Thiol-dependent DNA cleavage by aminomethylated Beaucage's reagent†

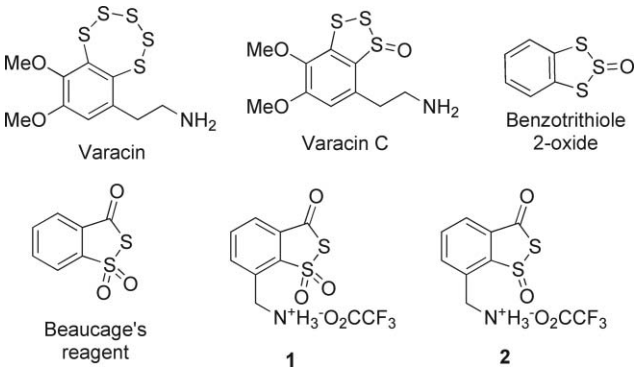
Jiahui Zheng,‡[a] Xiaoqian Liu,‡[a] Qing Yuan,[a] Yoon-Joo Shin,[b] Daekyu Sun[b] and Yixin Lu*[a,c]

Received 11th December 2009, Accepted 23rd January 2010
First published as an Advance Article on the web 3rd February 2010
DOI: 10.1039/b926217b

Aminomethylated Beaucage's reagent 1 was found to be more potent than 3*H*-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage's reagent) in causing DNA cleavage. The current study demonstrated the importance of the amino functionality in enhancing DNA-cleaving activities, and such findings may facilitate development of novel sulfur-containing DNA-cleaving molecules in cancer therapy.

Sulfur-containing cyclic organic compounds, with their unique chemical properties and potential synthetic applications,[1,2] as well as interesting biological and physiological activities,[3] have attracted much attention in recent years. These compounds include varacin[1,4] and lissoclinotoxin A,[5] which exhibit potent antibiotic and antifungal activities respectively. Varacin C, a new member of the varacin family, was discovered in the Far Eastern ascidian *Polycitor* sp. by Makarieva and coworkers in 1995.[6] Biological examination of varacin C revealed that it exhibits potent inhibitory activities against *Staphylococcus aureus*, *Candida albicans* and *Bacillus subtilus*.[6] The total synthesis of varacin C was first reported in 2002,[2c,7] and it was demonstrated that this antibiotic displayed potent thiol-dependent DNA-cleaving activity[8] that far exceeded that of varacin. It was further demonstrated that an acidic environment promoted the DNA-cleaving activities of varacin C.[7] Varacin C possesses a unique benzotrithiol 1-oxide functionality, which was believed to be responsible for its observed biological activities. In addition to the trithiol functional group, disulfide-derived functionalities have also been proven to be sufficient in causing DNA cleavage. Leinamycin, a natural product containing 3*H*-1,2-benzodithiol-3-one 1-oxide functionality, was found to exhibit DNA-cleaving activity.[9] Beaucage's sulfurizing agent, 3*H*-1,2-benzodithiol-3-one 1,1-dioxide, was found to lead to thiol-dependent DNA cleavage.[10] Some selective sulfur-containing organic molecules are shown in Scheme 1.

In our research program towards the discovery of efficient DNA-cleaving agents with potential applications in anticancer therapy, we were interested in evaluating the DNA-cleaving ability of novel sulfur-containing organic molecules. We reasoned that the presence of amino moiety in sulfur-containing compounds would result in better DNA cleaving ability compared to the parent



Scheme 1 Selected sulfur-containing cyclic organic molecules.

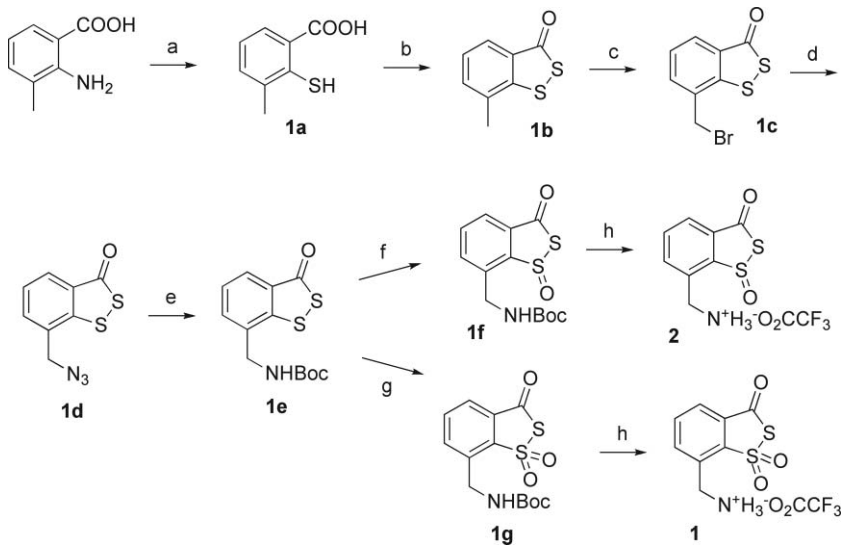
compounds without amino side chains, due to the favourable electrostatic interactions between the protonated amino group and the negatively charged phosphate backbone in DNA. Herein, we report the synthesis of aminomethylated Beaucage's reagent (**1**), and show that this novel sulfur-containing compound can cause efficient thiol-dependent DNA cleavage.

The synthesis of aminomethylated Beaucage's reagent **1** and its sulfoxide analogue **2** is outlined in Scheme 2. The synthesis of **1** began with the diazonium reaction on 2-amino-3-methyl benzoic acid following the procedure described by Allen and coworkers.[11] As the corresponding diazonium salt was unstable at elevated temperatures, the reaction temperature was strictly maintained below 5 °C. Upon its formation, the salt was treated immediately with sulfide to generate disulfide under strongly alkaline conditions. Using activated zinc in refluxing glacial acetic acid, the disulfide bond was cleaved off to yield thiol **1a**. The reaction of thioacetic acid with **1a** in concentrated sulfuric acid gave rise to the cyclic compound **1b**.[12] Radical bromination at the benzylic position was performed by treating **1b** with *N*-bromosuccinimide (NBS) and AIBN, yielding bromide **1c**. The subsequent reaction of **1c** with sodium azide in DMF then afforded the corresponding azide **1d** in good yield. Attempts to synthesize the corresponding amine from **1d** using triphenylphosphine following a typical Staudinger reduction[13] were unsuccessful. The Boc-protected product, **1e**, was then obtained with an *in situ* procedure,[14] albeit in low yield. *m*-Chloroperoxybenzoic acid (*m*-CPBA) was used to accomplish the S-oxidation of **1e** to produce the sulfoxide **1f**.[15] However, using a large excess of *m*-CPBA did not yield the corresponding sulfone.[16] We then took an alternative approach by employing dimethyldioxirane[16] as potential oxidizing agent. A one-pot procedure in which Oxone™ was added to a solution of **1e** in acetone led to the successful preparation of the sulfone **1g**. Finally, removal of the Boc protection in **1g** and **1f** afforded sulfone **1** and sulfoxide **2**, respectively.

[a]*Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore, 117543, Republic of Singapore. E-mail: chmlyx@nus.edu.sg; Fax: +65-6779-1691; Tel: +65-6516-1569*
[b]*Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, Tucson, AZ 85721, USA*
[c]*Medicinal Chemistry Program, Life Sciences Institute, National University of Singapore, Republic of Singapore*

† Electronic supplementary information (ESI) available: Experimental procedures, characterizations of synthetic compounds, and Figures of DNA cleavage experiments. See DOI: 10.1039/b926217b

‡ J. Z. and X. L. made equal contributions to this work.



Scheme 2 Synthesis of **1**. Reagents and conditions: (a) i) 1 eq. $NaNO_2$, conc. HCl, 0–5 °C; ii) 1.1 eq. Na_2S, 1.1 eq. S8, NaOH, rt, 2 h; iii) 4 eq. Zn, CH_3COOH, reflux, 16 h, 59%; (b) 1 eq. CH_3COSH, conc. H_2SO_4, 50 °C, 2 h, 94%; (c) 1.5 eq. NBS, 0.05 eq. AIBN, CCl_4, reflux, 50%; (d) 5 eq. NaN_3, DMF, 60 °C, 16 h, 85%; (e) 2.1 eq. Boc_2O, 3 eq. K_2CO_3, 1.2 PPh_3, CH_2Cl_2, rt, 19 h, 35%; (f) 1.5 eq. *m*-CPBA, CH_2Cl_2, –20 °C, 24 h, 60%; (g) 4 eq. Oxone, 12 eq. $NaHCO_3$, acetone, 0 °C, 24 h, 80%; (h) 10 eq. CF_3COOH, CH_2Cl_2, rt, 12 h, 100%.

It was shown in the previous reports[7,8] that varacin C and other benzotrithiole 2-oxides could efficiently cause single-stranded breaks in duplex DNA in the presence of excess thiol, as measured by conversion of circular supercoiled DNA (form I) to circular relaxed (form II) DNA. To examine the potential DNA cleaving activities of compound **1**, we incubated plasmid pBR322 with **1**, varying its concentrations from 5 μM to 100 μM, at pH 7.0 and in the presence of 2-mercaptoethanol, and the results are shown in Fig. 1.[17] Similar to varacin C[7] and 3*H*-1,2-benzodithiol-3-one 1,1-dioxide,[10] the DNA cleavage caused by **1** was also thiol-dependent. In the absence of thiol, compound **1** alone did not cause any DNA cleavage (Lanes 2 and 9). In the presence of thiol, sulfone **1** triggered DNA cleavage (Lanes 3 to 7). At a concentration of 80 μM (Lane 8), **1** led to very efficient cleavage of single-stranded DNA; the form I plasmid was almost completely converted to form II.

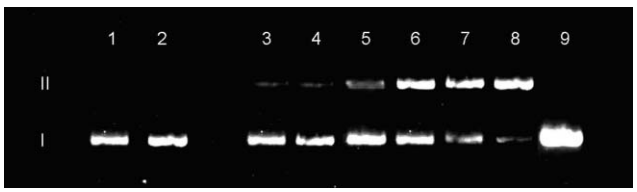


Fig. 1 Thiol-dependent DNA cleavage by **1** with various concentrations at pH 7.0. Assays were performed in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 μg of supercoiled pBR322 DNA in the presence or absence of compound **1** and 2-mercaptoethanol (total volume 20 μL). The reaction time was 12 h. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **1** (5 μM); Lane 3, **1** (5 μM) + thiol (25 μM); Lane 4, **1** (10 μM) + thiol (50 μM); Lane 5, pBR322 DNA with **1** (20 μM) + thiol (100 μM); Lane 6, pBR322 DNA with **1** (30 μM) + thiol (150 μM); Lane 7, pBR322 DNA with **1** (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with **1** (80 μM) + thiol (400 μM); Lane 9, pBR322 DNA with **1** (100 μM).

The pH dependence of compound **1**-triggered DNA cleavage was next examined, and plasmid pBR322 was incubated with **1** in different buffer solutions (pH ranging from 6 to 8). There was no significant enhancement on DNA-cleaving activities when the pH values were varied. These results are certainly very different from the varacin C-caused cleavage which could be effectively promoted under acidic conditions.[7] In order to achieve effective cleavage, reactions needed to be carried out for 12 h. Longer reaction time could lead to better yield of the cleaving products, but not very significantly.[18]

The DNA-cleaving activities of sulfoxide **2** were also investigated.[19] It was found that **2** was not an effective DNA-cleaving agent, even with concentration of **2** at 1 mM, no DNA cleavage was observed. This result suggested the presence of sulfone functional group, as well as disulfide bond, are important for the observed DNA-cleaving activities.

We next compared effectiveness of compound **1** and Beaucage's reagent in causing DNA cleavage, the results are shown in Fig. 2, and quantifications of DNA cleavages are summarized in Table 1. The extents of DNA cleavage caused by aminomethylated

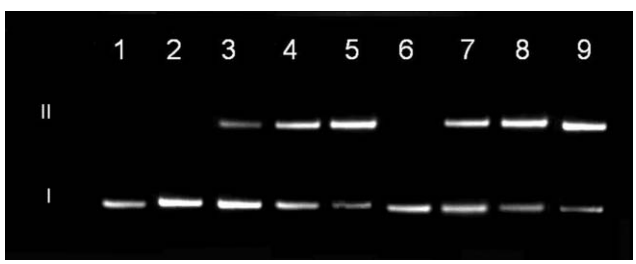


Fig. 2 Thiol-dependent cleavage by various concentrations of **1** at pH 7.0 compared to Beaucage's reagent (BR) at different concentrations. Lane 1, pBR322 DNA alone; Lane 2, pBR322 DNA with **1** (80 μM) without thiol; Lane 3, **1** (5 μM) + thiol (25 μM); Lane 4, **1** (30 μM) + thiol (150 μM); Lane 5, pBR322 DNA with **1** (80 μM) + thiol (400 μM); Lane 6, pBR322 DNA with BR (250 μM) but without thiol; Lane 7, pBR322 DNA with BR (50 μM) + thiol (250 μM); Lane 8, pBR322 DNA with BR (100 μM) + thiol (500 μM); Lane 9, pBR322 DNA with BR (250 μM) + thiol (1.25 mM).

Table 1 Intensity of form I and II DNA after treatments with **1** and Beaucage's reagent[a]

Entry	1	2	3	4	5	6	7	8	9
% of I	100	100	73	47	**23**	100	52	31	**24**
% of II	0	0	27	53	**77**	0	48	69	**76**

[a] Quantification of extent of DNA cleavage was done by measuring volumes of the bands on the photographs of ethidium bromide-stained agarose gels using Kodak Molecular Imaging Software (Standard Edition 5.00.40). The same experiment was repeated twice, and the reproducibility is generally with 10% range. See ESI for the full details.†

Beaucage's reagent (**1**) with concentrations of 5 μM, 30 μM and 80 μM are shown in lanes 2 to 5, respectively. Cleavages of DNA by Beaucage's reagent[10] are illustrated in lanes 7 to 9, with concentrations of Beaucage's reagent at 50 μM, 100 μM and 250 μM, respectively. Apparently, compound **1** is more efficient in causing DNA cleavage. In a quantitative comparison, compound **1** caused 77% cleavage of form I DNA at 80 μM (entry 5, Table 1), however, to reach similar level of cleavage (76%, entry 9, Table 1), a 250 μM of Beaucage's reagent needed to be used. These results clearly demonstrate that the presence of amino group effectively promotes DNA-cleaving activities.

In conclusion, we have successfully synthesized aminomethylated Beaucage's reagent **1**, and its sulfoxide analogue **2**. The DNA-cleaving activities of **1** and **2** have been investigated by examining their effectiveness in converting circular supercoiled DNA (form I) to the corresponding circular nicked form (form II). The presence of amino side chain in **1** improved its DNA cleaving activity, leading to substantial potency improvement comparing to its non-amino analogue (3*H*-1,2-benzodithiol-3-one 1-oxide). The sulfoxide **2**, however, was found to be ineffective in cleaving DNA. Our results suggested that combination of the amino group and sulfur-containing DNA-cleaving functionality could lead to the generation of novel effective DNA-cleaving agents, which may find potential applications in anticancer research. Further investigation to understand the origin of observed DNA-cleavage and to search for more potent sulfur-containing DNA-cleaving functionality is in progress in our laboratory.

Acknowledgements

The authors thank the National University of Singapore and the Biomedical Research Council (BMRC) of Singapore (R-143-000-349-305) for their generous financial support.

Notes and references

1 P. Ford and B. Davidson, *J. Org. Chem.*, 1993, **58**, 4522.
2 (*a*) R. Sato, S. Satoh and M. Saito, *Chem. Lett.*, 1990, 139; (*b*) R. Sato, A. Onodera, T. Goto and M. Saito, *Chem. Lett.*, 1989, 2111; (*c*) R. Sato, Y. Akutsu, T. Goto and M. Saito, *Chem. Lett.*, 1987, 2161.
3 (*a*) T. Chatterji and K. Gates, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1349; (*b*) S. Sivaramakrishnan and K. J. Gates, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3076; (*c*) H. Lim, K. Kubota, A. Kobayashi and F. Sugawara, *Phytochemistry*, 1998, **48**, 787.
4 (*a*) T. Chatterji and K. Gates, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 535; (*b*) F. D. Toste and W. J. Still, *J. Am. Chem. Soc.*, 1995, **117**, 7261; (*c*) V. Behar and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1993, **115**, 7017; (*d*) B. S. Davidson, T. F. Molinshi, L. R. Barrows and C. M. Ireland, *J. Am. Chem. Soc.*, 1991, **113**, 4709.
5 (*a*) M. Litaudon, F. Trigalo, M. Martin, F. Frappier and M. Guyot, *Tetrahedron*, 1994, **50**, 5323; (*b*) R. Davis, I. Sandoval, G. Concepcion, R. Rocha and C. Ireland, *Tetrahedron*, 2003, **59**, 2855.
6 T. N. Makarieva, V. A. Stonik, A. S. Dmitrenok, B. B. Grebnev, V. V. Isakov and N. M. Rebachyk, *J. Nat. Prod.*, 1995, **58**, 254.
7 A. Lee, J. Chen, D. Liu, T. Leung, A. Chan and T. Li, *J. Am. Chem. Soc.*, 2002, **124**, 13972.
8 A. Lee, A. Chan and T. Li, *Chem. Commun.*, 2002, 2112.
9 S. B. Behroozi, W. Kim and K. S. Gates, *J. Org. Chem.*, 1995, **60**, 3964.
10 L. Breydo and K. S. Gates, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 885.
11 C. F. H. Allen and D. D. MacKay, *Organic Syntheses, Coll. Vol. 2*, 1943, 580.
12 R. P. Iyer, L. R. Phillips, W. Egan, J. B. Regan and S. L. Beaucage, *J. Org. Chem.*, 1990, **55**, 4693.
13 B. Pal, P. Jaisankar and V. S. Giri, *Synth. Commun.*, 2004, **34**, 1317.
14 T. Yamaguchi, *Tetrahedron*, 1999, **55**, 1005.
15 A. G. Hortmann, A. J. Aron and A. K. Bhattacharya, *J. Org. Chem.*, 1978, **43**, 3374.
16 V. Marchán, M. Gibert, A. Messeguer, E. Pedroso and A. Grandas, *Synthesis*, 1999, 43.
17 All DNA cleavage experiments were performed twice. For a complete set of gels and quantifications of DNA cleavages, see ESI.
18 For gels showing DNA cleavage at various pH values and different reaction times, see ESI.
19 See ESI for the details.