Bronx STEM Scholars Program

$Re^{v}O$ Tripeptide Sequences using D-amino Acids:

Models to understand the structure and binding of ^{188}Re radiotherapeutic agents

Gladys Gutierrez, Ordy Gnewou, Alrasheed Althour, Donna McGregor, Gustavo Lopez

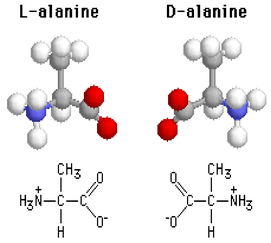
Department of Chemistry, Hostos Community College of the City University of New York

Department of Chemistry, Lehman College of the City University of New York, 250 Bedford Park Blvd. W. Bronx, NY 10468

BACKGROUND INFORMATION

- Cancer is the second leading cause of mortality in the U.S.
- 186Re and 188Re have already been used in radio-labeled peptides for targeted radiotherapy. These radioactive metals have shown favorable energy emission and decay properties.
- Peptides are convenient for drugs design because they can penetrate the tissues rapidly and clear the body as fast. Moreover, peptides exhibit low antigenicity, they can be produced in short sequences and can be synthesized and modified easily and at relatively low cost.

D vs L AMINO ACID (enantiomers)

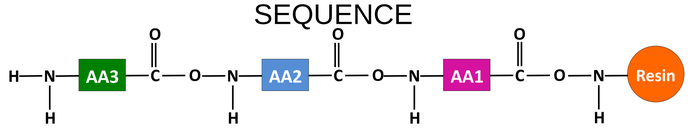


- Every amino acid (except glycine) can occur in two isomeric forms, because of the possibility of forming two different enantiomers (stereoisomers) around the central carbon atom.
- These are called L- and D- forms, analogous to left-handed and right-handed configurations.
- Only L-amino acids are manufactured in cells and incorporated into proteins. Some D-amino acids are found in the cell walls of bacteria, but not in bacterial proteins.
- Glycine, the simplest amino acid, has no enantiomers because it has two hydrogen atoms attached to the central carbon atom. Only when all four attachments are different can enantiomers occur.

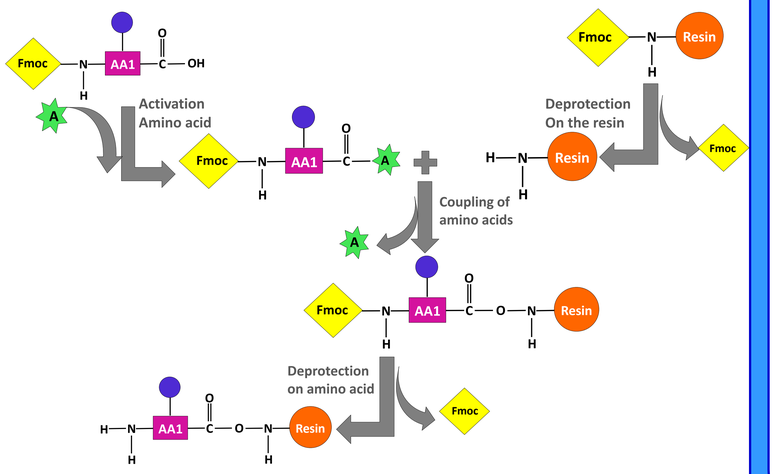
EXPERIMENTAL BIG PICTURE

Synthesize tri-peptides as chelates to investigate the features that bind to and stabilize Rhenium-188 as the radioactive metal in a targetted radiotherapeutic drug for the treatment of cancer.

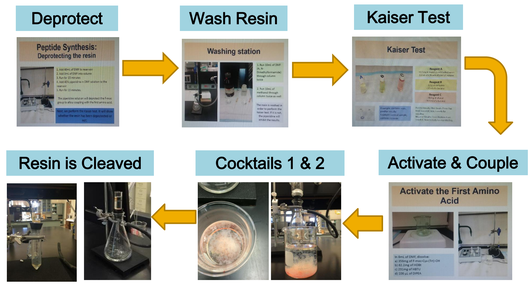
GRAPHICAL REPRESENTATION OF A TRIPEPTIDE SEQUENCE

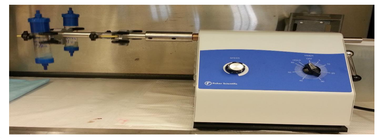


SOLID PHASE PEPTIDE SYNTHESIS



EXPERIMENTAL DETAILS



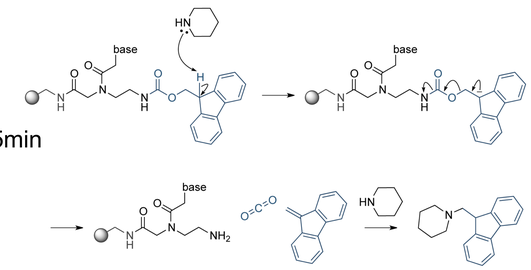


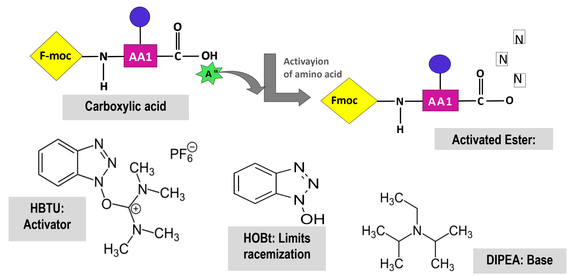
Shaker: simulates shaking by hand by rotating through 180°

- The shaker can hold 8 tubes at the time
- Instead of a 15 min deprotection and 4 hour coupling the shaker allows for a 10 min deprotection and a 3h coupling time.

STEP1: Deprotecting the Resin

- Make a 40 % piperdine in DMF solution
- Pass the solution over the column for 15min
- Wash the resin using 20 ml of DMF and Methanol



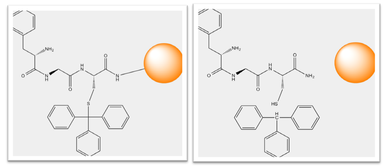


STEP 3: Amino Acid Activation

- 8ml of DMf
- 82.2ml of HOBt
- 231 mg of HBTU
- 106 ul of DIPEA
- 356 mg of Fmoc – cys

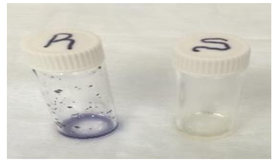
STEP 4: Cleave the resin

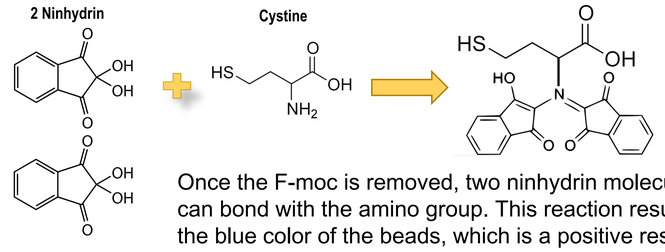
- Transfer dry resin in a vial
- Add the cocktail 1 into the dry resin and stir it for 3h
- Precipitated the solution in 150 ml of cold diethyl ether
- Filter the mixture and add cocktail 2 to the filtrate
- (repeat the process and get the second filtrate)
- Separated the peptide from the resin using a minimum amount of water



KAISER TEST EXPLAINED

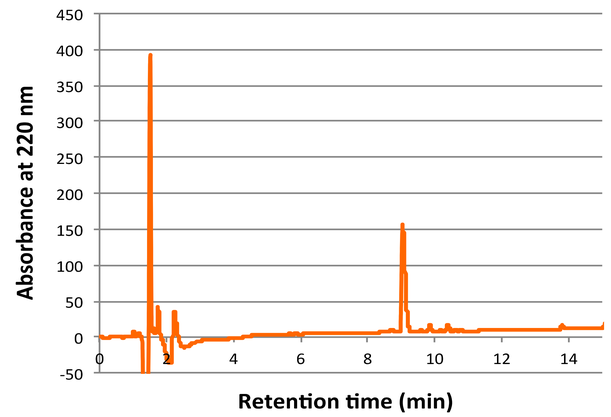
- Cleavage of the F-MOC protecting group frees the NH_2 for binding to the COOH of the amino acid ➔ **Positive test**: Blue beads
- Once an amino acid is attached the NH_2 group on the end is once again F-MOC protected. ➔ **Negative Test**: Clear beads.





Once the F-moc is removed, two ninhydrin molecules can bond with the amino group. This reaction results in the blue color of the beads, which is a positive result.

HPLC CHARACTERIZATION OF FGC TRIPEPTIDE SEQUENCE



ACKNOWLEDGEMENTS

Thanks to the STEM Scholars Program, Dr. Benjamin Burton-Pye from Hunter College, Professor Neal Phillip from BCC and Assistant Dean Felix Cardona from Hostos