Synthesis of Natural and Modified Trapoxins, Useful Reagents for Exploring Histone Deacetylase Function

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Abstract: Trapoxin, a cyclotetrapeptide isolated from the fungus *Helicoma ambiens*, profoundly affects mammalian cell growth and morphology. In this paper, we describe syntheses of trapoxin, [^{3}H]trapoxin, and K-trap, a trapoxin-based affinity reagent. These reagents have allowed us to achieve the first molecular characterization of histone deacetylase, trapoxin's cellular target.

Cancer results from a sequence of genetic changes that subvert the normal mechanisms of control over cell growth and morphology. The tools of molecular genetics have proven invaluable in the identification of genes whose mutation or loss results in an increased frequency of cellular transformation and cancer.[1] However, the biochemical basis of cell cycle and morphological deregulation has not been fully illuminated.

During the past ten years, several natural products have been isolated on the basis of their ability to revert the abnormal morphology of oncogenically transformed cell lines back to the "flat" morphology of the nontransformed, progenitor cell lines. This structurally diverse group of natural products includes FR901228,[2] depudecin,[3] radicicol,[4] and trapoxin B (**1**).[5] Trapoxin belongs to a small family of hydrophobic cyclotetrapeptides, all of which share three structural features: (1) a proline or pipecolinic acid residue; (2) at least one amino acid whose α-stereocenter is of the (*R*) configuration; and (3) a nonproteinogenic amino acid, (2*S*,9*S*)-2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe). Other members of this family include chlamydocin (**2**),[6] HC-toxin (**3**),[7] WF-3161 (**4**),[8] and Cyl-2 (**5**),[9] each projecting a variable array of hydrophobic side chains from a 12 atom cyclic backbone (Figure 1). Several groups have shown that reduction or hydrolysis of the epoxide results in a complete loss of biological activity, suggesting that trapoxin may irreversibly inactivate its receptor through covalent bond formation.[5,6,10] This notion has been challenged, however, with the recent isolation of a fungal metabolite that is structurally identical to chlamydocin, except that the (9*S*)-9,10-epoxide has been replaced by a (9*R*)-hydroxy group.[11] Although the activity of this compound toward mammalian cells has not been reported, it is active at 0.3 μM in a plant growth assay and thus raises interesting questions concerning the requirement of the epoxyketone element for biological activity.

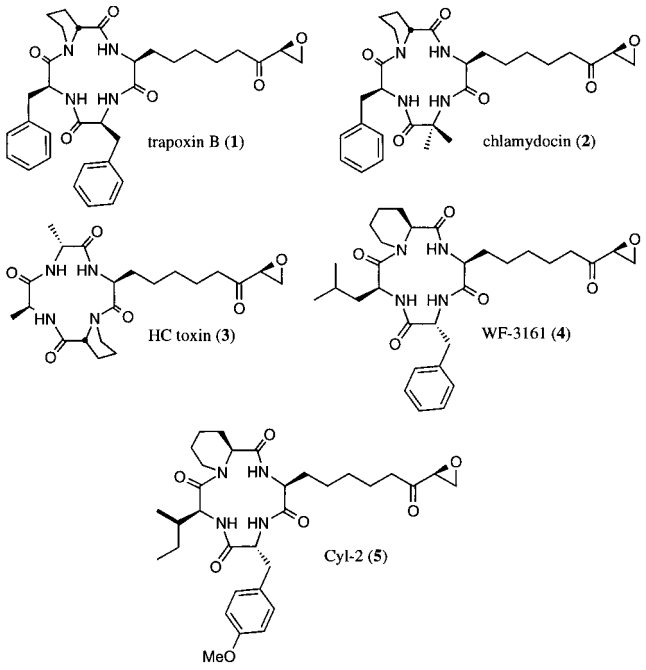

Figure 1. The Aoe cyclotetrapeptide family of natural products.

Subsequent to the publication of trapoxin's structure and detransformation activity, Yoshida and co-workers demonstrated that trapoxin potently and irreversibly inhibits a relatively uncharacterized enzymatic activity, histone deacetylase, which is present in crude cell lysates.[12] In addition, they showed that trapoxin blocks histone deacetylation in vivo at the same concentrations required for detransformation activity and in vitro histone deacetylase inhibition (<10 nM). These important observations placed trapoxin within another class of natural products, the histone deacetylase inhibitors,[13] which prior to the discovery of trapoxin, had only two members, butyric acid (**6**) and trichostatin A (**7**) (Figure 2). The cellular effects of trapoxin, trichostatin, and butyrate are strikingly congruent. These include, depending on the cell type, detransformation, cell cycle arrest, and differentiation, but whereas butyrate is only

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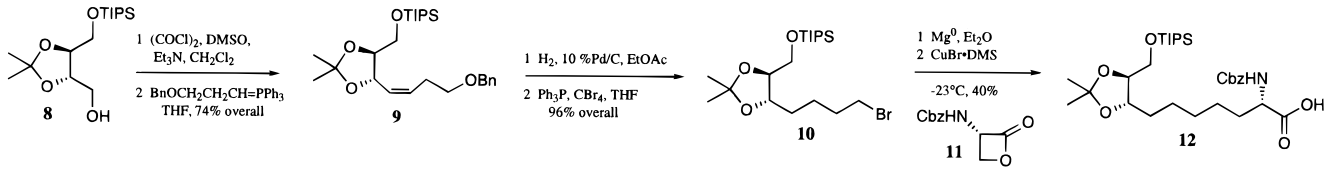
Scheme 1



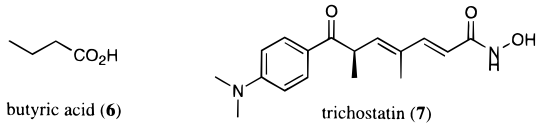


Figure 2. Butyric acid (**6**) and trichostatin (**7**), natural products that inhibit histone deacetylase and promote morphological reversion of transformed cells.

active at millimolar concentrations, both trichostatin and trapoxin function in the nanomolar concentration range. The fact that three disparate histone deacetylase inhibitors display parallel biological activities provides evidence that inactivation of this enzyme underlies most if not all of their cellular effects.

Although the reversible acetylation of specific lysine residues near the N-terminus of the core histones has been studied for over 30 years, more is known about the cellular consequences of these post-translational modifications than about the enzymes that catalyze the reactions.[14] As the nucleosome, composed of DNA wrapped around a histone octamer, is the fundamental structural unit of the eukaryotic genome, changes in histone acetylation are expected to play a role in the regulation of gene expression. Much correlative evidence exists to support this idea, but a molecular explanation of how histone acetylation affects transcription and particularly how patterns of acetylation are localized to discrete genomic domains, is lacking.

In order to explore the connections between histone acetylation, cell morphology, and cell growth, we have sought to identify and characterize trapoxin's molecular receptor. Our approach to this problem has been a chemical one, and therefore has relied upon a variety of synthetic trapoxin-based reagents. In this paper, we report the total syntheses of trapoxin B, [^{3}H]-trapoxin B, and an affinity reagent that we call K-trap, in which one of trapoxin's phenylalanine residues is replaced by a protected lysine residue. We have used the synthetic reagents described herein to purify trapoxin's biological target to homogeneity. The peptide sequence derived from the purified protein has allowed us to clone the human gene encoding the elusive histone deacetylase (HD1).[15]

Results and Discussion

Total Synthesis of Trapoxin B and [^{3}H]Trapoxin B. Numerous syntheses of Aoe-containing cyclotetrapeptides have appeared,[16−19] including an excellent synthesis of chlamydocin by Baldwin and co-workers.[20] In each of these syntheses, the correct choice of a linear tetrapeptide precursor proved crucial to the success of the cyclization. Our overall strategy was influenced by the chlamydocin (**2**) synthesis published by Schmidt and co-workers.[16] Thus, we planned a cyclization in which bond formation occurs between an N-terminal Aoe surrogate and C-terminal (*R*)-proline. It was in our approach to the Aoe residue that we diverged from previous investigators, as we needed an Aoe surrogate that would allow facile incorporation of a radioisotope.

Following the method of McDougal and co-workers,[21] we were able to quantitatively monosilylate the C_2 symmetric (+)-2,3-(*O*)-isopropylidene-L-threitol. The exposed primary alcohol of **8** was then oxidized under Swern conditions and homologated with the triphenylphosphorane prepared from benzyl 3-bromopropyl ether (Scheme 1). This reaction sequence provided olefin **9** in 74% yield. Simultaneous olefin hydrogenation and reductive debenzylation, followed by conversion of the alcohol to the bromide, furnished **10** in 96% overall yield. The Grignard reagent derived from **10** was transmetalated with CuBr/dimethyl sulfide complex and alkylated with Cbz serine β-lactone **11** under conditions similar to those developed by Vederas and co-workers.[22] This reaction afforded, albeit in modest yield, the fully functionalized Aoe surrogate **12**. Elaboration of the sensitive epoxyketone functionality was postponed until after the cyclization, at which time a radioisotope could be conveniently introduced.

The tripeptide **13** was coupled to acid **12** under the influence of EDC/HOBT (Scheme 2). Sequential deprotection of both termini proceeded to give the amino acid **14** in 76% yield for the three steps. After an exhaustive search, we found conditions that successfully promoted closure of the 12-membered ring tetrapeptide. Cyclization occurred in dilute DMF solution (0.2 mM) in the presence of DMAP (8 equiv) and BOP (6 equiv) and provided the pure cyclotetrapeptide alcohol **15** in 51% overall yield, following removal of the TIPS protecting group with TBAF. Use of DMF was essential, as all other solvents and solvent mixtures failed to effect this difficult cyclization. Our attempts to emulate Schmidt and co-workers' pentafluorophenyl ester method[16] were completely unsuccessful. In their total synthesis of chlamydocin, this method was used in a similar cyclization−the major, and apparently critical difference being the presence of an aminoisobutyric acid (Aib) residue adjacent to the Aoe residue in chlamydocin (**2**), as contrasted with the phenylalanine found at this position in trapoxin.

Conversion of **15** to trapoxin (**1**) and [^{3}H]trapoxin (**1***) was accomplished by the reaction sequence shown in Scheme 3. After formation of the primary tosylate (78% yield), the acetonide was removed with aqueous HCl. Epoxide formation with DBU/MeOH occurred rapidly at 0 °C to give **16** (62% yield, two steps). Based on the efficacy of the Moffatt oxidation in the final step of Schmidt's chlamydocin synthesis,[16] we treated epoxy alcohol **16** with the reagent combination DMSO, DCC, and catalytic dichloroacetic acid. These exceptionally mild conditions furnished totally synthetic trapoxin B (**1**) in 80% yield, its spectroscopic characteristics (^{1}H and ^{13}C NMR, $[\alpha]^{20}_D$,

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(15) Taunton, J.; Hassig, C. A.; Schreiber, S. L. *Science* **1996**, *272*, 408−411.
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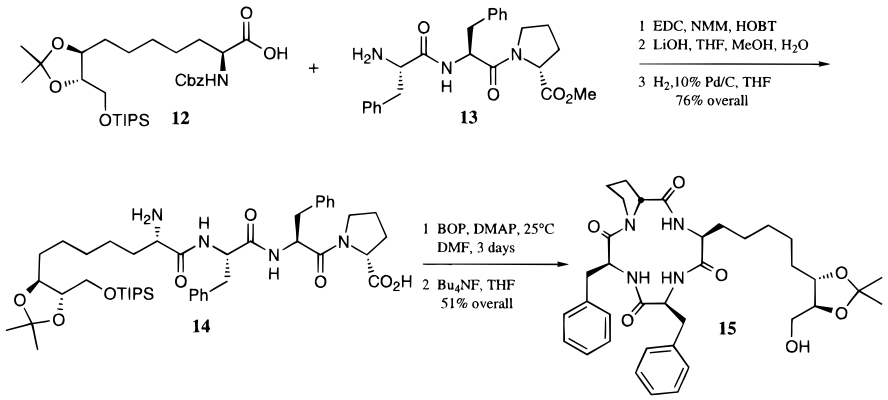
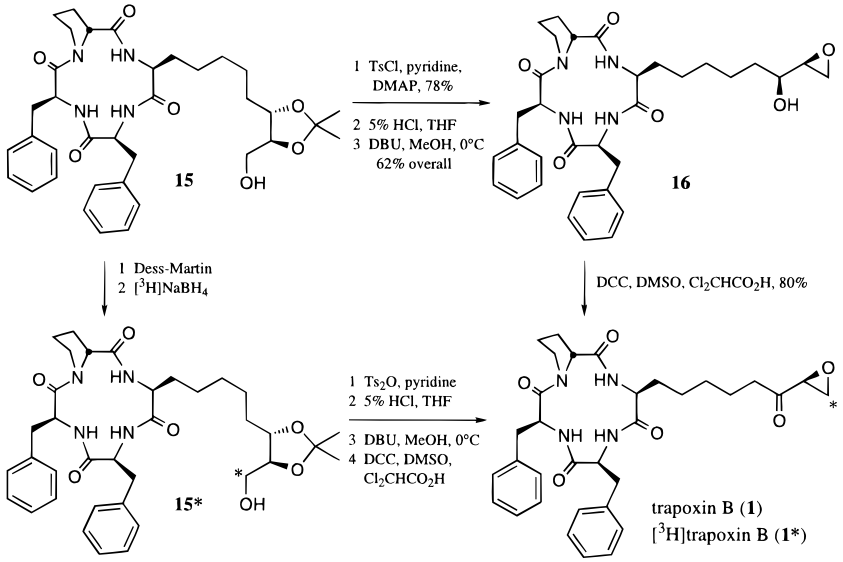
Scheme 2



Scheme 3



HRMS) and biological activities indistinguishable from those of the naturally derived substance.

Given that our primary motivation for pursuing the total synthesis of trapoxin lay in determining the molecular basis for its detransformation activity, it was essential that our route allow for radioisotope incorporation at a late stage. A major advantage of our synthesis as compared to previous syntheses of related Aoe-containing natural products rests in the intermediacy of acetonide alcohol **15**. The tritiated variant **15**** was obtained by oxidizing **15** with the Dess−Martin periodinane[23] and reducing the aldehyde with [^{3}H]$NaBH_4$ (Scheme 3). Compound **15**** was converted to [^{3}H]trapoxin (**1****) by using a reaction sequence similar to that used in the synthesis of trapoxin, which served as an excellent model system for the preparation of the tritiated compound. The identity of [^{3}H]trapoxin was established by the following criteria. (1) Starting with the $NaBH_4$ reduction, we performed a mock synthesis of [^{3}H]trapoxin using the same reaction conditions and concentrations as in the radioactive synthesis. The material so obtained was spectroscopically identical to naturally derived trapoxin B. (2) The TLC mobility of synthetic [^{3}H]trapoxin (>90% pure by TLC) was identical to that of the natural material. (3) [^{3}H]trapoxin was equipotent to natural and synthetic trapoxin in its ability to morphologically detransform *v*-sis NIH3T3 fibroblasts.[5]

Initial Characterization of a Trapoxin Binding Activity. We used [^{3}H]trapoxin to test for a binding activity in crude nuclear extracts prepared from bovine thymus.[24] Extracts were treated with 20 nM [^{3}H]trapoxin in the presence or absence of 400 nM unlabeled trapoxin for 30 min at 4 °C. A slurry of activated charcoal was then added to adsorb unbound trapoxin, leaving the specifically bound tracer in the supernatant.[25] Figure 3 shows the results of these experiments in which the existence of a trapoxin binding activity is clearly demonstrated. Simultaneous treatment of the extracts with 400 nM unlabeled trapoxin reduced the amount of [^{3}H]trapoxin recovered in the bound fraction to background levels and thus confirmed that the interaction detected by the assay is both specific and saturable. In each of these experiments, the residual tracer left in the supernatant most likely derives from nonspecific binding between [^{3}H]trapoxin and irrelevant, abundant proteins.

Because trichostatin A (**7**) displays a spectrum of biological activities nearly identical to that of trapoxin and is known to inhibit cellular histone deacetylase activity, we asked whether it could also antagonize [^{3}H]trapoxin in our charcoal assay. At a concentration of 1 μM, trichostatin fully competed with 20 nM [^{3}H]trapoxin (Figure 3). The combined data imply that the binding activity we detect is relevant to the cellular effects of both trapoxin and trichostatin and further suggest that the binding activity and the histone deacetylase activity are one and the same.

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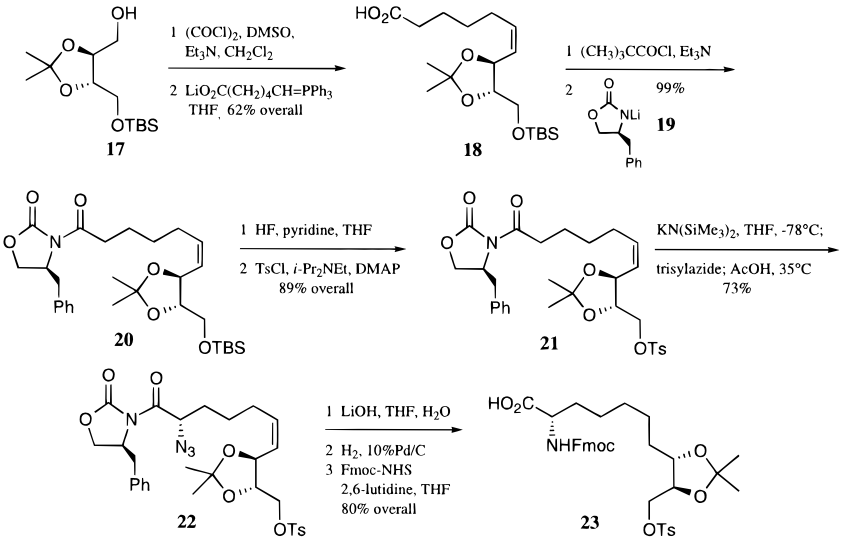
(25) *Receptor-Ligand Interactions: a Practical Approach*; Hulme, E. C., Ed.; Oxford University Press: New York, 1992.

Scheme 4



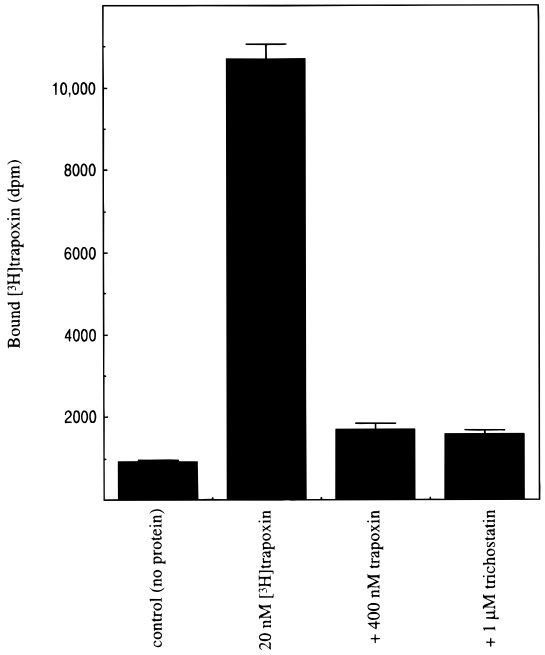


Figure 3. A trapoxin binding activity in nuclear extracts as revealed by a charcoal adsorption assay.

Synthesis of K-Trap and the K-Trap Affinity Matrix. [^{3}H]-trapoxin provided us with a means of detecting a binding entity in a complex mixture of macromolecules. Given that trapoxin's inhibition of histone deacetylase activity is irreversible at low nanomolar concentrations, it seemed plausible that we could specifically label the protein and follow it through several stages of a conventional (and most likely laborious) purification. However, we felt that an affinity-based purification would provide the most expeditious path to revealing the molecular identity of histone deacetylase. Hence we sought a versatile trapoxin-like molecule [cf. K-trap (**27**)] that would lend itself to the attachment of an affinity tag or a solid support.

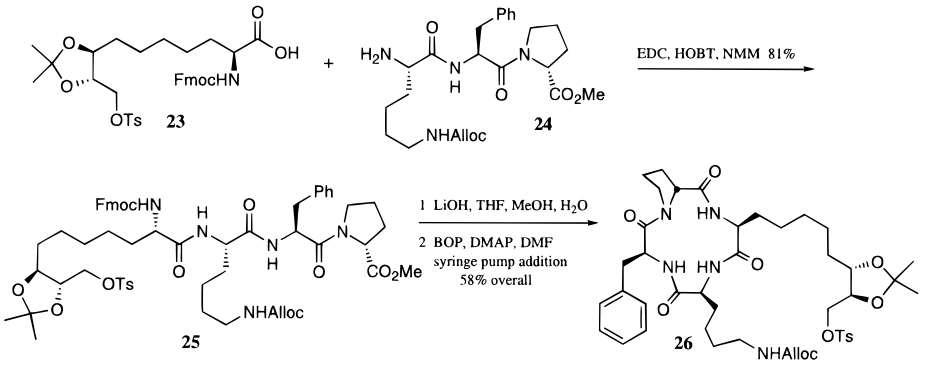
The decision to replace the phenylalanine directly adjacent to the Aoe residue with a protected lysine was based solely on the observation that a nonconservative change at this position, as seen in chlamydocin (**2**), does not lead to a decrease in potency. We found that in the fibroblast detransformation assay, chlamydocin was slightly more active than trapoxin B (our unpublished results). We could therefore be reasonably certain that the benzyl side chain was not required for detransformation activity. Our main synthetic concern involved the choice of a protecting group for the lysine ϵ-amine. As this protecting group would be the last to go, it would have to withstand acidic and basic conditions and be removed with reagents that would leave the epoxyketone function intact. Although the allyloxycarbonyl (Alloc) group fulfilled these criteria, it was incompatible with the Cbz group used to protect the Aoe surrogate **12** in our trapoxin synthesis. For this reason, and because we desired a more efficient synthesis of the Aoe fragment, we devised a second route that also was substantially different from previous syntheses of this amino acid. The route relies upon the Evans chiral auxiliary to set the α-amino stereocenter and makes use of the face selective electrophilic azidation of a chiral potassium enolate, a reaction developed in the Evans' laboratory.[26]

Starting with the same threitol derivative that was used in our trapoxin B synthesis, the mono-TBS ether **17** was prepared in quantitative yield (Scheme 4). After Swern oxidation of the primary alcohol, the Aoe carbon backbone was completed by coupling the unstable aldehyde with the ylide derived from bromohexanoic acid and triphenylphosphine. Attachment of the Evans (*S*)-oxazolidinone **19** to the resultant acid **18** furnished imide **20**, which was then converted into the tosylate **21** (88% overall yield). As expected, the electrophilic azidation of **21** was highly diastereoselective, affording (*S*)-azido imide **22** in 73% isolated yield. Imide hydrolysis followed by simultaneous hydrogenation of the azide and olefin gave the amino acid, which was protected as the Fmoc carbamate **23**. Although this sequence is one step longer than our previous route, it easily allows for the preparation of gram quantities of **23**. An additional improvement over our previous synthesis follows from the early installation of the tosyl group, which reduces by two the number of post-cyclization steps.

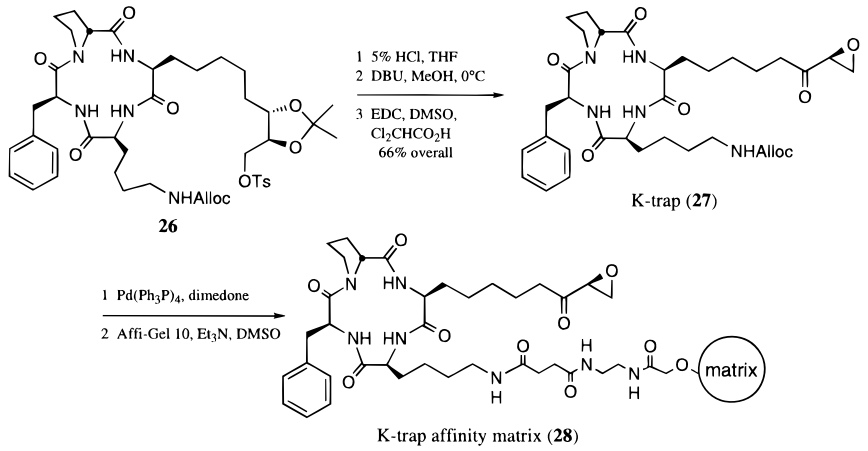
Standard peptide synthesis methods were used to assemble tripeptide **24**, which was then converted to the tetrapeptide **25** (Scheme 5). Concomitant removal of both the methyl ester and the Fmoc protecting groups was followed by BOP/DMAP mediated cyclization. Unexpectedly, the high dilution conditions used in the trapoxin B synthesis (Scheme 2) produced the cyclodimer as the major product. This problem was solved by syringe pump addition of the tetrapeptide to a solution of BOP (5.5 mM) and DMAP (7.4 mM) in dry DMF. After purification by reverse phase HPLC, cyclotetrapeptide **26** was obtained in 58% overall yield. Completion of the K-trap synthesis pro-

(26) Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011−30.

Scheme 5



Scheme 6



ceeded by analogy to the earlier trapoxin route (Scheme 6), except that water soluble EDC was used instead of DCC in the final Moffatt oxidation. Using this sequence of reactions, tens of milligrams of pure K-trap (**27**) were prepared. K-Trap and trapoxin exhibited virtually superimposable ^{1}H and ^{13}C NMR spectral features corresponding to their shared structural elements, and substitution of Alloc-lysine for phenylalanine had little effect on the ability of K-trap to inhibit histone deacetylase activity in vitro. However, K-trap was approximately ten times less potent than trapoxin in an in vivo cell growth assay (inhibition of [^{3}H]thymidine incorporation in MG-63 human osteosarcoma cells; our unpublished results). This loss of potency in vivo may reflect K-trap's decreased cell permeability and/or increased susceptibility to reductive inactivation in vivo.

K-Trap was deblocked with 5,5-dimethyl-1,3-cyclohexanedione (dimedone) and catalytic palladium(0).[27] The crude amine was purified by reverse phase HPLC, lyophilized and coupled immediately to Affi-Gel 10 (Biorad), a cross-linked agarose matrix displaying *N*-hydroxysuccinimide ester functional groups. In order to demonstrate that coupling had occurred without loss of biological activity, we performed the following simple experiment. Nuclear extracts were incubated with either the K-trap matrix (**28**) or an ethanolamine-capped control matrix for 12 h at 4 °C. The matrix beads were removed by centrifugation, and the supernatants were assayed for both [^{3}H]-trapoxin binding and histone deactylase activity.[15] Both activities were completely depleted by treatment with the K-trap matrix and yet were not affected by the control matrix (Figure 4).

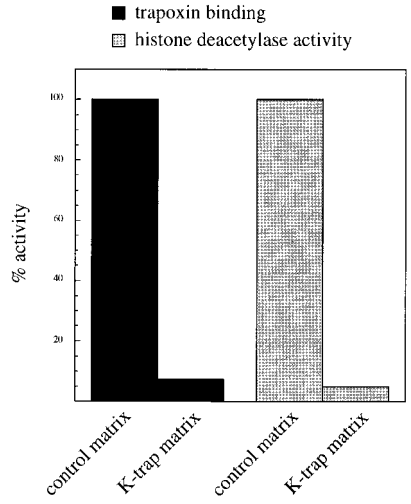


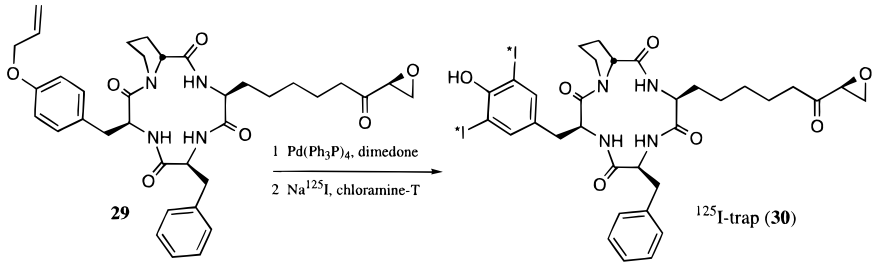
Figure 4. Specific depletion of trapoxin binding and histone deacetylase activities by the K-trap affinity matrix (**28**).

Detours and Dead-Ends: Synthesis of I-Trap. After completing the K-trap synthesis, we had all of the requisite tools for purifying histone deacetylase. Nevertheless, our first attempts to affinity purify the trapoxin receptor were frustrated by high levels of nonspecifically associated proteins. These initial experiments were carried out with a slightly modified form of the K-trap affinity matrix in which a cleavable disulfide linker had been appended to the solid support. A cleavable linker was employed because we had assumed that trapoxin irreversibly alkylated its target protein. However, concerns about an irreversible covalent bond soon evaporated with the synthesis of yet another trapoxin variant, I-trap (**30**) (Scheme 7).

We designed I-trap based on our conviction that a covalently labeled trapoxin target would be detected by SDS-PAGE/autoradiography if we used an isotopically labeled compound

(27) Kunz, H.; Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 436.

Scheme 7



with higher specific activity than [^{3}H]trapoxin. Previous attempts to visualize a [^{3}H]trapoxin covalent adduct had failed, and because autoradiographic detection of ^{125}I is several orders of magnitude more sensitive than tritium, a trapoxin variant bearing two ^{125}I atoms was synthesized. I-Trap (**30**) was synthesized by analogy to K-trap (**27**), except that in this case, we replaced the phenylalanine adjacent to proline in trapoxin (**1**) with tyrosine, protecting the oxidatively sensitive phenol as its allyl ether **29** (Scheme 7). Deprotection of **29** was followed by rapid and quantitative iodination of the phenol with NaI/chloramine-T.[28] I-Trap behaved similarly to K-trap in its ability to cause cell cycle arrest and to inhibit histone deacetylase activity in vitro (our unpublished results).

The reaction conditions used to prepare I-trap were modified with Na^{125}I to give a single compound by TLC that comigrated with the unlabeled compound (specific activity ~5000 Ci/mmol). A crude extract of nuclear proteins was treated with 1 nM ^{125}I-trap in the presence or absence of 1 μM cold trapoxin. Unbound I-trap was removed with charcoal as described above with [^{3}H]trapoxin, and proteins in the supernatant were treated with 2% sodium dodecyl sulfate (SDS) buffer and separated by polyacrylamide gel electrophoresis (PAGE; 12.5% gel). The resulting phosphorimage (Figure 5) shows a labeled band (lane 1) that is efficiently competed by coincubating with unlabeled trapoxin (lane 2). It is unlikely that this band corresponds to a labeled protein, since the labeled species comigrates exactly with free ^{125}I-trap and the dye front (lane 3). This experiment suggested, but did not prove, that trapoxin or a modified form of trapoxin, dissociates from its target protein under denaturing conditions, despite its ability to irreversibly inhibit histone deacetylase activity. Proof that this is indeed the case came from the successful elution of the purified (albeit denatured and therefore catalytically inactive) histone deacetylase from the K-trap affinity matrix (**28**), this time in the absence of a cleavable linker.[15]

We speculate that the active site nucleophile that reacts with trapoxin is also a good leaving group, for example, histidine, aspartate, or glutamate. In this model, nucleophilic attack occurs either α or β to the ketone, leading to a ring-opened intermediate that is stabilized by numerous active site interactions. Upon protein denaturation, these interactions disappear, causing the nucleophile/leaving group to be ejected via β-elimination or epoxide ring closure pathways. The model is reminiscent of enzyme inactivation by the chloromethylketone serine protease inhibitors, in which chloride is displaced by an active site histidine.[29] However, because the chloride leaving group of these inhibitors is lost to the aqueous milieu, no kinetically viable pathway exists for covalent bond reversal. Relevant to this issue is a chloromethylketone variant of chlamydocin (**4**) that was synthesized by Shute and co-workers and found to be nearly as active as chlamydocin in a cell proliferation assay ($IC_{50} \sim 10$ nM).[30] This reagent may enable the unequivocal identification of the active site residue that is alkylated by trapoxin and chlamydocin.

Purification and Cloning of the Trapoxin Target HD1, a Nuclear Histone Deacetylase. Both [^{3}H]trapoxin and the K-trap affinity matrix played important roles in our purification of histone deacetylase.[15] First, a crude nuclear extract from bovine thymus was fractionated by anion exchange chromatography, and the fractions were screened for [^{3}H]trapoxin binding activity. Analysis of the same fractions for histone deacetylase activity showed that the two activities precisely coeluted. These fractions were far from pure, however, and still contained hundreds of contaminating proteins. After affinity chromatography with the K-trap matrix, we obtained the pure (denatured) protein by SDS-PAGE. Peptide microsequencing revealed it to be homologous to a putative yeast protein, Rpd3p. Although Rpd3p exhibits no sequence homology to any protein of known function, the *RPD3* gene was shown to be required for the proper transcriptional regulation of a subset of inducible yeast genes.[31]

The peptide sequence information enabled us to obtain a polymerase chain reaction (PCR) product from a human complementary DNA (cDNA) library and, eventually, a full length cDNA clone encoding the histone deacetylase, which we call HD1. The predicted amino acid sequence is 60% identical to yeast Rpd3p and contains no obvious structural motifs. We have since shown that recombinant HD1 is a histone deacetylase and that its activity is inhibited by trapoxin and trichostatin. Currently, we are investigating its catalytic mechanism and substrate specificity as well its role in transcriptional regulation, cell growth, and cellular transformation.

Histone deacetylase resisted molecular characterization for over 30 years after Allfrey and co-workers first demonstrated its existence in crude nuclear extracts.[32] Through the chemical synthesis of a natural inhibitor and variants based on its structure, we were able to obtain the purified protein in two chromatographic steps. Our approach illustrates the power of synthetic organic chemistry as applied to a challenging cell biology problem.

Experimental Section

General Experimental Methods. All reactions were performed in flame or oven-dried glassware under a positive pressure of nitrogen or argon. Air and moisture sensitive compounds were introduced via syringe or cannula through a rubber septum. Tetrahydrofuran was distilled from potassium/benzophenone ketyl. Diethyl ether was distilled from sodium/benzophenone ketyl. Dichloromethane, toluene, diisopropylethylamine, triethylamine, acetonitrile, and hexamethyl-

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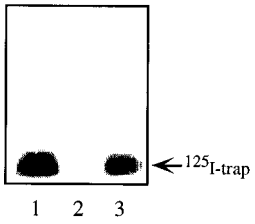

Figure 5. Reversibility of ^{125}I-trap binding under protein denaturing conditions. See text and Experimental Section for details.

disilazane were distilled from calcium hydride. Methanol was distilled from magnesium methoxide. Dimethylformamide was distilled from calcium hydride under reduced pressure and stored over 4-Å molecular sieves. Analytical and preparative thin layer chromatography was performed with E. Merck silica gel 60F glass plates, and flash chromatography made use of E. Merck silica gel 60 (230−400 mesh). Infrared spectra were recorded using a Nicolet 5PC FT-IR spectrometer. ^{1}H NMR spectra were obtained on either a Bruker AM-500 (500 MHz) or AM-400 (400 MHz). Combustion analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA).

(2*S*,3*S*)-4-[(Triisopropylsilyl)oxy]-2,3-(isopropylidenedioxy)butanol (8). To a solution of NaH (60% in oil, 2.56 g, 0.064 mol, 1 equiv) in 125 mL of THF at 0 °C was added a solution of (+)-2,3-(*O*)-isopropylidene-L-threitol (Lancaster, 10.4 g, 0.064 mol, 1 equiv) in 125 mL of THF. After warming to room temperature and stirring for 45 min, triisopropylsilyl chloride (13.7 mL, 0.064 mol, 1 equiv) was added over 15 min. After stirring for 3 h, the reacion was poured into saturated $NaHCO_3$ and extracted with diethyl ether. The organic fraction was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography (0−33% ether/hexanes) gave 20.4 g (100% yield) of silyl ether **8** as a clear oil: R_f 0.40 (4:1 hexanes/EtOAc); $[\alpha]^{20}_D$ +9.5° (*c* 0.017, CH_2Cl_2); IR (thin film) 3500 (br), 2944, 2869, 1464, 1381, 1369, 1248, 1217, 1084, 1071, 997, 884 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (dt, J = 7.8, 4.6 Hz, 1H), 3.96 (dd, J = 9.8, 3.9 Hz, 1H), 3.93−3.86 (m, 1H), 3.83−3.67 (m, 3H), 2.47−2.39 (m, 1H), 1.40 (s, 3H), 1.39 (s, 3H), 1.17−1.02 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.0, 80.4, 78.1, 64.2, 62.80, 27.0, 26.9, 17.8, 11.8; HRMS (FAB, NaI) Calcd for $C_{16}H_{34}O_4Si$ + Na 341.2124, found 341.2119. Anal. Calcd for $C_{16}H_{34}O_4Si$: C, 60.33; H, 10.76. Found: C, 60.20; H, 10.72.

(2*S*,3*S*)-1-[(Triisopropylsilyl)oxy]-7-(benzyloxy)-2,3-(isopropylidenedioxy)-4(*Z*)-heptene (9). A solution of DMSO (11.6 mL, 0.163 mol, 2.6 equiv) in 37 mL of CH_2Cl_2 was added via cannula to a cold (−78 °C) solution of oxalyl chloride (7.1 mL, 81.6 mmol, 1.3 equiv) in 203 mL of CH_2Cl_2. After stirring for 5 min, a solution of alcohol **8** (20 g, 62.8 mmol, 1 equiv) in 80 mL of CH_2Cl_2 was added dropwise. After stirring for 15 min, Et_3N (43.7 mL, 0.315 mol, 5.0 equiv) was added, and the reaction was warmed to room temperature and stirred for 1 h. The reaction mixture was diluted with 100 mL of water, and the organic fraction was separated and concentrated in vacuo. The residue was diluted with 200 mL of 50% hexanes/ether and washed with water (3 × 100 mL) and brine (50 mL). The combined aqueous fractions were extracted with ether (3 × 100 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude aldehyde was placed under high vacuum for 30 min and carried on directly to the next step.

A heterogeneous suspension of the phosphonium bromide derived from triphenylphosphine and benzyl 3-bromopropyl ether (46.3 g, 94.2 mmol, 1.5 equiv based on **8**) in 345 mL of THF was cooled to −78 °C and treated with *n*-BuLi (1.6 M in hexane, 58.9 mL, 94.2 mmol, 1.5 equiv). After stirring for 1 h at −23 °C, the reaction was cooled to −78 °C and treated with a solution of the crude aldehyde in 266 mL of THF. After 30 min at −78 °C and 45 min at 0 °C, the reaction mixture was diluted with hexanes and filtered through silica gel (66% hexanes/ether wash). The filtrate was concentrated in vacuo and purified by flash chromatography (100% hexanes to 100% ether gradient) to give 21 g of **9** (74% yield) as a clear oil: R_f 0.61 (5:1 hexanes/ether); $[\alpha]^{20}_D$ +0.7° (*c* 0.066, CH_2Cl_2); IR (thin film) 2944, 2867, 1464, 1454, 1379, 1368, 1244, 1148, 1103, 1082, 1016, 883, 862 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.24 (m, 5H), 5.75 (dt, J = 10.5, 7.4 Hz, 1H), 5.52 (tt, J = 10.5, 1.4 Hz, 1H), 4.83 (t, J = 8.5 Hz, 1H), 4.51 (s, 2H), 3.89 (dd, J = 11.0, 3.4 Hz, 1H), 3.80−3.69 (m, 2H), 3.51 (t, J = 6.8 Hz, 2H), 2.63−2.37 (m, 2H), 1.44 (s, 3H), 1.42 (s, 3H), 1.20−1.00 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 131.8, 128.3, 127.5, 127.5, 108.8, 81.8, 73.0, 72.8, 69.6, 61.9, 28.4, 27.3, 26.9, 17.9, 11.8; HRMS (FAB, NaI) Calcd for $C_{26}H_{44}O_4Si$ + Na 471.2906, found 471.2912. Anal. Calcd for $C_{26}H_{44}O_4Si$: C, 69.60; H, 9.88. Found: C, 69.50; H, 9.96.

(5*S*,6*S*)-1-Bromo-7-[(triisopropylsilyl)oxy]-5,6-(isopropylidenedioxy)heptane (10). A solution of olefin **9** (21.0 g, 0.047 mol) in 250 mL of EtOAc was treated with 10% Pd/C (8.4 g) and sparged with N_2 for 10 min. Hydrogen gas was then bubbled through the heterogeneous solution for 10 min and stirring was continued for 6 h under a blanket of H_2. The reaction mixture was filtered through Celite and concentrated to give 16.7 g (99% yield) of the saturated alcohol as a clear oil: R_f 0.16 (2:1 hexanes/ether); $[\alpha]^{20}_D$ −8.3° (*c* 0.047, CH_2Cl_2); IR (thin film) 3434 (br), 2944, 2869, 1462, 1379, 1370, 1246, 1217, 1092, 1069, 882 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (dt, J = 7.8, 3.6 Hz, 1H), 3.86 (dd, J = 10.0, 3.9 Hz, 1H), 3.78−3.66 (m, 2H), 3.64 (t, J = 6.3 Hz, 2H), 1.77−1.42 (m, 7H), 1.39 (s, 3H), 1.36 (s, 3H), 1.16−0.98 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 108.3, 80.9, 79.2, 64.2, 62.7, 33.1, 32.65, 27.4, 26.9, 22.3, 17.9, 11.8; HRMS (FAB, NaI) Calcd for $C_{19}H_{40}O_4Si$ + Na 383.2594, found 383.2568. Anal. Calcd for $C_{19}H_{40}O_4Si$: C, 63.28; H, 11.18. Found: C, 63.09; H, 11.22.

To a solution of the alcohol (4.0 g, 11.1 mmol, 1 equiv) and triphenylphosphine (5.8 g, 22.2 mmol, 2 equiv) in 140 mL of THF at room temperature was added CBr_4 (7.4 g, 22.2 mmol, 2 equiv). After stirring for 30 min, the reaction was diluted with hexanes and filtered through silica gel (33% ether/hexanes wash). Purification by flash chromatography (0−20% ether/hexanes) provided 4.6 g (97% yield) of bromide **10** as a clear oil: R_f 0.81 (2:1 hexanes/ether); $[\alpha]^{20}_D$ −7.2° (*c* 0.042, CH_2Cl_2); IR (thin film) 2944, 2867, 1462, 1379, 1370, 1242, 1094, 1069, 1013, 997, 884 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (dt, J = 7.5, 3.2 Hz, 1H), 3.88 (dd, J = 9.9, 3.8 Hz, 1H), 3.78−3.65 (m, 2H), 3.40 (t, J = 6.8 Hz, 2H), 1.95−1.85 (m, 2H), 1.77−1.50 (m, 4H), 1.39 (s, 3H), 1.36 (s, 3H), 1.17−0.98 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 108.4, 80.8, 79.1, 64.2, 33.5, 32.8, 32.6, 27.4, 26.9, 24.8, 17.9, 11.8; HRMS (FAB, NaI) Calcd for $C_{19}H_{39}BrO_3Si$ + H 423.1930, found 423.1926. Anal. Calcd for $C_{19}H_{40}BrO_3Si$: C, 53.89; H, 9.28. Found: C, 53.96; H, 9.26.

(2*S*,8*S*,9*S*)-*N*-(Benzyloxycarbonyl)-2-amino-10-[(triisopropylsilyl)oxy]-8,9-(isopropylidenedioxy)decanoic Acid (12). To magnesium turnings (14 mg, 3.26 mmol, 72 equiv based on **11**) was added 1,2-dibromoethane (1 μL) followed by a solution of bromide **10** (115 mg, 0.272 mmol, 6.0 equiv based on **11**) in 0.210 mL of ether over 5 min. After stirring for 2 h 40 min, the Grignard reagent was added to a cold (−23 °C) solution of β-lactone **11**[22] (10 mg, 0.045 mmol, 1 equiv) and CuBr/dimethyl sulfide complex (1.8 mg, 0.0085 mmol, 0.2 equiv) in 0.4 mL of THF and 20 μL of dimethyl sulfide. The reaction was stirred for 2 h 20 min at −23 °C and diluted with degassed ether (1 mL) followed by 1 N acetic acid (1 mL). After warming to 0 °C and stirring for 15 min, the solution was treated with glacial acetic acid (100 μL) and extracted with ether. The combined organic fractions were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography (5−10% MeOH/$CHCl_3$) gave 10 mg (40% yield) of acid **12** as a clear oil: R_f 0.24 (6% MeOH/CH_2Cl_2); $[\alpha]^{20}_D$ −15.3° (*c* 0.039, CH_2Cl_2); IR (thin film) 3321 (br), 2942, 2867, 1721, 1588, 1532, 1456, 1379, 1368, 1246, 1217, 1094, 1067, 884 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41−7.23 (m, 5H), 7.10 (br s, 1H), 5.01 and 4.98 (AB quartet, J = 12.6 Hz, 2H), 3.91−3.78 (m, 2H), 3.78−3.67 (m, 2H), 3.58 (dt, J = 7.8, 4.4 Hz, 1H), 1.72−1.14 (m, 10H), 1.28 (s, 3H), 1.26 (s, 3H), 1.13−0.95 (m, 21H); ^{13}C NMR (100 MHz, DMSO-d_6) (partial) δ 155.8, 137.2, 128.3, 127.6, 107.6, 80.84, 77.5, 65.2, 63.5, 54.7, 32.8, 31.8, 28.9, 27.3, 26.8, 25.7, 25.3, 17.8, 11.3; HRMS (FAB, NaI) Calcd for $C_{30}H_{51}NO_7Si$ + Na 588.3332, found 588.3340.

L-Phenylalanyl-L-phenylalanyl-D-proline Methyl Ester (13). An ice cold solution of L-phenylalanyl-D-proline methyl ester (721 mg, 1.66 mmol, 1 equiv) in 11.1 mL of CH_2Cl_2 was treated with *N*-methylmorpholine (NMM; 201 μL, 1.83 mmol, 1.1 equiv) followed by a solution of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (440 mg, 1.66 mmol, 1 equiv) in 13.8 mL of CH_2Cl_2. Solid 1-hydroxybenzotriazole (HOBT) (247 mg, 1.83 mmol, 1.1 equiv) was added, and stirring was continued for 15 min, whereupon 1-(3-dimethylaminopropyl)-3-ethyl-

carbodiimide (EDC) (351 mg, 1.83 mmol, 1.1 equiv) was added in one portion. The reaction was stirred at 0 °C for 2 h and at room temperature for 19 h. The reaction mixture was extracted with water, 1 N HCl, saturated $NaHCO_3$, and again with water. The organic fraction was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography (50−67% EtOAc/hexanes) provided 700 mg (81% yield) of the Boc tripeptide, which was quantitatively deblocked by stirring for 1 h in 50% TFA/CH_2Cl_2. Azeotropic removal of TFA with heptane provided the TFA salt of tripeptide **13** as a foam. The tripeptide was characterized as its Boc carbamate: R_f 0.26 (50% EtOAc/hexanes); $[\alpha]^{20}_D$ +33.9° (*c* 0.019, CH_2Cl_2); IR (thin film) 3287 (br), 2978, 1750, 1713, 1691, 1640, 1523, 1497, 1451, 1391, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (major rotamer) δ 7.30−7.10 (m, 10H), 6.80 (d, J = 8.0 Hz, 1H), 4.97 (d, J = 7.4 Hz, 1H), 4.88 (m, 1H), 4.38 (m, 1H), 4.27 (dd, J = 7.8, 4.0 Hz, 1H), 3.65 (s, 3H), 3.44 (m, 1H), 3.15−2.92 (m, 3H), 2.87 (dd, J = 12.8, 9.6 Hz, 1H), 2.60 (m, 1H), 1.95−1.72 (m, 3H), 1.55−1.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 170.4, 169.4, 155.1, 136.5, 136.1, 129.4, 129.4, 128.5, 128.5, 127.0, 126.8, 58.7, 52.3, 52.1, 46.7, 39.6, 28.9, 28.2, 24.3; HRMS (FAB, NaI) Calcd for $C_{29}H_{37}N_3O_6$ + Na 546.2580, found 546.2603.

(2*S*,8*S*,9*S*)-2-Amino-10-[(triisopropylsilyl)oxy]-8,9-(isopropylidenedioxy)-decanoyl-L-phenylalanyl-L-phenylalanyl-D-proline (14). An ice cold solution of tripeptide **13** (125 mg, 0.233 mmol, 1 equiv) in 1.6 mL of CH_2Cl_2 was treated with NMM (28 μL, 0.257 mmol, 1.1 equiv) followed by a solution of acid **12** (132 mg, 0.233 mmol, 1 equiv) in 2 mL of CH_2Cl_2. Solid HOBT (35 mg, 0.257 mmol, 1.1 equiv) was added, and stirring was continued for 15 min, whereupon EDC (49 mg, 0.257 mmol, 1.1 equiv) was added in one portion. After stirring for 1 h at 0 °C and 20 h at room temperature, the reaction was applied directly to a flash chromatography column. Elution with 60−67% EtOAc/hexanes gave 183 mg (81% yield) of the *N*-(benzyloxycarbonyl) tetrapeptide methyl ester as an amorphous solid: R_f 0.61 (2:1 EtOAc/hexanes); $[\alpha]^{20}_D$ +12.2° (*c* 0.024, CH_2Cl_2); IR (thin film) 3285 (br), 2942, 2867, 1748, 1723, 1638, 1536, 1455, 1368, 1217, 1175, 1094, 882 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (major rotamer) δ 7.40−6.98 (m, 15H), 6.85 (br s, 1H), 6.65 (d, J = 7.4 Hz, 1H), 5.24 (d, J = 8.2 Hz, 1H), 5.17−5.02 (m, 2H), 4.97−4.83 (m, 1H), 4.75−4.65 (m, 1H), 4.31 (dd, J = 7.5, 4.1 Hz), 4.17−4.07 (m, 1H), 3.90 (dt, J = 7.8, 3.7 Hz, 1H), 3.84 (dd, J = 10.2, 4.3 Hz, 1H), 3.77−3.61 (m, 3H), 3.69 (s, 3H), 3.53−3.42 (m, 1H), 3.16−2.86 (m, 5H), 2.71−2.60 (m, 1H), 1.97−1.69 (m, 4H), 1.68−1.17 (unresolved, 8H), 1.38 (s, 3H), 1.35 (s, 3H), 1.15−0.97 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$) (major rotamer) δ 172.0, 171.4, 169.6, 169.3, 156.1, 136.2, 136.1, 136.0, 129.4, 128.5, 128.4, 128.1, 128.0, 127.0, 126.9, 108.3, 81.0, 79.0, 67.0, 64.2, 58.7, 55.01, 52.4, 52.2, 46.8, 39.5, 38.2, 33.4, 32.4, 29.4, 28.9, 27.4, 26.9, 26.0, 25.4, 24.3, 17.9, 11.8; HRMS (FAB, NaI) Calcd for $C_{54}H_{78}N_4O_{10}$-Si + Na 993.5385, found 993.5388.

An ice cold solution of the Cbz tetrapeptide methyl ester (182 mg, 0.188 mmol, 1 equiv) in 3.2 mL of THF, 1.1 mL of MeOH, and 1.1 mL of water was treated with 1.0 N LiOH (563 μL, 0.563 mmol, 3.0 equiv). The reaction was stirred at 0 °C for 5 min and at room temperature for 2 h. The reaction mixture was concentrated in vacuo (room temperature), and the residue was dissolved in water and acidified with 1.0 N HCl to pH 3. The product was extracted into EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give an amorphous solid.

To this crude benzyl carbamate in 9.1 mL of THF was added 10% Pd/C (200 mg). The mixture was stirred under an atmosphere of H_2 for a total of 13.5 h. Filtration through Celite (MeOH wash) and concentration under reduced pressure gave 144 mg (94% yield) of deprotected tetrapeptide **14** as a foam.

***cyclo*[(2*S*,8*S*,9*S*)-2-Amino-10-hydroxy-8,9-(isopropylidenedioxy)-decanoyl-L-phenylalanyl-L-phenylalanyl-D-prolyl] (15).** A solution of tetrapeptide **14** (50 mg, 0.061 mmol, 1 equiv) in 300 mL of anhydrous DMF was treated with 4-(dimethylamino)pyridine (DMAP) (60 mg, 0.486 mmol, 8.0 equiv) followed by benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (161 mg, 0.364 mmol, 6.0 equiv). After stirring in the dark for 4 days, the solvent was removed by vacuum distillation (<40 °C), and the residue was dissolved in EtOAc and filtered through silica gel. The filtrate was concentrated, and the residue was rapidly purified by flash chromatography (2:1 EtOAc/hexanes) to give 39 mg of impure cyclotetrapeptide silyl ether, which was used immediately in the next reaction.

To a solution of the silyl ether (39 mg, 0.048 mmol, 1 equiv) in 4.8 mL of THF at room temperature was added TBAF (1.0 M in THF, 97 μL, 0.097 mmol, 2.0 equiv). The reaction was stirred for 1 h and concentrated (<25 °C) in vacuo to ca. 1 mL. Purification by flash chromatography (2:1 EtOAc/hexanes followed by 9:1 EtOAc/MeOH) gave impure product. Further purification by preparative TLC (2 × 0.5 mm plates; 5% MeOH/EtOAc) gave 20 mg (51% yield over two steps) of alcohol **15** as a white foam: R_f 0.56 (10% MeOH/EtOAc); $[\alpha]^{20}_D$ −99.5° (*c* 0.004, CH_2Cl_2); IR (thin film) 3289 (br), 2984, 2934, 2863, 1684, 1667, 1525, 1499, 1455, 1379, 1369, 1242, 1107, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 10.1 Hz, 1H), 7.33−7.00 (m, 10H), 6.71 (br d, J = 5.9 Hz, 1H), 6.57 (br d, J = 5.3 Hz, 1H), 5.15 (dt, J = 9.8, 6.3 Hz, 1H), 4.67 (d, J = 6.4 Hz, 1H), 4.15 (q, J = 8.2 Hz, 1H), 3.91−3.55 (m, 8H), 3.35−3.15 (m, 3H), 3.01 (dd, J = 13.0, 5.6 Hz, 1H), 2.28 (br d, J = 9.9 Hz, 1H), 2.23−2.05 (m, 2H), 1.90−1.15 (unresolved, 11H), 1.41 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 173.8, 172,81, 171.6, 137.0, 136.9, 129.1, 128.9, 128.7, 128.6, 126.9, 126.8, 108.6, 81.4, 76.9, 63.0, 62.1, 57.9, 54.1, 53.6, 46.9, 35.8, 35.1, 32.8, 29.2, 29.0, 27.4, 27.1, 25.8, 25.3, 24.9, 24.8; HRMS (FAB, NaI) Calcd for $C_{36}H_{48}N_4O_7$ + Na 671.3421, found 671.3425.

***cyclo*[(2*S*,8*S*,9*S*)-2-Amino-8-hydroxy-9,10-epoxy-decanoyl-L-phenylalanyl-L-phenylalanyl-D-prolyl] (16).** A solution of alcohol **15** (20 mg, 0.031 mmol, 1 equiv) in 0.62 mL of pyridine was treated with DMAP (ca. 1 mg) followed by tosyl chloride (11.8 mg, 0.062 mmol, 2.0 equiv). After stirring for 4 h, the solvent was removed. The crude product was dissolved in CH_2Cl_2 and applied to two preparative TLC plates (0.5 mm). Elution with 1% MeOH/EtOAc gave 19 mg (78% yield) of the tosylate as an amorphous solid: R_f 0.50 (2% MeOH/EtOAc); $[\alpha]^{20}_D$ −75.3° (*c* 0.003, CH_2Cl_2); IR (thin film) 3287 (br), 2934, 1663, 1524, 1497, 1455, 1368, 1242, 1213, 1190, 1177, 1098, 948 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 10.3 Hz, 1H), 7.35 (d, J = 8.2 Hz, 2H), 7.32−7.07 (m, 10H), 6.50 (d, J = 5.9 Hz, 1H), 5.15 (dt, J = 9.8, 6.6 Hz, 1H), 4.68 (d, J = 6.7 Hz, 1H), 4.16 (q, J = 8.8 Hz, 1H), 4.16−4.03 (m, 2H), 3.90−3.61 (m, 6H), 3.37−3.17 (m, 3H), 3.02 (dd, J = 13.9, 6.3 Hz, 1H), 2.45 (s, 3H), 2.28 (br d, J = 8.6 Hz, 1H), 2.23−2.07 (m, 2H), 1.88−1.15 (unresolved, 10H), 1.36 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 173.8, 172.8, 171.6, 145.0, 137.0, 136.9, 129.9, 129.5, 129.1, 128.9, 128.7, 128.6, 128.1, 126.9, 126.8, 109.4, 78.2, 77.8, 69.2, 63.1, 57.9, 54.1, 53.6, 47.0, 35.8, 35.2, 32.9, 29.2, 29.0, 27.3, 26.7, 25.7, 25.3, 24.9, 24.8, 21.7; HRMS (FAB, NaI) Calcd for $C_{43}H_{54}N_4O_9S$ + Na 825.3509, found 825.3497.

An ice cold solution of the acetonide tosylate (18 mg, 0.023 mmol) in 1 mL of THF was treated with 1 mL of 5% aqueous HCl. Upon warming to room temperature and stirring for 21 h, the THF was removed in vacuo (<25 °C). The aqueous solution was diluted with brine and extracted with CH_2Cl_2. The organic fraction was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by preparative TLC (0.5 mm plate; 2% MeOH/EtOAc) to give 13 mg (73% yield) of the diol tosylate as a clear oil.

To the diol (6.2 mg, 8.1 μmol, 1 equiv) in 1.6 mL of MeOH at 0 °C was added 1,8-diazabicylco[5.4.0]undec-7-ene (DBU) (1.0 M in THF, 41 μL, 41 μmol, 5.1 equiv). After stirring for 2 h, the reaction was filtered through silica gel (10% MeOH/EtOAc wash), and the filtrate was concentrated in vacuo. The crude product was purified by preparative TLC (0.25 mm plate; 10% MeOH/EtOAc) to give 4.1 mg (85% yield) of epoxy alcohol **16**: R_f 0.50 (10% MeOH/EtOAc); $[\alpha]^{20}_D$ −78.9° (*c* 0.002, CH_2Cl_2); IR (thin film) 3283 (br), 3029, 2930, 2859, 1686, 1624, 1527, 1497, 1455, 1250, 1113 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 10.3 Hz, 1H), 7.33−7.05 (m, 10H), 6.47 (d, J = 5.4 Hz, 1H), 5.16 (dt, J = 9.6, 6.5 Hz, 1H), 4.68 (d, J = 5.9 Hz, 1H), 4.16 (q, J = 8.2 Hz, 1H), 3.86 (dt, J = 9.5, 4.6 Hz, 1H), 3.79−3.62 (m, 3H), 3.58 (t, J = 4.6 Hz, 1H), 3.43 (q, J = 5.8 Hz, 1H), 3.35−3.17 (m, 3H), 3.06−2.92 (m, 2H), 2.83 (t, J = 4.6 Hz, 1H), 2.72 (dd, J = 4.6, 2.7 Hz, 1H), 2.35−2.10 (m, 2H), 2.00−1.15 (unresolved, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 173.7, 172.7, 171.54, 137.0, 136.8, 129.0, 128.8, 128.6, 128.5, 126.9, 126.8, 71.4, 63.1, 57.8, 55.3, 54.0, 53.49, 46.9, 45.1, 35.7, 35.0, 34.1, 29.0, 28.9, 25.2, 25.0,

24.9, 24.8; HRMS (FAB, NaI) Calcd for $C_{33}H_{42}N_4O_6$ + Na 613.3002, found 613.2994.

Trapoxin B (1). A solution of epoxy alcohol **16** (2.5 mg, 4.25 μmol, 1 equiv) in 0.4 mL of benzene and 40 μL of DMSO was treated with DCC (9 mg, 42.5 μmol, 10 equiv) followed by dichloroacetic acid (1.0 M benzene, 2.1 μL, 2.1 μmol, 0.5 equiv). After stirring for 2 h, more dichloroacetic acid (1.0 M benzene, 2.1 μL, 2.1 μmol, 0.5 equiv) was added, and stirring was continued for an additional 2 h. The reaction was filtered through silica gel (10% MeOH/EtOAc wash), and the filtrate was concentrated in vacuo. The residue was purified by preparative TLC (0.5 mm plate). Elution with 5% MeOH/EtOAc gave 2 mg (80% yield) of trapoxin B as a white solid: R_f 0.53 (10% MeOH/EtOAc); $[\alpha]^{20}_D$ −76.0° (*c* 0.50, MeOH) [lit.[5] $[\alpha]^{20}_D$ −75.3° (*c* 0.517, MeOH)]; IR (thin film) 3285 (br), 3061, 3028, 2928, 2856, 1684, 1525, 1499, 1455, 1343, 1306, 1262, 1242, 1113, 870, 742, 700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 10.3 Hz, 1H), 7.32−7.18 (m, 8H), 7.13 (d, J = 6.8 Hz, 2H), 7.08 (d, J = 10.3 Hz, 1H), 6.30 (d, J = 5.6 Hz, 1H), 5.16 (dt, J = 9.9, 6.3 Hz, 1H), 4.67 (d, J = 6.2 Hz, 1H), 4.14 (q, J = 7.9 Hz, 1H), 3.86 (dt, J = 9.2, 4.7 Hz, 1H), 3.78−3.64 (m, 2H), 3.42 (dd, J = 4.7, 2.4 Hz, 1H), 3.34−3.18 (m, 3H), 3.06−2.97 (m, 2H), 2.86 (dd, J = 5.9, 2.4 Hz, 1H), 2.41 (dt, J = 17.5, 6.9 Hz, 1H), 2.35−2.13 (m, 3H), 1.87−1.68 (m, 3H), 1.66−1.15 (unresolved, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.5, 175.1, 173.7, 172.7, 171.5, 137.0, 136.9, 129.1, 128.9, 128.6, 128.5, 126.9, 126.8, 63.2, 57.9, 53.9, 53.5, 53.4, 46.9, 46.0, 36.2, 35.8, 35.1, 28.8, 28.6, 25.2, 24.9, 24.8, 22.8; HRMS (FAB) Calcd for $C_{33}H_{40}N_4O_6$ + H 589.3026, found 589.3020.

[^{3}H]Trapoxin B (1*). A solution of alcohol **15** (2.3 mg, 3.6 μmol, 1 equiv) in 0.4 mL of CH_2Cl_2 was treated with the Dess−Martin reagent[23] (6 mg, 14.2 μmol, 3.9 equiv). After stirring for 1 h, the reaction was filtered through silica gel (10% MeOH/EtOAc wash) and concentrated. The residue was dissolved in CH_2Cl_2, and the filtration was repeated to give the crude aldehyde.

An ice cold solution of the aldehyde in 0.5 mL of THF was treated with [^{3}H]$NaBH_4$ (NEN; 500 μL of a 25 mCi/mL aqueous solution, 0.92 μmol, 13.6 Ci/mmol) and stirred for 1 h at 0 °C. The reaction mixture was extracted with EtOAc (4×), and the combined organic fractions were filtered through silica gel (10% MeOH/EtOAc wash). Purification on a small pipet column (silica gel; 0−10% MeOH/EtOAc) provided the [^{3}H]alcohol **15***, which was used immediately in the next reaction.

A solution of the **15*** in 200 μL of pyridine containing DMAP (ca. 1 mg) was treated with *p*-toluenesulfonic anhydride (1.0 M in CH_2Cl_2, 71 μL, 71 μmol). After stirring for 2 h, the reaction was filtered through silica gel (10% MeOH/EtOAc wash). The crude tosylate was purified exactly as in the previous step and used immediately in the next reaction.

The [^{3}H]acetonide tosylate was dissolved in 500 μL of THF, cooled to 0 °C, and treated with 500 μL of 5% aqueous HCl. After stirring at room temperature for 13 h, the reaction was cooled to 0 °C and neutralized with solid $NaHCO_3$. The product was extracted with EtOAc (5×), and the combined organic layers were filtered through silica gel (10% MeOH/EtOAc wash). After concentration in vacuo, the product was used immediately in the next reaction.

To an ice cold solution of the crude [^{3}H]diol tosylate in 400 μL of MeOH was added DBU (1.0 M in THF, 18 μL, 18 μmol). After stirring at 0 °C for 2 h, the reaction mixture was filtered through silica gel (10% MeOH/EtOAc wash), concentrated, and taken directly to the next step.

A solution of the [^{3}H]epoxy alcohol in 200 μL of benzene and 40 μL of DMSO was treated with DCC (8 mg, 35.5 μmol) followed by dichloroacetic acid (1.0 M in benzene, 3.6 μL, 3.6 μmol). After stirring for 2 h, the reaction mixture was filtered through silica gel (10% MeOH/EtOAc wash). Purification on a small pipet column (silica gel; 5-10% MeOH/EtOAc) gave [^{3}H]trapoxin B (**1***) (0.37 mg; specific activity 2.1 Ci/mmol). The compound was stored in DMSO stock solutions of 10 μM and 100 μM at −20 °C.

Charcoal Binding Assay with [^{3}H]Trapoxin. A crude nuclear extract was prepared from bovine thymus as described.[15] The dialyzed extract (0.5 mL in 25 mM tris pH 8, 10 mM NaCl, 10% glycerol) was treated at 4 °C with 20 nM [^{3}H]trapoxin (diluted from a 10 μM DMSO stock) with and without 400 nM unlabeled trapoxin (or 1 μM trichostatin, obtained from Wako Chemicals) for 30 min. A 10% (w/v) slurry (50 μL) of activated charcoal in 2.5% bovine serum albumin/phosphate buffered saline was added, and the mixture was vortexed briefly. After centrifugation at 14 000 rpm for 5 min, 0.35 mL of the supernatant was combined with 3 mL of Aquasol scintillation fluid (NEN) and counted with a Beckman LS 6500 scintillation counter. To determine the inherent background of the assay, a control experiment was performed in which 20 nM [^{3}H]trapoxin was incubated with 0.5 mL buffer (25 mM tris pH 8, 10 mM NaCl, 10% glycerol). The data in Figure 3 are the average values (with standard deviations) from three independent assays.

(2*S*,3*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]-2,3-(isopropylidenedioxy)-butanol (17). To an ice cold suspension of NaH (60% in oil, 1.22 g, 30.5 mmol, 1 equiv) in THF (60 mL) was added via cannula a solution of (+)-2,3-(*O*)-isopropylidene-L-threitol (4.95 g, 30.5 mmol, 1 equiv) in 50 mL of THF over 15 min. The suspension was allowed to stir for 45 min at room temperature, after which time *tert*-butyldimethylsilyl chloride (4.60 g, 30.5 mmol, 1 equiv) in 10 mL of THF was added. After stirring for 16 h, the reaction mixture was poured into 150 mL of saturated $NaHCO_3$ and extracted with ether (3 × 100 mL). The extracts were dried over $MgSO_4$, concentrated, and purified by flash chromatography (3:1 hexanes/EtOAc) to give 8.43 g of compound **17** (100% yield) as an oil: R_f 0.24 (1:4 EtOAc/hexanes); $[\alpha]^{20}_D$ +16.1° (*c* 2.4, $CHCl_3$); IR (thin film) 3480 (br), 2988, 2932, 2859, 1474, 1464, 1379, 1372, 1254, 1082, 837 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (m, 1H), 3.83−3.77 (m, 2H), 3.72 (dd, J = 11.6, 4.5 Hz, 1H), 3.65−3.59 (m, 2H), 2.66 (br s, 1H), 1.35 (s, 3H), 1.33 (s, 3H), 0.83 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.0, 80.0, 78.0, 63.6, 62.6, 27.0, 26.8, 25.8, 18.2, −5.56, −5.59; HRMS (CI, NH_3) Calcd for $C_{13}H_{28}O_4Si$ + NH_4 294.2101, found 294.2099.

(2*S*,3*S*)-10-[(*tert*-Butyldimethylsilyl)oxy]-8,9-(isopropylidenedioxy)-6(*Z*)-decenoic Acid (18). To a cooled (−78 °C) solution of oxalyl chloride (1.8 mL, 20.3 mmol, 1.3, equiv) in 50 mL of CH_2Cl_2 was added dropwise DMSO (2.9 mL, 41 mmol, 2.6 equiv) in 9 mL of CH_2Cl_2. Silyl threitol **17** (4.3 g, 15.6 mmol, 1 equiv) in 20 mL of CH_2Cl_2 was then added dropwise. After stirring for 20 min, triethylamine (10.8 mL, 78 mmol, 5 equiv) was added, and the reaction was allowed to warm to room temperature over a period of 1 h. The heterogeneous mixture was extracted with water, concentrated, redissolved in 50% hexanes/ether (200 mL), and extracted with water again (3 × 100 mL). The combined aqueous fractions were then extracted with 50% hexanes/ether, and the combined organic fractions were dried over Na_2SO_4 and concentrated. The crude aldehyde was dried azeotropically from benzene and used immediately.

In a separate flask, lithium hexamethyldisilazide was prepared by adding *n*-BuLi (1.6 M in hexanes, 25.4 mL, 40.6 mmol, 2.6 equiv based on **17**) to hexamethyldisilazane (8.6 mL, 40.6 mL, 2.6 equiv) in 50 mL of THF at 0 °C for 15 min. This solution was then transferred to a cooled (−20 °C) suspension of the phosphonium bromide derived from 6-bromohexanoic acid and triphenylphosphine (9.28 g, 20.3 mmol, 1.3 equiv). After 30 min, the mixture was cooled to −78 °C, and the aldehyde (see above) was added via cannula in 15 mL of THF. After stirring for 30 min at −78 °C and 1 h at room temperature, the reaction was quenched with water, and the organic solvents were removed in vacuo. The mixture was acidified with saturated $NaHSO_4$ and extracted with EtOAc. The organic fraction was washed with brine, dried over $MgSO_4$, and concentrated. Purification by flash chromatography (2:1 hexanes/EtOAc, 0−0.5% AcOH) provided the acid **18** (3.6 g, 62% yield) as an oil: R_f 0.22 (1:4 EtOAc/hexanes, 1% AcOH); $[\alpha]^{20}_D$ −0.76° (*c* 3.4, $CHCl_3$); IR (thin film) 3200 (br), 2932, 2859, 1740, 1711, 1252, 1146, 1080, 837, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (m, 1H), 5.40 (m, 1H), 4.75 (m, 1H), 3.78 (dd, J = 2.9, 8.1 Hz, 1H), 3.68−3.60 (m, 2H), 2.33 (t, J = 7.4 Hz, 2H), 2.3−2.0 (m, 2H), 1.5−1.4 (m, 2H), 1.41 (s, 3H), 1.40 (s, 3H), 0.88 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.6, 135.2, 126.9, 108.8, 81.6, 72.7, 61.4, 33.8, 28.9, 27.3, 27.2, 26.8, 25.8, 24.2, 18.3, −5.4, −5.5; HRMS (FAB, NaI) Calcd for $C_{19}H_{36}O_5Si$ + Na 395.2230, found 395.2236.

(4*S*)-4-Benzyl-3-[(8*S*,9*S*)-10-[(*tert*-butyldimethylsilyl)oxy]-8,9-(isopropylidenedioxy)-6(*Z*)-decenoyl]-2-oxazolidinone (20). To a cooled (−78 °C) solution of acid **18** (139 mg, 0.374 mmol, 1 equiv) and triethylamine (67 μL, 0.49 mmol, 1.3 equiv) was added pivaloyl chloride (51 μL, 0.41 mmol, 1.1 equiv). After stirring for 15 min at −78 °C and 45 min at 0 °C, the reaction was again cooled to −78 °C. To this

solution was added via cannula the lithiated oxazolidinone **19** in 1.5 mL of THF, which had been prepared at −78 °C by treating (*S*)-(−)-4-benzyl-2-oxazolidinone (119 mg, 0.67 mmol, 1.8 equiv based on **18**) with *n*-BuLi (2.3 M in hexane, 0.293 mL, 0.67 mmol, 1.8 equiv). After 15 min at −78 °C and 1.5 h at room temperature, the reaction was quenched with 5% $NaHSO_4$ (10 mL), and the solvents were removed in vacuo. The residue was extracted with EtOAc (3×), and the combined organic fractions were dried over Na_2SO_4 and concentrated. Purification by flash chromatography (5:1 hexanes/EtOAc) afforded the imide **20** (197 mg, 99% yield) as an oil: R_f 0.41 (1:4 EtOAc/hexanes); $[\alpha]^{20}_D$ +31.1° (*c* 0.80, $CHCl_3$); IR (thin film) 2930, 2857, 1786, 1701, 1499, 1385, 1250, 1213 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33−7.23 (m, 3H), 7.19 (m, 2H), 5.64 (m, 1H), 5.40 (m, 1H), 4.74 (m, 1H), 4.64 (m, 1H), 4.19−4.12 (m, 2H), 3.76 (m, 1H), 3.66−3.61 (m, 2H), 3.27 (dd, J = 10.0, 3.3 Hz, 1H), 2.97−2.86 (m, 2H), 2.73 (dd, J = 3.7, 9.6 Hz, 1H), 2.24−2.20 (m, 1H), 2.14−2.08 (m, 1H), 1.72−1.65 (m, 2H), 1.49−1.4 (m, 2H), 1.40 (s, 3H), 1.39 (s, 3H), 0.88 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 153.3, 135.2, 135.1, 129.3, 128.8, 127.2, 126.9, 108.6, 81.7, 72.8, 66.0, 61.6, 55.0, 37.8, 35.2, 29.0, 27.5, 27.2, 26.8, 25.8, 23.7, 18.2, −5.4, −5.5; HRMS (FAB, NaI) Calcd for $C_{29}H_{45}NO_6Si$ + Na 554.29140, found 554.2907.

(4S)-4-Benzyl-3-[(8*S*,9*S*)-10-[(*p*-toluenesulfonyl)oxy]-8,9-(isopropylidenedioxy)-6(*Z*)-decenoyl]-2-oxazolidinone (21). Hydrogen fluoride/pyridine complex (0.82 g) was cooled to 0 °C and treated with pyridine (2.3 mL) and THF (3.9 mL). This solution was then added to silyl ether **20** (2.7 g, 5.08 mmol, 1.0 equiv), and the resulting mixture was allowed to warm to room temperature. After 3 h, additional HF/pyridine/THF solution (5 mL) was added. The reaction was quenched 30 min later by the addition of saturated $NaHCO_3$ followed by extraction with ether (2 × 50 mL). After drying with $MgSO_4$, the combined organic fractions were concentrated and redissolved in dry CH_2Cl_2 (10 mL). Diisopropylethylamine (1.0 mL, 5.8 mmol, 1.1 equiv) was added, and the solution was cooled in ice. Tosyl chloride (1.1 g, 5.8 mmol, 1.1 equiv) and DMAP (0.124 g, 1.02 mmol, 0.2 equiv) were then added. After 4 h, more DMAP (total = 5.0 mmol, 1.0 equiv) and tosyl chloride (total = 7.5 mmol, 1.5 equiv) were added, and the reaction was stirred for a total of 15 h. The mixture was diluted with EtOAc (100 mL), washed with 5% $NaHCO_3$, dried over $MgSO_4$, and purified by flash chromatography (1:3 EtOAc/hexanes) to yield 2.57 g of tosylate **21** (89% yield) as an oil: R_f 0.19 (1:3 EtOAc/hexanes); $[\alpha]^{20}_D$ +32.9° (*c* 3.8, EtOAc); IR (thin film) 2936, 1778, 1700, 1599, 1454, 1360, 1177, 948, 820, 664 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.3 Hz, 2H), 7.3−7.1 (m, 7H), 5.64 (m, 1H), 5.29 (m, 1H), 4.7−4.5 (m, 2H), 4.15−4.05 (m, 3H), 3.98 (dd, J = 10.8, 4.7 Hz, 1H), 3.73 (m, 1H), 3.22 (dd, J = 13.4, 3.1 Hz, 1H), 3.0−2.7 (m, 2H), 2.70 (dd, J = 13.4, 9.6 Hz, 1H), 2.38 (s, 3H), 2.2−2.0 (m, 2H), 1.7−1.6 (m, 2H), 1.45−1.35 (m, 2H), 1.31 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 153.4, 144.8, 136.5, 135.3, 133.0, 129.8, 129.3, 128.9, 128.0, 127.2, 125.8, 109.7, 78.4, 73.1, 67.9, 66.2, 55.1, 37.9, 35.2, 28.9, 27.4, 27.1, 26.6, 23.8, 21.5; HRMS (FAB, NaI) Calcd for $C_{30}H_{37}NO_8S$ + Na 594.2137, found 594.2148.

(4S)-4-Benzyl-3-[(2*S*,8*S*,9*S*)-2-azido-10-[(*p*-toluenesulfonyl)oxy]-8,9-(isopro-pylidenedioxy)-6(*Z*)-decenoyl]-2-oxazolidinone (22). Tosylate **21** (2.57 g, 4.5 mmol, 1.0 equiv) was azeotropically dried with toluene under high vacuum and dissolved in dry THF (110 mL). To the cooled solution (−78 °C) was added potassium hexamethyldisilazide (0.67 M in toluene, 10.1 mL, 6.8 mmol, 1.5 equiv). After 30 min, a cooled solution (−78 °C) of 2,4,6-triisopropylbenzenesulfonylazide (2.09 g, 6.8 mmol, 1.5 equiv) in 19 mL of THF was added via cannula. Exactly 3 min later, AcOH (1.2 mL) was added, and the mixture warmed to 35 °C with a water bath. After 1.5 h, the mixture was concentrated and partitioned between EtOAc and 5% $NaHCO_3$ (50 mL each). The organic fraction was dried over Na_2SO_4, concentrated, and purified by flash chromatography (25% EtOAc/hexanes) to provide the azide **22** (2.0 g, 73% yield) as an oil: R_f 0.20 (1:2 EtOAc/hexanes); $[\alpha]^{20}_D$ +67.0° (*c* 3.5, EtOAc); IR (thin film) 2986, 2108, 1782, 1703, 1368, 1177, 982 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.4 Hz, 2H), 7.35−7.15 (m, 7H), 5.52−5.51 (m, 1H), 5.36 (m, 1H), 4.92 (dd, J = 9.2, 4.2 Hz, 1H), 4.647 (m, 1H), 4.59 (m, 1H), 4.25 (m, 1H), 4.18 (dd, J = 9.1, 2.9 Hz, 1H), 4.09 (dd, J = 10.8, 4.0 Hz, 1H), 4.01 (dd, J = 10.8, 4.6 Hz, 1H), 3.77 (m, 1H), 3.29 (dd, J = 13.4, 3.2 Hz, 1H), 2.81 (dd, J = 13.4, 9.4 Hz, 1H), 2.42 (s, 3H), 2.16 (m, 2H), 2.0−1.5 (m, 4H), 1.35 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 152.9, 145.0, 135.9, 134.8, 132.8, 129.8, 129.4, 129.0, 128.0, 127.4, 126.3, 109.8, 78.3, 73.3, 68.0, 66.6, 60.4, 55.3, 37.6, 30.7, 27.1, 27.0, 26.6, 25.9, 21.5; HRMS (FAB, NaI) Calcd for $C_{30}H_{36}N_4O_8S$ + Na 635.2151, found 635.2168.

(2*S*,8*S*,9*S*)-*N*-(Fluorenylmethoxycarbonyl)-2-amino-10-[(*p*-toluenesulfonyl)oxy]-8,9-(isopropylidenedioxy)decanoic Acid (23). To azide **22** (1.06 g, 1.73 mmol, 1.0 equiv) in 3:1 THF/water (40 mL) was added LiOH (0.124 g, 5.25 mmol, 3.0 equiv) at 0 °C. The reaction was quenched after 1 h with 1 N HCl (5.3 mL). The mixture was concentrated and extracted with EtOAc. After drying over $MgSO_4$ and concentrating in vacuo, the crude azido acid was dissolved in 9:1 THF/water (90 mL), treated with 10% Pd/C, and hydrogenated at 1 atm H_2 for 36 h. The black mixture was filtered through Celite and concentrated to give the crude amino acid. This compound was dissolved in 20 mL of 25% DMF/THF and treated at 0 °C with 2,6-lutidine (0.3 mL, 2.59 mmol, 1.5 equiv) and fluorenylmethyl-*N*-hydroxysuccinimidylcarbonate (0.87 g, 2.59 mmol, 1.5 equiv). After 1.5 h, water (20 mL) was added, and the volatile solvents removed in vacuo. The mixture was acidified with saturated $NaHSO_4$ to pH 2 and extracted with ether (3 × 30 mL). The organic phases were washed with water and brine and dried over $MgSO_4$. Purification by flash chromatography (4:5:1 hexanes/EtOAc/MeOH, 0−1% AcOH) gave Fmoc amino acid **23** (582 mg, 53% over three steps) as an oil: $[\alpha]^{20}_D$ −4.0° (*c* 1.4, $CHCl_3$); IR (thin film) 3250, 2986, 2938, 1719, 1522, 1366, 1177, 980 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.8−7.7 (m, 4H), 7.6−7.5 (m, 2H), 7.4−7.25 (m, 6H), 5.40 (d, J = 8.2 Hz, 1H), 4.55−4.35 (m, 3H), 4.20 (m, 1H), 4.08 (dd, J = 10.6, 4.0 Hz, 1H), 4.03 (dd, J = 10.6, 4.5 Hz, 1H), 3.8−3.75 (m, 2H), 2.40 (s, 3H), 1.95−1.83 (m, 1H), 1.8−1.65 (m, 1H), 1.55−1.45 (m, 2H), 1.5−1.2 (m, 6H), 1.33 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 156.1, 145.0, 143.8, 141.2, 132.6, 129.9, 128.0, 127.7, 127.1, 125.1, 120.0, 109.4, 78.1, 77.7, 69.1, 67.1, 53.7, 47.1, 32.7, 32.1, 28.9, 27.2, 26.6, 25.5, 25.0, 21.6; HRMS (FAB, NaI) Calcd for $C_{35}H_{41}NO_9S$ + Na 674.2400, found 674.2419.

N^{ϵ}-(Allyloxycarbonyl)-L-lysyl-L-phenylalanyl-D-proline Methyl Ester (24). The trifluoroacetate salt of L-phenylalanyl-D-proline methyl ester (566 mg, 1.45 mmol, 1 equiv) was combined with N^{α}-(*tert*-butoxycarbonyl)-N^{ϵ}-(allyloxycarbonyl)-L-lysine (481 mg, 1.45 mmol, 1 equiv), NMM (0.176 mL, 1.6 mmol, 1.1 equiv), HOBT (196 mg, 1.6 mmol, 1.1 equiv), and EDC (306 mg, 1.6 mmol, 1.1 equiv) in CH_2Cl_2 (18 mL). After 18 h, the crude mixture was purified directly by flash chromatography (67−80% EtOAc/hexanes) to give the tripeptide (559 mg, 64% yield) as an oil. Boc deprotection was carried out in 16 mL of 1:1 CH_2Cl_2/trifluoroacetic acid for 1 h. Solvents were removed, and the crude trifluoroacetate was concentrated several times from 50% heptane/CH_2Cl_2. This compound was characterized as the free base **24**: $[\alpha]^{20}_D$ +23.0° (*c* 3.9, $CHCl_3$); IR (thin film) 3320 (br), 2948, 2876, 1744, 1721, 1644, 1530, 1447, 1246; ^1H NMR (400 MHz, CD_3OD, mixture of rotamers) δ 7.32−7.17 (m, 5H), 5.91 (m, 1H), 5.28 (d, J = 17.2 Hz, 1H), 5.16 (d, J = 9.2 Hz, 1H), 4.93 (m, 1H), 4.50 (m, 2H), 4.25 (m, 1H), 3.86 (m, 1H), 3.66 (s, 3H), 3.2−2.9 (m, 6H), 2.0−1.3 (m, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 172.8, 172.2, 171.0, 169.9, 156.3, 136.3, 133.0, 129.4, 129.2, 128.3, 126.9, 126.6, 117.3, 65.2, 59.3, 58.6, 52.5, 52.2, 51.8, 46.8, 46.5, 40.5, 39.4, 37.4, 30.9, 29.5, 28.8, 24.3, 22.5, 21.9; HRMS (FAB, NaI) Calcd for $C_{25}H_{36}N_4O_6$ + Na 511.2533, found 511.2524.

(2*S*,8*S*,9*S*)-*N*-(Fluorenylmethoxycarbonyl)-2-amino-10-[(*p*-toluenesulfonyl)oxy]-8,9-(isopropylidenedioxy)decanoyl-N^{ϵ}-(allyloxycarbonyl)-L-lysyl-L-phenylalanyl-D-proline Methyl Ester (25). The trifluoroacetate salt of tripeptide **24** (155 mg, 0.252 mmol, 1.1 equiv) was combined with Fmoc amino acid **23** (145 mg, 0.229 mmol, 1.0 equiv), NMM (0.028 mL, 0.25 mmol, 1.1 equiv), HOBT (34 mg, 0.25 mmol, 1.1 equiv), and EDC (48 mg, 0.25 mmol, 1.1 equiv) in CH_2Cl_2 (3.2 mL). After 18 h, the crude mixture was purified directly by flash chromatography (0−2−5% MeOH/CH_2Cl_2) to give tetrapeptide **25** (206 mg, 81% yield) as an oil: R_f 0.45 (EtOAc); $[\alpha]^{20}_D$ +3.1° (*c* 0.91, $CHCl_3$); IR (film) 3298 (br), 2938, 1721, 1638, 1532, 1451, 1177, 984 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.8−7.7 (m, 4H), 7.7−7.6 (m, 2H), 7.4−7.1 (m, 11H), 7.95−7.8 (br, 1H), 5.9−5.75 (m, 2H), 5.45 (br s, 1H), 5.3−5.1 (m, 2H), 5.0−4.85 (br m, 1H),

4.6−4.15 (br m), 4.1−3.9 (m, 2H), 3.8−3.45 (br m), 3.61 (br s, 3H), 3.2−2.9 (br m, 4H), 2.75−2.65 (m, 1H), 2.39 (s, 3H), 1.9−1.2 (br m), 1.29 (br s, 3H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 172.0, 171.7, 170.5, 169.6, 156.4, 144.8, 143.8, 141.2, 136.0, 133.1, 132.8, 129.8, 129.3, 129.1, 128.4, 128.3, 127.9, 127.6, 127.0, 125.0, 119.8, 117.2, 109.2, 96.0, 78.0, 77.7, 69.1, 67.0, 65.2, 59.3, 58.6, 55.0, 53.3, 52.9, 52.4, 52.2, 52.1, 47.1, 46.7, 40.3, 39.3, 32.8, 32.3, 29.2, 29.0, 28.8, 27.2, 26.6, 25.5, 25.2, 24.2, 22.4, 22.1, 21.9, 21.5; MS (FAB, NaI) Calcd for $C_{60}H_{75}N_5O_{14}S$ + Na 1144, found 1144.

***cyclo*[(2*S*,8*S*,9*S*)-2-Amino-10-[(*p*-toluenesulfonyl)oxy]-8,9-(isopropylidenedioxy)-decanoyl-*N*$^{\epsilon}$-(allyloxycarbonyl)-L-lysyl-L-phenylalanyl-D-prolyl] (26).** To a THF/water solution (3:1, 2.6 mL) of tetrapeptide **25** (114 mg, 0.102 mmol, 1.0 equiv) was added 1 N LiOH (0.347 mL, 0.347 mmol, 3.4 equiv). After 2.5 h, the reaction was neutralized by the addition of 1 N HCl (0.347 mL), diluted with MeCN (10 mL), and extracted with hexanes (3 × 5 mL). The MeCN fraction was concentrated, azeotropically dried with benzene (3 × 2 mL), and dissolved in dry DMF (2.5 mL). This solution was added by syringe pump over 12 h to a DMF solution (110 mL) of BOP (270 mg, 0.61 mmol, 6 equiv) and DMAP (100 mg, 0.82 mmol, 8 equiv). After the addition was complete, the reaction was stirred for 2 h and the DMF was removed by vacuum distillation (bath temperature <40 °C). The residue was partitioned between EtOAc (50 mL) and 0.5 M citrate (pH 5, 20 mL) and washed with 5% K_2CO_3 and brine. The organic phase was dried over Na_2SO_4, concentrated, and dissolved in MeCN (2 mL). Purification by preparative reverse phase HPLC (Rainin Dynamax 300-A; 30−100% MeCN/0.1% aqueous TFA gradient over 30 min; 20 mL/min flow rate) provided cyclotetrapeptide **26** (51 mg, 58% yield) as a white solid: R_f 0.37 (5% MeOH/EtOAc); $[\alpha]^{20}_D$ −63.8° (*c* 0.65, $CHCl_3$); IR (thin film) 3297 (br), 2938, 1663, 1524, 1454, 1368, 1244, 1177 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.3−7.15 (m, 5H), 7.06 (d, J = 10.2 Hz, 1H), 6.60 (d, J = 6.0 Hz, 1H), 5.87 (m, 1H), 5.26 (dd, J = 17.2, 1.1 Hz, 1H), 5.17 (d, J = 10.3 Hz, 1H), 5.08 (m, 1H), 4.82 (br m, 1H), 4.65 (d, J = 7.5 Hz, 1H), 4.6−4.45 (m, 2H), 4.22 (m, 1H), 4.08 (dd, J = 10.8, 4.1 Hz, 1H), 4.03 (dd, J = 10.8, 4.4 Hz, 1H), 3.9−3.7 (m, 1H), 3.50 (m, 1H), 3.22−3.1 (m, 4H), 2.94 (dd, J = 13.5, 6.1 Hz, 1H), 2.43 (s, 3H), 2.35−2.25 (m, 1H), 2.25−2.05 (m, 4H), 1.8−1.2 (m, 15H), 1.33 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 174.2, 172.8, 171.5, 156.2, 145.0, 136.8, 133.0, 132.7, 129.9, 128.6, 128.0, 126.8, 117.5, 109.3, 78.1, 77.8, 77.2, 69.1, 65.4, 61.3, 57.8, 54.1, 53.4, 46.8, 40.5, 35,7, 32.8, 29.2, 29.0, 28.9, 28.8, 27.2, 26.6, 25.6, 25.3, 24.8, 23.5, 21.6; HRMS (FAB, NaI) Calcd for $C_{44}H_{61}N_5O_{11}S$ + Na 890.3986, found 890.4012.

K-Trap (27). Cyclotetrapeptide **26** (45 mg, 0.052 mmol) was dissolved in 1.0 mL of THF and treated with 1.0 mL of 6.2% aqueous HCl for 16 h. The reaction mixture was diluted with saturated $NaHCO_3$, extracted with CH_2Cl_2 (5×), and dried over Na_2SO_4. The crude diol was next dissolved in a mixture of DMSO (1 mL) and MeOH (5mL), to which was added DBU (25 μL, 0.16 mmol, 3.2 equiv) at 0 °C. After 1 h, the volume was reduced to 2 mL, and 20 mL of EtOAc was added and extracted sequentially with water, 0.5 M citrate (pH 5), half-saturated $NaHCO_3$, and brine. The aqueous fractions were separately back extracted with $CHCl_3$ (5 × 10 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated to give the crude epoxy alcohol, which was further dried by concentrating from toluene (2 × 0.5 mL).

To the epoxy alcohol in CH_2Cl_2 (3 mL) was added sequentially DMSO (0.25 mL), EDC (52 mg, 0.27 mmol, 8 equiv), and dichloroacetic acid (45 μL, 0.016 mmol, 0.5 equiv). After 8 h, the reaction was quenched with half-saturated $NaHCO_3$ (4 mL) and extracted with CH_2Cl_2 (4 × 5 mL). The combined organic fractions were dried over Na_2SO_4, concentrated, redissolved in MeOH (2 mL), and filtered through Celite. Purification by preparative HPLC (Rainin Dynamax 300-A; 0.1% aqueous TFA for 5 min, followed by a 0−50% MeCN gradient over 25 min; 18 mL/min flow rate) provided K-trap (**27**) as a white solid (22.5 mg, 66% yield over three steps): $[\alpha]^{20}_D$ −83.6° (*c* 0.45, $CHCl_3$); IR (thin film) 3293 (br), 2934, 1715, 1682, 1661, 1526, 1454, 1244; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.15 (m, 5H), 7.06 (d, J = 10.2 Hz, 1H), 6.60 (d, J = 5.4 Hz, 1H), 5.88 (m, 1H), 5.26 (d, J = 17.1 Hz, 1H), 5.18 (d, J = 10.3 Hz, 1H), 5.10 (m, 1H), 4.81 (br s, 1H), 4.65 (d, J = 6.9 Hz, 1H), 4.52 (m, 2H), 4.21 (m, 1H), 3.82 (m, 1H), 3.49 (m, 1H), 3.39 (m, 1H), 3.2−3.0 (m, 3H), 3.0−2.9 (m, 2H), 2.83 (dd, J = 5.7, 1.3 Hz, 1H), 2.40 (m, 1H), 2.4−2.1 (m, 5H), 1.9−1.7 (m, 2H), 1.7−1.4 (m, 6H), 1.4−1.2 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.5, 174.9, 174.2, 172.8, 171.6, 156.3, 136.8, 133.0, 129.0, 128.6, 126.8, 117.6, 77.2, 65.4, 61.4, 57.8, 54.0, 53.4, 46.9, 46.1, 40.5, 36.2, 35.7, 29.7, 29.2, 28.8, 28.6, 25.3, 24.9, 24.8, 23.6, 22.7; HRMS (FAB, NaI) Calcd for $C_{34}H_{47}N_5O_8$ + Na 676.3322, found 676.3354.

K-Trap Affinity Matrix (28). K-Trap (**27**) (1 mg, 1.5 μmol, 1 equiv) was concentrated to dryness from a CH_2Cl_2 stock solution in a 0.5 mL conical vial. A solution of 5,5-dimethyl-1,3-cyclohexanedione (0.06 M in THF, 0.2 mL, 12 μmol, 8 equiv) was added, and the mixture was degassed by three cycles of freeze−pump−thaw. A freshly prepared solution of tetrakis(triphenylphosphine)palladium[33] (0.01 M in degassed THF, 40 μL, 0.4 μmol, 0.27 equiv) was then added, and the reaction vial was immediately placed in a 35 °C water bath. After 30 min, the mixture was concentrated to dryness, dissolved in 1 mL of MeOH, and filtered through Celite, which was rinsed with 0.5 mL MeOH and 0.5 mL of water. Purification by semipreparative HPLC (2 × 1 mL injections; Beckman Ultrasphere ODS, 1 × 25 cm; 30% MeOH/0.1% aqueous TFA for 5 min, followed by 30−100% MeOH gradient over 25 min; 5 mL/min flow rate), afforded the amine as its TFA salt. This material was dissolved in a mixture of DMSO (0.1 mL) and THF (2 mL) and added to 1 mL of packed Affi-Gel 10 (Biorad), which had been washed with 10 mL of THF in a 5 mL polyethylene column (Biodrad). The slurry was treated with diisopropylethylamine (0.01 mL) and shaken for 2 h at room temperature. Ethanolamine (0.01 mL) was then added, and the slurry was shaken for an additional 3 h. The K-trap affinity matrix was washed with 15 mL of isopropyl alcoholl and stored as a 50% isopropyl alcohol slurry at −20 °C.

Charcoal Binding with ^{125}I-Trap Followed by SDS-PAGE. Nuclear extract (0.2 mL) was incubated with 1 nM ^{125}I-trap with and without 1 μM unlabeled trapoxin for 30 min at 4 °C. Charcoal was added and sedimented (see above), and 0.15 mL of the clear supernatant was treated for 30 min at room temperature with 50 μL of nonreducing sample buffer for sodium dodecylsulfate polyacrylamide gel electrophoresis (4× SDS-PAGE sample buffer: 8% SDS, 40% glycerol, 0.8% bromophenol blue, 0.25 M tris pH 6.8). As a control, pure ^{125}I-trap (0.5 nM, 0.15 mL) was processed identically for electrophoresis. Samples (40 μL) were electrophoresed (12.5% gel) until the dye front had migrated through 80% of the gel. The gel was stained with Coomasie brilliant blue, dried, and exposed to an imaging plate (Fuji). Images were processed with the Fujix BAS1000 image analyzer using MacBAS imaging software.

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