Pyridium-π Interaction Preserves the *N,S*-benzylidene Thioacetals (NTDs) in Acidolysis Enabling Efficient Protein Chemical Synthesis

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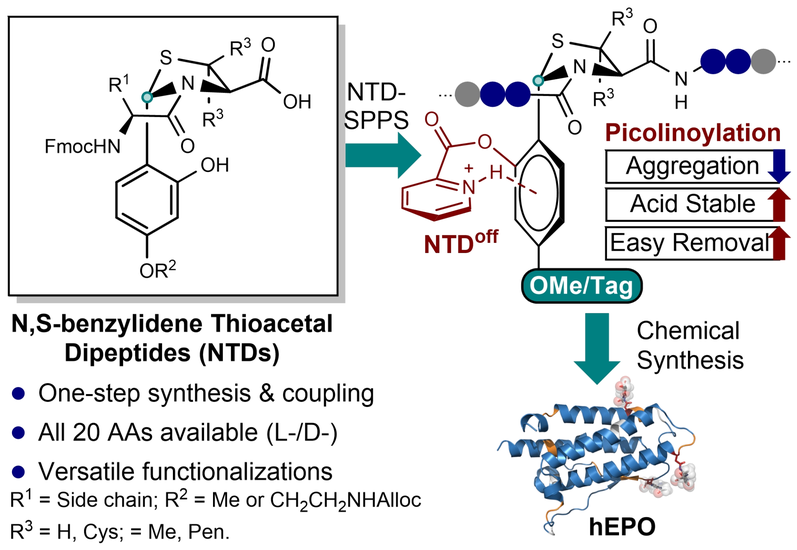
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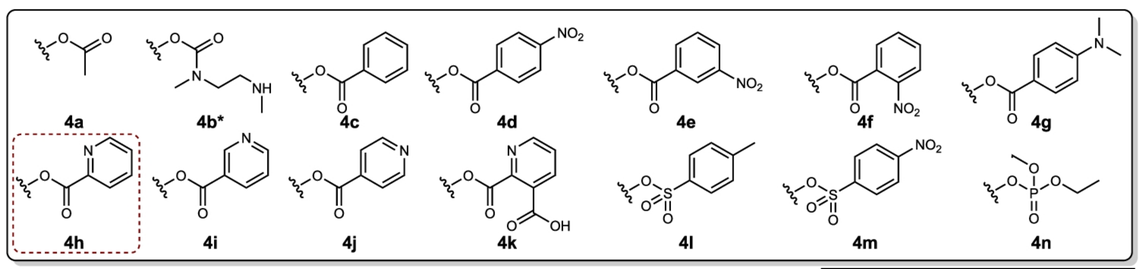
Introduction

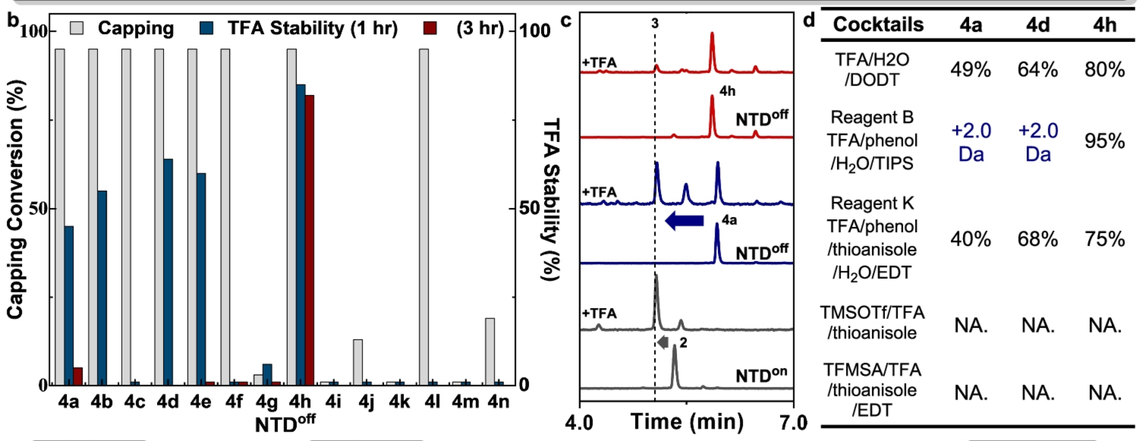
Li et al. reported the chemoselective serine/threonine ligation (STL) and cysteine/penicillamine ligation (CPL) where a C-terminal salicylaldehyde (SAL) esters of an unprotected peptide fragment is ligated with the N-terminal Ser/Thr/Cys/Pen residue of the other peptide fragment. The resulting ligation intermediates, *N,O/S*-benzylidene (thio)acetal, could serve as aggregation disruptors.[1] While such moieties could be introduced by ligation,[2,3] here, we report the synthesis and installation of *N,S*-benzylidene thioacetal dipeptides (NTDs) via standard SPPS. Moreover, we discovered a picolinoylation-based strategy to retain the NTD scaffold during global deprotection. This approach effectively suppresses aggregation both on-resin and in-solution. As a demonstration, we achieve the chemical synthesis of hEPO via a convergent route with NCL and STL incorporating the NTD chemistry.[4]



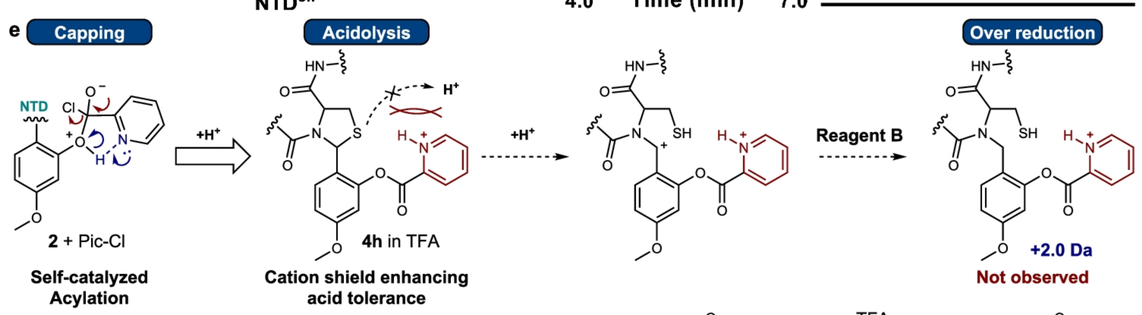
Picolinoylation enhances NTD stability in acidolysis

We incorporate an NTD scaffold in our model peptide. When the phenol group of NTD was capped by a series of acylation reagents (NTD^{off}), it will hopefully be retained during acidolysis. We found that picolinoyl-capped peptide showed superior acid-resistance, and a pyridinium-π coordination was proposed.





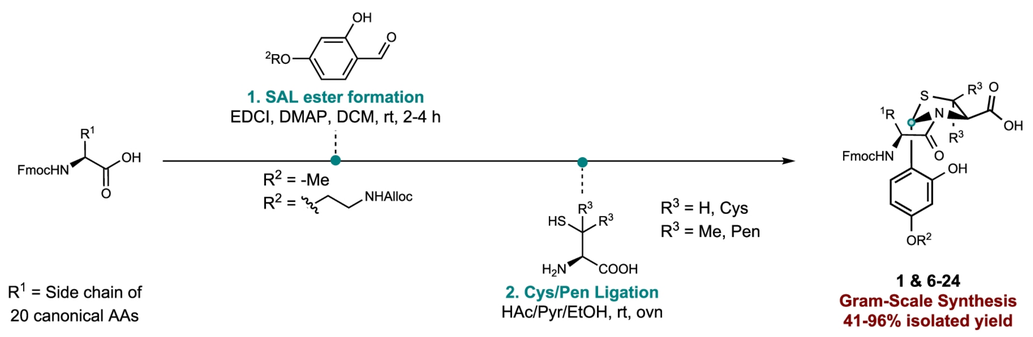
Cocktails	4a	4d	4h
TFA/H2O /DODT	49%	64%	80%
Reagent B TFA/phenol /H_2O/TIPS	+2.0 Da	+2.0 Da	95%
Reagent K TFA/phenol /thioanisole /H_2O/EDT	40%	68%	75%
TMSOTf/TFA /thioanisole	NA.	NA.	NA.
TFMSA/TFA /thioanisole /EDT	NA.	NA.	NA.



Peptide Sequence	Capping group	Conversion
Ac-AFA-IS(NBD^{off})- -APDTRPAPG-OH	Ac- (**5a**)	58%
	Pic- (**5b**)	89%
AcYGV-YT(NBD^{off})- -APDTRPAPG-OH	Ac- (**5c**)	42%
	Pic- (**5d**)	71%
AcG-VS(NBD^{off})-TAPPA- -YGVTSAPDTRPAPG-OH	Ac- (**5e**)	70%
	Pic- (**5f**)	87%
Fmoc-TT(NBD)^{off}- -APPA-OH	Ac- (**5g**)	0%
	Pic- (**5h**)	0%

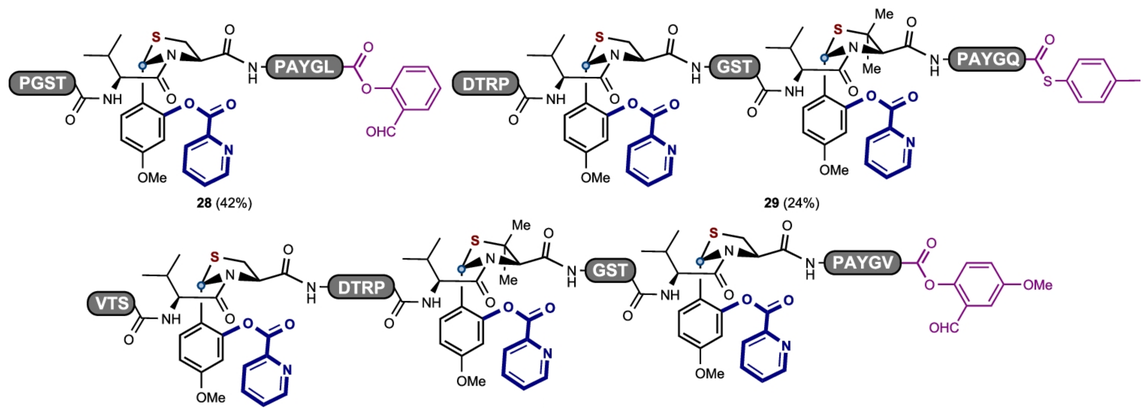
Preparation and installation of NTDs

NTD could be synthesized in a one-pot manner with satisfying isolated yields.



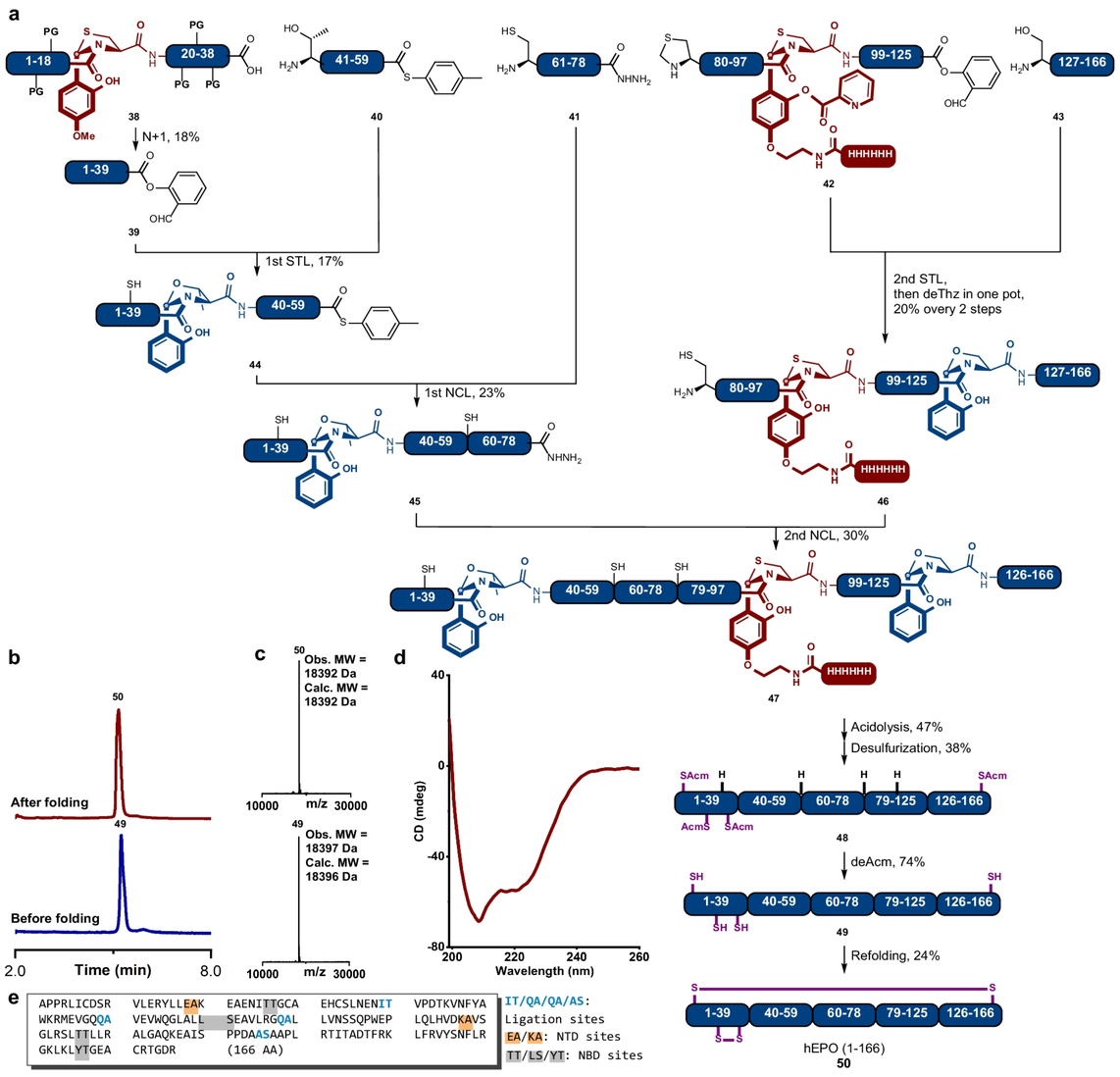
NTD can be efficiently incorporated into Fmoc-SPPS approximating other normal amino acid building blocks.

After completion of the whole peptide sequence, the NTDs are switched off by picolinoyl capping. Then the peptide undergoes either globally deprotection by TFA cocktails or soft cleavage with side-chain protected and then coupled to amino acid bearing SAL ester or thioester through N+1 strategy.[5]



Chemical synthesis of hEPO using NTD chemistry

hEPO is an important therapeutic hormone protein containing 166 amino acid residues and complicated glycosylation modification. We divided it into five fragments for a convergent synthesis adopting two NCL and two STL.



References

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