

# Supplementary Material

## A Novel Strategy for Bioconjugation: Synthesis and Preliminary Evaluation with Amphotericin B

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### General

All reactions were carried out in oven-dried glassware under an atmosphere of argon. CH<sub>2</sub>Cl<sub>2</sub> was passed through two 4 x 36 inch columns of anhydrous neutral A-2 alumina (8 x 14 mesh; Macherey und Nagel; activated under a flow of N<sub>2</sub> at 300 °C overnight) to remove water. All other solvents were ACS grade. Amphotericin B was purchased from Apollo Scientific (~90% purity). POPC (1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was from CHEMI S.p.A. All other chemicals were purchased from ABCR, Acros, Aldrich, COSMO S.p.A., Fluka, J. T. Baker, Merck, Novabiochem, Senn, Sigma, Strem SYNOPHARM and used without further purification.

The salts and the membrane components for the K<sup>+</sup>-selective electrode, valinomycin, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), bis(2-ethylhexyl) sebacate (DOS), tetradodecylammonium tetrakis(4-chlorophenyl)borate (ETH 500), poly(vinyl chloride) (PVC) of high molecular weight, and tetrahydrofuran (THF) were from Fluka Selectophore<sup>®</sup>. For selectivity measurements Suprapure KCl and NaCl from Merck were used. For other measurements NaCl puriss p.a. from J. T. Baker, and Titrisol<sup>®</sup> (HCl) from Merck were used.

Aqueous solutions were prepared with freshly deionized water (18.0 MΩcm specific resistance) obtained with a NANOpure reagent-grade water system (Barnstead).

Pyridine, Hünig's Base, and NEt<sub>3</sub> were distilled from KOH.

Chromatographic purification was performed as flash chromatography using Brunschwig silica 32-63, 60Å or Merck Silica 60 with 0.3 bar air pressure..

Solvent removal was performed by evaporation on a Büchi rotavapor at 40 °C.

TLC was performed on Merck silica gel 60 F<sub>254</sub> TLC glass plates and visualized with permanganate stain or CAM.

UV-VIS spectra were recorded as dmsol solutions in a Varian Cary 50 Conc UV-Visible Spectrophotometer.

A part of the NMR spectra were recorded by the NMR service at ETH Zürich.

<sup>1</sup>H-NMR spectra were recorded on a VARIAN Mercury 300 MHz spectrometer or on a Bruker DRX500 spectrometer in chloroform-d or DMSO-d<sub>6</sub>, all signals are reported in ppm with the solvent signal at 7.26 ppm or 2.50 ppm as standard. The data is being

reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved, br = broad signal, coupling constant(s) in Hz, integration).

<sup>13</sup>C-NMR spectra were recorded with <sup>1</sup>H-decoupling on a VARIAN Mercury 75 MHz spectrometer or on a Bruker DRX500 spectrometer in chloroform-d, or DMSO-d<sub>6</sub> all signals are reported in ppm with the internal signal at 77.0 ppm or 39.5 ppm as standard. Infrared spectra were recorded on a Perkin Elmer Spectrum RX-I FT-IR spectrophotometer using KBr pellets or Golden Gate single reflection diamond ATR. Mass spectrometric measurements were performed by the mass spectrometry service of the ETHZ on a Bruker Reflex MALDI-TOF using 2,5-dihydroxy benzoic acid as matrix (20 kV). ESI measurements were performed on a Finnigan/Thermoquest LCQ (3.5-5 kV).

## Liposome Preparation

The appropriate lipids (POPC, POPC/cholesterol 70/30 mol/mol, POPC/ergosterol 87/13 mol/mol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> in a 250 mL round bottom flask and the solvent was removed under reduced pressure (ca. 400 mbar) in a Rotavapor (Büchi, Switzerland). The thin lipid film was dried overnight at high vacuum, then hydrated with a 150 mM KCl, 5 mM HEPES (pH 7.4) buffer, in order to obtain a liposome suspension with approx. overall lipid concentration of 3 mM in the case of POPC and 5 mM in the cases of POPC/cholesterol and POPC/ergosterol. The suspension was sonicated under nitrogen atmosphere for 30 minutes in a bath sonicator (Bandelin Sonorex RK100H, 140 W, 35 kHz). Then the liposomes were sized by extrusion (The Extruder®, Lipex Biomembranes Inc., Vancouver, Canada), forcing the suspension to pass through two (stacked) polycarbonate membranes (Nuclepore® Whatman) of 400 nm, 200 nm, and finally 100 nm pore size (ten times for each pore size).

The resultant “100 nm” unilamellar liposomes (generally about 30 mL) were dialysed (Spectra/Por® Membranes MWCO 3,500; Spectrum) three times against 600 mL of 150 mM NaCl, 5 mM HEPES (pH 7.4) buffer. The actual phospholipid concentration was determined by measuring inorganic phosphate then the suspension was diluted with 150 mM NaCl, 5 mM HEPES (pH 7.4) buffer to 1 mM overall lipid concentration (phospholipid + sterols). For each efflux measurement 10 mL of this liposome suspension was placed in a small beaker.

After recording the amphotericin-induced potassium efflux, liposomes were lysed by adding sodium cholate (172 mg). The resulting reading (taken after 0.5 h) was used to quantify the 100% K<sup>+</sup>-release.

## ISE Membrane Preparation and Potentiometric Measurement

The membrane components (typically 300 mg of total mass) were dissolved in THF (3.0 mL) during ca. 2 h and poured into a glass ring (37 mm inner diameter) fixed on a glass support. After overnight evaporation of the solvent at rt, 5-mm disks were punched from the mother membrane (thickness, ca. 240 μm) and glued with THF to a plasticized PVC tubing (inner diameter 4 mm), mechanically fixed to a 1000-μL pipette tip. The membranes contained valinomycin (ca. 3.1, 9.2 mmol kg<sup>-1</sup>), DOS (ca. 150 mg), PVC (ca.

150 mg), KTFPB (ca. 1.1 mg, 4.2 mmol kg<sup>-1</sup>), ETH 500 (ca. 3.1 mg, 8.9 mmol kg<sup>-1</sup>). The inner filling solution consisted of 10<sup>-3</sup> M KCl. The ISEs were conditioned overnight in 10<sup>-3</sup> M KCl.

Potentiometric measurements were performed with a 16-channel electrode monitor (Lawson Labs Inc., Malvern, Pa 19355) in magnetically stirred solutions at ambient temperature (20–22 °C). Activity coefficients were obtained from the Debye–Hückel approximation and EMF values were corrected for liquid-junction potentials with the Henderson equation. The reference electrode was a Metrohm double junction Ag/AgCl reference electrode (Metrohm AG, CH-9010 Herisau, Switzerland, No. 6.0729.100) with 3 M KCl as reference electrolyte and 1 M LiOAc as bridge electrolyte. After each efflux measurement the reference electrode was washed twice with H<sub>2</sub>O and twice with 1 M LiOAc then refilled with 1 M LiOAc. This introduced an error in the absolute value measured but reduced the drift to approx. 1mV/min.

Prior to selectivity measurements, the ISEs were conditioned overnight in 10<sup>-2</sup> M NaCl. The sequence of the investigated ions was: Na<sup>+</sup>, H<sup>+</sup>, K<sup>+</sup>. For each of these ions, the membrane was conditioned in the corresponding 10<sup>-2</sup> M chloride solution (during 0.5 h), after which calibration curves were taken. The logarithmic selectivity coefficients were: -4.11 ± 0.04, and -4.35 ± 0.1 (SD, n=3), for Na<sup>+</sup> and H<sup>+</sup>, respectively.

## MIC

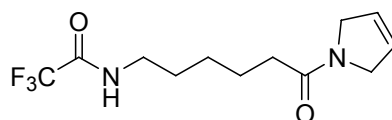
We determined the minimal drug concentration required to prevent growth of the yeast *Saccharomyces cerevisiae* as follows: For each amphotericin conjugate, several 100x stock solutions were prepared in DMSO. Each stock solution was then diluted 1:100 in YPD agar medium that had been cooled down to 50 °C, before pouring 3 mL of the mix into small petri dishes. Wildtype yeast cells (BY4741, a derivative of S288C) were grown in YPD medium, and approximately 10<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, and 10 cells were spotted on each plate. Colony formation was scored after incubation at 30 °C for 36-48 hours .

## Synthesis

The synthesis of compounds **19**, **20**, **2**, **21**, **22**, and **4** has already been reported: Zumbuehl, A.; Jeannerat, D.; Martin, S. E.; Sohrmann, M.; Stano, P.; Vigassy, T.; Clark, D. D.; Hussey, S. L.; Peter, M.; Peterson, B. R.; Pretsch, E.; Walde, P.; Carreira, E. M., An amphotericin B-fluorescein conjugate as a powerful probe for biochemical studies of the membrane. *Angewandte Chemie-International Edition* **2004**, 43, (39), 5181-5185.

It is repeated here for the purposes of completeness, following the request of a referee.

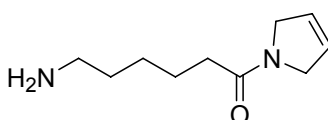
### *N*-[6-(2,5-Dihydro-pyrrol-1-yl)-6-oxo-hexyl]-2,2,2-trifluoro-acetamide (**19**)



To an ice-cooled solution of 6-(2,2,2-Trifluoro-acetyl-amino)-hexanoyl chloride (15.3 g; 62 mmol) and pyridine (6.3 mL, 78 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added 3-pyrroline (5.4 g; 78 mmol). After 5 min. the ice-bath was removed and the solution stirred for another 3 h. Then sat. NaHCO<sub>3</sub> was added and the water phase was twice extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic solvents were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and then the solvent was removed under reduced pressure. The resulting material was purified on a silica gel column (10% MeOH/ CH<sub>2</sub>Cl<sub>2</sub>) to give **19** as a white powder (11.9 g; 42.7 mmol; 69%).

R<sub>f</sub>: 0.89 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (aq.) 75/22/3; CAM). IR (KBr): 3417, 3212, 3072, 2942, 2912, 2875, 2850, 1716, 1638, 1618, 1562, 1478, 1459, 1411, 1388, 1356, 1282, 1214, 1189, 1151. <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): δ 5.84 (dd, *J* = 18.7 Hz, 6.2 Hz, 2H), 4.21 (s, 4H), 3.40 (dd, *J* = 13.1 Hz, 6.2 Hz, 2H), 2.28 (t, *J* = 6.8 Hz, 2H), 1.74-1.57 (m, 4H), 1.45-1.35 (m, 2H). <sup>13</sup>C (300 MHz, CDCl<sub>3</sub>): δ 171.0, 126.2, 124.7, 53.3, 52.9, 46.6, 45.7, 39.4, 34.3, 33.9, 28.4, 26.2, 24.4, 23.4. HRMS-MALDI (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>18</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub>: 279.1315; found: 279.1311.

### 6-Amino-1-(2,5-dihydro-pyrrol-1-yl)-hexan-1-one (**20**)

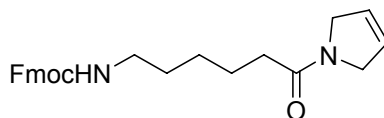


To a solution of (**19**) (11.9 g; 42.6 mmol) in MeOH (100 ml) and H<sub>2</sub>O (20 ml) was added K<sub>2</sub>CO<sub>3</sub> (11.2 g; 80.9 mmol). After stirring for 13h the mixture was filtered and the filtercake washed with additional MeOH. The solvent was removed under reduced pressure and the resulting solid was purified on a silica gel column

(CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (aq.) 75/22/3). The product was obtained as a white powder (6.40 g; 35.1 mmol; 82%).

R<sub>f</sub>: 0.36 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (aq.) 75/22/3; CAM). IR (KBr): 3409, 3083, 2939, 2864, 2132, 1686, 1638, 1614, 1461, 1358, 1324, 1202, 1176, 1133, 1002. <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): δ 5.76-5.64 (m, 2H), 4.08 (s, 4H), 2.90 (s, 3H), 2.62 (t, *J* = 6.8 Hz, 2H), 2.17 (t, *J* = 7.5, 2H), 1.57 (q, *J* = 7.5, 2H), 1.46-1.36 (m, 2H), 1.33-1.23 (m, 2H). <sup>13</sup>C (300 MHz, CDCl<sub>3</sub>): δ 171.3, 126.1, 124.7, 53.3, 52.8, 41.4, 34.1, 32.3, 26.5, 24.3. HRMS-ESI (*m/z*): [*M* + *H*]<sup>+</sup> calcd for C<sub>10</sub>H<sub>19</sub>N<sub>2</sub>O: 183.1492; found: 183.1495.

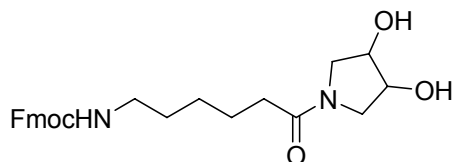
### [6-(2,5-Dihydro-pyrrol-1-yl)-6-oxo-hexyl]-carbamic acid 9-fluorenylmethyl ester (**2**)



To a mixture of **20** (6.39 g; 35.1 mmol) and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (17.7 g; 52.6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added pyridine (5.7 mL; 70 mmol). After stirring for 12 h sat. NaHCO<sub>3</sub> was added and extracted twice with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were washed with sat. NaCl and dried over Na<sub>2</sub>SO<sub>4</sub>. After removing the solvents under removed pressure the resulting material was purified on a silica gel column (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The product was obtained as a white powder (11.0 g; 27.1 mmol; 77%).

R<sub>f</sub>: 0.21 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5; CAM). IR (KBr): 3448, 3229, 3039, 2941, 2865, 1718, 1638, 1618, 1547, 1455, 1288, 1255, 1220, 1134, 1098, 1055, 1014. <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.41-7.26 (m, 4H), 5.87-5.76 (m, 2H), 5.09-5.00 (m, 1H), 7.59 (d, *J* = 6.8 Hz, 2H), 4.22 (s, 4H), 3.24-3.18 (m, 2H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.69 (q, *J* = 7.5 Hz, 2H), 1.59-1.34 (m, 4H). <sup>13</sup>C (300 MHz, CDCl<sub>3</sub>): δ 171.0, 156.3, 143.9, 141.1, 127.5, 126.9, 126.3, 125.0, 124.7, 119.8, 66.5, 53.4, 52.9, 47.3, 40.8, 34.2, 29.8, 26.5, 24.2. HRMS-MALDI (*m/z*): [*M* + Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>3</sub>: 427.1992; found: 427.1997.

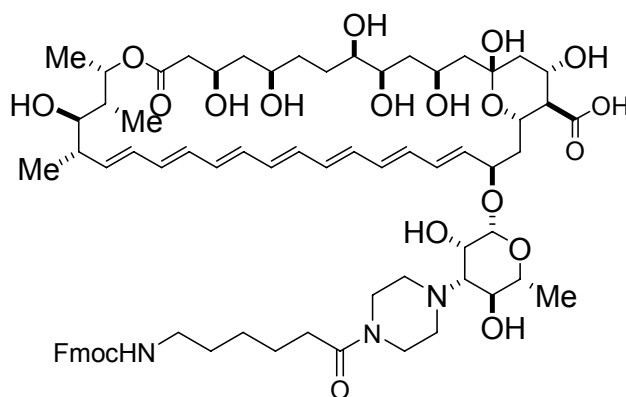
### [6-(3,4-Dihydroxy-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 9fluorenylmethyl ester (**21**)



To a solution of **2** (5.06 g; 12.5 mmol) in acetone (180 mL), H<sub>2</sub>O (60 mL), and *tert*-BuOH (12 mL) was added K<sub>2</sub>OsO<sub>4</sub>·2 H<sub>2</sub>O (430 mg; 1.3 mmol) and 4-methylmorpholine *N*-oxide (3.54 g; 26.2 mmol). After 16 h sodium hydrosulfite (2 g) and Florisil® (2 g) were added and the resulting mixture was stirred for 1 h. After filtration and removal of the solvents under reduced pressure the product was purified on a silica gel column (first 10% MeOH/ CH<sub>2</sub>Cl<sub>2</sub> 500mL; then changing to CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10/6/1). The product was obtained as white powder (3.6 g; 8.3 mmol; 66%).

R<sub>f</sub>: 0.18 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9/1; KMnO<sub>4</sub>). IR (KBr): 3404, 3337, 3074, 2935, 2882, 1963, 1920, 1880, 1808, 1697, 1616, 1599, 1547, 1466, 1449, 1378, 1359, 1330, 1297, 1268, 1242, 1170, 1138, 1110, 1059, 1018. <sup>1</sup>H (300 MHz, DMSO-d<sub>6</sub>): δ 7.88 (d, *J* = 7.5 Hz, 2H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.34-7.25 (m, 3), 4.95 (d, *J* = 5.0 Hz, 1H), 4.88 (d, *J* = 4.95 Hz, 1H), 4.37-4.18 (m, 3H), 4.07-3.94 (m, 2H), 3.56-3.51 (m, 1H), 3.39-3.33 (m, 1H), 3.25-3.14 (m, 2H), 3.00-2.93 (m, 2H), 2.17-2.12 (m, 2H), 1.51-1.35 (m, 4H), 1.29-1.19 (m, 2H). <sup>13</sup>C (300 MHz, DMSO-d<sub>6</sub>): δ 170.4, 155.8, 143.7, 140.5, 127.4, 126.8, 124.9, 119.9, 70.6, 69.2, 65.0, 50.4, 50.0, 46.7, 38.6, 33.2, 29.3, 26.0, 24.0. HRMS-MALDI (*m/z*): [M + Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>30</sub>N<sub>2</sub>NaO<sub>5</sub>: 461.2047; found: 461.2043.

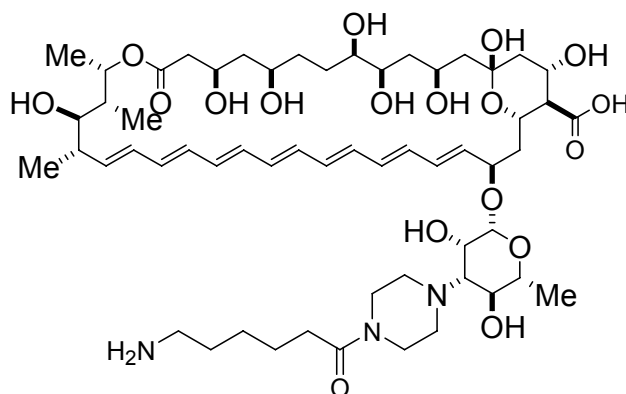
### Fmoc protected aminohexanoyl piperazinyl amphotericin B (**22**)



Silica gel (10 g) was suspended in CHCl<sub>3</sub> (80 mL). Slowly NaIO<sub>4</sub> (1.4 g; 6.5 mmol) in H<sub>2</sub>O (10 mL) was added to give a flocky suspension. To this a suspension of **21** (1.5 g; 3.4 mmol) was added. After 0.5 h the suspension was filtrated and the filtercake washed with CH<sub>2</sub>Cl<sub>2</sub> and EtOAc. The solvents were removed under reduced pressure. To the resulting solid amphotericin B (3.0 g; ca. 2.9 mmol) was added followed by DMF (60 mL). HCl (1 drop) and NaBH<sub>3</sub>CN (2.5 g; 4.0 mmol) were added and the suspension stirred overnight. Amberlite IRA-743 (2 g) was added and the suspension shaken for 1 h. After filtration the solvent was removed and the solid purified on a silica gel column (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10/6/1). The yellow product was washed thoroughly with water and lyophilized to give **22** as a yellow powder (2.4 g; 1.9 mmol; 63%).

R<sub>f</sub>: 0.70 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (DMSO, nm): 416, 392, 372, 354. IR (KBr): 3413, 3014, 2934, 1702, 1636, 1570, 1450, 1401, 1321, 1259, 1180, 1130, 1109, 1070, 1041, 1013. <sup>1</sup>H (500 MHz, DMSO-d<sub>6</sub>): δ 7.88 (d, J = 7.4 Hz, 2H), 7.68 (d, J = 7.3 Hz, 2H), 7.44-7.41 (m, 2H), 7.38-7.33 (m, 2H), 6.45-5.95 (m, 15H), 5.46-5.44 (m, 1H), 5.22 (br s, 1H), 4.38-4.21 (m, 8H), 4.06-3.81 (m, 2H), 3.46-3.42 (m, 8H), 3.18-3.10 (m, 3H), 2.97-2.84 (m, 4H), 2.84-2.75 (m, 2H), 2.38-1.92 (m, 11H), 1.71-0.72 (m, 20H). <sup>13</sup>C (125 MHz, DMSO-d<sub>6</sub>): δ 174.6, 171.1, 171.1, 156.5, 144.2, 142.8, 141.0, 139.7, 137.7, 137.2, 134.2, 134.0, 133.6, 133.5, 133.0, 132.8, 132.6, 132.4, 132.2, 131.6, 129.3, 129.1, 128.0, 127.7, 127.4, 125.5, 121.7, 120.4, 120.3, 110.1, 97.5, 97.4, 77.4, 74.8, 74.1, 74.0, 73.8, 73.7, 69.8, 69.5, 69.3, 68.0, 67.8, 67.7, 66.5, 65.5, 65.3, 57.2, 50.5, 50.1, 47.1, 46.3, 44.8, 44.3, 42.7, 42.2, 40.3, 36.2, 35.7, 35.2, 32.6, 29.4, 29.3, 26.4, 24.9, 18.9, 18.4, 17.3, 12.3. ESI (m/z): [M + H]<sup>+</sup> calcd for C<sub>72</sub>H<sub>102</sub>N<sub>3</sub>O<sub>20</sub>: 1328.7057; found: 1328.2.

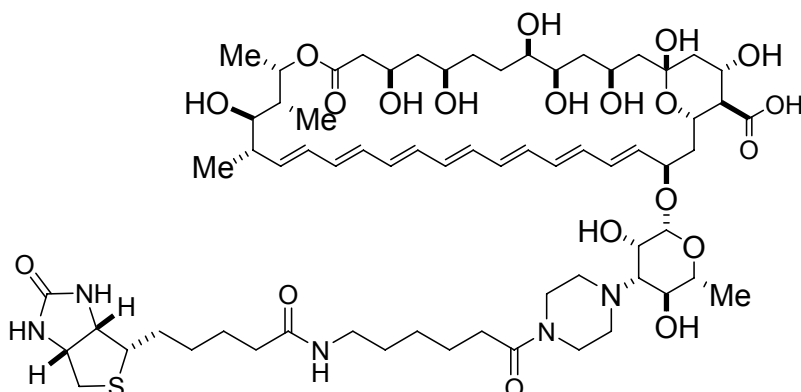
### Aminohexanoyl piperazinyl amphotericin B (**4**)



To a solution of **22** (1.0 g; 0.75 mmol) in DMSO (10 mL) was added piperidine (1 mL; 10 mmol). After 1 h the solution was slowly added to Et<sub>2</sub>O (500 mL) at r.t. The filtration yielded the crude product as a yellow powder (998 mg) which was of sufficient quality to be used directly in further reactions.

R<sub>f</sub>: 0.12 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH (aq.) 75/22/3; CAM). UV (DMSO, nm): 416, 392, 372, 354. IR (KBr): 3401, 3012, 2934, 1684, 1617, 1576, 1448, 1400, 1319, 1272, 1182, 1113, 1069, 1039, 1013. <sup>1</sup>H (500 MHz, DMSO-d<sub>6</sub>): δ 6.46-6.07 (m, 10H), 5.41 (s, 1H), 5.22 (s, 1H), 4.37-4.20 (m, 3H), 4.07 (s, 1H), 3.96-3.25 (m, 12H), 3.25-2.68 (m, 8H), 2.45-2.11 (m, 7H), 1.84-1.81 (m, 2H), 1.71-0.91 (m, 30H). <sup>13</sup>C (125 MHz, DMSO-d<sub>6</sub>): δ 176.1, 170.7, 170.6, 166.6, 137.6, 136.6, 134.0-130.6, 128.9, 128.2, 127.3, 121.3, 119.9, 99.3, 98.1, 96.8, 77.2, 75.9, 73.9, 73.4, 73.1, 72.6, 72.1, 72.0, 70.3, 70.2, 69.5, 69.2, 68.9, 68.7, 68.4, 67.7, 67.1, 67.0, 66.9, 66.2, 65.9, 65.5, 65.2, 62.7, 57.7, 50.0, 49.4, 46.4, 46.1, 44.3, 44.0, 43.6, 42.4, 42.0, 41.8, 34.8, 34.2, 32.2, 31.9, 31.8, 29.1, 28.2, 26.7, 25.4, 24.3, 24.2, 22.2, 21.7, 18.5, 18.4, 18.3, 18.1, 17.01, 16.9, 12.1, 11.9, 11.6. ESI (m/z): [M + H]<sup>+</sup> calcd for C<sub>57</sub>H<sub>92</sub>N<sub>3</sub>O<sub>18</sub>: 1106.6376; found: 1106.6.

## Biotinyl aminohexanoyl piperazinyl amphotericin B (**5**)



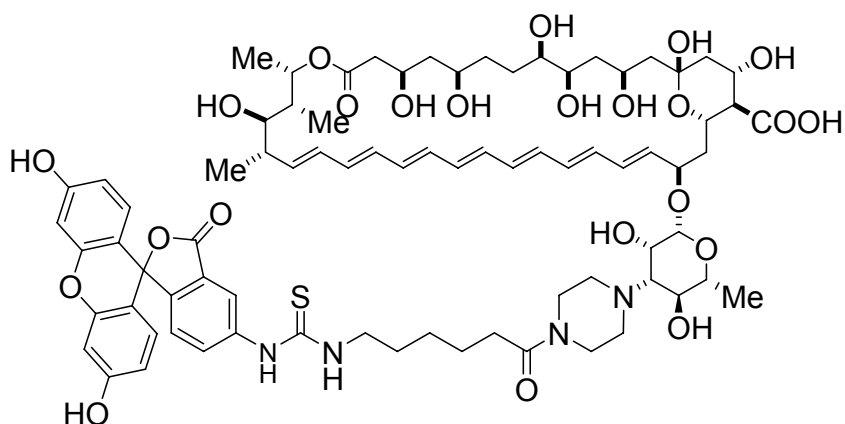
To a solution of aminohexanoyl piperazinyl amphotericin B (50.0 mg; 45.2 nmol) and (+)-biotin *N*-succinimidyl ester (12.5 mg; 36.6 nmol) in DMF (2 mL) was added  $\text{NET}_3$  (50.0  $\mu\text{L}$ ; 360 nmol). The resulting solution was stirred for 21 h whereupon the solvent was removed. The product was purified on a silica gel column ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10/6/1) to give **5** as a yellow powder (33.5 mg; 25.2 nmol; 56%).

$R_f$ : 0.46 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 416, 392, 372, 354. IR (KBr): 3400, 2930, 2859, 1696, 1654, 1637, 1570, 1560, 1458, 1438, 1400, 1331, 1239, 1177, 1111, 1070, 1012.  $^1\text{H}$  (500 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.46-6.42 (m, 1H), 6.37-6.21 (m, 9H), 6.18-5.96 (m, 6H), 5.46-5.42 (m, 1H), 5.21 (br s, 1H), 4.38-4.33 (m, 3H), 4.26-4.15 (m, 3H), 4.06-3.96 (m, 1H), 3.96-3.85 (m, 1H), 3.85-3.81 (m, 1H), 3.51-3.39 (m, 8H), 3.18-3.03 (m, 6H), 3.02-3.01 (m, 2H), 2.91-2.82 (m, 2H), 2.73-2.52 (m, 2H), 2.41-2.27 (m, 3H), 2.22-2.05 (m, 2H), 1.86-1.71 (m, 2H), 1.71-1.64 (m, 1H), 1.63-1.04 (m, 43H), 0.92-0.91 (m, 4H).

$^{13}\text{C}$  (125 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  175.1, 173.0, 172.3, 170.7, 170.6, 162.8, 136.7, 133.9, 133.7, 133.4, 133.1, 132.4, 132.4, 132.1, 131.8, 131.6, 131.1, 128.4, 97.3, 96.9, 74.8, 73.6, 73.4, 73.3, 69.5, 69.1, 69.0, 67.5, 67.0, 66.1, 65.4, 61.0, 59.1, 55.3, 50.2, 49.7, 48.4, 46.3, 46.0, 44.3, 43.9, 42.3, 42.1, 41.9, 41.8, 40.0, 39.7, 37.2, 35.1, 34.8, 32.2, 28.9, 28.7, 28.1, 27.9, 26.0, 25.2, 25.1, 24.5, 18.4, 18.0, 17.0, 16.9, 12.1, 11.9. MALDI-TOF ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{67}\text{H}_{105}\text{NaN}_5\text{O}_{20}\text{S}$ : 1354.7; found: 1354.3.



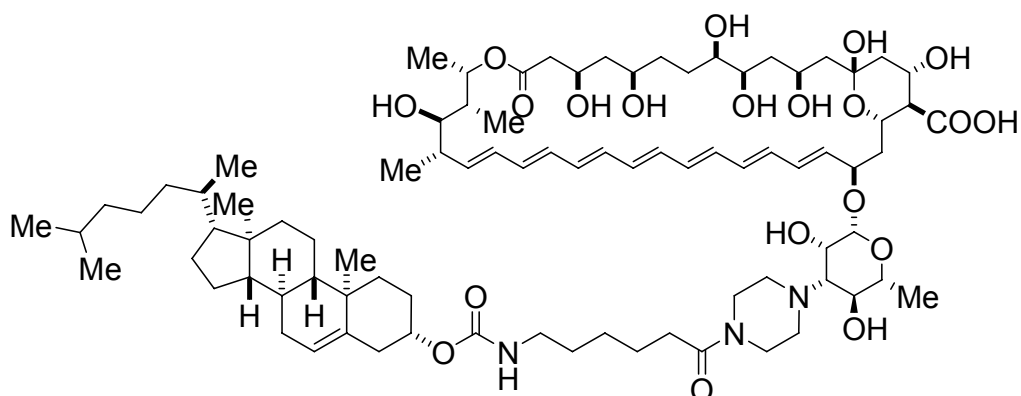
## Fluorescein piperazinyl amphotericin B (**6**)



To a solution of aminohexanoyl piperazinyl amphotericin B (99 mg, 91  $\mu\text{mol}$ ) and fluorescein isothiocyanate (35 mg, 91  $\mu\text{mol}$ ) in DMF (2 mL) was added  $\text{NEt}_3$  (49  $\mu\text{L}$ ; 360  $\mu\text{mol}$ ). After 32 h the solvent was removed under reduced pressure. The resulting product was purified on a silica gel column ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  18.5:6:1). (**6**) was isolated as a yellow powder (63 mg, 42  $\mu\text{mol}$ , 46%).

$R_f$ : 0.46 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 522, 417, 392, 372, 354. IR (KBr): 3400, 3013, 2930, 1718, 1685, 1637, 1591, 1560, 1507, 1466, 1385, 1320, 1263, 1210, 1181, 1112, 1070, 1012.  $^1\text{H}$  (600 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  8.35 (br s, 1H), 7.81–7.79 (m, 1H), 7.14 (d,  $J = 8.2$  Hz, 1H), 6.67–6.65 (m, 4H), 6.58–6.57 (m, 2H), 6.45–5.98 (m, 13H), 5.42 (br s, 1H), 5.21 (br s, 1H), 4.34–4.23 (m, 3H), 4.06–3.87 (m, 2H), 3.73–3.49 (m, 4H), 3.46–3.18 (m, 10H), 3.06–2.90 (m, 3H), 2.82–2.60 (m, 4H), 2.30–2.10 (m, 8H), 1.90–1.88 (m, 2H), 1.71–1.04 (m, 31H), 0.91 (d,  $J = 6.3$  Hz, 3H).  $^{13}\text{C}$  (125 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  180.1, 176.4, 170.7, 168.9, 162.6, 161.4, 152.5, 141.5, 137.3, 136.7, 133.9, 133.7, 133.5, 132.4, 132.1, 131.9, 131.6, 131.1, 129.2, 128.3, 124.4, 117.2, 113.7, 110.1, 102.2, 97.7, 96.9, 77.1, 76.6, 75.3, 73.7, 73.4, 69.1, 69.0, 67.6, 67.2, 66.1, 65.6, 65.5, 57.8, 50.3, 49.4, 48.4, 46.3, 46.0, 44.3, 44.0, 43.4, 42.3, 42.0, 41.8, 37.6, 35.8, 34.8, 34.2, 32.1, 30.8, 28.9, 28.0, 25.9, 24.6, 18.4, 18.0, 17.0, 16.9, 12.1, 11.9. MALDI-TOF (m/z):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{78}\text{H}_{163}\text{N}_4\text{O}_{23}\text{S}$ : 1495.6734; found: 1495.7.

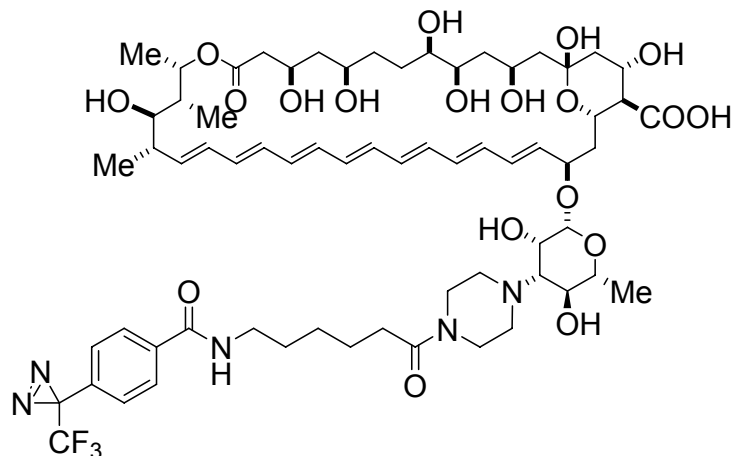
## Cholesteryl aminohexanoyl piperidinyl amphotericin B (7)



Cholesteryl chloroformate (40 mg, 50.0  $\mu\text{mol}$ ) was dissolved in DMF (2 mL). Then  $\text{NEt}_3$  (50  $\mu\text{L}$ , 300  $\mu\text{mol}$ ) and aminohexanoyl piperidinyl amphotericin B (50 mg, 50  $\mu\text{mol}$ ) were added. The resulting solution was stirred for 13 h. Then the solvent was removed under reduced pressure. The product was purified on a silica gel column ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  19:6:1) to give (7) as a yellow powder (20 mg, 2  $\mu\text{mol}$ , 33%).

$R_f$  = 0.74 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 417, 392, 372, 354. IR (Golden Gate): 3370, 2932, 2863, 2646, 1716, 1638, 1435, 1373, 1228, 1199, 1068, 1044, 1009.  $^1\text{H}$  (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  7.01 (s, 1H), 6.44–6.06 (m, 15H), 5.96–5.86 (m, 2H), 5.46–5.33 (m, 3H), 5.22 (s, 1H), 4.81–4.76 (m, 3H), 4.65 (s, 1H), 4.43–4.20 (m, 10H), 4.06–4.00 (m, 2H), 3.78 (s, 1H), 3.52–3.01 (m, 34H), 2.94–2.51 (m, 9H), 2.50–2.16 (m, 11H), 2.00–1.73 (m, 12H), 1.73–0.83 (m, 86H), 0.65 (s, 3H).  $^{13}\text{C}$  (125 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  174.3, 170.5, 170.2, 155.5, 139.7, 136.7, 136.5, 133.8, 133.6, 133.3, 133.1, 132.4, 132.1, 132.1, 131.8, 131.1, 128.6, 121.7, 97.1, 96.9, 77.1, 74.1, 73.7, 73.6, 73.5, 72.6, 69.5, 69.1, 68.7, 67.6, 67.5, 67.4, 66.1, 65.3, 64.9, 56.9, 56.0, 55.5, 50.2, 49.7, 49.4, 46.0, 45.9, 44.7, 44.2, 42.3, 41.9, 41.9, 41.8, 40.0, 38.8, 38.2, 36.6, 36.5, 36.0, 35.6, 35.1, 35.0, 32.2, 31.3, 31.2, 29.2, 28.9, 28.6, 28.5, 27.8, 27.7, 27.3, 26.0, 24.5, 23.8, 23.1, 22.6, 22.3, 22.0, 20.5, 18.9, 18.5, 18.4, 18.1, 17.0, 16.9, 13.9, 12.0, 11.6. ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{85}\text{H}_{135}\text{N}_3\text{NaO}_{20}$ : 1540.9537; found: 1540.80.

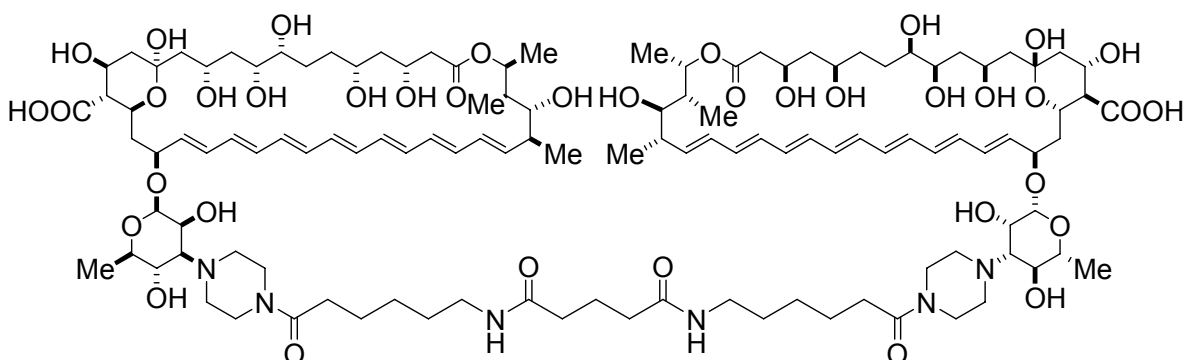
4-(3-trifluoromethyl-3-H-diazirin-3-yl)-benzoylaminohexanoyl piperazinyl amphotericin B (**8**)



To a solution of 4-(3-trifluoromethyl-3-H-diazirin-3-yl)-benzoate succinimide ester (15 mg, 45  $\mu$ mol, kindly supplied by Prof. Dr. J. Brunner) and amino-hexanoyl piperazinyl amphotericin B (49 mg, 45  $\mu$ mol) in DMF (2 mL) was added  $\text{NEt}_3$  (49  $\mu$ L; 360  $\mu$ mol). After 12 h the solvent was removed under reduced pressure. The resulting product was purified on a silica gel column ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  18.5:6:1). (**8**) was isolated as a yellow powder (33 mg, 25  $\mu$ mol, 55%).

$R_f$ : 0.70 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 417, 392, 372. IR (Golden Gate): 3334, 2932, 2646, 1824, 1690, 1630, 1561, 1443, 1403, 1318, 1285, 1238, 1183, 1154, 1111, 1068, 1010.  $^1\text{H}$  (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  8.64 (t,  $J = 5.5$  Hz, 1H), 7.94–7.92 (m, 2H), 7.38 (d,  $J = 8.1$  Hz, 2H), 6.47–6.05 (m, 10H), 5.96–5.91 (m, 2H), 5.46–5.41 (m, 1H), 5.21 (br s, 1H), 4.37 (br s, 1H), 4.28–4.06 (m, 3H), 4.04–3.98 (m, 2H), 3.80–3.70 (m, 25H), 3.52–3.40 (m, 6H), 3.27–3.24 (m, 2H), 3.10 (br s, 2H), 2.90–2.60 (m, 4H), 2.80 (d,  $J = 10.1$  Hz, 1H), 2.31–2.16 (m, 3H), 2.00–1.89 (m, 2H), 1.71–1.04 (m, 21H), 0.92 (d,  $J = 7.0$  Hz, 2H).  $^{13}\text{C}$  (125 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  174.3, 172.9, 170.7, 170.6, 165.3, 165.2, 136.8, 136.5, 136.0, 133.8, 133.6, 133.3, 133.1, 132.5, 132.3, 132.2, 131.9, 131.8, 131.1, 130.1, 128.7, 128.0, 126.3, 124.9, 122.8, 120.6, 118.4, 97.1, 77.0, 74.4, 73.6, 73.3, 69.4, 69.1, 69.0, 67.5, 67.4, 67.2, 66.1, 65.2, 65.0, 56.8, 50.1, 49.7, 45.9, 44.4, 43.9, 42.3, 41.8, 40.0, 36.8, 34.8, 32.2, 28.9, 28.6, 28.4, 28.1, 27.8, 27.5, 26.1, 25.1, 24.9, 24.5, 18.4, 18.0, 16.9, 11.9. ESI (m/z):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{66}\text{H}_{95}\text{F}_3\text{N}_5\text{O}_{19}$ : 1318.6573; found: 1318.6.

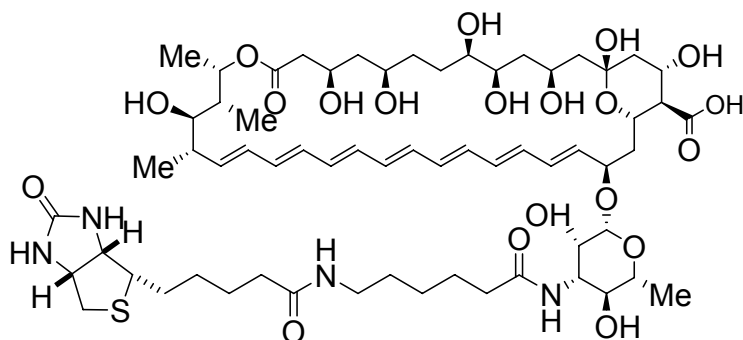
## Bis(diaminohexanoyl piperidyl amphotericin B) glutarate (**9**)



Di(N-succinimidyl)glutarate (6.5 mg, 21  $\mu\text{mol}$ ), and aminohexanoyl piperazinyl amphotericin B (50 mg, 50  $\mu\text{mol}$ ) were dissolved in DMF (1 mL). Then  $\text{NEt}_3$  (50  $\mu\text{L}$ , 0.3 mmol) was added. After 14 h the solvent was removed under reduced pressure. The product was purified on a silica gel column ( $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10/6/1) to give (**13**) as a yellow powder (20 mg, 8  $\mu\text{mol}$ , 40%).

$R_f$  = 0.35 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 417, 392, 372. IR (Golden Gate): 3321, 2932, 2646, 1716, 1634, 1569, 1434, 1373, 1272, 1217, 1197, 1106, 1047, 1003.  $^1\text{H}$  (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  7.77 (s, 1H), 6.47–6.06 (m, 11H), 5.97–5.92 (m, 1H), 5.85 (s, 1H), 5.46–5.41 (m, 2H), 5.21 (s, 1H), 4.80–3.99 (m, 9H), 3.79 (s, 1H), 3.58–3.01 (m, 20H), 3.00–2.71 (m, 2H), 2.59 (s, 1H), 2.50–2.17 (m, 7H), 2.16–1.86 (m, 5H), 1.72–1.67 (m, 2H), 1.56–1.01 (m, 27H), 0.91 (d,  $J$  = 7.0 Hz, 3H).  $^{13}\text{C}$  (125 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  174.4, 172.7, 171.5, 171.4, 170.5, 170.2, 136.7, 133.8, 133.6, 133.4, 133.1, 133.0, 132.3, 132.1, 131.8, 131.4, 131.1, 128.6, 97.1, 96.9, 77.1, 74.2, 73.7, 73.6, 73.5, 72.1, 70.0, 69.5, 69.2, 69.1, 68.8, 67.6, 67.5, 67.3, 66.1, 65.9, 65.3, 65.0, 56.9, 50.2, 49.7, 48.5, 47.5, 46.1, 45.9, 45.4, 44.7, 44.3, 42.3, 41.9, 37.1, 36.7, 35.0, 34.7, 32.2, 31.7, 29.7, 28.9, 28.5, 28.2, 26.1, 25.2, 25.1, 24.5, 24.0, 21.5, 21.1, 18.4, 18.1, 17.5, 17.0, 16.9, 12.1, 12.0, 11.6. MALDI-TOF (m/z):  $[(M + 2H)/2]^+$  calcd for  $\text{C}_{119}\text{H}_{188}\text{N}_6\text{O}_{38}$ : 1054.648; found: 1054.63.

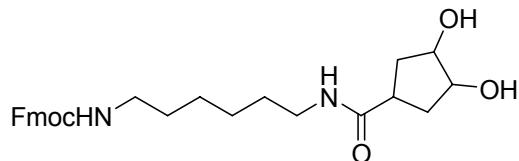
## Biotinyl aminohexanoyl amphotericin B (**10**)



To a solution of **16** (76 mg; 73 nmol) and (+)-biotine-N-succinimidylester (25 mg; 73 nmol) in DMF (2 mL) was added pyridine (100  $\mu$ L; 1.2 mmol). After 15 h the solvent was removed and the solid purified on a silica gel column (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 18.5:6:1 to CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1) to give **10** as a yellow powder (13.3 mg; 10.5 nmol; 14%).

R<sub>f</sub>: 0.37 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (DMSO, nm): 416, 391, 371. IR (KBr): 3422, 2919, 2847, 1694, 1641, 1550, 1453, 1381, 1314, 1262, 1175, 1108, 1057, 1013. <sup>1</sup>H (500 MHz, DMSO-d<sub>6</sub>):  $\delta$  7.77-7.75 (m, 1H), 7.75-7.61 (m, 1H), 6.47-6.06 (m, 14H), 5.98-5.93 (m, 1H), 5.80 (s, 1H), 5.46-5.41 (m, 1H), 5.34 (s, 1H), 5.21-5.10 (m, 1H), 4.81-4.64 (m, 6H), 4.47-4.30 (m, 3H), 4.29-4.24 (m, 1H), 4.21-4.19 (m, 1H), 4.14-4.06 (m, 1H), 4.06-3.95 (m, 1H), 3.67-3.46 (m, 3H), 3.16-3.00 (m, 8H), 2.89-2.80 (m, 1H), 2.58-2.54 (m, 2H), 2.28-2.03 (m, 8H), 1.87-1.84 (m, 2H), 1.72-1.62 (m, 1H), 1.59-1.03 (m, 36H), 0.91 (s,  $J = 7.0$  Hz, 3H). <sup>13</sup>C (125 MHz, DMSO-d<sub>6</sub>):  $\delta$  172.7, 172.5, 171.7, 170.5, 162.6, 136.8, 136.7, 133.8, 133.6, 133.4, 133.1, 132.7, 132.6, 132.3, 132.1, 131.8, 131.7, 131.1, 128.6, 97.0, 77.1, 74.9, 73.6, 73.5, 73.3, 69.9, 69.7, 69.3, 69.1, 68.9, 68.8, 67.6, 66.1, 65.9, 65.3, 60.9, 59.1, 56.2, 55.3, 54.7, 46.2, 44.6, 44.2, 42.3, 38.2, 37.2, 35.2, 35.1, 28.9, 28.2, 28.1, 27.9, 26.0, 25.2, 25.1, 25.0, 18.4, 18.0, 17.0, 16.9, 12.1, 12.0, 11.6. MALDI-TOF (m/z): [M + H]<sup>+</sup> calcd for C<sub>63</sub>H<sub>98</sub>N<sub>4</sub>O<sub>20</sub>S: 1262.6495; found: 1262.2.

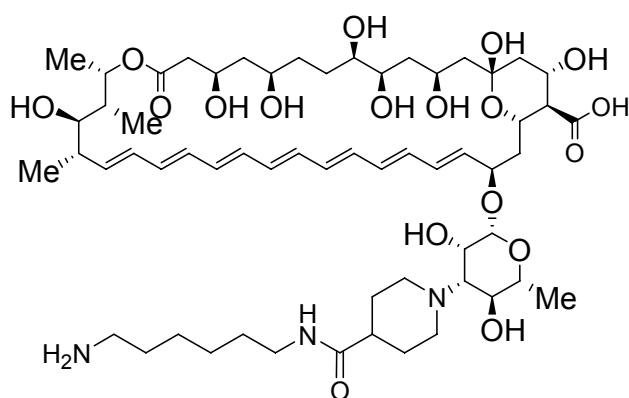
**{6-[(3,4-Dihydroxy-cyclopentanecarbonyl)-amino]-hexyl}-  
carbamic acid 9-fluoren-9-ylmethyl ester (11)**



To a solution of (**17**) (490 mg, 1.2 mmol) in acetone (15 mL), H<sub>2</sub>O (5 mL), and *tert*-BuOH (1 mL) was added K<sub>2</sub>OsO<sub>4</sub>·2 H<sub>2</sub>O (42 mg, 0.1 mmol) and 4-methylmorpholine *N*-oxide (230 mg, 1.7 mmol, Fluka, Buchs, Switzerland). After 14 h sodium hydrosulfite (2 g) and Florisil (2 g) were added and the resulting mixture was stirred for 1 h. After filtration and removal of the solvents under reduced pressure the product was purified on a silica gel column (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1). The product was obtained as white powder (140 mg, 0.31 mmol, 27 %).

R<sub>f</sub> = 0.21 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1; KMnO<sub>4</sub>). IR (KBr): 3468, 3325, 2939, 2862, 1687, 1613, 1540, 1475, 1450, 1258, 1223, 1144, 1114, 1086, 1044. <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): δ 7.88 (d, 2H, *J* = 7.5 Hz), 7.68 (d, 2H, *J* = 7.4 Hz), 7.43–7.38 (m, 2H), 7.34–7.25 (m, 2H), 4.36 (d, 2H, *J* = 3.7 Hz), 4.30–4.18 (m, 1 H), 3.89–3.87 (m, 2H), 3.02–2.88 (m, 4H), 2.85–2.77 (m, 1H), 1.78–1.61 (m, 4H), 1.37–1.22 (m, 8H). <sup>13</sup>C (300 MHz, DMSO-*d*<sub>6</sub>): δ 175.1, 155.8, 143.7, 140.5, 127.4, 126.8, 124.9, 119.9, 73.0, 65.0, 46.7, 34.9, 29.3, 29.1, 26.1, 25.9. HRMS-MALDI (*m/z*): [M + Na]<sup>+</sup> calcd for C<sub>27</sub>H<sub>34</sub>NaN<sub>2</sub>O<sub>5</sub>: 489.2360; found: 489.2365.

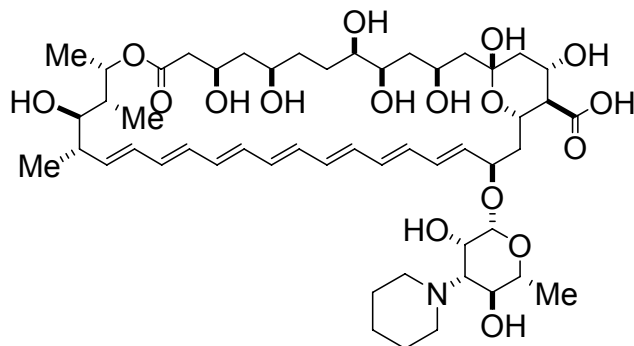
**Diaminohexanoyl pyridyl amphotericin B (12)**



To a solution of (**18**) (22.7 mg, 16.7 μmol) in DMSO (2 mL) was added piperidine (100 μL, 1 mmol). After 1 h the solution was slowly added to Et<sub>2</sub>O (250 mL). The filtration yielded the unpurified product as a yellow powder (17.2 mg, 15 μmol, 91 %).

R<sub>f</sub>: 0.00 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (DMSO, nm): 416, 392, 372, 354. IR (KBr): 3473, 3415, 1637, 1404, 1013. <sup>1</sup>H (600 MHz, DMSO-d<sub>6</sub>/D<sub>2</sub>O 10 %): δ 6.44–5.97 (m, 13H), 5.50–5.43 (m, 1H), 5.21 (br s, 1H), 4.52 (s, 1H), 4.44 (s, 1H), 4.31 (br s, 1H), 4.23–4.06 (m, 2H), 4.06–3.90 (m, 2H), 3.51–3.42 (m, 3H), 3.42–2.93 (m, 9H), 2.74–2.60 (m, 2H), 2.54–2.53 (m, 4H), 2.45–2.07 (m, 8H), 1.85–1.72 (m, 5H), 1.61–0.82 (m, 41H). <sup>13</sup>C (150 MHz, DMSO-d<sub>6</sub>/D<sub>2</sub>O 10 %): δ 175.8, 175.0, 173.7, 170.7, 136.7, 133.9, 133.7, 133.5, 133.3, 133.2, 132.4, 132.1, 131.9, 131.6, 131.4, 131.1, 97.8, 96.8, 77.0, 75.5, 73.6, 73.3, 73.0, 69.1, 69.0, 68.8, 68.6, 67.6, 67.4, 67.2, 66.3, 66.1, 65.8, 65.4, 49.7, 49.1, 46.4, 44.3, 44.1, 41.8, 38.0, 37.9, 34.8, 29.4, 29.1, 28.8, 28.6, 28.5, 27.2, 25.5, 25.3, 22.8, 18.4, 18.1, 17.2, 17.0, 16.9, 12.1, 11.9. MALDI-TOF (m/z): [M + Na]<sup>+</sup> calcd for C<sub>59</sub>H<sub>95</sub>N<sub>3</sub>NaO<sub>18</sub>: 1156.6508; found: 1156.0.

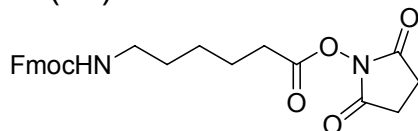
### Piperidyl amphotericin B (13)



Amphotericin B (200 mg, 0.2 mmol) and glutaric dialdehyde (25% in H<sub>2</sub>O, 620 μL, 1.7 mmol) were dissolved in DMF (5 mL) and MeOH (2 mL). To the solution was added (polystyrylmethyl)trimethylammonium cyanoborohydride (200 mg, 0.8 mmol) and a drop of 36% HCl. After 21 h the resin was filtered off, the solvents removed under reduced pressure, and the product was purified by silica gel column chromatography (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1). The product was obtained as a yellow powder (50 mg; 50 nmol; 25%).

R<sub>f</sub>: 0.37 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (dmsO, nm): 416, 392, 372, 353. IR (KBr): 3430, 2935, 1719, 1654, 1458, 1374, 1322, 1265, 1197, 1161, 1109, 1068, 1010. <sup>1</sup>H (500 MHz, DMSO-d<sub>6</sub>): δ 6.47–6.07 (m, 10 H), 5.97–5.93 (m, 1 H), 5.85 (s, 1 H), 5.46–5.38 (m, 2 H), 5.22–5.21 (m, 1 H), 4.79–4.65 (m, 4 H), 4.45 (s, 1 H), 4.37 (s, 1 H), 4.30 (s, 1 H), 4.24–4.17 (m, 1 H), 4.06–3.97 (m, 2 H), 3.86 (s, 1 H), 3.52–3.42 (m, 4 H), 3.17–3.09 (m, 4 H), 2.85–2.78 (m, 4 H), 2.50–2.49 (m, 2 H), 2.48–2.45 (m, 1 H), 2.38–2.36 (m, 1 H), 2.29–2.16 (m, 4 H), 2.04–2.02 (m, 1 H), 1.96–1.86 (m, 3 H), 1.74–1.71 (m, 1 H), 1.53–1.03 (m, 32 H), 0.92–0.91 (m, 3 H). <sup>13</sup>C (125 MHz, DMSO-d<sub>6</sub>): δ 174.7, 136.7, 136.6, 133.8, 133.6, 133.3, 133.1, 132.4, 132.3, 132.1, 131.8, 131.1, 128.6, 97.1, 96.9, 79.1, 77.1, 74.1, 73.7, 73.6, 74.5, 68.1, 68.8, 68.3, 68.2, 67.6, 66.8, 66.1, 65.3, 65.0, 57.9, 57.1, 55.9, 60.6, 46.0, 44.7, 44.3, 42.3, 42.2, 41.9, 40.3, 36.6, 35.0, 32.8, 28.9, 26.0, 24.3, 18.5, 18.4, 18.1, 17.1, 16.9, 12.0. ESI (m/z): [M + H]<sup>+</sup> calcd for C<sub>52</sub>H<sub>82</sub>NO<sub>17</sub>: 992.557; found: 992.27.

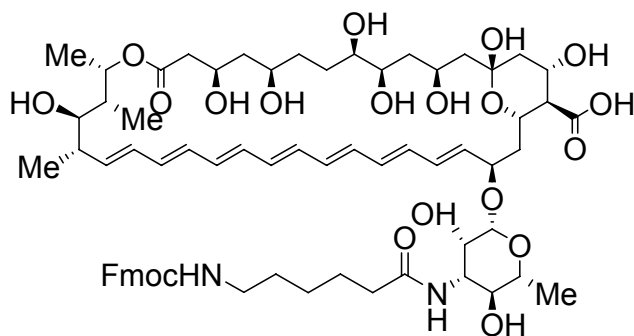
## 6-(9-*H*-Fluoren-9-ylmethoxycarbonylamino)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (**14**)



Fmoc-amino hexanoic acid (4.14 g; 11.7 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (80 mL). Then 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.36 g; 17.5 mmol), *N*-hydroxy succinimide (2.02 g; 17.5 mmol) and Hünig's base (4.10 mL; 23.4 mmol) were added. After 18 h the solution was washed thrice with H<sub>2</sub>O then with sat. NH<sub>4</sub>Cl and brine. After drying over Na<sub>2</sub>SO<sub>4</sub> the solvent was removed to give the product as a white powder (2.71 g; 6.01 mmol; 51%).

R<sub>f</sub>: 0.60 (in CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1; CAM). IR (KBr): 3363, 3018, 2938, 2863, 1820, 1787, 1742, 1728, 1695, 1654, 1532, 1451, 1369, 1259, 1210, 1145, 1064, 1006. <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>): δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.42-7.26 (m, 4H), 5.01-5.00 (m, 1H), 4.38 (d, *J* = 6.8 Hz, 2H), 4.21 (t, *J* = 6.8 Hz, 1H), 3.20 (q, *J* = 6.2 Hz, 2H), 2.78 (s, 4H), 2.63-2.58 (m, 2H), 1.81-1.72 (m, 2H), 1.59-1.27 (m, 4H). <sup>13</sup>C (300 MHz, CDCl<sub>3</sub>): δ 169.0, 168.3, 156.3, 143.9, 141.1, 128.6, 127.5, 126.9, 125.0, 120.9, 119.8, 107.8, 66.5, 47.3, 40.6, 30.9, 29.4, 25.7, 25.6, 24.3. HRMS-MALDI (*m/z*): [M + Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>26</sub>N<sub>2</sub>NaO<sub>6</sub>: 473.1689; found: 473.1687.

## Fmoc aminohexanoyl amphotericin B (**15**)



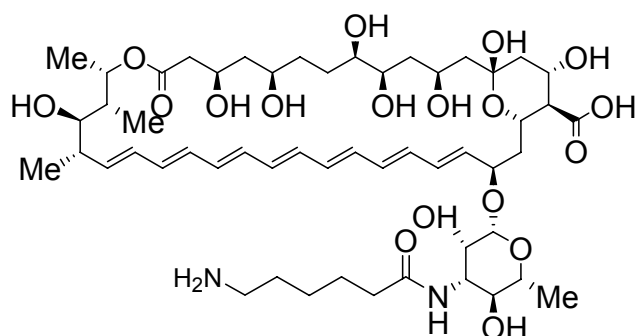
To a solution of amphotericin B (1.0 g; ~0.97 mmol) and **14** (500 mg; 1.1 mmol) in DMF (10 mL) was added pyridine (200 μL; 2.46 mmol). After 20 h the solvent was removed under reduced pressure. The resulting slurry was purified on a silica gel column (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 18.5:6:1 to CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1). **15** was isolated as a yellow powder (1.04 g; 0.82 mmol; 85%).

R<sub>f</sub>: 0.68 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (DMSO, nm): 416, 392, 372. IR (KBr): 3420, 2932, 1702, 1654, 1542, 1450, 1387, 1259, 1209, 1069, 1013. <sup>1</sup>H (500



MHz, DMSO- $d_6$ ):  $\delta$  7.88 (d,  $J = 7.5$  Hz, 4H), 7.69 (d,  $J = 7.4$  Hz, 3H), 7.43-7.40 (m, 3H), 7.34-7.31 (m, 4H), 7.25-7.22 (m, 1H), 6.47-6.23 (m, 10H), 6.18-6.16 (m, 2H), 6.12-6.07 (m, 2H), 5.98-5.93 (m, 1H), 5.82 (s, 1H), 5.46-5.42 (m, 1H), 5.33 (s, 1H), 5.21-5.20 (m, 1H), 4.80-4.62 (m, 6H), 4.44-4.31 (m, 3H), 4.30-4.19 (m, 3H), 4.07-3.98 (m, 2H), 3.69-3.60 (m, 1H), 3.58-3.32 (m, 8H), 3.18-3.03 (m, 5H), 2.97-2.93 (m, 3H), 2.81 (s, 2H), 2.66-2.63 (m, 1H), 2.51 (s, 1H), 2.37-2.30 (m, 1H), 2.30-2.11 (m, 5H), 2.04-1.96 (m, 1H), 1.94-1.87 (m, 1H), 1.74-1.72 (m, 1H), 1.64-1.49 (m, 9H), 1.41-1.24 (m, 15H), 1.16 (d,  $J = 5.5$  Hz, 3H), 1.12 (d,  $J = 6.4$  Hz, 3H), 1.04 (d,  $J = 6.3$  Hz, 3H), 0.92 (d,  $J = 7.1$  Hz, 3H).  $^{13}\text{C}$  (125 MHz, DMSO- $d_6$ ):  $\delta$  172.7, 172.5, 171.7, 170.5, 156.0, 143.9, 142.5, 140.6, 139.3, 137.3, 136.7, 133.8, 133.6, 133.4, 133.1, 132.6, 132.3, 132.1, 131.8, 131.7, 131.1, 128.8, 128.6, 127.5, 127.2, 126.9, 125.0, 124.1, 121.3, 120.0, 119.9, 109.6, 97.1, 96.9, 77.1, 73.7, 73.5, 73.3, 70.0, 69.2, 69.0, 68.8, 67.6, 66.2, 65.3, 65.1, 54.7, 46.7, 44.6, 44.2, 42.3, 42.0, 38.3, 35.3, 35.2, 35.0, 29.1, 29.1, 28.9, 26.0, 25.9, 25.3, 25.1, 25.0, 18.4, 18.0, 16.9, 12.0. MALDI-TOF ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{68}\text{H}_{94}\text{N}_2\text{NaO}_{20}$ : 1281.6298; found: 1282.0.

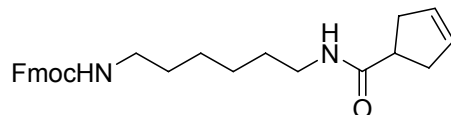
### Aminohexanoyl amphotericin B (**16**)



To a solution of **15** (390 mg; 0.31 mmol) in DMSO (3 mL) was added piperidine (1 mL; 10 mmol). After 1 h the solution was slowly added to  $\text{Et}_2\text{O}$  (250 mL). The filtration yielded the crude product as a yellow powder (320 mg; 310 nmol; 99 %).

$R_f$ : 0.10 (in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  10:6:1; CAM). UV (DMSO, nm): 416, 392, 372. IR (KBr): 3392, 2933, 2860, 1717, 1670, 1646, 1565, 1445, 1398, 1314, 1241, 1183, 1063, 1014.  $^1\text{H}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  6.44-6.15 (m, 7H), 6.15-6.11 (m, 2H), 6.11-6.05 (m, 2H), 5.45-5.40 (m, 2H), 5.40-5.21 (m, 2H), 4.54 (br s, 1H), 4.40-4.24 (m, 3H), 4.07-3.91 (m, 2H), 3.69-3.48 (m, 3H), 3.18-3.00 (m, 3H), 2.74-2.71 (m, 4H), 2.37-2.11 (m, 6H), 1.82-1.71 (m, 3H), 1.55-1.25 (m, 23H), 1.15-1.11 (m, 3H), 0.91 (d,  $J = 7.0$  Hz, 10H).  $^{13}\text{C}$  (100 MHz, DMSO- $d_6$ ):  $\delta$  172.8, 170.5, 136.2, 133.9, 133.7, 133.5, 133.2, 132.3, 132.0, 131.8, 131.6, 131.2, 96.7, 77.1, 73.9, 73.5, 73.3, 73.2, 69.5, 69.2, 68.8, 67.8, 66.2, 66.0, 65.5, 54.7, 45.6, 45.4, 44.6, 44.2, 42.4, 35.1, 34.9, 29.1, 27.6, 27.6, 26.1, 25.9, 25.2, 25.2, 24.6, 24.0, 24.0, 23.7, 18.4, 18.1, 17.0, 16.9, 12.1, 12.0. MALDI-TOF ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{53}\text{H}_{85}\text{N}_2\text{O}_{18}$ : 1037.5797; found: 1037.4.

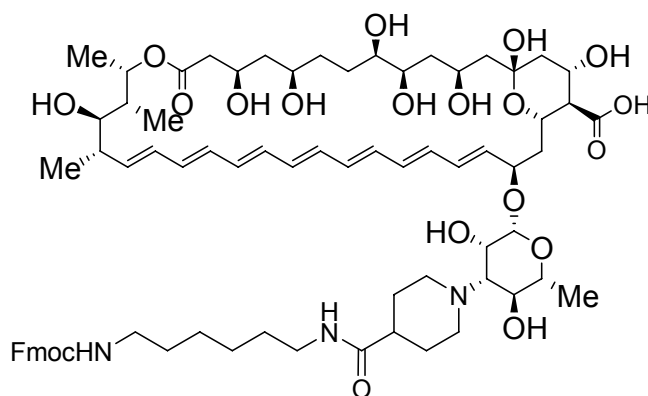
## 9H-fluoren-9-ylmethyl{6-[(3-cyclopenten-1-ylcarbonyl)-amino]hexyl}carbamate (**17**)



To a solution of 3-cyclopentene-1-carboxylic acid (0.50 g, 4.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) and DMF (7 drops) at 0 °C was slowly added oxalylchloride (1.90 mL, 22.3 mmol). After 13 h the solvent was removed and the resulting solid taken up in  $\text{CH}_2\text{Cl}_2$  (50 mL). At 0 °C pyridine (800  $\mu\text{L}$ , 9.9 mmol) and 1-Fmoc-1,6-diaminohexane·HCl (1.00 g; 2.66 mmol) were added. After 5 h sat.  $\text{NaHCO}_3$  was added, extracted twice with  $\text{CH}_2\text{Cl}_2$ , and dried over  $\text{Na}_2\text{SO}_4$ . After solvent removal the product was purified on a silica gel column (5% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give (**17**) as a white powder (550 mg, 1.3 mmol, 48 %).

$R_f$  = 0.40 (in  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1; CAM). IR (KBr): 3468, 3418, 3324, 3298, 3065, 2931, 2860, 1687, 1638, 1620, 1534, 1476, 1450, 1285, 1256, 1139, 1040.  $^1\text{H}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.77–7.75 (m, 2H), 7.61–7.58 (m, 2H), 7.42–7.26 (m, 4H), 5.76–5.59 (m, 2H), 5.02–4.89 (m, 1H), 4.49–4.32 (m, 2H), 4.27–4.13 (m, 1H), 3.30–3.02 (m, 4H), 2.98–2.85 (m, 1H), 2.68–2.50 (m, 4H), 1.59–1.39 (m, 4H), 1.39–1.06 (m, 4H).  $^{13}\text{C}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  176.2, 156.7, 144.2, 141.5, 129.4, 127.8, 127.2, 125.2, 120.1, 66.7, 47.5, 43.8, 40.9, 39.4, 37.2, 30.0, 29.7, 26.4, 26.2. HRMS-MALDI (m/z):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{27}\text{H}_{32}\text{NaN}_2\text{O}_3$ : 455.2305; found: 455.2309.

## Fmoc diaminohexanoyl pyridyl amphotericin B (**18**)



Silica gel (10 g) was suspended in  $\text{CHCl}_3$  (80 mL). Slowly  $\text{NaIO}_4$  (1.4 g, 6.5 mmol) in  $\text{H}_2\text{O}$  (10 mL) was added to give a floccy suspension. To this a suspension of (**11**) (100.0 mg, 214.5  $\mu\text{mol}$ ) was added. After 50 min the solvent was filtered and the filtercake washed with  $\text{CH}_2\text{Cl}_2$  and EtOAc. The solvents were removed under reduced pressure. To the resulting solid Amphotericin B (0.20 g, ca. 0.20 mmol) was added followed by DMF (10 mL). HCl (1 drop) and  $\text{NaBH}_3\text{CN}$  (200 mg, 0.3 mmol) were added and the

suspension stirred overnight. Amberlite IRA-743 (2 g) was added and the suspension shaken for 1 h. After filtration the solvent was removed and the solid purified on a silica gel column (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10/6/1). The yellow product was washed thoroughly with water and lyophilized to give (**18**) as a yellow powder (56 mg, 41 μmol, 24 %).

R<sub>f</sub> : 0.73 (in CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O 10:6:1; CAM). UV (DMSO, nm): 417, 392, 372, 354. IR (KBr): 3400, 3414, 2930, 1702, 1638, 1560, 1450, 1385, 1259, 1182, 1104, 1068, 1012. <sup>1</sup>H (600 MHz, DMSO-d<sub>6</sub>/D<sub>2</sub>O 10%): δ 7.94–7.83 (m, 4H), 7.67–7.63 (m, 2H), 7.49–7.27 (m, 6H), 6.45–6.08 (m, 10H), 5.96–5.88 (m, 2H), 5.44–5.22 (m, 1H), 4.88 (br s, 1H), 4.35–4.20 (m, 6H), 4.06–3.94 (m, 3H), 3.58–3.49 (m, 3H), 3.29–2.91 (m, 10H), 2.75 (s, 2H), 2.75 (s, 2H), 2.75–2.63 (m, 1H), 2.38 (s, 1H), 2.28–2.10 (m, 11H), 1.94–1.91 (m, 6H), 1.71–1.54 (m, 12H), 1.37–0.89 (m, 27H). <sup>13</sup>C (125 MHz, DMSO-d<sub>6</sub>/D<sub>2</sub>O 10%): δ 174.9, 171.3, 171.1, 163.1, 156.6, 144.2, 142.9, 141.1, 139.8, 137.8, 137.2, 134.2, 134.1, 133.7, 133.6, 133.0, 132.8, 132.7, 132.4, 132.3, 131.6, 129.5, 129.4, 129.1, 128.8, 128.7, 128.3, 128.1, 127.8, 127.7, 127.5, 127.2, 126.2, 126.1, 125.8, 125.5, 121.7, 120.5, 120.4, 110.1, 97.5, 97.4, 74.1, 73.8, 69.6, 69.5, 68.6, 68.1, 67.3, 66.6, 65.8, 65.6, 53.1, 52.9, 52.3, 50.0, 49.9, 47.1, 44.8, 44.4, 42.8, 42.4, 42.3, 42.1, 40.4, 40.0, 38.6, 36.3, 35.3, 33.5, 31.3, 29.6, 29.3, 26.3, 26.2, 24.2, 21.7, 18.9, 18.4, 17.3, 16.3, 14.4, 14.1, 12.4. MALDI-TOF (m/z): [M + H]<sup>+</sup> calcd for C<sub>74</sub>H<sub>106</sub>N<sub>3</sub>O<sub>20</sub>: 1357.6415; found: 1357.7.

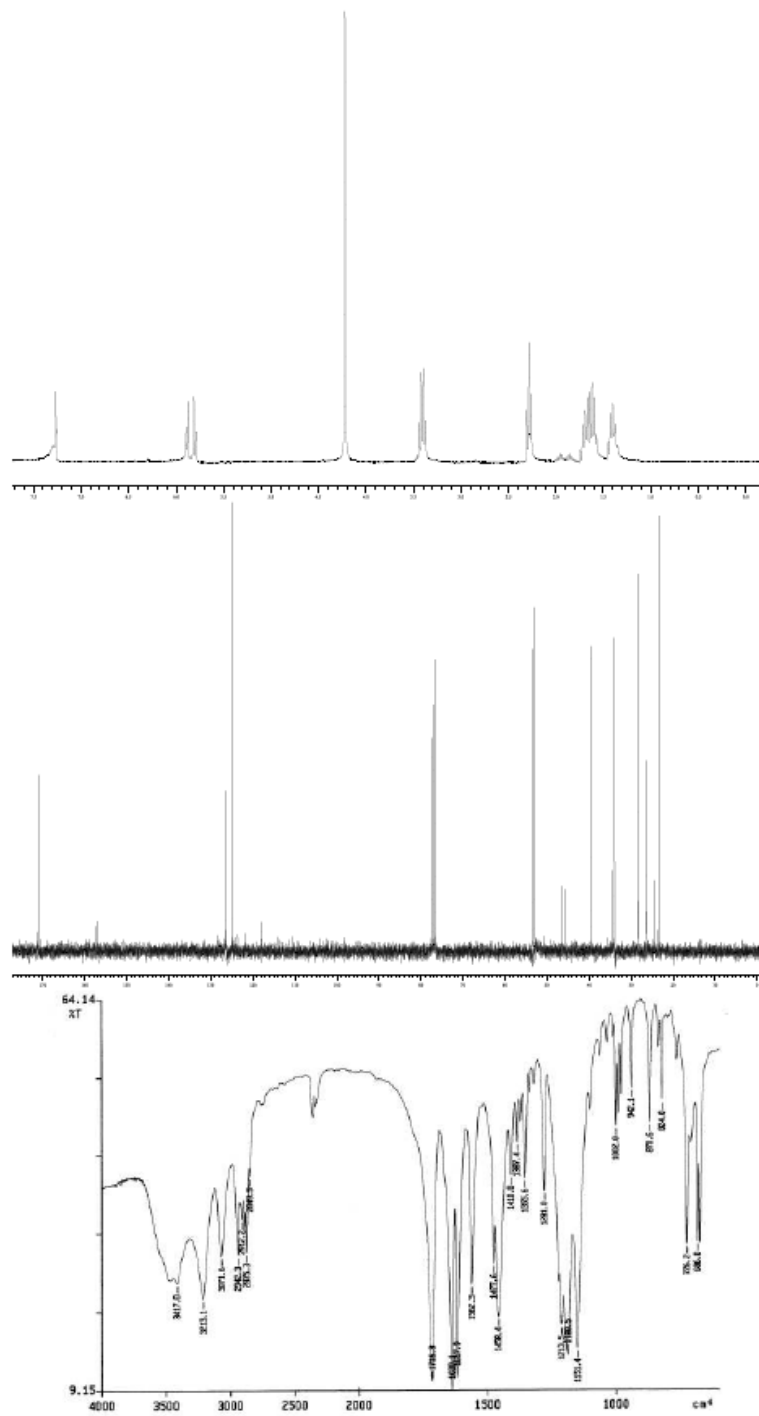


Figure 1. Spectra of *N*-[6-(2,5-Dihydro-pyrrol-1-yl)-6-oxo-hexyl]-2,2,2-trifluoro-acetamide (19).

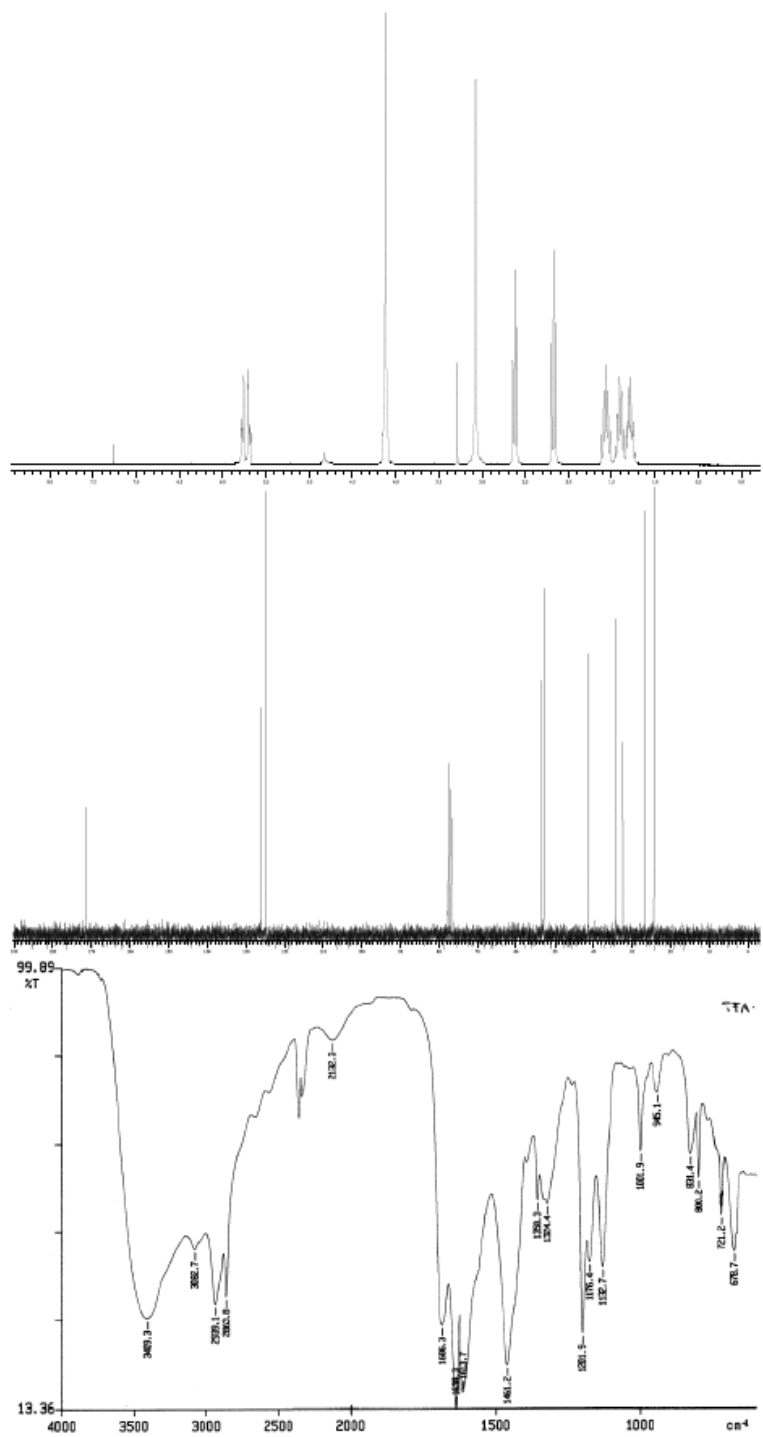
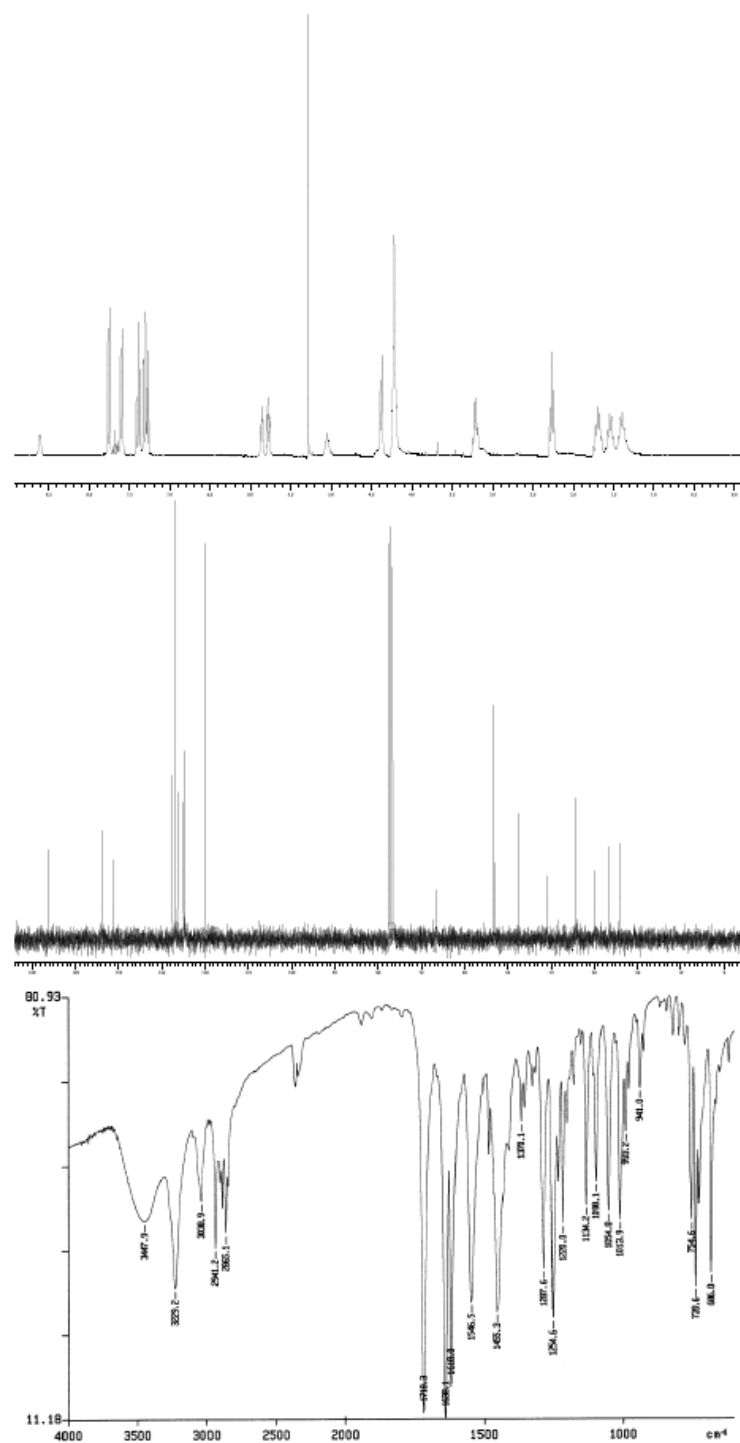
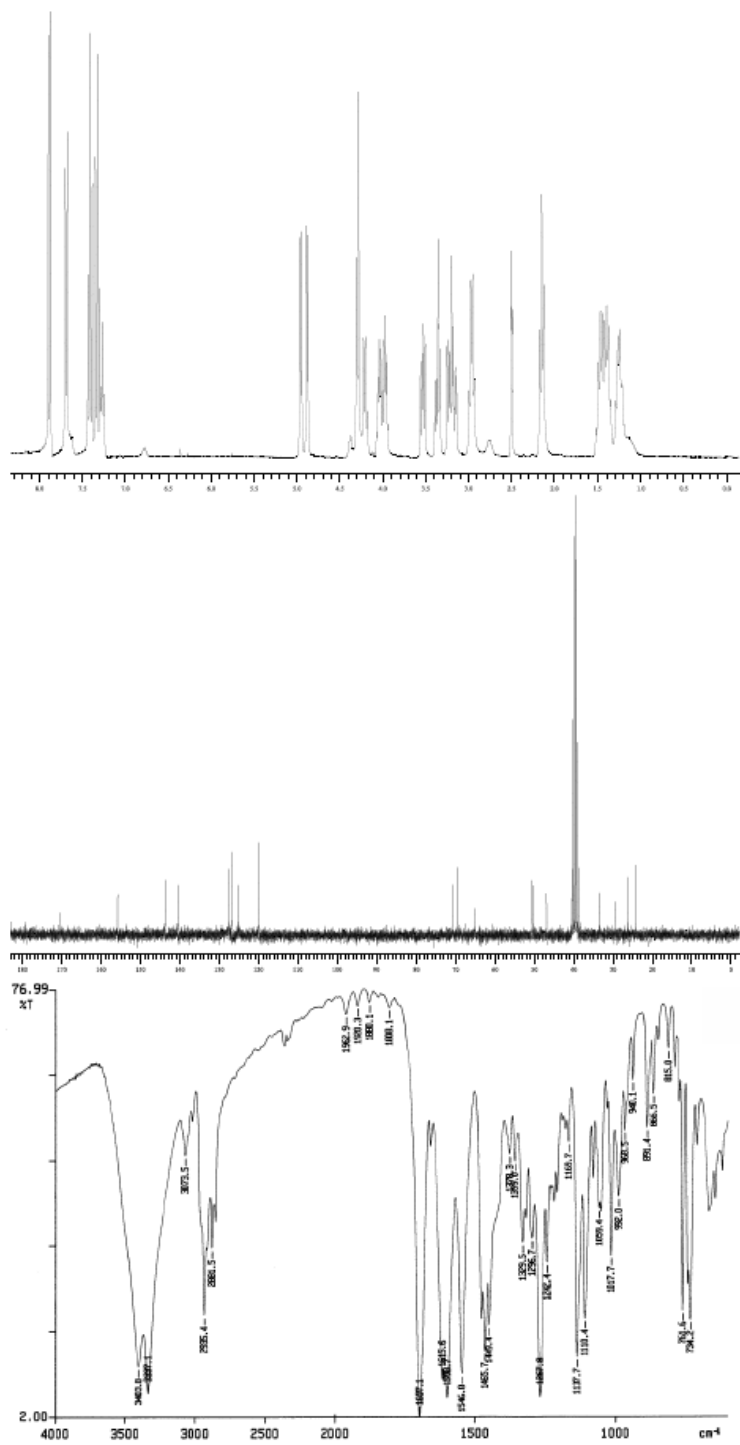


Figure 2. Spectra of 6-Amino-1-(2,5-dihydro-pyrrol-1-yl)-hexan-1-one (20)



**Figure 3.** Spectra of [6-(2,5-Dihydro-pyrrol-1-yl)-6-oxo-hexyl]-carbamic acid 9-fluorenylmethyl ester (2)



**Figure 4.** Spectra of [6-(3,4-Dihydroxy-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 9fluorenylmethyl ester (**21**)

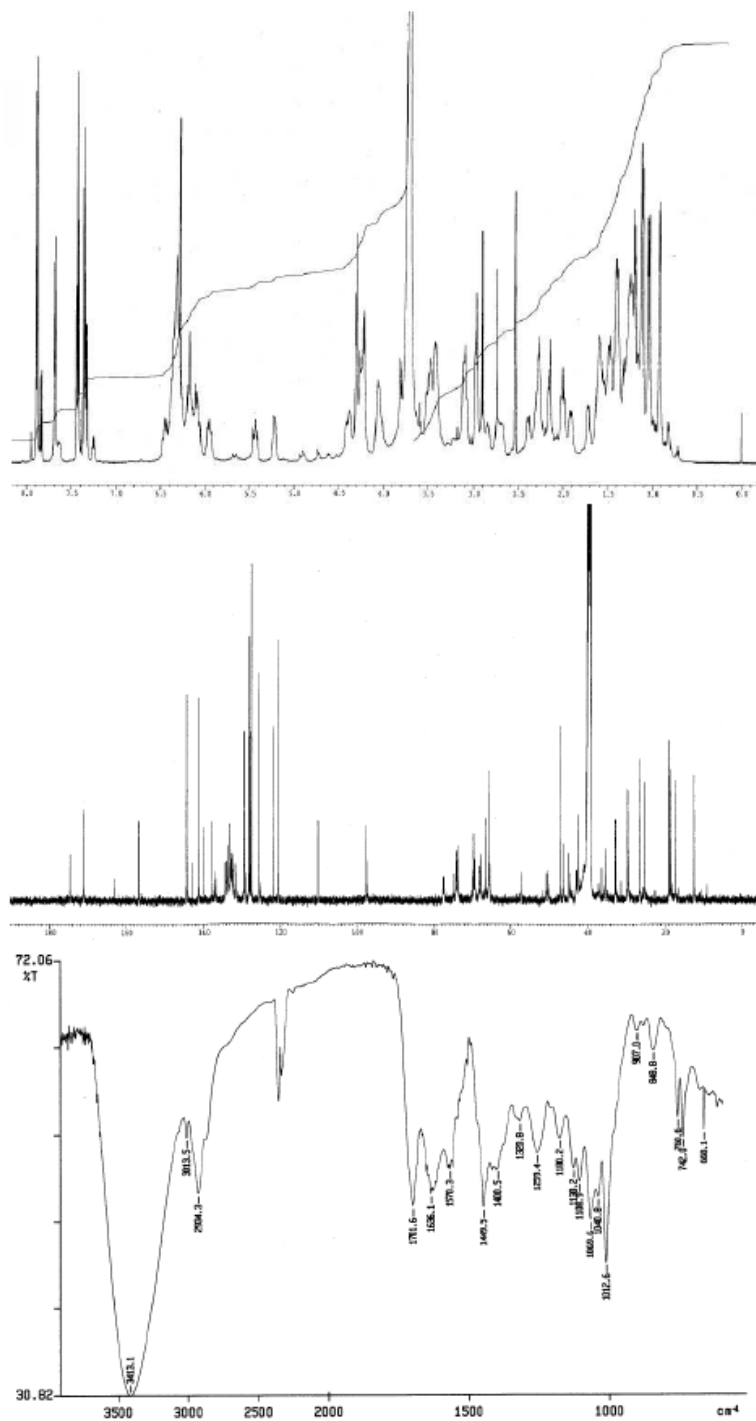


Figure 5. Spectra of Fmoc protected aminohexanoyl piperazinyl amphotericin B (22)



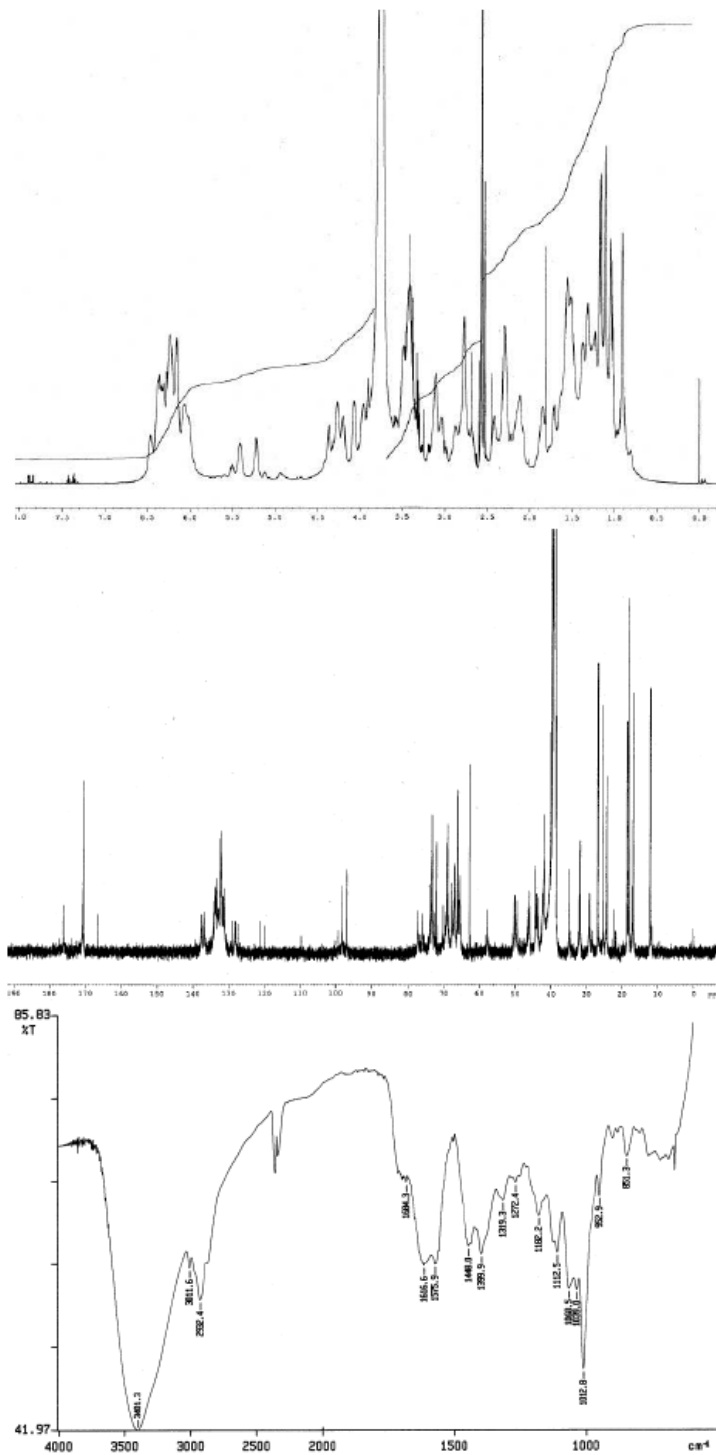


Figure 6. Spectra of Aminohexanoyl piperazinyl amphotericin B (4)

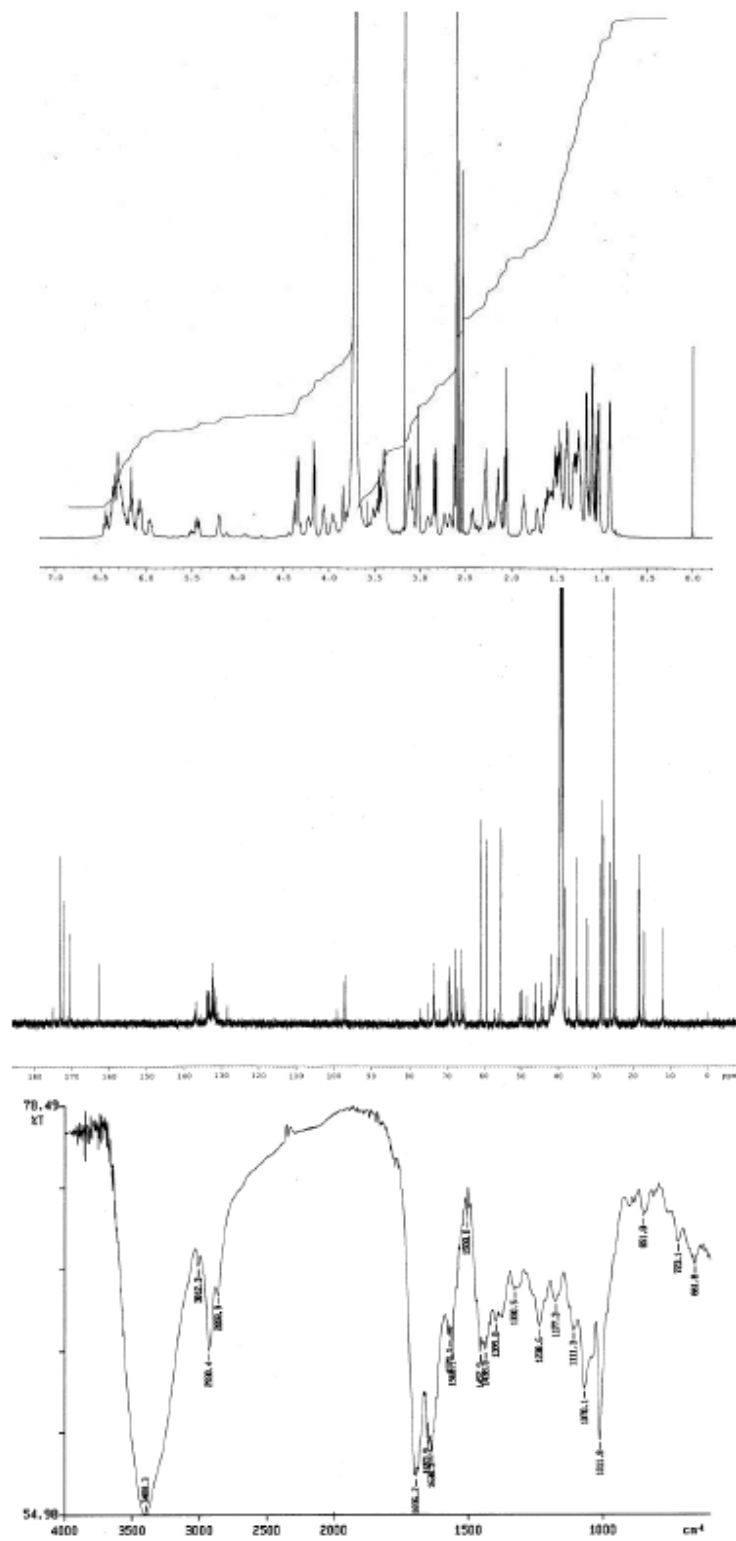


Figure 1. Spectra of biotinyl aminohexanoyl piperazinyl amphotericin B (5)

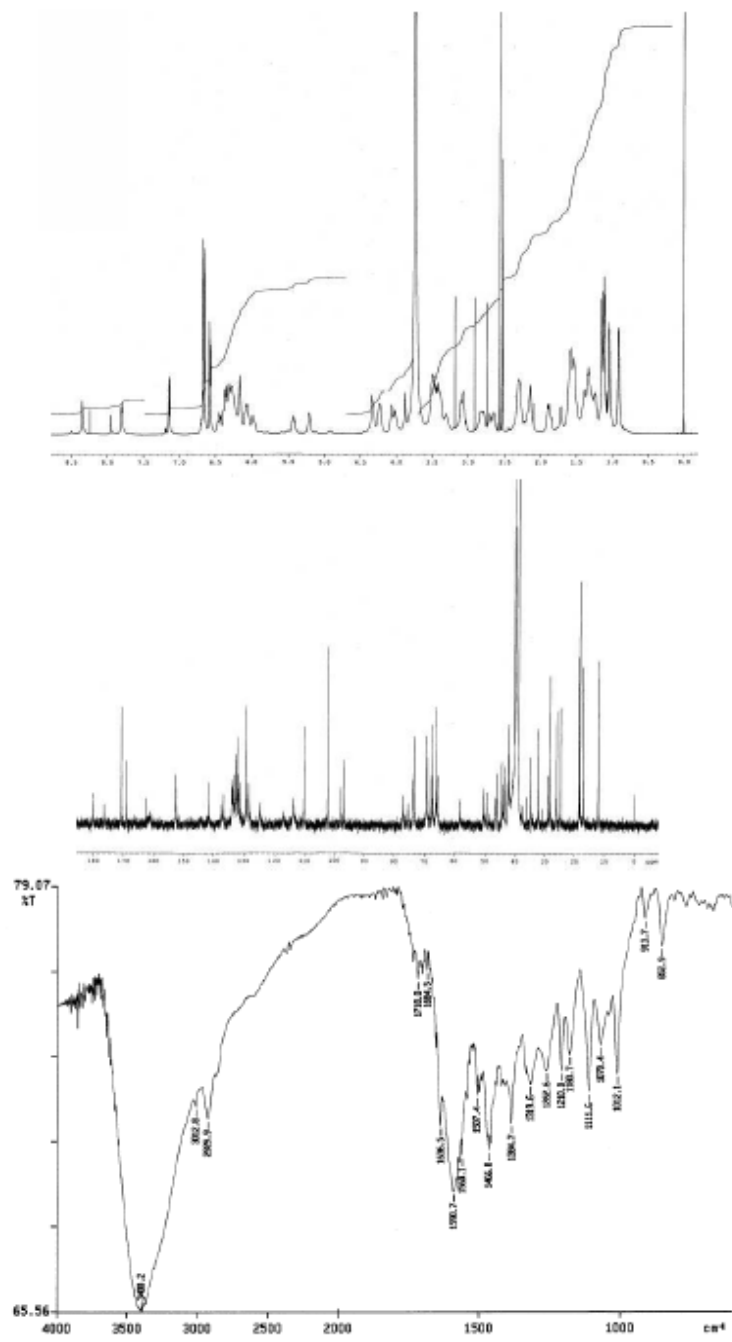


Figure 2. Spectra of fluorescein piperazinyl amphotericin B (6)

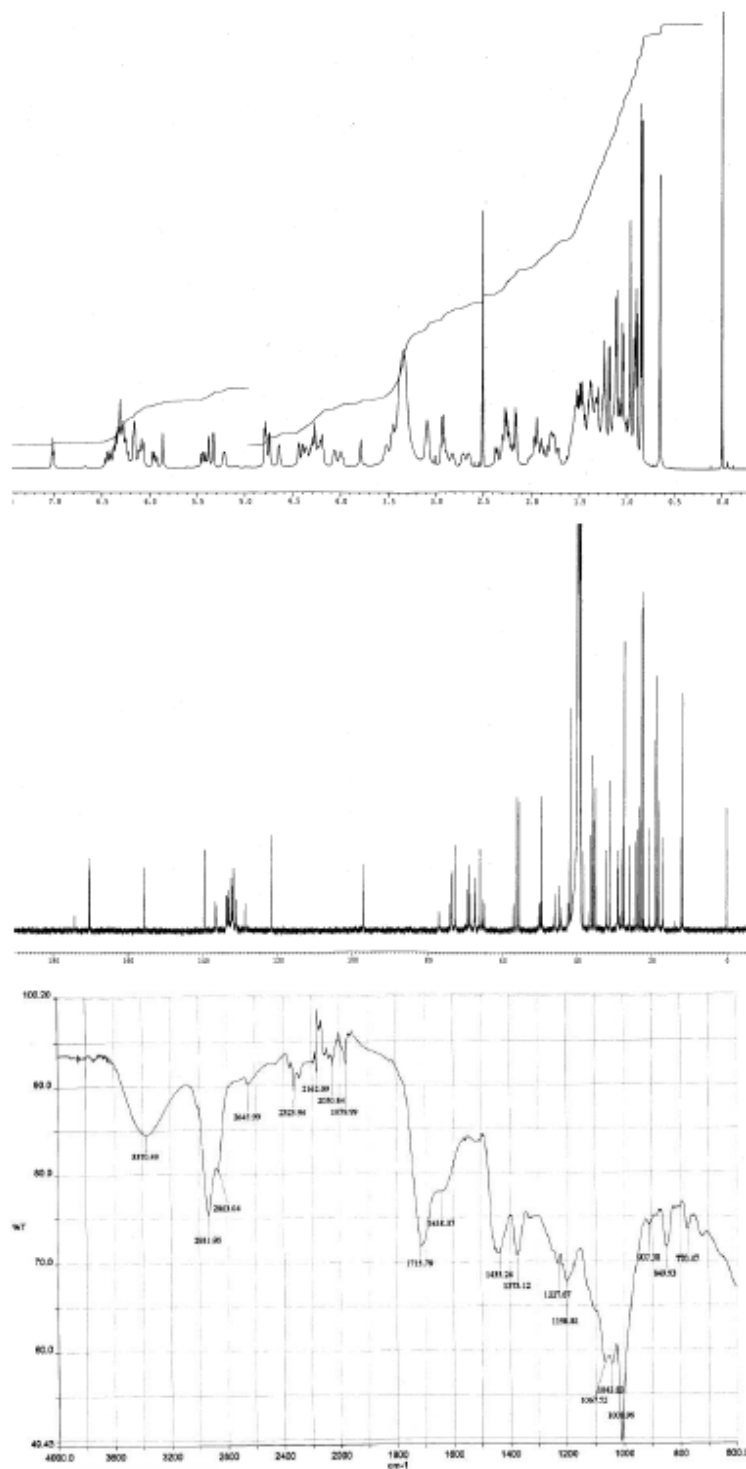
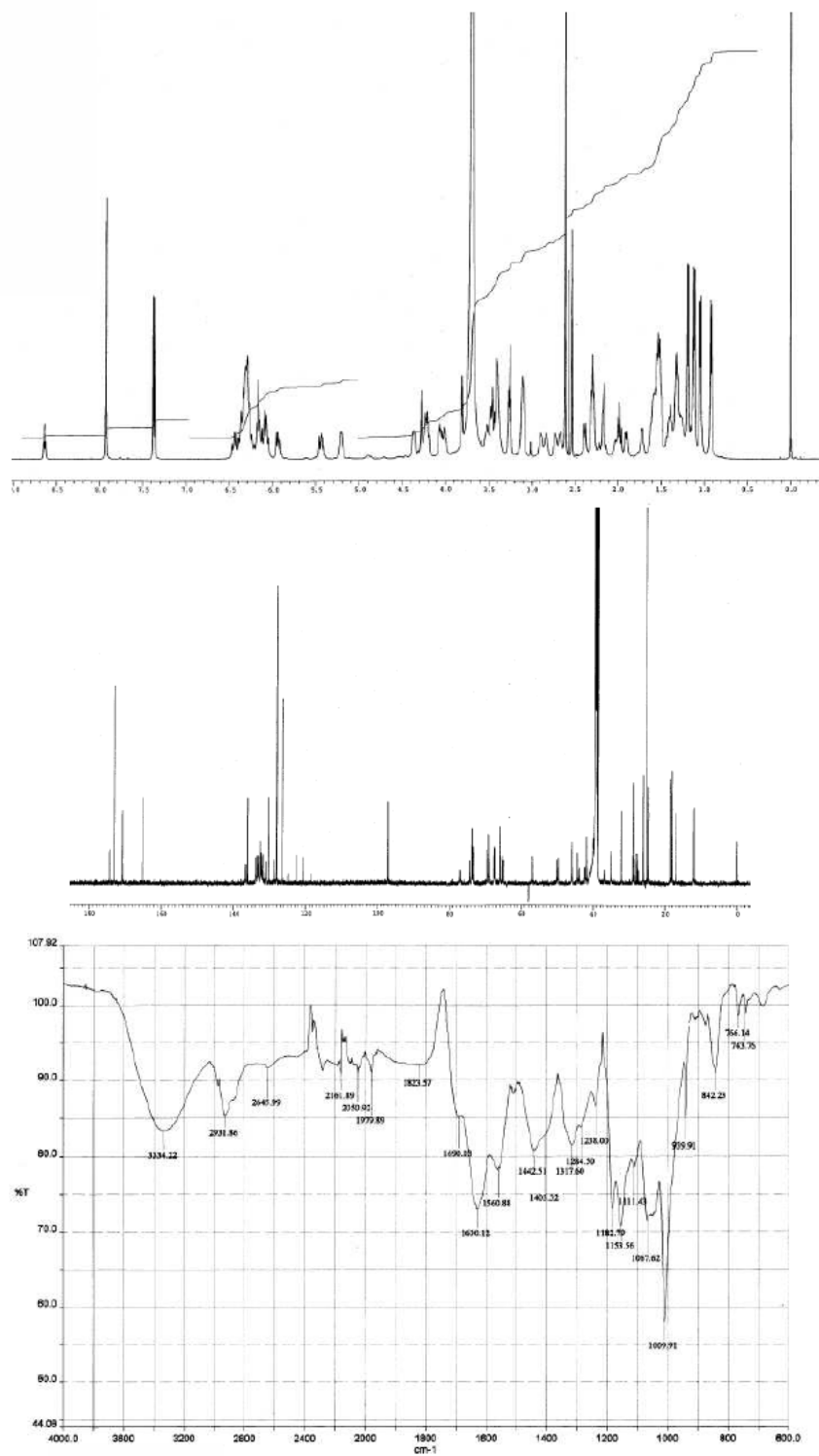
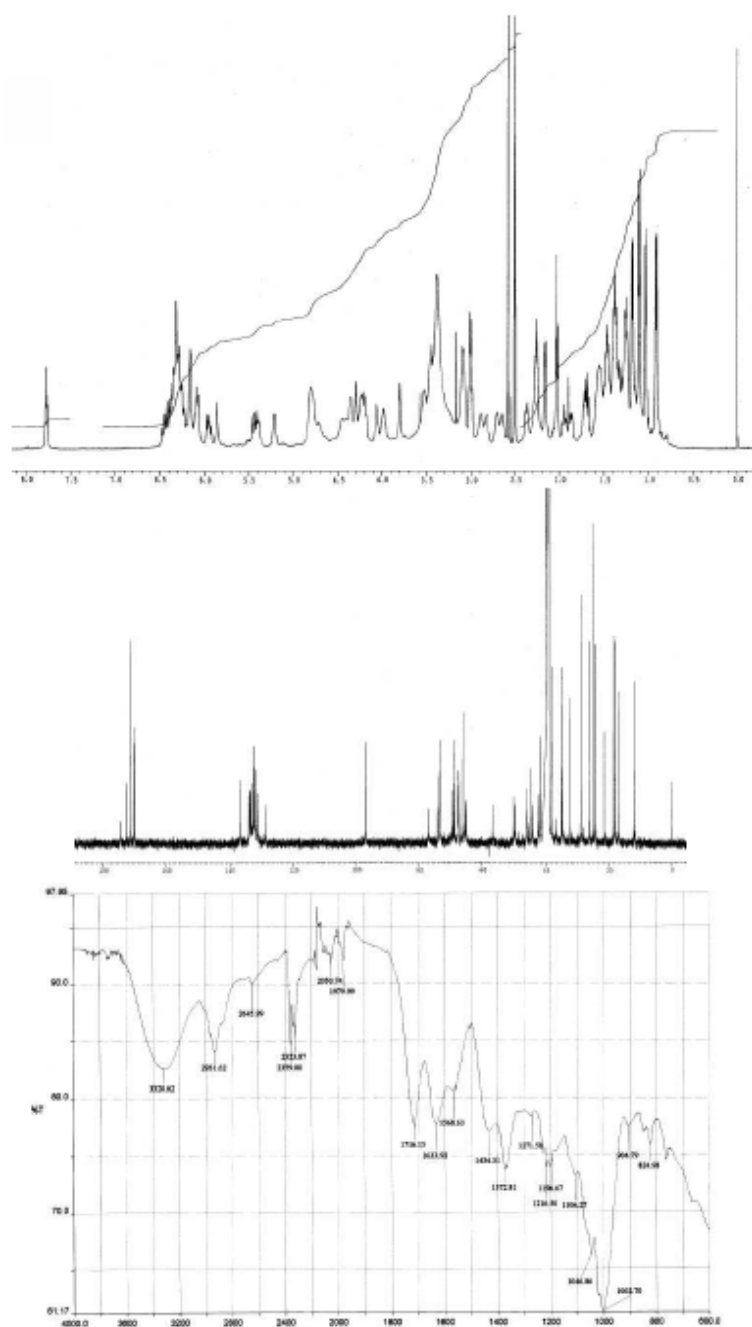


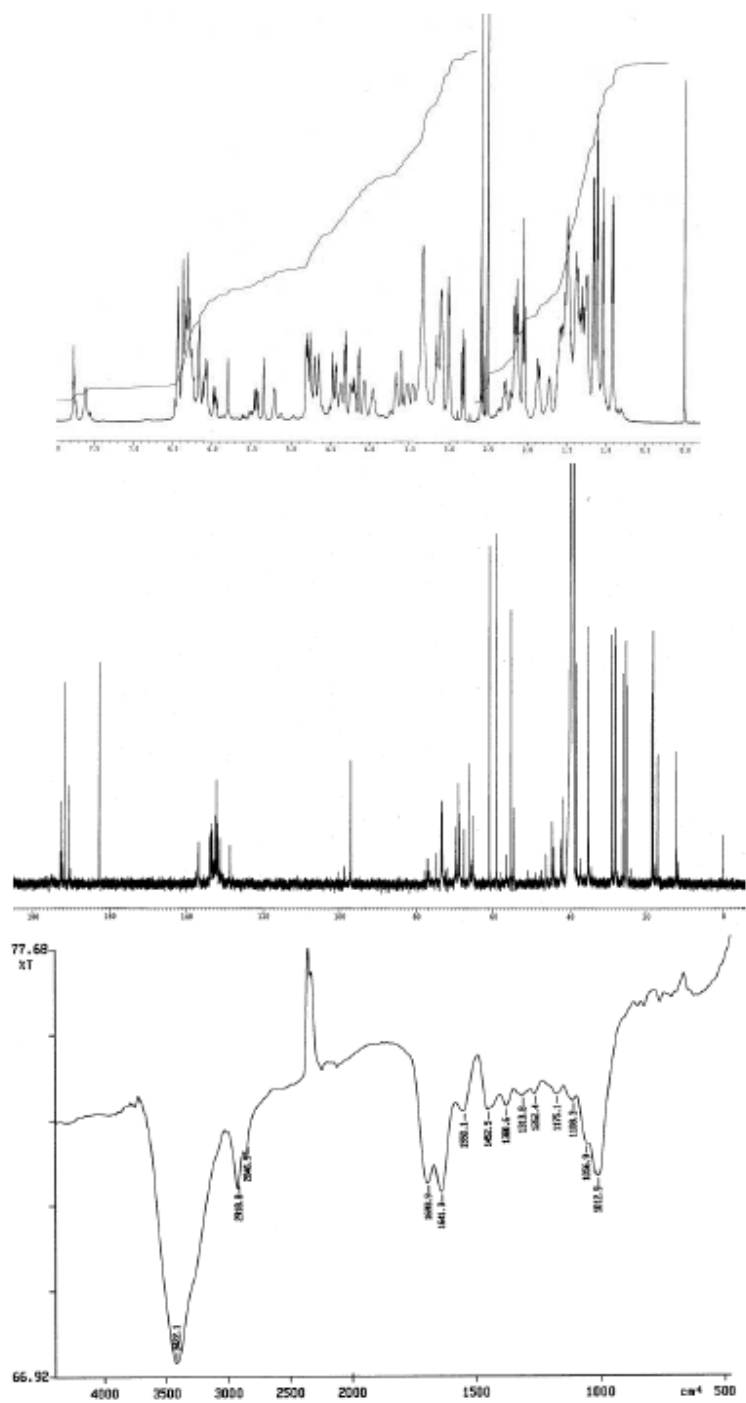
Figure 3. Spectra of cholesteryl aminohexanoyl piperidinyl amphotericin B (7)



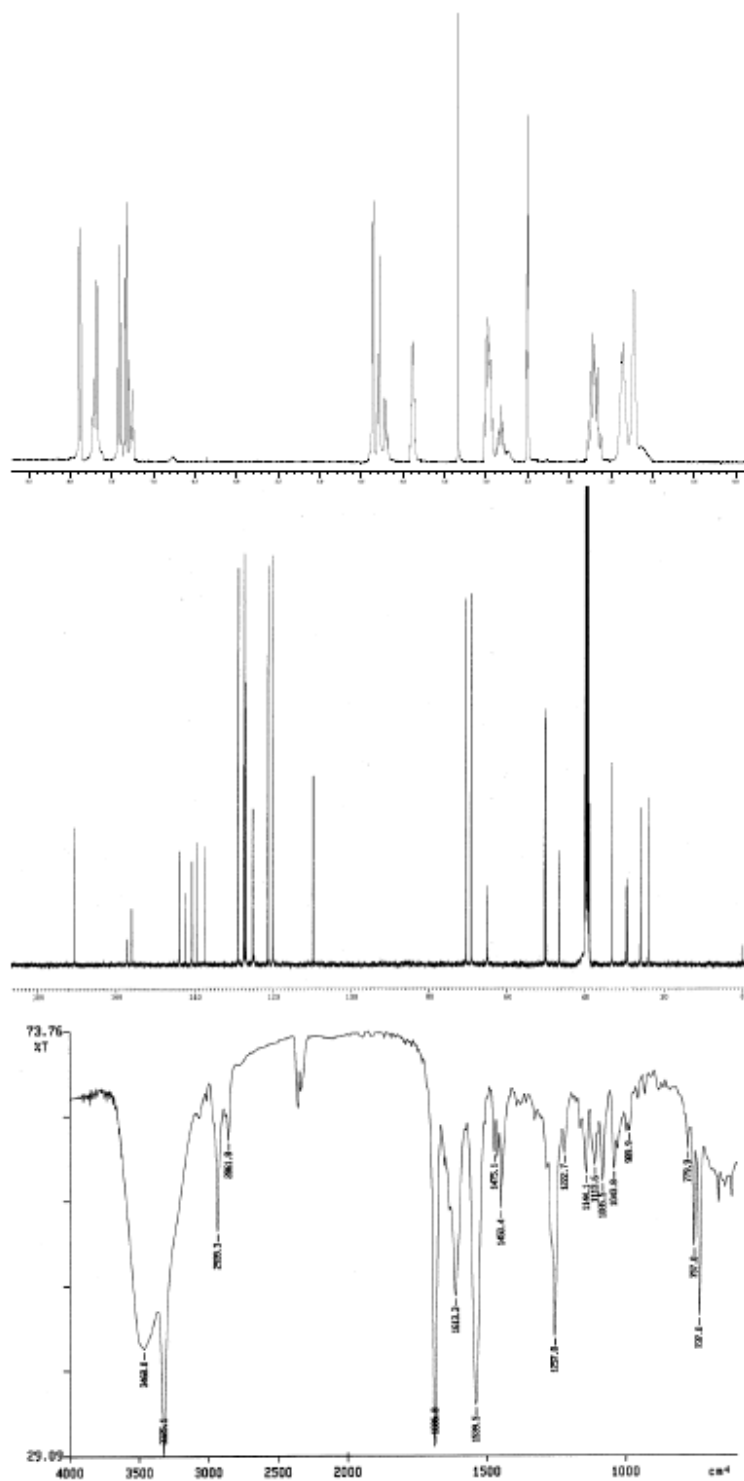
**Figure 4.** Spectra of 4-(3-trifluoromethyl-3-H-diazirin-3-yl) benzoylaminohexanoyl piperazinyl amphotericin B (**8**)



**Figure 5.** Spectra of bis(diaminohexanoyl piperidyl amphotericin B) glutarate (9)



**Figure 6.** Spectra of biotinyl aminohexanoyl amphotericin B (10)



**Figure 7.** {6-[(3,4-Dihydroxy-cyclopentanecarbonyl)-amino]-hexyl}-carbamic acid 9-fluoren-9-ylmethyl ester (**11**)



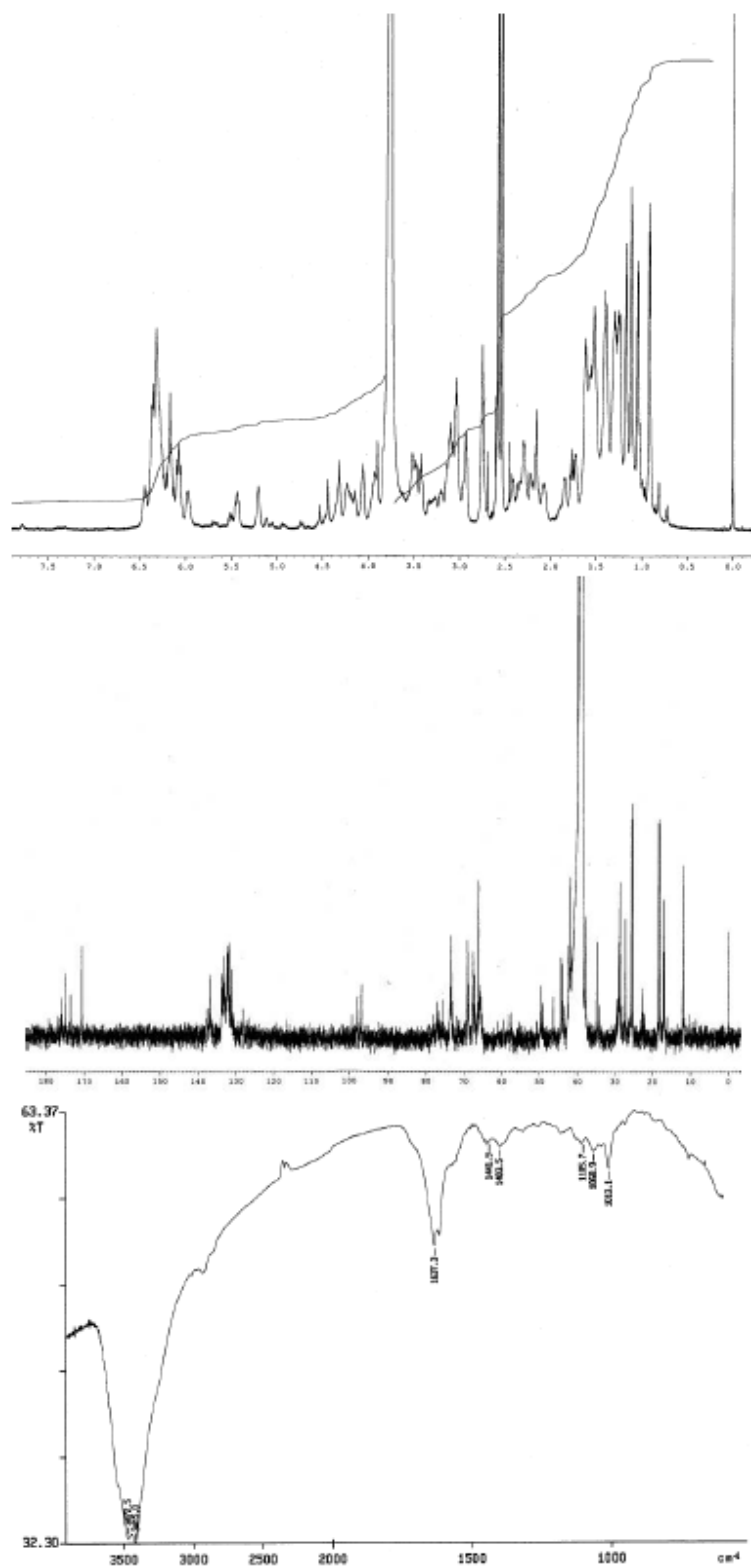


Figure 8. Spectra of diaminohexanoyl pyridyl amphotericin B (12)

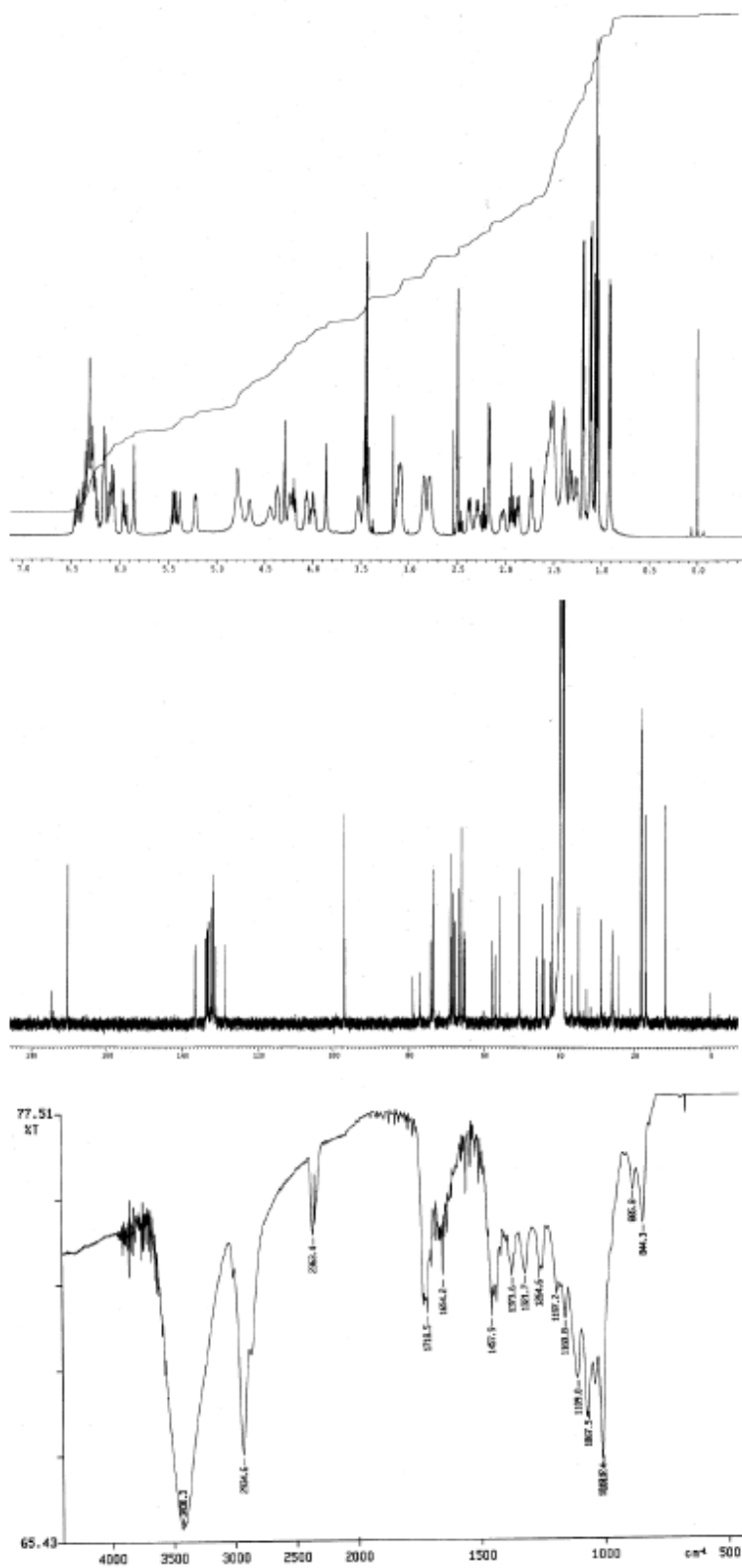
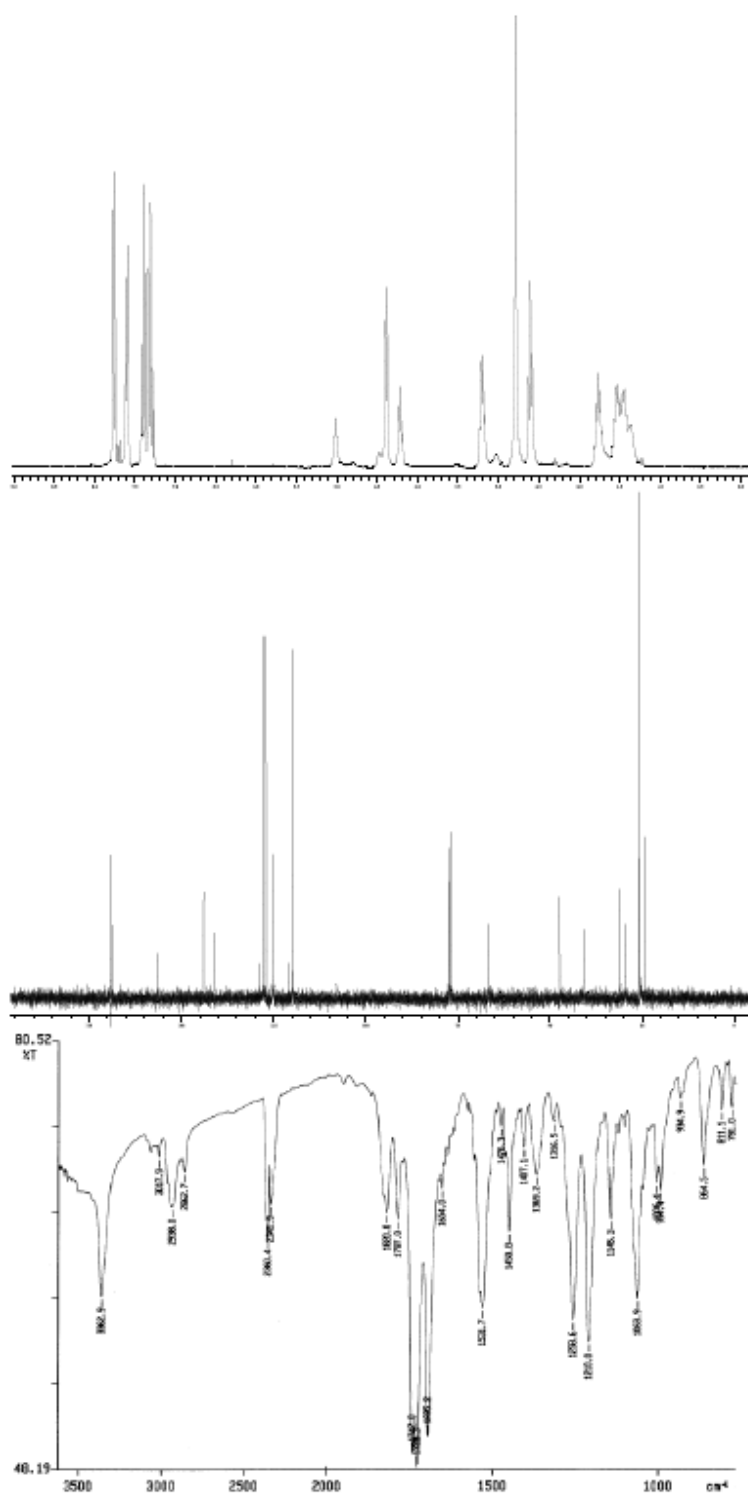
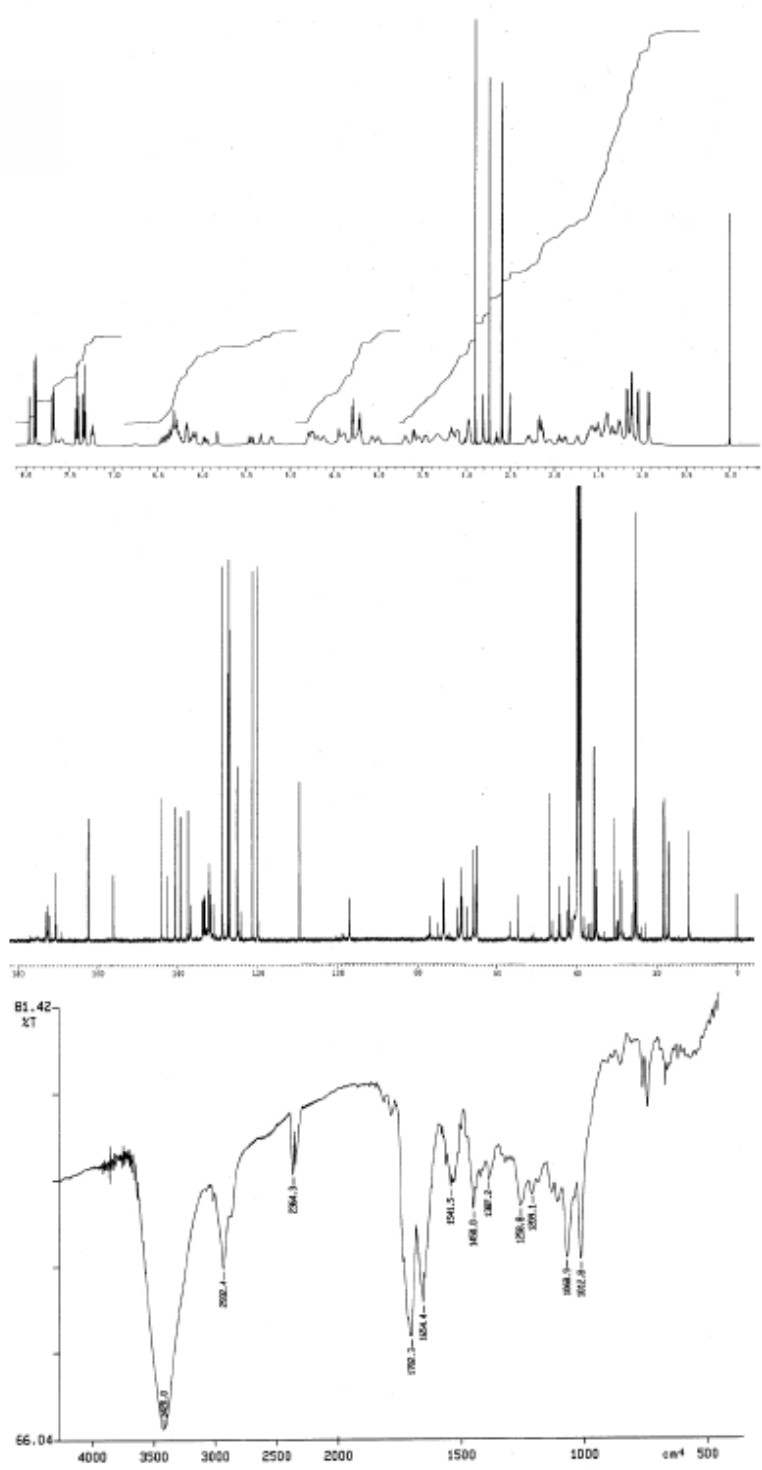


Figure 9. Spectra of Piperidyl amphotericin B (13)



**Figure 10.** Spectra of 6-(9-H-Fluoren-9-ylmethoxycarbonylamino)-hexanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (**14**)



**Figure 11.** Spectra of Fmoc aminohexanoyl amphotericin B (15)

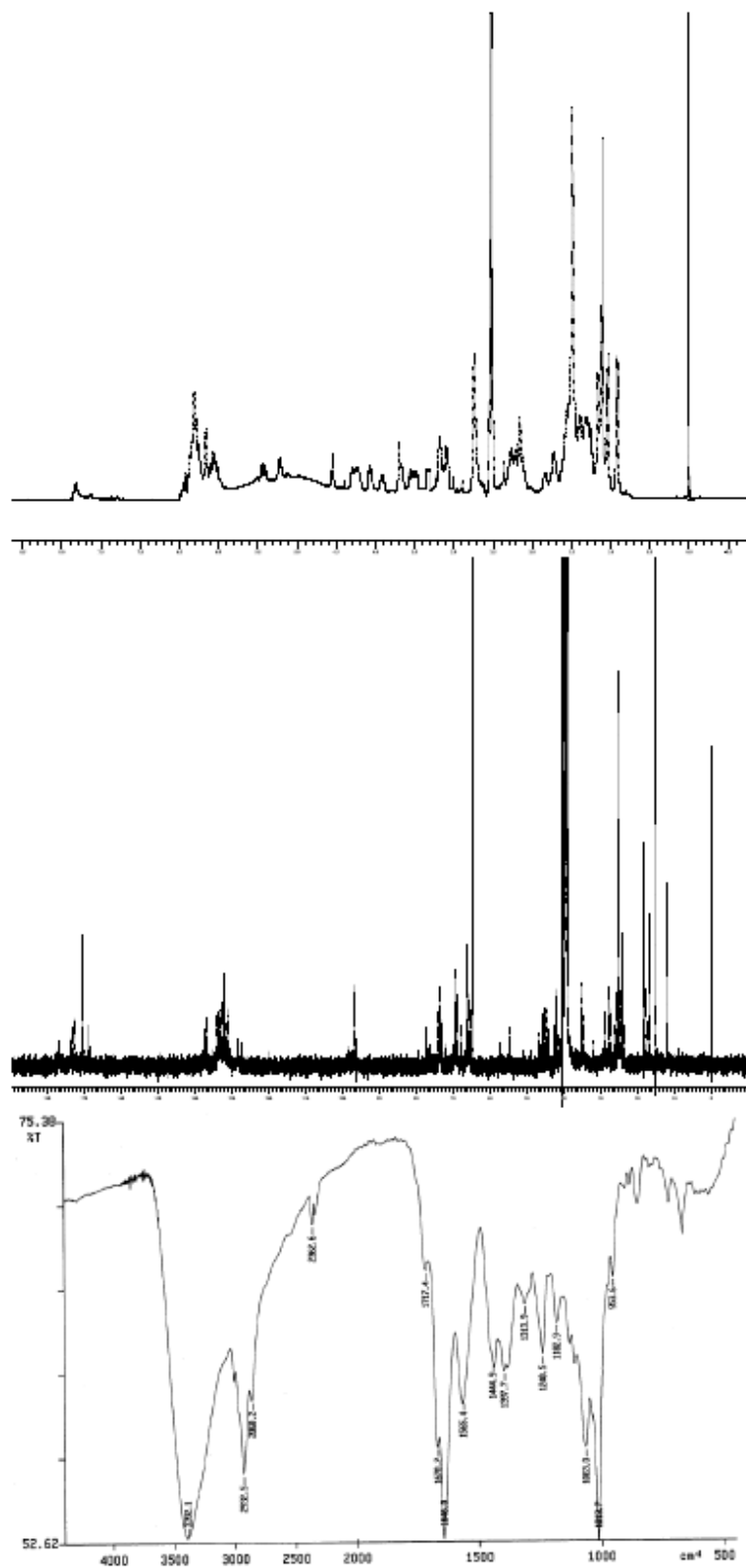


Figure 12. Spectra of aminohexanoyl amphotericin B (16)



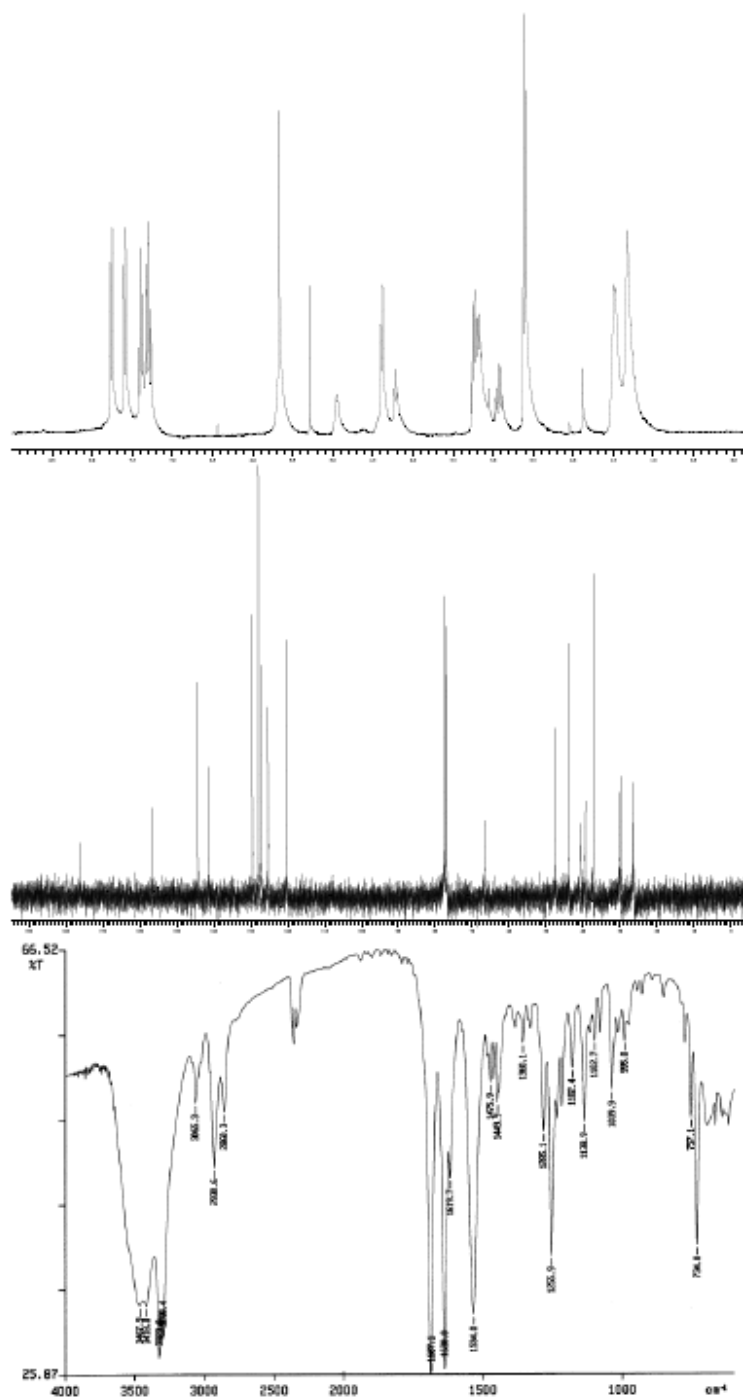
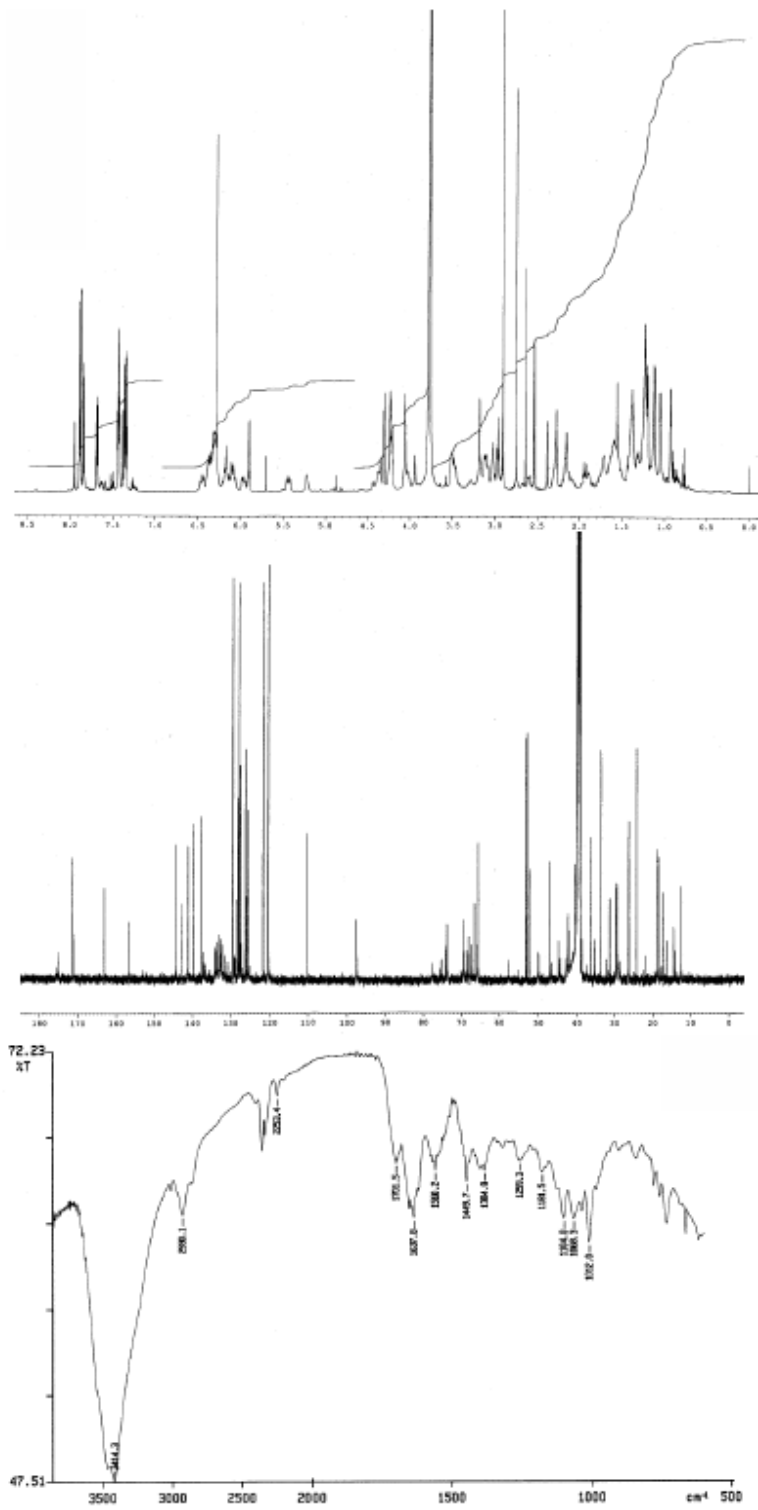


Figure 13.7 Spectra of 9H-fluoren-9-ylmethyl{6-[(3-cyclopenten-1-ylcarbonyl)-amino]hexyl} carbamate (17)



**Figure 14.** Spectra of Fmoc diaminohexanoyl pyridyl amphotericin B (18)