Combretastatin A-2 Synthetic Modifications

by

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ABSTRACT

Combretastatin A-4 (CA-4) represents one of the most promising antineoplastic and cancer vascular targeting stilbenes that have been isolated from the South African bush willow, *Combretum Caffrum* Kuntze. In order to further explore the bioactivity of this molecule, a diiodo derivative of CA-4, as well as its phosphate prodrug, was synthesized and analyzed for its biological activity; although only a scale up synthesis of this compound was performed herein for ongoing analysis. In general, no increased specificity was noted for the human cancer cell lines. Antiangiogenic properties were similar to the untreated control. The diiodocombstatin was active against *M. luteus*, and its phosphate prodrugs were very active against *N. gonorrhoeae*.

Combretastain A-2 is another biologically active stilbene isolated from *Combretum Caffrum* Kuntze. In an attempt to increase biological activity of this molecule both mono-iodo and diiodo derivatives have been partially synthesized. The initial step involving the iodination of piperonal utilizes a novel, cost effective and mild reaction. The iodo stilbenes were obtained via a Wittig reaction using phosphonium salts 25 and 27 along with 2,3-Bis-[tert-butyldimethylsiloxy]-4-methoxy benzaldehyde 29. Deprotection of the subsequent z-stilbenes, non-isolated mono-iodo stilbene and the diiodo 30 produced two synthetic objective z-stilbenes 16 and 17. Synthesis as well as biological analysis is ongoing.

ACKNOWLEDGEMENTS

I want to express my deep gratitude to Dr. G.R. Pettit for taking me on as a master's student and providing ongoing support while I studied and performed research. I am grateful to Dr. R. Dixon for teaching me laboratory techniques, to Drs. F. Hogan and C. Herald for constantly answering questions and keeping funds open for use. I want to thank Dr. J-C. Chapius for his biological evaluations, and Dr. J.C. Knight and Natasha Zolotov for their mass spec expertise. I am thankful to have worked with Dr. C. Pruis and to have received her support as well as the Chemistry Department's support while I studied at ASU. I am grateful to Drs. E. Skibo and A. Moore for their willingness to work with me on my committee and their support in obtaining a degree so much later than intended. I am aslo appreciative of the help that M. Mcdowell and T. Thornburgh have readily offered me in finishing this endeavor. I especially want to thank Dr. N. Melody for her constant mentoring throughout my stay at ASU as well as her generous friendship; without her, I would not have been able to complete the master's program.

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Cancer and Natural Products

1.1 Cancer in the United States

Cancer is a growing pandemic in the world. The American Cancer Society estimates that there will be 1.5 million (Table 1.1) new cases of cancer diagnosed within the United States alone in 2009.[1]

Table 1.1 Estimated U.S. Cancer Cases*

Cancer Type	Men	Cancer Type	Women
Prostate	25%	Breast	27%
Lung & bronchis	15%	Lung & bronchis	14%
Colon & rectum	10%	Colon & rectum	10%
Urinary bladder	7%	Uterine corpus	6%
Melanoma of skin	5%	Non-Hodgkin lymphoma	4%
Non-Hodgkin lymphoma	5%	Melanoma of skin	4%
Kidney & renal pelvis	5%	Thyroid	4%
Leukemia	3%	Kidney & renal pelvis	3%
Oral cavity	3%	Ovary	3%
Pancreas	3%	Pancreas	3%
All Other Sites	19%	All Other Sites	22%
Estimated Number	766,130	Estimated Number	713,220

*Estimates prepared by the American Cancer Society and do not include basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.[1]

In 2006, cancer accounted for close to one-quarter of the deaths in the U.S. and was exceeded only by heart disease (Table 1.2).[1] The probability of an American man developing cancer is one in two while the probability of an American woman developing cancer is one in three (Table 1.3).[1] Despite these discouraging

numbers, cancer survival rates are increasing[1] (Table 1.4) due to the progress in early detection as well as the progress in treatment.[2]

Table 1.2 U.S. Mortality, 2006

Rank	Cause of Death	No. of Deaths	% of all Deaths
1.	Heart Diseases	631,636	26.0
2.	Cancer	559,888	23.1
3.	Cerebrovascular diseases	137,119	5.7
4.	Chronic lower respiratory diseases	124,583	5.1
5.	Accidents (unintentional injuries)	121,599	5.0
6.	Diabetes mellitus	72,449	3.0
7.	Alzheimer disease	72,432	3.0
8.	Influenza & pneumonia	58,326	2.3
9.	Nephritis*	45,344	1.9
10.	Septicemia	34,234	1.4

*Includes Nephrote syndrome and nephrosis.
Source: U.S. Mortality Data 2006, National Center for Health Statistics, Center for Disease Control and Prevention, 2009.

Table 1.3 Lifetime Probability of Developing Cancer, Men/Women, US, 2003-2005*

Site in Men	Risk	Site in Women	Risk
All Sites+	**1 in 2**	**All Sites+**	**1 in 3**
Prostate	1 in 6	Breast	1 in 8
Lung and bronchus	1 in 13	Lung & bronchus	1 in 16
Colon and rectum	1 in 18	Colon & rectum	1 in 20
Urinary bladder$^{\delta}$	1 in 27	Uterine corpus	1 in 40
Melanoma$^{\tau}$	1 in 39	Non-Hodgkin lymphoma	1 in 53
Non-Hodgkin lymphoma	1 in 45	Urinary bladder$^{\delta}$	1 in 84
Kidney	1 in 57	Melanoma$^{\tau}$	1 in 58
Leukemia	1 in 67	Ovary	1 in 72
Oral Cavity	1 in 72	Pancreas	1 in 75
Stomach	1 in 90	Uterine cervix	1 in 145

*****For those free of cancer at beginning of age interval.
+All sites exclude basal and squamous skin cancers and in situ cancers except urinary bladder.
$^{\delta}$Includes invasive and in situ cancer cases.
$^{\tau}$Statistics for white men and women.
Source: DevCan: Probability of Developing or Dying of Cancer Software, Version 6.3.0 Statistical Research and Applications Branch, NCI, 2008. http://srab.cancer.gov/devcan

Table 1.4 Trends in Five-year Relative Survival (%)* Rates, US, 1975-2004

Site	1975-1977	1984-1986	1996-2004
All sites	**50**	**54**	**66**
Breast (female)	75	79	89
Colon	52	59	65
Leukemia	35	42	51
Lund and bronchus	13	13	16
Melanoma	82	87	92
Non-Hodgkin lymphoma	48	53	65
Ovary	37	40	46
Pancreas	3	3	5
Prostate	69	76	99
Rectum	49	57	67
Urinary bladder	74	78	81

*5-year relative survival rates based on follow up patients through 2005.
Source: Surveillance, Epidemiology, and End Results Program, 1975-2005, Division of Population Sciences, National Cancer Institute, 2008.

1.2 Natural Products' Contribution to Medicine

Natural products have played an extraordinarily valuable role in the drug discovery process. Some examples of natural products that have made a major impact on modern medicine include but are not limited to: quinidine, quinine, physostigmine, neostigmine, paclitaxel, colchicine, morphine, codeine and penicillin. Quinine (Figure 1.1) was isolated from the bark of the Cinchona tree and was one of the first anti-infective agents due to its efficacy against malaria.[3] Quinidine (Figure 1.2) is the diastereoisomer of quinine and is an important anti-arrhythmic drug.[3]

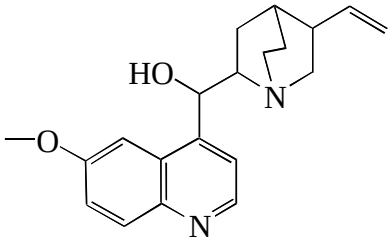

Figure 1.1 Quinine synthesis, **1944**[4]

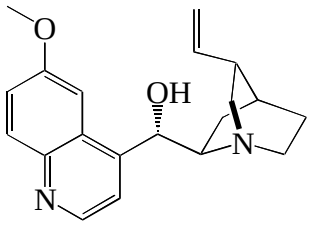

Figure 1.2 Quinidine synthesis, **1978**[5]

Figure 1.3 Cinchona Tree[6]

Physostigmine (Figure1.4) is a naturally occurring alkaloid isolated from the calabar bean (Figure 1.6) and Neostigmine (Figure 1.5) is its synthetic analog; both are important acetylcholinesterase inhibitors.[7]

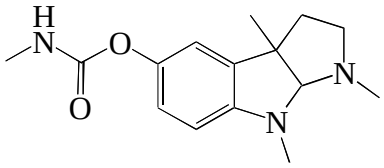

Figure 1.4 Physostigmine[7]

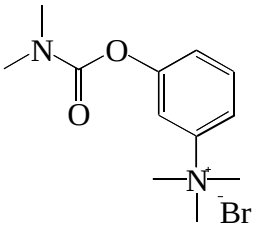

Figure 1.5 Neostigmine[7]

Figure 1.6 Calabar Bean[8]

Paclitaxel (Figure 1.7) is an unaltered natural product isolated from the Pacific yew tree (Figure 1.8) whose mechanism of derivation blocks de-polymerization of microtubules and is commonly used to treat ovarian, breast, lung, and colon cancer.[9]

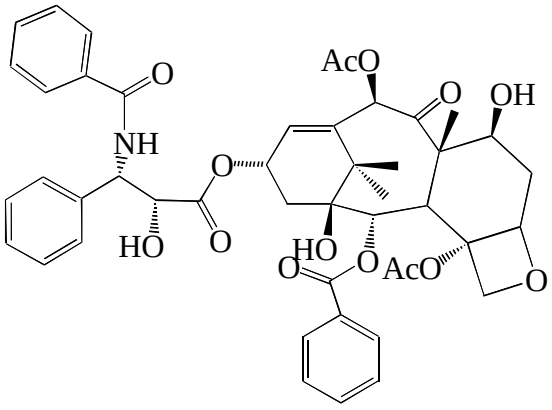

Figure 1.7 Paclitaxel[9]

Figure 1.8 Pacific Yew Tree[9]

Colchicine (Figure 1.9) was isolated from the Meadow Saffron (Figure 1.10) and is commonly used to treat gout and familial Mediterranean fever.[3]

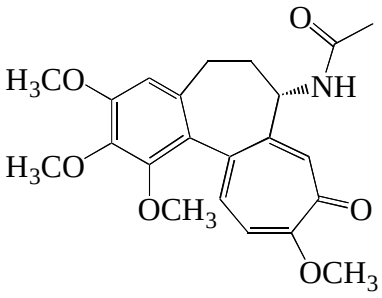

Figure 1.9 Colchicine, **1820**[10]

Figure 1.10 Meadow Saffron[11]

Morphine (Figure 1.11) was the first active alkaloid extracted from the opium poppy plant (*Papaver somnifer*) (Figure 1.12).[12] It acts directly on the central nervous system to relieve pain, therefore it is used as an analgesic psychoactive drug.[3] Codeine (Figure 1.11) was also isolated from the opium poppy plant but is more commonly synthesized from O-methylation of morphine.[3] Codeine is commonly used to treat a cough, diarrhea, mild to severe pain, and irritable bowel syndrome.[3]

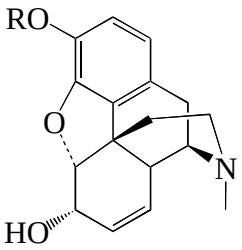

Figure 1.11 Morphine R=H, Codeine R=CH_3[12] **Figure 1.12** Opium Poppy Plant[12]

Penicillin (Figure 1.13) is the active constituent isolated from *Penicillium fungus* (Figure 1.14).[3] This discovery led to a dramatic change in medical history with the introduction of antibiotics. Antibiotics have had a huge impact on life expectancy as well as on quality of life. They play an important role as drugs in modern medicine and have also helped scientists understand the mechanisms by which these natural products exert their action. This biological understanding has allowed for the targeting of pathogens that would not likely have been possible without these biochemical probes.[3]

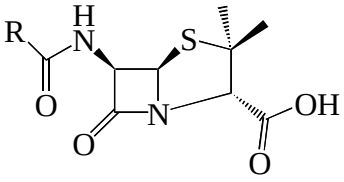

Figure 1.13 Core Structure of Penicillin[13]

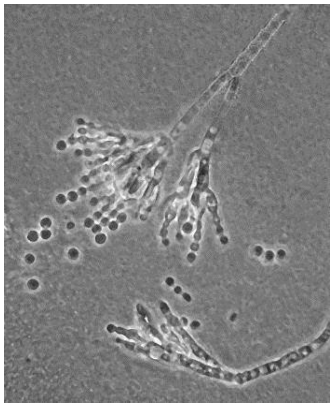

Figure 1.14 *Penicillium Fungi*[14]

Studies taken in 1996 revealed that over 60% of the approved drugs and pre-NDA candidates, excluding biologics, for anticancer and antiinfective agents from 1984-1995 are of natural origin. 62% of the 87 approved anticancer drugs are of natural origin or are modeled after natural product parents. 50 of the 299 pre-NDA anticancer drug candidates are the original natural product, while 48 are semisynthetic derivatives, and 30 are based on natural product models. Seven of the 93 new approved antiinfectives are natural products, 45 are semisynthetic derivatives, and seven are based on natural product models. This means that 63% of the approved antiinfectives are of natural origin.[15]

A more recent study, taken in 2006, showed that 63 of 81 anticancer drugs were either natural products or mimicked natural products in some form. The same study showed that ~50% of all small molecules approved as "New Chemical Entities" in the years 2000-2006 were derived from the natural products.[16]

The anticancer drugs Combretastatin A-4 phosphate **1** (CA4P) (Figure 1.15) and Combretastatin A-1 phosphate **2** (CA1P) (Figure 1.16) are two such NCEs. CA4P is the first well-established vascular-targeting anticancer drug.[17]

CA4P was synthesized[18] from combretastatin A-4 **1a** (Figure 1.17) which was isolated from the South African bushwillow, *Combretum Caffrum Kuntze*.[19] CA1P was likewise synthesized[20] from combretastatin A-1 **2a** (Figure 1.18) which was also isolated from *Combretum Caffrum Kuntze*.[21]

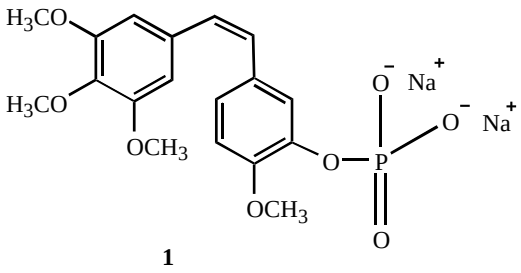

Figure 1.15 Combretastatin A4P

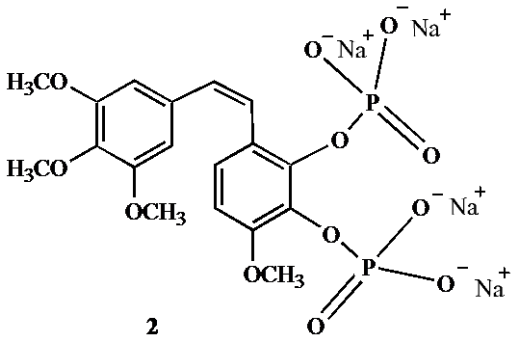

Figure 1.16 Combretastatin A1P

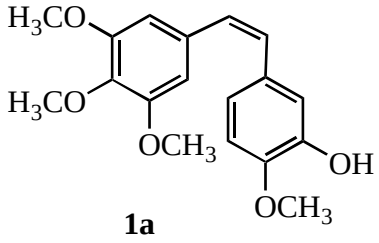

Figure 1.17 Combretastatin A-4

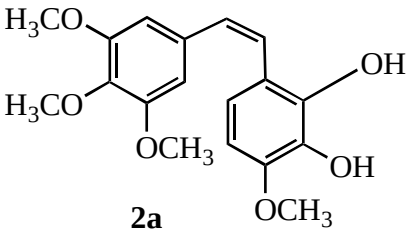

Figure 1.18 Combretastatin A-1

The South African bush willow, *Combretum Caffrum Kuntze*, has been the source of many biologically active compounds.[17] Combretastatin A-4P (ZYBRESTAT) has had success in phase II/III clinical trials evaluating the safety and efficacy of CA4P in combination with Paclitaxel and Carboplatin in comparison with Paclitaxel and Carboplatin against Anaplastic Thyroid Carcinoma.[22] CA4P is also involved in clinical trials as a treatment for solid tumors.[23] Combretastatin A-1 phosphate (OXi4503) is currently involved in

dose-escalating phase I and phase II studies evaluating its safety when administered to patients with advanced solid tumors.[24]

References

1. Cancer Statistics 2009. www.cancer.org/docroot/PRO/content/PRO_1_1_Cancer_Statistics_2009_Presentation.asp (accessed 10/14/09).

2. Cancer Trends Progress Report 2009. http://progressreport.cancer.gov/doc_detail.asp?pid=1&did=2007&chid=75&coid=727&mid=#trends (accessed 10/14/09).

3. Williams, David A., Lemke, Thomas. *Foye's Principles of Medicinal Chemistry*, 5th ed; Lippincott Williams & Wilkins: Baltimore, 2002; pp 30-33.

4. Woodward, R.B., Doering, W.E. The Total Synthesis of Quinine. *Journal of the American Chemical Society* **1944**, *66*, 849.

5. Gutzwiller, J.; Uskokovic, M.R. The Sterioselective Synthesis of Quinidine. *Journal of the American Chemical Society* **1978**, *100*, 576-581.

6. Buchler 2008. http://www.quinine-buchler.com/images/baumrindenernte.jpg (accessed 10/28/09).

7. Hieble, J.P.; Ruffolo, R.R. Pharmacology of neuromuscular transmission. In Munson P.L.; Mueller, R.A.; Breese, G.R., eds. Principles of pharmacology: basic concepts and clinical applications. New York. Chapman & Hall, **1975**, *145-159*, 1734.

8. Flora Delaterre Productions, LLC 2003. http://www.floradelaterre.com/uploads/RTEmagicC_julian_plant_small_02.jpg.jpg (accessed 10/28/09).

9. Wall, M.E.; Wani, M.C. Camptothecin and taxol: Discovery clinic. 13th ed. Bruce F. Cain Memorial Award Lecture. Cancer Res **1995**, *55*, 753-760.

10. Center for Invasive Species and Ecosystem Health, USDA Forest Service 2009. http://www.forestryimages.org/images/192x128/1208034.jpg (accessed 10/28/09).

11. Garden Herbs 2005-2009. http://www.gardenherbs.org/simples/saffron_meadow_cultivated.htm (accessed 10/28/09).

12. Serturner, F.W.A.F. *Journal of Pharmacological Arzte Apotheker Chemistry* **1806**, *14*, 47-93.

13. Berdy, J. ed. CRC Handbook of antibiotic compounds. Boca Raton, FL: CRC Press, **1980**.

14. University of Wisconsin-La Crosse. Tom Volk's Fungi 1995-2009. http://botit.botany.wisc.edu/toms_fungi/images/pen1.jpg (accessed 10/28/09).

15. Cragg, G.M. Natural Products in Drug Discovery and Development. *J. Nat. Prod.* 1997

16. Cragg, G.M., Newman, D.J. Natural Products as Sources of New Drugs over the last 25 Years. *Am. Chem. Soc.* 2007.

17. Pettit, G.R.; Singh, S. B.; Boyd, M.R.; Hansel, E.; Pettit, R.K.; Schmidt, J.M.; Hogan, F. Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatin A-4, A-5, and A-6. *Journal of Medicinal Chemistry* **1995**, *38*, 1666-1672. in Cragg, Gordon M.; Kingston, David G.I.; Newman, David J. *Anticancer Agents from Natural Products* CRC Press, Boca Raton, FL, 2005.

18. Pettit, G.R.; Temple, C.; Narayanan, V.L.; Ravi, Varma; Simpson, M.J.; Boyd, M.R.; Rener, G.A.; Bansal, N. Antineoplastic agents 322. Synthesis of combretastatin A-4 prodrugs. *Anti-Cancer Drug Design* **1995**, *10*, 299-309.

19. Pettit, G.R.; Singh, S.B.; Boyd, M.R.; Hansel, E.; Pettit, R.K.; Schmidt, J.M.; Hogan, F. Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatin A-4, A-5, and A-6. *Journal of Medicinal Chemistry* **1995**, *38*, 1666-1672.

20. Pettit, G.R.; Lippert, J.W. Antineoplatstic agents 429. Synthesis of the combretastatin A-1 and combretastatin B-1 prodrugs. *Anti-Cancer Drug Design* **2000**, *15*, 203-216.

21. Pettit, G.R.; Singh, S.B.; Niven, M.L.; Hamel, E.; Schmidt, J.M. Isolation, Structure and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived From *Combretum caffrum*. *Journal of Natural Products* **1987**, *50*, 119-131.

22. Cooney M.M.; Savvides P.; Agarwala S.S.; Wang D.; Flick S.; Bergant S.; Bhatka S.; Fu P.; Subbiah V.; Lavertu P.; Ortiz J.; Remick S. Phase II study of combretastatin A4 phosphate (CA4P) in patients with advanced anaplastic thyroid carcinoma. *Journal of Clinical Oncology*, **2006**, 24, 5580.

23. Bilenker, Joshua H.; Flaherty, Keith T.; Rosen, Mark; Davis, Lisa; Gallagher, Maryann; Stevenson, James P.; Sun, Weijing; Vaughn, David; Giantonio, Bruce; Zimmer, Ross; Schnall, Mitchell; O'Dwyer, Peter J. Phase I Trial of Combretastatin A-4 Phosphate with Carboplatin. *Clinical Cancer Research* **2005**, 11, 1527.

24. Patterson, D. et al.; OXi4503 Interim Phase I Results *American Society of Clinical Oncology* **2011**.

Chapter 2

Combretum Caffrum

2.1 Brief History of the Combretastatins

African traditional medicine utilizes some 24 species of the *Combretum* genus.[1] These plants are used to treat problems ranging from heart and worm remedies, to wound dressings, treatment for the mentally ill, scorpion stings[2], leprosy, and even cancer.[3] However, it was not until the 1970s that the United States National Cancer Institute (NCI), during its worldwide exploratory survey of plants, found that the root bark of the South African willow tree *Combretum Caffrum* had significant activity against the murine P388 lymphatic leukemia (ED_{50} 0.011 ug/mL).[4] Dr. G.R. Pettit and colleagues later isolated (using an astrocytoma bioassay procedure) the cancer cell growth inhibitor (-)-combretastatin (Figure 2.1) from C. Caffrum.[5,6]

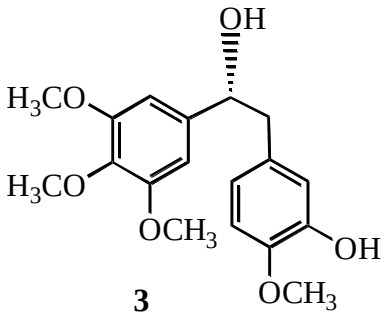

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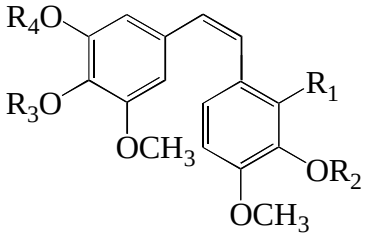
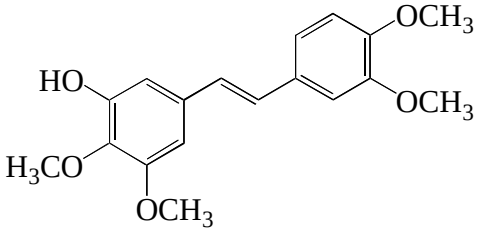
Figure 2.1 (-)-Combretastatin

(-)-Combretastatin (**3**) caused a significant rise in the mitotic index of L1210 cells, inhibited tubulin polymerization (IC_{50} 5-7 *u*M), stimulated tubulin-

dependent GTP hydrolysis, and competitively inhibited the binding of colchicines to tubulin [34% binding by colchicines in the presence of **3** (1:1 concentration ratio)].[7] The isolation and synthesis of (-)-combretastatin led our research group to isolate twenty related substances within what is now considered the combretastatins. The combretastatins consist of a group of stilbenes called the A-series (Figure 2.2),[8-11] four bibenzyl compounds called the B-series (Figure 2.3),[8, 9, 12] a phenanthraquinone called combretastatin C-1 (Figure 2.4),[13] and macrocyclic lactones called combretastatin D-1 and D-2 (Figure 2.5).[14, 15]





2a, R_1 = OH, R_2 = H, R_3 = R_4 = CH_3, A-1
P388 ED_{50} = 0.99 *u*g/mL; tubulin IC_{50} = 2-3 *u*M.[8]

2b, R_1 = R_2 = H, R_3 = R_4 = -CH_2-, A-2
P388 ED_{50} = 0.027 *u*g/mL; tubluin IC_{50} = 4-5 *u*M.[9]

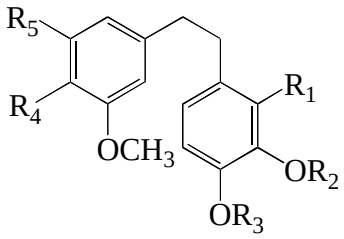
2c, R_1 = R_2 = H, R_3 = CH_3, R_4 = H, A-3
P388 ED_{50} = 0.026 *u*g/mL; tubulin IC_{50} = 4-5 *u*M.[9]

2d, R_1 = R_2 = H, R_3 = R_4 = CH_3, A-4
P388 ED_{50} = 0.0034 *u*g/mL; tubulin IC_{50} = 2-3 *u*M.[10]

2e, R_1 = H, R_2 = R_3 = CH_3, R_4 = H, A-5
P388 ED_{50} = 0.9 *u*g/mL;[11] tubulin IC_{50} = 75-100 *u*M.[16]

2f, A-6
P388 ED_{50} = 18 *u*g/mL;[16]
tubulin IC_{50} > 100 *u*M.[17]

Figure 2.2 Combretastatin A-Series



3a, R_1 = OH, R_2 = H, R_3 = CH_3, R_4 = R_5 = OCH_3, B-1

P388 ED_{50} = 2.7 *u*g/mL.[8]

3b, R_1 = R_2 = H, R_3 = CH_3, R_4 = OCH_3, R_5 = OH, B-2

P388 ED_{50} = 0.32 *u*g/mL;[9] tubulin IC_{50} = 40 *u*M.[16]

3c, R_1 = R_2 = R_3 = H, R_4 = R_5 = OCH_3, B-3

P388 ED_{50} = 0.4 *u*g/mL;[12] tubulin IC_{50} > 100 *u*M.[16]

3d, R_1 = R_2 = R_3 = R_4 = H, R_5 = OCH_3, B-4

P388 ED_{50} = 1.7 *u*g/mL;[12] tubulin IC_{50} > 100 *u*M.[16]

Figure 2.3 Combretastatin B-Series

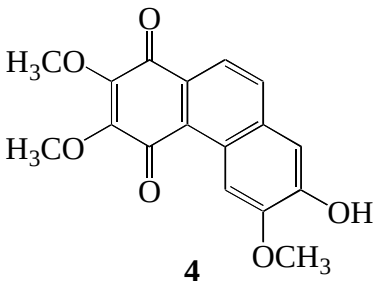


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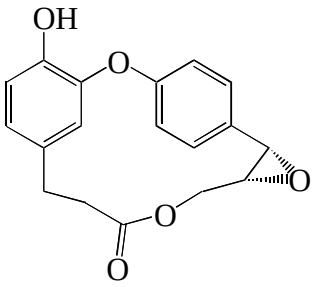
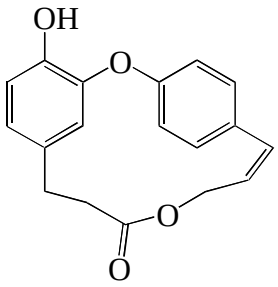
Figure 2.4 Combretastatin C-1

P388 ED_{50} = 2.2 *u*g/mL.[13]





5a, D-1 **5b**, D-2

Figure 2.5 Combretastatin D-Series

P388 ED_{50} = 3.3 *u*g/mL.[14] P388 ED_{50} = 5.2 *u*g/mL.[15]

Chapter 2.2 Combretastatin Structure Activity Relationships

Due to the simplicity of structure, relative simplicity of synthesis, and potency of the combretastatins, many derivatives have been synthesized and structure activity relationships been characterized. As the previous figures show, any major deviations from the original natural product resulted in a drop in cytotoxicity. The trans-stilbene also displays lower activity than the cis-stilbene. A very important discovery showed that the phosphate prodrug showed an increase in water solubility over the natural compound.[18]

The combretastatin series has produced many biologically active compounds. The ongoing SARS studies have helped scientists locate the necessary configurations and groups for increasing this activity. Combretastatin A-4P (**1**) is the most biologically promising stilbene isolated from *C. caffrum*.[11, 19, 20] It has proven to be a potent tubulin polymerization inhibitor, human cancer cell line inhibitor, and an effective vascular targeting agent.[11, 19, 20] Combretastatin A-1P (**2**) is also a very biologically promising stilbene.[20] Both are part of ongoing clinical trials for use as anticancer drugs.[21, 22] This success continues to promote

the study of the combretastatin compounds and their derivatives as well as drive new synthesis of novel derivatives.

References

1. Cragg, Gordon M.; Kingston, David G.I.; Newman, David J. *Anticancer Agents from Natural Products* CRC Press, Boca Raton, FL, 2005.

2. Watt, J.M. and Breyer-Brandwijk, M.G., *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd ed., E. and S. Livingston, London, 1962, 194.

3. Faulkner, D.J. *Natural Products Reports* **1994**, *11*, 355. Kobayashi, M.; Kitagawa, I. *Pure and Applied Chemistry* **1994**, 819. Konig, G.; Wright, A.D.; Sticher, O.; Angerhofer, C.K.; Pezzuto, J.M. *Planta Medicina* **1994**, *60*, 532. Davidson, B.S. *Chemical Reviews* **1993**, *93*, 1771.

4. Pettit, G.R.; Kamano, Y.; Fujii, Y.; Herald, C.L.; Inove, M.; Brown, P.; Gust, D.; Kitihara, K.; Schmidt, J.M.; Doubek, D.L.; Michel, C. *Journal of Natural Products* **1981**, *44*, 482.

5. Pettit, G.R.; Cragg, G.B.; Herald, D.L.; Schmidt, J.; Lohavanijay, P. Isolation and structure of combretastatin. *Canadian Journal of Chemistry* **1982**, *60*, 1374-1376.

6. Pettit, G.R.; Singh, S.B.; Gordon, C.M. Synthesis of Natural (-)-Combretastatin. *Journal of Organic Chemistry* **1985**, *50*, 3404-3406.

7. Pettit, G.R.; Cragg, G.M.; Herald, D.L.; Schmidt, J.M.; Lohavanuaya, P. *Canadian Journal of Chemistry*. **1982**, *60*, 1374. Pettit, G.R.; Singh, S.B.; Cragg, G.M. *Journal of Organic Chemistry* **1985**, *50*, 3404.

8. Pettit, G.R.; Singh, S.B.; Niven, M.L.; Hamel, E.; Schmidt, J.M. Isolation, Structure, and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived From *Combretum caffrum*. *Journal of Natural Products* **1987**, *50*, 119-131.

9. Pettit, G.R.; Singh, S.B. Isolation, Structure, and Synthesis of Combretastatin A-2, A-3, and B-2. *Canadian Journal of Chemistry* **1987**, *65*, 2390-2396.

10. Pettit, G.R.; Singh, S.B.; Hamel, E.; Lin, C.M.; Albert, D.S.; Garcia-Kendal, D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. *Experimentia* **1989**, *45*, 209-211.

11. Pettit, G.R.; Singh, S.B.; Boyd, M.R.; Hansel, E.; Pettit, R.K.; Schmidt, J.M.; Hogan, F. Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatin A-4, A-5, and A-6. *Journal of Medicinal Chemistry* **1995**, *38*, 1666-1672.

12. Pettit, G.R.; Singh, S.B.; Schmidt, J.M. Isolation, Structure, Synthesis, and Antimitotic Properties of Combretastatin B-3 and B-4 from *Combretum caffrum*. *Journal of Natural Products* **1988**, *51*, 517-527.

13. Singh, S.B.; Pettit, G.R. Isolation, Structure, and Synthesis of Combretastatin C-1. *Journal of Organic Chemistry* **1989**, *54*, 4105-4114.

14. Pettit, G.R.; Singh, S.B.; Niven, M.L. Isolation and Structure of Combretastatin D-1; A Cell Growth Inhibitory Macrocyclic Lactone from *Combretum caffrum*. *Journal of the American Chemical Society* **1988**, *110*, 8539-8540.

15. Singh, S.B.; Pettit, G.R. Antineoplastic Agents. 206. Structure of the Cytostatic Macrocyclic Lactone Combretastatin D-2. *Journal of Organic Chemistry* **1990**, *55*, 2797-2800.

16. Lin, C.M.; Singh, S.B.; Chu, P.S.; Dempcy, R.O.; Schmidt, J.M.; Pettit, G.R.; Hamel, E. *Molecular Pharmacology* **1988**, *34*, 200.

17. Medarde, M.; Clairac, R. P-L.; Lopez, J.L.; Feliciano, A.S. *Journal of Natural Products* **1994**, *57*, 1136.

18. Pettit, G.R.; Temple, C.; Narayana, V.L.; Varma, R.; Simpson, M.F.; Boyd, M.R.; Rener, G.A.; Bansal, N. Antineoplastic agents 322. Synthesis of combretastatin A-4 prodrugs. *Anti-Cancer Drug Design* **1995**, *10*, 299-309.

19. Pinney, K.C.; Jelinek, K.E.; Chaplin, D.J.; Pettit, G.R. *The Discovery and Development of the Combretastatins*, CRC Press, Boca Raton, FL, 2005.

20. Gaukroger, K. Structural requirements for the interaction of combretastatins with tubulin: how important is the trimethoxy unit? *Organic Biomolecular Chemistry* **2003**, *1*, 3033.

21. American Cancer Society 2009. http://www.cancer.org/docroot/PRO/content/PRO_1_1_Cancer_Statistics_2009_Presentation.asp (accessed 11/15/09).

22. National Cancer Institute 2009. http://progressreport.cancer.gov/doc_detail.asp?pid=1&did=2007&chid=75&coid=727&mid=#trends (accessed 11/15/09).

Chapter 3

Scale-up Synthesis of the Antineoplastic Agent Sodium 3,5-diiodo-4,4'dimethoxy-Z-stilbene 3'-*O*-phosphate

3.1 Introduction

The South African bush willow, *Combretum Caffrum* Kuntze, has been the source of many biologically active compounds. In collaboration with the NCI Natural Products Branch, Dr. G.R. Pettit and his research group continued a series of investigations of chemical constituents from the *C. Caffrum* collection.[1] This pursuit eventually led to the discovery of the Combretastatins, and more specifically the discovery of Combretastatin A-4 (**1a**).[2] Combretastatin A-4 is the most biologically promising stilbene isolated from *C. Caffrum*. It has proven to be a potent tubulin polymerization inhibitor, human cancer cell line inhibitor, and an effective vascular targeting agent.[1-3] Due to the success of Combretastatin A-4 in phase II clinical trials,[4] many structure activity relationship syntheses have been and continue to be pursued. This particular SAR study is based on the fact that CA4P (the phosphate prodrug of CA-4) had sufficient activity against thyroid cancer during phase I clinical trials.[5] The National Cancer Institute estimates that in 2007, there will be 33,550 new cases of thyroid cancer and 1,530 deaths due to thyroid cancer.[6] Papillary and follicular carcinoma are the major types of carcinoma of the thyroid gland while medullary (neuroendocrine) and anaplastic (very aggressive) are less common.[5] It has been found that both follicular and

anaplastic carcinomas are more common in populations residing in an area deficient in iodine.[5] In order to enhance the activity of CA-4P in thyroid carcinoma tissue, a diiodo structural (Figure 3.1) modification has been targeted for synthetic and biological evaluation.

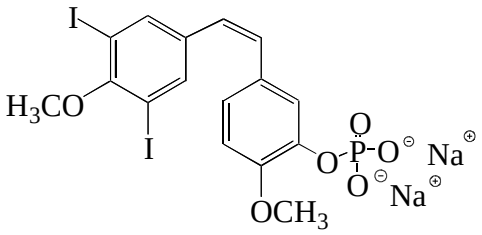


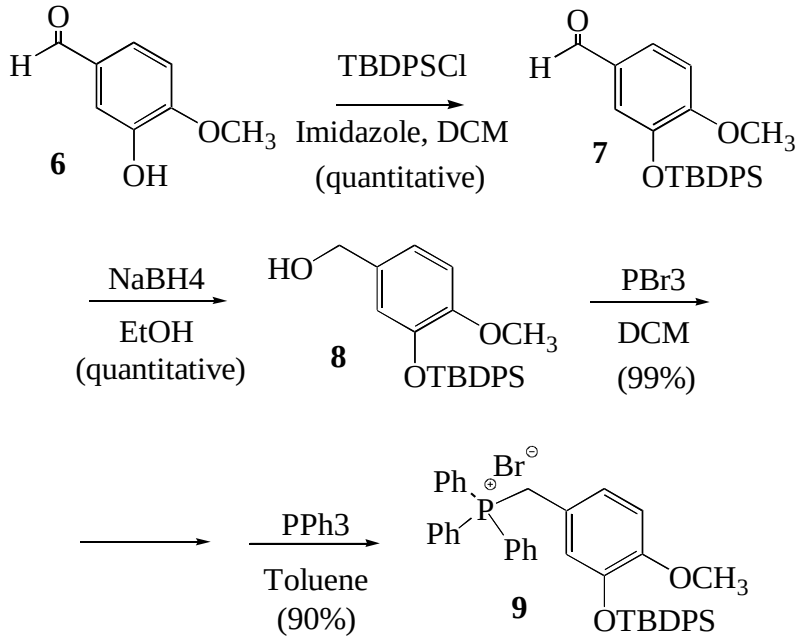
Figure 3.1 Sodium 3,5-diiodo-4,4'dimethoxy-Z-stilbene 3'-*O*-phosphate

3.2 Results and Discussion

The target compound was synthesized using a wittig reaction sequence with 3,5-diiodo-4-methoxy-benzaldehyde (**11**) with 3-O-tert-butyldiphenylsiloxy-4-methoxy-benzaldehyde (**9**).[6] Deprotection with tetrabutylammonium fluoride yielded the diiodo-stilbene (**13**). The phosphate group was introduced to the Z-isomer (**12**) using dibenzyl phosphate and carbon tetrachloride in the presence of diisopropylthylamine, N,N-dimethylaminopyridine to provide bisbenzylphosphate (**14**). The benzyl protecting group was then cleaved using bromotrimethylsilane and the resulting phosphoric acid was treated with sodium methoxide to yield the prodrug (**15**).

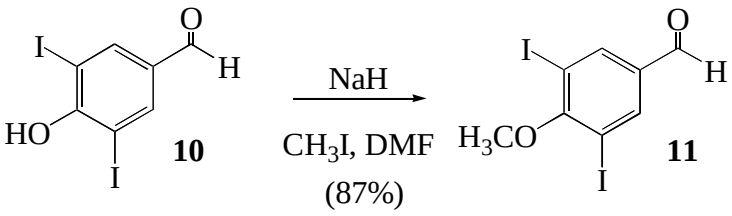
Scheme 3.1 Synthesis of Phosphonium Bromide (**9**)

A-Ring

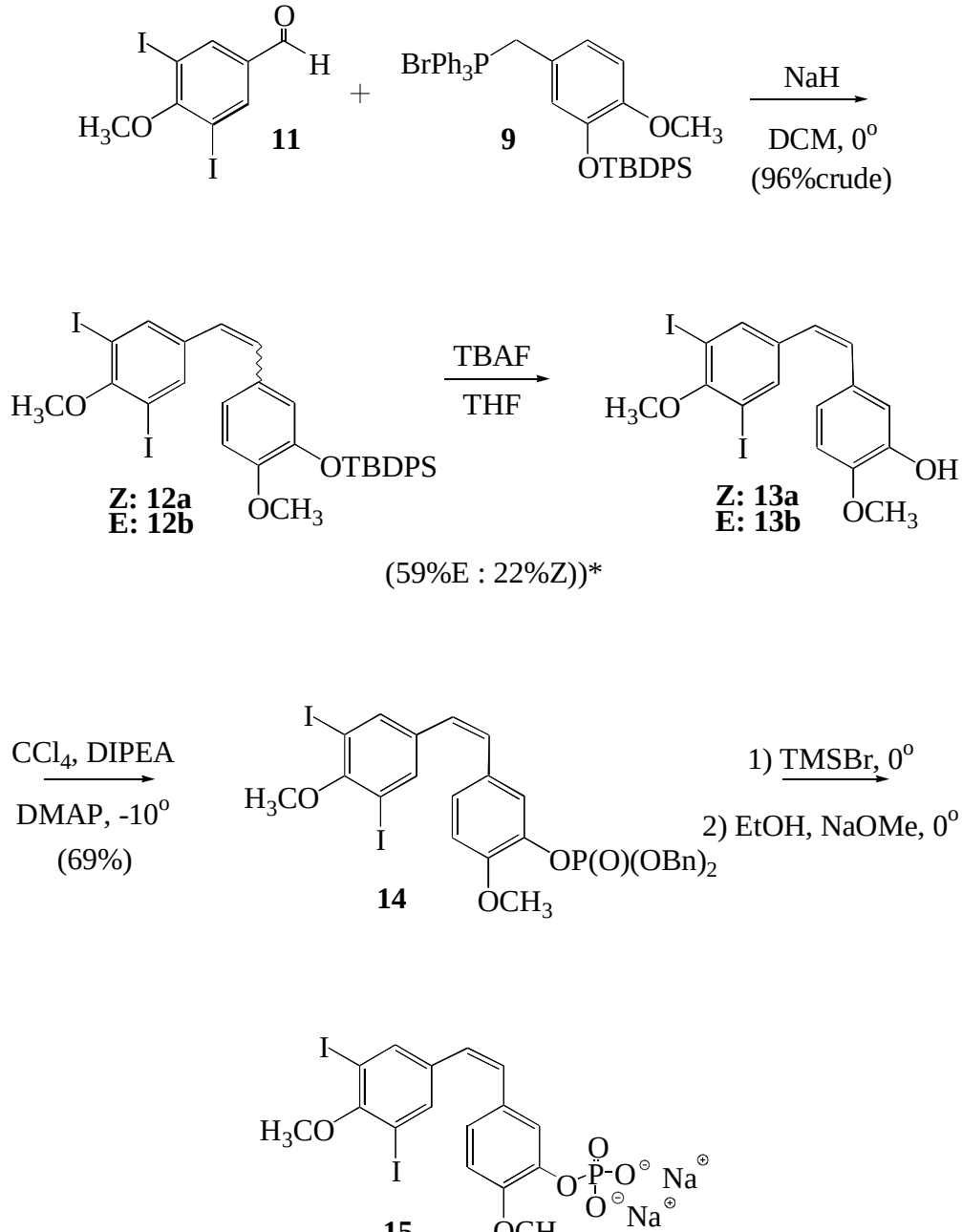


Scheme 3.2 Synthesis of Diiodo Benzaldehyde (**11**)

B-Ring



Scheme 3.3 Synthesis of 3,5-diiodo-3,4'-dimethoxy-Z-stilbene 3'-*O*-phosphate (**15**)



*The E/Z isomers were separated via column chromatography.

3.3 Conclusions

The scale up synthesis was straight forward and the reaction sequence was based on one as described by Dr. Heidi Rosenburg.[5] Recrystallization techniques needed to be improved for purification and to obtain better NMR data. In several cases the ^{1}H-NMR data was slightly different than the literature shifts and the coupling constants varied slightly as well. Further scale-up and purification is needed in order for biological testing to continue.

3.4 Materials and Methods

Isovanillin, anhydrous dicholoromethane (DCM), anhydrous toluene, dibenzylphosphite, triphenylphosphine, iodomethane, and carbon tetrachloride were obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI). 3,5-diiodo-4-hdroxybenzaldehyde was purchased from Lancaster Synthesis Inc. (Windham, NH). Diisopropylethylamine was obtained from Avocado Alfa Aesar (Ward Hill, MA). All other reagents were purchased from Acros Organics (Fisher Scientific, Pittsburg, PA).

All reactions were monitored by thin-layer chromatography using Analtech silica gel GHLF Uniplates visualized under long-wave and short-wave UV irradiation and by dipping into phosphomlybic acid in ethanol followed by heating. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Where appropriate, the crude products were separated by column chromatography, flash (230-400 mesh ASTM) or gravity (70-230 mesh ASTM) silica from E. Merck.

Melting points were measured using an electrothermal digital melting

point apparatus and are uncorrected. All 1H- and 13C-NMR spectra were obtained using Varian Gemini 300 MHz or varian unity 400 or 500 MHz instruments with CDCl3 (tetramethylsilane internal reference) as solvent unless otherwise noted. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

3.5 Experimental

3-O-tert-butyldiphenylsilyloxy – 4 – methoxy-benzaldehyde (7)

Imidazole (9.5 g, 132 mmol) was added to a heterogeneous mixture of isovanillin (**6**, 10 g, 65.7 mmol) in DCM at rt. The now homogeneous mixture was stirred for 5 mins and tert-butyldiphenylsilane (19.9 g, 72.3 mmol) was added. The mixture was left to stir for 18 hrs, and the reaction was terminated by the addition of water (50 mL) and extracted into DCM (50 mL). The organic layer was washed with 1 M HCl (50 mL), the phases separated and the organic layer washed with water (50 mL). The organic extract was dried, filtered and concentrated to yield a white solid (**7**, quantitative yield): ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.13 (s, C(CH3)3), 3.54 (s, OCH3), 6.79 (d, ArH, J = 8.4Hz), 7.27 (d, ArH, J = 1.8Hz), 7.37 (m, 7ArH), 7.68 (m, 4ArH), 9.65 (s, C(O)H).

3-O-tert-butyldiphenylsilyloxy-4-methoxy-benzyl alcohol (8)

Sodium borohydride (1.5 g, 39.7 mmol) was added to a solution of aldehyde (**7**) (12.8 g, 32.8 mmol) in EtOH (50 mL). The mixture was stirred for 18 hrs. The reaction was terminated by the addition of water (30 mL) and extracted into EtOAc (3 x 25 mL). The combined organic extracts were dried, filtered and concentrated to yield a colorless oil (**8**, quantitative yield): ^{1}HNMR

(300 MHz, $CDCl_3$) δ 1.11 (s, C(CH3)3), 3.51 (s, OCH3), 4.39 (s, CH2), 6.72 (d, ArH, J = 4.2Hz), 6.73 (d, ArH, J = 1.5), 6.82 (dd, ArH,, J = 8.4, 2.1), 7.37 (m, 6ArH), 7.70 (m, 4ArH).

3-*O*-tert-butyldiphenylsilyloxy-4-methoxy-benzyltriphenylphosphonium bromide (**9**)

Phosphorous tribromide (2.02 mL, 21.3 mmol) was added to a solution of alcohol (**8**, 15.8 g, 40.3 mmol) in DCM (250 mL). The mixture was left to stir for 1 hr and the reaction was terminated with 10% $NaHCO_3$ solution (200 mL), washed with water (75 mL) and extracted into DCM (3 x 75 mL). The combined organic layers were dried, filtered, and concentrated. Triphenylphosphine (0.634 g, 2.4mmol) was added to the resulting bromide in toluene (20 mL). The reaction mixture was placed in an oil bath and allowed to react under reflux at 120^{o}C for 1 hr. The heat source was removed and the reaction mixture was stirred for 18 hrs. The precipitate was collected and washed with toluene to yield a pure white solid (1.42 g, 90%): ^{1}HNMR (300 MHz, $CDCl_3$) δ 0.98 (s, C(CH3)3), 3.46 (s, OCH3), 5.02 (d, J_{pch}^{2} = 5.7Hz), 6.33 (t, J = 2.4Hz), 6.47 (d, J = 7.8Hz), 6.79 (dt, J = 2.4, 8.1Hz).

3,5-diiodo-4-methoxybenzaldehyde (**11)**

Sodium hydride (0.48g, 20 mmol, 60% dispersion in mineral oil) was slowly added to a cooled (0^{o}C) solution of 3,5-diiodo-4-hydroxybenzaldehyde (**10**, 5g, 13.37 mmol) in anhydrous DMF (50 mL). Iodomethane (3.33 ml, 53.5 mmol) was added, and stirring was continued at rt in the dark for 18 hrs. The reaction was terminated by the addition of water (50 mL), and the reaction

mixture was extracted with EtOAc-hexane (1:1, 3 x 50 mL). The combined organic extract was dried, filtered and concentrated. The residue was separated by column chromatography on silica gel using EtOAc-hexane (1:9) as eluent. The product was a white solid (**11**, 4.4g, 85%): mp 118-120^{o}C, (lit[12] mp 124^{o}C). ^{1}H-NMR (300 MHz, CDCl3) δ 3.91 (s, OCH3), 8.25 (s, 2ArH), 9.79 (s, CHO).

3,5-diiodo-4,4'-dimethoxy-3'-*O*-tert-butyl-diphenylsilyl-Z-stilbene (12a) and 3,5-diiodo-4,4'-dimethoxy-3'-*O*-tert-butyl-diphenylsilyl-E-stilbene (**12b**)

Phosphonium bromide **9** (0.624 g, 0.867 mmol) was dissolved in DCM at 0^{o}C. Sodium hydride (0.042 g, 1.75 mmol, 60% dispersion in mineral oil) was slowly added and the mixture gradually turned orange. The mixture was stirred for 20 mins. Aldehyde **11** (0.222 g, 0.572 mmol) was added and the mixture was stirred for 20 hrs. The reaction was terminated by adding water (20 mL) and extracted with DCM (3 x 20 mL). The organic extract was dried, filtered and concentrated. The oily residue was subjected to flash chromatography on silica gel using EtOAc-hexane (1:20) as eluent to give an isomeric mixture of the title compounds (96% yield, 0.41 g): Z-isomer (**12a**) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.85 (s, OCH3), 3.89 (s, OCH3), 5.54 (s, OH), 6.26 (d, J = 12Hz, ArH), 6.49 (d, J = 12Hz, ArH), 6.74 (s, ArH), 6.82 (s, ArH), 7.67 (s, ArH).

E-isomer (**12b**) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.86 (s, OCH3), 3.91 (s, OCH3), 5.62 (s, OH), 6.71 (d, J = 16.5Hz, ArH), 6.83 (d, J = 8.1Hz, ArH), 6.90 (d, J = 17.1Hz, ArH), 6.95 (d, J = 8.4Hz, ArH), 7.10 (d, J = 2.4Hz, ArH), 7.85 (s, 2ArH).

3,5-diiodo-4,4'-dimethoxy-3'-hydroxy-Z-stilbene (13a) and 3,5-diiodo-4,4'-dimethoxy-3'-hydroxy-E-stilbene (13b)

Tetrabutylammonium flouride (11.2 g, 11.2 mmol) was added to a solution of silyl ether **12a/12b** (7.55 g, 10.1 mmol) in THF (70 mL). The mixture was stirred under Ar in the dark for 20 min, and the reaction was terminated by the addition of water (70 mL). The product was extracted with EtOAc (3 x 75 mL), and the organic phase was dried, filtered and concentrated. The crude product was separated by silica gel column chromatography using 1:5 EtOAc-hexane as eluent to give stilbene **13a** as an oil (1.14 g, 22%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.84 (s, OCH3), 3.88 (s, OCH3), 5.59 (s, OH), 6.26 (d, J = 12Hz), 6.49 (d, J = 12Hz), 6.74 (s, 2ArH), 6.82 (s, ArH), 7.65 (s, 2ArH).

Further elution led to the E-stilbene **13b** (3.05 g, 59%) as a yellow solid**;** ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.86 (s, OCH3), 3.91 (s, OCH3), 5.65 (s, OH), 6.71 (d, ArH, J = 16Hz), 6.83 (d, ArH, J = 8.7Hz), 6.93 (d, ArH, J = 2.4 Hz), 6.96 (d, ArH, J = 2.4Hz), 7.10 (d, ArH, J = 1.8Hz), 7.85 (s, 2ArH).

Dibenzyl 3,5-diiodo-4,4'-dimethoxy-*Z*-stilbene 3'-*O*-phosphate (14)

A solution of *cis*-stilbene **13a** (0.372 g, 0.732 mmol) in acetonitrile (10 mL) was cooled to -10^{o}C. Carbon tetrachloride (0.71 mL, 7.32 mmol) was added, and the mixture was stirred for 10min at -10^{o}C in the dark. Diisopropylethylamine (255 *u*L, 1.46 mmol), and DMAP (0.089 g, 0.073 mmol) were added in rapid succession. After 1 min, dibenzylphosphite (194 *u*L, 0.878 mmol) was added, and the mixture was stirred for 1 hr at -10^{o}C. The reaction was terminated by the addition of 0.5 M KH2PO4 (10 mL) and extracted into EtOAc

(3 x 20 mL). The organic phase was dried, filtered and concentrated. The oily residue was separated by silica gel column chromatography using EtOAc-hexane 1:9 (1000 mL) and EtOAc-hexane 1:4 (2600 mL) as eluent to yield 0.39g, 69% of a pure oil (**14**): bp dec. 220^{o}C; R_f 0.57 (1:1 EtOAc-hexane); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.78 (s, OCH3), 3.81 (s, OCH3), 5.13 (s, CH2), 5.16 (s, CH2), 6.28 (d, ArH, J = 12Hz), 6.42 (d, ArH, J = 12Hz)**,** 6.78 (d, ArH, J = 8.7Hz)**,** 7.00 (d, ArH, J = 8.7Hz), 7.07 (s, ArH), 7.33 (s, 10ArH), 7.64 (s, 2ArH).

Sodium 3,5-diiodo-4,4'-dimethoxy-Z-stilbene 3'-*O*-phosphate (15)

Trimethylbromosilane (604 *u*L, 4.58 mmol) was added to a cooled (0^{o}C) solution of phosphate **14** in DCM (120 mL). After stirring for 45 min, the reaction was terminated with water (25 mL) and extracted into EtOAc (3 x 20 mL). The combined organic extracts were dried, filtered and concentrated to afford the phosphoric acid intermediate as a brown oil. After drying (high vacuum) over night, the oil was dissolved in EtOH (25 mL) and NaOMe (0.248 g, 4.6 mmol) was added. The mixture was stirred for 1 hr, and the precipitate was collected and washed with minimal EtOH to afford the sodium salt as a pure white powder (**15**, 0.835 g, 63%): mp 172^{o}C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.49 (s, OCH3), 3.89 (s, OCH3), 6.27 (d, ArH, J = 5.7Hz), 6.50 (d, ArH, J = 7.2Hz), 6.74 (s, 2ArH), 7.05 (s, ArH), 7.47 (s, ArH), 7.66 (s, ArH).

References

1. Pinney, K.C., Jelinek, K.E., Chaplin, D.J., Pettit, G.R., *The Discovery and Development of the Combretastatins*, CRC Press, Boca Raton, FL, 2005.

2. Pettit, G.R.; Singh, S.B.; Boyd, M.R.; Hamel, E.; Pettit, R.K.; Schmidt, J.M.; Hogan, F.; Antineoplastic agents. 291. Isolation and synthesis of combretastatins A-4, A-5, and A-6, *J. Med. Chem.*, 38,1666,1995.

3. Gaukroger, K., Structural requirements for the interaction of combretastatins with tubulin: how important is the trimethoxy unit? *Org. Biomol. Chem.* 1, 3033,2003.

4. OXiGENE, Inc. (Dec. 27, 2006). *Positive Results of Phase II Study of Combretastatin (CA4P) in Combination with Paclitaxel and Carboplatin in Patients with Advanced Imageable Malignancies.* Press Release.

5. Pettit, G.R. et al., *Antineoplastic Agents 548. Synthesis of Iodo- and Diiodocombstatin Phosphate Prodrugs.* Not yet published.

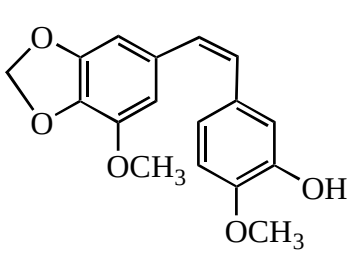
6. *Anticancer Drug Design* **2000**, *15*, 397-403.

Chapter 4

Iodination of Piperonal

4.1 Introduction

Combretastatin A-2 (CA2, Figure 4.1) is one of the original potent tubulin polymerization inhibitors, human cancer cell line inhibitors, and effective vascular targeting stilbenes[1, 2] isolated from the South African bush willow *Combretum caffrum*.[3] Due to the success of the CA-2 phosphate prodrug[4, 5] (Figure 4.2) many modifications have been synthesized based on CA-2.[6] In order to enhance the activity and better understand the structure activity relationships, synthesis of mono-iodo (Figure 4.3) and diiodo (Figure 4.4) derivatives were attempted herein, the phosphate prodrugs (Figure 4.5, 4.6) being the eventual targets. The initial step, iodination of piperonal (Figure 4.7), in this scheme presented some challenges due to its novelty and therefore will be discussed in detail.



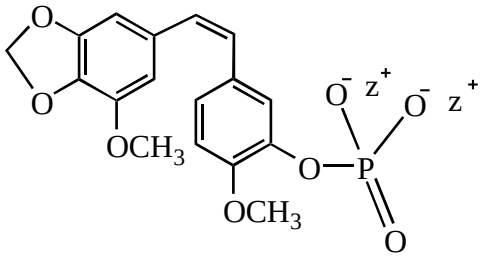
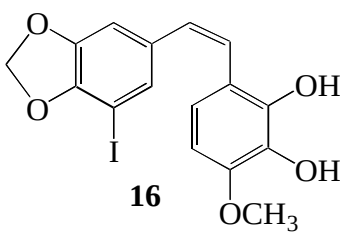


Figure 4.1 Combretastatin A-2 **Figure 4.2** CA2 Phosphate prodrug; z^+=Na, Li, K



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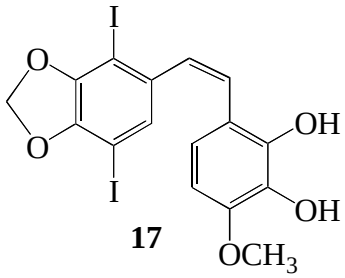

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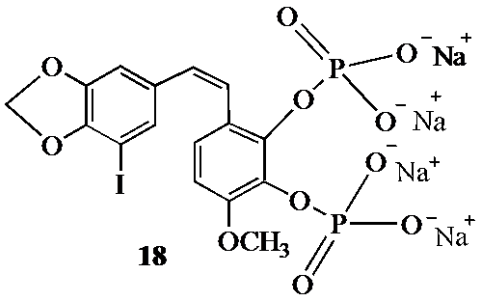
Figure 4.3 Mono-iodo derivative of CA-2 **Figure 4.4** Diiodo derivative of CA-2



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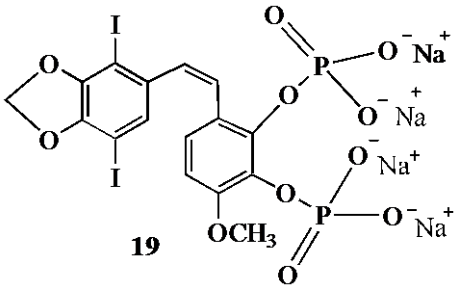

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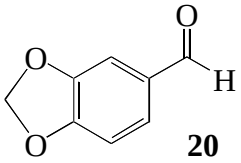
Figure 4.5 Mono-iodo CA-2 prodrug Z^+ = Li, Na, K

Figure 4.6 Diiodo derivative prodrug Z^+ = Li, Na, K



20

Figure 4.7 Piperonal

4.2 Solvent Directed Electrophilic Aromatic Substitution

A cost effective, mild and efficient, selective iodination of piperonal (**20**) was sought in order to obtain the mono- and diiodo derivatives of CA2. One promising research report reported selective iodination utilizing iodine in the presence of poly(4-vinylpyridine)-supported peroxodisulfate.[7] This was a two-step reaction: one to create the polymer, poly(4-vinylpyridine)-supported peroxodisulfate, and another to iodinate piperonal utilizing the polymer support. The supported polymer was created using very mild conditions culminating in an

efficient yield (98%). The iodination with the polymer reagent was also performed under mild conditions, however, no product was iodinated. Other iodination experiments were pursued.

Another promising, in fact the most promising, method employed the use of I_2 and [bis(trifluoroacetoxy]iodo) benzene (BTI, Figure 4.8) in either acetonitrile or methanol to iodinate activated alkyl aryl ketones.[8]

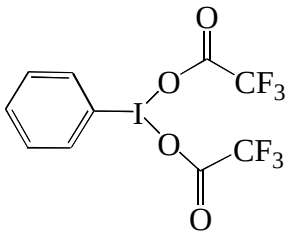


Figure 4.8 [Bis(trifluoroacetoxy)iodo] benzene (BTI)

Iodination of piperonal (**20**) was first attempted utilizing I_2 and BTI in acetonitrile and was successful in iodination. ^{1}H-NMR showed about a 53% conversion to the desired mono-iodo product (**21**) and 28% percent conversion to the desired diiodo product (**22**). Undesired iodinated products were also present in small amounts. Iodination of piperonal also attempted utilizing the aforementioned reagents and methanol as a solvent. Iodination appeared successful, but none of the desired compounds were attained. The same reaction was also attempted utilizing carbon tetrachloride as the solvent, however, no reaction occurred.

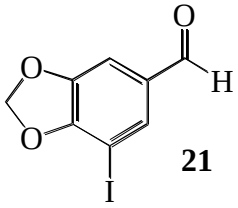


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Figure 4.9 3-Iodo Piperonal

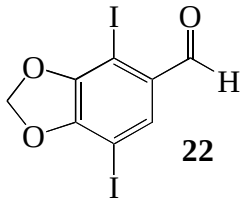

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Figure 4.10 2,5-Diiodo Piperonal

4.3 Attempts to Drive the Reaction towards the Mono-iodo Product (21) and the Diiodinated Product (22)

Even though iodination with I_2 and BTI was successful, it quickly became apparent that the products were not going to be easy to separate. In an attempt to drive the reaction to produce more favorable yields of exclusively mono-iodinated and exclusively diiodinated piperonal, so that separation might be efficiently achieved, the reaction conditions were altered and the results observed.

The reaction conditions that yielded favorable yields of mono-iodinated piperonal as reported above will be described first. The first scheme (scheme 4.1) included one equivalent of piperonal, 0.6 equivalents of I_2, 1.2 equivalents of BTI, and acetonitrile as solvent at room temperature. The reaction led to a 53% conversion of piperonal to the mono-iodinated product (**21**), 28% to the diiodinated product (**22**), and 18% of undesired products. Another set of reaction conditions that produced a favorable yield of mono-iodinated piperonal included one equivalent of piperonal, 0.6 equivalents of I_2, two equivalents of BTI, and acetonitrile as solvent at room temperature. These reaction conditions resulted in a 59% conversion of piperonal to the mono-iodinated product (**21**), 17% conversion to the diiodo product (**22**), and 24% of other products. The final set of reaction conditions that produced a favorable yield of mono-iodinated piperonal comprised one equivalent of piperonal, one equivalent of I_2, two and a half equivalents of BTI, and acetonitrile as solvent at 50^oC. These reaction conditions resulted in a 59% conversion of piperonal to the mono-iodinated product (**21**), a 13% conversion to the diiodinated product (**22**), and 28% of other products.

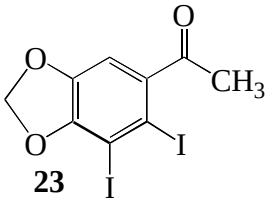
While the yield of mono-iodinated product (**21**) produced was favorable with the preceding methods, the amount of side products produced was unfavorable. Separation of the iodinated products was attempted utilizing many solvent systems in both flash and gravity silica, but to no real consequence. In an attempt to gain pure compounds at some point of the reaction sequence, the reaction was carried forward and separation attempted at each step. Separation was no easier at any step of the reaction sequence and so it is apparent that extensive HPLC methods are needed for this type of separation.

The reaction conditions that favored the production of the desired diiodinated product (**22**) include three different techniques. The first scheme was composed of one equivalent of piperonal, 1.8 equivalents of I_2, 3.4 equivalents of BTI, and acetonitrile as solvent at room temperature. These reaction conditions resulted in a 40% conversion of piperonal to the diiodo product (**22**), 37% conversion to the mono-iodo product (**21**), and 20% of other products. The second reaction scheme that favored the diiodinated product (**22**) began with one equivalent of piperonal, 0.6 equivalents of I_2, 1.2 equivalents of BTI, and acetonitrile at room temperature. The reaction was monitored by TLC and driven to completion by continuously adding I_2 and BTI until no starting material was present. This resulted in the addition of 1.7 equivalents of I_2 and 2.4 equivalents of BTI which consumed all the starting material and provided 54% conversion of piperonal to the diiodo product, 17% conversion to the mono-iodo product, and 18% of other products. Furthermore, there was an excess of I_2 in the reaction mixture that had to be dispelled with $Na_2S_2O_5$. The final reaction scheme

included one equivalent of piperonal, one equivalent of I_2, two equivalents of BTI, and acetonitrile as solvent at 50°C. This composition led to a 44% conversion of piperonal to the diiodo product (**22**), a 32% conversion to the mono-iodo product (**21**), and 25% of other products.

Elevating the temperature with less BTI than a reaction at room temperature seemed to force the reaction towards a diiodinated product. A reaction involving several equivalents of BTI with an elevated reaction temperature has yet to be performed, but would seem to favor the diiodinated product (**22**). As stated earlier, even though the reaction conditions could be altered to favor the diiodinated product (**22**), the existence of other iodinated products made separation impractical. Extensive HPLC techniques are needed for the separation of these iodinated products.

Interestingly, a reaction run utilizing methanol as a solvent produced entirely undesired iodinated products. While the structure(s) of these other products were not confirmed, mass spec and ^{1}H-NMR revealed that one is another diiodinated product (Figure 4.9) (**23**).



23

Figure 4.11 2,3-Diiodo Piperonal

4.4 Summary

The success of the combretastatins including combretastatin A-2 led to this SAR study which led to a cost effective and mild reaction to iodinate piperonal. This reaction was manipulated to drive the reaction to favor mono-iodination or diiodination. Unfortunately, efficient separation of the mono- (**21**) and diiodo (**22**) products from each other as well as from other products was not achieved. Extensive HPLC appeared needed for this endeavor. Hopefully, based on the preceding experiments, an even more efficient and reactive method can be achieved.

4.5 Experimental

General Mono-iodination Procedures

Method A: BTI (0.516 g, 1.2 mmol) was added to a solution of piperonal (**20**) (0.15 g, 1 mmol) and iodine (0.15 g, 0.6 mmol) in acetonitrile (5 mL). The mixture was stirred for 2 hrs and the reaction terminated by addition of (0.1 M) NaOH and extracted into DCM (3 x 5 mL). The combined organic layers were dried, filtered and concentrated to yield a reddish orange solid (0.43 g crude). ^{1}H-NMR revealed 59% mono-iodinated product (**21**), 32% diiodinated product (**22**), and 8% other products (**23**).

Method B: (This reaction was monitored by TLC using EtOAc-hexane 1:8 as eluent) BTI (0.142 g, 0.33 mmol) was added to a solution of piperonal (**20**, 0.1 g, 0.67 mmol) and iodine (0.1 g, 0.4 mmol) in acetonitrile (5 mL). The reaction mixture was stirred for 30 min, BTI (0.142 g, 0.33 mmol) was added; after stirring for 45 min BTI (0.142 g, 0.33 mmol) was added; following another

3.5 hrs BTI (0.142 g, 0.33 mmol) was again added. The reaction was stirred for 18 hrs, terminated by the addition of (0.1 M) NaOH (until pH7) and the product extracted into DCM (4 x 25 mL). The combined organic layers were dried, filtered and concentrated to yield a reddish orange oil (0.175 g crude). ^{1}H-NMR revealed 59% mono-iodinated product (**21**), 17% diiodinated product (**22**), and 24% other products (**23**).

Method C: BTI (0.361 g, 0.84 mmol) was added to a heated (50^{o}C) solution of piperonal (**20**) (0.1 g, 0.67 mmol) and iodine (0.178 g, 0.7 mmol) in acetonitrile (6 mL). The reaction mixture was stirred for 2 hrs at which time TLC revealed a significant amount of starting material remained. Another 0.84 mmol's of BTI was added and the mixture was stirred for another 2 hrs. The solvent was removed invacuo to yield a reddish brown solid. ^{1}H-NMR revealed 57% mono-iodinated product (**21**), 13% diiodinated product (**22**), and 31% other products (**23**).

General Diiodination Procedures

Method A: BTI (1.5 g, 3.4 mmol) was added to a solution of piperonal (**20**, 0.15 g, 1 mmol) and iodine (0.45 g, 0.1.8 mmol) in acetonitrile (7 mL). The mixture was stirred for 2 hrs and the reaction terminated by addition of (0.1 M) NaOH (until pH 7) and extracted into DCM (3 x 15 mL). The combined organic layers were dried, filtered and concentrated to yield a reddish orange solid (0.35 g, crude). ^{1}H-NMR revealed 40% conversion to the diiodo product (**22**) and 37% conversion to the mono-iodo product (**21**).

Method B: BTI (3.6 g, 8.37 mmol) was added to a solution of piperonal

(**20**, 1.05 g, 6.98 mmol) and iodine (1.07 g, 4.2 mmol) in acetonitrile (100 mL) at room temperature. The reaction mixture was stirred for 2 hrs, more iodine (0.7g, 2.8 mmol) was added and the mixture was stirred for 12 hrs. Additional BTI (0.27g, 0.63 mmol) was added and the reaction was left to stir for 2 hrs. At this point another equivalent of BTI (0.27g, 0.63 mmol) was added. After one hour the reaction had not yet reached completion so more iodine (0.77g, 3.0 mmol) and BTI (0.73g, 1.7 mmol) were added to the solution and the reaction mixture was stirred overnight. After 12 hrs, the reaction had not yet reached completion so more iodine (1.5g, 6.0 mmol) and BTI (3.4g, 8.0 mmol) were added. After 1.5 hours TLC revealed that conversion of starting material and mono-iodo to diiodo was partially successful. In an attempt to reach full conversion of mono-iodo to diiodo more iodine (0.75g, 3.0 mmol) and BTI (1.7g, 4.0 mmol) were added and stirring continued for 2 hrs. An excess of iodine was present in the reaction mixture so more BTI (0.85g, 2.0 mmol) was added and stirring continued another 2 hrs. The reaction was terminated with (0.1M) NaOH (until pH 1), the product extracted into DCM (3 x 100mL), dried, filtered, and concentrated to yield a dark purple liquid. Saturated $Na_2S_2O_5$ was added to the liquid to remove excess iodine. The remaining organic layer was a reddish-orange color (1.94g crude). ^{1}H-NMR revealed 54% conversion to diiodo product (**22**), 18% other products, and 17% mono-iodo product (**21**).

Method C: BTI (28.7g, 66.6 mmol) was added to a solution of piperonal (**20**, 5g, 33.3 mmol) and iodine (8.5g, 33.3 mmol) in acetonitrile at 50^{o}C. In 2 hrs the reaction was finished and the product concentrated (invacuo) to yield a deep

reddish brown solid. The product was placed in EtOAc and the solvent filtered to yield a grayish green solid (6.0g crude). ^{1}H-NMR revealed 44% conversion to diiodo product (**22**), 32% to mono-iodo product (**21**), and 25% other products.

References

1. Lin, C.M.; Singh, S.B.; Chu, P.S.; Dempcy, R.O.; Schmidt, J.M.; Pettit, G.R.; Hamel, E. *Molecular Pharmacology* **1988**, *34*, 200-208.

2. Lin, C.M.; Ho, H.H.; Pettit, G.R. Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicines to tubulin. *Biochemistry* **1989**,*28*, 6984-6991.

3. Pettit, G.R.; Singh, S.B. Isolation, structure, and synthesis of combretastatin A-2, A-3, and B-2. *Canadian Journal of Chemistry* **1987**, *65*, 2390-2396.

4. Pettit, G.R.; Temple, C. Jr.; Narayanan, V.L.; Varma, R.; Simpson, M.J.; Boyd, M.R.; Rener, G.A.; Bansal, N. Antineoplastic agents 322. Synthesis of combretastatin A-4 prodrug. *Anti-Cancer Drug Design* **1995**, *10*, 299-309.

5. Pettit, G.R.; Rhodes, M.R. Antineoplastic agents 389. New Synthesis of combretastatin A-4 prodrug. *Anti-Cancer Drug Design* **1998**, *13*, 183-191.

6. Pettit, G.R.; Moser, B.R.; Boyd, M.R.; Schmidt, J.M.; Pettit, R.K.; Chapuis, J.C. Antineoplastic Agents 460. Synthesis of combretastatin A-2 prodrugs. *Anti-Cancer Drug Design* **2002**, *16*, 185-193.

7. Tajik, H.; Mohammadpoor-Baltork, I.; Rasht-Abadi, H.R. Selective Para-Iodination of Methoxyarenes, Phenols, and Anilines with Iodine in the Presence of Poly(4-Vinylpyridine) Supported Peroxodisulfate. *Synthetic Communications* **2004**, *34*, 3579-3585.

8. Panunzi, B.; Rotiroti, L.; Tingoli, M.; Solvent directed electrophilic iodination and phenylselenenylation of activated alkyl aryl ketones. *Tetrahedron Letters* **2003**, *44*, 8753-8756.

Chapter 5

Partial Synthesis and Biological Evaluation of the Antineoplastic Agent 5-iodo-3,4-methylenedioxy-2',3'-dihydroxy-4'-methoxy-Z-stilbene (16) and 2,5-diiodo-3,4-methylenedioxy-2',3'-dihydroxy-4'-methoxy-Z-stilbene (17)

5.1 Introduction

Since complete separation of the iodinated piperonal was not achieved the synthesis began with mono and diiodo piperonal mixtures that led to several products at each step. However, prior to the wittig step, separation was almost complete and characterization was achieved at this point for both 5-iodo-3,4-methylenedioxy-2',3'-dihydroxy-4'-methoxy-Z-stilbene (**16**) and 2,5-diiodo-3,4-methylenedioxy-2',3'-dihydroxy-4'-methoxystilbene (**17**).

5.2 Results and Discussion

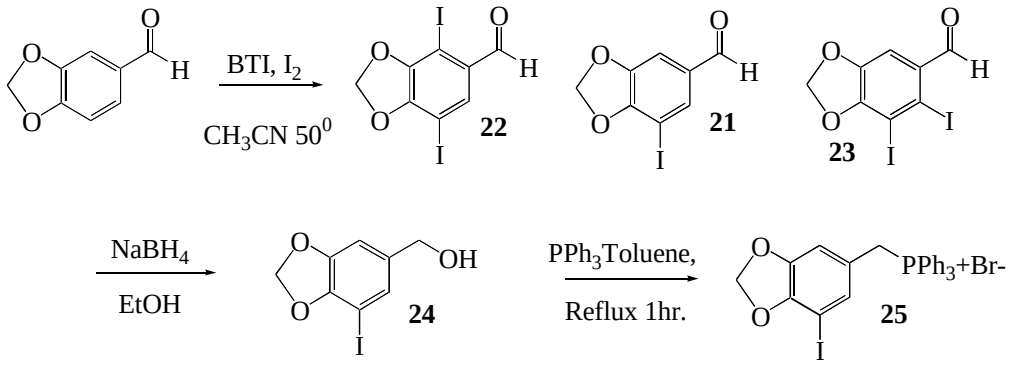
The A-rings, starting with either pure 5-iodo-piperanol (**21**) or 2,5-diiodo-piperanol (**22**) were obtained employing a solvent directed electrophilic aromatic substitution with BTI (Figure 4.8) as the key reagent. Many chromatographic column separations had to be employed in order to obtain a very small amount of each pure product for characterization. Since these are new compounds, they were tested against a panel of human cancer lines at this early point in the synthetic scheme (Table 5.1).

Table 5.1 Cancer Cell Biology for 5-iodo-piperonal (**21**) and 2,5-diiodo-piperonal (**22**)

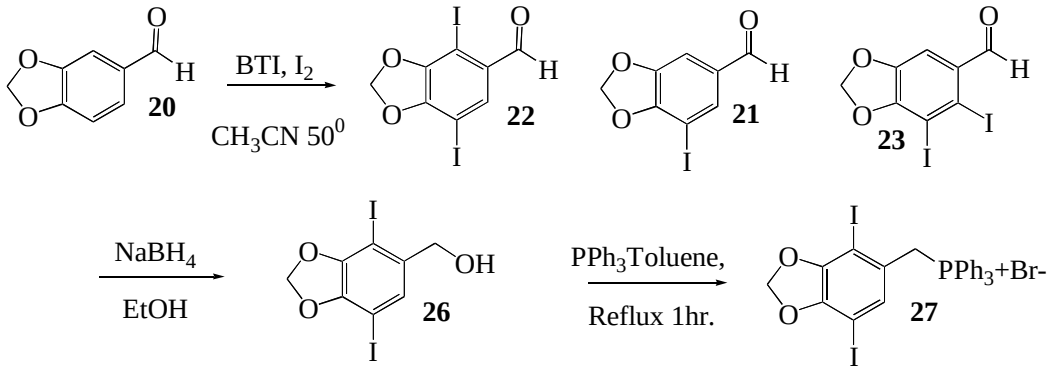
Compound	**BXPC-3**	**MCF-7**	**SF-268**	**NCI-H460**	**KM20L2**	**DU-145**
83F4 (mostly 21, 22, 23)	>10	3.1	5.6	29.9	>10	15.1
111F5-6 (21)	>10	>10	>10	>10	>10	>10
91F1 (21, mostly 22, 23)	2.3	2.3	2.8	3.5	>10	3.4
111F2 (22)	>10	4.9	4.7	6.3	>10	>10
83F3 (23)	>10	3.3	2.1	7.0	>10	5.7

The inhibitory properties for either of the mono-iodo samples is not ideal; however, it is interesting to note that the less pure sample (83F4) has increased inhibitory properties over the pure mono-iodo-piperonal **(21)**. The diiodo piperonal (**22**) was more inhibitory in general than the mono-iodo piperonal **(21).** Similarly to the mono-iodo piperonal, the diiodo sample that was not pure exhibited more inhibition than the pure sample. Furthermore, the undesired diiodo piperonal (**23**) exhibited more inhibition than expected.

The alcohols (**24**), (**26**) and the phosphonium bromides (**25**), (**27**) were synthesized utilizing standard reduction, bromination, and S_N2 procedures. Combustion and spectral analyses confirmed each of their structures.

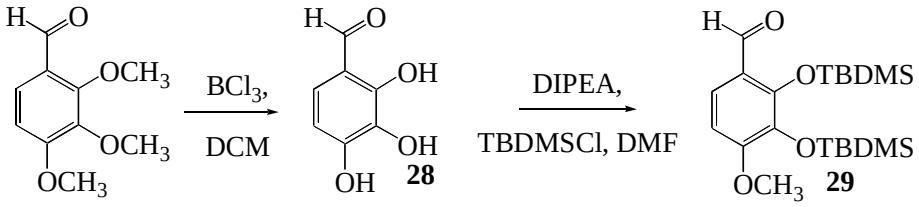


Scheme 5.1 Mono-iodination of Piperonal, Reduction, Bromination and Conversion to Phosphonium Bromide **25**



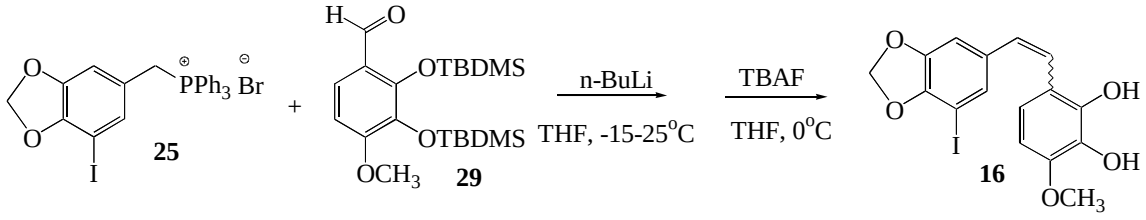
Scheme 5.2 Diiodination of Piperonal, Reduction and Conversion to Phosphonium Bromide **27**

The B-ring, starting with 2,3 dihydroxy-4-methoxy benzaldehyde, was synthesized utilizing a demethylation procedure. That was followed by a protection with TBDMS to produce the B-ring 2,3-Bis-[tert-butyldimethylsiloxy]-4-methoxy-benzaldehyde (**29**).



Scheme 5.3 Synthesis of the Protected B-ring **29**

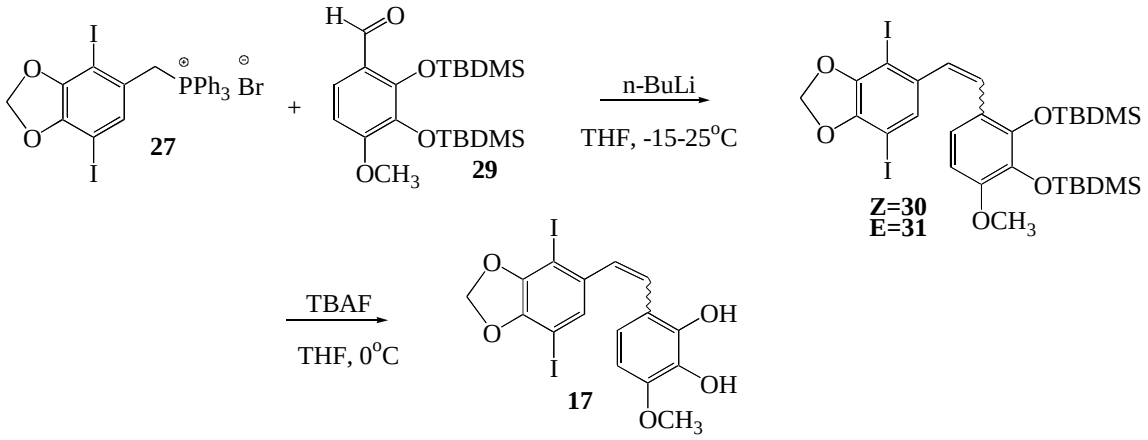
Due to time constraints, the key precursor of 5-mono-iodo-4'-methoxy-3,4-methylenedioxy-2',3'-dihydroxy-Z-stilbene (**16**) was not obtained in pure form. However it was synthesized utilizing a Wittig reaction sequence and subsequent deprotection to yield a Z/E mixture (Scheme 5.4). Despite the lack of separation, the Z/E mixture was tested against several human cancer cell lines and the findings summarized in Table 5.2. Again, activity is not ideal but the results are promising.



Scheme 5.4 Synthesis of 4,5-Bismethyl-3-iodo-CA-2 **16**

The same time constraints that restricted the separation of the mono-iodo stilbene (**16**) also restricted the separation of 2,5-diiodo-3,4-methylenedioxy-2',3'-dihydroxy-Z-stilbene (**17**) from it E-isomer. The diiodo-stilbenes were also synthesized utilizing the same Wittig reaction sequence and subsequent deprotection (Scheme 5.5). The protected Z/E mixture was tested against several human cancer cell lines and the findings summarized in Table 5.2. The results are very useful as they emphasize the pronounced reduction in cancer cell growth

inhibition when progressing from the fluoro-combretastatins to these iodo-combretastatins.



Scheme 5.5 Synthesis of 3,4-Bismethyl-2,5-diiodo-CA-2 **17**

Table 5.2 Cancer Cell Biology for 5-mono-iodo-4'-methoxy-3,4-methylenedioxy-2',3'-dihydroxy-stilbenes and 2,5-diiodo-4'-methoxy-3,4-methylendioxy-2',3'-*O*-tert-butyl-dimethylsilyl-stilbenes

Compound	**BXPC-3**	**MCF-7**	**MCF-10A**	**NCI-H460**	**SF268**	**DU-145**
149C (mostly Z 16, E 16)	>10	5.1	4.7	31.4	15.3	>10
145f2 (mostly 30, 31)	>10	>10	>10	>10	>10	>10

5.3 Conclusions

With the exception of the synthesis of novel compounds 21 and 22, the reaction sequence followed our general synthetic approach to the combretastatin class of stilbenes.[1] The results of the biological evaluations for the iodo/diiodo mixtures suggest future separation of the stilbenes (16) and (17) will lead to pure specimens with only modest cancer cell growth inhibition. However, the pure

stilbenes might have important anticancer activity when used against in-vivo thyroid cancer. 2,3-Diiodo piperonal (23) should be synthesized to provide its Z-stilbene and corresponding phosphate pro-drug in order to further evaluate it against human cancer and especially against thyroid cancer.

5.4 Materials and Methods

Anhydrous dicholoromethane (DCM), anhydrous toluene, dibenzyl phosphite, triphenylphosphine, and carbon tetrachloride were obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI). Diisopropylethylamine was obtained from Avocado Alfa Aesar (Ward Hill, MA). All other reagents were purchased from Acros Organics (Fisher Scientific, Pittsburg, PA).

All reactions were monitored by thin-layer chromatography using Analtech silica gel GHLF Uniplates visualized under long-wave and short-wave UV irradiation and by dipping into phosphomolybic acid in ethanol followed by heating. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Where appropriate, the crude products were separated by column chromatography, flash (230-400 mesh ASTM) or gravity (70-230 mesh ASTM) silica from E. Merck.

Melting points were measured using an electrothermal digital melting point apparatus and are uncorrected. All 1H- and 13C-NMR spectra were obtained using Varian Gemini 300 MHz or varian unity 400 or 500 MHz instruments with CDCl3 (tetramethylsilane internal reference) as solvent unless otherwise noted. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

5.5 Experimental

2,3-dihydroxy-4-methoxy-benzaldehyde (28)

Preparation of benzaldehyde **28** was repeated essentially as described (Pettit *et al*., 2000)[2] from 2,3,4-trimethoxybenzaldehyde (5 g) to yield a yellow solid which was recrystallized to afford yellow needles **28** (2.32 g, 54%): m.p. 115-116^{o}C [lit. 116-117^{o}C (Pettit *et al*., 1987)[3]]; (1:1 EtOAc-hexane); ^{1}HNMR (300 MHz, $CDCl_3$) δ 3.99 (s, 3H, OCH3), 5.46 (s, 1H, OH), 6.62 (d, 1H, J = 8.4 Hz, ArH), 7.15 (d, 1H, J = 8.4 Hz, ArH) 9.76 (s, 1H, CHO), 11.12 (s, 1H, OH).

2,3-Bis-[tert-butyldimethylsiloxy]-4-methoxybenzaldehyde (29)

Preparation of silyl ether **29** was repeated essentially as originally described (Pettit *et al.*, 1987)[3] from diphenol **28** (2.2 g). The reaction yielded a brown solid which was recrystallized from methanol to yield a white solid **29** (3.46 g, 67%); m.p. 73-74^{o}C [lit. 74.5-76^{o}C (Pettet *et al.*, 1987)[3]]; R_f 0.77 (1:1 EtOAc-hexane); ^{1}HNMR (300 MHz, $CDCl_3$) δ 0.14 (s, 12H, 4 x SiCH3), 0.99 (s, 9H, 3 x CH3), 1.04 (s, 9H, 3 x CH3), 3.84 (s, 3H, OCH3), 6.63 (d, 1H, J = 8.7 Hz, ArH), 7.49 (d, 1H, J = 8.7 Hz, ArH), 10.23 (s, 1H, CHO). HRMS calcd. for $C_{20}H_{36}O_4Si_2$ $[M+H]^+$ 397.22305, found 397.2030.

5-iodo-3,4-methylenedioxybenzaldehyde (21)

The product was separated by silica gel column chromatography using EtOAc-hexane 1:4 as eluent resulting in a cream solid **21** (359 mg, 19%): Rf 0.23 (1:8 EtOAc-hexane as eluent); ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.15 (2H, s, -OCH_2O-), 7.27 (1H, s, ArH), 7.69 (1H, s, ArH), 9.75 (1H, s, -CHO); ^{1}HNMR (500 MHz, $CDCl_3$) δ 6.14 (2H, s, -CH_2-), 7.27 (1H, s, ArH), 7.70 (1H, s, ArH)

9.75 (1H, s, -CHO); ^{13}CNMR (500 MHz, $CDCl_3$) δ 70.12, 101.73, 106.88, 133.40, 135.29, 136.50, 147.47, 188.94; HRMS calcd. for $C_8H_5IO_3$ $[M+H]^+$ 276.9362, found 276.9360.

5-iodo-3,4-methylenedioxy-benzyl alcohol (24)

$NaBH_4$ (2.45 g, 64.8 mmol) was added to a mixture of mono-iodo **21** and diiodo **22** aldehydes (14.91 g, 54 mmol) in ethanol (200 mL) and the reaction was left to stir for 23 hrs. The reaction was terminated by the addition of solid $NaHCO_3$, filtered and the solvent removed in vacuo to yield a cream solid. The products (1.0 g) were separated by flash silica gel column chromatography using ½% MeOH-DCM as eluent. The iodo alcohol resulted in a white solid: mp 161^{o}C; ^{1}HNMR (300 MHz, $CDCl_3$) δ 4.57 (2H, s, $-CH_2-$), 4.76 (1H, s, -OH), 6.02 (s, 2H, $-OCH_2O-$), 6.80 (1H, s, ArH), 7.13 (1H, s, ArH); ^{1}HNMR (500 MHz, $CDCl_3$) δ 4.56 (2H, s, $-CH_2-$), 4.72 (1H, s, -OH), 6.02 (1H, s, $-OCH_2O-$), 6.81 (1H, s, ArH), 7.14 (1H, s, ArH); ^{13}CNMR (500 MHz, $CDCl_3$) δ 64.65, 70.49, 100.87, 101.41, 107.83, 108.68, 128.86, 136.98. Analysis calcd. for $C_8H_7IO_3$: I, 45.64. Found: I, 46.19.

5-iodo-3,4-methylenedioxy-benzyl-triphyphenylphosphonium bromide (25)

Phosphorous tribromide (32.4 *u*L, 0.342 mmol) was added to a solution of iodo alcohol **24** (0.095 g, 0.342 mmol) in DCM (14 mL) and the reaction was stirred for 40 min. The reaction was terminated with 10% $NaHCO_3$ solution (2 x 50 mL), extracted into DCM (1 x 50 mL), the combined organic layers washed with water (1 x 50 mL), and the aqueous layer extracted with DCM (3 x 50 mL). The combined organic layers were dried, filtered and concentrated to yield a

white solid (0.227 g, 88.5%). Triphenylphosphine (0.192 g, 0.732 mmol) was added to a solution of the bromide (0.227g, 0.666 mmol) in toluene (10 mL) the reaction mixture heated at reflux for 1 hr. The reaction was then stirred at room temperature for 16 hrs. The product was collected and washed with toluene to yield a purple solid **25** (0.217 g, 54%): mp 195-242^{o}C (dec); ^{1}HNMR (500 MHz, CDCl3) δ 5.48 (2H, d, *J* = 14 Hz, -CH_2-), 5.94 (2H, s, -OCH_2O-), 6.56 (1H, s, ArH), 6.81 (1H, s, ArH), 7.67 (6H, s, ArH) 7.80 (9H, m, ArH); ^{13}CNMR MHz,$CDCl_3$)δ 31.18, 70.22, 101.17, 112.11, 112.14, 117.58, 118.26, 122.69, 122.76, 130.34, 130.44, 133.32, 133.37, 134.65, 134.73, 134.76, 135.24, 135.27, 146.74, 149.67. Analysis calcd. for $C_{26}H_{21}IBrO_2P$: C, 51.77; H, 3.51. Found: C, 50.08; H, 3.40.

5-iodo-4'-methoxy-3,4-methylenedioxy-2',3'-dihydroxy-Z-stilbene (16)

n-Butyl lithium (0.84 mL, 1.328 mmol) was added to a cooled (-25^{o}C) heterogeneous solution of phosphonium bromide **25** (0.2 g, 0.332 mmol) in THF (10 mL). The reaction was stirred for 20 min and aldehyde **29** (0.132 g, 0.332 mmol) was added. After 1 hr, the reaction temp was allowed to rise to rt and the reaction was stirred for another 18 hrs. The reaction was terminated by the addition of water (25 mL) and extracted into EtOAc (3 x 25 mL). The combined organic layers were dried, filtered and concentrated to yield the crude silyl ether. Tetrabutylammonium flouride (1.3 mL, 1.3 mmol) was added to a solution of crude silyl ether (0.374 g, 0.583 mmol) in THF (10 mL). The mixture was stirred under Ar in the dark for 1.5 hrs, and the reaction was terminated by the addition of water (10 mL). The product was extracted with EtOAc (3 x 15 mL), and the

organic phase was dried, filtered and concentrated to yield a brown oil. ^{1}HNMR (300 MHz $CDCl_3$) δ 2.85 (1H, s, OH), 3.15 (1H, s, OH), 3.83 (3H, s, OCH_3), 5.90 (2H, s, -OCH_2O-), 6.34 (1H, d, J = 6 Hz), 6.51 (1H, d, J = 6.6 Hz), 6.62 (1H, d, J = 8.4 Hz), 6.66 (1H, ArH), 6.72 (1H, s, ArH), 7.14 (1H, d, J = 9.3 Hz).

Synthesis of 2,5-diiodo-3,4-methylenedioxy-2',3'-dihydroxy-4'-methoxy stilbene

2,5-diiodo-3,4-methylenedioxybenzaldehyde (22)

The product was separated by silica gel column chromatography using EtOAc-hexane 1:8 as eluent resulting in a white solid: Rf 0.39 (1:8 EtOAc/hexane as eluent); ^{1}HNMR (300 MHz $CDCl_3$) δ 6.21 (2H, s, -OCH_2O-), 7.80 (1H, s, ArH), 9.83 (1H, s, -CHO). 1HNMR (500 MHz, $CDCl_3$) δ 6.21 (2H, s, -OCH_2O-), 7.80 (1H, s, ArH), 9.83 (1H, s, -CHO). ^{13}CNMR (500 MHz, $CDCl_3$) δ 75.78, 101.05, 109.24, 130.09, 135.24, 148.97, 152.29, 191.55. HRMS calcd. for $C_8H_4I_2O_3$ $[M+H]^+$ 402.8329, found 402.8357.

2,5-diiodo-3,4-methylenedioxybenzyl alcohol (26)

$NaBH_4$ (56.5 mg, 1.4 mmol) was added to a mixture of aldehyde **22** (502mg, 1.2 mmol) in EtOH (5 mL) under Ar at rt. The reaction was left to stir for 16 hours. The reaction was terminated with solid $NaHCO_3$, filtered and vacced down to yield a white solid (500 mg, 99% crude). The product was separated by silica gel column chromatography using ½% MeOH/DCM + 2 mL MeOH. The product was a white solid. ^{1}HNMR (300 MHz, $CDCl_3$) δ 4.59 (2H, s, -CH_2-), 5.02 (1H, s, OH), 6.10 (2H, s, -OCH_2-), 7.24 (1H, s, ArH). ^{1}HNMR (500 MHz, $CDCl_3$) δ 4.59 (2H, s, -CH_2-), 5.01 (1H, s, OH), 6.10 (2H, s, -OCH_2O-

), 7.25 (1H, s, ArH). ^{13}CNMR (500 MHz, $CDCl_3$) δ 67.78, 74.40, 100.50, 100.80, 130.18, 137.60, 147.69, 148.69. Analysis calcd. for $C_8H_6I_2O_3$: C, 23.79; H, 1.50; I, 62.83. Found: C, 23.91; H, 1.55; I, 63.43.

2,5-diiodo-3,4-methylenedioxybenzyltriphenylphosphonium bromide (27)

Phosphorous tri-bromide (125*u*L, 0.131 mmol) was added to a solution of alcohol **26** (97 mg, 0.262 mmol) in DCM (14 mL) under argon at room temperature and the reaction was left to stir for 20 hours. The reaction was terminated with 10% $NaHCO_3$ solution, extracted into DCM (3 x 10mL), dried ($MgSO_4$) and concentrated to yield a white solid (100 mg crude). Triphenylphosphine (62 mg, 0.236 mmol) was added to a solution of the resulting bromide (100 mg, 0.215 mmol) in toluene (5 mL) at room temperature. The reaction temperature was then elevated to react under reflux for two hours. The temperature was then reduced and the reaction was left to stir for 17 hours. The white precipitate was filtered to yield a white powdery solid (90 mg 57.5%): ^{1}HNMR (300 MHz, $CDCl_3$) δ 5.69 (2H, d, J_{PCH2} = 14.1 Hz, -CH_2-) 6.05 (2H, s, -OCH_2O-), 7.16 (1H, d, J = 3.0 Hz, ArH), 7.68 (6H, m, ArH), 7.75 (5H, m, ArH), 7.81 (4H, m, ArH). ^{1}HNMR (500 MHz, $CDCl_3$) δ 5.65 (1H, s, -CH_2-), 5.68 (1H, s, -CH_2-), 6.05 (2H, s, -OCH_2O-), 7.15 (2H, d, J = 2.1 Hz, ArH), 7.67 (6H, m, ArH), 7.74 (6H, m, ArH), 7.82 (3H, t, J = 4.4 Hz, ArH). ^{13}CNMR (500 MHz, $CDCl_3$) δ 33.53, 71.14, 71.17, 81.49, 81.54, 100.74, 105.0, 117.20, 117.88, 125.37, 125.44, 130.49, 130.59, 130.61, 133.81, 133.86, 134.73, 134.81, 134.83, 135.49, 135.51, 135.55, 148.16, 148.83. Analysis calculated for $C_{26}H_{20}I_2BrO_2P$: C, 42.83; H, 2.76; I, 34.81. Found C, 42.70; H, 2.73; I, 53.34.

2,5-diiodo-4'-methoxy-3,4-methylenedioxy-2',3'-*O*-tert-butyl-dimethylsilyl-Z-stilbene (30) and 2,5-diiodo-4'-methoxy-3,4-methylenedioxy-2',3'-*O*-tert-butyl-dimethylsilyl-E-stilbene (31)

n-Butyl lithium (3.7 mL, 2.3 mmol) was added to a cooled (-25^{o}C) heterogeneous solution of phosphonium bromide **27** (0.84 g, 1.15 mmol) in THF (50 mL). The reaction was stirred for 20 min and aldehyde **29** (0.46 g, 1.15 mmol) was added. After 1 hr, the reaction temp was allowed to rise to rt and the reaction was stirred for another 18 hrs. The reaction was terminated by the addition of water (50 mL) and extracted into EtOAc (4 x 50 mL). The combined organic layers were dried, filtered and concentrated to yield a brown oil (1.1 g, crude). The product was separated by silica gel column chromatography using EtOAc-hexane 1:19 as eluent resulting in a clear oil. Rf Z: 0.48; E: 0.41 (19:1 EtOAc/Hexane).

2,5-diiodo-4'-methoxy-3,4-methylenedioxy-2',3'-dihydroxy-Z-stilbene (17)

TBAF (1.0 mL, 1.0 mmol) was added to a cooled, stirred solution of protected stilbene (**30**) in anhydrous THF (7.5 mL) under argon. After 1.5 hrs. the reaction was terminated with H_2O (15 mL) and extracted into EtOAc (4 x 12 mL). The combined organic layers were dried, filtered and concentrated to yield a brown oil (367 mg, crude). ^{1}HNMR (300 MHz $CDCl_3$) δ 3.82 (3H, s, OCH3), 5.01 (1H, s, OH), 5.25 (1H, d, J = 11.4, ArH), 5.54 (1H, d, J = 17, ArH), 5.91 (1H, s, OH), 6.07 (2H, s, -OCH2O-), 6.69 (1H, d, J = 10.8), 6.75 (1H, d, J = 10), 6.98 (1H, s, ArH).

Further separation and characterization of these products was postponed due to the abrupt closing of the Cancer Research Institute and loss of our research laboratories as well as misplacement of product materials by the moving crews.

References

1. Pettit, G.R. et al., *Antineoplastic Agents 548. Synthesis of Iodo- and Diiodocombstatin Phosphate Prodrugs.* Not yet published.

2. Pettit, G.R.; Lippert, J.W. Antineoplatstic agents 429. Synthesis of the combretastatin A-1 and combretastatin B-1 prodrugs. *Anti-Cancer Drug Design* **2000**, *15*, 203-216.

3. Pettit, G.R.; Singh, S.B. Isolation, Structure, and Synthesis of Combretastatin A-2, A-3, and B-2. *Canadian Journal of Chemistry* **1987**, *65*, 2390-2396.

BIOGRAPHICAL SKETCH

Brindi Trickey-Platt was born in Safford, AZ, but grew up in St. Johns, AZ, where she graduated from high school in 2000. Brindi attended the Honors College at the University of Arizona for one year at which time she transferred to Southern Virginia University in Buena Vista, VA. She graduated from SVU in 2004 with a Bachelor of Arts in English; it is important to note that she minored in science taking courses such as chemistry, organic chemistry, and biochemistry during her undergraduate studies. In August 2005, Brindi entered the Graduate College at Arizona State University to pursue a master's degree in chemistry.