N-AMINOACYL DERIVATIVES OF POLYENE MACROLIDE ANTIBIOTICS AND THEIR ESTERS

J. J. KIM WRIGHT*, JAMES A. ALBARELLA, LARRY R. KREPSKI and DAVID LOEBENBERG

Chemical and Microbiological Research, Schering-Plough Corporation, 60 Orange Street, Bloomfield, New Jersey 07003, U.S.A.

(Received for publication April 23, 1982)

Amphotericin B continues to be the drug of choice for the treatment of deep-seated fungal infections despite the usual accompaniment of severe toxic side-reaction and there has been considerable interest in recent years in developing a safer drug within this class of antibiotic[1~8]. We wish to report the synthesis of *N*-aminoacyl derivatives of polyene esters which appear to represent a significant advance in this area[9]. They are more water-soluble and less toxic than the parent polyenes, but retain their antifungal potency both *in vitro*[9,10,11] and *in vivo*[12].

Our objective was the discovery of polyene derivatives that would form true solutions in water under physiological conditions, as we felt, as have others, that such derivatives would be less toxic and display different pharmacokinetics.

Antibiotic 67-121-C[13] (**1**), whose structure was established in our laboratories[14], was the initial subject of our modification program. The *N'*-glycyl derivative (**4**) of antibiotic 67-121-C was prepared from the *N'*-iodoacetate (**2**) by treatment with aqueous ammonium hydroxide (Scheme 1). This compound was found to be slightly more potent than the parent antibiotic. This was particularly significant as simple *N*-acyl derivatives (*e.g. N'*-acetyl) of antibiotic 67-121-C and of other polyene antibiotics have all proved to be much less potent than the parent compounds[15]. Thus it was clear that at least one amino group was necessary for full antifungal potency to be retained.

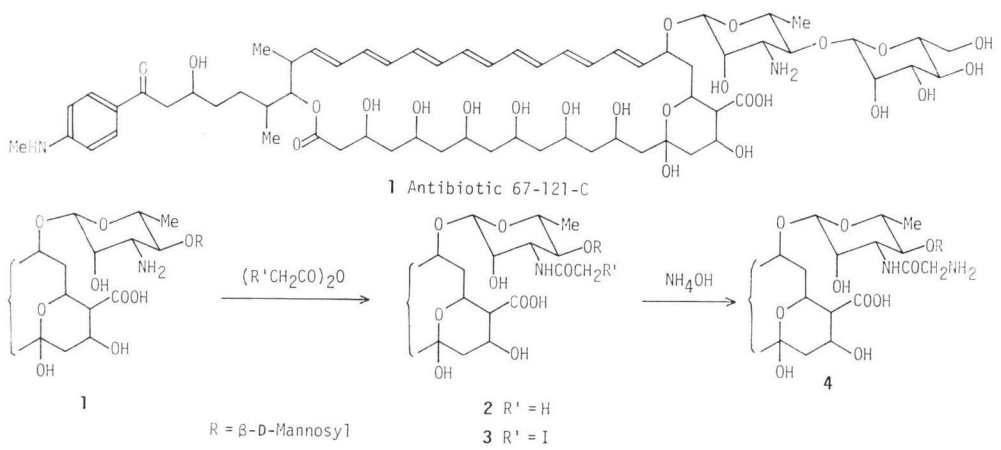
As derivatives of antibiotic 67-121-C were highly toxic, further studies were carried out on the non-aromatic heptaene, amphotericin B (**5**). Compounds in the Table were produced by acylating amphotericin B in dimethylsulfoxide (DMSO) with the active ester of the *N*-protected amino-acyl group. Esterification of the aglycone carboxylic acid group of the protected intermediate was achieved with the appropriate alkyl halide in excess, in the presence of a hindered tertiary amine or sodium carbonate.

The chemical reactivity and solubility properties of the polyene antibiotics make stringent demands upon the nature of the chemical operations performed on them; the difficulty of purifying intermediates or products requires reactions to be very efficient and rapid.

Our initial studies employed the trifluoroacetyl and the trichloroethoxycarbonyl blocking groups to protect the side-chain amino groups. Whilst the desired products could be, and indeed were, obtained, the conditions required for removing them did not meet the above criteria.

The fluorenemethoxycarbonyl (Fmoc) protect-

Scheme 1.



ing group was chosen for its highly lipophilic character allowing chromatographic separation of intermediates and for the mild conditions required for its removal. Although the original literature procedures[16] employed morpholine or ammonia for its removal, we have found conditions that are more suitable for the present application. Although these Fmoc derivatives had

Scheme 2.

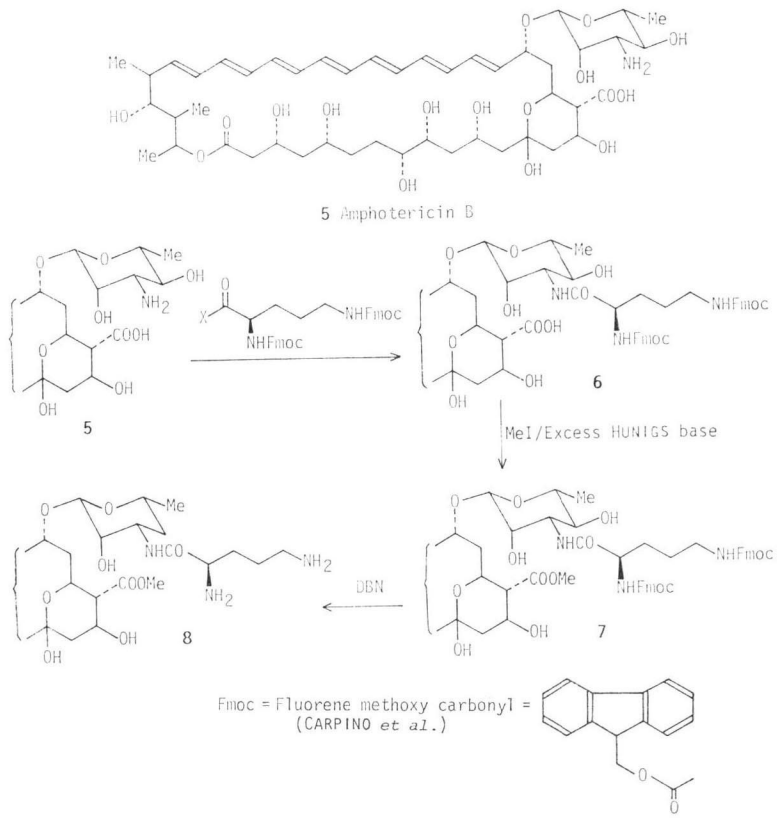


Fig. 1. UV Visible absorption spectra of Fungizone, AME and Sch 28191 in water.

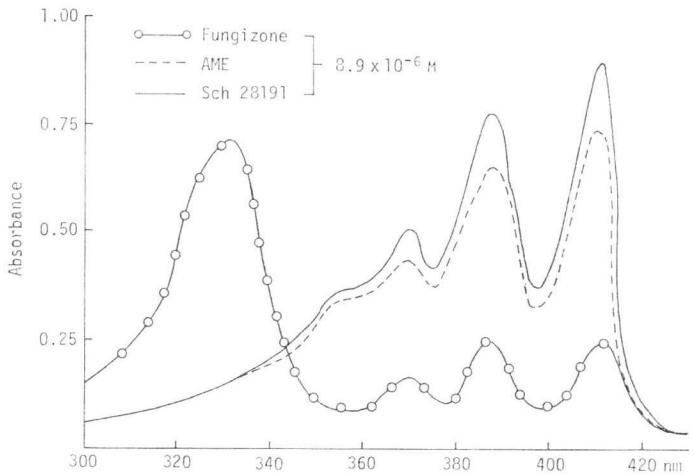
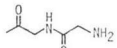
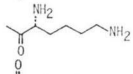
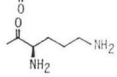
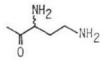
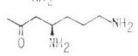
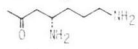
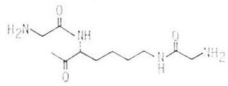
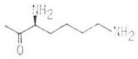
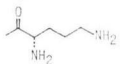
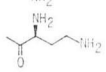
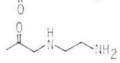
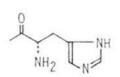
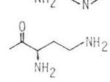
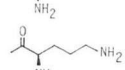
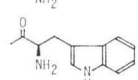
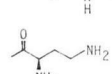


Table 1. *N*-Aminoacyl derivatives of amphotericin B.[a]

	Amino substituent	Ester	MIC (μg/ml, 48 hours)[b]		
			Candida albicans	Other yeasts	LD_{50}(mg/kg)[c]
Glycyl			0.025	0.058	80
β-Alanyl			0.048	0.130	70
Glycylglycyl			0.030	0.204	90
Aminoethylcarbamoyl			0.046	0.128	50
D-Lysyl			0.032	0.023	38
D-Ornithyl			0.050	0.051	45
DL-Diaminobutyryl			0.059	0.058	60
DL-Diaminopropionyl			0.155	0.223	83
D-β-Lysyl			0.036	0.044	25
L-β-Lysyl			0.036	0.028	30
Diglycyl-D-lysyl			0.042	0.094	60
L-Lysyl			0.046	0.027	38
L-Ornithyl			0.124	0.034	28
L-Diaminobutyryl			0.095	0.104	60
Aminoethylglycyl			0.033	0.061	—
Histidyl			0.083	0.105	65
DL-Aminobutyryl			0.115	0.122	70
D-Ornithyl			0.043	0.040	75
			0.170	0.171	50
D-Tryptophyl			0.246	0.584	90
D-Diaminobutyryl			0.051	0.050	75
D-Diaminobutyryl			0.089	0.058	110
Fungizone			0.049	0.102	5
AME			0.038	0.117	90

[a] Results of experiments done at various times.

[b] Average MICs of 17 strains of *C. albicans* or 7 strains of other yeasts done in S.D.B., pH 5.7, 28°C.

[c] Average, i.v. LD_{50}: done in groups of 5~10 mice each.

sufficient stability in the presence of tertiary amines (even up to 10% solutions in DMSO) to allow efficient methylation of the carboxylic acid salt with alkyl halides, removal was completely and rapidly effected with equimolar amounts of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (Scheme 2). This selectivity has enabled these synthetic transformations to be carried out under very mild conditions.

The compounds prepared from amphotericin B are listed in the Table. All aminoacyl derivatives except the aminoethylglycyl derivative were prepared by the above procedures; the last was prepared by treatment of the *N*-iodoacetyl derivative of the ester with ethylenediamine.

Those compounds with at least two amine groups in the side chain and with the aglycone carboxyl group in the form of its ester, were found to be the most promising. They form highly water-soluble salts at neutral pH with most acids. It is known that amphotericin B, even solubilized as its deoxycholate salt (Fungizone) is highly aggregated in aqueous medium and this is reflected in its absorption spectra (Fig. 1). In contrast, the acid salt of D-ornithyl amphotericin B methyl ester (Sch 28191) for example, appears to form a true solution.

Acute toxicity determinations led to the selection of D-ornithyl amphotericin B (**8**) methyl ester and D-lysyl amphotericin B methyl ester for further study. Both compounds retain the activity of the parent antibiotic *in vitro* and *in vivo*, and thus appear to represent a significant advance in this area.

Acknowledgements

We are pleased to acknowledge the encouragement of Dr. PETER DANIELS, Dr. ALAN MALLAMS and Dr. TATTANAHALLI NAGABHUSHAN and the fine technical support of Dr. RAULO PARMEGIANI, BARRY ANTONACCI and TAISA TOMAINE.

References

1) LECHEVALIER, H.; E. BOROWSKI, J. O. LAMPEN & C. P. SCHAFFNER: Water-soluble *N*-acetyl derivatives of heptaene macrolide antifungal antibiotics: microbiological studies. Antibiot. & Chemoth. 11: 640~647, 1961
2) SCHAFFNER, C. P. & E. BOROWSKI: Biologically active *N*-acyl derivatives of polyene macrolide antifungal antibiotics. Antibiot. & Chemoth. 11: 724~732, 1961
3) SCHAFFNER, C. P. & W. MECHLINSKI: Polyene macrolide derivatives. II. Physical-chemical properties of polyene macrolide esters and their water soluble salts. J. Antibiotics 25: 259~260, 1972
4) BRUZZESE, T.; M. CAMBIERI & F. RECUSANI: Synthesis and biological properties of alkyl esters of polyene antibiotics. J. Pharm. Sci. 64: 462~463, 1975
5) FALKOWSKI, L.; J. GOLIK, P. KOLODZIEJCZYK, J. PAWLAK, J. ZIELINSKI, T. ZIMINSKI & E. BOROWSKI: *N*-Glycosyl derivatives of polyene macrolide antibiotics. J. Antibiotics 28: 244~245, 1975
6) FALKOWSKI, L.; B. STEFANSKA, J. ZIELINSKI, E. BYLEC, J. GOLIK, P. KOLODZIEJCZYK & E. BOROWSKI: Methyl esters of trimethylammonium salts of polyene macrolide antibiotics. J. Antibiotics 32: 1080~1081, 1979
7) STEFANSKA, B.; E. BYLEC & E. BOROWSKI: The synthesis of amides of polyene macrolide antibiotics. J. Antibiotics 33: 103~104, 1980
8) WITZKE, N.: Water-soluble guanidine derivatives of polyene macrolides and the esters thereof. Eur. Pat. 31,722, 1981
9) This work was first disclosed at the 20th Internat. Conf. Antimicrob. Agents & Chemother., New Orleans, 1980; J. J. WRIGHT, J. ALBARELLA, L. R. KREPSKI & D. LOEBENBERG, Paper #472
10) OBLACK, D. L.; W. L. HEWITT & W. J. MARTIN: Susceptibility testing of yeasts with amphotericin derivatives. 20th Internat. Conf. Antimicrob. Agents & Chemother., Paper #469, New Orleans, 1980
11) SHADOMY, S.; G. WAGNER, D. M. DIXON & R. MAY: *In vitro* studies with polyene methyl esters. *Idem*, Paper #470
12) LOEBENBERG, D.; R. PARMEGIANI, G. H. MILLER & J. J. WRIGHT: Evaluation of *N*-D-ornithyl AME as a systemic antifungal agent. *Idem*, Paper #471
13) WAGMAN, G. H.; R. T. TESTA, M. PATEL, J. A. MARQUEZ, E. M. ODEN, J. A. WAITZ & M. J. WEINSTEIN. Antimicrob. Agents Chemother. 7: 457~461, 1975
14) WRIGHT, J. J.; D. GREEVES, A. K. MALLAMS & D. H. PICKER: Structural elucidation of heptaene macrolide antibiotics 67-121-A and 67-121-C. J. Chem. Soc. Chem. Comm. 1977: 710~712, 1977
15) MECHLINSKI, D. & C. P. SCHAFFNER: *N*-Acylation and esterification reactions with amphotericin B. J. Antibiotics 25: 256~258, 1972
16) CARPINO, L. A. & G. Y. HAN: The fluorenemethoxycarbonyl function; new base-sensitive amino-protecting group. J. Am. Chem. Soc. 92: 5748~5749, 1970