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Design, synthesis and antimicrobial properties of non-hemolytic cationic α-cyclopeptoids

Daniela Comegna [a], Monica Benincasa [b], Renato Gennaro [b], Irene Izzo [a,*], Francesco De Riccardis [a,*]

[a] Deparment of Chemistry, University of Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy
[b] Department of Life Sciences, University of Trieste, 34127 Trieste, Italy

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ABSTRACT

The synthesis and screening of neutral and cationic, linear and cyclic peptoids (*N*-alkylglycine peptidomimetics) is described. Structure–activity relationship studies show that the in vitro activities of the tested peptoids depend on both cyclization and decoration with cationic groups. The most powerful *N*-lysine cyclopeptoid derivatives showed good antifungal activity against *Candida albicans* (ATCC90029 and L21) and *Candida famata* (SA550, Amph B-resistant) and low hemolytic activity. The effects of the cyclic peptoids on membrane permeabilization were evaluated by the propidium iodide exclusion assay.

1. Introduction

The development of functionalised artificial scaffolds capable of mimicking pharmacologically relevant natural compounds is an area of vibrant interest both for synthetic and medicinal chemists.[1] One of the fields that poses more challenges and opportunities, from the molecular and therapeutic point of view, is that of innate immunity, which raises an effective, nonspecific, broad-spectrum antimicrobial response based on different cellular and molecular systems, including cationic antimicrobial peptides.[2] The antibiotic activities of these structurally diverse sets of peptides derive from their action on microbial cytoplasmic membranes. The model proposed by Shai–Matsuzaki–Huan[3] (SMH) presumes alteration and permeabilization of the phospholipid bilayer with irreversible damage of the critical membrane functions. The fact that peptides operating by the SMH mechanism kill microbes at micromolar concentrations[4] and the recent reports demonstrating that efficient membrane-active antibiotics can be prepared from monomers other than α-amino acids,[5] prompted the design and synthesis of facially amphiphilic peptides[2a] and peptidomimetics.[6] The conformational constraints of the proposed backbones, have been mainly achieved through periodic incorporation of bulky, α-chiral, side chains,[7] or use of rigid scaffolds.[5] Cyclization of linear peptide/peptoid precursors (as a mean to obtain conformational order), has been often neglected,[8] notwithstanding the fact that nature offers a vast assortment of powerful cyclic antimicrobial peptides.[9] However, macrocyclization of *N*-substituted glycines gives circular peptoids,[10] showing reduced conformational freedom[10,11] and excellent membrane-permeabilizing activity.[12]

In the present paper, we investigate the antimicrobial activities of five new cyclic cationic hexameric α-peptoids (**5**, **9**, **15**, **19**, **23**), correlating their efficacy with the linear cationic (**3**, **7**, **13**, **17**, **21**) and the cyclic neutral (**1**, **11**) counterparts (Fig. 1). The synthesized peptoids have been assayed against clinically relevant bacteria and fungi, including *Escherichia coli*, *Staphylococcus aureus*, amphotericin B-resistant *Candida albicans*, and *Cryptococcus neoformans*.[13] The purpose of this study is to explore the biological effects of the cyclization on positively charged oligomeric *N*-alkylglycines, with the idea to mimic the natural amphiphilic peptide antibiotics. The long-term aim of the effort is to find a key for the rational design of novel antimicrobial compounds using the finely tunable peptoid backbone.

2. Results and discussion

The exploration for possible biological activities of linear and cyclic α-peptoids, started with the assessment of the antimicrobial activity of the known[10a] *N*-benzyloxyethyl cyclohomohexamer **1** (Fig. 1, Block I). This neutral cyclic peptoid was considered a promising candidate in the antimicrobial assays for its high affinity to the first group alkali metals ($K_a \sim 10^6$ for Na^+, Li^+ and K^+)[10a] and its ability to promote Na^+/H^+ transmembrane exchange through ion-carrier mechanism,[14] a behavior similar to that observed for valinomycin, a well known K^+-carrier with powerful antibiotic activity.[15] However, determination of the MIC values showed that **1** did not exert any antimicrobial activity against a group of

* Corresponding authors. Tel.: +39 089 969552; fax: +39 089 969603.
E-mail addresses: iizzo@unisa.it (I. Izzo), dericca@unisa.it (F. De Riccardis).

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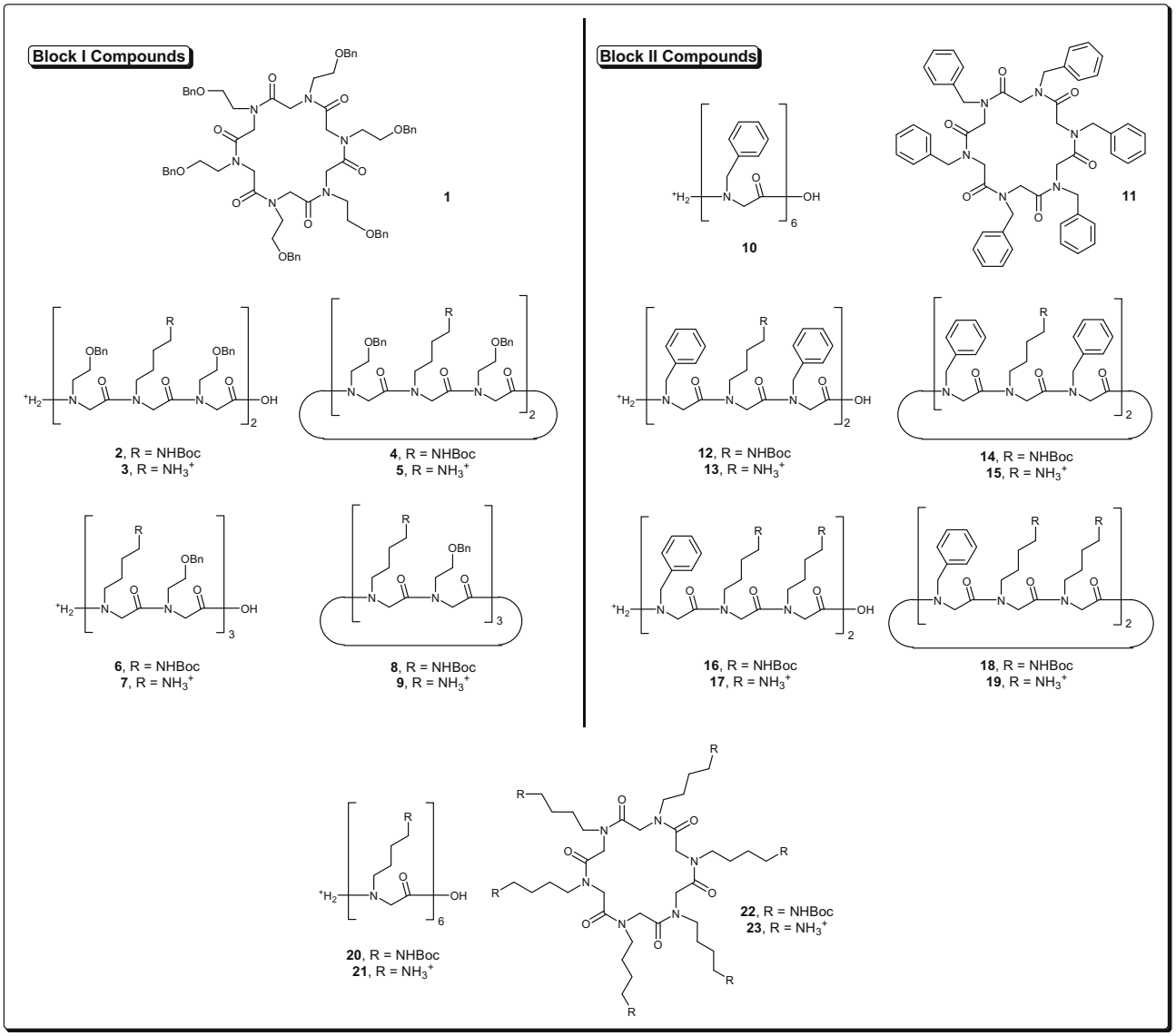


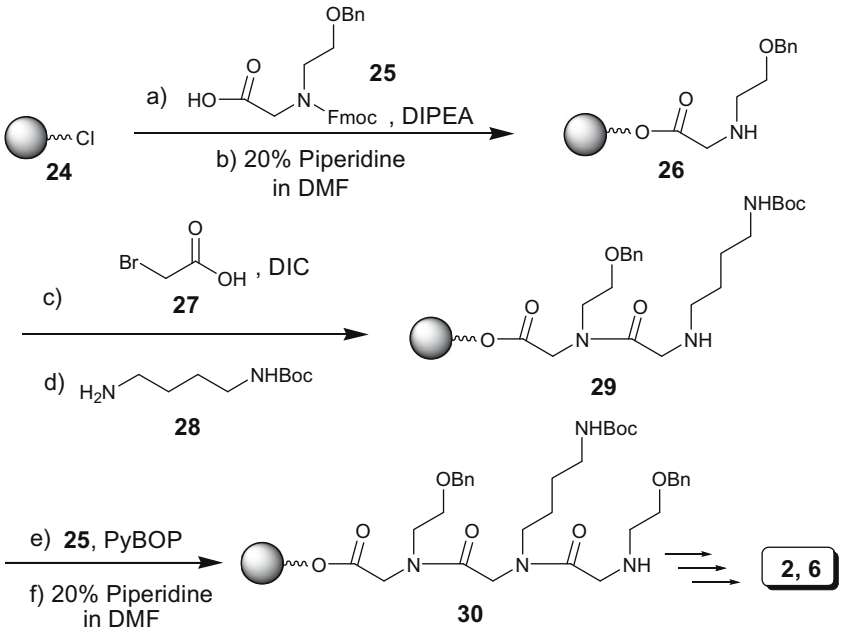
Figure 1. Structures of synthesized linear and cyclic peptoids. Bn = benzyl group; Boc = *t*-butoxycarbonyl group.

selected pathogenic fungi, and of Gram-negative and Gram-positive bacterial strains even at concentrations up to 1 mM (Table 1).

Detailed structure–activity relationship (SAR) studies[7,16] have revealed that the amphiphilicity of the peptides/peptidomimetics and the total number of positively charged residues, impact significantly on the antimicrobial activity. We, thus, reasoned that cationic versions of the cyclic α-peptoids **1** could represent interesting mimics of antimicrobial peptides and planned the synthesis of mixed *N*-(4-aminobutyl)/*N*-benzyloxyethyl hexameric α-peptoids **5**, and **9** (Fig. 1, Block I compounds). In our investigation, we also included the linear cationic precursors **3** and **7**, in order to evaluate the effect of macrocyclization on the antimicrobial activity.

The elaboration of the *t*-butoxycarbonyl protected Block I peptoids, was accomplished via an expeditious mixed 'monomer',[17] and 'submonomer',[18] approach, consisting in an alternate attachment of the *N*-fluorenylmethoxycarbonyl,*N*′-benzyloxyethyl glycine,[19] (**25**, Scheme 1), and a two step construction of the *N*-(4-aminobutyl) glycine remnant. The oligomerization of the units was performed on a 2-chlorotrityl resin (**24**). Once the construction of the linear oligomers was completed, they were cleaved from the resin, using a 1:1 solution of hexafluoroisopropanol (HFIP)/dichloromethane (the purity of the oligomers was >95%, RP-HPLC analysis). The slight acidity of the fluorinated secondary alcohol detached the hexamers from the 2-chlorotrityl groups, preserving the side chain *t*-butoxycarbonyl protection. Head-to-tail macrocyclizations of the linear *N*-substituted oligoglycines **2** and **6** (Block I compounds, Fig. 1), proceeded smoothly giving, under high dilution conditions (2.0×10^{-3} M) and in the presence of the efficient coupling agent benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), the cyclic peptoids **4** and **8**. The Boc-protected linear and cyclic precursors (**2**, **4**, **6**, and **8**) were deprotected with trifluoroacetic acid (TFA), to afford the couples **3**/**5** and **7**/**9**.

Cationic peptoids **3**, **5**, **7**, and **9** were tested against four pathogenic fungi and three clinically relevant bacterial strains. The antimicrobial activities are summarized in Table 1. The tests showed a marked increase of the antibacterial and antifungal activities with cyclization. The presence of charged amino groups also influenced the antimicrobial efficacy, as shown by the activity of the bi- and tricationic compounds **5** and **9**, when compared with the ineffective **1**.



Scheme 1. Mixed monomer/submonomer approach for the synthesis of oligomers **2** and **6**. DIPEA = diisopropylethylamine; DIC = *N,N′*-diisopropyl carbodiimide; PyBOP = benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate.

Encouraged by the promising results displayed by the *N*-Lys/*N*-benzyloxyethyl cyclopeptoids, we decided to move towards analogues produced with a fully sub-monomeric approach and, with the aim to have a better defined SAR, presenting a pairwise increase of cationic side-chains, interrupted by *N*-Phe residues (Block II compounds, Fig. 1). The solid-phase synthesis of these new peptoids proceeded uneventfully, giving, after detachment from the resin in the same condition reported for the congeners **2** and **6**, the linear oligomers **10**, **12**, and **16** (the purity of the oligomers was >95%, RP-HPLC analysis). High dilution (2.0×10^{-3} M) head-to-tail PyBOP-mediated macrocyclizations, gave the cyclic **11**, **14** and **18**. The four Boc-protected linear (**12** and **16**) and cyclic (**14** and **18**) precursors were deprotected with TFA, to yield the couples **13**/**15**, and **17**/**19**, respectively.

The synthesis of fully *N*-lysinated peptoids was also planned. The linear hexamer **20** was oligomerized on-resin and, once detached from the solid support, it was cyclized using the same conditions reported for the *N*-benzylated congeners, to give the fully protected homo-oligomer **22**. Both **20** and **22** were deprotected in the presence of TFA, to afford peptoids **21** and **23**.

Compounds **11**, **13**, **15**, **17**, **19**, **21** and **23** were tested against five pathogenic fungi and three bacterial strains. The MIC values are summarized in Table 2. The results of this second array of antimicrobial evaluations again demonstrated an increase of the antifungal activities when the cyclic arrangement was present (for instance, compare the results of linear **17** vs cyclic **19**) and weak antibacterial activity. It is interesting to note that the number of the amino groups inserted on the cyclic peptoids strongly influences the MIC value for the antifungal activity. In particular, compound **19** seems to have an optimal *N*-Lys/*N*-benzyloxyethyl residue ratio and, subsequently, a higher potency.

To be useful therapeutically, the active compounds should not be toxic to host cells. As a preliminary evaluation of the toxicity of **19** and **23**, which show the most potent antifungal activity among the peptoids analyzed in this work, their hemolytic activity was tested on 0.5% suspension of human red blood cells. Both compounds, tested at 100 μM concentration (approximately 10-fold higher than the best MIC values, Table 2), showed virtually no hemolytic activity, at variance with melittin, a well known hemolytic peptide used as a control, which lysed over 90% of the cells at 20 μM concentration. Although preliminary, these results are encouraging for the development of the cyclic peptoids under investigation, as they indicate that these compounds display a significant selectivity against fungal versus mammalian cells.

To understand the mechanism by which the peptoids act against bacterial and fungal cells, their effect on membrane integrity was evaluated by flow cytometry using the fluorescent probe propidium iodide (PI), which only enters cells with damaged membranes and stains their nucleic acids.[20]

The results obtained after treatment of *C. albicans* with different concentrations of the most active peptoids of the two series, **9** and **19**, are reported in Figure 2. Compounds **19**, at 100 μM concentration, permeabilized a significant percentage of the yeast cells after 120 min of incubation. The percentage of PI-positive cells increased up to 50% by prolonging the incubation time (Fig. 2A). The permeabilizing effect of compound **9** on *C. albicans* cells is less evident also at the maximal concentration used (Fig. 2B), and these results correlate with the different potency showed by the peptoids (Tables 1 and 2).

The effect of **9** on bacterial membrane integrity is shown in Figure 2C. No significant effect is evident on the *E. coli* membrane even after incubation for 180 min at a concentration of 250 μM. In contrast, the same dose of **9** caused an appreciable effect (>40% PI-positive cells) on *S. aureus* cells, already after 60 min incubation, indicating that this compounds interacts with a faster kinetics with the membrane of Gram-positive microorganisms.

3. Conclusions

Cyclization of amino acid-based molecules is a strategy that has been used by many organisms to produce compounds for defense, signaling and microbial competition. Compounds such as cyclosporin A and congeners are assembled by peptide synthetases; others are encoded by genes and ribosomally translated, such as members of the microcins and bacteriocins from bacteria, cyclotides from plants and theta-defensins from monkeys.[21] All these

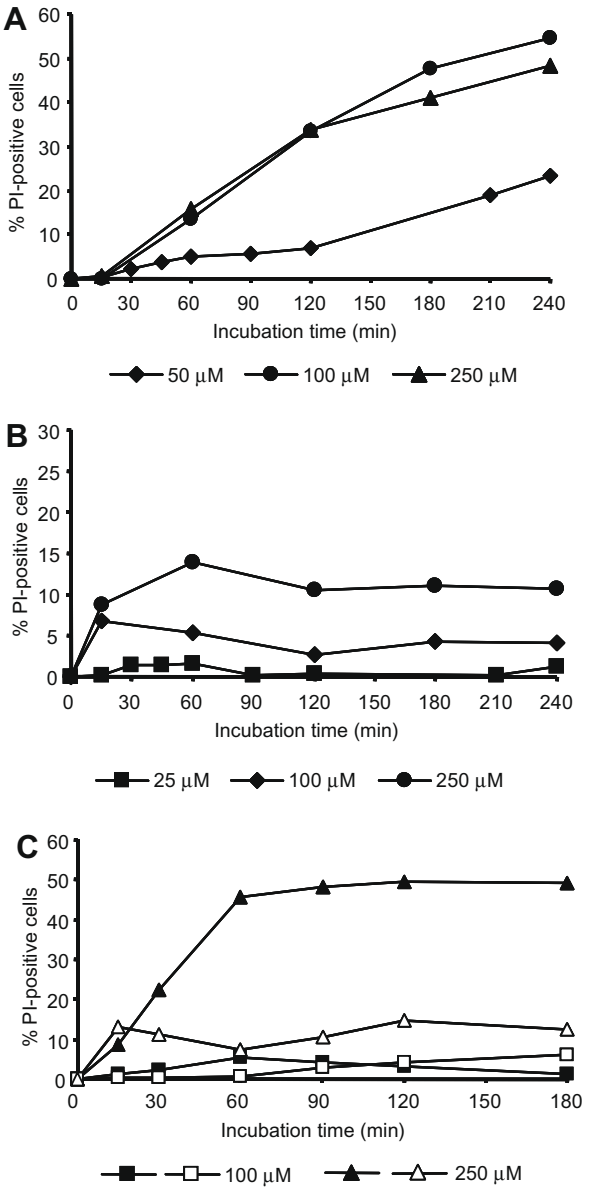


Figure 2. Flow cytometric evaluation of membrane integrity of fungal and bacterial cells after treatment with peptoids **9** and **19**. The percentage of fluorescent cells (% PI-positive cells) after incubation of *C. albicans* ATCC 90029 with **19** (A) and **9** (B), or after incubation of *S. aureus* (filled symbols) and *E. coli* (empty symbols) cells with **9** (C) is shown. Background values in the absence of peptoids (<3% of permeabilized cells) were subtracted to each peptoid-treated sample. Results are mean values of at least three independent determinations with similar results.

compounds exert a wide array of biological activities that range from regulation of microbial competitions to a potent microbicidal activity against bacteria, fungi and some viruses.[22,23]

Recent studies have suggested both a structural and functional role for the cyclic backbone. In fact, cyclization of the above mentioned peptides improves their stability, for instance by removing possible cleavage sites for exoproteases, as well as biological activity, as shown by the decreased biological activity of linear analogues of RTD-1 and cyclotides relative to the native peptides.[24]

In general the α-peptoids described in this study, which have a backbone of 18 atoms, are more active against fungi than bacteria. In addition, as observed for natural cyclic peptides, we found that the linear forms are less effective than their cyclic counterparts (compare for instance the antifungal activity of the linear compounds **7**, **17** and **21** with that of the respective cyclic forms **9**, **19** and **23**, Tables 1 and 2), in spite of a similar net positive charge. Finally, in the cyclic compounds, the antifungal potency appears to directly correlate with the net positive charge, as shown by compounds **19** and **23** that have the highest net positive charge (+3 and +5, respectively) and are also the most effective ones.

In conclusion, the synthesis, structural features, and antimicrobial activities of linear and cyclic homo- and heteroligomeric α-peptoids have been described. Two cyclic compounds in particular, **9** and **19**, showed good antifungal activity and no toxicity toward red blood cells at the concentrations tested. These results are the first indication that cyclic peptoids can represent new motifs on which to base artificial antibiotics. Investigations on the mechanism of action of structurally diverse cationic cyclopeptoids are currently underway.

4. Experimental section

4.1. Synthesis

4.1.1. General methods

All reactions involving air or moisture sensitive reagents were carried out under a dry argon or nitrogen atmosphere using freshly distilled solvents. Toluene and dichloromethane were distilled from calcium hydride under argon. Glassware was flame-dried (0.05 Torr) prior to use. When necessary, compounds were dried in vacuo over P_2O_5, by azeotropic removal of water with toluene under reduced pressure or by freeze-drying. Starting materials and reagents purchased from commercial suppliers were generally used without purification unless otherwise mentioned. Reactions were monitored by TLC on silica gel plates (0.25 mm) and visualized by UV light or by spraying with phosphomolybdic acid or ninhydrin solutions and drying. Flash chromatography was performed on Silica Gel 60 (particle size: 0.040–0.063 mm) and the solvents employed were of analytical grade. HPLC analysis were performed on a C_{18} reversed-phase analytical and preparative columns (Waters, μBondapak, 10 μm, 125 Å 3.9 mm × 300 mm and 7.8 × 300 mm, respectively) using a Modular HPLC System JASCO LC-NET II/ADC equipped with a JASCO Model PU-2089 Plus Pump and a JASCO MD-2010 Plus UV–vis multiple wavelength detector set at 220 nm. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure materials. The NMR spectra were recorded on Bruker DRX 400 (^{1}H at 400.13 MHz, ^{13}C at 100.03 MHz), Bruker DRX 300 (^{1}H at 300.10 MHz, ^{13}C at 75.48 MHz), and Bruker DRX 250 (^{1}H at

Table 1
MIC values of peptoids **1**, **3**, **5**, **7**, and **9**, against pathogenic fungi and Gram-positive/Gram-negative bacteria

Fungi[a]	**1**	**3**	**5**	**7**	**9**
C. albicans ATCC 90029	>10^3	360	196	>10^3	184
C. albicans L21	>10^3	360	196	681	184
C. neoformans ATCC 90112	>10^3	180	196	>10^3	184
C. neoformans L1	>10^3	90	98	349	92
Bacteria[a]					
E. coli ATCC 25922	>10^3	360	392	>10^3	383
Salmonella typhimurium ATCC 14028	>10^3	720	>792	>10^3	>766
Staphylococcus aureus ATCC 25923	>10^3	>720	392	>10^3	383

[a] The MIC value is defined as the lowest compound concentration resulting in a complete inhibition of visible growth after 48 h incubation at 30 °C for fungi and after 18 h incubation at 37 °C for bacteria. MIC values are in micromolar concentration to allow comparison of the same number of molecules per assay. To convert data to micrograms/milliliter, multiply the MIC value with the molecular weight/1000. Molecular weights of the compounds are as follows (as trifluoroacetate salts): **1**, 1147; **3**, 1381; **5**, 1249; **7**, 1432; **9**, 1300. The activity data of the cyclic cationic peptoids (**5** and **9**) are underlined.

Table 2
MIC values of peptoids **11**, **13**, **15**, **17**, **19**, **21** and **23**, against pathogenic fungi and Gram-positive/Gram-negative bacteria

Fungi[a]	11	13	15	17	19	21	23
C. albicans ATCC 90029	$>10^3$	$>10^3$	465	$>10^3$	12	$>10^3$	10
C. albicans L21	$>10^3$	$>10^3$	232	$>10^3$	12	500	5
C. neoformans ATCC 90112	$>10^3$	$>10^3$	232	$>10^3$	47	$>10^3$	$>10^3$
C. neoformans L1	$>10^3$	788	116	$>10^3$	99	$>10^3$	$>10^3$
C. famata SA550	n. t.	n. t.	n. t.	$>10^3$	12	$>10^3$	5
Bacteria[a]							
E. coli ATCC 25922	$>10^3$	$>10^3$	465	$>10^3$	786	$>10^3$	$>10^3$
Salmonella typhimurium ATCC 14028	$>10^3$	$>10^3$	465	$>10^3$	$>10^3$	$>10^3$	$>10^3$
Staphylococcus aureus ATCC 25923	$>10^3$	$>10^3$	465	$>10^3$	$>10^3$	$>10^3$	$>10^3$

[a] The MIC value is defined as the lowest compound concentration resulting in a complete inhibition of visible growth after 48 h incubation at 30 °C for fungi and after 18 h incubation at 37 °C for bacteria. The MIC values are in micromolar concentration to allow comparison of the same number of molecules per assay. To convert data to micrograms/milliliter, multiply the MIC value with the molecular weight/1000. Molecular weights of the compounds are as follows (as trifluoroacetate salts): **11**, 883; **13**, 1205; **15**, 1073; **17**, 1395; **19**, 1263; **21**, 1585; **23**, 1453. The activities of the cyclic cationic peptoids (**15**, **19** and **23**) are underlined. n.t.: not tested

250.13 MHz, ^{13}C at 62.50 MHz) spectrometers. Chemical shifts (δ) are reported in ppm relatively to the residual solvent peak (*CH*Cl$_3$, δ = 7.26, ^{13}CDCl$_3$, δ = 77.0; CD$_2$*H*OD, δ = 3.34, ^{13}CD$_3$OD, δ = 49.0; CD$_2$*H*CN: δ = 1.94; ^{13}CD$_3$CN: δ = 1.39 *HD*O, δ = 4.79, 1,4-dioxane [external standard for D$_2$O], δ = 67.19); and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet; br, broad. Coupling constants (*J*) are quoted in hertz. Homonuclear decoupling, for the complexed peptoid **14**, 2D NMR experiments such as COSY-45, ROESY, HSQC and HMBC were also performed for the full assignment of each signal. High resolution ESI-MS spectra were performed on a Q-Star Applied Biosystem mass spectrometer. ESI-MS analysis in positive ion mode was performed using a Finnigan LCQ Deca ion trap mass spectrometer (ThermoFinnigan, San Josè, CA, USA) and the mass spectra were acquired and processed using the Xcalibur software provided by Thermo Finnigan. Samples were dissolved in 1:1 CH$_3$OH/H$_2$O, 0.1 % formic acid, and infused in the ESI source by using a syringe pump; the flow rate was 5 μL/min. The capillary voltage was set at 4.0 V, the spray voltage at 5 kV, and the tube lens offset at −40 V. The capillary temperature was 220 °C. Data were acquired in MS^{1}and MS^n scanning modes. Zoom scan was used in these experiments.

4.2. Mixed monomer/submonomer solid-phase synthesis of linear peptoids 2 and 6

Linear peptoids **2** and **6** were synthesized by alternating submonomer solid-phase method[18] with standard manual Fmoc solid-phase peptide synthesis protocol.

Typically, 2-chlorotrityl chloride resin (2,α-dichlorobenzhydryl-polystyrene crosslinked with 1% DVB; 100–200 mesh; 1.55 mmol/g, 0.30 g, 0.46 mmol) was swelled in dry DMF (3 mL) for 45 min and washed twice with dry DCM (3 mL). A solution of *N*-Fmoc *N*-benzyloxyethyl glycine[10a] (**25**, 0.17 mmol in 3 mL of dry DCM) and DIPEA (0.68 mmol) was added on a shaker platform for 1.5 h at room temperature. The loaded resin was washed with dry DCM (3 mL), then with a mixture of DCM/MeOH/DIPEA (17:2:1) (2 × 3 mL) and finally with DMF (3 × 3 mL). Fmoc deprotection was performed twice with 20% piperidine in DMF (3 mL) for 3 min and 7 min, respectively, followed by extensive washes with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). The yield of the loading step was determined on the absorption of dibenzofulvene–piperidine adduct (λ_{max} = 301, ε = 7800 M^{-1} cm^{-1}). The incorporation of the *N*-(*N*-*tert*-butoxycarbonylbutyl)glycine (**26**) was performed by a sub-monomeric approach[18] (see below) while the *N*-(benzyloxyethyl)glycine was inserted by Fmoc solid-phase peptide synthesis method until the desired oligomer was obtained. According to Fmoc solid-phase synthesis, the coupling step was accomplished by incubating the resin with a solution of *N*-Fmoc N-alkylated glycine (0.68 mmol), PyBOP (0.66 mmol) and DIPEA (1.36 mmol) in dry DMF (2 mL) on a shaker platform for 1 h, followed by extensive washes with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). Once the coupling was complete the Fmoc group was deprotected with piperidine as already described and the resin washed again to prepare it for the next coupling. The cleavage was performed with 20% hexafluoro isopropanol (HFIP) solution in DCM (v/v). The final products were dissolved in 50% acetonitrile in HPLC grade water and analysed by RP-HPLC (purity >95% for all the oligomers, conditions: 5% → 100% B in 30 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 1.0 mL/min, 220 nm. C$_{18}$ reversed-phase analytical column [Waters, μBondapak, 10 μm, 125 Å, 3.9 mm × 300 mm]) and ESI mass spectrometry. The linear oligomers were subjected to the cyclization reaction without further purification.

4.2.1. Compound 2

White foam; ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C, mixture of rotamers): δ = 7.34–7.28 (m, 20H, Ar-H), 4.58 (d, J_{gem} = 11.8 Hz, 1H, –OC*H*HPh), 4.50 (d, J_{gem} = 11.2 Hz, 1H, –OC*H*HPh), 4.48 (d, J_{gem} = 12.1 Hz, 1H, –OC*H*HPh), 4.54–4.37 (m, 5H, –OC*H*$_2$Ph), 4.41–2.98 (m, 36H, –COC*H*$_2$N–, –C*H*$_2$CH$_2$OBn, –CH$_2$C*H*$_2$OBn, –NC*H*$_2$ (CH$_2$)$_3$-NHBoc, –C*H*$_2$NHBoc, overlapped), 1.76–1.27 (m, 26H, –NCH$_2$ (C*H*$_2$)$_2$CH$_2$NHBoc, –C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C, mixture of rotamers): δ = 173.5 (br s), 172.9 (br s), 171.0 (br s), 169.7, 169.4, 169.2, 168.7, 168.6, 168.4, 168.2, 168.0, 167.8, 167.5 (br s), 156.0 (br s) (CO), 138.0 (br s), 137.6 (br s), 137.5 (br s) (C$_{ipso}$-Bn), 128.6–127.4 (C-Ar), 78.7 (br s) (–*C*(CH$_3$)$_3$), 73.4, 73.2, 73.0, 72.9, 72.8 (–OCH$_2$Ph), 69.7 (br s), 69.5, 69.0, 68.7, 68.5, 68.3, 68.1, 67.9, 67.8 (br s), 67.3 (br s), 65.7, 65.6, 65.4, 64.8, 64.5 (br s) (–CH$_2$*C*H$_2$OBn), 53.4, 51.9 (br s), 51.7, 50.7, 50.5, 50.4, 49.9 (br s), 49.2, 48.9, 48.7, 48.5 (br s), 48.0 (br s), 47.7, 47. 5, 47.1, 46.1, 45.7, (–*C*OCH$_2$N–, –*C*H$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NHBoc), 41.7 (br s), 40.0 (br s), 39.9, 39.8 (–*C*H$_2$NHBoc), 28.3 (–C(*C*H$_3$)$_3$), 26.9, 26.4, 26.2 (br s), 24.9, 24.6, 24.4 (br s), 24.3, 23.8 (br s), 23.0 (-NCH$_2$-(*C*H$_2$)$_2$CH$_2$NHBoc); ESI MS: 1239.9 *m/z* [M+H$^+$].

4.2.2. Compound 6

White foam; ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C, mixture of rotamers): δ = 7.32–7.29 (m, 15H, Ar-H), 4.52 (d, J_{gem}=12.8 Hz, 1H, –OC*H*HPh), 4.51 (d, J_{gem} = 12.6 Hz, 1H, –OC*H*HPh), 4.49 (d, J_{gem} = 12.8 Hz, 1H, –OC*H*HPh), 4.46–2.89 (m, 39H, –OC*H*$_2$Ph, –COC*H*$_2$N–, –NC*H*$_2$CH$_2$OBn, –NCH$_2$C*H*$_2$OBn, –NC*H*$_2$(CH$_2$)$_3$NHBoc, –C*H*$_2$NHBoc overlapped), 1.44–1.28 (m, 39H, –C(C*H*$_3$)$_3$, –NCH$_2$(C*H*$_2$)$_2$ CH$_2$NHBoc); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C, mixture of rotamers): δ = 173.6, 173.4, 171.2, 170.8, 169.8, 169.5, 169.4, 169.2 (br s), 168.9 (br s), 168.4, 168.0 (br s), 167.4, 167.2, 166.7, 166.1, 156.4 (br s) (CO), 137.9 (br s), 137.5 (br s), 137.4 (br s) (C$_{ipso}$-Bn), 128.4–127.5 (C-Ar), 79.3 (br s) (–*C*(CH$_3$)$_3$), 73.6 (br s), 73.5, 72.9, (–OCH$_2$Ph), 67.5 (br s) (–CH$_2$*C*H$_2$OBn), 50.6, 50.4, 50.2, 49.8, 49.5, 49.2, 48.8 (br s), 48.6 (br s), 48.3 (br s), 48.0, 47.8, 47.6, 47.5 (–*C*OCH$_2$N–, –*C*H$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NHBoc), 40.0, 39.8 (br s), 39.6 (br s) (–*C*H$_2$NHBoc), 29.6, 29.0, 27.0, 26.9, 26.7, 26.4, 25.1, 24.6, 23.3, 23.1 (–NCH$_2$(*C*H$_2$)$_2$CH$_2$NHBoc), 28.3 (-C(*C*H$_3$)$_3$); ESI MS: 1276.8 *m/z* [M+H$^+$].

4.3. Sub-monomer solid-phase synthesis of linear peptoids 10, 12, 16 and 20

Linear peptoid oligomers **10**, **12, 16** and **20** were synthesized using a sub-monomer solid-phase approach[18] In a typical synthesis 2-chlorotrityl chloride resin (2,α-dichlorobenzhydryl-polystyrene crosslinked with 1% DVB; 100–200 mesh; 1.1 mmol/g, 0.30 g, 0.33 mmol) was swelled in dry DMF (3 mL) for 45 min and washed twice with dry DCM (3 mL). The first sub-monomer was attached onto the resin by adding 56 mg (0.40 mmol) of bromoacetic acid in dry DCM (3 mL) and 280 μL of DIPEA (1.6 mmol) on a shaker platform for 30 min at room temperature, followed by washing with dry DCM (3 mL) and then with DMF (3 × 3 mL). To the bromoacetylated resin was added a DMF solution (1 M, 3 mL) of the desired amine (benzylamine or the commercially available [Aldrich] *N*-*tert*-butoxycarbonyl-1,4-diaminobutane for the assembly of the *N*-(phenylmethyl)glycine and *N*-(*N*-*tert*-butoxycarbonylbutyl)glycine, respectively) the mixture was left on a shaker platform for 30 min at room temperature, then the resin was washed with DMF (3 × 3 mL). Subsequent bromoacetylation reactions were accomplished by reacting the aminated oligomer with a solution of bromoacetic acid in DMF (1.2 M, 2.5 mL) and 600 μL of DIC for 20 min at room temperature. The filtered resin was washed with DMF (3 × 3 mL) and treated again with the amine in the same conditions reported above. This cycle of reactions was iterated until the target oligomer was obtained.

The cleavage was performed by treating twice the resin, previously washed with DCM (3 × 3 mL), with 3 mL of 20% HFIP in DCM (v/v) on a shaker platform at room temperature for 30 min and 5 min, respectively. The resin was then filtered away and the combined filtrates were concentrated in vacuo. The final products were dissolved in 50% acetonitrile in HPLC grade water and analysed by RP-HPLC (purity >95% for all the oligomers, conditions: 5% → 100% B in 30 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 1.0 mL/min, 220 nm. C_{18} reversed-phase analytical column [Waters, μBondapak, 10 μm, 125 Å, 3.9 mm × 300 mm]) and ESI mass spectrometry. The linear oligomers were subjected to the cyclization reaction without further purification.

4.3.1. Compound 10

White foam; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.61–7.02 (m, 30H, Ar-H), 4.80–3.54 (m, 24H, –NC*H*$_2$Ph, –COC*H*$_2$N–, overlapped); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 173.7, 173.5, 173.0, 172.6, 172.2 (br s), 171.3, 170.8, 169.3, 169.0 (br s), 168.4 (br s), 168.3 (br s), 167.9 (br s), 167.3 (br s) (CO), 137.0–135.6 (C_{ipso}-Bn), 130.3–125.2 (C-Ar) 53.4 (br s), 52.2, 51.8 (br s), 51.7, 51.5, 51.4, 51.3, 51.2, 51.1, 50.9, 50.8, 50.6, 50.4, 50.2, 50.0, 49.8 (br s), 49.6, 49.1 (br s), 48.6 (br s), 48.5 (br s), 47.9, 47.8, 47.5, 47.4, 47.3, 47.2, 47.0, 48.8, 46.7, 46.6, 46.5 (–NC*H*$_2$Ph, –COC*H*$_2$N–); ESI MS: 901.7 *m/z* [M+H$^+$].

4.3.2. Compound 12

White foam; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.33–7.20 (m, 20H, Ar-H), 4.63–2.92 (m, 28H, –NC*H*$_2$Ph, –COC*H*$_2$N–, –NC*H*$_2$(CH$_2$)$_3$NHBoc, –C*H*$_2$NHBoc overlapped), 1.42–1.22 (m, 26H, –C(C*H*$_3$)$_3$, –NCH$_2$(C*H*$_2$)$_2$CH$_2$NHBoc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 172.3 (br s), 171.9 (br s), 169.3, 169.5, 169.0, 168.8 (br s), 168.5 (br s), 156.3 (br s) (CO), 135.3–133.2 (C_{ipso}-Bn), 129.4–126.1 (C-Ar), 79.5, 77.4 (-*C*(CH$_3$)$_3$), 52.2, 51.9 (br s), 51.6, 51.3, 51.1 (br s), 49.1, 48.8, 48.6, 48.3 (br s), 48.0 (br s), 47.5, 44.4, 44.0, 43.8, 41.2, 40.1, 39.8 (br s), 38.6, 27.0 (br s), 26.9, 26.6, 25.2 (br s), 24.9, 24.6, 23.3 (–NCH$_2$Ph, –COCH$_2$N–, –N(*C*H$_2$)$_4$NHBoc), 28.3 (–C(*C*H$_3$)$_3$); ESI MS: 1063.8 *m/z* [M+H$^+$].

4.3.3. Compound 16

White foam; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.75–7.16 (m, 10H, Ar-H), 5.07 (br s, 4H), 4.30–3.41 (m, 16H), 3.25 (br s, 6H), 2.99 (br s, 10H), 1.34 (br s, 52H); ^{13}C NMR (62.5 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 173.0 (br s), 170.8 (br s), 168.0 (br s), 156.3 (br s) (CO), 131.0–130.2 (C_{ipso}-Bn), 127.6 (br s) (C-Ar), 79.1 (br s) (–*C*(CH$_3$)$_3$), 50.2 (br s), 47.7 (br s), 39.8 (br s), 37.6 (br s), 35.3 (br s), 31.1 (br s), 30.2 (br s), 29.1 (br s), 27.9 (br s), 27.1 (br s), 25.1 (br s) (–NCH$_2$Ph, –COCH$_2$N–, –N(*C*H$_2$)$_4$NHBoc, –C(*C*H$_3$)$_3$); ESI MS: 1225.5 *m/z* [M+H$^+$].

4.3.4. Compound 20

White foam; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 4.40–3.78 (m, 12H), 3.52–3.20 (m, 10H), 3.15–2.90 (m, 14H), 1.69–1.23 (m, 78H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 172.7 (br s), 168.0 (br s), 156.4 (br s) (CO), 79.2 (br s) (–*C*(CH$_3$)$_3$), 48.1 (br s), 40.0 (br s), 29.6 (br s), 28.3 (br s), 27.2 (br s), 25.4 (br s), 24.6 (br s), 22.6 (br s) (–COCH$_2$N–, –N(*C*H$_2$)$_4$NHBoc, –C(*C*H$_3$)$_3$); ESI MS: 1387.9 *m/z* [M+H$^+$].

4.4. General cyclization reaction (synthesis of 4, 8, 11, 14, 18, 22)

A solution of the linear peptoid (**2**, **6**, **10**, **12**, **16**, **20**, 0.222 mmol), previously co-evaporated three times with toluene, was prepared under nitrogen in dry DMF (20 mL). The mixture was added drop-wise by syringe pump in 2 h to a stirred solution of PyBop (462 mg, 0.890 mmol) and DIPEA (230 μL, 1.320 mmol) in dry DMF (40 mL) at room temperature in anhydrous atmosphere. After 12 h the resulting mixture was concentrated in vacuo, diluted with CH_2Cl_2 (20 mL) and a solution of HCl (0.5 N, 20 mL). The mixture was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic phases were washed with water (12 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The cyclic products were dissolved in 50% acetonitrile in HPLC grade water and analysed by RP-HPLC (purity >85% for all the cyclic oligomers, conditions: 5% → 100% B in 30 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 1.0 mL/min, 220 nm. C_{18} reversed-phase analytical column [Waters, μBondapak, 10 μm, 125 Å, 3.9 mm × 300 mm]) and ESI mass spectrometry (zoom scan technique). The crude residues were purified by HPLC on a C_{18} reversed-phase preparative column, conditions: 20% → 100% B in 40 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 2.0 mL/min, 220 nm. The samples were dried in a falcon tube under low pressure.

4.4.1. Compound 4

White amorphous solid (154 mg, yield 57%). ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.36–7.18 (m, 20H, Ar-H), 4.85 (br s, 2H, –N*H*Boc), 4.58–4.44 (m, 4H, –OC*H*$_2$Ph), 4.38 (d, J_{gem} = 11.4 Hz, 1H, –OC*H*HPh), 4.36 (d, J_{gem} = 11.8 Hz, 1H, –OC*H*HPh), 4.30 (d, J_{gem} = 12.1 Hz, 1H, –OC*H*HPh), 4.29 (d, J_{gem} = 11.4 Hz, 1H, –OC*H*HPh), 3.80–2.89 (m, 36H, –COC*H*$_2$N–, –NC*H*$_2$CH$_2$OBn, –NCH$_2$C*H*$_2$OBn, –NC*H*$_2$(CH$_2$)$_2$C*H*$_2$NHBoc overlapped), 1.76–1.27 (m, 26H, –NCH$_2$(C*H*$_2$)$_2$CH$_2$NHBoc, –C(C*H*$_3$)$_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 170.2 (br s), 169.9 (br s), 169.4 (br s), 156.0 (br s) (CO), 137.8 (br s) (C_{ipso}-Bn), 129.3–126.3 (C-Ar), 78.7 (br s) (–*C*(CH$_3$)$_3$), 75.5 (br s), 73.0 (br s), 70.8 (br s), 70.2 (br s), 68.7 (br s), 67.1 (br s), 66.4 (br s), 65.9 (br s) (–OCH$_2$Ph, –CH$_2$CH$_2$OBn), 52.3, 52.1, 51.2, 51.0, 50.9, 50.2, 50.0, 49.2 (br s), 48.3, 47.9, 46.9, 46.1 (br s), 45.5, 43.8 (br s), 42.0, 39.9, 37.5 (–COCH$_2$N–, –CH$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NHBoc, –CH$_2$NHBoc), 31.2, 29.2 (br s), 28.6, 28.3, 27.2 (br s), 27.0, 26.1 (br s), 25.2, 24.8, 24.0 (br s) (–NCH$_2$(CH$_2$)$_2$CH$_2$NHBoc, –C(CH$_3$)$_3$); ESI MS: 1243.7 *m/z* [M+Na$^+$].

4.4.2. Compound 8

White amorphous solid (134 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.34–7.26 (m, 15H, Ar-H), 4.69 (br s, 3H, –N*H*Boc), 4.53–3.02 (m, 42H, –OCH_2Ph, –OCCH_2N–, –NCH_2CH$_2$OBn, –NCH$_2$CH_2OBn, –NCH_2(CH$_2$)$_2$CH_2NHBoc, overlapped), 1.51.1.25 (m, 39H, –C(CH_3)$_3$, –NCH$_2$(CH_2)$_2$CH$_2$NHBoc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 171.9 (br s), 171.6 (br s), 169.7 (br s), 169.2, 168.8, 167.7, 156.2 (br s), 156.0 (br s) (CO), 138.4, 138.3, 137.9 (br s), 137.6, 137.5 (C_{ipso}-Bn), 128.4–127.7 (C-Ar), 79.1 (br s) (–*C*(CH$_3$)$_3$), 74.0, 73.8, 73.5 (br s), 73.3, 73.2 (–OCH$_2$Ph), 69.9, 69.4, 68.8, 66.6 (–CH$_2$CH$_2$OBn), 51.2, 50.5, 49.6 (br s), 49.4, 49.0 (br s), 48.8, 48.5 (br s), 48.2, 48.0, 47.6 (br s), 46.6, 45.5, 45.3 (–COCH$_2$N–, –CH$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NHBoc), 40.3 (br s), 40.0 (br s) (–CH$_2$NHBoc), 34.4, 29.7, 27.0 (br s), 26.2 (br s), 26.0, 25.2 (br s), 24.0 (–NCH$_2$(CH$_2$)$_2$CH$_2$NHBoc), 28.4 (-C(CH$_3$)$_3$); ESI MS: 1280.9 *m/z* [M+Na$^+$].

4.4.3. Compound 11

White amorphous solid (90 mg, yield 46%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.40–7.06 (m, 30H, Ar-H), 4.86–3.25 (m, 24H, –NCH_2Ph, –COCH_2N–, overlapped); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 171.9, 171.3, 169.6, 168.9, 168.6, 167.9 (CO), 137.2–135.4 (C_{ipso}-Bn), 129.3–125.4 (C-Ar), 53.0 (br s), 51.6, 50.2 (br s), 48.9 (br s), 48.6, 48.4, 47.7, 47.5 (br s) (–NCH$_2$Ph, –COCH$_2$N–); ESI MS: 883.9 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 883.4183 [M+H]$^+$ (calcd for $C_{54}H_{55}N_6O_6$ 883.4178).

4.4.4. Compound 14

White amorphous solid (118 mg, yield 51%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers) δ: 7.49–7.01 (m, 20H, Ar-H), 5.39 (br s, 2H, –N*H*Boc), 4.86–2.93 (m, 28H, –NCH_2Ph, –COCH_2N–, –NCH_2(CH$_2$)$_3$NHBoc, –CH_2NHBoc, overlapped), 1.42–1.22 (m, 26H, –C(CH_3)$_3$, –NCH$_2$(CH_2)$_2$CH$_2$NHBoc); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 169.6, 169.4, 169.1, 168.7, 168.5, 168.4, 168.3, 168.1, 156.3 (br s) (CO), 135.3–133.2 (C_{ipso}-Bn), 129.4–126.1 (C-Ar), 79.5, 77.4 (–*C*(CH$_3$)$_3$), 53.0, 51.5, 51.4, 51.3, 51.1, 50.9, 50.8, 50.5, 50.4, 50.2 (br s), 49.8 (br s), 49.4 (br s), 49.1, 49.0, 48.8 (br s), 48.6, 48.0 (br s), 47.6, 47.4 (br s) (–NCH$_2$Ph, –COCH$_2$N–), 40.3 (br s), 40.0 (br s), 30.2 (br s), 30.0 (br s), 29.7 (br s), 29.4, 29.3, 27.3, 27.1, 26.9, 25.9, 25.7, 25.3 (br sbr s), 25.2, 25.1, 24.8 (br s), 22.6 (br s) (–N(CH_2)$_4$NHBoc), 28.3 (–C(CH_3)$_3$); ESI MS: 1045.9 *m/z* [M+H$^+$].

4.4.5. Compound 18

White amorphous solid (142 mg, 53 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.30–7.18 (m, 10H, Ar-H), 5.24–3.41 (m, 20H), 3.39–3.12 (m, 6H), 3.10–2.82 (m, 10H), 1.54–1.20 (m, 52H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 170.1 (br s), 168.8 (br s), 167.6 (br s), 165.6 (br s), 156.7 (br s), 156.3 (br s) (CO), 129.1, 128.2, 127.1, 126.2, 125.8 (C-Ar), 79.1 (br s) (–*C*(CH$_3$)$_3$), 53.4, 49.2 (br s), 46.2 (br s), 39.8 (br s), 29.6 (br s), 28.3 (br s), 27.2 (br s), 26.3 (br s), 26.2 (br s) (–NCH$_2$Ph, –COCH$_2$N–, –N(CH$_2$)$_4$NHBoc, –C(CH$_3$)$_3$); ESI MS: 1229.8 *m/z* [M+Na$^+$].

4.4.6. Compound 22

White amorphous solid (176 mg, 58% yield); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 5.06 (br s, 4H), 4.61 (br s, 2H), 4.48–3.58 (m, 12H), 3.50–3.18 (m, 10H), 3.15–2.28 (m, 14H), 1.72–1.31 (m, 78H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 172.3 (br s), 168.8 (br s), 156.6 (br s) (CO), 79.3 (br s) (-*C*(CH$_3$)$_3$), 49.6 (br s), 40.0 (br s), 29.7 (br s), 28.3 (br s), 27.2 (br s), 25.0 (br s) (–COCH$_2$N–, –N(CH$_2$)$_4$NHBoc, –C(CH$_3$)$_3$); ESI MS: 1392.0 *m/z* [M+Na$^+$].

4.5. Global deprotection of linear and cyclic peptoids (synthesis of 3, 5, 7, 9, 13, 15, 17, 19, 21, 23)

Typically, the *N-tert*-butoxycarbonyl protected peptoid (**2**, **4**, **6**, **8**, **12**, **14**, **16**, **18**, **20**, **22**, 0.030 mmol) was dissolved in a mixture of CH_2Cl_2/TFA 1:1 (1 mL) and stirred for 2 h at room temperature. The resulting solution was concentrated to 100 μL, under a nitrogen stream, and poured in cold diethyl ether (2 mL). The white precipitate was washed three times with cold diethyl ether (2 mL) and dried under high vacuum. The presence of the CF_3COOH resonances in the ^{13}C NMR spectrum depends on the concentration of the sample in the NMR experiment. Not all the samples showed evident $^{13}CF_3{}^{13}COOH$ NMR resonances, even though all the peptoids were isolated as trifluoroacetate salts.

4.5.1. Compound 3

White foam (41.4 mg, quantitative yield); ^{1}H NMR (300 MHz, D_2O, 25 °C, mixture of rotamers): δ = 7.40–7.32 (m, 20H, Ar-H), 4.59–2.90 (m, 44H, –OCCH_2N–, –NCH_2CH$_2$OBn, –NCH$_2$CH_2OBn, —NCH_2(CH$_2$)$_3$NH_3^+, —N(CH$_2$)$_3$CH_2NH_3^+ overlapped), 1.48 (br s, 8H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (75 MHz, D_2O, 25 °C, mixture of rotamers): δ = 172.7, 171.5, 170.2, 169.9 (br s), 169.7 (br s), 167.2, 167.1, 166.7, 165.8 (br s), 165.5 (br s), 163.3 (br s), 162.4 (br s), 162.2, 161.8 (CO), 162.6 (q, $^2J_{C,F}$ = 35 Hz, –COCF$_3$) 116.0 (q, $^1J_{C,F}$ = 289 Hz, –CF$_3$), 137.2 (br s), 136.8 (C_{ipso}-Bn), 128.4–128.0 (C-Ar), 72.8 (br s), 72.6 (br s), 72.2 (br s) (–OCH$_2$Ph), 67.1 (br s), 66.9 (br s), 66.6, 65.8, 65.6, 64.3 (br s), 64.0 (br s) (–CH$_2$CH$_2$OBn), 48.7, 48.4, 48.0, 47.4 (br s), 47.0 (br s), 46.9 (br s), 46.7 (–COCH$_2$N–, –CH$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NH$_2$), 38.7 (br s) (—CH$_2$NH_3^+), 23.8, 23.7 (br s), 23.1 (br s) (–NCH$_2$(CH$_2$)$_2$CH$_2$NH_3^+); ESI MS: 1039.4 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 1039.5873 [M+H]$^+$ (calcd for $C_{56}H_{79}N_8O_{11}$ 1039.5863).

4.5.2. Compound 5

White amorphous solid (37.5 mg, quantitative yield); ^{1}H NMR (250 MHz, CD_3OD, 25 °C, mixture of rotamers): δ = 7.58–7.29 (m, 20H, Ar-H), 4.82–2.78 (m, 44H, –COCH_2N, –NCH_2CH$_2$OBn, –NCH$_2$CH_2OBn, —NCH_2(CH$_2$)$_3$NH_3^+, —CH_2NH_3^+ overlapped), 1.90–1.41 (m, 8H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (62.5 MHz, D_2O/CD_3CN 1:1, 25 °C, mixture of rotamers): δ = 169.5 (br s), 169.2 (br s), 168.5 (br s) (CO), 137.3 (br s) (C_{ipso}-Bn), 127.9–127.3 (C-Ar), 72.2 (br s), 71.8 (br s) (–OCH$_2$Ph), 67.6, 67.3, 67.2, 67.1, 66.1, 65.0 (br s) 63.8, 62.9 (–CH$_2$CH$_2$OBn), 50.2 (br s), 49.3(br s), 48.9 (br s), 48.3 (br s), 47.5 (br s), 47.2 (br s), 47.1 (br s), 44.8 (br s), 38.4 (br s), 23.34 (br s), 23.0 (br s) (–COCH$_2$N–, –CH$_2$CH$_2$OBn, —N(CH$_2$)$_4$NH_3^+); ESI MS: 1021.4 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 1021.5751 [M+H]$^+$ (calcd for $C_{56}H_{77}N_8O_{10}$ 1021.5757).

4.5.3. Compound 7

White amorphous solid (43.0 mg, quantitative yield); ^{1}H NMR (400 MHz, D_2O, 25 °C, mixture of rotamers): δ = 7.40–7.34 (m, 15H, Ar-H), 4.55–2.90 (m, 42H, –OCH_2Ph, –COCH_2N–, –NCH_2–CH$_2$OBn, –NCH$_2$CH_2OBn, —NCH_2(CH$_2$)$_2$CH$_2$NH_3^+, —CH_2NH_3^+ overlapped), 1.75–1.45 (m, 12H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (100 MHz, D_2O, 25 °C, mixture of rotamers): δ = 172.6 (br s), 170.1 (br s), 170.0 (br s), 169.9 (br s), 169.8 (br s), 169.7 (br s), 169.6 (br s) (CO), 137.2 (br s) (C_{ipso}-Bn), 128.4–127.9 (C-Ar), 72.8 (br s), 72.7 (br s) (–OCH$_2$Ph), 67.0 (br s), 66.9 (br s), 65.7 (br s) (–CH$_2$CH$_2$OBn), 48.7 (br s), 48.1 (br s), 47.3 (br s), 47.0 (br s), 46.6 (br s) (–COCH$_2$N–, –CH$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NH$_2$), 38.7 (br s), 38.4 (br s) (—CH$_2$NH_3^+), 24.1, 23.7 (br s), 23.5 (br s), 23.3 (—NCH$_2$(CH$_2$)$_2$CH$_2$NH_3^+); ESI MS: 976.2 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 976.5875 [M+H]$^+$ (calcd for $C_{51}H_{78}N_9O_{10}$ 976.5866).

4.5.4. Compound 9

White amorphous solid (39.0 mg, quantitative yield); ^{1}H NMR (300 MHz, D_2O, 25 °C, mixture of rotamers): δ = 7.40–7.35 (m,

15H, Ar-H), 4.62–2.78 (m, 42H, –OCH_2Ph, –COCH_2N–, –NCH_2CH$_2$OBn, –NCH$_2$CH_2OBn, —NCH_2(CH$_2$)$_2$CH$_2$NH_3^+, —CH_2NH_3^+ overlapped), 1.71–129 (m, 12H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (75 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 169.5 (br s), 169.1, 168.9, 168.7, 168.2, 167.8 (CO), 137.2 (br s) (C$_{ipso}$-Bn), 128.4–127.5 (C-Ar), 72.8 (br s), 72.6 (br s), 72.2 (br s), 71.8 (–OCH$_2$Ph), 67.3 (br s), 67.0 (br s), 65.6 (br s) (–CH$_2$CH$_2$OBn), 51.2, 49.8, 49.6, 49.0 (br s), 48.5 (br s), 48.3 (br s), 48.0 (br s), 47.6 (br s), 47.3 (br s), 47.2 (br s), 46.8 (br s) (–COCH$_2$N–, –CH$_2$CH$_2$OBn, –NCH$_2$(CH$_2$)$_3$NH$_3^+$$_2$), 38.7 (br s) (–CH$_2NH_3^+$), 24.1, 23.7 (br s), 23.0 (–NCH$_2$(CH$_2$)$_2$CH$_2$NH$_3^+$); ESI MS: 958.5 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 958.5769 [M+H]$^+$ (calcd for C$_{51}$H$_{76}$N$_9$O$_9$ 958.5761).

4.5.5. Compound 13

White amorphous solid (36.1 mg, quantitative yield); ^{1}H NMR (400 MHz, CD$_3$CN, 25 °C, mixture of rotamers): δ = 7.33–7.20 (m, 20H, Ar-H), 4.60–2.92 (m, 28H, –NCH_2Ph, –COCH_2N–, —NCH_2(CH$_2$)$_3$NH_3^+, —CH_2NH_3^+ overlapped), 1.86–1.48 (m, 8H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (100 MHz, CD$_3$CN, 25 °C, mixture of rotamers): δ = 172.9, 171.8 (br s), 171.6, 171.2 (br s), 170.9 (br s), 170.6, 170.5, 170.3, 169.6, 168.5, 168.3, 168.0, 162.1 (br s) (CO), 139.6, 139.5, 139.3, 137.1, 137.0, 136.8, 136.7 (C$_{ipso}$-Bn), 131.8–129.5 (C-Ar), 53.2 (br s), 52.8, 52.3, 52.0, 51.8, 50.4 (br s), 49.4, 48.3, 47.0, 45.3, (–NCH$_2$Ph, –COCH$_2$N–), 40.8 (br s) 26.9, 26.7, 26.5, 26.4, 26.0, 25.2, 25.0 (—N(CH_2)$_4$NH_3^+); ESI MS: 864.0 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 863.4809 [M+H]$^+$ (calcd for C$_{48}$H$_{63}$N$_8$O$_7$ 863.4814).

4.5.6. Compound 15

White amorphous solid (32.2 mg, quantitative yield); ^{1}H NMR (400 MHz, CD$_3$CN, 25 °C, mixture of rotamers): δ = 7.52–7.12 (m, 20H, Ar-H), 4.81–2.96 (m, 28H, –NCH_2Ph, –COCH_2N–, —NCH_2(CH$_2$)$_3$NH_3^+, —CH_2NH_3^+, overlapped), 1.77–1.45 (m, 8H, —NCH$_2$(CH_2)$_2$CH$_2$NH_3^+); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C, mixture of rotamers): δ = 174.3, 171.9 (br s), 171.3 (CO), 138.4–137.3 (C$_{ipso}$-Bn), 130.3–127.2 (C-Ar), 53.9, 52.9 (br s), 52.2 (br s), 50.9 (br s), 50.6 (br s), 49.7 (br s), 49.5 (br s), (–NCH$_2$Ph, –COCH$_2$N–), 40.5 (br s), 40.3 (br s) (—NCH$_2$(CH$_2$)$_3$NH_3^+, —N(CH$_2$)$_3$CH$_2$NH_3^+), 25.8 (br s), 25.5 (br s) (—NCH$_2$(CH$_2$)$_2$CH$_2$NH_3^+); ESI MS: 846.0 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 845.4712 [M+H]$^+$ (calcd for C$_{48}$H$_{61}$N$_8$O$_6$ 845.4709).

4.5.7. Compound 17

White amorphous solid (31.0 mg, 74% yield); ^{1}H NMR (250 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 7.34–7.13 (m, 10H, Ar-H), 4.56–3.94 (m, 16H), 3.19 (br s, 6H), 2.82 (br s, 10H), 1.52–1.44 (m, 16H); ^{13}C NMR (62.5 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 172.1 (br s), 169.1 (br s), 168.4 (br s), 164.8 (br s) (CO), 161.6 (q, $^2J_{C,F}$ = 35 Hz, –COCF$_3$) 115.1 (q, $^1J_{C,F}$ = 290 Hz, –CF$_3$), 133.9 (br s),130.0 (br s), 129.4 (br s), 128.9 (br s), 127.4 (br s), 126.7 (br s), 125.6 (br s), 124.4 (br s) (C-Ar), 52.0 (br s), 49.0 (br s), 46.8 (br s), 45.1 (br s), 40.1 (br s), 37.8 (br s), 35.4 (br s), 32.0 (br s), 30.0 (br s), 28.7 (br s), 27.9 (br s), 22.7 (br s), 20.7 (br s) (–NCH$_2$Ph, –COCH$_2$N–, —N(CH$_2$)$_4$NH_3^+); ESI MS: 825.8 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 825.5339 [M+H]$^+$ (calcd for C$_{42}$H$_{69}$N$_{10}$O$_7$ 825.5345).

4.5.8. Compound 19

White amorphous solid (20.8 mg, 55% yield); ^{1}H NMR (400 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 7.42–7.15 (m, 10H, Ar-H), 4.78–3.85 (m, 16H), 3.29–3.10 (m, 6H), 2.90–2.76 (m, 10H), 1.59–1.37 (m, 16H); ^{13}C NMR (100 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 173.4 (br s), 169.5 (br s), 166.4 (br s), 165.6 (br s) (CO), 162.8 (q, $^2J_{C,F}$ = 35 Hz, –COCF$_3$) 116 (q, $^1J_{C,F}$ = 290.0 Hz, -CF$_3$), 130.2, 129.1, 128.8, 127.8, 127.3, 127.3, 126.7, 126.7, 126.2 (C-Ar), 51.0 (br s), 47.9 (br s), 43.9 (br s), 38.9 (br s), 38.6 (br s), 24.5 (br s), 23.9 (br s), 26.2 (br s) (–NCH$_2$Ph, –COCH$_2$N–, —N(CH$_2$)$_4$NH_3^+); ESI MS: 807.7 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 807.5244 [M+H]$^+$ (calcd for C$_{42}$H$_{67}$N$_{10}$O$_6$ 807.5240).

4.5.9. Compound 21

White amorphous solid (47.5 mg, quantitative yield); ^{1}H NMR (250 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 4.32–3.94 (m, 12H), 3.46–3.12 (m, 10H), 3.08–2.78 (m, 14H) 1.63–1.45 (br s, 24H); ^{13}C NMR (62.5 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 172.0 (br s), 169.2 (br s), 168.2 (br s), 164.9 (br s) (CO), 161.7 (q, $^2J_{C,F}$ = 36 Hz, -COCF$_3$) 115.0 (q, $^1J_{C,F}$ = 290 Hz, -CF$_3$), 48.9 (br s), 46.8 (br s), 40.0 (br s), 37.7 (br s), 35.5 (br s), 24.6 (br s), 22.8 (br s), 20.8 (br s) (–COCH$_2$N–, —N(CH$_2$)$_4$NH_3^+); ESI MS: 787.8 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 787.5871 [M+H]$^+$ (calcd for C$_{36}$H$_{75}$N$_{12}$O$_7$ 787.5876).

4.5.10. Compound 23

White amorphous solid (35.3 mg, 81% yield); ^{1}H NMR (400 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 4.80–3.50 (m, 12H), 3.41–3.02 (m, 10H), 3.02–2.81 (m, 14H) 1.78–1.39 (m, 24H); ^{13}C NMR (100 MHz, D$_2$O, 25 °C, mixture of rotamers): δ = 171.5 (br s), 170.6 (br s), 169.8 (br s), 168.9 (br s) (CO), 162.8 (q, $^2J_{C,F}$ = 35 Hz, -COCF$_3$) 116.3 (q, $^1J_{C,F}$ = 290 Hz, –CF$_3$), 50.9 (br s), 49.3 (br s), 48.1 (br s), 38.9 (br s), 25.3 (br s), 24.0 (br s) (–COCH$_2$N–, —N(CH$_2$)$_4$NH_3^+); ESI MS: 769.7 *m/z* [M+H$^+$]; HR-ESI MS: *m/z* 769.5767 [M+H]$^+$ (calcd for C$_{36}$H$_{73}$N$_{12}$O$_6$ 769.5771).

5. Biological activities

5.1. Bacterial and fungal strains and growth conditions

E. coli ATCC 25922, *Salmonella enterica* serovar *Typhimurium* ATCC 14028, and *S. aureus* ATCC 25923 were used for determining the antibacterial and the membrane permeabilizing activities of the peptoids. All strains were grown at 37 °C in Mueller–Hinton (MH) broth (DIFCO) and all tests were performed in the same medium using bacteria in logarithmic phase growth.

The fungal strains used in this study are the reference strains *C. neoformans* ATCC 90112 and *C. albicans* ATCC 90029, and 3 clinical strains isolated from immunocompromised patients (*C. albicans* L21, *C. famata* SA550 and *C. neoformans* L1). Fungi were grown on Sabouraud agar plates at 30 °C for 48 h. Fungal suspensions were then prepared by picking and suspending five colonies in 5 mL of sterile PBS. The turbidity of the conidial spore suspensions was measured at 600 nm and was adjusted in RPMI-1640 medium (Sigma–Aldrich) to obtain the appropriate cell density.

5.2. Antimicrobial assays

The antibacterial activity was evaluated by the broth microdilution susceptibility test following the guidelines of the National Committee for Clinical Laboratory Standards, to determine the Minimum Inhibitory Concentration (MIC) values. The MIC of each compound was determined as previously described.[13,25]

Briefly, twofold serial dilutions of each compound were prepared in 96-well polypropylene microplates (Sarstedt) in MH broth or RPMI-1640 medium to a final volume of 50 μL. Each series included a well without compound as a control. A total of 50 μL of the adjusted inoculum diluted in MH broth for bacteria (~2.5 × 10^5 cells/mL) or in RPMI-1640 medium for fungi (~5 × 10^4 cells/mL) was added to each well. Samples were then incubated at 37 °C overnight or 30 °C for 48 h, respectively, for bacteria o fungi. The MIC was taken as the lowest concentration of antimicrobial compound resulting in the complete inhibition of visible growth after incubation. Data are the mean of 4–6 independent determinations with values differing by one dilution at maximum.

5.3. Membrane permeabilization

Membrane permeabilization was determined by flow cytometry, measuring the propidium iodide (PI) uptake by bacterial or fungal cells.[13,26] Flow cytometric assays were based on detection of increased permeability of bacterial or fungal cells to propidium iodide (PI), a membrane impermeant DNA intercalating dye, following treatment with different compounds. Analyses were performed with a Cytomics FC 500 (Beckman-Coulter, Inc., Fullerton, CA, USA) equipped with an argon-cooled argon laser (488 nm, 5 mW) and standard system configuration for orange-filtered light detection (620 nm). All detectors were set on logarithmic amplification. Optical and electronic noise were eliminated by setting an electronic gating threshold on forward scattering detector, while the flow rate was kept at a data rate below 300 events/second to avoid cell coincidence. For each sample, at least 10,000 events were acquired and stored as list mode files.

For the analyses with bacteria, samples of 1×10^6 cells/mL were incubated in MH broth with the compounds at 37 °C for different times.

For the analyses with fungal cells, *C. albicans* or *C. neoformans* subcultured on Sabouraud agar were diluted in RPMI-1640 medium to give 1×10^5 cells/mL, and aliquots of the fungal suspension were then incubated with the compound at 30 °C for different times.

A 0.2 μm-filtered solution of PI (Sigma–Aldrich) was then added to the treated bacterial or fungal cells at a final concentration of 10 μg/mL, and samples were acquired after 4 min of incubation at 37 °C or 30 °C. All experiments were conducted in triplicate. Data analysis was performed with the WinMDI software (Dr J. Trotter, Scripps Research Institute, La Jolla, CA, USA). Data are expressed as means ± SD.

5.4. Hemolytic activity

Erythrocytes were prepared from freshly collected human blood anticoagulated with citrate-dextrose. The assays were performed in 10 mM phosphate buffer, pH 7.4, containing 139 mM NaCl, by incubating 0.5% (v/v) erythrocyte suspensions with 100 μM cyclopeptoids for 60 min at 37 °C. The reaction was stopped with cold buffer, and the supernatant was carefully collected after centrifugation at 10,000*g* for 1 min. The released hemoglobin was measured at 415 nm, and the percentage of hemolysis was determined as $(A_{sample} - A_{blank})/(A_{tot} - A_{blank}) \times 100$, where A_{blank} and A_{tot} correspond, respectively, to the hemolysis in the absence of the sample and to 100% hemolysis as obtained by addition of 0.2% Triton X-100.[27] Melittin at 20 μM was used as a positive control.

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Supplementary data

Synthesis, spectroscopic features, computational details and atom coordinates for the structure of compound **14** as a Na^+ complex are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.026.

References and notes

1. (a) Wu, Y.-D.; Gellman, S. *Acc. Chem. Res.* **2008**, *41*, 1231; (b) *Pseudo-Peptides in Drug Development*; Nielsen, P. E., Ed.; Wiley-VCH: Weinheim, 2004; (c) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244; (d) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699.
2. (a) Hancock, R. E. W.; Sahl, H.-G. *Nat. Biotechnol.* **2006**, *24*, 1551; (b) Zasloff, M. *Nature* **2002**, *415*, 389; (c) Shai, Y. *Biopolymers (Pept. Sci.)* **2002**, *66*, 236.
3. (a) Matsuzaki, K. *Biochim. Biophys. Acta* **1999**, *1462*, 1; (b) Yang, L.; Weiss, T. M.; Lehrer, R. I.; Huang, H. W. *Biophys. J.* **2000**, *79*, 2002; (c) Shai, Y. *Biochim. Biophys. Acta* **1999**, *1462*, 55.
4. (a) Giuliani, A.; Pirri, G.; Nicoletto, S. *Cent. Eur. J. Biol.* **2007**, *2*, 1; (b) Selsted, M. E.; Ouellette, A. J. *Nat. Immunol.* **2005**, *6*, 551; (c) Yang, D.; Biragyn, A.; Hoover, D. M.; Lubkowski, J.; Oppenheim, J. J. *Annu. Rev. Immunol.* **2004**, *22*, 181.
5. (a) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565; (b) Savage, P. B. *Eur. J. Org. Chem.* **2002**, 759.
6. (a) Ryge, T. S.; Frimodt-Møller, N.; Hansen, P. R. *Chemotherapy* **2008**, *54*, 152; (b) Lai, X.-Z.; Feng, Y.; Pollard, J.; Chin, J. N.; Rybak, M. Y.; Bucki, R.; Epand, R. F.; Epand, R. M.; Savage, P. B. *Acc. Chem. Res.* **2008**, 1233; (c) Ryge, T. S.; Hansen, P. R. *Bioorg. Med. Chem.* **2006**, *14*, 4444.
7. Chongsiriwatana, N. P.; Patch, J. A.; Czyzewski, A. M.; Dohm, M. T.; Ivankin, A.; Gidalevitz, D.; Zuckermann, R. N.; Barron, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2794.
8. Interesting examples are: (a) Motiei, L.; Rahimipour, S.; Thayer, D. A.; Wong, C.-H.; Ghadiri, M. R. *Chem. Commun.* **2009**, 3693; (b) Fletcher, J. T.; Finlay, J. A.; Callow, J. A.; Ghadiri, M. R. *Chem. Eur. J.* **2007**, *13*, 4008; (c) Au, V. S.; Bremner, J. B.; Coates, J.; Keller, P. A.; Pyne, S. G. *Tetrahedron* **2006**, *62*, 9373; (d) Fernandez-Lopez, S.; Kim, H.-S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452; (e) Casnati, A.; Fabbi, M.; Pellizzi, N.; Pochini, A.; Sansone, F.; Ungaro, R.; Di Modugno, E.; Tarzia, G. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2699; (f) Robinson, J. A.; Shankaramma, C. S.; Jetter, P.; Kienzl, U.; Schwendener, R. A.; Vrijbloed, J. W.; Obrecht, D. *Bioorg. Med. Chem.* **2005**, *13*, 2055.
9. (a) Craik, D. J.; Cemazar, M.; Daly, N. L. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 176; (b) Trabi, M.; Craik, D. J. *Trend Biochem. Sci.* **2002**, *27*, 132.
10. (a) Maulucci, N.; Izzo, I.; Bifulco, G.; Aliberti, A.; De Cola, C.; Comegna, D.; Gaeta, C.; Napolitano, A.; Pizza, C.; Tedesco, C.; Flot, D.; De Riccardis, F. *Chem. Commun.* **2008**, 3927; (b) Kwon, Y.-U.; Kodadek, T. *Chem. Commun.* **2008**, 5704; (c) Vercillo, O. E.; Andrade, C. K. Z.; Wessjohann, L. A. *Org. Lett.* **2008**, *10*, 205; (d) Vaz, B.; Brunsveld, L. *Org. Biomol. Chem.* **2008**, *6*, 2988; (e) Wessjohann, L. A.; Andrade, C. K. Z.; Vercillo, O. E.; Rivera, D. G.. In *Targets in Heterocyclic Systems*; Attanasi, O. A., Spinelli, D., Eds.; Italian Society of Chemistry, 2007; Vol. 10, pp 24–53; (f) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2007**, *129*, 3218; (g) Hioki, H.; Kinami, H.; Yoshida, A.; Kojima, A.; Kodama, M.; Taraoka, S.; Ueda, K.; Katsu, T. *Tetrahedron Lett.* **2004**, *45*, 1091.
11. (a) Chatterjee, J.; Mierke, D.; Kessler, H. *Chem. Eur. J.* **2008**, *14*, 1508; (b) Chatterjee, J.; Mierke, D.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 15164; (c) Nnanabu, E.; Burgess, K. *Org. Lett.* **2006**, *8*, 1259; (d) Sutton, P. W.; Bradley, A.; Farràs, J.; Romea, P.; Urpì, F.; Vilarrasa, J. *Tetrahedron* **2000**, *56*, 7947; (e) Sutton, P. W.; Bradley, A.; Elsegood, M. R.; Farràs, J.; Jackson, R. F. W.; Romea, P.; Urpì, F.; Vilarrasa, J. *Tetrahedron Lett.* **1999**, *40*, 2629.
12. Unciti-Broceta, A.; Diezmann, F.; Ou-Yang, C. Y.; Fara, M. A.; Bradley, M. *Bioorg. Med. Chem.* **2009**, *17*, 959.
13. Benincasa, M.; Scocchi, M.; Pacor, S.; Tossi, A.; Nobili, D.; Basaglia, G.; Busetti, M.; Gennaro, R. *J. Antimicrob. Chemother.* **2006**, *58*, 950.
14. De Cola, C.; Licen, S.; Comegna, D.; Cafaro, E.; Bifulco, G.; Izzo, I.; Tecilla, P.; De Riccardis, F. *Org. Biomol. Chem.* **2009**, *7*, 2851.
15. Clement, N. R.; Gould, J. M. *Biochemistry* **1981**, *20*, 1539.
16. Kourie, J. I.; Shorthouse, A. A. *Am. J. Physiol. Cell. Physiol.* **2000**, *278*, C1063.
17. Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S. C.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.
18. Zuckermann, R. N.; Kerr, J. N.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.
19. The *N*-fluorenylmethoxycarbonyl,*N*′-benzyloxyethyl glycine was synthesised in three steps, with an overall yield of 49%. See supporting information of Ref. 10a.
20. Pina-Vaz, C.; Sansonetty, F.; Rodrigues, A. G.; Costa-Oliveira, S.; Tavares, C.; Martinez-de-Oliveira, J. *Clin. Microbiol. Infect.* **2001**, *7*, 609.
21. Craik, D. J.; Daly, N. L.; Saska, I.; Trabi, M.; Rosengren, K. J. *J. Bacteriol.* **2003**, *185*, 4011.
22. Craik, D. J.; Cemazar, M.; Wang, C. K.; Daly, N. L. *Biopolymers* **2006**, *84*, 250.
23. Tran, D.; Tran, P.; Roberts, K.; Osapay, G.; Schaal, J.; Ouellette, A.; Selsted, M. E. *Antimicrob. Agents Chemother.* **2008**, *52*, 944.
24. Selsted, M. E. *Curr. Protein Pept. Sci.* **2004**, *5*, 365.
25. Benincasa, M.; Scocchi, M.; Podda, E.; Skerlavaj, B.; Dolzani, L.; Gennaro, R. *Peptides* **2004**, *25*, 2055.
26. Podda, E.; Benincasa, M.; Pacor, S.; Micali, F.; Mattiuzzo, M.; Gennaro, R.; Scocchi, M. *Biochim. Biophys. Acta* **2006**, *1760*, 1732.
27. Skerlavaj, B.; Benincasa, M.; Risso, A.; Zanetti, M.; Gennaro, R. *FEBS Lett.* **1999**, *463*, 58.