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# Versatile and Facile Synthesis of Diverse Semisynthetic Tetracycline Derivatives via Pd-Catalyzed Reactions

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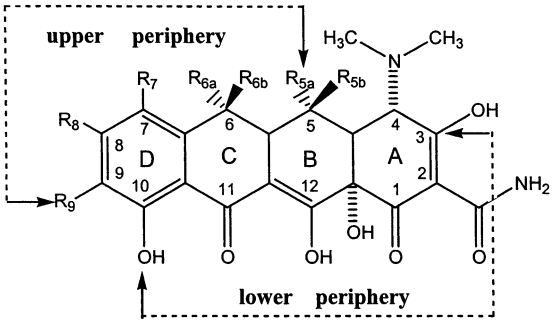
A diverse collection of tetracycline derivatives has been synthesized utilizing Heck, Suzuki, and other palladium-coupling reactions via tetracycline arenediazonium and iodoarene salts. Large numbers of tetracyclines are now possible via these reactions, including numerous upper periphery derivatives of doxycycline, minocycline, sancycline, and methacycline modified at positions C7, C9, and C6−C13 on the tetracycline naphthacene ring. Application of palladium-coupling reactions to the tetracyclines has yielded new tetracycline classes with differing structural attributes, greatly increasing the structural diversity of this family of antibiotics, one of the last of the early antibiotic families to be expanded by organic and medicinal chemistry.

## Introduction

While tetracyclines are known as potent antibacterial agents and have been studied extensively since their discovery in 1948, bacterial resistance to them has curtailed their clinical effectiveness.[1] Tetracyclines bear an intact naphthacene nucleus whose semisynthetic analogues may possess chemical modifications spanning the C5−C9 positions while the natural fermentation products derived from *Streptomyces* species chlortetracycline **1**, oxytetracycline **2**, and tetracycline **3** all possess a chemically labile $\beta$-6-OH group (Table 1).[2] The tetracyclines have had little semisynthesis and analoging compared to other classes of antibiotics,[3−5] perhaps due to their complex chemistry, chemical lability,[6] and lack of reactivity of earlier synthetic reagents with the tetracycline scaffold. Tetracyclines also present significant challenges for both their total synthesis and the semisynthesis of derivatives,[7] although the semisynthetics, doxycycline **4** and minocycline **5**, are widely used in medicine.[8,9]

**TABLE 1. Semisynthetic and Natural Tetracyclines and Designation of the Upper and Lower Peripheral Regions**




| | | $R_{5a}$ | $R_{5b}$ | $R_{6a}$ | $R_{6b}$ | $R_7$ | $R_8$ | $R_9$ |
|---|---|---|---|---|---|---|---|---|
| **1** | chlortetracycline | H | H | $CH_3$ | OH | Cl | H | H |
| **2** | oxytetracycline | OH | H | $CH_3$ | OH | H | H | H |
| **3** | tetracycline | H | H | $CH_3$ | OH | H | H | H |
| **4** | doxycycline | OH | H | $CH_3$ | H | H | H | H |
| **5** | minocycline | H | H | H | H | $N(CH_3)_2$ | H | H |
| **6** | sancycline | H | H | H | H | H | H | H |
| **7** | methacycline | OH | H | $=CH_2$ | | H | H | H |

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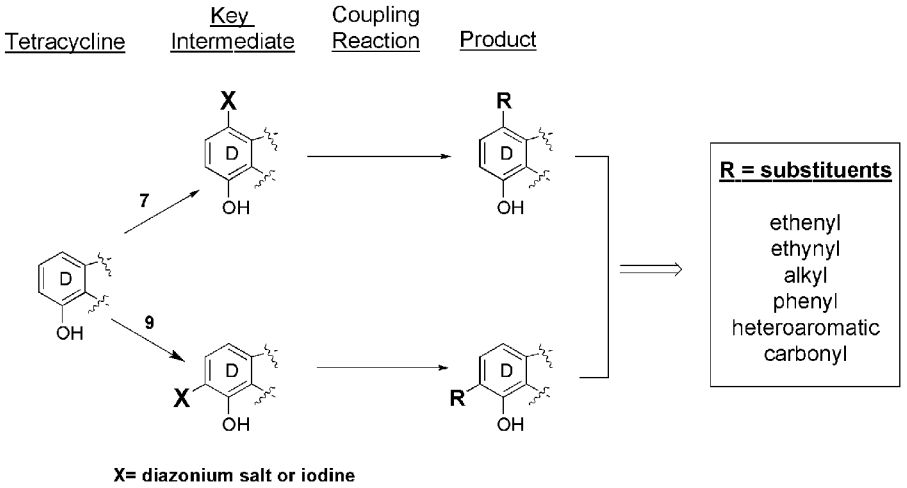
**SCHEME 1. Synthesis of D-Ring Tetracycline Derivatives via Key Reactive Intermediates**




The semisynthetic derivatives doxycycline **4**, minocycline **5**, sancycline **6**, and methacycline **7** lack the $\beta$-6-OH group and are generally stable to further chemical modification, while sancycline **6** is the simplest tetracycline possessing antibacterial activity and presents a core molecular scaffold devoid of C5−C9 chemical substituents.[10]

Structure−activity relationships based on observations of antibacterial activity subdivide the tetracycline naphthacene ring into two regions (see figure in Table 1): position modifications that when chemically modified decrease or eliminate altogether antibacterial activity, the lower peripheral region spanning carbons C10 to C3, and positions on the ring that may be chemically modified resulting in variable changes of antibacterial activity, the upper peripheral region spanning the C5−C9 carbons.[11] Antibacterial tetracyclines also possess a *4S*(α)-dimethylamino group at C4 necessary for optimal antibacterial activity, while epimerization to the unnatural 4*R*($\beta$) isomer decreases antibacterial activity, particularly against Gram-negative bacteria.[12,13] The epimerization process at the C4 position occurs during tetracycline metabolism in vivo,[14] pH changes,[6f] and under harsh chemical reaction conditions.[15]

One goal when producing semisynthetic tetracyclines is to use reagents that do not chemically modify the lower peripheral region or cause C4 epimerization, while chemically modifying the upper peripheral region and in particular the aromatic D-ring positions. Another goal is to facilitate the separation and isolation of tetracycline reactive intermediate regioisomers in high enough yield to continue with further reactions. We have found that by using Pd-catalyzed reactions[16] it is possible to produce a broad array of upper periphery modified and structurally diverse tetracyclines without affecting the lower peripheral region, causing tetracycline degradation and/or changing stereochemistry at C4.

We began by using tetracyclines lacking the 6α-OH group and added reactive functional groups on the D-ring at positions C7 and/or C9. Such reactive tetracyclines would allow the building of chemical diversity via the application of palladium-mediated reactions to doxycycline **4**, minocycline **5**, and sancycline **6** (Scheme 1). These new D-ring tetracyclines now possess alkene, alkyne, phenyl, heteroaryl, or carbonyl substituents. Palladium-catalyzed reactions also were used to modify the C6−C13 exocyclic bond of methacycline **7**, where phenylboronic acids coupled to produce C13 phenyl derivatives.

## Results

**Diazonium Salts of the Tetracyclines and Palladium Metal Based Chemistry.** Tetracycline diazonium salts[17] and iodotetracyclines[18] were produced to act as reactive intermediates (Scheme 1). Preparation of tetracycline arenediazonium salts from doxycycline **4** (Scheme 2) is possible by the electrophilic aromatic substitution of **4** with nitrating reagents to produce the 7- and 9-nitrodoxycyclines in a 1:4 ratio when using a slight excess of nitrating reagent.[5] Minocycline **5** yields

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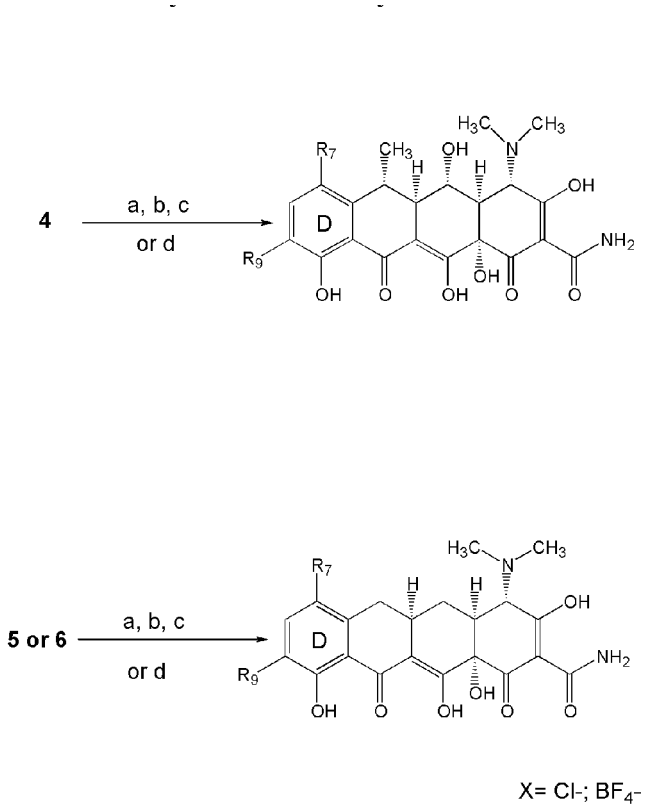
**SCHEME 2. Synthesis of Tetracycline Reactive Intermediates**[a,b]




| entry | $R_7$ | $R_9$ | Yield[b] |
|---|---|---|---|
| **4a** | $NH_2$ | H | (17) |
| **4b** | H | $NH_2$ | 82 |
| **4c** | $N_2$+X- | H | (99) |
| **4d** | H | $N_2$+X- | (99) |
| **4e** | I | H | (21) |
| **4f** | H | I | 80 |
| **4g** | I | I | 80 |
| **5a** | $N(CH_3)_2$ | $NH_2$ | 72 |
| **5b** | $N(CH_3)_2$ | $N_2$+X- | (99) |
| **5c** | $N(CH_3)_2$ | I | 69 |
| **6a** | $NH_2$ | H | (50) |
| **6b** | H | $NH_2$ | (50) |
| **6c** | $N_2$+X- | H | (99) |
| **6d** | H | $N_2$+X- | (99) |
| **6e** | I | H | 75 |
| **6f** | H | I | (50) |
| **6g** | I | I | 59 |

[a] Reagents and conditions: (a) $NaNO_3$, $H_2SO_4$; (b) $H_2$/10% Pd/C; (c) BuCN, MeOH, HCl, or BuCN, $HBF_4$; (d) NIS, $H_2SO_4$ or TFA. [b]Isolated yield (HPLC yield).

only 9-nitrominocycline[4] with use of similar reaction conditions (Scheme 2) while sancycline **6** produces a mixture of 7- and 9-nitrosancycline (Scheme 2) in a 1:1 ratio.[17] All 7- and 9-nitrotetracyclines and reaction mixtures were reduced to the corresponding amino groups by using $H_2$ as a reducing reagent with Pd/C or $PtO_2$ as a heterogeneous catalyst to yield the 7-$NH_2$ **4a** and 9-$NH_2$ **4b** derivatives of doxycycline, 9-$NH_2$ minocycline **5a**, and 7-$NH_2$ **6a** and 9-$NH_2$ **6b** derivatives of sancycline, respectively. Alternatively, 7-$NH_2$ sancycline **6a** can be prepared regiospecifically via reductive alkylation of 7-(*N*,*N*-dicarboxybenzyloxyhydrazino)sancycline followed by hydrogenation.[19]

The 7-$NH_2$ and 9-$NH_2$ tetracyclines undergo diazotization with HONO or *n*-butyl nitrite readily forming the 7- and 9-diazonium hydrochloride or tetrafluoroborate salts **4c**, **4d**, **5b**, **6c**, and **6d** in near quantitative yield.[18]

9-Diazonium doxycycline **4d** (Scheme 3) reacted readily with up to 2 equiv of α,β-unsaturated carbonyl reagents, such as acrylic acid or acrylic acid esters, in the presence of 5 mol % of $Pd(OAc)_2$ to yield 9-[3′(*E*)-propenoic acid] **8** or 9 -[3′(*E*)-propenoic acid] esters **9** of doxycycline. The salts also reacted with acrylonitrile and acrolein to form 9-[2′(*E*)-(1′-cyano)ethenyl] **10** and 9-[3′(*E*)-propenal] **11** doxycyclines. The reaction with styrene and substituted styrenes produced 9-[1′-(4″-phenyl)ethenyl] derivatives **12**−**14** in low to moderate yield without yield optimization. Ring-constrained alkenes such as cyclopentene reacted readily to form 9-(1′-cyclopentenyl)doxycycline **15**.

Palladium-catalyzed cross-coupling reactions between 9-diazonium salts **4d** and phenylboronic acids[22] were also used to synthesize numerous 9-aryl and heteroaryl derivatives of doxycycline **16**−**20** (Scheme 3).

9-Carboxylic acid doxycycline methyl ester **21** was also prepared from **4d** by using Pd-catalyzed carbonylation reactions [23] (Scheme 3). The reactions coupled carbon monoxide at ambient pressure or under high-pressure conditions (350 psi) and heat with $Pd(OAc)_2$ as a catalyst. In an alternative synthesis attempt of 9-carboxylic acid tetracyclines, the Sandmeyer reaction of arenediazonium ions with cyanide anion to form carboxylic acids did not work well with use of tetracyclines as starting materials.[24]

A C9 minocycline reactive intermediate was also prepared through diazotization, which reacted similarly to doxycycline diazonium salts. 9-Diazonium minocycline **5b** (Scheme 4) shows reactivity with olefins and catalysts yielding 9-styrylminocycline **22** and 9-[3′(*E*)-propenoic acid] esters of minocycline **23**.

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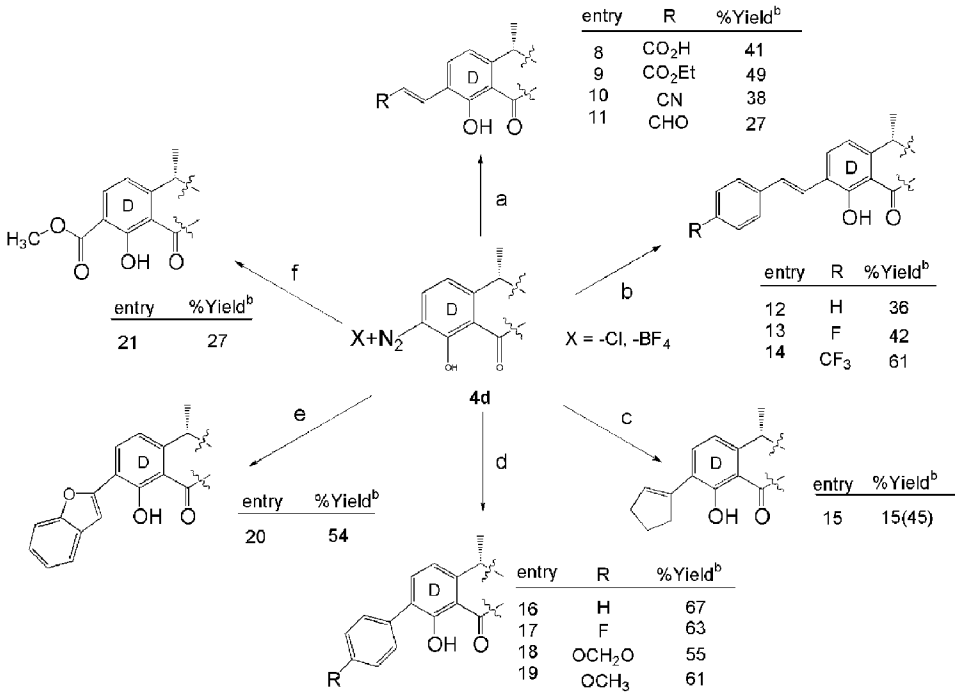
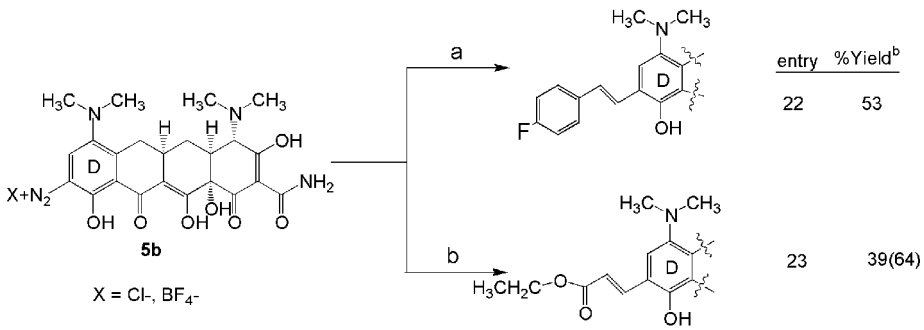
**SCHEME 3. Synthesis of C9 Doxycycline Derivatives via Tetracycline Diazonium Salts**[a,b]




[a] Reagents and conditions: (a) α,β-unsaturated carbonyl reagent, $Pd(OAc)_2$; (b) Ar-CH=$CH_2$, $Pd(OAc)_2$; (c) cyclopentene, $Pd(OAc)_2$; (d) Ar-$B(OH)_3$, $Pd(OAc)_2$; (e) Heteroaryl-$B(OH)_3$, $Pd(OAc)_2$; (f) CO, ROH, $Pd(OAc)_2$. [b]Isolated yield (HPLC yield).

**SCHEME 4. Synthesis of C9 Minocycline Derivatives via Tetracycline Diazonium Salts**[a,b]



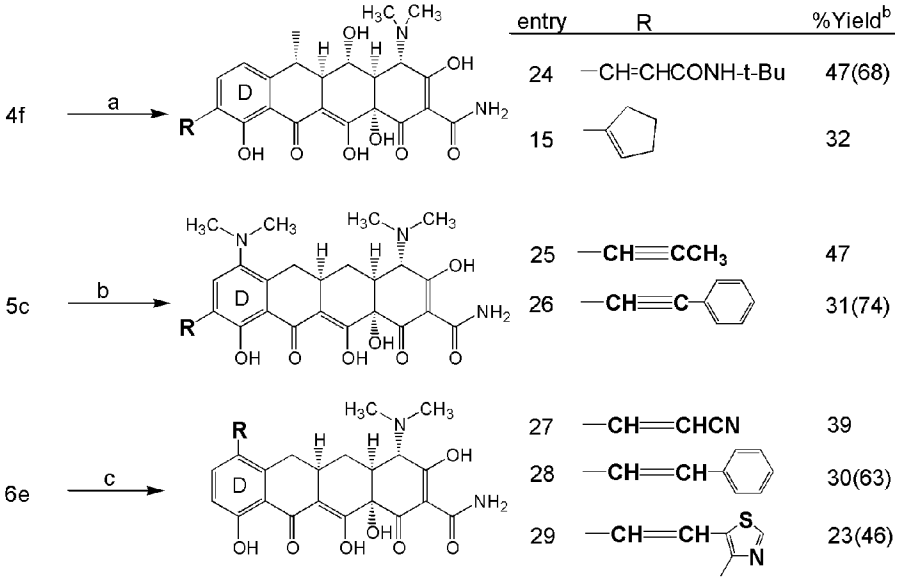

[a] Reagents: (a) Ar-CH=$CH_2$, $Pd(OAc)_2$; (b) α,β-unsaturated carbonyl reagent, $Pd(OAc)_2$. [b]Isolated yield (HPLC yield).

**7- and 9- Iodotetracyclines and Their Reactivity with Palladium Reagents.** While the diazonium salt chemistry applied to the tetracycline C7 and C9 positions led to the desired substituted tetracyclines, there were limitations of reactivity toward diverse sets of coupling reagents. An alternative semisynthesis of C7- and C9-substituted tetracyclines utilized electrophilic aromatic substitution(s) by iodine producing tetracyclines **4e**−**g**, **5c**, and **6e**−**g** (Scheme 2).[17,18] In concentrated $H_2SO_4$ and *N*-iodosuccinimide (NIS), C7 and C9 iodination products were produced as regioisomeric mixtures in a 1:1.5 ratio for doxycycline (**4e**, **4f**) and a 1:1 ratio for sancycline (**6e**, **6f**) facilitating the need for preparative separation and purification with use of C18 reversed-phase HPLC. Changing the reaction medium from concentrated $H_2SO_4$ to trifluoroacetic acid led to regioselective C7- and C9-iodinated products where doxycycline **4** was iodinated at the C9 position of **4f**, while sancycline **6** reacted only at C7 forming 7-iodosancycline **6e**. Elongation of the reaction time and the use of a 4-fold excess of NIS afforded 7,9-diiododoxycycline **4g** and 7,9-diiodosancycline **6g**, respectively. All iodinated tetracyclines **4e**−**g**, **5c**, **6e**−**g** were used to further explore their reactivity with palladium reagents (Scheme 2).

In general, we found that iodotetracyclines were an even more versatile platform for the semisynthesis of a diverse array of C7 and C9 monosubstituted and 7,9-disubstituted tetracyclines. We synthesized different structural classes of chemically modified tetracyclines, using doxycycline **4**, minocycline **5**, and sancycline **6** as starting scaffolds iodinated at positions C7 and C9, and grouped them as ethenyl, ethynyl, phenyl or heteroaryl, and carbonylation derivatives (Scheme 1). The Pd-coupling products could be further modified at other positions along the upper peripheral region to produce a structurally diverse library of tetracycline compounds, the representatives of which describe each separate synthetic pathway and are reported herein.

9-Ethenyldoxycyclines (Scheme 5) were prepared from 9-iododoxycycline **4f** by using the Heck coupling reaction

**SCHEME 5. Synthesis of Doxycycline, Minocycline, and Sancycline Derivatives via Iodotetracyclines[a,b]**




[a] Reagents and conditions: (a) α,β-unsaturated carbonyl reagent or R-CH-$CH_2$, $Pd(PPh_3)_4$, CuI, $Pd(OAc)_2$; (b) terminal alkyne, $Pd(PPh_3)_4$, CuI, $Pd(OAc)_2$; (c) R-CH=$CH_2$, $Pd(PPh_3)_4$ or Pd(*o*-tolyl)$_3$P, CuI, $Pd(OAc)_2$. [b]Isolated yield (HPLC yield).

procedures, catalysts $Pd(PPh_3)_4$ and/or $Pd(OAc)_2$, and CuI with unsubstituted and substituted alkenes in triethylamine and acetonitrile.[25] Coupling with *tert*-butyl acrylamide produced 9-(*tert*-butylacrylamido)doxycycline **24** in moderate unoptimized yield, while the 9-cycloalkenyl derivative was obtained by substituting cyclopentene as the coupling reagent **15**. The reaction of 9-iodominocycline **5c** with substituted terminal alkynes afforded 9-propynyl **25** and 9-phenylethynylminocycline **27**.

By using 7-iodosancycline **6e** numerous ethenyl and ethynyl derivatives were synthesized. Palladium-catalyzed coupling reactions of 7-iodosancycline **6e** in the presence of $Pd(Ph_3)_4$ and $Pd(OAc)_2$ with (*o*-tolyl)$_3$P and CuI produced 7-(acryl)sancyclines **27**, 7-styrylsancyclines **28**, and 7-heteroarylsancyclines **29** in moderate yield.

Symmetrical 7,9-disubstituted sancyclines were synthesized from 7,9-diiodosancycline **6g** (Scheme 6) and excess coupling reagent. Reaction of **6g** with trimethylsilylacetylene[26] (TMS-acetylene) followed by hydrolysis produced 7,9-diethynylsancycline **30** in good yield. Attempts to use acetylene as a coupling reagent resulted in numerous side and intractable products while the TMS-acetylene reaction went smoothly. The catalytic hydrogenation of **30** produced 7,9-diethylsancycline **31**.

Unsymmetrical 7,9-disubstituted products were synthesized by first conversion of 7-iodosancycline **6e** to a desired reaction product such as 7-ethylsancycline **32**.[27] 7-Derivatives were further iodinated at C9 by using NIS to produce 7-ethyl-9-iodo tetracycline **33**, which was consequently transformed by coupling reactions to produce 7-ethyl-9-ethynyl-disubstituted sancycline **34**.

9-Iodominocycline **5c** reacted with alkenes and alkynes under similar conditions producing 9-alkenyl- and 9-alkynylminocyclines, which upon catalytic hydrogenation yielded 9-alkylminocycline derivatives **35** and **36**.

7- and 9-substituted and 7,9-disubstituted phenyl and heteroaryl tetracyclines **18** and **37**−**47** were also readily synthesized via 9-iododoxycycline **4f**, 9-iodominocycline **5c**, and 7-iodosancycline **6e** (Scheme 7). 9-Iododoxycycline **4f** reacted under Suzuki coupling conditions of $Na_2CO_3$ and $Pd(OAc)_2$ in methanol with unsubstituted or substituted phenylboronic acids or heteroarylboronic acids to produce 9-phenyl or 9-heteroaryl doxycyclines **18**, **37**, and **38**.[27] 9-Phenyldoxycycline derivatives possessing amino group functionality were obtained by initial synthesis of 9-formylphenyldoxycycline **37**, followed by reductive amination with primary or secondary amines and sodium triacetoxyborohydride to afford **38**.[32] Similarly, the reaction of 9-iodominocycline **5c** afforded 9-phenylminocyclines **39** in high yield. 9-Formylphenyl minocycline **39** was further converted to 9-phenylmethylamine derivatives such as **40** by reductive amination.

7-Iodosancycline **6e**, under the same reaction conditions, produced numerous 7-phenylsancycline derivatives, whose scope was limited only by the commercial availability of phenylboronic acids or their synthesis (Scheme 7). 7-Phenylsancycline **41** was first synthesized, leading to further explorations of substituent space at all positions of the 7-phenyl ring. 7-Phenylmethylamine derivative **43** was synthesized by either of two previously described routes: first forming a 7-formylphenylsancycline **42** followed by conversion by reductive amination,[28] or by direct coupling of an aminomethyl phenylboronic acid with 7-iodosancycline **6e**. Both methods produced identical compounds, although direct coupling of ami-

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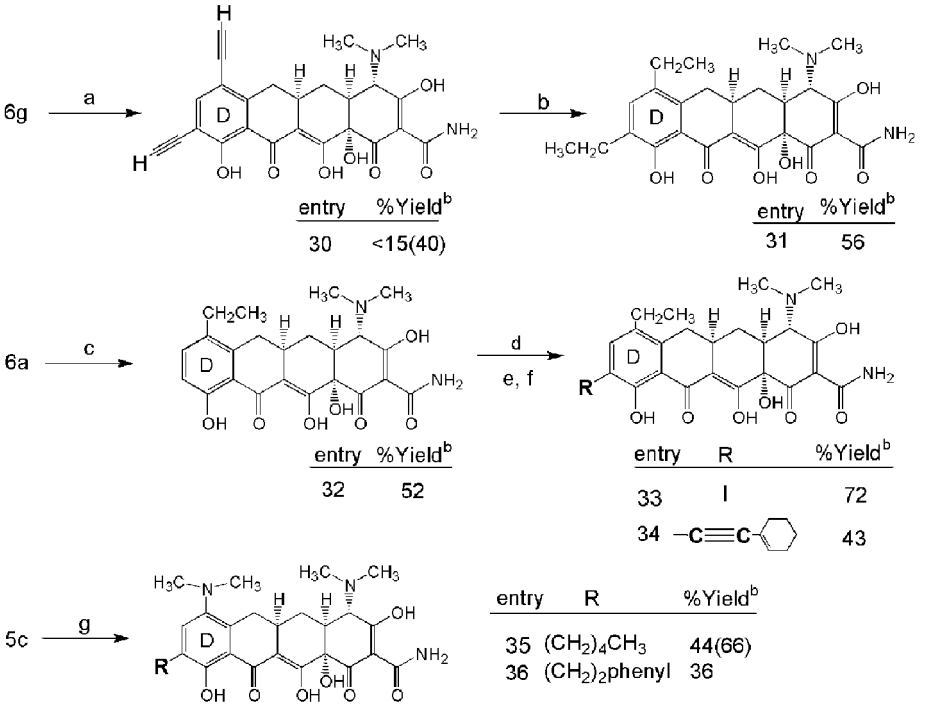
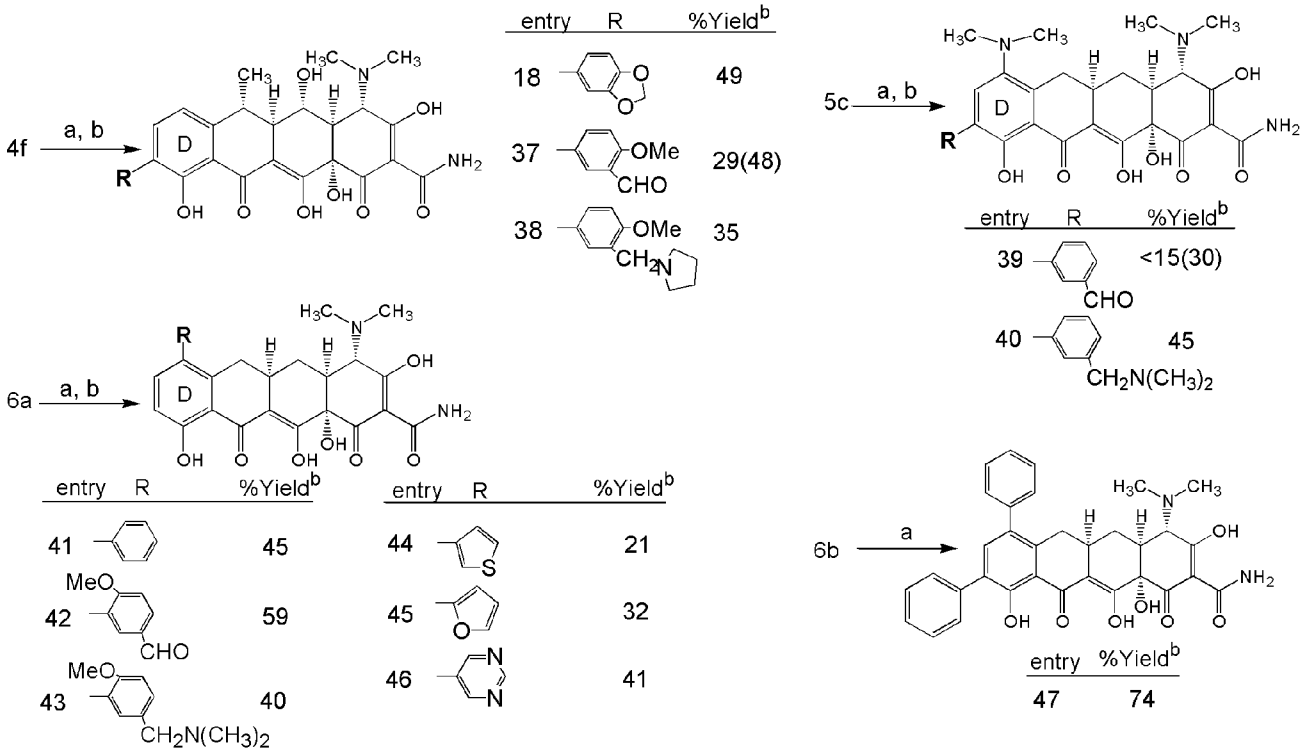
**SCHEME 6. Synthesis of C7 and C9 Disubstituted Sancycline Derivatives and C9 Alkyl Derivatives of Minocycline[a,b]**




[a] Reagents and conditions: (a) TMS-aceylene, $Pd(PPh_3)_4$ or Pd(*o*-tolyl)$_3$P, CuI, $Pd(OAc)_2$; (b) 10% Pd/C; (c) TMS-acetylene, $K_2CO_3$ followed by 10% Pd/C (d) TFA, MsOh, NIS; (e) HPLC; (f) cyclohexenyl acetylene, $Pd(PPh_3)_4$ or Pd(*o*-tolyl)$_3$P, CuI, $Pd(OAc)_2$; (g) terminal alkyne, $Pd(PPh_3)_4$ or Pd(*o*-tolyl)$_3$P, CuI, $Pd(OAc)_2$ followed by 10% Pd/C. [b]Isolated yield (HPLC yield).

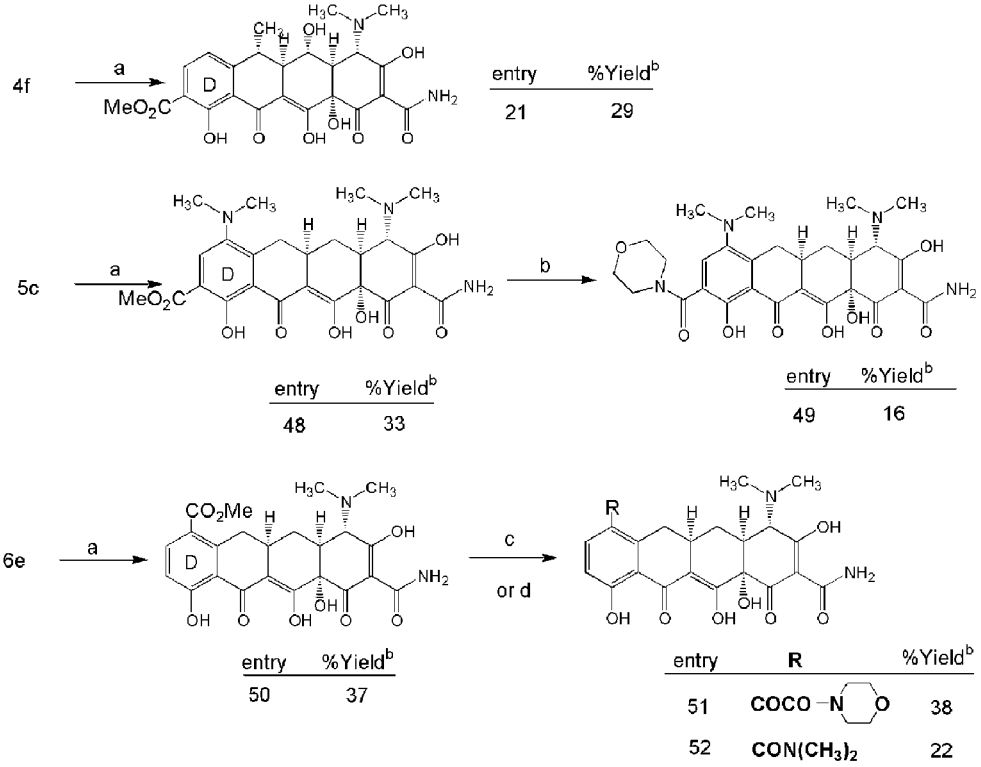
**SCHEME 7. Synthesis of C7 and C9 Tetracycline Derivatives via Iodotetracyclines[a,b]**




[a] Reagents and conditions: (a) Ar-B(OH)$_3$ or heteroaryl-B(OH)$_3$, $Na_2CO_3$, $Pd(OAc)_2$; (b) amine, $NaB(OAc)_3H$, 1,2-DCE. [b]Isolated yield (HPLC yield).

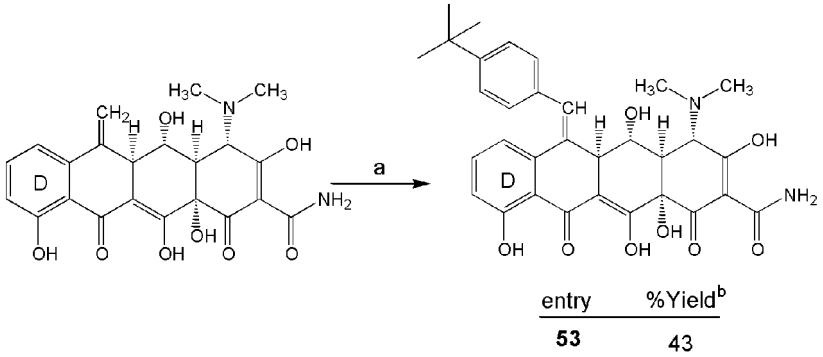
nomethylphenyl boronic acids resulted in higher yields and little or no epimerization of the C4 dimethylamino group. Heteroarylboronic acids possessing S-, O-, and N-containing heterocycles also coupled readily producing 7-heteroaryl sancyclines **44**−**46**. Symmetrical 7,9-diphenylsancycline **47** was also readily synthesized using 7,9-

**SCHEME 8. Carbonylation Reactions via Iodotetracyclines and Amide Formation[a,b]**




[a] Reagents and conditions: (a) CO, $Pd(OAc)_2$, ROH; (b) $Pd(PPh_3)_2Cl_2$, CO, morpholine; (c) NaOAc, CO, morpholine, $Pd(dppf)_2Cl_2$; (d) dimethylamine, TEA, HBTU, and DMF. [b]Isolated yield (HPLC yield).

**SCHEME 9. Synthesis of 13-Phenyl-Substituted Methacycline Derivatives[a,b]**




[a] Reagents: (a) 4-*tert*-butyl-Ar-$B(OH)_3$, $Pd(Cl)_2$ $CuCl_2$. [b]Isolated yield (HPLC yield).

diiodosancycline **6g**, coupling with excess reagents under Suzuki conditions.

9-Iododoxycycline **4f**, 9-iodominocycline **5c**, and 7-iodosancycline **6e** underwent coupling reactions with carbon monoxide (CO) with palladium catalysts to produce tetracycline carbonyl derivatives (Scheme 8).[29] The 9-carboxylic acid methyl ester of doxycycline **21** and minocycline **48** were readily synthesized from 9-iododoxycycline **4f** and 9-iodominocycline **5c**, respectively, and yielded products identical with the derivatives produced through the diazonium salt reactive intermediates. The carbonylation reaction conditions ranged from mild with carbon monoxide at atmospheric pressure and room temperature to more forcing conditions with high carbon monoxide pressure (350−500 psi) and heat (60−80 °C). The 9-substituted amide of minocycline **49** was then synthesized with use of standard reaction conditions. [30]

Similarly, 7-carboxylic acid esters of sancycline **50** were synthesized via carbonylation reactions, and the carboxylic acid was further converted to 7-carbonyl amides **51** and **52** (Scheme 8).

Pd-catalyzed reactions were next examined at the C6−C13 exocyclic double bond of methacycline **7**.[31] Methacycline **7** reacted with phenylboronic acids in the presence of $PdCl_2$ (Scheme 9) to yield the corresponding 13-phenylmethacycline derivatives, exemplified by 13-(4-*tert*-butylphenyl)methacycline **53** in moderate to good yield.[32]

## Discussion

In the past, the chemical modification of the tetracycline family of antibiotics was limited to C7 and C9 amine derivatization, producing reactive functional groups that could be further modified by electrophilic reagents or by

the production of diazonium salts of tetracyclines and subsequent reaction with nucleophiles.[17,32] 7-Amino derivatization has produced the antibiotic minocycline, while more recent work by Sum et al. has produced a new tetracycline, Tigecycline, a derivative of 9-aminominocycline.[4]

Position 7- and 9-aminotetracyclines were used as reactive intermediates to synthesize corresponding tetracycline D-ring derivatives by forming diazonium salts followed by palladium-catalyzed coupling with a wide variety of reagents possessing $\pi$ bond character. While these C7 and C9 tetracycline substitutions occurred readily to yield new structural classes of tetracyclines, the reaction of the tetracycline diazonium salts with Pd-based chemistries produced numerous side products and limited the number of reagents that could be coupled effectively and in satisfactory yield.

We increased the range of possible reaction products by producing iodotetracyclines at C7 and C9 that would remain stable to strong acidic conditions encountered during electrophilic aromatic substitution and would favor regioselective reaction at C7 or C9, depending on the starting tetracycline scaffold. While literature methods describe the synthesis of C7 and C9 iodotetracyclines,[33,34] inorganic acids generated complex mixtures of C7 and C9 and C7/C9 iodinated compounds needing preparative C18 reverse-phase HPLC. In a recent report based on early work begun in our laboratory on coupling of C7 sancycline with phenylboronic acids, it was shown that concentrated $H_2SO_4$ and NIS produced only the 7-iodosancycline intermediate, with no mention of an equal production of the 9-iodinated regioisomer.[34] The HPLC trace and $^1$H NMR spectra of the product of the reaction in $H_2SO_4$ (Figure 1, parts A and B, Supporting Information), shows that both C7 and C9 regioisomers are produced in approximately equal amounts, showing distinct peaks for both regioisomers, indicative of a mixture. While C7 and C9 iodo compound separation was not described, at first we separated the mixture by C18 reverse phase or other solid phases with increased regioisomer resolution abilities before carrying out further coupling reactions to avoid complex mixtures of C7 and C9 products. Unfortunately, in our hands silica gel chromatography even with chelation agents ($Na_2EDTA$) to suppress metal binding inherent in silica failed to resolve C7 and C9 regioisomers.

We overcame this problem by producing 7-iodosancycline or 9-iododoxycycline in high yield in regioisomerically pure form by changing the acidic solvent/catalyst from concentrated $H_2SO_4$ to trifluoroacetic acid. This eliminated the HPLC purification step, and produced pure compound, as followed by $^1$H NMR. The subsequent reaction of D-ring iodotetracyclines with palladium and phosphine ligands was more efficient than coupling with diazonium tetracyclines where, for example, Heck coupling utilizing iodotetracyclines generated the desired derivatives with fewer side products and in higher unoptimized yield. It was clearly evident that compared to diazonium salts, Pd(0)-catalyzed coupling of iodotetracyclines with palladium reagents and its reaction cycle occurs readily and can utilize a wide variety of Pd catalysts, unimpaired by the functional groups found within the tetracycline scaffold. Furthermore, some Pd-based reagents were found to degrade or modify the lower peripheral region or change stereochemical features at C4 under drastic chemical reaction conditions, whereas diazonium salts tended to cause more side products to occur. Under drastic carbonylation conditions of high pressure and temperature, both diazonium salts and iodotetracyclines caused C4 dimethylamine epimerization to occur, changing from the desired natural 4*S* epimer ($\alpha$) to the unnatural 4*R* ($\beta$).

## Conclusions

While numerous chemical problems plague tetracycline semisynthesis, using Pd-coupling reactions and tetracycline intermediates, new structural classes of tetracyclines substituted by alkenes, alkynes, phenyl, and heteroaryl groups have been achieved. Furthermore, carboxylic acid derivatives are also possible at C7 and C9 on the tetracycline scaffold and in yields substantial for further chemical modification and the production of further tetracycline classes.

We have also shown that the C6−C13 exocyclic double bond of methacycline is reactive toward Pd-based reagents and phenylboronic acids under standard reaction conditions, resulting in the formation of 13-phenyl derivatives, another new class of tetracyclines. Past modifications of methacycline at this position have been limited to the Markovnikov addition of mercaptans to the exocyclic double bond forming 13-thio derivatives of methacycline or the formation 1,2-diols via $OsO_4$-catalyzed reactions.[35,36]

We have applied more recent organic chemical reactions to the tetracycline family of compounds, greatly expanding the structural features along regions of the molecule that can be modified without damaging key molecular substructures necessary for biological activity. With use of tetracycline reactive diazonium and iodo intermediates and Pd-coupling reactions, it is now possible to produce new structurally diverse classes of tetracyclines, compounds formed from C−C bond-forming reactions at C7 and C9 on the tetracycline scaffold. It was also evident that the Pd-based reagents employed were stable and tolerant of the many functional groups present within the tetracycline nucleus, making them synthetically versatile for chemical modifications and structural changes along the tetracycline upper periphery.

With the generation of a large number of 7-, 9-, and 6−13 monosubstituted and 7- and 9-symmetrical and unsymmetrical disubstituted tetracyclines, their structure−activity relationships to antibacterial activity will be the subject of forthcoming publications. More recently, the biological activities of some tetracyclines have been described in eukaryotic systems, modulating inflammation, neurodegenerative, and cellular signaling pathways related to nitric oxide and cytokine production, further warranting the production of new tetracycline structural classes.[37]

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## Experimental Section

Reagents and catalysts were used without further purification. Reactions were monitored by HPLC with UV at 280 nm, using C18-reverse phase 4$\mu$ (50 × 4.5 mm) analytical columns. Method A used previously described binary solvent separations employing phosphate buffer with $Na_2EDTA$ at pH 4.5 and methanol on a linear gradient of 10% MeOH to 100% MeOH over 30 min at 1.6 mL/min.[3] Method B used a binary gradient of 0.1% TFA in water (Phase A) and 0.1% TFA in acetonitrile (Phase B) on a gradient from 1% Phase A at time 0 to 100 Phase B at 8 min at 1.6 mL/min. Method C utilized preparative HPLC separations employing C18 reverse-phase columns (250 × 42.4 mm) packed with C18-reverse phase 10$\mu$ media, using similar binary gradient conditions but at much higher flow rates of 40 to 60 mL/min, and are reported in minutes or minute-fraction ranges. Method D utilized preparative HPLC separations employing solid-phase columns (250 × 42.4 mm) packed with C18-reverse phase 10$\mu$ media or divinylbenzene (5−15 $\mu$m, 1000 Å), using similar binary solvent conditions of 0.1% TFA with a 0.1% TFA-acetonitrile gradient at higher flow rates of 40 to 60 mL/min. $^1$H NMR spectra were recorded at 300 or 400 MHz and the chemical shift values are expressed in $\delta$ values. Low- (ESI) and high-resolution spectra (FAB) were recorded while high-resolution EI mass spectra were via FAB analysis.

**General Procedures. I. Aminotetracycline Reactive Intermediates: [4*S*-(4α,12aα)]-9-Amino-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (4b).** 9-Nitro doxycycline hydrochloride was synthesized as previously described,[5] and a 1.0-g sample was hydrogenated in 40 mL of MeOH, 1 mL of concentrated HCl, and 100 mg of 10% Pd/C for 3 h under 30 psi of $H_2$. The solution was filtered through Celite and the filtrate evaporated in vacuo to obtain 0.9 g of the dihydrochloride salt as a yellow solid. Yield 82%. Analytical $R_t$ = 4.48, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.52 (d, 1H, $J$ = 8.08 Hz), 6.98 (d, 1H, $J$ = 8.08 Hz), 4.32 (s, 1H), 3.6 (dd, 1H), 2.98, 2.90 (each s, 3H), 2.84 (d, 1H), 2.72 (m, 1H), 2.59 (dd, 1H), 1.36 (d, 3H). HRMS calcd ($C_{22}H_{26}N_3O_8$ + H) 460.1720, found 460.1739.

**[4*S*-(4α,12aα)]-9-Amino-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (5a).** 9-Nitro minocycline hydrochloride was synthesized as previously described in the literature.[4] Catalytic hydrogenation of 16 g at 40 psi in 2-methoxyethanol and 2 N sulfuric acid with 1 g of 10% Pd/C afforded 9-aminominocycline sesquisulfate as a crude solid. The solid was dissolved in methanol (150 mL) and filtered through Celite, then added dropwise to ice-cold diethyl ether (2 L) with stirring. The resulting solid was filtered and washed with ether to give 13.5 g of compound in 72% yield. Analytical $R_t$ = 1.92, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.89 (s, 1H), 3.98 (s, 1H), 3.5 (dd, 1H), 3.07, 2.93 (m, 12H), 2.84 (d, 1H), 2.72 (m, 1H), 2.59 (dd, 1H), 1.56 (d, 3H).

**II. Diazonium Tetracycline Reactive Intermediates: [4*S*-(4α,12aα)]-9-(Diazonium)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (4d).** A flask was charged with 100 mg of compound **4b** and dissolved with 4 mL of 0.1 N HCl. The solution was cooled to 0 °C and 35 $\mu$L of *n*-butylnitrite was added with stirring. After 1 h, the bright red mixture was added dropwise to 100 mL of cold diethyl ether. The product was isolated by filtration under ether as an orange solid or utilized in situ for further reactions. Analytical $R_t$ = 1.95, Method A. Yield 99%. LC/MS (ESI) 472 (M + H).

**III. General Diazonium Reaction Procedures:** A 0.1-g sample of 9-diazonium salt generated in situ (HCl salt) or tetrafluoroborate salt was dissolved in MeOH or ACN and 0.05 equiv of $Pd(OAc)_2$ was added and up to 10% acetic acid. The reaction mixture was stirred for 5 min at room temperature, and 2 or more equiv of the desired reactant was added. The reaction was typically continued for 18 h. The catalyst was removed and the filtrate treated with activated charcoal and dried to give the crude product.

**[4*S*-(4α,12aα)]-9-[3′-(*E*)-Propenoic acid]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (8).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C to afford the product as a yellow powder in 41% yield. $R_t$ = 12.52. Analytical $R_t$ = 1.95, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.52−7.7 (m, 2H), 6.9 (d, 1H, $J$ = 8.03 Hz), 6.70 (d, 1H, $J$ = 8.03 Hz), 4.08 (s, 1H), 3.54 (dd, 1H), 2.99 (s, 6H), 2.90 (d, 1H), 2.72 (m, 1H), 2.70 (dd, 1H), 1.51 (d, 3H). HRMS calcd ($C_{25}H_{26}N_2O_{10}$ + H) 515.1667, found 515.1662.

**[4*S*-(4α,12aα)]-9-[3′-(*E*)-Ethylpropenoate]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (9).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C as a yellow solid in 49% yield, $R_t$ = 17.62. Analytical $R_t$ = 2.02, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.86 (d, 1H, $J$ = 18.0 Hz), 7.77 (d, 1H, $J$ = 8.0 Hz), 6.98 (d, 1H, $J$ = 8.0 Hz), 6.62 (d, 1H, $J$ = 18.0 Hz), 4.26 (m, 2H), 4.07 (s, 1H), 3.6 (dd, 1H), 2.86 (s, 6H), 2.84 (d, 1H), 2.72 (m, 1H), 2.59 (dd, 1H), 1.51 (d, 3H), 1.54 (m, 3H). HRMS calcd ($C_{25}H_{26}N_2O_{10}$ + H) 543.1980, found 543.1974.

**[4*S*-(4α,12aα)]-9-[2′-(*E*)-(1-Cyano)ethenyl]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (10).** Preparative C18 reverse-phase HPLC with use of Method C, Yield 38%. $R_t$ = 22.1. Analytical $R_t$ = 12.85, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.80 (d, 1H, $J$ = 17.9 Hz), 7.67 (d, 1H, $J$ = 8.0 Hz), 6.87 (d, 1H, $J$ = 8.0 Hz), 6.52 (d, 1H, $J$ = 17.9 Hz), 4.1 (m, 2H), 3.6 (dd, 1H), 2.69 (s, 6H), 2.45 (d, 1H), 2.42 (m, 1H), 1.48 (d, 3H). MS (FAB) 496 (M + H).

**[4*S*-(4α, 12aα)]-9-(3-Oxo-propenyl)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (11).** Preparative C18 reverse-phase HPLC with use of Method C. Yield 27%. $R_t$ = 17.3. Analytical $R_t$ = 9.42, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.90 (d, 1H, $J$ = 18.0 Hz), 7.82 (d, 1H, $J$ = 7.8 Hz), 7.02 (d, 1H, $J$ = 7.8 Hz), 6.64 (d, 1H, $J$ = 18.0 Hz), 4.41 (s, 1H), 3.53 (dd, 1H), 2.9−2.94 (d, 6H), 2.81 (d, 1H), 2.60 (dd, 1H), 1.56 (m, 1H), 1.30 (m, 3H). MS (FAB) 499 (M + H).

**[4*S*-(4α,12aα)]-9-[1′(*E*)-(2′-Phenyl)ethenyl]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (12).** Preparative C18 reverse-phase HPLC with use of Method C. Yield 36%. $R_t$ = 27−30. Analytical $R_t$ = 19.42, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.71 (d, 1H, $J$ = 8.0 Hz), 7.40 (d, 1H, $J$ = 24.0 Hz), 7.14 (m, 6H), 6.74 (d, 1H, $J$ = 8.0 Hz), 3.86 (s, 1H), 3.45 (d, 1H), 2.66 (s, 6H), 2.58 (d, 1H), 2.70 (m, 1H), 2.55 (d, 1H), 1.36 (m, 3H). MS (FAB) 547.1. HRMS calcd ($C_{30}H_{30}N_2O_8$ + H) 547.2082, found 547.2080.

**[4*S*-(4α,12aα)]-9-[1′(*E*)-(2′-(4″-Fluorophenyl)ethenyl)]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (13).** The purified product was obtained by C18 reverse-phase preparative reverse-phase HPLC with use of Method C. $R_t$ = 28−31. Yield 42%. Analytical $R_t$ = 19.5, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.79 (d, 1H, $J$ = 9.0 Hz), 7.52 (d, 1H, $J$ = 18.0 Hz), 7.02 (q, 4H), 6.96 (d, 1H, $J$ = 18.0 Hz), 6.93 (d, 1H, $J$ = 9.0 Hz), 4.49 (s, 1H), 3.53 (dd, 1H), 2.84−2.94 (s, each 3H), 2.78 (m, 1H), 2.47 (m, 1H), 1.44 (d, 3H). MS (FAB) 565.1 (M + H). HRMS calcd ($C_{30}H_{29}FN_2O_8$ + H) 565.2060, found 565.2049.

(37) For recent reviews regarding the eukaryotic properties of tetracyclines see: (a) Greenwald, R. A.; Golub, L. M. In *Tetracyclines in Biology, Chemistry and Medicine*; Nelson, M. L., Hillen, W., Greenwald, R. A., Eds; Birkhauser Verlag: Basel, Switzerland, 2001; p 199. (b) Greenwald, R. A.; Golub, L. M. In *Adv. Dental Res.* **1998**, *12*, 1.

**[4*S*-(4α,12aα)]-9-[1′(*E*)-(2′-(4″-Trifluorophenyl)ethenyl)]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (14).** The purified product was obtained by C18 reverse-phase preparative reverse-phase HPLC with use of Method C, $R_t$ = 35−37. Yield 61%. Analytical $R_t$ = 23.38, Method A. MS (FAB) 615.2 (M + H).

**[4*S*-(4α,12aα)]-9-(1′-cyclopentenyl)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (15).** HPLC yield 45%. The purified product was obtained by C18 preparative reverse-phase HPLC with use of Method C. $R_t$ range = 26−28. Isolated yield 15%. Analytical $R_t$ = 15.40, Method A. HRMS calcd ($C_{25}H_{26}N_2O_{10}$ + H) 511.2080, found 511.2064.

**IV. Reaction with Phenyl or Heteroaryl Boronic Acids: General Procedure.** A solution of diazonium salt (HCl or $HBF_4$) in MeOH or ACN (approximately 10 mg/mL) was cooled to 0 °C and 0.1 equiv of $Pd(OAc)_2$ was added. The mixture was stirred for 5 min, and 1 or more equiv of phenylboronic acid or heteroarylboronic acid was added and stirred for 6 h, warming to room temperature during the reaction. The catalyst was filtered through Celite, and the filtrate was dried to yield the crude reaction product.

**[4*S*-(4α,12aα)]-9-Phenyl-4-(dimethylamino)-3,5,10,12,-12a-pentahydroxy-6-methyl-1,11-dioxo -1,4,4a,5,5a,6,11,-12a-octahydro-naphthacene-2-carboxamide (16).** The purified product was obtained by preparative reverse-phase HPLC with use of Method C, $R_t$ = 41−43. Yield 67%. Analytical $R_t$ = 19.73, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.54 (m, 3H), 7.12 (m, 2H), 7.1 (d, 1H, $J$ = 8.0 Hz), 7.01 (d, 1H, $J$ = 8.0 Hz), 4.1 (s, 1H), 3.53 (dd, 1H), 2.80 (s, 6H), 2.56 (m, 1H), 2.53 (m, 1H), 1.33 (d, 3H). MS (FAB) 521.0 (M + H). HRMS calcd ($C_{28}H_{28}N_2O_8$ + H) 521.1928, found 521.1923.

**[4*S*-(4α,12aα)]-9-(4′-Fluorophenyl)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (17).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C. Yield 63%. Analytical $R_t$ = 19.9, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.54 (m, 3H), 7.18 (t, 2H), 7.04 (d, 1H, $J$ = 8.0 Hz), 4.39 (s, 1H), 3.53 (dd, 1H), 2.96 (d, 6H), 2.70 (m, 1H), 2.60 (m, 1H), 1.51 (d, 3H). HRMS ($C_{30}H_{29}FN_2O_8$ + H) calcd 539.1829, obsd 539.1819.

**[4*S*-(4α,12aα)]-9-[3,4-methylenedioxophenyl]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (18).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C, $R_t$ = 34−35. Yield 55%. Analytical $R_t$ = 20.0, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.45 (d, 1H, $J$ = 8.0 Hz), 7.05 (s, 1H), 6.96 (t, 3H), 6.82 (d, 1H, $J$ = 8.0 Hz), 5.96 (s, 2H), 4.44 (s, 1H), 2.95 (d, 6H), 2.73 (m, 1H), 2.60 (m, 1H), 1.49 (d, 3H). MS (FAB) 565.1 (M + H).

**[4*S*-(4α,12aα)]-9-(4-Methoxyphenyl)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (19).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C, $R_t$ = 33.97. Yield 61%. Analytical $R_t$ = 18.2, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.50 (m, 3H), 6.94 (m, 3H), 4.16 (m, 1H), 3.82 (d, 1H), 3.30 (s, 3H), 2.79 (s, 6H), 2.74 (m, 1H), 2.56 (m, 1H), 1.53 (d, 3H). MS (FAB) 551 (M + H).

**[4*S*-(4α,12aα)]-9-[Benzofuran-2-yl]-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (20).** The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C, $R_t$ = 39−42. Yield 54%. Analytical $R_t$ = 18.5, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.24 (d, 1H), 7.62−7.2 (br m, 6H), 4.41 (s, 1H), 3.59 (dd, 1H), 2.98 (br s, 6H), 2.73 (m, 1H), 2.62 (m, 1H), 1.63 (d, 3H). HRMS calcd ($C_{25}H_{26}N_2O_{10}$ + H) 561.1873, found 561.1854.

**[4*S*-(4α,12aα)]-9-(Carboxylic acid methyl ester)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (21).** A 100-mg sample of compound **4b** was diazotized in 0.1 N HCl/MeOH (3 mL) with 3 equiv of *n*-butylnitrite at room temperature. The diazonium salt was precipitated with $Et_2O$ and added to a reaction flask. All solvents were removed in vacuo and the flask was charged with 6 mL of anhydrous DMF. The flask was placed under Ar and 20 mg of $Pd(OAc)_2$ was added. Carbon monoxide (CO) was bubbled in slowly via syringe, changing the reaction color from dark red to brown over the course of 1 h. The solvent was removed and the crude product dissolved in MeOH, filtered through Celite, and purified by C18 reverse-phase HPLC with use of Method C, $R_t$ = 16−17. The material obtained (0.05 g) was refluxed in MeOH with 0.1% HCl for 12 h, and the solvent was removed in vacuo to yield the final product as a yellow solid. Yield 27%. Analytical $R_t$ = 12.4, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.16 (d, 1H), 7.10 (d, 1H), 4.15 (s, 1H), 3.6 (s, 3H), 3.55 (m, 1H), 3.0 (s, 6H), 2.70 (m, 1H), 2.61 (m, 1H), 1.6 (m, 3H). HRMS calcd ($C_{24}H_{26}N_2O_7$ + H) 503.1667, found 503.1662.

[4*S*-(4α,12aα)]-4,7-Bis-(dimethylamino)-9-[2-(4-fluoro-phenyl)-vinyl]-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,-11,12a-octahydro-naphthacene-2-carboxamide (22).

A 100- mg sample of compound **5a** was diazotized in 0.1 N $H_2SO_4$/MeOH (3 mL) with 4 equiv of *n*-butylnitrite and precipitated with $Et_2O$. The precipitate was dissolved in MeOH (3 mL), 3 equiv of 4′-F-phenylstyrene and 4.1 mg of $Pd(OAc)_2$ (10%) were added, and the reaction was stirred overnight. The solution was filtered through Celite, and the solvent was removed in vacuo to yield the crude product. Yield 53%. The purified product was obtained by preparative C18 reverse-phase HPLC with use of Method C. Analytical $R_t$ = 23.4, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.59 (s, 1H), 7.38 (br s, 1H), 7.13 (q, 4H), 7.10 (br s, 1H), 3.09 (m, 3H), 3.55 (dd, 1H), 3.09 (s, 6H). MS (FAB) 578 (M + H).

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-[3′-(*E*)-ethylpropenoate]-1,3,10,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (23).** HPLC yield 64%. The purified product was obtained by preparative reverse-phase HPLC with use of Method D. Isolated yield 39%. Analytical $R_t$ = 4.20, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.07 (s, 1H), 7.82 (d, 1H, $J$ = 19.2 Hz), 6.74 (d, 1H, $J$ = 19.2 Hz), 4.16 (s, 1H), 4.04 (m, 2H), 3.6 (br s, 1H), 3.13 (s, 6H), 2.94 (s, 6H), 2.74 (d, 1H), 2.43 (m, 1H), 1.58 (m, 1H), 1.21 (m, 3H). HRMS calcd ($C_{28}H_{33}N_3O_9$ + H) 556.2297, found 556.2290.

**V. General Procedures: Iodoarene tetracycline reactive intermediates: [4*S*-(4α,12aα)]-4-(Dimethylamino)-1,5,10,12,12a-pentahydroxy-9-iodo-6-methyl-3,11-dioxo-3,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (4f).** A 5.0-g sample of doxycycline monohydrate (10.8 mmol) was dissolved in 100 mL of trifluoroacetic acid (TFA) at room temperature and 2.6 g (11.88 mmol) of *N*-iodosuccinimide was added. After 5 h the TFA was removed in vacuo and the crude product triturated in methyl *tert*-butyl ether. After filtration the residue was recovered, dissolved in MeOH, and treated with activated charcoal to yield the TFA salt product as a yellow solid in 80% yield. Analytical $R_t$ = 15.92, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.93−7.96 (d, 1H), 6.67−6.69 (d, 1H), 4.03 (s, 1H), 3.65 (dd, 1H), 2.81 (s, 6H), 2.52 (m, 1H), 1.52 (m, 3H). HRMS calcd ($C_{24}H_{26}N_2O_7$ + H) 571.0577, found 571.0582.

**4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-iodo-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (5c).** A 30-g sample (56.5 mmol) of minocycline (2HCl) was slowly added to 200 mL of methanesulfonic acid and the amber solution was stirred at room temperature while 38 g (178.2 mmol) of *N*-iodosuccinimide (NIS) was added in aliquots over 3 h. The reaction was quenched in 2 L of ice water with 17.9 g of sodium thiosulfate

and rapidly stirred for 30 min. The aqueous layer was extracted with ethyl acetate before being poured onto sodium hydrogen carbonate (259.8 g, 3.1 M) containing 300 mL of *n*-butanol. The butanol layer was removed and the aqueous layer extracted with butanol. All organic extracts were combined and washed with $H_2O$ and once with brine. All solvents were removed in vacuo and the residue suspended in MeOH (600 mL) and anhydrous HCl gas was then bubbled in until dissolution. The solvent was removed in vacuo to a solid, and triturated with methyl *tert*-butyl ether. The solid was collected and washed with diethyl ether followed by hexane. The compound was dried in vacuo to yield 22.6 g of a light yellow powder in 69% yield. Analytical $R_t = 11.1$, Method B. $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 7.92−7.94 (s, 1H), 4.08 (s, 1H), 3.00 (s, 8H), 2.76 (s, 6H), 2.27 (m, 1H), 2.11 (m, 1H), 1.67 (m, 1H). HRMS calcd ($C_{23}H_{26}IN_3O_7$ + H) 584.0895, found 584.0889.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-7-iodo-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide (6e).** A 1.0-g sample of sancycline was dissolved in 25 mL of TFA that was cooled to 0 °C. *N*-Iodosuccinimide (1.2 equiv) was added and reacted for 40 min. The reaction was warmed to room temperature and reacted for 5 h. The reaction was then driven to completion by the stepwise addition of NIS (15-mg aliquots) until disappearance of sancycline was noted. Further additions of NIS resulted in the production of the 7,9-diiodo reaction product **6g**. The reaction was stopped by removal of the TFA in vacuo and the crude solid dissolved in MeOH (5 mL). The solution was added to cold, rapidly stirred diethyl ether and the precipitate collected. The solid was dissolved in MeOH, treated with activated charcoal, and filtered through Celite, and the solvent was removed in vacuo to produce a yellow solid in 75% yield. Analytical $R_t = 14.45$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.89 (d, 1H, $J = 8.86$ Hz), 6.67 (d, 1H, $J = 8.86$ Hz), 3.78 (s, 1H), 3.03 (s, 2H), 2.84 (s, 6H), 2.46 (m, 2H), 0.99 (m, 2H). HRMS calcd ($C_{21}H_{21}IN_2O_7$ + H) 541.0474, found 541.0472.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-7,9-diiodo-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxamide (6g).** Yield 59%. Analytical $R_t = 21.20$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.35 (s, 1H), 3.78 (s, 1H), 3.33 (s, 2H), 2.88 (s, 7H), 2.41 (m, 2H), 0.99−1.41 (m, 2H). HRMS calcd ($C_{24}H_{26}N_2O_7$ + H) 666.9438, found 666.9442.

**VI. General Procedures: Tetracycline Alkene and Alkyne Derivatives.** A 1-mmol sample of iodotetracycline, 50 mg of tetrakistriphenylphosphine palladium(0) catalyst or equivalent, 12 mg of $Pd(OAc)_2$, and 32 mg of CuI are dissolved in 10 mL of acetonitrile. Triethylamine (2−5 mL) and 3−5 mmol of alkene, styrene, or alkyne were added and the mixture was vigorously stirred between room temperature and 70 °C for 2−24 h. Filtration through Celite and removal of the solvent in vacuo produced crude products.

**[4*S*-(4α, 12aα)]-4-(Dimethylamino)-9-(2-*tert*-butylcarbamoyl-vinyl)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (24).** 9-Iodo doxycycline (0.9 g, TFA salt, **4f**, 1.48 mmol), $Pd(OAc)_2$ (120 mg, 0.53 mmol), and P(*o*-tolyl)$_3$ (276 mg, 0.9 mmol) with CuI (75 mg) were suspended in acetonitrile (40 mL) and 1 mL of TEA was added. The solution was degassed with $N_2$ and stirred at 60 °C for 2 h. HPLC yield 68%. The mixture was cooled to room temperature and filtered through Celite, and the crude product was obtained after solvent removal in vacuo. The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 47% yield. Analytical $R_t = 3.08$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.62 (d, 1H, $J = 21.0$ Hz), 7.52 (d, 1H, $J = 9.0$ Hz), 6.79 (d, 1H, $J = 9.0$ Hz), 6.57 (d, 1H, $J = 21.0$ Hz), 4.29 (s, 1H), 3.43 (dd, 1H), 2.82 (s, 3H), 2.73 (s, 3H), 2.46 (m, 1H), 1.35 (m, 3H), 1.13 (s, 9H). HRMS calcd ($C_{29}H_{35}N_3O_9$ + H) 570.2453, found 570.2453.

**[4*S*-(4α,12aα)]-9-(1′-Cyclopentenyl)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (15).** The purified product was obtained by preparative reverse-phase HPLC with use of Method C, $R_t = 22-24$. Yield 32%. Analytical $R_t = 15.36$, Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.35 (d, 1H, $J = 8.0$ Hz), 6.81 (d, 1H, $J = 8.0$ Hz), 6.45 (d, 1H, $J = 18.0$ Hz), 5.75 (d, 1H, $J = 18.0$ Hz), 3.46 (m, 1H), 2.90 (d, each 3H), 2.73 (m, 2H), 2.45 (m, 1H), 1.35 (m, 3H). HRMS calcd ($C_{25}H_{26}N_2O_{10}$ + H) 511.2080, found 511.2051.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-(prop-1-ynyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (25).** The reaction was performed in a Parr apparatus with all the reagents used for **24** except that propyne gas was added via a lecture bottle at −70 °C to **5c**, and the Parr apparatus was closed and then heated at 60 °C for 4 h. The purified product was obtained by reaction solution filtration, precipitation in diethyl ether, and further treatment with activated charcoal to yield the product as a yellow solid in 47% yield. Analytical $R_t = 8.99$, Method B. $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 8.23 (s, 1H), 4.20 (s, 1H), 3.13 (s, 6H), 3.08 (s, 6H), 2.89 (s, 6H), 2.71 (s, 3H), 2.45 (m, 1H), 1.74 (m, 1H). HRMS calcd ($C_{24}H_{26}N_2O_7$ + H) 496.2086, found 496.2070.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-(phenylethynyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (26).** HPLC yield 74%. The purified product was obtained by preparative reverse-phase HPLC with use of Method D. Isolated yield 31%. Analytical $R_t = 4.8$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.84 (s, 1H), 7.34 (m, 2H), 7.20 (s, 3H), 3.93 (s, 1H), 3.02 (s, 6H), 2.87 (s, 6H), 2.26−2.36 (m, 1H), 1.41−1.49 (m, 1H). HRMS calcd ($C_{31}H_{31}N_{23}O_7$ + H) 558.2240, found 558.2225.

**[4*S*-(4α,12aα)]-7-(2-(*E*/*Z*)-Cyanovinyl)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (27).** The purified products (*E* (66%) and *Z* (33%) isomers) were obtained by preparative reverse-phase HPLC with use of Method D as a mixture. Total yield 39%. Analytical $R_t = 2.69$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.79 (d, $J = 11.7$ Hz, *Z* isomer), 7.76 (m, 1H), 7.36 (d, $J = 16.3$ Hz, *E* isomer), 6.70 (m, 1H), 5.84 (d, $J = 16.3$ Hz, *E* isomer), 3.96 (s, 1H), 3.06 (m, 2H), 2.91 (s, 6H), 2.83 (s, 6H), 1.97−2.22 (m, 4H), 1.31−1.30 (m, 2H). HRMS calcd ($C_{24}H_{23}N_3O_7$ + H) 466.1619, found 466.1611.

**[4*S*-(4α,12aα)]-7-Styryl-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (28).** HPLC yield 63.2%. The purified product was obtained by preparative reverse-phase HPLC with use of Method D. Isolated yield 30%. Analytical $R_t = 3.98$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.69 (d, 1H), 7.42 (d, 2H), 7.12−7.40 (m, 4H), 7.15 (d, 1H, $J = 14.4$ Hz), 6.78 (d, 1H, $J = 14.4$ Hz), 3.99 (s, 1H), 3.1 (s, 1H), 2.95 (s, 6H), 2.85 (s, 6H), 2.30 (m, 1H), 2.08−2.15 (m, 4H), 1.55 (m, 2H). HRMS calcd ($C_{29}H_{28}N_2O_7$ + H) 517.1977, found 517.1967.

**[4*S*-(4α,12aα)]-7-[2-(4-Methyl-thiazol-5-yl)-vinyl]-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (29).** HPLC yield 46%. The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 23% yield. Analytical $R_t = 2.56$, Method D. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 9.63 (s, 1H), 7.91 (d, 1H, $J = 9.0$ Hz), 7.38 (d, 1H, $J = 21.1$ Hz), 7.06 (d, 1H, $J = 21.1$ Hz), 6.8 (d, 1H, $J = 9.0$ Hz), 4.13 (s, 1H), 2.98 (s, 3H), 2.83 (s, 3H), 2.61 (s, 3H), 2.43 (m, 1H), 1.64 (m, 2H). LC/MS (ESI), *m*/*z* 538.6.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-7,9-diethynyl-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4, 4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (30) and [4*S*-(4α,12aα)]-4-(Dimethylamino)-7,9-diethyl-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4, 4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (31).** A 300-mg sample of intermediate **6g** was dissolved in 20 mL of acetonitrile and 2.0 mL of triethylamine, 50.0 mg of $Pd(PPh_3)_4$, 50 mg of CuI, and 12.5 mg of $Pd(OAc)_2$ were added followed by 0.5 mL of trimethylsilylacetylene. The reaction was stirred at room

temperature for 4 h, filtered through a divinyl-benzene cartridge (25 g), then concentrated in vacuo to yield 280 mg of the crude material (monitored by LC/MS). The TMS group was removed by dissolving the crude material in MeOH, adding 250 mg of $K_2CO_3$ during 4 h of stirring. The mixture was filtered through a divinylbenzene cartridge, then the solvent was removed in vacuo to yield the 7,9-diethynyl derivative **30** in 40% yield by HPLC. A 250-mg sample of the material was dissolved in 25 mL of MeOH, 0.1 g of 10% Pd/C was added, and the reaction mixture was stirred overnight under 50 psi of $H_2$. The reaction mixture was filtered through Celite, concentrated to a solid, redissolved in MeOH (10 mL), then precipitated in cold diethyl ether to yield the product as a yellow solid in 56% overall yield. Analytical $R_t$ = 5.54, Method D. (**31**) $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.30 (s, 1H), 4.18 (s, 1H), 2.90−3.00 (br m, 12H), 2.45−2.55 (m, 4H), 2.37 (m, 2H), 1.45 (m, 4H), 1.15 (m, 6H). HRMS calcd ($C_{25}H_{30}N_2O_7$ + H) 471.2133, found 471.2125.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-7-ethyl-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (32).** A 5-g sample of 7-iodo sancycline **6e** (7.6 mmol), 50 mg of $Pd(OAc)_2$, 200 mg of $Pd(PPh_3)_4$, and CuI (200 mg) were added to a reaction flask and 30 mL of dry acetonitrile and 2 mL of triethylamine were added. The suspension was degassed with Ar, and 2 mL of triethylamine was added followed by 4.3 g of trimethylsilylacetylene (30.5 mmol). The reaction mixture was stirred for 3 h and filtered through Celite, and the solvent was removed in vacuo to yield the product as a crude material. Analytical $R_t$ = 2.83, Method D. The solid was dissolved in MeOH, 34 mg of $K_2CO_3$ per 50 mg of material was added, and the solution was stirred overnight. The mixture was filtered through a divinylbenzene cartridge (25 g), and the solvent was removed in vacuo. A 250-mg sample of the crude material was dissolved in 25 mL of MeOH, 0.1 g of 10% Pd/C or 0.15 g of $PtO_2$ was added, and the reaction mixture was stirred overnight under 50 psi of $H_2$. The reaction mixture was filtered through Celite, concentrated to a solid, redissolved in MeOH (20 mL), then precipitated in cold diethyl ether to yield the product as a yellow solid in 52% overall yield. Analytical $R_t$ = 2.95, Method D. $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 7.39 (d, 1H, $J$ = 9.0 Hz), 6.79 (d, 1H, $J$ = 9.0 Hz), 4.30 (s, 1H), 2.94 (br s, 6H), 2.80 (m, 2H), 2.61 (m, 3H), 2.25 (m, 3H), 1.07 (t, 3H). HRMS calcd ($C_{23}H_{26}N_2O_7$ + H) 443.1818, found 443.1818.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-7-ethyl-9-iodo-3,10,-12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (33).** Compound **32** (3.1 g, 6.33 mmol) was dissolved in methanesulfonic acid (15 mL) and NIS (1.68 g, 7.6 mmol) was added with stirring at room temperature for 4 h. The solid was then dissolved in isopropyl alcohol (10 mL) and dripped slowly into rapidly stirring, cold diethyl ether. The solid was collected and used as is for further reactions. Yield 72%. Analytical $R_t$ = 12.83, Method D. LC/MS (ESI), *m*/*z* 569.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-7-ethyl-9-cyclohex-1-enylethynyl-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,-4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (34).** A 0.5-g sample of compound **33** was dissolved in 20 mL of ACN, then 3 mL of triethylamine added followed by 50 mg of $Pd(Ph_3)_4$, 50 mg of $Pd(OAc)_2$, 50 mg of CuI, and 0.1 mL of cyclohexenyl acetylene. The mixture was stirred at 80 °C for 1 h, filtered through Celite, and the solvent removed in vacuo for purification via preparative C18-reverse phase HPLC with use of Method D. Yield 43%. Analytical $R_t$ = 13.4, Method B. $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 7.37 (s, 1H), 6.16 (t, 1H), 4.08 (s, 1H), 2.93−2.98 (d, 6H), 2.56 (m, 2H), 2.33 (m, 1H), 2.23 (m, 1H), 2.19 (m, 8H), 1.64 (m, 2H), 1.13 (m, 3H). HRMS calcd ($C_{31}H_{34}N_2O_7$ + H) 547.2444, found 547.2445.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-pentyl-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (35).** A 2-g sample of 9-iodominocycline **5c** (3.0 mmol), 20 mg of $Pd(OAc)_2$, 50 mg of $Pd(PPh_3)_4$, and CuI (50 mg) were added to a reaction flask and 6 mL of dry ACN and 0.4 mL of triethylamine were added. The suspension was degassed with Ar, and 0.4 mL of triethylamine was added followed by 2.1 g of 1-pentyne (14 mmol). The reaction mixture was stirred for 5 h and filtered through Celite, and the solvent was removed in vacuo to yield the product as a crude material. Analytical $R_t$ = 3.43, Method D. A 1.5-g sample of the crude material was dissolved in 50 mL of MeOH, 0.1 g of 10% Pd/C or 0.15 g of $PtO_2$ was added, and the reaction mixture was stirred overnight under 55 psi of $H_2$. The reaction mixture was filtered through Celite, concentrated to a solid, redissolved in MeOH (10 mL), then precipitated in diethyl ether to yield the product as a crude yellow solid in 66% yield by HPLC. The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 44% yield, $R_t$ = 18.4. Analytical $R_t$ = 4.36, Method B. $^1$H NMR ($CD_3$-OD, 300 MHz) $\delta$ 7.71 (s, 1H), 4.12 (s, 1H), 4.30 (s, 1H), 3.01−3.17 (d, 6H), 2.70 (m, 2H), 2.35 (m, 1H), 2.28 (m, 1H), 2.18 (m, 1H), 1.67 (m, 3H), 1.32 (m, 6H), 0.95 (5H). HRMS calcd ($C_{28}H_{37}N_3O_7$ + H) 528.2710, found 528.2717.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-9-phenethyl-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (36).** The purified product was obtained by preparative reverse-phase HPLC with use of Method D, in 36% yield. $R_t$ = 19−21. Analytical $R_t$ = 6.30, Method B. LC/MS (ESI), *m*/*z* 562.1.

**VII. General Procedures for 7 and 9 Phenyl Tetracycline Compounds.** C7 or C9 iodo tetracyclines (200 mg, 0.37 mmol) were combined with $Pd(OAc)_2$ (8.3 mg, 0.037 mmol) in methanol (15 mL) and degassed with Ar. The contents were purged with Ar while heating to 70 °C for 10 min. Separately, $Na_2CO_3$ (117 mg, 1.11 mmol in 5 mL of water) was purged with Ar for 10 min prior to addition by syringe into the reaction solution. This was followed by the addition of an Ar-degassed solution of phenylboronic acid (90 mg, 0.74 mmol in 5 mL of DMF). The reaction mixture was stirred at 70 °C, monitored via HPLC, and shown to be complete within 2 h. The solution was filtered through Celite and the solvent removed in vacuo to produce the crude material.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-(3,4-methylenedioxophenyl)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (18).** The purified product was obtained by preparative reverse-phase HPLC with use of Method D, isolated yield 49%. $R_t$ = 33−35. Analytical $R_t$ = 20.1. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.46 (d, 1H, $J$ = 8.9 Hz), 7.05 (s, 1H), 6.95 (m, 2H), 6.82 (d, 1H, $J$ = 8.9 Hz), 5.54 (s, 2H), 4.30 (s, 1H), 2.96 (s, 6H), 2.80 (d, 1H), 2.71 (m, 1H), 2.62 (dd, 1H), 1.43 (m, 3H). HRMS calcd ($C_{29}H_{28}N_2O_{10}$ + H) 565.1824, found 565.1828.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-9-(3-formyl-4-methoxy-phenyl)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (37**). Yield by HPLC 48%. The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 29% yield and used for further reductive amination. $R_t$ = 2.11. $^1$H NMR ($CD_3OD$, 300 MHz) 7.57 (s, 1H), 7.41, m, 2H), 6.69 (m, 2H), 4.30 (s, 1H), 3.80 (s, 3H), 3.45 (m, 1H), 2.82 (s, 6H), 2.45 (m, 1H), 1.43 (m, 3H). ESI-MS *m*/*z* (M + H) 579.1.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-9-(3-pyrrolidin-1-ylmethyl-phenyl)-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (38).** A suspension of **37** (200 mg, 0.3 mmol), pyrrolidine (53 mg, 0.7 mmol), and 1,2-dichloroethane (DCE, 5 mL) was added to a flask followed by sodium triacetoxyborohydride (160 mg, 0.72 mmol) and the solution was stirred at room temperature for 4 h. The reaction mixture was added to ice water (50 mL), and the crude product was extracted into ethyl acetate. The solvent was removed in vacuo and the crude product was purified via C18 preparative reverse-phase HPLC with use of Method D. Overall yield 35%. Analytical $R_t$ = 1.89. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.63 (s, 1H), 7.61 (d, 1H, $J$ = 9

Hz), 7.52 (d, 1H, $J$ = 9 Hz), 7.08 (d, 1H, $J$ = 9 Hz), 6.96 (d, 1H, $J$ = 9 Hz), 4.38 (s, 1H), 4.33 (s, 2H), 3.88 (s, 3H), 3.47 (m, 4H), 2.91 (s, 3H), 2.82 (s, 3H), 2.54 (m, 2H), 1.47 (m, 3H). HRMS calcd ($C_{34}H_{39}N_3O_9$ + H) 634.2764, found 634. 2749.

**[4*S*-[(4α,12aα)]-4,7-Bis(dimethylamino)-9-(3-formylphenyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,-12a-octahydro-naphthacene-2-carboxamide (39)**. A 500-mg sample of 9-iodominocycline **5c** (0.76 mmol) was dissolved in MeOH (10 mL) with 20 mg (0.08 mmol) of $Pd(OAc)_2$. The mixture was degassed with Ar, brought to reflux, and 1.6 mL of 2 M $Na_2CO_3$ added (3.2 mmol) along with 250 mg (1.67 mmol) of 2-formyl phenylboronic acid in 5 mL of DMF. The mixture was refluxed 2 h, cooled, and filtered through Celite. Yield by HPLC was approximately 30%. The solvent was removed, and the crude product was purified via C18 preparative reverse-phase HPLC with use of Method D followed by removal of the solvent and trituration with methyl *tert*-butyl ether. Yield <15%. Analytical $R_t$ = 2.09. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.93 (m, 2H), 7.65 (s, 1H), 7.5 (m, 1H), 7.45 (m, 1H), 4.0 (1H), 3.05 (s, 6H), 2.93 (s, 6H), 2.6−2.62 (m 2H), 2.1−2.3 (m, 2H), 1.63 (m, 1H). ESI-MS $m/z$ (M + H) 582.3.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-9-(3-((dimethylamino)methyl)phenyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (40).** Compound **39** (200 mg, 0.3 mmol), dimethylamine HCl (58 mg, 0.7 mM), and DCE (8 mL) were added to a reaction flask followed by sodium triacetoxyborohydride (151 mg, 0.7 mM) and the solution was stirred at room temperature for 2 h. The solvent was removed and the crude residue was dissolved in MeOH (5 mL) for preparative chromatography. The purified product was obtained by preparative reverse-phase HPLC with use of Method D. Yield 45%. Analytical $R_t$ = 2.09, Method D. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.15 (s, 1H), 7.98 (m, 1H), 7.93 (m, 1H), 7.57 (m, 2H), 4.42 (s, 2H), 4.19 (s, 1H), 3.36 (br d, 6H), 3.07 (s, 6H), 2.90 (s, 6H), 2.62 (m 2H), 2.2−2.45 (m, 2H), 1.69 (m, 1H). HRMS calcd ($C_{32}H_{38}N_4O_7$ + H) 591.2791, found 591.2780.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-7-phenyl-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (41).** 7-Iodosancycline·TFA (**6e**, 200 mg, 0.37 mmol) was combined with $Pd(OAc)_2$ (8.3 mg, 0.037 mmol) in methanol (15 mL). The contents were purged with Ar while heating to 70 °C for 10 min. Separately, $Na_2CO_3$ (117 mg, 1.11 mmol in 5 mL of water) was purged with Ar for 10 min prior to addition by syringe into the reaction solution, followed by the addition of an Ar-degassed solution of phenylboronic acid (90 mg, 0.74 mmol in 5 mL of DMF). The reaction mixture was stirred at 70°C in an Ar atmosphere for several hours. Upon completion of the reaction, the solution was filtered to remove catalyst, and the solvent was removed in vacuo. Solid material was dissolved in methanol (saturated with HCl), filtered, and purified by preparative HPLC. Purified material was reduced in vacuo to afford a yellow powder in 45% yield. Analytical Method A, $R_t$ = 16.3. $^1$H NMR ($CD_3OD$) $\delta$ 7.25−7.41 (m, 4H, m), 7.25 (m, 2H, d), 4.0 (s,1H), 2.93 (m, 10H), 2.4 (m, 2H), 1.84−1.91 (1H, m), 1.84−1.95 (m,1H), 1.5−1.75 (m, 2H). HRMS calcd ($C_{27}H_{26}N_2O_7$ + H) 491.1820, found 491.1818.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-7-(5-formyl-2-methoxy-phenyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,-5a,6,11,12a-octahydro-naphthacene-2-carboxamide (42).** This compound was prepared from 7-iodo-sancycline·TFA (**6e** 300 mg, 0.46 mmol), $Pd(OAc)_2$ (10 mg, 0.046 mmol), $Na_2CO_3$ (145 mg, 0.14 mmol), and 2-methoxy-4-formylphenylboronic acid (166 mg, 0.92 mmol) in methanol (20 mL). HPLC yield 52%. After HPLC purification (Method D) and salt exchange to the HCl salt, the desired compound in <15% yield was isolated as a pale yellow powder. $R_t$ = 4.51, Analytical Method B. $^1$H NMR ($CD_3OD$) $\delta$ 6.83−7.43 (m, 5H), 5.33 (1H, d, $J$ = 6 Hz), 4.01 (s,1H), 3.74 (d, 3H, $J$ = 15 Hz), 2.85−3.00 (m, 8H), 2.10−2.55 (m, 2H), 1.04−2.03 (m, 1H), 1.50 (q, 1H, $J$ = 12 Hz). HRMS calcd ($C_{29}H_{28}N_2O_9$ + H) 549.1875, found 549.1870.

**[4*S*-(4α, 12aα)]-4-(Dimethylamino)-7-(5-((dimethylamino)methyl)-2-methoxyphenyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (43).** 7-(5-Formyl-2-methoxyphenyl)sancycline (**42**, 1 g, 1.82 mmol) was slurried with dimethylamine·HCl (297 mg, 3.64 mmol) and DCE (40 mL) at room temperature. The reaction was stirred for 10 min and sodium triacetoxyborohydride (772 mg, 3.64 mmol) was added to the reaction solution. The reaction was complete within 2 h then quenched with methanol (20 mL), and solvent was removed in vacuo. The crude material was redissolved in methanol, filtered, and purified via preparative HPLC with use of Method D. The solvent was removed in vacuo to afford a yellow solid in 59% yield. $^1$H NMR ($CD_3OD$) $\delta$ 7.49 (m, 1H), 7.21−7.37 (M, 3H), 6.88 (M, 1H), 4.27 (d, 2H $J$ = 12 Hz), 3.98 (m, 8H), 3.74−3.82 (m, 3H), 2.91−2.96 (m, 7H), 2.86−2.82 (m, 7H), 2.21−2.61 (m, 2H), 1.81−2.09 (m, 1H), 1.31−1.51 (m, 1H). HRMS calcd ($C_{31}H_{35}N_3O_8$ + H) 578.2502, found 578.2488.

**7-(5-((Dimethylamino)methyl)-2-methoxyphenyl)sancycline (43).** 2-Methoxy-5-formylphenylboronic acid (200 mg, 1.11 mmol) was combined with dimethylamine·HCl (181 mg, 2.22 mmol), triethylamine (309 $\mu$L, 2.22 mmol), and DCE (10 mL) and the solution was stirred at room temperature for 5 min. Sodium triacetoxyborohydride (471 mg, 2.22 mmol) was added to the reaction and the mixture was stirred at room temperature for 2 h. The reaction solution was quenched with methanol (10 mL) and solvent was reduced in vacuo. Diethyl ether (100 mL) was added to the flask containing the dried reaction contents and the mixture was stirred at room temperature. The material was filtered and washed with hexanes and the precipitate was dried to produce 2-methoxy-5-benzyldimethylaminophenylboronic acid (150 mg, 64.6%). 7-Iodosancycline·TFA (**6e,** 235 mg, 0.36 mmol) was combined with $Pd(OAc)_2$ (8 mg, 0.036 mmol) and methanol (15 mL) under Ar. An aqueous solution of $Na_2CO_3$ (113 mg, 1.08 mmol), purged with Ar, was added to the reaction flask. 2-Methoxy-5-benzyldimethylaminophenylboronic acid (150 mg, 0.72 mmol) was dissolved in DMF:DMSO (6 mL:3 mL) and separately purged with Ar prior to addition via syringe to the reaction mixture. Upon reaction completion the solvent was removed in vacuo, diluted in methanol (3 mL), and purified. The material was reduced in vacuo to afford a yellow solid. Product was cooled to 0 °C and 20 mL of methanol (saturated with HCl) was added to form the HCl salt. The solvent was removed in vacuo to produce the compound as a yellow solid (50 mg, overall yield 40%). The product was shown to be epimerically pure by $^1$H NMR analysis in $CD_3OD$ and DMSO-$d_6$. $^1$H NMR ($CD_3OD$) $\delta$ 7.50−7.53 (m, 1H), 7.13−7.49 (m, 3H), 6.87−6.88 (m, 1H), 4.27 (d, 2H, $J$ = 12 Hz), 4.03 (d, 1H, $J$ = 6 Hz), 3.78 (m, 3H, $J$ = 24 Hz), 2.98−3.00 (m, 7H), 2.81−2.90 (m, 7H), 2.45−2.62 (m, 1H), 2.28−2.42 (m, 1H), 2.0−2.12 (m, 1H), 1.39−1.58 (m, 1H). HRMS calcd ($C_{31}H_{35}N_3O_8$ + H) 578.2502, found 578.2495.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-7-(thiophen-3-yl)-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (44).** The purified product was obtained by preparative reverse-phase HPLC with use of Method C. $R_t$ = 18−19. Yield 21%. Analytical Method B, $R_t$ = 12.05, $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.35 (m, 2H), 7.15 (d, 1H), 6.97 (d, 1H), 6.75 (d, 1H), 3.93 (s, 1H), 2.94 (6H, m), 2.36 (1H, m), 1.97 (1H, m), 1.93 (1H, m), 1.43 (1H, m). ESI-MS $m/z$ (M + H) 497.5.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-7-(furan-2-yl)-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (45).** The purified product was obtained by preparative reverse-phase HPLC with use of Method D. Yield 32%. $R_t$ = 12.5−14.0. Analytical Method B, $R_t$ = 5.23. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.62 (d, 1H), 7.47 (m, 1H), 6.83 (m, 1H), 6.43 (m, 1H), 6.34 (m, 1H), 3.98 (s, 1H), 3.16 (m, 2H), 2.86 (d, 6H), 2.45 (m, 1H), 2.05 (m, 1H), 1.56 (m, 1H). HRMS calcd ($C_{25}H_{24}N_2O_8$ + H) 481.1613, found 481.1612.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-7-(pyrimidin-5-yl)-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (46).** The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 41% yield. Analytical Method B, $R_t = 3.02$. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 9.13 (s, 1H), 8.79 (s, 2H), 7.37 (d, 1H, $J = 9$ Hz), 6.87 (d, 1H, $J = 9$ Hz), 3.91 (s, 1H), 2.86 (d, 6H), 2.48−2.58 (m, 1H), 1.91 (m, 1H), 1.43 (m, 1H). ESI-MS *m*/*z* (M + H) 493.5.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-7,9-diphenyl-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (47).** The compound was prepared as noted above except a 4-fold excess of phenylboronic acid was used along with 2-fold increases in $Pd(OAc)_2$ and $Na_2CO_3$ and extended reaction times (16 h). $R_t = 24.56$. Yield 74%. Analytical Method A. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.22−7.76 (br m, 12H), 4.02 (s, 1H), 2.94 (d, 6H), 2.50 (m, 1H), 2.01 (m, 1H), 1.43 (m, 1H). ESI-MS *m*/*z* (M + H) 567.7.

**VIII. General Procedures for Carbonylation Reactions: [4*S*-(4α,12aα)]-9-(Carboxylic acid methyl ester)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (21).** 9-Iododoxycycline-TFA (**4f**, 0.8 g, 1.1 mmol), NaOAc (0.64 g, 9.6 mmol), and $Pd(dppf)_2Cl_2$ (50 mg, 5 mol %) in 1 mL of $CH_2Cl_2$ were slurried in MeOH (20 mL) and sealed in a Parr apparatus. The reaction vessel was degassed with CO, then sealed at 450 psi and heated at 70 °C for 4 h. The solution was filtered through Celite, and the solvent removed in vacuo. The purified product was obtained by preparative reverse-phase HPLC with use of Method D in 29% yield. Analytical Method B, $R_t = 3.60$. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.11 (d, 1H, $J = 8.2$ Hz), 7.10 (d, 1H, $J = 8.2$ Hz), 4.45 (s, 1H), 3.95 (s, 3H), 3.61 (dd, 1H, $J = 9$ Hz, $J = 11.4$ Hz), 2.95 (br s, 6H), 2.87 (d, 2H, $J = 11.4$ Hz), 2.60 (dd, 1H), 1.59 (d, 3H). HRMS calcd ($C_{24}H_{26}N_2O_{10}$ + H) 503.1667, found 503.1660.

**[4*S*/4*R*-(4α,12aα)]-9-(Carboxylic acid methyl ester)-4,7-bis(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (48).** 9-Iodominocycline·2HCl salt (**5c**, 0.8 g, 1.17 mmol), NaOAc (0.64 g, 4 equiv), and $Pd(dppf)_2Cl_2$ (48 mg, 5 mol %) in $CH_2Cl_2$ (1 mL) were charged into a Parr apparatus along with CO at 450 psi. The reaction was stirred for 4 h at 80 °C. Workup as detailed with compound **21** provided compound as a yellow solid as an epimer mixture at C4 of ratio of 3:1 4*S*/4*R* in 33% isolated yield. Analytical Method B, $R_t = 3.03$. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.90−7.91 (s, 1H), 4.12 (s, 1H), 3.8 (s, 3H), 3.15−3.2 (d, 6H), 2.70 (m, 2H), 2.35 (m, 1H), 2.28 (m, 1H), 2.18 (m, 1H). HRMS calcd ($C_{25}H_{29}N_3O_9$ + H) 516.1984, found 516.1971.

**[4*S*-(4α,12aα)]-4,7-Bis(dimethylamino)-3,10,12,12a-tetrahydroxy-9-(morpholine-4-carbonyl)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (49).** 9-Iodominocycline·2HCl salt (**5c**, 0.8 g, 1.17 mmol) and $Pd(PPh_3)_2Cl_2$ (48 mg, 5 mol %) in morpholine (15 mL) were charged into a Parr apparatus along with CO at 150 psi and reacted overnight at 70 °C. The mixture was acidified with TFA, and the purified product was obtained by preparative reverse-phase HPLC with use of Method D to yield 16% (146 mg) of the 2TFA salt in an epimeric mixture of 65:35 *S*/*R* isomer at position C4. Analytical Method B, $R_t = 3.09$. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.70, 7.72 (2s, 1H), 4.00, 4.15 (2s, 1H), 3.80 (br s, 5H), 2.96−3.10 (m, 18H), 2.45 (t, 1H, $J = 16$ Hz), 2.12−2.32 (m, 1H), 1.6−1.78 (m, 1H). ESI-MS *m*/*z* (M + H) 599.60.

**[4*S*-(4α,12aα)]-7-(Carboxylic acid methyl ester)-4-(dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (50).** A Parr reactor was charged with 7-iodosancycline (**6e**, 0.654 g, 1 mmol), NaOAc (0.295 g, 3.6 mmol), MeOH (30 mL), and $Pd(dppf)_2Cl_2 \cdot CH_2Cl_2$ (40 mg, 5 mol %). The solution was degassed and pressurized with CO (300 psi) and the reaction stirred at 70 °C for 14 h. The solution was filtered and evaporated and the residue was subjected to preparative HPLC purification obtaining 0.215 g (37%) of the TFA salt as a mixture of 4*S*:4*R* epimers (7:3). Analytical $R_t = 3.37$, Method D. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.06 (d, 1H, $J = 8.9$ Hz), 6.92 (d, 1H, $J = 8.9$ Hz), 4.19, 4.83 (2s, 1H), 3.90−3.97 (m, 1H), 3.90 (s, 3H), 3.03 (s, 6H), 2.98−3.08 (m, 2H), 2.48 (t, 1H), 2.19−2.24 (m, 1H), 1.66 (q, 1H, $J = 11.5$ Hz). ESI-MS *m*/*z* (M + H) 473.15.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-7-(2-morpholin-4-yl-2-oxo-acetyl)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (51).** A Parr reactor was loaded with 7-carboxylic acid sancycline, prepared from **50** via ester hydrolysis in acid (0.654 g, 1 mmol), NaOAc (0.295 g, 3.6 mmol), MeOH (30 mL), and $Pd(dppf)_2Cl_2$ $CH_2Cl_2$ (40 mg, 5 mol %). The solution was pressurized with CO (300 psi). The reaction was stirred at 70 °C for 14 h. The solvent was removed and the residue was purified via preparative HPLC yielding 0.215 g (38%) of the product as a mixture 3:7 of 4*R*/4*S* epimers. The purified product was obtained by preparative reverse-phase HPLC with use of Method C. Analytical $R_t = 3.43$, Method B. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.87−7.83 (m, 1H), 7.01−6.98 (m, 1H), 4.10 (s, 1H), 3.97−2.94 (m, 18H), 2.53 (t, 1H), 2.26−2 (m, 1H), 1.75−1.56 (m, 1H). HRMS calcd ($C_{27}H_{29}N_3O_{10}$ + H) 556.1933, found 556.1925.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-3,10,12,12a-tetrahydroxy-7-(2-dimethylcarboxamide)-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (52).** A mixture of 7-sancycline carboxylic acid·TFA salt (200 mg, 0.35 mmol), dimethylamine hydrochloride (85 mg, 1.75 mmol), triethylamine (0.245 mL, 1.2 mmol), HBTU (0.4 g, 1.5 mmol), and DMF (1.5 mL) were stirred at rt for 48 h. The solvents were removed, and the residue was acidified with TFA followed by preparative HPLC with use of Method D to yield the product in 22% yield. $R_t = 3.47$, Analytical Method B. $^1$H NMR ($CD_3OD$, 300 MHz) 7.38 (d, 1H, $J = 8.3$ Hz), 6.92 (d, 1H, $J = 8.3$ Hz), 3.20−2.70 (m, 16H), 2.55−2.40 (m, 1H), 2.20−1.98 (m, 1H), 1.70−1.50 (m, 1H). HRMS calcd ($C_{24}H_{27}N_3O_8$ + H) 486.1878, found 486.1872.

**IX. General Procedure for Phenylboronic Acid Coupling to Methacycline.** Methacycline·HCl (1 equiv), $PdCl_2$ (0.14 equiv), and $CuCl_2$ (0.90 equiv) were dissolved in 20 mL of MeOH and refluxed under $N_2$ for 1 h. Phenylboronic acids (2 equiv) were added and further refluxed for 6−10 h. The reaction mixture was cooled and filtered through Celite, and the solvent was removed in vacuo to yield crude product. The purified product was obtained by preparative reverse-phase HPLC with use of Method C or D.

**[4*S*-(4α,12aα)]-4-(Dimethylamino)-6-(4-*tert*-butylbenzylidene)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxamide (53).** The purified product was obtained by preparative reverse-phase HPLC with use of Method C, $R_t = 16-18$. Yield 43%. Analytical Method A, $R_t = 12.77$. $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.7−7.9 (m, 3H), 7.04 (t, 2H), 7.54 (m, 2H), 7.0 (m, 1H), 6.6 (m, 1H), 6.03 (m, 1H), 4.1 (s, 1H), 2.99 (s, 6H), 2.44 (m, 1H), 2.3 (m, 1H), 1.89 (m, 1H). ESI-MS *m*/*z* (M + H) 575.2. HRMS calcd ($C_{28}H_{25}N_3O_{10}$ + H) 575.2405, found 575.2416.

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**Supporting Information Available:** Experimental data and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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