

out of airway cells, causing water to flow out and so thinning the airway's lining of mucus (Fig. 1). When CFTR is absent or inactive, as in people who have cystic fibrosis, the mucus thickens and accumulates, causing airway obstruction and repeated infections and inflammation¹⁴. Determining which lung cells express *CFTR* and are directly affected in people with cystic fibrosis has been difficult because expression of this gene seems to be complex and variable along the airways¹⁵. The current papers demonstrate that the gene's expression is not as random as it had seemed: the bulk of the *CFTR* mRNA detected was from the rare pulmonary ionocytes, each of which highly express the gene. People with cystic fibrosis can also experience gastrointestinal symptoms and infertility. Perhaps *CFTR*-expressing ionocytes will be discovered in organs involved in these problems, too.

Mice harbouring mutations in *Cftr* do not develop cystic fibrosis — a curious fact that has long hampered research into the disease. Montoro *et al.* found that cultured airway epithelial cells generated from *Foxl1*-mutant mice have low *Cftr* expression but, paradoxically, higher than normal *Cftr* activity. Differences in pulmonary ionocytes between mice and humans, such as compensatory expression of another chloride channel when *Cftr* expression is lost in mice, might explain both this paradox and why *Cftr*-mutant mice do not model the disease.

Although these results suggest that ionocytes have a key role in airway biology and cystic fibrosis, much work is still needed to define their physiological functions, the

role of CFTR in these functions, and how loss of CFTR causes or contributes to disease symptoms. Developing methods to genetically and pharmacologically manipulate ionocytes or replace them in model systems, and ultimately in patients, is another priority.

These papers provide excellent examples of how scRNAseq can transform long-established views of a tissue and a human disease. As scRNAseq tools improve and costs continue to drop, we will probably soon witness something similar for many human organs and diseases. ■

Kyle J. Travaglini and Mark A. Krasnow are in the Department of Biochemistry and Howard Hughes Medical Institute, Stanford, California 94305, USA.
e-mail: krasnow@stanford.edu

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This article was published online on 1 August 2018.

ORGANIC CHEMISTRY

A practical route to 3D molecular diversity

Cycloaddition reactions are powerful tools for synthesizing three-dimensional molecules, but their scope has been limited. A creative solution to this problem opens up opportunities for drug discovery. [SEE LETTER P.350](#)

WENBO YE & ANG LI

Reactions known as cycloadditions are unparalleled in their ability to construct ring-containing molecules in a way that precisely controls the geometric arrangement of groups attached to the carbon atoms in the molecules — that is, the reactions offer great stereoselectivity. The power of these reactions has been demonstrated in numerous syntheses of complex natural products^{1,2}. However, the scope of cycloadditions is limited to certain combinations of starting materials, which has restricted their use

for making libraries of compounds in drug-discovery programs³. On page 350, Chen *et al.*⁴ report a strategy that combines cycloadditions with another type of reaction, known as carbon–carbon cross coupling, to enable the modular and programmable preparation of cycloaddition-derived molecules.

Carbon–carbon (C–C) cross-coupling reactions are often used to form bonds between carbon atoms that are already part of a carbon–carbon double bond; such carbon atoms are said to have *sp*² orbital hybridization. Cross couplings between *sp*² carbons lend themselves to the modular synthetic routes

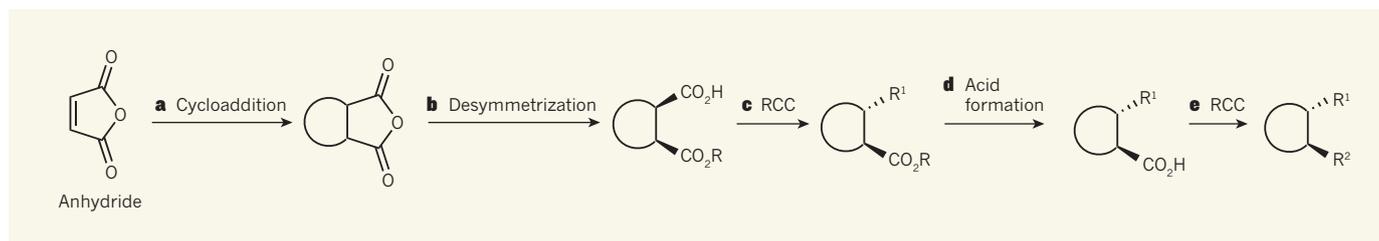


Figure 1 | A powerful strategy for making 3D molecules. Chen *et al.*⁴ report a practically simple procedure that allows access to products that cannot be made easily and directly using reactions known as cycloadditions. **a**, An anhydride starting material undergoes a cycloaddition to form a 3D scaffold. The ring represents several different ring structures. **b**, A desymmetrization reaction then forms an intermediate that contains a carboxylic acid (CO₂H), in which

the absolute stereochemistry (the geometric arrangement of groups) is fixed. Solid wedge bonds project above the plane of the page; R is methyl, benzyl or CH₂CH₂Si(CH₃)₃, and Si is silicon. **c**, The acid is replaced by a molecular appendage (R¹) in a radical cross-coupling (RCC) reaction. Broken wedges project below the plane of the page. **d**, e, A second acid group is generated (**d**), and is converted to a different appendage (R²) in another RCC reaction (**e**).

used to make compound libraries. This has resulted in the predominance of *sp*²-rich structures — which tend to be two-dimensional — in compounds tested for drug discovery. But 3D molecular structures can interact with biological targets in different ways from 2D ones, so are just as important for drug discovery. Three-dimensional molecules tend to be rich in *sp*³ carbons, which have a different orbital hybridization from *sp*² carbons, and have the capacity to form four single bonds.

A cycloaddition known as the Diels–Alder reaction provides one of the most efficient means of building *sp*³-rich ring systems. However, to achieve high reaction yields, stereoselectivity and regioselectivity (a preference to react at particular atoms) in Diels–Alder reactions, the electronic properties of the reactants should match. For example, in conventional Diels–Alder reactions, one of the reactants (known as a diene) should be electron-rich, whereas the other (the dienophile) should be electron-poor. This drastically reduces the number and diversity of Diels–Alder products that can be made. Chemistry students excitedly studying Diels–Alder reactions are often frustrated when they realize the restrictions involved. The constraints also prevent these reactions from being used in modular synthetic routes. Diels–Alder reactions have therefore been used much less often for drug discovery than have cross-coupling reactions³, and so their advantages for controlling the stereochemistry (the geometric arrangement of groups) of ring-containing molecules have not been fully exploited by medicinal chemists.

Workers from the same group as Chen *et al.* previously developed a type of cross-coupling reaction known as radical cross-coupling (RCC)^{5–9}. This is a useful tool for converting carboxylic acids (compounds that contain CO₂H groups) into products that contain alkyl, alkenyl, alkynyl or aryl groups (hydrocarbon groups that represent all the possible bonding geometries of carbon atoms). In the current paper, Chen and colleagues use carboxylic acids as a link that allows them to combine RCC and Diels–Alder reactions. The connection can be made because compounds known as anhydrides and esters serve as electronically

favourable dienophiles in Diels–Alder reactions, and can then be converted into acids to take part in various RCC reactions.

On the basis of this strategy, the authors devised a simple, modular, five-step sequence to generate molecules that have 3D structural complexity (Fig. 1). In the first step, a Diels–Alder reaction involving an anhydride or ester builds a 3D molecular scaffold. This is followed by a ‘desymmetrization’ reaction¹⁰, which generates a carboxylic acid and sets the absolute stereochemistry in the resulting product. In the third step, an RCC reaction replaces the acid group with a molecular appendage, producing an intermediate that is then hydrolysed to generate a second carboxylic acid. This is used in the final step: another RCC reaction, which introduces a second appendage.

The two RCC steps allow Diels–Alder-type products to be made that couldn’t be synthesized directly in a Diels–Alder reaction because the starting materials would have mismatched electronic properties. Another remarkable feature of this sequence is the clever mechanism of stereocontrol: the first appendage is attached to the molecular scaffold at an orientation that is governed by a nearby group produced during the desymmetrization reaction, and the orientation of the second appendage is governed by the orientation of the first. The final product therefore comprises mostly one isomer in which the two appendages are fixed in what is known as a *trans* orientation to each other.

Chen *et al.* went on to extend this chemistry from Diels–Alder reactions to three other types of cycloaddition reaction that construct rings formed of three, four or five atoms. Moreover, one of the RCC steps could be replaced with a reaction that allowed the formation of a carbon–nitrogen bond, rather than a C–C bond. The researchers suggest that bonds from carbon to other types of atom could also be made, to produce an even more structurally diverse set of final products.

The authors showcase their chemistry by using it to make natural products, pharmaceuticals and key intermediates used in the synthesis of such compounds. An example is the antipsychotic drug asenapine, which is usually manufactured as a mixture of two

mirror-image isomers (enantiomers). One of these is more effective as a drug than the other, but is difficult to synthesize as a single enantiomer. Starting from a symmetrical anhydride molecule, Chen *et al.* show that their strategy can be used to make this enantiomer in a practically simple, short synthetic route and in good overall yield, offering several advantages over the previously reported synthesis¹¹.

Chen and colleagues’ chemistry is easy to carry out in the laboratory. Our frustrated chemistry students can therefore immediately start to synthesize compounds that fall outside the conventional scope of Diels–Alder reactions described in their textbooks. All they have to do is to collect the anhydrides reported by the authors, the organic catalyst needed for the desymmetrization, the metal catalysts and the activating reagent used for RCC, and various other commercially available molecular building blocks (such as boronic acids). Some of these items will be used in every reaction sequence, and so the task would become even easier if a chemical-supplies company could market a kit that contained these items.

Chen and colleagues’ work will redefine how chemists think about synthesizing Diels–Alder-type products, and will find numerous applications for synthesizing molecules rich in *sp*³ carbons for drug discovery. The remaining challenges mainly concern the limitations of RCC. For example, the method can currently produce only a *trans* arrangement of the two appendages introduced in the RCC steps; to make the other arrangement, the stereochemical outcome of the second RCC reaction would need to be changed. If such issues can be addressed, then Chen and colleagues’ reactions will become even more powerful tools for synthesis than they already are. ■

Wenbo Ye and Ang Li are at the State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China. e-mail: ali@sioc.ac.cn

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50 Years Ago

Whatever advantages the distant future may offer from the manned exploration of space, it is clear that the present benefits of the space race are few and far between ... A fortnight's conference is being held ... under the title "Space Science and Technology —Benefits to Developing Countries". The introductory pamphlet ... describes in a style of sustained optimism the cornucopia of technological blessings which future satellite systems will rain down on the poor and rich alike. In the near future, reflector satellites will shed light on the night earth, the pamphlet says, and "by providing illumination for construction, lumbering, fishing and other outdoor industries, could conceivably have an important effect on the economic growth of the developing nations". The pamphlet does not discuss the catastrophic effect of such a satellite on biological rhythms, nor does it explain in what manner night-time fishing and lumbering will boost any nation's economy.

From *Nature* 17 August 1968

100 Years Ago

The entrance of the United States of America into the war has prompted Mr. A. Hansen to write to *Science* pointing out that the States possess no national floral emblem. France has its fleur-de-lis, England the rose, Scotland the thistle, but America has no flower with which it is associated in people's minds. Mr. Hansen points out the various characteristics required for a national flower, and comes to the conclusion that the columbine, which is in flower from April to July, is probably the most suitable for the purpose. The correspondence of the generic name *Aquilegia* with the Latin name of the eagle is also considered to be a point in its favour.

From *Nature* 15 August 1918

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This article was published online on 6 August 2018.

PARTICLE PHYSICS

Special relativity validated by neutrinos

Neutrinos are tiny, ghost-like particles that habitually change identity. A measurement of the rate of change in high-energy neutrinos racing through Earth provides a record-breaking test of Einstein's special theory of relativity.

MATTHEW MEWES

The existence of extremely light, electrically neutral particles called neutrinos was first postulated in 1930 to explain an apparent violation of energy conservation in the decays of certain unstable atomic nuclei. Writing in *Nature Physics*, the IceCube Collaboration¹ now uses neutrinos seen in the world's largest particle detector to scrutinize another cornerstone of physics: Lorentz invariance. This principle states that the laws of physics are independent of the speed and orientation of the experimenter's frame of reference, and serves as the mathematical foundation for Albert Einstein's special theory of relativity. Scouring their data for signs of broken Lorentz invariance, the authors carry out one of the most stringent tests of special relativity so far, and demonstrate how the peculiarities of neutrinos can be used to probe the foundations of modern physics.

Physicists generally assume that Lorentz invariance holds exactly. However, in the late 1990s, the principle began to be systematically challenged², largely because of the possibility that it was broken slightly in proposed theories of fundamental physics, such as string theory³. Over the past two decades, researchers have tested Lorentz invariance in objects ranging from photons to the Moon⁴.

The IceCube Collaboration instead tested the principle using neutrinos. Neutrinos interact with matter through the weak force — one of the four fundamental forces of nature. The influence of the weak force is limited to minute distances. As a result, interactions between neutrinos and matter are extremely improbable, and a neutrino can easily traverse through the entire Earth unimpeded. This poses a challenge for physicists trying to study these elusive particles, because almost every neutrino will simply pass through any detector completely unnoticed.

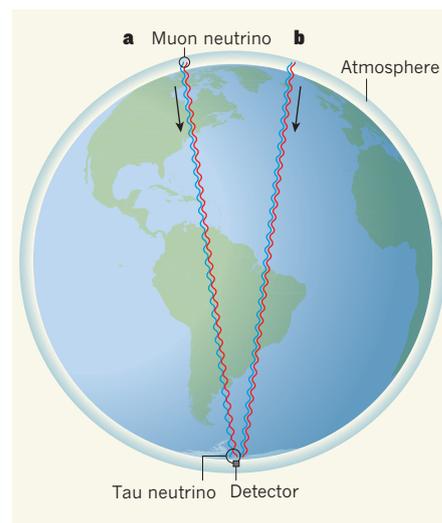


Figure 1 | Propagation of neutrinos through Earth. There are three known types of neutrino: electron, muon and tau. **a**, A muon neutrino produced in Earth's atmosphere can be thought of as the combination of two quantum-mechanical waves (red and blue) that are in phase — the peaks of the waves are observed at the same time. If a principle known as Lorentz invariance were violated, these waves could travel at different speeds through Earth's interior and be detected in the out-of-phase tau-neutrino state. **b**, The IceCube Collaboration¹ reports no evidence of such conversion, constraining the extent to which Lorentz invariance could be violated.

The IceCube Neutrino Observatory, located at the South Pole, remedies this problem by monitoring an immense target volume to glimpse the exceedingly rare interactions. At the heart of the detector are more than 5,000 light sensors, which are focused on 1 cubic kilometre (1 billion tonnes) of ice. The sensors constantly look for the telltale flashes of light that are produced when a neutrino collides with a particle in the ice.