

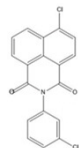


# Steric and catalytic effects of alcohols on the transamination of naphthalimides

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## Abstract



The 4-amino-1,8-naphthalimide fluorophore has long been recognized as a robust scaffold on which to build fluorescent labels for a wide range of applications, including fluorescent sensing of cations and anions, photochemical inactivation of enveloped viruses, and welding of tissues with visible light.

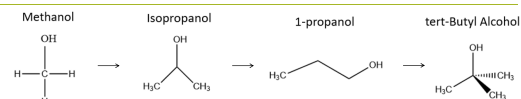
### The fluorophore has many desirable properties:

- It has a generally high fluorescence quantum yield that is not quenched by paramagnetic metal ions.
- It has a large (typically  $\geq 100$  nm) Stokes shift, which permits its use in fluorescence microscopy with minimal interference from scattering of the excitation radiation.
- It is highly resistant to photochemical bleaching.
- Its optimal excitation is in the visible, rather than the ultraviolet region.
- It is easy to manipulate synthetically.
- It possesses very low toxicity.

## Experimental Design

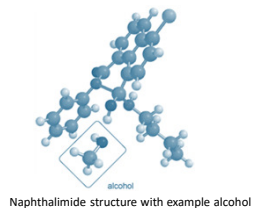
This experiment describes a systematic study of the effects of the size of alcohols on the transamination of *N*-(3-chlorophenyl)-4-chloro-1,8-naphthalimide.

Alcohols used to determine dependence of the observed rate constant in order of least to most sterically hindered



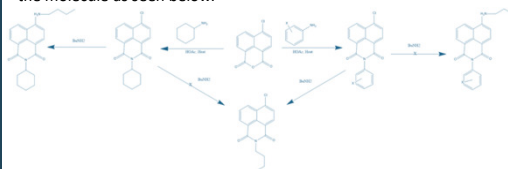
## Procedure

- A saturated solution is made with the naphthalimide in 2 mL of *n*-butylamine.
- A varying concentration of the chosen alcohol is added (0.5 M, 1 M, 1.5 M, 2 M, 2.5 M)
- Triethylamine is added to achieve a base volume of 5.00 mL.
- The reaction vessels are suspended in a water bath at 25.0°C.
- Aliquots (ca. 0.6 mL) are withdrawn and quenched in 10 mL of 12% aqueous hydrochloric acid.
- The insoluble organic matter is extracted with ethyl ether.
- The extracted dye is analyzed by  $^1\text{H}$  NMR spectroscopy.



## Theoretical

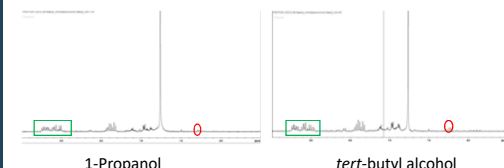
The mechanism below shows the known reaction pathway for naphthalimide dye in the presence of *n*-butylamine. When considering only  $pK_a$  effects, it is more favorable for the attacking group to displace the halogenated phenyl group before displacing the halogen on top of the molecule as seen below.



However we have found that with highly sterically hindered molecules (such as *tert*-butyl alcohol) the inability of the attacking group to reach the halogenated phenyl group results in an alternate mechanism. We now observe products in which the halogenated phenyl is replaced in addition to products where the halogen has been replaced. This alternate mechanism is illustrated below.



## Identifying Products with $^1\text{H}$ NMR



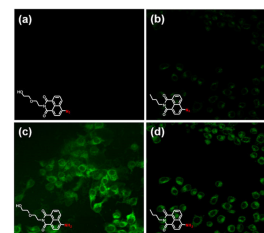
Above are  $^1\text{H}$  NMR spectra of aliquots taken at 180 minutes reacted with 1.0 mL of the chosen alcohols.

Circled in red we see a doublet present that shows an alternate product. It increases in size as the greater steric effects of the larger alcohol facilitates the formation of the alternate product.

Highlighted in green is the region where we see the proton resonances characteristic of naphthalimide dyes. As more of the alternate product is formed, we observe an increase in the number of peaks.

## Conclusions

A direct comparison of the NMR data between the unhindered methanol runs and the *tert*-butyl runs illustrates the presence of an alternate product.



<https://www.nature.com/articles/srep26203>

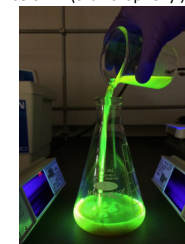
Computations performed in Spartan 10 showed that more sterically hindered alcohols are excised from the binding pocket, facilitating the formation of the alternate product.

In addition to observing the presence of an additional product in the NMR data, increased fluorescence was also witnessed indicating that the alternate product is a more fluorescent one.

## Future Implications

To further investigate the effects of steric hindrance on *N*-(3-chlorophenyl)-4-chloro-1,8-naphthalimide we plan to:

- Utilize spectrophotometry to quantitatively determine the increased fluorescence of the alternate products.
- Interpret  $^1\text{H}$  NMR data to determine a possible rate law for the alternate pathway.
- Investigate the alternate product's potential as a scaffold for biological fluorescent markers.



## References

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