The Total Synthesis of Eponemycin[1]

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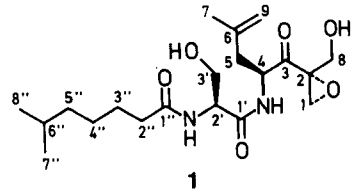
Eponemycin, an antibiotic with a highly potent and specific antitumor activity against B16 melanoma cells in vivo, has been synthesized. The framework of the western half of the molecule was built up from *N*-trityl-γ,δ-didehydroleucinal and the dilithium derivative LiO—CH_2—C(Li)=CH_2. In the final step, a vinyl ketone was oxidized nonstereoselectively to give a mixture of three isomers from which eponemycin was isolated in 40% yield.

The antibiotic eponemycin (**1**) exhibits a specific activity towards B16 melanoma cells and was first isolated from culture filtrates of *Streptomyces hygroscopicus* (No. P 247-71) in 1989.[2] The structure and configurations, with the exception of that at the epoxide ring, were established by degradation experiments and NMR analyses. The configuration at the epoxide ring, however, was not elucidated.[2]



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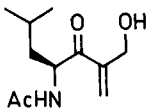

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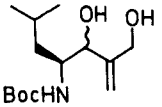


3

A putative intermediate in the biosynthesis of eponemycin is a 6-methylheptanoylseryl-γ,δ-didehydroleucine.

Similar to the strongly cytostatic cyclotetrapeptides chlamydocin[3] and WF-3161[4] of the chlamydocin group possessing an (*S*,*S*)-2-amino-8-oxo-9,10-epoxydecanoic acid unit in the ring, to the antitumor active, antibiotic agents of the manumycin group,[5] and to LL-C10037a,[6] the epoxy ketone group of eponemycin is the *locus minoris resistentiae*. The epoxy rings of oxiranyl ketones are very rapidly opened by nucleophiles in neutral media; thus, substances of this type are powerful alkylating agents.

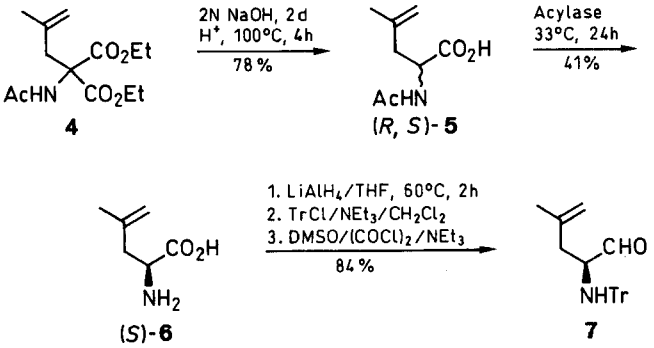
We have reported the total synthesis of eponemycin in a preliminary communication.[7] In the meantime a Japanese group synthesized 6,7-dihydroeponemycin and elucidated the configuration of the epoxide ring.[8] They reported the isolation and structure determination of the related antitumor antibiotic expoxomycin, which also has an epoxy ketone unit.[9] We now present the experimental details together with some improvements.

Two routes were considered for the synthesis of the sensitive epoxy ketone moiety. The first route involved the synthesis of a vinyl ketone and nucleophilic epoxidation in the final step; diastereoselective epoxidation could not be used since the electrophilic Sharpless reaction cannot be realized with electron-poor allylic alcohols such as **2**.

The second route comprised enantioselective construction of the corresponding epoxy alcohol by the Sharpless reaction and oxidation in the final step as employed in our syntheses of the antibiotics chlamydocin and WF-3161.

However, preliminary experiments with the model compound **3** revealed that a Sharpless oxidation of this diol was not possible, apparently due to the irreversible formation of a six-membered-ring titanium alcoholate. Furthermore, since the masking of a secondary hydroxy group appeared to be rather laborious, we selected the first route in anticipation of an advantageous optical induction by the neighbouring stereogenic centre.

For the construction of the "western" half of the molecule, we first prepared (*R*,*S*)-*N*-acetyl-γ,δ-didehydroleucine (**5**) by hydrolysis and decarboxylation of the malonic ester **4**,[10] the preparation of which was improved. Separation of the racemate by acylase gave rise to (*S*)-γ,δ-didehydroleucine (**6**), which was reduced to give (*S*)-γ,δ-didehydroleucinol; *N*-tritylation and oxidation yielded (*S*)-*N*-trityl-γ,δ-didehydroleucinal (**7**) (Scheme 1).

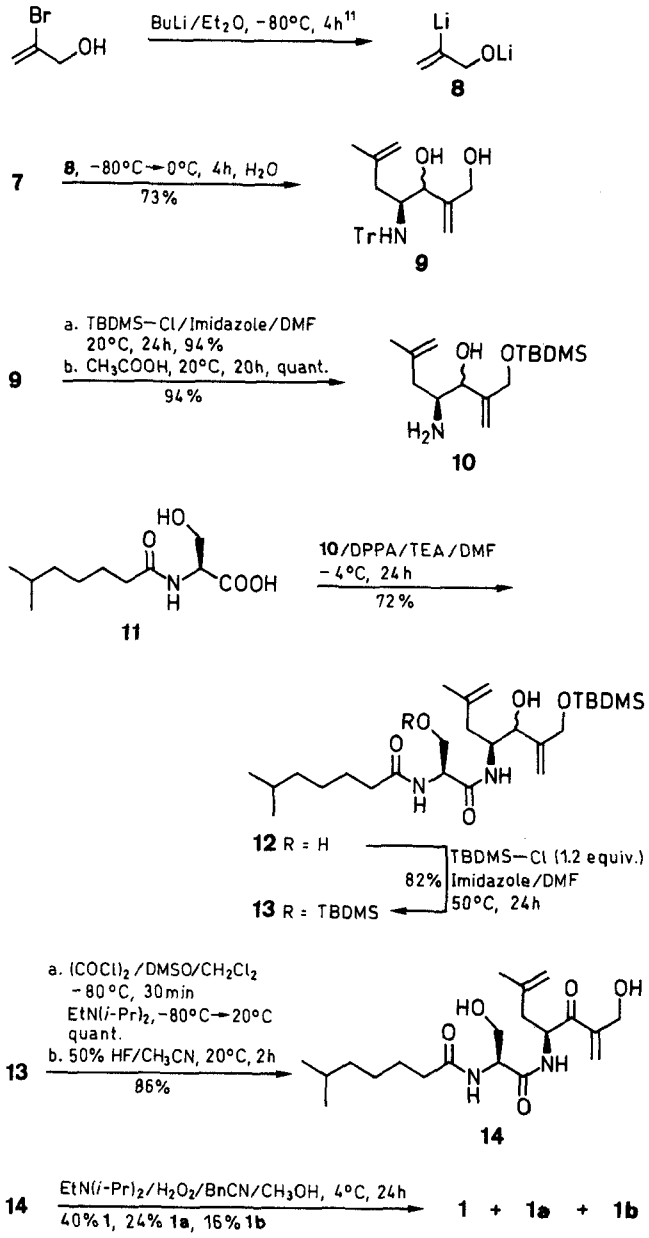


Scheme 1

The following sequence is illustrated in Scheme 2. Reaction of **7** with the dilithium reagent **8**[11] furnished a mixture of the two diastereoisomers **9**; this mixture was not separated because the two epimeric secondary alcohols were oxidized to a homogeneous ketone in a later step. We were not able to induce the dilithium derivative to react with (*Z*)-didehydroleucinal. Only the allyl esters were formed with *Z* amino acid chlorides. Attempts to obtain the lithium compound from 2-bromoallyl benzyl ether by halogen/lithium exchange were not successful.

Protection of the primary alcohol group and removal of the trityl protection gave compound **10** which was then coupled with 6-methylheptanoylserine (**11**) to furnish **12** as an epimeric mixture. Protection of the serine hydroxy group, oxidation of **13**, and deprotection yielded the ketone **14** – the substrate for epoxidation.

Of all our attempts to convert **14** into the corresponding epoxide, only base-catalyzed nucleophilic reactions pro-



Scheme 2

duced preparatively useful results. Peracid oxidation gave mono- and diepoxides, tungstenate-catalyzed oxidations failed, and merely 5% conversion was achieved after 5 days under Sharpless conditions. Alkaline workup of the latter reaction mixture furnished an epoxide with an NMR spectrum corresponding to that of the natural product in 30% yield; however, the optical rotation was completely different ($[\alpha]_D^{25}$ − 76.4°; $[\alpha]_D^{25}$ of eponemycin + 37.8°). We therefore assume that this product is 2-epi-eponemycin (**1a**).

When benzonitrile/H_2O_2/potassium hydrogen carbonate was used for the oxidation, three isomers were formed. After a preliminary purification by preparative HPLC, eponemycin (**1**) and 2-epi-eponemycin (**1a**) were obtained in the pure states by analytical HPLC. The third isomer **1b** still contained about 20% of **1a**; we suspect that **1b** is 2-epi-4-epi-eponemycin. After treatment of the mixture of **1a** + **1b** (20 + 80) with potassium hydrogen carbonate in methanol for 10 h, isomerization to a mixture comprising 80% **1a** and 20% **1b** had occurred. The fourth possible isomer, 4-epi-eponemycin, was not detected.

After oxidation with benzonitrile/H_2O_2/ethyl(diisopropyl)amine, a 40:24:16 mixture of **1**:**1a**:**1b** was obtained in a total yield of 80%. Thus, only slight epimerization at the 4-position takes place in the presence of the weakly basic amine.

The total yield of pure eponemycin thus amounted to 40% and the product was identical in all respects with the naturally occurring substance.

^{1}H NMR spectra were recorded on a Bruker WP 80 (80 MHz) and Bruker AC-F (250 MHz), using TMS as an internal standard. ^{13}C NMR spectra were recorded on a Bruker AC-F (63 MHz), using TMS as an internal standard. Optical rotations are determined with a Perkin-Elmer 241 polarimeter. Melting points (Reichert microscope) are uncorrected. TLC was performed on silica gel (Merck silica 60 F_{254} sheets) and medium pressure column chromatography (MPLC) used Merck LiChroprep Si 60 (15–25 μm). Analytical HPLC was conducted on a Pharmacia LKB instrument using either straight phase (Merck Hibar LiChrosorb Si 60, 5 μm) or chiral phase (Baker DNBPG/ionic). Preparative HPLC was performed on a Latek PRÄP 5000, using Merck Hibar LiChrosorb Si 60, 10 μm. Compounds **4–6**, **9** and **11–14** gave C,H,N ± 0.4%.

Ethyl 2-Acetamido-2-ethoxycarbonyl-4-methyl-4-pentenoic Acid (4):
To a stirred, boiling solution of diethyl acetamidomalonate (100 g, 0.46 mol) and Na (11.5 g, 0.5 mol) in abs. EtOH (600 mL), 2-methyl-2-propenyl chloride (58.7 mL, 0.6 mol) was added. Refluxing was continued for a further 2 h and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in a mixture of EtOAc (500 mL) and H_2O (200 mL). The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo* to a volume of 100 mL. Complete crystallization was achieved by adding hexane (600 mL) with vigorous stirring to give **4** as colourless crystals; yield: 118 g (95%); mp 90–91 °C; mp 92–93 °C from water.[10]

(*R*,*S*)-2-Acetamido-4-methyl-4-pentenoic Acid (5):
To a solution of **4** (100 g, 0.369 mol) in dioxane (500 mL), 2 M NaOH (500 mL) was added and the mixture was maintained at 50 °C for 2 d. The solution was concentrated and acidified with 6 M HCl to pH 1. The mixture was stored for complete crystallization in a freezer overnight. The precipitate was filtered off and decarboxylated by refluxing in water (300 mL) for 4 h. Cooling the solution for several hours yielded colourless needles; yield: 49.2 g (78%); mp 155–156 °C.

^{1}H NMR (250 MHz, CD_3OD): δ = 5.07 (br, 2H), 4.82 (s, 1H), 4.77 (s, 1H), 4.57 (dd, *J* = 9.8 Hz, *J* = 4.9 Hz, 1H), 2.57 (dd, *J* = 14.1 Hz, *J* = 4.9 Hz, 1H), 2.37 (dd, *J* = 14.1 Hz, *J* = 9.8 Hz, 1H), 1.96 (s, 3H), 1.75 (s, 3H).

^{13}C NMR (63 MHz, CD_3OD): δ = 175.4, 173.3, 142.4, 114.2, 52.1, 40.9, 22.4, 22.1.

(*S*)-γ,δ-Didehydroleucine (6), (*R*)-2-Acetamido-4-methyl-4-pentenoic Acid:
To a suspension of (*R*,*S*)-2-acetamido-4-methyl-4-pentenoic acid (3 g, 17.5 mmol) and phenol red (2–3 drops) in H_2O (100 mL), NH_4OH (25%) was added until a pH of 7.0–7.2 was reached and solution of the compound occured. To this solution, acylase I (5 mg, activity 720 μmol/mg protein from pork kidney[12]) was added and the mixture was maintained at 33 °C for 24 h. The reaction mixture was acidified with 6 M HCl to pH 1 and concentrated in vacuo (the temperature should not exceed 40 °C) to 200 mL. The unaffected (*R*)-2-acetamido-4-methyl-4-pentenoic acid was removed by EtOAc extraction (3 × 200 mL). The remaining aqueous solution was concentrated *in vacuo* to dryness. The amino acid was isolated by ion-exchange chromatography (Amberlite IR 120) to give **6** as a colourless solid; yield: 950 mg (42% calc. for **5**); mp > 210 °C (dec.); $[\alpha]_D^{20}$ − 30.9° (*c* = 1.04, H_2O).

^{1}H NMR (250 MHz, D_2O): δ = 5.03 (t, J = 1.5 Hz, 1 H), 4.94 (s, 1 H), 4.82 (s, 3 H), 3.88 (dd, J = 9.5 Hz, J = 4.6 Hz, 1 H), 2.72 (dd, J = 14.5 Hz, J = 4.6 Hz, 1 H), 2.54 (dd, J = 14.5 Hz, J = 9.5 Hz, 1 H), 1.81 (s, 3 H).

^{13}C NMR (63 MHz, D_2O): δ = 177.5, 143.0, 118.3, 55.5, 41.9, 23.6.

(*S*)-2-Amino-4-methyl-4-penten-1-ol:

To a suspension of (*R,S*)-2-acetamido-4-methyl-4-pentenoic acid (41 g, 0.24 mol) and phenol red (2–3 drops) in H_2O (1.3 L), NH_4OH (25%) was added until a pH of 7.0–7.2 was reached and solution of the compound occured. To this solution acylase I (50 mg, activity 720 μmol/mg protein from pork kidney)[12] was added and the mixture maintained at 33 °C for 24 h. The reaction mixture was acidified with 6 M HCl to pH 1 and concentrated *in vacuo* (the temperature should not exceed 40 °C) to 200 mL. The unreacted (*R*)-2-acetamido-4-methyl-4-pentenoic acid was removed by EtOAc extraction (10 × 200 mL). The remaining aqueous solution was treated with 6 M NaOH until pH 10 was reached then concentrated to dryness. Traces of H_2O were removed by azeotropic distillation with toluene. The obtained dry powder was added in small portions to a suspension of $LiAlH_4$ (25 g, 0.66 mol) in THF (500 mL). After refluxing for 2 h the reaction mixture was hydrolyzed by adding H_2O (40 mL) with vigorous stirring in an ice bath. The precipitated $Al(OH)_3$ was filtered off and extracted with boiling MeOH (3 × 200 mL). The filtrate and the MeOH extracts were combined and concentrated *in vacuo* to dryness. The colourless residue was treated with EtOAc (2 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated to give (*S*)-2-amino-4-methyl-4-penten-1-ol as a pale yellow oil which was used without further purification; yield: 11.3 g (41% calc. for **5**).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.01 (br, 4 H), 4.96 (s, 1 H), 3.70 (dd, J = 10.7 Hz, J = 4.5 Hz, 1 H), 3.52 (dd, J = 10.7 Hz, J = 6.9 Hz, 1 H), 3.16 (m, 1 H), 2.37 (dd, J = 13.6 Hz, J = 5.4 Hz, 1 H), 2.17 (dd, J = 13.6 Hz, J = 9.3 Hz, 1 H), 1.94 (s, 3 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 144.0, 113.5, 67.4, 51.4, 43.1, 22.5.

(*S*)-4-Methyl-2-tritylamino-4-penten-1-ol:

To a solution of (*S*)-2-amino-4-methyl-4-penten-1-ol (5.0 g, 43.4 mmol) in CH_2Cl_2 (50 mL), Et_3N (6.74 mL, 48.8 mmol) and Tr–Cl (12.12 g, 43.4 mmol) were added. The mixture was stirred at r.t. for 2 h and the solvent was removed *in vacuo*. The oily residue was dissolved in a mixture of EtOAc (100 mL) and H_2O (50 mL). The organic layer was extracted with H_2O (2 × 50 mL), dried ($MgSO_4$) and concentrated. The final purification was achieved by silica gel chromatography (eluent: hexane/EtOAc 8:2 + 0.1% pyridine) affording (*S*)-4-methyl-2-tritylamino-4-penten-1-ol as an almost colourless oil; yield: 13.2 g (85%); $[\alpha]_D^{20}$ − 13.5° (c = 1.81, $CHCl_3$).

$C_{25}H_{27}NO$	calc.	C 83.99	H 7.61	N 3.92
(357.5)	found	84.04	7.59	3.73

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.61–7.53 (m, 6 H), 7.34–7.18 (m, 9 H), 4.71 (s, 1 H), 4.98 (s, 1 H), 3.31 (dd, J = 10.9 Hz, J = 2.8 Hz, 1 H), 3.12 (dd, J = 10.9 Hz, J = 4.3 Hz, 1 H), 2.70 (m, 1 H), 2.02 (br, 3 H), 1.72 (dd, J = 13.2 Hz, J = 5.5 Hz, 1 H), 1.39 (s, 3 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 146.6, 143.3, 128.7, 127.9, 126.5, 113.3, 71.2, 63.4, 51.6, 42.1, 22.1.

(*S*)-4-Methyl-2-tritylamino-4-pentenal (7):

To a solution of $(COCl)_2$ (4.67 mL, 54.2 mmol) in CH_2Cl_2 (140 mL) at − 80 °C, DMSO (7.55 mL, 106.2 mmol) in CH_2Cl_2 (10 mL) was added over 5 min. After a further 10 min, a solution of (*S*)-4-methyl-2-tritylamino-4-penten-1-ol 12.7 g, 35.5 mmol) in CH_2Cl_2 (100 mL) was added slowly and stirring was continued for another 30 min. Then Et_3N (15 mL) was added and the reaction mixture was allowed to warm to r.t. The solution was poured into hexane (500 mL) and extracted with H_2O (3 × 200 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* furnishing **7** as a yellow oil which was used without further purification; yield: 12.44 g (98%).

(3*RS*,4*S*)-3-Hydroxy-2-hydroxymethyl-6-methyl-4-tritylamino-1,6-heptadiene (9):

To a solution of 2-bromo-3-propen-1-ol (14.38 g, 105 mmol) in Et_2O (360 mL) at − 80 °C BuLi (164 mL, 262.5 mmol, 1.6 M solution in pentane) was added dropwise. The solution was quickly warmed to 0 °C and stirred for a further 4 h and cooled to − 80 °C. Aldehyde **7** (12.44 g, 35 mmol) in Et_2O (40 mL) was added and stirring was continued for 1 h at − 80 °C then for 2 h in an ice bath. The reaction was hydrolyzed with H_2O (50 mL), the organic layer was separated, extracted with H_2O (30 mL) and dried ($MgSO_4$). Final purification was achieved by silica gel chromatography (eluent: hexane/EtOAc 7:3 then 1:1 + 0.1% pyridine) to give **9** as a pale sticky product consisting of a 1:1 mixture of two diastereomers; yield: 10.5 g (73%).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.60–7.50 (m, 6 H), 7.33–7.17 (m, 9 H), 5.23 (s, 0.5 H), 5.19 (s, 0.5 H), 5.11 (s, 0.5 H), 5.01 (s, 0.5 H), 4.74 (s, 1 H), 4.71 (s, 0.5 H), 4.64 (s, 0.5 H), 4.30–4.12 (m, 0.5 H), 4.08 (s, 0.5 H), 3.92 (s, 0.5 H), 3.86 (s, 0.5 H), 3.81 (s, 1 H), 3.2–2.5 (m, 3 H), 2.28–1.94 (m, 2 H), 1.80 (dd, J = 13.8 Hz, J = 3.9 Hz, 1 H), 1.31 (s, 2 H), 1.17 (s, 1 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 149.6, 147.5, 146.2, 146.1, 143.4, 143.0, 128.8, 127.9, 126.7, 114.1, 113.7, 112.6, 72.5, 71.8, 70.9, 70.4, 64.0, 53.9, 40.1, 37.7, 22.2, 21.6.

(3*RS*,4*S*)-2-*tert*-Butyldimethylsilyloxymethyl-3-hydroxy-6-methyl-4-tritylamino-1,6-heptadiene:

To a solution of **9** (9.3 g, 22.5 mmol) in DMF (50 mL) imidazole (3.35 g, 49.2 mmol) and TBDMS-Cl (3.39 g, 22.5 mmol) were added. The solution was stored for 24 h at r.t. then concentrated *in vacuo* to dryness. The residue was treated with EtOAc (100 mL) and the organic layer was washed successively with 0.5 M H_2SO_4 (2 × 30 mL), 1 M $KHCO_3$ (50 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Final purification was achieved by silica gel chromatography (eluent: hexane/EtOAc 8:2 + 0.1% pyridine) to give (3*RS*,4*S*)-2-*tert*-butyldimethylsiloxymethyl-3-hydroxy-6-methyl-4-tritylamino-1,6-heptadiene as a colourless oil; yield: 11.1 g (94%).

$C_{34}H_{45}NO_2Si$	calc.	C 77.37	H 8.59	N 2.65
(527.8)	found	77.37	8.72	2.57

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.61–7.50 (m, 6 H), 7.32–7.17 (m, 9 H), 5.32 (br, 0.5 H), 5.24 (s, 0.5 H), 5.13 (br, 0.5 H), 5.09 (s, 0.5 H), 4.76 (s, 1 H), 4.67 (s, 0.5 H), 4.62 (s, 0.5 H), 4.39 (dd, J = 10.0 Hz, J = 1.7 Hz, 0.5 H), 4.24 (s, 0.5 H), 3.90–3.70 (m, 2 H), 2.89 (br, 1 H), 2.68–2.61 (m, 1 H), 2.47–2.40 (m, 1 H), 2.37–1.78 (m, 2 H), 1.28 (s, 1.5 H), 1.14 (s, 1.5 H), 0.95–0.85 (m, 9 H), 0.05–(−0.02) (m, 6 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 149.6, 147.4, 146.3, 143.7, 129.0, 128.8, 128.7, 127.8, 126.5, 114.2, 113.5, 110.7, 109.9, 71,5, 70.8, 70.5, 70.1, 64.0, 63.8, 53.3, 53.2, 39.6, 37.4, 25.9, 25.8, 22.3, 21.6, 18.3, − 5.6, − 5.7.

(3*RS*,4*S*)-4-Amino-2-*tert*-butyldimethylsilyloxymethyl-3-hydroxy-6-methyl-1,6-heptadiene (10):

The detritylation was achieved by dissolving the foregoing trityl compound (11.1 g, 21 mmol) in HOAc (60 mL). The solution was stored for 12 h at r.t. and evaporated. The residue was dissolved in EtOAc (100 mL) and neutralized with 1 M $KHCO_3$ with vigorous stirring. The organic layer was separated, dried ($MgSO_4$) and concentrated to give **10** as a sticky yellow product which was used without further purification; yield: 11.5 g (quant.).

(*S*)-*N*-(6-Methylheptanoyl)serine Methyl Ester:

Through a suspension of *S*-serine methyl ester hydrochloride (4.0 g, 28.2 mmol) in CH_2Cl_2 (20 mL), a strong stream of ammonia was passed for 10 min. The mixture was filtered and the filtrate was concentrated *in vacuo*. To the residue in CH_2Cl_2 (20 mL), 6-methylheptanoic acid (3.46 g, 24 mmol) was added and, at − 20 °C, DCCD (4.96 g, 24 mmol). Stirring was continued for 10 h during which the mixture reached r.t. The precipitated urea was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in Et_2O (20 mL) and stored for 2 h at 0 °C. The precipitate was filtered off and the filtrate concentrated *in vacuo*. The final

purification was achieved by silica gel chromatography (eluent hexane/EtOAc 7:3 then EtOAc); yield: 5.1 g (86%); $[\alpha]_D^{20}$ + 19.5° (c = 1.50, CH_2Cl_2).

$C_{12}H_{23}NO_4$	calc.	C 58.75	H 9.45	N 5.71
(245.3)	found	58.80	9.53	5.83

^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.72 (d, J = 7.7 Hz, 1 H), 4.62 (ddd, J = 7.7 Hz, J = 3.9 Hz, J = 3.5 Hz, 1 H), 3.94 (dd, J = 11.2 Hz, J = 3.9 Hz, 1 H), 3.82 (dd, J = 11.2 Hz, J = 3.5 Hz, 1 H), 3.74 (s, 3 H), 2.24 (d, J = 7.3 Hz, 1 H), 2.21 (d, J = 7.9 Hz, 1 H), 1.64–1.41 (m, 3 H), 1.35–1.23 (m, 2 H), 1.19–1.06 (m, 2 H), 0.82 (d, J = 6.6 Hz, 6 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 174.0, 171.1, 63.1, 54.6, 52.6, 38.6, 36.5, 27.8, 27.0, 25.8, 22.5.

(*S*)-*N*-(6-Methylheptanoyl)serine (11):

To a solution of the foregoing methyl ester (5.0 g, 20.4 mmol) and H_2O_2 (0.5 mL, 30%) in THF (50 mL), 0.5 M LiOH (40.8 mL, pH < 10) was added dropwise. The mixture was concentrated *in vacuo* and the residue was dissolved in H_2O (30 mL). The aqueous layer was extracted with Et_2O (50 mL) then EtOAc (100 mL) was added to the aqueous layer and the pH was adjusted to 1 with 1 M HCl. The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo*; yield: 4.15 g (88%).

^{1}H NMR (250 MHz, CD_3OD): δ = 4.87 (s, 3 H), 4.43 (dd, J = 4.6 Hz, J = 4.4 Hz, 1 H), 3.83 (dd, J = 11.2 Hz, J = 5.0 Hz, 1 H), 3.74 (dd, J = 11.4 Hz, J = 5.1 Hz, 1 H), 2.22 (t, J = 7.2 Hz, 2 H), 1.60–1.43 (m, 3 H), 1.40–1.23 (m, 2 H), 1.18–1.09 (m, 2 H), 0.86 (d, J = 6.6 Hz, 6 H).

^{13}C NMR (63 MHz, CD_3OD): δ = 174.9, 171.9, 61.4, 54.5, 38.4, 35.4, 27.5, 26.6, 25.6, 21.5.

(3*RS*,4*S*)-2-*tert*-Butyldimethylsilyloxymethyl-3-hydroxy-4-[*N*-(6-methylheptanoyl)serylamino]-6-methyl-1,6-heptadiene (12):

To a solution of **10** (2.0 mmol) and **11** (500 mg, 2.2 mmol) in DMF (5 mL) at − 4 °C DPPA (476 µL, 2.2 mmol) in DMF (5 mL) and then Et_3N (307 µL, 2.2 mmol) in DMF (5 mL) were added. The reaction mixture was stirred for another 2 d at − 4 °C. The solvent was distilled off *in vacuo* and the residue was dissolved in a mixture of EtOAc (50 mL) and H_2O (30 mL). The organic layer was separated and extracted successively with 0.5 M H_2SO_4 (30 mL), 1 M $KHCO_3$ (30 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. Final purification was achieved by silica gel chromatography (eluent: hexane/EtOAc 7:3 then 1:1); yield: 720 mg (72%).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.94 (m, 1 H), 6.65 (d, J = 5.5 Hz, 1 H), 5.21 (s, 0.5 H), 5.13 (m, 1.5 H), 4.74 (m, 2 H), 4.42 (br, 1 H), 4.34–4.17 (m, 3.5 H), 3.92 (m, 1.5 H), 3.56 (m, 1 H), 2.41–2.11 (m, 4 H), 1.75 (s, 1.5 H), 1.71 (s, 1.5 H), 1.65–1.44 (m, 3 H), 1.29 (m, 2 H), 1.15 (m, 2 H), 0.91–0.84 (m, 15 H), 0.11–0.07 (m, 6 H).

(3*RS*,4*S*)-4-[*O*-*tert*-Butyldimethylsilyl-*N*-(6-methylheptanoyl)seryl-amino]-2-*tert*-butyldimethylsilyloxymethyl-3-hydroxy-6-methyl-1,6-heptadiene (13):

To a solution of **12** (580 mg, 1.16 mmol) in DMF (20 mL) TBDMS-Cl (211 mg, 1.39 mmol) and imidazole (216 mg, 3.6 mmol) were added. The mixture was maintained at 50 °C for 24 h. The solvent was removed under reduced pressure and the residue was dissolved in a mixture of EtOAc (30 mL) and H_2O (20 mL). The organic layer was washed successively with 0.5 M H_2SO_4 (20 mL), 1 M $KHCO_3$ (20 mL) and finally with brine (10 mL). The EtOAc extract was dried ($MgSO_4$), concentrated and purified by silica gel chromatography (eluent: hexane/EtOAc 1:1); yield: 600 mg (82%).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.86 (m, 0.5 H), 6.65 (d, J = 8.9 Hz, 0.5 H), 6.37 (d, J = 5.5 Hz, 0.5 H), 6.35 (d, J = 5.7 Hz, 0.5 H), 5.19 (s, 0.5 H), 5.10 (m, 1.5 H), 4.75 (m, 2 H), 4.44–4.08 (m, 5 H), 3.98 (m, 1 H), 3.51 (m, 1 H), 3.38 (br, 0.5 H), 2.68 (br, 0.5 H), 2.49–2.03 (m, 4 H), 1.75 (m, 3 H), 1.67–1.45 (m, 3 H), 1.39–0.99 (m, 4 H), 0.92 (s, 18 H), 0.92–0.84 (m, 6 H), 0.14–0.00 (m, 12 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 173.4, 173.2, 170.5, 170.3, 170.1, 148.3, 146.3, 142.2, 142.1, 127.9, 113.8, 113.4, 113.1, 111.6, 73.3, 64.8, 62.7, 54.0, 50.6, 49.8, 41.1, 40.9, 38.6, 37.8, 36.7, 31.0, 27.8, 27.0, 25.9, 22.6, 22.2, 18.3, 18.1, 0.0.

(4*S*)-4-[*O*-*tert*-Butyldimethylsilyl-*N*-(6-methylheptanoyl)-serylamino]-2-*tert*-butyldimethylsilyloxymethyl-6-methyl-3-oxo-1,6-heptadiene:

To a solution of $(COCl)_2$ (500 µl, 5.81 mmol) in CH_2Cl_2 (14 mL) at − 80 °C, DMSO (810 µl, 11.4 mmol) in CH_2Cl_2 (1 mL) was added over a period of 5 min. Stirring was continued for 10 min then **13** (2.44 g, 3.87 mmol) in CH_2Cl_2 (10 mL) was added slowly. After 30 min (i-Pr)$_2$NEt (1.5 mL) was added and the reaction mixture was allowed to warm to r.t. The solution was poured into hexane (50 mL) and washed successively with 0.5 M H_2SO_4 (20 mL), 1 M $KHCO_3$ (20 mL) and finally with brine (10 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*; yield: 2.43 g (quant.); $[\alpha]_D^{20}$ + 43.4° (c = 1.53, $CHCl_3$).

$C_{32}H_{62}N_2O_5Si_2$	calc.	C 62.90	H 10.23	N 4.58
(611.0)	found	63.13	10.14	4.51

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.42 (d, J = 8.3 Hz, 0.5 H), 7.14 (d, J = 8.3 Hz, 0.5 H), 6.33 (d, J = 6.3 Hz, 1 H), 6.26 (s, 1 H), 6.21 (dd, J = 1.8 Hz, J = 1.7 Hz, 1 H), 5.42 (ddd, J = 8.3 Hz, J = 4.9 Hz, J = 4.1 Hz, 1 H), 4.81 (s, 1 H), 4.80 (s, 1 H), 4.76–4.25 (m, 3 H), 4.01 (m, 1 H), 3.55 (m, 1 H), 2.51 (dd, J = 14.1 Hz, J = 4.5 Hz, 1 H), 2.22 (m, 3 H), 1.81 (s, 0.75 H), 1.74 (s, 0.75 H), 1.72 (s, 1.5 H), 1.68–1.41 (m, 3 H), 1.29 (m, 2 H), 1.11 (m, 2 H), 0.91 (s, 9 H), 0.90 (s, 9 H), 0.85 (d, J = 6.6 Hz, 6 H), 0.11 (s, 3 H), 0.10 (s, 3 H), 0.07 (s, 6 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 198.7, 173.2, 170.1, 145.5, 140.2, 124.6, 114.7, 62.6, 61.0, 53.8, 52.1, 41.4, 38.6, 36.7, 27.8, 27.0, 25.9, 22.6, 18.3, 18.2, − 5.5, − 5.6.

(4*S*)-2-Hydroxymethyl-[*N*-(6-methylheptanoyl)serylamino]-6-methyl-3-oxo-1,6-heptadiene (14):

To a solution of the foregoing compound (1.8 g, 2.86 mmol) in MeCN (30 mL), HF (50%, 1 mL; after 1 h again 1 mL) was added and stirring was continued for 3 h. The reaction mixture was neutralized with $KHCO_3$. The MeCN was distilled off and the residue was extracted with EtOAc (100 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was recrystallized from Et_2O; yield: 940 mg (86%); mp 101–103 °C; $[\alpha]_D^{20}$ + 3.5° (c = 1.28, $CHCl_3$).

HPLC: R_{t14} = 14.2 min (eluent: i-PrOH/hexane, 14:86) SiO_2 column; R_{t14} = 6.6 min (eluent: i-PrOH/hexane, 20:80) DNPG ionic.

^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.41 (d, J = 7.8 Hz, 1 H), 6.68 (d, J = 7.3 Hz, 1 H), 6.25 (s, 1 H), 6.14 (s, 1 H), 5.33 (ddd, J = 7.8 Hz, J = 4.7 Hz, J = 4.4 Hz, 1 H), 4.82 (s, 1 H), 4.73 (s, 1 H), 4.52 (m, 1 H), 4.37 (dd, J = 13.1 Hz, J = 5.7 Hz, 1 H), 4.27 (dd, J = 13.1 Hz, J = 5.7 Hz, 1 H), 4.00–3.88 (m, 1 H), 3.96 (s, 1 H), 3.56 (m, 1 H), 3.18 (dd, J = 6.1 Hz, J = 6.0 Hz, 1 H), 2.53 (dd, J = 14.3 Hz, J = 4.5 Hz, 1 H), 2.25 (m, 3 H), 1.74 (s, 3 H), 1.54 (m, 3 H), 1.29 (m, 2 H), 1.14 (m, 2 H), 0.85 (d, J = 6.6 Hz, 6 H).

^{13}C NMR (63 MHz, $CDCl_3$): δ = 199.9, 174.0, 170.6, 145.4, 140.2, 126.3, 114.6, 62.8, 61.7, 54.0, 52.2, 40.4, 38.6, 36.5, 27.8, 27.0, 25.8, 22.6, 22.2.

Eponemycin (1):

To a solution of **14** (20 mg, 0.053 mmol) in MeOH (0.5 mL), benzonitrile (6 µL, 0.06 mmol), Et(i-Pr)$_2$ (10 µL, 0.06 mmol) and H_2O_2 (7 µL, 30% solution in H_2O) were added at 0 °C. The reaction mixture was maintained at 4 °C for 24 h then the solvent was distilled off and the residue was dissolved in EtOAc (10 mL). The EtOAc layer was washed successively with 0.5 M $KHSO_4$ (2 × 5 mL), 1 M $KHCO_3$ (5 mL) and finally with brine (5 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by HPLC methods using first prep. HPLC (column Hibar LiChrosorb, eluent: i-PrOH/hexane 16:84) to give the pure isomer **1a** and the crude product **1**. The desired product **1** was isolated by analytical HPLC (column chiral phase DNBPG ionic, eluent i-PrOH/hexane 1:4); yield: 8.5 mg **1** (40%) and 5.1 mg **1a** (24%).

1: $[\alpha]_D^{25}$ + 37.8° {c = 0.404, MeOH, Lit. $[\alpha]_D^{24}$ + 32 ± 2° (c = 0.5, MeOH)}.

HPLC: R_{t1} = 17.7 min (eluent: i-PrOH/hexane, 14:86) SiO_2 column; R_{t1} = 7.8 min eluent: i-PrOH/hexane 20:80 DNPG ionic.

MS (70 eV): m/z = 399.2 (M^+ + H), 398.2 (M^+).

Table. ^1H and ^{13}C NMR Spectra of 2-epi-Eponemycin

Carbon	^1H NMR (250 MHz/$CDCl_3$) δ, J (Hz)	^{13}C NMR (63 MHz/$CDCl_3$) δ
1	3.12 (d, 4.9), 3.35 (d, 4.9)	49.5 (t)
2		62.4 (s)
3		207.1 (s)
4	4.61 (ddd, 10.3, 6.6, 3.9)	50.9 (s)
5	2.08 (dd, 14.0, 10.4), 2.58 (dd, 14.0, 3.6)	38.5 (t)
6		140.2 (s)
7	4.82 (s), 4.90 (s)	115.0 (s)
8	3.74 (dd, 12.6, 5.6), 4.20 (dd, 12.6, 4.9)	61.5 (t)
9	1.77 (s)	20.5 (q)
4-NH	7.13 (d, 6.7)	
1′		171.3 (s)
2′	4.46 (ddd, 7.4, 4.4, 3.3)	53.7 (d)
3′	3.59 (m), 4.04 (d, 11.4)	62.8 (t)
2′-NH	6.62 (d, 7.2)	
1″		174.4 (s)
2″	2.27 (t, 7.8)	35.4 (t)
3″	1.63 (m)	25.8 (t)
4″	1.33 (m)	27.0 (t)
5″	1.17 (m)	38.6 (t)
6″	1.53 (m)	27.8 (d)
7″	0.86 (d, 6.6)	21.6 (q)
8″	0.86 (d, 6.6)	21.6 (q)

HRMS: calc. for $C_{20}H_{34}N_2O_6$ 398.2414; found 398.2414.

For ^1H NMR and ^{13}C NMR see Lit.[2,13]

2-epi-Eponemycin (**1a**): $[\alpha]_D^{25} - 76.4°$ ($c = 0.45$, MeOH).

HPLC: $R_{t1a} = 13.0$ min (eluent: *i*-PrOH/hexane, 14:86) SiO_2 column; $R_{t1a} = 10.1$ min (eluent: *i*-PrOH/hexane 20:80) DNPG ionic.

MS (70 eV): $m/z = 398.2$ (M^+)

HRMS: calc. for $C_{20}H_{34}N_2O_6$ 398.2414; found 398.2413.

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