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Selective Functionalization of Antimycin A Through an *N*-Transacylation Reaction

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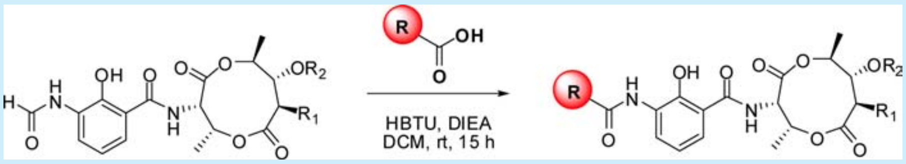
Supporting Information

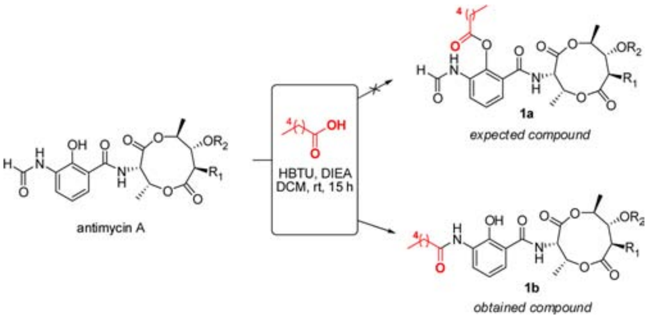


ABSTRACT: Acylation of 3-(*N*-formylamino)salicylic acids resulted in transacylation with loss of the formyl moiety. The reaction proceeds through a bis-*N*-acylated intermediate, which undergoes facile deformylation. This transacylation reaction has been employed for the site-specific functionalization of the mitochondrial poison antimycin A, affording several novel derivatives. The selective cytotoxicity of some of these derivatives toward cultured A549 human lung epithelial adenocarcinoma cells, in comparison with WI-38 normal human lung fibroblasts, illustrates one application of this transacylation reaction.

Antimycin A denotes a family of structurally related natural products produced by Streptomyces; they differ only by the aliphatic substituents appended to the bislactone moiety.[1] These compounds have been shown to inhibit the activity of complex III of the mitochondrial electron transport chain, resulting in inhibition of autophagy[2] and induction of apoptosis[3] in cultured mammalian cells. Accumulating evidence supports the involvement of mitochondrial bioenergetics and signaling in tumorigenesis and suggests that agents that target these functions may find utility in the chemotherapy of some tumors.[4] Several classes of molecules, including antimycin A, are of interest in this regard.[5] Hockenbery and co-workers described the effect of 2-methoxy antimycin A_3 as an experimental antitumor agent; this compound was reported to have minimal effects on the respiratory chain but to compete with a proapoptic Bak BH3 peptide for binding to recombinant Bcl-2. Consequently, cells expressing Bcl-x_L, which are usually resistant to anticancer drugs, were hypersensitive to antimycin A.[6] In addition to antimycin A, several types of other molecules targeting Bcl-2 or Bcl-x_L have been in (pre)clinical trials,[7] but no further efforts to develop therapeutic agents based on the structure of antimycin A have been described.

In an effort to develop new antimycin A derivatives by functionalization of the natural product,[8] we attempted the *O*-acylation of antimycin A, in analogy with the reported[6a] *O*-alkylation. As shown in Scheme 1, attempted *O*-acylation with hexanoic acid resulted instead in replacement of the *N*-formyl moiety. As a consequence we began to investigate this unexpected result. The *N*-transacylation of formylated aniline derivatives does not seem to have been reported using metal free methods. Literature examples of methods to effect the alteration of alkylcarboxamide structures involve metal catalysts such as palladium in aminocarbonylation reactions,[10] ruthenium catalyzed olefin hydrocarbamoylation reactions,[11] and more recently cobalt catalyzed activation of aldehydic C−H bonds.[12] Moreover, those reactions were all performed at high temperatures (>100 °C), reinforcing the unusual nature of the transformation outlined in Scheme 1, which occurred at room temperature.

Scheme 1. Acylation of Antimycin A: An Unexpected Result



Since the sample of antimycin A that we had employed was actually a mixture of congeners,[8] a simpler model system was employed initially to study the *N*-transacylation reaction. Scheme 2 presents the synthesis of an antimycin A model compound in three steps. Hydrogenation of 3-nitrosalicylic acid under pressure afforded the respective aniline derivative. Formylation of this derivative was performed using the conditions reported by Pettit et al.[13] to afford compound **2a** in 85% overall yield for the two steps. Amidation in the presence of *n*-butylamine gave model compound **2b** in 48% yield, along with the deformylated compound **2c** (44% yield). The latter was reformylated by treatment with formamide,

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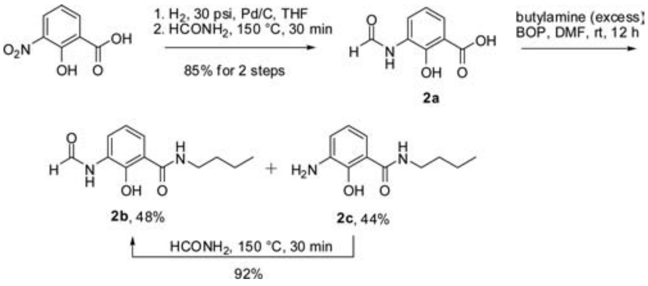
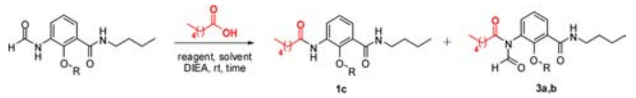
Scheme 2. Synthesis of AMA Model Compound 2b



exactly as described for the initial preparation of compound **2a**. With compound **2b** in hand, we studied its reactivity with activated esters of hexanoic acid under a variety of conditions (Table 1).

Table 1. Study of the Transacylation Reaction[a]



entry	R	time (h)	solvent	coupling reagent	yield **1c**[b] (%)	yield **3**[b,c] (%)
1	H	15	CH_2Cl_2	PyBrOP	39	37
2	H	24	CH_2Cl_2	PyBrOP	43	37
3[d]	H	30	CH_2Cl_2	PyBrOP	88	-
4	**H**	**15**	**CH_2Cl_2**	**HBTU**	**91**	-
5	H	15	CH_2Cl_2	BOP	84	-
6	H	15	THF	HBTU	68	20
7	H	24	THF	HBTU	86	-
8	H	15	DMF	HBTU	80	3
9	H	15	CH_3CN	HBTU	83	-
10	Bn	15	CH_2Cl_2	PyBrOP	-	14
11	Bn	15	CH_2Cl_2	HBTU	-	72

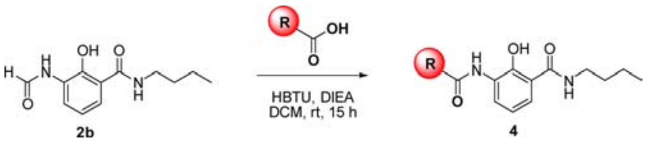
[a] All reactions were performed using DIEA (3 equiv) as base at a 0.2 M concentration of **2b** at room temperature under an argon atmosphere. [b] The yields represent pure compound isolated following purification on a silica gel column. [c] **3a**, R = H; **3b**, R = Bn. [d] Two equivalents of DIEA were added to the reaction mixture of entry 2 after 24 h, and the reaction was maintained for an additional 6 h until it was complete.

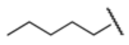
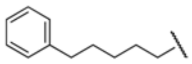
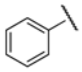
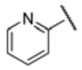
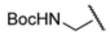
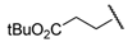
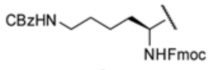
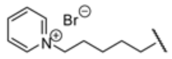
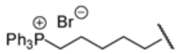
It was quickly apparent that the use of PyBrOP resulted in lower yields than was realized using HBTU or BOP. The latter provided the *N*-transacylation product **1c** in good yield after 15 h of reaction in CH_2Cl_2 at room temperature. It is important to note that the low yield of this product using PyBrOP was mainly a consequence of recovering significant amounts of *N*-formylated analogue **3**,[14] which did not happen using the other two coupling reagents. That the *N*-bisacylated species **3** is an intermediate on the reaction pathway from **2b** to **1c** was established by adding two additional equivalents of DIEA after 24 h of reaction (Table 1, entry 3); after an additional 6 h, **1c** was obtained in good yield, essentially equivalent to that obtained using HBTU (entry 4). The use of a solvent other than CH_2Cl_2 (e.g., THF, DMF, or CH_3CN) did not give significantly different results, indicating that the transacylation reaction should be compatible with a variety of organic solvents. Only a kinetic effect was observed, especially for THF, which required at least 24 h to achieve complete conversion. Important mechanistic information was obtained by using an *O*-benzylated analogue of **2b** as a starting material for the transacylation reaction. Even using the conditions that we determined to be optimal for **2b** (entry 4), the reaction appeared to stop at intermediate **3** (Table 1, entries 10 and 11). Clearly, the phenolic moiety is involved in the deformylation reaction leading from **3** to **1c** since in the absence of this free OH group no product was formed. While the precise mechanism for deformylation is presently unclear, it may be noted that incubation of 1 mM **3a** in a 1:2 mixture of acetonitrile and 10 mM phosphate buffer, pH 7.4, resulted in complete conversion to **1c** within 1 h.

In addition to its role in providing insights into the transacylation mechanism, it may be noted that **3a** represents a plausible prodrug for the final transacylated product, which may find utility in biological/therapeutic contexts.

The optimized reaction conditions (Table 1) were then applied to define the scope of this transacylation reaction. As shown in Table 2, ten structurally diverse carboxylic acids afforded the anticipated transacylation products (**1c**, **4a-i**) in isolated yields ranging from 85% to 99%, confirming the generality of the transformation.

Table 2. Scope of the Transacylation Reaction[a]

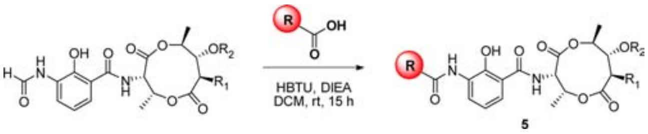


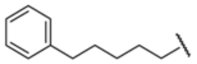
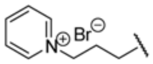
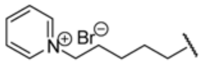
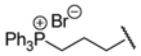
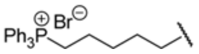
compound	R	product yield (%)
1c		91
4a		94
4b		99
4c		92
4d		89
4e	 BocHN	87
4f	 $tBuO_2C$	93
4g	 CBzHN NHFmoc	97
4h[b]	 $Br^{\ominus}$	85
4i	 $Ph_3P^{\oplus}$ $Br^{\ominus}$	89

[a] All reactions were performed using DIEA (3 equiv), **2b** (0.2 M), and HBTU (1.2 equiv) in CH_2Cl_2 at room temperature under an argon atmosphere for 15 h. [b] DMF was used as a solvent to optimize the solubility of the pyridinium salt.

The same reaction conditions were then employed for the attempted derivatization of antimycin A, using the commercially available mixture of this natural product.[8] The use of acetic acid and 6-phenylhexanoic acid afforded the expected products **5a** and **5b** in 75 and 99% yields, respectively (Table 3).[15] Numerous reports have established the ability of cationic functional groups to effect the delivery of small molecules to the mitochondria.[16] Since the mitochondria represent a major locus of action of antimycin A, triphenylphosphonium and pyridinium-containing carboxylic acids were prepared and used to provide access to antimycin A derivatives **5c**−**5f** in good yields.[15] Overall, the six antimycin A derivatives were obtained in yields ranging from 73 to 99%. The somewhat lower yields obtained for the derivatives containing a pyridinium moiety (**4h**, **5c**, and **5d**) were all associated with the use of DMF as solvent to facilitate dissolution of the carboxylic acid; this solvent was associated with a somewhat lower yield during the initial optimization experiments (Table 1, entry 8). The lower yields may also be related to small losses of material during the aqueous washing in the workup process.

Table 3. Application to the Selective *N*-Acylation of Antimycin A[a]



compound	R	product yield (%)
5a		75
5b		99
5c[b]		74
5d[b]		73
5e		90
5f		95

[a] All reactions were performed using DIEA (3 equiv), **2b** (0.2 M), and HBTU (1.2 equiv) in CH_2Cl_2 at room temperature under an argon atmosphere for 15 h. [b] DMF was used as a solvent to optimize the solubility of the pyridinium salt.

A preliminary investigation of the biological properties of AMA analogues **5a**−**5f** has been carried out, involving their cytotoxicity toward cultured mammalian cells. The assay involved a comparison of the effects of these compounds on A549 human lung epithelial adenocarcinoma cells, in comparison with WI-38 normal human lung fibroblasts. The results are reported in Figure 1. While limited differences in activity were noted for these compounds after 24 h of treatment, longer incubation times gave increasing divergence in expressed cytotoxicity among the individual analogues, and a clear selectivity for cytotoxicity toward cancer cells. These effects were especially pronounced for compound **5b**.

In summary, the convenient procedure described here for the functionalization of antimycin A should facilitate the further study of this mitochondrial poison, particularly at the levels of defining nonmitochondrial loci of action, and controlling trafficking to appropriate cellular compartments. It may also be noted that cyclodepsipeptides such as kitastatin 1 and respirantin,[13] as well as other natural products bearing the same 3-*N*-formylaminosalicylic acid moiety, might be amenable to functionalization using the same strategy.

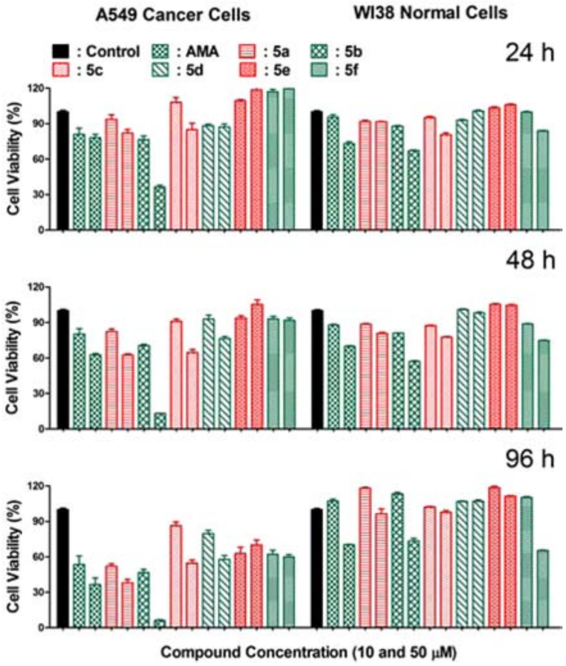


Figure 1. Cytotoxicity assays of compounds **5a**−**5f** in comparison with antimycin A for human lung cancer (A549) and normal lung fibroblast (WI-38) cells. Each of seven test compounds was employed at 10 *μ*M (left bar) and 50 *μ*M (right bar) concentrations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00882.

Experimental procedures and characterization data (PDF)
NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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(8) The commercial sample of antimycin A that we employed for our studies was actually a mixture consisting predominantly of antimycins A_1, A_2, A_3 and A_4, i.e. differing in the aliphatic substituents on the bislactone ring (cf Figure S1 of the Supporting Information).[9] For the experiments described here, we employed this mixture as received and assayed the product mixtures for their cytotoxic effects. Additionally, a sample of the commercial antimycin A mixture was separated by HPLC and the individual constituents were shown to have quite similar biological properties on the respiratory chain, and as cytotoxic agents.

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(14) The assignment of a *N,N*-bisacylated structure to compound **3a**, rather than a *N,O*-biosacylated species, was based both on the appearance of a phenolic H in the ^{1}H NMR spectra at ~ 13.1 ppm for entries 1, 2, 6 and 8 in Table 1, and on the fluorescence of compound **3a** (as compared with **3b**, entries 10 and 11 in Table 1), which requires the presence of a free phenolic OH group.

(15) In each case, high resolution mass spectrometric analysis of the isolated mixture revealed the presence of the expected molecular ion for each of the products derived from antimycins A_1, A_2, A_3 and A_4.

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