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Design and synthesis of a folate-receptor targeted diazepine-ring-opened pyrrolobenzodiazepine prodrug conjugate

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Abstract: Pyrrolobenzodiazepines (PBDs) and their dimers (bis-PBDs) have emerged as some of the most potent chemotherapeutic compounds and are currently under development as novel payloads in antibody-drug conjugates (ADCs). However, when used as stand-alone therapeutics or as warheads for small molecule drug conjugates (SMDCs), dose-limiting toxicities are often observed. As an elegant solution to this inherent problem, we designed and synthesized a diazepine-ring-opened bis-PBD prodrug (pro-PBD-PBD) folate conjugate lacking the one of the two imine moieties found in the corresponding free bis-PBD. Upon entering a targeted cell, cleavage of the linker system, including the hydrolysis of an oxazolidine moiety, results in the formation of a reactive intermediate which possesses a newly formed aldehyde as well as an aromatic amine. A fast and spontaneous intramolecular ring-closing reaction subsequently takes place as the aromatic amine adds to the aldehyde with the loss of water to give the imine, and as a result, the diazepine ring, thereby delivering the bis-PBD to the targeted cell. The *in vitro* and *in vivo* activity of this conjugate has been evaluated on folate receptor positive KB cells. Sub-nanomolar activity with good specificity and high cure rates with minimal toxicity have been observed.

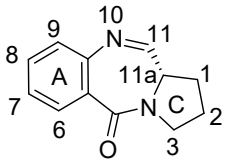
Keywords: pyrrolobenzodiazepine, prodrug, folate-receptor, conjugate, targeted therapy

Pyrrolo[2,1-c][1,4]benzodiazepine (PBD) antibiotics are a class of natural products produced by various *actinomycetes* bacteria and are known for their antibiotic and antitumor properties.[1] PBDs and their synthetic *C*-8/*C*-8'-tethered dimers (Figure 1) are sequence selective DNA-crosslinking alkylating agents. All PBD-based derivatives bind in a specific manner to DNA after recognition of a three-base-pair sequence in the minor grove. Insertion of the PBD dimer into the minor groove results in two interstrand cross-linking covalent aminal bonds,[2] formed as the result of the nucleophilic attacks of both exocyclic *N*-2/*N*-2' amines presented by two guanine (G) bases, integrated in the opposing DNA-strands, on the electrophilic *C*-11/C-11' imines of the PBD dimer (Figure 1B). Two additional hydrogen bonds are formed between the ring nitrogen *N*-3 of the 3'-adenines adjacent to the aminal-modified guanine and both *N*-10, *N'*-10 protons of the PBD units. The perfect fit of a PBD-dimer in the DNA minor grove results in negligible distortion of the DNA helix, thus potentially avoiding DNA-repair mechanisms and the common phenomenon of drug resistance.[3]

Since the discovery of natural PBDs, various synthetic PBD-monomers, PBD-hybrids, and PBD-dimers have been designed, prepared, and explored, improving our understanding of the mode of action of this important class of compounds.[4] A synthetic PBD-dimer, SJG-136 (Figure 1C), has undergone clinical evaluation as a monotherapy.[4b] In recent years, synthetic PBD-dimers have emerged as a new class of warheads in the field of antibody-drug conjugates (ADCs) and small molecule drug conjugates (SMDCs).[1e, 4d, 5]

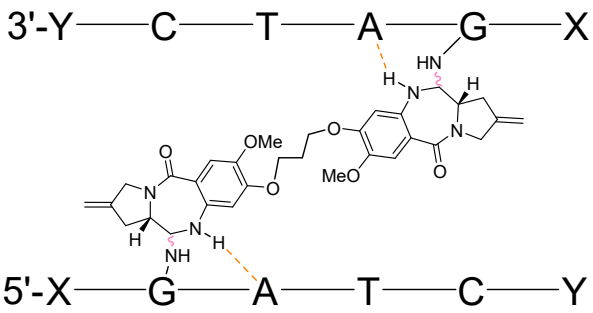
The folate receptor (FR) is a glycosylphosphatidylinositol anchored cell-surface glycoprotein which binds with high affinity to the vitamin folic acid (FA).[6] Upon binding, FA is transported into the cell *via* FR mediated endocytosis. Several rapidly growing cancers as well as activated macrophages associated with inflammation have been shown to overexpress the FR.[7] Targeting these nefarious cells can be achieved by covalently attaching FA to appropriate biologically active agents.[7b, 8] Several years ago, we synthesized a number of SMDCs linking FA through self-immolative linker systems to PBD dimers - conjugated primarily off one of the *C*-rings of the dimer (unpublished results). As constructed, these conjugates left PBD dimers with both of their reactive imines exposed at the end of peptidic chains. Unfortunately, although highly potent, these conjugates also displayed high (in some cases delayed) toxicities. Modification of the linker or spacer systems in this series of conjugates did little to improve the very tight therapeutic windows.

A.



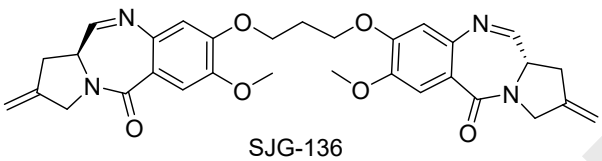
PBD imine form

B.



PBD dimer forming non-distorting interstrand cross-link in the DNA minor groove.

C.



SJG-136
(tethered PBD dimer)

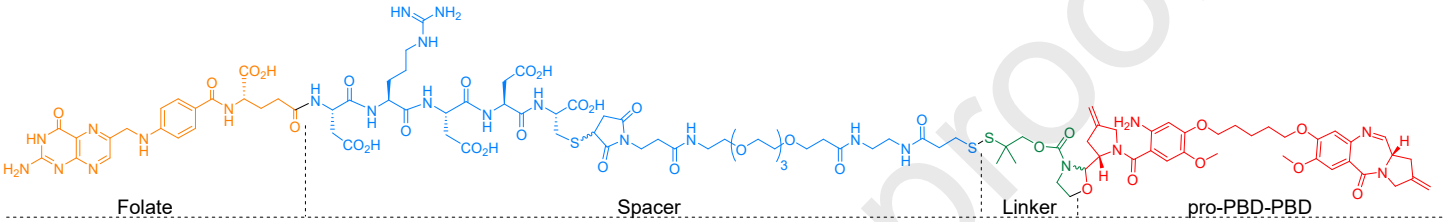
Figure 1. A) General structure of PBD; B) Representation of the insertion of a PBD dimer into the minor groove of DNA; C) Structure of PBD-dimer SJG-136.

We turned our attention to modifying the PBD dimer within the FA conjugate construct with the hope of reducing the observed off-target toxicities. Specifically, we believed that by protecting or masking the highly reactive *N*-10/*C*-11 imine of PBD, we could deliver this class of compounds to their targets much more efficaciously. This idea had been explored earlier. Thurston and Howard synthesized PBD prodrugs (pro-PBDs) and conjugates with cleavable linkers/side-chains at the *N*-10 position of PBD.[1e, 9] By doing so, they effectively blocked the imine moiety to prevent DNA alkylation or crosslinking. "Protection" in this manner significantly reduced the toxicities observed in a number of PBD prodrugs. Our design would find particular inspiration in the work of Jeffrey *et al.* who have shown that the heterocyclic iminium moiety in several anthracycline conjugates can be masked by replacing this moiety with its synthetic precursors: namely, an 1,3-oxazolidine carbamate and an amine.[10] The oxazolidine carbamate provided an attachment point for the anthracycline pro-drug to an antibody through one of several enzymatically labile linkers. A similar strategy could be applied to PDBs (Scheme 1). Upon entering a targeted cell, endosomal reductive cleavage of a disulfide bond and fragmentation of the linker system on a pro-PBD folate conjugate triggers the generation of a 1,3-oxazolidine. The latter, being hydrolytically labile, upon spontaneous reaction with water, forms a reactive aldehyde-amine intermediate. Subsequently, the aromatic amine in close proximity to the newly generated aldehyde, allows facile formation of the seven-membered carbinolamine ring. Spontaneous condensation results in the expected imine, thus generating the diazepine ring and providing *in situ* the PBD molecule. By applying this strategy, we were able to synthesize folate conjugates of a pro-PDB, a pro-PBD-pro-

PBD (both imine moieties in bis-PBD masked as 1,3-oxazolidine carbamates), a pro-PBD-diazepine-lactam hybrid, and a pro-PBD/Hoechst dye 33258 hybrid.[11]

Scheme 1. The concept of pro-PBDs as latent warheads for targeting cancer cells.

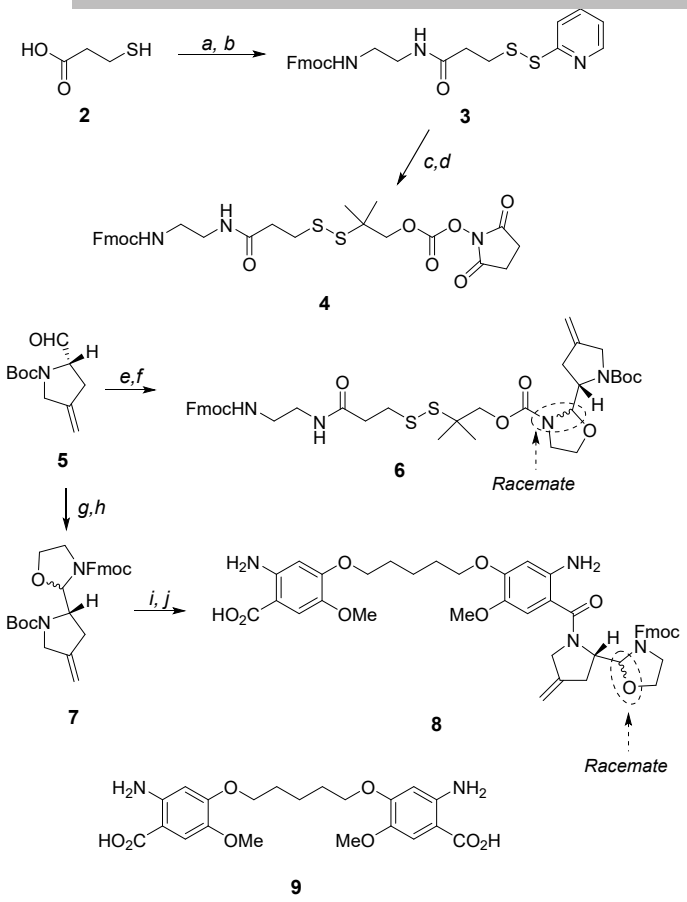
Herein, we report the structure, synthesis, and preliminary biological activity of folate-targeted, reductively-labile, pro-PBD-PBD conjugate **1** (Figure 2). Conjugate **1** delivers a highly active bis-PBD without the added complexity and instability of our earlier pro-PBD-pro-PBD conjugate which required two linker cleavages and subsequent releases to liberate bis-PBD.[11] The linker/spacer in **1** was optimized. Of note is the addition of a geminal-dimethyl functionality alpha to the disulfide bond on the linker. This structural feature is designed to stabilize the disulfide bond during circulation by providing steric hindrance. Once reduced in the endosome, however, the geminal-dimethyl increases the rate of linker immolation, as described by the Thorpe-Ingold effect.[12]



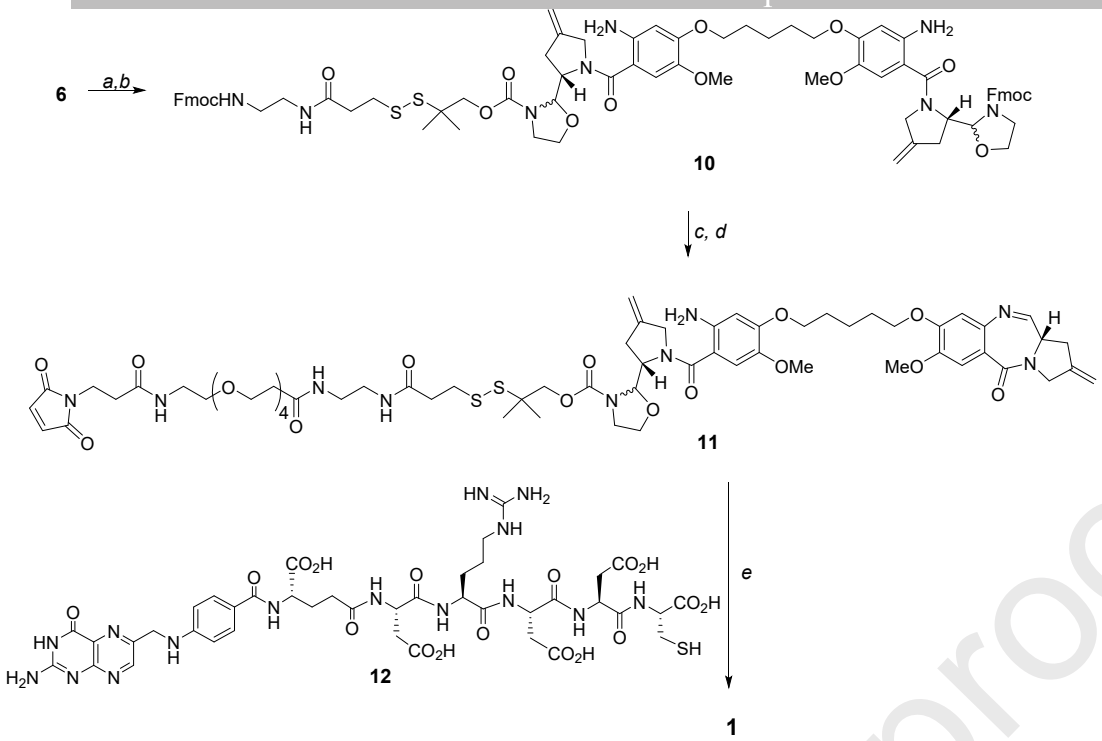
1

Figure 2. Folate-targeted, reductively-labile pro-PBD-PBD conjugate **1**.

Synthesis of **1** began by forming the linker and adjoining PBD *C*-ring (Scheme 2). 3-thiopropionic acid was treated with 2,2'-dithiopyridine to yield the resulting activated disulfanylcarboxylic acid. The acid was then treated with *N*-fluorenylmethyloxycarbonylethylenediamine (Fmoc-EDA), benzytriazol-1-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), and diisopropylethylamine (DIPEA) to give adduct **3**. Treatment of **3** with 2-mercapto-2-methyl-propanol followed by *N*,*N*'-disuccinimidyl carbonate (DSC) and pyridine resulted in activated carbonate **4**. (*S*)-*N*-tert-butoxy(Boc)-4-methylene-prolinal (**5**)[11] was treated with ethanolamine in the presence of 3Å molecular sieves resulting in the formation of the 1,3-oxazolidine which was captured/trapped upon addition of **4** with triethylamine (TEA) to give **6** as a mixture of enantiomers.[10a, 13] The other *C*-ring in the pro-PBD-PBD was similarly constructed (Scheme 2). Prolinal **5** was again treated with ethanolamine and magnesium sulfate, resulting in the formation of the 1,3-oxazolidine which was reacted further with Fmoc-Cl, resulting in adduct **7**.[10a, 13] Treatment of **7** with trifluoroacetic acid (TFA) followed by PyBOP promoted amide bond formation to the tethered bis-*A*-ring core (**9**),[11] resulted in the formation of **8**. After removal of the Boc protecting group on **6** with TFA, **8** was coupled with the free amine of **6** to yield adduct **10** as a diastereomeric mixture (Scheme 3). Treatment of **10** with diethylamine (DEA) resulted in the cleavage of both Fmoc protecting groups. The loss of the Fmoc moiety on the oxazolidine ring destabilizes the hemiaminal, ultimately resulting in the formation of the diazapene ring. Further treatment of the remaining free amine with maleimido-Peg_4-*N*-hydroxysuccimic acid ester (Mal-Peg_4-NHS) in the presence of TEA yields the maleimido adduct **11**. Michael addition of folate spacer **12**[14] to compound **11** in a solution of *N*,*N*-dimethylformamide (DMF) and ammonium bicarbonate (pH = 7) buffer (aq.) yields conjugate **1** as a mixture of diastereomers.



Scheme 2. Synthesis of Intermediate **8**. a) 2,2'-dipyridyldithiol (2 eq.), MeOH, 15 min., 68% yield; b) Fmoc-EDA (1 eq.), PyBOP (1 eq.), DIPEA (3 eq.), DMF, 5 min., 84% yield; c) 2-mercapto-2-methyl-propanol (1.3 eq.), $CHCl_3$, MeOH, 4 h., 60 °C, 90% yield; d) DSC (1.2 eq.), pyridine (1.25 eq.), ACN, 15 h., 95% yield; e) ethanolamine (0.9 eq.), 3Å molecular sieves, CH_2Cl_2, 4 h.; f) **4** (0.75 eq.), TEA (1.1 eq.), CH_2Cl_2, 2 h., 70% yield over two steps; g) ethanolamine (1.2 eq.), $MgSO_4$ (4.6 eq.), CH_2Cl_2, 1 h.; h) Fmoc-Cl (2 eq.), TEA (3 eq.), CH_2Cl_2, 16 h., 70% yield over two steps; i) TFA, CH_2Cl_2 (1:1), 30 min.; j) **9** (1.8 eq.), PyBOP (1.06 eq.), DIPEA (8 eq.), DMF/CH_2Cl_2 (1:1), 1 h., 60% yield over two steps.



Scheme 3. Synthesis of conjugate **1**. a) 30% TFA in CH_2Cl_2, 0 °C to rt, 1 h.; b) **8** (1 eq.), PyBOP (2 eq.), DIPEA (3 eq.), DMF, 3h., 85% yield over two steps; c) DEA, CH_2Cl_2 (1 : 2.5), 3h.; d) Mal-Peg_4-NHS (1.3 eq.), TEA (1.3 eq.), CH_2Cl_2, 1 h.; e) **12** (1.3 eq.), 50 mM NH_4HCO_3 (pH = 7) buffer (aq.), DMF, 2 h., 24% yield over 3 steps.

A model chemical release study was performed to verify compound **1** releases PBD dimer under reductive conditions found in internalized endosomes. **1** was treated with tris(2-carboxyethyl)phosphine (TCEP) in a pH = 6 phosphate buffer at 37 °C and the reaction was monitored by UPLC-MS (Scheme 4). Within five minutes of treatment with TCEP, **1** was completely reduced to the thiol terminating folate spacer **13** and pro-PBD-PBD linker remnant **14** as a mixture of diastereomers/rotamers. Further self-immolative release of the linker/1,3-oxazolidine carbonate from **14** over the course of 25 minutes resulted in the complete conversion to PBD dimer **15**.

Scheme 4. A) Chemical release of PBD dimer **15** from conjugate **1** upon treatment with TCEP; B) Chemical release as shown by UPLC traces (254 nm) at t = 0, 5, 15, and 30 min. (from bottom to top) post TCEP treatment. At t = 0, **1** is shown as a mixture of diastereomers. At t = 5 min., TCEP has completely reduced **1**. Thiol terminating folate spacer **13** and pro-PBD-PBD linker remnant **14** as a mixture of diastereomers/rotamers are readily apparent. A small amount of PBD dimer **15** is also visible at 2.37 min retention time. By t = 30 min., linker immolation is complete. **14** is no longer visible. Spacer **13** and free PBD dimer **15** are the two main remaining components of the reaction mixture.

Conjugate **1** has performed well in pre-clinical biological testing when compared against our previously reported folate-PBD conjugates (Figure 3).[11] For example, **1, 16, 17,** and **18** were evaluated *in vitro* on folate receptor positive KB (FR(+)-KB) cells with and without added folate competitor (Table 1 and Supplementary Data Figure 4). A sub-100 pM IC_{50} was obtained for **1** as a stand-alone agent, representing a 12-20 times improvement in activity compared to conjugates **16-18**. In the presence of 100-fold excess folic acid, the IC_{50}s for all conjugates increased roughly two orders of magnitude, thereby demonstrating the folate receptor targeted activity of these agents. The reduction in the IC_{50} observed for **1** under competitive FA binding conditions was inferior to that observed with compounds **16-18**. The activities of **1, 17,** and

18 against FR(+)-KB xenografts in female nude mice were also evaluated (Figure 4). Curative responses in 100% of the cohort dosed with 300 nmol/kg of **1** on a single dose a week schedule was observed. Very little weight loss (up to 96 days post tumor implantation) or major organ tissue degeneration was observed. Conjugates **17** and **18** were dosed at 2 μmol/kg on a single dose a week schedule. Even with the increased dose, **17** and **18** only slowed the tumor growth. Some weight loss was observed with **18**.

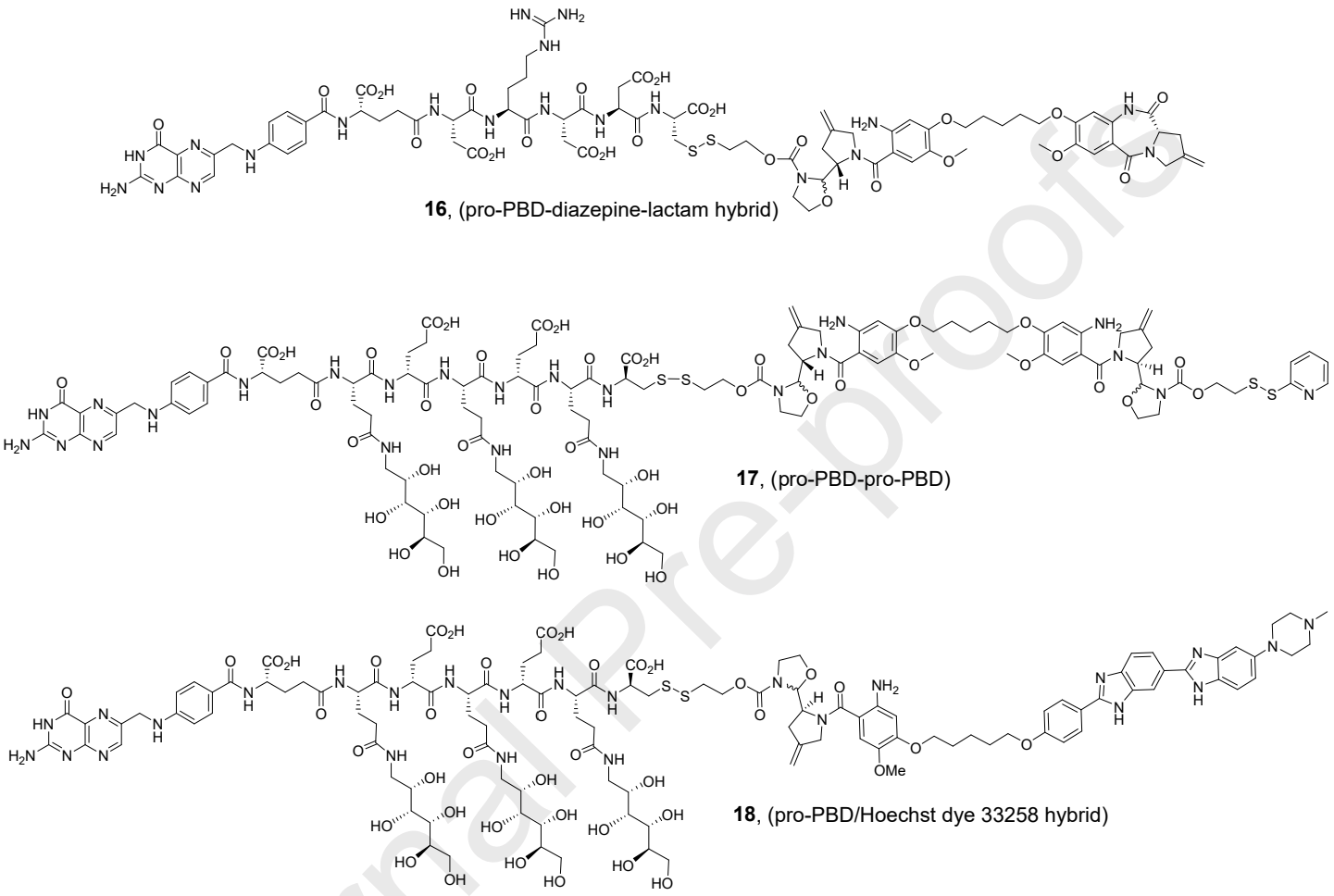


Figure 3. Structures of previously reported folate pro-PBD conjugates.

Table 1. Activity of pro-PBD-based folate conjugates on FR(+)-KB cells, 2 h pulse at 37 °C.

Compound	IC_{50} (nM)	IC_{50} with 100 μM FA (nM)
1	0.0522	3.78
16	1.12	696
17	1.31	184
18	0.658	84.3

With the synthesis of **1**, we have demonstrated another application of the 1,3-oxazolidine carbonate-iminium masking strategy toward the synthesis of a targeted prodrug form of a bis-PBD. The pre-clinical pharmacological studies which have been presented here, herald the potential of our approach. Ultimately, the totality of the pre-clinical data led us to select **1** for clinical development as the first-in-class pro-PBD-based SMDC. Additional information regarding the optimization of the linker and spacer as well as additional details relating to the activity of **1** in multiple cell lines and on varying dosing schedules will be reported in due course.

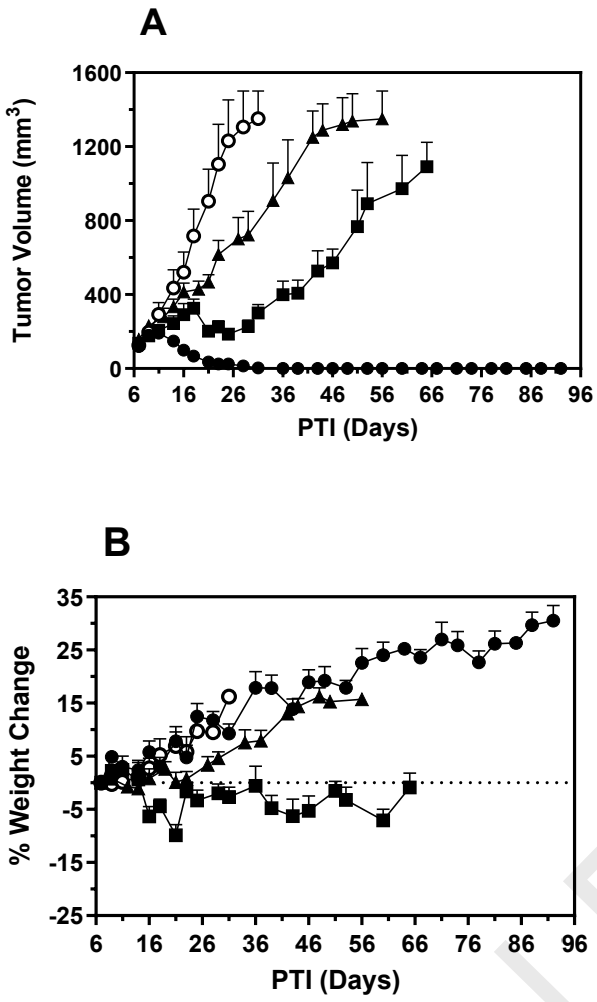


Figure 4. Antitumor (A) and weight change (B) effects of **1**, **18**, and **17** on FR expressing KB tumor *nu/nu* mice model. KB tumor cells were inoculated subcutaneously into *nu/nu* mice (1 x 10^6 cells) and therapy started on randomized animals with tumors in the 100–200 mm^3 (n = 5) range. ○, untreated controls; ●, **1**, 0.3 μmol/kg; ■, **18**, 2 μmol/kg; ▲, **17**, 2 μmol/kg; SIW x 2 weeks. Each curve shows the average volume of 3-5 tumors/animals.

References and Notes.

1. (a) Thurston, D. E.; Bose, D. S., Synthesis of DNA-Interactive Pyrrolo[2,1-c][1,4]benzodiazepines. *Chem. Rev.* **1994,** *94* (2), 433-65; (b) Thurston, D. E. Advances in the study of pyrrolo[2,1-c][1,4]benzodiazepine (PBD) antitumor antibiotics. In *Molecular Aspects of Anticancer Drug-DNA Interactions*, Neidel, S. and Waring, M. J., Eds. Macmillan Publishers Ltd, London: 1993; pp 54-88; (c) Leimgruber, W.; Stefanović, V.; Schenker, F.; Karr, A.; Berger, J., Isolation and characterization of anthramycin, a new antitumor antibiotic. *J. Am. Chem. Soc.* **1965,** *87* (24), 5791-5793; (d) Tendler, M. D.; Korman, S., 'Refuin': A non-cytotoxic carcinostatic compound proliferated by a thermophilic actinomycete. *Nature* **1963,** *199*, 501; (e) Mantaj, J.; Jackson, P. J. M.; Rahman, K. M.; Thurston, D. E., From anthramycin to pyrrolobenzodiazepine (PDB) - containing antibody-drug conjugates (ADCs). *Angew. Chem. Int. Ed.* **2017,** *56*, 462-488.

2. Mantaj, J.; Jackson, P. J. M.; Karu, K.; Rahman, K. M.; Thurston, D. E., Covalent bonding of pyrrolobenzodiazepines (PBDs) to terminal guanine residues within duplex and hairpin DNA fragments. *PLoS ONE* **2016,** *11* (4), e0152303/1-e0152303/26.

3. (a) Rahman, K. M.; Corcoran, D. B.; Bui, T. T. T.; Jackson, P. J. M.; Thurston, D. E., Pyrrolobenzodiazepines (PBDs) do not bind to DNA G-quadruplexes. *PLoS ONE* **2014,** *9* (8), e105021; (b) Kopka, M. L.; Goodsell, D. S.; Baikalov, I.;

Grzeskowiak, K.; Cascio, D.; Dickerson, R. E., Crystal structure of a covalent DNA-drug adduct: anthramycin bound to C-C-A-A-C-G-T-T-G-G and a molecular explanation of specificity. *Biochemistry* **1994,** *33* (46), 13593-610.

4. (a) Hartley, J. A.; Hochhauser, D., Small molecule drugs - optimizing DNA damaging agent-based therapeutics. *Curr. Opin. Pharmacol.* **2012,** *12* (4), 398-402; (b) Hartley, J. A., The development of pyrrolobenzodiazepines as antitumour agents. *Expert Opin. Invest. Drugs* **2011,** *20* (6), 733-744; (c) Antonow, D.; Thurston, D. E., Synthesis of DNA-Interactive Pyrrolo[2,1-c][1,4]benzodiazepines (PBDs). *Chem. Rev.* **2011,** *111* (4), 2815-2864; (d) Vlahov, I. Q., L.; Hahn, S.; Wang, K.; Santhapuram, H.; Felten, A.; Vaughn, J.; Leamon, C. Design, synthesis and early evaluation of hybrids of DNA minor groove binders and DNA-alkylating agents as warheads for small molecule drug conjugates (SMDCs) for targeted cancer therapies. **2016**, From Abstracts of Papers, 252nd ACS National Meeting & Exposition, Philadelphia, PA, United States, Philadelphia, PA, United States, August 21-26, MEDI-39.

5. (a) Saunders, L. R.; Bankovich, A. J.; Anderson, W. C.; Aujay, M. A.; Bheddah, S.; Black, K.; Desai, R.; Escarpe, P. A.; Hampl, J.; Laysang, A.; Liu, D.; Lopez-Molina, J.; Milton, M.; Park, A.; Pysz, M. A.; Shao, H.; Slingerland, B.; Torgov, M.; Williams, S. A.; Foord, O.; Howard, P.; Jassem, J.; Badzio, A.; Czapiewski, P.; Harpole, D. H.; Dowlati, A.; Massion, P. P.; Travis, W. D.; Pietanza, M. C.; Poirier, J. T.; Rudin, C. M.; Stull, R. A.; Dylla, S. J., A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Sci. Transl. Med.* **2015,** *7* (302), 302ra136; (b) 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., A potent anti-CD70 antibody-drug conjugate combining a dimeric pyrrolobenzodiazepine drug with site-specific conjugation technology. *Bioconjugate Chem.* **2013,** *24* (7), 1256-1263.

6. (a) Antony, A. C., Folate receptors. *Annu. Rev. Nutr.* **1996,** *16*, 501-521; (b) Locasale, J. W., Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat. Rev. Cancer* **2013,** *13*, 572-583.

7. (a) Elnakat, H.; Ratnam, M., Distribution, functionality and gene regulation of folate receptor isoforms: implications in targeted therapy. *Adv. Drug Delivery Rev.* **2004,** *56* (8), 1067-1084; (b) Lu, Y. J.; Parker, N.; Kleindl, P. J.; Cross, V. A.; Wollak, K.; Westrick, E.; Stinnette, T. W.; Gehrke, M. A.; Wang, K.; Santhapuram, H. K. R.; You, F.; Hahn, S. J.; Vaughn, J. F.; Klein, P. J.; Vlahov, I. R.; Low, P. S.; Leamon, C. P., Antiinflammatory activity of a novel folic acid targeted conjugate of the mTOR inhibitor everolimus. *Mol. Med.* **2015,** *21*, 584-596; (c) Parker, N.; Turk, M. J.; Westrick, E.; Lewis, J. D.; Low, P. S.; Leamon, C. P., Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal. Biochem.* **2005,** *338* (2), 284-293; (d) Ross, J. F.; Chaudhuri, P. K.; Ratnam, M., Differential regulation of folate receptor isoforms in normal and malignant tissues in vivo and in established cell lines. Physiologic and clinical implications. *Cancer* **1994,** *73* (9), 2432-2443; (e) Weitman, S. D.; Lark, R. H.; Coney, L. R.; Fort, D. W.; Frasca, V.; Zurawski, V. R.; Kamen, B. A., Distribution of the folate receptor GP38 in normal and malignant cell lines and tissues. *Cancer Res.* **1992,** *52* (12), 3396-3401.

8. (a) Edelman, M. J.; Harb, W. A.; Pal, S. E.; Boccia, R. V.; Kraut, M. J.; Bonomi, P.; Conley, B. A.; Rogers, J. S.; Messmann, R. A.; Garon, E. B., Multicenter trial of EC145 in advanced, folate-receptor positive adenocarcinoma of the lung. *J. Thorac. Oncol.* **2012,** *7* (10), 1618-1621; (b) Morris, R. T.; Joyrich, R. N.; Naumann, R. W.; Shah, N. P.; Maurer, A. H.; Strauss, H. W.; Uszler, J. M.; Symanowski, J. T.; Ellis, P. R.; Harb, W. A., Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted therapeutic (vintafolide) and companion SPECT-based imaging agent (99mTc-etarfolatide). *Ann. Oncol.* **2014,** *25* (4), 852-858; (c) Naumann, R. W.; Coleman, R. L.; Burger, R. A.; Sausville, E. A.; Kutarska, E.; Ghamande, S. A.; Gabrail, N. Y.; DePasquale, S. E.; Nowara, E.; Gilbert, L.; Gersh, R. H.; Teneriello, M. G.; Harb, W. A.; Konstantinopoulos, P. A.; Penson, R. T.; Symanowski, J. T.; Lovejoy, C. D.; Leamon, C. P.; Morgenstern, D. E.; Messmann, R. A., PRECEDENT: A randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer. *J. Clin. Oncol.* **2013,** *31* (35), 4400-4406; (d) Reddy, J. A.; Dorton, R.; Bloomfield, A.; Nelson, M.; Dircksen, C.; Vetzel, M.; Kleindl, P.; Santhapuram, H.; Vlahov, I. R.; Leamon, C. P., Pre-clinical evaluation of EC1456, a folate-tubulysin anti-cancer therapeutic. *Sci. Rep.* **2018,** *8* (1), 8943; (e) Vlahov, I. R.; Kleindl, P. J.; You, F., Design and synthesis of drug conjugates of vitamins and growth factors. In *Drug Delivery in Oncology*, Kratz, F.; Senter, P. D.; Steinhagen, H., Eds. Viley-VCH: New York, 2012; pp 1283-1322; (f) Vlahov, I. R.; You, F.; Santhapuram, H. K. R.; Wang, Y.; Vaughn, J. F.; Hahn, S. J.; Kleindl, P. J.; Fan, M. J.; Leamon, C. P., Design and regioselective synthesis of a new generation of targeted therapeutics. Part 3: Folate conjugates of aminopterin hydrazide for the treatment of inflammation. *Bioorg. Med. Chem. Lett.* **2011,** *21* (4), 1202-1205.

9. (a) Howard, P. W.; Masterson, L.; Tiberghien, A. C.; Flygare, J. A.; Gunzer, J. L.; Polakis, P.; Polson, A.; Raab, H. E.; Spencer, S. D. Pyrrolobenzodiazepines and conjugates therof. **2013**, US Patent 20130028917; (b) Thurston, D. E.; Wilson, P. T. Compounds. **2000**, WO00/12507.

10. (a) 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., Development and properties of beta-glucuronide linkers for monoclonal antibody-drug conjugates. *Bioconjugate Chem.* **2006,** *17* (3), 831-40; (b) Jeffrey, S. C.; Nguyen, M. T.; Andreyka, J. B.; Meyer, D. L.; Doronina, S. O.; Senter, P. D., Dipeptide-based highly potent doxorubicin antibody conjugates. *Bioorg. Med. Chem. Lett.* **2006,** *16* (2), 358-362.

11. Vlahov, I. R.; Qi, L. W.; Kleindl, P. J.; Santhapuram, H. K.; Felten, A.; Parham, G. L.; Wang, K.; You, F.; Vaughn, J. F.; Hahn, S. J.; Klein, H. F.; Vetzel, M.; Reddy, J. A.; Nelson, M.; Nicoson, J.; Leamon, C. P., Latent warheads for targeted cancer therapy: Design and synthesis of pro-pyrrolobenzodiazepines and conjugates. *Bioconjugate Chem.* **2017,** *28* (12), 2921-2931.

12. Beesley, R. M.; Ingold, C. K.; Thorpe, J. F., The formation and stability of spiro-compounds. Part I. Spiro-compounds from cyclo-hexane. *J. Chem. Soc. Trans.* **1915,** *107*, 1080-1106.

13. Tanaka, M.; Oishi, S.; Ohno, H.; Fujii, N., A Novel Oxazolidine Linker for the Synthesis of Peptide Aldehydes. *Int. J. Pept. Res. Ther.* **2007,** *13* (1), 271-279.

14. Vlahov, I. R.; Santhapuram, H. K. R.; Kleindl, P. J.; Howard, S. J.; Stanford, K. M.; Leamon, C. P., Design and regioselective synthesis of a new generation of targeted chemotherapeutics. Part 1: EC145, a folic acid conjugate of desacetylvinblastine monohydrazide. *Bioorg. Med. Chem. Lett.* **2006,** *16* (19), 5093-5096.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: