Metal-Binding Q-Proline Macrocycles

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Abstract

Herein, we introduce the efficient synthesis of Q-proline (Q-Pro) based, metal-binding macrocycles (QPM), which can display up to nine functional groups. Synthesis of eight QPM was achieved through standard Fmoc-SPPS and peptoid chemistry. QPM are disordered in the absence of a metal cation, as evidenced by NMR and a crystal structure of **QPM-3** obtained through racemic crystallization. Addition of metal cations cause these macrocycles to adopt ordered, uniform core structures regardless of the functional groups. Alkylation of QPM allows for addition of reactive functional groups as the final step in a synthesis. Interestingly, the addition of secondary functional groups to the hydantoin amide position (R_2) converts the proline ring from Cγ-endo to Cγ-exo, due to steric interactions.

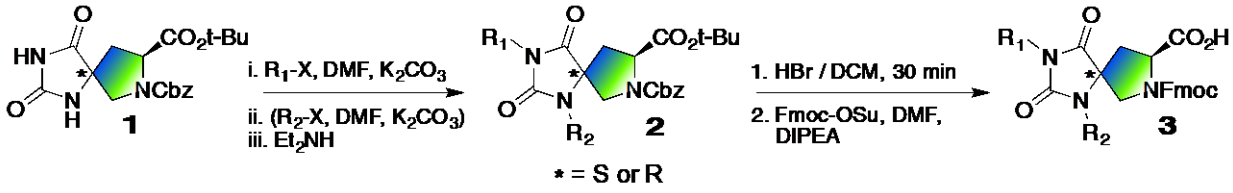
Introduction

Mimicking amino acids and peptides with unnatural amino acids or peptidomimetics has been a vital area of research for many decades. During this time, significant effort has been applied to using many different peptidomimetics, from beta-amino acids[1,2] and stapled peptides[3-6] to peptoids[7-10] and spiroligomers[11,12] to name a few. One particular amino acid of interest is proline, which is prevalent across a wide array of natural and synthetic structures,[13-17] with nonproteinogenic proline being used for conformationally rigid peptides, angiotensin converting enzyme inhibitors,[13] and asymmetric synthesis[15-17] among other applications.[17] Many nonproteinogenic prolines are functionalized at the 4-position of the pyrrolidine ring, synthetically accessed from L-trans-4-hydroxyproline.[14,17] Recently, we reported the synthesis of a new, conformationally-constrained quaternary residue, Q-proline (Q-Pro), that is also derived from L-trans-4-hydroxyproline.[18] The synthesis of Q-proline is straightforward even on multigram scale with minimal purification required. Q-pro residues have two stereocenters that are controlled in the synthesis, as well as two functional groups that are introduced after the stereochemistry is set. This allows us to synthesize large quantities of a stereochemically-pure, unfunctionalized building-block, and later attach functionality through simple alkylation chemistry to create Q-Pro residues. This decoupling of functional group installation from setting of stereochemistry allows the stereochemistry of the building block to be introduced, the diastereomers separated, and then functional groups are added, avoiding mixtures of stereoisomers that are difficult to separate. Given their pre-organized core, and the enormous diversity afforded by

incorporating two functional groups through simple alkylation reactions, these Q-Pro residues could prove useful in the development of peptidomimetics with new properties.

Results and Discussion

Starting from the stereochemically pure hydantoin **1**, we synthesized mono- and difunctionalized proline amino acids on a 10 gram scale of **1** as shown in **Figure 1**. A facile protecting group interchange from N-benzyloxycarbonyl (Cbz) to N-9-fluorenylmethyloxycarbonyl (Fmoc) afforded the compounds **4-15** (**QPro 1-12**) which were purified by reversed-phase flash chromatography (see **Figure SI-1**.) This synthesis over 3 steps yields the Q-prolines **4-15** in moderate to good overall yields (55-78%) from **1**.



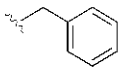
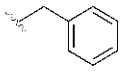
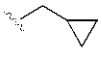
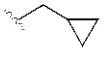
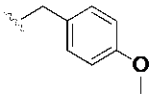
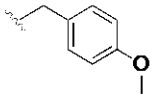
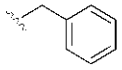
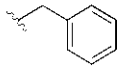
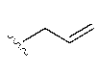
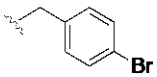
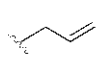
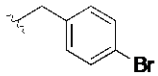
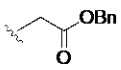
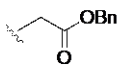
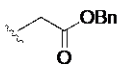
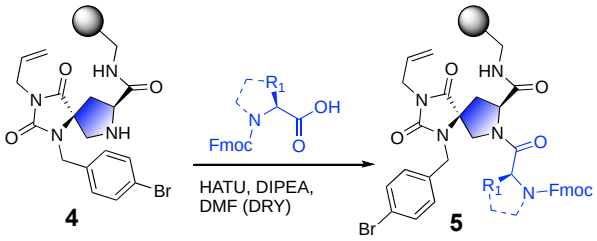
Name	Stereo-chem.	R_1	R_2	% Yield	Name	Stereo-chem.	R_1	R_2	% Yield
Q-Pro 1	S			75	**Q-Pro 7**	R			70
Q-Pro 2	S			75	**Q-Pro 8**	R			73
Q-Pro 3	S			70	**Q-Pro 9**	R			71
Q-Pro 4	S			78	**Q-Pro 10**	R			73
Q-Pro 5	S			58	**Q-Pro 11**	R			56
Q-Pro 6	S			53**	**Q-Pro 12**	S			53**

Figure 1. Synthesis of Q-prolines 1-12 from the stereochemically pure starting material **1.** **Q-Pro 6 and Q-Pro 12 were synthesized together and separated via Flash Chromatography, yield represents combined yields

The Q-proline derivatives were evaluated for standard Fmoc-SPPS. Q-Pro **5** was loaded onto rink amide resin to provide **4**, followed by quantitative Fmoc release. A proteinogenic amino acid (glycine, alanine, or proline) was then coupled to the resin bound Q-Pro (**Figure 2**) to synthesize dipeptide **5**. For proline, regardless of equivalents or double couplings (**Figure 2**, Trials 9-13), quantitative coupling to the resin bound Q-proline could not be achieved. Trials 11-13 are shown in **Figure SI-3A**, where a majority of the starting material **4** remains. We hypothesize this is due to steric clashes associated with the



Trial	Eq.	Res.	Time	Temp (℃)	2x Coup.	HPLC Est. % Comp.
1	3	Gly	1h	RT	No	100
4	3	Gly	1h	RT	Yes	100
7	3	Ala	1h	RT	No	100
8	3	Ala	1h	RT	Yes	100
9	2	Pro	15h	RT	No	50
10	5	Pro	15h	RT	No	60
11	2	Pro	1h	RT	Yes	25
12	3	Pro	1h	RT	Yes	50
13	4	Pro	1h	RT	Yes	50
14	2	Pro	1h	50	No	>98
15	2	Pro	2h	50	No	>98
16	2	Pro	1h	50	Yes	100
17	2	Pro	2h	50	Yes	100
27	3	Pro	3-2	60	No	>98
28	3	QP9	3-4	60	Yes	50
29	3	QP9	3-4	60*	Yes	>98
30	3	QP15	3-4	60*	Yes	>98

Figure 2. Coupling proteinogenic residues to a solid supported Q-proline residue at varied temperatures. Trials 27-30 were performed with a microwave reactor; time 3-2 denotes a 3 minute ramp to temp and a 2 minute hold at temp. Temp 60° for trials 29-30 signifies use of compressed air cooling during the hold. More trials shown in **Figure SI-2**, and explanation of estimated percent completion.

functional group on the amide of the hydantoin (**Figure SI-3B**). In previous studies,[19] a group on this amide displayed a steric blocking effect. Carrying out the coupling reactions at higher temperatures allows for quantitative couplings with proline (**Figure 2**, Trials 14-17). To facilitate more rapid syntheses of peptides incorporating hindered Q-Pro residues, we utilized a microwave reactor (**Figure 2**, Trials 27-30) based on protocols set forth in Murray and Gellman.[2] Proteinogenic proline was incorporated using a 3 min ramp to a hold

temperature of 60 °C, followed by a 2 min hold, which provided near quantitative coupling (yields > 98%.) We also attempted to couple two Q-Pro, as shown in Trial 28; however, with the same conditions utilized for proline, we only achieved approximately 50% coupling. By employing compressed air cooling during the hold time (Trials 29-30) – which forces continuous irradiation by the microwave to maintain temperature – we successfully coupled two Q-Pro residues with excellent yield.

The overarching goal of this work was to create a scaffold that adopts a well-defined 3-dimensional structure in solution. We predicted that by incorporating these Q-Pro residues into peptoid macrocycles, we could develop scaffolds that display functional groups above and around the periphery of the macrocyclic core. As shown in **Figure 3**, we synthesized the macrocycles by loading bromoacetic acid onto 2-Cl-trityl chloride resin using chemistry developed by Shin,[20] followed by standard Fmoc-SPPS or peptoid couplings to make mono-alkylated precursors. Macrocyclization of the linear precursors was achieved in yields ranging from 60-67% utilizing either a syringe pump or extreme dilution to provide the monoalkylated Q-proline macrocycles (For exact structures, see **Figure SI-5** or .) We applied the alkylation chemistry[18] to **QPM-3** (**Figure 4A**) to modify the amide functional group (R_2), synthesizing **QPM-5** and **QPM-6** (**Figure SI-5**.) This late stage alkylation is a unique property of Q-Pro derivatives, allowing for the addition of reactive functional groups to a molecule as the final step in the synthesis.

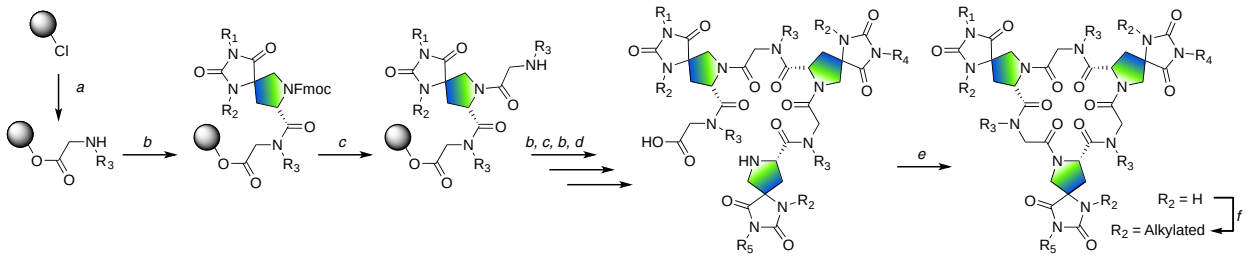


Figure 3. (A) Solid phase synthesis of Q-proline macrocycles on 2-Cl-Trt-Cl resin. *(a)* i. bromoacetic acid, DCM, DIPEA; ii. R_3-NH_2, DMF; *(b)* Q-Pro, HATU, DMF, DIPEA; *(c)* i. 20% Piperdine/DMF; ii. bromoacetic acid, DIC, DMF; iii. R_3-NH_2, DMF; *(d)* i. 20% Piperdine/DMF; ii. 30% HFIP/DCM; *(e)* DMF, PyAOP, DIPEA. *(f)* alkenyl or benzyl bromide, DMF, K_2CO_3

When injected on an HPLC, the macrocycles elute in multiple peaks at room temperature; however, by heating the column during elution (60 °C), the peaks coalesce, sometimes into a single peak. This suggests that at room temperature, these macrocycles might exist in multiple slowly-interconverting conformations. To determine if the conformations were interconverting at room temperature – or whether they were locked into a specific conformation – we isolated the corresponding HPLC peaks on an analytical column and left them in solution overnight. Upon reinjection, the samples equilibrated to provide a mixture of peaks, consistent with multiple slowly-interconverting conformations at room temperature.

Several research groups have shown that 6-residue (18-member) macrocycles containing proline exhibit multiple slowly-interconverting conformations at room temperature;[21-23] however, these conformations can be biased by internal steric interactions such as a well-placed methyl group[24] or via external sources such as cation-carbonyl interactions. Q-Pro containing macrocycles contain more functional groups than these previously described macrocycles. To test whether QPM could adopt well-defined structures in solution, we screened multiple metal triflates against **QPM-3**. The alkali triflates (lithium, sodium, and potassium) showed the best results for metal binding, each giving clean NMR spectra indicative of three-fold symmetry with 4 equiv. of the metal triflate, whereas other triflates (magnesium, zinc, calcium, scandium, copper and europium, **Figure SI-6**) did not provide symmetric structures of the macrocycles. To test the optimum conditions for obtaining single structures in solution, we varied the equiv of potassium triflate from 0.1 to 4.0 equiv. We found that 2.0 equiv of metal triflate was required to obtain a well-defined NMR spectrum (**Figure 4C**); however, simplification of the spectrum was noticeable after addition of just 0.5 equiv of potassium triflate. Furthermore, subsequent experiments demonstrated that after addition of potassium triflate, 4.75 hours are required for the spectra to become sharp and simple (**Figure 4D**, 285 min). We hypothesize this is due to the macrocycles having multiple, slowly-interconverting conformations in the absence of metal (as shown by HPLC), and that on the introduction of metal they become more ordered. The

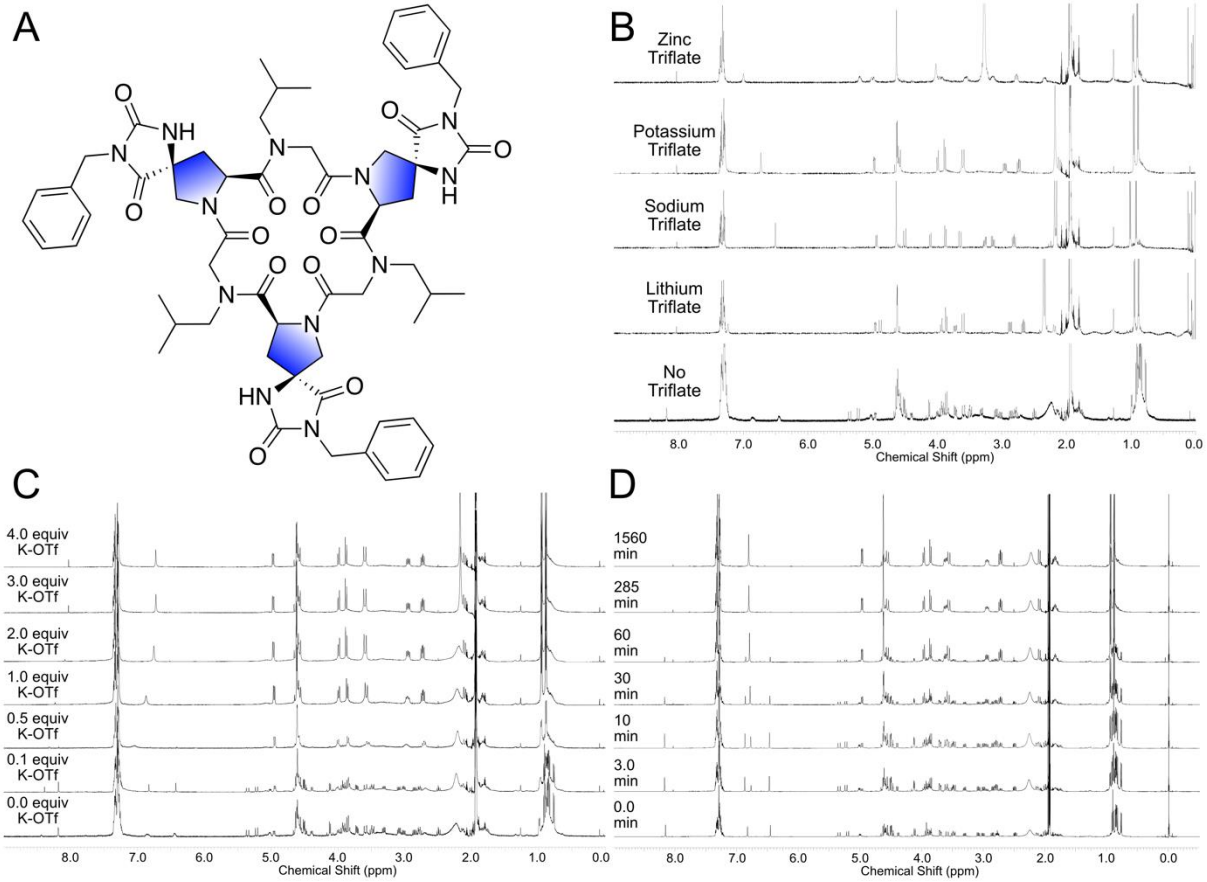


Figure 4. (A) Structure of **QPM-3;** (B) ^{1}H NMR of **QPM-3** with 4.0 equiv of various metal triflates; (C) ^{1}H NMR of **QPM-3** with various equiv of potassium triflate (K-OTf); (D) ^{1}H NMR of **QPM-3** with 4.0 equiv of potassium triflate with varied mix times.

five-hour time for the spectra to become ordered suggests a barrier to conversion of at least 17 kcal.

With the presumed ability to obtain single-conformation structures in solution of both symmetric and asymmetric macrocycles, we tested if changes in the functional groups at either the R_1 or R_3 positions would impart structural shifts of the Q-proline macrocycles. We obtained multiple 2D NMR spectra for each macrocycle (COSY, HSQC, HMBC, ROESY). An overlay of COSY spectra for **QPM-1**, **QPM-2**, and **QPM-3** show remarkable similarities in shifts for the α (4.98-5.02 ppm), β (2.10-2.16 ppm and 2.70-2.74 ppm), and δ (3.83 -3.87 ppm and 3.96-4.00 ppm) protons of the proline ring (**Figure SI-8**). Furthermore, ROESY data for each macrocycle corroborates these findings: for each macrocycle of the series, the proline α proton (group 1, HA) correlates to the "α" proton of the peptoid functional group (group 4, HA1). The amide proton of the hydantoin (R_2 = H) correlates to both a β proton (group 1, HB2) and a δ proton (group 1, HD1), indicating that the proline envelope most likely favors the Cγ-endo conformation for all the macrocycles. The COSY and ROESY data suggest that despite any functional group changes at the R_1 or R_3 positions, the overall structure of the macrocyclic core remains fairly uniform in the presence of an alkali cation.

The dialkylated macrocycles **QPM-5** through **QPM-7** show similar conformational flexibility and alkali-metal binding as the monoalkylated macrocycles. However, a marked difference in the proline envelope conformation for the dialkylated macrocycles was observed, as shown by a ROESY overlay of **QPM-3** and **QPM-5** (see **Figure SI-9**). Upon addition of any functional group to the R_2 position, the proline envelope flips into a Cγ-exo conformation, evidenced by the lack of any correlation between the new R_2 group and the β or δ protons of the proline ring; furthermore, a new correlation appears between a β proton (group 1, HB1) and a δ proton (group 1, HD2) that was not present in the monoalkylated macrocycles. We hypothesize that a steric clash exists between the new R_2 group and the proline carbonyl in the Cγ-endo conformation. The envelope flip suggests that rather than pointing towards the core of the macrocycle, the R_2 group is pointing away from the proline ring.

Following work pioneered by Stephen Kent on racemic crystallization of proteins,[25] we synthesized **QPM-8**, the enantiomer to **QPM-3**, with the rationale that an achiral, racemic complex has significantly more three-dimensional space groups available to it than does a chiral molecule. By synthesizing each macrocycle stereochemically pure, and then mixing equimolar quantities into ethyl acetate with hexanes as the diffusion solvent, we were able to obtain microcrystals of the racemate of **QPM-3** (**Figure 5**) in the absence of metal, a first

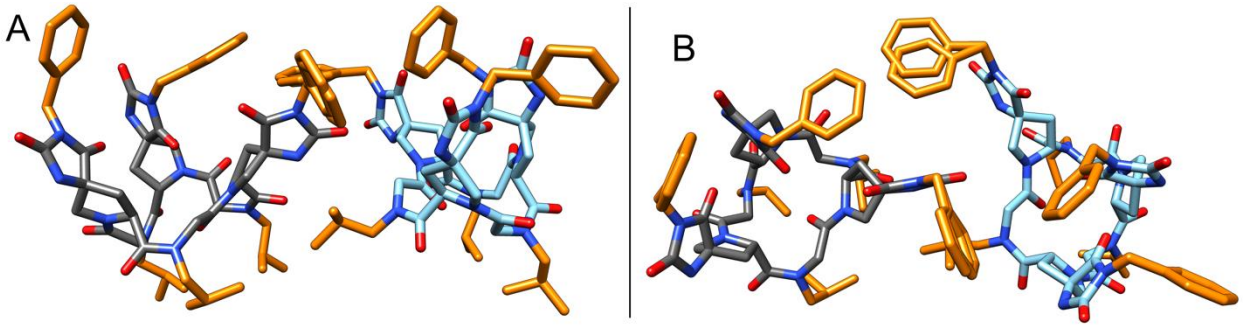


Figure 5. Racemic asymmetric unit of the crystal structure for **QPM-3** (grey) and its enantiomer (light-blue) with side chains shown in orange (solvent omitted for clarity). (A) Side view across the macrocycle (B) Top down view looking through the macrocyclic core.

for these types of peptoid macrocycles. The diversity of *cis* and *trans* amides seen in the crystal structure supports the idea that unbound macrocycles are highly disordered. When comparing the unbound vs. bound state, the core structure of the macrocycle goes through a ring inversion between the unbound crystal structure and the metal bound NMR structure. This ordering of macrocycles upon cation binding is consistent with simulation studies by Hurley, et al (Unpublished) in which ROE-restrained modeling [26] and directly observed cation association dynamics confirms an ordered, predominantly *trans*-amide, macrocycle conformation.

Conclusion

In summary, this work introduces Fmoc-protected Q-Pro amino acids, a modification of our previously work, which display two functional groups that are added after the stereochemistry has been set. These derivatives are synthesized on multigram scale with a single reversed-phase purification, and are readily incorporated into standard Fmoc-SPPS. The resultant peptidomimetics are disordered in the absence of a metal cation, as evidenced by the NMR and the racemic crystal structure which was obtained by synthesizing both enantiomers separately, then mixing equimolar quantities of each enantiomer. In the presence of a metal cation, these macrocycles adopt a uniform structure regardless of the functional groups appended to the molecules. Addition of a functional group to the hydantoin amide position converts the proline ring from a Cγ-endo to a Cγ-exo conformation, most likely due to steric strain. Future work will be focused on application of these new amino acids towards catalytic, peptidomimetic, and materials research.

Supporting Information

Supporting Information is available, including experimental procedures, analytical data (1H, 13C, HSQC, HMBC, and MS), and crystallographic information file.

Acknowledgements

J. D. N., J. A. W., and C. E. S. were supported by the Department of the Defense, Defense Threat Reduction Agency (HDTRA1-16-1-0047). V.A.V. and M.F.D.H. were supported by National Institutes of Health (NIH) grant 1R01GM123296. This work was supported in part by the National Institute of General Medical Sciences 1R01GM114358 of the NIH to RHGB. This research was supported in part by the National Science Foundation through major research instrumentation grant number 1625061, and by the US Army Research Laboratory under contract number W911NF-16-2-0189.

Experimental Details:

General Procedure 1 – Synthesis of Q-Proline residues.

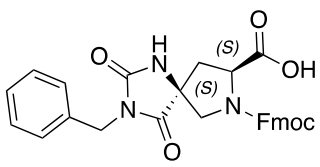
Step 1 – Alkylation: Reference 18.

Step 2 – Protecting group modification:

These compounds are deprotected using a 1:1 mixture of DCM/(33% HBr in AcOH) for 30 minutes, and the solvent removed *in vacuo*. The deprotected amino acid is dissolved in DMF and free based with 3 equiv of DIPEA, after which 1.1 equiv of Fmoc-OSu is added to the

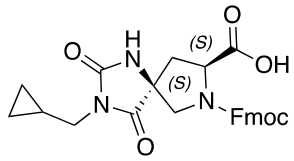
reaction. This is stirred for 2 hours, and the progress checked via HPLC-MS. Upon completion, the reaction is diluted with EtOAc, washed with ammonium chloride and brine, dried with Na_2SO_4, concentrated *in vacuo* to yield dark yellow solids, which are purified by reverse phase flash chromatography (5-95% acetonitrile in water, 0.1% formic acid). The fractions are combined, the acetonitrile removed *in vacuo*, and the product extracted from the residual aqueous mixture with EtOAc, rinsed with brine, and dried over Na_2SO_4. The EtOAc is removed *in vacuo*, to yield off white Fmoc protected amino acids **Q-Pro 1-12**.

QPro 1 (5S,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-benzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid
Using general procedure 1, 10.50 g **1** (2S, 4S), 0.88 equiv benzyl bromide, recovered 10.1 g pure (75%)



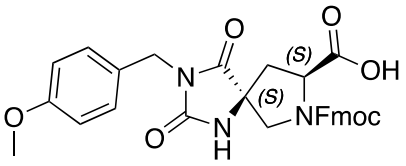
^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.22 (1H, dd, *J* = 13.9, 2.2), 2.88 (1H, m), 3.94 (1H, m), 3.97 (1H, d, *J* = 6.3), 4.21 (1H, m), 4.37 (1.5H, m), 4.55 (1H, m), 4.65 (0.5H, d, *J* = 9.5), 4.71 (2H, m), 7.33 (10H, m), 7.58 (4H, m), 8.58 (1H, s, rotameric); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 39.8, 40.8, 42.8, 42.9, 47.0, 47.1, 55.7, 56.4, 57.7, 58.2, 66.1, 67.2, 68.1, 76.8, 77.1, 77.3, 120.0, 125.0, 125.1, 127.0, 127.2, 127.7, 127.8, 128.4, 128.5, 128.6, 128.91, 128.93, 135.27, 141.2, 141.28, 141.29, 141.4, 143.5, 143.8, 143.9, 153.5, 154.0, 158.2, 158.3, 172.1, 172.3, 176.3, 176.4; HRMS-ESI: m/z calcd for $C_{29}H_{25}N_3O_6Na$ $(M+Na)^+$ 534.1636, found 544.1645

QPro 2 (5S,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid
Using general procedure 1, 10.50 g **1** (2S, 4S), 1.25 equiv of (bromomethyl)cyclopropane, recovered 9.79 g pure (75%)



^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 0.35 (2H, m), 0.52 (2H, m), 1.18 (1H, m), 2.26 (1H, d, *J* = 14.2), 2.89 (1H, m), 3.40 (1H, d, *J* = 7.3), 3.43 (1H, dd, *J* = 7.4, 2.7), 3.98 (2H, d, *J* = 14.5), 4.22 (1H, q, *J* = 7.0), 4.37 (1.5H, m), 4.51 (0.5H, dd, *J* = 10.7, 6.6), 4.65 (1H, d, *J* = 9.8, rotameric), 7.30 (5H), 7.60 (4H, m), 8.58 (1H, s, rotameric); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 3.9, 10.1, 39.9, 40.9, 44.0, 44.1, 47.0, 47.2, 55.8, 56.5, 57.8, 58.3, 66.1, 67.1, 68.17, 68.23, 120.02, 120.05, 125.0, 125.1, 125.2, 127.1, 127.15, 127.16, 127.2, 127.7, 127.8, 128.5, 141.2, 141.3, 141.4, 143.6, 143.8, 143.9, 153.6, 154.1, 158.7, 158.8, 172.5, 172.7, 176.4, 176.5; HRMS-ESI: m/z calcd for $C_{26}H_{25}N_3O_6Na$ $(M+Na)^+$ 498.1636, found 498.1624

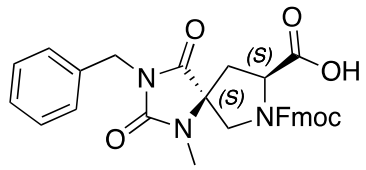
QPro 3 (5S,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid
Using general procedure 1, 10.50 g **1** (2S, 4S), 0.88 equiv 4-methoxybenzyl chloride, recovered 9.45 g pure (70%)



^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.18 (1H, d, *J* = 14.0), 2.85 (1H, dd, *J* = 9.8, 4.3), 3.78 (3H, s), 3.92 (2H, m), 4.19 (1H, m), 4.36 (2H, m, rotameric to 4.49), 4.62 (2H, m, rotameric to 4.53), 6.85 (2H, dd, *J* = 10.4, 8.9), 7.28 (6H, m), 7.49 (1H, d, *J = 7*.5), 7.57 (1H, d, *J* = 8.2), 7.70 (2H, m), 8.45 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 31.5, 36.6, 39.7, 40.8, 42.27, 42.31, 46.9, 47.1, 55.2, 55.3, 55.6, 56.4, 57.7, 58.3, 66.0, 67.1, 68.10, 68.16, 114.0, 114.2, 119.94, 119.96, 119.98, 124.9, 125.00, 125.05, 125.08, 127.0, 127.08, 127.11, 127.2, 127.5, 127.6, 127.72, 127.75, 127.76, 128.9, 130.03, 130.05, 130.2, 141.17, 141.22, 141.24, 141.3, 143.5, 143.7, 153.5, 154.0, 158.0, 158.2, 159.5, 159.6, 162.8, 172.1, 172.2, 176.3, 176.4; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_7Na$ $(M+Na)^+$ 564.1741, found 564.1755

QPro 4 (5*S*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-benzyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

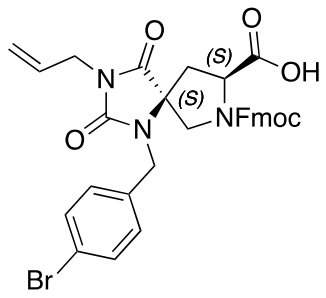
Using general procedure 1, 10.50 g **1** (2S, 4S), 0.88 equiv benzyl bromide, then 1.25 equiv iodomethane, recovered 11.2 g pure (78%)



^{1}H NMR (500 MHz, $CDCl_3$ rotamers present) 2.36 (1H, dd, *J* = 13.9, 7.5), 2.54 (1H, dd, *J* = 13.7, 8.9) 2.67 (4H, m), 3.59 (1H, d, *J* = 11.6), 3.78 (1H, d, *J* = 11.6), 4.17 (1H, t, *J* = 7.0), 4.37 (1H, d, *J* = 7.0), 4.44 (1H, dd, rotameric, *J* = 10.7, 7.0), 4.66 (2H,), 4.72 (1H, m), 7.31 (9H, m), 7.50 (2H, t, *J* = 7.2), 7.72 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$ or DMSO-d_6, rotamers present) 24.9, 25.0, 24.2, 31.8, 34.3, 35.6, 36.9, 42.6, 42.7, 46.9, 47.1, 49.6, 49.8, 57.8, 58.5, 66.1, 67.0, 68.3, 68.4, 119.9, 120.1, 124.9, 125.0, 125.09, 125.13, 127.1, 127.7, 127.80, 127.84, 128.11, 128.15, 128.45. 128.50, 128.57, 128.8, 135.7, 141.23, 141.25, 143.4, 143.5, 143.6, 155.1, 155.2, 163.5, 173.7, 174.0, 174.6; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_6Na$ $(M+Na)^+$ 548.1792, found 548.1784

QPro 5 (5*S*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-allyl-1-(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

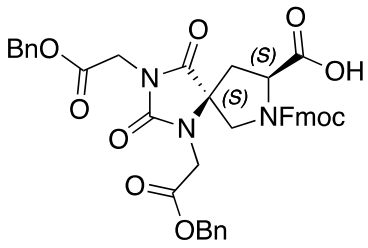
Using general procedure 1, 10.50 g **1** (2S, 4S), 0.88 equiv allyl bromide, then 1.25 equiv 4-bromobenzyl bromide, recovered 9.94 g pure (58%)



^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.39 (1H, dd, *J* = 13.6, 7.8), 2.52 (1H, dd, *J* = 13.7, 8.9), 3.40 (1H, d, *J* = 11.8), 3.64 (1H, d, *J* = 11.7), 4.18 (3H, m), 4.32 (1H, m), 4.40 (2H, m), 4.52 (1H, m), 4.67 (2H, m), 5.24 (2H, m), 5.87 (1H, m), 7.13 (2H, m), 7.26 (2H, m), 7.44 (6H, m), 7.72 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$ or DMSO-d_6, rotamers present) 14.2, 34.6, 41.3, 42.5, 46.8, 50.1, 58.1, 67.1, 68.1, 68.4, 118.7, 120.1, 122.1, 124.8, 124.9, 127.14, 127.17, 127.8, 127.9, 129.0, 129.2, 129.7, 130.6, 130.8, 131.7, 132.2, 135.9, 136.5, 141.22, 141.25, 143.3, 143.4, 155.4, 155.37, 155.39, 155.45, 173.5, 173.9; HRMS-ESI: m/z calcd for $C_{32}H_{28}BrN_3O_6Na$ $(M+Na)^+$ 652.1024, found 652.1024

QPro 6 (5*S*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-1,3-bis(2-(benzyloxy)-2-oxoethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

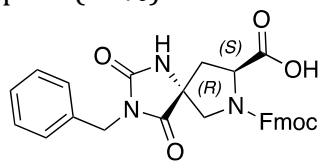
Using general procedure 1, 2.33 g **1** (2*S*, 4*S*), 1.3 equiv benzyl bromoacetate, recovered 1.59 g pure (53%)



^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.35 (1H, dd, *J* = 13.0, 7.5), 2.51 (1H, m), 3.56 (1H, d, *J* = 11.3), 3.84 (4H, m), 4.15 (5H, m), 4.43 (1H, t, *J* = 7.9), 5.02 (4H, m), 7.06 (2H, m), 7.23 (14H, m), 7.54 (2H, dd, *J* = 13.6, 7.5); ^{13}C NMR (125 MHz, $CDCl_3$ or DMSO-d_6, rotamers present) 35.7, 39.5, 41.0, 46.6, 50.5, 60.7, 67.4, 67.6, 67.9, 119.8, 125.1, 125.2, 127.0, 127.11, 127.6, 128.1, 128.28, 128.33, 128.50, 128.52, 128.59, 134.8, 135.0, 140.99, 141.03, 143.5, 143.7, 157.7, 155.6, 166.7, 169.1, 174.0, 177.7; HRMS-ESI: m/z calcd for $C_{40}H_{35}N_3O_{10}Na$ $(M+Na)^+$ 740.2215, found 740.2219

QPro 7 (5R,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-benzyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

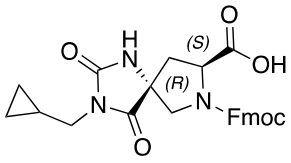
Using general procedure 1, 1.50 g **1** (2*S*, 4R), 0.75 equiv benzyl bromide, recovered 1.34 g pure (70%)



^{1}H NMR (500 MHz, DMSO-D_6, rotamers present) 2.27 (1H, dd, rotameric, *J* = 13.1, 9.6), 2.65 (1H, ddd, rotameric, *J* = 13.3, 8.0, 1.4), 3.63 (1H, dd, *J* = 16.1, 11.3), 3.77 (1H, dd, rotameric, *J* = 11.0, 1.3), 4.16 (1H, m), 4.29 (2H, m), 4.42 (0.5H, dd, *J* = 9.5, 7.9), 4.59 (2H, s, rotameric), 4.63 (0.5H, m), 7.35 (9H, m), 7.66 (1H, d, *J* = 7.6), 7.70 (1H, dd, *J* = 7.6, 3.8), 7.89 (2H, m), 9.07 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-D_6, rotamers present) 41.5, 46.5, 46.6, 55.6, 56.1, 57.8, 58.2, 59.8, 65.0 ,65.7, 67.1, 67.6, 120.2, 125.2, 125.3, 127.15, 127.20, 127.38, 127.43, 127.77, 127.81, 136.41, 136.44, 140.6, 140.7, 143.5, 143.6, 143.7, 153.5, 153.7, 155.4, 172.3, 172.38, 172.44, 172.90; HRMS-ESI: m/z calcd for $C_{29}H_{25}N_3O_6Na$ $(M+Na)^+$ 534.1636, found 544.16439

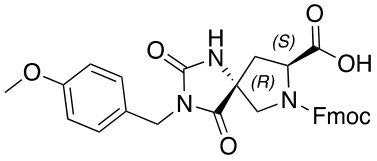
QPro 8 (5R,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(cyclopropylmethyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

Using general procedure 1, 10.50 g **1** (2S, 4R), 1.25 equiv of (bromomethyl)cyclopropane, recovered 9.52 g pure (73%)



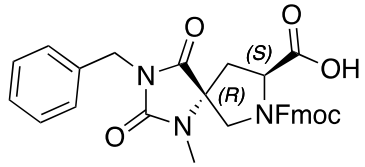
^{1}H NMR (500 MHz, DMSO-D_6, rotamers present) 0.27 (2H, m), 0.46 (2H, m), 1.06 (1H, m), 2.26 (1H, dd, rotameric, 13.2, 9.5), 2.60 (1H, ddd, rotameric *J* = 13.3, 8.1, 1.6), 3.26 (2H, dd, *J* = 6.9, 4.4), 3.62 (1H, m), 3.75 (1H, dd, rotameric, *J* = 11.3, 1.3), 4.16 (1H, m), 4.29 (2H, s), 4.41 (1H, dd, rotameric, 9.5, 7.9), 7.34 (2H, m), 7.44 (2H, m), 7.67 (1H, d, *J* = 7.3), 7.71 (1H, dd, *J* = 7.4, 3.0), 7.91 (2H, d, rotameric *J* = 7.6), 8.98 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-D_6, rotamers present) 3.5, 9.9, 42.6, 46.4, 46.6, 55.6, 56.1, 57.8, 58.2, 64.8, 65.6 67.1, 67.5, 120.2, 125.2, 125.3, 125.32, 125.41, 127.2, 127.7, 127.76, 127.8, 140.62, 140.67, 140.71, 143.50, 143.57, 143.64, 143.7, 153.5, 153.7, 155.6, 155.7, 172.4, 172.5, 172.9; HRMS-ESI: m/z calcd for $C_{26}H_{25}N_3O_6Na$ $(M+Na)^+$ 498.1636, found 498.1641

QPro 9 (5R,8S)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-3-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid
Using general procedure 1, 10.50 g **1** (2S, 4R), 0.75 equiv 4-methoxybenzyl chloride, recovered 9.49 g pure (71%)



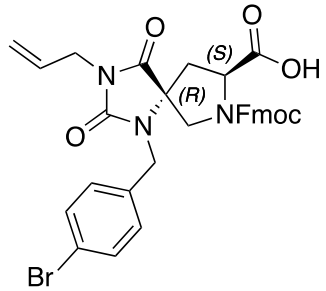
^{1}H NMR (500 MHz, DMSO-D_6, rotamers present) 2.25 (1H, dd, rotameric, *J* = 13.2, 9.8), 2.62 (1H, ddd, rotameric, *J* = 13.3, 7.9, 1.6), 3.62 (1H, d, rotameric, *J* = 11.2), 3.75 (3H, s, rotameric), 3.84 (1H, dd, *J* = 11.3, 1.6), 4.16 (1H, m), 4.29 (2H, s, rotameric), 4.41 (1H, dd, rotameric, *J* = 9.5, 7.9), 4.51 (2H, s, rotameric), 6.91 (2H, m), 7.21 (2H, dd, *J* = 8.7, 5.5), 7.39 (5H, m), 7.66 (1H, d, *J* = 7.3), 7.71 (1H, dd, *J* = 7.4, 3.3), 7.90 (2H, m), 9.03 (1H, s, rotameric); ^{13}C NMR (125 MHz, DMSO-D_6, rotamers present) 46.9, 47.1, 55.5, 56.0, 56.5, 58.3, 58.6, 65.4, 66.1, 67.6, 70.0, 114.4, 120.7, 125.7, 125.73, 125.8, 125.9, 127.7, 128.2, 128.23, 128.3, 128.9, 128.94, 129.3, 129.34, 141.10, 141.14, 141.2, 144.1, 144.2, 154.0, 154.2, 155.9, 159.1, 172.7, 172.82, 172.84, 173.4; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_7Na$ $(M+Na)^+$ 564.1741, found 564.1751

QPro 10 (5*R*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-(4-methoxybenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid
Using general procedure 1, 10.50 g **1** (2S, 4R), 0.75 equiv benzyl bromide, then 1.25 equiv iodomethane, recovered 10.4 g pure (73%)



^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.46 (1H, dd, *J* = 14.5, 9.3), 2.62 (1H, dd, *J* = 14.6, 7.0), 2.76 (3H, s, 3.56 (1H, d, rotameric to 3.82 ppm, *J* = 11.6), 3.77 (1H, d, *J* = 11.9), 4.21 (1H, t, *J* = 6.3), 4.33 (1H, m), 4.52 (2H, m), 4.62 (2H, s), 4.66 (1H, dd, *J* = 9.2, 7.0), 7.31 (9H, m), 7.52 (2H, m), 7.71 (2H, m) ; ^{13}C NMR (125 MHz, $CDCl_3$, rotamers present) 14.2, 25.43, 25.46, 25.55, 36.7, 37.9, 42.9, 43.0, 46.3, 47.1, 47.2, 53.1, 53.3, 57.8, 58.4, 67.5, 67.80, 67.82, 68.6, 72.9, 119.9, 120.00, 120.04, 120.2, 124.7, 124.8, 125.2, 127.1, 127.2, 127.4, 127.74, 127.77, 127.83, 127.87, 128.07, 128.14, 128.19, 128.4, 128.6, 128.76, 128.78, 135.5, 135.6, 141.20, 141.23, 141.27, 141.37, 141.42, 142.4, 143.25, 143.33, 143.6, 143.7, 153.7, 154.4, 154.69, 154.71, 168.7, 171.8, 172.2, 173.4, 174.1; HRMS-ESI: m/z calcd for $C_{30}H_{27}N_3O_6Na$ $(M+Na)^+$ 548.1792, found 548.1799

QPro 11 (5*R*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-benzyl-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid Using general procedure 1, 10.5 g **1** (2S, 4R), 0.75 equiv allyl bromide, then 1.25 equiv 4-bromobenzyl bromide, recovered 9.59 g pure (56%)

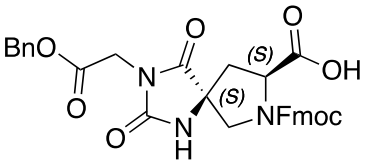


^{1}H NMR (500 MHz, $CDCl_3$, rotamers present) 2.39 (1H, ddd, *J* = 14.6, 9.2, 1.2), 2.60(1H, dd, *J* = 14.6, 7.6), 3.45 (1H, dd, rotameric to 3.83, *J* = 11.6, 1.2), 3.74 (1H, m), 4.11 (5H, m), 4.39 (1H, m), 4.54 (2H, m), 5.22 (2H, m), 5.84 (1H, m), 7.03 (2H, d, *J* = 8.2), 7.35 (6H, m), 7.48 (2H, m), 7.73 (2H, t, *J* = 8.4); ^{13}C NMR (125 MHz, $CDCl_3$ or

DMSO-d_6, rotamers present) 14.2, 37.5, 41.7, 43.5, 46.9, 47.0, 54.1, 58.3, 67.8, 69.2, 118.57, 118.59, 119.99, 120.04, 120.06, 122.1, 124.95, 124.99, 127.0, 127.10, 127.14, 127.16, 127.21, 127.6, 127.8, 127.9, 128.9, 129.0, 130.5, 130.6, 132.0, 132.1, 135.6, 141.29, 141.3, 143.3, 143.4, 153.9, 155.4, 171.2, 173.4; HRMS-ESI: m/z calcd for $C_{32}H_{28}BrN_3O_6Na$ $(M+Na)^+$ 652.1024, found 652.1029

QPro 12 (5*R*,8*S*)-7-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-allyl-1-(4-bromobenzyl)-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-8-carboxylic acid

Synthesized with **QPro 6**, separated by column chromatography, recovered 590 mg pure (53%)



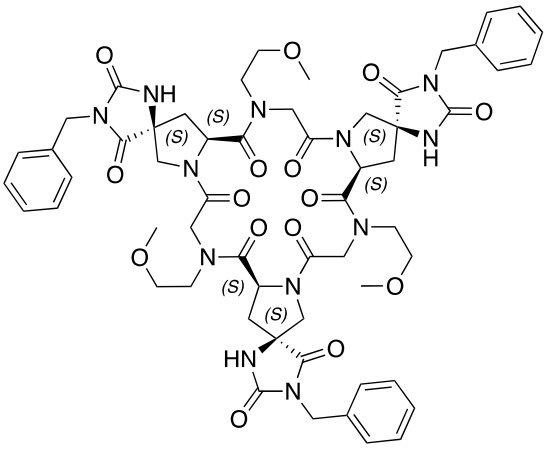
^{1}H NMR (500 MHz, $CDCl_3$ or DMSO-d_6) 2.26 (1H, d, *J* = 13.7), 2.87 (1H, td, *J* = 13.6, 9.8), 3.98 (2H, d, *J* = 5.8), 4.20 (1H, m), 4.36 (4H, m), 4.55 (1H, m), 5.19 (2H, d, *J* = 9.2), 7.28 (2H, m), 7.34 (7H, m), 7.51 (1H, d, *J* = 7.3), 7.58 (1H, t, *J* = 8.5), 7.71 (2H, m), 8.68 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$ or DMSO-d_6, rotamers present) 21.1, 39.7, 39.88, 39.9, 40.8, 46.9, 47.1, 55.7, 56.3, 57.6, 58.2, 60.4, 66.4, 67.5, 67.95, 67.98, 68.1, 68.2, 119.98, 119.99, 120.01, 124.93, 124.94, 125.05, 125.11, 127.07, 127.10, 127.13, 127.2, 127.7, 127.75, 127.79, 128.42, 127.44, 127.7, 128.8, 134.6, 141.2, 141.25, 141.34, 143.4, 143.5, 143.7, 143.8, 153.5, 156.0, 166.4, 172.0, 172.1, 176.3, 176.4; HRMS-ESI: m/z calcd for $C_{31}H_{27}N_3O_8Na$ $(M+Na)^+$ 592.1690, found 592.1702

General Procedure 2: Synthesis of macrocycles

75-150 mg of 2-Cl Trityl chloride resin (0.912 mmol/g) is loaded with 0.138 M bromoacetic acid (1.2 equiv) in DCM, with DIPEA (4 equiv). This reaction is stirred for 1 h, after which time the resin is rinsed repeatedly with DCM. An amine in DMF (1.0 mL, 1 M) is added to the resin and allowed to react for 1 h, followed by repeated rinsing of the resin with DMF and DCM (3x each). A preactivated solution of a Q-Pro residue (3 equiv) with HATU (3 equiv) and DIPEA (6 equiv) is prepared in 1.0 mL of DMF/DCM (1:1, anhydrous) 5 minutes prior to addition to the resin. This preactivated solution is added to the resin, and allowed to react overnight. The resin is then rinsed, and test-cleaved to check for complete addition to the resin. Fmoc deprotection is achieved with 2x 5 min additions of 20% piperidine in DMF, followed by repeated rinsing with DMF and DCM. The next residues are added using standard peptoid coupling procedures, another Q-Pro addition (double coupling required), another peptoid coupling, and a final Q-Pro addition (double coupling required). After the final Q-Pro addition, the Fmoc is removed with 20% piperidine, followed by vigorous rinsing with DMF, and then DCM to drive off any base. The macrocycle precursors are cleaved from resin using 30% hexafluoroisopropanol in dichloromethane, purified by reversed-phase flash chromatography (5-95% ACN in Water, 0.1% formic acid modifier), and lyophilized. The purified precursor is dissolved in anhydrous DCM (20 mL/mmol) with DIPEA (6 equiv), and added to a stirred mixture of PyAOP (3 equiv) in anhydrous DMF (30 mL/mmol) for a final solution concentration of 2 mM to promote intramolecular as opposed to intermolecular coupling. The reaction is stirred for 1 h, then checked via LCMS for completion. The macrocycle is diluted with ethyl acetate and washed with 3x sat. ammonium chloride, 3x sat. sodium bicarb, and 3x sat. sodium chloride, dried over sodium sulfate, and

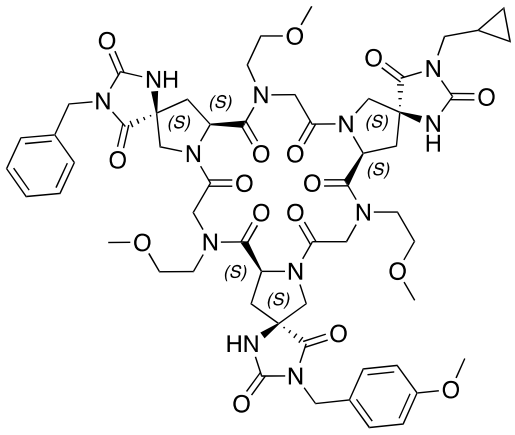
the solvent removed *in vacuo*. The resultant oil is purified via reversed-phase flash chromatography and lyophilized.

QPM-1



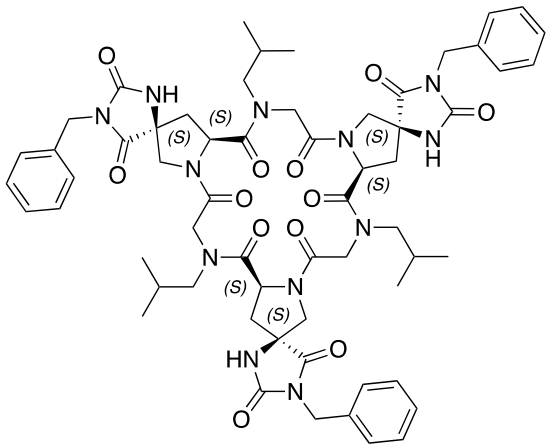
Using general procedure 2, 88 mg of 2-Cl-Trityl Chloride Resin, 2-methoxyethyamine, and **QPro-1**. Recovered 63 mg pure (67% yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 2.16 (3H, dd, *J* = 13.8, 1.8), 2.69 (3H, dd, *J* = 13.9, 9.6), 3.29 (9H, m), 3.39-3.41 (3H, m), 3.43 (3H, m), 3.50-3.53 (3H, m), 3.59 (3H, dd, *J* = 16.7), 3.75 (3H, m), 3.83 (3H, d, *J* = 10.9), 3.96 (3H, d, *J* = 10.9), 4.48 (3H, d, *J* = 16.9), 4.63 (6H, m), 5.02 (3H, dd, *J* = 9.5, 2.5), 6.79 (3H, s), 7.27-7.35 (15H, m); HRMS-ESI: m/z calcd for $C_{57}H_{66}N_{12}O_{15}Na$ $(M+Na)^+$ 1181.4463, found 1181.4676

QPM-2



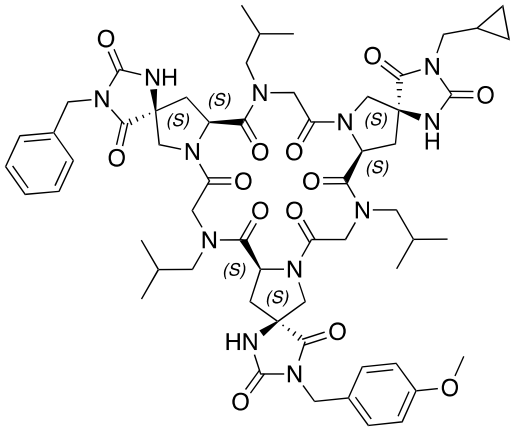
Using general procedure 2, 90 mg of 2-Cl-Trityl Chloride Resin, 2-methoxyethyamine, and **QPro-1, QPro-2,** and **QPro-3**. Recovered 56 mg pure (60% Yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.28 (2H, m), 0.47 (2H, m), 1.08 (1H, m), 2.14 (3H, m), 2.68 (3H, m), 3.31 (11H, m), 3.42 (3H, m), 3.46 (3H, m), 3.53 (3H, m), 3.62 (3H, d, *J* = 16.7), 3.75 (6H, s), 3.85 (3H, t, 10.2), 3.97 (3H, m), 4.55 (5H, m), 4.63 (2H, d, *J* = 5.0), 5.02 (3H, d, *J* = 9.5), 6.64 (1H, s), 6.72 (1H, s), 6.75 (1H, s), 6.87 (2H, m), 7.22 (2H, m), 7.31 (5H, m); HRMS-ESI: m/z calcd for $C_{55}H_{68}N_{12}O_{16}Na$ $(M+Na)^+$ 1175.4768, found 1175.4772

QPM-3



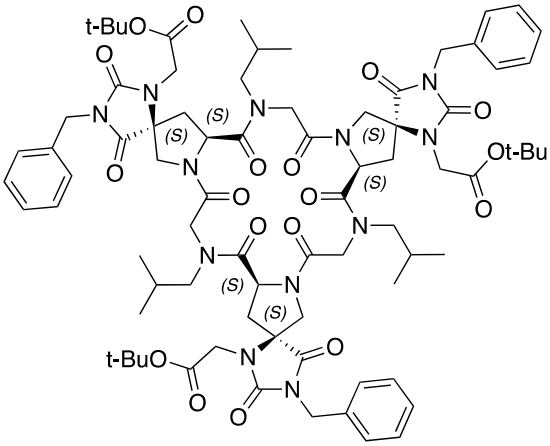
Using general procedure 2, 145 mg of 2-Cl-Trityl Chloride Resin, isobutylamine, and **QPro-1**. Recovered 101 mg (66% Yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.88 (9H, d, *J* = 6.4) 0.95 (9H, d, *J* = 6.4), 1.84 (3H, dt, *J* = 13.8, 7.0), 2.10 (3H, d, *J* = 14.0), 2.73 (3H, dd, *J* = 13.7, 9.8), 2.95 (3H, dd, *J* = 15.1, 8.7), 3.58 (3H, d, *J* = 16.8), 3.62 (3H, m), 3.87 (3H, d, *J* = 11.3), 3.99 (3H, d, *J* = 11.0), 4.57 (3H, d, *J* = 16.8), 4.63 (6H, m), 4.98 (3H, d, *J* = 8.8), 6.82 (3H, s), 7.32 (15H, m); HRMS-ESI: m/z calcd for $C_{60}H_{72}N_{12}O_{12}Na$ $(M+Na)^+$ 1175.5285, found 1175.5315

QPM-4



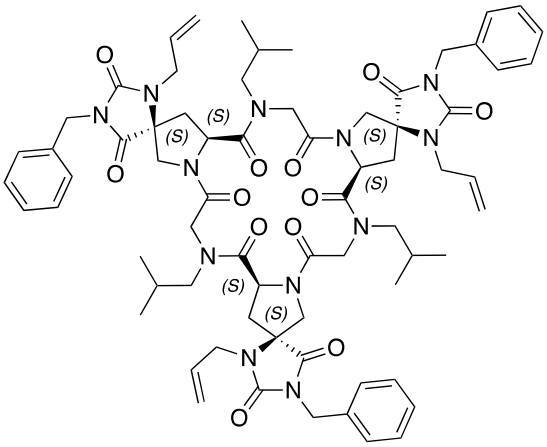
Using general procedure 2, isobutylamine, and **QPro-1, QPro-2,** and **QPro-3**. (60% Yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.29 (2H, m), 0.47 (2H, m), 0.89 (9H, d, *J* = 6.7), 0.96 (9H, d, *J* = 6.7), 1.08 (1H, m), 1.86 (3H, m), 2.10 (3H, m), 2.73 (3H, m), 2.96 (3H, dd, *J* = 15.0, 8.5), 3.3 (3H, m), 3.60 (2H, d, *J* = 16.8), 3.61 (3H, m), 3.76 (3H, s), 3.87 (3H, m), 3.99 (3H, m), 4.55 (2H, m), 4.58 (3H, m), 4.62 (2H, m), 4.98 (3H, d, *J* = 9.8), 6.72 (1H, s), 6.80 (1H, s), 6.83 (1H, s), 6.87 (2H, d, *J* = 8.9), 7.23 (2H, d, *J* = 8.9), 7.31 (5H, m); HRMS-ESI: m/z calcd for $C_{58}H_{74}N_{12}O_{13}K$ $(M+Na)^+$ 1185.5130, found 1185.5132

QPM-5



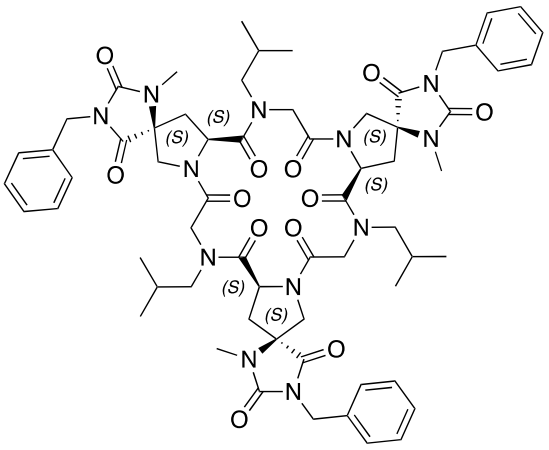
Using Step 1 of General Procedure 1, 30 mg of **QPM-3**, and tert-butyl bromoacetate. Purified by reversed phase flash chromatography (5-95% ACN in Water, 0.1% Formic Acid modifier) Recovered 31 mg (81% Step Yield; 53% Final Yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.87 (9H, d, *J* = 6.7) 0.95 (9H, d, *J* = 6.4), 1.40 (27H, s), 1.85 (3H, m), 2.07 (3H, dd, *J* = 13.4, 8.9), 2.56 (3H, dd, *J* = 13.6, 8.1), 3.03 (3H, dd, *J* = 15.0, 7.9), 3.51 (3H, dd, *J* = 15.3, 6.1), 3.63 (3H, d, *J* = 16.5), 3.80 (3H, m), 3.85 (3H, m), 3.97 (3H, m), 4.04 (3H, m), 4.35 (3H, d, *J* = 16.8), 4.66 (6H, m), 4.93 (3H, t, *J* = 8.4), 7.33 (15H, m); HRMS-ESI: m/z calcd for $C_{78}H_{102}N_{12}O_{18}K$ $(M+K)^+$ 1533.7067, found 1533.7043

QPM-6



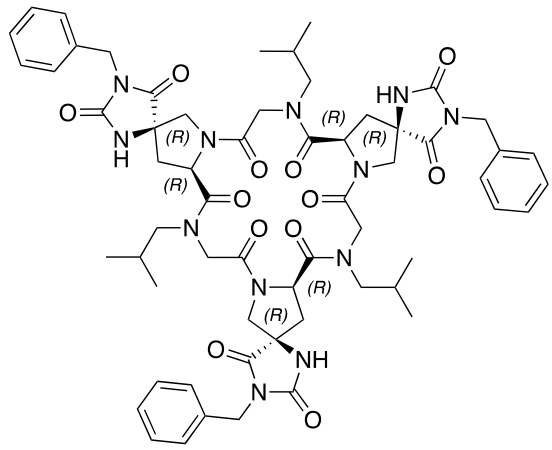
Using Step 1 of General Procedure 1, 30 mg of **QPM-3**, and allyl bromide. Purified by reversed phase flash chromatography (5-95% ACN in Water, 0.1% Formic Acid modifier) Recovered 27 mg (83% Step Yield; 55% Final Yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.87 (9H, d, *J* = 6.7) 0.95 (9H, d, *J* = 6.7), 1.84 (3H, dt, *J* = 13.8, 7.0), 2.17 (3H, dd, *J* = 13.4, 8.9), 2.52 (3H, dd, *J* = 13.4, 7.9), 3.02 (3H, dd, *J* = 15.0, 8.2), 3.54 (3H, dd, *J* = 14.5, 6.9), 3.60 (3H, d, *J* = 16.8), 3.78 (3H, d, *J* = 11.3), 3.93 (3H, d, *J* = 11.1), 3.94 (3H, m), 4.05 (3H,m), 4.37 (3H, d, *J* = 16.8), 4.63 (6H, m), 4.92 (3H, t, *J* = 8.4), 5.18 (6H, m), 5.87 (3H, m), 7.33 (15H, m); HRMS-ESI: m/z calcd for $C_{69}H_{84}N_{12}O_{12}K$ $(M+K)^+$ 1311.5963, found 1311.5975

QPM-7



Using general procedure 2, 85 mg of 2-Cl-Trityl Chloride Resin, isobutylamine, and **QPro-4**. Recovered 64 mg pure (65 %); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.88 (9H, d, *J* = 6.7) 0.96 (9H, d, *J* = 6.7), 1.84 (3H, dt, *J* = 13.8, 7.0), 2.15 (3H, dd, *J* = 13.4, 8.2), 2.50 (3H, dd, *J* = 13.4, 8.4), 2.88 (9H, s), 3.04 (3H, dd, *J* = 15.0, 8.2), 3.53 (3H, dd, *J* = 14.2, 6.6), 3.63 (3H, 16.8), 3.75 (3H, d, *J* = 11.3), 3.95 (3H, d, *J* = 11.0), 4.41 (3H, d, *J* = 16.8), 4.62 (6H, m), 4.94 (3H, t, *J* = 8.4), 7.33 (15H, m); HRMS-ESI: m/z calcd for $C_{63}H_{78}N_{12}O_{12}Na$ $(M+Na)^+$ 1217.5754, found 1217.5795

QPM-8



Using general procedure 2, 87 mg of 2-Cl-Trityl Chloride Resin isobutylamine, and the enantiomer of **QPro-1**. Recovered 58 mg pure (62% yield); ^{1}H NMR (500 MHz, CD_3CN, 4 equiv K-OTf) 0.91 (9H, d, *J* = 6.7) 1.01 (9H, d, *J* = 6.7), 1.84 (3H, m), 2.17 (3H, m), 2.81 (3H, dd, *J* = 13.9, 9.9), 3.13 (3H, dd, *J* = 14.8, 9.3), 3.25 (3H, dd, *J* = 15, 5.2), 3.63 (3H, d, *J* = 16.8), 3.86 (3H, d, *J* = 11.3), 4.08 (3H, m), 4.49 (3H, d, *J* = 16.8), 4.62 (6H, m), 4.93 (3H, d, *J* = 8.9), 6.57 (3H, s), 7.32 (15H, m); HRMS-ESI: m/z calcd for $C_{60}H_{72}N_{12}O_{12}Na$ $(M+Na)^+$ 1175.5285, found 1175.5303

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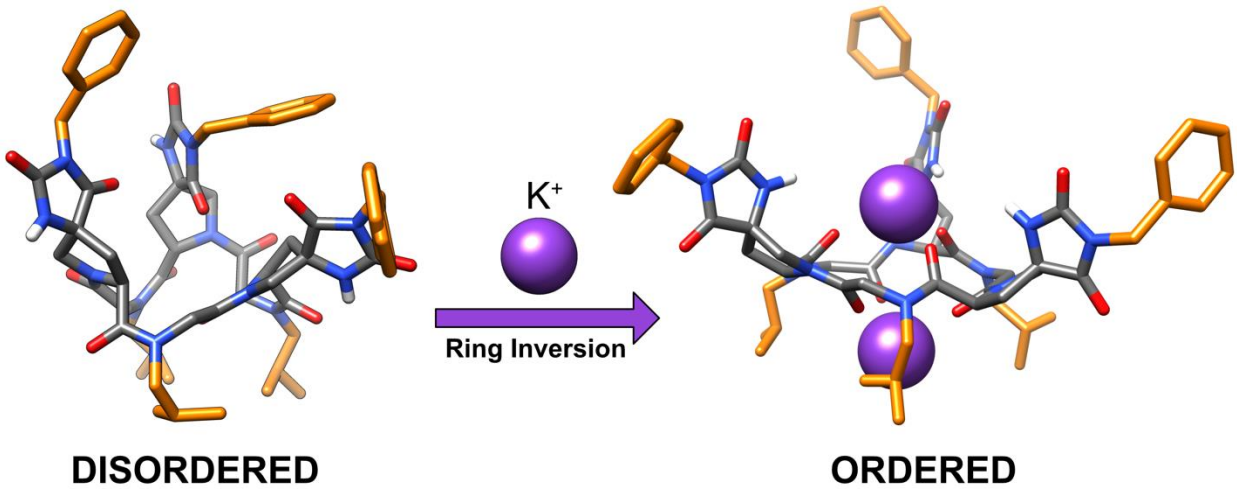
GRAPHICAL ABSTRACT



Q-Pro Macrocycles are disordered in the absence of a metal cation. With the addition of a cation, the macrocycles proceed through a ring inversion to a well-ordered species.