

Expanding the Toolkit of Synthetic Transformations Applied to Direct-to-Biology

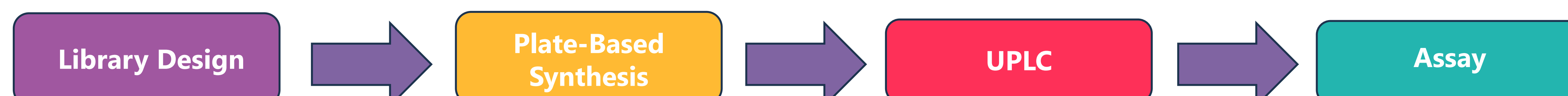
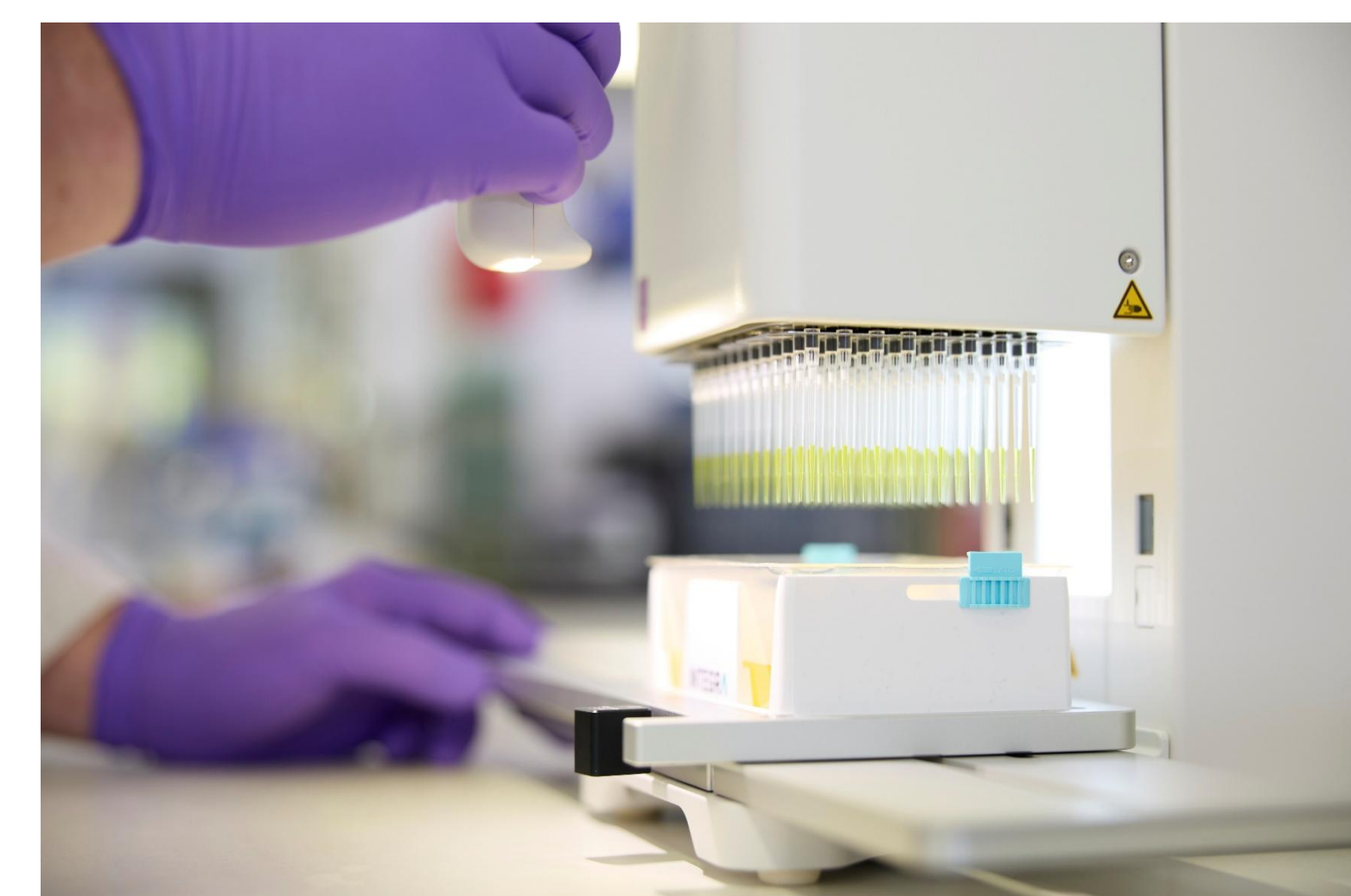


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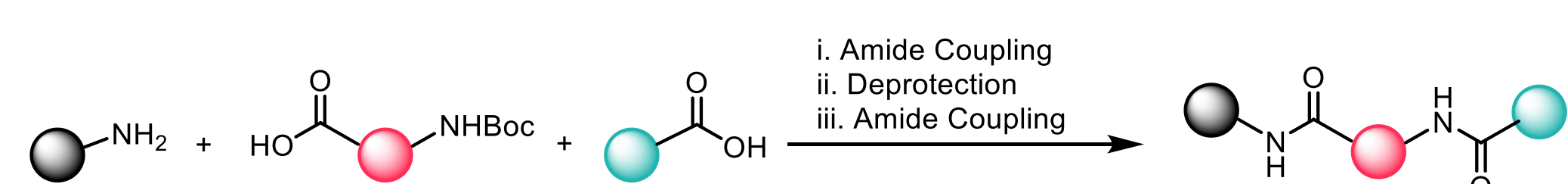
Introduction to Direct-to-Biology (D2B)

- In Direct-to-Biology (D2B) multiple molecules are synthesised in parallel in a plate-based format and the crude, un-purified reaction mixtures are screened directly in assays relevant for drug discovery.
- Using D2B, Structure-Activity Relationships can be generated far more quickly than using traditional synthesis.
- At Domainex, “D2B compatibility tests” check that reaction by-products and impurities are unlikely to affect assay performance, to ensure that reliable, high-quality data is ultimately generated by the D2B campaign.
- Our D2B experiments are performed in 384 well plates as standard, which enables us to run more reactions with less material – typically 30 mg is sufficient material to deliver 384 reaction products.
- D2B Workflow:



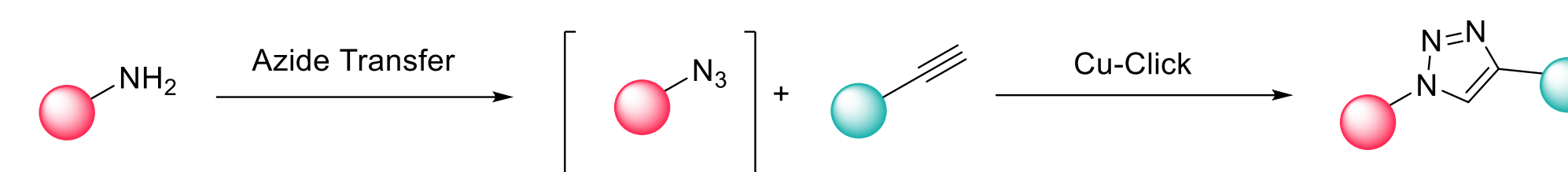
Validated D2B Transformations

Amide Coupling – Three Steps, One pot



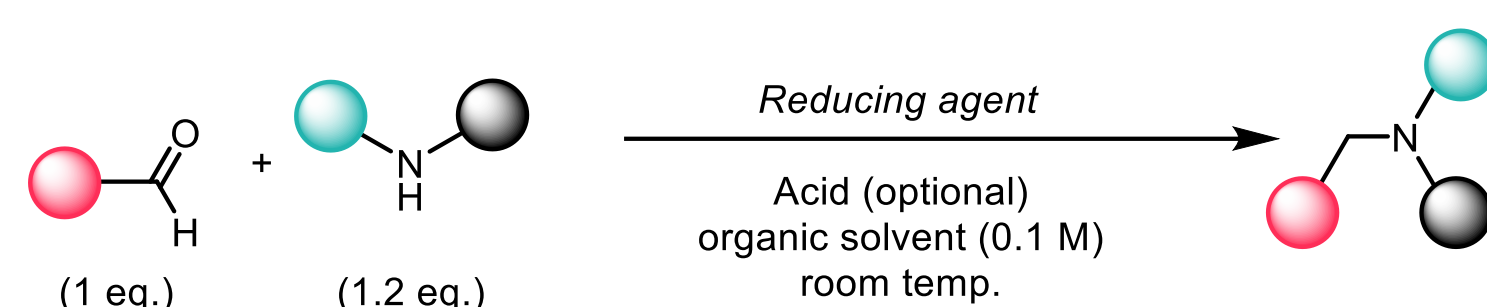
- Reaction conditions for the amide coupling, boc-deprotection, and second amide coupling in one-pot were found and used in a 384-well plate-based format
- Methodology used for multiple clients to synthesise hundreds of PROTACs®

One Pot Azide Formation and Copper Click Reaction



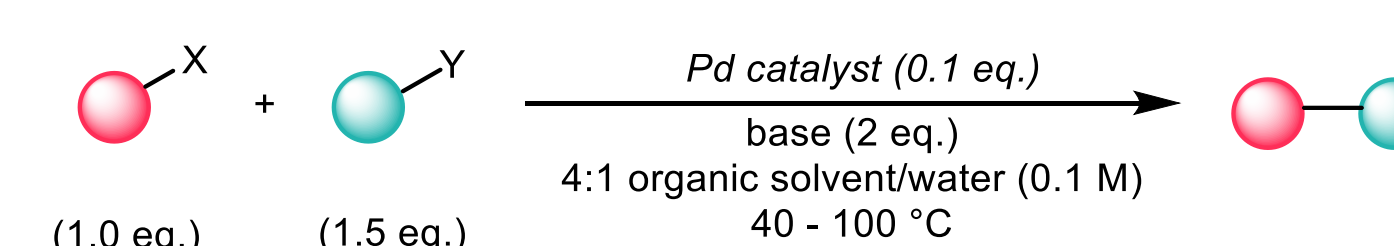
- Chemistry successfully applied to 12 different amine substrates and 12 different alkyne substrates in a plate-based format
- Applied to 36 partial PROTACs® from Domainex Partial PROTAC® library, product was observed in every well

Reductive Amination



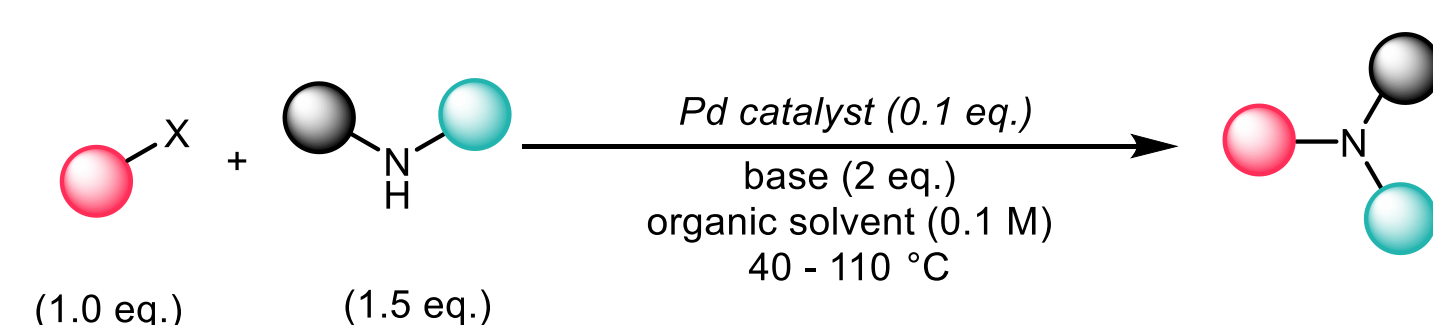
- Multiple building blocks screened in parallel to identify global optimal conditions
- 8 different aldehydes were reacted with 8 different amines
- 50% of the reactions gave desired product

Suzuki-Miyaura Cross Coupling



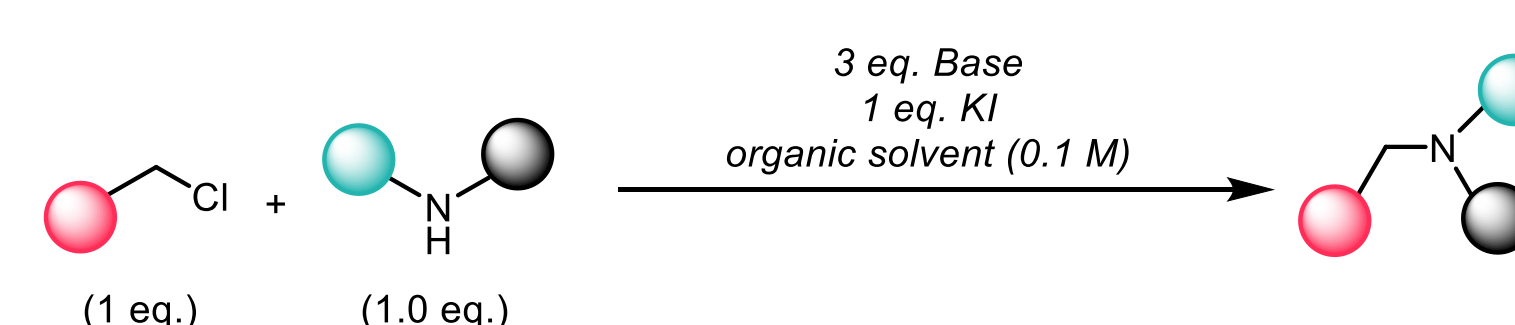
- Suzuki-Miyaura performed with 64 substrates using 6 different D2B compatible reaction conditions
- 40% of the 384 reactions gave desired product; over 150 product containing wells!

Buchwald-Hartwig Cross Coupling



- Reacted 8 different aryl halides with 8 different amines using 6 different reaction conditions on a single 384 well plate
- Every substrate combination gave desired product, except one unreactive amine

S_N2



- Chemistry successfully applied to partial PROTAC® substrates
- Crude S_N2 products were successfully boc-deprotected in a plate-based format

Direct-to-Biology Compatibility Assessment

	Amide	Reductive Amination	Suzuki-Miyaura	Buchwald-Hartwig	CuAAC	S _N 2
Chemistry Plate Successful	Y	Y	Y	Y	Y	Y
HiBiT	Y	Y	Y	Y	Y	Y
Cell-TitreGlo	Y	Y	Y	Y	Y	Y
Spectral Shift	Y	Y	Y	Y	N.T. ¹	N.T.
GCI	Y	Y	Y	Y	N.T.	N.T.

¹ N.T.: Not Tested



- D2B compatibility tests are a vital part of any D2B campaign, as they ensure that the crude reaction components do not interfere with the assay readout at the concentrations assessed
- D2B compatibility tests show that the optimised Amide coupling, Suzuki-Miyaura, Buchwald-Hartwig, Reductive Amination, Copper Click, and S_N2 reactions can be tolerated by multiple assay types
- Future work in this area will look to “fill in the gaps”, whilst also expanding the repertoire of our D2B-compatible synthetic methodologies such as photoredox

Conclusions

- Multiple reaction types shown to work in a plate-based format
- Multi-step transformations performed on a single plate
- D2B compatibility tests show that contemporary chemistries are tolerated by multiple assay types

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about our D2B drug discovery services, please contact: enquiries@domainex.co.uk