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Research Article

Chrysomycin A Derivatives for the Treatment of Multi-Drug-Resistant Tuberculosis

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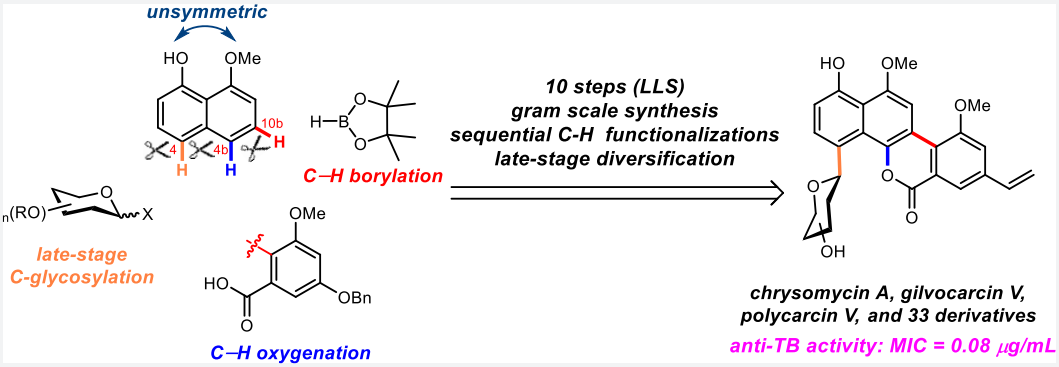

ABSTRACT: Tuberculosis (TB) is a life-threatening disease resulting in an estimated 10 million new infections and 1.8 million deaths annually, primarily in underdeveloped countries. The economic burden of TB has been estimated as approximately 12 billion USD annually in direct and indirect costs. Additionally, multi-drug-resistant (MDR) and extreme-drug-resistant (XTR) TB strains resulting in about 250 000 deaths annually are now widespread, increasing pressure on the identification of new anti-TB agents that operate by a novel mechanism of action. Chrysomycin A is a rare C-aryl glycoside first discovered over 60 years ago. In a recent high-throughput screen, we found that chrysomycin A has potent anti-TB activity, with minimum inhibitory concentration (MIC) = 0.4 μg/mL against MDR-TB strains. However, chrysomycin A is obtained in low yields from fermentation of *Streptomyces*, and the mechanism of action of this compound is unknown. To facilitate the mechanism of action and preclinical studies of chrysomycin A, we developed a 10-step, scalable synthesis of the isolate and its two natural congeners polycarcin V and gilvocarcin V. The synthetic sequence was enabled by the implementation of two sequential C−H functionalization steps as well as a late-stage C-glycosylation. In addition, >10 g of the advanced synthetic intermediate has been prepared, which greatly facilitated the synthesis of 33 new analogues to date. The structure−activity relationship was subsequently delineated, leading to the identification of derivatives with superior potency against MDR-TB (MIC = 0.08 μg/mL). The more potent derivatives contained a modified carbohydrate residue which suggests that further optimization is additionally possible. The chemistry we report here establishes a platform for the development of a novel class of anti-TB agents active against drug-resistant pathogens.

INTRODUCTION

Tuberculosis (TB) has become the number one life-threatening infectious disease, whose treatment is further complicated by the emergence of drug-resistant strains.[1] Over the past four decades, only two new drugs, bedaquiline[2] and delamanid,[3] have been approved by the FDA and EMA, respectively, for the treatment of MDR-TB. Considering the high attrition rate in clinical trials, more effective anti-TB drug candidates with distinct molecular scaffolds are in urgent need. Chrysomycin A (**1**) is an antitumor antibiotic first isolated from *Streptomyces* A-419 in 1955 as a mixture with chrysomycin B (**2**).[4] Recently, we rediscovered chrysomycin A (**1**) and its natural congeners from mining of a 10K actinobacteria genome sequences[5] and found that chrysomycin A showed promising antimicrobial activity against a number of Gram-positive strains and the MDR-TB strain with a minimum inhibitory concentration (MIC) of 0.4 μg/mL (see Tables S1 and S2). Kumar and co-workers also independently reported that chrysomycin A showed inhibitory activity against M. tb strains.[6] Congeneric chrysomycin C (**3**) was isolated from *Streptomyces sporoverrucosus* in 2013.[7] Chrysomycins A−C

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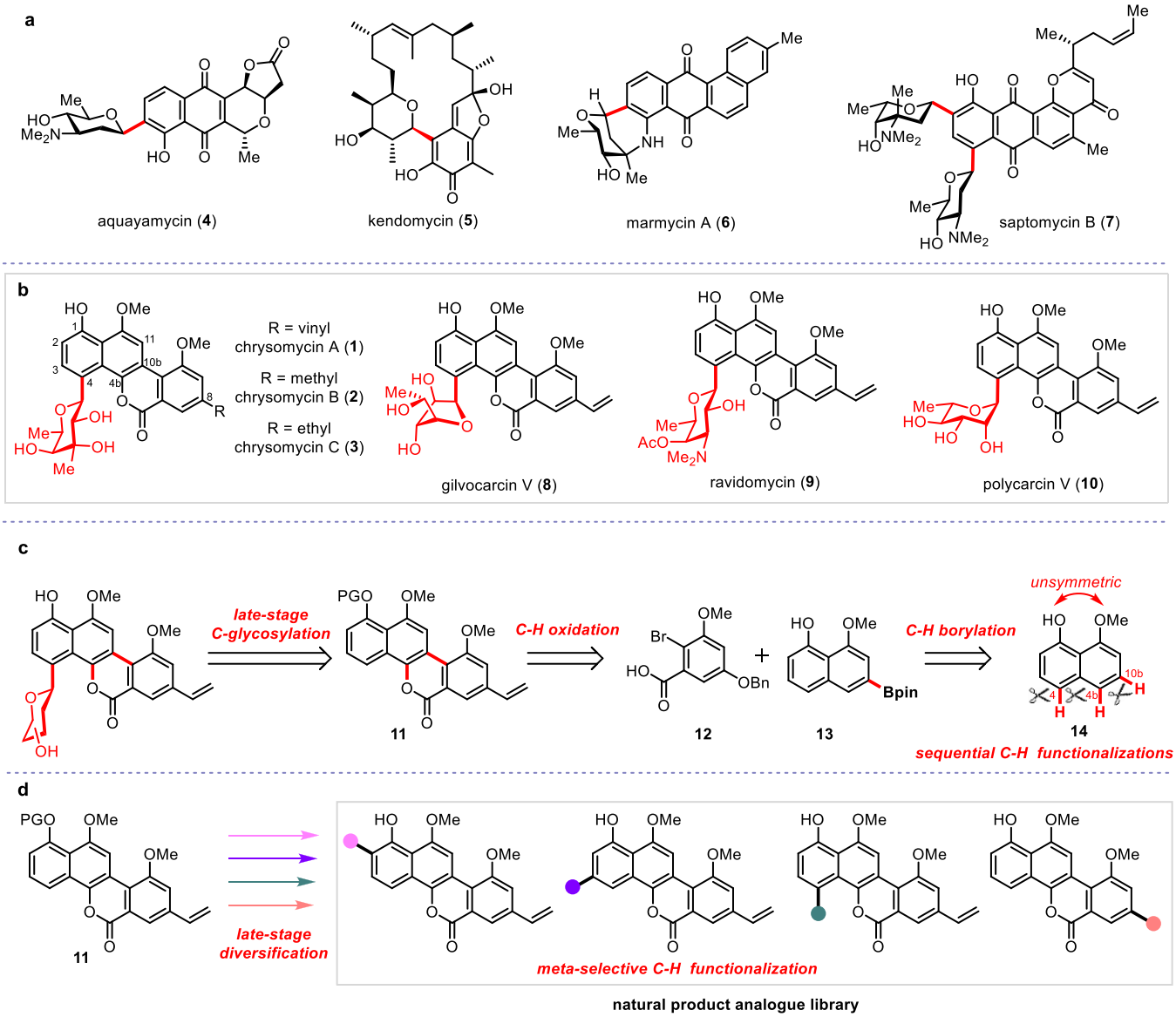

Figure 1. C-aryl glycoside natural products and synthetic plans for chrysomycin A and its analogues. (a) Representative bioactive C-aryl glycosides. (b) Structures of gilvocarcin family natural products. (c) Our bond disconnections of chrysomycin A. In our retrosynthetic analysis of chrysomycin A, both the sugar moiety and vinyl group were assembled at the late stage. The core structure of the chromophore was constructed through sequential regioselective C−H functionalizations. (d) Late-stage diversification of the natural product at multiple sites.

belong to the gilvocarcin family of C-aryl glycosides, which are a unique class of C-glycosilyated natural products and exhibit various important biological activities.[8] For instance, aquayamycin (**4**, Figure 1a), produced by *Streptomyces misawanensis*, displays notable antimicrobial activity against Gram positive bacteria.[9] Kendomycin (**5**) is a potent endothelin receptor antagonist and a calcitonin receptor agonist.[10] Marmycin A (**6**) and saptomycin B (**7**) show significant cytotoxicity against several tumor cell lines.[11,12] Gilvocarcin V (**8**), closely related to chrysomycin A, exhibits potent antitumor and antibacterial activity.[13] The sugar moieties are mainly located at the ortho-position of phenol in most C-glycosilyated natural products, which are often biogenetically attached through an initial O-glycosylation followed by a Fries-like rearrangement mediated by various glycosyltransferases.[14] Notably, chrysomycins possess the uncommon and unique para-substituted sugar moiety, and more investigations are required to fully elucidate the biological function of these rare para-substituted C-glycosides.

To our knowledge, no successful total synthesis of chrysomycins has been reported since the original discovery of these natural products 60 years ago. The Hart group attempted to synthesize chrysomycin B with a strategy to form the lactone in the presence of carbohydrate moiety at the C4 position but without success.[15] Attracted by the fascinating structure and potent anti-TB activity of chrysomycin A, we set out to accomplish its total synthesis that would serve as a sustainable source and platform for a structure−activity relationship study, which may facilitate the discovery of potential lead compounds for the treatment of TB. We aim to develop a scalable synthesis which will then allow for diverse late-stage transformations including direct C−H functionalization to rapidly access a wide range of analogues.

The landmark syntheses of other C8-vinyl gilvocarcin family members (Figure 1b) gilvocarcin V (**8**)[16] and ravidomycin

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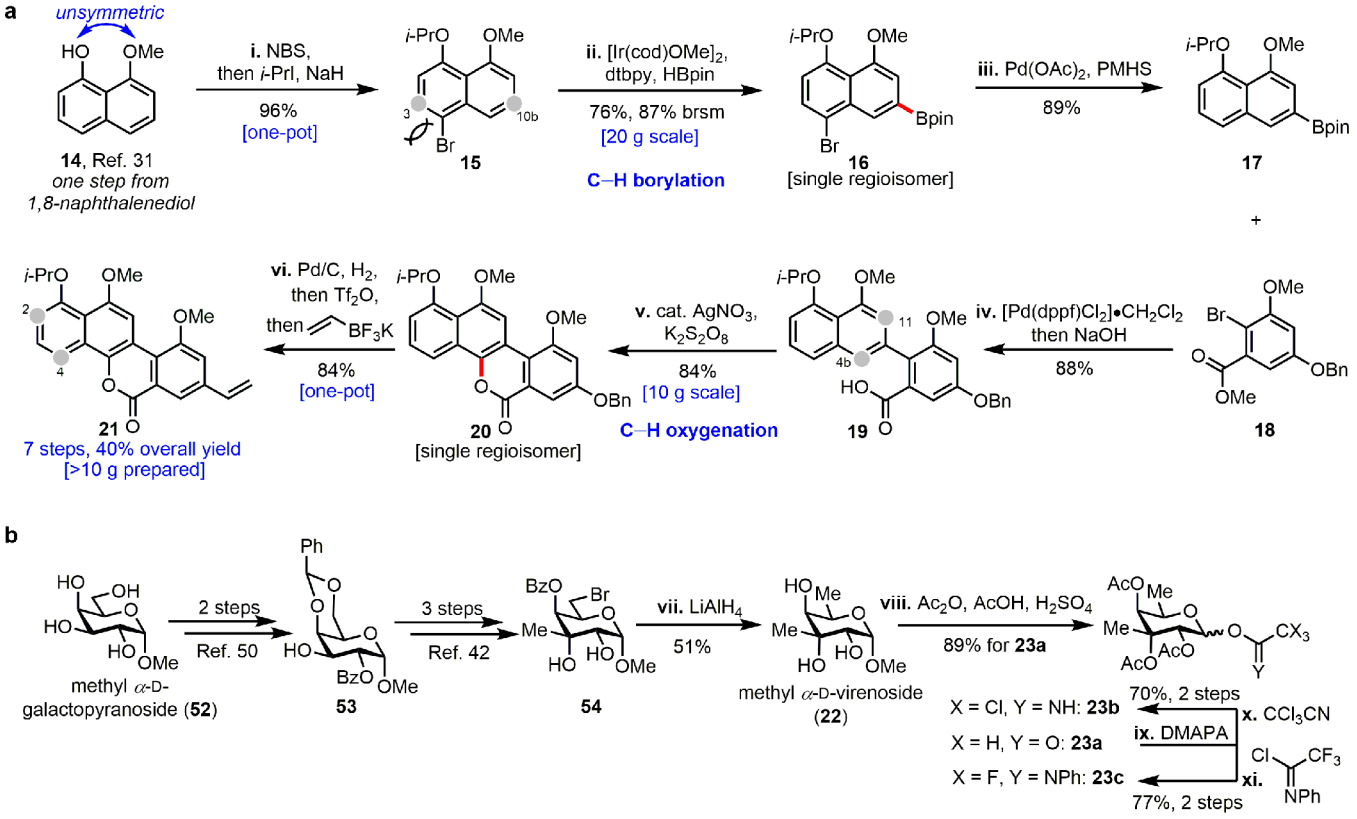

Figure 2. Concise and scalable syntheses of aglycon and glycosyl donors. (a) Regioselective C−H functionalizations enabled scalable preparation of the aglycon **21**. (b) Synthesis of glycosyl donors **23a−c**. Reagents and conditions are as follows: (i) NBS, MeCN, r.t.; *i*-PrI, NaH, DMF, 0 to 70 °C. (ii) $[Ir(cod)OMe]_2$, dtbpy, HBpin, hexane, 80 °C. (iii) $Pd(OAc)_2$, KF, PMHS, THF, H_2O, r.t. (iv) $[Pd(dppf)Cl_2]\cdot CH_2Cl_2$, KOH, MTBE/$H_2O$, 80 °C, then 40% NaOH (aq). (v) $K_2S_2O_8$, $AgNO_3$, MeCN/H_2O, 50 °C. (vi) Pd/C, H_2, MeOH, r.t.; Tf_2O, Et_3N, CH_2Cl_2, −78 °C; potassium vinyltrifluoroborate, $[Pd(dppf)Cl_2]\cdot CH_2Cl_2$, Et_3N, *n*-PrOH, reflux. (vii) $LiAlH_4$, THF, 50 °C. (viii) Ac_2O, AcOH, H_2SO_4, r.t. (ix) DMAPA, THF, 20 °C. (x) CCl_3CN, DBU, 4 Å MS, CH_2Cl_2, r.t. (xi) *N*-Phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, r.t.

(**9**)[17] were accomplished by the Suzuki group, and recently polycarcin V (**10**)[18] was synthesized by Minehan and co-workers. Their synthetic strategies all relied on the early-stage glycosylation prior to the construction of the chromophore backbone. However, these previous synthetic works all suffered from a long linear sequence (18−30 steps) which was not amenable for the preparation of diverse analogues. As this family of natural products is only distinguished by variation of side chains at C4 and C8, we aimed to develop a highly convergent synthetic route, which would allow late-stage installation of various substituents at both C4 and C8 positions and thus facilitate the derivatization of this family of natural products. We anticipate that this family of natural products could be accessed from **11** via late-stage C4 Friedel−Crafts type C-glycosylation, although this transformation could be conceivably challenging due to the fact that regioselectivity normally favors C2-selective C-glycosylation.[19,20]

RESULTS AND DISCUSSION

Retrosynthetic Analysis of Chrysomycin A. Inspired by recent advances in natural product synthesis using multiple C−H activation reactions,[21−28] we envision that the synthesis of **11** could be drastically simplified by adopting sequential regioselective C−H functionalizations of the unsymmetric monomethylated naphthalenediol **14** (Figure 1c). The C10b instead of C3 functionality (Figure 1c) would be built by intermolecular C−H borylation, while the C4b instead of C11 functionality would be installed by carboxyl-group-directed intramolecular C−H oxygenation (Figure 1c). Finally, late-stage and direct diversification of chrysomycin A via C−H functionalizations or other transformations would provide a more effective approach than de novo synthesis to generate various natural productlike analogues for a subsequent structure−activity relationship (SAR) study in order to generate potential lead compounds for drug discovery (Figure 1d).[29,30] Notably, the site-selective C−H functionalizations of complex molecules may also open the door to install various functional groups that are inaccessible with the traditional synthetic route.

Synthesis of the Aglycon 21. Our synthesis began with the C−H borylation at C10b (Figure 2a). Due to the asymmetric structure of **14**, we were able to install a removable bromine atom as a blocking group at C4 to constrict C3-borylation. Bromide **15** was prepared smoothly in 96% yield (one-pot bromination and protection) from **14** that was generated by monomethylation of the commercially available 1,8-naphthalenediol.[31] We then explored the selective catalytic borylation/dehalogenation sequence.[32] A commonly used boron source B_2pin_2 was first applied to the iridium-catalyzed C−H borylation[33] reaction in THF, but this condition gave no reaction. Only 4% of the desired **16** was obtained when the boron source was changed to HBpin. To our delight, the reaction was significantly improved when conducted in hexane even on a 20 g scale (76%, 87% yield brsm).[34] Remarkably, we also obtained a single regioisomer for this C−H borylation. The following Pd-catalyzed chemoselective hydrodebromina-

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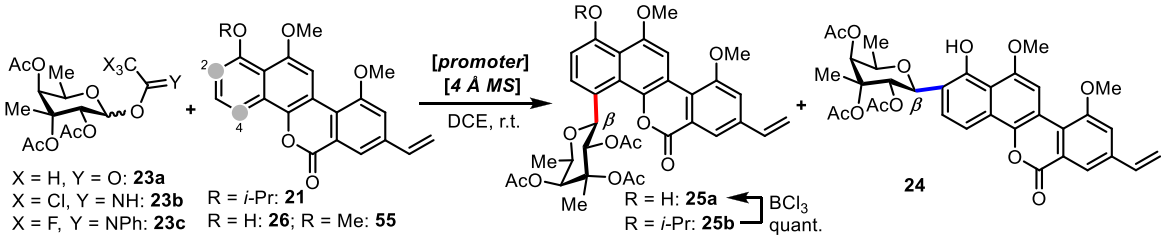
Table 1. Optimization of the C-Glycosylation Reaction



entry[a]	D/A	promoter (equiv)	T (°C)	4 Å MS (wt equiv)	**25**/**24**[b]	yield of **25**[c] (%)
1[d]	**23a**/**21**	$SnCl_4$ (9.0)	25	0	0/100	41[e]
2	**23a**/**21**	$SnCl_4$ (3.0)	25	0		<5
3	**23a**/**21**	$Et_2O{\cdot}BF_3$ (3.0)	25	4.0		0
4	**23a**/**21**	TMSOTf (3.0)	25	4.0		<5
5	**23a**/**21**	Cp_2ZrCl_2 (3.0), $AgClO_4$ (3.0)	25	4.0		0
6	**23a**/**21**	$SnCl_4$ (3.0), $AgClO_4$ (3.0)	25	4.0	60/40	14
7	**23a**/**21**	$SnCl_4$ (3.0)	25	4.0	72/28	25
8	**23a**/**21**	$SnCl_4$ (3.0)	15	4.0	52/48	<15
9	**23a**/**21**	$SnCl_4$ (3.0)	35	4.0	55/45	10
10[f]	**23a**/**21**	$SnCl_4$ (3.0)	25	4.0		0
11[g]	**23a**/**21**	$SnCl_4$ (3.0)	25	4.0	63/37	11
12	**23a**/**21**	$SnCl_4$ (3.0)	25	12.0	81/19	30
13	***23a*/*21***	*$SnCl_4$ (3.0)*	*25*	*20.0*	*>95/5*	*50*
14[h]	**23a**/**21**	$SnCl_4$ (3.0)	25	4.0	50/50	11
15	**23b**/**21**	$SnCl_4$ (3.0)	25	20.0	90/10	26
16	**23b**/**21**	TMSOTf (3.0)	25	20.0		<5
17	**23b**/**21**	TMSOTf (0.4)	25	20.0		<5
18	**23c**/**21**	$SnCl_4$ (3.0)	25	20.0	>95/5	41
19	**23c**/**21**	$SnCl_4$ (0.5)	25	20.0	>95/5	11[i]
20	**23a**/**55**	$SnCl_4$ (3.0)	25	12.0		<5[j]
21[d]	**23a**/**26**	$SnCl_4$ (9.0)	25	0	0/100	37[e]
22	**23a**/**26**	$SnCl_4$ (3.0)	25	4.0	0/100	<5[e]

[a]Conditions: **23** (1.0 equiv), aglycon (3.0 equiv), 4 Å MS, promoter (3.0 equiv), solvent (0.017 M), r.t. [b]Ratio determined by ^{1}H NMR of the crude reaction mixture. [c]Combined yield of **25a** and **25b**. [d]1.0 equiv of aglycon, 4.0 equiv of **23a**, and 9.0 equiv of $SnCl_4$ were used. [e]Isolated yield of **24**. [f]DCE/THF or MeCN was used as solvent. [g]Concentration = 0.034 M. [h]1.0 equiv of aglycon, 3.0 equiv of **23a**, and 3.0 equiv of $SnCl_4$ were used. [i]α-**25b** was also obtained in 19% yield. [j]Deprotection of 1-hydroxyl group did not happen. D/A = donor/acceptor.

tion proceeded smoothly by employing fluoride-activated PMHS[32] to deliver the requisite boronate ester **17** in 89% yield.

With **17** in hand, our attention was turned to the construction of the aglycon (Figure 2a). Suzuki−Miyaura cross-coupling using boronate ester **17** and bromide **18**[35] followed by hydrolysis of the methyl ester with 40% aqueous NaOH in one pot afforded acid **19** in 88% yield. With respect to the regioselectivity of the remote C−H oxygenation, both α- and β-oxygenation of 2-(naphthalen-2-yl)benzoic acid have been reported.[36,37] Encouraged by initial investigations using $Cu(OAc)_2$ and $[PhCO_2]_2$ in HFIP[38] to cyclize **19**, where C4b instead of C11 was specifically oxidized to generate ca. 10% of the desired product **20**, we changed the oxidant to the much cheaper $K_2S_2O_8$[39] and obtained **20** in 57% yield. The yield was further improved to 84% on the gram scale by the addition of a catalytic amount of $AgNO_3$.[39] At this point, the core of aglycon was fully constructed. The following functional group transformations assembled the vinyl group at C8: removal of the benzyl protecting group by hydrogenolysis, subsequent installation of the triflate, and Suzuki−Miyaura coupling employing potassium vinyltrifluoroborate[40] in one pot were achieved in 84% yield. Over 10 g of 1-*O*-isopropyldefucogilvocarcin V (**21**) was obtained in 40% overall yield from 1,8-naphthalenediol, which demonstrated the efficiency and scalability of this synthetic route compared to the previously reported approach.[41]

Syntheses of Glycosyl Donors. The carbohydrate portion of chrysomycin A is virenose, a branched-chained sugar which has rarely existed in natural products. The synthesis of methyl α-D-virenoside (**22**) was first accomplished in 1980.[42] We modified and improved the previous synthesis to efficiently generate **22** in good yield (Figure 2b).[42,50] Upon treatment with $AcOH/Ac_2O/H_2SO_4$, **22** underwent acetolysis to provide tetraacetate **23a**[43] as a mixture of anomers (α/β = 1/4) in 89% yield. The other two glycosyl donors trichloroacetimidate **23b** and *N*-phenyltrifluoroacetimidate **23c**[44] were also synthesized from **23a** (Figure 2b).

Survey of Reaction Conditions for the C-Glycosylation Reaction. After the two major building blocks for chrysomycin A were all obtained, the synthesis was then centered on the crucial late-stage C-glycosylation reaction. In early 1990s, Daves and co-workers first examined a late-stage C-glycosylation model for the gilvocarcin family natural product; however, they obtained a 1/1 ratio of α- and β-anomers.[19,20] In our case, challenges lay not only in the stereoselectivity of the anomeric center but also in the regioselectivity complicated by stereoelectronic effects of the fully elaborated chromophore. Daves' conditions were initially applied to the aglycon **21** (Table 1, entry 1) but afforded the

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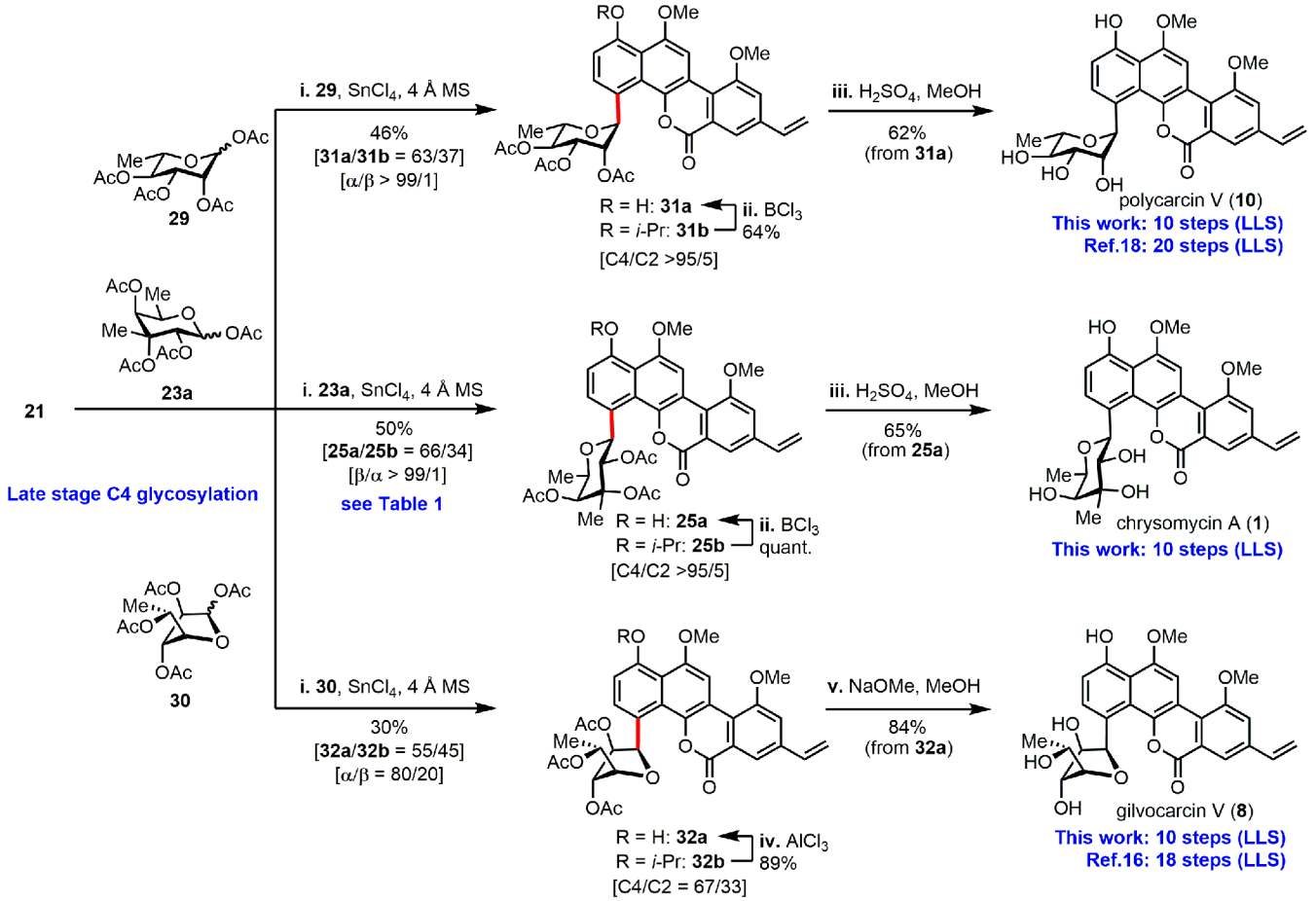

Figure 3. Total syntheses of chrysomycin A, polycarcin V, and gilvocarcin V through late-stage C-glycosylation. Reagents and conditions are as follows: (i) $SnCl_4$, 4 Å MS, DCE, r.t. (ii) BCl_3, CH_2Cl_2, −20 °C. (iii) 1.5 M H_2SO_4 in MeOH, 70 °C. (iv) $AlCl_3$, CH_2Cl_2, −20 °C. (v) NaOMe, MeOH, r.t.

C2-glycosylated product **24** exclusively with the removal of the *i*-Pr group (41% yield). The C4-glycosylated product **25** could only be isolated in a trace amount when excess aglycon **21** (relative to **23a**) and less promoter (3.0 equiv of $SnCl_4$) were used (Table 1, entry 2). The initial screening of various promoters did not improve the reaction outcome (Table 1, entries 3−6). Interestingly, in the $SnCl_4$-promoted reaction (Table 1, entry 7), **26** and **25a** were obtained where we speculated that the *i*-Pr group of both **21** and **25b** were removed under this condition. Alternatively, the *i*-Pr group of **25b** could be further deprotected with BCl_3 to afford **25a** in quantitative yield (Figure 3). When 4 Å MS was added to the reaction solution, we observed a significant improvement in the regioselectivity of C4-glycosylation. Higher loading of 4 Å MS was positively correlated to the improved regioselectivity and prevented the undesired removal of the *i*-Pr group in both **21** and the C4-glycosylated product **25b** (Table 1, entries 8−13). The optimized reaction was carried out with 20.0 wt equiv of 4 Å MS to afford excellent regioselectivity (**25**/**24** > 95/5) and 50% yield of **25** (Table 1, entry 13). Further screening of various glycosyl donors indicated that acetate **23a** was a better glycosyl donor than **23b** and **23c** (Table 1, entries 15−19). Notably, glycosylation turned out to be sluggish when the *i*-Pr group was replaced by the methyl group, presumably because the methyl group is more stable toward the deprotection condition (Table 1, entry 20). To further investigate the selectivity of C2 glycosylation, defucogilvocarcin V (**26**) was directly subjected to the conditions used in entries 1 and 7, which afforded 37% yield of **24** and a trace of **25**, respectively, as a single regioisomer (Table 1, entries 21 and 22).

Based on the above-mentioned results, a plausible mechanism of this $SnCl_4$-promoted C-glycosylation reaction is depicted in Scheme S1. There are three proposed reaction pathways to account for the observed different regioselectivities. The regioselective formation of C2-glycosylated product **24** from **26** (Table 1, entries 21 and 22) probably proceeds via the O- to C-glycoside rearrangement sequence that was previously proposed by Suzuki and co-workers[16,45] (path A). When much excess of $SnCl_4$ (9.0 equiv) was applied (entry 1), the *i*-Pr group of **21** was quickly removed to form **26**, which underwent path A as well. Comparing entry 7 with entry 22, we speculate that the C2-glycosylated product **24** could be generated via path B, in which the protected aglycon **21** attacks oxonium ion **27**, followed by Friedel−Crafts-type C2-glycosylation to yield intermediate **28**. After deprotection of the *i*-Pr group, **24** is obtained. In contrast, C4-glycosylated products **25** may only be formed via Friedel−Crafts-type C4-glycosylation of **21** (path C), which is sterically favored compared to C2-glycosylation. In this process, 4 Å MS blocked the removal of the *i*-Pr group of **21** by $SnCl_4$ (as observed) and thus presumably increased the proportion of the Friedel−Crafts reaction to afford a better yield of **25**. In addition, the C4-glycosylation was found to be highly *β*-selective due to the neighboring group participation effect.

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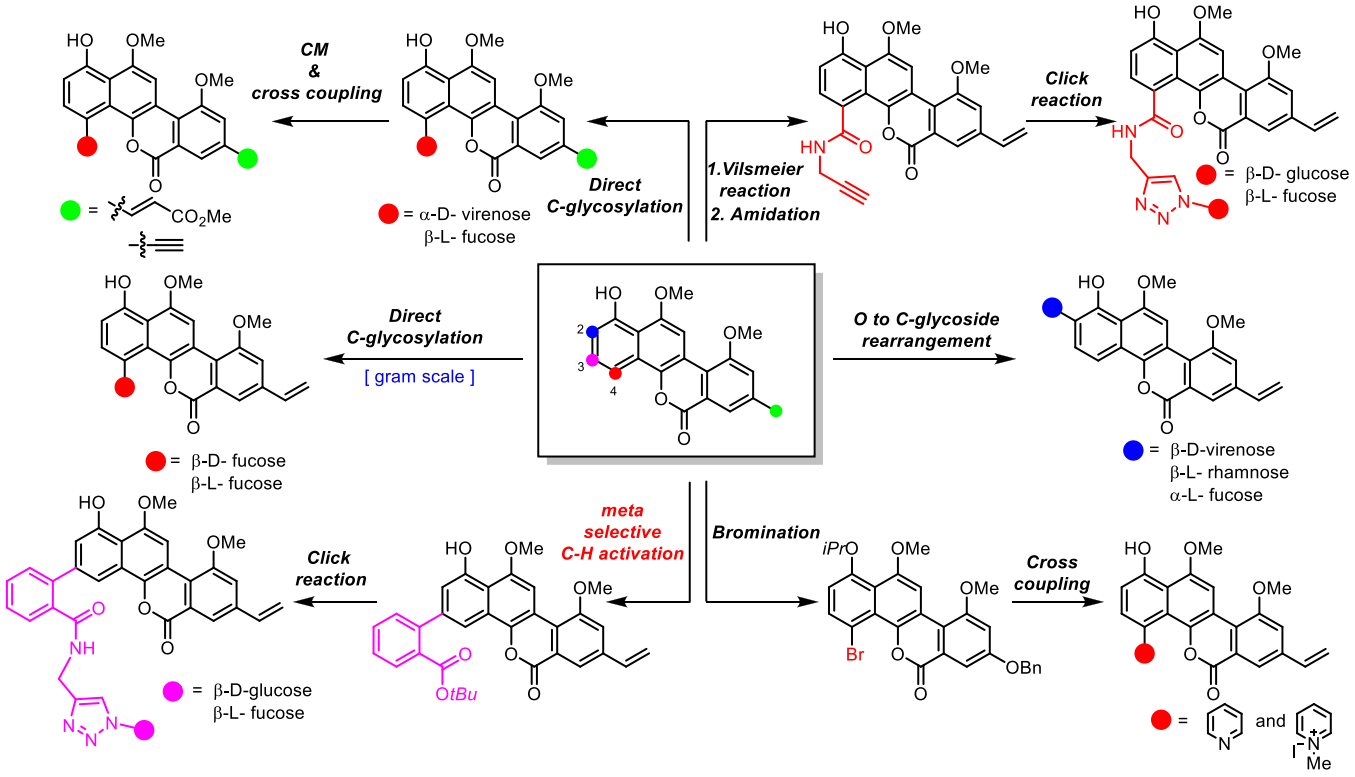

Figure 4. Methods used for late-stage diversification of chrysomycin A.

Synthesis of Chrysomycin A, Polycarcin V, and Gilvocarcin V. Finally, triacetate **25a** was subjected to the global deacetylation. Saponification of **25a** using diverse bases unexpectedly led to a complex of mono- or diacetate (Table S4). Fortunately, acid prompted deacetylation using 1.5 M of H_2SO_4 in MeOH cleanly afforded the desired chrysomycin A (**1**) in 65% yield (Figure 3). Overall, the total synthesis of chrysomycin A was accomplished in 10 steps (longest linear sequence, LLS) from the commercially available 1,8-naphthalenediol.

We next adapted our highly convergent and flexible synthetic route to prepare the other two gilvocarcin family members polycarcin V (**10**) and gilvocarcin V (**8**) (Figure 3). Using L-rhamnose tetraacetate (**29**) and D-fucofuranose tetraacetate (**30**) to react with chromophore **21**, C4-glycosylation products **31** and **32** were provided, respectively. Upon treatment with H_2SO_4/MeOH, **31a** was converted to polycarcin V (**10**) in 62% yield. Considering that the acidic conditions would cause anomerization and/or ring expansion of D-fucofuranose,[15] the basic condition using NaOMe in MeOH was applied for the synthesis of gilvocarcin V (**8**). As a result, polycarcin V and gilvocarcin V were also synthesized in 10 steps (LLS). Compared to the previously reported total syntheses, 20-step for polycarcin V,[18] 18-step for gilvocarcin V,[16] our work further demonstrated the remarkable efficiency of the sequential C−H functionalizations and late-stage C-glycosylation strategy.

Late-Stage Diversification of Chrysomycin A. This established synthetic route enabled us to further extensively explore SAR. A series of analogues of chrysomycin A were prepared through various late-stage functionalizations at C2, C3, C4, and C8 positions (Figure 4). O- to C-glycoside rearrangement was applied to install three different sugar units at the C2 position selectively (Figure 5a). Using **23a**, **33**, and **29** to react with defucogilvocarcin V (**26**), C2-glycosylation products **24**, **34**, and **35** were provided, respectively. Removal of the acyl group in **24** and **35** by acidic and basic conditions offered **36** and **38**, and upon treatment with BCl_3, **34** was converted to **37** in 67% yield. Olefin cross-metathesis and Sonogashira coupling were used to provide C8 alkynyl (**39**) and C8 methyl acrylate (**40**) analogues, respectively (Scheme S2). To increase the cell membrane permeability, positively charged lipophilic C4-noncarbohydrate analogue (**41**) was synthesized by the cross-coupling reaction[46] (Scheme S3). As for C4 functionalization, two commercially available 6-deoxy hexoses D- and L-fucose were installed regio- and stereoselectively by late-stage C-glycosylation to compare the anti-TB activity of these two enantiomers (Scheme S4). Naturally abundant natural sugars such as glucose and galactose were utilized as glycosyl donor candidates, but unfortunately none of them succeeded even with numerous attempts (Table S5). As an alternative strategy, we decided to incorporate a propargyl amide group into C4 as a handle to install various azide sugars through "click chemistry" (Figure 5b). Based on this design, the formyl group was first installed at the C4 position by Vilsmeier−Haack reaction to afford **42**, followed by Pinnick oxidation to afford carboxylic acid intermediate **43**. After amidation and deprotection, propygyl amide intermediate **45** was obtained. Finally, using "click chemistry", we introduced D-glucose and L-fucose, respectively.

We then turned our attention to the challenging C3 functionalization (Figure 5c). To the best of our knowledge, there are no methods reported for the selective meta-C-glycosylation on a phenol substrate. Moreover, in general very few conventional chemistries are available for the direct installation of any functional groups on the phenol meta position. Therefore, we planned to introduce a handle first via a meta-selective C−H functionalization strategy. Initial

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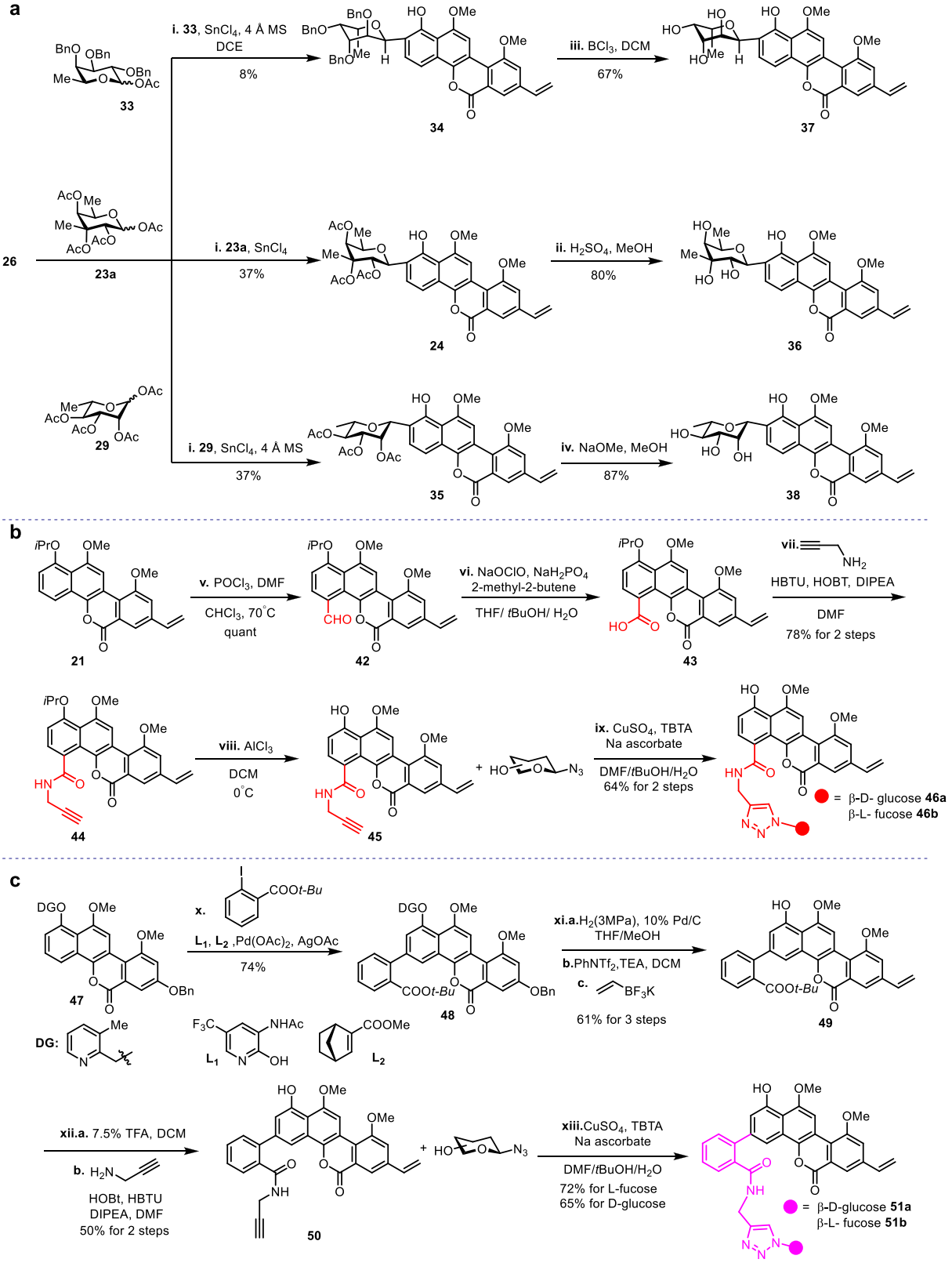

Figure 5. Late-stage diversification of chrysomycin A. (a) Synthesis of the C2 glycosylated derivatives. (b) Synthesis of the C4 hybrid derivatives. (c) Synthesis of the C3 hybrid derivatives via meta-selective C−H functionalization. Reagents and conditions are as follows: (i) $SnCl_4$, 4 Å MS, DCE, r.t. (ii) 1.5 M H_2SO_4 in MeOH, 70 °C. (iii) BCl_3, CH_2Cl_2, −78 °C. (iv) NaOMe, MeOH, r.t. (v) $POCl_3$, DMF, $CHCl_3$, 70 °C. (vi) NaOClO, NaH_2PO_4, 2-methyl-2-butene, THF/*t*BuOH/H_2O, r.t. (vii) 2-Propynylamine, HBTU, HOBt, DIPEA, DMF, r.t. (viii) $AlCl_3$, DCM, r.t. (ix) $CuSO_4$, TBTA, sodium ascorbate, DMF/*t*BuOH/H_2O, r.t. (x) *tert*-butyl 2-iodobenzoate, L_1, L_2, $Pd(OAc)_2$, AgOAc, r.t. (xi) Pd/C, H_2(3 MPa), THF/MeOH, r.t.; $PhNTf_2$, TEA, DCM, .r.t.; potassium vinyltrifluoroborate, $[Pd(dppf)Cl_2]\cdot CH_2Cl_2$, Et_3N, n-PrOH, reflux. (xii) 7.5% TFA, DCM, r.t.; prop-2-yn-1-amine, HOBT, HBTU, DIPEA, DMF, r.t. (xiii) *β*-Glycosyl azides, $CuSO_4$, TBTA, Na ascorbate, DMF/tBuOH/H_2O, r.t.

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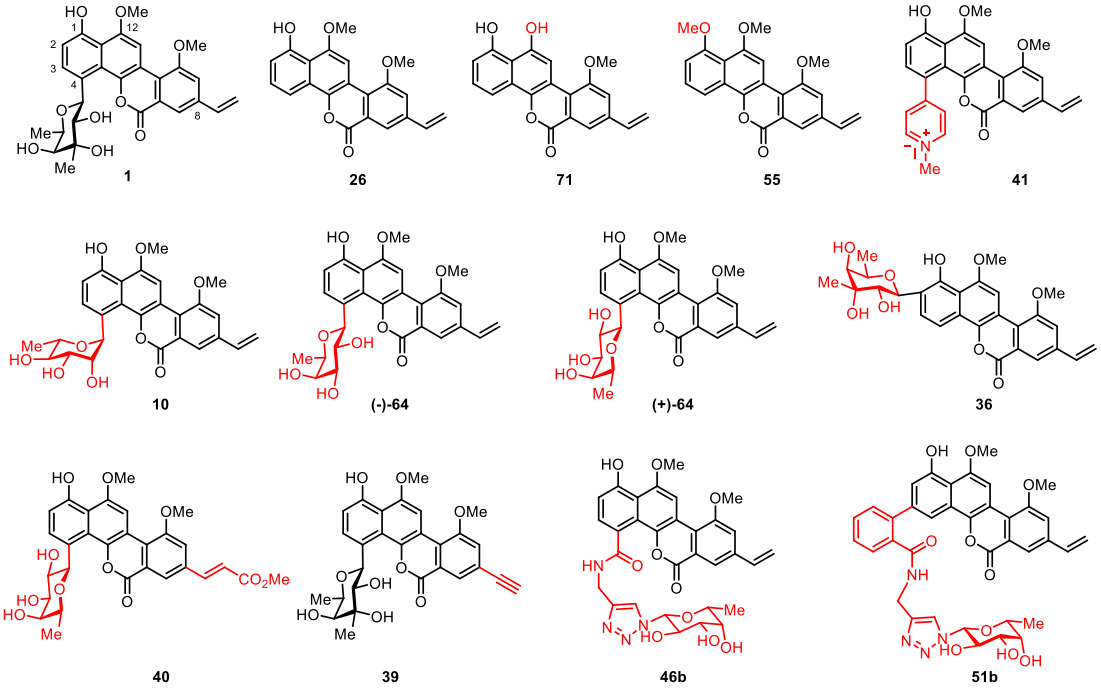
Table 2. MIC Values (μg/mL) for Chrysomycin A and Its Analogues against Various Mycobacterial Strains



Species	Strain	1	26	71	55	41	10	(-)-64	(+)-64	36	40	39	46b	51b	rifampicin	bedaquiline
M. bovis	BCG	0.4	5	>10	>10	5	0.32	0.32	0.08	0.16	0.64	2.5	>10	>10	0.02	——
M.tb	H37Rv	0.4	5	>10	>10	>10	0.16	0.16	0.08	0.16	0.64	0.32	>10	>10	0.02	0.04
	Hr1	0.4	5	>10	>10	>10	0.16	0.16	0.08	0.16	0.32	0.16	>10	>10	1	0.04
M.tb	Hr2	0.4	5	>10	>10	>10	0.16	0.32	0.32	0.16	>10	0.16	>10	>10	2	0.16
Clinical	Hr3	0.4	5	>10	>10	>10	0.16	0.16	0.16	0.16	0.64	0.32	>10	>10	0.5	0.08
isolates	Hr4	0.4	5	>10	>10	>10	0.16	0.16	0.16	0.16	0.64	0.32	>10	>10	1	0.32
	Hr5	0.4	5	>10	>10	>10	0.16	0.32	0.32	0.16	0.64	0.32	>10	>10	2	0.04

MIC colour scale (μg/mL): >10 | 5 | 2.5 | 2 | 1 | 0.64 | 0.5 | 0.4 | 0.32 | 0.16 | 0.08 | 0.04 | 0.02

attempts on C−H borylation and other nondirected C−H activation methods were unsuccessful (Table S6).[47,48] Fortunately, when a pyridine directing group was introduced,[49] a benzoic acid ester was successfully installed at the C3 position regioselectively to afford **48** in 74% yield. With this desired meta functionalized intermediate in hand, both the directing group and benzyl group were removed simultaneously by hydrogenolysis. After selective triflation and Suzuki coupling, **49** was generated in 61% yield. Then, propargyl amide **50** was obtained through deprotection and amidation in 50% overall yield. Finally, the Cu(I)-catalyzed "click" reaction afforded the desired C3 analogues **51a** and **51b** smoothly.

Study of Structure−Activity Relationship. More than 30 natural product analogues were synthesized and evaluated for antituberculosis activity against a panel of pathogens including the wild-type *M. tb* H37Rv strain and five clinically isolated rifampicin-resistant *M. tb* strains (Hr 1−5) (Table S1). We also used rifampicin, the widely applied first-line anti-TB drug, and bedaquiline, the latest second-line anti-TB drug, as positive control substrates. As a result, 6 compounds (**10**, (−)-**64**, (+)-**64**, **36**, **40**, **39**) showed promising antituberculosis activity (MIC < 1 μg/mL) against *M. tb* H37Rv and five rifampicin-resistant *M. tb* strains (Hr1−5) (Table 2). The sugar moiety was found to play an important role in the bioactivity, as seen in defucogilvocarcin V (**26**) (>10-fold loss of activity). Methylation of the 1-OH or demethylation of the 12-OMe was also found to be detrimental to activity. Polycarcin V (**10**), which showed an MIC of 0.16 μg/mL against M. tb, was more potent than chrysomycin A (**1**). A further change of the sugar portion to D- or L-fucose was also beneficial to activity. Interestingly, the β-L-fucosyl analogue (+)-**64** showed better activity than the β-D-fucosyl one (−)-**64**. Remarkably, (+)-**64**, the most active analogue, had 5-fold potency enhancement against *M. tb* compared to chrysomycin A (**1**). The C2-glycosylated analogue **36** displayed an MIC of 0.16 μg/mL against *M. tb*. Even more interestingly, comparing to bedaquiline, **36** showed comparable activity and inhibited the growth of five rifampicin-resistant *M. tb* strains (Hr 1−5) with the same MIC values (0.16 μg/mL), suggesting that the sugar moiety at the C2 position may play an important role to overcome the drug resistance. The C8-substituents were found to exert significant influence on the biological activity. For example, the C8-ethynyl analogue **39** exhibited comparable anti-TB activity to chrysomycin A (**1**) despite higher MIC against *M. bovis* BCG. (E)-C8-methyl acrylate analogue **40** was less potent than C8-vinyl analogue (+)-**64**. C4-pyridinium salt analogue **41**, C4 hybrid analogues **46b**, and C3 hybrid analogues **51b** lost activity. Overall, it is interesting and encouraging to note that both **36** and (+)-**64** display significant activity against these challenging rifampicin- or bedaquiline-resistant strains, which may offer promising drug leads for the treatment of MDR-TB.

CONCLUSION

In summary, the 10-step and gram-scale total synthesis of chrysomycin A has been accomplished in a highly convergent manner. Key features of the synthesis include two sequential C−H activation reactions and a late-stage C-glycosylation. The

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synthesis described in Figure 2a has provided >10 g of the advanced aglycon **21**. We furthermore demonstrated the late-stage diversification of natural products as a powerful strategy to generate natural product derivatives for drug discovery.[51] Accordingly, various late-stage diversification reactions, including the meta-selective C−H functionalization, have been applied to generate 33 new analogues efficiently. Extensive evaluation of the anti-TB activity of these compounds shed a light on the SAR of chrysomycin A and offered a more potent derivative (+)-**64** (5-fold enhancement) that showed a potential to overcome the drug resistance. We can envision that adapting the chemistry reported herein to create additional derivatives with optimized structures may help elucidate the mode of action and address an important area of unmet medical need.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.0c00122.

Experimental procedures, new compound characterization data, and biological evaluation data (PDF)

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Notes

The authors declare no competing financial interest.

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