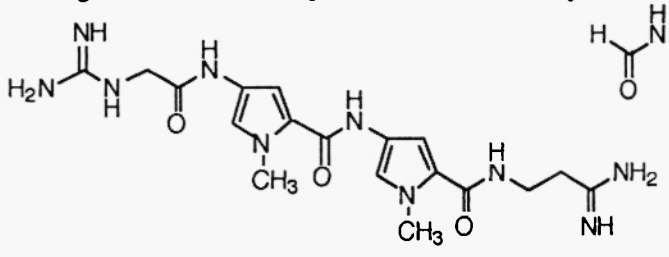
Practical Route to Introduce Lexitropsins Possessing A Natural Trisulfide Linker; Synthesis of *N*-(Lexitropsin-thiosulfenyl)phthalimides

Hirokazu Iida and J. William Lown*

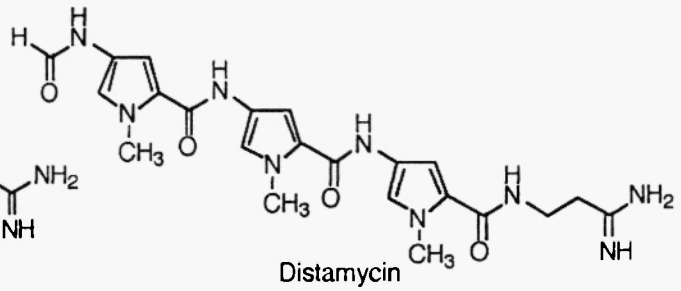
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada, T6G2G2

Abstract: Synthesis of practical reagent which may be used to introduce a lexitropsin possessing a trisulfide linker is described. As an application, the lexitropsin trisulfide was employed with podophyllotoxin to give a lexitropsin- podophyllotoxin conjugate connected with a trisulfide linker in good yield.

Studies on netropsin, distamycin and related compounds have led to the concept of lexitropsins, or information-reading oligopeptides (1). Predominantly 4-5 AT base pair sequences are recognized by netropsin and distamycin in the minor groove of DNA. Rational modifications have been made to these natural products to change their sequence specificity and binding characteristics (2). For example, replacement of one of the pyrrole rings with an imidazole, which can accept a hydrogen bond from G-2-NH_2, allows GC base pairs to be recognized (3,4). Recent developments of X-ray analysis have made it possible to determine the exact structure of certain DNA - lexitropsin conjugates. These and related results have encouraged many research groups to synthesize and examine the properties of lexitropsin - natural products conjugates (5,6). Our application of the lexitropsin concept to CC-1065 analogues led to certain cyclopropylpyrroloindole (CPI) - lexitropsin conjugates which exhibit up to 10,000 times higher potency than CC-1065 itself against KB human cancer cells (7). In the latter case, the sequence selectivity of the lexitropsin unit may play an important role for these conjugates to exhibit high or alternative expressions of bioactivity.

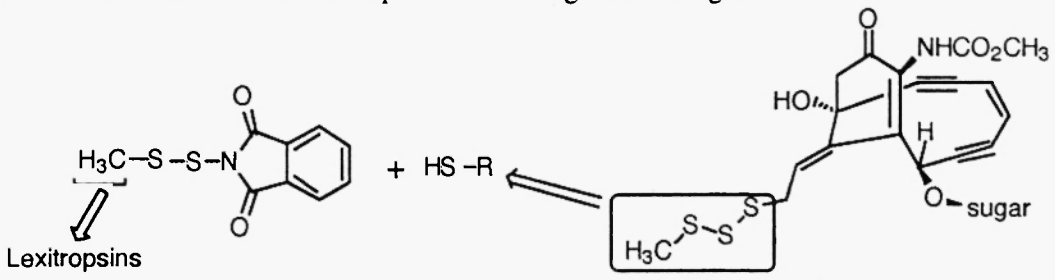


Netropsin



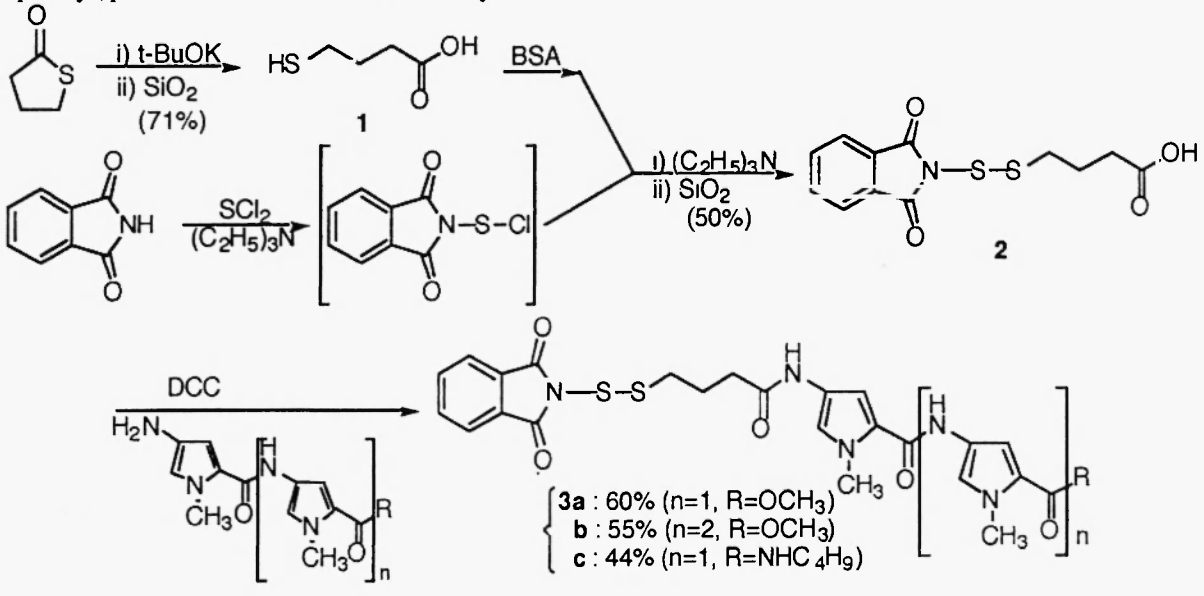
Distamycin

Recently, we have been interested in calicheamicins, which have extraordinary potency against murine tumors (8). During our research on calicheamicin chemistry, we focused on the presence of the trisulfide bond in calicheamicin. It was therefore reported that transformation of this trisulfide bond to a disulfide bond resulted in calicheamicin derivatives exhibiting lower reactivity (9). This result suggested the importance of the trisulfide link. To introduce this alkyl trisulfide group, *S*-methylsulfenylphthalimide was used in model reactions, and the yield in the reactions between thiol and this phthalimide reagent were high.

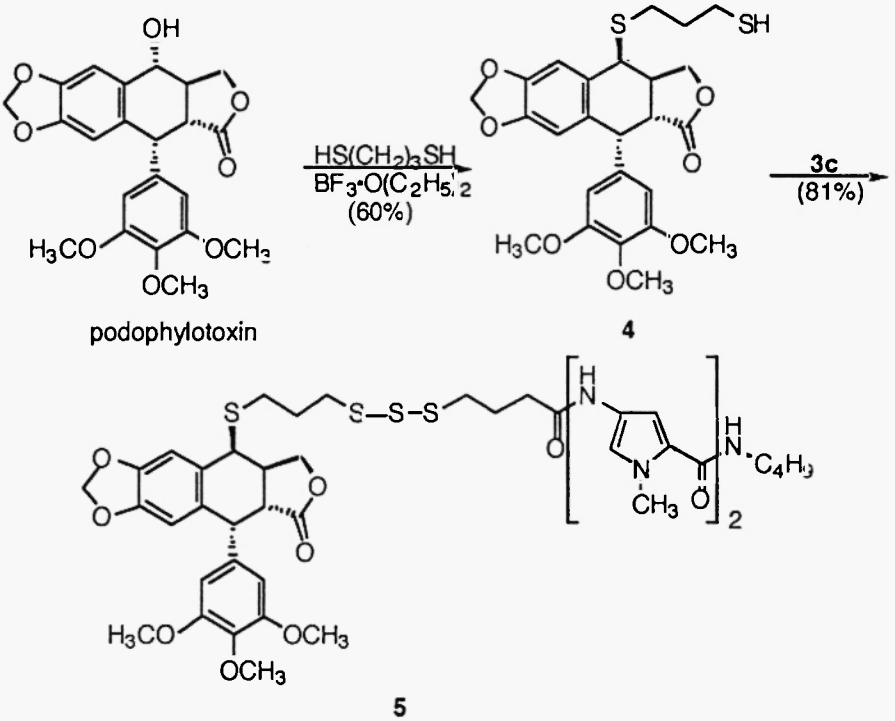


In connection with these studies, we have made some models of calicheamicin and other natural products-lexitropsin conjugates which are connected by trisulfide linkers. It was necessary to develop a practical route to introduce a lexitropsin moiety which had a trisulfide link. In addition we confirmed the facile route to introduce a lexitropsin trisulfide using (phthalimidesulfenyl)lexitropsin reagents, and accordingly report herein.

γ-Thiobutyric acid **1** was synthesized by ring opening of γ-thiobutyrolactone using potassium tert-butoxide in refluxing THF (71% yield). After protecting the acid moiety of **1** using bis(trimethylsilyl)acetamide (BSA), the free thiol was coupled to *N*-(*S*-chlorothio)phthalimide, which was synthesized *in situ* according to a literature procedure, in the presence of triethylamine in dichloromethane at room temperature to obtain *S*-[(phthalimido)-sulfenyl]-γ-thiobutyric acid **2** (over all 50 % yield) (10). This acid **2** was coupled with lexitropsin amines using DCC as coupling reagent in dichloromethane at room temperature to obtain of *N*-(lexitropsin-thiosulphenyl)phthalimides **3a-c** in 44-60 % yields.



In order to explore the usefulness of these new reagents, we applied them to the synthesis of representative podophyllotoxin - lexitropsin conjugates. Podophyllotoxin is a potent inhibitor of microtubule assembly and is a key intermediate for the synthesis of the clinical antitumor agents etoposide and teniposide (11). We have reported the synthesis of 4'-demethylepipodophyllotoxin-lexitropsin conjugates and their topoisomerase II inhibition activities, and those studies encouraged us to synthesize further additional types of podophyllotoxin - lexitropsin conjugates (12). The C-4 hydroxyl group of podophyllotoxin was allowed to react with 1,3-propanedithiol in the presence of trifluoroborane-diethyl ether complex in dichloromethane at -30 °C to afford a podophyllotoxin bearing a thiol group **4** (13). Thiol **4** was treated with (phthalimidesulfenyl)lexitropsin **3c** in refluxing dichloromethane to yield smoothly the corresponding podophyllotoxin -SSS- lexitropsin conjugate **5** (14) in 81% yield. This result illustrates the usefulness of *N*-(lexitropsin-thiosulphenyl)phthalimides reagents **3a-c** to introduce a trisulfide lexitropsin moiety.



In conclusion, we have confirmed a practical route to introduce a lexitropsin moiety possessing a trisulfide linker. We are applying this reagent to calicheamicin and will report the synthesis and properties of calicheamicin-SSS-lexitropsin conjugates in due course.

Acknowledgment

We thank Drs. D. L. J. Clive and Y. Bo at the Department of Chemistry, University of Alberta, for useful discussions. This research was supported by grants (to J.W.L.) from the Natural Sciences and Engineering Research Council of Canada, by the Department of Chemistry, University of Alberta.

References and Notes

1. J. W. Lown, Synthesis of Sequence-specific Agents: Lexitropsins. In: S. Niedle and M. Waring (Ed), Molecular Aspects of Anticancer Drug- DNA Interactions. Vol. 1. Boca Raton: CRC Press, 1993: pp 322, and references sited therein.
2. S. M. Sondhi, B. S. P. Reddy and J. W. Lown, Curr. Med. Chem. 4, 313 (1997).
3. J. W. Lown, K. Krowicki, U. G. Bhat, A. Skorobogaty, B. Ward and J. C. Dabrowiak, Biochemistry 25, 7408 (1986).
4. K. Kissinger, K. Krowicki, J. C. Dabrowiak and J. W. Lown, Biochemistry 26, 5590 (1987).
5. M. L. Kopka, D. S. Goodsell, G. W. Han, T. K. Chiu, J. W. Lown and R. E. Dickerson, Structure 5, 1033 (1997).
6. D. S. Goodsell, H. L. Ng, M. L. Kopka, J. W. Lown and R. E. Dickerson, Biochemistry 34, 16654 (1995).
7. H. Iida and J. W. Lown, Recent Research Developments in Organic Chemistry in press, and references sited therein.
8. M. D. Lee, T. S. Dunne, M. M. Siegel, C. C. Chang, G. O. Morton and D. B. Borders, J. Am. Chem. Soc. 109, 3464 (1987).
9. G. A. Ellestad, P. R. Hamann, N. Zein, G. O. Morton, M. M. Siegel, M. Pastel, D. B. Borders and W. J. McGahren, Tetrahedron Lett. 30, 3033 (1989).
10. P. Magnus, R. T. Lewis and F. Bennett, J. Chem. Soc., Chem. Commun. 916 (1989).
11. L. Chen, I. Zoulíková, J. Slaninová and G. Barany, J. Med. Chem. 40, 864 (1997).
12. Y. Damayanthi and J. W. Lown, Curr. Med. Chem. 5, 205 (1998).
13. R. Gupta, N. H. Al-Said, B. Oreski and J. W. Lown, Anticancer Drug Des. 11, 325 (1996).
14. Compound **5**: Calcd for $C_{45}H_{55}N_5O_{10}S_4$ 954.2910, found 954.2933 (M^++H); ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (3H, t, *J* = 7.0Hz), 1.30-1.45 (2H, m), 1.47-1.61 (2H, m), 2.07-2.22 (4H, m), 2.46 (2H, t, *J* = 7.0Hz), 2.60-2.73 (2H, m), 2.90-3.28 (5H, m), 3.29-3.37 (3H, m), 3.71 (6H, s), 3.78 (3H, s), 3.86 (3H, s), 3.87 (3H, s), 4.26 (1H, d, *J* = 4.2Hz), 4.38-4.44 (2H, m), 4.53 (1H, d, *J* = 5.0Hz), 5.93 (1H, d, *J* = 1.0Hz), 5.96 (1H, d, *J* = 1.0Hz), 6.12 (1H, t, *J* = 6.0Hz), 6.28 (2H, s), 6.40 (1H, d, *J* = 2.0Hz), 6.49 (1H, d, *J* = 2.0Hz), 6.58 (1H, d, *J* = 2.0Hz), 6.92 (1H, s), 7.07 (1H, s), 7.08 (1H, s), 7.67 (1H, s), 7.68 (1H, s);

Received on September 23, 1998