

The solid-phase synthesis of epothilone A (**1**) described here represents a new concept for the total synthesis of natural products, traces a highly efficient pathway to the naturally occurring epothilones, and opens the way for the generation of large combinatorial epothilone libraries. The biological results demonstrate that more potent microtubule binding analogues than the parent epothilones can be obtained (for example, compound **23**) by chemical synthesis. Furthermore, our findings point to lipophilic substituents rather than the epoxide moiety as important elements for binding activity. The role of the epoxide in the cytotoxicity of epothilones, however, still remains to be elucidated. □

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Evolution of the nitrogen cycle and its influence on the biological sequestration of CO₂ in the ocean

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Over geological time, photosynthetic carbon fixation in the oceans has exceeded respiratory oxidation of organic carbon. The imbalance between the two processes has resulted in the simultaneous accumulation of oxygen in, and drawdown of carbon dioxide from, the Earth's atmosphere, and the burial of organic carbon in marine sediments^{1–3}. It is generally assumed that these processes are limited by the availability of phosphorus^{4,5}, which is supplied by continental weathering and fluvial discharge^{5–7}. Over the past two million years, decreases in atmospheric carbon dioxide concentrations during glacial periods correlate with increases in the export of organic carbon from surface waters to the marine sediments^{8–11}, but variations in phosphorus fluxes appear to have been too small to account for these changes^{12,13}. Consequently, it has been assumed that total oceanic primary productivity remained relatively constant during glacial-to-interglacial transitions, although the fraction of this productivity exported to the sediments somehow increased during glacial periods^{12,14}. Here I present an analysis of the evolution of biogeochemical cycles which suggests that fixed nitrogen, not phosphorus, limits primary productivity on geological timescales. Small variations in the ratio of nitrogen fixation to denitrification can significantly change atmospheric carbon dioxide concentrations on glacial-to-interglacial timescales. The ratio of these two processes appears to be determined by the oxidation state of the ocean and the supply of trace elements, especially iron.

Globally, nitrogen and phosphorus are the two elements that potentially limit the biologically mediated carbon assimilation in the oceans by photoautotrophs. It is frequently argued that, as N₂ is abundant in both the ocean and atmosphere, and, in principle, can be biologically reduced to the equivalent of NH₃ by N₂-fixing cyanobacteria (that is, diazotrophs), nitrogen cannot be limiting on geological timescales^{4,15,16}. It then follows that phosphorus, which has no significant atmospheric source, must ultimately limit biological productivity. The underlying assumptions of these tenets should, however, be considered within the context of the evolution of biogeochemical cycles and the manifestations of those cycles in the contemporary ocean.

Virtually all fixed inorganic nitrogen in the contemporary ocean is oxidized to nitrate. Where did the nitrate come from? Although, in the Archaean atmosphere, electrical discharge or bolide impacts might have promoted NO formation from reaction between N₂ and CO₂, the yield for the reaction is low¹⁷. NH₃ in the Archaean atmosphere would have photodissociated, driven by ultraviolet radiation¹⁷; however, N₂ would have been stable and abundant^{17,18}. N₂ can be biologically reduced to NH₃ via the enzyme nitrogenase. Biological N₂ fixation is a strictly anaerobic process¹⁹, and the sequence of the genes encoding the catalytic subunits for nitrogenase is highly conserved in cyanobacteria and other eubacteria, strongly suggesting an ancient, common ancestral origin²⁰. The antiquity and homology of nitrogen fixation capacity also implies that fixed inorganic nitrogen in the Archaean and early Proterozoic oceans was scarce before the evolution of diazotrophic organisms; that is, there was strong evolutionary selection for N₂ fixation.

In contrast to fixed inorganic nitrogen, soluble phosphorus (largely as inorganic phosphate), in the Archaean oceans was probably relatively abundant in the mildly reducing environment of the time^{2,21}. Whereas apatite and other calcium-based and substituted solid phases of phosphate minerals precipitated in the primary formation of crustal sediments, secondary reactions of phosphate with Al and transition metals such as Fe are mediated at either low salinity or in sulphate-depleted waters (for example forming vivianite²²) low pH (for example forming strengite), or high oxidation states of the cations²³. Although these reactions would decrease the overall soluble phosphate concentration, the initial condition of the Archaean ocean probably had a very low reactive N:P ratio in the dissolved inorganic phase. As N₂ fixation proceeded, that ratio would have increased with a build-up of ammonium in the ocean interior. The accumulation of fixed nitrogen in the oceans would continue until the N:P ratio of the inorganic elements reached equilibrium with the N:P ratio of the sedimenting particulate organic matter (POM). Presumably, the latter ratio would approximate that of extant, nitrogen-fixing marine cyanobacteria, which is 16:1 by atoms^{4,24} or greater²⁵. In the absence of any substantial loss processes, the accumulation of the fixed inorganic nitrogen would ultimately have been constrained by the availability of phosphate.

The formation of nitrate from ammonium is sequentially catalysed through a nitrite intermediate by two groups of aerobic bacteria: one group oxidizes ammonium to nitrite, the second oxidizes nitrite to nitrate. Both processes require molecular oxygen; hence, nitrification must have evolved after the formation of free O₂ in the oceans by oxygenic photoautotrophs. Nitrification also provides reductant for the chemoautotrophic reduction of inorganic carbon, but is thermodynamically much less efficient than photosynthetic carbon fixation²⁶. Therefore, from a geological perspective, the conversion of ammonium to nitrate probably proceeded rapidly and provided a substrate (namely, NO₃⁻), that eventually could serve both as a source of nitrogen for photoautotrophs and as an electron acceptor for a diverse group of heterotrophic, anaerobic bacteria, the denitrifiers.

In the sequence of the three major biological processes that comprise the nitrogen cycle, denitrification must have been the last to emerge. This process, which permits the reduction of NO₃⁻ to (ultimately) N₂, occurs in the modern ocean in three major regions, namely, continental margin sediments, areas of restricted circulation such as fjords, and oxygen minima zones of perennially stratified seas^{27–30}. Denitrification seems to have evolved independently several times; the organisms and enzymes responsible for the pathway are highly diverse, and the pathway is found in both Archaeobacteria and Eubacteria³¹.

With the emergence of denitrification, a circuit for nitrogen was established between the atmosphere and ocean, where N₂ fixation provides a source of nitrogen for photoautotrophic production, but simultaneously, fixed nitrogen is removed from the oceans as N₂. The equilibrium between these two processes is bounded; the ratio of fixed inorganic nitrogen to dissolved inorganic phosphate in the ocean interior can only be less than, or at most equal to, that of the sinking flux of the two elements in POM.

There are three major inferences that can be drawn from the foregoing discussion. First, because the ratio of the sinking flux of particulate organic N and particulate P exceeds the N:P ratio of the dissolved pool of inorganic nutrients in the ocean interior, on average the upward flux of inorganic nutrients must be slightly enriched in P relative to N in relation to the elemental requirements of the photoautotrophs⁴. Hence, although there are some exceptions^{32,33}, at the present time, dissolved, inorganic fixed nitrogen generally limits primary production throughout most of the world oceans^{16,34–36}. Second, the N:P ratio of the dissolved pool of inorganic nutrients in the ocean interior was established by biological processes, not vice versa⁴. The average Redfield N:P ratio of

16:1 for particulate organic matter^{4,24} is an upper bound for the two elements in the dissolved inorganic phase in the ocean interior. In the contemporary ocean, the average N:P ratio of the dissolved inorganic nutrients in the ocean interior is ~14.7 by atoms³⁷, or less³⁸. The deficit in dissolved inorganic fixed nitrogen relative to soluble phosphate in the contemporary ocean implies a slight imbalance between nitrogen fixation and denitrification on time scales of ~10³ to 10⁴ years (ref. 39). Finally, if the net biologically mediated exchange of CO₂ between the atmosphere and ocean requires a change in either the concentration of limiting nutrients or the efficiency of their utilization⁵, and if dissolved inorganic nitrogen rather than phosphate limits productivity in the oceans, then it follows that the ratio of nitrogen fixation to denitrification has a critical role in determining the net biologically mediated exchange of CO₂ between atmosphere and ocean³⁹.

In the oligotrophic open ocean, the major nitrogen-fixing organisms are non-heterocystic cyanobacteria in the genus *Trichodesmium*⁴⁰. That there are no heterocystic marine cyanobacteria and few other planktonic, free-living marine diazotrophs suggests that some factor(s) have limited the abundance and speciation of these organisms in the oceans.

Trichodesmium spp. are obligate diazotrophs⁴¹, hence their distribution depends on the ability to synthesize nitrogenase. Nitrogenase contains two catalytic subunits, and requires Fe and Mo (or, in some organisms, V) to facilitate electron transfer reactions⁴². The catalytic activity per mole of Fe (that is, the rate of enzymatic turnover per mole of metal) is the lowest of any iron-containing enzyme involved in nitrogen metabolism⁴², and the iron requirement for diazotrophic growth is ~100-fold higher than that with fixed nitrogen⁴². The required transition metals, especially Fe, were relatively abundant under the mildly reducing conditions of the Archaean and early Proterozoic oceans^{2,43}, but the photosynthetic production of oxygen by cyanobacteria resulted in the precipitation of Fe(III) complexes, leaving much of the upper ocean with relatively low inventories of bioavailable iron². The loss in bioavailable iron seems to coincide with an evolutionary divergence of cyanobacteria, with the radiation of new marine species that were incapable of nitrogen fixation⁴⁴.

In the modern ocean, aeolian transport of continentally derived minerals is a significant, if not the most important, source of iron to the central ocean basins⁴⁵. In regions far removed from such fluxes, such as the South Pacific, the abundance of *Trichodesmium* seems to be extremely low, whereas in areas with higher fluxes, such as the North Atlantic, Indian Ocean, and the relatively iron-rich regions of the North Pacific, *Trichodesmium* blooms are much more frequent and extensive^{25,46}. The patterns of spatial and temporal distribution of *Trichodesmium* blooms have led to the suggestion that nitrogen fixation in the world oceans is limited by trace metals, especially iron^{25,47,48,68}. Whereas the role of iron in limiting photosynthesis in high-nitrate, low-chlorophyll regions of the world ocean has been established by direct experimental manipulation of iron concentrations^{49–51}, the larger role of iron (or other trace elements) in limiting nitrogen fixation in low-nutrient, low-chlorophyll regions of the subtropical ocean gyres might have a far greater impact on the biologically mediated net flux of atmospheric CO₂ into the oceans.

During recent glacial periods, the depression in sea level and corresponding reduction in continental shelf area, combined with a decrease in the intensity of water-column stratification in tropical regions⁵², seems to have resulted in decreased rates of denitrification relative to nitrogen fixation^{53,54}. Consequently, the ratio of dissolved inorganic nitrogen to phosphate could have 'caught up' with that of the sinking flux, effectively increasing the oceanic inventory of fixed inorganic nitrogen and simultaneously enhancing the effect of the biological CO₂ pump. During glacial periods, the net drawdown of atmospheric CO₂ would have been further accelerated by aeolian transport of minerals to the central ocean basins. The higher flux of iron to the oceans would not only have further stimulated the

biological pump by facilitating the biological utilization of preformed nutrients in the high-nitrate, low-chlorophyll regions⁵⁵, but, more importantly, would have stimulated N₂ fixation in the low-nutrient, low-chlorophyll regions. Given a C:N ratio of ~6.6 by atoms for the synthesis of new organic matter in the euphotic zone, and an average net deficit of dissolved inorganic nitrogen over phosphate in the modern ocean of 2.7 μmol kg⁻¹ (ref. 38), restoration of the N:P ratio to 16:1 in the ocean interior implies that during glacial periods the biological pump could have sequestered an additional 600 Pg C. This amount of carbon is approximately equal to the total global anthropogenic emissions since the beginning of the Industrial Revolution. Direct fertilization of the high-nitrate low-chlorophyll (HNLC) regions of the world oceans with iron would sequester about 140 Pg C (ref. 56). These two fluxes are additive.

Ice core records suggest that atmospheric CO₂ declined from ~290 to 190 μmol mol⁻¹ over a period of ~10,000 years during the last interglacial-glacial maximum (~20–100 kyr BP)⁵⁷. An equilibrium, three-box model calculation suggests that 800 Pg C would have to have been fixed by marine photoautotrophs if the ocean's biological pump were to account for the change in atmospheric CO₂. This model accounts for repartitioning of CO₂ between the atmosphere and the upper ocean, including internal adjustments in the equilibrium distributions of the major inorganic carbon species⁵⁸. The calculated change in atmospheric CO₂ would have required an addition of ~3 Tg fixed N per annum in addition to the utilization of nutrients in the HNLC regions. The net flux of fixed N requires only a small change in the global ratio of N₂ fixation to denitrification. Biological N₂ fixation in the contemporary ocean is poorly quantified; estimates range from ~20 Tg per annum for the entire ocean⁵⁹, to >50 Tg for the North Atlantic alone^{60,69}. The simultaneous decrease in denitrification during glacial periods would have further contributed to the net influx of fixed nitrogen in the oceans^{53,54,61}, requiring even less N₂ fixation to achieve the same result.

The enhancement of the effect of the biological pump in sequestering atmospheric CO₂ in the ocean interior by increasing the availability of fixed inorganic nitrogen would have exerted a positive climatic feedback. The initial (for example Milankovich⁶²) forcing would have led to increased cooling if atmospheric CO₂ were removed by a strengthening of the biological CO₂ pump³⁶. This process is self-limiting; when the N:P ratio of the dissolved inorganic nutrients caught up to that of the sinking flux of particulates, phosphorus would become limiting and the biological CO₂ pump would then approach a new steady state. During interglacial periods, however, a reduction in aeolian fluxes of trace elements could lead to a slight decrease in nitrogen fixation relative to denitrification³⁹ and to a slow readjustment towards higher atmospheric CO₂ levels. A nonlinear climate model, incorporating the effect of changes in the oceanic inventory of fixed nitrogen, has been developed that accounts for the termination of glacial cycles via the outgassing of the greenhouse gas, N₂O, as a consequence of increased denitrification in the oceans⁶³.

The concept that nitrogen limits the net effect of the biological CO₂ pump on geological timescales has been proposed previously^{28,36,63}. It has generally been assumed, however, that the increased flux of fixed nitrogen to the oceans during glacial periods was a consequence of continental weathering rather than an enhancement of nitrogen fixation by increased aeolian fluxes of trace elements. The tendency to overlook nitrogen fixation as a cause of variability in the biological pump apparently results from an historical misunderstanding of the nitrogen cycle in the ocean. Redfield⁴ was aware that the N:P ratio of dissolved inorganic nutrients in the ocean interior was lower than that required for photoautotrophic production; however, he concluded that nitrogen could not be limiting because biological N₂ fixation "is so active that there is no difficulty in assuming that it might serve in adjusting the

phosphorus:nitrogen ratio in the sea". That viewpoint was based on an understanding of nutrient cycles in lacustrine environments⁶⁴, where N₂ fixation is generally stimulated when fixed inorganic nitrogen becomes limiting. Redfield did not anticipate the possibility that, in the oceans, but not lakes, N₂ fixation is often limited by trace metals^{47,48}. Ironically, the concept of phosphorus limitation, which is generally embraced by geochemists (see, for example, ref. 65), cannot be supported by the bulk distribution of either dissolved inorganic N or P relative to the sinking flux of the particulate organic forms of these elements in the contemporary ocean, nor is it consistent with an analysis of the evolution of the nitrogen cycle in the ocean or with the sedimentary records that suggest enhanced biological organic fluxes and apparently lower rates of denitrification in low-latitude regions of the oceans during glacial maxima^{53,54,61,66,67}. Although phosphorus concentrations set an upper bound to carbon fixation, that bound is neither geochemically nor ecologically relevant as long as the sinking flux of N:P in organic matter exceeds that of the upwelling flux of inorganic nutrients, regardless of the relative turnover of the two elements. There is no geological evidence that phosphorus has ever actually limited primary production in the world's oceans.

The present, non-steady-state flux of CO₂ in the atmosphere will ultimately lead to a significant redistribution of carbon in the major reservoirs. The feedbacks induced by this potential forcing remain obscure⁶⁸. The processes described that affect the nitrogen cycle, and its potential influence on the biological pump, are complex. They involve the hydrological cycle as it affects the availability of continental sources of trace elements through desertification, thermal contrasts between the ocean and land as they affect aeolian transport vectors of iron or other trace metals, ocean circulation as it affects stratification and fluxes of phosphate and oxygen, as well as biological processes as they affect the transformation and fluxes of the key elements. At present, biogeochemical models do not adequately represent these interconnected processes, and consequently it is difficult to predict even the sign, let alone the magnitude, of the change in the ratio of nitrogen fixation/denitrification in the coming century. None the less, that this ratio has a key role in establishing atmospheric CO₂ concentrations is becoming increasingly clear^{36,39,53,54}, and cannot be ignored in ocean biogeochemical models^{5,12}. □

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Scour in large braided rivers and the recognition of sequence stratigraphic boundaries

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Alluvial scour into shallow marine sediments may be caused by the incision of a river adjusting to a new base level^{1–4} following a fall in sea level. The identification of such erosion surfaces^{1–3} has therefore been pivotal in the reconstruction of past sea-level changes from ancient sedimentary sequences^{1–14}. Here we report data from a study of the Jamuna river, Bangladesh, one of the world's largest modern braided rivers¹⁵, which illustrate that bed scour associated with channel confluences and bends alone can be substantial—as much as five times greater than the mean channel depth. Indeed, the basal erosion surfaces produced by such deep scours have characteristics similar to those of boundaries in some ancient sedimentary sequences that have been assumed to result from sea-level fall^{1–14}, potentially leading to radically different interpretations of past variation in base level and climate. We suggest that, to discount unambiguously the influence of fluvial scour in ancient sediments, the erosive boundary should be greater than five times the mean channel depth and extend for distances greater than the floodplain width. Ideally, it should be traceable between different basins.

Within ancient sedimentary successions, the interpretation of base-level fall, erosive 'sequence' boundaries and the accumulation of sediments in incised valley fills is commonly assessed using a number of diagnostic criteria^{3,5}. These are diachronous juxtaposition across the erosive sequence boundary of sediments deposited in different environments (such as fluvial sediments lying stratigraphically above shallow marine or shoreface deposits)^{1–3,5,7}; erosional relief on the sequence boundary that is significantly greater than the mean fluvial channel depth^{3–9}; a regionally extensive basal erosional surface^{3,7,9}; the presence of 'interfluvial' sediments, such as palaeosols, that are laterally correlative with the sequence boundary and characterize the valley margins^{3,6,7,10–12}; and an erosional boundary that can be correlated between basins^{1–4}, a criterion which is rarely applied^{8–10}.

Interpretation of some sequence boundaries, however, can be difficult where it is unclear what role is played by channel avulsion (the sudden switching of a river's course) and the prevailing alluvial sediment flux¹³, or by contemporary coastal erosion¹⁴. Additionally, a key factor in recognition of sequence boundaries is the depth of 'autocyclic' scour within alluvial channels which owes its origin to intrinsic channel flow and sediment transport processes¹⁶. Although many studies of ancient sediments have inferred sea-level fall when the depth of incision along the sequence boundary is larger than the estimated mean channel depth^{5,6,8}, such guidelines have not been tested critically with scour and channel change data from large¹⁵ modern rivers. Yet these data are essential both to verify the criteria used in the recognition of sequence boundaries and to provide margins of error for the interpretation of sea-level fall. Reliable scour data from braided rivers, such as those detailed here, may also have especial importance for the recognition of sequence boundaries, as the higher gradients of the alluvial plain generated following sea-level fall can favour formation of multichannel rivers^{5,9}.

One of the world's largest river channel confluences is between the sand-bedded Jamuna and Ganges rivers in Bangladesh. These rivers, which have braidplains up to 15 km wide, have a combined

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