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Synthesis of a major mitomycin C DNA adduct via a triaminomitosene

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ABSTRACT

We report here the synthesis of two amino precursors for the production of mitomycin C and 10-decarbamoylmitomycin C DNA adducts with opposite stereochemistry at C-1. The triamino mitosene precursors were synthesized in 5 steps from mitomycin C. In addition synthesis of the major mitomycin C-DNA adduct has been accomplished via coupling of a triaminomitosene with 2-fluoro-O^6-(2-*p*-nitrophenylethyl)deoxyinosine followed by deprotection at the N^2 and O^6 positions.

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The mitomycins are a family of antitumor antibiotics made by *Streptomyces*. One member of this family, mitomycin C (MC), is currently used to treat certain cancers.[1] Its cytotoxic and antitumor activity is attributed to its ability to alkylate DNA monofunctionally and bifunctionally, resulting in DNA monoadducts as well as interstrand and intrastrand cross-links (ICL).[2] 10-Decarbamoylmitomycin C (DMC) is a derivative of MC lacking the carbamate groups on C-10 (Fig. 1). As such, DMC was originally thought to only alkylate DNA monofunctionally. However, when EMT6 mouse mammary tumor cells were treated with DMC, both ICL and DNA monoadducts were produced.[3] The major adducts generated by MC and DMC have different stereochemistry at carbon 1: trans (or alpha, a) for MC and cis (or beta, b) for DMC. A rationale for this stereochemical preference of alkylation has recently been proposed.[4] In addition, biochemical responses to the two drugs are strikingly different.[5] Individual structure–activity relationships of multiple DNA adducts generated by a single agent have been investigated mostly in the case of organic mutagens and carcinogens.[6] In general, such studies utilize synthetic oligonucleotides bearing a specific adduct at a unique position of their base sequences.[7] The postoligomerization method[8] has been previously used to access such adducts. The MC and DMC adducts present an opportunity for similar studies, enabling in this case direct comparisons of biological effects induced by a different stereochemistry at carbon 1 (Fig. 1).

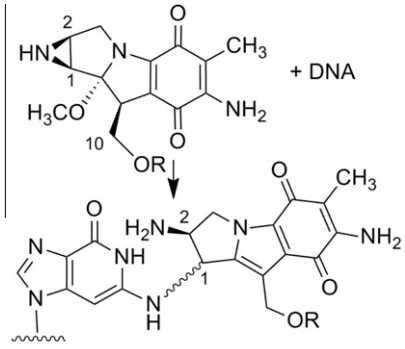

Figure 1. Major Mitomycin C (MC, R = $CONH_2$) adduct 1a = 1-alpha-Monoadduct (trans) and major Decarbamoyl Mitomycin C (DMC, R = H) adduct 1b = 1-beta-Decarbamoyl monoadduct (cis).

We have previously reported the synthesis of an MC metabolite (2,7-diaminomitosene) adduct both on the nucleoside and oligonucleotide levels by the postoligomerization method, the latter resulting in the synthesis of a site-specifically modified oligodeoxyribonucleotide.[9] This was the first application of this method to a DNA adduct of a complex natural product. We report here the synthesis of two amino precursors for the production of adducts **1a** and **1b**. In addition, the synthesis of monoadduct **1a**

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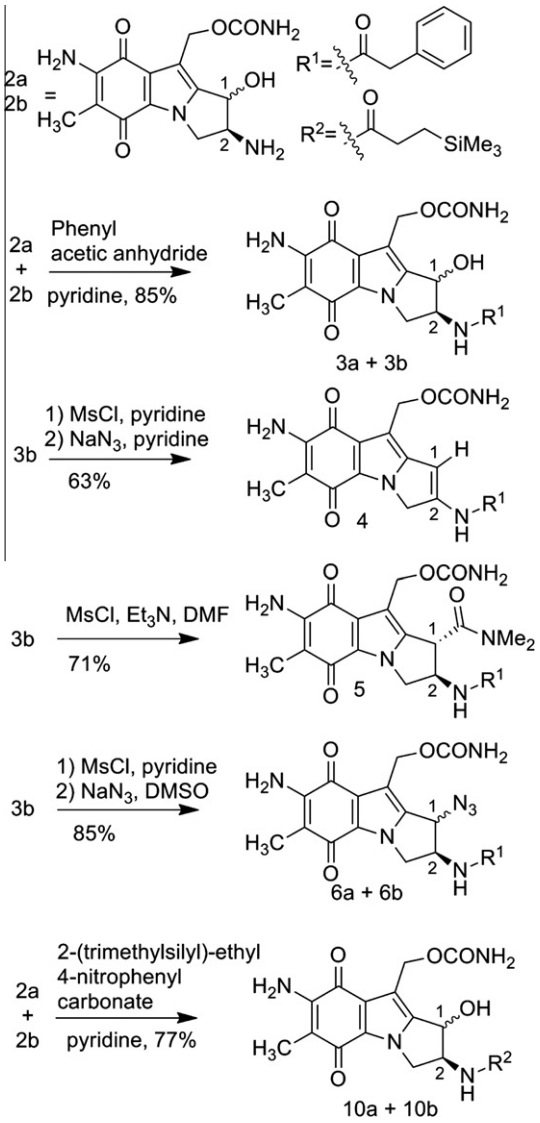
is described. The present alternative approach to adduct **1a** opens doors to the synthesis of MC and DMC-DNA adducts with beta stereochemistry. This is all the more essential as the biomimetic method developed for the production of these adducts is inefficient for the production of adducts with beta stereochemistry.[10]

For the synthesis of suitable stereoisomeric amino precursors **6a** (trans) and **6b** (cis), we needed to first protect the 2-amino position of the 2,7-diamino-1-hydroxymitosenes **2a** and **2b**. The amino hydrin precursors **2a** and **2b** (Scheme 1) are single enantiomers and were synthesized from Mitomycin C.[11] We initially used the phenylacetyl group[12] as a protective group (compounds **3a** (trans) and **3b** (cis)). Direct conversion of the 1-hydroxyl group to an azido group was first tested on compound **3b**. The reaction proved to be challenging. Use of the correct solvent was crucial. In the presence of pyridine, the elimination product **4** was obtained, as seen in Scheme 1. Use of DMF yielded the addition product **5**. However conversion to the azido group proceeded smoothly in DMSO to give a mixture of stereoisomers **6a** and **6b**. Reduction of the azido group to the amine was tried using different reaction conditions (H_2/Pd; Dithiothreitol/$FeSO_4$; Zn/NH_4Cl). The conversion was finally achieved via a Staudinger reaction in a methanol/ammonium hydroxide mixture. The reaction worked well for the trans isomer **6a**, and afforded the trans amine **7a**. However, attempts to convert the cis azide **6b** to the cis amine **7b** yielded a mixture of unidentified products. Coupling of precursor **7a** (trans) with 2-fluoro-O^6-(2-*p*-nitrophenylethyl)deoxyinosine[13] afforded intermediate **8a** and subsequent deprotection at the O^6 position using DBU yielded **9a** (Scheme 3). However, final deprotection at the 2 position using penicillin amidase failed repeatedly despite varied reaction conditions. Our focus shifted to another protecting group, the trimethylsilylethoxycarbonyl (teoc) group which can be removed under mild conditions.[14] The cis and trans teoc-protected 2,7-diamino-1-hydroxymitosene **10a** (trans) and **10b** (cis) (Scheme 1) were converted to the azido derivatives (using similar conditions as for the synthesis of **6a** and **6b** from **3b**) to give **11a** and **11b**. Reduction to the amines groups progressed efficiently in the case of the alpha amino precursor **11a**. However, the reaction was challenging in the case of the beta amino mitosene **11b**. (as previously observed for the conversion of **6b** to **7b**). In this case, the cis amine could be isolated but the yield and outcome of the reaction were inconsistent. Evidently, the cis azides **6b** and **11b** have a very different reactivity than the trans azides toward the Staudinger reduction.



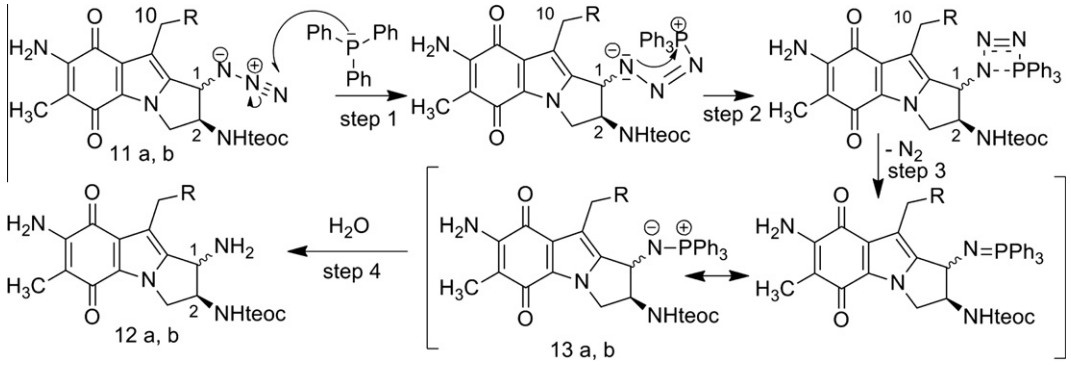
Scheme 1. Solvent effects for the synthesis of **6a** and **6b**. All trans compounds are labeled a and all cis compounds are labeled b.

We investigated the reason for the substrates' different reactivity through DFT calculations. The syntheses of compounds **12a** and **12b** (Scheme 3) were studied with quantum chemical calculations, using the Density Functional Theory method (DFT) as implemented by the Spartan program with the B3LYP method using the 6–31G** basis set.[15] The energies of the azides **11a** and **11b**, of the intermediate iminophosphoranes **13a**, **13b** and of the amine products **12a** and **12b** were calculated as well as the energies of each step. The structures investigated are depicted in Figures S9–S18 (Supplementary data). Table S1 (Supplementary data) shows the energies of all the structures optimized. Table 1 shows the energies of each step of the Staudinger reaction (Scheme 2). The cis compounds (azide **11 b**, amine **12b**) are somewhat more stable than the trans compounds (azide **11a**, amine **12a**), by 3.6 kcal/mol for the azides and 2.2 kcal/mol for the amines. This stability is due to some hydrogen bonds which are only present in the cis azides and amines. However, as shown experimentally, the cis azide is less reactive toward the Staudinger reduction. The thermodynamics of the reaction, with an energy slightly more favorable to the trans reaction is not sufficient to explain the experimental results. On the other hand, the attack of a water molecule on the iminophosphoranes **13a** and **13b** would encounter steric hindrance in the cis molecule. The resulting pseudo-transition states during the final hydrolysis (Figs. S12 and S17), show the trans structure to be more stable than the cis structure by approximately 5 kcal/mol. This difference and the steric hindrance can justify the trans reaction being more favorable than the cis reaction.

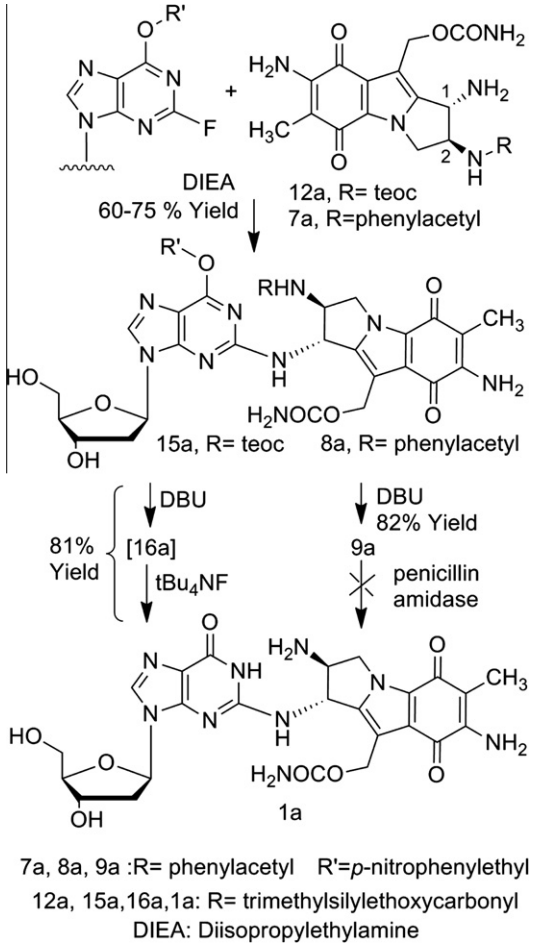
Next, we synthesized adduct **1a** through coupling of **12a** with 2-fluoro-O^6-(2-*p*-nitrophenylethyl)deoxyinosine. The nucleoside was deprotected at the O^6 position using DBU to afford **15a**. We previously reported that $ZnBr_2$ was a good reagent leading to complete and rapid deprotection of the teoc group at the nucleoside level for the synthesis of an MC metabolite (2,7-diaminomitosene).[9] Nonetheless, in the case of adduct **15a**, these conditions also cleaved the 10-carbamate group resulting in a mixture of products. Several other deprotection conditions were tried until finally, the use of

Table 1
Energies for the Staudinger reaction (kcal/mol)

	Step 1	Step 2	Step 3	Step 4	Total
12 b	3.3	−52.2	15.6	−52.2	−83.8
12 a	6	−60.2	14.3	−45.7	−86.5



Scheme 2. Staudinger reaction.



Scheme 3. Synthesis of the major MC-DNA adduct.

tetrabutylammonium fluoride afforded adduct **1a** (Scheme 3). UV[16] CD[17] and HRMS spectra were recorded and structure of **1a** was confirmed via co-elution with an authentic sample of **1a** (prepared according to Ref. 18). [18] The synthesis of the cis DNA adduct from **12b** was tried once. A product (less than 1 mg) was isolated from the coupling of **12b** with 2-fluoro-O^6-(2-*p*-nitrophenylethyl)deoxyinosine; however, it could not be successfully characterized. Given the scarcity of the starting material **12b**, we decided to reattempt the coupling once we develop a better approach to **12b**, the current approach being challenging.

In summary, we have developed new routes toward two precursors for the production of mitomycin C and 10-decarbamoylmitomycin C DNA adducts with opposite stereochemistry at C-1. We also have synthesized the major DNA-mitomycin C adduct at the nucleoside level. Adduct **1a** had previously been isolated using biomimetic methods but this is the first synthesis of this adduct.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.052.

References and notes

1. Verveij, J. D. H.; Pinedo, H. M. In *Cancer Chemotherapy*; Chabner, B. A., Collins, J. M., Eds.; Lippincott: Philadelphia, PA, 1990; p 382.
2. (a) Tomasz, M. *Chem. Biol.* **1995**, *2*, 575; (b) Palom, Y.; Belcourt, M. F.; Musser, S. M.; Sartorelli, A. C.; Rockwell, S.; Tomasz, M. *Chem. Res. Toxicol.* **2000**, *13*, 479.
3. Palom, Y.; Suresh Kumar, G.; Tang, L. Q.; Paz, M. M.; Musser, S. M.; Rockwell, S.; Tomasz, M. *Chem. Res. Toxicol.* **2002**, *15*, 1398.
4. Bueren-Calabuig, J. A.; Negri, A.; Morreale, A.; Gago, F. *Org. Biomol. Chem.* **2012**, *10*, 1543.
5. (a) Boamah, E. K.; Brekman, A.; Tomasz, M.; Natura Myeku, M.; Figueiredo-Pereira, M.; Hunter, S.; Meyer, J.; Bhosle, R. C.; Bargonetti, J. *Chem. Res. Toxicol.* **2010**, *23*, 1151; (b) Boamah, E. K.; White, D. E.; Talbott, K. E.; Arva, N. C.; Berman, D.; Tomasz, M.; Bargonetti, J. *ACS Chem. Biol.* **2007**, *2*, 399; (c) Abbas, T.; Olivier, M.; Lopez, J.; Houser, S.; Xiao, G.; Kumar, G. S.; Tomasz, M.; Bargonetti, J. *J. Biol. Chem.* **2002**, *277*, 40513.
6. Basu, A. K.; Essigmann, J. M. *Mutat. Res.* **1990**, *233*, 189.
7. Krishnegowda, G.; Sharma, A. K.; Krzeminski, J.; Gowda, A. S.; Lin, J. M.; Desai, D.; Spratt, T. E.; Amin, S. *Chem. Res. Toxicol.* **2011**, *24*, 967.
8. (a) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328; (b) Erlanson, D. A.; Chen, L.; Verdine, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 12583; (c) DeCorte, B. L.; Tsarouhtsis, D.; Kuchimanchi, S.; Cooper, M. D.; Horton, P.; Harris, C. M.; Harris, T. M. *Chem. Res. Toxicol.* **1996**, *9*, 630; (d) Cao, H.; Jiang, Y.; Wang, Y. *J. Am. Chem. Soc.* **2007**, *129*, 12123.
9. Champeil, E.; Paz, M. M.; Ladwa, S.; Clement, C.; Zatorski, A.; Tomasz, M. *J. Am. Chem. Soc.* **2008**, *130*, 9556.
10. (a) Kumar, S.; Lipman, R.; Tomasz, M. *Biochemistry* **1992**, *31*, 1399; (b) Borowy-Borowsky, H.; Lipman, R.; Tomasz, M. *Biochemistry* **1990**, *29*, 2999; Suresh Kumar, G.; Musser, S. M.; Cummings, J.; Tomasz, M. *J. Am. Chem. Soc.* **1996**, *118*, 9209.
11. Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307.
12. (a) Waldman, H.; Reidel, A. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 647; (b) Waldman, H. *Tetrahedron Lett.* **1988**, *29*, 1131.
13. Lee, H.; Hinz, M.; Stezowsky, J. J.; Harvey, R. G. *Tetrahedron Lett.* **1990**, *31*, 6773.
14. Ferreira, F.; Morvan, F. *Nucleosides Nucleotides Nucleic Acids* **2005**, *1009*, 24.
15. Spartan 06, Irvine, California, 92612.
16. Tomasz, M.; Chowdary, D.; Lipman, R.; Shimotakahara, S.; Veiro, D.; Walker, V.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6702.
17. Gargiulo, D.; Musser, S. S.; Yang, L.; Fukuyama, T.; Tomasz, M. *J. Am. Chem. Soc.* **1995**, *117*, 9388.
18. Tomasz, M.; Lipman, R.; Verdine, G. L.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337.