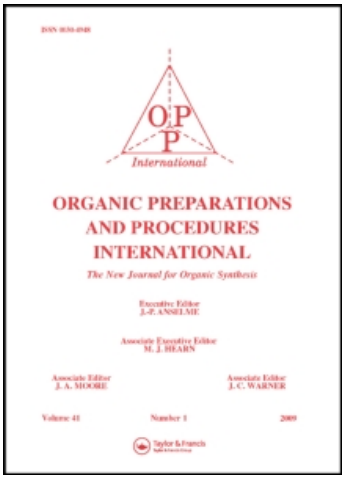

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Gurmit S. Gill[a]; Damian Grobelny[a]; Bernard Flynn[a]
[a] C/-Department of Chemistry, Iliad Chemicals PTY Limited, Vic, AUSTRALIA

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3. A. J. Moore, L. M. Goldenberg, M. R. Bryce, M. C. Petty, J. Moloney, J. A. K. Howard, M. J. Joyce and S. N. Port, *J. Org. Chem.*, **65**, 8269 (2000).

4. K. Bandyopadhyay, L. Shu, H. Liu and L. Echegoyen, *Langmuir*, **16**, 2706 (2000).

5. D. Shen, X. Wu, X. Liu, Q. Kang and S. Chen, *Microchemical Journal*, **63**, 322 (1999).

6. F. Eloy and R. Lenaers, *Chem. Rev.*, **62**, 155 (1962).

7. G. G. Urquhart, J. W. Gates, Jr., and R. Connor, *Org. Synth.*, **3**, 363 (1955).

8. J. H. Wynne, C. T. Lloyd and R. F. Cozzens, *Chemistry Lett.*, 926 (**2002**).

9. F. W. McLafferty and F. Tureceik, *"Interpretation of Mass Spectra"*, 4th Ed, p. 277, University Science Books, Sausalito, CA, 1993.

10. R. T. Bibart, K. W. Vogel and D. G. Drueckhammer, *J. Org. Chem.*, **64**, 2903 (1999).

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A PRACTICAL METHOD FOR PHOSPHORYLATION OF COMBRETASTATIN A-4 WITH PHOSPHORUS OXYCHLORIDE

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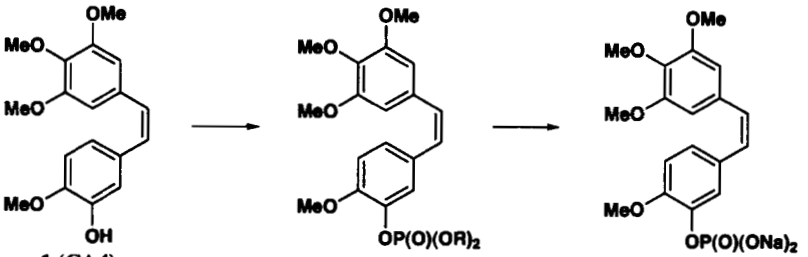
Gurmit S. Gill, Damian Grobelny* and Bernard Flynn

Iliad Chemicals PTY Limited
C/-Department of Chemistry
Latrobe University Vic 3086, AUSTRALIA
Email: dgrobelny@bionomics.com.au

Isolated from the South African tree *Combretum caffrum*, combretastatin A-4 **1** (**CA-4**)[1] is one of the most promising experimental anticancer drugs, targeting tumor vasculature.[2] The combretastatin A-4 disodium phosphate prodrug **3** (**CA-4P**) has emerged as the most useful form of this compound in preclinical *in vivo* models, as well as in clinical trials,[2a] due to the limited solubility of the parent molecule in water.[3]

The synthetic methods reported for **3** (**CA-4P**) (*Scheme 1*) involve the two step reaction of the phenolic group of **CA-4** with *in situ* generated dibenzyl chlorophosphite[3,5,6] or dibenzyl bromophosphite[4] followed by cleavage of the benzyl esters with iodotrimethylsilane[3,5,6] or bromotrimethylsilane.[4] In an alternative procedure, dibenzyl halophosphite is replaced by *bis*

(2,2,2-trichloroethyl) chlorophosphite, followed by removal of the trichloroethyl groups with zinc dust.[3,4] The synthesis of **3** (**CA-4P**) through intermediates **2c** and **2d** requires preparation of relevant starting dialkoxy(*N,N*-dialkylamino)phosphines and in addition, oxidation of the phosphine to phosphates **2c** and **2d** with *m*-chloroperbenzoic acid (MCPBA).[5,7] The multi-step character of the above methods, the need to use chromatography for purification of intermediates **2a-2d** and the formation of undesirable *trans*-isomer of **3** during deprotection of **2a** seem less than optimal.

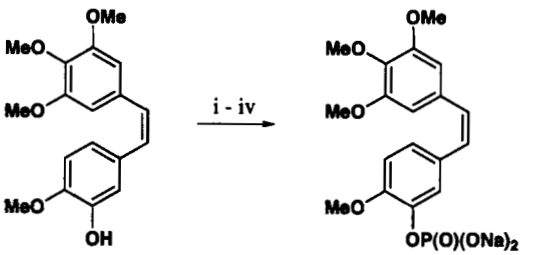


1 (CA4) **2a-d** **3 (CA4P)**

2a R = Bn; **2b** R = CH_2CCl_3; **2c** R = *t*-Bu; **2d** R = $CH_2CH_2Si(Me)_3$

Scheme 1

Phosphorylation of phenols with $P(O)Cl_3$ is by far the most direct method of preparation of phenyl phosphoric esters.[8] The reaction requires elevated temperature or presence of base to scavenge the evolving HCl. Pettit *et al.* have reported no reaction between **CA-4** and $P(O)Cl_3$ at room temperature, when trimethyl phosphate or tetrahydrofuran was used as a solvent. Increasing the reaction temperature lead to the polymeric material.[3] A successful $P(O)Cl_3$ phosporylation of **CA-4** has been recently claimed by Hua *et al.*[9] when the reaction was performed in an inert solvent in the presence of an acid scavenger. We report herein a variation of this method, which provides a simple and very efficient method of preparing disodium combretastatin A4 phosphate prodrug **3** (**CA-4P**) (*Scheme 2*).



1 (CA4) **3 (CA4P)**

i) $P(O)Cl_3$ (2.5 eq) in CH_2Cl_2, triethylamine (1.5 eq); ii) 0.5 N aq. NaOH, acetonitrile, iii) conc. HCl, acetonitrile; iv) 0.5 N aq. NaOH, methanol.

Scheme 2

This method is based on dropwise addition of a solution of **CA-4** and 1.5 eq of triethylamine in CH_2Cl_2 to a solution of freshly distilled $P(O)Cl_3$ (2.5 eq) in CH_2Cl_2 at –5-0°C, under N_2, followed by removal of excess of $P(O)Cl_3$ *in vacuo*.

The resultant dichlorophosphate intermediate was dissolved in acetonitrile and hydrolyzed to give **3** upon the rapid addition of its acetonitrile solution to excess of 0.5 N aqueous NaOH. The reverse addition should be avoided, due to formation of a small amount of the combretastatin A-4 diester of phosphoric acid. The NaCl formed during this process is easily removed by conversion of **3** (**CA-4**) into the phosphoric acid, collection of NaCl, followed by reconversion of the free acid to its disodium salt. This simple procedure gives pure **3** (**CA-4P**), with an overall yield over 95%, using an inexpensive phosphorylating agent, with no need for laborious chromatographic purification of the intermediates **2** and no traces of undesirable *trans*-isomer. Both *cis* and *trans* isomers of **CA-4P** have very different chemical shifts for aromatic as well as vinyl protons.[6] No traces of the *trans* isomer can be detected in the proton NMR spectra. In addition, both isomers have distinctive retention times in LC analysis and only one isomer can be identified (+99) in LC analysis of **CA-4P** samples synthesized by our method.

EXPERIMENTAL SECTION

All starting materials were purchased from Aldrich Chemical Co. Triethylamine was purified by distillation from CaH_2 and stored under N_2 over molecular sieves A4. $P(O)Cl_3$ was freshly distilled under reduced pressure prior to use and stored under dry N_2. Combretastatin A-4 was prepared from 3-hydroxy-4-methoxybenzaldehyde and 3,4,5-trimethoxyphenylacetic acid according to the reported procedure.[10] ^1H NMR were measured with a Brucker 300 MHz spectrometer. LC/MS analysis was conducted with ZorbaxC8 (4.6 x 150 mm) column using isocratic mobile phase containing 75% acetonitrile, 20% water and 5% ammonium formate solution, consisting of acetic acid (1 g), ammonium formate (0.315 g) in 33% aqueous methanol (1 liter). Detection was measured at 214 nm and constant flow rate of 0.5 mL/min. MS analysis was conducted with an Agilen 1100 Series MSD system. Melting points were measured with an *Electrothermal* melting point apparatus and are uncorrected. Elemental analyses were determined by *CMAS*, Belmont Victoria, Australia.

Combretastatin A-4 Disodium phosphate CA-4P: To a solution of freshly distilled $P(O)Cl_3$ (0.37 mL; 3.95 mmol) in anhydrous CH_2Cl_2 (1 mL) a mixture of combretastatin A-4[10] (0.5 g; 1.58 mmol) and anhydrous triethylamine (0.33 mlmL; 2.37 mmol) in CH_2Cl_2 (2 mL) was added dropwise at –5 - 0°C under N_2 with stirring. After stirring for 10 minutes at 0°C, the mixture was evaporated to dryness under reduced pressure. To facilitate a complete removal of excess $P(O)Cl_3$, the residue was suspended in anhydrous toluene (2 mL), stirred for 5 minutes and evaporated to dryness under reduced pressure. The process was repeated using anhydrous acetonitrile (2 mL). After evaporation of the solvent the residue was dried *in vacuo* for 30 minutes and suspended in anhydrous acetonitrile (4 mL). This was added immediately to a 0.5 N aqueous solution of NaOH (14 mL). The resulting mixture was evaporated to dryness under reduced pres-

sure and the residue was suspended in water (5 mL). After adjusting the pH of the solution to ~ 10 with 0.5 N NaOH, the resulting solution was filtered through a Whatman glass microfibre filter to remove any traces of solid material. The filtrate was concentrated to about 2 mL under reduced pressure and acidified to pH ~ 1 with concentrated HCl. To facilitate removal of water and excess of HCl the mixture was repeatedly diluted to 20 mL with acetonitrile and evaporated to dryness under reduced pressure (x 3) followed by dilution in CH_2Cl_2 (20 mL) and evaporation. The residue formed was suspended in a fresh portion of anhydrous CH_2Cl_2 and precipitated NaCl was collected. The filtrate was evaporated to dryness under reduced pressure to give 0.61 g (100% yield) of the phosphoric acid as a creamy hygroscopic foam, after drying *in vacuo* for 1 h. ^{1}H NMR ($CDCl_3$) 3.65 (s, 6H), 3.76 (s, 3H), 3.79 (s, 3H), 6.17 (broad singlet, 4H, 2 x OH + H_20), 6.41 (s, 2H), 6.43 (s, 2H), 6.76 (d, 1H, J = 8.5 Hz), 6.99 (dd, 1H, J = 1.76 Hz, 8.5 Hz), 7.13 (d, 1H, 1.76 Hz). The ^{1}H NMR spectrum of recovered NaCl in D_2O did not show any organic matter. The free acid (0.61 g; 1.58 mmol) was dissolved in methanol (4 mL) and the pH of the mixture was adjusted to ~10 by addition of 0.5 N NaOH and the resulting solution was evaporated to dryness under reduced pressure. The residue was suspended in 2-propanol (4 mL), stirred for 5 minutes at room temperature and collected. The solid was washed with fresh 2-propanol (2 mL), anhydrous diethyl ethyl ether (3 x 5 mL) and dried *in vacuo* until constant mass was achieved, to give 0.671g (96.4 % yield) of pure **CA-4P** as a colorless solid, mp. 238-242 °C (dec.), *lit.*[5] 190–195 (dec.). LC, retention time = 1.17 min; (+99 %); MS: 396.9 (MH_2 - $2Na^+$ + 1); 413.9 (MH - $2Na^+$ + $NH4^+$ + 1); ^{1}H NMR (D_2O) 3.59 (s, 6H), 3.65 (s, 3H), 3.72 (s, 3H), 4.66 (s, D_2O), 6.42 (d, 1H, J = 12.01 Hz), 6.55 (d, 1H, J = 12.01 Hz), 6.58 (s, 2H), 6.77 (m, 2H), 7.27 (s, 1H).[3-6]

Anal. Calcd for $C_{18}H_{19}Na_2O_8P{\bullet}H_2O$: Na, 10.03; P, 6.76. Found: Na, 10.28; P, 6.89.

REFERENCES

1. G. R. Pettit, S. B. Singh, E. Hamel, C. M. Lin, D. S. Alberts, and D. Garcia-Kendal, *Experientia*, **45**, 209 (1989).

2. a) J. Griggs, J. C. Metcalfe and R. Hesketh, *The Lancet Oncology*, **2**, 82 (2001) and references therein; b) R. T. Dorr, K., Dvorakova, K. Snead, D. S. Alberts, S. E. Salmon, and G. R. Pettit, *Investigational New Drugs*, **14**, 131 (1996); c) A. Dowlati, K. Robertson, M. Cooney, W. P. Petros, M. Stratford, J. Jesberger, N. Rafie, B. Overmoyer, V. Makkar, M. Stambler, A. Taylor, J. Waas, J. S. Lewin, K. R. McCrae, and S. C. Remick, *Cancer Research*, **62**, 3408 (2002).

3. G. R. Pettit, C. Temple, Jr, R. V. Narayanan, M. J. Simpson, M. R. Boyd, G. A. Renel and N. Bansal, *Anti-cancer Drug Design*, **10**, 299 (1995).

4. F. Seyedi, J. Gale, R. Haider, and J. Hoare, PCT Application No WO 02/06279 A1 (2002); CA **136**: 134622 (2002)

5. G. R. Pettit, and M. R. Rhodes, *Anti-cancer Drug Design,* **13**, 183 (1998).

6. G. R. Pettit, M. R. Rhodes, D. L. Herald, D. J. Chaplin, M. R. L. Stratford, E. Hamel, R. K., Pettit, J-C. Chapuis and D. Oliva, *Anti-cancer Drug Design,* **13**, 981 (1998).

7. S. B. Bedford, C. P. Quarterman, D. L. Rathbone, and J. A. Slack, *Bioorg. & Med. Chem. Lett.,* **6**, 157 (1996).

8. E. J. King, and T. F. Nicholson, *Biochemical J.,* **33**, 1182 (1939) and references therein.

9. W. Hua, D. Du H. Zhang, and Q. Wu, *Chinese Patent Application*, CN 1465580 A20040107; *CA* **142**: 316955 (2004).

10. K. Gaukroger, J. A. Hadfield, L. A. Hepworth, N. J. Lawrence, and A. T. McGown, *J. Org. Chem.*, **66**, 8135 (2001).