JOC The Journal of Organic Chemistry

Article

Assembly of complex macrocycles by incrementally amalgamating unprotected peptides with a designed four-armed insert

Brice H. Curtin, Francesco Manoni, Jiyong Park, Luke J Sisto, Yu-hong Lam, Michel Gravel, Anne Roulston, and Patrick G Harran

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.7b02958 • Publication Date (Web): 01 Mar 2018

ACS Publications

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

Assembly of complex macrocycles by incrementally amalgamating unprotected peptides with a designed four-armed insert

Brice H. Curtin,[†,§] Francesco Manoni,[†] Jiyong Park,[†,∫] Luke J. Sisto,[†] Yu-hong Lam,[†,◊] Michel Gravel,[‡] Anne Roulston,[‡] Patrick G. Harran[†]*

[†]Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, California 90095, USA.
[‡]Laboratory for Therapeutic Development, Rosalind and Morris Goodman Cancer Research Centre and Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada.

ABSTRACT: We describe the asymmetric synthesis of a highly-substituted ω-octynoic acid derivative and demonstrate its utility for generating complex macrocycles from unprotected peptides. The molecule harbors an isolated quaternary center that displays four uniquely functionalized arms, each of which can be reacted orthogonally in sequence as the molecule is integrated into peptide structure. These processing sequences entail 1) scaffold ligation 2) macrocyclization *via* internal aromatic alkylations or catalyzed etherifications 3) acyliminium ion mediated embedding of condensed heterocycles and 4) terminal alkyne derivatization or dimerization reactions. Numerous polycycles are prepared and fully characterized in this study. Factors that influence reaction efficiencies and selectivity are also probed. We construct a novel mimic of the second mitochondria derived activator of caspase using these techniques, wherein subtle variations in macrocycle connectivity have a marked impact on performance. In general, the chemistry is an important step towards facile, systematic access to complex peptidomimetics synthesized by directly altering the structure and properties of machine-made oligomers.

INTRODUCTION

Macrocycles have the potential to expand the scope of drug discovery due to their ability to scaffold extended pharmacophores.[1–5] This attribute is thought to facilitate interactions with protein surfaces, including those involved in biological signaling pathways. Signaling events mediated by protein-protein interactions (PPIs) provide the largest class of potential drug targets, but these are difficult to engage with small molecules.[6,7] That said, numerous PPIs are mediated by a short peptide sequence in one partner,[5] and that sequence therefore is a logical starting point for drug discovery.[8,9] However, poor transport properties and limited *in vivo* stability of small peptides are perennial challenges to this approach.[5,10] A step towards improved performance involves cyclization, as cyclic peptides can possess improved cellular permeability and stability relative to acyclic counterparts.[11,12] Conformational restriction affords defined topologies that can shield polar surface area while maintaining a desired binding motif. Numerous methods exist to form cyclic peptides, including conventional amidations as well as a range of alternate constructions developed more recently.[13–23] Many of these creative methods are both general and high yielding. Cyclic peptides can also be made in enormous numbers using nucleic acid encoding technologies.[24–26] The question is whether ring formation alone can sufficiently alter properties to afford valuable lead structures. While in certain instances the answer is yes,[27–30] we have been working under the assumption that, in general, it is not. To pursue further alterations in context of large ring structures, we have designed synthetic inserts that react incrementally with linear peptides to form macrocyclic composites. The goal is for the hybrid molecules to retain molecular recognition elements in the biopolymer while displaying that functionality

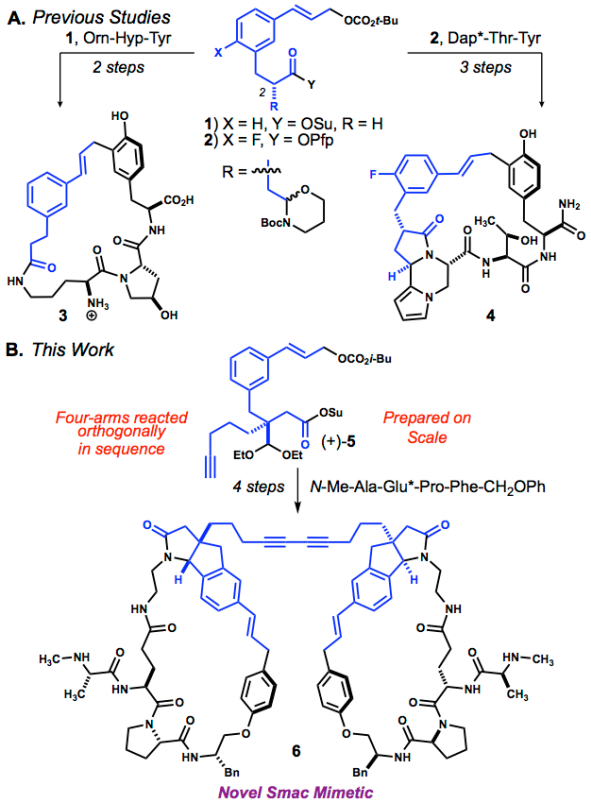


Figure 1. Molecular amalgamations made possible by increasingly capable scaffolding reagents. Reagents **1**, **2**, and (+)-**5** engage unprotected peptides in short processing sequences that generate complex products having defined shapes and altered properties.

as part of stable, amphipathic polycycles having defined shapes and improved pharmacological properties.[31–36] The scaffolding in our chemistry has evolved from a core cinnamyl alcohol motif. We have shown how this unit can support large ring forming reactions by exploiting the cinnamyl cation; generated either as a solvated ion pair under acidic conditions or as a metal stabilized complex. The latter allows us to synthesize macrocyclic ethers, amines and lactones while the former permits unique macrocyclizations *via* direct carbon-carbon bonding (*e.g.* **3**, Fig. 1A). In neither instance are protecting groups required on the peptide. As our scaffolding has become more functionalized, we have been able to sequence additional reactions with macrocyclization. For example, when cinnamyl carbonate containing propionic acid derivative **2** is used to acylate a pyrrolic derivative of Dap-Thr-Tyr, mild acidolysis (aq. AcOH) of the product converts the N-terminus into a pyrrolopiperazine *via* *N*-acyliminium ion cyclization.[35,36] Subsequent exposure to MsOH in $MeNO_2$ initiates internal Friedel-Crafts alkylation to afford a single regioisomeric macrocycle (**4**) in high yield. The secondary alcohol, primary carboxamide, phenol and disubstituted pyrrole are unaffected in the reaction, which occurs within minutes at room temperature.

Holding **2** constant, many variants of this three-step sequence can be executed on a range of functionalized oligomers. The operations are rapid and facile. We are currently generating numerous complex peptidomimetics in this manner. At the same time, our scaffold designs continue to evolve. We felt the methine hydrogen in **2** (namely C2-H) presented another opportunity. By substituting this position, not only would configurational stability at C2 be assured, we could also explore an entirely new series of experiments. If the fourth branch exhibited reactivity orthogonal to the other three, we could probe whether the initially formed macrocycles could be tagged, transannulated, or multimerized in sequence. The structures generated by such processes have little precedent and would position us to study their properties in detail. Importantly, this could include direct comparisons to products derived from manipulating individual peptide sequences with **1** and **2**. Here we describe important steps in this direction.

Based on results from model systems, we chose reagent **5** (Fig. 1B) as a target. This material retains all capabilities of **2** while adding a normal pentynyl chain at its lone chiral center. The alkyne was anticipated to be inert to macrocyclization and heteroannulation conditions used to react other functional groups in the molecule. It would also provide varied options for manipulating the resultant macrocycles. Before this idea could be tested, however, we faced a difficult synthetic problem. Initially we considered elaborating intermediates used to prepare **2**. Because this would have further lengthened a nine-step sequence, it was not attractive. Attention turned instead to *de novo* synthesis of **5**. Several tactics to generate this compound seemed plausible, including asymmetric conjugate addition of a formyl anion synthon to acrylic esters **8** (Fig. 2). Racemic intermediates could be prepared by adding nitromethane to **8**, but, in our hands, optically active products were elusive. Fortunately, ideas evolved quickly from **8**. Instead of adding a one-carbon nucleophile to **8**, we examined reacting a two-carbon electrophile with **9**. This involved attempts at catalyzing addition of an enol to nitroethylene.[37] This also proved challenging, but working with aldehyde **9** we recognized the enantiotopic faces of its capped enol (**10**, P = TBS) might be discriminated *via* asymmetric cyclopropanation.[38] This could generate an intermediate oxygenated cyclopropyl carboxylate (**11**), whose fragmentation (as shown) was expected to be facile. At about this same time, Marek *et al.* reported that directed carbometalation of chiral cyclopropenyl carboxylates followed by *in situ* oxidation gave optically active 4-oxobutyrate derivatives directly.[39] These reactions proceeded by way of species analogous to **11** and suggested a clear path to enantioenriched **5** beginning with cyclopropene **12** and an appropriate organometallic.

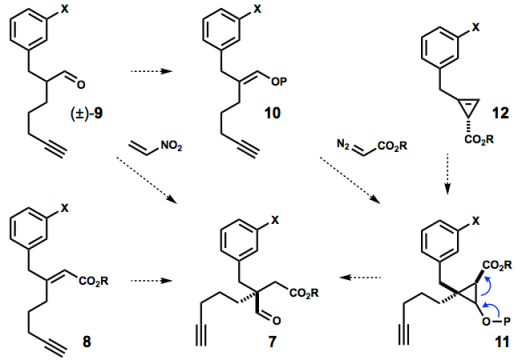


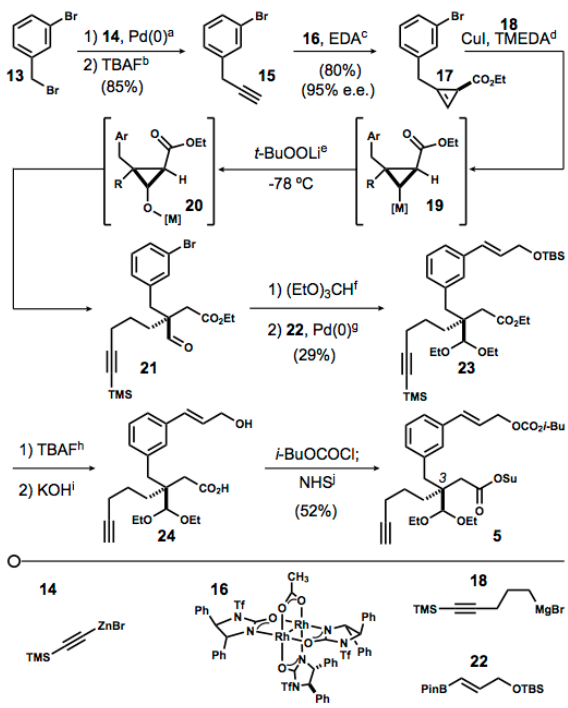
Figure 2. Routes contemplated to build a highly functionalized chiral quaternary center.

RESULTS & DISCUSSION

m-Bromophenyl propyne was synthesized from commercial 3-bromobenzyl bromide and trimethylsilyl acetylene using a Negishi protocol (Scheme 1).[40] Reaction of this material with ethyl diazoacetate in the presence of Corey's trisimidazolidinone dirhodium complex **16** (0.25 mol%)[41] afforded cyclopropene carboxylate (+)-**17** in 80% yield and 95% *e.e.* as judged by chiral supercritical fluid chromatography. *S* stereochemistry in this material was tentatively assigned by analogy to Corey's precedent, and later corroborated by NMR analyses of diastereomeric derivatives of downstream product (+)-**5** (*vide infra*).

With **17** in hand, we next examined copper-mediated carbometalation of its strained alkene. Procedures involving Grignard **18** and catalytic amounts of copper salts were not productive in our hands. However, when **17** was added slowly to superstoichiometric amounts of **18** (freshly prepared, 1.4 M in THF) and CuI at -40˚C, stirring for 30 min followed by quench with premixed NH_4Cl/NH_4OH cleanly generated cyclopropane **19** (R = 4-pentynyl, M = H). Diastereoselectivity appeared high and 2D-NOESY spectra of the material were consistent with the major isomer being that drawn (see SI). Sequencing the carbometalation with *in situ* oxidation was more challenging. Among the variety of oxidants examined, only lithium *t*-butyl peroxide proved effective.[39,42] An optimized protocol involved a pre-formed organocopper species prepared from **18** and CuI•TMEDA complex being used to carbometalate **17**. Temperature control while adding **17** was important such that organometallic species were largely dissolved at -40 ºC in THF. This was critical for scalability. Under such conditions, cyclopropene (+)-**17** was consumed within 30 min, whereupon the mixture was cooled to -78 ºC and carefully treated with anhydrous *t*-BuOOLi. After aqueous workup, aldehyde **21** was isolated directly, ostensibly *via in situ* fragmentation of a transient cyclopropanoxide (*i.e.* **20**). Compound **21** was difficult to purify without loss, and therefore crude material was treated with triethylorthoformate in the presence of catalytic TsOH. The resultant acetal was cross

Scheme 1. Enantioselective Synthesis of (+)-5

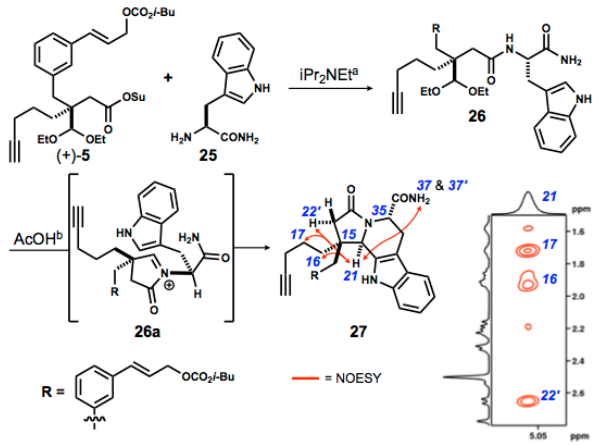


Reaction conditions: a) 0.2 mol% $Pd(DPEPhos)Cl_2$, 1.4 eq. **14**, THF, 22 ºC. b) 2.0 eq. AcOH, 2.0 eq. TBAF, THF, 22 ºC, 85% yield over two steps. c) 3.0 eq. **15**, 1 eq. ethyl diazoacetate, 0.25 mol% **16**, DCM, 80% yield, 95% *e.e.* d) 2.0 eq. **18**, 2.2 eq. TMEDA, 2.0 eq. CuI,; e) then 2.0 eq. *t*BuOOLi; 2:1 NH_4Cl/NH_4OH. f) 10 mol% *p*TSA, 3.0 eq. $(EtO)_3CH$, EtOH. g) 1 mol% $Pd(PPh_3)_4$, 1.2 eq. **22**, 3.0 eq. Na_2CO_3, 5:1 dioxane/H_2O, reflux, 29% yield over three steps. h) 2.5 eq. TBAF, THF, 0 ºC. i) 10 eq. KOH, 2:1 EtOH/H_2O, 50 ºC. j) 4.5 eq. *N*-methylmorpholine, 2.1 eq. *i*-BuOCOCl, DCM, -5 ºC; then 2.0 eq. *N*-hydroxysuccinimide, -5 ºC to 22 ºC, 12h, 52% over three steps, 94% *e.e.* EDA= ethyl diazoacetate; NHS= *N*-hydroxysuccinimide.

coupled with vinyl boronate **22** (See SI) using palladium catalysis to afford stable product **23** in 29% overall yield from **17**. Desilylation and saponification then afforded hydroxy acid **24**, which was reacted with excess *iso*-butyl chloroformate. The doubly acylated species formed *in situ* was partially decomposed with *N*-hydroxysuccinimide to afford target (+)-**5** in 52% isolated yield. This concise route to (+)-**5** gave access to our first four-armed scaffolding reagent on multi-gram scales.

Before exploring its utility, we further probed stereochemistry in (+)-**5**. The molecule was reacted with L-tryptophan carboxamide to give **26**. When that substance was dissolved in 80% aqueous AcOH, its acetal quickly (< 1 hr) decomposed to a mixture of diastereomeric hydroxy lactams. These gradually converted to pyrrolo-β-carboline **27** (*via* **26a**) over the next 12 hours. A single isomer of **27** was observed by ^1H NMR and HPLC. Its 2D-NOESY spectrum showed clear correlations between the carboxamide protons *H37,37'* and the *C21* methine hydrogen, which in turn correlated with methylene signals *H16* and *H17*. Literature precedent and our own studies indicated the configuration at *C35* would dictate stereochemistry at *C21*.[35,36,43,44] If true, the relative stereochemical relationships implied from NOE correlations would translate to (+)-**5** being *S*-configured, which was further consistent with the

Scheme 2. Corroborating the Stereochemical Assignment of (+)-5

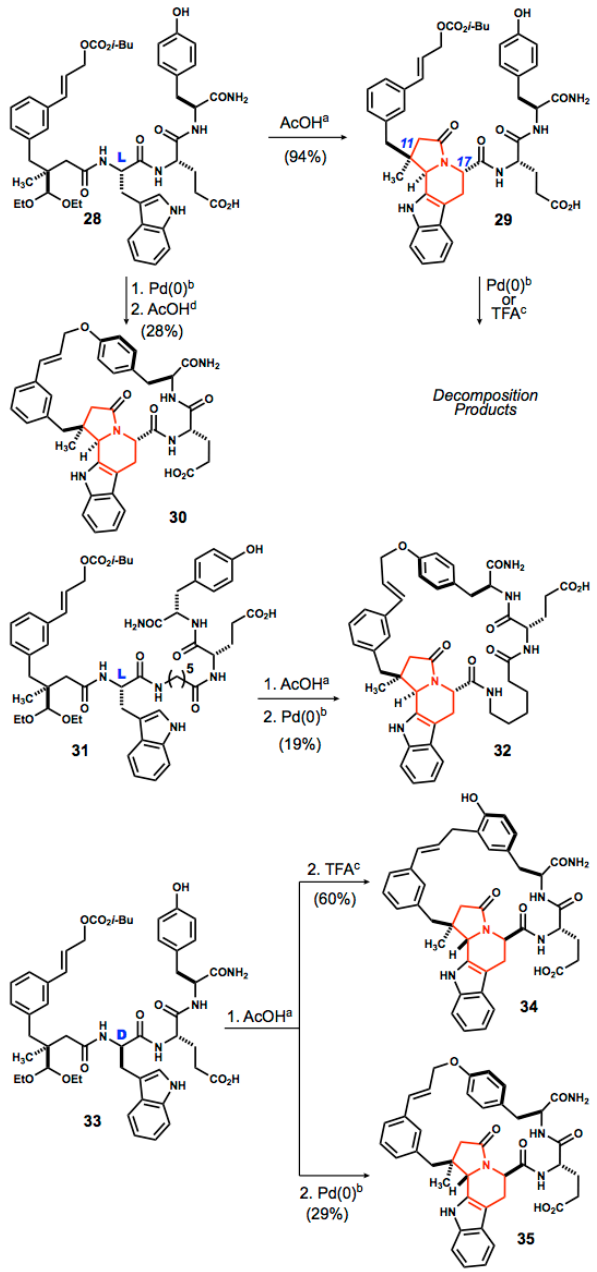


Reaction conditions: a) 1.0 eq. (+)-**5**, 1.5 eq. **25**, iPr_2NEt, DMF. b) 4:1 AcOH/H_2O, 22 ºC, 12 h, 51% yield over two steps. Inset: partial 2-D NOESY spectrum of **27** showing key correlations involving *H21*.

earlier assignment of stereochemistry in **17** made by analogy to Corey's results.

Initial macrocyclizations with this new scaffold were tested in the absence of the alkyne. An analog of (+)-**5** was prepared wherein the C3 pentynyl group was replaced by methyl (by substituting CH_3MgBr for **18** in Scheme 1, see Fig. S2). Acylating Trp-Glu-Tyr with this molecule gave **28**. Exposure of **28** to aqueous acetic acid gave Pictet-Spengler product **29** in high yield (Scheme 3). Interestingly, all attempts to macrocyclize this molecule by activating the cinnamyl carbonate under conditions developed previously led to decomposition.[33–35] Reversing the order of events solved this problem. Treatment of **28** with 5 mol% $Pd(PPh_3)_4$ in DMF at room temperature initiated a high yielding cycloetherification. When that product was dissolved in aqueous acetic acid, it slowly converted to polycycle **30** (90% conversion after 4 days at 25 ºC). Following preparative HPLC, analytically pure β-carboline **30** was isolated in 28% yield (three steps from **S5**). The 2-D NOESY spectrum showed correlations very similar to those observed in **27** (Scheme 2) and stereochemistry in **30** was thus assigned similarly. A possible explanation for the reluctance of **29** to participate in macrocyclization reactions was the conformational restriction imposed by the Pictet-Spengler process. It oriented the *C17* and *C11* β branches *anti* off of a rigid tetrahydroindolizinone core. The effect was marked. Substrate **31** was synthesized and harbored the same residues as **28**, but wherein an aminohexanoic acid spacer was inserted in between P1 and P2. Treatment with aqueous acetic acid smoothly initiated a Pictet-Spengler cyclization, but the system remained reluctant to macrocyclize. Only palladium-catalyzed cycloetherification was successful, and in that case product **32** was relatively unstable, presumably due to torsional strain present in the macrocycle. Attenuating this strain had a positive impact. Substrate **33** was synthesized. This molecule was the same as **28** except the tryptophan residue was D-configured. Acetic acid promoted Pictet-Spengler reaction within this molecule occurred cleanly and subsequent macrocyclizations were now facile. Treatment with either TFA in CH_3NO_2 or 5 mol% $Pd(PPh_3)_4$ in DMF gave regioisomeric macrocycles **34** and **35** in 60% and 29% yield, respectively.

Scheme 3. Probing Initial Macrocyclizations with a Three-Armed Model



Reaction conditions: a) 4:1 AcOH/H_2O, 22 ºC, 12 hours. b) 2.0 eq. Cs_2CO_3, 5 mol% $Pd(PPh_3)_4$, DMSO, 10 mM, 4 hours. c) 5 vol% TFA, CH_3NO_2, 5 mM, 2 hours. d) 4:1 AcOH/H_2O, 22 ºC, 4 days. Note: yields quoted throughout reflect analytically pure material isolated (prep-HPLC or SiO_2 chromatography) after full sequence beginning with (+)-**S5**.

Efficient macrocyclization *via* either internal Friedel-Crafts alkylation or Tsuji-Trost cinnamylation was consistent with earlier studies (*e.g.* Fig 1A) and reflective of the relative stereochemistry in the Pictet-Spengler product now positioning reactive termini *syn*, thereby favoring ring closures. The use of a D-configured P1 residue in conjunction with (+)-**5** to permit syntheses of unique structures such as **34** and **35** was an excellent outcome. It should be noted, however, the same result could in principle be achieved using all L-configured amino acids and the enantiomer of **5**.

Having established the new scaffold frame supported macrocyclizations, we turned to **5** and experiments to test the inertness of its alkyne to both palladium catalysis and acidolysis conditions used for large ring formations (Fig. 3). Acylation of D-Trp-Glu-Tyr with (+)-**5** and subsequent treatment with aqueous acetic acid followed by TFA in CH_3NO_2 (5 vol %) gave macrocycle **36** in 31% isolated yield over three steps. NOE correlations in **36** paralleled those observed in **27** and **34** and were fully consistent with the relative stereochemistry drawn. As we hoped, no products derived from reactions at the alkyne were detected, nor did the carboxylic acid or primary carboxamide interfere. The same three-step sequence beginning with **5** was repeated with D-Trp-Gln-Tyr, Pro-Ala-Lys(D-Trp)-Tyr, and D-Trp-Glu(tyramide) to yield macrocycles **37**–**39** in good per step average yields over three steps. We next prepared the *O*-linked regioisomer of **39** (namely **40**) by changing the third step in the processing sequence. Instead of treating with TFA in $MeNO_2$, the Pictet-Spengler product was exposed to 4 mol% allyl palladium chloride dimer, 10 mol% Xantphos, and stoichiometric Cs_2CO_3 in DMF.[34] The alkyne was again unaffected. The dipeptide D-Trp-AAP* having its C-terminus condensed with tyramine was readily processed with **5** to afford polycycle **41**. Both the alkyne and the primary azide were unaffected, opening the possibility for transannulations *via* Huisgen cycloadditions should that be desired in future iterations.

Each stage of engagement with **5** was designed to be flexible. Consistent with Meldal's results, the *N*-acyl iminium ion intermediates would react with a range of proximal π-basic aromatics.[35,36,43] For example, when D-3-MeOPhe-Thr(tyramide) was *N*-acylated with **5** and treated with aqueous acetic acid, two isomeric products (1:1) were formed. They were tentatively assigned as epimeric dihydropyrroloimidazole diones, although alternative structures could not be ruled out.[44] When those materials were treated with 5 vol % TFA in CH_3NO_2, Pictet-Spengler reaction and Friedel-Crafts macrocyclization occurred concomitantly to afford a single macrocyclization product (**42**) in good overall yield. Neither the alkyne nor the secondary alcohol were affected. In the case of D-Trp(5Br)-His(tyramide), its reaction with **5** gave a product that resisted Pictet-Spengler reaction in aqueous AcOH. However, addition of 10 vol % H_3PO_4 caused rapid cyclization. Notably, without degrading the cinnamyl carbonate. The product was then converted to macrocycle **43** by exposure to TFA in $MeNO_2$. Alternatively, palladium-catalyzed cycloetherifcation afforded macrocyclic cinnamyl ether **44**. The alkyne and the unprotected imidazole ring were unaffected by either process.

Lastly, in the course of these studies, we discovered what we believe is a unique macrocyclization process. When D-Trp-Cys(S*t*-Bu)(tyramide) was *N*-acylated with (+)-**5** and the product was dissolved in aq. AcOH, a Pictet-Spengler reaction occurred uneventfully. However, when that molecule was treated with TFA in $MeNO_2$, two products (~1:1) formed in good yield. One was the internal Friedel-Crafts alkylation product **45a**, as expected. The second lacked a *tert*-butyl group and its spectroscopic data were consistent with allylic disulfide containing macrocycle **45b**. This outcome was interpreted in terms of a solvated cinnamyl cation being captured by the distal sulfur of the disulfide and the incipient sulfonium ion extruding isobutylene. Macrocyclic allylic disulfides of this type may be manipulated in a host of ways by partial oxygenation reactions and/or sigmatropic rearrangements of derived

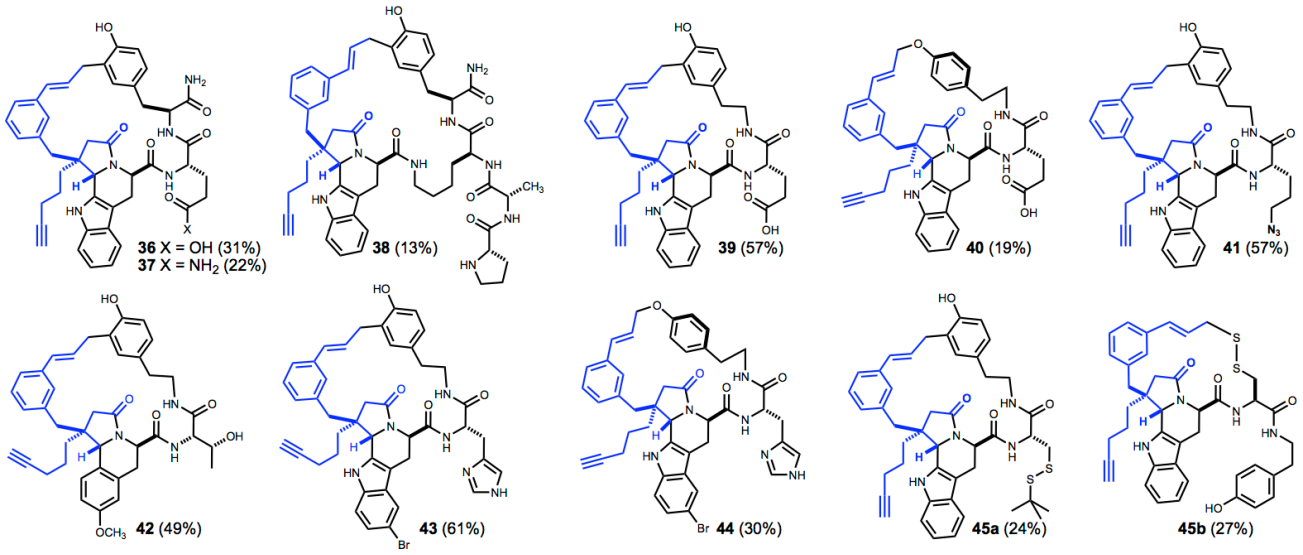


Figure 3. Macrocyclic products obtained by acylation of unprotected peptides with (+)-**5**, followed by *N*-acyliminium ion cyclization, and either acid-mediated Friedel-Crafts alkylation or palladium-catalyzed macrocycloetherification. Note: for yield calculations see Scheme 3.

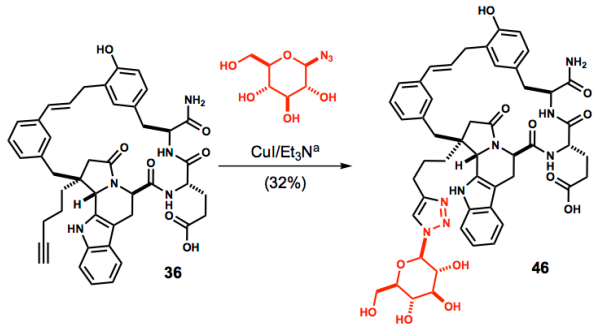
ylides. Towards this end, experiments to test the generality and efficiency of the macrocyclization reaction, both in the presence and absence of competing internal nucleophiles are ongoing.

In all processing sequences using (+)-**5** to date, the alkyne has been inert to chemistries used to synthesize macrocycles having imbedded condensed heterocycles. For the eleven structures depicted in Figure 3, and more, it was possible to integrate (+)-**5** into unprotected peptides using simple, telescoped three-step sequences followed by mass-guided preparative HPLC. Pure products were routinely isolated on tens of milligrams scales without incident.

While inert enough to be valuable in our schemes, the alkyne was certainly a handle for further manipulations. Many natural products are glycosylated and this feature can markedly alter solubility and transport properties, relative to the aglycon, in biological systems. Viewing our composite macrocycles as analogous to non-ribosomal peptides, a ready means to add sugars to these compounds was desirable. The alkyne made this trivial. For example, mixing polycycle **36** with commercial 1-azido-1-deoxy-β-D-glucopyranoside in the presence of catalytic copper iodide and triethylamine proceeded well to give unique glycoconjugate **46**. Several additional examples of this Huisgen cycloaddition are detailed in the supporting information. We have begun to examine passive membrane permeability in this series. Conventional wisdom suggests molecules of this type will have difficulty entering cells. We are interested in understanding this behavior deeply enough that we might eventually use our templates to facilitate permeability where it would otherwise not exist. In one of our first Caco-2 monolayer screens, compound **46** stood out as a substance displaying some passive permeability (see SI Table S2). While it is minimal relative to positive controls, the fact we observed any permeability for a molecule having as much exposed polar surface area as **46** is striking. Collaborations to explore structure / permeability relationships of peptidomimetic macrocycles in detail are ongoing. Alkyne functionalizations will greatly aid these studies, and we note that the triple bond may also be used for conjugation to cell penetrating peptides and serve as a linker site for assembly of antibody and/or protein drug conjugates.

A major goal for this program is to allow biologically active peptides to be molded directly into potent and stable lead

Scheme 4. Glycoconjugation *via* a Copper-Catalyzed Huisgen Cycloaddition



Reaction Conditions: a) 1.5 eq. azido sugar, 2.5 eq Et_3N, 10 mol% CuI, DMF, 22 ºC.

structures for further research. To initially demonstrate the potential of (+)-**5** in this context, we began with a familiar system. The second mitochondria derived activator of caspase (Smac) is a homodimeric protein secreted from mitochondria during programmed cell death.[45] Cytoplasmic Smac relieves inhibitor-of-apoptosis protein (IAP) mediated suppression of caspase activity. It binds avidly to X-chromosome encoded IAP, cellular IAP1 and cellular IAP2, and synergizes with both TRAIL and TNFα to potently induce caspase activation and apoptosis in human cancer cells.[45] Smac exploits a conserved tetrapeptide (AVPI) at its N-terminus to bind BIR domains within IAPs.[46] We had used traditional medicinal chemistry techniques earlier to develop a bivalent small molecule mimic of Smac.[47] That exercise went on to drive much research as well as clinical development programs.[48,49] However, it required several years of experiments. We were interested if the use of (+)-**5** might be able to generate Smac mimetic leads more quickly.

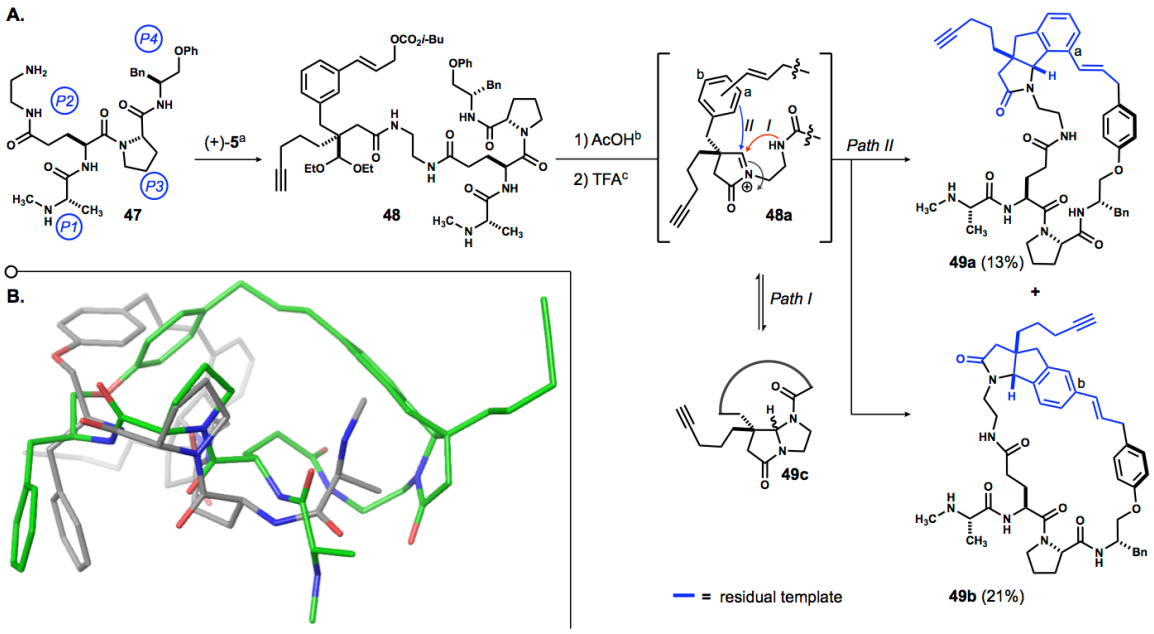


Figure 4. (A) Synthesis of macrocyclic Smac mimetic monomers. Reaction conditions: a) 1.0 eq. (+)-**5**, 1.5 eq. **47**, *i*Pr_2NEt, DMF. b) 4:1 AcOH/H_2O, 22 ºC, 12 h. c) 1:1 TFA/TFE, 5 mM in substrate, 22 ºC, 7h, 13% yield (**49a**) and 21% yield (**49b**). (B) Overlay of energy-minimized (*B3LYP-D3*) conformers of **49a** (gray) & **49b** (green), which orient their peptidyl segments differently within the composite structures.

In terms of caspase inhibition, it was known from *in vitro* peptide screens that the P2 position of AVPI was tolerant of side chain variations, and that an aromatic residue was preferred at P4.[47,50–52] We therefore prepared peptide **47**, wherein the P1 and P3 positions were unaltered. The P2 position was occupied by a glutamic acid derivative that provided both an attachment point for (+)-**5** and a means to generate an *N*-acyliminium ion from the composite. Lastly, L-*O*-phenyl phenylalaninol was placed at P4 such that it could participate in alkylative macrocyclizations while also displaying an aromatic side chain.

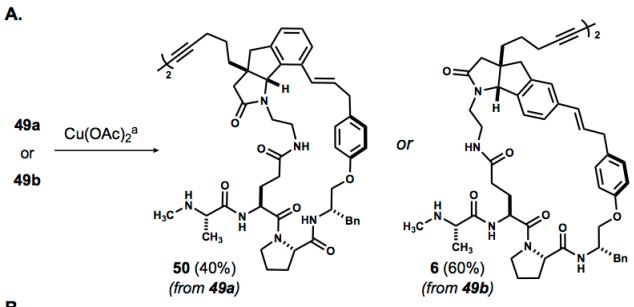
Treatment of **47** with (+)-**5** gave **48** in 71% yield and without competitive acylation of the N-terminus. Hydrolysis in aqueous acetic acid then generated hydroxy lactam intermediates. These species were concentrated to dryness, re-dissolved in trifluoroethanol and treated with TFA (1:1 final, 5 mM in substrate) at 25 ºC. This promoted an *N*-acyliminium ion cyclization and concomitant macrocyclization. The original expectation was that ion **48a** (Fig. 4) would be trapped by the adjacent amide to form a diacyl imidazolidine (*e.g.* **49c**, Fig. 4A). However, extensive NMR analyses (including HMBC and NOESY spectra) of the two isolated products showed them to be regioisomeric tetrahydroindenopyrrolones **49a** and **49b**. Similar to logic invoked for **42** (*vide supra*), this outcome was rationalized in terms of a transient diacyl imidazolidine (**49c**) giving way to more stable C-C bonded products *via* internal Friedel-Crafts alkylation. The closest aromatic ring to ion **48a** was that of the scaffold, and therefore **49a**/**b** were formed. To our knowledge, these macrocycles are without precedent. Moreover, from (+)-**5**, they were prepared and purified in less than 48 hours.

We were now positioned to study how subtle differences in ring connectivity might affect IAP binding and domain selectivity. *In silico* geometry optimization and conformational searches suggested that **49a** and **b** would display their peptide regions differently (Fig. 4B, see SI for computational details), although the relevance of this analysis to bound states was as yet unclear.

Despite structural homology, slight differences in BIR domain structures within IAPs have been leveraged to design cIAP-selective antagonists.[53,54] Because XIAP, cIAP1, and cIAP2 function independently, and differently, to block apoptosis, selective antagonists have been coveted as research tools.

Smac protein exists as a native dimer and, in the case of XIAP, binds simultaneously to adjacent BIR domains within its structure. We had exploited this previously by dimerizing monomeric BIR3 domain ligands, thereby achieving exceptional Smac mimicry.[47] Anticipating similar behavior, we oxidatively dimerized **49a** and **49b** *via* Glaser coupling. This involved treating their free-base forms with $Cu(OAc)_2$ and piperidine in 1:1 CH_3CN/MeOH at 70 ºC (Fig. 5A). Symmetric diynes **50** and **6** were isolated in 40% and 60% yields, respectively. Avidities for recombinant XIAP, cIAP1, and cIAP2 (BIR2-BIR3 domain constructs) were then evaluated by competitive binding using a fluorescence polarization (FP) assay (Fig. 5B). The same fluorescein labeled dimeric Smac peptide FP probe was employed in all experiments (see SI Fig. S5). Tetralogic's clinical compound Birinapant™ was used as a positive control.[55] Linear peptide **47** weakly displaced the FP probe from all three IAP constructs, although it did discriminate cIAP1 from cIAP2. Macrocyclic monomer **49a** was comparable to its precursor **47**, but macrocycle **49b** was not. As expected for a monomer,[56] it remained a poor competitor for XIAP, but it displaced the FP probe from cIAPs with low nanomolar efficacy. In fact, it was 13 times more effective than **49a** against both cIAP1 and cIAP2. This highlights a phenomena we did not fully appreciate, yet one that may be general for molecules of this type. Namely, that subtle variations in macrocycle topology and pharmacophore display can markedly alter performance.[57] Macrocyclic monomer **49b** also showed excellent cIAP1:XIAP selectivity (>250:1). The abil-

A.



B.

Compound	IC50 (nM) XIAP	cIAP1	cIAP2	cIAP1/XIAP
Peptide **47**	>5000	383 ± 165	2888 ± 393	>13:1
Monomer **49a**	>5000	261 ± 51	763 ± 226	>19:1
Dimer **50**	>5000	472 ± 217	717 ± 347	>11:1
*Monomer **49b***	*>5000*	20 ± 12	60 ± 28	*>250:1*
*Dimer **6***	1335 ± 1436	21 ± 5	42 ± 18	*65:1*
Birinapant	289 ± 12	28 ± 4	55 ± 11	10:1

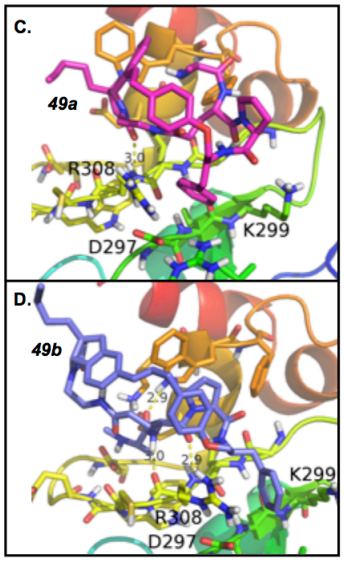


Figure 5. (A) Dimerization of macrocyclic monomers. Reaction Conditions: a) 1.0 eq. **49a** or **49b**, 7.0 eq. $Cu(OAc)_2$, 7.0 eq. piperidine, 1:1 MeOH/CH_3CN, 40% yield (**50**) or 60% yield (**6**). (B) Fluorescence polarization assay for competitive displacement of a labeled bivalent Smac-mimetic peptide from recombinant Bir2-Bir3 constructs of indicated IAP protein. Data is reported as IC_{50} values in nM (average of 2 biological and 2 technical replicates). (C) & (D) Final snapshots of 100 ns MD simulations of **49a** and **49b** bound to the BIR3 domain of cIAP1, respectively. Intermolecular hydrogen bonds are indicated in yellow (enlarged to show detail in SI Fig. S10).

ity of dimeric compounds **50** & **6** to compete for cIAP1/2 binding was similar to their respective monomers, a finding consistent with the 1:2 binding stoichiometry of Smac protein to these particular IAPs. Dimer **6**, on the other hand, was the only compound to show competitive binding to XIAP, while remaining able to potently displace the FP probe from cIAPs, especially cIAP1. The data above reflects competition IC_{50} values rather than direct binding constants although, for comparison, Birinipant is reported to bind to full length XIAP and cIAP1 with K_D = 45 nM and <1 nM, respectively.[55] The apparent discrimination of **49b** and **6** for cIAP1 over XIAP was fascinating and, along lines argued by others, may derive from minor variations in the P4 binding pockets on BIR domains within these proteins.[53,54] To probe this further we employed computational techniques. We studied the molecular dynamics (MD) of **49a**/**b** docked into the binding site of cIAP1 as well as XIAP (see SI for computational details). We found that **49b** buried 50 $Å^2$ more surface area compared to **49a** when averaged over the last 20 ns of a 100 ns MD simulation using cIAP1-BIR3 as the protein partner (Fig. 5 C & D). This observation suggested that **49b** interacts with cIAP1 more favorably than **49a** and correlated well with competitive binding data. Comparing MD simulations of ligated cIAP1 and XIAP explained the observed selectivity for cIAP1. The hydrophobic binding site in XIAP was unable to accommodate the P4 phenyl substituent, while in cIAP1 it provided a firm anchor for the ligand: after 75 ns of a 100 ns simulation, the phenyl substituent exits the XIAP hydrophobic pocket, which then leads to complex disengagement. Evident from MD data, the hydrophobic pocket of cIAP1 can accommodate a larger substituent relative to the constricted site in XIAP, presumably due to the steric demand of K206, which corresponds to G306 in cIAP1 (see SI Fig. S9 & S10).

The method of lead discovery in the above experiments was highly effective. Using scaffold (+)-**5** and an unprotected consensus peptide, we were able to rapidly generate unique macrocyclic ligands for protein surfaces. While this was a proof-of-principle exercise in a well-characterized system, we believe the chemistry has broad potential to create and optimize complex antagonists of protein-protein interactions, especially those mediated by a short-linear-interacting-motif (SLIM) in one partner.[9]

CONCLUSION

We have developed a short, scalable and enantioselective synthesis of our first four-armed scaffolding reagent. This molecule can be incrementally integrated into a range of oligomeric substrates, wherein the composite products are stable polycycles having defined conformations. By varying the order of events, ring forming modalities, and derivatization schemes, countless new complex structures are potentially available. From such collections the search for islands of useful pharmacological properties can proceed in ways not possible previously with increasingly intricate structures being made using multiple template generations (*i.e.* **1**, **2**, & (+)-**5**). Scaffold design and utilization within the project is continually advancing, and attempts to exploit the alkyne (and its homologs) in (+)-**5** for novel transannulation reactions are ongoing. Bridged macrocycles anticipated from those studies could bring yet another novel compound class into consideration.

EXPERIMENTAL SECTION

$Pd(DPEPhos)Cl_2$ was purchased from Strem. Catalyst **16** was prepared according to prior literature.[41] (5-bromopent-1-yn-1-yl)trimethylsilane was prepared according to prior literature.[58] *t*-Butylhydroperoxide ~5.5 M solution in decane was purchased from Aldrich and iodometrically titrated (c = 5.4 M).[59] Vinyl boronate **22** was prepared using a modified procedure using 1 mol% Schwartz's Reagent.[60] *N*-hydroxysuccinimide was azeotropically dried from benzene. Fmoc-5-bromo-D-tryptophan and Fmoc-3-methoxy-D-phenylalanine were synthesized by kinetic enzymatic resolution of their racemates according to published procedures.[61] L-Phenylalaninol was purchashed from Chem-Impex. Purification of acidolysis products was performed on an Agilent 1100/1200 HPLC system equipped with G1361A preparative pumps, a G1314A autosampler, a G1314A VWD, and a G1364B automated fraction collector. Analytical HPLC was performed using an identical system, but with a G1312A binary

pump. Mass spectra were recorded using an Agilent 6130 LC/MS system equipped with an ESI source. Stationary phase and gradient profile are noted for individual reactions below. NMR spectra were recorded on Brüker Avance (300, 400, 500 or 600 MHz) or DRX (500 MHz) spectrometers and calibrated according to the respective residual solvent peak. 2D-NMR data were acquired as previously detailed.[34] High-resolution mass spectra (HRMS) were obtained on a *Thermo Fisher Scientific Exactive Plus (orbitrap)* with IonSense ID-CUBE DART, on a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ *via* direct syringe pump injection, or on a *Waters LCT Premier with ACQUITY UPLC (ESI–TOF)*. Enantiomeric excess of cyclopropene **17** was assessed using a Mettler Toledo SFC equipped with a Chiralcel OJ-H column (4.6x250 mm, 5 µm) using 5% *i*-PrOH as co-solvent. Flow rate: 2.0 mL/min.

Peptide Synthesis: All peptides were synthesized via either standard Fmoc solid-phase peptide synthesis using Rink Amide MBHA resin (polystyrene, 1% DVB, 0.7 mmol/g) or Boc/Cbz solution-phase peptide synthesis.[34]

A. Acylation of peptide by template (+)-5 or (+)-S5: A round bottom flask was charged with peptide (1.35 eq.), DMF (1.0 M), and iPr$_2$NEt (4.0 – 6.0 eq.), followed by template (1.0 eq.). The reaction was heated to 40 ºC. Reaction progress was monitored by analytical HPLC-UV/MS. The reaction was diluted with EtOAc and washed thrice with $NaHCO_3$ followed by brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*.

B. Pictet-Spengler Annulation: Linear precursor was dissolved in a 4:1 mixture of AcOH/H_2O (0.2 M) and stirred until HPLC analysis confirmed reaction completion – typically 12 hours. The volatiles were removed and the residue was concentrated from acetonitrile (3x) followed by $CHCl_3$ (3x) to remove residual AcOH.

C. Friedel-Crafts Macrocyclization: A flask was charged with Pictet-Spengler product (1 eq.) and nitromethane (5 mM in substrate). The headspace was flushed with argon for 5 mins. TFA (5 vol%) was then quickly added. Reaction progress was monitored by analytical HPLC-MS.

D. Pd(0)-catalyzed Macrocyclization with Pd(PPh$_3$)$_4$ as catalyst: A flask was charged with Pictet-Spengler product (1 eq.), Cs_2CO_3 (2 eq.), and DMF (5 mM in substrate) and sparged for 30 minutes. $Pd(PPh_3)_4$ was then added as a solid, and the solution was sparged for another 5 minutes. Reaction progress was monitored by analytical HPLC-MS. After reaction completion, the reaction was diluted with EtOAc and washed with 3x NH_4Cl and 1x brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo.*

E. Pd(0)-catalyzed Macrocyclization with [PdCl(C$_3$H$_5$)]$_2$ / Xantphos: A flask was charged with Pictet-Spengler product (1 eq.), Cs_2CO_3 (2 eq.), and DMF (5 mM in substrate) and sparged for 30 minutes. In a glove bag, a flame-dried Schlenk tube was charged with $[PdCl(C_3H_5)]_2$ (9 mg) and Xantphos (37 mg). Outside of the glovebag, the Schlenk tube was charged with 9 mL of 1:1 THF/DMF, which had been separately sparged for 1 hour. The catalyst solution was stirred for 5 minutes under Ar and 4 mol% Pd was added to the reaction flask via syringe. Reaction progress was monitored by analytical HPLC-MS. After reaction completion, the reaction was diluted with EtOAc and washed with 3x NH_4Cl and 1x brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo.*

F. Copper(I)-Catalyzed Huisgen Cycloaddition:

A vial was charged with macrocyclic compounds (1 eq.), azidoglucopyranoside (1.5 eq.), and DMF (0.03 M). The solution was sparged for 10 minutes. In a separate vial, a stock solution of copper was prepared. Copper iodide was added to a vial and evacuated and backfilled with argon (3x). DMF (2 mL) was added and the suspension was sparged for 5 minutes. Et_3N (1 mL) was added to the copper suspension and mixed under sparge for 2 minutes until a homogeneous solution was achieved. The copper solution (10 mol% copper) was then added to the reaction flask. Reaction progress was monitored by analytical HPLC-MS. After reaction completion, the reaction was transferred to an HPLC vial. Desired product was isolated by semi-preparative HPLC purification – see details per example, in S.I.

***G. Dimerization of monovalent Smac-mimetics*:** The TFA-salt of macrocyclic monomer (1 eq.) was dissolved in 1 mL MeOH and treated with silica-bound carbonate (2 eq.) for 10 minutes. The suspension was filtered and washed 3x with 1 mL MeOH. The combined washes were concentrated *in vacuo* and reconstituted in 1:1 MeOH/CH_3CN (50 mM in substrate). The clear solution was treated with piperidine (7 eq.) and $Cu(OAc)_2$•H_2O (7 eq.); the vial was then capped and heated to 70 ºC. The reaction was monitored by HPLC and complete within 12 hours. The reaction was concentrated and purified (see S.I.).

(3-(3-Bromophenyl)prop-1-yn-1-yl)trimethylsilane

In a flame-dried flask under argon, (Trimethylsilyl)acetylene [15.8 mL, 112 mmol] in 80 mL of dry THF was treated with *n*-BuLi [44.8 mL, 112 mmol, c = 2.5 M] at -78 ºC. While the acetylide solution stirred, zinc(II) bromide [25.2 g, 112 mmol] was fused under vacuum then cooled to room temperature under argon. 80 mL of dry THF was then charged into the flask containing $ZnBr_2$. The $ZnBr_2$ solution was then cannulated into the acetylide solution at -78 ºC. The transmetalation was stirred for 30 min then treated with a solution of 3-bromobenzylbromide [20.0 g, 80 mmol] and Pd(DPEPhos)Cl_2 [115 mg, 0.160 mmol] in 80 mL of dry THF – catalyst loading can be increased to drop reaction time and temperature. The solution warmed to room temperature then heated to 35 ºC. Reaction monitored by ^{1}H-NMR. After 2 days, reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. Organic layer washed twice with sat. NH_4Cl, $NaHCO_3$, and 1x with brine. Dried with $MgSO_4$ and concentrated *in vacuo*. A portion of crude was purified by silica chromatography using hexanes as eluent. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.51 (t, J = 7.9 Hz, 1H), 7.38–7.36 (m, 1H), 7.29–7.26 (m, 1H), 7.19 (t, J = 7.9 Hz, 1H), 3.63 (s, 2H), 0.22 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 138.7, 131.1, 130.1, 129.9, 126.6, 122.7, 103.3, 87.8, 25.9, 0.2; HRMS (DART-Orbitrap) *m/z*: $[M-H]^-$ calc'd for $C_{12}H_{14}BrSi$ 265.0054; found 265.0062.

1-bromo-3-(propa-1,2-dien-1-yl)benzene

Crude (3-(3-Bromophenyl)prop-1-yn-1-yl)trimethylsilane [32 mmol] was dissolved in 30 mL of DCM and 20 mL of MeOH and treated with K_2CO_3 [13.3 g, 96 mmol] under argon. Reaction monitored by TLC. After 5 hours, the reaction was diluted with DCM and washed once with water. The aqueous layer was then extracted twice with DCM. The combined organic layers were dried with $MgSO_4$. The solvent was removed *in vacuo*. Purified on SiO_2 with hexanes. 1.0 g, 5.2 mmol, 16% yield over two steps (66% isolated yield of **15**). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.54 (br s, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 7.8 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 6.10 (t, J = 6.6 Hz, 2H), 5.19 (d, J = 6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 210.1, 136.4, 130.2, 129.9, 129.6, 125.4, 122.9, 93.1, 79.5; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for C_9H_8Br 194.9804; found 194.9792.

1-Bromo-3-(prop-2-yn-1-yl)benzene (15)

Crude (3-(3-Bromophenyl)prop-1-yn-1-yl)trimethylsilane [80 mmol] was dissolved in 400 mL of dry THF and treated with acetic acid [9.2 mL, 160 mmol] followed by slow addition of tetrabutylammonium fluoride [160 mL, 160 mmol] under argon. Reaction monitored by ^{1}H-NMR. After 15–30 min, THF was removed *in vacuo* and the residue reconstituted in diethyl ether. Organic layer washed twice with water, sat. $NaHCO_3$, and 1x with brine. Ether was completely removed *in vacuo*, the orange oil was dissolved in pentane, and passed through a plug of silica to remove residual tetrabutylammonium salts. Pentane was removed *in vacuo* and the colorless residue was distilled between 67–69 ºC at 4.7 torr. 12.0 g, 85% yield over two steps. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.54 (br s, 1H), 7.40–7.38 (m, 1H), 7.30–7.27 (m, 1H), 7.20 (t, J = 7.8 Hz, 1H), 3.59 (dd, J = 2.7, 0.5 Hz, 2H), 2.25 (t, J = 2.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 138.4, 131.0, 130.1, 1230.0, 126.6, 122.7, 81.1, 71.3, 24.5; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for C_9H_8Br 194.9804; found 194.9797.

(+)-Ethyl (*S*)-2-(3-bromobenzyl)cycloprop-2-ene-1-carboxylate (17)

A flame dried flask was charged with freshly distilled **15** [13.0 g, 66.6 mmol], catalyst **16** [76 mg, 0.056 mmol), and 320 mL of dry DCM. A solution of ethyl diazoacetate [2.5 g, 22.2 mmol] in 50 mL of dry DCM was added over 12 hours under an argon atmosphere *via* syringe pump. After addition, DCM was completely removed *in vacuo* and residue dissolved in 25 mL hexanes and loaded onto a

silica column. Column was eluted with hexanes until removal of starting alkyne. Column then eluted with a gradient from 2% → 6% EtOAc in hexanes. Product was obtained as a yellow oil [5.0 g, 17.5 mmol]. 80% yield, 95% *e.e.* $[\alpha]_D^{25}$ = +22.2º, c = 4.26, $CHCl_3$, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.37 (br s, 1H), 7.32 (d, *J* = 7.3 Hz, 1H), 7.16–7.11 (m, 2H), 6.47 (br s, 1H), 4.05 (q, *J* = 7.2 Hz, 2H), 3.79 & 3.72 (AB quartet, *J* = 17.6 Hz, 2H), 2.19 (br s, 1H), 1.17 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 176.0, 138.6, 131.8, 130.3, 130.1, 127.4, 122.7, 114.1, 96.5, 60.5, 31.1, 20.5, 14.5; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{13}H_{14}O_2Br$ 281.0172; found 281.0163.

(5-(Trimethylsilyl)pent-4-yn-1-yl)magnesium bromide (18)

A flask equipped with a condenser was charged with magnesium [2.24 g, 85.4 mmol] then flame-dried under vacuum then flushed with argon. After cooling, the flask was charged with 14 mL of dry THF and treated with an iodine crystal. The flask was then heated with a heatgun until complete dissolution of the orange iodine color. Neat (5-bromopent-1-yn-1-yl)trimethylsilane [9.36 g, 42.7 mmol] was then added at a rate sufficient to maintain a slight reflux. After complete addition, the system was heated to reflux for an additional 30 min. The Grignard reagent was measured to be 1.44 M by titration with menthol/phenanthroline.[62]

Ethyl (*S*)-3-(3-bromobenzyl)-3-formyl-8-(trimethylsilyl)oct-7-ynoate (21)

A flame-dried flask was charged with solid copper(I) iodide [6.25 g, 32.8 mmol] then evacuated and backfilled with argon 3x. The flask was charged with 131 mL of dry THF and TMEDA [5.4 mL, 36.1 mmol] and stirred at room temperature for 30 minutes then cooled to -45 ºC. Previously prepared Grignard reagent (**18**) [32.8 mmol] was added to the reaction flask and stirred an additional 30 minutes at -45 ºC. Cyclopropene (+)-**17** [4.6 g, 16.4 mmol] in 33 mL of dry THF was added to the reaction flask at -45 ºC and stirred for 30 minutes. In a separate flame-dried flask, *t*-butylhydroperoxide [6 mL, 32.8 mmol, c = 5.4 M] was dissolved in 82 mL of dry THF, cooled to -78 ºC, and treated with *n*-BuLi [13.4 mL, 33.6 mmol, c = 2.5 M]. After complete carbometalation as determined by TLC, the reaction flask was cooled to -78 ºC and treated with previously prepared *t*-BuOOLi *via* cannulation. The reaction was stirred at this temperature for one hour – significant decomposition was observed by ^{1}H-NMR at longer time points – then cannulated into a cold solution of 2:1 NH_4Cl / NH_4OH and extracted with EtOAc. The organic layer was washed 3x with water, 1x with brine, dried with $MgSO_4$, and concentrated *in vacuo* to give **21** as a green oil, which was carried forward without purification.

Ethyl (*S*)-2-(3-bromobenzyl)cycloprop-2-ene-1-carboxylate (S1)

A 1 mL aliquot of the above carbometalation was removed and quenched with 2:1 NH_4Cl/NH_4OH and worked up as above. pTLC to remove Grignard byproducts: 4% EtOAc in hexanes. Product was obtained as a colorless residue. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33–7.30 (m, 2H), 7.15–7.11 (m, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.90 & 2.84 (AB quartet, *J* = 15.3 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.66 (dd, *J* = 8.1, 5.7 Hz, 1H), 1.63–1.57 (m, 2H), 1.36–1.33 (m, 1H), 1.32–1.30 (m, 1H), 1.29 (t, *J* = 7.2 Hz, 3H), 1.28–1.25 (m, 1H), 1.00 (dd, *J* = 8.1, 4.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 176.0, 138.6, 131.8, 130.3, 130.1, 127.4, 122.7, 114.1, 96.5, 60.5, 31.1, 20.5, 14.5; HRMS (ESI-QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{21}H_{30}BrO_2Si$ 421.1193; found 421.1190.

Ethyl (*S*)-3-(3-bromobenzyl)-3-(diethoxymethyl)-8-(trimethylsilyl)oct-7-ynoate

Crude aldehyde ethyl (*S*)-2-(3-bromobenzyl)cycloprop-2-ene-1-carboxylate [16.4 mmol] was dissolved in 50 mL of dry ethanol then treated with ethyl orthoformate [8.2 mL, 49.2 mmol] and *p*-TSA [312 mg, 1.64 mmol]. The reaction was heated to 60 ºC and monitored by ^{1}H-NMR. The reaction was complete within 1 hour. Ethanol was removed *in vacuo* and the residue reconstituted in EtOAc then washed 3x with $NaHCO_3$ and 1x with brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. Acetal **S18** was carried forward without purification.

Ethyl (*S*,*E*)-3-(3-(3-((*tert*-butyldimethylsilyl)oxy)prop-1-en-1-yl)benzyl)-3-(diethoxymethyl)-8-(trimethylsilyl)oct-7-ynoate (23)

Dioxane and deionized water were sparged with argon for one hour. Crude acetal **S18** [16.4 mmol], vinyl boronate **22** [5.9 g, 19.7 mmol], and Na_2CO_3 [5.2 g, 49.2 mmol] were dissolved in 30 mL of 5:1 dioxane/water. The system was sparged for 15 min, charged with $Pd(PPh_3)_4$ [190 mg, 0.164 mmol], and sparged an additional 15 min. The system was then taken to reflux and monitored by ^{1}H-NMR. After two days, the reaction was complete. Dioxane was removed *in vacuo*, exchanged for EtOAc, and this solution was washed 3x with water and 1x with brine. The organic layer was dried with $MgSO_4$ and concentrated to dryness. The crude product was dissolved in hexanes and chromatographed using a gradient of 0% → 5% EtOAc in hexanes. Collected **23** [2.85 g, 4.73 mmol] as a colorless oil. 29% yield from **17**. $[\alpha]_D^{23}$ = +3.05º, c = 0.46, $CHCl_3$, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22–7.18 (m, 3H), 7.09 (ddd, *J* = 7.2, 1.4, 1.4 Hz, 1H), 6.55 (ddd, *J* = 15.9, 1.5, 1.5 Hz, 1H), 6.25 (ddd, *J* = 15.9, 5.0, 5.0 Hz, 1H), 4.34 (dd, *J* = 5.0, 1.7 Hz, 2H), 4.28 (s, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.82–3.74 (m, 2H), 3.51–3.41 (m, 2H), 2.91 & 2.82 (AB quartet, *J* = 13.4 Hz, 2H), 2.32 (s, 2H), 2.16 (dd, *J* = 6.7, 6.7 Hz, 2H), 1.71–1.48 (m, 6H), 1.28–1.19 (m, 11H), 0.94 (s, 9H), 0.13 (s, 9H), 0.11 (s, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 172.7, 138.7, 136.7, 130.1, 129.8, 129.3, 128.9, 128.1, 124.2, 108.2, 107.8, 84.4, 66.3, 66.2, 64.0, 60.1, 45.6, 39.6, 37.5, 33.3, 26.2, 26.1, 24.9, 23.7, 20.9, 18.6, 15.7, 15.7, 14.4, 0.3, -5.0; HRMS (QE Orbitrap) *m/z*: [M-*tert*-Bu]$^+$ calc'd for $C_{30}H_{49}O_5Si_2$ 545.3113; found 545.3115.

Ethyl (*S*,*E*)-3-(diethoxymethyl)-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)oct-7-ynoate (S19)

Pure **23** [2.29 g, 3.80 mmol] was dissolved in 40 mL of dry THF and cooled to 0 ºC. A solution of TBAF [9.5 mL, 9.50 mmol] was slowly added over 5 minutes. The reaction was monitored by TLC. After 30 minutes, THF was removed *in vacuo* and exchanged for EtOAc, and this solution was washed 3x with water and 1x with brine. Organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to provide **S19** as a yellow oil, which was carried forward without purification.

(*S*,*E*)-3-(Diethoxymethyl)-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)oct-7-ynoic acid (24)

Crude ethyl (*S*,*E*)-3-(diethoxymethyl)-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)oct-7-ynoate [3.80 mmol] was dissolved in 38 mL of 2:1 EtOH/H_2O and treated with KOH [2.13 g, 38.0 mmol]. The ensuing red solution was then heated to 50 ºC overnight. After stirring for 12 hours, the reaction was complete. Solvent was removed and the red oil was treated with 200 mL of 0.3 N NaH_2PO_4 and extracted 3x with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The red oil was carried forward without purification.

(+)-2,5-Dioxopyrrolidin-1-yl (*S*,*E*)-3-(diethoxymethyl)-3-(3-(3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)oct-7-ynoate (5)

Crude cinnamyl alcohol **24** [3.80 mmol] was dissolved in 7.6 mL of dry DCM, treated with *N*-methylmorpholine [1.88 mL, 17.1 mmol], and cooled to -5 ºC under argon. *i*-Butyl chloroformate [1.04 mL, 7.98 mmol] was then added over two minutes. The reaction was monitored by TLC for full conversion to the di-carbonate species. At this time, solid *N*-hydroxysuccinimide [875 mg, 7.60 mmol] was added to the reaction flask. The ice in the cold bath was replenished and the reaction was allowed to slowly warm overnight. Twelve hours after addition of NHS, solid DMAP [1.39 g, 11.4 mmol] was added to decompose byproduct, *i*-butyl succinimidyl carbonate. After stirring with DMAP for 10 min, the reaction was quenched with $NaHCO_3$ and extracted with EtOAc. The organic layer washed 2x with $NaHCO_3$ and 1x with brine, dried with $MgSO_4$, and concentrated *in vacuo*. The crude residue was dissolved in a minimum amount of 3:1 hexanes/$CHCl_3$ and loaded onto silica column. Elution with a gradient of 5% → 30% EtOAc/hexanes provided (+)-**5** [1.15 g, 1.96 mmol] as a colorless oil. 52% from **23**, 94% *e.e.* as determined by diastereomeric derivatization – see SI. $[\alpha]_D^{23}$ = +8.56º, c = 0.58, $CHCl_3$, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.28–7.21 (m, 3H), 7.15 (d, *J* = 7.3 Hz, 1H), 6.68 (d, *J* = 15.9 Hz, 1H), 6.30 (ddd, *J* = 15.9, 6.4, 6.4 Hz, 1H), 4.77 (d, *J* = 6.4 Hz, 2H), 4.34 (s, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.83–3.78 (m, 2H), 3.54–3.44 (m, 2H), 2.92 & 2.86 (AB quartet, *J* = 14.3 Hz, 2H), 2.84 (br s, 4H), 2.63 (s, 2H), 2.14 (ddd, *J* = 6.5, 6.5, 2.4 Hz,

2H), 2.00–1.95 (m, 1H), 1.76–1.55 (m, 5H), 1.25–1.19 (m, 6H), 0.95 (d, J = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 169.3, 167.7, 155.4, 138.1, 136.0, 135.0, 130.9, 129.5, 128.4, 124.8, 122.6, 107.7, 84.6, 74.3, 68.5, 68.5, 66.6, 66.3, 45.6, 39.3, 34.2, 33.1, 27.9, 25.7, 23.54, 19.3, 19.0, 15.6, 15.6; MS *m/z* HRMS (DART-Orbitrap) *m/z*: $[M-H]^-$ calc'd for $C_{32}H_{42}NO_9$ 584.2865; found 584.2871.

Ethyl (*S*)-3-(3-bromobenzyl)-3-methyl-4-oxobutanoate (S2)

A flame-dried flask was charged with solid copper(I) iodide [6.8 g, 35.7 mmol] then evacuated and backfilled with argon 3x. The flask was charged with 179 mL of dry THF and TMEDA [5.9 mL, 39.3 mmol] and stirred at room temperature for 30 minutes then cooled to -45 ºC. Methylmagnesium bromide [14.9 mL, 35.7 mmol] was added to the reaction flask and stirred an additional 30 minutes at -45 ºC. Cyclopropene (+)-**17** [5.0 g, 17.9 mmol] in 36 mL of dry THF was added to the reaction flask at -45 ºC and stirred for 30 minutes. In a separate flame-dried flask, *t*-butylhydroperoxide [7.9 mL, 42.8 mmol, c = 5.4 M] was dissolved in 107 mL of dry THF, cooled to -78 ºC, and treated with *n*-BuLi [21 mL, 44.6 mmol, c = 2.5 M]. After complete carbometalation as determined by TLC, the reaction flask was cooled to -78 ºC and treated with previously prepared *t*-BuOOLi *via* cannulation. The reaction was stirred at this temperature for one hour – significant decomposition was observed at longer reaction times – then cannulated into a cold solution of 2:1 NH_4Cl / NH_4OH and extracted with EtOAc. The organic layer was washed 3x with water, 1x with brine, dried with $MgSO_4$, and concentrated *in vacuo* to give a green oil, which was carried forward without purification.

Ethyl (*S*)-3-(3-bromobenzyl)-4,4-diethoxy-3-methylbutanoate

Crude ethyl (*S*)-3-(3-bromobenzyl)-3-methyl-4-oxobutanoate [17.9 mmol] was dissolved in 89 mL of dry ethanol then treated with ethyl orthoformate [8.9 mL, 53.6 mmol] and *p*-TSA [340 mg, 1.79 mmol]. The reaction was heated to 60 ºC and monitored by ^{1}H-NMR. The reaction was complete within 1 hour. Ethanol was removed *in vacuo*, and the residue was reconstituted in EtOAc then washed 3x with $NaHCO_3$ and 1x with brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. Acetal product [6.4 g, 16.4 mmol, 92% crude recovery] was carried forward without purification.

Ethyl (*S,E*)-3-(3-(3-((tert-butyldimethylsilyl)oxy)prop-1-en-1-yl)benzyl)-4,4-diethoxy-3-methylbutanoate (S3)

Dioxane and deionized water were sparged with argon for one hour. Crude ethyl (*S*)-3-(3-bromobenzyl)-4,4-diethoxy-3-methylbutanoate [16.4 mmol], vinyl boronate **22** [5.9 g, 19.7 mmol], and Na_2CO_3 [5.2 g, 49.2 mmol] were dissolved in 41 mL of 5:1 dioxane/water. The system was sparged for 15 min, charged with $Pd(PPh_3)_4$ [190 mg, 0.164 mmol], and sparged an additional 15 min. The system was then taken to reflux and monitored by ^{1}H-NMR. After two days, reaction was complete. Dioxane was removed *in vacuo*, exchanged for EtOAc, and washed 3x with water and 1x with brine. The organic layer was dried with $MgSO_4$ and concentrated to dryness. The crude product was dissolved in hexanes and chromatographed using a gradient of 0% → 5% EtOAc in hexanes. Collected product [2.28 g, 4.76 mmol] as a colorless oil. 29% yield from (+)-**17**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.24–7.18 (m, 3H), 7.05 (ddd, J = 7.2, 1.6, 1.6 Hz, 1H), 6.56 (ddd, J = 15.7, 1.9, 1.9 Hz, 1H), 6.26 (ddd, J = 15.8, 5.1, 5.1 Hz, 1H), 4.35 (dd, J = 5.1, 1.8 Hz, 2H), 4.30 (s, 1H), 4.13 (q, J = 7.2 Hz, 2H), 3.85–3.78 (m, 2H), 3.55–3.45 (m, 2H), 2.86 & 2.82 (AB quartet, J = 13.2 Hz, 2H), 2.35 & 2.28 (AB quartet, J = 14.9 Hz, 2H) 1.28–1.22 (m, 9H), 1.00 (s, 3H), 0.94 (s, 9H), 0.11 (s, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 172.9, 138.6, 136.8, 130.2, 129.8, 129.2, 129.0, 128.1, 124.2, 108.2, 66.7, 66.0, 64.1, 60.1, 42.9, 40.7, 39.4, 26.1, 19.8, 18.6, 15.7, 15.6, 14.4, -5.0. HRMS (QE Orbitrap) *m/z*: $[M+Na]^+$ calc'd for $C_{27}H_{46}O_5SiNa$ 501.3007; found 501.2991.

Ethyl (*S,E*)-4,4-diethoxy-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)-3-methylbutanoate

Pure ethyl (*S,E*)-3-(3-(3-((tert-butyldimethylsilyl)oxy)prop-1-en-1-yl)benzyl)-4,4-diethoxy-3-methylbutanoate [3.0 g, 6.27 mmol] was dissolved in 21 mL of dry THF and cooled to 0 ºC. A solution of TBAF [14.0 mL, 13.8 mmol] was slowly added over 5 minutes. The reaction was monitored by TLC. After 30 minutes, THF was removed *in vacuo* and exchanged for EtOAc, and the solution was washed 3x with water and 1x with brine. Organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to provide ethyl (*S,E*)-4,4-diethoxy-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)-3-methylbutanoate as a yellow oil, which was carried forward without purification.

(*S,E*)-4,4-Diethoxy-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)-3-methylbutanoic acid (S4)

Crude ethyl (*S,E*)-4,4-diethoxy-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)-3-methylbutanoate [6.27 mmol] was dissolved in 63 mL of 2:1 EtOH/H_2O and treated with KOH [3.5 g, 62.7 mmol]. The ensuing red solution was then heated to 50 ºC overnight. After stirring for 12 hours, the reaction was complete. Solvent was removed and the red oil was treated with 200 mL of 0.3 N NaH_2PO_4 and extracted 3x with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated *in vacuo*. The red oil was carried forward without purification.

(+)-2,5-Dioxopyrrolidin-1-yl (*S,E*)-4,4-diethoxy-3-(3-(3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-methylbutanoate (S5)

Crude (*S,E*)-4,4-Diethoxy-3-(3-(3-hydroxyprop-1-en-1-yl)benzyl)-3-methylbutanoic acid [1.58 mmol] was dissolved in 3.2 mL of dry DCM, treated with *N*-methylmorpholine [782 µL, 7.11 mmol], and cooled to -5 ºC under argon. *i*-Butyl chloroformate [431 µL, 3.32 mmol] was then added. The reaction was monitored by TLC for full conversion to the di-carbonate species. At this time, solid *N*-hydroxysuccinimide [364 mg, 3.16 mmol] was added to the reaction flask. The ice in the cold bath was replenished and the reaction was allowed to slowly warm overnight. Twelve hours after addition of NHS, solid DMAP [579 mg, 4.74 mmol] was added to decompose by-product, *i*-butyl succinimidyl carbonate. After stirring with DMAP for 10 min, reaction quenched with $NaHCO_3$ and extracted with EtOAc. Organic layer washed 2x with $NaHCO_3$ and 1x with brine, dried with $MgSO_4$, and concentrated *in vacuo*. The crude residue was dissolved in a minimum amount of 3:1 hexanes/$CHCl_3$ and loaded onto silica column. Elution with a gradient of 5% → 20% EtOAc/hexanes provided (+)-2,5-Dioxopyrrolidin-1-yl (*S,E*)-4,4-diethoxy-3-(3-(3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-methylbutanoate [615 mg, 1.15 mmol] as a white gum. 65% from ethyl (*S,E*)-3-(3-(3-((tert-butyldimethylsilyl)oxy)prop-1-en-1-yl)benzyl)-4,4-diethoxy-3-methylbutanoate, 89% *e.e.* as determined by diastereomeric derivatization – see SI. $[\alpha]_D^{23}$ = +15.71º, c = 0.56, $CHCl_3$, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.28–7.21 (m, 3H), 7.15 (d, J = 7.3 Hz, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.30 (ddd, J = 15.9, 6.4, 6.4 Hz, 1H), 4.77 (d, J = 6.4 Hz, 2H), 4.34 (s, 1H), 3.93 (d, J = 6.7 Hz, 2H), 3.83–3.78 (m, 2H), 3.54–3.44 (m, 2H), 2.92 & 2.86 (AB quartet, J = 14.3 Hz, 2H), 2.84 (br s, 4H), 2.63 (s, 2H), 2.14 (ddd, J = 6.5, 6.5, 2.4 Hz, 2H), 2.00–1.95 (m, 1H), 1.76–1.55 (m, 5H), 1.25–1.19 (m, 6H), 0.95 (d, J = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 169.2, 167.6, 155.3, 137.9, 135.9, 134.9, 130.8, 129.3, 128.3, 124.7, 122.5, 107.3, 74.2, 68.3, 66.7, 65.9, 43.1, 40.1, 36.2, 27.8, 25.6, 19.4, 18.9, 15.51, 15.48. HRMS (DART-Orbitrap) *m/z*: $[M-H]^-$ calc'd for $C_{28}H_{38}NO_9$ 532.2552; found 532.2599.

(*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-(2-oxo-2-(((*R*)-1-phenylethyl)amino)ethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S6)

Synthesized according to General Procedure A using (*R*)-(+)-phenylethylamine and (+)-**5** [77 µmol]. Obtained 20 mg [crude, 45%] of (*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-(2-oxo-2-(((*R*)-1-phenylethyl)amino)ethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate. The crude was then dissolved in 500 µL of $CDCl_3$ for ^{1}H-NMR analysis of diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34–7.31 (m, 4H), 7.28 (dd, J = 1.8, 1.8 Hz, 1H), 7.25–7.23 (m, 2H), 7.20 (dd, J = 7.5, 7.5 Hz, 1H), 7.16 (ddd, J = 7.4, 1.5, 1.5 Hz, 1H), 6.65 (ddd, J = 15.9, 1.2, 1.2 Hz, 1H), 6.34 (d, J = 7.3 Hz, 1H), 6.28 (ddd, J = 15.9, 6.4, 6.4 Hz, 1H), 5.07 (quint., J = 7.0 Hz, 1H), 4.77 (dd, J = 6.5, 1.3 Hz, 2H), 4.16 (s, 1H), 3.94 (d, J = 6.6 Hz, 2H), 3.73–3.65 (m, 2H), 3.47 (dd, J = 9.2, 7.0 Hz, 1H), 3.30 (dd, J = 9.0, 7.0 Hz, 1H), 2.85 & 2.72 (AB quartet, J = 13.4 Hz, 2H), 2.31 & 2.13 (AB quartet, J = 14.0 Hz, 2H), 2.18–2.13 (m, 1H), 2.01–1.95 (m, 1H), 1.95 (t, J = 2.7 Hz, 1H), 1.83–1.70 (m, 2H), 1.62–1.49 (m, 2H), 1.48 (d, J = 6.9 Hz, 1H), 1.15 (t, J = 7.0 Hz, 3H), 1.14 (t, J = 6.9 Hz,

3H), 0.95 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.2, 155.3, 143.8, 138.6, 135.7, 135.1, 131.0, 129.6, 128.7, 128.2, 127.3, 126.4, 124.5, 122.4, 108.6, 84.6, 74.3, 68.6, 68.4, 67.2, 65.5, 48.8, 45.9, 40.3, 39.6, 32.7, 27.9, 23.2, 21.9, 19.2, 19.0, 15.7, 15.6; MS *m/z*: $[M+Na]^+$ calc'd for $C_{36}H_{49}NO_6Na$ 614.3; found 614.4.

(*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-(2-oxo-2-(((*S*)-1-phenylethyl)amino)ethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S7)

Synthesized according to General Procedure A using (*S*)-(-)-phenylethylamine and (+)-**5** [77 µmol]. Obtained 22 mg [crude, 49%] of (*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-(2-oxo-2-(((*S*)-1-phenylethyl)amino)ethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate. The crude was then dissolved in 500 µL of $CDCl_3$ for ^{1}H-NMR analysis of diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35–7.32 (m, 4H), 7.28 (dd, *J* = 1.7, 1.7 Hz, 1H), 7.27–7.22 (m, 2H), 7.20–7.17 (m, 2H), 6.64 (ddd, *J* = 15.9, 1.2, 1.2 Hz, 1H), 6.38 (d, *J* = 7.4 Hz, 1H), 6.27 (ddd, *J* = 15.9, 6.5, 6.5 Hz, 1H), 5.07 (quint., *J* = 7.0 Hz, 1H), 4.76 (dd, *J* = 6.5, 1.3 Hz, 2H), 4.17 (s, 1H), 3.93 (d, *J* = 6.7 Hz, 2H), 3.74 (dd, *J* = 8.9, 6.9 Hz, 1H), 3.66 (dd, *J* = 9.2, 6.9 Hz, 1H), 3.42 (dd, *J* = 9.0, 7.0 Hz, 1H), 3.38 (dd, *J* = 9.1, 7.0 Hz, 1H), 2.87 & 2.74 (AB quartet, *J* = 13.4 Hz, 2H), 2.29 & 2.16 (AB quartet, *J* = 14.1 Hz, 2H), 2.10–2.02 (m, 1H), 2.01–1.94 (m, 1H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.78–1.69 (m, 2H), 1.61–1.45 (m, 3H), 1.47 (d, *J* = 6.9 Hz, 1H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.01 (t, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.2, 155.3, 143.8, 138.6, 135.7, 135.1, 131.1, 129.6, 128.7, 128.2, 127.3, 126.4, 124.5, 122.4, 108.6, 84.6, 74.3, 68.6, 68.4, 66.9, 65.8, 48.9, 45.9, 40.3, 39.5, 33.0, 27.9, 23.2, 21.9, 19.2, 19.0, 15.7, 15.6; MS *m/z*: $[M+Na]^+$ calc'd for $C_{36}H_{49}NO_6Na$ 614.3; found 614.4.

(*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-methyl-4-oxo-4-(((*R*)-1-phenylethyl)amino)butyl)phenyl)allyl isobutyl carbonate (S8)

Synthesized according to General Procedure A using (*R*)-(+)-phenylethylamine and (+)-**S5** [47 µmol]. Obtained 22 mg [crude, 87%] of (*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-methyl-4-oxo-4-(((*R*)-1-phenylethyl)amino)butyl)phenyl)allyl isobutyl carbonate. The crude was then dissolved in 500 µL of $CDCl_3$ for ^{1}H-NMR analysis of diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34–7.32 (m, 4H), 7.28–7.20 (m, 4H), 7.14 (ddd, *J* = 6.9, 1.5, 1.5 Hz, 1H), 6.66 (d, *J* = 15.9 Hz, 1H), 6.28 (ddd, *J* = 15.9, 6.5, 6.5 Hz, 1H), 6.03 (d, *J* = 7.8 Hz, 1H), 5.12 (quint., *J* = 7.2 Hz, 1H), 4.77 (dd, *J* = 6.5, 1.1 Hz, 2H), 4.22 (s, 1H), 3.94 (d, *J* = 6.2 Hz, 2H), 3.77–3.70 (m, 2H), 3.52–3.49 (m, 1H), 3.32–3.27 (m, 1H), 2.86 & 2.74 (AB quartet, *J* = 13.0 Hz, 2H), 2.25 & 2.09 (AB quartet, *J* = 13.9 Hz, 2H), 2.02–1.94 (m, 1H), 1.49 (d, *J* = 6.9 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 3H), 1.15 (t, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 6H), 0.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.1, 155.4, 143.6, 138.7, 135.7, 135.2, 131.2, 129.7, 128.7, 128.2, 127.4, 126.4, 124.5, 122.4, 108.5, 74.3, 68.5, 67.0, 65.4, 48.7, 43.2, 41.8, 41.2, 27.9, 21.8, 20.4, 19.1, 15.7, 15.6; MS *m/z*: $[M+Na]^+$ calc'd for $C_{32}H_{45}NO_6Na$ 562.3; found 562.4.

(*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-methyl-4-oxo-4-(((*S*)-1-phenylethyl)amino)butyl)phenyl)allyl isobutyl carbonate (S9)

Synthesized according to General Procedure A using (*S*)-(-)-phenylethylamine and (+)-**S5** [47 µmol]. Obtained 22 mg [crude, 87%] of (*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-methyl-4-oxo-4-(((*S*)-1-phenylethyl)amino)butyl)phenyl)allyl isobutyl carbonate. The crude was then dissolved in 500 µL of $CDCl_3$ for ^{1}H-NMR analysis of diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34–7.32 (m, 4H), 7.28–7.23 (m, 3H), 7.19 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.12 (ddd, *J* = 7.5, 1.2, 1.2 Hz, 1H), 6.64 (d, *J* = 15.9 Hz, 1H), 6.27 (ddd, *J* = 15.8, 6.5, 6.5 Hz, 1H), 6.01 (d, *J* = 7.8 Hz, 1H), 5.12 (quint., *J* = 7.1 Hz, 1H), 4.77 (dd, *J* = 6.5, 1.0 Hz, 2H), 4.25 (s, 1H), 3.94 (d, *J* = 6.7 Hz, 2H), 3.82–3.69 (m, 2H), 3.51–3.44 (m, 1H), 3.42–3.36 (m, 1H), 2.87 & 2.76 (AB quartet, *J* = 13.0 Hz, 2H), 2.23 & 2.09 (AB quartet, *J* = 13.9 Hz, 2H), 2.03–1.94 (m, 1H), 1.48 (d, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.15 (t, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 6H), 0.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.1, 155.4, 143.6, 138.7, 135.7, 135.1, 131.3, 129.7, 128.8, 128.2, 127.5, 126.4, 124.5, 122.4, 108.6, 74.3, 68.5, 66.7, 65.8, 48.7, 43.1, 41.8, 41.0, 27.9, 21.7, 20.7, 19.0, 15.7, 15.6; MS *m/z*: $[M+Na]^+$ calc'd for $C_{32}H_{45}NO_6Na$ 562.3; found 562.4.

(*E*)-3-(3-((*S*)-2-(2-(((*S*)-1-amino-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-(diethoxymethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (26)

Synthesized according to General Procedure A using L-tryptophan amide and (+)-**5** [51 µmol]. Obtained 30 mg [45 µmol, 87% crude recovery] of **26**.

(*E*)-3-(3-(((1*S*,5*S*,11b*R*)-5-carbamoyl-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (27)

Synthesized according to General Procedure B using crude material from the previous reaction. After reaction completion, solvent was removed, and the crude residue was dissolved in ~500 µL DMSO and purified by semi-preparative HPLC – see SI for details – to give 12 mg [21 µmol, 51% yield] of desired **27**. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.86 (s, 1H), 7.48 (s, 1H), 7.41 (d, *J* = 6.6 Hz, 1H), 7.41 (d, *J* = 6.6 Hz, 1H), 7.23 (d, *J* = 6.4 Hz, 1H), 7.14–7.11 (m, 1H), 7.12–7.09 (m, 1H), 7.09 (s, 1H), 7.00 (dd, *J* = 6.3, 6.3 Hz, 1H), 6.80 (d, *J* = 5.9 Hz, 1H), 6.71 (s, 1H), 6.46 (d, *J* = 15.5 Hz, 1H), 6.13–6.10 (m, 1H), 5.06 (s, 1H), 4.82 (d, *J* = 4.5 Hz, 1H), 4.68 (d, *J* = 4.1 Hz, 2H), 3.90 (d, *J* = 4.5 Hz, 2H), 3.35 (d, *J* = 15.0 Hz, 1H), 2.78 (s, 1H), 2.65 & 2.17 (AB quartet, *J* = 15.9 Hz, 2H), 2.55–2.52 (m, 1H), 2.33–2.31 (m, 1H), 2.33–2.31 (m, 1H), 2.24–2.20 (m, 1H), 2.21–2.19 (m, 1H), 1.95–1.90 (m, 1H), 1.92–1.89 (m, 1H), 1.88–1.83 (m, 1H), 1.74–1.70 (m, 1H), 1.60–1.56 (m, 1H), 0.9 (d, *J* = 4.9 Hz, 6H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.9, 171.6, 154.4, 137.2, 136.6, 135.0, 133.4, 130.0, 128.8, 128.5, 127.6, 125.8, 124.2, 122.8, 120.9, 118.3, 117.5, 111.1, 106.9, 84.2, 73.0, 71.1, 67.5, 59.2, 49.3, 45.3, 39.9, 38.6, 34.5, 26.9, 23.8, 22.9, 18.2, 18.0; HRMS (QE Orbitrap) *m/z*: $[M+Na]^+$ calc'd for $C_{35}H_{39}N_3O_5Na$ 604.2782; found 604.2783.

(S)-5-(((S)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((S)-2-((S)-4,4-diethoxy-3-(3-((E)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-methylbutanamido)-3-(1H-indol-3-yl)propanamido)-5-oxopentanoic acid (28)

Synthesized according to Procedure A beginning with 0.71 mmol of (+)-**S5**. Carried forward without purification.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((1*S*,5*S*,11b*R*)-1-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-1-methyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole-5-carboxamido)-5-oxopentanoic acid (29)

Synthesized according to Procedure B beginning with 58 mg of crude **28**. Chromatographed on SiO_2 with a gradient from 0% to 5% MeOH in $CHCl_3$ (0.5% AcOH). White Solid. 34 mg, [41 µmol, 94% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ12.07 (br s, 1H), 10.93 (s, 1H), 9.15 (s, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.30 (br s, 1H), 7.22 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.08 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.01–6.98 (m, 2H), 6.94–6.88 (m, 4 H), 6.63–6.54 (m, 4H), 6.26 (ddd, *J* = 15.9, 6.3, 6.3 Hz, 1H), 5.09 (d, *J* = 6.9 Hz, 1H), 5.01 (s, 1H), 4.72, (d, *J* = 6.1 Hz, 1H), 4.24 (ddd, *J* = 7.5, 7.5, 7.5 Hz, 1H), 4.08 (ddd, *J* = 7.9, 7.9, 7.9 Hz, 1H), 3.90 (d, *J* = 6.7 Hz, 2H), 2.82 (dd, *J* = 14.7, 6.5 Hz, 1H), 2.74 (dd, *J* = 13.6, 5.7 Hz, 1H), 2.61 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.41 (d, *J* = 15.8 Hz, 1H), 2.22–2.17 (m, 2H), 2.13–2.00 (m, 3H),1.90 (quint., *J* = 7.0 Hz, 1H), 1.86–1.80 (m, 1H), 1.75–1.65 (m, 1H), 1.42 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6, 126 MHz): δ174.1, 172.6, 172.5, 170.6, 170.2, 155.8, 154.5, 137.6, 136.8, 135.4, 133.6, 130.4, 130.1, 129.1, 128.8, 128.2, 127.5, 126.4, 124.5, 123.2, 121.2, 118.7, 117.9, 114.9, 111.4, 107.1, 73.4, 67.7, 62.7, 53.9, 52.2, 49.3, 42.6, 42.1, 36.6, 30.2, 27.3, 26.8, 23.5, 23.2, 18.7; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{45}H_{52}N_5O_{10}$ 822.3709; found 822.3676.

3-((8*S*,12*S*,15*S*,18*S*,*E*)-12-((1*H*-indol-3-yl)methyl)-18-carbamoyl-8-(diethoxymethyl)-8-methyl-10,13,16-trioxo-2-oxa-11,14,17-triaza-1(1,4),6(1,3)-dibenzenacyclononadecaphan-4-en-15-yl)propanoic acid (S16)

Synthesized according to Procedure E beginning with 53 mg of crude **28**. Carried forward without purification.

3-((1^1*S*,1^5*S*,1^{11b}*R*,10*S*,13*S*,*E*)-10-carbamoyl-1^1-methyl-1^3,12,15-trioxo-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14-diaza-1(1,5)-

indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclopentadecaphan-4-en-13-yl)propanoic acid (30)

Synthesized according to Procedure B using crude material from the previous reaction. Purified by preparative HPLC – see SI for conditions. White Solid. 11.4 mg [16 μmol, 28% yield over three steps]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.04 (br s, 1H), 10.87 (s, 1H), 8.32 (d, *J* = 9.2 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.38 (br s, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 7.11–7.01 (m, 5H), 7.04 (d, *J* = 8.9 Hz, 2H), 6.98–6.95 (m, 1H), 6.92 (d, *J* = 6.8 Hz, 1H), 6.75 (d, *J* = 8.7 Hz, 1H), 6.54 (d, *J* = 16.3 Hz, 1H), 6.25 (s, 1H), 5.98 (ddd, *J* = 16.3, 6.8, 4.0 Hz, 1H), 4.93 (s, 1H), 4.87 (ddd, *J* = 15.9, 4.0, 1.7 Hz, 1H), 4.80 (dd, *J* = 15.9, 6.8 Hz, 1H), 4.48–4.44 (m, 1H), 4.32 (d, *J* = 9.2 Hz, 1H), 4.13 (ddd, *J* = 9.1, 9.1, 4.8 Hz, 1H), 3.11 (dd, *J* = 14.5, 5.3 Hz, 1H), 2.76 (dd, *J* = 14.5, 4.3 Hz, 1H), 2.18 (d, *J* = 16.8 Hz, 1H), 2.10–2.07 (m, 2H), 2.01–1.94 (m, 1H), 1.82–1.76 (m, 1H), 1.64 (s, 3H), 1.62–1.56 (m, 1H), 1.54–1.48 (m, 1H), 1.41–1.35 (m, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 174.4, 174.0, 172.2, 171.9, 171.6, 170.1, 157.6, 137.5, 137.0, 134.5, 131.3, 130.7, 130.0, 129.3, 128.1, 127.7, 127.2, 126.5, 126.0, 125.0, 121.2, 118.7, 118.4, 115.0, 111.1, 106.0, 68.4, 52.0, 50.8, 48.6, 47.7, 43.2, 42.4, 36.3, 32.0, 27.1, 26.2, 23.7; HRMS (QE Orbitrap) *m/z*: [M+Na]$^+$ calc'd for $C_{40}H_{41}N_5O_7$ 704.3079; found 704.3071. **Contaminated with an unknown impurity. ^{13}C-NMR peaks chosen by analogy to Macrocycle **35****

(5*S*,9*S*,19*S*)-9-((1*H*-indol-3-yl)methyl)-19-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)-4-ethoxy-5-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-5-methyl-7,10,17-trioxo-3-oxa-8,11,18-triazadocosan-22-oic acid (31)

Synthesized according to Procedure A beginning with 0.22 mmol of (+)-**S5**. Carried forward without purification.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-(6-((1*S*,5*S*,11b*R*)-1-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-1-methyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole-5-carboxamido)hexanamido)-5-oxopentanoic acid (S17)

Synthesized according to Procedure B using crude material from the previous reaction. Carried forward without purification.

3-((1^1*S*,1^5*S*,1^{11b}*R*,10*S*,13*S*,*E*)-10-carbamoyl-1^1-methyl-1^3,12,15,22-tetraoxo-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14,21-triaza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclodocosaphan-4-en-13-yl)propanoic acid (32)

Synthesized according to Procedure D using 32 mg of crude **S17**. Purified by preparative HPLC – see SI for conditions. White Solid. 9 mg [11 μmol, 19% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.87 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.74 (dd, *J* = 5.6, 5.6 Hz, 1H), 7.63 (d, *J* = 8.1 Hz, 1H), 7.50 (br s, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 8.6, Hz, 1H), 7.17 (ddd, *J* = 7.7, 1.3, 1.3 Hz, 1H), 7.12 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.08 (br s, 1H), 6.96 (ddd, *J* = 7.9, 7.0, 0.8 Hz, 1H), 6.95–6.94 (m, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.88 (ddd, *J* = 7.9, 7.0, 0.8 Hz, 1H), 6.31 (dd, *J* = 1.4, 1.4 Hz, 1H), 6.23 (d, *J* = 16.1 Hz, 1H), 6.10 (ddd, *J* = 16.0, 5.8, 5.8 Hz, 1H), 5.01 (dd, *J* = 1.4, 1.4 Hz, 1H), 4.74 (d, *J* = 7.0 Hz, 1H), 4.65 (m, 2H), 4.40 (ddd, *J* = 9.9, 8.2, 3.4 Hz, 1H), 4.31 (ddd, *J* = 7.9, 7.9, 5.7 Hz, 1H), 3.21 (d, *J* = 15.1 Hz, 1H), 2.98 (dd, *J* = 14.0, 3.6 Hz, 1H), 2.96–2.93 (m, 2H), 2.78 (dd, *J* = 14.0, 10.0 Hz, 1H), 2.58 (ddd, *J* = 15.3, 6.3, 1.6 Hz, 2H), 2.51 & 2.37 (AB quartet, *J* = 15.8Hz, 2H), 2.39 (s, 2H), 2.24–2.15 (m, 2H), 1.98 (ddd, *J* = 14.9, 9.5, 5.7 Hz, 1H), 1.92–1.86 (m, 1H), 1.76 (ddd, *J* = 15.0, 9.5, 5.5 Hz, 1H), 1.74–1.69 (m, 1H), 1.45 (s, 3H), 1.37–1.31 (m, 1H), 1.29–1.24 (m, 1H), 1.22–1.17 (m, 1H), 1.17–1.13 (m, 1H), 0.93–0.87 (m, 2H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.9, 173.0, 172.0, 171.9, 171.0, 169.7, 156.8, 137.3, 136.6, 135.0, 132.1, 130.2, 129.9, 129.4, 129.3, 128.1, 127.7, 126.2, 124.4, 124.0, 121.0, 118.4, 117.5, 114.1, 111.1, 106.7, 67.6, 62.1, 54.1, 51.2, 49.1, 43.7, 42.3, 40.9, 37.7, 36.4, 35.0, 29.8, 28.1, 27.6, 25.6, 25.3, 24.6, 23.4; HRMS (QE Orbitrap) *m/z*: [M+H]$^+$ calc'd for $C_{46}H_{53}N_6O_8$ 817.3919; found 817.3927.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((*R*)-2-((*S*)-4,4-diethoxy-3-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-methylbutanamido)-3-(1*H*-indol-3-yl)propanamido)-5-oxopentanoic acid (33)

Synthesized according to Procedure A beginning with 0.20 mmol of (+)-**S5**. Carried forward without purification.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((1*S*,5*R*,11b*S*)-1-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-1-methyl-3-oxo-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole-5-carboxamido)-5-oxopentanoic acid (S18)

Synthesized according to Procedure B using the crude from the previous reaction. Carried forward without purification.

3-((1^1*S*,1^5*R*,1^{11b}*S*,9*S*,12*S*,*E*)-9-carbamoyl-7^6-hydroxy-1^1-methyl-1^3,11,14-trioxo-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-en-12-yl)propanoic acid (34)

Synthesized according to Procedure C using 6 mg of crude **S18**. Purified by preparative HPLC – see SI for conditions. White Solid. 3 mg [4.3 μmol, 60% yield over three steps]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.06 (br s, 1H), 10.64 (br s, 1H), 9.18 (s, 1H), 8.08 (d, *J* = 4.8 Hz, 1H), 7.42 (s, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.37 (br s, 1H), 7.36 (d, *J* = 7.4 Hz, 2H), 7.35 (dd, *J* = 7.3, 7.3 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.08 (dd, *J* = 7.9, 7.9 Hz, 2H), 6.98 (dd, *J* = 7.7, 7.7 Hz, 1H), 6.95 (br s, 1H), 6.86 (d, *J* = 1.7 Hz, 1H), 6.84 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 15.7 Hz, 1H), 6.32 (ddd, *J* = 15.6, 7.2, 7.2 Hz, 1H), 5.51 (s, 1H), 5.03 (d, *J* = 6.6 Hz, 1H), 4.19 (ddd, *J* = 8.1, 8.1, 3.8 Hz, 1H), 3.81–3.77 (m, 1H), 3.40 (dd, *J* = 15.6, 7.8 Hz, 1H), 3.39 (d, *J* = 13.8 Hz, 1H), 3.24 (dd, *J* = 15.5, 6.6 Hz, 1H), 3.15 (d, *J* = 15.2 Hz, 1H), 2.88 (dd, *J* = 15.2, 6.5 Hz, 1H), 2.78 (d, *J* = 13.8 Hz, 1H), 2.66 (dd, *J* = 13.9, 3.5 Hz, 1H), 2.58 (dd, *J* = 13.9, 8.4 Hz, 1H), 2.29 & 1.90 (AB quartet, *J* = 16.1 Hz, 2H), 2.15 (ddd, *J* = 16.3, 11.1, 5.3 Hz, 1H), 2.06 (ddd, *J* = 16.4, 11.1, 5.2 Hz, 1H), 1.83–1.77 (m, 1H), 1.68–1.62 (m, 1H), 0.81 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.9, 173.0, 171.1, 170.3, 153.3, 137.7, 137.7, 136.3, 130.8, 130.3, 130.0, 129.9, 129.3, 128.3, 128.1, 127.8, 127.7, 126.1, 125.4, 124.2, 120.8, 118.4, 117.1, 114.3, 111.1, 105.8, 57.0, 53.9, 52.1, 48.8, 41.9, 41.8, 41.2, 36.8, 32.4, 30.2, 26.7, 25.3, 23.7; HRMS (QE Orbitrap) *m/z*: [M+H]$^+$ calc'd for $C_{40}H_{42}N_5O_7$ 704.3079; found 704.3072.

3-((1^1*S*,1^5*R*,1^{11b}*S*,10*S*,13*S*,*E*)-10-carbamoyl-1^1-methyl-1^3,12,15-trioxo-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14-diaza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclopentadecaphan-4-en-13-yl)propanoic acid (35)

Synthesized according to Procedure D using 50 mg of crude **S18**. Purified by preparative HPLC using HCOOH as modifier – see SI for conditions. White Solid. 13 mg [18 μmol, 29% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 12.11 (br s, 1H), 11.05 (s, 1 H), 8.12 (d, *J* = 4.2 Hz, 1 H), 7.52 (d, *J* = 5.6 Hz, 1H), 7.52 (d, *J* = 5.6 Hz, 1 H), 7.40 (br s, 1H), 7.37 (d, *J* = 6.7 Hz, 1H), 7.36-7.34 (m, 1H), 7.34 (s, 1 H), 7.16 (d, *J* = 6.2 Hz, 2H), 7.13 (d, *J* = 6.7 Hz, 1 H), 7.11 (dd, *J* = 6.9, 6.9 Hz, 1H), 7.01 (d, *J* = 5.7 Hz, 1 H), 7.00 (dd, *J* = 6.7, 6.7 Hz, 1H), 6.90 (br s, 1H), 6.83 (d, *J* = 6.1 Hz, 2H), 6.71 (d, *J* = 15.7 Hz, 1 H), 6.38-6.36 (m, 1 H), 5.19 (s, 1 H), 5.08 (d, *J* = 4.1 Hz, 1 H), 4.92 (dd, *J* = 14.6, 5.0 Hz, 1H), 4.81 (d, *J* = 14.5 Hz, 1H), 4.09-4.07 (m, 1H), 3.70-3.67 (m, 1 H), 3.39 & 2.73 (AB quartet, *J* = 13.8 Hz, 2 H), 3.07 (d, *J* = 15.3 Hz, 1H), 2.90 (dd, *J* = 15.3, 4.2 Hz, 1H), 2.83 (d, *J* = 13.8 Hz, 1H), 2.60 (dd, *J* = 13.9, 9.2 Hz, 1H), 2.28-2.24 (m, 1H), 2.25 & 1.88 (AB quartet, *J* = 15.3 Hz, 2 H), 2.17-2.13 (m, 1H), 1.90-1.85 (m, 1H), 1.70-1.65 (m, 1 H), 0.74 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.8, 173.1, 171.3, 170.9, 170.6, 155.7, 137.5, 136.3, 135.7, 132.2, 131.2, 130.2, 130.1, 130.0, 129.6, 128.4, 126.1, 125.9, 123.0, 120.6, 118.5, 117.0, 114.8, 111.1, 105.3, 66.9, 56.8, 54.3, 48.3, 48.2, 42.0, 41.2, 40.9, 35.8, 30.3, 26.4, 26.0, 23.5; HRMS (QE Orbitrap) *m/z*: [M+H]$^+$ calc'd for $C_{40}H_{42}N_5O_7$ 704.3079; found 704.3057.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((*R*)-2-((*S*)-3-(diethoxymethyl)-3-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)oct-7-ynamido)-3-(1*H*-indol-3-yl)propanamido)-5-oxopentanoic acid (S19)

Synthesized according to Procedure A beginning with 0.57 mmol of (+)-**5**. Carried forward without purification.

(*S*)-5-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-4-((1*S*,5*R*,11b*S*)-1-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-oxo-1-(pent-4-

yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole-5-carboxamido)-5-oxopentanoic acid (S20)

Synthesized according to Procedure B using the crude material from the previous reaction. Carried forward without purification.

3-((1^{1}*S*,1^{5}*R*,1^{11b}*S*,9*S*,12*S*,*E*)-9-carbamoyl-7^{6}-hydroxy-1^{3},11,14-trioxo-1^{1}-(pent-4-yn-1-yl)-1^{2},1^{3},1^{5},1^{6},1^{11},1^{11b}-hexahydro-1^{1}*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-en-12-yl)propanoic acid (36)

Synthesized according to Procedure C using 280 mg of crude material from the previous reaction. Purified by SiO_2 chromatography 1→10% MeOH/$CHCl_3$ (0.1% AcOH). White Solid. 133 mg [176 µmol, 31% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 12.06 (br s, 1H), 10.59 (s, 1H), 9.17 (s, 1H), 8.05 (d, *J* = 6.6 Hz, 1H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.37 (s, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.37 (br s, 1H), 7.35 (dd, *J* = 7.4, 7.4 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 7.7 Hz, 1H), 7.08 (ddd, *J* = 7.6, 7.6, 0.8 Hz, 1H), 6.98 (dd, *J* = 7.6, 7.6 Hz, 1H), 6.95 (br s, 1H), 6.86 (d, *J* = 1.6 Hz, 1H), 6.84 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 1H), 6.49 (d, *J* = 15.7 Hz, 1H), 6.33 (ddd, *J* = 15.6, 7.2, 7.2 Hz, 1H), 5.60 (s, 1H), 5.02 (d, *J* = 6.6 Hz, 1H), 4.20 (ddd, *J* = 8.2, 8.2, 3.8 Hz, 1H), 3.80 (ddd, *J* = 9.3, 6.7, 4.4 Hz, 1H), 3.50 (dd, *J* = 15.6, 7.3 Hz, 1H), 3.34–3.32 (m (under water), 1H), 3.21 (dd, *J* = 15.6, 6.9Hz, 1H), 3.17 (d, *J* = 15.5 Hz, 1H), 3.17 (d, *J* = 15.4 Hz, 1H), 2.87–2.83 (m, 2H), 2.66 (dd, *J* = 13.9, 3.4 Hz, 1H), 2.59 (dd, *J* = 14.0, 8.3 Hz, 1H), 2.56 (dd, *J* = 2.6, 2.6 Hz, 1H), 2.19 (d, *J* = 16.4 Hz, 1H), 2.15 (ddd, *J* = 16.3, 11.3, 5.1 Hz, 1H), 2.09 (d, *J* = 16.4 Hz, 1H), 2.05 (ddd, *J* = 16.4, 11.3, 5.1 Hz, 1H), 1.91 (ddd, *J* = 6.6, 6.6, 2.5 Hz, 2H), 1.83–1.78 (m, 1H), 1.68–1.62 (m, 1H), 1.47–1.40 (m, 1H), 1.38–1.33 (m, 1H), 1.36–1.33 (m, 1H), 1.26–1.23 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.7, 172.7, 171.1, 171.0, 170.0, 153.2, 137.6, 137.5, 136.4, 130.5, 130.0, 130.0, 129.8, 129.5, 128.6, 128.4, 127.7, 127.5, 126.1, 125.4, 123.7, 120.5, 118.3, 116.9, 114.3, 110.9, 106.0, 83.8, 70.8, 57.3, 53.9, 52.0, 48.7, 44.1, 39.8, 38.1, 36.7, 34.7, 32.4, 30.0, 26.8, 25.1, 22.6, 18.1; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{44}H_{46}N_5O_7$ 756.3392; found 756.3391.

(*E*)-3-(3-((*S*)-2-(2-(((*R*)-1-(((*S*)-5-amino-1-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-1,5-dioxopentan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-(diethoxymethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S21)

Synthesized according to Procedure A beginning with 0.22 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((1*S*,5*R*,11b*S*)-5-(((*S*)-5-amino-1-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-1,5-dioxopentan-2-yl)carbamoyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (S22)

Synthesized according to Procedure B using 162 mg of crude **S21**. Carried forward without purification.

(1^{1}*S*,1^{5}*R*,1^{11b}*S*,9*S*,12*S*,*E*)-12-(3-amino-3-oxopropyl)-7^{6}-hydroxy-1^{3},11,14-trioxo-1^{1}-(pent-4-yn-1-yl)-1^{2},1^{3},1^{5},1^{6},1^{11},1^{11b}-hexahydro-1^{1}*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-9-carboxamide (37)

Synthesized according to Procedure C using the crude material from the previous reaction. Purified by preparative HPLC – see SI for conditions. White Solid. 27 mg [36 µmol, 22% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.51 (s, 1H), 9.20 (br s, 1H), 8.18 (d, *J* = 6.3 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.40 (s, 1H), 7.38 (br s, 1H), 7.37–7.34 (m, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 6.4 Hz, 1H), 7.31 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 6.8 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 1H), 7.08 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.00 (br s, 2H), 6.98 (dd, *J* = 7.6, 7.6 Hz, 1H), 6.85 (br s, 1H) , 6.84 (d, *J* = 8.5 Hz, 1H), 6.67 (d, *J* = 8.7 Hz, 1H), 6.49 (d, *J* = 15.6 Hz, 1H), 6.35 (ddd, *J* = 15.6, 7.1, 7.1 Hz, 1H), 5.59 (s, 1H), 5.03 (d, *J* = 6.4 Hz, 1H), 4.20–4.16 (m, 1H), 3.79–3.75 (m, 1H), 3.48 (dd, *J* = 15.8, 7.3 Hz, 1H), 3.33 & 2.83 (AB quartet, *J* = 13.9 Hz, 2H), 3.24 (dd, *J* = 15.8, 6.8 Hz, 1H), 3.20 (d, *J* = 15.9 Hz, 1H), 2.85 (dd, *J* = 15.9, 6.4 Hz, 1H), 2.66 (dd, *J* = 13.8, 2.5 Hz, 1H), 2.58 (dd, *J* = 2.5, 2.5 Hz, 1H), 2.54–2.50 (under DMSO) (m, 2H), 2.22 & 2.11 (AB quartet, *J* = 16.5 Hz, 2H), 2.08–2.01 (m, 1H), 1.98–1.93 (m, 1H), 1.92 (ddd, *J* = 6.1, 6.1, 2.3 Hz, 2H), 1.80–1.74 (m, 1H), 1.66–1.59 (m, 1H), 1.48–1.41 (m, 1H), 1.37–1.32 (m, 1H), 1.37–1.32 (m, 1H), 1.25–1.21 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.9, 173.0, 171.4, 171.1, 170.2, 153.2, 137.7, 137.4, 136.4, 130.7, 130.1, 129.9, 129.9, 129.4, 128.6, 128.4, 127.8, 127.6, 126.0, 125.4, 124.0, 120.8, 118.5, 117.3, 114.4, 111.1, 106.1, 83.9, 71.0, 57.4, 54.1, 52.6, 52.6, 48.8, 44.3, 39.9, 38.4, 36.8, 34.6, 32.2, 31.4, 25.0, 22.5, 18.1; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{44}H_{47}N_6O_6$ 755.3552; found 755.3576.

(*E*)-3-(3-((3*S*,6*S*,13*R*,17*S*)-13-((1*H*-indol-3-yl)methyl)-6-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)-17-(diethoxymethyl)-3-methyl-1,4,12,15-tetraoxo-17-(pent-4-yn-1-yl)-1-((*S*)-pyrrolidin-2-yl)-2,5,11,14-tetraazaoctadecan-18-yl)phenyl)allyl isobutyl carbonate (S23)

Synthesized according to Procedure A beginning with 0.43 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((1*S*,5*R*,11b*S*)-5-(((*S*)-6-(((*S*)-1-amino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-6-oxo-5-((*S*)-2-((*S*)-pyrrolidine-2-carboxamido)propanamido)hexyl)carbamoyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (S24)

Synthesized according to Procedure B using 368 mg of crude **S23**. Carried forward without purification.

(1^{1}*S*,1^{5}*R*,1^{11b}*S*,9*S*,12*S*,*E*)-7^{6}-hydroxy-1^{3},11,18-trioxo-1^{1}-(pent-4-yn-1-yl)-12-((*S*)-2-((*S*)-pyrrolidine-2-carboxamido)propanamido)-1^{2},1^{3},1^{5},1^{6},1^{11},1^{11b}-hexahydro-1^{1}*H*-10,17-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclooctadecaphan-4-ene-9-carboxamide (38)

Synthesized according to Procedure C using crude material from the previous reaction. Purified by preparative HPLC – see SI for conditions. Yellow solid. HCl-salt: 47 mg [49 µmol, 13% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.50 (s, 1H), 9.82 (br s, 1H), 8.73 (d, *J* = 7.1 Hz, 1H), 8.46–8.43 (m, 1H), 8.18 (d, *J* = 7.3 Hz, 1H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.56 (dd, *J* = 5.9, 5.9 Hz, 1H), 7.54 (s, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.37 (br s, 1H), 7.33–7.31 (m, 1H), 7.31 (dd, *J* = 7.7, 7.7 Hz, 1H), 7.20 (br s, 1H), 7.15–7.13 (m, 1H), 7.10 (dd, *J* = 8.1, 8.1 Hz, 1H), 7.00 (dd, *J* = 7.9, 7.9 Hz, 1H), 6.93 (d, *J* = 1.6 Hz, 1H), 6.84 (dd, *J* = 8.3, 1.6 Hz, 1H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.46 (ddd, *J* = 15.7, 6.6, 6.6 Hz, 1H), 6.35 (d, *J* = 15.9 Hz, 1H), 4.96 (s, 1H), 4.88 (d, *J* = 6.6 Hz, 1H), 4.39–4.34 (m, 1H), 4.33 (ddd, *J* = 7.0, 7.0, 7.0 Hz, 1H), 4.14 (dddd, *J* = 6.0, 6.0, 6.0, 6.0 Hz, 1H), 3.97 (ddd, *J* = 8.1, 8.1, 5.3 Hz, 1H), 3.47–3.44 (under water) (m, 1H), 3.46–3.43 (under water) (m, 1H), 3.35 (dd, *J* = 16.1, 6.9 Hz, 1H), 3.29 & 2.99 (AB quartet, *J* = 13.7 Hz, 2H), 3.18–3.12 (m, 2H), 2.94 (dd, *J* = 13.9, 6.1 Hz, 1H), 2.91 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.91 & 2.06 (AB quartet, *J* = 16.1 Hz, 2H), 2.78 (dd, *J* = 14.0, 8.9 Hz, 1H), 2.66 (dd, *J* = 15.7, 6.5 Hz, 1H), 2.66 (dd, *J* = 14.1, 5.8 Hz, 1H), 2.52 (dd, *J* = 2.4, 2.4 Hz, 1H), 2.28–2.22 (m, 1H), 1.88–1.86 (m, 2H), 1.84–1.78 (m, 2H), 1.82–1.78 (m, 1H), 1.62–1.58 (m, 1H), 1.42–1.35 (m, 1H), 1.32–1.28 (m, 2H), 1.32–1.28 (m, 1H), 1.25–1.21 (m, 1H), 1.24 (d, *J* = 7.0 Hz, 3H), 1.06–1.01 (m, 2H), 1.06–0.96 (m, 2H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 172.6, 172.2, 171.7, 170.7, 168.1, 167.5, 153.1, 137.5, 137.4, 136.5, 130.4, 129.5, 129.5, 129.3, 128.9, 128.5, 128.0, 127.4, 127.3, 126.0, 125.0, 124.0, 120.9, 118.4, 117.6, 114.3, 111.1, 106.9, 83.6, 70.9, 58.3, 57.4, 53.4, 53.2, 48.9, 48.3, 45.3, 44.6, 40.5, 39.1, 38.0, 36.3, 34.3, 32.2, 30.7, 29.3, 27.8, 23.3, 22.3, 22.2, 21.5, 18.0, 17.5; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{53}H_{63}N_8O_7$ 923.4814; found 923.4769.

(*S*)-4-((*R*)-2-((*S*)-3-(diethoxymethyl)-3-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)oct-7-ynamido)-3-(1*H*-indol-3-yl)propanamido)-5-((4-hydroxyphenethyl)amino)-5-oxopentanoic acid (S25)

Synthesized according to Procedure A beginning with 0.26 mmol of (+)-**5**. Carried forward without purification.

(*S*)-5-((4-hydroxyphenethyl)amino)-4-((1*S*,5*R*,11b*S*)-1-(3-((*E*)-3-((isobutoxycarbonyl)oxy)prop-1-en-1-yl)benzyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole-5-carboxamido)-5-oxopentanoic acid (S26)

Synthesized according to Procedure B using crude material from the previous reaction. Carried forward without purification.

3-((1^1*S*,1^5*R*,1^{11b}*S*,12*S*,*E*)-7^6-hydroxy-1^3,11,14-trioxo-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-en-12-yl)propanoic acid (39)

Synthesized according to Procedure C using 50 mg of crude **S26**. Purified by preparative TLC: 3% MeOH / 96.5%$CHCl_3$ / 0.5%AcOH. White solid. 20 mg [28 μmol, 57% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.66 (s, 1H), 9.21 (br s, 1H), 7.45 (s, 1H), 7.37 (dd, *J* = 5.7, 5.7 Hz, 1H), 7.37 (d, *J* = 7.3 Hz, 1H), 7.35 (dd, *J* = 5.8, 5.8 Hz, 1H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.34 (d, *J* = 5.8 Hz, 1H), 7.10 (d, *J* = 5.8 Hz, 1H), 7.07 (dd, *J* = 7.3, 7.3 Hz, 1H), 6.97 (dd, *J* = 7.3, 7.3 Hz, 1H), 6.79 (dd, *J* = 7.8 Hz, 1H), 6.75 (s, 1H), 6.69 (d, *J* = 7.8 Hz, 1H), 6.47 (d, *J* = 15.6 Hz, 1H), 6.38 (ddd, *J* = 15.6, 6.7, 6.7 Hz, 1H), 5.56 (s, 1H), 5.00 (d, *J* = 5.6 Hz, 1H), 3.62–3.57 (m, 1H), 3.49 (dd, *J* = 16.7, 7.0 Hz, 1H), 3.38–3.32 (m, 1H), 3.31 & 2.86 (AB quartet, *J* = 14.0 Hz, 2H), 3.26 (dd, *J* = 16.7, 6.8Hz, 1H), 3.21 (d, *J* = 15.1 Hz, 1H), 2.99–2.93 (m, 1H), 2.99–2.93 (m, 1H), 2.80 (dd, *J* = 15.1, 5.6 Hz, 1H), 2.58 (dd, *J* = 2.0, 2.0 Hz, 1H), 2.48–2.45 (m, 1H), 2.32 & 2.09 (AB quartet, *J* = 16.0 Hz, 2H), 2.01–1.88 (m, 2H), 1.92 (ddd, *J* = 6.4, 6.4, 2.0 Hz, 2H), 1.61–1.51 (m, 2H), 1.45–1.41 (m, 1H), 1.36–1.32 (m, 1H), 1.34–1.30 (m, 1H), 1.17 (ddd, *J* = 13.1, 13.1, 4.1 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 173.6, 171.6, 171.1, 169.4, 152.6, 137.7, 137.4, 136.1, 131.0, 129.9, 129.7, 129.5, 129.2, 128.9, 128.9, 128.2, 126.6, 126.0, 125.2, 123.6, 120.5, 118.3, 117.1, 114.2, 110.8, 105.7, 83.6, 70.8, 57.3, 52.7, 48.8, 44.2, 39.6, 39.4, 38.3, 34.4, 34.2, 31.5, 31.4, 26.9, 24.5, 22.5, 18.1; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{43}H_{45}N_4O_6$ 713.3334; found 713.3321.

3-((1^1*S*,1^5*R*,1^{11b}*S*,13*S*,*E*)-1^3,12,15-trioxo-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14-diaza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclopentadecaphan-4-en-13-yl)propanoic acid (40)

Synthesized according to Procedure D using 50 mg of crude **S26**. Purified by preparative TLC: 3% MeOH / 96.5%$CHCl_3$ / 0.5%AcOH. White solid. 8 mg [11 μmol, 19% yield over three steps]. ^{1}H NMR ($CDCl_3$, 500 MHz): δ11.06 (s, 1H), 8.23 (d, *J* = 6.9 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.45–7.42 (m, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 2.3 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.00 (dd, *J* = 7.7, 7.7 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 2H), 6.71 (d, *J* = 15.9 Hz, 1H), 6.33 (ddd, *J* = 15.9, 6.6, 4.8 Hz, 1H), 5.25 (s, 1H), 5.06 (d, *J* = 6.6 Hz, 1H), 4.90 (dd, *J* = 15.0, 7.0 Hz, 1H), 4.83 (dd, *J* = 15.0, 4.7 Hz, 1H), 4.11 (dd, *J* = 5.3, 5.3 Hz, 1H), 3.70–3.65 (m, 1H), 3.13 (d, *J* = 15.3 Hz, 1H), 3.01–2.96 (m, 1H), 2.90 (dd, *J* = 15.0, 7.0 Hz, 1H), 2.84 (d, *J* = 13.9 Hz, 1H), 2.74–2.67 (m, 1 H), 2.45–2.36 (m, 2H), 2.23–2.16 (m, 1H), 2.16 (d, *J* = 16.3 Hz, 1H), 2.08 (d, *J* = 16.2 Hz, 1H), 2.02–1.95 (m, 1H), 1.93 (ddd, *J* = 6.9, 6.9, 2.0 Hz, 2H), 1.80–1.73 (m, 1H), 1.70–1.62 (m, 1H), 1.43–1.23 (m, 6H), 1.12–1.06 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ173.8, 171.4, 171.2, 171.0, 155.8, 137.8, 136.7, 135.9, 132.9, 131.7, 131.5, 130.7, 130.04, 129.97, 128.8, 126.3, 126.2, 123.0, 121.1, 118.8, 117.3, 115.5, 111.5, 106.0, 84.2, 71.3, 67.6, 57.7, 52.6, 48.9, 45.2, 40.4, 38.0, 34.4, 34.0, 32.4, 30.5, 26.6, 26.1, 22.6, 18.2; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{43}H_{45}N_4O_6$ 713.3334; found 713.3324.

(*E*)-3-(3-((*S*)-2-(2-(((*R*)-1-(((*S*)-5-azido-1-((4-hydroxyphenethyl)amino)-1-oxopentan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-(diethoxymethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S27)

Synthesized according to Procedure A beginning with 0.19 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((1*S*,5*R*,11b*S*)-5-(((*S*)-5-azido-1-((4-hydroxyphenethyl)amino)-1-oxopentan-2-yl)carbamoyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (S28)

Synthesized according to Procedure B using crude material from the previous reaction. Carried forward without purification.

(1^1*S*,1^5*R*,1^{11b}*S*,12*S*,*E*)-12-(3-azidopropyl)-7^6-hydroxy-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (41)

Synthesized according to Procedure C using 55mg of crude **S28**. Purified by preparative HPLC – see SI for conditions. Pale yellow solid. 27 mg [37 μmol, 57% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.01 (s, 1H), 9.28 (br s, 1H), 7.81 (d, *J* = 7.0 Hz, 1H), 7.45 (dd, *J* = 5.3, 5.3 Hz, 1H), 7.44 (s, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 7.34 (dd, *J* = 7.2, 7.2 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.08 (dd, *J* = 7.7, 7.7 Hz, 1H), 6.98 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.80 (dd, *J* = 8.2 Hz, 1H), 6.76 (s, 1H), 6.70 (d, *J* = 8.2 Hz, 1H), 6.50 (d, *J* = 15.4 Hz, 1H), 6.39 (ddd, *J* = 15.4, 7.2, 7.2 Hz, 1H), 5.55 (s, 1H), 5.03 (d, *J* = 6.4 Hz, 1H), 3.69–3.66 (m, 1H), 3.50 (dd, *J* = 16.5, 7.1 Hz,1H), 3.39–3.35 (m,1H), 3.33 & 2.83 (AB quartet, *J* = 13.6 Hz, 2H), 3.26 (dd, *J* = 16.4, 6.8Hz, 1H), 3.17 (d, *J* = 15.3 Hz,1H), 3.13–3.04 (m, 2H), 3.02–2.94 (m, 1H), 2.83 (dd, *J* = 15.3, 6.3 Hz, 1H), 2.58 (dd, *J* = 2.5, 2.5 Hz, 1H), 2.54–2.52 (m,1H), 2.34 (ddd, *J* = 13.8, 10.7, 3.1 Hz, 1H), 2.25 & 2.09 (AB quartet, *J* = 16.3 Hz, 2H), 1.92 (ddd, *J* = 6.8, 6.8, 2.5 Hz, 2H), 1.45–1.39 (m,1H), 1.45–1.39 (m,1H), 1.38–1.31 (m, 4H), 1.24–1.18 (m, 1H), 1.19–1.13 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.3, 170.7, 169.8, 152.5, 137.3, 137.1, 136.3, 131.1, 129.9, 129.8, 129.3, 129.1, 128.8, 128.7, 128.2, 126.5, 125.9, 125.3, 123.4, 120.6, 118.2, 116.9, 114.1, 110.8, 105.9, 83.5, 70.7, 57.2, 52.0, 49.8, 48.8, 44.5, 39.5, 39.4, 38.0, 34.7, 34.6, 34.2, 31.5, 28.3, 24.8, 22.4, 17.9; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{43}H_{46}N_7O_4$ 724.3606; found 724.3592.

(*E*)-3-(3-((*S*)-2-(diethoxymethyl)-2-(2-(((*R*)-1-(((2*S*,3*R*)-3-hydroxy-1-((4-hydroxyphenethyl)amino)-1-oxobutan-2-yl)amino)-3-(3-methoxyphenyl)-1-oxopropan-2-yl)amino)-2-oxoethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S29)

Synthesized according to Procedure A beginning with 0.17 mmol of (+)-**5**. Carried forward without purification.

Pictet-Spengler Products S30a & b

Synthesized according to Procedure B using crude material from the previous reaction. Carried forward without purification.

(1^1*S*,1^5*R*,1^{10b}*S*,12*S*,*E*)-7^6-hydroxy-12-((*R*)-1-hydroxyethyl)-1^8-methoxy-1^1-(pent-4-yn-1-yl)-1^1,1^2,1^3,1^5,1^6,1^{10b}-hexahydro-10,13-diaza-1(1,5)-pyrrolo[2,1-*a*]isoquinolina-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (42)

Synthesized according to Procedure C using 26 mg of crude **S30**. Purified by preparative HPLC – see SI for conditions. White solid. 16 mg [24 μmol, 49% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.51 (d, *J* = 7.6 Hz, 1H), 7.48 (dd, *J* = 5.8, 5.8 Hz, 1H), 7.42 (s, 1H), 7.36-7.33 (m, 1H), 7.36-7.33 (m, 1H), 7.36-7.33 (m, 1H), 7.10 (d, *J* = 4.7 Hz, 1H), 6.85 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.70 (d, *J* = 8.2 Hz, 1H), 6.68 (s, 1H), 6.51 (d, *J* = 15.7 Hz, 1H), 6.38 (ddd, *J* = 15.6, 7.2 Hz, 1H), 5.37 (s, 1H), 5.04 (d, *J* = 6.4 Hz, 1H), 3.77-3.71 (m, 1H), 3.73-3.71 (m, 1H), 3.72 (s, 3 H), 3.48 (dd, *J* = 16.4, 7.0 Hz, 1H), 3.36 (dddd, *J* = 6.3, 6.3, 6.3, 6.3 Hz, 1H), 3.31 (dd, *J* = 16.5, 6.9 Hz, 1H), 3.16 (d, *J* = 13.9 Hz, 1H), 3.06-3.02 (m, 1H), 3.06 (d, *J* = 15.9 Hz, 1H), 2.90 (dd, *J* = 16.1, 6.1Hz, 1H), 2.85 (d, *J* = 14.0 Hz, 1H), 2.64 (t, *J* = 2.1 Hz, 1H), 2.54-2.48 (m, 1H), 2.45-2.41 (m, 2H), 2.39 (d, *J* = 16.8 Hz, 1H), 2.09 (d, *J* = 16.6 Hz, 1H), 1.92-1.90 (m, 2H), 1.39-1.29 (m,1H), 1.31-1.29 (m, 1H), 1.19-1.15 (m, 1H), 1.19-1.15 (m, 1H), 0.86 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.1, 169.5, 169.4, 157.1, 152.6, 137.5, 137.5, 133.8, 131.5, 130.1, 129.6, 129.2, 128.8, 128.3, 128.2, 126.7, 126.5, 125.6, 125.0, 123.9, 114.3, 113.9, 112.3, 83.8, 71.0, 66.1, 58.7, 58.2, 55.7, 48.5, 45.3, 40.8, 40.0, 38.8, 34.8, 34.3, 32.3, 31.4, 22.3, 19.7, 18.0; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{41}H_{46}N_3O_6$ 676.3381; found 676.3368.

(*E*)-3-(3-((*S*)-2-(2-(((*R*)-3-(5-bromo-1*H*-indol-3-yl)-1-(((*S*)-1-((4-hydroxyphenethyl)amino)-3-(1*H*-imidazol-4-yl)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-(diethoxymethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S31)

Synthesized according to Procedure A beginning with 0.17 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((1*S*,5*R*,11b*S*)-8-bromo-5-(((*S*)-1-((4-hydroxyphenethyl)amino)-3-(1*H*-imidazol-4-yl)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (S32)

Synthesized according to a modified Procedure B using crude material from the previous reaction: 10 vol% of conc. H_3PO_4 was added to the aqueous acetic acid solution. Carried forward without purification.

(1^1S,1^5R,$1^{11b}S$,12*S*,*E*)-12-((1*H*-imidazol-4-yl)methyl)-1^8-bromo-7^6-hydroxy-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (43)

Synthesized according to Procedure C using 43 mg of crude **S32**. Purified by preparative HPLC – see SI for conditions. White solid. TFA salt: 30 mg [33 μmol, 61% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.20 (s, 1H), 9.20 (s, 1H), 8.81 (s, 1H), 8.11 (d, *J* = 4.9 Hz, 1H), 7.46 (s, 1H), 7.44 (s, 1H), 7.42–7.40 (m, 1H), 7.37–7.35 (m, 1H), 7.36–7.35 (m, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 7.15 (s, 1H), 7.10 (d, *J* = 4.7 Hz, 1H), 6.81 (d, *J* = 7.2 Hz, 1H), 6.77 (s, 1H), 6.71 (d, *J* = 7.3 Hz, 1H), 6.49 (d, *J* = 15.7 Hz, 1H), 6.38 (ddd, *J* = 15.2, 6.5, 6.5 Hz, 1H), 5.49 (s, 1H), 5.01 (d, *J* = 4.5 Hz, 1H), 4.05–4.02 (m, 1H), 3.47 (dd, *J* = 16.1, 5.9 Hz, 1H), 3.38–3.34 (m, 1H), 3.32 (dd, *J* = 16.4, 6.1 Hz, 1H), 3.30 & 2.84 (AB quartet, *J* = 12.2 Hz, 2H), 3.07–3.03 (m, 1H), 2.95 (d, *J* = 15.3 Hz, 1H), 2.87–2.83 (m, 1), 2.78–2.75 (m, 1H), 2.77 (dd, *J* = 15.3, 4.6 Hz, 1H), 2.56 (br s, 1H), 2.50–2.48 (m, 1H), 2.39–2.36 (m, 1H), 2.26 & 2.10 (AB quartet, *J* = 16.4 Hz, 2H), 1.91 (very broad singlet, 2H), 1.40–1.37 (m, 1H), 1.32–1.29 (m, 1H), 1.32–1.29 (m, 1H), 1.17–1.13 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.4, 170.1, 169.8, 152.8, 137.5, 137.4, 135.1, 133.4, 131.5, 131.3, 130.2, 129.3, 129.2, 129.1, 128.9, 128.7, 128.3, 127.9, 126.7, 125.7, 123.8, 123.3, 119.4, 116.2, 114.4, 113.1, 111.1, 105.7, 83.8, 71.0, 57.3, 51.8, 48.5, 44.5, 39.8, 39.4, 38.0, 34.6, 34.1, 31.4, 26.4, 24.5, 22.4, 18.0; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{44}H_{44}BrN_6O_4$ 799.2602; found 799.2595.

(1^1S,1^5R,1^{11}b*S*,13*S*,*E*)-13-((1*H*-imidazol-4-yl)methyl)-1^8-bromo-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14-diaza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclopentadecaphan-4-ene-1^3,12,15-trione (44)

Synthesized according to Procedure E using 50 mg of crude Pictet-Spengler product (**S32**). Purified by preparative HPLC using HCOOH instead of TFA – see SI for conditions. White solid. 15 mg [19 μmol, 30% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.28 (s, 1H), 8.85 (s,1H), 8.45 (d, *J* = 7.2 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 1.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.34-7.32 (m, 1H), 7.34 (s,1H), 7.34 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.31 (s, 1H), 7.23 (dd, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 7.4 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 1H), 6.84 (d, *J* = 8.3 Hz, 1H), 6.66 (d, *J* = 16.1 Hz, 1H), 6.33 (ddd, *J* = 16.1, 5.7, 5.7 Hz, 1H), 5.15 (s, 1H), 5.06 (dd, *J* = 5.6, 1.4 Hz, 1H), 4.90 (dd, *J* = 15.0, 6.1 Hz, 1H), 4.85 (dd, *J* = 15.0, 4.4 Hz, 1H), 4.09 (ddd, *J* = 7.4, 7.4, 7.4 Hz, 1H), 3.23-3.19 (m, 1H), 3.22 & 2.85 (AB quartet, *J* = 13.8 Hz, 2H), 3.09-3.06 (m, 1H), 2.99 (d, *J* = 14.9, 5.8 Hz, 1H), 2.87-2.85 (m, 1H), 2.84-2.83 (m, 2H), 2.70-2.67 (m, 1H), 2.58 (dd, *J* = 2.1, 2.1 Hz, 1H), 2.41-2.37 (m, 1H), 2.18 & 2.10 (AB quartet, *J* = 16.4 Hz, 2H), 1.92 (ddd, *J* = 6.7, 6.7, 2.0 Hz, 2H), 1.40-1.30 (m, 2H), 1.28-1.25 (m, 1H), 1.06 (ddd, *J* = 12.7, 12.7, 3.7 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.1, 171.0, 170.0, 155.6, 137.4, 135.8, 135.1, 133.3, 132.5, 131.5, 131.3, 131.2, 130.0, 130.0, 129.1, 128.6, 127.9, 125.9, 123.3, 123.1, 119.3, 117.0, 115.4, 113.2, 111.3, 105.5, 83.9, 71.0, 67.3, 57.2, 51.9, 48.3, 45.0, 40.1, 38.5, 37.8, 34.1, 32.4, 26.1, 25.3, 22.4, 18.0; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{44}H_{44}BrN_6O_4$ 799.2602; found 799.2576.

(*E*)-3-(3-((*S*)-2-(2-(((*R*)-1-(((*R*)-3-(*tert*-butyldisulfaneyl)-1-((4-hydroxyphenethyl)amino)-1-oxopropan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)-2-(diethoxymethyl)hept-6-yn-1-yl)phenyl)allyl isobutyl carbonate (S33)

Synthesized according to Procedure A beginning with 0.17 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((1*S*,5*R*,11b*S*)-5-(((*R*)-3-(*tert*-butyldisulfaneyl)-1-((4-hydroxyphenethyl)amino)-1-oxopropan-2-yl)carbamoyl)-3-oxo-1-(pent-4-yn-1-yl)-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indol-1-yl)methyl)phenyl)allyl isobutyl carbonate (S34)

Synthesized according to Procedure B using crude material from previous reaction. Carried forward without purification.

(1^1S,1^5R,$1^{11b}S$,12*R*,*E*)-12-((*tert*-butyldisulfaneyl)methyl)-7^6-hydroxy-11-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (45a)

Synthesized according to Procedure C using crude material from previous reaction. Purified by preparative HPLC – see SI for conditions. Yellow solid. 32 mg [41 μmol, 24% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.96 (s, 1H), 9.10 (s, 1H), 7.87 (d, *J* = 7.3 Hz, 1H), 7.64 (dd, *J* = 5.7, 5.7 Hz, 1H), 7.45 (s, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.35-7.33 (m, 1H), 7.35-7.33 (m, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.08 (dd, *J* = 8.2, 8.2 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.78 (d, *J* = 8.3 Hz, 1H), 6.77 (s, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.46 (d, *J* = 15.7 Hz, 1H), 6.40 (ddd, *J* = 15.6, 6.6, 6.6 Hz, 1H), 5.59 (s, 1H), 5.04 (d, *J* = 6.4 Hz, 1H), 3.96 (ddd, *J* = 10.8, 7.3, 3.8 Hz, 1H), 3.51 (dd, *J* = 16.2, 6.7 Hz,1H), 3.36 & 2.81 (AB quartet, *J* = 14.3 Hz, 2H), 3.29 (d, *J* = 15.1 Hz,1H), 3.28-3.25 (m,1H), 3.24 (dd, *J* = 16.4, 5.8 Hz, 1H), 3.12-3.07 (m, 1H), 2.81 (dd, *J* = 15.0, 6.2 Hz, 1H), 2.73 (dd, *J* = 12.8, 3.6 Hz,1H), 2.63 (dd, *J* = 12.9, 10.9 Hz, 1H), 2.57 (t, *J* = 2.3 Hz, 1H), 2.56-2.52 (m,1H), 2.34 (ddd, *J* = 14.0, 11.0, 3.0 Hz, 1H), 2.24 & 2.08 (AB quartet, *J* = 16.2 Hz, 2H), 1.92-1.91 (m, 2H), 1.44-1.40 (m,1H), 1.36-1.31 (m,1H), 1.35-1.32 (m, 1H), 1.20-1.16 (m, 1H), 1.19 (s, 9H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.2, 169.8, 169.2, 152.8, 137.5, 137.4, 136.5, 131.0, 130.0, 129.7, 129.4, 129.3, 129.1, 128.9, 128.1, 126.9, 126.2, 125.6, 123.6, 120.6, 118.2, 117.3, 114.1, 110.9, 106.2, 83.8, 70.9, 57.3, 52.6, 48.9, 47.2, 44.3, 41.9, 39.7, 39.5, 38.1, 34.8, 34.2, 31.7, 29.1, 24.2, 22.6, 18.1; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{45}H_{51}N_4O_4S_2$ 775.3346; found 775.3365.

(1^1S,1^5R,$1^{11b}S$,10*R*,*E*)-*N*-(4-hydroxyphenethyl)-1^3,12-dioxo-1^1-(pent-4-yn-1-yl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7,8-dithia-11-aza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3)-benzenacyclododecaphan-4-ene-10-carboxamide (45b)

Purified by preparative HPLC from the above reaction – see SI for conditions. Pale yellow solid. 33 [46 μmol, 27% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.88 (s, 1H), 9.14 (br s, 1H), 7.93 (t, *J* = 5.6 Hz, 1H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.65 (s, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.38 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.21 (d, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 7.10 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.01 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 2H), 6.65 (d, *J* = 8.2 Hz, 2H), 6.58 (d, *J* = 15.4 Hz, 1H), 6.24 (ddd, *J* = 15.5, 9.1, 6.2 Hz, 1H), 5.25 (s, 1H), 4.93 (d, *J* = 5.8 Hz, 1H), 4.02 (ddd, *J* = 10.7, 6.8, 3.9 Hz, 1H), 3.62 (dd, *J* = 13.9, 9.2 Hz,1H), 3.46 (dd, *J* = 14.0, 6.2 Hz, 1H), 3.39 (d, *J* = 15.5 Hz,1H), 3.34 & 2.86 (AB quartet, *J* = 13.8 Hz, 2H), 3.14-3.03 (m, 2H), 2.76-2.74 (m, 1H), 2.73 (dd, *J* = 11.8, 11.8 Hz,1H), 2.57 (t, *J* = 2.3 Hz, 1H), 2.47 (t, *J* = 7.4 Hz, 2H), 2.37 (dd, *J* = 12.3, 3.6 Hz, 1H), 2.31 & 2.12 (AB quartet, *J* = 16.4 Hz, 2H), 1.94-1.92 (m, 2H), 1.51-1.45 (m, 1H), 1.43-1.39 (m, 1H), 1.35-1.32 (m, 1H), 1.35-1.32 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 171.4, 168.1, 168.0, 155.4, 137.3, 136.6, 136.6, 131.5, 130.5, 129.5, 129.2, 129.1, 128.3, 127.1, 127.0, 124.1, 124.0, 120.8, 118.3, 117.6, 114.7, 111.0, 106.8, 83.8, 70.9, 55.9, 52.8, 49.8, 43.4, 43.2, 40.7, 40.5, 39.9, 38.2, 35.1, 33.8, 22.8, 22.3, 18.1; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{41}H_{43}N_4O_4S_2$ 719.2720; found 719.2719.

3-((1^1S,1^5R,$1^{11b}S$,9*S*,12*S*,*E*)-9-carbamoyl-7^6-hydroxy-1^3,11,14-trioxo-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-en-12-yl)propanoic acid (46)

Synthesized according to Procedure F with 13 μmol of **36**. Purified by preparative HPLC – see SI for conditions. White solid. 4 mg [4.2 μmol, 32% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.73 (s, 1H), 7.48 (br s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.37–7.31 (m, 2H), 7.09 (d, *J* = 6.9 Hz, 1H), 7.08 (d, 7.4 Hz, 1H), 6.99 (d, *J* = 7.8 Hz, 1H), 6.97 (d, *J* = 4.9 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.77 (br s, 1H), 6.66 (*J* = 8.1 Hz, 1H), 6.52 (d, *J* = 15.4 Hz, 1H), 6.37 (ddd, *J* = 15.3, 7.2, 7.2 Hz, 1H), 5.67 (s, 1H), 5.34 (d, *J* = 9.4 Hz, 1H), 5.00 (d, *J* = 6.6 Hz, 1H), 4.14 (ddd, *J* = 7.9, 7.9, 2.7 Hz, 1H), 3.76–3.73 (m, 1H), 3.70–3.66 (m, 2H), 3.53–3.38 (m, 3H) 3.23–3.18 (m, 1H), 2.95–2.86 (m, 1H), 2.63–2.60 (m, 1H), 2.36 (dd, *J* = 7.6, 7.6 Hz, 2H), 2.22 (d, *J*

= 16.4 Hz, 1H), 2.15 (d, *J* = 16.0 Hz, 1H), 2.08–2.03 (m, 1H), 1.99–1.94 (m, 1H), 1.78–1.73 (m, 1H), 1.64–1.59 (m, 2H), 1.55–1.50 (m, 1H), 1.38–1.31 (m, 1H), 1.27–1.21 (m, 1H); HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{50}H_{57}N_8O_{12}$ 961.4091; found 961.4087.

(1^1S,1^5R,$1^{11b}S$,9*S*,12*S*,*E*)-12-(3-amino-3-oxopropyl)-7^6-hydroxy-1^3,11,14-trioxo-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^2,1^3,15,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-9-carboxamide (S10)

Synthesized according to Procedure F with 13 µmol of **37**. Purified by preparative HPLC – see SI for conditions. White solid. 5 mg [5.2 µmol, 40% yield]. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.85 (s, 1H), 7.47 (s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.4 Hz, 1H), 7.10 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.04 (dd, *J* = 7.5, 7.5 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 1H), 6.60–6.55 (m, 1H), 6.53 (d, *J* = 16.0 Hz, 1H), 5.77 (s, 1H), 5.32 (d, *J* = 9.2 Hz, 1H), 5.19 (d, *J* = 6.0 Hz, 1H), 4.17 (d, *J* = 10.7 Hz, 1H), 3.89 (d, *J* = 12.1 Hz, 1H), 3.86–3.83 (m, 1H), 3.83 (dd, *J* = 9.3, 9.3 Hz, 1H), 3.72 (d, *J* = 12.2, 5.2 Hz, 1H), 3.56–3.47 (m, 6H), 3.13 (d, 13.8 Hz, 1H), 2.95 (d, *J* = 16.4, 1H), 2.89 (dd, *J* = 15.4, 7.6 Hz, 1H), 2.75 (d, *J* = 13.3 Hz, 1H), 2.60–2.50 (m, 2H), 2.47 (d, *J* = 16.4 Hz, 1H), 2.17–2.08 (m, 2H), 1.81–1.74 (m, 1H), 1.72–1.63 (m, 3H), 1.42–1.32 (m, 3 H), 1.32–1.27 (m, 3H), 1.13–1.09 (m, 1H); ^{13}C NMR (CD_3OD, 126 MHz): δ 177.8, 176.7, 175.4, 174.2, 173.2, 154.6, 148.5, 140.0, 139.5, 137.9, 132.5, 131.0, 130.8, 130.5, 130.0, 129.9, 129.8, 128.9, 128.3, 127.5, 127.1, 127.0, 123.5, 122.4, 121.0, 119.5, 115.9, 112.1, 108.4, 89.4, 81.1, 78.5, 73.8, 70.9, 62.5, 60.9, 57.4, 56.6, 52.8, 43.4, 42.7, 37.2, 34.8, 32.7, 32.4, 27.6, 26.6, 24.7, 24.5; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{50}H_{58}N_9O_{11}$ 960.4250; found 960.4254.

(1^1S,1^5R,$1^{11b}S$,12*S*,*E*)-12-((1*H*-imidazol-4-yl)methyl)-1^8-bromo-7^6-hydroxy-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (S11)

Synthesized according to Procedure F with 13 µmol of **43**. Purified by preparative HPLC – see SI for conditions. White solid. TFA salt: 1.5 mg [1.3 µmol, 10% yield]. ^{1}H NMR (CD_3OD, 500 MHz): δ 8.63 (s, 1H), 7.56 (s, 1H), 7.53 (s, 1H), 7.50 (s, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.38 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.97 (s, 1H), 6.92 (s, 1H), 6.78 (d, *J* = 8.2 Hz, 1H), 6.62 (d, *J* = 8.1 Hz, 1H), 6.52 (br s), 5.50 (s, 1H), 5.36 (d, *J* = 9.1 Hz, 1H), 5.05 (d, *J* = 6.2, 1H), 4.28 (dd, *J* = 9.0, 5.2 Hz, 1H), 3.88 (d, *J* = 11.9 Hz, 1H), 3.83 (dd, *J* = 9.2, 9.2 Hz, 1H), 3.72 (dd, *J* = 12.1, 5.5 Hz, 1H), 3.57–3.47 (m, 4H), 3.42–3.83 (m, 1H), 3.22 (d, *J* = 15.7 Hz, 1H), 2.99–2.95 (m, 1H), 2.96 (d, *J* = 14.7 Hz, 1H), 2.84–2.77 (m, 1H), 2.56–2.43 (m, 5H), 2.28 (d, *J* = 16.8 Hz, 1H), 1.70–1.64 (m, 1H), 1.61–1.54 (m, 1H), 1.38–1.26 (m, 6H); ^{13}C NMR (CD_3OD, 126 MHz): δ 175.9, 171.9, 171.4, 154.4, 148.4, 140.4, 139.2, 136.9, 135.0, 132.4, 131.7, 131.3, 131.2, 131.2, 131.1, 131.0, 130.1, 130.0, 129.4, 128.6, 127.9, 125.8, 125.8, 122.4, 121.4, 118.1, 115.5, 114.1, 113.4, 108.1, 89.4, 81.1, 78.5, 73.9, 70.9, 62.4, 60.5, 53.2, 51.8, 47.5, 42.1, 41.8, 41.0, 36.0, 35.6, 33.1, 28.1, 26.6, 24.7, 24.4; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{50}H_{55}BrN_9O_9$ 1004.3301; found 1004.3259.

(1^1S,1^5R,$1^{10b}S$,12*S*,*E*)-7^6-hydroxy-12-((*R*)-1-hydroxyethyl)-1^8-methoxy-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^1,1^2,1^3,1^5,1^6,1^{10b}-hexahydro-10,13-diaza-1(1,5)-pyrrolo[2,1-*a*]isoquinolina-3,7(1,3)-dibenzenacyclotetradecaphan-4-ene-1^3,11,14-trione (S12)

Synthesized according to Procedure F with 15 µmol **42**. Purified by preparative HPLC – see SI for conditions. White solid. 9 mg [10 µmol, 67% yield]. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.63 (s, 1H), 7.50 (s, 1H), 7.34 (ddd, *J* = 8.1, 1.5, 1.5 Hz, 1H), 7.33 (d, *J* = 2.8 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 1H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.04 (ddd, *J* = 7.0, 1.5, 1.5 Hz, 1H), 7.01 (d, *J* = 2.2 Hz, 1H), 6.84 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.80, (dd, *J* = 8.1, 2.1 Hz, 1H), 6.74 (d, *J* = 2.6 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 15.9 Hz, 1H), 6.46 (ddd, *J* = 15.7, 6.2, 6.2 Hz, 1H), 5.51 (d, *J* = 9.3 Hz, 1H), 5.36 (s, 1H), 5.05 (dd, *J* = 6.3, 1.3 Hz, 1H), 3.89 (dd, *J* = 12.3, 2.0 Hz, 1H), 3.86 (dd, *J* = 9.1, 9.1 Hz, 1H), 3.78–3.71 (m, 3H), 3.65–3.59 (m, 1H), 3.57 (ddd, *J* = 4.8, 4.8, 1.7 Hz, 1H), 3.56–3.50 (m, 2H), 3.47–3.44 (m, 1H), 3.34 (dd, *J* = 15.6, 6.0 Hz, 1H), 3.28 (dd, *J* = 5.3, 5.3 Hz, 1H), 3.27–3.21 (m, 2H), 2.95 (dd, *J* = 16.3, 6.3 Hz, 1H), 2.92 (d, *J* = 14.2 Hz, 1H), 2.68 (d, *J* = 16.7 Hz, 1H), 2.62 (ddd, *J* = 14.0, 5.2, 5.2 Hz, 1H), 2.58–2.50 (m, 2H), 2.48–2.42 (m, 1H), 2.21 (d, *J* = 16.6 Hz, 1H), 1.63–1.54 (m, 1H), 1.49–1.40 (m, 1H), 1.36, (ddd, *J* = 13.0, 13.0, 3.8 Hz, 1H), 1.24 (ddd, *J* = 13.3, 13.3 4.5 Hz, 1H), 0.82 (d, *J* = 6.4 Hz, 1H); ^{13}C NMR (CD_3OD, 126 MHz): δ 176.0, 171.6, 171.6, 159.9, 154.4, 148.5, 140.1, 138.9, 135.3, 132.6, 131.3, 131.2, 131.0, 130.6, 130.1, 129.8, 128.8, 128.6, 128.1, 125.6, 125.4, 122.3, 115.5, 115.4, 114.6, 89.5, 81.1, 78.5, 73.9, 70.9, 68.2, 62.5, 61.3, 60.4, 55.8, 51.4, 48.2, 42.7, 41.3, 40.6, 36.7, 35.8, 33.4, 32.4, 26.7, 24.4, 19.7; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{47}H_{57}N_6O_{11}$ 881.4080; found 881.4085.

3-((1^1S,1^5R,$1^{11b}S$,12*S*,*E*)-7^6-hydroxy-1^3,11,14-trioxo-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-10,13-diaza-1(1,5)-indolizino[8,7-*b*]indola-3,7(1,3)-dibenzenacyclotetradecaphan-4-en-12-yl)propanoic acid (S13)

Synthesized according to Procedure F beginning with 15 µmol of **39**. Purified by preparative HPLC – see SI for conditions. White solid. 4 mg [4.4 µmol, 29% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.19 (d, *J* = 5.3 Hz, 1H), 7.47 (s, 1H), 7.46 (d, *J* = 5.6 Hz, 1H), 7.36 (ddd, *J* = 8.0, 1.2, 1.2 Hz, 1H), 7.34 (ddd, *J* = 8.1, 0.8, 0.8 Hz, 1H), 7.32 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.31 (s, 1H), 7.15 (ddd, *J* = 8.1, 7.2, 0.9 Hz, 1H), 7.05 (ddd, *J* = 7.8, 7.0, 0.8 Hz, 1H), 7.03 (ddd, *J* = 7. 1, 1.2, 1.2 Hz, 1H), 6.91 (d, *J* = 1.8 Hz, 1H), 6.73 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 6.51 (ddd, 15.8, 6.1, 6.1 Hz, 1H), 6.46 (d, *J* = 15.9 Hz, 1H), 5.45 (s, 1H), 5.24 (d, *J* = 9.0 Hz, 1H), 5.04 (d, *J* = 6.1 Hz, 1H), 3.87 (dd, *J* = 12.2, 1.9 Hz, 1H), 3.82 (dd, *J* = 9.1, 9.1 Hz, 1H), 3.73 (dd, *J* = 8.7, 4.4 Hz, 1H), 3.70 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.60 (dd, *J* = 15.1, 5.7 Hz, 1H), 3.56–3.51 (m, 1H), 3.54 (dd, *J* = 8.9, 8.9 Hz, 1H), 3.48 (d, *J* = 9.2 Hz, 1H), 3.42 (ddd, *J* = 13.5, 11.3, 4.1 Hz, 1H), 3.28 (d, *J* = 14.2 Hz, 1H), 3.21 (dd, *J* = 14.6, 7.0 Hz, 1H), 3.05 (ddd, *J* = 13.4, 4.4, 4.4 Hz, 1H), 2.94 (d, *J* = 14.1 Hz, 1H), 2.89 (d, *J* = 16.5 Hz, 1H), 2.81 (ddd, *J* = 13.4, 6.4, 1.8 Hz, 1H), 2.55–2.38 (m, 3H), 2.24 (d, *J* = 16.5 Hz), 1.94 (ddd, *J* = 17.6, 7.9, 4.3 Hz, 1H), 1.86 (ddd, *J* = 17.5, 8.3, 4.3 Hz, 1H), 1.69–1.55 (m, 2H), 1.45–1.38 (m, 2H), 1.32–1.25 (m, 3H); ^{13}C NMR (CD_3OD, 126 MHz): δ 177.9, 176.7, 172.7, 171.7, 154.5, 140.2, 138.8, 138.4, 131.9, 131.1, 130.9, 130.8, 130.7, 130.1, 129.9, 129.3, 128.1, 127.8, 125.1, 123.0, 122.5, 120.4, 119.0, 115.3, 112.5, 108.7, 89.3, 81.0, 78.5, 73.8, 70.9, 62.4, 59.5, 55.8, 51.8, 49.8, 49.6, 47.5, 42.3, 41.0, 40.2, 36.7, 35.7, 34.4, 30.9, 27.4, 26.7, 24.4, 23.6; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{49}H_{56}N_7O_{11}$ 918.4032; found 918.4036.

3-((1^1S,1^5R,$11^{1b}S$,13*S*,*E*)-1^3,12,15-trioxo-1^1-(3-(1-((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)-1*H*-1,2,3-triazol-4-yl)propyl)-1^2,1^3,1^5,1^6,1^{11},1^{11b}-hexahydro-1^1*H*-7-oxa-11,14-diaza-1(1,5)-indolizino[8,7-*b*]indola-3(1,3),8(1,4)-dibenzenacyclopentadecaphan-4-en-13-yl)propanoic acid (S14)

Synthesized according to Procedure F beginning with 11 µmol of **40**. Purified by preparative HPLC – see SI for conditions. White solid. 3 mg [3.3 µmol, 30% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.74 (d, *J* = 8.5 Hz, 1H), 7.77 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.41 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.27 (s, 1H), 7.26 (d, *J* = 6.8 Hz, 1H), 7.19–7.16 (m, 1H), 7.08 (dd, *J* = 7.5, 7.5, 0.8 Hz, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 15.9, 1H), 5.98 (ddd, 15.8, 7.3, 5.9 Hz, 1H), 5.47 (s, 1H), 5.15 (d, *J* = 9.2 Hz, 1H), 5.12 (d, *J* = 5.7 Hz, 1H), 4.97 (dd, *J* = 12.4, 7.2 Hz, 1H), 4.46 (ddd, *J* = 7.8, 7.8, 7.8 Hz, 1H), 4.31 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.86 (dd, *J* = 12.1 Hz, 1H), 3.84 (dd, *J* = 9.1, 9.1 Hz, 1H), 3.69 (dd, *J* = 12.1, 5.4 Hz, 1H), 3.54 (dd, *J* = 8.9, 8.9 Hz, 1H), 3.51 (ddd, *J* = 9.6, 5.4, 2.2 Hz, 1H), 3.47 (d, *J* = 8.9 Hz, 1H), 3.44–3.40 (m, 1H), 3.03 (ddd, *J* = 16.2, 5.9, 2.2 Hz, 1H), 2.98 (d, *J* = 12.3 Hz, 1H), 2.64 (dd, *J* = 7.1, 7.1 Hz, 2H), 2.57–2.52 (m, 1H), 2.55 (d, *J* = 16.8 Hz, 1H), 2.50–2.44 (m, 1H), 2.36 (d, *J* = 16.8 Hz, 1H), 1.91 (ddd, *J* = 16.5, 7.9, 3.3 Hz, 1H), 1.87–1.80 (m, 1H), 1.78–1.70 (m, 1H), 1.64–1.55 (m,

3H), 1.45 (ddd, *J* = 13.5, 13.5, 3.8 Hz, 1H), 1.34–1.23 (m, 3H); ^{13}C NMR (CD_3OD, 126 MHz): δ 173.7, 172.2, 171.6, 169.7, 155.5, 137.4, 137.1, 137.0, 132.8, 130.2, 129.7, 129.4, 129.3, 128.9, 127.6, 126.4, 126.2, 125.6, 121.7, 121.0, 119.2, 117.4, 114.8, 111.1, 106.7, 87.8, 79.6, 77.1, 72.3, 69.5, 63.9, 61.0, 57.8, 51.6, 50.8, 48.4, 44.2, 42.1, 40.8, 40.0, 36.0, 34.1, 28.7, 27.0, 25.3, 23.2, 22.8; MS *m/z*: $[M+H]^+$ calc'd for $C_{49}H_{56}N_7O_{11}$ 918.4; found 918.5. HRMS (QE Orbitrap) *m/z*: $[M+Na]^+$ calc'd for $C_{49}H_{55}N_7O_{11}Na$ 940.3852; found 940.3859.

***tert*-Butyl (*S*)-(1-phenoxy-3-phenylpropan-2-yl)carbamate**

Phenol [4.7 g, 50 mmol] in 48 mL of dry DMF was cannulated into a suspension of NaH [2.0 g, 50 mmol] in 48 mL of dry DMF and stirred for 5 min. *tert*-Butyl (*S*)-4-benzyl-1,2,3-oxathiazolidine-3-carboxylate 2,2-dioxide [40 mmol], dissolved in 96 mL of dry DMF, was cannulated into the reaction flask. The reaction was monitored for completion by ^{1}H-NMR. The reaction was quenched by addition of 0.25 N HCl, and the mixture was then diluted with EtOAc. The layers were separated, and the aqueous layer was extracted 2x with EtOAc. The combined organic layers were washed 2x with 1N HCl, 3x 1N NaOH, and 1x brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. Chromatographed on silica using 10% EtOAc/hexanes to provide *tert*-Butyl (*S*)-(1-phenoxy-3-phenylpropan-2-yl)carbamate as a white solid [6.2 g, 18.9 mmol]. 47% from commercial L-phenylalaninol (4 steps). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30–7.26 (m, 5H), 7.21 (d, *J* = 7.2 Hz, 2H), 6.97 (dd, *J* = 7.3, 7.3 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 4.96 (d, *J* = 8.3 Hz, 1H), 4.19–4.12 (m, 1H), 3.89 (dd, *J* = 9.4, 3.9 Hz, 1H), 3.86 (dd, *J* = 9.3, 3.5 Hz, 1H), 3.02 (dd, *J* = 13.2, 6.3 Hz, 1H), 2.98 (dd, *J* = 13.0, 8.3 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.7, 155.4, 137.9, 129.7, 129.6, 128.7, 126.6, 121.2, 114.6, 79.7, 67.7, 51.4, 37.9, 28.6; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{20}H_{26}NO_3$ 328.1907; found 328.1899.

(*S*)-1-phenoxy-3-phenylpropan-2-amine

Pure *tert*-Butyl (*S*)-(1-phenoxy-3-phenylpropan-2-yl)carbamate [6.2 g, 18.9 mmol] was dissolved in 160 mL DCM and cooled to 0 ºC. 40 mL of TFA was added to the reaction flask under argon. The reaction was monitored by TLC. After reaction completion, solvents were removed and the residue partitioned between ethyl ether and 1N NaOH; the layers were then separated. The ether layer was washed 2x with 1N NaOH and 1x with brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give (*S*)-1-phenoxy-3-phenylpropan-2-amine as a pale yellow, waxy solid [4.2 g, 18.5 mmol]. 98% yield. $[\alpha]_D^{23}$ = +18.5º, c = 1.34, $CHCl_3$. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.42–7.29 (m, 7H), 7.05 (ddd, *J* = 7.3, 1.0, 1.0 Hz, 1H), 7.02–6.98 (m, 2H), 3.99 (dd, *J* = 9.0, 4.3 Hz, 1H), 3.87 (dd, *J* = 8.9, 6.6 Hz, 1H), 3.51 (dddd, *J* = 7.7, 6.1, 6.1, 4.4 Hz, 1H), 3.00 (dd, *J* = 13.4, 5.7 Hz, 1H), 2.78 (dd, *J* = 13.3, 8.0 Hz, 1H), 1.55 (br s, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.8, 138.5, 129.4, 129.2, 128.5, 126.4, 120.8, 114.5, 72.1, 52.0, 40.6; HRMS (DART-Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{15}H_{18}NO$ 228.1383; found 228.1379.

(*E*)-3-(3-((3*S*,6*S*,16*S*)-16-(diethoxymethyl)-3-methyl-4,9,14-trioxo-16-(pent-4-yn-1-yl)-6-((*S*)-2-(((*S*)-1-phenoxy-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carbonyl)-2,5,10,13-tetraazaheptadecan-17-yl)phenyl)allyl isobutyl carbonate (48)

Synthesized according to Procedure A with 0.30 mmol of (+)-**5**. Carried forward without purification.

(*E*)-3-(3-(((3*S*)-2-hydroxy-1-(2-((*S*)-4-((*S*)-2-(methylamino)propanamido)-5-oxo-5-((*S*)-2-(((*S*)-1-phenoxy-3-phenylpropan-2-yl)carbamoyl)pyrrolidin-1-yl)pentanamido)ethyl)-5-oxo-3-(pent-4-yn-1-yl)pyrrolidin-3-yl)methyl)phenyl)allyl isobutyl carbonate (S35)

Synthesized according to Procedure B starting with 200 mg of crude material. Diastereomeric mixture was carried forward without purification.

(*S*)-*N*-((1^{3a}*S*,1^{8b}*R*,1^{12}*S*,8*S*,13*S*,*E*)-8-benzyl-1^2,10,12,16-tetraoxo-1^{3a}-(pent-4-yn-1-yl)-1^1,1^2,1^3,1^{3a},1^4,1^{8b}-hexahydro-6-oxa-9,17-diaza-1(8,1)-indeno[1,2-*b*]pyrrola-11(2,1)-pyrrolidina-5(1,4)-benzenacyclononadecaphan-2-en-13-yl)-2-(methylamino)propanamide (49a)

Synthesized with a modified General Procedure C with crude material from above: 1:1 TFA/TFE, 5 mM. Purified by preparative HPLC – see SI for conditions. TFA salt: 22 mg [23 µmol, 13% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.88 (d, *J* = 7.7 Hz, 1H), 8.84 (br s, 1H), 8.77 (br s, 1H), 7.56 (dd, *J* = 4.4, 4.4 Hz, 1 H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.26–7.24 (m, 1H), 7.26 (dd, *J* = 7.2, 7.2 Hz, 2H), 7.20 (d, *J* = 7.1 Hz, 2H), 7.19 (dd, *J* = 7.1, 7.1 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 7.13 (d, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 2H), 6.70 (d, *J* = 15.7 Hz, 1H), 6.56–6.51 (m, 1H), 48.3 (s, 1H), 4.62 (ddd, *J* = 6.7, 6.7, 6.7 Hz, 1H), 4.30–4.26 (m, 1H), 4.11 (dd, *J* = 8.9, 2.9 Hz, 1H), 3.90–3.89 (m, 1H), 3.79–3.77 (m, 1H), 3.79–3.76 (m, 1H), 3.75–3.73 (m, 1H), 3.55–3.49 (m, 2H), 3.37–3.34 (m, 1H), 3.34–3.29 (m, 1H), 3.00–2.97 (m, 1H), 2.97–2.94 (m, 1H), 2.92 & 2.78 (AB quartet, *J* = 16.8 Hz, 2H), 2.85–2.81 (m, 1H), 2.77–2.74 (m, 1H), 2.72 (dd, *J* = 2.3, 2.3 Hz, 1H), 2.62–2.57 (m, 1H), 2.49 (s, 3H), 2.37 & 2.32 (AB quartet, *J* = 16.3 Hz, 2H), 2.13–2.08 (m, 1H), 2.11 (br s, 2H), 2.01–1.97 (m, 1H), 1.98–1.94 (m, 1H), 1.91–1.87 (m, 1H), 1.78–1.76 (m, 2H), 1.74–1.70 (m, 1H), 1.57–1.52 (m, 1H), 1.54–1.51 (m, 1H), 1.49–1.44 (m, 2H), 1.39–1.30 (m, 2H), 1.33 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 172.7, 171.7, 171.2, 169.1, 168.6, 157.1, 144.5, 138.4, 137.0, 134.6, 131.7, 131.6, 129.6, 129.2, 128.8, 127.8, 126.6, 125.9, 123.5, 122.1, 114.9, 84.2, 71.3, 69.3, 68.4, 60.6, 55.8, 50.3, 49.1, 47.9, 46.6, 41.8, 41.2, 39.1, 37.5, 36.9, 36.2, 35.2, 31.0, 30.7, 28.9, 26.9, 24.1, 23.8, 18.1, 15.6; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{50}H_{61}N_6O_6$ 841.4647; found 841.4644.

(*S*)-*N*-((1^{3a}*S*,1^{8b}*R*,1^{12}*S*,8*S*,13*S*,*E*)-8-benzyl-1^2,10,12,16-tetraoxo-1^{3a}-(pent-4-yn-1-yl)-1^1,1^2,1^3,1^{3a},1^4,1^{8b}-hexahydro-6-oxa-9,17-diaza-1(6,1)-indeno[1,2-*b*]pyrrola-11(2,1)-pyrrolidina-5(1,4)-benzenacyclononadecaphan-2-en-13-yl)-2-(methylamino)propanamide (49b)

Isolated from previous reaction. TFA salt: 38 mg [40 µmol, 21% yield over three steps]. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.85 (br s, 1H), 8.75 (br s, 1H), 8.65 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 6.8 Hz, 1H), 7.86 (dd, *J* = 4.4, 4.4 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.31–7.28 (m, 1H), 7.27–7.25 (m, 1H), 7.25–7.24 (m, 1H), 7.22–7.21 (m, 1H), 7.21 (s, 1H), 7.11 (d, *J* = 8.3 Hz, 2H), 6.40–6.35 (m, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 4.55 (s, 1H), 4.34 (ddd, *J* = 9.2, 9.2, 2.1 Hz, 1H), 4.22 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.09–4.08 (m, 1H), 4.09–4.08 (m, 1H), 3.97 (dd, *J* = 12.9, 7.1 Hz, 1H), 3.80–3.77 (m, 1H), 3.45 (dd, *J* = 14.8, 3.4 Hz, 1H), 3.38–3.35 (m, 1H), 3.34–3.32 (m, 1H), 3.29–3.26 (m, 1H), 3.29–3.26 (m, 1H), 3.14 (ddd, *J* = 8.2, 8.2, 8.2 Hz, 1H), 2.97–2.93 (m, 1H), 2.95 & 2.82 (AB quartet, *J* = 16.4 Hz, 2H), 2.92–2.89 (m, 1H), 2.80–2.77 (m, 1H), 2.72 (dd, *J* = 2.0, 2.0 Hz, 1H), 2.54–2.51 (m, 1H), 2.53 (dd, *J* = 4.7, 4.7 Hz, 3H), 2.38 (s, 2H), 2.16–2.13 (m, 1H), 2.11 (ddd, *J* = 5.4, 5.4, 1.8 Hz, 2H), 2.07–2.04 (m, 1H), 1.91–1.87 (m, 1H), 1.81–1.75 (m, 1H), 1.62–1.58 (m, 1H), 1.60–1.53 (m, 2H), 1.49–1.45 (m, 1H), 1.42–1.38 (m, 1H), 1.42–1.32 (m, 2H), 1.36–1.32 (m, 1H), 1.30 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ 172.0, 171.4, 170.7, 168.4, 156.6, 143.9, 138.2, 137.9, 137.6, 132.2, 132.2, 131.0, 129.4, 129.0, 128.8, 128.0, 126.1, 125.9, 123.8, 122.8, 84.0, 71.1, 70.5, 68.2, 58.4, 55.6, 49.6, 48.8, 47.3, 45.9, 42.4, 41.6, 38.9, 37.4, 36.6, 36.3, 36.2, 31.1, 30.7, 29.3, 26.9, 23.6, 23.6, 18.0, 15.4; HRMS (QE Orbitrap) *m/z*: $[M+H]^+$ calc'd for $C_{50}H_{61}N_6O_6$ 841.4647; found 841.4644.

Dimer Product 50: Synthesized according to Procedure G using 10 mg of **49a**. Purified by preparative HPLC – see SI for conditions. White solid. Bis-TFA salt: 4.5 mg [2.4 µmol, 40% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.90 (d, *J* = 7.7 Hz, 1H), 8.81 (br s, 2H), 7.61 (dd, *J* = 4.7, 4.7 Hz, 1H), 7.49 (d, 7.8 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.28–7.23 (m, 3H), 7.23–7.17 (m, 3H), 7.14–7.12 (m, 4H), 6.87 (d, *J* = 8.2 Hz, 2H), 6.72 (d, *J* = 15.5 Hz, 1H), 6.53 (ddd, *J* = 15.5, 8.5, 5.2 Hz, 1H), 4.84 (s, 1H), 4.62 (dd, *J* = 14.0, 6.8 Hz, 1H), 4.31–4.24 (m, 1H), 4.10 (dd, *J* = 9.0, 3.2 Hz, 1H), 3.88 (dd, *J* = 9.5, 4.6 Hz, 1H), 3.78–3.72 (m, 2H), 3.55–3.45 (m, 2H), 3.02–2.93 (m, 1H), 2.95 (dd, *J* = 18.7, 4.6 Hz, 1H), 2.90–2.80 (m, 1H), 2.83 (dd, *J* = 13.8, 9.3 Hz, 1H), 2.79–2.76 (m, 2H), 2.37 (d, *J* = 16.2 Hz, 1H), 2.32 (d, *J* = 16.2 Hz, 1H), 2.23 (dd, *J* = 6.2, 6.2 Hz, 1H), 2.16–2.07 (m, 1H), 2.02–1.93 (m, 1H), 1.92–1.85 (m, 1H), 1.79–1.75 (m, 2H), 1.73–1.67 (m, 1H), 1.53–1.46 (m, 3H), 1.43–1.28 (m, 4H), 1.33 (d, *J* = 6.87 Hz, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz): δ 172.6, 171.8, 171.2, 168.8, 168.3, 157.2, 144.8, 138.5, 137.2, 134.9, 131.8, 131.7, 129.6, 129.2, 128.2, 128.1, 126.9, 126.1, 124.0, 122.4, 115.0, 77.9, 69.6,

68.6, 65.5, 60.7, 56.0, 50.3, 49.3, 48.4, 46.8, 42.2, 41.3, 40.4, 37.9, 37.2, 36.4, 35.8, 31.2, 30.8, 29.1, 27.2, 24.2, 23.8, 18.8, 15.6; HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calc'd for $C_{100}H_{118}N_{12}O_{12}Na$ 1702.8918; found 1702.8978.

Dimer Product 6: Synthesized according to Procedure G using 10 mg of **49b**. Purified by preparative HPLC – see SI for conditions. White solid. Bis-TFA salt: 6.8 mg [3.6 μmol, 60% yield]. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.81 (br s, 1H), 8.76 (br s, 1H), 8.65 (d, *J* = 7.7 Hz, 1H), 7.91 (d, *J* = 6.6 Hz, 1H), 7.88 (dd, *J* = 4.1, 4.1 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.37–7.34 (m, 1H), 7.36 (s, 1H), 7.29–7.22 (m, 5H), 7.20 (s, 1H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.87 (d, *J* = 8.3 Hz, 2H), 6.40–6.35 (m, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 4.54 (s, 1H), 4.33 (dd, *J* = 8.5, 8.5 Hz, 1H), 4.22 (dd, *J* = 8.1, 2.3 Hz, 1H), 4.09 (d, *J* = 7.9 Hz, 1H), 3.96 (dd, *J* = 12.7 Hz, 1H), 3.79–3.75 (m, 1H), 3.64–3.60 (m, 1H), 3.15–3.11 (m, 1H), 2.96–2.89 (m, 2H), 2.83–2.76 (m, 2H), 2.37 (br s, 2H), 2.24 (dd, *J* = 6.5, 6.5 Hz, 2H), 2.19–2.12 (m, 1H), 2.08–2.03 (m, 1H), 1.91–1.86 (m, 1H), 1.80–1.74 (m, 1H), 1.65–1.47 (m, 5 H) 1.41–1.30 (m, 4H), 1.30 (d, *J* = 7.0 Hz, 3H); HRMS (ESI-TOF) *m/z*: $[M+Na]^+$ calc'd for $C_{100}H_{118}N_{12}O_{12}Na$ 1702.8918; found 1702.8912.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website: *Fluorescence polarization assay, Caco-2 permeability screenings, computational procedures, & spectroscopic data (PDF).*

AUTHOR INFORMATION

Corresponding Author

* harran@chem.ucla.edu

Present Addresses

§ Koch Institute for Integrative Cancer Research at MIT, 500 Main Street, Cambridge, MA 02139

∫ Center for Catalytic Hydrocarbon Functionalization, Institute for Basic Science (IBS), Daejeon, 34141, Republic of Korea.

◊ Modeling and Informatics, Merck & Co., Inc., P. O. Box 2000, Rahway, NJ 07065

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Funding was provided by the NIH (CA184772 to P.G.H.), a grant from the University of California Cancer Research Coordinating Committee, the D. J. & J. M. Cram Endowment, the Human Frontier Science Program (LT001006/2015-C to F.M.), NSF Equipment Grant CHE-1048804, and NIH instrumentation grant 1S10OD016387-01. We thank Dr. Tristin Rose for helpful discussions and guidance. Dr. Noelle Williams (UT Southwestern Medical Center) is thanked for Caco2 permeability screens and expert advice. We also thank Professor Neil Garg and Lucas Morrill (UCLA) for use of their supercritical fluid chromatography instrument.

REFERENCES

(1) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discov.* **2008**, *7* (7), 608–624.

(2) Marsault, E.; Peterson, M. L. *J. Med. Chem.* **2011**, *54* (7), 1961–2004.

(3) Ivanov, A. A.; Khuri, F. R.; Fu, H. *Trends Pharmacol. Sci.* **2013**, *34* (7), 393–400.

(4) Gavenonis, J.; Sheneman, B. A.; Siegert, T. R.; Eshelman, M. R.; Kritzer, J. A. *Nat. Chem. Biol.* **2014**, *10* (9), 716–722.

(5) Tsomaia, N. *Eur. J. Med. Chem.* **2015**, *94*, 459–470.

(6) Wells, J. A.; McClendon, C. L. *Nature* **2007**, *450* (7172), 1001–1009.

(7) Watkins, A. M.; Arora, P. S. *Eur. J. Med. Chem.* **2015**, *94*, 480–488.

(8) Neduva, V.; Linding, R.; Su-Angrand, I.; Stark, A.; De Masi, F.; Gibson, T. J.; Lewis, J.; Serrano, L.; Russell, R. B. *PLoS Biol.* **2005**, *3* (12), 1–10.

(9) Van Roey, K.; Uyar, B.; Weatheritt, R. J.; Dinkel, H.; Seiler, M.; Budd, A.; Gibson, T. J.; Davey, N. E. *Chem. Rev.* **2014**, *114* (13), 6733–6778.

(10) Nevola, L.; Giralt, E. *Chem. Commun.* **2015**, *51* (16), 3302–3315.

(11) Bock, J. E.; Gavenonis, J.; Kritzer, J. A. *ACS Chem. Biol.* **2013**, *8* (3), 488–499.

(12) Yudin, A. K. *Chem. Sci.* **2015**, *6* (1), 30–49.

(13) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118* (40), 9606–9614.

(14) Blackwell, H. E.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1998**, *37* (23), 3281–3284.

(15) Isidro-Llobet, A.; Murillo, T.; Bello, P.; Cilibrizzi, A.; Hodgkinson, J. T.; Galloway, W. R.; Bender, A.; Welch, M.; Spring, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (17), 6793–6798.

(16) Beckmann, H. S. G.; Nie, F.; Hagerman, C. E.; Johansson, H.; Tan, Y. S.; Wilcke, D.; Spring, D. R. *Nat. Chem.* **2013**, *5* (October), 861–867.

(17) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *113* (24), 9391–9392.

(18) Giebel, L. B.; Cass, R. T.; Milligan, D. L.; Young, D. C.; Arze, R.; Johnson, C. R. *Biochemistry* **1995**, *34* (47), 15430–15435.

(19) Hill, R.; Rai, V.; Yudin, A. K. *J. Am. Chem. Soc.* **2010**, *132* (9), 2889–2891.

(20) Zaretsky, S.; Scully, C. C. G.; Lough, A. J.; Yudin, A. K. *Chem. Eur. J.* **2013**, *19* (52), 17668–17672.

(21) Frost, J. R.; Scully, C. C. G.; Yudin, A. K. *Nat. Chem.* **2016**, *8*, 1105–1111.

(22) Hopkins, B. A.; Smith, G. F.; Sciammetta, N. *Org. Lett.* **2016**, *18* (16), 4072–4075.

(23) Le, D. N.; Riedel, J.; Kozlyuk, N.; Martin, R. W.; Dong, V. M. *Org. Lett.* **2017**, *19* (1), 114–117.

(24) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305* (5690), 1601–1605.

(25) Li, X.; Gartner, Z. J.; Tse, B. N.; Liu, D. R. *J. Am. Chem. Soc.* **2004**, *126* (16), 5090–5092.

(26) Tse, B. N.; Snyder, T. M.; Shen, Y.; Liu, D. R. *J. Am. Chem. Soc.* **2008**, *130* (46), 15611–15626.

(27) White, T. R.; Renzelman, C. M.; Rand, A. C.; Rezai, T.; McEwen, C. M.; Gelev, V. M.; Turner, R. A.; Linington, R. G.; Leung, S. S. F.; Kalgutkar, A. S.; Bauman, J. N.; Zhang, Y.; Liras, S.; Price, D. A.; Mathiowetz, A. M.; Jacobson, M. P.; Lokey, R. S. *Nat. Chem. Biol.* **2011**, *7* (11), 810–817.

(28) Rand, A. C.; Leung, S. S. F.; Eng, H.; Rotter, C. J.; Sharma, R.; Kalgutkar, A. S.; Zhang, Y.; Varma, M. V.; Farley, K. A.; Khunte, B.; Limberakis, C.; Price, D. A.; Liras, S.; Mathiowetz, A. M.; Jacobson, M. P.; Lokey, R. S. *Medchemcomm* **2012**, *3* (10), 1282.

(29) Bockus, A. T.; Lexa, K. W.; Pye, C. R.; Kalgutkar, A. S.; Gardner, J. W.; Hund, K. C. R.; Hewitt, W. M.; Schwochert, J. A.; Glassey, E.; Price, D. A.; Mathiowetz, A. M.; Liras, S.; Jacobson, M. P.; Lokey, R. S. *J. Med. Chem.* **2015**, *58* (11), 4581–4589.

(30) Maianti, J. P.; McFedries, A.; Foda, Z. H.; Kleiner, R. E.; Du, X. Q.; Leissring, M. A.; Tang, W.-J.; Charron, M. J.; Seeliger, M. A.; Saghatelian, A.; Liu, D. R. *Nature* **2014**, *511* (7507), 94–98.

(31) Wei, Q.; Harran, S.; Harran, P. G. *Tetrahedron* **2003**, *59* (45), 8947–8954.

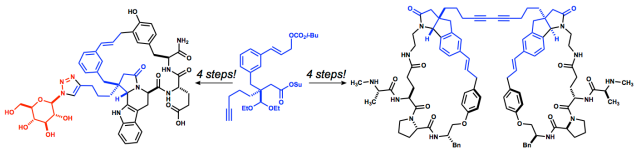
(32) Zhao, H.; Negash, L.; Wei, Q.; LaCour, T. G.; Estill, S. J.; Capota, E.; Pieper, A. A.; Harran, P. G. *J. Am. Chem. Soc.* **2008**, *130* (42), 13864–13866.

(33) Lawson, K. V.; Rose, T. E.; Harran, P. G. *Tetrahedron* **2013**, *69* (36), 7683–7691.

(34) Lawson, K. V; Rose, T. E.; Harran, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110* (40), E3753–E3760.

(35) Rose, T. E.; Lawson, K. V.; Harran, P. G. *Chem. Sci.* **2015**, *6* (4), 2219–2223.

(36) Rose, T. E.; Curtin, B. H.; Lawson, K. V.; Simon, A.; Houk, K. N.; Harran, P. G. *Chem. Sci.* **2016**, *7* (7), 4158–4166.
(37) Chi, Y.; Guo, L.; Kopf, N. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2008**, *130* (17), 5608–5609.
(38) Temme, O.; Taj, S.-A.; Andersson, P. G. *J. Org. Chem.* **1998**, *63* (17), 6007–6015.
(39) Delaye, P. O.; Didier, D.; Marek, I. *Angew. Chem. Int. Ed.* **2013**, *52* (20), 5333–5337.
(40) Qian, M.; Negishi, E. I. *Tetrahedron Lett.* **2005**, *46* (16), 2927–2930.
(41) Lou, Y.; Horikawa, M.; Kloster, R. A.; Hawryluk, N. A.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126* (29), 8916–8918.
(42) Zhang, D.; Ready, J. M. *Org. Lett.* **2005**, *7* (25), 5681–5683.
(43) Nielsen, T. E.; Meldal, M. *J. Org. Chem.* **2004**, *69* (11), 3765–3773.
(44) Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2005**, *7* (4), 599–610.
(45) Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. *Cell* **2000**, *102* (1), 33–42.
(46) Chai, J.; Du, C.; Wu, J. W.; Kyin, S.; Wang, X.; Shi, Y. *Nature* **2000**, *406* (6798), 855–862.
(47) Li, L.; Thomas, R. M.; Suzuki, H.; De Brabander, J. K.; Wang, X.; Harran, P. G. *Science* **2004**, *305*, 1471–1474.
(48) Bai, L.; Smith, D. C.; Wang, S. *Pharmacol. Ther.* **2014**, *144* (1), 82–95.
(49) Fulda, S. *Clin. Cancer Res.* **2015**, *21* (22), 5030–5036.
(50) Wu, G.; Chai, J.; Suber, T. L.; Wu, J.-W.; Du, C.; Wang, X.; Shi, Y. *Nature* **2000**, *408* (6815), 1008–1012.
(51) Zobel, K.; Wang, L.; Varfolomeev, E.; Franklin, M. C.; Elliott, L. O.; Wallweber, H. J. A.; Okawa, D. C.; Flygare, J. A.; Vucic, D.; Fairbrother, W. J.; Deshayes, K. *ACS Chem. Biol.* **2006**, *1* (8), 525–533.
(52) Sweeney, M. C.; Wang, X.; Park, J.; Liu, Y.; Pei, D. *Biochemistry* **2006**, *45* (49), 14740–14748.
(53) Ndubaku, C.; Varfolomeev, E.; Wang, L.; Zobel, K.; Lau, K.; Elliott, L. O.; Maurer, B.; Fedorova, A. V.; Dynek, J. N.; Koehler, M.; Hymowitz, S. G.; Tsui, V.; Deshayes, K.; Fairbrother, W. J.; Flygare, J. A.; Vucic, D. *ACS Chem. Biol.* **2009**, *4* (7), 557–566.
(54) Sun, H.; Lu, J.; Liu, L.; Yang, C. Y.; Wang, S. *ACS Chem. Biol.* **2014**, *9* (4), 994–1002.
(55) Allensworth, J. L.; Sauer, S. J.; Lyerly, H. K.; Morse, M. A.; Devi, G. R. *Breast Cancer Res. Treat.* **2013**, *137* (2), 359–371.
(56) Samuel, T.; Welsh, K.; Lober, T.; Togo, S. H.; Zapata, J. M.; Reed, J. C. *J. Biol. Chem.* **2006**, *281* (2), 1080–1090.
(57) Hruby, V. J.; Cai, M. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53* (1), 557–580.
(58) Davison, E. C.; Forbes, T.; Holmes, A. B.; Warner, J. A.; Road, L.; Cb, C. *Tetrahedron* **1996**, *52* (35), 11601–11624.
(59) Sharpless, K. B.; Verhoeven, T. R. *Aldrichimica Acta* **1979**, *12* (4), 63–74.
(60) Spicer, C. D.; Davis, B. G. *Chem. Commun.* **2013**, *49*, 2747–2749.
(61) Porter, J.; Dykert, J.; Rivier, J. *Int. J. Pept. Protein Res.* **1987**, *30* (1), 13–21.
(62) Lin, H.-S.; Paquette, L. a. *Synth. Commun.* **1994**, *24* (17), 2503–2506.


4 steps!
4 steps!