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Total Synthesis and Biological Evaluation of Tiancimycins A and B, Yangpumicin A, and Related Anthraquinone-Fused Enediyne Antitumor Antibiotics

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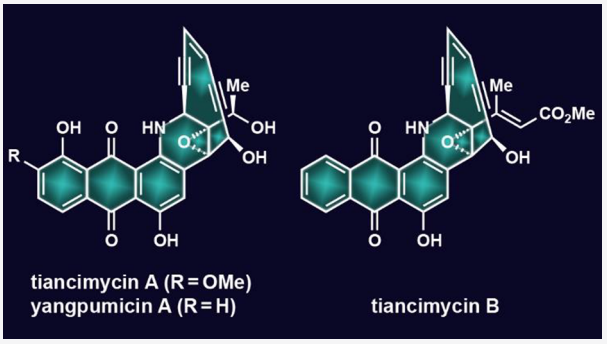
ABSTRACT: The family of anthraquinone-fused enediyne antitumor antibiotics was established by the discovery of dynemicin A and deoxydynemicin A. It was then expanded, first by the isolation of uncialamycin, and then by the addition to the family of tiancimycins A−F and yangpumicin A. This family of natural products provides opportunities in total synthesis, biology, and medicine due to their novel and challenging molecular structures, intriguing biological properties and mechanism of action, and potential in targeted cancer therapies. Herein, the total syntheses of tiancimycins A and B, yangpumicin A, and a number of related anthraquinone-fused enediynes are described. Biological evaluation of the synthesized compounds revealed extremely potent cytotoxicities against a number of cell lines, thus enriching the structure−activity relationships within this class of compounds. The findings of these studies may facilitate future investigations directed toward antibody−drug conjugates for targeted cancer therapies and provide inspiration for further advances in total synthesis and chemical biology.



1. INTRODUCTION

The enediyne family of antitumor antibiotics attracted considerable attention, initially due to their unprecedented structural features, biological potency, and novel mode of action[1] and subsequently because of their promise as potential payloads for antibody−drug conjugates (ADCs) as targeted cancer therapies.[2]

Dynemicin A (**1**, Figure 1)[3] and the closely related deoxydynemicin A (**2**, Figure 1)[4] were the first, and for more than a decade the sole, examples of anthraquinone-fused enediyne antitumor antibiotics. To this subclass of 10-membered enediynes was added, in 2005, uncialamycin (**3**, Figure 1),[5] a rather simpler member of the growing class. Despite its potent antibacterial activity and DNA-cleaving properties, the extremely small amounts (~300 μg) available from its natural source hindered uncialamycin's complete structural elucidation and detailed biological evaluation,[5] thus elevating it to an attractive total synthesis target. This challenge was later fully met by our group[6] and partially by others.[7] Recently, seeking to identify the genes involved in the biosynthesis of dynemicin A, genetic manipulation of the producing organism by C. A. Townsend et al. led to the identification of additional, partially oxygenated dynemicin A precursors (**4**, **5**, Figure 1).[8] Furthermore, motivated by the potential of anthraquinone-fused enediyne antibiotics as payloads for ADCs,[6c,9] B. Shen et al.[10] have surveyed a large number of actinomycetes in search of new members of this class of natural products. These efforts led to the discovery of several new congeners (**6**−**15**, Figure 1), thus enlarging significantly this family of bioactive molecules.[10a,b,d] It is interesting to note that, apart from yangpumicin A (**6**),[10b] tiancimycin A (**7**),[10a] and tiancimycin D (**10**),[10d] which were isolated from wild-type bacterial strains, the remaining tiancimycins [i.e., B (**8**), C (**9**), E (**11**), and F (**12**)] were produced through genetic manipulation of *Streptomyces* sp. CB03234,[10a,d] the tiancimycin A (**7**)-producing strain. It should also be noted that the existence of three of these new anthraquinone-fused enediyne antibiotics (i.e., **13**−**15**, Figure 1, in brackets) could only be inferred from isolation of the corresponding Bergman cycloaromatization[11] products.[10b,d] Interestingly, tiancimycin A (**7**)[10a,d] and yangpumicin A (**6**)[10b] were found to be even more cytotoxic than uncialamycin (**3**)[10a,b] against various human cancer cell lines.[10a,b,d]

A plethora of both experimental[3c,12] and theoretical[13] studies have shed light on the mode of cleavage of double-stranded DNA by dynemicin A (**1**). Based on its structural

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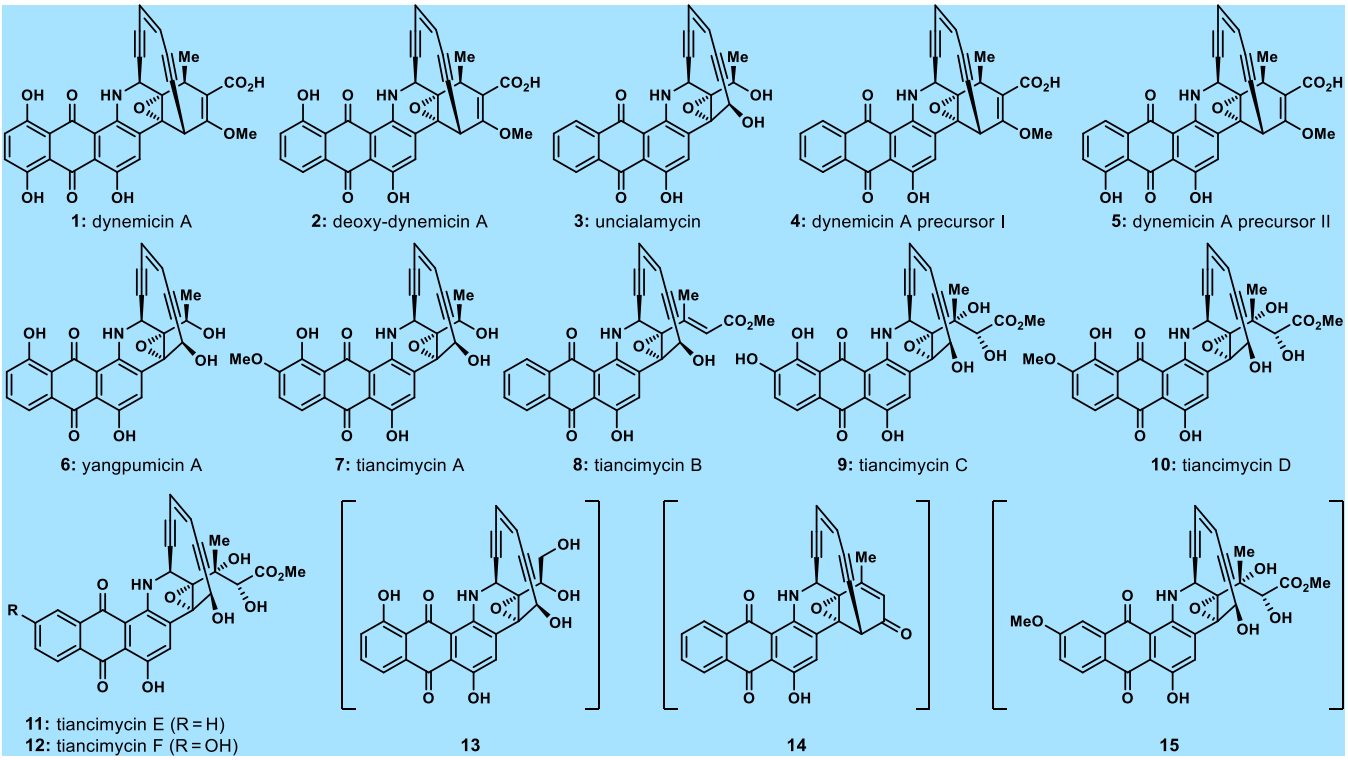

Figure 1. Naturally occurring anthraquinone-fused enediyne antitumor antibiotics. Bracketed structures (**13**–**15**) were inferred from their corresponding Bergman cycloaromatization products.

similarity with the most recent congeners (i.e., **3**–**12**, Figure 1) and the concurrent isolation of related cycloaromatized products,[6a,b,10b,d] the latter are anticipated to cleave double-stranded DNA by a mechanism similar to the one that has been proposed for the former.[12a,c] Thus, reduction of the anthraquinone core (**II** → **III**, Figure 2) would lead to opening of the epoxide ring. The strain release caused by the latter event would then trigger the Bergman cycloaromatization[11] of the enediyne moiety to form a reactive 1,4-benzenoid diradical species (**III** → **IV**, Figure 2), which could abstract a hydrogen atom from the DNA backbone, thus leading to the latter's cleavage (**IV** → **V**, Figure 2). Although the anthraquinone moiety of the molecule is capable of intercalation into double-stranded DNA,[12b,13a] studies on dynemicin A and related analogues by A. G. Myers et al. indicated that the *A* ring and C26 substituents that favor strong intercalative binding lead to diminished DNA cleavage.[12j,k] Thus, reduction and subsequent H-atom abstraction from DNA most likely occur at a minor-groove-inserted state.[13c,d] According to this proposed transient edgewise insertion of the anthraquinone moiety into the DNA minor groove, the N-substituted site of the anthraquinone moiety and C26 are expected to point toward the rim of DNA's minor groove (blue region in Figure 2), while the hydroxy-substituted site of the anthraquinone moiety should face toward the floor of the minor groove (green region in Figure 2).[13c,d]

Based on the above scenario, several notable structure–activity relationships (SARs) might be anticipated for this class of compounds: (a) The electronic nature of substituents on ring *A* could influence the redox potential of the anthraquinone moiety, and thus affect the initiation of the cascade that leads to DNA damage. (b) In parallel, substituents at C6/C7 and C8/C9 could potentially alter the equilibrium between the

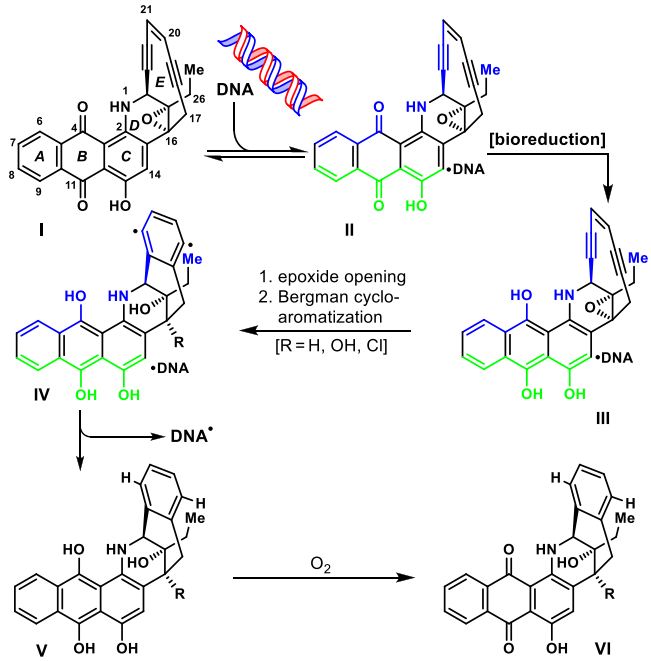

Figure 2. Presumed mechanism of DNA binding and cleavage by anthraquinone-fused enediynes. Only structural features shared by all anthraquinone-fused enediynes are depicted. The blue colored region of the molecule is presumed to face toward the rim of the DNA minor groove, while the green colored region is anticipated to face toward the floor of the minor groove during binding and cleavage.

intercalated and triggerable (i.e., minor groove inserted) states of the enediyne molecule through, for example, favorable interactions with the DNA deoxyribose phosphate backbone, or by exploiting the hydrogen-bonding potential of the DNA's

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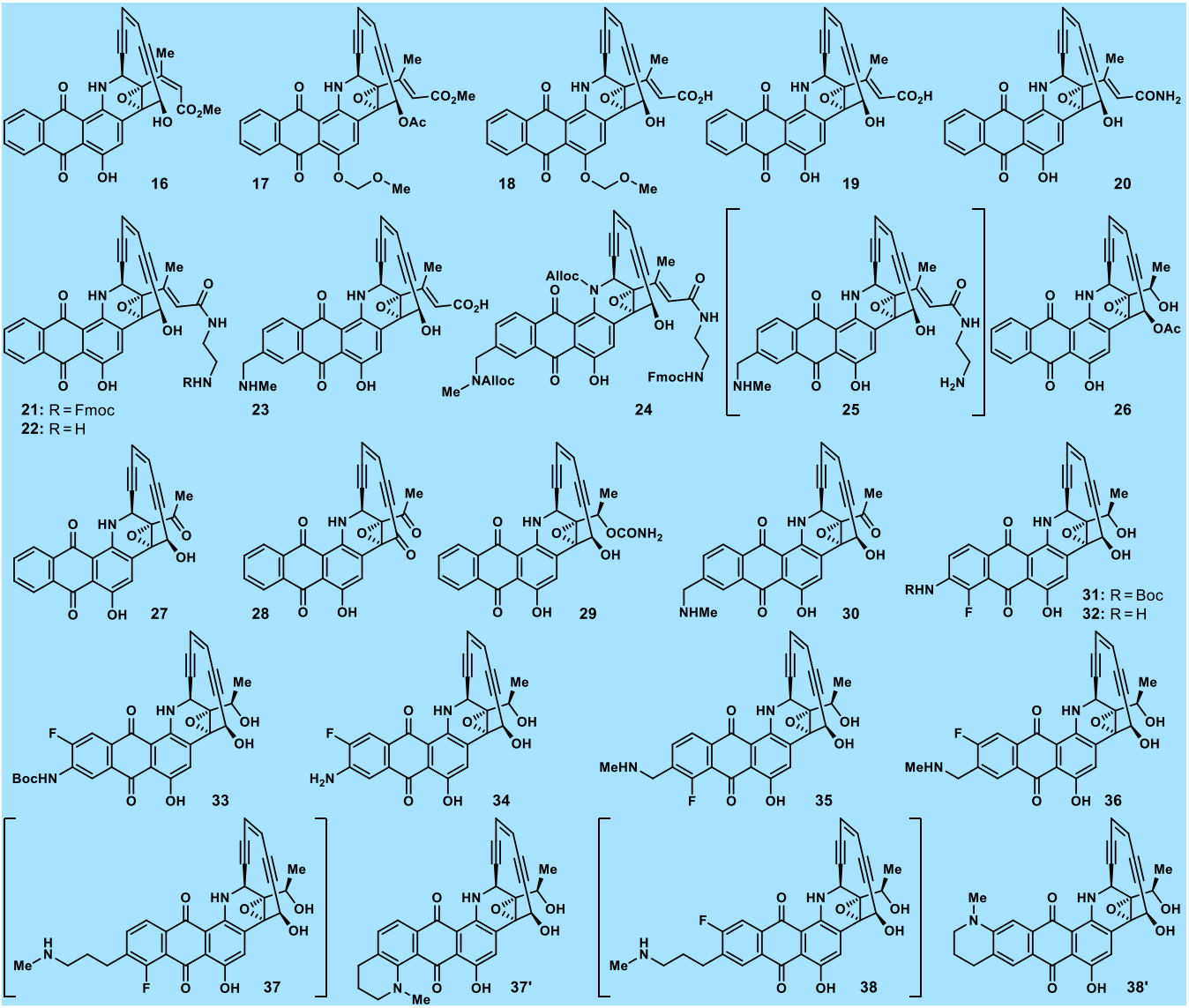

Figure 3. Designed and synthesized tiancimycin B and uncialamycin analogues. Targeted analogues **25**, **37**, and **38** were not reached; instead, analogues **24**, **37′**, and **38′** were obtained. Fmoc = 9-fluorenylmethyloxycarbonyl; Boc = *tert*-butoxycarbonyl.

minor groove floor, respectively. (c) As previously indicated by studies on dynemicin A models,[14] the oxidation state/substitution of the carbons adjacent to the epoxide ring (i.e., C17 and C26) might influence the biological activity since they could favor or hinder the consequential opening of this moiety. (d) In either the intercalation[9,13a] or insertion−intercalation[13c] mode of binding, uncharged substituents at C26 are not anticipated to interfere with the molecule's interaction with DNA, and thus might offer an alternative favorable site for linking to antibodies or other delivery systems.

Our prior endeavors toward the total synthesis of uncialamycin (**3**)[6] resulted in the development of an efficient and flexible synthetic route that provided access not only to uncialamycin itself but also to related analogues.[6c] Aiming to enrich the SARs within this class of compounds, and in order to facilitate further exploration of the potential of anthraquinone-fused enediyne antibiotics as payloads for ADCs, we initiated further studies within this field of investigation. Herein we report the application of our developed synthetic strategies and technologies to (a) the total syntheses of the naturally occurring yangpumicin A (**6**, Figure 1) and tiancimycins A and B (**7** and **8**, Figure 1); (b) the syntheses of related anthraquinone-fused enediyne antibiotics possessing the core framework of either tiancimycin B (i.e., analogues **16**−**25**, Figure 3) or uncialamycin (i.e., analogues **26**−**38′**, Figure 3) and different substituents on the ring *A* and positions C26 or C17, including fluorine residues, with interesting chemical and biological consequences; and (c) the results of the biological evaluation of a select number of the synthesized compounds against a series of cancer and related cell lines.

2. RESULTS AND DISCUSSION

2.1. Total Synthesis of Yangpumicin A (6), Tiancimycins A (7) and B (8), and Analogues 16−38′. According to our established approach toward uncialamycin,[6] the synthesis of the targeted anthraquinone-fused enediyne antibiotics bearing varying *A* ring substituents (i.e., **23**−**25**, and **29**−**37** and **38**, Figure 3; general structure **VII**, Figure 4), including the naturally occurring yangpumicin A (**6**, Figure 1) and tiancimycins A and B (**7** and **8**, Figure 1), was projected to rely

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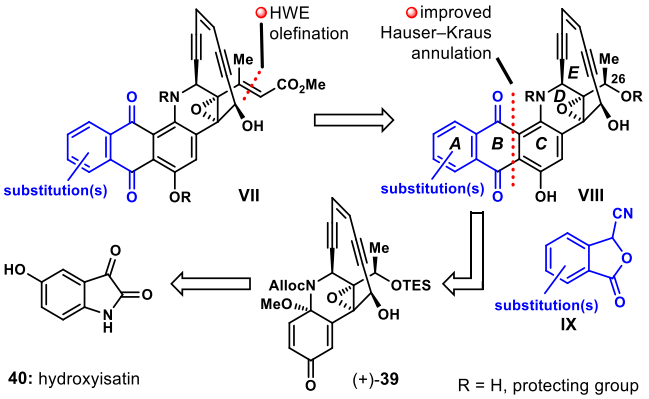
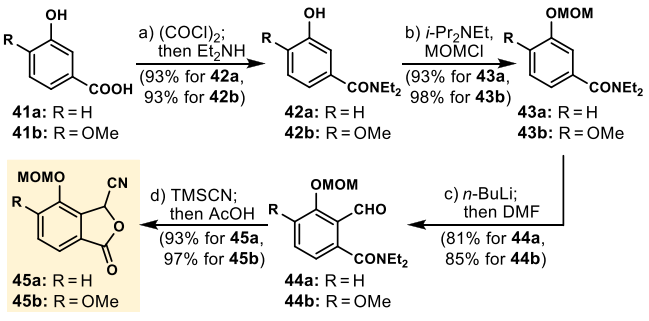


Figure 4. General retrosynthetic analysis of targeted anthraquinone antitumor antibiotics and analogues thereof. TES = triethylsilyl, Alloc = allyloxycarbonyl.

on the availability of the appropriately substituted 3-cyanophthalides **IX** (Figure 4) and their successful fusion via a Hauser–Kraus-type annulation,[15] with the Alloc-protected *p*-methoxy semiquinone aminal [(+)-**39**,[6a,c] Figure 4]. This key building block can be secured on large scale and in enantiomerically pure form from hydroxyisatin (**40**, Figure 4) as previously described.[6a,c] The synthesis of tiancimycin B (**8**, Figure 1) and related targeted analogues (i.e., **16**–**25**, Figure 3; general structure **VII**, Figure 4) was expected to rely on selective oxidation of the C26 hydroxyl group of the corresponding anthraquinone-fused enediynes (**VIII**, Figure 4) and subsequent Horner–Wadsworth–Emmons (HWE) olefination[16] of the resulting ketone.

Guided by the above retrosynthetic analysis, synthetic efforts were initially focused on the preparation of the appropriately substituted 3-cyanophthalides required for the synthesis of yangpumicin A (**6**) and tiancimycin A (**7**, Figure 1). Thus, commercially available 3-hydroxybenzoic acid (**41a**) and isovanillic acid (**41b**, Scheme 1) were converted to the corresponding diethyl amides **42a** (93% yield) and **42b** (93% yield), respectively, through exposure of their acid chlorides

Scheme 1. Synthesis of Cyanophthalides 45a and 45b[a]

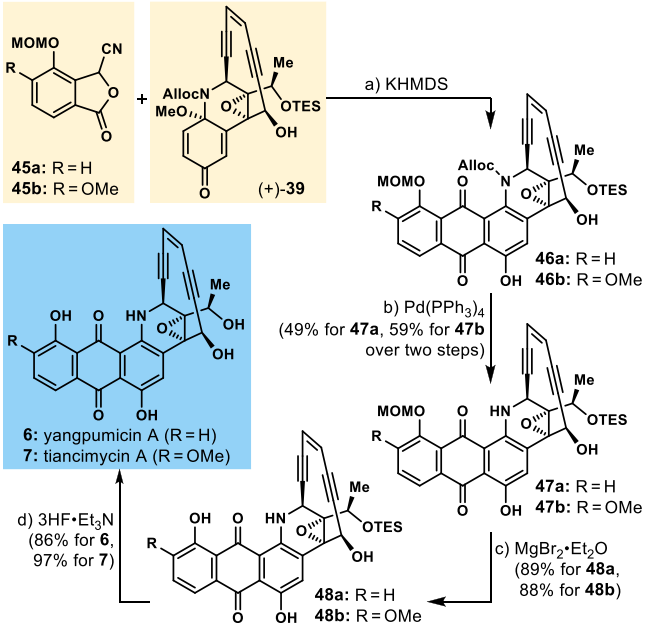


[a]Reagents and conditions: (a) $(COCl)_2$ (2.6 equiv), DMF (cat.), CH_2Cl_2, 0 to 25 °C, 12 h; then Et_2NH (4.6 equiv), THF, 0 to 25 °C, 12 h, **42a** (93%), **42b** (93%); (b) *i*-Pr_2NEt (3.0 equiv), MOMCl (3.0 equiv), CH_2Cl_2, 25 °C, 2–5 h, **43a** (93%), **43b** (98%); (c) TMEDA (1.1 equiv), *n*-BuLi (1.1 equiv), THF, −78 °C, 40 min; then DMF (1.2 equiv), −78 °C, 40–60 min, **44a** (81%), **44b** (85%); (d) TMSCN (1.3 equiv), KCN (0.2 equiv), 18-crown-6 (0.2 equiv), CH_2Cl_2, 0 °C, 4.5–5 h; then AcOH, 25 °C, 13–16 h, **45a** (93%), **45b** (97%). MOMCl = methoxymethyl chloride; TMEDA = tetramethylethylenediamine; TMSCN = trimethylsilyl cyanide.

$[(COCl)_2]$ to excess diethylamine. Subsequent protection of the phenol moiety as methoxymethyl (MOM) ether compounds **43a** (93% yield) and **43b** (98% yield), respectively, set the stage for the regioselective *ortho*-lithiation of the aromatic ring (*n*-BuLi) of the resulting intermediates, which, upon quenching with dimethylformamide, provided the corresponding formyl derivatives **44a** (81% yield) and **44b** (85% yield), respectively. Their exposure to TMSCN, in the presence of catalytic amounts of KCN and 18-crown-6, and then to AcOH secured the targeted substituted cyanophthalides **45a** (93% yield) and **45b** (97% yield), respectively, as shown in Scheme 1.

Annulation of MOM-protected cyanophthalides **45a** and **45b** with the readily obtainable[6a,c] *p*-methoxy-semiquinone aminal (+)-**39** (Scheme 2) with KHMDS led to the desired

Scheme 2. Total Synthesis of Yangpumicin A (6) and Tiancimycin A (7)[a]



[a]Reagents and conditions: (a) **45a** or **45b** (3.0 equiv), KHMDS (4.0 equiv), THF, −78 °C, 20 min; then (+)-**39** (1.0 equiv), −78 to 0 °C, 1.5 h; then 25 °C, 2.5 h (for **46a**), or (+)-**39** (1.0 equiv), −78 °C, 15 min; then 0 °C, 2 h (for **46b**); (b) $Pd(PPh_3)_4$ (0.07 equiv), morpholine (2.6 equiv), THF, 0 to 25 °C, 2.3–2.5 h, **47a** or **47b** (49% or 59% yield over two steps, respectively); (c) $MgBr_2{\cdot}Et_2O$ (3.0 equiv), THF, 0 °C, 3 h; then 25 °C, 15 min, **48a** (89%) or 0 to 25 °C, 1.75 h, **48b** (88%); (d) $3HF{\cdot}Et_3N$ (150 equiv), THF, 25 °C, 1 h, **6** (86%) or **7** (97%). KHMDS = potassium hexamethyldisilazide.

anthraquinones **46a** and **46b**, respectively, as shown in Scheme 2. The so-obtained fused products were more conveniently purified and characterized upon cleavage of the Alloc protecting group, by treatment of the corresponding crude annulation products (**46a**, **46b**) with catalytic amounts of $Pd(PPh_3)_4$ in the presence of morpholine. Thus, products **47a** and **47b** were obtained in good overall yield for the two steps [49% and 59%, respectively, based on (+)-**39**]. Finally, sequential removal of the MOM and TES protecting groups, by exposure to, initially, $MgBr{\cdot}Et_2O$ and then $3HF{\cdot}Et_3N$, furnished yangpumicin A (**6**) and tiancimycin A (**7**) in 77% and 85% overall yield from **47a** and **47b**, respectively, as summarized in Scheme 2.

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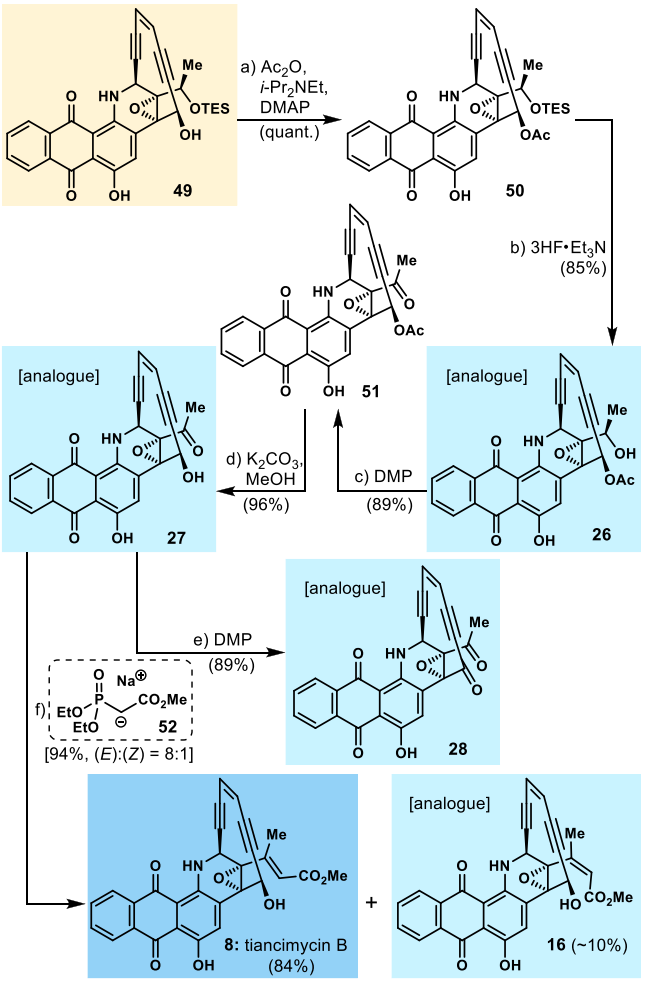
Seeking to secure a synthetic route to tiancimycin B (**8**) requiring the minimum protecting group manipulations, the TES-protected uncialamycin **49**[6c] (Scheme 3) was exploited as

Scheme 3. Total Synthesis of Tiancimycin B (8)[a]



[a]Reagents and conditions: (a) *i*-Pr_2NEt (2.0 equiv), DMAP (0.1 equiv), Ac_2O (2.0 equiv), CH_2Cl_2, 0 °C, 10 min, quant.; (b) 3HF·Et_3N (150 equiv), THF, 25 °C, 2 h, 85%; (c) DMP (2.5 equiv), CH_2Cl_2, 0 to 25 °C, 3 h, 89%; (d) K_2CO_3 (1.0 equiv), MeOH, 0 °C, 1.5 h, 96%; (e) DMP (2.5 equiv), CH_2Cl_2, 0 to 25 °C, 3 h, 89% (f) $(EtO)_2POCH_2COOMe$ (11 equiv), NaH (10.0 equiv), THF, −78 °C, 15 min; then **27**, −78 to 0 °C, 5 h, 84% (plus 10% of **16**). DMAP = 4-dimethylaminopyridine, DMP = Dess−Martin periodinane.

starting material. Thus, starting with **49**, a sequence involving selective acetylation of the propargylic hydroxyl group (*i*-Pr_2NEt, DMAP, Ac_2O, **50**, quant.) of the molecule, followed by cleavage of the TES protecting group (3HF·Et_3N, 85% yield) provided the targeted uncialamycin analogue **26**. Selective oxidation of the secondary alcohol of the latter in the presence of the free phenolic group to the corresponding ketone (**51**, 89% yield) by Dess−Martin periodinane, and finally careful methanolysis of the propargylic acetate (K_2CO_3, 96% yield) secured the targeted methyl ketone **27** as depicted in Scheme 3. HWE olefination of ketone **27** with **52** [$(EtO)_2POCH_2COOMe$, NaH] provided, after chromatographic purification, not only tiancimycin B [**8**, (*E*)-isomer, major product, 84% yield] but also the (*Z*)-isomer (**16**, minor product, ∼10% yield) as an analogue of the natural product. Further oxidation of analogue **27** with Dess−Martin periodinane led to the diketo analogue **28** (89% yield), as shown in Scheme 3.

Advanced synthetic intermediate **53**[6c] (Scheme 4) was employed as starting material for the synthesis of tiancimycin B analogues **17**−**22** and uncialamycin carbamate derivative **29** as summarized in Scheme 4. Thus, for the synthesis of analogue **29**, the phenolic group and C17 hydroxyl moiety of **53** were sequentially and selectively protected as methoxymethyl (MOM) ether and acetate, respectively, through treatment, first with MOMCl in the presence of *i*-Pr_2NEt, and then with Ac_2O and catalytic amounts of DMAP, to afford the corresponding fully protected Alloc derivative (**54**, 75% yield over two steps). The latter was subjected to TES-ether deprotection (3HF·Et_3N) to yield the expected hydroxyl intermediate and subsequent reaction of this alcohol with excess trichloroacetylisocyanate ($Cl_3CCONCO$), followed by concurrent quenching of excess reagent and methanolysis of the acetate protecting group with K_2CO_3 in MeOH, furnished carbamate **55** (72% yield over two steps). Uncialamycin analogue **29** was obtained from the latter upon sequential removal of the Alloc [$Pd(PPh_3)_4$, 96% yield] and MOM ($MgBr_2·Et_2O$, 87% yield) protecting groups under standard conditions, as shown in Scheme 4.

For the synthesis of tiancimycin B analogues **17**−**22**, the same intermediate **53** (see Scheme 4) was subjected, as above, to MOM and acetate protection steps, followed this time by cleavage of the Alloc protecting group to provide intermediate **56**, in 74% overall yield. Subsequent deprotection of the C26 alcohol within the latter (3HF·Et_3N), followed by oxidation with Dess−Martin periodinane delivered ketone **57** in 86% overall yield, setting the stage for the pending HWE olefination. Gratifyingly, the latter transformation proceeded, in this case, with exquisite stereoselectivity (compare **27** → **8** + **16**, Scheme 3 where the HWE reaction was not as exclusive), furnishing the targeted tiancimycin B analogue **17** as the sole product in 81% yield, as shown in Scheme 4. Treatment of the latter with LiOH in THF/H_2O (3:1, *v*/*v*) cleaved both the acetate protecting group and the methyl ester moiety, furnishing the MOM-protected tiancimycin B acid derivative **18** in 87% yield. The targeted free tiancimycin B acid (**19**) was obtained, in quantitative yield, from **18** upon treatment with $MgBr_2·Et_2O$. Its primary amide analogue (**20**) was secured by treatment with a 0.4 M solution of NH_3 in THF in the presence of EDCI·HCl, HOAt and *i*-Pr_2NEt (**19** → **20**, 74% yield) as shown in Scheme 4. To explore the feasibility of employing its carboxylic acid moiety as a linking functionality to construct linker-drugs and ADCs, analogue **19** was coupled with Fmoc-monoprotected 1,2-diaminoethane to yield amide **21** (74% yield), from which the targeted primary amine derivative **22** was liberated upon treatment with Et_2NH in DMSO in quantitative yield as depicted in Scheme 4.

Our experience with uncialamycin analogues revealed that introduction of a methylaminomethyl substitution at C8 (ring *A*, see structures **30**, **23**, and **24**, Scheme 5) not only provides an anchoring point for the construction of ADCs but also leads to derivatives of increased potency.[6c] In order to investigate whether such substitution might have similar effects on tiancimycin B-related analogues, derivatives **23**−**25** and **30** (see Figure 2) were targeted for synthesis as shown in Scheme 5. Their pursuit started from the known annulation product[6c] (structure not shown) of cyanophthalide **59**[6c] and *p*-methoxysemiquinone aminal (+)-**39**.[6a,c] Thus, and as shown

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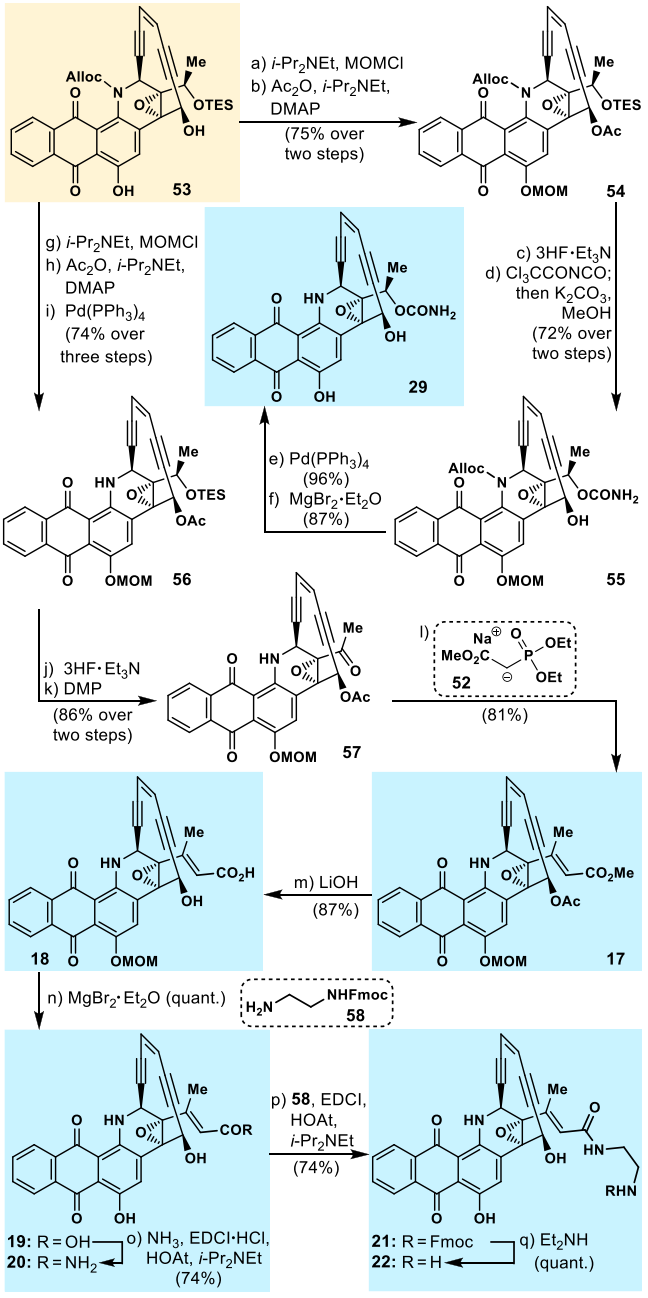
Scheme 4. Synthesis of Analogues 29 and 17−22[a]



[a]Reagents and conditions: (a) *i*-Pr_2NEt (3.0 equiv), MOMCl (3.0 equiv), CH_2Cl_2, 25 °C, 12 h; (b) *i*-Pr_2NEt (2.0 equiv), DMAP (0.1 equiv), Ac_2O (2.0 equiv), CH_2Cl_2, 0 °C, 0.5 h, 75% (over two steps); (c) 3HF·Et_3N (150 equiv), THF, 25 °C, 2 h; (d) $Cl_3CCONCO$ (2.5 equiv), $ClCH_2CH_2Cl$, 0 °C, 5 h; then K_2CO_3 (1.3 equiv), MeOH, 0 to 25 °C, 12 h, 72% (over two steps); (e) $Pd(PPh_3)_4$ (0.08 equiv), morpholine (2.6 equiv), THF, 0 to 25 °C, 2.5 h, 96%; (f) $MgBr_2·Et_2O$ (5.0 equiv), THF, 0 to 25 °C, 12 h, 87%; (g) *i*-Pr_2NEt (3.0 equiv), MOMCl (3.0 equiv), CH_2Cl_2, 25 °C, 12 h; (h) *i*-Pr_2NEt (2.0 equiv), DMAP (0.1 equiv), Ac_2O (2.0 equiv), CH_2Cl_2, 0 °C, 0.5 h; (i) $Pd(PPh_3)_4$ (0.08 equiv), morpholine (2.6 equiv), THF, 0 to 25 °C, 2.5 h, 74% (over three steps); (j) 3HF·Et_3N (150 equiv), THF, 25 °C, 2 h; (k) DMP (2.5 equiv), CH_2Cl_2, 0 to 25 °C, 12 h, 86% for the two steps; (l) $(EtO)_2POCH_2COOMe$ (6.0 equiv), NaH (5.0 equiv), THF, −78 °C, 15 min; then **57**, −78 to 0 °C, 5 h, 81%; (m) LiOH (10.0 equiv), THF/H_2O (3:1, *v*/*v*), 0 to 25 °C, 12 h, 87%; (n) $MgBr_2·Et_2O$ (5.0 equiv), THF, 0 to 25 °C, 12 h, quant; (o) EDCI·HCl (1.5 equiv), HOAt (1.5 equiv), 0.4 M NH_3 in THF (1.3 equiv), *i*-Pr_2NEt (5.0 equiv), CH_2Cl_2, 0 to 25 °C, 1 h, 74%; (p) EDCI·HCl (1.5 equiv), HOAt (1.5 equiv), $NH_2CH_2CH_2NHFmoc$ (1.3 equiv), *i*-Pr_2NEt (5.0 equiv), CH_2Cl_2, 0 to 25 °C, 1 h, 74%; (q) Et_2NH, DMSO, 25 °C, 15 min, quant. EDCI·HCl = N^3-(ethylcarbonimidoyl)-N^1,N^1-dimethyl-1,3-propanediamine hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole.

in Scheme 5, the resulting annulation product was first acetylated under the standard conditions (66% overall yield for the two steps) and then desilylated (3HF·Et_3N, 83% yield) to afford hydroxy acetate **61**, via intermediate **60**, as summarized in Scheme 5. Selective oxidation of alcohol **61** with DMP led to the triprotected analogue **62** in 86% yield. The latter precursor was converted to the targeted analogue **30** by sequential removal of the acetate (K_2CO_3, 81% yield) and Alloc [$Pd(PPh_3)_4$, morpholine, 71% yield] protecting groups. Alternatively, precursor **62** was employed as a starting material in an HWE olefination with **52** [$(EtO)_2POCH_2COOMe$, NaH] to produce, exclusively, the desired (*E*)-α,β-unsaturated methyl ester **64** in 82% yield. Exposure of **64** to LiOH induced concurrent cleavage of both the methyl ester and the acetyl group, leading to Alloc-protected hydroxy acid **65** (89% yield), from which the targeted tiancimycin analogue **23** was generated, in 78% yield, by removal of the Alloc protecting group through the action of $Pd(PPh_3)_4$ and morpholine. Carboxylic acid **65** was also successfully coupled with Fmoc-monoprotected 1,2-diaminoethane (**58**) in the presence of EDCI·HCl and HOAt to afford bis-protected amide **24** (80% yield). Interestingly, attempts to generate the targeted free primary amine analogue of **24** (i.e., **25** in Figure 2) under the standard Fmoc-deprotection conditions failed, in contrast to the results obtained in Scheme 4 (cf. **21** → **22**).

Substitution of a hydrogen atom with a fluorine residue within a molecule, especially in the vicinity of a basic functional group, can lead to potential drug candidates with improved pharmacokinetic profiles.[17] Furthermore, polar interactions between a fluoro-substituted ligand and its biological receptor can contribute to increased binding affinity, and thus potency, as compared to the affinity of its unsubstituted version.[18] These potentially beneficial effects prompted us to design and target of a series of fluoro-substituted uncialamycin analogues (**31**−**36**, Figure 2). The corresponding prerequisite fluoro-3-cyanophthalides were synthesized as shown in Schemes 6 (**69**, **70**, **75a**, and **75b**) and 7 (**82**, **83**, **88a**, and **88b**). Thus, the carbonyl moiety of commercially available 4-bromo-3-fluorobenzaldehyde (**66**, Scheme 6) was protected as an acetal with ethylene glycol (99% yield) and the resulting product was lithiated (LDA) regioselectively and reacted with methyl chloroformate to give aryl methyl ester bromide **67** in 79% yield. This versatile intermediate was utilized as precursor to required cyanophthalides (**69**, **70**, **75a**, and **75b**), as depicted in Scheme 6. Thus, Buchwald−Hartwig coupling[19] [$Pd(OAc)_2$, Xphos cat., Cs_2CO_3] of aryl bromide **67** with *tert*-butyl carbamate ($BocNH_2$, 97% yield), followed by acid-induced hydrolysis (PTSA, 78% yield) of the acetal moiety furnished *o*-formyl methyl benzoate derivative **68** as shown in Scheme 6 (center). Conversion of the latter to cyanophthalide **69** was achieved by exposure, first to TMSCN, KCN cat., 18-crown-6 cat., and then AcOH, in 60% overall yield as depicted in Scheme 6. Treatment of **69** with montmorillonite K 10 (MK10),[20] a mild reagent to remove the Boc group, allowed

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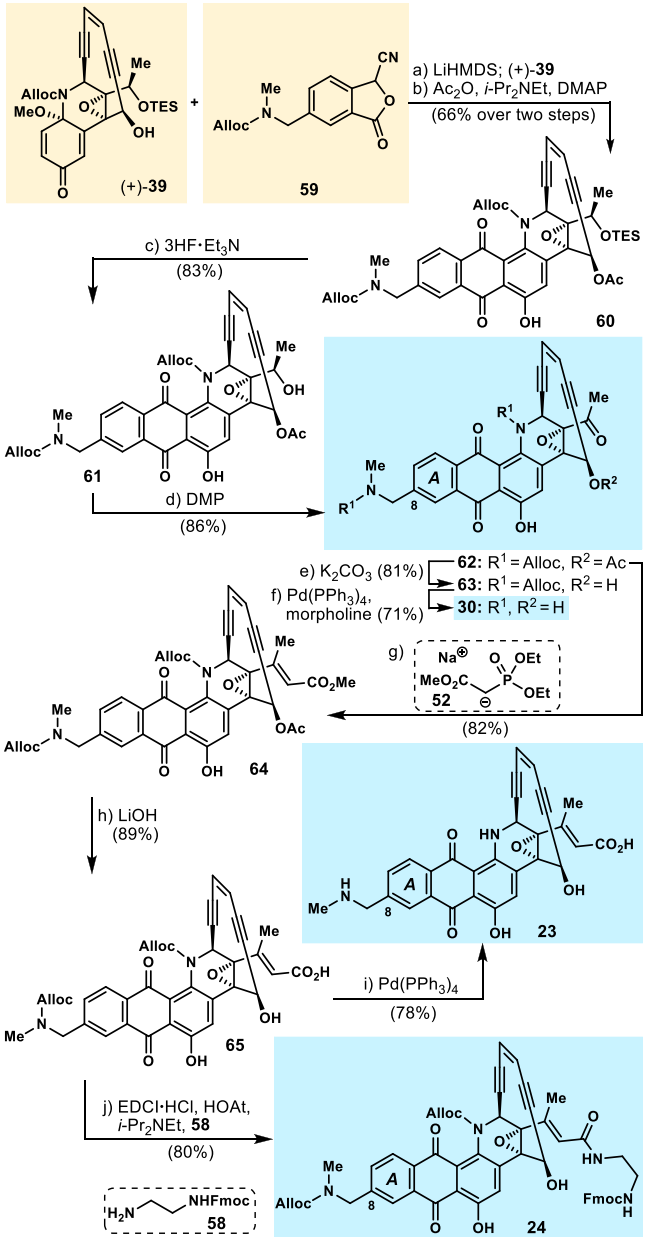
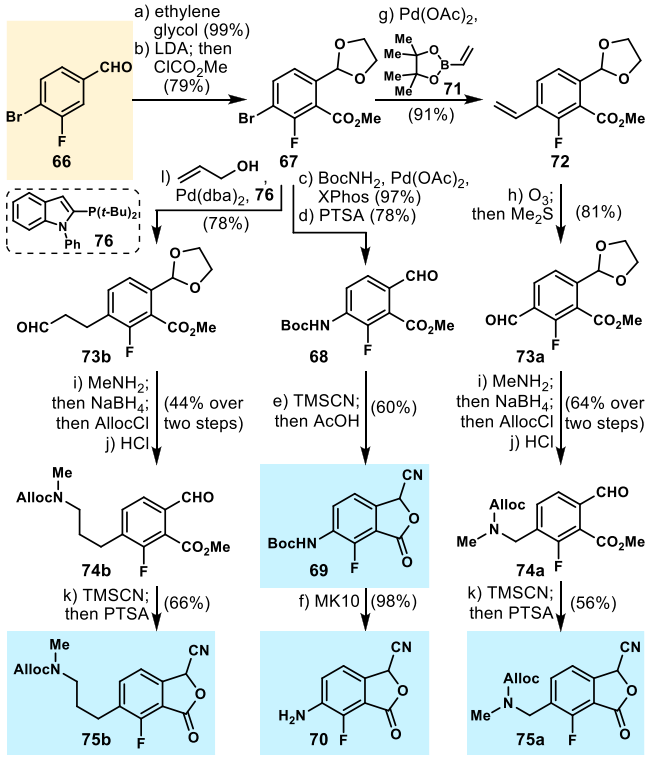
Scheme 5. Synthesis of Analogues 23, 24, and 30[a]



[a]Reagents and conditions: (a) **59** (3.0 equiv), LiHMDS (4.0 equiv), THF, −78 °C, 20 min; then (+)-**39** (1.0 equiv), −78 to 25 °C, 1 h; (b) *i*-Pr_2NEt (2.0 equiv), DMAP(0.1 equiv), Ac_2O (2.0 equiv), CH_2Cl_2, 0 °C, 10 min, 66% (over two steps); (c) 3HF·Et_3N (150 equiv), THF, 25 °C, 2 h, 83%; (d) DMP (2.5 equiv), CH_2Cl_2, 0 to 25 °C, 3 h, 86%; (e) K_2CO_3 (1.0 equiv), MeOH, 0 °C, 1.5 h, 81%; (f) $Pd(PPh_3)_4$ (0.2 equiv), morpholine (5.2 equiv), DMF, 0 to 25 °C, 2.5 h, 71%; (g) $(EtO)_2POCH_2COOMe$ (6.0 equiv), NaH (5.0 equiv), THF, −78 to 0 °C, 5 h, 82%; (h) LiOH (10.0 equiv), THF/H_2O (3:1, *v*/*v*), 0 to 25 °C, 12 h, 89%; (i) $Pd(PPh_3)_4$ (0.2 equiv), morpholine (5.2 equiv), DMF, 0 to 25 °C, 12 h, 78%; (j) EDCI·HCl (1.5 equiv), HOAt (1.5 equiv), **58** (1.3 equiv), *i*-Pr_2NEt (5.0 equiv), CH_2Cl_2, 0 to 25 °C, 1 h, 80%. LiHMDS = lithium hexamethyldisilazide.

the generation of the required free amino cyanophthalide **70**, in 98% yield. For the preparation of cyanophthlide **75a**, common precursor aryl bromide **67** (Scheme 6) was subjected to Suzuki coupling[21] [$Pd(OAc)_2$] with pinacol vinylborane (**71**) to give aryl vinyl compound **72** (91% yield) as shown in

Scheme 6. Synthesis of 7-Fluoro-3-Cyanophthalides 69, 70, 75a, and 75b[a]



[a]Reagents and conditions: (a) ethylene glycol (excess), PTSA (0.5 equiv), toluene, reflux, 12 h, 99%; (b) LDA (1.1 equiv), THF, −78 °C, 1 h; then $ClCO_2Me$ (1.1 equiv), −78 °C, 1 h, 79%; (c) $BocNH_2$ (1.2 equiv), $Pd(OAc)_2$ (3 mol%), XPhos (9 mol%), Cs_2CO_3 (1.4 equiv), dioxane, 100 °C, 8 h, 97%; (d) PTSA (0.5 equiv), acetone, 25 °C, 1 h, 78%; (e) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-crown-6 (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 1 h; then AcOH, 80 °C, 24 h, 60%; (f) MK10, DCE, reflux, 3 h, 98%; (g) pinacol vinylboronate (**71**, 1.1 equiv), $Pd(OAc)_2$ (5 mol%), Cs_2CO_3 (2.0 equiv), PPh_3 (0.2 equiv), 1,4-dioxane, 70 °C, 12 h, 91%; (h) O_3, CH_2Cl_2, −78 °C; then Me_2S (5.0 equiv), 25 °C, 1 h, 81%; (i) $MeNH_2$ (1.0 equiv), CF_3CH_2OH, 25 °C, 5 min; then $NaBH_4$ (1.2 equiv), 25 °C, 5 min; then $NaHCO_3$ (2.0 equiv), AllocCl (1.5 equiv), THF/H_2O (1:1, *v*/*v*), 25 °C, 40 min; (j) HCl (4 N, 15 equiv), THF, 25 °C, 4 h, **74a** (64%) or **74b** (44% over two steps, respectively); (k) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-crown-6 (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 4 h; then PTSA (0.5 equiv), AcOH, 80 °C, 12 h, **75a** (56%) or **75b** (66%); (l) allyl alcohol (1.1 equiv), $Pd(dba)_2$ (2 mol%), 2-(di-*tert*-butylphosphino)-1-phenylindole (**76**, 6 mol%), Cy_2NMe (1.1 equiv), DMF, 100 °C, 1 h, 78%. PTSA = *p*-toluenesulfonic acid, LDA = lithium diisopropylamide, XPhos = 2-dicyclohexyl-phosphino-2′,4′,6′-triisopropylbiphenyl, MK10 = montmorillonite K 10, DCE = 1,2-dichloroethane.

Scheme 6 (right). The latter was transformed to the desired product through a sequence involving (i) ozonolysis to afford aldehyde **73a** (81% yield); (ii) reductive amination with of **73a** $MeNH_2$ and $NaBH_4$; (iii) Alloc protection of the resulting amine, followed by HCl-induced hydrolysis of the acetal protecting group leading to aldehyde methyl ester **74a** (64% yield for the two steps); and finally (iv) TMSCN/PTSA-facilitated cyclization (TMSCN, PPTS, 56% yield) to generate the targeted cyanophthalide **75a**. Required cyanophathalide **75b** was constructed from intermediate aryl bromide **67** (see Scheme 6, top center) through a sequence involving: (i) Heck coupling[22] with allyl alcohol in the presence of $Pd(dba)_2$ and phosphine ligand **76** to give aldehyde **73b** (78% yield; note

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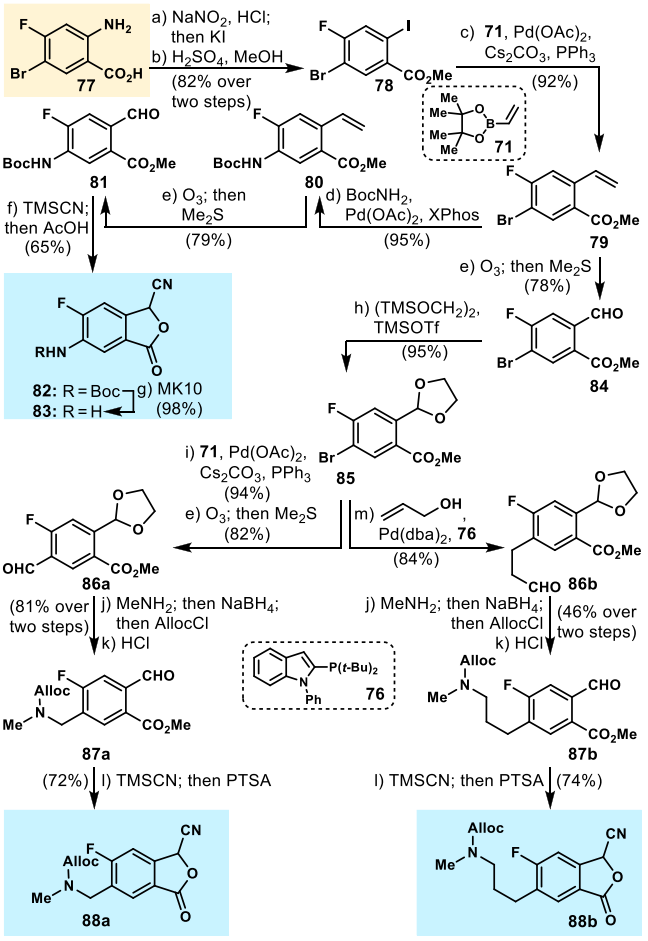
Scheme 7. Synthesis of 5-Fluoro-3-cyanophthalides 82, 83, 88a, and 88b[a]



[a]Reagents and conditions: (a) $NaNO_2$ (1.2 equiv), HCl (conc., 10.0 equiv), H_2O, 0 °C, 0.5 h; then KI (1.5 equiv), 0 °C, 1 h; then 90 °C, 0.5 h; (b) H_2SO_4 (2.0 equiv), MeOH, reflux, 12 h, 82% (over two steps); (c) pinacol vinylboronate (**71**, 1.1 equiv), $Pd(OAc)_2$ (5 mol %), Cs_2CO_3 (2.0 equiv), PPh_3 (0.2 equiv), 1,4-dioxane, 70 °C, 12 h, 92%; (d) $BocNH_2$ (1.2 equiv), $Pd(OAc)_2$ (3 mol%), XPhos (9 mol %), Cs_2CO_3 (1.4 equiv), dioxane, 100 °C, 8 h, 95%; (e) O_3, CH_2Cl_2, −78 °C; then Me_2S (5.0 equiv), 25 °C, 1 h, **81** (79%), **84** (78%) or **86a** (82%); (f) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-crown-6 (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 1 h; then AcOH, 80 °C, 24 h, 65%; (g) MK10, DCE, reflux, 3 h, 98%; (h) 1,2-bis(trimethylsilyloxy)-ethane [$(TMSOCH_2)_2$, 2.0 equiv], TMSOTf (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 4 h, 95%; (i) pinacol vinylboronate (**71**, 1.1 equiv), $Pd(OAc)_2$ (5 mol%), Cs_2CO_3 (2.0 equiv), PPh_3 (0.2 equiv), 1,4-dioxane, 80 °C, 18 h, 94%; (j) $MeNH_2$ (1.0 equiv), CF_3CH_2OH, 25 °C, 5 min; then $NaBH_4$ (1.2 equiv), 25 °C, 5 min; then $NaHCO_3$ (2.0 equiv), AllocCl (1.5 equiv), THF/H_2O (1:1, *v*/*v*), 25 °C, 40 min; (k) HCl (4 N, 15 equiv), THF, 25 °C, 4 h, **87a** (81%) or **87b** (46% yield over two steps, respectively); (l) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-crown-6 (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 4 h; then PTSA (0.5 equiv), AcOH, 80 °C, 12 h, **88a** (72%) or **88b** (74%); (m) allyl alcohol (1.1 equiv), $Pd(dba)_2$ (2 mol%), 2-(di-*tert*-butylphosphino)-1-phenylindole (**76**, 6 mol%), Cy_2NMe (1.1 equiv), DMF, 100 °C, 1 h, 84%.

concomitant isomerization/tautomerization of the allylic alcohol to the aldehyde moiety)[23] as shown in Scheme 6 (left); (ii) reductive amination of **73b** with $MeNH_2$ ($NaBH_4$) followed by Alloc protection (AllocCl) and acetal cleavage (aq. HCl) to form aldehyde **74b** (44% yield over two steps); and (iii) ring closure to form the cyanolactone ring as facilitated by TMSCN and PTSA (66% yield) to afford the targeted cyanophthalide building block (**75b**) as shown in Scheme 6.

For the preparation of the remaining required cyanophthalides (**82**, **83**, **88a**, and **88b**), commercially available 2-amino-4-fluoro-5-bromobenzoic acid (**77**) was employed as starting material as depicted in Scheme 7. Thus, aryl amine **77** was converted to its iodo counterpart by sequential treatment with $NaNO_2$, HCl and KI, followed by methyl ester formation (H_2SO_4, MeOH) to afford iodo-bromo-fluoro methyl ester **78** (82% yield over two steps). The latter was reacted with pinacol vinylboronate (**71**) in a Suzuki coupling[21] [$Pd(OAc)_2$, 92% yield] to give vinyl bromoaryl derivative **79**, whose Buchwald−Hartwig coupling[19] with $BocNH_2$ under standard conditions furnished Boc-protected aniline derivative **80** (95% yield) as shown in Scheme 7. Ozonolysis of the vinyl group within the latter (O_3; Me_2S, 79% yield), followed by ring closure of the resulting aldehyde methyl ester (TMSCN, KCN cat., 18-crown-6; AcOH) led to the desired fluoro cyanophthalide **82** (65% yield) and, upon removal of the Boc group from the latter (MK10, 98% yield), cyanophthalide **83**, as summarized in Scheme 7 (left). Through a second pathway, intermediate **78** was converted to common intermediate **85**, via **84** [ozonolysis (78% yield); followed by acetal formation, **85** (95% yield), Scheme 7, right], which was diverted through different routes to targeted cyanophthalides **88a** (left) and **88b** (right), as summarized in Scheme 7. Thus, the sequence toward cyanophthalide **88a** proceeded from **85** through Suzuki coupling with pinacol vinylboronate (**71**) under the standard conditions [$Pd(PPh_3)_4$; 94% yield], followed by ozonolysis to afford fluoro aldehyde **86a** (82% yield), whose reductive amination with $MeNH_2$, Alloc protection, and acetal hydrolysis led to aryl aldehyde methyl ester **87a** (81% overall yield from **86a**), as shown in Scheme 7. Finally, cyanophthalide **88a** was obtained from aldehyde methyl ester **87a** through the standard TMSCN/PTSA protocol, in 72% overall yield as summarized in Scheme 7. The two-carbon extended homologue of the latter, cyanophthalide **88b**, was synthesized in a similar manner from common intermediate **85** as depicted in Scheme 7. Thus, reaction of **85** with allyl alcohol in the presence of $Pd(dba)_2$ and phosphine ligand **76** furnished aldehyde **86b** (84% yield), whose conversion to the targeted cyanophthalide **88b** proceeded through intermediate **87b** under the same conditions, and in similar yields, as for the conversion of **86a** to **88a** via **87a**, as shown in Scheme 7.

With the prerequisite fluorocyanophthalides fragments in hand, their Hauser−Kraus-type annulation[15] onto the Alloc-protected *p*-methoxy semiquinone aminal (+)-**39** under the conditions previously established [i.e., LiHMDS, THF; then (+)-**39**, −78 to 25 °C],[6,15e] followed by either sequential removal of first the Alloc and then TES protecting groups (Procedure a, Scheme 8) or first removal of the TES protecting group and then concurrent global deprotection of both the aniline and amino functional groups (Procedure b, Scheme 8), provided fluorinated uncialamycin analogues **31**−**36**, respectively, as summarized in Scheme 8.

Interestingly, in the cases of targeted analogues **37** and **38**, our attempts to cleave the Alloc group and liberate the methylamino group in the last step (Procedure b, Scheme 8) resulted in exclusive formation of compounds **37′** and **38′**, respectively, apparently through an intramolecular displacement of the fluorine residue by the generated methylamino group under the reaction conditions. This reaction is

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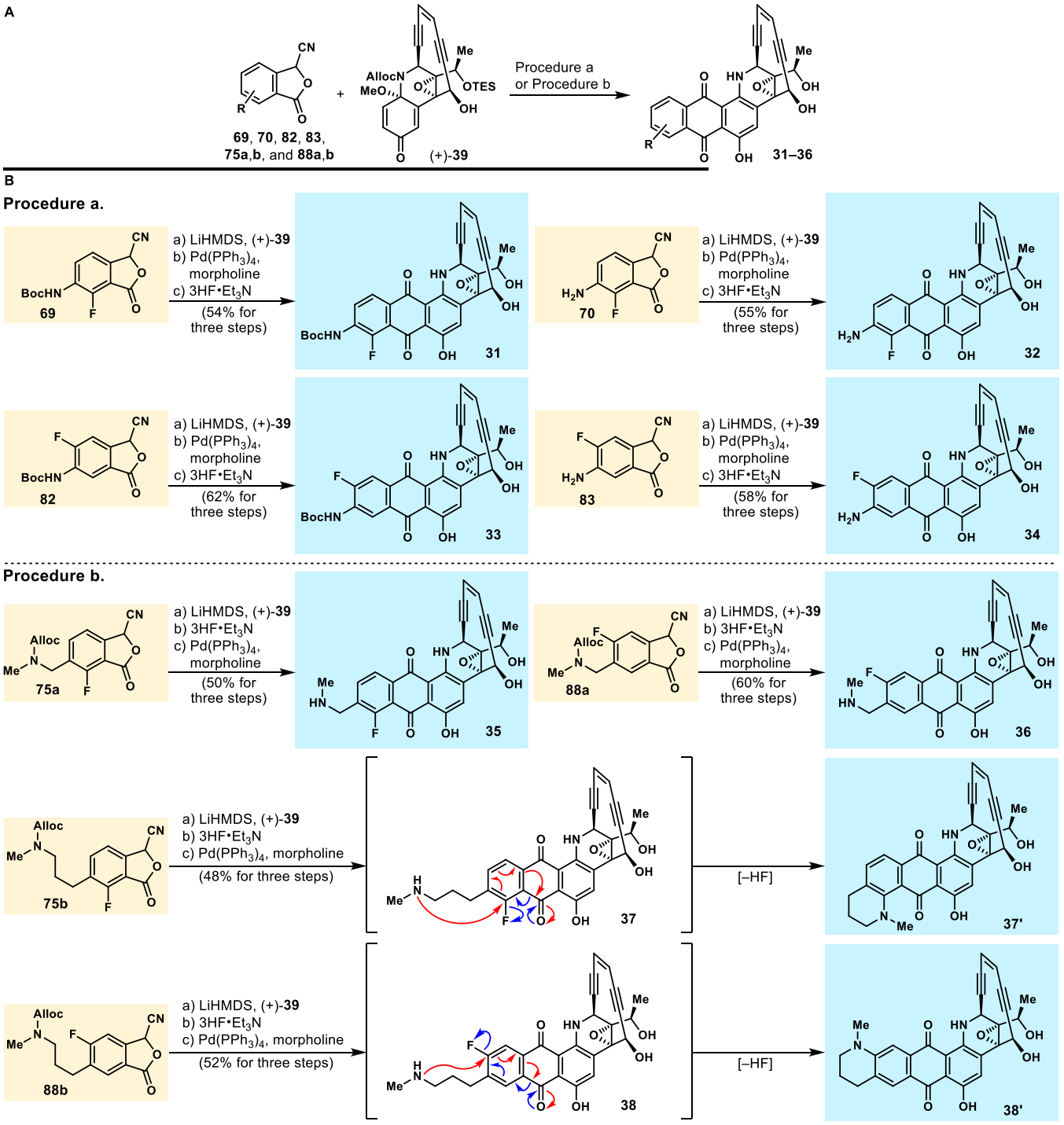
Scheme 8. Synthesis of Fluorinated Analogues 31−36, 37′, and 38′ [a]



[a]Reagents and conditions: **A.** General scheme for the preparation of analogues **31**−**36**, **37′** and **38′** from building blocks **69**, **70**, **82**, **83**, **75a**, **75b**, **88a**, and **88b**. **B.** Individual syntheses of fluorinated uncialamycin analogues **31**−**36**, **37′** and **38′** through procedures a and b. **Procedure a:** (a) LiHMDS (4.0 equiv), THF, −78 to 25 °C, 12 h; (b) $Pd(PPh_3)_4$ (0.1 equiv), morpholine (3.0 equiv), THF, 0 to 25 °C, 2 h; (c) 3HF·Et_3N (30.0 equiv), THF, 0 to 25 °C, 1.5 h. **Procedure b:** (a) LiHMDS (4.0 equiv), THF, −78 to 25 °C, 24 h; (b) 3HF·Et_3N (30.0 equiv), DMF, 0 to 25 °C, 4 h; (c) $Pd(PPh_3)_4$ (0.1 equiv), morpholine (3.0 equiv), DMF, 0 to 25 °C, 12 h.

presumably facilitated by activation of the fluoride residue through the electron-withdrawing effect of the conjugated carbonyl group on one hand, and the proximity effect of the amino group to the fluoride residue coupled with the formation of the six-membered ring of **37′** and **38′** on the other hand, as depicted in Scheme 8. The presumed mechanism for the conversion of **37** and **38** to **37′** and **38′**, respectively, is shown in Scheme 8B (bottom center). Needless to say, these serendipitous reactions leading to the unexpected and novel analogues **36′** and **37′** were of interest with regards to their biological profiles due to their unprecedented structures.

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2.2. Biological Evaluation of Synthesized Compounds. The synthesized anthraquinone-fused enediynes (natural products and designed analogues) were evaluated for their ability to inhibit the proliferation of the following cell lines: human embryonic kidney (HEK 293T), multidrug-resistant uterus sarcoma (MES SA/DX), and multidrug-resistant uterine sarcoma cell line with PGP inhibitor elacridar (MES SA/DXE) (Table 1), breast cancer cell line SK-BR-3, ovarian cancer cell line SKOV3, and cervical cancer cell line HeLa (Table 2). Uncialamycin (**3**) and *N*-acetyl calicheamicin γ_1^I were tested in parallel for direct comparison. Unlike *N*-acetyl calicheamicin γ_1^I, uncialamycin (**3**) is not a good substrate for PGP and is equally cytotoxic to MES SA/DX and MES SA/DXE cells. This multidrug pump resistance activity has been preserved among the other most active analogues [i.e., yangpumicin A (**6**), tiancimycin A (**7**), and analogues **27**, **30**, **35** and **36**, Table 1 and Figure 3]. The majority of the other designed analogues have demonstrated low nanomolar to sub-nanomolar IC_{50} values.

Notably, introduction of a secondary benzylic amine [i.e., 8-($MeNHCH_2$)-**3**] as a potential handle for ADC linker attachment resulted in a compound with cytotoxic properties very similar or even improved as compared to the parent natural product in the tested cell lines [IC_{50} values for 8-($MeNHCH_2$)-**3**: 6 pM (HEK293T); 15 pM (MES SA/DX); 4 pM (MES SA/DXE); 25 pM (SKBR3), 10 pM (SKOV3), 1.2 nM (HeLa)]. Furthermore, introduction of a single fluorine atom in the *ortho* position to the secondary benzylamine handle (cf. **35** and **36**) led to single digit picomolar cytotoxic activities in five out of the six cell lines evaluated. In comparison, fluoro aniline analogues **32** and **34** (Figure 3) were found to be 20−100-fold less cytotoxic as compared to their corresponding analogues **35** and **36**. These results also demonstrate that the combination of the potency enhancing effects of the C8-methylaminomethyl moiety and the fluorine residue at C7 or C9 of the molecule within the structures of **35** and **36** was apparently responsible for the extreme potencies exhibited by these analogues.

Interestingly, introduction of the potential linker attachment handles on the opposite side of the molecule [compounds **16** (carboxylic acid moiety derived from methyl ester) and **22** (primary amino group), Figure 3] resulted in a significant decrease in cytotoxicity (low nanomolar IC_{50} values) and significant increase in PGP efflux susceptibility (see Table 1).

Compound **19** (Figure 3) has shown no apparent cytotoxicity up to the highest concentration tested (100 nM), most likely due to the presence of the free carboxylic acid moiety and poor cell permeability. Similarly, compound **21** (Figure 3), that still contained an Fmoc protecting group, has shown no activity.

Novel analogues **37′** and **38′** showed differentiated cytotoxic activities, with compound **38′** being more potent and closely resembling the cytotoxic activity of uncialamycin (**3**), albeit

Table 1. In Vitro Cytotoxicity of Synthesized Anthraquinone-Fused Enediynes[a]

Compound	cell line		
	HEK 293T[b] IC_{50} (nM)	MES SA/DX[c] IC_{50} (nM)	MES SA/DXE[d] IC_{50} (nM)
3 (uncialamycin)	0.086	0.153	0.121
6 (yangpumicin A)	0.013	0.0207	0.013
7 (tiancimycin A)	0.030	0.064	0.037
8 (tiancimycin B)	0.404	0.661	0.599
NAC[e]	0.016	>100	0.024
16	4.853	9.503	9.511
17	>100	>100	>100
18	>100	>100	>100
19	>100	>100	>100
20	0.418	5.382	0.785
21	>100	>100	>100
22	2.627	>100	3.577
23	1.549	20.190	2.190
26	0.158	0.402	0.291
27	0.040	0.085	0.107
28	0.480	1.451	1.366
29	0.077	4.640	0.147
30	0.005	0.003	0.003
31	0.233	0.537	0.280
32	0.027	0.094	0.033
33	0.222	0.590	0.449
34	0.040	0.219	0.059
35	0.002	0.003	0.001
36	0.001	0.002	0.001
8-($MeNHCH_2$)-**3**[6c]	0.006	0.015	0.004
37′	0.855	2.459	0.820
38′	0.153	0.913	0.183

[a]For details of the biological assays, see Supporting Information. [b]Immortalized human embryonic kidney cell line. [c]Multidrug-resistant uterine sarcoma cell line. [d]Multidrug-resistant uterine sarcoma sell line with PGP inhibitor elacridar. [e]*N*-Acetyl calicheamicin γ_1^I.

Table 2. In Vitro Cytotoxicity of Select Synthesized Anthraquinone-Fused Enediynes[a]

Compound	cell line		
	SKBR3[b] IC_{50} (nM)	SKOV3[c] IC_{50} (nM)	HeLa[d] IC_{50} (nM)
NAC[e]	0.078	0.012	33.070
3	0.550	0.329	41.24
8-($MeNHCH_2$)-**3**[6c]	0.025	0.010	1.204
6	0.042	0.014	0.244
7	0.253	0.104	1.679
8	4.473	1.818	24.080
16	25.820	15.590	>100
20	7.628	3.749	97.71
21	>100	>100	>100
22	28.480	15.080	>100
23	25.880	11.330	>100
26	1.266	0.735	20.200
27	0.992	0.264	2.972
28	5.285	1.026	>100
29	0.989	0.392	38.560
30	0.009	0.002	0.092
31	3.157	1.996	>100
32	0.121	0.067	1.970
33	1.683	1.881	>100
34	0.298	0.197	5.129
35	0.007	0.002	0.052
36	0.004	0.001	0.042
37′	5.300	5.414	>100
38′	0.273	0.837	18.580

[a]For details of the biological assays, see Supporting Information. [b]Breast cancer cell line. [c]Ovarian cancer cell line. [d]Cervical cancer cell line. [e]*N*-Acetyl calicheamicin γ_1^I.

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being more susceptible to PGP efflux, while compound **37′** displayed low nanomolar potencies in five out of six cell lines evaluated.

Overall, cytotoxicity evaluation of the synthetic anthraquinone-fused enediynes have shown distinct SARs. Chemical modifications of the uncialamycin core allow for selective modulation of cytotoxic activity from low picomolar to high nanomolar IC_{50} values and also tune the analogue's susceptibility to PGP efflux.

3. CONCLUSION

Applying our streamlined synthetic strategies and methods, we synthesized the newly discovered uncialamycin congeners, tiancimycins A (**7**) and B (**8**), and yangpumicin A (**6**) and a series of novel designed analogues of these anthraquinone-type enediyne antitumor antibiotics. Biological evaluation of the synthesized natural and designed compounds revealed interesting and useful structure–activity relationships (see Figure 5B) and led to the discovery of extremely potent

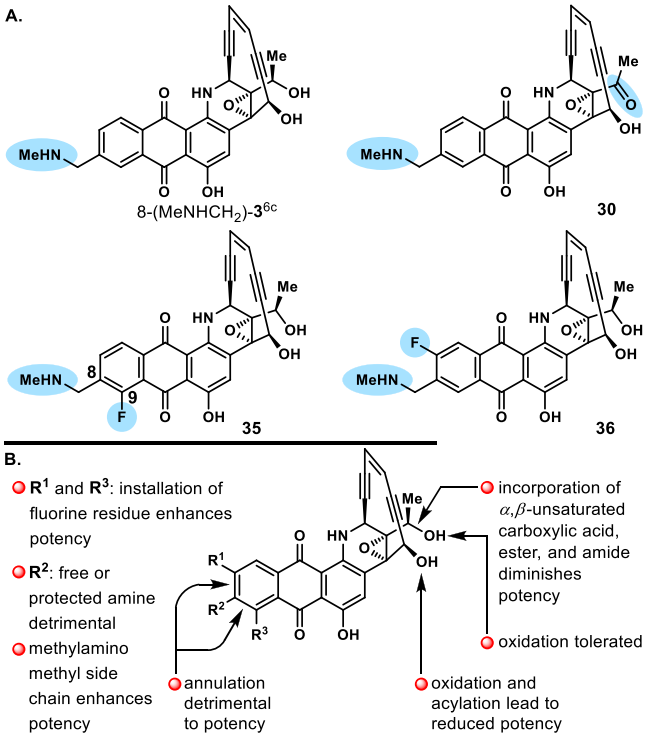

Figure 5. (A) Molecular structures of most potent uncialamycin analogues 8-(MeNHCH$_2$)-**3**,[6c] **30**, **35**, and **36**. (B) Summary of derived structure–activity relationships (SARs).

compounds against a number of cell lines, including the multidrug-resistant MES SA/DX cell line. Specifically, the 26-keto-8-methylamino methyl analogue **30**, 9-fluoro-8-methylamino methyl analogue **35**, and 7-fluoro-8-methylamino methyl analogue **36** (see Figure 5A for structures) were found to be more potent than their parent naturally occurring molecules possessing potencies down to low single digit picomolar IC_{50} values against the tested cell lines (see Table 1). These readily available analogues and others like them, and their derivatives, may serve as suitable payloads for antibody–drug conjugates and other possible delivery systems for personalized targeted cancer therapies. It should, however, be noted that potency is not necessarily the only criterium when evaluating payloads, sometimes it might be desirable to sacrifice potency in order to avoid unwanted side-effects caused by leakage of extremely potent payloads. The scalable reported synthetic strategies and technologies are destined to facilitate further studies in this relatively new oncology paradigm.[2e]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.9b12522.

Experimental procedures and characterization data for all compounds; cytotoxicity data (HEK 293T, MES SA DX, and MES SA DXE) (PDF)

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Notes

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