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Structurally homogeneous and heterogeneous synthetic combinatorial libraries

Viktor Krchňák*, Aleksandra S. Weichsel, Dasha Cabel, Zuzka Flegelova and Michal Lebl**

Selectide Corporation, a subsidiary of Hoechst Marion Roussel, 1580 E. Hanley Boulevard, Tucson, AZ 85737, U.S.A.

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Summary

We have designed and synthesized structurally homogeneous and heterogeneous nonpeptide libraries. Structurally homogeneous libraries are characterized by the presence of one common structural unit, a scaffold, in all library compounds (e.g. cyclopentane, cyclohexane, diketopiperazine, thiazolidine). In structurally heterogeneous libraries different organic reactions (acylation, etherification, reductive amination, nucleophilic displacement) were applied to connect bifunctional building blocks unrelated in structure (aromatic hydroxy acids, aromatic hydroxy aldehydes, amino alcohols, diamines, and amino acids). The focus of this communication is to document the use of bifunctional building blocks for the design and synthesis of structurally heterogeneous libraries of *N*-(alkoxy acyl)amino acids, *N,N'*-bis-(alkoxy acyl)diamino acids, *N*-acylamino ethers, *N*-(alkoxy acyl)amino alcohols, *N*-alkylamino ethers, and *N*-(alkoxy aryl)diamines.

Introduction

Currently, the main trend of synthetic combinatorial chemistry is to generate chemical diversity by designing and synthesizing libraries of small organic molecules. High-throughput screening of these libraries should enhance the probability of finding compounds with desired pharmacological properties and speed up the drug-discovery process (for recent reviews see Refs. 1–6). From the structural point of view, there are two different approaches for designing small-molecule combinatorial libraries: (i) structurally homogeneous libraries in which one common structural feature or scaffold is present for all library compounds (benzodiazepine [7–9], cyclopentane [10], cyclohexane [11], diketopiperazine [12–14], thiazolidine [15], pyrrolidine [16], benzylpiperazine [17], pyrrolidine [18]); and (ii) structurally heterogeneous libraries for which different organic reactions are applied to connect bifunctional, structurally unrelated building blocks.

A typical example of a bifunctional building block is an amino acid. In the case of peptide synthesis, the carboxyl group is used for attachment of the building block to the insoluble carrier. The second functionality, the amino group, serves for the attachment of the next building block, an α-amino acid. The repetition of amide bond formation using only α-amino acids leads to peptides, a class of compounds that have been explored from the very inception of combinatorial chemistry [19,20]. The two critical advantages of making libraries of peptides are: (i) well-documented chemistry on solid support; and (ii) availability of amino acids as building blocks. The most serious disadvantages, stressed already many times, are: (i) the limits in structural diversity provided by peptide libraries; and (ii) the unfavorable pharmacological properties of peptides as potential drugs. The use of structurally related building blocks, α-amino acids, results in a common backbone structure, which is responsible for the limited structural diversity of peptides. The only

*To whom correspondence should be addressed at: Houghten Pharmaceuticals Inc., 3550 General Atomics Court, San Diego, CA 92121, U.S.A.
**Present address: Houghten Pharmaceuticals Inc., 3550 General Atomics Court, San Diego, CA 92121, U.S.A.

Abbreviations: AcOH, acetic acid; DCE, dichloroethane; DCM, dichloromethane; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; DIC, diisopropyl carbodiimide; DIEA, diisopropylethylamine; DMAP, dimethylaminopyridine; DMF, dimethylformamide; Fmoc, fluorenylmethyloxycarbonyl; HOBt, *N*-hydroxybenzotriazole; MeCN, acetonitrile; MeOH, methanol; NaOH, sodium hydroxide; PEG/PS, polyethylene-grafted copolystyrene; PPh_3, triphenylphosphine; *t*-Bu, *tert*-butyl; TFA, trifluoroacetic acid; TG, TentaGel; THF, tetrahydrofuran.

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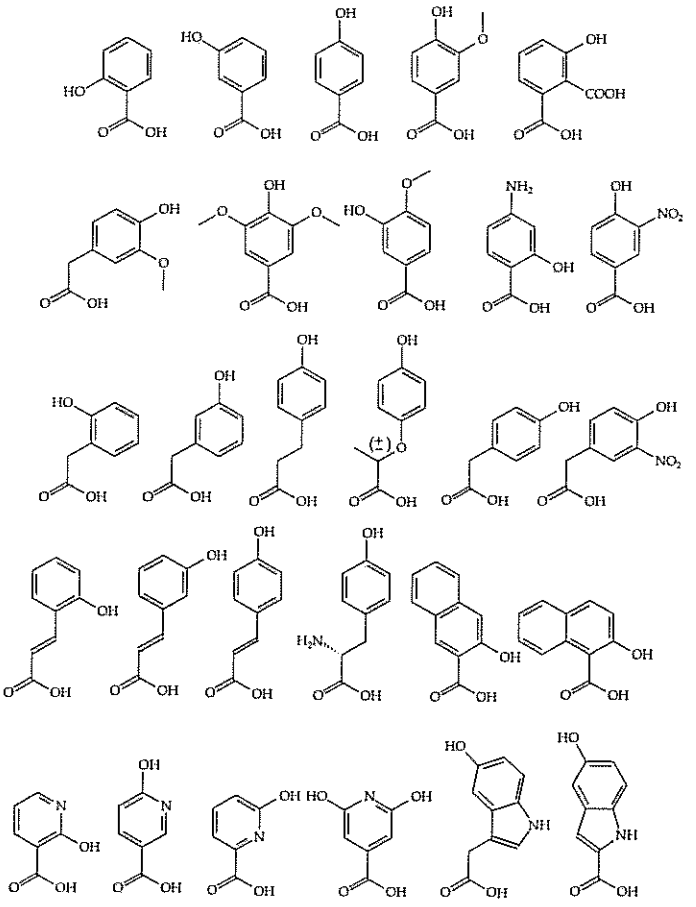

Fig. 1. Structure of aromatic hydroxy acids.

changing parameter of peptide libraries is the amino acid side chain. However, combination of α, β, γ, and δ amide bonds for backbone formation within one library produces a structurally heterogeneous backbone [21], thus dramatically changing the dissimilarity/diversity of compounds within one library.

To extend the limited diversity of peptide libraries and at the same time to keep the idea of joining bifunctional building blocks, we concentrated on finding different types of structurally unrelated bifunctional building blocks together with a set of compatible chemical reactions to link those building blocks together to form a library. We applied three main criteria for selecting new types of bifunctional building blocks: (i) polymer-supported chemistry should be available to link functional groups present on those building blocks; (ii) bifunctional building blocks should be commercially available or easy to prepare from available precursors, and (iii) linking chemical compounds should be compatible so that the different types of building blocks could be combined within one library to produce the greatest structural diversity. In this communication we describe the use of aromatic hydroxy acids, aromatic hydroxy aldehydes, amino alcohols, diamines, and amino acids as bifunctional building blocks for the design and synthesis of structurally heterogeneous libraries.

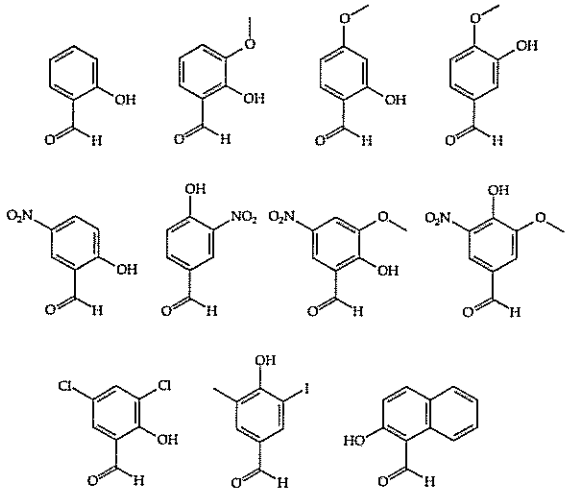

Fig. 2. Structure of aromatic hydroxy aldehydes.

Materials and Methods

Model compounds were synthesized on TentaGel-S-NH_2 130-μm resin, TentaGel-S-OH 130-μm resin (TG, Rapp Polymere, Tübingen, Germany), polyethylene-

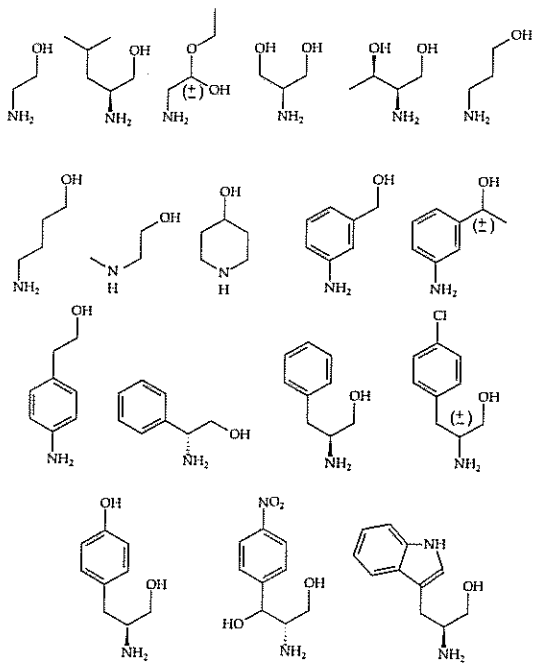

Fig. 3. Structure of amino alcohols.

grafted copolystyrene (contaning 1% divinylbenzene) 220-μm resin (PEG/PS, Millipore, Bedford, MA), or chlorotrityl polystyrene resin (Advanced ChemTech, Louisville, KY and Bachem Bioscience, King of Prussia, PA). Fluorenylmethyloxycarbonyl (Fmoc) amino acids with standard side-chain protecting groups (Cys protected by acetamidomethyl group) were obtained from Advanced ChemTech (Louisville, KY) or Propeptide (Vert-le-Petit, France). Twenty-eight aromatic hydroxy acids (Fig. 1), 11 aromatic hydroxy aldehydes (Fig. 2), 15 amino alcohols (Fig. 3), 14 diamines (Fig. 4), 30 carboxylic acids [2], 20 aldehydes [22], and 51 alcohols (Fig. 5) were used in randomizations. Diethyl azodicarboxylate (DEAD), diisopropyl azodicarboxylate (DIAD), diisopropyl carbodiimide (DIC), diisopropylethylamine (DIEA), dimethylaminopyridine (DMAP), *N*-hydroxybenzotriazole (HOBt), phenol, triphenylphosphine (PPh_3), piperidine, sodium triacetoxyborohydride, thioanisole, trifluoroacetic acid (TFA), and triethyl orthoformate were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). Anhydrous tetrahydrofuran (THF) was obtained from Aldrich. High-purity solvents (Baxter, McGaw Park, IL) were used without further purification.

Analytical HPLC was carried out on a Waters 625 LC system with a Waters 490E Programmable Multiwavelength Detector (Milford, MA) using a Vydac Peptide and Protein C_{18} analytical column (4.6 × 250 mm, 5 μm, 1 ml/min; The Separation Group, Hesperia, CA). The analytical gradient was run from water containing 0.07% TFA to 60% of acetonitrile (MeCN)/water in 30 or 60 min, or to 80% of MeCN in 40 min. UV/VIS Absorption spectra were recorded on a Hewlett Packard HP 8452A Diode-Array spectrophotometer (Palo Alto, CA) using a 1-cm quartz cuvette. Ion-spray mass spectra were obtained on a triple quadrupole PE-Sciex API III$^+$ mass spectrometer (Perkin-Elmer/Sciex, Thornhill, ON) with an articulated ion-spray sample inlet system.

Manual solid-phase synthesis

All reactions were carried out in a plastic syringe equipped with a sintered polypropylene disc at the bottom [23]. To wash the resin, the solvent, typically 3 ml of solvent per 1 ml of swollen resin, plus a small amount of air was drawn into the syringe, the syringe was shaken for 0.5 min and the solvent discharged. Reactions were performed using 3 ml of solution of reagents per 1 ml of swollen resin beads. The excess of reagents was always calculated with respect to the original substitution of the resin.

Preparation of linker-resin construct

TentaGel-S-NH_2 (1 g, 0.25 mmol/g, 130 μm average particle size) or PEG/PS hydrochloride (1 g, 0.58 mmol/g, 220 μm average particle size) was swollen in dimethylformamide (DMF) (swollen volume 5 ml/1 g), PEG/PS beads were neutralized by repeated 3-min treatment with 10% DIEA in DMF and washed five times with DMF. The iminodiacetic-acid-based double-cleavable linker (IdaDC linker; structure in Fig. 6, m.w. 1020, 3-fold excess) and HOBt (3-fold excess) were dissolved in DMF, activated by DIC (3-fold excess) and the solution was transferred to the resin. After overnight reaction the resin was checked for the presence of free amino groups by the ninhydrin test [24] and washed five times with DMF. If the test was positive, the entire coupling procedure was repeated.

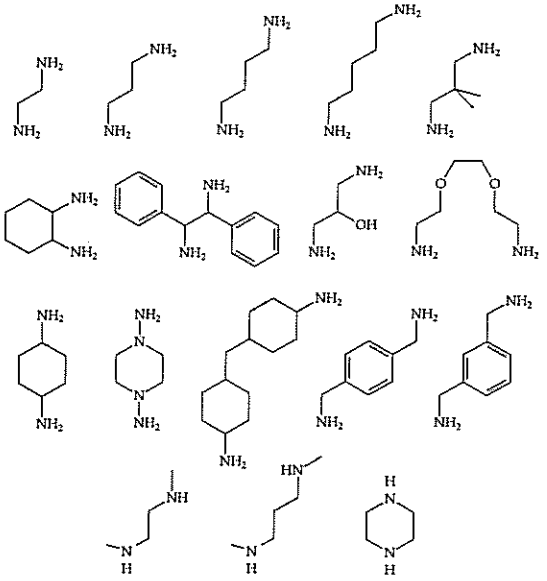

Fig. 4. Structure of diamines.

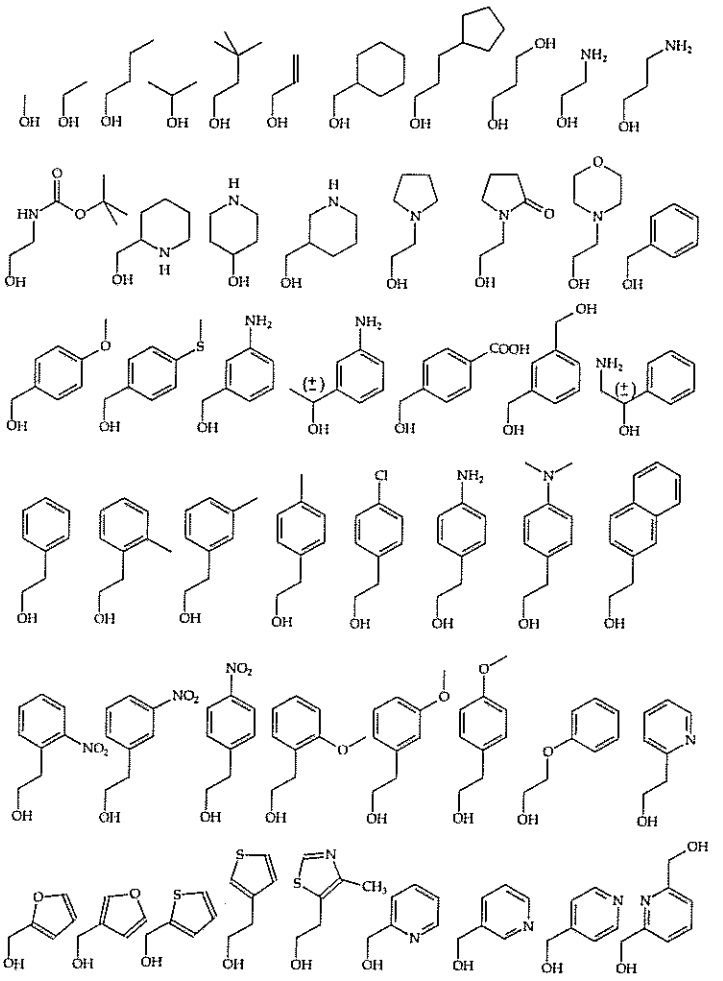

Fig. 5. Structure of alcohols.

Preparation of carboxylate resin

TentaGel-S-NH_2 resin was swollen in DMF, and then a 5-fold excess of glutaric anhydride in DMF was added to the resin and the slurry was shaken on the tumbler for 1 h. The resin was then washed five times with DMF and checked for the presence of free amino groups by the ninhydrin test [24]. If the test was positive the acylation was repeated.

Reverse esterification by Fmoc amino alcohols

The carboxylate resin (0.5 ml, ca. 100 mg) was washed five times with dry THF, then 0.5 ml of a 0.5-M solution of Fmoc-amino alcohol in THF and 0.25 ml of a 0.5-M PPh_3 in THF was added. The syringe with the resin was chilled in the freezer for 10 min and 0.25 ml 0.5 M DIAD in THF was added. The resin was placed in the freezer again for 15 min to cool down and the reaction was continued for 2 h at room temperature. After 2 h the resin was washed three times with THF and the reaction was repeated using half of the volume of reagents overnight. The resin was washed with DMF and the Fmoc group was cleaved.

Esterification of hydroxy-TentaGel by amino acids

The syringe was charged with TentaGel-S-OH (0.25 g, 0.25 mmol OH/g). The resin was washed three times with dry THF. Then 0.4 ml of a 0.5-M solution of Fmoc-protected amino acid in THF and 0.8 ml of a 0.5-M solution of PPh_3 in THF were added. The syringe was cooled to −10 °C in a DMF bath [25] and 0.6 ml of a 0.5-M solution of DEAD in THF was added. The syringe was rotated on a tumbler for 2 h. After esterification the resin was washed five times with DMF and the Fmoc group was cleaved.

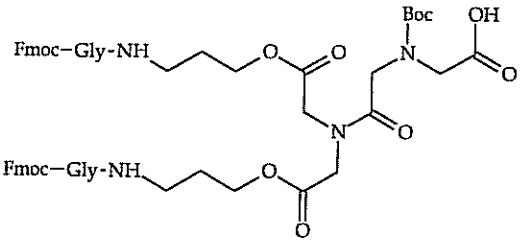

Fig. 6. Structure of iminodiacetic-acid-based double-cleavable linker (IdaDC linker).

TABLE 1
BIFUNCTIONAL ORGANIC COMPOUNDS RESULTING FROM COMBINATION OF FIVE FUNCTIONAL GROUPS

	Amine	Acid	Aldehyde	Alcohol	Phenol
Amine	*Diamines*				
Acid	*Amino acid*	*Diacid*			
Aldehyde	Amino aldehyde	*Carboxy aldehyde*	Dialdehyde		
Alcohol	*Amino alcohol*	Hydroxy acid	Hydroxy aldehyde	Diol	
Phenol	*Amino phenol*	*Aromatic hydroxy acids*	*Phenol aldehyde*	Hydroxy alkyl phenol	Aromatic diol

Compound types tested for library synthesis are in italics.

Attachment of building blocks to 2-chlorotrityl chloride resin

The syringe was charged with 100 mg of resin (Advanced ChemTech, substitution 1.7 mmol/g; or Bachem, substitution 1.15 mmol/g) and a 3-fold excess (0.5 mmol and 0.35 mmol, respectively) of diamine, alcohol or acid was dissolved in 1 ml of dichloroethane (DCE) and added to the resin. For the ester and ether formation, a 5-fold excess of DIEA with respect to acid or alcohol was added to the DCE solution before adding the solvent to the resin. All reactions were carried out at 60 °C for 2 h. Conversion of amines was 60–95%, for alcohols 55%, and for acids 75%.

Condensation of aromatic hydroxy acids

Aromatic hydroxy acid and HOBt were dissolved in DMF (0.5 mmol in 1 ml), DIC was added, the solution was transferred into the reactor, and the mixture was shaken for 1 h. The presence of free amino groups was checked [24]. If the test was positive, the resin was washed with DMF (three times) and the coupling was repeated.

Condensation of carboxylic acids

Carboxylic acid (3-fold excess) and HOBt (3-fold excess) were dissolved in DMF, DIC (3-fold excess) was added and the solution was transferred into the reactor. The mixture was shaken for 2–3.5 h and the presence of free amino groups was checked [24]. If the test was positive, the resin was washed five times with DMF and the coupling procedure was repeated.

Etherification of polymer-supported phenols by alcohols

The resin (ca 0.8 ml per syringe) was washed five times with dry THF. A solution of PPh_3 in THF (1.5 ml of a 1-M solution) and 3 mmol of alcohol was added and the slurry was shaken. Then, a solution of 1.5 mmol of DIAD in 0.2 ml of THF was added to the resin in four portions at 5-min intervals. The mixture was shaken overnight and then washed five times with DMF.

Reductive alkylation

The resin (0.2 ml, ca. 0.01 mmol of amino groups) was washed three times with DMF, then triethyl orthoformate (0.3 ml) and aldehyde (0.05 mmol) were added and the slurry was shaken vigorously for 5 h. Then the resin was washed three times with dichloromethane (DCM) and a 0.1-M suspension of sodium triacetoxyborohydride in DCM (0.5 ml, 0.05 mmol) was added. The mixture was shaken for 16 h. After the resin was washed with DMF (three times), both steps were repeated.

Fmoc deprotection

The resin was washed three times with DMF, treated with 50% piperidine in DMF twice for 5 min and then for 20 min, and washed six times with DMF. All washes were collected, the absorbance at 302 nm was measured and the Fmoc release calculated ($\varepsilon_{302} = 8100$).

Side-chain deprotection and final washes

The resin beads were washed with DMF (three times), DCM (three times), treated with TFA containing 5% *p*-cresol for 1 min and exposed to this mixture for 2 h. The resin was then washed with TFA (three times), DCM (five times), methanol (MeOH) (three times) and dried on a freeze-dryer overnight.

Cleavage of compounds from linkers

Double-cleavable IdaDC linker Resin beads were shaken in a syringe equipped with frit in 0.1 M ammonium acetate buffer, pH 7.5–8, for 2 h. The resin was washed three times with 80% aqueous MeCN, the washes were combined and lyophilized. The washed resin was then exposed to 5 ml of 0.5% sodium hydroxide (NaOH) for 2 h, the solution was acidified by acetic acid (AcOH) (pH 6–7) and the resin extracted with 80% aqueous MeCN. The combined washes were lyophilized.

Single-cleavable ester linker Resin beads were shaken in a syringe equipped with frit in 0.5% NaOH for 0.5 h. The resin was then washed three times with water and three times with 80% MeCN in water. Combined extracts were acidified by addition of AcOH (pH 3–4) and the resulting solution was lyophilized.

Single-cleavable trityl linker Resin beads were shaken in a syringe equipped with a frit in 95% TFA, 5% water for 0.5 h. The resin was filtered off, the filtrate diluted by water and the solution lyophilized.

Results and Discussion

One strategy for generating structural diversity in combinatorial chemistry focuses on synthesis of libraries of

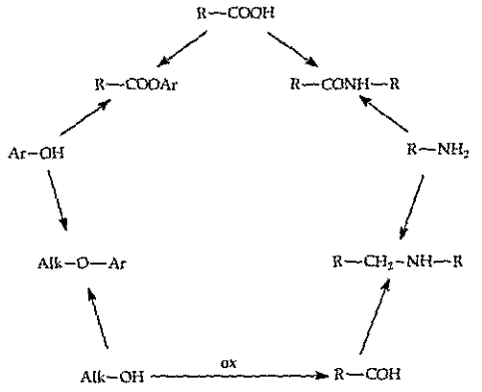

Fig. 7. Functional groups presented on selected bifunctional building blocks and products of reactions between two functionalities.

small organic compounds by connecting a variety of bifunctional building blocks. Organic reactions capable of connecting different functional groups have to be optimized for use on the solid phase, and in addition diverse sets of building blocks have to be tested. To achieve a high diversity of compounds for our structurally heterogeneous libraries, we searched for bifunctional building blocks for which the two functional groups do not have fixed positions. This is in contrast to peptides, for which the amino acid building blocks always have the amino group in the α-position with respect to the carboxyl group. To start building the battery of bifunctional building blocks we selected five types of functional groups to be present on those building blocks: amines, acids, aldehydes, alcohols and phenols. Fifteen different kinds of bifunctional reagents can be formed out of these five functionalities. They are listed in Table 1. We have selected six types of the most commonly available bifunctional building blocks for their use in design and synthesis of libraries. These include: aromatic hydroxy acids, aromatic hydroxy aldehydes, amino alcohols, diamines, and amino acids. We have not selected amino aldehydes, since few are commercially available and since the aldehyde function has to be protected. However, we have produced the same type of compounds from amino alcohols after attaching them via the amino group to the resin followed by oxidation of the hydroxyl groups [26,27]. Aliphatic hydroxy acids and hydroxy aldehydes are not commercially available in sufficient variety. We have used only a few examples of diols.

The following connections between two functional groups were used in our model libraries: (i) amide bond formation; (ii) reductive alkylation; (iii) esterification; and (iv) aryl ether formation. Bifunctional building blocks have been tested in the corresponding reactions on solid phase and those included in Figs. 1–4 provided acceptable purity and yield of product. We chose greater than 75% purity of expected product as a criterion for acceptance (estimated by analytical HPLC). The list is not final. We are still extending the sets of bifunctional building blocks. In Material and Methods the reaction conditions for each particular condensation are described. Only in the polymer-supported functional group transformation the oxidation of alcohols on the resin to form aldehydes was included. We used this reaction to provide polymer-supported carbonyl compounds for the Wittig–Horner–Emmons reactions [27]. We did not include these examples here. Figure 7 schematically depicts all reactions.

Bifunctional building blocks

Aromatic hydroxy acids The carboxyl function was employed for linking these reagents to the resin beads via amide bond formation. Since the activated carboxyl group can acylate unprotected hydroxyl groups, we prepared as a model 4-hydroxybenzoyl-Gly-OH, using both unprotected and *tert*-butyl (*t*Bu)-protected 4-hydroxybenzoic acid (DIC/HOBt activation and 1 h of reaction time). Both products provided the same HPLC profile, showing no sign of undesirable acylation of unprotected hydroxyl groups under these conditions [28]. However, only mild activation of the carboxyl group should be used to help avoid acylation of the phenols.

The polymer-supported phenolic hydroxyl groups were

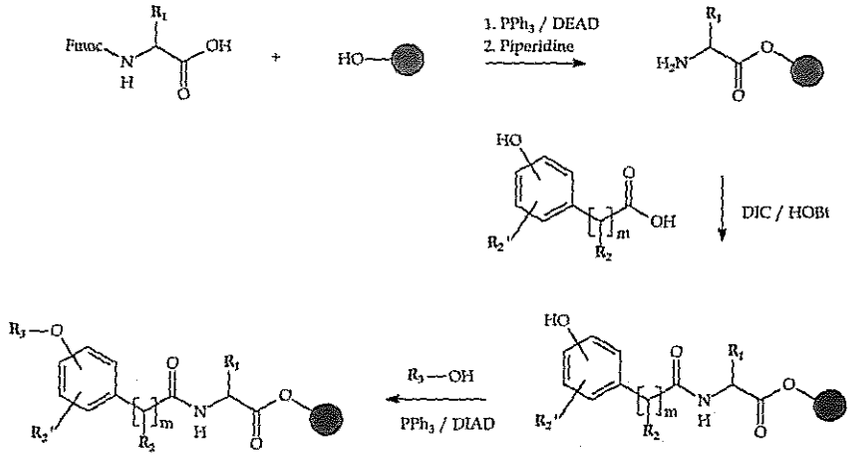

Fig. 8. Reaction scheme for a library of *N*-(alkoxy acyl)amino acids.

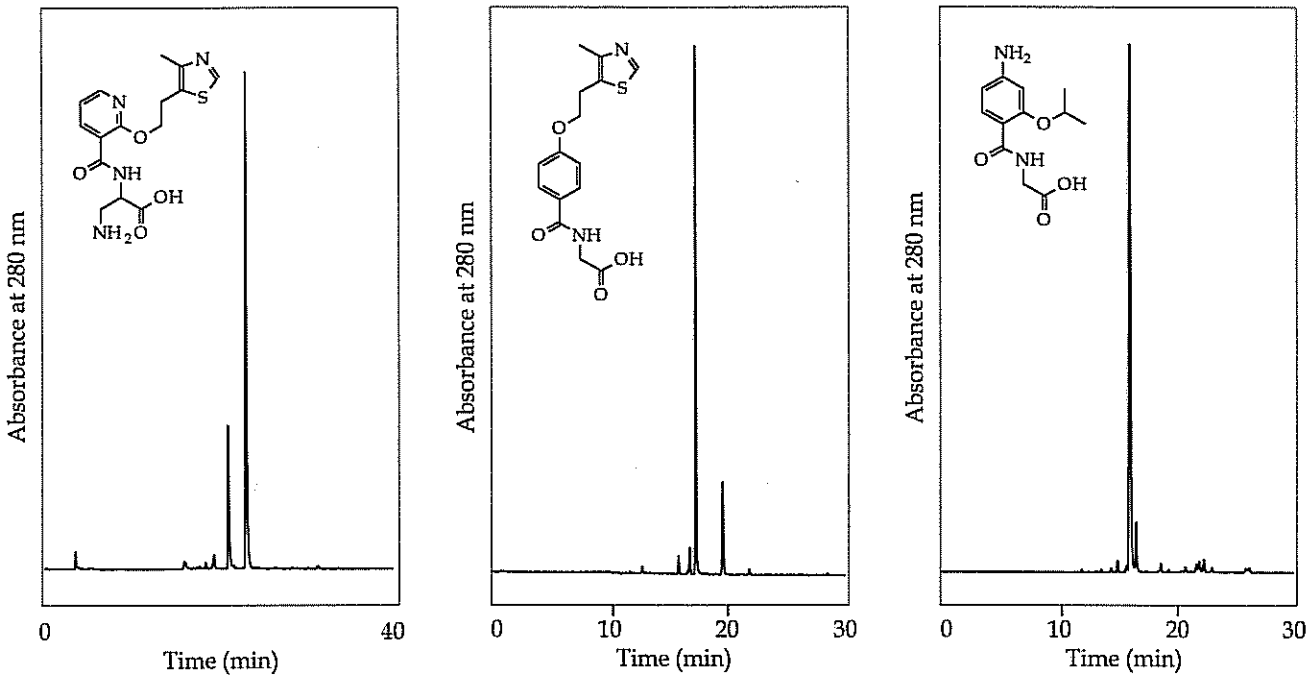

Fig. 9. HPLC traces of model compounds for a library of *N*-(alkoxy acyl)amino acids synthesized on hydroxy-TentaGel.

then reacted with alcohols under the conditions of Mitsunobu redox condensation [29,30]. The formation of aryl ethers on insoluble carrier has recently been described by us and others [28,31].

Aromatic hydroxy aldehydes Aromatic hydroxy aldehydes have been used in the same way as aromatic hydroxy acids; however, reductive alkylation was used to attach these building blocks to amino groups on the resin. We originally used sodium borohydride as a reducing agent [22,32]. Later we formed the Schiff base using triethyl orthoformate [33] and reduced it with sodium triacetoxyborohydride [13]. Resulting polymer-supported phenols reacted with alcohols in solution to produce aryl ethers [28,31].

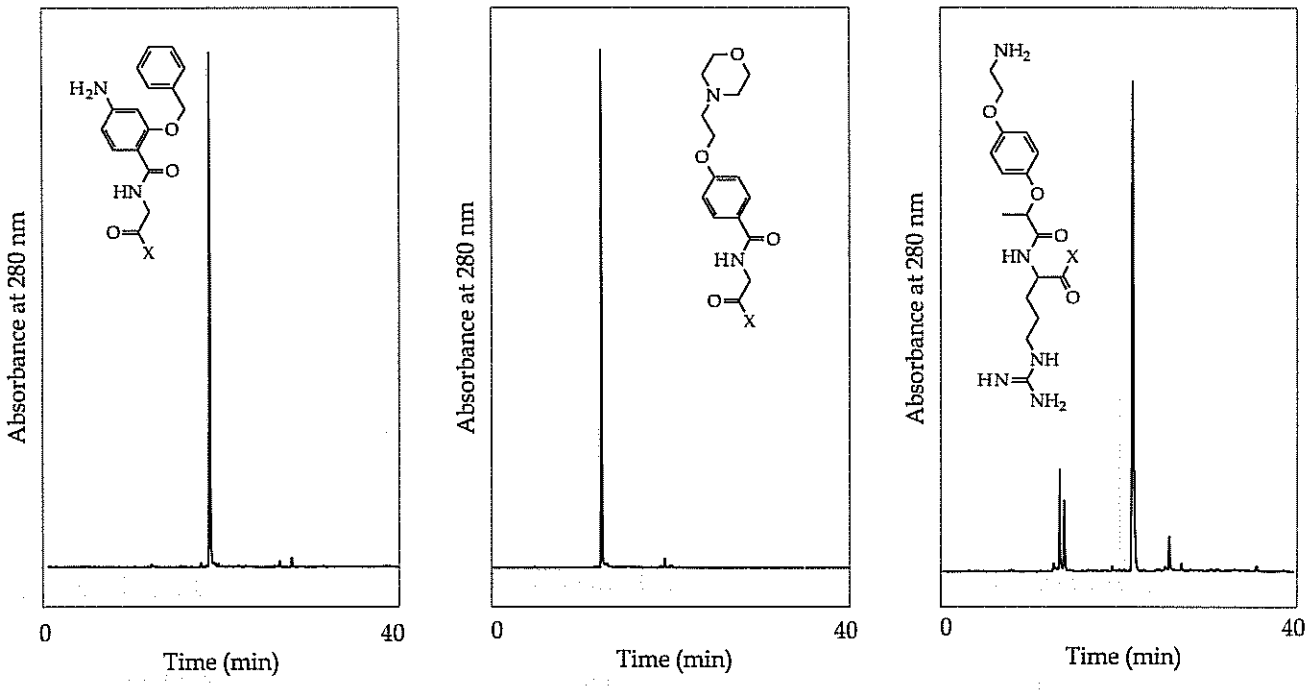

Fig. 10. HPLC traces of model compounds for a library of *N*-(alkoxy acyl)amino acids synthesized on IdaDC linker and PEG/PS 220 beads (X stands for hydroxypropylamide of glycine, present in all compounds).

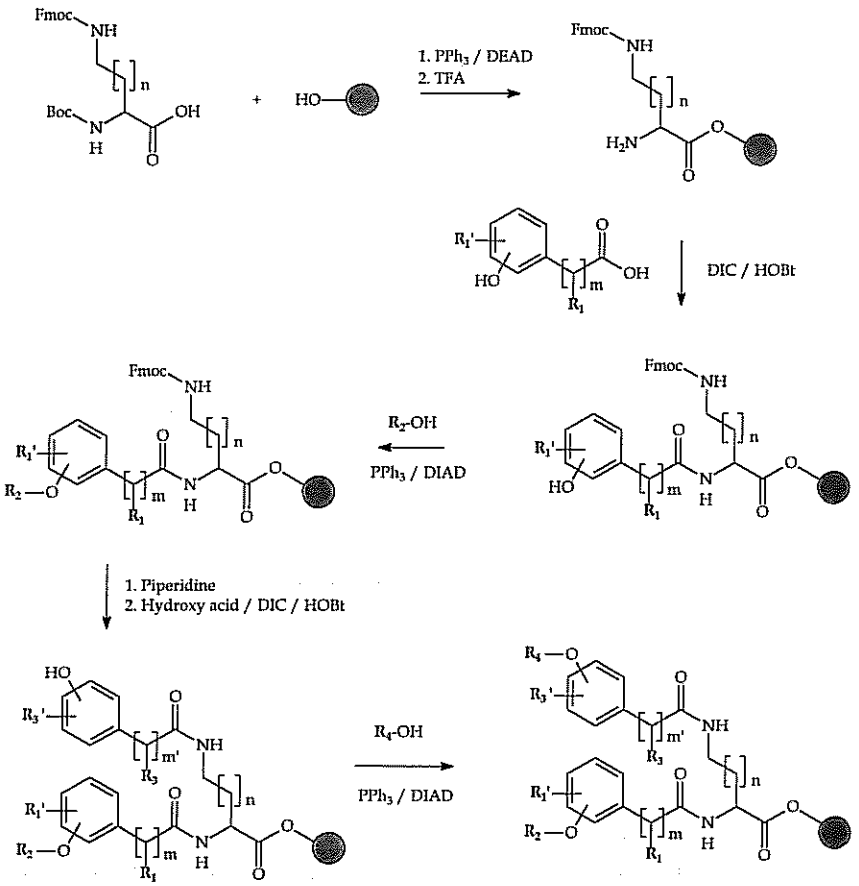

Fig. 11. Reaction scheme for a library of *N,N'*-bis(alkoxy acyl)diamino acids.

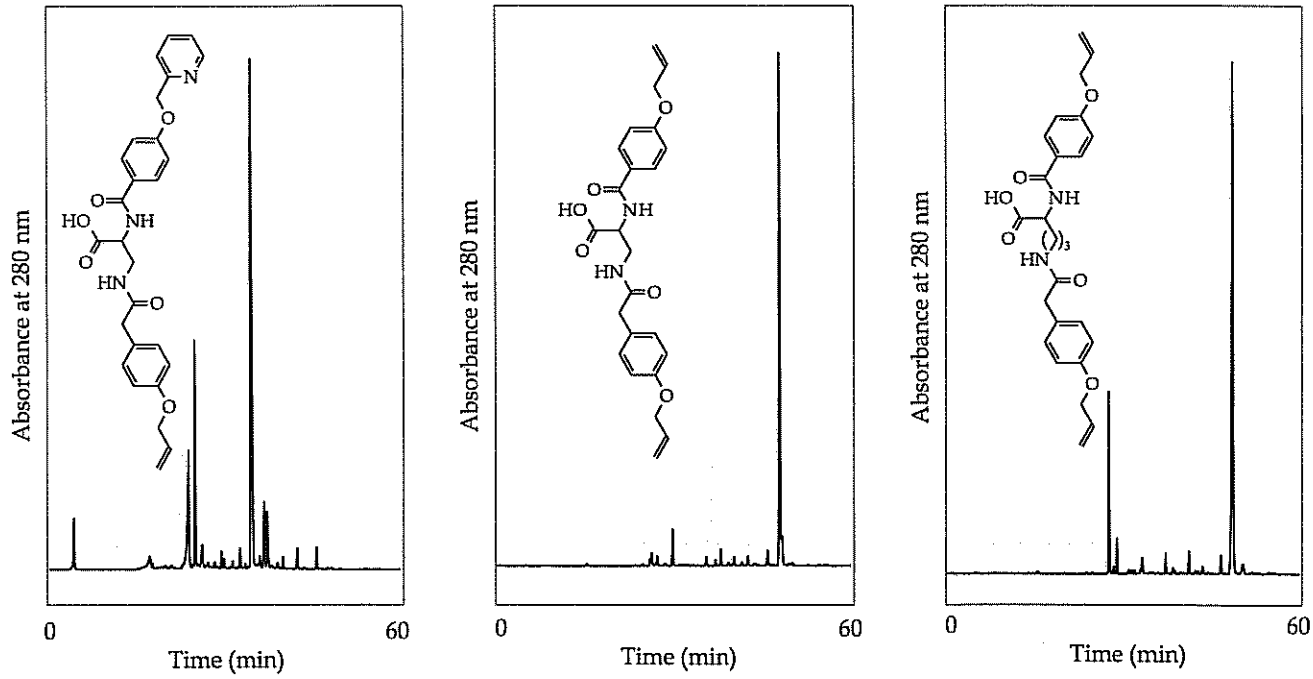

Fig. 12. HPLC traces of model compounds for a library of *N,N'*-bis(alkoxy acyl)diamino acids.

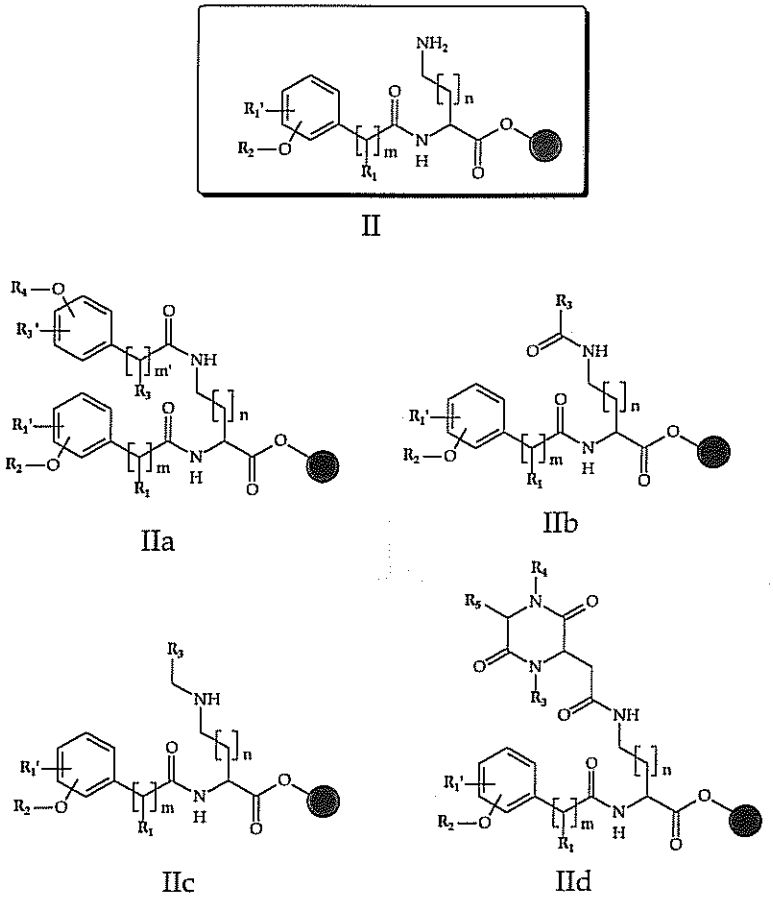

Fig. 13. Four various formats of *N*-(alkoxy acyl)diamino acid library. See text for details.

Amino alcohols Amino alcohols were attached to the resin beads either using the amino group or the hydroxyl group. In the former case amino alcohols were not protected. After attaching them to the resin, the hydroxyl groups were oxidized to aldehydes [26,27] for further reactions.

The attachment of amino alcohols via the hydroxyl group required protected amino functions for which we used the Fmoc group. In model libraries the amino alcohols were attached via the hydroxyl group to polymer-supported carboxyl groups (esterification), phenols (etherification), or to the trityl resin. After attachment and Fmoc group deprotection, the available amino groups were used for acylation or reductive alkylation.

Diamines In our examples the diamines were used in the first randomization by attachment directly to trityl

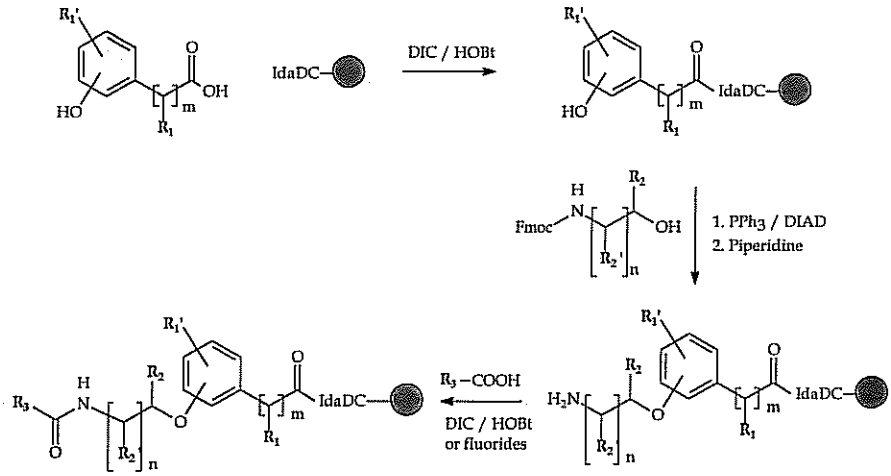

Fig. 14. Reaction scheme for a library of *N*-acylamino ethers.

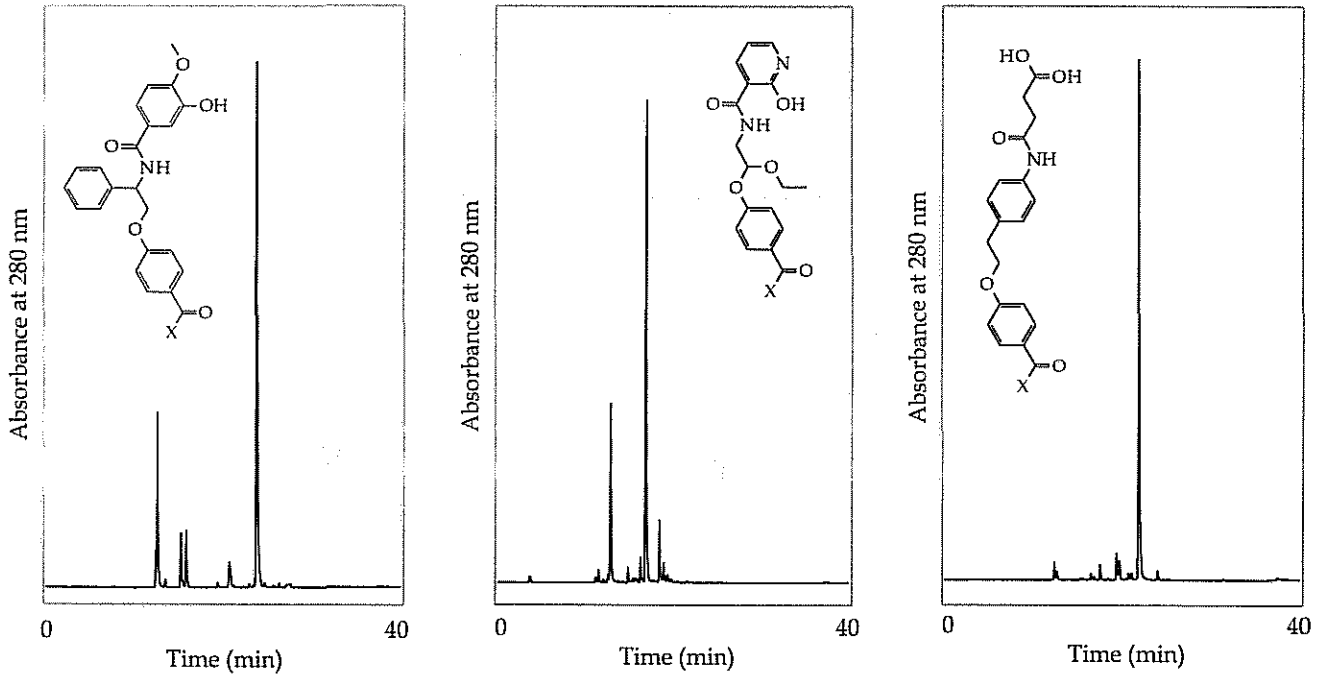

Fig. 15. HPLC traces of model compounds for a library of *N*-acylamino ethers (X = hydroxypropylamide of glycine, present in all compounds).

resin. In the case of unprotected symmetrical diamines the reaction was carried out with a large excess of diamine to favor formation of the monoalkylated species. Unsymmetrical diamines, e.g. commercially available diamino acids, selectively protected on one amino group, will yield the single-alkylated products.

Amino acids The amino acids were attached via their carboxyl group in the same way they are used in the synthesis of peptides. In a subsequent step, the amino groups have been either acylated or alkylated.

Model libraries

One can imagine a variety of ways to combine the described building blocks for library synthesis. We have documented the feasibility of the approach with examples of six library designs. With two exceptions, the library examples included three randomization steps. The reason for adopting three randomizations was practical: reasonable complexity could be produced while compounds would still be of low molecular weight. The libraries selected illustrate all of the reactions described, and in the production of the libraries all of the selected building blocks were used. For all libraries monofunctional building blocks were used in the last step of randomization.

We designed all libraries for the Selectide one-bead–

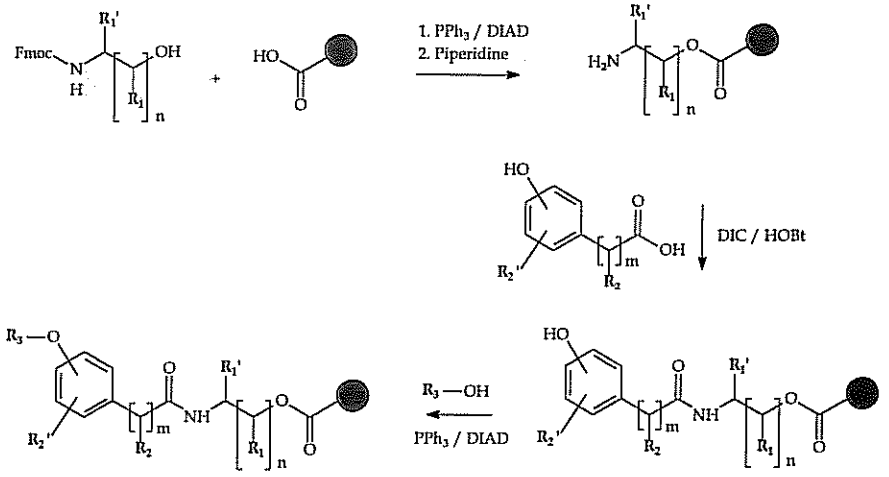

Fig. 16. Reaction scheme for a library of *N*-(alkoxy acyl)amino alcohols.

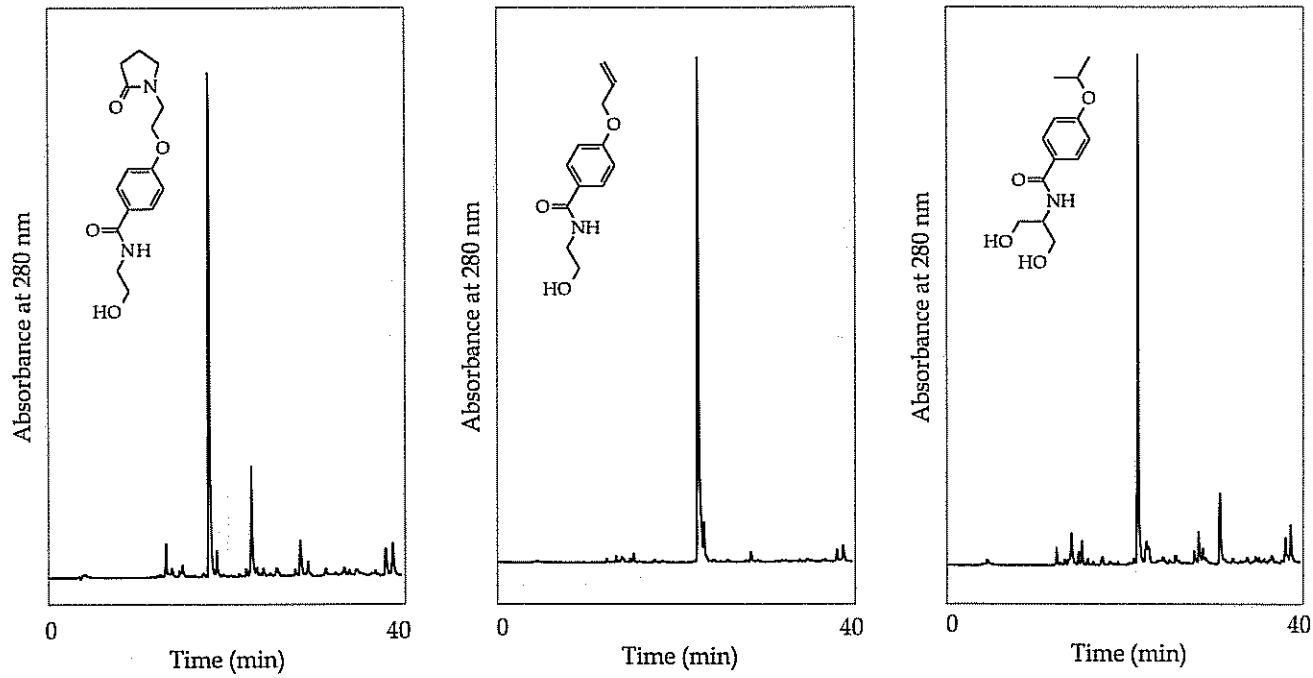

Fig. 17. HPLC traces of model compounds for a library of *N*-(alkoxy acyl)amino alcohols.

one-compound strategy [20] using the split/mix synthetic scheme [34] on cleavable linkers that allows the release of compounds into a solution [35]. Alternatively, the libraries could have been produced for use in on-bead binding assays. We used three different linkers in the libraries: (i) an iminodiacetic-acid-based double-cleavable linker (Ida-DC linker, Fig. 6) [36]; (ii) a single-cleavable ester-bond-containing linker; and (iii) a trityl linker [37,38].

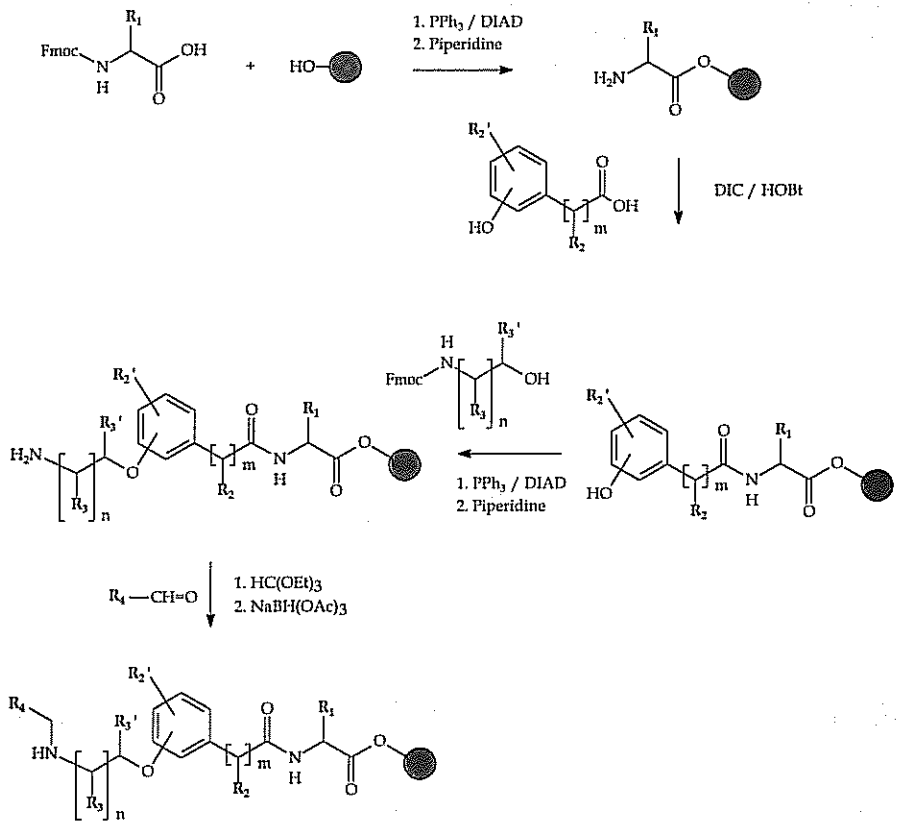

Fig. 18. Reaction scheme for a library of *N*-alkylamino ethers.

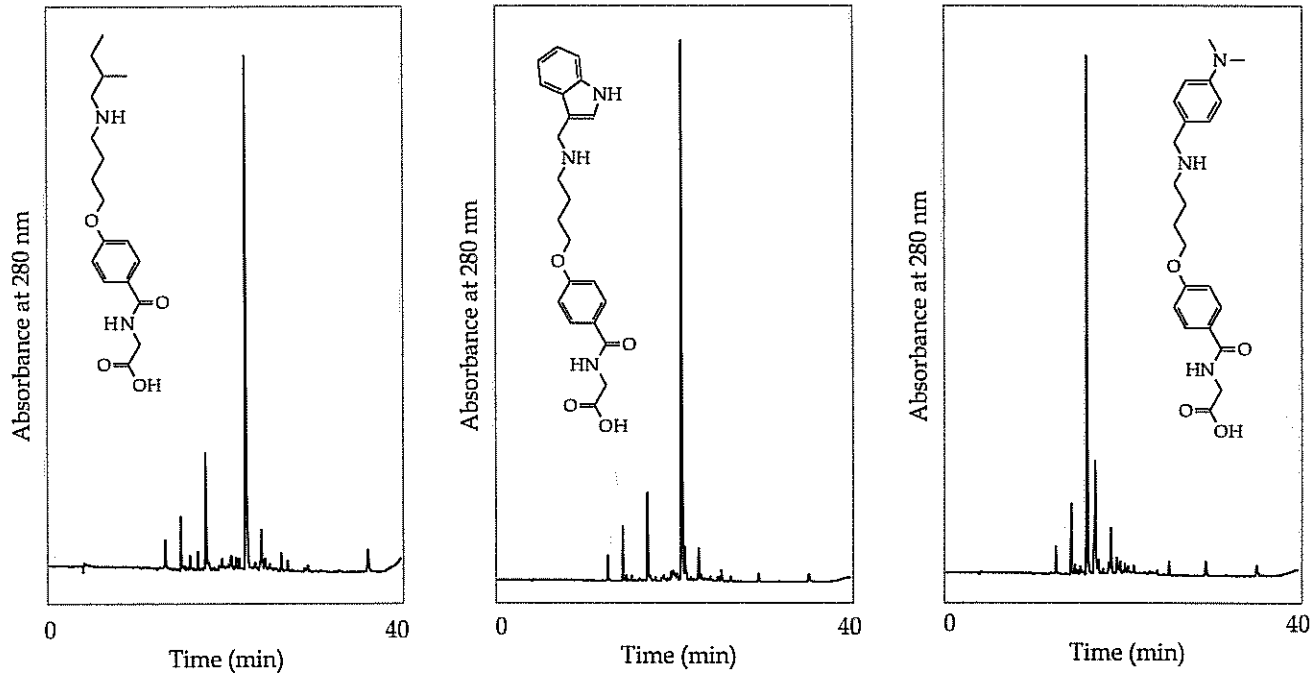

Fig. 19. HPLC traces of model compounds for a library of *N*-alkylamino ethers.

The IdaDC linker allows a two-stage release of defined amounts of compound into solution. An ester bond is used to attach compounds to both releasable arms. The ester bonds, however, are cleaved by two distinct mechanisms; the first by nucleophilic attack of an internal nucleophile, resulting in diketopiperazine formation, and the second via alkaline hydrolysis. All compounds were attached to the linker via an ester bond of Fmoc-Gly-NH-$(CH_2)_3$-OH, and when released to the aqueous solution they contain an identical carboxy terminus (hydroxypropylamide of glycine).

Diluted alkali or ammonia vapors were used to release compounds from the second linker used, the single-cleavable ester linker. The third linker, trityl, is compatible with strong nucleophiles, and compounds were cleaved using acids.

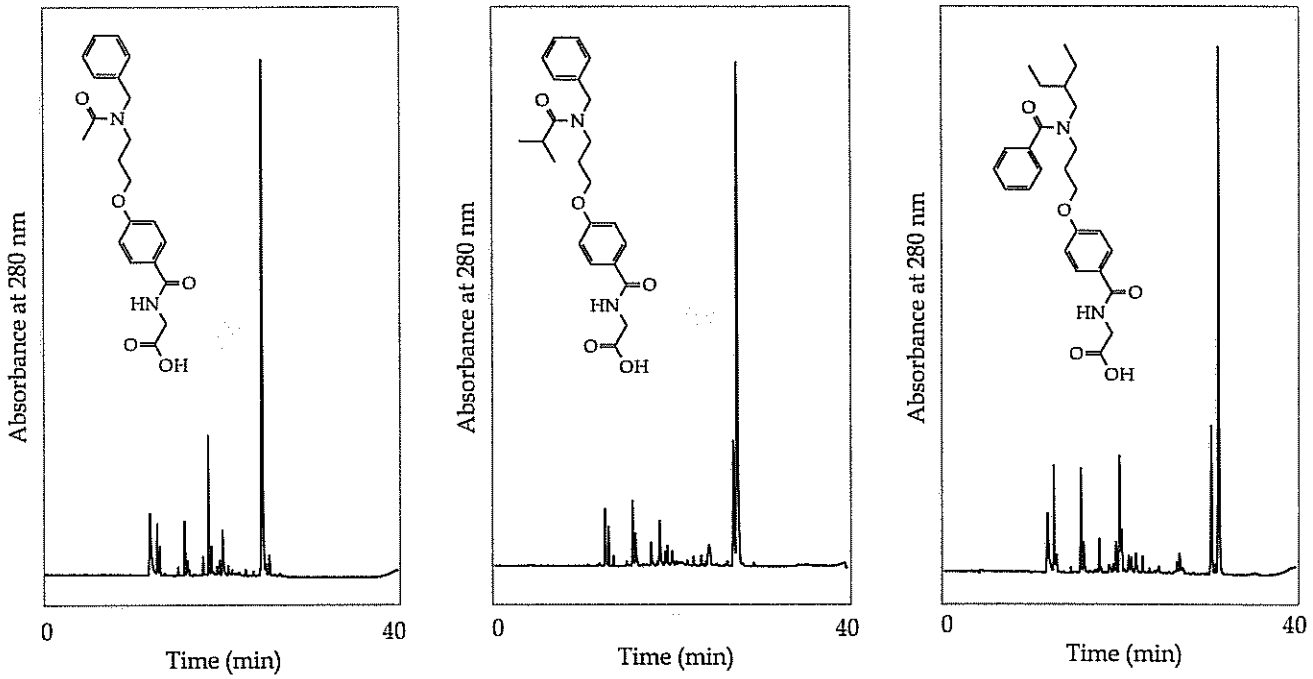

Fig. 20. HPLC traces of model compounds for a library of *N*-alkyl-*N*-acylamino ethers.

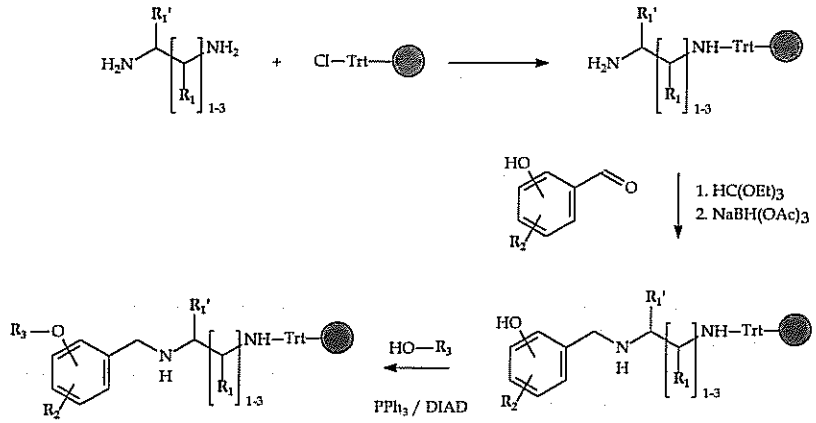

Fig. 21. Reaction scheme for a library of *N*-(alkoxy aryl)diamines.

We synthesized a number of model compounds to document the compatibility of chemical reactions in particular libraries. For each suggested library, all bifunctional building blocks listed in Figs. 1–4 have been tested and were shown to provide the expected product in acceptable purity. The purity of all model compounds was estimated by HPLC, all compounds corresponding to the main peak on the HPLC profile provided the expected signal on mass spectroscopy. NMR and MS/MS spectra of selected compounds were also measured. The synthesis of two libraries and the results found when the compounds were screened for a model biological target is also described; however, details of the MS/MS and biological results are presented elsewhere [39].

N-(Alkoxy acyl)amino acids The library was designed to be synthesized on both IdaDC linker as well as on hydroxy-TentaGel by direct esterification of hydroxyl groups with N-protected amino acids. The synthesis of the library included three randomization steps (Fig. 8):

(i) attachment of amino acids to the amino group of the IdaDC linker or the hydroxyl group of the resin beads;

(ii) coupling of aromatic hydroxy acids to the amino group of the amino acid;

(iii) etherification of the phenolic hydroxyl group with alcohols.

Not only alcohols (Fig. 5), but also N-Fmoc-protected amino alcohols (Fig. 3) were tested and could be used in the last randomization step. After the last randomization was finished, the side-chain protecting groups were cleaved by TFA. Since some ethers are acid-sensitive, we eliminated the corresponding alcohols. This included benzyl types of alcohols, in particular *p*-methoxybenzyl

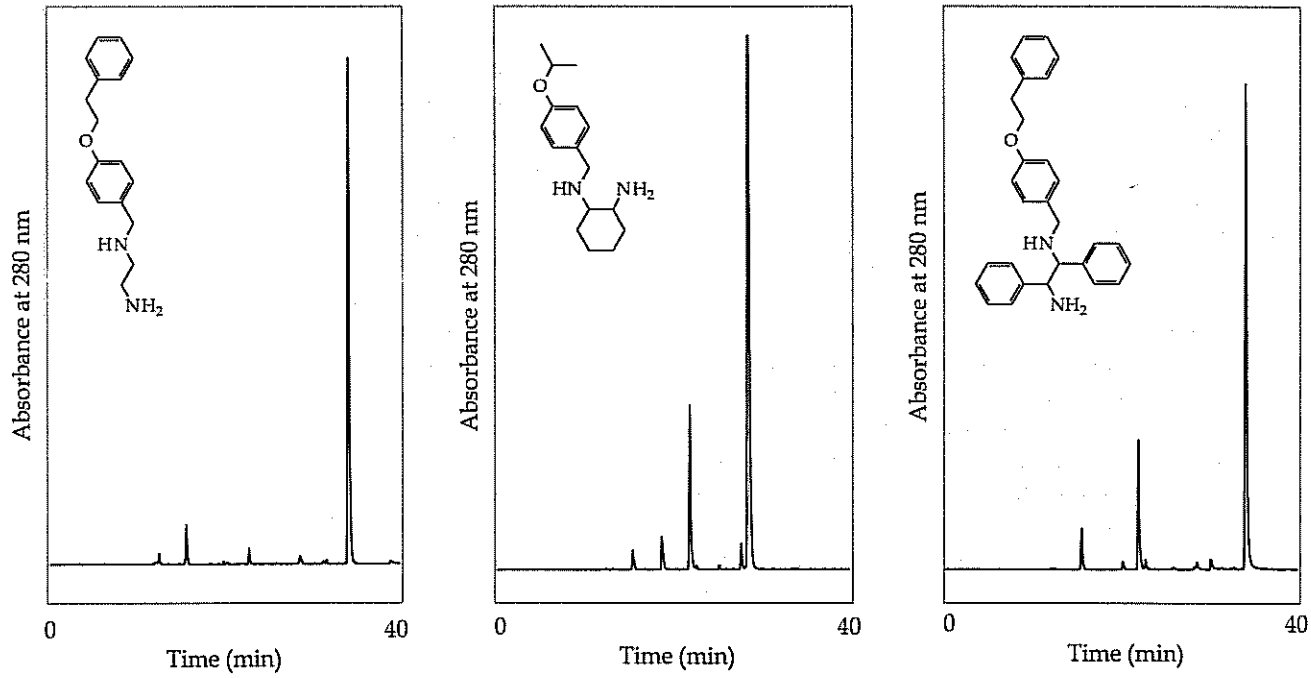

Fig. 22. HPLC traces of model compounds for a library of *N*-(alkoxy aryl)diamines.

and *p*-methylthiobenzyl alcohols. Alcohols that are not stable towards the final cleavage, such as furan-containing structures, had to be eliminated as well. Since the library was intentionally synthesized on double-cleavable linker (having the Boc protecting group on the iminodiacetic acid), it was not possible to eliminate the acidic treatment at the end of synthesis.

Before synthesizing the library, we synthesized a number of model compounds. Figure 9 documents the purity of crude material prepared on hydroxy-TentaGel, while Fig. 10 shows compounds synthesized on IdaDC linker. The first library composed of 4200 compounds was made on hydroxy-TentaGel using 20 proteinogenic amino acids, 10 aromatic hydroxy acids, and 21 alcohols [28,39]. The library was screened for binding to streptavidin and specific ligands were found. Their structures were elucidated by MS/MS experiments. The compounds were resynthesized, and binding was confirmed. Ligands were found that had been made from valine or leucine, *o*-hydroxybenzoic acid, and pyridinemethanol [39].

The second library has been synthesized on the IdaDC linker to provide double release of compounds into solution. An extended set of building blocks was used: 20 proteinogenic amino acids, 28 aromatic hydroxy acids, and 51 alcohols (complexity: 28 560 compounds).

N,N'-bis(Alkoxy acyl)diamino acids Model compounds for this library were synthesized on IdaDC linker as well as on hydroxy-TentaGel. There are four diamino acids commercially available: lysine, ornithine, diaminobutyric and diaminopropionic acids. In the synthesis of model compounds the α-amino group was protected by an Fmoc group. The Boc group was selected for the protection of the side-chain amino groups. The synthesis of the library consisted of four randomization steps (Fig. 11):

(i) coupling of aromatic hydroxy acids to the first amino group of the diamino acids;

(ii) etherification of phenolic hydroxyl groups with alcohols;

(iii) coupling of aromatic hydroxy acids to the second amino group of the diamino acid;

(iv) etherification of phenolic hydroxyl groups with alcohols.

Analytical HPLC profiles of model compounds are shown in Fig. 12. Both amino groups of the diamino acids were used for the same sequence of reactions. However, this library can be synthesized in different formats. Figure 13 shows the general structure of this family of libraries (structure II). Structure IIa shows the library described above. Three additional designs have the side-chain amino group either acylated (IIb), alkylated (IIc), or used to connect a heterocyclic moiety, a diketopiperazine (IId).

N-Acylamino ethers IdaDC linker again was used for the synthesis of model compounds. Synthesis of the library consisted of three randomization steps (Fig. 14):

(i) coupling of aromatic hydroxy acids to the amino group of the linker on the resin;

(ii) etherification of phenolic hydroxyl groups with N-protected amino alcohols;

(iii) coupling of carboxylic acids to the amino groups.

The analytical HPLC profiles of model compounds are shown in Fig. 15.

N-(Alkoxy acyl)amino alcohols This library was designed to provide compounds with a free hydroxyl group. Model compounds were synthesized on a single-cleavable ester linker; however, the carboxyl group was polymer-supported and the alcohol was in solution. Before the synthesis was started, a resin with available carboxyl groups was prepared by reacting TentaGel-S-NH_2 with the anhydride of glutaric acid. Synthesis of the library involved three randomization steps (Fig. 16):

(i) esterification of the polymer-supported carboxyl group with Fmoc-protected amino alcohols;

(ii) coupling of aromatic hydroxy acids to the amino groups;

(iii) etherification of phenolic hydroxyl groups with alcohols.

The first randomization involved ester-bond formation between the polymer-supported carboxylate and the hydroxyl groups of Fmoc-protected amino alcohols. We tested several esterification procedures, including: (i) HOBt/DIC/DMAP; (ii) HOBt/DIC in pyridine; and (iii) acid fluorides, but only the Mitsunobu redox condensation using DEAD and PPh_3 provided acceptable conversion. The extent of esterification was measured by quantification of the Fmoc release. The Mitsunobu esterification provided 70% conversion of polymer-supported carboxyl groups to esters, whereas the other three methods resulted in only marginal esterification (less than 10%).

The library synthesis ended with deprotection of side-chain protecting groups by TFA. The purity of the crude model compounds is shown in Fig. 17.

N-Alkylamino ethers We used hydroxy-TentaGel for the synthesis of the *N*-alkylamino ethers model compounds. Synthesis of the library consisted of four randomization steps (Fig. 18):

(i) coupling of amino acids to hydroxy-TentaGel;

(ii) reaction of aromatic hydroxy acids with polymer-supported amino groups;

(iii) etherification of phenolic hydroxyl groups with N-protected amino alcohols;

(iv) reductive alkylation of amino groups with aldehydes.

The analytical HPLC profiles of the model compounds are shown in Fig. 19. All resulting compounds have a secondary amine function and therefore one may wish to continue with a fifth reaction on the resin, e.g. acylation of the amine. The HPLC profiles of three such model compounds are shown in Fig. 20.

N-(Alkoxy aryl)diamines Model compounds were

synthesized on a trityl linker that allows release of compounds by treatment with acids. Synthesis of the library incorporated three randomization steps (Fig. 21):

(i) reaction of diamines with chlorotrityl resin;

(ii) reductive alkylation of amino groups with phenol aldehydes;

(iii) Mitsunobu aryl-ether formation with alcohols.

The analytical HPLC profiles of the model compounds are shown in Fig. 22. The synthesis may be continued, similarly as in the previous library, e.g. by acylating the secondary amino groups.

Conclusions

We have shown the usefulness of the production of structurally heterogeneous libraries with six sample library designs. From a limited set of five functional groups displayed on bifunctional building blocks, and by using mutually compatible chemical reactions to connect the functional groups in a variety of patterns, we have produced six quite diverse sets of compounds. Model compounds for all types of proposed libraries were produced in high yield, demonstrating the feasibility of the approach. We have obviously tested very few of the possible kinds of transformations of selected functionalities that could be imagined. Creativity in this respect is almost unlimited; David Weininger calculated that 'only' 10^{200} small organic compounds can be assembled [40].

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