Published in final edited form as:

Tetrahedron Lett. 2015 June 3; 56(23): 3101–3104. doi:10.1016/j.tetlet.2014.11.038.

A five-membered lactone prodrug of CBI-based analogs of the duocarmycins

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Abstract

The preparation, characterization and examination of the CBI-based 5-membered lactone **5** capable of serving as a prodrug or protein (antibody) conjugation reagent are disclosed along with its incorporation into the corresponding CC-1065 and duocarmycin analog **6**, and the establishment of their properties.

Keywords

CC-1065; duocarmycin; CBI; prodrug

CC-1065 (**1**)¹ and duocarmycin SA (**2**)² were the first isolated and are the two most widely recognized members of a class of exceptionally potent naturally occurring antitumor compounds that also include duocarmycin A (**3**)³ and yatakemycin (**4**)⁴ (Figure 1). They derive their antitumor properties from their sequence selective DNA alkylation properties, undergoing a stereoelectronically-controlled adenine N3 alkylation within the minor groove at specific sites within 4–5 base pair A–T rich sites.^{5,6} Extensive studies conducted not only with the natural products, but also their synthetic unnatural enantiomers,⁷ and a systematic series of key analogs have defined the fundamental features that control their DNA alkylation selectivity, efficiency, and catalysis, providing in a detailed understanding of the relationships between structure, reactivity, and biological activity.^{6,7,8}

In early studies, synthetic phenol precursors, which have yet to undergo the Winstein Ar-3' spirocyclization, were found to be equipotent to and indistinguishable from their cyclized cyclopropane containing counterparts in cell growth inhibition assays, DNA alkylation studies, and in vivo antitumor models. Due to this indistinguishable behavior, protection of the phenol provides an ideal site on which to prepare inactive prodrugs that can be cleaved in vivo releasing the active drug.⁹ Several classes of prodrugs that use this protection and release strategy have been disclosed based on a series of unique activation strategies

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including hydrolytic, reductive, oxidative, or enzymatic reactions.^{10–15} In the course of our recent studies in this area and although not the objective of the work,¹⁵ we had the opportunity to prepare, characterize and examine a CBI-based^{16,17} 5-membered lactone capable of serving as such a prodrug or protein (antibody) conjugation reagent. Herein, we disclose the synthesis of **5**, its incorporation into the corresponding CC-1065 and duocarmycin analog **6**, and the establishment of their properties (Figure 2).

Coupling of carboxylic acid **7**¹⁸ with *N*-methyl *O*-THP hydroxylamine provided **8** in excellent yield (Scheme 1, 90%). Transannular spirocyclization upon Mitsunobu activation of the secondary alcohol **8** afforded **9** (75%). Acid-catalyzed addition of chloride to the activated cyclopropane, effecting ring contraction of the fused piperidine to pyrrolidine, was carried out under conditions (1 N HCl, THF, 25 °C, 2 h) that also promoted the subsequent lactone formation in a single step without *N*-Boc deprotection, providing **5**¹⁹ directly in excellent yield (90%).

Acid-catalyzed *N*-Boc deprotection of **5** (4 N HCl, EtOAc, 25 °C, 1 h) followed by coupling of the crude hydrochloride salt **10** with **11** (1.2 equiv, 3 equiv of EDCI, 0.1 M DMF, 25 °C, 2 h) provided **6**¹⁹ (80%) in good yield (Scheme 2).

The chemical reactivity of the 5-membered lactone was probed by examining its reaction in pure MeOH (25 °C) and in pH 7 phosphate buffer (25% DMSO–phosphate buffer, 25 °C) where it displayed an interesting, somewhat divergent, behavior (Figure 3). Within 1 h in MeOH, approximately 50% of **5** was converted to the corresponding methyl ester **12**²⁰ derived from lactone methanolysis and the conversion was complete within 24 h. Extended exposure to the conditions led to slower generation of the spirocyclized methyl ester **13**²⁰ (25% at 3 d). In contrast to intuitive expectations, this reactivity was much more attenuated in pH 7 phosphate buffer.

Aside from characterizing the intrinsic reactivity of the 5-membered lactone toward mild nucleophiles, the studies suggest a novel protein conjugation strategy involving reaction of the lactone with an amine that can be conducted in water (buffer) where the lactone may selectively react with protein-linker amines in preference with water and that the initial *seco*-CBI-based product may not undergo rapid spirocyclization. Deliberate hydrolysis of **5** and **6** was accomplished in DMSO–H₂O (2:1, 0.006 and 0.002 M respectively, 80 °C, 24 h) to provide the corresponding *seco*-CBI derivatives **14** (51%) and **16** (65%), bearing the free carboxylic acid.

The cytotoxic activity of **5** and **6** were established in a cell growth inhibition assay enlisting a mouse leukemia cell line (L1210) that has been used historically to initially assess members of this class of candidate drugs. As anticipated and analogous to the behavior of the free drugs themselves, **5** was approximately 1000-fold less active than **6**. Consistent with the relatively slow release of the free drug and like the behavior of other hydrolytically released and labile prodrugs, both **5** and **6** were found to be potent inhibitors of cell growth, being only 5–10-fold less potent than the corresponding parent CBI-based free drug analogs (Figure 4). Interestingly, the corresponding carboxylic acids **14** and **16**, derived from water hydrolysis of the lactone with release of the free drug, were much less active in the cell-

based cell growth assay, suggesting their limited ability to penetrate either the cell or nucleus membrane.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA042056, DLB).

References and notes

1. Martin DG, Biles C, Gerpheide SA, Hanka LJ, Krueger WC, McGovren JP, Mizesak SA, Neil GL, Stewart JC, Visser J. J. Antibiot. 1981; 34:1119. [PubMed: 7328053]
2. Ichimura M, Ogawa T, Takahashi K, Kobayashi E, Kawamoto I, Yasuzawa T, Takahashi I, Nakano H. J. Antibiot. 1990; 43:1037. [PubMed: 2211354]
3. Takahashi I, Takahashi K, Ichimura M, Morimoto M, Asano K, Kawamoto I, Tomita F, Nakano H. J. Antibiot. 1988; 41:1915. [PubMed: 3209484]
4. Igarashi Y, Futamata K, Fujita T, Sekine A, Senda H, Naoki H, Furumai T. J. Antibiot. 2003; 56:107. [PubMed: 12715869]
5. For duocarmycin SA, see: Boger DL, Johnson DS, Yun W. J. Am. Chem. Soc. 1994; 116:1635. Wolfe AL, Duncan KK, Lajiness JP, Zhu K, Duerfeldt AS, Boger DL. J. Med. Chem. 2013; 56:6845. [PubMed: 23944748] . For yatakemycin, see: Parrish JP, Kastrinsky DB, Wolkenberg SE, Igarashi Y, Boger DL. J. Am. Chem. Soc. 2003; 125:10971. [PubMed: 12952479] Trzupek JD, Gottesfeld JM, Boger DL. Nature Chem. Biol. 2006; 2:79. [PubMed: 16415862] Tichenor MS, MacMillan KS, Trzupek JD, Rayl TJ, Hwang I, Boger DL. J. Am. Chem. Soc. 2007; 129:10858. [PubMed: 17691783] . For CC-1065, see: Hurley LH, Lee CS, McGovren JP, Warpehoski MA, Mitchell MA, Kelly RC, Aristoff PA. Biochemistry. 1988; 27:3886. [PubMed: 3408734] Boger DL, Johnson DS, Yun W, Tarby CM. Bioorg. Med. Chem. 1994; 2:115. [PubMed: 7922122] Boger DL, Munk SA, Zarrinmayeh H. J. Am. Chem. Soc. 1991; 113:3980. Boger DL, Zarrinmayeh H, Munk SA, Kito PA, Suntornwat O. Proc. Nat. Acad. Sci. U.S.A. 1991; 88:1431. Boger DL, Coleman RS, Invergo BJ, Sakya SM, Ishizaki T, Munk SA, Zarrinmayeh H, Kito PA, Thompson SC. J. Am. Chem. Soc. 1990; 112:4623. Boger DL, Munk SA, Zarrinmayeh H. J. Am. Chem. Soc. 1991; 113:3980. For duocarmycin A, see: Boger DL, Ishizaki T, Zarrinmayeh H, Munk SA, Kito PA, Suntornwat O. J. Am. Chem. Soc. 1990; 112:8961. Boger DL, Ishizaki T, Zarrinmayeh H. J. Am. Chem. Soc. 1991; 113:6645. Boger DL, Yun W, Terashima S, Fukuda Y, Nakatani K, Kito PA, Jin Q. Bioorg. Med. Chem. Lett. 1992; 2:759. Boger DL, Yun W. J. Am. Chem. Soc. 1993; 115:9872. Boger DL, Invergo BJ, Coleman RS, Zarrinmayeh H, Kito PA, Thompson SC, Leong T, McLaughlin LW. Chem.-Biol. Interactions. 1990; 73:29..
6. Reviews: Boger DL, Johnson DS. Angew. Chem. Int. Ed. Engl. 1996; 35:1438. Boger DL. Acc. Chem. Res. 1995; 28:20. Boger DL, Johnson DS. Proc. Natl. Acad. Sci. U.S.A. 1995; 92:3642. [PubMed: 7731958] Boger DL, Garbaccio RM. Acc. Chem. Res. 1999; 32:1043. Tichenor MS, Boger DL. Natural Prod. Rep. 2008; 25:220. MacMillan KS, Boger DL. J. Med. Chem. 2009; 52:5771. [PubMed: 19639994] Searcey M. Curr. Pharm. Des. 2002; 8:1375. [PubMed: 12052214] Tse WC, Boger DL. Chem. Biol. 2004; 11:1607. [PubMed: 15610844] .
7. (a) Boger DL, Coleman RS. J. Am. Chem. Soc. 1988; 110:1321. (b) Boger DL, Coleman RS. J. Am. Chem. Soc. 1988; 110:4796. (c) Boger DL, Machiya K. J. Am. Chem. Soc. 1992; 114:10056. (d) Boger DL, Machiya K, Hertzog DL, Kito PA, Holmes D. J. Am. Chem. Soc. 1993; 115:9025. (e) Boger DL, McKie JA, Nishi T, Ogiku T. J. Am. Chem. Soc. 1996; 118:2301. (f) Boger DL, McKie JA, Nishi T, Ogiku T. J. Am. Chem. Soc. 1997; 119:311. (g) Boger DL, Boyce CW, Garbaccio RM, Goldberg JA. Chem. Rev. 1997; 97:787. [PubMed: 11848889] (h) Tichenor MS, Kastrinsky DB, Boger DL. J. Am. Chem. Soc. 2004; 126:8396. [PubMed: 15237994] (i) Tichenor MS, Trzupek JD, Kastrinsky DB, Shiga F, Hwang I, Boger DL. J. Am. Chem. Soc. 2006; 128:15683. [PubMed:

- 17147378] (j) MacMillan KS, Nguyen T, Hwang I, Boger DL. *J. Am. Chem. Soc.* 2009; 131:1187. [PubMed: 19154178]
8. (a) Boger DL, Hertzog DL, Bollinger B, Johnson DS, Cai H, Goldberg J, Turnbull P. *J. Am. Chem. Soc.* 1997; 119:4977. (b) Boger DL, Bollinger B, Hertzog DL, Johnson DS, Cai H, Mesini P, Garbaccio RM, Jin Q, Kitos PA. *J. Am. Chem. Soc.* 1997; 119:4987. (c) Boger DL, Garbaccio RM. *Bioorg. Med. Chem.* 1997; 5:263. [PubMed: 9061191]
9. Wolkenberg SE, Boger DL. *Chem. Rev.* 2002; 102:2477. [PubMed: 12105933]
10. For Carzelesin, see: Li L, DeKoning TF, Kelly RC, Krueger WC, McGovren JP, Padbury GE, Petzold GL, Wallace TL, Ouding RJ, Prairie MD, Gebhard I. *Cancer Res.* 1992; 52:4904. [PubMed: 1516047] van Tellingen O, Nooijen WJ, Schaaf LJ, van der Valk M, van Asperen J, Henrar REC, Beijnen JH. *Cancer Res.* 1998; 58:2410. [PubMed: 9622082] . For KW-2189, see: Kobayashi E, Okamoto A, Asada M, Okabe M, Nagamura S, Asai A, Saito H, Gomi K, Hirata T. *Cancer Res.* 1994; 54:2404. [PubMed: 8162588] Nagamura S, Kanda Y, Kobayashi E, Gomi K, Saito H. *Chem. Pharm. Bull.* 1995; 43:1530. [PubMed: 7586077] .
11. CBI-based carbamate prodrugs: Boger DL, Boyce CW, Garbaccio RM, Searcey M, Jin Q. *Synthesis.* 1999:1505. Wang Y, Li L, Tian Z, Jiang W, Larrick J. *Bioorg. Med. Chem.* 2006; 14:7854. [PubMed: 16908174] Li LS, Sinha SC. *Tetrahedron Lett.* 2009; 50:2932. Wolfe AL, Duncan KK, Parelkar NK, Weir SJ, Vielhauer GA, Boger DL. *J. Med. Chem.* 2012; 55:5878. [PubMed: 22650244] .
12. For reductively activated prodrugs: Hay MP, Anderson RF, Ferry DM, Wilson WR, Denny WA. *J. Med. Chem.* 2003; 46:5533. [PubMed: 14640560] Hay MP, Sykes BM, Denny WA, Wilson WR. *Bioorg. Med. Chem. Lett.* 1999; 9:2237. [PubMed: 10465553] Tercel M, Atwell GJ, Yang S, Ashoorzadeh A, Stevenson RJ, Botting KJ, Gu Y, Mehta SY, Denny WA, Wilson WR, Pruijn FB. *Angew. Chem. Int. Ed.* 2011; 50:2606. Boger DL, Garbaccio RM. *J. Org. Chem.* 1999; 64:8350. [PubMed: 11674758] Townes H, Summerville K, Purnell B, Hooker M, Madsen E, Hudson S, Lee M. *Med. Chem. Res.* 2002; 11:248..
13. Glycosidic prodrugs: Tietze LF, Lieb M, Herzig T, Haunert F, Schuberth I. *Bioorg. Med. Chem.* 2001; 9:1929. [PubMed: 11425596] Tietze LF, Major F, Schuberth I. *Angew. Chem. Int. Ed.* 2006; 45:6574. Tietze LF, Schuster HJ, Schmuck K, Schuberth I, Alves F. *Bioorg. Med. Chem.* 2008; 16:6312. [PubMed: 18524605] .
14. For additional representative prodrugs: Zhao RH, Erickson HK, Leece BA, Reid EE, Goldmacher VS, Lambert JM, Chari RVJ. *J. Med. Chem.* 2012; 55:766. [PubMed: 22148292] Pors K, Loadman PM, Shnyder SD, Sutherland M, Sheldrake HM, Guino M, Kiakos K, Hartley JA, Searcey M, Patterson LH. *Chem. Commun.* 2011; 47:12062..
15. (a) Jin W, Trzupek JD, Rayl TJ, Broward MA, Vielhauer GA, Weir SJ, Hwang I, Boger DL. *J. Am. Chem. Soc.* 2007; 129:15391. [PubMed: 18020335] (b) Lajiness JP, Robertson WM, Dunwiddie I, Broward MA, Vielhauer GA, Weir SJ, Boger DL. *J. Med. Chem.* 2010; 53:7731. [PubMed: 20942408] (c) Wolfe AL, Duncan KK, Parelkar NK, Brown D, Vielhauer GA, Boger DL. *J. Med. Chem.* 2013; 56:4104. [PubMed: 23627265]
16. (a) Boger DL, Ishizaki T, Wysocki RJ Jr, Munk SA, Kitos PA, Suntornwat O. *J. Am. Chem. Soc.* 1989; 111:6461. (b) Boger DL, Ishizaki T, Kitos PA, Suntornwat O. *J. Org. Chem.* 1990; 55:5823. (c) Boger DL, Ishizaki T. *Tetrahedron Lett.* 1990; 31:793. (d) Boger DL, Wysocki RJ, Ishizaki T. *J. Am. Chem. Soc.* 1990; 112:5230. (e) Boger DL, Ishizaki T, Zarrinmayeh H, Kitos PA, Suntornwat O. *Bioorg. Med. Chem. Lett.* 1991; 1:55. (f) Boger DL, Munk SA. *J. Am. Chem. Soc.* 1992; 114:5487. (g) Boger DL, Yun W. *J. Am. Chem. Soc.* 1994; 116:5523. (h) Boger DL, Yun W, Cai H, Han N. *Bioorg. Med. Chem.* 1995; 3:761. [PubMed: 7582954] (i) Boger DL, Yun W. *J. Am. Chem. Soc.* 1994; 116:7996. (j) Parrish JP, Katrinsky DB, Stauffer F, Hedrick MP, Hwang I, Boger DL. *Bioorg. Med. Chem.* 2003; 11:3815. [PubMed: 12901927] (k) Parrish JP, Hughes TV, Hwang I, Boger DL. *J. Am. Chem. Soc.* 2004; 126:80. [PubMed: 14709069]
17. (a) Boger DL, Yun W, Teegarden BR. *J. Org. Chem.* 1992; 57:2873. (b) Boger DL, McKie JA. *J. Org. Chem.* 1995; 60:1271. (c) Boger DL, McKie JA, Boyce CW. *Synlett.* 1997:515. (d) Boger DL, Boyce CW, Garbaccio RM, Searcey M. *Tetrahedron Lett.* 1998; 39:2227. (e) Katrinsky DB, Boger DL. *J. Org. Chem.* 2004; 69:2284. [PubMed: 15049620] (f) Lajiness JP, Boger DL. *J. Org. Chem.* 2011; 76:583. [PubMed: 21192653] (g) Drost KJ, Cava MP. *J. Org. Chem.* 1991; 56:2240. (h) Aristoff PA, Johnson PD. *J. Org. Chem.* 1992; 57:6234. (i) Mohamadi F, Speez MM, Staten GS, Marder P, Kipka JK, Johnson DA, Boger DL, Zarrinmayeh H. *J. Med. Chem.* 1994; 37:232.

- [PubMed: 8295210] (j) Ling L, Xie Y, Lown JW. *Heterocycl. Commun.* 1997; 3:405.(k) Tietze LF, von Hof JM, Krewer B, Muller M, Major F, Schuster HJ, Schuberth I, Alvers F. *ChemMedChem.* 2008; 3:1946. [PubMed: 19021160]
18. Uematsu M, Boger DL. *J. Org. Chem.* 2014; 79:9699. [PubMed: 25247380]
19. For 5: yellow solid: $[\alpha]_{\text{D}}^{28}$ -41 (*c* 0.1, acetone); ^1H NMR (acetone- d_6 , 600 MHz) δ 8.34 (d, J = 8.4 Hz, 1H), 8.19–8.05 (br, 1H), 8.03 (d, J = 6.6 Hz, 1H), 7.91 (dd, J = 8.4, 6.6 Hz, 1H), 4.34–4.27 (m, 2H), 4.23 (dd, J = 11.4, 3.6 Hz, 1H), 3.95 (dd, J = 11.4, 7.2 Hz, 1H), 1.60 (s, 9H); ^{13}C NMR (acetone- d_6 , 150 MHz) δ 167.6, 152.9, 151.7, 145.9, 131.7, 129.3, 127.6, 126.7, 124.6, 122.6, 118.3, 99.0, 82.1, 54.1, 48.4, 41.4, 28.6; IR (film) ν_{max} 1784, 1704, 1401, 1327, 1137, 670 cm^{-1} ; ESI-TOF HRMS m/z 360.0997 ($\text{M}+\text{H}^+$, $\text{C}_{19}\text{H}_{18}\text{ClNO}_4$ requires 360.0997). For 6: yellow solid; $[\alpha]_{\text{D}}^{25}$ -327 (*c* 0.1, acetone); ^1H NMR (THF- d_8 , 600 MHz) δ 11.13 (s, 1H), 11.10 (s, 1H), 9.37 (s, 1H), 8.42 (s, 1H), 8.39 (d, J = 1.8 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 7.2 Hz, 1H), 7.82 (dd, J = 8.4, 7.2 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.47 (dd, J = 9.0, 1.8 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.20–7.17 (m, 2H), 7.03 (t, J = 7.2 Hz, 1H), 4.90 (dd, J = 10.8, 9.0 Hz, 1H), 4.79 (dd, J = 10.8, 3.0 Hz, 1H), 4.34 (ddd, J = 12.0, 8.4, 3.6 Hz, 1H), 4.09 (dd, J = 10.8, 3.6 Hz, 1H), 3.87 (dd, J = 10.8, 8.4 Hz, 1H); ^{13}C NMR (THF- d_8 , 150 MHz) 167.2, 161.7, 160.6, 151.6, 146.8, 138.3, 134.9, 133.5, 132.2, 131.4, 129.2, 129.10, 129.07, 127.8, 127.3, 124.9, 124.5, 123.1, 122.5, 120.8, 120.2, 119.9, 113.8, 112.9, 112.8, 107.3, 103.1, 101.2, 56.5, 47.8, 43.3; IR (film) ν_{max} 2923, 2854, 1738, 1367, 1230, 669 cm^{-1} ; ESI-TOF HRMS m/z 561.1330 ($\text{M}+\text{H}^+$, $\text{C}_{32}\text{H}_{21}\text{ClN}_4\text{O}_4$ requires 561.1324).
20. Boger DL, Hughes TV, Hedrick MP. *J. Org. Chem.* 2001; 66:2207. [PubMed: 11281757]

Figure 1.
Natural products.

Figure 2.
Lactone CBI prodrug or conjugation reagent.

Figure 3.
Chemical reactivity of the 5-membered lactone.

Compound	IC ₅₀ , L1210
5	370 nM
6	270 pM

(+)-N-Boc-CBI	80 nM
(+)-CBI-indole ₂	30 pM

14	2400 nM
16	6800 pM

Figure 4.
Cell growth inhibition (cytotoxic) activity.

Scheme 1.

Scheme 2.