chaperoning, while for others such interactions may be unproductive[3]. To make things more complex, the unfolded N-terminal domain of sHSPs can also bind substrates and contain alternative IXI motifs that compete for binding to the β4/β8 groove[4]. For example, in a recent report that uses different substrates, Alderson et al.[5] have shown that a disease-related mutation in the C-terminal IXI motif of HSP27 weakens the affinity for its own ACD, but leads to decreased (rather than increased) chaperone activity and more efficient recruitment of IXI-containing co-chaperones. The picture that emerges is a complex energy landscape that is sensitive to multiple contributing factors that have to be tuned 'just right' for different substrates. Such a scenario is not limited to sHSPs but seems to be a general theme in chaperones involved in anti-aggregation[6].

Even though native chemical ligation has been widely used to explore the aggregation and function of proteins, the work presented by Pratt and co-workers, provides a striking example of the use of this approach to unveil how the activity of a chaperone can be fine-tuned by O-GlycNAcylation at a specific site. While their study focuses on a single post-translational modification, sHSPs, like other proteins, are likely to contain several different modifications that occur simultaneously, and these combinations may enhance or perturb their activity in complex ways. In such cases, methods that exploit the powers of chemical approaches such as native chemical ligation or other strategies such as covalent tethering[7], are proving to be invaluable tools for understanding complex biological mechanisms such as protein self-assembly.

The exciting results presented by Pratt and co-workers[1] suggest that the enzymes responsible for adding O-GlcNAc to proteins (O-GlcNAc transferase), or for removing O-GlcNAc (glycosidase O-GlcNAcase) may be good targets for preventative strategies for amyloid diseases. However, before marching ahead, a key question remains unresolved: What is the fate of the aggregation-prone substrate after interaction with the chaperone? Pratt and co-workers report that interaction of α-synuclein and Aβ42 with O-GlyNAcylated sHSPs results in the formation of different aggregates, including short fibrils or amorphous aggregates. Whether formation of these products is protective or enhances amyloid-associated cytotoxicity remains an open question. Indeed, previous results have shown that amyloid toxicity and cell-to-cell spreading depends on fibril length[8]. Nevertheless, the finding that O-GlyNAcylation enhances chaperone activity in vitro and possibly also in the brain, opens the way for new therapeutic strategies to combat disease by targeting chaperones in a substrate-specific manner. ❐

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Competing interests
The authors declare no competing interests.

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AUTOMATED SYNTHESIS

Solid potential

Small-molecule drug discovery and development is limited by the ability of chemists to readily synthesize and purify new compounds with suitable chemical diversity. Now, a new twist on solid-phase chemical synthesis has enabled rapid and simplified synthesis of pharmaceutically relevant small molecules.

Mark S. Kerr and Kevin P. Cole

The discovery and development of new small-molecule therapeutic agents is a time-consuming endeavor that requires vast resources[1]. Traditional structure–activity relationship studies often feature late-stage diversification of a common core structure, often made in bulk to facilitate these efforts. Although this allows chemists to focus on the synthetic endgame and generate large numbers of compounds, it can reduce molecular diversity with respect to the core structure.

Once a new compound of interest is identified, it is imperative for the drug discovery team to have rapid access to gram quantities of the substrate in order to probe its pharmaceutical qualities and begin in vivo studies. The fields of synthetic peptides[2] and oligonucleotides[3] have long been dominated by solid-phase synthesis techniques, which provide advantages in terms of throughput, reduced impurity generation and ease of synthesis. Although elegant methods for the synthesis of small-molecule natural products on solid supports have also been reported[4], they have received considerably less attention than their biopolymeric counterparts. Now, writing in *Nature Chemistry*, a team in Singapore led by Saif Khan and Jie Wu have developed a method of rapidly generating small-molecule drug candidates de novo using a combination of solid-supported synthesis and automated continuous-flow techniques[5]. The test case chosen to demonstrate the potential of this method was the synthesis of CHK1 and CHK2 kinase inhibitor prexasertib[6] (Fig. 1a).

A system was constructed that features a column containing a polystyrene resin to which the substrate is attached. Reagent solutions were flowed through the bed to promote the desired chemical transformations; the system can be heated or cooled to adjust the reaction conditions.

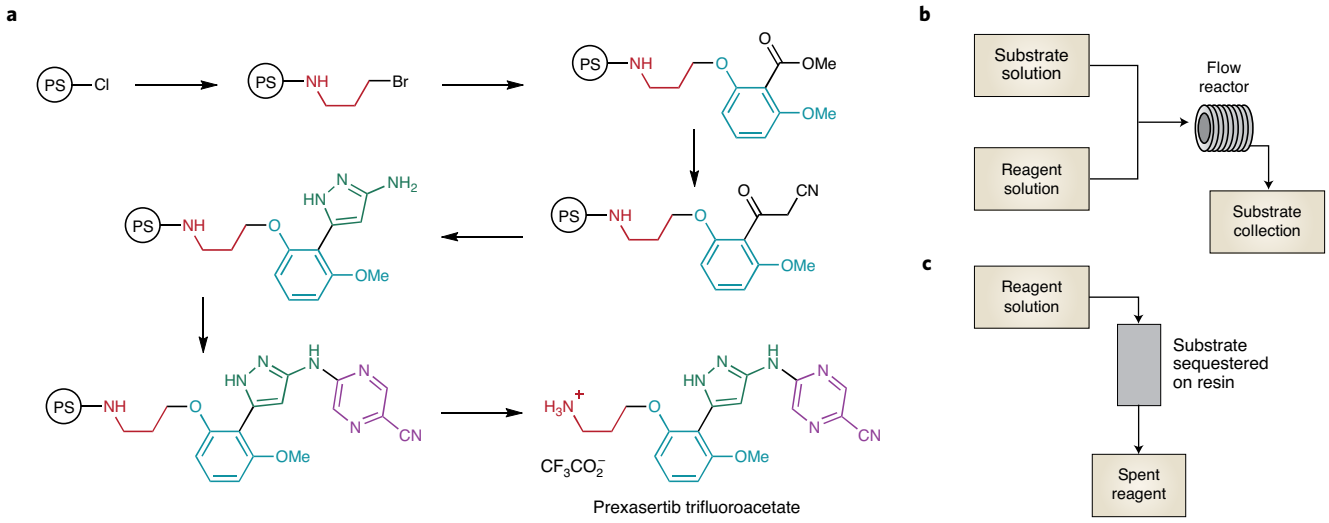

Fig. 1 | Solid-supported synthesis of prexasertib. a, Sequential functionalization of the resin in the continuous-flow system results in a growing target structure. Different colours illustrate the regions of the molecule that can readily be altered using this approach. PS, 2-chlorotrityl-functionalized polystyrene resin. **b**, Generalized schematic of a traditional flow chemistry setup, note that substrate flows in to and out of the reactor. **c**, Schematic of approach used in this work, note that the resin-bound substrate is sequestered in the reactor while reagents flow past.

The resin-bound substrate grows in both size and complexity as sequential transformations are conducted. While the "catch-and-release" approach is well known[7], the present approach conducts the entire synthesis on solid support with the control of computer automation. This differs from traditional flow chemistry, where the dissolved substrate flows through a series of reactors that can be connected to allow multiple unit operations to occur simultaneously. Here, the sequestered resin accommodates a single unit operation at a time (Fig. 1b,c).

To develop the fully automated synthetic process for prexasertib and its analogues, a three-phase approach was adopted. First, solution-phase proof-of-concept batch experiments using a 2-chlorotrityl protecting group were conducted to ensure the viability of the chemistry. Next, flask-based trials were run using the substrate loaded onto the resin. Finally, when the main reaction parameters — such as temperature, stoichiometry and exposure time — were determined, a resin column was then connected to the supporting infrastructure of tubing, pumps, switching valves, reagent vessels and computerized control system for the automated run. After the initial method development for prexasertib, each new analogue required only a brief study of the reaction conditions pertinent to the point of chemical diversification prior to the automated synthesis production run. Upon completion of the synthesis, the product is cleaved from the resin by treatment with trifluoroacetic acid (TFA) and a terminal purification by chromatography or crystallization was performed.

There are several intriguing potential advantages to this method. From a drug discovery perspective, once the system is established, there is no penalty to synthetic modifications early in the route, which should enable greater structural diversification during structure–activity relationship studies in medicinal chemistry. Instead of repeatedly running similar reactions to produce analogues, the chemist's time can be spent designing better molecules based on fresh assay data while the machine performs the more tedious synthetic tasks. In biopolymer chemistry, the repetitive sequence of monomer coupling and deprotection can lead to deletion impurities — where a desired monomer unit is absent — which are difficult to remove in the terminal purification. Although analogous impurities may exist with this method, the functional-group diversity is much greater, making propagation of the mistake less likely and rejection of the impurity more likely. Intermediate physical properties (such as poor solubility) are not a concern because crystallization is prevented by the resin. Intermediate purification is obviated, reducing potential loss in yield while saving both time and solvents. Logistically speaking, technology transfer between different laboratories should be greatly facilitated by the method's digital format. Unlike some flow chemistry platforms, this system should be universal and require no reconfiguration aside from changing feed solutions. The system also has a small footprint, requiring only half of a standard benchtop fume hood. Finally, it is noteworthy that the system successfully executes the automated synthesis without human intervention, as shown in Supplementary Video 1 (ref. [5]).

There are some aspects of this work that future research should help improve. Although the substrate loading is high (2 g of resin gave 635 mg of prexasertib TFA salt), the resins themselves are rather expensive. This may hinder the profitability of scaling up the method for small molecules, but resin costs could be driven down by increased demand or by recycling of the solid phase. And although an impressive array of reaction conditions were demonstrated, it is currently unclear whether conditions such as those required for liquid/liquid biphasic reactions, Pd-catalysed cross-couplings, or heterogeneously catalysed hydrogenation can be accommodated. It also appears that the up-front work required to set the system up for a particular reaction sequence is substantial, but this should decrease over time as the protocols become routine. Finally, analysis of the growing product during production may be challenging; Khan, Wu and co-workers demonstrate the use of infrared spectroscopy to characterize the on-resin product, which may also be useful for reaction monitoring.

Nevertheless, a six-step prexasertib synthesis was achieved in a remarkable 65%

overall isolated yield with a final purity of >99.9%. In addition, 23 prexasertib analogues were synthesized in overall yields of 13–70%, each requiring only 22–36 hours of synthesis time. These analogues display both early and late-stage diversification, showcasing the ability to readily alter any portion of a molecule to explore structure–activity relationships. The approach appears to be broadly applicable; Khan, Wu and co-workers estimate that 73% of the top 200 bestselling small-molecule drugs may be produced by this technique. Given this exciting potential, further improvements are expected that will help popularize and expand this approach. ❐

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Competing interests

The authors declare no competing interests.

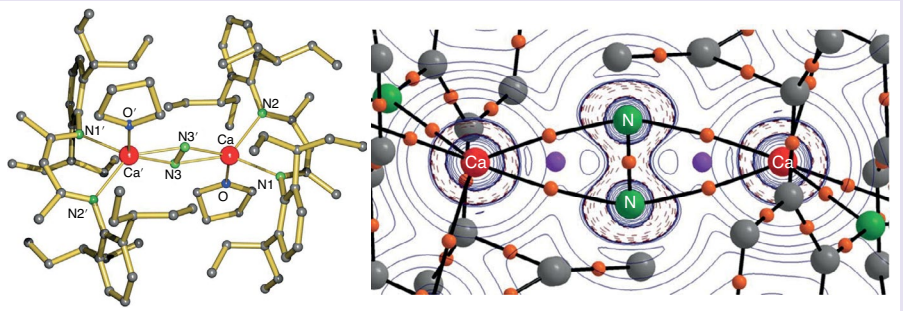
INORGANIC SYNTHESIS

Caught in a trap

Dinitrogen (N_2) — the most abundant gas in the Earth's atmosphere — is considered chemically inert, owing to the strength of its N≡N triple bond. Now, during the attempted synthesis of low-valent Ca(I) complexes, it has unexpectedly been found that the N≡N triple bond is reduced, resulting in the formation of bridging nitrogen complexes with intriguing reactivity (Rösch, B. et al. *Science* **371**, 1125–1128 (2021)).

The reduction of N_2 was observed by a team led by Sjoerd Harder at University Erlangen-Nürnberg during their attempted formation of Ca(I) complexes with sterically bulky diketiminate ligands (HC{C(CH_3)N[2,6-(3-pentyl)-phenyl]}$_2$) (BDI). Although they did not isolate the intended target, (BDI)Ca–Ca(BDI), the fixation and reduction of N_2 (present in the reaction as a supposedly inert atmosphere) led to the formation of (BDI)Ca(N_2)Ca(BDI) complexes.

The (BDI)Ca(N_2)Ca(BDI) complexes were formed in situ by the reaction of a strong reducing agent, K/KI, with (BDI)Ca(I_2)Ca(BDI). Addition of a coordinating solvent, either tetrahydrofuran (THF) or tetrahydropyran, led to the immediate formation of red-brown crystals. X-ray crystallographic analysis revealed the structure (pictured, left) with BDI ligands, coordinating THF molecules and a side-on bridging N_2 unit.



Credit: AAAS

Further inspection showed that the N–N bond length is elongated compared to the triple bond of N_2 and is similar to the N=N double bond length expected in the N_2^{2-} anion.

Collaborative computational studies with Gernot Frenking and his group at Philipps-Universität Marburg examined the nature of the electrostatic and orbital bonding interactions between the N_2^{2-} and the Ca(I) metal centres (pictured, right). Density functional theory calculations found the Ca(I)–N_2 to be highly ionic complexes, accompanied with significant π-backbonding from the *d* orbitals on the Ca(I) centre to the N_2^{2-} anion.

The reactivity of the (BDI)Ca(N_2)Ca(BDI) complexes highlights the strong reducing power of the N_2^{2-} anion, which is realized on release of nitrogen gas. Notably, reaction of (BDI)Ca(N_2)Ca(BDI) with H_2 gave the corresponding hydride complex, in marked contrast to the Mg(I) analogue, which does not react with H_2. Such Mg(I) complexes are typically good reducing agents, hence the stronger reducing power of the in-situ-generated Ca(I) species is promising and could broaden the range of reduction reactions that are possible using low-valent alkaline-earth metal complexes. ❐

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