ORGANIC LETTERS

2011
Vol. 13, No. 10
2786–2788

Asymmetric Enzymatic Glycosylation of Mitoxantrone

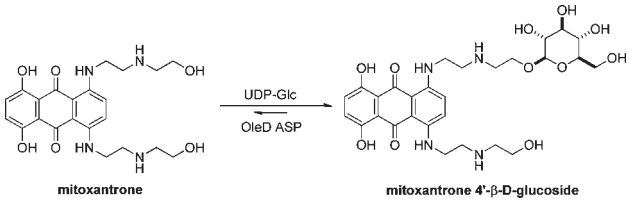
Maoquan Zhou and Jon S. Thorson*

Pharmaceutical Sciences Division, School of Pharmacy, Wisconsin Center for Natural Products Research, University of Wisconsin−Madison, 777 Highland Avenue, Madison, Wisconsin 53705, United States

jsthorson@pharmacy.wisc.edu

Received April 14, 2011

ABSTRACT



Using a uniquely promiscuous engineered glycosyltransferase (GT) derived from the macrolide-inactivating GT OleD, a single-step asymmetric glucosylation of one 'arm' of the drug mitoxantrone was efficiently achieved in high stereo- and regiospecificity. The synthesis, structural elucidation, and anticancer activity of the corresponding mitoxantrone 4′-β-D-glucoside are described.

The synthetic anthracenedione mitoxantrone (MXT) was developed as a drug in the 1970s and has been used in the treatment of metastatic breast cancer, acute myeloid leukemia, non-Hodgkin's lymphoma, and prostate cancer.[1] MXT is a topoisomerase II inhibitor, and its more recently recognized immunomodulatory properties have expanded the utility of this drug to include treatment for patients with secondary-progressive, progressive-relapsing, or worsening relapsing-remitting multiple sclerosis (MS).[2] In addition, MXT has demonstrated notable in vivo efficacy in animal models for rheumatoid arthritis[3] and was also recently found to be an efficient antituberculosis agent, functioning via inhibition of a specific mycobacterial kinase (PknB) which controls pathogen growth.[4] However, the side effects associated with MXT treatment, most notably irreversible cardiomyopathy, continues to spur efforts in generating analogues which achieve a broader therapeutic window (i.e., increased potency and reduced cardiotoxicity).[5]

Toward this goal, many anthracenedione analogs have been generated.[6,7] These studies have illuminated the impact of the C1 and C4 phenolic hydroxyl groups upon both anticancer potency and cardiotoxicity.[8] In general, substitution on the anthraquinone core diminished the activity. In addition, all nitrogen atoms were found to be critical as was the linker length between the nitrogen atoms and the

(1) (a) Van der Graaf, W.; Vries, E. D. *Anti-Cancer Drugs* **1990**, *1*, 109–125. (b) Weiss, R. B. *Oncology (Basel)* **1989**, *3*, 135–147. Legha, S. S. *Drugs Today* **1984**, *20*, 629–38.

(2) (a) Marriott, J. J.; Miyasaki, J. M.; Gronseth, G.; O'Connor, P. W. *Neurology* **2010**, *74*, 1463–1470. (b) Riahi, S.; Ganjali, M. R.; Dinarvand, R.; Karamdoust, S.; Bagherzadeh, K.; Norouzi, P. *Chem. Biol. Drug Des.* **2008**, *71*, 474–482. (c) Fox, E. *Clin. Ther.* **2006**, *28*, 461–474. (d) Scott, L. J.; Figgitt, D. P. *CNS Drugs* **2004**, *18*, 379–396. (e) Mazerski, J.; Martelli, S.; Borowski, E. *Acta Biochim. Pol.* **1998**, *45*, 1–11.

(3) Verdrengh, M.; Isaksson, O.; Tarkowski, A. *Rheumatology* **2005**, *44*, 183–186.

(4) Wehenkel, A.; Fernandez, P.; Bellinzoni, M.; Catherinot, V.; Barilone, N.; Labesse, G.; Jackson, M.; Alzari, P. M. *FEBS Lett.* **2006**, *580*, 3018–3022.

(5) (a) Fulbright, J. M.; Huh, W.; Anderson, P.; Chandra, J. *Curr. Oncol. Rep.* **2010**, *12*, 411–9. (b) Cavalletti, E.; Crippa, L.; Mainardi, P.; Oggioni, N.; Cavagnoli, R.; Bellini, O.; Sala, F. *Invest. New Drugs* **2007**, *25*, 187–195. (c) Evison, B. J.; Mansour, O.; Menta, E.; Phillips, D. R.; Cutts, S. M. *Nucleic Acids Res.* **2007**, *35*, 3581–3589.

(6) (a) Cheng, C. C.; Zee-Cheng, R. K. Y. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier: New York, 1983; Vol. p 20, 83 and references cited therein. (b) Zee-Cheng, R. K. Y.; Cheng, C. C. *J . Med. Chem.* **1978**, *21*, 291–294. (c) White, R. J.; Durr, F. E. *Invest. New Drugs* **1985**, *3*, 85–93.

(7) (a) Liu, Y.; Peacey, E.; Dickson, J.; Donahue, C. P.; Zheng, S.; Varani, G.; Wolfe, M. S. *J. Med. Chem.* **2009**, *52*, 6523–6526. (b) Johnson, M. G.; et al. *Bioorg. Med. Chem.* **1997**, *5*, 1469–1479. (c) Pors, K.; Shnyder, S. D.; Teesdale-Spittle, P. H.; Hartley, J. A.; Zloh, M.; Searcey, M.; Patterson, L. H. *J. Med. Chem.* **2006**, *49*, 7013–7023. (d) Krapcho, A. P.; Landi, J. J., Jr.; Shaw, K. J.; Phinney, D. G.; Hacker, M. P.; McCormack, J. J. *J. Med. Chem.* **1986**, *29*, 1370–1373.

(8) Removal of the hydroxyl groups lower cardiotoxicity as in ametantrone; see: Corbett, T. H.; Griswold, D. P., Jr.; Roberts, B. J.; Schabel, M., Jr. *Cancer Chemother. Pharmacol.* **1981**, *6*, 161–168.

10.1021/ol200977u
Published on Web 04/29/2011

basicity of terminal amines. Removal of one 'arm' was also found to be detrimental, and while a number of symmetrical disubstituted MXT conjugates have been generated (including amino acid,[9] galactose,[10] netropsin,[11] and protein[12]), the symmetry of MXT has proven to be a significant barrier to regioselective modification.[9–13] Herein we report that an engineered glycosyltransferase variant (OleD ASP)[14] surprisingly leads to the regio- and stereoselective modification of one 'arm' of MXT, providing a single unique MXT-glucoside that retains notable anticancer activity. This study reveals one of the first reported single-step asymmetric MXT modification strategies and highlights the synthesis of one of the only reported MXT monoglycosides to date. In addition, this work exposes an unexpected specificity for OleD ASP, a GT previously believed to primarily target aromatic nucleophiles.[14a,15]

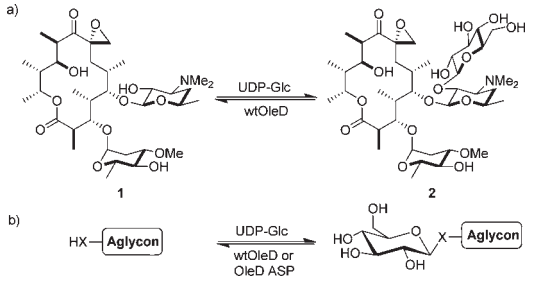


Figure 1. (a) Macrolide-inactivating reaction catalyzed by wtOleD (wild type OleD); (b) General *in vitro* reaction of OleDs with variant aglycons, X = O, S, NH, or NR.

Streptomyces antibioticus OleD catalyzes the transfer of glucose (from UDP-Glc) to various macrolide antibiotics as a means of self-resistance in macrolide-producing organisms (Figure 1a).[16] Directed evolution of OleD and subsequent screening for variants capable of glycosylating the model fluorescent coumarin acceptor 4-methyl umbelliferone led to the discovery of a triple mutant (OleD ASP) with a notably expanded donor and acceptor flexibility.[14] Preliminary LC-MS assessment of the OleD ASP acceptor flexibility revealed OleD ASP to glucosylate a diverse range of 'drug-like' scaffolds including anthraquinones, indolocarbozoles, polyenes, cardenolides, steroids, macrolides, β-lactams, and enediynes (Figure 1b).[17] Among the agents identified as putative substrates in this study, MXT leads to a monoglucosylated species based upon LC-MS. Based upon the established bias of OleD ASP toward aromatic nucleophiles, the putative OleD ASP-catalyzed MXT glucosylation was predicted to occur at C1 or C4. This postulation was also consistent with the known glucoronidation of C1 hydroxyl group as part of MXT metabolism in vivo.[18]

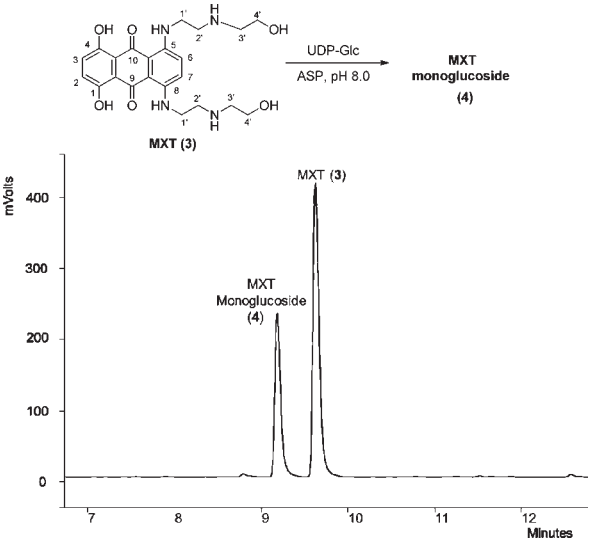


Figure 2. OleD ASP catalyzed glycosylation reaction of mitoxantrone and RP-HPLC analysis of pilot scale reaction. Assay and HPLC conditions are available in the Supporting Information.

To set the stage for a scale-up reaction to provide sufficient material for both full product characterization and biological evaluation, the pilot scale reaction conditions were first optimized. OleD variants (wt and ASP) were first overproduced and purified as previously described.[14] Figure 2 highlights the outcome of a representative reaction containing 0.25 mM MTX as the acceptor, 1.25 mM UDP-glucose (UDP-Glc) as the donor, 0.5 μg μL^{-1} purified OleD ASP as the catalyst in 50 mM Tris HCl (pH 8.0), and 5 mM $MgCl_2$ incubated at 25 °C for 16 h.[17] For the preparative scale reaction, MTX (5.6 mg, 12.6 μmol) was dissolved in 0.62 mL of DMSO and transferred to 50 mL of assay buffer solution (50 mM Tris HCl, 5 mM $MgCl_2$, pH

(9) (a) Hsin, L.-W.; Wang, H.-P.; Kao, P.-H; Lee, O.; Chen, W.-R; Chen, H.-W.; Guh, J.-H.; Chan, Y.-L.; Hi, C.-P.; Yang, M.-S.; Li, T.-K.; Lee, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1006–1014. (b) Zagotto, G.; Sissi, C.; Gatto, B.; Palumbo, M. *ARKIVOC* **2004**, *V*, 204–218.

(10) (a) Naleway, J. J.; Howard, R. A. Marker Gene Technologies, Inc., U.S. Patent 11/316,114, US 2006/0105915 A1, May 18, 2006. (b) Naleway, J. J.; Howard, R. A. Marker Gene Technologies, Inc., W.O. Patent 01/02020 A2, Jan. 11, 2001.

(11) Boitte, N.; Pommery, N.; Colson, P.; Houssier, C.; Waring, M. J.; Hénichart, J. P.; Bailly, C. *Anti-Cancer Drug Des.* **1997**, *12*, 481–501.

(12) Wirth, M.; Gabor, F.; Pittner, F.; Schalkhammer, T. *Scientia Pharmaceutica* **1996**, *64*, 737–744.

(13) Liu, W.-S.; Huang, Y.; Zhang, Z.-R. *Arch. Pharm. Res.* **2003**, *26*, 892–897.

(14) (a) Williams, G. J.; Zhang, C.; Thorson, J. S. *Nat. Chem. Biol.* **2007**, *3*, 657–662. (b) Williams, G. J.; Thorson, J. S. *Nat. Protoc.* **2008**, *3*, 357–62. (c) Williams, G. J.; Goff, R. D.; Zhang, C.; Thorson, J. S. *Chem Biol.* **2008**, *15*, 393–401.

(15) Yang, M.; Proctor, M. R.; Bolam, D. N.; Errey, J. C.; Field, R. A.; Gilbert, H. J.; Davis, B. G. *J. Am. Chem. Soc.* **2005**, *127*, 9336–7.

(16) (a) Quirós, L. M.; Carbajo, R. J.; Salas, J. A. *FEBS Lett.* **2000**, *476*, 186–189. (b) Quirós, L. M.; Carbajo, R. J.; Braña, A. F.; Salas, J. A. *J. Biol. Chem.* **2000**, *275*, 11713–20. (c) Bolam, D. N.; Roberts, S.; Proctor, M. R.; Turkenburg, J. P.; Dodson, E. J.; Martinez-Fleites, C.; Yang, M.; Davis, B. G.; Davies, G. J.; Gilbert, H. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5336–5341.

(17) Gantt, R. W.; Goff, R. D.; Williams, G. J.; Thorson, J. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 8889–8892.

(18) (a) Mian, J.; Mewes, K.; Ehninger, G.; Proksch, B.; Greger, B.; Waidelich, D.; Zeller, K.-P. *Cancer Res.* **1991**, *57*, 3427–3433. (b) Mewes, K.; Blanz, J.; Ehninger, G.; Gebhardt, R.; Zeller, K.-P. *Cancer Res.* **1993**, *53*, 5135–5142.

8.0). The reaction was initiated via addition of UDP-Glc (38 mg, 0.062 mmol) and 32 mg of OleD ASP. After 27 h of agitation at room temperature, the reaction was frozen and lyophilized to dryness. The residue was dissolved in methanol and subjected to HPLC purification to give MXT monoglucoside product (2.5 mg, 4.1 μmol) in 33% yield.

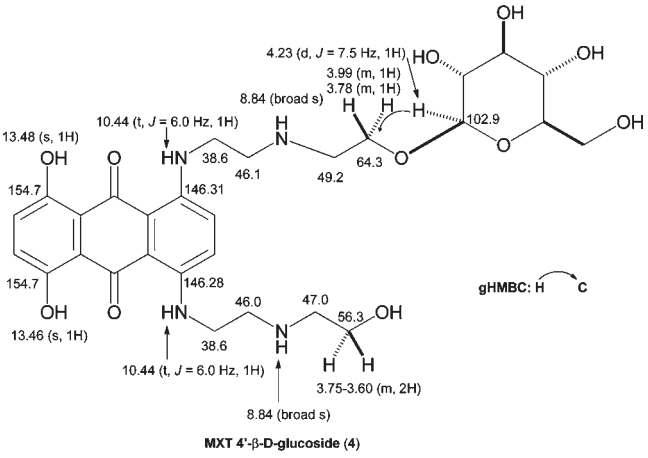


Figure 3. Key ^{1}H and ^{13}C NMR data and gHMBC correlations of MXT 4′-β-D-glucoside.

HiResMALDI-FTMS analysis of purified glucoside yielded an $[M + H]^+$ ion at m/z 607.26132 and $[M + Na]^+$ ion at m/z 629.24306, confirming a monoglucoside of mitoxantrone with a formula of $C_{28}H_{38}N_4O_{11}$ (calcd for $C_{28}H_{39}N_4O_{11}$, 607.26098; $C_{28}H_{38}N_4O_{11}Na$, 629.24293). ^{1}H and ^{13}C NMR along with gCOSY, TOCSY, gHSQCTOXY, gHMQC, and gHMBC data support the C4′ β-D-glucosidic structure presented in Figure 3 (see Supporting Information for complete ^{1}H and ^{13} C NMR assignments). The key evidence for C4′ glucosylation derives from the HMBC correlation between the anomeric proton and the C4′ carbon while the large coupling constant (7.5 Hz) of the anomeric proton (δ_H 4.23, doublet) serves as a key signature for the β-anomer. That OleD ASP catalysis led to asymmetric regioselective modification of a single MXT 'arm' was surprising given the bias for this evolved catalyst for aromatic nucleophiles.[14a,15]

(19) (a) Weymouth-Wilson, A. C. *Nat. Prod. Rep.* **1997**, *14*, 99–110. (b) Langenhan, J. M.; Griffith, B. R.; Thorson, J. S. *J. Nat. Prod.* **2005**, *68*, 1696–1711. (c) Krěn, V.; Řezanka, T. *FEMS Microbiol. Rev.* **2008**, *32*, 858–889. (d) Williams, G. J.; Gantt, R. W.; Thorson, J. S. *Curr. Opin. Chem. Biol.* **2008**, *12*, 556–564. (e) La Ferla, B.; Airoldi, C.; Zona, C.; Orsato, A.; Cardona, F.; Merlo, S.; Sironi, E.; D'Orazio, G.; Nicotra, F. *Nat. Prod. Rep.* **2011**, *28*, 630–648.

(20) (a) Fu, X.; Albermann, C.; Jiang, J.; Liao, J.; Zhang, C.; Thorson, J. S. *Nat. Biotechnol.* **2003**, *21*, 1467–1469. (b) Hoffmeister, D.; Yang, J.; Liu, L.; Thorson, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13184–9. (c) Fu, X.; Albermann, C.; Zhang, C.; Thorson, J. S. *Org. Lett.* **2005**, *7*, 1513–5. (d) Zhang, C.; Albermann, C.; Fu, X; Thorson, J. S. *J. Am. Chem. Soc.* **2006**, *128*, 16420–164211. (e) Zhang, C.; Moretti, R.; Jiang, J.; Thorson, J. S. *ChemBioChem* **2008**, *9*, 2506–2514. (f) Williams, G. J.; Yang, J.; Zhang, C.; Thorson, J. S. *ACS Chem. Biol.* **2011**, *6*, 95–100.

Table 1. Cancer Cell Line Cytotoxicity (nM)

compound	Hep3B	MCF7	MDA MB231	K562
MXT (**3**)	15 ± 2	2 ± 0.1	11 ± 0.6	490 ± 23
MXT-D-Glc (**4**)	54 ± 1	16 ± 0.3	44 ± 0.2	–[a]

[a] > Highest dose tested (1 μM).

The cytotoxicity of the MXT 4′-β-D-glucoside (**4**, Figure 3) was evaluated in three human cancer cell lines including liver (Hep3B), breast (MCF7 and MDA MB231), and leukemia (K562) with MXT (the parent compound) as the comparator (Table 1). Interestingly, the MXT β-D-glucoside retained notable potency, with IC_{50} values below a threshold of 60 nM (~4−8-fold the activity of the parent drug) in three out of four cell lines tested. This stands in marked contrast to prior glycosides reported, such as the mitoxantrone 4′,4′-β-di-D-galactoside, which was inactive.[10] Given that glycosylation of pharmaceutically important compounds can often markedly alter key pharmacological properties including drug pharmacokinetics and distribution,[19] it is reasonable to assume the MXT 4′-β-D-glucoside may offer the potential for a distinct toxicity profile from that of the parent drug.

In summary, this study is noteworthy both from the perspective of catalyst development as well as therapeutic lead development. From the perspective of catalyst development, this work uncovered a unique activity of an engineered glycosyltransferase (OleD ASP) that enabled the first regio- and stereoselective asymmetric glucosylation of a single 'arm' of the anticancer drug mitoxantrone (MXT). This efficient and specific single-step enzymatic reaction is also advantageous as it requires no protecting group or sugar activation manipulations. From a therapeutic lead perspective, this work highlighted the synthesis of the first monoglucoside of MXT and the first glycoside to show comparable anticancer activity to the parent. Given that glycosylation of pharmaceutically important compounds can often markedly alter corresponding pharmacokinetics and distribution, it is reasonable to assume that the MXT 4′-β-D-glucoside may offer a potentially beneficial toxicity profile which might be further optimized via glycodiversification methods.[20,21]

Acknowledgment. We thank the School of Pharmacy Analytical Instrumentation Center for analytical support and the Keck UWCCC Small Molecule Screening Facility for cancer cell line cytotoxicity screening. J.S.T. holds the Laura and Edward Kremers Chair in Natural Products. This work was supported by NIH AI52218.

Supporting Information Available. Experimental procedures, figures and characterization of compounds, and comprehensive assay data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

(21) UDP-Xyl, UDP-GlcNH$_2$, or UDP-GlcNHAc with OleD ASP led to conversions of < 5% while OleD mutant AIP (ref 14c) provided 16% conversion with UDP-Xyl.