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Article

Structure–Activity Relationships for the Marine Natural Product Sintokamides: Androgen Receptor N-Terminus Antagonists of Interest for Treatment of Metastatic Castration-Resistant Prostate Cancer

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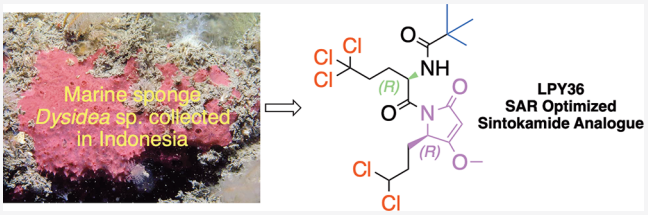
ABSTRACT: Synthetic analogues of the marine natural product sintokamides have been prepared in order to investigate the structure–activity relationships for the androgen receptor N-terminal domain (AR NTD) antagonist activity of the sintokamide scaffold. An *in vitro* LNCaP cell-based transcriptional activity assay with an androgen-driven luciferase (Luc) reporter was used to monitor the potency of analogues. The data have shown that the chlorine atoms on the leucine side chains are essential for potent activity. Analogues missing the nonchlorinated methyl groups of the leucine side chains (C-1 and C-17) are just as active and in some cases more active than the natural products. Analogues with the natural *R* configuration at C-10 and the unnatural *R* configuration at C-4 are most potent. Replacing the natural propionamide N-terminus cap with the more sterically hindered pivaloylamide N-terminus cap leads to enhanced potency. The tetramic acid fragment and the methyl ether on the tetramic acid fragment are essential for activity. The SAR optimized analogue **76** is more selective, easier to synthesize, more potent, and presumed to be more resistant to proteolysis than the natural sintokamides.



Prostate cancer (PC) is rare in men before age 40 and mostly encountered in men older than 65. It is the second leading cause of cancer death in American men after lung cancer.[1] Odds are that one man in nine will be diagnosed with PC in their lifetime, but since treatments for localized early stages of the disease are highly effective and the disease usually progresses slowly, most men diagnosed with PC will not die from it. The American Cancer Society estimates that there will be approximately 191 900 newly diagnosed cases and 33 330 deaths from PC in the USA in 2020.[1]

Localized PC is treated with radical prostatectomy surgery, radiation, or active surveillance.[2] Roughly 20–30% of patients receiving intent to cure treatments for localized PC will have recurrence of their disease as indicated by a rise in serum levels of prostate-specific antigen (PSA). The growth of most PC tumors is driven by the androgen receptor (AR), which is a ligand-activated transcription factor comprised of a ligand binding domain (LBD), a hinge region (HR), a DNA binding domain (DBD), and an N-terminal domain (NTD).[3] Patients whose disease has recurred after surgery or radiation are treated with androgen deprivation therapy (ADT) in order to reduce the levels of the androgens testosterone and 5α-dihydrotestosterone (DHT) in their bodies, thereby reducing activation of the AR by reducing its ligand titer. There are several types of ADT for treating PC, including orchiectomy (surgical castration), administration of luteinizing hormone releasing hormone (LHRH) agonists (chemical castration), the use of the CYP17 inhibitor abiraterone to prevent the synthesis of testosterone and DHT,[4] and administration of nonsteroidal antiandrogens such as bicalutamide and enzalutamide.[5] ADT effectively reduces serum levels of PSA and reduces tumor burden, leading to a significant period of progression-free disease.

Unfortunately, all current ADTs that target the AR LBD will eventually fail in patients with advanced disease, leading to metastatic castration-resistant prostate cancer (mCRPC), characterized by a rise in PSA levels and increased tumor burden.[6,7] mCRPC is lethal, and most men die from their cancer within 2–3 years of reaching this stage. Since PSA is an androgen-regulated gene that is dependent on AR activation, the rising PSA in mCRPC suggests continued AR transcriptional activity in the absence of testicular androgens.[1] AR splice variants that are missing the LBD and are constitutively active[7]

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are now recognized as an important source of androgen-independent AR transcriptional activity in mCRPC.[8] Transcriptional activity of both the AR splice variants and the full-length AR, with or without androgens, requires binding of coactivators such as CREB binding protein (CBP) to the activation function-1 (AF1) region of the androgen receptor N-terminal domain (AR NTD). Interleukin 6 (IL6) and protein kinase A (PKA) signal transduction pathways converge on the AF1 region of the AR NTD and are also known to drive transcriptional activity of full-length AR in the absence of androgens and may contribute to CRPC tumor growth. Sadar was the first to propose that small molecules able to bind to the AF1 region of the AR NTD should block activation resulting from binding of co-activating proteins such as CBP and signal and transducer and activator of transcription 3 (STAT3), IL6 signaling, and possible altered phosphorylation states of the AR NTD or interacting co-regulators and, therefore, overcome the most common mechanisms of ADT resistance that lead to mCRPC.[9]

We have used LNCaP human PC cells expressing a functional full-length AR that drives proliferation and gene expression in response to androgens or forskolin and have AR-driven luciferase (Luc) reporter gene constructs such as PSA (6.1 kb)-luciferase to screen a library of marine sponge extracts for the presence of AR NTD antagonists able to block AR transcriptional activity *in vitro*. To date, we have identified three distinct chemical scaffolds exemplified by niphatenones A (**1**) and B (**2**),[10] ralaniten (EPI-002) (**3**),[11,12] and the sintokamides A (**4**) to E (**8**)[13,14] that possess this novel AR NTD antagonist activity (Figure 1). The chemistry and biology of the niphatenone and ralaniten scaffolds have been reported in detail elsewhere.[10−12] EPI-506 (**9**) (ralaniten acetate), an acetate prodrug analogue of ralaniten, was evaluated in a phase I/II clinical trial for the treatment of mCRPC.[2,15] The trial was terminated due to lack of potency and metabolic stability of ralaniten acetate. However, there were patients in the trial with stable disease and drops in PSA, which provided preliminary clinical proof of concept for the AR NTD as a promising drug target for treatment of mCRPC.[15b]

Sintokamides A (**4**) to E (**8**) are a family of highly chlorinated modified dipeptides isolated from a *Dysidea* sp. of sponge collected in Indonesia.[13] They were the first small molecules reported to inhibit transactivation of the N-terminus of the AR in PC cells. Sintokamide A (**4**) does not inhibit the transcriptional activity of the closely related progesterone and glucocorticoid steroid receptors, indicating selective activity for blocking the AR.[15] The sintokamides have been shown to bind covalently in low yield to the AR AF1 region, and sintokamide A (**4**) inhibits the transcriptional activity of both AR splice variants and full-length AR. Consistent with these findings, sintokamide A (**4**) inhibits the growth of enzalutamide-resistant LNCaP95 PC cells driven by AR splice variants. Sintokamide A (**4**) caused regression of CRPC xenografts in mice via intratumoral administration and reduced expression of PSA in the tumors. Ralaniten (**3**) and sintokamide A (**4**) show additive inhibition of AR transcriptional activity, suggesting that they bind to different sites on the AR NTD AF1 region. Ralaniten (**3**) binds to the transcriptional activation unit 5 (tau5) region of the AF1, and Sadar has proposed that the sintokamides may bind to the tau1 region of the AR NTD.[14]

The *in vitro* and *in vivo* biological activities of sintokamide A (**4**) make it a promising lead compound for the development of a new structural class of "natural product inspired" drugs to treat mCRPC. In order to further the preclinical evaluation and development of the sintokamides, a synthetic program was undertaken to define and optimize the required structural elements of the sintokamide AR NTD blocking pharmacophore, generate simpler analogues that are as potent as the natural products, and develop a scalable synthetic route in order to solve the supply of compound for further *in vitro* and *in vivo* animal studies. The results of these synthetic efforts are described below.

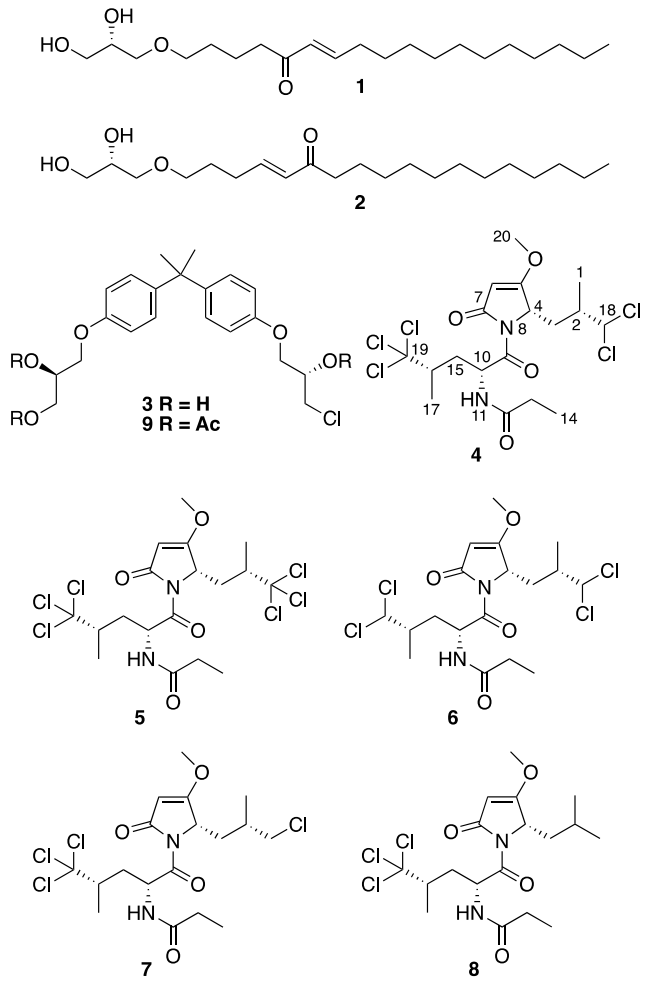

Figure 1. AR NTD antagonists.

RESULTS AND DISCUSSION

Synthesis of Sintokamide Analogues. There were no published synthetic routes to the sintokamides when we started our program. Total syntheses of sintokamides A (**4**), B (**5**), and E (**8**) by Gu and Zakarian[16] and a total synthesis of sintokamide C (**6**) by Xu, Ye, and co-workers[17] were reported while our work was in progress, and these syntheses provided useful guidance for our efforts.

The most striking chemical feature of the sintokamides is the presence of the chlorinated methyl groups on the leucine-derived side chain fragments of the modified dipeptide. Chlorination at C-18 and C-19 in the sintokamides generates stereogenic centers at C-2 and C-16 that do not exist in the nonchlorinated version of the dipeptide, increasing the number of possible stereoisomers in the natural products to 16 compared with four stereoisomers in the nonchlorinated

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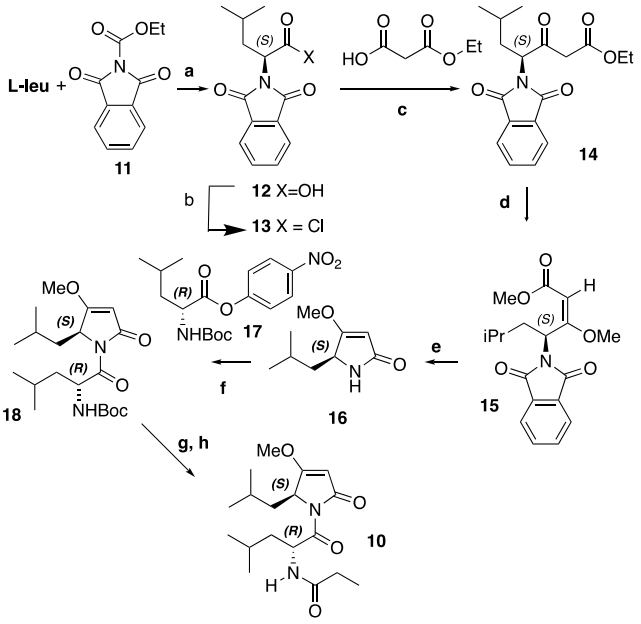
version. While there is methodology in the two published total syntheses[16,17] and other literature reports[18] that enables the enantioselective synthesis of the chiral chlorinated leucine side chain fragments, a drug candidate requiring the introduction of four independent stereogenic centers in high ee would be a challenge for scale-up manufacturing. Therefore, the first objectives of the synthetic program were (i) to see if chlorination in the side chain fragments was required for AR NTD blocking biological activity and (ii) to determine if simplifying the synthetic complexity of making chlorinated sintokamides by removing Me-1 and Me-17 to give 1,17-bisnorsintokamides that had only two stereogenic centers could still generate analogues with potency and selectivity comparable to the natural products. Secondary objectives were to examine the effects of different configurations at C-4 and C-10, the necessity of the tetramic acid core, and the possibility of adding different N-terminus capping residues to inhibit potential amide hydrolysis.

The synthesis of the nonchlorinated sintokamide (NCSTD) **10** shown in Scheme 1 followed Williard and de Laszlo's

Scheme 1. Synthesis of the Nonchlorinated Sintokamide 10 (NCSTD)[a]

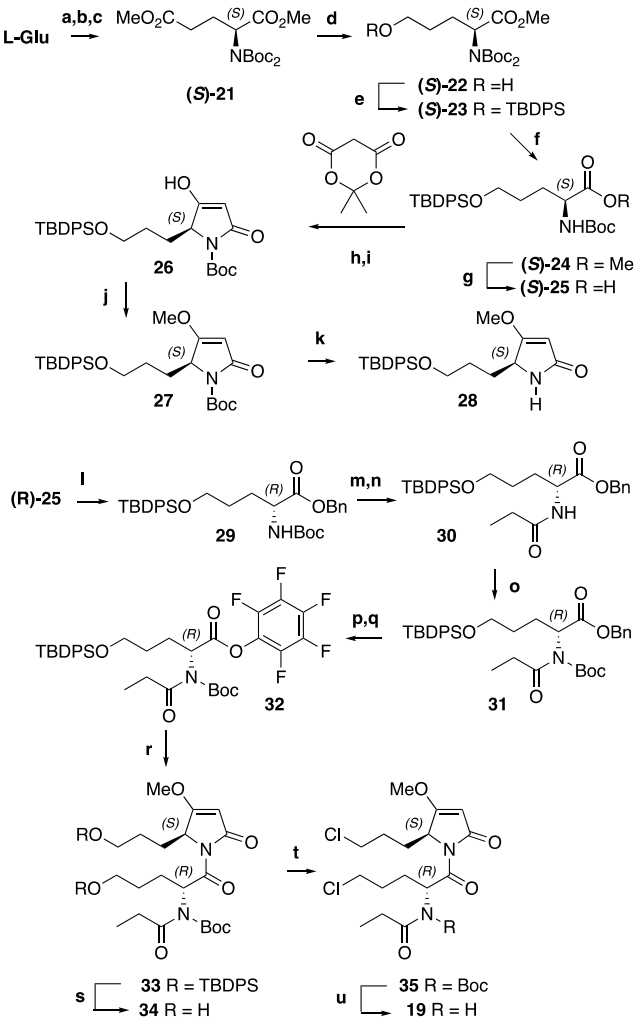


[a]Reagents and conditions: (a) Na_2CO_3; (b) $SOCl_2$; (c) *n*-BuLi, 2 equiv; (d) $HC(OCH_3)_3$, H^+; (e) H_2NNH_2; (f) *n*-BuLi; (g) TFA, CH_2Cl_2; (h) $(EtCO)_2O$, py.

published synthesis of the sponge natural product dysidin.[19] L-Leucine, *N*-carbethoxyphthalimide (**11**), and Na_2CO_3 were stirred in H_2O to give *N*-phthaloyl-L-leucine (**12**) in quantitative yield.[20] Reaction of acid **12** with $SOCl_2$ gave the acid chloride **13** in high yield. Homologation of acid chloride **13** with the dianion of monoethylmalonate[21] gave the desired *β*-ketoester **14**, which was methylated using trimethyl orthoformate and a catalytic amount of H_2SO_4 in MeOH to give enol ether **15**.[22] Treatment of **15** with refluxing hydrazine removed the phthaloyl protecting group, and subsequent cyclization in the same pot gave the tetramic acid **16**.[23] Deprotonation of **16** with *n*-butyllithium followed by reaction with the *p*-nitrophenylester of Boc-D-leucine **17**[24] gave the desired *N*-acylated tetramate **18**. Removal of the Boc protecting group in **18** with TFA followed by reaction with propionic acid anhydride in pyridine gave NCSTD (**10**).

The first chlorinated target molecule was (4*S*,10*R*)-18,19-dichloro-1,17-bisnorsintokamide (**19**), which has single chlorine atoms on each of the side chain appendages. Scheme 2 shows the synthetic route to **19**. The strategy was to use

Scheme 2. Synthesis of (4*S*,10*R*)-18,19-Dichloro-1,17-bisnorsintokamide (19)[a]



[a]Reagents and conditions: (a) MeOH, TMSCl; (b) Boc_2O, Et_3N; (c) Boc_2O, DMAP; (d) DIBAL-H; (e) TBDPS-Cl, imidazole; (f) LiBr; (g) 0.5 M $Ba(OH)_2$; (h) DCC, DMAP; (i) EtOAc, reflux; (j)$TMSCHN_2$; (k) TFA, CH_2Cl_2; (l) CbzCl, DMAP; (m) TFA; (n) C_2H_5COCl; (o) Boc_2O, DMAP; (p) H_2, Pd/C; (q) pentafluorophenol, DCC; (r) **28**, LiHMDS; (s) HF, py; (t) CCl_4, PPh_3; (u) TFA, CH_2Cl_2.

glutamic acid residues as synthons to make a 1,17-bisnorsintokamide intermediate **34** with primary alcohols on each side chain fragment that could be mono- or dichlorinated at a late stage in the synthesis after the 1,17-bisnorsintokamide scaffold had been assembled.

The synthesis of **19** started with the protected glutamic acid (*S*)-**21** prepared from (*S*)-glutamic acid using standard methodology. Reduction of the side chain methyl ester in **21** with DIBAL-H gave the primary alcohol **22**, which was

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silylated to give the TBDPS derivative **23**. Treatment of **23** with LiBr removed one of the Boc protecting groups to give **24**, and hydrolysis of the methyl ester in **24** with $Ba(OH)_2$ gave the carboxylic acid **25**. Activation of acid **25** with DCC followed by condensation with Meldrum's acid and refluxing a solution of the condensation product in EtOAc gave the Boc-protected tetramic acid **26**. Methylation of **26** with $TMSCHN_2$ gave the enol ether **27**, and removal of the Boc protecting group with TFA gave the tetramic acid **28**.

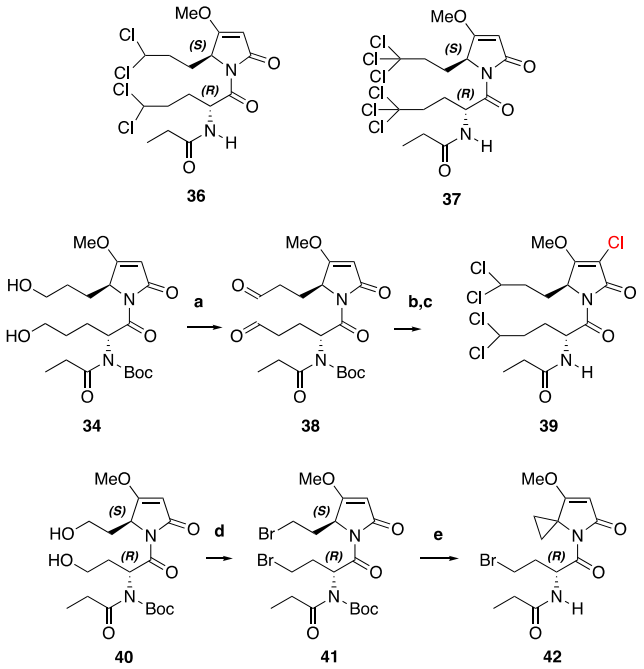
Synthesis of the second amino acid residue present in **19** started with the protected glutamic acid (*R*)-**25** prepared as described above for (*S*)-**25**. Reaction of acid (*R*)-**25** with CbzCl and DMAP gave the benzyl ester **29**.[25] Removal of the Boc protecting group in **29** with TFA followed by amide formation with propionyl chloride gave the propionamide **30**. Treatment of **30** with Boc_2O and DMAP gave the fully protected amino acid **31**. Hydrogenolysis converted the benzyl ester in **31** into the carboxylic acid, which was esterified with pentafluorophenol using DCC activation to give **32**.[26] Deprotonation of tetramic acid **28** using LiHMDS followed by addition to the pentafluorophenyl ester **32** gave the modified-dipeptide coupling product **33**. Removal of the TBDPS protecting group in **33** with HF in pyridine gave the desired 18,19-dihydroxy-1,17-bisnorsintokamide **34**, which was ready for chlorination. Diol **34** was subjected to standard Appel reaction conditions (CCl_4, PPh_3), resulting in the formation of the dichloro derivative **35**. Removal of the Boc protecting group in **34** with TFA gave the desired (4*S*,10*R*)-18,19-dichlororo-1,17-bisnorsintokamide (**19**).

With the dichloro analogue **19** in hand, we moved on to making 18,18,19,19-tetrachloro-1,17-bisnorsintokamide (**36**) and 18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide (**37**) (Scheme 3). Xu, Ye, and co-workers had shown that it was possible to convert an aldehyde functionality in the side chain of a sintokamide scaffold to a *gem*-dichloride functionality as a penultimate step in the total synthesis of sintokamide C.[17] As shown in Scheme 3, Dess Martin oxidation of the diol **34** proceeded to give the dialdehyde **38**, which corresponded to Xu, Ye, and co-workers' penultimate intermediate. Our attempt to chlorinate **38** using $P(OPh)_3/Cl_2$ followed by removal of the Boc protecting group with TFA did indeed generate the desired C-18 and C-19 *gem*-dichloro side chains in the product **39**. Unfortunately, **39** also had an unwanted chlorine atom at C-6 of the tetramic acid moiety, and we were not able to find conditions to prevent that unwanted chlorination, so an alternate approach to **36** was required.

A literature precedent showed that a trichloromethyl anion could displace a primary alkyl bromide to give a trichloromethyl alkyl fragment,[27] which seemed like a promising approach to the hexachloro-1,17-bisnorsintokamide **37**. In order to explore this reaction in the context of a synthesis of **37**, the intermediate **40** was prepared from *R* and *S* aspartic acid using the same methodology described above that was used to make **34** from *R* and *S* glutamic acid. Bromination of **40** using Appel reaction conditions (CBr_4, PPh_3) gave the desired dibromo compound **41**. Treatment of **41** with $CHCl_3$ and NaH in DMF as the literature had prescribed failed to generate any trichoromethyl-containing fragments, but instead gave the cyclopropane-containing product **42**, indicating that an alternate approach to **37** was also required.

The Zakarian route to the sintokamides,[16] which proceeds through a normal dipeptide, offered an efficient alternative

Scheme 3. Attempted Syntheses of 18,18,19,19-Tetrachloro-1,17-bisnorsintokamide (36) and 18,18,18,19,19,19-Hexachloro-1,17-bisnorsintokamide (37)[a]



[a]Reagents and conditions: (a) DMP; (b) $P(OPh)_3$, Cl_2; (c) TFA; (d) PPh_3, CBr_4; (e) $CHCl_3$, NaH, DMF.

approach to assembling the 1,17-bisnorsintokamide scaffold that had the advantage of making it possible to prepare analogues with different numbers of chlorine atoms on the two side chain fragments. Scheme 4 shows the application of this route to the synthesis of 18,18,19,19-tetrachloro-1,17-bisnorsintokamides (4*S*,10*R*)-**36** and (4*R*,10*R*)-**50**. The synthesis starts with preparation of the protected amino acids **43** and **45** from *S* and *R* glutamic acids, respectively. The key step in conversion of **43** and **45** to the protected amino acids **44** and **46** is the conversion of their side chain aldehydes to *gem*-dichloro functionalities by treatment with triphenylphosphite and chlorine.[28] The amino acids **44** and **46** were coupled with HOAt and EDCl to give the dipeptide **47**. The methyl ester in **47** was converted to the carboxylic acid by treatment with LiOH. Activation of the carboxylic acid by reaction with isopropenyl chloroformate and condensation of the resulting mixed anhydride with Meldrum's acid in the presence of DMAP, followed by refluxing the condensation product in MeCN, gave a mixture of the epimeric tetramic acids[16] that were methylated with $TMSCHN_2$ to give a mixture of **48** and **49**. Removal of the Boc protecting groups with TFA and capping the free amines via amide formation with propionyl chloride gave the desired (4*S*,10*R*)-18,18,19,19-tetrachloro-1,17-bisnorsintokamide **36** and its C-4 epimer **50**, which could be separated by Si gel chromatography.

The synthesis of (4*S*,10*R*)-18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide **37** and its C-4 epimer **57** shown in Scheme 5 follows the same dipeptide route used in the synthesis of the tetrachloro analogues **36** and **50** in all aspects except for the preparation of the protected trichloro amino acids **54** and **55**. Michael addition of the trichloromethyl anion to methyl acrylate[29] gave the 4,4,4-trichloromethylbutyrate **51** in good yield. Reduction of the ester **51** with DIBAL-H gave

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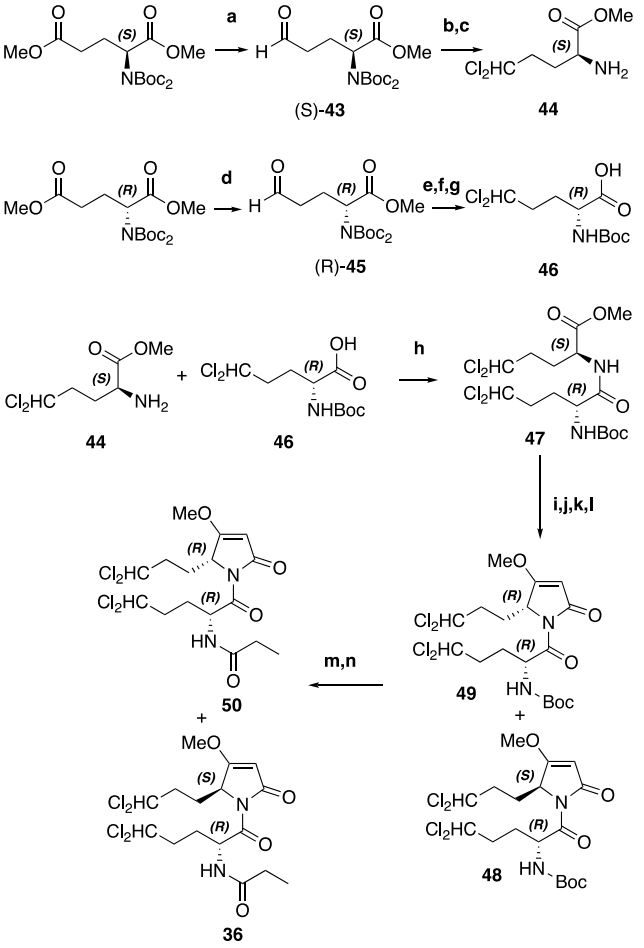
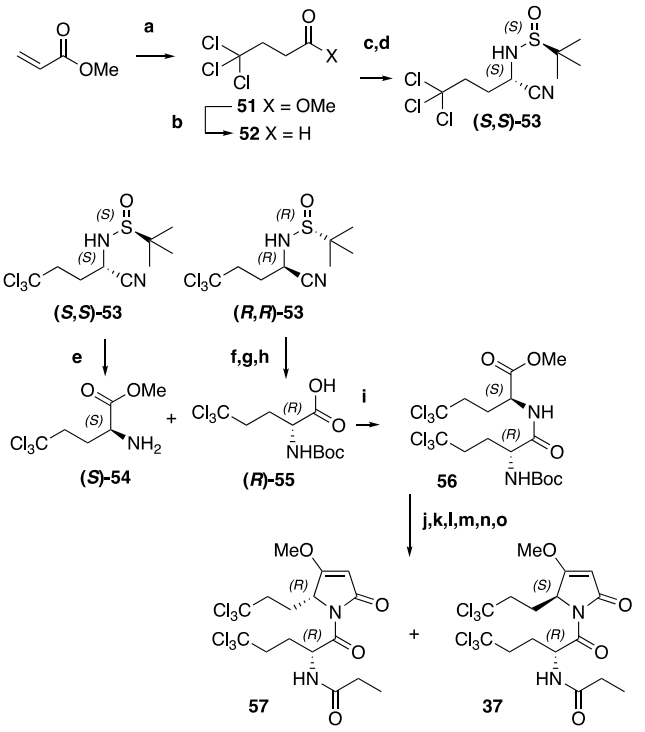
Scheme 4. Synthesis of (4*S*,10*R*)- and (4*R*,10*R*)-18,18,19,19-Tetrachloro-1,17-bisnorsintokamides 36 and 50[a]



[a]Reagents and conditions: (a) DIBAL-H; (b) $(PhO)_3P$, Cl_2, Et_3N; (c) TFA, CH_2Cl_2; (d) DIBAL-H; (e) $(PhO)_3P$, Cl_2, Et_3N; (f) LiBr; (g) 0.5 M $Ba(OH)_2$; (h) HOAt, EDCl; (i) 0.5 M LiOH; (j) Meldrum's acid, IPCF, DMAP; (k) MeCN, reflux; (l) $TMSCHN_2$; (m) TFA, CH_2Cl_2; (n) C_2H_5COCl, Et_3N.

Scheme 5. Synthesis of (4*S*,10*R*)-18,18,18,19,19,19-Hexachloro-1,17-bisnorsintokamide 37 and Its C-4 Epimer 57[a]



[a]Reagents and conditions: (a) $CHCl_3$, NaOH, $BnEt_3N^+Cl^-$; (b) DIBAL-H; (c) (*S*)-$NH_2SOC(CH_3)_3$, $CuSO_4$, CH_2Cl_2; (d) TMSCN, $Sc(OTf)_3$, CH_2Cl_2; (e) MeOH, HCl; (f) MeOH, HCl; (g) Boc_2O, $NaHCO_3$; (h) 0.5 M LiOH; (i) HOAt, EDCl; (j) 0.5 M LiOH; (k) Meldrum's acid, IPCF, DMAP; (l) MeCN, reflux; (m) $TMSCHN_2$; (n) TFA, CH_2Cl_2; (o) C_2H_5COCl, Et_3N.

the aldehyde **52**. Reaction of aldehyde **52** with (*S*)-*tert*-butanesulfinamide in the presence of a copper catalyst gave an *N*-*tert*-butanesulfinyl aldimine[30] that was converted to the nitrile (*S*,*S*)-**53** by treatment with trimethylsilylcyanide and scandium triflate in CH_2Cl_2. Methanolysis of nitrile (*S*,*S*)-**53** gave the methyl ester-protected amino acid (*S*)-**54**. Repeating the sequence using the (*R*)-*tert*-butanesulfinamide chiral auxiliary gave the nitrile (*R*,*R*)-**53**. Methanolyis of (*R*,*R*)-**53** gave the methyl ester (*R*)-**54**, which was Boc protected with Boc anhydride and hydrolyzed with LiOH to give the Boc-protected amino acid (*R*)-**55**. The protected amino acids (*S*)-**54** and (*R*)-**55** were converted into the desired (4*S*,10*R*)-18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide **37** and its C-4 epimer **57** as described above for **36** and **50**.

Using the methodology illustrated in Schemes 2, 4, and 5, we made a small library of 1,17-bisnorsintokamides with differing numbers of chlorine atoms on the two side chains, methylation or no methylation of the enol ether in the tetramic acid, differing N-terminus substituents, and different combinations of absolute configurations at C-4 and C-10 in order to probe additional aspects of the structure−activity relationship (SAR) for this scaffold. The compounds **58** to **76** that were made are shown in Figure 2.

Based on the bioactivity data discussed below, (4*R*,10*R*)-18,18,19,19,19-pentachloro-1,17-dinorsintokamide (**76**) [LPY36] was selected as the best candidate for *in vivo* evaluation in xenograft mouse models of mCRPC. In order to generate the amounts of **76** needed for animal studies, we optimized the synthesis of **76** for gram-scale production. Details are reported in the Supporting Information.

Structure−Activity Relationship for the Synthetic Sintokamide Analogues. The compounds synthesized to illuminate the SAR for the sintokamide scaffold were initially evaluated as AR NTD antagonists in a head-to-head "pairwise" or "small-group" fashion at either a single or a small number of concentrations in an LNCaP AR-driven transcriptional activity inhibition assay because (i) we needed relative biological activity data at each step of structural modification to guide our subsequent changes, (ii) there is sufficient variation in the results of this cell-based assay from experiment to experiment to make it difficult to effectively compare relative potencies using IC_{50} values determined in different experiments, and (iii) the assay could only handle small numbers of samples in each run due to limitations in producing large numbers of transfected cells at one time. Subsequently, IC_{50}'s of the most important compounds were determined in a single head-to-head experiment shown (see Figure 4) to confirm the SAR

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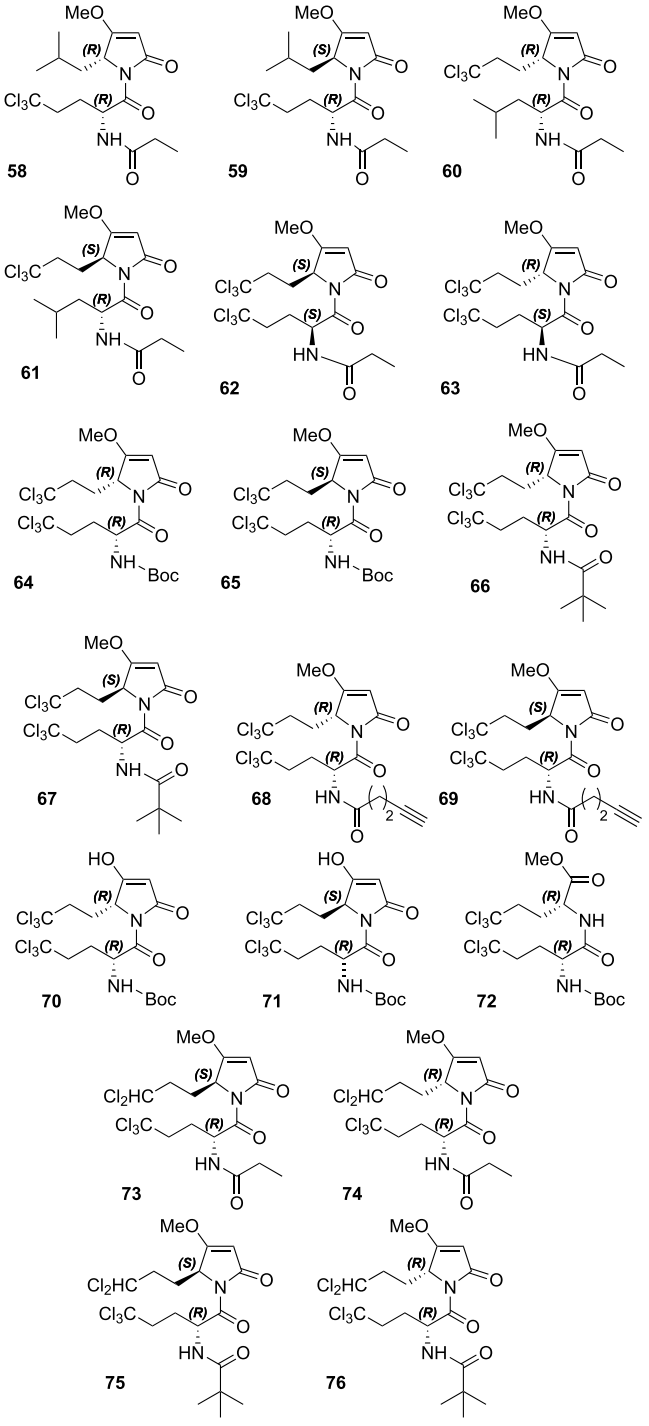

Figure 2. Synthetic 1,17-bisnorsintokamides **58**−**76**.

conclusions drawn from the results of the small group head-to-head assays shown in Figure 3.

Figure 3A shows that the positive controls bicalutamide and the penta- and hexachlorinated natural products sintokamide A (**4**) and sintokamide B (**5**) all effectively inhibited AR transcription at 5 or 10 μM, whereas NCSTD (**10**) was inactive at 5 μM and only very weakly active at 15 and 35 μM. These data demonstrated that the chlorine atoms in the natural sintokamides are an essential part of their AR NTD blocking pharmacophore. Compound **19** [LPY04], which is missing the C-1 and C-17 methyl groups in the natural sintokamide scaffold and has only one chlorine atom on each side chain appendage, was inactive at 10 μM (Figure 3A) and only weakly active at 25 and 50 μM, indicating that higher degrees of chlorination and/or the presence of the Me-1 and/or Me-17 groups were required for full potency. (4*S*,10*R*)-18,18,19,19-Tetrachloro-1,17-bisnorsintokamide **36** [LPY09] and its C-4 epimer **50** [LPY08] both showed clear dose−response curves (Figure 3A), but compared with the natural sintokamides A (**4**) and B (**5**), these compounds could be regarded as only weakly active. LPY08 (**50**), with the unnatural 4*R*,10*R* absolute configuration, was slightly more potent than LPY09 (**36**), which has the natural 4*S*,10*R* absolute configuration. (4*S*,10*R*)-18,18,18,19,19,19-Hexachloro-1,17-bisnorsintokamide **37** [LPY11] and its C-4 epimer **57** [LPY10] along with the hexachlorinated sintokamide B (**5**), as a positive control (Figure 3B), showed clear dose−response curves in the assay. Both **37** and **57** were slightly less potent than the most potent natural product sintokamide B (**5**). Interestingly, both LPY11 (**37**) (4*S*,10*R*) and LPY10 (**57**) (4*R*,10*R*) had nearly identical potencies at 5 μM. The data described above showed that chlorinated analogues of the 1,17-bisnorsintokamide scaffold were roughly as active as the natural products containing the branching methyl groups at C-2 and C-16, and they also suggested that at least one of C-18 or C-19 had to be trichlorinated for full potency.

Next we looked to answer whether trichlorination at only C-18 or C-19 on a norsintokamide scaffold was sufficient to generate full potency. (4*S*,10*R*)-18,18,18,19,19,19-Hexachloro-1,17-bisnorsintokamide **37** [LPY11], the bisnor analogue of the most potent natural product sintokamide B (**5**), was used as a reference compound. The epimeric analogues **58** [LPY12] and **59** [LPY13] with trichlorination only at C-19 and the epimeric analogues **60** [LPY17] and **61** [LPY18] with trichlorination at only C-18 were all similar in potency to each other and less potent than **5** [SINTB] at 20 μM in the same assay (Figure 3C). These data showed that trichlorination at either C-18 or C-19 is sufficient for activity but that maximum potency resulting from chlorination is only achieved by trichlorination at both C-18 and C-19 as found in LPY10 (**57**) and LPY11 (**37**).

The effect of absolute configuration at the two stereogenic centers C-4 and C-10 on the activity of the hexachlorinated analogues was probed by comparing the potency of the four stereoisomers of 18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide at 10 μM (Figure 3D). This comparison showed that the potencies of these four compounds were ranked **57** [LPY10] ≈ **37** [LPY11] > **62** [LPY20] ≈ **63** [LPY21], indicating that the natural (10*R*) configuration (**37** versus **62**; and **57** versus **63**) was clearly better than the unnatural (10*S*) configuration and that the (4*R*) and (4*S*) configurations (**37** versus **57**; and **62** versus **63**) were very similar.

We anticipated that the propionamide cap on the N-terminus of the natural sintokamides might be susceptible to proteolysis. Therefore, we examined the *N*-Boc and *N*-pivaloyl analogues **64** [LPY22], **65** [LPY23], and **66** [LPY26], which were presumed to be more resistant to proteolysis. All three of these compounds were more active at 20 μM than the reference compound sintokamide A (**4**) (Figure 3E). Removal of the methyl group from the enol ethers to give the tetramic acids **70** [LPY32] and **71** [LPY33] led to a significant reduction in activity compared with **64** and **65** (Figure 3E). The two dipeptides **72** [LPY34] and **56** [LPY35], completely missing the tetramic acid moiety, were only weakly active (Figure 3E).

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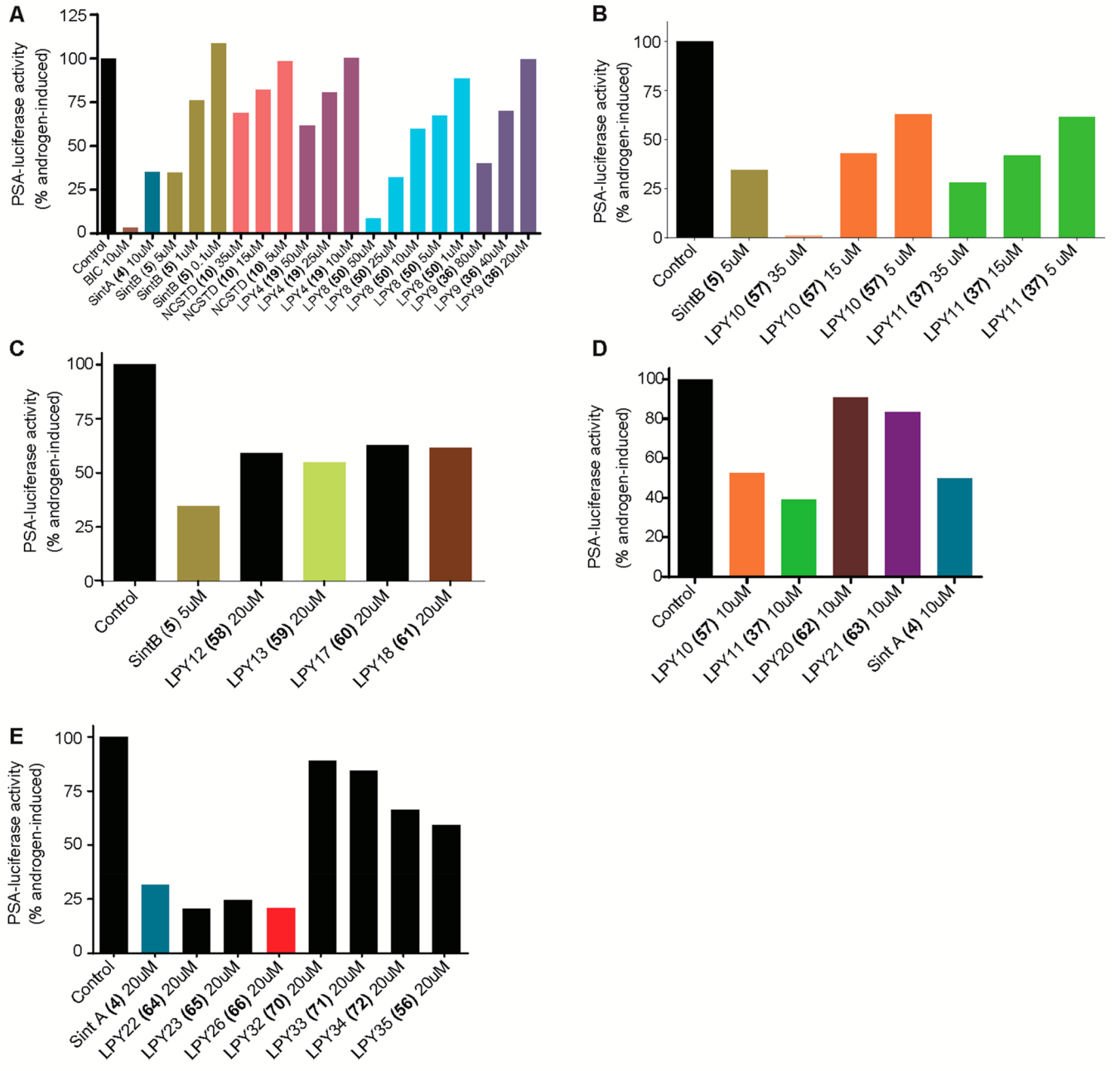

Figure 3. Effect of sintokamide analogues on AR transcriptional activity in LNCaP cells that were transiently transfected with PSA (6.1 kb) luciferase reporter and induced with 1 nM synthetic androgen (R1881). Representative data are shown.

In order to confirm the SAR conclusions described above, that were based on a single replicate and single or small numbers of concentrations in head-to-head comparisons of potency, the IC_{50}'s of several analogues were determined by conducting head-to-head dose−response curves in the PSA Luc transcriptional assay. Although the IC_{50} values shown in Figure 4 generally confirmed all of the SAR conclusions that were derived from single-replicate head-to-head evaluations, including the very weak activity of the nonchlorinated sintokamide scaffold NCSTD (**10**), the increase in potency in proportion to the number of chlorine atoms on the side chains (i.e., **57** > **37** > **36** > **19**), the preference for a natural C-10 *R* configuration and a C-4 *R* unnatural configuration (i.e., **57** > **37**), the need for chlorination at both C-18 and C-19 (i.e., **37** > **61** > **59**), and identification of LPY26 (**66**) as the most potent symmetrically chlorinated analogue, a number of more subtle features also emerged that were not recognized without IC_{50} values. The IC_{50} value comparison showed that LPY04 (**19**), with a 18,19-dichloro-1,17-bisnorsintokamide skeleton, and LPY09 (**36**), with a 18,18,19,19-tetrachloro-1,17-bisnorsintokamide skeleton, were both less active than the nonchlorinated sintokamide NCSTD (**10**), and LPY10 (**57**) and LPY11 (**37**), with 18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide skeletons, were less active than the natural sintokamides A (**4**) and B (**5**). This suggests that the C-1 and C-17 branching methyl groups are part of the natural product sintokamide pharmacophore. The pivaloyl N-terminus cap appears to compensate for the lipophilic interactions lost with

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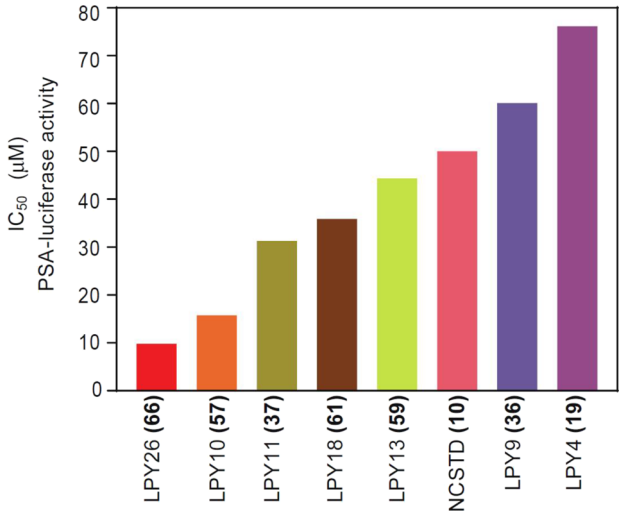

Figure 4. IC_{50}'s measured using the PSA-luciferase reporter for AR transcriptional activity in LNCaP cells induced with R1881 (1 nM) in the presence of test compounds for 48 h. Representative data are shown.

the 1,17-bisnor skeleton, making LPY26 (**66**) and LPY36 (**76**) (*vida infra*) as potent as or more potent than the natural product sintokamide A (**4**) in both the LNCaP Luc assay and the LNCaP proliferation assay.

A concern when a chemical scaffold is optimized using a negative readout cell-based phenotypic assay is whether or not the enhanced activity is still a result of binding to the desired cellular protein target or is caused by off-target cytotoxicity. Therefore, the two click chemistry probes **68** [LPY30] and **69** [LPY31], which have the same chlorination pattern as LPY26 (**66**), were used in streptavidin pull-down experiments to confirm that the 18,18,18,19,19,19-hexachloro-1,17-bisnorsintokamide scaffold binds to the full-length AR in LNCaP cells and to the AR NTD AF1 region in pure recombinant protein. The positive results confirming that **68** and **69** engage the AR NTD target have been published in detail elsewhere.[14]

Having identified compound **66** [LPY26], which has the same chlorination pattern as the most potent natural product sintokamide B (**5**) (Figure 3A), as the most potent hexachlorinated 1,17-bisnorsintokamide synthetic analogue, and anticipating it to be most resistant to proteolysis due to steric hindrance, it was selected for further evaluation. Consistent with previous reports, sintokamide A (**4**) selectively inhibited the proliferation of R1881-stimulated AR transcription-driven proliferation of LNCaP cells compared with its impact on the viability of PC3 and DU145 PC cells (Figure 5A).[14] Compound **66** showed little selectivity for blocking AR-driven proliferation compared with sintokamide A (**4**), and it decreased the viability of PC3 and DU145 PC cell lines, which do not express functional AR, suggesting off-target effects (Figure 5B). Therefore, we prepared the pentachlorinated 1,17-bisnorsintokamide analogues **76** [LPY36] and **75** [LPY37], which had the same chlorination pattern as sintokamide A (**4**) but had the pivaloyl N-terminus cap found in **66**. Unexpectedly, LPY36 (**76**) was more potent in the LNCaP transcriptional assay (IC_{50} 3.5 μM) than both LYP26 (**66**) (≈10 μM) and sintokamide A (**4**) (IC_{50} 6.5 μM) (Figures 4 and 6). LPY36 (**76**) also showed selectivity for

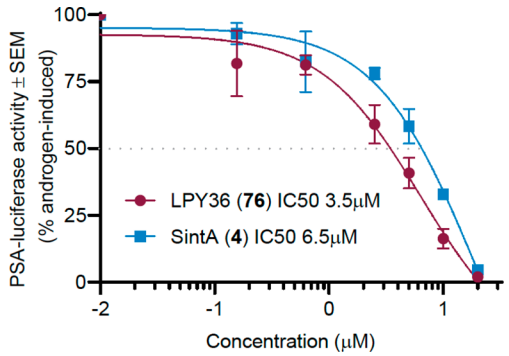

Figure 6. Dose-dependent inhibition of androgen-induced transcriptional activity of endogenous full-length AR in LNCaP cells by sintokamide A (**4**) versus LPY36 (**76**) using the PSA-luciferase reporter assay. Data represent mean ± SEM from n = 2 or 3 independent experiments.

inhibition of AR-driven proliferation of LNCaP cells compared with reduction of viability of PC3 and DU145 cells that was superior to that observed for the natural product sintokamide A (**4**) and LPY26 (**66**) [SintA (**4**) IC_{50}'s LNCaP 14 μM, PC3 29 μM, DU145 > 35 μM: LPY26 (**66**) IC_{50}'s LNCaP 12 μM, PC3 13 μM, DU145 20 μM: LPY36 (**76**) IC_{50}'s LNCaP 6.9 μM, PC3 28 μM, DU145 > 35 μM] (Figure 5).

CONCLUSION

The sintokamides are biosynthetically modified dipeptides isolated from extracts of an Indonesian marine sponge. They

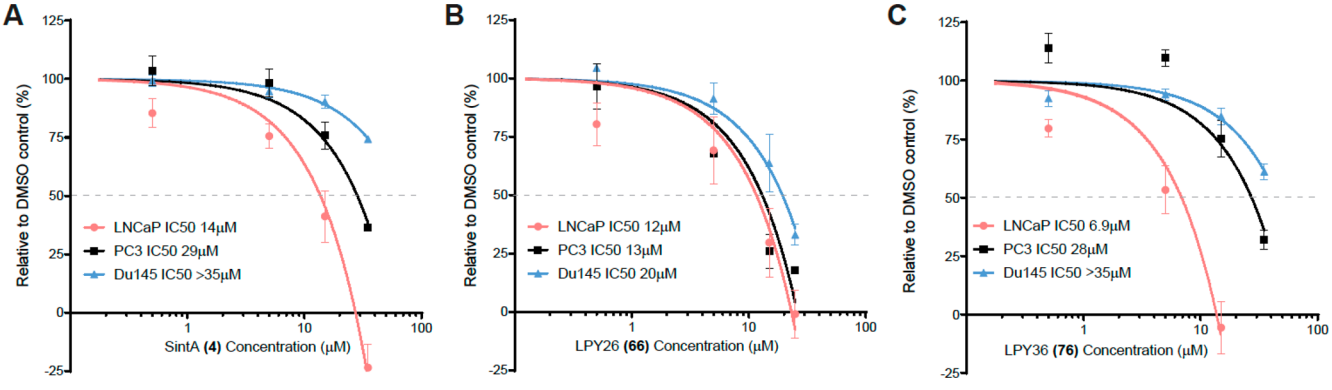

Figure 5. Effects of sintokamide compounds on androgen-dependent proliferation of LNCaP cells compared with PC3 and DU145 cell viability. (A) Sintokamide A (**4**); (B) LPY26 (**66**); and (C) LPY36 (**76**). Data represent mean ± SEM from n = 3 independent experiments.

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represent the first chemical scaffold reported to block the transcriptional activities of full-length and splice variant ARs in PC cells by binding to the AF1 region of the AR N-terminal domain (AR NTD).[13] They have provided preliminary *in vitro* and *in vivo* validation of the AR NTD as a new molecular target for drugs to treat mCRPC, and they represent a marine natural product chemical inspiration for the development of a new class of anticancer drugs that block the transcriptional activity of this target.[14] The first step in capitalizing on this inspiration is developing a detailed understanding of the SAR for the sintokamide AR NTD antagonist pharmacophore.

Three biosynthetic modifications of a Leu-Leu dipeptide generate the unique structural features of the sintokamide scaffold. These are mono-, di-, or trichlorination of a side chain methyl group in one or both of the two leucine-derived fragments and acetate extension of the C-terminus carboxylic acid followed by cyclization and methylation to give a methyltetramic acid moiety and an N-terminus propionamide cap. The SAR study described above has explored the role that each of these three modified peptide fragments play as components of the sintokamide AR NTD antagonist pharmacophore.

The sintokamide scaffold without any chlorine atoms on the side chain methyl groups was essentially inactive, and the activity was found to increase in proportion to the number of chlorine atoms added to the side chain methyls. Maximum potency was achieved when there was a trichloromethyl group at C-19 and a dichloro- or trichloromethyl at C-18 on the side chains. Therefore, the biosynthetic chlorination found in the sintokamides is an essential part of their AR NTD blocking pharmacophore. The presence of the chlorinated methyl groups in the natural products creates stereogenic centers at C-2 and C-16, which significantly increases the complexity of synthesizing enantiopure analogues. It was found that certain 1,17-bisnorsintokamides, for example, LPY26 (**66**) and LPY36 (**76**), that are missing the C-2 and C-16 stereogenic centers are as active as or more active than the natural products.

Synthetic analogues that were missing the C-terminus tetramic acid or even just the C-20 methyl ether on the tetramic acid were essentially inactive, demonstrating that this biosynthetic C-terminus modification of the basic Leu-Leu peptide was also an integral part of the AR NTD blocking pharmacophore. The SAR data showed that the natural *R* configuration at C-10 and the unnatural C-4 *R* configuration were preferred. Finally, it was found that replacing the propionamide N-terminus cap with a pivaloyl N-terminus cap increased the potency significantly, indicating that this lipophilic tail was an important part of the pharmacophore.

SAR using the PSA-luciferase assay to optimize the potency of the sintokamide scaffold initially yielded the synthetic analogue LPY26 (**66**) with the hexachlorination pattern of the most potent natural product sintokamide B (**5**). Unfortunately, LPY26 (**66**) lacked the on-target AR selectivity previously shown by sintokamide A (**4**) for AR-driven proliferation of LNCaP cells compared to PC3 and DU145 cells that do not contain AR. The 1,17-bisnor analogue LYP36 (**76**), which has the trichloro C-19 and dichloro C-18 chlorination pattern of the selective natural product sintokamide A (**4**) and the pivaloyl N-cap found in LPY26 (**66**), captured and exceeded both the on-target selectivity and potency of sintokamide A (**4**).

In summary, the current study has explored the SAR for the AR NTD blocking pharmacophore of the sintokamide scaffold. The results have shown that the chlorination, tetramic acid formation, and N-terminus acyl cap biosynthetic modifications of a Leu-Leu dipeptide created substructures in the natural products that are all important components of the pharmacophore. Guided by the SAR findings, a potency and target selectivity optimized synthetic analogue, LPY36 (**76**), has been identified that exceeds the potency and selectivity of the most promising natural product sintokamide A (**4**), is much easier to synthesize because of the removal of two of the four stereogenic centers in the natural product, and is presumed to be more resistant to proteolysis of the N-terminus cap. The synthetic route to LPY36 (**76**) has been optimized for preparation of the gram-scale quantities needed for *in vitro* and *in vivo* evaluation and further SAR optimization of this promising lead pharmacophore for the development of a new class of AR NTD blocking drugs to treat mCRPC. This work also highlights the importance of natural products as a source of highly effective lead compounds with unprecedented pharmacophores that modulate new experimental drug targets for untreatable human diseases.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-1010 polarimeter at room temperature (rt) and 589 nm (sodium D line) in $CHCl_3$ (g/100 mL). NMR spectra were obtained on Bruker Avance 400 direct, 400 inverse, 300 direct, or Bruker Avance 600 CryoProbe spectrometers at rt unless otherwise noted. ^1H and ^{13}C chemical shifts are referenced to the residual $CHCl_3$ (δ 7.24 ppm) and $CDCl_3$ (δ 77.0 ppm) solvent peaks. Electrospray ionization mass spectrometry (ESIMS) spectra were recorded on a Micromass LCT instrument. X-ray crystallography was performed on a Bruker X8 APEX CCD single-crystal X-ray diffraction instrument. All nonaqueous reactions were carried out in flame-dried glassware and under an argon or nitrogen atmosphere unless otherwise noted. Air- and moisture-sensitive liquid reagents were manipulated via a dry syringe. Anhydrous tetrahydrofuran (THF) was obtained from distillation over sodium. Other solvents and reagents were used as obtained from commercial sources without further purification. Flash column chromatography was performed using Silicycle Ultra-Pure silica gel (230−400 mesh). Analytical thin-layer chromatography (TLC) plates were aluminum-backed ultrapure silica gel 250 μm.

Synthesis and Purification. *(S)-2-(1,3-Dioxoisoindolin-2-yl)-4-methylpentanoic acid (**12**).* To a stirred solution of L-leucine (5.12 g, 39.0 mmol) and Na_2CO_3 (4.14 g, 39.0 mmol) in H_2O (40 mL) was added *N*-carbethoxyphathalimide **11** (8.55 g, 39.0 mmol) at rt. The reaction mixture was stirred at rt for an additional 2 h. HCl (6 M) was added dropwise to the reaction mixture placed in an ice−water bath until its pH was adjusted to 0. The aqueous layer was extracted with hexanes (3 × 100 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/acetone (3:1) to afford *N*-phthalimide-L-leucine **12** (10.2 g, 39.0 mmol) as a colorless oil in a quantitative yield: $[\alpha]^{20}_D$ −59.0 (*c* 0.50, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 11.32 (br s, 1H), 7.85 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.73 (dd, *J* = 5.4, 3.0 Hz, 2H), 4.99 (dd, *J* = 11.5, 4.2 Hz, 1H), 2.36 (ddd, *J* = 14.3, 10.0, 4.2 Hz, 1H), 1.95 (ddd, *J* = 14.3, 10.0, 4.2 Hz, 1H), 1.37−1.60 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 167.9, 134.4, 131.9, 123.8, 50.6, 37.2, 25.3, 23.3, 21.2; ESIMS *m*/*z* 260.0 $[M - H]^-$; HRESIMS *m*/*z* 284.0905 $[M + Na]^+$ (calcd for $C_{14}H_{15}NO_4Na$, 284.0899).

*Ethyl (S)-4-(1,3-Dioxoisoindolin-2-yl)-6-methyl-3-oxoheptanoate (**14**).* To a stirred solution of monoethyl malonate (450 mg, 3.41 mmol) in THF (5 mL) at −78 °C was added dropwise *n*-BuLi (3.7 mL, 7.40 mmol, 2.0 M in hexanes). The resultant white suspension was warmed gradually to −5 °C and then cooled to −78 °C again.

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The reaction mixture was stirred at −78 °C for 1 h. After addition of acid chloride **13** (535 mg, 1.91 mmol) in THF (2 mL) all at once, the reaction mixture was stirred at −78 °C for another 20 min, then poured onto a mixture of HCl (7 mL, 1 M) and ether (14 mL). The separated aqueous layer was extracted with ether (2 × 10 mL). The combined organic extract was washed with saturated $NaHCO_3$ (3 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/acetone (97:3) to afford homologous 1,3-diketone ester **14** (331 mg, 0.99 mmol) as a yellowish oil in a yield of 52%: $[\alpha]^{20}_D$ −26.5 (*c* 0.49, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.88 (dd, *J* = 5.6, 3.0 Hz, 2H), 7.76 (dd, *J* = 5.6, 3.0 Hz, 2H), 5.00 (dd, *J* = 11.3, 4.2 Hz, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.52 (s, 2H), 2.24 (ddd, *J* = 14.1, 10.0, 4.1 Hz, 1H), 1.91 (ddd, *J* = 14.1, 10.0, 4.1 Hz, 1H), 1.37−1.57 (m, 1H), 1.23 (t, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 168.0, 166.6, 134.6, 131.9, 123.8, 61.9, 57.7, 46.5, 36.5, 25.3, 23.5, 21.3, 14.2; ESIMS *m/z*: 354.0 $[M + Na]^+$; HRESIMS *m/z* 354.1316 $[M + Na]^+$ (calcd for $C_{18}H_{21}NO_5Na$, 354.1317).

*Methyl (S,E)-4-(1,3-Dioxoisoindolin-2-yl)-3-methoxy-6-methylhept-2-enoate (**15**).* To a stirred solution of β-keto ester **14** (186 mg, 0.56 mmol) in MeOH (5 mL) was added trimethyl orthoformate (0.25 mL, 2.24 mmol) and a drop of concentrated H_2SO_4 at rt. The reaction mixture was refluxed overnight. After addition of ether (80 mL), the organic layer was washed with saturated $NaHCO_3$ (3 × 10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/acetone (9:1 → 8:1) to afford the desired product *E*-enol ether methyl ester **15** (130 mg, 0.39 mmol) as a yellow oil in a yield of 70%: $[\alpha]^{20}_D$ −25 (*c* 0.04, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 5.4, 2.9 Hz, 2H), 7.71 (dd, *J* = 5.4, 2.9 Hz, 2H), 6.33 (dd, *J* = 11.4, 4.8 Hz, 1H), 5.05 (s, 1H), 3.74 (s, 3H), 3.63 (s, 3H), 2.66 (ddd, *J* = 13.1, 11.4, 3.8 Hz, 1H), 1.67 (ddd, *J* = 13.1, 11.4, 3.8 Hz, 1H), 1.49−1.60 (m, 1H), 1.00 (d, *J* = 6.6 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 168.7, 167.0, 134.0, 132.2, 123.4, 91.2, 56.3, 51.4, 50.2, 38.3, 25.6, 23.4, 21.2; ESIMS *m/z* 354.0 $[M + Na]^+$; HRESIMS *m/z* 354.1316 $[M + Na]^+$ (calcd for $C_{18}H_{21}NO_5Na$, 354.1317).

*(S)-5-Isobutyl-4-methoxy-1,5-dihydro-2H-pyrrol-2-one (**16**).* To a stirred solution of enol ether **15** (53 mg, 0.16 mmol) in MeOH (5 mL) was added excessive hydrazine hydrate (2 mL) at rt. The reaction mixture was refluxed overnight; then the solvent was removed *in vacuo*. After addition of CH_2Cl_2 (40 mL) and water (40 mL), the separated aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/MeOH (200:1) to afford methyl tetramate **16** (15 mg, 0.088 mmol) as a white solid in a yield of 55%: $[\alpha]^{20}_D$ +6.5 (*c* 1.52, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 6.24 (br s, 1H), 5.00 (d, *J* = 1.0 Hz, 1H), 4.06 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.79 (s, 3H), 1.70−1.83 (m, 1H), 1.64 (td, *J* = 9.2, 4.7 Hz, 1H),1.38 (td, *J* = 9.2, 4.7 Hz, 1H), 0.97 (d, *J* = 2.0 Hz, 3H), 0.95 (d, *J* = 2.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.2, 174.5, 93.3, 58.4, 56.2, 41.6, 25.6, 23.6, 22.0; ESIMS *m/z* 170.3 $[M + H]^+$; HRESIMS *m/z* 170.1177 $[M + H]^+$ (calcd for $C_9H_{16}NO_2$, 170.1181).

*4-Nitrophenyl (tert-Butoxycarbonyl)-D-leucinate (**17**).* To a stirred solution of Boc-D-leucine (878.9 mg, 3.80 mmol) and *p*-nitrophenol (581 mg, 4.18 mmol) in THF (20 mL) was added DCC (784.3 mg, 3.80 mmol) at 5 °C. The reaction mixture was stirred at rt overnight. After addition of hexanes (50 mL), the white precipitate formed was filtered off and then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/acetone (5:1 → 3:1) to afford active ester **17** (1.11 g, 3.125 mmol) as a colorless oil in a yield of 82%: $[\alpha]^{20}_D$ +24 (*c* 0.63, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 9.2 Hz, 2H), 7.31 (d, *J* = 9.2 Hz, 2H), 4.94 (d, *J* = 6.1 Hz, 1H), 4.52 (br s, 1H), 1.75−1.86 (m, 2H), 1.61−1.71 (m, 1H), 1.47 (s, 9H), 1.04 (d, *J* = 2.0 Hz, 3H), 1.02 (d, *J* = 2.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 155.7, 155.5, 145.6, 125.4, 122.5, 80.6, 52.7, 41.3, 28.5, 25.1, 23.0, 21.9; ESIMS *m/z* 375.1 $[M + Na]^+$; HRESIMS *m/z* 375.1534 $[M + Na]^+$ (calcd for $C_{17}H_{24}N_2O_6Na$, 375.1532).

*tert-Butyl ((R)-1-((S)-2-Isobutyl-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-4-methyl-1-oxopentan-2-yl)carbamate (**18**).* To a stirred solution of methyl tetramate **16** (11.2 mg, 0.066 mmol) in THF (2 mL) at −50 °C was added *n*-BuLi (32 μL, 0.066 mmol, 1.60 M in hexanes). The reaction mixture was stirred at −50 °C for 10 min. After dropwise addition of Boc-D-Leu-ONp **17** (25.6 mg, 0.073 mmol) in THF (2 mL), the reaction mixture was stirred at −50 °C for another 10 min. AcOH (0.1 mL) was added to the reaction mixture, and then the solvent was evaporated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:1) to afford coupling product **18** (12.0 mg, 0.031 mmol) as a white powder in a yield of 47%: $[\alpha]^{20}_D$ +66 (*c* 4.50, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.45 (td, *J* = 2.9, 1.9 Hz, 1H), 5.10 (br d, *J* = 8.0 Hz, 1H), 5.04 (s, 3H), 4.58 (t, *J* = 5.1 Hz, 1H), 1.75−1.88 (m, 6H), 1.46 (s, 9H), 1.32−1.40 (m, 1H), 1.04 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 6H), 0.89 (d, *J* = 5.8 Hz, 3H); ESIMS *m/z* 405.1 $[M + Na]^+$; HRESIMS *m/z* 405.2369 $[M + Na]^+$ (calcd for $C_{20}H_{34}N_2O_5Na$, 405.2365).

*N-((R)-1-((S)-2-Isobutyl-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-4-methyl-1-oxopentan-2-yl)propionamide (**10**).* Compound **18** (5.0 mg, 0.013 mmol) was dissolved in TFA/CH_2Cl_2 (1 mL, 1:3) and stirred at 0 °C for 10 min, then poured into an ammonia solution (10 mL, 25%). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified through a Sep-Pak silica (2 g) cartridge eluting with CH_2Cl_2/MeOH (98:2) to afford free amine (3.3 mg, 0.012 mmol) as a white powder in a yield of 90%: $[\alpha]^{20}_D$ +66.5 (*c* 4.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.05 (s, 1H), 4.60 (t, *J* = 5.1 Hz, 1H), 4.55 (dd, *J* = 9.5, 4.2 Hz, 1H), 3.87 (s, 3H), 1.83−1.92 (m, 2H), 1.76 (td, *J* = 13.3, 6.5 Hz, 1H), 1.54 (ddd, *J* = 13.4, 9.0, 4.3 Hz, 1H), 1.32 (td, *J* = 8.9, 4.8 Hz, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 2.6 Hz, 3H), 0.93 (d, *J* = 2.9 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H); ESIMS *m/z* 283.3 $[M + H]^+$.

To a stirred solution of free amine (1.0 mg, 0.0035 mmol) in pyridine (2 mL) was added propionyl anhydride (1.4 μL, 0.011 mmol) at rt. The reaction mixture was stirred at rt for 12 h. After addition of HCl (10 mL, 1 M), the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:1) to afford compound **10** (1.0 mg, 0.0029 mmol) as a colorless oil in a yield of 83%: $[\alpha]^{20}_D$ +50 (*c* 0.48, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 6.04 (d, *J* = 8.8 Hz, 1H), 5.75 (ddd, *J* = 10.7, 9.0, 2.9 Hz, 1H), 5.05 (s, 1 H), 4.57 (dd, *J* = 6.2, 4.0 Hz, 1H), 3.87 (s, 3H), 2.25 (q, *J* = 7.7 Hz, 2H), 1.80−1.83 (m, 3H), 1.75−1.79 (m, 1H), 1.59 (dt, *J* = 6.9, 3.6 Hz, 1H), 1.40 (dt, *J* = 6.9, 3.6 Hz, 1H), 1.17 (t, *J* = 7.5 Hz, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 6H), 0.88 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 181.0, 173.3, 173.1, 169.6, 93.6, 58.9, 58.9, 51.6, 41.7, 39.3, 29.9, 25.2, 24.3, 23.9, 23.8, 22.7, 21.4, 10.0; ESIMS *m/z* 361.4 $[M + Na]^+$; HRESIMS *m/z* 339.2281 $[M + H]^+$ (calcd for $C_{18}H_{31}N_2O_4$, 339.2284).

*Methyl (S)-2-(Bis(tert-butoxycarbonyl)amino)-5-hydroxypentanoate (**22**).* To a stirred solution of (*S*)-**21** (5.31 g, 14.1 mmol) in THF (30 mL) at −40 °C was added DIBAL-H (42.4 mL, 42.4 mmol, 1.0 M in hexanes) at a rate of 1.0 mL/min. The reaction mixture was stirred at −40 °C for 1 h. After successive addition of MeOH (40 mL), HCl (40 mL, 1 M), and Rochelle salt solution (40 mL, 1 M), the reaction mixture was stirred at rt for another 2 h. The separated aqueous layer was extracted with CH_2Cl_2 (4 × 40 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1 → 3:1) to afford primary alcohol (*S*)-**22** (2.26 g, 6.50 mmol) as a colorless oil in a yield of 46%: $[\alpha]^{20}_D$ −35 (*c* 3.53, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.86 (dd, *J* = 5.5, 8.8 Hz, 1H), 3.69 (s, 3H), 3.61−3.67 (m, 2H), 2.16−2.27 (m, 1H), 1.87−

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1.97 (m, 1H), 1.56−1.66 (m, 2H), 1.48 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 152.3, 83.3, 62.4, 58.0, 52.3, 29.5, 28.1, 26.6; ESIMS m/z 370.1 $[M + Na]^+$; HRESIMS m/z 370.1827 $[M + Na]^+$ (calcd for $C_{16}H_{29}NO_7Na$, 370.1842).

*Methyl (S)-2-(Bis(tert-butoxycarbonyl)amino)-5-((tert-butyldiphenylsilyl)oxy)pentanoate (**23**).* To a stirred solution of (*S*)-**22** (3.13 g, 9.00 mmol) in DMF (10 mL) at 0 °C were added *tert*-butyldiphenylsilyl chloride (3.7 mL, 14.2 mmol) and imidazole (2.97 g, 43.6 mmol). The reaction mixture was stirred at rt overnight; then DMF was removed *in vacuo*. After addition of HCl (15 mL, 1 M), the aqueous layer was extracted with diethyl ether (3 × 30 mL). The combined organic extract was washed with water (15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (12:1) to afford (*S*)-**23** (5.27 g, 8.99 mmol) as a colorless oil quantitatively: $[\alpha]^{20}_D$ −30 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 5.9 Hz, 4H), 7.35−7.47 (m, 6H), 4.90 (dd, *J* = 5.0, 9.4 Hz, 1H), 3.73 (s, 3H), 3.71 (t, *J* = 6.3 Hz, 2H), 2.20−2.33 (m, 1H), 1.97−2.08 (m, 1H), 1.60−1.70 (m, 2H), 1.51 (s, 18H), 1.08 (br s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 152.2, 135.7, 134.1, 129.8, 127.8, 83.2, 63.6, 58.3, 52.3, 29.6, 28.1, 27.0, 26.7, 19.4; ESIMS m/z 608.0 $[M + Na]^+$; HRESIMS m/z 608.3026 $[M + Na]^+$ (calcd for $C_{32}H_{47}NO_7SiNa$, 608.3019).

*Methyl (S)-2-((tert-Butoxycarbonyl)amino)-5-((tert-butyldiphenylsilyl)oxy)pentanoate (**24**).* To a stirred solution of (*S*)-**23** (4.40 g, 7.51 mmol) in MeCN (100 mL) at rt was added lithium bromide (3.04 g, 35.0 mmol). The reaction mixture was stirred 65 °C for 20 h; then the solvent was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (4:1) to afford (*S*)-**24** (3.47 g, 7.14 mmol) as a colorless oil in a yield of 95%: $[\alpha]^{20}_D$ +7.0 (*c* 0.85, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 6.7 Hz, 4H), 7.35−7.46 (m, 6H), 5.14 (d, *J* = 6.7 Hz, 1H), 4.34 (br s, 1H), 3.74 (s, 3H), 3.69 (t, *J* = 5.8 Hz, 2H), 1.88−2.01 (m, 1H), 1.72−1.83 (m, 1 H), 1.56−1.67 (m, 2H), 1.47 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 155.6, 135.7, 134.0, 129.8, 127.9, 80.0, 63.3, 53.5, 52.4, 31.8, 29.3, 28.5, 27.0, 19.4; ESIMS m/z 508.0 $[M + Na]^+$; HRESIMS m/z 508.2493 $[M + Na]^+$ (calcd for $C_{27}H_{39}NO_5SiNa$, 508.2495).

*(S)-2-((tert-Butoxycarbonyl)amino)-5-((tert-butyldiphenylsilyl)oxy)pentanoic acid (**25**).* To a stirred solution of (*S*)-**24** (1.94 g, 3.99 mmol) in THF (10 mL) at 0 °C was dropwise added lithium hydroxide (8.0 mL, 8.0 mmol, 1 M in H_2O) at a rate of 1.0 mL/min. The reaction mixture was stirred at 0 °C for 1 h and at rt for 3 h. After THF was removed *in vacuo*, to the reaction mixture was added 1 M HCl dropwise until its pH value was adjusted to pH = 3. The aqueous layer was extracted with EtOAc (5 × 15 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/MeOH (98:2) to afford acid (*S*)-**25** (1.18 g, 2.50 mmol) as a colorless oil in a yield of 63%: $[\alpha]^{20}_D$ +1.2 (*c* 1.57, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (br s, 1H), 7.68 (d, *J* = 6.7 Hz, 4H), 7.34−7.47 (m, 6H), 6.35, 5.18 (br s each, 1H), 4.35, 4.17 (br s each, 1H), 3.70 (t, *J* = 5.6 Hz, 2H), 1.97−2.06 (m, 1H), 1.78−1.87 (m, 1H), 1.61−1.74 (m, 2H), 1.46 (s, 9H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0 and 177.5, 155.8, 135.7, 133.9, 129.8, 127.9, 81.6 and 80.3, 63.3, 53.6 and 53.5, 29.3, 29.0, 28.5, 28.4, 27.0, 19.4 (31.8 s missing); ESIMS m/z 494.0 $[M + Na]^+$; HRESIMS m/z 494.2334 $[M + Na]^+$ (calcd for $C_{26}H_{37}NO_5SiNa$, 494.2339).

*tert-Butyl (S)-2-(3-((tert-Butyldiphenylsilyl)oxy)propyl)-3-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (**26**).* To a stirred solution of the carboxylic acid (*S*)-**25** (1.14 g, 2.42 mmol), Meldrum's acid 3.85 (348 mg, 2.41 mmol), and DMAP (430 mg, 3.52 mmol) in CH_2Cl_2 (5 mL) was added DCC (532 mg, 2.58 mmol) at 0 °C. The reaction mixture was stirred at rt for an additional 5 h, then poured onto cold EtOAc (50 mL). The white precipitate formed was filtered off. After successive washes with cold $KHSO_4$ solution (50 mL, 5%), cold water (50 mL), and cold brine (50 mL), the filtrate was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The yellowish crude residue was used directly for the next step without further purification.

The crude residue was refluxed in EtOAc (15 mL) for 1 h; then the solvent was removed *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (2:1) to afford **26** (580 mg, 1.17 mmol) as a colorless oil in a yield of 50%.

Due to the complexity of its NMR spectra, the structure of **26** was confirmed by mass spectrum data only. The correctness of the structural deduction was further proved by the product structures derived from **26**. ESIMS m/z 518.2 $[M + Na]^+$; HRESIMS m/z 496.2519 $[M + H]^+$ (calcd for $C_{28}H_{38}NO_5Si$, 496.2519).

*tert-Butyl (S)-2-(3-((tert-Butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (**27**).* To a stirred solution of **26** (63 mg, 0.127 mmol) in toluene/MeOH (10 mL, 4:1) at rt was dropwise added $TMSCHN_2$ (129 μL, 0.258 mmol, 2.0 M in hexanes). After addition of AcOH (0.1 mL), the reaction mixture was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (9:1 → 4:1) to afford **27** (65.7 mg, 0.128 mmol) as a colorless oil quantitatively: $[\alpha]^{20}_D$ +75 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 6.7 Hz, 4H), 7.34−7.46 (m, 6H), 5.07 (s, 1H), 4.51 (dd, *J* = 2.7, 5.5 Hz, 1H), 3.78 (s, 3H), 3.63 (m, 2H), 2.11−2.25 (m, 1H), 1.88−2.05 (m, 2H), 1.65−1.77 (m, 1H), 1.52 (s, 9H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 169.5, 149.5, 135.7, 134.0, 129.8, 127.8, 94.7, 82.7, 63.6, 59.8, 58.6, 34.1, 28.4, 27.0, 26.3, 25.6, 19.4; ESIMS m/z 532.3 $[M + Na]^+$; HRESIMS m/z 510.2680 $[M + H]^+$ (calcd for $C_{29}H_{40}NO_5Si$, 510.2676).

*(S)-5-(3-((tert-Butyldiphenylsilyl)oxy)propyl)-4-methoxy-1,5-dihydro-2H-pyrrol-2-one (**28**).* Compound **27** (35 mg, 0.0686 mmol) was dissolved in TFA/CH_2Cl_2 (1 mL, 25%) and stirred at 0 °C for 1 h. The solvent was evaporated *in vacuo*. After addition of saturated $NaHCO_3$ (10 mL), the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (7:1 → 100% EtOAc) to afford methyl tetramate **28** (23.6 mg, 0.057 mmol) as a colorless oil in a yield of 84%: $[\alpha]^{20}_D$ +14 (*c* 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 6.4 Hz, 4H), 7.35−7.47 (m, 6H), 5.75 (br s, 1H), 5.01 (s, 1H), 4.08 (br s, 1H), 3.78 (s, 3H), 3.68 (t, *J* = 5.8 Hz, 2H), 1.86−1.97 (m, 1H), 1.58−1.67 (m, 2H), 1.47−1.58 (m, 1H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4, 174.3, 135.8, 133.9, 129.9, 127.9, 93.9, 63.6, 58.5, 57.3, 28.8, 27.9, 27.1, 19.4; ESIMS m/z 410.2 $[M + H]^+$; HRESIMS m/z 410.2146 $[M + H]^+$ (calcd for $C_{24}H_{32}NO_3Si$, 410.2151).

*Benzyl (R)-2-((tert-butoxycarbonyl)amino)-5-((tert-butyldiphenylsilyl)oxy)pentanoate (**29**).* To a stirred solution of acid (*R*)-**25** (2.0 g, 4.24 mmol), TEA (0.76 mL, 5.46 mmol), and DMAP (52 mg, 0.42 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added benzyl chloroformate (0.76 mL, 5.32 mmol). The reaction mixture was stirred at 0 °C for 3 h. After addition of brine (10 mL), the separated aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (15:1 → 5:1) to afford **29** (2.20 g, 3.91 mmol) as a colorless oil in a yield of 92%: $[\alpha]^{20}_D$ +5.1 (*c* 1.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 7.0 Hz, 4H), 7.32−7.49 (m, 11H), 5.10−5.27 (m, 3H), 4.39, 4.22 (br s each, 1H), 3.67 (t, *J* = 5.6 Hz, 2H), 1.94−2.05 (m, 1H), 1.73−1.84 (m, 1H), 1.55−1.66 (m, 2H), 1.47 (s, 9H), 1.07 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 155.6, 135.7, 133.9, 129.8, 128.7, 128.5, 128.4, 127.8, 80.0, 67.1, 63.2, 53.6, 29.3, 28.5, 27.0, 19.4 (31.8 s missing); ESIMS m/z 584.2 $[M + Na]^+$; HRESIMS m/z 584.2808 $[M + Na]^+$ (calcd for $C_{33}H_{43}NO_5NaSi$, 584.2808).

*Benzyl (R)-5-((tert-Butyldiphenylsilyl)oxy)-2-propionamidopentanoate (**30**).* Compound **29** (1.76 g, 3.14 mmol) was dissolved in TFA/CH_2Cl_2 (30 mL, 25%) and stirred at 0 °C for 1 h. The solvent was evaporated *in vacuo*. The crude residue was used directly for the next step without further purification.

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To a stirred solution of the crude residue in THF (20 mL) at 0 °C was added TEA (0.56 mL, 4.00 mmol) and propionyl chloride (3.06 mL, 35.0 mmol). The reaction mixture was stirred at 0 °C for 3 h. After addition of saturated $NaHCO_3$ (20 mL), the separated aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1) to afford **30** (960 mg, 1.85 mmol) as a colorless oil in a yield of 59%: $[\alpha]^{20}_D$ +1.80 (*c* 1.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.0 Hz, 4H), 7.30−7.47 (m, 11H), 6.02 (d, *J* = 7.6 Hz, 1H), 5.18 (m, *J* = 6.4, 31.1 Hz, 2H), 4.70 (td, *J* = 5.2, 7.8 Hz, 1H), 3.64 (td, *J* = 2.1, 6.1 Hz, 2H), 2.23 (q, *J* = 7.6 Hz, 2H), 1.94−2.07 (m, 1H), 1.74−1.85 (m, 1H), 1.46−1.64 (m, 2H), 1.15 (t, *J* = 7.6 Hz, 3H), 1.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 172.8, 135.7, 133.8, 129.8, 128.8, 128.6, 128.4, 127.9, 67.3, 63.2, 52.1, 29.7, 29.2, 28.4, 27.0, 19.4, 9.9 (31.8 s missing); ESIMS *m*/*z* 540.2 $[M + Na]^+$; HRESIMS *m*/*z* 540.2546 $[M + Na]^+$ (calcd for $C_{31}H_{39}NO_4NaSi$, 540.2548).

*Benzyl (R)-2-(N-(tert-Butoxycarbonyl)propionamido)-5-((tert-butyldiphenylsilyl)oxy)pentanoate (**31**).* To a stirred solution of **30** (513 mg, 0.991 mmol), DIPEA (0.35 mL, 2.00 mmol), and DMAP (12 mg, 0.098 mmol) in THF (5 mL) at 0 °C was added Boc_2O (432 mg, 1.98 mmol). The reaction mixture was refluxed for 1 h. After addition of brine (5 mL), the separated aqueous layer was extracted with ether (3 × 20 mL). The combined ethereal extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (30:1 → 1:1) to afford **31** (391 mg, 0.632 mmol) as a colorless oil in a yield of 63%: $[\alpha]^{20}_D$ +9.4 (*c* 1.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 6.7 Hz, 4H), 7.30−7.45 (m, 11H), 5.33 (dd, *J* = 5.2, 9.1 Hz, 1H), 5.10−5.20 (m, *J* = 1.2, 27.4 Hz, 2H), 3.67 (t, *J* = 5.9 Hz, 2H), 2.90 (q, *J* = 7.1 Hz, 2H), 2.25−2.36 (m, 1H), 1.91−2.03 (m, 1H), 1.56−1.66 (m, 1H), 1.47−1.54 (m, 1H), 1.41 (s, 9H), 1.14 (t, *J* = 7.3 Hz, 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 170.8, 152.5, 135.7, 134.1, 129.8, 128.7, 128.4, 128.3, 127.8, 84.0, 67.0, 63.6, 55.9, 31.8, 29.6, 28.0, 27.1, 26.5, 19.4, 9.7; ESIMS *m*/*z* 640.2 $[M + Na]^+$; HRESIMS *m*/*z* 640.3076 $[M + Na]^+$ (calcd for $C_{36}H_{47}NO_6NaSi$, 640.3070).

*Perfluorophenyl (R)-2-(N-(tert-Butoxycarbonyl)propionamido)-5-((tert-butyldiphenylsilyl)oxy)pentanoate (**32**).* To a stirred solution of **31** (220 mg, 0.356 mmol) in MeOH (10 mL) was added Pd/C (50 mg, 10%). The reaction mixture was hydrogenated under atmospheric pressure at rt for 1.5 h. The catalyst was removed through a pad of Celite with MeOH (20 mL), and the filtrate was concentrated *in vacuo*. The crude residue was used directly for the next step without further purification.

To a stirred solution of the crude residue and pentafluorophenol (73.6 mg, 0.399 mmol) in EtOAc (5 mL) was added DCC (88.1 mg, 0.427 mmol) at 0 °C. The reaction mixture was stirred at rt for 3 h. After addition of hexanes (15 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/acetone (30:1) to afford active ester **32** (209 mg, 0.302 mmol) as a colorless oil in a yield of 84%: $[\alpha]^{20}_D$ +4.7 (*c* 1.27, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 6.8 Hz, 4H), 7.33−7.49 (m, 6H), 5.61 (dd, *J* = 5.5, 8.9 Hz, 1H), 3.70 (t, *J* = 6.0 Hz, 2H), 2.85−3.07 (m, 2H), 2.30−2.43 (m, 1H), 2.01−2.14 (m, 1H), 1.55−1.71 (m, 2H), 1.52 (s, 9H), 1.19 (t, *J* = 7.3 Hz, 3H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 167.0, 152.0, 135.8, 134.0, 129.8, 127.9, 85.2, 63.4, 55.3, 31.7, 29.3, 28.0, 27.1, 26.5, 19.4, 9.6; ^{19}F NMR (282 MHz, $CDCl_3$) δ −152.1 (d, *J* = 18.4 Hz, 2F), −158.2 (t, *J* = 22.9 Hz, 1F), −162.7 (t, *J* = 20.6 Hz, 2F); ESIMS *m*/*z* 716.3 $[M + Na]^+$; HRESIMS *m*/*z* 716.2454 $[M + Na]^+$ (calcd for $C_{35}H_{40}F_5NO_6NaSi$, 716.2443).

*tert-Butyl ((R)-5-((tert-Butyldiphenylsilyl)oxy)-1-((S)-2-(3-((tert-butyldiphenylsilyl)oxy)propyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)(propionyl)carbamate (**33**).* To a stirred solution of methyl tetramate **28** (297.5 mg, 0.726 mmol) in THF (5 mL) at −50 °C was dropwise added LiHMDS (0.54 mL, 0.54 mmol, 1.0 M in THF). After dropwise addition of **32** (252 mg, 0.363 mmol) in THF (2.5 mL), the reaction mixture was stirred at −40 °C for an additional 3 h. To the reaction mixture was added AcOH (0.5 mL); then the solvent was evaporated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (60:1 → 10:1) to afford coupling product **33** (168 mg, 0.182 mmol) as a colorless oil in a yield of 50%: $[\alpha]^{20}_D$ +46 (*c* 1.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56−7.76 (m, 8H), 7.31−7.46 (m, 12H), 5.83 (dd, *J* = 5.6, 9.0 Hz, 1H), 5.03 (s, 1H), 4.68 (dd, *J* = 2.7, 5.5 Hz, 1H), 3.80 (s, 3H), 3.68 (t, *J* = 6.5 Hz, 2H), 3.61−3.66 (m, 1H), 3.54−3.61 (m, 1H), 2.81 (q, *J* = 7.3 Hz, 2H), 2.14−2.29 (m, 2H), 1.97−2.14 (m, 3H), 1.68−1.80 (m, 1H), 1.55−1.66 (m, 2H), 1.49 (s, 9H), 1.13 (t, *J* = 7.5 Hz, 3H), 1.01−1.05 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.1, 177.5, 170.0, 169.5, 135.6, 153.6, 135.7, 134.2, 134.0, 129.8, 129.7, 127.8, 127.8, 94.0, 83.3, 64.0, 63.5, 59.6, 59.6, 58.8, 31.7, 30.1, 28.1, 27.3, 27.1, 27.0, 25.8, 25.7, 19.4, 10.0; ESIMS *m*/*z* 941.9 $[M + Na]^+$; HRESIMS *m*/*z* 941.4578 $[M + Na]^+$ (calcd for $C_{53}H_{70}N_2O_8Na^{28}Si_2$, 941.4568).

*tert-Butyl ((R)-5-Hydroxy-1-((S)-2-(3-hydroxypropyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)(propionyl)-carbamate (**34**).* To a stirred solution of **33** (166 mg, 0.180 mmol) in THF (5 mL) in a Nalgene bottle at 0 °C was added HF/pyridine complex (1 mL, 20 mmol, ∼70% HF in pyridine) in pyridine (1 mL). The reaction mixture was stirred at rt for 5 h, then cooled to 0 °C again. After carefully neutralizing with saturated $NaHCO_3$, the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1 → 1:4) to afford diol **34** (69.1 mg, 0.156 mmol) as a colorless oil in a yield of 86%: $[\alpha]^{20}_D$ +59 (*c* 0.47, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (dd, *J* = 6.5, 8.1 Hz, 1H), 5.06 (s, 1H), 4.71 (dd, *J* = 3.0, 5.5 Hz, 1H), 3.86 (s, 3H), 3.64−3.74 (m, 2H), 3.53−3.64 (m, 2H), 2.70−2.88 (m, 2H), 2.19−2.27 (m, 2H), 2.11−2.19 (m, 2H), 1.85−1.97 (m, 2H), 1.62−1.74 (m, 1H), 1.54−1.60 (m, 1H), 1.48−1.54 (m, 9H), 1.13 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.6, 177.7, 170.2, 169.8, 153.6, 93.9, 83.7, 62.2, 61.3, 59.2, 59.1, 58.7, 31.8, 29.3, 28.1, 26.2, 25.9, 25.5, 10.0; ESIMS *m*/*z*: 465.3 $[M + Na]^+$; HRESIMS *m*/*z* 465.2204 $[M + Na]^+$ (calcd for $C_{21}H_{34}N_2O_8Na$, 465.2213).

*tert-Butyl ((R)-5-Chloro-1-((S)-2-(3-chloropropyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)(propionyl)-carbamate (**35**).* To a stirred solution of deprotected coupling intermediate **34** (22 mg, 0.497 mmol) in CCl_4 (2 mL) at rt was added triphenyl phosphine (52.1 mg, 0.198 mmol). The mixture was refluxed overnight. After addition of hexanes (50 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/EtOAc (300:1) to afford bis-monochlorinated coupling intermediate **35** (14.5 mg, 0.030 mmol) as a colorless oil in a yield of 61%: $[\alpha]^{20}_D$ +71 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (t, *J* = 7.2 Hz, 1 H), 5.07 (s, 1H), 4.65 (dd, *J* = 3.1, 5.8 Hz, 1H), 3.87 (s, 3H), 3.52−3.65 (m, 2H), 3.50 (td, *J* = 2.7, 6.5 Hz, 2H), 2.81 (q, *J* = 7.4 Hz, 2H), 2.25−2.35 (m, 2H), 1.96−2.06 (m, 2H), 1.88−1.96 (m, 1H), 1.82 (m, 1H), 1.63−1.73 (m, 1H), 1.57−1.62 (m, 1H), 1.54 (s, 9H), 1.13 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 177.6, 169.8, 169.4, 153.5, 94.1, 83.8, 59.2, 59.1, 58.9, 44.9, 44.6, 31.7, 30.0, 28.2, 28.2, 27.0, 26.3, 10.0; ESIMS *m*/*z* 501.4 $[M + Na]^+$; HRESIMS *m*/*z* 501.1525 $[M + Na]^+$ (calcd for $C_{21}H_{32}N_2O_6Na^{35}Cl_2$, 501.1535).

*N-((R)-5-Chloro-1-((S)-2-(3-chloropropyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)propionamide (**19**).* Compound **35** (14.0 mg, 0.0292 mmol) was dissolved in TFA/CH_2Cl_2 (2 mL, 25%) and stirred at 0 °C for 1 h. The solvent was evaporated *in vacuo*. After addition of saturated $NaHCO_3$ (2 mL), the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (2:1 → 1:1) to afford **19** (9.4 mg, 0.0247 mmol) as a colorless oil in a yield of 85%: $[\alpha]^{20}_D$ +66 (*c* 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.23 (d, *J* = 8.2 Hz, 1H), 5.72 (td, *J* = 3.6, 8.4 Hz, 1H), 5.12 (s, 1H), 4.71

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(dd, J = 3.1, 5.6 Hz, 1H), 3.90 (s, 3H), 3.61−3.68 (m, 1H), 3.54−3.61 (m, 1H), 3.51 (t, J = 6.4 Hz, 2H), 2.27 (qd, J = 1.3, 7.6 Hz, 2H), 2.20−2.25 (m, 1H), 2.04−2.10 (m, 1H), 1.96−2.04 (m, 2H), 1.79−1.87 (m, 1H), 1.71−1.78 (m, 1H), 1.56−1.65 (m, 2H), 1.17 (t, J = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.2, 173.9, 172.2, 169.3, 94.2, 59.2, 58.7, 52.6, 44.7, 44.5, 31.0, 29.8, 29.0, 26.5, 26.2, 9.9; ESIMS m/z 379.3 $[M + H]^+$; HRESIMS m/z 401.1017 $[M + Na]^+$ (calcd for $C_{16}H_{24}N_2O_4Na^{35}Cl_2$, 401.1011).

*tert-Butyl ((R)-1-((S)-3-Methoxy-5-oxo-2-(3-oxopropyl)-2,5-dihydro-1H-pyrrol-1-yl)-1,5-dioxopentan-2-yl)(propionyl)carbamate (**38**).* To a stirred solution of diol **34** (9.4 mg, 0.0212 mmol) in CH_2Cl_2 (2 mL) at rt was added Dess−Martin periodinane (20 mg, 0.471 mmol). The mixture was stirred at rt for 0.5 h. After addition of CH_2Cl_2 (10 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/EtOAc (100:1) to afford **38** (4.7 mg, 0.106 mmol) as a colorless oil in a yield of 50%: $[\alpha]^{20}_D$ +85 (c 0.24, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.79 (s, 1H), 9.73 (s, 1H), 5.67 (dd, J = 5.6, 8.1 Hz, 1H), 5.06 (s, 1H), 4.65 (dd, J = 3.0, 5.6 Hz, 1H), 3.85 (s, 3H), 2.79 (q, J = 7.3 Hz, 3H), 2.62−2.69 (m, 1H), 2.52−2.58 (m, 2H), 2.46−2.51 (m, 1H), 2.39−2.45 (m, 1H), 2.28−2.36 (m, 2H), 2.24 (s, 1H), 1.53 (s, 9H), 1.13 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.7, 200.6, 178.8, 177.6, 169.6, 169.3, 153.2, 94.2, 84.1, 59.1, 59.0, 58.9, 41.3, 37.7, 31.8, 28.1, 23.2, 22.5, 9.9; ESIMS m/z 461.3 $[M + Na]^+$; HRESIMS m/z 439.2085 $[M + H]^+$ (calcd for $C_{21}H_{31}N_2O_8$, 439.2080).

*N-((R)-5,5-Dichloro-1-((S)-3-chloro-5-(3,3-dichloropropyl)-4-methoxy-2-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)-propionamide (**39**).* To a stirred solution of triphenyl phosphite (6 μL, 0.022 mmol) in CH_2Cl_2 (1 mL) at −20 °C was carefully bubbled chlorine gas until the solution just became bright yellow. After addition of a few drops of triphenyl phosphite, the solution was discharged. To this almost colorless solution at −20 °C were added **38** (2.4 mg, 0.005 mmol) in CH_2Cl_2 and TEA (6 μL, 0.045 mmol). The reaction mixture was stirred at rt overnight, then refluxed for 1 h. After addition of hexanes (5 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (250:1 → 99:1) to afford the crude product.

Using the procedure described above for preparing **19** from **35**, compound **39** (1.2 mg, 0.0024 mmol) was prepared from the crude residue as a colorless oil in a yield of 43% in two steps: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.14 (d, J = 6.66 Hz, 1H), 5.85 (br s, 1H), 5.78 (br s, 1H), 5.67 (br s, 1H), 4.68 (br s, 1H), 4.37 (br s, 3H), 2.47 (br s, 1H), 2.35−2.43 (m, 1H), 2.25−2.34 (m, 3H), 2.18 (br s, 2H), 1.98−2.11 (m, J = 11.2 Hz, 2H), 1.79−1.90 (m, 1H), 1.12−1.21 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.1, 171.5, 168.3, 165.5, 97.7, 72.8, 72.4, 60.5, 57.4, 52.5, 40.0, 37.0, 29.8, 29.7, 25.3, 9.9; ESIMS m/z 505.0 $[M + Na]^+$; HRESIMS m/z 502.9835 $[M + Na]^+$ (calcd for $C_{16}H_{21}N_2O_4Na^{35}Cl_5$, 502.9842).

*tert-Butyl ((R)-4-Hydroxy-1-((S)-2-(2-hydroxyethyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxobutan-2-yl)(propionyl)-carbamate (**40**).* Using the procedure described above for preparation of **34** from L-Glu and D-Glu, compound **40** (60 mg, 0.144 mmol) was prepared (208 mg, 0.233 mmol) as a colorless oil in a yield of 62%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1 → 1:4): $[\alpha]^{20}_D$ +83 (c 0.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (dd, J = 4.6, 9.7 Hz, 1H), 5.04 (s, 1H), 4.71 (t, J = 5.2 Hz, 1H), 3.87 (s, 3H), 3.69−3.78 (m, 1H), 3.65 (t, J = 5.8 Hz, 2H), 3.55−3.62 (m, 1H), 2.74−2.85 (m, 2H), 2.32−2.43 (m, 2H), 2.20−2.32 (m, 1H), 1.91−1.99 (m, 1H), 1.52 (s, 9H), 1.15 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 178.3, 170.9, 169.6, 153.3, 93.2, 84.3, 59.4, 59.1, 58.5, 57.8, 57.0, 34.4, 33.4, 31.9, 28.1, 10.0; ESIMS m/z 437.3 $[M + Na]^+$; HRESIMS m/z 437.1911 $[M + Na]^+$ (calcd for $C_{19}H_{30}N_2O_8Na$, 437.1900).

*tert-Butyl ((R)-4-Bromo-1-((S)-2-(2-bromoethyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxobutan-2-yl)(propionyl)-carbamate (**41**).* To a stirred solution of **40** (30 mg, 0.072 mmol) in CH_2Cl_2 (5 mL) were added CBr_4 (73 mg, 0.220 mmol) and PPh_3 (58 mg, 0.221 mmol) at rt. The reaction mixture was refluxed for 2 h. After addition of hexanes (50 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (8:1) to afford dibromide **41** (22 mg, 0.039 mmol) as a colorless oil in a yield of 55%: $[\alpha]^{20}_D$ +110 (c 0.27, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (dd, J = 6.4, 7.8 Hz, 1H), 5.07 (s, 1H), 4.65 (dd, J = 3.4, 5.8 Hz, 1H), 3.88 (s, 3H), 3.52 (dt, J = 1.5, 7.0 Hz, 2H), 3.22−3.39 (m, 2H), 2.80 (q, J = 7.1 Hz, 2H), 2.64−2.75 (m, 2H), 2.42−2.57 (m, 2H), 1.55 (s, 9H), 1.12 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.6, 177.7, 169.1, 169.1, 153.1, 94.2, 84.2, 59.1, 59.1, 58.8, 33.5, 33.3, 31.8, 30.2, 28.2, 26.3, 9.8; ESIMS m/z 563.0 $[M + Na]^+$; HRESIMS m/z 561.0228 $[M + Na]^+$ (calcd for $C_{19}H_{28}N_2O_6Na^{79}Br_2$, 561.0212).

*(R)-N-(4-Bromo-1-(7-methoxy-5-oxo-4-azaspiro[2.4]hept-6-en-4-yl)-1-oxobutan-2-yl)propionamide (**42**).* To a stirred solution of NaH (1.0 mg, 0.055 mmol, 60% in mineral oil) in DMF (5 mL) was added $CHCl_3$ (18 μL, 0.222 mmol) at −40 °C. The reaction mixture at that temperature was stirred for 10 min. To the trichloromethyl anion solution was added **41** (15 mg, 0.027 mmol) in DMF (1 mL) at −40 °C. The reaction mixture was stirred for an additional 2 h. After addition of water (0.5 mL) and EtOAc (15 mL), the organic layer was washed with saturated $NaHCO_3$ (3 × 5 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*.

The crude residue was dissolved in TFA/CH_2Cl_2 (2 mL, 25%) and stirred at 0 °C for 1 h, then was evaporated *in vacuo*. After addition of saturated $NaHCO_3$ solution (5 mL), the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (30:1) to afford **42** (2.1 mg, 0.0061 mmol) as a colorless oil in a yield of 22%: ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.30 (d, J = 8.3 Hz, 1H), 5.76 (dt, J = 3.2, 8.6 Hz, 1H), 5.15 (s, 1H), 3.86 (s, 1H), 3.55 (dt, J = 6.3, 9.8 Hz, 1H), 3.41 (dt, J = 5.5, 9.9 Hz, 1H), 2.51 (td, J = 3.7, 9.9 Hz, 1H), 2.26 (q, J = 7.5 Hz, 2H), 2.21−2.30 (m, 1H), 2.13−2.20 (m, 1H), 2.02−2.11 (m, 1H), 1.23−1.32 (m, 3H), 1.16 (dt, J = 0.5, 7.5 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.8, 173.7, 171.2, 170.1, 91.6, 58.8, 53.1, 46.3, 37.2, 29.9, 29.0, 11.2, 11.0, 9.9; ESIMS m/z 383.1 $[M + Na]^+$.

*Methyl (S)-2-(Bis(tert-butoxycarbonyl)amino)-5-oxopentanoate (**43**).* To a stirred solution of (*S*)-**21** (2.30 g, 6.12 mmol) in THF (20 mL) at −40 °C was added DIBAL-H (6.70 mL, 6.70 mmol, 1 M in hexanes) at a rate of 1.0 mL/min. The reaction mixture was stirred at −40 °C for 1 h. After successive addition of MeOH (6 mL), HCl (25 mL, 1 M), and Rochelle salt solution (25 mL, 1 M), the reaction mixture was stirred at rt for another 1 h. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (10:1) to afford 4,4,4-trichlorobutanal (*S*)-**43** (1.25 g, 3.61 mmol) as a colorless oil in a yield of 59%: $[\alpha]^{20}_D$ −43.4 (c 0.23, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.76 (s, 1H), 4.88 (dd, J = 5.1, 7.7 Hz, 1H), 3.71 (s, 3H), 2.55−2.64 (m, 1H), 2.45−2.55 (m, 2H), 2.11−2.21 (m, 1H), 1.49 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.1, 170.9, 152.1, 83.6, 57.5, 52.4, 40.7, 28.1, 22.7; ESIMS m/z 400.4 $[M + CH_3OH + Na]^+$; HRESIMS m/z 346.1872 $[M + H]^+$ (calcd for $C_{16}H_{28}NO_7$, 346.1866).

*Methyl (S)-2-Amino-5,5-dichloropentanoate (**44**).* To a stirred solution of triphenyl phosphite (1.73 mL, 6.60 mmol) in CH_2Cl_2 (5 mL) at −20 °C was carefully bubbled chlorine gas until the solution just became bright yellow. After addition of a few drops of triphenyl phosphite, the solution was discharged. To this almost colorless solution at −20 °C were added (*S*)-**43** (1.14 g, 3.30 mmol) in CH_2Cl_2 (3 mL) and TEA (1.84 mL, 13.2 mmol). The reaction mixture was stirred at rt overnight, then refluxed for 6 h. After addition of hexanes (200 mL), the white precipitate formed was filtered off; then the filtrate was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (20:1) to afford *gem*-dichloride (1.10 g, 2.74 mmol) as a colorless oil in a yield of 83%: $[\alpha]^{20}_D$ −32 (c 0.56, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81

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(dd, J = 5.0, 6.2 Hz, 1H), 4.87 (dd, J = 5.0, 9.3 Hz, 1H), 3.72 (s, 3H), 2.34−2.44 (m, 1H), 2.26−2.34 (m, 1H), 2.10−2.25 (m, 2H), 1.50 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 152.1, 83.7, 73.0, 57.1, 52.5, 40.4, 28.1, 26.5; ESIMS m/z 422.3 $[M + Na]^+$; HRESIMS m/z 422.1105 $[M + Na]^+$ (calcd for $C_{16}H_{27}NO_6Na^{35}Cl_2$, 422.1113).

The *gem*-dichloride (1.05 g, 2.62 mmol) was dissolved in TFA/CH_2Cl_2 (15 mL, 25%) and stirred at 0 °C for 1 h, then was evaporated *in vacuo*. After addition of a saturated $NaHCO_3$ solution (30 mL), the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (9:1) to afford free amine **44** (418 mg, 2.09 mmol) as a colorless oil in a yield of 80%: $[\alpha]^{20}_D$ +12 (c 0.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (t, J = 5.8 Hz, 1H), 3.74 (s, 3H), 2.24−2.46 (m, 2H), 1.94−2.10 (m, 1H), 1.71−1.89 (m, 1H), 1.45 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 73.3, 53.7, 52.4, 40.2, 31.0; ESIMS m/z 200.3 $[M + H]^+$; HRESIMS m/z 200.0241 $[M + H]^+$ (calcd for $C_6H_{12}NO_2{}^{35}Cl_2$, 200.0245).

*(R)-2-((tert-Butoxycarbonyl)amino)-5,5-dichloropentanoic Acid (**45**).* Using the procedure described above for the preparation of (*S*)-**43** from (*S*)-**21**, (*R*)-**45** (1.30 g, 3.76 mmol) was prepared from (*R*)-**21** (2.83 g, 7.53 mmol) as a colorless oil in a yield of 50%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (10:1): $[\alpha]^{20}_D$ +39 (c 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H), 4.89 (dd, J = 4.7, 9.3 Hz, 1H), 3.72 (s, 3H), 2.57−2.66 (m, 1H), 2.45−2.56 (m, 2H), 2.10−2.24 (m, 1H), 1.50 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2, 170.9, 152.2, 83.7, 57.5, 52.5, 40.7, 28.2, 22.7; HRESIMS m/z 346.1860 $[M + H]^+$ (calcd for $C_{16}H_{28}NO_7$, 346.1866).

*(R)-2-((tert-Butoxycarbonyl)amino)-5,5-dichloropentanoic Acid (**46**).* Using the procedure described above for the preparation of (*S*)-*gem*-dichloride from (*S*)-**43**, the (*R*)-*gem*-dichloride (869 mg, 2.17 mmol) was prepared from (*R*)-**45** (1.12 g, 3.24 mmol) as a colorless oil in a yield of 67%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (20:1): $[\alpha]^{20}_D$ +32 (c 0.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (t, J = 5.6 Hz, 1H), 4.90 (dd, J = 5.2, 9.1 Hz, 1H), 3.74 (s, 3H), 2.36−2.47 (m, 1H), 2.28−2.36 (m, 1H), 2.14−2.28 (m, 2H), 1.52 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 152.2, 83.8, 73.0, 57.1, 52.6, 40.4, 28.2, 26.5; ESIMS m/z 422.2 $[M + Na]^+$; HRESIMS m/z 422.1103 $[M + Na]^+$ (calcd for $C_{16}H_{27}NO_6Na^{35}Cl_2$, 422.1113).

To a stirred solution of methyl ester (750 mg, 1.87 mmol) in MeCN (50 mL) at rt was added lithium bromide (480 mg, 5.52 mmol). The reaction mixture was stirred 65 °C for 12 h; then the solvent was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (10:1) to afford the methyl ester of **46** (428 mg, 1.42 mmol) as a colorless oil in a yield of 76%: $[\alpha]^{20}_D$ −21 (c 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (t, J = 5.6 Hz, 1H), 5.14 (d, J = 7.0 Hz, 1H), 4.34 (br s, 1H), 3.74 (s, 3H), 2.16−2.34 (m, 2H), 2.05−2.16 (m, 1H), 1.82−1.93 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 155.5, 80.4, 72.8, 52.7, 52.5, 39.5, 29.3, 28.4; ESIMS m/z 322.2 $[M + Na]^+$; HRESIMS m/z 300.0775 $[M + H]^+$ (calcd for $C_{11}H_{20}NO_4{}^{35}Cl_2$, 300.0769).

To a stirred solution of the methyl ester (287 mg, 0.957 mmol) in H_2O/THF (5 mL, 1:1) at 0 °C was added $Ba(OH)_2$ (910 mg, 5.31 mmol). The reaction mixture was stirred at 0 °C for 1 h; then THF was removed *in vacuo*. Placed in an ice/water bath, to the reaction mixture was added 1 M HCl dropwise until its pH value was adjusted to pH = 3. The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with CH_2Cl_2/EtOAc (98:2) to afford **46** (191 mg, 0.667 mmol) as a colorless oil in a yield of 70%: $[\alpha]^{20}_D$ −15 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.54 (br s, 1H), 6.83 and 5.36 (d each, J = 5.7 Hz, 2H), 5.79 (t, J = 5.6 Hz, 1H), 4.34 and 4.13 (br s each, 1H), 2.21−2.35 (m, 2H), 2.07−2.18 (m, 1H), 1.83−1.97 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.4 and 176.4, 157.1 and 155.8, 82.4 and 80.7, 72.7, 53.7 and 52.4, 39.4, 28.3, 20.9; HRESIMS m/z 308.0435 $[M + Na]^+$ (calcd for $C_{10}H_{17}NO_4Na^{35}Cl_2$, 308.0432).

*Methyl (S)-2-((R)-2-((tert-Butoxycarbonyl)amino)-5,5-dichloropentanamido)-5,5-dichloropentanoate (**47**).* To a stirred solution of **46** (156 mg, 0.545 mmol), **44** (109 mg, 0.545 mmol), and HOAt (313 mg, 1.631 mmol) in THF (6 mL) at rt was added EDCI (253 mg, 1.635 mmol). The reaction mixture was stirred at rt overnight. After addition of HCl (10 mL, 1 M), the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (4:1) to give dipeptide **47** (193 mg, 0.412 mmol) as a light yellow oil in a yield of 76%: $[\alpha]^{20}_D$ +31 (c 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (d, J = 5.1 Hz, 1H), 5.82 (dt, J = 5.7, 8.1 Hz, 4H), 5.26 (d, J = 6.1 Hz, 1H), 4.64 (td, J = 4.6, 8.2 Hz, 1H), 4.26 (d, J = 3.6 Hz, 1H), 3.77 (s, 3H), 2.23−2.34 (m, 3H), 2.15−2.22 (m, 2H), 2.09−2.16 (m, 1H), 1.86−1.97 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.1, 171.7, 155.9, 80.8, 72.9, 72.6, 53.4, 53.0, 51.3, 39.6, 39.3, 28.8, 28.7, 28.5; HRESIMS m/z 489.0482 $[M + Na]^+$ (calcd for $C_{16}H_{26}N_2O_5Na^{35}Cl_4$, 489.0494).

*tert-Butyl ((R)-5,5-Dichloro-1-((S)-2-(3,3-dichloropropyl)-3-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)carbamate (**48**) and the Epimer **49**.* To a stirred solution of dipeptide **47** (172 mg, 0.369 mmol) in H_2O/THF (2 mL, 1:1) at 0 °C was added LiOH (1.48 mL, 0.739 mmol, 0.5 M in H_2O). The reaction mixture was stirred at 0 °C for 1 h, and then THF was removed *in vacuo*. To the reaction mixture placed in an ice water bath was added 1 M HCl dropwise until its pH value was adjusted to pH = 3. The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was used directly for the next step without further purification.

To a stirred solution of crude residue, Meldrum's acid (63.9 mg, 0.443 mmol), and DMAP (225.7 mg, 1.85 mmol) in CH_2Cl_2 (5 mL) at −10 °C was added IPCC (80 μL, 0.739 mmol) in CH_2Cl_2 (220 μL) at a rate of 10 μL/min. The reaction mixture was stirred at −10 °C for an additional 5 h and then poured into a $KHSO_4$ solution (10 mL, 5%). The separated aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The yellowish crude residue was refluxed in MeCN (20 mL) for 3 h; then the solvent was removed *in vacuo*. The crude residue was used directly for the next step without further purification.

To a stirred solution of the crude residue in toluene/MeOH (3 mL, 4:1) at rt was dropwise added $TMSCHN_2$ (0.18 mL, 0.369 mmol, 2.0 M in hexanes). After addition of AcOH (0.18 mL), the reaction mixture was dried *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (10:1 → 5:1) to afford **49** (41 mg, 0.084 mmol) as a colorless oil in a yield of 23% and **48** (47 mg, 0.095 mmol) as a colorless oil in a yield of 26%. **48**: $[\alpha]^{20}_D$ +77 (c 0.15, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.87 (t, J = 5.9 Hz, 1H), 5.75 (t, J = 5.6 Hz, 1H), 5.43 (t, J = 7.9 Hz, 1H), 5.27 (d, J = 8.2 Hz, 1H), 5.14 (s, 1H), 4.74 (br s, 1H), 3.91 (s, 3H), 2.42−2.49 (m, 1H), 2.30−2.40 (m, 2H), 2.10−2.18 (m, 2H), 2.00−2.07 (m, 2H), 1.73−1.80 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.9, 172.2, 169.1, 155.8, 94.3, 80.2, 73.0, 72.6, 59.4, 58.2, 53.8, 40.0, 37.2, 30.1, 28.5, 25.3; HRESIMS m/z 491.0667 $[M + Na]^+$ (calcd for $C_{18}H_{27}N_2O_5Na^{35}Cl_4$, 491.0674). **49**: $[\alpha]^{20}_D$ −48 (c 0.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85 (t, J = 5.6 Hz, 1H), 5.74 (t, J = 5.6 Hz, 1H), 5.32 (t, J = 7.9 Hz, 1H), 5.19 (d, J = 7.2 Hz, 1H), 5.14 (s, 1H), 4.69 (br s, 1H), 3.91 (s, 3H), 2.40−2.47 (m, 2H), 2.18−2.24 (m, 1H), 2.13−2.17 (m, 1H), 2.04−2.10 (m, 1H), 1.93−2.00 (m, 1H), 1.65−1.74 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.8, 172.6, 169.5, 155.9, 94.5, 80.4, 73.1, 73.0, 59.3, 58.6, 53.6, 40.1, 36.6, 28.7, 28.5, 25.2; HRESIMS m/z 491.0665 $[M + Na]^+$ (calcd for $C_{18}H_{27}N_2O_5Na^{35}Cl_4$, 491.0674).

*N-((R)-5,5-Dichloro-1-((S)-2-(3,3-dichloropropyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)propionamide (**36**).* Using the procedure described above for preparation of **30** from

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compound **29**, **36** (11.5 mg, 0.025 mmol) was prepared from **48** (18.1 mg, 0.036 mmol) as a colorless oil in a yield of 70%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:2 → 1:1): $[\alpha]^{20}_{D}$ +76 (*c* 0.06, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.26 (d, *J* = 8.2 Hz, 1H), 5.87 (dd, *J* = 4.9, 6.4 Hz, 1H), 5.76 (t, *J* = 5.4 Hz, 1H), 5.73 (td, *J* = 3.6, 8.7 Hz, 1H), 5.16 (s, 1H), 4.73 (dd, *J* = 3.1, 5.6 Hz, 1H), 3.92 (s, 3H), 2.41−2.47 (m, 1H), 2.34−2.41 (m, 1H), 2.26 (qd, *J* = 2.0, 7.5 Hz, 2H), 2.13−2.26 (m, 3H), 2.02−2.07 (m, 2H), 1.79−1.87 (m, 1H), 1.18 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.9, 174.0, 171.8, 169.0, 94.3, 73.0, 72.6, 59.4, 58.2, 52.2, 39.9, 37.1, 30.0, 29.8, 25.3, 9.9; HRESIMS *m*/*z* 447.0419 $[M + H]^+$ (calcd for $C_{16}H_{23}N_2O_4{}^{35}Cl_4$, 447.0412).

*N-((R)-5,5-Dichloro-1-((R)-2-(3,3-dichloropropyl)-3-methoxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)propionamide (**50**).* Using the procedure described above for the preparation of **30** from compound **29**, **50** (7.6 mg, 0.017 mmol) was prepared from **49** (14 mg, 0.028 mmol) as a colorless oil in a yield of 60%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1 → 3:2): $[\alpha]^{20}_{D}$ −62 (*c* 0.07, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.19 (d, *J* = 8.2 Hz, 1H), 5.85 (dd, *J* = 5.1, 6.7 Hz, 1H), 5.76 (dd, *J* = 4.4, 6.4 Hz, 1H), 5.57 (td, *J* = 3.6, 9.0 Hz, 1H), 5.15 (s, 1H), 4.70 (dd, *J* = 2.6, 5.6 Hz, 1H), 3.92 (s, 3H), 2.35−2.46 (m, 2H), 2.28−2.34 (m, 1H), 2.26 (qd, *J* = 2.0, 7.5 Hz, 2H), 2.10−2.19 (m, 3H), 1.92−2.00 (m, 1H), 1.73−1.82 (m, 1H), 1.17 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.8, 174.1, 171.9, 169.4, 94.5, 73.1, 73.0, 59.3, 58.6, 52.2, 40.1, 36.6, 29.7, 28.9, 25.2, 9.9; ESIHRMS *m*/*z* 469.0243 $[M + Na]^+$ (calcd for $C_{16}H_{22}N_2O_4Na^{35}Cl_4$, 469.0231).

*Methyl 4,4,4-Trichlorobutanoate (**51**).* To a vigorously stirred solution of NaOH (10 g, 0.25 mol) in water (10 mL) was successively added $BnEt_3N^+Cl^-$ (0.20 g, 0.878 mmol), methyl acrylate (4.0 mL, 44.4 mmol), and $CHCl_3$ (40 g, 500 mmol) at 0 °C. The reaction mixture was stirred at rt for 2 h. After addition of CH_2Cl_2 (50 mL) and water (50 mL), the separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Vacuum distillation of the crude residue gave methyl 4,4,4-trichlorobutanoate **51** (7.39 g, 36.0 mmol) as a colorless liquid in a yield of 81%. Alternatively, the crude residue could also be purified by silica gel flash chromatography eluting with hexanes/acetone (19:1) to afford **51**: bp 78 °C−80 °C/1 Torr (lit. bp 80 °C/0.3 Torr); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H), 2.95−3.13 (m, 2H), 2.68−2.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 98.7, 52.3, 50.0, 31.4; no EI- or ESIMS was detected.

*4,4,4-Trichlorobutanal (**52**).* To a stirred solution of methyl 4,4,4-trichlorobutanoate **51** (1.35 g, 6.57 mmol) in CH_2Cl_2 (30 mL) at −78 °C was added DIBAL-H (6.57 mL, 6.57 mmol, 1.0 M in hexanes) at a rate of 1.0 mL/min. The reaction mixture was stirred at −78 °C for 1 h. After successive addition of MeOH (7 mL), HCl (25 mL, 1 M), and Rochelle salt solution (25 mL, 1 M), the reaction mixture was stirred at rt for another 1 h. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (20:1) to afford 4,4,4-trichlorobutanal **52** (1.15 g, 5.78 mmol) as a colorless oil in a yield of 88%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 3.03−3.11 (m, 2H), 2.96−3.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.5, 98.9, 47.5, 41.2; no EI- or ESIMS was detected.

*(S)-2-Methyl-N-((S)-4,4,4-trichloro-1-cyanobutyl)propane-2-sulfinamide (**53**).* To a stirred solution of aldehyde **52** (1.42 g, 8.09 mmol) in CH_2Cl_2 (10 mL) were added (*S*)-(−)-*tert*-butanesulfinamide (0.98 g, 6.33 mmol) and $CuSO_4$ (3.88 g, 24.2 mmol) at rt. The reaction mixture was stirred at rt for 4 days. The catalyst was removed through a pad of Celite with CH_2Cl_2 (200 mL), and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (100:3) to afford the (*S*)-sulfinimine (2.0 g, 7.18 mmol) as a colorless oil in a yield of 90%: $[\alpha]^{20}_{D}$ +172 (*c* 0.68, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (br s, 1H), 3.03−3.15 (m, 2H), 2.93−3.03 (m, 2H), 1.18 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 98.9, 57.0, 50.2, 33.3, 22.5; HRESIMS *m*/*z* 277.9942 $[M + H]^+$ (calcd for $C_8H_{15}NO^{32}S^{35}Cl_3$, 277.9940).

To a stirred solution of the sulfinimine (1.20 g, 4.31 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added trimethylsilyl cyanide (1.17 mL, 8.61 mmol) and $Sc(OTf)_3$ (0.32 g, 0.64 mmol). The reaction mixture was stirred at 0 °C for 2 days and at rt for another 2 days. The reaction solution furnished pure diastereomeric cyanide intermediate (*S*,*S*)-**53** (1.13 g, 3.69 mmol) in a yield of 20% (86% for both diastereomers): $[\alpha]^{20}_{D}$ +23 (*c* 0.39, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.36 (d, *J* = 6.7 Hz, 1H), 4.15 (br s, 1H), 2.92 (t, *J* = 7.7 Hz, 2H), 2.34−2.47 (m, 2H), 1.28 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 118.5, 98.2, 57.6, 50.6, 45.0, 31.7, 22.7; HRESIMS *m*/*z* 305.0051 $[M + H]^+$ (calcd for $C_9H_{16}N_2O^{32}S^{35}Cl_3$, 305.0049).

*(R)-2-Methyl-N-((R)-4,4,4-trichloro-1-cyanobutyl)propane-2-sulfinamide (**53**).* $[\alpha]^{20}_{D}$ −28 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 (br s, 1H), 4.16 (br s, 1H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.28−2.52 (m, 2H), 1.28 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 118.5, 98.2, 57.8, 50.6, 45.2, 31.7, 22.8; ESIMS *m*/*z* 329.3 $[M + Na]^+$; HRESIMS *m*/*z* 305.0056 $[M + H]^+$ (calcd for $C_9H_{16}N_2O^{32}S^{35}Cl_3$, 305.0049).

*Methyl (S)-2-Amino-5,5,5-trichloropentanoate (**54**).* HCl gas was generated by dripping HCl (100 mL, concentrated) onto an anhydrous $CaCl_2$ granule (100 g). Into a stirred solution of (*S*,*S*)-**53** (579 mg, 1.77 mmol) in 10 mL of MeOH (10 mL) was bubbled HCl gas for 1 h. The solution was stirred at rt overnight. A saturated $NaHCO_3$ solution was added dropwise to the reaction mixture in an ice water bath until its pH value was adjusted to pH = 10. The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (5:1) to afford **54** (300 mg, 1.28 mmol) as a colorless oil in a yield of 73%: $[\alpha]^{20}_{D}$ +7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (s, 3H), 3.50 (dd, *J* = 4.9, 8.2 Hz, 1H), 2.84−2.97 (m, 1H), 2.65−2.84 (m, 1H), 2.10−2.30 (m, 1H), 1.87−2.10 (m, 1H), 1.58 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 99.7, 53.4, 52.4, 51.7, 31.6; ESIMS *m*/*z* 234.3 $[M + H]^+$; ESIHRMS *m*/*z* 233.9851 $[M + H]^+$ (calcd for $C_6H_{11}NO_2{}^{35}Cl_3$, 233.9855).

*(R)-2-((tert-Butoxycarbonyl)amino)-5,5,5-trichloropentanoic Acid (**55**).* Using the procedure described above for preparation of **54** from (*S*,*S*)-**53**, the amine (610 mg, 2.60 mmol) was prepared from (*R*,*R*)-**53** (1.10 g, 3.60 mmol) as a colorless oil in a yield of 72%. To a stirred solution of the amine (610 mg, 2.60 mmol), $NaHCO_3$ (436 mg, 5.19 mmol), in H_2O/THF (10 mL, 1:1) was added Boc_2O (573 mg, 2.62 mmol) at rt. The reaction mixture was refluxed overnight, and then THF was removed *in vacuo*. $NaHSO_4$ (1 M) was added dropwise to the reaction mixture in an ice water bath until its pH value was adjusted to pH = 3. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:2) to afford the *N*-Boc methyl ester intermediate (670 mg, 2.00 mmol) as a colorless oil in a yield of 77%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.17 (br s, 1H), 4.40 (br s, 4H), 3.78 (s, 3H), 2.63−2.90 (m, 2H), 2.34 (t, *J* = 12.3 Hz, 1H), 2.00−2.17 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 155.5, 99.2, 80.5, 52.9, 52.4, 51.2, 30.0, 28.5; ESIMS *m*/*z* 358.2 $[M + Na]^+$; HRESIMS *m*/*z* 356.0119 $[M + Na]^+$ (calcd for $C_{11}H_{18}NO_4NaCl_3$, 356.0203).

To a stirred solution of the *N*-Boc methyl ester intermediate (540 mg, 1.61 mmol) in H_2O/THF (2 mL, 1:1) at 0 °C was added LiOH (6.5 mL, 3.25 mmol, 0.5 M in H_2O). The reaction mixture was stirred at 0 °C for 1 h; then THF was removed *in vacuo*. HCl (1 M) was added dropwise to the reaction mixture in an ice water bath until its pH value was adjusted to pH = 3. The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:2) to afford **55** (505 mg, 1.57 mmol) as a colorless oil in a yield of 98%: $[\alpha]^{20}_{D}$ −25 (*c* 4.7, $CHCl_3$); ^{1}H NMR (400 MHz, MeOH-d_4) δ 5.09 (br s, 2H), 4.19 (dd, *J* = 4.7, 7.8 Hz,

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1H), 2.66−2.91 (m, 2H), 2.21−2.37 (m, 1H), 2.01−2.18 (m, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 174.9, 158.0, 100.6, 80.8, 53.6, 52.6, 50.0, 30.1, 28.8; ESIMS m/z 320.3 $[M + H]^+$; HRESIMS m/z 342.0044 $[M + Na]^+$ (calcd for $C_{10}H_{16}NO_4NaCl_3$, 342.0043).

*Methyl (S)-2-((R)-2-((tert-Butoxycarbonyl)amino)-5,5,5-trichloropentanamido)-5,5,5-trichloropentanoate (**56**).* Using the procedure described above for preparation of **47** from coupling **44** and **46**, **56** (150 mg, 0.279 mmol) was prepared from **54** (89 mg, 0.380 mmol) and **55** (122 mg, 0.380 mmol) as a colorless oil in a yield of 73%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (6:1 → 3:1): $[\alpha]^{20}_D$ +22 (*c* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97 (br s, 1H), 5.14 (d, *J* = 6.7 Hz, 1H), 4.66−4.78 (m, 1H), 4.30 (d, *J* = 2.0 Hz, 1H), 3.81 (s, 3H), 2.72−2.89 (m, 3H), 2.63−2.71 (m, 1H), 2.35−2.47 (m, 2H), 2.14−2.21 (m, 1H), 2.07−2.13 (m, 1H), 1.48 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 171.3, 155.9, 99.2, 98.9, 81.2, 53.3, 53.2, 51.3, 51.1, 51.1, 29.6, 29.3, 28.5; HRESIMS m/z 556.9713 $[M + Na]^+$ (calcd for $C_{16}H_{24}N_2O_5NaCl_6$, 556.9714).

*N-((R)-5,5,5-Trichloro-1-((R)-3-methoxy-5-oxo-2-(3,3,3-trichloropropyl)-2,5-dihydro-1H-pyrrol-1-yl)-1-oxopentan-2-yl)propionamide (**57**) and the R,S Epimer **37**.* Using the procedure described above for preparation of **36** from **47**, **37** (2.4 mg, 0.0047 mmol) was prepared from **56** (150 mg, 0.279 mmol) as a colorless oil in a yield of 1.7%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (7:1 → 3:2): $[\alpha]^{20}_D$ −28 (*c* 0.53, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.22 (d, *J* = 8.7 Hz, 1H), 5.70 (td, *J* = 3.6, 9.0 Hz, 1H), 5.19 (s, 1H), 4.75 (dd, *J* = 3.1, 5.6 Hz, 1H), 3.95 (s, 3H), 2.93−3.01 (m, 1H), 2.75−2.84 (m, 1H), 2.63−2.72 (m, 2H), 2.43−2.51 (m, 1H), 2.33−2.40 (m, 2H), 2.28 (qd, *J* = 3.3, 7.6 Hz, 2H), 1.91−1.99 (m, 1H), 1.17 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.6, 174.1, 171.7, 169.3, 99.5, 99.2, 94.7, 59.4, 58.3, 51.7, 51.6, 48.4, 30.2, 29.8, 26.1, 9.9; ESIMS m/z 539.2 $[M + Na]^+$; HRESIMS m/z 516.9608 $[M + H]^+$ (calcd for $C_{16}H_{21}N_2O_4{}^{35}Cl_5{}^{37}Cl$, 516.9603).

Under a similar procedure described above, **37** (3.0 mg, 0.0058 mmol) was prepared from **56** (150 mg, 0.279 mmol) as a colorless oil in a yield of 2.1%. The crude residue was purified by silica gel flash chromatography eluting with hexanes/EtOAc (3:2 → 1:1): $[\alpha]^{20}_D$ +28 (*c* 0.65, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.28 (d, *J* = 8.2 Hz, 1H), 5.79 (td, *J* = 3.6, 8.4 Hz, 1H), 5.19 (s, 1H), 4.79 (m, 1H), 3.95 (s, 3H), 2.98 (ddd, *J* = 4.6, 11.8, 14.3 Hz, 1H), 2.71−2.79 (m, 1H), 2.57−2.63 (m, 1H), 2.54 (dd, *J* = 6.1, 9.2 Hz, 2H), 2.42−2.49 (m, 1H), 2.34−2.41 (m, 1H), 2.30 (qd, *J* = 3.8, 7.6 Hz, 2H), 1.99−2.08 (m, 1H), 1.19 (t, *J* = 7.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.6, 174.0, 171.7, 168.9, 99.3, 98.9, 94.5, 59.5, 57.9, 51.9, 51.4, 48.9, 31.1, 29.9, 26.3, 9.9; ESIMS m/z 539.2 $[M + Na]^+$; ESIHRMS m/z 516.9598 $[M + H]^+$ (calcd for $C_{16}H_{21}N_2O_4{}^{35}Cl_5{}^{37}Cl$, 516.9603).

Cells and Plasmids. LNCaP cells and PSA(6.1kb)-Luc plasmids have been previously described.[11]

Transfection Assay. LNCaP cells (4×10^4 cell/well) plated into 24-well plates were transfected with PSA(6.1 kb)-luciferase reporter and pretreated the next day with serial dilutions or 10 μM of sintokamides analogues, before addition of synthetic androgen (1 nM) R1881 under serum-free and phenol red-free conditions. After 48 h of exposure, cells were harvested, and luciferase activity was measured and normalized to protein concentration. IC_{50} values were calculated using OriginPro 8.1 software (Northampton, MA, USA).

Cell Viability and Proliferation. A total of 2000 PC3 cells, 3000 DU145 cells, and 5000 LNCaP cells were seeded in 96-well plates in their respective media plus 0.5% FBS for 24 h. PC3 and DU145 cells were treated with compounds for 72 h. LNCaP cells were treated for 1 h with inhibitors before the addition of 0.1 nM R1881 for 4 days. Cell viability was measured using the Alamar blue cell viability assay (Invitrogen) following the manufacturer's protocol.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00921.

^{1}H and ^{13}C NMR data for synthetic intermediates and all analogues tested in the bioassays; details for the scale-up synthesis of **76**; details for X-ray diffraction analysis of (*R*,*R*)-**53**, (*S*,*S*)-**53**, and LPY26 (**66**) (PDF)

X-ray file (CIF)

X-ray file (CIF)

X-ray file (CIF)

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Notes

The authors declare the following competing financial interest(s): R. Andersen and M. Sadar have founders shares in ESSA Pharma. The sintokamide technology has been licensed to ESSA.

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■ DEDICATION

Dedicated to Dr. A. Douglas Kinghorn, The Ohio State University, for his pioneering work on bioactive natural products.

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