

Synthesis of ^{14}C Radio-labelled Combretastatin A-1 diphosphate ([Methyl- ^{14}C]-Combretastatin A-1 diphosphate)

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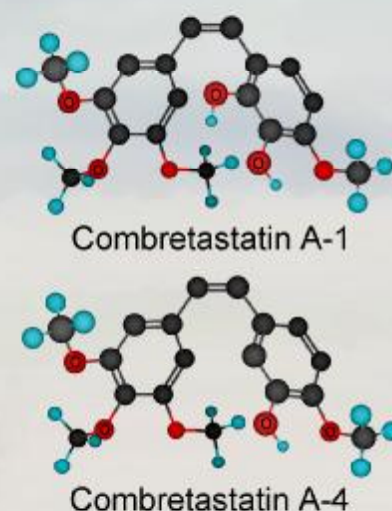
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Introduction

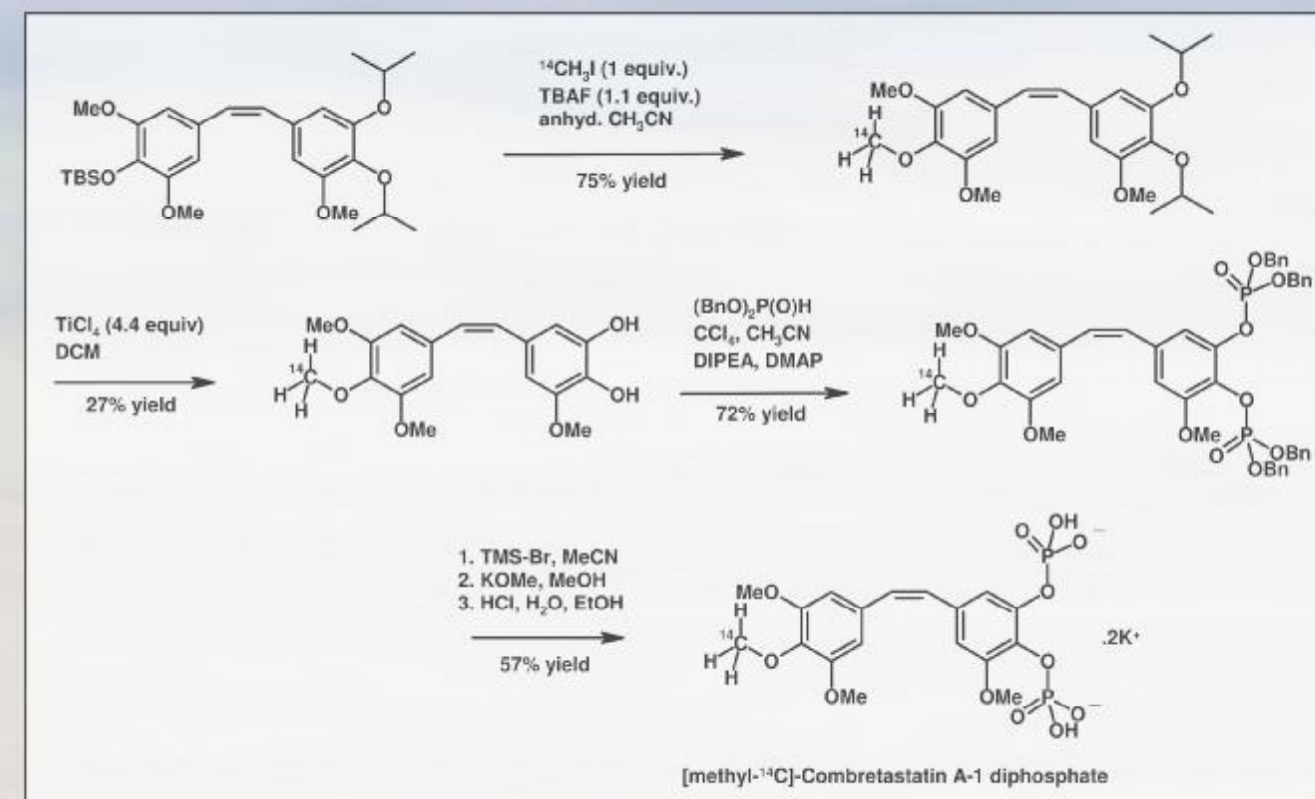
Combretastatin A-1 and Combretastatin A-4 are two microtubule depolymerising agents among a group of 17 related compounds isolated from the African bush willow tree *Combretum caffrum* by Pettit's group in the early 1980s¹.



OXiGENE Inc. (Waltham MA) is currently developing Combretastatin A-1 diphosphate (Oxi4503) and Combretastatin A-4 phosphate (ZybrestatTM) for the treatment of solid tumours². The mode of action of both compounds has been shown to be initial dephosphorylation, followed by binding of the free Combretastatin to the α , β -tubulin heterodimer in endothelial cells lining the tumour vasculature. This results in an irreversible process of microtubule depolymerisation, which, along with a cascade of cell signalling events, cause rapid blood flow shut down, and ultimately tumour necrosis³.

Chemistry

Almac Sciences recently completed a synthesis of [methyl- ^{14}C]-Combretastatin A-1 diphosphate using the route shown in Scheme 1. This route was established, unlabelled, by Professor Kevin Pinney's group in Baylor University⁴. Reaction of the orthogonally protected stilbene starting material with TBAF and one equivalent of [^{14}C]-methyl iodide gave the required selective introduction of the ^{14}C label. Removal of the two isopropyl groups was achieved using TiCl_4 /DCM under carefully controlled conditions. This was a capricious reaction, giving variable yields, particularly on a small scale. Use of carefully purified Step 1 product and a freshly opened bottle of TiCl_4 gave the desired product in good purity, albeit in low yield. Treatment of the deprotected Combretastatin core with dibenzyl chlorophosphate, generated



in situ from dibenzyl phosphite and carbon tetrachloride, gave the protected product. In our hands, commercially available dibenzyl phosphite did not perform well in this step and we prepared this reagent fresh for use in the radiosynthesis⁵. Finally, ester deprotection and salt formation yielded 20 mCi of the desired target in an overall radiochemical yield of 8.4% and with 97.5% chemical purity, 96.7% radiochemical purity, with a specific activity of 54.9 mCi/mmol.

Acknowledgements

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References

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