

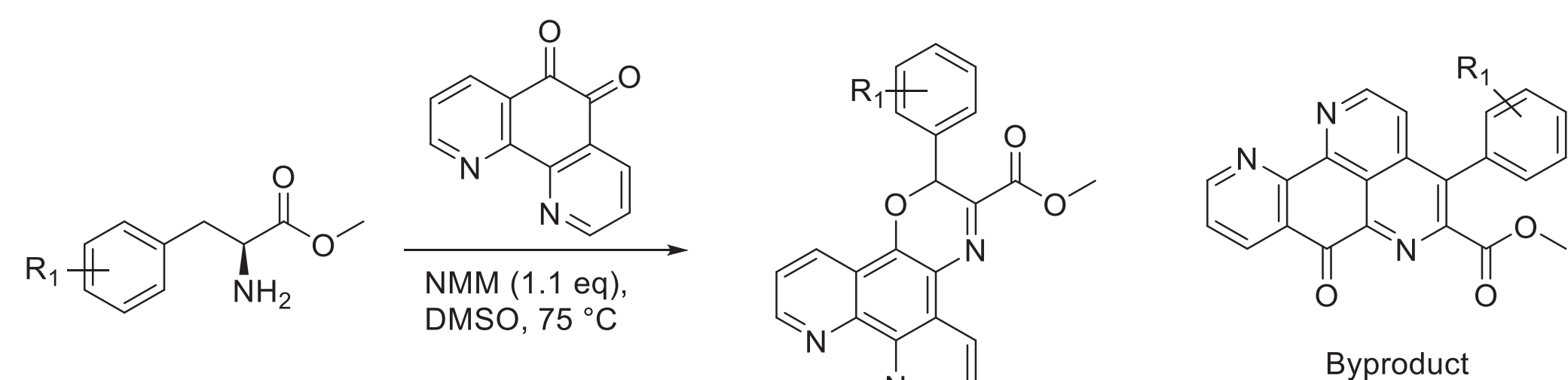
# Rapid Optimization and Synthesis of New Phenanthroline Amino Acid Ligands using Design of Experiments

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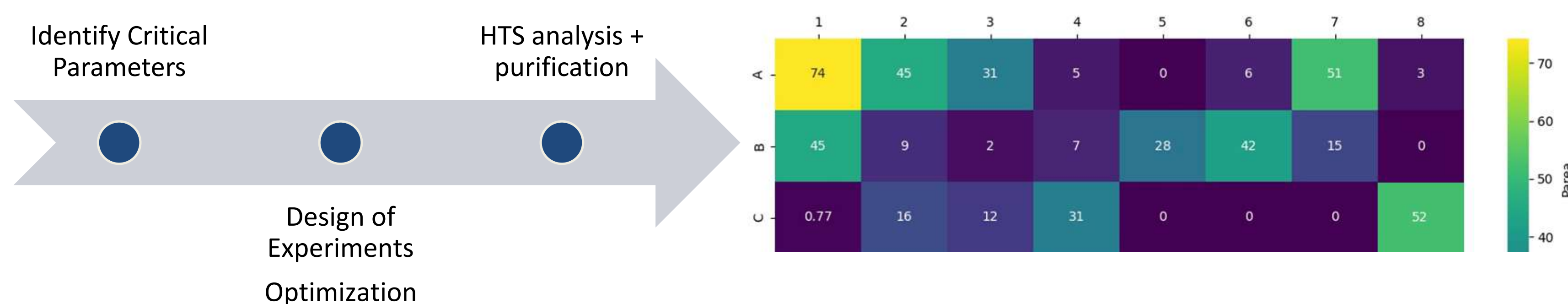
## Introduction

1,10-Phenanthroline-5,6-dione (phen) containing ligands are of interest as Cu chelators for biological applications such as DNA intercalation and as anti-cancer agents. Phenanthroline-amino acid ligand synthesis were first reported in 2013 by Kellett *et al.* through a simple Schiff base condensation of phenanthroline dione and an amino acid ester,<sup>1</sup> with a general procedure later published by Twamley *et al.* in 2019, Scheme 1.<sup>2</sup> Ag, Cu, and Mn complexes of these phen ligands have been reported and tested for their biological activity as DNA binders and as antimicrobials.<sup>1,3</sup>



Scheme 1: General synthesis of phenanthroline-amino acid ligands

At Charnwood Discovery, the synthesis of an expanded library of phen ligands was of interest and thus further understanding of the reaction's limits, robustness, and generality were required. Of concern was that the publication by Twamley *et al.* showed a significant quantity of byproduct formation for substrates containing electron withdrawing groups in the para position of the amino acid aromatic ring. We sought to rapidly investigate whether reaction yield, purity and selectivity could be improved through Design of Experiment (DoE) mediated optimization. The end goal was to synthesize a series of ligands using high throughput screening (HTS) employing a general method.



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## Solvent Screening

| Solvent           | % Product | % Byproduct |
|-------------------|-----------|-------------|
| DMSO              | 74        | 5           |
| DMF               | 65        | 4           |
| Toluene           | 11        | 2           |
| 1,4-Dioxane       | 40        | 5           |
| Isopropyl Acetate | 17        | 2           |
| MeCN              | 55        | 3           |
| 2-MeTHF           | 42        | 3           |

Table 1: Results for the initial solvent screen. % area

A brief solvent screen was conducted using the standard reported conditions for phen ligands: Tyrosine methyl ester HCl (1.0 eq), NMM (1.1 eq), DMSO (100 vol), 75 °C, 24 h.

DMSO gave the best conversion to product of all the solvents examined and was employed for the rest of the reaction optimization.

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## Other Amino Acids

Phenylalanine was then tested under the same reaction conditions as tyrosine. Surprisingly, a non-linear relationship of temperature vs. product was observed. Temperatures higher than 55 °C led to formation of predominantly by-product, Figure 2. Phenylalanine's only point of difference is the para-substituent, therefore the effect of aromatic ring substituent needed to be considered.

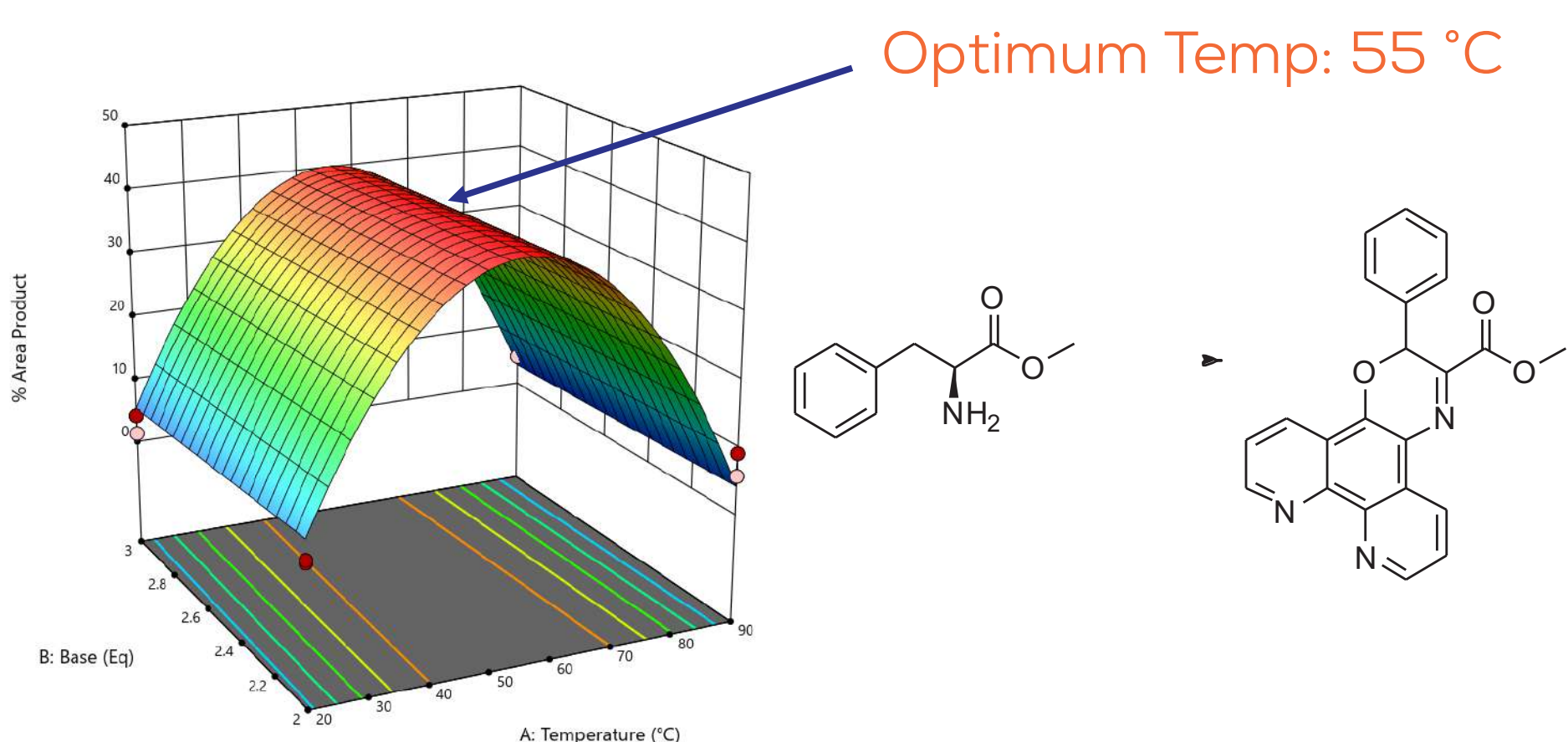


Figure 2: Phenylalanine reaction temperature dependence

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## Summary and Conclusion

In conclusion we have developed, through a design of experiments approach, a statistical model for the reaction of aromatic amino acid esters with 1,10-phenanthroline-5,6-dione for the generation of new phen ligands. The reaction model identified a significant relationship between amino acid p-Hammett parameter and temperature. This relationship was visually and mathematically modeled and has enabled a temperature optimized targeted synthesis of a variety of different ligands. In total, 25 new phen ligands have since been synthesized and purified using mass directed preparative HPLC. The total turnaround time was less than 10 days from inception to delivery.

References: [1] Chem. Commun., 2013, 49, 2341. [2] Dalton Trans., 2019, 48, 15283. [3] Biometals 35, 173–185 (2022).

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## Optimization of Temperature, Base eq., Amino Acid eq.

A 20-reaction DoE was conducted in a single Radleys 24-well reaction block. Parameters of interest were temperature, base and amino acid (aa) equivalents. A high, middle, and low value for each parameter were chosen. Tyrosine methyl ester HCl was again chosen as the amino acid of interest. Only temperature was noted as having a significant effect, with neither amino acid equivalents nor base equivalents significantly changing the reaction outcome, Figure 1.

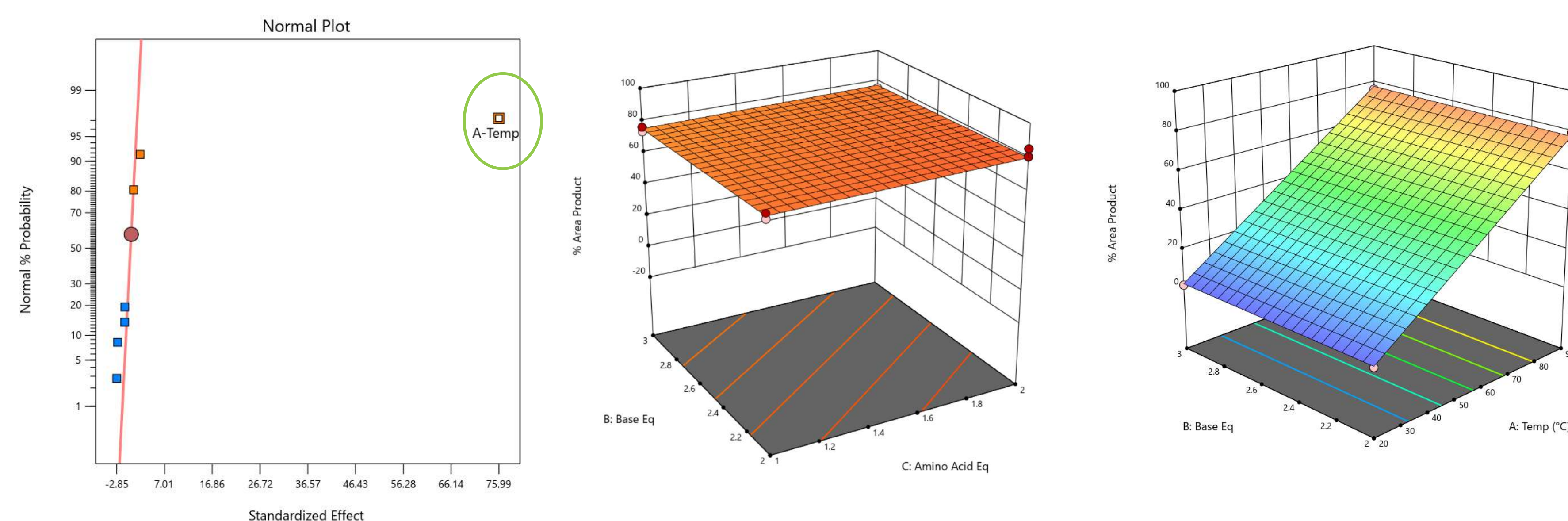


Figure 1: Left: normal plot showing standardized effect, note that only "A-Temperature" is considered a significant factor. Center: plot of aa eq. vs. base eq. for reactions conducted at 90 °C, no significant effect is observed. Right: plot of temperature vs. base eq. Only temperature has a significant effect on % product area.

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## Hammett Series

It was theorized that the Hammett series could be used to predict reaction outcomes / product distribution; more positive Hammett values giving poorer conversion to the desired phen ligands. This hypothesis was tested and validated by employing a range of para substituted aa's covering a range of Hammett values. The reaction was found to be substrate dependant; Hammett values of > +0.5 giving <5% product yield whereas more negative Hammett values gave a higher yield. Using the model of Hammett value vs. temperature, Figure 3, temperature tuned synthesis conditions were then applied to a range of amino acids to generate a series of new phen ligands.

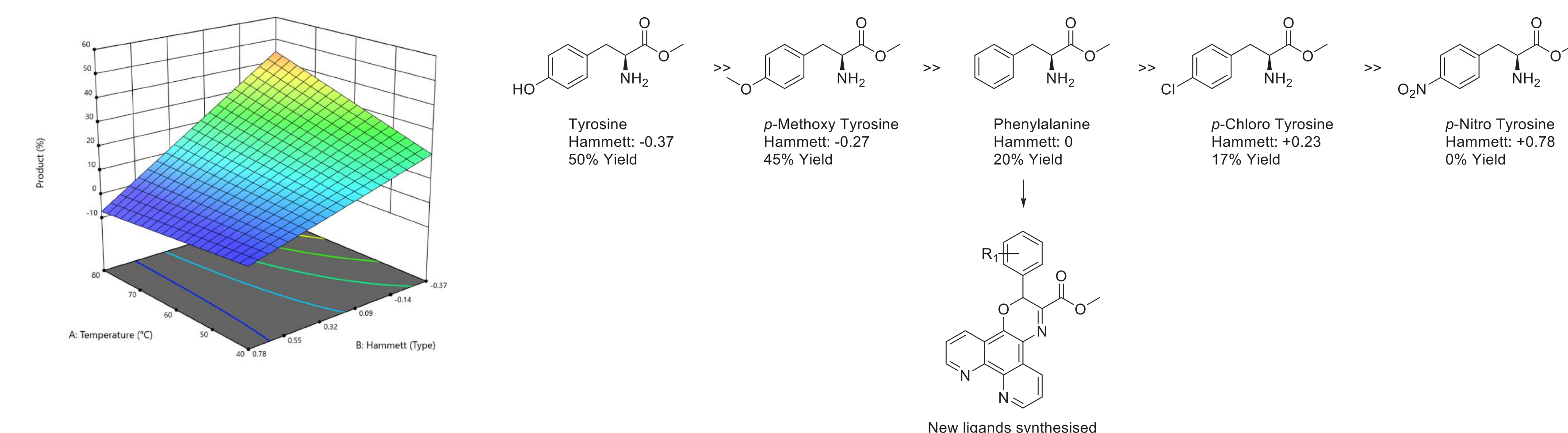


Figure 3: Left: Model of Hammett value vs. temperature. Right: Effects of Hammett values on product yield

