Synthesis and conformational study of truncated human calcitonin analogs

John W. Taylor, Qian Jin, Arunthathi Theivakumaran*
Dept. of Chemistry, Rutgers University
Wright-Rieman Laboratories, 610 Taylor Road
Piscataway, NJ 08854-8087

*Rutgers Undergraduate Research Fellow

Abstract

Calcitonin is a peptide hormone of 32 residues that plays a pivotal role in calcium-phosphorous metabolism; it is also believed to be a neuromodulator and/or neurotransmitter. In assays of certain *in vivo* hypocalcemic effects of calcitonins, the salmon and eel analogues are the most potent; human calcitonin (hCt) is among the least potent (100 times less potent than that of naturally occurring salmon or eel). Recent studies of the hypocalcemic effects of side-chained linked, cyclic human calcitonin analogues indicate that the residues at positions 17-21 play a pivotal role in determining this bioactivity of calcitonin. The cyclic nature of the peptide stabilizes the hypothesized β-turn at residues 18-19. The aim of this study is to understand the conformational requirements of synthesized hCT analogues in determining their hypocalcemic effects. Therefore, we are synthesizing truncated versions of these cyclic analogues for conformation studies in solution by nuclear magnetic resonance (NMR).

Introduction

Calcitonin is a peptide hormone of 32 residues that plays a pivotal role in calcium-phosphorous metabolism; it is also believed to be a neuromodulator and/or neurotransmitter. Exogenously administered calcitonin has been shown to reduce pain associated with Paget's disease and malignancy, by what is hypothesized to be a direct effect on the central nervous system

Calcitonin from species such as salmon and eel are the most effective classes of calcitonin; human calcitonin (hCt) is among the least potent (100 times less potent than that of salmon or eel). There is evidence that the bioactivity of hCt is strongly correlated with a β-turn/β-sheet conformation in the area of residues 17-21. By isolating a small region of the longer, constrained peptide (via a lactam bridge between residues 17 and 21) we can conclusively determine the structural information of the hypothesized turn region based on NMR studies

Figure 1. Chemical structure of synthesized peptide I including all protecting groups.

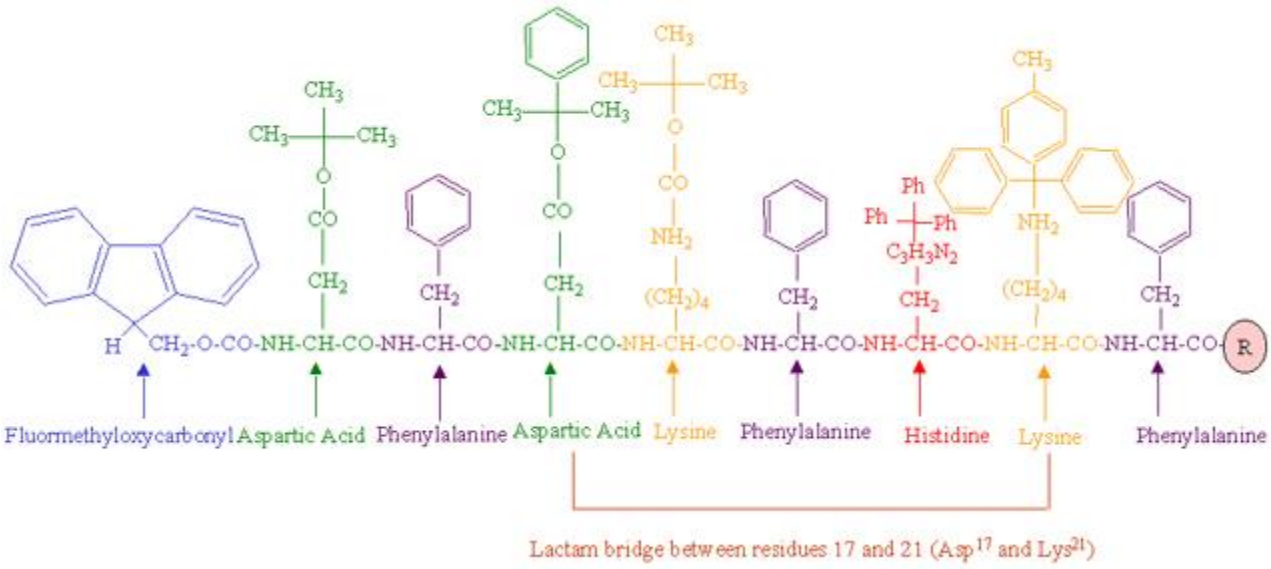


Figure 2a. Hypothesized conformation of synthesized peptide (peptide I)

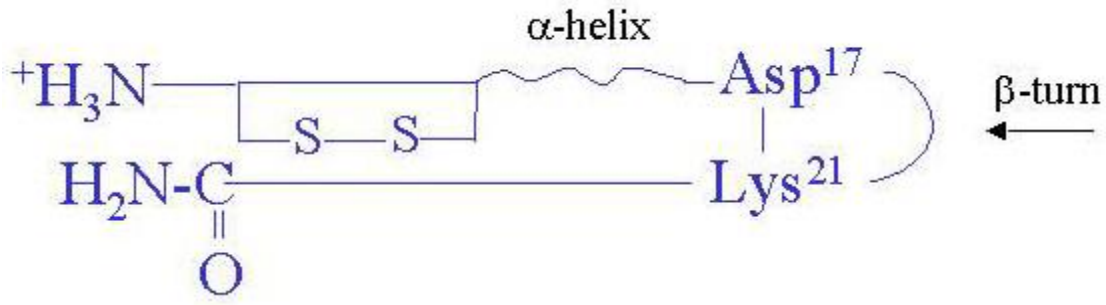


Figure 2b. Comparison of peptide I, acetyl-DFDKFHKF-amide naturally occurring in sCt (salmon calcitonin) and in hCt (human calcitonin) to chemically synthesized forms. A letter of the alphabet denotes each amino acid (i.e.: Q = glutamic acid, H = histidine, N = asparagine *etc*.). The lactam bridge between residues 17 and 21 is indicated in the hCt analogue structure, and is thought to stabilize the β-turn in amino acids 18 and 19 (see figure 2a).

SCt: *C*S*NLSTC*V*LG*[10]KLS*Q*ELH*K*LQ*[20]*TY*P*R*T*DV*G*A*G*[30]*T*P*-amide

hCt: *C*G*NLSTC*M*LG*[10]TYT*Q*DFN*K*FH*[20]*TF*P*Q*T*AI*G*V*G*[30]*A*P*-amide

hCt analogue: *C*G*NLSTC*M*LG*[10]TYT*Q*DF(D)[17]*K*FH[20](O)[21]F*P*Q*T*AI*G*V*G*[30]A*P*-amide

Figure 3. The α-helix in the peptide is thought to be responsible for N-terminal domain binding, as illustrated in this figure. Both the N-*terminus* and C-*terminus* regions of the peptide interact with receptor region that triggers signal transaction. The β-turn that is stabilized by a lactam bridge is thought to facilitate the latter interactions.

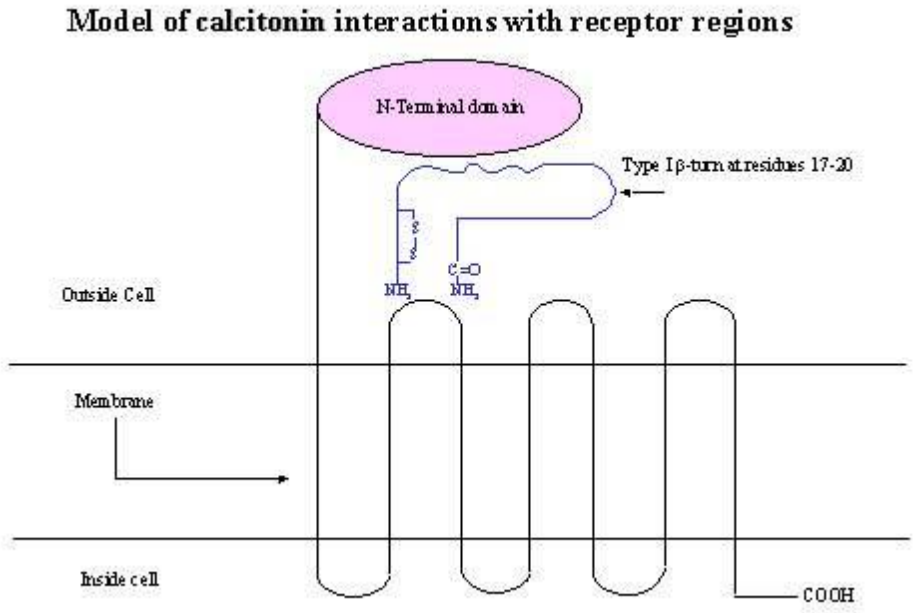
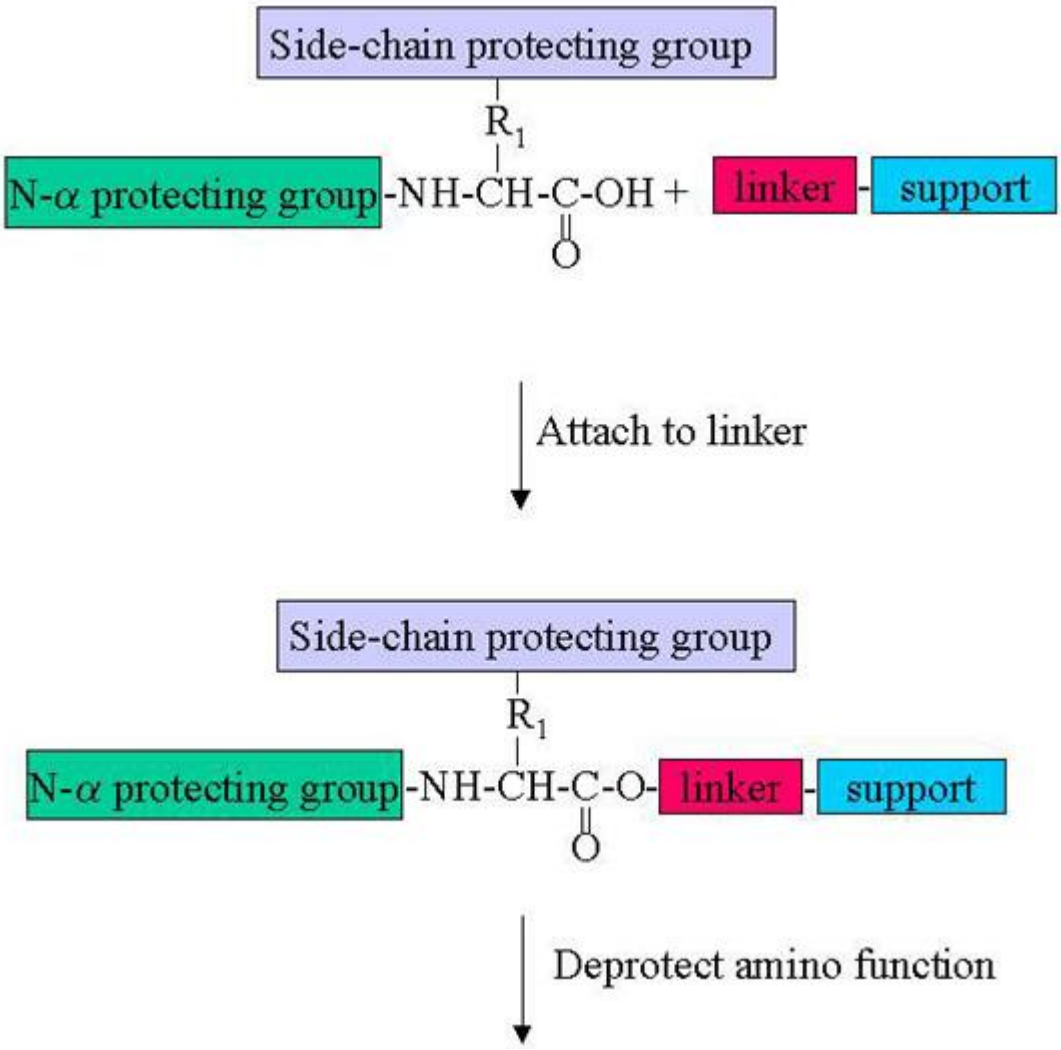


Figure 4. Solid phase peptide synthesis (or polymer-supported synthesis) is an important technique invented by R.B. Merrifield in 1963. The reactions are similar to that of ordinary synthesis, but in SPPS one of the reactants is anchored onto a solid polymer. The basic advantage of the polymer support techniques is that the polymer (with all chains attached to it) is insoluble in the solvents used and is therefore easily separated from all other reagents. Excess reagents, other reaction products, side products, and the solvents themselves are quickly washed away. This figure illustrates the chemical steps that are necessary in order to build up a peptide (small protein) consisting of any assembled sequence of amino acids on the polymer support.

General Scheme for Solid Phase Peptide Synthesis (SPSS)



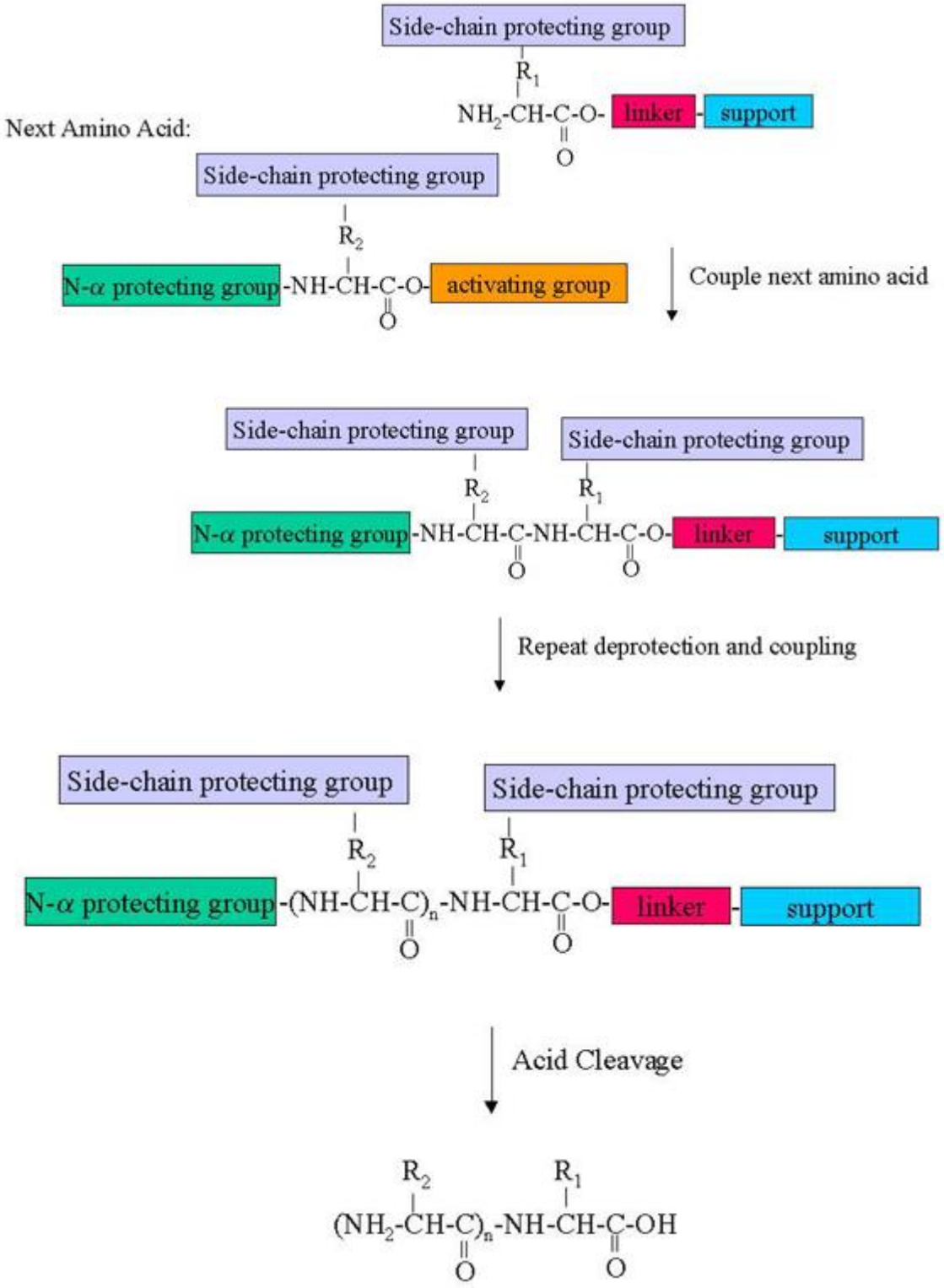
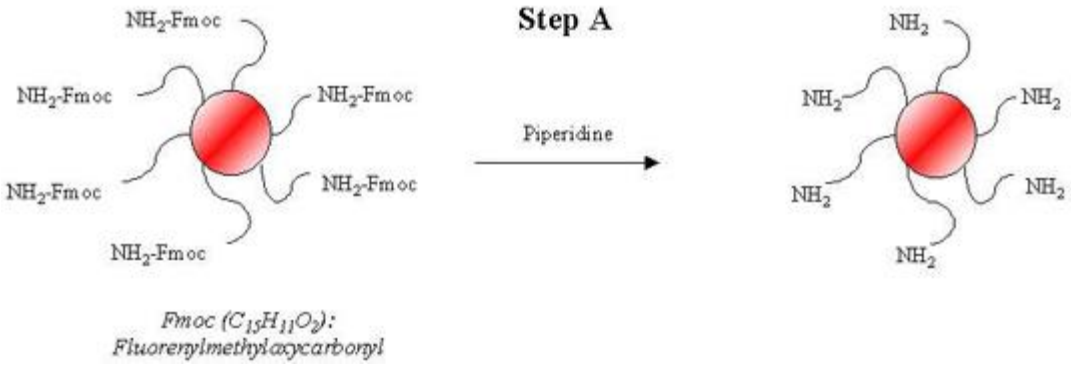
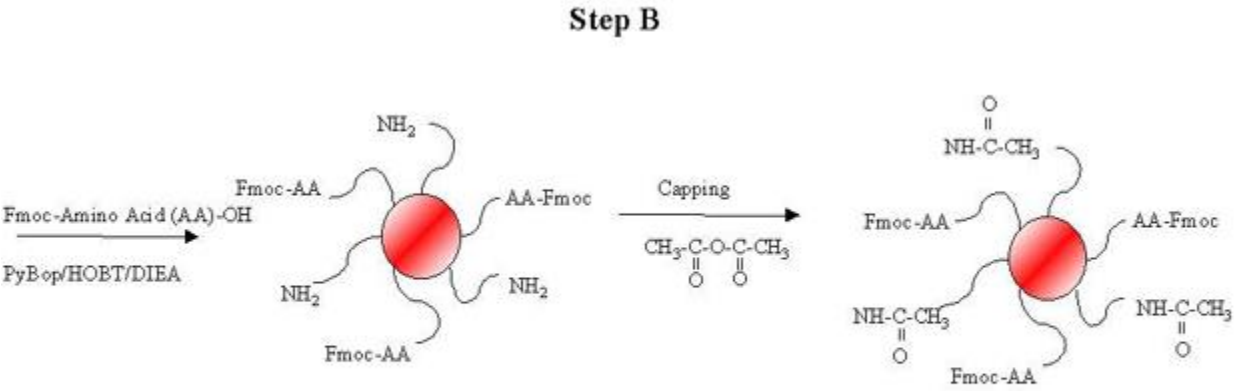

Side-chain protecting group
R1
NH2-CH-C-O-
linker
support
O
Next Amino Acid:
Side-chain protecting group
R2
N-α protecting group
-NH-CH-C-O-
activating group
O
Couple next amino acid
Side-chain protecting group
Side-chain protecting group
R2
R1
N-α protecting group
-NH-CH-C-NH-CH-C-O-
linker
support
O
O
Repeat deprotection and coupling
Side-chain protecting group
Side-chain protecting group
R2
R1
N-α protecting group
-(NH-CH-C)n-NH-CH-C-O-
linker
support
O
O
Acid Cleavage
R2
R1
(NH2-CH-C)n-NH-CH-C-OH
O
O

Figure 5.

Step A: Solid phase peptide synthesis starts with a polystyrene bead (denoted by the shaded red circle) that is Fmoc protected (NH_2-Fmoc). Reaction with piperidine (a base) deprotects the Fmoc functional group, yielding free amines that are ready to be coupled to an amino acid (*e.g.* phenylalanine).

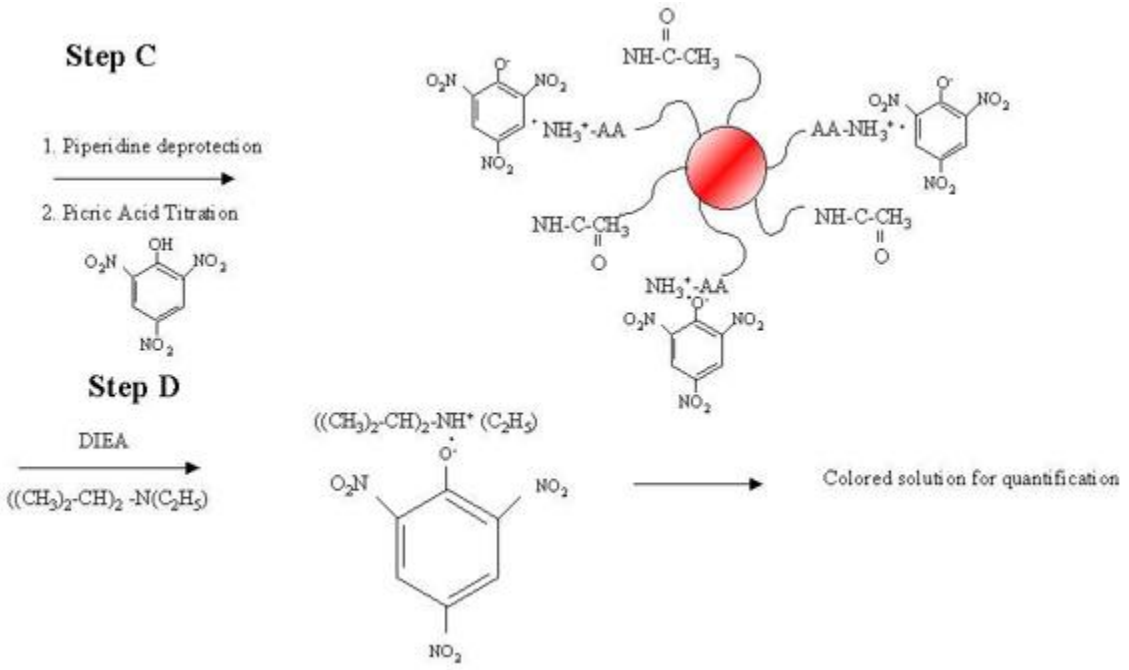


Step B: At this stage (when there are free amines), an amino acid can be coupled to the resin. PyBop, HOBT, and DIEA serve as coupling reagents in this process. Any excess free amines that have not been coupled to the amino acid are then "capped" using acetic anhydride. After this capping process, the substitution level (the actual amount of free amines on the resin) is determined using a picric acid titration. This substitution level will be the basis for determining the quantity of reagents and amino acid to be used in subsequent coupling reactions.



Steps C and D: In Step C, as in step A, the piperidine serves as a base that yields free amines. The picric acid can now

form an adduct with these free amines. In Step D: The DIEA competes for the picric acid with the NH_3^+ on the resin, washing the picric acid off for collection and quantification. By monitoring the UV absorbance of this compound at 358 nm, we can determine the amount of picric acid collected and, therefore, the substitution level using Beer's Law ($A = \varepsilon cb$, where A is absorbance, ε is the molar absorptivity with unit M^{-1} cm^{-1}, c is concentration in moles per liter (M), and b is the path length).



Results of Picric Acid Titration

Resin Mass (mg)	Absorbance (at 358 nm)	Substitution (mmol/g)
11	0.749	0.4300
14	0.918	0.4126
11	0.740	0.4244
		0.422 ± 0.009