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J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.7b01956 • Publication Date (Web): 21 Nov 2017

ACS Publications

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

DEVELOPMENT OF SPIROLIGOMER-PEPTOID HYBRIDS

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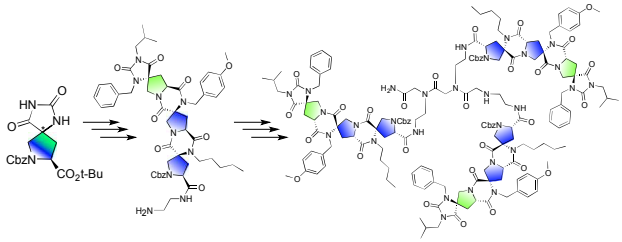
Abstract



Creating functional macromolecules that possess the diversity and functionality of proteins poses an enormous challenge, as this requires large, preorganized macromolecules to facilitate interactions. Peptoids have been shown to interact with proteins, and combinatorial libraries of peptoids have been useful in discovering new ligands for protein binding. We have created spiroligomer-peptoid hybrids that have a spirocyclic core that preorganizes functional groups in three-dimensional space. By utilizing spiroligomers, we can reduce the number of rotatable bonds between functional groups, while increasing the stereochemical diversity of the molecules. We have synthesized 15 new spiroligomer monomer amines that contain two stereocenters and three functional groups (67-84% yields from a common hydantoin starting material), as well as a spiroligomer trimer **25** with six stereocenters and five functional groups. These 16 amines were used to synthesize five first-generation spiroligomer-peptoids hybrids.

Introduction

To create functional macromolecules with the protein binding, catalytic, and membrane channel activities of folded proteins, it is necessary to create macromolecules that organize many diverse functional groups in pre-organized three-dimensional constellations. Many approaches to this challenge have been developed including peptide synthesis, stapled-peptides,[1,2] beta- and gamma-peptides,[3,4] triazolamers,[5,6] and N-amino peptides,[7,8] among many others. Two other

approaches are spiroligomers and peptoids, which are both peptidomimetic oligomers synthesized from readily available building blocks, with spiroligomers being synthesized from a unique set of bis-amino acid monomers derived from *trans*-4-hydroxy-*L*-proline,[9,10] while peptoids are typically synthesized from bromoacetic acid and a primary amine via a submonomer method.[11]

Peptoids have been studied for decades and have been utilized for a wide variety of purposes: Peptoids have been demonstrated as biologically active molecules ranging from protein secondary structure mimetics[12-16] to antimicrobial agents[17-19] and other therapeutics;[20] they have been used to construct nanomaterials such as nanotubes[21,22] and nanosheets;[23,24] and metal binding peptoids.[25-28] Peptoids are assembled using a very convenient submonomer approach, which allows for efficient synthesis of long oligomers with excellent yield and purity. Compared to peptides, the side chain is moved from the α-carbon to the backbone nitrogen creating a tertiary amide linkage. This removes the backbone chirality, any inter- or intrachain hydrogen bonding of the backbone, and leads to increased flexibility of the peptoid backbone relative to peptides due to rotational freedom around the ω dihedral.[29-31] To increase the degree of pre-organization, Kirshenbaum,[32] and others, have developed a macrocyclization strategy for the peptoid core.[18,19,33-38] These macrocycles range from only three residues in the peptoid core,[33] to six, eight, or more residues.[32] Peptoid containing macrocycles have been utilized for many applications, including protein inhibitors,[39,40] antimicrobials,[18] and antifreeze agents.[41]

Spiroligomers are excellent at pre-organizing functional groups, as the fused-spirocyclic structures do not allow any free rotation throughout the backbone.[9,42,43] Spiroligomers also benefit from having a large pool of side chains to utilize, as almost any aldehyde, ketone, alkyl halide, isocyanate, or amino acid can be incorporated to provide a functional group.[44,45] Functionalized, preorganized spiroligomers have been utilized for a variety of applications such as a transesterification catalyst,[46] a proline-aldol catalyst,[47] a spiroligomer that binds MDM2,[48] and a spiroligomer that forms supramolecular metal binding complexes,[49] among many others.

The submonomer synthesis of peptoids allows the incorporation of a large variety of functional groups as primary amines. The first peptoids that incorporated large side chains were

glucose-based glycopeptide mimetics,[50] with interest in these glycopeptoids ongoing.[51,52] Large side chains have also been incorporated into peptoids using a 3+2 Huisgen's cyclization (a click reaction),[53] after the displacement step with propargyl amine.[54-56]

Herein, we investigate linking complex, stereochemicaly-pure spiroligomers that display multiple functional groups through a peptoid backbone. This approach would provide new molecules for combinatorial screening of protein-protein interaction inhibitors and catalysts. To achieve this, we need to incorporate a protected primary amine in the spiroligomer synthesis, and we need to develop efficient conditions for integrating these complex amines into peptoids. In doing so, we can then combine any number of uniquely functionalized spiroligomers into any sequence of a peptoid for a variety of applications.

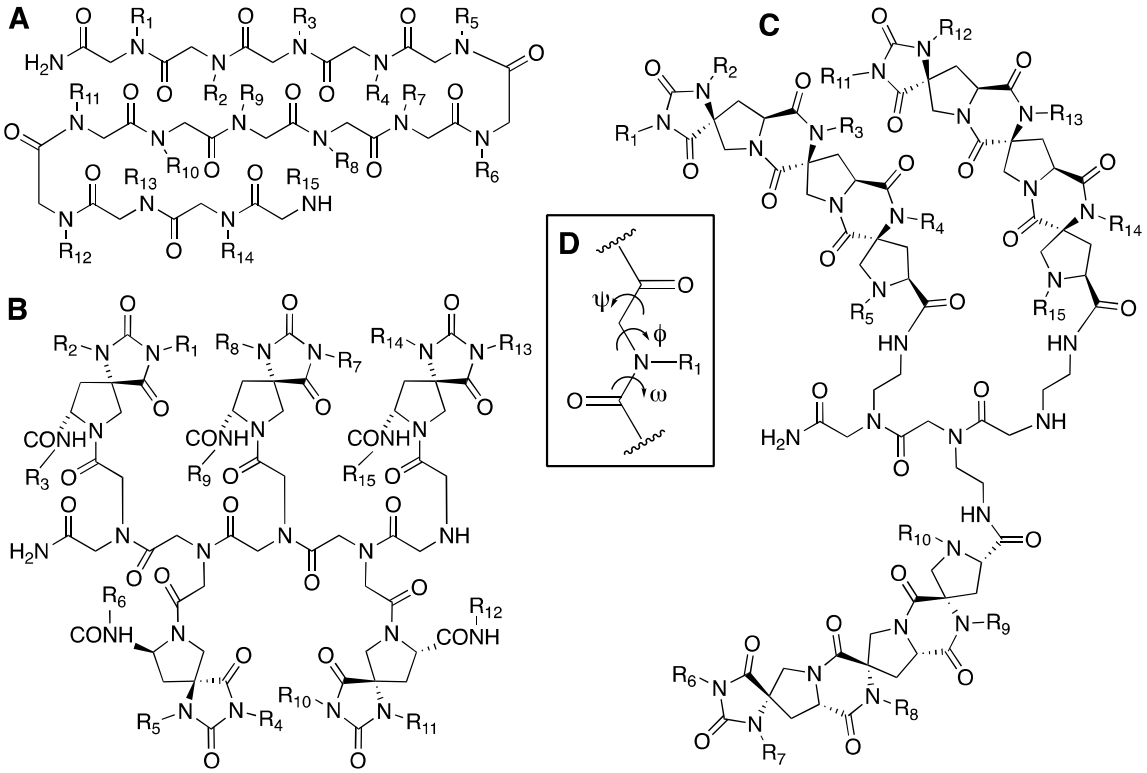


Figure 1. Three theoretical molecules displaying identical functional groups with increasing preorganization. (A) A theoretical peptoid 15-mer (B) A theoretical spiroligomer-peptoid hybrid 5-mer, incorporating five spiroligomers which each contain three functional groups and two stereocenters (15 total groups, 10 total stereocenters) (C) A spiroligomer-peptoid hybrid 3-mer, incorporating three spiroligomers which each contain five functional groups and six stereocenters (15 total groups, 18 total stereocenters). (D) Depiction of a peptoid showing omega, phi, and psi dihedral angles.

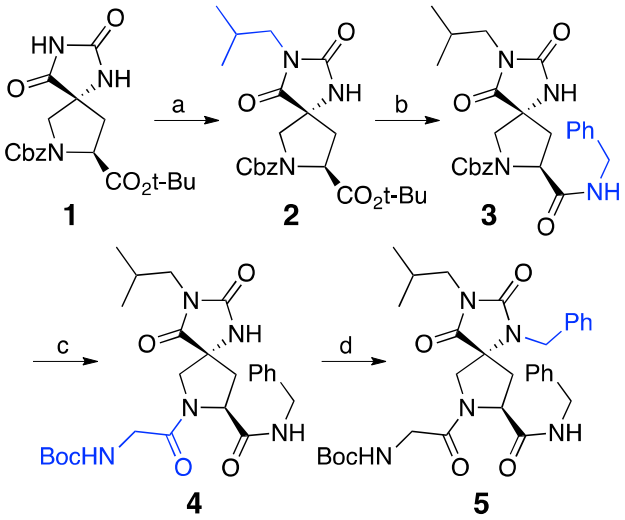
As shown in Figure 1A, a prototypical linear peptoid 15-mer has a very high ratio of rotatable bonds to functional groups. Each peptoid residue has a ϕ, ψ, and ω rotatable bond (Figure 1D), which means there are 42 rotatable bonds in the peptoid backbone. In comparison, a spiroligomer-peptoid hybrid like the one shown in Figure 1B incorporates fifteen functional groups and ten stereo-centers, yet it contains only 27 rotatable bonds in the peptoid backbone (a 35% reduction compared to the prototypical peptoid). Spiroligomers similar in size and functional group display to the amines for Figure 1B have previously been used by our group as small, organic catalysts.[47] If we were to incorporate a spiroligomer trimer into a peptoid (Figure 1C), fifteen functional groups can be incorporated with just three spiroligomer trimers containing eighteen total stereo-centers, and only 21 rotatable bonds in the backbone (a 50% reduction compared to the prototypical peptoid). For instance, between groups R_1-R_5 on the peptoid there are 12

rotatable bonds between residues in the peptoid backbone, whereas between groups R_1-R_5 on the spiroligomer, there are no rotatable bonds in the spiroligomer core. Spiroligomers similar in size and functional group display to the amines for Figure 1C have been shown to bind in the P53 groove of HDM2.[48] These hypothetical spiroligomer-peptoid hybrids represent a wide range of functional group and stereochemical diversity relative to flexibility, with increasing diversity and increasing pre-organization as the size of the spiroligomer is increased. Each of these functionalized spiroligomers approximates the structural complexity of a small-molecule fused-ring natural product containing two, four, six or more fused rings and several functional groups in precise three-dimensional constellations. Three to five of these spiroligomer domains can be displayed – in close proximity – on a peptoid chain and provide a molecule with a great deal of preorganization that has a large surface area which could potentially interact with other molecules, and at the same time display flexibility between the domains to accommodate different protein surfaces. One could imagine molecules like these binding to multiple shallow grooves on a protein surface through many non-covalent contacts. By incorporating the structural rigidity and stereochemical diversity of spiroligomers with the modular linking chemistry provided by peptoid synthesis, this should facilitate the rapid discovery of new molecules of interest for catalysis, disrupting protein-protein interactions, and exploring large surface area host-guest interactions.

RESULTS AND DISCUSSION

Synthesis of Spiroligomer Primary Amines

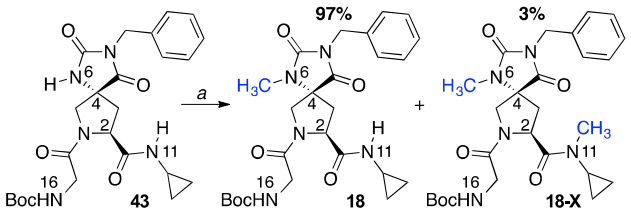
To facilitate the synthesis of spiroligomer-peptoid hybrids, we must first synthesize a variety of spiroligomers displaying primary amines. The building blocks of spiroligomers are the four stereoisomers of the proline hydantoin intermediate in spiroligomer bis-amino acid synthesis that we routinely synthesize on a 600 mmol scale (230g of mixed diastereomers and we have synthetic access to all four stereoisomers).[10] We have recently developed chemistry that allows us to directly functionalize the hydantoin by exploiting the varying reactivity between the imide and amide nitrogen atoms of the hydantoin.[45] As shown in Scheme 1, we can install a functional



Scheme 1: Synthesis of spiroligomer amine **5** from (2*S*, 4*R*) proline hydantoin **1**. a) i. DMF, K_2CO_3, 1-iodo-2-methylpropane; ii. Et_2NH; b) 1. TFA; 2. i. HOAT, EDCI, DMF, DCM; ii. benzylamine; c) 1. 1:1 HBr/DCM; 2. DMF, DCM, DIPEA, Boc-Gly-OAt; d) i. DMF, K_2CO_3, benzylbromide; ii. Et_2NH

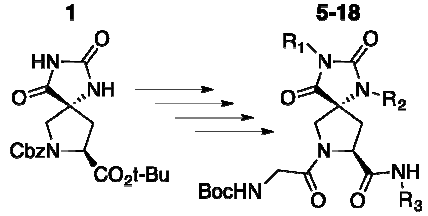
group onto the imide of hydantoin **1** via direct alkylation with either alkyl, allyl, or benzyl halides to form **2**. We then remove the *tert*-butyl protecting group with TFA, and couple a primary amine to form **3**. This is followed by removal of the Cbz protecting group and coupling an N-Boc protected amino acid to make **4**. Finally, another alkylation with either an allyl or benzyl halide to install a functional group on the amide of the hydantoin provides the final spiroligomer N-Boc protected amine **5**. We observe that the hydantoin amide is functionalized selectively in the presence of the secondary amide and secondary carbamate on **4**. When a small, highly reactive electrophile such

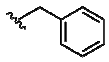
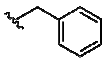
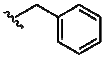
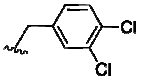
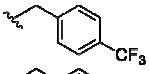
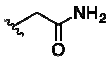
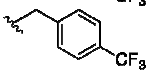
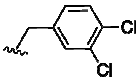
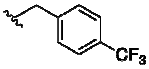
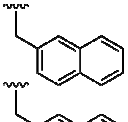
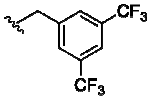
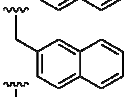
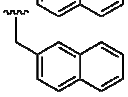
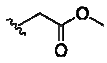
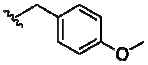
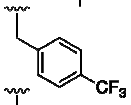
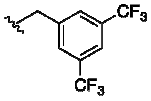
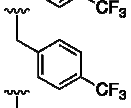
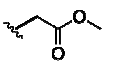
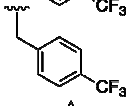
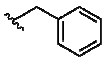
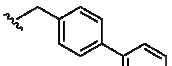
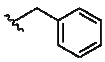
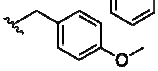
as methyl iodide is used, we observe less than 3% alkylation to what is most likely the secondary amide N11 (Scheme 2). The evidence that the alkylation is taking place selectively on the hydantoin amide was determined from the ^{1}H NMR for the alkylated products. If N6 were unalkylated, we would expect to see a triplet for NH16 and a singlet for NH6, yet we see two amide NH signals that show either a triplet for NH16 and a triplet for NH11 or a triplet for NH16 and a doublet for NH11, depending on whether the carbon attached to N11 is a methylene or methyne, respectively. This chemistry was used with varying electrophiles and amines to form the spiroligomer amines **6** to **18** shown in Table 1.



Scheme 2. *a*) DMF, 2 equiv CH_3-I, 2 equiv K_2CO_3, 24 hours. Methylation of N6 is the only alkylation that led to the formation of another product, which has the same mass as **18-X** and we presume that it has the structure shown above (this byproduct was not recovered)

Table 1. Spiroligomer amines synthesized from proline hydantoin **1**. Yield represents total yield of the amine from **1**.



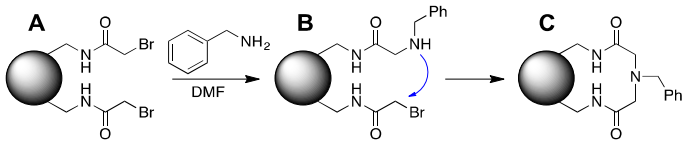
Cmpd	R_1	R_2	R_3	Yield
5				84%
6				69%
7				75%
8				67%
9				73%
10				76%
11				75%
12				73%
13				79%
14				76%
15				77%
16				75%
17				75%
18				71%

Testing the Minimum Requirements for Peptoid Chemistry

To make efficient use of these complex and synthetically derived amines we carried out a series of trials to determine the minimum amount of the amines required to achieve peptoid couplings while minimizing cross-linking (C-L) of the nascent peptiods on solid support. We varied the number of equivalents of amine relative to the resin loading on solid support and the concentration of amine required for peptoid chemistry to function reliably. We utilized commercially available benzylamine to obtain the baseline conditions for peptoid chemistry shown in Table 2, below. Varying the concentration of amine in the peptoid submonomer synthesis,[11,26] we determined that 3 equivalents of amine relative to resin loading (Rink Amide polystyrene, 0.63 mmol/g) with one equivalent of exogenous base (DIPEA; extra base lead to uncharacterized side products), at a concentration of 150 mM of benzylamine in DMF were the minimum equivalents and concentration that achieved efficient peptoid coupling and suppressed cross-linking to below 2%. Cross-linked products were observed and quantitated using HPLC-MS/UV-VIS. Any further decrease in equivalents or concentration resulted in significant cross-linking of the resin, whereby a secondary amine on resin would react with another bromoacetate on resin before an amine in solution could react, thereby terminating the sequence (shown in Scheme 3). On Rink Amide Tentagel® resin (loading 0.37 mmol/g), we found that we could lower the required concentration of amine to 125 mM, but the minimum number of equivalents required remained at 3 equivalents. A 2-Cl-Trityl chloride polystyrene resin (loading 1.1 mmol/g) required an increase in both the equivalents and concentration of benzylamine (5 equiv and 250 mM, respectively) to ensure efficient coupling and suppress cross-linking to less than 2%. These results were achieved at room temperature with four hour couplings.

Table 2. Minimum equivalents required for peptoid couplings on various resins

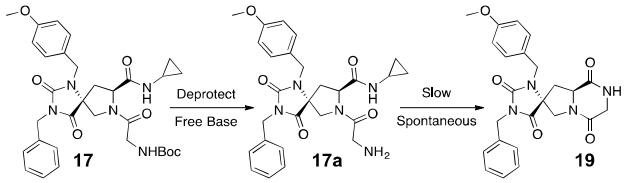
Trial	Equiv. Amine	Equiv. Base	Amine Conc. (mM)	Resin Loading (mmol/g)	% C-L (UV)
1	20	0	2000	0.63	< 1%
2	3	2	150	0.63	Side Product
3	3	1	150	0.63	< 2%
4	3	1	10	0.63	6-8%
5	3	1	30	0.63	6-8%
6	3	1	100	0.63	2-3%
7	2	1	100	0.63	2-3%
8	2	1	150	0.63	2-3%
9	3	1	125	0.37	< 2%
10	3	1	75	0.37	6-8%
11	3	1	150	1.1	8-10%
12	5	1	250	1.1	< 2%



Scheme 3. Benzylamine is combined with the resin (A); however, low concentration of amine in solution allows for an amine on resin to displace a neighboring bromine (B). This results in a crosslinked resin, and the termination of two peptoid sequences (C). C-L(UV) stands for cross-linking as determined using HPLC with UV detection.

Having determined the requirements of peptoid chemistry at sub molar concentrations, we proceeded to synthesize several variants of spiroligomer-peptoid hybrids. Any primary amine we use would first need to be Boc deprotected, as shown in Scheme 4, which was achieved with a 50% TFA / DCM mix, followed by concentration *in vacuo*. The free base of the amine **17** was obtained by extracting the trifluoroacetate salt with EtOAc and 1M KOH, followed by two washes with an equal volume of 1M KOH, which were back extracted with EtOAc. The Boc-protected amines are stable at room temperature for several years; however, if the free amine is left dry at room temperature or in solution, we observed slow, spontaneous formation of the diketopiperazine (DKP) **19**. To avoid this, the amine free-base was concentrated under reduced

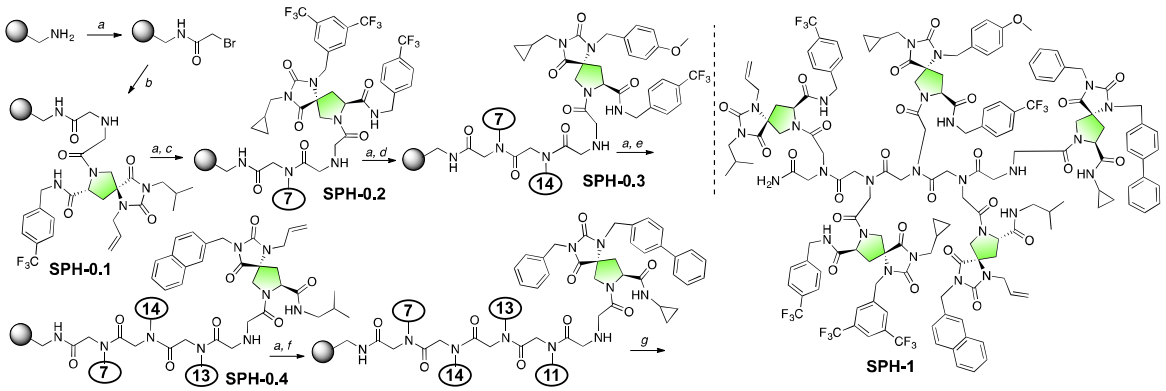
pressure to yield a foamy solid, which is stable for over a year (dry, -20 °C). Unreacted amine in the peptoid coupling can be recovered, protected, and stored.



Scheme 4. Generation of the spiroligomer free amine **17a** from **17**. Spontaneous DKP formation of spiroligomer free amines is possible at room temperature in solution.

Synthesis of Spiroligomer Peptoid Hybrids

Scheme 5 demonstrates the synthesis of spiroligomer-peptoid hybrid 1 (**SPH-1**) on Rink Amide polystyrene resin using standard submonomer synthesis and spiroligomer primary amines. This



Scheme 5. Peptoid synthesis of the spiroligomer-peptoid hybrid, **SPH-1** (*a*) Bromoacetic acid, DIC, DMF; (*b*) DMF, 150 mM **7**, 1 equiv DIPEA (*c*) DMF, 150 mM **14**, 1 equiv DIPEA; (*d*) DMF, 150 mM **15**, 1 equiv DIPEA; (*e*) DMF, 150 mM **11**, 1 equiv DIPEA; (*f*) DMF, 150 mM **16**, 1 equiv DIPEA; (*g*) TFA, neat.

spiroligomer-peptoid hybrid incorporates 15 functional groups and ten stereocenters on pre-organized spiroligomer side chains. HPLC chromatrograms documenting each elongation of **SPH-1** are highlighted in Figure 2. There is a solvent injection peak (denoted I) and a resin impurity (denoted as II) that are present in each chromatogram (Figure 2A-D, test cleavages) which do not change. The intended product III is the only other peak in the test cleavages. Figure 2E shows the crude HPLC chromatogram for **SPH-1**, which indicates a high level of fidelity for each coupling step. The peak around 2.8 min is from the final spiroligomer amine, which indicated longer washing steps were needed to recover unreacted amine from the resin. The spiroligomer-

peptoid hybrid can then be purified to remove the small impurities present in the crude material (Figure 2F-G).

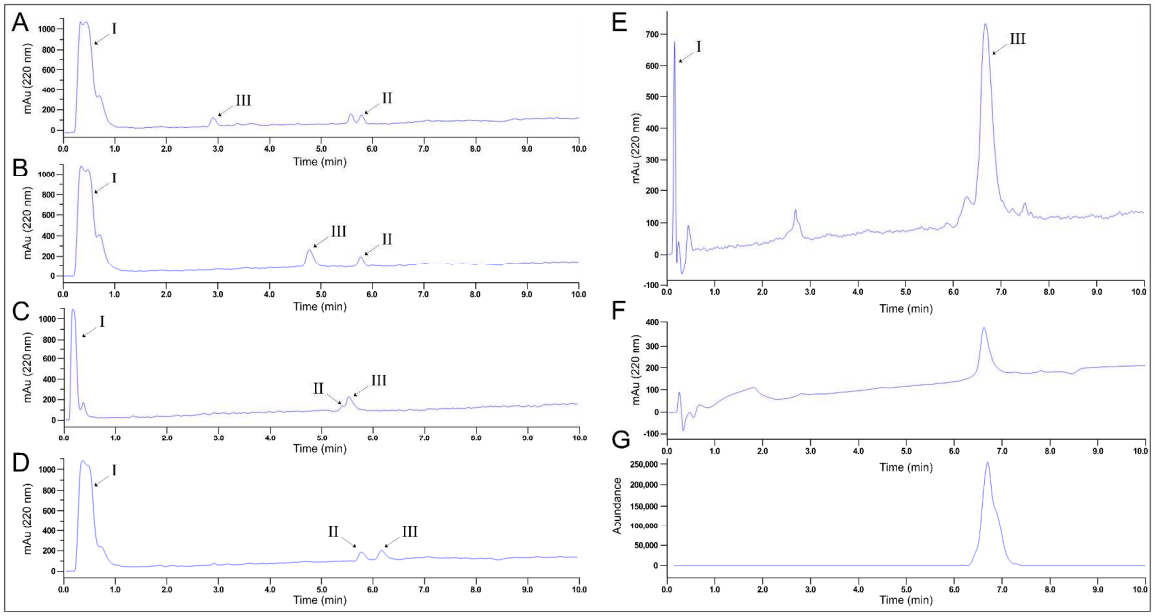


Figure 2. HPLC Chromatograms (A-F) following the synthesis of **SPH-1** (I = DMSO for A-D, 50% ACN/Water for E-G, II = resin impurity, III = desired product): (A) Test Cleavage of **SPH-0.1** (B) Test cleavage of **SPH-0.2** (C) Test cleavage of **SPH-0.3** (D) Test cleavage of **SPH-0.4** (E) Full resin cleavage for **SPH-1**, crude (F, G) HPLC-MS for purified **SPH-1**.

To test the fidelity of a spiroligomer-peptoid hybrid with increasing chain length, we synthesized both a 9-mer (**SPH-2**, 58% yield) and a 12-mer (**SPH-3**, 57%) incorporating 3 and 6 different amines, respectively (Figure 3A, B). Other spiroligomer-peptoid hybrids that we have synthesized include custom linkers incorporating propargyl (post-peptoid synthesis modification potential) or bromobenzyl side chains (the bromobenzyl group provides an easily identified mass spectroscopy signature due to the natural isotopic abundance of bromine). Installation of a c-terminal methionine residue to facilitate CNBr mediated cleavage from resin has been tested successfully on various spiroligomer-peptoid hybrids (Figure 3C, **SPH-4**, 22% yield). These types of hybrids would be most beneficial to those interested in on-resin screening for compounds, as it allows for the deprotection of any side chains, while leaving the peptoid still attached to the resin.

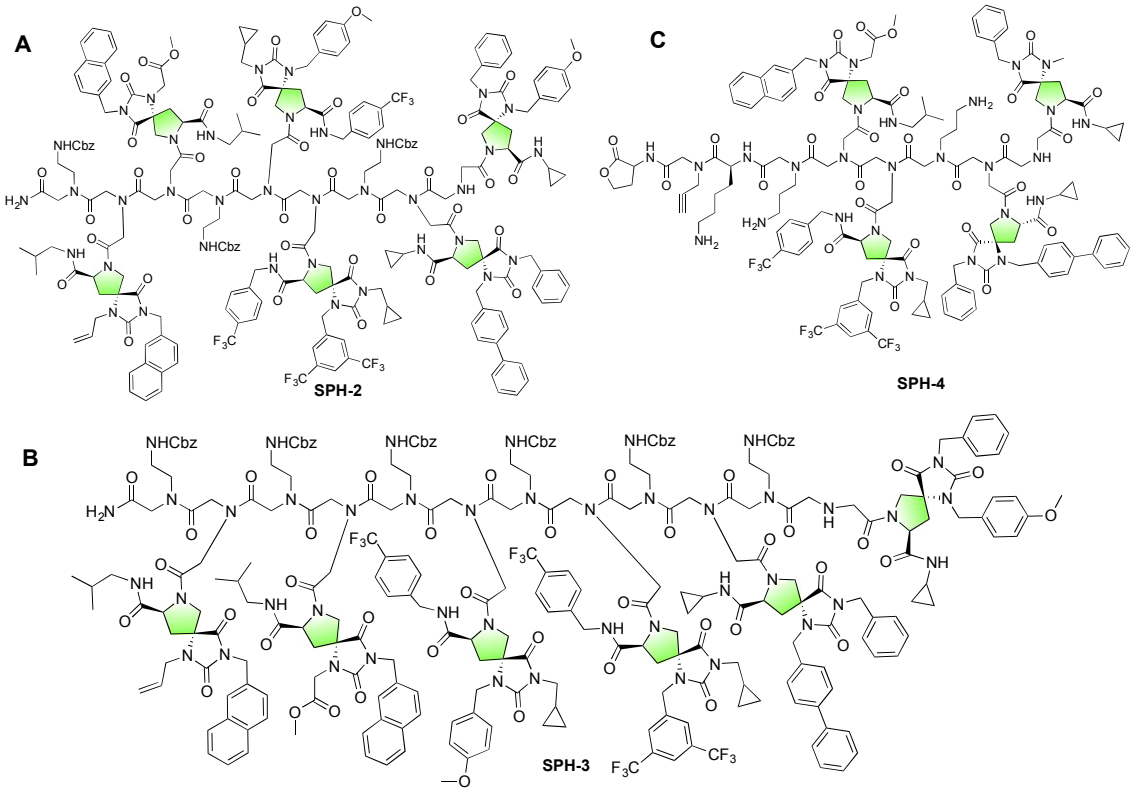
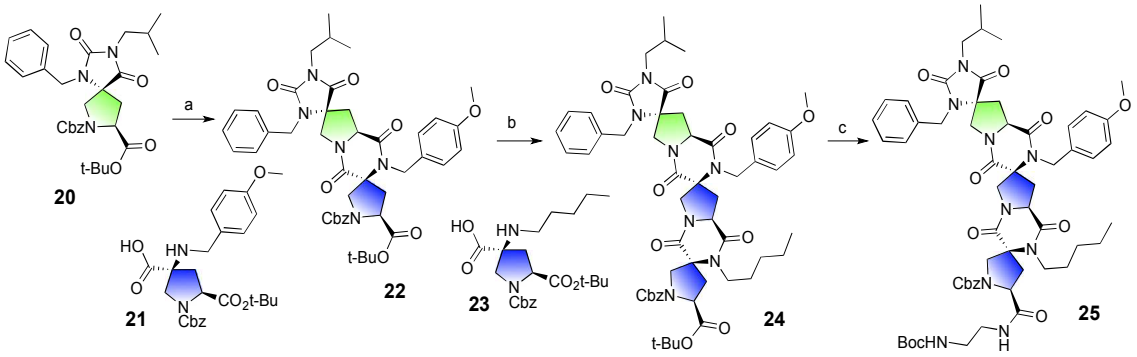


Figure 3. Three spiroligomer-peptoid hybrids of varying length, functional groups, and resin linker. (A) **SPH-2**, a peptoid 9-mer incorporating 6 spiroligomers (B**) SPH-3**, a peptoid 12-mer incorporating 6 spiroligomers (C) **SPH-4**, a hybrid peptoid containing 8 residues, four of which are spiroligomers, and an initial methionine residue for CNBr mediated cleavage.

Synthesis of a spiroligomer-peptoid hybrid containing a spiroligomer trimer

To further reduce the number of rotatable bonds in the peptoid backbone, we have synthesized a spiroligomer-peptoid hybrid containing three copies of a highly preorganized, spiroligomer trimer. Spiroligomer trimers have been demonstrated within our lab as designed catalysts[46] or for protein binding.[48] Incorporating large, structured macromolecules like these will be used to create even more functional, preorganized macromolecules. The trimer **25** was synthesized from a bifunctionalized proline hydantoin **20** and two bis-amino acids **21** and **23**, as shown in Scheme 6. The Cbz and *t*-Bu protected, dialkylated proline hydantoin **20** was treated with 1:1 DCM/(33% HBr/AcOH) for 30 minutes, after which the solvent was removed *in vacuo*, and the molecule left on a high vacuum pump overnight. Bis-amino acid **21** was preactivated

under inert conditions with EDC and HOAt in a 1:1 mix of anhydrous DMF/DCM for 1.5 h. This preactivation is achieved without base so the bis-amino acid does not react with itself. Deprotected compound **20** was dissolved in a minimal amount of anhydrous DMF, and added to the preactivated bis-amino acid **21** along with DIPEA. After stirring overnight, more EDC was added to the reaction to close the diketopiperazine ring to afford the spiroligomer dimer **22**. Spiroligomer dimer **22** is purified via flash chromatography (gradient from 0-100% ethyl acetate in hexanes) in 46% yield.



Scheme 6. Synthesis of the spiroligomer trimer **25** (a) i. 1:1 DCM/(33% HBr/AcOH); ii. (Preactivated **21**: HOAT, EDC, 1:1 dry DMF/DCM, 1.5 h), DMF, DIPEA; (b) i. 1:1 DCM/(33% HBr/AcOH); ii. (Preactivated **23**: HOAT, EDC, 1:1 dry DMF/DCM, 1.5 h), DMF, DIPEA; (c) i. 95:4:1 TFA/H_2O/TIPS, 1 h; ii. EDC, HOAT, 1:1 dry DMF/DCM, 1.5 h; iii. N-boc-ethylenediamine

Dimer **22** was deprotected with 1:1 DCM/(33% HBr/AcOH) for 30 minutes, the solvent removed *in vacuo*, and held under high vacuum overnight. Bis-amino acid **23** was pre-activated under inert conditions with EDC and HOAt in a 1:1 mix of anhydrous DMF/DCM for 1.5 h. This preactivation is achieved without base so the bis-amino acid does not react with itself. Dimer **22** was dissolved in a minimal amount of anhydrous DMF, and added along with DIPEA to the preactivated bis-amino acid **23** to afford the spiroligomer trimer **24**. Spiroligomer trimer **24** is then purified via flash chromatography (gradient from 0-100% ethyl acetate in hexanes) in 21% yield. Spiroligomer **24** can then be treated with a 95:4:1 TFA/H_2O/TIPS mixture to remove the *t*-Bu protecting group, then following the removal of the solvent, this spiroligomer is preactivated using EDC and HOAt in a 1:1 mix of dry DMF/DCM for 1.5 h. The preactivated spiroligomer was combined with N-Boc-ethylenediamine to provide spiroligomer trimer **25**, which was utilized to

make **SPH-5** shown in Figure 4 (25% purified yield) using standard peptoid synthesis. This spiroligomer trimer is, to the best of our knowledge, the largest amine utilized for the displacement step of a peptoid synthesis, containing five unique functional groups and six stereocenters per residue. This means the spiroligomer-peptoid hybrid **SPH-5** contains 15 functional groups and 18 stereocenters, over all of which we have total control.

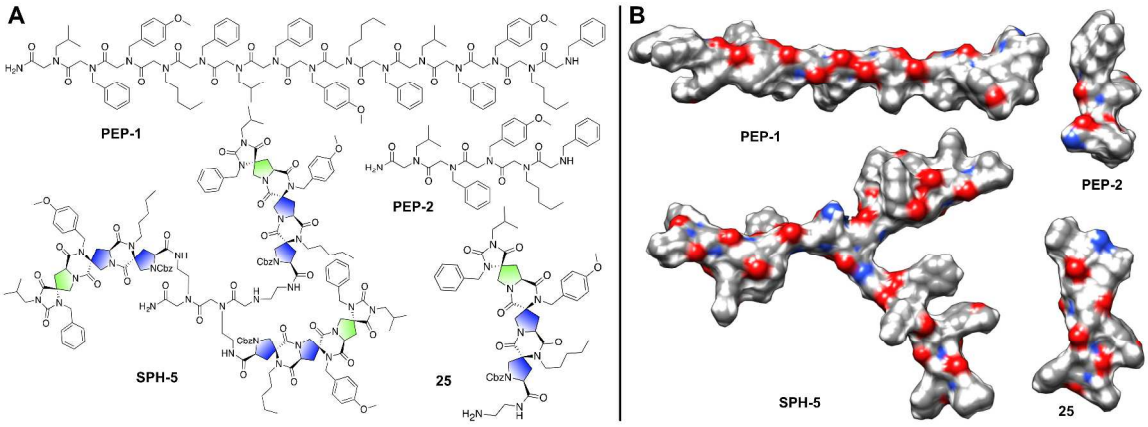


Figure 4. Comparisons of theoretical peptoids **PEP-1** (15mer) and **PEP-2** (5mer) vs **SPH-5** and amine **25**. (A) Chemdraws for **PEP-1**, **PEP-2**, **SPH-5**, and **25**. (B) CANDO models with solvent-excluded surface for **PEP-1** (surface area = 1743 Å^2), **PEP-2** (surface area = 628.3 Å^2), **SPH-5** (surface area = 2385 Å^2), and **25** (surface area = 774.6 Å^2).

By incorporating these large preorganized amines, we have successfully reduced the number of peptoid-backbone rotatable bonds by 50%. This was accomplished while increasing the potential surface area available for interactions as shown in Figure 4B, which depicts the solvent-excluded surface for two theoretical peptoids **PEP-1** and **PEP-2**, as well as hybrid **SPH-5** and its component amine **25**. When compared to a theoretical peptoid (**PEP-1**) with the same functional groups, **SPH-5** increases the total surface area by 33%. Similarly, when compared to a theoretical peptoid (**PEP-2**) with the same functional groups, amine **25** increases the total surface area by 23%. This shows that preorganization of functional groups on a spiroligomer-peptoid hybrid will not only reduce the number of backbone rotatable bonds, but it will also increase the potential surface area available for host-guest or protein-protein interactions. These large spiroligomer trimers, like the smaller monomers shown previously, can be used sequentially, or interspersed with smaller units, indicating that they would make excellent candidate amines for

future work. Furthermore, large spiroligomer trimers preorganize many more groups than the spiroligomer monomer amines or regular peptoid amines, thus reducing any entropic penalty that would be associated when binding a protein for example.

Conclusion

We have successfully synthesized 16 new spiroligomers containing three to five functional groups, two to six stereocenters, and a protected primary amine, which we have used to incorporate the spiroligomers into peptoids of varying lengths. To the best of our knowledge, this is the first reported synthesis which utilized large, preorganized, spirocyclic amines during the displacement step of peptoid synthesis. Notably, this work featured the use of spiroligomer trimer **25,** which organizes five functional groups on a spiroligomer backbone containing six stereocenters and no interior rotatable bonds. This spiroligomer was used to make the spiroligomer-peptoid hybrid **SPH-5**, which contains 18 stereocenters and 15 functional groups across three peptoid residues. A linear peptoid with the same number of functional groups would have 42 rotatable bonds in the backbone, whereas this hybrid has only 6 in the backbone and 15 in the linkers, a 50% reduction in the number of rotatable bonds for the same number of functional groups. This preorganization of functional groups will help facilitate the rapid discovery of novel structures for host-guest interactions, protein-protein interactions, and catalysis. Now that we can synthesize these new hybrid peptidomimetics, further work will be focused on developing spiroligomer-peptoid hybrids that display the appropriate side chains for these important interactions.

Experimental Details

General Procedure 1: Mono alkylation of P4srZBHyd

To a stirred mixture of **1** in DMF [100 mM] was added 0.75-0.85 equiv of alkyl, allyl, or benzyl halide (equiv dependent on salt content of the specific batch **1**, which can be determined utilizing an internal standard) along with 1.5 equiv of K_2CO_3. The reaction proceeded at room temperature

for 2-24 h, dependent on electrophile, and the progress checked via LCMS. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with Na_2SO_4, and concentrated *in vacuo* to yield **26-29** as foamy off-white to yellow solids. ^1H / ^{13}C NMR spectra for compounds **26**-**29** are available in the supporting information.

Compound 26 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate - Compound **26** was synthesized using General Procedure 1, with **1** [(2S, 4R) 7.5 mmol] and 2-(bromomethyl)naphthalene [6 mmol (0.80 equiv)]. Recovered yield was quantitative and the product used without purification. ^1H NMR (500 MHz, $CDCl_3$) 1.42 (9H, s, rotameric), 2.36 (1H, m), 2.48 (1H, m), 3.81 (2H, m), 4.45 (1H, m), 4.78 (1H, s, rotameric), 5.07 (2H, m), 7.25-7.84 (13H), 9.08 (1H, s, rotameric); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 27.8, 34.1, 39.6, 40.6, 42.8, 55.7, 58.4, 65.9, 66.8, 67.6, 82.4, 125.8, 126.2, 126.3, 126.4, 126.6, 126.8, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6, 128.7, 132.9, 133.0, 133.2, 135.1, 135.8, 136.0, 154, 7, 156.3, 169.8, 172.9; HRMS (ESI/Q-TOF) m/z: $(M+K)^+$ calcd for $C_{30}H_{31}N_3O_6K$ 568.1844, found 568.1840

Compound 27 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-benzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate - Compound **27** was synthesized using General Procedure 1, with **1** [(2S, 4R) 15 mmol] and benzyl bromide [12 mmol (0.80 equiv)]. Recovered yield was quantitative and the product used without purification. ^1H NMR (500 MHz, $CDCl_3$) 1.43 (9H, s, rotameric), 2.31 (1H, dd, rotameric, J = 14.5, 8.1), 2.49 (1H, ddd, J = 18.0, 13.4, 9.2), 3.84 (2H, m), 4.43 (1H, dt, J = 24.4, 8.6), 4.63 (2H, s), 5.08 (2H, m), 7.30 (11H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 27.9, 39.7, 40.7, 42.5, 55.7, 58.9, 65.5, 66.2, 67.6, 82.4, 127.7, 127.9, 128.1, 128.2, 128.5, 128.6, 128.8, 135.7, 136.0, 154.5, 155.9, 169.7, 171.7; HRMS (ESI/Q-TOF) m/z: $(M+K)^+$ calcd for $C_{26}H_{29}N_3O_6K$ 518.1688, found 518.1699

Compound 28 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate - Compound **28** was synthesized using General Procedure 1, with **1** [(2S, 4R) 7.5 mmol] and (bromomethyl)cyclopropane [6.0 mmol (0.80 equiv)]. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.33 (2H, d, J = 5.8), 0.49 (2H, d, J = 7.8), 1.13 (1H, m), 1.45 (9H, s, rotameric), 2.40 (1H, dd, rotameric, J = 14.3, 8.2), 2.54 (1H, ddd, J = 18.2, 13.4, 9.0), 3.35 (2H, dd, J = 7.2, 3.5), 3.81 (2H, m), 4.47 (1H, dt, J = 24.8, 8.3), 5.11 (2H, m), 7.32 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.7, 10.2, 27.9, 40.7, 43.7, 55.8, 58.9, 66.1, 67.6, 82.4, 127.9, 128.2, 128.5, 136.0, 154.5, 156.8, 169.7, 172.2 HRMS (ESI/Q-TOF) m/z: $(M+K)^+$ calcd for $C_{23}H_{29}N_3O_6K$ 482.1688, found 482.1693

Compound 29 - 7-benzyl 8-(*tert*-butyl) (5*R*,8*S*)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate – Compound **29** was synthesized using General Procedure 1, with **1** [(2S, 4R) 15 mmol] and 1-iodo-2-methylpropane [18 mmol (1.2 equiv)]. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.88 (6H, d, *J* = 6.7), 1.45 (9H, s, rotameric), 2.05 (1H, m), 2.38 (1H, m), 2.53 (1H, ddd, *J* = 18.2, 13.3, 9.2), 3.31 (2H, dd, *J* = 7.3, 3.1), 3.78 (2H, m), 4.48 (1H, dt, *J* = 24.4, 8.6), 5.11 (2H, m), 7.35 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.9, 27.8, 27.9, 39.8, 40.8, 46.1, 55.8, 56.0, 58.4, 58.9, 65.2, 65.9, 67.6, 82.4, 127.8, 127.9, 128.2, 128.5, 135.9, 136.0, 154.2, 154.5, 156.6, 156.8, 169.7, 169.9, 172.2, 172.4; HRMS (ESI/Q-TOF) m/z: $(M+K)^+$ calcd for $C_{23}H_{31}N_3O_6K$ 484.1844, found 484.1861

General Procedure 2: Deprotection of C2 t-Bu group and coupling

Each of **26-29** was placed in a RB flask and then treated with 95% TFA / TIPS for 1 h, with the reaction progress checked via LCMS. Upon successful deprotection of the *tert*-butyl group, the solvent was removed *in vacuo*, and placed on a high vacuum pump overnight. To an inert atmosphere RB flask containing the free acid in dry DMF/DCM (1:1 ratio, [100 mM]) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 1.5 equiv) and 1-Hydroxy-7-

azabenzotriazole (HOAT, 3 equiv), then stirred for 1 h. An amine (3 equiv) and DIPEA (2 equiv) were then added, and the reaction proceeded for 2 h, at which point the progress was checked via LCMS. Upon completion, the reaction was diluted with four times the reaction volume of EtOAc and washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution, and brine. The organic layer was dried with Na_2SO_4, and concentrated *in vacuo* to yield **30-35** as foamy yellow solids. ^{1}H / ^{13}C NMR spectra for compounds **30**-**35** are available in the supporting information.

Compound 30 benzyl (5*R*,8*S*)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate – Compound **30** was synthesized using General Procedure 2 and compound **26**, with HOAt (22.5 mmol), EDC (11.25 mmol), Isobutylamine (22.5 mmol), DIPEA (15 mmol), and 75 mL of 1:1 DMF/DCM - Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.82 (6H, d, *J* = 6.4), 1.89 (1H, m), 2.25 (1H, m), 2.64 (1H, m), 2.99 (1H, m), 3.05 (1H, sep, *J* = 6.5), 3.68 (1H, m), 3.84 (1H, d, *J* = 11.3), 4.59 (1H, m), 4.78 (2H, s, rotameric), 5.05 (2H, m), 7.02 (1H, m), 7.21 (5H, m), 7.44 (3H, m), 7.66 (1H, m), 7.78 (4H, m); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 20.0, 28.0, 28.1, 40.6, 41.5, 41.7, 46.0, 56.1, 56.5, 58.7, , 59.2, 65.1, 65.8, 66.2, 125.4, 125.5, 125.7, 125.8, 126.0, 126.3, 127.1, 127.5, 127.6, 127.7, 127.8, 128.2, 128.3, 132.2, 132.8, 134.0, 136.5, 136.6, 153.5, 152.7, 155.4, 155.5, 170.4, 170.7, 172.4; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{30}H_{33}N_4O_5$ 529.2445, found 529.2446

Compound 31 - benzyl (5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate - Compound **31** was synthesized using General Procedure 2 and compound **27**, with HOAt (22.5 mmol), EDC (11.25 mmol), cyclopropylamine (22.5 mmol), DIPEA (15 mmol), and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.30 (1H, m), 0.41 (1H, M), 0.58 (2H, dd, rotameric, *J* = 23.2, 7.1), 2.21 (1H, m), 2.33 (1H, m), 2.60 (1H, m), 3.64 (1H, dd, rotameric, *J* = 15.5, 11.4), 3.81 (1H, d, *J* = 11.3), 4.30 (1H, m), 4.55 (2H, d, *J* = 12.5), 5.05 (2H,

m), 7.30 (10H, m), 8.20 (1H, m), 9.16 (1H, m); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 5.4, 5.5, 5.6, 5.7, 22.3, 40.4, 41.3, 41.4, 56.0, 56.4, 58.6, 59.0, 65.1, 65.7, 66.2, 127.1, 127.2, 127.4, 127.5, 127.7, 127.8, 128.2, 128.4, 128.5, 128.6, 136.5, 136.6, 153.4, 153.6, 155.3, 155.4, 171.5, 171.8, 172.3; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{25}H_{26}N_4O_5Na$ 485.1795, found 485.1790

Compound 32 - benzyl (5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate - Compound **32** was synthesized using General Procedure 2 and compound **27**, with HOAt (22.5 mmol), EDC (11.25 mmol), Isobutylamine (22.5 mmol), DIPEA (15 mmol), and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.76 (6H, d, rotameric, J = 6.7) 1.62 (1H, sep, rotameric, J = 6.7), 2.22 (1H, dt, J = 12.8, 10.0), 2.38 (1H, m), 2.87 (2H, m), 3.66 (1H, dd, J = 17.0, 11.5), 3.84 (1H, d, rotameric, J = 11.5), 4.43 (1H, dd, rotameric, J = 10.0, 7.5), 4.56 (2H, d, J = 11.9), 5.05 (1H, d, J = 7.6), 5.07 (1H, d, J = 5.2), 7.30 (10H, m), 8.14 (1H, t, rotameric, J = 5.9), 9.22 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 19.42, 27.4, 27.5, 40.0, 40.9, 45.4, 55.5, 55.9, 58.1, 58.6, 64.5, 65.1, 65.6, 126.5, 126.6, 126.8, 126.9, 127.0, 127.2, 127.6, 127.8, 128.0, 135.9, 136.0, 152.9, 153.1, 154.8, 154.9, 169.8, 170.1, 171.8; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{26}H_{30}N_4O_5Na$ 501.2108, found 501.2110

Compound 33 - benzyl (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonane-7-carboxylate Compound **33** was synthesized using General Procedure 2 and compound **28**, with HOAt (22.5 mmol), EDC (11.25 mmol), 4-(trifluoromethyl)benzylamine (22.5 mmol), DIPEA (15 mmol), and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.25 (2H, m), 0.43 (2H, m), 1.04 (1H, m), 2.26 (1H, ddd, J = 13.0, 10.0, 7.2), 2.38 (1H, m), 3.23 (2H, dd, J = 11.0, 7.3), 3.67 (1H, d, rotameric, J = 11.2), 4.33 (1H, dd, J = 12.5, 5.8), 4.40 (1H, t, J = 6.1), 4.50 (1H, dd, rotameric, J = 9.9, 7.5), 5.07 (2H, m), 7.34 (6H, m), 7.50 (2H, m), 7.65 (1H, d, J = 7.9), 8.83 (1H, m), 9.10 (1H, s, rotameric); ^{13}C NMR (125 MHz,

DMSO-d_6, rotamers present) 3.4, 9.9, 40.3, 41.3, 41.7, 42.5, 56.1, 56.4, 58.9, 59.4, 64.9, 65.5, 66.3, 123.2, 125.0, 127.0, 127.3, 127.5, 127.6, 127.8, 128.2, 128.3, 136.5, 144.0, 153.7, 155.6, 155.7, 170.9, 171.1, 172.3; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{27}H_{27}F_3N_4O_5Na$ 567.1826, found 567.1827

Compound 34 - benzyl (5*R*,8*S*)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonane-7-carboxylate Compound **34** was synthesized using General Procedure 2 and compound **29**, with HOAt (22.5 mmol), EDC (11.25 mmol), 4-(trifluoromethyl)benzylamine (22.5 mmol), DIPEA (15 mmol), and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.83 (6H, d, *J* = 6.8), 1.94 (1H, m), 2.26 (1H, ddd, *J* = 13.0, 10.1, 8.1), 2.41 (1H, ddd, rotameric, *J* = 20.6, 13.1, 7.3), 3.18 (2H, dd, *J* = 10.2, 7.5), 3.65 (1H, d, rotameric, *J* = 11.4), 3.82 (1H, d, rotameric, *J* = 11.2), 4.33 (1H, dd, *J* = 10.8, 6.0), 4.40 (1H, t, *J* = 5.6), 4.48 (1H, dd, rotameric, *J* = 9.9, 7.5), 5.07 (2H, m), 7.34 (6H, M), 7.51 (2H, m), 7.66 (1H, d, rotameric, *J* = 8.2), 8.83 (1H, m), 9.10 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 19.8, 26.9, 40.4, 41.4, 41.7, 41.8, 45.3, 56.5, 58.9, 59.4, 64.8, 65.4, 66.3, 123.2, 123.3, 125.0, 125.1, 125.4, 127.0, 127.3, 127.6, 127.7, 127.9, 128.3, 128.4, 136.5, 136.6, 144.1, 144.3, 153.7, 153.8, 155.7, 155.8, 171.0, 171.2, 172.6; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{27}H_{29}F_3N_4O_5Na$ 569.1982, found 569.1986

Compound 35 - benzyl (5*R*,8*S*)-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate - Compound **35** was synthesized using General Procedure 2 and compound **29**, with 22.5 mmol HOAt, 11.25 mmol EDC, 22.5 mmol benzylamine, 15 mmol DIPEA, and 75 mL of 1:1 DMF/DCM. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, DMSO-d_6) 0.82 (6H, dd, *J* = 6.7, 4.6), 1.94 (1H, m), 2.24 (1H, m), 2.39 (1H, m), 3.18 (2H, dd, *J* = 10.4, 7.3), 3.66 (1H, m), 3.81 (1H, m), 4.27 (2H, m), 4.46 (1H, ddd, *J* = 17.5, 8.9, 7.5), 5.07 (2H, m), 7.20 (10H, m), 8.72 (1H, m), 9.10 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 19.9, 26.9, 40.5,

41.4, 42.0, 42.1, 45.3, 56.5, 58.9, 59.3, 64.8, 65.4, 66.3, 126.7, 127.0, 127.1, 127.6, 127.7, 127.9, 128.2, 128.3, 128.4, 136.6, 139.2, 153.6, 153.7, 155.7, 155.8, 170.7, 170.9, 172.6; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{26}H_{30}N_4O_5Na$ 501.2108, found 501.2113

General Procedure 3: Deprotection of Proline Cbz group

Each of **30-35** was added to a RB flask and reacted with 1:1 DCM/(33% HBr in AcOH) for 30 min, and the reaction progress checked via LCMS. Upon successful deprotection of the Cbz group, the solvent was removed *in vacuo* with the aid of toluene, and placed on a high vacuum pump overnight to afford **36-41**. ^1H / ^{13}C NMR spectra for compounds **36**-**41** are available in the supporting information.

Compound 36 - (5*R*,8*S*)-*N*-isobutyl-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **36** was synthesized using General Procedure 3 and compound **30**. Recovered yield was quantitative and the product used without purification. ^1H NMR (500 MHz, DMSO-d_6) 08.4 (6H, d, *J* = 6.7), 1.72 (1H, m), 2.23 (1H, dd, *J* = 13.4, 11.9), 2.69 (1H, dd, *J* = 13.4, 6.7), 2.93 (1H, m), 3.02 (1H, m), 3.53 (1H, d, *J* = 12.8), 3.67 (1H, d, *J* = 12.8), 4.53 (1H, dd, *J* = 11.6, 6.7), 4.72 (2H, m), 7.41 (2H, m), 7.50 (1H, m), 7.76 (1H, m), 7.90 (3H, m), 8.66 (1H, m), 9.01 (1H, s), 9.31 (1H, s), 9.91 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 20.0, 27.9, 34.6, 40.7, 42.0, 46.4, 48.6, 52.5, 55.0, 59.3, 65.7, 125.6, 125.9, 126.1, 126.4, 127.6, 127.7, 128.3, 128.7, 129.3, 132.3, 132.8, 133.8, 155.2, 165.9, 172.7; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{22}H_{27}N_4O_3$ 395.2078, found 395.2084

Compound 37 (5*R*,8*S*)-3-benzyl-*N*-cyclopropyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **37** was synthesized using General Procedure 3 and compound **31**. Recovered yield was quantitative and the product used without purification. ^1H NMR (500 MHz, DMSO-d_6) 0.47 (2H, m), 0.66 (2H, m), 2.21 (1H, dd, *J* = 13.4, 11.6), 2.56 (1H, dd, *J* = 13.3, 6.9), 2.7 (1H, tq, *J* = 7.3, 3.9) 3.49 (1H, d, *J* = 13.1), 3.62 (1H, d, *J* = 12.8), 4.42 (1H, dd, *J* = 11.6, 6.7),

4.55 (2H, s), 7.28 (5H, m), 8.73 (1H, d, *J* = 4.3), 8.96 (1H, s), 9.30 (1H, s), 9.88 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6,) 5.6, 21.1, 22.6, 40.5, 41.7, 48.6, 52.5, 59.2, 65.5, 127.3, 127.5, 128.6, 136.2, 150.1, 166.9, 172.6 HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{17}H_{21}N_4O_3$ 329.1608, found 329.1602

Compound 38 (5*R*,8*S*)-3-benzyl-*N*-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **38** was synthesized using General Procedure 3 and compound **32**. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, DMSO-d_6) 0.86 (6H, d, *J* = 7.6), 1.74 (1H, m), 2.22 (1H, dd, *J* = 13.4, 11.6), 2.69 (1H, dd, *J* = 13.4, 7.0), 2.95 (1H, m), 3.03 (1H, m), 3.54 (1H, d, *J* = 12.8), 3.66 (1H, d, *J* = 13.1), 4.57 (2H, s), 4.58 (1H, dd, obscured, *J* = 11.7, 6.7), 7.31 (5H, m), 8.70 (1H, t, *J* = 5.8), 9.01 (1H, s), 9.29 (1H, s), 9.96 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) 20.0, 20.1, 27.9, 35.6, 40.7, 41.7, 46.4, 48.6, 52.4, 59.3, 65.6, 127.3, 127.5, 128.3, 128.6, 136.2, 155.1, 165.9, 172.7; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{18}H_{25}N_4O_3$ 345.1921, found 345.1920

Compound 39 - (5*R*,8*S*)-3-(cyclopropylmethyl)-2,4-dioxo-*N*-(4-(trifluoromethyl)benzyl)-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **39** was synthesized using General Procedure 3 and compound **33**. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, DMSO-d_6) 0.24 (2H, m), 0.43 (2H, m), 1.02 (1H, m), 2.27 (1H, dd, *J* = 13.4, 11.6), 2.67 (1H, dd, *J* = 13.3, 6.9), 3.22 (2H, d, *J* = 7.0), 3.49 (1H, d, *J* = 13.1), 3.62 (1H, d, *J* = 13.1), 4.46 (2H, m), 4.65 (1H, dd, *J* = 11.4, 6.9), 7.51 (2H, d, *J* = 8.2), 7.69 (2H, d, *J* = 7.9), 8.89 (1H, s), 9.36 (1H, t, *J* = 6.0), 9.46 (1H, s), 9.80 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6) 3.5, 9.8, 40.4, 42.1, 42.8, 48.6, 52.5, 59.4, 65.4, 125.3, 125.4, 127.7, 127.9, 128.1, 143.3, 155.4, 166.3, 172.7; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{19}H_{22}F_3N_4O_3$ 411.1639, found 411.1638

Compound 40 - (5*R*,8*S*)-3-isobutyl-2,4-dioxo-*N*-(4-(trifluoromethyl)benzyl)-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **40** was synthesized using General

Procedure 3 and compound **34**. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, $CDCl_3$) 0.77 (6H, d, *J* = 6.4), 1.88 (1H, m), 2.49 (1H, m), 2.83, 1H, m), 3.16 (2H, m), 3.29 (1H, m), 3.91 (2H, m), 4.36 (1H, d, *J* = 11.0), 4.56 (1H, d, *J* = 9.2), 5.36 (1H, m), 7.35 (2H, d, *J* = 7.2), 7.47 (2H, d, *J* = 7.6), 8.31 (1H, m), 8.68 (1H, m), 9.45 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$) 19.8, 27.3, 33.6, 43.4, 46.5, 60.2, 65.9, 122.9, 125.0, 125.5, 127.7, 127.9, 128.8, 129.0, 129.6, 129.8, 141.2, 156.3, 167.3, 172.2; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{19}H_{24}F_3N_4O_3$ 413.1795, found 413.1794

Compound 41 (5*R*,8*S*)-*N*-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxamide - Compound **41** was synthesized using General Procedure 3 and compound **35**. Recovered yield was quantitative and the product used without purification. ^{1}H NMR (500 MHz, DMSO-d_6) 0.82 (6H, d, *J* = 6.7), 1.92 (1H, m), 2.24 (1H, dd, *J* = 13.3, 11.7), 2.65 (1H, dd, *J* = 13.3, 6.9), 3.17 (2H, d, *J* = 7.3), 3.49 (1H, d, *J* = 13.1), 3.61 (1H, d, *J* = 12.8), 4.36 (2H, dd, *J* = 5.8, 3.1), 4.60 (1H, dd, *J* = 11.6, 7.0), 7.29 (6H, m), 8.87 (1H, s), 9.21 (1H, t, *J* = 5.8); ^{13}C NMR (125 MHz, DMSO-d_6) 19.8, 26.9, 34.6, 40.5, 42.5, 45.5, 52.5, 59.3, 65.3, 127.1, 127.3, 128.4, 128.7, 129.3, 138.3, 155.4, 166.0, 172.9; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{18}H_{25}N_4O_3$ 345.1921, found 345.1921

General Procedure 4: Coupling of Boc-Gly-OH to 36-41

To an inert atmosphere RB flask containing 2 equiv of Boc-Gly-OH in dry DMF/DCM (1:1 ratio, [200 mM]) was added EDC (2 equiv) and HOAT (4 equiv) and stirred for 1 h. Afterwards, one of **36-41** in DMF [200 mM] and DIPEA (4 equiv) were added and the reaction stirred for another 2 h, at which point the progress was checked via LCMS. Upon completion, the reaction was diluted with four times the reaction volume of EtOAc and washed with saturated ammonium chloride solution, saturated sodium bicarbonate solution, and brine. The organic layer was then dried with Na_2SO_4, and concentrated *in vacuo* to yield **42-47** as foamy dark-yellow solids. These compounds were then purified by normal phase flash chromatography (0-5% MeOH in DCM) to

yield off-white foamy solids. ^{1}H / ^{13}C NMR spectra are available for compounds 42-47 in the supporting information.

Compound 42 - *tert*-butyl (2-((5*R*,8*S*)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate – Compound **42** was synthesized using General procedure 4, Boc-Gly-OH (15 mmol), HOAt (30 mmol), EDC (15 mmol), DIPEA (30 mmol), and compound **36**. Purified yield 3.23 g (78%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.80 (6H, m), 1.21 (9H, s, rotameric), 1.65 (1H, m), 2.24 (1H, dd, *J* = 12.8, 7.6), 2.47 (2H, m), 3.24 (1H, m), 3.73 (1H, dd, *J* = 17.1, 4.6), 3.95 (1H, m), 4.08 (1H, dd, *J* = 10.4), 4.43 (1H, m), 4.69 (1H, d, *J* = 14.6), 4.79 (1H, m), 4.82 (1H, d, *J* = 14.6), 5.77 (1H, m), 7.32 (1H, m), 7.46 (3H, m), 7.76 (4H, m), 8.18 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.0, 20.1, 25.3, 28.1, 28.3, 28.4, 40.9, 43.0, 46.7, 54.5, 59.7, 64.3, 66.5, 79.6, 126.4, 126.5, 127.7, 127.8, 128.1, 128.6, 132.9, 133.0, 133.1, 155.5, 156.5, 169.6, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{29}H_{37}N_5O_6Na$ 574.2636, found 574.2633

Compound 43 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Compound **43** was synthesized using General procedure 4, Boc-Gly-OH (15 mmol), HOAt (30 mmol), EDC (15 mmol), DIPEA (30 mmol), and compound **37** - Purified yield 2.94 g (81%); ^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 0.44 (1H, m), 0.52 (2H, m), 0.67 (1H, m), 1.31 (9H, s, rotameric), 2.17 (1H, m), 2.43 (1H, t, *J* = 11.7), 2.55 (1H, m), 3.76 (1H, dd, *J* = 17.1, 5.5), 3.84 (1H, d, *J* = 10.4), 4.06 (1H, d, *J* = 10.4), 4.22 (1H, m), 4.58 (1H, d, *J* = 14.6), 4.65 (1H, d, *J* = 14.6), 4.67 (1H, m), 5.80 (1H, s), 7.30 (5H, m), 7.45 (1H, m), 8.21 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 5.9, 6.7, 22.6, 28.2, 40.3, 42.7, 43.2, 54.9, 59.7, 66.4, 79.8, 128.1, 128.6, 128.8, 135.7, 155.6, 156.6, 171.1, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{24}H_{31}N_5O_6Na$ 508.2167, found 508.2165

Compound 44 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Compound **44** was synthesized using

General procedure 4, Boc-Gly-OH (15 mmol), HOAt (30 mmol), EDC (15 mmol), DIPEA (30 mmol), and compound **38**. Purified yield 2.87 g (78%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.85 (6H, m), 1.29 (9H, s, rotameric), 1.70 (1H, m), 2.22 (1H, dd, *J* = 12.5, 7.6), 2.45 (1H, t, *J* = 11.9), 2.47 (1H, m), 3.27 (1H, m), 3.75 (1H, dd, *J* = 17.1, 4.6), 3.99 (1H, m), 4.08 (1H, d, *J* = 9.8), 4.49 (1H, m), 4.55 (1H, d, *J* = 14.6), 4.67 (1H, d, *J* = 14.6), 4.82 (1H, dd, *J* = 10.1, 8.2), 5.79 (1H, m), 7.31 (6H, m), 8.17 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 25.3, 28.2, 28.3, 28.4, 41.0, 42.8, 43.1, 46.7, 54.4, 59.7, 60.4, 66.5, 79.6, 128.2, 128.7, 128.8, 135.7, 155.5, 156.5, 169.5, 171.4 HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{25}H_{35}N_5O_6$ Na 524.2480, found 524.2487

Compound 45 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Compound **45** was synthesized using General procedure 4, Boc-Gly-OH (15 mmol), HOAt (30 mmol), EDC (15 mmol), DIPEA (30 mmol), and compound **39 –** Purified yield 3.64 g (86% Yield); ^{1}H NMR (500 MHz, $CDCl_3$) 0.25 (2H, d, *J* = 4.6), 0.44 (2H, m), 1.04 (1H, m), 1.33 (9H, s, rotameric), 2.07 (1H, m), 2.45 (1H, t, *J* = 11.9), 3.22 (1H, dd, *J* = 14.0, 7.3), 3.33 (1H, dd, *J* = 14.0, 7.0), 3.83 (1H, dd, *J* = 17.1, 5.2), 3.92 (1H, m), 4.11 (1H, m), 4.34 (2H, m), 4.62 (1H, dd, *J* = 15.4, 6.6), 4.89 (1H, m), 5.82 (1H, s), 7.42 (2H, d, *J* = 8.2), 7.54 (2H, d, *J*, = 8.2), 8.08 (1H, s), 8.30 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 4.1, 10.1, 21.1, 25.3, 28.2, 42.9, 43.2, 43.8, 54.8, 60.1, 60.4, 66.3, 79.9, 123.0, 125.2, 125.4, 125.5, 127.6, 129.4, 129.7, 142.2, 156.1, 156.6, 170.3, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{26}H_{32}F_3N_5O_6$ Na 590.2197, found 590.2196

Compound 46 - *tert*-butyl (2-((5*R*,8*S*)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Compound **42** was synthesized using General procedure 4, Boc-Gly-OH (30 mmol), HOAt (60 mmol), EDC (30 mmol), DIPEA (60 mmol), and compound **40** - Purified yield 6.82 g (91%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.82 (6H, d, rotameric, *J* = 6.7), 1.33 (9H, s, rotameric), 1.96 (1H, m),

2.02 (1H, m), 2.44 (1H, t, *J* = 11.9), 3.12 (1H, dd, *J* = 13.1, 8.2), 3.29 (1H, dd, *J* = 13.4, 6.7), 3.78 (1H, dd, *J* = 17.1, 5.2), 3.92 (1H, m), 4.07 (1H, m), 4.17 (1H, m), 4.56 (1H, dd, *J* = 15.1, 7.2), 4.86 (1H, m), 5.81 (1H, s), 7.26 (5H, m), 7.85 (1H, m), 8.36 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 19.7, 19.8, 25.3, 27.1, 28.2, 40.7, 43.1, 43.3, 45.8, 54.5, 59.9, 66.1, 79.7, 127.3, 127.3, 128.5, 138.0, 156.0, 156.5, 169.9, 170.7, 171.7; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{25}H_{35}N_5O_6$ Na 524.2480, found 524.2487

Compound 47 - *tert*-butyl (2-((5*R*,8*S*)-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Compound **42** was synthesized using General procedure 4, Boc-Gly-OH (15 mmol), HOAt (30 mmol), EDC (15 mmol), DIPEA (30 mmol), and compound **41** – Purified Yield 3.50 g (82%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.84 (6H, d, rotameric, *J* = 6.7), 1.32 (9H, s), 2.00 (2H, m), 2.16 (1H, s), 2.46 (1H, t, *J* = 11.9), 3.17 (1H, dd, *J* = 13.1, 7.9), 3.31 (1H, dd, *J* = 13.4, 7.0), 3.81 (1H, dd, *J* = 16.9, 5.2), 4.08 (1H, d, *J* = 10.7), 4.32 (2H, m), 4.57 (1H, dd, *J* = 15.6, 6.4), 4.88 (1H, m), 5.72 (1H, s), 7.4 (2H, d, *J* = 8.2), 7.54 (2H, d, *J* = 8.2), 7.99 (1H, m), 8.30 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 19.7, 19.8, 27.2, 28.2, 31.0, 40.3, 42.9, 43.3, 46.0, 54.9, 60.1, 66.1, 80.0, 123.0, 125.1, 125.4, 125.5, 127.5, 129.5, 129.7, 142.2, 156.1, 156.6, 170.4, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{26}H_{34}F_3N_5O_6$ Na 592.2353, found 592.2354

General Procedure 5: Alkylation of 42-47 to Synthesize 2-15

To a stirred mixture of one of **42-47** in DMF [100 mM] was added 1.05 equiv of allyl or benzyl halide along with 1.5 equiv of K_2CO_3. The reaction proceeded at room temperature for 14-24 h, and the progress checked via LCMS. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with Na_2SO_4, and concentrated *in vacuo* to yield **5-18** as tan to yellow foamy solids. UHPLC (C18, 5-100% ACN in Water) and ^{1}H / ^{13}C NMR spectra are available in the supporting information.

Spiroligomer Amine 5 - *tert*-butyl (2-((5*R*,8*S*)-1-benzyl-8-(benzylcarbamoyl)-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate – Spiroligomer Amine **5** was synthesized using General Procedure 5, compound **46**, and benzyl bromide (1.05 equiv). Purified yield 1.10 g (93%). ^{1}H NMR (500 MHz, $CDCl_3$) 0.94 (6H, d, *J* = 6.7), 1.39 (9H, s, rotameric), 2.16 (1H, m), 2.36 (1H, dd, *J* = 14.5, 9.0), 2.44 (1H, dd, *J* = 17.1, 4.3), 3.03 (1H, dd, *J* = 14.6, 8.5), 3.27 (1H, dd, *J* = 16.9, 5.3), 3.4 (1H, t, *J* = 3.5), 3.44 (2H, d, rotameric, *J* = 7.6), 3.80 (1H, d, *J* = 11.6), 4.05 (1H, d, *J* = 16.2), 4.37 (1H, d, rotameric, *J* = 5.5), 4.46 (1H, d, rotameric, *J* = 6.4), 4.76 (1H, t, *J* = 8.7), 5.00 (1H, t, *J* = 4.4), 5.04 (1H, d, *J* = 16.2), 7.07 (1H, t, *J* = 5.8), 7.18 (2H, d, *J* = 7.3), 7.25 (3H, m), 7.33 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 20.0, 21.0, 24.8, 24.9, 27.3, 28.3, 36.5, 42.7, 43.7, 44.1, 46.8, 54.6, 60.0, 63.8, 69.3, 80.0, 80.8, 120.7, 127.1, 127.4, 127.5, 128.3, 128.7, 129.1, 129.3, 137.0, 137.8, 151.3, 156.1, 168.3, 169.0, 171.5 ; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{41}N_5O_6Na$ 614.2949, found 614.2955

Spiroligomer Amine 6 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-1-(3,4-dichlorobenzyl)-8-(isobutylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **6** was synthesized using General Procedure 5, compound **44**, and 3,4-dichlorobenzyl bromide (1.05 equiv). Purified yield 1.16 g (88%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.87 (6H, d, *J* = 6.7), 1.43 (9H, s, rotameric), 1.75 (1H, m), 2.24 (1H, ddd, *J* = 14.7, 9.1, 1.2), 2.96 (1H, dd, *J* = 17.1, 4.6), 3.05 (3H, m), 3.45 (1H, dd, *J* = 17.1, 5.2), 3.52 (1H, d, *J* = 11.6), 3.84 (1H, d, *J* = 11.9), 4.21 (1H, d, *J* = 16.2), 4.63 (1H, t, *J* = 8.5), 4.75 (3H, m), 5.14 (1H, t, *J* = 4.7), 6.70 (1H, t, *J* = 5.5), 7.06 (1H, d, *J* = 6.4), 7.36 (7H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 28.3, 28.4, 36.0, 43.1, 43.2, 43.3, 47.2, 54.5, 59.7, 69.5, 80.2, 126.6, 128.3, 128.6, 128.7, 128.9, 129.1, 129.2, 130.0, 131.1, 132.6, 133.3, 135.2, 135.4, 137.2, 151.7, 155.7, 168.2, 168.6, 170.9 ; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{39}Cl_2N_5O_6Na$ 682.2170, found 682.2161

Spiroligomer Amine 7 - *tert*-butyl (2-((5*R*,8*S*)-1-allyl-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **7** was synthesized using General Procedure 5, compound **47**, and allyl

bromide (1.05 equiv). Purified yield 804 mg (92%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.80 (6H, d, *J* = 6.7), 1.39 (9H, s, rotameric), 2.07 (1H, m), 2.44 (1H, dd, *J* = 14.3, 9.5), 2.95 (1H, dd, *J* = 14.5, 7.5), 3.36 (2H, d, rotameric, *J* = 7.3), 3.82 (4H, m), 3.97 (1H, d, *J* = 1.16), 4.14 (1H, dd, rotameric, *J* = 16.5, 4.9), 4.46 (1H, d, rotameric, *J* = 5.8), 4.53 (1H, d, rotameric, *J* = 6.1), 4.83 (1H, t, *J* = 8.2), 5.18 (2H, m), 5.32 (1H, t, *J* = 4.6), 5.84 (1H, dddd, *J* = 16.9, 10.5, 6.5, 5.2), 7.32 (1H, t, *J* = 6.0), 7.40 (2H, d, *J* = 8.2), 7.57 (2H, d, *J* = 7.9); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 27.3, 28.2, 36.1, 43.1, 43.6, 46.6, 54.3, 60.2, 68.8, 80.5, 118.1, 125.5, 125.6, 127.7, 133.4, 142.1, 156.3, 156.0, 168.7, 169.5, 172.1; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{29}H_{38}F_3N_5O_6Na$ 632.2666, found 632.2655

Spiroligomer Amine 8 - *tert*-butyl (2-((5*R*,8*S*)-1-(2-amino-2-oxoethyl)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **8** was synthesized using General Procedure 5, compound **47**, and 4-(trifluoromethyl)benzyl bromide (1.05 equiv). Purified yield 770 mg (82%); ^{1}H $CDCl_3$ (500 MHz, $CDCl_3$) 0.87 (6H, t, *J* = 6.7), 1.31 (9H, s, rotameric), 2.02 (1H, tq, *J* = 13.4, 6.6), 2.16 (1H, m), 2.47 (1H, dd, *J* = 13.3, 9.6), 3.32 (2H, d, *J* = 7.3), 3.81 (4H, m), 4.19 (1H, d, *J* = 11.3), 4.42 (3H, m), 4.83 (1H, m), 5.88 (1H, s), 6.62 (1H, s), 7.36 (2H, d, *J* = 7.6), 7.51 (2H, d, *J* = 7.9), 7.83 (1H, s, rotameric), 8.23 (1H, s, rotameric); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.6, 9.9, 19.8, 25.3, 27.3, 28.2, 28.3, 37.2, 42.6, 42.9, 43.7, 46.8, 54.9, 60.1, 64.4, 69.1, 80.7, 122.0, 122.9, 125.1, 125.4, 127.5, 127.7, 129.6, 155.8, 157.0, 170.0, 170.3, 170.9; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{28}H_{37}F_3N_6O_7Na$ 649.2568, found 649.2566

Spiroligomer Amine 9 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,4-dichlorobenzyl)-3-isobutyl-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **9** was synthesized using General Procedure 5, compound **47**, and 3,4-Dichlorobenzyl bromide (1.05 equiv). Purified yield 971 mg (89%). ^{1}H NMR (500 MHz, $CDCl_3$) 0.93 (6H, d, *J* = 7.0), 1.37 (9H, s, rotameric), 2.12 (1H, m), 2.35 (1H, dd, *J* = 14.2, 9.6), 2.52 (1H, q, *J* = 7.3), 2.98 (1H, m), 3.43 (3H, m), 3.53 (1H, m), 3.90 (1H, d, *J* =

11.6), 4.23 (1H, d, *J* = 16.5), 4.41 (1H, d, rotameric, *J* = 5.8), 4.50 (1H, d, rotameric, *J* = 6.1), 4.74 (1H, t, *J* = 8.4), 4.82 (1H, d, *J* = 16.2), 5.17 (1H, t, *J* = 4.9), 7.09 (1H, dd, *J* = 8.2, 1.8), 7.34 (4H, m), 7.44 (1H, m), 7.56 (1H, d, *J* = 7.9); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 19.9, 27.3, 28.2, 28.3, 36.3, 43.0, 43.1, 43.2, 46.8, 46.9, 54.5, 56.5, 59.9, 69.2, 80.5, 123.0, 125.6, 126.6, 127.6, 128.1, 129.2, 130.1, 130.6, 131.2, 135.6, 133.3, 137.4, 142.0, 156.0, 156.1, 168.5, 169.2, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{33}H_{38}Cl_2F_3N_5O_6Na$ 750.2043, found 750.2042

Spiroligomer Amine 10 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,5-bis(trifluoromethyl)benzyl)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **10** was synthesized using General Procedure 5, compound **42**, and 3,5-bis(trifluoromethyl)benzyl bromide (1.05 equiv). Purified yield 1.88 g (97%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.85 (6H, d, *J* = 6.7), 1.41 (9H, s, rotameric), 1.73 (1H, dquin, *J* = 13.5, 6.8), 2.09 (1H, dd, *J* = 14.5, 9.0), 3.01 (2H, m), 3.12 (1H, dd, *J* = 14.8, 7.8), 3.19 (1H, dd, *J* = 17.1, 5.2), 3.42 (1H, dd, *J* = 17.1, 4.9), 3.64 (1H, d, *J* = 11.6), 3.88 (1H, d, *J* = 11.9) 4.53 (1H, t, *J* = 8.2), 4.61 (1H, d, *J* = 16.8), 4.78 (1H, d, *J* = 16.5), 4.93 (2H, m), 5.14 (1H, t, *J* = 4.7), 6.71 (1H, t, *J* = 5.8), 7.50 (3H, m), 7.82 (7H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.0, 20.1, 28.2, 28.3, 35.8, 43.0, 43.5, 43.6, 47.2, 54.3, 59.4, 69.5, 80.3, 122.3, 122.9, 124.0, 126.1, 126.4, 126.5, 127.5, 127.7, 128.0, 128.9, 132.4, 132.6, 132.7, 133.1, 133.3, 139.9, 155.8, 156.0, 168.3, 168.4, 170.9; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{38}H_{41}F_6N_5O_6Na$ 800.2853, found 800.2864

Spiroligomer Amine 11 - *tert*-butyl (2-((5*R*,8*S*)-1-allyl-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **11** was synthesized using General Procedure 5, compound **42**, and allyl bromide (1.05 equiv). Purified yield 1.41 g (96%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.89 (6H, d, *J* = 6.7), 1.42 (9H, s, rotameric), 1.78 (1H, m), 2.34 (1H, dd, *J* = 14.5, 9.0), 3.06 (3H, m), 3.73 (2H, m), 3.84 (2H, m), 3.91 (1H, d, *J* = 11.6), 4.10 (1H, dd, *J* = 16.5, 5.2), 4.72 (1H, t, *J* = 8.4), 4.84 (2H, m), 5.15 (2H,

m), 5.30 (1H, t, *J* = 4.4), 5.81 (1H, ddd, *J* = 16.5, 11.6, 6.3), 6.73 (1H, t, *J* = 5.8), 7.48 (3H, m), 7.82 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 28.3, 28.4, 36.1, 43.3, 43.6, 47.2, 54.4, 60.1, 69.2, 80.2, 118.2, 126.3, 126.4, 127.7, 128.0, 128.7, 133.0, 133.2, 133.3, 154.9, 155.8, 168.3, 168.9, 171.5; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{41}N_5O_6Na$ 614.2949, found 614.2951

Spiroligomer Amine 12 - methyl 2-((5*R*,8*S*)-7-((*tert*-butoxycarbonyl)glycyl)-8-(isobutylcarbamoyl)-3-(naphthalen-2-ylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-1-yl)acetate - Spiroligomer Amine **12** was synthesized using General Procedure 5, compound **42**, and methyl-bromoacetate (1.05 equiv). Purified yield 1.44 g (93%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.88 (6H, d, *J* = 6.7), 1.41 (9H, s), 1.78 (1H, m), 2.41 (1H, dd, *J* = 14.5, 9.0), 2.99 (1H, m), 3.07 (2H, m), 3.73 (3H, s), 3.77 (2H, m), 3.90 (2H, m), 4.09 (1H, m), 4.34 (1H, d, *J* = 18.3), 4.68 (1H, t, *J* = 8.5), 4.86 (2H, m), 5.24 (1H, t, *J* = 4.3), 6.76 (1H, t, *J* = 5.8), 7.48 (3H, m), 7.82 (4H, m),; ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.1, 20.2, 28.3, 28.4, 36.0, 41.9, 43.4, 47.1, 52.9, 54.5, 59.6, 69.4, 80.2, 126.2, 126.3, 126.4, 127.7, 127.9, 128.1, 128.8, 132.7, 133.0, 133.2, 155.2, 155.9, 168.8, 168.9, 169.4, 170.9; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{41}N_5O_8Na$ 646.2847, found 646.2848

Spiroligomer Amine 13 - *tert*-butyl (2-((5*R*,8*S*)-3-(cyclopropylmethyl)-1-(4-methoxybenzyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **13** was synthesized using General Procedure 5, compound **45**, and 4-methoxybenzyl chloride (1.05 equiv). Purified yield 1.58 g (92%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.39 (2H, m), 0.55 (2H, m), 1.21 (1H, m), 1.35 (9H, s), 2.45 (1H, dd, *J* = 14.5, 9.3), 2.63 (1H, dd, *J* = 16.8, 4.3), 2.97 (1H, dd, *J* = 14.6, 7.9), 3.34 (1H, dd, *J* = 16.8, 5.5), 3.47 (3H, m), 3.78 (3H, s), 3.83 (1H, d, *J* = 11.6), 4.05 (1H, d, *J* = 15.9), 4.42 (1H, d, rotameric, *J* = 5.8), 4.51 (1H, d, rotameric, *J* = 6.1), 4.81 (1H, t, *J* = 8.5), 4.97 (1H, d, *J* = 16.2), 5.04 (1H, t, *J* = 4.7), 6.87 (2H, d, *J* = 8.5), 7.13 (2H, d, *J* = 8.5), 7.29 (1H, t, *J* = 5.8), 7.37 (2H, d, *J* = 8.2), 7.56

(2H, d, *J* = 8.2); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present)3.8, 3.9, 10.1, 28.2, 28.3, 36.3, 43.0, 43.1, 43.5, 44.4, 54.6, 55.4, 60.2, 69.4, 80.2, 114.4, 125.5, 125.6, 127.6, 128.5, 128.8, 142.1, 155.8, 155.9, 159.5, 168.5, 169.5, 171.7; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{34}H_{40}F_3N_5O_7Na$ 710.2772, found 710.2778

Spiroligomer Amine 14 - *tert*-butyl (2-((5*R*,8*S*)-1-(3,5-bis(trifluoromethyl)benzyl)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **14** was synthesized using General Procedure 5, compound **45**, and 3,5-bis(trifluoromethyl)benzyl bromide (1.05 equiv). Purified yield 1.74 g (88%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.39 (2H, m), 0.56 (2H, m), 1.22 (1H, m), 1.38 (9H, s, rotameric), 2.22 (1H, dd, *J* = 14.3, 9.2), 3.04 (1H, dd, *J* = 14.6, 7.3), 3.21 (1H, dd, *J* = 16.8, 5.2), 3.46 (1H, dd, *J* = 16.9, 5.2) 3.49 (2H, d, *J* = 7.3), 3.67 (1H, d, *J* = 11.6), 3.93 (1H, d, *J* = 11.9), 4.42 (1H, d, rotameric, *J* = 5.8), 4.47 (1H, d, rotameric, *J* = 6.1), 4.60 (1H, d, *J* = 16.8), 4.66 (1H, t, *J* = 8.2), 4.83 (1H, d, *J* = 16.8), 5.16 (1H, t, *J* = 5.0), 7.29 (1H, t, *J* = 5.5), 7.35 (2H, d, rotameric, *J* = 7.9), 7.56 (2H, d, *J* = 7.9), 7.72 (2H, s, rotameric), 7.84 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.8, 10.0, 28.1, 35.9, 43.0, 43.2, 43.3, 44.6, 54.2, 59.6, 69.3, 80.5, 121.8, 122.3, 123.0, 124.0, 125.5, 125.6, 127.4, 127.6, 128.2, 132.4, 132.7, 140.0, 142.0, 156.0, 156.3, 168.6, 169.0, 171.3; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{35}H_{36}F_9N_5O_6Na$ 816.2414, found 816.2412

Spiroligomer Amine 15 methyl 2-((5*R*,8*S*)-7-((*tert*-butoxycarbonyl)glycyl)-3-(cyclopropylmethyl)-2,4-dioxo-8-((4-(trifluoromethyl)benzyl)carbamoyl)-1,3,7-triazaspiro[4.4]nonan-1-yl)acetate - Spiroligomer Amine **15** was synthesized using General Procedure 5, compound **45**, and methyl bromoacetate (1.05 equiv). Purified yield 1.42 g (89%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.35 (2H, m), 0.52 (2H, m), 1.15 (1H, m), 1.36 (9H, s, rotameric), 2.50 (1H, dd, *J* = 14.3, 9.2), 2.94 (1H, dd, *J* = 14.6, 7.6), 3.42 (2H, d, *J* = 7.3), 3.72 (1H, m), 3.72 (3H, s), 3.83 (3H, m), 3.95 (1H, d, *J* = 11.6), 4.15 (1H, *J* = 11.3), 4.40 (1H, d, *J* = 18.3), 4.46 (1H, d, rotameric, *J* = 5.8), 4.52 (1H, d, rotameric, *J* = 6.1), 4.79 (1H, t, *J* = 8.5), 5.28 (1H, t, *J* = 5.0), 7.39

(2H, d, *J* = 7.9), 7.57 (2H, d, *J* = 8.2); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.8, 3.9, 10.0, 28.2, 35.9, 41.8, 43.1, 43.6, 44.4, 52.9, 54.5, 59.7, 69.1, 80.5, 123.6, 125.2, 125.5, 125.6, 127.6, 128.2, 142.1, 155.5, 156.2, 169.2, 169.5, 169.6, 171.4; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{29}H_{36}F_3N_5O_8Na$ 662.2408, found 662.2415

Spiroligomer Amine 16 - *tert*-butyl (2-((5*R*,8*S*)-1-([1,1'-biphenyl]-4-ylmethyl)-3-benzyl-8-(cyclopropylcarbamoyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **16** was synthesized using General Procedure 5, compound **43**, and 4-(bromomethyl)biphenyl (1.05 equiv). Purified yield 1.51 g (93%); ^{1}H NMR (500 MHz, $CDCl_3$) 0.50 (2H, m), 0.71 (2H, m), 1.34 (9H, s, rotameric), 2.38 (1H, dd, *J* = 14.2, 9.0), 2.58 (1H, dd, *J* = 16.8, 4.3), 2.66 (1H, tq, *J* = 7.2, 3.7), 2.96 (1H, dd, *J* = 14.4, 8.6), 3.32 (1H, dd, *J* = 16.8, 5.5), 3.45 (1H, d, *J* = 11.6), 3.79 (1H, d, *J* = 11.3), 4.13 (1H, d, *J* = 16.2), 4.68 (1H, t, *J* = 8.5), 4.76 (2H, m), 4.96 (1H, t, *J* = 4.9), 5.01 (1H, d, *J* = 16.2), 6.84 (1H, m), 7.25 (2H, d, *J* = 7.9), 7.35 (4H, m), 7.42 (4H, m), 7.55 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 28.2, 28.3, 36.3, 43.0, 43.3, 43.8, 54.5, 60.0, 69.5, 80.1, 126.8, 127.0, 127.7, 128.2, 128.5, 128.6, 128.7, 128.9, 135.6, 135.7, 140.0, 141.3, 155.6, 155.7, 168.2, 170.4, 171.2; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{37}H_{41}N_5O_6Na$ 674.2949, found 674.2935

Spiroligomer Amine 17 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-1-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **17** was synthesized using General Procedure 5, compound **43**, and 4-methoxybenzyl chloride (1.05 equiv). Purified yield 1.39 g (92%). ^{1}H NMR (500 MHz, $CDCl_3$) 0.50 (2H, m), 0.71 (2H, m), 1.42 (9H, s, rotameric), 2.33 (1H, dd, *J* = 14.5, 9.0), 2.61 (1H, dd, *J* = 16.8, 4.3), 2.67 (1H, tq, *J* = 7.2, 3.7), 2.93 (1H, dd, *J* = 14.7, 8.2), 3.31 (1H, dd, *J* = 16.8, 5.2), 3.39 (1H, d, *J* = 11.6), 3.76 (3H, s) 3.77 (1H, m), 4.03 (1H, d, *J* = 15.9), 4.65 (1H, t, *J* = 8.5), 4.73 (2H, m), 4.91 (1H, d, *J* = 16.2), 5.08 (1H, t, *J* = 4.6), 6.84 (3H, m), 7.09 (2H, d, *J* = 8.5), 7.34 (3H, m), 7.4 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 28.3, 2=36.2, 43.0, 43.2, 43.5, 54.4, 55.3, 60.0, 69.5, 80.0, 114.4, 128.2, 128.5, 128.7, 128.9, 135.6, 155.6, 159.5, 168.2,

170.4, 171.3; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{39}N_5O_7Na$ 628.2742, found 628.2734

Spiroligomer Amine 18 - *tert*-butyl (2-((5*R*,8*S*)-3-benzyl-8-(cyclopropylcarbamoyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)-2-oxoethyl)carbamate - Spiroligomer Amine **18** was synthesized using General Procedure 5, compound **43**, and iodomethane(1.05 equiv). Purified yield 1.09 g (88%). ^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 0.54 (2H, m), 0.75 (2H, m), 1.43 (9H, s), 2.35 (1H, dd, *J* = 14.3, 9.2), 2.66 (1H, d, *J* = 7.3), 2.70 (1H, tq, *J* = 7.2, 3.7), 2.89 (3H, s), 3.76 (2H, m), 3.90 (2H, m), 1.94 (2H, s), 4.72 (1H, m), 5.40 (1H, t, *J* = 4.7), 6.83 (1H, m), 7.32 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 6.3, 6.4, 22.8, 25.7, 28.3, 35.2, 42.9, 43.4, 53.3, 59.8, 68.7, 80.3, 128.1, 128.4, 128.6, 128.8, 135.6, 154.8, 156.0, 168.6, 170.5, 171.6; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{25}H_{33}N_5O_6Na$ 522.2323, found 522.2325

Synthesis of the spiroligomer trimer 4.21

Compound 20 - 7-benzyl 8-(tert-butyl) (5R,8S)-1-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7,8-dicarboxylate

To a stirred mixture of **1** [(2S, 4R) 5.0 mmol] in DMF [100 mM] was added 2-iodo-1-methyl-propane (1.1 equiv) along with K_2CO_3 (2.0 equiv). The reaction proceeded at room temperature for 24 h, and the progress checked via LCMS. After completion, benzyl bromide (1.0 equiv) was added and the reaction stirred for another 24 hours. The reaction was diluted with four times the reaction volume of EtOAc and washed with water, saturated ammonium chloride solution, and brine. The organic layer was dried with Na_2SO_4, and concentrated *in vacuo* to yield **20 (**2.21 g purified, 84%) as a foamy off-white solid; ^{1}H NMR (500 MHz, $CDCl_3$); 0.93 (6H, d, *J* = 6.7), 1.42 (9H, s, rotameric), 2.11 (1H, dq, *J* = 13.5, 7.0), 2.38 (1H, dd, *J* = 9.2, 1.5, rotameric), 2.44 (1H, t, *J* = 14.4, rotameric), 3.39 (2H, t, *J* = 7.2), 3.72-3.88 (2H, m), 4.35 (1H, m), 4.67-4.86 (2H, m), 5.06 (2H, m), 7.15-7.37 (10H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present); 19.9, 27.8, 27.9, 38.2, 39.4, 44.2, 46.6, 54.3, 58.8, 59.5, 67.4, 68.3, 69.2, 82.2, 127.0, 127.2, 127.3, 128.0, 128.1,

128.2, 128.3, 128.4, 128.5, 128.8, 128.9, 136.0, 136.9, 153.7, 156.0, 169.7, 172.1; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{30}H_{37}N_3O_6Na$ 558.2575, found 558.2574

Compound 52 - (5*R*,8*S*)-1-benzyl-3-isobutyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

Compound **20** was reacted with 25 mL of 1:1 DCM/(33% HBr in AcOH) for 30 min, and the reaction progress checked via LCMS. Upon successful deprotection of the Cbz group, the solvent was removed *in vacuo* with the aid of toluene, and placed on a high vacuum pump overnight to afford **52.** Recovered yield was quantitative and the product used without purification; ^{1}H NMR (500 MHz, $CDCl_3$) 0.85 (6H, d, *J* = 6.7), 1.97 (1H, dquin, *J* = 13.7, 7.0), 2.11 (1H, dd, *J* = 14.0, 7.6), 2.21-2.25 (1H, m), 3.04 (1H, d, *J* = 11.9), 3.21 (1H, d, *J* = 11.9), 3.25 (1H, d, *J* = 7.3), 3.76 (1H, dd, *J* = 8.5, 7.6), 4.55-4.67 (2H, m), 7.26-7.37 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$) 20.3, 27.5, 38.6, 40.5, 43.2, 54.0, 60.3, 70.3, 127.5, 127.7, 129.0, 138.4, 156.2, 173.5, 175.5; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{18}H_{24}N_3O_4$ 346.1761, found 346.1764

Compound 21 (3S,5S)-1-((benzyloxy)carbonyl)-5-(tert-butoxycarbonyl)-3-((4-methoxybenzyl)amino)pyrrolidine-3-carboxylic acid

(3*S*,5*S*)-3-amino-1-((benzyloxy)carbonyl)-5-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (13 mmol) was dissolved in 100 mL of MeOH along with *p*-anisaldehyde (10 mmol) and stirred for 1 hour, after which $NaCNBH_3$ (14 mmol) was added and reacted for 3 hours. After completion, the solvent was removed *in vacuo*, the resulting solid was redissolved in deionized (DI) H_2O, and the amino acid precipitated upon neutralization with dropwise addition of 2M HCl. Precipitate was collected by vacuum filtration and dried on a lyophilizer (3.72 g (7.68 mmol) recovered, 77% yield); ^{1}H NMR (500 MHz, DMSO-d_6) 1.28 (9H, s, rotameric), 1.43 (1H, d, rotameric, *J* = 3.4), 2.09 (1H, ddd, *J* = 18.1, 12.6, 7.4), 2.65 (1H, ddd, *J* = 20.9, 12.2, 8.2), 3.46 (1H, t, *J* = 10.2), 3.71 (5H, m), 3.92 (1H, dd, *J* = 10.5, 8.9), 4.22 (1H, t, rotameric, *J* = 7.8), 5.06 (2H, m), 6.87 (2H, m), 7.32 (8H, m); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 27.9, 28.1, 28.1, 28.2, 37.5, 38.3, 48.3, 53.9, 54.5, 55.5, 59.4, 59.7, 63.0, 66.5, 66.7, 67.0, 67.9, 80.5, 81.0, 81.2, 114.0, 127.8,

127.9, 128.2, 128.3, 128.4, 128.7, 128.8, 128.9, 130.4, 130.5, 137.0, 137.3, 154.0, 154.3, 159.1, 159.2, 170.9, 171.3, 173.2, 173.3; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{26}H_{34}N_2O_7$ 485.2282, found 485.2287

Compound 22 - 1''-benzyl 5''-(tert-butyl) (3'S,4R,5''S,8a'S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',2,4',5-tetraoxotetrahydro-4'H,6'H-dispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',3''-pyrrolidine]-1'',5''-dicarboxylate

Compound **21 (**700 mg) was preactivated with HOAt [979 mg (6 eq)] and EDC [253 mg (1.1 eq)] in 21 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which all of **52** and DIPEA (0.63 mL) dissolved in 7 mL of DMF were added and reacted overnight with stirring. Subsequently, 575 mg (2.5 eq) of EDC-HCl were added and the reaction stirred for another 4 hours. The reaction was diluted with EtOAc, and washed with saturated solutions of NH_4Cl, $NaHCO_3$, and brine, dried over Na_2SO_4, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 478 mg (46% yield) of **22**; ^1H NMR (500 MHz, $CDCl_3$); 0.96 (6H, d, *J* = 6.7), 1.47 (9H, rotameric), 2.08-2.19 (2H, m), 2.53 (1H, dd, *J* = 14.2, 8.7), 2.55-2.59 (1H, m), 2.89 (1H, dd, *J* = 14.3, 7.6), 3.40-3.42 (1H, m), 3.43 (2H, dd, *J* = 7.5, 0.8), 3.78 (3H, s), 3.85 (1H, t, *J* = 10.7), 4.07 (1H, dd, *J* = 13.6, 2.3), 4.11-4.16 (1H, m), 4.18-4.26 (2H, m), 4.40-4.49 (1H, m), 4.65 (1H, d, *J* = 4.1, rotameric), 4.72-4.78 (1H, m), 4.89-5.00 (1H, m), 5.03-5.11 (1H, m), 5.15-5.19 (1H, m), 6.80-6.85 (2H, m), 7.10-7.18 (2H, m), 7.27-7.42 (10H, m) ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 20.0, 27.4, 27.7, 28.0, 36.5, 38.9, 39.3, 43.9, 45.7, 46.6, 50.2, 50.5, 51.8, 55.2, 57.4, 57.6, 57.7, 57.9, 65.66, 65.73, 66.4, 67.3, 67.45, 67.53, 82.3, 114.3, 114.4, 126.9, 127.0, 127.6, 127.9, 128.4, 128.6, 128.9, 129.3, 135.5, 136.2, 136.3, 136.6, 153.9, 154.5, 156.4, 159.0, 165.2, 167.7, 169.9, 170.1, 173.3; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{44}H_{51}N_5O_9Na$ 816.3579, found 816.3589

Compound 53 - (3'S,4R,5''S,8a'S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',2,4',5-tetraoxotetrahydro-4'H,6'H-dispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',3''-pyrrolidine]-5''-carboxylic acid

22 was treated with 1:1 DCM/(33% HBr/AcOH) for 30 minutes, after which the solvent was removed *in vacuo*, and the molecule left on a high vacuum pump overnight to give the deprotected spiroligomer dimer; ^{1}H NMR (500 MHz, DMSO-d_6) 0.88 (6H, dd, *J* = 6.7, 1.8), 1.99 (1H, dquin, *J* = 13.7, 7.0), 2.14 (1H, t, *J* = 12.8), 2.60 (2H, m), 2.79 (1H, dd, *J* = 13.4, 6.7), 3.27 (2H, d, *J* = 7.3), 3.42 (3H, m), 3.53 (1H, dd, *J* = 11.9, 6.7), 3.72 (3H, s), 3.94 (1H, d, *J* = 12.8), 4.43 (1H, d, *J* = 16.8), 4.62 (1H, d, *J* = 16.5), 4.81 (2H, m), 4.96 (1H, d, *J* = 16.5), 6.86 (2H, d, *J* = 8.8), 7.17 (2H, d, *J* = 8.5), 7.28 (1H, m), 7.39 (4H, m); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 20.3, 20.4, 27.5, 35.6, 38.3, 42.9, 44.8, 46.2, 48.1, 51.3, 55.5, 57.0, 59.6, 65.7, 69.5, 114.4, 127.4, 127.9, 129.2, 130.2, 138.4, 156.3, 158.7, 165.5, 168.3, 168.8, 174.8; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{32}H_{37}N_5O_7Na$ 626.2585, found 626.2587

Compound 23 - (3S,5S)-1-((benzyloxy)carbonyl)-5-(tert-butoxycarbonyl)-3-(pentylamino)pyrrolidine-3-carboxylic acid

(3*S*,5*S*)-3-amino-1-((benzyloxy)carbonyl)-5-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (13 mmol) was dissolved in 100 mL of MeOH along with valeraldehyde (10 mmol) and stirred for 1 hour, after which $NaCNBH_3$ (14 mmol) was added and reacted for 3 hours. After completion, the solvent was removed *in vacuo*, the resulting solid was redissolved in DI H_2O, and the amino acid precipitated upon neutralization with dropwise addition of 2M HCl. Precipitate was collected by vacuum filtration and dried on a lyophilizer, 3.56 g (8.21 mmol) recovered, 82% yield; ^{1}H NMR (500 MHz, DMSO-d_6) 0.84 (4H, m), 1.29 (16H, m), 1.46 (1H, m), 2.04 (1H, ddd, rotameric, *J* = 17.1, 12.2, 7.9), 2.62 (2H, m), 3.44 (1H, m), 3.87 (1H, dd, *J* = 10.1, 3.7), 4.28 (1H, t, rotameric, *J* = 8.3), 5.04 (2H, m), 7.33 (5H, m); ^{13}C NMR (125 MHz, DMSO-d_6, rotamers present) 13.8, 13.9, 14.0, 14.3, 19.0, 21.0, 21.8, 22.1, 27.2, 27.3, 27.4, 27.6, 27.7, 27.8, 28.3, 31.5, 31.7, 36.2, 36.9, 44.3, 53.5, 59.0, 59.4, 66.1, 66.2, 67.0, 80.5, 80.7, 127.3, 127.4, 127.7, 127.8, 128.2, 128.3, 128.4, 136.5, 136.8, 153.5, 153.8, 170.5, 170.9, 171.0; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{23}H_{35}N_2O_6$ 435.2490, found 435.2481

Compound 24 - 1'''-benzyl 5'''-(tert-butyl) (3'S,3''S,4R,5'''S,8a'S,8a''S)-3-benzyl-1-isobutyl-2'-(4-methoxybenzyl)-1',1'',2,4',4'',5-hexaoxo-2''-pentyloctahydro-4'H,4''H,6'H,6''H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7''-pyrrolo[1,2-a]pyrazine-3'',3'''-pyrrolidine]-1''',5'''-dicarboxylate

Compound **23** [370 mg (1.2 equiv)] was preactivated with HOAt [563 mg (6 equiv)] and EDC [143 mg (1.1 equiv)] in 15 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which **53** [420 mg (1.0 equiv)] and DIPEA [366 uL (5 equiv)] dissolved in 5 mL of DMF were added and reacted overnight with stirring. Subsequently, EDC [330 mg (2.5 eq)] was added and the reaction stirred for another 4 hours. The reaction was diluted with EtOAc, and washed with saturated solutions of NH_4Cl, $NaHCO_3$, and brine, dried over Na_2SO_4, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 148 mg (21% yield) of **20**; ^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 0.92 (9H, m), 1.29 (10H, m), 1.46 (4H, m), 1.59 (1H, m), 1.98 (1H, m), 2.13 (2H, m), 2.20 (1H, m), 2.61 (3H, m), 2.85 (1H, dd, *J* = 14.3, 7.3), 3.12 (1H, m), 3.38 (1H, d, *J* = 12.8), 3.42 (2H, d, *J* = 7.6), 3.64 (1H, m), 3.75 (3H, m), 3.82 (1H, d, *J* = 11.6), 4.05 (4H, m), 4.25 (2H, m), 4.37 (2H, m), 4.56 (1H, d, *J* = 16.2), 4.83 (1H, d, *J* = 16.5), 4.94 (1H, m), 5.06 (1H, d, *J* = 12.2), 5.19 (2H, m), 6.82 (2H, m), 7.12 (2H, m), 7.33 (10H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 13.9, 14.0, 20.0, 22.2, 22.3, 22.6, 27.4, 27.7, 27.9, 29.0, 29.3, 36.2, 38.7, 39.7, 43.4, 43.7, 45.8, 46.6, 49.5, 51.1, 51.7, 55.2, 55.9, 57.4, 57.7, 65.4, 66.5, 67.5, 67.5, 82.1, 114.5, 127.1, 127.7, 127.9, 128.35, 128.46, 128.48, 128.52, , 128.9, 129.4, 136.0, 136.5, 154.1, 156.3, 159.1, 164.7, 165.5, 166.6, 167.1, 170.5, 173.6; HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{55}H_{67}N_7O_{11}Na$ 1024.4791, found 1024.4784

Compound 54 - (3'S,3''S,4R,5'''S,8a'S,8a''S)-3-benzyl-1'''-((benzyloxy)carbonyl)-1-isobutyl-2'-(4-methoxybenzyl)-1',1'',2,4',4'',5-hexaoxo-2''-pentyloctahydro-4'H,4''H,6'H,6''H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7''-pyrrolo[1,2-a]pyrazine-3'',3'''-pyrrolidine]-5'''-carboxylic acid

Spiroligomer trimer **24** was treated with a 95:4:1 TFA/H_2O/TIPS mixture to remove the *t*-Bu protecting group and afford compound **54;** ^{1}H NMR (500 MHz, $CDCl_3$) 0.85 (3H, m), 0.93 (6H, d,

J = 6.6), 1.26 (6H, m), 1.56 (1H, m), 2.12 (1H, tt, *J* = 13.7, 6.9), 2.22 (2H, m), 2.55 (1H, m), 2.65 (2H, m), 2.80 (1H, dd, *J* = 13.2, 6.6), 3.06 (1H, t, *J* = 11.2), 3.40 (3H, m), 3.63 (1H, m), 3.74 (3H, m), 3.85 (1H, m), 4.02 (3H, m), 4.18 (1H, m), 4.26 (1H, m), 4.37 (3H, m), 4.59 (1H, m), 4.70 (1H, m), 4.91 (1H, m), 5.14 (2H, m), 6.80 (2H, m), 7.09 (2H, d, *J* = 8.2), 7.32 (10 H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 10.2, 11.7, 12.2, 13.9, 14.1, 17.3, 17.6, 19.4, 19.94, 19.96, 22.17, 22.22, 27.4, 28.9, 29.3, 36.1, 38.0, 38.2, 43.6, 45.9, 46.6, 49.5, 51.5, 55.2, 56.0, 57.3, 57.6, 65.5, 66.6, 67.4, 68.1, 114.4, 127.1, 127.2, 127.4, 127.6, 127.67, 127.72, 128.2, 128.3, 128.4, 128.6, 128.9, 129.3, 135.8, 136.6, 155.6, 156.4, 159.1, 164.5, 165.6, 167.3, 167.6, 173.8**;** HRMS (ESI/Q-TOF) m/z: $(M+Na)^+$ calcd for $C_{51}H_{59}N_7O_{11}Na$ 968.4165, found 968.4133

Compound 25 - benzyl (3'S,3''S,4R,5'''S,8a'S,8a''S)-3-benzyl-5'''-((2-((tert-butoxycarbonyl)amino)ethyl)carbamoyl)-1-isobutyl-2'-(4-methoxybenzyl)-1',1'',2,4',4'',5-hexaoxo-2''-pentyloctahydro-4'H,4''H,6'H,6''H-trispiro[imidazolidine-4,7'-pyrrolo[1,2-a]pyrazine-3',7''-pyrrolo[1,2-a]pyrazine-3'',3'''-pyrrolidine]-1'''-carboxylate

All of **54** was preactivated with HOAt [82 mg (4 eq)] and EDC [400 mg (2 eq)] in 6.0 mL of anhydrous 2:1 DCM/DMF for 1.5 h, after which N-Boc-Ethylenediamine [143 uL (5 eq)] was added and reacted overnight with stirring. The reaction was diluted with EtOAc, and washed with saturated solutions of NH_4Cl, $NaHCO_3$, and brine, dried over Na_2SO_4, and rotovapped. The foamy solid was then purified via normal phase flash chromatography to afford 127 mg (46% yield) of **25**; ^{1}H NMR (500 MHz, $CDCl_3$) 0.87 (3H, t, *J* = 6.8), 0.92 (6H, d, *J* = 6.6), 1.30 (6H, m), 1.40 (9H, s), 1.57 (1H, m), 2.11 (1H, dt, *J* = 13.7, 7.0), 2.18 (1H, dd, *J* = 12.9, 11.3), 2.40 (1H, dd, *J* = 11.7, 7.3), 2.57 (3H, m), 2.82 (1H, dd, *J* = 14.0, 7.7), 3.12 (5H, m), 3.37 (4H, m), 3.68 (5H, m), 4.01 (3H, m), 4.10 (1H, m), 4.23 (2H, d, *J* = 16.4), 4.35 (2H, m), 4.56 (1H, d, *J* = 16.1), 4.77 (1H, d, *J* = 16.4), 4.93 (1H, m), 5.17 (2H, m), 6.79 (2H, t, *J* = 9.5), 7.08 (2H, d, *J* = 8.2), 7.18 (1H, s, broad), 7.32 (10H, m); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 14.0, 20.0, 22.3, 25.3, 28.4, 28.9, 19.4, 36.3, 38.6, 40.2, 43.4, 43.8, 45.8, 46.6, 49.4, 50.9, 51.8, 55.2, 55.9, 57.5, 58.9, 65.5, 66.6, 67.4, 67.9, 79.3, 114.4, 127.1, 127.2, 127.7, 127.9, 128.2, 128.49, 128.54, 128.9, 129.4, 136.0,

136.6, 156.0, 156.3, 159.1, 165.1, 165.3, 166.7, 167.3, 170.7, 173.6; HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{58}H_{74}N_9O_{12}$ 1088.5451, found 1088.5495

Peptoid Synthesis

Peptoid SPH-1

54 mg of Rink amide (0.63 mmol / g) resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **7** (3 equiv, previously deprotected) in DMF was added to the resin and reacted overnight with stirring. The bromoacetic acid addition and subsequent amine additions were repeated for amines **14, 13, 11,** and **16**. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. **SPH-1** was purified by reverse phase flash chromatography, recovered 59 mg (55% yield) HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{152}H_{161}F_{15}N_{26}O_{26}$ 3049.1676; Found: 3049.1543

Peptoid SPH-2

60 mg of Rink amide (0.63 mmol / g) resin was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which N-(Z)-Ethylenediamine-HCl (10 equiv) in DMF (freebased with DIPEA) was reacted with the resin for 1 h. The resin was drained and washed repeatedly with DCM and DMF, followed by standard peptoid submonomer synthesis with 150 mM **11** (3 equiv, previously deprotected) in DMF as the amine, and stirred overnight. The resin was subsequently rinsed with DMF and DCM, and then the next round of peptoid coupling proceeded as the first, except with the use amine **12** (3 equiv, previously deprotected). Another round of peptoid coupling follows,

with the use of N-(Z)-Ethylenediamine-HCl (10 equiv) as the amine for 1 h. The bromoacetic acid addition and subsequent amine additions were repeated for amines **13, 14, 16,** and **17**, with the N-(Z)-Ethylenediamine-HCl amine residue after every two spiroligomer amine additions. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. **SPH-2** was purified by reverse phase flash chromatography, recovered 102 mg (59% yield) HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{220}H_{235}F_{12}N_{37}O_{43}$ 4310.7148; Found: 4310.6912

Peptoid SPH-3

60 mg of Rink amide resin (0.63 mmol / g) was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which N-(Z)-Ethylenediamine-HCl (10 equiv) in DMF (freebased with DIPEA) was reacted with the resin for 1 h. The resin was drained and washed repeatedly with DCM and DMF, followed by standard peptoid submonomer synthesis with 150 mM **11** (3 equiv, previously deprotected) in DMF was added to the resin and reacted overnight with stirring. The resin was subsequently rinsed with DMF and DCM, and then the next round of peptoid coupling proceeded as the first, except with the use of N-(Z)-Ethylenediamine-HCl (10 equiv) as the amine for 1 h (10 equiv of DIPEA was used to freebase the amine prior to addition). The bromoacetic acid addition and subsequent amine additions were repeated for amines **12, 13, 14, 16,** and **17**, with the N-(Z)-Ethylenediamine-HCl amine residue interspersed after each spiroligomer amine. After the final amine addition, the resin was rinsed with DMF and DCM, and the resin treated with neat TFA to cleave the peptoid from the resin. **SPH-3** was purified by reverse phase flash chromatography, recovered 113 mg (57% yield) HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{256}H_{277}F_{12}N_{43}O_{52}$ Target Mass: 5013.0161; Found 5012.9707

Peptoid SPH-4

100 mg of Tentagel-NH_2 (S) resin (0.37 mmol / g) was rinsed multiple times with DCM and DMF. 3 equiv of Fmoc-Met-OH and HATU in 0.6 mL of NMP with 6 equiv of DIPEA were then added to the resin and stirred for 1 hour (Standard SPPS). The resin was treated with 20% piperidine in DMF 2x15 min, and rinsed with DMF and DCM. 800 uL of 1.3 M Bromoacetic acid in DMF was reacted with 200 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 0.3 mL of a 1M solution of propargylamine was added to the resin and stirred for 1 h (standard peptoid coupling). Fmoc-Lys(Boc)-OH was coupled to the resin using standard SPPS, Fmoc-deprotected, and followed by an N-substituted, boc protected ornithine derivative (N-Orn-Boc) with standard peptoid coupling. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **12** (3 equiv, previously deprotected) in DMF was added to the resin and reacted overnight with stirring. This was repeated for addition of amine **14**. Standard peptoid coupling was used to add another (N-Orn-Boc), and finally two more round of spiroligomer peptoid reactions using amines **16** and **18**. The resin was washed repeatedly with DMF and DCM, then treated with 1:1 TFA/DCM to remove the Boc protecting groups. The resin was washed exhaustively with water, then treated with a 7:3 Formic Acid/H_2O mixture containing 30 mg of cyanogen bromide to cleave the peptoid from the resin. **SPH-4** was purified by reverse phase flash chromatography, 19 mg recovered (22% yield). HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{142}H_{163}F_9N_{28}O_{28}$ 2879.2048; Found: 2879.1845

Peptoid SPH-5

15 mg of Rink amide resin (0.63 mmol / g) was swelled with DCM, and then treated with 20% Piperidine in DMF 2x15 min, and subsequently rinsed with DCM and DMF. 400 uL of 1.3 M Bromoacetic acid in DMF was reacted with 100 uL of DIC for 5 minutes, added to the resin, and allowed to react for 30 min. The resin was drained and washed repeatedly with DCM and DMF, after which 150 mM **25** (3 equiv, previously deprotected) in DMF was added to the resin and stirred overnight. The resin was drained into a small vial containing 1.05 equiv of Boc_2O (relative to the mmol of amine) to reprotect the spiroligomer trimer. This peptoid coupling was repeated

with amine **25** two more times, the resin washed exhaustively with DMF and DCM, then treated with neat TFA, followed by 1:1 TFA/DCM, and finally DCM to give 7.5 mg of purified **SPH-5** (25% yield). HRMS (ESI/Q-TOF) m/z: $(M+H)^+$ calcd for $C_{165}H_{198}N_{28}O_{33}$ 3099.4676; Found: 3099.4803

Acknowledgements

The project or effort depicted is sponsored by the Department of the Defense, Defense Threat Reduction Agency (HDTRA1-16-1-0047). The content of the information does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred.

Supporting Information

Supporting information containing ^{1}H NMR, ^{13}C NMR, and HPLC data is available free of charge at pubs.acs.org.

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