Smallest Peptoids with Antiproliferative Activity on Human Neoplastic Cells

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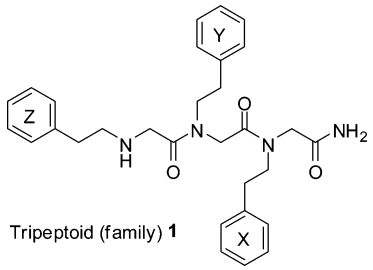
Libraries of new, small peptoid monomers and dipeptoids were synthesized and assayed for antiproliferative activity against representative human neoplastic cell lines. The C-terminal *N*-alkyl amide peptoids are cytotoxic and are the smallest peptoids reported to have such activity. These compounds were conveniently synthesized on a BAL resin. Owing to their structure, the peptoids did not suffer from DKP formation, a problematic side reaction typically observed in peptide and peptoid synthesis.

Introduction

Oligomers of *N*-alkylglycines, also known as peptoids, are a very attractive family of peptidomimetics for drug discovery. Peptoids mimic the biological activity of natural peptides, but are stable to proteolysis.[1,2] This advantage can be explained by the fact that in peptoids, the side chain is located on the nitrogen atom, rather than on the α-carbon as in peptides. Moreover, peptoids can readily be synthesized on solid-phase using the method of Zuckermann et al., which is based on the assembly of two submonomers: a haloacetic acid and a primary amine.[3] These reactions are highly automatable, facilitating the design and synthesis of peptoid libraries, whether of single compounds or compound mixtures. Peptoid libraries have been prepared via split-and-mix[4] and positional scanning[5] chemistries and screened for a wide range of biological activities such as Tat/TAR RNA inhibition,[6−8] gene delivery,[9] antimicrobial,[10−12] analgesic,[13,14] multidrug resistance,[15,16] anti-endotoxin,[17] and anticancer.[18]

In an ongoing research program performed in one of our laboratories, the tripeptoid family **1** (Figure 1) was shown to have pro-apoptotic and other activities.[18] Herein we focus on the identification of cytotoxic peptoid monomers and dipeptoids, which we hoped would have higher activity and solubility and less unspecific toxicity than some of the aforementioned tripeptoids.

To the best of our knowledge, no active dimers of *N*-alkylglycines have been described in the literature to date. In fact, the smallest peptoids reported are trimers.[12] A possible explanation for the scarcity of dipeptoids in the literature is their tendency to form diketopiperazines (DKPs).[19] Formation of DKPs, which are cyclic dipeptides, is highly influenced by amino acid structure. For example, amino acids that easily adopt an amide bond in the *cis*-configuration, such as *N*-alkylamino acids, favor cyclization.[20,21] Hence, special care should be taken to avoid this side reaction.



Tripeptoid (family) **1**

Figure 1. Chemical structure of the tripeptoid family **1**. Chemical diversity at specific positions is defined here as X, Y, and Z.

Described herein are the preparation, screening for cytotoxicity against selected representative human neoplastic cell lines, and resulting SAR of two libraries of new mono- and dipeptoids.

Results and Discussion

Design and Synthesis of the Peptoid Libraries. All libraries were synthesized following the methodology of Zuckermann et al. with slight modifications, as indicated in Scheme 1.[3] The dipeptoids with unsubstituted carboxamides at the C-terminal were prepared on Rink-resin. Alternatively, C-terminal *N*-substituted carboxamides were prepared using a similar strategy but on BAL-resin.[22,23] In this case, the first amine was incorporated onto the aldehyde-based resin by reductive amination using $NaCNBH_3$ as reducing agent. In the acylation step, the best results for the formation of the active symmetric anhydride of the chloroacetic acid were obtained with DCM. The urea byproduct formed in the pre-activation is insoluble in DCM and was thus removed by single filtration before reaction. Amines were added in the presence of triethylamine (TEA) to neutralize the hydrochloric acid formed in situ. Good yields and purity, as observed by HPLC, were obtained after two treatments (2 × 5 h). The final cleavage was accomplished using TFA−H_2O (95:5) for 1 h at 25 °C.

As amines, 3,3-diphenylpropylamine (**2**), 2-(4-methoxyphenyl)ethylamine (**3**), and 2-(aminomethyl)tetrahydrofuran (**4**) were used because they showed interesting biological activity in previous studies as inducers of apoptosis (Figure 2).[18]

For the dipeptoids synthesized using the Rink resin (R_1 = H), amine **2** was introduced either as R_2 or R_3. A total of five dipeptoids were therefore prepared and characterized (Figure 3).

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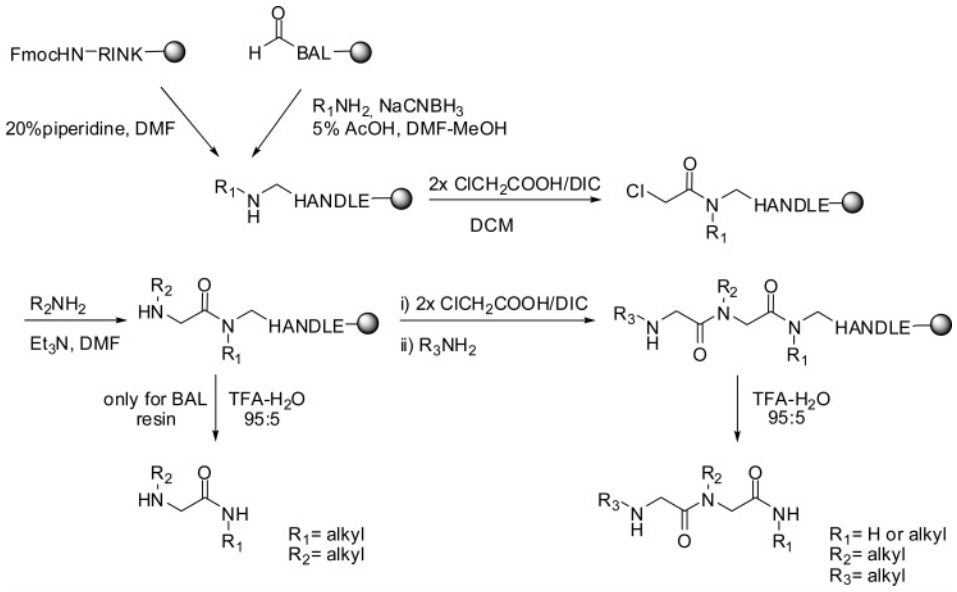
Scheme 1. Synthesis of the Peptoid Libraries



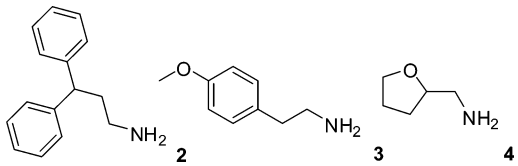


Figure 2. Primary amines used in the design and synthesis of the libraries.

As the peptoid backbone can be considered as a scaffold for aromatic systems, we sought to prepare a series of peptoids substituted at the C-terminal amide (R_1 = alkyl, Scheme 1). Thus, two dipeptoids and two *N*-alkylglycine amides (the latter are monomers and can thus be considered as *N*-substituted monopeptoids) with **2** at the C-terminal were prepared on BAL resin (Scheme 1). The structures of the dipeptoids are shown in Figure 4.

DKP Formation Studies. Finally, cyclic dipeptoids, which are *N*-alkyl-DKPs, were synthesized (Figure 5). This was easily accomplished by taking advantage of the tendency of *N*-alkyldipeptides to form DKPs via cyclization.[20]

To optimize the minimization and the formation of DKPs and to improve the understanding of the stability of these *N*-alkyl-DKPs, we first studied the formation of DKP-01. As DKPs can be formed either in basic[24] or acidic[25] media, three

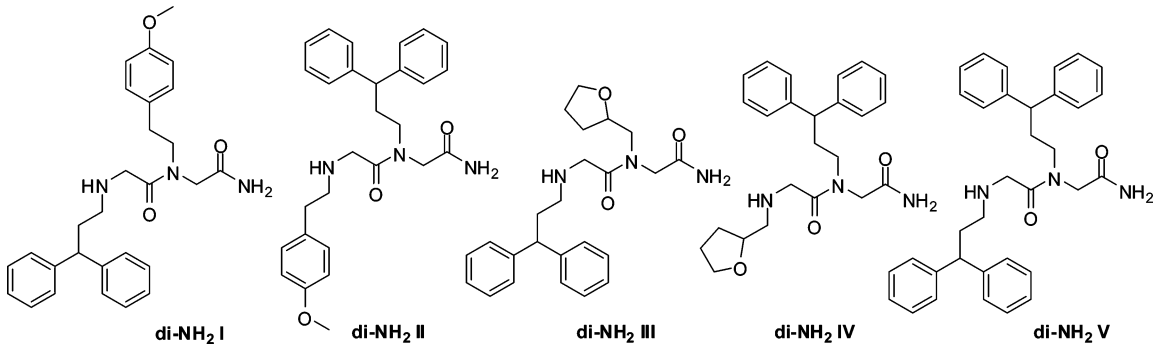


Figure 3. Chemical structures in the first dipeptoids library.

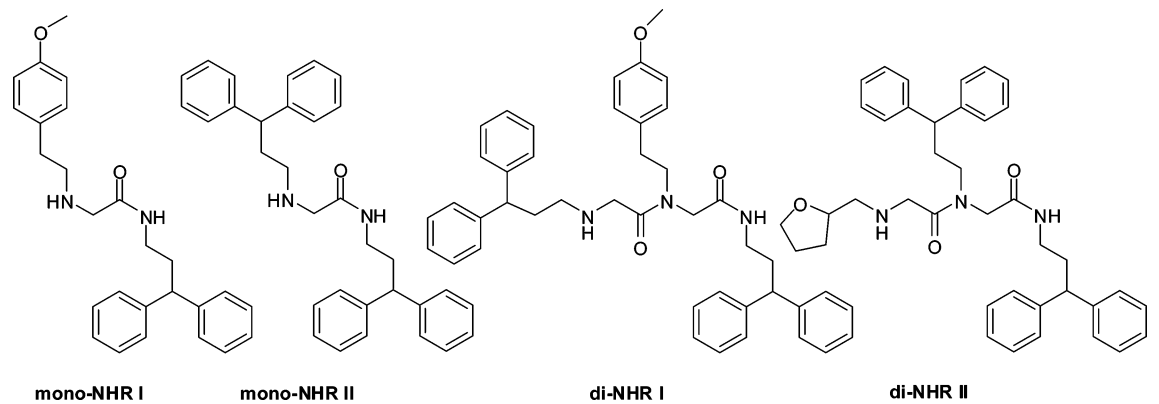


Figure 4. Chemical structures in the second dipeptoids library.

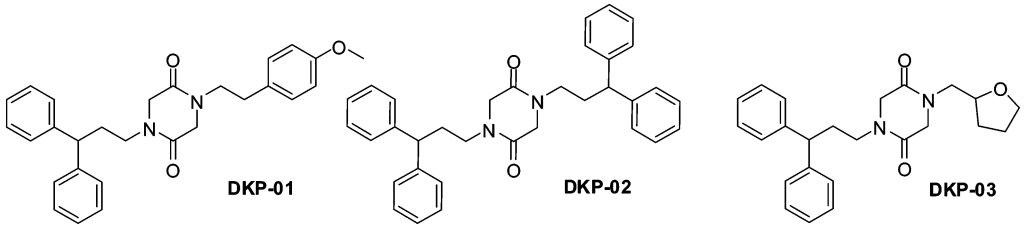


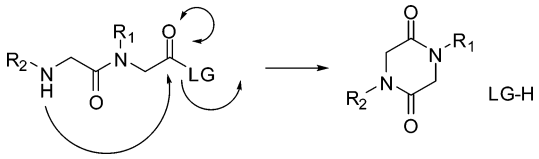
Figure 5. Chemical structures of the *N*-alkyl-DKPs synthesized.

Table 1. DKP Formation Study at $t = 0$ h and at $t = 24$ h

peptoid		DKP formation at $t = 0$ h	DKP formation at $t = 24$ h
di-NH_2 I	H_2O-CH_3CN	<2%	<2%
	10% $NaHCO_3$	37%	98%
	10% TFA	4%	53%
di-NH_2 II	H_2O-CH_3CN	<2%	<2%
	10% $NaHCO_3$	31%	100%
	10% TFA	9%	49%
di-NHR I	H_2O-CH_3CN	ND[a]	ND
	10% $NaHCO_3$	ND	ND
	10% TFA	ND	ND

[a] ND: not detected by HPLC.

Scheme 2. DKP Formation by a Nucleophilic Addition/Elimination Mechanism



conditions were tested: H_2O-CH_3CN (1:1), 10% $NaHCO_3$ in H_2O-CH_3CN (1:1), and 10% TFA in H_2O-CH_3CN (1:1). Peptoids (di-NH_2 I, di-NH_2 II, and di-NHR I) were dissolved in the above mixtures and left to stir for 24 h. DKP formation was followed by HPLC (see the Supporting Information). The results are shown in Table 1.

Peptoids di-NH_2 I and di-NH_2 II were both stable in neutral aqueous media. However, in basic or acidic media, their corresponding DKPs formed rapidly: in basic conditions, conversions were quantitative after 24 h. In contrast, no DKP formation was observed with peptoid di-NHR I in any of the conditions.

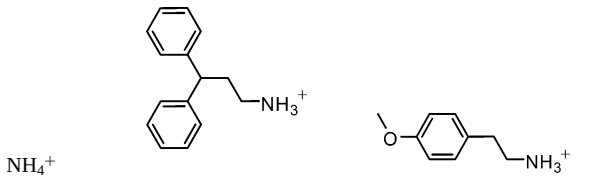
DKP formation involves nucleophilic attack of the terminal carbonyl group by the terminal amino group, followed by displacement of a leaving group (LG), as shown in Scheme 2.

Therefore, the reaction depends on the nucleophilicity of the amino group and the quality of the LG. Peptoids di-NH_2 I and di-NH_2 II, which have the same LG (NH_2) but differ in their nucleophiles (3,3-diphenylpropylamine (**2**) and 2-(4-methoxyphenyl)ethylamine (**3**), respectively), did not exhibit any differences in DKP formation. However, di-NH_2 I and di-NHR I, which contain distinct LGs but the same nucleophile, differed dramatically in DKP formation. These results support the notion that the characteristics of the LG are critical for DKP formation. The results were confirmed for the formation of DKP-03, whereby di-NH_2 III and di-NH_2 IV also gave the expected DKPs in contrast to di-NHR II, which did not react.

To analyze these results in terms of LG quality, the pK_a of each protonated LG was calculated (Table 2).

Although the pK_a values did not differ greatly, ammonia is a weaker base than the other alkyl-amines and, hence, a stronger acid. In addition, the kinetic effect should also be highlighted because the carbonyl moiety is more easily accessible in a primary amide bond, whereas it is partially masked in an alkyl-amide bond, thereby hindering nucleophilic attack. Thus, both parameters led us to conclude that NH_2 is clearly a better leaving group than the aforementioned alkylamines.

Table 2. pK_a Values of Ammonia and of Primary Amines **2** and **3** According to the pK_{alc} Module[a]



NH_4^+		
pKa: 9.24	pKa: 9.96	pKa: 9.85

[a] PALLAS, version 2.0, CompuDrug.

Table 3. In Vitro Cytotoxicity of the Peptoid Libraries in Adherent Cell Lines

	cytotoxicity GI_{50}[a] (μM)		
peptoid	MDA-MB-231	A549	HT29
Trimer I	2.72	2.57	2.27
Trimer II	1.32	1.41	1.41
di-NH_2 I	19.1	N.D.[b]	9.14
di-NH_2 II	9.36	9.36	9.14
di-NH_2 III	N.D.	N.D.	N.D.
di-NH_2 IV	N.D.	N.D.	N.D.
di-NH_2 V	3.08	3.08	1.64
mono-NHR I	2.73	3.73	1.89
mono-NHR II	3.46	3.46	3.03
di-NHR I	1.02	1.02	1.04
di-NHR II	1.06	1.06	0.94
DKP-01	N.D.	N.D.	N.D.
DKP-03	N.D.	N.D.	N.D.

[a] All assays were carried out in triplicate at 10 concentrations. The 30 points obtained were used for curve calculation and, hence, GI_{50} values determination using LIMS (Laboratory Information Management Systems) software from PharmaMar. [b] N.D. = not detected at the experimental conditions.

DKPs were then synthesized in basic medium (see above), using an adaptation of a literature procedure.[26] All reactions were monitored by HPLC, and the conversions were quantitative. However, the very hydrophobic DKP-02 was insoluble in all the aqueous media assayed as well as in $H_2O-DMSO$ mixtures and so was discarded for biological purposes.

Cytotoxicity Screening of the Peptoid Libraries. A panel of human tumor cell lines was used to evaluate the cytotoxicity of the peptoid libraries using a conventional colorimetric assay (see Experimental Section). The cell growth assays comprised three different adherent cell lines (MDA-MB-231 breast adenocarcinoma, A-549 lung carcinoma NSCL, and HT-29 colon carcinoma) and two non-adherent cell lines (Jurkat T-lymphoid and HL60 promyelocytic leukemia).

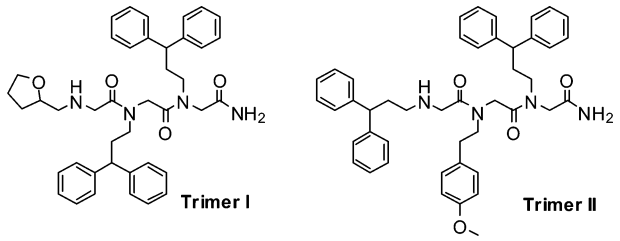
GI_{50} values (i.e., the drug concentration at which 50% of cell growth is inhibited) were determined after 72 h of continuous exposure of the adherent (Table 3) and nonadherent cell lines (Table 4) to the test molecules. The tripeptoids Trimer I and II, derived from family **1** and used in another project (for more

Table 4. In Vitro Cytotoxicity of the Peptoid Libraries in Nonadherent Cell Lines

peptoid	cytotoxicity GI_{50}[a] (μM) JURKAT	HL60
Trimer I	21.3	3.55
Trimer II	6.68	21.8
di-NH_2 I	33.9	31.1
di-NH_2 II	27.3	29.1
di-NH_2 III	N.D.[b]	N.D.
di-NH_2 IV	N.D.	N.D.
di-NH_2 V	10.5	13.2
mono-NHR I	18.0	10.6
mono-NHR II	10.2	12.6
di-NHR I	3.75	2.94
di-NHR II	5.83	23.0
DKP-01	N.D.	N.D.
DKP-03	N.D.	N.D.

[a] All assays were carried out in triplicate at 10 concentrations. The 30 points obtained were used for curve calculation and hence GI_{50} values determination using LIMS (Laboratory Information Management Systems) software from PharmaMar. [b] N.D. = not detected at the experimental conditions.

details, see ref 17, in which Trimers I and II appear as peptoids 4 and 7, repectively), were included in the test as positive controls.



As seen in Table 3, several of the test peptoids had at least moderate cytotoxicity. Among the di-NH_2 series of C-terminal primary amide dipeptoids, there was a clear relationship between aromaticity and activity: compound V was the most active, while compounds I, II, III, and IV were less or inactive. Whereas compound V contains two diphenyl groups, the others only have one, suggesting the importance of the diphenyl group for cytotoxicity. Moreover, compounds I and II, which contain a methoxyphenyl, were more active than III and IV, which instead contain a tetrahydrofuranyl.

In contrast, all of the C-terminal *N*-alkyl amide peptoids were cytotoxic. Peptoids di-NHR I and di-NHR II showed the same activity as the control tripeptoids. Compounds mono-NHR I and mono-NHR II exhibited moderate activities, similar to or even higher than their dipeptoid analogs (di-NH_2 II and di-NH_2 V, respectively), even though they are formed by only a single *N*-alkyl glycine amide. Dipeptoids di-NHR I and di-NHR II contain two diphenyl groups, which, as mentioned above, are key residues for the activity. di-NHR I also contains an extra aromatic group. Thus, the peptoids synthesized on BAL resin, which are smaller than their primary amide analogs but have the same number of substituents, had equal or greater original activities. DKPs 01 and 03 were also tested in these cell lines, but did not exhibit any cytotoxicity.

Several of the compounds synthesized also exhibited cytotoxicity in nonadherent cell lines (see Table 4). Although values of cytotoxicity for these cell lines were generally lower, the data is consistent with the results discussed above. Thus, di-NH_2 peptoids did not present any relevant activity, with the exception of compound di-NH_2 V, which was again the most active of its family. The C-terminal *N*-alkyl amides peptoids were more cytotoxic than their primary amide analogs. Interestingly, the peptoid di-NHR II was specific for JURKAT cell lines. Trimer II was also more active against JURKAT cells. In contrast, Trimer I was six times more active against HL60 cells than against JURKAT cells. DKPs 01 and 03 were also tested in these cell lines but did not show any cytotoxicity, as observed for the adherent cell lines.

Table 5. Antimitotic Activity of Select Peptoids in HeLa Cells

code	antimitotic activity IC_{50}[a] (μM)
di-NH_2 V	0.154
mono-NHR I	0.994
mono-NHR II	0.035
di-NHR I	0.025

[a] All assays were carried out in triplicate at 10 concentrations. The 30 points obtained were used for curve calculation and hence IC_{50} values determination using LIMS (Laboratory Information Management Systems) software from PharmaMar.

According to previously published results,[18] the Trimer family **1** is able to arrest G1 and induce apoptosis in several human cancer cell lines. As the peptoids in the libraries are derived from this family, we performed another assay using HeLa cells to determine if they also exhibited antimitotic activity. Several of the most active peptoids from above showed a high level of antimitotic activity (see Table 5).

The fact that none of the inactive peptoids (di-NH2 I, di-NH2 II, di-NH2 III, and di-NH2 IV) showed antimitotic activity reinforces the idea that the active ones may somehow interfere with the cell cycle and/or the apoptosis machinery. Nevertheless, further experiments are required to determine the mechanism of action of these molecules, which is outside of the scope of the present work. To discard any possible interaction with the DNA of the tumor cells used, peptoids were incubated with pBR322 DNA plasmid, and their interaction was analyzed by electrophoresis. The electrophoretic mobility pattern showed that none of the peptoids were able to bind to the DNA (see the Supporting Information).

Solubility Assay. The aqueous solubility of the peptoid libraries and the two tripeptoids was evaluated by turbidity measurements. The peptoids were dissolved in water−DMSO (4:1), and their absorbance at 620 nm was analyzed at different concentrations (0.05−5000 μM). Interestingly, the library peptoids were much more water soluble than the reference tripeptoids. All peptoids from the libraries showed greater than 10-fold solubility compared to Trimer I and greater than 20-fold solubility compared to Trimer II.

Conclusions

To the best of our knowledge, this is the first report of a dipeptoid or *N*-alkyl glycine amide with antiproliferative activity. In fact, such compounds showed to be cytotoxic and antimitotic in a panel of representative human neoplastic cell lines. The observation that C-terminal *N*-alkyl amide peptoids do not form DKPs suggests that other bioactive tripeptoid families described in the literature could also be developed into DKP-free, bioactive peptoid monomers and dipeptoids. The present results also highlight the utility of BAL resins for the synthesis of peptoid monomers and dipeptoids. The insertion of an alkyl group at the C-terminal amide provides an extra group for biological interaction without increasing the length of the peptoid chain, which also leads to marked improvements in aqueous solubility.

Experimental Section

General. Solvents, amines, and other reagents were purchased from commercial suppliers and used without further purification.

HPLC was performed using a Waters Alliance 2695 chromatography system with a PDA 995 detector, a reverse-phase Symmetry C_{18} (4.6 × 150 mm) 5-μm column, and linear gradient (MeCN with 0.036% TFA into H_2O with 0.045% TFA). The system was run at a flow rate of 1.0 mL/min from 0% to 100% over 15 min. HPLC-MS was performed using a Waters Alliance 2796 with a UV/vis detector 2487 and ESI-MS Micromass ZQ (Waters) chromatography system, a reversed-phase Symmetry 300 C_{18} (3.9 × 150 mm) 5-μm column, and H_2O with 0.1% formic acid and MeCN with 0.07% formic acid as mobile phases. Mass spectra were recorded on a MALDI Voyager DE RP time-of-flight (TOF) spectrometer and high-resolution mass spectra (HRMS) were obtained at the Mass Spectrometry Service of the University of Santiago de Compostela (Spain). The ^{1}H and ^{13}C NMR spectra of peptoids were recorded using a Varian Mercury 400 apparatus (400 MHz, $CDCl_3$ or CD_3OD). The conformations of the peptoids led to the observation of complex absorption (ca), the assignments of which were confirmed by gCOSY and gHSQC experiments.

Solid-Phase Synthesis of Peptoid Libraries. Peptoids were manually synthesized on solid phase in polypropylene syringes, each of which was fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Washes between reaction steps were done with DMF and DCM, using 10 mL of solvent/g of resin per wash. The dipeptoid amide library was synthesized on Rink amide aminomethyl resin (100 mg, 0.7 mmol/g). The Fmoc group was initially removed by treatment with piperidine−DMF (1:4) for 20 min. The alkyl-amide library was synthesized on BAL aminomethyl resin (100 mg, 1 mmol/g).

Reductive Amination on BAL Resin. The primary amine (5 equiv) was dissolved in a mixture of MeOH−DMF (1:1) and added to the resin, which was pre-activated with a mixture of MeOH−DMF−AcOH (47.5:47.5:5). Solid $NaCNBH_3$ (5 equiv) was then added to the imine, and the resin was stirred overnight at room temperature. Reaction completion was confirmed using the Vazquez test.[27]

Acylation Steps. Acylation steps were accomplished after 2 × 1 h treatment at room temperature with chloroacetic acid (20 equiv) and DIC (10 equiv) in DCM. The resulting insoluble urea was filtered off before adding the mixture to the resin. The reaction was monitored by the Kaiser[28] or de Clercq[29] tests.

Insertion of Primary Amines. The chloromethyl intermediate was aminated by adding the primary amine (5 equiv) in the presence of TEA (5 equiv) in DMF (2 × 5 h treatment). The reaction was monitored using the chloranil[30] or de Clercq tests and quantified by HPLC.

Cleavage of the Peptoids. The resin was washed with DCM (3 × 1), dried, and treated with TFA−H_2O (19:1) for 1 h. TFA was then removed by evaporation, and peptoids were dissolved in H_2O−MeCN (1:1), filtered, and lyophilized. Peptoids were purified by semipreparative HPLC when purities obtained were below 80%.

Characterization. Peptoid libraries were characterized by ^{1}H NMR, ^{13}C NMR, HPLC, HPLC-MS, and MALDI-TOF and/or HRMS.

[*N*-(3,3-Diphenylpropyl)glycyl]-*N*-(4-methoxyphenethyl)glycinamide (di-NH$_2$ I). ^{1}H NMR ($CDCl_3$): 7.25−7.16 (m, 10H), 6.95 (d, J = 8.2 Hz, 2H), 6.74 (d, J = 8.2 Hz, 2H), 3.88 (ca, *CH*Ph$_2$), 3.81−3.32 (ca, 2 × CH_2CON), 3.67 (s, OCH_3), 3.32 (ca, *CH*$_2$CH$_2$-PhOMe), 2.66 (ca, CH$_2$*CH*$_2$PhOMe), 2.66 (ca, *CH*$_2$CH$_2$CHPh$_2$), 2.37 (ca, CH$_2$*CH*$_2$CHPh$_2$). ^{13}C NMR ($CDCl_3$): 171.34 ($CONH_2$), 166.33 (CO), 158.72 (C_{Ar}), 142.85 (2 × C_{Ar}), 129.85 (CH_{Ar} × 2), 129.22 (C_{Ar}), 128.76 (CH_{Ar} × 4), 127.50 (CH_{Ar} × 4), 126.74 (CH_{Ar} × 2), 114.38 (CH_{Ar} × 2), 55.16 (OCH_3), 50.62 (*CH*$_2$CONH$_2$) 49.24 (*CH*$_2$CON), 48.47 (*CH*Ph$_2$), 47.39 (*CH*$_2$CH$_2$PhOMe), 46.92 (*CH*$_2$-CH$_2$CHPh$_2$), 33.07 (CH$_2$*CH*$_2$PhOMe), 31.24 (CH$_2$*CH*$_2$CHPh$_2$). Purity of 98% (t_R = 9.53 min). HRMS [M + H]$^+$ calcd for $C_{28}H_{33}N_3O_3$, 460.2595; found, 460.2592.

[*N*-(4-Methoxyphenethyl)glycyl]-*N*-(3,3-diphenylpropyl)glycinamide (di-NH$_2$ II). ^{1}H NMR ($CDCl_3$): 7.27−7.13 (m, 10H), 7.07 (d, J = 8.2 Hz, 2H), 6.80 (d, J = 8.2 Hz, 2H), 3.88 (ca, *CH*Ph$_2$), 3.74 (s, OCH_3), 3.70−3.47 (ca, 2 × CH_2CON), 3.16 (ca, *CH*$_2$CH$_2$CHPh$_2$), 2.98 (ca, *CH*$_2$CH$_2$PhOMe), 2.94 (ca, CH$_2$*CH*$_2$-PhOMe), 2.29 (ca, CH$_2$*CH*$_2$CHPh$_2$). ^{13}C NMR ($CDCl_3$): 171.62 ($CONH_2$), 166.21 (CO), 158.93 (C_{Ar}), 143.51 (2 × C_{Ar}), 129.95 (CH_{Ar} × 2), 129.14 (CH_{Ar} × 4), 128.35 (C_{Ar}), 127.88 (CH_{Ar} × 4), 127.05 (CH_{Ar} × 2), 114.51 (CH_{Ar} × 2), 55.47 (OCH_3), 55.47 (*CH*$_2$-CONH$_2$), 49.40 (*CH*$_2$CON), 48.19 (*CH*Ph$_2$), 47.64 (*CH*$_2$CH$_2$-PhOMe), 47.61 (*CH*$_2$CH$_2$CHPh$_2$), 33.77 (CH$_2$*CH*$_2$CHPh$_2$), 31.36 (CH$_2$*CH*$_2$PhOMe). Purity of 82% (t_R = 9.56 min). HRMS [M + H]$^+$ calcd for $C_{28}H_{33}N_3O_3$, 460.2595; found, 460.2594.

[*N*-(3,3-Diphenylpropyl)glycyl]-*N*-(4-tetrahydrofurfuryl)glycinamide (di-NH$_2$ III). ^{1}H NMR ($CDCl_3$): 7.25−7.15 (m, 10H), 4.14 (ca, *CH*Ph$_2$), 4.01 (ca, OCH), 3.77−3.67 (ca, OCH_2), 3.63−3.60 (ca, CH_2CON, OCH*CH*$_2$N), 3.29 (ca, CH_2CON), 2.98 (ca, *CH*$_2$-CH$_2$CHPh$_2$), 2.59 (ca, CH$_2$*CH*$_2$CHPh$_2$), 1.92−1.39 (ca, OCH$_2$-CH$_2$*CH*$_2$), 1.85 (ca, OCH$_2$*CH*$_2$). ^{13}C NMR ($CDCl_3$): 172.09 ($CONH_2$), 167.05 (CO), 143.29 (2 × C_{Ar}), 128.98 (CH_{Ar} × 4), 127.93 (CH_{Ar} × 4), 126.91 (CH_{Ar} × 2), 76.69 (OCH), 68.40 (OCH_2), 53.46 (*CH*$_2$CONH$_2$), 50.47 (OCH*CH*$_2$N), 48.86 (*CH*Ph$_2$), 48.24 (*CH*$_2$CON), 47.34 (*CH*$_2$CH$_2$CHPh$_2$), 31.51 (CH$_2$*CH*$_2$CHPh$_2$), 29.13 (OCH$_2$CH$_2$*CH*$_2$), 25.83 (OCH$_2$*CH*$_2$). Purity of 84% (t_R = 8.63 min). HRMS [M + H]$^+$ calcd for $C_{24}H_{31}N_3O_3$, 410.2438; found, 410.2437.

[*N*-(4-Tetrahydrofurfuryl)glycyl]-*N*-(3,3-diphenylpropyl)glycinamide (di-NH$_2$ IV). ^{1}H NMR (CD_3OD): 7.34−7.13 (m, 10H), 4.09 (ca, OCH), 4.00 (ca, CH_2CONH_2), 3.97 (ca, *CH*Ph$_2$) 3.90−3.81 (ca, OCH_2), 3.35−3.25 (m, *CH*$_2$CH$_2$CHPh$_2$), 3.31 (ca, CH$_2$-CON) 3.13−2.93 (m, OCH*CH*$_2$N), 2.40−2.32 (m, CH$_2$*CH*$_2$CHPh$_2$), 2.10−1.60 (m, OCH$_2$CH$_2$*CH*$_2$), 1.95 (m, OCH$_2$*CH*$_2$). ^{13}C NMR (CD_3OD): 172.97, 172.39 ($CONH_2$), 167.33, 166.79 (CO), 145.77, 145.37 (2 × C_{Ar}), 129.85, 129.59 (CH_{Ar} × 4), 128.85, 128.80 (CH_{Ar} × 4), 127.71, 127.41 (CH_{Ar} × 2), 75.29 (OCH), 69.55 (OCH_2), 52.52 (OCH*CH*$_2$N), 50.61 (*CH*Ph$_2$), 49.59 (*CH*$_2$CONH$_2$), 48.50 (*CH*$_2$CON), 48.24 (*CH*$_2$CH$_2$CHPh$_2$), 34.56 (CH$_2$*CH*$_2$CHPh$_2$), 30.28 (OCH$_2$CH$_2$*CH*$_2$), 26.43 (OCH$_2$*CH*$_2$). Purity of 98% (t_R = 8.57 min). HRMS [M + H]$^+$ calcd for $C_{24}H_{31}N_3O_3$, 410.2438; found, 410.2438.

[*N*-(3,3-Diphenylpropyl)glycyl]-*N*-(3,3-diphenylpropyl)glycinamide (di-NH$_2$ V). ^{1}H NMR ($CDCl_3$): 7.24−7.13 (m, 20H), 3.91 (ca, *CH*Ph$_2$ (C_t)), 3.75 (ca, *CH*Ph$_2$), 3.75 (ca, CH_2CONH_2), 3.20 (CH_2CON), 3.06 (ca, *CH*$_2$CH$_2$CHPh$_2$(C_t)), 2.64 (ca, *CH*$_2$CH$_2$-CHPh$_2$), 2.41 (ca, CH$_2$*CH*$_2$CHPh$_2$), 2.22 (ca, CH$_2$*CH*$_2$CHPh$_2$ (C_t)). ^{13}C NMR ($CDCl_3$): 171.19 ($CONH_2$), 165.92 (CO), 143.18 (2 × C_{Ar}), 142.89 (2 × C_{Ar}), 128.86 (CH_{Ar} × 4), 128.81 (CH_{Ar} × 4), 127.61 (CH_{Ar} × 8), 127.48 (CH_{Ar} × 2), 126.81 (CH_{Ar} × 2), 49.19 (*CH*$_2$CONH$_2$), 48.65 (*CH*Ph$_2$ (C_t)), 47.79 (*CH*Ph$_2$), 47.39 (*CH*$_2$-CON), 47.06 (*CH*$_2$CH$_2$CHPh$_2$), 46.86 (*CH*$_2$CH$_2$CHPh$_2$(C_t)), 33.45 (CH$_2$*CH*$_2$CHPh$_2$(C_t)), 31.20 (CH$_2$*CH*$_2$CHPh$_2$). Purity of 88% (t_R = 10.72 min). HRMS [M + H]$^+$ calcd for $C_{34}H_{37}N_3O_2$, 520.2959; found, 520.2958.

***N*-(4-Methoxyphenethyl)glycin[*N*-(3,3-diphenylpropyl)]amide (mono-NHR I).** ^{1}H NMR ($CDCl_3$): 7.25−7.12 (m, 10H), 7.09 (d, J = 8.2 Hz, 2H), 6.82 (d, J = 8.2 Hz, 2H), 3.91 (ca, *CH*Ph$_2$), 3.76 (s, OCH_3), 3.74−3.71 (ca, CH_2CON), 3.17 (ca, *CH*$_2$-CH$_2$CHPh$_2$), 3.17 (ca, *CH*$_2$CH$_2$PhOMe), 2.97 (ca, CH$_2$*CH*$_2$PhOMe), 2.23 (ca, CH$_2$*CH*$_2$CHPh$_2$). ^{13}C NMR ($CDCl_3$): 164.50 (CO), 158.95 (C_{Ar}), 143.93 (2 × C_{Ar}), 129.76 (CH_{Ar} × 2), 128.59 (CH_{Ar} × 4), 127.67 (CH_{Ar} × 4), 127.17 (C_{Ar}), 126.42 (CH_{Ar} × 2), 114.48 (CH_{Ar} × 2), 55.24 (OCH_3), 49.72 (*CH*$_2$CH$_2$PhOMe), 48.83 (*CH*Ph$_2$), 48.83 (*CH*$_2$CON), 38.89 (*CH*$_2$CH$_2$CHPh$_2$), 34.58 (CH$_2$*CH*$_2$CHPh$_2$), 31.53 (CH$_2$*CH*$_2$PhOMe). Purity of 90% (t_R = 10.09 min). HRMS [M + H]$^+$ calcd for $C_{26}H_{30}N_2O_2$, 403.2380; found, 403.2381.

***N*-(3,3-Diphenylpropyl)glycin[*N*-(3,3-diphenylpropyl)]amide (mono-NHR II).** ^{1}H NMR ($CDCl_3$): 7.25−7.12 (m, 20H), 3.91−3.87 (ca, 2 × *CH*Ph$_2$), 3.53 (ca, CH_2CON), 3.11 (ca, *CH*$_2$-CH$_2$CHPh$_2$(C_t)), 2.85 (ca, *CH*$_2$CH$_2$CHPh$_2$), 2.45 (ca, CH$_2$*CH*$_2$-CHPh$_2$), 2.18 (ca, CH$_2$*CH*$_2$CHPh$_2$ (C_t)). ^{13}C NMR ($CDCl_3$): 164.71 (CO), 144.19 (2 × C_{Ar}), 142.82 (2 × C_{Ar}), 129.08 (CH_{Ar} × 4), 128.83 (CH_{Ar} × 4), 127.90 (CH_{Ar} × 4), 127.71 (CH_{Ar} × 4), 127.12 (CH_{Ar} × 2), 126.66 (CH_{Ar} × 2), 49.12 (*CH*Ph$_2$), 48.94 (CH_2CON), 48.68 (*CH*Ph$_2$), 47.68 (*CH*$_2$CH$_2$CHPh$_2$), 39.11 (*CH*$_2$CH$_2$CHPh$_2$(C_t)), 34.76 (CH$_2$*CH*$_2$CHPh$_2$(C_t)), 31.87 (CH$_2$*CH*$_2$CHPh$_2$). Purity of 89%

(t_R = 11.23 min). HRMS $[M + H]^+$ calcd for $C_{32}H_{34}N_2O$, 463.2744; found, 463.2747.

[*N*-(3,3-Diphenylpropyl)glycyl]-*N*-(4-methoxyphenethyl)glycin[*N*-(3,3-diphenylpropyl)]amide (di-NHR I). ^{1}H NMR (CD_3OD): 7.32−7.13 (m, 20H), 7.11 (d, J = 8.2 Hz, 2H), 6.81 (d, J = 8.2 Hz, 2H), 4.04−3.95 (ca, 2 × $CHPh_2$), 3.73−3.70 (br s, OCH_3), 4.00−3.47 (ca, 2 × CH_2CON), 3.51 (m, CH_2CH_2PhOMe), 3.14 (m, $CH_2CH_2CHPh_2(C_t)$), 2.78 (m, CH_2CH_2PhOMe), 2.73 (m, $CH_2CH_2CHPh_2$), 2.50−2.31 (m, $CH_2CH_2CHPh_2$), 2.27 (m, $CH_2CH_2CHPh_2$ (C_t)). ^{13}C NMR (CD_3OD): 170.33, 169.82 ($CO(C_t)$), 167.32, 166.96 (CO), 160.22, 159.89 (C_{Ar}), 145.93 (2 × C_{Ar}), 144.69 (2 × C_{Ar}), 131.55 (C_{Ar}), 131.30, 130.78 (CH_{Ar} × 2), 129.85 (CH_{Ar} × 4), 129.56 (CH_{Ar} × 4), 128.90 (CH_{Ar} × 4), 128.72 (CH_{Ar} × 4), 127.86 (CH_{Ar} × 2), 127.36 (CH_{Ar} × 2), 115.38, 115.07 (CH_{Ar} × 2), 55.79 (OCH_3), 51.47 (CH_2CON), 50.83 (CH_2CON), 50.12 ($CHPh_2$), 49.87 ($CHPh_2$), 48.52 (CH_2CH_2PhOMe), 47.97 ($CH_2CH_2CHPh_2$), 39.47 ($CH_2CH_2CHPh_2(C_t)$), 36.1 ($CH_2CH_2CHPh_2$ (C_t)), 34.10, 33.67 (CH_2CH_2PhOMe), 32.62 ($CH_2CH_2CHPh_2$). Purity of 91% (t_R = 12.05 min). HRMS $[M + H]^+$ calcd for $C_{43}H_{47}N_3O_3$, 654.3690; found, 654.3688.

[*N*-(4-Tetrahydrofurfuryl)glycyl]-*N*-(3,3-diphenylpropyl)glycin[*N*-(3,3-diphenylpropyl)]amide. (di-NHR II). ^{1}H NMR (CD_3OD): 7.31−7.10 (m, 20H), 4.15−4.07 (ca, OCH), 4.01−4.00 (ca, 2 × CH_2CON), 3.97−3.94 (ca, 2 × $CHPh_2$), 3.80−3.73 (ca, OCH_2), 3.33−3.19 (m, $CH_2CH_2CHPh_2(C_t)$), 3.17−2.83 (m, $OCHCH_2N$), 3.12 (m, $CH_2CH_2CHPh_2$), 2.37−2.26 (m, $CH_2CH_2CHPh_2$), 2.24 (m, $CH_2CH_2CHPh_2$ (C_t)), 2.08−1.57 (m, $OCH_2CH_2CH_2$), 1.93 (m, OCH_2CH_2). ^{13}C NMR (CD_3OD): 170.34, 169.93 ($CO(C_t)$), 167.30, 166.76 (CO), 145.90 (2 × C_{Ar}), 145.38 (2 × C_{Ar}), 129.88 (CH_{Ar} × 4), 129.62, 129.56 (CH_{Ar} × 4), 128.92, 128.88 (CH_{Ar} × 4), 128.85, 128.78 (CH_{Ar} × 4), 127.74 (CH_{Ar} × 2), 127.45, 127.36 (CH_{Ar} × 2), 75.29 (OCH), 69.56 (OCH_2), 52.62, 52.53 ($OCHCH_2N$), 51.00 (CH_2CON), 50.29 ($CHPh_2$), 50.06 ($CHPh_2$), 49.58 (CH_2CON), 48.25 ($CH_2CH_2CHPh_2$), 39.64, 39.44 ($CH_2CH_2CHPh_2(C_t)$), 36.12 ($CH_2CH_2CHPh_2$ (C_t)), 34.60, 33.93 ($CH_2CH_2CHPh_2$), 30.27 ($OCH_2CH_2CH_2$), 26.44 (OCH_2CH_2). Purity of 91% (t_R = 11.49 min). HRMS $[M + H]^+$ calcd for $C_{39}H_{45}N_3O_3$, 604.3534; found, 604.3531.

Solid-Phase Synthesis of the DKPs. DKP-01 and DKP-03 were obtained from peptoids di-NH_2 I and di-NH_2 III, respectively, as follows: The crude peptoids (**di-NH_2 I**, 5 mg, 0.01 mmol; **di-NH_2 III**, 4.5 mg, 0.01 mmol) were dissolved in 1 mL of a 10% solution of $NaHCO_3$ in H_2O-CH_3CN (1:1) and stirred for 24 h. The solvent was then lyophilized, and the residues were extracted with DCM (3 × 2 mL). The organic solvent was removed by evaporation, and the peptoids were dissolved in H_2O−MeCN (1:1) and lyophilized again to afford the expected DKPs as white solids (**DKP-01**, 2.2 mg, 50%; **DKP-03**, 1.3 mg, 33%).

***N*-(3,3-Diphenylpropyl)-*N′*-(4-methoxyphenethyl)-2,5-piperazinedione (DKP-01).** Purity of 85%, t_R = 12.48 min. HRMS $[M + H]^+$ calcd for $C_{28}H_{31}N_2O_3$, 443.2329; found, 443.2325.

***N*-(3,3-Diphenylpropyl)-*N′*-(4-tetrahydrofurfuryl)-2,5-piperazinedione (DKP-03).** Purity of 99%, t_R = 11.13 min. HRMS $[M + H]^+$ calcd for $C_{24}H_{28}N_2O_3$, 393.2173; found, 393.2171.

Solubility Assay. The peptoids from the library were dissolved at 10 different concentrations (0.05−5000 μM) in water−DMSO (4:1) in a 96-well plate. The turbidity of the peptoid library at the different concentrations was measured at 620 nm in a microplate reader (Thermolabsystem Multiskan Ascent).

Cell Growth Inhibition Assay. A colorimetric assay using sulforhodamine B (SRB) was adapted to perform a quantitative measurement of cell growth and viability, following a previously described method.[31] The cells were seeded in 96-well microtiter plates at 5 × 10^3 cells/well in aliquots of 195 μL of RPMI medium and were left to grow in drug-free medium for 18 h to allow attachment to the plate surface. Samples were then added in aliquots of 5 μL (dissolved in DMSO−H_2O, 3:7). After 72 h of exposure, the antitumor effect was measured by the SRB methodology: cells were fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and were incubated for 60 min at 4 °C. Plates were washed with deionized H_2O and dried, and 100 μL of SRB solution (0.4 wt %/vol in 1% acetic acid) was added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried, and the bound stain was dissolved with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analyses were automatically generated by LIMS implementation. Assays were done in a dose−response manner at 10 different concentrations (from 10 μg/mL with 1:2.5 dilutions to 0.0026 μg/mL). Although concentrations were adjusted in mg/mL, GI_{50} values were calculated in molarity. For the nonadherent cell lines, this rank was fine-tuned and concentrations used were from 0 to 100 μM. All assays were run in triplicate, and the curves were automatically adjusted with 30 points by nonlinear regression using "Prism 3.03" (GraphPad) software.

Antimitotic Assay. The mitotic ratio of the cell cultures (percentage of cells arrested in mitosis) was estimated using a specific 96-well microplate immunoassay that quantitatively detects a specific mitotic marker. HeLa cells (h-cervix carcinoma, ATCC# CCL-2) were incubated for 18 h in the presence or absence of the peptoids being tested. Afterward, cells were washed with PBS and lysed on ice in 75 μL of freshly prepared lysis buffer (1 mM EGTA (pH 7.5), 0.5 mM PMSF, and 1 mM $NaVO_3$) for 30 min. An aliquot of the cell extract (60 μL) was transferred to a high-binding surface ELISA plate and dried in a speed-vac for 2 h at room temperature. Plates were then blocked in 100 μL of PBS-1% BSA for 30 min at 30 °C and sequentially incubated with anti-MPM2 primary mouse monoclonal antibody (Upstate Biotechnology, cat #05-368) for 18 h at 4 °C and appropriate peroxidase-conjugated secondary antibody for 1 h at 30 °C. After intensive washing in 0.02% Tween-20, the peroxidase reaction was performed using 30 μL of TMB (3,3′,5,5′-tetramethyl-benzidine) for 30 min at 30 °C. The reaction was quenched with 30 μL of a 4% H_2SO_4 solution. The assay was quantified by measuring the O.D. at 450 nm in a microplate spectrophotometer. The results are expressed as IC_{50} (i.e., the concentration of sample at which 50% of mitosis in treated cell cultures is arrested as compared to untreated cultures).

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Supporting Information Available: Chromatograms of DKP formation studies and gel electrophoresis analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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