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Distributed Drug Discovery: Synthesis of Unnatural Amino Acids as Potential Antimalarial Drugs

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Distributed Drug Discovery:

Synthesis of Unnatural Amino Acids as Potential Antimalarial Drugs

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Of the Requirements for

Honors Scholar

by

Amanda Dugan

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This Project Thesis, directed and approved by the candidate's committee, has been accepted by the Honors College of Abilene Christian University in partial fulfillment of the requirements for the distinction

HONORS SCHOLAR
[or: HONORS ASSOCIATE]

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Dr. Jason Morris, Dean of the Honors College

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Date

Advisory Committee

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Dr. Bruce Hopkins, Committee Chair

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Dr. Greg Powell, Committee Member

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Dr. Cynthia Powell, Committee Member

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Dr. Kim Pamplin, Department Head

Through the collaboration of many institutions across the globe, the Distributed Drug Discovery project founded at Indiana University-Purdue University Indianapolis seeks to aid in the development of drugs for the developing world. In response to two antimalarial assay hits, our team at Abilene Christian University has synthesized many unnatural amino acid analogs using resin-based combinatorial chemistry. Proton nuclear magnetic resonance spectroscopy has been used to characterize the compounds and thin layer chromatography to determine purity. All compounds were purified on hypersep cyanosilica columns.

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Introduction

"Distributed Drug Discovery" (D^3) is a concept developed by a team led by William L. Scott at Indiana University-Purdue University Indianapolis (IUPUI) to address some of the larger problems encountered in drug discovery in relation to underdeveloped nations.[1] One of these problems is the pharmaceutical industry develops drugs for economic gain, thus diseases plaguing the developing world are often ignored.[1] One of the goals of D^3 is collaboration between non-profit organizations and institutions that can focus on these neglected diseases.

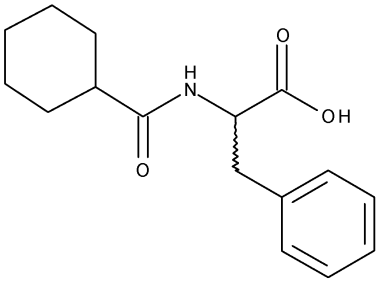
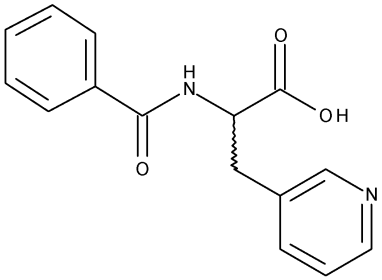
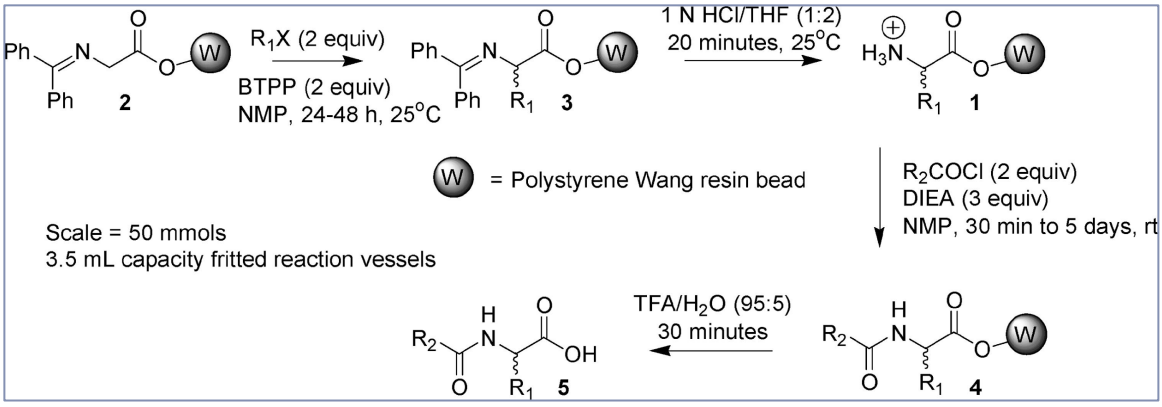
Antimalarial compounds from Screening	
qHTS-1	qHTS-2
	
Potency (⌈M): 10.4179	2.9632

Figure 1. Two amino acid analogs that demonstrated biological activity in a validated assay testing for inhibition of apicoplast formation in Plasmodium falciparum.[3]

In this collaboration, the large workload of the drug discovery process can be distributed to many smaller groups working with one another as well as aiding in the educational process through real-world application of drug synthesis in a global project.[1] Combinatorial chemistry[2] is employed to aid in the drug discovery process along with inexpensive equipment and procedures, that were developed specifically for this effort.[3] There are three main stages in the D^3 project: molecule library planning/computational analysis, chemical synthesis, and biochemical screening.[1] The first stage involves

creating virtual catalogues of potential drug molecules based on known compounds, which possess the desired biological activity (Fig. 1). The second stage involves synthesizing the potential drug molecules. The third stage is the biological screening of the synthesized molecules for activity. Our research focuses on the second stage through the use of resin-based chemistry with the Bill-board apparatus developed at IUPUI to simplify the small-scale parallel synthesis of multiple compounds. The general reaction scheme for this synthesis can be seen in Scheme 1 and an example Bill-board reaction in Figure 2. The details of the actual synthesis are covered in the experimental section.



Scheme 1. Benzophenone imine Gly-Wang resin alkylation-acylation reaction scheme used to create unnatural amino acid analogs.[3]

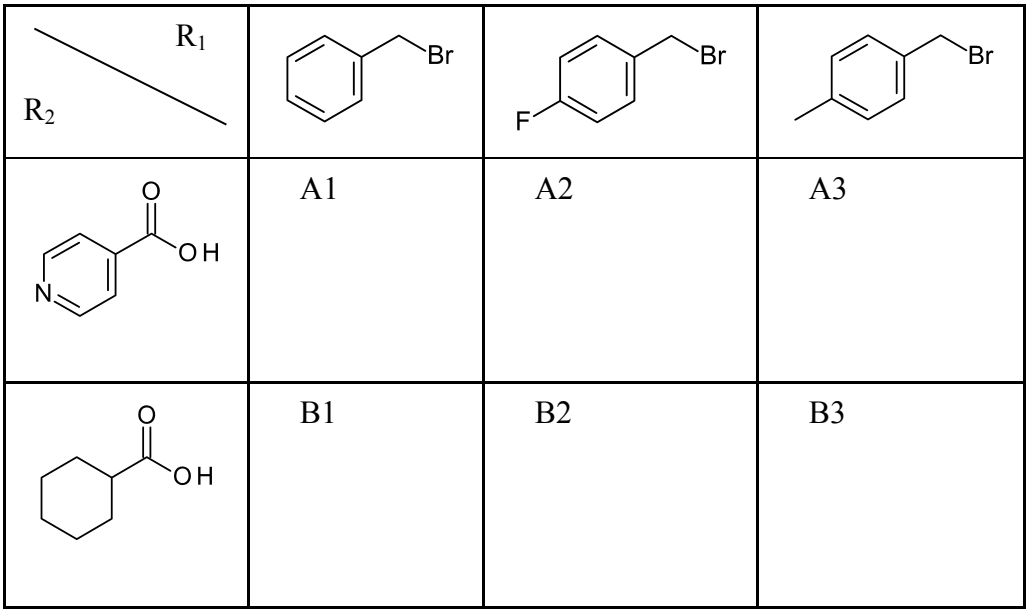
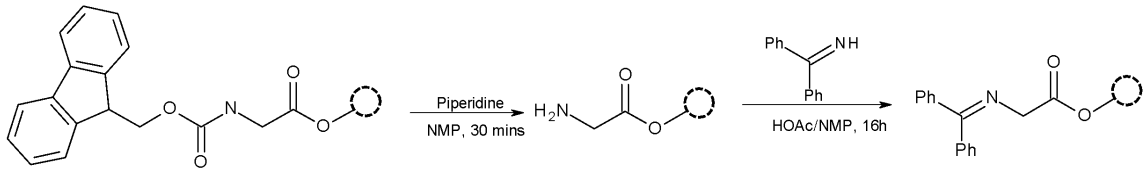


Figure 2. Sample 2x3 reaction array for combinatorial chemistry on Bill-board apparatus. The symbols A1 through B3 represent the chemical compound synthesized in that reaction vessel.

Discussion

In order to use resin-based chemistry, the Fmoc protecting group on the resin-bound glycine must be replaced with a benzophenone imine group for our reactions (Scheme 2). This benzophenone imine group helps to facilitate the alkylation of the alpha carbon on the resin molecule. The procedure for producing this resin is detailed in the experimental section. The resin is synthesized in the lab, as the preparation is less expensive than buying the resin already prepared. The resin prepared for this work had comparable results to the resin previously prepared by another student in a small 4 x 4 test plate. This prepared resin was used for all of the syntheses in this report.



Scheme 2. Preparation of Fmoc Gly-Wang resin for benzophenone imine Gly-Wang resin.[5]

With the prepared resin, our team pursued the synthesis of compound analogs of the two molecules that were active in the antimalarial screen. The alkylating (R_1) and acylating (R_2) reagents were chosen to allow for the synthesis of compounds that vary in molecular properties in order to explore the structure-activity relationships for this class of compounds. This work was focused on bicyclic compounds as both alkylating and acylating reagents. There was little success in using certain bicyclic alkylating reagents (ACU-ACD-29 A2, A3, B2, B3), especially 2-(bromomethyl) naphthalene. The TLC on A2 and B2 had many additional spots and the NMR was also complex, revealing that the reaction did not proceed selectively. The same effect was noted on the A3 and B3 compounds but there were not as many byproducts, the alkylation with 1-(bromomethyl) naphthalene seeming to be more successful.

With the mixed results of the bicyclic alkylating reagent plate, bicyclic acylating reagents were tested next. The first plate (ACU-ACD-45 A1-B3) indicated very little side product on TLC. The NMR spectra also indicated relatively pure product. As far as could be observed from the characterization done, these compounds were very successful in synthesis. The same was noted for ACU-ACD-69 with a few slight possible impurities. The high yields, with most being above quantitative, likely indicate that either solvent was still present when the weights were obtained or there were impurities present.

Bicyclic compounds used for alkylation were revisited with plate ACU-ACD-85. In this trial, the amount of 2-(bromomethyl) naphthalene was increased to 3 equivalents to see if increasing the concentration would facilitate the reaction for better results in A2 and B2. In A3 and B3, the alkylation was catalyzed by the addition of KI to react *in situ* with the 2-(bromomethyl) naphthalene to replace the bromine with iodine to act as a

better leaving group. The results of this plate were mixed, as there seemed to be two products seen on thin layer chromatography (TLC) overlapping each other. The NMR spectra look relatively clean with a few possible impurities. Another indication of the presence of impurities were the greater than quantitative yields for this plate.

Compound purifications were carried out using cyanosilica stationary phase chromatography cartridges. Results are reported in the table below and the general procedure can be found in the results section. Overall, the procedure used was relatively successful with 10 compounds successfully purified. All compounds except for ACD-29 compounds and ACD-45-A compounds were successfully purified. ACD-29 compounds were not purified due to previous complications with a different silica column.[7] Purifications were then halted until new cartridges could be obtained. These hypersep cyanosilica columns were then tested with a previously made compound and noted to be successful in the purification process.[8] There were some issues with the ACD-85-B compounds as well as ACD-45-B1. ACD-45-B1 seemed to have changed properties after being stored for a long period of time. This compound would not dissolve in the 5% methanol/dichloromethane solution in which the other compounds dissolved to be loaded on the column. The compound initially appeared to dissolve until a yellow, greasy looking substance began to form. Even the addition of extra methanol did not dissolve this substance and the purification was abandoned. The ACD-85-B compounds were successfully transferred onto the silica columns, however, there was not a significant return of product. Even after washing the column with a polar solvent, there was no further product was removed from the column. Due to these results, it is suggested that the compound likely decomposed on the column. The procedure that is included in the

experimental section became the standard procedure for all purifications due to the large success in returning product for most purifications. The results of the purifications are reported below in Table 1.

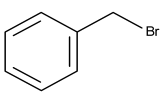
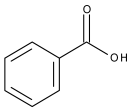
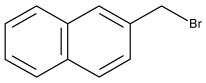
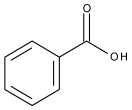
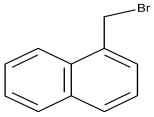
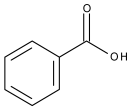
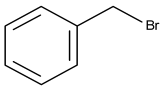
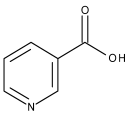
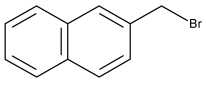
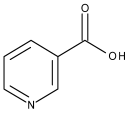
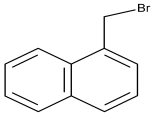
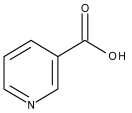
Some of the alkylating and acylating reagents employed in these syntheses were synthesized in lab either due to the cost of the reagent or the reagent being unavailable commercially. The first reagent synthesis was 4-(methoxyphenyl)methyl methanesulfonate as previously attempted by another student.[6] Unfortunately the same issue occurred as previously encountered as the 4-(methoxyphenyl) methanol reacted with itself to form an ether. The synthesis was abandoned as it is unknown how to solve the issue and to obtain the methanesulfonate.

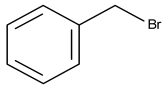
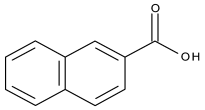
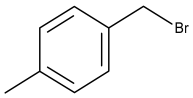
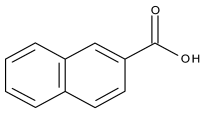
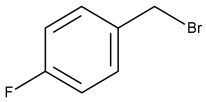
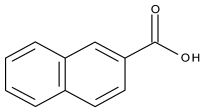
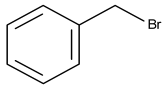
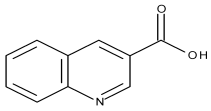
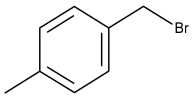
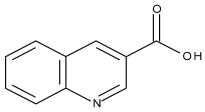
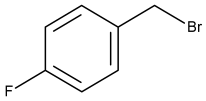
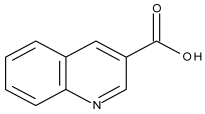
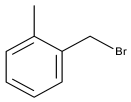
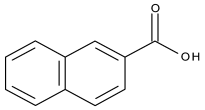
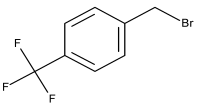
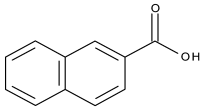
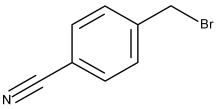
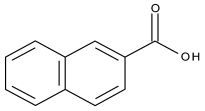
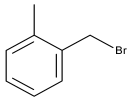
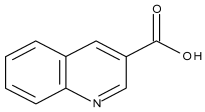
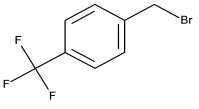
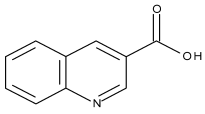
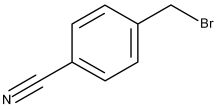
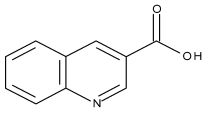
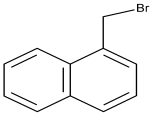
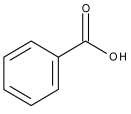
Another student previously tried to synthesize the bromine derivative of 4-(chloromethyl) anisole, but it was unstable.[9] The chlorine derivative was attempted, but due to adding triethylamine in error, the thionyl chloride did not react with the 4-methoxybenzyl alcohol. The recovered alcohol with a slight amount of triethylamine was used to attempt the synthesis again without additional triethylamine, but the reaction was unsuccessful, as the alcohol still did not react.

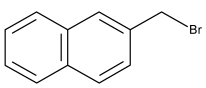
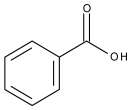
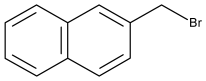
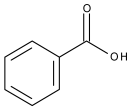
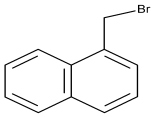
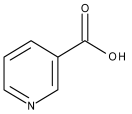
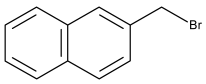
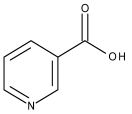
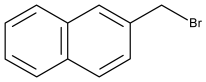
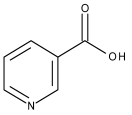
The final synthesis attempted was 3-pyridyl methanol from nicotinic acid. The first procedure used sulfuric acid in methanol to synthesize methyl nicotinate before using sodium borohydride to reduce to the alcohol.[10] The esterification in the first step of this procedure was successful with the greatest yield at about 47%. However, with the reduction, results from the literature procedure could not be reproduced. The second procedure used $BF_3 \bullet Et_2O$ with sodium borohydride to reduce the nicotinic acid to 3-pyridyl methanol.[11] Most of the product obtained was decomposed THF and the desired

product was not noted through characterization with ^{1}H NMR. Another product was noted with the decomposed THF, but could not be readily identified. This product was separated from the decomposed THF through column chromatography with silica gel. This by-product seemed to have changing properties as it would first be a very thick oil that would solidify after a while. Other characteristics were noted to change during the synthesis such as change in color of the aqueous layer from yellow to colorless/pale yellow. Even after isolating this other product, it could not be readily characterized. It is possible that the product is an unstable borate compound.

Table 1. Alkylating and acylating reagents used for each amino acid analog synthesis. See Scheme 1 for core amino acid structure.[13] *Compound serial numbers are reported in the format of "institution-student initials-page number-Bill-board position."

Serial Number*	Alkylating Reagent, R_1	Acylating Reagent, R_2	crude % yield	% Return
ACU-ACD-29-A1			37.6	not purified[15]
ACU-ACD-29-A2			46.6	0%
ACU-ACD-29-A3			98.8	not purified[15]
ACU-ACD-29-B1			84.7	not purified[15]
ACU-ACD-29-B2			70.9	0%
ACU-ACD-29-B3			>100	not purified[15]

Serial Number*	Alkylating Reagents, R_1	Acylating Reagents, R_2	crude % yield	% return
ACU-ACD-45-A1			>100	not purified[16]
ACU-ACD-45-A2			>100	not purified[16]
ACU-ACD-45-A3			88.7	not purified[16]
ACU-ACD-45-B1			>100	not purified[17]
ACU-ACD-45-B2			>100	19.4
ACU-ACD-45-B3			>100	13.3
ACU-ACD-69-A1			98.8	34.7
ACU-ACD-69-A2			96.9	34.5
ACU-ACD-69-A3			>100	31.3
ACU-ACD-69-B1			>100	15.7
ACU-ACD-69-B2			>100	26.8
ACU-ACD-69-B3			>100	16.2
ACU-ACD-85-A1			>100	29.7

Serial Number*	Alkylating Reagents, R_1	Acylating Reagents, R_2	crude % yield	% return
ACU-ACD-85-A2			>100	30.2[18]
ACU-ACD-85-A3			>100	
ACU-ACD-85-B1			>100	0
ACU-ACD-85-B2			>100	0[18]
ACU-ACD-85-B3			>100	

Conclusion

The bicyclic ring systems work as better acylating reagents than alkylating reagents. If used as alkylating reagents, the use of a catalyst such as KI or increasing the concentration helped to facilitate the alkylation, though more methodology development is needed. Altogether, there were 24 amino acid analogs synthesized. ACD-29 A2, A3, B2, and B3 had many impurities and the syntheses were not successful. Other plates were purified to varying degrees of success. Overall, the 10 compounds successfully purified and 3 additional compounds are ready to be sent for biological assay. Since this project is relatively new, it is unsure what the results of the biological assay will report. However, better selectivity for creating more potent molecules for possible antimalarial analogs can be determined from the results of the biological assay.

While there are no conclusions to exactly what is occurring in the 3-pyridyl methanol synthesis, identification of what is happening can be used to make necessary

changes to the procedure to get more desirable results. The main issue may lie with the borate not hydrolyzing properly and more success may be found with quenching the reaction at a warmer temperature instead of in the ice bath. Altogether 4 reagent syntheses were attempted and were unsuccessful.

Experimental

Preparation of Benzophenone imine Gly-Wang resin[5]

5.08g (3.56 mmol) Fmoc Gly-Wang resin (from Creosalus, loading factor 0.7 mmol/g) was added to a 100 mL fritted peptide reaction vessel and washed with 42 mL of 30% piperidine in 1-methyl-2-pyrrolidinone (NMP). Another 42 mL of the 30% solution was added and contents shaken for 30 minutes at room temperature. Waste was drained from the vessel and resin washed with 5 x 40 mL NMP. Benzophenone imine (5.97 mL, 35.6 mmol) in 42 mL NMP was added to the vessel along with 1.77 mL acetic acid. The contents were shaken for 18 hours at room temperature. The solution was drained and the resin washed with 5 x 40 mL NMP, 5 x 40 mL CH_2Cl_2, and then dried under a vacuum. A total of 4.74 g (93% yield) was collected and placed in a vacuum desiccator for storage.

Resin-based Unnatural Amino Acid Synthesis

Alkylation - Approximately 71.0 mg of Benzophenone Imine Gly-Wang Resin (loading factor 0.7 mmol/g) was weighed out for each reaction vessel (six total) and placed in each vessel. The Bill-board was placed on the drain tray and each vessel was washed with 3 x 3 mL of 1-methyl-2-pyrrolidinone (NMP). The screw caps with clean septa were placed on the bottom of each vessel. Alkylating reagent **1** [0.5 mL of 0.2 M solution in NMP, 100 μmol, 2 equivalent] was added to A1 and B1. Alkylating reagent **2** [0.5 mL of 0.2 M solution in NMP, 100 μmol, 2 equivalent] was added to A2 and B2.

Alkylating reagent **3** [0.5 mL of 0.2 M solution in NMP, 100 µmol, 2 equivalent] was added to A3 and B3. The base, tert-butylimino-tri(pyrrolidino)phosphorane (BTPP) [0.5 mL of 0.2 M solution in NMP, 100 µmol, 2 equivalent], was added to all six reaction vessels. The tops were sealed with screw tops and septa. The Bill-board was then placed on the orbital shaker for 48 to 72 hours at room temperature.

Hydrolysis - The Bill-board was removed from the orbital shaker, inverted and the bottom caps removed from the vessels. The board was then reverted and placed on the drain tray and top caps removed to allow alkylating reagents and solutions to drain. The resin was then washed with 1 x 3 mL tetrahydrofuran (THF). The bottoms of the vessels were recapped with clean caps. To each vessel, 2.5 mL of a 1N solution of HCl/THF (1:2) was added. The tops were capped and the Bill-board was placed back on the shaker for 20-30 minutes at room temperature. The Bill-board was removed, caps removed as done previously. On the drain tray, waste was removed before the resin was washed with 1 x 3 mL THF each vessel.

Acylation - Two different acylating methods were used depending on whether the acylation reagent was an acid chloride or a carboxylic acid.

For an acid chloride, the resin bound compound was washed with 1 x 3 mL NMP each vessel and the bottoms of the vessels recapped. Acylating reagent **A** [0.5 mL of 0.3 M solution in NMP, 150 µmol, 3 eq] was added to the three vessels in row A (A1-3). Acylating reagent **B** [0.5 mL of 0.3 M solution in NMP, 150 µmol, 3 eq] was added to the three vessels in row B (B1-3). Then DIEA (N,N-diisopropylethylamine) [0.5 mL of 0.3 M solution in NMP, 150 µmol, 3 eq] was added to all six vessels. The tops of the

vessels were capped and the Bill-board was placed on the orbital shaker for 48-72 hours at room temperature.

For a carboxylic acid, the resin was washed with 3 x 2.5 mL of a 0.2 M solution of DIEA in NMP and 2 x 2.5 mL NMP. The bottoms were recapped. Acylating reagent **A** [1.0 mL of 0.25 M **A** and 0.25 M HOBt (hydroxybenzotriazole) in NMP, 250 μmol, 5 eq for both **A** and HOBt] was added to the three vessels in row A (A1-3). Acylating reagent **B** [1.0 mL of 0.25 M in 0.25 M HOBt (hydroxybenzotriazole) in NMP, 250 μmol, 5 eq for both **B** and HOBt] was added to the three vessels in row B (B1-3). The coupling reagent N,N-diisopropylcarbodiimide (DIC) [0.5 mL of 0.5 M in NMP, 250 μmol, 5 eq] was added to all six reaction vessels. The tops of the vessels were recapped and the Bill-board was placed on the orbital shaker for 48-72 hours at room temperature.

Cleavage of Products from Resin - Each reaction vessel was washed with 2 x 3 mL NMP, 2 x 3 ml THF, and 3 x 3 mL dichloromethane (DCM). The bottoms of the vessel were recapped and 2 mL of a trifluoroacetic acid (TFA), water solution (95:5 TFA/H_2O) was added to each vessel. The vessels were capped and the Bill-board was placed on the orbital shaker for 30 minutes. The filtrate was collected in tared vials by inverting the Bill-board, removing the bottom caps, putting a vial over each vessel, placing the collection rack over the vials, and reverting the Bill-board. The resin was washed with 2 mL TFA solution and 2 mL DCM for each vessel. The vials in the collection rack were placed in an apparatus that allows for the evaporation of solvents under a stream of nitrogen. This system was connected to a bubbler to capture evaporated TFA in a solution of 2 M NaOH. Once the samples were dried down, they were transferred to be heated slightly under vacuum to ensure all solvents were removed. After

this, the samples were characterized with 1H NMR to analyze the compounds' structure to ensure that the alkylation and acylation were successful.

Purification of Unnatural Amino Acids

The standard procedure for purifications was as follows. The desired product was dissolved in 0.3 mL 5% methanol/dichloromethane and loaded onto the silica column and allowed to dry. Then the first fraction was collected with 2 mL of hexanes. Fractions 2-3 were collected with 1 mL 75/25 hexanes/acetone. Varying amounts of fractions were collected with the same 75/25 solution as previously. The number of fractions required was different for each compound.[11] If necessary, the polarity of the solution was increased as needed for additional fractions by increasing the percentage of acetone in the solvent. Each purification was monitored by TLC to note when different products were coming out of the column and when the purification was complete and no other product was being removed from the column. Once the purification was complete, the solvent was allowed to evaporate. Once evaporated, a full TLC was done to compare different fractions to a sample of the original products before purification to determine which fractions contained the desired product. Once the potential desired product was found, the appropriate fractions were combined in NMR solvent ($CDCl_3$ and CD_3OD) and a H^1NMR was run to confirm that the desired product was recovered. After it was confirmed that the desired product was obtained, the product dissolved in the solvent was transferred to a tared vial and the solvent allowed to evaporate. Once all solvent evaporated, the vial could be massed to determine the return of product.

^{1}H NMR Data for Unnatural Amino Acids

All compounds synthesized were characterized with ^{1}H NMR with a Varian EM360A NMR spectrometer at 60 MHz in $CDCl_3$ and CD_3OD. The spectra tended to have similar characteristics with the methyne and methylene protons usually indicative of our compounds. There is some variability depending on the substituent. The methyne proton usually appeared as a triplet or sometimes a doublet of doublets with a chemical shift of approximately 4.7 to 5.2 PPM from TMS. The methylene protons usually appeared as a doublet of doublets with a chemical shift of approximately 3.2-3.7 PPM from TMS. The below compounds are representative of the other compounds synthesized.

ACU-ACD-29 A3: ™ 8.16-7.23 (m, 12 H), 4.92 (t, 1 H), 3.41 (m, 2 H).[11]

ACU-ACD-29 B3: ™ 8.87 (m, 2 H), 8.43-7.42 (m, 9 H), 5.11 (t, 1 H), 3.76 (m, 2 H).

ACU-ACD-45 A2: ™ 8.23 (s, 1H), 7.82-7.30 (m, 10 H), 5.04 (t, 1 H), 3.22 (d, 2 H), 2.30 (s, 3 H).

ACU-ACD- 45 B2: ™ 9.65 (s, 1 H), 9.26 (s, 1 H), 8.56-8.17 (m, 8 H), 5.33 (d, 1 H), 3.54 (d, 2 H), 2.56 (s, 3 H).

ACD-ACD-69 A1: ™ 8.56 (s, 1 H), 8.42-7.36 (m, 10 H), 5.38 (t, 1 H), 3.67 (d, 2 H), 2.73(s, 3 H).

ACU-ACD-69 B1: ™ 9.32 (s, 1 H), 8.93 (s, 1 H), 8.44-7.81 (m, 8 H), 5.02 (d, 1 H), 3.23 (d, 2H), 2.32 (s, 3 H).

ACU-ACD-85 A3: ™ 7.90-7.36 (m, 12 H), 5.15 (t, 1 H), 3.41 (m, 2 H).[7]

ACU-ACD-85 B3: ™ 9.05 (s, 1 H), 8.76 (m, 1 H), 8.40 (1 H), 8.29 (s, 1 H), 7.88-7.35 (m, 7 H), 5.15 (d, 1 H), 3.44 (m, 2 H).

References and Notes

1. Scott, W. L.; O'Donnell, M. J. *Distributed Drug Discovery, Part 1: Linking Academia and Combinatorial Chemistry to Find Drug Leads for Developing World Diseases*. J. Comb. Chem., **2009**, *11*, 3-13.

2. Combinatorial chemistry is a laboratory technique in which many different molecules can be synthesized simultaneously, employing the same chemical steps in multiple reaction vessels. Frequently, as in this work, a matrix array of reaction vessels is employed. Two chemical structural elements are varied along the x and y axes of the array, creating a unique compound in each vessel.

3. Scott, W. L.; O'Donnell, M. J. *Distributed Drug Discovery, Part 2: Global Rehearsal of Alkylating Agents for the Synthesis of Resin-Bound Unnatural Amino Acids and Virtual D^3 Catalogue Construction*. J. Comb. Chem., **2009**, *11*, 14-33.

4. *Primary qHTS for delayed death inhibitors of the malarial parasite plastic, 96 hour incubation – BioAssay Summary* [Web]. PubChem BioAssay 504834. National Center for Biotechnology Information, 2013.

5. Procedure from personal correspondence with IUPUI.

6. Collins, Alexander Dru, Unpublished Research Notebook. Abilene Christian University. ADC-70

7. See in unpublished research notebooks ACD-43 and RWR-9,17,33

8. See ACD-97

9. Pruett, Cliff Harrison, Unpublished Research Notebook. Abilene Christian University. CHP-87

10. C. Shripanavar et al. *Der Pharmacia Lettre* **3**(4) 264-266 (2011)

11. S. Cho et al. *Bull. Korean Chem. Soc.* **2004**, Vol. 25, No. 3 407-409

12. CD_3OD solvent peak overlaying sample peak.

13. Crude yields that were purified through cyanosilica columns.

14. Dugan, Amanda, Unpublished Research Notebook. Abilene Christian University. See various purification procedures.

15. These compounds were remade in plate ACD-85 and those were purified.

16. These compounds will be sent off for biological assay as the compounds appear relatively pure from TLC and NMR. High initial yields may be from solvent present.

17. Compound did not dissolve in solvent and could not be purified.

18. These products were combined for the purification since the same molecule was formed.