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Heterogeneous Phase Transfer Catalysis in Solid Phase Syntheses of *Anth*-Cyclic Tetrapeptides

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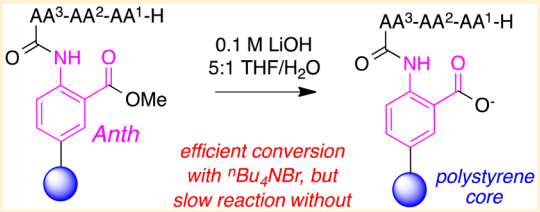
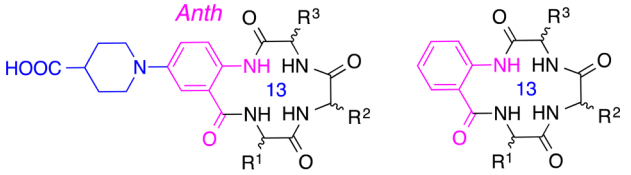
Supporting Information

ABSTRACT: This study features solid phase syntheses of cyclic tetrapeptides containing anthranilic acid (*Anth*) on relatively inexpensive resins derived from polystyrene. It proved to be difficult to hydrolyze a supported *Anth*-methyl ester *unless* a phase transfer catalyst was added to facilitate transport of hydroxide into the swollen hydrophobic gel state of the resin. We suggest this may be an under-appreciated strategy for improving syntheses on polystyrene supports.

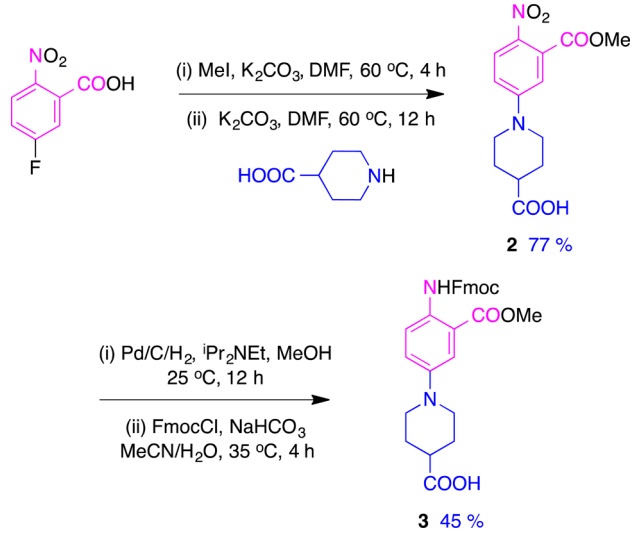


Tetraalkylammonium salts are widely used for liquid−liquid phase transfer catalysis,[1,2] but not to ferry reagents across a *solid*−liquid interface. We came across a useful example of this kind of heterogeneous phase transfer catalysis when developing a route to the 13-ring cyclic tetrapeptides **1** on a polystyrene-based support.



Solution phase access tp the *Anth*-containing cyclic tetrapeptides **A** has recently been developed in our laboratories,[3] but that unfunctionalized *Anth*-residue does not have a functional group to enable attachment to a solid phase. Consequently, the first task in this study was to make a modified anthranilic acid (fragment in blue for structure **1**). Use of piperidine-4-carboxylic acid as a nucleophile in SNAr displacement of fluoride from methyl 5-fluoro-2-nitrobenzoate gave the linked system **2** (Scheme 1).[4] It was important to use a *methyl* benzoate rather than another ester (e.g., allyl) because pilot reactions showed coupling onto methyl anthranilates were significantly easier than if a larger ester were used (see Supporting Information, Reaction S1). Hydrogenation of the nitro-group and then reaction with Fmoc-Cl gave **3**. *N*-Protected amino acid **3** was used as a nucleophile to displace chloride from chlorotrityl polystyrene resin to give the corresponding supported diester (Scheme 2). Substoichiometric amounts of acid **3** were used relative to the available loading sites on the resin, and any unreacted electrophilic centers on the polymer were capped as indicated. Quantitative UV-detection of Fmoc-cleavage products[5,6] indicated the loading of the resin with **3** was 0.2 mmol/g. Thus, this strategy conserved

Scheme 1. Synthesis of a Modified Anthranilic Acid Derivative



the most valuable component (**3**) and reduced the loading of the resin to levels that are conducive to intramolecular cyclization over intermolecular processes.[7,8] Conventional couplings for Fmoc-based amino acids led to the linear precursor **B** that may be represented as H-Glu(ᵗBu)-Phe-Ile-*Anth*'.

Table 1 outlines some of the attempts that were made to achieve the methyl ester hydrolysis of the supported linear peptide (see Supporting Information for all the conditions attempted). Cleavage of the products from the resin under mildly acidic conditions enabled the degree of conversion of the

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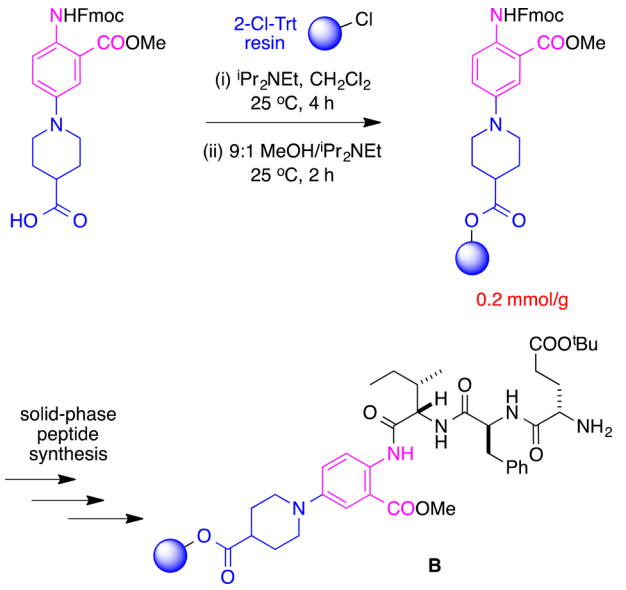
Scheme 2. Solid Phase Syntheses of Linear *Anth*-Containing Peptides

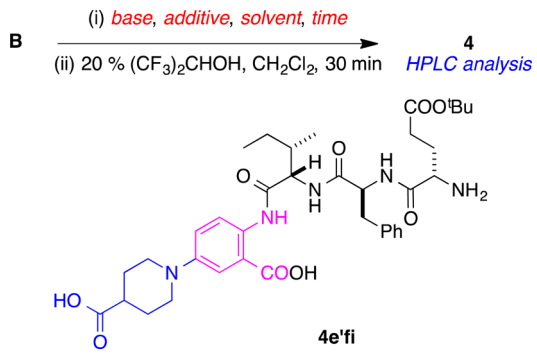


methyl ester to the side-chain-protected linear peptide **4e′fi** {*ie* the linear peptide containing Glu(^{t}Bu), Phe, and Ile } to be analyzed by HPLC.

Entries 1−4 in Table 1 show there was poor conversion to the desired product **4** even after an extended time (12 h); this is consistent with others who found it necessary to use 72 h to obtain a reasonable conversion of a polystyrene-supported methyl ester to the corresponding carboxylic acid.[9] In THF/MeOH (entry 5) the reaction was faster, but the product purity was compromised (two significant byproducts by HPLC, one of these was from hydrolysis of the ^{t}Bu-ester as concluded from LC-MS). However, the reaction rate increased dramatically when tetra-*n*-butyl ammonium bromide was added to the THF/H_2O conditions (entry 6), so much, in fact, that the hydrolysis was complete in 1 h (entry 7). At 1 h of reaction time, the less hydrophobic salts Et_4NBr and $BnNEt_3Cl$ gave much less conversion than nBu_4NBr (entries 8 and 9). Tetra-*n*-hexyl ammonium bromide gave only marginally less conversion than nBu_4NBr (compare entries 7 and 10), which was slightly more efficient than cetrimonium bromide, ($^nC_{16}H_{33}$)N-(CH_3)$_3$Br (entry 11), and triphenyl phosphonium salts gave very poor conversion (entries 12 and 13). Overall, these data indicate symmetrical long-chain tetraalkyl ammonium salts are preferred, and unsymmetrical analogs are marginally inferior. Phenyl phosphonium salts are ineffective for the featured reaction, and cations having several aromatic rings do not appear to be able to permeate into the resin effectively.

Table 2 shows hydrolysis product *purities* at 100% conversion when the optimized conditions were used (*nb*, Table 1 is different because it shows *conversions*). Little variation in the product purities was observed as the constituent amino acids were varied between combinations of Ile (**i**), Phe (**f**), Glu(^{t}Bu) (**e′**), Val (**v**), Ala (**a**), Ser(^{t}Bu) (**s′**), Tyr(^{t}Bu) (**y′**), Arg(Pbf) (**r′**), Cys(Acm) (**c′**), and His(Tr) (**h′**). NMR analysis of the crude material after cleavage of **4e′fi** indicated that the carboxylate was paired with a tetra-*n*-butyl ammonium cation. Throughout, there was no evidence of cleavage of *tert*-butyl side-chain esters, and premature (*ie* in the base-mediated step)

Table 1. Phase Transfer Catalysts and Methyl Ester Hydrolysis on Polystyrene Beads



entry	base (concn/M)	solvent (ratio)	time (h)	additive	conversion (%)
1	LiOH (0.1)	THF/H_2O (5:1)	12	–	40
2	LiOH (0.2)	THF/H_2O (5:1)[a]	12	–	30
3	KOH (0.2)	THF/H_2O (10:1)	12	–	15
4	LiOH (0.1)	THF/H_2O (2:1)	12	–	8
5	LiOH (0.1)	THF/MeOH (5:1)	12	–	80[b]
6	LiOH (0.1)	THF/H_2O (5:1)	12	$^nBu_4NBr$	>95
7	**LiOH (0.1)**	**THF/H_2O (5:1)**	**1**	**$^nBu_4NBr$**	**>95**
8	LiOH (0.1)	THF/H_2O (5:1)	1	Et_4NBr	25
9	LiOH (0.1)	THF/H_2O (5:1)	1	$BnNEt_3Cl$	42
10	LiOH (0.1)	THF/H_2O (5:1)	1	Hex_4NBr	89
11	LiOH (0.1)	THF/H_2O (5:1)	1	($^nC_{16}H_{33}$) $N(CH_3)_3Br$	65
12	LiOH (0.1)	THF/H_2O (5:1)	1	Ph_4PBr	5
13	LiOH (0.1)	THF/H_2O (5:1)	1	Ph_3PEtBr	6

[a]Two phases. [b]Impurities identified by HPLC.

cleavage of the ester link from the trityl polystyrene was not observed by HPLC.

Cyclization of tetrapeptide precursors to relatively small, and therefore strained, 13-membered rings is a difficult transformation, and much optimization was required to achieve this for the supported intermediates **C**. For instance, when HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxid hexafluorophosphate)[10,11] was used the predominant product was the tetramethylguanidine.

After experimenting with 35 sets of reagents and conditions (see Supporting Information Table S2) those indicated in Table 3 were selected. In practice, it is important to follow a "staged" coupling procedure wherein (i) the acid is activated using DCC/HOAt and *N*-methyl morpholine (NMM) for 2 h; (ii) the coupling agents and byproducts that are in solution are washed away; (iii) the resin is resuspended in NMM/DMF for 48 h to allow the cyclization to proceed in an environment that does not contain byproducts and excess reagents from the activation step; and (iv) operations (i)−(iii) are repeated. If the excess coupling agents were not removed in step (ii) then significant amounts of linear *N*-terminal guanidines formed.

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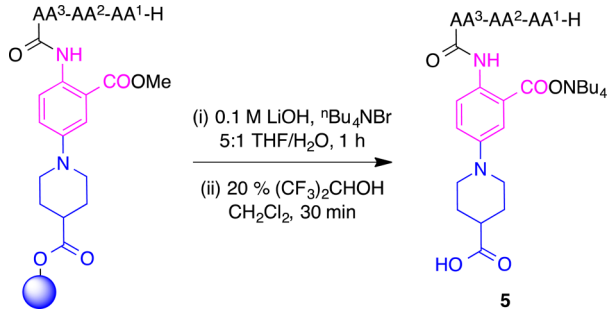
Table 2. Efficient Methyl Ester Hydrolysis on a Polystyrene Support



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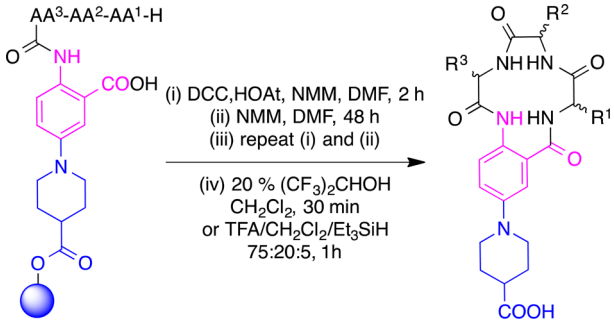
entry	sequence	HPLC purity (%)
1	LLL-**4e′fi**	92
2	LLL-**4aaf**	95
3	LLL-**4vs′y′**	91
4	DDL-**4y′s′v**	89
5	DDL-**4e′af**	86
6	DLL-**4fae′**	90
7	LLD-**4ar′c′**	83
8	DDL-**4h′e′c′**	81

Table 3. Formation of the Featured *Anth*-Containing Cyclic Tetrapeptides 1



1

entry	sequence	HPLC purity (%)	isolated yield (%)
1[a]	LLL-**1vsy**	81	45
2[a]	DDL-**1ysv**	87	35
3[a]	DDL-**1eaf**	82	41
4[a]	DLL-**1fae**	76	39
5[b]	LLD-**1ar′c′**	71	31
6[b]	DDL-**1h′e′c′**	75	29

[a]Final step: TFA-based cleavage. [b]Final step: HFIP-based.

Staged couplings of this kind are at least uncommon in the literature.

Overall, the above procedure to obtain the cyclic tetrapeptides was difficult, probably because the desired cyclization reaction is only slightly more favorable than several possible competing processes. Nevertheless, Table 3 lists the products that were isolated under the optimized conditions, without (TFA-based conditions) or with (1,1,1,3,3,3-hexafluoropropanol, HFIP)[12] side-chain protection.

In conclusion, tetra-*n*-butylammonium bromide was pivotal in the supported methyl ester hydrolyses described here. Use of ammonium salts in solid phase syntheses is rare; examples we found include $^{n}Bu_4NI$/18-crown-6 in loading a sodium alkoxide onto a bromomethylene polystyrene resin,[13] $Me_4NHB(OAc)_3$ in a reductive amination,[14] $^{n}Bu_4NOH$ featuring a TOSMIC reaction,[15] $^{n}Bu_4NOH$ as a base in a cyclization reaction,[16] $^{n}Bu_4NIO_4$ in an oxidation reaction,[17] and in an electrolysis of a supported substrate.[18] Optimization of the conditions for the hydrolysis greatly facilitated synthesis of the strained cyclic tetrapeptides **1**, and we suggested that similar strategies warrant wider consideration in optimization of reactions on polystyrene supports.

EXPERIMENTAL SECTION

General Procedures. All solution phase reactions were carried out under an inert atmosphere (nitrogen or argon where stated). Glassware for anhydrous reactions was dried in an oven at 140 °C for a minimum of 6 h prior to use. Solid phase syntheses were carried out in plastic fritted syringes. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at a high commercial quality (typically 97% or higher) and used without further purification. Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV. Flash column chromatography was performed using silica gel (230−400 mesh). ^{1}H and ^{13}C spectra were recorded on a 400 MHz spectrometer and were calibrated using residual nondeuterated solvent as an internal reference. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, p = pentet, br = broad singlet, dd = doublet of doublet. Melting points were recorded on an automated melting point apparatus and are uncorrected. All of the HPLC analyses were carried out with UV detection monitored at 254 nm. Analytical reversed-phase HPLC analyses were performed with a 250 mm × 4.6 mm C-18 column using gradient conditions (10−90% acetonitrile in water, flow rate = 0.75 mL/min, injection volume = 30 μL).

General Procedure for the Syntheses of 3. MeI (1.25 mL, 20 mmol) was added dropwise to a mixture of 5-fluoro-2-nitrobenzoic acid (3.70 g, 20 mmol) and K_2CO_3 (2.76 g, 20 mmol) in 100 mL of DMF at room temperature. The mixture was stirred at 60 °C for 4 h under N_2. Then K_2CO_3 (6.90 g, 50 mmol) was added, followed by the addition of piperidine-4-carboxylic acid (3.10 g, 24 mmol) in one portion. The mixture was further stirred at 60 °C under N_2 for 12 h. The reaction mixture was cooled to room temperature, and DMF was removed under vacuum. 1 M $KHSO_4$ aqueous solution was added slowly while stirring to acidify the crude mixture until the pH was adjusted to 3−4. The precipitated yellow solid product was filtered and washed with water (20 mL × 3) and then with CH_2Cl_2 (5 mL × 2). After drying, compound **2** was obtained as a yellow solid (4.74 g, 77% yield).

To a solution of **2** (3.08 g, 10 mmol) and DIPEA (1.71 mL, 10 mmol) in methanol (100 mL, 0.1 M) under nitrogen was added 10 wt % Pd/C (1.02 g, 0.1 equiv. Pd). The reaction was placed under an atmosphere of hydrogen (1 atm, balloon) for 12 h. After the reaction finished, the flask was purged with N_2. The reaction mixture was filtered over a Celite pad and concentrated to afford the product. The product was dissolved in 100 mL of MeCN/H_2O (1:1 mixture), and $NaHCO_3$ (1.68 g, 20 mmol) was added. The mixture was stirred at room temperature for 5 min followed by the addition of Fmoc-Cl (3.88 g, 15 mmol) in one portion. The mixture was stirred at 35 °C for 4 h, and the mixture was concentrated under vacuum to remove MeCN. 0.1 M aqueous HCl was added to adjust the pH of the mixture to 3−4, and the solution was extracted with chloroform (50 mL × 5). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to give the crude product. The crude material was purified with flash chromatography (1% MeOH in CH_2Cl_2 to 3% MeOH in CH_2Cl_2) to give the pure product as a white solid (2.2 g, 44%).

*1-(3-(Methoxycarbonyl)-4-nitrophenyl)piperidine-4-carboxylic Acid (**2**).* Yellow solid, 4.74 g, 77%; mp = 214.9−216.0 °C; ^{1}H NMR (400 MHz, DMSO) δ 7.99 (d, J = 9.1 Hz, 1H), 7.15−7.04 (m, 2H), 4.04−3.97 (m, 2H), 3.83 (s, 3H), 3.19−3.10 (m, 2H), 2.64−2.53 (m,

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1H), 1.98−1.87 (m, 2H), 1.62−1.52 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 175.9, 167.6, 153.9, 133.7, 132.5, 127.5, 113.8, 112.1, 53.3, 46.5, 27.6; HRMS (ESI-TOF) m/z calcd for $C_{14}H_{15}N_2O_6$ $(M-H)^-$ 307.0930; found 307.0933.

*1-(4-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(methoxycarbonyl)phenyl)piperidine-4-carboxylic Acid (**3**).* White solid, 2.2 g, 44%; mp = 194.8−195.8 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.80 (s, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 7.4 Hz, 2H), 7.49−7.41 (m, 2H), 7.39−7.28 (m, 3H), 7.24 (dd, J = 9.1, 2.6 Hz, 1H), 4.46 (d, J = 6.8 Hz, 2H), 4.32 (t, J = 6.7 Hz, 1H), 3.83 (s, 3H), 3.63−3.54 (m, 2H), 2.83−2.66 (m, 2H), 2.43−2.36 (m, 1H), 1.96−1.88 (m, 2H), 1.69−1.62 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 176.3, 168.1, 153.7, 147.1, 144.2, 141.2, 131.8, 128.2, 127.6, 125.5, 122.7, 122.3, 120.6, 119.1, 117.1, 66.5, 52.8, 48.9, 47.0, 28.0; HRMS (ESI-TOF) m/z calcd for $C_{29}H_{27}N_2O_6$ $(M-H)^-$ 499.1869; found 499.1875.

Solid Phase Synthesis of Cyclic Peptides 1. *Loading of Linker onto 2-Cl-Trityl Resin.* 2-Cl-Trt resin (200 mg, 1.4 mequiv/g) was shaken with anhydrous CH_2Cl_2 (4 mL) in a fritted syringe for 30 min. Then the CH_2Cl_2 was removed, and a mixture of **3** (22 mg, 0.044 mmol) and DIPEA (68 μL, 0.4 mmol) in CH_2Cl_2 (2 mL) was added into the syringe, followed by shaking at room temperature for 12 h. The remaining reactive site was blocked with MeOH/DIPEA (9:1 v/v) for 30 min, and the beads were washed with CH_2Cl_2 3 times, MeOH, and then DMF 3 times.

Coupling with Amino Acids and Fmoc Deprotection. Fmoc protection groups were deprotected by treating the bead with 20% piperidine in DMF for 1 min, followed by the second treatment with 20% piperidine in DMF for 15 min. The beads were washed with DMF 6 times after the second treatment.

Coupling reactions with the first amino acids were carried out twice with 5 equiv of Fmoc amino acid, 5 equiv of HATU, and 10 equiv of NMM at 0.6 M concentration in DMF for 1 h. For the other coupling reactions, 3 equiv of Fmoc amino acid, 3 equiv of HBTU, and 6 equiv of DIPEA were used at 0.2 M concentration in DMF for 1 h at room temperature. The beads were washed with DMF 6 times after the coupling reaction, and a few beads were subjected to a chloranil test (for the first coupling reaction) or Kaiser test (for the other coupling reactions) to confirm the completion of the coupling reaction.

Methyl Ester Hydrolysis. After the last Fmoc deprotection step, the resin was washed with DMF 3 times, MeOH 2 times, and THF 3 times. A mixture of THF/0.5 M LiOH aqueous solution (5:1) and 3 equiv of ^{n}Bu$_4$NBr was added to the resin, and the mixture was shaken at room temperature for 1 h. The beads were washed with THF 3 times, MeOH 2 times, and DMF 3 times. The materials on a small sample of beads were cleaved with the method described in Cleavage From Solid Support Method 1 and analyzed by reversed-phase HPLC for purity.

Cleavage from Solid Support Method 1. The peptide was cleaved off the bead by treating the beads with HFIP/CH_2Cl_2 (1:4 v/v) for 30 min at room temperature. After filtration, the solvents were removed under vacuum, and the crude material was dried under high vacuum to give the crude product. The crude material was analyzed by reversed-phase analytical HPLC for its purity.

Cleavage from Solid Support Method 2. The peptide was cleaved off the bead by treating the beads with TFA/CH_2Cl_2/Et_3SiH (75:20:5) for 1 h at room temperature. After filtration, the solvents were removed under vacuum and the crude material was dried under high vacuum to give the crude product. The crude material was analyzed by reversed-phase analytical HPLC for its purity.

On-Bead Cyclization. The deprotected linear peptide on resin was activated with 3 equiv of DCC, 3 equiv of HOAt, and 6 equiv of NMM at 0.06 M concentration in DMF at room temperature for 2 h to give the HOAt ester of the linear peptide. Then the beads were filtered and shaken with 6 equiv of NMM at 0.06 M concentration in DMF at room temperature for 48 h to enable the cyclization of the activated linear peptides. The activation−cyclization cycle was repeated, and then the material on the bead was cleaved with the method described in Cleavage From Solid Support Method 1 for protected cyclic peptides and Cleavage From Solid Support Method 2 for deprotected cyclic peptides and analyzed by reversed-phase HPLC for purity. The crude product was purified with reversed-phase prep-HPLC (10%−50% MeCN/water containing 0.1% TFA), and the fractions containing the product were lyophilized to give the pure product as white solids.

*1-((3S,6S,9S)-3-(4-Hydroxybenzyl)-6-(hydroxymethyl)-9-isopropyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic Acid (LLL-**1vsy**).* White amorphous solid, 10.7 mg, 45%; ^{1}H NMR (400 MHz, DMSO) δ 9.16 (s, 1H), 8.73 (d, J = 7.0 Hz, 1H), 8.44 (d, J = 5.8 Hz, 1H), 8.09 (d, J = 9.0 Hz, 1H), 7.15−6.92 (m, 5H), 6.63 (d, J = 8.4 Hz, 2H), 4.39−4.30 (m, 1H), 3.97−3.90 (m, 1H), 3.68−3.53 (m, 5H), 3.16−3.07 (m, 1H), 2.94−2.74 (m, 3H), 2.46−2.36 (m, 1H), 2.07−1.86 (m, 3H), 1.80−1.59 (m, 2H), 1.05 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.3, 171.0, 170.9, 170.2, 169.1, 158.7, 156.0, 130.9, 129.4, 129.1, 127.3, 122.3, 118.8, 115.5, 115.3, 64.3, 61.2, 56.8, 55.3, 49.9, 49.3, 33.8, 28.6, 27.9, 20.8, 19.5; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{38}N_5O_8$ $(M+H)^+$ 596.2720; found 596.2734.

*1-((3S,6R,9R)-9-(4-Hydroxybenzyl)-6-(hydroxymethyl)-3-isopropyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic Acid (DDL-**1ysv**).* White amorphous solid, 8.3 mg, 35%; ^{1}H NMR (400 MHz, DMSO) δ 9.04 (s, 1H), 8.85 (d, J = 4.9 Hz, 1H), 8.27 (d, J = 5.7 Hz, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 7.13−7.00 (m, 3H), 6.78 (s, 1H), 6.70 (d, J = 8.4 Hz, 2H), 4.59−4.49 (m, 1H), 4.05−3.94 (m, 1H), 3.74−3.52 (m, 4H), 3.28 (dd, J = 10.0, 4.9 Hz, 1H), 2.93−2.86 (m, 2H), 2.86−2.73 (m, 2H), 2.45−2.40 (m, 1H), 2.07−1.86 (m, 3H), 1.77−1.59 (m, 2H), 1.02 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.2, 172.0, 171.3, 169.7, 169.5, 158.7, 156.6, 130.5, 127.7, 127.0, 121.9, 118.8, 117.6, 115.5, 114.7, 63.1, 60.7, 59.7, 54.1, 49.4, 49.0, 35.3, 29.2, 27.7, 27.6, 19.8, 19.6; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{38}N_5O_8$ $(M+H)^+$ 596.2720; found 596.2695.

*1-((3S,6R,9R)-3-Benzyl-9-(2-carboxyethyl)-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic Acid (DDL-**1eaf**).* White amorphous solid, 9.7 mg, 41%; ^{1}H NMR (400 MHz, DMSO) δ 9.23 (s, 1H), 8.99 (d, J = 6.0 Hz, 1H), 8.44 (d, J = 5.2 Hz, 1H), 8.11 (d, J = 9.7 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.37−7.15 (m, 5H), 7.10−7.05 (m, 2H), 4.53−4.46 (m, 1H), 4.28−4.16 (m, 1H), 4.03−3.94 (m, 1H), 3.67−3.59 (m, 2H), 3.05−2.94 (m, 2H), 2.86−2.77 (m, 2H), 2.47−2.35 (m, 3H), 2.07−1.77 (m, 4H), 1.77−1.60 (m, 2H), 1.05 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.3, 174.3, 172.3, 171.4, 169.9, 169.7, 138.4, 129.4, 128.7, 127.4, 126.9, 121.7, 119.4, 118.5, 115.3, 58.3, 57.4, 55.3, 49.2, 49.1, 47.9, 36.8, 30.8, 27.9, 25.5, 17.4; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{36}N_5O_8$ $(M+H)^+$ 594.2564; found 594.2583.

*1-((3S,6S,9R)-9-Benzyl-3-(2-carboxyethyl)-6-methyl-2,5,8,11-tetraoxo-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic Acid (DLL-**1fae**).* White amorphous solid, 9.3 mg, 39%; ^{1}H NMR (400 MHz, DMSO) δ 9.19 (d, J = 8.0 Hz, 1H), 9.07 (s, 1H), 8.44 (d, J = 7.2 Hz, 1H), 7.85 (d, J = 8.9 Hz, 1H), 7.69 (d, J = 9.3 Hz, 1H), 7.37−7.14 (m, 5H), 7.06−6.91 (m, 1H), 6.60 (d, J = 2.5 Hz, 1H), 4.51−4.37 (m, 1H), 4.36−4.29 (m, 1H), 4.17−4.09 (m, 1H), 3.58−3.50 (m, 2H), 3.13 (dd, J = 13.7, 5.6 Hz, 1H), 2.99 (dd, J = 13.2, 9.2 Hz, 1H), 2.80−2.70 (m, 2H), 2.46−2.39 (m, 1H), 2.31−2.25 (m, 2H), 2.15−2.01 (m, 1H), 1.95−1.79 (m, 3H), 1.72−1.58 (m, 2H), 1.27 (d, J = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.3, 174.3, 172.3, 170.2, 169.8, 169.4, 139.0, 129.9, 128.6, 126.7, 126.1, 123.0, 122.7, 118.3, 114.5, 62.2, 56.1, 55.5, 54.0, 50.7, 48.6, 34.9, 30.7, 27.8, 26.3, 17.7; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{35}N_5O_8Na$ $(M+Na)^+$ 616.2383; found 616.2367.

*1-((3S,6S,9S)-3-(((Acetamidomethyl)thio)methyl)-9-methyl-2,5,8,11-tetraoxo-6-(3-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic Acid (LLD-**1ar′c′**).* White amorphous solid, 11.1 mg, 31%; ^{1}H NMR (400 MHz, DMSO) δ 9.35 (s, 1H), 8.99 (d, J = 5.2 Hz, 1H), 8.58−8.51 (m, 2H), 8.10 (d, J = 8.9 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.13−7.07 (m, 2H), 6.88−6.30 (m, 4H), 4.48−4.40 (m, 1H), 4.31−4.18 (m, 2H),

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4.18−4.08 (m, 1H), 4.01−3.95 (m, 1H), 3.10−3.01 (m, 2H), 2.99−2.91 (m, 4H), 2.82 (t, J = 11.2 Hz, 2H), 2.48 (s, 3H), 2.43 (s, 3H), 2.02 (s, 3H), 1.97−1.88 (m, 3H), 1.86−1.78 (m, 4H), 1.73−1.65 (m, 2H), 1.61−1.39 (m, 10H), 1.36 (d, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.2, 173.4, 172.2, 169.9, 169.5, 168.9, 158.8, 157.9, 156.5, 137.7, 131.9, 127.1, 124.8, 121.9, 118.8, 116.7, 115.5, 86.8, 57.1, 53.8, 52.3, 49.4, 49.2, 42.9, 41.3, 31.8, 29.4, 28.8, 27.8, 25.9, 23.0, 19.4, 18.0, 16.2, 12.7; HRMS (ESI-TOF) m/z calcd for $C_{41}H_{58}N_9O_{10}S_2$ $(M + H)^+$ 900.3748; found 900.3719.

*1-((3R,6R,9R)-3-(((Acetamidomethyl)thio)methyl)-6-(3-(tert-butoxy)-3-oxopropyl)-2,5,8,11-tetraoxo-9-((1-trityl-1H-imidazol-4-yl)methyl)-2,3,4,5,6,7,8,9,10,11-decahydro-1H-benzo[k][1,4,7,10]tetraazacyclotridecin-13-yl)piperidine-4-carboxylic aAcid (DDL-**1h′e′c′**).* White amorphous solid, 11.4 mg, 29%; ^{1}H NMR (400 MHz, DMSO) δ 9.18 (s, 1H), 8.96 (d, J = 5.6 Hz, 1H), 8.80 (s, 1H), 8.73 (d, J = 5.1 Hz, 1H), 8.52 (t, J = 6.2 Hz, 1H), 8.08 (d, J = 9.1 Hz, 1H), 7.63 (d, J = 8.6 Hz, 1H), 7.51−7.39 (m, 9H), 7.34−6.95 (m, 9H), 4.49−4.40 (m, 1H), 4.39−4.33 (m, 1H), 4.28−4.19 (m, 3H), 3.59−3.49 (m, 2H), 3.20−3.11 (m, 2H), 3.03−2.92 (m, 2H), 2.81−2.69 (m, 2H), 2.43−2.32 (m, 1H), 2.11−2.01 (m, 2H), 1.93−1.75 (m, 5H), 1.77−1.55 (m, 4H), 1.35 (s, 9H); ^{13}C NMR (101 MHz, DMSO) δ 176.2, 171.9, 171.6, 170.9, 169.9, 169.5, 168.8, 158.7, 158.4, 148.2, 140.7, 137.3, 129.8, 129.2, 129.1, 128.2, 128.0, 127.1, 121.8, 118.5, 115.2, 80.1, 77.9, 57.0, 56.8, 52.1, 49.0, 48.9, 41.3, 31.6, 28.2, 28.0, 27.0, 23.0; HRMS (ESI-TOF) m/z calcd for $C_{53}H_{61}N_8O_9S$ $(M + H)^+$ 985.4282; found 985.4306.

A tetramethylguanidine side-product was formed when 3.0 equiv of HATU were used as the coupling reagent. The side-product was cleaved from the bead with Cleavage From Solid Support Method 2.

1-(3-Carboxy-4-((5S,8S,11S)-3-(Dimethylamino)-11-(4-hydroxybenzyl)-8-(hydroxymethyl)-5-isopropyl-2-methyl-6,9-dioxo-2,4,7,10-tetraazadodec-3-en-12-amido)phenyl)piperidine-4-carboxylic Acid. ^{1}H NMR (400 MHz, DMSO) δ 10.90 (s, 1H), 9.15 (s, 1H), 8.39 (d, J = 7.4 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 9.1 Hz, 1H), 7.45 (d, J = 2.92 Hz, 1H), 7.40 (d, J = 7.2 Hz, 1H), 7.24 (dd, J = 9.2, 3.0 Hz, 1H), 7.04−6.98 (m, 2H), 6.64−6.59 (m, 2H), 4.54−4.29 (m, 2H), 3.89−3.83 (m, 1H), 3.71−3.62 (m, 1H), 3.61−3.53 (m, 3H), 3.04−2.67 (m, 16H), 2.44−2.36 (m, 1H), 2.08−2.00 (m, 1H), 1.96−1.88 (m, 2H), 1.71−1.60 (m, 2H), 0.97 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.3, 170.3, 170.2, 169.6, 161.9, 156.3, 147.0, 132.5, 130.4, 130.3, 127.9, 122.4, 122.0, 118.6, 117.5, 115.5, 63.6, 62.0, 56.7, 55.2, 48.8, 36.8, 31.3, 31.0, 28.0, 19.2, 18.9; HRMS (ESI-TOF) m/z calcd for $C_{35}H_{50}N_7O_9$ $(M + H)^+$ 712.3670; found 712.3647.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01475.

Copies of ^{1}H NMR, ^{13}C NMR, and HPLCs for selected intermediate and all final compounds **1**; conditions tried for the optimization of methyl ester hydrolysis and on-bead cyclization (PDF)

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Notes

The authors declare no competing financial interest.

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