189. Synthesis of Lipid Derivatives of Colchicine

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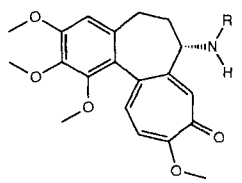
The synthesis of glycero-lipids linked to colchicine derivatives is reported. The lipid structures are designed to perform two-dimensional crystallization experiments with tubulin, the structural subunit protein of microtubules.

Introduction. – Colchicine, a plant alkaloid extracted from *Colchicum automnale* L. in the early thirties, is certainly one of the oldest and the most studied inhibitor of microtubule-mediated processes *in vivo* [1], and has been used in the treatment of acute gout, familial Mediterranean fever and liver cirrhosis [2]. Colchicine binds to soluble tubulin heterodimer and inhibits microtubule assembly substoichiometrically [3]. It has been shown that incorporation of liganded tubulin into microtubules induces a conformational change that prevents incorporation of additional liganded or unliganded dimers [4]. Actually, three-dimensional structural analyses on tubulin either in microtubules [5], double rings [6], or Zn-induced sheets [7] provide data on functional protein, but no description of conformationally modified colchicine-liganded tubulin is available.

To attend a structural analysis of colchicine-liganded tubulin by two-dimensional crystallization and electron crystallography [8], we prepared lipid derivatives of colchicine. Once spread into monolayers at the air/water interface, these derivatives should promote an in-plane concentration of the protein introduced into the sub-phase with further two-dimensional crystallization of the liganded immobilized tubulin. Herein, we describe the synthesis of these specifically designed lipid derivatives of colchicine.

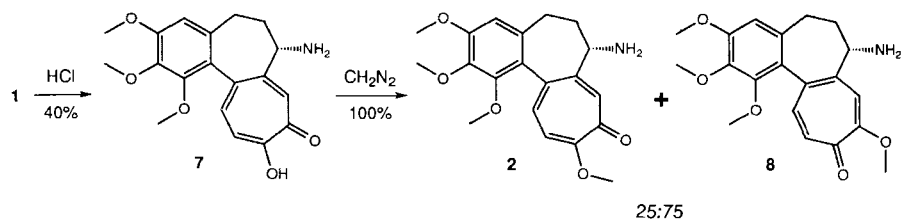
Due to its antimitotic properties, colchicine has been extensively studied, and a number of analogs have been synthesized, providing interesting structure-activity-related informations [9]. When aiming to anchor the tubulin molecule at a surface, results of chromatography experiments with the protein are especially valuable and allow pointing out pertinent hypotheses about the way colchicine can be attached to a matrix, mostly preserving its biological properties [10]. As a matter of fact, results in the literature indicate that the acetamido moiety in colchicine can be chemically modified without substantial loss of activity. Accordingly, colchicine derivatives **3**–**6** were prepared in order to evaluate their biological properties prior to target any structure of a lipid derivative of the drug.



1 R = Ac, Colchicine
2 R = H, Deacetylcolchicine
3 R = BnO–$(CH_2)_4$–CO
4 R = BnO–$(CH_2)_2$–O–CH_2–CO
5 R = BnO–$(CH_2)_5$
6 R = BnO–$(CH_2)_2$–O–$(CH_2)_2$

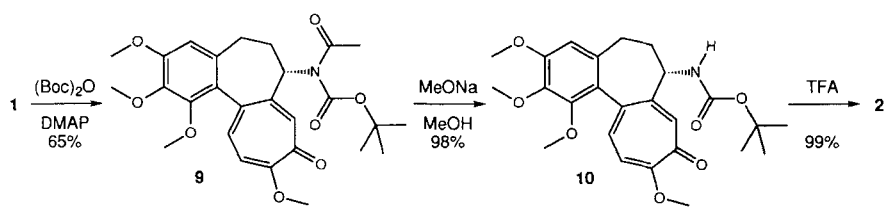
All these compounds share the same precursor, deacetylcolchicine (**2**), that is available from colchicine [11]. However, the direct acid hydrolysis of the amide described in the literature occurs with the loss of the Me group on the tropone moiety (*Scheme 1*). The resulting trimethyl colchicinic acid **7** has to be further methylated, but isomerization of the tropone ring provides a mixture of **2** and isodeacetylcolchicine **8** in a 25:75 ratio. Deacetylcolchicine (**2**) can be separated from its isomer and is obtained with an overall yield of 10% from **1**. More recently, a modification of that synthetic scheme, involving an intermediate protection of the amino group in **7** as a trifluoroacetamide, afforded a slightly higher overall yield (12.7%) [12].

Scheme 1



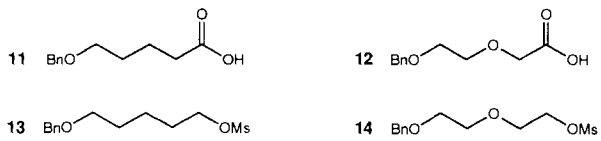
Results. – We have developed an alternative route to compound **2** using *Grieco*'s methodology for the hydrolysis of amide (*Scheme 2*) [13]. This strategy straightforwardly provides **2** in a high yield.

Scheme 2

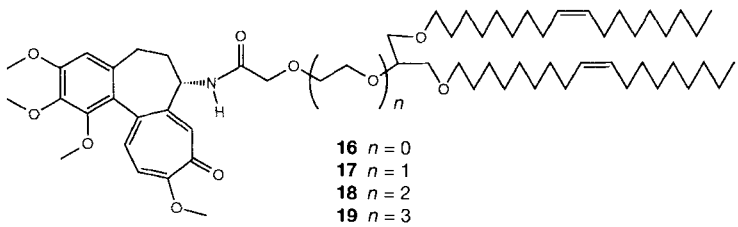


Colchicine (**1**) is transformed into *tert*-butyl carbamate **9** in a 65% yield. A portion of intact starting alkaloid **1** (24%) is recovered during purification of **9**. The carbamate **9** is smoothly decomposed into the Boc-protected deacetylated compound **10** using MeONa in MeOH. The reaction is nearly quantitative, so is the removal of the Boc protection that lead to compound **2**. The overall yield from consumed colchicine (**1**) is 83%. Purification of **9**, **10**, and **2** by chromatography on silica gel is efficient and trouble-free.

Acylated compounds **3** and **5** are obtained by coupling **2** with the corresponding carboxylic acids **11** and **12** using *N*,*N*′-dicyclohexylcarbodiimide as condensation reagent and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). *N*-Alkylated derivatives of deacetylcolchicine (**2**), **4**, and **6**, are prepared, in lower yield, by reacting with adequate mesylates **13** and **14**. The substitution reaction is conducted in MeCN using Et_3N stoichiometrically and a catalytic amount of NaI. Surprisingly, when running the reaction in DMF partial isomerization of the tropone ring was observed.

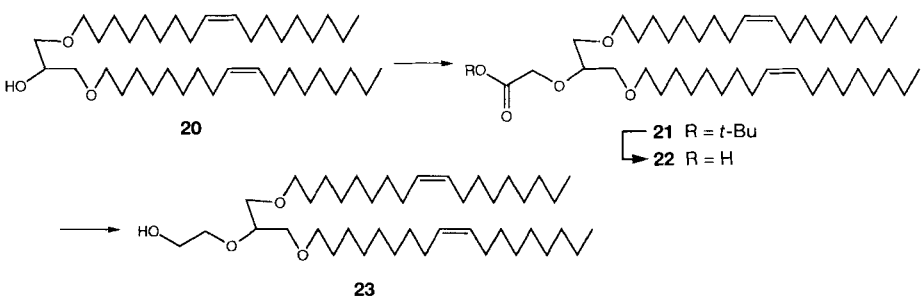


Binding experiments with tubulin reveal that all compounds **3**–**6** are good ligands for the protein, satisfactorily inhibiting microtubules polymerization [14]. So, final lipid structures **16**–**19** were targeted according to purely practical considerations. Acyl derivatives were perferred to alkyl compounds because of the higher yields obtained in the



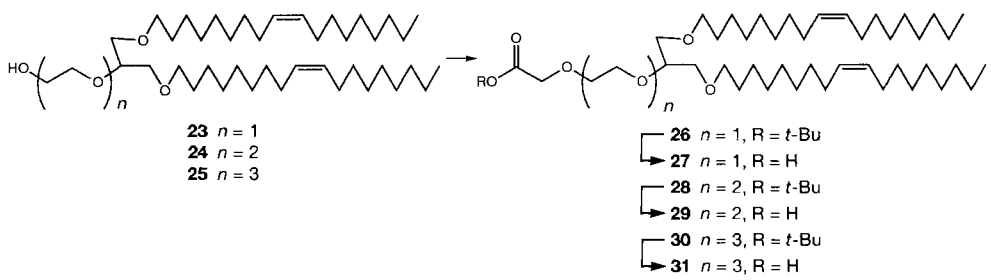
coupling reaction between deacetylcolchicine (**2**) and carboxylic acids **11** and **12** when compared to the reaction with the corresponding mesylates **13** and **14**. The structural requirements with respect to the lipid moiety have been already extensively discussed [8] [15], and they are principally responsible for the mechanical behavior of the subsequent monolayers. The required lipid precursors bearing a free carboxylic-acid function were prepared sequentially from compound **20** as described in [16] (*Scheme 3*).

Scheme 3



Compound **22**, the first member of the series, is directly obtained by the nucleophilic attack of the sodium alkoxide of alcohol **20** on *tert*-butyl bromoacetate. Hydrolysis of the resulting ester **21** affords the acid **22**. On the other hand, reduction of **21** with $LiAlH_4$ leads to alcohol **23** that can be in its turn involved in a *Williamson* coupling with *tert*-butyl bromoacetate to yield ester **26** and, after hydrolysis, the acid **27** (*Scheme 4*). The same reaction sequence is applied to alcohols **24** and **25** [16] to prepare the two higher homologs of the series, **29** and **31**, respectively. The final coupling reaction between the lipids **22**, **27**, **29**, and **31** and deacetylcolchicine (**2**) is realized essentially using the method described above for the preparation of compounds **3** and **4**.

Scheme 4



Biological properties of compounds **16–19** are now under investigation, and crystallization experiments with tubulin are in progress.

The authors are thankful to *A. Valleix* for running mass spectra.

Experimental Part

General. THF and Et_2O were distilled over Na/benzophenone and CH_2Cl_2 over CaH_2, just before use. Reactions were monitored by TLC (*Merck* precoated plates 0.25 mm, silica gel *60* F_{254}, 0.040–0.060 mm, 230–400 mesh ASTM). Liquid chromatography: on silica gel *60* (*Merck*, 0.040–0.060 mm, 230–400 mesh ASTM). ^{1}H- and ^{13}C-NMR spectra: *Bruker-WP-200-Sy* spectrometer; chemical shifts δ in ppm relative to an internal reference (^{1}H: $CHCl_3$ at 7.27 ppm or CD_2HOD at 3.35 ppm; ^{13}C: $CDCl_3$ at 77.0 ppm), J in Hz. IR Spectra: *Perkin-Elmer-1600-FT* spectrometer; absorption values in cm^{-1}. MS: *Finnigan-4600* quadrupole instrument and *Varian MAT 311* GC-coupled instrument (HR-MS).

N-*[(*tert-*Butoxy)carbonyl]colchicine* (**9**). Di(tert-butyl) pyrocarbonate (3.08 g, 14.1 mmol) is added by portions to a mixture of colchicine (**1**; 1.02 g, 2.5 mmol), Et_3N (0.4 ml, 2.8 mmol), and 4-(dimethylamino)pyridine (DMAP; 0.34 g, 2.8 mmol) in CH_2Cl_2 (10 ml) that is stirred for 12 h at r.t. Solvent is removed *in vacuo*, and the residue is purified by chromatography (AcOEt) to yield **9** (0.83 g, 65%) as a yellow solid, and intact **1** (0.24 g, 24%). TLC (AcOEt/MeCOMe 8:2): R_f 0.5. IR (CH_2Cl_2): 2938.1, 1737.7, 1682.4, 1619.8, 1588.8, 1487.8, 1462.2, 1370.2, 1324.2, 1252.8, 1142.5, 1094.0, 1022.5. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.54 (*s*, 1 H); 7.17 (*d*, J = 10.6, 1 H); 6.73 (*d*, J = 10.6, 1 H); 6.51 (*s*, 1 H); 5.12 (*dd*, J = 5.8, 11.9, 1 H); 3.94 (*s*, 3 H); 3.90 (*s*, 3 H); 3.87 (*s*, 3 H); 3.63 (*s*, 3 H); 2.70–2.37 (*m*, 3 H); 2.25 (*s*, 3 H); 2.02–1.84 (*m*, 1 H); 1.53 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 179.2; 163.8; 153.3; 153.2; 151.0; 148.8; 141.5; 137.5; 134.3; 133.9; 132.8; 126.2; 111.6; 106.9; 84.6; 61.4; 61.2; 57.6; 56.1; 55.9; 50.2; 32.3; 30.0; 27.7; 25.5. CI-MS (NH_3): 517 ($[M + NH_4]^+$).

N-*[(*tert-*Butoxy)carbonyl]deacetylcolchicine* (**10**). Compound **9** (0.48 g, 0.96 mmol) and MeONa (0.19 g, 3.52 mmol) in MeOH (10 ml) are stirred for 30 min at 0°. The mixture is neutralized by adding solid NH_4Cl, before the solvent is evaporated. The crude residue is chromatographed (AcOEt/MeCOMe 10:0 to 8:2) to yield **10** (0.43 g, 98%) as a pale-yellow solid. TLC (AcOEt/MeCOMe 8:2): R_f 0.5. IR (CH_2Cl_2): 3278.3, 2935.9, 1708.0, 1588.8, 1567.5, 1487.8, 1460.9, 1252.9, 1168.5, 1138.9, 1092.5. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.49 (*s*, 1 H); 7.23 (*d*, J = 10.7, 1 H); 6.79 (*d*, J = 10.7, 1 H); 6.51 (*s*, 1 H); 5.06 (*d*, J = 7.6, 1 H); 4.42–4.35 (*m*, 1 H); 3.97 (*s*, 3 H); 3.90 (*s*, 3 H); 3.87 (*s*, 3 H); 3.62 (*s*, 3 H); 2.51–2.16 (*m*, 3 H); 1.72–1.63 (*m*, 1 H); 1.33 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 190.3; 179.5; 154.3; 153.3; 151.1; 141.5; 136.0; 135.0; 134.2; 131.1; 125.6; 112.0; 107.1; 79.8; 61.5; 61.3; 60.4; 56.2; 56.0; 53.0; 37.6; 29.9; 29.2; 28.2. CI-MS (NH_3): 475 ($[M + NH_4]^+$).

Deacetylcolchicine (**2**). Carbamate **10** (0.41 g, 0.89 mmol) is stirred for 3 h in CH_2Cl_2 (10 ml) with CF_3COOH (1 ml). Toluene (20 ml) is added, and the mixture is reduced *in vacuo* and purified on silica gel to yield **2** (0.32 g, 99%) as a yellow solid. Anal. data are consistent with those described in [17]. TLC (CH_2Cl_2/EtOH 9:1): R_f 0.2.

N-*[5-(Benzyloxy)pentanoyl]deacetylcolchicine* (**3**). Deacetylcolchicine (**2**; 40 mg, 0.11 mmol), *5-(benzyloxy)pentanoic acid* (**11**; 46 mg, 0.22 mmol), DCC (80 mg, 0.39 mmol), and DMAP (3 mg, 0.02 mmol) are stirred in CH_2Cl_2 (1 ml) at r.t. Compound **2** is completely consumed after 1 h, and after 2 h, the solvent is removed, and the residue is purified by chromatography (AcOEt/MeCOMe/EtOH 8:2:0 to 8:2:1) to yield **3** (61 mg, 66%). TLC (CH_2Cl_2/EtOH) 9:1): R_f 0.5. IR (CH_2Cl_2): 3272.0, 2933.7, 2857.9, 1654.3, 1615.1, 1588.4, 1563.7, 1487.7, 1460.0,

1349.6, 1322.4, 1252.1, 1178.6, 1140.3, 1094.8, 1018.0. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.45 (*s*, 1 H); 7.30 (*m*, 5 H); 7.29 (*d*, J = 8.9, 1 H); 7.06 (*d*, J = 6.9, 1 H); 6.81 (*d*, J = 8.9, 1 H); 6.51 (*s*, 1 H); 4.62 (*ddd*, J = 5.8, 6.9, 11.9, 1 H); 4.45 (*s*, 2 H); 3.97 (*s*, 3 H); 3.93 (*s*, 3 H); 3.89 (*s*, 3 H); 3.64 (*s*, 3 H); 3.46 (*t*, J = 5.9, 2 H); 2.43–2.16 (*m*, 5 H); 1.70–1.56 (*m*, 5 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 179.4; 172.4; 163.9; 153.3; 151.6; 141.5; 138.4; 136.4; 135.1; 134.1; 130.7; 128.3; 127.7; 127.5; 125.6; 112.3; 107.2; 100.1; 72.9; 70.2; 61.5; 61.3; 56.2; 56.0; 52.0; 36.6; 35.8; 29.8; 28.9; 22.4. CI-MS (NH_3): 537 ($[M + NH_4]^+$).

N-{*[2-(Benzyloxy)ethoxy]acetyl*}*deacetylcolchicine* (**4**). Compound **4** (51 mg) is obtained in 65% yield from **2** following the procedure described for **3**. TLC (CH_2Cl_2/EtOH 9:1): R_f 0.6. IR (CH_2Cl_2): 3325.7, 2934.6, 2858.1, 1675.1, 1617.1, 1589.4, 1568.7, 1487.9, 1461.0, 1349.8, 1322.8, 1252.4, 1179.4, 1140.5, 1094.9, 1018.6. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.60 (*d*, J = 7.4, 1 H); 7.43–7.24 (*m*, 5 H); 7.37 (*s*, 1 H); 7.22 (*d*, J = 10.7, 1 H); 6.77 (*d*, J = 10.7, 1 H); 6.46 (*s*, 1 H); 4.61 (*AB*, J_{AB} = 11.1, $\Delta\nu$ = 33.7, 2 H); 4.55 (*m*, 1 H); 3.98 (*s*, 3 H); 3.94 (*AB*, J_{AB} = 2.1, $\Delta\nu$ = 0.7, 2 H); 3.92 (*s*, 3 H); 3.88 (*s*, 3 H); 3.75 (*m*, 4 H); 3.62 (*s*, 3 H); 2.37–2.90 (*m*, 3 H); 2.02–1.82 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 194.3; 179.3; 169.7; 153.3; 150.4; 142.0; 137.4; 136.0; 134.6; 134.2; 131.4; 128.6; 128.4; 127.9; 125.7; 111.8; 107.2; 97.5; 71.2; 69.9; 69.4; 61.4; 56.2; 56.0; 51.4; 36.1; 33.5; 30.0. CI-MS (NH_3): 539 ($[M + NH_4]^+$).

N-*[5-(Benzyloxy)pentyl]deacetylcolchicine* (**5**). Compound **2** (45 mg, 0.12 mmol), *5-(benzyloxy)pentyl methanesulfonate* **13** (92 mg, 0.33 mmol), Et_3N (47 µl, 0.33 mmol), and NaI (4 mg, 0.02 mmol) are stirred in refluxing MeCN for 16 h. The solvent is evaporated, and the crude residue is chromatographed (CH_2Cl_2/EtOH 10:0 to 9:1) to yield **5** (21 mg, 31%). TLC (CH_2Cl_2/EtOH 8:2): R_f 0.5. IR (CH_2Cl_2): 2936.6, 2856.2, 1715.8, 1614.8, 1588.1, 1571.5, 1487.1, 1460.0, 1395.8, 1344.9, 1319.7, 1281.5, 1248.3, 1137.6, 1094.1, 1018.0. ^{1}H-NMR (CD_3OD, 200 MHz): 7.81 (*s*, 1 H); 7.42 (*d*, J = 10.7, 1 H); 7.32 (*m*, 5 H); 7.22 (*d*, J = 10.7, 1 H); 6.75 (*s*, 1 H); 4.48 (*s*, 2 H); 4.03 (*s*, 3 H); 3.92 (*s*, 3 H); 3.91 (*m*, 1 H); 3.90 (*s*, 3 H); 3.62 (*s*, 3 H); 3.47 (*t*, J = 6.4, 2 H); 2.59–2.25 (*m*, 5 H); 1.71–1.32 (*m*, 7 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 179.7; 163.8; 153.1; 151.3; 141.1; 138.5; 137.0; 135.2; 134.5; 132.3; 128.3; 127.6; 127.4; 125.6; 111.7; 107.1; 72.8; 70.2; 61.3; 60.8; 56.2; 56.0; 47.8; 38.7; 30.3; 30.0; 29.5; 23.9. CI-MS (NH_3): 551 ($[M + NH_4]^+$).

N-{*2-[2-(Benzyloxy)ethoxy]ethyl*}*deacetylcolchicine* (**6**). Compound **6** (19 mg) is obtained in 33% yield from **2** and *2-[2-(benzyloxy)ethoxy]ethyl methanesulfonate* **14** following the procedure described for **5**. TLC (CH_2Cl_2/EtOH 9:1): R_f 0.6. IR (CH_2Cl_2): 2928.4, 2855.6, 1614.9, 1588.5, 1571.5, 1487.1, 1461.2, 1395.4, 1345.6, 1248.5, 1136.5, 1094.0, 1018.6. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.86 (*s*, 1 H); 7.34–7.27 (*m*, 5 H); 7.21 (*d*, J = 10.6, 1 H); 6.78 (*d*, J = 10.6, 1 H); 6.52 (*s*, 1 H); 4.55 (*s*, 2 H); 3.99 (*s*, 3 H); 3.92 (*s*, 3 H); 3.90 (*s*, 3 H); 3.60 (*s*, 3 H); 3.59–3.50 (*m*, 6 H); 3.35 (*dd*, J = 6.2, 10.7, 1 H); 2.66–2.18 (*m*, 5 H); 1.72–1.64 (*m*, 1 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 179.8; 163.8; 153.1; 151.0; 150.6; 138.2; 136.9; 135.2; 134.4; 132.6; 128.3; 127.7; 125.6; 111.6; 107.1; 73.1; 70.4; 70.3; 69.3; 65.8; 61.2; 60.8; 56.2; 56.0; 47.4; 38.4; 30.2. CI-MS (NH_3): 553 ($[M + NH_4]^+$).

tert-*Butyl 2-*{*1-[(Z)-Octadec-9-enyloxy]-2-*{*[(Z)-octadec-9-enyloxy]methyl*}*ethoxy*}*acetate* (**21**). Alcohol **20** (1.50 g, 2.52 mmol), *tert*-butyl bromoacetate (0.37 ml, 2.52 mmol) and HMPA (2 ml) are stirred at 0° in THF (20 ml). NaH 60% in oil (0.15 g, 3.75 mmol) is added in portions, and the mixture is refluxed for 18 h. It is then cooled to r.t., and additional portions of *tert*-butyl bromoacetate (0.37 ml, 2.52 mmol) and NaH (0.08 g, 1.90 mmol) are added before refluxing for 24 h. The mixture is decomposed at 0° with a sat. NH_4Cl soln. and extracted with Et_2O. The org. layer is washed with H_2O, brine, then dried (Na_2SO_4), and reduced *in vacuo*. The residue is chromatographed on silica gel (Et_2O/hexane 5:95) to yield **21** (1.15 g, 64%). TLC (Et_2O/hexane 2:8): R_f 0.7. IR (CH_2Cl_2): 2921.2, 2855.8, 1746.8, 1719.6, 1458.0, 1365.4, 1310.9, 1256.3, 1223.6, 1147.8. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, J = 4.7, 4 H); 4.19 (*s*, 2 H); 3.73 (*m*, 1 H); 3.57 (*d*, J = 4.9, 4 H); 3.43 (*t*, J = 6.6, 4 H); 2.00 (*m*, 8 H); 1.60–1.53 (*m*, 4 H); 1.48 (*s*, 9 H); 1.30–1.25 (*m*, 44 H); 0.89 (*t*, J = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 170.0; 129.8; 129.7; 81.6; 78.1; 71.6; 71.2; 68.3; 31.8; 29.7; 29.6; 29.2; 28.0; 27.9; 26.0; 22.6; 14.0. CI-MS (NH_3): 724 ($[M + NH_4]^+$). CI-MS (CH_4): 707.4 ($[M + H]^+$).

Compounds **26** (0.39 g, 81%), **28** (0.54 g, 63%), and **30** (0.43 g, 61%) are obtained following the same procedure, starting from **23**, **24**, and **25**, resp.

tert-*Butyl 2-*{*2-*{*1-[(Z)-Octadec-9-enyloxy]-2-*{*[(Z)-octadec-9-enyloxy]methyl*}*ethoxy*}*ethoxy*}*acetate* (**26**). TLC (Et_2O/hexane 2:8): R_f 0.4. IR (CH_2Cl_2): 2919.2, 2848.7, 1748.6, 1460.7, 1360.8, 1219.8, 1125.7. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, J = 4.7, 4 H); 4.03 (*s*, 2 H); 3.81 (*t*, J = 5.3, 2 H); 3.70 (*t*, J = 5.3, 2 H); 3.62 (*m*, 1 H); 3.50 (*d*, J = 4.2, 4 H); 3.43 (*t*, J = 6.6, 4 H); 2.04–1.98 (*m*, 8 H); 1.62–1.51 (*m*, 4 H); 1.48 (*s*, 9 H); 1.32–1.24 (*m*, 44 H); 0.88 (*t*, J = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 170.5; 129.8; 129.7; 81.7; 78.4; 71.6; 70.9; 68.5; 66.6; 31.8; 29.7; 29.6; 29.5; 29.3; 28.1; 27.1; 26.1; 22.6; 14.0. CI-MS (NH_3): 768 ($[M + NH_4]^+$).

tert-*Butyl 2-*{*2-*{*2-*{*1-[(Z)-Octadec-9-enyloxy]-2-*{*[(Z)-octadec-9-enyloxy]methyl*}*ethoxy*}*ethoxy*}*ethoxy*}*acetate* (**28**). TLC (Et_2O/hexane 1:1): R_f 0.4. IR (CH_2Cl_2): 2921.2, 2855.8, 1746.8, 1730.4, 1460.1, 1365.4, 1294.6, 1251.0, 1223.7, 1125.6. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, J = 4.7, 4 H); 4.02 (*s*, 2 H); 3.82–3.60 (*m*, 9 H); 3.49

(*d*, *J* = 5.9, 4 H); 3.43 (*t*, *J* = 6.6, 4 H); 2.03–1.98 (*m*, 8 H); 1.62–1.51 (*m*, 4 H); 1.48 (*s*, 9 H); 1.33–1.25 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 169.7; 129.9; 129.8; 81.4; 78.5; 71.7; 70.9; 70.8; 70.6; 69.7; 69.1; 31.9; 29.8; 29.7; 29.5; 29.3; 28.1; 27.2; 26.1; 22.7; 14.1. CI-MS (NH_3): 812 ($[M + NH_4]^+$).

tert-*Butyl 2-{2-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}-ethoxy}ethoxy}acetate* (**30**). TLC (Et_2O/hexane 1:1): R_f 0.2. IR (CH_2Cl_2): 2931.0, 2840.7, 1748.6, 1460.7, 1366.7, 1225.6, 1143.4, 1119.9. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, *J* = 4.7, 4 H); 4.03 (*s*, 2 H); 3.77–3.61 (*m*, 13 H); 3.49 (*d*, *J* = 5.8, 4 H); 3.43 (*t*, *J* = 6.5, 4 H); 2.03–1.98 (*m*, 8 H); 1.56–1.43 (*m*, 4 H); 1.47 (*s*, 9 H); 1.29–1.25 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 169.5; 129.8; 129.7; 81.3; 78.4; 71.6; 70.9; 70.8; 70.7; 70.6; 70.5; 69.7; 69.0; 31.8; 29.7; 29.6; 29.4; 29.2; 28.0; 27.1; 26.1; 22.6; 14.0. CI-MS (NH_3): 856 ($[M + NH_4]^+$), 839.4 ($[M + H]^+$).

2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}acetic Acid (**22**). Ester **21** (470 mg, 0.66 mmol) is stirred for 30 min in CH_2Cl_2 (1 ml) with CF_3COOH (0.1 ml) at r.t. Solvent is removed *in vacuo*, and the residue is purified by chromatography (Et_2O/hexane 2:8 to 10:0) to yield **22** (362 mg, 84%). TLC (Et_2O): R_f 0.7. IR (CH_2Cl_2): 2924.4, 2854.3, 1769.3, 1735.7, 1465.6, 1376.2, 1118.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.34 (*t*, *J* = 4.6, 4 H); 4.27 (*s*, 2 H); 3.68 (*m*, 1 H); 3.49 (*t*, *J* = 6.4, 4 H); 2.00 (*m*, 8 H); 1.59 (*m*, 4 H); 1.28 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 172.0; 129.8; 129.7; 80.4; 71.9; 70.6; 68.7; 31.8; 31.7; 29.7; 29.6; 29.4; 29.3; 29.2; 29.1; 28.8; 27.1; 25.9; 22.5; 13.9. CI-MS (NH_3): 668 ($[M + NH_4]^+$).

Compounds **27** (0.22 g, 87%), **29** (0.37 g, 80%), and **31** (0.26 g, 81%) are obtained following the same procedure, starting from **26**, **28**, and **30**, resp.

2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}acetic Acid (**27**). TLC (CH_2Cl_2/EtOH 9:1): R_f 0.3. IR (CH_2Cl_2): 3405.3, 2921.3, 2846.0, 1697.0, 1610.9, 1460.4, 1433.5, 1202.2, 1137.7. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, *J* = 4.7, 4 H); 4.08 (*s*, 2 H); 3.84–3.80 (*m*, 2 H); 3.74–3.71 (*m*, 3 H); 3.53 (*d*, *J* = 4.8, 4 H); 3.45 (*t*, *J* = 6.6, 4 H); 2.03–1.99 (*m*, 8 H); 1.55–1.29 (*m*, 48 H); 0.89 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 174.5; 129.9; 129.8; 78.5; 71.9; 71.7; 70.0; 69.9; 68.2; 31.9; 29.8; 29.7; 29.6; 29.5; 29.3; 29.2; 29.0; 27.2; 27.1; 26.2; 22.6; 14.0. CI-MS (NH_3): 712 ($[M + NH_4]^+$).

2-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}ethoxy}acetic Acid (**29**). TLC (CH_2Cl_2/EtOH 9:1): R_f 0.3. IR (CH_2Cl_2): 3426.4, 2911.7, 2855.7, 1694.7, 1616.4, 1454.1, 1325.4, 1202.3, 1129.5. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.34 (*t*, *J* = 4.8, 4 H); 4.04 (*s*, 2 H); 3.79–3.61 (*m*, 9 H); 3.49 (*d*, *J* = 4.7, 4 H); 3.42 (*t*, *J* = 6.6, 4 H); 2.03–1.96 (*m*, 8 H); 1.58–1.52 (*m*, 4 H); 1.31–1.25 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 174.5; 129.9; 78.6; 71.6; 70.6; 69.9; 69.5; 68.3; 31.9; 29.8; 29.7; 29.6; 29.5; 29.3; 29.2; 29.0; 27.2; 27.1; 26.2; 22.6; 14.0. CI-MS (NH_3): 756 ($[M + NH_4]^+$).

2-{2-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}ethoxy}ethoxy}-acetic Acid (**31**). TLC (CH_2Cl_2/EtOH 9:1): R_f 0.3. IR (CH_2Cl_2): 3409.9, 2932.2, 2852.6, 1694.8, 1605.3, 1456.0, 1431.1, 1326.6, 1321.9, 1202.2, 1137.5. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.35 (*t*, *J* = 4.7, 4 H); 3.97 (*s*, 2 H); 3.80–3.65 (*m*, 13 H); 3.48 (*d*, *J* = 4.9, 4 H); 3.38 (*t*, *J* = 6.6, 4 H); 2.07–1.94 (*m*, 8 H); 1.62–1.23 (*m*, 48 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 174.5; 129.9; 129.8; 78.3; 71.6; 70.7; 70.3; 70.1; 70.0; 69.9; 69.2; 68.3; 31.9; 29.8; 29.7; 29.6; 29.5; 29.3; 29.2; 29.0; 27.2; 27.1; 26.2; 22.6; 14.0. CI-MS (NH_3): 800 ($[M + NH_4]^+$).

2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethanol (**23**). To **21** (1.10 g, 1.55 mmol) in Et_2O is added $LiAlH_4$ (88 mg, 2.32 mmol) at 0°. The mixture is stirred for 30 min, and excess reagent is decomposed by addition of a few drops of MeOH before filtration over *Celite*. The filtrate is reduced *in vacuo* and purified by chromatography (Et_2O/hexane 1:3) to yield **23** (0.88 g. 89%). TLC (Et_2O/hexane 1:1): R_f 0.4. IR (CH_2Cl_2): 3466.0, 2910.3, 2855.8, 1458.0, 1370.8, 1120.2. ^{1}H-NMR ($CDCl_3$, 200 MHz): 5.34 (*t*, *J* = 4.8, 4 H); 3.75–3.65 (*m*, 4 H); 3.61–3.53 (*m*, 1 H); 3.48–3.41 (*m*, 8 H); 2.05–1.96 (*m*, 8 H); 1.59–1.46 (*m*, 4 H); 1.30–1.25 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 129.8; 129.7; 78.5; 72.2; 71.6; 71.2; 62.0; 31.8; 29.7; 29.5; 29.4; 29.2; 28.9; 27.1; 26.0; 22.6; 14.0. CI-MS (NH_3): 654 ($[M + NH_4]^+$).

2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}acetyldeacetylcolchicine (**16**). Carboxylic acid **22** (49 mg, 75 µmol), **2** (27 mg, 75 µmol), DCC (75 mg, 363 µmol), and DMAP (8 mg, 65 µmol) are stirred for 6 h at r.t. in CH_2Cl_2 (2 ml). Solvent is removed *in vacuo*, and the residue is purified by prep. TLC (AcOEt) to afford **16** (28 mg, 37%). TLC (AcOEt): R_f 0.5. IR (CH_2Cl_2): 2922.1, 2842.9, 1737.5, 1675.4, 1618.9, 1584.9, 1460.6, 1245.8, 1138.4, 1093.2. ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.06 (*d*, *J* = 6.7, 1 H); 7.42 (*s*, 1 H); 7.25 (*d*, *J* = 10.8, 1 H); 7.05 (*s*, 1 H); 6.77 (*d*, *J* = 10.8, 1 H); 6.53 (*s*, 1 H); 5.34 (*t*, *J* = 4.6, 4 H); 4.67 (*m*, 1 H); 4.05 (*AB*, J_{AB} = 4.4, $\Delta\nu$ = 7.9, 2 H); 3.97 (*s*, 3 H); 3.94 (*s*, 3 H); 3.90 (*s*, 3 H); 3.75 (*m*, 1 H); 3.65 (*s*, 3 H); 3.53–3.45 (*m*, 8 H); 2.58–2.42 (*m*, 3 H); 2.28–2.08 (*m*, 1 H); 2.05–1.95 (*m*, 8 H); 1.66–1.52 (*m*, 4 H); 1.35–1.24 (*m*, 44 H); 0.88 (*t*, *J* = 6.7, 6 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 179.4; 170.3; 164.0; 153.4; 151.3; 150.5; 141.7; 136.0; 134.6; 134.0; 131.3; 129.9; 129.8; 126.0; 111.7; 107.3; 79.3; 71.9; 70.8; 70.5; 69.0; 61.4; 56.2; 53.4; 51.6; 36.6; 31.9; 29.9; 29.7; 29.5; 29.3; 29.2; 29.0; 27.2; 26.0; 22.6; 14.0. HR-MS: $C_{61}H_{99}NO_9$: calc.: 989.7320; found: 989.7373.

Compounds **17** (56 mg, 67%), **18** (53 mg, 62%), and **19** (36 mg, 42%) are obtained in the same way from **27**, **29**, and **31**, resp.

N-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}ethoxy}acetyldeacetylcolchicine (**17**). TLC (AcOEt): R_f 0.5. IR (CH_2Cl_2): 2919.2, 2848.7, 1739.6, 1678.1, 1613.5, 1584.1, 1460.7, 1249.1, 1137.5, 1096.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 8.34 (*d*, J = 6.6, 1 H); 7.39 (*s*, 1 H); 7.27 (*d*, J = 10.4, 1 H); 6.79 (*d*, J = 10.4, 1 H); 6.53 (*s*, 1 H); 5.34 (*t*, J = 4.8, 4 H); 4.66 (*m*, 1 H); 4.20–4.04 (*m*, 2 H); 3.98 (*s*, 3 H); 3.94 (*s*, 3 H); 3.90 (*s*, 3 H); 3.84 (*t*, J = 5.0, 2 H); 3.70 (*t*, J = 5.0, 2 H); 3.65 (*s*, 3 H); 3.55 (*d*, J = 4.2, 4 H); 3.43 (*t*, J = 6.7, 4 H); 2.56–2.35 (*m*, 3 H); 2.21–2.14 (*m*, 1 H); 2.05–1.94 (*m*, 8 H); 1.63–1.22 (*m*, 48 H); 0.88 (*t*, J = 6.6, 6 H). HR-MS: $C_{63}H_{103}NO_{10}$: calc.: 1033.7582; found: 1033.7536.

N-{2-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}ethoxy}-ethoxy}acetyldeacetylcolchicine (**18**). TLC (AcOEt): R_f 0.5. IR (CH_2Cl_2): 2919.2, 2848.7, 1725.1, 1678.1, 1613.5, 1584.1, 1460.7, 1249.1, 1119.9, 1096.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.57 (*d*, J = 5.8, 1 H); 7.40 (*s*, 1 H); 7.27 (*d*, J = 10.6, 1 H); 6.79 (*d*, J = 10.6, 1 H); 6.53 (*s*, 1 H); 5.34 (*t*, J = 5.3, 4 H); 4.71–4.62 (*m*, 1 H); 4.14 (*AB*, J_{AB} = 4.0, $\Delta\nu$ = 32.9, 2 H); 3.98 (*s*, 3 H); 3.95 (*s*, 3 H); 3.90 (*s*, 3 H); 3.81–3.70 (*m*, 9 H); 3.65 (*s*, 3 H); 3.50 (*d*, J = 3.9, 4 H); 3.42 (*t*, J = 6.6, 4 H); 2.57–2.35 (*m*, 3 H); 2.32–2.15 (*m*, 1 H); 2.07–1.96 (*m*, 8 H); 1.64–1.21 (*m*, 48 H); 0.88 (*t*, J = 6.6, 6 H). HR-MS: $C_{65}H_{107}NO_{11}$: calc.: 1077.7844; found: 1077.7867.

N-{2-{2-{2-{2-{1-[(Z)-Octadec-9-enyloxy]-2-{[(Z)-octadec-9-enyloxy]methyl}ethoxy}ethoxy}ethoxy}-ethoxy}ethoxy}acetyldeacetylcolchicine (**19**). TLC (AcOEt): R_f 0.5. IR (CH_2Cl_2): 2919.2, 2860.5, 1725.1, 1678.1, 1619.3, 1584.1, 1460.7, 1255.0, 1137.5, 1119.9, 1096.4. ^{1}H-NMR ($CDCl_3$, 200 MHz): 7.50 (*d*, J = 5.7, 1 H); 7.39 (*s*, 1 H); 7.27 (*d*, J = 10.5, 1 H); 6.78 (*d*, J = 10.5, 1 H); 6.53 (*s*, 1 H); 5.34 (*t*, J = 5.2, 4 H); 4.71–4.61 (*m*, 1 H); 4.12–3.90 (*m*, 2 H); 3.98 (*s*, 3 H); 3.94 (*s*, 3 H); 3.90 (*s*, 3 H); 3.70–3.62 (*m*, 13 H); 3.65 (*s*, 1 H); 3.47 (*d*, J = 3.7, 4 H); 3.41 (*t*, J = 6.6, 4 H); 2.59–2.35 (*m*, 3 H); 2.33–2.15 (*m*, 1 H); 2.07–1.95 (*m*, 8 H); 1.64–1.21 (*m*, 48 H); 0.88 (*t*, J = 6.6, 6 H). HR-MS: $C_{67}H_{111}NO_{12}$: calc.: 1121.8106; found: 1121.8087.

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