Published in final edited form as:

Tetrahedron Lett. 2017 February 15; 58(7): 597–601. doi:10.1016/j.tetlet.2016.12.047.

Acetone promoted 1,4-migration of an alkoxycarbonyl group on a syn-1,2-diamine

Tanya Napolitano^a, Shu-Yuan Cheng^{a,d}, Brooke Nielsen^a, Christopher Choi^a, William Aguilar^a, Manuel M. Paz^c, Anne-Marie Sapse^{a,b}, and Elise Champeil^{a,b,*}

^aJohn Jay College of Criminal Justice, New York, 524 west 59th Street, New York, NY, 10019, USA

^bPh.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA

^cDepartamento de Química Orgánica, Facultade de Química, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

^dPh.D. Program in Biochemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA

Abstract

A 2-protected cis-amino mitosene undergoes an irreversible acetone promoted isomerization and converts to the 1-isomer. Kinetic studies and DFT calculations of the reaction are reported. An organocatalytic mechanism is proposed, involving a covalent intermediate formed by reaction of the mitosene and acetone.

Graphical Abstract

*Corresponding Author: Elise Champeil Tel: (001) 6465574502; fax: (001) 2126213739, Eechampeil@jjay.cuny.edu.

Supplementary data

Supplementary files are provided with the following information: 1) Optimized structures from DFT calculations for compounds **1**, **2**, **X** and **Y** (figures S1 to S4); 2) NMR data: NOESY, HSQC, COSY and DEPT spectra for compound **2** (figures S5 to S8), ¹H NMR spectrum for compound **X** (figure S9), UV and CD data for compound **2** (figure S10, S11), ¹H NMR for compound **2** at variable temperature (figure S12–S13), HRMS spectra for compound **2** and **1** (figure S14, S15); 3) Kinetic calculations: decay of **1** in pure DMSO-*d*₆ and decay of **1** in DMSO-*d*₆/acetone-*d*₆ (10/1); 4) Experimental.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Mitosene; Alkoxy carbonyl migration; Acetone; Organocatalytic mechanism

Introduction

Mitomycin C (MC)¹ is an antitumor antibiotic used clinically to treat stomach, anal and lung cancers² and is the center of numerous research endeavors^{1–12}. Its cytotoxicity is believed to be primarily due to DNA interstrand crosslinks with a trans stereochemistry (α -ICLs). Decarbamoyl mitomycin C (DMC), a MC analog lacking the carbamate on C10, generates the same α -ICL as MC but also yields a major unique stereoisomeric ICL with a cis stereochemistry (beta isomer, β -ICL, Fig. 1). Both α - and β ICLs are the lesions primarily responsible for the cytotoxicities of MC and DMC¹³. Though both lesions share similar features, DMC is more toxic to human cancer cells than MC and induces apoptosis efficiently in a p53 independent manner¹⁴.

Access to mitosene-alpha and mitosene-beta DNA-adducts is essential to elucidate if and how the local DNA structure of these adducts is responsible for the different biochemical responses displayed by the two drugs. Such substrates have been previously used to investigate the mechanisms involved in the repair of mitomycin C mono- and interstrand cross-linked DNA adducts⁶ and to determine how MC and DMC induce p53-independent p21^{WAF1/Cip1} activation in p53 proficient and p53 deficient cancer cells¹⁵.

MC-DNA adducts (α -ICLs) are easily obtained *via* a biomimetic route, contrary to DMC-DNA adducts (β -ICLs). We have recently developed a route to an amino-mitosene with cis-stereochemistry which can be used to generate a cis MC-DNA monoadduct *via* post-oligomerization synthesis (scheme 1)¹⁶. This chemistry paves the way to the synthesis of β -ICLs.

Synthesis of the cis-amino mitosene precursor **1** led to an unforeseen discovery: during the reduction of the azide precursor *via* a Staudinger reaction, the desired cis-triamino mitosene **1** was often accompanied by a novel mitosene **2** with similar mass and spectroscopic characteristics as **1**.

Unexpected reactions and rearrangements from cis-amino mitosenes have been previously described. Relevant to the findings presented here is the *O* to *N* migration of the acetyl group from the cis-acetoxyaminomitosene to the cis-acetylamino-hydroxymitosene during the solvolysis of MC with glacial acetic acid¹⁷ and the *O* to *N* migration of an acyl group in the reaction of MC with trifluoroacetic acid (scheme 2)¹⁸.

Additionally, an oxazolidinone derivative of cis-1-hydroxy-2,7-diaminomitosene resulting from an intramolecular reaction between the adjacent 1,2 cis carbonate and amino groups was recently characterized (scheme 3)¹⁹.

Results and discussion

In line with these previous findings, we report here the acetone promoted migration of a 2-trimethylsilylethoxycarbonyl (teoc) group on the cis-triaminomitosene **1**, yielding the novel mitosene **2** (scheme 4).

Compound **2** was first detected during the isolation of **1** by chromatography. HRMS analysis revealed a molecular formula and molecular weight identical to that of the desired cis-triamino mitosene strongly suggesting that compound **2** was an isomer of **1**. Due to the limited availability of MC we devoted some time to maximize the yield of the novel adduct for NMR characterization. The formation of **2** was observed only when acetone was used for the isolation of **1**. This prompted us to investigate if acetone itself was involved in the formation of **2**. A solution of **1** in methanol and DMSO was prepared without using acetone and was monitored by NMR. Compound **1** was stable and no trace of **2** was observed. Upon addition of acetone, the disappearance of **1** and formation of **2** were observed. This proved that acetone was involved in the conversion of **1** to **2** and allowed us to synthesize enough **2** for characterization. The UV spectrum of the novel compound showed the maximum absorbance at 252 and 310 nm characteristics of the mitosene chromophore (supplementary information, fig. S10). The relative stereochemistry of many 1-substituted 2-aminomitosenes has also been assigned using circular dichroism, using the sign of the Cotton effect at 520–550 nm as diagnosis²⁰. The CD spectrum of **2** followed this general rule as **2** exhibited a weak positive cotton effect around 550 nm as expected for a cis configuration (supplementary information, fig. S11).

The ¹H and ¹³C NMR in DMSO-*d*₆ of the new mitosene showed the expected peaks for all hydrogens and carbons. NOESY, HSQC, COSY and DEPT experiments allowed the correct assignment of all carbons and protons (supplementary information; fig. S5 to S8).

Noteworthy is the dramatic upfield shift (3.25 ppm) of the signal for the amide proton in the ¹H NMR spectrum upon migration of the teoc group from N₂ to N₁ along with the downfield shift of H₁ and H₂ signals (Fig. 2). In addition, splitting of the amide signal at a 2:1 ratio indicates that **2** exist as rotamers in solution, this latter phenomenon likely due to the newly hindered free rotation around the NH-C(O) bond (Fig. 2). Variable temperature NMR experiments show a coalescence of the 2 signals at T=60 ° C (Fig. S12). NOE and COSY experiments showed both direct and through-space correlations between the amide proton and H₁, confirming the position of the teoc group on N₁.

We then investigated the kinetics of formation of **2** both in pure acetone-*d*₆ and in DMSO-*d*₆: acetone-*d*₆ (1:10) by NMR spectroscopy. The formation of **2** was not a direct mechanism as expected. Instead a third compound, compound **X**, was transiently observed but could not be isolated (scheme 4). The concentrations of the 3 species in solution (**1**, **2** and **X**) were calculated over time using qNMR (Mnova software; Mestrelab) in both solvents (pure acetone-*d*₆ and DMSO-*d*₆: acetone-*d*₆) (Fig. 3). The formation of **2** in pure acetone-*d*₆ consists of two consecutive steps (with *k*_{1obsA} and *k*_{2obsA} being the associated rate constants). The first step is extremely fast, (*i.e.* all of the **1** initially present is rapidly converted into **X**) and appears to follow a first order rate with *k*_{1obsA} = 0.145 min⁻¹ (supplementary material, kinetic-calculations). Compound **X** is then converted to **2** and the

rate of the formation of **2** matches the rate of decomposition of **X**. In DMSO-*d*₆: acetone-*d*₆ (10:1), the rate of conversion of **1** to **X** is much slower than in acetone-*d*₆ with a first-order rate constant of 0.0004 min⁻¹ (supplementary material, kinetic-calculations), indicating that the rate of the first step is dependent on the concentration of acetone. This data evidences that acetone reacts with the mitosene in the first step of the reaction.

Suggested mechanism

Kinetic observations prove that acetone is involved in the migration of the teoc group and that the mechanism involves an unstable intermediate **X**, that is further converted to **2**. We propose an organocatalytic mechanism to explain this transformation, involving a covalent adduct **X**, formed by the reaction of **1** with acetone. The most plausible structures for **X** would be either a hemiaminal or an imine derivative at N₁ of **1**. ¹H NMR analysis of **X** (t=30 minutes, in acetone-*d*₆) provides some information about its structure (supplementary material, Fig. S 9). The signal for H₁ is strongly deshielded (δ 5.15) compared to **1** (δ 4.45) but remains a doublet. The splitting patterns for H₂ (m; δ 4.83) and H₃ (d,d; δ 4.59) signals remain the same as for compound **1** however, both signals are slightly deshielded (by 0.2 ppm for H₂ and by 0.1 ppm for H₃). Finally, the amide proton appears as a doublet and is shifted 0.3 ppm upfield compared to **1**. The mechanism is better explained through a hemiaminal intermediate stabilized by hydrogen bonds (Fig. 5). The reverse reaction is not observed (acetone promotes the conversion of **1** to **2**, but does not promote the conversion of **2** to **1**), therefore complex **Y** must be quickly and irreversibly converted to **2**.

DFT calculations were performed to evaluate the energies of **1**, **2**, **X** and **Y** in the gas phase and their optimized structures are shown (Fig. S1, S2, S3, S4, supplementary material, optimized structures). Optimized structure **1** is more stable than **2** by 2 Kcal/mole and optimized structure **X** has the same energy as the sum of energies of **2** and acetone (table 1). This indicates that the suggested mechanism is plausible. The optimized structure of compound **X** show that hydrogen bonding is possible between the hemiaminal hydrogen H* and three hydrogen atoms, N₁, N₂ and O₁ (H*-N₁ = 2.28 Å, H*-N₂ = 2.30 Å and H*-O₁ = 2.59 Å) (scheme 5). However, for H bonding to occur, the 3 atoms involved in the bonding need to be aligned. This is only the case with O- H*-N₂. The alkoxycarbonyl migration may be facilitated by the presence of this strong hydrogen bond which makes the amide nitrogen N₂ a better leaving group than the alkoxycarbonyl group, since N₂ is partially protonated. The conformation of this intermediate also facilitates the reaction by placing the 2 reacting atoms (N₁ and C(O)) in close proximity (d N₁-C(O)=3.87 Å)

Conclusion

In conclusion, we have discovered a novel mitosene resulting from the acetone catalyzed migration of a trimethylsilylethoxycarbonyl (teoc) group between adjacent syn amino groups. This migration is exclusively observed when acetone is present and proceeds *via* an unstable intermediate. Kinetics experiments revealed a complex mechanism with two consecutive steps, the rate of the first step being dependent on the concentration of the starting material and acetone. A mechanism is proposed to explain this transformation based on kinetic studies, DFT calculations and NMR observations. This mechanism involves the

formation of a transient hemiaminal intermediate formed by the reaction of acetone with the N₁ amino group of the mitosene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institute of Health (grant 5SC3GM105460) and the Program for Research Initiatives for Science Majors (PRISM) at John Jay College of Criminal Justice.

References and Notes

1. Hata T, Sano Y, Sugawara R, Matsumae A, Kanamori K, Shima T, Hoshi T. *J Antibiotics*, Ser A. 1956; 9:141–146.
2. a) Verweij, J., Pinedo, H. Cancer chemotherapy and biological modifiers. In: Pinedo, HM, Chabner, BA., Longo, DL., editors. Annual 11. Elsevier Science Publishers B. V; Amsterdam: 1990. p. 67b) Chabner, BA., Amrein, PC., Druker, BJ., Michaelson, MD., Mitsiades, CS., Goss, PE., Ryan, DP., Ramachandra, S., Richardson, PJ., Supko, JG., Wilson, WH. Antineoplastic Agents. In: Brunton, LL, Lazo, JS., Parker, KL., editors. Goodman & Gilman's *The Pharmacological Basis of Therapeutics*. McGraw-Hill Publishers; New York: 2005. p. 1315–1403.
3. Borowy-Borosky H, Lipman R, Tomasz M. *Biochemistry*. 1990; 29:2999–3006. [PubMed: 2110821]
4. Paz MM, Ladwa S, Champeil E, Liu Y, Rockwell S, Boamah EK, Bargonetti J, Callahan J, Roach J, Tomasz M. *Chem, Res Toxicol*. 2008; 21:2370–2378. [PubMed: 19053323]
5. Champeil E, Paz MM, Ladwa S, Clement C, Zatorski A, Tomasz M. *J Am Chem Soc*. 2008; 130:9556–9565. [PubMed: 18588303]
6. Weng M-W, Zheng Y, Jasti VP, Champeil E, Tomasz M, Wang Y, Basu AK, Tang M-S. *Nucl Acid Res*. 2010; 38:6976–6984.
7. Champeil E, Paz MM, Lukasiewicz E, Kong W, Watson S, Sapse AM. *Bioorg Med Chem Lett*. 2012; 22:7198–7200. [PubMed: 23079525]
8. Wiedner SD, Vedejs E. *Org Lett*. 2010; 12:4030–4033. [PubMed: 20738104]
9. Chamberland S, Grischow S, Sherman DH, Williams RM. *Org Lett*. 2009; 11:791–794. [PubMed: 19161340]
10. Paz MM, Zhang X, Jun Lu J, Holmgren A. *Chem Res Toxicol*. 2012; 25:1502–1511. [PubMed: 22694104]
11. Williams AL, Srinivasan JM, Johnston JN. *Org Lett*. 2006; 8:6047–6049. [PubMed: 17165926]
12. Kern N, Hoffmann M, Blanc A, Weibel JM, Pale P. *Org Lett*. 2013; 15:836–839. [PubMed: 23350973]
13. Palom Y, Suresh Kumar G, Tang LQ, Paz MM, Musser SM, Rockwell S, Tomasz M. *Chem Res Toxicol*. 2002; 15:1398–1406. [PubMed: 12437330]
14. Boamah EK, Brekman A, Tomasz M, Natura Myeku M, Figueiredo-Pereira M, Hunter S, Meyer J, Bhosle RC, Bargonetti J. *Chem Res Toxicol*. 2010; 23:1151–1162. [PubMed: 20536192]
15. Cheng SY, Seo J, Huang BT, Napolitano T, Champeil E. *Int J Oncol*. 2016; 49:1815–1824. [PubMed: 27666201]
16. Champeil E, Cheng SY, Huang BT, Conchero-Guisan M, Martinez T, Paz MM, Sapse AM. *Bioorg Chem*. 2016; 65:90–99. [PubMed: 26894558]
17. (a) Iyengar BS, Remers WA. *J Med Chem*. 1985; 28:963–967. [PubMed: 3925148] (b) Taylor WG, Remers WA. *J Med Chem*. 1975; 18:307. [PubMed: 1133823]
18. Verdine GL, McGuinness BF, Nakanishi K, Tomasz M. *Heterocycles*. 1987; 25:577–587.
19. Paz MM. *Bioorg Med Chem Lett*. 2010; 20:31–34. [PubMed: 19954979]

20. Tomasz M, Jung M, Verdine G, Nakanishi K. J Am Chem Soc. 1984; 106:7367–7370.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Acetone promoted isomerization of an amino mitosene
- Kinetic studies and DFT calculations
- Organocatalytic mechanism

Figure 1.
Mitomycin C (MC) and Decarbamoyl Mitomycin C (DMC) interstrand cross links.

Figure 2.
 ^1H NMR of **1** (bottom) and **2** (top) in $\text{DMSO}-d_6$ (ppm).

Figure 3. Concentration of **1**, **2** and **X** (mol/L) versus time a) in acetone- d_6 , b) in DMSO- d_6 with 10% acetone- d_6

Scheme 1. Synthesis of a beta MC-DNA monoadduct *via* a cis-triaminomitosene.

Scheme 2.

Acyl migration on cis-acyloxymitosenes to form *N*-acylmitosenes (R=CH₃, CF₃).

Scheme 3.

Formation of an oxazolidinone derivative of cis-1-hydroxy-2,7-diaminomitosenes.

Scheme 4.
Acetone assisted migration of the teoc group.

Scheme 5.

Possible mechanism for the formation of **2** and hypothetical structure for **X** and **Y**.

Table 1

Energies of all species involved in scheme 5

Compounds	Energies (au)
1	−1755.00784
2	−1755.00451
X	−1948.16863
Y	−1948.15729
acetone	−193.16431