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Electronic Tuning of Site-Selectivity

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Abstract

Site-selective functionalizations of complex small molecules can generate targeted derivatives with exceptional step-efficiency, but general strategies for maximizing selectivity in this context are rare. Here we report that site-selectivity can be tuned by simply modifying the electronic nature of the reagents. A Hammett analysis is consistent with linking of this phenomenon to the Hammond postulate: electronic tuning to a more product-like transition state amplifies site-discriminating interactions between a reagent and its substrate. This strategy transformed a minimally site-selective acylation reaction into a highly selective and thus preparatively useful one. Electronic tuning of both an acylpyridinium donor and its carboxylate counterion further promoted site-divergent functionalizations. With these advances, a range of modifications to just one of the many hydroxyl groups appended to the ion channel-forming natural product amphotericin B was achieved. Thus, electronic tuning of reagents represents an effective strategy for discovering and optimizing site-selective functionalization reactions.

Introduction

Structurally complex small molecules have an extraordinary capacity to perform a wide range of useful functions. Accessing structural derivatives of these compounds, however, represents a major bottleneck in efforts to understand and/or optimally harness this capacity. Site-selective functionalization represents a frontier synthesis strategy with outstanding potential for addressing this limitation.[1–7] Site-selective acylation has emerged as a particularly promising approach for accessing derivatives of polyhydroxylated natural products, with the capacity for exceptional step-efficiency relative to total synthesis.[1,8–18] Current strategies for achieving selectivity in this context include modifying the steric and/or stereochemical features of the acylating reagents,[1,8–16] or utilizing lipase enzymes.[17,18] However, suboptimal site-selectivities too often limit the preparative utility of this approach. New strategies for maximizing site-selectivity or enabling the development of reagents that can override substrate bias to achieve site-divergent functionalizations stand to

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address these important limitations and thereby have a major impact on small molecule science.

In order for site-selective reactions to occur under kinetic control, information encoded in distinct local chemical environments within a substrate must be effectively translated into substantial differences in the energies of the corresponding transition states for functionalization. The Hammond postulate predicts that as a reaction becomes less exothermic, the corresponding transition state will become more product-like.[19] As a consequence, any potentially site-discriminating interactions between reagents and substrates should be amplified, thus leading to enhanced site-selectivities. Although the Hammond postulate has classically been invoked to explain a variety of different trends in reactivity,[20] a general approach for engaging this phenomenon to maximize site-selective functionalizations of complex small molecules has not, to the best of our knowledge, been previously reported.

We found inspiration for this approach in the context of asymmetric catalysis, where electronic tuning of reagents can have a substantial impact.[21–24] For example, with the Jacobsen epoxidation, i.e., more electron-releasing salen ligands lead to substantially increased enantioselectivities.[21] Mechanistic studies of this phenomenon are consistent with the Hammond postulate, i.e., electron-releasing ligands create milder manganese-based oxidants which, in turn, react via more product-like transition states.[22] This presumably amplifies the enantiotopic face-discriminating interactions between the chiral oxidant and the prochiral olefin thus yielding higher levels of enantioselectivity.[22] Guided by the logic outlined above and this encouraging precedent, we hypothesized that increasing the electron-richness of an acyl donor would lead to an increase in the product-like nature of the transition state for rate-limiting acyl transfer,[25–27] resulting in amplified site-discriminating interactions between an acyl donor and a polyol substrate and thus greater site-selectivity (Fig. 1a).

The decahydroxylated natural product amphotericin B (AmB) (Fig. 1b) represents an outstanding platform for testing this hypothesis, and recent advances have made the hydroxyl group at C2' a particularly important target for site-selective acylation (Fig. 1b).[28–35] Specifically, in contrast to the widely accepted channel model,[31–38] AmB primarily kills yeast via simply binding ergosterol,[30] a lipid that is vital for many aspects of yeast physiology. Competitive binding of cholesterol in human cells likely plays an important role in the substantial toxicity of this clinically vital antifungal agent.[29] Sterol binding is also critical for formation of the AmB-based ion channel,[29] a prototype for the development of small molecules that might replicate the function of deficient protein ion channels that underlie human diseases. For all of these reasons, gaining an atomistic understanding of AmB-sterol interactions represents a critical goal.

The mycosamine appendage is required for binding both ergosterol and cholesterol,[29] but the specific role(s) played by the three functional groups at C2', C3', and C4' remain unclear. In a leading model,[31–35] the C2'-OH is predicted to form a hydrogen bond with the 3β-OH on the A-ring of the sterol (Fig. 1b). If C2'-selective acylation could be achieved, orthogonal protection of the remaining hydroxyl groups and subsequent cleavage of the C2' ester would

generate a uniquely exposed hydroxyl group at this position. This site could then be selectively manipulated in a variety of ways to generate derivatives designed to more deeply probe the fundamental underpinnings of AmB function (Fig. 1c).

Separating constitutional isomers of highly complex small molecules can be very challenging. This classic problem is magnified substantially in the case of AmB because the physical properties of this natural product, including its amphipathicity and poor solubility in many organic solvents, make the utilization of standard chromatographic techniques ineffective in many cases. Because of this, and the fact that multiple grams of mono-acylated material would be needed to prepare site-selectively modified derivatives of AmB via the sequence outlined in Fig. 1c, a highly site-selective acylation of the C2' hydroxyl group was required.

As described below, electronic tuning proved to be an exceptionally effective strategy for solving this challenging problem. This methodology enables the rational transformation of a minimally site-selective reaction into a highly selective one that is preparatively useful. Importantly, this approach can be applied to increase site-selectivities even if the factors underlying site-discrimination in a specific case are unknown. We further report the surprising discovery that concomitant electronic tuning of both the acylpyridinium ion and its counterion can lead to site-divergent functionalization reactions. Collectively, these findings establish electronic tuning as a strategy for discovering and optimizing site-selective functionalizations of complex small molecules.

Results

To enable our search for a C2'-selective acylation of AmB, we first protected the C3' amine and C41 carboxylic acid as the corresponding phenylacyl amide[30] and methyl ester.[39] With the goal of biasing acylation towards the C2' position, we then selectively masked the hydroxyl groups at C13, C3/C5, and C9/C11 as a methyl ketal[28,40] and *p*-methoxybenzylidene acetals, respectively[30,41] (see Supplementary Information). Collectively these manipulations provided scalable access to derivative **1**, possessing five unprotected secondary hydroxyl groups. Of these remaining sites, steric considerations suggested that the C2'-hydroxyl group might be the most accessible to an acylating reagent.

When **1** was exposed to a standard set of acylating conditions [4-dimethylaminopyridine (DMAP), one equivalent of acetic anhydride (**2a**), diisopropylethylamine (DIPEA) in THF], a complex mixture containing many different mono-, bis-, and tris-acylated products was observed by HPLC (Table 1, entry 1). Analysis of this product mixture revealed that it contained only 2% of the C2' monoacylated product **3a**.

We thus attempted to achieve site-selective acylation at C2' via screening a large collection of commercial lipase enzymes under a wide range of reaction conditions and using many different acyl donors. However, while some encouraging results were obtained, these enzymatic reactions suffered from low conversions, lack of scalability, and/or poor reproducibility.

An established strategy for enhancing site-discrimination is to increase the steric bulk of the acylating reagent,[1,42,43] thereby increasing its sensitivity towards subtle differences in the local steric environments of different alcohols appended to the substrate. Following this approach, we evaluated a series of anhydrides with increasing steric bulk. However, little improvement in site-selectivity was observed with propionic (Supplementary Information) or isobutyric anhydride (**2b**) (Table 1, entry 2), and no conversion was observed for pivalic anhydride (Supplementary Information). Thus, steric modifications of the anhydride donors were unable to improve site-selectivity with this substrate.

Intrigued by reports that changing the counterion of acylpyridinium complexes can also impact site-selectivity,[16,44] we next surveyed the analogous series of sterically modified acyl chlorides. Treatment of **1** with acetyl chloride (**2c**) again provided a complex mixture of acylated products (Table 1, entry 3), further demonstrating that the site-discriminating features of **1** were subtle, and if there was an inherent preference for reactivity at C2', then this preference was small. Pivaloyl chloride led to no reactivity. Encouragingly, however, when we increased the steric bulk of the acyl chloride donor in the form of isobutyryl chloride **2d**, we observed the somewhat selective formation of the major product **3b** (Table 1, entry 4, 48% site-selectivity). Although **3b** could not be separated from this complex mixture of products via standard silica gel chromatography, carefully optimized preparative HPLC provided a few milligrams of purified material. Characterization by multidimensional ^{1}H NMR analysis and high resolution mass spectrometry established that **3b** was monoacylated at C2' (Supplementary Information).

Albeit an important step forward, we were unable to develop a practical process for purifying intermediate **3b** on larger scale. Thus, it was ultimately not possible to transform this moderately site-selective acylation into a preparatively useful process. Faced with the need to substantially improve this site-selectivity, we considered the hypothesis that electronic tuning of the acyl donor might have an impact. As shown in Fig. 1a, the Hammond postulate predicts that increasing the electron–richness of the acyl donor will increase the product-like nature of the transition state of rate-limiting acyl transfer. As a result, the site-discriminating interactions between the acyl donor and the polyol substrate should be magnified. This, in turn, should lead to larger differences in the activation energies for acylations of different hydroxyl groups and thus greater site-selectivity.

To test this hypothesis, we alternatively employed electronically tunable *para*-substituted benzoyl chlorides as acyl donors under otherwise identical reaction conditions. The electron-deficient *p*-nitrobenzoyl chloride (**2e**) provided only a modest 39% site-selectivity for formation of the corresponding C2' acylated product **3c** (Table 1, entry 5). In contrast, simply switching to the much more electron-rich *p*-*N*,*N*-dimethylaminobenzoyl chloride (**2f**) donor provided the desired C2' acylated product **3d** with an outstanding site-selectivity of 72% (Table 1, entry 6).

To systematically evaluate whether this effect is primarily attributable to the electronic nature of the acyl donor, we performed a Hammett study[45] with a series of sterically similar *para*-substituted benzoyl chlorides. Importantly, control experiments confirmed that for all of these donors acyl transfer was irreversible and the rate of background acylation in the

absence of DMAP was negligible (Supplementary Information). Thus, the ratio of site-isomers (C2'/other) is attributable to kinetic selectivity for acyl transfer from the corresponding acylpyridinium complexes to one hydroxyl group versus the others. In turn, with a lack of correction for the minor contributions of multiple acylations noted, this ratio of site isomers is a function of the difference in energies of the transition states of the corresponding acylation reactions ($\Delta\Delta G^{\ddagger}$).

As predicted by the analysis in Fig. 1a, C2' site-selectivity progressively decreased as the electron-withdrawing capacity of the *para*-substituent increased (Fig. 2a). A Hammett plot of log[ratio of site-isomers (C2'/other)] vs. σ_{para} revealed a linear correlation with a negative slope (rho = −0.395) (Fig. 2b). A complementary prediction of the analysis presented in Fig. 1a is that the reaction rate should also exhibit a linear correlation with σ_{para}, but in the opposite direction. Specifically, as the electron-withdrawing character of the substituent on the aryl ring increases, the reaction rate should also increase. We tested this prediction directly by determining the relative initial rates for the same five reactions and in fact observed a linear and positive correlation between log(initial rate) and σ_{para} (Fig. 2c). Combining these experiments, a plot of C2' selectivity vs. initial rate (Fig. 2d) also revealed a linear correlation, as collectively predicted by the Hammond analysis presented in Fig. 1a. These results demonstrate that electronic tuning of reagents can have a substantial impact on site-selective functionalization of a complex small molecule substrate, and that this effect is consistent with the Hammond postulate.

With this concept established, an interesting observation from our earlier studies caused us to further question whether electronic tuning might also contribute to another frontier challenge in the area of site-selectivity, i.e., the development of reagent based site-divergent functionalization reactions. Specifically, although neither reaction was highly site-selective, we noted that acylations with isobutyryl chloride **2d** (Table 1, entry 4) and the corresponding anhydride **2b** (Table 1, entry 2) produced different outcomes.[16,44] As described above, the major product derived from **2d** is mono-acylated at C2'. In contrast, HPLC purification and multidimensional NMR characterization of the mixture of products formed from anhydride **2b** revealed a nearly stoichiometric mixture of derivatives mono-acylated at C4' and C15 (Supplementary Information).

We recognized that electronically modified derivatives of benzoic anhydrides would translate into concomitant electronic tuning of both the acylpyridinium ion intermediate and its associated carboxylate counterion. Because both of these components are thought to play a role during rate-limiting acyl transfer,[25–27] we anticipated that electronic tuning of these anhydrides might also have a substantial impact on site-selectivity. Due to the combinatorial and potentially competing nature of the effects, however, the specific outcome was difficult to predict in this case. When we reacted **1** with either the electron-rich *p*-tertbutylbenzoic anhydride **2g** (σ_{para} t-Bu = −0.20) or its electron-deficient counterpart *p*-nitrobenzoic anhydride **2h**, a very interesting pair of stereodivergent acylation reactions were observed. Specifically, with the electron-rich anhydride **2g** site-selective acylation of the C4' hydroxyl group is the primary pathway yielding **4a** as the major product (Table 2, entry 1). In contrast, utilization of the electron-poor anhydride **2h** caused a remarkable turnover in site-selectivity, with a new major product **5b** resulting from selective acylation at C15 (Table 2,

entry 2). It has been demonstrated that chiral catalysts can be used to achieve reagent-based site-divergent functionalizations of complex small molecules.[1,6,8–11] Importantly, we note that all of the site-divergent functionalizations shown in this work were achieved using only achiral reagents. Thus, electronic tuning has the potential to provide a highly complementary alternative approach for reagent-based site-divergent functionalizations of complex small molecule substrates.

Having established electronic tuning as a strategy for the development of site-selective functionalization reactions, we returned to the initial goal of selectively modifying the C2' position of AmB. A survey of various electron-rich benzoyl donors revealed that *p*-tertbutylbenzoyl chloride provided an optimized combination of C2' site-selectivity (66%), conversion (68%), and ease of purification of the monoacylated product **3e** by standard silica gel chromatography. Importantly, both this reaction and chromatographic purification proved to be readily scalable, providing more than 3 grams of purified **3e** (45% isolated yield) from a single run. Thus, electronic tuning can transform a minimally site-selective reaction into a highly selective and preparatively useful process.

With efficient and scalable access to monoacylated derivative **3e** in hand, unique exposure and subsequent functionalization of the C2' hydroxyl was readily achieved according to the plan outlined in Fig. 1c. Specifically, as shown in Fig. 3, concomitant protection of the four remaining hydroxyl groups as the corresponding diethylisopropylsilyl (DEIPS) ethers was followed by facile cleavage of the aryl ester at C2' with KCN in MeOH to yield **6**. As demonstrated with this transformation, another important benefit of the electronic tuning approach is that it allows relatively mild conditions to be employed to achieve deacylation. This stands in contrast to the much more forcing conditions typically required to remove sterically encumbered acyl groups, which can lead to competitive decomposition of complex small molecule substrates.

Compound **6**, having a uniquely exposed hydroxyl group at C2', has proven to be a highly versatile intermediate. For example, despite the presence of the very sensitive polyene macrolide core, efficient deoxygenation at C2' to form **7** was achieved via nucleophilic displacement of the axial C2'-hydroxyl group to generate the equatorial iodide[46] followed by a novel AgOAc-mediated reductive deiodination with $NaBH_4$.[47] Alternatively, epimerization at C2'[34] to yield **8** was readily achieved using standard Mitsunobu conditions. This approach also provides unique access to AmB-small molecule conjugates[48] linked via the C2' hydroxyl group. For example, a molecule of ergosterol was tethered to **6** via simple esterification with acid chloride intermediate **9** to form the novel heterodimer **10**, which is reminiscent of the predicted structure of the AmB-ergosterol complex (Fig. 1b).

Discussion

We have thus far only demonstrated the electronic tuning strategy with AmB, and the generality of this approach remains to be determined.[49] However, because our studies are consistent with linking of this effect to the Hammond postulate, and the Hammond postulate is broadly applicable to many reactions, our results suggest that electronic tuning may represent a general strategy for improving site-selective functionalizations of complex small

molecules. For example, it may be possible to combine this approach with a range of other nucleophilic catalysts[1,8–15] en route to highly optimized site-selective acylations and/or other group transfer processes.[50,51] Moreover, this same approach should be applicable to a wide range of other substrate-based site-selective transformations[1–7] as long as the corresponding functionalization reagents can be tuned electronically. In addition, because increasing the product-like nature of a transition state should increase the consequences of site-discriminating interactions regardless of their origins, this strategy should be applicable even in cases where the specific site-discriminating interactions are not understood. Of course, if the specific underpinnings can be elucidated, then a combination of electronic tuning and rational design of the reagent would potentially be synergistic.

Electronic tuning might also prove to be generally useful in the development of reagent-based site-divergent functionalization reactions. For example, if two different acylation catalysts produce modest levels of site-divergency with the same acid chloride, electronic tuning of the acid chloride should lead to parallel optimization of both site-selectivities, thereby yielding highly optimized site-divergency. Moreover, we found that site divergency can also be achieved via concomitant electronic tuning of both an acylpyridinium ion and its carboxylate counterion, which is accessible by simply modifying the corresponding readily available anhydride donors.

It is also highly notable that all of the site-divergent acylation reactions described herein involve only the use of achiral acylating reagents and catalysts. These findings suggest that physical and/or mechanistic features independent of stereochemistry stand ready to impact site-divergent functionalizations of complex small molecules in ways that exceed the current level of understanding and utilization.

In conclusion, the rate-limiting step in small molecule science is still all too often the synthesis of targeted derivatives. Site-selective functionalization represents a synthetic strategy with an exceptional level of theoretical efficiency, but the challenge in reducing this approach to practice with complex small molecule substrates can be substantial. Electronic tuning can enable the development of highly practical and synthetically useful site-selective functionalization reactions. This strategy may therefore facilitate advanced understanding and harnessing of the still largely untapped functional potential that complex small molecules possess.

Methods

Full experimental details, procedures, and characterization for new compounds are included in the Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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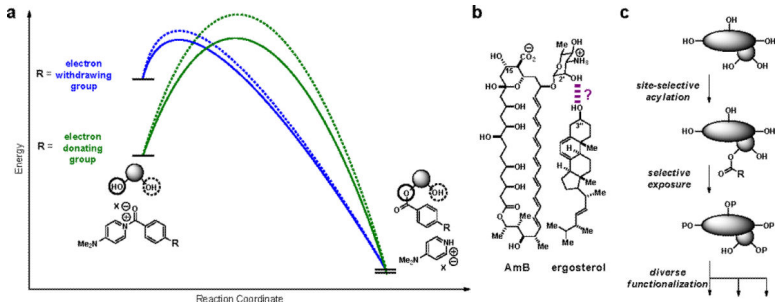
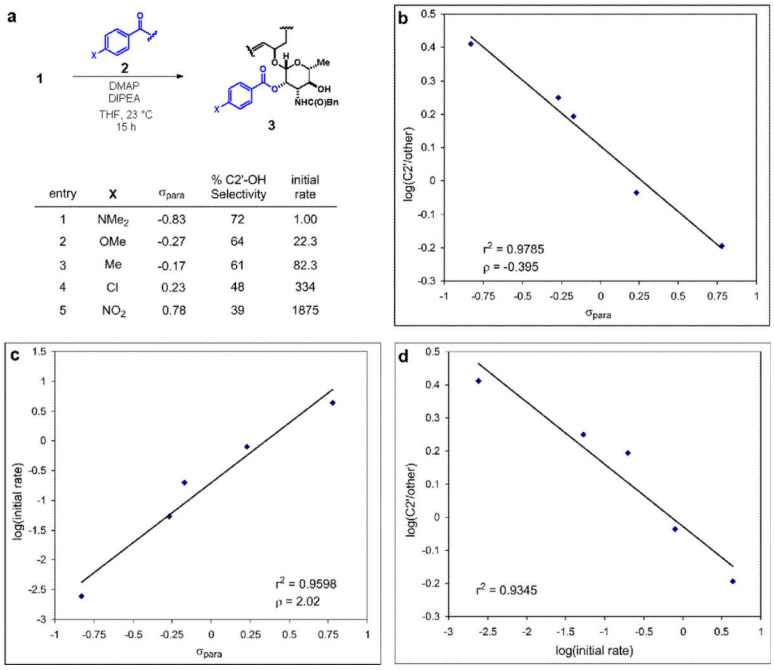


Figure 1. Overview of an approach to functionalize AmB to probe a possible interaction with ergosterol

a, The Hammond postulate applied to site-selectivity. More electron-rich acylpyridinium ions are predicted to react via a more product-like transition state in which the site-discriminating interactions between the acylating reagent and the polyol substrate are magnified. These enhanced interactions increase the difference in activation energies ($\Delta\Delta G^{\ddagger}$) for the acylation of one hydroxyl group (solid line) versus another (dashed line). **b,** Amphotericin B (AmB) and a putative interaction between the hydroxyl group at C2' and ergosterol. **c,** A strategy for site-selective functionalization of just one of many distinct secondary hydroxyl groups appended to AmB.



entry	X	σ_{para}	% C2'-OH Selectivity	initial rate
1	NMe_2	-0.83	72	1.00
2	OMe	-0.27	64	22.3
3	Me	-0.17	61	82.3
4	Cl	0.23	48	334
5	NO_2	0.78	39	1875

Figure 2. Analysis of acylation reactions with para-substituted benzoyl chlorides

a, A Hammett study of site-selective acylation. As the electron-withdrawing capacity of the substituent increased, the selectivity decreased and the rate increased. **b**, A Hammett plot of the log of the ratio of the product monoacylated at C2' to all other products as a function of σ_{para}. **c**, A Hammett plot of the log of the initial rate as a function of σ_{para}. **d**, A plot of the ratio of site isomers as a function of the initial rate. Values for the %C2'-OH selectivity, ratio of site isomers, and initial rate all represent the average of three trials.

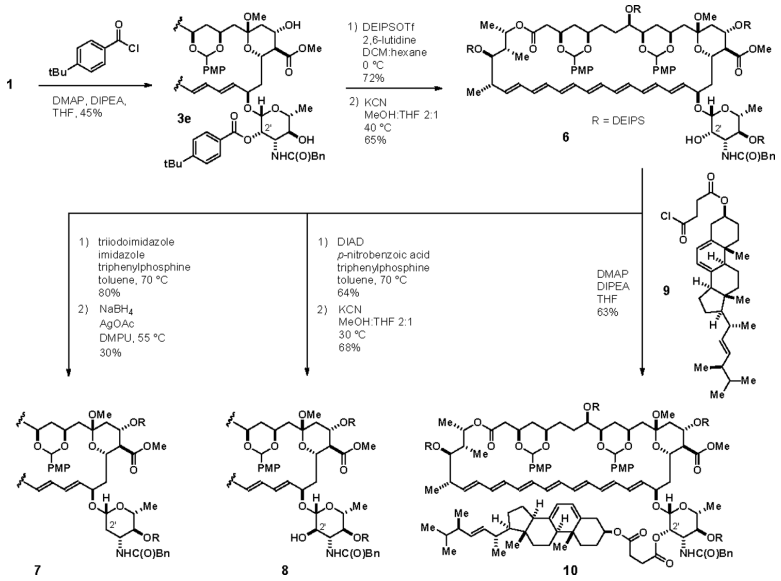
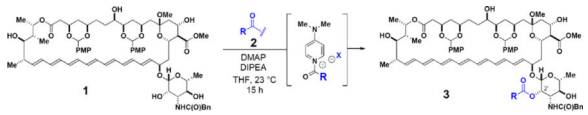
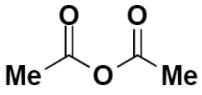
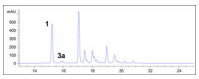
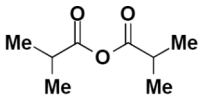
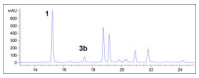
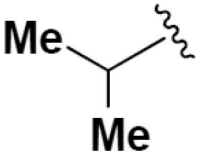
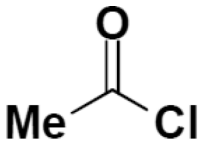
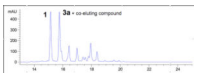
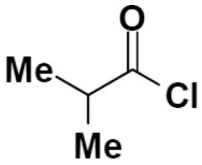
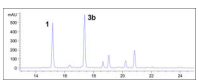
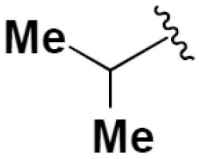


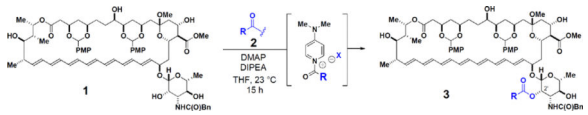
Figure 3. Selective functionalizations at the C2' position of AmB

Electronic tuning of the acyl donor enables selective acylation at the C2' hydroxyl of intermediate **1**. The acyl group acts as a temporary protecting group that is removed after orthogonal protection of the remaining hydroxyl groups leaving only the C2' hydroxyl exposed. The C2' hydroxyl group can then undergo a variety of functionalizations such as deoxygenation, stereochemical inversion, or acylation to form AmB-small molecule conjugates.

Table 1



entry	acyl donor(2)	hplc trace	C2' selectivity	R
1	 2a		2%	 3a
2	 2b		5%	 3b
3	 2c		26%	 3a
4	 2d		48%	 3b



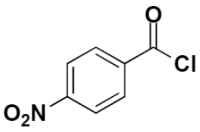
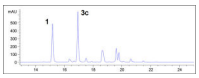
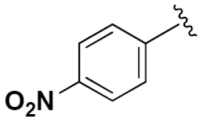
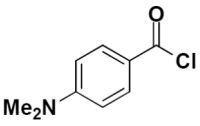
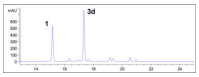
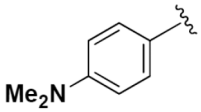
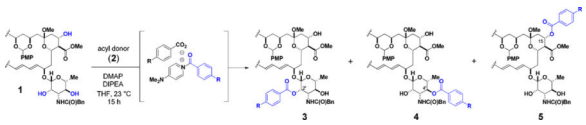
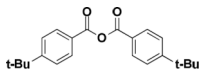
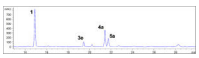
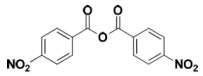
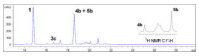
entry	acyl donor(2)	hplc trace	C2' selectivity	R
5	 2e		39%	 3c
6	 2f		72%	 3d

TAble 2



entry	acyl donor (2)	hplc trace	Ratio of products
1	 **2g**		**3e** (C2') **4a** (C4') **5a** (C15) (R = t-Bu) 1.0 4.4 2.2
2	 **2h**		**3c** (C2') **4b** (C4') **5b** (C15) (R = NO_2) 1.0 5.0 15.0