Pergamon

0960-894X(95)00580-3

# SYNTHESIS OF WATER-SOLUBLE PRODRUGS OF THE CYTOTOXIC AGENT COMBRETASTATIN A4.

Simon B Bedford, Charmaine P Quarterman, Daniel L Rathbone* and John A Slack
Aston Molecules Ltd, 10 Holt Court South, Aston Science Park, Birmingham B7 4EJ, UK.

Roger J Griffin
*Department of Chemistry, Bedson Building, University of Newcastle,
Newcastle upon Tyne NE1 7RU, UK.*

Malcolm F G Stevens
Cancer Research Laboratories, University of Nottingham, University Park, Nottingham NG7 2RD, UK

ABSTRACT: Water-soluble phosphate and glycine carbamate prodrugs of the cytotoxic agent Combretastatin A4 (1) have been prepared. The phosphate prodrug was degraded slowly in plasma at 37°C. The degradation was accelerated by the addition of alkaline phosphatase.

Combretastatin A4 **1** has been extracted from the bark of a South American tree *Combretum caffrum* and its total synthesis is also reported[1,2]. This substance, which bears a structural resemblance to colchicine **2**, is an inhibitor of tubulin polymerisation thereby exerting an antineoplastic effect by inhibiting mitosis and microtubule assembly. Combretastatin A4 exhibited excellent cytotoxicity against murine P388, L1210 lymphocytic leukemia cells and various human cell lines.[2] Despite this initial promise as an anti-cancer agent Combretastatin A4 was prevented from entering phase I clinical trial owing to its limited solubility in pharmaceutically acceptable solvents.
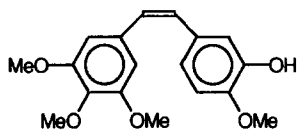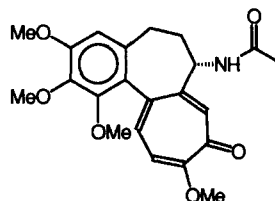

Combretastatin A4 (1)   Colchicine (2)

In this paper we report the synthesis of several derivatives of Combretastatin A4 in an attempt to create a prodrug having increased aqueous solubility and pharmaceutically acceptable properties. Recently, Brown *et al*[3] have reported the synthesis of water-soluble glycosides of Combretastatin A4, two of which have potent cytotoxic properties. Pettit *et al*[4] reported the synthesis of a series of water soluble prodrugs of Combretastatin A4, in particular the 3-O-phosphate derivative.

The obvious position available for prodrug modification in Combretastatin A4 is the hydroxyl function. Before such chemistry could be undertaken a supply of Combretastatin A4 was required. Pettit *et al*[1,2,5,6] have published a linear six-step synthesis with an overall yield of 19%, wherein the key step was construction of the stilbene by a Wittig reaction (Scheme 1).
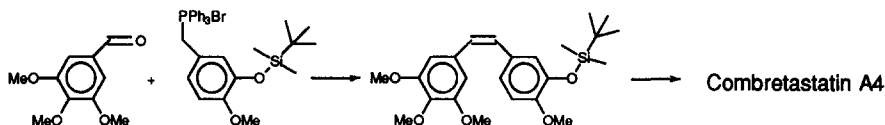
Scheme 1

As an improvement to this we have reversed the components of the Wittig reaction in order to maximise the bulk of the aldehyde component (Scheme 2). There is precedent to hope that large steric bulk in the aldehyde component would favour the production of the desired Z-isomer in the stilbene product.[6]
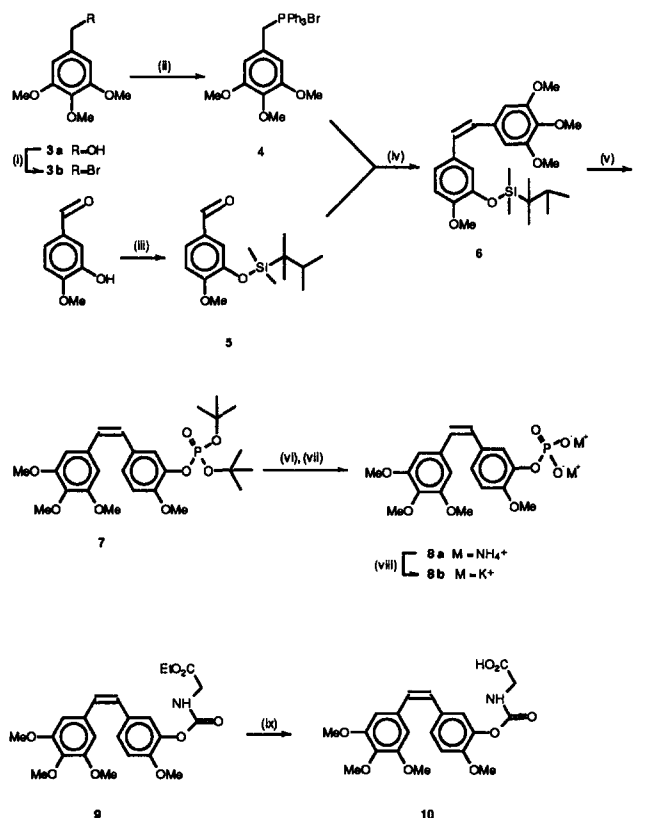
Scheme 2

Reaction conditions: (i) LiBr/TMSCl/CH$_3$CN (ii) PPh$_3$/PhMe (iii) *t*-hexylSi(Me)$_2$Cl/Et$_3$N/THF (iv) *n*-BuLi/THF (v) (*n*-Bu)$_4$NF/THF (vi) TFA/CH$_2$Cl$_2$ (vii) NH$_3$aq/EtOH (viii) Dowex 50 X8 (K$^+$) (ix) H$_2$SO$_4$/H$_2$O/THF/60°C

3,4,5-Trimethoxybenzyl alcohol **3a** was brominated and converted immediately into the triphenylphosphonium salt **4**. 3-Hydroxy-4-methoxybenzaldehyde was silylated to give 4-methoxy-3-(thexyldimethylsilyloxy)-benzaldehyde **5**. This was coupled to the ylide of the phosphonium salt from above to give a 1/1 mixture of the silylated Combretastatin A4 and its E-isomer. The desired Z-isomer **6** was separated by chromatography and deprotected to give Combretastatin A4 **1** in an overall yield of 26% (Scheme 2).

Two derivatives of Combretastatin A4 were prepared as potential water-soluble prodrugs of the parent compound. Combretastatin A4 was phosphorylated *via* di-*tert*-butyl *N,N*-diethylphosphoramidite[7] followed by oxidation with MCPBA to give Combretastatin A4 phosphate bis *t*-butyl ester **7** in 77% yield which was hydrolysed and isolated as the ammonium salt **8a** in 78% yield and then converted to the potassium salt **8b** in 55% yield. Combretastatin A4 was also converted into the glycine carbamate ethyl ester derivative **9** in 86% yield by reaction with ethyl isocyanatoacetate, and then hydrolysed to give Combretastatin A4 glycine carbamate **10** in 84% yield.

Although the phosphate and the glycyl carbamate of Combretastatin A4 were in hand the synthetic route employed presented a considerable obstacle to the preparation of larger quantities of these derivatives because of the time-consuming separation of the Z and E-stilbenes. A direct synthesis of Combretastatin A4 phosphate was undertaken, whereby it was intended that 3-hydroxy-4-methoxybenzaldehyde would be derivatised as the bulky bis-*t*-butylphosphate triester **15** prior to coupling with the other half of the molecule *via* a Wittig reaction. (Scheme 3)
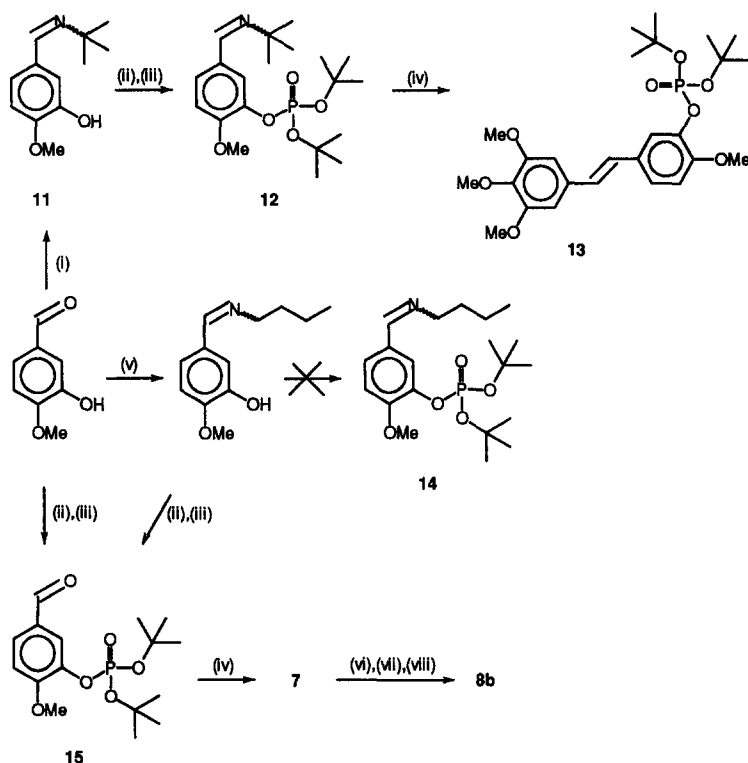
Scheme 3

Reaction conditions: (i) *t*-BuNH₂/PTSA/PhMe (ii) Et₂NP(OBu-*t*)₂/tetrazole/THF (iii) MCPBA/-70°C (iv) *n*-BuLi/4
(v) *n*-BuNH₂/PTSA/PhMe (vi) TFA/CH₂Cl₂ (vii) NH₃aq/EtOH (viii) Dowex 50 X8 (K⁺)

Phosphorylation of 3-hydroxy-4-methoxybenzaldehyde proved to be difficult and only very small quantities of the phosphate triester 15 could be isolated. It was thought that the carbonyl group was interfering with the phosphorylation and so this was converted to the t-butyl imine 11 with a view to protecting the carbonyl function during the phosphorylation reaction, and later regeneration for the Wittig coupling (Scheme 3). The t-butyl imine 11 was converted to the imine-phosphate triester 12 in a one-pot two-step procedure by reaction with di-t-butyl N,N-diethylphosphoramidite and tetrazole in THF at room temperature followed by MCPBA at -70°C. Compound 12 was subjected to various conditions in order to generate the free aldehyde 15 but generally the imine function remained intact whereas the phosphate triester suffered hydrolysis. Therefore the imine was used directly as a carbonyl equivalent in a Wittig reaction with 3,4,5-trimethoxybenzyl triphenylphosphonium ylid. Unfortunately, the exclusive stilbene product from this reaction was the unwanted E-isomer of Combretastatin A4 phosphate bis-t-butyl ester 13. Here the steric bulk of the t-butyl imine appeared to be dominant, forcing the production of an E product. As a result it was thought that the n-butyl imine-phosphate triester 14 would be a more favourable substrate in the Wittig reaction for the synthesis of a Z-stilbene. Consequently, an attempt was made to prepare 14 following the same procedure as for 12 but the compound isolated was in fact the aldehyde 15. This, when reacted with 3,4,5-trimethoxybenzyl triphenylphosphonium ylid, gave a 4/1 mixture of Combretastatin A4 phosphate bis t-butyl ester 7 and its E-isomer 13. The isomer mixture was treated with trifluoroacetic acid in dichloromethane to cleave the t-butyl groups. The product was isolated as the ammonium salt and then converted to the potassium salt 8b using cationic exchange resin. This was recrystallised once to give Combretastatin A4 phosphate potassium salt and its E-isomer in a 9/1 ratio.

The solubility of the ammonium salt of Combretastatin A4 phosphate was approximately 2.8mg/ml. The salt was stable in unbuffered aqueous solution and was degraded in vitro to Combretastatin A4 when incubated with acid phosphatase and alkaline phosphatase. It was degraded slowly in plasma at 37°C. Degradation was accelerated by the addition of alkaline phosphatase. The potassium salt (soluble at ca 5mg/ml in unbuffered aqueous solution) was administered intravenously to male Balb/c mice at doses of 50, 100 and 150mg/kg. These doses were equivalent to approximately 33, 67 and 100mg/kg of free Combretastatin A4. No adverse effects were noted following bolus intravenous administration of 50 and 100mg/kg. However, the higher dose of 150mg/kg proved to be acutely toxic in 60% of the mice dosed. All mice which survived the injection remained well with no other toxicity being observed for the duration of the study which was terminated after 28 days.

References

1. Pettit G. R., Singh S. B., Boyd M. R., Hamel E., Pettit R. K., Schmidt J. M. and Hogan F., J. Med. Chem., 1995, 38, 1666-1672

2. Pettit G. R., Singh S. B., Hamel E., Lin C. M., Alberts D. S. and Garcia-Kendall D., Experientia, 1989, 45, 209-211

3. Brown R. T., Fox B. W., Hadfield J. A., McGown A. T., Mayalarp S. P., Pettit G. R. and Woods J. A., J. Chem. Soc. Perkin Trans. 1, 1995, 577-581

4. Pettit G. R., Temple C., Narayanan V. L., Varma R., Simpson M. J., Boyd M. R., Rener G. A. and Bansal N., Anti-Cancer Drug Design, 1995, 10, 299-309

5. Pettit G. R., Singh S. B. and Cragg G. M., J. Org. Chem., 1985, 50, 3404-3406

6. Pettit G. R., Singh S. B. and Niven M. L., J. Nat. Products, 1987, 50, 119-131

7. Johns R. B. and Perich J. W., Synthesis, 1988, 142