Libraries of small compact structures based on *N*-acyl-*N*-alkylamino acids

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Introduction

Combinatorial libraries have proven to be a powerful technique to identify lead compounds with desired biological properties [1,2]. The peptide backbone, however, predetermines the conformational space covered by peptide libraries and, thus, the complexity of such libraries can only be varied by cyclization and by using a set of both natural and non-natural amino acids. To map more diverse space we have to modify the positions of randomization or the backbone (scaffold); non-peptidic libraries accomplish this demand. Using the 'split and mix' procedure we have synthesized libraries based on *N*-acyl-*N*-alkylamino acids, where three units (amino acid, aldehyde, and carboxylic acid) were randomized.

Results and Discussion

Reductive alkylation
Schiff base formation was achieved by repeated exposure of the amino group of the resin-bound amino acid to aldehyde in two different solvent systems (DMF/1% AcOH, DCM/methanol/1% AcOH); using $NaCNBH_3$ we then reduced the double bond that had been formed. Depending on the reactivity of the aldehydes used, the reaction was carried out either by addition of reductive agent to the reaction mixture, or in a separate step after washing the aldehyde, or by performing the reduction step in the presence of a small amount of aldehyde. After fine tuning the reaction conditions we chose a set of 20 aldehydes for library synthesis.

Acylation of the secondary amino group
Based on results of acylation of resin-bound N-alkylated amino acid with a set of carboxylic acids we chose 50 acids for library synthesis and carried out the acylation using either HOBt esters, symmetrical anhydrides or PyBrOP in the presence of DIEA.

Library construction
We have synthesized two types of libraries in both releasable and non-releasable formats using the split and mix synthesis protocol (Fig. 1).

(a) After coupling a set of amino acids to the solid support we alkylated the alpha amino group of these acids using a reductive alkylation procedure; in the last step we acylated the secondary amine formed by alkylation by a set of carboxylic acids.

(b) In the first step of library synthesis we coupled a set of diaminocarboxylic acids to a resin-bound single releasable, double cleavable linker. After acylation of the amino group of these acids with a set of carboxylic acids, we alkylated the side-chain amino group in the next randomization. The last reaction used for library construction was acylation of the secondary side-chain amine.

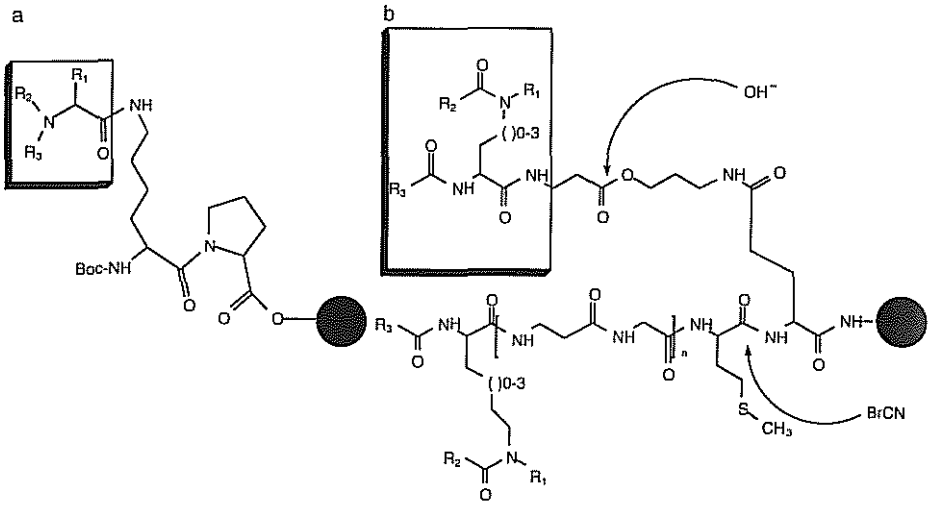

Fig. 1. Structure of N-acyl-N-alkylamino (a) and N^{α}-acyl-N^{ω}-acyl-N^{ω}-alkylamino acid (b) based libraries.

Screening of library and structure determination of active compounds

We have screened samples of the *N*-acyl-*N*-alkylamino library against streptavidin, anti-β-endorphin monoclonal antibody and thrombin. Ligands were identified in the streptavidin assay. We have confirmed the specificity of binding by using a competition assay with biotin.

The structure of the compounds was determined by mass spectrometry (MS and MS/MS). Using a program that can generate the building block composition of a particular molecular weight, we determined the building block used in the synthesis of a particular compound.

Conclusions

We have prepared small libraries of organic compounds based on reductive alkylation of the amino group of amino acids and acylation by carboxylic acids of the secondary amino group generated by the alkylation. We demonstrated that a small non-peptidic library can be used to identify ligands of significant affinity for a relatively large protein molecule. In addition we have illustrated that mass spectrometry offers an alternative to coding methods for the identification of structures of non-peptidic compounds.

References

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