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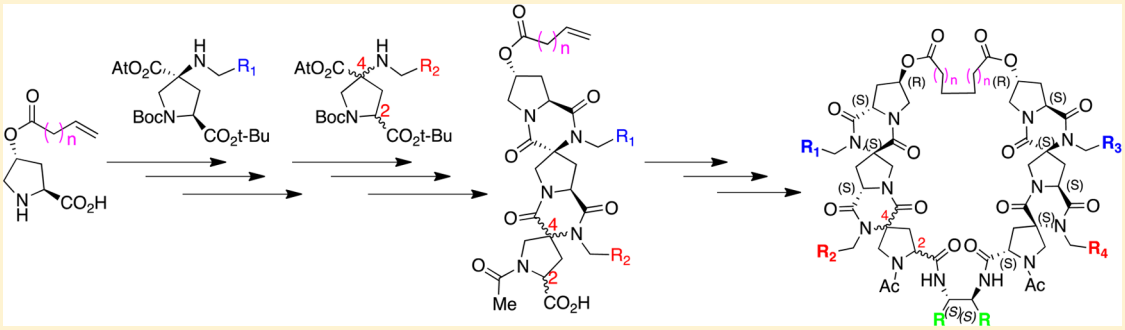
Synthesis of Spiroligomer-Containing Macrocycles

Qingquan Zhao and Christian E. Schafmeister*

Department of Chemistry, Temple University, 1901 North 13th Street, Philadelphia, Pennsylvania 19122, United States

Supporting Information



ABSTRACT: We demonstrate the synthesis and characterization of the solution conformations of a collection of functionalized spiroligomer-based macrocycles. These macrocycles contain 14 independently controllable stereocenters and four independently controllable functional groups on a highly preorganized scaffold. These molecules are being developed to display complex, preorganized surfaces for binding proteins and to create enzyme-like active sites. In this work, we demonstrate the convergent synthetic approach to this new class of macrocycles and demonstrate that the conformational properties of these molecules can be changed by altering the configuration stereocenters within the backbone.

INTRODUCTION

Macrocyclic molecules have received significant attention because of their potential applications in molecular recognition, catalysis, material science, and pharmaceutical development. A number of preorganized macrocyclic scaffolds bearing well-defined structures have been developed, including calixarenes,[1−6] cyclodextrin,[7−11] resorcinarenes,[12−17] conjugated aromatic systems,[18−23] and Schiff base macrocycles,[24−37] among others.[38−48] However, site-specifically incorporating multiple and diverse functional groups, such as catalytic groups or protein binding groups, within these scaffolds is often challenging due the inherent symmetry of many of the most studied macromolecules. In other cases, such as some Schiff base macrocycles,[25,34,35] the cyclic scaffolds are assembled from monomers in one step, making it difficult to append multiple functional groups in more than one specific positions. Proteins demonstrate that the presentation of diverse functional groups in preorganized three-dimensional constellations can give rise to tremendous capabilities, such as the catalysis in enzyme active sites and molecular recognition in protein binding sites. Therefore, new classes of synthetic macromolecular scaffolds that can present preorganized constellations of functional groups are highly desirable for the development of catalytic and molecular recognition applications.

Toward this goal, we have developed a collection of cyclic, stereochemically pure monomers called "bis-amino acids" that couple through pairs of amide bonds to form spiro- ladder oligomers named "spiroligomers".[49] Along the way, a new amide bond forming reaction, called "acyl-transfer-coupling",[50] was developed and has been applied to the sequential formation of functionalized penta- and hexasubstituted diketopiperazines between pairs of functionalized bis-amino acids.[51] Herein, we report a facile synthesis of spiroligomer-based macrocyclic scaffolds, whose structure and functional group presentation is controlled by the sequence and stereochemistry of the component monomers. In addition, NMR studies showed that one macrocycle exists in a small number of preferred conformational isomers, and they exchange slowly on the NMR time scale at 298 K. The barrier between two of these isomers can be partially overcome at the slightly higher temperature of 333 K. Considering the 14 independently configurable stereocenters in the backbone and the four configurable functional groups on the macrocycles, the macromolecules presented here represent a few examples of a virtual library containing millions of different conformationally restrained, functionally diverse macrocycles with unexplored and diverse properties. The synthetic methodology we present here provides an effective path for the development of these molecules for future applications.

RESULTS AND DISCUSSION

Synthesis of Building Blocks: Preactivated *pro4* Amino Acids 11−17. The general synthetic approach to preactivated, alkylated *pro4* amino acids like **11−17** has been described in previous reports from our group.[49,51] Taking

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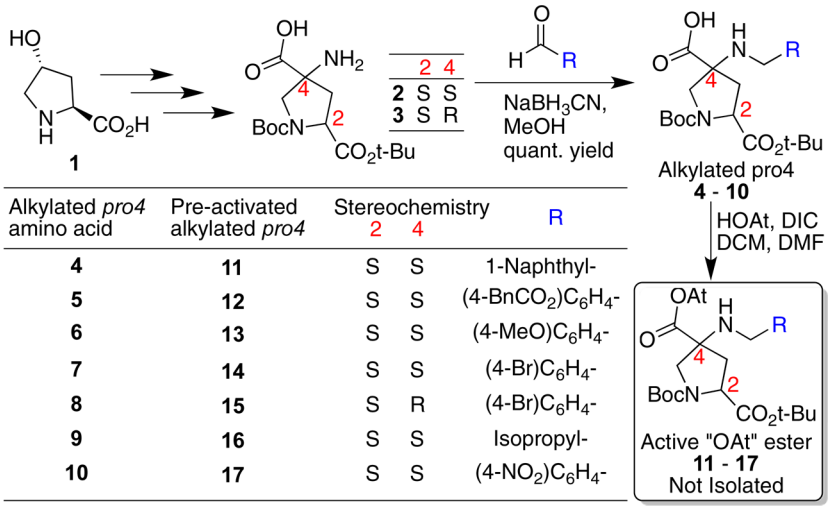
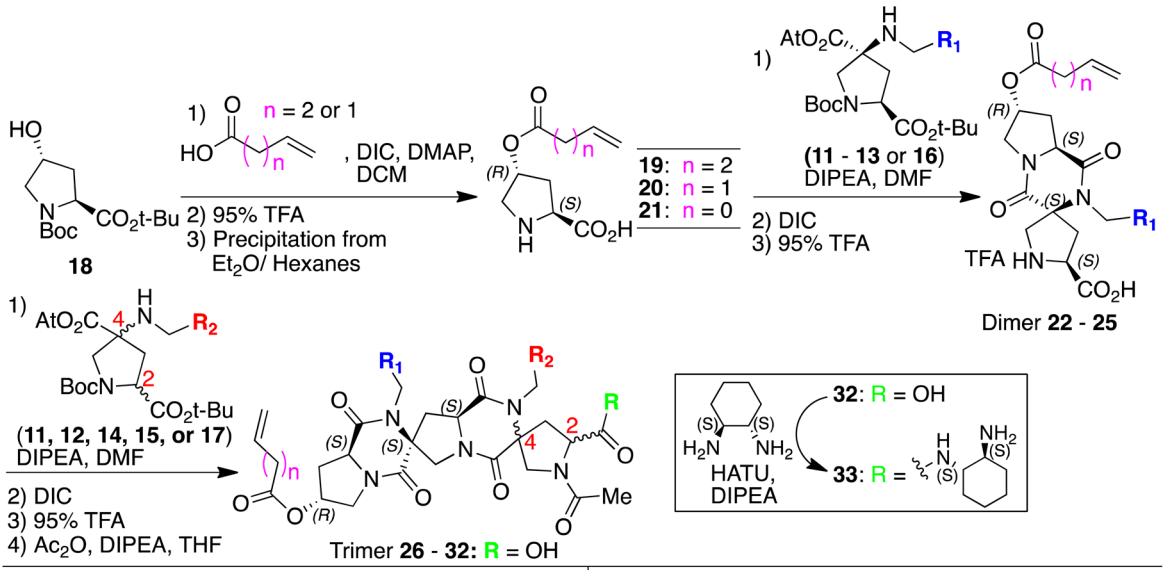
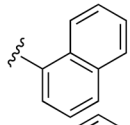
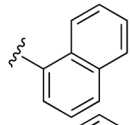
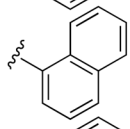
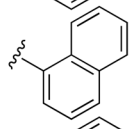
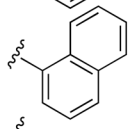
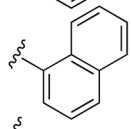
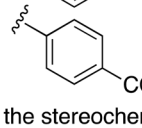
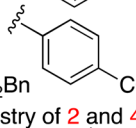
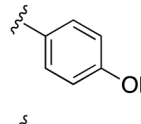
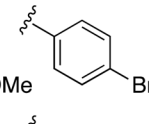
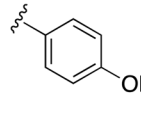
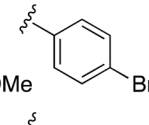
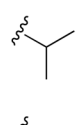
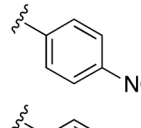
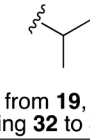
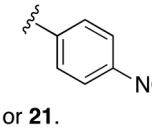
Scheme 1. Synthesis of Preactivated Alkylated *pro4* Amino Acids



Alkylated *pro4* amino acid	Pre-activated alkylated *pro4*	Stereochemistry 2	4	R
4	**11**	S	S	1-Naphthyl-
5	**12**	S	S	$(4\text{-BnCO}_2)C_6H_4$-
6	**13**	S	S	$(4\text{-MeO})C_6H_4$-
7	**14**	S	S	$(4\text{-Br})C_6H_4$-
8	**15**	S	R	$(4\text{-Br})C_6H_4$-
9	**16**	S	S	Isopropyl-
10	**17**	S	S	$(4\text{-NO}_2)C_6H_4$-

Scheme 2. Synthesis of Spiroligomer Trimer



Entry	Trimer[a]	n	R_1	R_2	Overall Yield (%)[b]
1	**26**	2			39
2	**27**	1			67
3	**28**	0			19
4	**29**	2	 CO_2Bn	 CO_2Bn	33
5	**30**	2	 OMe	 Br	44
6	**31**[c]	2	 OMe	 Br	38
7	**32**	2		 NO_2	-[d]
8	**33**	2		 NO_2	19

[a] Unless notified, the stereochemistry of 2 and 4 are both "S". [b] Isolated yield from **19**, **20** or **21**.
[c] Stereochemistry of 2 is "S" and 4 is "R". [d] yield was calculated after converting **32** to **33**.

compound **11** as a representative example, free amine **2** was reductively alkylated overnight using 2-naphthylaldehyde and sodium cyanoborohydride in methanol at room temperature, with a quantitative yield as determined by LC−MS (Scheme 1).

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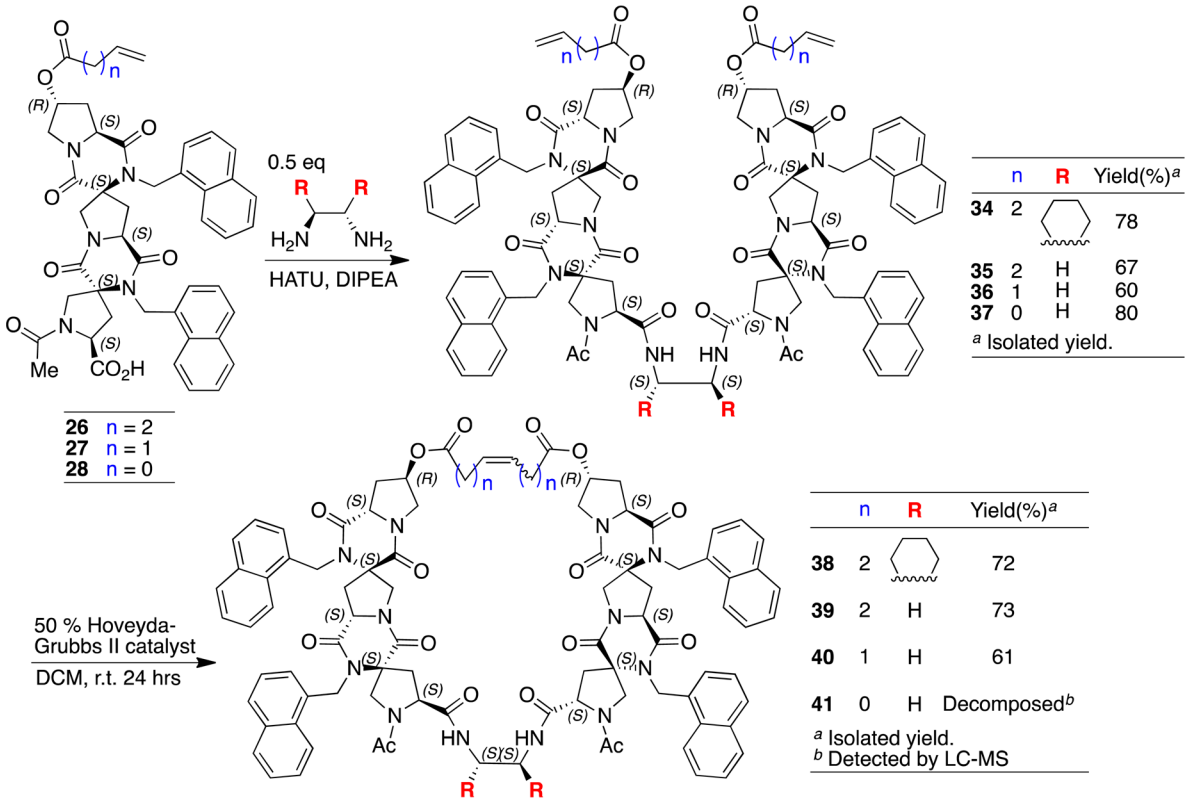
Scheme 3. Macrocycle Formation through an Olefin Metathesis Reaction



	n	R	Yield(%)[a]
34	2		78
35	2	H	67
36	1	H	60
37	0	H	80

[a] Isolated yield.

	n	R	Yield(%)[a]
38	2		72
39	2	H	73
40	1	H	61
41	0	H	Decomposed[b]

[a] Isolated yield.
[b] Detected by LC-MS

The resulting *N*-alkylated amino acid **4** was treated with 6 equiv of 1-hydroxy-7-azabenzotriazole (HOAt) and 1 equiv of *N*,*N*′-diisopropylcarbodiimide (DIC) in dichloromethane/DMF (2:1) at room temperature for 2 h. Under these conditions, the resulting oxy-7-azabenzotriazole (−OAt) ester **11** will not polymerize with its own secondary amine because of the extremely hindered nature of the secondary amine on the quaternary carbon center.[51] Instead, under dry atmosphere, the activated carboxylate species will remain in solution for many minutes, long enough to perform nucleophilic coupling with other added nucleophiles. By following this scheme, alkylated *pro4* amino acids **5**−**10** were prepared with quantitative yields and preactivated as oxy-7-azabenzotriazole (−OAt) esters **12**−**17** for assembly into functionalized spiroligomers.

Synthesis of Spiroligomer Trimers. Spiroligomer **26** was synthesized starting from intermediate **19** in seven steps with an overall 39% yield (Scheme 2, entry 1). Compound **19** was prepared by coupling 4-pentenoic acid with 1 equiv of *N*-Boc, CO_2-*tert*-butyl-protected hydroxylproline **18** at room temperature, followed by removal of the protecting groups and precipitation of the amino acid **19** from diethyl ether/hexanes (1:1) solution. Amino acid **19** was then treated with 1.2 equiv of preactivated *pro4* amino acid **11** as described above under basic conditions at room temperature. The coupling likely proceeds via multiple pathways including direct acylation of the prolyl amine of **19** and a new reaction mechanism that we have previously described called "acyl-transfer coupling".[50] The hexasubstituted diketopiperazine intermediate **22** was formed after addition of 2.0 equiv of DIC followed by removal of the *N*-Boc and *tert*-butyl ester protecting groups using 95% TFA/2.5%TIPS/2.5% H_2O. The dimer intermediate **22** was then treated with another 1.2 equiv of preactivated *pro4* amino acid **11** under basic conditions, followed by addition of DIC and removal of the protecting groups. The resulting amino acid residue was protected with acetic anhydride to provide acyl-protected spiroligomer trimer **26**.

The synthesis of these spiroligomer trimers is extremely versatile. Every stereocenter can be controlled and every functional group can be substituted. Starting from **20** and **21**, trimer **27** and **28** with shorter alkene linkers were obtained by following the identical synthetic route as that for trimer **26** (Scheme 2, entry 2 and 3). Trimer **29** was prepared with a 33% overall yield by following the same protocol as trimer **26** except alkylated *pro4* monomer **12** was used in place of **11** (Scheme 2, entry 4). Different side-chain groups (R_1 and R_2) can be incorporated by sequential addition of alkylated *pro4* monomers (Scheme 2, entries 5−8) bearing different functional groups derived from appropriate aldehydes. The shape of the trimer, as well as the relative positions of side chain groups in three-dimensional space, can be controlled by utilizing *pro4* monomers with different stereochemistry (Scheme 2, entries 5 and 6).

Macrocycle Formation through a Metathesis Reaction. Trimers **26**−**28** were coupled with 0.5 equiv of either ethylenediamine or diaminocyclohexane to provide dialkenes **34**−**37** (Scheme 3). The metathesis ring closure ring of **34** was not achieved using Grubbs I, II or Hoveyda−Grubbs I catalysts either at room temperature or under dichloromethane reflux. With 50% Hoveyda−Grubbs II catalyst in dichloromethane at room temperature, the metathesis reaction of **34** proceeded smoothly to afford **38** in 72% yield. Although both *E* and *Z* configurations may be formed as products, only one peak was observed in the C_{18} reversed-phase LC−MS chromatograph. The *E*/*Z* configuration of the alkene could not be determined conclusively; therefore, the next step was to reduce it to the alkane for NMR analysis. Similarly, macrocycles **39** and **40** were

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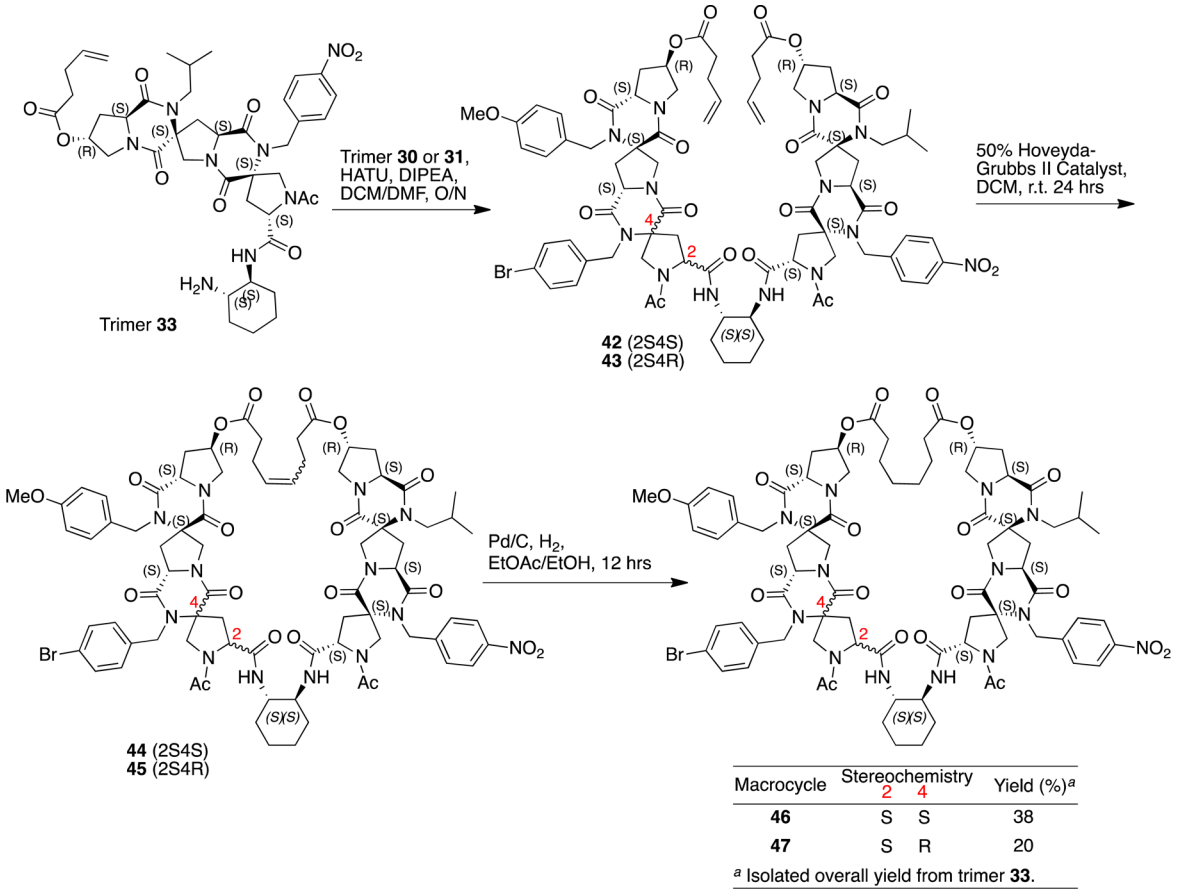
Scheme 4. Synthesis of Non-C_2-Symmetric Tetrasubstituted Macrocycles 46 and 47



Macrocycle	Stereochemistry 2	4	Yield (%)[a]
46	S	S	38
47	S	R	20

[a] Isolated overall yield from trimer **33**.

formed under the same conditions from the corresponding precursors **35** and **36**. The metathesis reaction of substrate **37** failed with decomposition, as observed by LC–MS.

Synthesis of Tetrasubstituted Macrocycles 46 and 47. With an olefin metathesis pathway to the macrocyles developed, a modification of the synthesis was carried out to produce non-C_2-symmetric tetrasubstitued macrocycles. Slow addition of 1-[dis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) activated trimer **32** into 6 equiv of diaminocyclohexane in DMF resulted in only one of the amines of the diamine being acylated by the spiroligomer segment, with the other primary amine free (Scheme 2, entries 7 and 8). The primary amine **33** was coupled with trimer **30** or **31** to form the dialkene intermediate **42** or **43**, which was then subjected sequentially to metathesis reaction and hydrogenation using H_2 catalyzed by palladium on carbon to produce macrocycles **46** and **47** (Scheme 4). Since trimers were incorporated sequentially into the scaffold, this synthesis allows them to be different from each other and enables the synthesis of completely asymmetric macrocycles.

Conformational Analysis of Macrocyclic 46 and 47. A series of NMR experiments including ^{1}H spectra, COSY, ROESY, as well as the ^{1}H, ^{13}C heteronuclear experiments HMQC and HMBC were performed to determine if the macrocyclic spiroligomers **46** and **47** displayed well-defined structures or if they were dynamic in benzene-d_6. Nearly all the ^{1}H and ^{13}C signals were assigned as shown in the Supporting Information. In addition, computational conformational searching using the AMBER94 force field using the Molecular Operating Environment (MOE) was performed to identify the lowest energy conformers of these two molecules.

Macrocycle **46** (Figure 1A) is composed of four *pro4* (2*S*,4*S*) monomers having three aromatic (M, N, and P) and one isopropyl (Q) groups as side chains. The structures of individual functionalized spiroligomer segments and the presentation of functional groups M and N (as well as P and Q) are well-defined by virtue of the ladder structure of spiroligomers. In these experiments, we were interested to observe if the spiroligomer segments have any predisposition to align with each other because of the cross-links across the two ends of the segments. The conformational searches of macrocycle **46** suggest that they are quite dynamic. This can be seen in two conformers extracted from a molecular dynamics run followed by energy minimization of compound **46** (Figure 1C). The side chains of segment MN are dynamic and sample multiple rotamers. In the dynamics simulations, the MN segment moves quite freely relative to the PQ segment because the hydrocarbon linker formed using the metathesis reaction is relatively long and flexible. The 4-bromobenzyl group (M) and 4-nitrobenzyl group (P) have a strong tendency to sit on opposite faces of the macrocycle ring with a distance of about 10.6 Å. In the ROESY NMR experiment, cross-peaks between the protons on the side chains and the spiroligomer segments that they are directly attached to were observed and were consistent with the ladder-molecule structure of the spiroligomer segments (Figure 1B); however, no ROESY cross-peaks were observed between hydrogens from any atoms on one spiroligomer segment to its neighboring segment. The lack of observable ROESY correlations between side-chains can be

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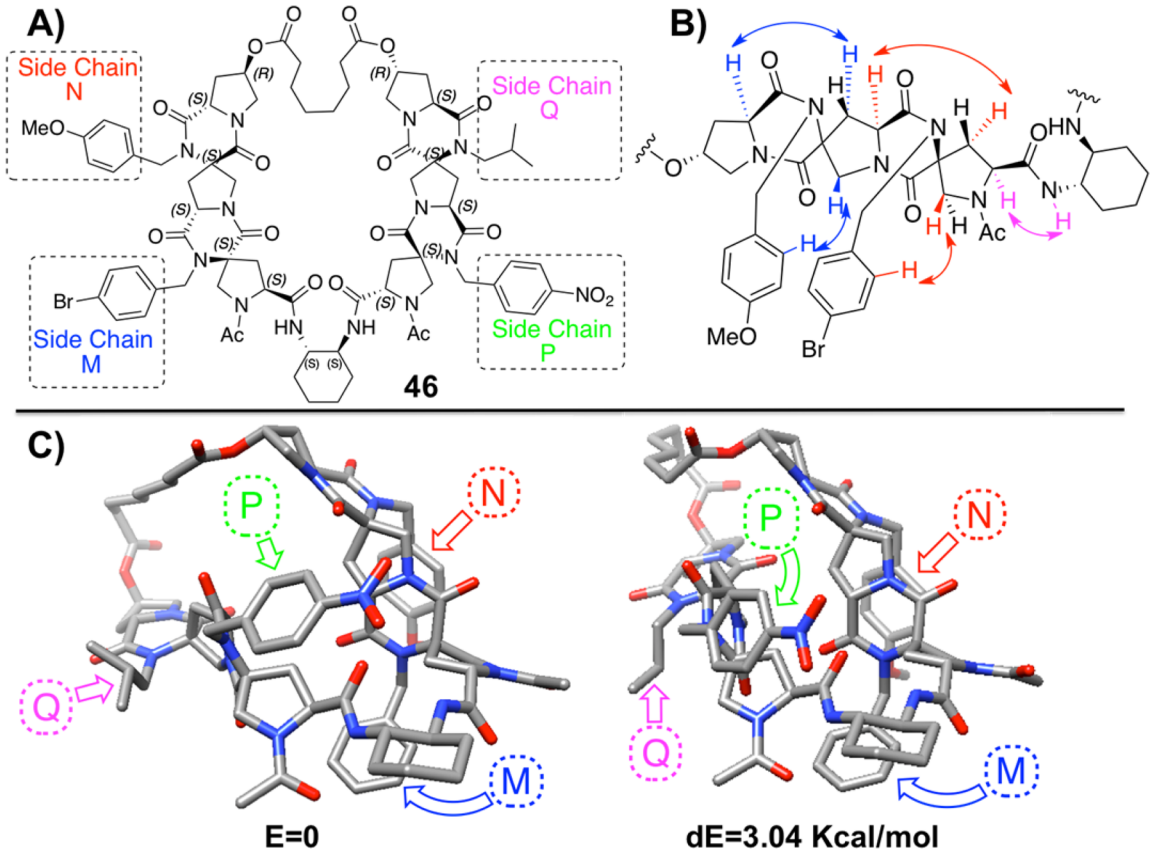

Figure 1. (A) Structure of macrocycle **46**; (B) ROESY correlations observed in NMR experiment are shown using blue, red, and magenta arrows; (C) two low energy conformers of **46** after energy minimization (ring M was treated as a benzyl group because Br atom is not recognized in AMBER94 force field).

understood in terms of the dynamic nature of macrocycles **46** as suggested by the molecular dynamics simulations.

Macrocycle **47** has the identical bonded structure as that of **46** except one *pro4* (2S4R) monomer was incorporated rather than a *pro4* (2S4S) monomer in one segment. This has a large consequence to the modeled structure of **47** relative to **46** in that it flips the side chains M and N to the same face of the macrocycle as P and Q. Thus, macrocycle **47** is a diastereomer of **46** that differs in only one of the 14 stereocenters, and therefore, **46** and **47** are two diastereomers of a set of 16384 total stereoisomers. Molecular dynamics simulations of **47** indicate that **47** is also highly dynamic but involves very different side-chain presentations than **46**.[52]

An unanticipated conformational isomerism was observed in the COSY NMR experiment of compound **47** in benzene-d_6 as shown in Figure 2. At 298 K, the coupling between BCHG and BCHB1 (or BCHB2) was split into two separate cross-peaks (Figure 2A), both of which coupled with one carbon resonance (Figure 2B). At 333 K, the coupling between these two protons coalesced into one single cross peak as shown in Figure 2D. Although a slightly different chemical shift was observed due to the increased temperature (Figure 2C), it suggests that compound **47** exists in a restricted set of conformations that exchange slowly on the NMR time-scale at 298 K.

The restricted conformational flexibility of **47** and the slowly exchanging conformational isomerism is also visible in the aromatic area in the COSY spectrum as shown in Figure 3. At 298 K, unlike the single cross-peak shown in macrocycle **46** (Figure 3A), the coupling between HM3 and HM2 on the *p*-

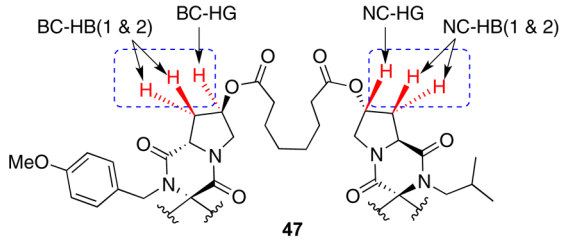

Figure 2. Observation of slow exchange on the NMR time scale between at least two conformations in **47** at 298 K that coalesce at 333 K- protons in aliphatic carbon (labeling rules are discussed in Supporting Information).

bromobenzyl group (ring M) was split into three separate cross-peaks (Figure 3B). At 333 K, this coupling partially coalesced into two cross-peaks (Figure 3C). It is noteworthy that the *p*-bromobenzyl group (side chain M) is very close to the stereocenter that is different between **46** and **47**. The chemical shift of protons in the 4-methoxybenzyl group (ring N), which was on the same spiroligomer fused ring scaffold, moved upfield after flipping the stereocenter, whereas the chemical shift of protons in the 4-nitrobenzyl group (ring Q) are unchanged (Figure 3D). These observations suggest that compound **47** has a restricted set of conformational isomers, and they exchange slowly on the NMR time scale at 298 K. The barrier between two of these isomers can be partially overcome at a slightly higher temperature (333 K). This demonstrates that changing just one stereocenter out of the 14 stereocenters of the backbone can lead to different conformational properties

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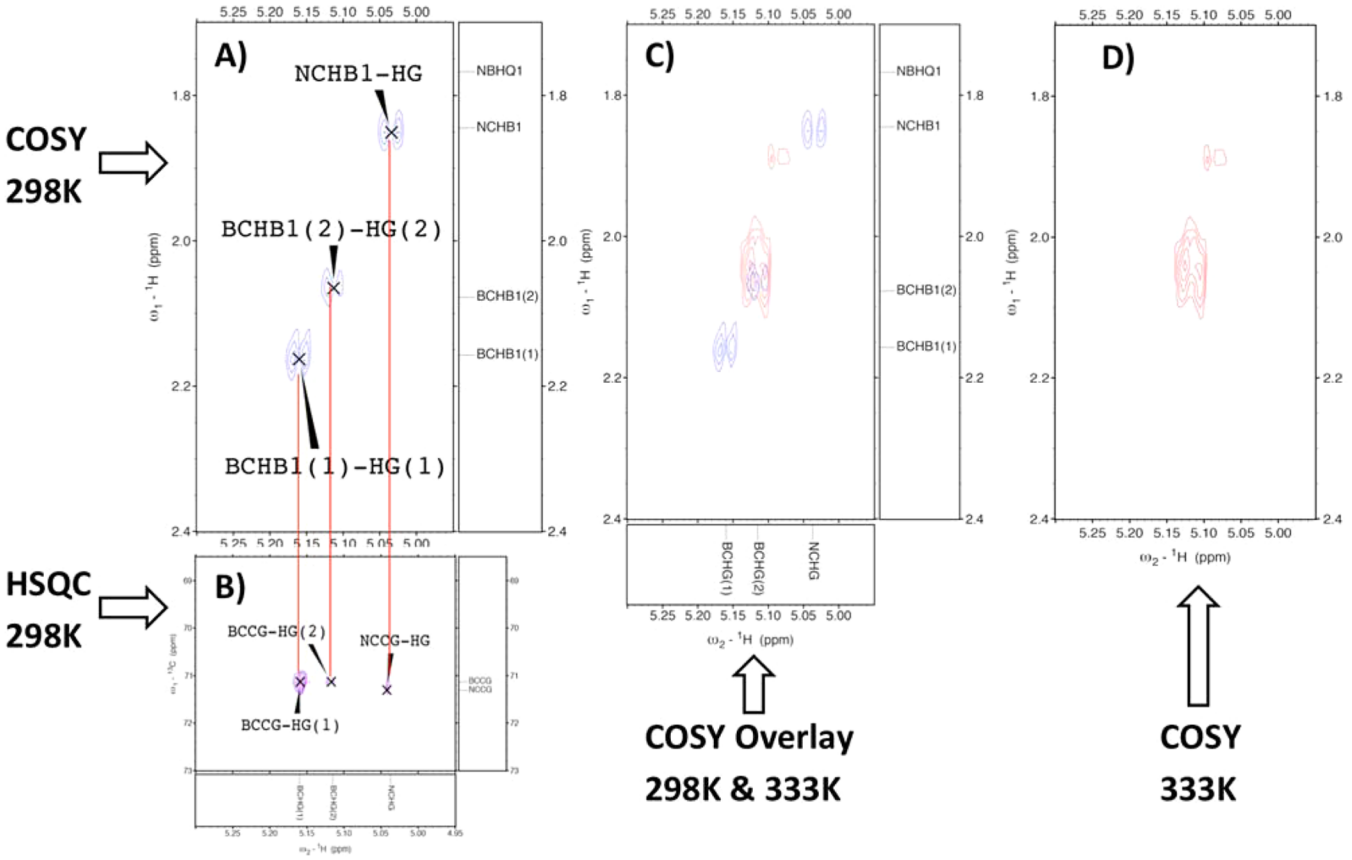

Figure 3. Observation of slow exchange in the ^{1}H COSY on the NMR time-scale between well-defined conformations in macrocycle **47** at 298 K, some of which coalesce at 333 K; the protons are in aromatic rings.

of the macrocycles. In the future, we plan to incorporate shorter and more constrained linkers as well as careful design of the stereocenters of the spiroligomer segments to reduce the dynamics of the macrocycles and bias the structure to one well-defined three-dimensional structure that presents the functional groups in constellations that enable them to bind protein surfaces[53] and accelerate chemical reactions.[54−56]

CONCLUSION

In summary, a synthetic protocol of macrocyclic spiroligomers using cross-olefin metathesis coupling was developed, and a series of tetrafunctionalized macromolecules were synthesized. The NMR study of compounds **46** and **47** indicated that they are conformationally dynamic, and this is consistent with molecular dynamics calculations using the AMBER94 force field. Compound **47** exhibits a limited number of conformational isomers as demonstrated by well-defined cross peaks in the correlations isomers, and they exchange slowly on the NMR time scale at 298 K. The barrier between two of these isomers can be partially overcome at the slightly higher temperature (333 K). The characters of the similar macrocycles with shorter linkers are currently under investigation. This synthetic methodology also provides a path to create more spiroligomer based macromolecules to create molecular surfaces and pockets that will be used to develop applications in supramolecular molecular recognition and catalysis.

EXPERIMENTAL SECTION

General Synthetic Procedure of Alkylated *pro4* Amino Acid 4−10. The synthesis of *pro4* amino acids **2** and **3** has been previously described in the literature.[49] A general synthetic procedure-reductive alkylation of *pro4* amino acids is described as follows: To a solution of (3*S*,5*S*)-3-amino-1,5-bis(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid **2** (300 mg to 2 g scale, 1.0 equiv) in methanol (10 mL per mmol of **2**), an aldehyde chosen from the table in Scheme 1 (1.2 equiv) was added and stirred for 30 min at room temperature. Sodium cyanoborohydride (1.5 equiv) was then added and stirred for 20 h at room temperature until the reaction was complete, as monitored by LC−MS. The reaction mixture was concentrated under vacuum and the resulting residue was dissolved in 50 mL of H_2O. The pH was adjusted to 7 by dropwise addition of 2 N HCl. Crude alkylated *pro4* amino acid (**4−10**) was obtained by vacuum filtration with quantitative yield. (Note: this reaction is a peak-to-peak reaction as determined by LC−MS, and the products formed were used directly without further purification in the next steps, so the purified isolated yields were not calculated. For characterization purposes, alkylated amino acids were purified by reversed- phase C_{18} high-performance liquid chromatography using a 5−95% H_2O/acetonitrile gradient).

(3S,5S)-1,5-Bis(tert-butoxycarbonyl)-3-((naphthalen-1-ylmethyl)-amino)pyrrolidine-3-carboxylic acid, ***4:*** ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 8.20 (d, J = 8.0 Hz, 1H), 7.88 (dd, J = 7.8, 1.5 Hz, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.54−7.46 (m, 3H), 7.41 (dd, J = 8.0, 7.2 Hz, 1H), 4.16 (dd, J = 8.5, 6.7 Hz, 1H), 4.08 (q, J = 12.9 Hz, 2H), 3.93 (d, J = 10.6 Hz, 1H), 3.33 (d, J = 10.6 Hz, 1H), 2.74 (s, 1H), 2.00 (dd, J = 12.7, 6.6 Hz, 1H), 1.39 (s, 9H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K) δ 175.3, 171.5, 153.6, 136.5, 133.8, 132.0, 128.7, 127.7, 126.5, 126.2, 125.8, 125.7, 124.3, 80.6, 79.2, 59.6, 55.2, 46.7, 28.5, 28.3, 28.1; HRMS-ESI m/z calcd for $C_{26}H_{34}N_2O_6$ $(M + H)^+$ 471.2490, found 471.2488.

(3S,5S)-3-((4-((Benzyloxy)carbonyl)benzyl)amino)-1,5-bis(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid, ***5:*** ^{1}H NMR (500 MHz, DMSO-d_6, rt, rotamers observed) δ 8.05 (s, 1H), 7.98−7.85 (m, 2H), 7.55−7.41 (m, 4H), 7.40−7.30 (m, 3H), 5.33 (s, rotamers, 2H), 4.15 (t, rotamers, J = 7.3 Hz, 1H), 3.98−3.56 (m, 3H), 3.33 (d,

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J. Org. Chem. 2015, 80, 8968−8978

rotamers, J = 10.6 Hz, 1H), 2.67 (dd, rotamers, J = 11.9, 9.0 Hz, 1H), 2.04 (dd, rotamers, J = 12.0, 6.1 Hz, 1H), 1.40 (s, 9H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6, rt, rotamers observed) δ 174.8, 171.4, 171.1, 167.6, 166.0, 165.6, 153.8, 153.4, 149.0, 146.2, 146.0, 137.7, 136.7, 136.4, 132.9, 129.9, 129.6, 129.5, 128.94, 128.85, 128.7, 128.6, 128.5, 128.3, 126.8, 81.5, 80.8, 80.6, 79.7, 79.3, 68.2, 67.2, 66.9, 66.5, 62.9, 59.3, 54.7, 48.6, 38.8, 38.1, 28.5, 28.4, 28.3, 28.1; HRMS-ESI m/z calcd for $C_{30}H_{38}N_2O_8$ $(M + H)^+$ 555.2701, found 555.2699.

(3S,5S)-1,5-Bis(tert-butoxycarbonyl)-3-((4-methoxybenzyl)-amino)pyrrolidine-3-carboxylic acid, ***6****:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 7.24−7.21 (m, 2H), 6.87−6.84 (m, 2H), 4.14 (dd, J = 8.6, 6.4 Hz, 1H), 3.84 (d, J = 10.9 Hz, 1H), 3.74 (s, 3H), 3.60 (dd, J = 27.7, 12.6 Hz, 2H), 3.31 (d, J = 10.9 Hz, 1H), 2.66 (dd, J = 10.8, 9.8 Hz, 1H), 1.99 (dd, J = 12.9, 6.4 Hz, 1H), 1.41 (s, 9H), 1.39 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K) δ 174.2, 171.3, 159.0, 153.5, 132.0, 129.7, 114.1, 80.9, 79.4, 67.2, 59.3, 55.6, 54.7, 48.4, 38.7, 28.5, 28.1; HRMS-ESI m/z calcd for $C_{23}H_{34}N_2O_7$ $(M + H)^+$ 451.2439, found 451.2438.

(3S,5S)-3-((4-Bromobenzyl)amino)-1,5-bis(tert-butoxycarbonyl)-pyrrolidine-3-carboxylic acid, ***7****:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 7.47−7.43 (m, 2H), 7.29−7.26 (m, 2H), 4.13 (dd, J = 8.7, 6.2 Hz, 1H), 3.82 (d, J = 10.8 Hz, 1H), 3.61 (dd, J = 31.8, 13.5 Hz, 2H), 3.28 (d, J = 10.9 Hz, 1H), 2.70−2.61 (m, 1H), 1.98 (dd, J = 13.0, 6.1 Hz, 1H), 1.39 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6, rt, rotamers observed) δ 174.3, 171.4, 171.0, 165.1, 153.8, 153.4, 139.5, 131.3, 130.8, 81.0, 80.8, 79.5, 67.8, 66.9, 59.2, 54.6, 48.1, 38.7, 37.9, 28.5, 28.4, 28.1; HRMS-ESI m/z calcd for $C_{22}H_{31}BrN_2O_6$ $(M + H)^+$ 499.1438, found 499.1443.

(3R,5S)-3-((4-Bromobenzyl)amino)-1,5-bis(tert-butoxycarbonyl)-pyrrolidine-3-carboxylic acid, ***8****:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 7.45 (d, J = 8.3 Hz, 2H), 7.26 (d, J = 8.3 Hz, 2H), 4.16 (t, J = 8.1 Hz, 1H), 3.69 (d, J = 13.9 Hz, 1H), 3.59 (d, J = 14.0 Hz, 1H), 3.55 (broad, 2H), 2.39−2.30 (m, 1H), 2.23−2.14 (m, 1H), 1.42 (s, 9H), 1.37 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6, rt, rotamers observed) δ 177.4, 176.1, 174.9, 173.3, 173.2, 173.1, 171.7, 171.3, 168.2, 166.6, 154.3, 153.8, 153.5, 153.3, 151.3, 140.2, 139.9, 131.3, 130.6, 120.1, 99.4, 80.9, 80.7, 79.3, 68.3, 67.5, 61.4, 59.3, 59.1, 28.3, 28.0; HRMS-ESI m/z calcd for $C_{22}H_{31}BrN_2O_6$ $(M + H)^+$ 499.1438, found 499.1436.

(3S,5S)-1,5-Bis(tert-butoxycarbonyl)-3-(isobutylamino)-pyrrolidine-3-carboxylic acid, ***9****:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 4.12 (t, J = 7.3 Hz, 1H), 3.81 (d, J = 10.6 Hz, 1H), 3.26 (d, J = 10.6 Hz, 1H), 2.63 (broad, 1H), 2.34 (dd, J = 10.9, 6.4 Hz, 1H), 2.29−2.21 (m, 1H), 1.91 (dd, J = 12.3, 6.2 Hz, 1H), 1.67−1.56 (m, 1H), 1.44 (s, 9H), 1.39 (s, 9H), 0.88 (dd, J = 6.2, 2.9 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K, aliphatic carbon signals overlap) δ 174.0, 171.5, 171.2, 80.8, 79.3, 59.4, 54.7, 52.7, 28.5, 28.1, 20.7; HRMS-ESI m/z calcd for $C_{19}H_{34}N_2O_6$ $(M + Na)^+$ 409.2309, found 409.2316.

(3S,5S)-1,5-Bis(tert-butoxycarbonyl)-3-((4-nitrobenzyl)amino)-pyrrolidine-3-carboxylic acid, ***10****:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 8.14−8.10 (m, 2H), 7.63−7.59 (m, 2H), 4.15 (dd, J = 8.7, 6.0 Hz, 1H), 3.87−3.73 (m, 3H), 3.31 (d, J = 10.9 Hz, 1H), 2.68 (dd, J = 10.4, 9.5 Hz, 1H), 2.01 (dd, J = 13.0, 6.0 Hz, 1H), 1.40 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6, rt, rotamers observed) δ 174.6, 171.1, 170.7, 153.6, 153.3, 149.0, 148.9, 146.7, 129.3, 123.4, 80.8, 80.7, 79.33, 79.28, 67.7, 66.8, 59.0, 54.7, 54.6, 48.0, 38.8, 37.9, 28.4, 28.2, 27.9; HRMS-ESI m/z calcd for $C_{22}H_{31}N_3O_8$ $(M + H)^+$ 466.2184, found 466.2188.

(2S,4R)-4-(Pent-4-enoyloxy)pyrrolidine-2-carboxylic Acid, ***19****.* At room temperature, alcohol **18** (synthesized from 4-hydroxyproline,[57,58] 1.5 g, 5.2 mmol, 1.05 equiv), 4-pentenoic acid (500 mg, 5.0 mmol, 1.0 equiv), and DMAP (61 mg, 0.5 mmol, 0.1 equiv) were dissolved in 50 mL of DCM. To this solution was added DIC (862 μL, 5.5 mmol, 1.1 equiv) dropwise. The reaction was stirred at room temperature overnight until completion as detected by TLC with I_2 stain. The reaction was then quenched with 50 mL of satd NH_4Cl (aq), and the product was extracted with DCM (3 × 100 mL). The combined organic layers were washed subsequently with satd NH_4Cl (aq), satd $NaHCO_3$ (aq), and brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was purified through silica gel chromatography with 0−15% EtOAc/hexanes as eluting solvent to give a white solid intermediate (1.7 g, 4.6 mmol, 92% yield). The intermediate (1.5 g, 4.1 mmol) was then treated with 50 mL of 95% TFA/2.5% TIPS/2.5% H_2O at room temperature for 2 h. Removal of the solvent produced a light yellow oil, which was then added dropwise into 100 mL of ether/hexanes (1:1) and stirred for 30 min. The white precipitate formed was then filtered and dried under high vacuum to provide the white solid powder **19** as a TFA salt (1.02 g, 76% yield): ^{1}H NMR (500 MHz, DMSO-d_5, rt) δ 5.80 (ddt, J = 16.7, 10.2, 6.4 Hz, 1H), 5.26 (t, J = 4.8 Hz, 1H), 5.04 (ddd, J = 17.2, 3.4, 1.6 Hz, 1H), 4.98 (ddd, J = 10.3, 3.1, 1.3 Hz, 1H), 4.17 (dd, J = 10.2, 7.8 Hz, 1H), 3.50 (dd, J = 13.0, 4.8 Hz, 1H), 3.22 (d, J = 13.0 Hz, 1H), 2.43−2.35 (m, 2H), 2.32−2.23 (m, 3H), 2.23−2.16 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6, rt) δ 172.2, 170.1, 137.4, 116.0, 73.3, 59.0, 50.8, 35.1, 33.1, 28.6; HRMS-ESI m/z HRMS-ESI m/z calcd for $C_{10}H_{15}NO_4$ $(M + H)^+$ 214.1074, found 214.1080.

(2S,4R)-4-(But-3-enoyloxy)pyrrolidine-2-carboxylic Acid, ***20****.* Compound **20** was prepared by following the identical synthesis procedure as compound **19** except 4-pentenoic acid was changed to 3-butenoic acid: ^{1}H NMR (500 MHz, DMSO-d_6, rt) δ 10.05 (s, 1H), 9.01 (s, 1H), 5.85 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.29 (t, J = 4.8 Hz, 1H), 5.17−5.09 (m, 2H), 4.45 (t, J = 8.6 Hz, 1H), 3.56 (d, J = 10.2 Hz, 1H), 3.32 (d, J = 12.9 Hz, 1H), 3.11 (ddt, J = 6.9, 4.0, 1.4 Hz, 2H), 2.38 (ddt, J = 8.0, 3.3, 1.4 Hz, 1H), 2.34−2.26 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6, rt) δ 170.6, 170.1, 130.7, 118.8, 72.9, 58.1, 50.9, 38.5, 34.4; HRMS-ESI m/z HRMS-ESI m/z calcd for $C_9H_{13}NO_4$ $(M + Na)^+$ 222.0737, found 222.0738. Compound **21** has been reported in the previous literature.[59,60]

General Synthetic Procedure of Spiroligomer Trimer. A representative synthesis of trimer **26** is described as below: At room temperature, *pro4* amino acid **4** (940 mg, 2.0 mmol, 1.0 equiv) and HOAt (1.6 g, 12.0 mmol, 6.0 equiv) were dissolved in 30 mL of DMF/DCM (2:1) in a flame-dried 100 mL round-bottom flask. To this solution was added DIC (344 μL, 2.2 mmol, 1.1 equiv) dropwise, and the reaction was stirred at room temperature for 2 h. To the reaction with intermediate **11** formed in situ were then added the premixed **19** (556 mg, 1.7 mmol, 0.85 equiv) and DIPEA (883 μL, 5.1 mmol, 2.55 equiv) in 10 mL of DMF in one portion. The reaction was stirred for 15 h, followed by the addition of DIC (626 μL, 4.0 mmol, 2.0 equiv), and it stirred for an additional 2 h until the DKP closure was complete as detected by LC−MS. The reaction mixture was then quenched by the addition of satd NH_4Cl (aq) (40 mL) and extracted with EtOAc (100 mL + 2 × 50 mL). The combined organic portion was washed sequentially with 3× satd NH_4Cl (aq), 3× satd $NaHCO_3$ (aq), and 2× brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the yellow solid residue was treated with 50 mL of 95% TFA/2.5% TIPS/2.5% H_2O at room temperature for 2 h. Removal of the solvent under vacuum produced a yellow oil crude product, which was then added dropwise into 200 mL of ether/hexanes (1:1) and stirred for 30 min. The precipitate formed was then filtered and dried under high vacuum to give the light yellow solid dimer **22** as a TFA salt (1.49 g). The product was used in the next step without further purification: HRMS-ESI m/z calcd for $C_{27}H_{30}N_3O_6^+$ $(M + H)^+$ 492.2129, found 492.2121.

At room temperature, amino acid **4** (553 mg, 1.18 mmol, 1.0 equiv) and HOAt (964 mg, 7.08 mmol, 6.0 equiv) were dissolved in 18 mL of DMF/DCM (2:1) in a flame-dried 100 mL round-bottom flask. To this solution was added DIC (203 μL, 1.29 mmol, 1.1 equiv) dropwise, and the reaction was stirred at room temperature for 2 h. To the reaction were then added the premixed crude dimer **22** (606 mg, theoretically 1.0 mmol, 0.85 equiv) and DIPEA (520 μL, 3.0 mmol, 2.55 equiv) in 6 mL DMF in one portion. The reaction was stirred for 15 h followed by the addition of DIC (369 μL, 2.35 mmol, 2.0 equiv) and stirred for an additional 2 h until the DKP closure was complete as indicated by LC−MS. The reaction mixture was then quenched by addition of satd NH_4Cl (aq) (40 mL), and product was extracted with EtOAc (100 mL + 2 × 50 mL). The combined organic portion was washed sequentially with 3× satd NH_4Cl (aq), 3× satd $NaHCO_3$ (aq), and 2× brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the yellow solid residue was treated

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with 50 mL of 95% TFA/2.5% TIPS/2.5% H_2O at room temperature for 2 h. Removal of the solvent under vacuum produced a crude product as a dark yellow oil, which was then added dropwise into 200 mL of ether/hexanes (1:1) and stirred for 30 min. The precipitate formed was then filtered and dried under high vacuum to give the crude brown solid trimer as a TFA salt (1.03 g). The crude product was used in the next acyl protection step without further purification.

The crude product obtained above (883 mg, theoretically 1 mmol, 1.0 equiv) was dissolved in 20 mL of THF, followed by the addition of DIPEA (520 μL, 3.0 mmol, 3.0 equiv) and Ac_2O (142 μL, 1.5 mmol, 1.5 equiv). The reaction was stirred overnight until completion as indicated by LC−MS. The reaction mixture was then quenched by addition of satd NH_4Cl (aq) (40 mL), and the product was extracted with EtOAc (100 mL + 2 × 50 mL). The combined organic portion was washed sequentially with 3× satd NH_4Cl (aq) and 2× brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the crude product **26** was obtained as a brown solid. The pure trimer **26** (190 mg) was obtained after purification by reversed-phase C_{18} column using 5−95% H_2O/acetonitrile gradient as the eluting solvent with an overall 39% isolated yield from the starting building block **19**.

*(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′,2″-bis(naphthalene-1-ylmethyl)-1′,1″,4′,4″ tetraoxo-7″-(pent-4-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic acid, **26**:* ^{1}H NMR (500 MHz, DMSO-d_6, 350 K, rotamer observed) δ 8.12 (d, *J* = 8.1 Hz, 2H), 8.02−7.96 (m, 1H), 7.96−7.90 (m, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 8.2 Hz, 1H), 7.63−7.57 (m, 2H), 7.57−7.52 (m, 2H), 7.46 (dd, *J* = 7.9, 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 6.9 Hz, 1H), 6.99 (s, 1H), 5.86 (ddt, *J* = 16.7, 10.3, 6.4 Hz, 1H), 5.50 (d, *J* = 17.5 Hz, 1H), 5.37 (t, *J* = 4.8 Hz, 1H), 5.31−5.24 (m, 2H), 5.08 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.01 (ddd, *J* = 10.3, 3.1, 1.4 Hz, 1H), 4.94 (d, *J* = 17.5 Hz, 1H), 4.82 (dd, *J* = 10.1, 7.3 Hz, 1H), 4.59−4.48 (m, 1H), 4.15 (dd, *J* = 13.5, 5.4 Hz, 1H), 4.03 (s, *J* = 7.6 Hz, 1H), 3.99 (d, *J* = 12.4 Hz, 1H), 3.76 (dd, *J* = 12.8, 3.9 Hz, 1H), 3.55 (d, *J* = 13.6 Hz, 1H), 3.34 (dd, *J* = 35.3, 12.7 Hz, 1H), 2.96 (dd, *J* = 13.2, 6.7 Hz, 1H), 2.70 (dd, *J* = 21.0, 9.3 Hz, 1H), 2.60 (dd, *J* = 13.4, 9.8 Hz, 1H), 2.47 (d, *J* = 7.1 Hz, 2H), 2.44−2.21 (m, 6H), 1.80 (s, *J* = 12.6 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K, rotamer observed) δ 173.0, 172.3, 169.2, 168.8, 168.6, 166.2, 165.7, 137.5, 134.3, 133.8, 133.7, 133.3, 130.5, 129.1, 128.9, 127.8, 127.3, 126.7, 126.6, 126.4, 126.2, 125.9, 125.8, 123.6, 123.3, 122.2, 115.8, 71.9, 71.1, 68.2, 67.6, 62.2, 58.8, 57.0, 56.8, 52.7, 51.4, 51.2, 50.6, 44.5, 43.9, 42.7, 38.2, 36.1, 34.2, 33.5, 28.7, 22.3; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 21.8 min; HRMS-ESI *m*/*z* calcd for $C_{46}H_{45}N_5O_9$ $(M + H)^+$ 812.3290, found 812.3290.

*(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-7″-(but-3-enoyloxy)-2′,2″-bis(naphthalene-1-ylmethyl)-1′,1″,4′,4″tetraoxododecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic Acid, Trimer **27**.* Starting from amino acid **20** (356 mg, 1.14 mmol), trimer **27** was synthesized through dimer intermediate **23** by following the identical synthetic procedure as that of trimer **26** with an overall 67% isolated yield: ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 8.16−8.03 (m, 2H), 7.98 (t, *J* = 8.7 Hz, 2H), 7.89−7.81 (m, 2H), 7.62−7.55 (m, 4H), 7.50−7.39 (m, 2H), 7.23−7.02 (m, 2H), 5.94 (ddt, *J* = 17.0, 10.3, 6.8 Hz, 1H), 5.52 (d, *J* = 17.5 Hz, 1H), 5.40 (t, *J* = 4.8 Hz, 1H), 5.36 (d, *J* = 17.4 Hz, 1H), 5.22 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.18 (dd, *J* = 10.2, 1.4 Hz, 1H), 5.03 (broad, 1H), 4.94 (d, *J* = 17.3 Hz, 1H), 4.83 (dd, *J* = 10.1, 7.4 Hz, 1H), 4.64 (dd, *J* = 10.2, 6.9 Hz, 1H), 4.41−4.28 (m, 1H), 4.18 (dd, *J* = 13.5, 5.4 Hz, 1H), 4.15−4.08 (m, 1H), 4.01 (d, *J* = 12.5 Hz, 1H), 3.89 (d, *J* = 12.2 Hz, 1H), 3.78 (d, *J* = 12.5 Hz, 1H), 3.56 (d, *J* = 13.6 Hz, 1H), 3.48 (dd, *J* = 15.8, 13.1 Hz, 1H), 3.19 (dd, *J* = 6.7, 0.8 Hz, 2H), 3.18−3.11 (m, 1H), 2.99 (dd, *J* = 13.2, 6.7 Hz, 1H), 2.78 (dd, *J* = 12.8, 6.5 Hz, 2H), 2.49−2.35 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K, 5 aromatic and 3 aliphatic carbon signals were missing probably because of overlap) δ 170.80, 170.79, 168.7, 168.6, 168.4, 165.6, 165.1, 133.8, 133.2, 133.0, 131.0, 130.4, 130.3, 129.1, 127.7, 126.8, 126.6, 126.3, 125.9, 123.3, 122.9, 122.7, 122.2, 118.8, 71.4, 68.3, 67.5, 56.9, 56.7, 52.7, 52.2, 50.6, 49.8, 43.9, 38.8, 38.1, 36.0; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 21.0 min; HRMS-ESI *m*/*z* calcd for $C_{45}H_{43}N_5O_9$ $(M + H)^+$ 798.3134, found 798.3167.

*(3S,5S,7′R,8a′S)-7′-(But-3-enoyloxy)-2′-(naphthalen-1-ylmethyl)-1′,4′-dioxohexahydro-1′H-spiro[pyrrolidine-3,3′-pyrrolo[1,2-a]pyrazine]-5-carboxylic acid, dimer intermediate **23**:* HRMS-ESI *m*/*z* calcd for $C_{26}H_{27}N_3O_6$ $(M + H)^+$ 478.1973, found 478.1970.

*(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′,2″-bis(naphthalene-1-ylmethyl)-1′,1″,4′,4″tetraoxo-7″-(prop-2-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic Acid, Trimer **28**.* Starting from amino acid **21** (160 mg, 0.53 mmol), trimer **28** was synthesized through dimer intermediate **24** by following the identical synthetic procedure as that of trimer **26** with an overall 19% isolated yield: ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 8.14−8.09 (m, 2H), 7.99−7.96 (m, 1H), 7.94−7.92 (m, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 7.79 (d, *J* = 8.2 Hz, 1H), 7.62−7.58 (m, 2H), 7.57−7.50 (m, 4H), 7.47 (dd, *J* = 8.1, 7.3 Hz, 1H), 7.42−7.35 (m, 1H), 7.15 (d, *J* = 7.1 Hz, 1H), 6.43 (dd, *J* = 17.3, 1.4 Hz, 1H), 6.21 (dd, *J* = 17.3, 10.5 Hz, 1H), 5.98 (dd, *J* = 10.5, 1.4 Hz, 1H), 5.50 (d, *J* = 17.5 Hz, 1H), 5.45 (td, *J* = 4.2, 1.1 Hz, 1H), 5.36−5.26 (m, 2H), 5.26−5.13 (m, 2H), 4.95 (d, *J* = 17.4 Hz, 1H), 4.87 (dd, *J* = 10.0, 7.5 Hz, 1H), 4.53 (dd, *J* = 10.7, 6.7 Hz, 1H), 4.20 (dd, *J* = 13.6, 5.4 Hz, 1H), 4.05 (s, 1H), 4.00 (d, *J* = 12.4 Hz, 1H), 3.77 (d, *J* = 12.4 Hz, 1H), 3.59 (d, *J* = 13.6 Hz, 1H), 3.31 (d, *J* = 12.8 Hz, 1H), 2.99 (dd, *J* = 13.2, 6.8 Hz, 1H), 2.71−2.67 (m, 1H), 2.65−2.61 (m, 1H), 2.48−2.43 (m, 2H), 1.80 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K) δ 173.4, 169.3, 168.7, 168.5, 166.1, 165.6, 165.4, 134.1, 133.8, 133.7, 133.2, 132.2, 130.4, 129.1, 128.8, 128.7, 127.7, 127.3, 126.7, 126.6, 126.4, 126.2, 125.9, 125.8, 125.7, 123.51, 123.46, 123.3, 122.2, 71.4, 67.5, 66.6, 62.0, 56.9, 56.7, 52.7, 51.1, 50.6, 44.4, 43.9, 42.5, 38.1, 36.0, 22.2; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 20.3 min; HRMS-ESI *m*/*z* calcd for $C_{44}H_{41}N_5O_9$ $(M + H)^+$ 784.2977, found 784.2959.

*(3S,5S,7′R,8a′S)-7′-(Acryloyloxy)-2′-(naphthalen-1-ylmethyl)-1′,4′-dioxohexahydro-1′H-spiro[pyrrolidine-3,3′-pyrrolo[1,2-a]pyrazine]-5-carboxylic acid, dimer intermediate **24**:* HRMS-ESI *m*/*z* calcd for $C_{25}H_{25}N_3O_6$ $(M + H)^+$ 464.1816, found 464.1794.

*(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′,2″-bis[[4-[(benzyloxy)carbonyl]phenyl]methyl]-1′,1″,4′,4″-tetraoxo-7″-(pent-4-enoyloxy)-dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic Acid, Trimer **29**.* Starting from amino acid **19** (279 mg, 0.85 mmol), the synthesis of trimer **29** followed the general trimer synthesis procedure with corresponding equivalence except changing the 1-naphthyl-*pro4* amino acid **4** to benzyl carboxylate *pro4* **5** with an overall 33% isolated yield: ^{1}H NMR (500 MHz, $CDCl_3$, rt) δ 8.06−8.01 (m, 4H), 7.40−7.34 (m, 10H), 7.22−7.17 (m, 2H), 7.15 (t, *J* = 6.5 Hz, 2H), 5.82 (ddt, *J* = 16.6, 10.2, 6.3 Hz, 1H), 5.46 (dd, *J* = 9.0, 4.5 Hz, 1H), 5.36−5.31 (m, 6H), 5.29−5.17 (m, 2H), 5.11−5.00 (m, 2H), 4.65−4.34 (m, 4H), 4.24 (dd, *J* = 25.9, 16.9 Hz, 1H), 4.08 (t, *J* = 10.8 Hz, 1H), 4.06−4.00 (m, 1H), 3.96−3.71 (m, 1H), 3.70−3.51 (m, 2H), 2.88 (dd, *J* = 12.9, 6.3 Hz, 1H), 2.66 (dd, *J* = 13.5, 6.5 Hz, 2H), 2.46 (q, *J* = 7.3 Hz, 2H), 2.40 (dd, *J* = 13.2, 6.3 Hz, 2H), 2.37−2.26 (m, 3H), 1.99 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$, rt) δ 172.9, 172.1, 168.2, 167.9, 165.8, 165.7, 165.0, 164.5, 164.2, 142.0, 141.4, 136.2, 135.9, 135.8, 130.6, 130.2, 129.9, 128.7, 128.6, 128.4, 128.3, 128.1, 126.2, 126.1, 116.0, 70.2, 69.2, 67.8, 67.1, 66.9, 66.8, 62.7, 58.0, 57.1, 56.3, 53.0, 52.0, 50.0, 49.3, 46.0, 43.6, 38.2, 36.3, 33.4, 28.7, 22.3; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 24.1 min; HRMS-ESI *m*/*z* calcd for $C_{54}H_{53}N_5O_{13}$ $(M + H)^+$ 980.3713, found 980.3708.

*(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′-[(4-bromophenyl)methyl]-2″-[(4-methoxyphenyl)methyl]-1′,1″,4′,4″-tetraoxo-7″-(pent-4-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic Acid, Trimer **30**.* Starting from amino acid **19** (509 mg, 1.56 mmol) and following the general synthesis procedure of trimer **26** except changing the *pro4* amino acid **4** to the corresponding **6** and **7**, trimer **30** (560 mg, 44% overall isolated yield) was obtained as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 7.51 (d, *J* = 8.4 Hz, 2H), 7.21−7.12 (m, 2H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.91−6.86 (m, 2H), 5.85 (ddt, *J* = 16.7, 10.3, 6.4

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J. Org. Chem. 2015, 80, 8968−8978

Hz, 1H), 5.36−5.27 (m, 1H), 5.07 (dtd, J = 17.2, 3.7, 1.6 Hz, 1H), 5.00 (dddd, J = 6.7, 2.8, 2.2, 1.1 Hz, 1H), 4.92 (d, J = 16.3 Hz, 1H), 4.78 (broad, 1H), 4.73 (dd, J = 15.1, 7.4 Hz, 1H), 4.60−4.53 (m, 1H), 4.50 (dd, J = 10.5, 6.8 Hz, 1H), 4.38 (d, J = 16.3 Hz, 1H), 4.31−4.09 (m, 2H), 4.02 (dd, J = 13.5, 5.3 Hz, 1H), 3.95−3.90 (m, 1H), 3.84 (d, J = 12.2 Hz, 1H), 3.74 (s, 3H), 3.46 (d, J = 13.5 Hz, 1H), 3.31−3.22 (m, 1H), 3.01−2.68 (m, 4H), 2.47−2.43 (m, 2H), 2.38−2.32 (m, 4H), 1.92−1.83 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, rt, rotamers observed) δ 173.1, 172.33, 172.26, 169.4, 168.8, 168.7, 168.5, 168.4, 165.3, 164.6, 158.8, 158.6, 137.5, 137.3, 131.8, 131.3, 131.0, 130.0, 129.1, 128.7, 127.6, 120.6, 120.5, 120.4, 115.9, 114.3, 71.1, 67.4, 66.9, 66.5, 56.7, 56.1, 55.5, 55.4, 52.5, 51.4, 50.6, 49.9, 49.2, 46.6, 45.1, 44.6, 43.2, 37.4, 37.2, 35.7, 33.1, 28.7, 23.5, 22.5, 22.3, 21.9, 21.6, 19.4, 19.2, 19.0; HRMS-ESI m/z HRMS-ESI m/z calcd for $C_{39}H_{42}BrN_5O_{10}$ $(M + H)^+$ 820.2188, found 820.2118.

(3R,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′-[(4-bromophenyl)methyl]-2″-[(4-methoxyphenyl)methyl]-1′,1″,4′,4″-tetraoxo-7″-(pent-4-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-carboxylic Acid, Trimer ***31***. Starting from amino acid **19** (273 mg, 0.83 mmol) and following the general trimer synthesis procedure of **26** except changing the *pro4* amino acid **4** to the corresponding **6** and **8**, trimer **31** (260 mg, 38% overall isolated yield) was obtained as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.57−7.50 (m, 1H), 7.48−7.42 (m, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.85 (ddt, J = 16.7, 10.3, 6.3 Hz, 1H), 5.36−5.29 (m, 1H), 5.08 (ddd, J = 17.2, 3.4, 1.7 Hz, 1H), 5.01 (ddd, J = 10.3, 3.0, 1.4 Hz, 1H), 4.81−4.63 (m, 2H), 4.61−4.17 (m, 4H), 4.16−3.82 (m, 4H), 3.74 (s, 3H), 3.63−3.33 (m, 2H), 3.26−2.57 (m, 4H), 2.48−2.43 (m, 2H), 2.41−2.29 (m, 4H), 1.72 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6, rt, rotamers observed) δ 173.0, 172.8, 172.3, 169.4, 169.0, 168.4, 165.5, 165.4, 164.4, 163.6, 158.8, 137.4, 131.5, 131.3, 130.1, 128.9, 128.7, 127.8, 120.3, 116.0, 114.5, 71.2, 68.8, 67.0, 58.1, 57.7, 56.8, 56.3, 56.1, 55.5, 52.6, 50.3, 50.0, 46.8, 46.0, 45.8, 44.7, 37.6, 37.4, 35.9, 35.0, 33.3, 28.8, 22.2, 21.5, 19.3; HRMS-ESI m/z calcd for $C_{39}H_{42}BrN_5O_{10}$ $(M + H)^+$ 820.2188, found 820.2208.

(3R,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-5-[[(1S,2S)-2-aminocyclohexyl]carbamoyl]-2″-(2-methylpropyl)-2′-[(4-nitrophenyl)methyl]-1′,1″,4′,4″-tetraoxododecahydro-1″H-dispiro-[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-7″-yl Pent-4-enoate, ***33***. Starting from amino acid **19** (557 mg, 1.7 mmol) and following the general trimer synthesis procedure of **26** except changing the alkylated *pro4* **4** to the corresponding **9** and **10**, crude trimer **32** (780 mg, theoretically 1.08 mmol) was obtained as a brown solid, which was used in the next step without further purification. In a flame-dried round-bottom flask, crude trimer **32** (780 mg, theoretically 1.08 mmol, 1.0 equiv) and HATU (616 mg, 1.62 mmol, 1.5 equiv) were dissolved in 20 mL of dry 20 DMF/DCM (1:1) at room temperature, followed by the addition of DIPEA (561 μL, 3.24 mmol, 3.0 equiv) in one portion. The reaction mixture was stirred for 10 min and then slowly added to another flame-dried round-bottom flask containing diaminocyclohexane (740 mg, 6.48 mmol, 6.0 equiv) in 15 mL of DMF within 2 h. The reaction was stirred overnight at room temperature until completion as indicated by LC−MS. The reaction mixture was then quenched by addition of satd NH_4Cl (aq) (50 mL), and the product was extracted with EtOAc (100 mL × 3). The combined organic portion was washed sequentially with 2 × 20 mL brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the crude residue was purified by reversed-phase C_{18} column using a 5−95% H_2O/acetonitrile gradient as eluting solvent to give the pure product **33** as a yellow colored powder (270 mg, 0.33 mmol, 19% overall isolated yield from **19**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (d, J = 8.7 Hz, 2H), 7.92−7.78 (m, 1H), 7.61−7.42 (m, 2H), 5.84 (ddt, J = 16.7, 10.3, 6.4 Hz, 1H), 5.29 (t, J = 4.7 Hz, 1H), 5.06 (ddd, J = 17.2, 3.3, 1.6 Hz, 1H), 4.99 (ddd, J = 10.3, 3.0, 1.3 Hz, 1H), 4.81−4.68 (m, 1H), 4.64 (dd, J = 10.8, 6.6 Hz, 1H), 4.49 (s, 1H), 4.37−4.18 (m, 1H), 4.10 (d, J = 12.1 Hz, 1H), 4.01 (d, J = 12.2 Hz, 1H), 3.97 (dd, J = 13.5, 5.3 Hz, 1H), 3.53 (dd, J = 14.3, 8.2 Hz, 1H), 3.41 (d, J = 13.5 Hz, 1H), 3.01 (dd, J = 14.2, 6.7 Hz, 1H), 2.89 (s, 2H), 2.82 (dd, J = 13.0, 6.8 Hz, 1H), 2.46−2.41 (m, 2H), 2.36−2.30 (m, 3H), 2.26−2.22 (m, 1H), 2.14−2.02 (m, 1H), 2.00−1.88 (m, 3H), 1.80−1.74 (m, 1H), 1.73−1.65 (m, 2H), 1.38−1.30 (m, 1H), 1.26−1.19 (m, 2H), 0.86 (dd, J = 22.1, 6.7 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6, 350 K, from HSQC and HMBC, overlap observed) δ 171.3, 171.2, 170.8, 168.4, 168.0, 167.9, 167.3, 164.7, 164.4, 145.3, 136.7, 136.3, 136.1, 136.0, 126.6, 122.6, 114.6, 114.5, 114.3, 70.3, 70.1, 69.9, 69.8, 69.4, 65.6, 65.5, 57.3, 55.7, 55.6, 55.4, 55.2, 55.0, 54.5, 52.4, 50.7, 50.5, 50.0, 48.3, 47.4, 44.2, 44.1, 36.2, 36.1, 34.5, 34.3, 31.7, 29.3, 29.1, 28.4, 27.3, 26.9, 22.3, 20.8, 18.7; HRMS-ESI m/z calcd for $C_{41}H_{54}N_8O_{10}$ $(M + H)^+$ 819.4036, found 819.4030.

General Synthetic Procedure of Macrocycles 38−40. A representative synthesis of macrocycle **38** is described as follows: In a flame-dried round-bottom flask, trimer **26** (41 mg, 0.05 mmol, 1.0 equiv) and HATU (23 mg, 0.06 mmol, 1.2 equiv) were dissolved in dry 1.65 mL DMF/DCM (10:1) at room temperature followed by the addition of DIPEA (21 μL, 0.12 mmol, 2.4 equiv) in one portion. The reaction mixture was stirred for 10 min and then slowly added to another flame-dried round bottle containing diaminocyclohexane (2.57 mg, 0.023 mmol, 0.45 equiv) in 0.5 mL of DMF (this solution was prepared from a stock solution of 25.7 mg diaminocyclohexane in 5 mL DMF) within 2 h. The reaction was stirred overnight at room temperature until completion as indicated by LC−MS. The reaction mixture was then quenched by addition of satd NH_4Cl (aq) (10 mL), and the product was extracted with EtOAc (50 mL). The combined organic portion was washed sequentially with 3× satd NH_4Cl (aq) and 2× brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the crude residue was purified by a reversed-phase C_{18} column using 5−95% H_2O/acetonitrile gradient as eluting solvent to give pure product **34** as white powder (30 mg, 0.017 mmol, 78% isolated yield).

Compound **34** (17 mg, 0.01 mmol, 1.0 equiv) in 4 mL of DCM was degassed using bubbling argon for 5 min, followed by the addition of Hoveyda−Grubbs Catalyst II (3.1 mg, 5 × 10^{-3} mmol, 0.5 equiv) in one portion. The reaction was stirred under inert atmosphere for 24 h until completion as indicated by LC−MS. The solvent was removed under vacuum, and the residue was purified through reversed-phase C_{18} column using 5−95% H_2O/acetonitrile gradient as eluting solvent to give pure macrocycle **38** as a white powder (12 mg, 7.2 × 10^{-3} mmol, 72% isolated yield).

(3S,5S,7′S,7″R,8′aS,8′aS,8″aS)-5-[[(1S,2S)-2-[[(3S,5S,7′S,7″R,8′aS,-8″aS)-1-acetyl-2′,2″-bis(naphthalene-1-ylmethyl)-1′,1″,4′,4″-tetraoxo-7″-(pent-4-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″bis(bis(pyrrolo[1,2-1]piperazine)]-5-yl]amido]cyclohexyl]-carbamoyl]-1-acetyl-2″,2″-bis(naphthalene-1-ylmethyl)-1′,1″,4′,4″-tetraoxododecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-7″-yl pent-4-enoate, ***34:*** ^{1}H NMR (500 MHz, DMSO-d_6, 350 K) δ 8.11 (t, J = 4.1 Hz, 3H), 8.03 (s, 2H), 7.97 (d, J = 9.3 Hz, 2H), 7.95 (d, J = 6.6 Hz, 2H), 7.86−7.80 (m, 4H), 7.63−7.48 (m, 9H), 7.47−7.41 (m, 4H), 7.24−7.04 (m, 4H), 5.83 (qd, J = 12.1, 6.1 Hz, 2H), 5.50 (d, J = 17.4 Hz, 2H), 5.46−5.34 (m, 3H), 5.29 (s, 1H), 5.06 (d, J = 17.2 Hz, 1H), 5.00 (d, J = 9.5 Hz, 1H), 4.97−4.66 (m, 6H), 4.54 (broad s, 2H), 4.22 (broad s, 1H), 4.03 (broad s, 1H), 3.99 (d, J = 12.5 Hz, 2H), 3.78 (d, J = 12.5 Hz, 2H), 3.66−3.30 (m, 4H), 2.98 (dd, J = 13.1, 6.7 Hz, 2H), 2.83−2.63 (m, 4H), 2.47−2.07 (m, 16H), 1.80−1.44 (m, 12H), 1.14 (broad s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.4, 172.1, 170.7, 168.6, 168.3, 165.6, 162.8, 137.4, 133.8, 133.2, 133.0, 130.5, 130.3, 129.1, 127.8, 126.7, 126.4, 125.9, 123.3, 123.0, 122.8, 122.2, 119.0, 116.7, 115.8, 114.4, 112.2, 70.9, 67.5, 56.9, 56.6, 52.6, 50.7, 44.0, 38.3, 36.1, 33.4, 32.1, 31.6, 29.4, 29.2, 29.0, 28.9, 28.7, 27.4, 26.2, 24.6, 22.4, 14.1; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 26.5 min; HRMS-ESI m/z calcd for $C_{98}H_{100}N_{12}O_{16}$ $(M + 2H)/2^+$ 851.8779, found 851.8763.

Macrocycle ***38:*** ^{1}H NMR (500 MHz, $CDCl_3$, rt) δ 8.05 (d, J = 8.3 Hz, 2H), 7.99−7.86 (m, 6H), 7.80 (t, J = 7.5 Hz, 4H), 7.56 (broad s, 8H), 7.41 (broad s, 4H), 7.02 (d, J = 6.6 Hz, 2H), 6.95 (d, J = 6.6 Hz, 2H), 6.85 (s, 2H), 5.69 (dd, J = 23.7, 17.1 Hz, 4H), 5.51 (d, J = 24.6 Hz, 4H), 4.94 (d, J = 17.3 Hz, 2H), 4.76 (d, J = 16.7 Hz, 2H), 4.62 (dd, J = 16.5, 10.2 Hz, 4H), 4.46 (t, J = 8.4 Hz, 2H), 4.32−4.05 (m, 4H), 3.93 (d, J = 13.2 Hz, 2H), 3.84 (d, J = 11.4 Hz, 2H), 3.66 (d, J =

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13.9 Hz, 2H), 3.52 (s, 2H), 3.40 (d, J = 11.4 Hz, 2H), 3.07−2.77 (m, 3H), 2.78−2.62 (m, 3H), 2.62−2.53 (m, 2H), 2.53−2.21 (m, 10H), 1.73−1.56 (m, 12H), 1.21−1.05 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$, rt) δ 172.1, 170.2, 169.8, 168.1, 167.6, 165.7, 165.4, 133.9, 133.8, 131.8, 130.6, 130.2, 130.1, 129.3, 128.9, 128.5, 128.2, 126.7, 126.5, 126.3, 126.2, 125.4, 125.2, 122.7, 122.0, 121.7, 121.5, 70.2, 67.9, 67.4, 57.1, 56.4, 54.7, 53.5, 53.2, 52.0, 50.6, 44.2, 44.0, 38.5, 36.6, 35.5, 33.8, 31.7, 29.7, 27.5, 24.2, 22.3; LC−MS analysis at 274 nm (C_{18} reversed-phase, 30 min, 5−95% H_2O/acetonitrile with 0.1% formic acid) t_R = 25.5 min; HRMS-ESI m/z calcd for $C_{96}H_{96}N_{12}O_{16}$ (M + 2H)/2$^+$ 837.8622, found 837.8607.

Macrocycle ***39***. Starting from trimer **26** (82 mg, 0.1 mmol, 1.0 equiv), macrocycle **39** was obtained through intermediate **35** by following the identical procedure as macrocycle **38** except changing diaminocyclohexane to 1,2-ethanediamine (2.7 mg, 0.045 mmol, 0.45 equiv) (49% overall isolated yield, 67% in the first step and 73% in the second step): LC−MS analysis at 274 nm (C_{18} reversed-phase, 30 min, 5−95% H_2O/acetonitrile with 0.1% formic acid) t_R = 24.9 min; HRMS-ESI m/z calcd for $C_{92}H_{90}N_{12}O_{16}$ (M + H)$^+$ 1619.660, found 1619.664.

Macrocycle ***40***. Starting from trimer **27** (110 mg, 0.138 mmol, 1.0 equiv), macrocycle **40** was obtained through intermediate **36** by following the identical procedure as macrocycle **38** except changing diaminocyclohexane to 1,2-ethanediamine (3.7 mg, 0.062 mmol, 0.45 equiv) (36% overall isolated yield, 60% in the first step and 61% in the second step): LC−MS analysis at 274 nm (C_{18} reversed-phase, 30 min, 5−95% H_2O/acetonitrile with 0.1% formic acid) t_R = 23.5 min; HRMS-ESI m/z calcd for $C_{90}H_{86}N_{12}O_{16}$ (M + 2H)/2$^+$ 796.8231, found 796.8251. Intermediate **36**: HRMS-ESI m/z calcd for $C_{92}H_{90}N_{12}O_{16}$ (M + 2H)/2$^+$ 810.3372, found 810.3347.

(3S,5S,7′S,7″R,8′aS,8″aS)-5-[[2-[(3S,5S,7′S,7″R,8′aS,8″aS)-1-Acetyl-2′,2″-bis(naphthalen-1-ylmethyl)-1′,1″,4′,4″-tetraoxo-7″-(prop-2-enoyloxy)dodecahydro-1″H-dispiro[pyrrolidine-3,3′:7′,3″-bis(pyrrolo[1,2-a]piperazine)]-5-ylformamido]ethyl]carbamoyl)-1-acetyl-2′,2″-bis(naphthalen-1-ylmethyl)-1′,1″,4′,4″-tetraoxododecahydro-1″H-dispiro[pyrrolidine3,3′:7′,3″bis(pyrrolo[1,2-a]piperazine)]-7″-yl Prop-2-enoate, Compound ***37***. Starting from trimer **28** (20 mg, 0.025 mmol, 1.0 equiv) and 1, 2-ethanediamine (0.68 mg, 0.011 mmol, 0.45 equiv), compound **37** (14 mg, 8.8 × 10^{-3} mmol, 80% yield) was obtained by following the same general procedure as for **35**: HRMS-ESI m/z calcd for $C_{90}H_{86}N_{12}O_{16}$ (M + 2H)/2 796.8231, found 796.8254. The metathesis reaction of compound **37** resulted in a HPLC trace that could not be interpreted and no mass corresponding to the product was found.

Synthetic Procedure of Macrocycle 46. At room temperature, trimer **30** (60 mg, 0.073 mmol, 1.0 equiv) and HATU (56 mg, 0.147 mmol, 2.0 equiv) were dissolved in 1.5 mL of anhydrous DMF/DCM (2:1) in a flame-dried vial, followed by the addition of DIPEA (51 μL, 0.293 mmol, 4.0 equiv) in one portion. The reaction mixture was stirred for 10 min and transferred dropwise to another flame-dried vial containing amine **33** (60 mg, 0.073 mmol, 1.0 equiv) in 1 mL of anhydrous DMF under inert atmosphere. The reaction mixture was stirred overnight at room temperature until completion as indicated by LC−MS. The reaction mixture was then quenched by addition of satd NH_4Cl (aq) (10 mL), and the products were extracted with EtOAc (50 mL). The combined organic portion was washed sequentially washed with 3× satd NH_4Cl (aq), 3× satd $NaHCO_3$ (aq), and 2× brine and dried over anhydrous Na_2SO_4. After filtration and removal of solvent under vacuum, the crude residue was purified by reversed-phase C_{18} column using 5−95% H_2O/acetonitrile gradient as eluting solvent to give pure dialkene as white powder **42** (70 mg, 0.043 mmol, 59% isolated yield). The dialkene intermediate **42** was then subjected to the metathesis reaction as described in synthesis of macrocycle **38** to give macrocycle **44** with an 80% isolated yield. The hydrogenation of **44** with palladium on activated carbon with H2 balloon afforded pure macrocycle **46** as a white powder with an 80% isolated yield: ^{1}H NMR (500 MHz, C_6D_6, rt, TFA-*d* added) δ 7.99 (d, J = 8.2 Hz, 2H), 7.47−7.41 (m, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.36−7.29 (m, 1H), 7.04 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.09−4.78 (m, 3H), 4.76−4.65 (m, 3H), 4.61 (d, J = 15.5 Hz, 1H), 4.57−4.28 (m, 7H), 4.24 (d, J = 12.5 Hz, 1H), 4.11 (d, J = 12.5 Hz, 1H), 4.06−3.97 (m, 2H), 3.96−3.89 (m, 1H), 3.85−3.78 (m, 1H), 3.76−3.70 (m, 2H), 3.69−3.63 (m, 1H), 3.57−3.40 (m, 4H), 3.30 (s, 3H), 3.16−2.96 (m, 2H), 2.83 (dd, J = 13.9, 6.3 Hz, 1H), 2.75−2.67 (m, 1H), 2.64−2.50 (m, 2H), 2.49−2.41 (m, 2H), 2.41−2.30 (m, 2H), 2.24−2.10 (m, 4H), 1.96 (d, J = 9.9 Hz, 4H), 1.93−1.87 (m, 2H), 1.86−1.77 (m, 2H), 1.72−1.65 (m, 1H), 1.55−1.43 (m, 5H), 1.37−1.26 (m, 8H), 1.19−1.07 (m, 8H), 0.96−0.88 (m, 4H); ^{13}C NMR (126 MHz, C_6D_6, rt, collected from HSQC and HMBC, overlapping observed) δ 171.4, 169.7, 169.3, 159.4, 147.6, 142.2, 132.5, 127.9, 127.8, 126.9, 126.7, 124.2, 114.8, 70.9, 67.4, 67.0, 57.5, 57.4, 56.9, 56.8, 56.0, 55.9, 54.7, 53.4, 53.2, 51.1, 50.6, 50.1, 49.5, 46.2, 46.0, 45.8, 36.3, 35.7, 35.5, 35.2, 35.1, 34.9, 33.4, 31.3, 31.2, 29.7, 28.7, 19.1, 19.0, 18.7, 13.9; NMR including COSY, HSQC, HMBC, and ROESY, and characterization are included in the Supporting Information; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 22.2 min; HRMS-ESI m/z calcd for $C_{78}H_{92}BrN_{13}O_{19}$ (M + H)$^+$ 1594.5889, found 1594.5886.

Macrocycle ***47***. Starting from trimer **33**, macrocycle **47** was obtained with a 20% overall isolated by following the synthesis of macrocycle **46** except changing trimer **30** to **31**: ^{1}H NMR (500 MHz, C_6D_6, rt, TFA-*d* added, several conformations observed) δ 7.45 (dd, J = 18.8, 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.27−7.20 (m, 1H), 7.13−7.08 (m, 2H), 7.04−6.99 (m, 2H), 6.82 (dd, J = 22.9, 8.2 Hz, 2H), 6.76−6.67 (m, 3H), 4.90−4.67 (m, 6H), 4.66−4.59 (m, 1H), 4.55−4.46 (m, 2H), 4.44−4.34 (m, 2H), 4.32−4.26 (m, 1H), 4.26−4.13 (m, 3H), 4.12−4.03 (m, 2H), 4.03−3.86 (m, 3H), 3.86−3.76 (m, 2H), 3.75−3.60 (m, 3H), 3.59−3.46 (m, 3H), 3.43 (d, J = 13.8 Hz, 1H), 3.28−3.16 (m, 2H), 3.15−3.06 (m, 3H), 3.06−2.61 (m, 6H), 2.55−2.37 (m, 4H), 2.16−2.08 (m, 4H), 1.99−1.90 (m, 3H), 1.88 (s, 3H), 1.82−1.74 (m, 4H), 1.60−1.50 (m, 4H), 1.07 (m, 16H, overlap with grease and impurities, not identified); ^{13}C NMR (126 MHz, C_6D_6, rt, collected from HSQC and HMBC, overlapping observed) δ 181.9, 179.2, 176.1, 174.3, 173.5, 170.3, 166.2, 165.5, 159.6, 150.0, 149.4, 148.3, 147.8, 142.6, 139.2, 133.4, 131.3, 130.7, 130.0, 127.8, 124.7, 123.1, 87.6, 74.3, 72.3, 67.9, 67.6, 67.4, 64.6, 53.9, 50.6, 47.6, 46.8, 46.1, 35.5, 35.2, 34.9, 33.9, 32.6, 31.7, 31.5, 30.5, 29.8, 29.3, 29.0, 28.0, 27.2, 24.8, 23.3, 21.8, 19.7, 19.5, 14.5; NMR including COSY, HSQC, HMBC and ROESY, and characterization are included in the Supporting Information; LC−MS analysis at 274 nm (C_{18} reversed-phase, 40 min, 5−100% H_2O/acetonitrile with 0.1% formic acid) t_R = 21.9 min; HRMS-ESI m/z calcd for $C_{78}H_{92}BrN_{13}O_{19}$ (M + H)$^+$ 1594.5889, found 1594.5915.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01109.

General information, ^{1}H and ^{13}C NMR spectra, and LCMS spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: meister@temple.edu.

Notes

The authors declare no competing financial interest.

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