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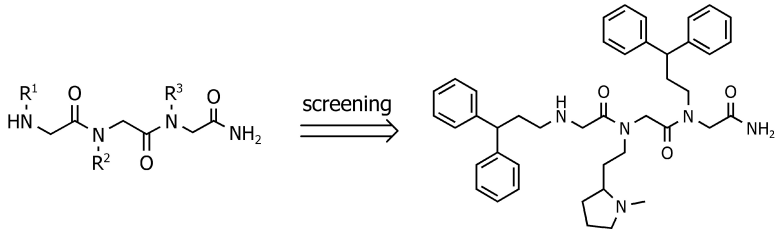

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A Positional Scanning Combinatorial Library of Peptoids As a Source of Biological Active Molecules: Identification of Antimicrobials

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A positional scanning library of *N*-alkylglycine trimers (peptoids) containing over 10 000 compounds has been synthesized on solid phase. The synthetic pathway involved the use of the submonomer strategy and a set of 22 commercially available primary amines as a chemical diversity source. The unbiased nature of the library allowed its screening against a variety of biological targets, leading to the identification of individual peptoids exhibiting remarkable biological activities (García-Martínez, C. et al. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2374. Montoliu, et al. *J. Pharm. Exp. Therap*. **2002**, *302*, 29. Planells-Cases, R., et al. *J. Pharm. Exp. Therap*. **2002**, *302*, 163). In the present work, the screening of this library against a panel of Gram-positive and Gram-negative bacteria led to the identification of different compounds exhibiting antimicrobial activity.

Introduction

Oligomers of *N*-alkylglycines, also known as peptoids, constitute a family of nonnatural molecules attractive for the drug discovery process because of their broad variety of biological activities and to the proteolytic stability that they exhibit.[1] In addition, the modular scaffold of these compounds made them amenable for combinatorial strategies. In this context, the pioneering work done by Zuckermann and co-workers yielded the development of two solid-phase-based complementary approaches for the preparation of *N*-alkylglycine oligomers. The first one involved the condensation of suitably activated *N*-Fmoc-protected *N*-alkylglycines. This route implies the previous preparation of a set of protected N-substituted glycine monomers.[2] The second approach consisted of the systematic assembly of two readily available submonomers, that is, an α-haloacetyl moiety and a primary amine.[3,4] The advantages of this approach are that the α-haloacetyl monomer is common to all backbone elongation cycles and that the availability of primary amines expands the diversity that can be explored. On the other hand, Liskamp and co-workers have also described an alternative solution phase approach for the synthesis of *N*-alkylglycine oligomers.[5] These procedures can be easily automated; consequently, peptoid libraries using the split-and-mix format have been used for the identification of high-affinity ligands for membrane receptors of special relevance.[6−8] Furthermore, the broad molecular diversity achievable together with the inherent conformational flexibility of these molecules opened their applications to target the disruption of large macromolecular complexes, such as protein−protein, protein−nucleic acid and protein/membrane interactions. In this context, Hamy et al. reported the discovery of a Tat/TAR RNA inhibitor that suppresses HIV-1 replication,[9,10] and Murphy et al. described a combinatorial approach to the discovery of cationic peptoid reagents for gene delivery.[11] Peptoid libraries have been also used in one of the nowadays most active research fields, the identification of new antibacterial compounds needed to overcome the wide spread of drug-resistant microorganisms.[12,13]

Because of the modular composition of both peptides and peptoids, it was questioned whether libraries of peptoids could be synthesized and screened in a positional scanning format. The availability of a peptoid library in such a format will open the use of the same library in different biological targets for the identification of hit compounds. An inherent advantage of this approach will rely on avoiding the biological-assay-directed synthesis of sublibraries required in the split-and-mix procedure. In fact, the concept of positional scanning libraries was initially introduced by Houghten et al.[14] for peptide-based libraries to shorten the time required for the identification of active compounds. The approach was successful for the identification of active compounds.[15] It was also demonstrated that using the same chemical diversity in two different formats, split-and-mix and positional scanning, compounds of the same nature that inhibited the biological activity of cytolytic toxins were obtained.[16]

In the present contribution, the design and synthesis of an unbiased library of *N*-alkylglycine trimers containing over

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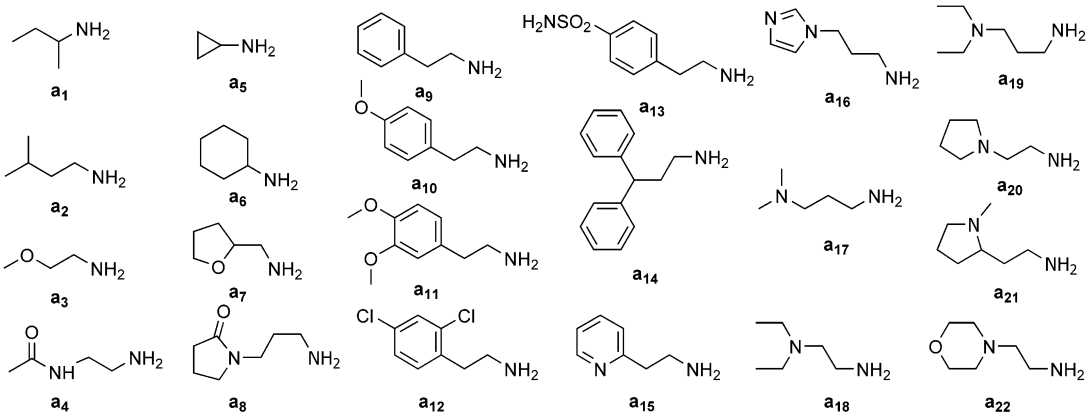


Figure 1. Primary amines used for the synthesis of the *N*-alkylglycine trimers library.

10 000 peptoids constructed using the positional scanning format as well as the identification of compounds with antimicrobial activity is reported. Furthermore, the same library when screened in membrane receptors allowed the identification of potent analgesic compounds that block the VR1 channel[17] and NMDA receptor open channel blockers[18] with promising properties for the treatment of neurodegenerative diseases. The library was also screened in a whole-cell-based assay and led to the identification of molecules with in vivo neuroprotectant activity.[19]

Results and Discussion

Design and Synthesis of a Library of *N*-Alkylglycines in a Positional Scanning Format. The dimension of combinatorial libraries, particularly that of controlled mixtures, is still a subject of debate. From the thousands and even millions of compounds contained in early peptide libraries, these numbers have been progressively reduced for other oligomers and small organic molecules, mainly as a result of synthetic requirements. However, one can still look to incorporate in the library a large representation of functional groups guided by several criteria. Then the library complexity could be controlled, ensuring enough chemical diversity to accomplish the final purpose, that is, the identification of biologically active compounds. In this sense, it seems that a library of trimers of *N*-alkylglycines in a positional scanning format could fulfill such requirements. For the synthesis of this peptoid library, the submonomer synthetic strategy was used.[3,4] To select the appropriate diversity and following chemical and pharmacophoric criteria, a set of 22 commercially available primary amines were chosen to give a library of 22^3, that is, 10 648 peptoids. As shown in Figure 1, although most of the primary amines had an $-CH_2-CH_2-NH_2$ residue to warrant similar reactivities toward the haloacetyl moiety, the selection procedure ensured chemical diversity in terms of hydrophobic and lipophilic substituents, aromatic residues, polar chains, and hydrogen-bond acceptors. In addition, the incorporation of primary amines bearing an additional tertiary amino group was contemplated. This family of amines, which can provide supplementary protonatable sites to the peptoid, had not been utilized in previously reported peptoid libraries, with the exception of the morpholine $\mathbf{a_{22}}$ or the pyridine $\mathbf{a_{15}}$ derivatives.

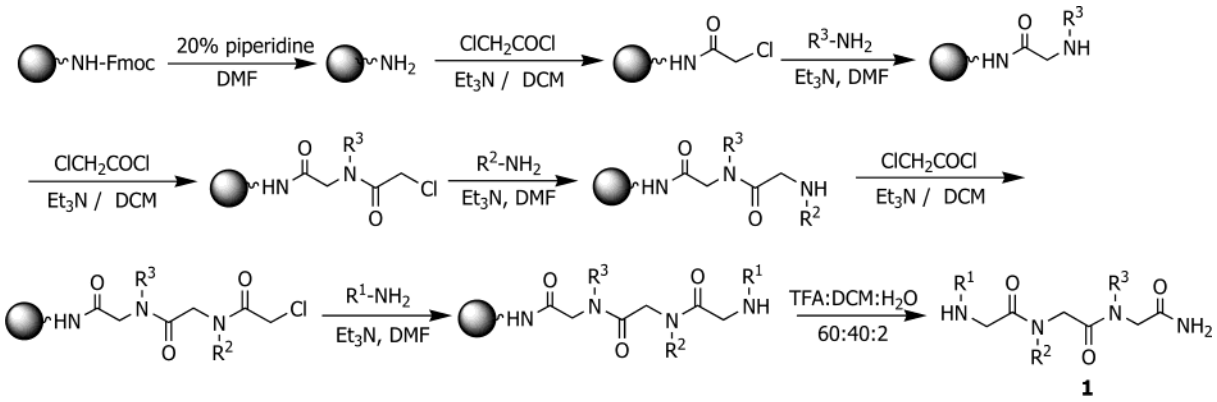
The positional scanning format demands that the different building blocks employed, in this case the primary amines, exhibit comparable reactivities. To this end, a representative set of the selected amines was assayed for reactivity with chloroacetamide. From the results obtained, it was inferred that the large excess of primary amines required for the solid-phase amination steps would overcome the reactivity differences found within the different amines tested. According to the positional scanning format, the library was divided into three sublibraries (OXX, XOX, and XXO, where O represents a defined diversity position, whereas X refers to a preequilibrated mixture of all 22 amines). A total of 10 648 compounds in 66 controlled mixtures were then synthesized using solid-phase chemistry. The eight-step synthetic sequence employed is shown in Scheme 1.

The solid support was confined in sealed tea bags.[20] Thus, after the initial release of the Fmoc protecting group from the Rink amide resin, successive steps of acylation with chloroacetyl chloride, followed by the corresponding amination of the chloromethyl intermediate using the specific primary amine or the mixture of the 22 amines, were conducted. All reaction steps were carried out in duplicate to warrant maximum conversion. Peptoids were released from the resin by using a 60:40:2 trifluoroacetic acid−dichloromethane−water mixture.

In addition, a "reporter peptoid" containing three different 2-aryl-substituted ethylamines to facilitate UV monitoring was introduced in the synthetic protocol. The MS analysis at different synthetic stages of the synthesis showed the presence of the expected peptoid fragments, and the RP-HPLC profile of the final product showed a major peak that accounted for 70% of the total peak profile. On the other hand, the dry weight analysis of the 66 lyophilized mixtures showed a regular distribution, suggesting a high probability that all library components should be present.

Screening of the Library of *N*-Alkylglycine Trimers for the Identification of Antimicrobial Compounds. The positional scanning format allows the direct determination of key residues at each library position,[14] speeding up the identification of bioactive compounds. In this case, the library of *N*-alkylglycine trimers is composed of three single-position sublibraries, each representing the same diversity of compounds but differing from one to another by the location of the defined position. Thus, when used in concert, the data

Scheme 1. Solid-Phase Synthesis of the Library of *N*-Alkylglycine Trimers



derived from each positional sublibrary yield information about the most active amino residue at each position. This information is used to prepare individual sequences that are expected to exhibit biological activity. In this context, the screening of the library of trimers of *N*-alkylglycines resulted in the direct and rapid identification of two potent receptor channel antagonists that also have had activity in model animal test assays.[17] Likewise, a further screening of this library allowed the one-step identification of an NMDA receptor open-channel blocker with in vitro and in vivo neuroprotective activity.[18] Finally, in an assay based in the direct observation of cerebellar neurons' survival subjected to toxic glutamate treatment, two peptoids that prevented the glutamate-induced exocytotoxic neurodegeneration in vitro and in vivo were identified.[19]

Bacterial resistance to existing drugs is a growing process that is reaching alarming levels,[21,22] and there is a general call for the development of new antimicrobial leads, preferably with a broad spectrum activity against Gram-positive bacteria that currently show the highest levels of resistance. A growth inhibition assay against a panel of Gram-positive and Gram-negative bacteria including a clinical isolated strain was included (see the Experimental Section). It was possible to identify from each positional sublibrary active mixtures defined by different amines. Figure 2 shows, as an example, the antimicrobial activity of the library against a representative strain of each group of bacteria. The main criteria initially imposed in the biological assay for the library deconvolution were a broad spectrum activity and selectivity against Gram-positive bacteria. Thus, amines $\mathbf{a_{11}}$, $\mathbf{a_{12}}$, and $\mathbf{a_{14}}$ for the N-terminal position, $\mathbf{a_{21}}$ for the central position, and $\mathbf{a_2}$ and $\mathbf{a_6}$ for the C-terminal position were selected. As mentioned above, this information should be enough to define active individual peptoids. Consequently, the six peptoids shown in Figure 3 had to be synthesized. However, in contrast to that which occurred in the synthesis of the individual peptoids addressed to the vanilloid receptor and neuroprotectants,[17,19] here, an unexpected synthetic behavior was observed.

Evaluation of the Side Reaction and Synthesis of Model 2,5-Piperazinedione (DKP) Derivatives. During the synthesis leading to the six defined peptoids shown in Figure 3a, it was observed that after the amination with $\mathbf{a_{21}}$ and subsequent acylation step, the third amination reaction did not take place with the expected conversion yields. The

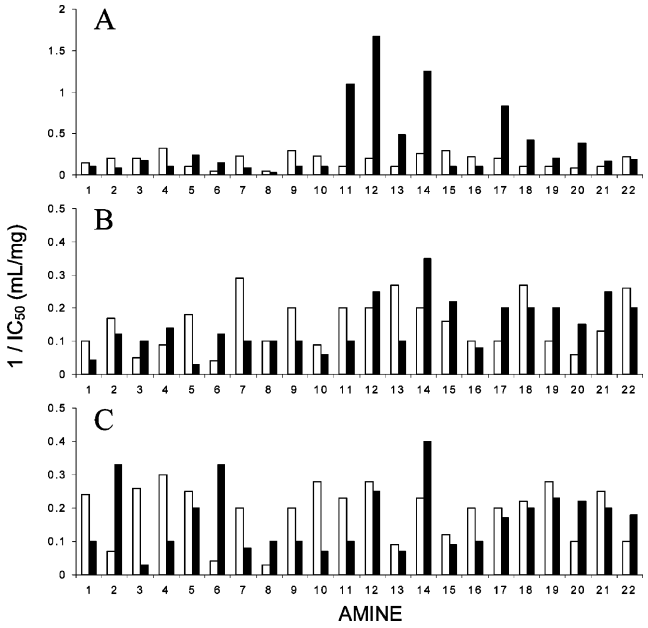


Figure 2. Screening of the library of *N*-alkylglycine trimers for the identification of antimicrobial compounds. As an example, the antimicrobial activity profile of the library mixtures is shown for two of six bacterial strains assayed (*Staphyloccocus aureus* methicillin-resistant SAMR10 and *Pseudomonas aeruginosa* ATCC 9721, black and white bars, respectively). Each bar in each panel represents the inverse IC_{50} value for each peptoid mixture, with the *x* axis representing the defined amine ("O" position). The IC_{50} was determined for each of the three separate positional libraries: (A) OXX, (B) XOX, and (C) XXO.

analysis of the crude reaction mixture showed the presence of the corresponding ammonium compounds **2a** and **2b** as major components (Figure 3b). It should be noted that amine $\mathbf{a_{21}}$ has an additional tertiary amino group. Therefore, a study was undertaken to clarify the influence of such amines in the synthetic pathway and their potential relevance with regard to the validation of the peptoid library.

To facilitate this study, the synthesis of 2,5-piperazinedione derivatives (DKP) was considered a more feasible tool (Table 1). In comparison with peptoids, DKPs are structures that are more amenable to chromatographic and spectroscopic analysis. Thus, the scope of the synthetic procedure involving two consecutive acylation and amination steps followed by the release from the resin and basic treatment to favor the cyclization of the expected dimer peptoid to the DKP derivative was examined.[23] A set of primary amines bearing

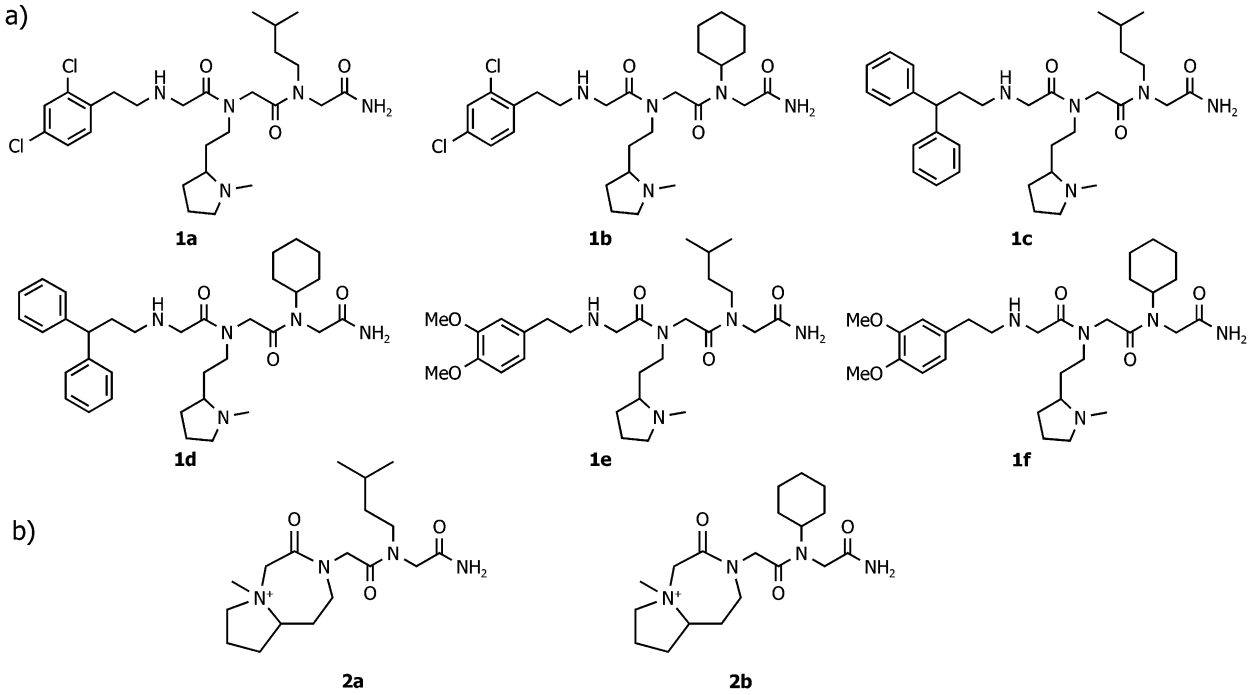


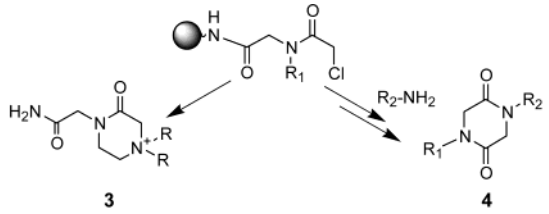
Figure 3. (a) Individual *N*-alkylglycine trimers rescued from the library as potential antimicrobial compounds. (b) Cyclic tetraalkylammonium derivatives formed as side products.

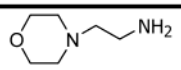
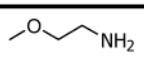
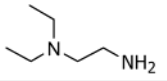
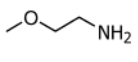
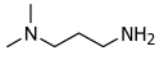
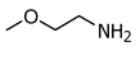
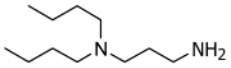
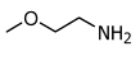
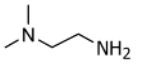
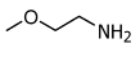
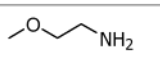
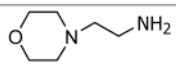
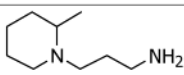
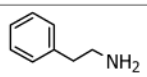
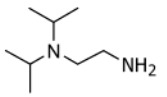
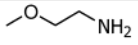
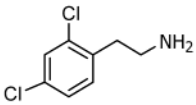
an additional tertiary amino group was used in the first amination step, and standard β-substituted ethylamines were used in the second amination. The NMR analysis of the products showed that primary amines bearing the additional nitrogen atom that were employed for the synthesis of the peptoid library (i.e., amines $\mathbf{a_{15}}-\mathbf{a_{22}}$; Figure 1) did not give the expected DKP **4** (Table 1, entries 1−5), but rather, yielded the corresponding cyclic ammonium derivative **3**.[24] However, when amines in entry 1 were interchanged, the DKP **4a** was obtained (Table 1, entry 6). Finally, the use of amines bearing sterically hindered tertiary amino groups larger than those used in the synthesis of the peptoid library, such as 1-(3-aminopropyl)-2-methylpiperidine and 2-(*N*,*N*-(bis)isopropyl)aminoethylamine, afforded the corresponding DKP formation (Table 1, entries 7−9). Taken together, these results indicated that the tertiary amino group was playing a crucial role in the prevention of the DKP formation through a direct participation in a competitive reaction with the α-chloro intermediate. The optimization of this side reaction has been developed for the construction of a library of this class of derivatives (Masip, I., et al., unpublished results).

The transfer of the above finding to the peptoid library led to anticipation that the competitive intramolecular cyclization would have been feasible for those cases in which the six above-mentioned amines ($\mathbf{a_{17}}-\mathbf{a_{22}}$) had been introduced at the C terminal or at the internal position (Scheme 2). Conversely, the use of these amines at the N-terminal position did not have to cause any problems. These results suggested that the peptoid library would contain more products than predicted. Nevertheless, it should be also noted that this side reaction was difficult to foresee in the preliminary workup procedures carried out before the synthesis of the library. In addition, as mentioned above, the use of amine $\mathbf{a_{22}}$ at the C-terminal position of trimeric peptoids had been reported, with no indication of this eventual cyclization problem.[13] In any case, future work on the synthesis of individual peptoids involving the introduction of such "conflictive" amines at the C-terminal or at the internal position should contemplate the use of the activated *N*-Fmoc-protected *N*-alkylglycines coupling procedure.

Synthesis and Biological Activity of Individual Peptoids. Because of the occurrence of amine $\mathbf{a_{21}}$ in all six individual peptoids that had to be synthesized, the formation of the corresponding cyclic tetraalkylammonium derivatives was observed (cf., Figure 3). Therefore, both the individual peptoid and the side product were purified by semipreparative HPLC and assayed. The six individual peptoids were initially tested for growth inhibition against six bacterial species. The four most active peptoids, namely **1a**, **1b**, **1c**, and **1d** were selected for further assays, and Table 2 shows the MIC, IC_{50}, and MBC values obtained. Nonetheless, the cyclic tetraalkylammonium side products were also tested, and no antimicrobial activity was detected. Compound **1d** showed the best antimicrobial profile under the initial criteria imposed at the library deconvolution (broad spectrum activity and selectivity against Gram-positive bacteria). However, at this point, it was unclear whether such criteria could mask active amines that showed low selectivity between Gram-positive and Gram-negative bacteria, but could exhibit increased antimicrobial activity with respect to **1d**. In this sense, the initial screening (Figure 2) showed that $\mathbf{a_{14}}$, although being less selective than $\mathbf{a_2}$ and $\mathbf{a_6}$ at the C-terminal position, showed higher activity against Gram-positive bacteria. Hence, compound **1g** (Figure 4) was synthesized, purified and biologically evaluated (Table 3). Two potent antimicrobial *N*-alkylglycine trimers recently identified by Goodson et al. (CHIR29498 and CHIR32133; cf., Figure 4)[12] that are currently in preclinical phase, were synthesized for comparative purposes (Table 3). All the antimicrobial parameters evaluated for compound **1g** suggested that there is an increase

Table 1. Influence of the Structure of Primary Amines in the Solid-Phase Synthesis of 2,5-Piperazinediones[a]



Entry	1st amine (R_1)	2nd amine (R_2)	Cmpd
1			**3a**
2			**3b**
3			**3c**
4			**3d**
5			**3e**
6			**4a**
7			**4b**
8			**4c**
9			**4d**

[a] For experimental details and description of compounds, see the Supporting Information.

in the antimicrobial activity when it is compared to the original **1d** compound. In addition, such activity is closely similar to that showed by the well-characterized Chiron derivatives (Tables 2 and 3).

Conclusion

With a set of 22 commercially available primary amines as chemical diversity source, a positional scanning library of *N*-alkylglycine trimers containing over 10 000 compounds has been synthesized on solid phase. The unbiased nature of the library allowed its screening against a variety of biological targets, leading to the identification of individual peptoids exhibiting remarkable biological activities.[17,19] In the present study, compounds that exhibit antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria have been identified. At the initial screening of the library, one of the selection criteria was based on the selectivity between Gram-positive and -negative bacteria. Actually, the defined peptoids coming out of the library deconvolution showed modest selectivity (Table 2), although the antimicrobial activity was only moderate. This fact could point to the inherent difficulties in obtaining compounds with high antimicrobial activity and with the capability of clearly differentiating between Gram-positive and -negative bacteria. However, the most promising of the initially defined peptoids served well as a hit, and its antimicrobial activity was increased by 1 order of magnitude by one-point amine substitution (compound **1g**). Moreover, this result corroborates the similarities between peptoid libraries synthesized either with the split-and-mix or the positional scanning format, as evidenced in early studies with peptide libraries.[16] Thus, using a positional scanning library, compounds with biological activity and chemical profile similar to those reported by Goodson et al.[12] from an split-and-mix peptoid library have been identified (Table 3 and Figure 4).

Nevertheless, the introduction of primary amines bearing an additional tertiary amino group into the library could lead to the formation of side products derived from an intramolecular cyclization promoted by this tertiary amine. However, the purified cyclized compounds had no antimicrobial activity and did not affect the library's biological deconvolution.

Peptoids are simple molecules, susceptible to broad structural manipulation and, therefore, leadlike property optimization. Consequently, before those strategies looking for increasing complexity in the hit rate, which allows less room for optimization of the druglike profile, the use of peptoid libraries can establish a valuable link between modern drug discovery technologies and the currently revisited historical lead approach.[25,26] On the other hand, the great conformational mobility of these compounds allows the envisioning of each peptoid as a small dynamic combinatorial library.[27] The price that must be paid is the difficulty of identifying the active conformer among all those existing in the conformational space in equilibrium. Work in this direction is in progress, and preliminary results suggest that conformationally more rigid analogues preserving the original biological activity could be obtained.

Experimental Section

General. Solvents, amines, and other reagents were purchased from commercial suppliers and used without further purification. The NMR spectra of peptoids were recorded on a Varian Inova 500 apparatus (^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz), whereas those of DKPs and cyclic ammonium derivatives were registered on a Varian 300 Unity machine (^{1}H NMR, 300 MHz; ^{13}C NMR, 75 MHz). The assignments of absorptions observed for the different peptoids were confirmed by gDQCOSY and gHCQC experiments. For the case of peptoids and in some of the cyclic ammonium derivatives, different conformations were observed. In addition, when clearly differentiated, diastereotopic proton atoms are indicated. The HPLC analyses were carried out using a Hewlett-Packard series 1100 (UV detector 1315A) modular system using a Kromasil 100 C8 (15 × 0.46 cm, 5 μm) column, with CH_3CN-H_2O mixtures containing 0.1% TFA at 1 mL/min as mobile phase and monitoring at 220 nm. High-resolution mass spectra (HRMS−FAB) were carried out at the Mass Spectrometry Service of the University of Córdoba (Spain).

Synthesis of the Peptoid Library. The synthesis of the library was carried out on a 1% cross-linked polystyrene resin

Scheme 2. Side Reaction Leading to the Formation of the Cyclic Tetraalkylammonium Derivatives

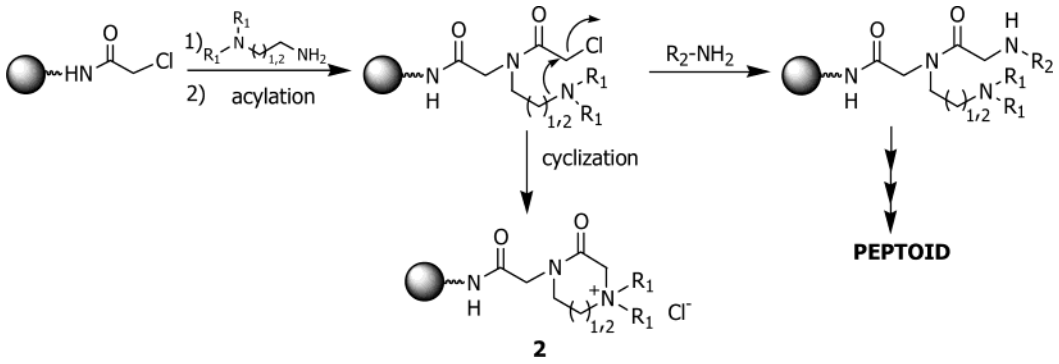


Table 2. Activity of Individual Peptoids Derived from the Library of *N*-Alkylglycine Trimers

	MIC (mg/mL)				IC_{50} (mg/mL)				MBC (mg/mL)			
strain	1a	1b	1c	1d	1a	1b	1c	1d	1a	1b	1c	1d
E. coli ATCC 25922	>2	2	>2	1	1	0.60	>2	0.10	>2	2	>2	1
P. aeruginosa ATCC 9721	0.25	>2	>2	0.50	0.10	0.13	2	0.13	>2	>2	>2	>2
A. calcoaceticum ATCC 19606	>2	>2	>2	1	1.20	0.32	>2	0.54	>2	>2	>2	1
S. aureus ATCC 25923	>2	0.50	1	0.50	>2	0.50	0.09	0.25	>2	1	1	0.5
S. aureus SAMR10	>2	0.50	2	0.50	>2	0.40	0.15	1	>2	1	2	1
E. faecalis ATCC 29212	2	2	0.50	1	>2	0.90	0.18	0.10	2	2	1	1

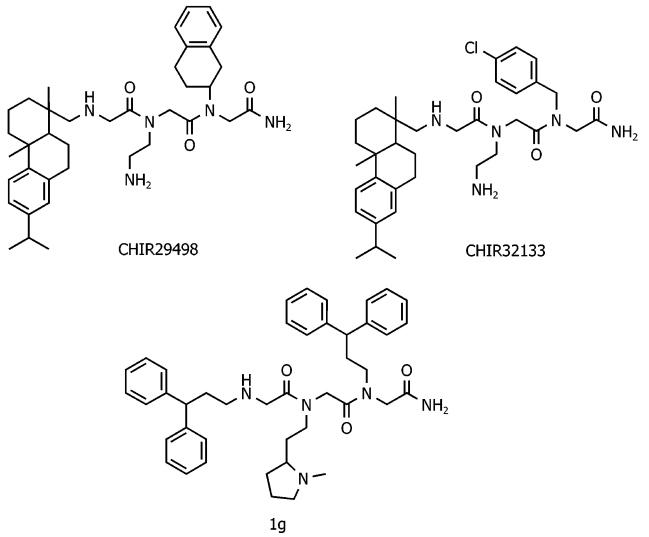


Figure 4. Structures of compounds **1g** (antimicrobial lead outlined in the present study) and two potent antimicrobial *N*-alkylglycine trimers recently identified by Goodson et al.[12]

bearing the Fmoc-protected Rink amide linker (AM RAM) (0.7 mmol/g, Rapp Polymer; Germany) using a HS501 Digital IKALabortechnik stirrer. The eight-step synthesis protocol was similar to that previously described for individual peptoids,[3] although it was slightly modified in order to adapt it to the positional scanning format.[14] All treatments were carried out in duplicate. Concerning the control of reactions, the TNBS test was conducted to monitor the initial Fmoc deprotection and the chloranil test for checking the acylation and amination steps.

The 66 tea bags of the library, in addition to six extra tea bags for reaction control, were filled with 0.7 g of Rink amide resin (load of 0.7 mmol/g, 0.49 mmol) and manipulated in three groups, one per sublibrary ($3 \times (22 + 2)$). Common reactions on the tea bags (deprotection, acylation, and the introduction of a random diversity position) were carried out in 1-L polypropylene bottles, and amination reactions at the defined positions were performed in 50-mL polypropylene pots.

Deprotection. A solution containing 720 mL of 20% piperidine in DMF was added to each one of three 1-L bottles containing the tea bags, and the mixture was stirred for 30 min. The resin was drained and washed with DMF ($3 \times$ 720 mL), ^{i}PrOH (3×720 mL), and CH_2Cl_2 (3×720 mL).

Acylation. The resin was treated with a solution of chloroacetyl chloride (19 mL, 20 equiv/bag) and triethylamine (33 mL, 20 equiv/bag) in 720 mL of CH_2Cl_2 per bottle for 90 min on an ice bath. The resin was drained and washed with CH_2Cl_2 (3×720 mL), ^{i}PrOH (3×720 mL), and DMF (3×720 mL).

Amination Conditions. (a) Defined Positions. The tea bags corresponding to the defined position and the control bags were separated and introduced individually into 50-mL bottles. The corresponding primary amine (20 equiv, 9.8 mmol) and triethylamine (1.36 mL, 20 equiv, 9.8 mmol) in 30 mL of DMF were added to the appropriate tea bag, and the mixture was stirred for 3 h at room temperature. Afterward, the tea bag was drained and washed with DMF (3×30 mL), ^{i}PrOH (3×30 mL), and CH_2Cl_2 (3×30 mL).

(b) Random Position. The other tea bags corresponding to the sublibraries with a random position were maintained together in 1-L bottles. An equimolar mixture of the 22 primary amines (20 equiv/amine, 0.91 equiv amine/bag) and triethylamine (30 mL, 20 equiv/bag) in 660 mL of DMF were added to the bags, and the mixture was stirred for 3 h at room temperature. Afterward, the tea bags were drained and washed with DMF (3×720 mL), ^{i}PrOH (3×720 mL), and CH_2Cl_2 (3×720 mL).

Cleavage. The resin was treated with a mixture of 60:40:2 (v/v/v) TFA/DCM/H_2O for 30 min at room temperature. The cleavage mixture was filtered, all filtrates were pooled,

Table 3. Antimicrobial Parameters Determined for Compound **1g** and for CHIR29498 and CHIR32133

	MIC (mg/mL)			IC_{50} (mg/mL)			MBC (mg/mL)		
strain	1g	CHIR 29498	CHIR 32133	1g	CHIR 29498	CHIR 32133	1g	CHIR 29498	CHIR 32133
E. coli ATCC 25922	0.062	0.05	0.025	0.01	0.023	0.015	0.5	0.05	0.05
P. aeruginosa ATCC 9721	0.031	0.05	0.050	0.018	0.09	0.025	2	0.05	0.05
A. calcoaceticum ATCC 19606	0.062	0.05	0.025	0.028	0.02	0.012	0.5	0.05	0.05
S. aureus ATCC 25923	0.031	0.025	0.012	0.013	0.012	0.008	0.125	0.05	0.05
S. aureus SAMR10	0.031	0.025	0.012	0.015	0.01	0.006	0.125	0.05	0.025
E. faecalis ATCC 29212	0.031	0.05	0.025	0.015	0.027	0.012	0.125	0.05	0.025

and the solvent was removed by evaporation under reduced pressure followed by lyophilization. The recovered average weight for all mixtures was ~70%. Finally, all of the 66 mixtures were redissolved in 10% DMSO at 10 mg/mL and stored at −20 °C until use.

Synthesis of Individual Peptoids. Individual *N*-alkylglycine trimers were synthesized following the eight-step procedure described above with slight modifications, that is, use of a 5-equiv excess of reagents and reactions carried out in 10-mL polypropylene syringes. The cyclic tetraalkylammonium derivatives obtained as side producs in the synthesis of individual peptoids were separated and purified from the reaction crude by semipreparative HPLC using a Kromasil C8 (25 × 2 cm, 5 μm) column, with CH_3CN-H_2O mixtures containing 0.1% TFA at 5 mL/min as mobile phase.

[*N*-(2,4-Diclorophenethyl)glycyl]-[*N*-[2-(2-(1-methylpyrrolidinyl))ethyl]glycyl]-*N*-(3-methylbutyl)glycinamide (1a). ^{1}H NMR (500 MHz, CD_3OD): 7.52 (bs, 1H), 7.39 (d, 2H), 7.35 (d, 2H), 4.55−4.1 (ca, 2 × $COCH_2N$), 4.06 (ca, *CH*Ph_2), 4.00−3.85 (ca, $COCH_2N$), 3.7−3.6 (A), 3.1−3.0 (B) (ca, *CH*$_2$$NCH_3$), 3.45−3.35 (ca, $CH_3NCHCH_2CH_2N$), 3.28 (ca, *CH*$_2$$CH_2Ph$), 3.18 (ca, CH_2CH_2Ph), 2.91, 2.87 (ds, CH_3N), 2.51 (m, $NCHCH_2CH_2$ (A)), 2.18 (A), 1.84 (B) ($CHCH_2$-CH_2), 2.1−1.95 ($CHCH_2CH_2CH_2N$), 1.75−1.68 ($NCHCH_2$-CH_2 (B)), 1.65−1.6 (*CH*$(CH_3)_2$), 1.55, 1.38 (N*CH*$_2$$CH_2$), 0.98, 0.91 (dd, J = 6.5 Hz, 2 × CH_3). ^{13}C NMR (125 MHz, CD_3OD): 173.04, 172.69 (CO), 170.61, 170.01 (CO), 168.53, 168.46 (CO), 135.94 (C_{Ar}), 135.19 (C_{Ar}), 134.28 (C_{Ar}), 133.3 (CH_{Ar}), 130.55 (CH_{Ar}), 128.96 (CH_{Ar}), 68.51, 68.47 (*CH*NCH_3), 57.27, 57.23 (*CH*$_2$$NCH_3$), 50.45 ($COCH_2N$), 50.24 ($COCH_2N$), 49.1 ($COCH_2N$), 47.85, 47.91 ($CH_2CH_2$-NH), 46.49, 46.24 (N*CH*$_2$$CH_2$), 40.30, 40.15 ($CH_3N$), 38.19, 37.30 (N*CH*$_2$$CH_2$), 30.78, 30.72 (Ph*CH*$_2$$CH_2$), 30.48, ($CHCH_2$-$CH_2CH_2N$), 30.09, 29.84 ($CHCH_2CH_2N$), 27.31, 27.14 (*CH*$(CH_3)_2$), 22.89, 22.79 ($CH_3$ × 2), 22.48 ($CHCH_2CH_2$-CH_2N). HRMS: Calcd. for $C_{26}H_{42}N_5O_3{}^{35}Cl_2$, 542.2666; found, 542.2666. Calcd. for $C_{26}H_{42}N_5O_3{}^{35}Cl^{37}Cl$, 544.2635; found, 544.2669.

[*N*-(2,4-Dichlorophenetyl)glycyl]-[*N*-[2-(2-(1-methylpyrrolidinyl))ethyl]glycyl]-*N*-(cyclohexyl)glycinamide (1b). ^{1}H NMR (500 MHz, CD_3OD): 7.51 (bs, 1H), 7.39 (d, 2H), 7.35 (d, 2H), 4.55−4.1 (ca, 2 × $COCH_2N$), 4.00−3.85 (ca, $COCH_2N$), 3.7−3.6 (ca, *CH*$_2$$NCH_3$ (A), *CH*$(CH_2)_2$), 3.45−3.35 (ca, $CH_3NCHCH_2CH_2N$), 3.27 (ca, *CH*$_2$$CH_2Ph$), 3.18 (ca, CH_2CH_2Ph), 3.15−3.10 (ca, *CH*$_2$$NCH_3$ (B)), 2.91, 2.87 (ds, CH_3N), 2.51 (m, $NCHCH_2CH_2$ (A)), 2.18 (ca, $CHCH_2$-CH_2, (A)), 2.1−1.99 (ca, $CHCH_2CH_2CH_2N$), 1.89−1.82 (ca, $CHCH_2CH_2$ (B), $(CH_2)_2$CH) (A)), 1.8−1.7 (ca, $NCHCH_2$-CH_2 (B)), 1.7−1.6 (ca, $CH_2(CH_2)_2$), 1.46 (ca, $(CH_2)_2$CH) (B)), 1.34 (ca, $CH_2(CH_2)_2$). ^{13}C NMR (125 MHz, CD_3OD): 173.51, 173.47 (CO), 170.35, 169.73 (CO), 168.56, 168.51 (CO), 135.94 (C_{Ar}), 135.13 (C_{Ar}), 134.29 (C_{Ar}), 133.33 (CH_{Ar}), 130.51 (CH_{Ar}), 128.93 (CH_{Ar}), 68.51, 68.42 (*CH*N-CH_3), 57.66, 55.94 (N*CH*$(CH_2)_2$), 57.22, 57.17 (*CH*$_2$$NCH_3$), 50.84 ($COCH_2N$), 50.43 ($COCH_2N$), 48.8 ($COCH_2N$), 47.74 ($CH_2CH_2NH$), 46.44, 46.11 (N*CH*$_2$$CH_2$), 40.29, 40.05 ($CH_3N$), 32.11 (*CH*$_2$$CH$*CH*$_2$), 30.98 (*CH*$_2$$(CH_2)_2$), 30.77 (Ph*CH*$_2$$CH_2$), 30.48, ($CHCH_2CH_2CH_2N$), 30.08, 29.74 ($CHCH_2CH_2N$), 26.82, 26.62 (*CH*$_2$$CH_2$*CH*$_2$), 22.48 ($CHCH_2CH_2CH_2N$). HRMS: Calcd. for $C_{27}H_{42}N_5O_3{}^{35}Cl_2$, 554.2665; found, 554.2667. Calcd. for $C_{27}H_{42}N_5O_3{}^{35}Cl^{37}Cl$, 556.2635; found, 556.2648.

[*N*-(3,3-Diphenylpropyl)glycyl]-[*N*-[2-(2-(1-methylpyrrolidinyl))ethyl]glycyl]-*N*-(3-methylbutyl)glycinamide (1c). ^{1}H NMR (500 MHz, CD_3OD): 7.30 (ca, 8H), 7.194 (m, 2H), 4.55−4.1 (ca, 2 × $COCH_2N$), 4.04 (ca, *CH*Ph_2), 3.95−3.8 (ca, $COCH_2N$), 3.7−3.6 (A), 3.1−3.0 (B) (ca, *CH*$_2$$NCH_3$), 3.39−3.31 (ca, $CH_3NCHCH_2CH_2N$, NCH_2CH_2CH), 2.96 (t, J = 8 Hz, *CH*$_2$$CH_2CHPh_2$), 2.87, 2.82 (ds, CH_3N), 2.48 (m, *CH*$_2$$CHPh_2$, $NCHCH_2CH_2$ (A)), 2.18 (A), 1.8 (B) ($CHCH_2$-CH_2), 2.1−1.99 ($CHCH_2CH_2CH_2N$), 1.7−1.6 ($NCHCH_2CH_2$ (B), *CH*$(CH_3)_2$), 1.55, 1.38 (N*CH*$_2$$CH_2$), 0.96, 0.89 (dd, J = 6.5 Hz, 2 × CH_3). ^{13}C NMR (125 MHz, CD_3OD): 172.66 (CO), 170.57, 169.99 (CO), 168.60, 168.54 (CO), 144,75 (C_{Ar}), 129.79 (CH_{Ar} × 4), 128.71 (CH_{Ar} × 4), 127.78 (CH_{Ar} × 2), 68.52, 68.49 (*CH*NCH_3), 57.27, 57.21 (*CH*$_2$$NCH_3$), 50.39 ($COCH_2N$), 50.09 ($COCH_2N$), 49.9 (*CH*$Ph_2$), 48.75 ($COCH_2N$), 47.95, 47.90 ($CH_2CH_2NH$), 47.81 (N*CH*$_2$$CH_2$-CH), 46.42, 46.17 ($NCH_2CH_2$), 40.35, 40.20 ($CH_3N$), 38.16, 37.27 (N*CH*$_2$$CH_2$), 32.52, 32.43 (*CH*$_2$$CHPh_2$), 30.55, 30.46 ($NCHCH_2CH_2$), 30.07, 29.82 ($CHCH_2CH_2N$), 27.32, 27.14 (*CH*$(CH_3)_2$), 22.89, 22.79 ($CH_3$ × 2), 22.48 ($CHCH_2CH_2$-CH_2N). HRMS: Calcd. for $C_{33}H_{50}N_5O_3$, 564.3914; found, 564.3921.

[*N*-(3,3-Diphenylpropyl)glycyl]-[*N*-[2-(2-(1-methylpyrrolidinyl))ethyl]glycyl]-*N*-(cyclohexyl)glycinamide (1d). ^{1}H NMR (500 MHz, CD_3OD): 7.30 (ca, 8H), 7.20 (m, 2H), 4.55−4.1 (ca, 2 × $COCH_2N$), 4.03 (ca, *CH*Ph_2), 3.95−3.8 (ca, $COCH_2N$), 3.7−3.6 (ca, *CH*$_2$$NCH_3$ (A), *CH*$(CH_2)_2$), 3.39−3.31 (ca, $CH_3NCHCH_2CH_2N$), 3.15−3.10 (ca, *CH*$_2$-NCH_3 (B)), 2.97 (t, J = 8 Hz, *CH*$_2$$CH_2CHPh_2$), 2.87, 2.82 (ds, CH_3N), 2.49 (m, *CH*$_2$$CHPh_2$, $NCHCH_2CH_2$ (A)), 2.18 (A), 1.85 (B) ($CHCH_2CH_2$), 2.1−1.99 ($CHCH_2CH_2CH_2N$), 1.89−1.78 (ca, $CHCH_2CH_2$ (B), $(CH_2)_2$CH) (A)), 1.74−1.60 (ca, $NCHCH_2CH_2$ (B), $CH_2(CH_2)_2$), 1.44 (ca, $(CH_2)_2$CH) (B)), 1.35 (ca, *CH*$_2$$(CH_2)_2$). ^{13}C NMR (125 MHz, CD_3OD): 173.52, 173.42 (CO), 170.35, 169.71 (CO), 168.63, 168.60

(CO), 144.76 (C_{Ar}), 129.77 (CH_{Ar} × 4), 128.71 (CH_{Ar} × 4), 127.75 (CH_{Ar} × 2), 68.55, 68.47 (*CH*NCH_3), 57.68, 56.033 (N*CH*$(CH_2)_2$), 57.68, 57.23 (*CH*$_2$$NCH_3$), 50.83 ($COCH_2N$), 50.41 ($COCH_2N$), 49.91 (*CH*$Ph_2$), 48.83 ($COCH_2N$), 47.93, 47.82 ($CH_2$*CH*$_2$NH), 46.40, 46.11 (N*CH*$_2$$CH_2CH$), 40.11 ($CH_3N$), 32.37 (*CH*$_2$$CHPh_2$), 32.12 ((*CH*$_2$)$_2$CH), 31.00 (*CH*$_2$-$(CH_2)_2$), 30.53 (NCH*CH*$_2$$CH_2$), 30.09, 29.76 (CH*CH*$_2$$CH_2N$), 26.84, 26.60 ((*CH*$_2$)$_2$$CH_2$), 22.48 ($CHCH_2$*CH*$_2$$CH_2N$).

[*N*-(3,3-Diphenylpropyl)glycyl]-[*N*-[2-(2-(1-methylpyrrolidinyl))ethyl]glycyl]-*N*-(3,3-diphenylpropyl)glycinamide (1g). ^{1}H NMR (500 MHz, CD_3OD): 7.35 (ca, 8H), 7.2 (m, 2H), 4.5−4.1 (ca, 2 × $COCH_2N$), 4.09 (ca, 2 × *CH*Ph_2), 3.9−3.8 (ca, $COCH_2N$), 3.7−3.6 (A), 3.1−3.0 (B) (ca, *CH*$_2$$NCH_3$), 3.4−3.3 (ca, CH_3N*CH*CH_2*CH*$_2N$), 2.96 (ca, 2 × *CH*$_2$$CH_2CHPh_2$), 2.8 (ds, CH_3N), 2.45 (m, 2 × *CH*$_2$-$CHPh_2$, NCH*CH*$_2$$CH_2$ (A)), 2.18 (A), 1.8 (B) (CH*CH*$_2$$CH_2N$), 2.1 ($CHCH_2$*CH*$_2$$CH_2N$), 1.65 (NCH*CH*$_2$$CH_2$ (B)). ^{13}C NMR (125 MHz, CD_3OD): 174.8 (CO), 170.8 (CO), 168.6, 168.27 (CO), 142.97 (C_{Ar}), 128.94 (CH_{Ar} × 8), 127.51 (CH_{Ar} × 8), 126.92 (CH_{Ar} × 4), 67.04 (*CH*NCH_3), 56.18 (*CH*$_2$$NCH_3$), 49.18 ($COCH_2N$), 48.89 ($COCH_2N$), 48.63 (*CH*$Ph_2$), 48.33 ($COCH_2N$), 47.70 ($CH_2$*CH*$_2$NH), 47.38 (N*CH*$_2$$CH_2CH$), 40.4 ($CH_3N$), 31.57 (*CH*$_2$$CHPh_2$), 29.55 (NCH*CH*$_2$$CH_2$), 28.90 (CH*CH*$_2$$CH_2N$), 21.63 ($CHCH_2$*CH*$_2$$CH_2N$). HRMS: Calcd. for $C_{43}H_{54}N_5O_3$, 688.4227; found, 688.4239.

6-[(*N*-(3-Methylbutyl)-*N*-((carbamoyl)methyl)carbamoyl)methyl]-6-aza-8a-azonia-8a-methyl-7-oxo-perhydroazulene trifluoroacetate (2a). ^{1}H NMR (300 MHz, CD_3OD): 4.8−4.6 (ca, CO*CH*$_2$$N^+$), 4.3−3.9 (ca, 2 × CO*CH*$_2$N, CHN^+), 3.8 (ca, CH_2N^+), 3.4−3.3 (CH_3N^+, 2 × N*CH*$_2$$CH_2$), 2.4−2.0 (ca, *CH*$_2$$CH$*CH*$_2$*CH*$_2$), 1.57 (m, CH*CH*$_2$), 1.4 (m, *CH*$(CH_3)_2$), 0.95 (dd, 2 × CH_3). ^{13}C NMR (75 MHz, CD_3OD): 169.7 ($CONH_2$), 165.8 (CO), 165.7 (CO), 75.4 (CHN^+), 70.08 (CH_2N^+), 61.11 (CO*CH*$_2$$N^+$), 52.32 ($H_2$-NCO*CH*$_2$), 49.8 ($CO$*CH*$_2$N), 49.19 ($CH_3N^+$), 47.56 (N*CH*$_2$-$CH_2CH$), 45.30 (N*CH*$_2$$CH_2$), 37.97 (NCH$_2$*CH*$_2$CH), 30.75 (*CH*$_2$CHN), 27.17 ($CH(CH_3)_2$), 26.46 ($CH_2$*CH*$_2$$CH_2$), 22.77 (2 × CH_3), 19.53 (NCH$_2$*CH*$_2$CH). HRMS: Calcd. for $C_{18}H_{33}N_4O_3$, 353.2553; found, 353.2548.

6-[(*N*-(3-Cyclohexyl)-*N*-((carbamoyl)methyl)carbamoyl)methyl]-6-aza-8a-azonia-8a-methyl-7-oxo-perhydroazulene trifluoroacetate (2b). ^{1}H NMR (300 MHz, CD_3OD): 4.8−4.6 (ca, CO*CH*$_2$$N^+$), 4.3−3.9 (ca, 2 × CO*CH*$_2$N, CHN^+), 3.8 (ca, CH_2N^+), 3.6 (ca, *CH*$(CH_2)_2$), 3.4−3.3 (CH_3N^+, N*CH*$_2$$CH_2$), 2.4−2.0 (ca, *CH*$_2$$CH$*CH*$_2$*CH*$_2$), 1.87 (m, 2 × *CH*$_2$), 1.67 (m, *CH*$_2$$(CH_2)_2$), 1.42 (m, 2 × CH_2). ^{13}C NMR (75 MHz, CD_3OD): 174.4 ($CONH_2$), 169.9 (CO), 165.9 (CO), 75.4 (CH), 70.08 (CH_2N^+), 61.17 (CO*CH*$_2$$N^+$), 57.86 (N*CH*$(CH_2)_2$), 52.66 ($H_2$NCO*CH*$_2$), 49.8 ($CO$*CH*$_2$N), 49.28 ($CH_3N^+$), 45.43 (N*CH*$_2$$CH_2$), 31.95 (2 × CH_2), 30.96 (*CH*$_2$CHN), 26.8 (*CH*$_2$$(CH_2)_2$), 26.46 ($CH_2$*CH*$_2$$CH_2$), 26.16 (2 × CH_2), 19.54 (NCH$_2$*CH*$_2$CH). HRMS: Calcd. for $C_{19}H_{33}N_4O_3$, 365.2553; found, 365.2554.

6-[(*N*-(3′,3′-Diphenylpropyl)-*N*-((carbamoyl)methyl)-carbamoyl)methyl]-6-aza-8a-azonia-8a-methyl-7-oxo-perhydroazulene trifluoroacetate (2c). ^{1}H NMR (300 MHz, CD_3OD): 4.8−4.6 (ca, CO*CH*$_2$$N^+$), 4.3−3.9 (ca, 2 × CO*CH*$_2$N, CHN^+, *CH*Ph_2), 3.8 (ca, CH_2N^+), 3.19 (t, N*CH*$_2$-CH_2), 3.0 (s, CH_3N^+), 2.3 (m, *CH*$_2$$CHPh_2$), 2.2−2.0 (ca, *CH*$_2$-$CH$*CH*$_2$*CH*$_2$). ^{13}C NMR (75 MHz, CD_3OD): 170.03 ($CONH_2$), 166.25 (CO), 165.7 (CO), 144.98 (C_{Ar}), 129.77 (CH_{Ar} × 4), 128.59 (CH_{Ar} × 4), 127.57 (CH_{Ar} × 2), 75.4 (CH), 69.99 (CH_2N^+), 60.89 (CO*CH*$_2$$N^+$), 52.32 ($H_2$NCO*CH*$_2$), 51.69 ($Ph_2$*CH*), 49.62 ($CH_3N^+$), 49.41 ($COCH_2N$), 48.58 (N*CH*$_2$-$CH_2$), 45.48 (N*CH*$_2$$CH_2CH$), 33.97 (*CH*$_2$CHN), 33.38 ($Ph_2$-CH*CH*$_2$), 25.94 ($CH_2$*CH*$_2$$CH_2$), 19.32 (NCH$_2$*CH*$_2$CH). HRMS: Calcd. for $C_{28}H_{37}N_4O_3$, 477.2866; found, 477.2867.

Antimicrobial Assays. The unbiased peptoid library was screened in a 96-well format in a growth-inhibition assay against a panel of three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and a clinical isolate *Staphylococcus aureus* methicillin-resistant SAMR10) and three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9721, and *Acinetobacter calcoaceticum* ATCC 19606). The exponential phase from an overnight culture grown at 37 °C in Mueller Hinton broth at a final concentration of (1−6) × 10^4 colony-forming units/mL was used in all assays. Bacterial suspension in 2× broth was added to the mixtures at concentrations varying from 5 to 0.6 mg/mL (or from 2 to 0.006 mg/mL for individual peptoids) derived from serial 2-fold dilutions (the final DMSO concentration was below 1%, and appropriate DMSO controls showed no effect on bacterial growth). The plates were incubated overnight at 37 °C. The relative percentage growth of the bacteria found for each mixture was determined by measuring absorbance at 405 nm. For the individual peptoids, the antimicrobial parameters evaluated were the minimum inhibitory concentration (MIC), the IC_{50}, and the minimum bactericidal concentration (MBC), determined as described.[28]

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Supporting Information Available. Experimental details and spectral data for cyclic ammonium derivatives **3** and 2,5-piperazinediones **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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