

Peptoids: A modular approach to drug discovery

(chemical diversity/peptide libraries/*N*-alkylglycine/*N*-substituted glycine)

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ABSTRACT Peptoids, oligomers of *N*-substituted glycines, are described as a motif for the generation of chemically diverse libraries of novel molecules. Ramachandran-type plots were calculated and indicate a greater diversity of conformational states available for peptoids than for peptides. The monomers incorporate *t*-butyl-based side-chain and 9-fluorenylmethoxycarbonyl α -amine protection. The controlled oligomerization of the peptoid monomers was performed manually and robotically with *in situ* activation by either benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate or bromotris(pyrrolidino)phosphonium hexafluorophosphate. Other steps were identical to peptide synthesis using α -(9-fluorenylmethoxycarbonyl)amino acids. A total of 15 monomers and 10 oligomers (peptoids) are described. Preliminary data are presented on the stability of a representative oligopeptoid to enzymatic hydrolysis. Peptoid versions of peptide ligands of three biological systems (bovine pancreatic α -amylase, hepatitis A virus 3C proteinase, and human immunodeficiency virus transactivator-responsive element RNA) were found with affinities comparable to those of the corresponding peptides. The potential use of libraries of these compounds in receptor- or enzyme-based assays is discussed.

Broad screening of compound libraries, of broths grown from soil samples, and of synthetic intermediates has been a fruitful method for discovery of lead compounds in pharmaceutical and agrochemical research (e.g., ref. 1). With the advent of automated chemical methods for solid-phase peptide and nucleotide synthesis, and of molecular biological methods for protein and nucleic acid synthesis, the stage has been set for the generation of new kinds of compound libraries, namely, collections of oligomeric biomolecules (2–14). Such libraries have been used to map epitopes for antibody binding, to discern ribonucleotide sequences with specific binding or catalytic activity, and to provide initial leads in receptor-based assays. Advantages of these oligomeric molecules are an almost limitless diversity as a result of their modular structure, the ease with which they can be synthesized and sequenced, and their inherent biological relevance. On the other hand, the metabolic instability of peptides and nucleotides and their poor absorption characteristics mean that any lead sequence will require extensive modification before *in vivo* activity can be expected.

Many of these problems could be avoided if an alternative, modular system was devised, with a basis set of “unnatural” monomers and a method for their controlled oligomerization. A host of chemically and pharmaceutically interesting subunits or modules would generate a diverse and novel set of

heteropolymers. Once an interesting compound has been identified from a library of such nonpeptide polymers, it can serve as a lead for drug discovery, further along the road to a metabolically stable drug. Optimized analogs of a lead compound could then be developed rapidly due to the modular synthetic nature of these compounds.

Here we describe the development of oligomeric *N*-substituted glycines as a motif for the generation of chemically diverse libraries. We refer to these oligomers as “peptoids,” in recognition of their conceptual lineage. They are also an example of compounds given this name previously by Farmer and Ariens (15) and Horwell (16). In this report, the rationale behind this selection is described, along with general routes to the synthesis of a variety of protected *N*-substituted glycines and their assembly into peptoid oligomers.

MATERIALS AND METHODS

Computation. Model structures were built with the Insight-II package from Biosym (San Diego) using standard libraries, and structures were minimized using the CVFF force-field (17) as implemented in the Discover molecular mechanics program, also from Biosym. A constant dielectric with $\epsilon = 1$ was used in all calculations. Energies were obtained by rotating torsion angles through 360° in 30° increments with the desired values set with the Rotors option, and held at those values with the Torsion Forcing option. Each structure was minimized using the VA09A minimization protocol.

General Synthesis. 4-[2',4'-Dimethoxyphenyl (9-fluorenylmethoxycarbonylaminoethyl)]phenoxy resin [Rink amide resin (18), 100–200 mesh, 1% crosslinked with divinylbenzene] was used for the preparation of C-terminal amides. Other chemicals for peptide synthesis were from Advanced ChemTech or Nova Biochem (San Diego) and used as received. Other chemicals and solvents were obtained from Fluka or Aldrich and were used without further purification. Trypsin, chymotrypsin, papain, and *p*-nitrophenyl α -D-maltotriose were purchased from Boehringer Mannheim. α -Amylase (isozyme I) was from Sigma. Pepsin and thermolysin were from Calbiochem.

All synthetic compounds and intermediates gave ¹H NMR and mass spectra consistent with the proposed structures. Reversed-phase (RP-HPLC) was performed on a Hewlett-Packard 1090 equipped with a diode-array detector, using gradient elution (solvent A, water with 0.1% trifluoroacetic

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Abbreviations: FAB, fast atom bombardment; Fmoc, 9-fluorenylmethoxycarbonyl; PyBOP, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBroP, bromotris(pyrrolidino)phosphonium hexafluorophosphate; RP, reversed-phase; TAR, transactivator-responsive element.

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acid; solvent B, acetonitrile) with 0.1% trifluoroacetic acid through a Vydac (Hysperia, CA) C₁₈ RP-HPLC column. Fast atom bombardment (FAB) mass spectra were obtained in either nitrobenzyl alcohol or thiolglycerol matrices at the University of California mass spectrometry facilities (Berkeley and San Francisco) or at Mass Search (Modesto, CA).

Synthesis of a Representative Monomer via Reductive Amination. β -Alanine *t*-butyl ester hydrochloride (10.0 g, 55.1 mmol) and glyoxylic acid hydrate (5.47 g, 59.5 mmol) were dissolved in water (100 ml) and the pH was titrated to 6.0 with 1 M NaOH. Catalyst (10% Pd/C, 400 mg) was added and the mixture was placed in a Parr (Moline, IL) hydrogenator under hydrogen for 24 hr, at which point no further reduction in pressure was noted. The catalyst was removed by filtration and washed with water, and the filtrate was brought to pH 9.5 with 1 M NaOH. Acetone was added (100 ml), followed by a slow addition of a solution of *N*-(9-fluorenylmethoxycarbonyl)succinimide (Fmoc-OSu, 18.6 g, 55.1 mmol) in 100 ml of acetone. The mixture was maintained at pH 9.5 with periodic addition of 1 M NaOH. After stirring overnight, the mixture was concentrated under reduced pressure to remove the acetone, and the remaining aqueous solution was washed three times with 50 ml of a 1:1 mixture of ether and hexanes. The aqueous layer was acidified to pH 2.5 with aqueous citric acid and extracted with ethyl acetate. The organic layer was dried over Na₂SO₄ and evaporated, and the residue was recrystallized from ethyl acetate/hexanes to give 14.6 g (62% yield) of Fmoc-Nglu(OBu^t)-OH (where Nglu is *N*-carboxyethylglycine; see Table 1).

Oligomer Synthesis. Standard solid-phase peptide synthesis techniques were used with *in situ* activation by either benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) (19) or bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP) (20). The standard synthesis cycle involved swelling the resin in either dichloromethane or *N,N*-dimethylformamide, deprotection with 20% (vol/vol) piperidine in dimethylformamide, thorough washing with the reaction solvent, reaction with the activated amino acid for either a single or a double cycle, thorough washing, and in most cases, capping unreacted amino groups with acetic anhydride and pyridine in dichloromethane. The differences in the protocols for the two activating agents are given below.

Protocol A. PyBOP was used in the robotic assembly of compounds 1–6 (see Table 2) with a Zymate XP laboratory automation system (Zymark, Hopkinton, MA) (21). The Fmoc monomer, PyBOP, and 1-hydroxybenzotriazole were added in a 5-fold molar excess to resin-bound amino groups at a final concentration of 0.3 M each, in dimethylformamide. Diisopropylethylamine was then added (0.6 M). The reaction was allowed to proceed for 30 min at room temperature with mixing provided by an intermittent stream of argon through the frit of the reaction vessel. The acylation was repeated, and the unreacted amines were capped with acetic anhydride.

Protocol B. PyBroP was used in the manual syntheses of compounds 7–10. The Fmoc-protected monomer and PyBroP were added to the peptoid resin in a 3-fold molar excess to resin-bound amino groups, for a final concentration of 0.1 M in dichloromethane. *N*-Methylmorpholine or diisopropylethylamine was then added (0.3 M). The reaction was allowed to proceed for 1 hr at room temperature with good mixing on a rotary shaker. A single acylation step was used in this protocol. Capping with acetic anhydride was used in the preparation of compound 7.

The substitution level of the coupled resin was determined by removing the *N*-terminal Fmoc group quantitatively and measuring the absorbance at 300 nm of the piperidine/dibenzofulvene adduct. The dried resin (1–5 mg) was weighed into a small tube and mixed with 1 ml of a 20% piperidine/dimethylformamide solution for 25 min. The tube was cen-

trifuged for 3 min. An aliquot (50 μ l) of the supernatant was diluted with 1 ml of acetonitrile. The absorbance at 300 nm was recorded vs. a standard of the same dilution of 20% piperidine/dimethylformamide in acetonitrile and the substitution level was determined from the empirically derived value of 7040 M⁻¹cm⁻¹ for the extinction coefficient of the adduct.

Removal of the side-chain protecting groups and cleavage of the oligomer from the resin were accomplished by treatment with a mixture of 82.5% trifluoroacetic acid, 5% phenol, 5% water, 5% thioanisole, and 2.5% ethanedithiol (10 ml per 500 mg of resin) for 2 hr at room temperature. The resulting solution was concentrated to \approx 1 ml with a stream of nitrogen, diluted with 10 ml of 10% aqueous acetic acid, and extracted three times with diethyl ether. The peptoids were generally obtained as powders after lyophilization of the acid solution.

Assay for Resistance to Proteinases. Compound 6 (1 mg/ml) was incubated with papain, chymotrypsin, or thermolysin (\approx 20 μ g/ml) in 100 mM ammonium bicarbonate buffer overnight. The specific conditions or alterations were as follows: chymotrypsin, pH 7.8, 21°C; papain, 1 mM 2-mercaptoethanol, 0.1 mM EDTA, pH 7.8, 37°C; and thermolysin, 1 mM CaCl₂, pH 7.8, 45°C. The reaction mixtures were analyzed by C₁₈ RP-HPLC and the retention time and concentration were compared with those of the starting material.

Assay for Inhibition of α -Amylase. Bovine pancreatic α -amylase was assayed at 30°C in 25 mM Hepes, pH 7.0/30 mM NaCl/1 mM CaCl₂, with *p*-nitrophenyl α -D-maltotriose as substrate ($\Delta\epsilon_{405} = 9500$) (22).

Assay for Inhibition of Hepatitis A Virus Proteinase. Recombinant hepatitis A virus 3C proteinase was expressed in *Escherichia coli* in a soluble, active form (23). Purity of the enzyme samples was >80% as determined by PAGE. Proteinase concentrations were determined by the bicinchoninic acid (BCA) protein assay (Pierce) with bovine serum albumin as a standard. Proteinase activity was determined with the peptide Ac-ELRTQSFS-NH₂.

Assay for Binding to Transactivator-Responsive Element (TAR) RNA of Human Immunodeficiency Virus Type 1. TAR RNA binding constants of the peptoid 10 and the peptide Ac-YKKKRKKKA-NH₂ were estimated by a gel shift assay using ³²P-labeled TAR (24, 25). Specificity of RNA binding was determined by measuring the change in circular dichroism (CD) upon peptide binding to wild-type TAR or to a TAR mutant containing a single nucleotide substitution in the three-nucleotide bulge (U²³ \rightarrow C) (ref. 24; R.T. and A.D.F., unpublished data). CD spectra were recorded on an Aviv (Lakewood, NJ) model 62DS spectropolarimeter at RNA concentrations of 30 μ g/ml.

RESULTS AND DISCUSSION

In devising an alternative to the natural polymers, we postulated five desirable attributes for any modular system: (i) the monomers should be straightforward to synthesize in large amounts, (ii) the monomers should have a wide variety of functional groups presented as side chains off of the oligomeric backbone, (iii) the linking chemistry should be high yielding and amenable to automation, (iv) the linkage should be resistant to hydrolytic enzymes, and (v) the monomers should be achiral.

The schematic comparison of peptides and peptoids provided in Fig. 1 shows the similarities in the spacing of the side chains and the carbonyl groups, and the differences in the chirality of the two monomers. That these peptoids are simply isomers of peptides should not obscure the important differences in stereochemical and conformational characteristics of the two oligomers. These differences are shown in Fig. 2, which depicts the Ramachandran-type plots (26) for three model systems: Ac-Gly-NMe₂, Ac-Ala-NMe₂, and Ac-

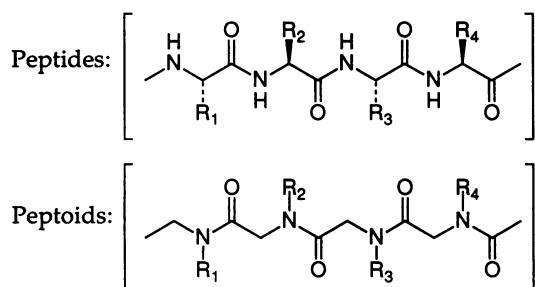


FIG. 1. Schematic comparison between peptides and peptoids showing the similarity of spacing of the side chains, and the lack of stereochemistry of the peptoid monomers.

Sar-NMe₂ [sarcosine (Sar), *N*-methylglycine, was used as a model for *N*-substituted glycines].

The traditional computational model of a dipeptide was altered to the dimethylamide derivative as a model of either a peptidyl peptoid or a "dipeptoid." As expected, the Ramachandran plot for Ac-Gly-NMe₂ (Fig. 2A) shows a significant amount of conformational flexibility for glycine. Two relatively shallow minima are found near $\phi, \psi = (-120^\circ, +90^\circ)$ and $(+120^\circ, -90^\circ)$. This calculation compares well with that reported for Ac-Gly-NHMe (27). Conformations with a *cis* peptide bond between the acetyl group and the glycine are higher in energy than the corresponding *trans* conformations.

The plot of Ac-Ala-NMe₂ (Fig. 2B) shows the dramatic effect of an *N*-alkyl group on the ψ angle of *L*-ala. The second *N*-methyl group significantly restricts the conformation to a region where $\phi < 0^\circ$ and $\psi > 60^\circ$. This result is consistent with those reported for *L* amino acids preceding a proline (27), with the obvious exception that the disubstituted amide bond precludes formation of helices. In contrast, the conformational profile of Ac-Sar-NMe₂ (Fig. 2C) looks very similar to that of Ac-Gly-NMe₂, although the former can adopt low-energy conformations with either a *cis* or a *trans* amide bond between the acetyl group and the sarcosine. In the *trans* conformation, the added steric bulk of the methyl group limits the ϕ, ψ space available, effectively eliminating conformations in either the lower left or the upper right quadrant of ϕ, ψ space. The two minima at $\phi, \psi = (-120^\circ, +90^\circ)$ and $(+120^\circ, -90^\circ)$ are considerably narrower than in the case of Ac-Gly-NMe₂. Two higher-energy minima at $(+60^\circ, +60^\circ)$ and $(-60^\circ, -60^\circ)$ arise from conformers with a *cis* peptide bond. The conformational profiles of other peptoid monomers have been computed and they are qualitatively similar to that of Ac-Sar-NMe₂, suggesting that the backbone of peptoids, like oligomers of *L* amino acids, is largely unaffected by side chain.

These calculations suggest that there will be a greater diversity of conformational states for a peptoid than for a peptide. In addition, the absence of substituted backbone carbons and of amide hydrogens removes the intramolecular CO...HN hydrogen bonds and changes the steric interactions that induce secondary structure in peptides. These

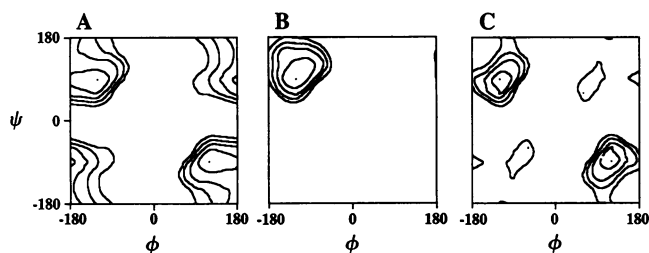


FIG. 2. Ramachandran-type plots for Ac-Gly-NMe₂ (A), Ac-Ala-NMe₂ (B), and Ac-Sar-NMe₂ (C). Sar, sarcosine (*N*-methylglycine).

features may serve to increase further the conformational space that can be explored by the peptoids.

As for any chemically synthesized modular system, the choice of functional groups is virtually limitless. We drew the initial set of modules from the functional groups corresponding to the natural amino acids (Table 1). Additional or alternative choices could be a selection of traditional pharmacophores (28, 29) or of functional groups with unusual steric or electronic properties. Cyclic and branched peptoids can be generated to explore additional diversity. The intention is to provide a system in which diversity can be maximized and in which synergistic interactions are made possible.

In the synthesis of the protected monomers, side-chain protection is incorporated prior to formation of the *N*-substituted glycine monomers. Three principal routes are used in their syntheses (Fig. 3), with the path of choice dictated primarily by the availability of the appropriate side-chain derivative. A number of *N*-substituted glycines have been reported—e.g., as substitutes for proline in inhibitors of human leukocyte elastase and angiotensin-converting enzyme (30). However, these compounds have relatively simple side-chain functionality that does not require an orthogonal protection strategy. We have found that the most generally useful synthetic method is route A: reductive amination of a side-chain amine with glyoxylic acid (31) (Table 1). A permutation of this method was used in the preparation of Nade, using a side-chain aldehyde with glycine methyl ester under similar conditions. This method is convenient because stoichiometric amounts of reagents are used and purification is straightforward. Alternative routes are alkylation of a side-chain amine with chloroacetic acid (route B), or in the case of Ngl, Michael addition of glycine to acrylamide (route C) (32). After formation of the *N*-substituted glycines, the α -amino group is protected as the Fmoc derivative for storage and subsequent use.

t-Butyl-based groups are used to protect side chains containing amino, carboxyl, hydroxyl, and indole moieties; guanidine and imidazole moieties are blocked with 2,2,5,7,8-pentamethylchroman-6-sulfonyl (33) and trityl groups, re-

Table 1. Synthesis of *N*-alkylglycines as peptoid modules

Side chain (R)	Protection*	Designator†	Method‡
CH ₃		Nala	§
CH ₂ CH ₂ CH ₂ N=C(NH ₂) ₂	Pmc	Narg	A
CH ₂ CO ₂ H	Bu'	Nasp	A
CH ₂ CH ₂ CO ₂ H	Bu'	Nglu	A
CH ₂ CH ₂ CONH ₂		Ngl	C
CH ₂ CH ₂ -(4-imidazolyl)	Trt	Nhhis	A [¶]
CH ₂ CH(CH ₂) ₂		Nleu	B
CH ₂ CH ₂ NH ₂	Boc	Naeg	B
CH ₂ C ₆ H ₅		Nphe	§
CH ₂ CH ₂ OH	Bu'	Nhser	A
CH ₂ CH ₂ -(3-indolyl)	Boc	Nhtrp	A
CH ₂ CH ₂ - <i>p</i> -C ₆ H ₄ OH	Bu'	Nhtyr	A
CH ₂ CH ₂ -(1-adamantyl)		Nade	A [¶]
CH ₂ CH(C ₆ H ₅) ₂		Nbhm	A
CH ₂ CH ₂ CH(C ₆ H ₅) ₂		Nbhe	A

*Side-chain protecting group introduced prior to assembly of monomer. Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Bu', *t*-butyl; Trt, trityl; Boc, butoxycarbonyl.

†See text for explanation of the designators.

‡A, RCH₂NH₂ + O=CHCO₂H or RCHO + H₂NCH₂CO₂Me, H₂/Pd; B, RNH₂ + ClCH₂CO₂H; C, H₂NCOCH=CCH₂ + H₂NCH₂CO₂H.

§Commercially available.

¶NaBH₃CN was used in place of H₂/Pd.

||Naeg, *N*-aminoethylglycine; Nade, *N*-adamantylethylglycine; Nbhm, *N*-benzhydrylmethylglycine; Nbhe, *N*-benzhydrylethylglycine.

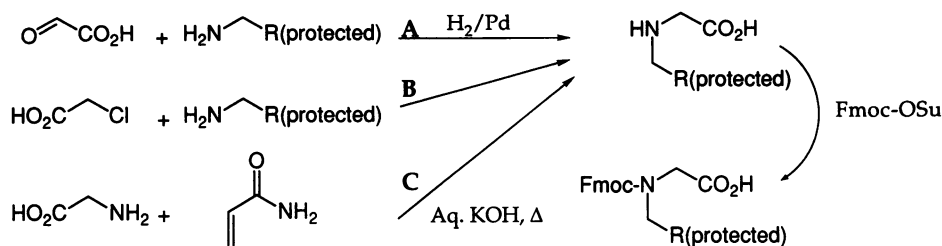


FIG. 3. General routes for synthesis of peptoid monomers.

spectively. This scheme was chosen for its orthogonality to the Fmoc protection of the α -amino group and its compatibility with the global deprotection chemistry implemented on a robotic synthesizer (21).

The methods used for assembly of representative peptoid monomers are listed in Table 1. For convenience, we have given the monomers designations corresponding to the amino acids with similar functionality, with the prefix N. For Nhser, Nhhis, Nhtrp, and Nh Tyr, direct correspondence would lead to analogs of limited chemical stability; in these cases, the peptoid monomer corresponds to the homologous amino acid. In the case of Naeg (*N*-aminoethylglycine) the side-chain amino group is shorter than for the common amino acid lysine. Three "unnatural" monomers were synthesized with adamantyl and benzhydryl functional groups, Nade, Nbhbm, and Nbhe. *N*-Benzylglycine (Nphe) and *N*-methylglycine (sarcosine, Nala) are commercially available.

The methods for oligomerization of the peptoid monomers are broadly based on the techniques developed for solid-phase peptide synthesis. A number of modifications are necessary, since difficulties are often encountered with *N*-alkylamino acids; e.g., proline and other *N*-alkylamino acids often couple poorly under standard conditions (e.g., ref. 34). Additionally, when conventional ester resins are used for the preparation of peptides with C-terminal prolines as free acids, diketopiperazine formation often preempts introduction of the third residue unless special precautions are taken. As an alternative, resins with bulky linking groups for the attachment ester can minimize this problem (35, 36).

The couplings of Nphe to Nphe, Nasp, and Nglu bound to Rink amide resin (18) were used to optimize the protocol for peptoid coupling; yields were determined by quantitation of the chromophore released upon removal of the Fmoc protecting group. Conventional carbodiimide coupling reagents proved to be less effective than PyBOP (19) and PyBroP (20),

which were developed specifically for coupling hindered or N^α -disubstituted acids. These reagents gave high (80–100%) yields of coupled products (data not shown). In addition, PyBOP is compatible with automation because of its solvent stability (21). The other steps in the sequence (removal of Fmoc protection at each step, cleavage from the resin, side-chain deprotection, etc.) proceed under the usual conditions for peptide synthesis. A system is thus available for the multistep assembly of oligopeptoids (Table 2), and by extension, oligopeptoid and peptide hybrids and mixtures, by manual as well as robotic methods.

Oligopeptoids exhibit chromatographic (RP-HPLC) and solubility properties that are similar to those of the analogous peptides^{||} and often are eluted as single, well-defined peaks (Fig. 4), in spite of the potential for *cis*–*trans* isomerism about the amide linkages. The major components of the syntheses have been identified as the desired oligopeptoids by mass spectral analysis.

Preliminary experiments have been conducted on several *N*-substituted glycine oligopeptoids to explore relative susceptibility to peptidases. Compound 6, a representative of the present series, is not cleaved by chymotrypsin, papain, or thermolysin. This result is perhaps expected in view of the substituted nitrogens (37).

The primary application envisaged for oligopeptoids is in the screening of diverse mixtures for identification of a favorable array of functional groups for binding to an enzyme or receptor. Because the peptide and peptoid backbones have different hydrogen bonding and conformational characteristics, one may not expect a direct translation of the sequence of a peptide ligand into a peptoid mimic. Indeed, to the extent that such a translation is conceivable, from the alignment of Fig. 1, it is apparent that the retro peptoid sequence is required if both the side chains and carbonyl groups are to be in register with the corresponding peptide.

Table 2. Synthesis of representative oligopeptoids

Compound*	Protocol†	Analysis‡
1 Gly-Nleu-Nhser-Nala-Naeg-Naeg-Nleu	A	FAB
2 Nleu-Naeg-Naeg-Nala-Nhser-Nleu-Gly	A	FAB
3 Nleu-Pro-Naeg-Nhser-Nhser-Nglu	A	MS–MS
4 Nglu-Nhser-Nhser-Naeg-Pro-Nleu	A	MS–MS
5 Nhser-Nphe-Nhser-Nglu-Nhser-Nala-Nleu-Nglu	A	FAB
6 Nglu-Nleu-Nala-Nhser-Nglu-Nhser-Nphe-Nhser	A	MS–MS
7 Nhser-Nphe-Nhser-Nala-Nhser-Nala-Nleu-Nglu	B	FAB
8 Nhtrp-Narg-Nh Tyr	B	FAB
9 Nh Tyr-Narg-Nhtrp	B	FAB
10 Naeg-Naeg-Naeg-Narg-Naeg-Naeg-Naeg-Naeg	B	FAB

*Peptoids were synthesized on Rink amide resin; all peptoids were *N*-acetylated and C-amidated.

†See *Materials and Methods*.

‡FAB–MS was performed at the University of California, Berkeley, or at Mass Search; tandem mass spectrometry (MS–MS) was performed at the University of California, San Francisco. In addition to molecular weight verification, preliminary work has shown that individual oligopeptoids can be sequenced with MS–MS (A. Burlingame, K. Medzihradzsky, and R.J.S., unpublished data).

^{||}For example, both the peptide Ac-SFSQSALE-NH₂ and its peptoid relative 5 were prepared; on a C₁₈ RP-HPLC column eluted with a 5–65% acetonitrile/water gradient in 0.1% trifluoroacetic acid over 30 min, the peptide and peptoid had retention times of 18.77 and 18.46 min, respectively.

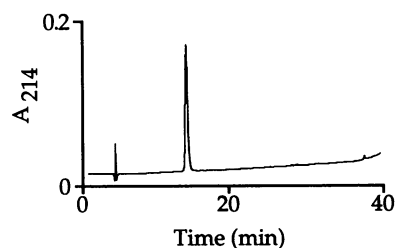


FIG. 4. Analytical RP-HPLC trace of purified 1, Ac-Gly-Nleu-Nhser-Nala-Naeg-Naeg-Nleu-NH₂. Conditions: Vydac C₁₈, 1.0 ml/min; solvent A, 0.1% trifluoroacetic acid in water; solvent B, 0.1% trifluoroacetic acid in acetonitrile; elution with a linear gradient of 2% B/min after a 2-min wash with A.

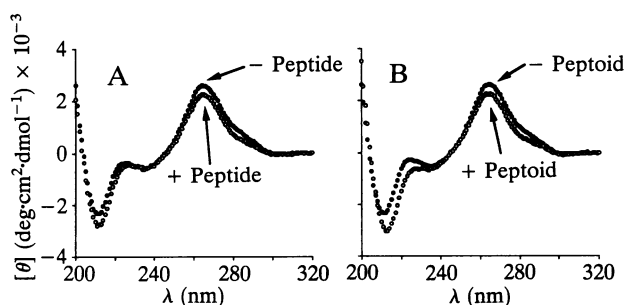


FIG. 5. CD spectra of peptide (Ac-YKKRKKKA-NH₂) and peptoid (10)-TAR RNA complexes. (A) Wild-type TAR in the absence (●) or presence (○) of the peptide. (B) Wild-type TAR in the absence (●) or presence (○) of the peptoid. All complexes were at 1:1 stoichiometry. The change in the spectrum near 265 nm indicates a conformational change in TAR (R.T. and A.D.F., unpublished data).

An indication of this alignment may indeed be found in the affinity of a tripeptide and the corresponding tripeptoid inhibitors of the glycolytic enzyme α -amylase. Ac-WRY-OMe, a fragment of the proteinaceous inhibitor tendamistat, binds with a K_i value of 100 μ M (T. Guo and P.A.B., unpublished data); the corresponding peptoids show similar affinity, with the retro sequence 9 (K_i 200 μ M) preferred slightly over the direct-translation peptoid 8 (K_i 350 μ M). In another example, both forward, 6, and retro, 5, peptoid analogs of the consensus substrate (Ac-ELATQSFS-NH₂) of the hepatitis A virus 3C protease (23, 38) were prepared, with the "conservative" substitution of Nhsr for threonine. While the retro peptoid, 5 (K_i 2 mM), competed with affinity comparable to that of the peptide substrate (K_m 2.1 mM), the forward peptoid, 6, showed no inhibition at 10 mM concentration.

Short peptides from the human immunodeficiency virus Tat protein bind specifically to TAR RNA, requiring a single arginine residue surrounded by at least three basic amino acids (25). A gel shift assay was used to compare the TAR binding affinity of the Tat RNA-binding-domain peptide Ac-YKKRKKKA-NH₂ with that of a closely related peptoid, 10. The K_d values estimated for the peptoid and peptide were similar ($\leq 2 \mu$ M), although precise affinities could not be measured because the complexes partially dissociated during electrophoresis. To determine the specificity of RNA binding, CD spectra of peptoid and peptide complexes with wild-type TAR and with a TAR mutant were recorded. Specific peptide-RNA binding has been shown to induce a change in TAR conformation that can be detected by CD (ref. 24; R.T. and A.D.F., unpublished data). Both peptide and peptoid bind specifically as seen from the change in the CD spectrum near 265 nm with wild-type TAR (Fig. 5), which is characteristic of such a conformational change. The CD spectrum of the TAR mutant in the region of 265 nm was unchanged in the presence of either the peptoid or the peptide (data not shown). Thus, the peptoid analog of the Tat RNA-binding domain mimics the activity of its peptide counterpart.

The synthesis of a diverse collection of N-substituted glycines as peptoid monomers and the subsequent development of the chemistry required for their automated assembly provides a modular system for the creation of unusual compound libraries. We anticipate that such libraries, and the individual peptoids that are identified through screening them, will provide exciting leads for drug design. By virtue of their resistance to enzymatic degradation, these lead compounds will be well along the way toward new pharmaceuticals.

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