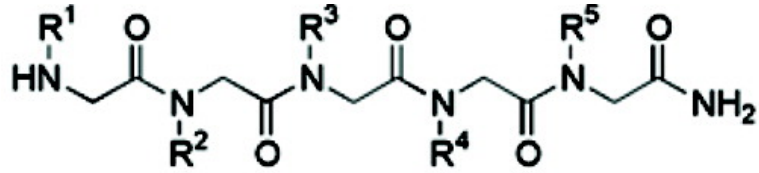
JOURNAL OF combinatorial CHEMISTRY

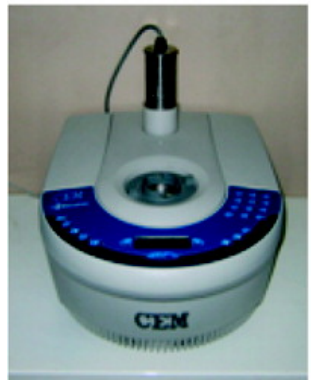
Article

Synthesis of a Positional Scanning Library of Pentamers of *N*-Alkylglycines Assisted by Microwave Activation and Validation via the Identification of Trypsin Inhibitors

Joaquim Messeguer, Nuria Corte#s, Nuria Garci#a-Sanz, Gloria Navarro-Vendrell, Antonio Ferrer-Montiel, and Angel Messeguer

J. Comb. Chem., **2008**, 10 (6), 974-980• DOI: 10.1021/cc800144x • Publication Date (Web): 11 October 2008





More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Synthesis of a Positional Scanning Library of Pentamers of *N*-Alkylglycines Assisted by Microwave Activation and Validation via the Identification of Trypsin Inhibitors

Joaquim Messeguer,[†] Nuria Cortés,[†] Nuria García-Sanz,[‡] Gloria Navarro-Vendrell,[†] Antonio Ferrer-Montiel,[‡] and Angel Messeguer*,[†]

Department of Chemical and Biomolecular Nanotechnology, Instituto de Química Avançada de Catalunya (IQAC), CSIC, Jordi Girona 18, 08034 Barcelona, Spain, and Instituto Biología Molecular y Celular, Universidad Miguel Hernández. 03202 Elche, Spain

Received August 27, 2008

A positional scanning library of 625 *N*-alkylglycine pentamers has been synthesized on solid-phase, employing a set of 10 commercially available primary amines as a source of chemical diversity. The iterative synthetic steps were carried out in tea bags and accelerated by using microwave assisted organic synthesis (MAOS). The reactivity study of the primary amines used as diversity sources led to determine their relative reactivity values and equireactivity factors, which were applied to the library synthesis to ensure comparable concentrations of all final oligomers in the mixtures. This library was validated by the screening, deconvolution, and identification of trypsin inhibitors. These compounds are of potential interest for controlling the intracellular transport of TRPV1 channel.

Introduction

Oligomers of N-substituted glycines, also known as peptoids, are a family of non-natural molecules with a broad variety of biological activities, thus making them attractive candidates for drug discovery. These compounds exhibit enhanced stability and bioavailability toward proteolysis relative to natural peptides.[1] Moreover, peptoids have a modular scaffold that makes them amenable to combinatorial strategies. The most-employed method for the preparation of peptoids is the solid-phase submonomer procedure developed by Zuckermann et al.[2,3] This method involves an iterative acylation reaction, with an α-haloacetyl moiety that is common to all backbone elongation processes and an iterative amination reaction employing the broad commercial availability of primary amines. Because the synthesis of peptoids can be easily automated, the split-and-mix format have been used to prepare peptoid libraries that have allowed the identification of high-affinity ligands for membrane receptors[1,4−6] and new antibacterial compounds.[7,8] More recently, the development of peptoids as drug carriers[9] or, in the case of cyclic derivatives, as inhibitors of integrins, have been reported.[10] Furthermore, the inherent conformational flexibility of these oligomers has led to their use for the disruption of protein−protein, protein−nucleic acid, and protein−membrane interactions.[11−13]

Houghten et al. introduced the concept of positional scanning format to synthesize solid-phase peptide-based libraries.[14,15] In addition to shortening the time required for the identification of active compounds, this format allows the use of the same library for the identification of hit compounds in different biological targets, thus avoiding the biological-assay-directed synthesis of sublibraries required in the split-and-mix procedure. In previous work carried out in our group, *N*-alkylglycine trimers identified from two combinatorial libraries constructed under the positional scanning format became promising active compounds against different biological targets: TRPV1 channel[16] and NMDA receptor open-channel blockers,[17] peptoids with in vivo neuroprotectant activity,[18] compounds exhibiting antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria,[19] modulators of the multidrug resistance phenotype (MDR),[20,21] Gram-negative lipopolysaccharide (LPS) neutralizing compounds,[22] or inhibitors of the apoptosome formation.[23]

However, from the practical point of view, solid-phase reaction rates are slower (up to 100-fold) than their solution-phase analog reactions.[24] This is an important point to optimize for the synthesis of a library and also for the resynthesis of the hits obtained after the deconvolution process. In this context, the application of microwave-assisted organic synthesis (MAOS) has been revealed as a powerful tool to enhance reaction rates in homogeneous and heterogeneous media. In recent years, MAOS has been applied to a wide range of organic reactions, resulting in a reduction in synthesis time, an improvement in product yield, or both.[25] In the peptoids field, Kodadek et al. reported the use of a domestic MAOS oven to accelerate the acylation and amination reactions in the synthesis of peptoids.[26] More recently, Blackwell et al. improved that procedure in terms

* To whom correspondence should be addressed. Phone: 34 93 400 61 21. Fax: 34 93 204 59 04. E-mail: ampqob@iiqab.csic.es.
[†] Institute of Chemical and Environmental Research (IQAC).
[‡] Universidad Miguel Hernández.

10.1021/cc800144x CCC: $40.75
Published on Web 10/11/2008

of safety and reproducible methodology by using a commercial MAOS reactor.[27]

Consequently, MAOS has become an attractive tool for acceleration of the preparation of combinatorial libraries. However, to take advantage of the time-saving aspect of MAOS, together with the benefits of solid-phase synthesis, it is important to determine the best suitable reaction vessel, particularly, when the construction of large libraries is pursued, to circumvent the one reaction at a time irradiation sequence. In this context, some authors have developed a strategy in multimode microwave reactors based on cellulose supports[28,29] or on multiwell plates,[30−32] although these techniques have the limitation of working at small scale. In case of monomode microwave reactors, Gellman et al. have worked with syringes provided with polyethylene frits inside a glass MAOS reaction vessel containing DMF and employing nitrogen flux for stirring.[33,34] However, this method does not permit to work with sealed MAOS vessels or adapt reflux condensers to the reaction vessel.

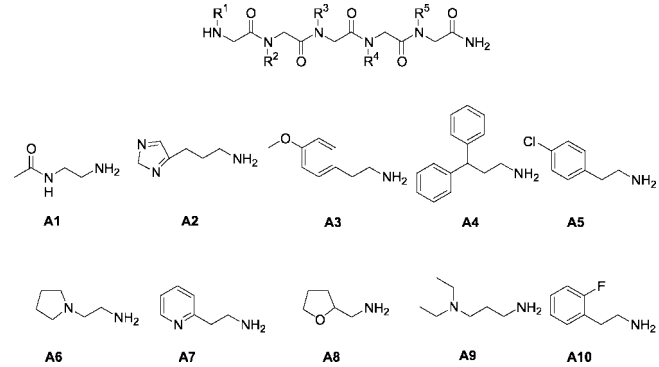
We report herein the design and synthesis of an optimized positional scanning combinatorial library of *N*-alkylglycine pentamers, by using MAOS for achieving high conversion rates and tea bags as reaction vessels. According to the requirements of the positional scanning format, a previous study of the building blocks employed in the chemical diversity was carried out to ensure comparable reactivities. Finally, the validation of this library by its screening for the identification of inhibitors of trypsin is also presented.

Results and Discussion

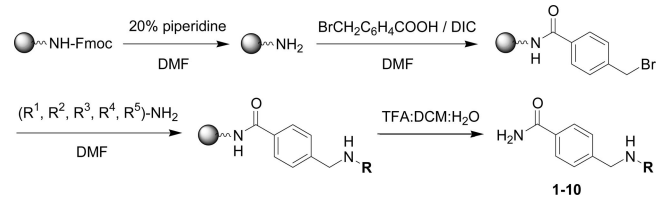
Design and Microwave-Assisted Synthesis of a Library of *N*-Alkylglycines in a Positional Scanning Format. The submonomer synthetic strategy was used for the synthesis of the peptoid library.[2,3] Therefore, the chemical diversity of the *N*-alkylglycine pentamers was introduced by primary amines in the amination steps. Criteria of hydrophobicity, lipophilicity, aromatic residues, polar chains, and hydrogen-bond acceptors were evaluated to select the appropriate diversity. To avoid the undesired side-reactions observed in a previous library of *N*-alkylglycine trimers synthesized in our group,[19] amines with an additional tertiary amino group in their structure were employed only for the N-terminal position of the pentamers. We also considered the most bioactive structures derived from the screening of our previous peptoid libraries.[16−19,21,22] Scheme 1 shows the set of 10 commercially available primary amines chosen for the library.

Amines **A1**−**A5** were introduced at R^2, R^3, and R^4 diversity positions, whereas amines **A6**−**A10** were introduced at the R^5 diversity position. The R^5 position was fixed with amine **A1** to provide pentamers with acceptable solubility. According to the positional scanning format, the library was divided into four sublibraries: OXXX, XOXX, XXOX, and XXXO, in which O represents a defined diversity position and X refers to a random position. Because the positional scanning format demands that the different building blocks employed in the diversity positions exhibit comparable reactivity, a previous study of the primary amines selected for the chemical diversity was carried out. With regards to MAOS, a careful study was also performed to determine the best reaction conditions.

Scheme 1. (Top) General Structure of a *N*-Alkylglycine Pentamer, Where R^1, R^2, R^3, R^4, and R^5 Represent the Chemical Diversity Employed for the Peptoid Library, and (Bottom) the 10 Primary Amines Selected for the Construction of the Library



Scheme 2. Synthesis of the Benzamides Employed for the Reactivity Studies on the Amines Used for the Construction of the Library



(a). Optimization of MAOS for *N*-Alkylglycine Pentamers. Toward this aim, different variables were considered, including solvents, reagent equivalents and concentration, reaction time, power, and temperature. We concluded that an efficient submonomer synthesis of a *N*-alkylglycine pentamer was achieved by mild acylation reaction conditions (0.4 M bromoacetic acid and 0.4 M *N*,*N*′-diisopropylcarbodiimide in DMF, 150 W, 35 °C, 30 s), whereas more severe conditions were required for the amination steps (0.4 M amine in DMF, 150 W, 90 °C, 1.5 min). These conditions were similar to those reported by Blackwell et al.,[35] although the reagent concentration was drastically reduced in our synthesis. This MAOS optimization was carried out in the same reaction vessel (a 50 mL round bottomed flask) used for the synthesis of the library and with the solid support confined in sealed tea bags. Moreover, the reaction conditions established for one tea bag in the flask were transferred to the real situation in the library (five tea bags per flask), obtaining satisfactory results with comparable purities and yields for the products isolated from all tea bags.

(b). Reactivity Study of the Primary Amines. A simple methodology based on HPLC was established to evaluate the reactivity of primary amines in the amination step. Reactions were carried out on solid-phase via the sequence shown in Scheme 2.

Thus, after the initial release of the Fmoc protecting group from the Rink amide resin, an acylation reaction with 4-bromomethylbenzoic acid was conducted. This acid replaced the bromoacetic acid used in the synthesis of the library and allowed UV monitoring of the resulting com-

Table 1. Relative Reactivity Values and Equireactivity Factors Determined for the 10 Primary Amines Used in the Model Amination Reaction Shown in Scheme 2[a]

group A			group B		
amine	relative reactivity	equireactivity factor	amine	relative reactivity	equireactivity factor
A1	0.73	1.37	**A6**	1.00	1.00
A2	1.00	1.00	**A7**	0.46	2.18
A3	0.95	1.05	**A8**	0.47	2.15
A4	0.78	1.28	**A9**	0.90	1.11
A5	0.76	1.32	**A10**	0.31	3.24

[a] **A2** and **A6** were taken as the internal reference amine for each group, respectively.

Table 2. Relative Reactivity Values Determined for the 10 Primary Amines, After Application of the Equireactivity Factors in the Amination Reaction Shown in Scheme 2[a]

group A		group B	
amine	relative reactivity	amine	relative reactivity
A1	0.98	**A6**	1.00
A2	1.00	**A7**	0.89
A3	1.01	**A8**	0.95
A4	0.98	**A9**	0.97
A5	1.00	**A10**	0.87

[a] **A2** and **A6** were taken as the internal reference amine for each group, respectively.

pounds. The reaction conditions used in the amination step under study were the same employed for the construction of the library. Thus, an equimolar mixture of the five primary amines was added to the resin, and MAOS was used to accelerate the amination reaction (150 W, 90 °C, hold 1.5 min). The resulting benzamides were released from the resin and analyzed by HPLC using *N*-3,3-diphenylpropylacetamide as external standard. Then, the concentration ratio of the benzamides with respect to the internal reference for each group (**A2** for group A and **A6** for group B) afforded the relative reactivity values and the equireactivity factors for the amines of both groups (Table 1).

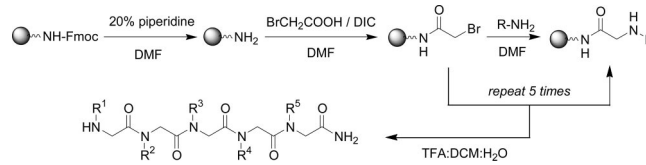
In accordance with the equireactivity factors obtained, primary amines were tested again following the same procedure, but in the amination step, the resin was treated with an equireactive mixture of the five amines, for each group. From the HPLC analysis of the corresponding crude reaction mixtures, the concentration of each benzamide derivative was calculated. As above, the ratio of benzamides with respect to the internal reference for each group afforded the relative reactivity values for the amines of both groups (Table 2).

These results showed comparable benzamide concentrations in the crude reaction mixtures. Likewise, a previous reactivity study carried out under room temperature conditions showed similar reactivity values for the selected primary amines. Taken together, this reactivity test confirmed that the use of amines concentrations in accordance with their equireactivity factors results in comparable amounts of the expected products in the final mixtures.

(c). Synthesis of the Peptoid Library. The *N*-alkylglycine pentamer library was synthesized in a twelve-step synthetic sequence (Scheme 3).

The library was organized in 20 controlled mixtures, separated in 4 sublibraries containing 125 compounds per

Scheme 3. MAOS-Assisted Solid-Phase Synthesis of the Library of Pentamers of *N*-Alkylglycines



mixture for a total of 625 library compounds. The Rink amide resin was confined in 20 sealed tea bags, and the Fmoc protecting groups were released from the resin. Successive steps of acylation with bromoacetic acid, followed by the corresponding amination of the bromomethyl intermediate using the specific primary amine or the equireactive mixture of the 5 amines, were conducted. Apart from the initial deprotection, all reactions were carried out under MAOS in accordance with the experimental conditions described above. Finally, peptoids were released from the resin by using a 60:40:2 trifluoroacetic acid/dichloromethane/water mixture. The MS analysis (flow injection analysis, FIA) of the 20 mixtures showed a Gaussian distribution of the 125 compounds present in each mixture (for a representative example, see Figure 1). These profiles suggest that all the library compounds were present in comparable concentrations in the different mixtures. It should be noted that the overall time required for the synthesis of this library was approximately 10 h (not including the cleavage step), in contrast with the 4−5 days required for an equivalent synthesis carried out without MAOS activation.

Identification of Library Components Active As Inhibitors of Trypsin. The library was validated through the screening, deconvolution, and identification of compounds active as inhibitors of trypsin. This enzyme acts as an activator of the protease-activated receptor (PAR) family that plays a key role as trasducers of proteinase-mediated signaling in inflammation and immune responses.[36] Thus, potent and selective inhibitors of this protease may have an important impact on wound healing, psoriaris, pancreatitits, and other inflammatory maladies.[37] Screening of the peptoid library was carried out using an in vitro assay based on

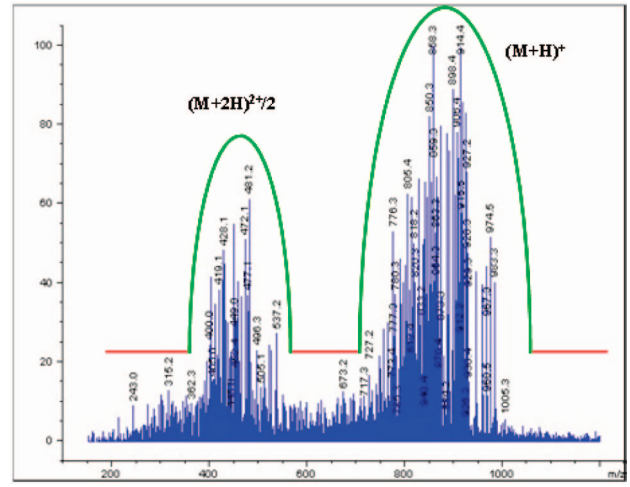


Figure 1. Profile of the mass spectra values for a representative mixture of the *N*-alkylglycine pentamers library. The Gaussian distribution of *m*/*z* and (*m*/*z*)/2 values are indicated.

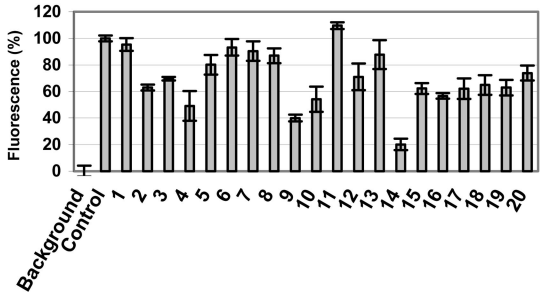


Figure 2. Screening of the library of *N*-alkylglycine pentamers for inhibition of trypsin (for details, see Experimental Section). The individual bars in the panel represent inhibition capacity for each mixture (at 0.2 mg/mL), with the *x*-axis representing the defined amine (_O_position), for the five separate positional sublibraries: $OXXXR^5$, $XOXXR^5$, $XXOXR^5$, $XXXOR^5$, and $XXXXR^5$. Mixtures 2, 4, 9, 10, and 14−18 were selected for deconvolution according to their activity and cytotoxicity. Values for background and negative control are also indicated.

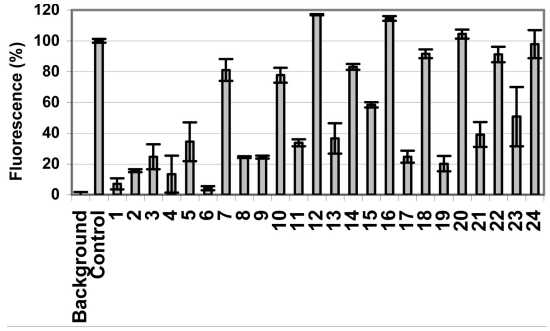


Figure 3. Screening of the 24 *N*-alkylglycine pentamers selected from the deconvolution for inhibition of trypsin (for details, see Experimental Section). The individual bars in the panel represent inhibition capacity for each pentamer (at 0.1 mg/mL). Values for background and negative control are also indicated.

measuring the enzymatic activity on a fluorescently labeled substrate. This assay is readily adapted to a HTS format, thus allowing the efficient screening of combinatorial libraries. Defined mixtures from the library revealed the presence of trypsin inhibitors. As shown in Figure 2, quantification of the inhibitory activity of the mixtures at 0.2 mg/mL led to the selection of the most active mixtures diversity positions 2 and 4 for R^1 (N-terminal position sublibrary $OXXXR^5$), 9 and 10 for R^2 ($XOXXR^5$), 14 and 15 for R^3 ($XXOXR^5$), and 16−18 for R^4 ($XXXOR^5$).

These amines were selected because of their relatively high activity and low cytotoxicity. When considered together, the screening data provided sufficient information for the identification of the 24 compounds in the library. These compounds were independently synthesized, and their inhibitory activity of trypsin was evaluated at 0.1 mg/mL (Figure 3). In addition, the dose response of the compounds was obtained. Furthermore, the enzymatic selectivity of more active compounds was determined by evaluating their effect on the endopeptidase elastase (Figure 1 and Supporting Information). Pentamers **11**−**13** (bars 11, 17, and 19, respectively in this figure) (Scheme 4), displayed the lowest inhibitory efficacy and, therefore, the highest selectivity toward trypsin. Full details regarding the in vitro and in vivo activity and toxicity studies of these peptoid hits will be reported elsewhere.

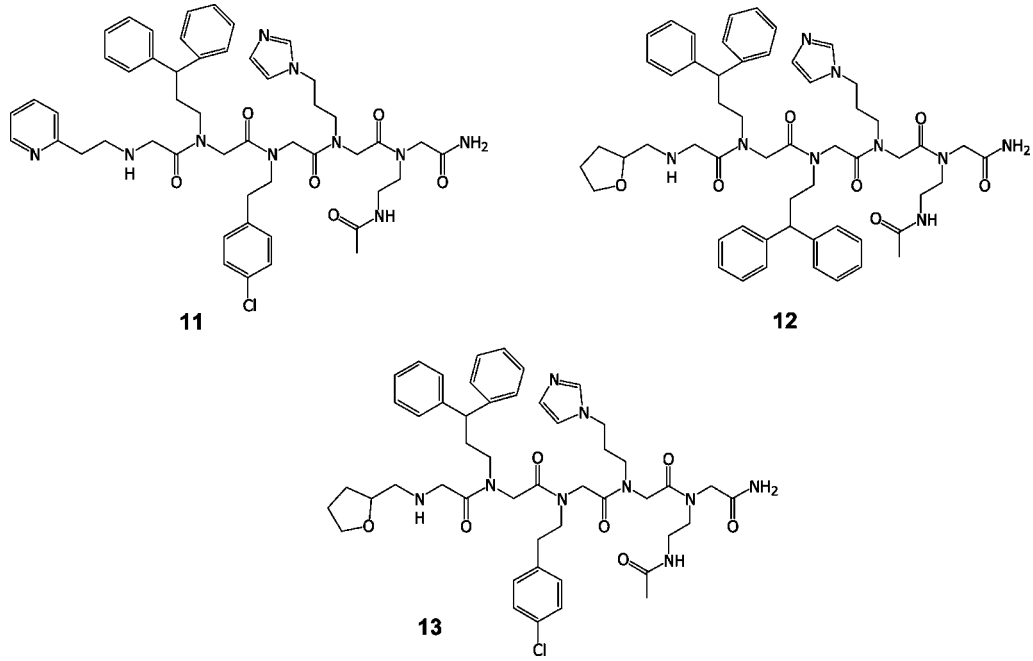
In conclusion, a positional scanning library of 625 *N*-alkylglycine pentamers has been synthesized on solid-phase supports, employing a set of 10 commercially available primary amines as a source of chemical diversity. The iterative synthetic steps were accelerated by using MAOS, and to the best of our knowledge, this is the first report on the construction of a positional scanning library of controlled mixtures using the tea bags methodology under this activation procedure. In addition, a careful reactivity study of the primary amines used as diversity sources allowed determination of their relative reactivity values and equireactivity factors, which were applied to the library synthesis to ensure that comparable concentrations of final oligomers in the mixtures will be obtained. This library was validated by the screening, deconvolution, and identification of active oligomers as trypsin inhibitors.

Experimental Section

General. Solvents, amines, and other reagents were purchased from commercial suppliers and were used without further purification. MAOS-assisted reactions were performed in a monomode CEM Discover microwave reactor, except for the primary amine capture by a scavenger resin used in the preparation of N-substituted aminobenzamides, which was carried out in a LGMS-197H domestic MAOS oven. The NMR spectra were recorded on a Varian Inova 500 apparatus (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz), Mercury 400 apparatus (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz), and Unity 300 apparatus (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz). The RP-HPLC analyses were performed with a Hewlett-Packard Series 1100 (UV detector 1315A) modular system using a reverse-phase Kromasil 100 C8 (25 × 0.46 cm, 5 μm) column, with CH_3CN-buffer ammonium formate (20 mM, pH 5.0) mixtures at 1 mL/min as mobile phase and monitoring at 220 nm. Semipreparative RP-HPLC was performed with a Waters (Milford, MA, U.S.A.) system using a Kromasil 100 C8 (250 × 20 cm, 5 μm) column, with CH_3CN/H_2O mixtures containing 0.1% TFA at 10 mL/min as mobile phase. The GC analyses were performed in a Hewlett-Packard 5890 Series II (FID detector) system using a SPB-5 capillary column. High-resolution mass spectra (HRMS-FAB) were carried out at the Mass Spectrometry Service of the University of Santiago de Compostela (Spain) and at the Mass Spectrometry Service of the IQAC. The melting points (mp) were determined in a Reichert-Jung 286238 system with a Crison T-637 probe and are uncorrected.

Synthesis of the Peptoid Library. The synthesis of the library was carried out on a 1% cross-linked polysterene resin bearing the Fmoc-protected Rink amide linker AM RAM (0.79 mmol/g, Rapp Polymer; Germany). The twelve-step protocol was similar to that previously described.[19,21] The TNBS test was used to monitor the initial Fmoc deprotection and the chloranil test for checking the acylation and amination steps. The 20 tea bags of the library were filled with 100 mg of Rink amide resin (0.79 mmol/g load, 0.079 mmol). The common initial Fmoc deprotection was carried out by placing the tea bags in a 500 mL polypropylene bottle, whereas for the subsequent steps, the tea bags were divided

Scheme 4. Structure of the Peptoid Hits Identified As Trypsin Inhibitors[a]



[a] Compounds **11**−**13** correspond to bars 11, 17, and 19, respectively in Figure 3.

in four groups (one per sublibrary), and the reactions were performed in a 50 mL round bottomed flask.

1. Deprotection. A solution of 250 mL of 20% piperidine in DMF was added to the 500 mL bottle containing the 20 tea bags, and the mixture was stirred for 30 min at 20 °C. The resin was drained and washed with DMF (3 × 150 mL), ^{i}PrOH (3 × 150 mL), and CH_2Cl_2 (3 × 150 mL). The treatment was carried out in duplicate.

2. Acylation. The tea bags corresponding to the first sublibrary (numbered 1−5) were placed in the 50 mL round-bottomed flask containing a magnetic stirring bar and were treated with a solution of bromoacetic acid (2.4 mmol/bag, 30 equiv) and *N*,*N*′-diisopropylcarbodiimide (2.4 mmol/bag, 30 equiv) in 30 mL of DMF. The flask was placed inside the monomode microwave cavity equipped with a Dimroth condenser, and the resin was irradiated at atmospheric pressure (150 W, 35 °C, hold 30 s). Then, the tea bags were placed in a 20 mL polypropylene bottle, and the resin was drained and washed with DMF (3 × 10 mL), ^{i}PrOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). This treatment was repeated for the other three sublibraries (tea bags numbered 6−10, 11−15, and 16−20, respectively).

Amination Conditions. (a). Defined positions. The tea bags corresponding to the sublibrary with a defined position were separated and introduced individually in a 50 mL round-bottomed flask. The desired primary amine (4 mmol) in 10 mL of DMF was added, and the resulting mixture was irradiated (150 W, 90 °C, hold 1.5 min). The tea bag was placed in a 20 mL polypropylene bottle, and the resin was drained and washed with DMF (3 × 10 mL), ^{i}PrOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL).

(b). Random Positions. The tea bags corresponding to the sublibraries with a random position were placed in 50 mL round-bottomed flasks (one flask per sublibrary). An equireactive mixture of the five primary amines (2.4 mmol/bag, 30 equiv) was added, and the resulting mixture was irradiated (150 W, 90 °C, hold 1.5 min). Then, the tea bags were placed in a 20 mL polypropylene bottle, and the resin was drained and washed with DMF (3 × 10 mL), ^{i}PrOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL).

3. Cleavage. The tea bags were placed individually in 15 mL polypropylene tubes, and the resin was treated with a mixture of 60:40:2 (v/v/v) TFA/CH_2Cl_2/H_2O for 30 min at room temperature. The cleavage mixtures were filtered, and the solvent was removed under reduced pressure, followed by lyophilization. Finally, all 20 mixtures were stored at −20 °C until use. The different mixtures were analyzed by HPLC-MS; Figure 1 shows a representative example of the Gaussian distribution of peaks at the average M^+ and $M^+/2$ expected values for one of the mixtures.

Synthesis of Individual Peptoids. Individual *N*-alkylglycine pentamers were synthesized following the twelve-step procedure described above with slight modifications (e.g., use of 5 equiv excess of reagents), and the reactions were carried out in 10 mL polypropylene syringes. Compounds were purified from the crude reaction mixture by semi-preparative HPLC using a Kromasil C8 (25 × 2 cm, 5 μm) column, CH_3CN/H_2O mixtures containing 0.1% TFA as mobile phases, and a flow rate of at 5 mL/min. Selected bioactive pentamers **11**−**13** (Scheme 4) were fully characterized. The occurrence of different conformations resulted in very complex spectra. The absorptions assigments were elucidated by additional gDQCOSY experiments. The signals described are for major conformers.

[*N*-(2-(2′-Pyridinyl)ethyl)glycyl]-[*N*-(3′,3′-diphenylpropyl)glycyl]-[*N*-(2-(4′-chlorophenyl)ethyl)glycyl]-[*N*-(3-(1′-imidazolyl)propyl)glycyl]-*N*-(2-acetamide)ethyl)glycinamide (11): ^{1}H NMR (500 MHz, CD_3OD) δ 8.88 (1 H, H_{Imid}), 8.60 (1 H, H_{pyr}), 7.81 (1 H, H_{pyr}), 7.64 (1 H, H_{Imid}), 7.48 (1 H, H_{Imid}), 7.34−7.16 (14H H_{Ar} + 2H H_{pyr}), 4.54−3.71 (10 H,

$COCH_2N$), 4.25 (2 H, $NCH_2CH_2CH_2$), 3.92 (1 H, Ph_2CH), 3.56 (2H, CH_2CH_2Ph), 3.48 (2H, $NCH_2CH_2CH_2$), 3.45−3.31 (4H, $CH_2CH_2NHCO + CH_2CH_2Pyr$), 3.35−3.14 (2H, CH_2CH_2CH), 3.23−3.13 (4H, $CH_2CH_2NHCO + CH_2CH_2Pyr$), 2.89−2.80 (2 H, CH_2CH_2Ph), 2.39−2.18 (2 H, CH_2CH), 2.08 (2 H, $CH_2CH_2CH_2$), 1.94 (3H, $COCH_3$); ^{13}C NMR (100 MHz, CD_3OD): δ 171.0 (CO), 167.6 (CO), 162.3 (CO), 161.9 (CO), 161.5 (CO), 161.1 (CO),157.2 (C_{Pyr}), 148.2 (CH_{Pyr}), 145.9 (C_{Ar}), 145.5 (C_{Ar}), 141.6 (CH_{Pyr}), 139.2 (C_{Ar}), 136.5 ($NCH_{Imid}N$), 132.3 ($C_{Ar}Cl$), 131.7 (2 × CH_{Ar}), 129.6 (4 × CH_{Ar}), 128.9 (4 × CH_{Ar}), 127.7 (2 × CH_{Ar}), 127.4 (2 × CH_{Ar}), 126.1 (CH_{Pyr}), 124.8 (CH_{Pyr}), 123.4 (NCH_{Imid}), 121.0 (NCH_{Imid}), 51.2 (CH_2CONH_2), 51.0 ($NHCH_2CO$), 50.7 (Ph_2CH), 50.4 (NCH_2CO), 50.3 (NCH_2CO), 50.2 (NCH_2CO), 47.9 ($CH_2CH_2Pyr + NCH_2CH_2CH_2$), 47.6 ($NCH_2CH_2Ph + NCH_2CH_2$ NH), 46.1 ($NCH_2CH_2CH_2$), 39.2 ($Ph_2CHCH_2CH_2$), 38.2 ($CH_3CONHCH_2$), 34.4 (CH_2Pyr), 33.9 (CH_2Ph), 32.6 (Ph_2CHCH_2), 29.6 ($CH_2CH_2CH_2$), 22.8 (CH_3CO); HRMS calcd for $C_{50}H_{61}ClN_{10}O_6$ 933.4542 $(M + H)^+$, found 933.4546.

[*N*-(2′-Tetrahydrofuranyl)methyl)glycyl]-[*N*-(3′,3′-diphenylpropyl)glycyl]-[*N*-(3′,3′-diphenylpropyl)glycyl]-[*N*-(3-(1′-imidazolyl)propyl)glycyl]-*N*-(2-acetamide)ethyl)glycinamide (12): ^1H NMR (500 MHz, CD_3OD) δ 8.86 (1 H, H_{Imid}), 7.56 (1 H, H_{Imid}), 7.46 (1 H, H_{Imid}), 7.31−7.10 (20 H, H_{Ar}), 4.54−3.67 (10 H, $COCH_2N$), 4.18 (2 H, $NCH_2CH_2CH_2$), 4.16 (1 H, OC*H*), 4.14−4.10 (2H, Ph_2CH), 3.86 (2H, OCH_2), 3.80 (2H, NCH_2CH_2NH), 3.54−3.38 (4H, $NCH_2CH_2NH + CH_2CH_2CH_2$), 3.35−3.21 (4H, $Ph_2CHCH_2CH_2$), 3.13−3.10 (1H, $CHCH_{a,b}NH$), 2.95−2.88 (1H, $CHCH_{b,a}NH$), 2.44−2.28 (4H, Ph_2CHCH_2), 2.10 (1H, $OCHCH_{a,b}$), 2.06 (2 H, $NCH_2CH_2CH_2$), 1.98 (2H, OCH_2CH_2), 1.96−1.90 (3H, $COCH_3$), 1.59 (1H, $OCHCH_{b,a}$); ^{13}C NMR (100 MHz, CD_3OD) δ 171.0 (CO), 167.6 (CO), 163.2 (CO), 163.1 (CO), 162.6 (CO), 162.5 (CO), 146.0 (2 × C_{Ar}), 145.6 (2 × C_{Ar}), 136.6 (NCH_{Imid} N), 129.8 (8 × CH_{Ar}), 129.6 (8 × CH_{Ar}), 127.5 (4 × CH_{Ar}), 123.4 (NCH_{Imid}), 121.1 (NCH_{Imid}), 75.2 (O*CH*), 69.6 (OCH_2), 65.0 ($CHCH_2NH$), 52.5 (2 × Ph_2CH), 50.8 (CH_2CONH_2), 50.6 ($NHCH_2CO$), 50.4 (NCH_2CO), 50.3 (NCH_2CO), 50.2 (NCH_2CO), 47.9 ($NCH_2CH_2CH_2$), 47.7 (NCH_2CH_2NH), 46.1 ($NCH_2CH_2CH_2$), 39.1 ($Ph_2CHCH_2CH_2$), 38.2 ($CH_3CONHCH_2$), 34.6 (Ph_2CHCH_2), 34.4 (Ph_2CHCH_2), 30.8 ($OCHCH_2$), 30.2 ($CH_2CH_2CH_2$), 26.5 (OCH_2CH_2), 22.8 (CH_3CO); HRMS calcd for $C_{55}H_{69}N_9O_7$ 968.5398 $(M + H)^+$, found 968.5400.

[*N*-(2′-Tetrahydrofuranyl)methyl)glycyl]-[*N*-(3′,3′-diphenylpropyl)glycyl]-[*N*-(2-(4′-chlorophenyl)ethyl)glycyl]-[*N*-(3-(1′-imidazolyl)propyl)glycyl]-*N*-(2-acetamide)ethyl)glycinamide (13): ^1H NMR (500 MHz, CD_3OD) δ, 8.86 (1 H, H_{Imid}), 7.64 (1 H, H_{Imid}), 7.51 (1 H, H_{Imid}), 7.32−7.14 (14 H, H_{Ar}), 4.55−3.66 (10 H, $COCH_2N$), 4.28−4.22 (2 H, $NCH_2CH_2CH_2$), 4.17 (1 H, OC*H*), 3.98−3.95 (1H, Ph_2CH), 3.90−3.79 (1H, OCH_2), 3.56 (2H, CH_2CH_2Ph), 3.53−3.47 (2H, $NCH_2CH_2CH_2$), 3.49 (2H, NCH_2CH_2NH), 3.37 (2H, NCH_2CH_2NH), 3.34−3.07 (2H, $Ph_2CHCH_2CH_2$), 2.95−2.86 (2H, $CHCH_2NH$), 2.80 (2 H, CH_2CH_2Ph), 2.30−2.26 (2H, Ph_2CHCH_2), 2.11 (1H, $OCHCH_{a,b}$), 2.09 (2 H, $CH_2CH_2CH_2$), 1.99−1.90 (3H, $COCH_3$), 1.95 (2H, OCH_2CH_2), 1.31 (1H, $OCHCH_{b,a}$); ^{13}C NMR (100 MHz, CD_3OD) δ 171.0 (CO), 167.5 (CO), 162.4 (CO), 162.0 (CO), 161.7 (CO), 161.4 (CO), 145.9 (C_{Ar}), 145.5 (C_{Ar}), 139.2 (C_{Ar}), 136.5 ($NCH_{Imid}N$), 132.2 ($C_{Ar}Cl$), 131.6 (2 × CH_{Ar}), 129.9 (4 × CH_{Ar}), 128.9 (4 × CH_{Ar}), 127.7 (2 × CH_{Ar}), 127.4 (2 × CH_{Ar}), 123.4 (NCH_{Imid}), 121.0 (NCH_{Imid}), 75.2 (O*CH*), 69.6 (OCH_2), 64.2 ($CHCH_2NH$), 52.6 (Ph_2CH), 52.4 (CH_2CONH_2), 51.1 ($NHCH_2CO$), 50.5 (NCH_2CO), 50.4 (NCH_2CO), 50.3 (NCH_2CO), 48.2 ($NCH_2CH_2CH_2$), 48.1 (NCH_2CH_2Ph), 47.9 (NCH_2CH_2NH), 46.1 ($NCH_2CH_2CH_2$), 39.1 ($Ph_2CHCH_2CH_2$), 38.2 ($CH_3CONHCH_2$), 34.4 (CH_2Ph), 33.9 (Ph_2CHCH_2), 30.8 ($OCHCH_2$), 30.3 ($CH_2CH_2CH_2$), 26.5 (OCH_2CH_2), 22.9 (CH_3CO); HRMS calcd for $C_{48}H_{62}ClN_9O_7$ 912.4539 $(M + H)^+$, found 912.4537.

Screening of the Peptoid Library. Library mixtures were resuspended in 5% DMSO at a 10 mg/mL concentration and assayed at 0.2 mg/mL (mixtures with R1 fixed were screened also at 0.3 mg/mL to detect differences in activity). Mixtures were preincubated in 96-well microplates containing trypsin (0.2 units, TPCK treated from bovine pancreas, T1426, Sigma) for 1 h at 22 °C, under stirring. Thereafter, the substrate (BODIPY-FL casein, EnzChek Protease Assay Kit, Molecular Probes) was added to the incubation mixtures at a 5 μg/mL concentration, and incubation was prolonged for 1 h at 20 °C, under stirring, and protected from light. The nonfluorescent substrate is digested by trypsin-releasing fluorescent fragments that were monitorized using a FLUOstar galaxy apparatus (BMG), using the 485 and 530 nm filters for excitation and emission, respectively. The results obtained are shown in Figure 2. A similar experimental paradigm was used for measuring the inhibitory activity of individual peptoids. Compound stocks were prepared at 10 mg/mL in 5% DMSO. Dose−response curves were obtained by measuring the inhibitory activity at increasing concentrations of the peptoids. Dose−response relationships were fitted to a Michaelis−Menten binding isotherm to obtain the concentration that inhibits half of the maximal response (IC_{50}) and steepness of the curve.

Acknowledgment. Financial support from Ministery of Education and Science (Grants CTQ 2005-00995 and SAF2006-02580) are acknowledged. Fellowships to N.C. from the Generalitat de Catalunya and to G.N. from CSIC are also acknowledged.

Supporting Information Available. Synthesis of benzamides in solution, preparation of N-substituted aminobenzamides **1**−**10**, synthesis of the external standard for the calibration curves for benzamides **1**−**10**, reactivity study of the primary amines, and assay for inhibition of elastase (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657–2662.
(2) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646–10647.
(3) Figliozzi, G. M.; Goldsmith, R.; Ng, S. C.; Banville, S. C.; Zuckermann, R. N. *Methods Enzymol.* **1996**, *267*, 437–447.
(4) Gibbons, J. A.; Hancock, A. A.; Vitt, C. R.; Knepper, S.; Buckner, S. A.; Brune, M. E.; Milicic, I.; Kerwin, J. F.; Richter, L. S.; Taylor, E. W.; Spear, K. L.; Zuckermann, R. N.; Spellmeyer, D. C.; Braeckman, R. A.; Moos, W. H. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 885–899.

(5) Heizmann, G.; Hildebrand, P.; Tanner, H.; Ketterer, S.; Pansky, A.; Froidevaux, S.; Beglinger, C.; Eberle, A. N. *J. Recept. Signal Transduction Res.* **1999**, *19*, 449–466.
(6) Udugamasooriya, D. G.; Dineen, S. P.; Brekken, R. A.; Kodadek, T. *J. Am. Chem. Soc.* **2008**, *130*, 5744–5752.
(7) Goodson, B.; Ehrhardt, A.; Ng, S.; Nuss, J.; Johnson, K.; Giedlin, M.; Yamamoto, R.; Moos, W. H.; Krebber, A.; Ladner, M.; Giacona, M. B.; Vitt, C.; Winter, J. *Antimicrob. Agents Ch.* **1999**, *43*, 1429–1434.
(8) Ng, S.; Goodson, B.; Ehrhard, A.; Siani, M.; Winter, J. *Bioorg. Med. Chem.* **1999**, *7*, 1781–1785.
(9) Schröder, T.; Niemeier, N.; Afonin, S.; Ulrich, A. S.; Krug, H. F.; Bräse, S. *J. Med. Chem.* **2008**, *51*, 376–379.
(10) Vercillo, O. E.; rade, C. K. Z.; Wessjohann, L. A. *Org. Lett.* **2008**, *10*, 205–208.
(11) Daelemans, D.; Schols, D.; Witvrouw, M.; Pannecouque, C.; Hatse, S.; VanDooren, S.; Hamy, F.; Klimkait, T.; DeClercq, E.; Vandamme, A. M. *Mol. Pharmacol.* **2000**, *57*, 116–124.
(12) Hamy, F.; Felder, E. R.; Heizmann, G.; Lazdins, J.; Aboulela, F.; Varani, G.; Karn, J.; Klimkait, T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3548–3553.
(13) Murphy, J. E.; Uno, T.; Hamer, J. D.; Cohen, F. E.; Dwarki, V.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1517–1522.
(14) Pinilla, C.; Appel, J. R.; Blanc, P.; Houghten, R. A. *Biotechniques* **1992**, *13*, 901–902.
(15) Dooley, C. T.; Houghten, R. A. *Life Sci.* **1993**, *52*, 1509–1517.
(16) García-Martínez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; Peña, E.; Sanchez-Baeza, F.; Carbonell, T.; Felipe, C.; Pérez-Payá, E.; Belmonte, C.; Messeguer, A.; Ferrer-Montiel, A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2374–2379.
(17) Planells-Cases, R.; Montoliu, C.; Humet, M.; Fernández, A. M.; García-Martínez, C.; Valera, E.; Merino, J. M.; Pérez-Payá, E.; Messeguer, A.; Felipo, V.; Ferrer-Montiel, A. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 163–173.
(18) Montoliu, C.; Humet, M.; Canales, J-J.; Burda, J.; Planells-Cases, R.; Sánchez-Baeza, F.; Carbonell, T.; Pérez-Payá, E.; Messeguer, A.; Ferrer-Montiel, A.; Felipo, V. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 29–36.
(19) Humet, M.; Carbonell, T.; Masip, I.; Sánchez-Baeza, F.; Mora, P.; Cantón, E.; Gobernado, M.; Abad, C.; Pérez-Payá, E.; Messeguer, A. *J. Comb. Chem.* **2003**, *5*, 597–605.
(20) Abad-Merin, M. J.; Cortés, N.; Masip, I.; Pérez-Payá, E.; Ferragut, J. A.; Messeguer, A.; Ferrer-Montiel, A. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 112–120.
(21) Masip, I.; Cortés, N.; Abad, M. J.; Guardiola, M.; Pérez-Payá, E.; Ferragut, J.; Ferrer-Montiel, A.; Messeguer, A. *Bioorg. Med. Chem.* **2005**, *13*, 1923–1929.
(22) Mora, P.; Masip, I.; Cortés, N.; Marquina, R.; Merino, R.; Merino, J.; Carbonell, T.; Mingarro, I.; Messeguer, A.; Pérez-Payá, E. *J. Med. Chem.* **2005**, *48*, 1965–1968.
(23) Malet, G.; Martín, A.; Orzáez, M.; Vicent, M. J.; Masip, I.; Sanclimens, G.; Mingarro, I.; Ferrer-Montiel, A.; Messeguer, A.; Fearnhead, H. O.; Pérez-Payá, E. *Cell Death Differ.* **2006**, *13*, 1523–1532.
(24) Li, W. B.; Yan, B. *J. Org. Chem.* **1998**, *63*, 4092–4097.
(25) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284.
(26) Olivos, H. J.; Prasanna, G. A.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057–4059.
(27) Blackwell, H. E. *Org. Biomol. Chem.* **2003**, *1*, 1251–1255.
(28) De Luca, L.; Giacomelli, G.; Porcheddu, A.; Salaris, M.; Taddei, M. *J. Comb. Chem.* **2003**, *5*, 465–471.
(29) Bowman, M. D.; Jeske, R. C.; Blackwell, H. E. *Org. Lett.* **2004**, *6*, 2019–2022.
(30) Nüchter, M.; Ondruschka, B. *Mol. Diversity* **2003**, *7*, 253–264.
(31) Alcázar, J. *J. Comb. Chem.* **2005**, *7*, 353–355.
(32) Murray, J. K.; Gellman, S. H. *J. Comb. Chem.* **2006**, *8*, 58–65.
(33) Murray, J. K.; Farooqi, B.; Sadowsky, J. D.; Scalf, M.; Freund, W. A.; Smith, L. M.; Chen, J.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13271–13280.
(34) Murray, J. K.; Gellman, S. H. *Org. Lett.* **2005**, *7*, 1517–1520.
(35) Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521–1524.
(36) Steinhoff, M.; Duddenkotte, J.; Shpacovitch, V.; Rattenholl, A.; Moormann, C.; Vergnolle, M.; Luger, T. A.; Hollenberf, M. D. *Endocr. Rev.* **2005**, *26*, 1–43.
(37) Ramachandran, R.; Hollenberg, M. D. *Br. J. Pharmacol.* **2008**, *153*, S263–S282.

CC800144X