Synthesis of Water-soluble Sugar Derivatives of Combretastatin A-4

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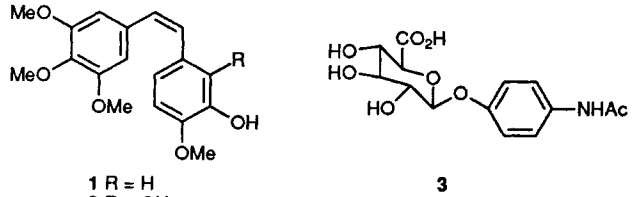
Three sugar derivatives (**6**, **7** and **13**) of the antimitotic agent combretastatin A-4 **1** have been synthesized. Two of these water-soluble derivatives (**6**, **13**) showed strong cytotoxicity to the murine P388 cell line.

Combretastatin A-4 **1**, a natural product isolated from *Combretum caffrum*, is currently being investigated under the sponsorship of the Cancer Research Campaign Clinical Trials scheme. This stilbene **1** has been shown to be one of the most potent inhibitors of tubulin assembly yet discovered. A close structural analogue, Combretastatin A-1 **2**, has also been shown to alter tubulin dynamics, a mechanism of action common to the clinically useful *Vinca* alkaloid class of anti-cancer agents. These agents are known to belong to the group of compounds that show extensive cross-resistance in multidrug-resistance (MDR) cell lines.

A major problem encountered with combretastatin A-4 **1** has been its insolubility. So far no suitable formulation has been developed so as to allow the commencement of clinical trials. Herein we describe the synthesis of three water-soluble derivatives of combretastatin A-4 **1**.



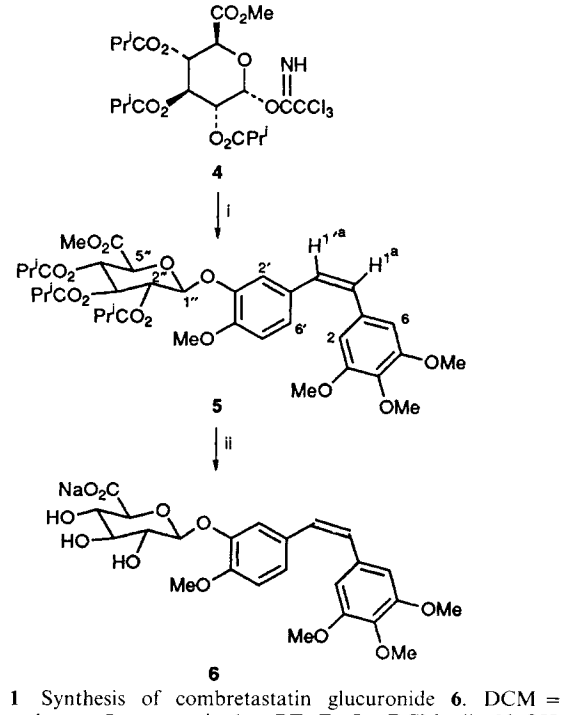
Structures of compounds **1**–**3**

Drugs such as morphine and paracetamol conjugate to glucuronic acids *in vivo* to afford paracetamol β-D-glucuronide **3**[1,2] and morphine 3- and 6-β-D-glucuronide.[3-6] Morphine 6-glucuronide is now thought to be the active analgesic.[7] The methodology developed[8,9] to synthesize the conjugate glycoside **3** *via* C-1 imidate **4** was used to prepare β-D-melibioside and β-D-glucuronide derivatives of combretastatin A-4 **1** whilst a protected bromoglucose was used to synthesize a combretastatin β-D-glucoside.

Results and Discussion

Methyl (trichloroacetimidoyl 2,3,4-tri-*O*-isobutyryl-α-D-glucopyranosid)uronate **4** was allowed to react with the phenol **1** in the presence of boron trifluoride–diethyl ether to afford the protected combretastatin glucuronide **5** in 68% yield. Deprotection of compound **5** using aq. sodium hydroxide, followed by chromatography on Sephadex G15, gave the required combretastatin β-D-glucuronide **6** as a chromatographically pure gum in 77% yield (Scheme 1).

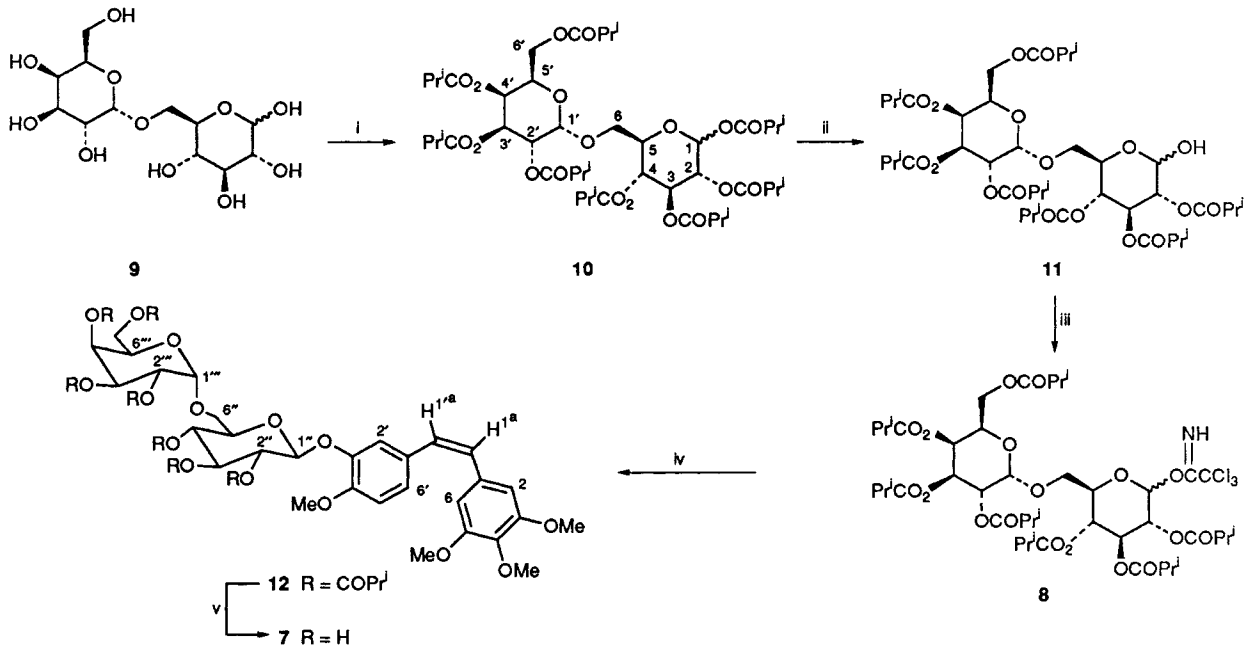
Combretastatin melibioside **7** was prepared in a similar fashion from a melibiose C-1 imidate **8**. Briefly, melibiose **9** was treated with isobutyryl chloride in the presence of pyridine to



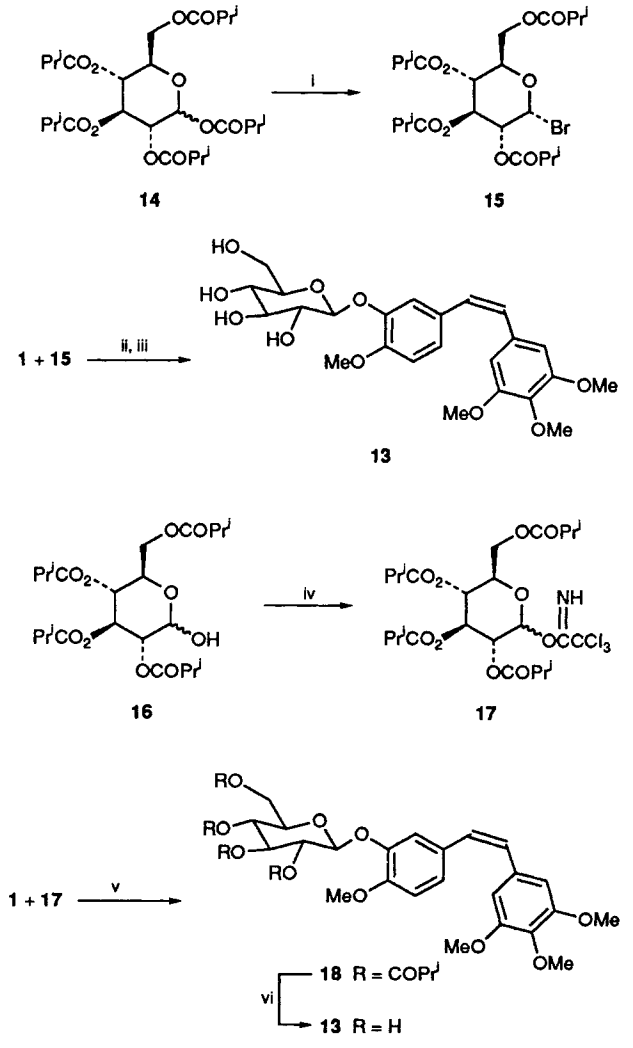
Scheme 1 Synthesis of combretastatin glucuronide **6**. DCM = dichloromethane. *Reagents:* i, **1** + BF_3·Et_2O, DCM; ii, NaOH, MeOH.

give the octaisobutyryl ester of melibiose, compound **10**, as an α/β mixture (6:1 from NMR analysis) in excellent yield. Selective removal of the ester from the anomeric hydroxy group was achieved by treatment of the octaisobutyryl ester **10** with gaseous ammonia, and afforded the heptaisobutyrate of the hemiacetal, compound **11**, in 64% yield. Treatment of the hemiacetal **11** with trichloroacetonitrile followed by filtration through silica gel gave the ester-protected melibiose C-1 imidate **8** as a mixture of α/β anomers in excellent yield. On account of its complexity the NMR spectrum of compound **8** could not be fully characterised but showed the expected shift in the position of the C-1 anomeric proton. Reaction of combretastatin A-4 **1** with imidate **8** in the presence of boron trifluoride–diethyl ether yielded the desired combretastatin melibioside **12** as indicated by fast-atom bombardment (FAB) mass spectrometry and ^1H NMR spectroscopy. Saponification of heptaester **12** with aq. sodium hydroxide, followed by chromatography on Sephadex G15, afforded combretastatin melibioside **7** as a water-soluble gum (Scheme 2).

Combretastatin glucoside **13** was prepared from glucose in



Scheme 2 Synthesis of combretastatin melibioside **7**. DCM = dichloromethane, Py = pyridine. *Reagents:* i, Pr^iCOCl, Py, DCM; ii, NH_3, DCM, MeOH; iii, CCl_3CN, K_2CO_3, DCM; iv, **1** + $BF_3{\cdot}Et_2O$; v, NaOH, MeOH.



Schemes 3 and 4 Synthesis of combretastatin glucoside **13**. DCM = dichloromethane, AcOH = acetic acid. *Reagents:* i, 30% HBr–AcOH, DCM; ii, 1 mol equiv. of 5% LiOH, MeOH; iii, 5 mol equiv. of 5% aq. LiOH, MeOH; iv, CCl_3CN, K_2CO_3, DCM; v, $BF_3{\cdot}Et_2O$; vi, LiOH

Table 1 Effect of compounds **1**, **6**, **7** and **13** on the growth of P388 mouse leukaemia cells, the multidrug resistant subline P388PR8/22, A2780 human ovarian tumour cells and the multidrug resistant subline A2780/ADR. Values shown are the concentrations (μmol dm^{-3}) required to cause a 50% decrease in cell growth.

	Cell lines			
	P388	P388PR/22	A2780	A2780/ADR
1	0.002 6	0.001 2	0.000 72	0.000 84
6	0.28	nd	nd	nd
7	> 50	> 50	> 50	> 50
13	3.8	8.3	12.8	> 50

nd = not determined.

four steps as follows. Pentaisobutyrylglucose **14**, prepared in high yield from D-glucose and isobutyryl chloride, was treated with 30% hydrogen bromide in acetic acid to give the α-bromo sugar **15**. Coupling of combretastatin A-4 **1** with the bromoglucose **15** with 1 mol equiv. of lithium hydroxide in methanol followed by hydrolysis with 5 mol equiv. of lithium hydroxide produced the desired combretastatin β-D-glucoside **13** in 80% yield (Scheme 3). This glucoside **13** was also prepared using the imidate chemistry as described above (Scheme 4). Briefly, ammonolysis of pentaester **14** yielded an α/β anomer mixture of the tetraester hemiacetal **16** which was converted into the imidate **17** in high yield. Acid-catalysed coupling of imidate **17** with the phenol **1** gave the ether **18**, which afforded combretastatin β-D-glucoside **13** on hydrolysis.

All three combretastatin sugar derivatives (**6**, **7**, **13**) were tested for *in vitro* cytotoxicity in a P388 murine leukaemia cell line. The melibioside **7** and the glucuronide **6** were also tested in the P388PR8/22 MDR and in the A2780 human ovarian cell lines. Two of the sugar derivatives (**6**, **13**) showed cytotoxic activity (Table 1) but were not as active as the parent phenol **1**.

Experimental

All solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Hexanes refer to a light petroleum fraction boiling at 40–60 °C. NMR spectra were recorded on

Bruker AC300 or Varian Unity 500 spectrometers and are referenced to tetramethylsilane. *J* Values are given in Hz. Optical rotations were measured on a Optical Activity AA-100 polarimeter; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were recorded on a Kratos Concept mass spectrometer at an ionising energy of 70 eV; for CI spectra ammonia was the reagent gas, and for FAB spectra the matrix used was 3-nitrobenzyl alcohol. IR spectra were determined on a Perkin-Elmer 1710 FT spectrometer. UV spectra were determined on a Shimadzu UV-260 spectrophotometer. Mps were measured on a Kofler block and are uncorrected. TLC was carried out on plates coated with silica $60F_{254}$.

Methyl (Combretastatinyl 2,3,4-Tri-O-isobutyryl-β-D-glucopyranosid)uronate **5**.—To a solution of combretastatin A-4 **1** (500 mg, 1.58 mmol) and the imidate **4** (178 mg, 3.17 mmol) in anhydrous dichloromethane (15 cm^3) at < 0 °C (ice–NaCl) under N_2 was added boron trifluoride–diethyl ether (400 mm^3, 3.17 mmol) and the mixture allowed to warm to room temperature and stirred for 3 days. The resulting red solution was diluted with dichloromethane, washed successively with saturated aq. sodium hydrogen carbonate and brine, and dried, and the solvent was removed under reduced pressure. The glucuronate **5** was isolated as a solid (770 mg, 68%) after chromatography on silica gel with dichloromethane–ethyl acetate (9:1) as eluent. Trituration with dichloromethane afforded the *title ester* **5** as needles, mp 127–131 °C; R_f 0.55 [dichloromethane–ethyl acetate (9:1)]; ν_{max}(film)/cm^{-1} 3372, 3320, 3246, 1694 and 1609; λ_{max}(EtOH)/nm 230, 244, 288 and 314 (no base shift on addition of NaOH); δ_H($CDCl_3$–D_2O) 7.04 (1 H, d, *J* 2, 2′-H), 7.00 (1 H, dd, *J* 8 and 2, 6′-H), 6.76 (1 H, d, *J* 8, 5′-H), 6.49 (2 H, s, 2- and 6-H), 6.48 (1 H, d, *J* 12, 1a-H), 6.46 (1 H, d, *J* 12, 1a-H), 5.39–5.25 (3 H, m, 2″-, 3″- and 4″-H), 4.94 (1 H, d, *J* 7, 1″-H), 3.97 (1 H, d, *J* 8, 5″-H), 3.97, 3.78, 3.71 and 3.70 (15 H, 4 × s, 4 × OMe, 1 × CO_2Me), 2.58–2.42 (3 H, m, C*H*Me$_2$) and 1.37–0.85 (18 H, m, CHMe$_2$) [(FAB) M$^+$, 716.3044. $C_{37}H_{48}O_{14}$ requires M, 716.3044].

Combretastatinyl β-D-Glucopyranosiduronic Acid Sodium Salt **6**.—To a solution of combretastatin glucuronate **5** (0.73 g, 1.02 mmol) in methanol (18 cm^3) was added 5% aq. sodium hydroxide (3.26 cm^3) and the mixture was stirred overnight. Removal of the solvent under reduced pressure, followed by chromatography on Sephadex G15 with water as eluent, afforded the sodium salt **6** (424 mg) as a yellow solid containing a trace amount of sodium isobutyrate (NMR spectroscopy); R_f 0.59 [ethyl acetate–methanol–water–acetic acid (60:30:9:1)]; λ_{max}(water)/nm 240 and 288 (no base shift with NaOH); δ_H(D_2O–[$^2H_6$]acetone) 7.17 (1 H, d, *J* 2, 2′-H), 7.00 (1 H, dd, *J* 8 and 2, 6′-H), 6.96 (1 H, d, *J* 8, 5′-H), 6.62 (2 H, s, 2- and 6-H), 6.59 (1 H, d, *J* 12, 1a-H), 6.52 (1 H, d, *J* 12, 1′a-H), 4.76 (1 H, d, *J* 7.5, 1″-H), 3.88, 3.79 and 3.71 (12 H, 3 × s, 4 × OMe) [(FAB) 537.1341 (M$^+$ + Na). $C_{24}H_{27}Na_2O_{11}$ requires *m/z* 537.1349].

Melibiose Octaisobutyrate **10**.—To a vigorously stirred suspension of α-melibiose hydrate **9** (5.0 g, 13.9 mmol) in a mixture of pyridine (12 cm^3) and dichloromethane (30 cm^3) at −8 °C (ice–NaCl) was added isobutyryl chloride (17.3 cm^3, 0.167 mol) over a period of 35 min whilst the temperature of the mixture during this very exothermic reaction was kept below 15 °C. The mixture was then allowed to warm to room temperature and was stirred overnight. The resulting suspension was diluted with dichloromethane, washed successively with 1 mol dm^{-3} HCl, saturated aq. sodium hydrogen carbonate and brine, and dried and the solvent was removed under reduced pressure to leave a pale yellow oil. Trituration with hexanes afforded the crude product **10** as a solid (15.4 g) as an α/β anomeric mixture (6:1, by NMR spectroscopy); R_f 0.60 [hexane–ethyl acetate (3:1)]; δ_H($CDCl_3$) 6.28 (1 H, d, *J* 3.5, 1-H$^\alpha$), 5.69 (1 H, d, *J* 8, 1-H$^\beta$), 5.54 (1 H, t, *J* 10, 3-H), 5.50 (1 H, d, *J* 3, 1′-H), 5.41 (1 H, dd, *J* 10 and 3, 2′-H), 5.23 (1 H, t, *J* 10, 4-H), 5.13–5.07 (1 H, m, 3′-H), 5.04 (1 H, dd, *J* 10 and 3.5, 2-H), 4.25–4.15 (2 H, m, 6-H$_2$), 4.07–3.95 (2 H, m, 5- and 5′-H), 3.69 (1 H, dd, *J* 12 and 4.5, 6′-H), 3.48 (1 H, dd, *J* 12 and 1.5, 6′-H), 2.72–2.35 (8 H, m, C*H*Me$_2$) and 1.25–1.06 (48 H, m, CH*Me*$_2$); *m/z* (FAB) 901 (M − H).

Heptaisobutyrylmelibiose Imidate **8**.—Into a solution of the crude melibiose octaisobutyrate **10** (12.0 g) in a mixture of dichloromethane (20 cm^3) and methanol (20 cm^3) at −8 °C (ice–NaCl) was bubbled gaseous ammonia during 20 min. The mixture was stirred at room temperature for 18 h, more ammonia was passed through for 30 min, and the mixture was stirred for a further 30 min. Evaporation off of the solvent and chromatography of the residue on silica gel with hexanes–ethyl acetate (3:1) as eluent afforded the heptaisobutyrate **11** as an oil (7.4 g, 64%), R_f 0.38 [hexanes–ethyl acetate (3:1)]; ν_{max}(film)/cm^{-1} 3474 (OH).

To a solution of the hemiacetal **11** (7.4 g, 8.9 mmol) in anhydrous dichloromethane (85 cm^3) were added trichloroacetonitrile (2.23 cm^3, 22.3 mmol) and anhydrous potassium carbonate (3.7 g, 26.7 mmol) and the mixture was stirred overnight. The solution was filtered through a pad of silica gel and the product was isolated by elution with diethyl ether (250 cm^3). Evaporation off of the solvent afforded the gummy imidate **8** as an α/β (3:2) mixture (7.8 g, 89%), R_f 0.46 and 0.59 [hexanes–ethyl acetate (3:1)]; δ_H($CDCl_3$) 9.22 [1 H, s, NH (β)], 8.88 [1 H, s, NH (α)], 6.47 (1 H, d, *J* 3.5, 1-H$^\alpha$) and 5.84 (1 H, d, *J* 8, 1-H$^\beta$).

Heptaisobutyryl Ester of Combretastatin Melibioside **12**.—To a solution of combretastatin A-4 **1** (100 mg, 0.316 mmol) and melibiose imidate **8** (1.24 g, 1.266 mmol) in dichloromethane (5 cm^3) in the dark at < 0 °C (ice–NaCl) under nitrogen was added boron trifluoride–diethyl ether (40 mm^3, 0.316 mmol) and the mixture was allowed to warm to room temperature overnight. The resulting red solution was diluted with dichloromethane, washed successively with saturated aq. sodium hydrogen carbonate and brine, and dried, and the solvent was removed under reduced pressure to give compound **12** R_f 0.35 [hexanes–ethyl acetate (3:1)], δ_H 6.97 (1 H, d, *J* 2, 2′-H), 6.94 (1 H, dd, *J* 9 and 2, 6′-H), 6.74 (1 H, d, *J* 9, 5′-H), 6.51 (2 H, s, 2- and 6-H), 6.46 (1 H, d, *J* 12, 1a-H), 6.43 (1 H, d, *J* 12, 1′a-H) and 5.60 (1 H, t, *J* 10, 3″-H) [(FAB) M$^+$, 1130.5337. $C_{58}H_{82}O_{22}$ requires M, 1130.5298].

Combretastatin Melibioside **7**.—To a solution of the crude protected combretastatin melibioside **12** (681 mg) in methanol (17 cm^3) cooled in ice–salt was added 5% aq. sodium hydroxide (3.38 cm^3) and the mixture was allowed to warm to room temperature overnight. Removal of the solvent under reduced pressure, followed by chromatography on Sephadex G15 with water as eluent, afforded combretastatin melibioside **7** as a yellow gum (382 mg), λ_{max}(water)/nm 238 and 290 (no base shift on addition of NaOH); δ_H(D_2O) 6.98 (1 H, d, *J* 2, 2′-H), 6.88 (1 H, dd, *J* 8 and 2, 6′-H), 6.82 (1 H, d, *J* 8, 5′-H), 6.62 (1 H, d, *J* 12, 1a-H), 6.60 (2 H, s, 2- and 6-H), 6.55 (1 H, d, *J* 12, 1′a-H), 4.85 (1 H, d, *J* 3, 1‴-H), 4.40 (1 H, d, *J* 8, 1″-H) and 3.81, 3.74 and 3.65 (12 H, 3 × s, 4 × OMe) [(FAB) 663.2220 (M + Na$^+$). $C_{30}H_{40}NaO_{15}$ requires *m/z* 663.2265].

1,2,3,4,6-*Penta*-O-*isobutyryl*-D-*glucopyranose* **14**.—A suspension of D-glucose (48.0 g, 0.267 mol) in pyridine (200 cm^3) was heated on a steam-bath for 1.5 h and then was allowed to cool to −7 °C (ice–NaCl–MeOH). To a mechanically stirred suspen-

sion of the above mixture was added a solution of isobutyryl chloride (180 cm^3) in dichloromethane (200 cm^3) over a period of 4.5 h at such a rate as to keep the reaction temperature below 10 °C. After half of the isobutyryl chloride–dichloromethane solution had been added, the reaction mixture was diluted with dichloromethane (200 cm^3). When the addition was complete, the mixture was diluted with dichloromethane (600 cm^3), washed successively with 1 mol dm^{-3} HCl (3 × 200 cm^3) and saturated aq. sodium hydrogen carbonate (3 × 200 cm^3). The organic phase was separated and added to 0.880 aq. ammonia (200 cm^3). The two-phase mixture was stirred for 20 min and the aqueous layer was decanted. The dichloromethane solution was washed with brine, dried, and filtered, and the solvent was removed under reduced pressure to leave a yellow gum which solidified. Recrystallisation from ethanol afforded a 1:1 α/β mixture of *glucose pentaisobutyrate* **14** as crystals (119 g, 84%), mp 52–54 °C (Found: C, 59.1; H, 8.0. $C_{26}H_{42}O_{11}$ requires C, 58.9; H, 7.90%); ν_{max}(film)/cm^{-1} 2976, 2879, 1754, 1472 and 1389; *m/z* (CI) 548 (M + 18). Both anomers can be separated by chromatography on silica gel with hexanes–ethyl acetate (3:1) as eluent: α-anomer, mp 76–78 °C (from EtOH); $[\alpha]_D$ + 70 (*c* 28 mg cm^{-3}, $CHCl_3$); δ_H($CDCl_3$) 6.60 (1 H, d, *J* 4, 1-H), 5.61 (1 H, t, *J* 9.5, 3-H), 5.20 (1 H, t, *J* 9.5, 4-H), 4.83 (1 H, dd, *J* 9.5 and 4, 2-H), 4.31 (1 H, ddd, *J* 9.5, 4.8 and 2.4, 5-H); 4.26 (1 H, dd, *J* 11 and 4.8, 6-H), 4.14 (1 H, dd, *J* 11 and 2.4, 6-H), 2.65–2.41 (5 H, m, C*H*Me_2) and 1.20–1.05 (30 H, m, CH*Me*$_2$); β-anomer, mp 106 °C (from EtOH); $[\alpha]_D$ +40 (*c* 32 mg cm^{-3} $CHCl_3$); δ_H($CDCl_3$) 5.73 (1 H, d, *J* 8, 1-H), 5.32 (1 H, t, *J* 9.5, 3-H), 5.18 (1 H, dd, *J* 9.5 and 8, 2-H), 5.17 (1 H, t, *J* 9.5, 4-H), 4.23 (1 H, dd, *J* 12.5 and 4.5, 6-H), 4.11 (1 H, dd, *J* 12.5 and 2, 6-H), 3.85 (1 H, ddd, *J* 9.5, 4.5 and 2, 5-H), 2.65–2.42 (5 H, m, 5 × C*H*Me_2) and 1.18 (30 H, m, 5 × CHMe_2).

2,3,4,6-*Tetra*-O-*isobutyryl*-α-D-*glucopyranosyl Bromide* **15**.—To a solution of 1,2,3,4,6-penta-*O*-isobutyryl-D-glucopyranose (1:1 α/β mixture) **14** (5.0 g, 9.4 mmol) in dichloromethane (10 cm^3) at < 0 °C (ice–NaCl) was added 30% hydrogen bromide in acetic acid (10 cm^3). After warming to room temperature overnight, the resulting solution was evaporated to dryness under reduced pressure. The residue was redissolved in dichloromethane, washed successively with cold, saturated aq. sodium hydrogen carbonate and brine, and dried, and the solvent was removed under reduced pressure. Recrystallisation from ethanol afforded the *α-bromide* **15** as crystals (2.46 g, 50%), mp 67–69 °C; R_f 0.8 [$CHCl_3$–MeOH (9:1)]; $[\alpha]_D$ +189 (*c* 17 mg cm^{-3}, $CHCl_3$) (Found: C, 50.5; H, 6.9; Br, 15.3. $C_{22}H_{35}BrO_9$ requires C, 50.6; H, 6.7; Br, 15.1%); ν_{max}(film)/cm^{-1} 2976, 1747, 1471, 1390 and 1248; δ_H($CDCl_3$) 6.61 (1 H, d, *J* 4, 1-H), 5.62 (1 H, t, *J* 10, 3-H), 5.21 (1 H, t, *J* 10, 4-H), 4.84 (1 H, dd, *J* 10 and 4, 2-H), 4.39–4.23 (2 H, m, 5- and 6-H), 4.15 (1 H, dd, *J* 12.5 and 1.5, 6-H), 2.68–2.42 (4 H, m, 4 × C*H*Me_2) and 1.21–0.97 (24 H, m, 4 × CHMe_2); *m/z* (FAB) 523 (M + H), 443, 373, 285, 197 and 71.

2,3,4,6-*Tetra*-O-*isobutyryl*-D-*glucopyranose* **16**.—Into a solution of D-glucose pentaisobutyrate **14** (1:1 α/β mixture) (57.46 g, 0.108 mol) in a mixture of dichloromethane (1.2 dm^3) and MeOH (800 cm^3) cooled in ice–salt was passed gaseous NH_3 over a period of 4 h and the mixture was left to react overnight at ambient temperature. On each of the following days, more gaseous NH_3 was bubbled through for 2–3 h. The solvent was then removed under reduced pressure, the residue was redissolved in dichloromethane, the solution was washed successively with water and brine, and dried, and the solvent was evaporated off. The crude mixture was chromatographed on silica gel with hexanes–ethyl acetate (2:1) as eluent to give the tetra ester **16** as a gum (14.0 g, 28%) as an α/β mixture of hemiacetals. Trituration with hexanes gave the pure α-hemiacetal, mp 85–87 °C; $[\alpha]_D$ +38 (*c* 15 mg cm^{-3}, $CHCl_3$); R_f 0.38 [hexanes–ethyl acetate (2:1)]; ν_{max}(film)/cm^{-1} 3469, 2976, 1749, 1472, 1390 and 1251; δ_H($CDCl_3$) 5.60 (1 H, t, *J* 10, 3-H), 5.46 (1 H, d, *J* 4, 1-H$^{\alpha}$), 5.31 (1 H, d, *J* 9, 1-H$^{\beta}$), 5.14 (1 H, t, *J* 10, 4-H), 4.91 (1 H, dd, *J* 10 and 4, 2-H), 4.28 (1 H, ddd, *J* 10, 4 and 2, 5-H), 4.21–4.10 (2 H, m, 6-H_2), 2.69–2.37 (4 H, m, 4 × C*H*Me_2) and 1.30–1.04 (24 H, m, 4 × CHMe_2); *m/z* (EI) 460, 443, 373, 355, 285 and 197; *m/z* (CI) 478 (M + 18).

2,3,4,6-*Tetra*-O-*isobutyryl*-α-D-*glucopyranosyl Trichloroacetimidate* **17**.—To a solution of the α/β hemiacetal **16** (3.74 g, 8.13 mmol) in dichloromethane (45 cm^3) at room temperature was added trichloroacetonitrile (2.46 cm^3, 3.52 g, 24.39 mmol) and the solution was stirred for 10 min. Anhydrous potassium carbonate (3.93 g, 28.46 mmol) was added and 20 h later the mixture was filtered through a pad of silica gel and the product was eluted with diethyl ether. The filtrate was concentrated under reduced pressure to afford a mixture of α/β anomers (1:1 by NMR spectroscopy) of the imidate **17** as a gum (4.79 g, 98%), R_f 0.71 and 0.64 [hexanes–ethyl acetate (2:1)]; ν_{max}(film)/cm^{-1} 3300, 2976, 1749, 1679, 1471, 1388 and 1248; δ_H($CDCl_3$) 8.79 and 8.75 (2 H, 2 × s, 2 × NH), 6.27 (1 H, d, *J* 4, 1-H), 5.32 (1 H, dd, *J* 10 and 9.5, 3-H), 5.17 (1 H, t, *J* 10, 4-H), 5.16 (1 H, dd, *J* 9.5 and 4, 2-H), 4.24–4.13 (2 H, m, 6-H), 4.07 (1 H, ddd, *J* 10, 5 and 2, 5-H), 2.67–2.35 (4 H, m, 4 × C*H*Me_2) and 1.27–1.00 (24 H, m, 4 × CH*Me*$_2$); *m/z* (EI) 460, 443, 355 and 286; *m/z* (CI) 588, 568, 478, 443 and 355.

Combretastatinyl 2,3,4,6-*Tetra*-O-*isobutyryl*-β-D-*glucopyranoside* **18**.—To a solution of combretastatin A-4 **1** (200 mg, 0.63 mmol) and the glucose imidate **17** (2.296 g, 3.8 mmol) in dichloromethane (10 cm^3), at room temperature under nitrogen, was added boron trifluoride–diethyl ether (477 mm^3, 3.8 mmol) and the mixture was stirred overnight in the dark. The resulting purple solution was diluted with dichloromethane, washed successively with saturated aq. sodium hydrogen carbonate and brine, and dried, and the solvent was removed under reduced pressure. Chromatography on silica gel with dichloromethane–ethyl acetate as eluent (20:1 to 8:1) gave the protected glucoside **18** as a solid (435 mg, 91%), mp 193–198 °C; R_f 0.85 [dichloromethane–ethyl acetate (4:1)]; ν_{max}(film)/cm^{-1} 2975, 2939, 2877 and 1749; λ_{max}(EtOH)/nm 324, 240 and 225 (no base shift on addition of NaOH); δ_H 7.09 (1 H, d, *J* 2, 2′-H); 7.02 (1 H, dd, *J* 2 and 8, 6′-H), 6.79 (1 H, d, *J* 8, 5′-H), 6.54 (2 H, s, 2- and 6-H), 6.49 (1 H, d, *J* 12, 1a-H), 6.46 (1 H, d, *J* 12, 1′a-H), 5.30 (1 H, t, *J* 9.5, 3″-H), 5.16 (1 H, t, *J* 9.5, 4″-H), 5.00 (1 H, dd, *J* 9.5 and 8, 2″-H), 4.86 (1 H, d, *J* 8, 1″-H), 4.23 (1 H, dd, *J* 12 and 4.5, 6″-H), 4.16 (1 H, dd, *J* 12 and 2, 6′-H), 3.96, 3.91 and 3.86 (12 H, 3 × s, 4 × OMe), 3.80–3.70 (1 H, m, 5″-H), 2.68–2.42 (4 H, m, 4 × C*H*Me_2) and 1.25–1.08 (24 H, m, 4 × CH*Me*$_2$); *m/z* 758 (M^+), 443 and 316.

Combretastatin Glucoside **13**.—To a solution of combretastatin A-4 **1** (205 mg, 0.65 mmol) in 2.4% methanolic lithium hydroxide (1.4 cm^3) was added the bromo sugar **15** (0.339 g, 0.65 mmol). After 2.5 h more methanolic lithium hydroxide (1.4 cm^3) was added and the mixture was stirred for 16 h. Another aliquot of methanolic lithium hydroxide (4.2 cm^3) was added, the mixture was left for 4 h, and the solvent was removed under reduced pressure. The mixture was separated on Sephadex G-10 with water as eluent to afford *combretastatin glucoside* **13** as a solid (249 mg, 80%), mp 92–95 °C (Found: C, 58.1; H, 6.3. $C_{24}H_{30}O_{10}$·H_2O requires C, 58.1; H, 6.5%); R_f 0.54 [ethyl acetate–methanol–water–acetic acid (60:30:9:1)]; λ_{max}(water)/nm 220, 240 and 286 (no shift on addition of NaOH); δ_H(D_2O) 6.97 (1 H, br s, 2′-H), 6.96 (1 H, dd, *J* 8 and 2, 6′-H), 6.91 (1 H, br s, 5′-H), 6.58 (2 H, br s, 2- and 6-H), 6.56 (1 H, d, *J* 12, 1a-H), 6.51 (1 H, d, *J* 12, 1′a-H), 4.40 (1 H, d, *J* 8, 1″-H) and

3.79, 3.71 and 3.63 (12 H, 3 × s, 4 × OMe) [(FAB) M^+, 478.1846. $C_{24}H_{30}O_{10}$ requires M, 478.1839].

Cytotoxicity testing was performed by using an MTT assay.[10]

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References

1 B. B. Brodie and J. Axelrod, *J. Pharmacol.*, 1948, **94**, 29.
2 B. B. Brodie and J. Axelrod, *J. Pharmacol.*, 1949, **97**, 58.
3 L. A. Woods, *J. Pharmacol. Exp. Ther.*, 1954, **112**, 158.
4 J. M. Fujimoto and E. L. Way, *J. Pharmacol. Exp. Ther.*, 1957, **121**, 340.
5 J. M. Fujimoto and E. L. Way, *J. Am. Pharm. Assoc., Sci. Ed.*, 1958, **47**, 273.
6 K. Yoshimura, K. Oguri and H. Tsukamoto, *Chem. Pharm. Bull.*, 1968, **16**, 2114.
7 R. Osborne, P. Thompson, S. Joel, D. Trew, N. Patel and M. Slevin, *Br. J. Clin. Pharmacol.*, 1992, **34**, 130.
8 F. Scheinmann, K. W. Lumbard, R. T. Brown and S. P. Mayalarp, *Int. Pat.*, 1993, WO 93/3051.
9 R. T. Brown, S. P. Mayalarp, A. T. McGown and J. A. Hadfield, *J. Chem. Res. (S)*, 1993, 496.
10 J. M. Edmondson, L. S. Armstrong and A. O. Martinez, *J. Tissue Culture Methods*, 1988, **11**, 15.

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