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Topoisomerase I-mediated DNA cleavage as a guide to the development of antitumor agents derived from the marine alkaloid lamellarin D: triester derivatives incorporating amino acid residues

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Abstract—The marine alkaloid lamellarin D (LAM-D) has been recently characterized as a potent poison of human topoisomerase I endowed with remarkable cytotoxic activities against tumor cells. We report here the first structure–activity relationship study in the LAM-D series. Two groups of triester compounds incorporating various substituents on the three phenolic OH at positions 8, 14 and 20 of 6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one pentacyclic planar chromophore typical of the parent alkaloid were tested as topoisomerase I inhibitors. The non-amino compounds in group A showed no activity against topoisomerase I and were essentially non cytotoxic. In sharp contrast, compounds in group B incorporating amino acid residues strongly promoted DNA cleavage by human topoisomerase I. LAM-D derivatives tri-substituted with leucine, valine, proline, phenylalanine or alanine residues, or a related amino side chain, stabilize topoisomerase I–DNA complexes. The DNA cleavage sites detected at T↓G or C↓G dinucleotides with these molecules were identical to that of LAM-D but slightly different from those seen with camptothecin which stimulates topoisomerase I-mediated cleavage at T↓G only. In the DNA relaxation and cleavage assays, the corresponding Boc-protected compounds and the analogues of the non-planar LAM-501 derivative lacking the 5–6 double bond in the quinoline B-ring showed no effect on topoisomerase I and were considerably less cytotoxic than the corresponding cationic compounds in the LAM-D series. The presence of positive charges on the molecules enhances DNA interaction but melting temperature studies indicate that DNA binding is not correlated with topoisomerase I inhibition or cytotoxicity. Cell growth inhibition by the 41 lamellarin derivatives was evaluated with a panel of tumor cells lines. With prostate (DU-145 and LN-CaP), ovarian (IGROV and IGROV-ET resistant to ecteinascidin-743) and colon (LoVo and LoVo-Dox cells resistant to doxorubicin) cancer cells (but not with HT29 colon carcinoma cells), the most cytotoxic compounds correspond to the most potent topoisomerase I poisons. The observed correlation between cytotoxicity and topoisomerase I inhibition strongly suggests that topoisomerase I-mediated DNA cleavage assays can be used as a guide to the development of superior analogues in this series. LAM-D is the lead compound of a new promising family of antitumor agents targeting topoisomerase I and the amino acid derivatives appear to be excellent candidates for a preclinical development.

1. Introduction

Many natural products are used to combat cancer. The chemotherapeutic arsenal includes drugs extracted from plants such as etoposide and taxol, or drugs derived from plant alkaloids such as the camptothecin (CPT) derivatives topotecan and irinotecan. Antibiotics are equally well represented because several of the most frequently used anticancer drugs, daunomycin and bleomycin, for example, originate from Streptomyces. The sea world is also a rich source of anticancer agents. The marine environment has provided many lead compounds, the bryostatins, aplidine and kahalalide F to cite only a few of them.[1–5] The most advanced anti-

Keywords: Lamellarin; Topoisomerase I; DNA interaction; Cytotoxicity; Anticancer drugs.
Abbreviations: CPT, Camptothecin; LAM-D, Lamellarin D.
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cancer drug issued from the sea is probably ET-743 (Yondelis®, trabectidin), a tetrahydroisoquinoline alkaloid produced by the tunicate *Ecteinascidia turbinata*.[6,7]

PharmaMar, the spanish company which develops ET-743, aplidine and kahalalide F, has characterized a large number of marine compounds of potential interest for the treatment of cancer. One of the novel drug candidate is the hexacyclic alkaloid lamellarin D (LAM-D) initially isolated from a prosobranch mollusc of the genus *Lamellaria*[8] and subsequently found in ascidians.[9] Over 30 lamellarins have been isolated and interestingly, some of them including LAM-D, show equally potent cytotoxic activities against both multi-drug resistant (MDR) and their corresponding parental cell lines.[10] Very recently, we have identified topoisomerase I as a molecular target for LAM-D and this important discovery has prompted us to exploit the LAM-D pharmacophore for the development of topoisomerase I-targeted anticancer agents. Molecular and cellular studies revealed that LAM-D potently stabilizes topoisomerase I–DNA covalent complexes so as to promote the formation of DNA single strand breaks. In parallel, camptothecin-resistant cell lines expressing a mutated *top1* gene were found to be cross-resistant to LAM-D.[11] In sharp contrast, the synthetic analogue LAM-501 lacking the 5–6 double bond in the quinoline B-ring was found to be totally inactive against topoisomerase I and considerably less cytotoxic than the parent natural alkaloid. The 5–6 double bond seems to be an essential element for the poisoning of topoisomerase I and the antiproliferative activity. Here we have investigated further the importance of this structural element by comparing the activity of two series of lamellarin analogues bearing a 6H-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one pentacyclic planar chromophore typical of the parent alkaloid or a 5,6-dehydro non-planar chromophore as found in the analogue LAM-501. The newly synthesized lamellarin derivatives are all triesters and differ by the nature of the substituent introduced on the three phenolic OH at positions 8, 14 and 20. Two sets of compounds were studied. Compounds in group A (Chart 1) refer to the non-amino derivatives and include various ester with aryl groups or diethylphosphono and methylsulfone groups. Compounds in group B (Chart 2) refer to cationic derivatives with diverse amino acid substituents (such as Leu, Val, Ala, Pro, Trp, Phe). The results of our first structure–activity relationship study in the LAM-D series, aimed at identifying the structural elements implicated in the anti-topoisomerase I activity, confirm that the lamellarins represent a new and promising series of topoisomerase I inhibitors. The correlation between the capacity of the drugs to stimulate topoisomerase I-mediated DNA cleavage and their cytotoxic potential is a good augur for the development of antitumor agents.

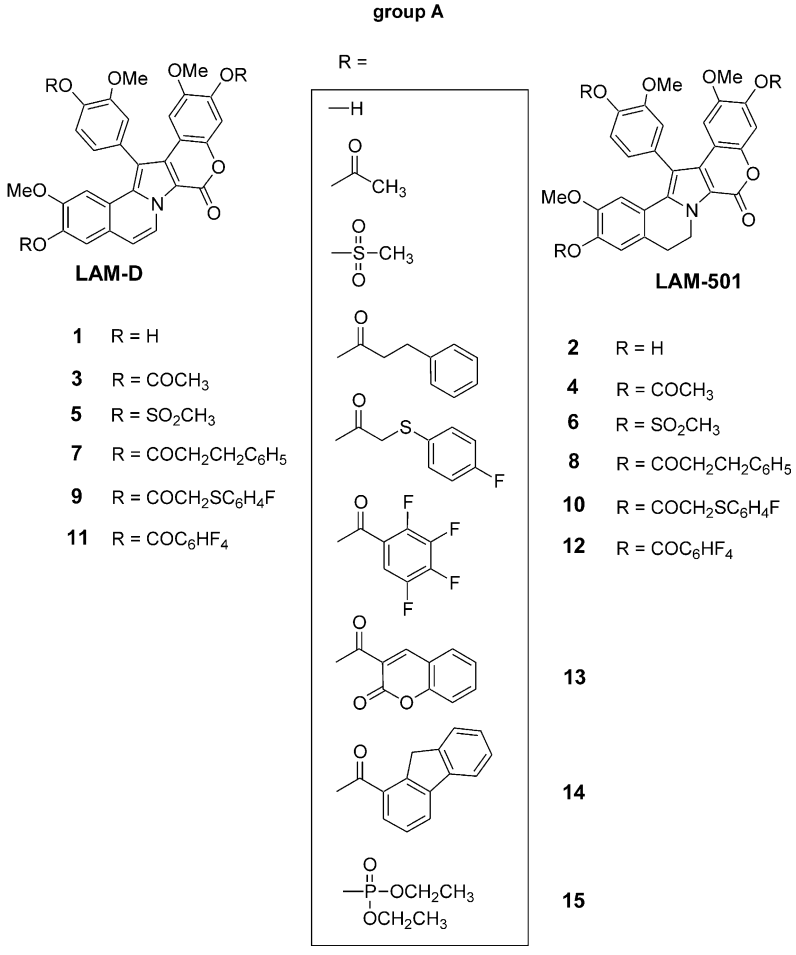


Chart 1.

2. Results

2.1. Chemistry

The synthesis of LAM-D (**1**) and LAM-501 (**2**) was achieved by [3 + 2] cycloaddition reaction of the corresponding dihydroisoquinoline **44** with ester **43** (Scheme 1) following the procedure described previously.[12] Compounds in group A (Chart 1) were obtained (Scheme 2) by reaction of LAM-501 (**2**) with the corresponding acid using EDC as a coupling reagent or the acid chloride with triethylamine. Treatment of the ester derivatives with DDQ afforded the derivatives of LAM-D (**1**). Compounds in group B (Chart 2) were obtained in a similar way (Scheme 3) by reaction of LAM-501 (**2**) with N-protected aminoacid using EDC as a coupling reagent, oxidation with DDQ to obtain

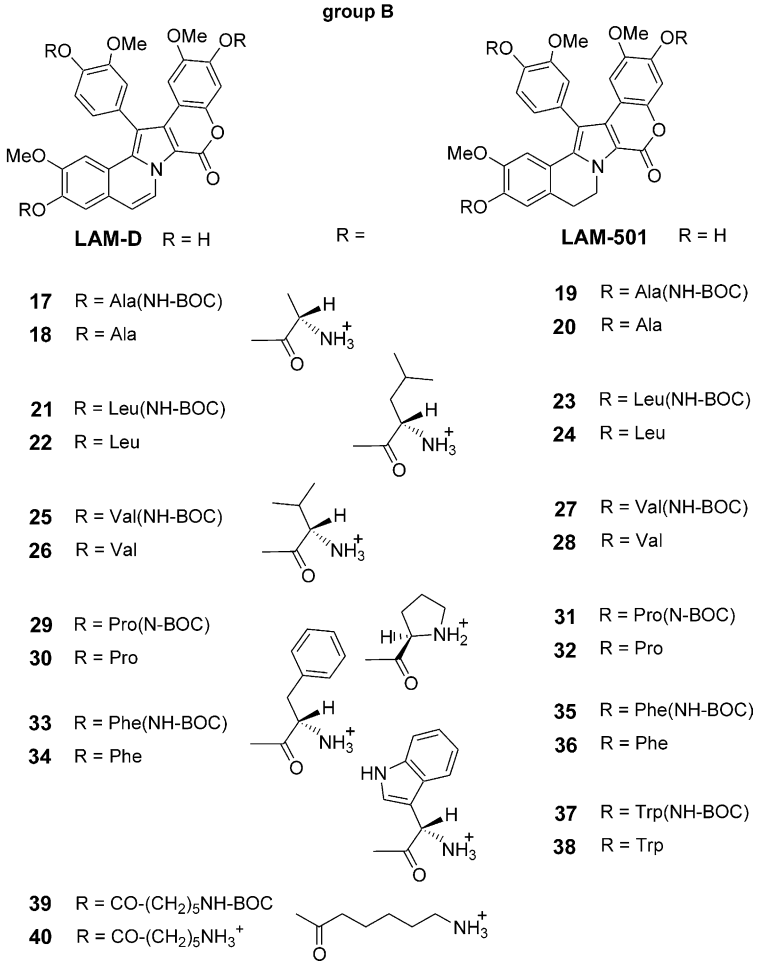
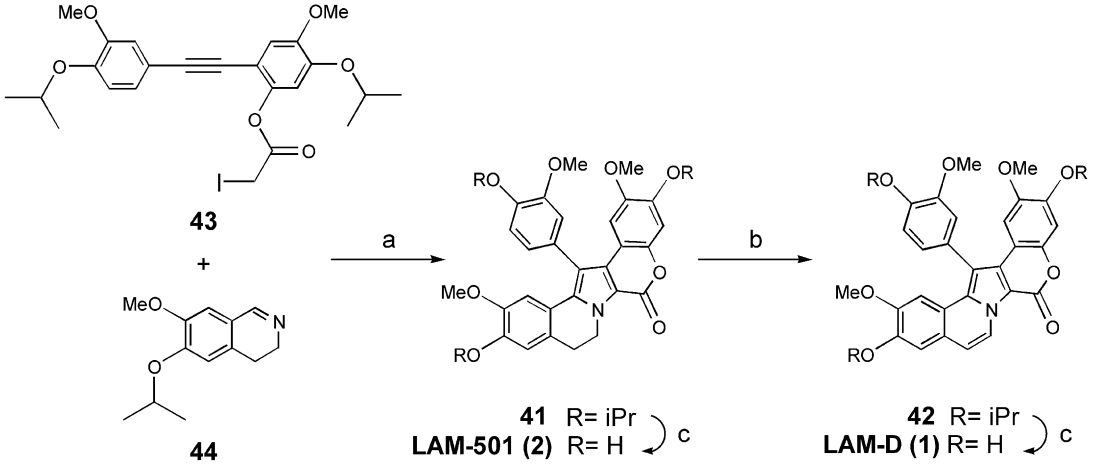


Chart 2.

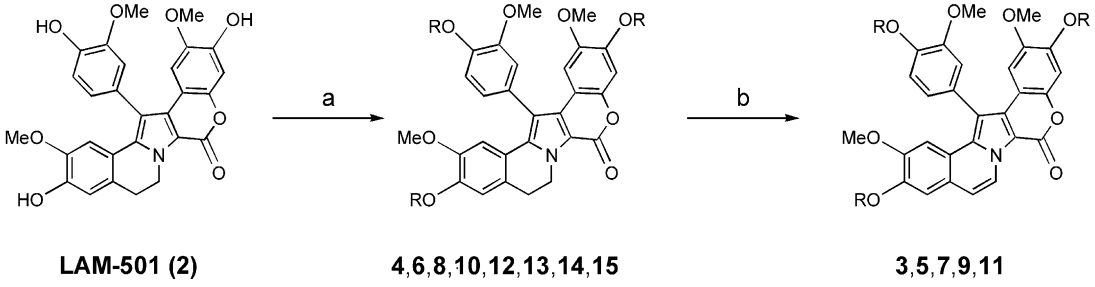


Scheme 1. Reagents: (a) (i) $ClCH_2CH_2Cl$, 23 °C; (ii) DIPEA, 85 °C; (b) DDQ, $CHCl_3$, reflux; (c) $AlCl_3$, CH_2Cl_2, 23 °C.

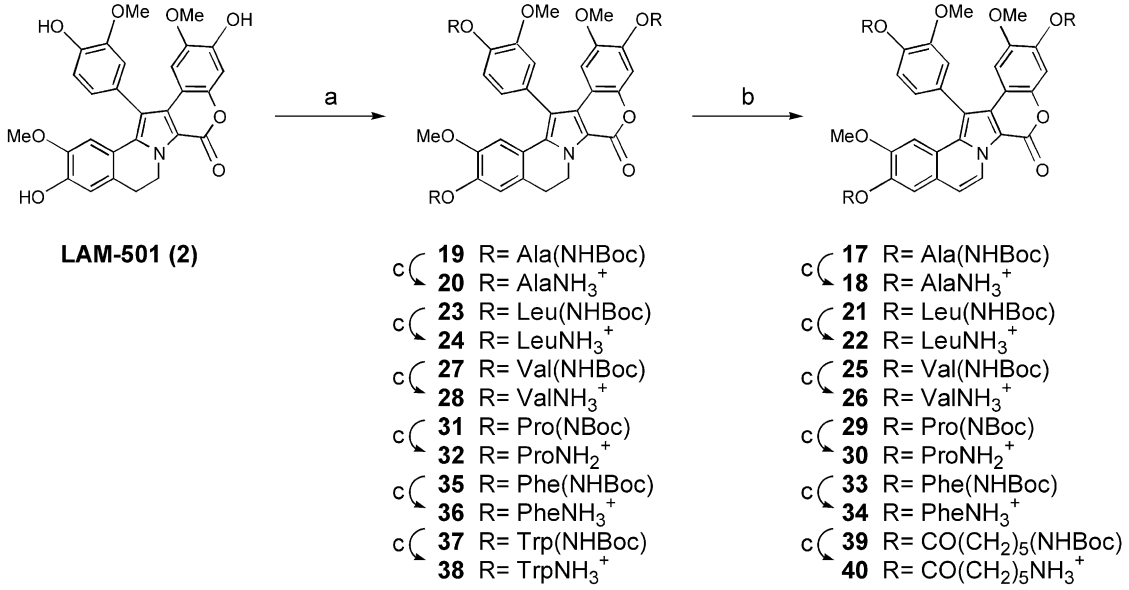
the derivatives of LAM-D (**1**) and deprotection of the amino group with trifluoroacetic acid or acetyl chloride in MeOH to afford the cationic derivatives.

2.2. Topoisomerase I inhibition

Two assays, based on DNA relaxation and DNA cleavage,[13] were used evaluate the effects of the lamellarin analogues on the catalytic activity of human topoisomerase I. In the first assay, a supercoiled plasmid DNA was relaxed with topoisomerase I in the absence or presence of the test compounds, each tested at 1 μM. DNA relaxation products were then resolved by gel electrophoresis on agarose gels containing ethidium bromide to stain the DNA. The results are presented in Figure 1. The alkaloid camptothecin, used as a positive control, strongly stabilizes the cleaved complex with topoisomerase I. Similarly, the intensity of the band corresponding to nicked DNA is significantly amplified in the presence of LAM-D indicating that this natural product also stabilizes DNA–topoisomerase I covalent complexes. This functional assay is useful to identify the topoisomerase I poisons among the various analogues synthesized. Compounds in group A are all inactive against topoisomerase I. All the uncharged esters, be it a small acetyl substituent (**3**) or with a bulkier 2,3,4,5-acetotetrafluorophenyl (**11**) group, show no effect on DNA cleavage by the enzyme. The analogous compounds with a 5–6 saturated bond (**2**,**4**,**6**,**8**,**12**) were also inactive in this assay. Different ester groups, coumarin (**13**), 1-aceto-(9*H*-fluoren-1-yl) (**14**), and diethylphosphate (**15**), were incorporated on the three phenoxy positions of LAM-D but no topoisomerase I inhibitor could be identified. The first design was unsuccessful and therefore we adopted another strategy which consisted to incorporate a cationic substituent with the rational that a positively charged group might facilitate interaction with DNA phosphate and possibly the target enzyme. Different cationic groups, mostly amino acid residues, were incorporated at the three phenoxy positions of LAM-D. Compounds of group B (Chart 2) include Ala, Leu, Val, Pro and Phe derivatives and a few analogous molecules such as the aminopentyl derivative **39**. In all cases, we tested the uncharged Boc-protected intermediates and the final cationic products. Here again, in most cases the compounds were prepared in the LAM-D (C5–C6 double bond) and LAM-501 (C5–C6 single bond) series. A marked inhibition of topoisomerase I was observed with the positively charged molecules **18** (Ala), **22** (Leu), **26** (Val), **30** (Pro) and **34** (Phe) but not with the corresponding NH-Boc derivatives or the non-planar C5–C6 analogues (Fig.



Scheme 2. Reagents: (a) RCO_2H, EDC·HCl, DMAP, or RCOCl, Et_3N, CH_2Cl_2, 23 °C; (b) DDQ, $CHCl_3$, reflux.



Scheme 3. Reagents: (a) BocHNaaCO_2H, EDC·HCl, DMAP, CH_2Cl_2, 23 °C; (b) DDQ, $CHCl_3$, reflux; (c) TFA, CH_2Cl_2, or $ClCOCH_3$/MeOH, EtOAc, 23 °C.

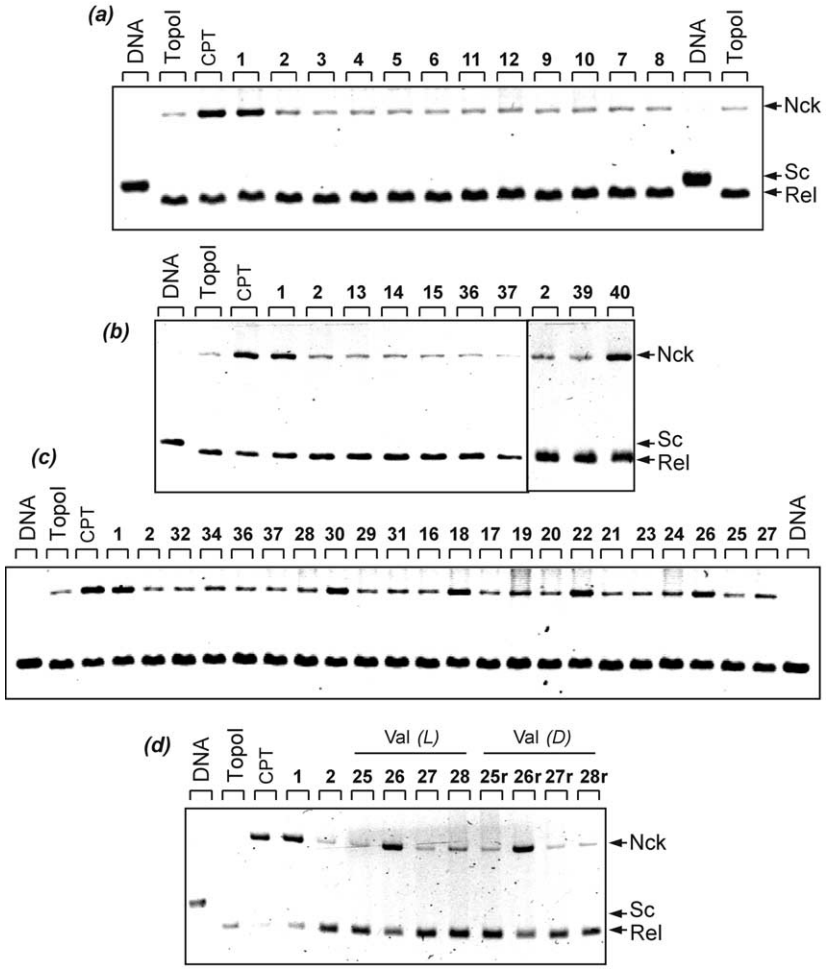


Figure 1. Effect of the lamellarin derivatives on the stabilisation of covalent DNA–topoisomerase I complexes. Native supercoiled pLAZ3 DNA (lane DNA) was incubated with topoisomerase I in the absence (lane TopoI) or presence of the indicated compound. Lanes marked CPT refer to camptothecin (10 μM). LAM-D (**1**) and its derivatives were tested at 1 μM. DNA samples were separated by electrophoresis on agarose gels containing ethidium bromide (1 μg/mL). Gel were photographed under UV light. Nck, nicked; Rel, relaxed; Sc, supercoiled.

1c). The Phe derivative is significantly less potent than the other amino acid derivatives which are all more or less equally effective at inhibiting topoisomerase I. The stereospecificity was investigated with the Val derivatives for which we compared the activity of the (L) (**25**–**28**) and (D) (**25r**–**28r**) isomers but, as shown in Figure 1d, there was no difference between the two series. Compound **26** and **26r** both stimulated DNA cleavage by the enzyme. No effect was observed with the Boc-proteted analogues in the C5–C6 double stranded (**25**, **25r**) or C5–C6 single-stranded (**27**, **28**, **27r**, **28r**) series. The amino compound **40** was also found to inhibit topoisomerase I (Fig. 1b).

Concentration-dependent measurements were performed with each of the positive compounds identified and a few representative gels comparing the anti-topoisomerase I activity of LAM-D (**1**) with the three analogues Val(D) (**26r**), Pro (**30**) and the amino compound **40** are shown in Figure 2. This later compound is equally efficient to **1** in terms of stimulation of DNA cleavage by topoisomerase I and is also equally cytotoxic (see below). In all cases, the dose–response analysis confirmed that the cationic LAM-D analogues potently inhibit the enzyme.

It is clear that the introduction of an amino acid functionality on the phenolic OH groups at positions 8, 14 and 20 of LAM-D is not detrimental to topoisomerase I inhibition. The extent of topoisomerase I-mediated DNA cleavage is fully maintained when a Leu, Val, Ala or Pro residue is incorporated on the LAM-D skeleton whereas a non charged group abolishes the anti-topoisomerase I activity. A phenylalanine residue is much less favorable than a proline or an alanine residue for example. The observations that the incorporation of a cationic group promoted topoisomerase I inhibition suggested that the enhanced capacity of the drugs to bind to DNA could be responsible for a better enzyme inhibition. To verify this idea, melting temperature measurements were carried out with the polymer poly(dAT)$_2$ and for each compound we calculated the ΔTm values ($T\mathrm{m}^{\text{drug–DNA complex}} - T\mathrm{m}^{\text{DNA alone}}$). Poly(dAT)$_2$ which melt at a low temperature ($41 \pm 1\,^{\circ}\mathrm{C}$ in BPE buffer) affords a sensitive determination of the DNA binding capacity of the studied molecules. As indicated in Table 1, the Ala (**18**) and Pro (**30**) derivatives markedly stabilize the polymer against heat denaturation with ΔTm values $> 10\,^{\circ}\mathrm{C}$ and this correlates with their potent anti-topoisomerase I activity. In contrast, the Phe derivative (**34**) showed no effect on DNA thermal stability and this

may be the reason why this compound is more weakly active against topoisomerase I compared to the other amino acid derivatives. Enhanced DNA binding seems to facilitate topoisomerase I inhibition but tight binding to DNA is not required for enzyme inhibition since the natural product LAM-D shows little affinity for DNA despite its potent topoisomerase I poisoning capacity.

A second assay, based on the cleavage of a radiolabeled DNA substrate by topoisomerase I, was used to confirm

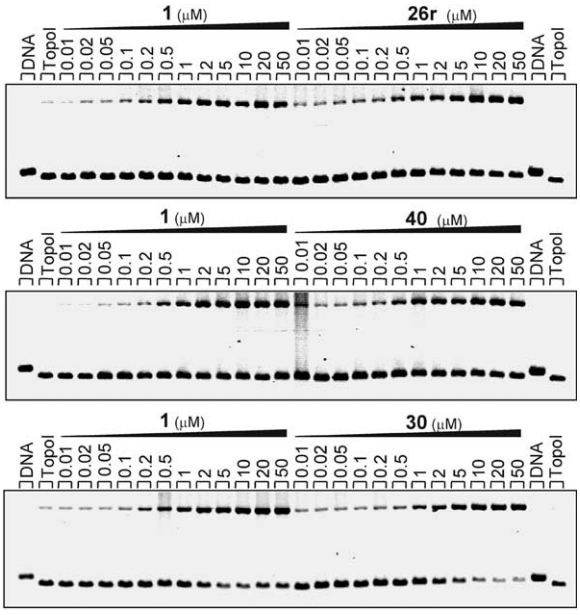


Figure 2. Concentration-dependent effect for the inhibition of topoisomerase I by LAM-D (**1**) or the cationic derivatives **26r**, **30** and **40**. All concentrations are given in μM. Other details as for Figure 1.

Table 1. Topoisomerase I inhibition, DNA binding and cytotoxicity

	Nicked DNA (%)[a]	ΔTm (°C)[b]	GI_{50} (nM)[c]
LAM-D (**1**)	25.7	2.9	10.9
LAM-501 (**2**)	12.3	0.0	2990
18	29.7	13.3	16.2
19	7.0	6.8	3650
22	31.1	4.5	18.0
23	9.2	1.0	4000
26	33.6	8.0	10.8
27	6.7	4.1	1600
30	24.6	12.0	18.6
31	13.4	5.3	2120
34	31.4	0.0	25.2
36	10.0	0.9	1630
38	6.2	0.0	913
40	27.6	4.1	15.1

[a] Extent of topoisomerase I-mediated DNA cleavage measured with the lamellarin derivatives (1 μM each). The% of nicked DNA (form II) was determined by densitometry. Band intensities from three gels such as those shown in Figure 1 were compiled for the quantitative analysis.

[b] Variation of the melting temperature (ΔTm) of helix-to-coil transition of poly(dAT)$_2$ in the presence of the lamellarin derivatives. *T*m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) using 20 μM poly(dAT)$_2$ (nucleotide concentration) and 20 μM drug.

[c] Drug concentration that causes 50% growth inhibition of DU-145 metastatic cells. Values were calculated from dose–response curves.

that the cationic lamellarin derivatives do effectively function as topoisomerase I poisons. A 117-bp DNA restriction fragment uniquely end-labeled at the 3′ end was subjected to cleavage by topoisomerase I in the presence of the different compounds and the resulting DNA cleavage products were resolved on sequencing-type polyacrylamide gels. A typical gel is presented in Figure 3. The advantage of this assay is to detect the cleavage sites and to locate their positions with nucleotide resolution, providing thus information on the site selectivity of cleavage.

From the two typical gels shown in Figure 3a and b, it is easy to identify the compounds that induce DNA cleavage by topoisomerase I. The reference drug CPT produces three sites at nucleotide positions 26, 48 and 81 which all three correspond to T↓G sites. Cleavage at TG sites in the presence of CPT is believed to result from the interaction of topoisomerase I with the T residue combined with the stacking of the CPT molecule with the adjacent G residue.[14,15] A fourth weak site can be detected at the top of the gels (T↓G107). The sequence selectivity profiles are slightly different with the lamellarin analogues. LAM-D is less efficient than CPT for topoisomerase I-mediated DNA cleavage at sites T↓G48 and T↓G81 but it induces an additional cleavage site at C↓G73. This likely reflects a different mode of interaction with the topoisomerase I–DNA covalent complexes, as recently discussed.[11] Cleavage profiles identical to that of **1** were obtained with the cationic derivatives such as **22** (Leu), **26** (Val), **30** (Pro) and **34** (Phe) but not with the corresponding NH-Boc derivatives or the non-planar C5–C6 analogue (Fig. 3a). The amino compound **40** was also found to stimulate DNA cleavage by the enzyme and here also we found no difference between the (L) (**26**) and (D) (**26d**) Val isomers (Fig. 3b). The results are thus entirely consistent with those obtained by the relaxation assay and therefore validate the conclusion that the cationic lamellarin derivatives potently inhibit topoisomerase I.

2.3. Cytotoxicity

Seven human tumor cell lines were used to determine the cytotoxicity of the lamellarin derivatives: HT29 human colon carcinoma cells, LoVo human colon lymph node metastasis cells and the corresponding LoVo-Dox cells resistant to doxorubicin, ovarian cells sensitive (IGROV) or resistant (IGROV-ET) to ecteinascidin-743, and the prostate tumor cells LN-CaP and DU-145. A colorimetric assay was used up to estimate GI_{50} values, that is, the drug concentration which causes 50% cell growth inhibition after 72 h continuous exposure to the test molecules. We have shown recently that LAM-D exhibits a pronounced cytotoxic effect toward prostatic cells, in particular the DU-145 metastatic cells which are androgen-insensitive.[11] This cell line was used as a reference to compare the different compound. Table 1 reports the GI_{50} values for each compound along with the effect on topoisomerase I and DNA binding activity. To quantify topoisomerase I poisoning, we measured the extent of DNA cleavage in the presence of the drug at 1 μM (% nicked DNA).

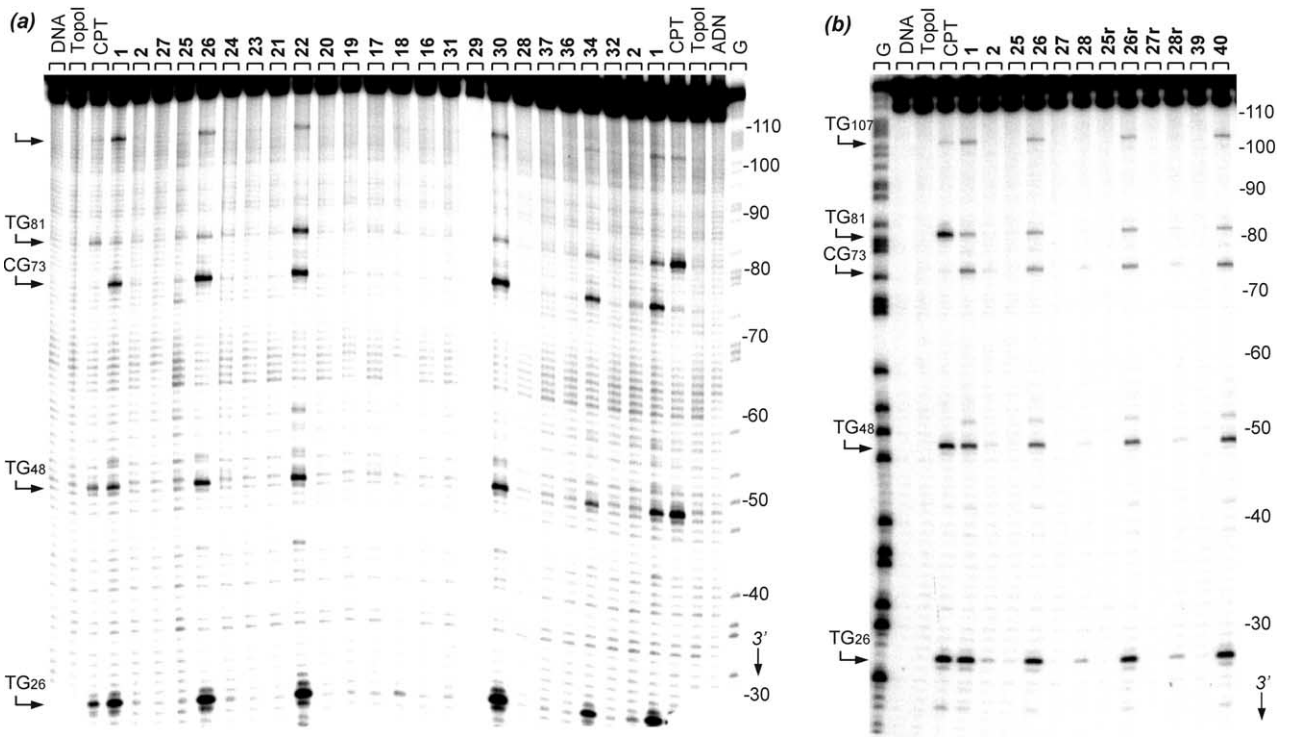


Figure 3. Cleavage of the 117 bp DNA fragment by topoisomerase I in the presence of the lamellarin derivatives. The 3′-end labeled fragment was incubated in the absence (lane TopoI) or presence of the test drug at 50 μM. CPT was used at 20 μM. Topoisomerase I cleavage reactions were analyzed on a 8% denaturing polyacrylamide gel. Numbers at the right side of the gels show the nucleotide positions, determined with reference to the guanine tracks labeled G. Arrows point to the five main cleavage sites.

Compounds **18**, **22**, **26**, **30**, **34** and **40** were found to be equally efficient as **1** at stimulating DNA cleavage by topoisomerase I. This effect is well correlated with the GI_{50} data. Indeed, GI_{50} values are in the 10–25 nM range with these compounds whereas the other molecules inactive or weakly active against topoisomerase I all give GI_{50} values in the 1–4 μM range. In contrast, the DNA binding activity, measured through the extent of stabilization of poly(dAT)$_2$ against heat denaturation (ΔTm values in Table 1), is not correlated with cytotoxicity or topoisomerase I inhibition. LAM-D and compound **22** both give low ΔTm values, 2.9 and 4.5 °C respectively, and compounds **18** and **30** give high ΔTm values, 13.3 and 12.0 °C respectively, but these four compounds are equally cytotoxic and represent potent topoisomerase I poison. Nevertheless, if DNA binding is not sufficient to confer activity against topoisomerase I in this series, it may be useful because the compounds which do not stabilize duplex DNA, such as **23**, **34** and **38**, are inactive. DNA binding is certainly not a good criterion to predict cytotoxicity in this series. On the opposite, topoisomerase I inhibition (i.e., enzyme-mediated DNA cleavage) may represent a useful indicator of the drug antiproliferative activity.

The relationship between topoisomerase I poisoning and cytotoxicity was investigated further with the other cell lines. GI_{50} values determined with each type of cells were plotted against the extent of DNA cleavage measured with the different compounds. The graphics in Figure 4a shows a satisfactory relation between the enzyme inhibitory activity and the cytotoxicity toward the two prostatic cell lines DU-145 (metastatic and androgen-insensitive) and LN-CaP (hormonally responsive but non metastatic). In both cases, the most cytotoxic compounds (low GI_{50} values) generally correspond the most potent inhibitors of topoisomerase I. A similar conclusion can be drawn when looking at the LoVo human colon cells and IGROV ovarian cells. In most cases (but not all) the compounds which strongly stimulate DNA cleavage by topoisomerase I were the most potent cytotoxic agents (Fig. 4b and c). The use of cells resistant to doxorubicin (LOVO-DOX) or to ecteinascidin 743 (IGROV-ET) gave the same results. Here again, we found that the less cytotoxic molecules were generally devoid of activity against topoisomerase I whereas inhibition of the enzyme often coincided with a marked cytotoxic potential. These data showing a correlation between topoisomerase I inhibition and cytotoxicity are extremely important as they strongly suggest that the topoisomerase I-mediated DNA cleavage assay can be used as a guide to the development of superior analogues in this series. This observation may greatly facilitate the optimization of the LAM-D lead compound. However, one should bear in mind that no general rule can be drawn. This is illustrated with the HT29 human colon carcinoma cell line which is poorly sensitive to LAM-D[11] and with which we could not detect any correlation between cytotoxicity and topoisomerase I inhibition (Fig. 4d).

In conclusion, our SAR study reveals that LAM-D derivatives represent a novel family of topoisomerase I-targeted antitumor agents. The 5–6 double bond in the quinoline B-ring of LAM-D is absolutely required to maintain an activity against topoisomerase I and a

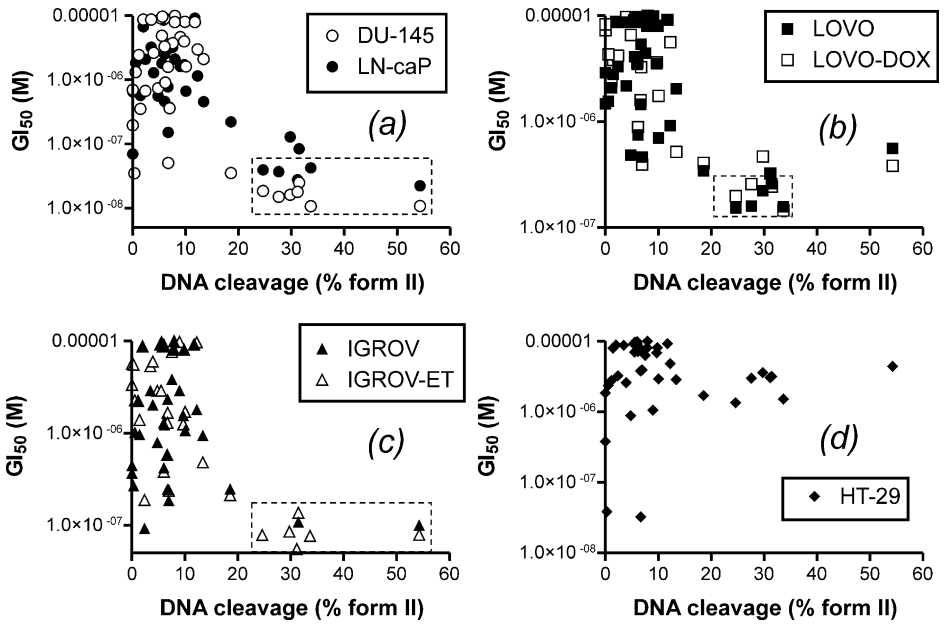


Figure 4. Correlation between cytotoxicity and topoisomerase I inhibition. In each graph the extent of topoisomerase I-mediated DNA cleavage measured with each compound at 1 μM is plotted as a function of the GI_{50} values determined with the indicated cell lines (see text for details on the origin of the tumor cells). In panels (a), (b) and (c), note that the most cytotoxic molecules are generally the most potent topoisomerase I poisons (dashed box).

potent cytotoxic action. The three phenolic OH at positions 8, 14 and 20 are also important structural elements of the LAM-D structure but they can be substituted with amino acid derivatives without loss of activity. The cationic proline (**30**) and valine (**26**) derivatives have been selected for a preclinial development to evaluate their antitumor activity in vivo.

3. Experimental

3.1. Chemistry

3.1.1. General methods. ^1H and ^{13}C NMR spectra were recorded on a Varian AC300 instrument (300 MHz for ^1H, 75 MHz for ^{13}C) using $CDCl_3$, CD_3OD or $(CD_3)_2SO$. Chemical shifts were reported in ppm (δ scale) relative to Me_4Si as an internal standard, and all J values were in Hz. Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. All air- and water-sensitive reactions were performed in flame-dried glassware under a positive pressure of argon. Thin-layer chromatography was carried out on SDS precoated silica gel 60 F_{254} plates. The spots were visualized with UV light (254 and 366 nm). Column chormatography was performed using the indicated solvents on silica gel 60 (0.040–0.063 mm, SDS). Mass spectra (ESI and APCI) were recorded on a HP Series 1100.

3.1.2. 5,6-dihydro-3,11-Diisopropoxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6*H*-[1]Benzopyrano[4′,3′:4,5]pyrrolo[2,1-a]isoquinolin-6-one (41). 6-Isopropoxy-7-methoxy-3,4-dihydroisoquinoline **44** (1.06 g, 4.83 mmol) was added to a solution of Iodo-acetic acid 5-isopropoxy-2-(4-isopropoxy-3-methoxy-phenylethynyl)-4-methoxy-phenyl ester **43** (2.30 g, 4.27 mmol) in anhydrous 1,2-dichloroethane (40 mL) under Argon atmosphere. The reaction mixture was stirred for 8 h at 23 °C turning to an orange solution. At this time, diisopropylamine (DIPEA, 0.75 mL, 4.31 mmol) was added and the resulting brown solution stirred at 85 °C for 32 h. The reaction mixture was cooled to 23 °C, silica gel (6 g) was added and the solvent removed under reduced pressure. The resulting crude was purified by chromatography on silica gel (hexane:CH_2Cl_2:Et_2O, 5:5:2) to afford **41** as a pale yellow solid (1.27 g, 47%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.08–7.04 (m, 3H), 6.92 (s, 1H), 6.76–6.74 (m, 2H), 6.67 (s, 1H), 4.87–4.71 (m, 2H), 4.65–4.48 (m, 3H), 3.82 (s, 3H), 3.42 (s, 3H), 3.33 (s, 3H), 3.09 (t, J = 6.6 Hz, 2H), 1.41–1.36 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 151.2, 148.5, 147.2, 146.9, 146.8, 146.4, 145.8, 135.9, 128.5, 128.1, 126.3, 123.3, 120.1, 116.8, 114.8, 114.6, 114.5, 113.6, 110.3, 109.1, 104.8, 103.4, 71.7, 71.3, 71.2, 56.1, 55.4, 55.0, 42.3, 28.5, 22.0 (2C), 21.8, 21.8, 21.7 (2C). MS (ESI) m/z: 628 $(M+1)^+$. R_f: 0.28 (hexane:CH_2Cl_2:Et_2O, 5:5:2).

3.1.3. 3,11-Diisopropoxy-14-(4-isopropoxy-3-methoxyphenyl)-2,12-dimethoxy-6*H*-[1]Benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (42). A suspension of **41** (301 mg, 0.480 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 139 mg, 0.611 mmol) in $CHCl_3$ (10 mL) was refluxed for 2 h. The mixture was cooled at 23 °C then filtered through Celite, and washed with CH_2Cl_2. The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane:EtOAc, 2:1) to give **42** (283 mg, 94%). ^1H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, J = 7.3 Hz, 1H), 7.29–7.17 (m, 2H), 7.12–7.10 (m, 2H), 7.04–7.02 (m, 2H), 6.98 (s, 1H), 6.76 (s, 1H), 4.70–4.56 (m, 3H), 3.84 (s, 3H), 3.44 (s, 3H), 3.43 (s, 3H), 1.44–1.39 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 151.2, 150.0, 148.3, 147.7, 147.0, 146.4, 146.3, 134.2,

129.2, 128.6, 124.6, 123.8, 122.9, 118.8, 116.7, 114.9, 112.1, 110.8, 110.2, 109.8, 107.6, 105.5, 105.3, 103.2, 71.6, 71.3, 71.0, 56.0, 55.3, 55.0, 21.8 (3C), 21.7, 21.7, 21.6. MS (ESI) m/z: 648 $(M+23)^+$, 626 $(M+1)^+$. R_f: 0.35 (hexane:EtOAc, 2:1).

3.1.4. LAM-D (1). A suspension of **42** (271 mg, 0.433 mmol) and $AlCl_3$ (462 mg, 3.465 mmol) in anhydrous CH_2Cl_2 (4 mL) was stirred at 23 °C for 5.5 h under Argon atmosphere. H_2O was added, then HCl 2 M until pH 1–2, the resulting aqueous solution was extracted with CH_2Cl_2 (3×), dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (EtOAc, 100%) to afford **LAM-D (1)** as a pale yellow solid (92 mg, 43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.81 (s, 1H), 9.32 (s, 1H), 8.98 (d, $J=7.3$ Hz, 1H), 7.22–6.98 (m, 6H), 6.85 (s, 1H), 6.70 (s, 1H), 3.75 (s, 3H), 3.36 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.3, 148.7, 148.5, 148.3, 147.8, 146.8, 146.3, 144.6, 134.1, 129.2, 128.9, 125.5, 124.7, 123.9, 117.6, 116.4, 115.1, 113.9, 112.3, 111.5, 110.8, 106.4, 105.7, 105.4, 103.7, 56.0, 55.1, 54.5. MS (APCI) m/z: 500 $(M+1)^+$. R_f: 0.60 (EtOAc).

3.1.5. LAM-501 (2). A suspension of **41** (1.422 g, 2.265 mmol) and $AlCl_3$ (1.208 g, 9.061 mmol) in anhydrous CH_2Cl_2 (43 mL) was stirred at 23 °C for 2.5 h under Argon atmosphere. MeOH (20 mL) was added and the solvent evaporated under reduced pressure. The brown residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 20:1 to 10:1 to 5:1) to afford **LAM-501 (2)** as a pale brown solid (1.11 g, 97%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.64 (s, 1H), 9.41 (s, 1H), 9.24 (s, 1H), 7.01 (s, 1H), 6.99 (d, $J=8.1$ Hz, 1H), 6.88 (d, $J=8.4$ Hz, 1H), 6.78 (s, 1H), 6.73 (s, 1H), 6.67 (s, 1H), 6.59 (s, 1H), 4.58 (t, $J=6.5$ Hz, 2H), 3.73 (s, 3H), 3.34 (s, 3H), 3.25 (s, 3H), 2.99 (t, $J=6.4$ Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.3, 148.5, 147.1, 146.9, 146.5, 146.0, 145.7, 144.4, 135.9, 127.7, 127.1, 125.5, 123.4, 118.1, 116.3, 115.3, 114.7, 114.3, 112.2, 109.2, 108.8, 105.1, 103.6, 56.0, 55.0, 54.7, 42.0, 27.5. MS (ESI) m/z: 524 $(M+23)^+$. R_f: 0.55 (CH_2Cl_2:MeOH 10:1).

3.1.6. Compound 3. A suspension of **4** (20 mg, 0.032 mmol) and DDQ (15 mg, 0.064 mmol) in $CHCl_3$ (2 mL) was refluxed for 24 h. The mixture was cooled to 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 40:1) to give **3** (12 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (d, $J=7.3$ Hz, 1H), 7.39 (s, 1H), 7.30 (d, $J=7.7$ Hz, 1H), 7.25–7.22 (m, 3H), 7.14 (s, 1H), 7.05 (d, $J=7.5$ Hz, 1H), 6.81 (s, 1H), 3.84 (s, 3H), 3.45 (s, 6H), 2.37 (s, 3H), 2.34 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 168.7, 168.7, 155.0, 152.4, 151.0, 147.8, 145.4, 140.9, 140.3, 139.7, 134.2, 133.5, 128.2, 124.1, 123.8, 123.7, 123.6, 123.1, 120.7, 115.7, 115.0, 112.8, 112.3, 112.2, 109.1, 106.4, 106.1, 56.2, 55.7, 55.6, 20.6 (3C). MS (ESI) m/z: 626 $(M+1)^+$. R_f: 0.40 (CH_2Cl_2:MeOH, 100:1).

3.1.7. Compound 4. A solution of **LAM-501 (2)** (40 mg, 0.08 mmol) and Ac_2O (0.5 mL, 5.289 mmol) in pyridine (1 mL) was stirred at 23 °C under Argon atmosphere for 4 h. The resulting pale yellow solution was washed with HCl 1 N (2×10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 50:1 to 40:1) to give **4** as a white solid (38 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, $J=7.9$ Hz, 1H), 7.15–7.09 (m, 3H), 6.95 (s, 1H), 6.79 (s, 1H), 6.69 (s, 1H), 4.92–4.71 (m, 2H), 3.81 (s, 3H), 3.43 (s, 3H), 3.35 (s, 3H), 3.12 (t, $J=6.7$ Hz, 2H), 2.35 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 168.8, 168.6, 155.1, 152.2, 149.9, 147.7, 144.9, 140.0, 139.4, 138.9, 135.0, 134.0, 127.1, 125.9, 125.7, 123.9, 123.2, 122.6, 116.0, 115.9, 114.9, 114.6, 112.0, 109.7, 105.4, 56.2, 55.7, 55.5, 42.5, 28.1, 20.6 (3C). MS (ESI) m/z: 628 $(M+1)^+$. R_f: 0.32 (CH_2Cl_2:MeOH, 100:1).

3.1.8. Compound 5. A suspension of **6** (25 mg, 0.034 mmol) and DDQ (15 mg, 0.068 mmol) in $CHCl_3$ (2 mL) was refluxed for 77 h. The mixture was cooled to 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 80:1) to give **5** (17 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (d, $J=7.5$ Hz, 1H), 7.66 (s, 1H), 7.60 (d, $J=8.6$ Hz, 1H), 7.33–7.30 (m, 3H), 7.18 (s, 1H), 7.06 (d, $J=7.7$ Hz, 1H), 6.76 (s, 1H), 3.96 (s, 3H), 3.49 (s, 6H), 3.37 (s, 3H), 3.24 (s, 3H), 3.21 (s, 3H). MS (APCI) m/z: 734 $(M+1)^+$. R_f: 0.33 (CH_2Cl_2:MeOH, 80:1).

3.1.9. Compound 6. To a suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol) in anhydrous CH_2Cl_2 (2 mL) under Argon at 0 °C, Et_3N (83 μL, 0.5982 mmol) and methanesulfonyl chloride (47 μL, 0.5982 mmol) were added. The resulting mixture was stirred at 23 °C for 6 h, then quenched with H_2O and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with saturated aqueous solution of $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The resulting residue was purified on silica gel (CH_2Cl_2:MeOH, 80:1) to afford **6** as a pale yellow solid (47 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, $J=8.1$ Hz, 1H), 7.30 (s, 1H), 7.23–7.20 (m, 2H), 7.17 (d, $J=1.6$ Hz, 1H), 6.75 (s, 1H), 6.65 (s, 1H), 4.99–4.90 (m, 1H), 4.71–4.61 (m, 1H), 3.92 (s, 3H), 3.46 (s, 3H), 3.38 (s, 3H), 3.34 (s, 3H), 3.19 (s, 3H), 3.18 (s, 3H), 3.14 (t, $J=6.0$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.5, 152.8, 150.1, 148.0, 144.6, 138.0, 137.7, 136.9, 135.4, 134.4, 126.5, 126.4, 126.3, 125.6, 124.4, 123.3, 117.0, 115.8, 115.4, 115.2, 113.5, 109.9, 105.6. MS (ESI) m/z: 736 $(M+1)^+$. R_f: 0.33 (CH_2Cl_2:MeOH, 80:1).

3.1.10. Compound 7. A suspension of **8** (23 mg, 0.026 mmol) and DDQ (12 mg, 0.051 mmol) in $CHCl_3$ (2 mL) was refluxed for 21 h. The mixture was cooled to 23 °C then filtered through Celite and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 100:1) to give **7** (16

mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (d, J = 7.3 Hz, 1H), 7.39–7.20 (m, 20H), 7.08–7.03 (m, 2H), 6.80 (s, 1H), 3.79 (s, 3H), 3.42 (s, 6H), 3.16–3.06 (m, 6H), 3.00–2.90 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.7, 170.6, 155.1, 152.4, 151.0, 147.7, 145.4, 140.9, 140.2, 140.1 (2C), 139.7, 134.2, 133.5, 128.5 (6C), 128.4 (2C), 128.4 (4C), 128.2, 126.4, 126.4 (2C), 124.0, 123.8, 123.6, 123.6, 123.1, 120.7, 115.6, 115.0, 112.8, 112.3, 112.1, 109.0, 106.4, 106.1, 56.2, 55.7, 55.6, 35.4 (3C), 30.9, 30.8 (2C). MS (APCI) m/z: 896 $(M+1)^+$. R_f: 0.25 (CH_2Cl_2:MeOH, 200:1).

3.1.11. Compound 8. To a suspension of **LAM-501 (2)** (25 mg, 0.050 mmol) in anhydrous CH_2Cl_2 (2 mL), 4-(dimethylamino)pyridine (DMAP, 4 mg, 0.030 mmol), pyridine (24 μL, 0.300 mmol) and hydrocinnamoyl chloride (45 μL, 0.300 mmol) were added. The reaction mixture was stirred under Argon atmosphere at 23 °C for 22 h, then diluted with EtOAc (50 mL) and washed with H_2O (2×20 mL) and saturated aqueous solution of $NaHCO_3$ (2×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 200:1 to 100:1) to give **8** as a white solid (31 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.21 (m, 15H), 7.13–7.02 (m, 4H), 6.87 (s, 1H), 6.77 (s, 1H), 6.68 (s, 1H), 4.92–4.83 (m, 1H), 4.79–4.70 (m, 1H), 3.76 (s, 3H), 3.39 (s, 3H), 3.32 (s, 3H), 3.13–3.04 (m, 8H), 2.97–2.87 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.7, 170.6, 155.1, 152.2, 149.8, 147.7, 144.9, 140.1 (3C), 140.0, 139.4, 138.9, 135.0, 133.9, 128.5 (6C), 128.4 (6C), 127.1, 126.4, 126.4, 126.4, 125.9, 125.6, 123.8, 123.1, 122.6, 116.0, 115.9, 114.9, 114.6, 111.9, 109.7, 105.4, 56.1, 55.7, 55.5, 42.4, 35.5 (3C), 30.9, 30.9, 30.8, 28.0. MS (ESI) m/z: 898 $(M+1)^+$. R_f: 0.25 (CH_2Cl_2:MeOH, 200:1).

3.1.12. Compound 9. A suspension of **10** (26 mg, 0.026 mmol) and DDQ (9 mg, 0.039 mmol) in $CHCl_3$ (1.5 mL) was refluxed for 40 h. The mixture was cooled at 23 °C, filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 200:1 to 100:1) to give **9** (17 mg, 65%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.21 (d, J = 7.3 Hz, 1H), 7.58–7.50 (m, 6H), 7.31 (s, 1H), 7.21–7.16 (m, 4H), 7.10–7.01 (m, 8H), 6.75 (s, 1H), 3.87 (s, 2H), 3.84 (s, 2H), 3.81 (s, 2H), 3.78 (s, 3H), 3.37 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6, 167.5 (2C), 162.6 (d, $J_{C\text{-}F}$ = 248.3, 3C), 154.9, 152.3, 150.8, 147.6, 145.4, 140.6, 140.0, 139.5, 134.6, 133.9, 133.8, 133.8, 133.7, 133.6, 133.4, 129.5, 129.3, 128.1, 123.8, 123.6, 123.2, 120.5, 116.4 (6C), 116.1 (6C), 115.9, 115.1, 112.8, 112.3, 112.0, 109.1, 106.4, 106.2. MS (ESI) m/z: 1026 $(M+23)^+$, 1004 $(M+1)^+$. R_f: 0.35 (CH_2Cl_2).

3.1.13. Compound 10. A suspension of **LAM-501 (2)** (25 mg, 0.05 mmol), (4-Fluorophenyl)thioacetic acid (56 mg, 0.30 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 58 mg, 0.30 mmol) and DMAP (4 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred under Argon atmosphere at 23 °C for 2 h. The resulting pale yellow solution was washed with H_2O (10 mL), the aqueous phase was extracted with CH_2Cl_2 (10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (hexane:EtOAc, 60:40) to give **10** as a white solid (47 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57–7.49 (m, 6H), 7.16–7.00 (m, 10H), 6.86 (s, 1H), 6.74 (s, 1H), 6.64 (s, 1H), 4.89–4.85 (m, 1H), 4.75–4.70 (m, 1H), 3.85 (s, 2H), 3.79 (s, 4H), 3.75 (s, 3H), 3.34 (s, 3H), 3.28 (s, 3H), 3.09 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 167.5, 167.4, 164.2, 160.9, 154.9, 152.1, 149.7, 147.5, 144.8, 139.7, 139.2, 138.6, 134.9, 134.3, 133.8, 133.7, 133.6, 133.6, 129.3, 129.3, 126.9, 125.9, 125.8, 123.6, 123.1, 122.3, 116.4 (6C), 116.2, 116.1 (6C), 115.8, 115.0, 114.7, 111.7, 109.7, 105.5, 56.1, 55.6, 55.4, 42.4, 37.5 (3C), 27.8. MS (ESI) m/z: 1006 $(M+1)^+$. R_f: 0.40 (hexane:EtOAc, 60:40).

3.1.14. Compound 11. A suspension of **12** (20 mg, 0.019 mmol) and DDQ (9 mg, 0.039 mmol) in CCl_4 (2 mL) was refluxed for 7 h. The mixture was cooled at 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 100:1) to give **11** (15 mg, 75%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, J = 7.5 Hz, 1H), 7.84–7.75 (m, 3H), 7.54 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.37–7.35 (m, 2H), 7.30 (s, 1H), 7.26 (s, 1H), 7.08 (d, J = 7.3 Hz, 1H), 6.90 (s, 1H), 3.87 (s, 3H), 3.52 (s, 3H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7 (3C), 154.8, 152.3, 150.8, 148.2, 147.6, 145.4, 140.3, 139.8, 139.2, 135.0, 133.4, 128.0, 124.0, 123.9, 123.8, 123.7, 123.2, 120.7, 116.2, 115.3, 113.8, 113.6, 112.8, 112.4, 112.1, 109.2, 106.6, 106.3, 56.4, 55.9, 55.8. MS (ESI) m/z: 1050 $(M+23)^+$, 1028 $(M+1)^+$. R_f: 0.63 (CH_2Cl_2).

3.1.15. Compound 12. A suspension of **LAM-501 (2)** (25 mg, 0.050 mmol), 2,3,4,5-tetrafluorobenzoic acid (58 mg, 0.30 mmol), EDC·HCl (58 mg, 0.30 mmol) and DMAP (4 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred under Argon atmosphere at 23 °C for 6 h. The resulting pale yellow solution was washed with H_2O (10 mL), the aqueous phase was extracted with CH_2Cl_2 (10mL), the combined organic phases were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 200:1) to give **12** as a white solid (33 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81–7.74 (m, 3H), 7.36 (d, J = 8.1 Hz, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 7.19 (s, 1H), 7.10 (s, 1H), 6.86 (s, 1H), 6.78 (s, 1H), 4.97–4.91 (m, 1H), 4.82–4.77 (m, 1H), 3.83 (s, 3H), 3.48 (s, 3H), 3.41 (s, 3H), 3.17 (t, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6 (3C), 154.9, 152.1, 149.7, 147.5, 144.9, 139.5, 138.9, 138.3, 134.9, 134.7, 126.9, 126.2, 126.1, 123.7, 123.3, 122.5, 116.5, 115.9, 115.2, 114.9, 113.8, 113.5, 111.9, 109.9, 105.6, 56.3, 55.9, 55.7, 42.5, 28.1. MS (APCI) m/z: 1030 $(M+1)^+$. R_f: 0.50 (CH_2Cl_2:MeOH, 200:1).

3.1.16. Compound 13. A suspension of **LAM-501 (2)** (25 mg, 0.050 mmol), coumarin 3-carboxylic acid (38 mg, 0.200 mmol), EDC·HCl (38 mg, 0.200 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4 mL) was stirred under Argon atmosphere at 23 °C for 3.5 h. The resulting pale yellow solution was washed with H_2O (10 mL) and saturated aqueous solution of $NaHCO_3$ (10 mL). Both aqueous phases were extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 50:1 to 40:1) to give **13** as a white solid (41 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.79 (s, 1H), 8.75 (s, 1H), 7.72–7.65 (m, 6H), 7.42–7.34 (m, 7H), 7.26–7.21 (m, 3H), 7.23 (s, 1H), 6.85 (s, 1H), 6.81 (s, 1H), 4.93–4.86 (m, 1H), 4.78–4.69 (m, 1H), 3.84 (s, 3H), 3.50 (s, 3H), 3.44 (s, 3H), 3.15 (br t, 2H). MS (ESI) *m/z*: 1040 $(M+23)^+$. R_f: 0.24 (CH_2Cl_2:MeOH, 50:1).

3.1.17. Compound 14. A suspension of **LAM-501 (2)** (25 mg, 0.05 mmol), 9H-fluorene-4-carboxylic acid (63 mg, 0.30 mmol), EDC·HCl (58 mg, 0.30 mmol) and DMAP (4 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred under Argon atmosphere at 23 °C for 2 h. The resulting pale yellow solution was washed with H_2O (10 mL), the aqueous phase was extracted with CH_2Cl_2 (10 mL), the combined organic phases were dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 200:1) to give **14** as a white solid (26 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56–8.52 (m, 2H), 8.46–8.43 (m, 1H), 8.18–8.11 (m, 3H), 7.77–7.74 (m, 3H), 7.58–7.56 (m, 3H), 7.50–7.30 (m, 13H), 7.21 (s, 1H), 7.04 (s, 1H), 6.93 (s, 1H), 5.04–4.95 (m, 1H), 4.92–4.83 (m, 1H), 3.96 (s, 6H), 3.93 (s, 3H), 3.62 (s, 3H), 3.54 (s, 3H), 3.25 (t, $J=6.5$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 166.0, 166.0, 155.2, 152.6, 150.2, 148.0, 145.2, 145.2, 145.1, 144.2, 144.2, 144.2, 141.5, 141.3, 140.4, 139.9, 139.8, 139.2, 135.2, 134.3, 129.5, 129.3, 129.1, 129.0, 127.7, 127.3, 126.9, 126.8, 126.1, 126.1, 126.0, 125.9, 125.4, 125.1, 125.1, 125.0, 124.7, 124.6, 124.1, 123.4, 122.8, 116.3, 116.1, 115.1, 114.8, 112.2, 109.9, 105.7, 56.3, 55.9, 55.7, 42.6, 37.0 (3C), 28.2. MS (ESI) *m/z*: 1100 $(M+23)^+$. R_f: 0.47 (CH_2Cl_2).

3.1.18. Compound 15. To a suspension of **LAM-501 (2)** (15 mg, 0.030 mmol) in anhydrous CH_2Cl_2 under Argon atmosphere, Et_3N (17 μL, 0.120 mmol) and diethyl chlorophosphate (18 μL, 0.120 mmol) were added and the mixture was stirred at 23 °C. After 4.5 h, two more equivalents of Et_3N (9 μL, 0.060 mmol) and diethyl chlorophosphate (9 μL, 0.060 mmol) were added and the mixture stirred at 23 °C overnight. The mixture was concentrated under reduced pressure and the residue purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 30:1 to 15:1) to give **15** as a white solid (20 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (dd, $J=8.1$, 1.5 Hz, 1H), 7.29 (d, $J=1.5$ Hz, 1H), 7.18 (s, 1H), 7.10 (dd, $J=8.1$, 1.6 Hz, 1H), 7.06 (s, 1H), 6.71 (s, 1H), 6.64 (s, 1H), 4.94–4.86 (m, 1H), 4.72–4.63 (m, 1H), 4.34–4.18 (m, 12H), 3.84 (s, 3H), 3.44 (s, 3H), 3.36 (s, 3H), 3.09 (br t, 2H), 1.44–1.31 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 151.7 (d, $J_{C\text{-}P}=4.5$ Hz), 149.3 (d, $J_{C\text{-}P}=5.5$ Hz), 147.4 (d, $J_{C\text{-}P}=5.0$ Hz), 144.9, 139.9 (d, $J_{C\text{-}P}=7.1$ Hz), 139.6 (d, $J_{C\text{-}P}=6.5$ Hz), 139.1 (d, $J_{C\text{-}P}=7.6$ Hz), 135.0, 133.0, 127.0, 126.2, 124.4, 123.2, 122.6, 122.6, 121.1, 115.5, 114.9, 114.8, 110.5, 109.8, 105.6, 64.7, 64.7, 64.7 (3C), 64.6, 56.2, 55.8, 55.5, 42.4, 28.1, 16.2, 16.1 (3C), 16.0, 16.0. MS (ESI) *m/z*: 910 $(M+1)^+$. R_f: 0.23 (CH_2Cl_2:MeOH, 30:1).

3.1.19. Compound 17. A suspension of **19** (63 mg, 0.062 mmol) and DDQ (21 mg, 0.093 mmol) in $CHCl_3$ (5 mL) was refluxed for 2 h. The mixture was cooled at 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (hexane:EtOAc, 50:50) to give **17** (58 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, $J=7.3$ Hz, 1H), 7.44–7.32 (m, 2H), 7.25–7.18 (m, 4H), 7.07 (d, $J=7.5$ Hz, 1H), 6.79 (d, $J=7.5$ Hz, 1H), 5.11–5.09 (m, 3H), 4.64–4.60 (m, 3H), 3.81 (s, 3H), 3.44 (s, 6H), 1.63–1.55 (m, 9H), 1.49 (s, 9H), 1.47 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 171.3, 171.1, 155.0, 154.8, 152.2, 150.8, 147.6, 145.3, 140.6, 140.0, 139.4, 134.4, 133.3, 128.0, 127.9, 123.9, 123.7, 123.7, 123.7, 123.6, 123.0, 120.6, 115.7, 115.1, 112.7, 112.2, 112.0, 108.9, 106.3, 106.1, 80.0 (3C), 56.2 (2C), 55.8, 55.7, 55.7, 55.5, 28.3 (9C), 18.6 (3C). MS (ESI) *m/z*: 1035 $(M+23)^+$, 1013 $(M+1)^+$. R_f: 0.43 (hexane:EtOAc, 50:50).

3.1.20. Compound 18. TFA (1 mL) was added to a solution of **17** (15 mg, 0.015 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 5 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **18** as a white solid (16 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 9.03–8.99 (m, 1H), 7.63–7.60 (m, 2H), 7.52 (dd, $J=8.0$, 1.6 Hz, 1H), 7.39–7.27 (m, 2H), 7.18–7.15 (m, 2H), 6.85 (d, $J=9.2$ Hz, 1H), 4.53–4.36 (m, 3H), 3.93 (s, 3H), 3.48 (s, 6H), 1.80 (d, $J=7.1$ Hz, 3H), 1.74 (d, $J=7.3$ Hz, 3H), 1.71–1.68 (m, 3H). MS (ESI) *m/z*: 713 $(M+1)^+$.

3.1.21. Compound 19. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Ala-OH (75 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 2 h. The resulting pale yellow solution was washed with H_2O (10 mL) and HCl 0.1 N (10 mL) and the aqueous phases were extracted with CH_2Cl_2 (10 mL). The organic phases were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (hexane:EtOAc, from 2:1 to 1:1) to give **19** as a white solid (81 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.09 (m, 4H), 6.97 (s, 1H), 6.75 (d, $J=7.7$ Hz, 1H), 6.67 (d, $J=10.1$ Hz, 1H), 5.12 (br s, 2H), 4.89–4.85 (m, 1H), 4.70–4.55 (m, 3H), 3.78 (s, 3H), 3.40 (s, 3H), 3.33 (s, 3H), 2.03 (br t, 2H), 1.58 (d, $J=7.1$ Hz, 3H), 1.52 (d, $J=7.1$ Hz, 6H),

1.47 (s, 9H), 1.45 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 155.0, 152.0, 149.7, 147.4, 144.8, 139.7, 139.1, 138.6, 135.0, 134.1, 126.9, 126.8, 126.0, 125.9, 125.7, 123.7, 123.1, 122.4, 116.1, 115.8, 114.9, 114.7, 111.7, 109.6, 105.4, 79.9, 60.3, 56.1, 55.7, 55.7, 55.5, 55.4, 49.2, 42.3, 28.2, 27.9, 21.0, 18.5, 14.1. MS (ESI) *m/z*: 1037 $(M+23)^+$, 1015 $(M+1)^+$. R_f: 0.44 (hexane:EtOAc, 50:50).

3.1.22. Compound 20. TFA (1 mL) was added to a solution of **19** (15 mg, 0.0148 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 2 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **20** as a white solid (17 mg, quant.) ^{1}H NMR (300 MHz, CD_3OD) δ 7.46–7.44 (m, 2H), 7.28–7.27 (m, 1H), 7.19 (s, 1H), 7.16 (s, 1H), 6.87 (d, $J=8.8$ Hz, 1H), 6.78 (d, $J=10.0$ Hz, 1H), 4.75 (t, $J=6.2$ Hz, 2H), 4.77–4.37 (m, 3H), 3.87 (s, 3H), 3.45 (s, 3H), 3.38 (s, 3H), 3.16 (t, $J=6.2$ Hz, 2H), 1.77 (d, $J=6.9$ Hz, 3H), 1.71–1.67 (m, 6H). MS (ESI) *m/z*: 715 $(M+1)^+$.

3.1.23. Compound 21. A suspension of **23** (52 mg, 0.046 mmol) and DDQ (16 mg, 0.068 mmol) in $CHCl_3$ (5 mL) was refluxed for 35 h. The mixture was cooled at 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 100:1) to give **21** (38 mg, 73%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (d, $J=7.3$ Hz, 1H), 7.42 (s, 1H), 7.32 (d, $J=7.9$ Hz, 1H), 7.25–7.17 (m, 4H), 7.05 (d, $J=7.7$ Hz, 1H), 6.79 (d, $J=5.9$ Hz, 1H), 4.98–4.96 (m, 3H), 4.62–4.56 (m, 3H), 3.80 (s, 3H), 3.44 (s, 3H), 3.43 (s, 3H), 1.87–1.64 (m, 9H), 1.49 (s, 9H), 1.46 (s, 18H), 1.06–0.99 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (4C), 155.3, 155.0, 152.3, 150.9, 147.7, 145.4, 140.7, 140.1, 139.6, 134.4, 128.1, 124.0, 123.8, 123.6, 123.2, 120.7, 115.8, 115.1, 112.8, 112.3, 112.2, 109.1, 106.4, 106.2, 80.0 (3C), 56.2 (2C), 55.8 (2C), 55.7, 52.2, 41.7 (2C), 41.5, 28.3 (9C), 24.8 (3C), 23.0, 22.9 (3C), 21.9. MS (ESI) *m/z*: 1161 $(M+23)^+$, 1139 $(M+1)^+$. R_f: 0.45 (CH_2Cl_2:MeOH, 50:1).

3.1.24. Compound 22. TFA (1 mL) was added to a solution of **21** (15 mg, 0.013 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 5 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **22** as a white solid (19 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 9.15–9.12 (m, 1H), 7.64 (d, $J=2.7$ Hz, 1H), 7.58–7.52 (m, 2H), 7.40–7.29 (m, 2H), 7.26–7.22 (m, 2H), 6.89 (d, $J=7.3$ Hz, 1H), 4.45–4.31 (m, 3H), 3.90 (s, 3H), 3.48 (s, 3H), 3.48 (s, 3H), 2.11–1.79 (m, 9H), 1.13–1.06 (m, 18H). MS (ESI) *m/z*: 839 $(M+1)^+$.

3.1.25. Compound 23. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Leu-OH·H_2O (99 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 3 h. The resulting pale yellow solution was washed with H_2O (10 mL) and HCl 0.1 N (10 mL) and the aqueous phases were extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (hexane:EtOAc, 2:1) to give **23** as a white solid (77 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24–7.08 (m, 4H),6.99 (s, 1H), 6.76 (d, $J=7.0$ Hz, 1H), 6.69 (d, $J=8.4$ Hz, 1H), 4.94–4.86 (m, 3H), 4.78–4.68 (m, 1H), 4.63–4.50 (m, 2H), 4.37–4.26 (m, 2H), 3.78 (s, 3H), 3.41 (s, 3H), 3.34 (s, 3H), 3.12 (br t, 2H), 1.90–1.60 (m, 9H), 1.45 (s, 27H), 1.05–0.95 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6 (2C), 171.4, 155.6, 155.4, 155.1, 152.1, 149.7, 147.6, 144.8, 139.8, 139.2, 138.7, 135.0, 134.1, 127.0, 127.0, 126.0, 125.7, 123.8, 123.1, 122.5, 116.1, 115.8, 114.9, 114.7, 111.9, 109.7, 105.5, 80.0 (3C), 56.1, 55.7, 55.5, 53.1, 52.2 (2C), 42.4, 41.5 (3C), 28.3 (9C), 28.0, 24.7 (2C), 22.9, 22.8 (4C), 21.8 (2C). MS (ESI) *m/z*: 1163 $(M+23)^+$, 1141 $(M+1)^+$. R_f: 0.26 (hexane:EtOAc, 2:1).

3.1.26. Compound 24. TFA (1 mL) was added to a solution of **23** (15 mg, 0.013 mmol) in CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 5 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **24** as a white solid (14 mg, 88%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.47–7.41 (m, 2H), 7.29–7.24 (m, 2H), 7.16 (s, 1H), 6.87 (d, $J=8.2$ Hz, 1H), 6.80 (d, $J=10.1$ Hz, 1H), 4.42–4.29 (m, 3H), 3.92–3.87 (m, 2H), 3.85 (s, 3H), 3.45 (s, 3H), 3.37 (s, 3H), 3.18 (t, $J=6.2$ Hz, 2H), 2.14–1.61 (m, 9H), 1.12–0.98 (m, 18H). MS (ESI) *m/z*: 841 $(M+1)^+$.

3.1.27. Compound 25. A suspension of **27** (49 mg, 0.045 mmol) and DDQ (20 mg, 0.089 mmol) in CCl_4 (2 mL) was refluxed for 5 h. The mixture was cooled to 23 °C then filtered through Celite and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 100:1 to 50:1) to give **25** (43 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (d, $J=7.3$ Hz, 1H), 7.40 (s, 1H), 7.30–7.13 (m, 5H), 7.04 (d, $J=7.3$ Hz, 1H), 6.79 (d, $J=7.5$ Hz, 1H), 5.10–5.06 (m, 3H), 4.56–4.48 (m, 3H), 3.81 (s, 3H), 3.43 (s, 6H), 2.45–2.32 (m, 3H), 1.49 (s, 9H), 1.47 (s, 18H), 1.14–1.00 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4 (3C), 155.7, 155.0, 152.3, 150.9, 147.6, 145.4, 140.6, 140.0, 139.4, 134.5, 133.5, 130.9, 128.8, 128.2, 128.1, 124.1, 123.8, 123.6, 123.2, 120.8, 115.9, 115.1, 112.8, 112.3, 112.2, 109.1, 106.4, 106.2, 80.0 (3C), 58.5, 56.0, 55.6, 55.6, 55.5, 55.5, 31.3 (2C), 31.2, 28.3 (9C), 19.2, 19.1, 17.2 (2C), 17.1 (2C). MS (ESI) *m/z*: 1119 $(M+23)^+$, 1097 $(M+1)^+$. R_f: 0.33 (CH_2Cl_2:MeOH, 100:1).

3.1.28. Compound 26. TFA (1 mL) was added to a solution of **25** (15 mg, 0.014 mmol) in CH_2Cl_2 (3 mL) at

0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 4 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **26** as a white solid (21 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 9.09–9.04 (m, 1H), 7.62–7.51 (m, 3H), 7.41–7.32 (m, 2H), 7.23–7.18 (m, 2H), 6.88 (d, J = 9.0 Hz, 1H), 4.36 (d, J = 4.4 Hz, 1H), 4.31 (d, J = 4.4 Hz, 1H), 4.24 (t, J = 4.4 Hz, 1H), 3.92 (s, 3H), 3.48 (s, 6H), 2.62–2.43 (m, 3H), 1.29–1.19 (m, 18H). MS (APCI) m/z: 797 $(M+1)^+$.

3.1.29. Compound 27. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Val-OH (87 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 4 h. The resulting pale yellow solution was washed with H_2O (10 mL) and saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (10 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 100:1 to 50:1) to give **27** as a white solid (87 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.13 (m, 2H), 7.09 (s, 2H), 6.96 (s, 1H), 6.76 (d, J = 7.5 Hz, 1H), 6.68 (d, J = 9.5 Hz, 1H), 5.08–5.05 (m, 3H), 4.91–4.69 (m, 2H), 4.52–4.46 (m, 3H), 3.77 (s, 3H), 3.40 (s, 3H), 3.32 (s, 3H), 3.18 (br t, 2H), 2.42–2.38 (m, 3H), 1.48 (s, 9H), 1.45 (s, 18H), 1.11–0.98 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (3C), 155.6, 154.9, 152.1, 149.7, 147.5, 144.8, 139.7, 139.1, 138.5, 134.9, 134.2, 126.9, 126.0, 125.7, 123.8, 123.1, 122.5, 116.1, 115.8, 115.0, 114.6, 111.9, 109.6, 105.4, 79.9 (3C), 58.5, 56.0, 55.6, 55.3, 55.3, 53.4, 42.4, 31.2 (3C), 28.3 (9C), 28.0, 19.1 (2C), 17.1 (4C). MS (ESI) m/z: 1121 $(M+23)^+$, 1099 $(M+1)^+$. R_f: 0.35 (CH_2Cl_2:MeOH, 50:1).

3.1.30. Compound 28. TFA (1 mL) was added to a solution of **27** (15 mg, 0.0136 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 4 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **28** as a white solid (16 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 7.47–7.44 (m, 2H), 7.28 (d, J = 8.1 Hz, 1H), 7.19 (s, 1H), 7.15 (s, 1H), 6.90–6.78 (m, 2H), 4.77 (br t, 2H), 4.32 (s, 1H), 4.24 (s, 2H), 3.87 (s, 3H), 3.45 (s, 3H), 3.37 (s, 3H), 3.17 (br t, 2H), 2.54–2.46 (m, 3H), 1.26–1.17 (m, 18H). MS (ESI) m/z: 799 $(M+1)^+$.

3.1.31. Compound 29. A suspension of **31** (45 mg, 0.041 mmol) and DDQ (19 mg, 0.082 mmol) in CCl_4 (2 mL) was refluxed for 17 h. The mixture was cooled at 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 30:1) to give **29** (30 mg, 67%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27–9.23 (m, 1H), 7.47–7.36 (m, 1H), 7.26–7.08 (m, 6H), 6.84–6.78 (m, 1H), 4.56–4.49 (m, 3H), 3.80 (s, 3H), 3.66–3.47 (m, 6H), 3.43 (s, 6H), 2.40–2.29 (m, 6H), 2.04–1.98 (m, 6H), 1.49 (s, 27H). MS (ESI) m/z: 1091 $(M+1)^+$. R_f: 0.31 (CH_2Cl_2:MeOH 30:1).

3.1.32. Compound 30. TFA (1 mL) was added to a solution of **29** (15 mg, 0.014 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 2 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **30** as a white solid (19 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 8.96–8.90 (m, 1H), 7.67–7.52 (m, 3H), 7.40–7.24 (m, 2H), 7.13–7.08 (m, 2H), 6.84–6.80 (m, 1H), 4.86–4.67 (m, 3H), 3.95 (s, 3H), 3.55–3.43 (m, 12H), 2.66–2.35 (m, 6H), 2.27–2.14 (m, 6H). MS (ESI) m/z: 791 $(M+1)^+$.

3.1.33. Compound 31. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Pro-OH (86 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 4 h. The resulting pale yellow solution was washed with H_2O (10 mL) and saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 100:1 to 50:1) to give **31** as a white solid (74 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16–7.13 (m, 2H), 7.06–7.03 (m, 2H), 6.90 (s, 1H), 6.78–6.63 (m, 2H), 4.95–4.62 (m, 2H), 4.56–4.46 (m, 3H), 3.78 (s, 3H), 3.69–3.43 (m, 6H), 3.40 (s, 3H), 3.33 (s, 3H), 3.11 (br t, 2H), 2.40–2.26 (m, 6H), 2.08–1.90 (m, 6H), 1.47 (s, 27H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.8, 170.7, 155.0, 154.3, 153.7, 153.7 (2C), 152.1, 149.7, 147.6, 144.8, 139.8, 139.3, 138.7, 134.1, 125.9, 125.6, 124.0, 123.4, 123.1 (2C), 122.7, 122.2, 116.0, 114.7, 111.6, 109.6, 105.3, 80.1, 80.0, 79.8, 58.9 (2C), 56.1, 55.7, 55.6, 55.5, 46.5, 46.3 (2C), 42.4, 31.5, 30.9, 29.9, 28.3 (9C), 28.0, 24.2, 23.4, 22.5. MS (ESI) m/z: 1115 $(M+23)^+$, 1093 $(M+1)^+$. R_f: 0.18 (CH_2Cl_2:MeOH, 50:1).

3.1.34. Compound 32. TFA (1 mL) was added to a solution of **31** (15 mg, 0.014 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred at 23 °C for 3 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **32** as a white solid (17 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 7.49–7.44 (m, 2H), 7.29–7.17 (m, 3H), 6.88–6.75 (m, 2H), 4.79–4.68 (m, 3H), 3.89 (s, 3H), 3.52–3.38 (m, 12H), 3.15 (br t, 2H), 2.63–2.35 (m, 6H), 2.25–2.15 (m, 6H). MS (ESI) m/z: 793 $(M+1)^+$.

3.1.35. Compound 33. A suspension of **35** (50 mg, 0.040 mmol) and DDQ (18 mg, 0.080 mmol) in $CHCl_3$ (2 mL)

was refluxed for 22 h. The mixture was cooled to 23 °C then filtered through Celite, and washed with CH_2Cl_2 (50 mL). The filtrated was concentrated under reduced pressure and the residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 80:1) to give **33** (43 mg, 86%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (d, J = 7.3 Hz, 1H), 7.37–7.20 (m, 20H), 7.08–7.03 (m, 2H), 6.80 (d, J = 2.2 Hz, 1H), 5.29–5.02 (m, 3H), 4.90–4.88 (m, 3H), 3.85 (s, 3H), 3.44 (s, 6H), 3.41–3.23 (m, 6H), 1.46 (s, 9H), 1.43 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 170.0, 169.9, 155.1 (2C), 154.9, 152.3, 150.9, 147.6, 145.3, 140.5, 139.9, 139.3, 135.8 (2C), 134.5, 133.4, 125.0 (9C), 128.6 (6C), 128.1, 128.0, 127.1 (2C), 124.0, 123.7, 123.6, 123.1, 120.7, 115.8, 115.1, 112.8, 112.3, 112.1, 109.1, 106.4, 106.1, 80.1 (3C), 56.2 (2C), 55.7, 55.6, 55.5, 54.4, 38.1 (3C), 28.2 (9C). MS (ESI) m/z: 1263 $(M+23)^+$, 1241 $(M+1)^+$. R_f: 0.56 (CH_2Cl_2:MeOH, 50:1).

3.1.36. Compound 34. TFA (1 mL) was added to a solution of **33** (15 mg, 0.012 mmol) in CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred to 23 °C for 1 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **34** as a white solid (20 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 9.08 (d, J = 7.5 Hz, 1H), 7.60 (d, J = 2.2 Hz, 1H), 7.53 (s, 1H), 7.49–7.36 (m, 17H), 7.30 (d, J = 3.5 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.08 (d, J = 4.8 Hz, 1H), 6.87 (d, J = 4.2 Hz, 1H), 4.76 (t, J = 7.0 Hz, 1H), 4.70 (t, J = 6.9 Hz, 1H), 4.63 (t, J = 6.2 Hz, 1H), 3.95 (s, 3H), 3.47 (s, 6H), 3.61–3.36 (m, 6H). MS (ESI) m/z: 941 $(M+1)^+$.

3.1.37. Compound 35. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Phe-OH (106 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 6 h. The resulting pale yellow solution was washed with H_2O (10 mL) and saturated aqueous solution of $NaHCO_3$ (10 mL) and both aqueous phases were extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 100:1) to give **35** as a white solid (87 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.26 (m, 15H), 7.16 (br s, 2H), 7.10 (s, 1H), 7.05 (s, 1H), 6.91 (s, 1H), 6.78–6.68 (m, 2H), 4.99 (t, J = 8.6 Hz, 2H), 4.88–4.72 (m, 6H), 3.81 (s, 3H), 3.41 (s, 3H), 3.34 (s, 3H), 3.30–3.12 (m, 8H), 1.44 (s, 9H), 1.43 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 169.9 (2C), 155.0, 154.9, 152.0, 149.7, 147.5, 144.7, 139.6, 139.0, 138.4, 135.8 (2C), 134.8, 134.2, 129.4 (9C), 129.2, 128.5 (6C), 127.1 (2C), 126.9, 125.9, 125.7, 123.8, 123.1, 122.5, 116.1, 115.8, 114.9, 114.7, 111.8, 109.7, 105.4, 80.0 (3C), 56.1, 55.6, 55.4, 54.3 (3C), 42.3, 38.0 (3C), 28.2 (9C), 27.9. MS (ESI) m/z: 1265 $(M+23)^+$. R_f: 0.65 (CH_2Cl_2:MeOH, 30:1).

3.1.38. Compound 36. TFA (1 mL) was added to a solution of **35** (15 mg, 0.012 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred to 23 °C for 2.5 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **36** as a white solid (17 mg, quant.). ^{1}H NMR (300 MHz, CD_3OD) δ 7.44–7.35 (m, 17H), 7.26 (d, J = 8.1 Hz, 1H), 7.10 (d, J = 1.6 Hz, 1H), 7.06 (s, 1H), 6.88 (d, J = 3.5 Hz, 1H), 6.78 (d, J = 3.5 Hz, 1H), 4.79–4.70 (m, 3H), 4.63 (t, J = 6.7 Hz, 2H), 3.89 (s, 3H), 3.53–3.26 (m, 6H), 3.44 (s, 3H), 3.36 (s, 3H), 3.16 (t, J = 6.7 Hz, 2H). MS (ESI) m/z: 965 $(M+23)^+$, 943 $(M+1)^+$.

3.1.39. Compound 37. A suspension of **LAM-501 (2)** (50 mg, 0.0997 mmol), Boc-L-Trp-OH (121 mg, 0.3988 mmol), EDC·HCl (76 mg, 0.3988 mmol) and DMAP (7 mg, 0.0598 mmol) in anhydrous CH_2Cl_2 (4.3 mL) was stirred under Argon atmosphere at 23 °C for 4 h. The resulting pale yellow solution was washed with H_2O (10 mL) and aqueous saturated solution of $NaHCO_3$ (10 mL). The combined aqueous phases were extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, from 30:1 to 15:1) to give **37** as a white solid (115 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.28 (s, 2H), 7.68–7.62 (m, 3H), 7.39–7.36 (m, 3H), 7.26–7.07 (m, 12H), 6.90 (s, 1H), 6.72 (s, 1H), 6.65 (br s, 2H), 5.15–5.12 (m, 2H), 5.00–4.59 (m, 6H), 3.75 (s, 3H), 3.52–3.28 (m, 12H), 3.00 (br t, 2H), 1.43 (s, 27H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.4, 170.4, 155.3 (2C), 154.9, 152.0, 149.6, 147.5, 144.6, 139.6, 139.0, 138.4, 136.1 (3C), 134.9, 134.0, 127.7 (3C), 126.8, 125.9, 125.5, 123.8, 123.1 (3C), 122.5, 122.0 (3C), 119.5 (3C), 118.6 (3C), 116.0, 115.8, 114.7, 111.7, 111.3 (3C), 109.5 (3C), 105.3, 80.0 (3C), 56.0 (2C), 55.6, 55.4, 54.4 (2C), 42.3, 28.2 (12C), 27.7. MS (ESI) m/z: 1382 $(M+23)^+$. R_f: 0.13 (CH_2Cl_2:MeOH, 30:1).

3.1.40. Compound 38. TFA (1 mL) was added to a solution of **37** (25 mg, 0.018 mmol) in CH_2Cl_2 (3 mL) at 0 °C under Argon atmosphere. The reaction mixture was stirred to 23 °C for 1.5 h. The solvent was evaporated under reduced pressure and, in order to eliminate the remaining TFA, the mixture was treated with CH_2Cl_2 (3×15 mL) and evaporated to dryness to give **38** as a white solid (27 mg, quant.) ^{1}H NMR (300 MHz, CD_3OD) δ 7.68 (d, J = 8.1 Hz, 1H), 7.62 (s, 1H), 7.60 (s, 1H), 7.44 (s, 1H), 7.42 (s, 3H), 7.34 (s, 1H), 7.30 (s, 1H), 7.29 (s, 1H), 7.21–7.16 (m, 5H), 7.12–7.09 (m, 3H), 6.98 (s, 1H), 6.85 (d, J = 2.0 Hz, 1H), 6.77–6.76 (m, 2H), 4.73–4.69 (m, 3H), 4.60 (br t, 2H), 3.87 (s, 3H), 3.77–3.34 (m, 6H), 3.43 (s, 3H), 3.34 (s, 3H). MS (ESI) m/z: 1060 $(M+1)^+$.

3.1.41. Compound 39. A suspension of **LAM-D (1)** (300 mg, 0.601 mmol), 6-*tert*-Butoxycarbonylamino-hexanoic acid (834 mg, 3.604 mmol), EDC·HCl (691 mg, 0.3.604 mmol) and DMAP (44 mg, 0.360 mmol) in CH_2Cl_2 anh. (50 mL) was stirred under Argon atmosphere at 23 °C for 3 h. The resulting pale yellow solution

was diluted with CH_2Cl_2 (50 mL), washed with H_2O (100 mL) and aqueous saturated solution of $NaHCO_3$ (100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The residue was purified by chromatography on silica gel (CH_2Cl_2:MeOH, 40:1) to give **39** as a white solid (608 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (d, $J = 7.3$ Hz, 1H), 7.38 (s, 1H), 7.29–7.13 (m, 5H), 7.07 (d, $J = 7.5$ Hz, 1H), 6.80 (s, 1H), 4.56 (bs, 3H), 3.82 (s, 3H), 3.44 (s, 6H), 3.20–3.13 (m, 6H), 2.66–2.56 (m, 6H), 1.84–1.75 (m, 6H), 1.60–1.44 (m, 39H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.2, 155.9, 155.0, 152.4, 151.0, 147.7, 145.4, 140.9, 140.3, 139.8, 134.1, 133.5, 128.2, 124.0, 123.8, 123.6, 123.5, 123.0, 120.6, 115.5, 115.0, 112.7, 112.2, 112.1, 108.9, 106.3, 106.1, 79.0(3C), 56.2, 55.7, 55.6, 40.3(3C), 33.8(2C), 33.7, 29.7(3C), 28.4(9C), 26.2, 26.1(2C), 24.5(2C), 24.5. MS (ESI) m/z: 1161.8 $(M+23)^+$. R_f: 0.30 (CH_2Cl_2:MeOH, 40:1).

3.1.42. Compound 40. A solution of **39** (503 mg, 0.442 mmol) in a 3.0 M solution of HCl in EtOAc was stirred at 23 °C for 30 min. The resulting suspension was filtered and the solid washed with EtOAc and *n*-hexane to give **40** as a white solid (389 mg, 93%). ^{1}H NMR (300 MHz, CD_3OD) δ 9.07 (d, $J = 7.3$ Hz, 1H), 7.49 (s, 2H), 7.39 (d, $J = 8.1$ Hz, 1H), 7.31–7.28 (m, 2H), 7.16–7.11 (m, 2H), 6.86 (s, 1H), 3.88 (s, 3H), 3.46 (s, 6H), 3.02–2.94 (m, 6H), 2.73–2.60 (m, 6H), 1.88–1.75 (m, 12H), 1.54–1.52 (m, 6H). MS (ESI) m/z: 839 $(M+1)^+$.

3.2. Drugs and chemicals

CPT was purchased from Sigma Chemical Co. All drug solutions were prepared in DMSO at 5 mM and then further diluted with water. The final DMA concentration never exceeded 0.3% (v/v) in the cleavage reactions. Under these conditions DMSO which is also used in the controls, does not affect the topoisomerase activity. The stock solutions of drugs were kept at −20 °C and freshly diluted to the desired concentration immediately prior to use. All other chemicals were analytical grade reagents.

3.3. Melting temperature studies

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of *T*m measurements, 12 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm pathlength) were heated by circulating water. The measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20–100 °C with a heating rate of 1 °C/min. The 'melting' temperature *T*m was taken as the mid-point of the hyperchromic transition.

3.4. DNA relaxation experiments

Recombinant topoisomerase I protein was produced and purified from baculovirus infected Sf9 cells.[16] Supercoiled pLAZ3 DNA (0.25 μg) was incubated with 3 units human topoisomerase I at 37 °C for 1 h in relaxation buffer (50 mM Tris pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/mL. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed at room temperature for 2 h at 120V in 1% agarose gels containing ethidium bromide (1 μg/mL). After electrophoresis, gels were washed and photographed under UV light.[13]

3.5. Purification of the DNA restriction fragment and radiolabeling

The 117-bp DNA fragment was prepared by 3′-[^{32}P]-end labeling of the EcoRI-PvuII double digest of the pBS plasmid (Stratagene) using α-[^{32}P]-dATP (Amersham, 3000 Ci/mmol) and AMV reverse transcriptase (Roche). The labeled digestion products were separated on a 6% polyacrylamide gel under non-denaturing conditions in TBE buffer (89 mM Tris–borate pH 8.3, 1 mM EDTA). After autoradiography, the requisite band of DNA was excised, crushed and soaked in water overnight at 37 °C. This suspension was filtered through a Millipore 0.22 μm filter and the DNA was precipitated with ethanol. Following washing with 70% ethanol and vacuum drying of the precipitate, the labeled DNA was re-suspended in 10 mM Tris adjusted to pH 7.0 containing 10 mM NaCl.

3.6. Sequencing of topoisomerase I-mediated DNA cleavage sites

Each reaction mixture contained 2 μL of 3′-end [^{32}P] labeled DNA (~1 μM), 5 μL of water, 2 μL of 10X topoisomerase I buffer, 10 μL of drug solution at the desired concentration (50 μM final concentration). After 10 min incubation to ensure equilibration, the reaction was initiated by addition of 2 μL (20 units) topoisomerase I. Samples were incubated for 45 min at 37 °C prior to adding SDS to 0.25% and proteinase K to 250 μg/mL to dissociate the drug-DNA–topoisomerase I cleavable complexes. The DNA was precipitated with ethanol and then resuspended in 5 μL of formamide-TBE loading buffer, denatured at 90 °C for 4 min then chilled in ice for 4 min prior to loading on to the sequencing gel. DNA cleavage products were resolved by polyacrylamide gel electrophoresis under denaturing conditions (0.3 mm thick, 8% acrylamide containing 8 M urea). After electrophoresis (about 2.5 h at 60 Watts, 1600 V in TBE buffer, BRL sequencer model S2), gels were soaked in 10% acetic acid for 10 min, transferred to Whatman 3MM paper, and dried under vacuum at 80 °C. A Molecular Dynamics 425E PhosphorImager was used to collect data from the storage screens exposed to dried gels overnight at room temperature. Base line-corrected scans were analyzed by integrating all the densities between two selected boundaries using ImageQuant version 3.3 software. Each resolved band was assigned to a particular bond within the DNA fragment by comparison of its position relative to

sequencing standards generated by treatment of the DNA with dimethylsulphate followed by piperidine-induced cleavage at the modified guanine residues.

3.6.1. Cell growth inhibition assay: screening. A colorimetric assay using sulforhodamine B (SRB) has been adapted for a quantitative measurement of cell growth and viability, following a previously described method.[17,18] Cells were seeded in 96-well microtiter plates, at 5×10^3 cells per well in aliquots of 195 μL of RPMI medium, and they are allowed to attach to the plate surface by growing in drug free medium for 18 h. Afterward, samples are added in aliquots of 5 μL (dissolved in DMSO/H_2O 3:7). After 72 h exposure, the antitumor effect is measured by the SRB methodology: cells are fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubating for 60 min at 4 °C. Plates are washed with deionized water and dried; 100 μL of SRB solution (0.4% wt/vol in 1% acetic acid) is added to each microtiter well and incubated for 10 min at room temperature. Unbound SRB is removed by washing with 1% acetic acid. Plates are air dried and bound stain is solubilized with Tris buffer. Optical densities are read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis are generated automatically by LIMS implementation. Using control OD values (C), test OD values (T) and time zero OD values (T_0), the drug concentration that causes 50% growth inhibition (GI_{50} value) was calculated from the equation: $100\times[(T-T_0)/C-T_0)_-]=50$.

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