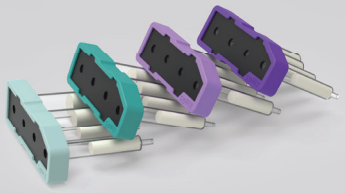
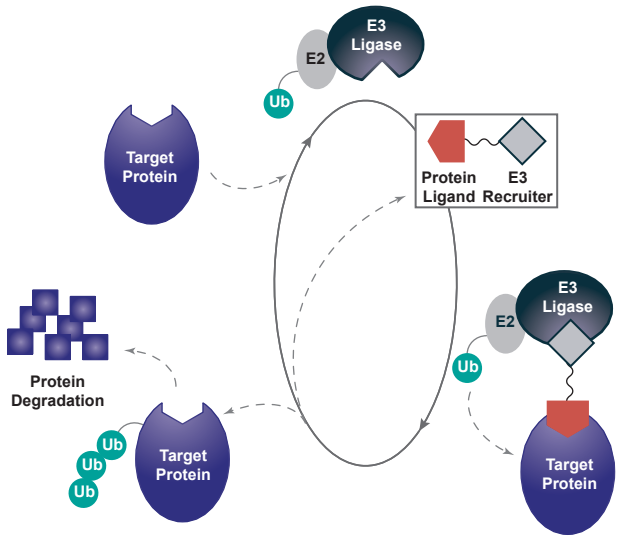
Case Study

Rapid, efficient PROTAC synthesis using automated, cartridge-based chemistry



Role of protein degraders in drug discovery

Bifunctional PROteolysis TArgeting Chimeras (PROTACs) have emerged as a promising new modality for drug discovery. Capable of both binding to a target protein and recruiting a ubiquitin E3 ligase, the PROTAC is able effect the tagging of the specific protein for subsequent proteasomal degradation. This approach has several advantages compared to the traditional protein inhibition strategy. Due to the catalytic nature of the degradation process, lower concentration of the PROTAC, compared to the protein binder itself, can be therapeutically effective. In addition, degredation is not reliant on the inhibition of a specific binding site on the protein and thus much lower affinity protein binders can be transformed into effective PROTACs. As such, the PROTAC approach offers the potential to develop therapeutics for previously undruggable targets.[1]



PROTAC-mediated Protein Degradation

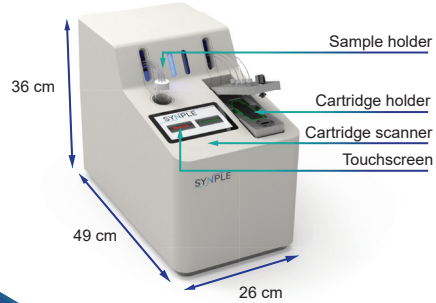
Challenges in PROTAC synthesis

Despite the promise that the protein degradation approach holds, it is not without its challenges. Aside from all the pharmacological and profiling challenges that relate to the testing of new PROTACs, the synthesis of such modalities can be incredibly challenging. In small molecule discovery, most, albeit not all, chemists are accustomed to preparing molecules that meet the criteria for drug-likeness, i.e. those that fall roughly within the Lipinski parameters, whereas PROTACs are typically much larger and have different physical properties, making the synthesis, handling and purification of the intermediate and final products far more challenging. Ultimately, the preparation of PROTACs requires time, dedication, expertise and can be very costly. This increased synthetic effort inevitably comes at the expense of other projects as, due to personnel, space, and cost limitations, it is not always possible to simply find more resources in order to execute all projects of interest.

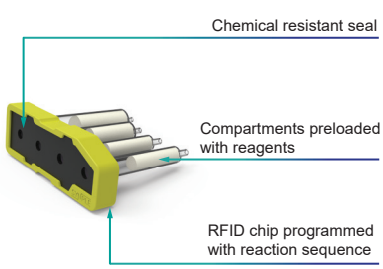
Automation eases resource constraints

Since bringing more chemists in house is not always a feasible option and competition for outsourced resource at CROs is increasing, discovery research is looking towards automation as a means to increase productivity. .

Automated Synthesizer

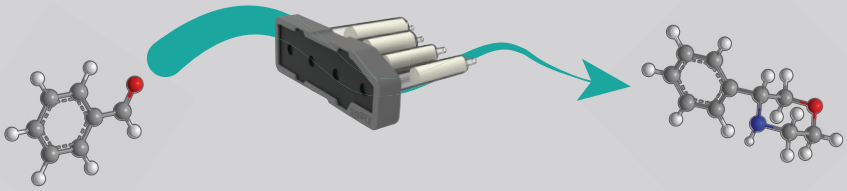


Reaction Cartridge



Technology Benefits

[1] Gao, H.; Sun, X.; Rao, Y. PROTAC Technology: Opportunities and Challenges, ACS Med. Chem. Lett. **2020**, 11, 237-240. DOI:10.1021/acsmedchemlett.9b00597



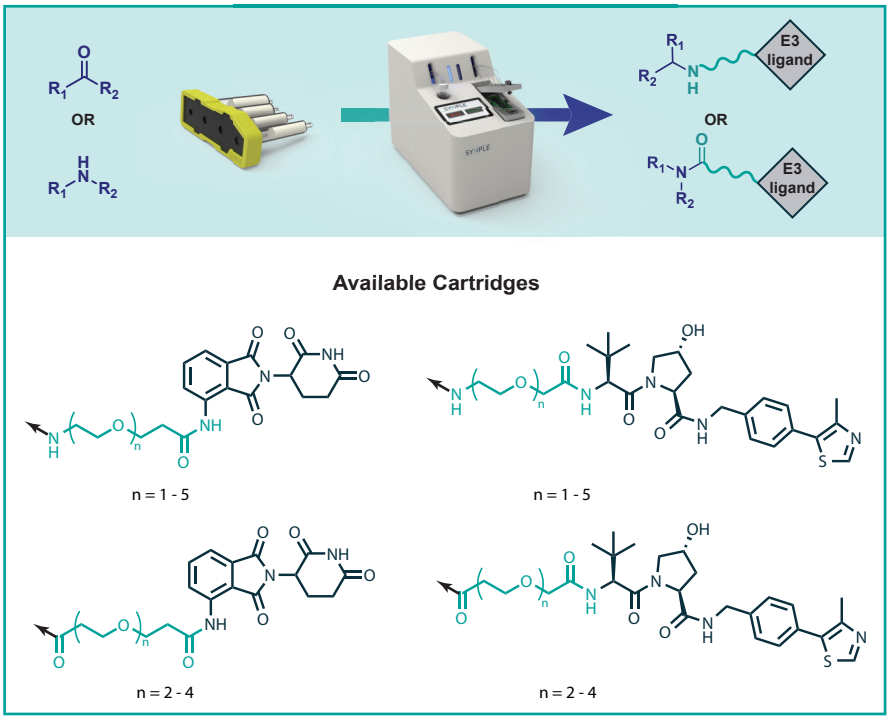
Enabling tools like the Synple, with its small footprint, time- and cost-saving benefits, offer the option to have machines run chemical reactions alongside the chemists, meaning that the same chemists can support more projects in parallel.

Charles River automates PROTAC synthesis

Like most researchers, medicinal chemists at Charles River Laboratories in Cambridge, UK are also faced with an increasing number of demands on their time. Always keen to embrace new technologies to enable their chemists to do more faster, they recently demonstrated the advantages Synple's technology offers in enabling multiple projects to be pursued in parallel.

As part of a collaborative project with M4K Pharma, in which Charles River Laboratories provided in kind support for the discovery of a treatment for DIPG, an aggressive brain tumour affecting children, the team sought to prepare a range of PROTACs based on ALK2 binders.

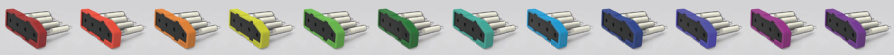
To effect this initial exploration as efficiently as possible, chemists at Charles River looked to automate the synthesis of PROTACs for this project by using Synple's easy to use, quick to set up, bench-top automated synthesis system, along with the prefilled partial PROTAC cartridges and highly optimised synthesis protocols. In this respect, a number of ALK2 PROTACs were designed based on Synple's commercially available partial PROTAC cartridges.



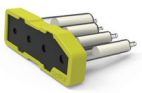
PROTAC design for cartridge-based synthesis

The physicochemical properties of each ALK2 PROTAC design were subsequently calculated and the team selected analogues for synthesis based on two main criteria – permeability and varied linker length. As such, they chose to make those with the lowest number of hydrogen bond donors and acceptors, and the lowest polar surface area and molecular weight. In addition, they selected analogues that would enable them to explore the effect of changing the linker length.

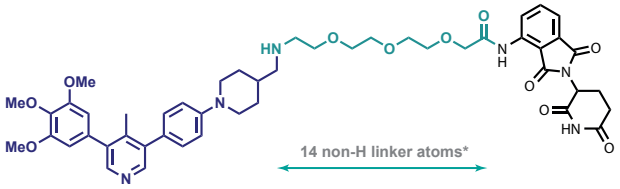
Each ALK2 PROTAC was easily prepared since all the difficult to make and handle partial PROTAC reagents are already contained within the cartridge in the desired amount. All the chemist needed to do was add the ALK2 binder to the sample vial, scan the PROTAC cartridge on the machine to load the method and then press "go", returning only at the end to collect the product. Thanks to the technology, chemists at Charles River were able to rapidly prepare these analogues with minimal resource and in a time frame that would not have been possible had this been done manually.



SYNPLE

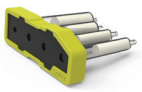


SYNPLE Reagent Cartridge
PROTAC-CRBN-PEG3
P003 / P103

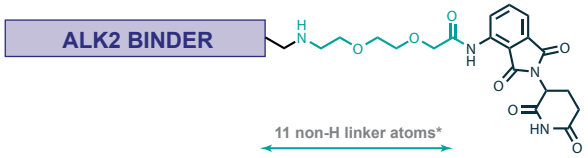


MK4-PEG3-POM

MW:	892
# HBD:	3
TPSA:	196
logD @ 7.4:	2.6

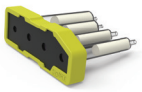


SYNPLE Reagent Cartridge
PROTAC-CRBN-PEG2
P002 / P102

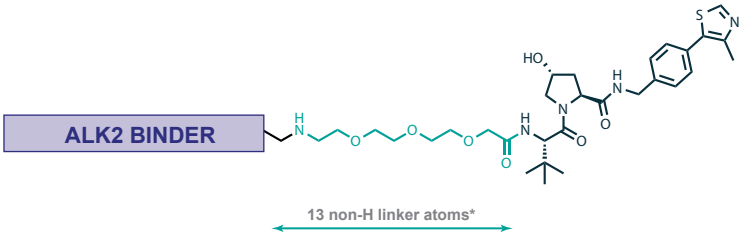


MK4-PEG2-POM

MW:	848
# HBD:	3
TPSA:	187
logD @ 7.4:	1.3



SYNPLE Reagent Cartridge
PROTAC-VHL-PEG3
P023 / P123



MK4-PEG3-VHL

MW:	1050
# HBD:	4
TPSA:	195
logD @ 7.4:	3.3

* number of non-H atoms between ALK2 piperidine and E3 recruiter

Prioritising synthesis with Synple

For many discovery chemists, an ideal day would involve successfully completing all planned lab work, catching up with colleagues to exchange ideas, and of course, receiving great data that validates the current hypothesis! However, the reality can look somewhat different. A morning meeting could mean it is not possible to start planned reactions on time or even worse they might need to be delayed until another day. A meeting might also overrun, leaving reactions running for longer than ideal. A chemist's best made plans can be disrupted by other factors too. Synthetic chemistry can be unpredictable, often requiring close monitoring, and can thus end up taking far longer than planned, often meaning that the time to interact with colleagues, discuss projects and share ideas is sacrificed.

Preparation (1 molecule)	Batch PROTAC synthesis	SYNPLE
Hands - on time	3 h	5 min
Labour costs	300 CHF[1]	8 CHF
Reagent / cartridge costs	300 CHF[2]	170 CHF
Cost saving per reaction	0 CHF	422 CHF

1. Based on costs of 100 CHF / h. 2. Based on commercial price of 0.1 mmol Pomalidomide-PEG3-NH2.xHCl

Synple's technology offers the opportunity to prioritise synthesis – in only 5 minutes a reaction can be safely initiated. Overrunning meetings are not an issue for the Synple – workup and isolations are automatically initated following the reaction, leaving the product there to collect at the first opportunity. An automated washing sequence also ensures that the machine is cleaned and ready to use again at the touch of a button – no excessive glassware washing! Whatever other demands are placed on a chemists' time, automated enabling tools, like the Synple, ensure that new molecules still get made and discovery research continues to move forwards.

Additional information

Detailed information about reaction procedures, conditions and scope for all Synple reactions can be downloaded as application notes from our homepage. For any questions please contact us:

info@synplechem.com www.synplechem.com

Download reaction application notes

Visit Synple Chem Homepage