# SYNTHESIS AND ANTIFUNGAL SELECTIVITY OF NEW DERIVATIVES OF AMPHOTERICIN B MODIFIED AT THE C-13 POSITION

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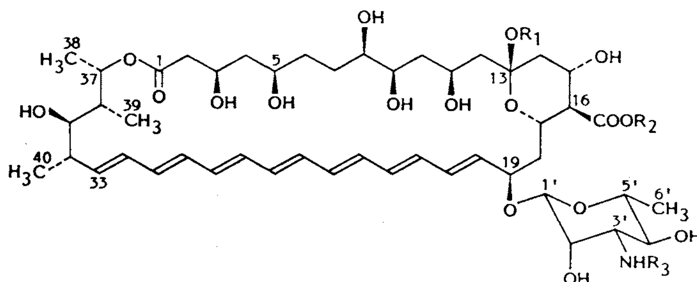
The syntheses of the first amphotericin B derivatives to be modified solely at the C-13 hemiketal position are described. Selective functionalisation at this position is facilitated by use of the allyl ester as a C-16 carboxylate protecting group on the amphotericin B nucleus. In *in vitro* tests all compounds showed markedly reduced haemolytic activity against mammalian erythrocytes while two of the novel 13-alkoxy derivatives retained good antifungal activity.

The polyene macrolide antibiotic amphotericin B[1] (**1**) is still the drug of choice for the treatment of many serious systemic fungal infections in man[2], particularly with immunocompromised patients. However, its therapeutic utility is impaired by a variety of severe side effects, especially nephrotoxicity[3].

A fundamental cause of the drug's insufficient antifungal selectivity is its ability to perturb both fungal and mammalian cellular membranes by interaction with endogenous sterols[3], principally ergosterol in fungi and cholesterol in mammals. Over the last twenty years a range of amphotericin B analogues[4] have been prepared with the primary aim of reducing the toxicity of the parent antibiotic. Chemical manipulation has concentrated almost exclusively on both the 16-carboxylate functionality, which has been derivatised as esters, amides[5] and a hydrazide[6], and on the mycosamine amino group, where a variety of modifications have been carried out including the preparation of *N*-alkyl[7] and *N*-acyl[8] analogues. Many of these derivatives have demonstrated reduced haemolytic activity against mammalian erythrocytes compared to amphotericin B in tests *in vitro* and two compounds in particular, amphotericin B methyl ester (**2**)[9] and *N*-ornithyl amphotericin B methyl ester[10] (**3**) were markedly less




1 $R_1 = R_2 = R_3 = H$
2 $R_1 = H$, $R_2 = CH_3$, $R_3 = H$
3 $R_1 = H$, $R_2 = CH_3$, $R_3 =$ D-ornithyl
4 $R_1 = CH_3$, $R_2 = R_3 = H$
5 $R_1 = CH_2CH_2OH$, $R_2 = R_3 = H$
6 $R_1 = CH_2CH_2CH_3$, $R_2 = R_3 = H$
7 $R_1 = H$, $R_2 = CH_3$, $R_3 = COCH_3$

nephrotoxic both in animal models and, in the case of **2**, in clinical trials. However, both compounds have been rejected clinically, primarily because of neurotoxicity.

Chemical modification at other positions in the amphotericin B molecule have involved macrocyclic degradation, masking of the polyhydroxy moiety[11~13], removal of the polyene unit[14] and opening of the tetrahydropyranyl ring[15]. All these operations radically alter the structural components of the molecule regarded as optimal for antifungal activity and no compounds of pharmaceutical interest have emerged from them.

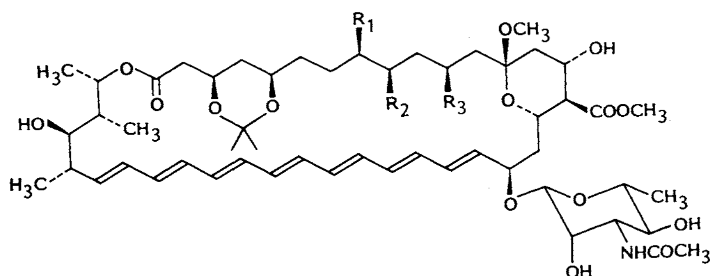
In this report we describe the synthesis of the first amphotericin B analogues exclusively functionalised at the C-13 position. These derivatives (**4**, **5**, **6** and **17**), all of which retain the parent mycosamine and 16-carboxylate groups, show interesting antifungal activity combined with a pronounced reduction in haemolytic activity against mammalian erythrocytes *in vitro*.

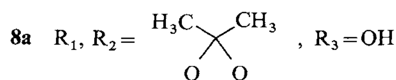
## Synthesis

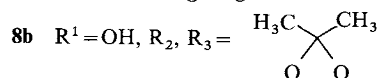
The polyhydroxy portion of the macrocyclic ring of amphotericin B is thought to be actively involved in the disruption of eukaryotic membranes, both fungal and mammalian, though the detailed biochemical role of this hydrophilic part of the antibiotic has not been rigorously established[14,16]. We aimed to modify this C-3 to C-14 polyol moiety at one defined chemically practical position, and to explore the effect of such structural changes on biological properties.

The C-13 hemiketal stands out as the one hydroxyl in this part of the molecule at which selective reaction should be readily achieved. *N*-Acetyl amphotericin B methyl ester (**7**) had been converted to the bis-acetonide 13-methyl ketals[12] (**8a** and **8b**) and also to the 13-anhydro derivative[17] (**19**) *via* persilyl intermediate (**18**). However, the functionalities masking the 3′-amino and 16-carboxyl groups of these derivatives (**8** and **19**) cannot be cleanly removed from the macrolide nucleus. We required more readily removable protecting groups for these two positions, as masking of the 3′-amino group facilitates acid-catalysed anomeric exchange at the hemiketal (C-13), and esterification of the 16-carboxylate group facilitates chromatographic purification of amphotericin derivatives in general. We chose the 3′-*N*-9-fluorenylmethoxycarbonyl (*N*-Fmoc) and 16-allyl ester groups respectively prior to carrying out a series of C-13 hemiketal modifications, as follows.

Reaction of *N*-Fmoc amphotericin B[18] (**9**) with allyl bromide and Hunig's base gave the allyl ester

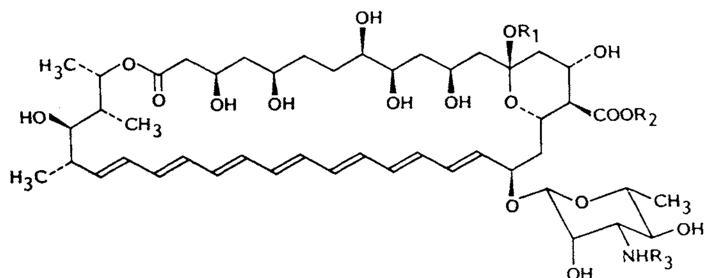




**8a** $R_1$, $R_2$= $H_3C$ $CH_3$ O O , $R_3$=OH

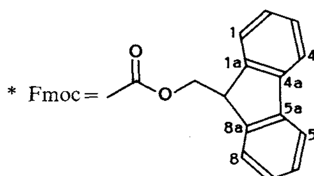

**8b** $R^1$=OH, $R_2$, $R_3$= $H_3C$ $CH_3$ O O

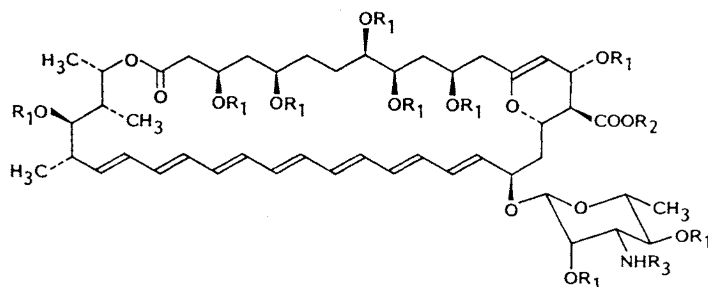
(**10**), which was converted by acid-catalysed anomeric exchange with the appropriate alcohol in tetrahydrofuran to the 13-methoxy (**11**), 13-hydroxyethoxy (**12**) and 13-propyloxy (**13**) ketals. In turn, each of these derivatives was deprotected to the corresponding free 3′-amino, 16-carboxylate analogues (**4**, **5** and **6**), firstly by removal of the *N*-Fmoc group with piperidine to give intermediates (**14**, **15** and **16**), and finally by cleavage of the allyl ester with pyrrolidine and catalytic tetrakis (triphenylphosphine) palladium[19]. This last procedure had not been utilised previously in polyene macrolide chemistry.

Reaction of 13-hydroxy allyl ester (**10**) with trimethylsilyl trifluoromethanesulphonate and




**9** $R_1 = R_2 = H$, $R_3 = Fmoc$*
**10** $R_1 = H$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**11** $R_1 = CH_3$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**12** $R_1 = CH_2CH_2OH$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**13** $R_1 = CH_2CH_2CH_3$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**14** $R_1 = CH_3$, $R_2 = CH_2CH{=}CH_2$, $R_3 = H$
**15** $R_1 = CH_2CH_2OH$, $R_2 = CH_2CH{=}CH_2$, $R_3 = H$
**16** $R_1 = CH_2CH_2CH_3$, $R_2 = CH_2CH{=}CH_2$, $R_3 = H$




**17** $R_1 = R_2 = R_3 = H$
**18** $R_1 = Si(CH_3)_3$, $R_2 = CH_3$, $R_3 = COCH_3$
**19** $R_1 = H$, $R_2 = CH_3$, $R_3 = COCH_3$
**20** $R_1 = Si(CH_3)_3$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**21** $R_1 = H$, $R_2 = CH_2CH{=}CH_2$, $R_3 = Fmoc$
**22** $R_1 = H$, $R_2 = CH_2CH{=}CH_2$, $R_3 = H$

Table 1. Antifungal activity *in vitro* of C-13 modified amphotericin B analogues.

| Organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | (1) | (4) | (5) | (6) | (17) |
| *Candida albicans* 73/079 | 1 | 1 | 2 | 16 | 16 |
| *C. parapsilosis* 937A | 2 | 8 | 4 | >32 | 16 |
| *Cryptococcus neoformans* 451 | 0.5 | 1 | 1 | 8 | 4 |
| *Aspergillus niger* 57A | 2 | 2 | 8 | 16 | 8 |
| *A. fumigatus* | 2 | 2 | 8 | 8 | 8 |

MIC: Minimum inhibitory concentration against inocula of $10^3$ cells/ml organism (except $10^4$ spores/ml *Aspergillus*) in SABOURAUD's broth, read after 2 days at 37°C; microtitre.

Table 2. Haemolytic activity *in vitro* of C-13 modified amphotericin B analogues.

| | (1) | (4) | (5) | (6) | (17) |
|---|---|---|---|---|---|
| $EH_{50}$ (μg/ml) | 1 to 3 | 42 | >256 | >256 | 185 |

$EH_{50}$: Concentration of compound required to reduce by 50% turbidity (OD) of $3.3 \times 10^7$ cells/ml of horse erythrocytes in phosphate buffered saline. Read after 1 hour at 37°C; microtitre.

2,6-lutidine[17] effected dehydration of the hemiketal concomitant with silylation of all other hydroxyl groups. The reaction product (**20**) was directly desilylated (hydrogen fluoride in pyridine: tetrahydrofuran) to the 13-anhydro derivative (**21**). Subsequent removal of the *N*-Fmoc and allyl ester protecting groups as above afforded the parent 13-anhydroamphotericin B (**17**) *via* intermediate ester (**22**).

## Results and Discussion

The antifungal activity *in vitro* of the C-13 modified amphotericin B analogues (**4**, **5**, **6** and **17**) is shown as MIC data in Table 1. Their haemolytic activity against mammalian erythrocytes, an indication of their potential mammalian toxicity[14], is shown in Table 2. Most notable is the markedly reduced haemolytic activity of all the derivatives compared to amphotericin B (**1**) itself, with two of the compounds inert under the test conditions. While one of these, the 13-*O*-propyl derivative (**6**) shows much reduced activity *in vitro* against the test fungi, the 13-*O*-methyl (**4**) and 13-*O*-hydroxyethyl (**5**) analogues possess good antifungal activity, approximately 2-fold less than that of amphotericin B (**1**). These compounds are the first amphotericin B analogues which both retain the 3′-amino, 16-carboxylate zwitterion of amphotericin B and show significantly greater antifungal selectivity than the parent molecule. It is also of interest that unlike amphotericin B (**1**), the 13-*O*-methyl derivative (**4**) possessed considerable water solubility (≥30 mg/ml).

## 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 parasilosis* or *Cryptococcus neoformans* to a final concentration of $10^3$ cells/ml, or with spore suspensions of *Aspergillus fumigatus* or *Aspergillus niger* 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 2-fold dilution series of the compounds in PBS in microtitre plates, final concentration $3.3 \times 10^7$ cells/ml. Plates were incubated at 37°C for 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

UV spectra were recorded on a Kontron Uvikon 810 spectrometer. IR spectra were recorded on a Bio-Rad FTS-7 spectrometer. NMR spectra were recorded at 400 MHz ($^1$H) and 100 MHz ($^{13}$C D.E.P.T135) on a Bruker AM 400 MHz instrument, with $Me_4Si$ as internal reference. FAB mass spectra were recorded on a JEOL SX-102 mass spectrometer. Product purities were also assayed by HPLC (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; normal phase: Waters Radial-Pak Nova-Pak column, 405 nm UV detection, ratios of ethyl acetate in *n*-hexane as mobile phase). Preparative chromatography was carried out using Kieselgel 60 (finer than 230 mesh ASTM) at medium pressure.

Full spectroscopic and experimental data are provided below for the complete synthetic route to 13-*O*-(2-hydroxyethyl)amphotericin B (**5**). NMR assignments are made not only by comparison with the established literature[20] but also by comparison with the absolute assignments made for compound (**15**) by 2D methods ($^1$H and $^{13}$C-$^1$H COSY). The corresponding 13-*O*-methyl (**4**) and 13-*O*-propyl (**6**) analogues were synthesised and fully characterised by essentially the same methods, using the appropriate alcohol as cosolvent during the anomeric exchange reaction, in place of ethylene glycol. The conversion of 13-anhydro intermediate (**21**) to the parent derivative (**17**) again parallelled the described deprotection of 13-*O*-(2-hydroxyethyl) intermediate (**12**) to the target compound (**5**). The dehydration of 13-hydroxy derivative (**10**) to the corresponding 13-anhydro intermediate (**21**) is detailed with full spectroscopic data at the close of this section, below. NMR assignments were again correlated with further 2D data obtained for the 16-methoxycarbonyl analogue of **21**.

Representative Synthetic Route to a 13-Alkoxy Amphotericin B Derivative, 13-*O*-(2-Hydroxyethyl)amphotericin B (**5**)

*N*-(9-Fluorenylmethoxycarbonyl)amphotericin B, Allyl Ester (**10**)

*N*-(9-Fluorenylmethoxycarbonyl)amphotericin B (**9**)[18] (5.88 g, 5.13 mmol) in DMF (150 ml) was sequentially treated with methanol (15 ml), diisopropylethylamine (3.8 ml, 21.5 mmol) and allyl bromide (15 ml), and the mixture stirred for 4 hours. The solution was added to ether (4 litres) and the precipitate filtered. The solids were then stirred for 10 minutes in water and refiltered and dried *in vacuo* to give a residue purified by chromatography on silica (methylene chloride - MeOH, 10 : 1), affording title product (**10**) (3.40 g, 56%), UV $\lambda_{max}^{MeOH}$ nm (ε) 404 (130,000), 381 (116,000), 362 (72,000); IR $\nu_{max}$ (Nujol) cm$^{-1}$ 3400, 1725, 1705; $^1$H NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$) characteristic signals include δ 1.16 (3H, d, $J$=7.1 Hz, 39-$CH_3$), 1.24 (3H, d, $J$=6.4 Hz, 40-$CH_3$), 1.35 (3H, d, $J$=6.4 Hz, 38-$CH_3$), 1.48 (3H, d, $J$=6.05 Hz, 6'-$CH_3$), 2.36 (1H, dd, $J$=16.8, 2.75 Hz, 1 of 2-H), 2.49 (1H, dd, $J$=16.8, 9.6 Hz, 1 of 2-H), 2.60 (1H, t, $J$=10.4 Hz, 16-H), 3.37 (1H, dd, $J$=9.5, 1.9 Hz, 35-H), 3.59 (1H, m, 5'-H), 3.76 (1H, t, $J$=9.7 Hz, 4'-H), 3.86 (1H, m, 9-H), 3.96 (1H, m, 5-H), 4.07 (1H, dd, $J$=10.2, 2.9 Hz, 3'-H), 4.23 (2H, m, 2'-H and Fmoc -$OCH_2$*CH*), 4.36 (2H, m, Fmoc -*O*$CH_2$), 4.78 (1H, s, 1'-H), 5.25 (1H, br d, $J$=10.5 Hz, 1 of allyl -CH=$CH_2$), 5.5 (2H, complex, 33-H and 1 of allyl -CH=$CH_2$), 5.63 (1H, m, 37-H), 6.04 (1H, m, allyl -$CH_2$-*CH*=), 7.30 (2H, t, $J$=7.4 Hz, Fmoc 2, 7-H), 7.42 (2H, t, $J$=7.5 Hz, Fmoc 3, 6-H), 7.71 (2H, m, Fmoc 1, 8-H), 7.84 (2H, d, $J$=7.5 Hz, Fmoc 4, 5-H); $^{13}$C NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$) δ 12.57 (C-39), 17.33, 18.49, 19.18 (C-38, 6', 40), 31.52 (C-7), 36.35 (C-6), 38.93 (C-18), 40.89 (C-10), 41.41, 44.08 (C-34, 36), 42.81, 44.83, 45.26 47.57 (C-2, 4, 12, 14), 48.25 (Fmoc -$OCH_2$*CH*), 58.32, 58.46 (C-3', 16), 67.58, 68.50 (Fmoc and allyl -$CO_2CH_2$), 66.8~77.5 (C-3, 5, 8, 9, 11, 15, 17, 19, 37, 2', 4', 5'), 79.33 (C-35), 98.80 (C-13), 99.04 (C-1'), 118.63 (allyl CH=$CH_2$), 120.83 (Fmoc C-4, 5), 126.23 (Fmoc C-1, 8), 128.03, 128.61 (Fmoc C-2, 7; 3, 6), 130.61~135.03 (unassigned -*CH*=), 137.35, 137.59 (C-20, 33), 142.26 (Fmoc C-4a, 5a), 145.10, 145.20 (Fmoc C-1a, 8a), 158.30 (Fmoc *C*=*O*), 172.52 (C-1), 173.93 (allyl $CO_2$); MS (NOBA Na matrix) *m*/*z* 1,208 (MNa, $C_{65}H_{87}NO_{19}$+Na).

*N*-(9-Fluorenylmethoxycarbonyl)-13-*O*-(2-hydroxyethyl)amphotericin B, Allyl Ester (**12**)

The allyl ester (**10**) (2.51 g, 2.16 mmol) in dry THF (25 ml): ethylene glycol (25 ml) was treated in an ice-bath with camphorsulphonic acid (0.145 g, 0.58 mmol). After 30 minutes stirring, ethyl acetate and

sodium bicarbonate solution were added. The organic layer was separated, water-washed, dried ($Na_2SO_4$) and evaporated to give a residue which was chromatographed on silica (methylene chloride - MeOH, 10 : 1) to give the title product (**12**) (1.31 g, 50%), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) 406 (96,000), 383 (88,000), 363 (58,000); IR $\nu_{max}$ (THF) $cm^{-1}$ 3400~3500, 1725; $^1H$ NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$) characteristic signals include $\delta$ 1.15 (3H, d, $J$=7.1 Hz, 39-$CH_3$), 1.25 (3H, d, $J$=6.5 Hz, 40-$CH_3$), 1.34 (3H, d, $J$=6.3 Hz, 38-$CH_3$), 1.48 (3H, d, $J$=5.9 Hz, 6'-$CH_3$), 2.42 (1H, dd, $J$=16.6, 3.6 Hz, 1 of 2-H), 2.53 (1H, dd, $J$=16.6, 8.7 Hz, 1 of 2-H), 2.64 (1H, t, $J$=10.6 Hz, 16-H), 3.76 (1H, t, $J$=9.6 Hz, 4'-H), 3.98 (1H, m, 5-H), 4.07 (1H, dd, $J$=10.2, 2.9 Hz, 3'-H), 4.23 (2H, m, 2'-H and Fmoc -$OCH_2$*CH*), 4.38 (2H, d, $J$=7.7 Hz, Fmoc -*$OCH_2$*), 4.78 (1H, s, 1'-H), 5.29 (1H, br d, $J$=10.5 Hz, 1 of allyl -CH=*$CH_2$*), 5.41 (1H, m, 37-H), 5.49 (1H, dm, $J_{max}$=17 Hz, 1 of allyl -CH=*$CH_2$*), 5.69 (1H, dd, $J$=14.4, 9.3 Hz, 33-H), 6.04 (1H, m, allyl -$CH_2$-*CH*=), 6.15 (1H, dd, $J_{max}$=14.4 Hz, 20-H), 7.31 (2H, t, $J$=7.4 Hz, Fmoc 2, 7-H), 7.43 (2H, t, $J$=7.4 Hz, Fmoc 3, 6-H), 7.71 (2H, m, Fmoc 1, 8-H), 7.85 (2H, d, $J$=7.5 Hz, Fmoc 4, 5-H); $^{13}C$ NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$) $\delta$ 12.21 (C-39), 17.91, 18.29, 18.96 (C-38, 6', 40), 30.13 (C-7), 35.95 (C-6), 38.3 (C-18), 41.68~44.69 (C-34, 36, 2, 4, 10, 12, 14), 57.57, 58.11 (C-3', 16), 62.34, 63.01 (-*$OCH_2CH_2$*OH), 66.60~78.99 (C-3, 5, 8, 9, 11, 15, 17, 19, 35, 37, 2', 4', 5', Fmoc and allyl -$CO_2$*$CH_2$*), 99.99 (C-1'), 102.36 (C-13), 119.09 (allyl CH=*$CH_2$*), 120.94 (Fmoc C-4, 5), 126.27 (Fmoc C-1, 8), 128.21, 128.81 (Fmoc C-2, 7; 3, 6), 130.64~135.07 (unassigned -*CH*=), 137.16, 137.55 (C-20, 33), 142.59 (Fmoc C-4a, 5a), 145.31, 145.40 (Fmoc C-1a, 8a), 158.71 (Fmoc *C*=*O*), 172.55 (C-1), 174.08 (allyl *$CO_2$*); MS (TDE Na matrix) $m/z$ 1,252.8 (MNa, $C_{67}H_{91}NO_{20}$+Na).

13-*O*-(2-Hydroxyethyl)amphotericin B, Allyl Ester (**15**)

The 13-hydroxyethoxy derivative (**12**) (1.25 g, 1.01 mmol) in DMSO (10 ml) and MeOH (3 ml) was treated at room temperature with piperidine (0.21 ml, 2.1 mmol). After 100 minutes, MeOH (10 ml) was added and the mixture poured into ether (2 litres). The precipitate was filtered and dried *in vacuo* to give title product (**15**) (0.94 g, 92%), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) 406 (147,000), 383 (121,000), 364 (77,000); IR $\nu_{max}$ (Nujol) $cm^{-1}$ 3400, 1720; $^1H$ NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$) confirmed by 2D NMR, $\delta$ 1.14 (3H, d, $J$=7.2 Hz, 39-$CH_3$), 1.24 (3H, d, $J$=6.5 Hz, 40-$CH_3$), 1.33 (3H, d, $J$=6.3 Hz, 38-$CH_3$), 1.44 (3H, d, $J$=5.8 Hz, 6'-$CH_3$), 1.60 (1H, dt, $J_{max}$=14.0 Hz, 1 of 4-H), 1.65~1.80 (5H, complex, 1 of 4-H, 6-$CH_2$-, 1 of 7-H, 1 of 10-H), 1.78 (1H, dd, $J$=13.2, 11.5 Hz, 14 axial-H), 1.90~2.05 (6H, complex, 12-$CH_2$-, 1 of each of 7-H, 10-H, 18-H; 36-H), 2.19 (1H, dd, $J$=13.6, 7.1 Hz, 1 of 18-H), 2.40 (1H, dd, $J$=16.6, 3.6 Hz, 1 of 2-H), 2.52 (1H, dd, $J$=16.6, 8.7 Hz, 1 of 2-H), 2.54 (1H, m, 34-H), 2.62 (1H, t, $J$=10.5 Hz, 16-H), 2.66 (1H, dd, $J$=13.2, 4.8 Hz, 14 equatorial-H), 2.79 (1H, dd, $J$=9.2, 3.1 Hz, 3'-H), 3.43 (1H, m, 5'-H), 3.46 (1H, t, $J$=9.1 Hz, 4'-H), 3.51 (1H, dd, $J$=9.0, 2.7 Hz, 35-H), 3.52 (1H, m, 8-H), 3.62 (1H, m, 1 of hydroxyethoxy-H), 3.81 (1H, m, 9-H), 3.86 (3H, m, other hydroxyethoxy-H), 3.97 (1H, m, 5-H), 4.13 (1H, dd, $J$=3.0, 0.9 Hz, 2'-H), 4.31 (1H, m, 11-H), 4.44 (1H, m, 3-H), 4.49 (1H, m, 17-H), 4.64 (1H, td, $J$=10.8, 4.6 Hz, 15-H), 4.70 (1H, d, $J$=1.0 Hz, 1'-H), 4.75 (2H, dt, $J$=5.4, 1.5 Hz, allyl -$CO_2$*$CH_2$*-), 4.80 (1H, br t, $J$=6.8 Hz, 19-H), 5.28 (1H, dq, $J$=10.5, 1.4 Hz, 1 of allyl CH=*$CH_2$*), 5.38 (1H, dq, $J$=6.2, 4.5 Hz, 37-H), 5.47 (1H, dq, $J$=17.2, 1.6 Hz, 1 of allyl CH=*$CH_2$*), 5.68 (1H, dd, $J$=14.5, 9.2 Hz, 33-H), 6.04 (1H, ddt, $J$=17.2, 10.5, 5.4 Hz, allyl -*OCH*), 6.14 (1H, dd, $J$=14.5, 6.1 Hz, 20-H), 6.32~6.53 (12H, complex, 21 to 32-H); $^{13}C$ NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$), confirmed by 2D NMR, $\delta$ 12.3 (C-39), 17.9 (C-38), 18.4 (C-6'), 18.8 (C-40), 30.5 (C-7), 36.1 (C-6), 37.9 (C-18), 41.6 (C-10), 42.2 (C-36), 42.5 (C-34), 43.1 (C-14), 43.4 (C-2), 44.5 (C-12), 44.8 (C-4), 57.6 (C-16), 57.7 (C-3'), 61.9, 62.8 (-*$OCH_2CH_2$*OH), 65.9 (allyl -*$OCH_2$*), 67.3 (C-15), 67.6 (C-17), 67.7 (C-11), 68.4 (C-3), 71.3 (C-37), 71.8 (C-2'), 72.0 (C-5), 74.5 (C-9), 74.6 (C-4'), 74.7 (C-5'), 75.7 (C-8), 76.0 (C-19), 78.3 (C-35), 100.1 (C-1'), 102.1 (C-13), 118.5 (allyl -CH=*$CH_2$*), 129.9 (C-21), 132.3~134.8 (unassigned -*CH*=), 133.4 (allyl -$CH_2$-*CH*=$CH_2$), 135.5 (C-20), 137.7 (C-33), 171.9 (C-1), 173.6 (allyl *$CO_2$*); MS (TDE Na matrix) $m/z$ 1,030 (MNa, $C_{52}H_{81}O_{18}N$+Na).

13-*O*-(2-Hydroxyethyl)amphotericin B (**5**)

The allyl ester (**15**) (0.60 g, 0.60 mmol) in THF (35 ml), flushed with nitrogen, was treated at room temperature with pyrrolidine (0.15 ml, 1.77 mmol) and tetrakis (triphenylphosphine) palladium (0.08 g, 0.08 mmol). After 1 hour, the precipitate was collected by centrifugation and washed with THF. The solids were reprecipitated from MeOH (5): THF (1)-ether and dried *in vacuo* gave the title product (**5**), 0.42 g (73%), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) 406 (114,000), 383 (106,000), 364 (67,000); IR $\nu_{max}$ (Nujol) $cm^{-1}$ 3500~2600, 1720; $^1H$ NMR (1 : 1 pyridine-$d_5$ - MeOH-$d_4$), characteristic signals include $\delta$ 1.15 (3H, d,

$J$=7.1 Hz, 39-$CH_3$), 1.24 (3H, d, $J$=6.5 Hz, 40-$CH_3$), 1.27 (3H, d, $J$=6.3 Hz, 38-$CH_3$), 1.44 (3H, d, $J$=6.1 Hz, 6′-$CH_3$), 2.40 (1H, dd, $J$=16.6, 3.5 Hz, 1 of 2-H), 3.72 (1H, t, $J$=9.6 Hz, 4′-H), 4.40 (1H, d, $J$=3.0 Hz, 2′-H), 4.67 (1H, m, 15-H), 4.83 (1H, m, 19-H), 4.92 (1H, s, 1′-H), 5.43 (1H, m, 37-H), 5.65 (1H, m, 33-H), 6.19 (1H, dd, $J$=14.0, 6.3 Hz, 20-H); $^{13}$C NMR (1 : 1 pyrdine-$d_5$-MeOH-$d_4$) $\delta$ 12.39 (C-39), 17.83, 18.29, 18.99 (C-38, 6′, 40), 30.59 (C-7), 36.21 (C-6), 38.61 (C-18), 41.61 (C-10), 42.13, 42.74 (C-34, 36), 43.36, 44.37 44.75, 45.96 (C-2, 4, 12, 14), 57.21, 60.75 (C-16, 3′), 62.13, 63.38 (-O$CH_2CH_2$OH), 67.49~78.34 (C-3, 5, 8, 9, 11, 15, 17, 19, 35, 37, 2′, 4′, 5′), 99.48 (C-1′), 102.04 (C-13), 129.66~134.98 (unassigned -*CH*=), 137.40, 137.58 (C-20, 33), 171.93 (C-1), 180.12 (-$CO_2$-); MS (TDE Na matrix) $m/z$ 990 (MNa, $C_{49}H_{77}O_{18}N+Na$). Traces of pyrrolidine present in the above product may be removed by passage through HP20 in aqueous THF.

Conversion of *N*-(9-Fluorenylmethoxycarbonyl)amphotericin B, Allyl Ester (**10**) to *N*-(9-Fluorenylmethoxycarbonyl)-13-14-anhydroamphotericin B, Allyl Ester (**21**)

To a slurry of the allyl ester (**10**) (1.69 g, 1.43 mmol) in methylene chloride (25 ml) in a cold water bath was added 2,6-lutidine (2.77 ml, 23.9 mmol) and trimethylsilyl trifluoromethanesulphonate (3.53 ml, 18.3 mmol). After 15 minutes stirring at room temperature, the solution was evaporated and hexane (500 ml) added. Solids were triturated, filtered off and washed with hexane. The filtrate was evaporated to give *N*-(9-fluorenylmethoxycarbonyl)-3,5,8,9,11,15,35,2′,4′-nona-*O*-trimethylsilyl-13,14-anhydroamphotericin B, allyl ester (**20**) (2.50 g), used directly without further purification as follows. The crude product (**20**) (2.50 g, 1.37 mmol) in THF (40 ml) was stirred under nitrogen in a plastic bottle at room temperature. A solution of hydrogen fluoride in pyridine; THF (53.4 ml of a 2.06 M stock solution of hydrogen fluoride, prepared from 14.64 g 70% hydrogen fluoride in pyridine (Aldrich), diluted with pyridine (90 ml) and made up to 250 ml with THF) was added by plastic syringe. After 4 hours, the solution was poured into hexane-ether (1 : 1, 4 litres). The precipitate was filtered and copiously washed with ether. Evacuation gave a solid which was chromatographed on silica (methylene chloride-MeOH, 12 : 1) to afford the title product (**21**), (0.94 g, 56% overall from (**10**)), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) 407 (149,000), 383 (134,000), 364 (83,000); IR $\nu_{max}$ (Nujol) cm$^{-1}$ 3300, 1710; $^1$H NMR (1 : 1 pyridine-$d_5$-MeOH-$d_4$) characteristic signals include $\delta$ 1.16 (3H, d, $J$=7.1 Hz, 39-$CH_3$), 1.25 (3H, d, $J$=6.6 Hz, 40-$CH_3$), 1.34 (3H, d, $J$=6.3 Hz, 38-$CH_3$), 1.48 (3H, d, $J$=6.05 Hz, 6′-$CH_3$), 2.49 (1H, dd, $J$=17.0, 9.6 Hz, 1 of 2-H), 2.86 (1H, br t, $J$=10 Hz, 16-H), 3.58 (1H, m, 5′-H), 3.78 (1H, t, $J$=9.7 Hz, 4′-H), 3.87 (1H, m, 9-H), 3.97 (1H, m, 5-H), 4.07 (1H, dd, $J$=10.2, 2.8 Hz, 3′-H), 4.23 (2H, m, Fmoc -O$CH_2$*CH* and 2′-H), 4.38 (2H, d, $J$=7.2 Hz, Fmoc -O$CH_2$), 4.76 (1H, s, 1′-H), 4.94 (1H, br s, 14-H), 5.27 (1H, br d, $J$=11.8 Hz, 1 of allyl CH=$CH_2$), 5.47 (1H, dm, $J$=17.2, 1.4 Hz, 1 of allyl CH=$CH_2$), 5.61 (1H, dd, $J$=14.1, 9.8 Hz, 33-H), 6.06 (2H, m, 20-H and allyl O$CH_2$*CH*=), 7.30 (2H, t, $J$=7.4 Hz, Fmoc 2, 7-H), 7.42 (2H, t, $J$=7.4 Hz, Fmoc 3, 6-H), 7.72 (2H, m, Fmoc 1, 8-H), 7.85 (2H, d, $J$=7.4 Hz, Fmoc 4, 5-H); $^{13}$C NMR (1 : 1 pyridine-$d_5$-MeOH-$d_4$) $\delta$ 12.58 (C-39), 17.71 (C-38), 18.58 (C-6′), 18.99 (C-40), 31.32 (C-7), 36.35 (C-6), 37.76 (C-18), 40.80 (C-10), 41.87 (C-36), 43.18, 44.48 (C-2, 12), 43.59 (C-34), 48.05 (Fmoc -O$CH_2$*CH*), 54.50 (C-16), 58.34 (C-3′), 66.19, 67.40 (Fmoc and allyl -O$CH_2$), 67.51 (C-15), 68.68, 69.14 (C-3, 11), 70.87, 71.40, 71.72 (C-2′, 4′, 37), 72.50, 73.08 (C-5, 17), 74.93, 75.39, 75.50 (C-5, 8, 9), 76.75 (C-19), 78.84 (C-35), 99.50 (C-1′), 104.24 (C-14), 118.80 (allyl -CH=$CH_2$), 120.74 (Fmoc C-4, 5), 126.13 (Fmoc C-1, 8), 127.91, 128.49 (Fmoc C-2, 7; 3, 6), 131.68~137.63 (unassigned -*CH*=), 142.09 (Fmoc C-4a, 5a), 144.94, 145.07 (Fmoc C-8a, 1a), 152.69 (C-13), 158.15 (Fmoc *C*=*O*), 172.07 (C-1), 173.55 (allyl $CO_2$); MS (TDE Na matrix) $m/z$ 1,191.0 (MNa, $C_{65}H_{85}NO_{18}+Na$).

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