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# A Remarkable Series of Vinblastine Analogues Displaying Enhanced Activity and an Unprecedented Tubulin Binding Steric Tolerance: C20' Urea Derivatives

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## Abstract

A systematic series of previously inaccessible key C20' urea and thiourea derivatives of vinblastine were prepared from 20'-aminovinblastine that was made accessible through a unique Fe(III)/$NaBH_4$-mediated alkene functionalization reaction of anhydrovinblastine. Their examination defined key structural features of the urea-based analogues that contribute to their properties and provided derivatives that match or exceed the potency of vinblastine by as much as 10-fold in cell-based functional assays, which is directly related to their relative tubulin binding affinity. In contrast to expectations based on apparent steric constraints of the tubulin binding site surrounding the vinblastine C20' center depicted in an x-ray co-crystal structure, remarkably large C20' urea derivatives are accommodated.

## INTRODUCTION

Vinblastine (**1**) and vincristine (**2**) are the most widely recognized members of the Vinca alkaloids as a result of their clinical use as antitumor drugs, and their discovery represent one of the earliest important contributions that plant-derived natural products have made to cancer chemotherapy (Figure 1).[1] Originally isolated from the leaves of *Catharanthus roseus* (L) G. Don in trace quantities,[2,3] vinblastine and vincristine were among the first small molecules shown to bind tubulin and to inhibit microtubule formation and mitosis.[1,4] Due to their clinical importance, low natural abundance, and structural complexity, they have been the subject of extensive and continuing biological and synthetic investigations.[4,5,6]

We previously reported the total synthesis of vinblastine[6] and its extension to the total synthesis of related natural products including vincristine and key analogues that utilizes a one-pot, two-step, biomimetic Fe(III)-promoted single electron oxidative coupling of catharanthine and vindoline and a subsequent Fe(III)/$NaBH_4$-mediated in situ alkene oxidation to generate vinblastine directly.[7,8,9] Recently, we detailed the results of investigation of the Fe(III)/$NaBH_4$-mediated free radical oxidation of the anhydrovinblastine trisubstituted alkene used to introduce the vinblastine C20' tertiary alcohol,[6b] extending the reaction to provide a simple method for direct functionalization of unactivated alkenes.[10,11] In these studies, the broad alkene substrate scope was defined, the exclusive Markovnikov addition regioselectivity was established, the excellent functional group tolerance was

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**Supporting Information**
Cytotoxic activity data for the L1210 cell line for all compounds reported. This material is available free of charge via the Internet at http://pubs.acs.org.

revealed, alternative free radical traps were introduced, the Fe(III) salt and initiating hydride source were examined, and remarkably mild reaction conditions (0–25 °C, 5–30 min) were introduced that are relatively insensitive to the reaction parameters. The interest in this Fe(III)/$NaBH_4$-mediated reaction emerged not only from its use in accessing vinblastine, but the opportunity it presented for the late-stage, divergent[12] preparation of otherwise inaccessible vinblastine analogues incorporating alternative C20' functionality. Although this site is known to be critical to the properties of vinblastine[13] and is found deeply embedded in the tubulin bound complex (Figure 1),[14] the prior exploration of C20' substituent effects has been limited to semi-synthetic *O*-acylation of the C20' alcohol or its elimination and subsequent alkene reduction or superacid-catalyzed additions.[15] These invariably led to substantial reductions in biological potency of the resulting derivative, albeit with examination of only a limited number of key analogues. Consequently, in the course of the development of the Fe(III)/$NaBH_4$-mediated alkene functionalization reaction, its use was extended to the preparation of a series of key vinblastine analogues bearing alternative C20'-functionality. Those of initial interest included the C20' azide **6** and amine **7**, both of which proved to be approximately 100-fold less potent than vinblastine (**1**) and 10-fold less potent than 20'-deoxyvinblastine (**3**). However, acylation of the C20' amine improved activity 10-fold and installation of the unsubstituted C20' urea **11** or thiourea **12** provided compounds that nearly matched the potency of vinblastine itself (Figure 1). Herein and based on these observations, we report a systematic exploration of C20' amine, urea, and thiourea derivatives of vinblastine that have not only provided C20' urea-based analogues that substantially exceed the potency of vinblastine, but that also exhibit good activity against a Pgp-overexpressing, vinblastine-resistant tumor cell line. Just as remarkably and in contrast to expectations based on the steric constraints of the tubulin binding site surrounding the vinblastine C20' center as depicted in the x-ray co-crystal structure of a tubulin bound complex,[14] large C20' urea derivatives are accommodated, exhibiting potent functional activity in cell-based proliferation assays and effectively binding tubulin.

## RESULTS AND DISCUSSION

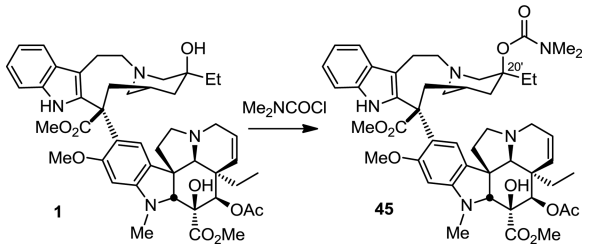
### Chemistry

The targeted vinblastine C20' urea analogues were prepared by two methods (Scheme 1). The first method (Method 1) involved treating the recently accessible 20'-aminovinblastine (**7**)[10] with available isocyanates to provide the corresponding ureas. In the instances when the isocyanates were not readily available, Method 2 was used. This entailed treating 20'-aminovinblastine (**7**) with *p*-nitrophenylchloroformate to provide the activated carbamate **13**, which was then treated with a series of amines to provide the additional C20' urea analogues.

The vinblastine C20' thiourea analogues were prepared also using two complementary methods (Scheme 2). Thus, treatment of 20'-aminovinblastine (**7**) with a small series of commercially available isothiocyanates (Method 3) versus isocyanates provided the corresponding C20' thiourea analogues. Alternatively, treatment of 20'-isothiocyanovinblastine (**5**),[10] made from the reaction of 20'-aminovinblastine (**7**) and carbon disulfide (82%) or that is available upon direct Fe(III)/$NaBH_4$-mediated functionalization of anhydrovinblastine (KSCN),[10] with available amines (Method 4) also provided a set of C20' thiourea vinblastine analogues.

A series of *N,N*-disubstituted C20' urea and thiourea vinblastine analogues were also prepared using Method 2 and 4 and commercially available *N,N*-disubstituted amines. Additionally, the carbamate analogue **45** was synthesized for direct comparison with what

proved to be the potent C20' urea and thiourea derivatives. Thus, vinblastine was treated with the *N,N*-dimethylcarbamyl chloride to provide the C20' carbamate **45** (eq 1).




(1)

## Biological Activity

The C20' substituted vinblastine analogues were examined for cell growth inhibitory activity against the HCT116 (human colon cancer), and HCT116/VM46 (resistant human colon cancer) tumor cell lines, the latter of which exhibits resistance (100-fold) to vinblastine through overexpression of Pgp.[16,17] As we reported, the unsubstituted urea **11** on which the studies are based approached but did not match the potency of vinblastine. The results of the examination of the systematically varied monosubstituted C20' urea derivatives are summarized in Figure 2 alongside those of **1** and **11**. Terminal *N*-alkyl substituents were not only well tolerated, but provided significant enhancements in activity, improving on the potency of **11** and providing derivatives that substantially surpass that of vinblastine itself. Most notable of these are the urea derivatives **14–18**, bearing small *N*-alkyl substituents, some of which exhibited $IC_{50}$s of 700–800 pM against HCT116, improving activity against HCT116 10-fold relative to **11** and substantially surpassing the potency of vinblastine itself (ca. 10-fold). Even the larger *N*-alkyl derivatives **19** and **21** matched or slightly surpassed the activity of vinblastine and only **20**, bearing the large *t*-butyl substituent, experienced a small, but surprisingly modest loss in activity given expectations. Introduction of a polar group that can serve as either a H-bond donor or acceptor on the alkyl substituent in **22** maintained the activity observed with other small alkyl substituents in the HCT116 cell line; however, it had a deleterious effect in the potency against the resistant cell line ($IC_{50}$ >1000 nM, HCT116/VM46), similar to that seen in the parent urea **11**. The cell growth inhibition activity against the L1210 (mouse leukemia) tumor cell line was also measured and the results were qualitatively and quantitatively ($IC_{50}$) nearly identical to those observe with the HCT116 cell line providing $IC_{50}$ values for the urea derivatives that matched or exceeded the activity of vinblastine itself (see Supporting Information for L1210 cytotoxic activity data).

The examination of the monosubstituted C20' urea derivatives bearing *N*-aryl substituents (**23–30**) proved even more unexpected. All exhibited cell growth inhibitory activity at levels exceeding the parent urea **11**, matching or surpassing the potency of vinblastine itself. Electron-withdrawing or electron-donating substituents on the parent *N*-phenyl urea **23** are well tolerated and although no strongly polar substituents were examined, it is notable that the *p*-methoxy substituent proved to be among the best of the *p*-substituents. As a result, the impact of a *m*- and *o*-methoxy phenyl substituent was also examined. Significantly, **29** and **30** bearing *N*-(*m*-methoxyphenyl) and *N*-(*o*-methoxyphenyl) urea substituents respectively, exhibited exceptional activity, substantially exceeding the potency of vinblastine nearly 10-fold ($IC_{50}$ 770 pM vs 6.8 nM, HCT116) and displaying uniquely potent activity against the vinblastine-resistant HCT116 cell line ($IC_{50}$ = 80 and 65 nM, HCT116/VM46). This activity

along with that of **15** and **18** represents a 10-fold improvement over vinblastine and may represent activity at a level of clinical significance.

Placement of a one or two methylene spacer between the phenyl ring and urea nitrogen (**31** and **32**) maintained activity with both derivatives matching the activity of both **23** and vinblastine itself. Replacement of the phenyl ring in **31** with a heteroaromatic ring (furan or pyridine) provided **33** and **34**, which displayed comparable activity to the parent **31**, matching or slightly exceeding the potency of vinblastine. All these observations are unexpected given the apparent steric constraints of the tubulin binding site surrounding the vinblastine C20' center observed in the x-ray crystal structure of a tubulin bound complex.[14] To further probe just how much space may be available to a C20' derivative, the rigid *N*-biphenyl urea **35** was prepared and examined. Remarkably, it displayed cell growth inhibitory activity at a level indistinguishable from vinblastine (Figure 3). This result indicates that sterically demanding modifications to such C20' urea derivatives are possible even beyond what we have probed herein. As a result and in addition to improvements in potency, this may be a superb site for modulating the physical and chemical properties of the drug that impact additional features including Pgp efflux,[18] in vivo drug distribution, selective cellular uptake, and metabolism.

A series of thiourea derivatives were also examined (Figure 4). Although the unsubstituted thiourea derivative **12** approached the activity of the corresponding urea **11** in our original studies ($IC_{50}$ = 7.7 vs 7.5 nM, HCT116), the monosubstituted *N*-alkyl or *N*-aryl derivatives **36** and **37** proved to be 3- to 4-fold less active than the corresponding ureas **21** and **23** (HCT116). However, it is notable that the activity difference between sensitive and vinblastine-resistant cell lines diminishes in this thiourea series (15- to 25-fold vs 100-fold), suggesting they may be transported by Pgp somewhat less effectively.[18]

A small, but important series of *N,N*-disubstituted ureas and thioureas were also examined in order to establish whether the terminal urea nitrogen could be fully substituted or whether the derivatives require or benefit from the presence of an N–H H-bond donor (Figure 5). Remarkably, all the *N,N*-disubstituted ureas exhibited potent cell growth inhibitory activity matching or surpassing the activity of vinblastine and indicating that a terminal H-bond donor site is not important to their functional activity. However, both **39** and **40** were less active than the corresponding monosubstituted urea derivatives **14** and **15**. Ureas with cyclic substituents on the terminal nitrogen, **41** and **42,** exhibited a similar potency to the acyclic derivatives **39** and **40**. An analogous observation was made with the *N,N*-dimethyl thiourea derivative **43**, which approached the potency of vinblastine but exhibited activity slightly lower than the corresponding *N,N*-dimethyl urea **38**. Interestingly, and like the thiourea derivatives **36** and **37**, **43** and especially the urea derivatives **39** and **42** exhibited a diminished activity difference between sensitive and vinblastine-resistant HCT116 cell lines (10-fold vs 100-fold). Finally, comparison of the *N,N*-dimethyl urea or thiourea **39** and **43** with the *N,N*-dimethylcarbamate **44** (>1000-fold less active) clearly illustrates the distinction and importance of the C20' amine versus C20' alcohol functionalization, suggesting the H-donor capabilities of the former may be important.

Although less pronounced, but as detailed in our initial work,[10] the amide **9** and methyl carbamate **10** were found to be more than 10-fold less active than the urea **11**, further highlighting a unique role the urea terminal nitrogen plays in potentiating the activity (Figure 6). As a result and given the size of substituents tolerated, even the intermediate *p*-nitrophenylcarbamate **13** used to prepare the ureas herein was tested and proved to be a surprisingly effective agent ($IC_{50}$ = 55 nM, HCT116), matching the activity of the methyl carbamate **10**.

Finally, two additional C20' amines (**45** and **46**) were prepared by reductive amination ($H_2CO$ (5 equiv), $NaBH_3CN$ (20 equiv), THF, 4 h, 32% (**45**) NHMe, 33% (**46**) $NMe_2$) of 20'-aminovinblastine (**7**) in order to establish the generality of the observations made with the unsubstituted primary amine **7** itself (Figure 7). Although the C20' methylamine proved more potent than **7**, it was still 10-fold less active than vinblastine and the C20' dimethylamine derivative **46** was the least active of the C20' amines, suggesting the importance of a H-bond donor with regard to biological activity.

Finally, the 20'-(methylamino)vinblastine (**45**) was enlisted to establish whether the active urea derivatives require or benefit from the H-bond donor site of the derivatized C20' amine. Thus, treatment of **45** with ethyl isocyanate provided the *N*-methyl urea **47** for comparison with **15** (Figure 8). There was a 700-fold decrease in activity between **15** and **47** (HCT116), clearly illustrating the importance of a H-bond donor site on the C20' position. This observation clearly suggests that the incorporation of a urea functionality maintains a key H-bond site directly attached to the C20' position and that it best approximates the acidity of the vinblastine C20' alcohol, while allowing for further functionalization on the urea terminal nitrogen that maintains or in many cases improves the activity of the compound.

In recent work, the incorporation of a fluorine atom at the 10' position provided a potent molecule (**48**) with an 8-fold improvement in activity over vinblastine itself.[9a] We were interested in determining whether the incorporation of both the 10'-F substituent and a C20' urea would have an additive effect in enhancing the potency of vinblastine. An analogue **49** with both functionalities was prepared and evaluated (Figure 9). Only a modest improvement in potency was observed in **49** relative to **15** and **48**, suggesting that these modifications are not fully additive. Nonetheless, the modified vinblastine **49** is at least 10-fold more potent than vinblastine exhibiting a sub nanomolar $IC_{50}$ for cell growth inhibition (620 pM, HCT116) and a nearly 10-fold improved activity against a vinblastine-resistant cell line ($IC_{50}$ = 70 nM, HCT116/VM46).

## Binding to Tubulin

Given the apparent steric constraints of the tubulin binding site surrounding the vinblastine C20' center (Figure 1) and the size of the C20' urea substituents that support and improve on the functional potency of vinblastine itself in the cytotoxic cell growth assays, it was not clear whether these effects could be related to their target (tubulin) binding affinity or derived from their impact on other properties of the molecules (e.g., cell permeability, metabolism, solubility). As a result, two representative C20' urea derivatives **35** and **39** were examined in a well-established tubulin binding assay conducted by measuring the competitive displacement of $^3$H-vinblastine from porcine tubulin (Figure 10).[19] Notably, **35** contains the large biphenyl urea substituent yet matches the functional activity of vinblastine, whereas **39** bears the much smaller *N,N*-dimethylurea whose functional activity slightly exceeds that of vinblastine (≤ 3-fold). Importantly, these binding studies confirmed that **39** binds tubulin with a slightly better affinity than vinblastine and further established that even **35**, bearing the large biphenyl substituent, remarkably binds with an affinity matching or even slightly exceeding that of vinblastine. Thus, the effects of the urea **39** as well as **35** observed in the functional assays correlate directly with their target tubulin binding affinities. These unanticipated observations with **35** highlight that the vinblastine interaction with tubulin surrounding the C20' center is flexible and capable of reorganization to accommodate even a very large substituent. It is notable that this site is adjacent to the nucleotide binding site involving the T5 loop in the N-terminal β1 tubulin nucleotide binding domain and adjacent to the H6 helix and H6–H7 loop that links the nucleotide binding domain to the intermediate domain. It is likely this region is capable of significant

reorganization to accommodate the binding of **35** or that the urea substituent may extend into the nucleotide binding site and displace a bound nucleotide.

## CONCLUSIONS

A remarkable series of previously inaccessible C20' urea derivatives of vinblastine were prepared and found to match or substantially exceed the potency of vinblastine in functional cell-based growth inhibition assays. In addition to defining structural features of the urea required for or potentiating their activity that are directly related to their relative tubulin binding affinity, the studies established an unprecedented steric tolerance for the size of a C20' substituent. A H-bond donor on the C20' position was unequivocally shown to be an important feature of the potent vinblastine analogues. Although this site is known to be critical to the properties of vinblastine and is located deeply embedded in the tubulin bound complex where such substituents would be apparently sterically constrained, the studies revealed that sterically demanding ureas are not only tolerated but that functionalization of this site offers a superb opportunity for enhancing potency as much as 10-fold. In addition to improvements in potency, this may also be a superb site for modulating the physical and chemical properties of the drug that impact additional features including Pgp efflux, in vivo drug distribution, selective cellular uptake, and metabolism.

## EXPERIMENTAL SECTION

### General Procedures

All commercial reagents were used without further purification unless otherwise noted. THF was distilled prior to use. All reactions were performed in oven-dried (200 °C) glassware and under an inert atmosphere of anhydrous Ar unless otherwise noted. Column chromatography was performed with silica gel 60. TLC was performed on Whatman silica gel (250 μm) $F_{254}$ glass plates and spots visualized by UV. PTLC was performed on Whatman silica gel (250 and 500 μm) $F_{254}$ glass plates. Optical rotations were determined on a Rudolph Research Analytical Autopol III automatic polarimeter using the sodium D line (λ = 589 nm) at room temperature (23 °C) and are reported as follows: $[\alpha]^{D}_{23}$, concentration (*c* = g/100 mL), and solvent. FT-IR spectroscopy was recorded on a Nicolet 380 FT-IR instrument. $^{1}$H NMR was recorded on a Bruker 600 MHz spectrometer. Chemical shifts are reported in ppm from an internal standard of residual $CHCl_3$ (δ 7.26 for $^{1}$H). Proton chemical data are reported as follows: chemical shift (δ), multiplicity (ovlp = overlapping, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant, and integration. High resolution mass spectra were obtained on an Agilent ESI-TOF/MS using Agilent ESI-L low concentration tuning mix as internal high resolution calibration standards. The purity of each tested compound (>95%) was determined on an Agilent 1100 LC/MS instrument using a ZORBAX SB-C18 column (3.5 mm, 4.6 mm × 50 mm, with a flow rate of 0.75 mL/min and detection at 220 and 254 nm) with a 10–98% acetonitrile/water/0.1% formic acid gradient (two different gradients).

### General methods for the synthesis of ureas

**Method 1—**A solution of 20'-aminovinblastine (**7**, 3.5 mg, 0.004 mmol) in THF (3 mL) was treated with an isocyanate (0.008 mmol). The reaction mixture was stirred for 2 h at 25 °C and then was quenched with the addition of distilled $H_2O$ (3 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$ and the combined organic extracts were washed with saturated aqueous NaCl (3 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided the urea (**15**, **19**–**21**, **23**–**32**, and **34**–**35**); yields (35–98%). Isocyanates used include: ethyl isocyanate, *n*-butyl isocyanate, *t*-butyl isocyanate, cyclohexyl isocyanate, phenyl isocyanate, benzyl

isocyanate, phenethyl isocyanate, *o*-methoxyphenyl isocyanate, *m*-methoxyphenyl isocyanate, *p*-methoxyphenyl isocyanate, *p*-fluorophenyl isocyanate, *p*-chlorophenyl isocyanate, *p*-tolyl isocyanate, *p*-trifluoromethylphenyl isocyanate, furfuryl isocyanate and *p*-biphenyl isocyanate.

**Method 2—**A solution of 20'-aminovinblastine (**7**, 5.7 mg, 0.007 mmol) in THF (3 mL) was treated with 4-nitrophenyl chloroformate (2.1 mg, 0.011 mmol, 1.5 equiv). The reaction mixture was stirred for 4 h at 25 °C and then was concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH = 95:5) provided **13** (4.6 mg, 67%, off white solid). A solution of **13** (4.0 mg, 0.004 mmol) in THF (3 mL) was treated with an amine (0.008 mmol). The reaction mixture was stirred for 1 h at 25 °C and then was quenched with the addition of distilled $H_2O$ (3 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$, and the combined organic extracts were washed with saturated aqueous NaCl (3 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided the urea (**14**, **16**–**18**, **22**, **33**, and **39**–**42**); yields (40–99%). The amines used include: methylamine, propylamine, isopropylamine, cyclopropylamine, dimethylamine, diethylamine, 2-(aminomethyl)pyridine, morpholine, piperidine, and ethanolamine.

### General methods for the synthesis of thioureas

**Method 3—**A solution of 20'-aminovinblastine (**7**, 8.0 mg, 0.010 mmol) in THF (4 mL) was treated with an isothiocyanate (0.031 mmol). The reaction mixture was stirred for 2 h at 25 °C and then was quenched with the addition of distilled $H_2O$ (3 mL), the mixture was extracted with 10% MeOH in $CH_2Cl_2$, and the combined organic extracts were washed with saturated aqueous NaCl (3 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided the thiourea (**37**); yields (70%). The isothiocyanate used: phenylisothiocyanate.

**Method 4—**A solution of 20'-aminovinblastine (**7**, 8.2 mg, 0.010 mmol) in THF (3 mL) was treated with carbon disulfide (915 μL, 15 mmol). The reaction mixture was stirred for 13 h and then was quenched with the addition of distilled $H_2O$ (5 mL), the mixture was extracted with 10% MeOH in $CH_2Cl_2$, and washed with saturated aqueous NaCl (2 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided **5** (7.0 mg, 82%, white solid).[10] A solution of 20'-isothiocyanovinblastine (**5**, 6.0 mg, 0.007 mmol) in THF (3 mL) was treated with an amine (0.011 mmol). The reaction mixture was stirred for 1 h at 25 °C and then was quenched with the addition of distilled $H_2O$ (3 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$, and the combined extracts were washed with saturated aqueous NaCl (3 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided the thiourea (**36**, **38**, and **43**); yields (26–92%). The amines used include: cyclohexylamine, 4-fluorophenethylamine and dimethylamine.

**(R = 20'-NHCO$_2$C$_6$H$_4$NO$_2$) 13:** Yield: 67%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.87 (br s, 1H), 8.11 (d, *J* = 9.2 Hz, 2H), 8.04 (br s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.18–7.14 (m, 1H), 7.14–7.08 (m, 2H), 6.76 (d, *J* = 9.1 Hz, 2H), 6.63 (s, 1H), 6.11 (s, 1H), 5.85 (dd, *J* = 10.0, 3.9 Hz, 1H), 5.48 (s, 1H), 5.30 (d, *J* = 9.8 Hz, 1H), 4.00 (t, *J* = 13.2 Hz 1H), 3.81 (s, 3H), 3.79 (s, 3H), 3.72 (s, 1H), 3.63 (s, 3H), 3.60–3.55 (m, 4H), 3.43 (d, *J* = 13.6 Hz, 1H), 3.37 (dd, *J* = 15.9, 4.5 Hz, 1H), 3.35–3.26 (m, 2H), 3.20–3.13 (m, 2H), 2.98 (d, *J* = 13.8 Hz, 1H), 2.85–2.77 (m, 2H), 2.70 (s, 3H), 2.46–2.42 (m, 1H), 2.35 (d, *J* = 11.9 Hz, 1H), 2.24 (d, *J* = 13.0 Hz, 1H), 2.20–2.13 (m, 1H), 2.11 (s, 3H), 1.85–1.76 (m, 2H), 1.64 (d, *J* = 14.8 Hz, 1H), 1.47–1.40 (m, 2H), 1.37–1.29 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H), 0.83 (t, *J* = 7.4 Hz,

3H); IR (film) $\nu_{max}$ 3467, 2924, 1736, 1218 $cm^{-1}$; HRESI-TOF *m/z* 975.4492 ($C_{53}H_{62}N_6O_{12}$ + $H^+$, required 975.4498); $[\alpha]_D^{23}$ −13 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCONHCH$_3$) 14:** Yield: 85%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.97 (br s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.19–7.13 (m, 1H), 7.13–7.07 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.0, 4.4 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 10.7 Hz, 2H), 4.54 (br s, 1H), 4.33 (br s, 1H), 3.84 (t, *J* = 13.2 Hz 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.60 (s, 3H), 3.39–3.36 (m, 2H), 3.32–3.21 (m, 3H), 3.20–3.10 (m, 2H), 2.86 (d, *J* = 4.8 Hz, 3H), 2.83 (d, *J* = 16.6 Hz, 1H), 2.71 (s, 3H), 2.68 (s, 1H), 2.58 (d, *J* = 13.8 Hz, 1H), 2.45 (dd, *J* = 16.9, 10.5 Hz, 1H), 2.38 (d, *J* = 12.8 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 1.85–1.74 (m, 4H), 1.69 (d, *J* = 13.7 Hz, 2H), 1.44–1.30 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3539, 2870, 1721, 1554, 1461, 1223, 1039, 712 $cm^{-1}$; HRESI-TOF *m/z* 867.4631 ($C_{53}H_{62}N_6O_{12}$ + $H^+$, required 867.4651); $[\alpha]_D^{23}$ −1.5 (*c* 0.05, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$CH$_3$) 15:** Yield: 95%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 7.98 (br s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.19–7.14 (m, 1H), 7.14–7.07 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 9.9, 4.0 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 9.8 Hz, 1H), 4.59 (br s, 1H), 4.29 (br s, 1H), 3.83 (t, *J* = 12.0 Hz, 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.60 (s, 3H), 3.37 (d, *J* = 15.0 Hz, 2H), 3.34–3.19 (m, 4H), 3.18–3.13 (m, 2H), 2.83 (d, *J* = 16.0 Hz, 1H), 2.71 (s, 3H), 2.68 (s, 1H), 2.57 (d, *J* = 13.1 Hz, 1H), 2.47–2.43 (m, 1H), 2.38 (d, *J* = 13.4 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 1.86–1.73 (m, 4H), 1.68 (d, *J* = 14.3 Hz, 2H), 1.41–1.38 (m, 4H), 1.19 (t, *J* = 7.2 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3463, 2921, 1739, 1500, 1459, 1228, 1039, 739 $cm^{-1}$; HRESI-TOF *m/z* 881.4797 ($C_{49}H_{64}N_6O_9$ + $H^+$, required 881.4808); $[\alpha]_D^{23}$ +5.4 (*c* 0.08, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$CH$_2$CH$_3$) 16:** Yield: 40%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 7.98 (br s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.18–7.14 (m, 1H), 7.14–7.08 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.1, 4.3 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 9.8 Hz, 1H), 4.63 (br s, 1H), 4.28 (br s, 1H), 3.83 (t, *J* = 12.0 Hz, 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.60 (s, 3H), 3.37 (dd, *J* = 15.9, 4.7 Hz, 2H), 3.34–3.27 (m, 2H), 3.27–3.11 (m, 4H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.71 (s, 3H), 2.68 (s, 1H), 2.57 (d, *J* = 13.8 Hz, 1H), 2.47–2.42 (m, 1H), 2.38 (d, *J* = 13.1 Hz, 1H), 2.24–2.15 (m, 2H), 2.09 (s, 3H), 1.87–1.75 (m, 4H), 1.60–1.52 (m, 6H), 1.40–1.34 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3436, 2952, 1739, 1547, 1459, 1243, 1032, 755 $cm^{-1}$; HRESI-TOF *m/z* 895.4953 ($C_{50}H_{66}N_6O_9$ + $H^+$, required 895.4964); $[\alpha]_D^{23}$ +4.0 (*c* 0.05, $CHCl_3$).

**(R = 20'-NHCONHCH(CH$_3$)$_2$) 17:** Yield: 59%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 8.00 (br s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.17–7.08 (m, 3H), 6.65 (s, 1H), 6.09 (s, 1H), 5.87 (dd, *J* = 10.1, 3.7 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 9.3 Hz, 1H), 4.29 (br s, 1H), 4.14 (br s, 1H), 3.96–3.91 (m, 1H), 3.81–3.77 (m, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.74 (s, 1H), 3.73–3.71 (m, 1H), 3.60 (s, 3H), 3.37 (d, *J* = 14.8 Hz, 2H), 3.34–3.26 (m, 2H), 3.23 (t, *J* = 11.9 Hz, 1H), 3.18–3.11 (m, 2H), 2.83 (d, *J* = 16.0 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.56 (d, *J* = 13.6 Hz, 1H), 2.47–2.43 (m, 1H), 2.36 (d, *J* = 14.4 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 1.86–1.73 (m, 4H), 1.47–1.42 (m, 1H), 1.37–1.29 (m, 3H), 1.20 (dd, *J* = 6.4, 2.5 Hz, 6H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3471, 3323, 2923, 1741, 1459, 1239, 1041 $cm^{-1}$; HRESI-TOF *m/z* 917.4761 ($C_{50}H_{66}N_6O_9$ + $Na^+$, required 917.4783); $[\alpha]_D^{23}$ +6.1 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCONHCH(CH$_2$)$_2$) 18:** Yield: 70%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.86 (br s, 1H), 8.02 (br s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.18–7.13 (m, 1H), 7.13–7.07 (m, 2H), 6.62 (s, 1H), 6.10 (s, 1H), 5.85 (dd, *J* = 10.2, 4.4 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 6.6 Hz, 1H), 5.17 (s, 1H), 4.66 (s, 1H), 3.91 (t, *J* = 13.8 Hz, 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.56

(s, 3H), 3.41–3.13 (m, 6H), 3.08–3.00 (m, 2H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.77 (br s, 1H), 2.71 (s, 3H), 2.70–2.68 (m, 1H), 2.67 (s, 1H), 2.63 (d, *J* = 13.5 Hz, 1H), 2.47–2.43 (m, 1H), 2.38 (d, *J* = 12.1 Hz, 1H), 2.24 (d, *J* = 14.2 Hz, 1H), 2.19 (dd, *J* = 12.9, 7.7 Hz, 1H), 2.15 (s, 1H), 2.11 (s, 3H), 1.86–1.70 (m, 4H), 1.52–1.49 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H), 0.76 (t, *J* = 7.3 Hz, 3H), 0.70–0.69 (m, 2H), 0.50–0.48 (m, 2H); IR (film) $\nu_{max}$ 3629, 2952, 1740, 1506, 1458, 1245, 998, 748 $cm^{-1}$; HRESI-TOF *m/z* 893.4792 ($C_{50}H_{64}N_6O_9 + H^+$, required 893.4808); $[\alpha]_D^{23}$ +19 (*c* 0.09, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$CH$_2$CH$_2$CH$_3$) 19:** Yield: 45%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 7.98 (br s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.18–7.13 (m, 1H), 7.13–7.07 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.2, 4.4 Hz, 1H), 5.46 (s, 1H), 5.31 (d, *J* = 11.1 Hz, 1H), 4.56 (br s, 1H), 4.27 (br s, 1H), 3.80 (s, *J* = 1.6 Hz, 6H), 3.74 (s, 1H), 3.60 (s, 3H), 3.37 (dd, *J* = 15.9, 4.7 Hz, 2H), 3.31–3.20 (m, 4H), 3.15 (br s, 2H), 3.09 (dd, *J* = 14.5, 7.2 Hz, 4H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.71 (s, 3H), 2.68 (s, 1H), 2.55 (d, *J* = 13.8 Hz, 1H), 2.47–2.43 (m, 1H), 2.38 (d, *J* = 12.1 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 2.07 (s, 1H), 1.86–1.72 (m, 4H), 1.56–1.49 (m, 2H), 1.38–1.33 (m, 4H), 0.93 (t, *J* = 7.3 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H); IR (film) $\nu_{max}$ 3544, 2952, 1736, 1501, 1458, 1240, 1030, 761 $cm^{-1}$; HRESI-TOF *m/z* 909.5106 ($C_{51}H_{68}N_6O_9 + H^+$, required 909.5121); $[\alpha]_D^{23}$ +14 (*c* 0.06, $CHCl_3$).

**(R = 20'-NHCONHC(CH$_3$)$_3$) 20:** Yield: 45%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.99 (br s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.19–7.14 (m, 1H), 7.13–7.07 (m, 2H), 6.65 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.1, 3.9 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 10.2 Hz, 1H), 4.54 (br s, 1H), 4.10 (br s, 1H), 3.83 (t, *J* = 14.1 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.74 (s, 1H), 3.60 (s, 3H), 3.40–3.22 (m, 5H), 3.17–3.12 (m, 2H), 2.71 (s, 3H), 2.68 (s, 1H), 2.54 (d, *J* = 13.6 Hz, 1H), 2.47–2.42 (m, 1H), 2.35 (d, *J* = 13.4 Hz, 1H), 2.21–2.14 (m, 2H), 2.11 (s, 3H), 1.87–1.75 (m, 2H), 1.73–1.62 (m, 4H), 1.49–1.41 (m, 2H), 1.38 (s, 9H), 1.37–1.31 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3457, 2941, 1741, 1556, 1458, 1244, 1036, 741 $cm^{-1}$; HRESI-TOF *m/z* 909.5128 ($C_{51}H_{68}N_6O_9 + H^+$, required 909.5121); $[\alpha]_D^{23}$ +3.0 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCONHC$_6$H$_{11}$) 21:** Yield: 51%, Method 1.$^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 7.99 (br s, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.20–7.14 (m, 1H), 7.14–7.07 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.3, 4.1 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 9.3 Hz, 1H), 4.53 (br s, 1H), 4.22 (br s, 1H), 4.02 (d, *J* = 9.0 Hz, 1H), 3.83 (t, *J* = 12.9 Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.74 (s, 1H), 3.60 (s, 3H), 3.50–3.45 (m, 1H), 3.39–3.21 (m, 3H), 3.16–3.12 (m, 2H), 2.83 (d, *J* = 16.1 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.55 (d, *J* = 13.5 Hz, 1H), 2.47–2.42 (m, 1H), 2.37 (d, *J* = 12.7 Hz, 1H), 2.21–2.14 (m, 2H), 2.11 (s, 3H), 2.04–1.90 (m, 2H), 1.86–1.74 (m, 4H), 1.64–1.59 (m, 2H), 1.37–1.31 (m, 6H), 1.20–1.02 (m, 6H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3460, 2919, 1738, 1556, 1459, 1242, 1033, 752 $cm^{-1}$; HRESI-TOF *m/z* 935.5276 ($C_{53}H_{70}N_6O_9 + H^+$, required 935.5277); $[\alpha]_D^{23}$ +3.8 (*c* 0.09, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$CH$_2$OH) 22:** Yield: 60%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.78 (br s, 1H), 7.99 (br s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.19–7.09 (m, 3H), 6.69 (s, 1H), 6.08 (s, 1H), 5.89–5.85 (m, 1H), 5.48 (s, 1H), 5.31 (d, *J* = 11.8 Hz, 1H), 4.59 (br s, 1H), 3.83–3.72 (m, 4H), 3.80 (s, 3H), 3.79 (s, 3H), 3.76 (s, 1H), 3.57 (s, 3H), 3.57–3.54 (m, 1H), 3.42–3.37 (m, 3H), 3.34–3.29 (m, 1H), 3.26–3.17 (m, 4H), 3.08–3.03 (m, 1H), 2.87 (br s, 1H), 2.82 (d, *J* = 16.0 Hz, 1H), 2.72 (s, 3H), 2.68 (s, 1H), 2.60–2.42 (m, 1H), 2.33–2.17 (m, 1H), 2.14–2.09 (m, 1H), 2.11 (s, 3H), 1.97–1.77 (m, 2H), 1.75–1.64 (m, 3H), 1.50–1.45 (m, 1H), 1.38–1.34 (m, 2H), 1.16 (dd, *J* = 14.3, 4.6 Hz, 2H), 0.83–0.81 (m, 3H), 0.77 (t, *J* = 7.1

Hz, 3H); IR (film) $\nu_{max}$ 3399, 2927, 1738, 1502, 1458, 1232, 1040 $cm^{-1}$; HRESI-TOF *m/z* 897.4753 ($C_{49}H_{64}N_6O_{10} + H^+$, required 897.4756); $[\alpha]_D^{23}$ −14 (*c* 0.2, $CHCl_3$).

**(R = 20'-NHCONHC$_6$H$_5$) 23:** Yield: 87%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ $^1$H NMR (600 MHz, $CDCl_3$) δ 9.87 (br s, 1H), 7.98 (br s, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.16–7.14 (m, 1H), 7.13–7.05 (m, 3H), 6.62 (s, 1H), 6.09 (s, 1H), 5.85 (dd, *J* = 10.3, 4.6 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 9.4 Hz, 1H), 4.80 (s, 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.60 (s, 3H), 3.39–3.35 (m, 2H), 3.32–3.28 (m, 1H), 3.22–3.18 (m, 1H), 3.10 (s, 3H), 2.82 (d, *J* = 16.3 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.59 (d, *J* = 13.8 Hz, 1H), 2.47–2.42 (m, 1H), 2.36–2.28 (m, 2H), 2.25–2.13 (m, 2H), 2.11 (s, 3H), 2.04 (s, 1H), 1.85–1.76 (m, 3H), 1.71–1.65 (m, 2H), 1.64–1.54 (m, 2H), 1.24–1.20 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3468, 2940, 1742, 1501, 1460, 1237, 1031, 760 $cm^{-1}$; HRESI-TOF *m/z* 929.4807 ($C_{53}H_{64}N_6O_9 + H^+$, required 929.4807); $[\alpha]_D^{23}$ +16 (*c* 0.9, $CHCl_3$).

**(R = 20'-NHCONH(4-fluorophenyl)) 24:** Yield: 47%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.81 (br s, 1H), 7.97 (br s, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.42 (dd, *J* = 8.8, 4.8 Hz, 2H), 7.18–7.14 (m, 1H), 7.12–7.08 (m, 2H), 7.01 (t, *J* = 8.6 Hz, 2H), 6.63 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 12.1 Hz, 1H), 4.71 (br s, 1H), 3.80 (s, 6H), 3.75 (s, 1H), 3.61 (s, 3H), 3.40–3.36 (m, 2H), 3.32–3.28 (m, 1H), 3.21–3.06 (m, 4H), 2.82 (d, *J* = 16.5 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.59 (d, *J* = 13.7 Hz, 1H), 2.47–2.42 (m, 1H), 2.34 (d, *J* = 12.7 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 1.85–1.75 (m, 4H), 1.59–1.50 (m, 4H), 1.23–1.21 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3490, 2921, 1745, 1509, 1455, 1228, 1043, 736 $cm^{-1}$; HRESI-TOF *m/z* 947.4726 ($C_{53}H_{63}FN_6O_9 + H^+$, required 947.4713); $[\alpha]_D^{23}$ −4.4 (*c* 0.08, $CHCl_3$).

**(R = 20'-NHCONH(4-chlorophenyl)) 25:** Yield: 63%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.80 (br s, 1H), 7.95 (br s, 1H), 7.40 (d, *J* = 7.9 Hz, 2H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.19–7.07 (m, 5H), 6.65 (s, 1H), 6.08 (s, 1H), 5.86 (dd, *J* = 10.2, 4.5 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 12.1 Hz, 1H), 4.81 (br s, 1H), 3.79 (s, 6H), 3.75 (s, 1H), 3.60 (s, 3H), 3.36 (d, *J* = 15.7 Hz, 2H), 3.31–3.26 (m, 2H), 3.22–3.15 (m, 2H), 3.01–2.91 (m, 1H), 2.82 (d, *J* = 15.7 Hz, 1H), 2.72 (s, 3H), 2.67 (s, 1H), 2.61–2.55 (m, 2H), 2.49–2.43 (m, 2H), 2.21–2.14 (m, 2H), 2.10 (s, 3H), 1.83–1.74 (s, 4H) 1.36–1.27 (m, 4H), 1.16–1.04 (m, 2H), 0.82 (t, *J* = 7.4 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3376, 2923, 1736, 1493, 1455, 1240, 1090, 765 $cm^{-1}$; HRESI-TOF *m/z* 963.4407 ($C_{53}H_{63}ClN_6O_9 + H^+$, required 963.4418); $[\alpha]_D^{23}$ +21 (*c* 0.07, $CHCl_3$).

**(R = 20'-NHCONH(4-methylphenyl)) 26:** Yield: 40%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.86 (br s, 1H), 7.99 (br s, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.18–7.07 (m, 3H), 6.61 (s, 1H), 6.08 (s, 1H), 5.85 (d, *J* = 5.9 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.3 Hz, 1H), 4.74 (br s, 1H), 4.30 (br s, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.74 (s, 1H), 3.67–3.62 (m, 3H), 3.61 (s, 3H), 3.40–3.34 (m, 2H), 3.31–3.27 (m, 1H), 3.20–3.16 (m, 1H), 2.81 (d, *J* = 15.7 Hz, 1H), 2.71 (s, 3H), 2.66 (s, 1H), 2.58 (d, *J* = 13.3 Hz, 1H), 2.46–2.41 (m, 1H), 2.32 (s, 3H), 2.24–2.14 (m, 2H), 2.11 (s, 3H), 2.03 (s, 1H), 1.87 (d, *J* = 13.8 Hz, 2H), 1.84–1.76 (m, 2H), 1.63–1.53 (m, 4H), 1.38–1.34 (m, 2H), 1.21 (dd, *J* = 14.5, 5.7 Hz, 2H), 0.81 (t, *J* = 7.6 Hz, 3H), 0.78 (t, *J* = 7.6 Hz, 3H); IR (film) $\nu_{max}$ 3369, 2912, 1722, 1507, 1445, 1230, 1017, 729 $cm^{-1}$; HRESI-TOF *m/z* 943.4949 ($C_{54}H_{66}N_6O_9 + H^+$, required 943.4964); $[\alpha]_D^{23}$ +36 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCONH(4-trifluoromethylphenyl)) 27:** Yield: 60%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.82 (br s, 1H), 7.94 (br s, 1H), 7.58 (d, *J* = 8.6 Hz, 2H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.18–7.14 (m, 1H), 7.13–7.07 (m, 2H), 6.65 (s, 1H), 6.09 (s,

1H), 5.87 (dd, *J* = 10.0, 4.2 Hz, 1H), 5.46 (s, 1H), 5.31 (d, *J* = 10.3 Hz, 1H), 4.99 (br s, 1H), 3.90–3.84 (m, 2H), 3.80 (s, 6H), 3.75 (s, 1H), 3.67–3.63 (m, 1H), 3.61 (s, 3H), 3.40–3.36 (m, 2H), 3.33–3.29 (m, 1H), 3.27–3.20 (m, 1H), 3.13–3.10 (m, 1H), 2.83 (d, *J* = 15.8 Hz, 1H), 2.72 (s, 3H), 2.68 (d, *J* = 11.9 Hz, 1H), 2.60 (d, *J* = 13.8 Hz, 1H), 2.48–2.43 (m, 1H), 2.40 (d, *J* = 13.3 Hz, 1H), 2.33–2.24 (m, 2H), 2.20–2.15 (m, 1H), 2.10 (s, 3H), 2.01 (s, 1H), 1.90–1.87 (m, 2H), 1.84–1.75 (m, 2H), 1.66 (d, *J* = 14.9 Hz, 2H), 1.40–1.32 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 6H); IR (film) $\nu_{max}$ 3334, 2931, 1716, 1537, 1472, 1232, 1023, 745 $cm^{-1}$; HRESI-TOF *m/z* 997.4685 ($C_{54}H_{63}F_3N_6O_9 + H^+$, required 997.4681); $[\alpha]_D^{23}$ −18 (*c* 0.04, $CHCl_3$).

**(R = 20'-NHCONH(4-methoxyphenyl)) 28:** Yield: 36%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.80 (br s, 1H), 8.01 (br s, 1H), 7.38 (d, *J* = 7.9 Hz, 1H), 7.24–7.20 (m, 1H), 7.18–7.09 (m, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.39 (s, 1H), 6.34 (s, 1H), 6.08 (s, 1H), 5.88 (dd, *J* = 10.0, 4.7 Hz, 1H), 5.41 (s, 1H), 5.30 (d, *J* = 10.1 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.04 (t, *J* = 12.0 Hz, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77 (s, 1H), 3.66 (s, 3H), 3.65–3.58 (m, 2H), 3.52–3.48 (m, 1H), 3.38–3.33 (m, 2H), 3.31–3.27 (m, 1H), 3.02–2.93 (m, 2H), 2.86–2.79 (m, 2H), 2.73 (s, 3H), 2.66 (s, 1H), 2.48–2.41 (m, 1H), 2.18–2.14 (m, 2H), 2.11 (s, 3H), 2.04–1.97 (m, 1H), 1.83–1.74 (m, 2H), 1.35–1.28 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H), 0.79 (t, *J* = 7.3 Hz, 3H); IR (film) $\nu_{max}$ 3447, 2899, 1742, 1507, 1459, 1234, 1036, 736 $cm^{-1}$; HRESI-TOF *m/z* 959.4917 ($C_{54}H_{66}N_6O_{10} + H^+$, required 959.4913); $[\alpha]_D^{23}$ −9.4 (*c* 0.04, $CHCl_3$).

**(R = 20'-NHCONH(3-methoxyphenyl)) 29:** Yield: 50%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.82 (br s, 1H), 7.96 (br s, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.19 (t, *J* = 8.1 Hz, 1H), 7.16–7.13 (m, 1H), 7.12–7.06 (m, 3H), 6.99 (d, *J* = 8.6 Hz, 1H), 6.62 (s, 1H), 6.61 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 1H), 5.85 (dd, *J* = 10.2, 4.6 Hz, 1H), 5.46 (s, 1H), 5.30 (d, *J* = 9.9 Hz, 1H), 4.82 (s, 1H), 3.79 (s, 6H), 3.78 (s, 3H), 3.74 (s, 1H), 3.66–3.62 (m, 3H), 3.59 (s, 3H), 3.38–3.35 (m, 2H), 3.31–3.27 (m, 1H), 3.22–3.18 (m, 1H), 3.15–3.10 (m, 1H), 3.03 (d, *J* = 13.3 Hz, 2H), 2.82 (d, *J* = 16.1 Hz, 1H), 2.71 (s, 3H), 2.66 (s, 1H), 2.58 (d, *J* = 13.8 Hz, 1H), 2.47–2.40 (m, 1H), 2.34 (d, *J* = 13.0 Hz, 1H), 2.22–2.14 (m, 2H), 2.10 (s, 3H), 1.84–1.76 (m, 3H), 1.75–1.69 (m, 2H), 1.38–1.31 (m, 2H), 1.23–1.21 (m, 1H), 0.81 (ovlp t, *J* = 7.4 Hz, 3H), 0.80 (ovlp t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3483, 2985, 1745, 1501, 1454, 1228, 1033, 760 $cm^{-1}$; HRESI-TOF *m/z* 959.4905 ($C_{54}H_{66}N_6O_{10} + H^+$, required 959.4913); $[\alpha]_D^{23}$ −19 (*c* 0.05, $CHCl_3$).

**(R = 20'-NHCONH(2-methoxyphenyl)) 30:** Yield: 35%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 8.07 (d, *J* = 9.1 Hz, 1H), 8.01 (br s, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.17–7.12 (m, 1H), 7.13–7.07 (m, 2H), 7.05–6.95 (m, 2H), 6.88–6.86 (m, 1H), 6.64 (s, 1H), 6.10 (s, 1H), 5.85 (dd, *J* = 10.0, 4.7 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.1 Hz, 1H), 5.13–5.10 (m, 1H), 4.76 (br s, 1H), 4.57 (br s, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.74 (s, 1H), 3.56 (s, 3H), 3.40–3.36 (m, 2H), 3.34–3.20 (m, 2H), 3.14–3.07 (m, 2H), 2.71 (s, 3H), 2.67 (s, 1H), 2.60 (d, *J* = 13.7 Hz, 1H), 2.50–2.42 (m, 1H), 2.39 (d, *J* = 12.9 Hz, 1H), 2.27 (d, *J* = 13.7 Hz, 1H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 2.09–1.99 (m, 2H), 1.84–1.76 (m, 4H), 1.50–1.39 (m, 2H), 1.38–1.28 (m, 2H), 0.81 (ovlp t, *J* = 7.4 Hz, 3H), 0.80 (ovlp t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3451, 2919, 1730, 1531, 1461, 1257, 952, 721 $cm^{-1}$; HRESI-TOF *m/z* 959.4909 ($C_{54}H_{66}N_6O_{10} + H^+$, required 959.4913); $[\alpha]_D^{23}$ +24 (*c* 0.03, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$C$_6$H$_5$) 31:** Yield: 96%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 7.98 (br s, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.17–7.08 (m, 4H), 6.62 (s, 1H), 6.09 (s, 1H), 5.85 (dd, *J* = 10.3, 4.6 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 9.4 Hz, 1H), 4.68 (s, 1H), 4.48–4.31 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.74 (s, 1H), 3.60 (s, 3H), 3.38–3.33 (m, 2H), 3.31–3.26 (m, 1H), 3.22–3.18 (m, 1H),

3.10 (s, 3H), 2.83 (d, *J* = 16.3 Hz, 1H), 2.70 (s, 3H), 2.67 (s, 1H), 2.59 (d, *J* = 13.8 Hz, 1H), 2.47–2.42 (m, 1H), 2.36–2.28 (m, 2H), 2.25–2.13 (m, 2H), 2.11 (s, 3H), 2.04 (s, 1H), 1.83–1.71 (m, 3H), 1.71–1.65 (m, 2H), 1.64–1.54 (m, 2H), 1.24–1.20 (m, 2H), 0.83 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3454, 2992, 1737, 1501, 1459, 1231, 1033, 729 $cm^{-1}$; HRESI-TOF *m/z* 943.4950 ($C_{54}H_{66}N_6O_9 + H^+$, required 943.4964); $[\alpha]_D^{23}$ +29 (*c* 0.3, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$CH$_2$C$_6$H$_5$) 32:** Yield: 98%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.97 (br s, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.17–7.08 (m, 6H), 6.39 (s, 1H), 6.07 (s, 1H), 5.89 (dd, *J* = 10.3, 4.6 Hz, 1H), 5.39 (s, 1H), 5.32 (d, *J* = 9.4 Hz, 1H), 4.52 (s, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.74 (s, 1H), 3.65 (s, 3H), 3.64–3.63 (m, 2H), 3.49–3.43 (m, 1H), 3.37–3.32 (m, 1H), 3.31–3.19 (m, 1H), 3.10 (s, 3H), 2.99 (d, *J* = 15 Hz, 1H), 2.95–2.89 (m, 2H), 2.72 (s, 3H), 2.67 (s, 1H), 2.58–2.50 (m, 1H), 2.45–2.37 (m, 1H), 2.40–2.30 (m, 2H), 2.25–2.13 (m, 2H), 2.11 (s, 3H), 2.00–1.94 (m, 1H), 1.84–1.70 (m, 4H), 1.71–1.65 (m, 2H), 1.64–1.54 (m, 2H), 1.34–1.28 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3448, 2927, 1742, 1555, 1461, 1236, 1038, 749 $cm^{-1}$; HRESI-TOF *m/z* 957.5106 ($C_{55}H_{68}N_6O_9 + H^+$, required 957.5121); $[\alpha]_D^{23}$ −17 (*c* 0.06, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$(2-pyridyl)) 33:** Yield: 79%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 8.52 (s, 1H), 8.04 (br s, 1H), 7.66 (t, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.18–7.08 (m, 3H), 6.67 (s, 1H), 6.09 (s, 1H), 5.87–5.83 (m, 1H), 5.54 (br s, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.4 Hz, 1H), 4.62–4.52 (m, 2H), 3.87–3.83 (m, 1H), 3.80 (s, 6H), 3.74 (s, 1H), 3.57 (s, 3H), 3.39–3.28 (m, 3H), 3.23–3.17 (m, 1H), 3.10–3.02 (m, 1H), 2.83 (d, *J* = 15.0 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.57 (d, *J* = 13.8 Hz, 1H), 2.48 (q, *J* = 9.5 Hz, 1H), 2.35 (d, *J* = 14.6 Hz, 1H), 2.28 (d, *J* = 9.8 Hz, 1H), 2.21–2.16 (m, 1H), 2.11 (s, 3H), 1.99 (s, 1H), 1.45–1.32 (m, 1H), 1.86–1.70 (m, 8H), 1.27–1.22 (m, 2H), 0.80 (t, *J* = 6.8 Hz, 3H), 0.73 (t, *J* = 7.1 Hz, 3H); IR (film) $\nu_{max}$ 3375, 2925, 1737, 1503, 1459, 1230, 1039 $cm^{-1}$; HRESI-TOF *m/z* 944.4913 ($C_{53}H_{65}N_7O_9 + H^+$, required 944.4916); $[\alpha]_D^{23}$ +2.7 (*c* 0.2, $CHCl_3$).

**(R = 20'-NHCONHCH$_2$(2-furyl)) 34:** Yield: 96%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.98 (br s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.35 (s, 1H), 7.17–7.08 (m, 3H), 6.66 (s, 1H), 6.31 (d, *J* = 9.0 Hz, 2H), 6.21 (br s, 1H) 6.09 (s, 1H), 5.86 (dd, *J* = 9.8, 4.7 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.0 Hz, 1H), 4.40–4.37 (m, 2H), 3.80 (s, 6H), 3.74 (s, 1H), 3.59 (s, 3H), 3.40–3.28 (m, 2H), 3.26–3.20 (m, 1H), 3.22–3.18 (m, 1H), 3.10–3.00 (m, 3H), 2.83 (d, *J* = 17.3 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.55 (d, *J* = 14.2 Hz, 1H), 2.47–2.42 (m, 1H), 2.35 (d, *J* = 12.8 Hz, 1H), 2.25–2.15 (m, 1H), 2.11 (s, 3H), 2.02 (s, 1H), 1.85–1.76 (m, 3H), 1.71–1.65 (m, 4H), 1.37–1.20 (m, 4H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.72 (t, *J* = 7.5 Hz, 3H); IR (film) $\nu_{max}$ 3388, 2925, 1739, 1504, 1459, 1230, 1039 $cm^{-1}$; HRESI-TOF *m/z* 933.4736 ($C_{52}H_{64}N_6O_{10} + H^+$, required 933.4756); $[\alpha]_D^{23}$ +11 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCONH(4-biphenyl)) 35:** Yield: 44%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.82 (br s, 1H), 7.98 (br s, 1H), 7.58–7.51 (m, 6H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.17–7.13 (m, 1H), 7.13–7.06 (m, 2H), 6.64 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.4, 4.8 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 10.2 Hz, 1H), 4.82 (br s, 1H), 3.82 (t, *J* = 13.8 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.75 (s, 1H), 3.61 (s, 3H), 3.39–3.36 (m, 2H), 3.32–3.28 (m, 1H), 3.19 (t, *J* = 11.4 Hz, 1H), 3.14–3.06 (m, 1H), 3.02 (d, *J* = 12.5 Hz, 2H), 2.82 (d, *J* = 15.7 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.60 (d, *J* = 13.8 Hz, 2H), 2.47–2.42 (m, 1H), 2.35 (d, *J* = 13.0 Hz, 1H), 2.23 (d, *J* = 14.2 Hz, 1H), 2.21–2.14 (m, 1H), 2.11 (s, 3H), 1.82–1.77 (m, 4H), 1.73–1.65 (m, 4H), 1.37–1.34 (m, 1H), 0.82 (t, *J* = 7.4 Hz,

6H); IR (film) $\nu_{max}$ 3444, 2967, 1735, 1523, 1459, 1240, 1039, 744 $cm^{-1}$; HRESI-TOF *m/z* 1005.5122 ($C_{59}H_{68}N_6O_9 + H^+$, required 1005.5121); $[\alpha]_D^{23}$ −8.8 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCSNHC$_6$H$_{11}$) 36:** Yield: 26%, Method 4. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 8.03 (br s, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.16–7.14 (m, *J* = 7.0 Hz, 1H), 7.12–7.06 (m, 2H), 6.62 (s, 1H), 6.10 (s, 1H), 5.84 (dd, *J* = 10.1, 4.1 Hz, 1H), 5.47 (s, 2H), 5.35–5.33 (m, 1H), 5.29 (d, *J* = 9.1 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.73 (s, 1H), 3.67–3.63 (m, 2H), 3.60 (s, 3H), 3.44 (s, 1H), 3.39–3.33 (m, 2H), 3.32–3.26 (m, 2H), 3.24–3.14 (s, 2H), 3.09 (q, *J* = 7.7 Hz, 1H), 2.81 (d, *J* = 14.7 Hz, 1H), 2.70 (s, 3H), 2.65 (s, 1H), 2.62–2.60 (m, 1H), 2.47–2.42 (m, 1H), 2.38–2.26 (m, 3H), 2.24–2.14 (m, 3H), 2.10 (s, 3H), 2.08–1.97 (m, 5H), 1.83–1.66 (m, 4H), 1.44–1.36 (m, 4H), 1.20–1.06 (m, 2H), 0.87 (t, *J* = 6.9 Hz, 3H), 0.81 (t, *J* = 6.9 Hz, 3H); IR (film) $\nu_{max}$ 3467, 2912, 1727, 1506, 1456, 1230, 1087, 774 $cm^{-1}$; HRESI-TOF *m/z* 951.5051 ($C_{53}H_{70}N_6O_8S + H^+$, required 951.5048); $[\alpha]_D^{23}$ +9.2 (*c* 0.2, $CHCl_3$).

**(R = 20'-NHCSNHC$_6$H$_5$) 37:** Yield: 70%, Method 3. $^1$H NMR (500 MHz, $CDCl_3$) δ 9.82 (br s, 1H), 8.09 (br s, 1H), 7.54 (m, 4H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.24–7.19 (m, 1H), 7.19–7.14 (m, 2H), 6.60 (s, 1H), 6.25 (s, 1H), 6.18 (s, 1H), 5.92 (dd, *J* = 10.0, 4.3 Hz, 1H), 5.56 (s, 1H), 5.37 (d, *J* = 10.0 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.80 (s, 1H), 3.70 (s, 3H), 3.49–3.32 (m, 4H), 3.17–3.07 (m, 2H), 2.94–2.80 (m, 3H), 2.78 (s, 3H), 2.65–3.62 (s, 1H), 2.52–2.43 (m, 1H), 2.33–2.22 (m, 4H), 2.19 (s, 3H), 1.94–1.80 (m, 2H), 1.44–1.37 (m, 3H), 1.14 (d, *J* = 9.2 Hz, 1H), 0.88 (t, *J* = 6.3 Hz, 6H); IR (film) $\nu_{max}$ 3449, 2930, 1737, 1498, 1458, 1231, 1039, 751 $cm^{-1}$; HRESI-TOF *m/z* 945.4573 ($C_{53}H_{64}N_6O_8S + H^+$, required 945.4579); $[\alpha]_D^{23}$ +8.1 (*c* 0.2, $CHCl_3$).

**(R = 20'-NHCSNHCH$_2$CH$_2$(4-fluorophenyl)) 38:** Yield: 91%, Method 4. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 8.01 (br s, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.26–7.24 (m, 2H), 7.17–7.08 (m, 3H), 6.97–6.94 (m, 2H), 6.64 (s, 1H), 6.12 (s, 1H), 5.85 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.75 (br s, 1H), 5.48 (s, 1H), 5.30 (d, *J* = 10.2 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77–3.74 (m, 3H), 3.61 (s, 3H), 3.60 (s, 1H), 3.42–3.35 (m, 2H), 3.32–3.28 (m, 1H), 3.25–3.10 (m, 3H), 3.02–2.97 (m, 1H), 2.96–2.91 (m, 1H), 2.83–2.80 (m, 1H), 2.72 (s, 3H), 2.66 (s, 1H), 2.55 (d, *J* = 13.7 Hz, 1H), 2.47–2.42 (m, 1H), 2.32 (d, *J* = 13.8 Hz, 1H), 2.22–2.17 (m, 2H), 2.11 (s, 3H), 1.84–1.65 (m, 4H), 1.54–1.44 (m, 2H), 1.37–1.22 (m, 5H), 0.82 (t, *J* = 7.2 Hz, 3H), 0.74 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3468, 2961, 1737, 1507, 1461, 1226, 1038 $cm^{-1}$; HRESI-TOF *m/z* 991.4790 ($C_{55}H_{67}N_6O_8S + H^+$, required 991.4798); $[\alpha]_D^{23}$ +6.6 (*c* 0.2, $CHCl_3$).

**(R = 20'-NHCON(CH$_3$)$_2$) 39:** Yield: 99%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 7.98 (br s, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.17–7.13 (m, 1H), 7.12–7.07 (m, 2H), 6.62 (s, 1H), 6.09 (s, 1H), 5.86 (dd, *J* = 10.0, 5.1 Hz, 1H), 5.46 (s, 1H), 5.30 (d, *J* = 10.3 Hz, 1H), 4.68 (s, 1H), 3.90 (t, *J* = 14.7 Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.73 (s, 1H), 3.58 (s, 3H), 3.38–3.35 (m, 2H), 3.31–3.27 (m, 2H), 3.23–3.19 (m, 2H), 3.17–3.12 (m, 2H), 3.05 (s, 6H), 2.82 (d, *J* = 16.0 Hz, 1H), 2.70 (s, 3H), 2.67 (s, 1H), 2.58 (d, *J* = 13.9 Hz, 1H), 2.47–2.42 (m, 1H), 2.38 (d, *J* = 13.1 Hz, 1H), 2.23–2.14 (m, 2H), 2.10 (s, 1H), 1.87–1.74 (m, 4H), 1.69 (d, *J* = 13.9 Hz, 2H), 1.40–1.33 (m, 4H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3472, 2955, 1723, 1500, 1459, 1258, 1038, 777 $cm^{-1}$; HRESI-TOF *m/z* 881.4790 ($C_{49}H_{64}N_6O_9 + H^+$, required 881.4808); $[\alpha]_D^{23}$ −50 (*c* 0.04, $CHCl_3$).

**(R = 20'-NHCON(CH$_2$CH$_3$)$_2$) 40:** Yield: 88%, Method 2. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.87 (br s, 1H), 8.02 (br s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.18–7.13 (m, 1H), 7.13–7.07 (m, 2H), 6.62 (s, 1H), 6.10 (s, 1H), 5.85 (dd, *J* = 10.2, 4.1 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.2 Hz, 1H), 4.35 (br s, 1H), 3.85 (t, *J* = 12.7 Hz, 2H), 3.79 (s, 6H), 3.74 (s, 1H), 3.59 (s, 3H),

3.51–3.45 (m, 1H), 3.40–3.20 (m, 3H), 3.17–3.12 (m, 1H), 2.83 (d, *J* = 16.2 Hz, 1H), 2.71 (s, 7H), 2.58 (d, *J* = 13.7 Hz, 1H), 2.47–2.42 (m, 1H), 2.39 (d, *J* = 12.7 Hz, 1H), 2.23 (d, *J* = 14.4 Hz, 1H), 2.20–2.14 (m, 1H), 2.11 (s, 3H), 2.08 (s, 1H), 1.88–1.67 (m, 4H), 1.37–1.32 (m, 1H), 1.29–1.25 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 6H), 0.81 (t, *J* = 7.3 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3486, 2940, 1741, 1520, 1458, 1233, 1042, 709 $cm^{-1}$; HRESI-TOF *m/z* 909.5130 ($C_{51}H_{68}N_6O_9$ + $H^+$, required 909.5121); $[\alpha]_D^{23}$ −38 (*c* 0.08, $CHCl_3$).

**(R = 20'-NHCO-(morpholine)) 41:** Yield: 99%, Method 2. $^1H$ NMR (600 MHz, $CDCl_3$) δ 9.84 (br s, 1H), 8.06 (s, 1H), 7.99 (br s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.19–7.14 (m, 1H), 7.13–7.07 (m, 2H), 6.62 (s, 1H), 6.10 (s, 1H), 5.85 (dd, *J* = 10.1, 4.1 Hz, 1H), 5.47 (s, 1H), 5.30 (d, *J* = 10.1 Hz, 1H), 4.48 (s, 1H), 3.85 (d, *J* = 10.9 Hz, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.79–3.77 (m, 1H), 3.77–3.64 (m, 11H), 3.62–3.56 (m, 4H), 3.48–3.44 (m, 1H), 3.38–3.36 (m, 1H), 3.33–3.05 (m, 2H), 2.83 (d, *J* = 16.1 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.60 (d, *J* = 13.6 Hz, 1H), 2.47–2.42 (m, 1H), 2.40 (d, *J* = 12.5 Hz, 1H), 2.29–2.22 (m, 2H), 2.20–2.15 (m, 2H), 2.11 (s, 3H), 1.90–1.74 (m, 2H), 1.70–1.67 (m, 2H), 1.37–1.32 (m, 2H), 0.81 (t, *J* = 7.3 Hz, 3H), 0.76 (t, *J* = 7.5 Hz, 3H); IR (film) $\nu_{max}$ 3448, 2919, 1740, 1538, 1449, 1247, 1032, 711 $cm^{-1}$; HRESI-TOF *m/z* 923.4897 ($C_{51}H_{66}N_6O_{10}$ + $H^+$, required 923.4913); $[\alpha]_D^{23}$ −8.0 (*c* 0.1, $CHCl_3$).

**(R = 20'-NHCO-(piperidine)) 42:** Yield: 34%, Method 2. $^1H$ NMR (600 MHz, $CDCl_3$) δ 9.85 (br s, 1H), 8.02 (br s, 1H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.17–7.14 (m, 1H), 7.11–7.09 (m, 2H), 6.59 (s, 1H), 6.09 (s, 1H), 5.84 (dd, *J* = 10.1, 4.1 Hz, 1H), 5.45 (s, 1H), 5.29 (d, *J* = 10.4 Hz, 1H), 4.45 (s, 1H), 3.82 (t, *J* = 14.7 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.73 (s, 1H), 3.59 (s, 3H), 3.52–3.48 (m, 2H), 3.43–3.40 (m, 2H), 3.38–3.34 (m, 2H), 3.31–3.26 (m, 4H), 3.18–3.12 (m, 2H), 2.82 (d, *J* = 16.6 Hz, 1H), 2.70 (s, 3H), 2.66 (s, 1H), 2.47–2.38 (m, 4H), 2.28–2.20 (m, 2H), 2.18–2.14 (m, 2H), 2.10 (s, 3H), 2.07 (s, 1H), 1.90–1.76 (m, 4H), 1.69 (s, 1H), 1.35–1.27 (m, 5H), 0.80 (t, *J* = 7.3 Hz, 3H), 0.75 (t, *J* = 7.3 Hz, 3H); IR (film) $\nu_{max}$ 3458, 2958, 1791, 1509, 1466, 1251, 1061, 737 $cm^{-1}$; HRESI-TOF *m/z* 921.5103 ($C_{52}H_{68}N_6O_9$ + $H^+$, required 921.5121); $[\alpha]_D^{23}$ −9.2 (*c* 0.02, $CHCl_3$).

**(R = 20'-NHCSN($CH_3$)$_2$) 43:** Yield: 71%, Method 4. $^1H$ NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.98 (br s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.18–7.13 (m, 1H), 7.12–7.08 (m, 2H), 6.64 (d, *J* = 3.9 Hz, 1H), 6.10 (s, 1H), 5.86 (dd, *J* = 10.0, 5.1 Hz, 1H), 5.54 (s, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 10.0 Hz, 1H), 4.70–4.67 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.75 (s, 1H), 3.59 (s, 3H), 3.44 (s, 6H), 3.38 (d, *J* = 12.2 Hz, 2H), 3.31 (d, *J* = 4.7 Hz, 1H), 3.27–3.15 (m, 3H), 3.14–3.03 (m, 2H), 2.83 (d, *J* = 15.2 Hz, 1H), 2.72 (s, 3H), 2.70 (s, 1H), 2.67 (s, 1H), 2.54 (d, *J* = 13.7 Hz, 1H), 2.48–2.43 (m, 1H), 2.38 (d, *J* = 14.1 Hz, 1H), 2.23–2.16 (m, 2H), 2.11 (s, 3H), 1.96 (d, *J* = 15.2 Hz, 1H), 1.85–1.74 (m, 2H), 1.38–1.29 (m, 4H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3458, 2963, 1728, 1536, 1474, 1235, 1061, 737 $cm^{-1}$; HRESI-TOF *m/z* 897.4559 ($C_{49}H_{64}N_6O_8S$ + $H^+$, required 897.4579); $[\alpha]_D^{23}$ −56 (*c* 0.07, $CHCl_3$).

**(R = 20'-OCON($CH_3$)$_2$) 44:** Yield: 88%. $^1H$ NMR (600 MHz, $CDCl_3$) δ 9.86 (br s, 1H), 8.02 (br s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.17–7.12 (m, 1H), 7.12–7.07 (m, 2H), 6.61 (s, 1H), 6.09 (s, 1H), 5.84 (dd, *J* = 10.2, 4.1 Hz, 1H), 5.46 (s, 1H), 5.29 (d, *J* = 10.0 Hz, 1H), 4.34 (s, 1H), 3.84 (t, *J* = 12.7 Hz, 1H), 3.79 (s, 6H), 3.74 (s, 1H), 3.58 (s, 3H), 3.47 (dd, *J* = 14.6, 7.2 Hz, 2H), 3.40–3.19 (m, 4H), 3.16–3.12 (m, 2H), 2.82 (d, *J* = 16.2 Hz, 1H), 2.70 (s, 9H), 2.66 (s, 1H), 2.57 (d, *J* = 13.7 Hz, 1H), 2.46–2.41 (m, 1H), 2.38 (d, *J* = 12.7 Hz, 1H), 2.22 (d, *J* = 14.4 Hz, 1H), 2.20–2.13 (m, 1H), 2.10 (s, 3H), 2.07 (s, 1H), 1.86–1.76 (m, 2H), 1.72 (d, *J* = 14.5 Hz, 2H), 1.38–1.30 (m, 2H), 0.80 (t, *J* = 7.3 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3472, 2969, 1731, 1539, 1459, 1224, 1048, 740 $cm^{-1}$; HRESI-TOF *m/z* 882.4642 ($C_{49}H_{63}N_5O_{10}$ + $H^+$, required 882.4648); $[\alpha]_D^{23}$ −64 (*c* 0.03, $CHCl_3$).

**(R = 20'-NHCH$_3$) 45:** A solution 20'-aminovinblastine (8.8 mg, 0.011 mmol) in THF (3 mL) was treated with a 37% formaldehyde in water solution (4 μL, 0.05 mmol). The reaction mixture was stirred for 4 h at 25 °C and then was treated with sodium cyanoborohydride (12 mg, 0.20 mmol). The reaction mixture was stirred for 1 h at 25 °C and then was quenched with distilled $H_2O$ (3 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$, and the combined organic extracts were washed with saturated aqueous NaCl (3 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided **45** (2.8 mg, 32%, off white solid) and **46** (2.9 mg, 33%, off white solid). For **45**: $^1$H NMR (600 MHz, $CDCl_3$) δ 9.87 (br s, 1H), 8.01 (br s, 1H), 7.51 (d, *J* = 7.5 Hz, 1H), 7.18–7.14 (m, 1H), 7.11–7.08 (m, 2H), 6.58 (br s, 1H), 6.09 (s, 1H), 5.85 (dd, *J* = 9.9, 4.0 Hz, 1H), 5.46 (s, 1H), 5.30 (d, *J* = 9.8 Hz, 1H), 4.07–3.91 (m, 2H), 3.80 (s, 3H), 3.79 (s, 3H), 3.73 (s, 1H), 3.61 (s, 3H), 3.36 (dd, *J* = 16.3, 4.6 Hz, 1H), 3.31–3.26 (m, 1H), 3.12–3.04 (m, 2H), 2.83 (d, *J* = 16.1 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 3H), 2.46–2.41 (m, 1H), 2.37–2.31 (m, 1H), 2.31–2.20 (m, 1H), 2.17–2.13 (m, 1H), 2.11 (s, 3H), 1.87–1.73 (m, 2H), 1.18–1.08 (m, 2H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 3487, 2953, 1729, 1505, 1461, 1239, 1037, 735 cm$^{-1}$; HRESI-TOF *m/z* 824.4576 ($C_{47}H_{61}N_5O_8$ + $H^+$, required 824.4593); $[\alpha]_D^{23}$ −114 (*c* 0.03, $CHCl_3$).

**(R = 20'-N(CH$_3$)$_2$) 46:** $^1$H NMR (600 MHz, $CDCl_3$) δ 9.89 (br s, 1H), 7.98 (br s, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.17–7.11 (m, 3H), 6.57 (br s, 1H), 6.10 (s, 1H), 5.85 (dd, *J* = 9.5, 4.3 Hz, 1H), 5.46 (s, 1H), 5.30 (d, *J* = 10.1 Hz, 1H), 3.97–3.92 (m, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.73 (s, 1H), 3.61 (s, 3H), 3.38–3.27 (m, 4H), 3.20 (d, *J* = 13.8 Hz, 1H), 3.17–3.12 (m, 1H), 2.84 (d, *J* = 16.2 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 3H), 2.46–2.41 (m, 1H), 2.38 (s, 6H), 2.31–2.29 (m, 1H), 2.18–2.14 (m, 1H), 2.11 (s, 3H), 1.87–1.73 (m, 6H), 1.49–1.42 (m, 2H), 1.36–1.31 (m, 2H), 0.83–0.78 (m, 6H); IR (film) $\nu_{max}$ 3404, 2951, 1734, 1501, 1459, 1227, 1037, 731 cm$^{-1}$; HRESI-TOF *m/z* 838.4752 ($C_{48}H_{63}N_5O_8$ + $H^+$, required 838.4749); $[\alpha]_D^{23}$ +16 (*c* 0.01, $CHCl_3$).

**(R = 20'-NMeCONHCH$_2$CH$_3$) 47:** Yield: 95%, Method 1. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.83 (br s, 1H), 7.95 (br s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.16–7.09 (m, 3H), 6.66 (s, 1H), 6.08 (s, 1H), 5.86 (dd, *J* = 9.9, 3.9 Hz, 1H), 5.48 (s, 1H), 5.31 (d, *J* = 10.6 Hz, 1H), 4.54 (br s, 1H), 4.29 (br s, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.74 (s, 1H), 3.70–3.65 (m, 1H), 3.57 (s, 3H), 3.43–3.37 (m, 2H), 3.33–3.18 (m, 5H), ), 3.05–3.02 (m, 2H), 2.98 (br s, 3H), 2.82 (d, *J* = 16.1 Hz, 1H), 2.71 (s, 3H), 2.67 (s, 1H), 2.56 (d, *J* = 14.2 Hz, 1H), 2.47–2.43 (m, 1H), 2.36 (d, *J* = 11.6 Hz, 1H), 2.24–2.16 (m, 2H), 2.11 (s, 3H), 1.87–1.75 (m, 3H), 1.38–1.32 (m, 5H), 1.19 (t, *J* = 7.2 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H), 0.79 (t, *J* = 7.3 Hz, 3H); IR (film) $\nu_{max}$ 3462, 3400, 2926, 1735, 1504, 1460, 1243, 1039 cm$^{-1}$; HRESI-TOF *m/z* 895.4956 ($C_{50}H_{66}N_6O_9$ + $H^+$, required 895.4964); $[\alpha]_D^{23}$ +17 (*c* 0.1, $CHCl_3$).

**Compound 49:** Iron(III) chloride hexahydrate (55 mg, 0.21 mmol) was added to a solution of (−)-vindoline (19 mg, 0.041 mmol) and 10'-fluorocatharanthine[9a] (15 mg, 0.041 mmol) in $CF_3CH_2OH$ (0.1 mL), aqueous 0.1 N HCl (1.0 mL) and $H_2O$ (1.0 mL) at 23 °C under Ar. The reaction mixture was stirred for 2 h at 23 °C. Meanwhile, in a separate flask, a mixture of iron(III) oxalate hexahydrate (198 mg, 0.41 mmol) in degassed $H_2O$ (40 mL) was cooled to 0 °C and placed under Ar. $CsN_3$ (215 mg, 1.23 mmol) was added to the mixture at 0 °C, followed by the vindoline coupling solution and $NaBH_4$ (31 mg, 0.81 mmol) in $H_2O$ (1 mL). The resulting mixture was stirred for 30 min before being quenched by addition of 28–30% aqueous $NH_4OH$ (4 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$, the organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH:$Et_3N$ = 97:3:3) provided 10'-fluoro-20'-azidovinblastine (**50**, 2.4 mg, 7%, white solid), and 10'-fluoro-20'-azidoleurosidine (6.0 mg, 17%, white solid). A solution of compound **50** (2.4 mg, 0.0028 mmol) in THF/$H_2O$ (1/1 mL) was treated with $CoCl_2$•

$6H_2O$ (35 mg, 0.15 mmol) followed by $NaBH_4$ (17 mg, 0.45 mmol). The reaction mixture was stirred for 2 h before being quenched with the addition of saturated $NaHCO_3$ (1 mL). The mixture was extracted with 10% MeOH in $CH_2Cl_2$, and washed with saturated aqueous NaCl (1 mL). The organic layer was dried over $Na_2SO_4$, and concentrated under reduced pressure. PTLC ($SiO_2$, EtOAc:MeOH = 90:10) provided 10'-fluoro-20'-aminovinblastine (**51**, 1.6 mg, 70%, white solid). A solution **51** (1.6 mg, 0.0019 mmol) in THF (3 mL) was treated with ethyl isocyanate (2 μL 0.025 mmol). The reaction mixture was stirred for 4 h at 25 °C and then was concentrated under reduced pressure. PTLC ($SiO_2$, $CH_2Cl_2$:MeOH = 92:8) provided **49** (1.0 mg, 59%, white solid). **50:** $^1$H NMR (600 MHz, $CDCl_3$) δ 9.76 (br s, 1H), 8.00 (br s, 1H), 7.39 (dd, *J* = 8.8, 5.1 Hz, 1H), 6.86–6.82 (m, 1H), 6.76 (dd, *J* = 9.6, 2.4 Hz, 1H), 6.56 (s, 1H), 6.10 (s, 1H), 5.86 (dd, *J* = 10.3, 4.6 Hz, 1H), 5.48 (s, 1H), 5.29 (d, *J* = 10.2 Hz, 1H), 3.93 (t, *J* = 14.1 Hz, 1H), 3.82–3.76 (m, 1H), 3.80 (s, 6H), 3.72 (s, 1H), 3.63 (s, 3H), 3.40–3.33 (m, 2H), 3.30–3.25 (m, 2H), 3.13–3.07 (m, 1H), 2.95 (d, *J* = 14.4 Hz, 1H), 2.83–2.73 (m, 3H), 2.69 (s, 3H), 2.66 (s, 1H), 2.62 (s, 1H), 2.43–2.40 (m, 2H), 2.25 (d, *J* = 13.9 Hz, 1H), 2.18–2.14 (m, 1H), 2.10 (s, 3H), 2.07 (s, 1H), 1.85–1.77 (m, 2H), 1.63–1.52 (m, 2H), 1.47–1.41 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H), 0.78 (t, *J* = 7.4 Hz, 3H); IR (film) $\nu_{max}$ 2925, 2109, 1734, 1614, 1460, 1228, 1038 $cm^{-1}$; HRESI-TOF *m/z* 854.4218 ($C_{46}H_{56}FN_7O_8$ + $H^+$, required 854.4247); $[\alpha]_D^{23}$ −6.0 (*c* 0.08, $CHCl_3$). **51**: $^1$H NMR (600 MHz, $CDCl_3$) δ 9.81 (br s, 1H), 8.00 (br s, 1H), 7.40–7.37 (m, 1H), 6.89–6.85 (m, 1H), 6.78 (d, *J* = 9.2 Hz, 1H), 6.51 (s, 1H), 6.10 (s, 1H), 5.89 (dd, *J* = 10.1, 4.0 Hz, 1H), 5.45 (s, 1H), 5.31 (d, *J* = 10.1 Hz, 1H), 3.92 (t, *J* = 14 Hz, 1H), 3.82–3.77 (m, 1H), 3.81 (s, 6H), 3.74 (s, 1H), 3.63 (s, 3H), 3.38 (d, *J* = 16.0, 4.7 Hz, 2H), 3.32–3.27 (m, 1H), 3.10–3.05 (m, 2H), 2.84 (d, *J* = 15.7 Hz, 1H), 2.75–2.68 (m, 3H), 2.71 (s, 3H), 2.64 (s, 1H), 2.50–2.45 (m, 1H), 2.17 (d, *J* = 3.4 Hz, 1H), 2.11 (s, 3H), 2.08 (s, 1H), 2.05 (s, 1H), 1.87–1.75 (s, 4H), 1.42–1.15 (m, 6H), 0.88 (t, *J* = 7.4 Hz, 3H), 0.78 (t, *J* = 7.3 Hz, 3H); HRESI-TOF *m/z* 828.4311 ($C_{46}H_{58}FN_5O_8$ + $H^+$, required 828.4311); $[\alpha]_D^{23}$ −23 (*c* 0.10, $CHCl_3$). **49:** $^1$H NMR (600 MHz, $CDCl_3$) δ 9.77 (br s, 1H), 7.95 (br s, 1H), 7.39 (dd, *J* = 8.8, 5.2 Hz, 1H), 6.86 (t, *J* = 9.4 Hz, 1H), 6.77 (d, *J* = 9.3 Hz, 1H), 6.58 (s, 1H), 6.09 (s, 1H), 5.88 (dd, *J* = 9.8, 4.6 Hz, 1H), 5.47 (s, 1H), 5.31 (d, *J* = 11.4 Hz, 1H), 4.41 (br s, 1H), 4.21 (br s, 1H), 3.84–3.77 (m, 2H), 3.80 (s, 6H), 3.75 (s, 1H), 3.61 (s, 3H), 3.40 (dd, *J* = 17.0, 5.2 Hz, 2H), 3.35–3.19 (m, 4H), 3.16–3.08 (m, 2H), 2.82 (d, *J* = 14.9 Hz, 1H), 2.71 (s, 3H), 2.65 (s, 1H), 2.57 (d, *J* = 14.1 Hz, 1H), 2.47–2.43 (m, 1H), 2.39 (d, *J* = 12.8 Hz, 1H), 2.22–2.17 (m, 2H), 2.11 (s, 3H), 1.87–1.75 (m, 4H), 1.69 (d, *J* = 14.6 Hz, 2H), 1.43–1.38 (m, 3H), 1.19 (t, *J* = 7.1 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); HRESI-TOF *m/z* 899.4713 ($C_{49}H_{63}FN_6O_9$ + $H^+$, required 899.4713).

**Cell growth inhibition assay:** Compounds were tested for their cell growth inhibition of L1210 (ATCC #CCL-219, mouse lymphocytic leukemia, see Supporting Information) cells, HCT116 (ATCC #CCL-247, human colorectal carcinoma) cells, and HCT116/VM46 (a vinblastine-resistant strain of HCT116) cells in culture. A population of cells (> $1 \times 10^6$ cells/mL as determined with a hemocytometer) was diluted with an appropriate amount of Dulbecco-modified Eagle Medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco) to a final concentration of 30,000 cells/mL. To each well of a 96-well plate (Corning Costar), 100 μL of the cell-media solution was added with a multichannel pipette. The cultures were incubated at 37 °C in an atmosphere of 5% $CO_2$ and 95% humidified air for 24 h. The test compounds were added to the plate as follows: test substances were diluted in DMSO to a concentration of 1 mM and 10-fold serial dilutions were performed on a separate 96-well plate. Fresh culture medium (100 μL) was added to each well of cells to constitute 200 μL of medium per well followed by 2 μL of each test agent. Compounds were tested in duplicate (n = 2–8 times) at six concentrations between 0–1,000 nM or 0–10,000 nM. Following addition, cultures were incubated for an additional 72 h.

A phosphatase assay was used to establish the $IC_{50}$ values as follows: the media in each cell was removed and 100 μL of phosphatase solution (100 mg phosphatase substrate in 30 mL 0.1 M NaOAc, pH 5.5, 0.1% Triton X-100 buffer) was added to each well. The plates were incubated at 37 °C for either 5 min (L1210) or 15 min (HCT116 and HCT116/VM46). After the given incubation time, 50 μL of 0.1 N NaOH was added to each well and the absorption at 405 nm was determined using a 96 well plate reader. As the absorption is directly proportional to the number of living cells, the $IC_{50}$ values were calculated and the reported values represent of the average of 4–16 determinations (SD ±10%).

**Tubulin binding competition assay:** The competitive displacement of $^3$H-vinblastine (obtained from Moravek Biochemicals, Inc.) from purified porcine tubulin (tubulin and general tubulin buffer obtained from Cytoskeleton, Inc.) was measured using a procedure previously described.[19] As described, a 100 μL sample of tubulin solution diluted with 850 μL buffer was incubated with 25 μL of $7.2 \times 10^{-5}$ M $^3$H-vinblastine for 15 min at 37 °C, after which 25 μL of $7.2 \times 10^{-5}$ M unlabeled alkaloid was added and incubation continued for 60 min. Tubulin-bound $^3$H-vinblastine was adsorbed onto DEAE filter paper and counted directly. Millipore Steriflip filter units were used to wash the filter paper with buffer under mild suction.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## ABBREVIATIONS USED

**Pgp** P-glycoprotein

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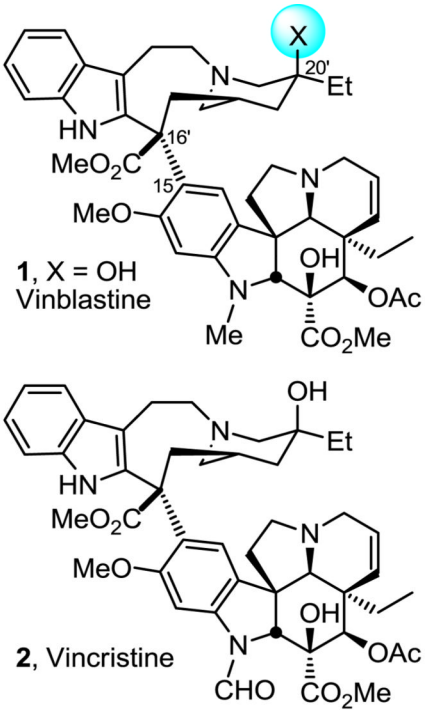



| compound | HCT116, $IC_{50}$ (nM) |
|---|---|
| X = OH, **1** | 6.8 |
| X = H, **3** | 60 |
| X = SCN, **4** | 550 |
| X = NCS, **5** | 530 |
| X = $N_3$, **6** | 690 |
| X = $NH_2$, **7** | 600 |
| X = NHCHO, **8** | 85 |
| X = $NHCOCH_3$, **9** | 90 |
| X = $NHCO_2CH_3$, **10** | 75 |
| X = $NHCONH_2$, **11** | 7.5 |
| X = $NHCSNH_2$, **12** | 7.7 |

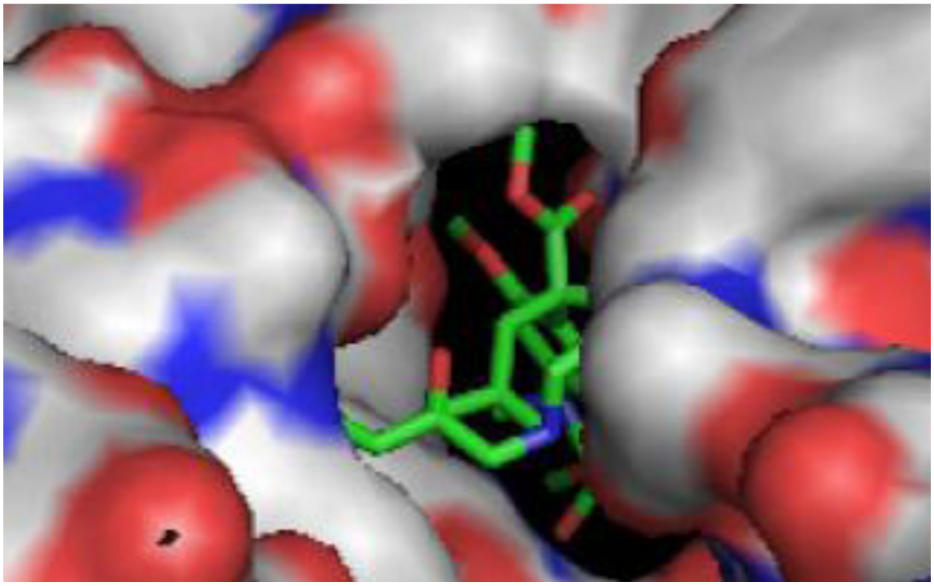
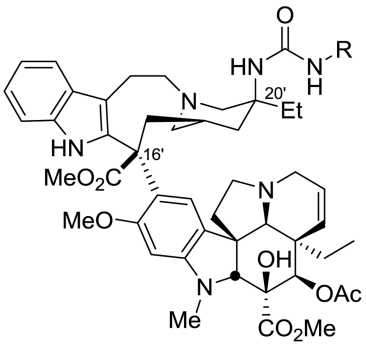
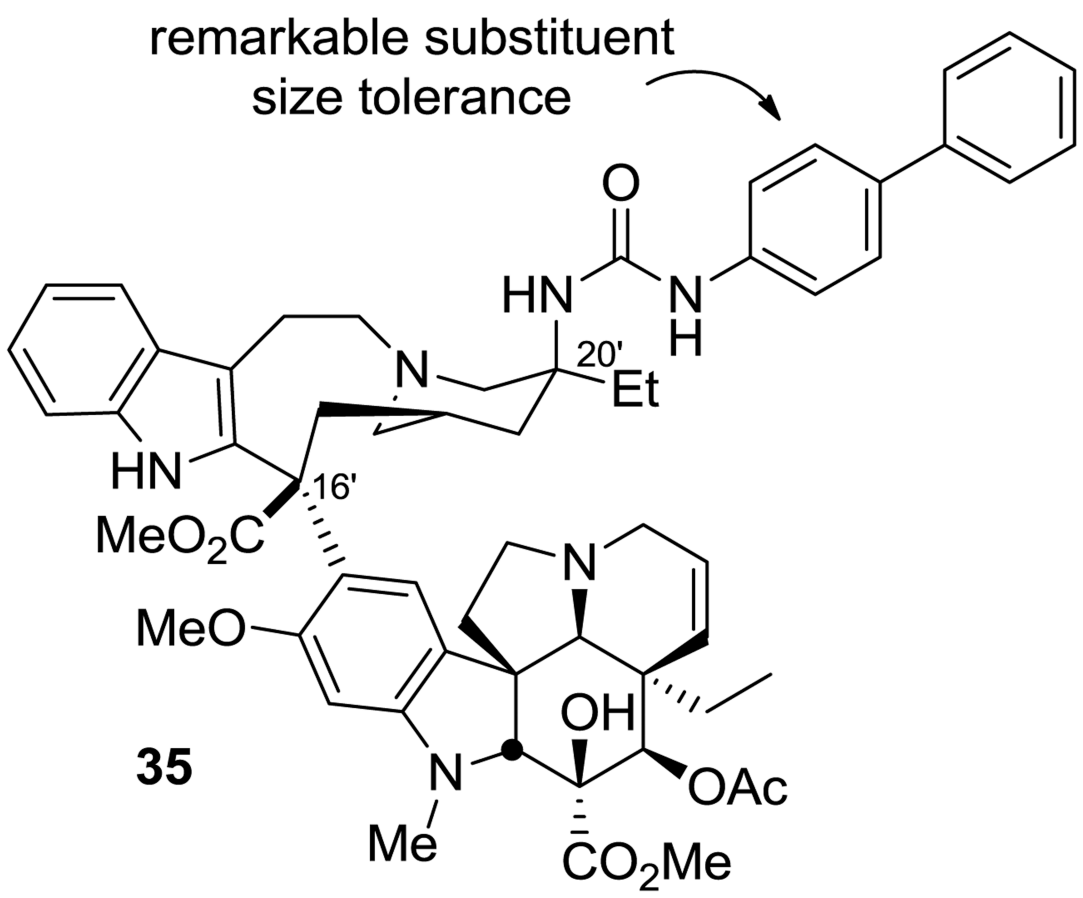


**Figure 1.**
Top: Natural products and initial C20' vinblastine analogues. Bottom: x-ray structure of vinblastine bound to tubulin highlighting the region surrounding vinblastine C20' site (PDB ID: 1Z2B)[14].



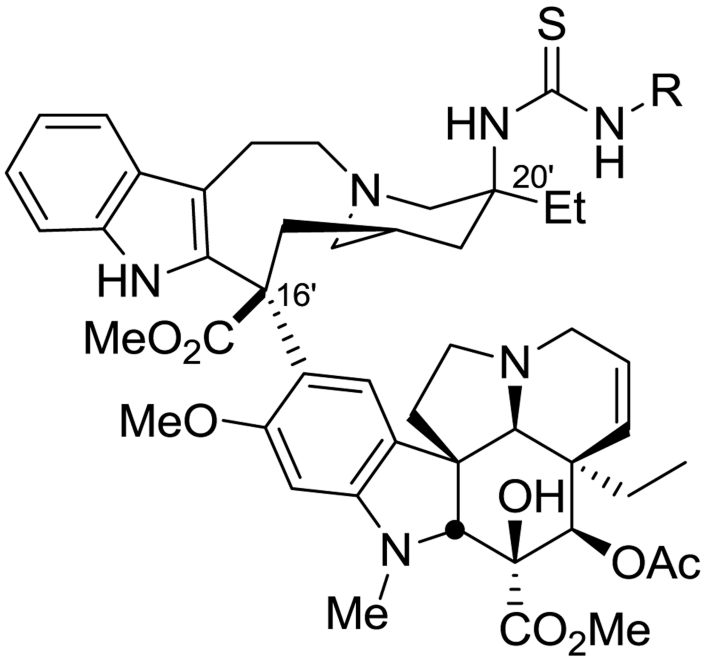

| Compound | $IC_{50}$ (nM) HCT116 | HCT116/VM46 |
|---|---|---|
| Vinblastine (**1**) | 6.8 | 600 |
| R = H (**11**) | 7.5 | 4400 |
| **Alkyl** | | |
| R = methyl (**14**) | 0.82 | 530 |
| R = ethyl (**15**) | 0.73 | 90 |
| R = *n*-propyl (**16**) | 2.7 | 220 |
| R = *i*-propyl (**17**) | 5.7 | 530 |
| R = cyclopropyl (**18**) | 0.73 | 85 |
| R = *n*-butyl (**19**) | 4.6 | 270 |
| R = *t*-butyl (**20**) | 20 | 670 |
| R = cyclohexyl (**21**) | 5.4 | 450 |
| R = $CH_2CH_2OH$ (**22**) | 8.8 | >1000 |
| **Aryl** | | |
| R = $C_6H_5$ (**23**) | 5.1 | 390 |
| R = *p*-$C_6H_4F$ (**24**) | 3.9 | 400 |
| R = *p*-$C_6H_4Cl$ (**25**) | 6.7 | 590 |
| R = *p*-$C_6H_4CH_3$ (**26**) | 4.8 | 330 |
| R = *p*-$C_6H_4CF_3$ (**27**) | 7.1 | 610 |
| R = *p*-$C_6H_4OCH_3$ (**28**) | 2.0 | 230 |
| R = *m*-$C_6H_4OCH_3$ (**29**) | 0.77 | 80 |
| R = *o*-$C_6H_4OCH_3$ (**30**) | 0.77 | 65 |
| R = $CH_2C_6H_5$ (**31**) | 7.3 | 740 |
| R = $CH_2CH_2C_6H_5$ (**32**) | 6.3 | 590 |
| R = $CH_2$(2-pyridyl) (**33**) | 5.6 | 670 |
| R = $CH_2$(2-furyl) (**34**) | 5.1 | 530 |

**Figure 2.**
Activity of vinblastine C20' urea analogues



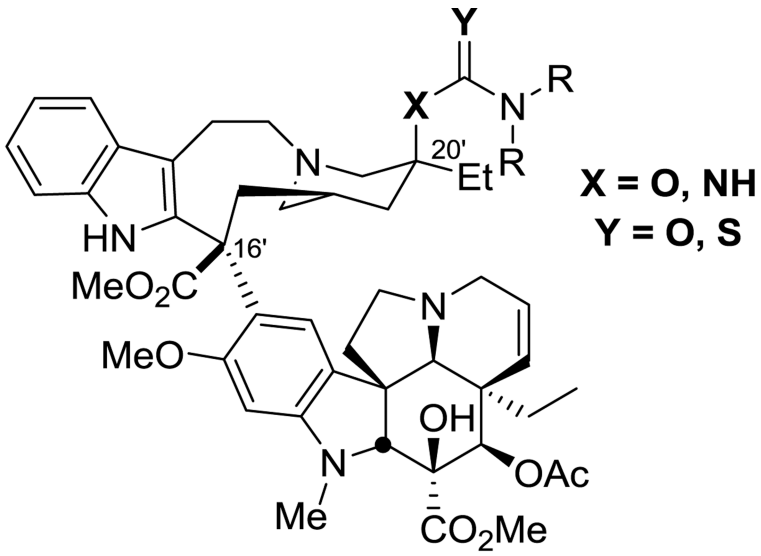

| Compound | $IC_{50}$ (nM) | |
|---|---|---|
| | HCT116 | HCT116/VM46 |
| **1** | 6.8 | 600 |
| **35** | 6.9 | 470 |

**Figure 3.**
Unprecedented C20' steric tolerance



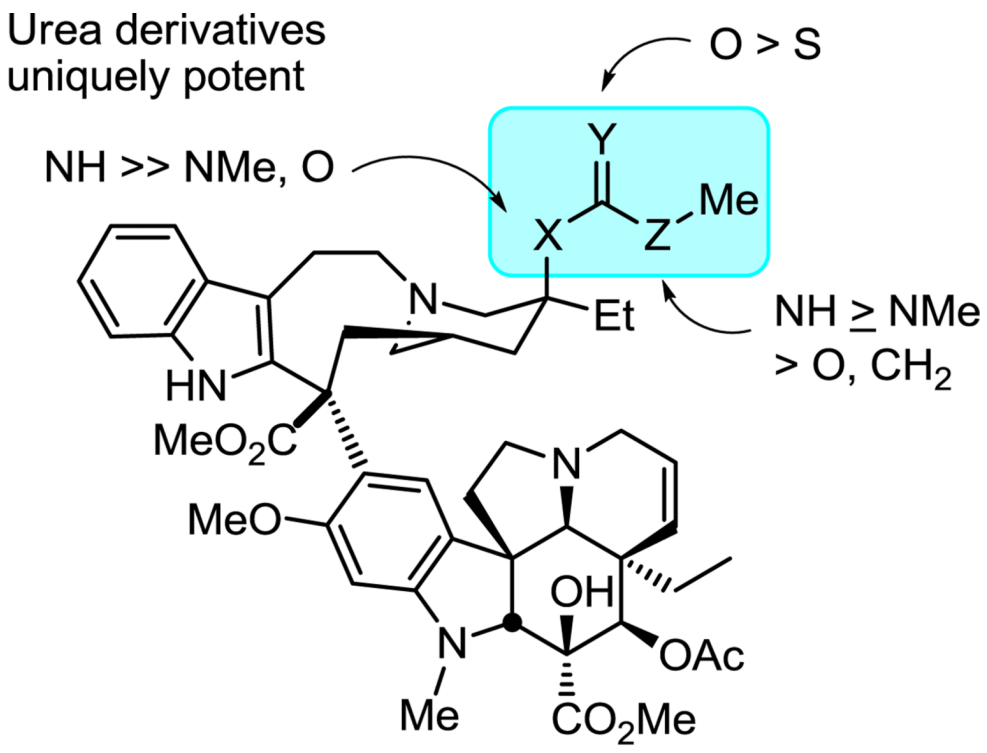

| Compound | $IC_{50}$ (nM) | |
|---|---|---|
| | HCT116 | HCT116/VM46 |
| Vinblastine | 6.8 | 600 |
| R = H | 7.7 | 2000 |
| R = $C_6H_{11}$ (**36**) | 20 | 520 |
| R = $C_6H_5$ (**37**) | 40 | 650 |
| R = $CH_2CH_2(4\text{-}FC_6H_4)$ (**38**) | 60 | 750 |

**Figure 4.**
Activity of vinblastine C20' thiourea analogues



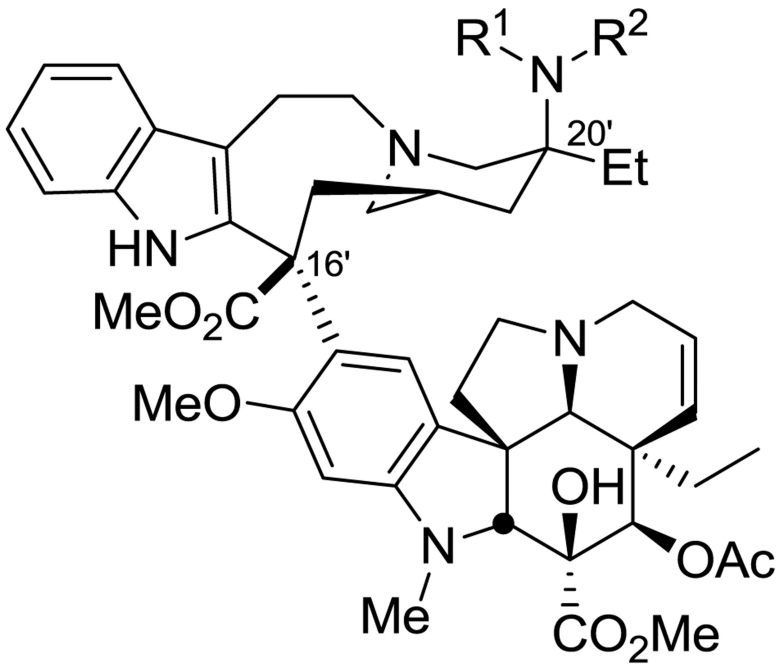

| Compound | $IC_{50}$ (nM) HCT116 | HCT116/VM46 |
|---|---|---|
| Vinblastine | 6.8 | 600 |
| **X = NH, Y = O** | | |
| R = H (**11**) | 7.5 | 4400 |
| R = $CH_3$ (**39**) | 2.8 | 80 |
| R = $CH_2CH_3$ (**40**) | 6.7 | 450 |
| R = Morpholine (**41**) | 4.5 | 360 |
| R = Piperidine (**42**) | 3.9 | 50 |
| **X = NH, Y = S** | | |
| R = H | 7.7 | 2000 |
| R = $CH_3$ (**43**) | 8.7 | 250 |
| **X = O, Y = O** | | |
| R = $CH_3$ (**44**) | 4700 | 9100 |

**Figure 5.**
Activity of vinblastine C20' disubstituted urea and thiourea analogues



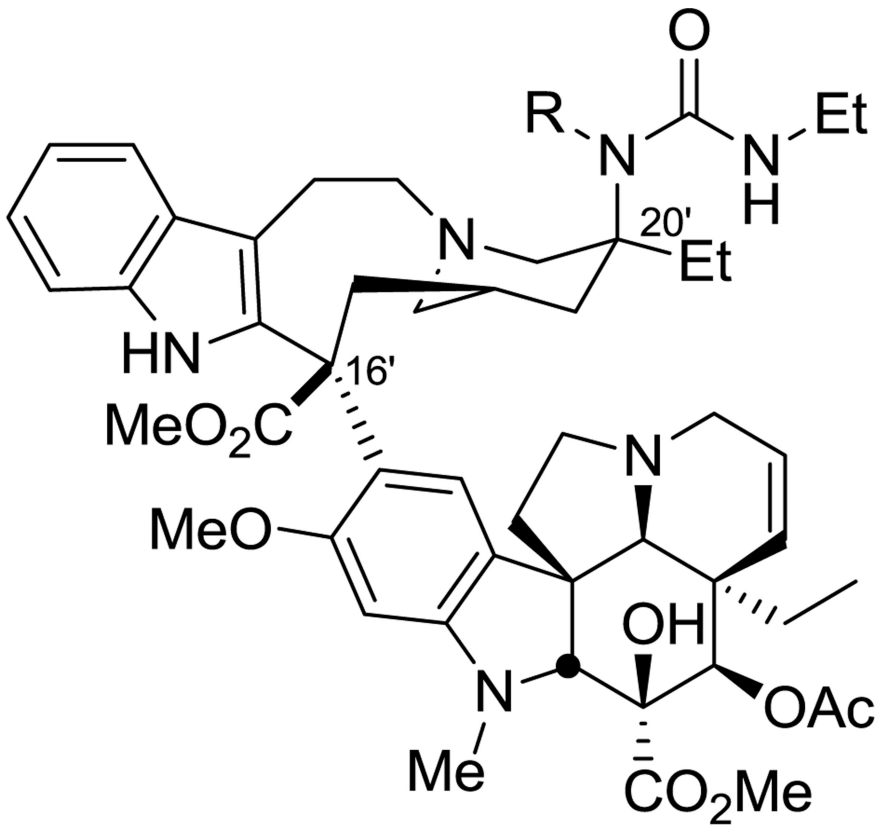

| compd | X | Y | Z | $IC_{50}$ (nM) HCT116 |
|---|---|---|---|---|
| **1** | | | | 6.8 |
| **39** | NH | O | NMe | 2.8 |
| **43** | NH | S | NMe | 8.7 |
| **10** | NH | O | O | 75[10] |
| **9** | NH | O | – | 90[10] |
| **44** | O | O | NMe | 4700 |

**Figure 6.**
Importance of the C20' amine versus alcohol functionalization and distinctions between urea/thiourea versus carbamate/amide derivatives



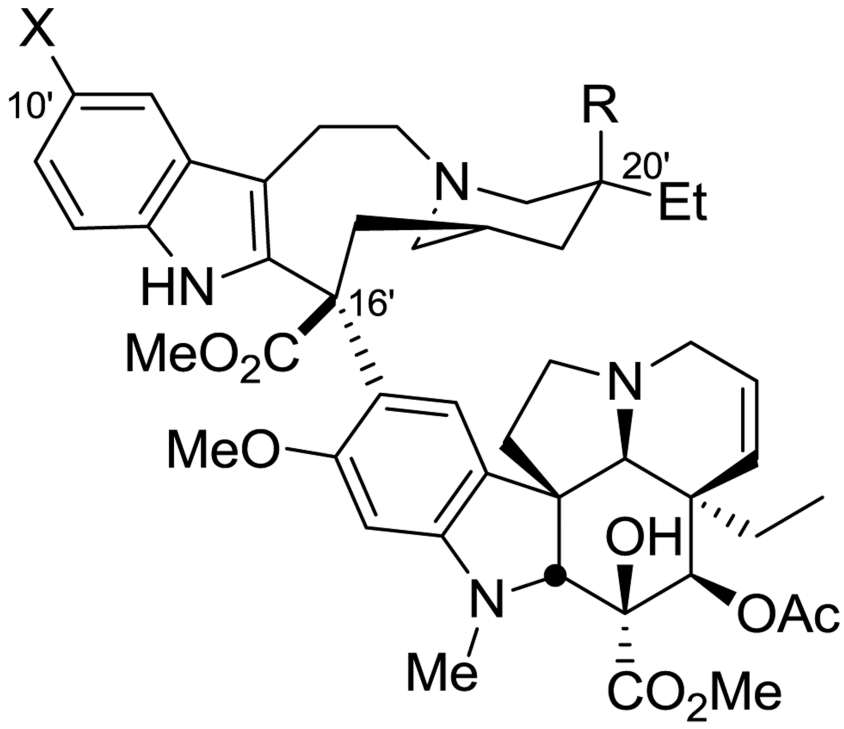

| Compound | $IC_{50}$ (nM) HCT116 | HCT116/VM46 |
|---|---|---|
| Vinblastine | 6.8 | 600 |
| $R^1$ and $R^2$ = H (**7**) | 600 | >10000 |
| $R^1$ = H $R^2$ = $CH_3$ (**45**) | 60 | 8500 |
| $R^1$ and $R^2$ = $CH_3$ (**46**) | 980 | >10000 |

**Figure 7.**
Activity of C20' amines



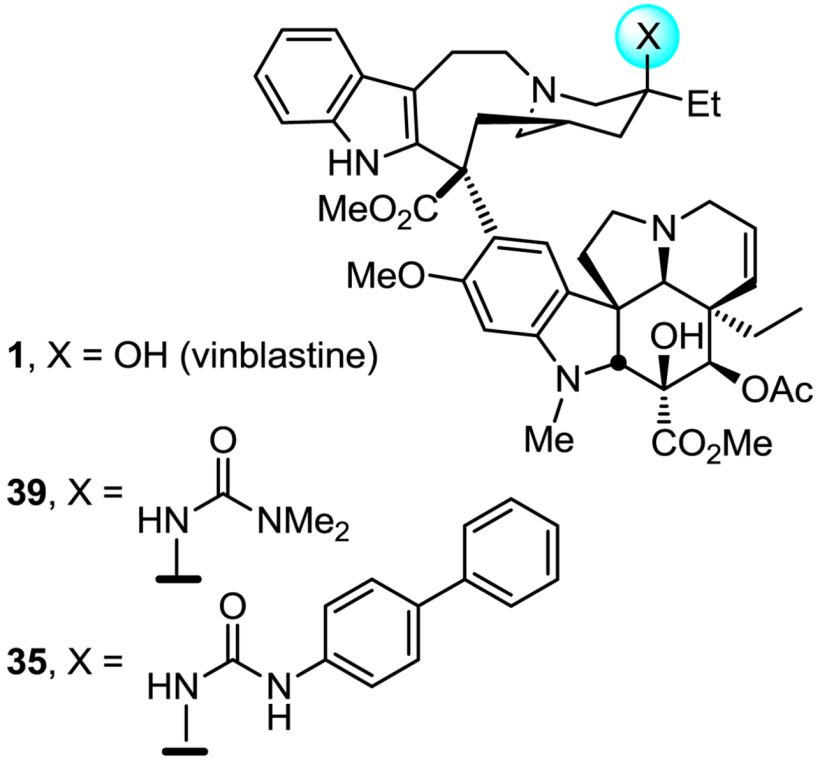

| Compound | $IC_{50}$ (nM) HCT116 | HCT116/VM46 |
|---|---|---|
| R = H (**15**) | 0.73 | 90 |
| R = Me (**47**) | 520 | 8500 |

**Figure 8.**
Impact of C20' nitrogen H-bond donor




| Compound | $IC_{50}$ (nM) | |
|---|---|---|
| | HCT116 | HCT116/VM46 |
| R = OH, X = H (**1**) | 6.8 | 600 |
| R = NHCONHEt, X = H (**15**) | 0.73 | 90 |
| R = OH, X = F (**48**) | 0.80 | 80 |
| R = NHCONHEt, X = F (**49**) | 0.62 | 70 |

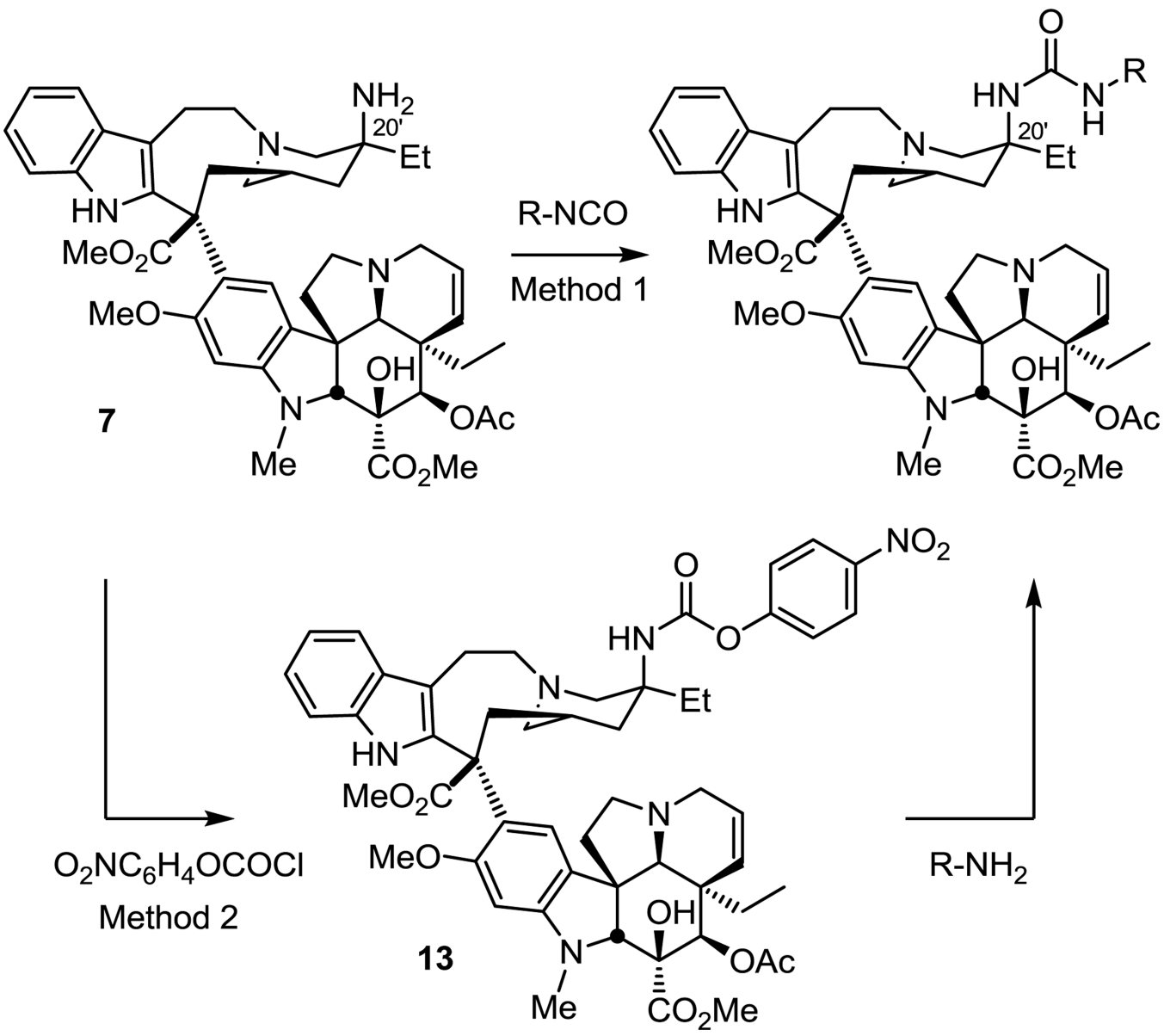
**Figure 9.**
Urea derivative of 10'-fluorovinblastine



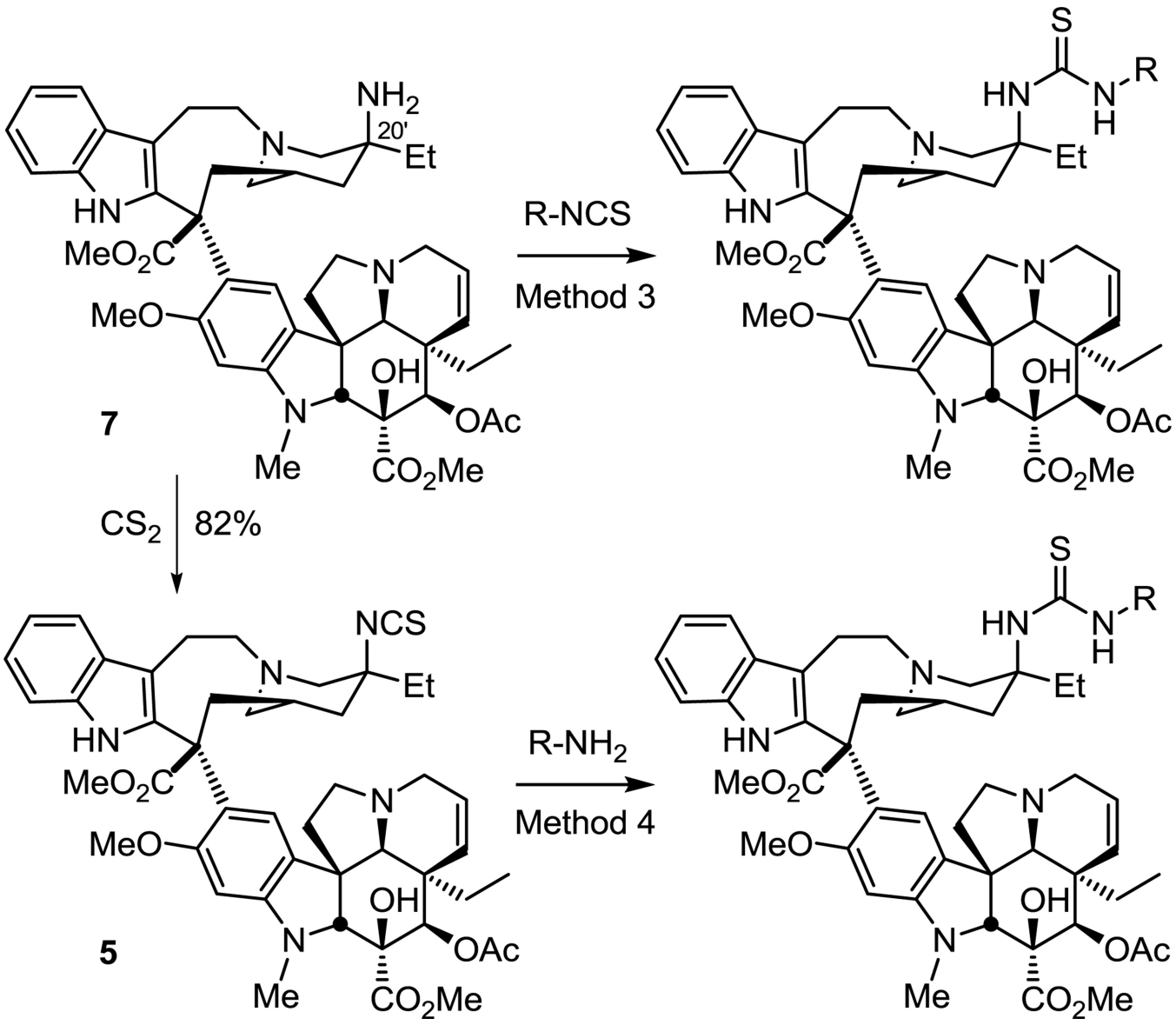

| compd | % $^3$H-vinblastine remaining bound[a] | HCT116 $IC_{50}$ (nM) |
|---|---|---|
| **1** | 50.0 | 6.8 |
| **39** | 45.1 ± 3.5 | 2.8 |
| **35** | 48.5 ± 6.9 | 6.9 |

[a]Competitive binding of ligand vs [$^3$H]VBL (1:1) measuring the remaining bound [$^3$H]VBL. Average of 3 repeat determination, normalized to have dpm (25 μL VLB + 25 μL [$^3$H]VLB) = 50.0%.

**Figure 10.**
Tubulin binding assay




**Scheme 1.**




**Scheme 2.**