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A Robust Platform for the Synthesis of New Tetracycline Antibiotics

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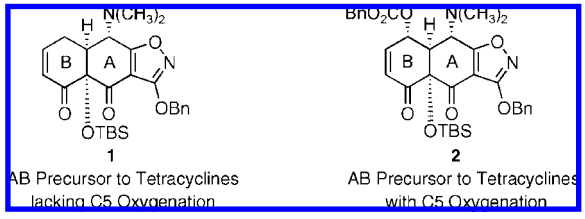
Abstract: Tetracyclines and tetracycline analogues are prepared by a convergent, single-step Michael−Claisen condensation of AB precursor **1** or **2** with D-ring precursors of wide structural variability, followed by removal of protective groups (typically in two steps). A number of procedural variants of the key C-ring-forming reaction are illustrated in multiple examples. These include stepwise deprotonation of a D-ring precursor followed by addition of **1** or **2**, in situ deprotonation of a D-ring precursor in mixture with **1** or **2**, and in situ lithium−halogen exchange of a benzylic bromide D-ring precursor in the presence of **1** or **2**, followed by warming. The AB plus D strategy for tetracycline synthesis by C-ring construction is shown to be robust across a range of different carbocyclic and heterocyclic D-ring precursors, proceeding reliably and with a high degree of stereochemical control. Evidence suggests that Michael addition of the benzylic anion derived from a given D-ring precursor to enones **1** or **2** is quite rapid at −78 °C, while Claisen cyclization of the enolate produced is rate-determining, typically occurring upon warming to 0 °C. The AB plus D coupling strategy is also shown to be useful for the construction of tetracycline precursors that are diversifiable by latter-stage transformations, subsequent to cyclization to form the C ring. Results of antibacterial assays and preliminary data obtained from a murine septicemia model show that many of the novel tetracyclines synthesized have potent antibiotic activities, both in bacterial cell culture and in vivo. The platform for tetracycline synthesis described gives access to a broad range of molecules that would be inaccessible by semisynthetic methods (presently the only means of tetracycline production) and provides a powerful engine for the discovery and, perhaps, development of new tetracycline antibiotics.

Introduction

In prior research we showed that cyclohexenones **1** and **2** can be transformed into 6-deoxytetracycline antibiotics using a sequence of as few as three chemical steps.[1]



The first and key step of the sequence forms the C ring of the tetracyclines by a Michael−Claisen cyclization reaction, a potentially general means for constructing tetracycline analogues widely variant in the left-hand or D-ring portion of the molecule.[2] Here, we expand upon our original findings, describing the synthesis of more than 50 different tetracyclines and tetracycline analogues, many of which are active in inhibiting the growth of cultured Gram-positive and Gram-negative bacteria, including tetracycline-resistant strains. We provide detailed protocols for the key cyclization reaction in its various forms and discuss features of stereochemistry, chemical efficiency, and mechanism. We also report minimum inhibitory concentration values for selected analogues in a number of different bacterial strains, as well as preliminary in vivo data obtained in a mouse septicemia model using a tetracycline-sensitive strain of *Staphylococcus aureus*.

Background

The first tetracycline antibiotic was discovered in 1948, when Benjamin Duggar of Lederle Laboratories isolated the natural product chlorotetracycline (Aureomycin, **3**) from the culture broth of a novel species of *Streptomyces*.[3] Within two years, a research team from Chas. Pfizer and Co. had isolated a second natural tetracycline, oxytetracycline (Terramycin, **4**),[4] and in

(1) Charest, M. G.; Lerner, C. D.; Brubaker, J. D.; Siegel, D. R.; Myers, A. G. *Science* **2005**, *308*, 395–398.

(2) As pointed out by a reviewer, the second step of the key C-ring-forming reaction sequence is more properly designated as an internal Claisen reaction, rather than a Dieckmann reaction, as we previously described it; for discussion, see: (a) Hauser, C. R.; Swamer, F. W.; Adams, J. T. *Org. React. (N.Y.)* **1954**, *8*, 59. (b) Schaefer, J. P.; Bloomfield, J. J. *Org. React. (N.Y.)* **1967**, *15*, 1. For brevity, this sequence is referred to here as a Michael−Claisen cyclization.

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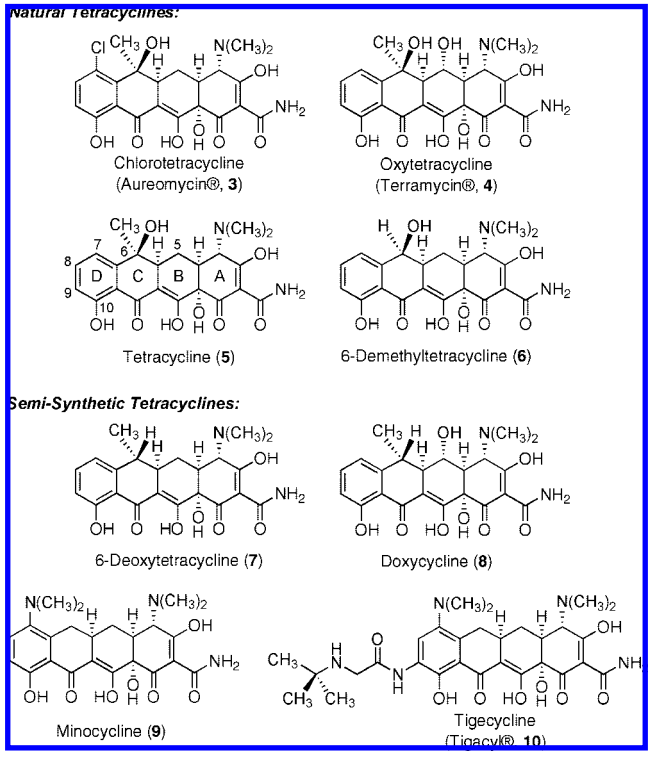

1953, tetracycline itself (**5**) was prepared from chlorotetracycline by catalytic hydrogenolysis of the carbon−chlorine bond, a transformation discovered by Lloyd Conover of Pfizer.[5] Subsequently, tetracycline was found to be a natural product,[6] and later still, Lederle researchers isolated 6-demethyltetracyclines (see structure **6**) from culture broths of a mutant strain of *Streptomyces*.[7] Conover has provided detailed and insightful accounts of research efforts leading to new tetracyclines specifically and antibiotics more generally.[8] All tetracyclines approved for human or veterinary use are fermentation products or are derived from fermentation products by semisynthesis. This is also true of most β-lactam and all macrolide antibiotics. Tracing the paths of human efforts to produce new antibiotics from natural products not accessible by synthesis reveals an evolutionary process marked by specific, impactful discoveries. In the case of the tetracyclines, Pfizer scientists achieved a major enabling advance approximately 10 years after the class had been identified when they demonstrated that the C6-hydroxyl group of the natural products oxytetracycline (**4**), tetracycline (**5**), and 6-demethyltetracycline (**6**) could be removed reductively.[9] The 6-deoxytetracyclines produced, including 6-deoxytetracycline itself (**7**), were found to be more stable than the parent compounds, yet they retained broad-spectrum antibacterial activity. The important and now generic antibiotics doxycycline (**8**; Pfizer, 1967) and minocycline (**9**; Lederle, 1972) followed as a consequence, the latter arising from the additional discovery that electrophilic aromatic substitution at C7 becomes possible when the more stable 6-deoxytetracyclines are used as substrates.[10] Decades later, a team of Wyeth scientists led by Frank Tally synthesized 7,9-disubstituted tetracycline derivatives, leading to the discovery of the antibiotic tigecycline (Tigacyl, **10**; U.S. approval 2005).[11]

Tigacyl is one of only three new antibiotics to be brought to market in the United States in the past three years, and the only broad-spectrum agent.[12] Diminishing economic incentives and increasing regulatory hurdles have led many pharmaceutical companies to discontinue efforts to develop new antibiotics, raising concern among public health officials, particularly with the emergence of antibiotic-resistant bacterial strains in community settings. While careful management of the use of existing antibiotics in society is warranted, it would seem unwise to abandon the search for new agents, given the diversity of bacteria and their capacity to evolve rapidly. Attempts to develop antibacterial agents with novel targets have met with little success.[13] As a consequence, many research programs seeking to discover new antibiotics have been refocused toward the modification of agents in proven classes, such as the tetracyclines, with an emphasis on overcoming bacterial resistance. While the innovations of chemists seeking to modify the structure of naturally occurring tetracyclines have been extraordinary, the slowing pace of discovery in this area is evident.

From the time that the structures of the tetracycline antibiotics were first revealed by Woodward and collaborators,[14] many laboratories have sought to develop a practical route for their synthesis. In 2003, an expert opinion of the patent literature summarized the state of the art, concluding, "the original effort of Woodward has survived as the basic strategy for the total synthesis of this series and at greater than 25 steps is clearly not to be considered as practical. ... we believe there is ample justification to explore new total synthetic and convergent synthetic routes that take full advantage of the body of chemistry that has become available since Woodward's original effort." [15] Among the many developments since Woodward and coworkers first synthesized sancycline (6-deoxy-6-demethyltetra-

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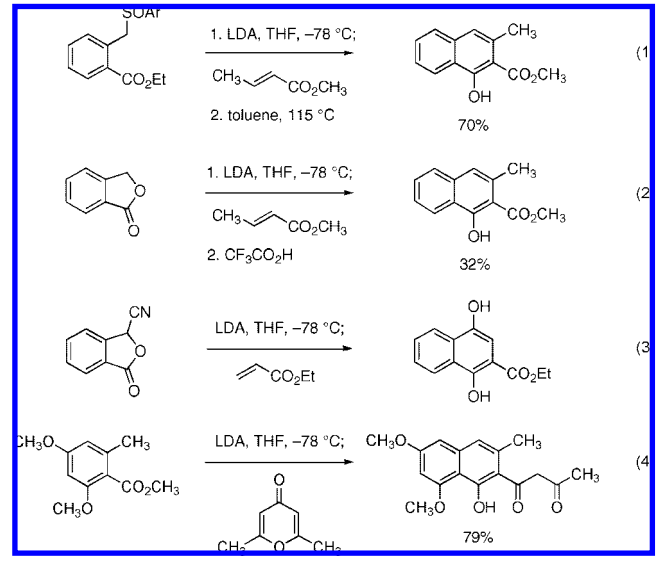
cycline) in 1962,[16] one of particular note, and of relevance to the results described herein, is Stork and Hagedorn's strategy for protection of the vinylogous carbamic acid group of the A ring of the tetracyclines as a 3-benzyloxyisoxazole group; subsequent deprotection occurs under mild (hydrogenolytic) conditions.[17]

Strategically, the original route developed by Woodward and collaborators for the synthesis of sancycline employed a "left-to-right" or D→A mode of construction. The Shemyakin and Muxfeldt research groups adopted a similar directionality in their remarkable syntheses of tetracycline (**5**; 1967) and terramycin (**4**; 1968), respectively, using a bicyclic CD-ring precursor as starting material.[18,19] With the benefit of more than 50 years of structure−activity relationship data, as well as X-ray crystal structures of tetracycline bound to the bacterial ribosome (its putative target),[20] the left-to-right mode of construction used in these pioneering synthetic efforts can be seen to present a practical disadvantage to the discovery of new tetracycline antibiotics, for the D ring has emerged as one of the most promising sites for structural variation. This was a primary consideration guiding our initial retrosynthetic analysis of the tetracycline class, leading us to focus upon disconnection of the C ring. Thus, we envisioned assembling tetracyclines by a convergent coupling of D- and AB-ring precursors. Although model studies suggested that both Diels−Alder and Michael−Claisen cyclization reactions might be used to form the C ring,[21] only the latter proved successful with the AB precursors that we later targeted and synthesized (**1** and **2**).

Michael−Claisen and Michael−Dieckmann reaction sequences have been widely employed to construct naphthalene derivatives since 1978, when three different cyclization protocols were introduced by independent research groups. Hauser and Rhee used a sulfoxide-stabilized *o*-toluate ester anion as the nucleophilic component in a Michael−Dieckmann cyclization reaction with methyl crotonate (eq 1). In this case, aromatization occurred upon thermal elimination of phenylsulfenic acid.[22] The use of phthalide and cyanophthalide anions as nucleophilic components was described by Broom and Sammes (eq 2)[23] and Kraus and Sugimoto (eq 3),[24] respectively. Formal loss of water and hydrogen cyanide, respectively, led to naphthoate ester products in these procedures. In 1979, the Weinreb and Staunton research groups first reported that simple *o*-toluate ester anions (unsubstituted at the benzylic position) undergo Michael−Claisen cyclization reactions with β-methoxycyclohexenones and γ-pyrones to form naphthyl ketones (see eq 4 for one example), a sequence sometimes referred to as the Staunton−Weinreb annulation.[25]



There is also precedent for the formation of non-aromatic six-membered rings by Michael−Claisen and Michael−Dieckmann reaction sequences.[23,26,27] With few exceptions,[27] stereochemical features of these cyclization reactions have not been discussed, frequently because they were of little consequence (aromatization followed cyclization). The Michael−Claisen cyclizations detailed below are unusual in their stereochemical complexity, stereocontrol, and efficiency. In 2000, while our studies were in progress, Tatsuta and co-workers reported a synthesis of (−)-tetracycline (34 steps, 0.002% yield) that employed an early stage Michael−Claisen cyclization reaction to form an aromatic C-ring precursor, which was dearomatized later in the sequence.[28] Since 2005, our laboratory has reported two different routes to synthesize the AB precursor **1** in optically active form; the more recent of these was scaled to prepare >40 g of crystalline product in one batch.[29] Here we provide details of the different protocols that can be used to construct the C

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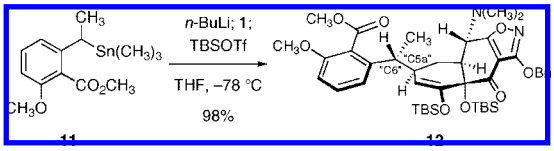
ring of the tetracyclines and exemplify these protocols with the preparation of a number of novel substances with antibacterial properties.

Results

A critical early experiment in our attempts to assemble the C ring of the tetracyclines by a Michael−Claisen cyclization reaction provided both direction and mechanistic insight. As depicted in Scheme 1, treatment of a solution of organostannane **11**[30] in tetrahydrofuran (THF) with *n*-butyllithium at −78 °C, followed by transfer of this mixture to a solution of enone **1**, also at −78 °C, and subsequent quenching with *tert*-butyldimethylsilyl triflate (TBSOTf), afforded the Michael addition product **12** as a single stereoisomer in 98% yield.

Scheme 1. Michael Addition of a Benzylic Anion (Generated by Tin−Lithium Exchange) to Enone **1**, Followed by Trapping of the Resultant Enolate with *tert*-Butyldimethylsilyl Triflate



Crystallization of the chromatographically purified product from hexanes at −30 °C provided a single crystal suitable for X-ray analysis (mp 146 °C). Inspection of the crystal structure (Figure 1) revealed that the stereochemistry of positions "C5a" and "C6" of the adduct **12** corresponded to that of 6-deoxytetracycline (**7**) and doxycycline (**8**), which was fortuitous given that as many as four diastereomers could have been formed in the Michael addition reaction. The product that is formed apparently arises from selective addition of the nucleophile to the concave face of the enone. This selectivity may arise as a consequence of stereoelectronic factors (pseudoaxial addition to the enone) and/or steric effects (addition of the nucleophile to the π-face opposite the *tert*-butyldimethylsilyloxy substituent, which is also axial; see the space-filling model of enone **1** depicted in Figure 1).

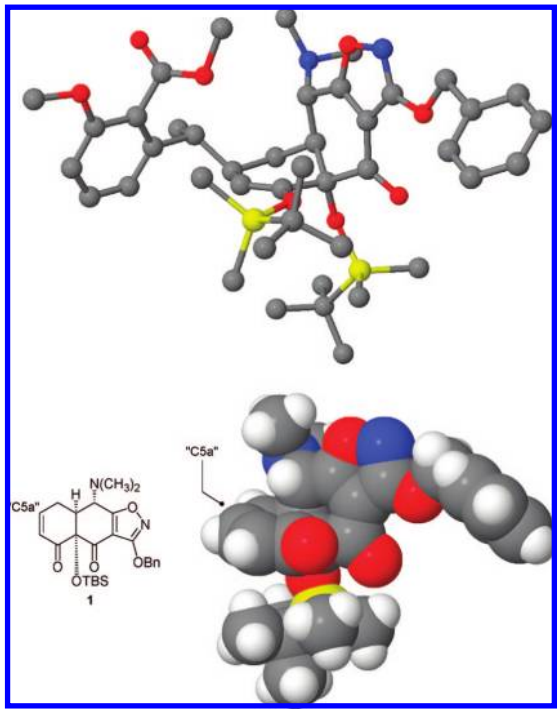
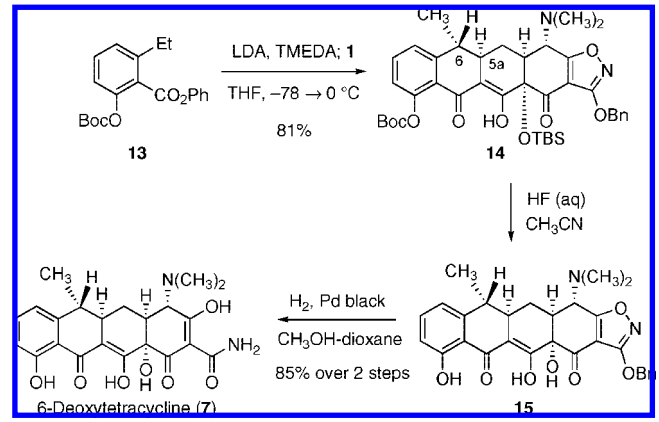


Figure 1. Crystal structures of the Michael addition product **12** and the enone **1**, presented as ball and stick and space-filling models, respectively.

Directed by this key finding, we proceeded to strategically modify the D-ring precursor, with three objectives: (1) to activate the ester toward Claisen cyclization (which we did not observe with the methyl ester substrate **11**), (2) to obviate the use of organotin intermediates, and (3) to mask the C10 phenoxy substituent with a protective group more labile than methyl. These objectives were achieved using the *tert*-butoxycarbonyl-protected phenyl ester substrate **13** (Scheme 2).[31,32]

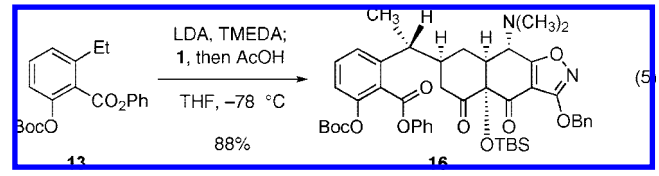
Scheme 2. Synthesis of 6-Deoxytetracycline (**7**) by Michael−Claisen Cyclization Using the *tert*-Butoxycarbonyl-Protected Phenyl Ester **13** as the D-Ring Precursor and a Stepwise Protocol for Addition of the Base and Enone **1**, Followed by Deprotection



Deprotonation of **13** (3 equiv) with lithium diisopropylamide (LDA, 4 equiv) at −78 °C in the presence of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA, 4 equiv) afforded a deep red solution of the corresponding *o*-toluate ester anion; addition of a solution of the enone **1** (1 equiv) and slow warming of the resulting mixture to 0 °C over 3 h provided the Michael−Claisen cyclization product **14** in 81% yield in diastereomerically pure form after isolation by reverse-phase high-performance liquid chromatography (rp-HPLC). A minor diastereomer (<4%), believed to be epimeric at C6, was isolated separately.

Thus, Michael addition occurs with >20:1 stereoselectivity at C6, in the sense indicated in Scheme 2, and appears to proceed with complete stereocontrol at C5a (attack upon a single diastereoface of the enone). These observations have held consistently in more than 40 different C-ring-forming cyclization reactions examined to date, with two (closely related) exceptions, discussed below.

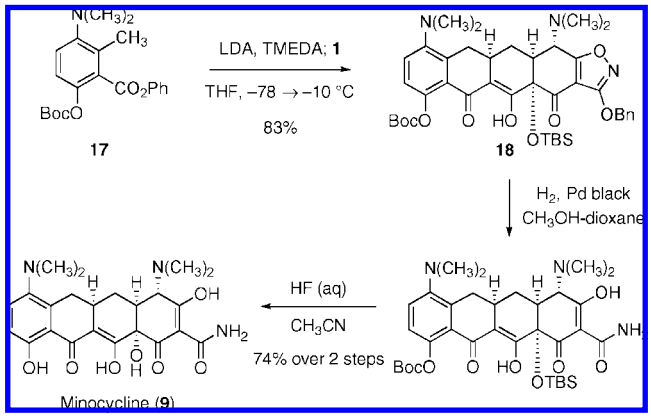
There is little question that the transformation of enone **1** to the 6-deoxytetracycline precursor **14** proceeds by a stepwise mechanism, involving sequential Michael and Claisen reactions, for the intermediate Michael adduct can be intercepted by protonation with acetic acid at −78 °C to give the keto ester **16** in 88% yield (eq 5).[33]



Indeed, we have observed across a range of different D-ring precursors that Michael addition is relatively rapid at −78 °C, while Claisen cyclization proceeds more slowly, typically upon warming to 0 °C. Thus, we view Claisen cyclization and not Michael addition as rate-determining. It is evident from these observations that C-ring formation cannot occur by Diels−Alder cycloaddition of an *o*-quinonemethide intermediate, which is hypothetically a mechanistic alternative to Michael−Claisen cyclization.

With the establishment of an effective protocol for construction of the C ring, a two-step deprotection sequence was employed to transform the cyclization product **14** into 6-deoxytetracycline (Scheme 2). The *tert*-butoxycarbonyl and *tert*-butyldimethylsilyl protective groups were removed upon treatment of **14** with hydrofluoric acid in acetonitrile at 23 °C (2 days). Hydrogenolysis of the crude reaction product (**15**) in the presence of a palladium catalyst under an atmosphere of hydrogen in methanol−dioxane at 23 °C and subsequent purification by rp-HPLC then afforded 6-deoxytetracycline (**7**) in 85% yield.[17] As the examples below will demonstrate, this two-step deprotection protocol has been found to be widely applicable, though in some instances it is advantageous to invert the ordering of the two steps. In general, the *tert*-butoxycarbonyl group is cleaved relatively rapidly in the deprotection step employing hydrofluoric acid, while the tertiary *tert*-butyldimethylsilyl ether undergoes protodesilylation more slowly.

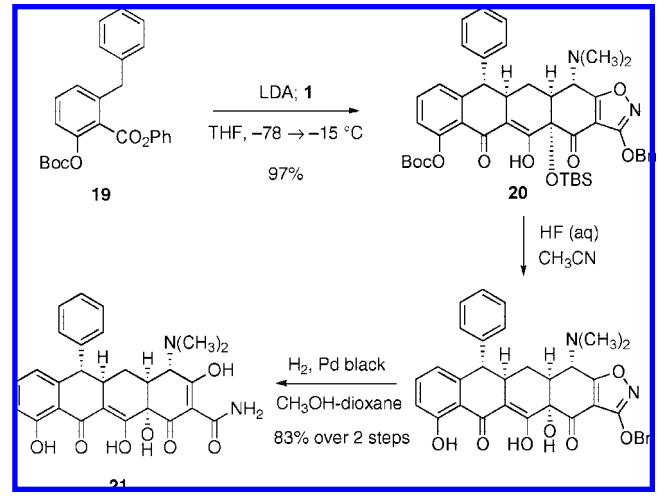
Scheme 3. Synthesis of Minocycline (**9**) by Michael−Claisen Cyclization Using the *tert*-Butoxycarbonyl-Protected Phenyl Ester **17** as the D-Ring Precursor and a Stepwise Protocol for Addition of the Base and Enone **1**, Followed by Deprotection



The conditions developed for the cyclization reaction depicted in Scheme 2, sequential deprotonation of a D-ring precursor followed by addition of the enone **1**, have been effective for the synthesis of a number of known tetracyclines as well as novel tetracycline analogues variant within or near the D ring. For example, treatment of phenyl ester **17**[34] (3 equiv, Scheme 3) with LDA (3 equiv) in the presence of TMEDA (6 equiv) at −78 °C, followed by addition of enone **1** (1 equiv) and warming of the resulting mixture to −10 °C, provided the Michael−Claisen cyclization product **18** in 83% yield after purification by rp-HPLC. Two-step deprotection and purification by rp-HPLC then afforded minocycline (**9**) in 74% yield.

Among the modified D-ring precursors we have investigated, substrates with benzylic anion-stabilizing groups were found to undergo particularly efficient cyclization reactions. For example, addition of enone **1** (1 equiv) to a solution of the stabilized anion derived from substrate **19** (3 equiv, Scheme 4),[32] followed by warming of the resulting mixture to −15 °C, provided the Michael−Claisen cyclization product **20** in 97% yield after purification by rp-HPLC. Standard deprotection of **20** then afforded the novel tetracycline analogue 6-(*S*)-phenylsancycline (**21**), which was found to effectively inhibit the growth of a number of Gram-positive bacteria, including tetracycline-resistant strains (vide infra), prompting the synthesis of a series of 6-aryl-substituted tetracyclines (see Table 1).[35] It is worthy of note that more than half of the cyclization reactions presented in the tables below were attempted only once.

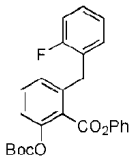
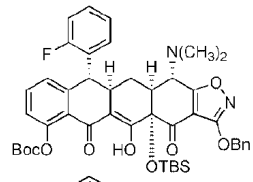
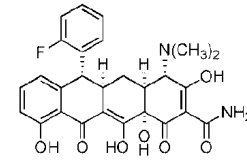
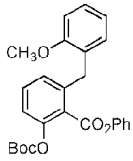
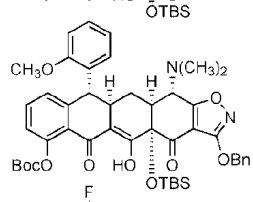
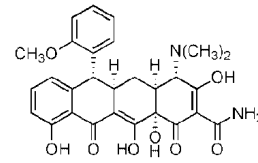
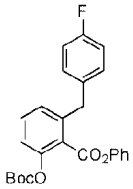
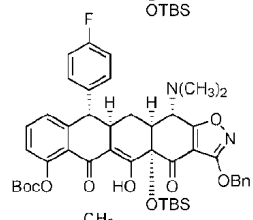
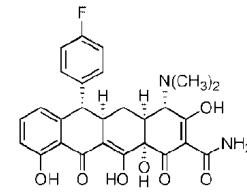
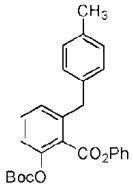
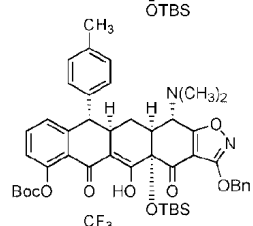
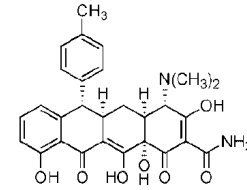
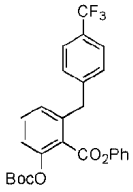
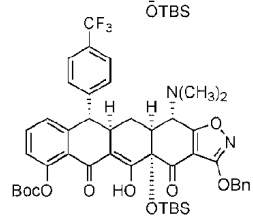
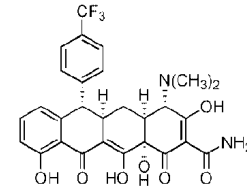
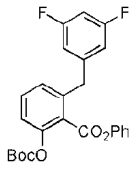
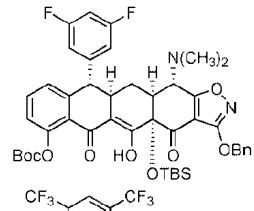
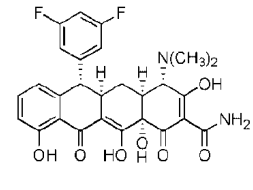
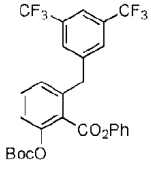
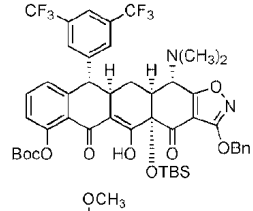
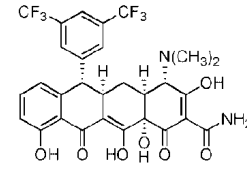
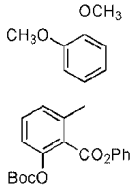
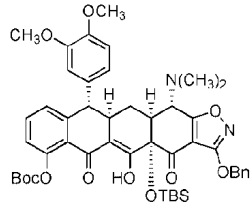
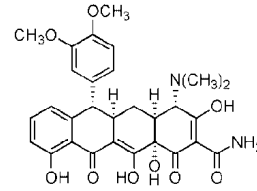
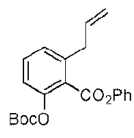
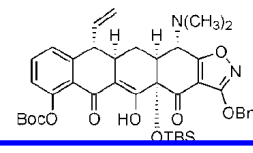
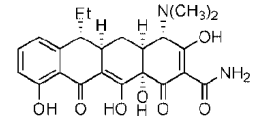
Scheme 4. Synthesis of 6-(*S*)-Phenylsancycline (**21**) by Michael−Claisen Cyclization Using the *tert*-Butoxycarbonyl-Protected Phenyl Ester **19** as the D-Ring Precursor and a Stepwise Protocol for Addition of the Base and Enone **1**, Followed by Deprotection



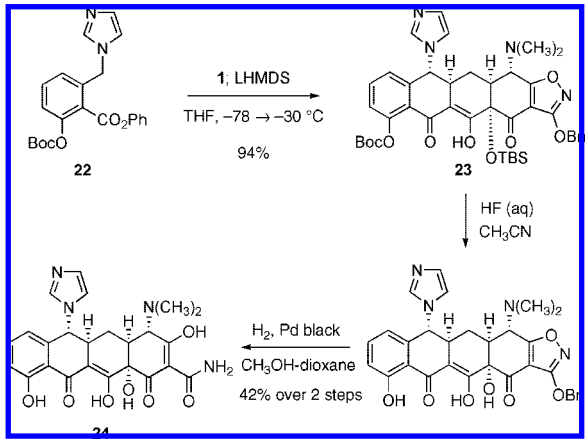
Other notable features of the cyclization reactions of D-ring precursors with benzylic anion-stabilizing groups include the fact that additives such as TMEDA were typically not required and that stoichiometries of just over 1 equiv of a given D-ring precursor frequently sufficed to achieve a high yield of cyclization product. We also found that benzylic deprotonation could be conducted with the weaker base lithium bis(trimethylsilyl)-amide (LHMDS) in lieu of the standard base LDA, and, using *N*-imidazoyl substrate **22** (Scheme 5), the important observation was made that condensation could be achieved with high efficiency by an in situ deprotonation protocol, which is to say by addition of base to the D-ring precursor in the presence of

(30) Carpenter, T. A.; Evans, G. E.; Leeper, F. J.; Staunton, J.; Wilkinson, M. R. *J. Chem. Soc., Perkin Trans. I* **1984**, 1043–1051.
(31) Phenyl ester activation in toluate ester condensations is precedented: (a) White, J. D.; Nolen, E. G., Jr.; Miller, C. H. *J. Org. Chem.* **1986**, *51*, 1150–1152 (see also ref 27c).
(32) Bennetau, B.; Mortier, J.; Moyroud, J.; Guesnet, J.-L. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1265–1271.
(33) For other examples of the isolation of uncyclized Michael adducts, see refs 23, 25c, 25d, and 26b.
(34) Phenyl ester **17** was synthesized in six steps from ethyl 6-methylsalicylate; see: (a) Olah, G. A.; Malhotra, R.; Narang, S. C. *Nitration, Methods and Mechanisms;* VCH Publishers Inc.: New York, 1980. (b) Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, *26*, 839–842. (c) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
(35) Leading references for the synthesis of D-ring precursors to 6-aryl-substituted tetracyclines: (a) Miyaura, N.; Suzuki, A *Chem. Rev.* **1995**, *95*, 2457–2483. (b) Kotha, S.; Mandal, K. *Eur. J. Org. Chem.* **2006**, *23*, 5387–5393. (c) Wright, S. W.; Hageman, D. L.; McClure, L. D. *J. Org. Chem.* **1994**, *59*, 6095–6097. (d) Osyanin, V. A. ; Purygin, P. P.; Belousova, Z. P. *Russ. J. Gen. Chem.* **2005**, *75*, 111–117. (e) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van der Eycken, E. *Org. Lett.* **2004**, *6*, 4223–4225.

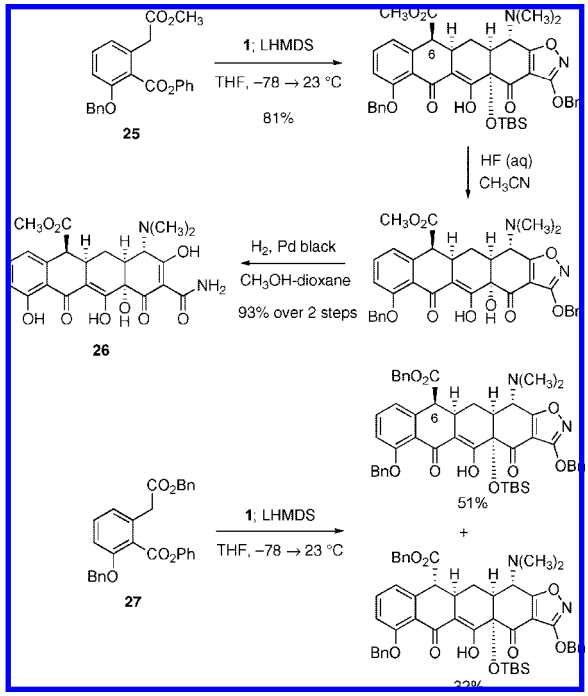
Table 1. Synthesis of 6-Substituted Tetracyclines by Michael−Claisen Cyclization Using D-Ring Precursors with Anion-Stabilizing Substituents in the Benzylic Position and a Stepwise Protocol for Addition of the Base and Enone **1**, Followed by Deprotection [(i) HF (aq), CH_3CN; (ii) H_2, Pd/C (or Pd black), CH_3OH−dioxane]

D-ring Precursor	Cyclization Conditions	Cyclization Product	Cyclization Yield	Tetracycline	Deprotection Yield
	LDA; **1** −78 → −10 °C		84%		71%
	LDA; **1** −78 → −5 °C		57%		70%
	LDA; **1** −78 → −10 °C		79%		72%
	LDA; **1** −78 → −10 °C		79%		83%
	LDA, DMPU; **1** −78 → −10 °C		81%		72%
	LDA, DMPU; **1** −78 → −10 °C		37%		59%
	LDA, DMPU; **1** −78 → −10 °C		78%		79%
	LDA; **1** −78 → −5 °C		74%		64%
	LHMDS, HMPA; **1** −78 → −10 °C		49%		79%

Scheme 5. Synthesis of 6-(*R*)-*N*-Imidazoylsancycline (**24**) by Michael−Claisen Cyclization Using the *tert*-Butoxycarbonyl-Protected Phenyl Ester **22** and Enone **1** as Substrates, with Deprotonation in Situ, Followed by Deprotection



Scheme 6. Synthesis of 6-(*S*)-Carbomethoxysancycline (**26**) by Michael−Claisen Cyclization Using the Methyl Phenyl Diester **25** and Enone **1** as Substrates, with Deprotonation in Situ, Followed by Deprotection[a]



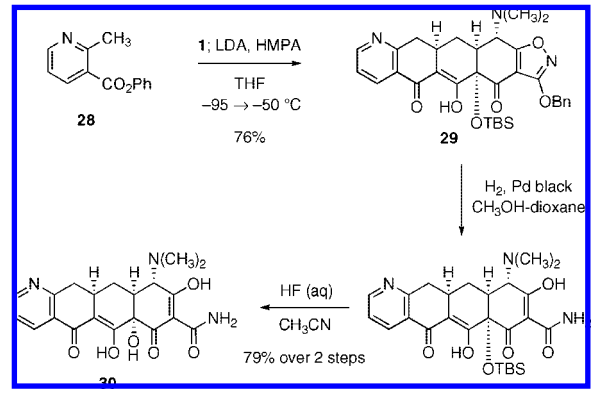
[a] In one experiment conducted with the corresponding benzyl phenyl diester **27** as the D-ring precursor, a diastereomeric mixture of cyclization products was obtained.

enone **1**. Thus, treatment of a mixture of phenyl ester **22** (1.3 equiv) and enone **1** (1 equiv) with an excess of LHMDS (3.3 equiv) at −78 °C, followed by warming of the resulting mixture to −30 °C over 90 min, provided the cyclization product **23** in 94% yield (Scheme 5); removal of protective groups then provided 6-(*R*)-*N*-imidazoylsancycline (**24**, 42% yield over two steps).

In situ deprotonation with LHMDS was also effective in bringing about cyclization of the methyl phenyl diester substrate **25** with enone **1**, as well as cyclization of the corresponding benzyl phenyl diester substrate **27**, as depicted in Scheme 6. In both cases, the major product of cyclization was epimeric at C6 relative to the major products of all other cyclization reactions we have studied (these providing the two exceptions referred to above), which we attribute to epimerization at C6 after cyclization. We have not yet conducted the rigorous studies necessary to establish if the product ratios were kinetically or thermodynamically determined in these examples.

The in situ deprotonation protocol has proven to be effective for the Michael−Claisen cyclization reactions of a number of different D-ring precursors containing benzylic anion-stabilizing substituents (see Table 2). Because of its greater experimental convenience, this has become the preferred method for the synthesis of tetracycline analogues substituted at the C6-position.

Scheme 7. Synthesis of 7-Aza-10-deoxysancycline (**30**) by Michael−Claisen Cyclization Using Phenyl Ester **28** and Enone **1** as Substrates, with Deprotonation in Situ, Followed by Deprotection



D-ring heterocyclic analogues of tetracyclines had not been made before, so far as we are aware, and their construction by semisynthesis cannot be easily imagined. In targeting analogues of this type, we initially chose to explore the synthesis of the D-ring pyridine derivative 7-aza-10-deoxysancycline (**30**, Scheme 7). As we previously reported, in situ deprotonation of phenyl ester **28** (4 equiv) with LDA (5 equiv) at −95 °C in the presence of enone **1** (1 equiv) and hexamethylphosphoramide (HMPA, 10 equiv), followed by warming to −50 °C, afforded the Michael−Claisen cyclization product **29** in 76% yield (Scheme 7).[1] Claisen ring-closure was notably more facile in this example than in others we have studied, proceeding to completion in less than 1 h upon warming to −50 °C. 7-Aza-10-deoxysancycline (**30**) was then obtained in 79% yield after removal of protective groups.

Another novel class of tetracyclines that we have explored is the pentacyclines (Scheme 8). This required that we develop

Scheme 8. Synthesis of the Pentacycline **33** by Michael−Claisen Cyclization Using the Phenyl Bromomethylnaphthoic Acid Ester **31** and Enone **1** as Substrates, and an in Situ Protocol for Lithium−Halogen Exchange, Followed by Deprotection

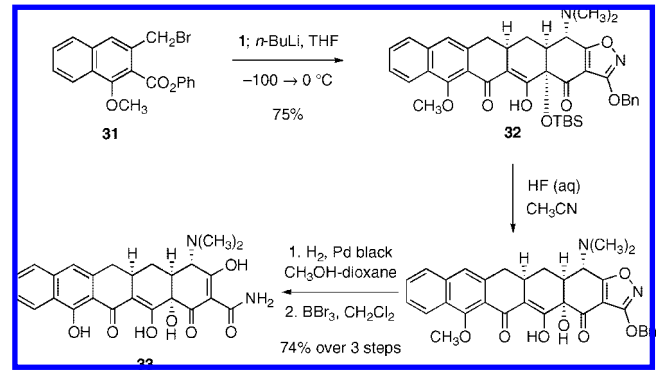
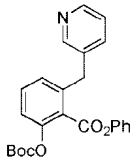
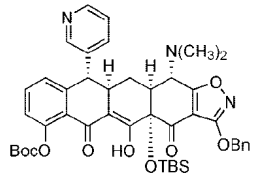
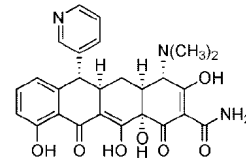
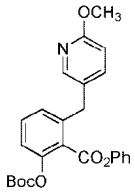
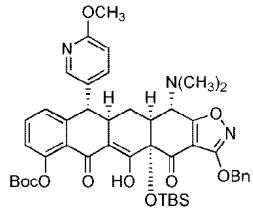
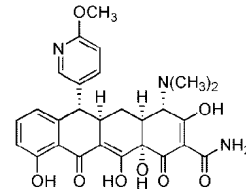
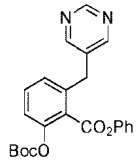
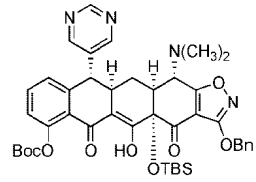
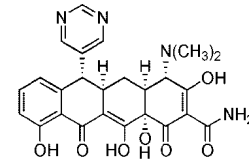
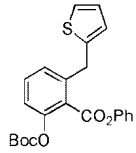
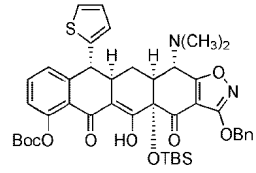
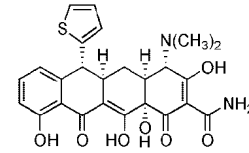
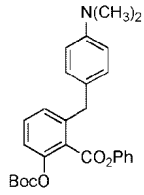
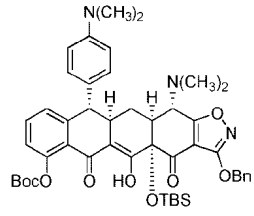
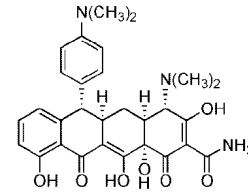
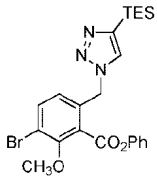
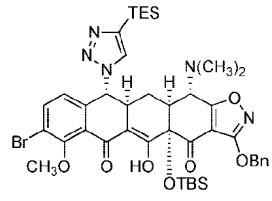
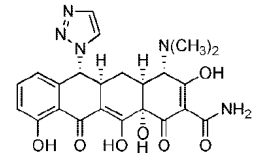
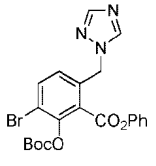
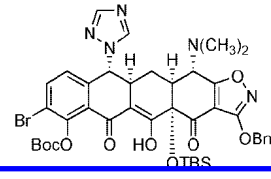
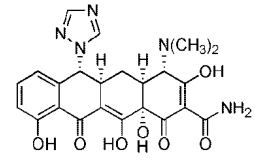


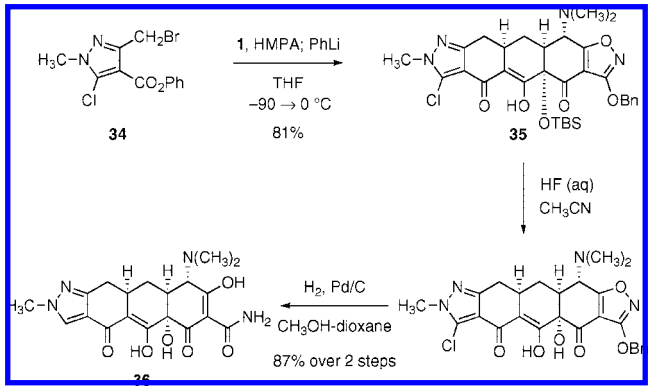
Table 2. Synthesis of 6-Substituted Tetracyclines by Michael−Claisen Cyclization Using D-Ring Precursors with Anion-Stabilizing Substituents in the Benzylic Position and Enone **1** as Substrates, with Deprotonation in Situ, Followed by Deprotection [(i) HF (aq), CH_3CN; (ii) H_2, Pd/C, CH_3OH−dioxane][37]

D-ring Precursor	Cyclization Conditions	Cyclization Product	Cyclization Yield	Tetracycline	Deprotection Yield
	**1**; LDA −78 → −10 °C		58%		58%
	**1**; LDA −78 → −10 °C		72%		60%
	**1**; LDA −78 → −10 °C		44%		23%
	**1**; LDA −78 → −10 °C		83%		98%
	**1**; LDA, TMEDA −78 → −10 °C		25%		64%
	**1**; LHMDS −78 → −10 °C		72%		43%
	**1**; LHMDS −78 → −10 °C		38%		65%

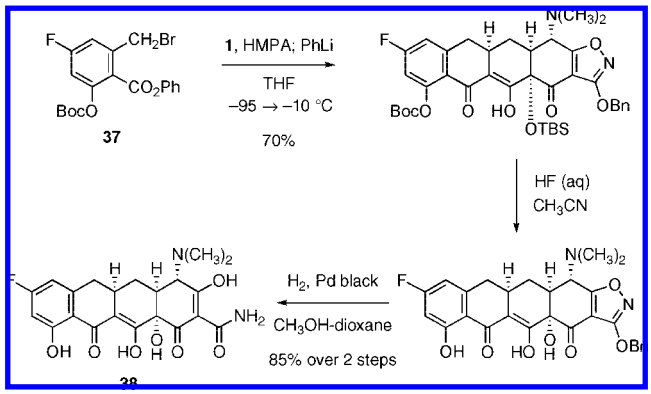
chemistry suitable for Michael−Claisen cyclization of naphthoate ester DE-ring precursors,[22−24] as the protocols that we had used to this point were found to be largely ineffective with these substrates.

Lithium−halogen exchange is rarely employed to form benzyllithium reagents in organic synthesis, due to the propensity of the benzyl halide substrates to engage in Wurtz-type coupling reactions in the presence of an alkyllithium reagent.[36]

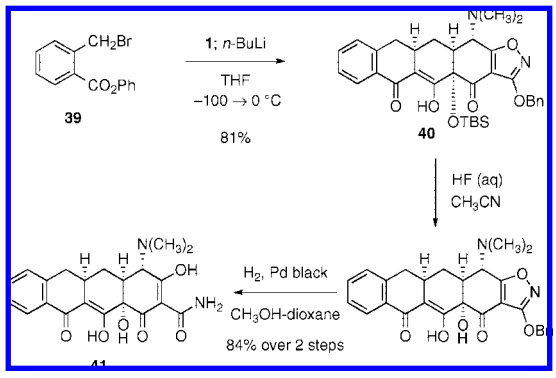
Scheme 9. Synthesis of a Pyrazole Analogue (**36**) by Michael−Claisen Cyclization Using the Benzylic Bromide **34** and Enone **1** as Substrates, and an in Situ Protocol for Lithium−Halogen Exchange, Followed by Deprotection



Scheme 10. Synthesis of 8-Fluorosancycline (**38**) by Michael−Claisen Cyclization Using Benzylic Bromide **37** and Enone **1** as Substrates, and an in Situ Protocol for Lithium−Halogen Exchange, Followed by Deprotection



Scheme 11. Synthesis of 10-Deoxysancycline (**41**) by Michael−Claisen Cyclization Using Phenyl 2-(Bromomethyl)benzoate (**39**) and Enone **1** as Substrates, with Deprotonation in Situ, Followed by Deprotection



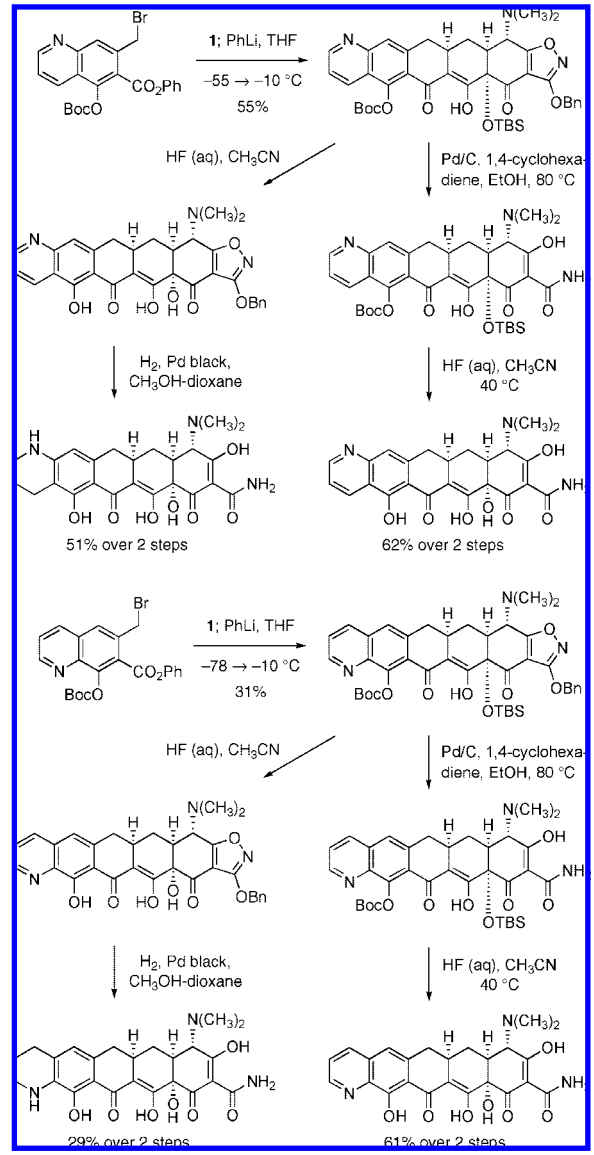
In a surprising transformation, treatment of a solution of phenyl bromomethylnaphthoic acid ester **31** (4 equiv) and enone **1** (1 equiv) with *n*-butyllithium (4 equiv) at −100 °C, followed by warming to 0 °C, provided the Michael−Claisen cyclization product **32** in 75% yield (Scheme 8).[1] A three-step deprotection sequence then afforded pentacycline **33** in 74% yield.

(36) (a) Parham, W. E.; Jones, L. D.; Sayed, Y. A. *J. Org. Chem.* **1978**, *41*, 1184–1186. (b) Berk, S. C.; Yeh, M. C. P.; Jeong, N.; Knochel, P. *Organometallics* **1990**, *9*, 3053–3064.

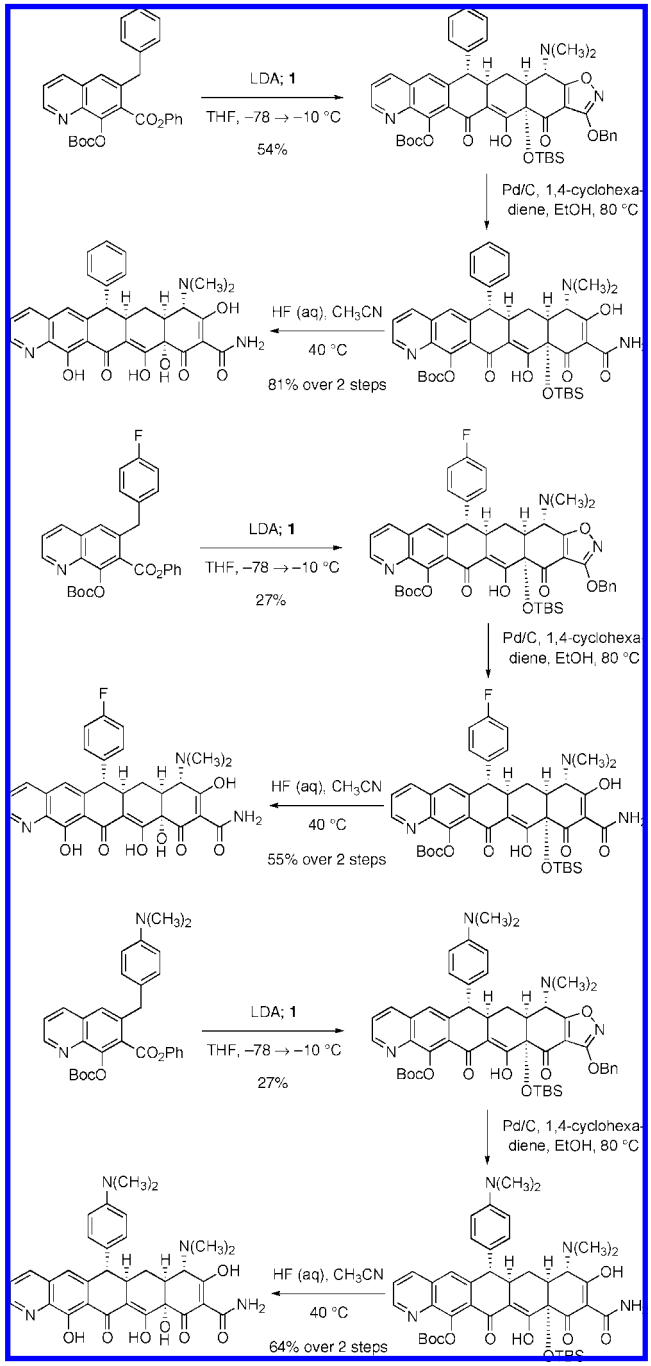
The in situ lithium−halogen exchange protocol developed for the synthesis of the pentacycline **33** has proven to be generally effective for the synthesis of a number of quite different tetracycline analogues. In many cases we have adopted the procedural modification of substituting the less reactive reagent phenyllithium for *n*-butyllithium in the lithium−halogen exchange reaction. For example, addition of phenyllithium to a solution of pyrazole **34** (3 equiv) and enone **1** (1 equiv) containing HMPA (6 equiv) at −90 °C, followed by warming to 0 °C over 2 h, provided the Michael−Claisen cyclization product **35** in 81% yield (Scheme 9). Removal of the protective groups led to concomitant cleavage of the heteroaryl carbon−chlorine bond during hydrogenolysis, affording the tetracycline analogue **36** containing a D-ring pyrazole (87% yield over two steps).

A very similar procedure produced 8-fluorosancycline (**38**) from the benzyl bromide **37** (Scheme 10). Like the D-ring pyrazole analogue **36**, the 8-fluorotetracycline analogue **38**

Scheme 12. Synthesis of Pentacyclines with Heterocyclic E Rings by Michael−Claisen Cyclizations Using Bromomethylquinolines and Enone **1** as Substrates, and an in Situ Protocol for Lithium−Halogen Exchange, Followed by Deprotection



Scheme 13. Synthesis of 6-Aryl-Substituted Heterocyclic Pentacyclines by Michael−Claisen Cyclizations Using Benzylically Substituted Quinolines as DE-Ring Precursors and a Stepwise Protocol for Addition of the Base and Enone **1**, Followed by Deprotection



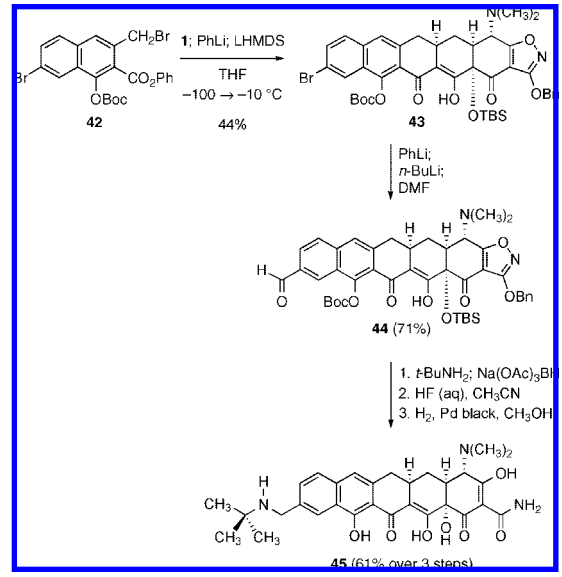
would have been difficult, if not impossible, to prepare by semisynthetic methods.

Lithium−halogen exchange was also employed for the synthesis of 10-deoxysancycline (**41**, Scheme 11). As we reported previously, treatment of a solution of phenyl 2-(bromomethyl)benzoate (**39**, 4 equiv) and enone **1** (1 equiv) with *n*-butyllithium (4 equiv) at −100 °C, followed by warming to 0 °C over 30 min, provided the Michael−Claisen cyclization product **40** in 81% yield.[1,38] The typical two-step deprotection sequence then transformed the cyclized product **40** into 10-deoxysancycline (**41**) in 84% yield.

Bromomethylquinoline derivatives[39] were also employed as DE-ring precursors in Michael−Claisen cyclization reactions with enone **1**, providing pentacycline precursors with heterocyclic E rings (Scheme 12). The typical two-step deprotection sequence afforded tetrahydroquinoline products in these examples, while the use of modified conditions led to deprotection without reduction of the pyridine E-ring. Cyclization reactions of quinoline substrates with 6-aryl substituents were also investigated,[35b] using stepwise deprotonation conditions (Scheme 13).

Thus far, each different cyclization reaction described has produced a single tetracycline analogue. It is worth noting explicitly that the convergent coupling strategy employed to prepare individual tetracycline analogues can also be used to target structures that serve as branch points to large numbers of analogues, versatile structures such as the aryl bromide **43** or the aldehyde **44** (Scheme 14). Both of these pentacycline precursors were targeted for synthesis.

Scheme 14. Synthesis of the Diversifiable Pentacycline Precursors **43** and **44** by Michael−Claisen Cyclization Using the Benzylic Bromide **42** and Enone **1** as Substrates, with an in Situ Protocol for Selective Lithium−Halogen Exchange[a]



[a] Further transformation of **44** by a reductive amination reaction provides a route to alkylaminomethylpentacyclines, as illustrated by the synthesis of the *tert*-butylaminomethylpentacycline **45**.

Michael−Claisen cyclization of the benzylic bromide **42**[40] with enone **1** was successfully achieved by in situ lithium−halogen exchange using phenyllithium, but not *n*-butyllithium. Thus, treatment of a mixture of the bis-bromide **42** (3 equiv) and enone **1** (1 equiv) with phenyllithium (3 equiv) at −100 °C,

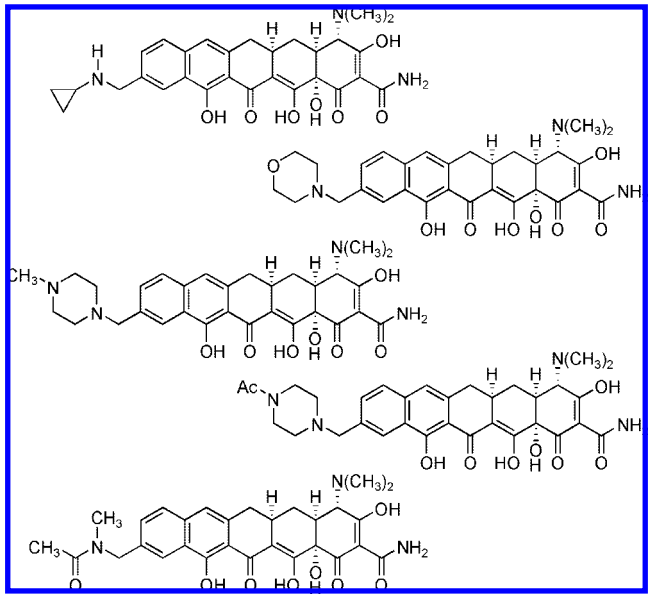
(37) The use of a methyl ether protecting group in the penultimate entry of Table 2 required one additional deprotection step (BBr_3, CH_2Cl_2, −78 °C → 23 °C) following the typical two-step sequence.

(38) Both the stepwise and in situ protocols for deprotonation−cyclization were also effective for the synthesis of the Michael−Claisen cyclization product **40** from phenyl *o*-toluate and enone **1**, though yields were lower.

(39) Van Leusen, A. M.; Terpstra, J. W. *Tetrahedron Lett.* **1981**, *22*, 5097–5100.

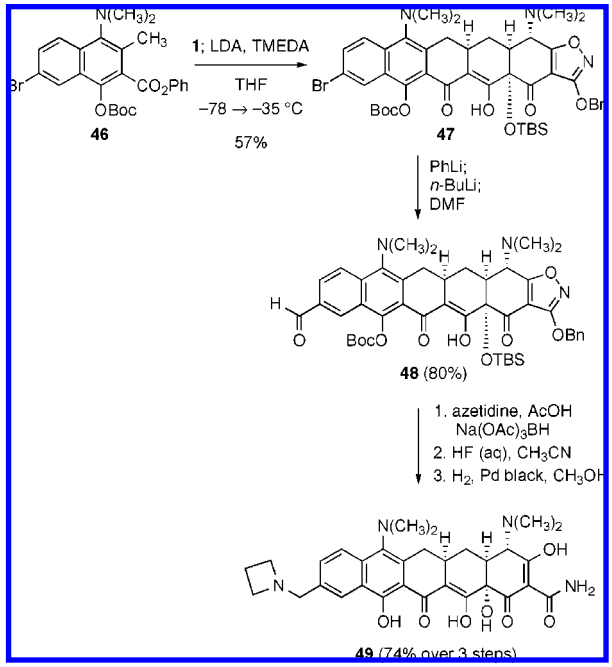
(40) Synthesized from 6-bromophthalide and methyl crotonate: Broom, N. J. P.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 465–470.

Chart 1. Alkylaminomethylpentacyclines Synthesized from Aldehyde **44** by Reductive Amination Followed by Deprotection[a]



[a] See Supporting Information for experimental details.[42]

Scheme 15. Synthesis of the Diversifiable Pentacycline Precursors **47** and **48** by Michael−Claisen Cyclization Using the Phenyl Naphthoate Ester **46** and Enone **1** as Substrates, with Deprotonation in Situ[a]



46 **47** **48** (80%) **49** (74% over 3 steps)

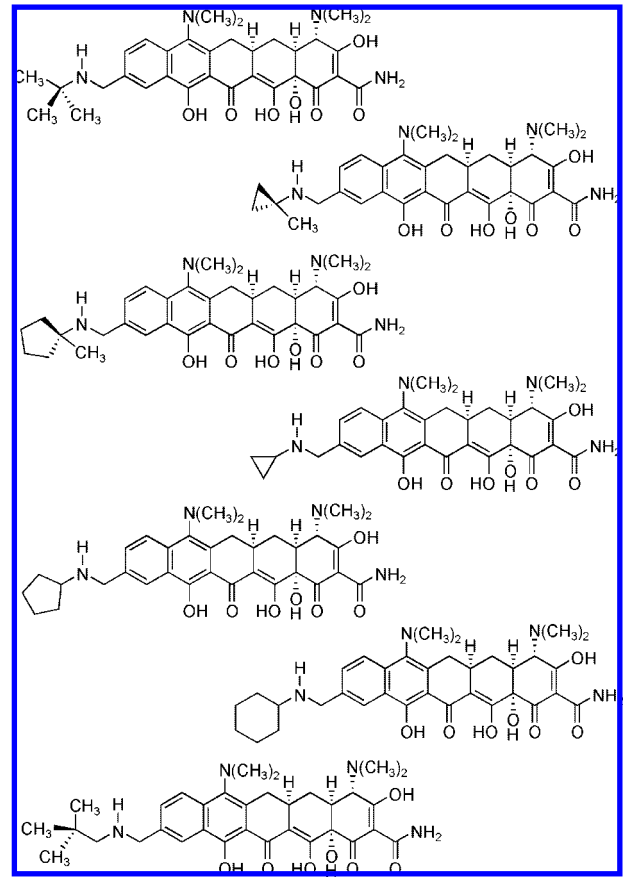
[a] Further transformation of **48** by a reductive amination reaction provides a route to 7-dimethylamino-alkylaminomethylpentacyclines, as illustrated by the synthesis of the 7-dimethylamino-azetidinylmethylpentacycline **49**.

followed by addition of LHMDS (1 equiv) and warming of the resulting mixture to −10 °C, provided the cyclization product **43** in 44% yield (Scheme 14).[41] Although the yield

(41) The yield of the Michael−Claisen cyclization product **43** was somewhat lower (28%) when LHMDS was omitted from the reaction.

(42) The final compound in Chart 1 was prepared by *N*-acetylation after reductive amination with methylamine.

Chart 2. 7-Dimethylamino-alkylaminomethylpentacyclines Synthesized from Aldehyde **48** by Reductive Amination Followed by Deprotection[43]

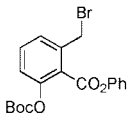
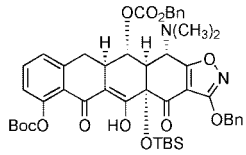
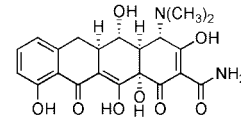
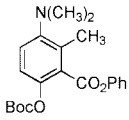
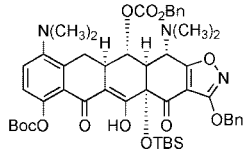
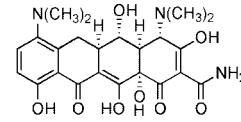
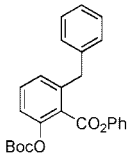
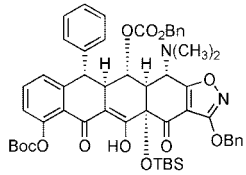
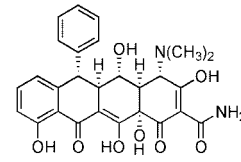
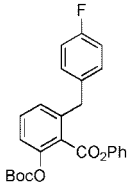
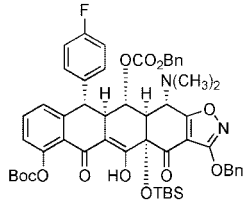
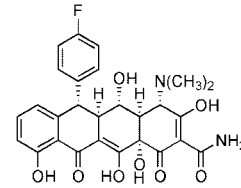
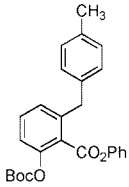
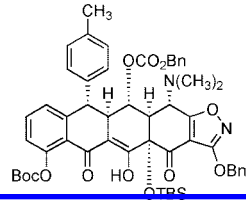
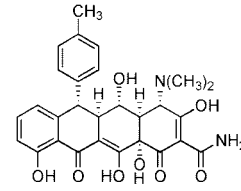


of product in this cyclization reaction was moderate, it remained consistent during scale-up and allowed for sufficient quantities of **43** to be produced to explore late-stage diversification. The aryl bromide intermediate **43** was treated sequentially with phenyllithium and *n*-butyllithium to effect deprotonation and lithium−halogen exchange, respectively, and the resulting dianion was formylated with *N,N*-dimethylformamide (DMF) to give the aldehyde **44**; reductive amination with *tert*-butylamine and subsequent deprotection afforded *tert*-butylaminomethylpentacycline **45** in 61% yield after purification by rp-HPLC. The use of a number of different amines in the reductive amination reaction led to the synthesis of various alkylaminomethylpentacycline analogues from the common intermediate **44** (Chart 1).

We also successfully pursued the synthesis of a parallel series of diversifiable pentacycline precursors containing a dimethylamino substituent at C7, as in minocycline (**9**) and tigecycline (**10**, see Scheme 15). In the case of the dimethylamino-substituted naphthoate ester **46**, it was possible to conduct cyclization by in situ deprotonation, in the presence of enone **1**, forming the dimethylamino-substituted aryl bromide **47** in 57% yield. Subjection of **47** to deprotonation

(43) 7-Dimethylamino-alkylaminomethylpentacyclines were initially synthesized from a DE-ring precursor in which the phenolic hydroxyl group was protected as a methyl ether (necessitating a three-step deprotection sequence); it was later found that the corresponding DE-ring precursor containing a *tert*-butoxycarbonyl protecting group (compound **46**) was also an effective cyclization substrate (allowing for standard two-step deprotection, see Supporting Information for details).

Table 3. Synthesis of 5-Hydroxytetracyclines by Michael−Claisen Cyclization Using a Number of Different D-Ring Precursors and Enone **2** as Substrates, Followed by Deprotection[a]

D-ring Precursor	Cyclization Conditions	Cyclization Product	Cyclization Yield	Deprotection Conditions	Tetracycline	Deprotection Yield
	**2**; PhLi, LHMDS −78 → 0 °C		88%	B		58%
	LDA, TMEDA; **2** −78 → −10 °C		62%	C		50%
	LDA; **2** −78 → −10 °C		39%	A		82%
	LDA; **2** −78 → −10 °C		88%	A		73%
	LDA; **2** −78 → 0 °C		66%	A		65%

[a] Deprotection conditions: **A**, (i) HF (aq), CH_3CN; (ii) H_2, Pd-black, CH_3OH−dioxane; **B**, (i) HF (aq), CH_3CN; (ii) H_2, Pd/C, CH_3OH−dioxane; **C**, (i) H_2, Pd-black, CH_3OH−dioxane; (ii) HF (aq), CH_3CN.

and lithium−halogen exchange, and formylation of the resulting dianion with DMF, gave the pentacycline precursor aldehyde **48** in 80% yield; reductive amination with azetidine and subsequent deprotection afforded azetidinylmethylpentacycline **49** in 74% yield after purification by rp-HPLC (Scheme 15). As with aldehyde **44** above, reductive amination of **48** could be conducted using a number of different amines, providing various 7-dimethylamino-alkylaminomethylpentacyclines upon deprotection (see Chart 2).

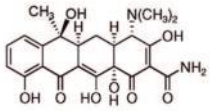
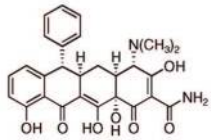
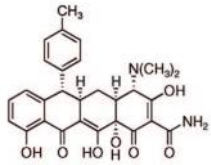
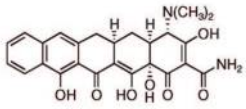
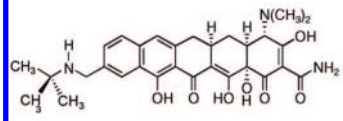
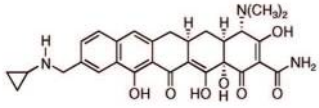
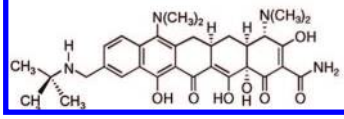
An alternative approach to diversification involved reduction of aldehyde **44** with sodium triacetoxyborohydride, mesylation of the resulting primary alcohol with methanesulfonic anhydride, and then nucleophilic displacement (see Scheme 16). When imidazole was used as the nucleophile, substitution afforded the *N*-imidazoylmethyl product **50** (59% yield over two steps); removal of protective groups afforded the corresponding pentacycline analogue **51** in 40% yield.

As we have previously shown, by variation of our original synthetic route to the enone **1** the corresponding C5-benzyloxycarbonyloxy substituted enone **2** (see Introduction) can be prepared in gram amounts.[1] This in turn has enabled the synthesis of a number of C5-hydroxytetracyclines (Scheme 17 and Table 3). Like the corresponding cyclization reactions with enone **1**, Michael−Claisen condensations with enone **2** proceed with uniformly high stereoselectivity. For example, addition of enone **2** (1 equiv) to a solution of the *o*-toluate ester anion derived from phenyl ester **13** (4.5 equiv) at −78 °C, followed by warming of the resulting mixture to 0 °C over 2 h, provided the Michael−Claisen cyclization product **52** in 79% yield in diastereomerically pure form after purification by rp-HPLC (Scheme 17). A minor diastereomer, believed to be 6-epi-**52**, was isolated separately (<7% yield). Doxycycline (**8**) was obtained in 90% yield after removal of protective groups and purification by rp-HPLC.[1]

Antibacterial Activities

Minimum inhibitory concentrations (MICs) were determined in whole-cell antimicrobial assays using a panel of tetracycline-sensitive and tetracycline-resistant Gram-positive and Gram-negative bacteria. The results for selected com-

Table 4. Minimum Inhibitory Concentration (MIC) Values for Selected Tetracycline Analogues (μg/mL)[a]

	SA 100	SA 2147	SA 757	SA 2011	EF 708	EF 1092	SP 1195	SP 911	EC 102	EC 2271	EC 119	EC 121	AB 1630	PA 103	HI 1224
Resistance:		M	M, T	M, T		V, T		P, T		T		T		Mult	A, T
 Tetracycline	1	0.5	64	32	0.5	>64	0.25	64	1	64	2	64	1	32	16
	0.25	0.12	0.5	0.12	0.12	0.5	<0.06	<0.06	>16	>16	>16	8	0.25	>16	2
	0.25	0.25	0.5	0.25	0.5	0.5	0.25	0.25	>32	>32	ND	ND	8	>32	>32
	0.25	0.25	0.5	0.25	0.25	0.5	0.25	0.25	>32	>32	ND	ND	2	>32	64
	1	1	1	0.5	0.5	0.5	0.25	0.25	>32	>32	>32	>32	8	>32	0.5
	1	0.5	2	0.5	0.12	2	<0.06	0.5	0.5	1	1	0.25	2	>32	0.06
	0.5	0.25	2	0.5	<0.06	1	<0.06	0.5	0.25	1	1	0.25	0.5	>32	0.06
	0.5	0.25	1	0.25	<0.06	0.5	<0.06	0.5	0.5	1	2	0.12	1	32	0.25

[a] Organisms in red are antibiotic-resistant. Abbreviations: A, ampicillin; M, methicillin; P, penicillin; V, vancomycin; T, tetracycline; Mult, multiple. Organisms: SA, *S. aureus*; EF, *E. faecium*; SP, *S. pneumoniae*; EC, *E. coli*; AB, *A. baumanii*; PA, *P. aeruginosa*; HI, *H. influenzae*.

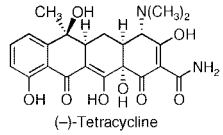
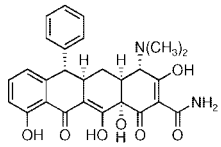
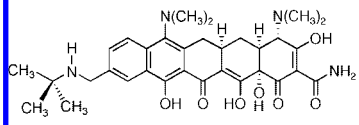
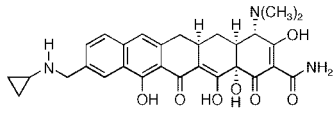
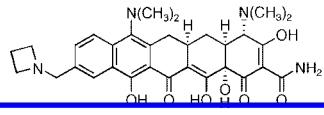
pounds are shown in Table 4. Two promising new series of tetracycline antibiotics emerge from this study: 6-aryltetracyclines, which are active in both tetracycline-sensitive and tetracycline-resistant Gram-positive strains, and alkylaminomethylpentacyclines, which show good activity in both tetracycline-sensitive and tetracycline-resistant Gram-positive and Gram-negative organisms.

Several compounds were selected for further study in a mouse septicemia model to determine efficacy in vivo (Table 5). Mice were inoculated intraperitoneally with an LD_{90-100} bolus of *S. aureus* (Smith). One hour later, tetracycline analogues were administered intravenously at dose levels ranging from 0.3 to 30 mg/kg. Mortality was monitored once daily for 7 days. All mice survived at the highest level of dosing (30 mg/kg) for each of the compounds tested, indicating that the threshold of acute toxicity in mice is greater than this value. The 6-phenyltetracycline analogue showed some efficacy in this murine model (ED_{50} 10.7 mg/kg), while the alkylaminomethylpentacyclines were more potent, with efficacy similar to that of tetracycline (ED_{50} 1.7−2.8 mg/kg).

Conclusion

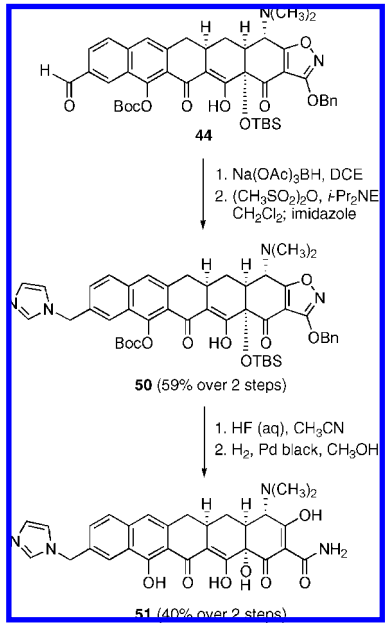
The results described illustrate the application of a general AB plus D strategy for the synthesis of more than 50 tetracyclines and tetracycline analogues. C-ring formation was

Table 5. In Vivo Efficacy of Selected Tetracycline Analogues in a Tetracycline-Sensitive Strain of *S. aureus*[a]

Compound	Number of Mice	Number of Deaths at Dose of					ED_{50} (mg/kg)
		30 mg/kg	10 mg/kg	3 mg/kg	1 mg/kg	0.3 mg/kg	
 (–)-Tetracycline	6		0	0	2	6	0.97
	6	0	5	6	6	6	10.71
	6	0	0	1	6	6	2.80
	6	0	0	1	5	6	1.73
	6	0	0	1	5	6	1.73

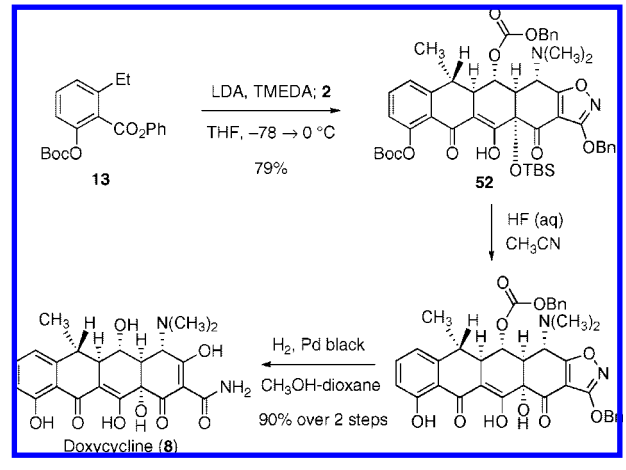
[a] Mice were inoculated intraperitoneally with an LD_{90-100} bolus of *S. aureus* (Smith) (3.5×10^5 CFU/mouse) in 0.5 mL of BHI broth containing 5% mucin. Tetracycline analogues were administered intravenously to test animals at 1 h after bacterial inoculation. Mortality was monitored once daily for 7 days.

Scheme 16. An Alternative Route to Diversification of the Aldehyde **44**, Involving Reduction, Activation, and Nucleophilic Displacement, As Exemplified by the Synthesis of the *N*-Imidazoylmethylpentacycline **51**



achieved by a stereocontrolled Michael–Claisen cyclization reaction employing one or more of the protocols detailed herein. The examples discussed demonstrate that structural

Scheme 17. Synthesis of Doxycycline (**8**) by Michael–Claisen Cyclization Using the *tert*-Butoxycarbonyl-Protected Phenyl Ester **13** as the D-Ring Precursor and a Stepwise Protocol for Addition of the Base and Enone **2**, Followed by Deprotection



modification of both AB and D-ring components allows for the preparation of individual tetracyclines, as well as for the synthesis of tetracycline precursors that are readily diversified by late-stage transformations. In many cases a single cyclization attempt provided, after deprotection, sufficient material for antibacterial screening against a panel of Gram-positive and Gram-negative organisms. For compounds of interest, reactions could then be readily scaled to provide amounts necessary for further evaluation in assays such as a

murine septicemia model. A number of novel structural classes were explored, including D-heteroaryl tetracyclines, pentacyclines, and E-heteroaryl pentacyclines. The platform for tetracycline synthesis described gives access to a broad range of molecules that would be inaccessible by semisynthetic methods (presently the only means of tetracycline production) and provides a powerful engine for the discovery and, perhaps, development of new tetracycline antibiotics.

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Supporting Information Available: Detailed experimental procedures for Michael−Claisen cyclization reactions and deprotection sequences, and characterization data for all tetracycline analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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