A Versatile Strategy for the Derivatisation of the Carboxy Group of Amphotericin B: The Preparation of C-16 Ketone Analogues

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The carboxy group of amphotericin B **1** has been converted into a range of ketone analogues **12–15** *via* a strategy based on the use of protecting groups to facilitate derivatisation of **1**.

The polyene macrolides are an important class of biologically active natural products.[1] One such compound, amphotericin B (AmB; **1**) is widely used for the treatment of serious, systemic fungal infections.[2] However, its use is associated with a number of undesirable properties such as nephrotoxicity and poor solubility.[2] Two approaches have been used to address these problems. Novel formulations of the drug, *e.g.* liposomal AmB,[3] have shown reduced toxicity relative to the parent compound. Alternatively, the derivatisation of AmB **1** to produce less toxic and more water-soluble analogues is an approach adopted by ourselves and others.[4] Previous studies to produce analogues of **1** have concentrated on relatively simple derivatives of the 16-carboxylic acid and the 3′-amine functionalities. In general, the range of reactions carried out at these positions has been limited by the highly functionalised nature of AmB **1** together with its poor solubility in many common organic solvents. In this communication we describe a strategy which uses readily removable protecting groups to overcome these complications† and results in a more versatile approach towards derivatisation of the carboxy group of AmB **1**. The approach is exemplified by the preparation of some novel 16-ketone derivatives of AmB **1**.

Scheme 1 illustrates a three-stage protection of AmB **1** to produce the versatile intermediates **3** and **8**. Trifluoroacetylation of the 3′-amine of **1** with CF_3CO_2Et[6] followed by acid-catalysed exchange of the anomeric hydroxy group at C-13 (PPTS, methanol, tetrahydrofuran)[7] gave the intermediate **2** (73% from **1**). Complete silylation of the secondary hydroxy groups in **2** was effected with triethylsilyl trifluoromethanesulfonate‡ (14 equiv. 2,6-lutidine, CH_2Cl_2) to afford **8** (38% after column chromatography).§ These conditions also induced some elimination of the 13-methoxy group to give **3** (21%), which could be readily separated from **8**. We have previously reported the elimination[9] of a 13-hydroxy group under similar conditions. Switching the solvent for the silylation reaction to hexane gave only the 13-methoxy substituted product **8** (61%). Both **3** and **8** proved to be useful for our derivatisation studies.

The preparation of 16-ketone derivatives required activation of the 16-carboxy group for subsequent reaction with Grignard reagents. This was accomplished by conversion of **3** and **8**, respectively, into 2-pyridyl thioesters[10] **4** (76%) and **9** (94%) with 2-pyridylthio chloroformate–NEt_3[11] (Scheme 1). The 2-pyridyl thioester in the vinyl ether derivative **4** was found to be more reactive than that in **9** and was regarded as the first choice substrate for ketone synthesis. Addition of phenyl, pyrrol-2-yl[12] and vinyl Grignard reagents to **4** gave ketones **5–7**, respectively, in 68–82% yield.¶ The formation of **7** presumably arises from 1,4-addition of a second mole of Grignard reagent to the initially formed α,β-unsaturated ketone. Further reaction of the desired ketone was also a problem when **4** was treated with methylmagnesium bromide and the tertiary alcohol **11** became the major product. The desired 16-methyl ketone could be prepared by taking advantage of the lower reactivity of the 13-methoxy substituted derivative **9**. Addition of methylmagnesium bromide (16 equiv.) to **9** gave the 16-acetyl derivative **10** (68%). Presumably, the conformational change in the 6-membered ring resulting from introduction of the 13-methoxy substituent into **9** increases the steric hindrance sufficiently at C-16 to suppress overaddition.

The ketones **5–7** and **10** were deprotected to the amphotericin B analogues in three stages. Desilylation occurred readily with HF–pyridine (THF, methanol, pyridine). Regeneration of the 13-hydroxy group either by hydration of the 13,14-double bond, or in the case of the 16-methyl ketone by anomeric exchange, was carried out in each case by treatment with PPTS in THF–water. Finally, the trifluoroacetyl group was removed with aqueous NH_3 in THF–methanol to produce the AmB analogues **12–15** in overall yields of 37–51% for the three steps.‖ The ketones can be converted into water-soluble salts by reaction of the 3′-amine with stoichiometric quantities of an acid.[13]

The methodology described here permits a versatile entry to novel 16-acyl derivatives **12–15** of AmB which displayed good *in vitro* antifungal activity and reduced nephrotoxicity relative to the parent compound. Further details will be reported elsewhere.

Experimental

Synthesis of Pyrrol-2-yl Ketone **6**.—A solution of pyrrolylmagnesium bromide was prepared by treating pyrrole (0.443 g, 0.458 cm^3, 6.60 mmol) in THF (7 cm^3) at 0 °C with ethylmagnesium bromide (2.0 mol dm^{-3} in solution in THF; 2.35 cm^3, 4.70 mmol). After being stirred at 0 °C for 5 min and room temperature for 5 min, the mixture was recooled to 0 °C and transferred *via* a cannula with the aid of additional THF (5 cm^3) to a stirred solution of 2-pyridyl thioester **4** (0.500 g, 0.235 mmol) in THF (7 cm^3) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 0.5 h and then cautiously quenched with saturated aqueous ammonium chloride. The

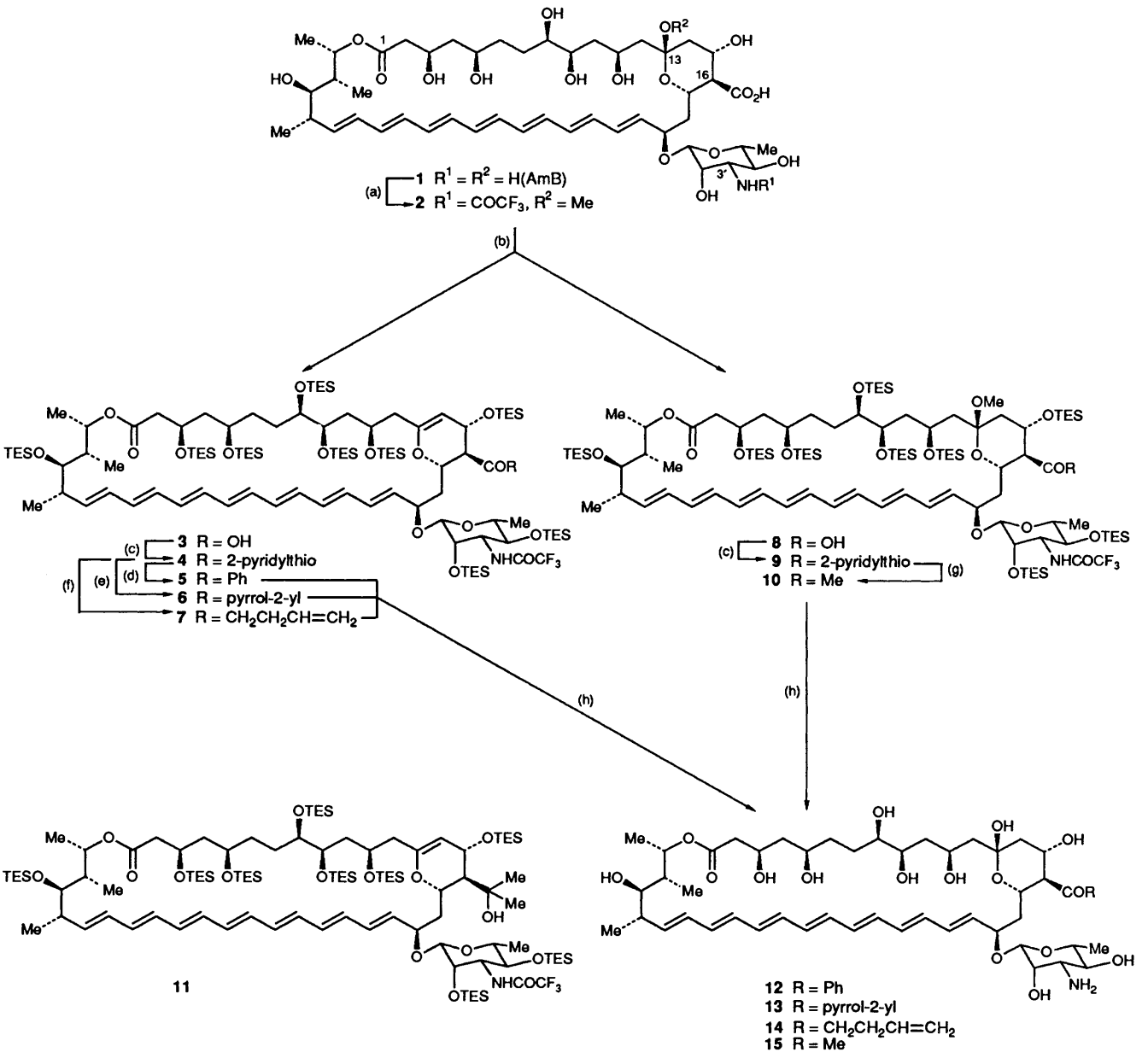
† During synthetic studies, Nicolaou[5] recognised the need to derivatise amphotericin B in order to facilitate development of its chemistry.

‡ Previously the trimethylsilyl (TMS) and *tert*-butyldimethylsilyl (TBDMS) groups have been used for hydroxy group protection in AmB **1** derivatives. Both have drawbacks; the TMS is labile to column chromatography[4a] and the TBDMS has proved difficult to remove under mild conditions.[8]

§ Compounds **3–15** were characterised by ^{13}C NMR, ^1H NMR (including 2D NMR for **6** and **12**), IR, UV and FAB MS.

¶ In some reactions, partial deprotection of the trifluoroacetamide was observed. In these cases the crude isolated product was treated with $(CF_3CO)_2O$ and triethylamine in CH_2Cl_2 to reprotect the amine.

‖ All the ketones **12–15** contained 5–10% of another product (HPLC/NMR). We believe that in each case this is an isomer caused by rearrangement of the macrocyclic lactone and will be the subject of a future publication. This product can be reconverted into the desired ketone under acidic conditions.



Scheme 1 *Reagents and conditions:* (a) i, CF_3CO_2Et, Pr^iNEt_2, DMF, 25 °C, overnight; ii, PPTS (6–8 equiv.), MeOH, THF (3:1), 25 °C, 1–2 h, 73% from **1**; (b) $Et_3SiOSO_2CF_3$ (14 equiv.), 2,6-lutidine (18 equiv.), CH_2Cl_2, 25 °C, 1 h, **3**, 21%; **8**, 38% (or substituting hexane for CH_2Cl_2, 25 °C, 16–24 h gave **8** only, 61%); (c) NEt_3, 2-pyridylthio chloroformate, diethyl ether, 0 °C, 1 h, **4**, 76%; **9**, 94%; (d) i, PhMgBr (15 equiv.), THF, 0 °C, 0.5 h; ii, $(CF_3CO)_2O$, NEt_3, CH_2Cl_2, 0 °C, 1–2 h, 73% (see footnote ¶); (e) pyrrol-2-ylmagnesium bromide (20 equiv.), THF, 0 °C, 0.5 h, 82%; (f) i, vinylmagnesium bromide (16 equiv.), THF, 0 °C, 1 h; ii, $(CF_3CO)_2O$, NEt_3, CH_2Cl_2, 0 °C, 1 h, 68% (see footnote ¶); (g) MeMgBr (15 equiv.), THF, 0 °C, 5–6 h, 68%; (h) i, HF–pyridine, THF, MeOH, pyridine, 25 °C, 16–24 h; ii, PPTS (6–8 equiv.), THF, H_2O, 25 °C, 1–2 h; iii, aq. NH_3, THF, MeOH, 25 °C, 6–12 h, 37–51%. Abbreviations used here and in text: DMF = *N*,*N*-dimethylformamide, PPTS = pyridinium toluene-*p*-sulfonate, THF = tetrahydrofuran, TES = triethylsilyl.

product was extracted into diethyl ether and the combined extracts were dried ($MgSO_4$) and evaporated. Purification of the residue by flash column chromatography on silica gel with 5% ethyl acetate in hexane gave **6** (0.401 g, 82%) as a yellow–orange foam; ν_{max}(thin film)/cm^{-1} 3415, 3370, 2950, 2905, 2875, 1730, 1670, 1620, 1525, 1455, 1410, 1375, 1235, 1165, 1070, 1005, 870, 835 and 760; λ_{max}(hexane)/nm 408, 384, 365 and 346; *m*/*z* (FAB/3-nitrobenzyl alcohol, sodium matrix) 2100 (MNa^+); δ_H[400 MHz; $(CD_3)_2CO$] 11.03 (1 H, s), 7.37 (1 H, d, *J* 8.5*), 7.23 (1 H, m), 7.06 (1 H, m), 6.57–6.10 (13 H, series of m), 5.97 (1 H, dd, *J* 6.6, 15.4), 5.54 (1 H, dd, *J* 9.4, 14.8), 4.78 (1 H, d, *J* 8.6), 4.68 (1 H, d, *J* 1.7), 4.66 (1 H, m), 4.55 (1 H, t, *J* 7.0), 4.37 (1 H, ddd, *J* 2.5, 7.1, 11.0), 4.35 (1 H, s), 4.21 (1 H, m), 4.15 (1 H, m), 4.02 (1 H, m), 3.93 (1 H, s), 3.92 (1 H, m), 3.86 (1 H, dd, *J* 2.9, 8.6), 3.76 (1 H, m), 3.72 (1 H, m), 3.64 (1 H, t, *J* 8.7), 3.44 (1 H, dd, *J* 8.6, 11.0), 3.19 (1 H, dq, *J* 6.2, 8.5), 2.60 (1 H, dd, *J* 5.3, 17.2), 2.53 (1 H, dd, *J* 7.5, 17.2), 2.43 (1 H, m), 2.39 (1 H, m), 2.10–1.60 (11 H, series of m), 1.51 (1 H, m), 1.20 (3 H, d, *J* 6.2), 1.18 (3 H, d, *J* 6.0), 1.12–0.90 (87 H, series of m) and 0.85–0.35 (54 H, series of m); δ_C[100.6 MHz; $(CD_3)_2CO$] 191.2, 170.5, 157.3 (J_{CF} 36.4), 154.2, 139.1, 135.4, 135.3, 135.2, 135.1, 135.0, 134.4, 133.6, 132.8, 132.7, 132.6, 132.4, 131.5, 131.0, 130.7, 127.1, 118.9, 117.3 (J_{CF} 287.9), 111.2, 103.2, 99.2, 76.9, 76.8, 76.5, 74.4 (2 coincident

* *J* Values in Hz.

peaks), 73.53, 73.50, 73.2, 72.4, 71.2, 70.5, 69.3, 67.3, 57.4, 53.6, 48.2, 45.7, 43.7, 41.4, 40.8, 40.3, 36.7, 35.3, 27.7, 19.9, 19.2, 19.1, 11.2, 7.74, 7.67, 7.54, 7.36, 7.31, 7.25, 7.16, 6.19, 6.15, 5.94, 5.91, 5.85, 5.71 and 5.36.

Commercial solutions of Grignard reagents were used for the preparation of **5**, **7** and **10**.

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