careful to point out, inhibition by siRNAs is effective in mammalian cells, but gene expression is not eliminated completely as it is in *Drosophila* cells. Further, siRNA techniques in mammalian cells have some of the same drawbacks associated with antisense RNA, another technique used to prevent expression of particular genes. In both cases, success depends on the cell type, as well as on the level of expression of the gene to be targeted. That apart, however, RNAi has repeatedly proven itself to be more robust than antisense techniques: it works more often, and typically decreases expression of a gene to lower levels, or eliminates it entirely. And, as Tuschl and colleagues show, even in mammalian cells, siRNAs are effective at concentrations that are several orders of magnitude below the concentrations typically used in antisense experiments.

One of the most important aspects of the new work is the further research it will inspire. Although RNAi works in mouse eggs and embryos[11,12], scientists have been reluctant to invest time in applying it to other mammalian cells because of reported problems[13,14]. Now we will see studies aimed at optimizing the use of siRNAs, as well as at understanding why conventional RNAi, with longer dsRNA, works in eggs and embryos. Might these cells lack the non-specific pathway?

The RNAi technique has had a huge impact in studies of non-mammalian systems. Use of siRNA in mammalian cells could be just as far-reaching, with the applications extending to functional genomics and therapeutics. But various technical issues must be addressed, especially for large-scale applications. For instance, dsRNA can be delivered to *C. elegans* by feeding or soaking, but effective delivery of siRNAs to mammalian cells will not be so simple. The analysis of *C. elegans* phenotypes is aided by short generation times and a wealth of information about the worm's morphology and behaviour; developing rapid ways to screen mammalian cells, or whole organisms, will take some time and thought.

So far I have discussed RNAi as a technique. But of course the pathway does not exist in cells solely to make life easier for scientists. RNAi is a natural biological pathway, albeit one we don't quite understand yet. Especially for those with a long-standing interest in the roles of dsRNA, Tuschl and colleagues' paper is interwoven with information about how RNAi coexists with previously characterized dsRNA pathways. This is especially interesting because dsRNA-binding proteins are usually not sequence specific and will bind any dsRNA. A single dsRNA can interact with proteins of different pathways so that the pathways compete. The different ratios of specific to nonspecific inhibition observed by the authors are probably telling us something about the particular constellation of dsRNA-binding proteins in the different cell types and how they compete with RNAi. Regardless of that, the new study shows that one way dsRNA pathways can coexist is to require different lengths of dsRNA. This is good news for cells — and for researchers. ■

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Chemistry

Synthetic lessons from quinine

Steven M. Weinreb

As the oldest, naturally occurring, treatment for malaria, quinine has been a target for synthetic chemistry for 150 years. At last, modern techniques provide full control over the synthetic molecule.

Nowadays the isolation and structure determination of a new biologically significant, naturally occurring molecule stirs the interest of synthetic chemists as well as biologists. In many cases, such a discovery is quickly followed by a laboratory synthesis. Organic chemists now have the ability and tools to synthesize virtually any large and complex natural product, but there are a few 'classical' compounds that have been revisited by the synthetic community time and time again. One such compound is the alkaloid quinine, chiefly used to treat malaria. In the *Journal of the American Chemical Society*, Gilbert Stork and co-workers[1] show that chemical 'firsts' are still possible — even when dealing with a natural product that has been used in medicine for over 300 years, and which has been a prime target for synthetic organic chemists for 150 years.

The Countess of Chinchon, the consort of the Spanish Viceroy of Peru, first popularized quinine as a treatment for malaria in the early seventeenth century. Natural quinine and related alkaloids are extracted from the bark of *Cinchona* trees. These alkaloids were first isolated in pure form in the early nineteenth century, but the structures of the major compounds were not worked out for another 100 years. Like many drugs, quinine ($C_{20}H_{24}N_2O_2$) can exist in several structural forms. It contains 'asymmetric' carbon atoms, which are connected to four different chemical groups and so allow the overall structure to be arranged in different ways. For quinine, 16 such stereoisomers are possible, but only one corresponds to the active form of the drug (Fig. 1, overleaf).

Because the supply of quinine depended on the political vagaries of the producing countries — especially during wartime — organic chemists soon became interested in synthesis as a way to ensure a constant supply of the drug. Early attempts to synthesize quinine are legendary. In 1856, even before the structure of quinine was elucidated, William Henry Perkin[2] naively attempted to prepare the alkaloid by oxidizing allyltoluidine ($C_{10}H_{13}N$). In the course of this work he inadvertently produced the first synthetic dye, which was the main event responsible for the founding of the organic chemical industry.

When detailed structural information on the *Cinchona* alkaloids became available in the twentieth century, more rational approaches to quinine synthesis were devised. In 1944, two Harvard chemists, R. B. Woodward and William Doering, announced the first formal synthesis of quinine[3], despite the limited synthetic and spectroscopic tools available at that time. Although they did not actually make quinine itself, they succeeded in synthesizing an intermediate compound known as homomeroquinene (Fig. 1). This compound is a degradation product of quinine, and had reportedly been converted back to the alkaloid by Paul Rabe in 1918 (although that account is now in dispute)[1,4]. So Woodward and Doering had developed a synthetic route to quinine insofar as they assumed that Rabe's part of the sequence would work. But their synthesis of homomeroquinene was inefficient, largely because they could not control the spatial arrangement of atoms around the asymmetric carbons. This meant they actually generated several stereoisomers of homomeroquinene, which then

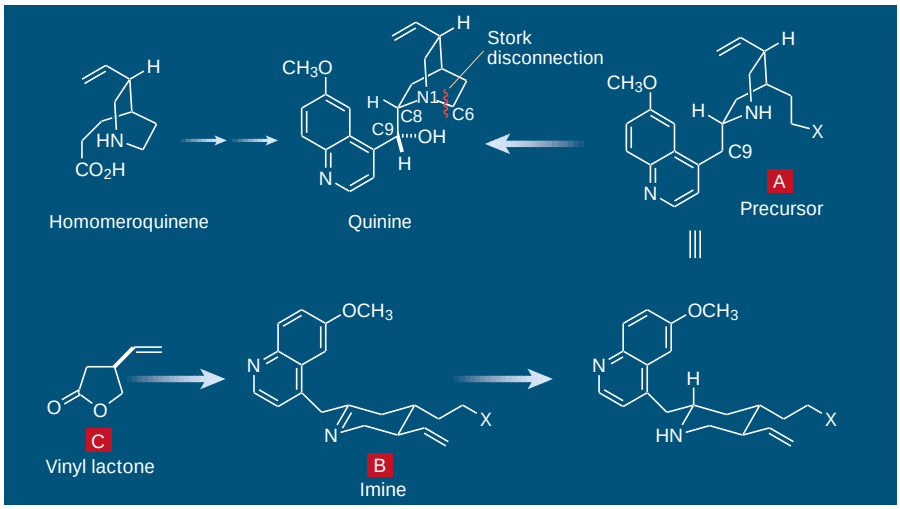

Figure 1 **Routes to synthetic quinine — the oldest known treatment for malaria. A famous wartime synthesis of quinine was claimed by Woodward and Doering[3] in 1944, in response to limited supplies of the natural drug. But they achieved only a partial synthesis of the compound — making the intermediate homomeroquinene and relying on a previously reported sequence for turning this into the final product. Later work[5] produced a full synthesis of quinine, but it could not control the crucial arrangement of atoms around two central carbons (C8 and C9). This meant that several structural variations (stereoisomers) of quinine were produced, which then had to be separated from the active drug. Stork and colleagues[1] have now succeeded in synthesizing quinine by a different route, which involves a more complex intermediate (A), but which yields the correct configuration at C8 and C9.**

had to be put through a tedious separation process.

Researchers got closer to a full stereoselective synthesis of quinine in the 1970s, when Milan Uskoković and co-workers at Hoffmann-La Roche developed a considerably more efficient strategy that controlled most — but still not all — configurations of the asymmetric carbons in the molecule[5,6]. The Uskoković approach was similar to that of Woodward and Doering in the sense that a homomeroquinene derivative was a pivotal intermediate. The Hoffmann-La Roche synthesis produced equal amounts of quinine and its stereoisomer quinidine, which differs from quinine in the arrangement of the bonds around two carbon atoms (C8 and C9 in Fig. 1). Quinidine is also a naturally occurring *Cinchona* alkaloid, and is sometimes used to treat heart conditions.

Normally, organic chemists design the synthesis of a complex molecule by working backwards from the final product in order to come up with intermediates that are simpler and easier to make[7]. In other words, one works in a 'retrosynthetic' direction, conceptually breaking key bonds (disconnection) with the intention of forming those strategic bonds in the laboratory. Before Stork's work[1], quinine syntheses had all been based on a strategy involving the key retrosynthetic disconnection of the bond between C8 and N1, effectively making homomeroquinene the intermediate (Fig. 1). Stork, an acclaimed master of organic synthesis who has worked intermittently on quinine for over 55 years, recognized that disconnecting the C6–N1 bond in the alkaloid could offer complete stereochemical control over the intermediate and final products.

Why was this approach not previously investigated? At first glance, this disconnection produces an intermediate compound (A in Fig. 1) that is almost as complex as quinine itself, and so would be difficult to make. Although this conclusion may have been valid several years ago, by using modern synthetic methodology, Stork has designed a direct and efficient route to A by way of a stereoselective reduction of the imine (B). In turn, B can be prepared in a pure isomeric form in about ten simple steps from the readily available vinyl lactone (C). In the final step, the necessary functionality and stereochemistry is cleanly introduced at C9 by an auto-oxidation.

This last step was based on an important observation previously made by the Uskoković group[5]. None of the steps in the Stork synthesis is completely new, but their arrangement is what makes this an elegant and efficient strategy. In addition, the Stork paper is written with an insight and historical perspective (as well as correcting some myths) rarely seen in the primary chemical literature, and should be required reading for all students of organic chemistry.

Although quinine is still used worldwide to treat malaria, it is slowly being supplanted by newer drugs. Along with the fact that the alkaloid is still readily obtained from natural sources, it is unlikely that total synthesis of quinine will ever provide an economical supply of the drug. Nonetheless, the lessons in synthetic methodology and strategy learned over the years from the many chemists who have worked on quinine have been significant, and it seems unlikely that the Stork synthesis will be the final word in this area. ■

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Carbon cycle

Fertile forest experiments

Eric A. Davidson and Adam I. Hirsch

Long-term experiments under realistic conditions are beginning to deliver data on how forests — or at least some forests — will react to increasing levels of CO_2 in the atmosphere.

The global experiment of increasing atmospheric CO_2 concentrations by burning fossil fuels has neither a control nor replicates. So it is difficult to quantify how much faster the world's forests might be growing under high CO_2 conditions. Higher levels of CO_2 can clearly make plants grow better. But will Earth's vegetation absorb from the atmosphere, and retain, much of the CO_2 pouring out of our exhaust pipes and smoke stacks? If it does, then the threat of global warming from increasing CO_2 would be less severe. Current estimates of annual terrestrial plant uptake of carbon attributable to 'CO_2 fertilization' are within the range $0.5–2.0 \times 10^{15}$ g, which is about 8–33% of annual fossil-fuel emissions[1]. What are needed of course, are controlled, replicated experiments under realistic field conditions to help reduce the uncertainty of these figures.

Papers in this issue by Oren *et al.*[2] and Schlesinger and Lichter[3] describe just that — well-designed studies to test the effects of CO_2 fertilization, in these cases at the scale of a forest stand in the United States. The data