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Natural Product Diversification Using a Non-natural Cofactor Analogue of *S*-Adenosyl-L-methionine

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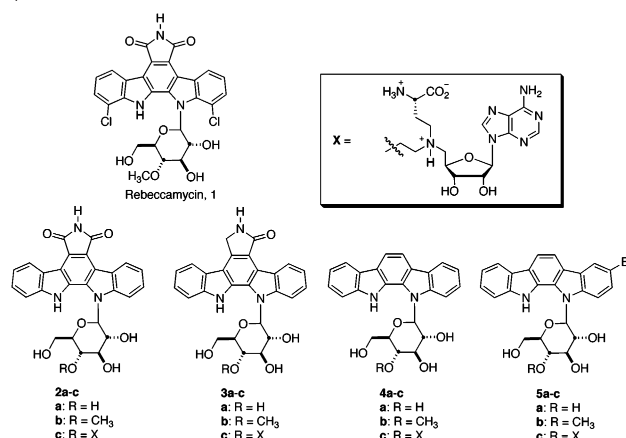
Natural products represent an unparalleled source of molecular diversity in drug discovery and complement emerging molecular sources, such as combinatorial libraries.[1] Yet there remains an urgent need to identify new chemotypes as leads for effective drug development in many therapeutic areas. Among the challenges facing efforts to diversify natural products is the need to remain competitive with synthetic and combinatorial libraries. Significant advances have been made on this front by exploiting the tailoring enzymes involved in natural product biosynthesis; new structures bearing non-natural substituents and substitution patterns are the result. Natural product oxidases, acetylases, carbamoyltransferases, and glycosyltransferases (GTs) all represent tailoring enzymes responsible for structural refinement of natural product skeletons.[2] All provide opportunities for natural product diversification. In particular, GTs have been very cleverly exploited to generate new natural product variants with new bioactivities.[3] Among such strategies, glycorandomization provides natural or engineered GTs access to unnatural sugars, the consequence being enzymatic modification of natural products with non-natural glycoside moieties.[4]

Natural product methyltransferases (MTases) represent another important class of tailoring enzymes.[2,3] For instance, the clinically significant avermectins, rapamycin, erythromycin, and rebeccamycin (**1**) all bear *S*-adenosyl-L-methionine (SAM)-derived methyl groups.[5] Rebeccamycin and related indolocarbazole alkaloids are of particular biosynthetic interest because of their tremendous therapeutic potential for cancer and neurodegenerative diseases.[6] Indeed, much elegant work has focused on the elucidation and utilization of the enzymes responsible for rebeccamycin biosynthesis, including rebeccamycin MTase (RebM).[7] In the presence of non-natural cofactors, such MTases might enable a powerful new means of rebeccamycin diversification.

We show here that RebM efficiently produces new rebeccamycin analogues upon presentation with synthetic cofactor **7**.[8] The 4′-OH ordinarily methylated in a number of RebM substrates (Scheme 1) undergoes MTase-dependent linkage to **7** through the probable agency of SAM mimic **8** (Figure 1).[9] Thus, the combination of natural product MTases and **7** allows a new means by which to increase the diversity of natural product analogues for drug discovery efforts.

We have relied primarily on didechloro analogue **2a** for structural and functional studies of RebM (Scheme 1). Demethylated **3a**−**5a** and 4′-O-methylated analogues **3b**−**5b** also have been useful for studies of RebM. Importantly, the efficiency of **2a** and **3a** methylation by RebM is far superior to that observed with the more highly truncated **4a** and **5a** (Supporting Information). We thus selected indolocarbazole **2a** as the primary substrate for studies of RebM and **7**. Although **7** and related congeners are known MTase-directed DNA alkylating agents, the ability of these synthetic cofactors to be used by alternative MTase classes has not been documented.[8,10]

Scheme 1. Rebeccamycin and Related Analogues Used to Investigate RebM Compatibility with Synthetic Cofactor **7** (Figure 1)



RebM-mediated modification of **2a** was monitored by RP-HPLC (Figure 2). To start, we noted that treatment of **2a** with **7** in the presence of denatured RebM yielded no discernible change to **2a**; **7** appears to lack the capacity for nonspecific alkylation of **2a**. That RebM is functional prior to denaturation is shown in panel B; reaction of **2a**, **6**, and RebM provides the 4′-O-methyl product **2b** with a slower retention time than **2a**. The addition of the methyl group was confirmed by mass spectrometry (Figure 2). In contrast, reaction of **2a** with RebM and **7** affords a faster mobility substance (panel C). The mass of this product is consistent with nucleoside

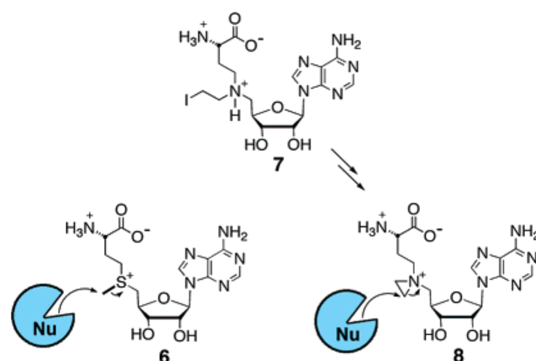


Figure 1. Depiction of MTase-promoted biomolecule (Nu) alkylations with SAM (**6**) and *N*-mustard **7** (through the intermediacy of **8**).

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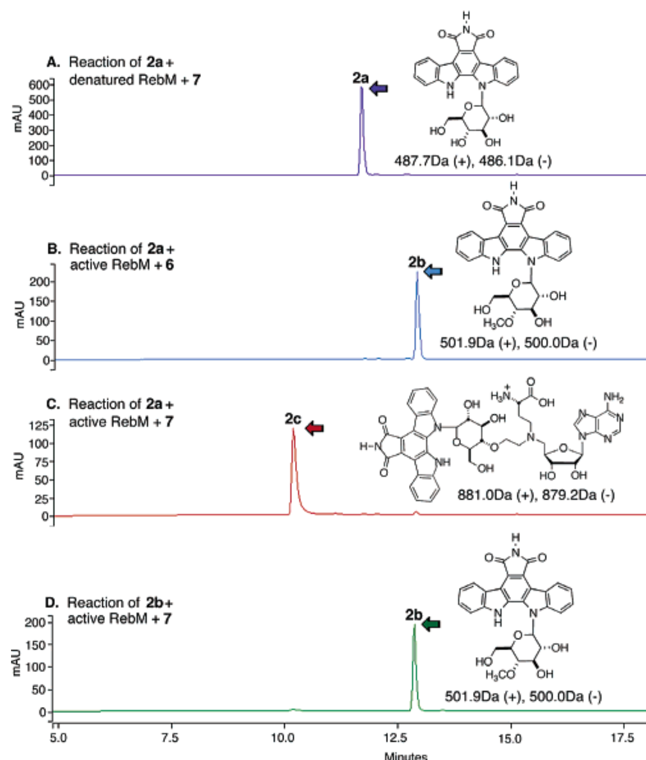

Figure 2. RP-HPLC analysis of RebM reactions of **2a** or **2b** with SAM or **7**. Structures shown indicate reaction products. Reactions were 50 μM in **2a** (or **2b**), 20 μM RebM, and 100 μM SAM (or **7**). All reactions were conducted at 30 °C for 4 h. Panel A: reaction of **2a**, heat denatured RebM and **7**. Panel B: reaction of **2a**, RebM, and SAM. Panel C: reaction of **2a**, RebM, and **7**. Panel D: reaction of **2b**, RebM, and **7**. Product masses are shown for both positive and negative modes (ESI).

conjugate **2c** as are MS/MS fragmentation patterns and ^{1}H NMR data (Supporting Information). Comparison of panels B and C indicates that RebM is just as amenable to exploitation of **7** as a cofactor as it is to SAM over the 4 h reaction time examined.[11]

N-Mustard **7** is highly active, and **2a** presents more than just one potential site of modification. How then can the regiochemistry of **2a** alkylation with **7** be addressed? We sought to answer this using 4′-O-methylated substrate **2b**. Substrate alkylation with **7** at sites other than the 4′-OH would be expected to proceed with comparable efficiencies for both **2a** and **2b**. However, 4′-OH methylation would prevent this position's enzyme-dependent linkage to **7**. Panel D shows that, in fact, 4′-O-methylation abrogates RebM-mediated attachment to cofactor **7**. Thus, RebM-mediated alkylations of **2a** with SAM and synthetic cofactor **7** share the same regiochemistry.

On the strength of these findings, we evaluated RebMs' ability to modify **3a**−**5a** with **7**. In all cases, RebM used cofactor **7** as efficiently as SAM (**6**) (Supporting Information). In particular, results obtained using substrate **3a** closely resemble those shown for **2a**. Both indolocarbazole substrates are almost quantitatively alkylated using RebM with either SAM or **7** after 4 h at 30 °C (Supporting Information).

5′-Aziridinyl adenylates and related *N*-mustards are MTase-dependent DNA modifying agents that can enable abiotic chemoselective ligations following MTase chemistry.[10,12] Thus, they represent potentially powerful tools with which to answer questions about the biological importance of methylation. *However, to date, such agents have only been shown to be compatible with DNA MTases*. It is therefore highly significant that **7** can be used by a natural product MTase en route to new rebeccamycin analogues. The combination of **7** and related congeners along with natural product MTases may thus afford a new means of enhancing structural diversity among natural product-like libraries. Rebeccamycin's topoisomerase I inhibitor activity and the established importance of its glycoside moiety in providing the agent's useful cytotoxic effects provide motivation for our continued study of the chemistry and biology of nucleoside conjugates **2c**−**5c** as well as the application of **7** and related congeners to other natural product MTases.

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Supporting Information Available: RebM overexpression and purification procedures, reaction conditions for reactions involving RebM, SAM, and **7**, and RP-HPLC analyses (with mass spectral data) and for reactions involving **3a**−**5a**. Characterization data for **2c** and experiments involving differing RebM concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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