Synthesis and Cytotoxic Evaluation of Arimetamycin A and Its Daunorubicin and Doxorubicin Hybrids

Eric D. Huseman, Jo Ann W. Byl, Scott M. Chapp, Nathan D. Schley, Neil Osheroff, and Steven D. Townsend

ABSTRACT: The arimetamycin A glycan governs the compound’s cytotoxicity (IC_{50}). To study this branched, deoxy-amino disaccharide, we designed and synthesized a modified acyl donor that underwent glycosylation with three anthracycline aglycones: steffimycinone, daunorubicinone, and doxorubicinone. The result of the approach was a synthesis of arimetamycin A and two novel hybrid anthracyclines. Each molecule exhibited enhanced cytotoxicity in comparison to the parent anthracyclines, steffimycin B, daunorubicin, and doxorubicin. An orienting mechanistic evaluation revealed that the daunorubicin hybrid inhibits the ability of human topoisomerase II α to relax negatively and positively supercoiled DNA.

INTRODUCTION

The arimetamycins are a family of anthracycline natural products isolated following the heterologous expression of bacterial eDNA from soil.\(^1\)\(^,\)\(^2\) They demonstrated varying levels of antiproliferative activity across a panel of four cancer cell lines with arimetamycin A (AMA, 1) emerging with nanomolar cytotoxicity (IC_{50}) while arimetamycin B−C (AMB−AMC, 2−3) exhibited micromolar activity (Figure 1a). Furthermore, AMA is more cytotoxic than the clinical anthracycline chemotherapeutics daunorubicin (DNR, 5)\(^3\) and doxorubicin (DOX, 6)\(^4\) and maintained its nanomolar activity against the multi-drug-resistant (MDR) lung cancer line H69AR,\(^5\) a line with known anthracycline resistance due to its overexpression of the multidrug resistance protein 1 (MRP1) efflux pump\(^6\)\(^−\)\(^8\) (Figure 1b).

Structurally, the arimetamycins possess a common steffimycin aglycone, indicating that their glycans serve as the activity determining domain. AMB and AMC feature neutral \(L\)-olivose-based sugars and are similar to steffimycin (4) which is decorated by \(L\)-rhamnose (Figure 1a).\(^9\)\(^−\)\(^13\) In contrast, AMA bears a disaccharide composed of the rare branched deoxy-amino sugars \(L\)-brasiliose and \(L\)-lemonose, making it one of the few steffimycin anthracyclines to incorporate an amino sugar\(^12\)\(^−\)\(^15\) and the only known anthracycline with two branched dimethylamino sugars (Figure S1).

Due to its potent cytotoxicity and unique, activity modulating glycan, we chose AMA (1) as a target for chemical synthesis. Furthermore, as various disaccharide analogues of DNR and DOX have demonstrated promising biological activity,\(^16\)\(^−\)\(^19\) we hypothesized that merging the AMA disaccharide with the aglycone of these clinically useful anthracyclines would generate potent hybrid compounds. Biological evaluation of these hybrids and a comparison to 1 would indicate if the activity associated with the AMA disaccharide is specific to the natural steffimycin aglycone or is generally applicable to various anthracycline aglycones. To these ends, we describe herein the synthesis and initial biological evaluation of 1, DNR−AMA hybrid 7, and DOX−AMA hybrid (8) (Figure 1c).

RESULTS AND DISCUSSION

Retrosynthetic Analysis. The synthesis of AMA (1) and hybrids 7 and 8 is a formidable synthetic challenge for several reasons. First, the installation of the two \(\alpha\)-linkages must occur selectively despite the presence of the \(\text{syn} \ C3 \) methyl groups. Thus, each glycosylation would need to override an impending 1,3-diaxial interaction. Second, amine dimethylation requires careful orchestration. A postglycosidation methylation strategy must account for the susceptibility of the \(C7\)−\(C1\)′ benzyl ether linkage to hydrogenolysis\(^20\)\(^,\)\(^21\) and the lability of the \(C13 \alpha\)-hydroxy-ketone of the DNR and DOX aglycones to hydride reduction.\(^22\) Conversely, a preglycosylation methylation
approach requires the use of a polar glycosyl donor featuring two dimethyl amines. While not unprecedented, interaction between a Brønsted or Lewis acid promoter and the basic amines complicates the reaction. Lastly, reaction conditions must accommodate the glycosidic linkage instability inherent to deoxy sugars as compared to their fully oxygenated counterparts.

At the planning stage, we proposed forming both α-glycosidic linkages using thioglycoside donors activated under Hirama’s AgPF$_6$ mediated α-selective methodology as work from Wang, Sun, and co-workers demonstrated that these conditions are effective for coupling C3 branched donors with deoxy sugars and daunorubicine (10). Strategically, we elected to form the disaccharide prior to the introduction of the aglycones as this would enable divergent access to synthetic targets 1, 7, and 8. Finally, we planned to methylate the amines after glycosidation to the aglycones to avoid incompatibility between the basic amines and the glycosidation promotor.

To implement this strategy, we would derive aglycones semisynthetically via the hydrolysis of commercially available steffimycin B (18), daunorubicin (5), and doxorubicin (7), respectively (Scheme 1). We would obtain disaccharide donors from common disaccharide intermediate which would arise from the glycosylation of L-brasiliose acceptor and L-lemonose donor. As L-brasiliose and L-vancosamine differ only in the substitution of the C3 amine, protecting group manipulation and degradation of vancomycin-HCl (15) would provide access to acceptor 14. For donor 16, we would optimize our previously described de novo synthesis from L-threonine (17) as this approach proved inefficient for the preparation of large amounts of 16. Our route drew inspiration from previous

Figure 1. Structures of synthetic targets 1, 7, and 8.

Scheme 1. Retrosynthetic Analysis for the Divergent Synthesis of AMA (1), DNR–AMA Hybrid 7, and DOX–AMA Hybrid 8.

*Pg = protecting group; LG = leaving group.
syntheses of d-lemonose as well as related amino sugars D-AAT, l-kedarosamine, and D-callipeltose.

Synthesis of Common Disaccharide Intermediate 13.
We recently described the synthesis of L-lemonose thioglycoside in 10 steps from D-threonine (17, Scheme 2a). Our approach featured the gram-scale synthesis of vinylogous ester which underwent diastereoselective (≥20:1) 1,2-addition with CH₃Li to establish the C3 tetrasubstituted center. After silyl protection (TBSOTf) of the resulting allylic alcohol to give silyl ether, a 3-step sequence of glycal hydration...

Scheme 2. Gram-Scale Synthesis of L-Lemonose Thioglycoside 16

Scheme 3. Gram-Scale Synthesis of Common Disaccharide Intermediate 13

b. Minor β-Linked Glycosylation Product 25

c. Verification of 13's α-Linkage by XRD
Scheme 4. Initial Efforts to Synthesis DNR–AMA Hybrid 7 Leading to the Rational Design of 4th Generation Donor 12d

a. Summary of Initial Glycosidation Attempts

\[
\begin{array}{cccc}
\text{Entry} & R^1 & R^2 & \text{Temp.} & \text{Yield*} \\
1^\text{st} & \text{SPh} & H & -8^\circ \text{C} \rightarrow \text{rt} & \text{trace} \\
2^\text{nd} & \text{OH} & \text{H} & \text{PhSO}_2 & -78^\circ \text{C} \rightarrow -40^\circ \text{C} & \text{trace} \\
3^\text{rd} & \text{OTBS} & \text{TBS} & \text{BF}_3 \cdot \text{OEt}_2 & -78^\circ \text{C} & 12\% (88\%) \\
4^\text{th} & \text{OTBS} & \text{TBS} & \text{BF}_3 \cdot \text{OEt}_2 & -78^\circ \text{C} \rightarrow -60^\circ \text{C} & 35\% (>95\%) \\
5^\text{th} & \text{OTBS} & \text{TBS} & \text{BF}_3 \cdot \text{OEt}_2 & -78^\circ \text{C} \rightarrow -50^\circ \text{C} & 59\% (90\%) \\
\end{array}
\]

*Note: yield after FCC; yield based on recovered donor (4.12g) given in parentheses.

b. Unsuccessful Elaboration of 28 to DNR-AMA Hybrid (7)

(c) Rational Design of 4th Generation Donor 12d

16 \text{Hg(OAc)}_2, \text{amionic acetylation (Ac}_2\text{O), and thioglycosidation (PhSH, BF}_3 \cdot \text{OEt}_2) \text{yielded } \alpha/\beta-16 (\geq 20:1 \alpha/\beta). \text{Interestingly, } \alpha-16 \text{deviated from the expected } \text{C}_4 \text{ conformation as indicated by the } ^1\text{H} \text{coupling constant and NOEY analyses.}

While this initial route provided sufficient donor for orienting disaccharide glycosylation studies, the inefficiency of the glycal hydration (51% yield) limited material throughput. Gratifyingly, we found that the hydration proceeded in 70% yield on \textit{sans}-TBS glycal 21 to give diol 22 (Scheme 2b). Furthermore, we found that we could subject crude 21 to the hydration, allowing isolation of 22 in 64% yield over 2 steps on a gram scale from 19. Ensuing bis-silyl protection (TBSOTf) followed by 2-fold exposure to excess PhSH and TMSOTf concluded our second generation approach to thioglycoside 16 which was free of elimination product 20 as assessed by $^1\text{H}$ NMR.

Regarding the two-step nature of the thioglycosylation, crude $^1\text{H}$ NMR indicates that the first round of PhSH/TMSOTf treatment generates a complex mixture consisting of hydrolysis product $\alpha/\beta-24$ (52%) and thioglycoside $\alpha/\beta-16$ (43%) along with trace amounts of starting material $\alpha/\beta-23$ (4%) and elimination product 20 (1%) (Scheme 2d,e, entry 1). This mixture completely converges to thioglycoside $\alpha/\beta-16$ upon resubjection to the reaction conditions, suggesting that reducing sugar $\alpha/\beta-24$ and glycal 20 are competent substrates for this reaction (Scheme 2d,e, entry 2). The independent treatment of pure $\alpha/\beta-24$ to similar reaction conditions resulted in full conversion to thioglycoside $\alpha/\beta-16$, thus verifying the reducing sugar as a competent substrate (Scheme 2d,e, entry 3). In contrast, the exposure of pure glycal 20 gave only 20% conversion to $\alpha/\beta-16$, providing inconclusive evidence as to whether the complete disappearance of 20 upon resubjection is a result of its conversion to the thioglycoside or simply the result of material loss during workup or the limits of NMR detection (Scheme 2d,e, entry 4).

Having established a scalable synthesis of donor $\alpha/\beta-16$, we initiated the synthesis of acceptor 14. Known Cbz protection of commercially available vancomycin-HCl (15) and subsequent treatment with methanolic HCl provided an inconsequential mixture of methyl glycosides (Scheme 3a). Ensuing hydrolysis followed by chemoselective silyl protection (TBSCl) afforded $\beta$-pyranose 14.

With grams of each monosaccharide building block in hand, we proceeded to study their glycosylation. Subjection of acceptor 14 and excess donor $\alpha/\beta-16$ to Hirama-like conditions (AgPF$_6$, TTPB) gave a $\geq$20:1 mixture of coeluting disaccharides 13 and 25 after column chromatography (Scheme 3a,b, Tables S3 and S4). Initial inspection of major product 13's $J_{\text{H1-"H2}}$ coupling constants ($J = 7.2$, 2.9 Hz) indicated the formation of the undesired $\beta$-anomer. The measurement of the $J_{\text{C1-"H1}}$ coupling constant ($J = 162$ Hz) provided further evidence for a $\beta$-linkage ($\alpha$; $J_{\text{C1-"H1}} \sim 170$ Hz; $\beta$; $J_{\text{C1-"H1}} \sim 160$ Hz). Recalling our experience with the conformationally deviant $\alpha$-16 (Scheme 2a), we acquired a NOEY spectrum of 13. This experiment revealed correlations between H1′ ↔ C3'Me, H1′ ↔ C3'TBS$_{\text{Bu}}$ and H2′$_{\text{pro-S}}$ ↔ H4′, allowing us to deduce that the nonreducing lemonose sugar of 13 had undergone a chair flip to the unexpected $\text{C}_4$ conformation (Scheme 3a). The axial position of H1′ allowed us to reconcile the NOEY data with the $J_{\text{H1-"H2}}$ and $J_{\text{C1-"H1}}$ values and conclude that we had in fact synthesized the desired $\alpha$-linked 13 on the gram scale. Synthesis and single-crystal XRD of crystalline derivative 26 confirmed this NMR-based assertion (Scheme 3c).

Regarding the selectivity of the glycosylation event, NMR analysis of the CDA$_3$ soluble crude materials coupled with the high isolated yield (82%) for the desired $\alpha$-linked 13 led us to conservatively conclude that 13 was formed in a $\geq$4:1 ratio to any other disaccharide component (Figure S4). The NMR analysis of minor, coeluting disaccharide 25 revealed the presence of a $\beta$-linkage (as confirmed by $J_{\text{H1-"H2}}$; $J_{\text{C1-"H1}}$, and NOEYS analyses) and, to our surprise, a glycal resulting from formal elimination of the anomic silyl ether (Scheme 3b). As 25 constituted such a minor component of the bulk material ($\geq$20:1 13:25), we progressed the mixture forward without further purification.

Synthesis of AMA (1) and Hybrids 7 and 8. Having successfully united the monosaccharide building blocks, we
turned our attention to joining the disaccharide with aglycones 9−11. Due to the relatively low cost of DNR (5) as compared to steffimycin B (18) and the absence of a complicating 1° alcohol as in the doxorubicin aglycone (11), we began our glycosidation studies using daunorubicinone (10, obtained in 1 step from 5·HCl)49 as our acceptor. Unfortunately, glycosidation of our initially targeted thioglycoside donor 12a with 10 under Sun and Wang’s modified Hirama conditions (AgPF6, TTBP)19,32−34 yielded, at most, trace amounts of the desired glycosidated product 27 (Scheme 4a, entry 1). An attempted dehydrative glycosidation50−52 with reducing sugar 12b proved similarly ineffective (Scheme 4a, entry 2). Gratifyingly, activation of TBS donor 12c with BF3·OEt2 at −78 °C afforded an isolable amount of glycosylated product 28 (12% yield), and further optimization increased the yield to 59% (Scheme 4a, entries 3−5); though nontraditional, silyl ether donors have proven effective in the α-selective synthesis of other anthracyclines.53−56 Unfortunately, the elaboration of 28 to DNR−AMA hybrid 7 faltered at its final step as the 3° TBS ether of 29 proved recalcitrant to deprotection (Scheme 4b). Efforts to overcome this problem by removing the silyl ether prior to the amine deprotection−methylation sequence or employing a donor analogous to 12c with an unprotected 3° alcohol were unsuccessful.

To obviate the need for postglycosylation manipulation, we designed fourth generation donor 12d featuring an unprotected 3° alcohol and two dimethylamines (Scheme 4c). For the anomeric leaving group, we elected to retain the TBS ether from third generation donor 12c due to its previously demonstrated success (Scheme 4a, entries 3−5). Furthermore, we posited that the lipophilic silyl group would counteract the anticipated polarity of the two dimethyl amino sugars, easing purification and allowing us to conduct the glycosylation in standard organic solvents.

The synthesis of fourth generation donor 12d began with the bis-silyl deprotection (TBAF, 50 °C) of 13 followed by selective anomeric resilylation (TBSCl) to give 32 (1:9 α/β) (Scheme 5a). Ensuing transfer hydrogenolysis (NH4CO2H, Pd/C) unmasked both primary amines,57 setting the stage for the glycosylation in standard organic solvents.
HCHO, NaBH$_3$CN) gave a mixture featuring oxazolidine as its major component (Figure S5). Presumably, ring closure occurs via intramolecular capture of a C$^4$N-imine(iminium) species by C$^3$OH; similar oxazolidine species have been observed for syn-1,2-amino alcohols under Eschweiler–Clarke conditions. This oxazolidine proved resistant to further reduction, opening fully only with the use of acid (AcOH) and excess reductant to give donor $\beta$-30. Under these forcing conditions, we also observed the formation of a side product resulting from cyanide addition (tentatively assigned as 34) that could be separated from the desired $\beta$-30 by preparatory HPLC (Scheme 5b, Figure S6).61

Moving forward to the key event, glycosylation with excess 8 provided a mixture of the desired DNR–AMA hybrid 7 and unactivated 30 (Scheme 3a). Normal- and reverse-phase separation proved ineffective at resolving this mixture. Gratifyingly, we could leverage the phenols unique to 7 to separate the compounds by anion exchange chromatography. Following RP-HPLC purification to remove trace impurities, we isolated pure DNR–AMA hybrid (7·2TFA) with the desired $\alpha$-C$^7$O–C$^1$′ linkage as verified by $^1$H multiplicity (absence of a large $^3$J$_{H1′-H2′}$ coupling constant indicative of an axial–axial coupling pathway), $^1$J$_{CH}$ (170 Hz), and ROESY (absence of strong H$1′$ ↔ C$3′$Me and H$1′$ ↔ H$5′$ correlations) (Scheme 5c, Tables S3). Interestingly, ROESY analysis revealed that the lemonose residue of 7·2TFA does not solely occupy the C$1$ conformation (Scheme 5d); rather, it exists in conformational equilibrium and/or an intermediate conformation not depicted in Scheme 5d.

At this point, we had isolated a quantity of 7·2TFA (1.8 mg) sufficient for initial biological evaluation (vide infra). Nevertheless, we found ourselves synthetically unsatisfied with the...
we designed an acyl donor. Lewis acid mediated activation would occur more readily for anomeric leaving group (Scheme 5e). We hypothesized that conversion of donor to product and ease purification of the disaccharide donor would, respectively, enable greater relaxation assay using theoretical gels. Cartoon explanation of inhibition of the TopII DNA relaxation assay using theoretical gels.

Table 1. Cytotoxicity Evaluation of Synthetic AMA (1) and Hybrids 7, 8, and 37

| entry | compound | HCT116 (colony)* | MDA-MB231 (breast)* | H69AR (lung/MDR)* |
|-------|----------|-----------------|---------------------|-------------------|
| 1     | stefimmicin B (18) | 2250           | 3690                | 4800              |
| 2     | synthetic AMA (1)  | 230°           | 320°                | 90°               |
| 3     | DNR (5)         | 210            | 80                  | 1250              |
| 4     | DNR–AMA Hybrid (7f) | 90          | 90                  | 200               |
| 5     | DOX (6)         | 90             | 470                 | 14 900            |
| 6     | DOX–AMA hybrid (8) | 40          | <30°                | 30                |
| 7     | DOX–C14–AMA hybrid (37) | 330     | 970                 | 1010              |

Isolation Report [IC_{50} (nM)] (Ref 1,2)

| Cell line (cancer type). | MDR = multidrug resistant. | TC_{50} lower than lowest evaluated concentration (30 nM).
|-------------------------|---------------------------|------------------------|
| 8 | natural AMA (1) | 2.5 | 164 | 67 |
| 9 | DNR (5) | 6.8 | 330 | 4800 |
| 10 | DOX (6) | 22.5 | 970 | 18 400 |

Figure 2. Cartoon explanation of inhibition of the TopII DNA relaxation assay using theoretical gels.

low-yielding (ca. 4%) final transformation (Scheme 5a). We hypothesized that increasing the reactivity and lipophilicity of the disaccharide donor would, respectively, enable greater conversion of donor to product and ease purification, both of which would increase the isolated yield of 7-2TFA. To this end we designed fifth generation donor 12e featuring a novel anomeric leaving group (Scheme 5e). We hypothesized that Lewis acid mediated activation would occur more readily for an acyl donor than it would for the corresponding silyl ether. However, we also reasoned that the silyl group of 12d had lent lipophilicity to an otherwise polar disaccharide and thereby imparted it with solubility in organic solvent. Joint consideration of these factors led to the design of fifth generation donor 12e featuring a novel anomeric leaving group incorporating an acyl glycoside functionalized with a highly lipophilic, UV active TBDPS-protected phenol.

Starting from diol 30, Steglich esterification (EDC-HCl, DMAP) with carboxylic acid 35 was followed by deprotection (NH_{4}CO_{2}H, Pd/C) and reductive amination (aq. HCHO, AcOH, pyr. BH_{3}) to provide β-12e (Scheme 5f); the use pyr. BH_{3} rather than NaBH_{4}CN prevented the formation of a cyano addition product analogous to the proposed 34 (Scheme 6a). A comparison of LCMS retention times revealed that, as anticipated, β-12e exhibited increased lipophilicity as compared to 12d and 7 (Figures S7 and S8).

Finally, BF_{3}·OEt_{2} mediated activation of excess 12e (1.1 equiv) in the presence of daunorubicinone (8) gave 7-2TFA in a modest ca. 20% isolated yield, a 5-fold increase as compared to fourth-generation donor β-12d (Scheme 6b, Table S4).

Having increased the yield of DNR–AMA hybrid 7, we turned our attention to the synthesis of DOX–AMA hybrid 8. Hydrolysis of commercial doxorubicin-HCl (6-HCl) followed by chemoselective silyl protection (TBSCI) of the α hydroxyl group gave known, C14-protected doxorubicinone acceptor 11a. Proceeding to the glycosidation, we exposed a mixture of fifth generation donor 12e and excess acceptor 11a to BF_{3}·OEt_{2} (Scheme 6b). To our surprise, LCMS showed a mass corresponding to glycosidated product sans the TBS protecting group. Following HPLC purification, we isolated DOX–C14–AMA hybrid 37 in 18% yield along with coeluting fractions containing multiple species (Table S5). Hypothesizing that the more robust tert-butyldiphenyl silyl ether would withstand the glycosidation conditions, we synthesized 14-O-TBDPS-protected donor 11b. Glycosidation of fifth generation donor 12e and acceptor 11b occurred without noticeable amounts of TBDPS protection as judged by LCMS. As the mass corresponding to the desired glycosidated product coeluted with unreacted donor 12e, we took the crude material into silyl deprotection (HF·pyridine) to give DOX–AMA hybrid 8 in 41% yield over 2 steps (Table S6).

Finally, we turned our attention to the synthesis of arimetamycin A. Starting from stefimmicin B (18), hydrolysis was accomplished using a solution of aq. HCl in dioxanes at 90 °C (Scheme 6b). The aglycone was taken forward and subjected to BF_{3}·OEt_{2} mediated glycosylation with 12e to provide arimetamycin A (1), albeit in 4% yield over the two steps after HPLC purification. Importantly, the spectral data for 1 matched that provided in the isolation report (Table S7).

Biological Evaluation. Having achieved the synthesis of our three synthetic targets (i.e., 1, 7, and 8) as well as DOX–C14–AMA hybrid 37, we evaluated the cytotoxicity of each anthracycline against a panel of three cancer cell lines (TC_{50}, XTT staining, Table 1 and Table S9). Synthetic AMA (1, entry 2) proved substantially more cytotoxic than its parent arimetamycin stefimmicin B (18, entry 1). In the case of DNR (5, entry 3) and DOX (6, entry 5), exchange of their monosaccharides for the AMA disaccharide generally resulted in enhanced cytotoxicity (entries 3 vs 4 and 5 vs 6, respectively), particularly for DOX-AMA hybrid 8, which proved more cytotoxic than synthetic AMA (1) across all three cell lines. Intriguingly, the C14 glycosylated DOX (entry 7) also showcased impressive cytotoxicity given that the activity determining domain is located at the incorrect site. Lastly, synthetic AMA (1) along with hybrids 7 and 8 possessed sub-micromolar cytotoxicity against MDR cell line H69AR.1,5

Next, we turned our attention to gaining a preliminary understanding of the mechanism of action. Due to material throughput, we chose to evaluate hybrid 7. Since their discovery, numerous mechanisms of action have been
proposed for the antitumor anthracyclines. Historically, the most widely accepted mechanism is topoisomerase IIα (TopIIα) poisoning leading to double strand DNA breaks while recent studies have demonstrated that DOX also effects nucleosome dynamics.

For our preliminary mechanistic studies, we assessed the ability of DNR−AMA hybrid to inhibit the human topoisomerase IIα (hTopIIα) mediated relaxation of negatively and positively supercoiled (−SC and + SC, respectively) circular DNA using the DNA relaxation assay developed by Osheroff, Shelton, and Brutlag. This assay takes advantage of supercoiled DNA’s accelerated migration through agarose as compare to relaxed DNA. As such, a theoretical gel of individual topoisomers in various states of relaxation ranging from fully supercoiled (lane 1) to fully relaxed (lane 5) would give the banding pattern seen in Figure 2a. Taking this forward to a theoretical TopII inhibition assay, the addition of the TopII enzyme results in the full conversion of the fully supercoiled DNA to relaxed DNA topoisomers (lane 1 vs 2, Figure 2b). The addition of increasing amounts of TopII inhibitor decreases the enzyme’s ability to relax the supercoiled DNA, resulting in a dose dependent decrease in relaxed topoisomers and an increase in fully supercoiled DNA (lanes 3→4→5, Figure 2b). Finally, we note that all lanes in Figure 2b contain nicked DNA, a single strand break byproduct from the synthesis of supercoiled DNA.

Moving to the experimental data, we found that the addition of increased amounts of 7 led to the loss of relaxed DNA topoisomers, indicating an inhibition of the topoisomerase IIα-catalyzed relaxation (Figures 3ai and 3ci, lanes 10→13 vs 2). However, 7 does not restore electrophoretic mobility to the level of untreated SC DNA (Figures 3ai and 3ci, lanes 10→13

Figure 3. Biological evaluation of DNR−AMA hybrid 7-2tFA.
vs 1). We hypothesize that this discrepancy results from intercalation as intercalation is known to reduce electrophoretic mobility.27,78 A comparison with DNR (5) indicated that hybrid 7 is marginally less effective at inhibiting the relaxation of −SC DNA (Figure 3a vs Figure 3b) and that both compounds inhibit +SC with largely the same effectiveness (Figure 3c vs Figure 3d).

**CONCLUSION**

In conclusion, we have synthesized arimetamycin A (1) as well as hybrids 7, 8, and 27 in a divergent manner from a common disaccharide intermediate (12e). The synthesis featured a gram-scale Hira-ma glycosylation that united two branched deoxy-amino sugars (9 and 11) and a final glycosylation optimized through the rational design of a novel acyl donor (12e). This is the first example of a complex glycoconjugate synthesis wherein a glycosyl donor incorporating two N,N-dimethyl amine moieties was implemented. Cytotoxicity studies indicate that incorporation of the AMA glycan enables the anthracyclines to overcome the anthracycline resistance of the H69AR lung cancer cell line. Interestingly, pairing the AMA glycan with the doxorubicin aglycone produced a DOX-AMA hybrid (8) more potent than AMA itself across the three studied cell lines. Preliminary mechanistic studies demonstrated that DNR–AMA hybrid 7 inhibits topoisomerase IIα-catalyzed DNA relaxation in a manner comparable to DNR (5) and is hypothesized to be a DNA intercalator. Future studies will assess the ability of AMA (1) and its hybrids to induce DNA double strand breaks (DSBs) and chromatin damage, as a recent study showed that N,N-dimethylation of DOX (6) results in a compound that does not cause DSBs yet retains the ability to damage chromatin and maintains its cytotoxicity; most importantly, this dimethylated derivative did not prove cardiotoxic in a mouse model.79 Results in this regard will be reported in due course.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.1c00040.

Synthetic procedures, characterization data, cytotoxicity data, DNA relaxation data, X-ray crystallographic data, NMR spectra, and a table of abbreviations (PDF)

Compound 26 (CCDC 2043036) (CIF)

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**Author Contributions**

E.D.H. and S.D.T. designed the synthesis of 1-2TFA, 7-2TFA, and 8-2TFA. E.D.H. carried out the synthesis of 1-2TFA, 7-2TFA, and 8-2TFA and analyzed the relevant data with input from S.D.T. J.A.W.B. carried out the hTopIIα relaxation studies. J.A.W.B. and N.O. analyzed the results of the hTopIIα relaxation studies. S.M.C. obtained single-crystal XRD data for 26 and analyzed the data with input from N.D.S. The manuscript was drafted by E.D.H. and S.D.T. The manuscript was revised by E.D.H., N.D.S., N.O., and S.D.T. All authors have given approval to the final version of the manuscript.

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**Notes**

The authors declare no competing financial interest.

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