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Macrolactamization versus Macrolactonization: Total Synthesis of FK228, the Depsipeptide Histone Deacetylase Inhibitor†

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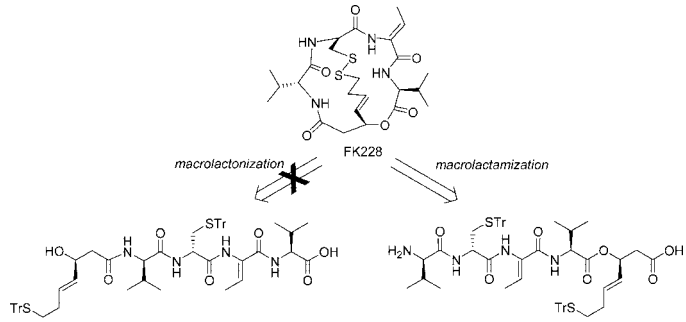

The cyclic depsipeptide FK228 is the only natural product histone deacetylase (HDAC) inhibitor that has advanced to clinical trials as an anticancer agent. While currently obtained by fermentation, total synthesis is an attractive alternative that will facilitate the preparation of unnatural analogues. The previous total syntheses of FK228 featured macrocylization by ester bond formation from a *seco*-hydroxy acid. Such routes are operationally jeopardized by the steric hindrance of the carboxylic acid and the sensitivity of the allylic alcohol toward elimination. We report a strategically different approach whereby the ester bond is formed intermolecularly at an early stage and macrocyclization is efficiently achieved by amide bond formation.

Introduction

The reversible acetylation[1] of lysine residues represents an important mode of protein posttranslational modification. As it converts a basic primary amine protonated at physiological pH to a neutral acetamide, it has major consequences for protein structure and function. It is of particular significance in eukaryotic histone proteins, where the pattern of posttranslational modification is part of the "epigenetic code" that regulates gene transcription or repression. Small molecule inhibitors of either the acetylation catalyzed by histone acetyltransferases (HATs) or the deacetylation catalyzed by histone deacetylases (HDACs) are thus of value as chemical probes of biological function, and as therapeutic agents. To date, drug discovery efforts have focused on the zinc-dependent HDACs,[2] of which there are 11 (HDAC1−11) in the human genome.

Zinc-dependent HDACs are metallohydrolases sharing a highly conserved catalytic domain containing an active site zinc ion. HDAC inhibitors typically contain a "warhead" that reversibly binds to the catalytic zinc atom, linked by a spacer to a "cap" that extends beyond the substrate-binding channel into the enzyme's surface exposed "rim". One can envision two extremes of HDAC inhibitors depending on the relative importance of warhead and cap binding to the enzyme. Merck's vorinostat, the first HDAC inhibitor to reach the market, is a

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† Abbreviations used: Dhb, dehydrobutyrine; HAT, histone acetyltransferase; HATU, 2-(1*H*-7-azabenzotriazol-1-yl)--1,1,3,3-tetramethyluronium hexafluorophosphate; HDAC, histone deacetylase; MSNT: 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole; PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; TMSE: (trimethylsilyl)ethyl.

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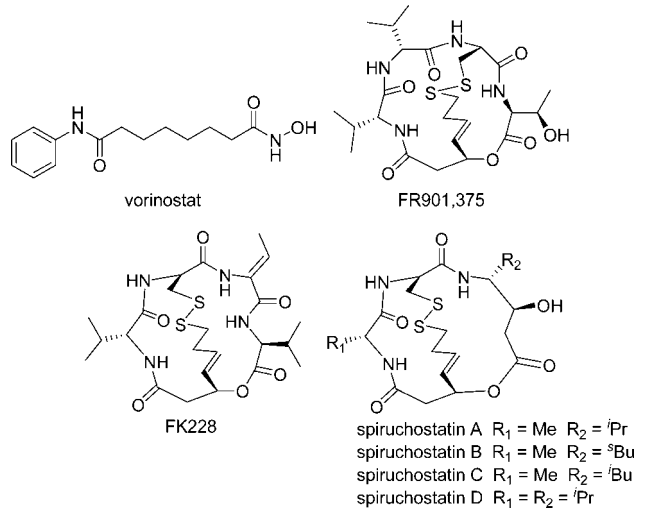
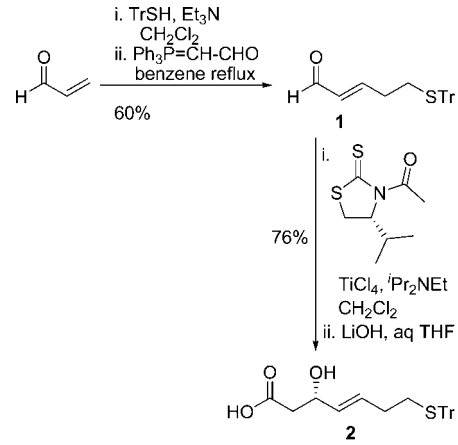


FIGURE 1. Structures of vorinostat and the bicyclic depsipeptide HDAC inhibitors.

classic example[3] with a potent active site zinc-binding hydroxamic acid and a minimal cap. At the other end are compounds with relatively weak zinc-binding groups that compensate by having large caps engaged in significant binding interactions with the enzyme rim. These are primarily natural products, a historically well established source of biologically active compounds.[4]

Natural product HDAC inhibitors with large caps are cyclic peptides and depsipeptides that can be further subdivided into two families. The first, exemplified by trapoxin, HC-toxin and apicidin, are cyclic tetrapeptides containing a mixture of L- and D-amino acids and cyclic amino acids such as proline or pipecolic acid. Both these features alleviate the ring strain associated with cyclic tetrapeptides containing all L or all D amino acids. The second family comprises bicyclic depsipeptides[5] (Figure 1) isolated from bacterial fermentation extracts. These bicyclic depsipeptide HDAC inhibitors are larger macrocycles compared to the cyclic tetrapeptides and are hence less stereochemically constrained. Typically, the amino acids are of D-stereochemistry, and the backbone of these natural products can be mapped in a "retro-inverso"[6] manner onto that of the enzyme substrate. Unlike the tetrapeptides, the bicyclic depsipeptides are prodrugs, with the disulfide bridge undergoing intracellular reduction and the resulting butenylthiol side chain then serving as the active site binding group. Although this is a weaker warhead compared to a hydroxamic acid, the affinity of the macrocyclic scaffold for the enzyme leads to significantly higher overall potency than vorinostat. Furthermore, as the enzyme rim is less conserved than the active site, there is the potential for depsipeptides to discriminate between the eleven HDAC isoforms. This is indeed the case, and the depsipeptides have pronounced selectivity[7] for the so-called Class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8), which fortuitously happen to be the isoforms most heavily implicated[8] in cancer and other proliferative disease conditions.

SCHEME 1. Synthesis of the Carboxylic Acid Fragment



Nature has thus far revealed only six examples of the bicyclic depsipeptides and five structurally related azumamides[9] which contain a carboxylic acid or carboxamide warhead in place of the thiol. It is a moot point if these natural products are best-in-class, and they offer limited opportunities for semisynthetic modification. Thus, the discovery of additional analogues that may be superior has to rely on de novo total synthesis. Despite FK228's progression to human clinical trials, synthetic analogues were unknown until we recently reported the first structure−activity relationships of the natural product.[10]

The earliest total synthesis of a bicyclic depsipeptide HDAC inhibitor was of FK228, reported in 1996 by Simon and coworkers.[11] After nearly a decade of inactivity, there followed in rapid succession the synthesis of FR901,375 by the Wentworth-Janda group,[12] spiruchostatins by us[13] and others,[14] and a second[15] FK228 synthesis by Williams. A key challenge in these syntheses is the preparation of the common β-hydroxy-γ,δ-unsaturated-ζ-thio-carboxylic acid, containing four functional groups and a stereogenic center within a seven carbon atom framework. All the early routes converged on the asymmetric acetate aldol reaction of aldehyde **1** (Scheme 1), readily obtained[12] in two steps from acrolein by the Wentworth-

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Janda synthesis. The desired aldol was first achieved by Simon[11] using Carreira's reagent-based conditions in 99% yield and >98% enantiomeric excess. However, neither Wentworth-Janda nor ourselves could reliably reproduce this result, and we opted for substrate-based control with oxazolidinone[16] auxiliaries. As the Evans auxiliary is not diastereoeselective in acetate aldols,[17] Wentworth−Janda used the 2-chloroacetyl enolate followed by reductive removal of the chloride after the aldol reaction. In our group, we used the 4-alkylthiazolidinethione Fujita−Nagao auxiliary which does give high stereoselectivity in acetate aldols.[18] In their later synthesis,[14a] Doi−Takahashi used Seebach's quaternary oxazolidinone as an alternative solution. Meanwhile, Katoh[14b] and Williams[15] devised nonaldol but lengthier routes respectively relying on the chiral pool and Noyori asymmetric reduction.

From a practical point of view, the thiazolidinedione aldol sequence is concise, providing **2** in four steps from acrolein and we have routinely performed the synthesis on multigram scale. As it is an auxiliary-based diastereoselective approach, the aldol impurity is a diastereomer easily removed by conventional chromatography. In contrast, a reagent-based enantioselective approach would need a chiral separation from the undesired minor enantiomer. Furthermore, in our aldol route the auxiliary can be recovered after hydrolysis and advantageously recycled.

A second key operation is the macrocylization to form the cyclodepsipeptide and disulfide rings. Simon selected the ester bond for macrolide cyclization[19] from the linear *seco*-hydroxy acid, but a variety of carboxyl activating conditions gave only recovered starting material or side-products. They state that Keck's modification of Steglich's carbodiimide esterification did afford the cyclic depsipeptide, but in a "disappointingly low" yield. Faced with this obstacle, they switched to an "umpolung" macrocyclization by activation of the alcohol under Mitsunobu conditions and displacement by the carboxylate. As the allylic alcohol is susceptible to elimination, extensive optimization of this reaction was needed and ultimately furnished the product in 62% yield. A similar Mitsunobu macrolactonization was employed in the Wentworth−Janda FR901,375 synthesis, occurring in 59% yield. In our spiruchostatin A synthesis, we returned to carboxyl activation of the linear hydroxy acid, and the popular Yamaguchi conditions were found to give the cyclic depsipeptide in a respectable 53% yield. In their second generation approach,[14a] Doi−Takahashi improved this further by using the more reactive Shiina reagent.[20] The Shiina reagent enables the macrolactonization to proceed under milder room temperature conditions thus minimizing the formation of side-products.

Once the cyclodepsipeptide is formed, the second disulfide macrocycle is readily installed by oxidative conditions. Interestingly, this sequence of macrolactonization followed by disulfide bond formation mirrors the final stages of the biosynthesis, as suggested[21] by analysis of the FK228 producing gene cluster. Reversing the order of macrocyclizations appears to be counterproductive in the laboratory. Thus, Doi−Takahashi were unsuccessful[14a] in macrolactonization of a monocycle with the disulfide bridge in place, although modeling suggests the existence of low energy conformers suitably oriented for cyclization.

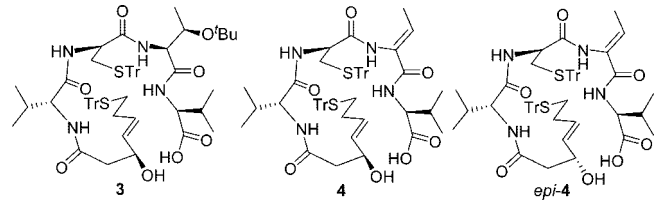

FIGURE 2. Compounds tested in macrolactonization reactions.

Results and Discussion

We have prepared over a hundred FK228 and spiruchostatin analogues by the Shiina macrolactonization, with every case being successful. Given this background, the total synthesis of FK228 itself by the same methodology seemed trivial but proved to be surprisingly insurmountable. The attempted Shiina macrolactonization of **3** or **4** (Figure 2) did not yield cyclized product in detectable quantities. With hindsight, we believe this failure is due to high steric hindrance at the carboxylic acid undergoing macrocyclization. In these compounds, there is an adjacent isopropyl group whereas the corresponding intermediate in our spiruchostatin A total synthesis is unencumbered at the α-position. Although we had made analogues with substitution at this position such as methyl and benzyl, none had involved α-branching like the isopropyl group in FK228. Apparently, this difference in steric demand is sufficient to prevent the cyclization.

Needing a supply of FK228 for our biological studies, we then expended considerable effort in repeating Simon's total synthesis.[11] Since his Mitsunobu macrolactonization involves inversion of the alcohol, this meant that we had to go back and prepare *epi*-**4**, the epimer of **4** at the alcohol chiral center. The Mitsunobu reaction of *epi*-**4**, when carried out under Simon's conditions, *was* productive and resulted in the desired cyclodepsipeptide. Nevertheless, due to the large excess of Ph_3P (25 equiv), diisopropyl azodicarboxylate (20 equiv) and TsOH (5 equiv), the product is a very minor component of the reaction mixture. Chromatographic purification eventually provided 10−20% of the depsipeptide, a significant decrease over Simon's reported yield. Overall, we decided the Simon synthesis was not practical for scale-up, as a large amount of the precious late stage intermediate *epi*-**4** is sacrificed in the macrocyclization. Similar findings were reported by Williams,[15] who also repeated the Simon macrolactonization in their FK228 synthesis. Despite numerous trials, the cyclization yield was only 24%.

These results prompted us to explore a new route wherein the difficult ester bond is installed at an earlier stage, and macrocyclization performed by lactamization rather than lactonization. Indeed, the literature on cyclic depsipeptides suggests that amide bond formation is often easier than ester bond formation for the macrocyclization step.[22] Nevertheless, in the total syntheses of depsipeptide HDAC inhibitors, all previous

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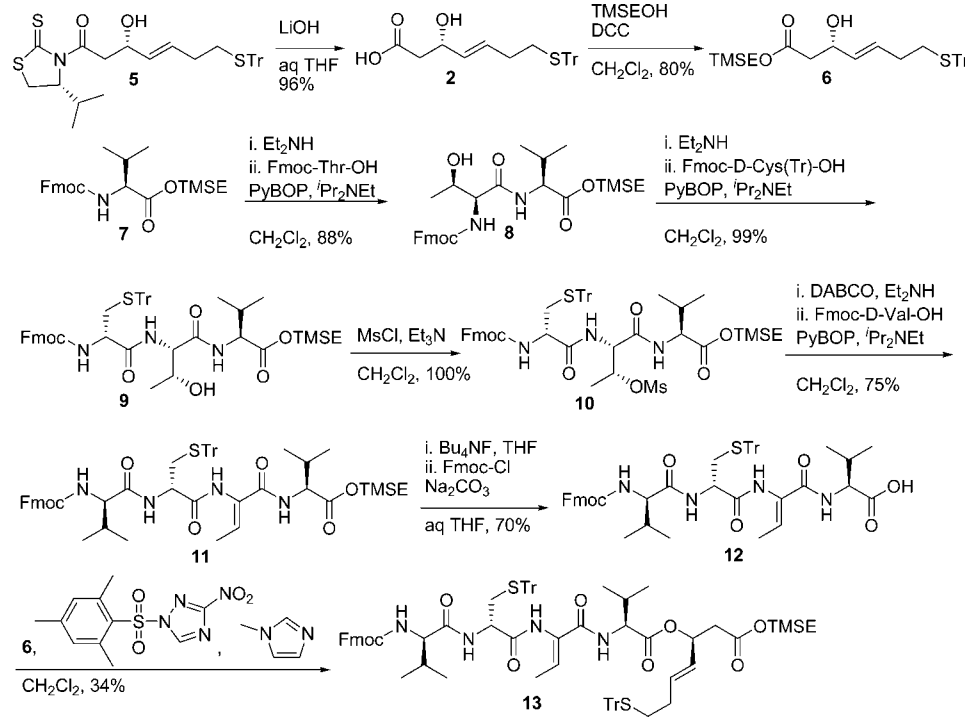
SCHEME 2. Synthesis of a Linear Depsipeptide



routes[11−15] have selected macrolactonization instead of macrolactamization. One reason is the initial success reported by Simon, suggesting that macrolactonization is a viable strategy. Second, macrolactamization requires a more complex set of protecting groups, as will be seen below.

Our synthesis began with the aldol product **5** (Scheme 2). Hydrolysis of the auxiliary releases β-hydroxy acid **2**, which was protected as the 2-(trimethylsilyl)ethyl (TMSE) ester **6.** The choice of ester was dictated by the need for later deprotection to be accomplished under neutral conditions and orthogonality to other functionality in the molecule. For the peptide fragment, Fmoc-L-valine was similarly protected as TMSE ester **7**. Since the deprotection mechanism involves reaction at silicon rather than the carbonyl, this was expected to minimize issues with steric hindrance from the valine side chain. Following Fmoc deprotection, sequential coupling with Fmoc-L-threonine and Fmoc-D-cysteine gave dipeptide **8** and tripeptide **9** respectively. Peptide **9** was subjected to mesylation to give **10**, followed by elimination and coupling with Fmoc-D-valine to provide tetrapeptide **11**. The TMSE ester was next deprotected by fluoride. As this was accompanied by cleavage of the Fmoc protecting group, the crude reaction mixture was treated with Fmoc-Cl, affording **12** in 70% overall yield. The key intermolecular esterification with the β-hydroxy ester **6** was then investigated, and proved to be as challenging as the intramolecular macrolactonization of **3** or **4**. Best results were obtained with 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) which furnished **13** in a modest 34% yield, accompanied by the revovery of 42% unreacted alcohol **6**. Prolonged reaction times did not help, instead leading to epimerization.

Our observations indicate that esterification of FK228 precursors by carboxyl activation is a difficult process, whether it is intermolecular as in **12** or an intramolecular macrocylization as in **3** and **4**. Since valine itself is not known to be so recalcitrant, this suggests that the poor reactivity is not only due to the isopropyl group but is further exacerbated by the rest of the peptide sequence. The solution would then be to carry out this ester bond formation at the very beginning. The new route began with the carbodiimide-mediated coupling between Fmoc-L-valine and **6**. This early stage esterification proceeded in high yield to furnish diester **14** (Scheme 3), confirming that valine by itself is significantly more reactive than the homologated peptides. Meanwhile, starting from D-cysteine, the tripeptide Fmoc-D-Val-D-Cys(Tr)-Dhb-OH **18** was prepared by stepwise peptide coupling and introduction of the Dhb side chain by elimination from threonine.

The Fmoc group of **14** was removed, and the amine coupled with tripeptide **18** to furnish linear depsipeptide **13** (Scheme 4). This new route proceeded in high yields throughout and was a significant improvement over the late stage esterification of **12**. With practical quantities of **13** in hand, the orthogonal Fmoc and TMSE protecting groups were then removed to unmask the linear *seco*-amino acid. The crucial macrolactamization using HATU (2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) as condensing agent afforded depsipeptide **19** at the first attempt, and reliably occurred in >70% yield in subsequent experiments. In these reactions, we did not observe significant byproducts resulting from guanidinylation, a known issue with HATU.[23] Although not investigated, the macrocylization yields may be further improved by switching to coupling reagents such as 7-azabenzotriazol-1-yloxy-tris-(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) that avoid this side reaction.[24]

The successful cyclization of **13** confirms that the amide-bond forming strategy toward the FK228 macrocycle is far superior to ester-bond formation. It avoids the difficulties associated with macrolactonization whether by carboxylic acid or alcohol activation, due to the sterically hindered nature of

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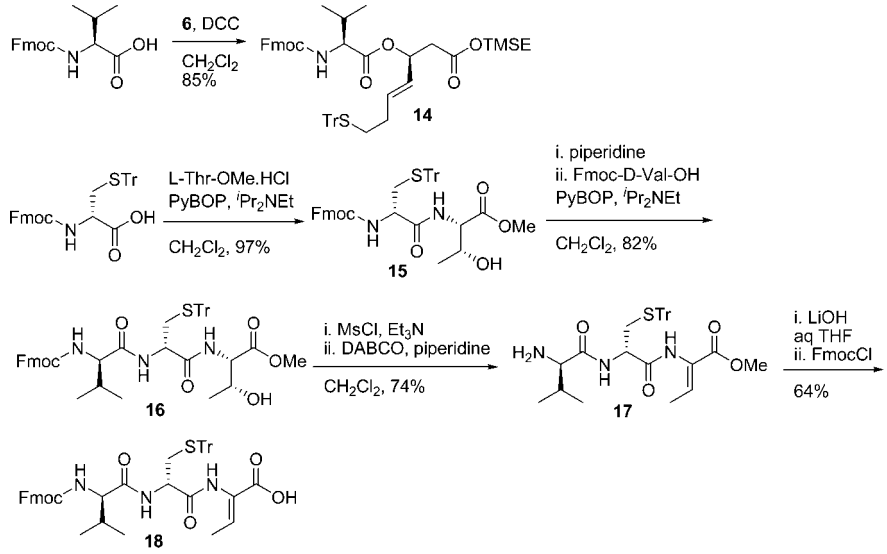
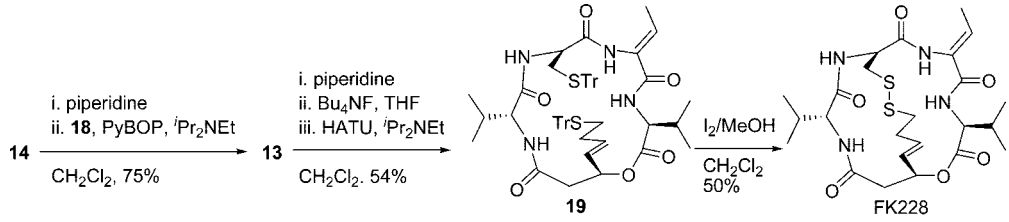
SCHEME 3. Synthesis of Depsipeptide and Peptide Fragments for Intermolecular Coupling



SCHEME 4. Final Stages of the FK228 Total Synthesis



the former and the sensitivity of the latter toward elimination. The NMR spectrum of depsipeptide **19** indicated the presence of the tetrabutylammonium cation (from the TBAF deprotection of the ester in **13**). We believe this may be due to encapsulation of the cation by the macrocyclic scaffold. The cyclodepsipeptide was taken forward, and oxidative disulfide bond formation provided pure FK228 after silica column chromatography. Synthetic FK228 was identical in physical properties to a sample of naturally isolated material, and spectroscopic data matched that reported in the literature. Furthermore, synthetic FK228 showed equal activity to the natural product as an inhibitor of cell growth and HDAC enzymes in vitro.

In summary, we have completed the first total synthesis of FK228 using amide bond formation for the key macrocylization step. Our route is convergent, taking six steps from acrolein to depsipeptide **14** and seven steps from Fmoc-D-Cys(Tr)-OH to peptide **18**, followed by their union and a further four steps to complete the total synthesis of FK228. Importantly, this bypasses the difficulties encountered in macrolactonization for less reactive substrates, as is certainly true for FK228 and likely to be the case for unnatural analogues with similarly bulky substituents proximal to the carboxylic acid. The successful route hinged upon early formation of the difficult ester bond, and the choice of orthogonal protecting groups to facilitate selective manipulation of advanced intermediates.

The total synthesis of natural products is considered a major touchstone of organic chemistry. At the same time, it is an activity that is rarely put to the test of verifiability common to many scientific endeavors. The difficulties we and others have encountered in repeating either Simon's enantioselective aldol for the preparation of **2**, or the Mitsunobu macrolactonization of *epi*-**4**, well illustrate the subjective elements of complex molecule total synthesis and the difficulty of reproducing these in a new environment. We believe that our second-generation FK228 synthesis circumvents these obstacles, and the route described here was successfully transferred to a contract research organization for a gram scale synthesis of FK228.

Experimental Section

(4*E*,3*S*)-3-Hydroxy-7-mercapto-[*S*-triphenylmethyl]-4-heptenoic acid (2). To a stirred solution of **5** (3.80 g, 6.76 mmol) in THF (120 mL) cooled in an ice bath was added a solution of LiOH (324 mg, 13.53 mmol) in water (40 mL). After stirring for 1 h, the solution was acidified to pH 2−3 with 1 M aq HCl, and diluted with EtOAc (300 mL). The phases were separated, and the aqueous phase extracted with EtOAc (150 mL × 3). The combined organic phases were dried (Na_2SO_4), concentrated, and purified by flash chromatography (eluent: EtOAc/Hexane = 1/3−3/2) to provide **2** (2.71 g, 96%) as a white solid: $[\alpha]^{25}_D$ −4.4 (c 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14−7.49 (m, 15H), 5.51−5.64 (m, 1H), 5.42 (dd, 1H, J = 15.4, 6.4 Hz), 4.39−4.50 (m, 1H), 2.46−2.59 (m, 2H), 2.14−2.27 (m, 2H), 2.01−2.13 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 176.7, 145.0, 131.8, 130.9, 129.7, 128.0, 126.8, 68.6, 66.8, 41.3, 31.5, 31.4; MS *m*/*z* 417 (M-H$^+$).

(*E*)-(*S*)-3-Hydroxy-7-tritylsulfanyl-hept-4-enoic Acid 2-Trimethylsilanyl-ethyl Ester (6). To a stirred solution of **2** (300 mg, 0.71 mmol) and trimethlysilylethanol (0.41 mL, 2.84 mmol) cooled in an ice bath was added DMAP (5 mg) and DCC (161 mg, 0.78 mmol). After stirring for 2 h, the solution was filtered and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/10−1/5) to give **6** (296 mg, 80%) as a colorless liquid: IR 3400, 1729, 1249 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.38 (m, 6 H), 7.15−7.26 (m, 9 H), 5.53 (m, 1 H), 5.37 (dd, 1H, J = 6.0, 15.2 Hz), 4.38 (m, 1 H), 4.15 (t, 2 H, J = 8.9 Hz), 2.42 (m, 2 H), 2.17 (m, 2 H), 2.04 (m, 2 H), 0.94 (m, 2

H), 0.00 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 145.1, 132.2, 130.3, 129.7, 128.0, 126.7, 68.7, 66.7, 63.2, 41.7, 31.6, 31.5, 17.5, −1.4. MS *m/z* 542 (M + Na^+); HRMS *m/z* calcd for [M + Na]$^+$ $C_{31}H_{38}NaO_3SSi$ 541.2203, found 541.2201.

(*S*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyric Acid 2-Trimethylsilanyl-ethyl Ester (7). To a stirred solution of Fmoc-L-Val-OH (3.4 g, 10 mmol) and trimethylsilylethanol (2.0 mL, 14 mmol) in anhydrous dichloromethane (20 mL) cooled in an ice bath was added a solution of DCC (3.1 g, 15 mmol) and DMAP (122 mg, 1 mmol) in anhydrous dichloromethane (30 mL). After stirring for 2 h, the solution was filtered, concentrated and purified by flash column chromatography (eluent: EtOAc/Hexane = 1/10) to give **7** (4.8 g, 100%) as a colorless oil: $[\alpha]^{25}_D$ −2.3 (c, 0.76, $CHCl_3$); IR 2957, 1715 cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, 2 H, *J* = 7.50 Hz), 7.62 (d, 2H, *J* = 7.14 Hz,), 7.29−7.47 (m, 4H), 5.33 (d, 1H, *J* = 8.78 Hz,) 4.35−4.49 (m, 2H), 4.21−4.35 (m, 3H), 2.11−2.28 (m, 1H), 1.02−1.09 (m, 2H), 1.06 (d, 1H), 1.00 (d, 3H, *J* = 7.41 Hz), 0.94 (d, 3H, *J* = 6.9 Hz,), 0.07 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.3, 156.3, 144.0, 141.5, 127.8, 127.2, 125.2, 120.1, 67.2, 63.8, 59.3, 47.4, 31.5, 19.1, 17.7, 17.7, −1.4; MS *m/z* 463 (M + Na^+), 903 (2 M + Na^+).

(*S*)-2-[(2*S*,3*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-butyrylamino]-3-methyl-butyric Acid 2-Trimethylsilanyl-ethyl Ester (8). At room temperature, to a stirred solution of **7** (4.4 g, 10 mmol) in dichloromethane (50 mL) was added diethylamine (10 mL). After stirring for 8 h, the solution was concentrated, and the residue purified by flash chromatography (eluent: EtOAc/Hexane = 1/10−1/2, then MeOH/CH_2Cl_2 = 1/20−1/10) to give the free amine (2.19 g) as a colorless oil. To a stirred solution of Fmoc-L-Thr-OH (3.4 g, 10 mmol) and the above amine (2.19 g) in anhydrous dichloromethane (50 mL) cooled in an ice-bath was added PyBOP (5.7 g, 11 mmol) and *N*,*N*-diisopropylethylamine (2.1 mL, 12 mmol). After stirring for another 0.5 h, the solution was warmed to room temperature, stirred for 1.5 h, followed by addition of sat aq NH_4Cl (20 mL). The phases were separated, and the aqueous phase extracted with dichloromethane (25 mL × 3). The combined organic phases were washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/5−1/2) to give **8** (4.8 g, 88%) as a colorless oil: $[\alpha]^{25}_D$ −35 (c, 0.56, $CHCl_3$); IR 3372 (br), 1731 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, 2 H, *J* = 7.3 Hz), 7.54 (d, 2 H, *J* = 7.6 Hz), 7.35 (t, 2 H, *J* = 7.2 Hz), 7.26 (t, 2 H, *J* = 7.2 Hz), 6.93 (d, 1 H, *J* = 8.1 Hz), 5.81 (d, 1 H, *J* = 7.7 Hz), 4.32−4.48 (m, 4 H), 4.15−4.25 (m, 4 H), 2.15 (m, 1 H), 1.14 (d, 3 H, *J* = 6.2 Hz), 0.96 (t, 2 H, *J* = 8.4 Hz), 0.88 (d, 3 H, *J* = 6.9 Hz), 0.84 (d, 3 H, *J* = 6.9 Hz), 0.00 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 171.1, 156.9, 143.8, 141.4, 127.8, 127.2, 125.1, 120.1, 67.4, 67.0, 63.9, 58.3, 57.5, 47.2, 30.9, 191.1, 18.0, 17.7, 17.6, −1.5; MS *m/z* 542 (M + H^+), 564 (M + Na^+).

(*S*)-2-{(2*S*,3*R*)-2-[(*S*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-butyrylamino}-3-methyl-butyric Acid 2-Trimethylsilanyl-ethyl Ester (9). At room temperature, to a stirred solution of compound **8** (4.69 g, 8.70 mmol) in anhydrous acetonitrile (45 mL) was added diethylamine (4.5 mL). After stirring for 4 h, the solution was concentrated, and the residue dissolved in dichloromethane and concentrated again. The procedure was repeated once, and the residue dried by high vacuum to give the crude amine (4.04 g) as an oil. To a suspension of Fmoc-D-Cys(Tr)-OH (5.35 g, 9.13 mmol) and the above amine in anhydrous dichloromethane (50 mL) and acetonitrile (5 mL) cooled in an ice-bath was added PyBOP (4.98 g, 9.57 mmol) and *N*,*N*-diisopropylethylamine (1.91 mL, 10.96 mmol). The solution was warmed to room temperature slowly and stirred overnight. The reaction mixture was worked up following the general procedure, and purified by flash chromatography (eluent: EtOAc/Hexane = 1/10−1/2.5) to give compound **9** (6.98 g, 91%) as a white solid: $[\alpha]^{25}_D$ −29.7 (c, 0.83, $CHCl_3$); mp 80−83 °C; IR 3307 (brs), 1647 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (m, 2 H), 7.54 (m, 2 H), 7.28−7.42 (m, 7 H), 7.14−7.26 (m, 12 H), 7.00 (d, 1 H, *J* = 8.7 Hz), 6.80 (d, 1 H, *J* = 7.7 Hz), 5.14 (d, 1 H, *J* = 7.2 Hz), 4.25−4.40 (m, 5 H), 4.14−4.24 (m, 3 H), 3.80 (q, 1 H, *J* = 6.9 Hz), 2.62 (d, 2 H, *J* = 6.9 Hz), 2.10 (m, 1 H), 1.04 (d, 3 H, *J* = 6.2 Hz), 0.92 (m, 2 H), 0.79 (t, 6 H, *J* = 6.9 Hz), 0.0 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 171.2, 170.7, 144.4, 143.9, 143.7, 141.4, 129.6, 128.2, 127.8, 127.2, 127.1, 125.1, 120.0, 67.5, 67.3, 66.2, 63.8, 57.5, 56.8, 54.3, 47.2, 34.1, 30.8, 19.1, 18.1, 17.7, 17.6, −1.5; MS *m/z* 909 (M + Na^+); HRMS *m/z* calcd for [M + Na]$^+$ $C_{51}H_{59}N_3NaO_7SSi$ 908.3735, found 908.3726.

(*S*)-2-{(2*S*,3*R*)-2-[(*S*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-methanesulfonyloxy-butyrylamino}-3-methyl-butyric Acid 2-Trimethylsilanyl-ethyl Ester (10). To a stirred solution of compound **9** (5.0 g, 5.64 mmol) in anhydrous dichloromethane (50 mL) cooled in an ice bath was added DMAP (40 mg), Et_3N (1.5 mL, 10.75 mmol) and MsCl (0.64 mL, 7.72 mmol). After stirring for 30 min, sat aq $NaHCO_3$ (15 mL) was added followed by dichloromethane (20 mL). The phases were separated, and the aqueous phase extracted with dichloromethane (30 mL × 3). The combined organic phases were washed with sat aq NH_4Cl (25 mL) and brine (25 mL) separately, dried (Na_2SO_4), and concentrated to give crude **10** (5.48 g, quantitative) as a light yellow solid: $[\alpha]^{25}_D$ −12.3 (c, 0.58, $CHCl_3$); mp 75−80 °C; IR 1651, 1175 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (dd, 2 H, *J* = 4.7, 7.3 Hz), 7.55 (m, 2 H), 7.33−7.46 (m, 8 H), 7.16−7.32 (m, 11 H), 6.98 (d, 1 H, *J* = 8.0 Hz), 6.54 (d, 1 H, *J* = 8.4 Hz), 5.28 (m, 1 H), 5.03 (d, 1 H, *J* = 6.9 Hz), 4.62 (dd, 1 H, *J* = 3.3, 8.4 Hz), 4.30−4.42 (m, 3 H), 4.08−4.20 (m, 3 H), 3.48 (m, 1 H), 3.00 (s, 3 H), 2.68 (m, 2 H), 2.14 (m, 1 H), 1.37 (d, 3 H, *J* = 6.2 Hz), 0.95 (m, 2 H), 0.90 (d, 3 H, *J* = 3.6 Hz), 0.88 (d, 3 H, *J* = 3.6 Hz), 0.00 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 171.0, 167.8, 144.3, 144.0, 143.7, 141.5, 141.4, 129.7, 128.3, 127.9, 127.2, 125.2, 125.1, 120.1, 67.7, 67.5, 63.7, 58.1, 56.6, 54.7, 47.2, 38.2, 33.3, 31.0, 19.2, 18.1, 18.0, 17.6, −1.4; MS *m/z* 965 (M + H^+), 982 (M + NH_4^+), 987 (M + Na^+); HRMS *m/z* calcd for [M + Na]$^+$ $C_{52}H_{61}N_3NaO_9S_2Si$ 986.3511, found 986.3482.

(*S*)-2-((*Z*)-2-{(*S*)-2-[(*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-3-tritylsulfanyl-propionylamino}-but-2-enoylamino)-3-methyl-butyric Acid 2-Trimethylsilanyl-ethyl Ester (11). To a stirred solution of compound **10** (5.40 g, 5.6 mmol) in anhydrous dichloromethane (50 mL) cooled in an ice bath was added DABCO (6.28 g, 56 mmol). After stirring for 30 min, the solution was warmed to room temperature, and was stirred for 1.5 h before diethylamine (2.9 mL) was added. After stirring for another 3.5 h, the solution was concentrated, and purified by flash chromatography (eluent: EtOAc/Hexane = 1/10−1/2, then MeOH/dichloromethane = 1/40−1/20) to give the amine (3.24 g, 90%) as a colorless oil: $[\alpha]^{25}_D$ = 1.8 (c, 0.70, $CHCl_3$). To a stirred solution of Fmoc-D-Val-OH (1.7 g, 5.0 mmol) and the above amine (2.95 g, 4.56 mmol) cooled in an ice bath was added PyBOP (2.85 g, 5.48 mmol) and *N*,*N*-diisopropylethylamine (1.20 mL, 6.89 mmol). After stirring for 70 min, the reaction mixture was worked up following the general procedure. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/4−1/1.5) to give **11** (4.02 g, 83%) as a white solid: $[\alpha]_D^{25}$ 7.0 (c, 0.78, $CHCl_3$); mp 97−100 °C; IR 3289, 1643, 1495 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, 2 H, *J* = 7.3 Hz), 7.50 (d, 2 H, *J* = 7.7 Hz), 7.31−7.42 (m, 9 H), 7.10−7.26 (m, 11 H), 6.60−6.71 (m, 2 H), 6.38 (brs, 1 H), 5.35 (brs, 1 H), 4.46 (dd, 1 H, J_1 = 5.5, 8.4 Hz), 4.34 (dd, 1 H, *J* = 7.2, 10.5 Hz), 4.18−4.32 (m, 1 H), 4.06−4.17 (m, 3 H), 3.90−4.05 (m, 2 H), 2.82 (m, 1 H), 2.62 (m, 1 H), 2.08 (m, 2 H), 1.66 (d, 3 H, *J* = 6.9 Hz), 0.82−0.98 (m, 14 H), 0.00 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 171.8, 168.7, 164.1, 156.9, 144.3, 143.9, 143.7, 141.5, 131.5, 129.6, 129.0, 128.2,

127.9, 127.2, 127.1, 125.1, 120.1, 67.6, 67.3, 63.4, 60.6, 58.0, 53.2, 47.3, 32.9, 31.3, 30.8, 19.4, 19.2, 18.3, 17.8, 17.6, 13.4, −1.4; MS ESI *m/z* 968 (M + H^+), 990 (M + Na^+).

(*S*)-2-((*Z*)-2-{(*S*)-2-[(*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-3-tritylsulfanyl-propionylamino}-but-2-enoylamino)-3-methyl-butyric Acid (12). At room temperature, to a stirred solution of **11** (1.5 g, 1.55 mmol) in anhydrous THF (8 mL) was added 1 M TBAF in THF (4.65 mL). After stirring for 7.5 h, the solution was cooled in an ice bath, followed by the addition of sat aq $NaHCO_3$ (2 mL), Na_2CO_3 (164 mg, 1.55 mmol) and Fmoc-Cl (481 mg, 1.86 mmol). After stirring for 2 h, the solution was acidified to pH 4−5 with 1 M aq HCl. The phases were separated, and the aqueous phase extracted with EtOAc (20 mL × 5). The combined organic phases were dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/Hexane = 1/5−1/1.5, then MeOH/dichloromethane = 1/20−1/10) to give **12** (0.94 g, 70%) as a white solid: $[\alpha]^{25}_D$ 8.0 (c, 0.63, $CHCl_3$); mp 118−120 °C; IR: 3277, 1651, 1504 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90 (brs, 1 H), 7.70 (d, 2 H, J = 7.7 Hz), 7.45 (d, 2 H, J = 7.7 Hz), 7.05−7.36 (m, 20 H), 6.80−6.90 (m, 2 H), 6.67 (m, 1 H), 5.43 (d, 1 H, J = 6.6 Hz), 4.28−4.40 (m, 2 H, 4.20 (m, 1 H), 4.00−4.12 (m, 2 H), 3.89 (brs, 1 H), 2.75 (m, 1 H), 2.60 (m, 1 H), 2.00−2.12 (m, 2 H), 1.58 (d, 3 H, J = 6.9 Hz), 0.75−0.92 (m, 12 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 172.0, 169.3, 164.7, 156.9, 144.2, 143.8, 143.5, 141.3, 133.1, 129.4, 128.1, 127.8, 127.1, 127.0, 125.0, 120.0, 67.4, 60.6, 58.9, 53.1, 47.1, 32.8, 30.8, 30.3, 19.1, 18.3, 13.5; MS *m/z* 866 (M-H^+); HRMS *m/z* calcd for [M + $Na]^+$ $C_{51}H_{54}N_4NaO_7S$ 889.3605, found 889.3613.

(*E*)-(*S*)-3-((*S*)-2-{(*Z*)-2-[(*S*)-2-((*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino)-3-tritylsulfanyl-propionylamino]-but-2-enoylamino}−3-methyl-butyryloxy)-7-[(Z)-2-eth-(E)-ylidene-1,1-diphenyl-hexa-3,5-dienylsulfanyl]-hept-4-enoic Acid 2-Trimethylsilanyl-ethyl Ester (13). To a stirred solution of **12** (100 mg, 0.12 mmol) and **6** (74 mg, 0.14 mmol) in anhydrous dichloromethane (4 mL) cooled in an ice bath was added MSNT (90 mg, 0.30 mmol) and 1-methylimidazole (28 uL, 35 mmol). After stirring for 5 h, the solution was warmed to room temperature and stirred for 100 min, followed by addition of sat aq NH_4Cl (5 mL). The phases were separated, and the aqueous phase extracted with dichloromethane (10 mL × 3). The combined organic phases were washed with brine (5 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/5 - 1/1.5) to give recovered **6** (30.6 mg, 42%) and **13** (53.6 mg, 34%) as a white solid: $[\alpha]^{25}_D$ −0.97 (c, 0.52, $CHCl_3$); mp 95−100 °C; IR 3306, 1731, 1644, 1489 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, 2 H, J = 7.5 Hz), 7.52 (d, 2 H, J = 7.1 Hz), 7.12−7.40 (m, 35 H), 6.62 (m, 2 H), 6.29 (brs, 1 H), 5.52−5.65 (m, 2 H), 5.32 (dd, 1 H, J = 7.1 Hz, 15.6 Hz), 5.24 (m, 1 H), 4.48 (dd, 1 H, J = 5.5 Hz, 8.5 Hz), 4.40 (dd, 1 H, J = 7.1, 10.8 Hz), 4.23 (m, 1 H), 4.00−4.13 (m, 4 H), 3.92 (m, 1 H), 2.89 (m, 1 H), 2.58−2.70 (m, 2 H), 2.50 (dd, 1 H, J = 6.2, 15.8 Hz), 1.94−2.18 (m, 6 H), 1.68 (d, 3 H, J = 6.0 Hz), 0.75−0.95 (m, 14 H), 0.00 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 170.8, 169.8, 168.6, 163.9, 156.9, 145.0, 144.3, 141.4, 133.6, 129.7, 129.6, 128.3, 128.0, 127.9, 127.2, 127.1, 126.7, 125.1, 125.1, 120.1, 71.6, 67.6, 67.4, 66.7, 63.2, 60.6, 57.6, 53.1, 47.3, 39.8, 32.9, 31.5, 31.4, 31.2, 30.7, 19.4, 19.1, 18.0, 17.8, 17.4, 13.7, −1.4; MS *m/z* 1390 (M + Na^+).

(*E*)-(*S*)-7-[(*Z*)-2-Eth-(*E*)-ylidene-1,1-diphenyl-hexa-3,5-dienylsulfanyl]-3-[(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyryloxy]-hept-4-enoic Acid 2-Trimethylsilanyl-ethyl Ester (14). To a stirred solution of Fmoc-L-Val-OH (297 mg, 0.875 mmol) and **2** (378 mg) in anhydrous dichloromethane (8 mL) cooled in an ice bath was added DAMP (8 mg) and DCC (196 mg, 0.95 mmol). After stirring for 3 h, the solution was filtered and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/10−1/5) to give **14** (522 mg, 85%) as a colorless oil: $[\alpha]^{25}_D$ 15 (c, 0.69, $CHCl_3$); IR 1712, 1219 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, 2 H, J = 7.5 Hz), 7.57 (m, 2 H), 7.20−7.38 (m, 19 H), 5.58−5.70 (m, 2 H), 5.34 (dd, 1 H, J = 7.5, 15.5 Hz), 5.25 (d, 1 H, J = 9.0 Hz), 4.30−4.42 (m, 2 H), 4.16 − 4.29 (m, 2 H), 4.05−4.15 (m, 2 H), 2.64 (dd, 1 H, J = 8.0, 16.0 Hz), 2.53 (dd, 1 H, J = 5.5, 12.0 Hz), 2.08 −2.20 (m, 3 H), 2.01 (m, 2 H), 0.90−0.98 (m, 2 H), 0.89 (d, 3 H, J = 6.5 Hz), 0.78 (d, 3 H, J = 7.0 Hz), 0.00 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 169.8, 156.3, 145.0, 144.8, 144.1, 144.0, 141.5, 134.2, 129.7, 128.0, 127.9, 127.2, 126.8, 125.3, 120.1, 72.0, 67.2, 66.8, 63.3, 58.9, 47.4, 39.8, 31.6, 31.5, 31.3, 19.1, 17.5, 17.5, −1.4; MS *m/z* 858 (M + NH^+), 863 (M + Na^+); HRMS *m/z* calcd for [M + $Na]^+$ $C_{51}H_{57}NNaO_6SSi$ 862.3568, found 862.3546.

(2*S*,3*R*)-2-[(*S*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-tritylsulfanyl-propionylamino]-3-hydroxy-butyric Acid Methyl Ester (15). To a stirred solution of Fmoc-D-Cys(Tr)-OH (4.00 g, 6.83 mmol) in anhydrous dichloromethane (50 mL) cooled in an ice bath was added PyBOP (4.00 g, 7.68 mmol), *N*,*N*-diisopropylethylamine (3.00 mL, 17.22 mmol), and then L-Thr-OMe.HCl (1.27 g, 7.49 mmol). After stirring for 1.5 h, the reaction mixture was worked up following the general procedure. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/2−2/1) to give **15** (4.62 g, 97%) as a white solid: $[\alpha]^{25}_D$ 0.6 (c, 0.75, $CHCl_3$); mp 90−95 °C; IR 3356 (brs), 1727, 1667, 1216 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (t, 2 H, J = 7.2 Hz), 7.56 (d, 2 H, J = 7.5 Hz), 7.36−7.48 (m, 8 H), 7.16−7.30 (m, 11 H), 6.56 (d, 1 H, J = 9.0 Hz), 5.08 (d, 1 H, J = 7.5 Hz), 4.49 (dd, 1 H, J = 2.5, 8.5 Hz), 4.37 (d, 2 H, J = 7.0 Hz), 4.18−4.27 (m, 2 H), 3.88 (q, 1 H, J = 6.5 Hz), 3.69 (s, 3 H), 2.67 (d, 2 H, J = 6.5 Hz), 1.14 (d, 3 H, J = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.5, 144.4, 143.8, 143.7, 141.4, 129.6, 128.2, 127.8, 127.2, 127.0, 125.1, 120.1, 68.2, 67.5, 67.2, 57.3, 54.2, 52.7, 47.2, 34.0, 20.1; MS *m/z* 723 (M + Na^+); HRMS *m/z* calcd for [M + $Na]^+$ $C_{42}H_{40}N_2NaO_6S$ 723.2499, found 723.2487.

(2*S*,3*R*)-2-{(*S*)-2-[(*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-3-tritylsulfanyl-propionylamino}-3-hydroxy-butyric Acid Methyl Ester (16). At room temperature, to a stirred solution of **15** (4.62 g, 6.59 mmol) in anhydrous dichloromethane (32 mL) was added piperidine (3.2 mL, 32.4 mmol). After stirring for 70 min, the solution was concentrated, and the residue purified by flash chromatography (eluent: EtOAc/Hexane = 1/4−1/2, then MeOH/dichloromethane = 1/10) to give an amine (2.95 g) which was used in the next step directly. To a stirred solution of the above amine and Fmoc-D-Val-OH (2.23 g, 6.59 mmol) in anhydrous dichloromethane (50 mL) cooled in an ice bath was added PyBOP (3.80 g, 7.30 mmol) and *N*,*N*-diisopropylethylamine (1.72 mL, 9.87 mmol). After stirring overnight at room temperature, the reaction mixture was worked up following the general procedure. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/2−2/1) to give **16** (4.30 g, 82%) as a white solid: $[\alpha]^{25}_D$ 5.0 (c, 0.50, $CHCl_3$); mp 148−150 °C; IR 3357 (brs), 1727, 1667, 1216, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$:CD_3OD = 9:1) δ 7.76 (d, 2 H, J = 7.3 Hz), 7.60 (dd, 2 H, J = 7.3, 11.7 Hz), 7.17−7.42 (m, 19 H), 4.22−4.45 (m, 5 H), 4.19 (t, 1 H, J = 6.3 Hz), 3.90 (d, 1 H, J = 5.5 Hz), 3.67 (s, 3 H), 2.67 (dd, 1 H, J = 5.4, 12.6 Hz), 2.57 (dd, 1 H, J = 8.4, 12.6 Hz), 2.05 (m, 1 H), 1.16 (d, 3 H, J = 6.6 Hz), 0.95 (d, 3 H, J = 6.9 Hz), 0.93 (d, 3 H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD = 9:1) δ 171.9, 171.4, 170.6, 157.4, 144.3, 143.8, 143.5, 141.2, 129.4, 127.9, 127.7, 127.0, 126.7, 124.9, 119.9, 67.5, 67.1, 66.9, 61.0, 58.0, 52.3, 52.3, 47.0, 33.1, 30.5, 19.5, 19.0, 17.7; MS *m/z* 822 (M + Na^+); HRMS *m/z* calcd for [M + $Na]^+$ $C_{47}H_{49}N_3NaO_7S$ 822.3183, found 822.3167.

(*Z*)-2-[(*S*)-2-((*R*)-2-Amino-3-methyl-butyrylamino)-3-tritylsulfanyl-propionylamino]-but-2-enoic Acid Methyl Ester (17). To a stirred solution of **16** (4.2 g, 5.25 mmol) in anhydrous dichloromethane (45 mL) cooled in an ice bath was added DMAP (32 mg, 0.26 mmol), Et_3N (1.2 mL, 8.60 mmol) and methanesulfonyl chloride (0.53 mL, 6.82 mmol). After stirring for 1 h, the reaction was quenched by adding sat aq NH_4Cl (20 mL). The phases were separated, and the aqueous phase extracted with dichloromethane (25 mL × 3). The combined organic phases were washed with sat aq NH_4Cl (15 mL) and brine (15 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/2−2/1) to give the mesylate of **16** (3.81 g, 83%) as a white solid: $[\alpha]_D^{25}$ 26.8 (c, 0.52, $CHCl_3$); mp 158−160 °C. To a stirred solution of the mesylate (3.50 g, 3.98 mmol) in anhydrous dichloromethane (40 mL) cooled in an ice bath was added DABCO (4.47 g, 39.8 mmol). After stirring for 10 min, the solution was warmed to room temperature and stirred for another 1.5 h. Piperidine (2.0 mL) was then added, and the reaction mixture stirred for 40 min. The solution volume was concentrated to one-third, and the residue purified by flash chromatography (eluent: EtOAc/Hexane = 1/3−1/2, then MeOH/dichloromethane = 1/20−1/10) to give **17** as a white glass (1.99 g, 89%): $[\alpha]^{25}_D$ 28.7 (c, 0.52, $CHCl_3$); IR 3312 (brs), 1651, 1489, 1273 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, 1 H, J = 8.0 Hz), 7.58 (s, 1 H), 7.18−7.46 (m, 15 H), 6.74 (q, 1 H, J = 7.0 Hz), 4.15 (q, 1 H, J = 7.0 Hz), 3.69 (s, 3 H), 3.22 (d, 1 H, J = 4.0 Hz), 2.67 (d, 2 H, J = 7.0 Hz), 2.24 (m, 1 H), 1.70 (d, 3 H, J = 7.0 Hz), 0.96 (d, 3 H, J = 7.0 Hz), 0.80 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 168.6, 164.6, 144.6, 134.3, 129.7, 128.1, 126.9, 126.0, 67.2, 60.2, 52.4, 52.3, 33.0, 30.9, 19.7, 16.3, 14.7; MS m/z 560 (M + H^+), 582 (M + Na); HRMS m/z calcd for [M + $Na]^+$ $C_{32}H_{37}N_3NaO_4S$ 582.2397, found 582.2404.

(*Z*)-2-{(*S*)-2-[(*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]-3-tritylsulfanyl-propionylamino}-but-2-enoic Acid Methyl Ester (18). To a stirred solution of **17** (1.04 g, 1.85 mmol) in THF (12 mL) cooled in an ice bath was added an aqueous solution of LiOH (109 mg, 4.55 mmol) in H_2O (4 mL). After stirring for 5 h, the solution was neutralized by adding 1 M aq HCl, followed by addition of $NaHCO_3$ (311 mg, 3.7 mmol) and Fmoc-Cl (622 mg, 2.40 mmol). After stirring for 1 h, the solution was acidified to pH 3−4 with 1 M HCl, and diluted with EtOAc (20 mL). The phases were separated, and the aqueous phase extracted with EtOAc (15 mL × 3). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/1, then MeOH/dichloromethane = 1/30−1/15) to give **18** (0.91 g, 64%) as a white solid: $[\alpha]^{25}_D$ 33.8 (c, 0.61, $CHCl_3$); IR 3295 (brs), 1678, 1651, 1237 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, 2 H, J = 7.5 Hz), 7.45−7.55 (m, 3 H), 6.90−7.36 (m, 20 H), 6.78 (q, 1 H, J = 7.0 Hz), 5.87 (d, 1 H, J = 7.5 Hz), 4.32 (m, 1H), 4.20 (m, 1 H), 4.02−4.12 (m, 2 H), 3.91 (brs, 1 H), 2.59 (d, 2 H, J = 6.0 Hz), 1.87 (m, 1 H), 1.64 (d, 3 H, J = 7.0 Hz), 0.75 (brs, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 166.7, 156.7, 144.4, 144.0, 143.9, 141.4, 136.1, 129.6, 128.2, 127.8, 127.2, 127.0, 125.4, 120.1, 67.4, 67.3, 60.3, 52.8, 52.7, 47.3, 32.9, 31.5, 19.2, 18.0, 15.0; MS m/z 791 (M + Na^+), 767 (M + H^+); HRMS m/z calcd for [M + $Na]^+$ $C_{46}H_{45}N_3NaO_6S$ 790.2921, found 790.2918.

(*E*)-(*S*)-3-((*S*)-2-{(*Z*)-2-[(*S*)-2-((*R*)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino)-3-tritylsulfanyl-propionylamino]-but-2-enoylamino}-3-methyl-butyryloxy)-7-[(*Z*)-2-eth-(*E*)-ylidene-1,1-diphenyl-hexa-3,5-dienylsulfanyl]-hept-4-enoic Acid 2-Trimethylsilanyl-ethyl Ester (13) (Second Method). To a stirred solution of **14** (362 mg, 0.43 mmol) in anhydrous dichloromethane (5 mL) cooled in an ice bath was added piperidine (0.64 mL, 6.45 mmol). After stirring for 30 min, the solution was warmed to room temperature, stirred for 1 h, and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/5, then MeOH/dichloromethane = 1/20−1/10) to give the free amine (0.24 g, 89%) as a solid. To a stirred solution of **18** (342 mg, 0.445 mmol) and the above amine (230 mg, 0.372 mmol) in anhydrous dichloromethane (6 mL) cooled in an ice bath was added PyBOP (232 mg, 0.445 mmol) and *N*,*N*-diisopropylethylamine (0.1 mL, 0.574 mmol). After stirring for 1 h, the solution was warmed to room temperature and stirred overnight. The reaction mixture was worked up following the general procedure, and the residue purified by flash chromatography (eluent: EtOAc/Hexane = 1/3−1/1) to give **13** (356 mg, 84%) as a white solid: $[\alpha]^{25}_D$ −1.12 (c, 0.4, $CHCl_3$), other data as above.

(3*S*,9*S*,12*R*,16*S*)-6-Eth-(*Z*)-ylidene-3,12-diisopropyl-16-((*E*)-4-tritylsulfanyl-but-1-enyl)-9-tritylsulfanylmethyl-1-oxa-4,7,10,13-tetraaza-cyclohexadecane-2,5,8,11,14-pentaone (19). At room temperature, to a stirred solution of **13** (305 mg, 0.223 mmol) in anhydrous dichloromethane (5 mL) was added piperidine (0.5 mL). After stirring for 1 h, the solution was concentrated, and the residue purified by flash chromatography (eluent: EtOAc/Hexane = 1/3−1/1, then MeOH/dichloromethane = 1/20−1/10) to give the amine (182 mg, 70%) as an oil. At room temperature, to a stirred solution of the above amine (174 mg, 0.152 mmol) in anhydrous THF (4 mL) was added 1 M TBAF (0.22 mL, 0.220 mmol). After stirring for 1 h 40 min, the solution was concentrated, and the residue dried by high vacuum. The crude amino acid was dissolved in anhydrous dichloromethane (35 mL). To a vigorously stirred solution of HATU (144 mg, 0.378 mmol) and *N*,*N*-diisopropylethylamine (0.10 mL, 0.574 mmol) in anhydrous dichloromethane (40 mL) was added the preprepared solution above slowly over 4 h. After stirring overnight at room temperature, sat aq NH_4Cl (20 mL) was added and the phases separated. The aqueous phase was extracted with dichloromethane (20 mL × 3), and the combined organic phases were washed with brine (20 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/Hexane = 1/4−1/1−2/1) to give compound **19** as a white solid (120 mg, 77%): ^1H NMR (400 MHz, $CDCl_3$:CD_3OD = 9:1) δ 7.20−7.42 (m, 30 H), 6.37 (q, 1 H, J = 7.0 Hz), 5.78 (m, 1 H), 5.56 (m, 1 H), 5.36 (dd, 1 H, J = 7.5, 15.6 Hz), 4.04 (d, 1 H, J = 7.5 Hz), 3.83 (d, 1 H, J = 9.0 Hz), 3.60 (m, 1 H), 2.84 (dd, 1 H, J = 8.5, 13.1 Hz), 2.62−2.70 (m, 2 H), 2.45 (m, 1 H), 2.06−2.30 (m, 4 H), 2.00 (m, 2 H), 1.89 (m, 1 H), 0.95 (d, 6 H, J = 6.5 Hz), 0.88 (d, 3 H, J = 7.0 Hz), 0.81 (d, 3 H, J = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD = 9:1) δ 177.1, 173.0, 172.2, 170.7, 170.4, 144.8, 144.2, 132.7, 129.5, 129.4, 128.0, 127.9, 127.8, 126.9, 126.6, 118.9, 71.9, 67.4, 66.6, 60.4, 58.9, 53.8, 40.2, 32.0, 31.9, 31.1, 30.3, 29.6, 19.2, 19.0, 18.9, 18.4, 12.8; MS m/z: 1028 (M + H^+), 1045 (M + NH^+), 1050 (M + Na^+).

(*E*)-(1*S*,4*S*,10*S*,21*R*)-7-Eth-(*Z*)-ylidene-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraaza-bicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentaone (FK228). At room temperature, to a vigorously stirred solution of iodine (284 mg, 1.12 mmol) in MeOH/dichloromethane (1/10, 220 mL) was added a solution of **19** (115 mg, 0.112 mmol) in MeOH/dichloromethane (1/10, 110 mL) over 1 h. After stirring for 30 min, the solution was cooled down in an ice bath, and 0.1 M $Na_2S_2O_3$ (25 mL) added followed by brine (25 mL). The phases were separated, and the aqueous phase extracted with dichloromethane (50 mL × 3). The combined organic phases were washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (eluent: 1/2−/10/1) to give FK228 (30 mg, 50%) as a white solid: $[\alpha]^{25}_D$ 34 (c, 0.17, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$:CD_3OD=9:1) δ 6.20 (q, 1 H, J = 7.0 Hz), 5.50−5.60 (m, 3 H), 4.55 (dd, 1 H, J = 5.0, 11.0 Hz), 4.37 (d, 1 H, J = 4.0 Hz), 3.83 (m, 1 H), 2.95−3.05 (m, 3 H), 2.80−2.90 (m, 1 H), 2.70 (m, 1 H), 2.45−2.58 (m, 3 H), 2.22 (m, 1 H), 2.05 (m, 1 H), 1.59 (d, 3 H, J = 7.0 Hz), 0.98 (d, 3 H, J = 7.0 Hz), 0.96 (d, 3 H, J = 7.0 Hz), 0.88 (d, 3 H, J = 7.0 Hz), 0.85 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD=9:1) δ 172.4,

171.6, 169.4, 168.5, 165.8, 130.4, 129.8, 129.3, 70.3, 62.5, 58.3, 56.7, 37.9, 37.7, 34.7, 31.7, 30.1, 29.0, 19.2, 19.0, 18.4, 18.2, 13.2; MS *m*/*z* 541 ($M + H^+$), 563 ($M + Na^+$).

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Supporting Information Available: General experimental methods and NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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