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# A Convergent Enantioselective Route to Structurally Diverse 6-Deoxytetracycline Antibiotics

**Mark G. Charest, Christian D. Lerner, Jason D. Brubaker, Dionicio R. Siegel, Andrew G. Myers***

**Complex antibiotics based on natural products are almost invariably prepared by semisynthesis, or chemical transformation of the isolated natural products. This approach greatly limits the range of accessible structures that might be studied as new antibiotic candidates. Here we report a short and enantioselective synthetic route to a diverse range of 6-deoxytetracycline antibiotics. The common feature of this class is a scaffold of four linearly fused rings, labeled A through D. We targeted not a single compound but a group of structures with the D ring as a site of structural variability. A late-stage, diastereoselective C-ring construction was used to couple structurally varied D-ring precursors with an AB precursor containing much of the essential functionality for binding to the bacterial ribosome. Five derivatives were synthesized from benzoic acid in yields ranging from 5 to 7% over 14 to 15 steps, and a sixth, (–)-doxycycline, was synthesized in 8.3% yield over 18 steps.**

The limitations of chemical synthesis frequently present a substantial obstacle to the development and discovery of new antibiotics and of pharmaceutical agents in general. The problem is nowhere more evident than among the structurally complex natural products tetracycline (**1**) and erythromycin. Both agents have proven highly effective in treating a wide range of bacterial infections, but decades of clinical use have led to the emergence of widespread bacterial resistance and, as a result, a need for the development of new antibiotics (*1*–*3*). The approach to the synthesis of varied structures in these classes has changed little in more than 50 years of research and is largely restricted to a process of semisynthesis, whereby the natural product is isolated and then modified, although approaches based on modified biosynthetic pathways are under development (*4*–*6*).

The tetracycline class of molecules is characterized by a carbon skeleton composed of four linearly fused six-membered carbon rings, conventionally labeled A through D (Fig. 1A). Among the derivatives accessed by semisynthesis, those with the hydroxyl group removed from carbon 6 of the C ring have shown particular clinical promise. These 6-deoxytetracyclines are considerably more resistant to degradation than their 6-hydroxy counterparts, and they show equal or greater potencies in antibacterial assays (*7*, *8*). The clinical efficacy of 6-deoxytetracyclines such as doxycycline (**2**) and minocycline (**3**) argues for a broad evaluation of 6-deoxytetracyclines. Unfortunately, the elaboration of natural tetracyclines is greatly limiting in terms of scope, and a general synthetic route to diverse tetracyclines has been elusive.

Here we report a short and efficient route for the synthesis of enantiomerically pure members of the 6-deoxytetracyclines from benzoic acid. The route we describe allows for the synthesis of 6-deoxytetracyclines (both with and without a hydroxyl group at C5) by a late-stage coupling reaction of the AB precursors **4** or **5** (Figs. 1B and 2) and provides access to a wide range of 6-deoxytetracyclines with modified D rings, as illustrated by the preparation of (–)-doxycycline (**2**), (–)-6-deoxytetracycline (**6**), the D-ring heterocyclic derivatives **7** and **8**, 10-deoxysancycline (**9**), and the pentacycline derivative **10** (see Fig. 3 for structures). The advantage of the late-stage

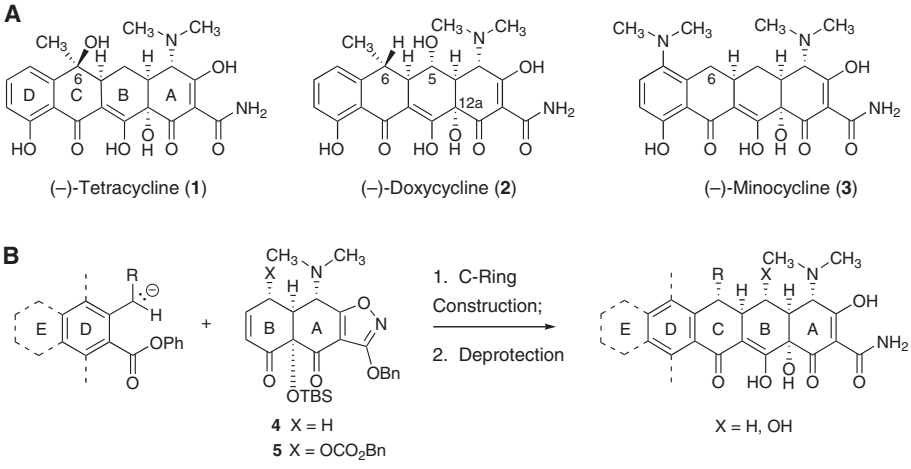


**Fig. 1.** (**A**) Chemical structures of tetracycline antibiotics. (–)-Tetracycline (**1**) was first produced semisynthetically, by hydrogenolysis of the fermentation product aureomycin (7-chlorotetracycline), but later was discovered to be a natural product and is now produced by fermentation (*8*). (–)-Doxycycline (**2**) and minocycline (**3**) are clinically important non-natural antibiotics and are both manufactured by multistep chemical transformations of fermentation products (semisynthesis) (*8*). (**B**) A generalized Michael-Dieckmann reaction sequence that forms the C ring of tetracyclines from the coupling of structurally varied carbanionic D-ring precursors with either of the AB precursors **4** or **5**.

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C-ring construction that we report [AB + D → ABCD (Fig. 1B)] is that much of the polar functionality known to play a role in the binding of tetracyclines to the bacterial ribosome lies within the AB fragment (*9*, *10*). At the same time, wide structural variation on or near the D ring has been cited as a means to overcome bacterial resistance. For example, the advanced clinical candidate tigecycline (*11*), which bears a substituted D ring, is reported to be one of the most promising new antibiotics under evaluation by the U.S. Food and Drug Administration (*12*).

Previous approaches to the synthesis of the tetracycline antibiotics have proceeded by stepwise assembly of the ABCD ring system, beginning with D or CD precursors, and are typically low-yielding. Examples are the Woodward synthesis of (±)-6-deoxy-6-demethyltetracycline (sancycline, 25 steps, ~0.002% yield) (*13*); the Shemyakin synthesis of (±)-12a-deoxy-5a,6-anhydrotetracycline (*14*); and the Muxfeldt synthesis of (±)-5-oxytetracycline (terramycin, 22 steps, 0.06% yield) (*15*); as well as the synthesis of (–)-tetracycline itself from the A-ring precursor D-glucosamine (34 steps, 0.002% yield) (*16*). The most efficient construction of the tetracycline ring system thus far is the synthesis of (±)-12a-deoxytetracycline by the Stork laboratory (16 steps, 18 to 25% yield) (*17*). In this case, however, the absence of a hydroxyl group at a fusion point of the A and B rings (C12a) is associated with greatly reduced antimicrobial activity (*18*), and late-stage introduction of this group has not been practical (*13*–*17*). We therefore introduced the C12a hydroxyl group in the first step of our sequence (Fig. 2) and used the stereogenic center produced in that step to elaborate all others in the target molecule.

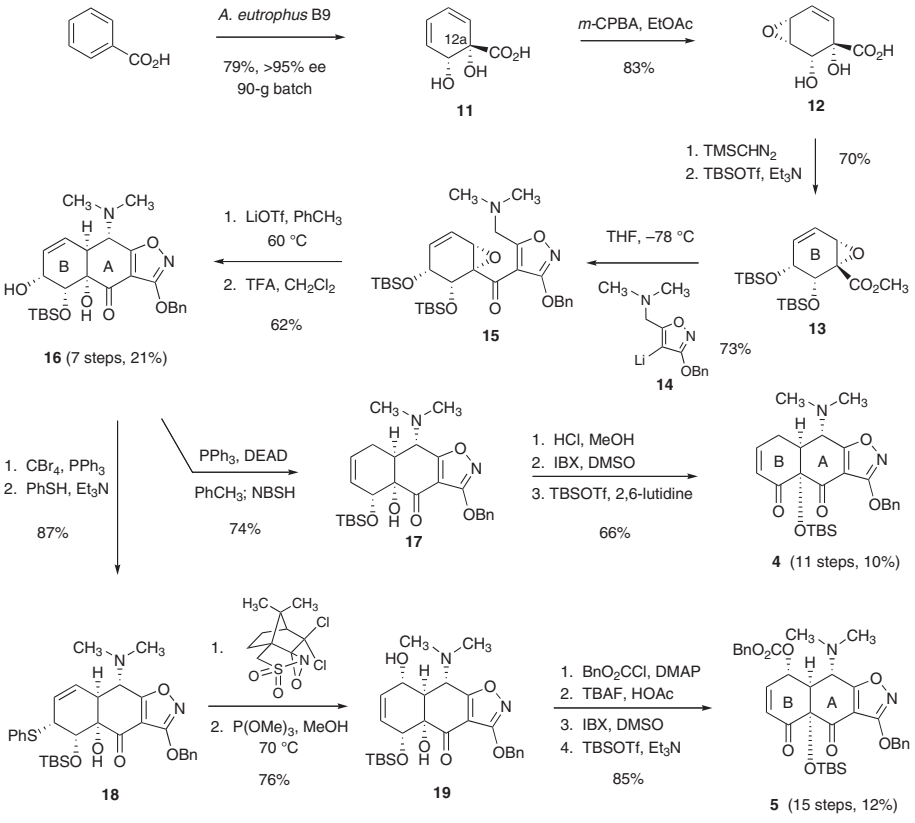
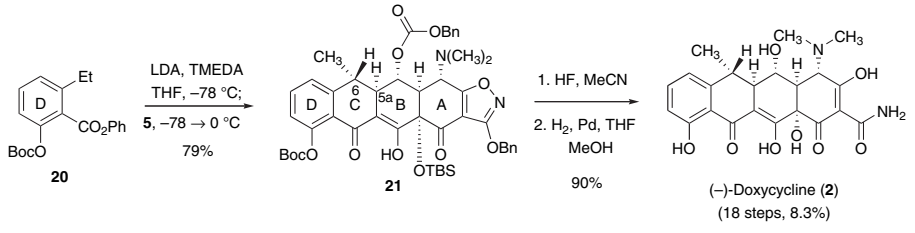



**Fig. 2.** Benzoic acid is transformed in seven steps to the key bicyclic intermediate **16**. This product is then used to prepare the AB precursor enone **4** by the four-step sequence shown or to enone **5**, the AB precursor to 6-deoxy-5-hydroxytetracycline derivatives, by the eight-step sequence shown.




**Scheme 1.**

Our synthesis (Fig. 2) was initiated by whole-cell microbial dihydroxylation of benzoic acid (which would become the B ring of the targeted 6-deoxytetracyclines) using a mutant strain of *Alcaligenes eutrophus* (*19*, *20*), producing the diol **11** with >95% enantiomeric excess in 79% yield (a 90-g batch, ~13 g/liter). Hydroxyl-directed epoxidation of the microcrystalline product (**11**, *m*-chloroperbenzoic acid) provided the α-oriented epoxide **12** in 83% yield; esterification of this product with trimethylsilyldiazomethane, followed by bis-silylation and concomitant epoxide isomerization in the presence of *tert*-butyldimethylsilyl triflate (three equivalents), afforded the epoxy ester **13** in 70% yield (*20*).

In constructing the A ring, we protected the vinylogous carbamic acid function (the right side of the final A ring as drawn) as a 5-benzyloxyisoxazole group, developed by Stork and Haggedorn for that purpose (*21*). 3-Benzyloxy-5-dimethylaminomethylisoxazole, prepared on the mole scale by a simple four-step sequence from glyoxylic acid (*22*, *23*), was deprotonated at C4 with *n*-butyllithium, and the resulting organolithium reagent (**14**) was then added to the epoxy ester **13**, forming the ketone **15** (73%). In a key step of the synthesis, closure of the A ring was achieved by warming of the ketone **15** with lithium triflate (5 mole %) at 60°C, followed by selective removal of the allylic silyl ether of the rearranged product by means of trifluoroacetic acid. The tricyclic AB precursor **16** was isolated in 62% yield after purification by flash-column chromatography. We believe that the transformation of **15** to **16** involves initial $S_N$-prime opening of the allylic epoxide by the *N*,*N*-dimethylamino group, followed by ylide formation and [2,3]-sigmatropic rearrangement, a process that is reminiscent of the Sommelet-Hauser rearrangement (*24*). Compound **16** possesses the requisite cis stereochemistry of the AB fusion, as well as an α-oriented *N*,*N*-dimethylamino substituent (confirmed by x-ray crystallographic analysis of a derivative), and serves as a common intermediate for the synthesis of both the AB precursor enone **4** (four steps, 49% yield) and the AB precursor to 5-α-hydroxy-6-deoxytetracyclines, enone **5** (eight steps, 56% yield), as detailed below.

Conversion of intermediate **16** to enone **4** first required migration of the B-ring double bond, with concurrent reduction of the hydroxyl group. This reductive transposition (*25*) was achieved using triphenylphosphine, diethyl azodicarboxylate, and *o*-nitrobenzenesulfonyl hydrazide (added last, in a procedural variant), affording the transposed cycloalkene **17** in 74% yield. Hydrolysis of the silyl ether protective group within **17** (using HCl and methanol), oxidation of the resulting allylic alcohol (using *o*-iodoxybenzoic acid in dimethylsulfoxide) (*26*), and protection of the remaining (tertiary) carbinol with *tert*-butyldimethylsilyl trifluoro-

methanesulfonate and 2,6-lutidine (*27*) then provided the enone **4** in 66% yield (three steps) after flash-column chromatography.

By a somewhat longer but slightly more efficient sequence, the intermediate **16** could also be transformed into the enone **5**, the AB precursor to 5-α-hydroxy-6-deoxytetracyclines (Fig. 2). This sequence began with the replacement of the secondary hydroxyl group of **16** with a thiophenyl group to form **18**, with net stereochemical retention. Next, diastereoselective sulfoxidation with a chiral oxidant (*28*) (99:1 selectivity) and Mislow-Evans rearrangement (*29*) produced the allylic alcohol **19** in 66% yield over the four steps. High diastereoselectivity in the sulfoxidation step was essential, because only one diastereomer (the major isomer under the conditions specified, stereochemistry not determined) underwent efficient thermal rearrangement. After protection of the allylic alcohol **19** by means of benzyl chloroformate, a sequence nearly identical to the final three steps of the synthesis of **4** was employed to transform the resulting benzyl carbonate into the enone **5** in 85% yield (56% net yield over the eight steps from **16**).

From these precursors, 6-deoxytetracyclines were assembled with all requisite functionality and stereochemistry in a single operation. Enones **4** or **5** were coupled with a range of different carbanionic D-ring precursors in a Michael-Dieckmann reaction sequence (*30*) that forms two carbon-carbon bonds and the C ring of the 6-deoxytetracyclines (Figs. 1B and 3). The process is perhaps best illustrated in detail by the three-step synthesis of (–)-doxycycline from the AB precursor **5** (Scheme 1). The D-ring precursor in this case (**20**) was synthesized in 42% yield over five steps from anisic acid. Deprotonation of **20** (4.5 equivalents) at the benzylic position (C6 in the product) was achieved with lithium diisopropyl amide in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in tetrahydrofuran at –78°C. The enone **5** was then added to the solution and the temperature was raised to 0°C. The tetracyclic coupling product **21** was isolated as a single diastereomer in 79% yield after purification by reverse-phase high-performance liquid chromatography (RP-HPLC). Removal of the protective groups (90% yield over two steps) and purification by RP-HPLC afforded (–)-doxycycline hydrochloride in 8.3% net yield over 18 steps from benzoic acid.

A remarkable feature of the convergent coupling reaction that produces the tetracyclic product **21** is its stereoselectivity. Although, in theory, four diastereomeric products can be formed by stereochemical variation of carbons 5a and 6 (the former being a BC fusion point), only one emerged in significant yield, and it matched the configuration (5a*R*, 6*R*) of the known biologically active 6-deoxytetracyclines. A minor diastereomeric impurity, believed to be 6-epi-**21**, was also isolated in separate RP-HPLC fractions (<7% yield). Michael-Dieckmann cyclization sequences (*30*) and condensations of *o*-toluate anions in particular (*31*–*33*) are extensively precedented in synthesis but not with the high degree of diastereoselectivity seen here.

Phenyl ester activation in toluate condensations is also precedented, though in a system that forms a fully aromatized cyclization product (*34*). We observed that the presence of the phenyl ester group of the D-ring precursor **20** was essential for successful cyclization to occur. Anions derived from D-ring

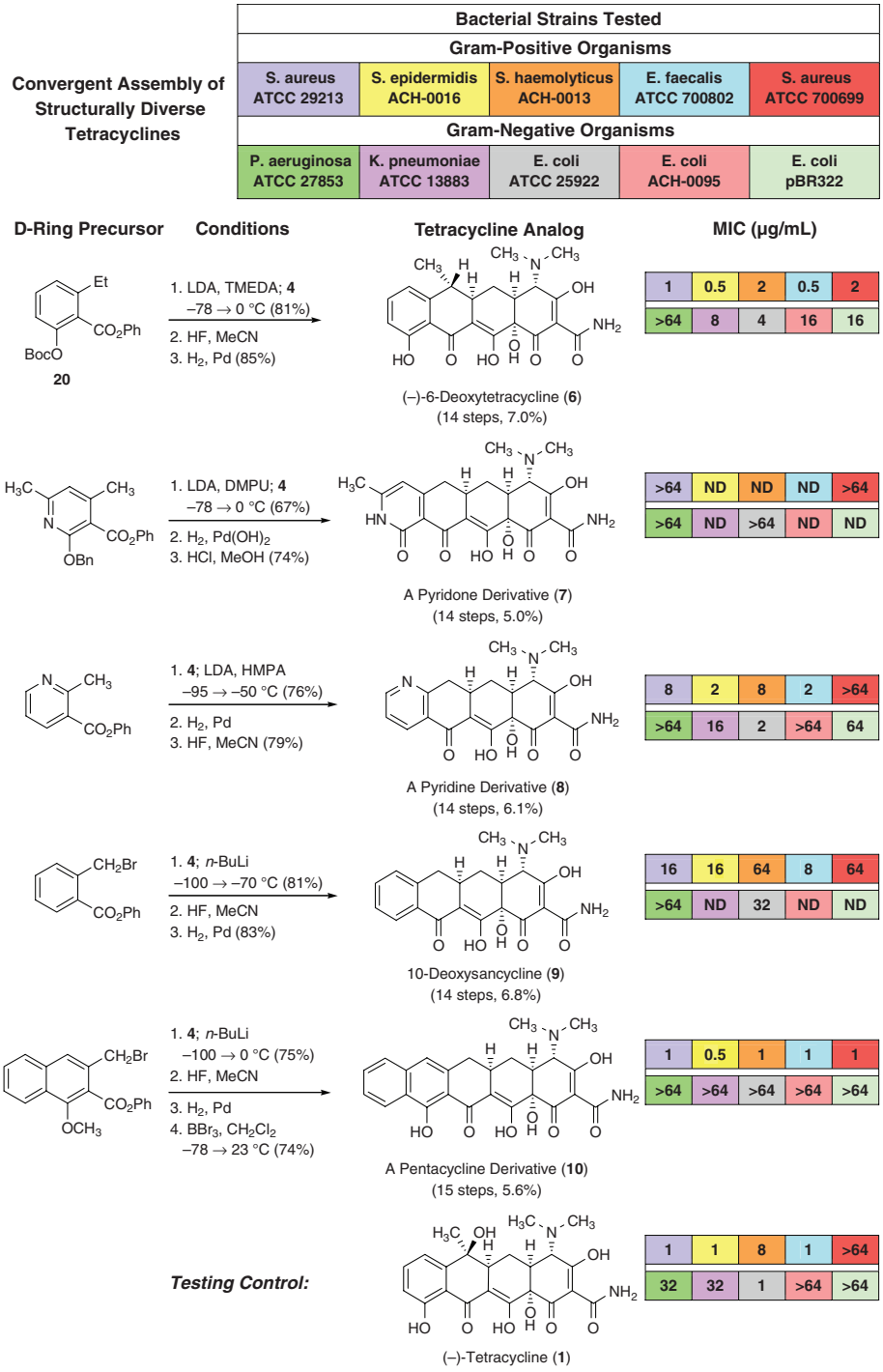



**Fig. 3.** Synthesis of structurally diverse 6-deoxytetracyclines by coupling of structurally diverse D-ring precursors and AB precursors **4** or **5**. The number of steps and overall yields from benzoic acid are shown in parentheses below each structure synthesized. Shown also are MIC values (in μg/ml) for whole-cell antibacterial testing of each analog against five Gram-positive and five Gram-negative microorganisms. Corresponding MICs for tetracycline (**1**), a testing control, appear at the bottom.

precursors containing simple alkyl esters underwent Michael addition, but the resulting adducts did not cyclize. Perhaps even more remarkable than the condensation that produces **21** is the parallel transformation of **20** with the enone **4** (Fig. 3, entry 1), which forms (–)-6-deoxytetracycline (**6**) in protected form with >20:1 diastereoselectivity, in 81% yield after purification by RP-HPLC (diastereomerically pure, a minor diastereomer, epimeric at C6, was also isolated separately). It appears that additions to **4** and **5** proceed almost exclusively by addition to one face of each enone (the top face as drawn in Fig. 1B), producing C5a stereochemistry corresponding to that of natural tetracyclines, although why this should be the case is not obvious.

Efficient and stereoselective condensations were not restricted to the *o*-toluate anion derived from the D-ring substrate **20**. In all, we prepared six 6-deoxytetracycline variants (Fig. 3). In each case, it was necessary to optimize the specific conditions for *o*-toluate anion generation and trapping. For the synthesis of products **8**, **9**, and **10** (Fig. 3), anion generation was best conducted in situ, in the presence of the enone **4**, either by selective deprotonation (**8**) or by lithium-halogen exchange (**9** and **10**). A number of potentially competing nonproductive reaction sequences might have intervened during in situ anion generation (such as enolization of **4**); the observed efficiencies of the transformations are surprising in light of this. It is also noteworthy that in situ anion generation permits the use of *o*-toluates lacking an *o*-alkoxy substituent (such as used in the synthesis of **8** and **9**), substrates that are known to be problematic from prior studies (*35*). Also, lithium-halogen exchange reactions of benzylic halides (such as used in the synthesis of **9** and **10**) had previously been considered impracticable (*36*, *37*).

The efficiencies of the synthetic sequences we report have allowed for the preparation of sufficient quantities of each tetracycline analog for antibacterial testing, using standard serial-dilution techniques (in 5- to 20-mg amounts). Minimum inhibitory concentrations (MICs) were determined for each analog in whole-cell antimicrobial assays using five Gram-positive and five Gram-negative organisms (Fig. 3). Thus far, the pentacycline derivative **10** has shown the most promising antibacterial properties, with activity equal to or greater than tetracycline in each of the Gram-positive strains examined, including strains with resistance to tetracycline, methicillin, and vancomycin. Although this finding is noteworthy, it is very likely that antibiotics with even greater potencies and/or improved pharmacological properties will emerge with further exploration of the complex chemical space now made accessible by the versatile synthetic route described.

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# Hypoxia, Global Warming, and Terrestrial Late Permian Extinctions

**Raymond B. Huey* and Peter D. Ward**

**A catastrophic extinction occurred at the end of the Permian Period. However, baseline extinction rates appear to have been elevated even before the final catastrophe, suggesting sustained environmental degradation. For terrestrial vertebrates during the Late Permian, the combination of a drop in atmospheric oxygen plus climate warming would have induced hypoxic stress and consequently compressed altitudinal ranges to near sea level. Our simulations suggest that the magnitude of altitudinal compression would have forced extinctions by reducing habitat diversity, fragmenting and isolating populations, and inducing a species-area effect. It also might have delayed ecosystem recovery after the mass extinction.**

A catastrophic extinction marks the end of the Permian (*1*, *2*) and is attributed to an acute climate crisis, among other causes (*3*–*5*). However, background extinction rates and ecosystem turnover were elevated throughout much of the Late Permian (*6*, *7*), and recovery after extinction was slow (*1*, *2*). Thus, environmental degradation likely occurred both before and after the final catastrophe, perhaps caused by major shifts in atmospheric chemistry (*8*). Indeed, modeling, isotope, and paleontological evidence (*9*–*13*) suggests that $O_2$ levels plummeted in the Late Permian and Early Triassic (Fig. 1A) and would have restricted the supply of $O_2$ to organisms. At the same time, $CO_2$ levels were rising (Fig. 1A), and climate warming (*14*) would have increased metabolic demand for $O_2$. Severe hypoxia was inevitable (*9*, *15*, *16*).

Here, we explore a biogeographic consequence of presumed low $O_2$ levels during the Late Permian and Triassic: Terrestrial animals