



*A Reliable and Innovative Partner
to Speed up Your Success*

Expertise

Amino Acids & Peptides

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About Granlen

Granlen is a preclinical contract research organization that specializes in the “*more difficult*” chemistries of nucleosides, peptides, and phosphorous chemistry. Our research facility is located in Zhengzhou, China and we offer project management and business development support from San Diego. We can offer made-to-order compound libraries, hit-to-lead optimization, medicinal chemistry, and a wide range of chemistry services including custom synthesis, intermediates, references compounds, metabolites and impurities, route scouting, process development, and scale-up. We can provide nucleosides, peptide conjugates, disaccharides, haptens, antigens, and bioreagents. Heterocyclic chemistry is easy and always a welcome change. Teams at Granlen are also specialized in macrocyclic compounds used as super-chelating and MRI contrast agents.

Apart from our expertise in chemistry, as a preclinical CRO we offer an integrated development platform in order to speed projects toward clinical trials. We have developed relationships with some specialized facilities that may be able to assist with your preclinical and clinical development.



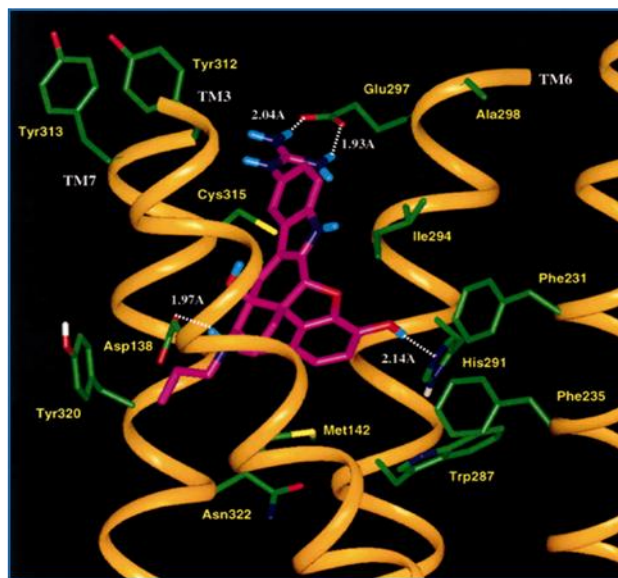
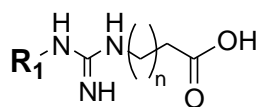
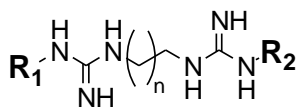
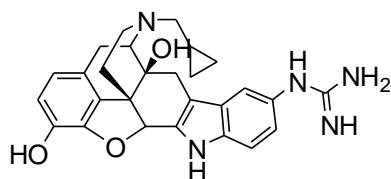
Our management team has led several compounds into clinical trials and we have expertise in every step toward Phase I. In conjunction with our drug discovery services we provide expertise in:

- Nucleosides, nucleotides, nucleic acids, and libraries
- Di-nucleotides, aminoacylated dinucleotides, triphosphates, methylene-phosphonates, and other organophosphorus derivatives
- Novel amino acids, dipeptides, and glycopeptides
- Bio-reagents, bio-materials, immunogens
- Macrocyclic compounds as super-chelating and MRI contrast agents

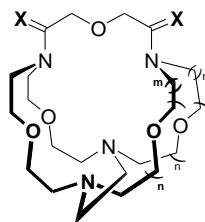
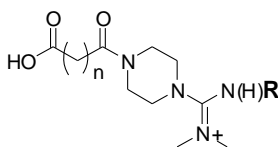
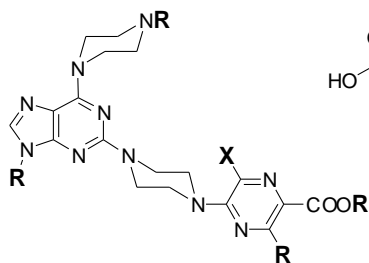
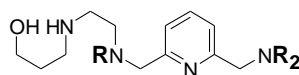
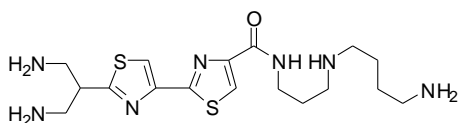


Polyamines

Our management team has invented techniques applicable to the synthesis of guanidines from more unreactive amines and has demonstrated simple methods for the preparation of multiple guanidines within the same molecule. The synthesis of carboxylic acids attached to guanidines has also proven quite facile. This work is technically challenging because orthogonal protection is required in order to prevent guanidine nitrogens from condensing with adjacent protective groups.



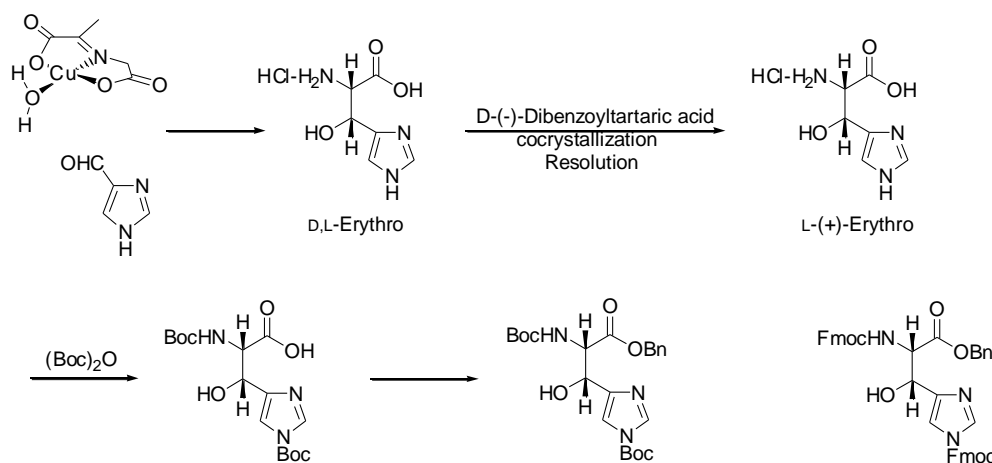
We have also prepared numerous examples of polybasic compounds, of which a small sampling are shown here. Synthesis of such challenging targets has required development of mixed protective group strategies and extensive purification expertise. Many such compounds require great care in handling as they may decompose when not in a buffered or acidic environment. All have proven to be quite stable as acid salts.



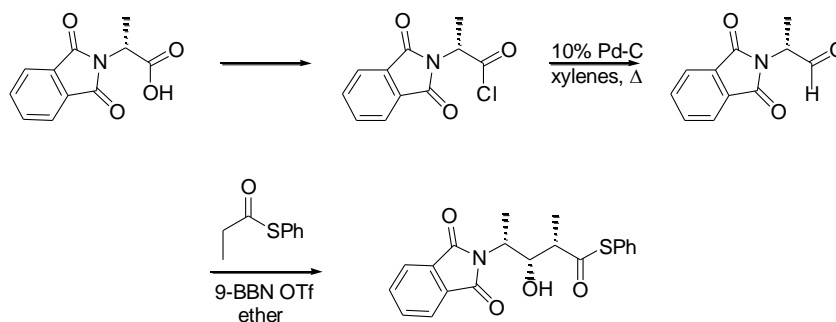


Amino Acids

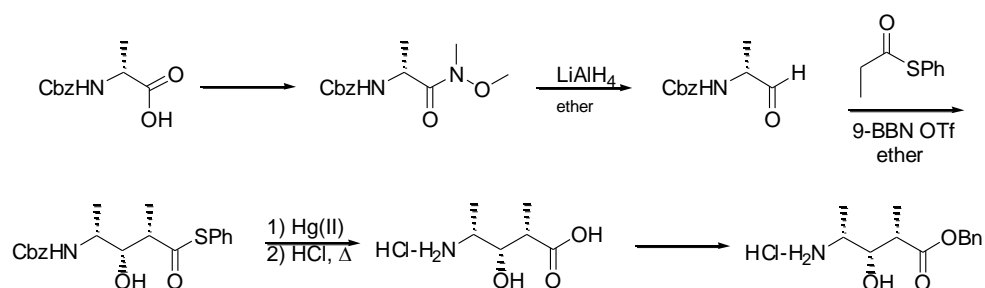
The amino acid β -hydroxyhistidine has been prepared previously. It is commonly prepared as a D,L-*erythro* monohydrochloride salt after condensation of 4-formylimidazole and *N*-pyruvylideneglycinatocopper (II). We have resolved this mixture by diastereomeric co-crystallization with D-(-)-dibenzoyltartaric acid. The L-(+)-*erythro* amino acid was further modified by converting it to the di-Boc protected benzyl ester. We have also prepared the orthogonally protected Fmoc- β -hydroxyhistidine benzyl ester.



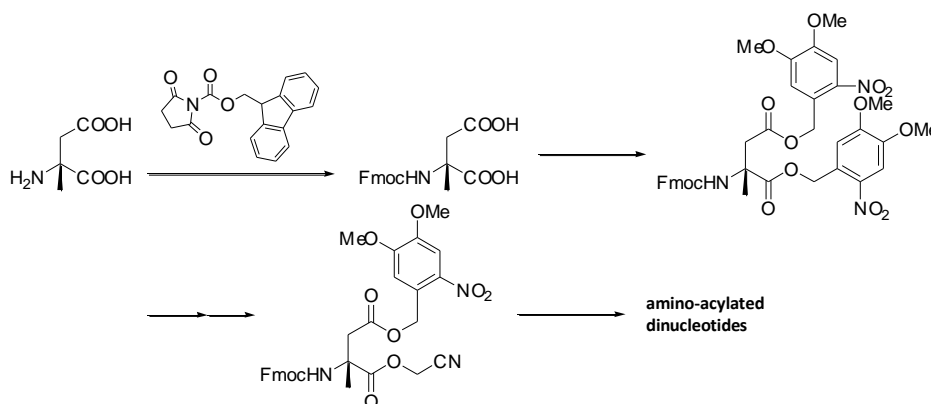
N-Phthaloyl-protected alanine has been converted to the corresponding acid chloride and reduced to the chiral aldehyde using a Rosenmund reduction. The key to effectively performing this reaction without racemization proved to be bubbling hydrogen gas through the heated reaction in such a way that the solvent was not evaporated and liberated HCl was removed from the reaction. A thermodynamically controlled aldol reaction was then used to set the adjacent two stereocenters in high de.



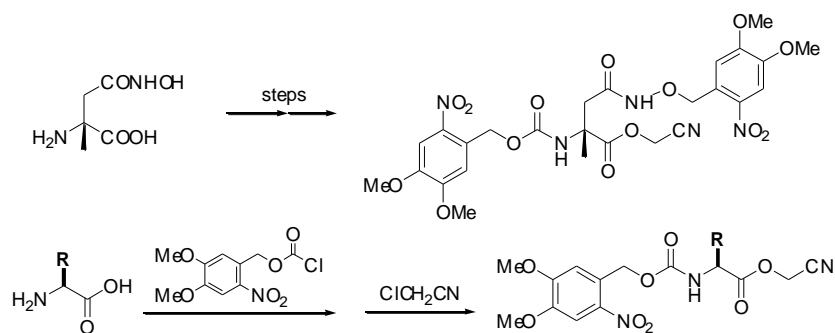
Due to the operational difficulties of the described Rosenmund reduction we explored an alternative strategy using Weinreb amides. The resulting chiral α -alkyl aldehyde proved to be more prone to racemization, however the resulting pentanoate recovered after aldol condensation could be crystallized to improve the de of intermediate amino acid.



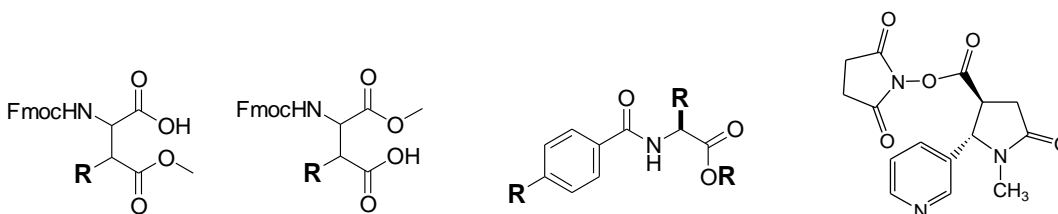
As shown below we have also prepared modified aspartic acid derivatives. The well protected aspartic acid analogues were activated into COOCH_2CN ester for their reaction with the dinucleotide pdDpA. The resulting amino-acylated pdCpA (dinucleotides) were ligated onto t-RNA for protein modifications. The NVOC groups were used as a protecting group during synthesis, and then removed using light after the AA was incorporated into the protein dihydrofolate reductase through modified aminoacylated t-RNAs.



The synthetic methodology was applied to several related amino acids in order to provide a range of modified proteins.

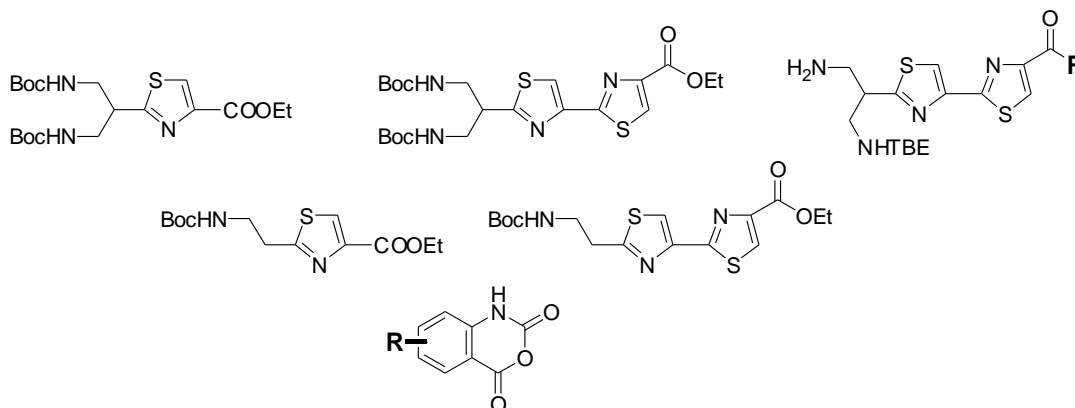


In addition to the described projects we have prepared a range of simple amino acids for various studies. Some general structures are shown below. The hippuric acid derivatives were synthesized for the metabolite studies of a potential anticancer drug. The nicotine carboxylic acid derivative was also synthesized for its conjugation.



Heterocyclic Amino Acids

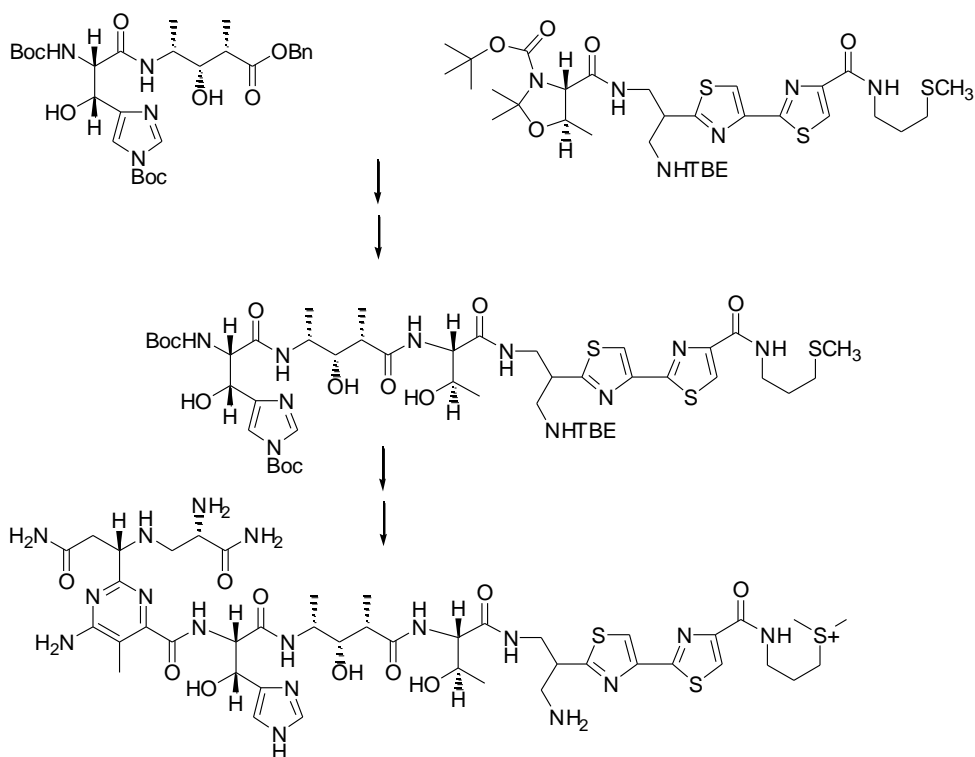
In addition to experience with hydantoins, thiohydantoins, creatinine, and related cycles we have prepared a number of thiazole amino acid derivatives. We have also prepared a number of isatoic anhydride derivatives.



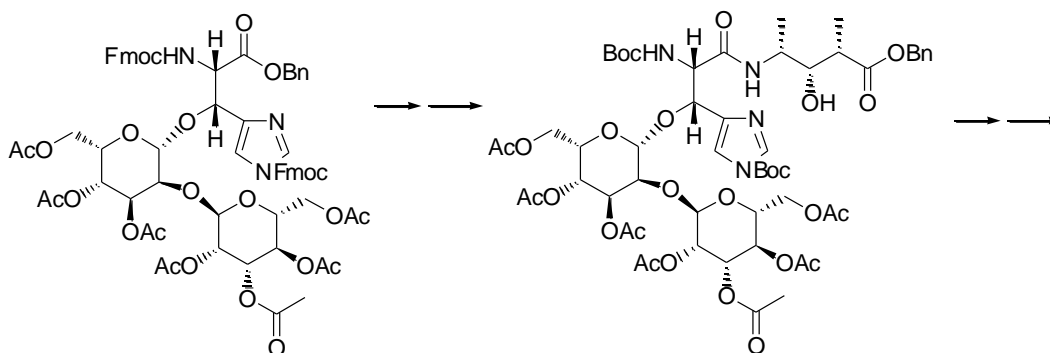
Peptides, Natural Products, and Derivatives

While we have performed a great deal of relevant work in the areas of amino acid and peptide synthesis, we are not able to disclose all of the work we have done.

As detailed on the next page, we have coupled many of the previously described amino acids in order to prepare di- and tripeptides for the synthesis of deglycobleomycin and deglycobleomycin analogs as well as bleomycin and bleomycin analogs. As detailed on the following page, one of the classes of bleomycin analogs prepared was an aminomethyl-deglycobleomycin analog. This was prepared achiral and diastereomeric resolution was demonstrated at both the tripeptide and pentapeptide stage using chiral HPLC. The acetone derivative shown was prepared to facilitate diastereomeric resolution.

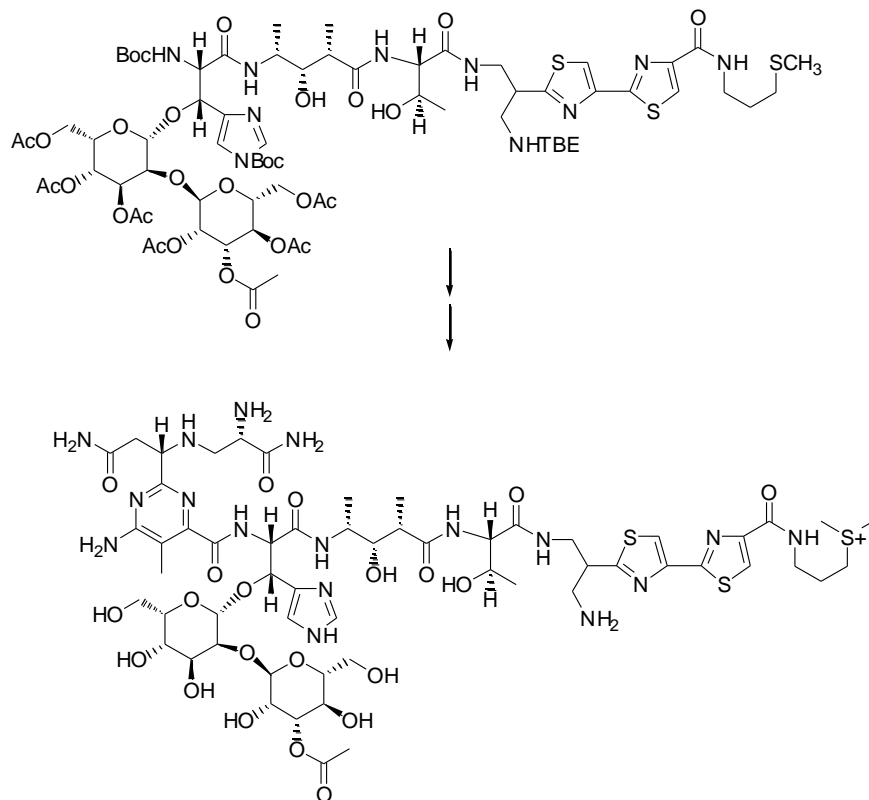


The aminomethyl-deglycobleomycin derivatives were prepared in order to evaluate their ability to modulate the supercoiling of ds-plasmid DNA. We found that the sugars on the native bleomycin were requisite for this effect and we synthesized the glycosylated bleomycin analogs via two synthetic routes. The Koenigs-Knorr reaction was performed using silver triflate with Boc-protected glycopeptides and the more aggressive TMS-triflate catalyst were used with the corresponding Fmoc-protected glycopeptide. The bleomycin disaccharide was also prepared by both the traditional Koenigs-Knorr route as well as a phosphonate coupling approach.





In addition to the aminomethyl bleomycin shown below we have also prepared other bleomycins and have suffered through the difficulties of purification by CM-Sephadex as well as by HPLC.



Summary

The management and employees of Granlen have significant expertise in the areas of amino acid synthesis, peptide formation, peptide conjugates, peptidomimetics, and glycopeptides. As alluded to within this document, we also have significant expertise in the areas of nucleoside chemistry and phosphorous chemistry. In a bulleted fashion, the team at Granlen can assist with:

- Design and synthesis of amino acid derivatives
- Orthogonal protection and deprotection strategies for peptides and peptidomimetics
- Substituted guanidine derivatives
- Di-, tri- and larger peptide syntheses
- Glycopeptides, and
- Peptide conjugates