by chemical transformation of existing peptide libraries. As shown in Scheme 1, the peralkylation and/or the reduction of the amide bonds can generate completely different classes of compounds: peralkylated amide peptidomimetics and various polyamine compounds.[13,16] As an example, we have reported a soluble peptidomimetic SCL made up of 57,000 compounds having a dipeptide scaffold, with each amide hydrogen replaced with different alkyl groups.[13]



Scheme 1. Generation of peptidomimetic libraries through chemical modification of existing peptide library: (A) reduction of the amide carbonyls; (B) peralkylation of the amide nitrogens; (C) reduction of peralkylated amide nitrgens.

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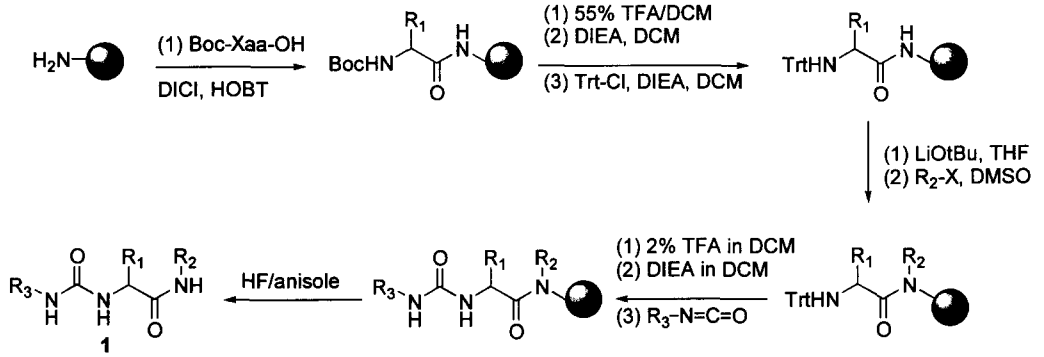
PII: S0960-894X(98)00412-0

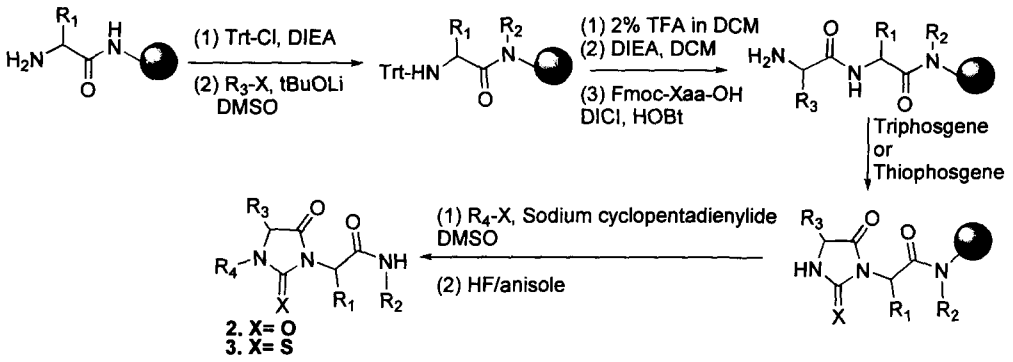
In recent years, our combinatorial chemistry focus has broadened to include libraries of small acyclic and heterocyclic compounds. We report here part of our ongoing efforts toward the synthesis of SCLs of small organic and heterocyclic compounds using the "libraries from libraries" approach.[16]

A linear urea library **1** has been prepared. The reaction of a resin-bound amino acid with an individual preformed isocyanate affords the linear urea in good yield. As shown in Scheme 2, an isocyanate is generated by slowly adding a substituted amine to a solution of triphosgene in anhydrous DCM in the presence of diisopropylethylamine (DIEA). The condensation of the resulting isocyanate with resin-bound amino acid amides generates linear ureas. In order to increase the number and range of compounds, selective N-alkylation was performed on the resin-bound amino acid amide. Following the individual synthesis of controls, a mixture-based combinatorial library of 125,000 linear *N,N'*-disubstituted ureas was prepared. This SCL has been tested for opioid activity at the mu, delta, kappa, and sigma opioid receptors (manuscript in preparation). Following the deconvolution of this library, individual compounds having IC_{50} values of 1 to 5 nM for the sigma receptor have been found.



Scheme 2. Solid-phase synthesis of linear urea library **1**.

Substituted heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents.[17,19] For these reasons, a great deal of effort has been directed over the past two to three years towards the synthesis these types of compounds on the solid phase. We have focused on the synthesis and design of heterocyclic compounds using peptides and peptidomimetics as starting materials.[20,21] For example, we have developed a simple synthetic route to the solid-phase synthesis of individual hydantoin and thiohydantoin compounds and libraries from resin-bound dipeptides. This synthetic approach entailed the reaction of the free N-terminal amino group of resin-bound dipeptides with phosgene or thiophosgene. This led to the intermediate isocyanate or thioisocyanate that further reacted intramolecularly with the peptide to form the five-member ring hydantoin or thiohydantoin. In order to increase the number and the class of available compounds, we have selectively alkylated the resin-bound amide, followed by the remaining reactive nitrogen, to generate a dialkylated hydantoin library (Scheme 3).



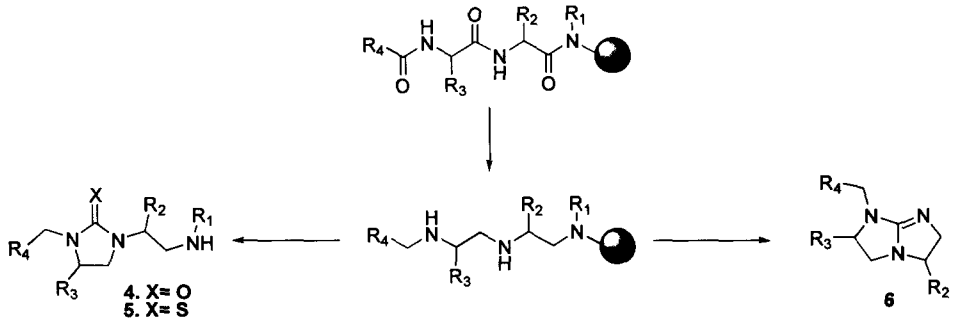
Scheme 3. Solid-phase synthesis of dialkylated hydantoin **2** and thiohydantoin **3** libraries.

Using 54 different amino acids for the first site of diversity (R_1), 60 different amino acids for the second site of diversity (R_2), and four different alkyl groups, a library of 38 880 compounds (54 × 60 × 3 × 4) has been prepared. This library was examined in a sigma opioid radioreceptor binding assay (radioligand = [^{3}H] pentazocine). Initial screening of the mixtures revealed the importance of basic or N-benzylated hydrophobic amino acids in the R_1 position (IC_{50} values in the 1 to 10 μM range) and N-benzylated basic amino acids in the R_2 position (IC_{50} vaules in the 100–500 nM range). From these results, 12 individual hydantoins were synthesized and tested. The resulting individual hydantoins showed significant improvement in their binding affinities compared to the mixtures, with two of the individual di-alkylated hydantoins having IC_{50} values close to 60 nM (Table 1).

R_1	L-Ile	L-Nva	L-Nva	L-Ile	D-Cha	D-Cha	L-Ile	D-Cha	L-Nva	L-Nva	D-Cha	L-Ile
R_2	Bn	Bn	Bn	Bn	H	H	Bn	H	Bn	Bn	H	Bn
R_3	L-Lys	D-Lys	L-Lys	D-Lys	L-Lys	D-Lys	L-Orn	L-Orn	L-Orn	7-Aha	7-Aha	7-Aha
R_4	Bn	Bn	Bn	Bn	Bn	Bn	Bn	Bn	Bn	Bn	Bn	Bn
IC_{50} (nM)	62	64	80	125	189	254	333	406	435	2417	3630	4615

Table 1. IC_{50} values for the individual di-alkylated hydantoins against [^{3}H] pentazocine (sigma opioid receptor).

As described above, the "libraries from libraries" approach has been successfully used for the generation of peralkylated peptide and polyamine SCLs derived from existing peptide SCLs.[13,16] This approach has also recently been applied for the generation of cyclic urea **4**, thiourea **5** and bicyclic guanidine **6** libraries (Scheme 4).

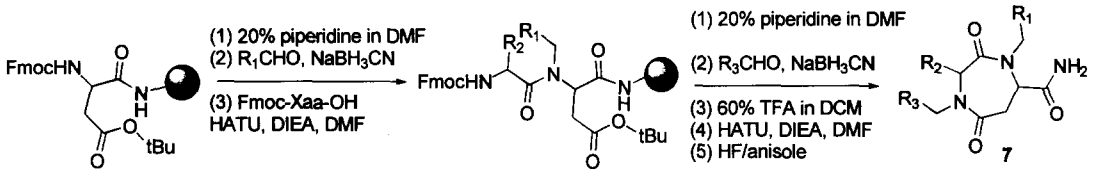


Scheme 4. Solid-phase synthesis of cyclic urea, cyclic thiourea, and bicyclic guanidine libraries.

Modified dipeptide SCLs having four positions of diversity have been selected as starting materials for the solid-phase synthesis of cyclic urea and thiourea libraries.[20] The complete reduction of the amide carbonyls in an N-alkylated dipeptide SCL with diborane yielded a triamine SCL having two secondary amine groups. The treatment of this triamine SCL with carbonyldiimidazole or thiocarbonyldiimidazole affords the corresponding cyclic urea and thiourea in good yield and high purity. Using this approach and following the initial synthesis of individual control compounds, four PS-SCLs were generated from acylated dipeptide N-alkylamide SCLs having either a methyl or benzyl group on the resin-linked C-terminal amide.

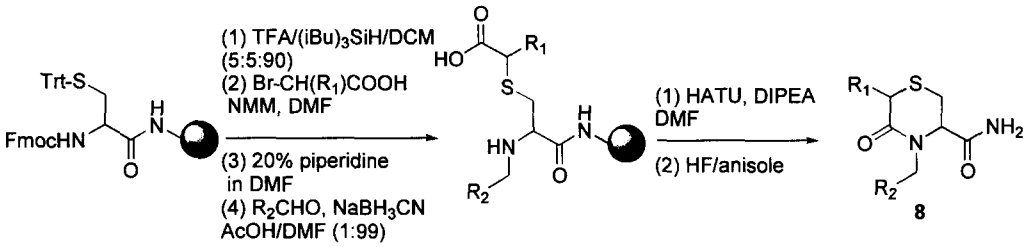
Similar to the synthesis of cyclic ureas and thioureas, the treatment of a resin-bound N-acyl dipeptide (R_1= H) with diborane affords a triamine having three available secondary amine functionalities. Following reaction with thiocarbonyldiimidazole, intermediate cyclic thioureas were initially formed as described above. In contrast to previous synthetic routes, however, the presence of a third secondary amine allowed the reaction to proceed to a protonated bicyclic guanidine in good yield and high purity. Using 49 amino acids for the first site of diversity, 51 amino acids for the second site, and 41 carboxylic acids for the third, a library of 102,459 (49 × 51 × 41) bicyclic guanidines has been synthesized in the positional scanning format.[6] The screening of this library in a radioreceptor assay selective for the kappa opiate receptor led to the identification of individual compounds showing excellent binding affinity (IC_{50} = 37 nM; manuscript in preparation).

A number of diazepine derivatives **7** were synthesized by the reduction of the imine formed between an aldehyde and the α-amino group of *p*-methylbenzhydrylamine resin-bound aspartic acid using $NaBH_3CN$ in 1% HOAc/DMF.[21] The resulting secondary amine was coupled to Fmoc amino acids by double coupling with HATU.[22] The success of such a coupling step depends strongly on the incoming amino acid. Once the dipeptide was formed, the Fmoc protecting group was removed and a second reductive alkylation was carried out using the same conditions. Following cleavage of the *t*-butyl group, the thermodynamically favorable coupling of the resulting secondary amine to the side chain of aspartic acid was readily accomplished in the presence of HATU. Forty different diazepines were prepared, and in most cases their HPLC purities were greater than 80% (Scheme 5).



Scheme 5. Solid-phase synthesis of diazepine derivatives 7.

We also designed an approach for the solid phase synthesis of 2,4,5-trisubstituted thiomorpholine-3-ones **8** from a resin-bound protected cysteine (Scheme 6). Starting from MBHA resin, N-α-Fmoc-S-trityl-L-cysteine is coupled in the presence of diisopropylcarbodiimide (DICI) and hydroxybenzotriazole (HOBt). Following cleavage of the trityl (Trt) group with 5% trifluoroacetic acid (TFA) in DCM in the presence of 5% of $(i\text{Bu})_3\text{SiH}$, the resin-bound Fmoc-cysteine is reacted with a range of different α-bromo α-alkyl carboxylic acids in DMF in the presence of N-methyl morpholine (NMM).



Scheme 6. Solid-phase synthesis of thiomorpholinone derivatives **8**.

Poor purity was obtained for bulky R_1 groups such as phenyl or isopropyl; however, excellent results were obtained with bromoacetic acid (R_1= H), 2-bromopropionic acid (R_1= Me), and 2-bromovaleric acid (R_1= Et). Following Fmoc removal with 20% piperidine in DMF, reductive alkylation of the free amine occurred in the presence of an aldehyde and sodium cyanoborohydrate ($NaBH_3CN$). The formation of thiomorpholinone occurred via intramolecular amidation using HATU as coupling reagent.

We have found that peptides are versatile precursors for the solid phase synthesis of individual and combinatorial libraries of acyclic and heterocyclic compounds. Using the "libraries from libraries" concept, modified dipeptides have been successfully used for the solid-phase synthesis of a wide range hetrocyclic synthetic combinatorial libraries.

Acknowledgments: The authors thank Eileen Weiler for her editorial assistance. This work was funded by: National Science Foundation Grant No. CHE-9520142; NIDA Grant No. DA09410; and Trega Biosciences, Inc.

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