SUPPORTING INFORMATION

Broad substrate specificity of the amide synthase in *S. hygroscopicus* – new 20-membered macrolactones derived from geldanamycin

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1. General information

Related to geldanamycin **4** is reblastatin which is saturated at C4-C5 and which has a benzene chromophore instead of a benzoquinone or a hydroquinone moiety.[S1] Importantly, reblastatin shows lower cytotoxicity than geldanamycin while having a higher affinity for Hsp90.[S1] As several of the metabolites described in this paper are structurally closely related to reblastatin their naming is based on reblastatin.

^{1}H NMR spectra were recorded at 400 MHz with a BRUKER Avance-400 or at 500 MHz with a BRUKER DRX-500 and cryo TCI probe at 283 or 323K. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra were recorded at 100 MHz with a BRUKER Avance-400 and at 125 MHz with a BRUKER DRX-500. The multiplicities refer to the resonances in the off-resonance decoupled spectra and were elucidated using phase-sensitive HSQC experiments. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), q = quartet (methyl), t = triplet (methylene). Chemical shift values of ^{1}H and ^{13}C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard.[S2] For interpretation of geldanamycin derivative spectra, supporting ^{1}H-^{1}H correlation (COSY) and ^{1}H-^{13}C correlation (phase-sensitive HSQC, HMBC) experiments were performed. ^{13}C-chemical shifts of the diluted probes were extracted from the 2D-^{13}C correlation spectra. For determination of the macrolactone carbamate moiety variable temperature ^{1}H-NMR as well as ^{15}N-edited HSQC spectra in THF-d_8 were recorded. NMR-spectra of reblastatin derivatives were accumulated in THF-d_8 at an increased temperature of 323K leading to averaged conformational ensembles. Mass spectra were obtained alternatively with a type VG Autospec (EI) spectrometer at 75 eV (MICROMASS), with a type LCT (ESI) (MICROMASS) equipped with a lockspray dual ion source in combination with a WATERS Alliance 2695 LC system, or with a type Q-TOF premier (MICROMASS) spectrometer (ESI mode) in combination with a WATERS Acquity

UPLC system equipped with a WATERS Acquity UPLC BEH C18 1.7 µm (SN 01473711315545) column (solvent A: water + 0.1 % {v/v} formic acid, solvent B: MeCN or MeOH {given in experimental part} + 0.1 % {v/v} formic acid; flow rate = 0.4 mL/min; gradient {t [min]/solvent B [%]}: {0/5} {2.5/95} {6.5/95} {6.6/5} {8/5}; retention times {r_t} given in the experimental part). Ion mass signals (*m/z*) are reported as values in atomic mass units. Analytical thin-layer chromatography was performed using precoated silica gel 60 F254 plates (MERCK, Darmstadt) and the spots were visualized with UV light at 254 nm or alternatively by staining with ninhydrin, permanganate or 4-methoxybenzaldehyde solutions. Flash column chromatography was performed on MACHERY-NAGEL silica gel (particle size = 40-63 µm). Size exclusion chromatography was performed with Sephadex® LH-20 stationary phase (500 · Ø20 mm) and methanol as eluent. Isolation of geldanamycin/reblastatin derivatives was achieved by preparative high performance liquid chromatography using a MERCK HITACHI LaChrom system (pump L-7150, interface D-7000, diode array detector L-7450 {λ = 220-400 nm, preferred monitoring at λ = 248 nm}) with columns mentioned in the experimental part. TRENTECReprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm. Operating conditions and retention times (t_R) are reported in the experimental part.
Melting points were measured using either a SRS OptiMelt apparatus or an ELECTROTHERMAL IA 9200 instrument and are reported uncorrected. Commercially available reagents, chromatography type or dry solvents were used as received or purified by standard techniques according to the literature. Preparation of aminobenzoic acids **7**[S1], **18**, **20** and **22** are described below. Aminobenzoic acids **24** and **26** are commercially available.

2. Fermentation of *S. hygroscopicus* mutant K390-61-1

General parameters: *S. hygroscopicus* mutant K390-61-1 was stored as stock cultures at 4°C on R5-agar plates[S3] in the refrigerator. The strain was grown on a R5 agar plate at 30°C for 7 days in a HERAEUS incubator. A single colony of the K390-61-1 mutant from this 7 day-old agar plate was used to inoculate the GYP precultures which were incubated at 28°C for 2 days. Precultures were prepared in GYP-medium (40 mL/flask, 2.5 g/L yeast extract, 10 g/L peptone, 10 g/L glucose, 3 g/L xanthan gum, distilled water).[S4] Main cultures were prepared in GP-medium (40 g/L glucose, 2.5 g/L peptone, 2.5 g/L tryptone, 5 g/L oatmeal, 2.5 g/L yeast extract, 3 g/L xanthan gum, distilled water).[S5] Liquid cultures were incubated in a NEW BRUNSWICK SCIENTIFIC Innova 4900 gyratory multi-shaker at 28°C with vigorous shaking (180 rpm) in 500 mL Erlenmeyer flasks with a baffle. Main cultures were inoculated with 1 mL preculture per 30 mL culture broth. After 7 d of fermentation the production cultures were extracted twice with EtOAc. The EtOAc extracts were concentrated, and the residue was dissolved in MeOH (1 ml) and used directly for ESI-MS analysis.
Cultivation parameters. Cultures were shaken for 1 day at 28°C before addition of mutasynthons was initiated. For novel mutasynthons, productivity of the strain was monitored by parallel feeding of mutasynthons of known acceptance (e.g., natural synthon: 3-amino-5-hydroxybenzoic acid hydrochloride (**1**)). Mutasynthons were dissolved in DMSO/water (preferably 1:1, mutasynthon-hydrochlorides require less DMSO; volume of feeding solution not exceeding 10 % {v/v} with respect to the recipient culture) and sterilized by filtration. Mutasynthons were added to production cultures (preferably 1.25 mmol/L of culture volume) either portion-wise (4 portions, 24 hour interval) or preferably continuously (drop-wise; for large-scale fermentations) over the time-course of 4 days using autoclavable, syringe pumpdriven feeding capillaries (BRAINTREE SCIENTIFIC BS-9000-8 syringe pump with UPCHURCH SCIENTIFIC high-purity Teflon® PFA tubing {1/16" OD, 0.1" ID} and Tefzel® connectors). Shaking was continued to a total cultivation time of 7-8 days. For detection of

novel products from test cultures, samples of the culture broth (200 µL) were mixed with ethanol (200 µL), centrifuged (20800*g, 3 min, 4°C) and the clear supernatant subjected to UPLC-ESI-MS analysis. Failing detection of novel products, the culture broth was extracted three times with ethyl acetate, dried over $MgSO_4$, concentrated *in vacuo*, filtered over silica gel with ethyl acetate and the solvent removed *in vacuo*. The residue was dissolved in methanol (1 mL) and subjected to UPLC-ESI-MS analysis. For isolation of novel products from large-scale fermentation, the combined fermentation broth was extracted with ethyl acetate as described above, and the crude extract was subjected to a sequence of chromatographic purifications based on silica gel chromatography, size-exclusion chromatography and/or reversed phase-HPLC as described for each new metabolite in detail below.

3. Cell proliferation assay

Permanent cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (L-929 ACC2; KB-3-1 ACC 158; A-431 ACC 91) or American Type Culture Collection (ATCC) (SK-OV-3 HTB-77; PC-3 CRL-1435). Primary human umbilical vein endothelial cells (HUVEC) are from LONZA. Cells were grown in the culture media recommended by the supplier. Growth inhibitory effects of substances were measured in microtiter plates. Sixty µL of serial dilutions of the test compounds were added to 120 µL aliquots of a cell suspension (50.000/mL) in 96-well plates and incubated at 37 °C and 10% CO_2 for 5 days. MTT [3(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] was used to measure growth and viability of cells which are capable of reducing it to a violet formazan product. Twenty µL of MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg/mL. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL isopropanol containing 0.4% hydrochloric acid. The microtiter plates were measured at 590 nm using an ELISA plate reader. All experiments were carried out in parallel duplicates, the percentage of metabolic activity per well was calculated as the mean with respect to the controls set to 100%. The IC_{50} was derived from concentration-dependent activity curves.

4. Heatshock protein 90 (Hsp-90) assay

Purification of human Hsp90α. The *E. coli* (strain BL21plys, Invitrogen) was used to host the plasmid pET15.1 containing the human HSP90α gene cloned into the frame encoding an N-terminal hexahistidine and a TEV protease cleavage site.[S6] All protein purification steps were carried out at 4 °C and protease inhibitors were freshly added to lysis buffers to minimize the degradation of recombinant proteins by endogenous proteases. All buffers contained 6 mM β-mercaptoethanol (ME). Cell pellets were subjected to lysis by two French Press cycles at 12,000 p.s.i. The debris was adjusted to 100 ml by addition of lysis buffer containing 500 mM KCl, 2 mM imidazole, 10% glycerol, 5 mM ME, 5 µl protease inhibitor (sigma P8465), 20 mM Tris-HCl, pH 8.0. Cellular debris was removed by centrifugation at 25,000xg for 50 min at 4 °C. The soluble protein in the supernatant was further incubated with 5 ml metal chelating resin Talon (Clontech) upon incubation for 2 hours on ice. The resin was filled into a column and unbound protein was removed by 15-fold volumes of resin with buffer A (500 mM KCl, 2 mM imidazole, 6 mM ME, 20 mM Tris-HCl, pH 8.0) and followed by a washing step with 2 volumes of resin with buffer B (1 M KCl, 2 mM imidazole, 1 mM Mg-ATP, 0.1% Tween20, 6 mM ME, 20 mM Tris-HCl, pH 8.0). Bound proteins were eluted into 10 ml of 500 mM KCl, 250 imidazole, 6 mM ME and 20 mM Tris-HCl, pH 8.0. The N-terminal tag was removed by TEV protease (Promega) and dialysis was conducted for 24 h against 1 l buffer composed of 20 mM Tris pH 8.0 , 20 mM KCl, 6 mM mercaptoethanol (ME) and 10% glycerol at 4°C. Further purification included anion exchange chromatography

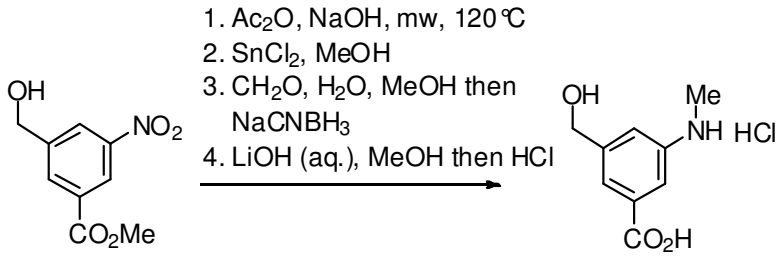
on a Mono Q column in 20 mM Tris-HCl pH 8, and 6 mM ME using a linear gradient of 0 to 0.5 M KCl. The protein was concentrated in a YM50 centrifugal filter device (Amicon) and purified by SEC 16/60 chromatography in a buffer system composed of 20 mM Tris-HCl pH 8, 500 mM KCl and 6 mM ME. Human Hsp90 was concentrated and exchanged by dialysis into 20 mM Tris pH 7.5, 50 mM KCl, 6 mM ME and 10% glycerol and samples were frozen at a concentration of 2 mg ml^{-1} in liquid nitrogen and stored at -80°C.

GM-FITC binding/competition assays. Binding of FITC labeled geldanamycin (GM-FITC, Biomol) was estimated by fluorescence polarization (FP) with 480 nm for excitation and 530 nm for emission at a grating ratio of 0.9 using a Berthold Mithras 970 microplate reader. Polarization was calculated as described by Kim et al.[S7] FP measurements were performed in 10 nM GM-FITC, 2-5% DMSO, 50 mM KCl, 1 mM DTT, 5 mM $MgCl_2$, 20 mM Na_2MoO_4, 0.01% NP40 detergent, 0.1 mg/ml BSA, 20 mM HEPES-KOH, pH 7.3 and 30-50 nM HSP90. The dose-response curve for the binding of 10 nM geldanamycin-FITC conjugate to human Hsp90α was determined by incubating different amounts of purified protein with geldanamycin-FITC at 4° C. Fluorescence polarization was measured after 16 h. Data were imported into Origin 6.0 to determine the dissociation constant from a dose-response curve as a function of the Hsp90α concentration using the logistic function (y = A2 + (A1-A2)/(1 + (x/x0)p), using the parameters A1= 0, A2 = 185, x0 = 2.3 ± 0.11303 and p = 1.18094 ± 0.07511. The tracer had a Kd of 2.3 nM for Hsp90a. Signal intensities obtained from fluorescence polarization measurements were calculated as mP values using the equation: mP = 1000 × [(IS - ISB) - (IP - IPB)]/[(IS - ISB) + (IP - IPB)], where IS is the parallel emission intensity measurement and IP is the perpendicular emission intensity. ISB and ISP are the corresponding background values (buffer). Measured intensities for a "blank sample" that does not contain the polarization label was: IS = 474075.67 and for IP = 106103E6; in contrast, "samples" that are labeled gave intensities in the range of IS = 4.97549E6 to 2.00363E7 and IP = 2.95068E6 to 8.88856E6 (E stands for exponent).

5. Preparation of mutasynthons

For the preparation of 3-amino-5-hydroxymethyl-benzoic acid (**7**) see ref. S1.

3-Hydroxymethyl-5-N-methylaminobenzoic acid hydrochloride (18)



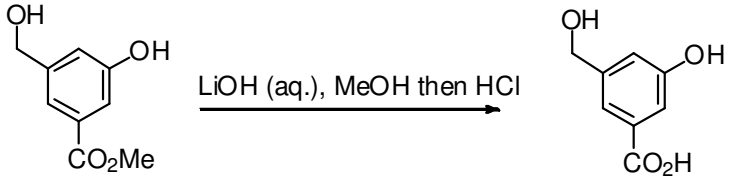
18

A microwave vial charged with 2.5 g 3-hydroxymethyl-5-nitrobenzoic acid methyl ester (11.84 mmol, 1 eq.), 54 mg solid NaOH (1.35 mmol, 0.1 eq.) and 2.22 mL acetic anhydride (23.68 mmol, 2 eq.) were heated for 4 min at 120°C in a microwave oven. After cooling to room temperature water and ethyl acetate were added. The aqueous layer was extracted with ethyl acetate twice, dried ($MgSO_4$) and evaporated under reduced pressure to yield 2.99 g of 3-acetoxymethyl-5-nitrobenzoic acid methyl ester **S1** (11.84 mmol, quant.) as a light yellow solid.[S8]

To a solution of 3.5 g 3-acetoxymethyl-5-nitrobenzoic acid methyl ester **S1** (13.82 mmol, 1 eq.) in 100 mL MeOH 15.6 g $SnCl_2 \cdot 2\ H_2O$ (41.47 mmol, 3 eq.) were added and the resulting solution was heated under reflux for 3 h. After cooling to room temperature sodium bicarbonate solution was added and the mixture was extracted with ethyl acetate (three times).

The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to yield 3.05 g 3-acetoxymethyl-5-aminobenzoic acid methyl ester **S2** (13.68 mmol, 99%) as a colorless solid.
Formalin (1.5 mL) was added to a methanolic solution (30 mL) of 2.8 g 3-acetoxymethyl-5-aminobenzoic acid methyl ester **S2** (12.54 mmol, 1 eq.) at 0°C. The resulting mixture was stirred at this temperature for 0.5 h. $NaBH_3CN$ (0.788 g, 12.54 mmol, 1 eq.) was added in portions and the solution was stirred for 1 h at 0°C and for 2 h at room temperature. The reaction was hydrolyzed by addition of 20 mL of an ammonium chloride solution, extracted with ethyl acetate (three times), dried ($MgSO_4$) and evaporated under reduced pressure. Purification by column chromatography yielded 1.05 g 3-acetoxymethyl-5-methylaminobenzoic acid methyl ester **S3** as a colorless solid (4.39 mmol, 35%).[S9]
Twenty mL of 1M aqueous LiOH solution (23.05 mmol, 5 eq.) were added to a solution of 0.9 g 3-hydroxymethyl-5-methylaminobenzoic acid methyl ester **S3** (4.61 mmol, 1 eq.) in 10 mL MeOH and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was acidified with 2M HCl and evaporated under reduced pressure. The crude product was recrystallized from 6M HCl to yield 0.87 g of 3-hydroxymethyl-5-methylaminobenzoic acid hydrochloride **18** (4.02 mmol, 87%) as light brownish solid.
^{1}H-NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm): δ 7.71 (s, 1H), 7.69 (s, 1H), 7.49 (s, 1H), 4.55 (s, 2H), 2.84 (s, 3H,) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6, DMSO-d_6= 39.52 ppm): δ 166.7 (s), 145.0 (s), 141.7 (s), 131.9 (s), 123.5 (d), 121.5 (d), 118.4 (d), 62.1 (t), 34.2 (q) ppm; **HRMS (ESI)**: M^-: calculated: 180.0661 found: 180.0667; **melting point**: 176°C.

3-Hydroxy-5-hydroxymethyl-benzoic acid (20)



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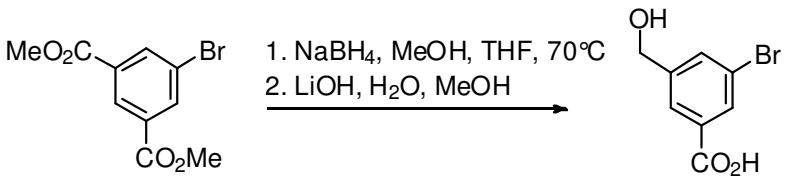
3-Hydroxy-5-hydroxymethyl-benzoic acid methyl ester (303 mg, 1.66 mmol, 1eq.) was dissolved in 5 mL methanol, 8.3 mL of a 1M LiOH-solution (8.31 mmol, 5 eq.) were added and stirring was continued for 2 h at room temperature. Afterwards, the mixture was acidified to pH 3 with 2M HCl and extracted with ethyl acetate several times. The combined organic layers were dried ($MgSO_4$) and evaporated to yield 279 mg of 3-hydroxy-5-hydroxymethyl-benzoic acid **20** (1.66 mmol, quant.) as a colorless solid.
^{1}H-NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm): δ 9.66 (br.s, 1H), 7.35 (s, 1H, J = 1.8, 1.0 Hz), 7.19 (s, 1H, J = 2.2, 1.8 Hz), 6.95 (s, 1H, J = 2.2, 1.0 Hz), 5.23 (br.s, 1H), 4.46 (s, 2H), 3.37 (br.s, 1H) ppm; **^{13}C-NMR** (100 MHz, DMSO-d_6, DMSO-d_6= 39.52 ppm): δ 167.4 (s) 157.3 (s), 144.6 (s), 131.7 (s), 118.0 (d), 117.7 (d), 114.2 (d), 62.5 (t) ppm; **HRMS (ESI)**: M^-: calculated: 167.0344 found: 167.0350; **melting point:** 127°C.

3-Bromo-5-hydroxymethyl-benzoic acid (22)



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5-Bromoisophthalic acid dimethyl ester (1.00 g, 5.66 mmol, 1 eq.) was dissolved in 30 mL THF, 3 mL MeOH and 139 mg $NaBH_4$ (5.66 mmol, 1eq.) were added and stirring was continued for 1.5 h at 70°C.
Afterwards, the mixture was hydrolyzed with sodium bicarbonate solution and extracted with ethyl acetate (three times). The combined organic layer was dried ($MgSO_4$) and evaporated. Purification by column chromatography yielded 766 mg of 3-bromo-5-hydroxymethyl-benzoic acid methyl ester **S4** (3.13 mmol, 86%) as a colorless solid. This material (700 mg, 2.86 mmol, 1 eq.) was dissolved in 10 mL MeOH, 10.0 mL of an aqueous 1M LiOH-solution (10.0 mmol, 3.5 eq.) were added and stirring was continued for 2 h at room temperature. Then, the mixture was acidified to pH 4 with 2M HCl, the MeOH was evaporated and the resulting precipitate was filtered and dried *in vacuo* to yield 640 mg of 3-bromo-5-hydroxymethyl-benzoic acid **22** (2.77 mmol, 97%) as a colorless solid.
^{1}H-NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm): δ 13.31 (br.s, 1H), 7.90 (dd, 1H, *J* = 1.7, 0.8 Hz), 7.89 (dd, 1H, *J* = 2.5, 0.8 Hz), 6.95 (dd, 1H, *J* = 2.5, 1.7 Hz), 5.49 (br.s, 1H), 4.55 (s, 2H) ppm; **^{13}C-NMR:** (100 MHz, DMSO-d_6, DMSO-d_6= 39.52 ppm): δ 166.1 (s), 146.1 (s), 133.1 (d), 132.9 (s), 129.9 (d), 126.1 (d), 121.5 (s), 61.7 (t) ppm; **HRMS (ESI)**: M^-: calculated: 228.9500 found: 228.9507; **melting point:** 182.5°C.

5. Mutasynthetic experiments

18-Hydroxymethyl reblastatin derivatives 8-12 and macrolactones 13-15. The mutasynthetic generation of 18-hydroxymethyl reblastatin derivatives **8-12** and macrolactones **13-15** was achieved by addition of 3-amino-5-hydroxymethyl-benzoic acid (**7**) to cultures of *S. hygroscopicus* mutant K390-61-1 according to the protocol described above. Mutasynthon **7** (0.53 g, 3.20 mmol) was fed to cultures with a combined starting volume of 1600 mL (2.00 mmol/L) by continuous addition. After extractive work-up, the concentrated crude extract was purified by silica gel column chromatography using ethyl acetate, followed by purification via Sephadex LH-20 and reversed phase HPLC (*vide infra*).
In total, eight novel metabolites were isolated (**8**: 0.5 mg, 0.94 µmol, 0.3 mg/L; **9**: 1.3 mg, 2.37 µmol, 0.7 mg/L; **10**: 2.8 mg, 5.13 µmol, 1.7 mg/L; **11**: 1.0 mg, 1.78 µmol, 0.7 mg/L; **12**: 0.9 mg, 1.56 µmol, 0.6 mg/L; **13**: 3.4 mg, 6.38 µmol, 2.3 mg/L; **14**: 2.3 mg, 4.02 µmol, 1.5 mg/L; **15**: 0.5 mg, 0.87 µmol, 0.3 mg/mL.

17-Demethoxy-18-hydroxymethyl-18-deoxyreblastatin (8). Reversed phase HPLC. 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 57.04 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 67.25 min; 3rd purification: TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 46.50 min.
17-O-Demethyl-18-hydroxymethyl-18-deoxyreblastatin (9). Reversed phase HPLC**:** 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 57.04 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 63.00 min; 3rd purification: TRENTEC Reprosil 100 CN 5

µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 37.50 min.

17-O-Demethyl-18-hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (10). Reversed phase HPLC. 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 57.04 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 63.00 min; 3rd purification: TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 27.00 min.

18-Hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (11). Reversed phase HPLC: 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 74.00 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 67.00 min.

18-Hydroxymethyl-21-hydroxy-4,5-dehydro-18-deoxyreblastatin (12). Reversed phase HPLC: 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 57.04 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 67.25 min; 3rd purification: TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 37.00 min.

Macrolactone 13. 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 83.00 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 78.20 min.

Macrolactone 14. 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 87.00 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 75.80 min.

Macrolactone 15. 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 25 mm, with guard column, 30 mm * 20 mm; gradient MeOH:H_2O = 60:40 → 100 % MeOH in 110 min, flow rate 15 mL/min; r_t: 76.00 min; 2nd purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 71.00 min.

Macrolactone 19. The mutasynthetic generation of macrolactone **19** was achieved by addition of 3-hydroxymethyl-5-methylaminobenzoic acid hydrochloride **18** to cultures of *S. hygroscopicus* mutant K390-61-1 according to the fermentation protocol described above. Benzoic acid derivative **18** (1.22 g, 5.61 mmol) was fed to cultures with a combined starting volume of 2.8 L (2.0 mmol/L) by continuous addition. After extractive work-up, the concentrated crude extract was purified by silica gel column chromatography using ethyl

acetate, followed by purification via Sephadex LH-20 and reversed phase HPLC (*vide infra*). In total, one novel derivative was isolated (1.0 mg, 1.84 µmol, 0.3 mg/L).
Reversed phase HPLC purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 86.59 min.)
Macrolactone 21. The mutasynthetic generation of macrolactone **21** was achieved by addition of 3-hydroxy-5-hydroxymethyl-benzoic acid **20** to cultures of *S. hygroscopicus* mutant K390-61-1 according to the fermentation protocol described above. Mutasynthon **20** (0.4 g, 2.38 mmol) was fed to cultures with a combined starting volume of 1.2 L (2.0 mmol/L) by continuous addition. After extractive work-up, the concentrated crude extract was purified by silica gel column chromatography using ethyl acetate, followed by purification via Sephadex LH-20 and reversed phase HPLC (*vide infra*). In total, one novel derivative was isolated (3.2 mg, 6.02 µmol, 2.7 mg/L).
Reversed phase HPLC: 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 82.56 min; 2nd purification: TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 57.82 min.)

Macrolactone 23. The mutasynthetic generation of macrolactone **23** was achieved by addition of 3-bromo-5-hydroxymethyl-benzoic acid **22** to cultures of *S. hygroscopicus* mutant K390-61-1 according to the fermentation protocol described above. Mutasynthon **22** (0.74 g, 3.20 mmol) was fed to cultures with a combined starting volume of 1.6 L (2.00 mmol/L) by continuous addition. After extractive work-up, the concentrated crude extract was purified by silica gel column chromatography using ethyl acetate, followed by purification via Sephadex LH-20 and reversed phase HPLC (*vide infra*). In total, one novel derivative was isolated (0.6 mg, 1.01 µmol, 0.4 mg/L). Reversed phase HPLC: 1st purification: TRENTEC Reprosil-Pur 120 C18 AQ 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 95.25 min; 2nd purification: TRENTEC Reprosil 100 CN 5 µm, 250 mm * 8 mm, with guard column, 40 mm * 8 mm; gradient MeCN:H_2O = 5:95 → 100 % MeCN in 100 min, flow rate 2.5 mL/min; r_t: 67.81 min.)

Spectroscopic data of mutaproducts
17-Demethoxy-18-hydroxymethyl-18-deoxyreblastatin (8).
^{1}HNMR (500 MHz, 283K, MeOD, MeOH = 3.31 ppm): δ 7.07 (br. s, 1H, 19-H), 6.92 (s, 1H, 17-H), 6.71 (s, 1H, 21-H), 5.73 (br. s, 1H, 3-H), 5.23 (d, 1H, J = 7.5 Hz, 9-H), 4.89 (d, 1H, J = 7.3 Hz, 7-H), 4.58 (d, 1H, J = 14.1 Hz, benzyl-$CH_{2a/b}$), 4.55 (d, 1H, J = 14.1 Hz, benzyl-$CH_{2a/b}$), 3.59-3.58 (m, 1H, 11-H), 3.43 (s, 3H, 6-OMe), 3.35 (s, 3H, 12-OMe), 3.31 (under MeOH, 1H, 6-H), 3.09-3.07 (m, 1H, 12-H), 2.80 (dd, 1H, J = 13.1, 4.1 Hz, 15-H_a), 2.49 (dd, 1H, J = 13.1, 5.5 Hz, 15-H_b), 2.40-2.34 (m, 1H, 10-H), 2.33-2.25 (m, 1H, 4-H_a), 2.14-2.06 (m, 1H, 4-H_b), 2.03-1.95 (m, 1H, 14-H), 1.82 (s, 3H, 2-Me), 1.68-1.58 (m, 2H, 13-H), 1.36 (s, 3H, 8-Me), 1.25-1.17 (m, 1H, 5-H_a), 1.16-1.09 (m, 1H, 5-H_b), 1.03 (d, 3H, J = 6.5 Hz, 10-Me) 0.77 (d, 3H, J = 4.3 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, 283K MeOD, MeOH = 49.0 ppm): δ 174.3 (s, C-1), 159.1 (s, $COONH_2$), 143.6 (s, C-18), 140.1 (s, C-16), 137.3 (s,

C-20), 136.3 (d, C-3), 134.4 (d, C-9), 132.8 (s, C-2), 131.3 (s, C-8), 126.5 (d, C-17), 125.6 (d, C-21), 119.4 (d, C-19), 1 83.7 (d, C-7), 82.0 (d, C-12), 80.9 (d, C-6), 74.4, (d, C-11), 64.7 (t, benzyl-$CH_{2a/b}$), 59.9 (q, C-6 OMe), 57.0 (q, C-12 OMe), 43.8 (t, C-15), 36.0 (d, C-10), 31.2 (t, C-13), 30.9 (d, C-5), 26.2 (d, C-14), 24.4 (t, C-4), 21.2 (q, C-14 Me), 18.2 (q, C-10 Me), 13.9 (q, C-2 Me), 12.1 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{45}N_2O_7$ $[M+H]^+$: 533.3227 found: 533.3251.

17-O-Demethyl-18-hydroxymethyl-18-deoxyreblastatin (9). ^{1}HNMR (500 MHz, T = 323K, THF-d_8, THF-d_7 = 1.73 ppm): δ 8.29 (br.s, 1H, NH), 8.05 (s, 1H, phen.OH), 7.12 (br.s, 1H, 21-H), 6.74 (d, 1H, J = 1.4 Hz, 19-H), 5.89 (t, 1H, J = 6.0 Hz, 3-H), 5.70 (br.s, 2H, NH_2), 5.34 (d, 1H, J = 9.5 Hz, 9-H), 5.07 (d, 1H, J = 5.7 Hz, 7-H), 4.95 (t, 1H, J = 5.1 Hz, benzyl-OH), 4.71 (dd, 1H, J = 5.1, 13.1 Hz, benzyl-$CH_{2a/b}$), 4.66 (dd, 1H, J = 5.1, 13.1 Hz, benzyl-$CH_{2a/b}$), 3.41 (dt, 1H, J = 3.4, 8.8 Hz, 6-H), 3.50 (ddd, 1H, J = 3.9, 3.9, 8.0 Hz, 11-H) 3.36 (s, 3H, 6-OMe), 3.32 (s, 3H, 12-OMe), 3.30-3.26 (m, 1H, 6-H), 3.23 (d, 1H, J = 4.1 Hz, 11-OH), 3.15 (td, 1H, J = 3.9, 8.0 Hz, 12-H), 2.71 (dd, 1H, J = 6.0, 13.9 Hz, 15-H_a), 2.52-2.48 (m, 1H, 10-H), 2.44 (dd, 1H, J = 6.0, 13.9 Hz, 15-H_b) 2.35- 2.27 (m, 1H, 4-H_a), 2.17- 2.10 (m, 1H, 4-H_b), 1.98-1.93 (m, 1H, 14-H), 1.78 (s, 3H, 2-Me), 1.70-1.65 (m, 1H, 13-H_a), 1.51 (s, 3H, 8-Me) 1.46–1.39 (m, 2H, 5-H), 1.32- 1.24 (m, 1H, 13-H_b), 0.99 (d, 3H, J = 6.6 Hz, 10-Me) 0.89 (d, 3H, J = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, T = 323K, THF-d_8 = 25.5 ppm): δ 170.8 (s, C-1), 157.3 (s, $COONH_2$), 152.5 (s, C-17), 134.6 (d, C-3), 134.0 (s, C-2), 133.4 (d, C-9), 132.5 (s, C-20), 131.6 (s, C-8), 128.9 (s, C-16), 126.9 (s, C-18), 125.7 (d, C-21), 119.1 (d, C-19), 83.1 (d, C-12), 81.8 (d, C-6), 81.1 (d, C-7), 75.4 (d, C-11), 64.7 (t, benzyl-$CH_{2a/b}$), 58.8 (q, C-6 OMe), 57.3 (q, C-12 OMe), 36.5 (t, C-15), 35.4 (d, C-10), 35.1 (t, C-13), 32.6 (d, C-14), 31.1 (t, C-5), 25.0 (t, C-4), 20.4 (q, C-14 Me), 17.0 (q, C-10 Me), 13.7 (q, C-2 Me), 13.0 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{44}N_2O_8Na$ $[M+Na]^+$: 571.2995 found: 571.2988.

17-O-Demethyl-18-hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (10). ^{1}HNMR (500 MHz, T = 323K, THF-d_8, THF-d_7 = 1.73 ppm): δ 8.67 (s, 1H, NH), 8.53 (br.s, 1H, 17-OH), 8.00 (s, 1H, 21-H), 7.11 (d, 1H, J = 11.5 Hz, 3-H), 6.79 (d, 1H, J = 1.8 Hz, 19-H), 6.56 (d, 1H, J = 11.5 Hz, 4-H), 5.72-5.66 (m, 1H, 5-H), 5.72-5.66 (m, 1H, 9-H), 5.72-5.66 (m, 2H, NH_2) 5.06 (s, 1H, 7-H), 4.47 (d, 2H, J = 6.0 Hz, benzyl-$CH_{2a/b}$), 4.40 (d, 1H, J = 9.3 Hz, 6-H), 4.06 (br.s, 1H, 11-OH), 3.78 (t, 1H, J = 6.0 Hz, benzyl-OH), 3.61-3.55 (m, 1H, 11-H), 3.35 (s, 3H, 6-OMe) 3.35-3.32 (m, 1H, 12-H), 3.16 (s, 3H, 12-OMe), 2.86 (dd, 1H, J = 13.5, 3.2 Hz, 15-H_a), 2.80-2.75 (m, 1H, 10-H), 2.44 (dd, 1H, J = 13.5, 9.0 Hz, 15-H_b), 1.98 (s, 3H, 2-Me), 1.93-1.86 (m, 1H, 14-H), 1.79-1.73 (m, 1H, 13-H_a), 1.73 (under THF, 3H, 8-Me) 1.69- 1.65 (m, 1H, 13-H_b), 1.02 (d, 3H, J = 6.6 Hz, 10-Me) 0.96 (d, 3H, J = 7.1 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, T = 323K, THF-d_8 = 25.5 ppm): δ 168.8 (s, C-1), 157.2 (s, $COONH_2$), 144.4 (s, C-17), 137.4 (s, C-2), 135.33 (s, C-8), 135.30 (s, C-18), 135.30 (d, C-5), 133.1 (d, C-9), 129.1 (s, C-20), 127.7 (s, C-16), 127.4 (d, C-4), 126.3 (d, C-3), 124.7 (d, C-21), 117.3 (d, C-19), 82.6 (d, C-12), 82.4 (d, C-6), 81.9 (d, C-7), 75.4 (d, C-11), 65.3 (t, benzyl-$CH_{2a/b}$), 57.3 (q, C-12 OMe), 56.7 (q, C-6 OMe), 41.9 (t, C-15), 36.3 (t, C-13), 33.7 (d, C-10), 29.7 (d, C-14), 23.2 (q, C-14 Me), 13.2 (q, C-8 Me), 13.1 (q, C-10 Me), 13.0 (q, C-2 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{42}N_2O_8Na$ $[M+Na]^+$: 569.2839 found: 569.2843.

18-Hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (11). ^{1}HNMR (500 MHz, T = 323K, THF-d_8, THF-d_7 = 1.73 ppm): δ 8.67 (s, 1H, NH), 8.02 (br s, 1H, 21-H), 7.09 (d, 1H, J = 11.2 Hz, 3H), 6.72 (d, 1H, J = 1.4 Hz, 19-H), 6.54 (dd, 1H, J = 11.5, 11.2 Hz, 4-H), 5.72-5.67 (m, 1H, 5-H), 5.72-5.67 (m, 1H, 9-H), 5.72-5.67 (m, 2H, NH_2) 5.03 (s, 1H, 7-H), 4.37 (d, 1H, J = 9.4 Hz, 6-H) 4.30 (s, 2H, benzyl-$CH_{2a/b}$), 3.58 (under THF, 1H, 11-H), 3.35 (s, 3H, 12-OMe) 3.34-3.32 (m, 1H, 12-H), 3.25 (s, 3H, 17-OMe), 3.16 (s, 3H, 6-OMe), 2.86-2.83 (m, 1H, 15-H_a), 2.81-2.76 (m, 1H, 10-H), 2.44-2.39 (m, 1H, 15-H_b), 2.01 (s, 3H, 2-Me), 1.91-1.83 (m, 1H, 14-H), 1.79-1.73 (m, 1H, 13-H_a), 1.73 (under THF, 3H, 8-Me), 1.73 (under THF, 1H, 11-OH), (under THF, 1H, benzyl-OH), 1.69- 1.64 (m, 1H, 13-H_b), 0.98 (d, 3H, J = 6.6 Hz, 10-Me) 0.93 (d, 3H, J = 7.0 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, T = 323K, THF-d_8 = 25.5 ppm): δ 168.6 (s, C-1), 158.8 (s, $COONH_2$), 137.4 (s, C-2), 135.4 (d, C-5), 132.8 (d, C-9), 131.9 (s, C-17), 130.7 (s, C-18), 127.3 (d, C-4), 126.2 (d, C-3), 125.7 (d, C-19), 118.4 (d, C-21), 82.6 (d, C-12), 81.8 (d, C-6), 80.1 (d, C-7), 76.6 (d, C-11), 75.5 (t, benzyl-$CH_{2a/b}$), 57.6 (q, C-17 OMe), 57.3 (q, C-12 OMe), 56.9 (q, C-6 OMe), 42.2 (t, C-15), 36.4 (t, C-13), 34.0 (d, C-10), 29.6 (d, C-14), 23.0 (q, C-14 Me), 13.4 (q, C-8 Me), 13.1 (q, C-10 Me), 13.0 (q, C-2 Me) ppm (C-8, C-16 and C-20 could not be assigned beyond doubt); HRMS (ESI): calculated for $C_{30}H_{44}N_2O_8Na$ $[M+Na]^+$: 583.2995 found 583.3002.

18-Hydroxymethyl-21-hydroxy-4,5-dehydro-18-deoxyreblastatin (12). ^{1}HNMR (500 MHz, T = 323K, THF-d_8, THF-d_7 = 1.73 ppm): δ 8.56 (br s, 1H, NH), 7.71 (br s, 1H, H-19), 7.32 (s, 1H, 21-OH), 7.01 (br s, 1H, 3-H), 6.51 (t, 1H, J = 11.3 Hz, 4-H), 5.76-5.61 (m, 1H, 5-H), 5.76-5.61 (m, 1H, 9-H), 5.76-5.61 (m, 2H, NH_2) 5.02 (s, 1H, 7-H), 4.53 (d, 1H, J = 12.1 Hz, benzyl-$CH_{2a/b}$), 4.49 (d, 1H, J = 12.1 Hz, benzyl-$CH_{2a/b}$), 4.39-4.31 (m, 1H, 6-H) 3.58-3.54 (m, 1H, 11-H), 3.33 (s, 3H, 6-OMe) 3.32 (s, 3H, 17-OMe), 3.32-3.29 (m, 1H, 12-H), 3.16 (s, 3H, 12-OMe), 2.79 (dd, 1H, J = 13.6, 8.8 Hz, 15-H_a), 2.82-2.74 (m, 1H, 10-H), 2.62 (dd, 1H, J = 13.6, 3.6 Hz, 15-H_b), 1.99 (s, 3H, 2-Me), 1.94-1.89 (m, 1H, 14-H), 1.79-1.73 (m, 1H, 13-H_a), 1.73 (under THF, 3H, 8-Me) 1.73- 1.66 (m, 1H, 13-H_b), 1.00 (d, 3H, J = 6.5 Hz, 14-Me) 0.92 (d, 3H, J = 7.1 Hz, 10-Me) ppm; ^{13}C-NMR (125 MHz, T = 323K, THF-d_8 = 25.5 ppm): δ 169.5 (s, C-1), 157.2 (s, $COONH_2$), 151.8 (s, C-17), 147.8 (s, C-21), 137.2 (s, C-2), 135.3 (d, C-5), 133.2 (d, C-9), 131.3 (d, C-19), 127.3 (d, C-4), 126.2 (d, C-3), 121.6 (s, C-8), 119.2 (s, C-18), 117.0 (s, C-16), 114.8 (s, C-20), 82.8 (d, C-12), 82.5 (d, C-6), 82.0 (d, C-7), 75.2 (d, C-11), 74.2 (t, benzyl-$CH_{2a/b}$), 57.7 (q, C-6-OMe), 57.2 (q, C-17 OMe), 56.7 (q, C-12 OMe), 36.0 (t, C-13), 33.6 (d, C-10), 33.4 (t, C-15), 30.1 (d, C-14), 23.7 (q, C-14 Me), 13.1 (q, C-8 Me), 13.0 (q, C-2 Me), 11.8 (q, C-10 Me) ppm; HRMS (ESI): calculated for $C_{30}H_{44}N_2O_9Na$ $[M+Na]^+$: 599.2945 found: 599.2943.

Macrolactone 13. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 6.68 (tq, 1H, J = 7.3, 1.4 Hz, 3-H), 6.55 (t, 1H, J = 1.3 Hz, 17-H), 6.51 (d, 1H, J = 1.3 Hz, 19-H), 6.51 (d, 1H, J = 1.3 Hz, 21-H), 5.49 (dq, 1H, J = 10.2, 1.4 Hz, 9-H), 5.07 (d, 1H, J = 11.8 Hz, arom.-CH_{2a}), 4.95 (d, 1H, J = 8.4 Hz, 7 H), 4.87 (under H_2O, 1H, arom. CH_{2b}), 3.47 (s, 3H, 12-OMe), 3.41 (dt, 1H, J = 3.4, 8.8 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.27 (s, 3H, 6-OMe), 3.19 (ddd, 1H, J = 2.5, 6.0, 9.7 Hz, 12-H), 2.61-2.56 (m, 1H, 10-H), 2.61-2.56 (m, 1H, 15-H_a), 2.41-2.35 (m, 1H, 15-H_b), 2.41-2.35 (m, 1H, 4-H_a), 2.27- 2.19 (m, 1H, 4-H_b), 1.88-1.81 (m, 1H, 14-H), 1.85 (s, 3H, 2-Me), 1.58-1.52 (m, 1H, 13-H_a), 1.58 (s, 3H, 8-Me), 1.47–1.28 (m, 1H, 5-H_a), 1.41- 1.28 (m, 1H, 5-H_b), 1.47-1.28 (m, 1H, 13-H_b), 0.97 (d, 3H,

J = 6.7 Hz, 10-Me), 0.90 (d, 3H, *J* = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 169.3 (s, C-1), 159.2 (s, $COONH_2$), 148.7 (s, C-18), 143.7 (s, C-16), 143.3 (d, C-3), 138.1 (s, C-20), 136.7 (d, C-9), 131.1 (s, C-8), 129.1 (s, C-2), 120.2 (d, C-17), 117.5 (d, C-21), 114.1 (d, C-19), 84.9 (d, C-7), 82.1 (d, C-6), 81.7 (d, C-12), 76.3 (d, C-11), 67.8 (t, arom.-CH_2), 59.8 (q, C-12 OMe), 57.1 (q, C-6 OMe), 45.5 (t, C-15), 37.1 (t, C-13), 34.8 (d, C-10), 33.3 (d, C-14), 31.5 (t, C-5), 25.6 (t, C-4), 20.7 (q, C-14 Me), 15.1 (q, C-10 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{44}N_2O_7Na$ $[M+Na]^+$: 555.3046 found: 555.3043.

Macrolactone 14. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 7.43 (t, 1H, *J* = 1.6 Hz, 19 H), 7.30 (t, 1H, *J* = 1.6 Hz, 21-H), 6.98 (s, 1H, 17-H), 6.72 (dt, 1H, *J* = 1.4, 7.5 Hz, 3-H), 5.50 (dd, 1H, *J* = 1.0, 10.2 Hz, 9-H), 5.16 (d, 1H, *J* = 12.1 Hz, arom.-CH_{2a}), 5.02 (d, 1H, *J* = 12.1 Hz, arom.-CH_{2b}), 4.97 (d, 1H, *J* = 8.5 Hz, 7-H), 3.50 (s, 3H, 12-OMe), 3.43 (dt, 1H, *J* = 3.5, 8.7 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.31 (s, 3H, 6-OMe), 3.22 (m, 1H, 12-H), 2.72 (dd, 1H, *J* = 6.7, 13.1 Hz, 15-H_a), 2.60 (tdd, 1H, *J* = 6.6, 10.3, 12.4 Hz, 10-H), 2.47 (dd, 1H, *J* = 7.2, 13.2 Hz, 15-H_b), 2.44-2.37 (m, 1H, 4-H_a), 2.30- 2.22 (m, 1H, 4-H_b), 2.14 (s, 3H, acetate), 1.93-1.89 (m, 1H, 14-H), 1.88 (s, 3H, 2-Me), 1.58-1.55 (m, 1H, 13-H_a), 1.56 (s, 3H, 8-Me) 1.53–1.46 (m, 1H, 5-H_a), 1.46-1.42 (m, 1H, 5-H_b), 1.37-1.31 (m, 1H, 13-H_b), 1.00 (d, 3H, *J* = 6.7 Hz, 10-Me) 0.92 (d, 3H, *J* = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 171.7 (s, acetate), 169.1 (s, C-1), 159.2 (s, $COONH_2$), 143.7 (s, C-16), 143.4 (d, C-3), 140.0 (s, C-18), 138.1 (s, C-20), 136.6 (d, C-9), 131.2 (s, C-8), 129.1 (s, C-2), 125.8 (d, C-21), 121.7 (d, C-17), 118.5 (d, C-19), 84.8 (d, C-7), 82.1 (d, C-6), 81.9 (d, C-12), 76.3 (d, C-11), 67.4 (t, arom.-CH_2), 59.8 (q, C1-2 OMe), 57.2 (q, C-6 OMe), 45.3 (t, C-15), 37.0 (t, C-13), 34.9 (d, C-10), 33.4 (d, C-14), 31.4 (t, C-5), 25.6 (t, C-4), 23.8 (q, acetate), 20.6 (q, C-14 Me), 15.3 (q, C-10 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{31}H_{46}N_2O_8Na$ $[M+Na]^+$: 597.3152 found 597.3171.

Macrolactone 15. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 7.23 (t, 1H, *J* = 1.6 Hz, 19-H), 7.10 (t, 1H, *J = 1.6 Hz,* 21-H), 6.86 (t, 1H, *J* = 1.6 Hz, 17-H), 6.69 (tq, 1H, *J* = 7.5, 1.4 Hz, 3-H), 5.48 (dq, 1H, *J* = 10.0, 1.4 Hz, 9-H), 5.14 (d, 1H, *J* = 12.1 Hz, arom.-CH_{2a}), 5.00 (d, 1H, *J* = 12.1 Hz, arom.-CH_{2b}), 4.94 (d, 1H, *J* = 8.8 Hz, 7-H), 3.47 (s, 3H, 12-OMe), 3.41 (dt, 1H, *J* = 3.4, 8.6 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.29 (s, 3H, 6-OMe), 3.21-3.18 (m, 1H, 12-H), 2.68 (dd, 1H, *J* = 6.8, 13.1 Hz, 15-H_a), 2.58 (qd, 1H, *J* = 6.1, 16.7 Hz, 10-H), 2.44 (dd, 1H, *J* = 7.2, 13.2 Hz, 15-H_b), 2.40-2.35 (m, 1H, 4-H_a), 2.27- 2.22 (m, 1H, 4-H_b), 1.89-1.84 (m, 1H, 14-H), 1.86 (d, 3H, *J* =1.4 Hz, 2-Me), 1.57-1.51 (m, 1H, 1-3H_a), 1.54 (d, 3H, *J* = 1.4 Hz, 8-Me) 1.47–1.40 (m, 2H, 5-H), 1.39-1.36 (m, 1H, 13-H_b), 0.98 (d, 3H, *J* = 6.7 Hz, 10-Me) 0.90 (d, 3H, *J* = 6.5 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 169.2 (s, C-1), 159.4 (s, $COONH_2$), 143.6 (d, C-3), 143.4 (s, C-16), 138.0 (s, C-20), 136.7 (d, C-9), 131.2 (s, C-8), 129.1 (s, C-2), 124.4 (d, C-17), 120.9 (d, C-21), 117.7 (d, C-19), 84.8 (d, C-7), 82.1 (d, C-6), 81.8 (d, C-12), 76.3 (d, C-11), 67.5 (t, arom.-CH_2), 59.8 (q, C-12 OMe), 57.2 (q, C-6 OMe), 45.4 (t, C-15), 37.1 (t, C-13), 34.9 (d, C-10), 33.3 (d, C-14), 31.4 (t, C-5), 25.6 (t, C-4) 15.2 (q, C-10 Me), 14.5 (q, C-14 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm (C-18 and C-urea could not be assigned beyond doubt); HRMS (ESI): calculated for $C_{30}H_{46}N_3O_8[M+H]^+$: 576.3285, found: 576.3288.

Macrolactone 19. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 6.68 (tq, 1H, J = 7.4, 1.4 Hz, 3-H), 6.50 (t, 1H, J = 1.3 Hz, 21-H), 6.40 (t, 1H, J = 1.3 Hz, 17-H), 6.40 (t, 1H, J = 1.3 Hz, 19-H), 5.48 (dd, 1H, J = 10.2, 1.3 Hz, 9-H), 5.09 (d, 1H, J = 12.0 Hz, arom.-CH_{2a}), 4.95 (d, 1H, J = 12.0 Hz, arom.-CH_{2b}), 4.94 (d, 1H, J = 8.7 Hz, 7-H), 3.47 (s, 3H, 12-OMe), 3.41 (td, 1H, J = 8.7, 3.3 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.27 (s, 3H, 6-OMe), 3.21-3.16 (m, 1H, 12-H), 2.76 (s, 3H, NH*Me*) 2.61-2.56 (m, 1H, 10-H), 2.59 (dd, 1H, J = 13.1, 7.3 Hz, 15-H_a), 2.41-2.36 (m, 1H, 4-H_a), 2.39 (dd, 1H, J = 13.1, 7.0 Hz, 15-H_b), 2.27- 2.19 (m, 1H, 4-H_b), 1.88-1.83 (m, 1H, 14-H), 1.85 (d, 3H, J = 1.4 Hz, 2-Me), 1.57-1.53 (m, 1H, 13-H_a), 1.54 (d, 3H, J = 1.3 Hz, 8-Me) 1.46–1.36 (m, 2H, 5-H), 1.33-1.29 (m, 1H, 13-H_b), 0.97 (d, 3H, J = 6.7 Hz, 10-Me) 0.90 (d, 3H, J = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 169.4 (s, C-1), 159.2 (s, $COONH_2$), 151.5 (s, C-18), 143.6 (s, C-16), 143.3 (d, C-3), 138.0 (s, C-20), 136.7 (d, C-9), 131.1 (s, C-8), 129.2 (s, C-2), 119.0 (d, C-21), 114.6 (d, C-17), 111.1 (d, C-19), 84.9 (d, C-7), 82.1 (d, C-6), 81.8 (d, C-12), 76.3 (d, C-11), 68.0 (t, benzyl-$CH_{2a/b}$), 59.8 (q, C-12 OMe), 57.1 (q, C-6 OMe), 45.7 (t, C-15), 37.1 (t, C-13), 34.8 (d, C-10), 33.3 (d, C-14), 31.5 (t, C-5), 25.6 (t, C-4), 20.7 (q, C-14 Me), 15.1 (q, C-10 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{30}H_{47}N_2O_7$ $[M+H]^+$: 547.3383 found: 547.3390.

Macrolactone 21. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 6.70-6.68 (m, 1H, 3-H), 6.68 (t, 1H, J = 1.3 Hz, 21-H), 6.56 (dd, 1H, J = 1.5, 1.3 Hz, 19-H), 6.55 (dd, 1H, J = 1.5, 1.3 Hz, 17-H), 5.48 (d, 1H, J = 10.2 Hz, 9-H), 5.10 (d, 1H, J = 12.0 Hz, benzyl-$CH_{2a/b}$), 4.96 (d, 1H, J = 12.0 Hz, benzyl-$CH_{2a/b}$), 4.94 (d, 1H, J = 8.7 Hz, 7-H), 3.47 (s, 3H, 12-OMe), 3.41 (td, 1H, J = 8.6, 3.4 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.30 (s, 3H, 6-OMe), 3.19 (ddd, 1H, J = 9.6, 5.8, 2.2 Hz, 12-H), 2.63 (dd, 1H, J = 12.9, 6.9 Hz, 15-H_a), 2.58 (dqd, 1H, J = 10.2, 6.4, 6.2 Hz, 10-H), 2.41- 2.37 (m, 1H, 4-H_a), 2.39 (dd, 1H, J = 13.0, 7.1 Hz, 15-H_b), 2.27- 2.20 (m, 1H, 4-H_b), 1.87-1.82 (m, 1H, 14-H), 1.85 (s, 3H, 2-Me), 1.57-1.51 (m, 1H, 13-H_a), 1.54 (s, 3H, 8-Me), 1.45–1.36 (m, 2H, 5-H), 1.31 (ddd, 1H, J =14.3, 9.2, 2.1 Hz, 13-H_b), 0.98 (d, 3H, J = 6.4 Hz, 10-Me), 0.89 (d, 3H, J = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 169.2 (s, C-1), 159.2 (s, $COONH_2$), 158.4 (s, C-18), 144.4 (s, C-16), 143.3 (d, C-3), 138.6 (s, C-20), 136.7 (d, C-9), 131.2 (s, C-8), 129.1 (s, C-2), 121.4 (d, C-21), 117.0 (d, C-17), 113.7 (d, C-19), 84.8 (d, C-7), 82.1 (d, C-6), 81.8 (d, C-12), 76.3 (d, C-11), 67.5 (t, benzyl-$CH_{2a/b}$), 59.8 (q, C-12 OMe), 57.1 (q, C-6 OMe), 45.4 (t, C-15), 37.0 (t, C-13), 34.9 (d, C-10), 33.3 (d, C-14), 31.4 (t, C-5), 25.6 (t, C-4), 20.6 (q, C-14 Me), 15.2 (q, C-10 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{43}NNaO_8[M+Na]^+$: 556.2886 found: 556.2875.

Macrolactone 23. ^{1}HNMR (500 MHz, T = 295K, MeOD, MeOH = 3.31 ppm): δ 7.34 (dd, 1H, J = 1.7, 1.6 Hz, 19-H), 7.30 (dd, 1H, J = 1.7, 1.1 Hz, 17-H), 7.20 (dd, 1H, J = 1.6, 1.1 Hz, 21-H), 6.71 (tq, 1H, J = 7.5, 1.5 Hz, 3-H), 5.47 (dd, 1H, J = 10.0, 1.0 Hz, 9-H), 5.17 (d, 1H, J = 12.3 Hz, benzyl-$CH_{2a/b}$), 5.04 (d, 1H, J = 12.3 Hz, benzyl-$CH_{2a/b}$), 4.87 (under H_2O, 1H, 7-H), 3.47 (s, 3H, 6-OMe), 3.41 (td, 1H, J = 8.7, 3.5 Hz, 6-H), 3.31 (under MeOH, 1H, 11-H) 3.31 (under MeOH, 3H, 12-OMe), 3.19 (ddd, 1H, J = 9.4, 5.4, 2.2 Hz,12-H), 2.74 (dd, 1H, J = 13.1, 6.5 Hz, 15-H_a), 2.56 (ddq, 1H, J = 10.0, 6.6, 6.5 Hz, 10-H), 2.44 (dd, 1H, J = 13.1, 7.5 Hz, 15-H_b), 2.42-2.36 (m, 1H, 4-H_a), 2.29-2.22 (m, 1H, 4-H_b), 1.89-1.83 (m, 1H, 14-H), 1.86 (d, 3H, J = 1.5 Hz, 2-Me), 1.54 (d, 3H, J = 1.2 Hz, 8-Me), 1.53-1.49 (m, 1H, 13-H_a),

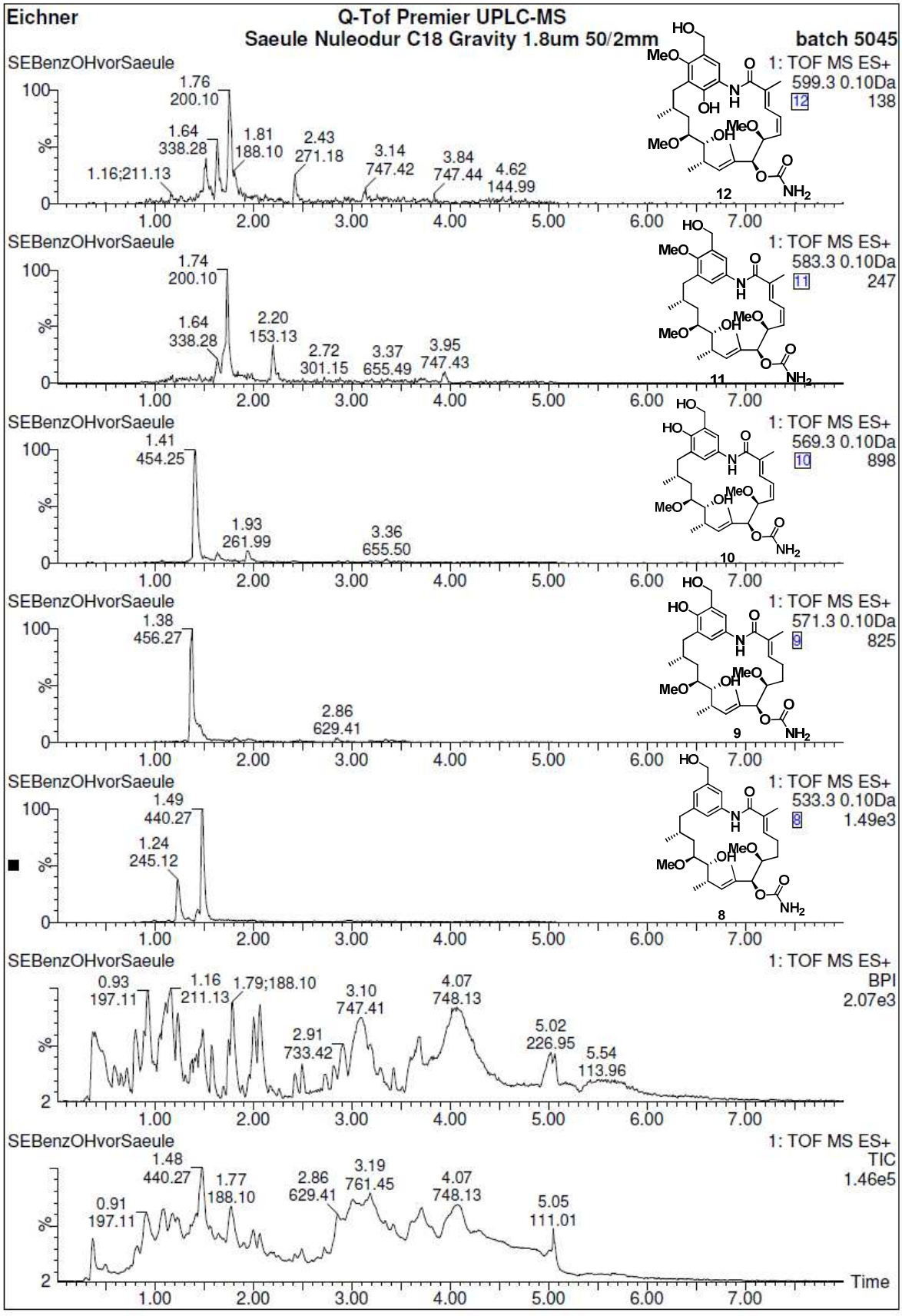
1.46–1.38 (m, 2H, 5-H), 1.34-1.29 (m, 1H, 13-H_b), 0.99 (d, 3H, J = 6.5 Hz, 10-Me) 0.87 (d, 3H, J = 6.6 Hz, 14-Me) ppm; ^{13}C-NMR (125 MHz, MeOD = 49.0 ppm): δ 168.9 (s, C-1), 159.2 (s, $COONH_2$), 145.5 (s, C-16), 143.6 (s, C-3), 140.0 (s, C-20), 136.5 (d, C-9), 133.1 (d, C-17), 131.2 (s, C-8), 129.6 (d, C-19), 129.0 (s, C-2), 128.9 (d, C-21), 122.9 (s, C-18), 84.7 (d, C-7), 82.0 (d, C-6), 82.0 (d, C-12), 76.1 (d, C-11), 66.5 (t, benzyl-$CH_{2a/b}$), 59.9 (q, C-6 OMe), 57.3 (q, C-12 OMe), 44.8 (t, C-15), 36.8 (t, C-13), 35.0 (d, C-10), 33.4 (d, C-14), 31.4 (t, C-5), 25.7 (t, C-4), 20.5 (q, C-14 Me), 15.5 (q, C-10 Me), 12.5 (q, C-2 Me), 11.6 (q, C-8 Me) ppm; HRMS (ESI): calculated for $C_{29}H_{42}NO_7BrNa$ $[M+Na]^+$: 618.2042 found: 618.2042.

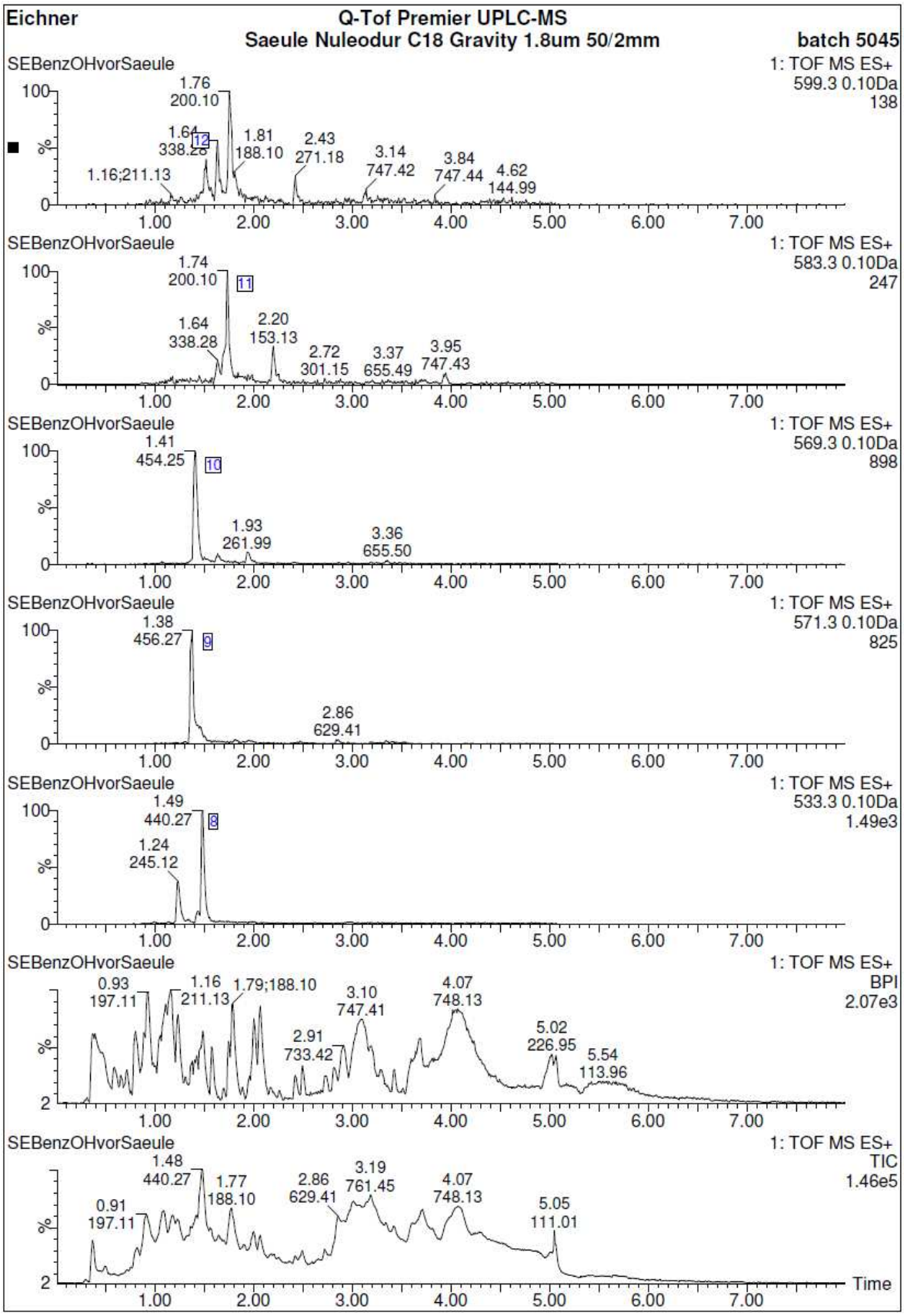
Macrolactone 25.
HRMS (ESI): calculated for $C_{29}H_{43}NO_7Na$ $[M+Na]^+$: 540.2937, found: 540.2938.

Macrolactone 30.
HRMS (ESI): calculated for $C_{29}H_{44}N_2O_7Na$ $[M+Na]^+$: 555.3046, found: 555.3039.

Molecular Modelling
GdmF (and also Asm9) has been modeled using the crystal structure coordinates of arylamine N-acetyltransferase 1 of *Mesorhizobium loti* (PDB: 2bsz). The 3D protein structure of GdmF was simulated using SWISS-MODEL.[S10] Molecular dynamics simulations were carried out using the AMBER10 software package. All calculations used explicit solvent and the protein was surrounded by an octahedral periodic box of TIP3P water molecules to a minimum distance of 10 Å from the solute before equilibration using a standard multistate protocol.[S11] Residue-specific structure RMS was calculated using the PTRAJ module available within AMBER.[S12] Secondary structure analysis was performed using the Dictionary of Secondary Structure of Protein (DSSP).[S13] Seco-progeldanamycin and the hydroxymethyl derivative were parameterized using ANTECHAMBER (GAFF) and modeled into the catalytic site of GdmF using a distance restraint (5 Å) with linear increasing energy penalty between C-1 and the Cys72 sulphur atom. After equilibration the proton at the sulphur atom (Cys72) was manually deleted before introducing a covalent bond between the sulphur atom and C-1. The equilibrated constructs (GdmF covalently bound to seco-progeldanamycin or the hydroxymethyl derivative) served as starting coordinates to simulate the macrocyclization reaction.

7. Chromatographic analysis and MS data from the fermentation with mutasynthon 7 (numbers of compounds are inserted).

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200.10
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338.28
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188.10
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271.18
3.14
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3.84
747.44
4.62
144.99
1.16;211.13
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1.74
200.10
11
1.64
338.28
2.20
153.13
2.72
301.15
3.37
655.49
3.95
747.43
SEBenzOHvorSaeule
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454.25
10
1.93
261.99
3.36
655.50
SEBenzOHvorSaeule
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825
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456.27
9
2.86
629.41
SEBenzOHvorSaeule
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1.49
440.27
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1.24
245.12
SEBenzOHvorSaeule
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BPI
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0.93
197.11
1.16
211.13
1.79;188.10
3.10
747.41
4.07
748.13
2.91
733.42
5.02
226.95
5.54
113.96
SEBenzOHvorSaeule
1: TOF MS ES+
TIC
1.46e5
1.48
440.27
1.77
188.10
2.86
629.41
3.19
761.45
4.07
748.13
0.91
197.11
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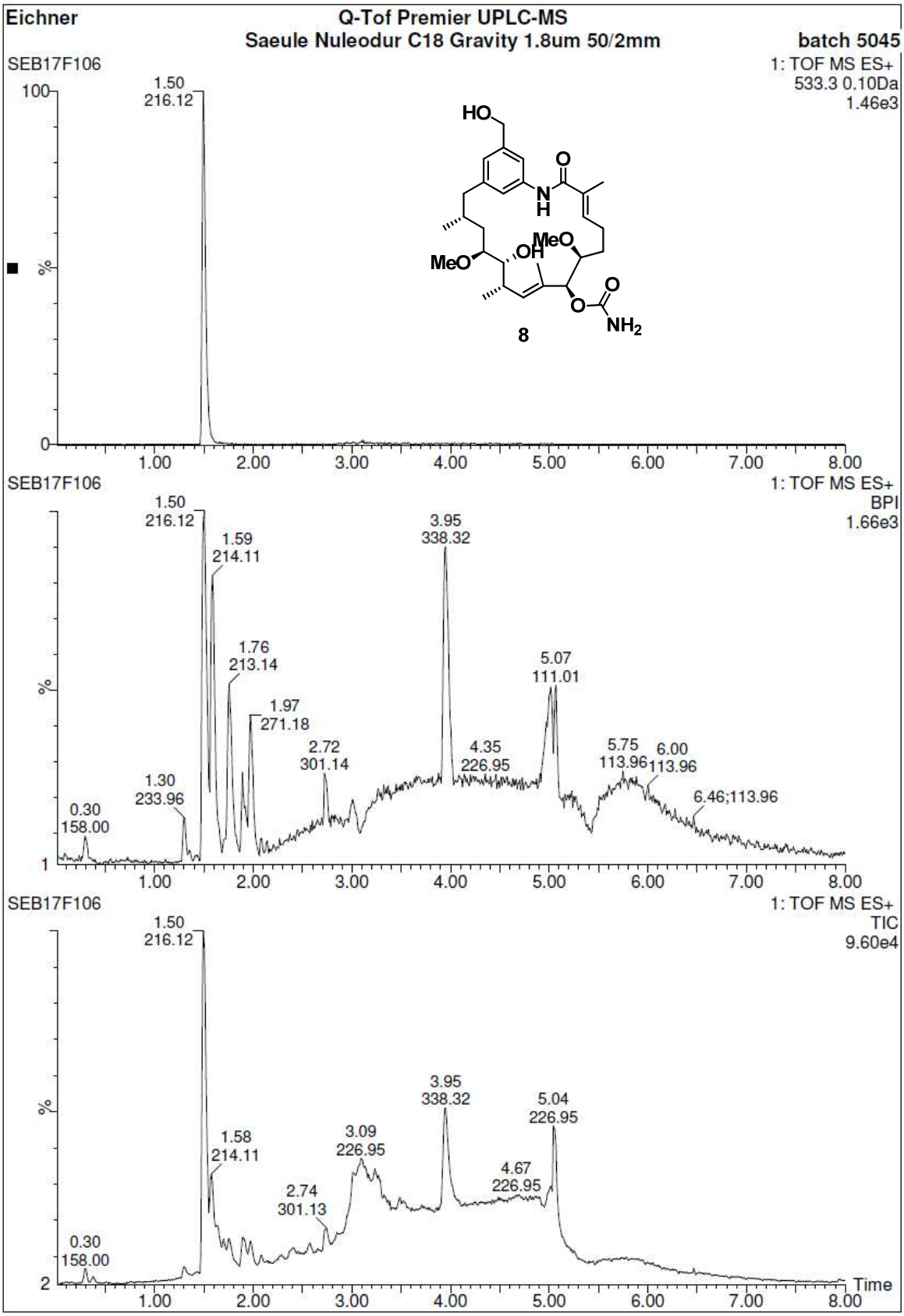
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Q-Tof Premier UPLC-MS
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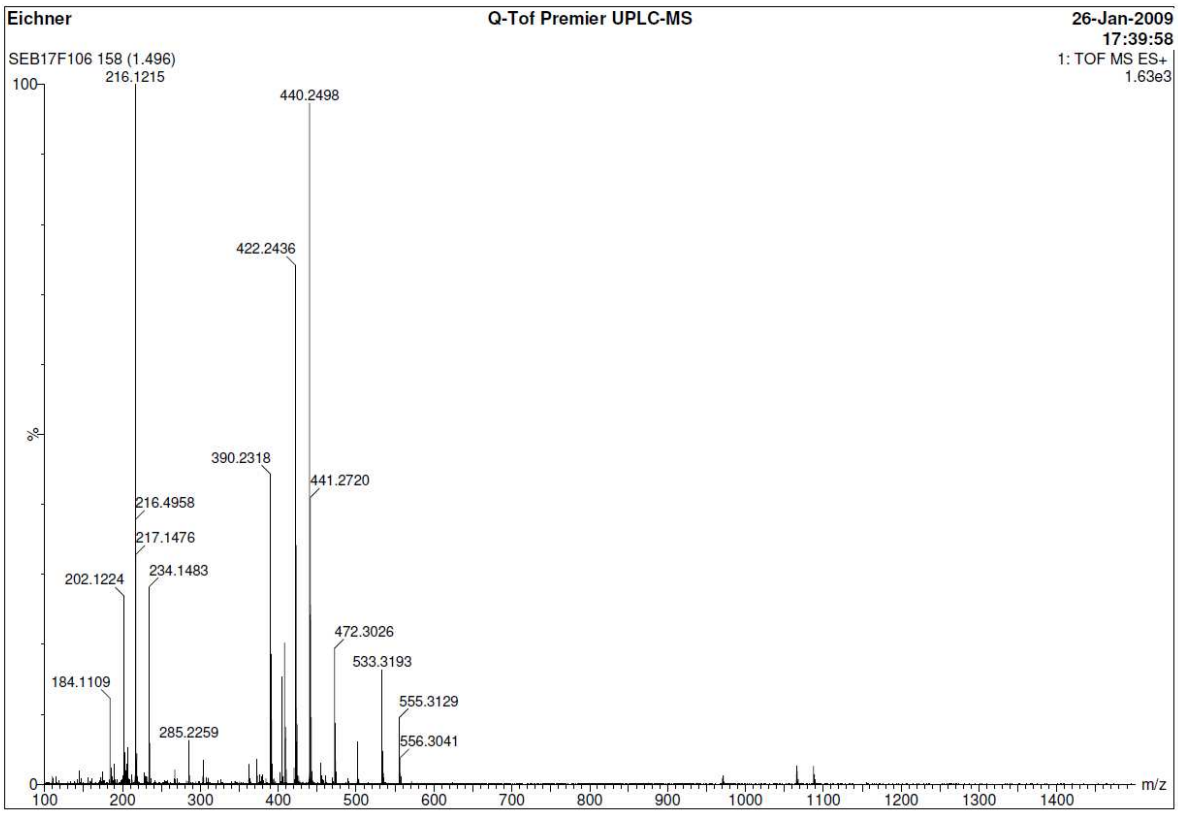
Time


Eichner
Q-Tof Premier UPLC-MS
26-Jan-2009
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472.3026
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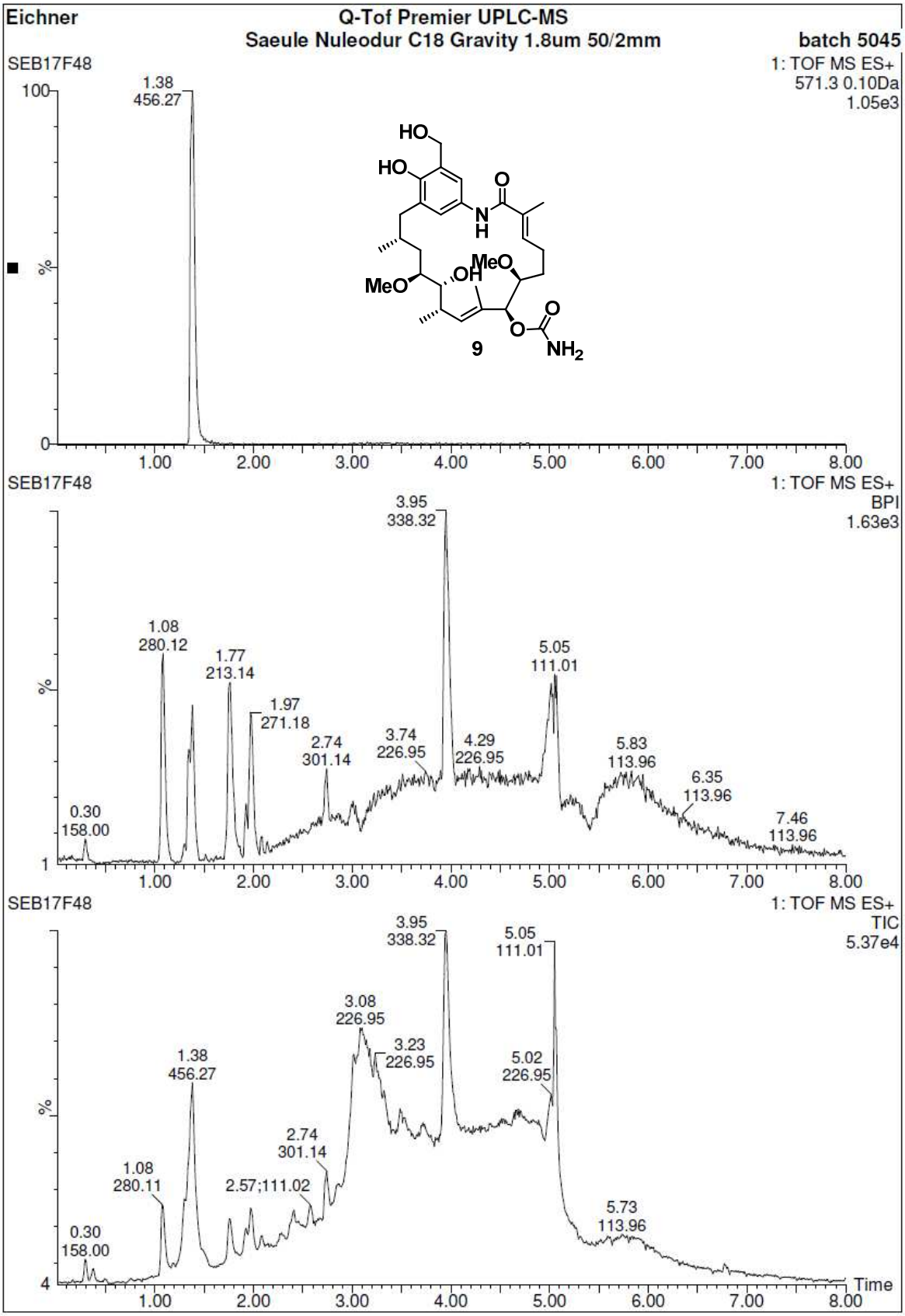
m/z


Eichner
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456.27
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HO
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N
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MeO
OH
MeO
O
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NH2
9
SEB17F48
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3.74
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226.95
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280.11
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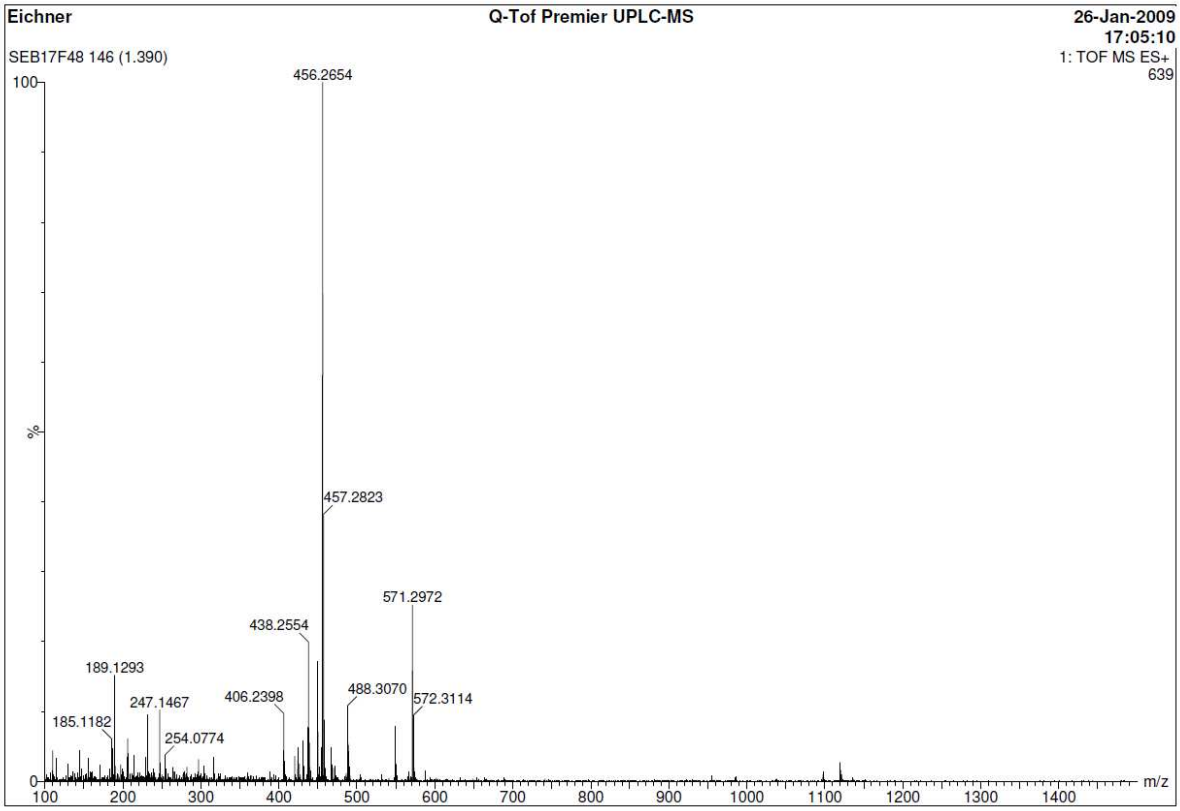
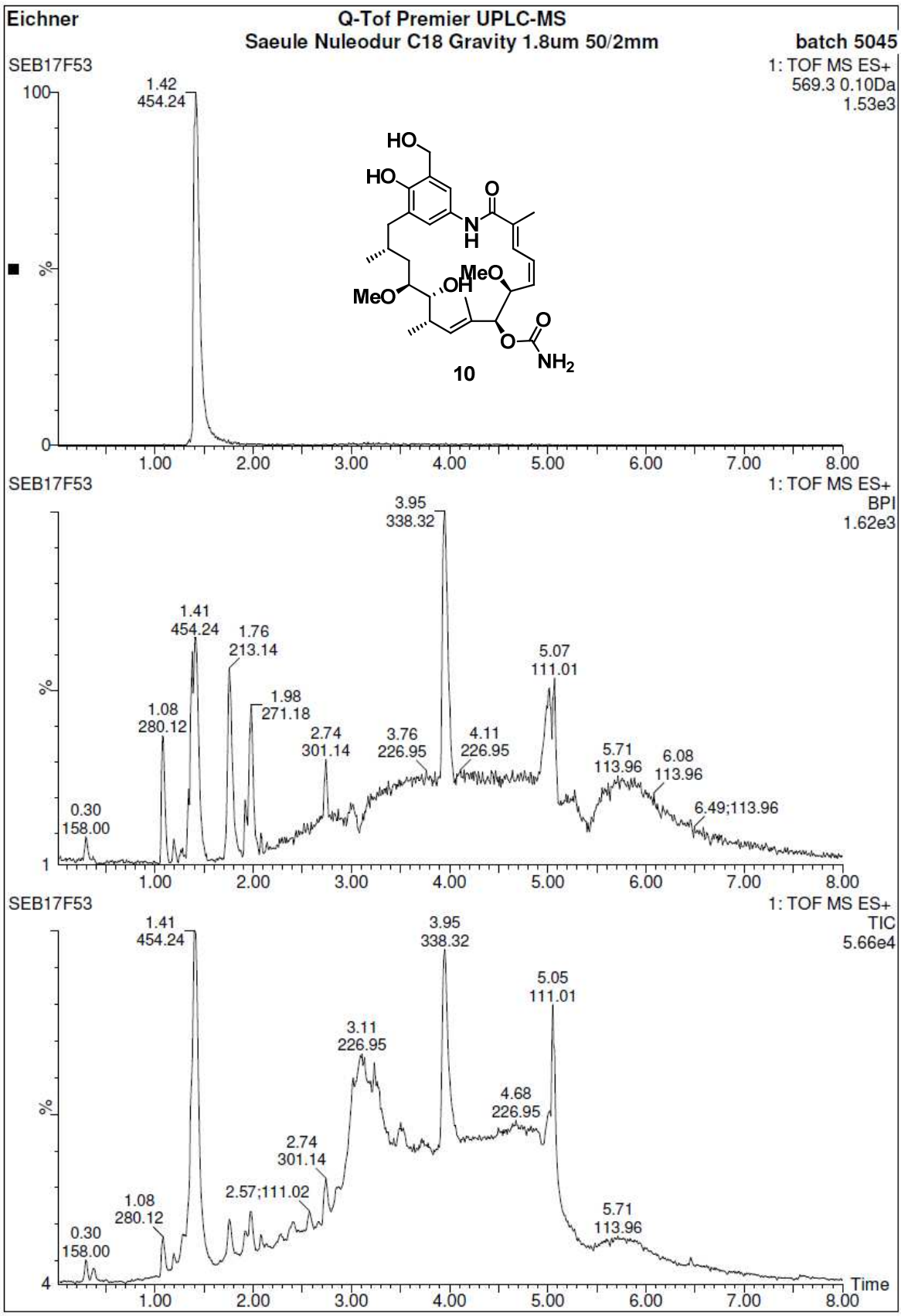
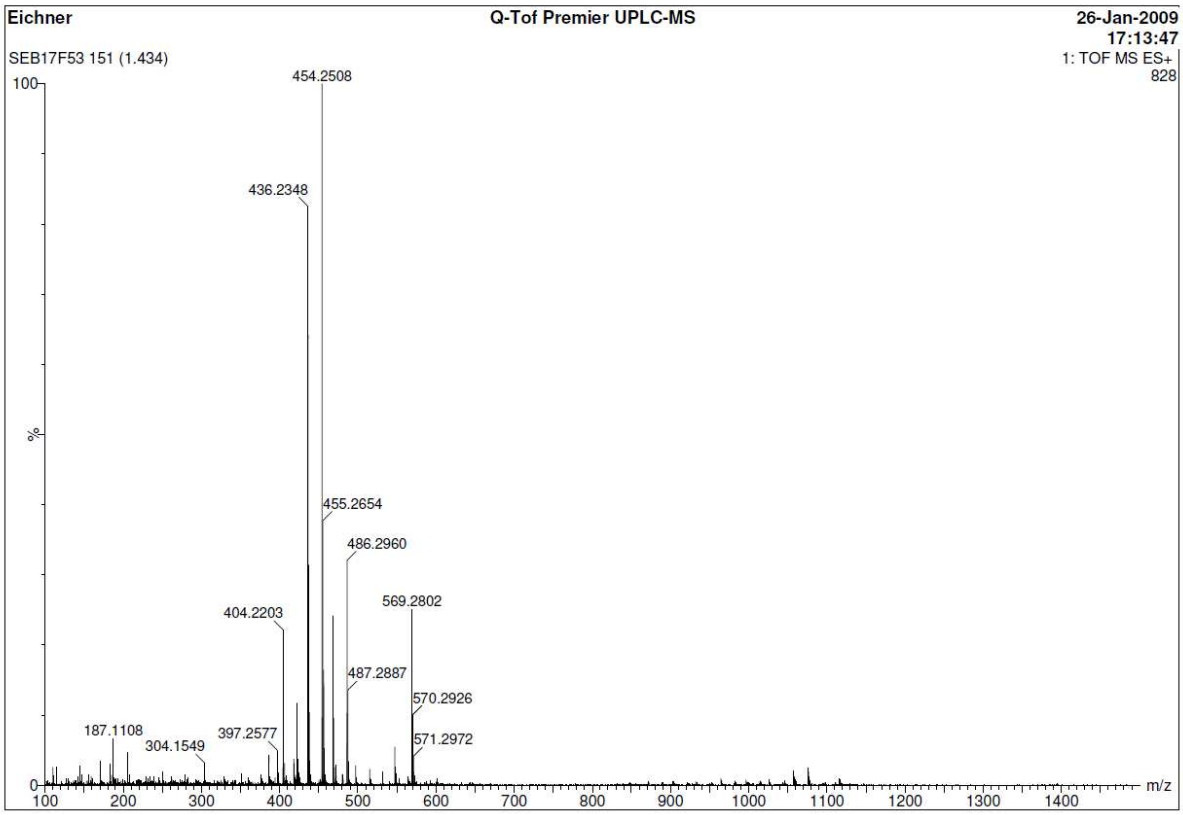
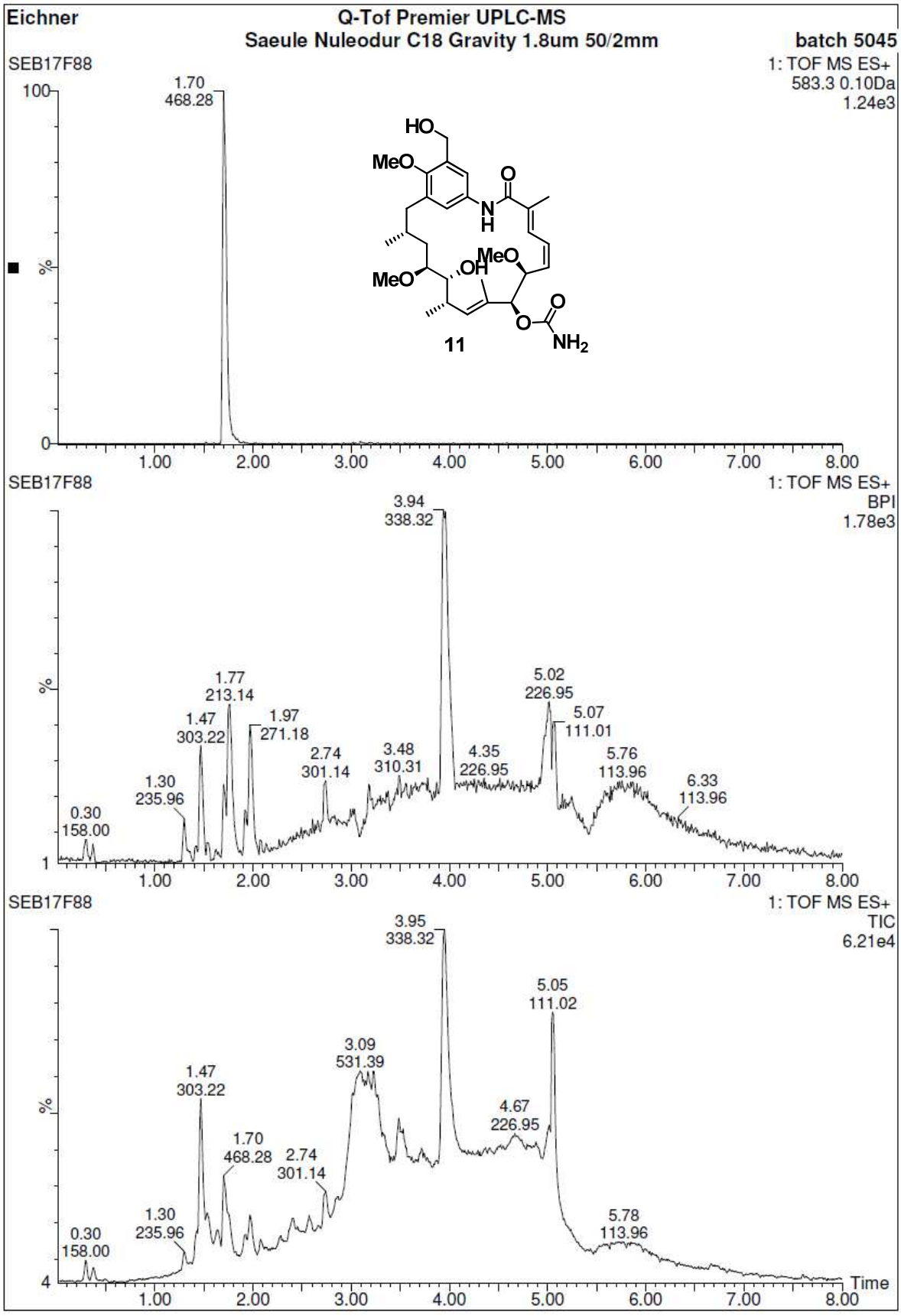
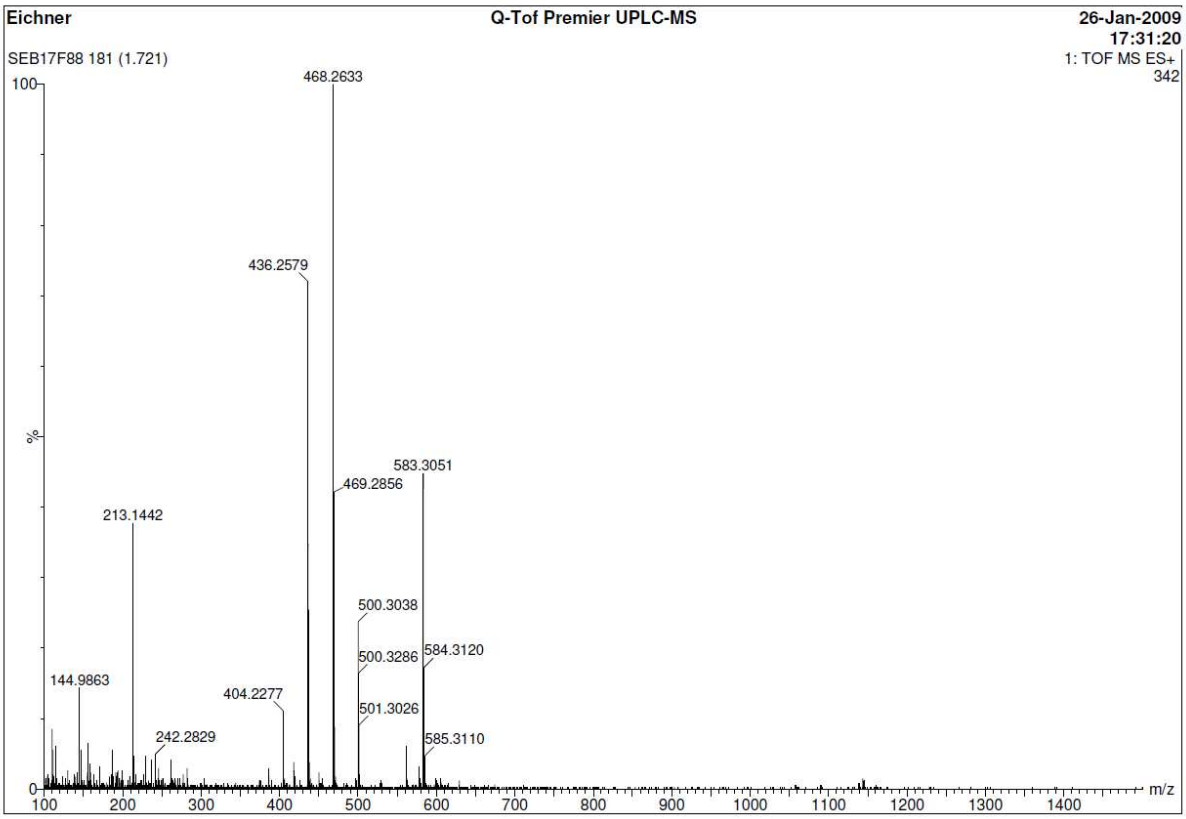
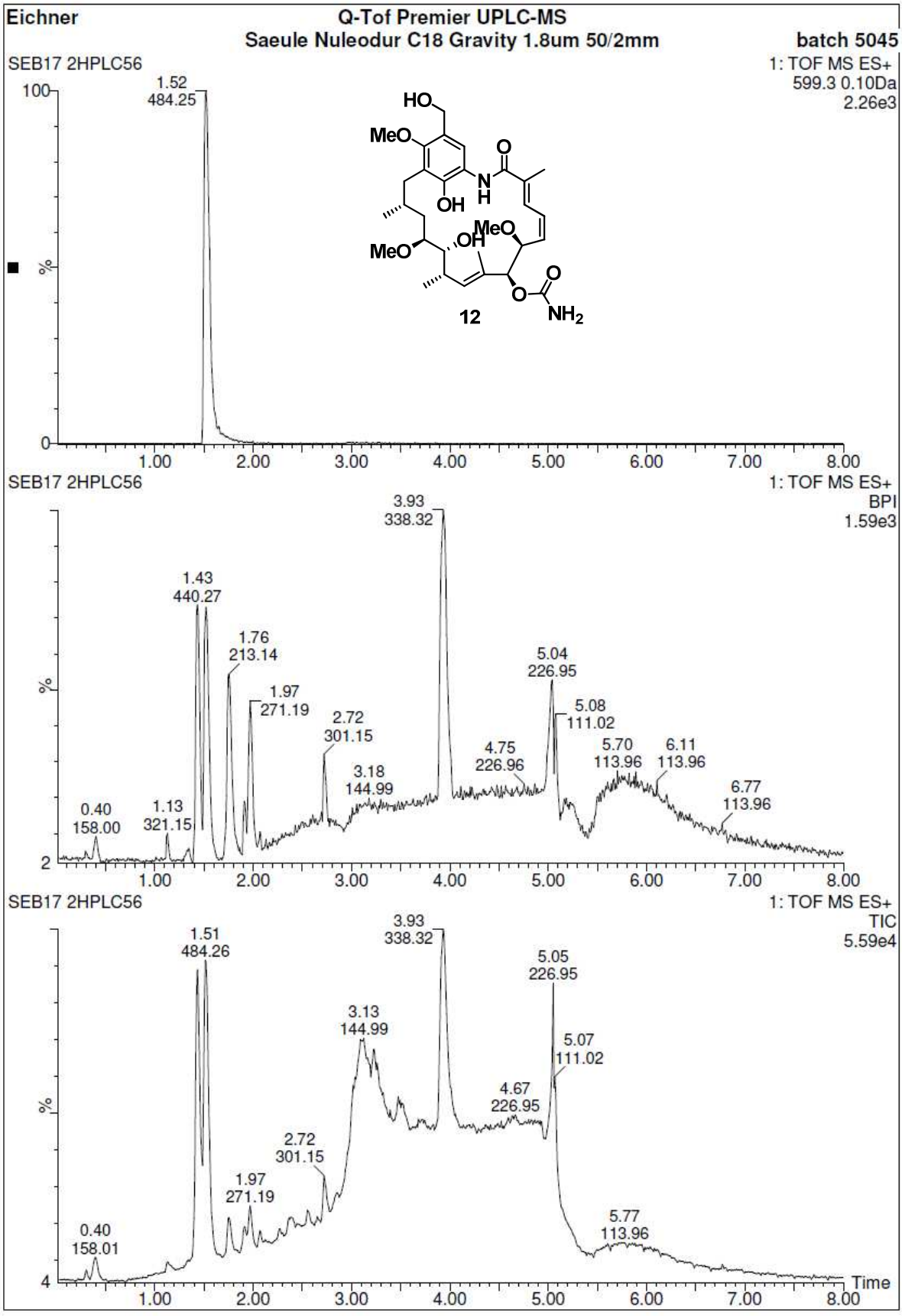

Eichner
Q-Tof Premier UPLC-MS
26-Jan-2009
17:05:10
SEB17F48 146 (1.390)
1: TOF MS ES+
639
456.2654
457.2823
571.2972
438.2554
189.1293
247.1467
406.2398
488.3070
572.3114
185.1182
254.0774
100
%
0
m/z
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400

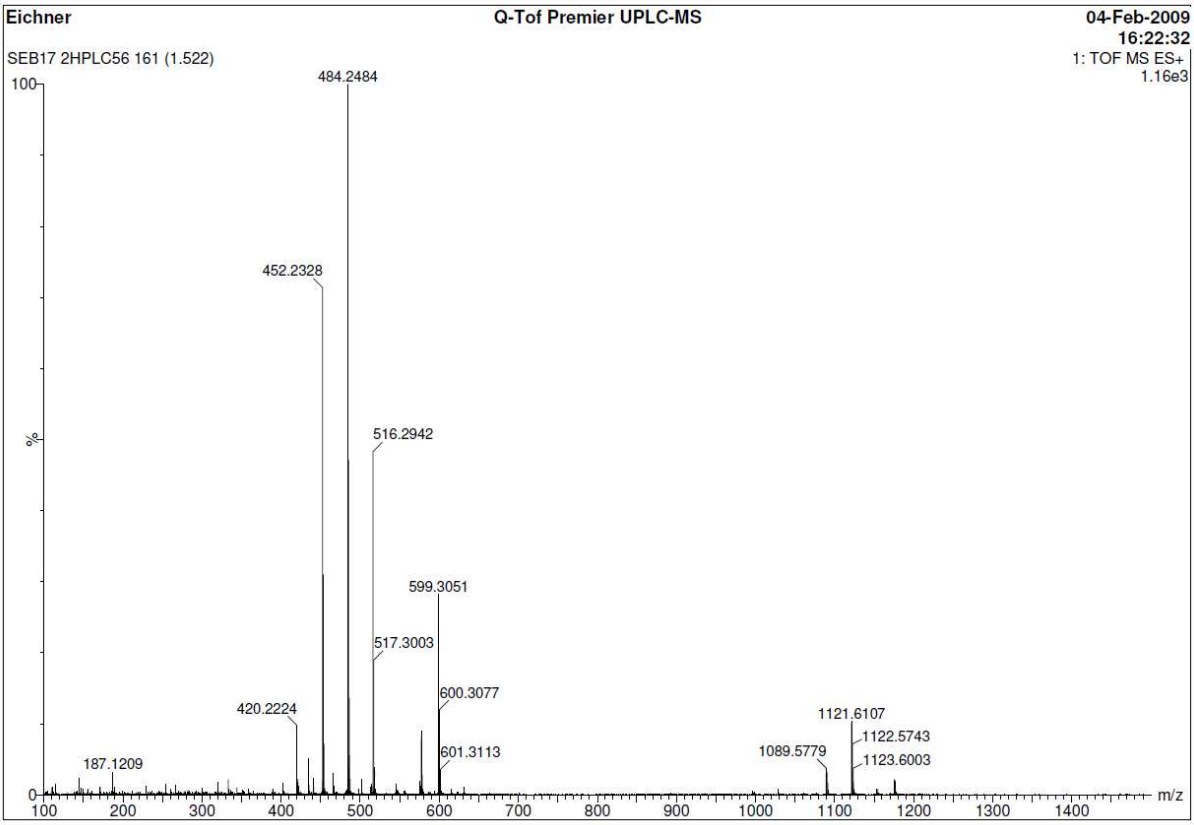

Eichner
Q-Tof Premier UPLC-MS
Saeule Nuleodur C18 Gravity 1.8um 50/2mm
batch 5045
SEB17F53
1: TOF MS ES+
569.3 0.10Da
1.53e3
1.42
454.24
HO
HO
O
N
H
MeO
OH
MeO
O
O
NH2
10
SEB17F53
1: TOF MS ES+
BPI
1.62e3
3.95
338.32
1.41
454.24
1.76
213.14
5.07
111.01
1.08
280.12
1.98
271.18
2.74
301.14
3.76
226.95
4.11
226.95
5.71
113.96
6.08
113.96
0.30
158.00
6.49;113.96
SEB17F53
1: TOF MS ES+
TIC
5.66e4
1.41
454.24
3.95
338.32
5.05
111.01
3.11
226.95
4.68
226.95
2.74
301.14
2.57;111.02
1.08
280.12
5.71
113.96
0.30
158.00
Time
%
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00

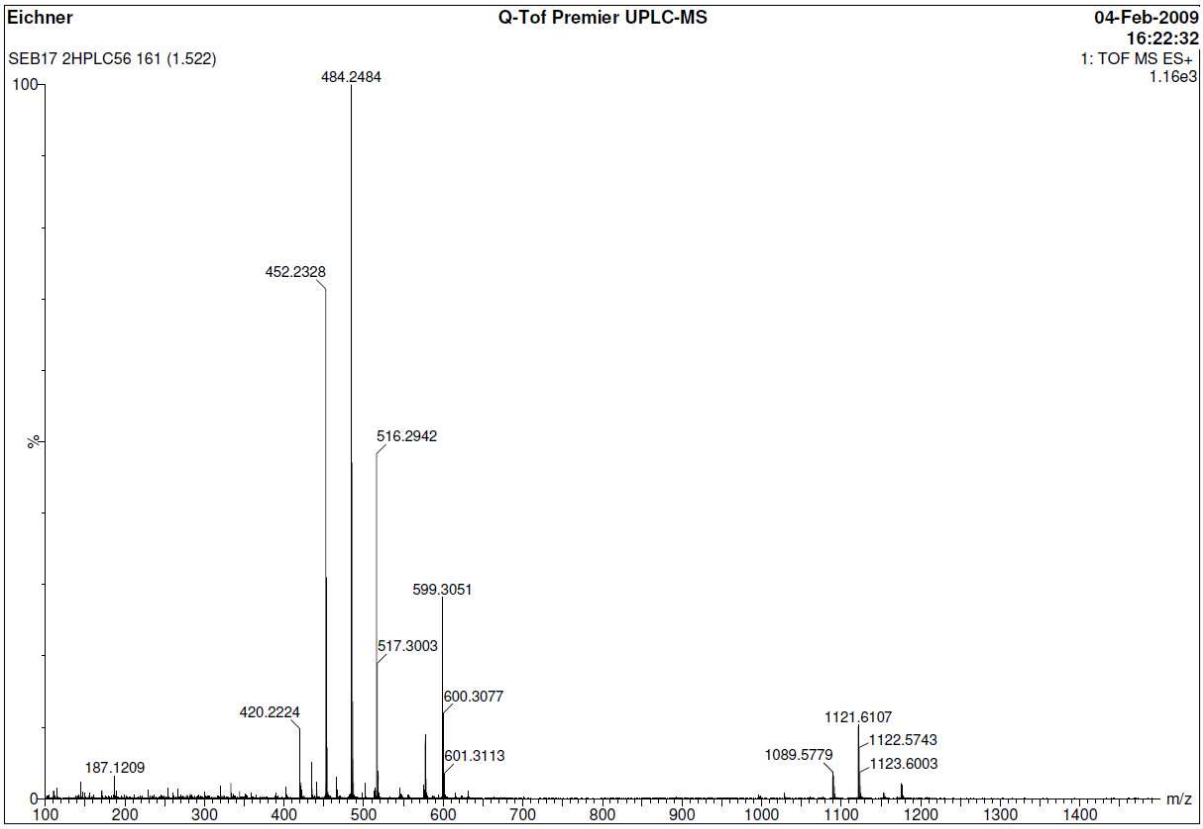

Eichner
Q-Tof Premier UPLC-MS
26-Jan-2009
17:13:47
SEB17F53 151 (1.434)
1: TOF MS ES+
828
100
%
0
454.2508
436.2348
455.2654
486.2960
569.2802
404.2203
487.2887
570.2926
571.2972
187.1108
304.1549
397.2577
m/z
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400


Eichner
Q-Tof Premier UPLC-MS
Saeule Nuleodur C18 Gravity 1.8um 50/2mm
batch 5045
SEB17F88
1: TOF MS ES+
583.3 0.10Da
1.24e3
1.70
468.28
HO
MeO
O
N
H
MeO
MeO
OH
O
O
NH2
11
SEB17F88
1: TOF MS ES+
BPI
1.78e3
3.94
338.32
1.77
213.14
1.47
303.22
1.97
271.18
2.74
301.14
3.48
310.31
4.35
226.95
5.02
226.95
5.07
111.01
5.76
113.96
6.33
113.96
1.30
235.96
0.30
158.00
SEB17F88
1: TOF MS ES+
TIC
6.21e4
3.95
338.32
5.05
111.02
3.09
531.39
1.47
303.22
4.67
226.95
1.70
468.28
2.74
301.14
5.78
113.96
1.30
235.96
0.30
158.00
%
Time
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00


Eichner
Q-Tof Premier UPLC-MS
26-Jan-2009
17:31:20
SEB17F88 181 (1.721)
1: TOF MS ES+
342
100
%
0
468.2633
436.2579
583.3051
469.2856
213.1442
500.3038
500.3286
584.3120
144.9863
404.2277
501.3026
242.2829
585.3110
m/z
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400

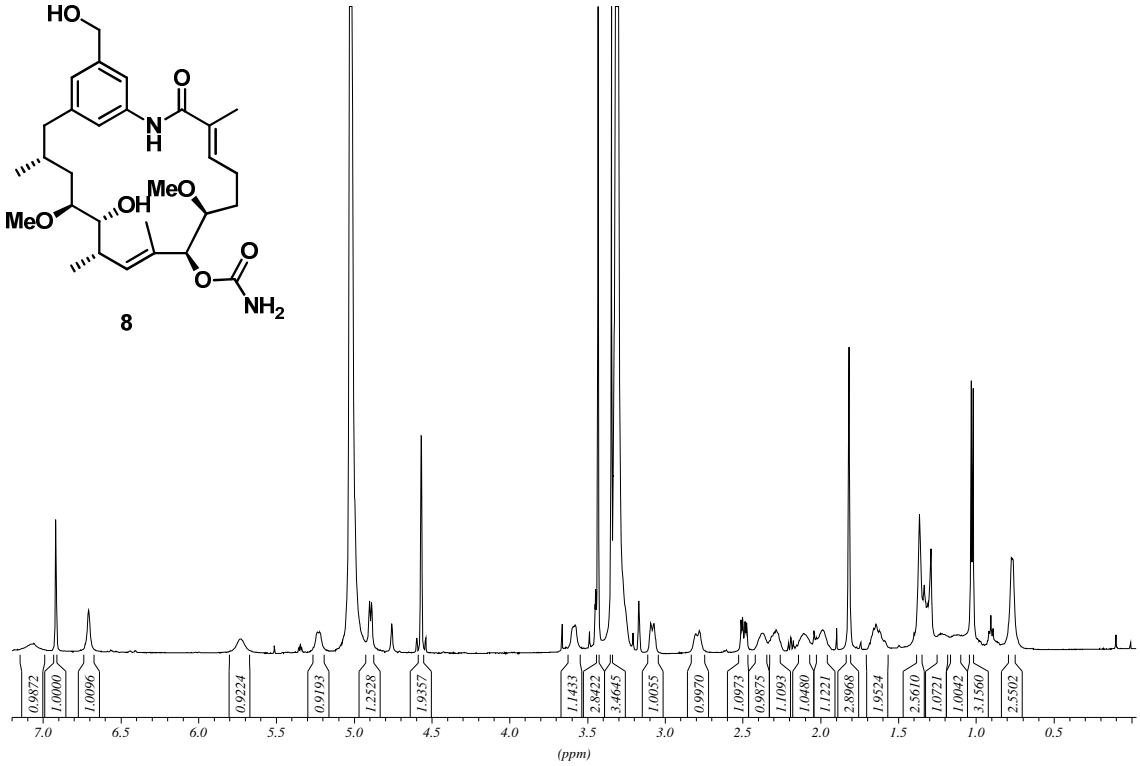

Eichner
Q-Tof Premier UPLC-MS
Saeule Nuleodur C18 Gravity 1.8um 50/2mm
batch 5045
SEB17 2HPLC56
1: TOF MS ES+
599.3 0.10Da
2.26e3
1.52
484.25
HO
MeO
O
N
H
OH
MeO
OH
MeO
O
O
NH2
12
SEB17 2HPLC56
1: TOF MS ES+
BPI
1.59e3
3.93
338.32
1.43
440.27
1.76
213.14
1.97
271.19
2.72
301.15
5.04
226.95
5.08
111.02
4.75
226.96
5.70
113.96
6.11
113.96
3.18
144.99
6.77
113.96
0.40
158.00
1.13
321.15
SEB17 2HPLC56
1: TOF MS ES+
TIC
5.59e4
1.51
484.26
3.93
338.32
5.05
226.95
3.13
144.99
5.07
111.02
4.67
226.95
2.72
301.15
1.97
271.19
5.77
113.96
0.40
158.01
Time

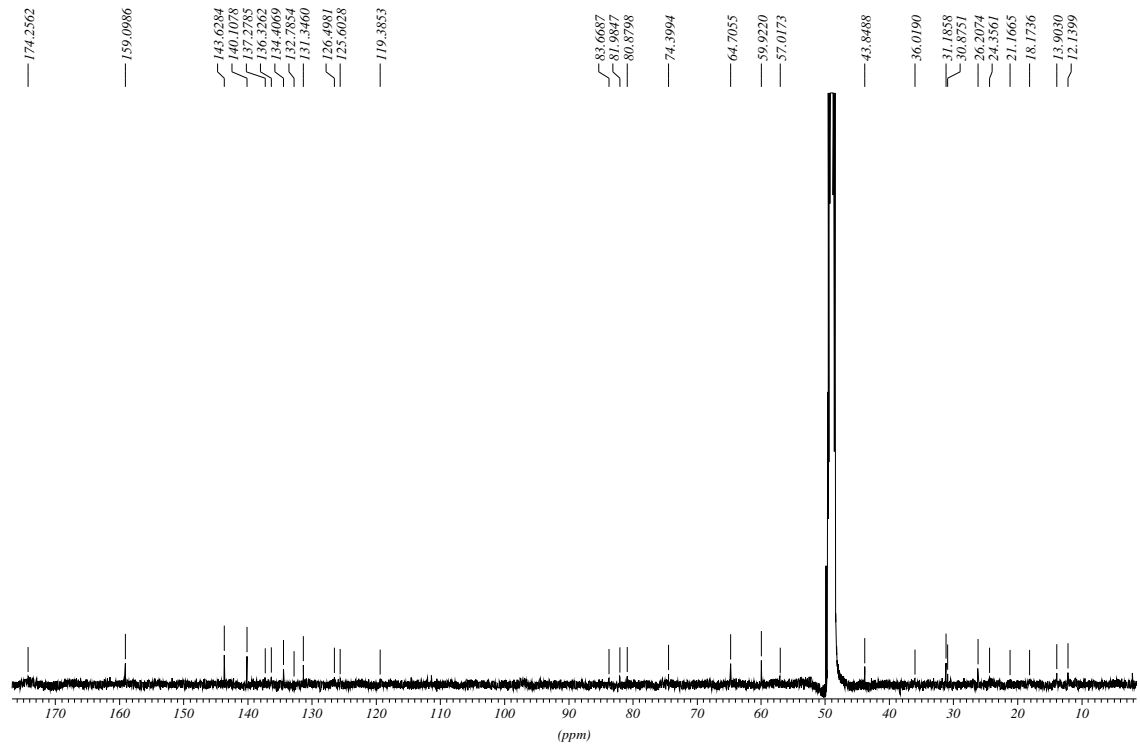

Eichner
Q-Tof Premier UPLC-MS
04-Feb-2009
16:22:32
SEB17 2HPLC56 161 (1.522)
1: TOF MS ES+
1.16e3
484.2484
452.2328
516.2942
599.3051
517.3003
600.3077
420.2224
601.3113
187.1209
1121.6107
1122.5743
1089.5779
1123.6003
%
m/z


Eichner
Q-Tof Premier UPLC-MS
04-Feb-2009
16:22:32
SEB17 2HPLC56 161 (1.522)
1: TOF MS ES+
1.16e3
484.2484
452.2328
516.2942
599.3051
517.3003
600.3077
420.2224
601.3113
187.1209
1121.6107
1122.5743
1089.5779
1123.6003
%
m/z

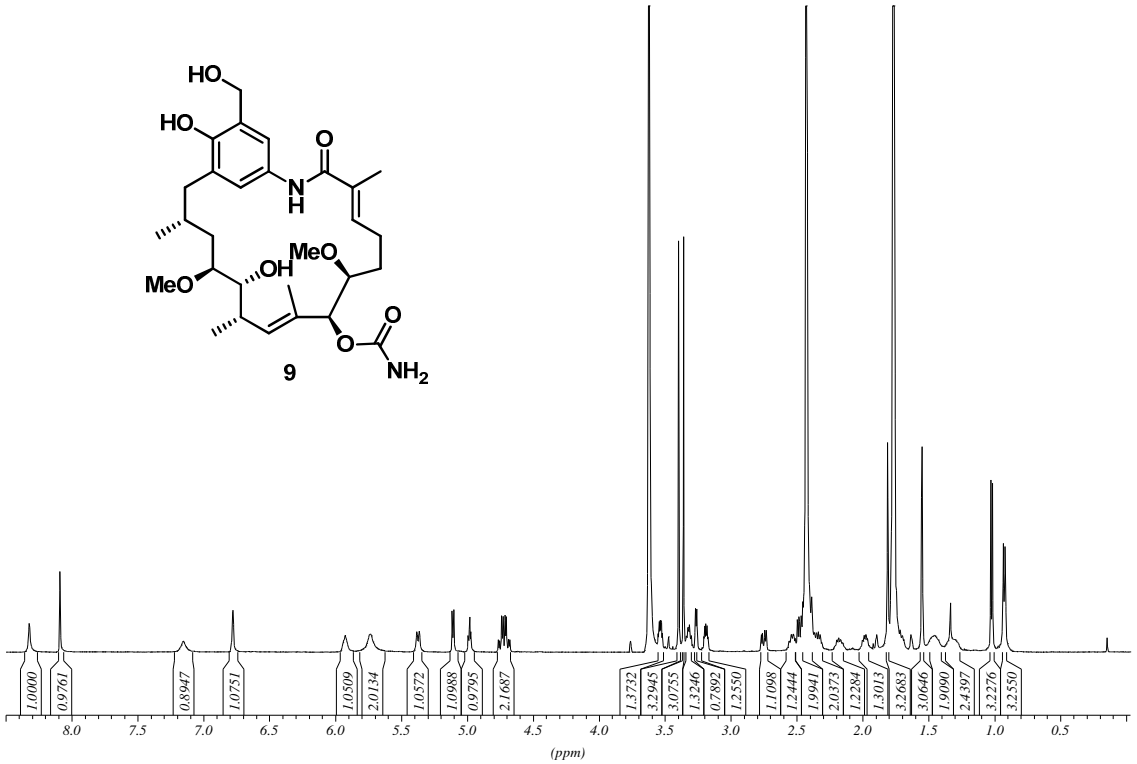
8. ^{1}H-, ^{13}C-NMR and MS spectra of geldanamycin derivatives

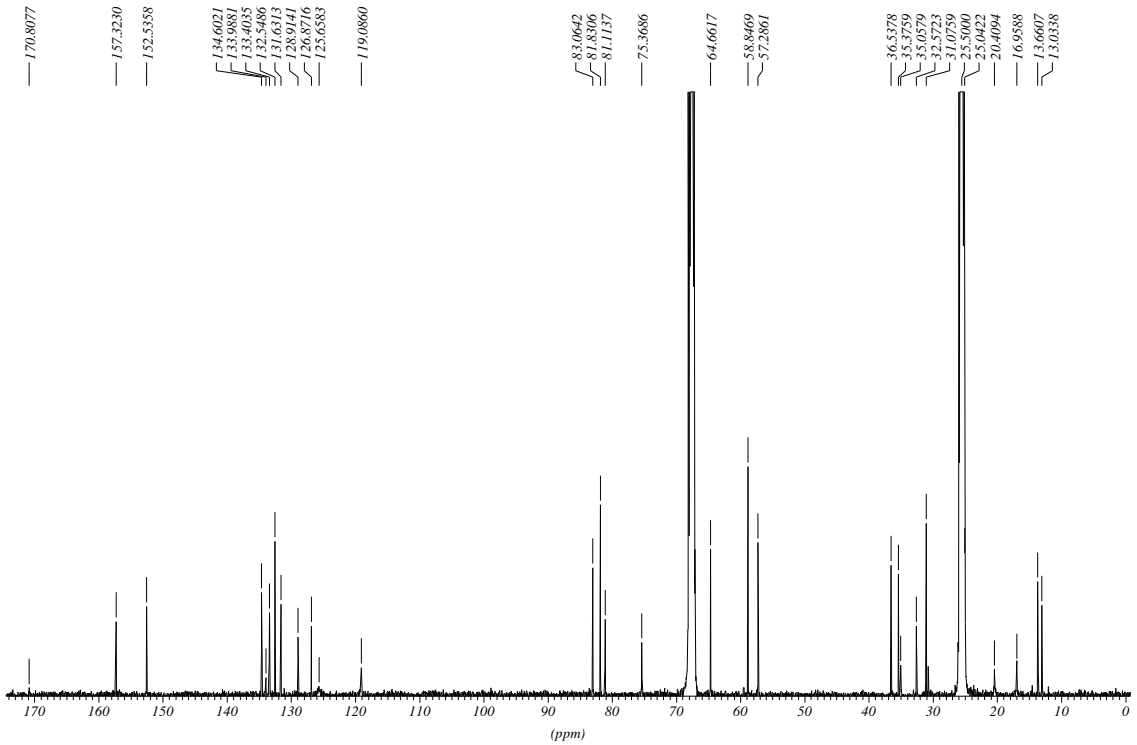
17-Demethoxy-18-hydroxymethyl-18-deoxyreblastatin (8).



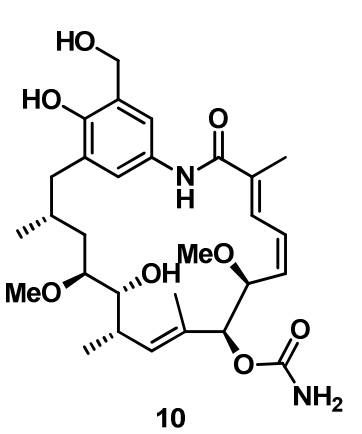


17-O-Demethyl-18-hydroxymethyl-18-deoxyreblastatin (9)

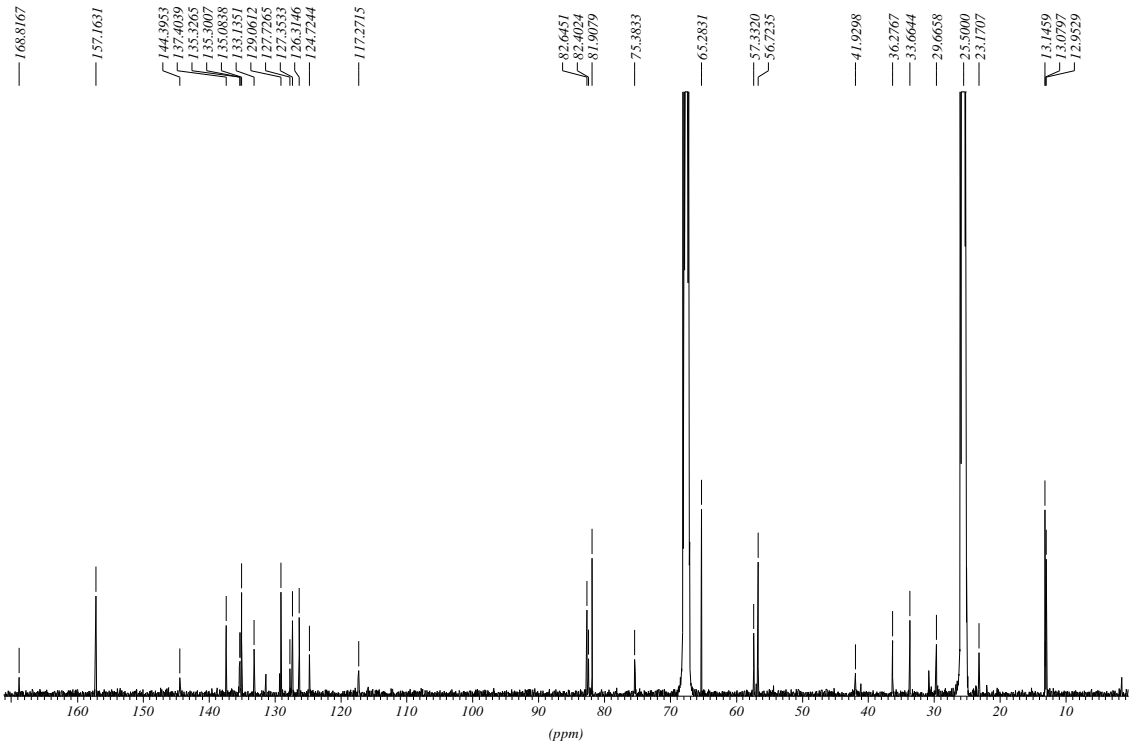




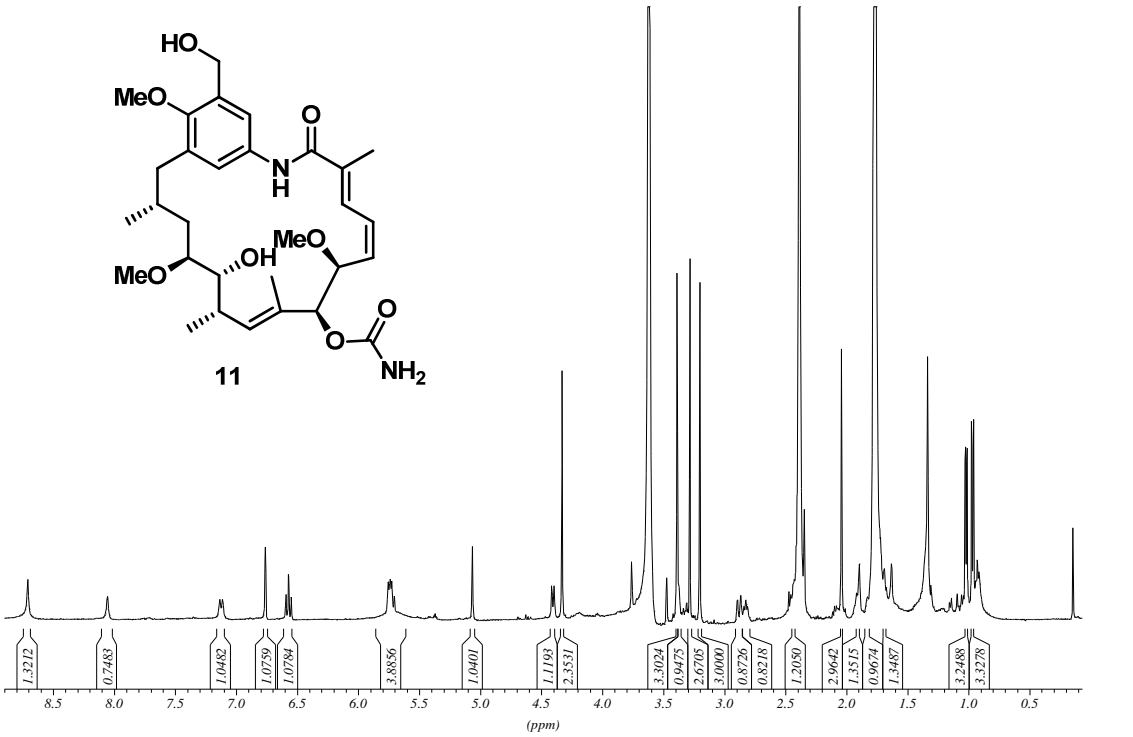
17-O-Demethyl-18-hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (10)

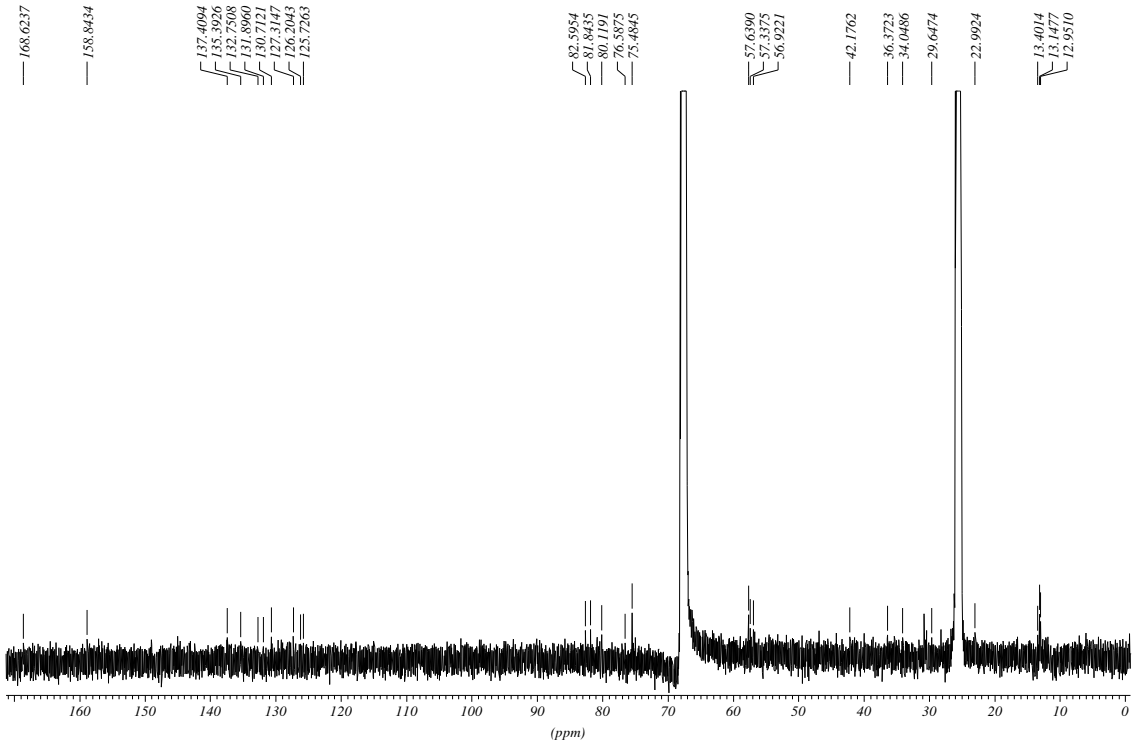


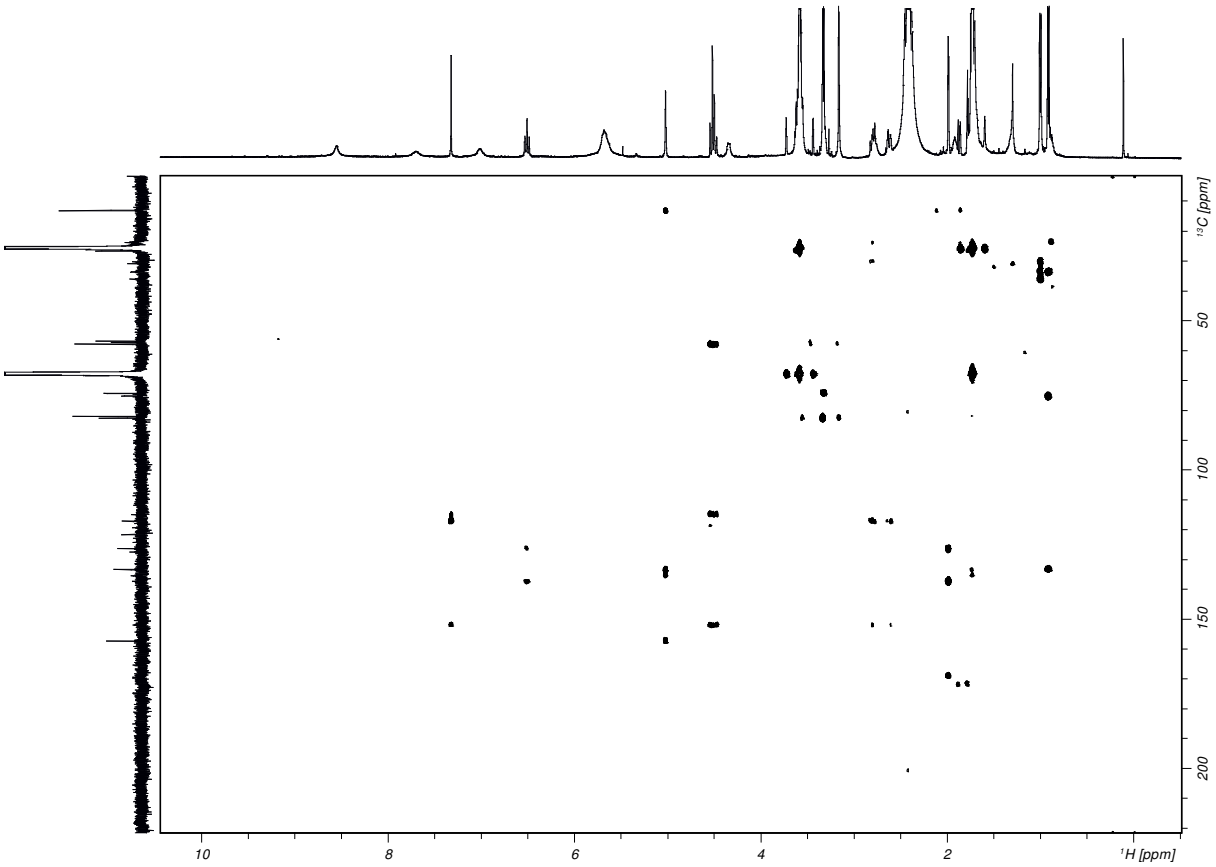
10

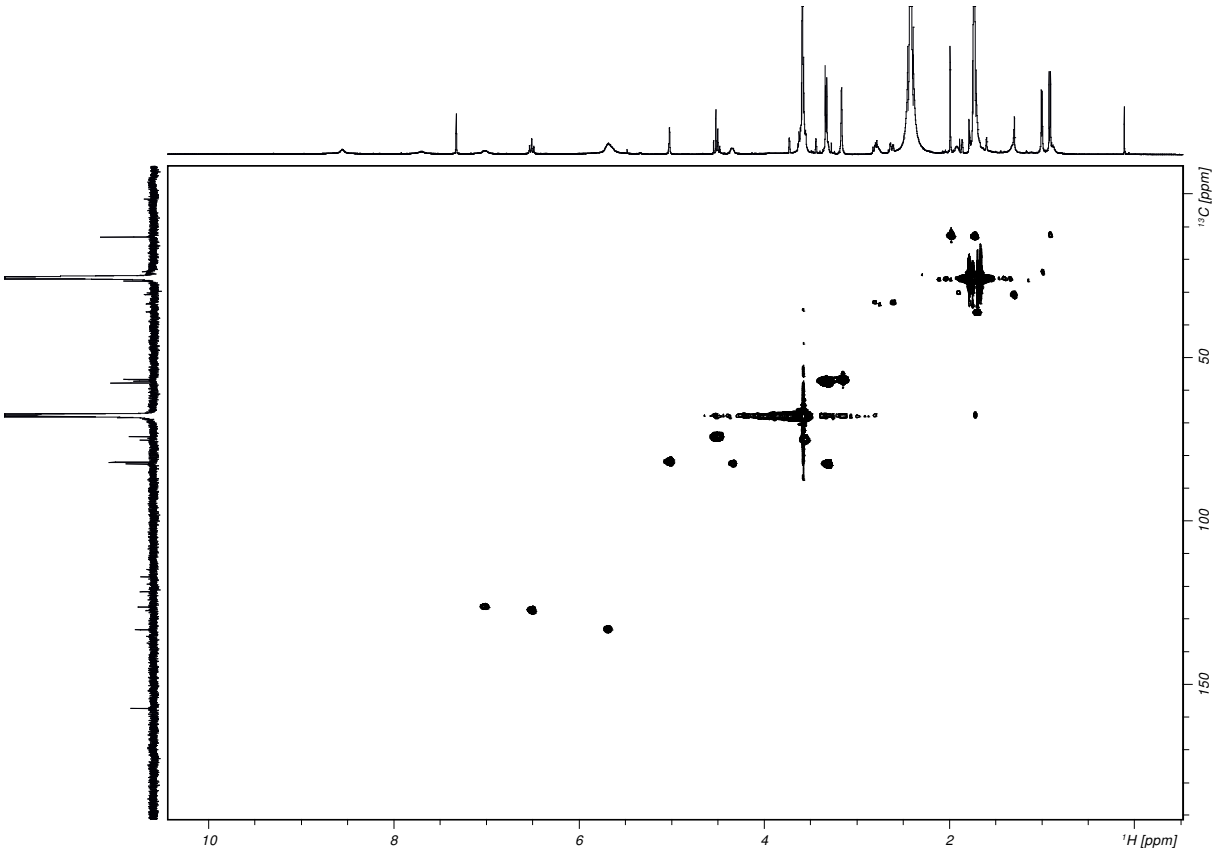


18-Hydroxymethyl-4,5-dehydro-18-deoxyreblastatin (11)

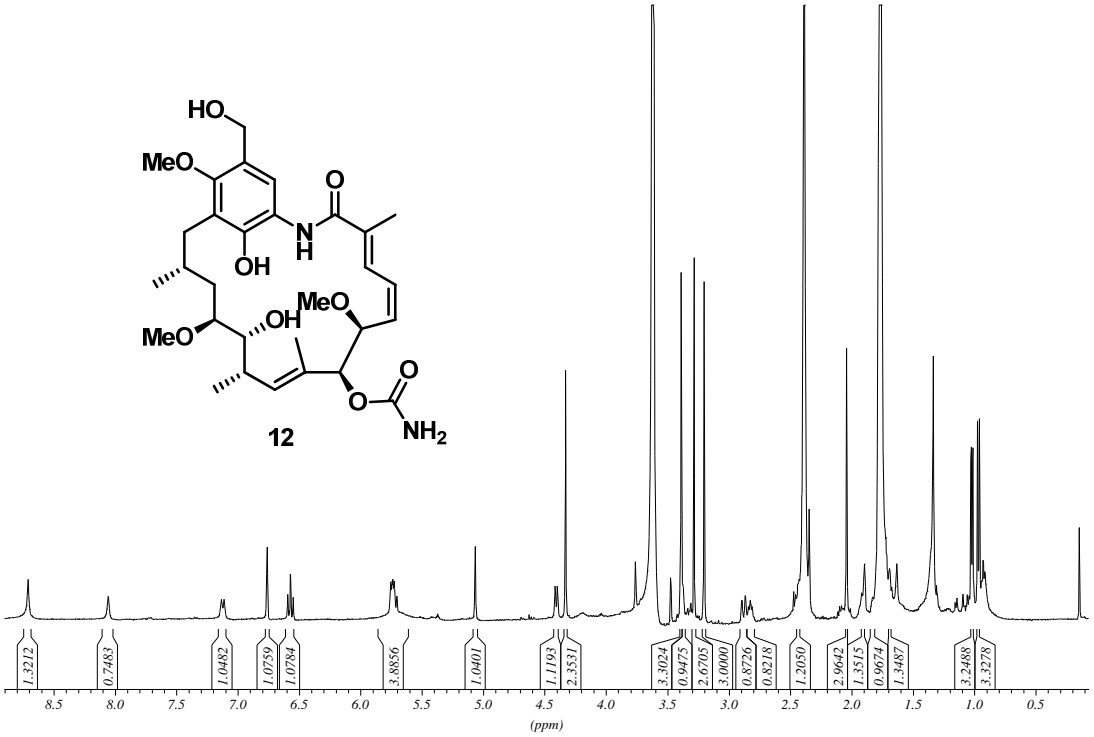


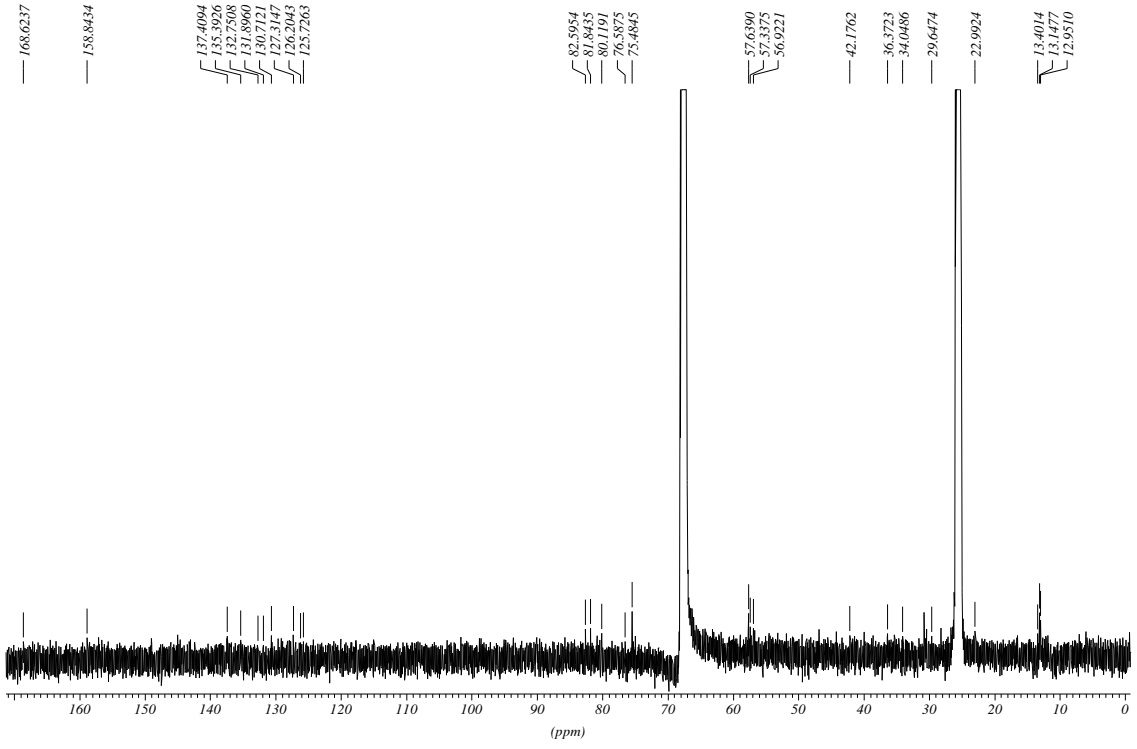


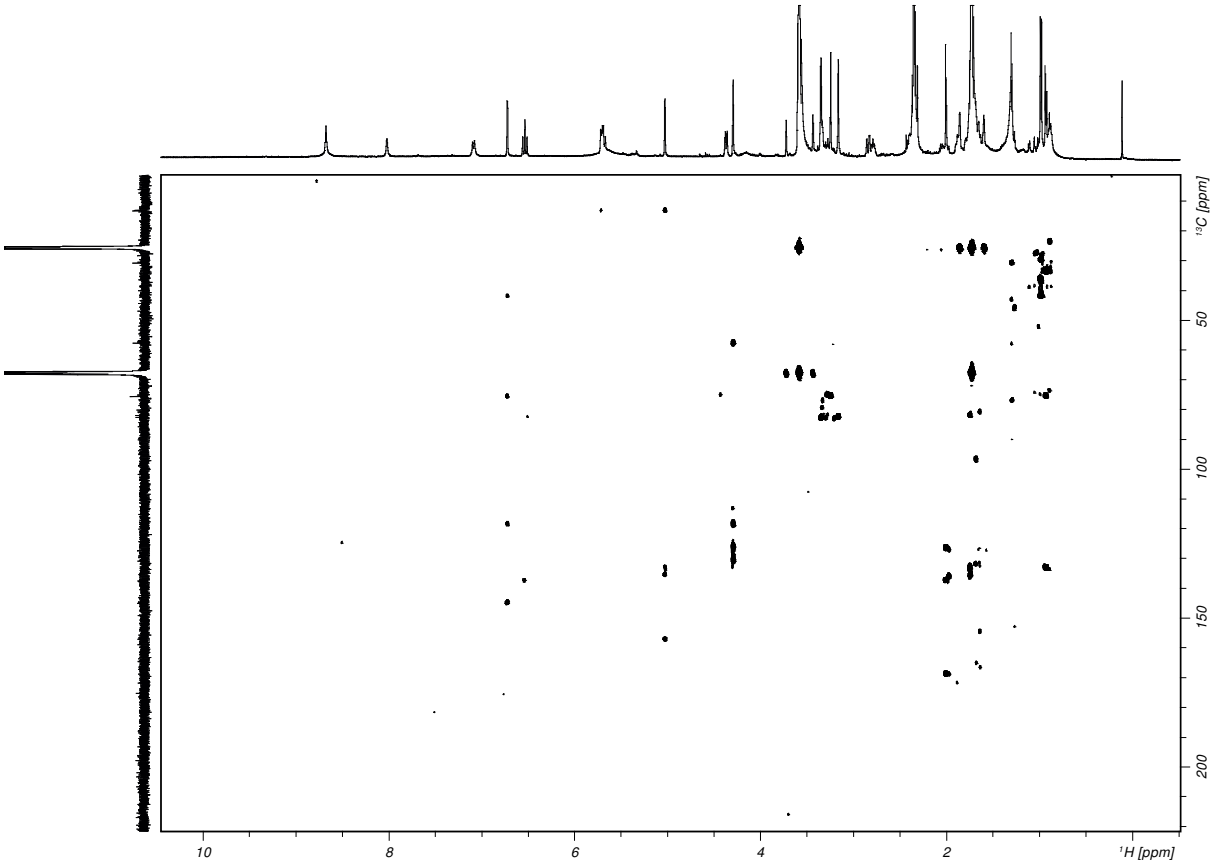

10
8
6
4
2
¹H [ppm]
50
100
150
200
¹³C [ppm]

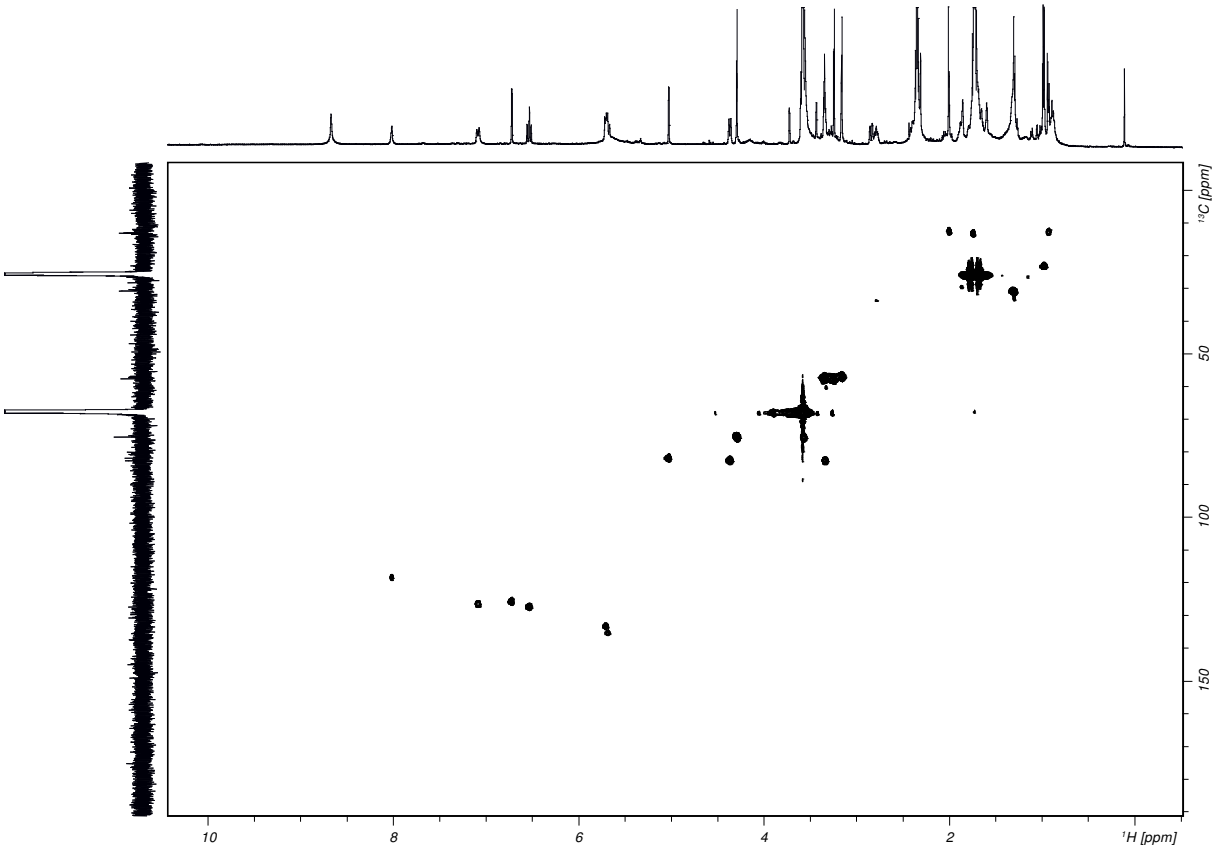

10
8
6
4
2
¹H [ppm]
50
100
150
¹³C [ppm]

18-Hydroxymethyl-21-hydroxy-4,5-dehydro-18-deoxyreblastatin (12)

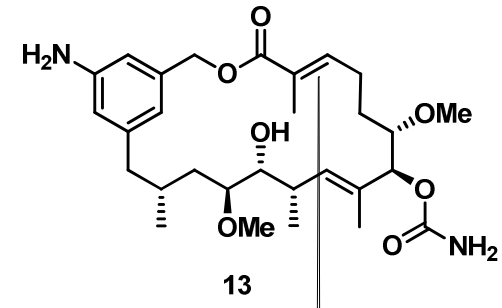




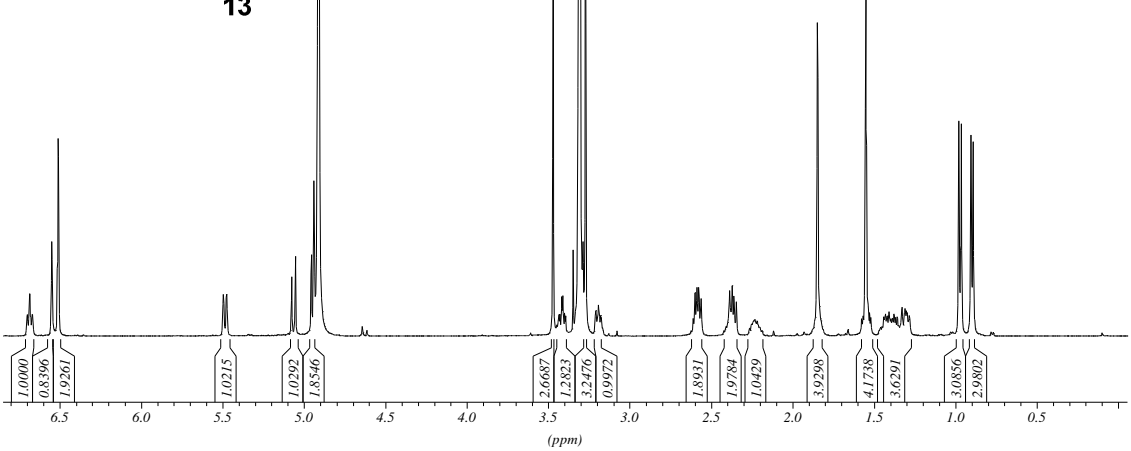

10
8
6
4
2
^{1}H [ppm]
50
100
150
200
^{13}C [ppm]

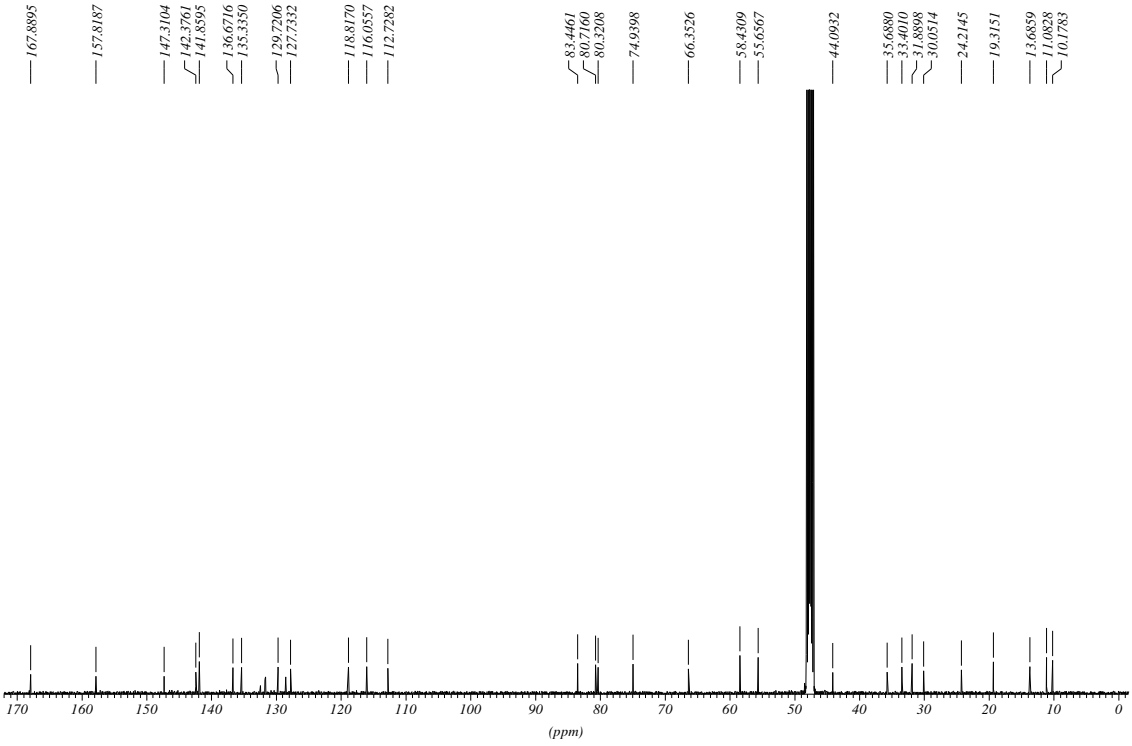

10
8
6
4
2
^{1}H [ppm]
50
100
150
^{13}C [ppm]

Macrolactone 13

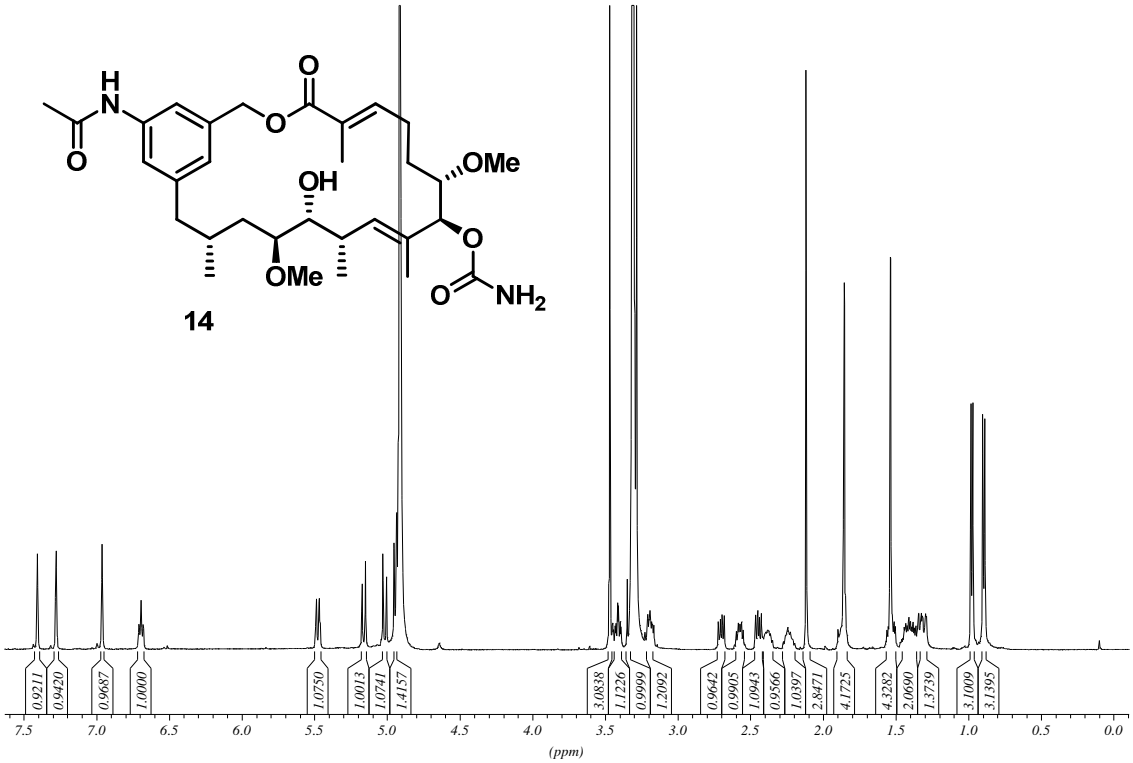


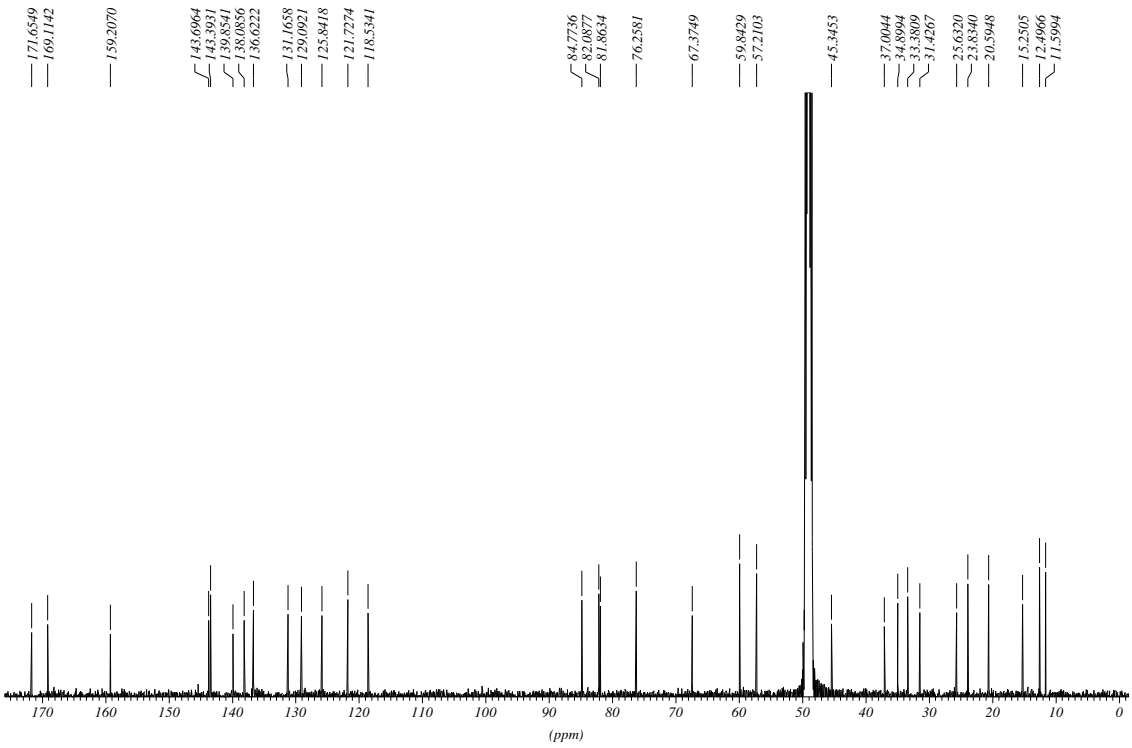
13



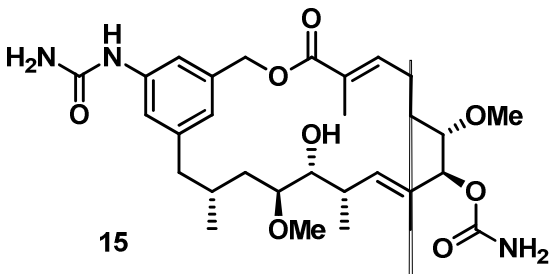


Macrolactone 14

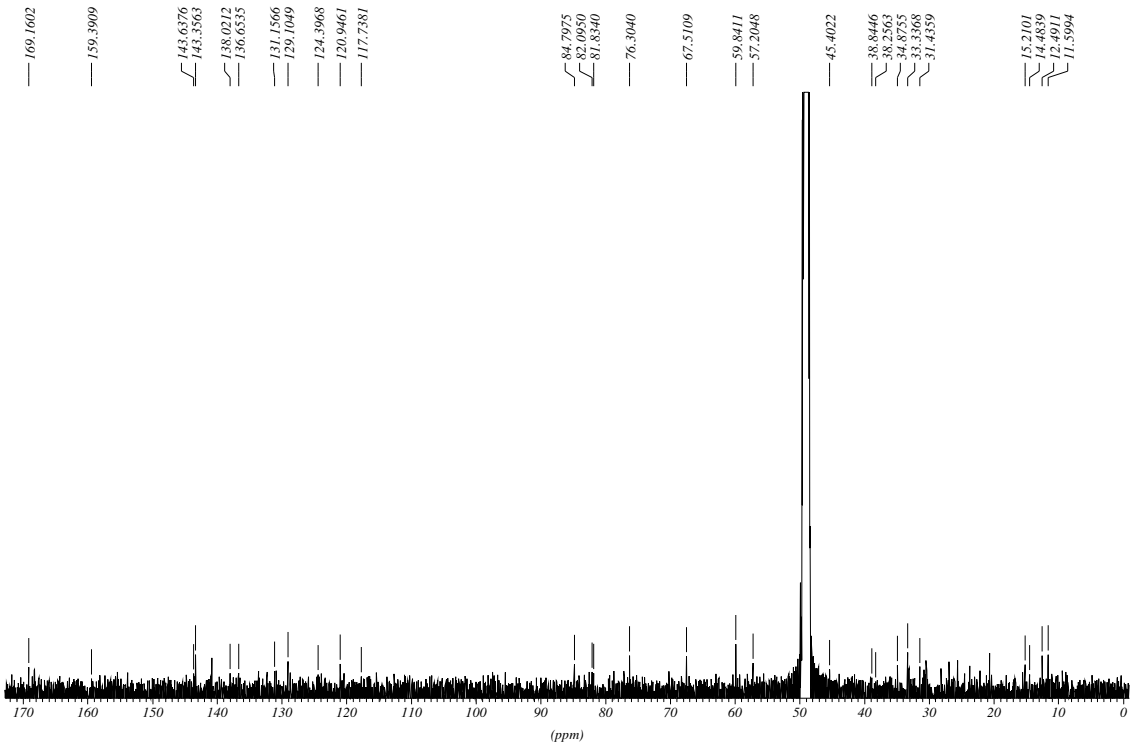


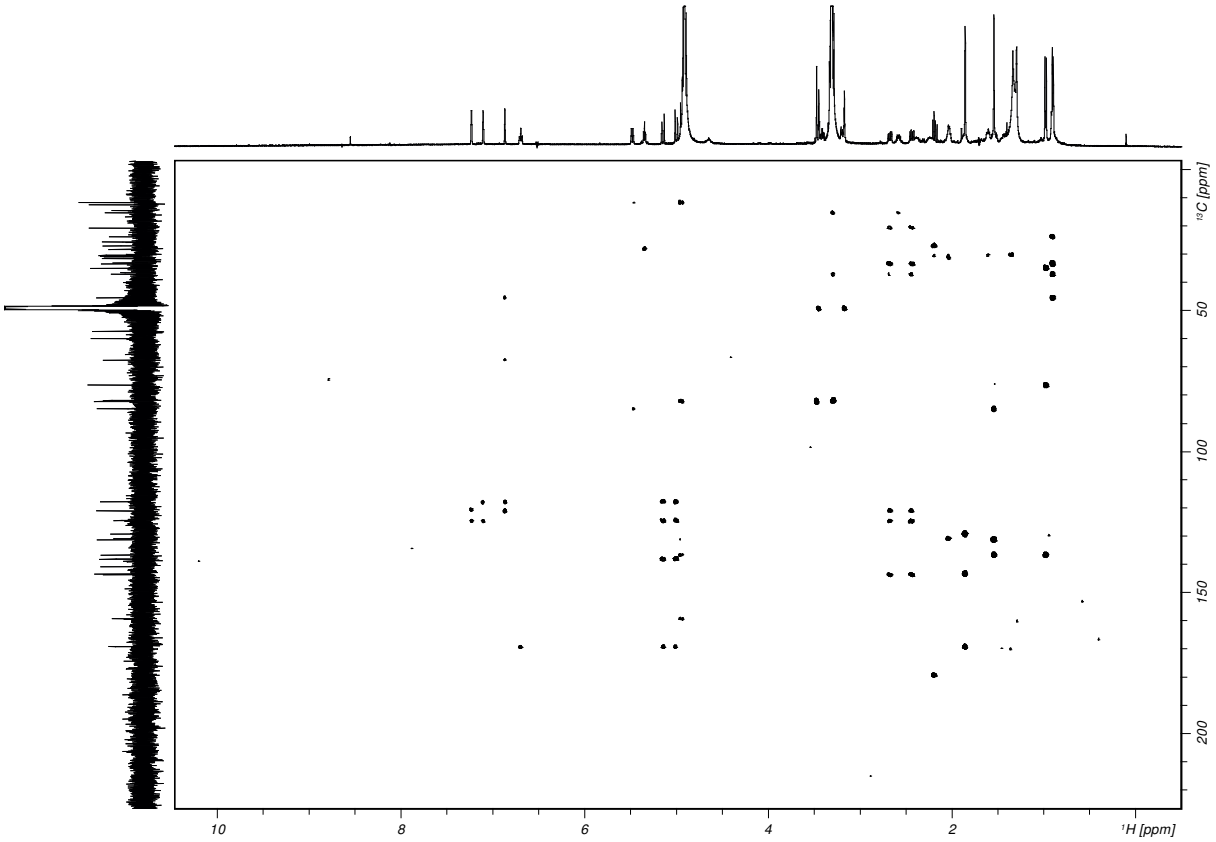


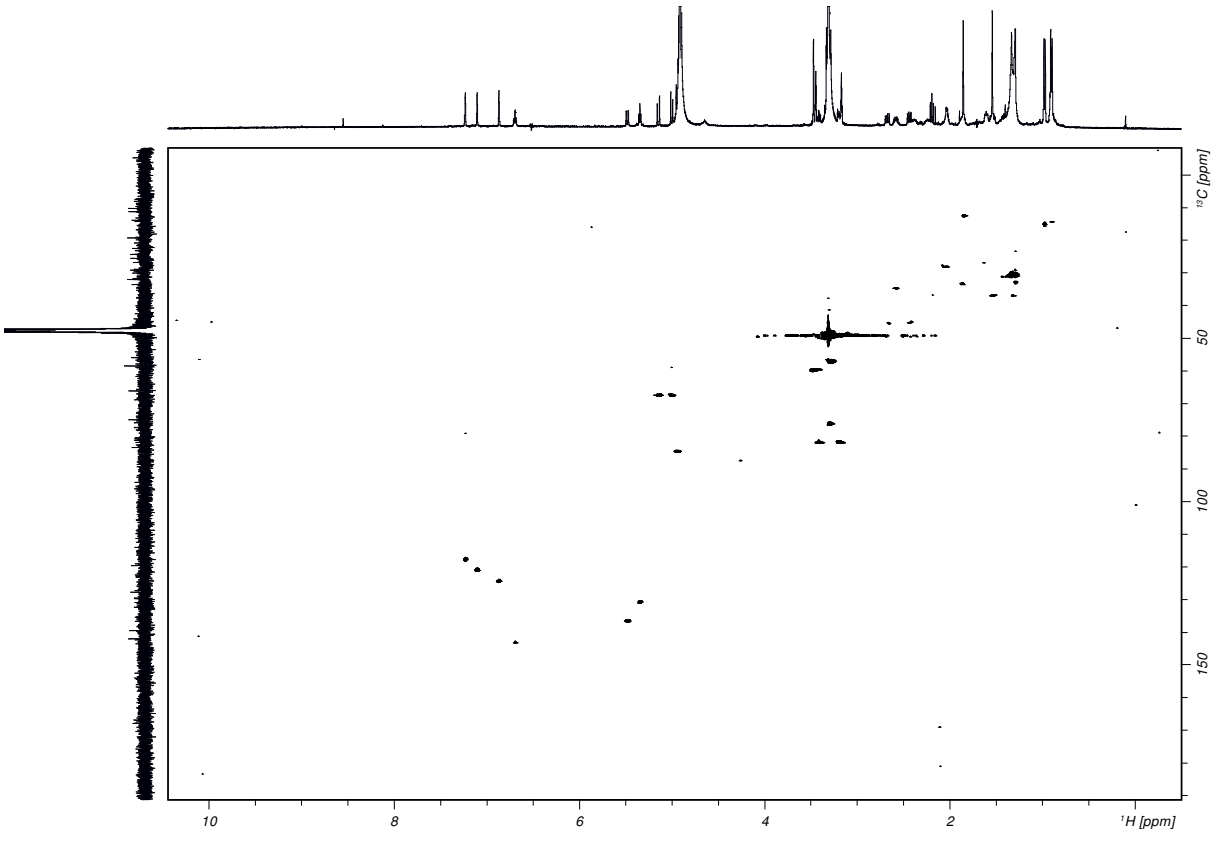
Macrolactone 15



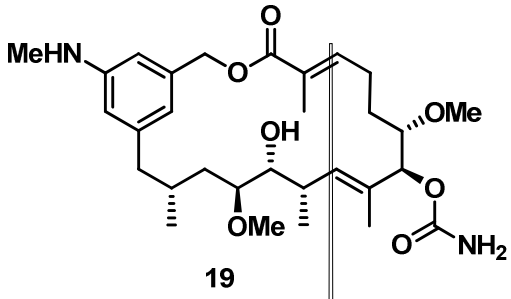
15



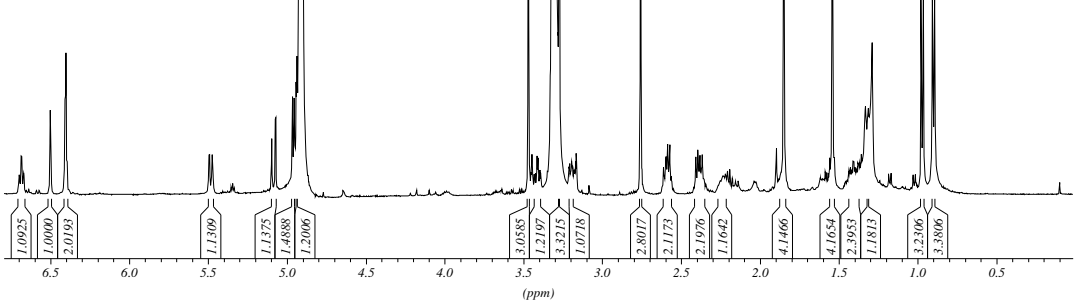

10
8
6
4
2
¹H [ppm]
50
100
150
200
¹³C [ppm]

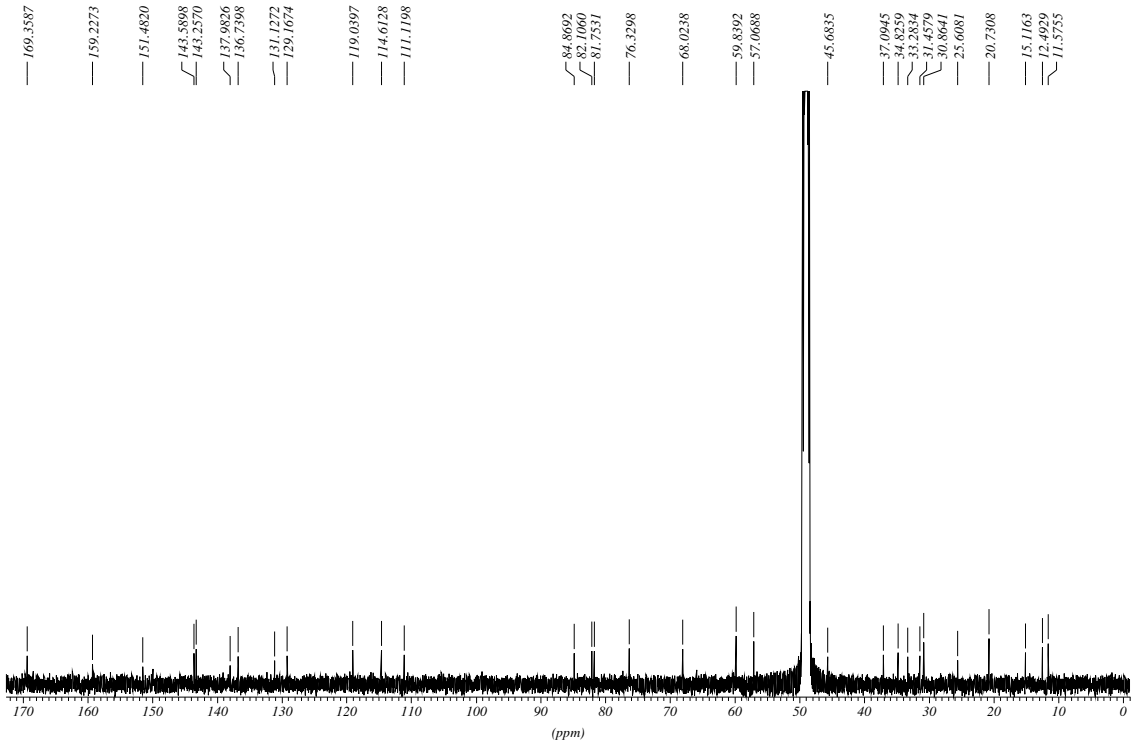

10
8
6
4
2
¹H [ppm]
50
100
150
¹³C [ppm]

Macrolactone 19

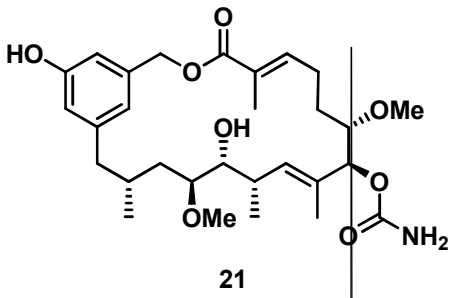


19

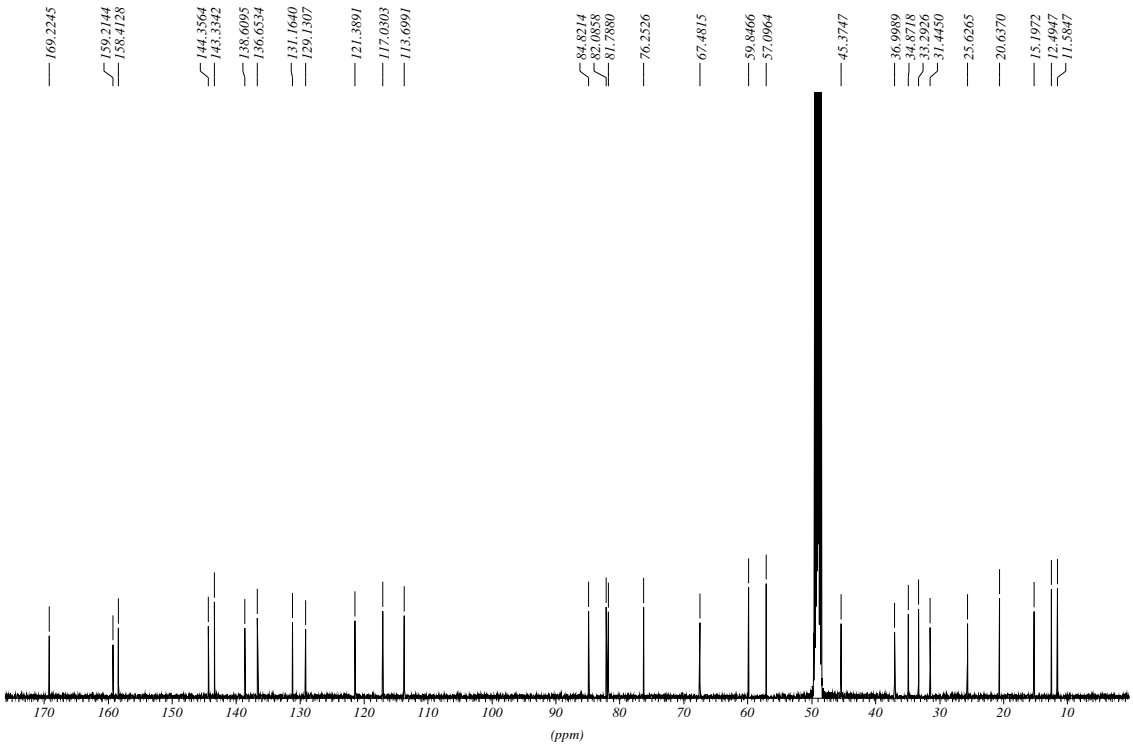




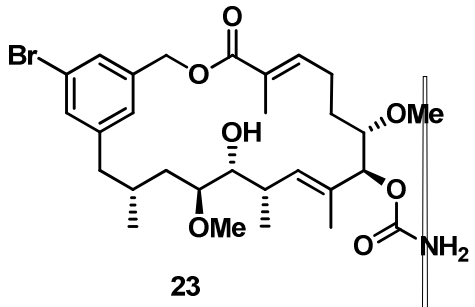
Macrolactone 21



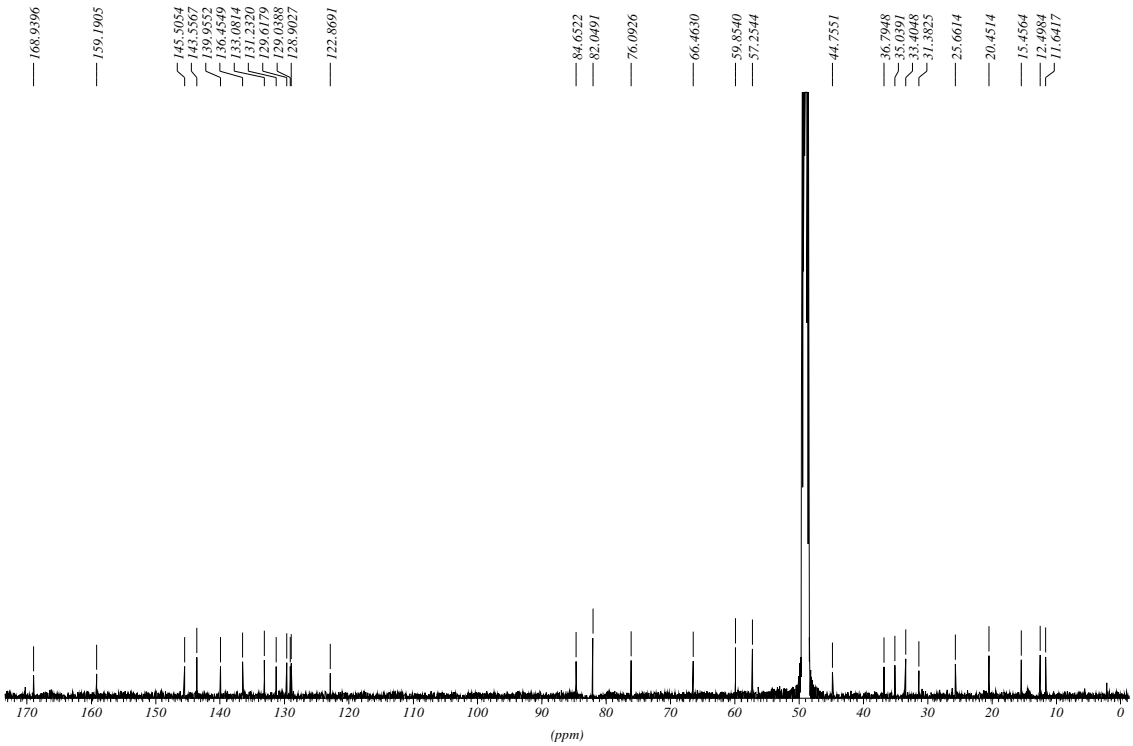
21



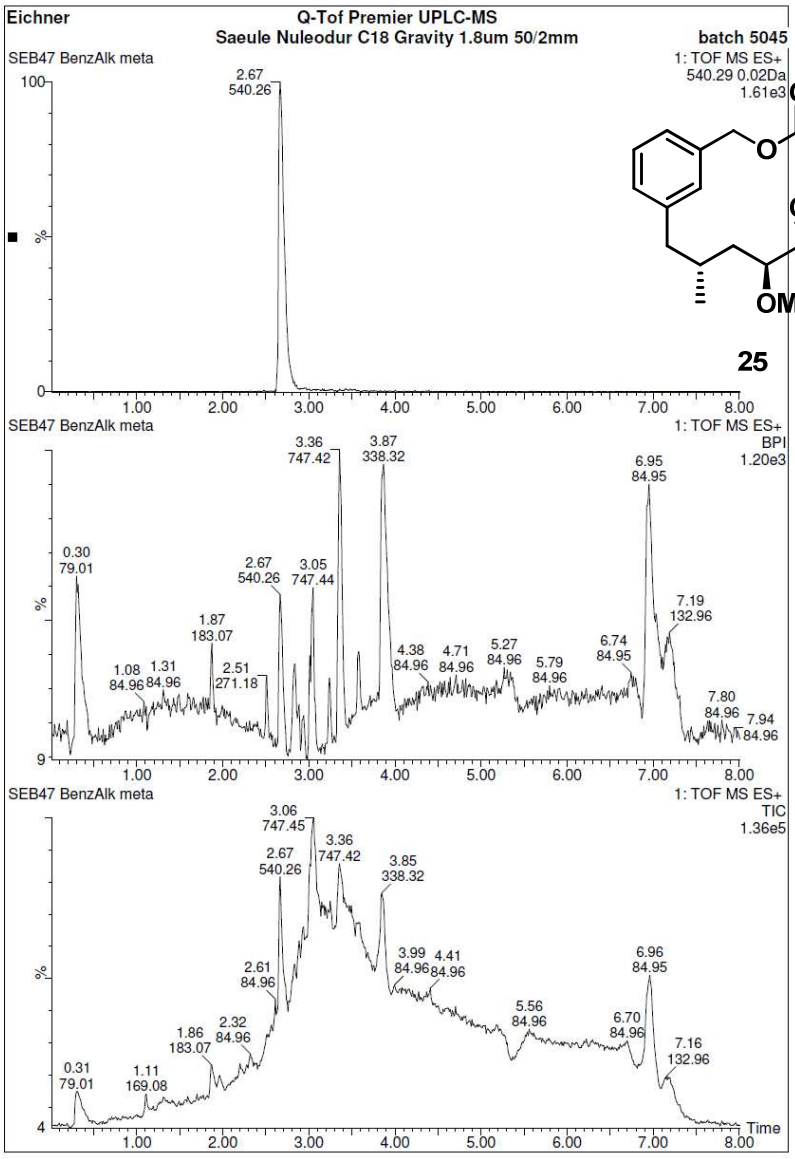
Macrolactone 23

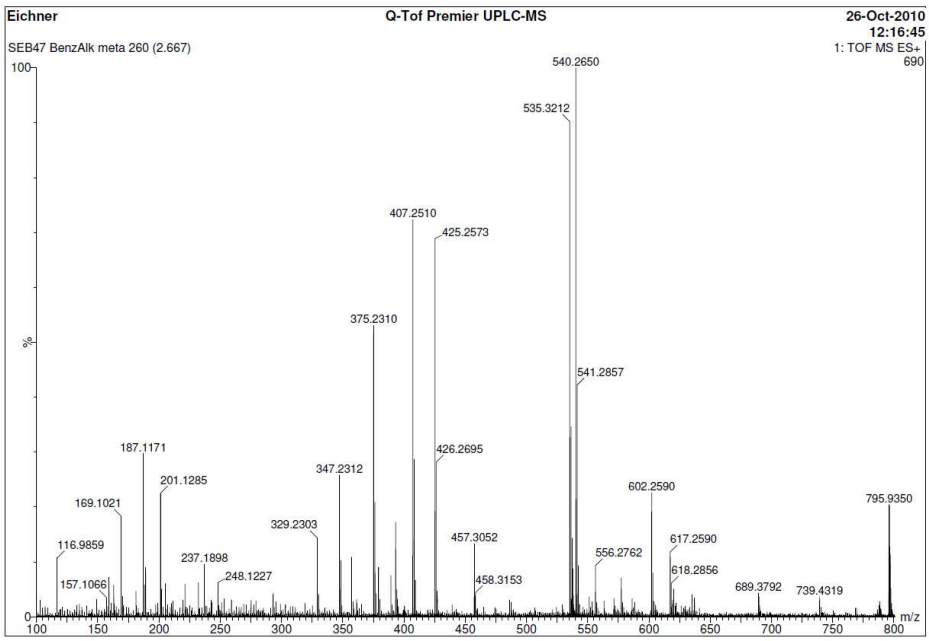


23



Macrolactone 25





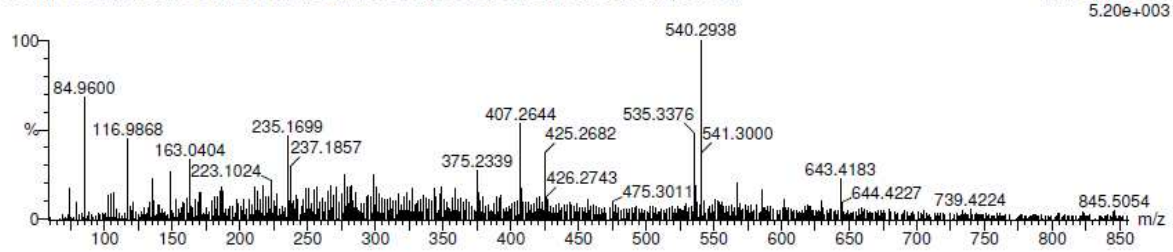
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 51.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
539 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-80 N: 0-3 O: 0-10 Na: 0-1

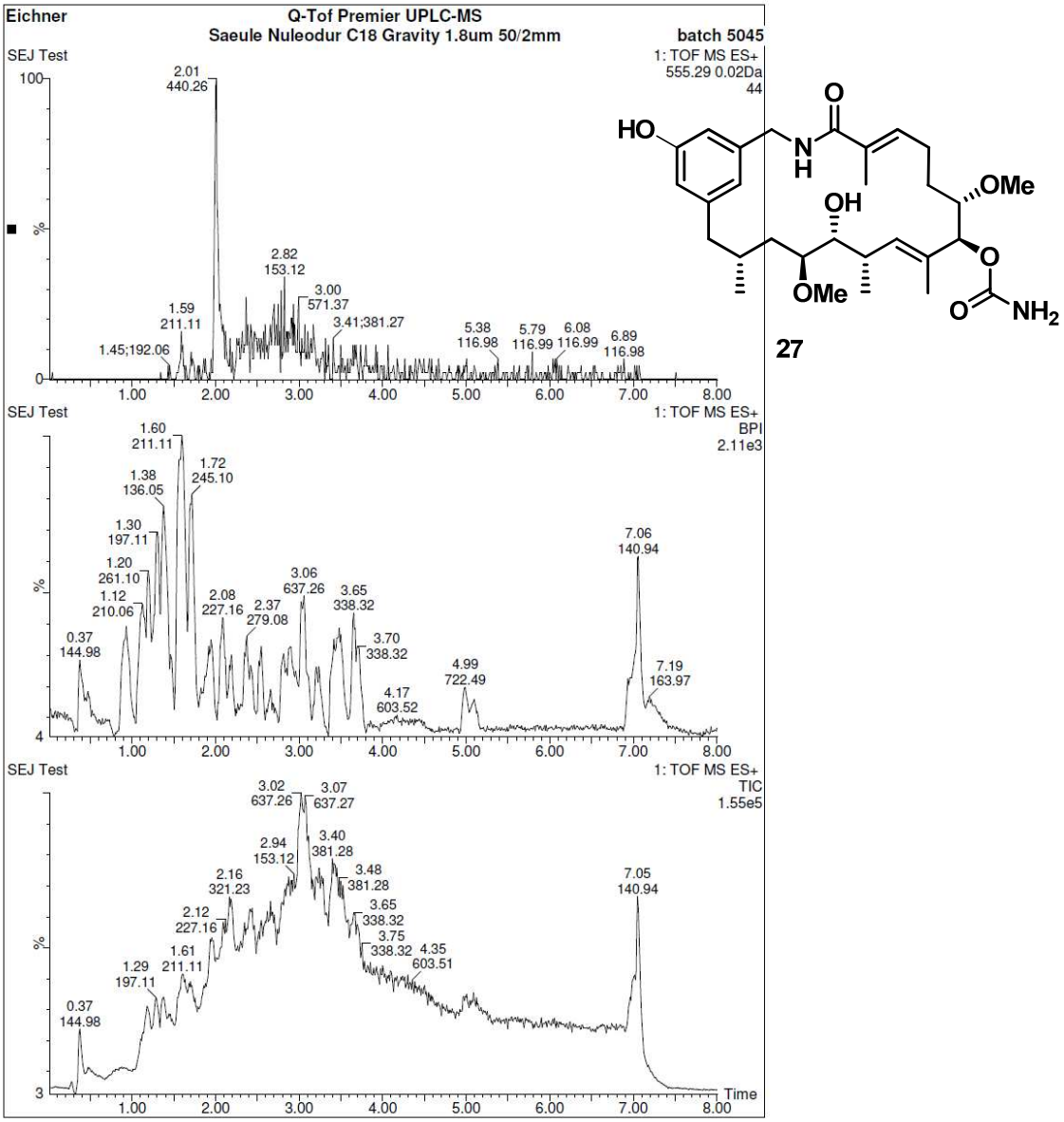
Eichner Q-Tof Premier UPLC-MS 26-Oct-2010 12:16:45

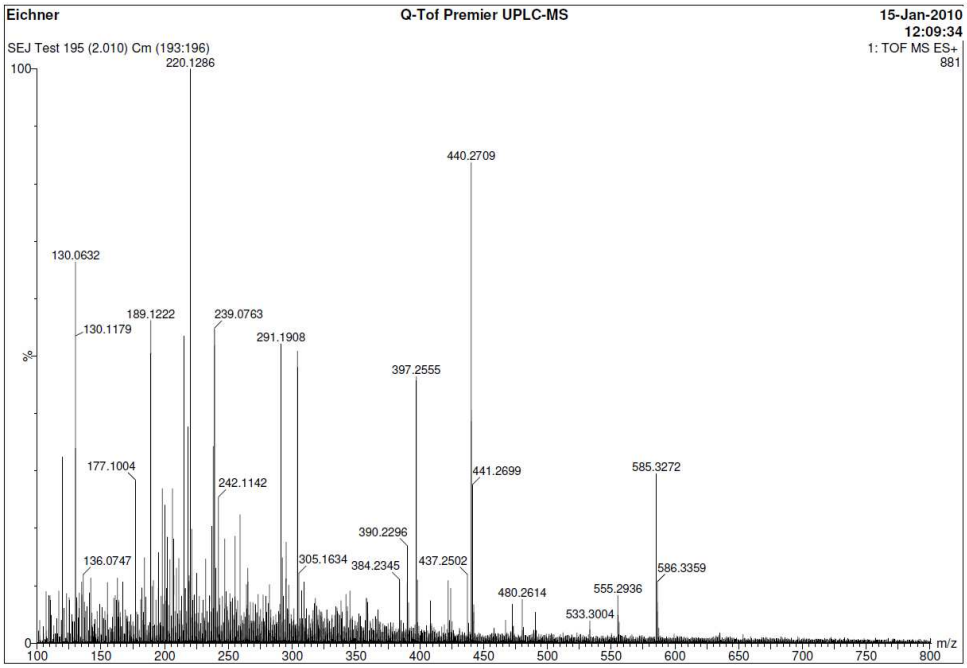
SEB47 BenzAlk meta 266 (2.731) AM (Cen,5, 70.00, Ar,8000.0,556.28,0.70,LS 5); Cm (266:271) 1: TOF MS ES+ 5.20e+003



Minimum:				-1.5		
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
540.2938	540.2937	0.1	0.2	8.5	34.2	C29 H43 N O7 Na
	540.2921	1.7	3.1	7.5	54.0	C26 H42 N3 O9
	540.2961	-2.3	-4.3	11.5	18.7	C31 H42 N O7

Macrolactam 27

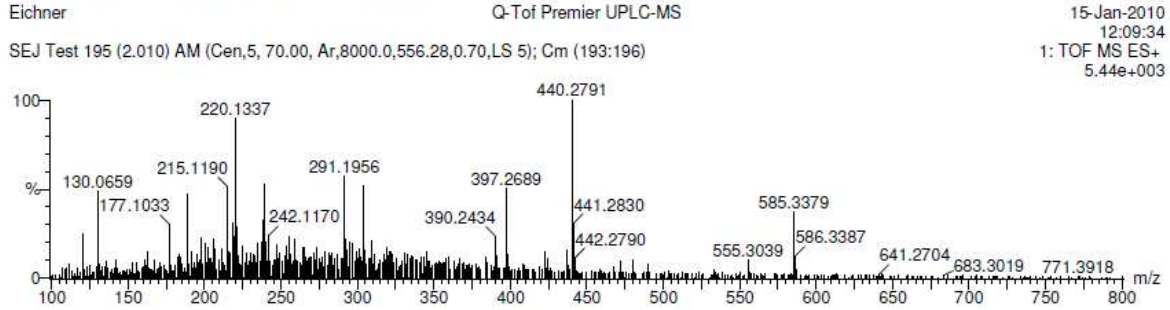




Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 51.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
552 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-80 N: 0-3 O: 0-10 Na: 0-1



Minimum:				-1.5		
Maximum:		6.0	5.0	51.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
555.3039	555.3046	-0.7	-1.3	8.5	37.6	C29 H44 N2 O7 Na
	555.3028	1.1	2.0	21.5	35.5	C41 H40 Na
	555.3052	-1.3	-2.3	24.5	35.8	C43 H39
	555.3012	2.7	4.9	20.5	34.4	C38 H39 N2 O2

9. References

S1 a) Stead, P.; Latif, S.; Blackaby, A. P.; Sidebottom, P. J.; Deakin, A.; Taylor, N. L.; Life, P.; Spaull, J.; Burrell, F.; Jones, R.; Lewis, J.; Davidson, I.; Mander, T.; *J. Antibiot.***2000**, *53*, 657-663; b) Takatsu, T.; Ohtsuki, M.; Muramatsu, A.; Enokita, R.; Kurakata, S. I.; *J. Antibiot.* **2000**, *53*, 1310-1312.

S2 Gottlieb, H. E.; Kotlyar, V.; Nudelman, A.; *J. Org. Chem.* **1997**, *62*, 7512-7515.

S3 Kieser, T.; Bibb, J.; Buttner, M. J.; Chater, K. F.; Hopwood, D. A.; *Practical Streptomyces genetics, The John Innes Foundation* **2000**, Norwich, United Kingdom.

S4 DeBoer, C.; Dietz, A.; *J. Antibiot*. **1976**, *29*, 1182-1188.
S5 Knobloch, T.; Harmrolfs, K.; Taft, F.; Thomaszewski, B.; Sasse, F.; Kirschning, A.; *ChemBioChem*. **2011**, *12*, 540-547.
S6 a) Street, T. O.; Lavery, L. A.; Agard, *Mol. Cell* **2011**, *42*, 96-105; b) Daniel R.; Southworth, D. R; Agard, D. A., *Mol Cell.* **2008**, *32*, 631–640.
S7 Kim, J.; Felts, S.; Llauger, L.; He, H.; Huezo, H.; Rosen, N.; Chiosis, G., *J. Biomol. Screen* **2004**, 9, 375-381.
S8 a) Rajabi, F.; Saidi, M. R.; *Syn. Comm.* **2005**, *35*, 483-491; b) Bandgar, B. P.; Kasture, S. P.; Kamble, V. T.; *Syn. Comm.* **2001**, *31*, 2255-2259.
S9 Watanabe, T.; Suzuki, T.; Umezawa, Y.; Takeuchi, T.; Otsuka, M.; Umezawa, K.; *Tetrahedron* **2000**, *56*, 741-752.
S10 Arnold K.; Bordoli, L.; Kopp, J.; Schwede T., *Bioinformatics*, **2006**, *22*, 195-201.
S11 Shields, G.C., Laughton, C. A.; Orozco, M.; *J. Am. Chem. Soc.* **1997**, *119*, 7463–7469.
S12 Case, D. A. et al. AMBER 10, University of California, San Francisco, **2008**.
S13 Kabsch, W.; Sander, C.; *Biopolymers* **1983**. *22*, 2577-637.