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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED Enantiomeric Seperation of 2-(1-naphthoxyl)-N,N-diethyl-propionamide and Similar Compounds on Pirkle-type High Performance Liquid Chromatography Chiral Stationary Phases

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Enantiomeric Separation of 2-(1-naphthoxyl)-N,N-diethylpropionamide and Similar Compounds on Pirkle-type High Performance Liquid Chromatography Chiral Stationary Phase

by

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ABSTRACT

The enantiomers of 2-(1-naphthoxyl)-N,N-diethyl propionamide as well as similar tertiary amides may be separated on Pirkle-type 3,5-dinitrobenzoyl amino acid Chiral Stationary Phases (CSP's) on High Performance Liquid Chromatography (HPLC). This thesis examines the synthesis of 2-(1-naphthoxyl)-N,N- diethyl propionamide (Napropamide) and related compounds, and their separability on these CSP's. Differences in steric hindering on the amide nitrogen and the chiral center carbon were examined to get a better understanding of the chiral recognition mechanism for this type of amides. The data indicated a good degree of separation on 3,5-dinitrobenzoyl- (L)-phenylglycene, (DNB-PGL), -Leucene, (DNB-LEU), and -Phenylalanine, (DNB-PHE), while a considerable decrease was observed for other amino acid derivatives. Increasing steric bulk on the solutes also displayed a decrease in selectivity and column retention for most cases, although there was an exception with the DNB-PGL column and the 2-(1-naphthoxyl0-N,N-diethylbutionamide, which showed better separation than the smaller chain Napropamide.

HISTORY

2-(1-naphthoxyl)-N,N-diethylpropionamide, common name Napropamide, product name Devrinol, (Stauffer Chemical Company), has been studied extensively in the past two decades for its herbicidal activity in preventing the growth of a wide variety of weeds and grasses. Napropamide is a soil-treating herbicide which prevents root growth of grasses as well as inhibiting photosynthesis, and RNA and protein synthesis in these weeds. It is a product of the Stauffer Chemical Company, and was synthesized mainly by a method by C. K. Tseng.[1]

Napropamide is useful for a wide variety of grasses and weeds, and is also easily metabolized by many plants such as tomatoes[2] or fruit trees, where it is converted into water soluble metabolites.

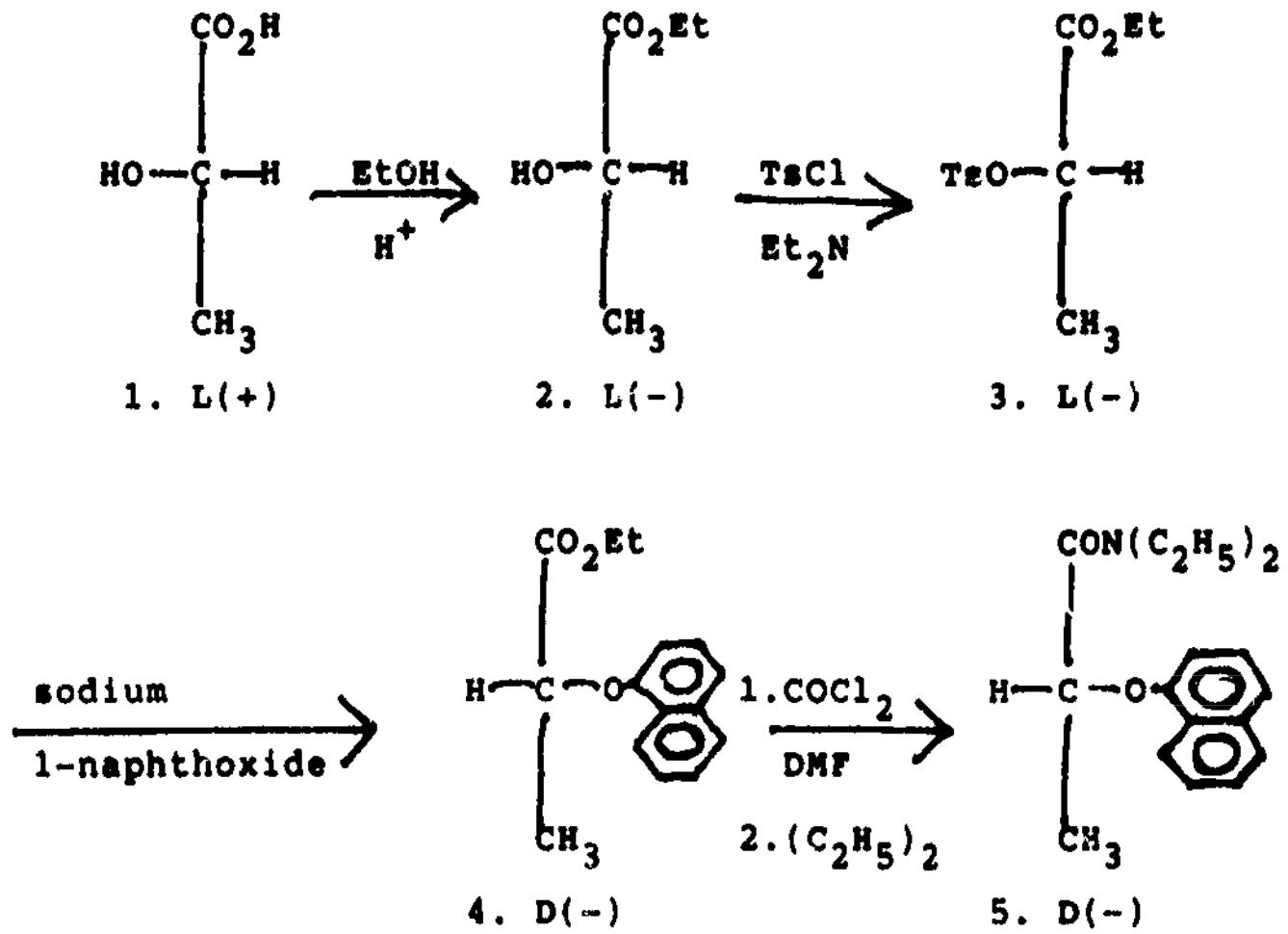
Napropamide was first introduced as a herbicide in 1966[3] and was part of an extensive study of 1-naphthoxylacetamides for use as herbicides.[5] Experiments showed that more sterically hindered amides had reduced activity. Studies at this point were mainly concerned with the racemic mixture of compounds.

Although both enantiomers of this and related compounds are active herbicides, there has been some examination of the

optically pure enantiomers. The Stauffer Chemical Company devised a synthetic method for preparing the optically pure isomers and examined the D and L configurations for their respective herbicidal activity.[1] It was found that the D configuration was approximately eight times more active than the L configuration in controlling crabgrass, foxtail, and watergrass weeds. Also, the racemic mixture was found to be half as active as the pure D configuration.

Research was also found that examined the relative inhibitions of weed photosynthesis, RNA synthesis, and protein synthesis.[4] The D and L configurations were slightly different in levels of inhibition. However, experiments also showed that the D isomer was more detrimental to corn root by a ten-fold value in concentration. This favored the use of the L isomer for this case.

Since both enantiomers have specific advantages for different cases, a separation of the two isomers provides an ideal tool for synthesis. The Stauffer Chemical Company has devised a chiral synthesis scheme to make optically pure D-Napropamide from L[+]lactic acid in four steps. The scheme is presented here.



1. L(+) 2. L(-) 3. L(-)

4. D(-) 5. D(-)

This scheme represents a long and costly, as well as difficult, approach to get only one optically pure species. Also even with the best conditions, there would still be a small degree of racemization.

However, with the growing interest and use of High Performance Liquid Chromatography, (HPLC), we have an easier method to separate the racemic mixture. Pirkle-type Chiral Stationary Phases (CSP's) have been successfully employed for a wide range of chiral separations.[6,7] Especially useful CSP's are the 3,5-DNB-amino acid phases. These have been used

to separate a variety of functionality from alcohols to amides.

The Pirkle columns incorporate a "three-point" attractive interaction scheme as a recognition model for the optical isomers. This usually involves two sites of hydrogen bonding between the solute and the CSP, as well as one pi-pi interaction between the aromatic substituents on the solute and, in this case, the 3,5-DNB ring. This "three point" interaction scheme was first introduced by Dagliesh[10] in various studies he performed. However, for this case there is no amide hydrogen to make the third interactions[11] the solute.

Other experiments have come up with successful separations of enantiomers using only two points of interaction. These models have two NH--O=C interactions with stereochemical dependence as the method of recognition. It is possible that this case could employ such a model, however, with one NH--O=C interaction and one pi-pi interaction. The elution order would be dependent on the steric bulk created by the different enantiomers.

There are two sites of steric bulk in Napropamide, the carbon chain on the carbonyl and the substituents on the amide nitrogen. These are varied in this examination to see the effects on separation by CSP's.

For herbicidal activity, varying steric size has a

degree of influence on activity. Research has suggested that similar compounds to Napropamide have various degrees of activity.[5] The metabolic degradation of uninhibited "good" plants however, is less affected since the metabolic process mainly on the naphthoxyl substituent. For this reason, it was advantageous to examine different relatives of the Napropamide compound.

RESULTS AND DISCUSSION

Napropamide was synthesized according to a method developed more recently than the aforementioned synthesis.[8] The Stauffer Chemical Company has made many synthetic approaches to napropamide in recent years. The one used produces napropamide from 2-Bromo-N,N-diethyl propionamide in the presence of aqueous sodium hydroxide. This method differs from the traditional sysnthesis which reacts diethylamine with the 1-(1-naphthoxyl)-propionic acid, since the formation of this acid has low yields with respect to 1-naphthol, the most expensive reactant. The reaction employed here will have yields reaching 95%, where the old method could only produce a 50% yield with many undesirable impurities. The details of this synthesis are presented in the experimental section.

Since 2-Bromo-N,N-diethyl propionamide was not available, it was synthesized by a process developed particularly for this type of compounds,[9] from a reaction of diethylamine and 2-Bromo-propionic acid with the addition of aluminum chloride as a reaction promoter. The only change made here was the use of the ethyl ester instead of the acid, but this did not seem to affect reactivity.

The compounds synthesized for this analysis were napropamide, 2-(1-naphthoxyl)-N,N-diethyl butionamide,

2-(1-naphthoxyl)-N,N-diethyl heptamide, 2-(1-naphthoxyl)-N,N-diphenyl propionamide, 2-(1-naphthoxyl)-N-isobutyl propionamide, and 2-(1-naphthoxyl)-N,N-diethyl undecamide. The last two substances were not found to be in good purity and resolution on 3,5-dinitrobenzoyl-(L)-Phenylglycine was not within measurable parameters, so they were omitted from the analysis.

Napropamide was tested on a series of DNB-(L)-amino acid columns which were provided by DR. Pirkle and associates. The compound was in a solution of methylene chloride. The mobile phase was a mixture of isopropyl alcohol in hexane, the percent IPA by volume was varied during tests from 10%, 5%, and 1%. The results of these preliminary runs are in table I.

TABLE I

2-(1-NAPHTHOXYL)-N,N-DIETHYLPROPIONAMIDE

3,5-DINITROBENZOYL-(L)-AMINO ACID CHIRAL STATIONARY PHASE

AMINO ACID	K'_1 (MIN)	K'_2	ALPHA	MOBILE PHASE %IPA/HEXANE
(1)Phenylglycine	27.2	39.2	1.44	1%
(2)valine	12.8	13.6	1.35	1%
(3)Phenylglycine	48.6	68.8	1.40	1%
(4)Leucine	26.8	36.2	1.35	1%
Alanine	11.2	-	1.00	5%
B-Naphthyl Glycine	11.2	12.2	1.09	5%
$(MeO)_2PO$-Phenylglycine	21.2	-	1.00	5%
N-Methylaminopropylleucine	14.4	15.6	1.10	1%

* Samples which displayed good results at %5 IPA/Hexane were measured at 1% for more accurate results.

The napropamide peaks were determined by a series of tests. Since there were small amounts of impurities in this sample, the napropamide was determined to be the last

substance eluted from the columns because of the high retention displayed by amides. Later purification and characterization by Nuclear Magnetic Ressonance confirmed this assumption. The two peaks that were suggested to be enantiomers were compared to a single peak that was obtained when a racemic column was employed.

The compounds were detected with an ultraviolet detector which was tuned to detect the aromatic behavior displayed here by the naphthoxyl substituent. Two wavelengths of ultraviolet light were used, 280nm and 254nm, so that comparisons could be made of peak height of enantiomers when the racemic columns of the CSP's were not available. While other substances will display different amounts of absorbtion at different wavelengths, optical enantiomers would retain the same ratio of intensities since their bonded structure has not changed.

From the data in table I, four CSP's were chosen which had the highest alpha (α) values, (seperation values). Alpha is the ratio of the retention time of the second enantiomer (k_2'), and the retention time of the first enantiomer, (k_1'). The CSP's used were the phenylglycine, leucine, phenylalanine, and valine DNB derivative columns. These four columns were then used to analyze the other compounds that were synthesized so that a better understanding of the nature of the seperation could be established.

These compounds were examined by HPLC and the results of this analysis are listed in table II.

TABLE II

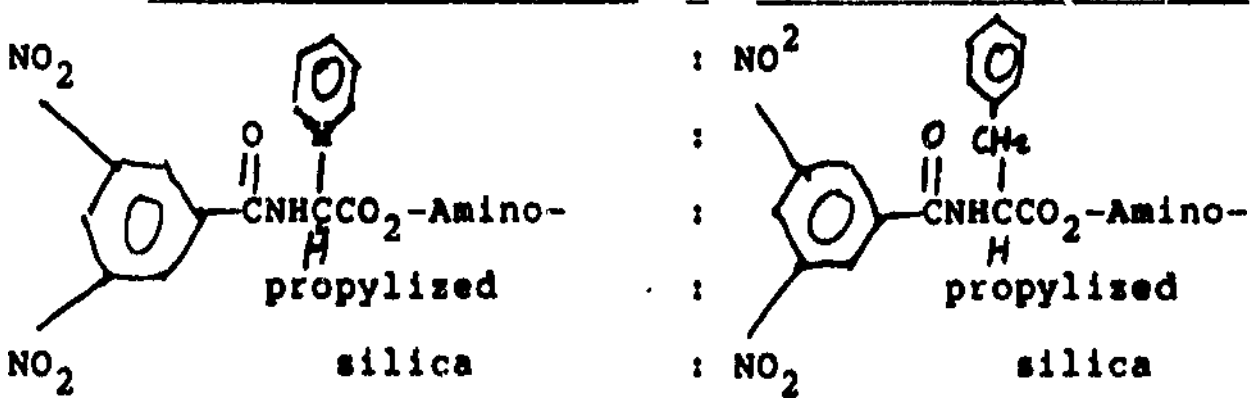
COLUMNS ARE REFERRED TO BY THEIR NUMBERS FROM TABLE I

	COLUMN USED				
SOLUTE	(1)	(2)	(3)	(4)	
Mapropamide	27.2	12.8	48.6	26.8	k'_1
	39.2	13.6	68.8	36.2	k'_2
	1.44	1.35	1.40	1.35	alpha
2-(1-naphthoxyl)-	15.8	6.1	24.0	16.4	
N,N-diethyl-	25.4	-	31.2	17.2	
butionamide	1.61	1.00	1.30	1.05	
2-(1-naphthoxyl)-	4.4	-	5.2	4.0	
N,N-diethyl-	4.5	-	5.4	-	
heptamide	1.03	-	1.05	1.00	
2-(1-naphthoxyl)-	4.6	5.9	6.0	5.0	
N,N-diphenyl-	5.4	-	6.2	5.4	
propionamide	1.17	1.00	1.01	1.08	

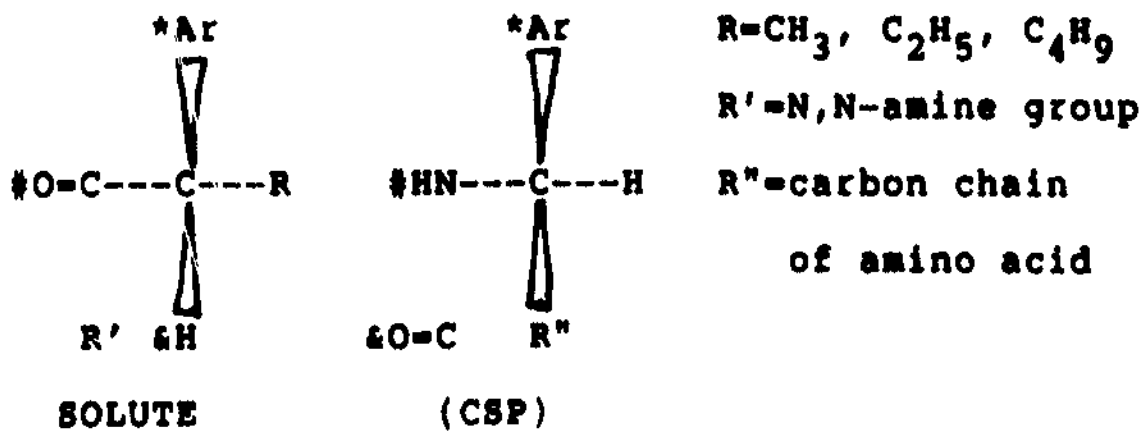
2-(1-naphthoxyl)-N-isobutyl-propionamide	analysis of this compound by NMR showed only trace amounts of product

Napropamide showed the best alpha values overall except for the one instance where the butionamide showed an alpha of 1.61 on the DNB-phenylglycine column and napropamide only showed an alpha of 1.44. This observation was unique and restricted to this column. The most similar column to the phenylglycine was the phenylalanine, which showed a loss of seperation of 0.10 with the change from propyl to butyl chains. The structures of these compounds are shown below.

<u>3,5-DNB-Phenylglycine</u> : <u>3,5-DNB-Plenylalanine</u>



The proposed mechanism for interaction between CSP's and solutes would involve a C;O--HN association and a pi-pi interaction in the aromatic substrates.



The aromatic interaction is labelled (*), and the C:O--HN interaction is labelled (#). A third interaction that is possible is between the alpha carbon's hydrogen on the solute and the carboxylic acid's carbonyl of the CSP, (&). This model would leave the enantiomeric selectivity to the steric blocking of the R group of the solute and the R" or hydrogen of the CSP.

When larger R groups are used, two developements occur, the k's (capacity factors of solutes) decrease, and the alpha values decrease. (Except for the mentioned case.) This trend reflects the effect of increasing the steric hindering on retention, the larger the carbon chain, the more of a tendency of the solute to behave like a hydrocarbon and less like an amide.

For selectivity, alpha values would be expected to increase as R is increased. This effect however competes with many factors. One factor is that when retention time is decreased substantially, seperation will also decrease. This

is especially exhibitted by the diphenyl-amide, and the diethyl-heptamide. For these compounds, column retention with the present mobil phases was so low that seperation was difficult to measure.

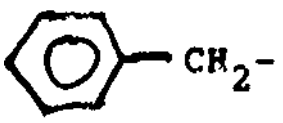
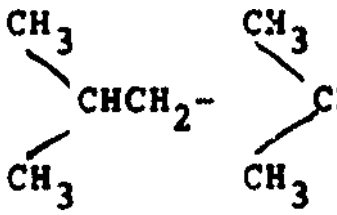
Another factor that would affect seperability is the structure of the CSP. The size and shape of the R" group on the chiral center will determine whether the R group of the solute has sufficiently increased to increase the value of alpha. For Phenylglycine, the addition of CH_2 to the R group is sufficient to increase alpha enough to compensate for the decrease of k_1'. On the other three columns, however, the addition does not make a significant enough difference to raise alpha above what is lost from the k' decrease.

The common trend for amides on these Pirkle-type CSP's is that the k' values decrease and the alpha values increase as the R groups of the solute becomes larger.[7] those amides tested, however, always had at least one amide hydrogen which was actively involved in the selective interaction with the CSP. For this case, however, this interaction does not exist, so that the trend is that alpha will also decrease as R increases. The one exception where phenylglycine is used has a CSP with a phenyl group bonded directly to the chiral , where the phenyl group was directly bonded to the chiral carbon center while eas the other phases had at least one CH_2 bonded to the center carbon. This case could be the reason

for the exclusive rise in alpha as the R group increased.

when the amide group changed, from diethylamide to diphenyl amide, there was a sharp decrease in retention, and only a small degree of seperation depending on the column used. Because of this, the diphenyl compound does not present a good example of amide effects on selectivity. It does show that increased steric hindering on the amide, or a mixture of that and the aromatic effect of the phenyl groups on the amide, will decrease the solutes affinity for these columns.

One final point to be discussed here is the effect of the R" group of the CSP to the retention and seperation. R"'s for the four columns tested and their structures are shown below.

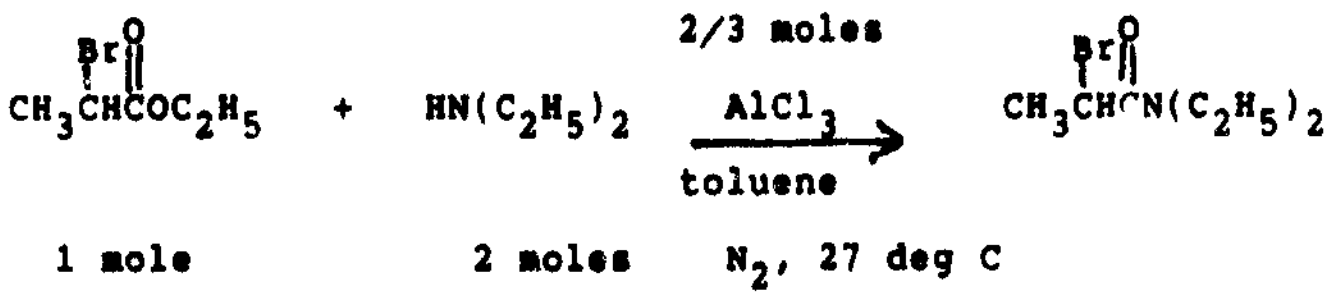
R"	Phenylglycine	Phenylalanine	Leucine	Valine
Structure				

From the data in table II, one notices that the R" groups with aromatic substituents will exhibit larger alpha values while k' retention is similar with Leucine in most cases. This however, can be an effect of the size of the R" group, and need not be a result of the fact that an aromatic group was involed. Other analysises of this sort did not treat aromaticity as a major factor.[6,7]

EXPERIMENTAL

As was mentioned earlier, the synthesis of these compounds was followed from a revised method developed by the Stauffer Chemical Company.[8] This synthesis was preceded by another adapted synthesis of 2-Bromo-N,N-diethylpropionamide (or relevant species depending on which compound was synthesized.)

This synthesis was also developed by the Stauffer Chemical Company.[9] It follows this general reaction scheme. (The species shown here is the synthesis for napropamide. The other compounds were synthesized in the same way, only making changes in sample weights to retain stoichiometric equivalence.)



In a three necked reaction flask equipped with a thermometer, nitrogen sweep, and magnetic stirrer was placed 2 grams ethyl-2-bromopropionate (.01 moles), 1.46 grams diethylamine (.01 moles), and 10 ml toluene. The flask was

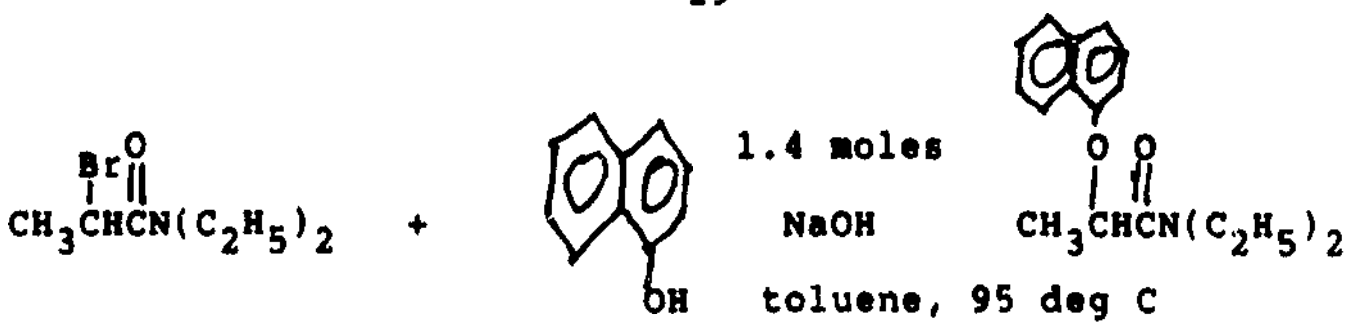
then equipped with a Gooch tube connected to a flask containing .9 grams aluminum chloride (.0067 moles). The aluminum chloride was added while the mixture was stirred over a period of 13 minutes while maintaining a temperature of 27 degrees celcius with an ice bath. The reaction was allowed to run for an additional 50 minutes and was monitored by thin layer chromatography using methylene chloride as a solvent.

The reaction mixture was added to 20 ml of 3 M HCl where the temperature of the mixture rose to 35 degrees celcius. The organic phase was separated with a separatory funnel and the aqueous phase washed with 20 ml toluene. The organic extracts were combined and washed with 20 ml saturated NaCl aqueous solution, dried over magnesium sulfate, and rotovapped free of solvent.

This reaction yielded 2.01 grams of 2-bromo-N<N-diethyl propionamide with slight impurities. (70% yield). 1 gram was used for the second reaction.

The synthesis of the other compounds were made by the following model changing only the amount of ester to compensate for the change in molecular weights of the different amides. Yields and purity became poorer as the R group became larger.

The second reaction made napropamide from the bromo-amide and 1-naphthol. It follows this scheme.



1 mole 1.1 moles

In a three necked reaction flask equipped with a thermometer, a condensor, nitrogen sweep, and magnetic stirrer was placed 1 gram 2-bromo-N,N-diethylpropionamide (.0045 moles), .71 grams 1-naphthol (.005 moles), and 10 ml toluene. The mixture was stirred and .0063 moles of a 50% solution of NaOH (aqueous) was slowly added. The solution temperature increased by 10 degrees celcius and turned black during the addition.

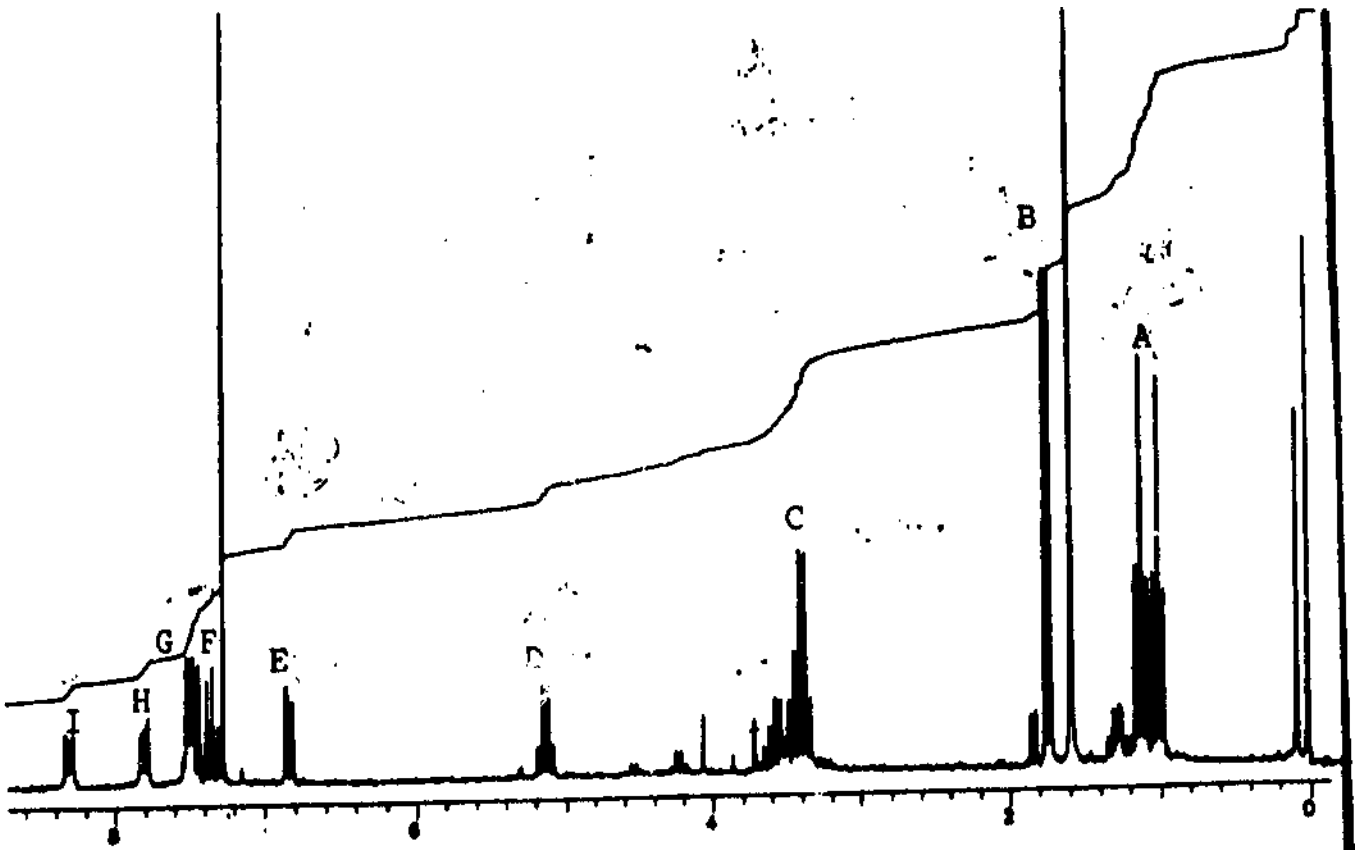
The reaction was heated to reflux (95 degrees C)and sodium chloride began to seperate. The reaction was run for four hours, being monitored by TLC with a 10% methanol/methylene chloride solvent mixture. The reaction seemed to reach completion after only 2 hours.

The solution was then cooled to 50 degrees C and 20 ml water was added at this temperature. The organic phase and the interphase was separated and reheated to this temperature. 20 ml of 12.5% NaOH solution was added and a clean separation occured. The organic phase was dried over magnesium sulfate and rotovapped free ov solvent.

To further purify the product, flash chromatography was

performed on silica using first methylene chloride to remove unreacted 1-naphthol, and then a 5% solution of methanol in methylene Chloride to obtain the product. The solvent was evaporated under .1 mm Hg vacuum. The purity was estimated to be 95%.

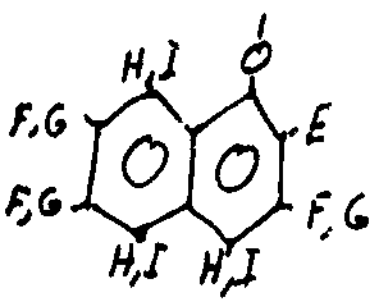
The purity of the product was determined by HPLC and the structure was confirmed by NMR. The NMR spectrum is shown below.



NMR of 2-(1-naphthoxyl)-N,N-diethylpropionamide

The following peaks were analized and compared to a reference spectrum of a metabolite of this compound.[2]

A.	(2) Triplets	1.0,1.1, (J=7.0)	6H's	$NCH_2C\underline{H}_3$
B.	Doublet	1.75, (J=7.0)	3H's	$O{=}CC\underline{H}CH_3$
C.	Quartet	2.4, (J=7.0)	4H's	NCH_2CH_3
D.	Quartet	5.15, (J=6.0)	1 H	$O{=}CC\underline{H}CH_3$
E.	Doublet	6.85, (J=8.0)		
F,G.	Multiplet	7.2-7.6		
H.	Multiplet	7.8		
I.	Multiplet	8.3		



The other compounds made with this synthesis were

also characterized by HPLC and NMR. Each of the compounds will be presented here with their respective changes from the spectrum of Napropamide already shown here.

2-(1-naphthoxyl)-N,N-diethylbutionamide:

Peak B was changed, instead there were two sets of peaks.

B_1,	Triplet	1.2, (J=7.0)	3H's	$O=CCHCH_2C\underline{H}_3$
B_2,	Multiplet,	2.15		$O=CCHC\underline{H}_2CH_3$

Peak D was also shifted to 4.85

2-(1-naphthoxyl)-N.N-diethylheptamine:

Peak B was changed. There were several peaks in place of it.

B_1,	Triplet,	1.2, (J=7.0)	3H's	$O=CCH(CH_2)C\underline{H}_3$
B_2,	Multiplets	1.8-2.4		$O=CCH(C\underline{H}_2)CH_3$

Peak D was shifted to 4.75

2-(1-naphthoxyl)-N,N-diphenylpropionamide:

A.	Doublet	1.8, (J=7.0)	3H's	$O=CCHC\underline{H}_3$
B,	Quartet	4.25, (J=6.0)	1 H	$O=CC\underline{H}CH_3$
C,	Multiple peaks	6.9-7.6, 7.8, 8.3	Aromatic	

hydrogens from 1-naphthoxyl- and diphenyl groups, 17H's

2-(1-naphthoxyl)-N-isobutylpropionamide:

Analysis by NMR showed only trace amounts in sample, sample was also found to be insoluble in $CDCl_3$, so this substance was not ised in data analysis along with 2-(1-naphthoxyl)-

N,N-diethylundecamide, which showed poor results on HPLC retention. Since the yield of this compound was low and the impurities difficult to remove, it was not analyzed and instead, data from other disubstituted amides, (with one amide hydrogen,) was examined from literature.[7] It did not seem necessary to synthesize the compound via another route.

Analysis by HPLC was performed on microliter samples of methylene chloride solutions. They displayed acceptible separations of napropamide and compounds that were very similar to it. As carbon chain substituents became larger however, separation values decreased and became impractical after only a few CH_2 groups. This indicated that size was an important factor for the recognition model.

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