

# Synthesis and characterization of Petasis-Ugi ligand for antibody purification

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## INTRODUCTION

Synthetic mixed-mode and affinity ligands are agents used in bioseparation processes for the capture of biological targets. These ligands are rationally designed and produced by combinatorial chemistry using simple and fast procedures as multicomponent one-pot reactions (e.g. Ugi reaction) [1–3].

Our group has shown that multicomponent chemistries can be combined yielding new scaffolds for combinatorial chemistry as the tandem Petasis-Ugi reaction and previously reported the design and production of an affinity adsorbent using a Fab-Ligand from one-pot reaction [2,4].

## WHY SYNTHETIC AFFINITY LIGANDS FOR PURIFICATION?



Low-cost



Stable and robust



Simple and fast production



Tailor-Made

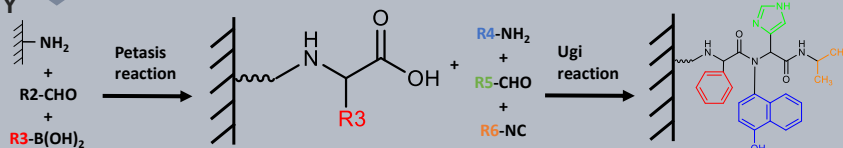
Synthetic ligand from Petasis-Ugi reaction

Fab-Ligand [4]:

- High selectivity for Immunoglobulins
- Mammalian and avian sources
- Fab fragments

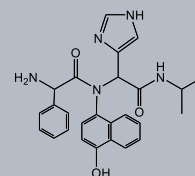
## AIM AND NOVELTY

Previously: Synthesis on the solid support



In this work:

Synthesis in liquid-phase using one-pot-reaction



## METHODS

1

### One-pot synthesis of Fab-Ligand

- One-pot reaction in liquid phase
- Removal of the BOC group

2

### Coupling on a Commercial Resin

3

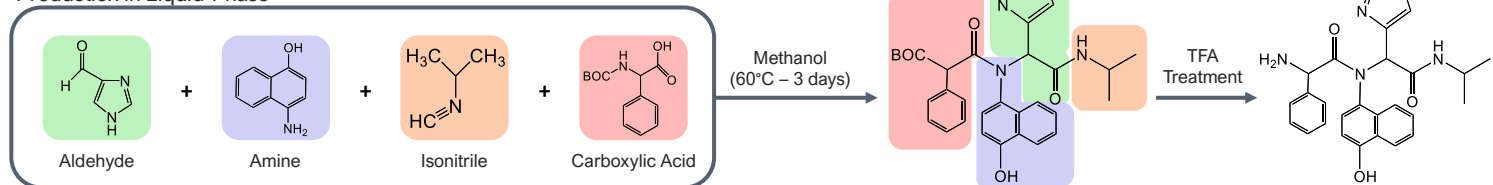
### Purifying IgG from Human Plasma

## RESULTS

### 1.1 One-pot synthesis of Fab-Ligand

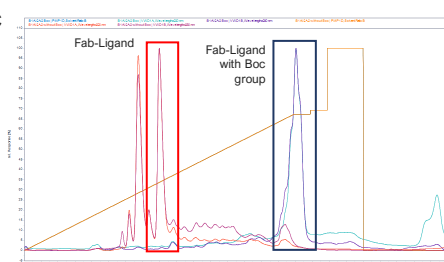
The synthesis of Fab-ligand was carried out in a one-pot reaction lasting 3 days (fast production).

Production in Liquid-Phase

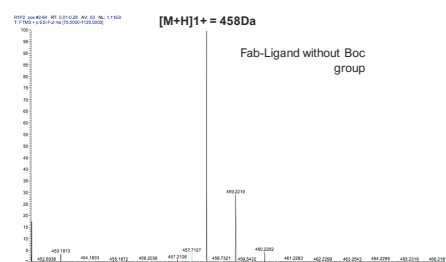


After Fab-Ligand was synthesized, it was purified by High-Performance Liquid Chromatography (HPLC) and its identity was verified by mass spectrometry (ESI-MS).

HPLC

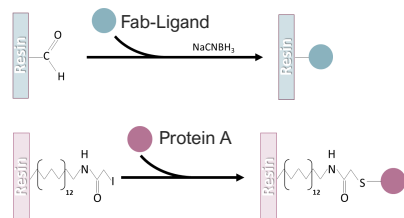


ESI-MS

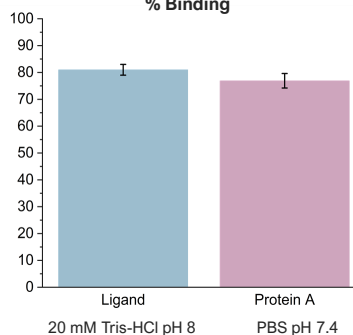


### 1.2 Coupling on a Commercial Resin and Purifying IgG from Human Plasma

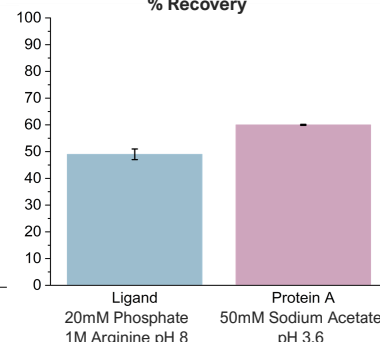
After the synthesis and purification, the Fab-Ligand and a standard Protein A were conjugated in a commercial resin. The commercial resin functionalized with the Fab-Ligand was then tested for the purification of IgG from Human Plasma.



% Binding



% Recovery



## CONCLUSIONS

- The new Petasis-Ugi ligand was chemically synthesized in liquid-phase, purified and characterized.
- It was possible to immobilize the ligand in a matrix.
- The ligand is functional for IgG from human plasma purification.

## ACKNOWLEDGEMENTS

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## REFERENCES

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