Looking Glass Chemistry

Mirror-image molecules don't always act like twins, so chemists, drug designers, and materials scientists are devising choosy ways to make them

With its molecule-making virtuosity, each cell in a chemist's body, even a bacterium in the gut, serves up a nagging reminder that laboratory chemists are the least skilled members of the living kingdom at assembling atoms into precise spatial arrangements. And nowhere do chemists have more catching up to do than in the arena of so-called chiral molecules—molecules that come in mirror-image, or enantiomeric, structures that are to each other what right hands are to left hands. Try as you might, there is just no way to superimpose these kinds of mirror images. The crucial molecules of life—among them the building blocks of DNA and proteins—are chiral, and life's chemical reactions are famously choosy about their enantiomeric participants. One enantiomer of the compound limonene smells like lemons; the other like oranges. One enantiomer of the drug thalidomide is a sedative; the other produces tragic birth defects. Which is why drug designers, among others, simply have to know one hand from another—and why chemists, with the zeal of underdogs, are striving to match nature's knack for putting forth the correct molecular hand.

What's new, you say; chemists have long recognized the significance of chirality. But acting on this knowledge—that is, obtaining a single-enantiomer drug or other compound—traditionally has required a complex and costly process to isolate one enantiomer from a racemate, the equal mix of enantiomeric twins that laboratory syntheses of chiral molecules almost always produce. The obvious alternative—making a single enantiomer from scratch—has been out of reach for all but a handful of industrially important processes, says Paul J. Reider, executive director of process research at Merck Sharp & Dohme Research Laboratories in Rahway, New Jersey. And yet, as chemist K. Barry Sharpless of the Scripps Research Institute in La Jolla puts it, "More and more, people want to get the perfect reaction"—a cheap, efficient route to one enantiomeric product and not the other one.

Chemists already can carry out a few transformations that fit the bill, but they need dozens more before they can hope to join the enantiochemical big leagues with bacteria and liver cells. Still, as the early fruits of their basic research move into industry, the cost of producing single-enantiomer drugs and other products is dropping. Moreover, many compounds now synthesized as a mixture of enantiomers should become available in enantiomerically pure forms, opening the way to safer and more targeted drugs and agrichemicals, such as herbicides and moth attractants, and less expensive and more abundant flavor and fragrance compounds. And, as a bonus, enantiomeric control also promises to touch fields far from biology. Light and electric fields also "feel" a molecule's handedness, which suggests that the single-handed products of enantiochemistry could lead to new materials for optical and optoelectronic technologies.

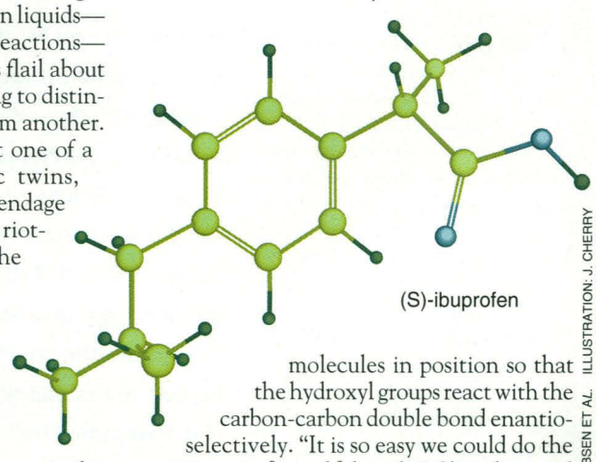
Chemists will have to spend a lot of time in the laboratory before they reach this enantiomeric heaven. To picture the challenge chemists face along the way, think of a chiral molecule like lactic acid, a metabolic product in overworked muscles and certain anaerobic bacteria. In each enantiomer, four chemical appendages—a hydrogen atom, a methyl group (CH_3), a hydroxyl group (OH), and a carboxylate group (COOH)—are all linked to a central carbon atom like fingers attached to the palm of a hand. But their spatial arrangement differs between the enantiomers, like that of the fingers on opposite hands. To make one enantiomer of a particular molecule, chemists have to find a way to attach the appendages in the appropriate arrangement. The problem has been that in liquids—the usual medium for chemical reactions—the precursors of chiral molecules flail about in a spherical blur, leaving nothing to distinguish one part of the molecule from another.

A steady hand. To make just one of a chiral molecule's enantiomeric twins, chemists have to attach each appendage to a specific side of the precursor's riotously moving structure. Unless the precursor is somehow immobilized, success is about as likely as flipping a coin millions upon millions of times and always coming up heads.

Biology manages the feat with enzymes, which are adept at holding their target molecules in a particular orientation because both they and their targets are chiral. Thus, many synthetic chemists are striving to develop catalysts that have the enantioselectivity of an enzyme but are more versatile—able to do the same kind of precise chemistry on a broader range of reactants.

One popular strategy for weighting the enantiomeric coin is to build new catalysts containing so-called chiral inducing agents: chiral compounds, ultimately derived from natural sources, that can endow a catalyst with geometric selectivity. In effect, chemists following this strategy borrow a trick from nature. In so doing, they've managed to boost the odds of synthesizing one enantiomer over another dramatically, for certain reactions. In some cases, the enantiomeric enrichment has reached 95% and more, and chemists are extending such selectivity to more and more reactions.

In the 8 May *Journal of Organic Chemistry*, for example, Sharpless and a team of Scripps co-workers describe their progress toward a reaction for adding a pair of hydroxyl (OH) groups to olefins, a pharmaceutically important class of molecules containing one or more carbon-carbon double bonds, with nearly complete enantioselectivity. At the heart of the process is a pair of yellow powders, which Sharpless says look rather like the powdered drink mix Tang, consisting of an osmium salt, one or the other of two chiral inducing agents derived from the bark of the cinchona bush, and generous helpings of other reactants. When mixed in a solution with olefins, the osmium and cinchona derivatives form chiral catalysts that hold olefin molecules in position so that the hydroxyl groups react with the carbon-carbon double bond enantioselectively. "It is so easy we could do the reaction on [a park] bench," Sharpless said during an outdoor interview.



SOURCE: JACOBSEN ET AL. ILLUSTRATION: J. CHERRY

In spite of that success, Sharpless is the first to admit that it was partly a shot in the dark; mirror-image chemistry, he says, suffers from a dearth of detailed theoretical insight that would guide researchers in their quest for new enantioselective catalysts. "We can't

Government Smiles on One-Handed Drugs

When one mirror-image form, or enantiomer, of a drug can cure what ails you and the other is at best useless baggage, drug companies have plenty of financial incentive to find ways to produce just the active form. But both the Food and Drug Administration (FDA) and the Patent Office are adding a little extra inducement to move enantioselective chemistry along a little faster.

"They have yet to say so explicitly, but there is no question that the FDA viewpoint is, 'If you do not make a new drug in a single enantiomer form, you had better have a very good excuse,'" says John Scott, a research manager for Hoffmann-LaRoche. In fact, the agency expects to finalize in the next few months a policy statement or at least a set of industry guidelines that will officially make single enantiomer drug development the standard, according to Charles Kumkumian, assistant director in the chemistry division of the FDA's office of drug evaluation. The FDA's counterparts around the world are now debating similar measures in an effort to "harmonize" the drug approval process globally, he adds.

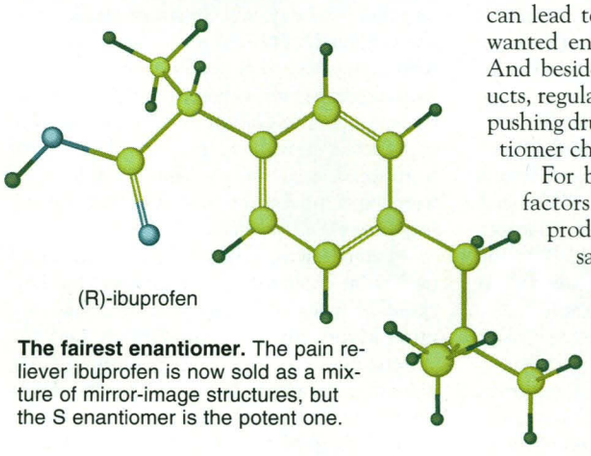
Besides the stick wielded by the FDA, there's also a carrot offered by the Patent Office. A drug company can patent a single-enantiomer form of an existing racemic (mixed-enantiomer) compound as an entirely new drug, providing the company can show that its single enantiomer candidate actually confers some kind of therapeutic benefit such as increased efficacy or potency. For the small biotechnology company Sepracor Inc. of Marlborough, Massachusetts, which specializes in enantiochemistry, that provision opens a tempting niche. Sepracor president Timothy Barberich says his company's aggressive legal experts have already filed 40 patent applications for single-enantiomer forms of existing and new drugs. And on 15 April, the company was issued a patent for the use of S-fluoxetine, a single-enantiomer form of Prozac, the leading antidepression drug, now sold as a racemic mixture of fluoxetine enantiomers by Eli Lilly & Company. When Lilly's patent for racemic fluoxetine expires in 2001, Sepracor and its licensees could have exclusive market control of the enantiomeric fluoxetine until nearly 2010, Barberich says.

And when it comes to developing entirely new drugs, it's single enantiomers all the way. Says David Coffen, director of chemistry research at Hoffmann-LaRoche, "It will be a rare and vanishing situation where a racemate is brought to the market."

–I.A.

rationally predict things," concurs long-time enantiochemist Albert Myers of Colorado State University. So this month Sharpless is embarking on a trial-and-error search for new chiral-selective reactions. He expects to switch on a robotic system that will systematically mix and match metal compounds, such as the osmium salts, with different chiral inducing groups, all under varying temperature and pH. "This is brute force Edisonianism," he admits.

But automated reaction testing like Sharpless' makes sense, says Eric Jacobsen of the University of Illinois, because "it's so easy to miss an important result." A few degrees could mean the difference between a run-of-the-mill racemic mixture and the prize: an enantiomerically pure product. Jacobsen has had a little luck himself, he says. Three years ago, he and his colleagues happened onto manganese-based asymmetric catalysts that give a chiral spin to an important drug-building reaction known as epoxidation. "We found this asymmetric reaction that brought enormous attention to us," Jacobsen says. "It got me every award you can imagine and tenure in 3 years."



The fairest enantiomer. The pain reliever ibuprofen is now sold as a mixture of mirror-image structures, but the S enantiomer is the potent one.

Such basic research is finding an appreciative audience in the pharmaceutical business. There's ample incentive for synthesizing drugs with a single handedness, Merck's Reider notes. "Although the vast majority of drugs are sold as racemates, there is a growing sense in drug companies that these are only 50% pure." Underlying that conviction is the fact that only one enantiomer of many drugs does the intended therapeutic job. Though the other enantiomer sometimes enhances the drug's action, more often it's useless ballast that, set loose in a patient's body, can lead to side effects. At worst, the unwanted enantiomer can be outright toxic. And besides the potential for better products, regulatory and legal incentives are also pushing drug companies toward single-enantiomer chemistry (see box).

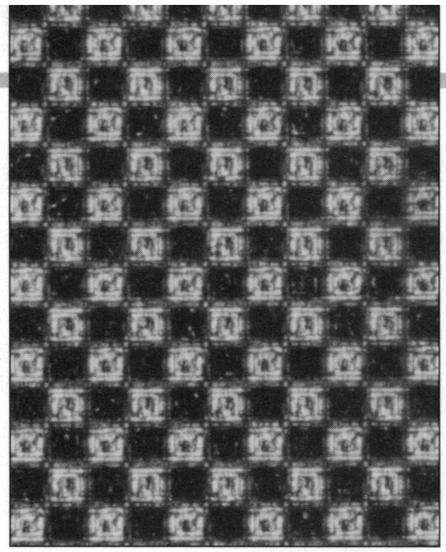
For big companies like Merck, those factors are making single-enantiomer products a major focus of their plans, says Reider. And one small biotech company is trying to enter the pharmaceutical big leagues by specializing in enantiomerically pure products. In the mid-1980s, Sepracor Inc., based in Marlborough, Massachusetts, recognized the stumbling block presented by the difficulty and expense of separating subtly different enantiomers, says Robert Bratzler, general manager of the company's chiral chemistry division. "Our idea was to overcome that impediment."

The company started out 8 years ago by developing enzyme-laced membranes that convert conventionally synthesized racemic mixtures into enantiomerically pure chemical intermediates—the building blocks for single enantiomer drugs and other products. But now Sepracor is also pursuing "an inherently more efficient" strategy, Bratzler says. Building on work by Sharpless, Jacobsen, and many others, he says, "We are developing [in-house] and acquiring technology to synthesize the one enantiomer directly."

The pharmaceutical industry isn't the only arena where single-enantiomer chemistry is stirring excitement. Enantiochemist John Brown of Oxford University thinks that enantiomers, the first hints of which emerged in the early 19th century due to their ability to rotate the plane of polarized light, will spawn a whole realm of applications in optolectronics and photonics.

Enantiomers on display. David M. Walba of the University of Colorado and his colleagues are already giving substance to Brown's prediction. Using enantioselective reactions, they have synthesized a variety of chiral linear organic molecules that, in solution, form so-called ferroelectric liquid crystals—the same kind of material that is responsible for the display of a digital watch or a laptop computer. Under the influence of an electric field, the molecules in a liquid crystal line up, affecting the passage of light and thereby creating the light and dark spots of the display. By creating the liquid crystal molecules as single enantiomers, Walba's team has gained additional control over how they stack to form layers of liquid crystal. The payoff: materials with greater sensitivity to electric fields, which could open the way to higher resolution displays and more efficient and faster-switching optical transistors, light valves, and other photonic components.

Displaytech Inc., a company Walba and



DISPLAYTECH INC.

Chirality on display. A single enantiomer liquid crystal is sensitive enough to read out the 1s and 0s on a computer memory chip.

his colleagues founded in 1986 to develop their materials, has already demonstrated some of that promise. Displaytech's integrated circuit-ferroelectric liquid crystal (IC-FLC), still at the experimental stage, can display pixels just 60 microns on side. "This device is basically a RAM [random access memory chip] with FLC on top," Walba says. "The 1s and 0s written to the RAM by a computer are turned into bright and dark pixels by the FLC."

The growing finesse at making single enantiomers of chiral molecules is also fueling basic research, some of which is coming full circle to the original impetus for enantiochemistry: life's own chiral nature. In an audacious extension of mirror-image chemistry, peptide chemists have been working on making mirror-image forms of naturally occurring proteins and enzymes. They expect that their synthetic enantiomeric biomolecules will catalyze the same reactions as the originals but on the enantiomerically opposite substrates. A question emerges from such work: Might there exist somewhere now, or in the future, inverse biological organisms whose molecular dance of life twists the other way and whose sentient forms greet each other with a shake of left hands?

–Ivan Amato

AIDS

HIV Comes in Five Family Groups

Virologist Gerald Myers looks at the rapidly mutating AIDS virus and sees Darwinian evolution on "fast-forward." So divergent have strains of the virus become that researchers have all but abandoned hope of developing a single vaccine effective against all of them. So, taking a cue from flu vaccine research, Myers has spent the last several years cataloguing known strains of HIV, to determine whether it might be feasible to concoct multiple-strain, cocktail vaccines like those used in flu.

The effort has yielded two surprises. While flu comes in three major families, Myers, director of the HIV sequencing project at Los Alamos National Laboratory, has found that strains of HIV seem to cluster in at least five—and possibly more—distinct families, not just the broad Western and African groupings that researchers have accepted for nearly a decade. "It would be wrong of us to think there are only two types of virus to be confronted by vaccines and antivirals," Myers told geneticists recently at a symposium at Stanford University.

Just as unexpected is Myers' finding that HIV isolates from Gabon seem to fit into all five groups he has identified. This, he told the Stanford symposium, suggests that West-Central Africa—and particularly Gabon—may be the "epicenter or source of AIDS in the world." That conclusion is drawing flak even though Myers' data have not yet been published.

Myers and his team of Los Alamos researchers began collecting sequences of HIV genes in GenBank computers in the mid-1980s, when the sequences first became available. Myers and his co-workers have worked out a genetic family tree with multiple branches for the predominant form of the AIDS virus, HIV-1. The computer analysis traces genetic changes in the *gag* and *env* genes, showing which of the strains are most closely related and likely to have emerged from common ancestors.

Late last year, Myers said, the research team realized that HIV-1 viruses cluster into recognizable "families." Within each family, the AIDS viruses differ from one another by only 10% to 20%—but the five families of viruses differ from one another by at least 30%. Strains from the United States and Europe, among others, fall into the first group; Brazilian and Zairian strains in the second;

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>
> **–Gerald Myers**

Zambian and Somalian strains in the third; Taiwanese strains in the fourth; and strains from Uganda, the Ivory Coast, and Kenya fall in the fifth.

Virologist Howard Temin of the University of Wisconsin, who has followed Myers' research, says these findings are an important contribution to vaccine research. "We need to know what strains are out there to know what vaccines to make," Temin said. If strains of the virus can, indeed, be grouped into broad families, researchers may be able to test candidate vaccines against reference strains from each grouping.

But, despite Nobelist Temin's endorsement, the suggestion that HIV-1 may have originated in Gabon threatens to rekindle an acrimonious, decade-old debate over the origin of the epidemic. Western scientists' contentions that AIDS originated in Africa have repeatedly drawn accusations of racism from African government officials and scientists, and Myers' new claim is already prompting an outcry. And the arguments of the skeptics are not based purely on political correctness.

Most epidemiologists would agree that Gabon, an equatorial country on the Atlantic Coast of Africa, would seem an unlikely place for the epidemic to have started. It has one of the lowest AIDS infection rates among African nations, with about 1.8% of its 1.2 million population infected with the virus. It is bordered on the north by Guinea and Camaroon, with infection rates of less than 5%. But to the east, Gabon is bordered by the Congo, which has an infection rate of 7%. Countries such as Uganda, Zaire, Kenya, and the Ivory Coast have infection rates ranging from 7% to 28% in urban areas, according to U.S. Bureau of the Census statistics. No surprise, then, that Guy Eboumy, a Gabonese diplomat in Washington, challenged Myers' claim, arguing that the AIDS toll in his country does not support the theory. "If the epidemic began in Gabon, I would expect more people to be infected."

The diplomat is supported by AIDS researcher Jay Levy, a professor of medicine at the University of California, San Francisco, who argues that it is unlikely that the virus would spread from Gabon to the Congo without spreading in Gabon first. "Of course, social factors may have prevented the virus from spreading locally so that the infections were exported, but I would find that surprising," Levy told *Science*.

Nevertheless, Chin-Yih Ou, the chief molecular biologist at the Centers for Disease Control's AIDS division, who has supplied Myers with African viruses, said the genetic evidence and the computer analysis, at least so far, appear to back up Myers' claims on the groupings of the virus and on the anomalous position of the Gabon strains. However, he adds a strong note of caution: "We need to sequence more viruses to confirm his findings."

–Steve Sternberg

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