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Flexible Synthesis and Evaluation of Diverse Anti-Apicomplexa Cyclic Peptides

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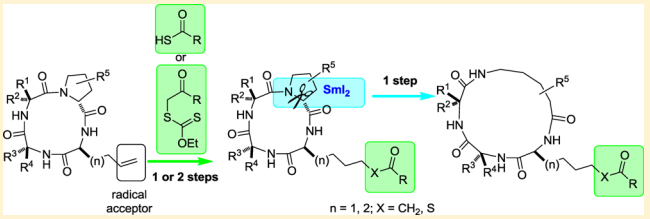
Supporting Information

ABSTRACT: A modular approach to synthesize anti-Apicomplexa parasite inhibitors was developed that takes advantage of a pluripotent cyclic tetrapeptide scaffold capable of adjusting appendage and skeletal diversities in only a few steps (one to three steps). The diversification processes make use of selective radical coupling reactions and involve a new example of a reductive carbon−nitrogen cleavage reaction with SmI_2. The resulting bioactive cyclic peptides have revealed new insights into structural factors that govern selectivity between Apicomplexa parasites such as *Toxoplasma* and *Plasmodium* and human cells.



INTRODUCTION

The Apicomplexa phylum of protozoa includes important human pathogens such as *Plasmodium falciparum* and *Toxoplasma gondii*, responsible for malaria and toxoplasmosis, respectively. Targeting epigenetic mechanisms is an emerging and attractive area for therapeutic applications, particularly those involved in histone modifications.[1] Histone deacetylase (HDAC) holds a prominent position as a drug target due to its role in the regulation of the dynamic post-translational level of acetylation in lysine residues located at the histone tail. The inhibition of this key event in chromatin remodeling results in alteration of gene expression regulation.[2] This approach has been validated by the discovery of selective histone deacetylase inhibitors (HDACis) against Apicomplexa parasites such as *P. falciparum*.[3,4] For many years, naturally occurring cyclic tetrapeptides or depsipeptides have served as rich sources for the discovery of new HDACis.[5] Apicidin (Figure 1) was the first described HDACi that exhibits an inhibition effect on Apicomplexa parasite proliferation, albeit with low selectivity.[4a] FR235222,[6] another cyclic tetrapeptide isolated in 2003 from the fermentation broth of the fungi *Acremonium*, was reported to exhibit immunosuppressive properties and HDAC inhibitions. Most recently, the close natural product analogue AS1387392 (R = H)[7] was reported to display a better oral absorption with a plasma concentration higher than that of FR235222.

Recently, Hakimi and co-workers showed that FR235222 was highly effective against different Apicomplexa.[8] With *P. falciparum* and *berghei* parasites, the growth and differentiation were blocked in red blood cells. FR235222 also inhibits *T. gondii* intracellular growth at a very low nanomolar level (ED_{50} = 9.7 nM) and is much more potent than pyrimethamine, a current toxoplasmosis drug, evaluated under the same assay conditions. At sublethal concentrations, this molecule induces *T. gondii* differentiation from replicative (tachyzoite) to cystogenic (bradyzoite) form. Importantly, *T. gondii* HDAC3 (*Tg*HDAC3), a class I HDAC zinc-dependent enzyme, was identified as the target of FR235222. Moreover, the infectious capacity of Toxoplasma cysts was abolished when they were treated with FR235222, broadening the therapeutic applications from acute to chronic toxoplasmosis, particularly for immunocompromised patients.[9]

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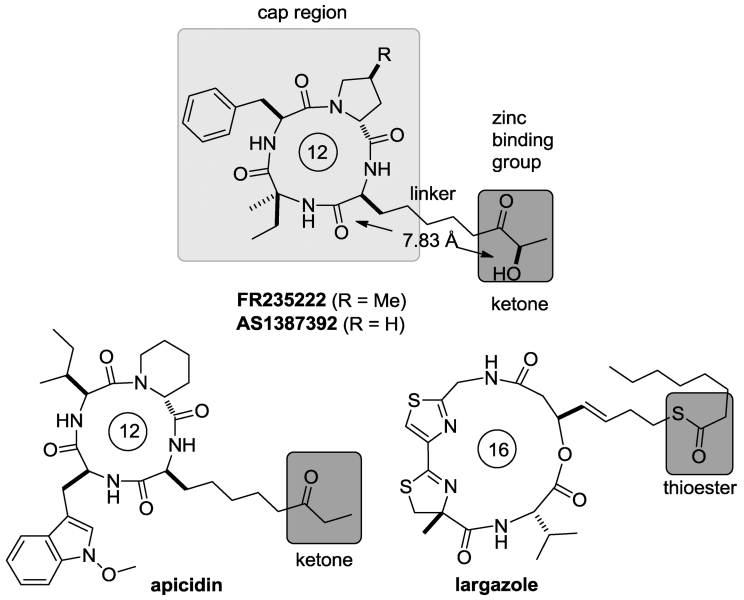
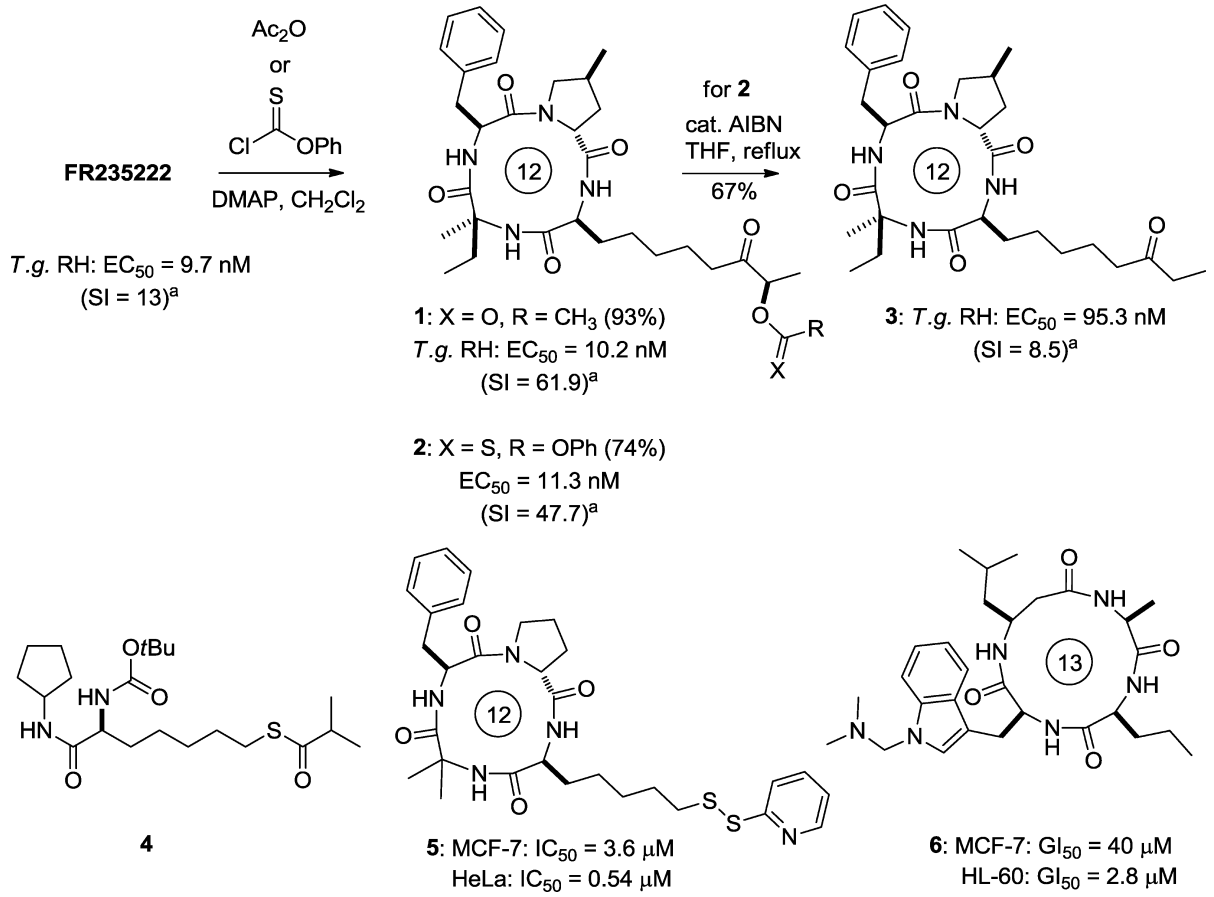


Figure 1. Natural cyclic tetrapeptides and depsipeptides HDACi.

Scheme 1. Synthetic Analogues and Their Biological Activities on Cells[a]



[a]Selectivity index (SI): EC_{50} human foreskin fibroblasts (HFF)/EC_{50} *T. gondii* RH strain (*T.g.* RH). MCF-7 (breast cancer), HeLa (cervical cancer), HL-60 (acute promyelocytic leukemia).

With regard to their key pharmacophoric features, these natural products can be divided into two regions (Figure 1), the cyclic peptide part (cap region) involved in the recognition or cell permeability[10] and the zinc binding group (ZBG), both parts being joined by a hydrophobic linker of about 7.8 Å length. Carbonyl groups are found to be a privileged ZBG in many HDACi. Recently, we reported modifications of FR235222 at its ZBG by converting its chiral hydroxyl group located at the

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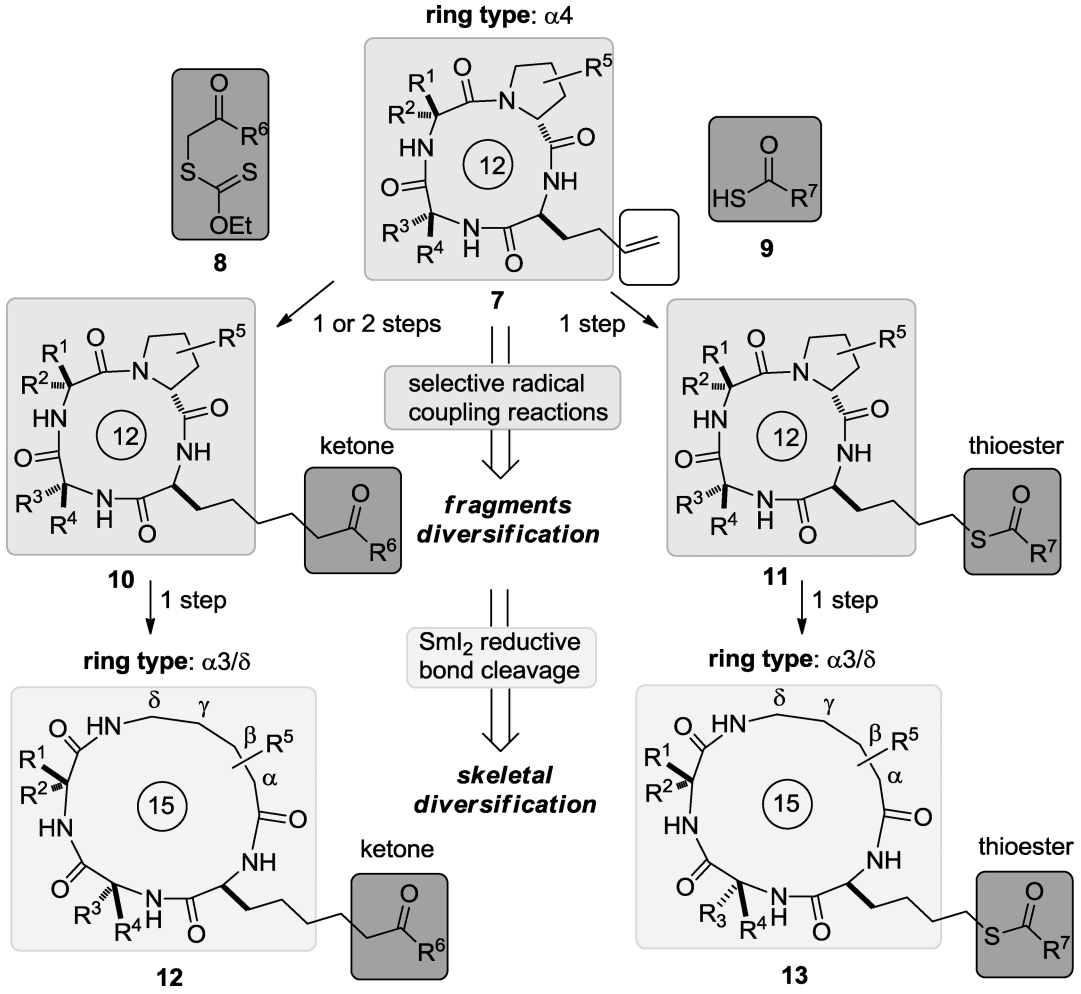
Scheme 2. Connecting Bio-Relevant Analogues by Short Reaction Pathways



position α to the ketone group to either acetate (**1**) or thioacetate (**2**) derivatives (Scheme 1).[9] These products appeared to be significantly less toxic to human foreskin fibroblasts (HFF) while keeping efficiency at a low nanomolar concentration against *T. gondii*. Conversely, removal of these chiral hydroxyl groups to give **3**, having the apicidin-like ZGB (ethyl ketone), resulted in reduced potency against *T. gondii*. The possibility of altering such selectivity warrants the development of further new carbonyl ZBGs. Thioester, which is found in the HDACi natural cyclic depsipeptide largazole[11] (Figure 1) or synthetic HDACi (**4**),[12] is a novel and emergent masked ZBG. Like the disulfide group (e.g. **5**[13]), thioester is expected to act as a prodrug group by the delivery in cell medium of a free thiol capable of chelating the zinc cation in the catalytic pocket.[14] The exploration of the cyclic peptide part by iterative variations of amino acid residues has been reported along the conventional α_4 ring system (12-membered ring)[15] or with enlarged β-amino acid containing scaffolds such as α_3/β and α_2/β_2 (13- or 14-membered ring).[16] The results showed that larger cyclic α_3/β pseudotetrapeptides can give rise to selective HDACis.[17] Moreover, it was recently demonstrated that the cyclic α_3/β-pseudotetrapeptide **6** without ZBG can retain HDACi activity by itself by interacting with the active-site opening.[18]

Our current interest in exploring and improving cyclic tetrapeptide HDACis as potent antitoxoplasmosis agents prompted us to conceive a concise and modular pathway to synthesize existing or new cyclic tetrapeptides closely related to FR235222. Moreover, short adjustable/customizable syntheses capable of constructing structural extensions through fragments and/or skeletal variations according to the diversity-oriented synthesis (DOS) principles[19,20] are highly attractive in drug design, making an ever-growing diverse and relevant bioactive library easily accessible.

Our strategy rests on the design of cyclic tetrapeptide building blocks **7** (Scheme 2), presenting two major features for diversification: a terminal olefin group as a generic acceptor for the grafting of a collection of an assortment of carbonyl xanthates **8** or thioacids **9** ZBG (fragment diversity) using known radical reactions and a cyclic tetrapeptide scaffold capable of increasing readily its ring size in only one step (skeletal diversity). In the course of our work, we found indeed that our cyclic tetrapeptide scaffold was potent for delivering a new enlarged ring system, promoted by a novel reductive samarium diiodide (SmI_2) bond cleavage at the proline unit of **10** and **11**. Previous DOS approaches had already employed bond cleavage to achieve the increment of diversity through the formation of new skeletons by oxidative[21] or retro-Diels–Alder reactions.[22] Taken together, these selective reactions bring modularity and flexibility and connect each analogue to each parent structure in not more than one or two steps. This short distance between derivatives should allow a fast navigation and selection between analogues, avoiding the synthesis of tremendous numbers of intermediates. These reactions provide a new, convenient, and direct means to combine fragments and skeletal diversities onto bioactive cyclic

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Scheme 3. Syntheses of cyclic Tetrapeptide (−)-17 and the Collection of Carbonyl Xanthate Fragments 8

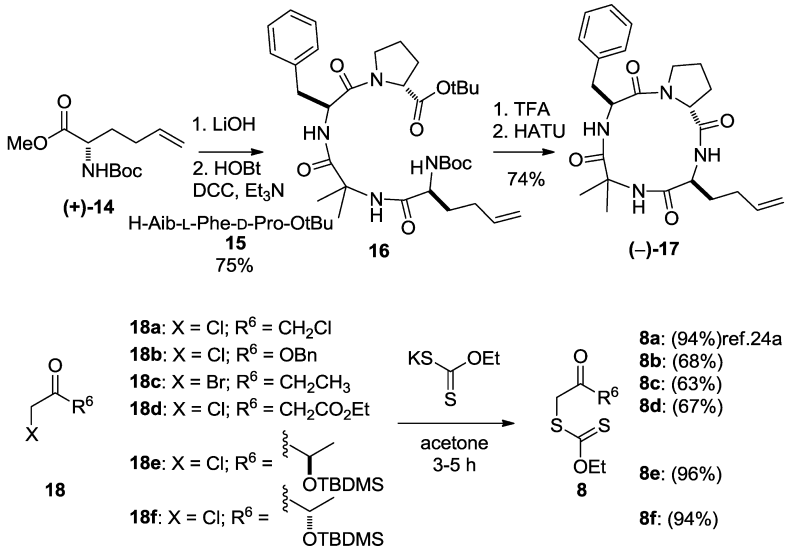
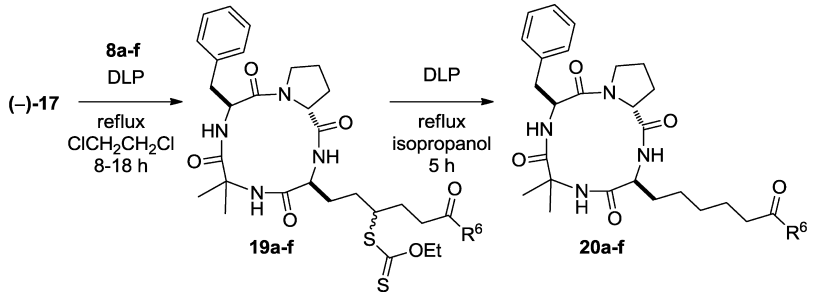


Table 1. Synthesis of Carbonyl ZBG Cyclic Tetrapeptides 20



entry	R^6	**19** (yield, %)	**20** (yield, %)
1	CH_2Cl	**19a** (65)	**20a** (92)
2	OBn	**19b** (90)	**20b** (69%)
3	CH_2CH_3	**19c** (−)	**20c** (50)[a]
4	CH_2CO_2Et	**19d** (−)	**20d** (52)[a]
5	(*R*)-CHCOTBDMSCH$_3$	**19e** (96)	**20e** (60)
6	(*S*)-CHCOTBDMSCH$_3$	**19f** (95)	**20f** (59)

[a]Overall yield in a "one vessel" reaction.

peptides. As a result, this collection of novel analogues by their evaluation on cell assays has shed light on new structural features that will help in the design of more potent and selective antiparasitic agents.

RESULTS AND DISCUSSION

To gain a better understanding of the positive contribution to the antiparasitic activity from each part of the molecule, we first planned to identify the best ZBG. Previous grafting strategies of carbonyl ZBG onto cyclic peptide or depsipeptide scaffolds have mainly involved cross-metathesis reactions. However, this assembling process usually proceeded with moderate yields due to competitive homocoupling reactions.[14,23] In addition, the xanthate ZBG intermediates **8a−f**[24] (Scheme 3) were easily obtained by reacting potassium ethyl xanthate with commercially available α-halogen carbonyls **18a−d** or with chiral precursors **18e,f** accessible within a few steps.[25] Radical transfer of either xanthates (Zard's reaction)[26] or thioacid derivatives with **7** would allow the preparation of **10** and **11** more selectively.

The cyclic tetrapeptide (−)-**17** was taken as a model and was prepared from the *N*-(Boc)-homoallylglycine (+)-**14**[27] and tripeptide **15**[28] in an overall yield of 56%. When (−)-**17** was reacted with different xanthates **8** (Table 1) in refluxing 1,2-dichloroethane and in the presence of dilauroyl peroxide (DLP), the radical transfer reaction allowed xanthates **19** to be obtained in good yields (Table 1, entries 1 and 2 and entries 5 and 6). The reductive removal of the resulting xanthate group was accomplished by refluxing **19** with DLP in isopropyl alcohol to yield the final cyclic tetrapeptides **20**. Alternatively, this conversion in two steps can be performed in the same vessel by removing the solvent 1,2-dichloroethane once **19** was formed and by replacing it by isopropyl alcohol (entries 3 and 4).

The α-hydroxyl ketones cyclic tetrapeptides **21**[29] and **23**[29d] were obtained from **20e,f** by silyl deprotection, and **21** was further converted into the corresponding acetylated product **22**[29b] (Scheme 4). Hemisynthesis from FR235222 has focused on adding an aliphatic acyl group to the α-hydroxyl ketone ZBG to give **24**, which mimics the thioester group in largazole.

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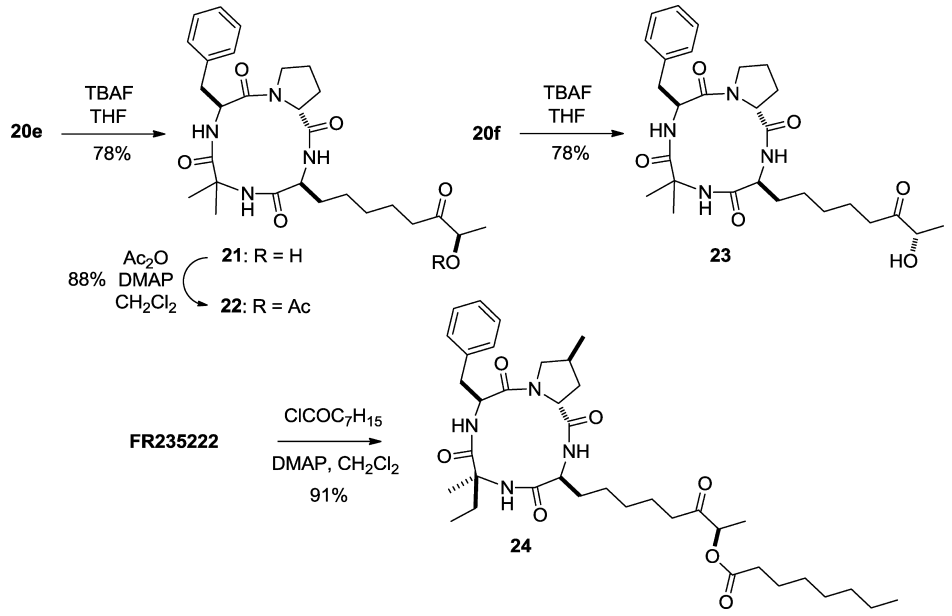
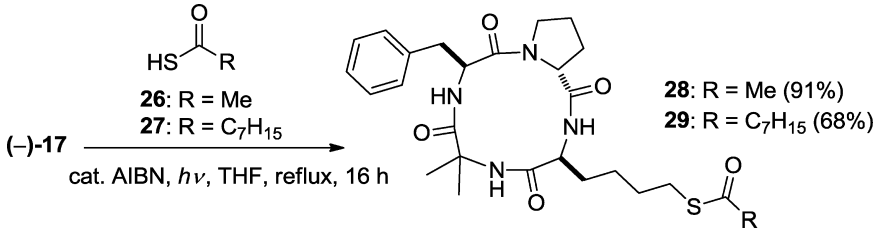
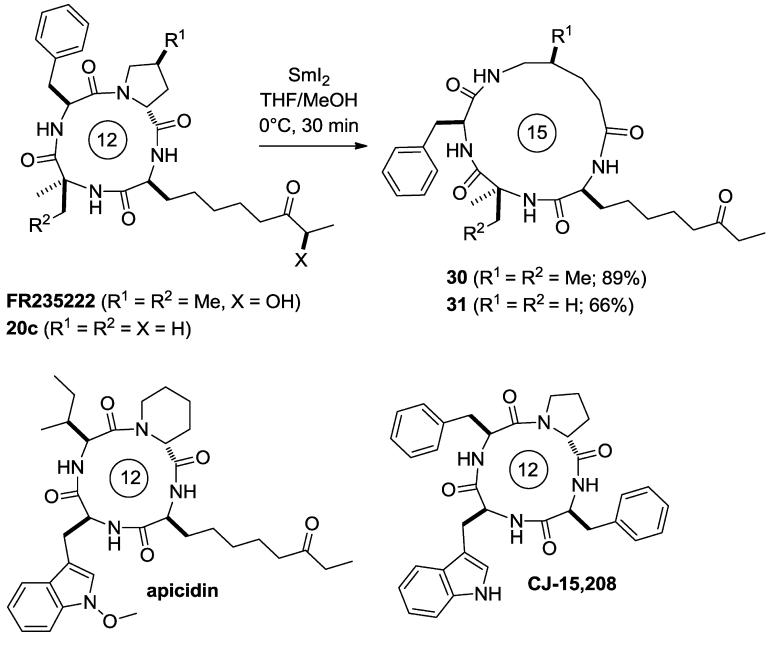
Scheme 4. Further Carbonyl ZBG Analogues Derived from 20e,f and FR235222



Scheme 5. Thioacetylation by Photoreaction of (−)-17 To Give Thioester Analogues



Scheme 6. Cyclic Tetrapeptide Scaffolds Tested under the Reductive Carbon−Nitrogen Cleavage Conditions



Alternatively, the terminal olefin in the cyclic tetrapeptide (−)-**17** can selectively react with thioacids **26** and **27**[30] under photoreaction conditions using AIBN as radical initiator to give the corresponding thioesters **28** and **29**, respectively, in good yields (Scheme 5).

In our attempts to directly remove the hydroxyl group from FR235222 to obtain **3** (see Scheme 1) by reductive deoxygenation using SmI_2,[31] an unexpected reductive carbon−nitrogen cleavage reaction simultaneously took place in the 3-methylproline core. Instead of the desired product **3**, the

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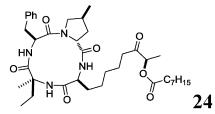
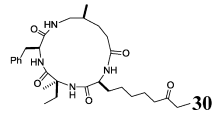
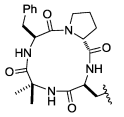
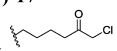
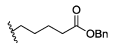
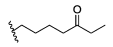
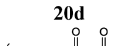
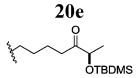
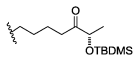
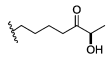
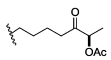
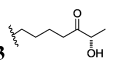
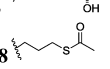
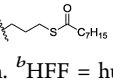
Table 2. In Vitro Proliferation Assays

entry	Products	%RH cells inhibition	EC_{50} RH[a] strain (nM)	EC_{50} HFF[b] strain (nM)	SI (EC_{50}HFF/EC_{50}RH)
1	**apicidin**	100	15	165	11
2	**FR235222**	100	9.7	127	13.1
3	 **24**	100	14.3	66.8	4.7
4	 **30**	22	-	-	

[a]RH = type 1 *T. gondii* RH strain. [b]HFF = human foreskin fibroblast.

Table 3. In Vitro Proliferation Assays

entry	Products	%RH cells inhibition at 90 nM	EC_{50} RH[a] strain (nM)	EC_{50} HFF[b] strain (nM)	SI (EC_{50}HFF/ EC_{50}RH)
					
1	**(−)-17** 	0	-	-	
2	**20a** 	28	-	-	
3	**20b** 	0	-	-	
4	**20c** 	52	172	243	1.4
5	**20d** 	0	-	-	
6	**20e** 	22	-	-	
7	**20f** 	20	-	-	
8	**21** 	100	28.6	648	22.7
9	**22** 	100	17	256	15
10	**23** 	40	47	909	14.6
11	**28** 	0	-	>4000[c]	
12	**29** 	0	-	-	

[a]RH = type 1 *T. gondii* RH strain. [b]HFF = human foreskin fibroblast. [c]No inhibition effect at 4 μM.

enlarged cyclic structure **30** was obtained in good yield (Scheme 6) in 30 min at 0 °C. In comparison with the FR235222 structure, **30** displays a ^{13}C NMR spectrum (DEPT 135) that clearly shows a change in the ratio of CH and CH_2 with the lack of a CH and the appearance of a new CH_2 at 34.1 ppm. High-resolution mass spectrometry (ESI+) confirms the reduction of FR235222 to give **30** by the presence of major peaks for $[M + Na]^+$ (100%) and $[M + H]^+$ (10%). Works by Honda showed that SmI_2 promotes reductive deamination of α-amino carbonyl groups in the presence of HMPA and a proton source such as methanol.[32] To the best of our knowledge, clean reductive deamination of proline located in a peptide backbone has never been reported. In addition, the use of HMPA in our case was unnecessary. To define the scope of this reaction, we applied the same conditions for the analogue **20c**, which lacks a methyl group on the proline unit. The enlarged ring system **31** was obtained in a fair yield of 66%. With CJ-15,208,[33] which lacks a quaternary center in the cyclopeptide backbone, conversion was much slower, as shown by TLC monitoring. However, increasing the reaction time up to 3 h resulted in product decomposition. It seems that this reaction has to proceed quickly to avoid any side reactions. In contrast, for apicidin with its piperidine motif fused within the 12-membered ring, no reaction occurred and only starting material was recovered. The effectiveness of this pyrrolidine reductive ring cleavage under SmI_2 conditions seems to proceed efficiently through a breaking strain induced by the constrained 12−5-membered ring system having a quaternary center.

Having in hand this first generation of analogues with diverse ZBGs and two kinds of ring sizes (12- and 15-membered rings), their potency to inhibit cell proliferation in vitro with *T. gondii*

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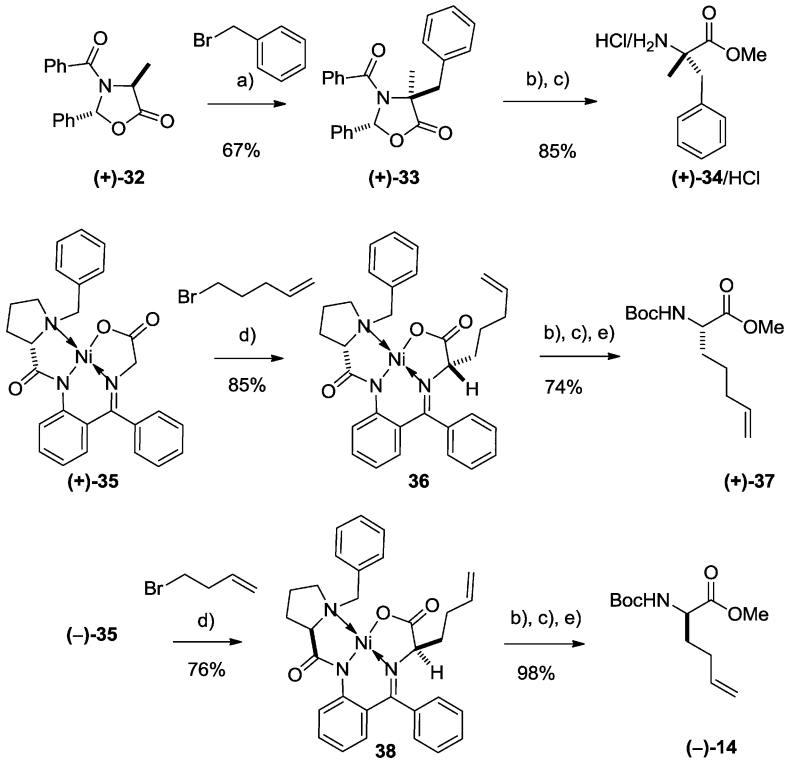
Scheme 7. Syntheses of Homochiral α-Amino Methyl Ester[a]



[a]Reagents and conditions: (a) LHMDS, THF, −78 °C; (b) HCl; (c) $SOCl_2$, MeOH; (d) NaOH, CH_3CN, 4 h; (e) Boc_2O, Et_3N, CH_3CN.

RH strain was evaluated (Tables 2 and 3). A screening at 90 nM was conducted to distinguish molecules having high or low efficacy. The new ester analogue **24** derived from FR235222 bearing the same aliphatic acyl group found in largazole (Table 2, entry 3), although being highly active (EC_{50} 14.3 nM), showed no improvement in term of selectivity index. The α_4 cyclic tetrapeptide of (−)-**17** alone (without ZBG) showed no activity (Table 3, entry 1), whereas α_3/β cyclic scaffolds lacking a ZBG can be potent.[18] Analogues derived from (−)-**17** and having either the new α-chloro ketone **20a** (entry 2), the benzyl ester **20b** (entry 3), the new β-keto ester **20d** (entry 5), the silyl protected α-hydroxyl ketones **20e**,**f** (entries 6 and 7) and the thioesters **28** and **29** (entries 11 and 12) turned out to be also inactive or weakly active at this concentration. To check further putative activity on human cells, the compound **28** was tested on human foreskin fibroblast strain (HFF) and no inhibition was detected even at 4 μM (entry 11). The results obtained with these thioesters confirmed previous works by Nishino and co-workers, who demonstrated that the bioactive masked thiol ZBG with disulfide derivatives such as **5** (see Scheme 1) requires a longer aliphatic linker.[34] For the other analogues that induced cell inhibition higher than 40% at 90 nM with *T. gondii* RH strain, their EC_{50} values on *T. gondii* RH and HFF strains were investigated to determine their efficiency and their selectivity indexes. Again, the acetylated (*R*)-hydroxyl ketone ZBG from analogue **22** was identified as being the most efficient ZBG against *T. gondii* (EC_{50} = 17 nM, entry 9). Analogue **21** (entry 8), having an (*R*)-hydroxyl ketone configuration, showed efficiency slightly higher than that of its (*S*)-hydroxyl ketone diastereomer **23** (entry 10), demonstrating a preferential configuration for a better antiparasitic profile. Finally, the potencies of each cyclopeptide scaffold to act as an antiparasitic agent can be compared, given that apicidin, **20c**, **3**, and **30** share the same ZBG (ethyl ketone). The best cyclopeptide framework is apicidin (EC_{50} = 15 nM; Table 2, entry 1), followed by FR235222 (**3**; EC_{50} = 95.3 nM, see Scheme 1) and the synthetic model (**20c**; EC_{50} = 172 nM, Table 3, entry 4), whereas the enlarged 15-membered ring **30** (Table 2, entry 4) exhibited only a weak inhibitory effect.

We next turned our attention to the modification of residues located at the cyclic tetrapeptide part. New homochiral α-amino acids were synthesized. Quaternary α-amino methyl esters (+)-**34** and (−)-**34** were readily obtained according to Seebach and Mutter procedures[35] by derivation of their corresponding chiral synthons **32**.[36] For the tertiary α-amino acids (+)-**37** and (−)-**14**[37] (Scheme 7), we chose the Belokon' procedure,[38] which offers a more general and practical access to each kind of configuration.

Aromatic groups were found in many synthetic or natural HDACis, suggesting a critical and specific hydrophobic interaction involved in their contact with the enzyme external surface that provides a stabilizing interaction for such complexes.[13] To evaluate the contribution of hydrophobic phenyl residues, we synthesized the three novel cyclic building blocks **47**−**49** by replacing the existing phenyl group with a cyclohexyl group or by adding an additional phenyl group at the Aib position with a D or L configuration (Scheme 8). We chose a convergent and more modular pathway by coupling dipeptides **39**−**41** with dipeptides **42** and **43** to give the linear tetrapeptides **44**−**46**. Cyclization of **44** gave **47** in a fair yield of 48%, whereas **48** and **49** were obtained in lower yields of 22% and 36%, respectively.[39] The cyclic tetrapeptide building block (+)-**17**, having a reverse configuration, was obtained from dipeptides (+)-**39** and (−)-**42**.

With regard to our previous results, the (*R*)-hydroxyl ketone head was chosen as the standard ZBG. The xanthate **8e** was

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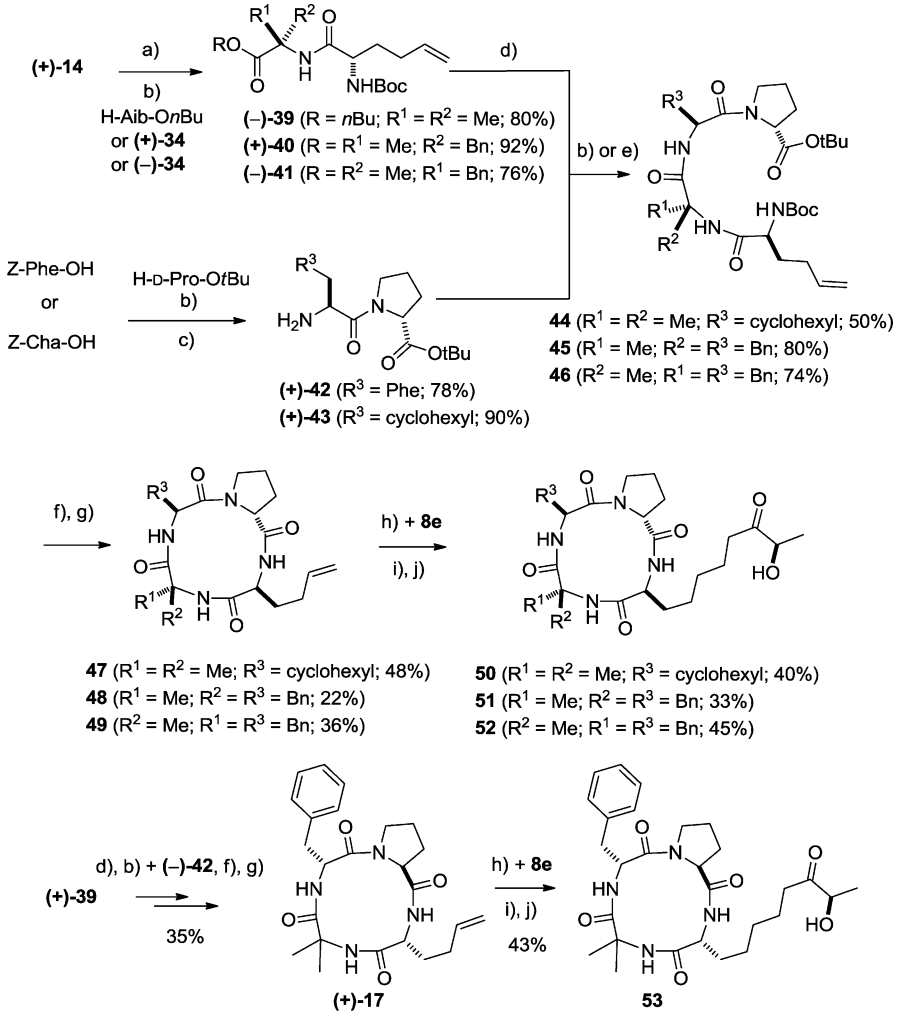
Scheme 8. Convergent Synthesis of New Cyclic Building Blocks 47−49 and (+)-17 and Their Derivations To Give the Carbonyl ZBG Cyclic Derivatives 50−53[a]



[a]Reagents and conditions: (a) LiOH, THF/H_2O; (b) HOBt, DCC, Et_3N, DMF, 18 h; (c) H_2, Pd/C, acetic acid; (d) LiOH, THF/MeOH/H_2O; (e) HOBt, EDC, *N*-methylmorpholine, DMF, 18 h; (f) TFA, 0 °C, 3 h; (g) HATU, DIEA, DMF, 18 h; (h) DLP, 1,2-dichloroethane, reflux; (i) DLP, isopropyl alcohol, reflux; (j) TBAF, THF.

selectively grafted on **47−49** and (+)-**17**. Subsequent reductive xanthate removal and silyl deprotection afforded the new analogues **50−52** and **53**. The cyclic peptide **56**,[40] differing from (−)-**17** by only one extra methylene group in the aliphatic chain, was also obtained according to our strategy (Scheme 9). This building block gave access to new thioester analogues **57** and **58** with the desired length in the aliphatic linker.[12,13] The corresponding thiol analogue **59** was obtained from **58** by removal of the acetyl group followed by dithiothreitol (DTT) reduction of the disulfide bond formed by the resulting dimer.[34] The enlarged cyclic depsipeptide thioester analogue **60** was readily obtained by treating **57** with SmI_2.

These new analogues were evaluated on *T. gondii* RH and HFF strains (Table 4). The cyclic tetrapeptides **50−52** showed no inhibition at 90 nM, indicating that the lack (**50**, entry 1) or the presence of too many (**51** and **52**, entries 2 and 3) hydrophobic aromatic groups disfavors activity against the parasite. A decreased activity on the HFF strain was also observed. Only a preferential configuration found in the product **51** retained significant activity on human cells (EC_{50} = 438 nM). The analogue **53** (entry 4), which has on one side the cyclic tetrapeptide with a configuration reverse to that of natural HDCAi and on the other side the most efficient (*R*)-hydroxyl ketone ZBG, offers the opportunity to interrogate cell penetrating mechanisms. An active membrane permeability process that involves the chiral recognition of FR235222 may prevent penetration of **53** into cells. This compound was indeed active at 90 nM on the RH strain, with a good EC_{50} value of 98 nM, and showed a weaker impact on human cells (EC_{50} = 735 nM on the HFF strain). A passive membrane permeability property by virtue of the cyclic tetrapeptide unit could explain this cell permeability,[10] although a nonchiral selective and active process cannot be ruled out. From a pharmacodynamic point of view, this result also pointed out the configuration tolerance for ligands (substrates) interacting with the enzyme.

With the analogues **57−60**, we wanted to check whether new largazole-like analogues with a longer aliphatic chain could confer inhibiting properties against *T. gondii*. Surprisingly, the thioesters **57** and **58** showed no activity on *T. gondii* at 90 nM while keeping a good inhibition on human cell proliferation (Table 4, entries 5 and 6). Under these test conditions, the thioester ZBG with the optimized aliphatic chain length exhibits a reversal of selectivity

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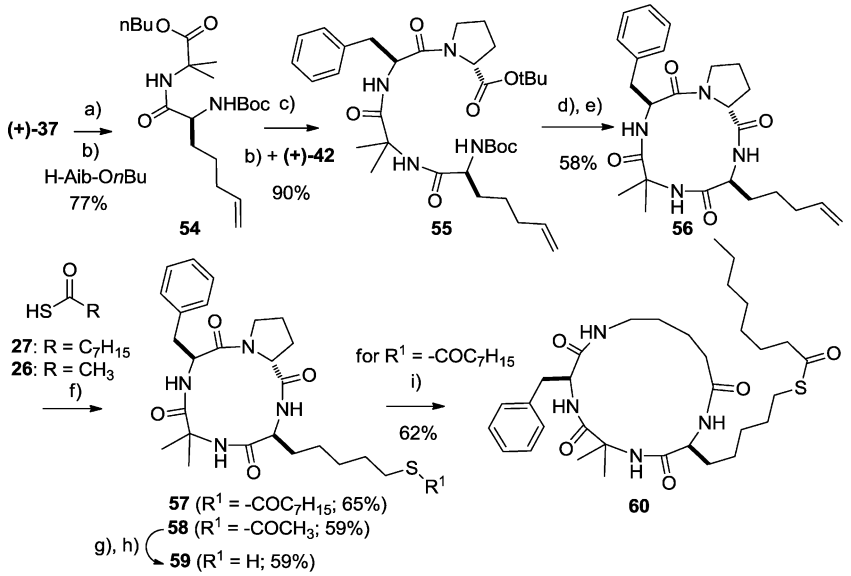
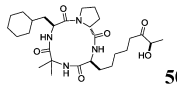
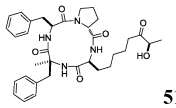
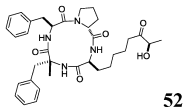
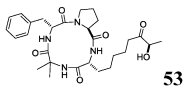
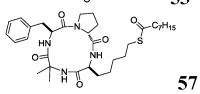
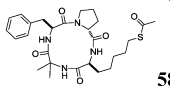
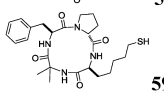
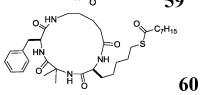
Scheme 9. [a]



[a]Reagents and conditions: (a) LiOH, THF/H_2O; (b) HOBt, DCC, Et_3N, DMF, 18 h; (c) LiOH, THF/MeOH/H_2O; (d) TFA, 0 °C, 3 h; (e) HATU, DIEA, DMF, 18 h; (f) AIBN cat., THF, reflux, light; (g) NH_3 in MeOH, DMF. 67 h; (h) DTT, DMF, 37°C, 24 h; (i) SmI_2, THF/MeOH, 0 °C, 30 min.

Table 4. In Vitro Proliferation Assays with New Analogues

entry	Products	%RH cells inhibition at 90 nM	EC_{50} RH[a] strain (nM)	EC_{50} HFF[b] strain (nM)
1	 **50**	0	-	>4000[c]
2	 **51**	0	-	438
3	 **52**	0	-	5900
4	 **53**	40	98	735
5	 **57**	0	-	82
6	 **58**	0	-	120
7	 **59**	0	-	nd
8	 **60**	0	-	>4000[c]

[a]RH = Type 1 *T. gondii RH* strain. [b]HFF = human foreskin fibroblast. [c]No inhibition effect at 4 μM.

for human cell versus *T. gondii* parasite. The putative in vivo active species, the free thiol analogue **59**, has no effect on *T. gondii* and a weak activity on HFF, very likely due to its instability in cell medium.

Again, the ring size contribution of the cyclic peptide part can be evaluated by using **60** as a comparative element. We observed that **60** has no effect on the RH strain at 90 nM and has lost activity on HFF (entry 8), demonstrating the critical role of a more constrained and smaller cyclic peptide structure for efficient parasitic inhibition. To confirm the reversal of selectivity for **57** and **59**, these products and the most representative antiparasitic ZBG ketone analogues **1**, **21** and **22** were also evaluated against *P. falciparum* 3D7 strain, Jurkat cell line, and erythroblast cells (K-562) (Table 5).

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Table 5

entry	product	IC_{50} *P. falciparum* 3D7 strain (nM)	CC_{50} h.e. K-562 (nM)	IC_{50} Jurkat cell line (nM)
1	**1**	7.1	17	48
2	**21**	17.4	18	48
3	**22**	22.1	61	127
4	**57**	9450	67	40
5	**59**	6200	723	763

Once again, for the thioester (**57**) and thiol (**59**) analogues (Table 5, entries 4 and 5), we observed a dramatic decrease in efficiency against Apicomplexa parasite such as *Plasmodium falciparum*, whereas the activity remains high (7.1−22.1 nM) for the analogues **1**, **21**, and **22** (entries 1−3). Against the Jurkat cell line and erythroblast cells, the high activity observed with **1**, **21**, **22**, and **57**, and to a lesser extent **59**, correlates well with the previous results obtained with HFF (Table 4).

CONCLUSION

The design and discovery of short postmodifications on a pluripotent cyclopeptide scaffold have given an easy access to useful and relevant functional and skeletal diversifications. Such an approach addresses the issue of extracting maximum biological information with a limited number of molecules. The number of synthetic steps is minimized by maintaining connectivity between each synthetic and bioactive intermediates. By targeting Apicomplexa inhibition with our assortment of cyclic peptides, we have gained a better insight into important molecular features affecting selectivity between species. This work has confirmed that the (*R*)-hydroxyl ketone ZBG and its acetylated analogue connected to a cyclic tetrapeptide backbone are the most potent and selective ZBGs against *T. gondii*. In contrast, the substitution with thioester ZBG located at the proper distance from the peptide results in high affinity against human cells, while showing no or little effect on *T. gondii* RH and *P. falciparum* strains. This inversion in selectivity with thioester ZBG is significant and deserves further investigations to better understand the mechanisms underlying this new phenotype. During this work, we also focused our attention on the remarkable properties arising from this natural cyclic tetrapeptide scaffold. In particular, the strain in this ring system has revealed new reactivity with SmI_2. We believe that this constrained preorganized structure is essential for bioactivity, as the new analogues (**30** and **60**) arising from this ring expansion reaction showed a decrease or loss of cell bioactivity in comparison to their analogues with a 12-membered ring (**3** and **57**, respectively). This gives rise to questions as to whether the cell permeability or ligand−protein interaction is affected by this structural change. Indeed, ligand−protein interactions can also benefit from the reduced entropic cost of a more constrained system. Another noteworthy feature of this cyclic peptide scaffold seems to be its good cell permeation, regardless of their configuration (**53** compared to **21**), which may also contribute to the nanomolar efficacy in cell assays. Other structural features deserve attention as well. From the cyclic tetrapeptide part, involved in cell permeability and also in ligand−protein recognition, the benzyl residue on the phenylalanine is beneficial, as its change to a greatly hindered aliphatic ring (cyclohexyl group) led to loss of bioactivity (**21** versus **50**). In contrast, the presence of too many benzyl groups on the cyclopeptide seems to be detrimental. We have also confirmed that the methyl group of the proline found in FR235222 is not necessary for the antiparasitic effect. Future directions to improve the selectivity between species will mainly explore new modulations on new positions of the cyclic tetrapeptide scaffold to increase the structural diversity of our analogues. Further analogue optimizations to better target Apicomplexa and novel synthetic strategies to increase diversity are now underway.

EXPERIMENTAL SECTION

General Considerations. All reagents were used as purchased from commercial suppliers. Solvents were purified or dried by conventional methods prior to use. For reactions performed under anhydrous conditions, glassware was oven-dried and reactions were performed under an argon atmosphere. Reactions were monitored by thin-layer chromatography on precoated aluminum sheets (silica gel 60, F_{254}). Flash column chromatography was performed on silica gel 0.04−0.063 mm (230−400 mesh). ^1H and ^{13}C NMR spectra were recorded at room temperature in deuterated solvents. Chemical shifts δ are given relative to TMS as internal standard or relative to the solvent ^{13}C $\delta(CDCl_3)$ 77.23 ppm. Accurate mass measurements (HRMS) were carried out on a TOF spectrometer.

(−)-(*R*)-8-[(3*S*,6*R*,9*S*,13*S*,14a*R*)-9-Benzyl-6-ethyl-6,13-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]-tetraazacyclododecin-3-yl]-3-oxooctan-2-yl Acetate (1). To FR235222 (20 mg, 0.036 mmol) in CH_2Cl_2 (2 mL) were added acetic anhydride (5 mg, 0.049 mmol), Et_3N (7.5 mg, 0.072 mmol), and DMAP (9 mg, 0.072 mmol) at 0 °C. The solution was stirred for 1 h at 0 °C and 16 h at room temperature. EtOAc was added, and the mixture was washed with a saturated solution of NH_4Cl. The organic layer was dried ($MgSO_4$) and was removed in vacuo. After flash chromatography (silica gel, 2% MeOH/CH_2Cl_2), the product **1** (20 mg, 93%) was isolated as a colorless film: R_f = 0.18 (2% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −87.5° (*c* = 2.4, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3538, 3293, 2929, 2864, 1728, 1680, 1621, 1520, 1433, 1237, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.84 (t, *J* = 7.4 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 1.25−1.35 (m, 7H), 1.36 (m, 1H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.53−1.66 (m, 3H), 1.81 (m, 1H), 2.13 (s, 3H), 2.16 (m, 1H), 2.28−2.56 (m, 4H), 2.63 (m, 1H), 2.73 (dd, *J* = 9.6, 8.1 Hz, 1H), 2.96 (dd, *J* = 13.5, 6.0 Hz, 1H), 3.24 (dd, *J* = 13.5, 9.9 Hz, 1H), 4.06 (dd, *J* = 9.6, 7.6 Hz, 1H), 4.21 (ddd, *J* = 10.2, 7.6, 7.6 Hz, 1H), 4.67 (d, *J* = 6.5 Hz, 1H), 5.08 (q, *J* = 7.1 Hz, 1H), 5.16 (ddd, *J* = 10.1, 9.9, 6.0 Hz, 1H), 5.87 (s, 1H, NH), 7.16 (d, *J* = 10.2 Hz, 1H, NH), 7.19−7.29 (m, 5H), 7.57 (d, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 8.6 (CH_3), 16.3 (CH_3), 18.3 (CH_3), 21.0 (CH_3), 22.5 (CH_3), 23.1 (CH_2), 25.5 (CH_2), 28.3 (CH_2), 28.8 (CH_2), 28.9 (CH_2), 33.0 (CH), 33.2 (CH_2), 35.9 (CH_2), 38.2 (CH_2), 53.5 (CH), 54.0 (CH_2), 54.6 (CH), 58.2 (CH), 63.2 (C), 74.8 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 170.6 (C), 172.0 (C), 173.2 (C), 174.4 (C), 175.8 (C), 207.7 (C); LRMS (ESI+) *m*/*z* (%) 621 (100) $[M + Na]^+$, 599 (15); HRMS (ESI+) *m*/*z* calcd for $C_{32}H_{46}N_4O_7Na$ 621.3264, found 621.3257.

(−)-*O*-(*R*)-8-[(3*S*,6*R*,9*S*,13*S*,14a*R*)-9-Benzyl-6-ethyl-6,13-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*]-[1,4,7,10]tetraazacyclododecin-3-yl]-3-oxooctan-2-yl *O*-Phenyl Carbonothioate (2). Phenyl chlorothiono carbonate (34 mg, 0.2 mmol) was added to a solution of FR235222 (83 mg, 0.15 mmol) in CH_2Cl_2 (2 mL) at 0 °C, followed by DMAP (38 mg, 0.32 mmol). The mixture was stirred for 1 h at 0 °C and at room temperature for 16 h. After solvent evaporation, the residue was directly purified by flash chromatography (silica gel, 25% EtOAc/cyclohexane) to give **2** (76 mg, 74%) as a colorless film: R_f = 0.15 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −71.9° (*c* = 1.1, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3303, 2924, 1735, 1683, 1662, 1627, 1525, 1274, 1222, 1207 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.83 (t, *J* = 7.4 Hz, 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 1.25−1.50 (m, 8H), 1.57 (d, *J* = 7.2 Hz, 3H), 1.59−1.71 (m, 3H), 1.80 (m, 1H), 2.15 (m, 1H), 2.31 (m, 1H), 2.37 (m, 1H), 2.45−2.77 (m, 3H), 2.73 (dd, *J* = 9.6, 8.0 Hz, 1H), 2.96 (dd, *J* = 13.5, 6.0 Hz, 1H), 3.24 (dd, *J* = 13.5, 9.9 Hz, 1H), 4.06 (dd, *J* = 9.6, 7.6 Hz, 1H), 4.19 (ddd, *J* = 10.0, 7.6, 7.6 Hz, 1H), 4.66 (dd, *J* = 7.8, 1.6 Hz, 1H), 5.16 (ddd, *J* = 10.1, 9.9, 6.0 Hz, 1H), 5.56 (q, *J* = 7.2 Hz, 1H), 5.78 (s, 1H, NH), 7.13−7.34 (m, 9H, NH), 7.43 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.54 (d, *J* = 10.1 Hz, 1H, NH);

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^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 8.6 (CH_3), 16.6 (CH_3), 18.3 (CH_3), 22.6 (CH_3), 23.0 (CH_2), 25.6 (CH_2), 28.0 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 33.1 (CH), 33.2 (CH_2), 35.9 (CH_2), 38.4 (CH_2), 53.6 (CH), 54.1 (CH_2), 54.6 (CH), 58.2 (CH), 63.2 (C), 83.4 (CH), 122.0 (2 × CH), 126.9 (CH), 127.0 (CH), 128.8 (2 × CH), 129.3 (2 × CH), 129.8 (2 × CH), 137.3 (C), 153.6 (C), 172.1 (C), 173.3 (C), 174.4 (C), 175.9 (C), 194.8 (C), 206.7 (C); LRMS (ESI+) m/z (%) 715 (100) $[M + Na]^+$, 693 (25) $[M + H]^+$; HRMS (ESI+) m/z calcd for $C_{37}H_{48}N_4O_7NaS$ 715.3141, found 715.3154.

(−)-(3*S*,6*R*,9*S*,13*S*,14a*R*)-9-Benzyl-6-ethyl-6,13-dimethyl-3-(6-oxooctyl)decahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (3). To the product **2** (4.4 mg, 6.4 μmol) in a degassed solution of toluene (1 mL) were added nBu$_3$SnH (5.5 mg, 19 μmol) and AIBN (catalytic). The mixture was warmed at 100 °C under argon for 3 h. The solvent was removed in vacuo, and the residue was directly purified by flash chromatography (10% KF w/w in silica gel, 30% EtOAc/cyclohexane) to give the product **3** (2.3 mg, 67%) as a colorless film: R_f = 0.12 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −117.6° (c = 1.3, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3307, 2925, 1714, 1682, 1662, 1628, 1524, 1456, 1378, 1248, 914 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.84 (t, J = 7.4 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 1.05 (t, J = 7.3 Hz, 3H), 1.23−1.42 (m, 8H), 1.52−1.65 (m, 3H), 1.80 (m, 1H), 2.15 (m, 1H), 2.27−2.46 (m, 6H), 2.62 (m, 1H), 2.73 (dd, J = 9.7, 8.0 Hz, 1H), 2.96 (dd, J = 13.5, 6.0 Hz, 1H), 3.24 (dd, J = 13.5, 9.9 Hz, 1H), 4.06 (dd, J = 9.7, 7.6 Hz, 1H), 4.19 (ddd, J = 10.2, 7.6, 7.6 Hz, 1H), 4.67 (dd, J = 8.0, 2.0 Hz, 1H), 5.16 (ddd, J = 10.1, 9.9, 6.0 Hz, 1H), 5.77 (s, 1H, NH), 7.14 (d, J = 10.2 Hz, 1H, NH), 7.18−7.32 (m, 5H), 7.54 (d, J = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 8.1 (CH_3), 8.6 (CH_3), 18.3 (CH_3), 22.6 (CH_3), 23.8 (CH_2), 25.6 (CH_2), 28.1 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 33.1 (CH), 33.3 (CH_2), 35.9 (CH_2), 36.1 (CH_2), 42.4 (CH_2), 53.6 (CH), 54.1 (CH_2), 54.6 (CH), 58.2 (CH), 63.3 (C), 126.9 (CH), 128.8 (2 × CH), 129.3 (2 × CH), 137.3 (C), 172.1 (C), 173.2 (C), 174.4 (C), 175.9 (C), 211.9 (C); LRMS (ESI+) m/z (%) 563 (100) $[M + Na]^+$; HRMS (ESI+) m/z calcd for $C_{30}H_{44}N_4O_5Na$ 563.3209, found 563.3209.

General Procedure for 8: Example with Benzyl 2-(Ethoxycarbonothioylthio)acetate (8b). To a solution of benzyl 2-chloroacetate **18b** (2.76 g, 15 mmol) in acetone (50 mL) was added at 0 °C a solution of potassium ethyl xanthate (2.60 g, 16 mmol) in acetone (50 mL). The mixture was stirred for 4 h at room temperature. The organic solvent was removed in vacuo and CH_2Cl_2 was added. The organic layer was washed with brine, dried ($MgSO_4$) and then evaporated. After Kugelrohr distillation (13 mbar, 180−200 °C), the product **8b** (2.74 g, 68%) was isolated as a pale yellow oil (for **8e** and **8f**, purification was performed by flash chromatography): R_f = 0.21 (5% EtOAc/cyclohexane); IR ν_{max} (film) 2982, 1740, 1615, 1498, 1455, 1376, 1232, 1150 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.35 (t, J = 7.1 Hz, 3H), 3.93 (s, 2H), 4.58 (q, J = 7.1 Hz, 2H), 5.18 (s, 2H), 7.32−7.36 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.7 (CH_3), 37.9 (CH_2), 67.6 (CH_2), 70.8 (CH_2), 128.5 (CH), 128.6 (2 × CH), 128.7 (2 × CH), 135.4 (C), 167.9 (C), 212.4 (C); LRMS (ESI+) m/z (%) 293 (100) $[M + Na]^+$; HRMS (ESI+) m/z calcd for $C_{12}H_{14}O_3NaS_2$ 293.0282, found 293.0281.

*O-Ethyl S-2-Oxobutyl carbonodithioate (**8c**):* Product **8c** was purified by distillation (Kugelrohr, 2.2 mbar, 100 °C): 63% yield (pale yellow oil); IR ν_{max} (film) 2980, 1729, 1717, 1457, 1376, 1224, 1113, 1052 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.11 (t, J = 7.1 Hz, 3H), 1.42 (t, J = 7.1 Hz, 3H), 2.65 (q, J = 7.1 Hz, 2H), 4.00 (s, 2H), 4.63 (q, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 7.9 (CH_3), 13.9 (CH_3), 35.4 (CH_2), 45.3 (CH_2), 71.0 (CH_2), 204.0 (C), 213.5 (C); LRMS (ESI+) m/z (%) 215 (100) $[M + Na]^+$.

*Ethyl 4-(ethoxycarbonothioylthio)-3-oxobutanoate (**8d**):* Product **8d** was purified by distillation (Kugelrohr, 2.2 mbar, 150 °C): 67% yield (pale yellow oil); IR ν_{max} (film) 2983, 1745, 1616, 1376, 1225, 1113, 1048 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.30 (t, J = 7.1 Hz, 3H), 1.42 (t, J = 7.1 Hz, 3H), 3.65 (s, 2H), 4.12 (s, 2H), 4.20 (q, J = 7.1 Hz, 2H), 4.64 (q, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.9 (CH_3), 14.3 (CH_3), 45.6 (CH_2), 48.3 (CH_2), 61.8 (CH_2), 71.2 (CH_2), 166.9 (C), 196.3 (C), 213.0 (C); LRMS (ESI+) m/z (%) 273 (100) $[M + Na]^+$.

*(−)-(R)-S-3-(tert-Butyldimethylsilyloxy)-2-oxobutyl O-ethyl carbonodithioate (**8e**):* Product **8e** was purified by flash chromatography (5% ether/cyclohexane): 96% yield (pale yellow oil); R_f = 0.25 (4% ether/cyclohexane); $[\alpha]_D^{20}$ = −7.3° (c = 1.15, $CHCl_3$); IR ν_{max} (film) 2955, 2931, 1735, 1728, 1363, 1224, 1112, 1051, 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.13 (s, 3H), 0.14 (s, 3H), 0.95 (s, 9H), 1.38 (d, J = 6.8 Hz, 3H), 1.41 (t, J = 7.1 Hz, 3H), 4.29 (d, J = 17.9 Hz, 1H), 4.34 (q, J = 6.8 Hz, 1H), 4.35 (d, J = 17.9 Hz, 1H), 4.62 (q, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.9 (CH_3), −4.4 (CH_3), 13.9 (CH_3), 18.1 (C), 21.0 (CH_3), 25.9 (2 × CH_3), 42.8 (CH_2), 70.6 (CH_2), 75.0 (CH), 205.7 (C), 213.6 (C); LRMS (ESI+) m/z (%) 345 (100) $[M + Na]^+$, 323 (5); HRMS (ESI+) m/z calcd for $C_{13}H_{26}O_3NaSiS_2$ 345.0990, found 345.0987.

*(+)-(S)-S-3-(tert-Butyldimethylsilyloxy)-2-oxobutyl O-ethyl carbonodithioate (**8f**):* Product **8f** was purified by flash chromatography (5% ether/cyclohexane), 94% yield (pale yellow oil): R_f = 0.25 (4% ether/cyclohexane); $[\alpha]_D^{20}$ = +7.4° (c = 1.2, $CHCl_3$); IR ν_{max} (film) 2960, 2930, 1735, 1725, 1363, 1232, 1110, 1048, 835 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.13 (s, 3H), 0.14 (s, 3H), 0.95 (s, 9H), 1.38 (d, J = 6.8 Hz, 3H), 1.41 (t, J = 7.1 Hz, 3H), 4.29 (d, J = 17.9 Hz, 1H), 4.34 (q, J = 6.8 Hz, 1H), 4.35 (d, J = 17.9 Hz, 1H), 4.62 (q, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.9 (CH_3), −4.4 (CH_3), 13.9 (CH_3), 18.1 (C), 21.0 (CH_3), 25.9 (2 × CH_3), 42.8 (CH_2), 70.6 (CH_2), 75.0 (CH), 205.7 (C), 213.6 (C); LRMS (ESI+) m/z (%) 345 (100) $[M + Na]^+$, 323 (8); HRMS (ESI+) m/z calcd for $C_{13}H_{26}O_3NaSiS_2$ 345.0990, found 345.0989.

(+)-(*S*)-Methyl 2-(*tert*-butoxycarbonylamino)hex-5-enoate ((+)-14). Into zinc powder (2.48 g, 37.9 mmol) in dry DMF (28 mL) was injected 1,2-dibromoethane (0.17 mL) under argon. The mixture was stirred for 20 min at room temperature. TMSCl (50 μL) was injected, and the solution was stirred at 60 °C for 30 min. (*R*)-Methyl 2-(*tert*-butoxycarbonylamino)-3-iodopropanoate[41] (2.0 g, 6.08 mmol) in DMF (8 mL) was added dropwise. The solution was stirred for 20 min at 60 °C. LiCl (587 mg, 13.8 mmol) and CuCN (619 mg, 6.9 mmol) in DMF (6.5 mL) were injected at −55 °C, and the mixture was warmed with stirring for 10 min at 0 °C. The solution was cooled again to −55 °C, and allyl bromide (1.05 mL, 12 mmol) was injected. After 5 min, the mixture was warmed to 0 °C and then stirred for 2 h at this temperature. The unreacted zinc was removed by Celite filtration, and the filtrate was quenched with a saturated aqueous NH_4Cl solution. The product was extracted with EtOAc. The combined organic layer was washed with brine, dried ($MgSO_4$), and evaporated. After flash chromatography (silica gel, 8% EtOAC/cyclohexane), the product (+)-**14** (1.38 g, 93%) was isolated as a colorless oil: R_f = 0.25 (10% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +20.5° (c = 1.0, $CHCl_3$); IR ν_{max} (film, CH_2Cl_2) 3358, 2978, 1745, 1716, 1518, 1453, 1366, 1249, 1163, 914 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.45 (s, 9H), 1.74 (m, 1H), 1.90 (m, 1H), 2.12 (m, 2H), 3.74 (s, 3H), 4.32 (m, 1H), 5.00 (bd, J = 10.3 Hz, 1H), 5.05 (bd, J = 17.0 Hz, 1H), 5.18 (bd, J = 7.2 Hz, 1H, NH), 5.79 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 28.4 (3 × CH_3), 29.6 (CH_2), 32.0 (CH_2), 52.3 (CH_3), 53.0 (CH), 79.8 (C), 115.7 (CH_2), 137.0 (CH), 155.4 (C), 173.4 (C); LRMS (ESI+) m/z (%) 266 (100) $[M + Na]^+$, 210 (43), 166 (38); HRMS (ESI+) m/z calcd for $C_{12}H_{21}NO_4Na$ 266.1368, found 266.1374.

(−)-(3*S*,9*S*,14a*R*)-9-Benzyl-3-(but-3-enyl)-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone ((−)-17). To a cooled solution of tripeptide **15** (H-Aib-L-Phe-D-Pro-O-*t*-Bu; 1.38 g, 3.42 mmol) and methyl (*S*)-2-(benzyloxycarbonylamino)-5-hexenoate ((+)-**14**; 784 mg, 3.42 mmol) in dry DMF (7 mL) were added HOBt·H_2O (525 mg, 3.89 mmol), DCC (850 mg, 3.8 mmol), and triethylamine (0.5 mL), and the mixture was stirred at room temperature overnight (18 h). DMF was evaporated, and the residue was diluted with EtOAc and washed with 10% citric acid, 4% sodium carbonate, and brine. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (silica gel, 1.5% MeOH/CH_2Cl_2) to give the corresponding tetrapeptide **16** (1.58 g, 75%) as a colorless film: R_f = 0.09 (2% MeOH/CH_2Cl_2); LRMS (ESI+) m/z (%) 637 (100) $[M + Na]^+$, 615 (20) $[M + H]^+$; HRMS (ESI+) m/z calcd for $C_{33}H_{50}N_4O_7Na$ 637.3577, found 637.3567. This tetrapeptide (1.57 g, 2.55 mmol) was dissolved in TFA (7 mL) at 0

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°C and stirred for 3 h at this temperature. After evaporation, the product was precipitated in dry ether to give after filtration the deprotected product (1.08 g, 74%) as a TFA salt. To a solution of this tetrapeptide trifluoroacetic acid (900 mg, 1.57 mmol) in DMF (140 mL) were added HATU (666 mg, 1.75 mmol) and DIEA (0.81 mL) in five aliquots with a time interval of 30 min under vigorous stirring. Then the reaction mixture was stirred for 1 h at room temperature. The DMF was evaporated, and EtOAc was added. After it was washed with a solution of 10% citric acid followed by 4% $NaHCO_3$, the organic phase was dried ($MgSO_4$) and evaporated. After flash chromatography (silica gel, 1% MeOH/CH_2Cl_2), the cyclotetrapeptide (−)-**17** (512 mg, 74%) was isolated as a white foam: R_f = 0.17 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −110.0° (*c* = 0.84, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3303, 2930, 1678, 1663, 1630, 1663, 1529, 1427, 1251, 1181, 913 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.31 (s, 3H), 1.67−1.82 (m, 6H), 1.92 (m, 1H), 2.09 (m, 2H), 2.16 (m, 1H), 2.30 (m, 1H), 2.94 (dd, *J* = 13.5, 5.8 Hz, 1H), 3.19−3.28 (m, 2H), 3.86 (m, 1H), 4.30 (ddd, *J* = 10.1, 7.5, 7.5 Hz, 1H), 4.66 (m, 1H), 4.97 (m, 1H), 5.00 (m, 1H), 5.17 (ddd, *J* = 10.1, 10.0, 5.8 Hz, 1H), 5.78 (ddt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 6.18 (s, 1H, NH), 7.16−7.28 (m, 6H, NH), 7.61 (d, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.4 (CH_3), 24.6 (CH_2), 24.8 (CH_2), 26.3 (CH_3), 28.2 (CH_2), 29.6 (CH_2), 35.7 (CH_2), 46.7 (CH_2), 53.2 (CH), 53.7 (CH), 57.6 (CH), 58.5 (C), 115.5 (CH_2), 126.5 (CH), 128.4 (2 × CH), 128.9 (2 × CH), 136.9 (C), 136.9 (CH), 171.5 (C), 172.6 (C), 174.2 (C), 175.4 (C); LRMS (ESI+) *m*/*z* (%) 463 (100) $[M + Na]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{32}N_4O_4Na$ 463.2321, found 463.2316.

General Method To Obtain 19. *Method A. Example with (S)-{1-[(3S,9S,14aR)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]-7-chloro-6-oxoheptan-3-yl} O-Ethyl Carbonodithioate (**19a**).* A solution of cyclotetrapeptide (−)-**17** (75 mg, 0.17 mmol) and carbonyl xanthate **2a** (72 mg, 0.34 mmol) in 1,2-dichloroethane (0.3 mL) was refluxed for 30 min under argon. Dilauroyl peroxide (7 mg) was added every 2 h (five to seven times). After completion (8−18 h at reflux), the solvent was removed in vacuo and the residue was directly purified by flash chromatography (silica gel, 40% EtOAc/cyclohexane for **19a**) to give **19a** (72 mg, 65%) as a colorless film: R_f = 0.16 (40% EtOAc/cyclohexane); IR ν_{max} (film) 3302, 2925, 1734, 1678, 1663, 1628, 1525, 1435, 1390, 1217, 1111, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.35 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.62−1.96 (m, 10H), 2.07−2.23 (m, 2H), 2.32 (m, 1H), 2.76 (m, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.26 (dd, *J* = 13.5, 10.2 Hz, 1H), 3.76 (m, 1H), 3.86 (m, 1H), 4.08 (s, 2H), 4.22 (m, 1H), 4.65 (q, *J* = 7.1 Hz, 2H), 4.66 (m, 1H), 5.17 (m, 1H), 5.92 (s, 1H, NH), 7.15 (bd, *J* = 10.1 Hz, 1H, NH), 7.19−7.32 (m, 5H), 7.47 (bd, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.0 (CH_3), 23.7 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 26.7 (CH_3), 28.0 (CH_2), 29.9 (CH_2), 31.1 (CH_2), 36.0 (CH_2), 37.0 (CH_2), 47.2 (CH_2), 48.4 (CH_2), 50.7 (CH), 53.6 (CH), 54.3 (CH), 58.0 (CH), 59.0 (C), 70.5 (CH_2), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.1 (C), 173.1 (C), 174.1 (C),175.7 (C), 202.0 (C), 214.3 (C); LRMS (ESI+) *m*/*z* (%) 675 (100) $[M + Na]^+$, 653 (32); HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{41}N_4O_6ClNaS_2$ 675.2054, found 675.2053.

*S-(R)-1-[(3S,9S,14aR)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]-7-(tert-butyldimethylsilyloxy)-6-oxo-octan-3-yl O-ethyl carbonodithioate (**19e**).* According to method A and starting from (−)-**17** (60 mg, 0.14 mmol) and xanthate **8e** (110 mg, 0.34 mmol), the product **19e** (100 mg, 96%), was obtained as a colorless film (flash chromatography: 0.8% MeOH/CH_2Cl_2): R_f = 0.10 (1% MeOH/CH_2Cl_2); IR ν_{max} (thin film, CH_2Cl_2) 3474, 3295, 2920, 2855, 1712, 1666, 1628, 1525, 1438, 1251, 1111 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.07 (s, 6H), 0.91 (s, 9H), 1.28 (m, 3H), 1.34 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.62−1.88 (m, 9H), 1.92−2.11 (m, 2H), 2.18 (m, 1H), 2.33 (m, 1H), 2.76 (m, 2H), 2.96 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.27 (dd, *J* = 13.5, 10.1 Hz, 1H), 3.77 (m, 1H), 3.86 (m, 1H), 4.14 (q, *J* = 6.7 Hz, 1H), 4.22 (m, 1H), 4.63 (q, *J* = 7.1 Hz, 2H), 4.66 (m, 1H), 5.17 (m, 1H), 5.96 (s, 1H, NH), 7.15 (d, *J* = 10.1 Hz, 1H, NH), 7.18−7.30 (m, 5H), 7.51 (bd, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.8 (CH_3), −4.5 (CH_3), 14.0 (CH_3), 18.2 (C), 21.1 (CH_3), 23.7 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 25.9 (3 × CH_3), 26.4 (CH_2), 26.7 (CH_2), 27.6 (CH_2), 31.4 (CH_2), 34.5 (CH_2), 36.0 (CH_2), 47.2 (CH_2), 51.1 (CH), 53.6 (CH), 54.3 (CH), 58.0 (CH), 59.0 (C), 70.2 (CH_2), 75.0 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.1 (C), 173.0 (C), 174.1 (C), 175.8 (C), 213.5 (C), 214.4 (C); LRMS (ESI+) *m*/*z* (%) 785 (90) $[M + Na]^+$, 763 (70), 413 (100); HRMS (ESI+) *m*/*z* calcd for $C_{37}H_{58}N_4O_7NaSiS_2$ 785.3414, found 785.3407.

General Method To Obtain 20 from 19. *Method B. Example with (−)-(3S,9S,14aR)-9-Benzyl-3-(7-chloro-6-oxoheptyl)-6,6-dimethyldecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (**20a**).* A solution of product **19a** (12 mg, 0.018 mmol) in isopropyl alcohol (1 mL) was refluxed for 5 h with DLP (11 mg, 0.027 mmol). After evaporation and direct flash chromatography (silica gel, 30% EtOAc/cyclohexane), the product **20a** (9 mg, 92%) was obtained as a colorless film: R_f = 0.07 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −80° (*c* = 1.0, $CHCl_3$); IR ν_{max} (film) 3304, 2928, 1733, 1678, 1663, 1628, 1525, 1434, 1390, 1179 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.29−1.34 (m, 7H), 1.63 (m, 2H), 1.69−1.87 (m, 7H), 2.17 (m, 1H), 2.32 (m, 1H), 2.56 (t, *J* = 7.3 Hz, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.21 (dd, *J* = 10.0, 7.0 Hz, 1H), 3.26 (dd, *J* = 13.5, 10.0 Hz, 1H), 3.86 (m, 1H), 4.07 (s, 2H), 4.19 (ddd, *J* = 10.2, 7.6, 7.6 Hz, 1H), 4.67 (m, 1H), 5.16 (ddd, *J* = 10.1, 10.0, 5.7 Hz, 1H), 5.95 (s, 1H, NH), 7.11 (bd, *J* = 10.1 Hz, 1H, NH), 7.19−7.29 (m, 5H), 7.51 (bd, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.4 (CH_2), 23.7 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 25.4 (CH_2), 26.7 (CH_3), 28.8 (CH_2), 28.9 (CH_2), 36.0 (CH_2), 39.6 (CH_2), 47.1 (CH_2), 48.3 (CH_2), 53.6 (CH), 54.4 (CH), 57.9 (CH), 59.0 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 175.0 (C), 175.8 (C), 202.7 (C); LRMS (ESI+) *m*/*z* (%) 555 (100) $[M + Na]^+$, 533 (10); HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{37}N_4O_5ClNa$ 555.2350, found 555.2343.

*Benzyl 6-[(3S,9S,14aR)-9-benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]-4-(ethoxycarbonothioylthio)hexanoate (**19b**).* According to method A (8 h reflux) and starting from (−)-**17** (100 mg, 0.23 mmol) and xanthate **8b** (92 mg, 0.34 mmol), the product **19b** (145 mg, 90%) was obtained as a colorless film (flash chromatography: 1% MeOH/CH_2Cl_2): R_f = 0.06 (1% MeOH/CH_2Cl_2); IR ν_{max} (film) 3308, 2940, 1734, 1681, 1663, 1629, 1525, 1454, 1436, 1215, 1111, 1049, 910 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.34 (s, 3H), 1.41 (t, *J* = 7.1 Hz, 3H), 1.65−1.81 (m, 8H), 1.86−2.00 (m, 2H), 2.05−2.26 (m, 2H), 2.32 (m, 1H), 2.52 (m, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.26 (dd, *J* = 13.5, 10.1 Hz, 1H), 3.79 (m, 1H), 3.86 (m, 1H), 4.21 (m, 1H), 4.62 (q, *J* = 7.1 Hz, 2H), 4.66 (m, 1H), 5.12 (s, 2H), 5.17 (m, 1H), 5.92 (s, 1H, NH), 7.13 (bd, *J* = 10.2 Hz, 1H, NH), 7.19−7.39 (m, 10H), 7.47 (bd, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.0 (CH_3), 23.7 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 26.4 (CH_2), 26.7 (CH_3), 29.5 (CH_2), 30.8 (CH_2), 31.7 (CH_2), 36.0 (CH_2), 47.2 (CH_2), 50.6 (CH), 53.6 (CH), 54.3 (CH), 58.0 (CH), 59.0 (C), 66.7 (CH_2), 70.3 (CH_2), 126.9 (CH), 128.5 (2 × CH), 128.8 (2 × CH), 128.9 (2 × CH), 129.2 (3 × CH), 136.0 (C), 137.2 (C), 172.1 (C), 172.9 (C), 173.0 (C), 174.1 (C), 175.8 (C), 214.1 (C); LRMS (ESI+) *m*/*z* (%) 733 (100) $[M + Na]^+$, 711 (46), 463 (25); HRMS (ESI+) *m*/*z* calcd for $C_{36}H_{46}N_4O_7NaS_2$ 733.2706, found 733.2709.

*S-(S)-1-[(3S,9S,14aR)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]-7-(tert-butyldimethylsilyloxy)-6-oxo-octan-3-yl O-ethyl carbonodithioate (**19f**).* According to method A and starting from (−)-**17** (80 mg, 0.18 mmol) and xanthate **8f** (145 mg, 0.45 mmol), the product **19f** (126 mg, 95%) was obtained as a colorless film (flash chromatography: 0.8% MeOH/CH_2Cl_2): R_f = 0.1 (1% MeOH/CH_2Cl_2); IR ν_{max} (thin film, CH_2Cl_2) 3463, 3305, 2955, 2932, 1715, 1671, 1625, 1522, 1444, 1257, 1216, 1111, 1047, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.07 (s, 6H), 0.91 (s, 9H), 1.26 (m, 3H), 1.35 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.60−1.86 (m, 9H), 1.90−2.08 (m, 2H), 2.17 (m, 1H), 2.33 (m, 1H), 2.76 (m, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.26 (dd, *J* = 13.5, 10.1 Hz, 1H), 3.77 (m, 1H), 3.86 (m, 1H), 4.13 (q, *J* = 6.7 Hz, 1H), 4.24 (m, 1H), 4.63 (q, *J* = 7.1 Hz, 2H), 4.67 (m, 1H), 5.17 (m, 1H), 6.00 (bs, 1H, NH), 7.15 (d, *J* = 10.1 Hz, 1H, NH), 7.18−7.30 (m, 5H), 7.50 (bd, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.9 (CH_3), −4.5 (CH_3), 14.0 (CH_3), 18.2 (C), 21.1

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(CH_3), 23.7 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 25.9 (3 × CH_3), 26.4 (CH_2), 26.6 (CH_3), 27.6 (CH_2), 31.2 (CH_2), 34.4 (CH_2), 36.0 (CH_2), 47.1 (CH_2), 51.0 (CH), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 70.2 (CH_2), 75.0 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.1 (C), 175.8 (C), 213.6 (C), 214.3 (C); LRMS (ESI+) m/z (%) 785 (100) $[M + Na]^+$, 763 (30); HRMS (ESI+) m/z calcd for $C_{37}H_{58}N_4O_7NaSiS_2$ 785.3414, found 785.3431.

*(−)-Benzyl 6-[(3S,9S,14aR)-9-benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecin-3-yl]hexanoate (**20b**).* According to method B and starting from **19b** (124 mg, 0.17 mmol), the product **20b** (69 mg, 69%) was obtained as a colorless film (flash chromatography: silica gel, 1% MeOH/CH_2Cl_2): R_f = 0.17 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −75.0° (c = 1.4, $CHCl_3$); IR ν_{max} (film) 3309, 2934, 1735, 1678, 1663, 1629, 1525, 1424, 1257, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.25−1.40 (m, 7H), 1.65 (m, 2H), 1.72 (m, 1H), 1.77−1.87 (m, 6H), 2.17 (m, 1H), 2.29 (m, 1H), 2.35 (t, J = 7.5 Hz, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.19−3.29 (m, 2H), 3.86 (ddd, J = 10.3, 8.6, 4.6 Hz, 1H), 4.18 (ddd, J = 10.2, 7.6, 7.6 Hz, 1H), 4.65 (m, 1H), 5.11 (s, 2H), 5.16 (ddd, J = 10.2, 10.1, 5.7, 1H), 5.90 (s, 1H, NH), 7.09 (bd, J = 10.2 Hz, 1H, NH), 7.17−7.40 (m, 10H), 7.51 (bd, J = 10.3 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.7 (CH_3), 24.86 (CH_2), 24.93 (CH_2), 25.2 (CH_2), 25.4 (CH_2), 26.7 (CH_3), 28.9 (2 × CH_2), 34.3 (CH_2), 36.0 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 66.3 (CH_2), 126.9 (CH), 128.4 (3 × CH), 128.7 (2 × CH), 128.8 (2 × CH), 129.2 (2 × CH), 136.2 (C), 137.2 (C), 172.0 (C), 173.0 (C), 173.6 (C), 174.5 (C), 175.8 (C); LRMS (ESI+) m/z (%) 613 (100) $[M + Na]^+$, 591 (18); HRMS (ESI+) m/z calcd for $C_{33}H_{42}N_4O_6Na$ 613.3002, found 613.2991.

*(−)-(3S,9S,14aR)-9-Benzyl-3-[(R)-7-(tert-butyldimethylsilyloxy)-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (**20e**).* According to method B and starting from **19e** (71 mg, 0.093 mmol), the product **20e** (36 mg, 60%) was obtained as a colorless film (flash chromatography: silica gel, 0.8% MeOH/CH_2Cl_2): R_f = 0.32 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −47° (c = 2.3, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3520, 3314, 2925, 2853, 1716, 1678, 1630, 1525, 1427, 1246, 1165, 1117, 836 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.07 (s, 6H), 0.91 (s, 9H), 1.26−1.34 (m, 7H), 1.34 (s, 3H), 1.55−1.73 (m, 4H), 1.75 (m, 2H), 1.77 (s, 3H), 2.17 (m, 1H), 2.32 (m, 1H), 2.47−2.64 (m, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.21 (m, 1H), 3.26 (dd, J = 13.5, 10.1 Hz, 1H), 3.86 (m, 1H), 4.13 (q, J = 6.8 Hz, 1H), 4.19 (ddd, J = 10.1, 7.6, 7.6 Hz, 1H), 4.66 (bd, J = 7.8 Hz, 1H), 5.16 (m, 1H), 6.03 (s, 1H, NH), 7.12 (d, J = 10.1 Hz, 1H, NH), 7.18−7.29 (m, 5H), 7.55 (bd, J = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.8 (CH_3), −4.5 (CH_3), 18.2 (C), 21.1 (CH_3), 22.9 (CH_2), 23.2 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 26.0 (3 × CH_3), 26.7 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 36.0 (CH_2), 36.9 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.6 (CH), 58.0 (CH), 59.0 (C), 75.1 (CH), 126.9 (CH), 128.8 (2 × CH), 129.3 (2 × CH), 137.3 (C), 172.0 (C), 173.0 (C), 174.6 (C), 175.9 (C), 214.4 (C); LRMS (ESI+) m/z (%) 665 (17) $[M + Na]^+$, 551 (100); HRMS (ESI+) m/z calcd for $C_{34}H_{54}N_4O_6NaS_i$ 665.3710, found 665.3717.

*(−)-(3S,9S,14aR)-9-Benzyl-3-[(S)-7-(tert-butyldimethylsilyloxy)-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (**20f**).* According to method B and starting from **19f** (114 mg, 0.15 mmol), the product **20f** (57 mg, 59%) was obtained as a colorless film (flash chromatography: silica gel, 0.8% MeOH/CH_2Cl_2): R_f = 0.32 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −77.8° (c = 1.1, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3515, 3311, 2920, 2850, 1718, 1677, 1631, 1526, 1427, 1251, 1170, 1117, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.07 (s, 6H), 0.91 (s, 9H), 1.25−1.34 (m, 10H), 1.55 (m, 2H), 1.63 (m, 2H), 1.76 (m, 2H), 1.77 (s, 3H), 2.17 (m, 1H), 2.32 (m, 1H), 2.47−2.64 (m, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.21 (m, 1H), 3.26 (dd, J = 13.5, 10.1 Hz, 1H), 3.86 (m, 1H), 4.13 (q, J = 6.8 Hz, 1H), 4.20 (ddd, J = 10.1, 7.6, 7.6 Hz, 1H), 4.67 (bd, J = 7.6 Hz, 1H), 5.17 (m, 1H), 6.11 (bs, 1H, NH), 7.15 (d, J = 10.1 Hz, 1H, NH), 7.18−7.29 (m, 5H), 7.57 (bd, J = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −4.9 (CH_3), −4.5 (CH_3), 18.2 (C), 21.1 (CH_3), 22.9 (CH_2), 23.1 (CH_2), 23.8 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 25.9 (3 × CH_3), 26.6 (CH_3), 29.8 (CH_2), 29.9 (CH_2), 36.0 (CH_2), 36.9 (CH_2), 47.1 (CH_2), 53.6 (CH), 54.6 (CH), 58.0 (CH), 58.9 (C), 75.1 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.5 (C), 175.8 (C), 214.3 (C); LRMS (ESI+) m/z (%) 665 (100) $[M + Na]^+$, 643 (30); HRMS (ESI+) m/z calcd for $C_{34}H_{54}N_4O_6NaS_i$ 665.3710, found 665.3705.

General Method To Obtain 20 from (−)-17. *Method C. Example with **20e**.* A solution of cyclotetrapeptide (−)-**17** (31 mg, 0.07 mmol) and carbonyl xanthate **8e** (45 mg, 0.14 mmol) in 1,2-dichloroethane (0.2 mL) was refluxed for 30 min under argon. Dilauroyl peroxide (3 mg) was added every 2 h (five to seven times). After completion (18 h reflux), the solvent was removed in vacuo and isopropyl alcohol (1.5 mL) was added. After the mixture was refluxed for 30 min under argon, DLP (28 mg, 0.07 mmol) was added and the solution was refluxed for 1 h. Additional DLP (14 mg, 0.035 mmol) was then added again, and the solution was refluxed for 3 h. After evaporation, the residue was directly purified by flash chromatography (silica gel, 25% EtOAc/cyclohexane) to give the product **20e** (22 mg, 48%).

*(−)-(3S,9S,14aR)-9-Benzyl-6,6-dimethyl-3-(6-oxooctyl)decahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (**20c**).* According to method C and starting from (−)-**17**, product **20c** was obtained after flash chromatography (silica gel, 30% EtOAc/cyclohexane) as a colorless film: 50% yield; R_f = 0.1 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −65.0° (c = 0.47, $CHCl_3$); IR ν_{max} (film) 2923, 1716, 1683, 1540, 1522, 1457, 1177 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.05 (t, J = 7.3 Hz, 3H), 1.25−1.34 (m, 4H), 1.34 (s, 3H), 1.51−1.70 (m, 4H), 1.77 (m, 2H), 1.77 (s, 3H), 2.18 (m, 1H), 2.32 (m, 1H), 2.39 (t, J = 7.3 Hz, 2H), 2.41 (q, J = 7.3 Hz, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.22 (m, 1H), 3.26 (dd, J = 13.5, 9.9 Hz, 1H), 3.86 (m, 1H), 4.18 (m, 1H), 4.66 (bd, J = 8.0 Hz, 1H), 5.16 (ddd, J = 10.1, 10.1, 5.8 Hz, 1H), 5.89 (s, 1H, NH), 7.08 (d, J = 10.2 Hz, 1H, NH), 7.18−7.31 (m, 5H), 7.51 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 8.1 (CH_3), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.6 (CH_2), 26.7 (CH_3), 29.0 (CH_2), 29.1 (CH_2), 29.9 (CH_2), 36.0 (CH_2), 36.1 (CH_2), 42.4 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.6 (C), 175.9 (C), 211.8 (C); LRMS (ESI+) m/z (%) 535 (100) $[M + Na]^+$, 437 (33); HRMS (ESI+) m/z calcd for $C_{28}H_{40}N_4O_5Na$ 535.2896, found 535.2896.

*(−)-Ethyl 8-[(3S,9S,14aR)-9-benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-a][1,4,7,10]tetra-azacyclododecin-3-yl]-3-oxooctanoate (**20d**).* According to method C and starting from (−)-**17**, product **20d** was obtained after flash chromatography (silica gel, 35% EtOAc/cyclohexane) as a colorless film: 52% yield; R_f = 0.05 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −71.0° (c = 0.68, $CHCl_3$); IR ν_{max} (film) 3305, 2928, 1741, 1714, 1681, 1666, 1629, 1529, 1434, 1315, 1232, 1178, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.28 (t, J = 7.1 Hz, 3H), 1.25−1.34 (m, 4H), 1.34 (s, 3H), 1.54−1.67 (m, 4H), 1.77 (m, 2H), 1.77 (s, 3H), 2.17 (m, 1H), 2.32 (m, 1H), 2.53 (t, J = 7.3 Hz, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.26 (dd, J = 13.4, 10.0 Hz, 1H), 3.42 (s, 2H), 3.86 (m, 1H), 4.17 (m, 1H), 4.20 (q, J = 7.1 Hz, 2H), 4.66 (bd, J = 7.8 Hz, 1H), 5.16 (ddd, J = 10.1, 10.1, 5.8 Hz, 1H), 5.92 (s, 1H, NH), 7.09 (d, J = 10.2 Hz, 1H, NH), 7.19−7.32 (m, 5H), 7.51 (d, J = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.3 (CH_3), 23.3 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.5 (CH_2), 26.7 (CH_3), 28.8 (CH_2), 28.9 (CH_2), 36.0 (CH_2), 43.0 (CH_2), 47.2 (CH_2), 49.5 (CH_2), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 61.6 (CH_2), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 167.4 (C), 172.0 (C), 173.0 (C), 174.5 (C), 175.8 (C), 202.9 (C); LRMS (ESI+) m/z (%) 593 (100) $[M + Na]^+$, 521 (20); HRMS (ESI+) m/z calcd for $C_{30}H_{42}N_4O_7Na$ 593.2951, found 593.2942.

(−)-(3S,9S,14aR)-9-Benzyl-3-[(R)-7-hydroxy-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-a][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (21). Three drops of TBAF (1 M in THF, 20 μL) were added to a solution of **20e** (12.6 mg, 0.0196 mmol) in THF (1 mL). The mixture was stirred for 30 min at room temperature. After evaporation, the residue was directly purified by flash chromatography (silica gel, 2% MeOH/CH_2Cl_2) to give **21** (8.1 mg, 78%): R_f = 0.16 (2% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −98° (c = 0.58, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3288, 2920, 2850, 1710, 1666, 1625, 1523, 1426, 1368, 1187 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.26−1.35 (m, 4H), 1.34 (s, 3H), 1.38 (d, J = 7.1 Hz, 3H), 1.60−

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1.69 (m, 4H), 1.77 (m, 2H), 1.77 (s, 3H), 2.18 (m, 1H), 2.33 (m, 1H), 2.40−2.57 (m, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.21 (m, 1H), 3.27 (dd, J = 13.5, 10.1 Hz, 1H), 3.86 (m, 1H), 4.19 (m, 1H), 4.24 (q, J = 7.1 Hz, 1H), 4.66 (bd, J = 7.7 Hz, 1H), 5.17 (ddd, J = 10.1, 10.1, 5.8 Hz, 1H), 6.00 (s, 1H, NH), 7.13 (d, J = 10.2 Hz, 1H, NH), 7.18−7.30 (m, 5H), 7.51 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 23.5 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.4 (CH_2), 26.7 (CH_3), 28.9 (CH_2), 29.0 (CH_2), 36.0 (CH_2), 37.5 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 72.8 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.1 (C), 173.0 (C), 174.5 (C), 175.8 (C), 212.6 (C); LRMS (ESI+) m/z (%) 551 (100) $[M + Na]^+$, 529 (10); HRMS (ESI+): m/z calcd for $C_{28}H_{40}N_4O_6Na$ 551.2846, found 551.2847.

(−)-(*R*)-8-(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl)-3-oxooctan-2-yl Acetate (22). To a stirred solution of **21** (9.7 mg, 0.018 mmol) in CH_2Cl_2 (1 mL) were added DMAP (catalytic amount) and Ac_2O (5.5 mg, 0.054 mmol) at 0 °C. The mixture was stirred overnight at room temperature. After solvent evaporation, the residue was directly purified by flash chromatography (40% EtOAc/cyclohexane) to give **22** (9.2 mg, 88%) as a colorless film: R_f = 0.09 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −73.1° (c = 0.9, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3518, 3293, 2929, 1723, 1679, 1625, 1528, 1425, 1368, 1233, 1082 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.25−1.34 (m, 4H), 1.34 (s, 3H), 1.39 (d, J = 7.1 Hz, 3H), 1.54−1.67 (m, 4H), 1.77 (m, 2H), 1.77 (s, 3H), 2.14 (s, 3H, Ac), 2.18 (m, 1H), 2.32 (m, 1H), 2.42 (m, 1H), 2.51 (m, 1H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.23 (m, 1H), 3.26 (dd, J = 13.5, 10.1 Hz, 1H), 3.86 (m, 1H), 4.19 (m, 1H), 4.66 (bd, J = 7.7 Hz, 1H), 5.08 (q, J = 7.1 Hz, 1H), 5.16 (ddd, J = 10.1, 10.1, 5.8 Hz, 1H), 6.02 (s, 1H, NH), 7.12 (d, J = 10.2 Hz, 1H, NH), 7.20−7.30 (m, 5H), 7.53 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 16.3 (CH_3), 21.0 (CH_3), 23.1 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.5 (CH_2), 26.7 (CH_3), 28.9 (CH_2), 29.0 (CH_2), 36.0 (CH_2), 38.2 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 74.8 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 170.6 (C), 172.0 (C), 173.0 (C), 174.6 (C), 175.9 (C), 207.8 (C); HRMS (ESI+) m/z calcd for $C_{30}H_{42}N_4O_7Na$ 593.2946, found 593.2946.

(−)-(3*S*,9*S*,14a*R*)-9-Benzyl-3-[(*S*)-7-hydroxy-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (23). TBAF (1 M in THF, 34 μL) was added to a solution of **20f** (21.5 mg, 0.0334 mmol) in THF (1.5 mL). The mixture was stirred for 30 min at room temperature. After evaporation, the residue was directly purified by flash chromatography (2% MeOH/CH_2Cl_2) to give **23** (13.7 mg, 78%): R_f = 0.09 (60% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −65.3° (c = 0.98, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3290, 2925, 2840, 1716, 1666, 1625, 1525, 1424, 1188 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.25−1.35 (m, 4H), 1.34 (s, 3H), 1.38 (d, J = 7.1 Hz, 3H), 1.62−1.69 (m, 4H), 1.77 (m, 2H), 1.77 (s, 3H), 2.17 (m, 1H), 2.32 (m, 1H), 2.38−2.58 (m, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.22 (m, 1H), 3.26 (dd, J = 13.5, 10.1 Hz, 1H), 3.86 (m, 1H), 4.19 (m, 1H), 4.22 (q, J = 7.1 Hz, 1H), 4.67 (bd, J = 7.7 Hz, 1H), 5.16 (ddd, J = 10.1, 10.1, 5.8 Hz, 1H), 6.12 (bs, 1H, NH), 7.17 (d, J = 10.2 Hz, 1H, NH), 7.20−7.30 (m, 5H), 7.54 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 23.5 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.5 (CH_2), 26.7 (CH_3), 28.9 (CH_2), 29.0 (CH_2), 36.0 (CH_2), 37.5 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 72.8 (CH), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.1 (C), 173.0 (C), 174.5 (C), 175.8 (C), 212.6 (C); LRMS (ESI+) m/z (%) 551 (45) $[M + Na]^+$, 242 (100); HRMS (ESI+): m/z calcd for $C_{28}H_{40}N_4O_6Na$ 551.2846, found 551.2847.

(−)-(*R*)-8-[(3*S*,6*R*,9*S*,13*S*,14a*R*)-9-Benzyl-6-ethyl-6,13-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]-tetraazacyclododecin-3-yl]-3-oxooctan-2-yl Octanoate (24). To a solution of FR235222 (42.6 mg, 0.077 mmol) in dry CH_2Cl_2 (2 mL) were added octanoyl chloride (22 mg, 0.13 mmol) and DMAP (36 mg, 0.29 mmol). The mixture was stirred for 18 h and was quenched by a saturated solution of $NaHCO_3$. The product was extracted with EtOAc. The organic layer was dried ($MgSO_4$), and the solvent was concentrated in vacuo. After flash chromatography (silica gel, 25% EtOAc/cyclohexane), the product **24** (47.5 mg, 91%) was isolated as a colorless film: R_f = 0.30 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −88.6° (c = 1.0, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3444, 2933, 2855, 1720, 1646, 1630, 1523, 1421, 1384, 1164 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.83 (t, J = 7.4 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H), 1.24−1.35 (m, 15H), 1.36 (m, 1H), 1.38 (d, J = 7.1 Hz, 3H), 1.51−1.71 (m, 5H), 1.81 (m, 1H), 2.16 (m, 1H), 2.27−2.45 (m, 5H), 2.51 (dt, J = 17.4, 7.2 Hz, 1H), 2.62 (m, 1H), 2.73 (dd, J = 9.7, 8.1 Hz, 1H), 2.96 (dd, J = 13.5, 6.5 Hz, 1H), 3.24 (dd, J = 13.5, 9.8 Hz, 1H), 4.06 (dd, J = 9.6, 7.6 Hz, 1H), 4.20 (ddd, J = 10.2, 7.6, 7.6 Hz, 1H), 4.67 (d, J = 6.5 Hz, 1H), 5.08 (q, J = 7.1 Hz, 1H), 5.16 (ddd, J = 10.2, 9.9, 6.0 Hz, 1H), 5.86 (s, 1H, NH), 7.15 (d, J = 10.2 Hz, 1H, NH), 7.18−7.31 (m, 5H), 7.56 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 8.6 (CH_3), 14.2 (CH_3), 16.3 (CH_3), 18.3 (CH_3), 22.5 (CH_3), 22.8 (CH_2), 23.1 (CH_2), 25.0 (CH_2), 25.5 (CH_2), 28.0 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 31.8 (CH_2), 33.0 (CH), 33.2 (CH_2), 34.2 (CH_2), 35.9 (CH_2), 38.2 (CH_2), 53.5 (CH), 54.0 (CH_2), 54.6 (CH), 58.1 (CH), 63.2 (C), 74.6 (CH), 126.8 (CH), 128.7 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.2 (C), 173.4 (C), 174.3 (C), 175.8 (C), 207.9 (C); HRMS (ESI+) m/z calcd for $C_{38}H_{58}N_4O_7Na$ 705.4203, found 705.4200.

(−)-(*S*)-4-[(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl]butyl Ethanethioate (28). To a solution of (−)-**17** (32 mg, 0.073 mmol) in dry THF (10 mL) was added thioacetic acid **26** (22 mg, 0.29 mmol). The mixture was refluxed for 30 min under argon. A catalytic amount of AIBN was added, and the mixture was stirred at reflux for 16 h while the reaction mixture was held up to the light. After evaporation, the residue was directly purified by flash chromatography (1% MeOH/CH_2Cl_2) to give **28** (34 mg, 91%) as a colorless film: R_f = 0.12 (1% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −85.1° (c = 1.1, $CHCl_3$); IR ν_{max} (film) 3307, 2934, 1684, 1630, 1528, 1428, 1274, 1187, 915 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.34 (s, 3H), 1.39 (m, 2H), 1.53−1.71 (m, 4H), 1.72 (m, 5H), 2.11−2.22 (m, 2H), 2.32 (s, 3H), 2.85 (t, J = 7.2 Hz, 2H), 2.95 (dd, J = 13.4, 5.6 Hz, 1H), 3.23 (m, 1H), 3.26 (m, 1H), 3.86 (m, 1H), 4.21 (m, 1H), 4.68 (m, 1H), 5.17 (s, 1H), 6.02 (s, 1H, NH), 7.13 (d, J = 10.1 Hz, 1H, NH), 7.19−7.29 (m, 5H), 7.53 (d, J = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.7 (CH_3), 24.8 (CH_2), 24.9 (CH_2), 25.1 (CH_2), 26.6 (CH_2), 28.6 (CH_2), 28.9 (CH_2), 29.33 (CH_2), 30.8 (CH_3), 35.9 (CH_2), 47.1 (CH_2), 53.6 (CH), 54.4 (CH), 57.9 (CH), 58.9 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.4 (C),175.8 (C), 196.0 (C); LRMS (ESI+) m/z (%) 539 (100) $[M + Na]^+$, 517 (10); HRMS (ESI+) m/z calcd for $C_{26}H_{36}N_4O_5NaS$ 539.2304, found 539.2302.

(−)-(*S*)-4-[(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl]butyl Octanethioate (29). To a solution of (−)-**17** (42 mg, 0.095 mmol) in dry THF (12 mL) was added thioacid **27** (61 mg, 0.38 mmol). The mixture was refluxed for 30 min under argon. A catalytic amount of AIBN was added, and the mixture was stirred at reflux for 16 h while the reaction mixture was held up to the light. After evaporation, the residue was directly purified by flash chromatography (1% MeOH/CH_2Cl_2) to give **29** (38.7 mg, 68%) as a colorless film: R_f = 0.19 (8% EtOAc/CH_2Cl_2); $[\alpha]_D^{20}$ = −56.5° (c = 1.2, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3297, 2945, 2921, 2855, 1687, 1658, 1620, 1524, 1420, 1225, 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.88 (t, J = 6.9 Hz, 3H), 1.23−1.33 (m, 10H), 1.34 (s, 3H), 1.58 (m, 2H), 1.60−1.70 (m, 5H), 1.77 (s, 3H), 1.78 (m, 2H), 2.17 (m, 1H), 2.53 (t, J = 7.5 Hz, 2H), 2.85 (t, J = 7.3 Hz, 2H), 2.95 (dd, J = 13.5, 5.7 Hz, 1H), 3.18−3.31 (m, 2H), 3.86 (m, 1H), 4.19 (dt, J = 10.2, 7.6 Hz, 1H), 4.66 (m, 1H), 5.16 (dt, J = 10.2, 5.7 Hz, 1H), 6.00 (s, 1H, NH), 7.12 (d, J = 10.2 Hz, 1H, NH), 7.17−7.35 (m, 5H), 7.51 (d, J = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.3 (CH_3), 22.8 (CH_2), 23.8 (CH_3), 24.9 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 25.9 (CH_2), 26.6 (CH_3), 28.6 (CH_2), 28.7 (CH_2), 29.1 (2 × CH_2), 29.5 (CH_2), 31.8 (CH_2), 36.0 (CH_2), 44.4 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.4 (CH), 58.0 (CH), 59.0 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.1 (C), 173.0 (C), 174.4 (C), 175.8 (C), 199.9 (C); HRMS (ESI+) m/z calcd for $C_{32}H_{48}N_4O_5NaS$ 623.3243, found 623.3244.

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(−)-(3*S*,6*R*,9*S*,14*S*)-3-Benzyl-6-ethyl-6,14-dimethyl-9-(6-oxooctyl)-1,4,7,10-tetraazacyclopentadecane-2,5,8,11-tetraone (30). FR235222 (15 mg, 0.027 mmol) in a degassed solution of THF/methanol (1:1; 1 mL) under argon was cooled to 0 °C. SmI_2 (0.1 M in THF)[42] was injected until the persistence of a deep blue coloration (about 3 mL). The mixture was stirred for 30 min at 0 °C. A THF/H_2O solution (1:1) was added, and the product was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$). After evaporation, the residue was purified by flash chromatography (silica gel, 3% MeOH/CH_2Cl_2) to give **30** (13 mg, 89%) as a colorless film: R_f = 0.3 (4% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −56° (*c* = 1.3, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3309, 2933, 2864, 1707, 1638, 1520, 1454, 1260, 1180 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.46 (t, *J* = 7.4 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.06 (t, *J* = 7.3 Hz, 3H), 1.25−1.42 (m, 8H), 1.42−1.79 (m, 6H), 1.80−2.03 (m, 2H), 2.08 (m, 1H), 2.25 (m, 1H), 2.40−2.47 (m, 4H), 2.68 (m, 1H), 2.88 (dd, *J* = 14.2, 11.0 Hz, 1H), 3.49 (m, 1H), 3.54 (m, 1H), 3.80 (m, 1H), 4.74 (m, 1H), 6.18 (s, 1H, NH), 6.44 (bd, *J* = 6.8 Hz, 1H, NH), 6.55 (bd, *J* = 9.5 Hz, 1H, NH), 7.19 (m, 1H), 7.26−7.27 (m, 4H), 7.63 (bd, *J* = 7.6 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 7.9 (CH_3), 8.1 (CH_3), 18.3 (CH_3), 21.6 (CH_3), 23.7 (CH_2), 26.3 (CH_2), 28.8 (CH_2), 29.2 (CH_2), 29.7 (CH_2), 31.6 (CH_2), 33.2 (CH), 34.1 (CH_2), 36.2 (CH_2), 37.7 (CH_2), 42.2 (CH_2), 44.3 (CH_2), 54.7 (CH), 55.2 (CH), 61.7 (C), 126.7 (CH), 128.6 (2 × CH), 129.3 (2 × CH), 138.2 (C), 171.7 (C), 172.6 (C), 173.2 (C), 175.0 (C), 211.9 (C); LRMS (ESI+) *m*/*z* (%) 565 (100) $[M + Na]^+$, 543 (10) $[M + H]^+$, 291 (8); HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{46}N_4O_5Na$ 565.3366, found 565.3364.

(−)-(3*S*,9*S*)-3-Benzyl-6,6-dimethyl-9-(6-oxooctyl)-1,4,7,10-tetraazacyclopentadecane-2,5,8,11-tetraone (31). According to the procedure used for **30** and starting from **20c** (5 mg, 0.098 mmol), **31** (3.3 mg, 66%) was obtained after flash chromatography (silica gel, 4% MeOH/CH_2Cl_2) as a colorless film: R_f = 0.10 (4% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −56.4° (*c* = 0.33, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3311, 2927, 2855, 1646, 1547, 1452, 1361, 1266 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.05 (t, *J* = 7.3 Hz, 3H), 1.25−1.45 (m, 9H), 1.45−1.86 (m, 9H), 2.05−2.21 (m, 2H), 2.39−2.46 (m, 4H), 3.12 (m, 1H), 3.17 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.42 (m, 1H), 3.46 (dd, *J* = 14.0, 4.9 Hz, 1H), 3.91 (m, 1H), 4.49 (m, 1H), 6.19 (d, *J* = 7.3 Hz, 1H, NH), 6.44 (s, 1H, NH), 6.50 (m, 1H, NH), 7.16−7.30 (m, 5H), 7.37 (bd, *J* = 8.4 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 8.05 (CH_3), 22.1 (CH_2), 23.4 (CH_2), 24.8 (CH_3), 25.7 (CH_3), 25.9 (CH_2), 27.6 (CH_2), 28.7 (CH_2), 29.8 (CH_2), 35.9 (CH_2), 36.2 (CH_2), 36.6 (CH_2), 38.0 (CH_2), 42.1 (CH_2), 54.9 (CH), 55.8 (CH), 58.0 (C), 126.7 (CH), 128.6 (2 × CH), 129.5 (2 × CH), 138.4 (C), 171.6 (C), 173.3 (C), 173.9 (C), 175.1 (C), 212.0 (C); HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{42}N_4O_5Na$ 537.3047, found 537.3046.

(+)-(2*R*,4*S*)-3-Benzoyl-4-benzyl-4-methyl-2-phenyl-1,3-oxazolidin-5-one ((+)-33). To a solution of hexamethyldisilazane (5.7 mL, 26.7 mmol) in THF (25 mL) was added *n*-butyllithium (2.5 M in hexane, 7.8 mL, 19.6 mmol) at −78 °C. After 5 min at this temperature, the reaction mixture was warmed to 0 °C for 30 min and then cooled to −78 °C again. A solution of (+)-(2*R*,4*S*)-3-benzoyl-4-methyl-2-phenyl-1,3-oxazolidin-5-one ((+)-**32**;[36] 5.0 g, 17.8 mmol) in THF (60 mL) was slowly added under nitrogen to this reaction mixture. After the mixture was stirred for 3 h at this temperature, a solution of benzyl bromide (2.76 mL, 23.1 mmol) in THF (50 mL) was slowly added. The reaction mixture was warmed to room temperature over 4 h and was stirred overnight. The solvent was evaporated in vacuo, and the residue was dissolved in 10% NH_4Cl and extracted with dichloromethane. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. After flash chromatography (silica gel, 10% EtOAc/cyclohexane), the product (+)-**33** (4.42 g, 67%) was isolated as a white solid: R_f = 0.48 (20% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +246.9° (*c* = 2.5, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3554, 1795, 1650, 1447, 1393, 1358, 1226, 1170, 1032 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.18 (s, 3H), 3.38 (d, *J* = 13.6 Hz, 1H), 3.94 (d, *J* = 13.6 Hz, 1H), 5.64 (s, 1H), 6.65 (d, *J* = 7.0 Hz, 2H), 6.75 (d, *J* = 7.4 Hz, 2H), 7.02−7.11 (m, 4H), 7.15−7.22 (m, 2H), 7.33−7.39 (m, 2H), 7.40−7.45 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 24.6 (CH_3), 41.3 (CH_2), 65.6 (C), 90.6 (CH), 126.0 (2 × CH), 127.0 (2 × CH), 128.0 (CH), 128.5 (2 × CH), 128.6 (2 × CH), 129.1 (2 × CH), 129.8 (CH), 129.9 (CH), 130.2 (2 × CH), 136.1 (C), 136.2 (C), 136.4 (C), 169.3 (C), 175.0 (C); LRMS (ESI+) *m*/*z* (%) 394 (10) $[M + Na]^+$, 372 (100) $[M + H]^+$, 238 (15); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{22}NO_3$ 372.1594, found 372.1598.

(−)-(2*S*,4*R*)-3-Benzoyl-4-benzyl-4-methyl-2-phenyl-1,3-oxazolidin-5-one ((−)-33). The product (−)-**33** was obtained as a white solid following the experimental procedure described above, starting from (−)-(2*S*,4*R*)-3-benzoyl-4-methyl-2-phenyl-1,3-oxazolidin-5-one ((−)-**32**):[36] $[\alpha]_D^{20}$ = −251.1° (*c* = 1.2, $CHCl_3$); LRMS (ESI+) *m*/*z* (%) 394 (8) $[M + Na]^+$, 372 (100) $[M + H]^+$, 238 (15); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{22}NO_3$ 372.1594, found 372.1598.

(+)-(2*S*)-Methyl-2-amino-2-methyl-3-phenylpropanoate Hydrochloride ((+)-34/HCl). A suspension of oxazolidinone (+)-**33** (1.50 g, 4.04 mmol) in concentrated HCl (18 mL) was refluxed under nitrogen overnight. After the mixture was cooled, the precipitate was removed by filtration and the filtrate was evaporated in vacuo. The residue was then dissolved in methanol (12 mL), and thionyl chloride (0.88 mL, 12.1 mmol) was slowly added at 0 °C. The reaction mixture was stirred overnight at room temperature and was then concentrated in vacuo. The oily residue was precipitated in Et_2O to give the salt of the amino ester (+)-**34** (789 mg, 85%) as a white solid: $[\alpha]_D^{20}$ = +8.5° (*c* = 2.5, MeOH); IR ν_{max} (thin film) 3408, 2075, 1654 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ (ppm) 1.64 (s, 3H), 3.17 (d, *J* = 12.0 Hz, 1H), 3.29 (d, *J* = 12.0 Hz, 1H), 3.83 (s, 3H), 7.20−7.23 (m, 2H), 7.32−7.51 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 22.6 (CH_3), 44.2 (CH_2), 54.0 (CH_3), 62.1 (C), 129.3 (CH), 130.2 (2 × CH), 131.4 (2 × CH), 134.5 (C), 172.3 (C); LRMS (ESI+) *m*/*z* (%) 194 (100) $[M + H]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{11}H_{16}NO_2$ 194.1176, found 194.1177.

(−)-(2*R*)-Methyl-2-amino-2-methyl-3-phenylpropanoate Hydrochloride ((−)-34/HCl). The product (−)-**34**/HCl was obtained as a white solid (725 mg, 78%) following the experimental procedure described above, starting from (−)-**33** (1.50 g, 4.04 mmol): $[\alpha]_D^{20}$ = −8.1° (*c* = 1.1, MeOH); LRMS (ESI+) *m*/*z* (%) 194 (100) $[M + H]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{11}H_{16}NO_2$ 194.1176, found 194.1178.

***(R)*-Glycine Nickel (*R*)-2-[*N*-(*N'*-Benzylpropyl)amino]-benzophenone pent-1-ene Complex (36).** To a suspension of (+)-**35** (9.9 g, 19.8 mmol) and NaOH (2.0 g, 50 mmol) in acetonitrile (100 mL) was added 5-bromopent-1-ene (4.4 g, 29.72 mmol). The mixture was stirred at room temperature for 4 h. An aqueous solution of 0.1 N HCl (155 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 100 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The product **36** (9.5 g, 85%) was obtained after flash chromatography (silica gel, 30% acetone/CH_2Cl_2): $[\alpha]_D^{20}$ = +6.1° (*c* = 1.0, $CHCl_3$); R_f = 0.4 (30% acetone/CH_2Cl_2); IR ν_{max} (film) 2957, 1671, 1637, 1589, 1439, 1330, 1256, 1165, 1063, 913, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.77−1.58 (m, 1H), 2.11−1.87 (m, 4H), 2.13−2.19 (m, 2H), 2.31−2.17 (m, 1H), 2.60−2.45 (m, 1H), 2.81−2.71 (m, 1H), 3.49 (m, 2H), 3.57 (d, *J* = 12.7 Hz, 1H), 3.91 (dd, *J* = 8.1, 3.5 Hz, 1H), 4.42 (d, *J* = 12.7 Hz, 1H), 4.95 (m, 2H), 5.72 (tdd, *J* = 16.9, 10.2, 6.7 Hz, 1H), 6.64 (m, 2H), 6.91 (d, *J* = 7.43 Hz, 1H), 7.12 (m, 1H), 7.18 (m, 1H), 7.26 (m, 1H), 7.34 (m, 2H), 7.42−7.47 (m, 2H), 7.51 (m, 2H), 8.05 (m, 2H), 8.13 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.7 (CH_2), 24.6 (CH_2), 30.8 (2 × CH_2), 33.3 (CH_2), 34.8 (CH_2), 57.1 (CH_2), 63.2 (CH), 70.3 (CH), 115.3 (CH), 120.7 (CH), 123.7 (CH), 126.5 (CH), 127.2 (CH), 127.6 (2 × CH), 128.9 (4 × CH), 129.7 (2 × CH), 131.6 (C), 132.1 (CH), 133.3 (CH), 133.8 (CH), 137.7 (2 × C), 142.3 (C), 170.3 (C), 179.3 (C), 180.3 (C); HRMS (ESI+) *m*/*z* calcd for $C_{32}H_{33}N_3NaNiO_3$ 588.1512 found 588.1512.

(+)-(2*S*)-*tert*-Butyl 1-(Methoxycarbonyl)hex-4-enylcarbamate ((+)-37). According to the procedure reported for (−)-**14** (vide infra) and starting from **36** (9.48 g, 16.75 mmol), the product (+)-**37** (3.19, 12.4 mmol, 74%) was obtained after flash chromatography (5% EtOAc/cyclohexane): R_f = 0.15 (5% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +11.45° (*c* = 0.2, $CHCl_3$); IR ν_{max} (film, CH_2Cl_2) 3359, 2977, 1743, 1718, 1520, 1450, 1366, 1249, 1163, 915 cm^{-1}; NMR (400 MHz, $CDCl_3$) δ (ppm) 1.34 (s, 11H), 1.53 (m, 1H), 1.71 (m, 1H), 1.96 (m, 2H), 3.63 (s, 3H), 4.18 (dd, *J* = 12.9, 7.5 Hz, 1H), 4.86 (m, 2H), 5.16 (d, *J* = 8.12 Hz, 1H, NH), 5.66 (m, 1H); ^{13}C NMR (100 MHz; $CDCl_3$) δ

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(ppm) 24.5 (CH_2), 27.2 (3 × CH_3), 31.9 (CH_2), 33.0 (CH_2), 51.9 (CH_3), 53.2 (CH), 79.4 (C), 114.9 (CH_2), 137.8 (CH), 155.3 (C), 173.1 (C); LRMS (ESI+) m/z (%) 258 (100) $[M + H]^+$; HRMS (ESI+) m/z calcd for $C_{13}H_{23}NO_4Na$ 280.1519, found 280.1520.

(*S*)-Glycine Nickel (*S*)-2-[*N*-(*N*′-Benzylpropyl)amino]benzophenone But-1-ene Complex (38). According to the procedure reported for **36** and starting from (−)-**35** (6.36 g, 12.8 mmol) and 4-bromobut-1-ene (2.44 g, 19.52 mmol), the product **38** (5.33 g, 9.67 mmol, 76%) was obtained after flash chromatography (silica gel, 30% acetone/CH_2Cl_2): $[\alpha]_D^{20}$ = −9.9° (c = 1.0, $CHCl_3$), R_f = 0.4 (30% acetone/CH_2Cl_2); IR ν_{max} (film) 2957, 1672, 1637, 1589, 1438, 1330, 1257, 1165, 1063, 913, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.72 (m, 1H), 1.82 (dd, J = 6.5, 1.4 Hz, 1H), 2.08 (m, 1H), 2.17 (m, 1H), 2.29 (m, 1H), 2.49 (m, 1H), 2.74 (m, 2H), 3.48 (m, 2H), 3.57 (d, J = 12.7 Hz, 1H), 3.90 (dd, J = 8.6, 3.5 Hz, 1H), 4.43 (dd, J = 12.7, 5.6 Hz, 1H), 4.87 (dd, J = 10.2, 1.7 Hz, 1H), 4.96 (qd, J = 17.1, 1.5 Hz, 1H), 5.49 (m, 1H), 6.64 (m, 2H), 6.92 (d, J = 7.4 Hz, 1H), 7.16 (m, 2H), 7.26 (m, 1H), 7.35 (m, 2H), 7.42−7.48 (m, 2H), 7.51 (m, 2H), 8.05 (m, 2H), 8.12 (d, J = 8.3 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.9 (CH_2), 29.5 (CH_2), 30.9 (2 × CH_2), 35.1 (CH_2), 57.2 (CH_2), 63.2 (CH), 70.4 (CH), 115.9 (CH), 120.9 (CH), 123.8 (CH), 126.6 (CH), 127.4 (CH), 127.6 (2 × CH), 128.9 (2 × CH), 129.0 (2 × CH), 129.8 (2 × CH), 131.6 (C), 132.2 (CH), 133.3 (CH), 13 3.8 (CH), 136.6 (2 × C), 142.3 (C), 170.5 (C), 179.3 (C), 180.5 (C); HRMS (ESI+) m/z calcd for $C_{31}H_{31}N_3NaNiO_3$ 574.1611, found 574.1611.

(+)-(2*R*)-*tert*-Butyl 1-(methoxycarbonyl)pent-4-enylcarbamate ((+)-14). To a suspension of compound **38** (5.3 g, 9.67 mmol) in MeOH (80 mL) was added 2 M aqueous HCl (100 mL), and the mixture was stirred under reflux for 2 h. The green solution was cooled to room temperature and basified to pH 9−10 with 25% ammonia solution. After extraction with CH_2Cl_2 the aqueous layer was concentrated in vacuo to afford a green solid. Thionyl chloride (1.2 mL, 14.81 mmol) was added dropwise to a suspension of the following green solid (1.25 g, 9.67 mmol) in dry methanol (100 mL) under nitrogen at 0 °C, and the reaction mixture was stirred under reflux for 3 h. The solvent was removed under reduced pressure, and the solid residue was dissolved in acetonitrile (75 mL) and was placed in an ice/water bath. Triethylamine (2.10 mL, 14.81 mmol) and di-*tert*-butyl dicarbonate (3.23 g, 14.81 mmol) were added, and the suspension was stirred for 16 h. The solvent was removed under reduced pressure, and the residue was dissolved in water (50 mL) and EtOAc (75 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an oil which was purified by silica gel column chromatography (5% EtOAc/cyclohexane) to afford (+)-**14** (2.30 g, 9.47 mmol, 98%): $[\alpha]_D^{20}$ = −19.2° (c = 1, $CHCl_3$); HRMS (ESI+) m/z calcd for $C_{12}H_{21}NO_4Na$ 266.1368, found 266.1368.

(−)-(*S*)-Butyl 2-{2-[(*tert*-Butoxycarbonyl)amino]hex-5-enamido}-2-methylpropanoate ((−)-39). To a solution of (+)-**14** (959 mg, 3.95 mmol) in THF (15 mL) was added LiOH (434 mg, 9.88 mmol) in water (15 mL). The mixture was stirred at room temperature for 2−3 h until completion. A 5% aqueous solution of H_3PO_4 was added until pH 3, and the organic phase was extracted with EtOAc, dried ($MgSO_4$), and evaporated to give the corresponding carboxylic acid (915 mg, 4 mmol). To a cooled solution of the following residue and H-Aib-O*n*Bu (636 mg, 4 mmol) in dry DMF (20 mL) were added HOBt·H_2O (540 mg, 4 mmol), DCC (929 mg, 4.80 mmol), and Et_3N (0.6 mL), and the mixture was stirred at room temperature overnight (18 h). DMF was evaporated, and the residue was diluted with EtOAc and washed with 10% citric acid, 4% sodium carbonate, and brine. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (silica gel, 20% EtOAc/cyclohexane) to give the corresponding dipeptide (−)-**39** (1.17 g, 3.16 mmol, 80%): $[\alpha]_D^{20}$ = −17.0° (c = 1.0, $CHCl_3$); R_f = 0.23 (20% EtOAc/cyclohexane); IR ν_{max} (film, CH_2Cl_2) 3358, 2978, 2890, 1745, 1750, 1518, 1453, 1366, 1220, 1070, 914 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.82 (t, J = 7.4 Hz, 3H), 1.26 (m, 2H), 1.32 (ds, 9H), 1.41 (ds, 6H), 1.50 (m, 2H), 1.61 (m, 1H), 1.77 (m, 1H), 2.04 (dd, J = 13.7, 6.7 Hz, 2H), 4.00 (m, 2H), 4.1 (td, J = 12.0, 5.9 Hz, 1H), 4.90 (m, 2H), 5.57−5.79 (m, 2H, NH), 7.28 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.5 (CH_3); 18.9 (CH_2), 24.5 (CH_3), 25.0 (CH_3), 28.2 (3 × CH_3), 29.6 (CH_2), 30.4 (CH_2), 31.7 (CH_2), 53.7 (CH), 56.0 (C), 64.9 (CH_2), 79.4 (C), 115.1 (CH_2), 137.4 (CH), 155.7 (C), 171.5 (C), 174.0 (C); HRMS (ESI+) m/z calcd for $C_{19}H_{34}N_2O_5Na$ 393.2365, found 393.2365.

(+)-(*R*)-Butyl 2-{2-[(*tert*-Butoxycarbonyl)amino]hex-5-enamido}-2-methylpropanoate ((+)-39). According to the procedure reported for (−)-**39**, and starting from (−)-**14** (1.0, 4.11 mmol), the product (+)-**39** was obtained (1.23 g, 81% yield): $[\alpha]_D^{20}$ = +16.5° (c = 1.0, $CHCl_3$); HRMS (ESI+) m/z calcd for $C_{19}H_{34}N_2O_5Na$ 393.2365, found 393.2360.

(+)-(2*R*,5*S*)-Methyl 2-Benzyl-5-[(*tert*-butoxycarbonyl)amino]-2-methyl-4-oxonon-8-enoate ((+)-40). To a solution of methyl ester (+)-**14** (655 mg, 2.69 mmol) in THF (10 mL) was added a solution of LiOH (161 mg, 6.73 mmol) in water (10 mL). After it was stirred for 2 h at room temperature, the reaction mixture was quenched by addition of a solution of 5% H_3PO_4 until pH 3. After extraction with EtOAc, the organic layer was dried over $MgSO_4$ and evaporated to give the corresponding carboxylic acid. The hydrochloride salt of the amino ester (+)-**32** (789 mg, 3.43 mmol) was neutralized using a saturated aqueous solution of $NaHCO_3$, and the free amine was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. To a solution of carboxylic acid and free amine in dichloromethane (10 mL) was added DCC (555 mg, 2.69 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature, the precipitate was filtered off, and the filtrate was concentrated in vacuo. After flash chromatography (silica gel, 10% EtOAc/cyclohexane), the product (+)-**40** (1.0 g, 92%) was isolated as a colorless oil: R_f = 0.48 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +5.8° (c = 1.8, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3313, 2974, 1740, 1666, 1523, 1249, 1168, 1123 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.47 (s, 9H), 1.65−1.72 (m, 4H), 1.92−2.02 (m, 1H), 2.16 (q, J = 7.2 Hz, 2H), 3.22 (d, J = 13.5 Hz, 1H), 3.55 (d, J = 13.5 Hz, 1H), 3.80 (s, 3H), 4.02−4.12 (m, 1H), 4.95−5.11 (m, 3H, NH), 5.82 (ddt, J = 17.0, 10.2, 6.6 Hz, 1H), 6.71 (bs, 1H, NH), 7.06 (dd, J = 7.8, 2.0 Hz, 2H), 7.21−7.29 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.3 (CH_3), 28.5 (3 × CH_3), 29.9 (CH_2), 31.8 (CH_2), 41.7 (CH_2), 52.8 (CH_3), 54.5 (CH), 61.3 (C), 80.2 (C), 115.9 (CH_2), 127.2 (CH), 128.5 (2 × CH), 130.0 (2 × CH), 136.2 (C), 137.4 (CH), 155.7 (C), 171.5 (C), 174.2 (C); LRMS (ESI+) m/z (%) 405 (58) $[M+H]^+$, 349 (100) $[M - t\text{-Bu} + 2H]^+$, 305 (69) $[M - \text{Boc} + 2H]^+$; HRMS (ESI+) m/z calcd for $C_{22}H_{33}N_2O_5$ 405.2384, found 405.2386.

(−)-(2*S*,5*S*)-Methyl 2-Benzyl-5-[(*tert*-butoxycarbonyl)amino]-2-methyl-4-oxonon-8-enoate ((−)-41). According to the procedure reported for (+)-**40** and starting from (+)-**14** (420 mg, 1.73 mmol) and (−)-**34**/HCl (594 mg, 2.59 mmol), the product (−)-**41** (532 mg, 76%) was obtained after flash chromatography as a colorless oil (silica gel, 10% EtOAc/cyclohexane): R_f = 0.55 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −14.4° (c = 1.6, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3415, 1650, 1527, 1258, 1168, 1119 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.46 (s, 9H), 1.63 (s, 3H), 1.64−1.73 (m, 1H), 1.81−1.93 (m, 1H), 2.10 (q, J = 7.0 Hz, 2H), 3.28 (d, J = 13.6 Hz, 1H), 3.46 (d, J = 13.6 Hz, 1H), 3.79 (s, 3H), 4.00−4.12 (m, 1H), 4.99−5.07 (m, 2H), 5.08−5.16 (m, 1H), 5.80 (ddt, J = 17.0, 10.3, 6.6 Hz, 1H), 6.64 (bs, 1H, NH), 7.08 (dd, J = 8.0, 1.4 Hz, 2H), 7.24−7.32 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.3 (CH_3), 28.4 (3 × CH_3), 29.8 (CH_2), 31.8 (CH_2), 41.6 (CH_2), 52.8 (CH_3), 54.3 (CH), 60.9 (C), 80.0 (C), 115.8 (CH_2), 127.2 (CH), 128.5 (2 × CH), 130.1 (2 × CH), 136.1 (C), 137.4 (CH), 155.7 (C), 171.5 (C), 174.2 (C); LRMS (ESI+) m/z (%) 405 (63) $[M + H]^+$, 349 (100) $[M - t\text{-Bu} + 2H]^+$, 305 (73) $[M - \text{Boc} + 2H]^+$; HRMS (ESI+) m/z calcd for $C_{22}H_{33}N_2O_5$ 405.2385, found 405.2384.

(+)-(*S*)-*tert*-Butyl 1-[(*R*)-2-Amino-3-phenylpropanoyl]-pyrrolidine-2-carboxylate ((+)-42). To a solution of (*Z*)-Phe-OH (2.88 g, 9.6 mmol) and H-L-Pro-O-*t*-Bu (2.0 g, 9.6 mmol) in DMF (20 mL) were added at 0 °C HOBt·H_2O (1.5 g, 11.1 mmol), DCC (2.38 g, 11.5 mmol), and Et_3N (1,43 g, 9.6 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. DMF was evaporated, and the residue was diluted with EtOAc and washed with 10% citric acid, 4% sodium carbonate, and brine. The organic phase was dried ($MgSO_4$) and evaporated. After flash chromatography (silica gel, 2% MeOH/CH_2Cl_2), the corresponding dipeptide was obtained (4.0 g,

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8.84 mmol, 92%). To a solution of this protected dipeptide in acetic acid (18 mL) was added Pd/C 10% (520 mg, 0.48 mmol), and the mixture was stirred under H_2 at room temperature overnight. The mixture was filtered on Celite, washed with EtOAc, and concentrated in vacuo. After flash chromatography (silica gel, 3% MeOH/CH_2Cl_2), the product (+)-**42** (2.39 g, 85%) was isolated as a colorless film: $[\alpha]_D^{20}$ = +43.0° (*c* = 1.0, $CHCl_3$); R_f = 0.23 (5% MeOH/CH_2Cl_2); IR ν_{max} (film, CH_2Cl_2) 3358, 2978, 2929, 1724, 1646, 1454, 1364, 1254, 1152, 1090, 910, 849, 756, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.39 (s, 9H), 1.49 (m, 1H), 1.71−1.90 (m, 3H), 2.62 (m, 1H), 2.74 (m, 2H), 3.44 (m, 1H), 3.72 (dd, *J* = 7.9, 6.8 Hz, 1H), 4.18 (dd, *J* = 8.3, 3.9 Hz, 1H), 7.05−7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 24.3 (CH_2), 27.8 (3 × CH_3), 28.7 (CH_2), 42.0 (CH_2), 46.4 (CH_2), 54.7 (CH), 59.3 (CH), 81.0 (C), 126.5 (CH), 128.2 (2 × CH), 129.2 (2 × CH), 137.5 (C), 171.1 (C), 173.1 (C).

(−)-(*R*)-*tert*-Butyl 1-[(*S*)-2-Amino-3-phenylpropanoyl]-pyrrolidine-2-carboxylate ((−)-42). According to the procedure reported for (+)-**42** and starting from (*Z*)-D-Phe-OH (3.0 g, 10.0 mmol) and H-Pro-O-*t*-Bu (2.1 g, 10.0 mmol), the protected and unprotected dipeptides (−)-**42** were subsequently obtained in yields of 92% (4.1 g) and 79% (2.17 g), respectively: $[\alpha]_D^{20}$ = −42.6° (*c* = 1.0, $CHCl_3$); IR ν_{max} (film, CH_2Cl_2) 3358, 2978, 2929, 1724, 1646, 1454, 1364, 1254, 1152, 1090, 910, 849, 756, 694 cm^{-1}; HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{26}N_2O_3Na$ 341.1841, found 341.1838.

(*R*)-*tert*-Butyl 1-[(*S*)-2-Amino-3-cyclohexylpropanoyl]pyrrolidine-2-carboxylate ((+)-43). According to the procedure reported for (+)-**42**, and starting with (*Z*)-Cha-OH (1.0 g, 3.28 mmol) and H-D-Pro-O-*t*-Bu (679 mg, 3.28 mmol), the protected and unprotected dipeptides (+)-**43** were subsequently obtained in yields of 93% (1.4 g) and 97% (964 mg), respectively: $[\alpha]_D^{20}$ = +67.2° (*c* = 1.0, $CHCl_3$); R_f =0.21 (20% EtOAc/cyclohexane); IR ν_{max} (film, CH_2Cl_2) 2974, 2925, 2851, 1723, 1638, 1442, 1364, 1217, 1148, 841 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.70−0.91 (m, 2H), 1.03 (m, 1H), 1.18 (m, 2H), 1.26 (m, 1H), 1.36 (s, 9H), 1.57 (m, 4H), 1.75 (m, 1H), 1.79−1.92 (m, 3H), 2.04 (m, 2H), 3.37 (m, 1H), 3.51 (m, 1H), 3.60 (m, 1H), 4.01 (dd, *J* = 14.3, 7.1 Hz, 1H), 4.23 (dd, *J* = 8.3, 3.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 24.6 (CH_2), 25.9 (CH_2), 26.2 (CH_2), 26.4 (CH_2), 27.9 (3 × CH_3), 28.9 (CH), 32.5 (CH_2), 33.9 (CH_2), 34.2 (CH_2), 42.4 (CH_2), 46.8 (CH_2), 50.4 (CH), 59.6 (CH), 81.0 (C), 171.3 (C), 174.9 (C); LRMS (ESI+) *m*/*z* (%) 325 (100) $[M + H]^+$, 269 (75), 649 (20); HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{32}N_2NaO_3$ 347.2305, found 347.2301.

(−)-(3*S*,9*S*,14a*R*)-9-Cyclohexan-3-(but-3-enyl)-6,6-dimethyl-decahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (47). To a solution of the dipeptide (−)-**39** (120 mg, 0.32 mmol) in THF/H_2O/MeOH (1/1/0.5, 2.5 mL) was added LiOH (36 mg, 0.82 mmol), and the mixture was stirred at room temperature for 2 h. A 5% aqueous solution of H_3PO_4 was added until pH 3, and the organic phase was extracted with EtOAc, dried ($MgSO_4$), and evaporated to give the corresponding unprotected dipeptide. To a cooled solution of this dipeptide and (*Z*)-Cha-D-Pro-O-*t*-Bu ((+)-**43**; 131 mg, 0.32 mmol) in dry DMF (5 mL) were added HOBt·H_2O (44 mg, 0.32 mmol), DCC (80 mg, 0.39 mmol), and Et_3N (45 μL), and the mixture was stirred at room temperature overnight (18 h). DMF was evaporated, and the residue was diluted with EtOAc and washed with 10% citric acid, 4% sodium carbonate, and brine. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (silica gel, 20% EtOAc/cyclohexane) to give the corresponding tetrapeptide **44** (101 mg, 50%): HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{50}N_4O_7Na$ 637.3577, found 637.3577. This tetrapeptide was dissolved in TFA (1 mL) at 0 °C and stirred for 3 h at this temperature. After evaporation, the product was precipitated in dry ether to give after filtration the unprotected product. The tetrapeptide trifluoroacetic acid, HATU (75 mg, 0.19 mmol), and DIEA (0.1 mL) were added in five aliquots with a time interval of 30 min under vigorous stirring in DMF (100 mL). Then the reaction mixture was stirred for 18 h at room temperature. The DMF was evaporated, and EtOAc was added. After washing with a solution of 10% citric acid followed by 4% $NaHCO_3$, the organic phase was dried ($MgSO_4$) and evaporated. After flash chromatography (silica gel, 30% EtOAc/cyclohexane), the cyclic tetrapeptide **47** (35 mg, 48%) was isolated as a white foam: $[\alpha]_D^{20}$ = −80.0° (*c* = 0.5, $CHCl_3$); R_f = 0.23 (20% EtOAc/cyclohexane); IR ν_{max} (film, CH_2Cl_2) 330, 2921, 2852, 1650, 1630, 1585, 1458, 1380, 719 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.87−1.01 (m, 2H), 1.07−1.28 (m, 4H), 1.34 (s, 3H), 1.54−1.63 (m, 2H), 1.64−1.75 (m, 5H), 1.77 (s, 3H), 1.79−1.97 (m, 4H), 2.09 (m, 2H), 2.26 (m, 1H), 2.39 (m, 1H), 3.51 (ddd, *J* = 10.2, 7.4, 7.4 Hz, 1H), 3.97 (ddd, *J* = 10.2, 8.4, 8.4 Hz, 1H), 4.23 (ddd, *J* = 10.3, 7.5, 7.5 Hz, 1H), 4.74 (dd, *J* = 7.9, 2.0 Hz, 1H), 4.88−5.09 (m, 3H), 5.77 (ddd, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.92 (s, 1H, NH), 7.13 (d, *J* = 10.3 Hz, 1H), 7.31 (d, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.8 (CH_2), 25.0 (CH_2), 25.4 (CH_2), 26.3 (CH_3), 26.6 (CH_3), 26.8 (CH_2), 28.3 (CH_2), 29.8 (CH_2), 33.4 (CH), 33.5 (CH_2), 34.1 (CH_2), 34.6 (CH), 37.1 (CH_2), 47.3 (CH_2), 50.3 (CH), 54.0 (CH), 58.0 (CH), 59.0 (C), 115.9 (CH_2), 137.2 (CH), 172.1 (C), 173.7 (C), 174.4 (C), 175.8 (C); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{38}N_4O_4Na$ 469.2785, found 469.2781.

(−)-(3*S*,6*S*,9*S*,14a*R*)-6,9-Dibenzyl-3-(but-3-enyl)-6-methyl-decahydropyrrolo[1,2-*α*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (48). To a solution of methyl ester (+)-**40** (610 mg, 1.51 mmol) in THF (10 mL) was added a solution of LiOH (90 mg, 3.77 mmol) in water (10 mL). The reaction mixture was stirred overnight at room temperature, and the reaction was quenched by addition of a solution of 5% H_3PO_4 until pH 3. After extraction with EtOAc, the organic layer was dried over $MgSO_4$ and evaporated to give the corresponding carboxylic acid. To a solution of this carboxylic acid and amine (+)-**42** (480 mg, 1.51 mmol) in DMF (4.5 mL) were added HOBt·H_2O (245 mg, 1.81 mmol), EDC (347 mg, 1.81 mmol), and *N*-methylmorpholine (0.33 mL, 3.02 mmol). The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was dissolved in EtOAc, and the organic layer was washed with 10% citric acid, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. After flash chromatography (silica gel, 20−30% EtOAc/cyclohexane), the product **45** (832 mg, 80%) was isolated as a colorless film: R_f = 0.38 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +24.1° (*c* = 1.1, $CHCl_3$); LRMS (ESI+) *m*/*z* (%) 713 (8) $[M + Na]^+$, 691 (100) $[M + H]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{39}H_{55}N_4O_7$ 691.4065, found 691.4065. The tetrapeptide **45** (150 mg, 0.22 mmol) was dissolved in TFA (2 mL) at 0 °C and stirred for 3 h at this temperature. After evaporation, the product was precipitated in dry ether. To a solution of this tetrapeptide trifluoroacetic acid in DMF (13 mL) were added HATU (91 mg, 0.24 mmol) and DIEA (0.11 mL, 0.63 mmol) in five aliquots with a time interval of 30 min under vigorous stirring. The reaction mixture was stirred overnight. The solvent was then removed, and EtOAc was added. After it was washed with a solution of 10% citric acid followed by 5% $NaHCO_3$, the organic layer was dried over $MgSO_4$ and evaporated. After flash chromatography (silica gel, 30−40% EtOAc/cyclohexane), the cyclotetrapetide **48** (25 mg, 22%) was isolated as a white foam: R_f = 0.34 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −134.7° (*c* = 0.7, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3375, 1626, 1654, 1532, 1430, 1246, 993, 911 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.22 (s, 3H), 1.75−1.92 (m, 4H), 2.01−2.27 (m, 3H), 2.34−2.41 (m, 1H), 3.14 (dd, *J* = 13.8, 7.0 Hz, 1H), 3.29−3.42 (m, 2H), 3.42 (d, *J* = 13.9 Hz, 1H), 3.70 (d, *J* = 13.9 Hz, 1H), 3.87−3.95 (m, 1H), 4.25−4.32 (m, 1H), 4.73−4.77 (m, 1H), 5.02−5.09 (m, 2H), 5.27−5.36 (m, 1H), 5.77−5.87 (m, 1H), 5.89 (bs, NH), 7.08−7.10 (m, 2H), 7.20 (d, *J* = 10.3 Hz, NH), 7.22−7.47 (m, 8H), 7.91 (d, *J* = 10.3 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.4 (CH_3), 25.3 (CH_2), 27.1 (CH_2), 28.5 (CH_2), 29.8 (CH_2), 36.0 (CH_2), 40.7 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.1 (CH), 58.1 (CH), 62.9 (C), 116.1 (CH_2), 127.0 (CH), 127.1 (CH), 128.4 (2 × CH), 128.9 (2 × CH), 129.3 (2 × CH), 130.4 (2 × CH), 135.6 (C), 137.2 (CH), 137.3 (C), 171.9 (C), 173.0 (C), 174.8 (C), 175.4 (C); LRMS (ESI+) *m*/*z* (%) 517 (100) $[M + H]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{37}N_4O_4$ 517.2809, found 517.2808.

(−)-(3*S*,6*R*,9*S*,14a*R*)-6,9-Dibenzyl-3-(but-3-enyl)-6-methyl-decahydropyrrolo[1,2-*α*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (49). According to the procedure reported for **48** and starting from dipeptide (−)-**41** (504 mg, 1.25 mmol) and dipeptide (+)-**42** (397 mg, 1.25 mmol), the tetrapeptide **46** (635 mg, 74%) was obtained after flash chromatography (silica gel, 20−30% EtOAc/

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cyclohexane) as a colorless oil: R_f = 0.14 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +33.8° (*c* = 1.1, $CHCl_3$); LRMS (ESI+) *m*/*z* (%) 691 (100) $[M + H]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{39}H_{55}N_4O_7$ 691.4065, found 691.4060. Starting from the tetrapeptide **46** (480 mg, 0.70 mmol), the cyclotetrapeptide **49** (80 mg, 36%) was obtained after flash chromatography (silica gel, 20−30% EtOAc/cyclohexane) as a white foam: R_f = 0.36 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −41.5° (*c* = 1.4, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3309, 2921, 2851, 1679, 1625, 1527, 1450, 1262, 739, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.65−1.86 (m, 6H), 1.88−1.98 (m, 1H), 2.02−2.21 (m, 3H), 2.28−2.35 (m, 1H), 2.99 (d, *J* = 14.1 Hz, 1H), 3.01 (dd, *J* = 13.4, 5.7 Hz, 1H), 3.19 (d, *J* = 14.1 Hz, 1H), 3.22−3.34 (m, 2H), 3.80−3.87 (m, 1H), 4.22 (dt, *J* = 15.1, 7.6 Hz, 1H), 4.66−4.71 (m, 1H), 4.94−4.98 (m, 1H), 4.99−5.01 (m, 1H), 5.20 (dt, *J* = 10.1, 5.8 Hz, 1H), 5.77 (ddt, *J* = 16.5, 10.8, 6.8 Hz, 1H), 6.10 (bs, NH), 7.08 (d, *J* = 10.3 Hz, NH), 7.23−7.42 (m, 10H), 7.48 (d, *J* = 10.2 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 22.1 (CH_3), 25.0 (CH_2), 27.1 (CH_2), 28.1 (CH_2), 29.9 (CH_2), 36.1 (CH_2), 44.0 (CH_2), 47.0 (CH_2), 53.8 (CH), 53.9 (CH), 58.0 (CH), 61.8 (C), 116.0 (CH_2), 126.9 (CH), 127.4 (CH), 128.6 (2 × CH), 128.8 (2 × CH), 129.3 (2 × CH), 131.2 (2 × CH), 135.5 (C), 137.1 (CH), 137.2 (C), 171.8 (C), 172.9 (C), 174.5 (C), 175.8 (C); LRMS (ESI+) *m*/*z* (%) 517 (100) $[M + H]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{37}N_4O_4$ 517.2809, found 517.2806.

(−)-(3*S*,6*S*,9*S*,14a*R*)-6,9-Dibenzyl-3-[(*R*)-7-hydroxy-6-oxooctyl]-6-methyldecahydropyrrolo[1,2-*α*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (51). A solution of cyclotetrapeptide **48** (87 mg, 0.17 mmol) and carbonyl xanthate **8e** (109 mg, 0.34 mmol) in 1,2-dichloroethane (0.3 mL) was refluxed for 30 min under argon. Dilauroyl peroxide (DLP, 8 mg) was added every 1 h (five to seven times). After the mixture was refluxed overnight, the solvent was removed in vacuo and the residue was directly purified by flash chromatography (silica gel, 20−30% EtOAc/cyclohexane) to give the xanthate intermediate (82 mg, 58%) as a colorless film: R_f = 0.35 (40% EtOAc/cyclohexane); LRMS (ESI+) *m*/*z* (%) 839 (100) $[M + H]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{43}H_{63}N_4O_7S_2Si$ 839.3902, found 839.3892. A solution of this intermediate (75 mg, 0.09 mmol) in isopropyl alcohol (4 mL) was refluxed for 5 h with DLP (50 mg). After evaporation and direct flash chromatography (silica gel, 30−40% EtOAc/cyclohexane), the silylated intermediate (40 mg, 62%) was obtained as a colorless film: R_f = 0.40 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −90.1° (*c* = 0.8, $CHCl_3$); LRMS (ESI+) *m*/*z* (%) 719 (100) $[M+H]^+$; HRMS (ESI+): *m*/*z* calcd for $C_{40}H_{59}N_4O_6Si$ 719.4198, found 719.4190. TBAF (1 M in THF, 42 μL) was added to a solution of the silylated intermediate (30 mg, 0.042 mmol) in THF (1.5 mL). The mixture was stirred for 30 min at room temperature. After evaporation, the residue was directly purified by flash chromatography (1% MeOH/CH_2Cl_2) to give **51** (23 mg, 91%) as a colorless oil: R_f = 0.24 (4% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −117.0° (*c* = 0.9, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3297, 2921, 2847, 1674, 1654, 1523, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.21 (s, 3H), 1.25−1.41 (m, 4H), 1.41 (d, *J* = 7.1 Hz, 3H), 1.61−1.98 (m, 7H), 2.17−2.26 (m, 1H), 2.33−2.62 (m, 3H), 3.13 (dd, *J* = 13.8, 7.0 Hz, 1H), 3.29−3.40 (m, 2H), 3.43 (d, *J* = 13.7 Hz, 1H), 3.69 (d, *J* = 13.7 Hz, 1H), 3.86−3.94 (m, 1H), 4.29 (q, *J* = 7.1 Hz,1H), 4.76 (dd, *J* = 7.9, 2.4 Hz, 1H), 5.27−5.36 (m, 1H), 6.01 (s, 1H, NH), 7.04−7.10 (m, 2H), 7.20−7.36 (m, 9H), 7.91 (d, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 23.3 (CH_2), 23.5 (CH_3), 24.9 (CH_2), 25.2 (CH_2), 25.5 (CH_2), 29.0 (CH_2), 29.8 (CH_2), 36.0 (CH_2), 37.5 (CH_2), 40.7 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.7 (CH), 58.0 (CH), 62.9 (C), 72.8 (CH), 126.9 (CH), 127.1 (CH), 128.4 (2 × CH), 128.9 (2 × CH), 129.3 (2 × CH), 130.4 (2 × CH), 135.6 (C), 137.2 (C), 172.0 (C), 173.0 (C), 174.9 (C), 175.4 (C), 212.6 (C); LRMS (ESI+) *m*/*z* (%) 627 (6) $[M + Na]^+$, 605 (100) $[M + H]^+$, 517 (8); HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{45}N_4O_6$ 605.3334, found 605.3326.

(−)-(3*S*,9*S*,14a*R*)-9-(Cyclohexylmethyl)-3-[(*R*)-7-hydroxy-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]-tetraazacyclododecine-1,4,7,10-tetraone (50). According to the procedure reported for **51** and starting from cyclic tetrapeptide **47** (36 mg, 0.081 mmol) and carbonyl xanthate **8e** (65 mg, 0.2 mmol), the xanthate intermediate (40.5 mg, 65%) was obtained after flash chromatography (silica gel, 20−30% EtOAc/cyclohexane). Starting from this xanthate intermediate (20 mg, 0.026 mmol), the next silylated intermediate (10.5 mg, 62%) was obtained after flash chromatography (silica gel, 0.8% MeOH/CH_2Cl_2) as a colorless film: R_f = 0.30 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −64.7° (*c* = 0.8, $CHCl_3$); HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{60}N_4NaO_6Si$ 671.416615, found 671.417433. Starting from this silylated intermediate (10.5 mg, 0.016 mmol), the product **50** (10.3 mg, 100%) was obtained after flash chromatography (silica gel, 1% MeOH/CH_2Cl_2) as a colorless oil: R_f = 0.30 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −78.0° (*c* = 1.0, $CHCl_3$); IR ν_{max} (film, CH_2Cl_2) 3465, 3281, 2983, 2925, 2860, 1724, 1643, 1458, 1368, 1225, 1152, 1368, 907, 841, 747, 703 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.02−0.84 (m, 2H), 1.10−1.24 (m, 2H), 1.26 (m, 2H), 1.26−1.32 (m, 3H), 1.34 (s, 3H), 1.38 (d, *J* = 7.1 Hz, 3H), 1.63−1.57 (m, 7H), 1.73−1.64 (m, 4H), 1.77 (s, 3H), 1.78−1.97 (m, 3H), 2.33−2.14 (m, 1H), 2.40−2.55 (m, 2H), 3.51 (td, *J* = 10.2, 7.4 Hz, 2H), 3.98 (ddd, *J* = 10.2, 8.5, 4.8 Hz, 1H), 4.27−4.14 (m, 2H), 4.73 (dd, *J* = 7.9, 2.0 Hz, 1H), 4.97 (ddd, *J* = 10.0, 8.3, 7.2 Hz, 1H), 5.93 (s, 1H, NH), 7.12 (d, *J* = 10.2 Hz, 1H, NH), 7.29 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 23.5 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.4 (CH_2), 25.5 (CH_2), 26.3 (CH_3), 26.6 (CH_2), 26.8 (CH_3), 28.9 (CH_2), 29.0 (CH_2), 29.9 (CH), 33.4 (CH_2),, 36.6 (CH_2), 34.7 (CH_2), 37.2 (CH_2), 37.5 (CH_2), 47.3 (CH_2), 50.3 (CH), 54.5 (CH), 58.0 (C), 59.0 (CH), 72.8 (CH), 172.2 (C), 173.7 (C), 174.4 (C), 175.8 (C) 212.6 (C); LRMS (ESI+) *m*/*z* (%) 535 (100) $[M + H]^+$, 225 (70); HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{46}N_4O_6Na$ 557.3305, found 557.3310.

(−)-(3*S*,6*R*,9*S*,14a*R*)-6,9-Dibenzyl-3-[(*R*)-7-hydroxy-6-oxooctyl]-6-methyldecahydropyrrolo[1,2-*α*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (52). According to the procedure reported for **51** and starting from cyclic tetrapeptide **49** (70 mg, 0.14 mmol), the xanthate intermediate (80 mg, 70%) was obtained after flash chromatography (silica gel, 20−30% EtOAc/cyclohexane) as a colorless film: R_f = 0.36 (40% EtOAc/cyclohexane); LRMS (ESI+) *m*/*z* (%) 856 (100) $[M + H_2O + H]^+$, 839 (46) $[M + H]^+$, 391 (35); HRMS (ESI+): *m*/*z* calcd for $C_{43}H_{63}N_4O_7S_2Si$ 839.3902, found 839.3897. Starting from this xanthate intermediate (75 mg, 0.09 mmol), the next silylated intermediate (49 mg, 76%) was obtained after flash chromatography (silica gel, 30% EtOAc/cyclohexane) as a colorless film: R_f = 0.42 (40% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −13.6° (*c* = 1.9, $CHCl_3$); LRMS (ESI+) *m*/*z* (%) 736 (100) $[M + H_2O + H]^+$, 719 (69) $[M + H]^+$, 391 (86); HRMS (ESI+) *m*/*z* calcd for $C_{40}H_{59}N_4O_6Si$ 719.4198, found 719.4190. Starting from this silylated intermediate (30 mg, 0.04 mmol), the product **52** (21 mg, 85%) was obtained after flash chromatography (silica gel, 1% MeOH/CH_2Cl_2) as a colorless oil: R_f = 0.24 (4% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −32.1° (*c* = 0.7, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3305, 2925, 2851, 1674, 1527, 1450, 1258, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.25−1.36 (m, 4H), 1.42 (d, *J* = 7.1 Hz, 3H), 1.60−1.71 (m, 4H), 1.71−1.86 (m, 5H), 2.08−2.17 (m, 1H), 2.28−2.36 (m, 1H), 2.38−2.57 (m, 2H), 3.00 (m, 2H), 3.17−3.24 (m, 2H), 3.29 (dd, *J* = 13.4, 10.1 Hz, 1H), 3.78−3.86 (m, 1H), 4.15−4.25 (m, 1H), 4.28 (q, *J* = 7.0 Hz,1H), 4.69 (dd, *J* = 7.6, 1.8 Hz, 1H), 5.19 (ddd, *J* = 10.1, 10.1, 5.8 Hz,1H), 6.18 (s, 1H, NH), 7.09 (d, *J* = 10.2 Hz, 1H, NH), 7.23−7.40 (m, 10H), 7.45 (d, *J* = 10.1 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 22.9 (CH_2), 23.5 (CH_3), 24.9 (CH_2), 25.0 (CH_2), 25.4 (CH_2), 28.8 (CH_2), 29.0 (CH_2), 36.2 (CH_2), 37.5 (CH_2), 43.9 (CH_2), 47.0 (CH_2), 53.9 (CH), 54.4 (CH), 58.1 (CH), 61.9 (C), 72.8 (CH), 127.0 (CH), 127.4 (CH), 128.6 (2 × CH), 128.8 (2 × CH), 129.3 (2 × CH), 131.3 (2 × CH), 135.6 (C), 137.2 (C), 172.0 (C), 173.0 (C), 174.6 (C), 175.8 (C), 212.6 (C); LRMS (ESI+) *m*/*z* (%) 605 (100) $[M + H]^+$; HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{45}N_4O_6$ 605.3334, found 605.3330.

(+)-(3*R*,9*R*,14a*S*)-9-Benzyl-3-(but-3-enyl)-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone ((+)-17). According to the procedure reported for **47** and starting from dipeptide (+)-**39** (1.11 g, 3.06 mmol) and dipeptide (−)-**42** (1.08 g, 2.68 mmol), the corresponding tetrapeptide (1.12 g, 68%) was obtained after flash chromatography (silica gel, 20% EtOAc/cyclohexane) as a white foam: HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{50}N_4O_7Na$ 637.3577, found 637.3577. This tetrapeptide was converted into cyclic tetrapeptide (+)-**17** (350 mg, 51%, flash chromatography: silica gel, 20% EtOAc/cyclohexane) as a white foam:

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$[\alpha]_D^{20}$ = +109° (*c* = 1, $CHCl_3$); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{32}N_4O_4Na$ 463.2321, found 463.2321.

(+)-(3*R*,9*R*,14a*S*)-9-Benzyl-3-[(*R*)-7-hydroxy-6-oxooctyl]-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (53). According to the procedure reported for **51** and starting from cyclotetrapeptide (+)-**17** (63.6 mg, 0.14 mmol) and carbonyl xanthate **8e** (112 mg, 0.35 mmol), the xanthate intermediate (60 mg, 57%) was obtained after flash chromatography (silica gel, 20−30% EtOAc/cyclohexane) as a colorless film. Starting from this xanthate intermediate (48 mg, 0.063 mmol), the next silylated intermediate (30 mg, 75%) was obtained after flash chromatography (silica gel, 20% EtOAc/cyclohexane) as a colorless film: R_f = 0.30 (30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = +78.5° (*c* = 0.8, $CHCl_3$); HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{54}N_4O_6NaSi$ 665.3710, found 665.3705. Starting from this silylated intermediate (11.8 mg, 0.018 mmol), the product **53** (10.3 mg, 100%) was obtained after flash chromatography (silica gel, 1% MeOH/CH_2Cl_2) as a colorless oil: $[\alpha]_D^{20}$ = +64.7° (*c* = 0.43, $CHCl_3$); R_f = 0.16 (2% MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.22−1.30 (m, 4H), 1.34 (s, 3H), 1.38 (dd, *J* = 7.1 Hz, 3H), 1.57−1.69 (m, 4H), 1.77 (m, 2H), 1.77 (m, 3H), 2.18 (m, 1H), 2.33 (m, 1H), 2.38−2.58 (m, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.11−3.39 (m, 2H), 3.8 (m, 1H), 4.21 (m, 2H), 4.66 (d, *J* = 7.3 Hz, 1H), 5.16 (ddd, *J* = 10.1, 10.1, 5.8 Hz, 1H), 6.0 (s, 1H, NH), 7.13 (d, *J* = 10.2 Hz, 1H, NH), 7.17−7.31 (m, 5H), 7.50 (d, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.1 (CH_3), 23.5 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.4 (CH_2), 26.7 (CH_3), 28.9 (CH_2), 29.0 (CH_2), 36.0 (CH_2), 37.5 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C, C9), 72.8 (CH, C24), 126.9 (CH, C17), 128.8 (2 × CH, 2 × C15), 129.2 (2 × CH, 2 × C16), 137.2 (C), 172.1 (C), 173.0 (C), 174.5 (C), 175.8 (C), 212.6 (C); LRMS (ESI+) *m*/*z* (%) 551 (100) $[M + Na]^+$, 529 (10); HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{40}N_4O_6Na$ 551.2846, found 551.2847.

(+)-(*S*)-Butyl 2-{2-[(*tert*-Butoxycarbonyl)amino]hept-6-enamido}-2-methylpropanoate (54). According to the procedure reported for (−)-**39**, and starting from (+)-**37** (135 mg, 0.53 mmol) and H-Aib-O-*n*-Bu (84.3 mg, 0.53 mmol), the product **54** (157 mg, 77%) was obtained as a colorless film: $[\alpha]_D^{20}$ = −16.9° (*c* = 1.0, $CHCl_3$); R_f = 0.23 (20% EtOAc/cyclohexane); IR ν_{max} (film, CH_2Cl_2) 3358, 2978, 2890, 1745, 1750, 1518, 1453, 1366, 1220, 1070, 914 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.91 (t, *J* = 7.4 Hz, 3H), 1.35 (m, 2H), 1.41 (m, 2H), 1.43 (s, 9H), 1.51 (s, 6H), 1.60 (m, 3H), 1.78 (m, 1H), 2.05 (m, 2H), 4.09 (m, 2H), 4.95 (m, 2H), 5.27 (d, *J* = 7.8 Hz, 1H), 5.75 (tdd, *J* = 16.9, 10.2, 6.7 Hz, 1H), 7.0 (s, 1H, NH); ^{13}C NMR (100 MHz; $CDCl_3$) δ (ppm) 13.8 (CH_3), 19.1 (CH_2), 24.7 (CH_2), 24.8 (CH_2), 26.9 (CH_3), 28.4 (3 × CH_3), 30.6 (CH_3), 32.1 (CH_2), 33.4 (CH_2), 54.4 (CH), 56.4 (C), 65.3 (CH_2), 79.8 (C), 114.9 (CH_2), 138.2 (CH), 155.9 (C), 171.4 (C), 174.4 (C); HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{36}N_2NaO_5$ 407.2516, found 407.2515.

(−)-(3*S*,9*S*,14a*R*)-9-Benzyl-3-(pent-3-enyl)-6,6-dimethyldecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecine-1,4,7,10-tetraone (56). According to the procedure reported for **47** and starting from dipeptide **54** (157 mg, 0.4 mmol) and (+)-**42** (161 mg, 0.4 mmol), the tetrapeptide (220 mg, 90%) was obtained after flash chromatography (silica gel, 30% EtOAc/cyclohexane) as a white foam: HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{52}N_4O_7Na$ 651.3728, found 651.3721. This tetrapeptide (220 mg, 0.38 mmol) was converted into cyclic tetrapeptide **56** (100 mg, 58%, flash chromatography: silica gel, 30% EtOAc/cyclohexane) as a white foam: $[\alpha]_D^{20}$ = −86.6° (*c* = 1, $CHCl_3$); R_f = 0.22 (30% EtOAc/cyclohexane); IR ν_{max} (film, CH_2Cl_2) 3304, 2930, 1678, 1663, 1630, 1663, 1529, 1428, 1251, 1181, 914 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.33 (s, 3H), 1.55 (m, 2H), 1.61−1.79 (m, 5H), 1.84 (m, 1H), 1.99 (m, 2H), 2.09 (m, 1H), 2.23 (m, 1H), 2.25 (m, 1H), 2.85 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.10−3.20 (m, 2H), 3.76 (m, 1H), 4.10 (td, *J* = 10.3, 7.6, 7.6 Hz, 1H), 4.56 (dd, *J* = 7.7, 2.3 Hz, 1H), 4.85−4.94 (m, 2H), 5.68 (td, *J* = 10.1, 10.0, 5.8 Hz, 1H), 5.80 (dt, *J* = 16.8, 10.2, 6.6 Hz, 1H), 6.99 (s, 1H, NH), 7.07−7.22 (m, 6H, NH), 7.41 (d, *J* = 10.26 Hz, 1H, NH); ^{13}C NMR (100 MHz; $CDCl_3$) δ (ppm) 23.8 (CH_3), 24.9 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 26.7 (CH_3), 28.6 (CH_2), 33.5 (CH_2), 36.0 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.7 (CH), 58.0 (CH), 59.0 (C), 115.3 (CH_2), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.3 (C), 138.2 (CH), 172.0 (C), 173.0 (C), 174.5 (C), 175.8 (C); HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{34}N_4O_4Na$ 477.2472, found 477.2469.

(−)-(*S*)-4-[(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl]pentyl Octanethioate (57). To a solution of **56** (92.2 mg, 0.20 mmol) in dry THF (10 mL) was added thioacetic acid **27** (129.5 mg, 0.89 mmol). The mixture was refluxed for 30 min under argon. A catalytic amount of AIBN was added, and the mixture was stirred at reflux for 16 h while the reaction mixture was held up to the light. After evaporation, the residue was directly purified by flash chromatography (silica gel, 1% MeOH/CH_2Cl_2) to give **57** (80 mg, 65%) as a colorless film: R_f = 0.12 (1% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −60.7° (*c* = 0.7, $CHCl_3$); IR ν_{max} (film) 3296, 2945, 2920, 2855, 1689, 1658, 1620, 1524, 1420, 1225, 1176 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.88 (t, *J* = 5.9, Hz, 3H), 1.25−1.33 (m, 10H), 1.34 (s, 3H), 1.41 (m, 2H), 1.56 (m, 2H), 1.59−1.69 (m, 2H), 1.71−1.86 (m, 7H), 2.18 (m, 1H), 2.32 (m, 1H), 2.50 (t, *J* = 7.5 Hz, 2H), 2.85 (t, *J* = 7.3 Hz, 2H), 2.95 (dd, *J* = 13.5, 1H), 3.16−3.30 (m, 2H), 3.86 (dt, *J* = 10.1, 4.6 Hz, 1H), 4.18 (td, *J* = 10.2, 7.6 Hz, 1H), 4.66 (d, *J* = 5.7 Hz, 1H), 5.16 (dt, *J* = 10.1, 5.8 Hz, 1H), 5.93 (s, 1H, NH), 7.09 (d, *J* = 10.2 Hz, 1H, NH), 7.13−7.35 (m, 5H), 7.51 (d, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz; $CDCl_3$) δ (ppm) 14.3 (CH_3), 22.8 (CH_2), 23.8 (CH_3), 25.0 (CH_2), 25.2 (CH_2), 25.9 (CH_2), 26.7 (CH_3), 28.6 (CH_2), 28.8 (CH_2), 29.0 (CH_2), 29.1 (2 × CH_2), 29.6 (CH_2), 31.8 (CH_2), 34.0 (CH_2), 36.0 (CH_2), 44.4 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.5 (C), 175.8 (C), 199.9 (C); HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{50}N_4NaO_5S$ 637.3394, found 637.3387.

(−)-(*S*)-4-[(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl]pentyl Ethanethioate (58). To a solution of **56** (10 mg, 0.022 mmol) in dry THF (10 mL) was added thioacetic acid **26** (7 mg, 0.092 mmol). The mixture was refluxed for 30 min under argon. A catalytic amount of AIBN was added, and the mixture was stirred at reflux for 16 h while the reaction mixture was held up to the light. After evaporation, the residue was directly purified by flash chromatography (silica gel, 1% MeOH/CH_2Cl_2) to give **58** (7 mg, 59%) as a colorless film: R_f = 0.12 (1% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −54.3° (*c* = 0.7, $CHCl_3$); IR ν_{max} (film) 3307, 2934, 1684, 1630, 1528, 1428, 1274, 1187, 915 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.24 (m, 2H), 1.34 (s, 3H), 1.40 (m, 2H), 1.57 (m, 2H), 1.61 (s, 3H), 1.77−1.87 (m, 5H), 2.17 (m, 1H), 2.32 (s, 3H), 2.85 (t, *J* = 7.2 Hz, 2H), 2.95 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.25 (m, 1H), 3.75 (m, 1H), 3.85 (m, 1H), 4.18 (td, *J* = 10.3, 7.6, 7.6 Hz, 1H), 4.66 (dd, *J* = 7.7, 2.3 Hz, 1H), 5.16 (dt, *J* = 10.1, 10.1, 5.8 Hz, 1H), 5.95 (s, 1H, NH), 7.09 (d, *J* = 10.3 Hz, 1H, NH), 7.18−7.34 (m, 5H), 7.50 (d, *J* = 10.2 Hz, 1H, NH); ^{13}C NMR (100 MHz; $CDCl_3$) δ (ppm) 23.8 (CH_3), 25.0 (CH_2), 25.3 (2 × CH_2), 26.7 (CH_3), 28.6 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 30.9 (CH_3), 36.1 (CH_2), 47.2 (CH_2), 53.7 (CH), 54.6 (CH), 58.0 (CH), 59.1 (C), 126.9 (CH), 128.8 (2 × CH), 129.3 (2 × CH), 137.3 (C), 172.1 (C), 173.0 (C), 174.5 (C), 175.9 (C), 196.2 (C); HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{38}N_4NaO_5S$ 553.2450, found 553.2455.

(−)-(*S*)-4-[(3*S*,9*S*,14a*R*)-9-Benzyl-6,6-dimethyl-1,4,7,10-tetraoxotetradecahydropyrrolo[1,2-*a*][1,4,7,10]tetraazacyclododecin-3-yl]pentyl Ethanethioate (59). The cyclic tetrapeptide **58** (63 mg, 0.12 mmol) was dissolved in DMF (2 mL), NH_3/MeOH (1.2 mL of 7 N solution) was added, and the mixture was kept at room temperature under an argon atmosphere for 67 h. DMF was evaporated in vacuo, and the crude product was purified using flash chromatography (silica gel, 50% EtOAc/cyclohexane) to give a mixture of monomer and dimer (45 mg) as a colorless film. From this mixture, 31 mg was dissolved in DMF (1 mL), dithiotreitol (98 mg, 0.63 mmol) was added, and the mixture was kept at 37 °C under an argon atmosphere for 24 h. DMF was evaporated in vacuo, and the product was extracted with EtOAc and washed with water. After evaporation, the crude product was purified using column chromatography (alumina, 99.75/0.25 CH_2Cl_2/MeOH) to give **59** (18 mg, 0.037 mmol, 59%) as a colorless film: R_f = 0.24 (silica gel, 30% EtOAc/cyclohexane); $[\alpha]_D^{20}$ = −92.5° (*c* = 1.1, $CHCl_3$); IR ν_{max} (film, CH_2Cl_2) 3309, 3284, 2929, 2358, 2337, 1659,

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1626, 1524, 1422 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.25−1.34 (m, 7H), 1.38−1.46 (m, 2H), 1.58−1.65 (m, 1H), 1.72−1.87 (m, 6H), 2.16−2.21 (m, 1H), 2.29−2.34 (m, 1H), 2.52 (q, J = 7.3 Hz, 2H), 2.95 (dd, J = 13.4, 5.8 Hz, 1H), 3.19−3.29 (m, 2H), 3.48 (s, 1H, SH), 3.83−3.89 (m, 1H), 4.19 (td, J = 10.2, 7.6, 7.6 Hz, 1H), 4.66−4.68 (m, 1H), 5.16 (td, J = 10.1, 5.7, 5.7 Hz, 1H), 6.02 (s, 1H, NH), 7.13 (d, J = 10.4 Hz, 1H, NH), 7.19−7.30 (m, 5H), 7.52 (d, J = 10.4 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 23.8 (CH_3), 24.6 (CH_2), 24.9 (CH_2), 25.2 (CH_2), 26.7 (CH_3), 28.1 (CH_2), 29.0 (CH_2), 29.9 (CH_2), 33.9 (CH_2), 36.0 (CH_2), 47.2 (CH_2), 53.6 (CH), 54.5 (CH), 58.0 (CH), 59.0 (C), 126.9 (CH), 128.8 (2 × CH), 129.2 (2 × CH), 137.2 (C), 172.0 (C), 173.0 (C), 174.5 (C), 175.8 (C); LRMS (ESI+) m/z (%) 489 (100) $[M + H]^+$; HRMS (ESI+) m/z calcd for $C_{25}H_{37}N_4O_4S$ 489.2530, found 489.2530.

(−)-(*S*)-{5-[(2*S*,8*S*)-8-Benzyl-5,5-dimethyl-3,6,9,15-tetraoxo-1,4,7,10-tetraazacyclopentadecan-2-yl]pentyl} Octanethioate (60). According to the procedure used for **30** and starting from **57** (9.7 mg, 0.0157 mmol), **60** (6.0 mg, 62%) was obtained after flash chromatography (3% MeOH/CH_2Cl_2) as a colorless film: R_f = 0.10 (3% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ = −42.8° (c = 0.6, $CHCl_3$); IR ν_{max} (thin film, CH_2Cl_2) 3313, 2925, 2859, 1646, 1548, 1450, 1360, 1266, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.88 (t, J = 6.8 Hz, 3H), 1.25−1.30 (m, 12H), 1.31 (s, 3H), 1.32 (s, 3H), 1.34−1.85 (m, 10H), 2.06−2.24 (m, 2H), 2.54 (t, J = 7.4 Hz, 2H), 2.85 (t, J = 7.3 Hz, 2H), 3.06−3.19 (m, 2H), 3.39−3.49 (m, 2H), 3.91 (m, 1H), 4.50 (m, 1H), 6.28 (m, 1H, NH), 6.42 (s, 1H, NH), 6.50 (m, 1H, NH), 7.15−7.29 (m, 5H), 7.37 (m, 1H, NH); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 14.3 (CH_3), 22.1 (CH_2), 22.8 (2 × CH_2), 24.8 (CH_3), 25.5 (CH_2), 25.6 (CH_3), 25.9 (CH_2), 27.6 (CH_2), 28.2 (CH_2), 28.5 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 30.0 (CH_2), 31.8 (CH_2), 35.8 (CH_2), 36.7 (CH_2), 38.0 (CH), 44.4 (CH_2), 55.0 (CH), 55.6 (CH), 58.0 (C), 126.7 (CH), 128.6 (2 × CH), 129.5 (2 × CH), 138.4 (C), 171.6 (C), 173.2 (C), 173.9 (C), 175.2 (C), 200.4 (C); LRMS (ESI+) m/z (%) 617 (100) $[M + H]^+$; HRMS (ESI+) m/z calcd for $C_{33}H_{53}N_4O_{5S}$ 617.3731, found 617.3729.

■ ASSOCIATED CONTENT

Supporting Information

Text and figures giving ^1H and ^{13}C NMR spectra and biological evaluation conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Biel, M.; Wascholowski, V.; Giannis, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 3186−3216. (b) Cole, P. A. *Nat. Chem. Biol.* **2008**, *4*, 590−597. (c) Thaler, F.; Minucci, S. *Expert Opin. Drug Discovery* **2011**, *6*, 393−404. (d) Fierz, B.; Muir, T. W. *Nat. Chem. Biol.* **2012**, *8*, 417−427.

(2) (a) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discovery* **2006**, *5*, 769−784. (b) Marks, P. A.; Xu, W. S. *J. Cell. Biochem.* **2009**, *107*, 600−608. (c) Marks, P. A. *Biochim. Biophys. Acta, Gene Regul. Mech.* **2010**, *1799*, 717−725. (d) Andrews, K. T.; Gupta, A. P.; Tran, T. N.; Fairlie, D. P.; Gobert, G. N.; Bozdech, Z. *PLoS One* **2012**, *7*, e31847.

(3) For reviews see (a) Andrews, K. T.; Tran, T. N.; Wheatley, N. C.; Fairlie, D. P. *Curr. Top. Med. Chem.* **2009**, *9*, 292−308. (b) Andrews, K. T.; Haque, A.; Jones, M. K. *Immunol. Cell Biol.* **2012**, *90*, 66−77.

(4) (a) Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; Allocco, J. J.; Cannova, C.; Meinke, P. T.; Colletti, S. L.; Bednarek, M. A.; Singh, S. B.; Goetz, M. A.; Bombrowski, A. W.; Polishook, J. D.; Schmatz, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13143−13147. (b) Dow, G. S.; Chen, Y.; Andrews, K. T.; Cardiha, D.; Gerena, L.; Gettayacamin, M.; Johnson, J.; Li, Q.; Melendez, V.; Obaldia, N., III; Tran, T. N.; Kozikowski, A. P. *Antimicrob. Agents Chemother.* **2008**, *52*, 3467−3477. (c) Agbor-Enoh, S.; Seudieu, C.; Davidson, E.; Dritschilo, A.; Jung, M. *Antimicrob. Agents Chemother.* **2009**, *53*, 1727−1734. (d) Patel, V.; Mazitschek, R.; Coleman, B.; Nguyen, C.; Urgaonkar, S.; Cortese, J.; Barker, R. H., Jr.; Greenberg, E.; Tang, W.; Bradner, J. E.; Schreiber, S. L.; Duraisingh, M. T.; Wirth, D. F.; Clardy, J. *J. Med. Chem.* **2009**, *52*, 2185−2187. (e) Marfurt, J.; Chalfein, F.; Prayoga, P.; Wabiser, F.; Kenangalem, E.; Piera, K. A.; Fairlie, D. P.; Tjitra, E.; Anstey, N. M.; Andrews, K. T.; Price, R. N. *Antimicrob. Agents Chemother.* **2011**, *55*, 961−966. (f) Sumanadasa, S. D. M.; Goodman, C. D.; Lucke, A. J.; Skinner-Adams, T.; Sahama, I.; Haque, A.; Do, T. A.; McFadden, G. I.; Fairlie, D. P.; Andrews, K. T. *Antimicrob. Agents Chemother.* **2012**, *56*, 3849−3856.

(5) Newkirk, T. L.; Bowers, A. A.; Williams, R. M. *Nat. Prod. Rep.* **2009**, *26*, 1293−1320.

(6) Mori, H.; Abe, F.; Furukawa, S.; Furukawa, S.; Sakai, F.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 80−86.

(7) Sasamura, S.; Sakamoto, K.; Takagaki, S.; Yamada, T.; Takase, S.; Mori, H.; Fujii, T.; Hino, M.; Hashimoto, M. *J. Antibiot.* **2010**, *63*, 633−636.

(8) Bougdour, A.; Maubon, D.; Baldacci, P.; Ortet, P.; Bastien, O.; Bouillon, A.; Barale, J.-C.; Pelloux, H.; Menard, R.; Hakimi, M.-A. *J. Exp. Med.* **2009**, *206*, 953−966.

(9) Maubon, D.; Bougdour, A.; Wong, Y.-S.; Brenier-Pinchart, M.-P.; Curt, A.; Hakimi, M.-A.; Pelloux, H. *Antimicrob. Agents Chemother.* **2010**, *54*, 4843−4850.

(10) Rezai, T.; Bock, J. E.; Zhou, M. V.; Kalyanaraman, C.; Lokey, R. S.; Jacobson, M. P. *J. Am. Chem. Soc.* **2006**, *128*, 14073−14080.

(11) Taori, K.; Paul, V. J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 1806−1807.

(12) (a) Suzuki, T.; Kouketsu, A.; Itoh, Y.; Hisakawa, S.; Maeda, S.; Yoshida, M.; Nakagawa, H.; Miyata, N. *J. Med. Chem.* **2006**, *49*, 4809−4812. (b) Itoh, Y.; Suzuki, T.; Kouketsu, A.; Suzuki, N.; Maeda, S.; Yoshida, M.; Nakagawa, H.; Miyata, N. *J. Med. Chem.* **2007**, *50*, 5425−5438.

(13) Huang, D.; Li, X.; Sun, L.; Xiu, Z.; Nishino, N. *J. Pept. Sci.* **2012**, *18*, 242−251.

(14) Ying, Y.; Taori, K.; Kim, H.; Hong, J.; Luesch, H. *J. Am. Chem. Soc.* **2008**, *130*, 8455−8459.

(15) Shivashimpi, G. M.; Amagai, S.; Kato, T.; Nishino, N.; Maeda, S.; Nishino, T. G.; Yoshida, M. *Bioorg. Med. Chem.* **2007**, *15*, 7830−7839.

(16) Montero, A.; Beierle, J. M.; Olsen, C. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2009**, *131*, 3033−3041.

(17) Olsen, C. A.; Ghadiri, M. R. *J. Med. Chem.* **2009**, *52*, 7836−7846.

(18) Vickers, C. J.; Olsen, C. A.; Leman, L. J.; Ghadiri, M. R. *ACS Med. Chem. Lett.* **2012**, *3*, 505−508.

(19) (a) Burke, M. D.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58. (b) Nielsen, T. E.; Schreiber, S. L. *Angew. Chem., Int. Ed.* **2008**, *47*, 48−56. (c) Galloway, W. R. J. D.; Isidro-Llobet, A.; Spring, D. R. *Nat. Commun.* **2010**, *1*, Gal1/1−Gal1/13.

(20) Peuchmaur, M.; Wong, Y.-S. *Comb. Chem. High Throughput Screening* **2008**, *11*, 587−601.

(21) Kopp, F.; Stratton, C. F.; Akella, L. B.; Tan, D. S. *Nat. Chem. Biol.* **2012**, *8*, 358−365.

(22) (a) Kumar, N.; Kiuchi, M.; Tallarico, J. A.; Schreiber, S. L. *Org. Lett.* **2005**, *7*, 2535−2538. (b) Schomberg, D.; Thielmann, M.; Winterfeldt, E. *Tetrahedron Lett.* **1985**, *26*, 1705−1706.
(23) (a) Terracciano, S.; Di Micco, S.; Bifulco, G.; Gallinari, P.; Riccio, R.; Bruno, I. *Bioorg. Med. Chem.* **2010**, *18*, 3252−3260. (b) Souto, J. A.; Vaz, E.; Lepore, I.; Poppler, A.-C.; Franci, G.; Alvarez, R.; Altucci, L.; de Lera, A. R. *J. Med. Chem.* **2010**, *53*, 4654−4667.
(24) (a) De Greef, M.; Zard, S. Z. *Org. Lett.* **2007**, *9*, 1773−1776. (b) Charrier, N.; Zard, S. Z. *Angew. Chem., Int. Ed.* **2008**, *47*, 9443−9446.
(25) Nebot, J.; Romea, P.; Urpi, F. *J. Org. Chem.* **2009**, *74*, 7518−7521.
(26) (a) Quiclet-Sire, B.; Zard, S. Z. *Top. Curr. Chem.* **2006**, *264*, 201−236. (b) Quiclet-Sire, B.; Zard, S. Z. *Chem. Eur. J.* **2006**, *12*, 6002−6016.
(27) (a) Dunn, M. J.; Jackson, R. F. W.; Pietruszka, J.; Turner, D. *J. Org. Chem.* **1995**, *60*, 2210−2215. (b) Kaul, R.; Surprenant, S.; Lubell, W. D. *J. Org. Chem.* **2005**, *70*, 3838−3844. (c) Shimasaki, Y.; Kiyota, H.; Sato, M.; Kuwahara, S. *Tetrahedron* **2006**, *62*, 9628−9634.
(28) Bhuiyan, M. P. I.; Kato, T.; Okauchi, T.; Nishino, N.; Maeda, S.; Nishino, T. G.; Yoshida, M. *Bioorg. Med. Chem.* **2006**, *14*, 3438−3446.
(29) (a) Gupta, S.; Peiser, G.; Nakajima, T.; Hwang, Y.-S. *Tetrahedron Lett.* **1994**, *35*, 6009−6012. (b) Tani, H.; Fujii, Y.; Nakajima, H. *Phytochemistry* **2001**, *58*, 305−310. (c) Rodriquez, M.; Bruno, I.; Cini, E.; Marchetti, M.; Taddei, M.; Gomez-Paloma, L. *J. Org. Chem.* **2006**, *71*, 103−107. (d) Gomez-Paloma, L.; Bruno, I.; Cini, E.; Khochbin, S.; Rodriquez, M.; Taddei, M.; Terracciano, S.; Sadoul, K. *ChemMedChem* **2007**, *2*, 1511−1519.
(30) Souto, J. A.; Vaz, E.; Lepore, I.; Poppler, A.-C.; Franci, G.; Alvarez, R.; Altucci, L.; de Lera, A. R. *J. Med. Chem.* **2010**, *53*, 4654−4667.
(31) Molander, G. A.; Hahn, G. *J. Org. Chem.* **1986**, *51*, 1135−1138.
(32) (a) Honda, T.; Ishikawa, F. *Chem. Commun.* **1999**, 1065−1066. (b) Honda, T. *Heterocycles* **2011**, *83*, 1−46.
(33) Ross, N. C.; Kulkarni, S. S.; McLaughlin, J. P.; Aldrich, J. V. *Tetrahedron Lett.* **2010**, *51*, 5020−5023.
(34) Nishino, N.; Jose, B.; Okamura, S.; Ebisusaki, S.; Kato, T.; Sumida, Y.; Yoshida, M. *Org. Lett.* **2003**, *5*, 5079−5082.
(35) (a) Seebach, D.; Naef, R. *Helv. Chim. Acta* **1981**, *64*, 2704−2708. (b) Nebel, K.; Mutter, M. *Tetrahedron* **1988**, *44*, 4793−4796. (c) Tomooka, K.; Sakamaki, J.; Harada, M.; Wada, R. *Synlett* **2008**, 683−686.
(36) Hsiao, Y.; Hegedus, L. S. *J. Org. Chem.* **1997**, *62*, 3586−3591.
(37) Rodriguez, A.; Miller, D. D.; Jackson, R. F. W. *Org. Biomol. Chem.* **2003**, *1*, 973−977.
(38) (a) Belokon', Y. N.; Zelter, I. E.; Bakhmutov, V. I.; Saporovskaya, M. B.; Ryzhov, M. G.; Yanovsky, A. I.; Struchkov, Y. T.; Belikov, V. M. *J. Am. Chem. Soc.* **1983**, *105*, 2010−2017. (b) Belokon', Y. N.; Bulychev, A. G.; Vitt, S. V.; Struchkov, Y. T.; Batsanov, A. S.; Timofeeva, T. V.; Tsyryapkin, V. A.; Ryzhov, M. G.; Lysova, L. A.; Bakhmutov, V. I.; Belikov, V. M. *J. Am. Chem. Soc.* **1985**, *107*, 4252−4259. (c) Collet, C.; Bauchar, P.; Danion-Bougot, R.; Danion, D. *Tetrahedron: Asymmetry* **1998**, *9*, 2121−2131. (d) Belokon', Y. N.; Tararov, V. I.; Maleev, V. I.; Savel'eva, T. F.; Ryzhov, M. G. *Tetrahedron: Asymmetry* **1998**, *9*, 4249−4252.
(39) Nishino and co-workers reported similar low yields during the cyclization of tetrapeptides having hindered quaternary amino acids placed in the backbone.[15]
(40) Islam, M. S.; Bhuiyan, M. P. I.; Islam, M. N.; Nsiama, T. K.; Oishi, N.; Kato, T.; Nishino, N.; Ito, A.; Yoshida, M. *Amino Acids* **2012**, *42*, 2103−2110.
(41) Jackson, R. F. W.; Perez-Gonzalez, M. *Org. Synth.* **2005**, *81*, 77−88.
(42) "Homemade" or commercially available SmI_2 (0.1 M in THF by Aldrich) works equally well.