ORGANIC LETTERS

2009
Vol. 11, No. 4
791−794

# Synthesis of Potential Early-Stage Intermediates in the Biosynthesis of FR900482 and Mitomycin C

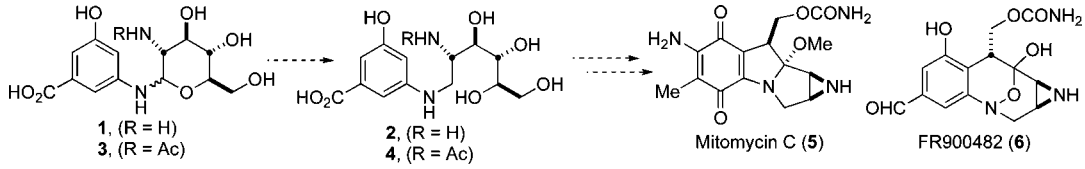
**Stephen Chamberland,[†] Sabine Grüschow,[‡] David H. Sherman,[‡] and Robert M. Williams*[,†,§]**

*Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, Life Sciences Institute and Departments of Medicinal Chemistry, Chemistry, Microbiology & Immunology, The University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan 48109-2216, and University of Colorado Cancer Center, Aurora, Colorado 80045*

*rmw@lamar.colostate.edu*

**Received November 14, 2008**

## ABSTRACT




**Beyond the identification of 3-amino-5-hydroxybenzoic acid (AHBA) and D-glucosamine as biosynthetic precursors to mitomycin C (5) and FR900482 (6), little is known about the pathway Nature uses to prepare these antitumor antibiotics. To gain some insight into their biosynthesis, amino acids 1 and 2 as well as C-2 *N*-acetylated derivatives 3 and 4 were prepared. Preparation of these putative biosynthetic intermediates and *N*-acetylcysteamine thioester analogues 28 and 29 should enable confirmation of their involvement in FR900482 and mitomycin C biosynthesis.**

Mitomycin C (MMC, Figure 1) has been employed successfully as a cancer chemotherapeutic for nearly three decades, but use of this drug often causes harmful side effects that are associated with hematotoxicity.[1−3] One of the modes of action by which MMC displays cytotoxicity is through a bioreductive DNA alkylation and an interstrand cross-linking reaction, a process that has been thoroughly studied.[3] A more recently isolated structural relative of MMC is FR900482, which has been shown to exhibit even more potent bioreductive mitosene-based DNA cross-linking activity than MMC.[4,5] In contrast to MMC, FR900482 and several semisynthetic relatives (such as FK317) lack the quinone moiety responsible for the production of adventitious and harmful superoxide during bioreductive MMC activation.[6,7] We have also demonstrated that FR900482 and its derived

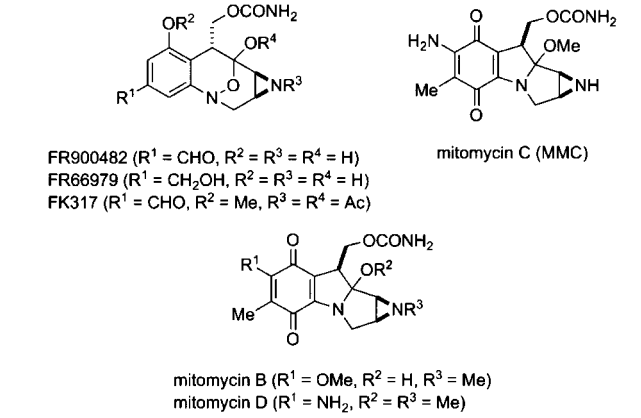



**Figure 1.** Structures of the mitomycins, FR900482, and congeners.

[†] Colorado State University.
[‡] The University of Michigan.
[§] University of Colorado Cancer Center.

(1) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723–2795.
(2) Denny, W. A. In *Cancer Chemotherapeutic Agents*; Foye, W. A., Ed.; American Chemical Society: Washington, DC, 1995; pp 491−493.
(3) Tomasz, M. *Chem. Biol.* **1995**, *2*, 575–579.
(4) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477–2495.

10.1021/ol802631c CCC: $40.75 
**Published on Web 01/22/2009**

semisynthetic relative, FK317, cross-link the HMG-A1 oncoproteins to chromosomal DNA in human cells.[5,8] The interesting biological activity and unique structures of these agents have also attracted considerable interest in the total synthesis of FR900482 and derivatives.[9]

In addition to shared aspects of their mechanism of biological activity, the structural similarity between MMC and FR900482 suggests that these antitumor antibiotics share a common biosynthetic origin. Indeed, biogenetic studies revealed that these agents originate from 3-amino-5-hydroxybenzoic acid (AHBA) and D-glucosamine (Figure 2).[10]

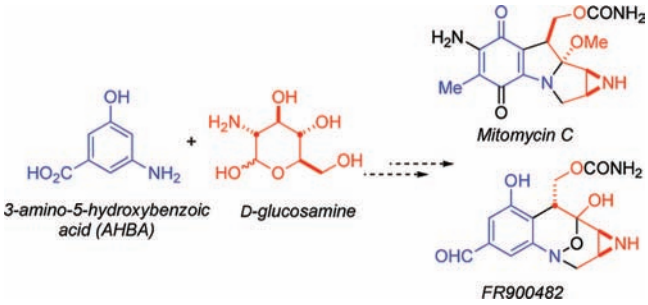


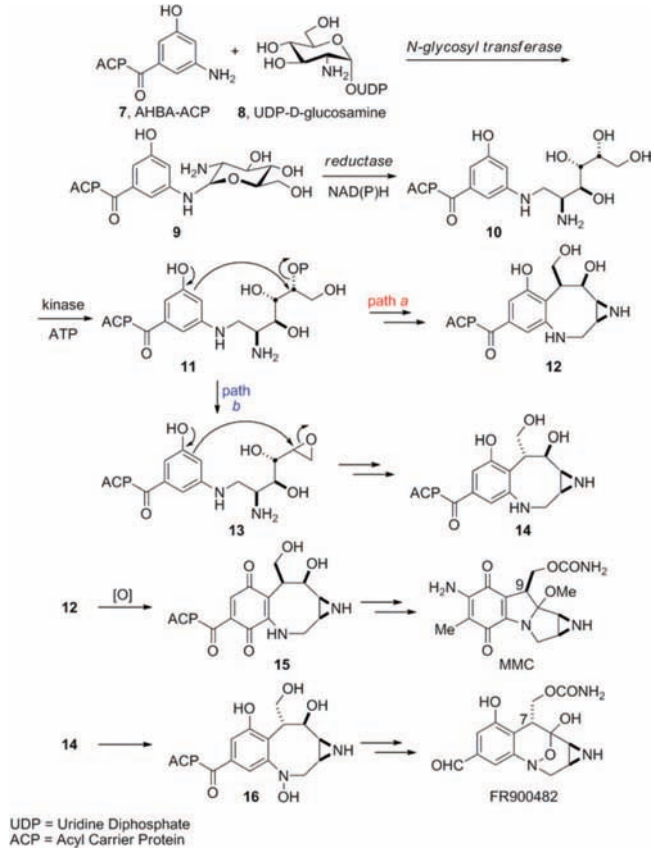

**Figure 2.** Incorporation of AHBA and D-glucosamine into mitomycin C and FR900482.

Beyond these early investigations, little is known regarding the details of how these simple precursors are assembled into the complex architectures characteristic of these structurally unique agents.[11]

A unified biogenesis for the formation of MMC and FR900482 has been partially formulated by Sherman and co-workers and is further elaborated herein (Scheme 1).[12] A preliminary step in the biogenesis of MMC and FR900482 likely involves MitE, an acyl AMP ligase, and MmcB, a discrete acyl carrier protein.[13,14]

We propose that MitE catalyzes the reaction between AHBA-AMP and MmcB to provide an AHBA−ACP conjugate (**7**).[15] Once this substance is formed, *N*-glycosylation of activated D-glucosamine derivative **8** with ACP-bound amine **7** may afford the first committed biosynthetic intermediate, *N*-glycoside **9**.[11] Another enzyme critical for mitomycin biosynthesis, MitB, has been implicated as the glycosyl transferase responsible for the in vivo assembly of *N*-glycoside **9**.[12,13] Next, ring-opening and reduction of *N*-glycoside **9** would afford the corresponding amine **10**.[12,13]

**Scheme 1.** Unified Biosynthesis of FR900482 and MMC




An alternative pathway for joining the two halves may involve the biosynthetic equivalent of an intermolecular Friedel−Crafts alkylation of ACP-ligated AHBA with a suitably activated derivative of D-glucosamine, but this possibility appears unlikely.[12,13] Of particular intrigue in the overall biogenesis of MMC and FR900482 is the striking difference in the stereochemistry of the C9 (MMC number-

(5) Williams, R. M.; Rajski, S. R.; Rollins, S. B. *Chem. Biol.* **1997**, *4*, 127–137.

(6) Masuda, K.; Makamura, T.; Shimomura, K.; Shibata, T.; Terano, H.; Kohsaka, M. *J. Antibiot.* **1988**, *41*, 1497–1499.

(7) Sartorelli, A. C.; Pristos, C. A. *Cancer Res.* **1986**, *46*, 3528–3532.

(8) (a) Beckerbauer, L.; Tepe, J. J.; Eastman, R. A.; Mixter, P.; Williams, R. M.; Reeves, R. *Chem. Biol.* **2002**, *8*, 427–441. (b) Beckerbauer, L.; Tepe, J. J.; Cullison, J.; Reeves, R.; Williams, R. M. *Chem. Biol.* **2000**, *7*, 805–812.

(9) For total syntheses of FR900482, see: (a) Suzuki, M.; Kambe, M.; Tokuyama, H.; Fukuyama, T. *J. Org. Chem.* **2004**, *69*, 2831–2843. (b) Judd, T. C.; Williams, R. M. *J. Org. Chem.* **2004**, *69*, 2825–2830, and references cited therein.

(10) (a) Floss, H. G. *Nat. Prod. Rep.* **1997**, *14*, 433–452. (b) Fujita, T.; Takase, S.; Otsuka, T.; Terano, H.; Kohsaka, M. *J. Antibiot.* **1988**, *41*, 392–394. (c) Kibby, J. J.; Rickards, R. W. *J. Bacteriol.* **1981**, *34*, 605–607. (d) Anderson, M. G.; Kibby, J. J.; Rickards, R. W.; Rothschild, J. M. *J. Chem. Soc., Chem. Commun.* **1980**, 1277–1278. (e) Hornemann, U.; Eggert, J. H. *J. Antibiot.* **1975**, *28*, 841–843. (f) Hornemann, U.; Kehrer, J. P.; Nunez, C. S.; Ranieri, R. L. *J. Am. Chem. Soc.* **1974**, *96*, 320–322. (g) Hornemann, U.; Aikman, M. J. *J. Chem. Soc., Chem. Commun.* **1973**, 88–89. (h) Hornemann, U.; Cloyd, J. C. *J. Chem. Soc., Chem. Commun.* **1971**, 301–302.

(11) Namiki, H. N.; Chamberland, S.; Gubler, D. A.; Williams, R. M. *Org. Lett.* **2007**, *9*, 5341–5344.

(12) Sherman, D. H.; Mao, Y.; M.; Varoglu, M.; He, M.; Sheldon, P. Mitomycin biosynthetic gene cluster. U.S. Patent 6,495,348, December 17, 2002.

(13) (a) Mao, Y.; Varoglu, M.; Sherman, D. H. *Chem. Biol.* **1999**, *6*, 251–263. (b) Mao, Y.; Varoglu, M.; Sherman, D. H. *J. Bacteriol.* **1999**, *181*, 2199–2208.

(14) Sherman, D. H. Personal communication, 2007.

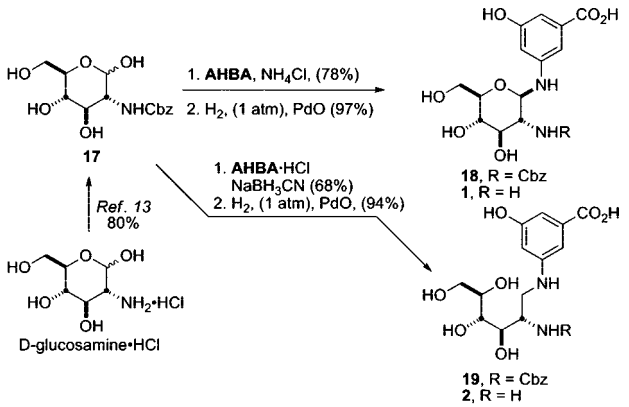
(15) Sherman, D. H.; Li, S. Unpublished results, 2007.

(16) (a) Kalmanowa, M.; Sokolowski, J. *Rocz. Chem., Ann. Soc. Chim. Pol.* **1976**, *50*, 1175–1189. (b) Bertho, A.; Koziollek, D. *Chem. Ber.* **1959**, *92*, 627–637. (c) Bertho, A.; Koziollek, D. *Chem. Ber.* **1954**, *87*, 934–940. (d) Weygand, F.; Perkow, W.; Kuhner, P. *Chem. Ber.* **1951**, *84*, 594–602. (e) Kuhn, R.; Strobele, R. *Chem. Ber.* **1937**, *70B*, 773–787.

ing) and C7 (FR900482 numbering) benzylic stereocenter in MMC and FR900482, which has potentially powerful biosynthetic implications.

To confirm whether the more plausible *N*-glycosylation is responsible for the union of precursors **7** and **8**, we prepared AHBA/D-glucosamine adduct **1** (Scheme 2). Lit-

**Scheme 2.** Synthesis of Putative Biosynthetic Intermediates **1** and **2**

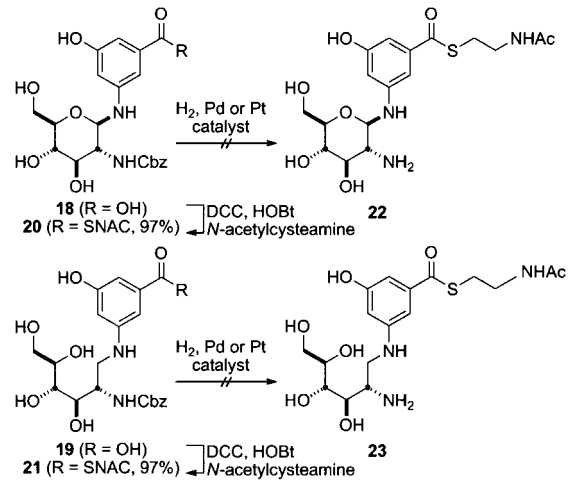



erature precedent for the synthesis of unprotected *N*-aryl glycosides is extremely limited.[16] Nonetheless, these methods were amenable to the preparation of compound **1**. In practice, condensation of *N*-Cbz-D-glucosamine (**17**)[17] and AHBA[18] under Brønsted acid catalysis afforded *N*-glycoside **18** exclusively in the $\beta$-configuration (Scheme 2). Subsequent cleavage of the *N*-Cbz moiety using catalytic hydrogenolysis with palladium(II) oxide cleanly provided the requisite amino acid **1**.[19] Putative biosynthetic intermediate **2** was prepared in a two-step, one-flask *N*-glycosylation/reductive amination reaction sequence enlisting *N*-Cbz-D-glucosamine (**17**) and AHBA·HCl in the presence of sodium cyanoborohydride to provide amine **19**.[20] Unmasking the amine functionality using catalytic hydrogenolysis provided amino acid **2** in a fashion analogous to that applied to the preparation of amine **1**.[18]

To facilitate chemoenzymatic analysis of the proposed ACP-complexed biosynthetic intermediates with cloned, functionally expressed biosynthetic enzymes MitB, MitE, and putative reductase MitF, we prepared *N*-acetylcysteamine (SNAC) thioester analogues of compounds **1** and **2** (Scheme 3).[12,13,21] Although SNAC ester derivatives of Cbz-protected *N*-aryl glycosides **18** and **19** could be prepared, the thioester moiety was incompatible with standard Pd- or Pt-mediated hydrogenolysis conditions used to deprotect the Cbz group. Furthermore, repeated efforts to reveal the C-2 amino functionality in analogues possessing a photolabile Nvoc (6-nitroveratryl) carbamate were similarly unsuccessful.

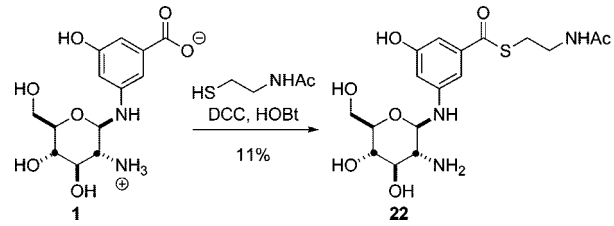
Because purification and handling of these polar reaction products proved to be quite onerous, we found in the *tert*-butoxycarbonyl (Boc) group a potential masking agent for the C-2 amino functionality that could undergo facile cleavage without the production of byproducts that require separation, thereby obviating extensive manipulation to

**Scheme 3.** Attempts To Prepare SNAC Thioester Analogues **21** and **26** of Amines **13** and **15**, Respectively




isolate the pure product. Assembly and deprotection of *N*-Boc-protected and reduced amine **28** readily provided the free amine **23**; however, acid-sensitive *N*-glycoside **26** was incompatible with standard conditions employed to deprotect the Boc-carbamate, as well as a plethora of alternative conditions that were explored (Scheme 5). To circumvent this problem, an alternative approach was necessary; namely, coupling amino acid **1** to *N*-acetylcysteamine using DCC afforded the desired thioester **22**, albeit in low yield (Scheme 4).

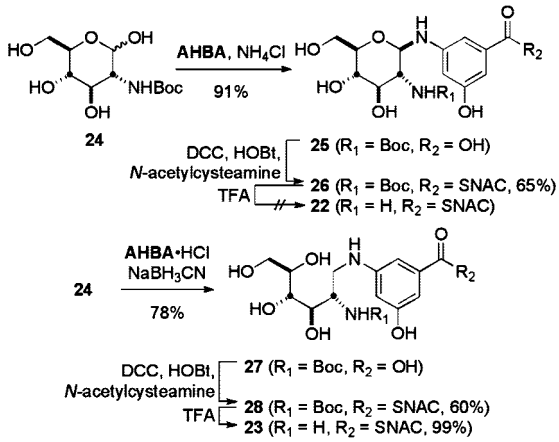
**Scheme 4.** SNAC Thioester Preparation



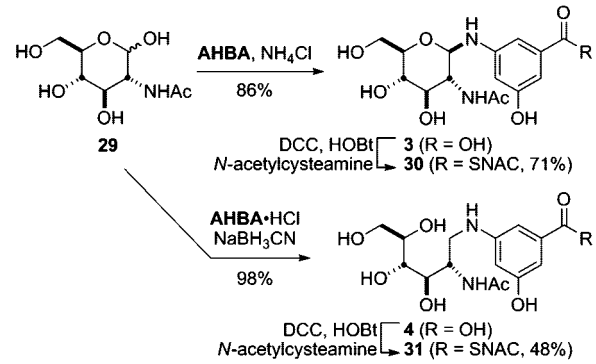

In addition to complex *N*-aryl glycosides of 2-amino-2-deoxyglucose, we considered biosynthetic intermediates bearing an acetyl residue on the C-2 nitrogen atom. Recent studies on the biosynthesis of the antibiotics teicoplanin and butirosin suggest that a common strategy for incorporating

(17) van der Berg, R. J. B. H.; Noort, D.; van der Marel, G. A.; van Boom, J. H.; Benschop, H. P. *J. Carbohydr. Chem.* **2002**, *21*, 167–188.
(18) Herlt, A. J.; Kibby, J. J.; Rickards, R. W. *Aust. J. Chem.* **1981**, *34*, 1319–1324.
(19) Brieskorn, C. H.; Hamm, R. *Arch. Pharm. (Weinheim)* **1986**, *319*, 673–682.
(20) Kolter, T.; Dahl, C.; Giannis, A. *Liebigs Ann.Chem.* **1995**, 625–629.
(21) (a) Ehmann, D. E.; Trauger, J. W.; Stachelhaus, T.; Walsh, C. T. *Chem. Biol.* **2000**, *7*, 765–772. (b) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253–1255. (c) Cane, D. E.; Yang, C.-C. *J. Am. Chem. Soc.* **1987**, *109*, 1255–1257.

**Scheme 5.** Attempts To Prepare SNAC Thioesters **22** and **23** from *N*-Boc-D-glucosamine (**24**)




**Scheme 6.** Preparation of SNAC Thioester Analogues **36** and **37** Incorporating *N*-Acetyl-D-glucosamine




a glucosamine moiety into secondary metabolites utilizes the abundant primary metabolite uridine diphospho-*N*-acetyl-D-glucosamine (UDP-GlcNAc).[22] Consequently, we have reassessed the function of early MMC biosynthetic enzyme *MitC* and now believe this protein may be involved in deacetylation of an early adduct of AHBA and D-glucosamine.[14,21] These findings, in conjunction with the 30% sequence identity found at the C-terminus of putative mitomycin glycosyltransferase MitB with UDP-GalNAc-polypeptide *N*-acetylgalactosaminyltransferase, stimulated our desire to analyze *N*-acetylated sugar derivatives.[13b] The presence of an *N*-acetyl group at the C-2 position prior to biosynthetic *N*-glycosylation is also chemically sound, since neighboring group participation can increase the rate of synthetic glycosylation reactions. Scheme 6 illustrates the facile approach used to prepare 2-*N*-acetyl-2-deoxy-D-glucosamine analogues.

The requisite substrates **3** and **4** were prepared as detailed in Scheme 6 and converted into their corresponding SNAC esters **30** and **31** in synthetically useful yields.

To establish whether amino acids **1** and **2** or *N*-acetylated analogues **3** and **4** are biosynthetic intermediates leading to the production of mitomycin C and FR900482, we must now assess how these potential substrates interact with the biosynthetic machinery of the respective *Streptomyces* spp., the organisms from which these antitumor agents were isolated. Sherman and co-workers have identified many of the genes believed to be responsible for the biosynthesis of FR900482 and the mitomycins in *Streptomyces* spp.[13a] We are currently examining whether compound **22** or **30** is a product of MitB catalysis and if compound **23** or **31**, respectively, follows in the biosynthetic pathway via MitF.

In conclusion, we have prepared several potential substrates believed to be early biosynthetic intermediates to FR900482 and the mitomycins in a straightforward fashion. Along with enabling ongoing biosynthetic studies, the availability of these putative biosynthetic intermediates may reveal additional, unforseen aspects of the biochemical production of mitomycin C and FR900482. For example, access to the various substrates described herein and more advanced potential biosynthetic intermediates may elucidate the role of enzymes implicated in the pathway, but whose function has not yet been ascertained. Studies to assess these issues are in progress, and we will report our findings in due course.

**Acknowledgment.** We gratefully acknowledge the National Institutes of Health (CA51975). Mass spectra were obtained on instruments supported by NIH Shared Instrumentation Grant GM49631. We dedicate this paper to Thomas A. and Margaret A. Vanderslice Professor of Chemistry T. Ross Kelly of Boston College on the occasion of his 65th birthday. We are grateful to Dr. John Greaves of the University of California, Irvine, for mass spectrometric data.

**Supporting Information Available:** Experimental procedures and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL802631C

(22) (a) Truman, A. W.; Huang, F.; Llewellyn, N. M.; Spencer, J. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 1462–1464. (b) Truman, A. W.; Robinson, L.; Spencer, J. B. *ChemBioChem* **2006**, *7*, 1670–1675.