Synthesis of a C2-Aryl-Pyrrolo[2,1-*c*][1,4]benzodiazepines Monomer Enabling the Convergent Construction of Symmetrical and Non-symmetrical Dimeric Analogs

Robert V. Kolakowski, Thomas D. Young, Philip H. Howard, Scott C. Jeffrey, Peter D. Senter

PII: S0040-4039(15)00958-2
DOI: http://dx.doi.org/10.1016/j.tetlet.2015.05.116
Reference: TETL 46388

To appear in: *Tetrahedron Letters*

Received Date: 1 May 2015
Revised Date: 26 May 2015
Accepted Date: 29 May 2015

Please cite this article as: Kolakowski, R.V., Young, T.D., Howard, P.H., Jeffrey, S.C., Senter, P.D., Synthesis of a C2-Aryl-Pyrrolo[2,1-*c*][1,4]benzodiazepines Monomer Enabling the Convergent Construction of Symmetrical and Non-symmetrical Dimeric Analogs, *Tetrahedron Letters* (2015), doi: http://dx.doi.org/10.1016/j.tetlet.2015.05.116

Synthesis of a C2-Aryl-Pyrrolo[2,1-*c*][1,4]benzodiazepines Monomer Enabling the Convergent Construction of Symmetrical and Non-symmetrical Dimeric Analogs.

Robert V. Kolakowski[1*], Thomas D. Young[1], Philip H. Howard[2], Scott C. Jeffrey[1], Peter D. Senter[1].

[1] Seattle Genetics, Inc. 21823-30th Drive SE, Bothell, WA 98021.

[2] ADC Products Ltd., The QMB Innovation Centre, 42 New Road, London, E1 2AX, United Kingdom

Keywords. Pyrrolobenzodiazapines, Antibody-Drug Conjugates, Payloads, Drug-Linker, Anti-cancer

Corresponding author: Robert V. Kolakowski

Postal address; Seattle Genetics, Inc. 21823-30th Drive SE, Bothell, WA 98021.

Phone; 1.425.527.4776

Fax; 1.425.527.4001

E-mail; rkolakowski@seagen.com

Abstract: We disclose the synthesis of a versatile C2-aryl-pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) monomer that enables the late-stage diversification and synthesis of PBD dimers. The monomer unit was elaborated and dimerized utilizing different tethers to yield symmetric and non-symmetrical C2-aryl-PBD dimers. The dimers were tested for cytotoxic activity on a number of cancer cell lines and showed potent cytotoxicity in the nanomolar to picomolar range. The synthesis of PBD monomers enables the modular construction of PBD free drugs and drug-linkers that may have application for the development of antibody-drug conjugations.

Introduction: Antibody-drug conjugates, (ADCs) have emerged as important therapeutics for treating cancer as evidenced by the FDA approval of Adcetris™, Kadcyla™ and the over 35 ADCs[1-4] currently in clinical trials.[5-7] A distinctive feature of this approach for cancer therapy is that antibody targeting has the potential of improving drug activity through antigen mediated delivery, and at the same time, lowering systemic toxicity of the anti-cancer payload. Recent studies have highlighted the impact the payload[8] and linker[9-10] can have on ADC efficacy and tolerability.

The majority of the ADCs currently under evaluation deliver antimitotic agents, either auristatin or maytansine analogs, as their cytotoxic payloads. While antimitotics hold considerable promise as ADC payloads, there is a strong rationale for new drug classes with alternative and complementary mechanisms to further extend ADC technology. Recent work with payloads such as amanitins (RNA-polymerase II inhibitors),[11-12] spliceostatins (spliceosome inhibitors),[13-14] and ultrapotent doxorubicin analogs (topoisomerase II inhibitors)[15-16] are a few examples of the many promising chemotypes under active investigation.

We have recently described a highly potent class of pyrrolobenzodiazepine (PBD) dimers for ADCs.[17-18] PBD dimers were designed from monomeric natural products[19-22] which include anthramycin **1**, sibiromycin **2** and tomaymycin **3** Figure 1. These natural products are DNA minor groove binders with GC sequence specificity and potent cytotoxic activity in the nM IC_{50} range. They are known to alkylate DNA moieties preferentially at guanine residues through their reactive imine functionalities .[23-24] Synthetic PBD dimers, such as SJG-136 (**4**), were shown to be even more potent than the PBD monomers and to have GATC and the GAAT sequence specificities resulting in DNA cross-linking .[25-27] Dimeric PBDs can have IC_{50} values in the pM range. Notably, the C2-aryl derivatives are among the most potent members in the class.[28-32]

To better evaluate the properties of this potent class of cytotoxic agents for ADCs, we set out to develop a modular synthesis of the PBD dimers that would enable us to prepare both symmetric and non-symmetric analogs. Past syntheses of PBD dimers have introduced a bridging linkage between the monomeric units

early in the synthesis, in order to avoid late-stage protecting group manipulation.[33-35] While this method has utility, it is best suited for symmetrical PBD dimers. Approaches to monomeric PBDs, such as Chen et. al.,[36] utilize a similar disconnection strategy, but lack the proper protection at the C8 position for late-stage dimerization. Kamal et. al.[37] demonstrated the feasibility of a late-stage dimerization strategy but their analogs lacked C2 substitution, which was found empirically to be an important element for high potency. Herein, we demonstrate a synthesis that leverages late-stage monomer diversification via a Suzuki coupling reaction and subsequent monomer dimerization that can prepare both symmetric and non-symmetric substituted analogs.[38-39] This synthetic strategy introduces diversity in the final steps of PBD dimer synthesis, allowing the efficient evaluation of the structure activity relationship (SAR) at the PBDs point of linker attachment and of the C2 aryl groups.

We began the synthesis of the key intermediate, PBD monomer **18**, (Scheme 1) with chlorobenzyl protection of methyl vanillate, **5,** to give **6** in high yield. The use of claycop,[40] (copper nitrate deposited on K-10 clay), regioselectively nitrated **6** to provide **7** in a 96 % yield. The methyl ester of **7** was saponified using an aqueous sodium hydroxide solution in THF to afford the acid **8**, in good yield. After an aqueous wash, **8** was taken on without further purification and treated with oxalyl chloride and catalytic amount of DMF to convert **8** to the corresponding acid chloride *in situ*, further treatment of the acid chloride with TBS protected pyrrolidine diol **9** yielded amide **10**. After extensive optimization, then nitro group of **10** could be reduced and the phenol deprotected under hydrogenating conditions with Pd/C and H_2 to provide aniline **11** in a 78 % yield. Intermediate **11** was subjected to a Ley oxidation[41] to give ketone **12** in a 90 % yield which was protected with TBDPSCl in DMF to provide **13**. The aniline moiety could then be capped with TROC-Cl to cleanly yield **14**. Acidic removal of the TBS group with PTSA gave the primary alcohol **15** in an 88% yield. With our primary alcohol **15** in hand, we sought to carry out an oxidative cyclization of **15** to form the 7-membered diazepine ring system, compound **16**. Ley oxidation with TPAP, Dess-Martin oxidation,[42] and PCC[43] all gave different amounts of over-oxidized lactam product, instead of the desired lactol, **16**. However, the Swern oxidation effectively oxidized the

alcohol to the lactol with one equivalent of oxalyl chloride at – 78 °C. Both acetylation of **16** and enol triflation of **17** proceeded smoothly to yield our key point of diversity, intermediate **18**. The Suzuki coupling of **18**[44] with *p*-methoxyphenyl boronic acid gave **19** in good yield. Finally, TBAF was employed to remove the phenolic TBDPS group from **19** to yield the desired monomeric intermediate **20**, ready for dimerization.

To validate this approach, we sought to prepare and evaluate the biological activity of several analogs including the well-studied C2-*para*-methoxy-phenyl dimer SG2202, compound **24** (Scheme 2).[45] To this end, monomer **20** was alkylated with 0.5 equivalents of 1,3-dibromopropane (**21a**) using K_2CO_3 in DMF to yield the **22**, in a 86 % yield. Global TROC deprotection with the Pb-Cd[46] couple yielded SG2202 (**24**) in a good yield. The synthesis of a symmetric PBD dimer **25**, with an aniline group in the *para*-C2 aryl position, utilized the *para*-methoxy monomer **20** which was dimerized with 1,3-bis(bromomethyl)-5-nitrobenzene (**21b**) to yield **23**. Both the TROC groups and the nitro group could be reduced in a one-pot procedure utilizing Pb-Cd to yield **25** after silica gel purification.

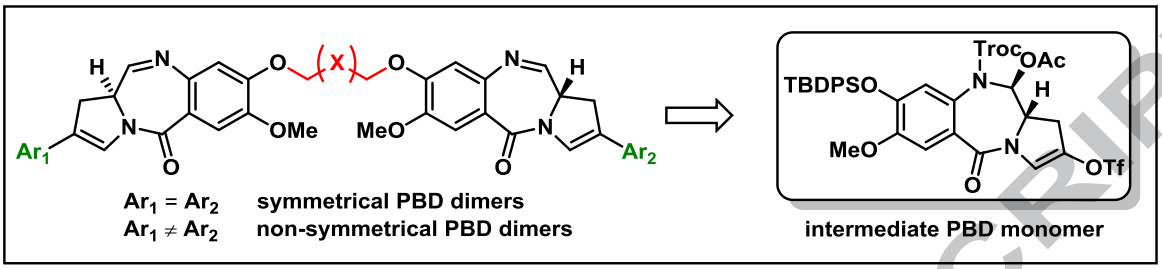
To demonstrate the feasibility of synthesizing non-symmetrical PBD dimers with this approach, we converted enol triflate **18** to the PBD monomer **28** (Scheme 3). The *para*-aniline monomer **26** was synthesized from the Suzuki coupling of **18** with 4-aminophenylboronic acid pinacol ester. The *para*-aniline group of **26** was protected with TROC-Cl and the protected phenol was TBDPS deprotected with TBAF to yield **28** in a 87 % yield for the two steps. In parallel, the *para*-methoxy monomer **20** was alkylated with an excess of 1,3-dibromopropane (**21a**) to yield the alkyl bromide **27** in a 82 % yield. Alkylation of **27** with **28** in Cs_2CO_3 and DMF yielded 80 % of the protected non-symmetric dimer **29**. Global TROC deprotection with the Pb-Cd couple gave **30** in a 58 % yield.

Compounds, **24**, **25**, and **30** were tested in an *in vitro* cellular potency assay (Table 1) on a panel of cancer cell lines. **24** and **30** gave IC_{50} values comparable to literature values,[45] in the nM to pM range.[47] The inclusion of the bridging aniline moiety in the symmetric dimer, **25**, did not appear to perturb the

biological potency. Compound **25** was approximately 10-fold more potent than compound **24** and the increased potency of **25** is consistent with the finding that PBD dimers possessing a 5-carbon connecting units are generally more potent than the 3-carbon spaced analogs.[48]

Conclusions: With the synthesis of the versatile monomeric analog **18,** we were able to efficiently synthesize both symmetric and non-symmetrical PBD dimers and the analogs synthesized were highly potent on cancer cell lines. The method disclosed here enables the evaluation of a variety of PBD dimers as payloads for ADCs.

ACKNOWLEDGMENTS: We would like to thank Arnaud Tiberghien and Luke Masterson for previous contributions to the synthesis of PBDs and useful discussions throughout the course of this work.



Abstract Figure

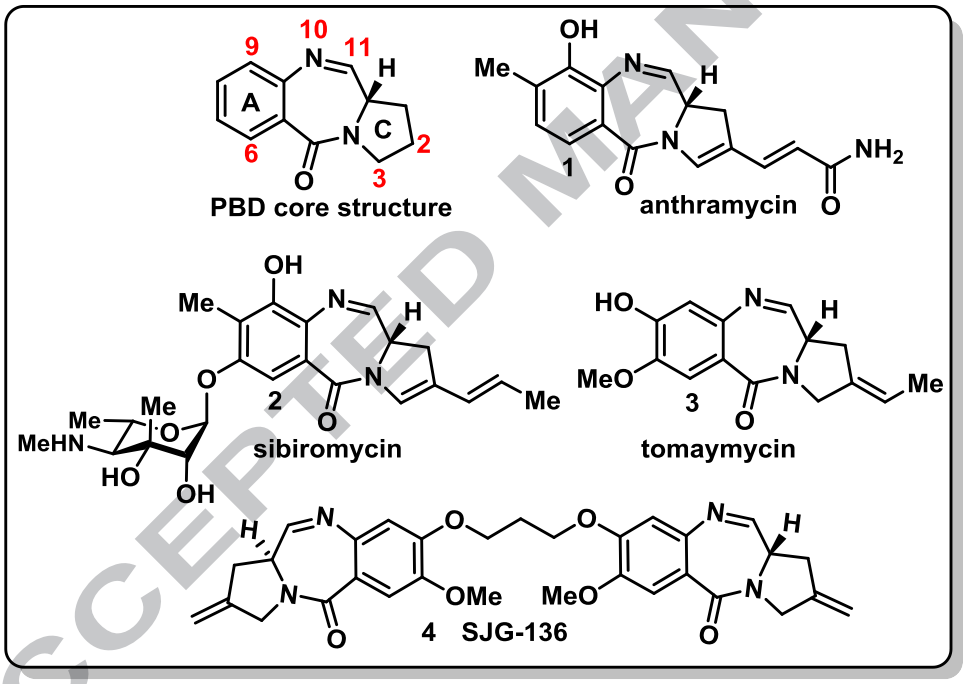
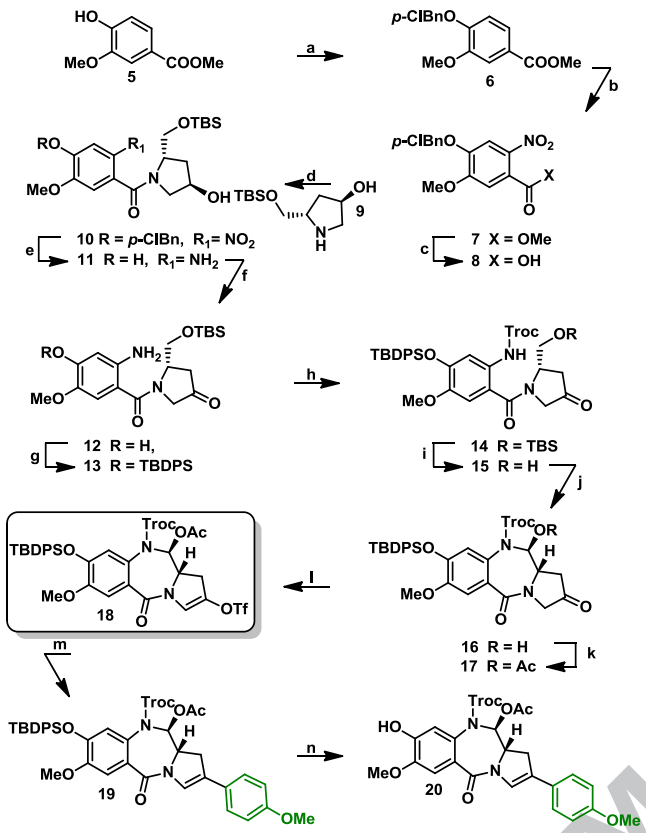
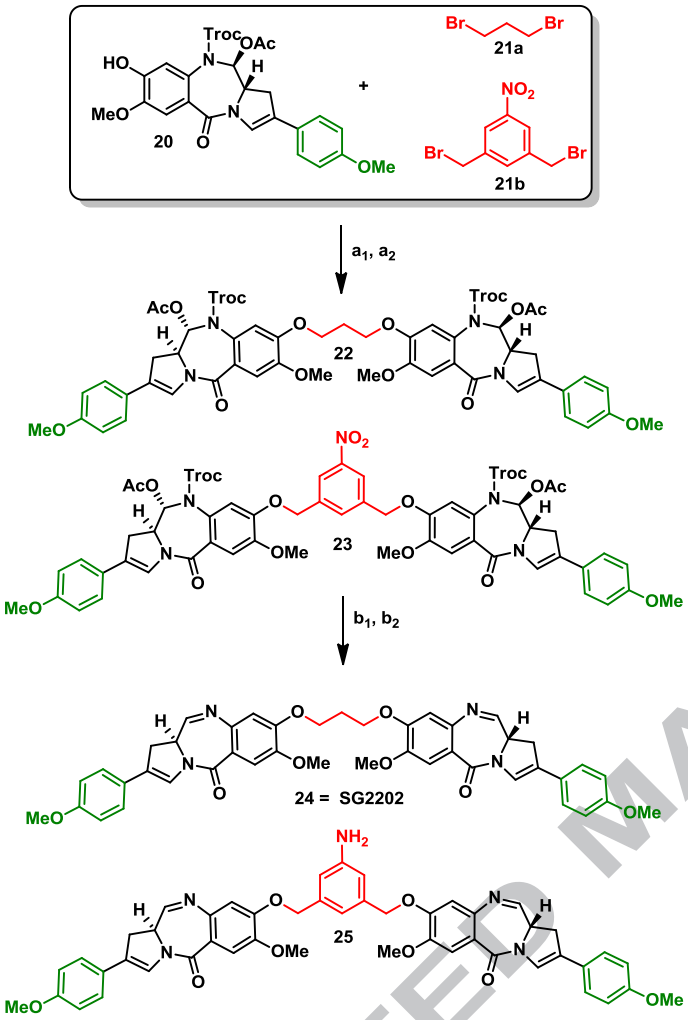


Figure 1. Related pyrrolo[1.4]benzodiazepine natural products and dimers.

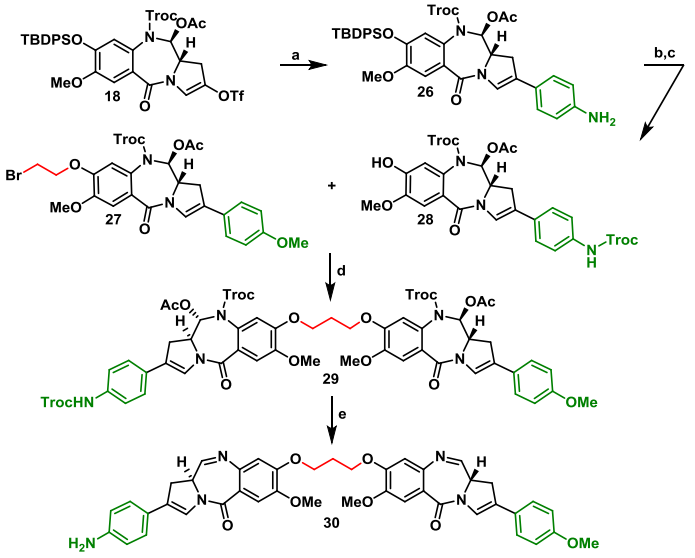


Scheme 1:

(a) *p*-ClBnBr (1.1 equiv.), K_2CO_3, tol., rt, 95 %, (b) $Cu(NO_3)_2$ on K-10 clay 1:1 v/v, DCM, Ac_2O, 0 °C, 96 %, (c) NaOH, THF:MeOH 1:1, rt, 89%, (d) $(COCl)_2$ (1.5 equiv.), cat. DMF, DCM, 0 °C, then, rt, **9** (1.0 equiv.) 91 %, (e) 10 % Pd/C, (0.05 equiv.), H_2, MeOH, DMF, 3 h, rt, 78 %, (f) TPAP (0.05 equiv.), NMO (3.0 equiv.), DCM, rt, 90 %, (g) TBDPSCl (1.2 equiv.), imidazole (equiv 1.2), DMAP (equiv. 0.1), DMF, rt, 86 %, (h) TROC-Cl (equiv. 1.1), pyr. (5.0 equiv.), DCM, rt, 98 %, (i) PTSA (1.3 equiv), MeOH, 50 °C, 88 %, (j) (1.0 equiv.), $(COCl)_2$, DCM, -78 °C, then (5 equiv.), Hünig's Base, -78 °C to rt, 1 h, 72 %, (k) Ac_2O, pyr. (5.0 equiv.), rt, 84 %, (l) Tf_2O (3.0 equiv.), 2,6-lutidine (5.0 equiv.), -45 °C, 94 %, (m) *p*-$MeO(C_6H_4)B(OH)_2$, $Pd(P(Ph)_3)_4$, (0.01 equiv.), rt, K_2CO_3, H_2O:EtOH:tol (10:1:2), 93 %, (n) TBAF (1.2 equiv.), THF, rt, 91 %.



Scheme 2: (a_1) **20** (1.0,equiv.), K_2CO_3, DMF, **21 a** (0.5 equiv.), rt, 86 % of **22,** (a_2) **20** (1.0,equiv.), K_2CO_3, DMF, **21 b** (0.5 equiv.), rt, 74 % of **23,** (b_1) **22**, Pb-Cd 1:10 v/v (5 equiv.), **24**, THF:NH_4OAc (1 M, *aq*.) 1:1, rt, 68 %, (b_2) **23**, Pb-Cd 1:10 v/v (3 equiv.),THF:NH_4OAc (1 M, *aq*.) 1:1, rt, **25**, 48 %.



Scheme 3: (a) $NH_2(C_6H_4)$B-pinacol ester, $Pd(P(Ph)_3)_4$, (0.01 equiv.), K_2CO_3, H_2O:EtOH:tol (10:1:2), rt, 84 %, (b) TROC-Cl (equiv. 1.1), DCM, pyr., rt, 97 %, (c) TBAF, THF, 93 %, (d) **27** (1.3 equiv.), Cs_2CO_3, DMF, yields **29** in 80% (e) **29** and Pb-Cd (3 equiv.), THF:NH_4OAc (1 M, *aq.*) 1:1, yields **30** in 58 %.

compounds	786-0	Caki-1	HEL92.1.7	HL60cy	MCF-7	TF-1a
24	0.25	0.11	0.032	0.017	1	1
25	0.030	0.038	0.0074	0.0093	0.3	0.2
30	0.4	0.2	0.024	0.013	1	1

Table 1: Free drug *in vitro* activity against cancer cell lines. The cells were treated with the test articles for 48 h in duplicate. IC_{50} values were determined by Cell Titer Glo and are reported in nM concentrations.

1 Okeley, N. M.; Alley, S. C.; Senter, P. D. *Hematol. Oncol. Clin. North Am.* **2014**, *28*, 13.

2 Sievers, E. L.; Senter, P. D. *Annu. Rev. Med.* **2013**, *64*, 15.

3 Sliwkowski, M. X.; Mellman, I. *Science* **2013**, *341*, 1192.

4 Lambert, J. M.; Chari, R. V. *J. Med. Chem.* **2014**, *57*, 6949.

5 Drachman, J. G.; Senter, P. D. *Hematology Am Soc Hematol Educ Program* **2013**, *2013*, 306.

6 Sassoon, I.; Blanc, V. *Methods Mol. Cell. Biol.* **2013**, *1045*, 1.

7 Beck, A.; Reichert, J. M. *mAbs* **2014**, *6*, 15.

8 Chari, R. V.; Miller, M. L.; Widdison, W. C. *Angew. Chem.* **2014**, *53*, 3796.

9 Lyon, R. P.; Setter, J. R.; Bovee, T. D.; Doronina, S. O.; Hunter, J. H.; Anderson, M. E.; Balasubramanian, C. L.; Duniho, S. M.; Leiske, C. I.; Li, F.; Senter, P. D. *Nat. Biotechnol.* **2014**, *32*, 1059.

10 Zhao, R. Y.; Erickson, H. K.; Leece, B. A.; Reid, E. E.; Goldmacher, V. S.; Lambert, J. M.; Chari, R. V. *J. Med. Chem.* **2012**, *55*, 766.

11 Davis, M. T.; Preston, J. F., 3rd *Science* **1981**, *213*, 1385.

12 Moldenhauer, G.; Salnikov, A. V.; Luttgau, S.; Herr, I.; Anderl, J.; Faulstich, H. *J. Natl. Cancer Inst.* **2012**, *104*, 622.

13 Eustaquio, A. S.; Janso, J. E.; Ratnayake, A. S.; O'Donnell, C. J.; Koehn, F. E. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E3376.

14 Dirico, K. J.; Eustaquio, A. S.; Green, M. E.; He, H.; He, M.; Koehn, F. E.; O'donnell, C. J.; Puthenveetil, S.; Ratnayake, A. S.; Subramanyam, C.; WO 2014068443 A1, 2014.

15 Jeffrey, S. C.; Andreyka, J. B.; Bernhardt, S. X.; Kissler, K. M.; Kline, T.; Lenox, J. S.; Moser, R. F.; Nguyen, M. T.; Okeley, N. M.; Stone, I. J.; Zhang, X.; Senter, P. D. *Bioconjugate Chem.* **2006**, *17*, 831.

16 Beria, I.; Caruso, M.; Flygare, J. A.; Lupi, V.; Perego, R.; Polakis, P.; Polson, A.; Salsa, M.; Spencer, S. D.; Valsasina, B.; WO 2010009124 A3, 2010.

17 Jeffrey, S. C.; Burke, P. J.; Lyon, R. P.; Meyer, D. W.; Sussman, D.; Anderson, M.; Hunter, J. H.; Leiske, C. I.; Miyamoto, J. B.; Nicholas, N. D.; Okeley, N. M.; Sanderson, R. J.; Stone, I. J.; Zeng, W.; Gregson, S. J.; Masterson, L.; Tiberghien, A. C.; Howard, P. W.; Thurston, D. E.; Law, C. L.; Senter, P. D. *Bioconjugate Chem.* **2013**, *24*, 1256.

18 Kung Sutherland, M. S.; Walter, R. B.; Jeffrey, S. C.; Burke, P. J.; Yu, C.; Kostner, H.; Stone, I.; Ryan, M. C.; Sussman, D.; Lyon, R. P.; Zeng, W.; Harrington, K. H.; Klussman, K.; Westendorf, L.; Meyer, D.; Bernstein, I. D.; Senter, P. D.; Benjamin, D. R.; Drachman, J. G.; McEarchern, J. A. *Blood* **2013**, *122*, 1455.

19 Leimgruber, W.; Batcho, A. D.; Schenker, F. *J. Am. Chem. Soc.* **1965**, *87*, 5793.

20 Arima, K.; Kosaka, M.; Tamura, G.; Imanaka, H.; Sakai, H. *J. Antibiot.* **1972**, *25*, 437.

21 Brazhnikova, M. G.; Kovsharova, I. N.; Konstantinova, N. V.; Mezentsev, A. S.; Proshliakova, V. V. *Antibiotiki* **1970**, *15*, 297.

22 Leber, J. D.; Hoover, J. R. E.; Holden, K. G.; Johnson, R. K.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 2992.

23 Burger, A. M.; Loadman, P. M.; Thurston, D. E.; Schultz, R.; Fiebig, H. H.; Bibby, M. C. *J. Chemother.* **2007**, *19*, 66.

24 Antonow, D.; Kaliszczak, M.; Kang, G. D.; Coffils, M.; Tiberghien, A. C.; Cooper, N.; Barata, T.; Heidelberger, S.; James, C. H.; Zloh, M.; Jenkins, T. C.; Reszka, A. P.; Neidle, S.; Guichard, S. M.; Jodrell, D. I.; Hartley, J. A.; Howard, P. W.; Thurston, D. E. *J. Med. Chem.* **2010**, *53*, 2927.

25 Alley, M. C.; Hollingshead, M. G.; Pacula-Cox, C. M.; Waud, W. R.; Hartley, J. A.; Howard, P. W.; Gregson, S. J.; Thurston, D. E.; Sausville, E. A. *Cancer Res.* **2004**, *64*, 6700.

26 Hartley, J. A.; Spanswick, V. J.; Brooks, N.; Clingen, P. H.; McHugh, P. J.; Hochhauser, D.; Pedley, R. B.; Kelland, L. R.; Alley, M. C.; Schultz, R.; Hollingshead, M. G.; Schweikart, K. M.;

Tomaszewski, J. E.; Sausville, E. A.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Cancer Res.* **2004**, *64*, 6693.

27 Ahmed Kamal, M. K. R., Ajay Kumar Srivastava and Y. V. V. Srikanth *Pyrrolobenzodiazepines as Sequence Selective DNA Binding Agents*; Intech, 2012. pp Chp. 6.

28 Bose, D. S.; Thompson, A. S.; Ching, J.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939.

29 Wang, J. J.; Hill, G. C.; Hurley, L. H. *J. Med. Chem.* **1992**, *35*, 2995.

30 Jenkins, T. C.; Hurley, L. H.; Neidle, S.; Thurston, D. E. *J. Med. Chem.* **1994**, *37*, 4529.

31 Rahman, K. M.; Thompson, A. S.; James, C. H.; Narayanaswamy, M.; Thurston, D. E. *J. Am. Chem. Soc.* **2009**, *131*, 13756.

32 Howard, P. W.; Gregson, S. J.; CA 2558195 A1, 2005.

33 Thurston, D. E.; Bose, D. S. *Chem. Rev.* **1994**, *94*, 433.

34 J. Gregson, S.; W. Howard, P.; E. Thurston, D.; C. Jenkins, T.; R. Kelland, L. *Chem. Comm.* **1999**, 797.

35 Antonow, D.; Thurston, D. E. *Chem. Rev.* **2011**, *111*, 2815.

36 Chen, Z.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1547.

37 Kamal, A.; Ramesh, G.; Srinivas, O.; Ramulu, P.; Laxman, N.; Rehana, T.; Deepak, M.; Achary, M. S.; Nagarajaram, H. A. *Biorg. Med. Chem.* **2004**, *12*, 5427.

38 Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2007**, *72*, 4293.

39 Newman, D. J. *J. Med. Chem.* **2008**, *51*, 2589.

40 Gigante, B.; Prazeres, A. O.; Marcelo-Curto, M. J.; Cornelis, A.; Laszlo, P. *J. Org. Chem.* **1995**, *60*, 3445.

41 Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, *1994*, 639.

42 Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.

43 Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, *16*, 2647.

44 Miyaura, N.; Suzuki, A. *J. Chem. Soc., Chem. Commun.* **1979**, 866.

45 Howard, P. W.; Chen, Z.; Gregson, S. J.; Masterson, L. A.; Tiberghien, A. C.; Cooper, N.; Fang, M.; Coffils, M. J.; Klee, S.; Hartley, J. A.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6463.

46 Dong, Q.; Eric Anderson, C.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 5681.

47 The *para*-chloro group inductively deactivates the aryl ring allowing for regioselective nitration.

48 Gregson, S. J.; Howard, P. W.; Gullick, D. R.; Hamaguchi, A.; Corcoran, K. E.; Brooks, N. A.; Hartley, J. A.; Jenkins, T. C.; Patel, S.; Guille, M. J.; Thurston, D. E. *J. Med. Chem.* **2004**, *47*, 1161.