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PAPER

Elaboration of vinblastine hybrids using a reactive *in situ* generated *N*-carboxyanhydride†

Claire Rannoux, Fanny Roussi,* Marie-Thérèse Martin and Françoise Guéritte

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Hybrids of vinblastine and phomopsin, designed by a molecular modelling study, were elaborated in order to target tubulin. The key step of the synthesis (fragmentation and insertion of vindoline) was mediated by an internal *N*-carboxyanhydride (or *O*-acylcarbamate). This reaction was diastereospecific and addition of silver salts could reverse the diastereoselectivity. Even if the synthesized compounds are inactive, this synthesis represents an original example of a C–N fragmentation mediated by a *N*-carboxyanhydride.

Introduction

Microtubules play a key role in many cellular functions such as cell division, as they form the mitotic spindle along which chromosomes migrate during mitosis. These polymers of α and β tubulin heterodimers are constantly in dynamic instability. Some drugs can alter this dynamic and tubulin binding molecules are today one of the most important classes of anticancer agents. Among these antimitotic drugs, vinca alkaloids[1] are commonly used in cancer chemotherapy, inhibiting tubulin polymerization into microtubules.

Their precise binding site in the so-called vinca domain was determined a few years ago[2] and it was shown that it partially overlaps with that of phomopsin A **4**[3] (a cyclopeptide natural compound). In order to explore the vinca domain and to elaborate new acute derivatives, we have recently synthesized vinca–phomopsin hybrids, linking the hexahydrophomopsin lateral chain to the tertiary amine of the cleavamine moiety of anhydrovinblastine and vinorelbine.[4] Albeit very active, we showed that, in the tubulin binding site, the phomopsin lateral chain of these hybrids was not oriented like the native compound but was extending in another direction.

So as to elaborate new hybrids with a correct orientation of this peptide side chain, we designed, by molecular modelling studies (SYBYL 7.3), new hybrids of type **5** (Fig. 1). These modelling experiments showed that low energy conformers of **5** superimposed correctly with phomopsin and vinblastine in their active conformations (Fig. 2).

In these compounds, a simplified cleavamine moiety (*i.e.*, suppression of cycle D) is used as a template to keep the vindoline and phomopsin side chain in a correct orientation.

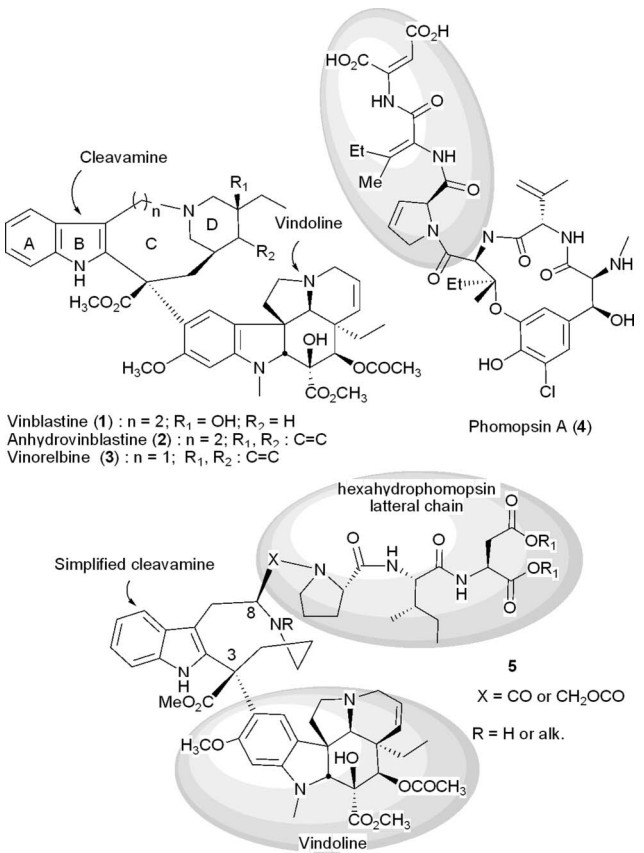


Fig. 1 Some vinca alkaloids **1–3**, phomopsin A **4** and target-simplified vinca–phomopsin A hybrids **5**.

These hybrids may be elaborated starting from L-tryptophan, that could provide the desired (*S*) absolute configuration at C-8. The formation of the central nine membered ring and the stereoselective insertion of vindoline at C3 could be performed simultaneously using a modified procedure of that developed by

Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, Avenue de la terrasse, 91198 Gif sur Yvette, France. E-mail: roussi@icsn.cnrs-gif.fr; Fax: +33 (0)1 69 07 72 47; Tel: +33 (0)1 69 82 31 21

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of all compounds. See DOI: 10.1039/c0ob01065k

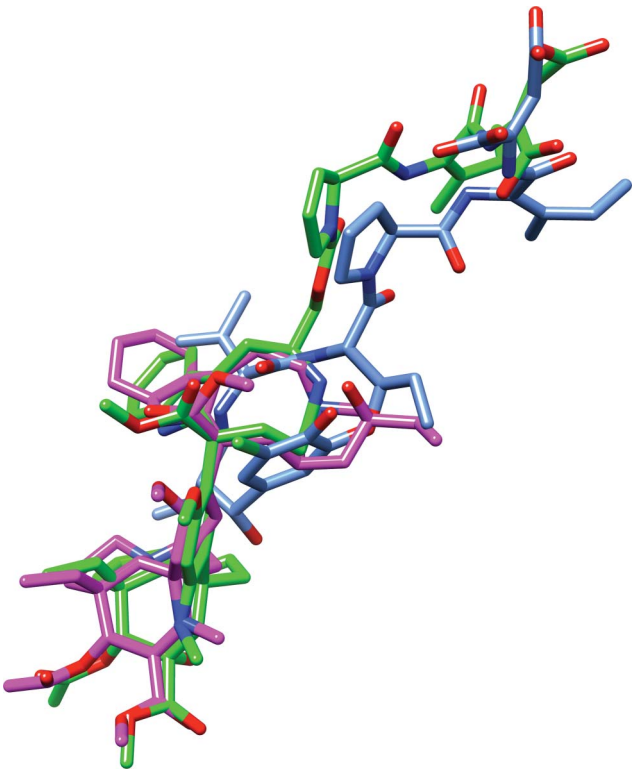


Fig. 2 Superimposition of phomospin A **4** (blue) and vinblastine **1** (magenta) in their active conformation in tubulin with a low energy conformer of hybrid **5** (green) with R = H and X = CH_2OCO.

Magnus and colleagues[5,6] in their very elegant and powerful total synthesis of vinblastine. Indeed, these authors have shown that the coupling of vindoline to a precursor of cleavamine **6** could be achieved, in a few days, by trapping an intermediate delocalized cation, generated *via* a C–N fragmentation using *p*-nitrobenzyl chloroformate. They nicely showed that the regiochemistry and the *S*/*R* ratio of the formed bond is dependent on the rate of the capture of the iminium ion before its racemization. This rate, in turn, depends on the temperature and the dielectric constant of the solvent in the reaction (Fig. 3).

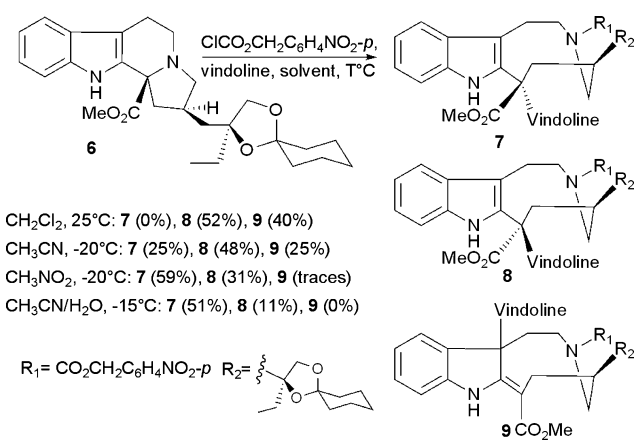


Fig. 3 Magnus synthesis of vinblastine.

By analogy with these results, we reasoned that, starting from compound **14**, we could generate the transient iminium ion **12** from intermediate **13**, bearing a *N*-carboxyanhydride (NCA) function (Fig. 4).

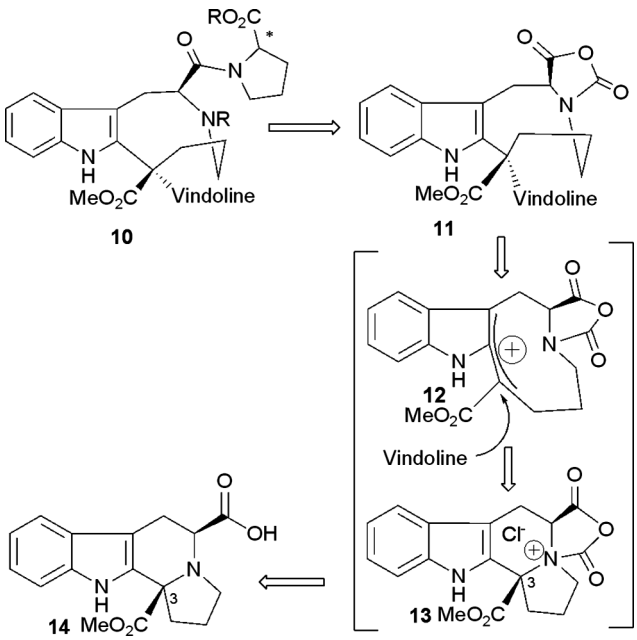


Fig. 4 Envisaged retrosynthetic pathway.

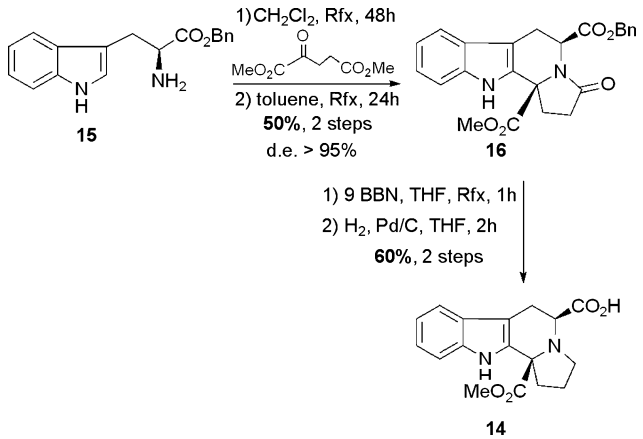
To our knowledge, an *N*-carboxyanhydride has, so far, never been used to induce a C–N fragmentation.[7] The choice of this group is, nevertheless, strategic in our case as it would play a triple role and would serve:

- as a protecting group of the acid function,
- as an activating group to fragment the C3–N bond to generate the macrocyclic cation **12** that could be captured by vindoline to form the expected **11**,
- as a template on compound **11** for the direct addition of the peptide chain of phomopsin.

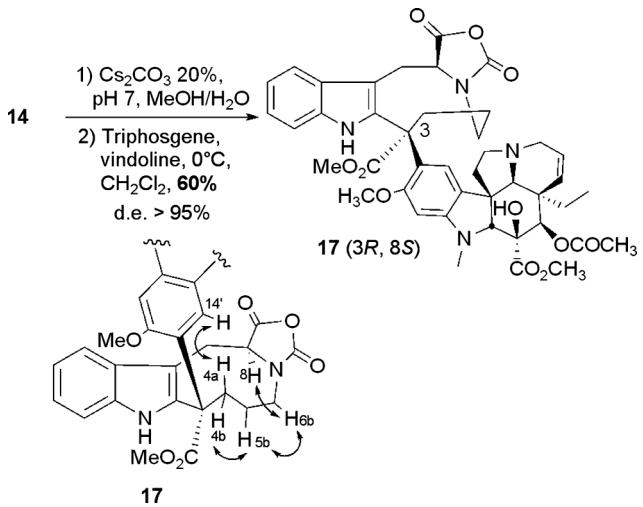
Results and discussion

Compound **14** was elaborated in four steps from L-tryptophan benzyl ester (Scheme 1). Magnus *et al.* have already prepared the first intermediate **16**,[8] as a mixture of two diastereomers (43% and 7%) by a one pot Pictet–Spengler/lactamization reaction with dimethyl α-ketoglutarate in refluxing THF for 72 h. In our case, we found that using a two-step procedure (Pictet–Spengler in refluxing dichloromethane, 48 h), then lactamization in toluene (reflux, 24 h) allowed the selective formation of the *cis*-diaxial adduct **16** (50% yield for two steps). The lactam function was thus selectively reduced using 9 BBN[9] and hydrogenolysis of the benzyl ester furnished the corresponding acid **14** (Scheme 1).

With this compound in hand, the formation of the transient intermediate **13** was next studied (Scheme 2). The formation of the NCA turned out to be quite difficult because of the congested environment around nitrogen: it was unable to react even with small and reactive 1-chloroethylchloroformate. Using standard procedures to generate this anhydride (triphosgene alone,[10] or with pyridine or triethylamine[11]) only resulted in recovering starting



Scheme 1



Scheme 2

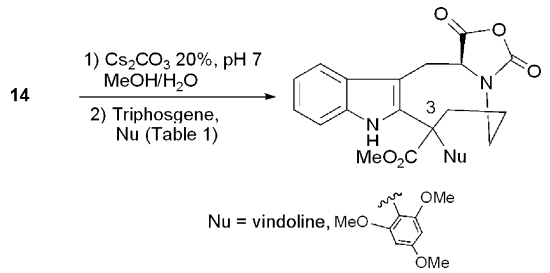
material. Finally, a "pre-activation" of the acid function as a cesium salt[12] allowed a clean and total conversion of **14** when reacted with 1/3 equivalent of triphosgene and vindoline. The use of a NCA significantly enhanced the rate of this functionalization: transformation of intermediate **14** was completed in a few hours. After fast flash chromatography, an insertion compound **17** was isolated (Scheme 2).

Complete NMR assignments confirmed that insertion of vindoline occurred at C3, thus validating the NCA strategy. In addition, the insertion compound was obtained as only one diastereomer. The absolute configuration of the new quaternary center C3 was determined using NOESY experiments. Particularly, NOEs between H8, H6b, H5b and H4b, and between H4a and H14′ accounted for a 3*R* configuration. This configuration was quite surprising as it resulted from an attack of vindoline exclusively on the more hindered side of intermediate cation **12**, *syn* to the NCA function. Various experimental parameters were screened, in order to reverse this selectivity (Scheme 3, Table 1). Nevertheless, neither modification of the solvent (entry 2), nor decreased temperature (entries 3 and 4) could modify the selectivity of the attack, contrary to Magnus's observations. These very disappointing results were, however, tempered by those observed when replacing vindoline with more reactive trimethoxybenzene (TMB). In that case, when

Table 1

Entry	Ar	Solvent	Temp	Additive	Cpd	Yield[b]
1	Vindoline	CH_2Cl_2	0 °C	/	**17** (3*R*)	50%
2	Vindoline	CH_3CN	0 °C	/	**17** (3*R*)	60%
3	Vindoline	CH_2Cl_2	–40 °C	/	**17** (3*R*)	ND
4	Vindoline	CH_3CN	–40 °C	/	**17** (3*R*)	ND
5	TMB[a]	CH_3CN	25 °C	/	**18** (3*R*), (3*S*) 0.4/1[c]	30%

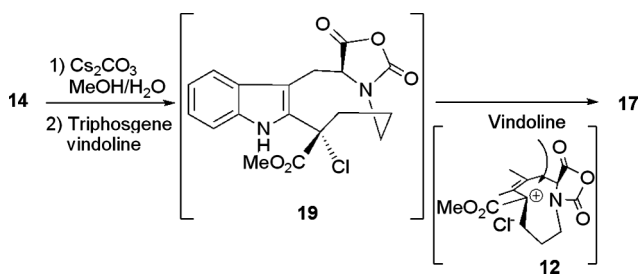
[a] TMB = trimethoxybenzene. [b] Yield of isolated compounds after purification by flash chromatography. [c] Absolute configuration of major and minor isomers could not be determined because of absence of NOEs between TMB and the macrocycle.



Scheme 3

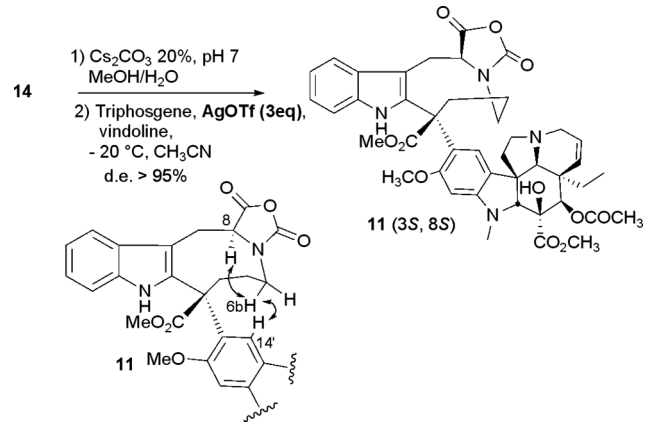
the reaction was performed at room temperature in acetonitrile, a mixture of 3*R* and 3*S* **17** was formed. That result clearly indicates that TMB can partially trap intermediate **12** from "below", even at room temperature.

Furthermore, the fast formation of a transient intermediate, that was slowly replaced by final compound **17**, could be detected when the reaction, performed with vindoline, was followed by NMR. These observations led us to assume the following hypothesis (Scheme 4): highly reactive cation **12** may be trapped by chlorine (derived from degradation of triphosgene) leading to unstable **19**[13] that could not be isolated. Compound **19** may then slowly regenerate cation **12**. While leaving, Cl^- may form an intimate ion pair[14] with **12**, and may block its α-face, which could allow the β-face to be attacked by vindoline and could explain the observed diastereoselectivity.

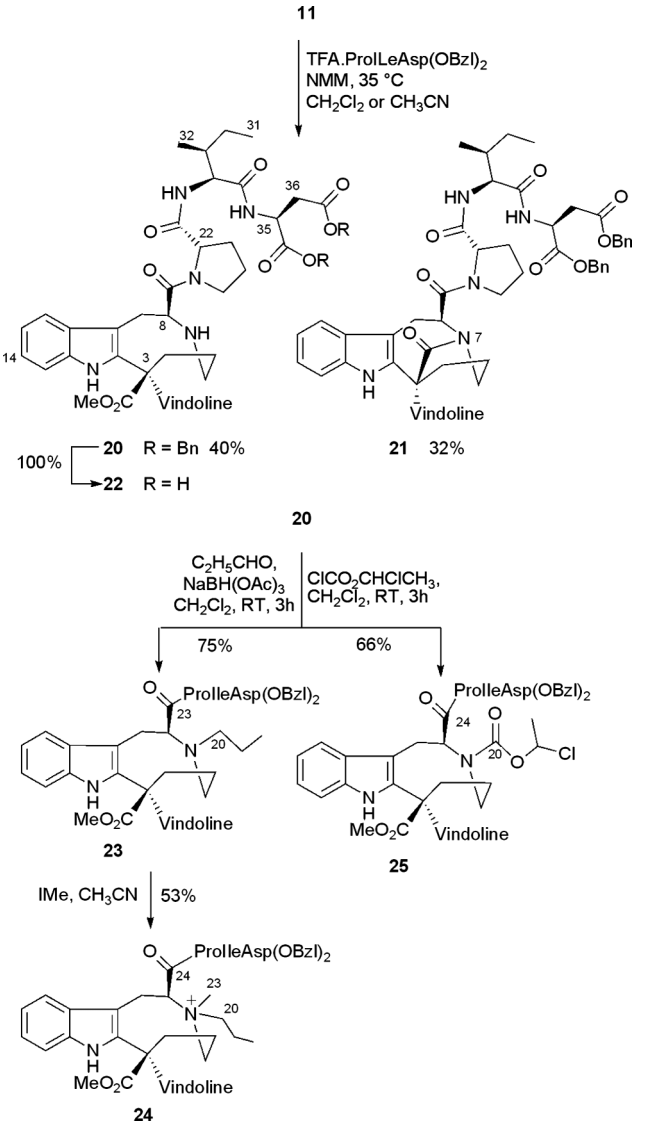


Scheme 4

With more reactive trimethoxybenzene, a competition between chlorine and TMB could account for the formation of both diastereomers. To validate this mechanistic hypothesis, the reaction was performed with vindoline in acetonitrile and an excess of AgOTF (3 eq.) in order to trap Cl^- as silver salts (Scheme 5). In these conditions, we were pleased to obtain exclusively the desired compound **11** with 91% yield. Observed NOEs between H8, H6 and H14′ confirmed a 3*S* configuration resulting in a



Scheme 5



Scheme 6

direct insertion of vindoline on the α-face. The observed total diastereoselectivity was based on the generation of a hyper-reactive NCA intermediate that not only permitted induction of the C3–N bond fragmentation but also ensured a total chirality transfer from the substrate to the C3 center. That strategy represents an improvement to the Magnus methodology.[6] In addition, even if only the 3*S* diastereomer was useful in our case, the easy switch over of the coupling selectivity simply by adding silver salts makes this route very attractive and conceptually interesting.

The ring opening of the *N*-carboxyanhydride was next performed on compound **11**, using a three amino acid peptide chain corresponding to that of the protected lateral chain of hexahydrophomopsin (Scheme 6). As expected, the peptide bond was formed simply by stirring **11** and the NH free proline of the small peptide in acetonitrile for a few hours. Nevertheless, the high nucleophilicity of N7 led also to the formation of the undesired lactam **21** by reaction with methyl ester on C3. This side reaction occurred at room temperature, whereas the temperature had to be raised to 35 °C to complete the opening of the NCA. Nevertheless, the expected compound **20** could be isolated in a reasonable yield (40%). Deprotection of aspartic acid furnished compound **22** in a quantitative yield. In addition, in order to decrease the high nucleophilicity of N7, that could affect the potential biological activity of the hybrids, a reductive amination was performed on **20** with propionaldehyde, to provide **23** that was converted to the quaternary ammonium salt **24**. In parallel, compound **20** was reacted with chloroacetyl chloride to provide carbamate **25**.

NMR spectra clearly indicated that compounds **20**, **22–25** were in equilibrium between several conformers both at low and room temperature. NOE experiments confirmed that these were due to the flexibility of the macrocycle. For example, one conformer of compound **23** presented NOEs between H8, H20, H9 and H12, whereas another one showed NOEs between H14′, H6, H5 and H4. This high flexibility of the macrocycle may promote generation of the conformers that were predicted by molecular modelling experiments to superimpose with phomopsin and vinblastine in their active conformation (Fig. 2).

The activity of compounds **20–25** on tubulin polymerization assays, as well as their cytotoxicity on KB, MCF7 and MCF7R cell lines, was evaluated. It was found that none of these compounds showed a significant microtubule assembly inhibitory activity or cytotoxicity. These biological results were very disappointing and demonstrate, once again,[15] that modifications on the cleavamine moiety of vinblastine-type compounds can have a dramatic impact on activity.

Conclusions

Based on molecular modelling studies, we have elaborated phomopsine–vinblastine hybrids to target tubulin. The key step of this synthesis, *i.e.*, the insertion of vindoline, proceeds rapidly with a complete diastereoselectivity due to an intramolecular activation of a pyrrolo-β-carboline using a *N*-carboxyanhydride. Unfortunately, these molecules are inactive, in spite of the flexibility of the macrocycle of the resulting compounds, which is expected to favour the interaction with tubulin. In turn, this work is, to our knowledge, the first example of a use of a *N*-carboxyanhydride as an activating group for a C–N fragmentation. This example offers new prospects for the NCA chemistry beyond peptides synthesis.

Experimental section

Chemistry

General methods. Anhydrous acetonitrile was purchased from Aldrich. Anhydrous THF was dried and deoxygenated by distillation from sodium/benzophenone under argon. CH_2Cl_2 was distilled from P_2O_5 under argon. Reactions were monitored by thin layer chromatography (TLC) with SDS 0.25 mm silica gel 60 F254. TLC plates were visualized by exposure to UV light (254 nm) and stained with Salkowski reagent or ammonium molybdate. Flash column chromatographies were performed using normal phase silica gel (60 Å, 40–63 μm) or neutral alumina (70–230 mesh ASTM). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC300 or Avance 600 or ARX500 spectrometer. Chemical shifts (δ) are reported in ppm relative to chloroform for ^{1}H NMR (δ = 7.24 ppm) and for ^{13}C NMR (δ = 77.26 ppm) or relative to acetonitrile for ^{1}H NMR (δ = 1.94 ppm) and for ^{13}C NMR (δ = 1.40 ppm). NMR attributions were based on HMQC, HMBC and COSY correlations. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constant in Hz, attribution. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum BX FT-IR instrument at 22 °C. $[\alpha]_D$ were measured using a JASCO P-1010 instrument. HRMS were recorded by ElectroSpray Ionisation (ESI) with a ThermoQuest AQA Navigator spectrometer.

(+)-5-Benzyl-11b-methyl (5*S*,11b*R*)-3-oxo-2,3,6,11-tetrahydro-1*H*-indolizino[8,7b]indole-5,11b(5*H*)-dicarboxylate 16. Dimethyl-2-oxoglutarate (2.21 mL, 15.2 mmol) was added to a solution of L-tryptophan benzyl ester (3.74 g, 12.7 mmol) in CH_2Cl_2. The mixture was heated at reflux for 2 days. The solvent was removed under reduced pressure and the crude Pictet–Spengler product was dissolved in toluene and heated at reflux for one more day. After removal of the solvent, the mixture was dissolved in EtOAc and washed with saturated sodium bicarbonate solution. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel (heptane/EtOAc: 50/50) to give compound **16** as the only diastereoisomer (2.66 g, 50% over 2 steps). $[\alpha]_D^{22}$ +55 (*c* 0.5 in $CHCl_3$), $[\alpha]_{D\ lit.}$[8] +58 (*c* 1.0 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3376, 2952, 1742 and 1685; δ_H (500 MHz; $CDCl_3$) 2.20 (m, 1H), 2.49 (dd, 1H, *J* 16.6 and 9.2 Hz), 2.65 (dd, 1H, *J* 12.8 and 8.4 Hz), 2.91 (m, 1H), 3.10 (dd, 1H, *J* 16.0 and 7.4 Hz), 3.32 (d, 1H, *J* 16.0 Hz), 3.73 (s, 3H), 5.00 (s, 2H), 5.54 (d, 1H, *J* 7.2 Hz), 7.09–7.39 (m, 7H), 7.37 (d, 1H, *J* 7.9 Hz), 7.46 (d, 1H, *J* 7.5 Hz), 8.60 (s, 1H); δ_C(75 MHz, $CDCl_3$) 24.4, 30.9, 34.3, 49.6, 52.8, 63.9, 66.9, 105.7, 111.5, 118.6, 119.7, 122.6, 126.0, 127.8, 128.2, 128.5, 130.9, 135.1, 137.0, 170.2, 172.3, 175.1; *m*/*z* (ESI) 441.1421 (M + Na$^+$; $C_{24}H_{22}N_2O_5Na$ requires 441.1426).

(+)-(5*S*,11b*R*)-11b-methoxycarbonyl-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7b]indole-5-carboxylic acid 14. A flask was charged with compound **16** (2.66 g, 6.36 mmol), 9-BBN (2.33 g, 9.53 mmol) and anhydrous THF (40 mL). The mixture was heated at reflux. When a TLC plate indicated that compound **16** was consumed, the reaction mixture was cooled under argon and ethanolamine (575 μL, 9.53 mmol) was added. The solution was stirred for 10 min and the solvent was removed under reduced pressure. Ether was added to the residue, the resulting mixture was stirred 30 min and filtered through Celite to remove the boran complex insoluble in ether. After removal of the solvent, the product was purified by flash chromatography on silica gel (heptane/EtOAc: 50/50 → heptane/EtOAc: 30/70) to give the expected compound (1.54 g, 60%); $[\alpha]_D^{22}$ +43 (*c* 0.5 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3384, 2949 and 1725; δ_H (300 MHz; $CDCl_3$) 1.78 (m, 1H), 1.97 (m, 1H), 2.12 (m, 1H), 2.45 (m, 1H), 2.97 (m, 1H), 3.11 (dd, 1H, *J* 15.8 and 1.9 Hz), 3.17 (dd, 1H, *J* 15.9 and 5.7 Hz), 3.35 (m, 1H), 3.76 (s, 3H), 4.24 (dd, 1H, *J* 5.6 and 1.93 Hz), 4.89 (d, 1H, *J* 12.4 Hz), 4.97 (d, 1H, *J* 12.4 Hz), 7.20–6.97 (m, 7H), 7.33 (d, 1H, *J* 8.0 Hz), 7.45 (d, 1H, *J* 7.9), 8.65 (s, 1H); δ_C(75 MHz, $CDCl_3$) 19.8, 23.9, 42.1, 55.9, 53.0, 58.2, 64.3, 66.6, 106.1, 111.4, 118.5, 119.5, 122.3, 126.6, 128.5, 128.1, 128.0, 134.5, 135.8, 136.9, 172.5, 176.2; *m*/*z* (ESI) 404.1816 (M + H$^+$; $C_{24}H_{25}N_2O_4$ requires 405.1814).

10% Pd/Carbon (293 mg, 0.276 mmol), was added to a solution of reduced compound (1.11 g, 2.76 mmol) in anhydrous THF. The mixture was stirred for 3 h under an H_2 atmosphere and filtered through a pad of Celite to afford quantitatively compound **14** (867 mg, 100%); $[\alpha]_D^{22}$ +38 (*c* 0.5 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3056, 2951, 2849 and 1729; δ_H (300 MHz; $CDCl_3$) 2.04–1.78 (m, 3H), 2.69 (m, 1H), 2.97 (m, 1H), 3.07 (dd, 1H, *J* 15.6 and 6.4), 3.41 (dd, 1H, *J* 15.6 and 3.8 Hz), 3.51(m, 1H), 3.85 (s, 3H), 3.86 (m, 1H), 7.10 (t, 1H, *J* 7.6), 7.18 (t, 1H, *J* 7.7 Hz), 7.32 (d, 1H, *J* 8.0 Hz), 7.52 (d, 1H, *J* 7.9 Hz), 8.42 (s, 1H); δ_C(75 MHz, $CDCl_3$) 19.5, 24.0, 41.2, 53.9, 55.2, 60.5, 66.0, 107.4, 111.3, 119.0, 120.0, 122.9, 126.4, 131.1, 136.4, 174.3, 175.3; *m*/*z* (ESI) 337.1164 (M + Na$^+$; $C_{17}H_{18}N_2O_4Na$ requires 337.1164).

Compound 17. Compound **14** (110 mg, 0.35 mmol), was dissolved in a 9/1 mixture of methanol and water (650 μL/65 μL). A 20% solution of cesium carbonate was added dropwise until the pH reached 7. The solvent was removed under vacuum and the residue was carefully dried by the addition of acetonitrile (3 × 1 mL) to form an azeotrope. The residue and vindoline (53 mg, 0.12 mmol, 1/3 equiv.), were dissolved in anhydrous acetonitrile (1 mL) and cooled to 0 °C. A solution of triphosgene (35 mg, 0.12 mmol, 1/3 equiv.) in dry acetonitrile (600 μL), was added dropwise to the mixture and the reaction was stirred for 5 h at 0 °C. After removal of the solvent, the product was purified by flash chromatography on silica gel (heptane/EtOAc: 2/8) to give compound **17** as one diastereoisomer (55 mg, 0.07 mmol, 60% yield); $[\alpha]_D^{22}$ +5.5 (*c* 0.1 in $CHCl_3$); ν_{max}(film)/cm^{-1} 2921, 2852, 1839, 1774 and 1731; δ_H (600 MHz; CD_3CN) 0.59 (t, 3H, *J* 7.3 Hz, 21′-H), 0.99 (sextuplet, 1H, *J* 7.1 Hz, 20′-H), 1.53 (m, 1H, 5-H), 1.55 (q, 1H, *J* 7.1 Hz, 20′-H), 1.94 (s, 3H, 30′-H), 2.12 (m, 1H, 5-H), 2.18 (m, 2H, 11-H), 2.19 (m, 1H, 4-H), 2.44 (t, 1H, *J* 13.7 Hz, 6-H), 2.57 (s, 3H, 24′-H), 2.69 (q, 1H, *J* 8.5 Hz, 10′-H), 2.88 (d, 1H, *J* 16 Hz, 8′-H), 2.94 (s, 1H, 19′-H), 3.35 (m, 1H, 10′-H), 3.39 (m, 1H, 9-H), 3.43 (m, 1H, 6-H), 3.46 (m, 1H, 9-H), 3.48 (m, 1H, 8′-H), 3.55 (s, 1H, 2′-H), 3.57 (m, 1H, 4-H), 3.67 (s, 3H, 27′-H), 3.68 (s, 3H, 19-H), 3.70 (s, 3H, 23′-H), 4.29 (d, 1H, *J* 10.9 Hz, 8-H), 5.17 (s, 1H, 4′-H), 5.23 (d, 1H, *J* 10.4 Hz, 6′-H), 5.86 (dd, 1H, *J* 10.3 and 4.4 Hz, 7′-H), 6.11 (s, 1H, 17′-H), 7.03 (t, 1H, *J* 7.7 Hz, 13-H), 7.12 (t, 1H, *J* 7.9 Hz, 14-H), 7.38 (d, 1H, *J* 7.7 Hz, 12-H), 7.41 (s, 1H, 14′-H), 7.48 (d, 1H, *J* 7.9 Hz, 15-H), 9.91 (s, 1H, 1-H); δ_C(150 MHz; CD_3CN) 8.1 (21′-C), 21.2 (30′-C), 24.0 (5-C),

27.8 (9-C), 31.8 (4-C), 31.9 (20′-C), 39.1 (24′-C), 42.8 (6-C), 43.9 (5′-C), 44.9 (11′-C), 51.8 (10′-C), 51.8 (8′-C), 52.6 (27′-C), 53.1 (3-C), 53.1 (19-C), 54.1 (12′-C), 56.6 (23′-C), 62.2 (8-C), 66.8 (19′-C), 77.5 (4′-C), 80.5 (3′-C), 84.3 (2′-C), 95.1 (17′-C), 105.0 (10-C), 112.3 (15-C), 119.3 (12-C), 120.3 (13-C), 120.6 (14′-C), 122.9 (14-C), 125.3 (15′-C), 125.6 (7′-C), 125.7 (13′-C), 127.5 (11-C), 131.4 (6′-C), 135.4 (16-C), 137.3 (2-C), 152.8 (22-C), 153.5 (18′-C), 158.2 (16′-C), 170.5 (20-C), 171.5 (29′-C), 173.0 (25′-C), 176.1 (17-C); *m/z* (ESI) 797.3427 (M + H^+; $C_{43}H_{49}N_4O_{11}$ requires 797.3398).

Compound 18. Compound **14** (80 mg, 0.25 mmol), was dissolved in a 9/1 mixture of methanol/water (550 μL/55 μL). A 20% solution of cesium carbonate was added dropwise until the pH reached 7. The solvent was removed under vacuum and the residue was dried by forming an azeotrope with acetonitrile. The residue and trimethoxybenzene (43 mg, 0.25 mmol, 1 equiv.), were dissolved in anhydrous acetonitrile (700 μL), and cooled to 0 °C. A solution of triphosgene (25.2 mg, 0.085 mmol, 1/3 equiv.), in acetonitrile (600 μL), was added dropwise to the mixture and the reaction was stirred for 5 h at 0 °C. After removal of the solvent, the product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOH 97/3 → 94/6) to give compound **18** as a mixture of diastereoisomers (dr: 1/0.4, 39 mg, 0.08 mmol, 30% yield); major diastereomer: ν_{max}(film)/cm^{-1} 3408, 2927 and 1727; δ_H (500 MHz; CD_3CN) 1.22 (m, 1H, 5-H), 1.82 (m, 1H, 5-H), 2.08 (m, 1H, 4-H), 3.35 (m, 2H, 4-H, 6-H), 3.42 (s, 3H, 8′-H or 12′-H), 3.45 (br s, 3H, 19-H), 3.43 (m, 1H, 6-H), 3.54 (m, 1H, 9-H), 3.66 (m, 1H, 9-H), 3.78 (s, 3H, 8′-H or 12′-H), 3.87 (s, 3H, 10′-H), 4.37 (d, 1H, *J* 11.8 Hz, 8-H), 6.34 (s, 2H, 3′-H, 5′-H), 7.07 (t, 1H, *J* 7.8 Hz, 13-H), 7.13 (t, 1H, *J* 8.0 Hz, 14-H), 7.32 (d, 1H, *J* 8.0 Hz, 15-H), 7.48 (d, 1H, *J* 7.8 Hz, 12-H), 9.01 (s, 1H, 1-H); δ_C(75 MHz; CD_3CN)/3-C and 1′-C not visible/25.3 (5-C), 28.9 (9-C), 31.1 (4-C), 43.9 (6-C), 53.2 (19-C), 56.5 (10′-C), 56.7 (12′-C and 8′-C), 63.4 (8-C), 94.0 (3′-C and 5′-C), 106.4 (10-C), 112.0 (15-C), 118.8 (12-C), 120.4 (13-C), 123.4 (14-C), 129.0 (11-C), 129.3 (2-C), 136.9 (16-C), 153.1 (22-C), 161.1 (4′-C), 162.4 (2′-C and 6′-C), 171.2 (20-C), 175.0 (17-C); *m/z* (ESI) 496.2 (M + H^+).

Compound 11. Compound **14** (296 mg, 0.94 mmol), was dissolved in a 9/1 mixture of methanol/water (200 μL/2 mL). A 20% solution of cesium carbonate was added dropwise until the pH reached 7. The solvent was removed under vacuum and the residue was dried by forming an azeotrope with acetonitrile. The residue, vindoline (215 mg, 0.47 mmol, 1/2 equiv.) and silver trifluoromethane sulfonate (726 mg, 2.82 mmol, 3 equiv.) were dissolved in anhydrous acetonitrile (4 mL) and cooled to −20 °C. A solution of triphosgene (93 mg, 0.31 mmol, 1/3 equiv.), in acetonitrile (1 mL), was added dropwise to the mixture and the reaction was stirred overnight at −20 °C. The mixture was filtered through Celite to remove silver salts and the product was filtered through silica gel (CH_2Cl_2/EtOH: 97/3) to give compound **11** as only one diastereoisomer (319 mg, 0.40 mmol, 91% yield). $[\alpha]_D^{22}$ +30 (*c* 0.1 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3450, 2921, 2851, 1838, 1760 and 1747; δ_H (500 MHz; CD_3CN; T 333 K) 0.56 (t, 3H, *J* 6.8 Hz, 21′-H), 1.21 (m, 1H, 20′-H), 1.27 (m, 1H, 5-H), 1.56 (m, 1H, 20′-H), 1.84 (m, 1H, 5-H), 1.97 (s, 3H, 30′-H), 2.16 (m, 2H, 11′-H), 2.47 (m, 1H, 4-H), 2.54 (m, 1H, 10′-H), 2.57 (s, 3H, 24′-H), 2.76 (s, 1H, 19′-H), 2.78 (d, 1H, *J* 16.9 Hz, 8′-H), 2.89 (t, 1H, *J* 13.6 Hz, 6-H), 3.02 (t, 1H, *J* 14.1 Hz, 4-H), 3.33 (td, 1H, *J* 9.7 Hz, 5.0 Hz, 10′-H), 3.41 (dd, 1H, *J* 16.9 and 5.3 Hz, 8′-H), 3.52 (m, 1H, 6-H), 3.63 (s, 3H, 27′-H), 3.65 (s, 1H, 2′-H), 3.66 (m, 2H, 9-H), 3.72 (s, 3H, 19-H), 3.75 (s, 3H, 23′-H), 4.42 (m, 1H, 8-H), 5.25 (m, 1H, 6′-H), 5.29 (s, 1H, 4′-H), 5.83 (dd, 1H, *J* 9.9 Hz and 5.0 Hz, 7′-H), 6.29 (s, 1H, 17′-H), 6.96 (s, 1H, 14′-H), 7.07 (t, 1H, *J* 7.9 Hz, 13-H), 7.13 (t, 1H, *J* 7.8 Hz, 14-H), 7.32 (d, 1H, *J* 7.8 Hz, 15-H), 7.51 (d, 1H, *J* 7.9 Hz, 12-H), 8.72 (s, 1H, 1-H); δ_C(75 MHz; CD_3CN) 8.7 (21′-C), 21.4 (30′-C), 25.3 (5-C), 29.6 (9-C), 32.2 (4-C), 32.2 (20′-C), 39.1 (24′-C), 44.3 (6-C), 44.5 (5′-C), 45.6 (11′-C), 51.8 (10′-C), 51.9 (8′-C), 52.8 (27′-C), 53.1 (19-C), 54.6 (12′-C), 56.4 (3-C), 57.0 (23′-C), 63.0 (8-C), 67.6 (19′-C), 77.8 (4′-C), 81.0 (3′-C), 84.6 (2′-C), 95.6 (17′-C), 108.7 (10-C), 112.6 (15-C), 118.9 (12-C), 120.8 (13-C), 122.2 (15′-C), 123.5 (14-C), 124.4 (14′-C), 125.6 (7′-C), 125.7 (13′-C), 129.6 (11-C), 131.6 (6′-C), 136.0 (2-C), 136.6 (16-C), 153.2 (22-C), 154.7 (18′-C), 159.7 (16′-C), 171.3 (20-C), 171.7 (29′-C), 173.2 (25′-C), 175.5 (17-C); *m/z* (ESI) 797.3400 (M + H^+; $C_{43}H_{49}N_4O_{11}$ requires 797.3398).

Compounds 20 and 21. Compound **11** (101 mg, 0.13 mmol), the trifluoroacetate salt of the peptide ProIleAspOBzl$_2$ (121 mg, 0.19 mmol, 1.5 equiv.) and *N*-methyl morpholine (21 μL, 0.19 mmol, 1.5 equiv.) were stirred in acetonitrile at 35 °C for 48 h to give, after treatment and purification by preparative layer chromatography (CH_2Cl_2/EtOH: 93/7), compounds **20** (65 mg, 0.05 mmol) and **21** (51 mg, 0.04 mmol) with 40% and 32% yield, respectively. Compound **20**: $[\alpha]_D^{22}$ +21 (*c* 0.5 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3326, 2922, 2852 and 1737; δ_H (600 MHz; CD_3CN) 0.51 (t, 3H, *J* 7.0 Hz, 21′-H), 0.84 (t, 3H, *J* 7.3 Hz, 31-H), 0.91 (d, 1H, *J* 6.9 Hz, 32-H), 1.07 (m, 1H, 5-H), 1.16 (m, 1H, 30-H), 1.24 (m, 1H, 20′-H), 1.47 (m, 2H, 5-H, 30-H), 1.60 (m, 1H, 20′-H), 1.89 (m, 2H, 24-H, 29-H), 1.96 (s, 3H, 30′-H), 2.02 (m, 1H, 24-H), 2.10 (m, 2H, 23-H), 2.21 (m, 2H, 11′-H), 2.31 (m, 1H, 4-H), 2.48 (m, 1H, 10′-H), 2.64 (m, 1H, 6-H), 2.69 (s, 3H, 24′-H), 2.74 (m, 2H, 8′-H, 19′-H), 2.87 (m, 1H, 6-H), 2.92 (t, 1H, *J* 5.6 Hz, 36-H), 3.04 (m, 1H, 4-H), 3.25 (m, 2H, 9-H), 3.31 (m, 1H, 10′-H), 3.37 (dd, 1H, *J* 16.1 and 4.8 Hz, 8′-H), 3.56 (s, 3H, 19-H), 3.62 (s, 1H, 2′-H), 3.66 (s, 3H, 27′-H), 3.63 (m, 1H, 8-H), 3.66 (m, 1H, 25-H), 3.70 (s, 3H, 23′-H), 4.29 (dd, 1H, *J* 8.6 and 5.3 Hz, 28-H), 4.47 (dd, 1H, *J* 7.7 and 3.9 Hz, 22-H), 4.84 (td, 1H, *J* 8.1 and 6.1 Hz, 35-H), 5.06 (s, 1H, 48-H), 5.09 (s, 1H, 39-H), 5.23 (d, 1H, *J* 10.2 Hz, 6′-H), 5.27 (s, 1H, 4′-H), 5.80 (dd, 1H, *J* 10.2 and 5.0 Hz, 7′-H), 6.27 (s, 1H, 17′-H), 6.97 (t, 1H, *J* 7.8 Hz, 13-H), 7.03 (t, 1H, *J* 8.0 Hz, 14-H), 7.04 (s, 1H, 14′-H), 7.15 (d, 1H, *J* 8.6 Hz, 27-H), 7.23 (d, 1H, *J* 8.0 Hz, 15-H), 7.26–7.36 (m, 11H, 34-H, 41-H, 42-H, 43-H, 44-H, 45-H, 50-H, 51-H, 52-H, 53-H, 54-H), 7.48 (d, 1H, *J* 7.8 Hz, 12-H), 8.38 (s, 1H, 1-H); δ_C(150 MHz; CD_3CN) 8.4 (21′-C), 12.1 (31-C), 16.2 (32-C), 21.3 (30′-C), 25.4 (30-C), 25.9 (24-C), 27.8 (5-C), 29.1 (23-C), 31.2 (9-C), 32.0 (20′-C), 33.4 (4-C), 37.0 (36-C), 38.2 (29-C), 39.0 (24′-C), 43.9 (5′-C), 45.3 (11′-C), 46.9 (6-C), 48.5 (25-C), 50.0 (35-C), 51.6 (8′-C, 10′-C), 52.6 (27′-C), 52.7 (19-C), 54.2 (12′-C), 56.4 (3-C), 56.7 (23′-C), 58.5 (28-C), 60.8 (8-C), 61.4 (22-C), 66.9 (19′-C), 67.4 (48-C), 68.0 (39-C), 77.5 (4′-C), 80.5 (3′-C), 84.5 (2′-C), 95.4 (17′-C), 112.0 (15-C), 112.16 (10-C), 118.9 (12-C), 119.9 (13-C), 122.4 (14-C), 122.8 (15′-C), 124.6 (14′-C), 125.5 (13′-C), 125.5 (7′-C), 129.1, 129.2, 129.22, 129.23, 129.56, 129.59 (41-C, 42-C, 43-C, 44-C, 45-C, 50-C, 51-C, 52-C, 53-C, 54-C), 130.3 (11-C), 131.3 (6′-C), 135.6 (2-C), 135.9 (16-C), 137.0 (49-C), 137.2 (40-C), 154.2 (18′-C), 159.6 (16′-C), 171.3 (46-C), 171.4 (37-C), 171.5 (29′-C), 172.0 (33-C), 172.6 (26-C), 173.1 (25′-C), 174.9 (20-C), 175.0 (17-C); *m/z* (ESI) 1276.6182 (M + H^+;

$C_{71}H_{86}N_6O_{15}$ requires 1276.6189). Compound **21**: $[\alpha]_D^{22}$ −15.8 (*c* 0.5 in $CHCl_3$); ν_{max}(film)/cm^{-1} 3292, 2960, 2923, 2853 and 1737; δ_H(300 MHz, CD_3CN) 0.05 (t, 3H, *J* 7.2 Hz, 21′-H), 0.76–0.92 (m, 6H, 29-H, 30-H, 20′-H), 1.11 (m, 1H, 28-H), 1.31 (m, 1H, 20′-H), 1.42 (m, 1H, 28-H), 1.73 (m, 1H, 5-H), 1.90 (s, 3H, 30′-H), 1.94 (m, 1H, 27-H), 1.91–2.09 (m, 5H, 22-H, 21-H, 5-H), 2.16 (d, 2H, 11′-H), 2.17 (m, 1H, 10′-H), 2.28 (s, 1H, 19′-H), 2.45 (d, 1H, J 17.4 Hz, 8′-H), 2.52 (m, 1H, 4-H), 2.63 (m, 1H, 4-H), 2.68 (s, 3H, 24′-H), 2.84 (t, 1H, *J* 11.6 Hz, 6-H), 2.91 (dd, 2H, *J* 6.2 and 3.9 Hz, 34-H), 3.07 (dd, 1H, *J* 15.5 and 6.7 Hz, 9-H), 3.28-3.16 (m, 2H, 8′-H, 10′-H), 3.32 (d, 1H, *J* 11.5 Hz, 6-H), 3.43 (dd, 1H, *J* 15.5 and 10.7 Hz, 9-H), 3.54 (s, 1H, 2′-H), 3.60 (m, 1H, 23-H), 3.68 (s, 3H, 27′-H), 3.79 (m, 1H, 8-H), 3.83 (s, 3H, 23′-H), 3.86 (m, 1H, 23-H), 4.23 (dd, 1H, *J* 8.6 and 5.6 Hz, 26-H), 4.45 (t, 1H, *J* 4.9 Hz, 20-H), 4.84 (dt, 1H, *J* 8.1 and 6.1 Hz, 33-H), 4.97–5.13 (m, 6H, 4′-H, 6′-H, 37-H, 46-H), 5.64 (dd, 1H, *J* 10.2 and 5.1 Hz, 7′-H), 6.31 (s, 1H, 17′-H), 6.36 (s, 1H, 14′-H), 6.91 (d, 1H, 25-H), 7.00 (t, 1H, *J* 7.8 Hz, 13-H), 7.13 (t, 1H, *J* 7.8 Hz, 14-H), 7.25–7.39 (m, 11H, 32-H, 39-H, 40-H, 41-H, 42-H, 43-H, 48-H, 49-H, 50-H, 51-H, 52-H), 7.42 (d, 1H, *J* 7.9 Hz, 15-H), 7.54 (d, 1H, *J* 7.8 Hz, 12-H), 9.57 (s, 1H, 1-H); δ_C (75 MHz, CD_3CN) 7.6 (21′-C), 12.0 (29-C), 16.2 (30-C), 21.2 (30′-C), 22.4 (9-C), 23.6 (5-C), 25.5 (28-C), 26.1 (21-C), 29.3 (22-C), 31.5 (20′-C), 37.1 (34-C), 37.6 (27-C), 38.0 (4-C), 39.5 (24′-C), 43.7 (5′-C), 44.3 (11′-C), 48.2 (23-C), 49.9 (33-C), 51.3 (8′-C), 51.6 (10′-C), 52.4 (6-C), 52.6 (27′-C), 53.9 (12′-C), 56.5 (23′-C), 57.9 (3-C), 58.6 (26-C), 62.5 (20-C), 65.2 (8-C), 66.2 (19′-C), 67.3 (37-C), 67.9 (46-C), 77.31 (4′-C), 80.6 (3′-C), 84.2 (2′-C), 95.7 (17′-C), 110.1 (10-C), 111.9 (15-C), 119.3 (12-C), 119.9 (13-C), 122.7 (14-C), 123.7 (14′-C), 123.85 (15′-C), 123.9 (13′-C), 125.4 (7′-C), 129.02 (49, C51-C), 129.05 (40-C, C42-C), 129.12 (11-C), 129.15 (50-C), 129.2 (41-C), 129.5 (39, C43, C48, C52-C), 131.3 (6′-C), 135.9 (2-C), 136.7 (16-C), 136.9 (38-C), 137.1 (47-C), 154.7 (18′-C), 159.9 (16′-C), 171.3 (35-C, C44-C), 171.4 (29′-C), 171.6 (18-C), 172.1 (31-C), 173.0 (25′-C), 173.2 (24-C), 182.6 (17-C); *m*/*z* (ESI) 1244.5918 (M + H$^+$; $C_{70}H_{81}N_7O_{14}$ requires 1244.5920).

Compound 22. 10% Pd/carbon (8 mg, 0.008 mmol), was added to a solution of compound **20** (55 mg, 0.04 mmol) in anhydrous THF. The mixture was stirred for 2 days under an H_2 atmosphere and filtered through a pad of Celite to afford quantitatively compound **22** (43 mg, 100%); *Apparent peaks* δ_H (600 MHz; MeOD) 0.50 (m, 3H, 21′-H), 0.97 (t, 3H, *J* 7.3 Hz, 31-H), 1.07 (d, 1H, *J* 6.9 Hz, 32-H), 1.41–1.30 (m, 3H, 5-H, 30-H, 20′-H), 1.62–1.74 (m, 3H, 5-H, 30-H, 20′-H), 1.93–2.05 (m, 2H, 24-H, 29-H), 2.07 (s, 3H, 30′-H), 2.15–2.25 (m, 2H, 23-H, 24-H), 2.26 (m, 2H, 11′-H), 2.32 (m, 1H, 23-H), 2.59 (m, 1H, 10′-H), 2.77 (s, 3H, 24′-H), 2.79 (m, 1H, 19′-H), 2.88 (m, 1H, 36-H), 3.15 (m, 1H, 8′-H), 3.27 (m, 2H, 6-H), 3.53 (m, 1H, 10′-H), 3.61 (m, 1H, 8′-H), 3.64 (m, 1H, 9-H), 3.70 (s, 1H, 2′-H), 3.73 (s, 3H, 19-H), 3.82 (s, 3H, 27′-H), 3.83 (m, 1H, 8-H), 3.84 (m, 2H, 25-H), 3.84 (s, 3H, 23′-H), 4.42 (d, 1H, *J* 7.2 Hz, 28-H), 4.44–4.74 (m, 2H, 22-H, 35-H), 5.39 (m, 1H, 6′-H), 5.44 (s, 1H, 4′-H), 5.90 (dd, 1H, *J* 10.2 and 5.0 Hz, 7′-H), 6.38 (s, 1H, 17′-H), 6.99 (s, 1H, 14′-H), 7.08 (t, 1H, *J* 8.0 Hz, 14-H), 7.15 (t, 1H, *J* 7.8 Hz, 13-H), 7.37 (d, 1H, *J* 8.0 Hz, 15-H), 7.71 (m, 1H, 12-H); δ_C(150 MHz; MeOD) 8.6 (21′-C), 11.9 (31-C), 16.2 (32-C), 20.9 (30′-C), 23.9 (5-C), 26.0 (30-C), 26.3 (24-C), 27.9 (9-C), 30.8 (23-C), 32.3 (20′-C), 37.6 (36-C), 38.6 (29-C), 39.0 (24′-C), 44.2 (5′-C), 44.8 (11′-C), 49.3 (6-C), 49.3 (27-C), 50.7 (35-C), 51.4 (8′-C, 10′-C), 53.2 (19-C, 23′-C), 54.3 (12′-C), 56.5 (25-C), 59.6 (28-C), 61.5 (22-C), 77.1 (4′-C), 81.2 (3′-C), 83.5 (2′-C), 95.7 (17′-C), 112.4 (15-C), 121.0 (14-C), 123.5 (13-C), 124.5 (14′-C), 170.4 (29′-C), 173.2 (37-C, 39-C), 174.2 (17-C); *m*/*z* (ESI) 1096.5212 (M + H$^+$; $C_{57}H_{74}N_7O_{15}$ requires 1096.5243).

Compound 23. Freshly distilled propionaldehyde (116 μL, 1.61 mmol, 10 equiv.) and sodium triacetoxyborohybride (68 mg, 0.32 mmol, 2 equiv.) were added to a solution of **20** (202 mg, 0.16 mmol, 1 equiv.) in anhydrous CH_2Cl_2 (2 mL). After stirring for 3 h at room temperature, the solvent was evaporated to dryness and the crude product was purified by preparative layer chromatography (CH_2Cl_2/EtOH: 93/7) to give compound **23** (160 mg, 0.12 mmol, 75%); $[\alpha]_D^{22}$ +11 (*c* 0.1 in $CHCl_3$); ν_{max}(film)/cm^{-1} 2959, 1739 and 1658; δ_H(600 MHz, CD_3CN) 0.48 (t, 3H, *J* 7.3 Hz, 21′-H), 0.59 (t, 3H, *J* 7.3 Hz, 22-H), 0.84 (m, 3H, 34-H), 0.87 (m, 3H, 35-H), 1.12 (m, 1H, 33-H), 1.18 (m, 2H, 21-H), 1.21 (m, 1H, 20′-H), 1.44 (m, 1H, 33-H), 1.59 (m, 1H, 20′-H), 1.83 (m, 1H, 32-H), 1.95 (m, 2H, 5-H), 1.97 (s, 3H, 30′-H), 1.93 (m, 1H, 27-H), 1.97 (m, 1H, 26-H), 2.03 (m, 1H, 27-H), 2.10 (m, 1H, 26-H), 2.16 (m, 1H, 4-H), 2.17 (m, 2H, 11′-H), 2.36 (m, 1H, 20-H), 2.45 (m, 1H, 10′-H), 2.47 (m, 1H, 4-H), 2.69 (m, 1H, 19′-H), 2.70 (m, 3H, 24′-H), 2.72 (m, 1H, 8′-H), 2.94 (dd, 1H, *J* 5.6 and 4.9 Hz, 39-H), 3.19 (m, 1H, 9-H), 3.24 (m, 1H, 6-H), 3.31 (m, 1H, 10′-H), 3.37 (dd, 1H, *J* 16.7 and 4.6 Hz, 8′-H), 3.48 (m, 2H, 6-H, 9-H), 3.56 (s, 3H, 19-H), 3.62 (s, 1H, 2′-H), 3.71 (s, 3H, 27′-H), 3.71 (m, 1H, 28-H), 3.73 (s, 3H, 23′-H), 3.81 (m, 1H, 28-H), 3.86 (m, 1H, 8-H), 4.24 (m, 1H, 31-H), 4.47 (m, 1H, 25-H), 4.84 (m, 1H, 38-H), 5.07 (s, 2H, 51-H), 5.10 (s, 2H, 42-H), 5.24 (d, 1H, *J* 10.3 Hz, 6′-H), 5.27 (s, 1H, 4′-H), 5.81 (dd, 1H, *J* 10.2 and 4.6 Hz, 7′-H), 6.30 (s, 1H, 17′-H), 6.93 (s, 1H, 14′-H), 6.98 (t, 1H, *J* 7.6 Hz, 13-H), 7.03 (t, 1H, *J* 7.6, 14-H), 7.21 (m, 1H, 15-H), 7.29–7.40 (m, 12H, 30-H, 37-H, 44-H, 45-H, 46-H, 47-H, 48-H, 53-H, 54-H, 55-H, 56-H, 57-H), 7.49 (d, 1H, *J* 7.6 Hz, 12-H), 8.33 (s, 1H, 1-H); δ_C (150 MHz, CD_3CN) 8.5 (21′-C), 12.0 (22-C), 12.1 (34-C), 16.2 (35-C), 21.2 (5-C), 21.3 (30′-C), 22.3 (21-C), 25.5 (33-C), 25.8 (27-C), 28.7 (9-C), 29.0 (26-C), 31.9 (20′-C), 34.2 (4-C), 37.0 (39-C), 38.2 (32-C), 39.1 (24′-C), 43.8 (5′-C), 45.2 (11′-C), 48.6 (28-C), 50.0 (38-C), 51.0 (6-C), 51.5 (8′-C), 51.0 (10′-C), 52.7 (27′-C), 52.8 (19-C), 54.2 (12′-C), 56.6 (3-C), 56.6 (23′-C), 57.9 (20-C), 58.7 (31-C), 61.4 (25-C), 64.9 (8-C), 66.8 (19′-C), 67.4 (51-C), 68.0 (42-C), 77.4 (4′-C), 80.7 (3′-C), 84.4 (2′-C), 95.1 (17′-C), 112.0 (15-C), 112.6 (10-C), 119.0 (12-C), 119.9 (13-C), 122.3 (14-C), 123.2 (15′-C), 124.7 (14′-C), 125.0 (13′-C), 125.4 (7′-C), 129.1, 129.2, 129.5, 129.6 (44-C, 45-C, 46-C, 47-C, 48-C, 53-C, 54-C, 55-C, 56-C, 57-C), 130.3 (11-C), 131.3 (6′-C), 134.9 (2-C), 135.9 (16-C), 136.9 (52-C), 137.1 (43-C), 154.0 (18′-C), 159.1 (16′-C), 171.5 (40-C, 49-C), 171.6 (29′-C), 172.7 (29-C), 173.1 (25′-C), 175.0 (23-C), 175.8 (17-C); *m*/*z* (ESI) 1318.6621 (M + H$^+$; $C_{74}H_{92}N_7O_{15}$ requires 1318.6645).

Compound 24. Methyl iodide (29 μL, 0.47 mmol, 10 equiv.) was added to a solution of **23** (60 mg, 0.05 mmol) in anhydrous acetonitrile (1 mL). After stirring for 48 h at room temperature, the solvent was evaporated and the crude product was purified by preparative layer chromatography (CH_2Cl_2/EtOH: 93/7) to give compound **24** (33 mg, 0.025 mmol, 53%) and some recovered starting material (14 mg, 0.01 mmol); $[\alpha]_D^{22}$ −37 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 3454, 2963, 1737 and 1653; δ_H(300 MHz, CD_3CN) 0.46–0.55 (m, 3H, 21′-H), 0.55–0.64 (m, 3H, 22-H), 0.89 (t, 1H, *J* 7.3 Hz, 35-H), 0.93 (d, 3H, *J* 6.9 Hz, 36-H), 1.14–1.27 (m, 3H, 5-H, 34-H, 20′-H), 1.44–1.69 (m, 5H, 5-H, 21-H, 34-H, 20′-H),

1.88 (m, 1H, 33-H), 1.98 (s, 3H, 30′-H), 2.01–2.35 (m, 6H, 11′-H, 27-H, 28-H), 2.60 (m, 1H, 10′-H), 2.77 (m, 1H, 8′-H), 2.75 (s, 3H, 24′-H), 2.84 (s, 1H, 19′-H), 2.91 (m, 1H, 4-H), 2.96 (d, 1H, *J* 5.9 Hz, 40-H), 3.07 (m, 2H, 20-H), 3.14 (s, 3H, 23-H), 3.26–3.43 (m, 3H, 4-H, 8′-H, 10′-H), 3.53 (d, 1H, *J* 17.5 Hz, 9-H), 3.65 (s, 3H, 19-H), 3.66 (s, 1H, 2′-H), 3.73 (s, 3H, 27′-H), 3.80 (s, 3H, 23′-H), 3. 58–3.85 (m, 4H, 6-H, 29-H), 3.91 (dd, 1H, *J* 17.7 and 8.7 Hz, 9-H), 4.30 (m, 1H, 32-H), 4.59–4.72 (m, 2H, 8-H, 26-H), 4.88 (m, 1H, 39-H), 5.11 (d, 2H, *J* 4.4 Hz, 52-H), 5.13 (d, 2H, *J* 3.8 Hz, 43-H), 5.22 (s, 1H, 4′-H), 5.27 (d, 1H, *J* 10.4 Hz, 6′-H), 5.83 (dd, 1H, *J* 10.2 and 5.0 Hz, 7′-H), 6.34 (s, 1H, 17′-H), 6.84 (s, 1H, 14′-H), 7.07–7. 22 (m, 3H, 13-H, 14-H, 31-H), 7.30 (d, 1H, *J* 8.0 Hz, 38-H), 7. 32–7. 49 (m, 11H, 15-H, 45-H, 46-H, 47-H, 48-H, 49-H, 54-H, 55-H, 56-H, 57-H, 58-H), 7.80 (d, 1H, *J* 7.9 Hz, 12-H), 8.84 (s, 1H, 1-H); δ_C (75 MHz, CD_3CN)/4-C, 10-C, 11-C not visible/8.4 (21′-C), 10.7 (22-C), 12.0 (35-C), 1626.2 (9-C),.0 (36-C), 17.1 (21-C), 21.2 (30′-C), 22.1 (5-C), 25.7 (28-C, 34-C), 30.1 (27-C), 31.8 (20′-C), 37.1 (40-C), 38.2 (33-C), 38.9 (24′-C), 43.7 (5′-C), 45.4 (11′-C), 49.9 (39-C, 29-C), 51.0, 51.4 (8′-C, 10′-C), 52.7 (27′-C), 53.2 (19-C), 54.3 (12′-C), 56.1 (3-C), 56.6 (23′-C), 58.8 (32-C), 61.6 (26-C), 63.0 (6-C), 66.1 (19′-C), 67.4 (52-C), 68.0 (20-C, 43-C), 71.6 (8-C), 77.5 (4′-C), 80.7 (3′-C), 84.2 (2′-C), 95.1 (17′-C), 112.6 (15-C), 119.2 (12-C), 120.9 (13-C), 121.0 (15′-C), 123.7 (14-C), 124.1 (14′-C), 125.3 (13′-C), 125.5 (7′-C), 129.15, 129.24, 129.3, 129.6 (45-C, 46-C, 47-C, 48-C, 49-C, 54-C, 55-C, 56-C, 57-C, 58-C), 131.3 (6′-C), 134.5 (2-C), 136.5 (16-C), 136.9 (53-C), 137.1 (44-C), 154.4 (18′-C), 159.0 (16′-C), 166.6, 171.4, 171.47, 171.53, 171.9, 172.9 (24-C, 30-C, 37-C, 41-C, 50-C, 25′-C, 29′-C), 175.0 (17-C); *m/z* (ESI) 1332.6791 (M^+; $C_{75}H_{94}N_7O_{15}$ requires 1332.6808).

Compound 25. 1-Chloroethylchloroformate (mixture of enantiomers) (34 μL, 0,31 mmol, 5 equiv.) was added to a solution of **20** (78 mg, 0.063 mmol) in anhydrous dichloromethane (0,7 mL). After stirring for 3 h at room temperature, the solvent was evaporated and the crude product was purified by preparative layer chromatography (CH_2Cl_2/EtOH: 93/7) to give compound **25** as two diastereomers (rd : 1/0.4) (66%); *Major diastereomer* :$[\alpha]_D^{22}$ –34 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 2927, 1740, 1365, 1217 and 768; δ_H(300 MHz, CD_3CN) 0.73 (m, 3H, 21′-H), 0.87 (t, 1H, *J* 7.4 Hz, 35-H), 0.91 (d, 3H, *J* 6.9 Hz, 36-H), 1.18 (m, 2H, 5-H, 34-H), 1.27 (m, 1H, 20′-H), 1.31 (d, 1H, *J* 5.7 Hz, 23-H), 1.47 (m, 1H, 34-H), 1.62 (m, 1H, 20′-H), 1.72 (m, 1H, 5-H), 1.89 (m, 1H, 28-H), 1.95 (m, 2H, 28-H, 33-H), 1.97 (s, 3H, 30′-H), 2,02 (m, 2H, 27-H), 2.10 (m, 1H, 11′-H), 2.41 (m, 1H, 4-H), 2.50 (td, 1H, *J* 12.4 and 5.2 Hz, 11′-H), 2.65 (m, 1H, 10′-H), 2.67 (s, 3H, 24′-H), 2.79 (d, 1H, *J* 15.4 Hz, 8′-H), 2.86 (s, 1H, 19′-H), 2.93 (m, 1H, 4-H), 2.93 (m, 1H, 40-H), 3.05 (m, 1H, 6-H), 3.14 (m, 1H, 9-H), 3.25 (m, 2H, 29-H, 10′-H), 3.31 (dd, 1H, *J* 16.1 and 5.1 Hz, 8′-H), 3.41 (m, 1H, 6-H), 3.48 (s, 3H, 19-H), 3.58 (s, 1H, 2′-H), 3.63 (m, 1H, 9-H), 3.70 (s, 3H, 27′-H), 3.72 (m, 1H, 29-H), 3.76 (s, 3H, 23′-H), 4.24 (dd, 1H, *J* 8.3 and 5.9 Hz 32-H), 4.39 (m, 1H, 26-H), 4.84 (m, 1H, 39-H), 5.05 (s, 2H, 52-H), 5.09 (s, 2H, 43-H), 5.23 (m, 1H, 8-H), 5.25 (s, 1H, 4′-H), 5.29 (d, 1H, *J* 10.3 Hz, 6′-H), 5.81 (dd, 1H, *J* 10.2 and 5.3 Hz, 7′-H), 6.15 (d, 1H, *J* 5.6 Hz, 22-H), 6.26 (s, 1H, 17′-H), 6.92 (s, 1H, 14′-H), 7.03 (m, 2H, 13-H, 14-H), 7.11 (d, 1H, *J* 7.7 Hz, 15-H), 7.30 (m, 10H, 45-H, 46-H, 47-H, 48-H, 49-H, 54-H, 55-H, 56-H, 57-H, 58-H), 7.59 (d, 1H, *J* 7.6 Hz, 12-H), 8.21 (s, 1H, 1-H); δ_C (150 MHz, CD_3CN) 9.2 (21′-C), 12.0 (35-C), 16.2 (36-C), 21.2 (30′-C), 22.0 (5-C), 25.2 (23-C), 25.6 (34-C), 25.9 (28-C), 27.7 (9-C), 29.4 (27-C), 32.0 (20′-C), 33.1 (4-C), 37.1 (40-C), 37.8 (33-C), 39.2 (24′-C), 43.9 (5′-C), 45.2 (11′-C), 48.9 (6-C), 48.0 (29-C), 50.0 (39-C), 50.9, 51.0 (8′-C, 10′-C), 52.6 (19-C, 27′-C), 54.5 (12′-C), 56.7 (23′-C), 57.2 (3-C), 57.4 (8-C), 58.7 (32-C), 62.2 (26-C), 66.1 (19′-C), 67.4 (52-C), 68.0 (43-C), 77.5 (4′-C), 80.7 (3′-C), 83.3 (22-C), 85.0 (2′-C), 94.9 (17′-C), 111.1 (10-C), 112.1 (15-C), 118.3 (12-C), 118.9 (13-C), 122.5 (15′-C), 123.0 (14-C), 124.5 (13′-C), 125.5 (14′-C), 126.9 (7′-C), 129.1, 129.2, 129.3, 129.6 (45-C, 46-C, 47-C, 48-C, 49-C, 54-C, 55-C, 56-C, 57-C, 58-C), 130.6 (11-C), 131.4 (6′-C), 133.8 (2-C), 135.7 (16-C), 137.0 (53-C), 137.1 (44-C), 153.0 (20-C), 154.1 (18′-C), 158.9 (16′-C), 170.5 (24-C), 171.3, 171.5, 171.6 (29′-C, 41-C, 50-C), 172.0 (37-C), 172.6 (30-C), 173.0 (25′-C), 175.5 (17-C); *m/z* (ESI) 1382.6017 (M^+; $C_{74}H_{89}N_7O_{17}Cl$ requires 1382.6003). *Minor diastereomer* : $[\alpha]_D^{22}$ –60 (*c* 0.1, $CHCl_3$); ν_{max}(film)/cm^{-1} 2927, 1740, 1365, 1217 and 768; δ_H(300 MHz, CD_3CN) 0.57–0.67 (m, 6H, 23-H, 21′-H), 0.89 (t, 1H, *J* 7.4 Hz, 35-H), 0.92 (d, 3H, *J* 6.9 Hz, 36-H), 1.19 (m, 1H, 34-H), 1.31–1.42 (m, 2H, 5-H, 20′-H), 1.49 (m, 1H, 34-H), 1.65 (m, 1H, 20′-H), 1.78 (m, 1H, 5-H), 1.89 (m, 1H, 28-H), 1.91–1.99 (m, 2H, 28-H, 33-H), 1.99 (s, 3H, 30′-H), 2.03 (m, 2H, 27-H), 2.13 (m, 1H, 11′-H), 2.36–2.50 (m, 1H, 4-H, 10′-H, 11′-H), 2.58 (s, 1H, 19′-H), 2.60 (d, 1H, *J* 15.4 Hz, 8′-H), 2.71 (s, 3H, 24′-H), 2.89 (m, 1H, 4-H), 2.95 (m, 1H, 40-H), 2.96 (m, 1H, 6-H), 3.16 (d, *J* 15.7 Hz, 1H, 9-H), 3.25 (m, 1H, 29-H), 3.36 (m, 1H, 10′-H), 3.41 (dd, 1H, *J* 15.8 and 5.6 Hz, 8′-H), 3.49 (s, 3H, 19-H), 3.49 (m, 1H, 6-H), 3.61 (m, 1H, 9-H), 3.62 (s, 1H, 2′-H), 3.71 (s, 3H, 27′-H), 3.75 (s, 3H, 23′-H), 3.78 (m, 1H, 29-H), 4.25 (dd, 1H, *J* 8.3 and 5.9 Hz 32-H), 4.38 (m, 1H, 26-H), 4.85 (m, 1H, 39-H), 5.06 (s, 2H, 52-H), 5.10 (s, 2H, 43-H), 5.19 (m, 1H, 8-H), 5.27 (s, 1H, 4′-H), 5.33 (d, 1H, *J* 10.3 Hz, 6′-H), 5.84 (dd, 1H, *J* 10.2 and 5.3 Hz, 7′-H), 5.98 (m, 1H, 22-H), 6.27 (s, 1H, 17′-H), 6.63 (s, 1H, 14′-H), 6.95–7.08 (m, 2H, 13-H, 14-H), 7.17 (d, 1H, *J* 7.7 Hz, 15-H), 7.27–7.37 (m, 10H, 45-H, 46-H, 47-H, 48-H, 49-H, 54-H, 55-H, 56-H, 57-H, 58-H), 7.61 (d, 1H, *J* 7.6 Hz, 12-H), 8.32 (s, 1H, 1-H); δ_C (150 MHz, CD_3CN) 8.7 (21′-C), 12.0 (35-C), 16.2 (36-C), 21.3 (30′-C), 22.2 (5-C), 24.3 (23-C), 25.6 (34-C, 28-C), 27.5 (9-C), 29.5 (27-C), 31.8 (20′-C), 33.5 (4-C), 37.1 (40-C), 37.8 (33-C), 38.7 (24′-C), 43.7 (5′-C), 44.9 (11′-C), 48.7 (6-C), 48.2 (29-C), 50.0 (39-C), 51.8, 51.9 (8′-C, 10′-C), 52.7 (19-C, 27′-C), 54.4 (12′-C), 56.7 (23′-C), 57.2 (3-C), 57.7 (8-C), 58.7 (32-C), 62.2 (26-C), 67.1 (19′-C), 67.4 (52-C), 68.0 (43-C), 77.4 (4′-C), 80.8 (3′-C), 83.9 (22-C), 84.5 (2′-C), 94.7 (17′-C), 111.1 (10-C), 112.1 (15-C), 119.2 (12-C), 120.1 (13-C), 122.0 (15′-C), 122.9 (14-C), 124.2 (13′-C), 125.4 (7′-C), 126.3 (14′-C), 129.1, 129.2, 129.3, 129.6 (45-C, 46-C, 47-C, 48-C, 49-C, 54-C, 55-C, 56-C, 57-C, 58-C), 130.5 (11-C), 131.3 (6′-C), 134.8 (2-C), 137.3 (16-C), 137.0 (53-C), 137.2 (44-C), 153.2 (20-C), 154.0 (18′-C), 159.1 (16′-C), 170.2 (24-C), 171.3, 171.5, 171.6 (29′-C, 41-C, 50-C), 172.1 (37-C), 172.6 (30-C), 172.8 (25′-C), 175.4 (17-C); *m/z* (ESI) 1382.6025 (M^+; $C_{74}H_{89}N_7O_{17}Cl$ requires 1382.6003).

Computational procedures. All calculations were performed on a PC workstation. The modeling study was performed using Sybyl 7.3 software. The MMFF94 force field was used for minimization and partial charge calculations, a dielectric constant of 1.0 being employed. Compound **5** was subjected to an unrestrained molecular dynamics simulation at 1600 K for 20 000 fs. Conformations were sampled every 100 fs during the simulation resulting in 200

randomized structures. Each of these conformers was minimized and compared with others with a RMS of 0.3 Å. The obtained structures were ranked according to energy. They were analyzed using Sybyl 7.3 software and superimposition of conformers was based on the backbone atoms of vinblastine.

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