# *Synthesis and Applications of Site-Specific Analogue of Farnesyltransferase in Reversible Immobilization*

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Sponsored by Undergraduate Research Opportunities Program, Department of Chemistry

## Background

### I. Protein Farnesylation

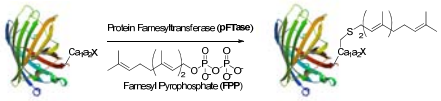


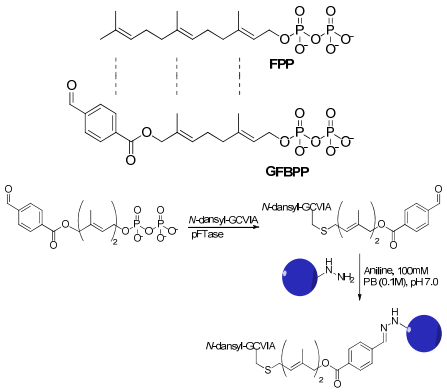

**Fig. 1** Attachment of farnesyl pyrophosphate to a protein with C-terminal sequence of $Ca_1a_2X$ (CaaX-box), where C is cysteine; a1 and a2 are small aliphatic amino acids; and X is an amino acid most influential on specificity[1]

- Moderate tolerance of changes on FPP terminal methyl group; allow **functionalization** of FPP analogues
- **High site-specific** farnesylation at terminal thiol group on CaaX-box; only allow reaction with CaaX-box protein

### II. Protein Immobilization

- Protein immobilization onto solid surfaces applicable to manufacture of: functional protein microarrays, biosensors, continuous flow reactors, etc.
- Enzymatic biological immobilization **useful** due to **high selectivity**, reactivity under **mild conditions** to reduce protein degradation

## Hypothesis




- Functionalize FPP analogue; formylbenzoate moiety at terminal methyl group of geranyl pyrophosphate to synthesize **G**eranyl **F**ormyl**B**enzoate **Pyroph**ate (**GFBPP**)
- Characterize enzymatic activity of pFTase and GFBPP ($K_m$, $V_{max}$, $K_{cat}$, etc.) on the model fluorescence peptide, *N*-dansyl-GCVIA, using continuous fluorescence assay
- Immobilize prenylated *N*-dansyl-GCVIA onto hydrazine functionalized beads, determine immobilization efficiency

## References

1. *J. Lipid Res.*, **2006**, 47, pp 681 – 699
2. *J. Org. Chem.* **1996**, *61*, pp 7738 – 7745
3. *Chem. Comm.*, **2010**, *46*, pp 8998 – 9000.

## Acknowledgements

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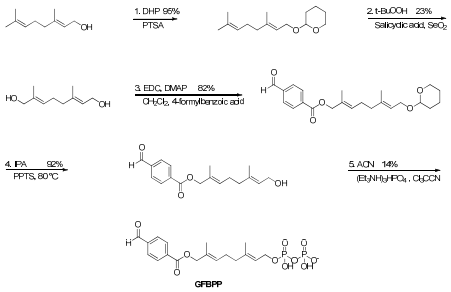
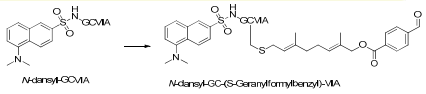
## Methods - Synthesis




**GFBPP** synthesized in five steps starting from commercially available Geraniol (CAS# 106-24-1)

1. Alcohol moiety on geraniol is protected from side reactions via dihydropyran
2. Protected geraniol is regio-selectively oxidized at C-8 to a terminal alcohol
3. Protected geranyl alcohol is coupled w/4-formylbenzoic acid using EDC
4. Protecting THP group removed to result in alcohol
5. Alcohol displacement with $[(n\text{-}Bu)_4N]_3HP_2O_7$ results in diphosphate of interest, **GFBPP**; purification by ion-exchange chromatography/ RP-HPLC
6. **GFBPP** characterized by $^1H$ NMR, $^{13}C$ NMR, $^{31}P$ NMR, and MS/GC

## Methods: Enzymatic Activity[3]

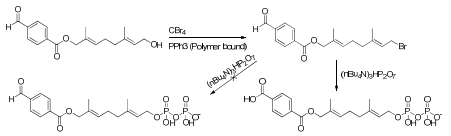



- *N*-dansyl-GCVIA is a environmental-sensitive fluorophore peptide – increased hydrophobicity of its molecular environment is positively correlated with observed fluorescence intensity (FI)
- 50 mM Tris · HCl, pH 7.5, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5.0 mM DTT, 2.4 μM *N*-dansyl-GCVIA, 0.040 % (*w/v*) *n*-dodecyl-s-D-maltoside, 80 nM PFTase, and varying concentrations of **GFBPP** (0-10 μM).
- Equilibrated at 30 °C for 5 min, initiated by the addition of PFTase, and monitored for increase in fluorescence ($\lambda_{excitation}$ = 340 nm, $\lambda_{emitted}$ = 505 nm, slit widths = 10 nm for both) for approximately 10 min.
- Data collected in intensity units per min, converted to peptide (nM) per sec

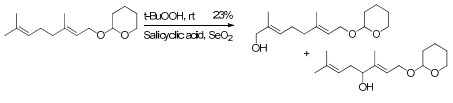
## Results/Discussion

### Problems encountered

- Original method for attaching diphosphate moiety onto substrate resulted in complete conversion to carboxylic acid; new method reduced number of steps by one and resulted in higher yield

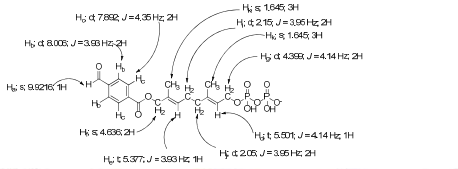


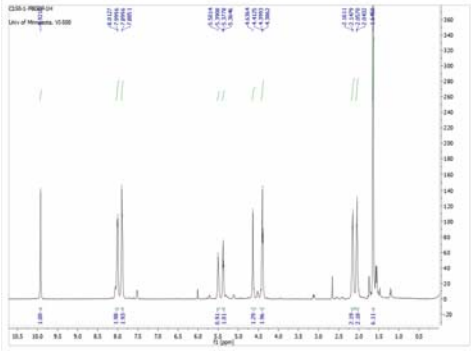

- Oxidation step results in ~1:1 mole:mole of two diastereomers; more selectivity (~2:1 mole:mole) when run in -20 °C for longer duration (~48 hours)




## Results/Discussion (continued)

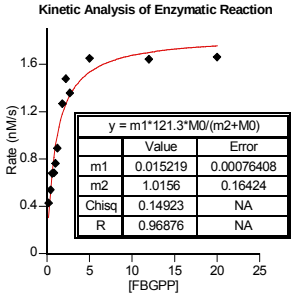
### $^1H$ NMR Analysis of GFBPP





**$^1H$ NMR** spectral evidence for characterization of GFBPP. Final product >90% pure from analysis, used for characterization of **GFBPP** and pFTase enzymatic properties

### Enzymatic Activity Evaluation




| Compound | $k_{cat}(s^{-1})$ | $K_m$ | $k_{cat}/K_m$ ($s^{-1}$ $\mu M^{-1}$) | $(k_{cat}/K_m)_{rel}$ |
|---|---|---|---|---|
| **FPP** | 0.52 | 1.71 | 0.30 | 1 |
| **FBGPP** | 0.015 | 1.02 | .015 | 0.05 |

Evaluated to be a good alternative substrate for pFTase, with relative $k_{cat}/K_m$ values of 0.05. $k_{cat}$ value for **GFBPP** shows relatively slower substrate in comparison to **FPP**. $K_m$ value of **GFBPP**, however, is relatively lower than that of **FPP**, resulting in a 20-fold decrease in catalytic efficiency. **GFBPP**, which contains a phenyl ring, showed tight binding to pFTase active site than **FPP** with its $K_m$ value of 1.8, indicating phenyl group's substantial effect on binding characteristics of substrate to pFTase active site. Previous studies by Distefano group[2] shows the same result: The substrates containing a phenyl group have high binding affinity to the active site and consequent small turnover numbers.

## Conclusion

GFBPP shown to be a capable, tight binding alternative substrate to pFTase. Aldehyde functional moiety will prove to be of excellent use for reversible immobilization assays using hydrazine beads as previously shown. Two modifications in synthesis result in facile method of making additional diphosphate analogs and more selective oxidation to a terminal alcohol.

**Future work** lies in actual immobilization experiments using hydrazine beads, and evaluating reversibility of immobilized proteins from a solid surface.