

# SYNTHESIS OF NEW SUBTYPE SELECTIVE INHIBITORS OF THE GABA TRANSPORTERS DERIVED FROM SNAP-5114



**SAPIENZA**  
UNIVERSITÀ DI ROMA

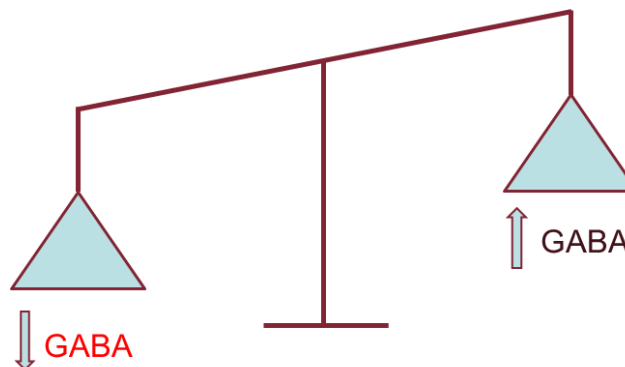
**Facoltà di Farmacia e Medicina**  
**Corso di Laurea in Chimica e Tecnologia Farmaceutiche**  
**Tesi Sperimentale in Chimica Farmaceutica**  
**a.a. 2014/2015**

**Laureanda: Davia Prischich**  
**Matricola: 1245037**

**Relatore: Prof. Rino Ragno**  
**Correlatore: Prof. Dr. K. T. Wanner**



## DISORDERS ASSOCIATED TO DISFUNCTIONS OF THE INHIBITORY NEUROTRANSMISSION

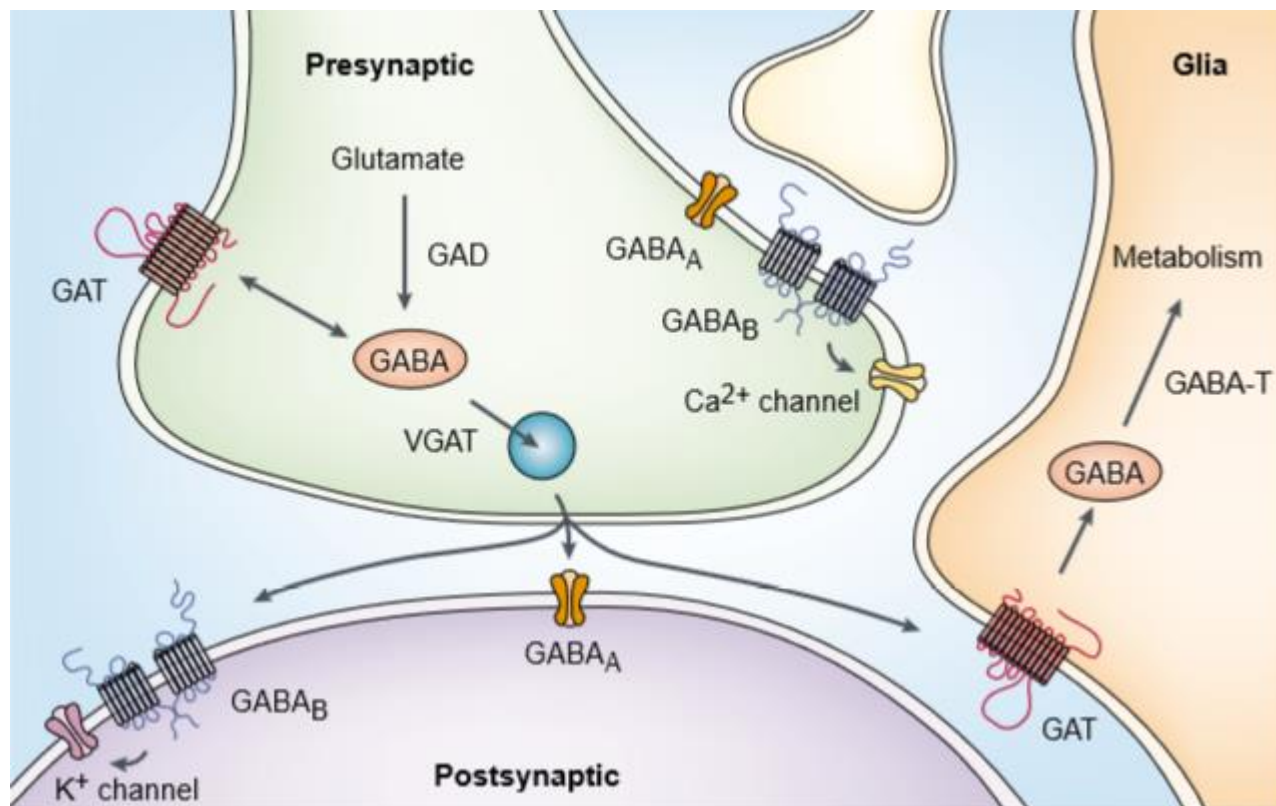


1. Epilepsy
2. Alzheimer's disease
3. Neuropathic pain
4. Huntington's Chorea
5. Schizophrenia
6. Anxiety and depression



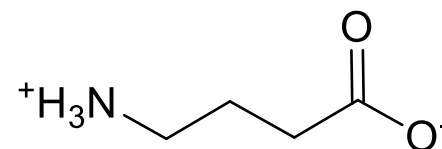
# GABAergic Neurotransmission

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## γ-aminobutyric acid

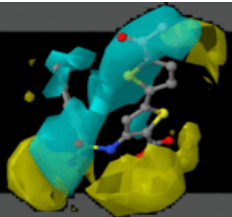
is the main inhibitory neurotransmitter in the mammalian central nervous system



**GABA**

Representation of the GABAergic synapse (Owens & Kriegstein, 2002)

Reduction of neuronal excitability is mediated by activation of both ionotropic (GABA<sub>A</sub> and GABA<sub>C</sub>) and metabotropic (GABA<sub>B</sub>) receptors.



# GABAergic Neurotransmission

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## DRUGS TO ENHANCE GABAERGIC NEUROTRANSMISSION

1. **GABA<sub>A</sub> receptors agonists**
2. **GABA<sub>A</sub> positiv allosteric modulators**
3. **GABA analogues (GAD modulators)**
4. **GABA-T inhibitors**
5. **GAT inhibitors**

- Barbiturates
- Benzodiazepines
- Gabapentin
- Vigabatrin
- **Tiagabine**

Inhibits neuronal and glial intracellular uptake of GABA

↑ **synaptic GABA [ ]**

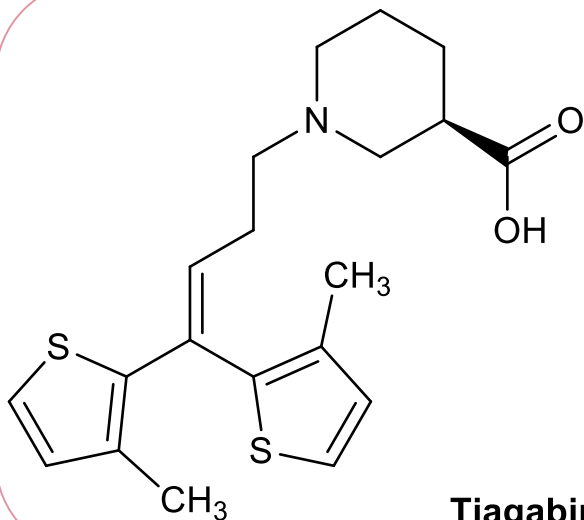
### GABA transporters nomenclature across species

Rat	rGAT-1	rBGT-1	rGAT-2	rGAT-3
Human	hGAT-1	hBGT-1	hGAT-2	hGAT-3
Mouse	mGAT1	mGAT2	mGAT3	mGAT4



# mGAT Selective Inhibitors

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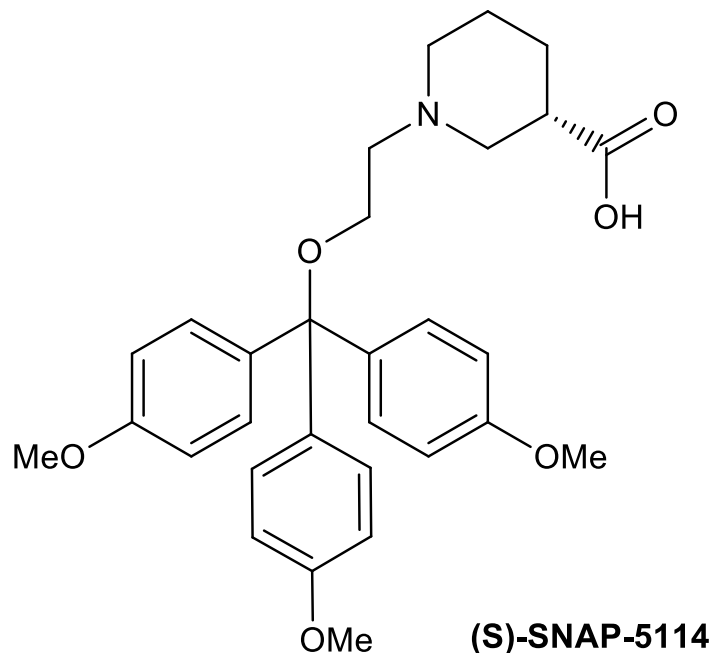
**Tiagabine**

## Side effects:

- Diarrhea
- Dizziness
- Asthenia
- Nervousness
- Tremor
- Depression
- Exacerbation of absence seizures

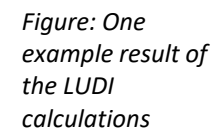
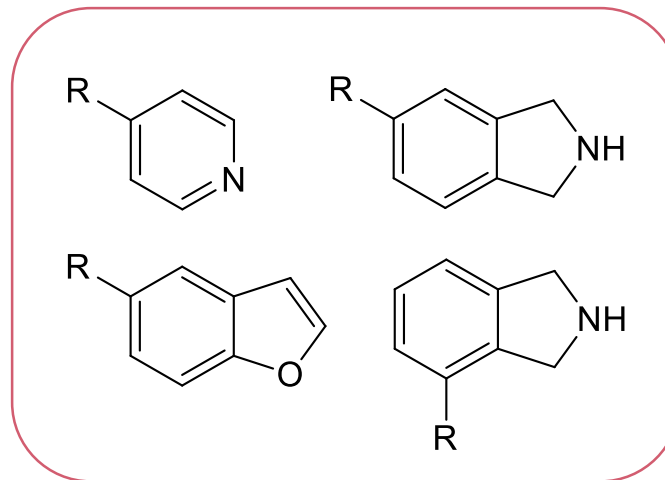
## GABA uptake inhibition IC<sub>50</sub> (μm)

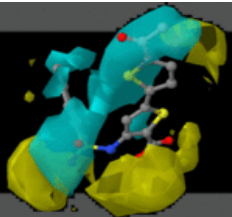
	mGAT1	mGAT2	mGAT3	mGAT4
Tiagabine	0.11	>100	>100	800
(S)-SNAP-5114	388	140	21	5



**(S)-SNAP-5114**



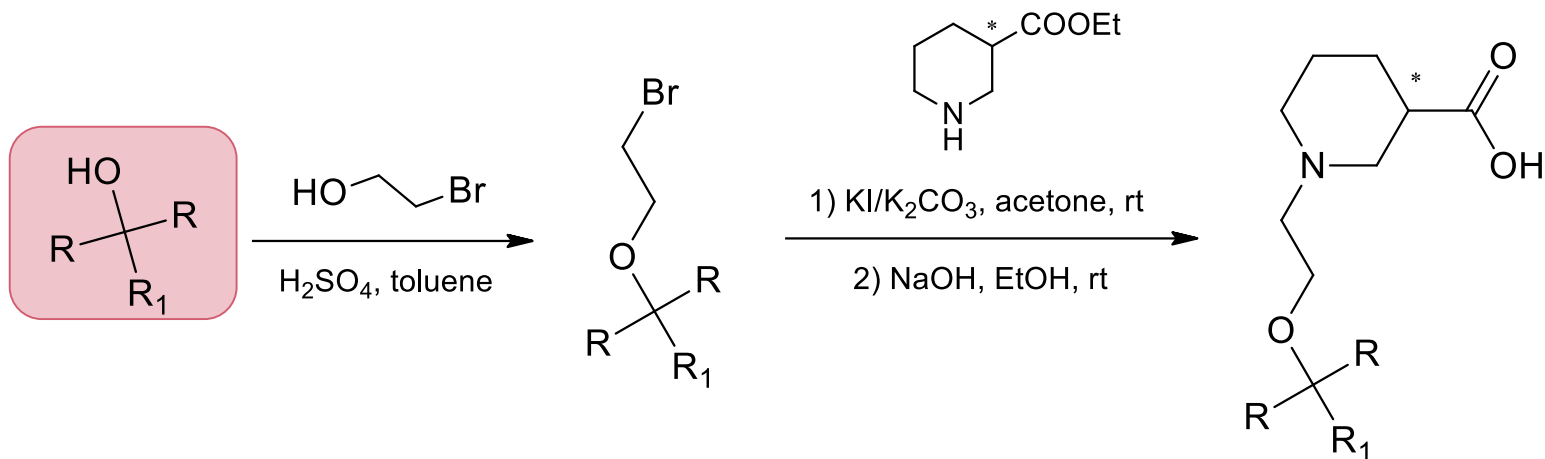




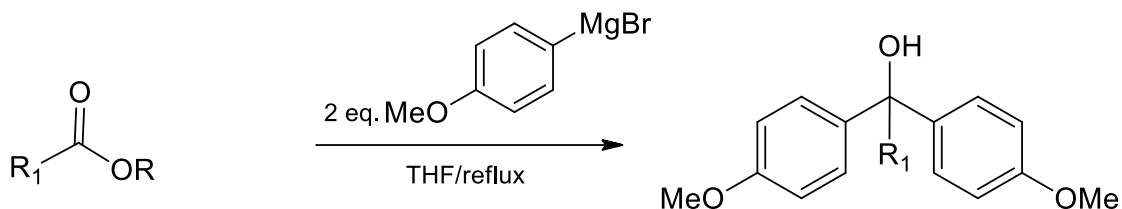
# SNAP-5114 analogues synthesis

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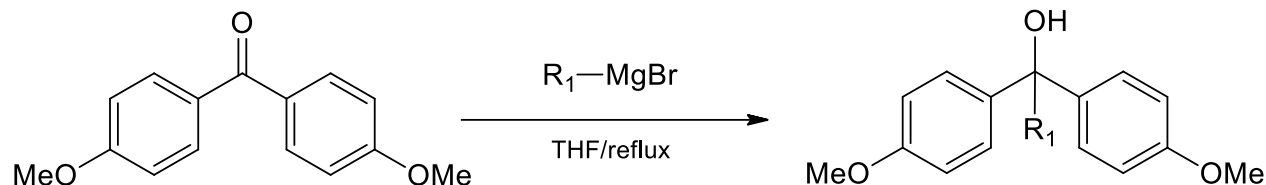
## SYNTHETIC PLAN TO SNAP-5114 ANALOGUES



### Method A



### Method B

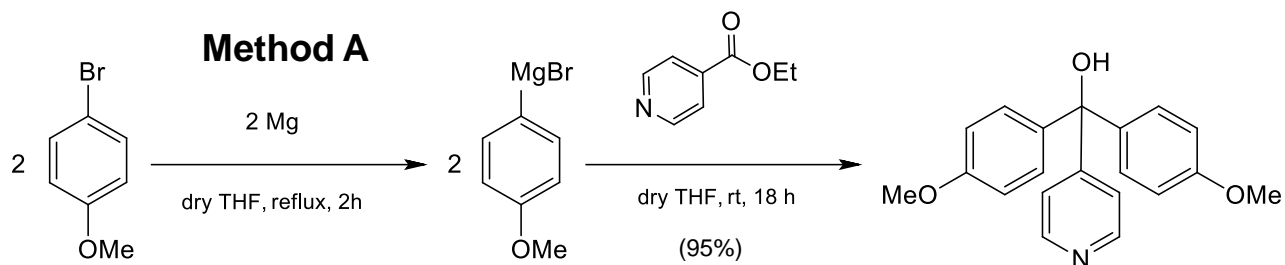


**SYNTHESIS OF THE  
SUBSTITUTED TRIARYL  
ALCOHOLS FROM  
GRIGNARD REAGENTS**

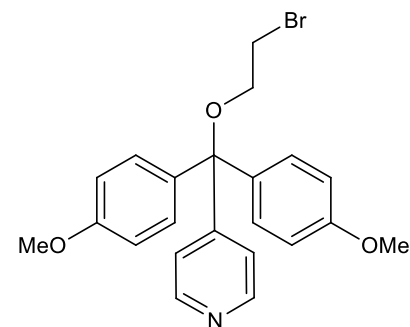
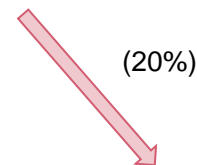


# SNAP-5114 analogues synthesis

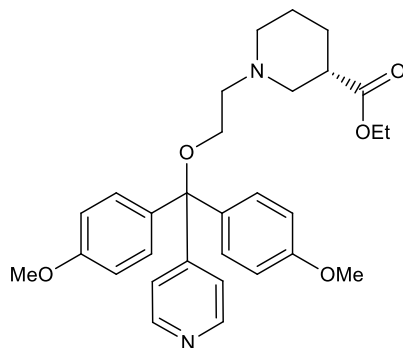
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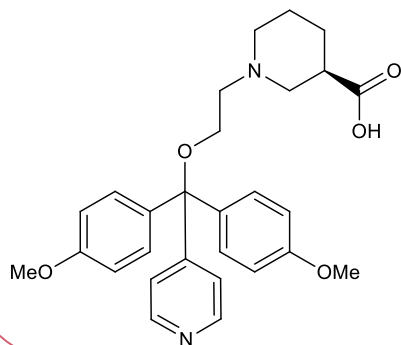
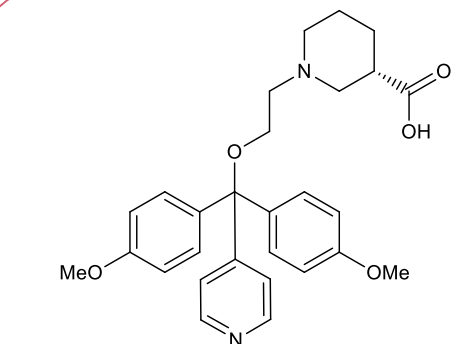
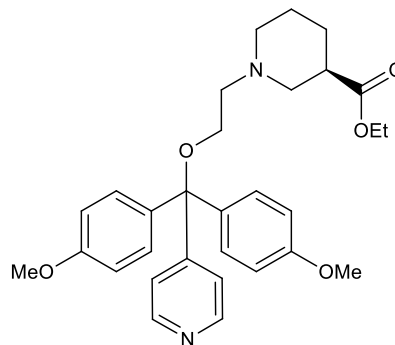
**Limiting  
step**



(64%)



(56%)



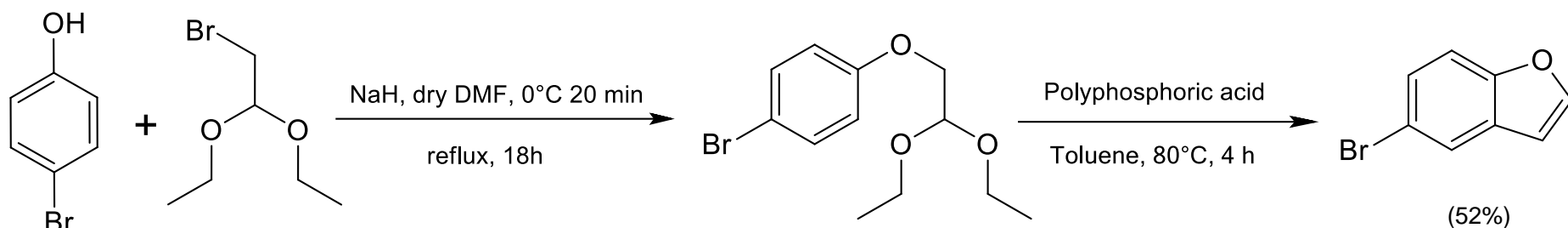




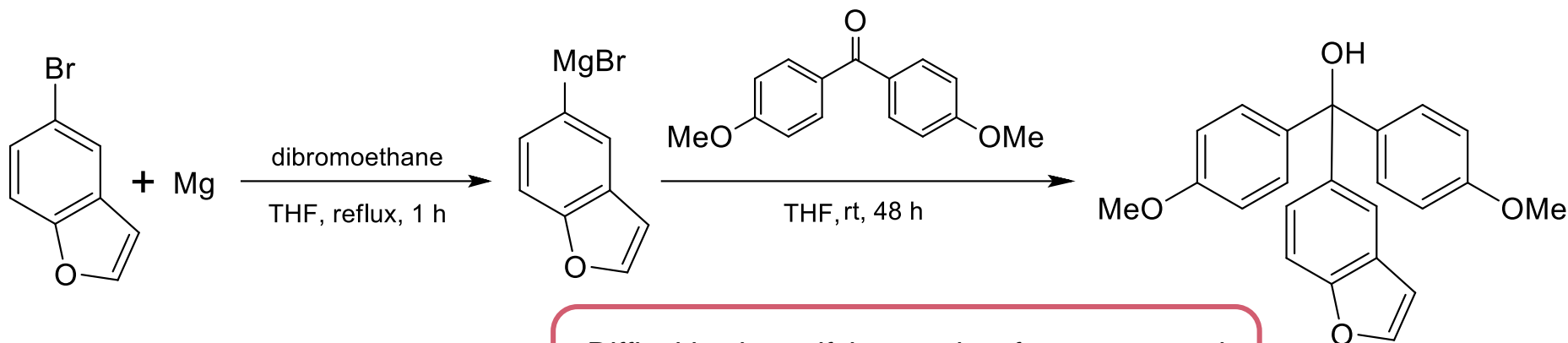
# SNAP-5114 analogues synthesis

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## SYNTHESIS OF 5-BROMO-BENZOFURAN



## GENERATION OF THE BENZOFURAN-5-YL 3<sup>a</sup> ALCOHOL



Difficulties in purifying product from unreacted substrate because of the same Rf



## PURIFICATION OF THE BENZOFURAN SUBSTITUTED ALCOHOL

### 1. Optimization of reaction conditions

- a) Lower temperature to diminish side products formation
- b) Attempt at using a different solvent
- c) Benzofuran magnesium bromide in excess compared to the equivalents of ketone substrate

### 2. Crude product purification through sequential MPLC runs

### 3. Crystallization

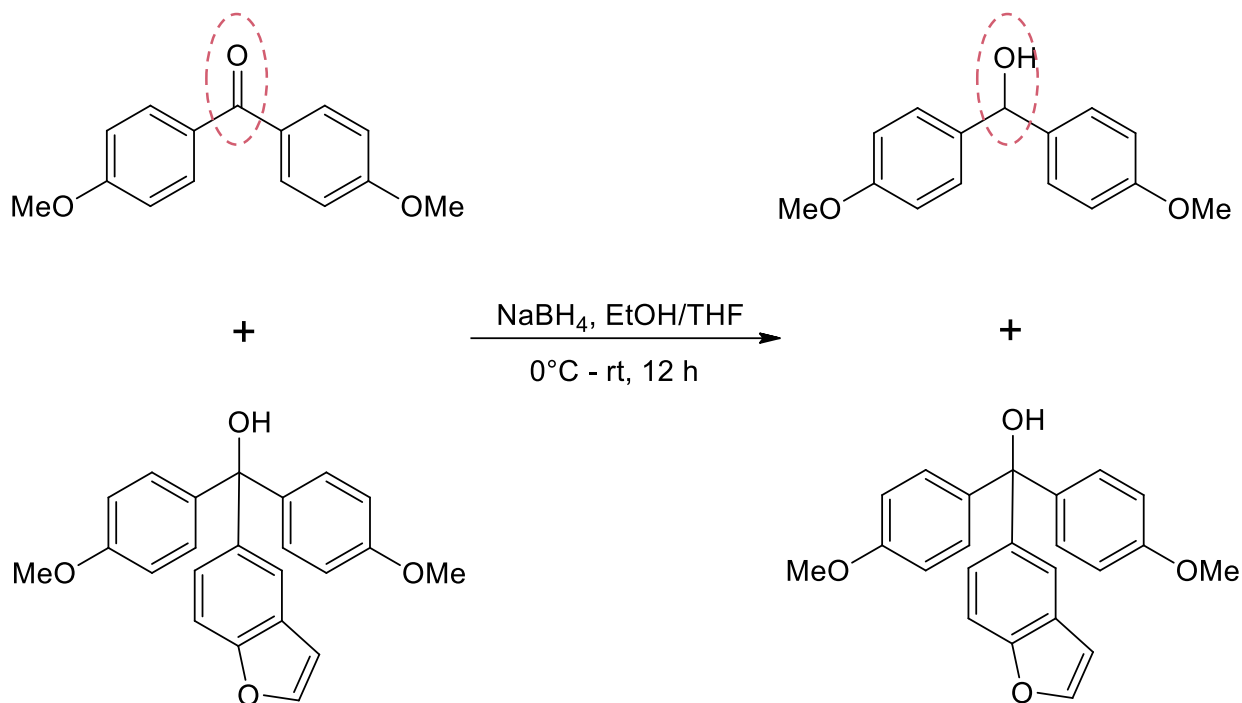
### 4. Selective reduction of unreacted ketone substrate



# SNAP-5114 analogues synthesis

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## SELECTIVE REDUCTION OF UNREACTED KETONE SUBSTRATE

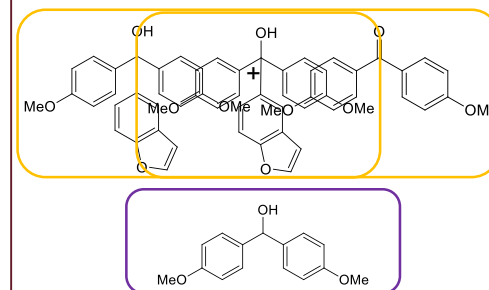


### In conclusion:

possibility to use a combination of methods to reach the triaryl alcohol in sufficient amount and purity

### TLC plate

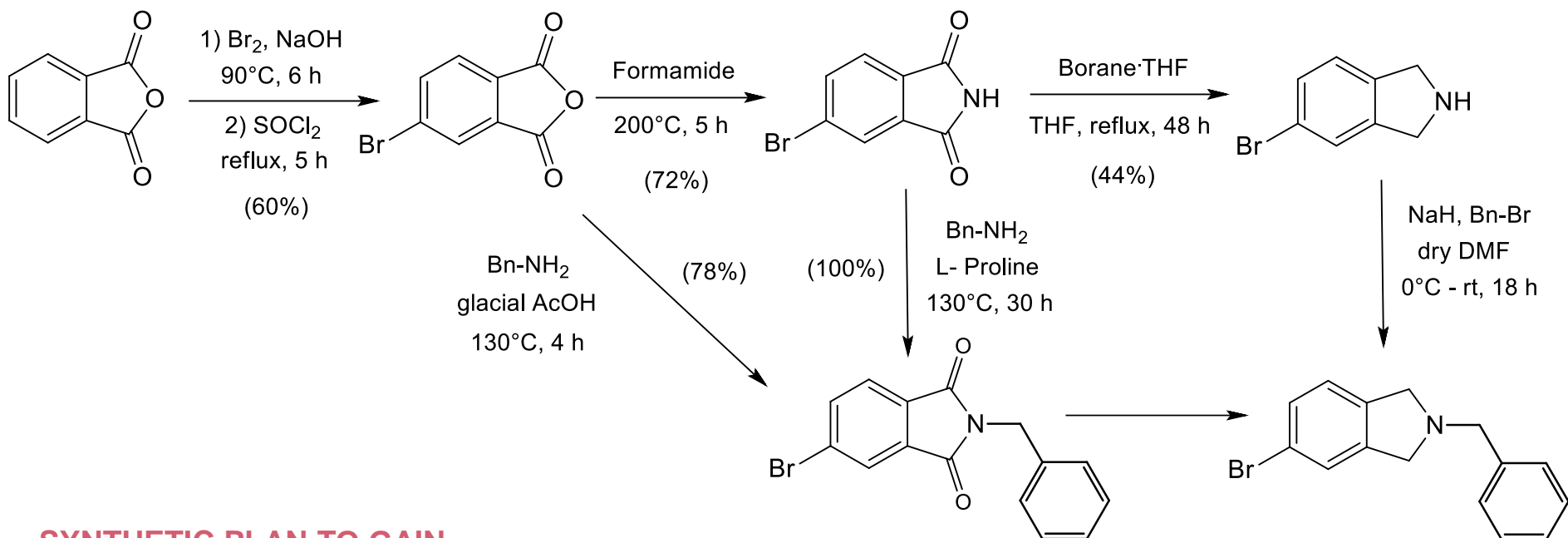
Isohexan:EtOAc (8:2)





# SNAP-5114 analogues synthesis

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SYNTHETIC PLAN TO GAIN

5-BROMO-ISOINDOLINE

AND N-BENZYL-5-BROMO-

ISOINDOLINE

**N-benzyl 5-bromo phthalimide reduction:**

a) 1 M Borane · THF complex, THF, reflux, 18 h

b) 1 M  $\text{LiAlH}_4$  in THF, 0°C – rt, 18h



# SNAP-5114 analogues synthesis

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## SYNTHETIC PLAN TO GAIN

## N-BENZYL-4-BROMO-

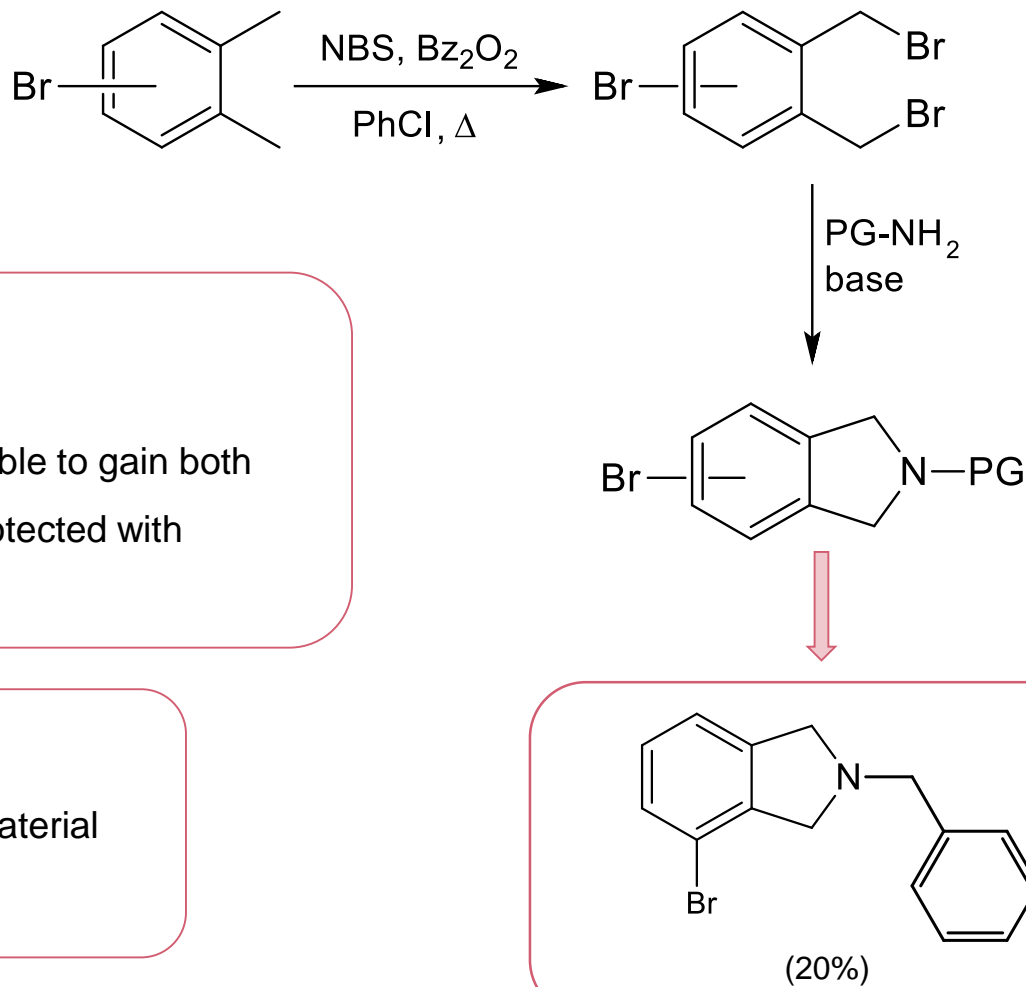
## ISOINDOLINE

### Advantages:

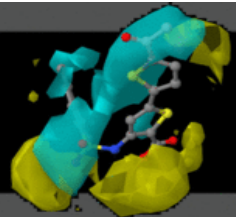
- Only two synthetic steps
- More flexible approach applicable to gain both 4- and 5-bromoisoindoline protected with different PGs

### Drawbacks:

- Higher cost of the starting material
- Low yield (20%)

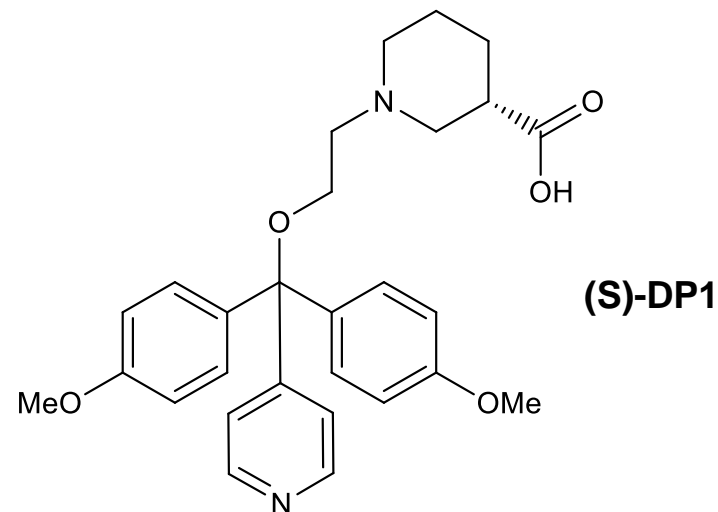
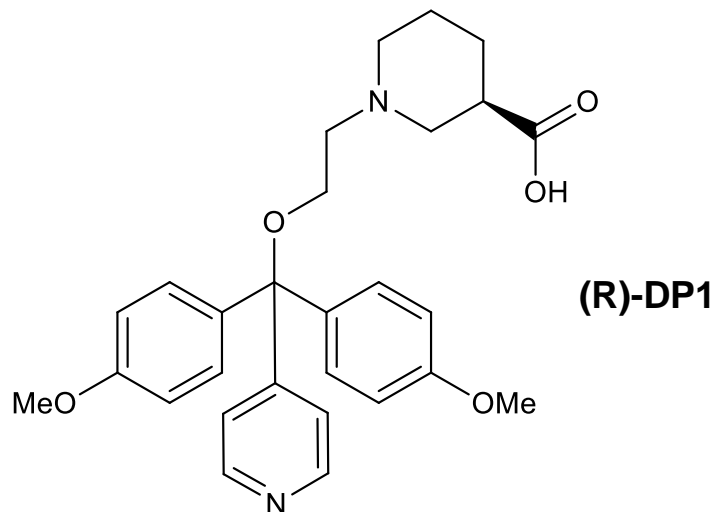






# Biological evaluation

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## GABA uptake inhibition IC<sub>50</sub> (μm)

	mGAT1	mGAT2	mGAT3	mGAT4
Tiagabine	0.11	>100	>100	800
(S)-SNAP-5114	388	140	21	5
(R)-DP1				
(S)-DP1				



*Thanks for the attention*



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