Synthesis and Antifungal Activity of C-16 Oximino and Vinyl Amphotericin B Derivatives

DAVID F. CORBETT*, DAVID K. DEAN, ALEXANDER R. GREENLEES and DAVID T. MACPHERSON

SmithKline Beecham Pharmaceuticals,
Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, UK

(Received for publication January 24, 1995)

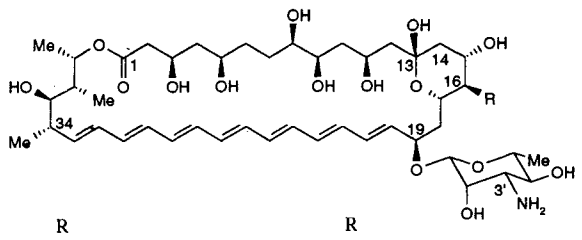
Several new C-16 oximino and vinyl derivatives of amphotericin B are described. They are prepared by the reaction of a suitably protected amphotericin B C-16 aldehyde with hydroxylamine derivatives and Wittig reagents, respectively, followed by sequential removal of the protecting groups. The compounds possess potent antifungal activity *in vitro*, similar to or in some cases superior to that of amphotericin B itself. With the exception of the C-16 (*Z*)-methoxime, the new derivatives do not show significantly reduced haemolytic activity against mammalian erythrocytes compared with amphotericin B.

Although the polyene antibiotic amphotericin B (**1**) remains the drug of choice to combat serious systemic fungal infections, multiple side effects caused by its mammalian toxicity continue to compromise its clinical use[1]. Consequently, for many years there has been a quest to improve the antifungal selectivity of the antibiotic, and recently certain liposomal and lipid formulations of the drug[2] have shown the promise of reduced toxicity and have begun to make a clinical impact. As well as the search for new formulations, there have been many efforts to improve the therapeutic ratio of amphotericin B by chemical modification of the parent structure. Until recently, because of the multifunctional and amphiphilic nature of the molecule, chemistry was restricted to simple manipulations of the mycosamine C-3′ amino group (*e.g. N*-acylation, *N*-alkylation) and of the C-16 carboxylic acid (*e.g.* ester and amide formation)[3,4]. Nevertheless, derivatives with markedly reduced nephrotoxicity were identified[3], and two of these, the C-16 methyl ester and its C-3′ *N*-D-ornithyl analogue, were developed further[5] but failed to reach the clinic because of neurotoxicity. Subsequently, in these[6] and other[7] laboratories, the development of selective protection group strategies for functionalities present in amphotericin B has increased the scope of chemistry possible, allowing the synthesis of a range of novel analogues[6,8~12] modified at C-13 and C-14, as well as C-16. While various amphotericin B derivatives have shown the promise of increased antifungal selectivity[6,10], the C-16 modified analogues have the added advantage of potential water solubility when formulated as their C-3′ amine salts[13]. Of our previously described amphotericin B C-16 analogues, which include ketones[6,9] and substituted methyl derivatives[6], the 16-hydroxymethyl derivative (**2**)[6,8] possessed a sufficiently increased therapeutic ratio to be selected for further development[14,15].

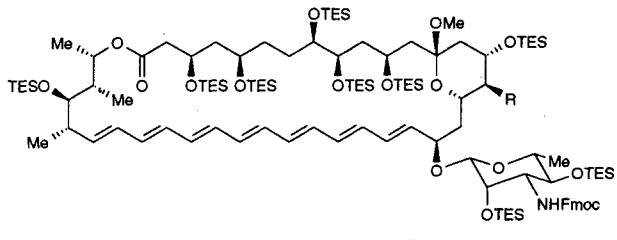
A chemical intermediate in one of the synthetic routes to **2** is the protected amphotericin B C-16 hydroxymethyl derivative (**11**), which could be readily converted by Swern oxidation to the corresponding aldehyde (**12**)[6], a potentially versatile intermediate for further chemical modification at C-16. The protecting groups present in **12** [9-fluorenylmethoxycarbonyl (Fmoc) for the mycosamine amino moiety, methyl acetal for the C-13 anomeric centre, and triethylsilyl (TES) for the remaining nine hydroxy groups] were developed for a range of amphotericin B modifications[6] and can be removed selectively to liberate the parent functionalities necessary for optimum biological activity. We now describe the conversion of the aldehyde (**12**) into the novel amphotericin B C-16 oximes (**3**~**5**) and vinyl derivatives (**6**~**8** and **10**), which possess potent *in vitro* antifungal activity against clinically important pathogens.

Chemistry

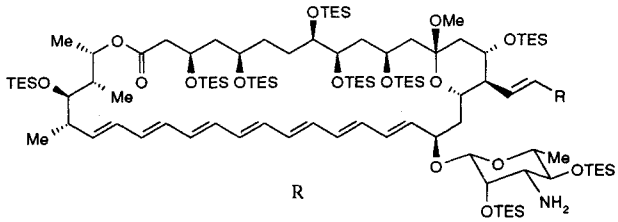
The reaction of amphotericin B with methoxylamine at the C-13 hemiacetal to afford a ring-opened C-13 methoxime, with much reduced antifungal activity, has been reported by both BOROWSKI[16] and ourselves[6]. However, as anticipated, protection of this position as the methyl acetal in **12** allowed reaction of the C-16 aldehyde with methoxylamine hydrochloride to afford



R	R
1 CO_2H Amphotericin B	6 $CH{=}CH_2$
2 CH_2OH	7 (*E*)-CH=CH-CO_2Me
3 (*E*)-CH=N-OMe	8 (*E*)-CH=CH-CH_2OH
4 (*Z*)-CH=N-OMe	9 (*E*)-CH=CH-$CO_2CH_2CH{=}CH_2$
5 (*E*)-CH=N-OH	10 (*E*)-CH=CH-CO_2H



R	R
11 CH_2OH	16 (*Z*)-CH=N-OH
12 CHO	17 $CH{=}CH_2$
13 (*E*)-CH=N-OMe	18 (*E*)-CH=CH-CO_2Me
14 (*Z*)-CH=N-OMe	19 (*E*)-CH=CH-CH_2OH
15 (*E*)-CH=N-OH	20 (*E*)-CH=CH-$CO_2CH_2CH{=}CH_2$



R
21 H
22 CO_2Me

TES: triethylsilyl Fmoc: 9-fluorenylmethoxycarbonyl

the (*E*)- and (*Z*)-methoximes (**13** and **14**) (isomer ratio 3:1) in 74% yield. The isomers could be separated by silica-gel chromatography and were distinguished by ^1H and ^{13}C NMR chemical shift differences [$\delta_{C\text{-}16}$ 51.2, $\delta_{H\text{-}16}$ 2.16, $\delta_{HC=N}$ 7.24 ppm for (*E*)-isomer; $\delta_{C\text{-}16}$ 47.7, $\delta_{H\text{-}16}$ 3.08, $\delta_{HC=N}$ 6.57 ppm for (*Z*)-isomer], consistent with literature observations for aldoximes[17,18]. A similar reaction of the aldehyde (**12**) with hydroxylamine hydrochloride gave the oximes (**15**, 93% and **16**, 4%), with a significantly lower proportion of the (*Z*)-isomer in this case.

The aldehyde (**12**) also successfully participated in Wittig reactions, although the phosphoranes employed were sufficiently basic to cause partial cleavage of the Fmoc group from the mycosamine amino function. Thus, reaction of methylenetriphenylphosphorane with **12** in benzene at room temperature for 3 hours gave a separable mixture of the fully protected C-16 vinyl derivative (**17**, 39%) and the corresponding free amine (**21**, 31%). The stabilised ylid, (methoxycarbonylmethylene)triphenylphosphorane, required 30 hours reaction with **12** in refluxing benzene to afford the unsaturated ester (**18**, 47%) along with the amine (**22**, 45%). Only the expected (*E*)-isomer was formed, as confirmed by the ^1H NMR coupling constant in **18** ($J_{CH=CH}$ 15.6 Hz). The free amines can be recycled to the *N*-Fmoc derivatives by reaction with *N*-(Fmoc-oxy)succinimide if desired. Selective reduction of the ester moiety in **18** was achieved with lithium borohydride to furnish the 3-hydroxypropenyl derivative (**19**) in 79% yield. Reduction of **18** with diisobutyl aluminium hydride was not selective and led to cleavage of the macrocyclic lactone in addition to ester reduction.

The oximes (**13**~**15**) and vinyl derivatives (**17**~**19**) were all deprotected to the corresponding amphotericin B analogues (**3**~**8**) in a three stage process. Desilylation of the hydroxyl groups with hydrogen fluoride-pyridine complex, followed by acid catalysed anomeric exchange of the 13-methoxyl function with water, and finally, removal of the *N*-Fmoc group with piperidine gave the fully deprotected compounds (**3**~**8**) in yields of 37~89% over the three steps. An attempt to liberate the free acid (**10**) by LiOH mediated hydrolysis of the methyl ester (**7**) was unsuccessful, though a similar conversion of amphotericin B methyl ester to its carboxylic acid has been reported[7]. This problem was circumvented by use of the allyl ester protecting group[10], which is conveniently removed in the presence of tetrakis(triphenylphosphine)palladium. Thus, the C-16 unsaturated allyl ester (**20**) was obtained by reaction of the aldehyde (**12**) with the appropriate phosphorane, and subjected to the above deprotection sequence to yield the unsaturated ester (**9**).

Deallylation of **9** with the catalytic Pd(0) and pyrrolidine afforded the C-16 unsaturated acid analogue (**10**) of amphotericin B in 49% yield.

Results and Discussion

In vitro antifungal activities of the amphotericin B C-16 oximes (**3**~**5**) and vinyl derivatives (**6**~**8** and **10**) against representative strains of *Candida*, *Cryptococcus* and *Aspergillus* are shown in Table 1. The hydroximino derivative (**5**) is more potent than amphotericin B (**1**), though the methoximes (**3** and **4**) are a little less active. Both the vinyl analogue (**6**) and the unsaturated ester (**7**) also compare favourably with amphotericin B, but whereas the unsaturated acid (**10**) has reduced activity compared to **1**, the alcohol (**8**) is close to the hydroxymethyl derivative (**2**) in potency.

Haemolytic activity of amphotericin B derivatives against mammalian erythrocytes has been used as one possible indicator of their potential toxicity[19]. Table 2 shows that most of the new derivatives are as haemolytic as amphotericin B or slightly less so, with the exception of the (*Z*)-methoxime (**4**) which is significantly less haemolytic than its (*E*)-isomer (**3**), whilst maintaining similar antifungal activity. These structure-activity relationships suggest that the desired level of antifungal potency and reduction in haemolytic activity might be achieved in the (*Z*)-isomer of the (*E*)-hydroxyiminomethyl derivative (**5**), but unfortunately this compound was not readily available owing to the very low (*Z*):(*E*)-isomer ratio of the intermediates (**15** and **16**). The least haemolytic of the vinyl derivatives is the unsaturated alcohol (**8**), which shows a similar *in vitro* profile to the alcohol (**2**) and thus may be worthy of further evaluation.

In summary, we have demonstrated the utility of the amphotericin B aldehyde (**12**) in extending the scope of C-16 derivatisation, and have further probed antifungal activity-selectivity relationships at this position. While a number of the new amphotericin B C-16 oximes and vinyl derivatives possess improved *in vitro* antifungal activity over the parent, the most active compounds (**5**~**7**) are also highly haemolytic, suggesting that their overall selectivity profiles will not offer further advantages over derivatives such as the previously reported C-16 hydroxymethyl compound (**2**)[14,15].

Experimental

Biological Assays

Antifungal activity (MIC): Compounds were diluted two-fold in SABOURAUD's liquid medium (Lab M) in wells of a microtitre plate. Wells were then inoculated with cell suspensions of *Candida albicans*, *Candida parapsilosis* or *Cryptococcus neoformans* to a final concentration of 10^5 cells/ml or with spore suspensions of *Aspergillus fumigatus* to a final concentration of 10^4 spores/ml. Total volume in each well was 100 μl. Plates were incubated at 37°C for 2 days and the turbidity of each well then assessed visually. MIC were taken as the lowest concentration of compound which produced marked inhibition of growth.

Haemolytic Activity (EH_{50}): Tests for haemolytic activity were performed with horse erythrocytes harvested by centrifugation from defibrinated blood. Erythrocytes were washed in phosphate-buffered saline (PBS) and then added to a two-fold dilution series of the compounds in PBS in microtitre plates, final concentration 3.3×10^7 cells/ml. Plates were incubated at 37°C for

Table 1. Antifungal activity *in vitro* of amphotericin B C-16 oximes and vinyl derivatives.

Organism	MIC (μg/ml)								
	(1)*	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(10)
Candida albicans 73/079	4	2	4	8	0.5	2	2	4	8
C. parapsilosis 937A	4	2	8	4	2	2	4	4	16
Cryptococcus neoformans 451	2	1	4	4	1	0.5	0.5	1	4
Aspergillus fumigatus	4	1	8	8	1	0.5	1	1	4

MIC: Minimum inhibitory concentration against inocula of 10^5 cells/ml organism (except 10^4 spores/ml *Aspergillus*) in SABOURAUD's broth, read after 48 hours at 37°C; microtitre. * Mean result from several tests in parallel with new derivatives.

Table 2. Haemolytic activity *in vitro* of amphotericin B C-16 oximes and vinyl derivatives.

	(1)*	(2)*	(3)	(4)	(5)	(6)	(7)	(8)	(10)
EH_{50} (μg/ml)	3.2	6.5	5.5	105	2.9	2.8	2.7	11.8	10.9

EH_{50}: Concentration of compound required to reduce by 50% turbidity (OD) of 3.3×10^7 cells/ml of horse erythrocytes in phosphate buffered saline. Read after 1 hour at 37°C; microtitre. * Mean result from several tests in parallel with new derivatives.

1 hour and then the turbidity of the cell suspensions measured at 492 nm in a microtitre plate reader. The concentration of compound which would cause a 50% decrease in turbidity of the whole cell suspension was then calculated and recorded as the EH_{50}.

Chemistry

Compounds were characterised by ^1H NMR, ^{13}C NMR, IR, UV, MS and HPLC. UV spectra were recorded on a Kontron Uvikon 810 spectrometer. IR spectra were recorded on either a Perkin-Elmer 298 or a Bio-Rad FTS-7 spectrometer. ^1H NMR spectra were recorded at 400 MHz on a Bruker AMX 400 instrument, with Me_4Si as internal reference. ^{13}C NMR were recorded at either 100.6 MHz on a Bruker AMX 400 instrument or at 67.8 MHz on a Jeol GX270 instrument. NMR assignments were made by comparison with the literature[10] and also by comparison with the absolute assignments made for compounds (**13** and **14**) by ^1H-^1H COSY, and for **13** by ^{13}C-^1H COSY. FAB mass spectra were recorded on a Jeol SX-102 mass spectrometer using a 3-nitrobenzyl alcohol-sodium (NOBA Na) matrix unless otherwise stated. Product purities were assayed by HPLC (non-silylated compounds, reverse phase: Waters Radial-Pak C-18 Nova-Pak column, variable ratios of 0.05 M NaH_2PO_4 buffer at pH 3.5 in methanol, 405 nm UV detection; silylated derivatives, normal phase: Waters Radial-Pak Nova-Pak silica column, variable ratios of ethyl acetate in *n*-hexane as mobile phase, 405 nm UV detection). Preparative chromatography was carried out using Kieselgel 60 (230~400 mesh ASTM).

Full experimental and spectroscopic data are provided below for representative Wittig and oxime forming reactions together with details for a typical deprotection sequence and for compounds (**10** and **19**). Variations in reaction conditions for other analogues are indicated below the appropriate experiments.

Representative Oxime Formation

N-(9-Fluorenylmethoxycarbonyl)-13-*O*-methyl-3,5,8,-9,11,15,35,2',4'-nona-*O*-triethylsilyl-16-decarboxy-16-hydroxyiminomethylamphotericin B. (*E*)- and (*Z*)-isomers (**15** and **16**)

A solution of the 16-formyl derivative[6] (**12**) (404 mg, 0.186 mmol) in EtOH (27 ml)-THF (9 ml) was treated with anhydrous sodium acetate (447 mg, 5.58 mmol) and hydroxylamine hydrochloride (120 mg, 1.86 mmol). The mixture was stirred at room temperature for two hours, and then filtered through Celite and the filtrate was concentrated. Purification by chromatography on silica gel (*n*-hexane-ethyl acetate, 9 : 1) gave two products. The faster running product was the (*E*)-oxime (**15**) (368 mg, 91%) and the slower running product was the (*Z*)-oxime (**16**) (21 mg, 5%).

(*E*)-Oxime (**15**), UV $\lambda_{max}^{n\text{-hexane}}$ nm ($E_{1\,cm}^{1\%}$) 408 (642), 384 (585), 365 (352), 346 (162); IR ν_{max} (thin film) cm^{-1} 3450~3400, 1735, 1715; ^1H NMR (acetone-d_6) δ 9.90 (1H, s, exchanges with D_2O, NOH), 7.87 (2H, d, J=7.5 Hz), 7.71 (2H, d, J=7.3 Hz), 7.43 (2H, t, J=7.5 Hz), 7.40 (1H, d, J=7.3 Hz, CH=N), 7.34 (2H, m), 6.54~6.11 (12H, series of m), 5.97 (1H, dd, J=15.6, 5.0 Hz, 20-H), 5.53 (1H, d, J=9.5 Hz, 3'-NH), 5.50 (1H, dd, J=14.9, 9.3 Hz, 33-H), 4.65 (2H, m, 19-H, 37-H), 4.56 (1H, s, 1'-H), 4.51 (1H, dd, J=10.5, 6.4 Hz, 1 of Fmoc-CH_2), 4.41 (1H, dd, J=10.5, 6.3 Hz, 1 of Fmoc-CH_2), 4.27~4.13 (4H, m, Fmoc-CH_2CH, 3-H, 11-H, 15-H), 4.03~3.93 (3H, m, 2'-H, 5-H,17-H), 3.86 (1H, m, 35-H), 3.73~3.56 (3H, m, 3'-H, 8-H, 9-H), 3.49 (1H, t, J=9.1 Hz, 4'-H), 3.34 (1H, dq, J=8.4, 6.2 Hz, 5'-H), 3.12 (3H, s, OCH_3), 2.59 (2H, d, J=7.3 Hz, 2-H), 2.44 (1H, m, 34-H), 2.32~2.16 (2H, m, 16-H, 18-H), 2.12~1.45 (14H, series of m), 1.25 (3H, d, J=6.1 Hz, 6'-CH_3), 1.18 (3H, d, J=6.0 Hz, 38-CH_3), 1.10~0.85 (87H, m, 39-CH_3, 40-CH_3 and $SiCH_2CH_3$ × 27), 0.82~0.45 (54H, m, $SiCH_2$ × 27); ^{13}C NMR (acetone-d_6) 170.5 (C-1), 157.1 (Fmoc C=O), 151.3 (CH=NOH), 145.0, 142.2, 139.5, 135.9, 135.7, 135.3, 134.5, 134.0, 132.44, 132.35, 132.2, 132.0, 131.3, 130.7, 128.5, 127.9, 125.9, 125.8, 120.8, 101.4, 98.4, 76.7, 76.3, 75.8, 74.7, 74.1, 73.7, 72.9, 71.2, 70.1, 67.6, 67.5, 67.4, 58.5, 51.0 (C-16), 48.2, 48.0, 45.9, 44.5, 43.5, 43.0, 41.6, 40.8, 35.6, 35.4, 27.6, 20.0, 19.3, 19.0, 11.2, 7.7~5.7 ($SiCH_2CH_3$); MS (NOBA Na matrix) *m/z* 2208.5 (MNa), 2185.5 (M, $C_{117}H_{212}N_2O_{18}Si_9$). (*Z*)-Oxime (**16**), UV $\lambda_{max}^{n\text{-hexane}}$, nm ($E_{1\,cm}^{1\%}$) 408 (555), 384 (526), 365 (328), 347 (157); IR ν_{max} (thin film) cm^{-1} 3445~3000, 1735; ^1H NMR (acetone-d_6) 10.20 (1H, s, exchanges with D_2O, NOH), 7.87 (2H, d, J=7.5 Hz), 7.69 (2H, d, J=7.5 Hz), 7.43 (2H, t, J=7.5 Hz), 7.34 (2H, t, J=7.4 Hz), 6.57~6.11 (13H, series of m), 6.50 (1H, dd, J=15.4, 5.8 Hz, 20-H), 5.51 (1H, dd, J=14.7, 9.5 Hz, 33-H), 5.34 (1H, d, J=9.8 Hz, NH), 4.77~4.64 (2H, m, 37-H, 19-H), 4.56 (1H, s, 1'-H), 4.49 (1H, dd, J=10.4, 6.4 Hz, 1 of Fmoc-CH_2), 4.36 (1H, dd, J=10.4, 6.4 Hz, 1 of Fmoc-CH_2), 4.29~4.09 (4H, m, Fmoc-CH_2CH, 3-H, 11-H, 15-H), 4.00 (1H, m, 5-H), 3.90~3.80 (3H, m, 2'-H, 17-H, 35-H), 3.75~3.58 (3H, m, 3'-H, 8-H, 9-H), 3.45 (1H, t, J=9.0 Hz, 4'-H), 3.30 (1H, m, 5'-H), 3.18 (1H, m, 16-H), 3.15 (3H, s, OCH_3), 2.57 (2H, d, J=6.5 Hz, 2-H), 2.44 (1H, m, 34-H), 2.17~1.48 (15H, series of m), 1.23 (3H, d, J=6.1 Hz, 6'-CH_3), 1.18 (3H, d, J=6.0 Hz, 38-CH_3), 1.10~0.83 (87H, series of m, 39-CH_3, 40-CH_3, $SiCH_2CH_3$ × 27), 0.80~0.46 (54H, series of m, $SiCH_2CH_3$ × 27); ^{13}C NMR (acetone-d_6) 170.7 (C-1), 156.4 (Fmoc C=O), 150.8 (CH=NOH), 145.1, 145.0, 142.2, 139.2, 135.5, 135.4, 134.7, 133.9, 133.4, 132.8, 132.7, 132.5, 132.3, 131.4, 131.3, 130.8, 128.5, 127.9, 125.8, 125.7, 120.8, 101.5, 98.9, 76.7, 74.5, 74.2, 74.0, 73.4, 73.3, 71.1, 69.0, 67.5, 67.4, 67.3, 58.2, 48.2, 48.1, 47.9, 46.8 (C-16), 45.5, 44.5, 43.5, 42.9, 41.6, 41.0, 36.8, 35.8, 27.5, 19.9, 19.2, 19.0, 11.3, 7.7~5.9 ($SiCH_2CH_3$); MS (NOBA Na matrix) *m/z* 2222.5 (MNa, $C_{118}H_{214}N_2O_{18}Si_9$+Na).

The methoximes (**13**) (55%) and (**14**) (19%) were prepared from (**12**) and methoxylamine hydrochloride in a similar fashion, but the reaction time was increased to

24 hours.

Representative Wittig Reaction

N-(9-Fluorenylmethoxycarbonyl)-13-*O*-methyl-3,5,8,-9,11,15,35,2',4'-nona-*O*-triethylsilyl-16-decarboxy-16-[(*E*)-2-methoxycarbonylvinyl]amphotericin B (**18**)

The 16-formyl derivative (**12**) (670 mg, 0.3 mmol) in benzene (50 ml) and (methoxycarbonylmethylene)triphenylphosphorane (2.5 g, 7.5 mmol), under nitrogen, were stirred under reflux for 30 hours. After cooling, the solvent was evaporated, *n*-hexane (300 ml) was added, triphenylphosphine oxide was removed by filtration, and the filtrate was concentrated. Purification of the residue by chromatography on silica gel (*n*-hexane - EtOAc, 9 : 1) gave the title product (**18**) (316 mg, 47%), UV $\lambda_{max}^{n\text{-hexane}}$ nm ($E_{1\,cm}^{1\%}$) 408 (570), 384 (540), 364 (330), 346 (165); IR ν_{max} (KBr) cm^{-1} 3400, 1730; ^1H NMR (acetone-d_6) characteristic signals include δ 7.86 (2H, d, J=7.4 Hz, Fmoc 4-H, 5-H), 7.69 (2H, d, J=7.4 Hz, Fmoc 1-H, 8-H), 7.42 (2H, d, J=7.4 Hz, Fmoc 3-H, 6-H), 7.35 (2H, d, J=7.4 Hz, Fmoc 2-H, 7-H), 6.72 (1H, dd, J=15.6, 10.1 Hz, C*H*=CHCO$_2$Me), 6.04 (1H, dd, J=15.6, 5.9 Hz, 20-H), 5.98 (1H, d, J=15.6 Hz, CH=C*H*CO$_2$Me), 5.50 (1H, dd, J=14.7, 9.5 Hz, 33-H), 5.32 (1H, d, J=9.8 Hz, 3'-NH), 4.70 (1H, m, 37-H), 4.60 (1H, m, 19-H), 4.53 (1H, b, 1'-H), 4.49 (1H, dd, J=10.3, 6.5 Hz, 1 of Fmoc-CH$_2$), 4.34 (1H, dd, J=10.3, 6.5 Hz, 1 of Fmoc-CH$_2$), 4.25 (2H, m, Fmoc-CH$_2$C*H*, 3-H), 4.20~3.99 (3H, m, 5-H, 11-H, 15-H), 3.92 (1H, b, 2'-H), 3.83 (1H, dd, J=8.9, 2.2 Hz, 35-H), 3.72 (3H, s, CO$_2$CH$_3$), 3.45 (1H, t, J=9 Hz, 4'-H), 3.25 (1H, m, 5'-H), 3.17 (3H, s, 13-OCH$_3$), 1.23 (3H, d, J=6.2 Hz, 6'-CH$_3$), 1.18 (3H, d, J=6.1 Hz, 38-CH$_3$), 1.10~0.85 (87H, m, SiCH$_2$C*H*$_3$ × 27, 39-CH$_3$, 40-CH$_3$), 0.75~0.50 (54H, m, SiCH$_2$ × 27); ^{13}C NMR (acetone-d_6) δ 170.7 (C-1), 166.3 (*C*O$_2$CH$_3$), 156.3 (Fmoc C=O), 145.1 (Fmoc C-1a, 8a), 142.2 (Fmoc C-4a, 5a), 139.3 (C-33), 135.5~130.0 (unassigned *C*H= and CH=*C*HCO$_2$CH$_3$), 128.5, 127.9 (Fmoc C-2, 7; 3, 6), 125.8 (Fmoc C-1, 8), 120.6 (Fmoc C-4, 5), 101.4 (C-13), 98.7 (C-1'), 76.7~67.4 (C-3, 5, 8, 9, 11, 15, 17, 19, 35, 37, 2', 4', 5'), 67.3 (Fmoc-CH$_2$), 58.1 (C-3'), 54.5, (C-16), 51.7 (CO$_2$*C*H$_3$), 48.3, 48.1, (13-OCH$_3$, Fmoc-CH$_2$*C*H), 47.9, 44.5, 43.5, 42.9, 41.6 (C-2, 4, 10, 12, 14), 45.6 (C-36), 41.0 (C-34), 36.5, 35.7 (C-6, 18), 27.5 (C-7), 19.9, 19.2, 17.0 (C-38, 40, 6'), 11.3 (C-39), 7.7~5.5 (Si*C*H$_2$*C*H$_3$); MS (NOBA Na matrix) m/z 2249.5 (MNa, $C_{120}H_{215}NO_{19}Si_9$+Na); Further elution gave the corresponding 3'-amino compound (**22**) (268 mg, 45%), MS (NOBA Na matrix) m/z 2027.5 (MNa, $C_{105}H_{205}NO_{17}Si_9$+Na).

The corresponding 16-vinyl compound (**17**) (39%) and 16-[(*E*)-2-allyloxycarbonylvinyl derivative (**20**) (36%) were prepared from (**12**) in a similar way with methylenetriphenylphosphorane (10 equiv., benzene, room temperature, 3 hours-generated from methyltriphenylphosphonium bromide and *n*-butyl lithium) and (allyloxycarbonylmethylene)triphenylphosphorane (29 equiv., benzene, reflux, 22 hours), respectively.

N-(9-Fluorenylmethoxycarbonyl)-13-*O*-methyl-3,5,8,-9,11,15,35,2',4'-nona-*O*-triethylsilyl-16-decarboxy-16-[(*E*)-3-hydroxypropenyl]amphotericin B (**19**)

The 16-[(*E*)-2-methoxycarbonylvinyl] derivative (**18**) (650 mg, 0.29 mmol) in dry ether (30 ml) under nitrogen was treated at room temperature with lithium borohydride (63 mg, 2.9 mmol). After 18 hours, the solution was cooled to 5°C and saturated ammonium chloride solution was added. EtOAc was added and the organic extract was washed with water and brine, dried ($MgSO_4$) and concentrated. Purification of the residue by chromatography on silica gel (*n*-hexane - EtOAc, 9 : 1) gave the title product (**19**) (503 mg, 79%), UV $\lambda_{max}^{n\text{-hexane}}$ nm ($E_{1\,cm}^{1\%}$) 408 (610), 384 (560), 365 (335), 346 (160); IR ν_{max} (KBr) cm^{-1} 3400, 1730; ^1H NMR (acetone-d_6) characteristic signals include δ 7.88 (2H, d, J=7.4 Hz, Fmoc 4-H, 5-H), 7.71 (2H, d, J=7.4 Hz, Fmoc 1-H, 8-H), 7.43 (2H, d, J=7.4 Hz, Fmoc 3-H, 6-H), 7.35 (2H, d, J=7.4 Hz, Fmoc 2-H, 7-H), 5.93 (1H, dd, J=15.7, 4.5 Hz, 20-H), 5.73 (1H, dt, J=15.3, 5.0 Hz, CH=C*H*-CH$_2$OH), 5.49 (1H, dd, J=14.9, 9.9 Hz, 33-H), 5.44 (1H, d, J=10.1 Hz, 3'-NH), 5.40 (1H, dd, J=15.3, 9.5 Hz, C*H*=CHCH$_2$OH), 4.7~4.6 (2H, m, 19-H, 37-H), 4.54 (1H, b, 1'-H), 4.53 (1H, dd, J=10.5, 6.3 Hz, 1 of Fmoc-CH$_2$), 4.39 (1H, dd, J=10.5, 6.3 Hz, 1 of Fmoc-CH$_2$), 3.95 (1H, b, 2'-H), 3.87 (1H, dd, J=8.9, 2.6 Hz, 35-H), 3.80~3.60 (5H, m, CH=CHC*H*$_2$OH, 8-H, 9-H, 3'-H), 3.46 (1H, t, J=9.0 Hz, 4'-H), 3.10 (3H, s, 13-OCH$_3$), 1.24 (3H, d, J=6.0 Hz, 6'-CH$_3$), 1.18 (3H, d, J=6.0 Hz, 38-CH$_3$), 1.15~0.85 (87H, m, SiCH$_2$C*H*$_3$ × 27, 39-CH$_3$, 40-CH$_3$), 0.80~0.55 (54H, m, SiCH$_2$ × 27); ^{13}C NMR (acetone-d_6) δ 170.5 (C-1), 157.0 (Fmoc C=O), 145.1, 145.0 (Fmoc C-1a, 8a), 142.3 (Fmoc C-4a, 5a), 139.5 (C-33), 135~130 (unassigned *C*H= and CH=*C*HCH$_2$-OH), 128.5, 127.9 (Fmoc C-2, 7; 3, 6), 125.9 (Fmoc C-1, 8), 120.8 (Fmoc C-4, 5), 101.3 (C-13), 98.5 (C-1'), 76.7~67.5 (C-3, 5, 8, 9, 11, 15, 17, 19, 35, 37, 2', 4', 5'), 67.4 (Fmoc-CH$_2$), 62.8 (CH=CH*C*H$_2$OH), 58.3 (C-3'), 54.6 (C-16), 49.1 (13-OCH$_3$, Fmoc-CH$_2$*C*H), 49.2, 44.6, 43.5, 42.8, 41.8 (C-2, 4, 10, 12, 14), 46.0 (C-36), 40.8 (C-34), 35.6, 35.2 (C-6, 18), 27.7 (C-7), 20.1, 19.4, 19.0 (C-38, 40, 6'), 11.2 (C-39), 7.7~5.8 (Si*C*H$_2$*C*H$_3$); MS (NOBA Na matrix) m/z 2221.5 (MNa, $C_{119}H_{215}NO_{18}Si_9$+Na).

Representative Deprotection Sequence

16-Decarboxy-16-(*E*)-hydroxyiminomethylamphotericin B (**5**)

A mixture of the (*E*)-oxime (**15**), (368 mg, 0.168 mmol) in THF (4 ml) - MeOH (1 ml) and hydrogen fluoride-pyridine (7 ml of a solution of 11.4 g of hydrogen fluoride-pyridine and 80 ml of pyridine made up to 200 ml with THF) was stirred overnight at room temperature in a plastic bottle. The solution was added to ether-*n*-hexane (500 ml, 1 : 1) and the precipitated product was collected by filtration, washed with ether and dried.

This product was stirred at room temperature in THF (6 ml) - water (1 ml) with pyridinium *p*-toluenesulfonate (273 mg, 1.09 mmol). After 1.75 hours, triethylamine (144 mg, 0.198 ml, 1.42 mmol) was added and the THF was removed on the rotary evaporator. The aqueous residue was added to water (600 ml) and the precipitated product was collected by filtration, washed with water and dried. The resulting product was stirred at room temperature in DMSO (7 ml) - MeOH (2 ml) and treated with piperidine (23 mg, 0.027 ml, 0.269 mmol). After 1.5 hours, the mixture was diluted with MeOH (5 ml) and added to ether (1 liter). The precipitated product was collected by filtration, washed with ether and dried. Purification by chromatography on silica gel (eluting with the lower phase of a 4:3:3 mixture of chloroform - methanol - 0.880 ammonia solution) gave the title compound (**5**) as a yellow powder (118 mg, 76% from **15**), UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 406 (1,602), 382 (1,416), 363 (869), 345 (393); IR ν_{max} (KBr) cm^{-1} 3415 (broad), 1713, 1631; ^1H NMR (1:1, methanol-d_4 - pyridine-d_5) δ 7.59 (1H, d, J=8.0 Hz, CH=N), 6.67~6.25 (13H, series of m), 5.64 (1H, m, 37-H), 5.49 (1H, dd, J=14.8, 10.2 Hz, 33-H), 4.80 (1H, s, 1'-H), 4.73 (1H, m, 19-H), 4.69~4.60 (2H, m, 11-H, 17-H), 4.50~4.37 (2H, m, 3-H, 15-H), 4.23 (1H, d, J=3.0 Hz, 2'-H), 3.96 (1H, m, 5-H), 3.86 (1H, d, J=10.8 Hz, 9-H), 3.58 (1H, t, J=9.3 Hz, 4'-H), 3.53 (1H, m, 5'-H), 3.44 (1H, m, partially masked by solvent, 8-H), 3.36 (1H, dd, J=9.5, 1.8 Hz, 35-H), 3.05 (1H, dd, J=9.5, 3.1 Hz, 3'-H), 2.64~2.30 (6H, series of m), 2.18~1.38 (13H, series of m), 1.43 (3H, d, J=5.9 Hz, 6'-CH_3), 1.36 (3H, d, J=6.4 Hz, 38-CH_3), 1.24 (3H, d, J=6.4 Hz, 40-CH_3), 1.17 (3H, d, J=7.2 Hz, 39-CH_3); ^{13}C NMR (1:1, methanol-d_4 - pyridine-d_5) 172.4 (C-1), 151.6 (CH=NOH), 137.6, 137.3, 134.9, 134.8, 134.3, 134.2, 134.0, 133.9, 133.8, 133.6, 133.5, 133.3, 133.0, 130.6, 98.9, 98.6, 79.1, 77.2, 76.3, 75.3, 74.7, 74.1, 72.4, 71.2, 70.5, 69.8, 68.7, 67.0, 66.5, 57.6, 52.0 (C-16), 47.6, 45.5, 44.9, 44.0, 42.8, 41.3, 40.9, 37.8, 36.3, 31.6, 19.1, 18.3, 17.3, 12.6; MS (thiodiethanol Na matrix) *m/z* 945.5 (MNa, $C_{47}H_{74}N_2O_{16}$+Na).

Compounds **3** (80%), **4** (52%), **6** (53%), **7** (65%), **8** (37%) and **9** (89%) were prepared in an analogous manner from the fully protected intermediates **13**, **14**, **17**, **18**, **19** and **20**, respectively.

16-[(*E*)-2-Carboxyvinyl]-16-decarboxyamphotericin B (**10**)

The allyl ester (**9**) (60 mg, 0.06 mmol) in THF (4 ml) - MeOH (0.4 ml) - DMSO (3 drops), flushed with nitrogen, was treated at room temperature with pyrrolidine (0.022 ml, 0.258 mmol) and tetrakis(triphenylphosphine)-palladium (7.0 mg, 0.006 mmol). After 1 hour the THF was evaporated and the mixture was poured into ether (200 ml). The precipitate was filtered, washed with acetone and ether and dried. Purification of the residue by chromatography on silica (chloroform - MeOH - 0.880 ammonia solution, 2:2:1) gave the title product (**10**) (28 mg, 49%), UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 405 (1,120), 382 (990), 363 (602), 344 (280); IR ν_{max} (KBr) cm^{-1} 3400, 1717; ^1H NMR (3:1, DMSO-d_6 - methanol-d_4) characteristic signals include δ 6.00 (1H, dd, J=15.0, 9.0 Hz, 20-H), 5.88 (1H, d, J=15.6 Hz, CH=C*H*CO_2H), 5.42 (1H, dd, J=14.2, 10.2 Hz, 33-H), 5.28 (1H, m, 37-H), 4.43 (1H, s, 1'-H), 3.89 (1H, b, 2'-H), 1.20 (3H, d, J=5.6 Hz, 6'-CH_3), 1.14 (3H, d, J=6.2 Hz, 38-CH_3), 1.07 (3H, d, J=6.2 Hz, 40-CH_3), 0.95 (3H, d, J=6.9 Hz, 39-CH_3); ^{13}C NMR (3:1, DMSO-d_6 - methanol-d_4) δ 171.2, 170.6 (C-1, CO_2H), 143.4 (*C*H=CHCO$_2$H), 137~129.7 (unassigned *C*H= and CH=*C*HCO$_2$H), 97.6 (C-1'), 97.5 (C-13), 77.9~66.3 (C-3, 5, 8, 9, 11, 15, 17, 19, 35, 37, 2', 4', 5'), 56.0, 53.4 (C-16, 3'), 46.7, 44.8, 44.7, 42.3 (C-2, 4, 12, 14), 43.1 (C-36), 40.2 (C-34), 39.9, 37.3, 35.5 (C-6, 10, 18), 29.9 (C-7), 18.7, 17.8, 17.0 (C-38, 40, 6'), 12.2 (C-39); MS (NOBA Na matrix) *m/z* 972.5 (MNa, $C_{49}H_{75}NO_{17}$+Na).

Acknowledgements

We wish to thank P. HUNTER, E. CARTER, R. NICHOLAS, P. MURDOCK, L. WILLIAMS and G. SCOTT-WOOD for the biological results. We are indebted to S. A. READSHAW for valuable assistance in the interpretation of NMR spectra.

References

1) GALLIS, H. A.; R. H. DREW & W. W. PICKARD: Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. 12: 308~329, 1990
2) BRAJTBURG, J.; W. G. POWDERLY, G. S. KOBAYASHI & G. MEDOFF: Amphotericin B: delivery systems. Antimicrob. Agents Chemother. 34: 381~384, 1990
3) SCHAFFNER, C. P.: Amphotericin B derivatives. *In* Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents. *Ed.*, R. A. FROMTLING, pp. 595~618, Prous Science Publishers, Barcelona, 1987
4) CHERON, M.; B. CYBULSKA, J. MAZERSKI, J. GRZYBOWSKA, A. CZERWINSKI & E. BOROWSKI: Quantitative structure-activity relationships in amphotericin B derivatives. Biochem. Pharmacol. 37: 827~836, 1988
5) HOEPRICH, P. D.; N. M. FLYNN, M. M. KAWACHI, K. K. LEE, R. M. LAWRENCE, L. K. HEATH & C. P. SCHAFFNER: Treatment of fungal infections with semi-synthetic derivatives of amphotericin B. Ann. N.Y. Acad. Sci. 544: 517~546, 1988
6) MACPHERSON, D. T.; D. F. CORBETT, B. J. COSTELLO, M. J. DRIVER, A. R. GREENLEES, W. S. MACLACHLAN, C. T. SHANKS & A. W. TAYLOR: Adventures in polyene macrolide chemistry: the derivatisation of amphotericin B. *In* Recent Advances in the Chemistry of Anti-infective Agents. *Ed.*, P. H. BENTLEY & R. PONSFORD, pp. 205~222, Royal Society of Chemistry Special Publication No. 119, 1993
7) NICOLAOU, K. C. & W. W. OGILVIE: The total synthesis of amphoteronolide B and amphotericin B. Chemtracts-Organic Chemistry 3: 327~349, 1990
8) DRIVER, M. J.; A. R. GREENLEES, W. S. MACLACHLAN, D. T. MACPHERSON & A. W. TAYLOR: Synthesis of 16-decarboxy-16-hydroxymethyl amphotericin B—a novel antifungal agent. Tetrahedron Lett. 33: 4357~4360, 1992

9) DRIVER, M. J.; A. R. GREENLEES & D. T. MACPHERSON: A versatile strategy for the derivatisation of the carboxy group of amphotericin B: the preparation of C-16 ketone analogues. J. Chem. Soc., Perkin Trans. 1 1992: 3155~3157, 1992
10) TAYLOR, A. W.; B. J. COSTELLO, P. A. HUNTER, W. S. MACLACHLAN & C. T. SHANKS: Synthesis and antifungal selectivity of new derivatives of amphotericin B modified at the C-13 position. J. Antibiotics 46: 486~493, 1993
11) COSTELLO, B. J.; M. J. DRIVER, W. S. MACLACHLAN & A. W. TAYLOR: Stereoselective syntheses of the 14-hydroxy epimers of amphotericin B methyl ester. J. Chem. Soc., Perkin Trans. 1 1993: 1829~1830, 1993
12) TAYLOR, A. W. & D. T. MACPHERSON: Preparation of a novel C-13 thioacetal derivative of amphotericin B. Tetrahedron Lett. 35: 5289~5292, 1994
13) CZERWINSKI, A.; T. ZIENIAWA, E. BOROWSKI & L. G. MICOSSI: Amphotericin B 2-morpholinoethylamide diaspartate, a new water soluble derivative of the antibiotic. Synthesis and biological properties. J. Antibiotics 43: 680~683, 1990
14) HUNTER, P.; E. CARTER, P. MURDOCK, L. WILLIAMS & G. SCOTT-WOOD: Comparative activity of a new polyene, BRL 49594A and amphotericin B against fungal and mammalian cells. Abstracts of Papers of 32nd Intersci. Conf. on Antimicrob. Agents Chemother., No. 1042, Anaheim, California, 1992
15) HUNTER, P.; P. MURDOCK, G. RANDALL, S. ANTHONY, S. JONES, A. EVERETT, C. WINCH & I. BURBIDGE: Comparative activity *in vivo* and toxicity of a new polyene, BRL 49594A and amphotericin B. Abstracts of Papers of 32nd Intersci. Conf. on Antimicrob. Agents Chemother., No. 1043, Anaheim, California, 1992
16) CZERWINSKI, A.; W. A. KÖNIG, P. SOWINSKI, L. FALKOWSKI, J. MAZERSKI & E. BOROWSKI: Amphotericin B *O*-methyl oxime. Synthesis and biological properties. J. Antibiotics 43: 1098~1100, 1990
17) KARABATSOS, G. J. & R. A. TALLER: Structural studies by nuclear magnetic resonance—XV. Conformations and configurations of oximes. Tetrahedron 24: 3347~3360, 1968
18) HAWKES, G. E.; K. HERWIG & J. D. ROBERTS: Nuclear magnetic resonance spectroscopy. Use of ^{13}C spectra to establish configurations of oximes. J. Org. Chem. 39: 1017~1028, 1968
19) HAMMOND, S. M.: Biological activity of polyene antibiotics. Prog. Med. Chem. 14: 105~179, 1977