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Drug design (STAT5 modulators), development (Glyceollin I) and improvement (Esmolol Plus)

Michael Reese
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A Thesis

Entitled

**Drug Design (STAT5 Modulators), Development (Glyceollin I)
and Improvement (Esmolol Plus)**

By

Michael Reese

Submitted as partial fulfillment of the requirements for the
Master of Science Degree in Medicinal Chemistry

Adviser: Paul W. Erhardt, Ph.D.

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College of Graduate Studies

The University of Toledo

December 2009

An Abstract of
**Drug Design (STAT5 Modulators), Development (Glyceollin I)
and Improvement (Esmolol Plus)**

Michael Reese

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Master of Science Degree in Medicinal Chemistry

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This thesis encompasses several different research activities that can contribute to advances within the pharmaceutical arena. The first part of my thesis describes synthetic work directed toward improving the clinical profile of a marketed drug called ‘esmolol’ by developing only its active enantiomer. The second part of the thesis describes my contributions toward the overall scale-up synthesis of a promising anticancer agent called ‘glyceollin I,’ which was undertaken in a team effort. The final part of the thesis describes my efforts to assemble an initial library of compounds that can be used to probe the STAT5 pathway for the potential inhibition of prostate cancer.

Acknowledgements

This thesis could not have been possible without the support, financially and intellectually, that I have received while attending the University of Toledo.

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List of Abbreviations

ACN	Acetonitrile
BnBr	Benzyl Bromide
(-)-DAG	(-)-2,3,4,6-di-O-isopropylidene-2-keto-L-gulonic acid
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DNA	Deoxyribonucleic Acid
(-)-DTT	(-)-O,O'-di-p-toluoyl-L-tartaric acid
ee	Enantiomeric Excess
Et ₂ O	Ethyl Ether
EtOAc	Ethyl Acetate
EtOH	Ethanol
FDA	Food and Drug Administration
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
JAK	Janus Kinase
mCPBA	<i>meta</i> -Chloroperoxybenzoic Acid
MEK	Methyl Ethyl Ketone
MeOH	Methanol
MOM	Methoxymethyl
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
RT	Room Temperature
SAR	Structure Activity Relationship
SERMs	Selective Estrogen Receptor Modulators
STAT	Signal Transducer and Activator of Transcription
TBDMS	<i>tert</i> -butyldimethylsilyl
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

Chapter 1

Esmolol Plus

1.1 Introduction

Esmolol hydrochloride (**5**), Scheme 1, is an ultra-short acting, cardioselective β -adrenergic receptor blocker that is marketed in its racemic form for the intravenous treatment of hypertension, angina pectoris and cardiac arrhythmias.⁽¹⁾ Generally, the cardiac β -blocking activity of β -blockers resides in the (S)-enantiomer. Esmolol has a single chiral center and as expected, its (S)-enantiomer possesses much greater affinity for binding to the β -adrenergic receptor than its (R)-enantiomer.⁽²⁾

Esmolol belongs to a class of drugs known as ‘soft drugs’ which are rapidly hydrolyzed to their inactive metabolites. Esmolol was designed to be rapidly hydrolyzed by the ubiquitous esterases found in the body, to methanol and its acid metabolite (Figure 1) therefore placing it into the soft drugs realm.⁽³⁾ By intent, it has an extremely short half-life that is useful in critical care situations by intravenous infusion. Full recovery from β -blockade is observed within 18-30 minutes post infusion. The pharmacokinetic parameters displayed by esmolol after administration to patients averaged 2 min for the distribution half-life, 9 min for the elimination half-life, a 3.4 L/kg volume of distribution, and total clearance of 285 mL/kg/min.⁽⁴⁾

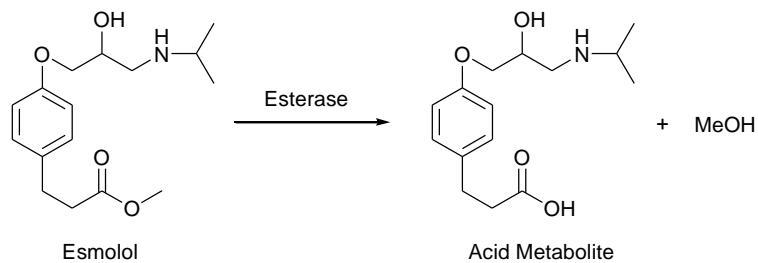
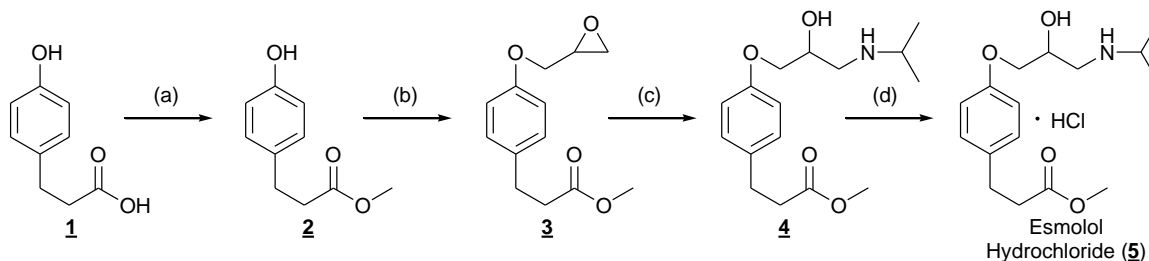


Figure 1. Chemical structure of esmolol and its metabolite.

Many older drugs are marketed as their racemic mixtures but it is now well understood that it is most desirable to market drugs in their pure enantiomeric forms. The current synthesis scheme to produce esmolol employs the use of epichlorohydrin to install the alcohol group into the compound at its chiral center (Scheme 1).



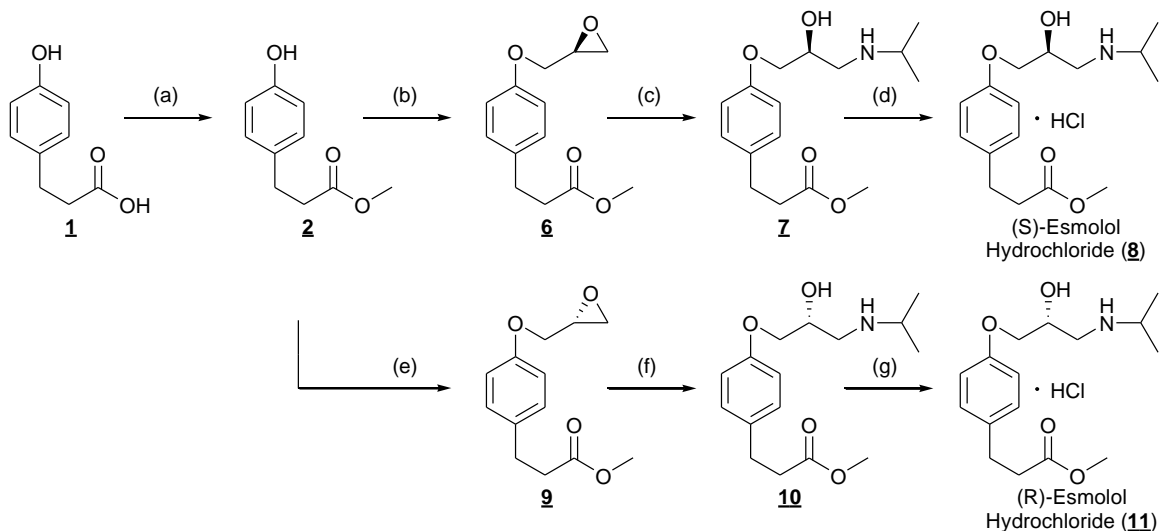
Scheme 1. Synthetic route used to prepare esmolol hydrochloride (**5**) from 3-(4-Hydroxyphenyl)-propionic acid (**1**). Conditions and yields: (a) HCl, MeOH, reflux, 24 h, 78%; (b) Epichlorohydrin, K₂CO₃, MEK, reflux, 20 h, 85%; (c) Isopropylamine, MeOH, reflux, 4 h, 100%; (d) MeOH·HCl, MEK, 67%.

In order to synthesize the enantiomerically pure forms of esmolol, enantiomerically-pure epichlorohydrin is used. This is not an optimal industry scale process because of the high cost of using enantiomerically-pure epichlorohydrin. Our lab, as well as others, have used enantiomerically-pure epichlorohydrin to produce both the (S)- and (R)-esmolol enantiomers.⁽⁵⁾ In addition, we have devised a different synthetic approach that takes advantage of hydrogenation over palladium/carbon while still utilizing the asymmetric epichlorohydrin in order to obtain each enantiomerically pure acid metabolite. In other studies conducted with the use of racemic and both enantiomerically pure isomers of esmolol, diastereomeric salt crystallization studies were

performed based on the procedure set forth by Patil, G. et. al,⁽²⁾ which states that from (\pm)-esmolol each enantiomerically pure isomer can be favorably crystallized in the presence of a diastereomeric salt while the other isomer does not crystallize and remains in solution.

1.2 Synthesis of (S)- and (R)-Esmolol Hydrochloride

Enantiomerically pure esmolol hydrochloride was synthesized using chiral epichlorohydrin in order to instill the necessary stereochemistry of the hydroxyl group via a stereochemically defined epoxide moiety. The same overall procedure was followed as in Scheme 1 except for the use of enantiomerically-pure epichlorohydrin. Scheme 2 shows the synthetic procedure followed to obtain (S)- and (R)-esmolol hydrochloride.



Scheme 2. Synthetic scheme to prepare (S)- and (R)-esmolol hydrochlorides from 3-(4-Hydroxyphenyl)-propionic acid (**1**) utilizing (R)- and (S)-epichlorohydrin, respectively. Conditions and yields: (a) HCl, MeOH, reflux, 24 h, 78%; (b) (R)-epichlorohydrin, K₂CO₃, MEK, reflux, 20 h, 81%; (c) Isopropylamine, MeOH, reflux, 4 h, 100%; (d) MeOH·HCl, MeOH, 44%; (e) (S)-epichlorohydrin, K₂CO₃, MEK, reflux, 20 h, 77%; (f) Isopropylamine, MeOH, reflux, 4 h, 100%; (g) MeOH·HCl, MeOH, 29%

The synthesis of each enantiomer of esmolol begins with the use of 3-(4-hydroxyphenyl)-propionic acid (**1**) from which a methyl ester is made through the use of

methanol and concentrated HCl to afford **2**. (R)- And (S)-epichlorohydrin are then used in separate reactions with potassium carbonate and methyl ethyl ketone to create an epoxide ether linkage with (S)- and (R)-stereochemistry, respectively, for **6** and **9**. The rest of the synthesis is mirrored exactly for both enantiomers after the attachment of the epoxide ring. The epoxide intermediate is then reacted with isopropylamine which opens the ring to form the free amine products, **7** and **10**. The free amine is converted to an HCl salt by the use of methanolic HCl that is prepared by bubbling HCl gas into anhydrous methanol while cooled over an ice bath. After the hydrochloride salt is formed, the product is crystallized using methanol and ethyl ether to afford the final compounds, **8** and **11** in an 28% and 17% overall yield for 4 steps, respectively.

During these syntheses the question was raised as to which epoxide intermediate stereoisomer is obtained when reacting (R)- and (S)-epichlorohydrin with the phenolic hydroxyl group. These questions were answered through HPLC and optical rotation studies of the epoxide intermediates (**6**, **9**) coupled with the results for the esmolol final products (**8**, **11**) all of which are summarized in Table 1. Based on these results it was concluded that (R)-epichlorohydrin gives the (S)-esmolol product while (S)-epichlorohydrin gives the (R)-esmolol product. Through examination and literature reviews, the stereochemistry is not an inversion, but rather proceeds through a Payne-like opening of the epoxide ring shown in Figure 2. ⁽⁶⁾

Table 1. HPLC and optical rotation values found for the epoxide intermediates (**6,9**) and esmolol hydrochloride (**8,11**).^a

Compound	HPLC (%ee)	[α]	[α] Lit. Values
6	88.44%	+8.5°	+7.9° ⁽⁵⁾
9	90.02%	-8.2°	N/A
8	97.72%	-19.4°	-19.6° ⁽²⁾
11	ca. 95.00% ^b	+21.2°	+19.1° ⁽²⁾

^a HPLC was performed on a reverse phase chiral column and optical rotations were obtained on a 589nm λ Na D line at a concentration of 1.0 g/100 ml in MeOH. ^b Additional runs necessary.

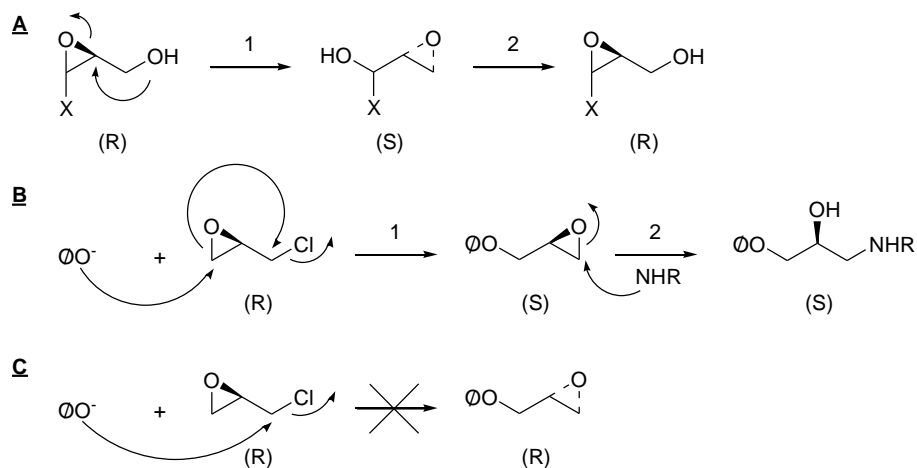


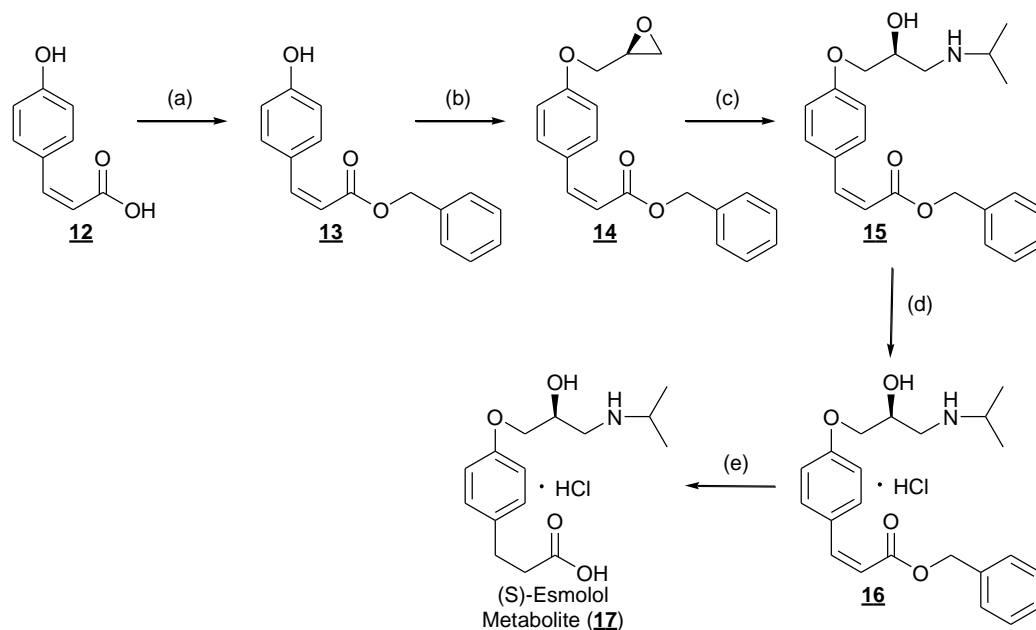
Figure 2. Payne-like opening of epichlorohydrin during the esmolol synthesis. Pathway A shows a true Payne rearrangement as it pertains to how an epoxy alcohol becomes isomerized when treated with aqueous base. Pathway B shows a Payne-like isomerization of the epoxide when epichlorohydrin is attacked by a phenolate anion. Pathway C shows an alternate reaction that can also lead to an epoxy intermediate.⁽⁶⁾

As seen in Figure 2, the reaction of epichlorohydrin with the phenolate anion (**2**) is expected to follow pathway B. Pathway C could account for the reason why there is a small percentage of the opposite enantiomer that is visualized during the HPLC methods leading to calculations of the % ee.

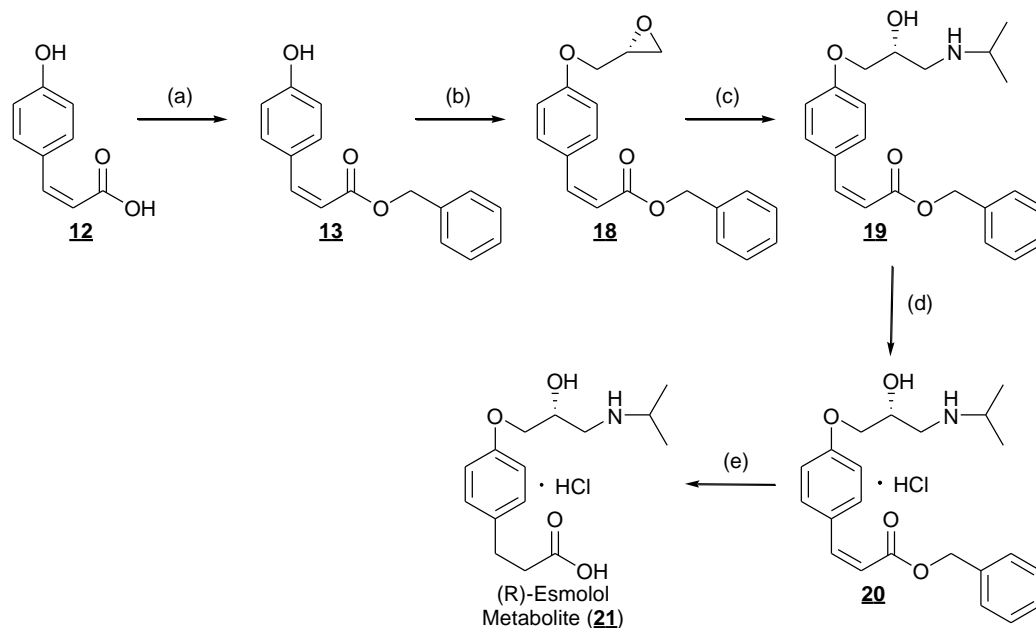
1.3 Synthesis of (S)- and (R)-Esmolol Acid Metabolites

During our investigations of esmolol there was an increasing need for the acid metabolites. A synthetic route was devised, shown in Schemes 3 and 4, which took advantage of hydrogenation to simultaneously reduce a double bond as well as remove a benzyl group that was used to protect the acid as its ester. This synthesis utilizes commercially available *p*-coumaric acid (**12**) to start the synthesis and eventually provides the acid metabolite in one extra step when compared to the esmolol synthesis.

The theory behind this scheme was to utilize hydrogenation to reduce the double bond and deprotect the benzyl ester during the same reaction in order to afford the esmolol acid metabolites (**17**, **21**). The synthesis of each enantiomer of the acid metabolite uses *p*-coumaric acid (**12**) from which a benzyl ester is made through the use of benzyl bromide, potassium bicarbonate and dimethylformamide. The initial product mixture is applied to a silica column to remove the excess benzyl bromide to afford (**13**).⁽⁷⁾ (R)- And (S)-epichlorohydrin are then each used in conjunction with potassium carbonate and acetone to create the epoxide ether linkage with (S)- and (R)-stereochemistry, respectively, for **14** and **18**. The rest of the synthesis is mirrored exactly for both enantiomers after the attachment of the epoxide ring. Each epoxide intermediate was then reacted with isopropylamine in methyl ethyl ketone to afford the free amine intermediates **15** and **19**. The free amines were subjected to a methyl ethyl ketone-HCl solution where HCl gas is previously bubbled into methyl ethyl ketone cooled in an ice bath until the pH \approx 1-2. The methyl ethyl ketone-HCl solution and methyl ethyl ketone were added in a 1:1 ratio to the free amine oil. Each solution was evaporated and dried under reduced pressure as a prelude to crystallization. The latter was accomplished by



Scheme 3. Synthetic scheme to prepare the (S)-esmolol acid metabolite (**17**) from *p*-coumaric acid (**12**). Conditions and yields: (a) BnBr (1.5 eq), KHCO₃, DMF, 40 °C, 15 h, 80%; (b) *R*-epichlorohydrin, K₂CO₃, Acetone, reflux, 48 h, 45%; (c) Isopropylamine, MEK, reflux, 40 h, 98%; (d) MEK·HCl, MEK, 95%; (e) H₂, Pd/C, EtOAc, 35 psi, 18 h, 75%.



Scheme 4. Synthetic scheme to prepare the (R)-esmolol acid metabolite (**21**) from *p*-coumaric acid (**12**). Conditions and yields: (a) BnBr (1.5 eq), KHCO₃, DMF, 40 °C, 15 h, 80%; (b) *S*-epichlorohydrin, K₂CO₃, Acetone, reflux, 24 h, 62%; (c) Isopropylamine, MEK, reflux, 16 h, 100%; (d) MEK·HCl, MEK, 97%; (e) H₂, Pd/C, EtOAc, 35 psi, 18 h, 74%.

dissolving the evaporated residues in a minimal amount of methyl ethyl ketone and adding ethyl ether until the solution turned turbid, after which each flask was placed at 4°C to afford **16** and **20**. These products were reacted using hydrogenation over palladium-carbon to reduce the double bond and deprotect the benzyl ester to free the carboxylic acid for the final products **17** and **21** in a 25% and 36% overall yield over 5 steps, respectively.

As with the esmolol scheme described in Section 1.2, the stereochemical integrity and ee of the acid metabolite scheme was also verified using HPLC and optical rotation. These compounds did not have any literature reported values so there was not a standard value to base our values on. Table 2 illustrates the HPLC and optical rotation data obtained for the epoxide intermediates (**14**, **18**) as well as for the acid metabolites (**17**, **21**).

Table 2. HPLC and optical rotation values found for the epoxide intermediates (**14**, **18**) and acid metabolites (**17**, **21**).^a

Compound	HPLC (%ee)	$[\alpha]^b$
14	80.68%	+8.4°
18	81.86%	-6.7°
17	84.06%	-13.8°
21	91.26%	+16.7°

^aHPLC was performed on a reverse phase chiral column and optical rotations were obtained on a 589nm λ Na D line at a concentration of 1.0 g/100 ml in MeOH. ^bNo literature values have previously been reported for any of these intermediates or acid metabolites of the esmolol enantiomers.

While experimenting with crystallization methods on the free amine intermediates (**15**, **19**) it was found that transesterification of the ester moiety occurs easily as seen in Figure 3. As with the esmolol crystallization scheme, which uses methanolic HCl for the

HCl salt formation, the same was tried for the acid metabolite scheme. It was witnessed that the benzyl group is replaced with a methyl through the transesterification of the ester group at the end of the molecule. The HCl used to acidify the methanol enhances the ability to attack the carbonyl group of the ester and displace the benzyl group. Through NMR studies the benzyl group peaks at 7.48-7.34 ppm have disappeared and a peak for the methyl group at 3.70 ppm is instead seen while still retaining the double bond.

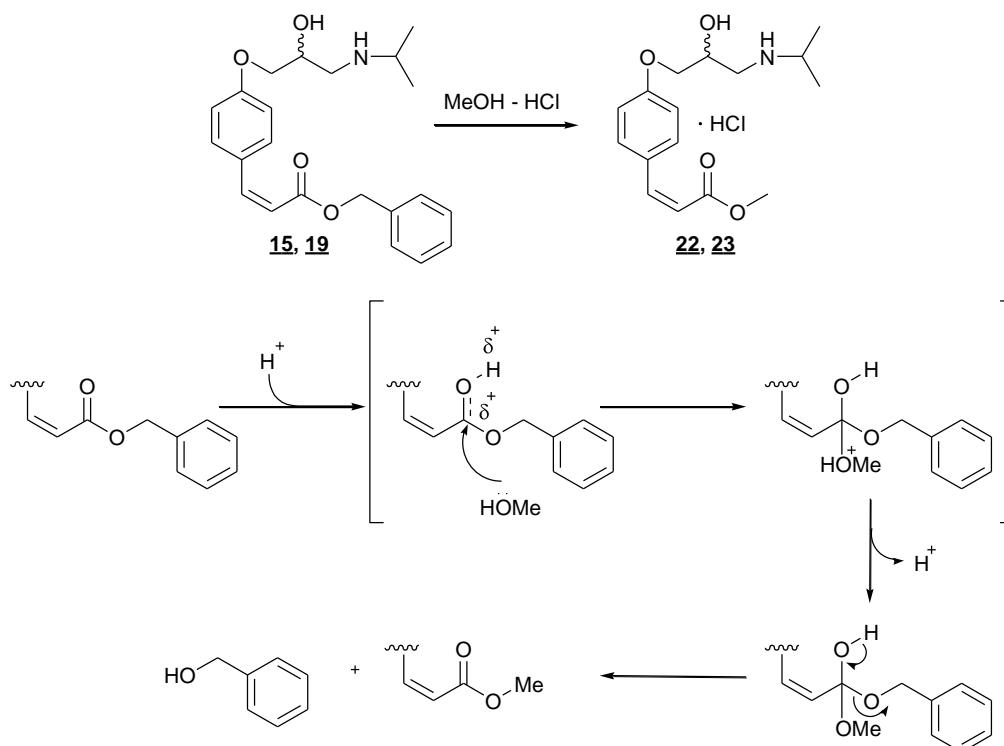
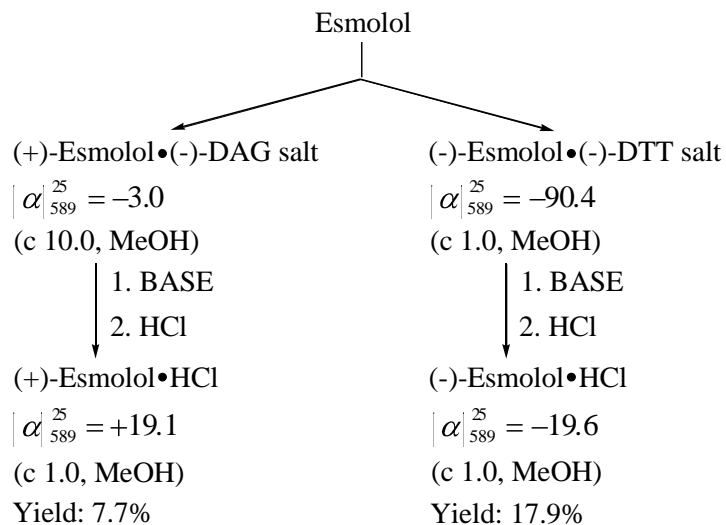


Figure 3. Acid catalyzed transesterification of a benzyl protected ester moiety with MeOH·HCl. Where **15** and **22** are the (S)-enantiomer and **19** and **23** are the (R)-enantiomer.

1.4 Diastereomeric Salt Studies using Esmolol

As mentioned previously, it is most desirable to market drugs as their pure enantiomeric forms instead of their racemic mixtures. Two common methods for preparing pure enantiomeric forms involve: (i) asymmetric synthesis which has been described earlier; and, (ii) optical resolution.⁽²⁾ In 1988, a group from Dupont issued a

patent on the resolution of (\pm)-esmolol into (+) and (-) isomers. They followed the procedure set forth in Scheme 5. This procedure has been performed in our lab to try to recreate the author's results but so far efforts have not proved promising.



Scheme 5. Procedure for the resolution of (\pm)-esmolol with diastereomeric salts to (+)- and (-)-esmolol. (-)-DAG: (-)-2,3,4,6-di-O-isopropylidene-2-keto-L-gulonic acid. (-)-DTT: (-)-O,O'-di-p-toluoyl-L-tartaric acid.⁽²⁾

During the procedure diastereomeric salts are formed in order to exploit physicochemical differences that the latter have such that one isomer might crystallize out while the other stays in solution. The free amine of (\pm)-esmolol is reacted with (-)-2,3,4,6-di-O-isopropylidene-2-keto-L-gulonic acid [(-)-DAG] in a 1:1 mixture of acetone and water. The (-)-DAG salt is treated with sodium hydroxide and then treated with methyl ethyl ketone and acidified with HCl. Ethyl ether is then added as an attempt to crystallize pure (+)-esmolol. In order to crystallize the other isomer, the free amine of (\pm)-esmolol is reacted with (-)-O,O'-di-p-toluoyl-L-tartaric acid [(-)-DTT] using acetone as the solvent. The resulting (-)-DTT salt is collected and treated with sodium hydroxide, dissolved in methyl ethyl ketone, acidified with HCl, and then ethyl ether is added in an attempt to crystallize pure (-)-esmolol. So far these procedures have been followed

exactly as stated in the patent but have not been able to produce the same results as their claims.

To further examine the feasibility of this patented process, the intermediate diastereomeric salts have been prepared using the pure (-)- and (+)-enantiomers of esmolol, respectively. Again, as above, no promising results have been seen with the use of pure enantiomeric free amines. The now single diastereomeric salts will not even crystallize out of solution when following the procedure exactly as reported.

1.5 Future Directions

The increasing need for enantiomerically pure drugs in the pharmaceutical market makes it very advantageous to devise a scheme that is industrially applicable in order to make the (S)-(-)-esmolol hydrochloride. In the past, chiral (benzyl)isopropylamine auxiliaries were used analogously to the isopropylamine attack on the epoxide ring to favor one enantiomer over the other through steric hindrance.^(8, 9) Expanding on the benzylamine auxiliaries may become beneficial to completing an industry applicable synthesis. It would also be interesting to try to utilize other diastereomeric salts that may be able to resolve the crystallization of (\pm)-esmolol more reproducibly.

Chapter 2

Development of (-)-Glyceollin I Process Chemistry

2.1 Introduction

Natural products have been of high interest to the pharmaceutical industry for many years. In China's early history, they used herbal supplements like ginkgo and echinacea to try to remedy illnesses and diseases. Of recent interest have been the phytoalexins that are produced within soybeans as a defense mechanism to various insults, such as that from attack by cyst root nematodes.⁽¹⁰⁾ Glyceollins (Glys) are members of this family of phytoalexins and are produced as a mixture of three family members.⁽¹¹⁾ It has been shown that these Glys exhibit antiestrogenic activity, have anticancer properties and may behave as selective estrogen receptor modulators (SERMs).^(12, 13, 14) To date there have been three Glys reported. Their structures are depicted in Figure 4.

Glyceollin I is the most prevalent of the three family members produced in soybeans when elicited by stress.⁽¹¹⁾ According to results produced in our lab and in conjunction with our collaborators, Gly I also has the most promising drug profile in targeting breast cancer. With this information it prompted our lab to undertake the first total synthesis of Gly I.^(15, 16) This 14-step synthesis, Scheme 6, had previously been completed on small scale in low overall yields. With the promising results that Gly I has

exhibited toward breast cancer treatment, it was taken upon our lab to complete a team-oriented multi-gram scale total synthesis to support the testing needed to further develop Gly I as a potential drug candidate.

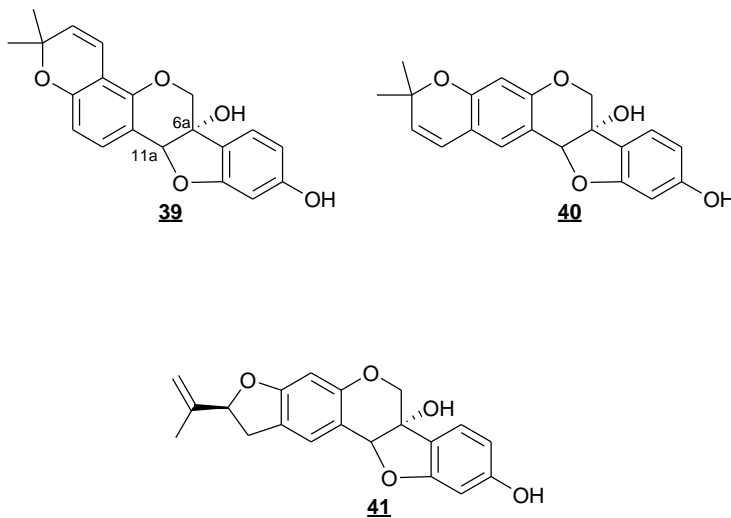
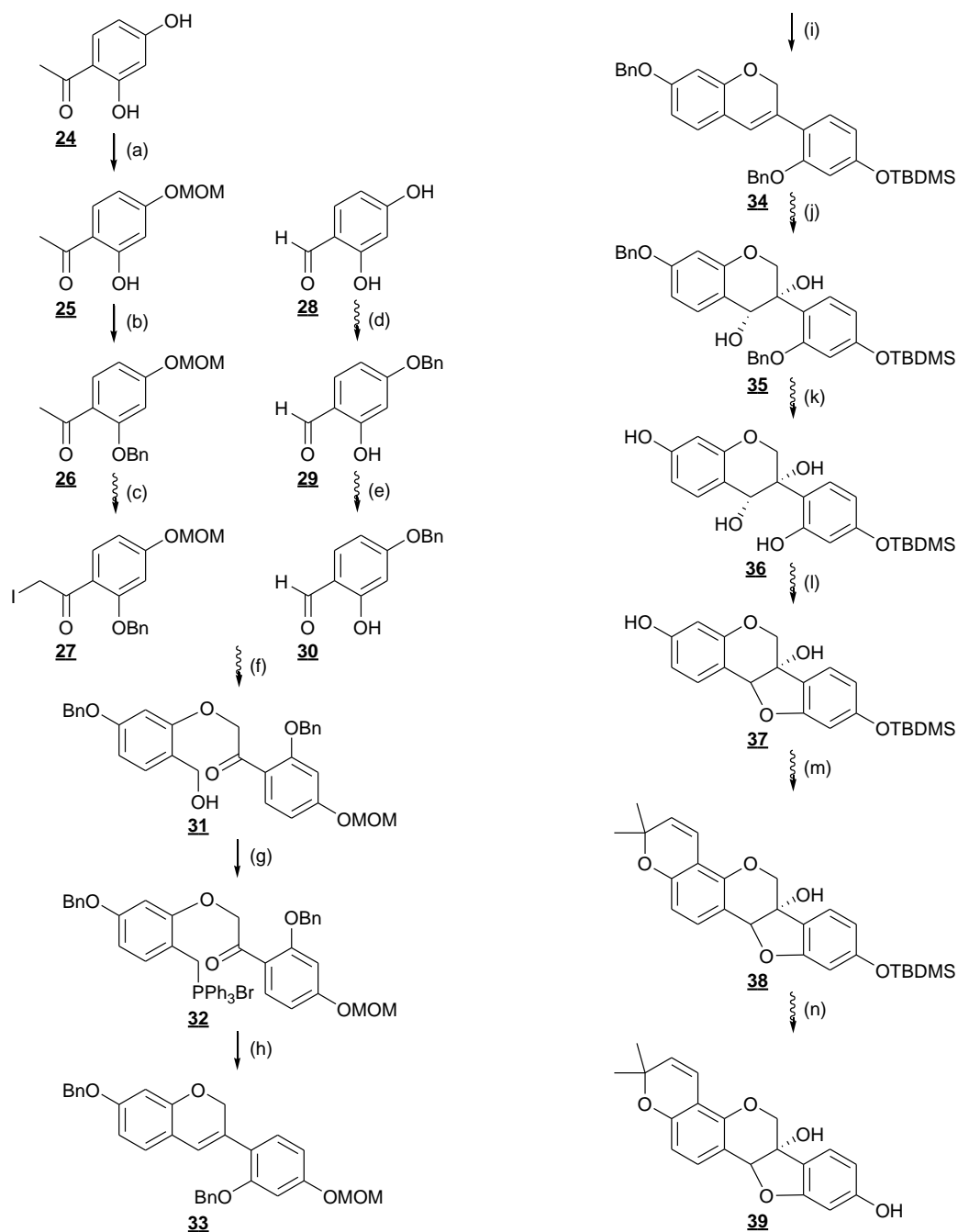


Figure 4: Structures of the Glyceollins I (**39**), II (**40**), and III (**41**).

2.2 Multi-gram Synthesis of Glyceollin I

The promising anticancer properties of the natural product Glyceollin I (Gly I) prompted a multi-gram total synthesis in an effort to produce about 2 grams of material. In the beginning of the project a team of four lab members was assembled to tackle the 14-step total synthesis. A timeline for this effort leading to a FDA-approved clinical trial that included obtaining pharmacokinetic data as well as *in vitro* and *in vivo* studies, was devised wherein 6 months was allotted for the synthetic effort. During the entire synthesis three lab members worked on the project throughout while two others helped during the times when most needed. Scheme 6 shows the 14-step synthesis. The solid arrows represent the steps that were personally conducted by me and the ‘wavy’ lined arrows



Scheme 6. Synthetic Scheme for (-)-Glyceollin I (**39**). Solid arrows depict reactions personally completed while ‘wavy’ lined arrows depict reactions completed by other team members. Conditions and yields: (a) (i) Dimethoxymethane, Zn(OAc)₂, EtOAc, Acetyl Chloride, RT, 3 h (ii) **24**, DIPEA, EtOAc, RT, 18 h, 100%; (b) BnBr, K₂CO₃, Acetone, reflux, 72 h, 72%; (c) SelectfluorTM, I₂, DCM:MeOH (1:6), RT, 16 h, 61%; (d) BnBr, NaHCO₃, ACN, reflux, 48 h, 91%; (e) NaBH₄, EtOH, -20°C, 4 h, 60%; (f) 4 Å MS, K₂CO₃, Acetone, reflux, 16 h, 65%; (g) PPh₃•HBr, ACN, RT, 1 h; (h) *t*-BuOK, MeOH, reflux, 24 h, 58% (**32** to **33**); (i) (i) PPh₃•HBr, DCM, RT, 1 h (ii) TEA, TBDMS-Cl, RT, 14 h, 63%; (j) (DHQD)₂PHAL, OsO₄, DCM, -20°C, 18 h, 90%; (k) Pd/C, H₂, 35 psi, EtOH, RT, 6 h, 93%; (l) 4 Å MS, cat. Polymeric base, EtOH, 90°C, 20 h, 49%; (m) 1,1-Diethoxy-3-methyl-2-butene, 3-picoline, *p*-Xylene, 120°C, 20 h, 57% ; (n) Et₃N•3HF, Pyridine, DCM, RT, 5 h, 84%.

represent the reactions that the other team members completed. Many reactions in the preliminary stages required a step-wise scale-up that started at small scale reactions of <1 g and eventually led to larger scale reactions, especially during the beginning of the synthesis where initial steps exceeded 100 g scales.

The descriptions of the following steps are the reactions that have been personally completed during this team oriented synthetic effort. The synthesis towards Gly I began with the protection of the *para*-hydroxy of 2',4'-dihydroxyphenone (**24**) with a MOM group. During the reaction, MOM-Cl was produced *in situ* using dimethoxymethane reacted with acetyl chloride which was added dropwise in the flask. To this *in situ* made reagent, 200 g of **24** was added to the reaction after which diisopropylethylamine was added dropwise and allowed to react for 18 hours to afford **25**. The *ortho*-hydroxy was then protected using benzyl bromide with potassium carbonate to afford **26**. After compounds **27** and **30** were coupled together by one of the other team members, a Wittig reaction utilizing triphenylphosphine hydrobromide was used in order to create one of the rings needed in the backbone of Gly I. Compound **31** was reacted with triphenylphosphine hydrobromide in acetonitrile and allowed to react for one hour to afford the Wittig salt, **32**. Upon scale-up of this reaction it was noticed that when one equivalent of triphenylphosphine hydrobromide was added to the reaction mixture at one time, the product, visualized by NMR, produced a new singlet peak at 10.58 ppm and lacked the peak splitting pattern of the MOM group. It was seen that after work-up 80-90% of the MOM deprotected material was recovered. To avoid this deprotection, triphenylphosphine hydrobromide was added in five separate 0.2 equivalent fractions 15-20 minutes apart. This could easily be monitored because **31** has limited solubility in

acetonitrile whereas **32** is very soluble such that by the last addition of the 0.2 equivalent portion, the reaction mixture became a clear solution. Once **32** was in hand and NMR studies showed less than 2% deprotection of the MOM group, it was subjected to potassium *t*-butoxide in methanol and allowed to reflux for 24 hours in order for the Wittig reaction to occur and provide **33**.

The question was raised whether or not the MOM group might be deprotected at the end of the synthesis while still retaining the hydroxyl group at the *6a*-position by using this gentle method. Others explored that possibility which, in the end, was not fruitful. This is because the *6a*-position is very sensitive to harsh conditions (especially highly acidic or basic) and can readily lead to the dehydroxylated unsaturated product between the *6a*- and *11a*-positions of the ring junction. Earlier studies have shown that MOM was difficult to remove and that had resulted in the need to change protecting groups at this synthetic step of the overall synthesis.⁽¹⁵⁾ The final reaction conditions that I completed culminated in a one-pot reaction using triphenylphosphine hydrobromide to deprotect the MOM group after which TBDMS-Cl was added along with triethylamine to reprotect the *para*-hydroxyl group with a TBDMS so as to afford **34**. Close to 125 g of **34** was made from 1.5-2 kg of **24** and **28** combined.

The Gly I project proved to be very difficult at times and showed that “*nothing is ever simple.*”⁽¹⁷⁾ There were many difficulties throughout the project that had to be quickly resolved by ideas proposed by the team members of this project. For example, reaction (c) required the commercial reagent Selectfluor,TM but not every bottle produced a successful reaction even from the same lot numbers. This elicited a 5 g batch test reaction of each bottle before use with many bottles failing to give successful reactions.

Next was the MOM deprotection observed at step (g) with the use of triphenylphosphine hydrobromide. After testing some different reaction solvents it was decided to try to add the reagent in fractioned portions and those studies resulted in the five 0.2 equivalent portions approach which ultimately produced the most beneficial results. Reaction (j) probably gave the team the biggest challenge because some batches of osmium tetroxide were not working. In addition, the chiral ligand needed for this step [(DHQD)₂PHAL] was sold out across the entire U.S. while 200 g was still needed to complete these reactions. After a method was obtained for synthesizing the chiral ligand, a triple quality control protocol was set in-place for verifying batches of **34** with known osmium tetroxide and chiral ligand, verifying osmium tetroxide with known batches of **34** and chiral ligand, and verifying the synthesized chiral ligand with known batches of **34** and osmium tetroxide.⁽¹⁸⁾ After this situation was solved, the reaction procedure developed quite nicely with the benefit of improved yields. The team effort had a total of five team members working on it at any one time with three of these members being constantly involved in the project. As a synthetic team, about 500 reactions were completed for the total synthesis of ca. 5 g of (-)-Gly I in a 34 week period of time.

2.3 Future Directions

One of the future directions for the lab is to create analogs to try to expand the structure-activity relationship data of the binding pocket that Gly I fits into while also trying to synthesize an analytical standard of Gly II (**40**) and III (**41**). Another main focus is on soy harvests from fields within Ohio to try and extract mainly pure Gly I, as well as Gly II and III. Finally, there will be additional testing of the intermediates produced

during the synthesis for any activity against breast cancer, as well as the possibility of selective estrogen receptor modulators. The arena for the glyceollins and intermediates in their role against breast cancer are proving to be a very exciting path being driven by our lab.

Chapter 3

Design of STAT5 Small Molecule Inhibitors

3.1 Introduction

The signal transducers and activators of transcription (STAT) consists of seven family members: STAT 1, 2, 3, 4, 5a, 5b and 6.⁽¹⁹⁾ For the past few years, researchers have been experimenting to elucidate the individual roles of each of the family members. It has been shown that many of the members exhibit unique functions in signal transduction through members of the cytokine receptor superfamily.⁽¹⁹⁾ Utilizing the knowledge obtained from each of these family members, in turn, allows them to serve as potential targets for different types of cancers and diseases. Our collaborators have shown that STAT5 is a potential therapeutic target for prostate cancer.⁽²⁰⁾

STAT5 consists of two highly confluent forms which are 94-kDa STAT5a and 92-kDa STAT5b.⁽²⁰⁾ STAT5a and STAT5b (STAT5) are cytoplasmic proteins that act as both signaling proteins and nuclear transcription factors. Activation of STAT5 starts with the phosphorylation of a specific tyrosine residue in the carboxy-terminal domain by a tyrosine kinase of the JAK protein family.^(20, 21, 22) After this phosphorylation, the STAT5 dimerize and translocate to the nucleus where they bind to specific gene promoters.⁽²⁰⁾

STAT5 proteins are divided into five domains, shown in Figure 5. The N-terminal domain is involved in stabilizing interactions between two STAT5 dimers to form tetramers, which are needed for maximum transcriptional activation of weak promoters.⁽²³⁾ After this domain is the coiled-coil domain which facilitates protein-protein interactions important for transcriptional regulation.⁽²⁴⁾ The DNA binding domain mediates the direct binding of STAT5 to DNA which is stabilized by the adjacent linker domain.⁽²⁵⁾ The portions of both the STAT5a and 5b proteins that are the most similar are their SH2 domains which mediate STAT dimerization and receptor recruitment.⁽²⁶⁾ The carboxy terminus has a transactivation domain which varies in both length and sequence among all the STAT family members. The carboxy terminus binds co-activators and is explicitly involved in the initiation of transcription.⁽²⁷⁾

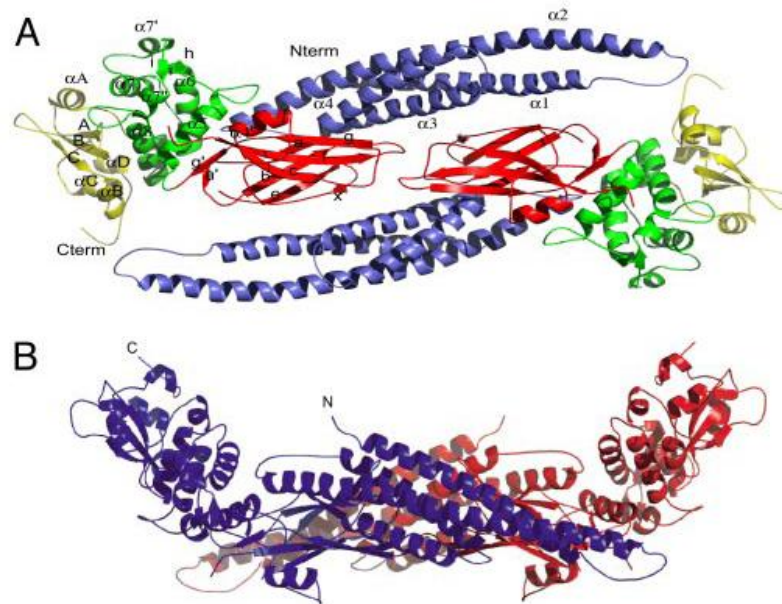


Figure 5. STAT5 domains and structure. Ribbon diagram of the STAT5a dimer. **A**, top view. The color coding is according to the domain: the N-terminal (Nterm) four-helix bundle (blue), the β -barrel domain (red), the linker domain (green), and the SH2 domain (yellow), C-terminal (Cterm). **B**, side view (the dyad running parallel to the displayed plane). The color coding is according to each monomer.⁽²⁸⁾

Activation of STAT5 in prostate cancer can predict early prostate cancer recurrence because STAT5 is active in prostate cancer but not in normal prostate cells.⁽²⁹⁾ It has been found that prolactin, a local mitogen produced during prostate cancer, activates STAT5 and promotes the growth of cancer cells.^(30, 31) Our collaborators have shown in prostate cancer that the activation of STAT5 and prolactin expression is associated with a high histological grade of cancer.⁽²⁹⁾ Studies have shown that mice which overexpress prolactin develop a large amount of prostate enlargement, whereas mice that do not express prolactin have smaller prostates than even their wild-type controls.^(32, 33) With these results, a dominant-negative mutant of STAT5a, which blocks both of the STAT5's, was created and shows that inhibition of STAT5 induces apoptotic death of human prostate cancer cells *in vitro*.⁽³⁴⁾

The results showing that inhibition of STAT5 can lead to death of prostate cancer cells, suggest that STAT5 is a viable target for prostate cancer prevention and treatment. If a small molecule can be synthesized to block the dimerization and activation of STAT5, the resulting death of prostate cancer cells could lead to a good candidate for a drug against prostate cancer.

3.2 Using STAT3 Compounds as Starting Points

With some similarities between STAT3 and STAT5, a literature search was completed by our collaborators to try to discover what types of compounds have been used to inhibit STAT3. A list of compounds was formulated with the hope that one of these compounds could provide a lead scaffold into what STAT5 needs in regard to small molecule inhibition. Figure 6 shows the list of compounds that would be desired. Of the

seven compounds listed, only one was picked to try to synthesize and test. The compound denoted as “*static*,” **43** (stat three inhibitory compound), was the scaffold used to try to create some structure activity relationships (SAR) for the binding pocket of STAT5.

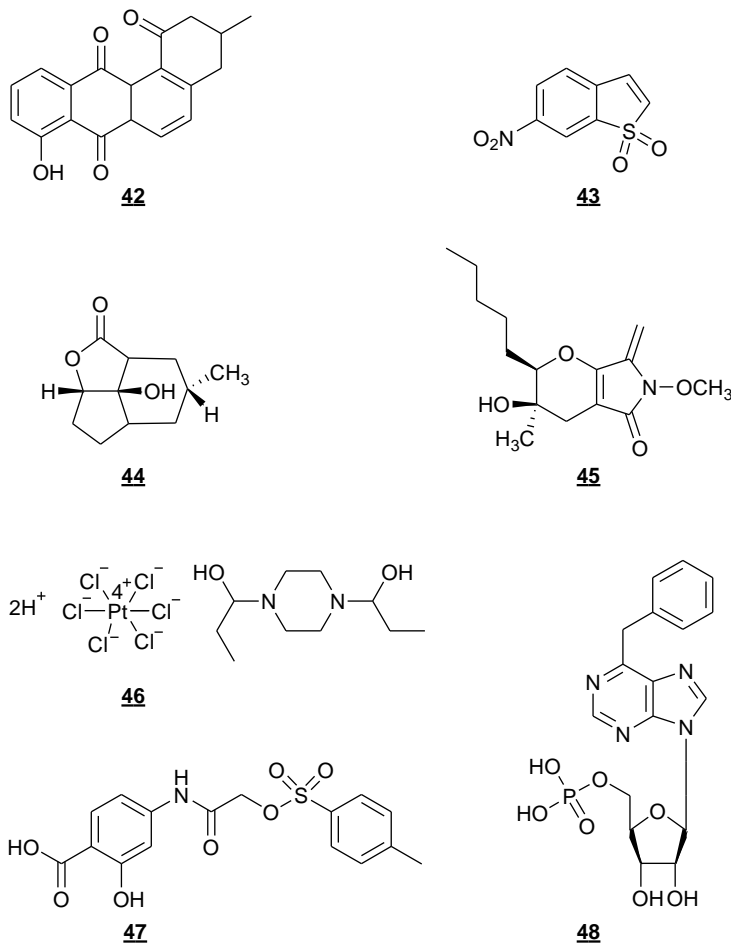


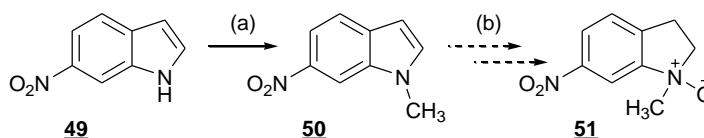
Figure 6. Structures of compounds that have been shown to inhibit STAT3. ^(35, 36, 37, 38, 39, 40)

Among the compounds listed by our collaborator, all had some activity toward the inhibition of the STAT3 pathway. Based on results from our collaborator, compounds **42** and **48** were excluded from any further discussions. With a few compounds left to choose from, **43** was picked to be the first scaffold to develop SAR data and **47** was selected as our next choice. Compound **43** was itself able to be purchased from Sigma-Aldrich. A few compounds were designed to try to explore the necessity of the different groups on

43, namely the nitro group, the double bond in the five membered ring, and the sulfonyl group. Compounds were designed to examine each of these factors individually, but there was not enough time to complete the work with this series in conjunction with the three aspects of my research activities.

3.3 Synthesis of Initial STAT5 Small Molecule Inhibitor Members

The only STAT5 analog that was synthesized was a nitroindole derivative to try to exploit the sulfonyl group in **43**. Scheme 7 shows the synthetic scheme followed to prepare the nitroindole derivative.



Scheme 7. Synthetic scheme to prepare nitroindole derivative **51**. Conditions and yields: (a) K_2CO_3 , Dimethylcarbonate, DMF, reflux, 2 h, 77%; (b) Reduction followed by oxidation.

The rationale behind the synthesis of **51** was to examine the relationship between the sulfonyl group in **43** and replace it with an N-oxide that might display similar electronic features. 6-Nitroindole, **49**, was reacted with dimethylcarbonate and potassium carbonate in dimethylformamide to produce the N-methylated nitroindole product **50**.⁽⁴¹⁾ The next set of reactions will look into the reduction of the double bond in the five membered ring followed by N-oxidation to obtain **51**.

When the synthesized product and its intermediates are in hand, they will be sent to our collaborators for testing along with **43** that has already shown good STAT3 inhibition. Other compounds have been purchased that relate to the above nitroindole scheme and will also look at the SAR of **43**. These compounds are shown in Figure 7 and

they will be sent for testing as well. Even though this represents a very small library of compounds, the information that the testing may show will provide initial insights toward the next level of SAR manipulations that should then be completed in more detail in the future.

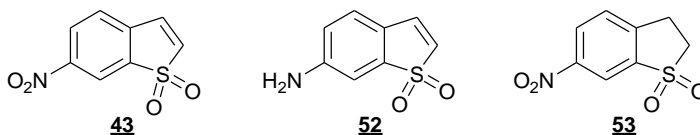


Figure 7. Compounds purchased from commercial suppliers to be tested against STAT5.

3.4 Future Directions

Although not much synthesis was completed for this part of the project, the initial probe of the structure activity relationships for the STAT5 pathway has begun. There are many more paths that can be taken to try to design more compounds to be tested. Through a recent literature screen done by our collaborators, eight compounds were identified that had good activity against STAT3. With this knowledge, each of these compounds could be synthesized in the lab and tested to see if any are active against the STAT5/JAK2 assay that our collaborators have devised. If any show signs of activity, then more intensive SAR studies can be developed and tested. The synthesized and purchased compounds will be screened against our collaborators STAT5 assay to see if they inhibit any activity. With the knowledge obtained from this testing, appropriate decisions will be made to either create more analogs or to try another lead structure.

Chapter 4

Experimental Section

Materials and Methods

All Reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich or Fisher Scientific) and were used without purification. Thin-layer chromatography (TLC) was done on 250 μ fluorescent TLC plates acquired from VWR and visualized by using UV light or iodine vapor. Normal phase flash column chromatography was performed using silica gel (200-425 mesh 60 Å pore size) and ACS grade solvents. Chromatography eluents were assessed by TLC and fractions containing desired material were initially evaporated under reduced pressure using a Büchi rotavapor either by water aspirator vacuum or by vacuum achieved with a Welch dry-vacuum pump (Model 2027). Temperatures never exceeded 60 °C during solvent removal. A Welch high-vacuum pump (Model 1405B-01) was used in many cases to further remove final traces of solvents. Chiral HPLC was performed on a Waters HPLC 2695 instrument with dual λ absorbance detector using a chiralcel OD column purchased from Chiral Technologies, Inc. Melting points (mp) were recorded on a Electrothermal digital apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on either an INOVA600 or Varian VXR5-400 instrument and were referenced using residual non-deuterated solvent as an internal standard. Coupling constants are expressed in Hertz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t

= triplet, q = quarter, m = multiplet, bs = broad singlet, dd = doublets of doublet.

Elemental analyses were performed by combustion analysis at Atlantic Microlab, Inc.

Methyl 3-(*p*-hydroxyphenyl)propionate (2**)**

3-(4-Hydroxyphenyl)-propionic acid (0.9884 g, 5.95 mmol) in anhydrous methanol (10 ml) and 10 drops conc. HCl was heated at reflux for 24 hours. The reaction mixture was evaporated to dryness and toluene (10 ml) was added to take up the oil. The toluene organic phase was washed with water (3 x 5 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to provide **2** (0.8421 g, 78%) as a clear oil; TLC R_f 0.58 [EtOAc:DCM (1:15)]; ¹H NMR (600 MHz, CDCl₃) δ 7.05-7.04 (d, 2H, *J* = 8.4 Hz), 6.76-6.74 (d, 2H, *J* = 14.4 Hz), 3.67 (s, 3H), 2.89-2.86 (t, 2H, *J* = 7.8 Hz), 2.62-2.59 (t, 2H, *J* = 7.8 Hz).

(±)-Methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (3**)**

A mixture of methyl 3-(*p*-hydroxyphenyl)propionate (5.05 g, 28.0 mmol), epichlorohydrin (6.57 ml, 83.8 mmol), K₂CO₃ (5.8 g, 42.0 mmol) and MEK (42.5 ml) was heated at reflux for 20 hours. The reaction mixture was filtered and evaporated under reduced pressure. The resulting oil was taken up in toluene (22 ml) and washed consecutively with water (11 ml), 1N NaOH (2 x 11 ml) and water (2 x 11 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to provide **3** (5.62 g, 85%) as a clear oil; TLC R_f 0.72 [EtOAc:DCM (1:15)]; ¹H NMR (400 MHz, CDCl₃) δ 7.11-7.10 (d, 2H, *J* = 9.0 Hz), 6.85-6.83 (d, 2H, *J* = 8.4 Hz), 4.20-4.18 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 3.0 Hz), 3.94-3.92 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 5.4 Hz), 3.66 (s, 3H), 3.35-3.33 (m, 1H), 2.90-2.87 (m, 3H), 2.75-2.74 (dd, 1H, ²*J* =

4.8 Hz, $^3J = 2.4$ Hz), 2.60-2.58 (t, 2H, $J = 8.4$ Hz); Anal. Calcd. for C₁₃H₁₆O₄: C, 66.09; H, 6.83; Found: C, 65.86; H, 6.77.

(±)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate (4)

A mixture of methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (5.04 g, 21.3 mmol), isopropylamine (12.9 ml, 151.4 mmol) and MeOH (13 ml) was heated at reflux for 4 hours. The reaction mixture was evaporated to dryness, toluene (20 ml) was added and again evaporated under reduced pressure to provide **4** (6.28 g, 100%) as a yellow oil; TLC R_f 0.05 [EtOAc:DCM (1:15)]; ¹H NMR (600 MHz, CDCl₃) δ 7.11-7.09 (d, 2H, $J = 8.4$ Hz), 6.84-6.83 (d, 2H, $J = 9.0$ Hz), 4.07-4.05 (m, 1H), 3.96-3.94 (t, 2H, $J = 5.4$ Hz), 3.66 (s, 3H), 2.93-2.87 (m, 4H), 2.77-2.74 (dd, 1H, $^2J = 12.6$ Hz, $^3J = 8.4$ Hz), 2.60-2.58 (t, 2H, $J = 8.4$ Hz), 2.54 (bs, 1H), 1.13-1.12 (d, 6H, $J = 6.0$ Hz); Anal. Calcd. for C₁₆H₂₅NO₄: C, 65.06; H, 8.53; N, 4.74; Found: C, 64.77; H, 8.31; N, 4.49.

(±)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate hydrochloride (5) [(±)-Esmolol]

HCl gas was bubbled into anhydrous methanol over an ice-bath to provide MeOH·HCl with a pH ≈ 1. Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate (6.28 g, 21.3 mmol) was dissolved in anhydrous MeOH (23 ml) after which MeOH·HCl (23 ml) was added and the pH of the resulting solution was ~3-4. The solvents were evaporated to dryness and the resulting oil was taken up in toluene (20 ml) and evaporated under reduced pressure. The oil was taken up in a minimal amount of warm MEK and placed in the refrigerator overnight. Crystals formed were filtered,

washed with cold MEK and dried to provide **5** (4.74 g, 67%) as a white granular solid, mp 84-86 °C [Lit.⁽⁴²⁾ 85-86 °C]; TLC R_f 0.44 [MeOH:DCM (1:10)]; ¹H NMR (600 MHz, CDCl₃) δ 9.65 (s, 1H), 8.49 (s, 1H), 7.07-7.05 (d, 2H, *J* = 11.4 Hz), 6.80-6.78 (d, 2H, *J* = 12.0 Hz), 4.62-4.58 (m, 1H), 4.05-4.03 (dd, 1H, ²*J* = 9.6 Hz, ³*J* = 4.2 Hz), 3.95-3.93 (dd, 1H, ²*J* = 9.6 Hz, ³*J* = 6.0 Hz), 3.64 (s, 3H), 3.44-3.40 (m, 1H), 3.30-3.27 (m, 1H), 3.15-3.10 (m, 1H), 2.87-2.84 (t, 2H, *J* = 7.8 Hz), 2.57-2.55 (t, 2H, *J* = 8.4 Hz), 1.49-1.46 (dd, 6H, ²*J* = 9.6 Hz, ³*J* = 6.6 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 173.48, 156.83, 133.43, 129.43, 114.70, 69.71, 65.82, 51.72, 48.25, 36.07, 30.21, 19.17; Anal. Calcd. for C₁₆H₂₆ClNO₄: C, 57.91; H, 7.90; N, 4.22; Found: C, 57.94; H, 7.89; N, 4.21.

(S)-(+)-Methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (6**)**

A mixture of methyl 3-(*p*-hydroxyphenyl)propionate (0.9079 g, 5.04 mmol), R-epichlorohydrin (1.43 ml, 18.4 mmol), K₂CO₃ (1.12 g, 8.1 mmol) and MEK (9 ml) was heated at reflux for 20 hours. The reaction mixture was filtered and evaporated under reduced pressure. The resulting oil was taken up in toluene (6 ml) and washed consecutively with water (3 ml), 1N NaOH (2 x 3 ml), and water (2 x 3 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to provide **6** (0.96 g, 81%) as a clear oil; TLC R_f 0.73 [EtOAc:DCM (1:15)]; HPLC 88.44% ee; [α]_D²⁵ +8.5° (c = 1 in MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.12-7.11 (d, 2H, *J* = 8.4 Hz), 6.85-6.84 (d, 2H, *J* = 8.4 Hz), 4.21-4.18 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 3.0 Hz), 3.95-3.92 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 6.0 Hz), 3.66 (s, 3H), 3.36-3.34 (m, 1H), 2.91-2.88 (m, 3H), 2.76-2.75 (dd, 1H, ²*J* = 4.8 Hz, ³*J* = 2.4 Hz), 2.61-2.58 (t, 2H, *J* = 7.8 Hz); Anal. Calcd. for C₁₃H₁₆O₄: C, 66.09; H, 6.83; Found: C, 65.95; H, 6.85.

(S)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate (7)

A mixture of (S)-(+)-methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (0.8848 g, 3.7 mmol), isopropylamine (4.9 ml, 57.5 mmol) and MeOH (7 ml) was heated at reflux for 4 hours. The reaction mixture was evaporated and the resulting oil taken up in toluene (10 ml) which was then evaporated under reduced pressure to provide **7** (1.10 g, 100%) as a clear yellow oil; TLC R_f 0.22 [MeOH:DCM (1:10)]; $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.10-7.09 (d, 2H, $J = 8.4$ Hz), 6.84-6.82 (d, 2H, $J = 9.0$ Hz), 4.07-4.04 (m, 1H), 3.95-3.94 (t, 2H, $J = 5.4$ Hz), 3.66 (s, 3H), 2.91-2.84 (m, 4H), 2.81 (s, 1H), 2.76-2.72 (dd, 1H, $^2J = 12.0$ Hz, $^3J = 8.4$ Hz), 2.60-2.57 (t, 2H, $J = 7.8$ Hz), 1.12-1.10 (d, 6H, $J = 6.6$ Hz).

(S)-(-)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate hydrochloride (8) [(S)-(-)-Esmolol]

HCl gas was bubbled into anhydrous methanol over an ice-bath to provide MeOH·HCl with a pH \approx 1. (S)-methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate (0.4826 g, 1.63 mmol) was dissolved in anhydrous MeOH (2.5 ml) after which MeOH·HCl (2.5 ml) was added and the pH of resulting solution was \sim 3-4. The solvents were evaporated to dryness and the resulting oil was taken up in toluene (3 ml) and evaporated under reduced pressure. The oil was taken up in a minimal amount of warm MEK and placed in the refrigerator overnight. Crystals formed were, filtered, washed with cold MEK and dried to provide **8** (0.2378 g, 44%) as a white granular solid. mp 85-87 °C [Lit.⁽²⁾ 93-94 °C]; TLC R_f 0.44 [MeOH:DCM (1:10)]; HPLC 97.72% ee; $[\alpha]_D^{25}$ -19.4° ($c = 1$ in MeOH); $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 9.73 (s, 1H), 8.51 (s, 1H), 7.10-7.09 (d, 2H, $J = 9.0$ Hz), 6.82-6.81 (d, 2H, $J = 9.0$ Hz), 4.62-4.60 (m, 1H), 4.08-4.06 (dd, 1H, $^2J = 9.6$ Hz, $^3J = 4.2$ Hz), 3.98-3.95 (dd, 1H, $^2J = 9.6$ Hz, $^3J = 6.0$

Hz), 3.66 (s, 3H), 3.45-3.42 (m, 1H), 3.34-3.31 (m, 1H), 3.15-3.13 (m, 1H), 2.89-2.87 (t, 2H, $J = 7.8$ Hz), 2.60-2.57 (t, 2H, $J = 7.8$ Hz), 1.51-1.49 (dd, 6H, $^2J = 8.4$ Hz, $^3J = 6.6$ Hz).

(R)-(-)-Methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (9**)**

A mixture of methyl 3-(*p*-hydroxyphenyl)propionate (1.000 g, 5.55 mmol), *S*-epichlorohydrin (1.57 ml, 20.02 mmol), K_2CO_3 (1.23 g, 8.9 mmol) and MEK (9.6 ml) was heated at reflux for 20 hours. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The resulting oil was taken up in toluene (6 ml) and washed consecutively with water (3 ml), 1N NaOH (2 x 3 ml) and water (2 x 3 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to provide **9** (1.003 g, 77%) as a clear oil; TLC R_f 0.73 [EtOAc:DCM (1:15)]; HPLC 90.02% ee; $[\alpha]_D^{25} -8.2^\circ$ ($c = 1$ in MeOH); 1H NMR (600 MHz, $CDCl_3$) δ 7.12-7.11 (d, 2H, $J = 8.4$ Hz), 6.85-6.84 (d, 2H, $J = 8.4$ Hz), 4.21-4.18 (dd, 1H, $^2J = 11.4$ Hz, $^3J = 3.6$ Hz), 3.95-3.92 (dd, 1H, $^2J = 10.8$ Hz, $^3J = 5.4$ Hz), 3.66 (s, 3H), 3.36-3.34 (m, 1H), 2.91-2.88 (m, 3H), 2.76-2.75 (dd, 1H, $^2J = 4.8$ Hz, $^3J = 2.4$ Hz), 2.61-2.59 (t, 2H, $J = 7.8$ Hz); Anal. Calcd. for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83; Found: C, 65.88; H, 6.79.

(R)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]propionate (10**)**

A mixture of (R)-(-)-methyl 3-[*p*-(2,3-epoxypropoxy)phenyl]propionate (0.9715 g, 4.1 mmol), isopropylamine (5.4 ml, 63.4 mmol) and MeOH (8 ml) was heated at reflux for 4 hours. The reaction mixture was evaporated to dryness and the resulting oil was

taken up in toluene (10 ml) and evaporated under reduced pressure to provide **10** (1.2166 g, 100%) as a clear yellow oil; TLC R_f 0.24 [MeOH:DCM (1:10)]; ¹H NMR (600 MHz, CDCl₃) δ 7.10-7.09 (d, 2H, *J* = 9.0 Hz), 6.84-6.82 (d, 2H, *J* = 9.0 Hz), 4.05-4.02 (m, 1H), 3.96-3.91 (m, 2H), 3.65 (s, 3H), 2.89-2.81 (m, 5H), 2.78 (bs, 1H), 2.74-2.70 (dd, 1H, ²*J* = 12.0 Hz, ³*J* = 8.4 Hz), 2.60-2.57 (t, 2H, *J* = 7.8 Hz), 1.10-1.09 (d, 6H, *J* = 6.6 Hz).

(R)-(+)-Methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl] propionate hydrochloride (11**) [(R)-(+)-Esmolol]**

HCl gas was bubbled into anhydrous methanol over an ice-bath to provide MeOH·HCl with a pH ≈ 1. (R)-methyl 3-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl] propionate (0.8939 g, 3.03 mmol) was dissolved in anhydrous MeOH (2.5 ml) after which MeOH·HCl (2.5 ml) was added and the pH of the resulting solution was ~3-4. The solvents were evaporated to dryness and the resulting oil was taken up in toluene (3 ml) and evaporated under reduced pressure. The oil was taken up in a minimal amount of warm MEK and allowed to stand in the refrigerator overnight. Crystals formed were filtered, washed with cold MEK and dried to provide **11** (0.2953 g, 29%) as a white granular solid. mp 85-89 °C [Lit.⁽²⁾ 93-94 °C]; TLC R_f 0.45 [MeOH:DCM (1:10)]; HPLC 95% ee; [α]²⁵_D +21.2° (c = 1 in MeOH); ¹H NMR (600 MHz, CDCl₃) δ 9.72 (s, 1H), 8.50 (s, 1H), 7.10-7.09 (d, 2H, *J* = 8.4 Hz), 6.82-6.81 (d, 2H, *J* = 8.4 Hz), 4.62-4.60 (m, 1H), 4.08-4.06 (dd, 1H, ²*J* = 9.6 Hz, ³*J* = 4.2 Hz), 3.98-3.95 (dd, 1H, ²*J* = 9.6 Hz, ³*J* = 6.0 Hz), 3.66 (s, 3H), 3.45-3.43 (m, 1H), 3.34-3.31 (m, 1H), 3.15-3.13 (m, 1H), 2.89-2.87 (t, 2H, *J* = 7.8 Hz), 2.60-2.57 (t, 2H, *J* = 7.8 Hz), 1.51-1.48 (dd, 6H, ²*J* = 9.0 Hz, ³*J* = 6.6 Hz).

Benzyl 4-hydroxycinnamate (13)

p-Coumaric acid (1.0032 g, 6.1 mmol) was dissolved in DMF (10 ml) after which KHCO_3 (0.73 g, 7.29 mmol) was added and the mixture was stirred for several minutes at room temperature. BnBr (1.1 ml, 9.26 mmol) was added and the mixture was warmed to 40°C with a water bath while monitoring by TLC [EtOAc:Hex (1:2)]. The reaction was allowed to run for 15-18 hours and upon completion was added to water (30 ml) and extracted with EtOAc (3 x 15 ml). The organic layer was subsequently washed with 10% NaHCO_3 and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The oil was applied to a silica column (ca. 100 g) EtOAc and Hex (1:2) was used as eluent to separate excess BnBr from product. The product fractions were collected and evaporated under reduced pressure to provide **13** (1.2485 g, 80%) as a white solid, mp 88-90 °C [Lit.⁽⁷⁾ 90-92 °C]; TLC R_f 0.43 [EtOAc:Hex (1:2)]; ^1H NMR (600 MHz, CDCl_3) δ 7.69-7.66 (d, 1H, $J = 16.2$ Hz), 7.43-7.33 (m, 7H), 6.85-6.84 (d, 2H, $J = 8.4$ Hz), 6.36-6.34 (d, 1H, $J = 15.6$ Hz), 5.74 (s, 1H), 5.25 (s, 2H).

(S)-(+)-Benzyl 4-(2,3-epoxypropoxy)cinnamate (14)

A mixture of benzyl 4-hydroxycinnamate (0.4975 g, 1.96 mmol), R-epichlorohydrin (1.1 ml, 14.02 mmol), K_2CO_3 (1.3 g, 9.4 mmol) and acetone (10 ml) was heated at reflux for 48 hours. The reaction mixture begins as a clear solution and with the addition of base turns yellow. The reaction was filtered and evaporated under reduced pressure to dryness. The oil was taken up in a minimal amount of Et_2O and placed in the refrigerator overnight to afford white crystals. The crystals were filtered, washed with cold Et_2O and dried under reduced pressure to provide **14** (0.2715 g, 45%) as white

crystals, mp 73.4-79.3 °C; TLC R_f 0.57 [Hex:EtOAc (1:1) buffered in 0.1% TEA]; HPLC 80.68% ee, [α]_D²⁵ +8.4° (c = 1 in MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.69-7.66 (d, 1H, *J* = 16.2 Hz), 7.48-7.46 (d, 2H, *J* = 9.0 Hz), 7.42-7.37 (m, 4H), 7.35-7.34 (m, 1H), 6.93-6.91 (d, 2H, *J* = 8.4 Hz), 6.38-6.35 (d, 1H, *J* = 16.2 Hz), 5.24 (s, 2H), 4.28-4.26 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 2.4 Hz), 3.98-3.95 (dd, 1H, ²*J* = 11.4 Hz, ³*J* = 6.0 Hz), 3.38-3.36 (m, 1H), 2.93-2.92 (t, 1H, *J* = 4.8 Hz), 2.78-2.76 (dd, 1H, ²*J* = 4.8 Hz, ³*J* = 3.0 Hz); Anal. Calcd. for C₁₉H₁₈O₄: C, 73.53; H, 5.85; Found: C, 73.66; H, 5.80.

(S)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate (15)

A mixture of (S)-(+)-benzyl 4-(2,3-epoxypropoxy)cinnamate (0.2906 g, 0.94 mmol), isopropylamine (1.63 ml, 19.1 mmol) and MEK (4 ml) was heated at reflux for 40 hours. At 24 hours, additional isopropylamine (0.5 ml) was added to the reaction since it was not complete by TLC [Hex:EtOAc (1:1)]. Once completed, the reaction mixture was evaporated under reduced pressure to provide 15 (0.3346 g, 97%) as a clear oil; TLC R_f 0.05 [Hex:EtOAc (1:1) buffered in 0.1% TEA]; ¹H NMR (600 MHz, CDCl₃) δ 7.67-7.64 (d, 1H, *J* = 16.2 Hz), 7.48-7.32 (m, 7H), 6.89-6.88 (d, 2H, *J* = 8.4 Hz), 6.36-6.33 (d, 1H, *J* = 16.2 Hz), 5.23 (s, 2H), 4.44 (bs, 1H), 4.14-4.13 (d, 1H, *J* = 4.8 Hz), 4.08-4.06 (m, 1H), 4.01-3.98 (m, 1H), 3.67-3.66 (t, 1H, *J* = 9.0 Hz), 3.50-3.48 (dd, 1H, ²*J* = 9.0 Hz, ³*J* = 6.0 Hz), 3.25 (bs, 1H), 3.18-3.16 (m, 1H), 3.01 (m, 1H), 1.36 (bs, 6H), 1.21-1.20 (d, 1H, *J* = 7.2 Hz); Anal. Calcd. for C₂₂H₂₇NO₄: C, 71.52; H, 7.37; N, 3.79; Found: C, 70.92; H, 7.39; N, 3.58.

(S)-(-)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate hydrochloride
(16)

HCl gas was bubbled into MEK over an ice-bath to provide MEK·HCl with a pH \approx 1. (S)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate (0.3335 g, 0.90 mmol) was dissolved in anhydrous MEK (6 ml) after which MEK·HCl (6 ml) was added and the pH of the resulting solution was \sim 3-4. The solvents were evaporated to dryness and the resulting oil was taken up in toluene (6 ml) and evaporated under reduced pressure. The oil was taken up in a minimal amount of warm MEK and allowed to stand in the refrigerator overnight. No crystals formed therefore a brown oil was collected to provide **16** (0.3493 g, 95%); TLC R_f 0.38 [MeOH:DCM (1:10)]; ^1H NMR (600 MHz, CDCl_3) δ 9.62 (s, 1H), 8.51 (s, 1H), 7.67-7.64 (d, 1H, $J = 15.6$ Hz), 7.44-7.33 (m, 7H), 6.88-6.87 (d, 2H, $J = 9.0$ Hz), 6.36-6.33 (d, 1H, $J = 16.2$ Hz), 5.24 (s, 2H), 4.66-4.65 (m, 1H), 4.12-4.10 (dd, 1H, $^2J = 9.6$ Hz, $^3J = 4.2$ Hz), 4.02-4.00 (dd, 1H, $^2J = 9.6$ Hz, $^3J = 6.0$ Hz), 3.47-3.43 (m, 1H), 3.30-3.27 (m, 1H), 3.18-3.14 (m, 1H), 2.36 (s), 1.50-1.47 (dd, 6H, $^2J = 10.8$ Hz, $^3J = 6.6$ Hz), 1.42-1.41 (dd, 1H, $^2J = 6.6$ Hz, $^3J = 6.0$ Hz).

(S)-(-)-3-[4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]-propionic acid (17)

To a cooled mixture of (S)-(-)-benzyl-4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate hydrochloride (0.0605 g, 0.15 mmol) in EtOAc (10 ml) was added 10% Pd/C (0.0649 g) and reacted at RT under H_2 gas at 35 psi for 18 h. Once complete, the reaction mixture was filtered through a pad of celite to remove the Pd/C and the filtrate was evaporated under reduced pressure to provide **17** (0.0354 g, 75%); TLC R_f 0.14 [MeOH:DCM (1:10)]; HPLC % ee; $[\alpha]_D^{25} -13.8^\circ$ (c = 1, MeOH); ^1H NMR (600 MHz,

D₂O) δ 7.05-7.03 (d, 2H, J = 8.4 Hz), 6.77-6.75 (d, 2H, J = 8.4 Hz), 4.09-4.08 (d, 1H, J = 5.4 Hz), 3.93-3.85 (m, 2H), 3.30-3.28 (m, 1H), 3.12-3.10 (d, 1H, J = 13.2 Hz), 3.05-3.01 (t, 1H, J = 10.2 Hz), 2.69-2.67 (t, 2H, J = 7.2 Hz), 2.47-2.45 (t, 2H, J = 7.8 Hz), 1.14 (s, 6H), 1.09-1.08 (d, 1H, J = 6.6 Hz); ¹³C NMR (100 MHz, D₂O) δ 178.50, 156.52, 134.02, 129.68, 114.95, 69.72, 65.78, 51.19, 49.00, 46.86, 35.99, 29.67, 18.42, 17.99.

(S)-(-)-Methyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate hydrochloride
(22)

A mixture of (S)-(-)-benzyl-4-(2,3-epoxypropoxy)cinnamate (0.232 g, 0.75 mmol), isopropylamine (1.3 ml, 15.3 mmol) and MeOH (2 ml) was heated at reflux for 4 hours. Once completed, the reaction mixture was evaporated and toluene (5 ml) was added and evaporated under reduced pressure to provide **15** (0.303 g, 100%) as a clear oil; TLC R_f 0.08 [Hex:EtOAc (1:1) buffered in 0.1% TEA]. (S)-benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate was solubilized in a minimal amount of anhydrous MeOH and the same volume of MeOH-HCl was added to the solution. The solution was evaporated to an off-white solid and dissolved in a minimal amount of MeOH. Et₂O was added dropwise until the solution became turbid after which it was allowed to stand in the refrigerator overnight. The white crystals formed were filtered, washed with cold Et₂O, and dried under reduced pressure to provide **22** (0.183 g, 74%), mp 181-192.5 °C [Lit.⁽⁴⁴⁾ 183.3 °C (racemic)]; TLC R_f 0.24 [MeOH:DCM (1:10)]; HPLC 48.64% ee; ¹H NMR (600 MHz, DMSO-d₆) δ 8.76 (s, 1H), 8.52 (s, 1H), 7.71-7.69 (d, 2H, J = 8.4 Hz), 7.64-7.61 (d, 1H, J = 15.6 Hz), 7.01-7.00 (d, 2H, J = 9.0 Hz), 6.53-6.50 (d, 1H, J = 16.2 Hz), 5.94-5.93 (d, 1H, J = 4.8 Hz), 4.21-4.18 (m, 1H), 4.04-4.03 (d, 2H, J = 5.4 Hz), 3.70 (s,

3H), 3.38 (m, 1H), 3.14-3.11 (m, 1H), 2.98-2.96 (m, 1H), 1.26-1.23 (m, 6H); Anal. Calcd. for: C, 58.27; H, 7.33; N, 4.25; Found: C, 58.36; H, 7.52; N, 4.27.

(R)-(-)-Benzyl 4-(2,3-epoxypropoxy)cinnamate (18)

A mixture of benzyl 4-hydroxycinnamate (1.2238 g, 4.8 mmol), S-epichlorohydrin (2.75 ml, 35.1 mmol), K₂CO₃ (3.25 g, 23.5 mmol) and acetone (25 ml) was heated at reflux for 24 hours. The reaction mixture begins as a clear solution and with the addition of base turns yellow. The reaction mixture was filtered and evaporated under reduced pressure to a clear oil which solidified upon standing at room temperature. This oil/solid was taken up in a minimal amount of Et₂O with a minimal amount of heating and placed in the refrigerator overnight for crystallization. The crystals formed were filtered, washed with cold Et₂O and dried under reduced pressure to provide of **18** (0.9186 g, 62%) as white crystals, mp 77-79.3 °C; TLC R_f 0.54 [Hex:EtOAc (1:1) buffered in 0.1% TEA]; HPLC 81.86% ee; [α]_D²⁵ -6.7° (c = 1 in MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.69-7.67 (d, 1H, *J* = 15.6 Hz), 7.48-7.46 (d, 2H, *J* = 9.0 Hz), 7.42-7.33 (m, 5H), 6.93-6.91 (d, 2H, *J* = 9.0 Hz), 6.38-6.35 (d, 1H, *J* = 15.6 Hz), 5.24 (s, 2H), 4.28-4.26 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 3.0 Hz), 3.98-3.95 (dd, 1H, ²*J* = 10.8 Hz, ³*J* = 5.4 Hz), 3.38-3.35 (m, 1H), 2.93-2.92 (t, 1H, *J* = 4.8 Hz), 2.78-2.76 (dd, 1H, ²*J* = 4.8 Hz, ³*J* = 2.4 Hz); Anal. Calcd. for C₁₉H₁₈O₄: C, 73.53; H, 5.85; Found: C, 73.62; H, 5.79.

(R)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate (19)

A mixture of (R)-(-)-benzyl 4-(2,3-epoxypropoxy)cinnamate (0.4747 g, 1.53 mmol), isopropylamine (2.75 ml, 32.3 mmol) and MEK (8 ml) was heated at reflux for

16 hours. Once completed, the reaction mixture was evaporated under reduced pressure to provide **19** (0.591 g, 105%) as a clear oil which solidified to an off-white solid upon standing; TLC R_f 0.07 [Hex:EtOAc (1:1) buffered in 0.1% TEA]; ¹H NMR (600 MHz, CDCl₃) δ 7.68-7.65 (d, 1H, *J* = 16.2 Hz), 7.48-7.33 (m, 7H), 6.90-6.88 (d, 2H, *J* = 9.0 Hz), 6.36-6.34 (d, 1H, *J* = 15.6 Hz), 5.23 (s, 2H), 4.33-4.32 (m, 1H), 4.14-4.13 (d, 1H, *J* = 4.8 Hz), 4.07-4.04 (m, 1H), 4.01-3.98 (m, 1H), 3.67-3.66 (t, 1H, *J* = 9.0 Hz), 3.50-3.48 (dd, 1H, ²*J* = 9.0 Hz, ³*J* = 6.0 Hz), 3.14-3.12 (t, 1H, *J* = 6.6 Hz), 3.10-3.07 (dd, 1H, ²*J* = 12.6 Hz, ³*J* = 3.6 Hz), 2.95-2.91 (dd, 1H, ²*J* = 12.6 Hz, ³*J* = 9.6 Hz), 1.30-1.28 (t, 6H, *J* = 6.6 Hz), 1.21-1.20 (d, 1H, *J* = 7.2 Hz); Anal. Calcd. for C₂₂H₂₇NO₄: C, 71.52; H, 7.37; N, 3.79; Found: C, 71.27; H, 7.53; N, 3.73.

(R)-(+)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate hydrochloride
(20)

HCl gas was bubbled into MEK over an ice-bath to provide MEK·HCl with a pH ≈ 1. (R)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate (0.5872 g, 1.59 mmol) was dissolved in anhydrous MEK (9 ml) after which MEK·HCl (9 ml) was added and the pH of the resulting solution was ~3-4. The solvents were evaporated to dryness and the resulting oil was taken up in toluene (10 ml) and evaporated under reduced pressure. The oil was taken up in a minimal amount of warm MEK and allowed to stand in the refrigerator overnight. No crystals formed therefore a brown oil was collected to provide **20** (0.6235 g, 97%); TLC R_f 0.39 [MeOH:DCM (1:10)]; ¹H NMR (600 MHz, CDCl₃) δ 9.61 (s, 1H), 8.53 (s, 1H), 7.66-7.63 (d, 1H, *J* = 15.6 Hz), 7.43-7.32 (m, 7H), 6.88-6.86 (d, 2H, *J* = 8.4 Hz), 6.35-6.32 (d, 1H, *J* = 16.2 Hz), 5.23 (s, 2H), 4.65-4.64 (m,

1H), 4.12-4.09 (dd, 1H, $^2J = 9.6$ Hz, $^3J = 4.2$ Hz), 4.02-3.99 (dd, 1H, $^2J = 10.2$ Hz, $^3J = 6.0$ Hz), 3.47-3.43 (m, 1H), 3.29-3.26 (m, 1H), 3.15-3.14 (m, 1H), 1.49-1.46 (dd, 6H, $^2J = 10.8$ Hz, $^3J = 6.6$ Hz), 1.42-1.41 (d, 1H, $J = 6.0$ Hz).

(R)-(+)-3-[4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]-propionic acid (21)

To a cooled mixture of (R)-(+)-benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy] cinnamate hydrochloride (0.0542 g, 0.13 mmol) in EtOAc (10 ml) was added 10% Pd/C (0.070 g) and reacted at RT under H₂ gas at 35 psi for 18 h. Once complete, the reaction mixture was filtered through a pad of celite to remove the Pd/C and the filtrate was evaporated under reduced pressure to provide **21** (0.0312 g, 74%); TLC R_f 0.15 [MeOH:DCM (1:10)]; HPLC % ee; $[\alpha]_D^{25} +16.7^\circ$ (c = 1, MeOH); ¹H NMR (600 MHz, D₂O) δ 7.04-7.03 (d, 2H, $J = 8.4$ Hz), 6.76-6.75 (d, 2H, $J = 7.8$ Hz), 4.08-4.07 (d, 1H, $J = 4.8$ Hz), 3.92-3.85 (m, 2H), 3.30-3.26 (m, 1H), 3.12-3.10 (d, 1H, $J = 12.6$ Hz), 3.04-3.01 (t, 1H, $J = 10.2$ Hz), 2.69-2.66 (t, 2H, $J = 7.2$ Hz), 2.46-2.43 (t, 2H, $J = 7.8$ Hz), 1.14 (s, 6H), 1.08-1.07 (d, 2H, $J = 6.6$ Hz); ¹³C NMR (100 MHz, D₂O) δ 178.44, 156.53, 134.00, 129.69, 114.96, 69.73, 66.79, 51.21, 46.86, 35.96, 29.65, 19.89, 18.43, 18.01.

(R)-(+)-Methyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate hydrochloride (23)

A mixture of (R)-(+)-benzyl 4-(2,3-epoxypropoxy)cinnamate (0.525 g, 1.69 mmol), isopropylamine (3.0 ml, 35.2 mmol) and MeOH (5 ml) was heated at reflux for 4 hours. Once completed, the reaction mixture was evaporated and toluene (10 ml) was added and evaporated under reduced pressure to provide a clear oil; TLC R_f 0.15

[Hex:EtOAc (1:1) buffered in 0.1% TEA]. (R)-Benzyl 4-[2-hydroxy-3-(isopropylamino)propoxy]cinnamate was solubilized in a minimal amount of anhydrous MeOH after which MeOH·HCl was added to the solution. The solution was evaporated to an off-white solid and dissolved in a minimal amount of anhydrous MeOH. Et₂O was added dropwise until the solution became turbid after which it was allowed to stand in the refrigerator overnight. The white crystals formed were filtered, washed with cold Et₂O, and dried under reduced pressure to provide **23** (0.296 g, 53%), mp 178.2-192.9 °C; [Lit.⁽⁴⁴⁾ 183.3 °C (racemic)]; TLC R_f 0.28 [MeOH:DCM (1:10)]; HPLC 25.68% ee; ¹H NMR (600 MHz, DMSO-d₆) δ 8.95 (bs, 1H), 8.60 (bs, 1H), 7.70-7.69 (d, 2H, *J* = 8.4 Hz), 7.64-7.61 (d, 1H, *J* = 16.2 Hz), 7.01-7.00 (d, 2H, *J* = 9.0 Hz), 6.53-6.50 (d, 1H, *J* = 15.6 Hz), 5.95-5.94 (d, 1H, *J* = 4.8 Hz), 4.24-4.21 (m, 1H), 4.05-4.04 (d, 2H, *J* = 4.8 Hz), 3.70 (s, 3H), 3.34-3.31 (m, 1H), 3.14-3.10 (m, 1H), 2.99-2.96 (m, 1H), 1.27-1.24 (dd, 6H, ²*J* = 9.6 Hz, ³*J* = 6.6 Hz); Anal. Calcd. for: C, 58.27; H, 7.33; N, 4.25; Found: C, 58.17; H, 7.49; N, 4.18.

2-Hydroxy-4-(methoxymethyl)-acetophenone (25**)**

To a solution of dimethoxymethane (175 ml, 1.98 mol) in a 2L 3-necked flask was added EtOAc (350 ml) and Zn(OAc)₂ (42.88 mg) while stirring at room temperature. Acetyl chloride (140 ml, 1.97 mol) was placed in an addition funnel and added to the reaction mixture at a dropwise rate over 30 min. The reaction self-heats to 40-45 °C and then re-cools to ambient temperature over 2-3 hours after which the addition funnel is rinsed 3-4 times with EtOAc (~100 ml). The reaction mixture is then cooled to 0 °C in an ice bath. A separate solution of 2', 4'-dihydroxyacetophenone (200 g, 1.31 mol) in

EtOAc (615 ml) was prepared by heating to 45 °C. It was then re-cooled to room temperature and added to the initial reaction mixture. DIPEA (285.25 ml, 1.64 mol) was placed in an addition funnel and added to reaction at a dropwise rate while maintaining a temperature below 20 °C. As the DIPEA is added to the reaction, a white cloud will be produced inside the flask and then fade after 30 min. After addition, the mixture was stirred at room temperature for 18 hours. Once complete, TLC [Hex:EtOAc (5:1)], water (300 ml) was added by rinsing the funnel and condenser and the mixture stirred at room temperature for another 1-2 hours. Then the inside of the reaction flask was rinsed with water and added 1M NaOH solution (100 ml) and continued stirring for 15 minutes and transferred the mixture to a 2L separation funnel and repeated the extraction with 1M NaOH (2 x 250 ml). The organic phases were collected, dried over anhydrous sodium sulfate and evaporated to provide **25** (252.88 g) as an oil. A back extraction of the aqueous layer was also conducted with DCM (3 x 100 ml). These organic phases were collected, dried over anhydrous sodium sulfate and evaporated to provide additional **25** (26.65 g) as an oil. TLC R_f 0.4 [Hex:EtOAc (5:1)]; ¹H NMR (600 MHz, CDCl₃) δ 7.66-7.65 (d, 1H, *J* = 9.0 Hz), 6.59-6.59 (d, 1H, *J* = 2.4 Hz), 6.55-6.54 (dd, 1H, ²*J* = 8.4 Hz, ³*J* = 2.4 Hz), 5.21 (s, 2H), 3.48 (s, 3H), 2.57 (s, 3H), -0.72 (s, -OH) solvent peaks (EtOAc) δ 4.12, 2.05, 1.26.

1-(2-Benzyloxy-4-methoxymethoxy-phenyl)-ethanone (26**)**

A mixture of 2-hydroxy-4-(methoxymethyl)-acetophenone (118.07 g, 0.602 mol), K₂CO₃ (89.02 g, 0.644 mol), BnBr (107.74 ml, 0.907 mol) and acetone (1180 ml) was heated at reflux for 72 hours in a 2L flask while monitoring the reaction by TLC

[Hex:EtOAc (5:1)]. Once completed, the KBr salt formed during the reaction was filtered on a Büchner funnel and the solvent was evaporated to an oil. After which the oil was crystallized by dissolving in anhydrous MeOH (500ml) and allowing the solution to stand overnight at 0 °C. Red-pinkish crystals formed were collected and dried under reduced pressure to provide **26** (123.66 g, 72%), mp 71-72 °C [Lit.⁽¹⁵⁾ 70-71 °C]; TLC R_f 0.31 [Hex:EtOAc (5:1)]; ¹H NMR (400 MHz, CDCl₃) δ 7.83-7.81 (d, 1H, *J* = 8.4 Hz), 7.45-7.36 (m, 5H), 6.69-6.67 (m, 2H), 5.20 (s, 2H), 5.14 (s, 2H), 3.48 (s, 3H), 2.56 (s, 3H).

**{4-Benzyloxy-2-[2-(2-benzyloxy-4-methoxymethoxy-phenyl)-2-oxo-ethoxy]-benzyl}-
triphenyl-phosphonium bromide (**32**)**

A turbid solution of 2-(5-benzyloxy-2-hydroxy-phenoxy)-1-(2-benzyloxy-4-methoxy methylphenyl)-ethanone (30.10 g, 0.0585 mol) in anhydrous ACN (1 L) was prepared under anhydrous conditions in a 2-neck 2-L flask at RT. Then PPh₃·HBr (20.06 g, 0.0584 mol in total) was added in five increasing equivalents: 3.09 g, 3.49 g, 3.78 g, 4.05 g, and 5.65 g to total the 1 equivalent needed for the reaction and was reacted for 1-2 hours while continuously monitored by TLC [DCM:MeOH (15:1)]. Throughout the reaction the mixture turns from a turbid to a clear solution which is an indicator that the reaction is complete. Once completed, the ACN was evaporated under reduced pressure at room temperature to provide quantitative yields of **32** (49.12 g) as a white solid. This crude product was used directly in the next reaction without further purification, TLC R_f 0.30 [DCM:MeOH (15:1)]; ¹H NMR (600 MHz, DMSO-d₆) δ 10.58 (s, -OH), 7.80-7.76 (m, 4H), 7.75-7.73 (d, 1H, *J* = 9 Hz), 7.63-7.57 (m, 12H), 7.54-7.53 (d, 2H, *J* = 7.2 Hz), 7.41-7.33 (m, 8H), 7.24 (t, 1H, *J* = 7.2 Hz), 6.95-6.93 (dd, 1H, ²*J* = 9.0 Hz, ³*J* = 3.0 Hz),

6.91-6.91 (d, 1H, $J = 1.8$ Hz), 6.78-6.76 (dd, 1H, $^2J = 9.0$ Hz, $^3J = 2.4$ Hz), 6.54-6.52 (dd, 1H, $^2J = 9.0$ Hz, $^3J = 2.4$ Hz), 6.09-6.08 (d, 1H, $J = 1.8$ Hz), 5.32 (s, 2H), 5.22 (s, 2H), 4.97 (s, 2H), 4.87-4.84 (d, 2H, $J = 14.4$ Hz), 4.47 (s, 2H), 3.39 (s, 3H), 2.08 (ACN)

2', 7-Dibenzyloxy-4'-(methoxymethoxy)-isoflav-3-ene (33)

{4-Benzyloxy-2-[2-(2-benzyloxy-4-methoxymethoxy-phenyl)-2-oxo-ethoxy]-benzyl}-triphenyl-phosphonium bromide (49.12 g, 0.0585 mol), used directly from the previous step, was dissolved in anhydrous MeOH (1.5 L) within a 2-L 2-neck flask. Potassium tert-butoxide (13.13 g, 0.117 mol) was added and the mixture was heated at reflux for 18–24 hours while monitoring by TLC [Hex:EtOAc (5:1)]. During the reaction, product precipitates and needs good stirring throughout the duration of the reaction. Once complete, the reaction is allowed to cool to room temperature, the solid product is collected by filtration and dried under reduced pressure to provide 33 (16.41 g, 58% over two steps) as a white material. The mother liquor of the reaction was also evaporated to a solid and extracted using a minimal amount of DCM to separate the KBr salt which is formed during the reaction. The KBr salt was removed by filtration and MeOH is added to the DCM until turbid (usually 10 – 15x the volume of DCM). The turbid mixture was placed at 0 °C overnight after which the resulting crystals were collected by filtration and washed with cold MeOH to provide additional 33 (ca. 5 g), mp 132-134 °C [Lit.⁽¹⁵⁾ 132-134 °C]; TLC R_f 0.43 [Hex:EtOAc (5:1)]; ^1H NMR (600 MHz, Acetone- d_6) δ 7.53-7.51 (d, 2H, $J = 7.2$ Hz), 7.48-7.46 (d, 2H, $J = 7.8$ Hz), 7.42-7.38 (m, 4H), 7.35-7.31 (m, 2H), 7.30-7.28 (d, 1H, $J = 8.4$ Hz), 7.03-7.01 (d, 1H, $J = 8.4$ Hz), 6.82-6.81 (d, 1H, $J = 2.4$ Hz), 6.70-6.68 (dd, 1H, $^2J = 8.4$ Hz, $^3J = 1.8$ Hz), 6.62 (s, 1H), 6.58-6.57 (dd, 1H, $^2J = 8.4$

Hz, $^3J = 2.4$ Hz), 6.47-6.47 (d, 1H, $J = 2.4$ Hz), 5.22 (s, 2H), 5.15 (s, 2H), 5.10 (s, 2H), 4.93-4.93 (d, 2H, $J = 1.2$ Hz), 3.44 (s, 3H), 2.84 (water).

2', 7-Dibenzyloxy-4'-(*t*-butyldimethylsilyl)-isoflav-3-ene (34)

A mixture of 2', 7-Dibenzyloxy-4'-(methoxymethoxy)-isoflav-3-ene (19.61 g, 40.8 mmol), anhydrous DCM (200 ml) and $\text{PPh}_3\cdot\text{HBr}$ (17.58 g, 51.2 mmol) was stirred for 1 hour at RT while monitoring by TLC R_f 0.34 [Hex:EtOAc (2:1)]. TEA (10 ml) and TBDMS-Cl (7.59 g, 50.4 mmol) were added and stirred at room temperature for 14 hours. All solvents were evaporated at 30 °C and then the residue was further placed under a vacuum oil pump for 1-2 hours. After which DCM (2 L), oven-dried silica (340 g, dried overnight at 120 °C in oven and cooled under vacuum) and TFA (5 ml) were added and the mixture was gently stirred for 0.5 hours while monitoring the reaction by TLC [Hex:DCM (1:1) was used to confirm disappearance of PPh_3]. The oven-dried silica was filtered to give a green, transparent solution. To separate the base line spot seen by TLC, a silica pad filtration was employed. A 3–3.5 cm pad of silica with a piece of filter paper on top was placed inside a 10 cm diameter x 23 cm length coarse fritted funnel. With the aid of a diaphragm vacuum pump the green solution was filtered through this silica pad to give a colorless, transparent solution. The pad was rinsed with DCM (2 x 100 ml) while being careful to exclude any colored bands from passing through the frit into the solution. All solvents were evaporated to a clear oil which was taken up in a minimal amount of DCM followed by the addition of MeOH until the solution became turbid [DCM:MeOH (1:5)]. This solution provided crystals after standing overnight at 0 °C. The crystals were collected by filtration and dried under reduced pressure to provide 34 (14.15 g, 63% over

two steps) as a white solid, mp 103.5-106.7 °C [Lit.⁽¹⁵⁾ 106-107 °C]; TLC R_f 0.56 [Hex:DCM (1:1)]; ¹H NMR (600 MHz, Acetone-d₆) δ 7.51-7.46 (dd, 4H, ²J = 19.2 Hz, ³J = 7.2 Hz), 7.42-7.38 (m, 4H), 7.35-7.33 (m, 2H), 7.26-7.25 (d, 1H, J = 8.4 Hz), 7.03-7.02 (d, 1H, J = 8.4 Hz), 6.63-6.62 (d, 2H, J = 3.0 Hz), 6.59-6.57 (dd, 1H, ²J = 8.4 Hz, ³J = 3.0 Hz), 6.53-6.51 (dd, 1H, ²J = 8.4 Hz, ³J = 2.4 Hz), 6.48-6.47 (d, 1H, J = 2.4 Hz), 5.63 (s, DCM, solvent), 5.16 (s, 2H), 5.10 (s, 2H), 4.94 (s, 2H), 0.98 (s, 9H), 0.21 (s, 6H); Anal. Calcd. for C₃₅H₃₈O₄Si: C, 76.30; H, 6.95; Found: C, 76.05; H, 6.90.

N-methyl-6-nitroindole (50)

A mixture of 6-nitroindole (0.2511 g, 1.55 mmol), K₂CO₃ (0.05 g, 0.36 mmol), dimethylcarbonate (0.28 ml, 3.29 mmol) and DMF (1 ml) was heated at reflux for 2 hours. After completion, the reaction mixture was cooled to about 5 °C over an ice bath and ice cold water (2 ml) was added slowly. The product precipitated as a bright yellow solid during the addition. The product was washed with water (1 ml) and dried in a heated vacuum dessicator at 60 °C to provide 51 (0.2104 g, 77%) as a yellow solid, mp 81.2-84.6 °C; TLC R_f 0.67 [Hex:EtOAc (2:1)]; ¹H NMR (400 MHz, CDCl₃) δ 8.33-8.33 (d, 1H, J = 1.2 Hz), 8.03-8.00 (dd, 1H, ²J = 8.8 Hz, ³J = 2.0 Hz), 7.67-7.65 (d, 1H, J = 8.8 Hz), 7.35-7.34 (d, 1H, J = 3.2 Hz), 6.60-6.59 (d, 1H, J = 3.2 Hz), 3.91 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 134.75, 133.43, 120.89, 115.03, 106.55, 102.32, 33.48; Anal. Calcd. for C₉H₈N₂O₂: C, 61.36; H, 4.58; N, 15.90; Found: C, 61.27; H, 4.47; N, 15.78.

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Appendix A

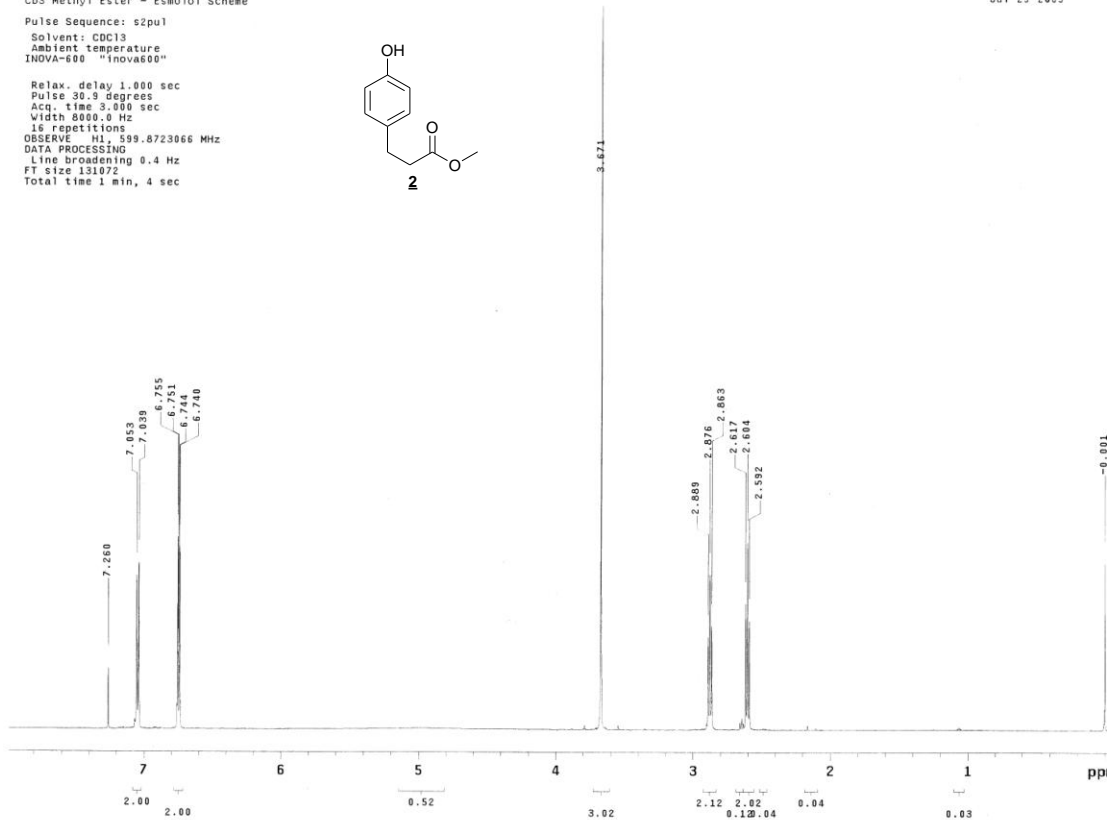
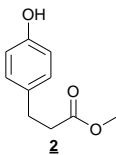
NMR Spectra for Synthesized Compounds

CD3 Methyl Ester - Esmolol Scheme

Jul 29 2009

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Ambient temperature
INNOVA-600 "inova600"

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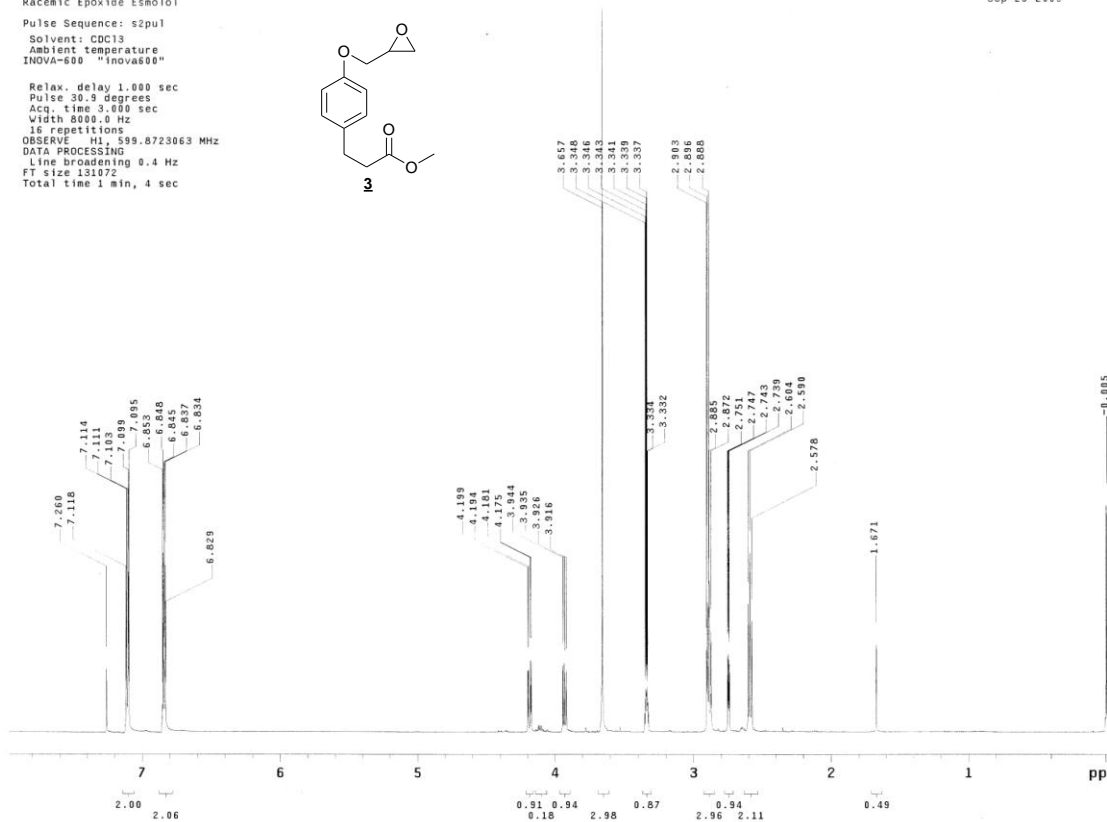
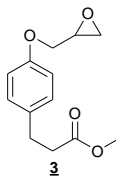


Racemic Epoxide Esmolol

Sep 29 2009

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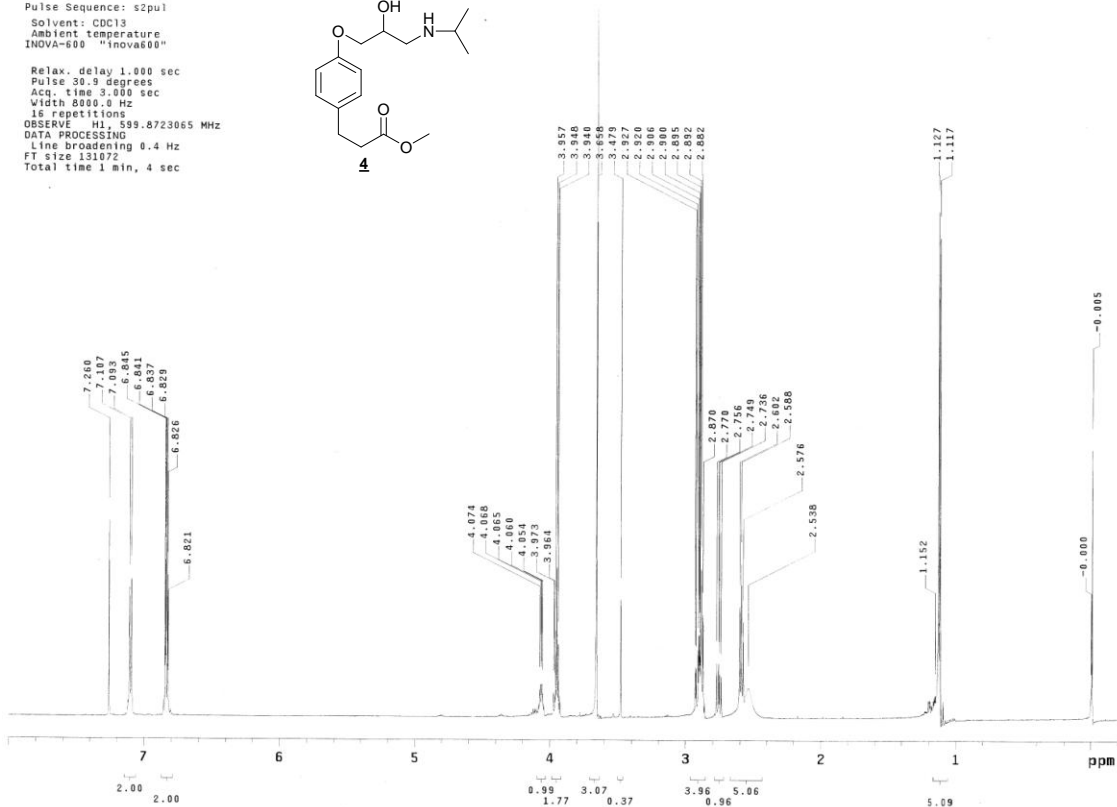
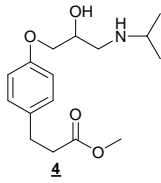
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Racemic Free Amine Esmolol
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 Ambient temperature
 INOVA-600 "inova600"

Sep 28 2009

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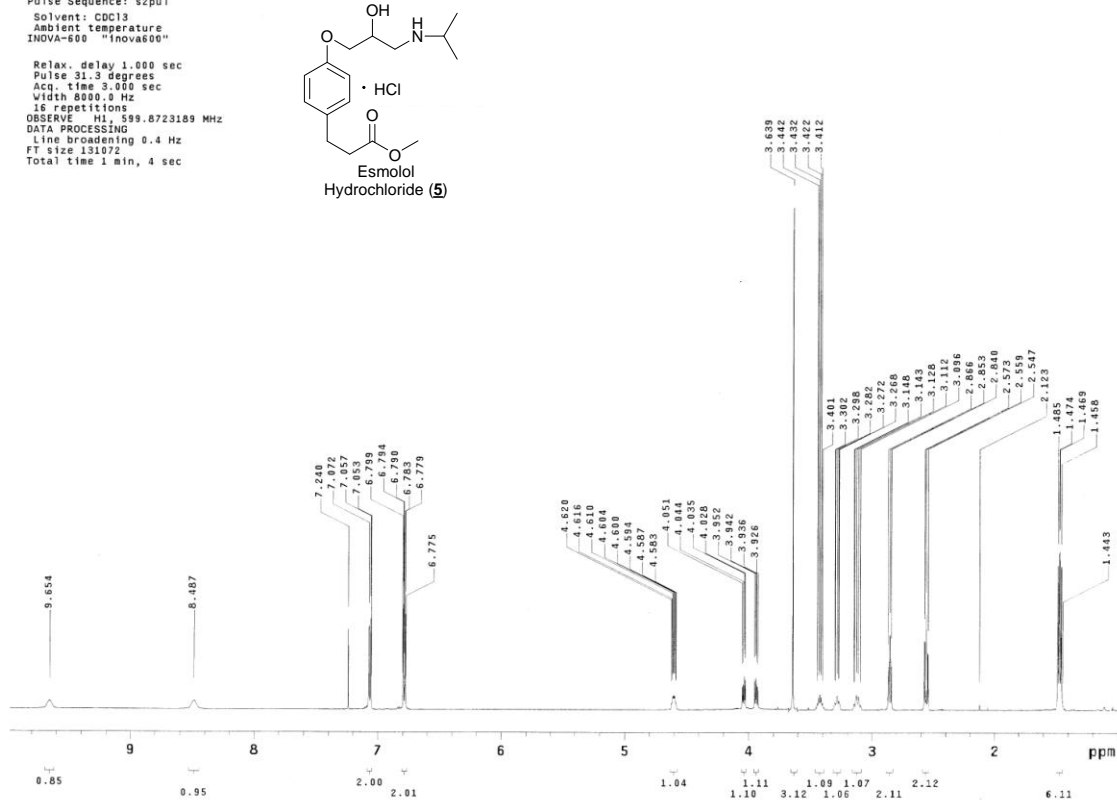
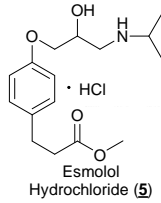


STANDARD PROTON PARAMETERS

Jul 11 2007

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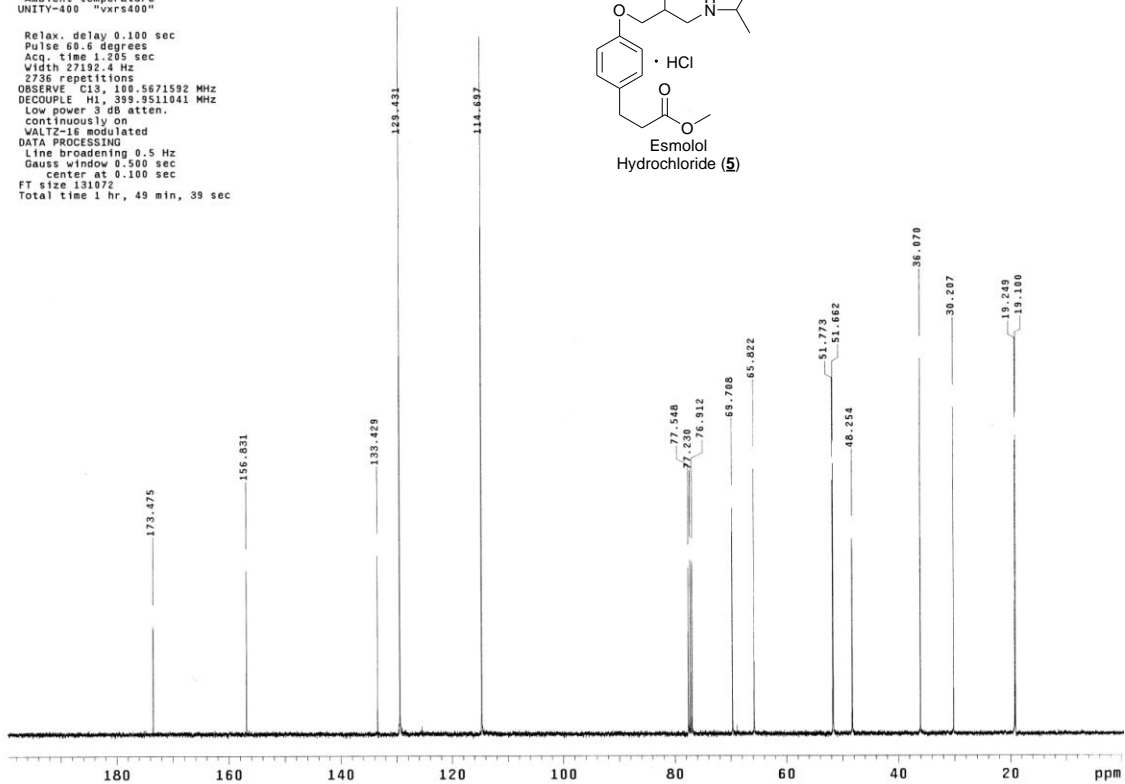
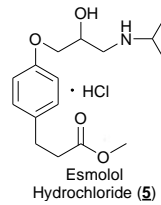
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 Ambient temperature
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Nov 5 2009

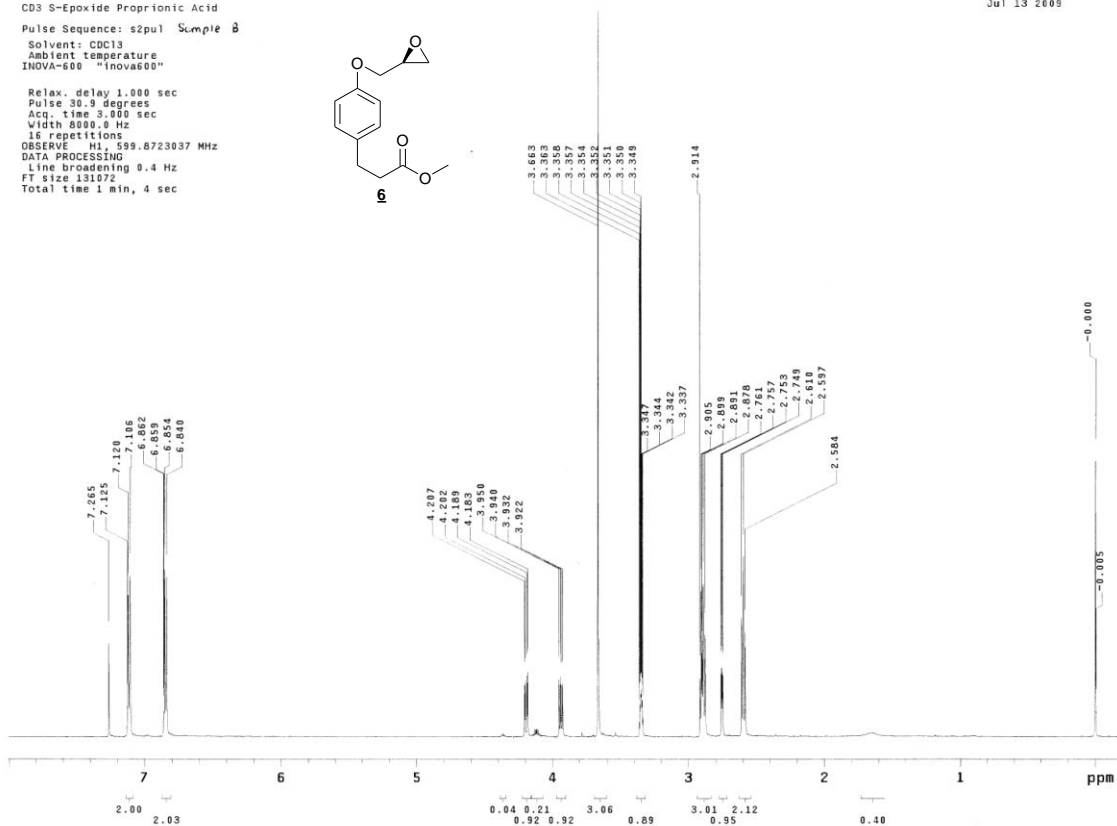
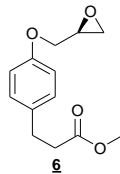
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 2736 repetitions
 OBSERVE C13, 100.6271592 MHz
 DECOUPLE H1, 399.9511041 MHz
 Low power 3 dB atten.
 continuously on
 WALTZ-16 modulated
 DATA PROCESSING
 Line broadening 0.5 Hz
 Gauss window 0.500 sec
 center at 0.100 sec
 FT size 131072
 Total time 1 hr, 49 min, 39 sec



CD3 S-Epoxyde Propionic Acid
 Pulse Sequence: s2pul Sample B
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Jul 13 2009

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.860 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723037 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec

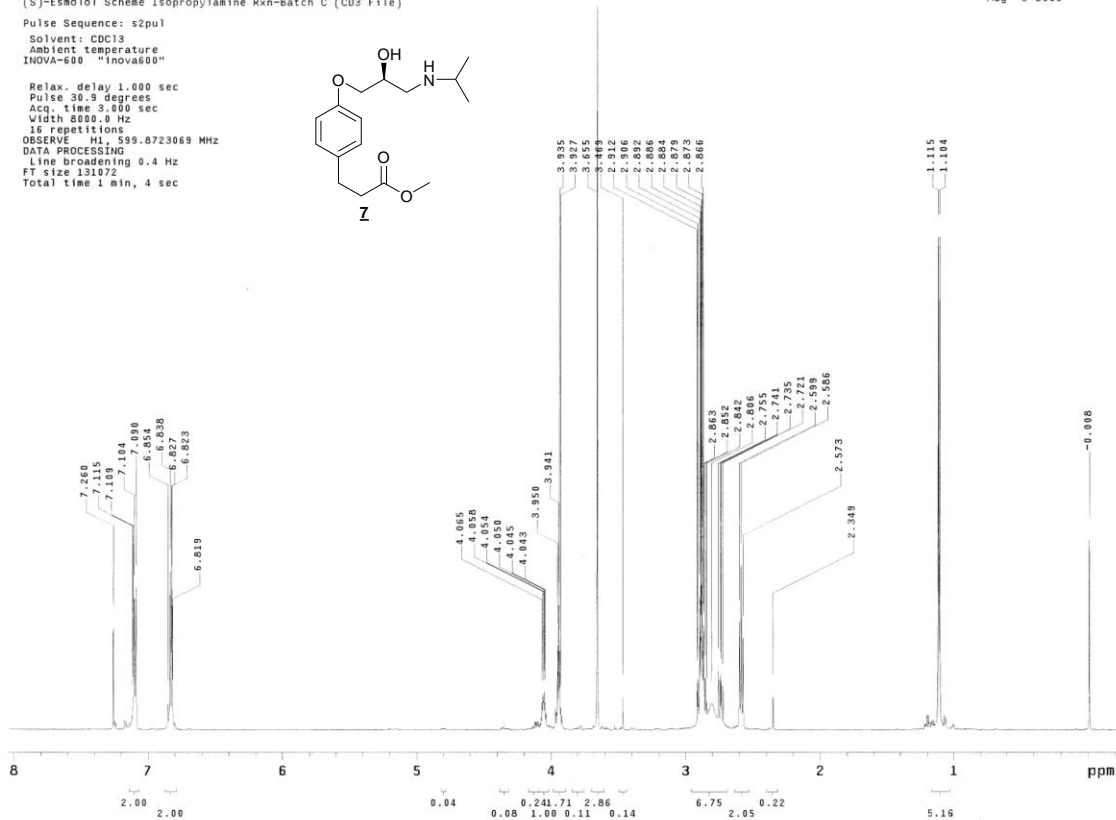
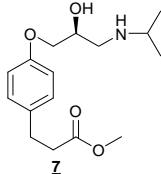


(S)-Esmolol Scheme Isopropylamine Rxn-Batch C (CD3 File)

Aug 5 2009

Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
INNOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.9 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 599.8723069 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec

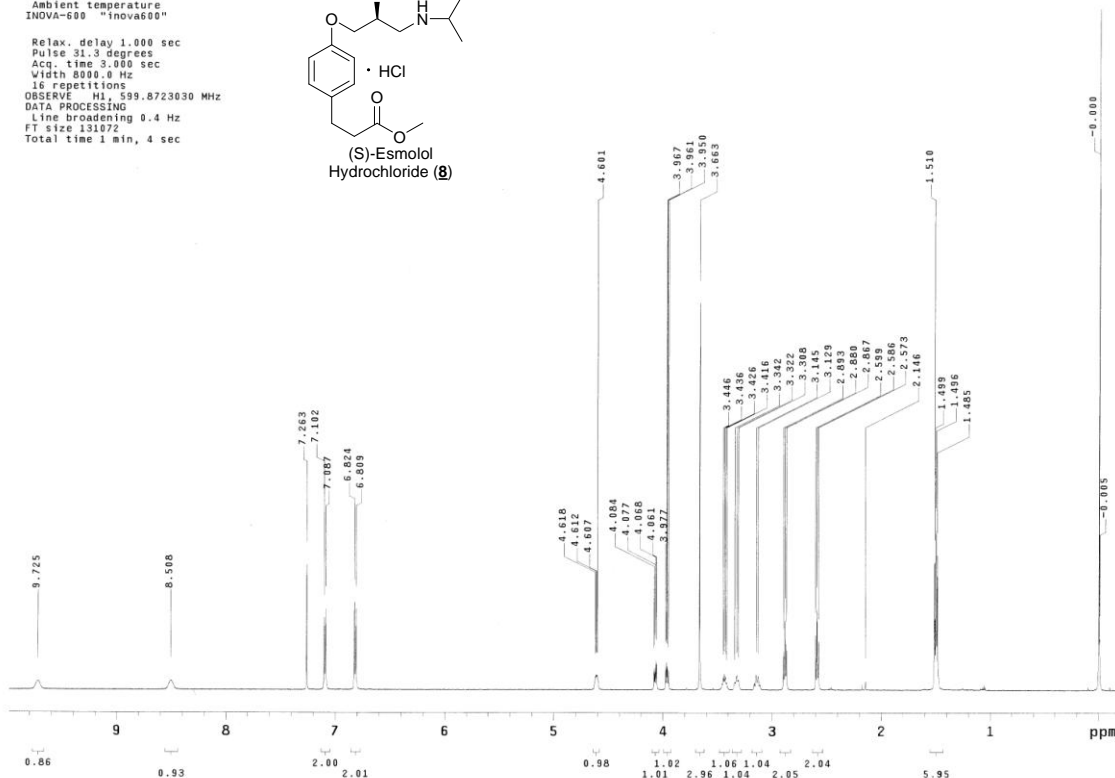
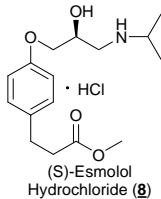


STANDARD PROTON PARAMETERS

Feb 18 2008

Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
INNOVA-600 "inova600"

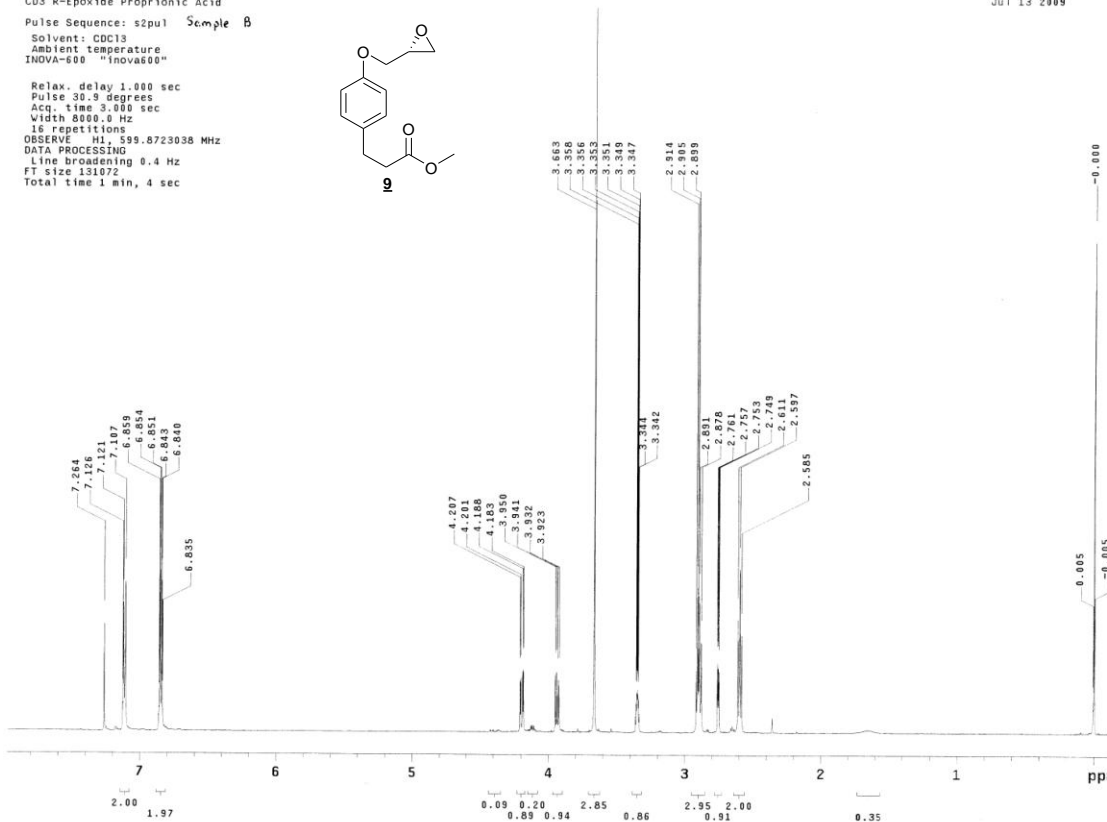
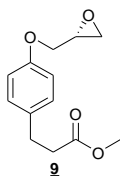
Relax. delay 1.000 sec
Pulse 31.3 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 599.8723030 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec



CD3 R-Epoxyde Propionic Acid
 Pulse Sequence: s2pul Sample B
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Jul 13 2009

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723038 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec

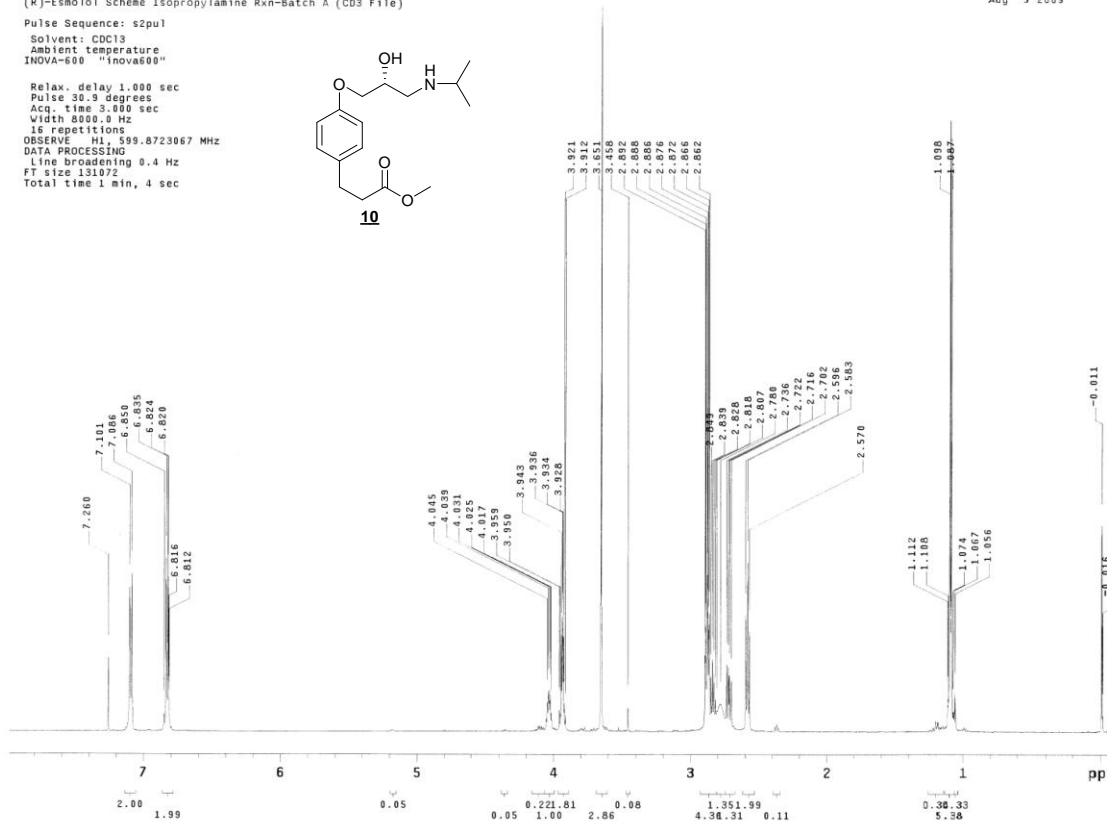
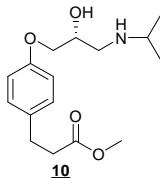


(R)-Esmolol Scheme Isopropylamine Rxn-Batch A (CD3 File)

Aug 5 2009

Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

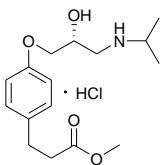
Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723067 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec



STANDARD PROTON PARAMETERS

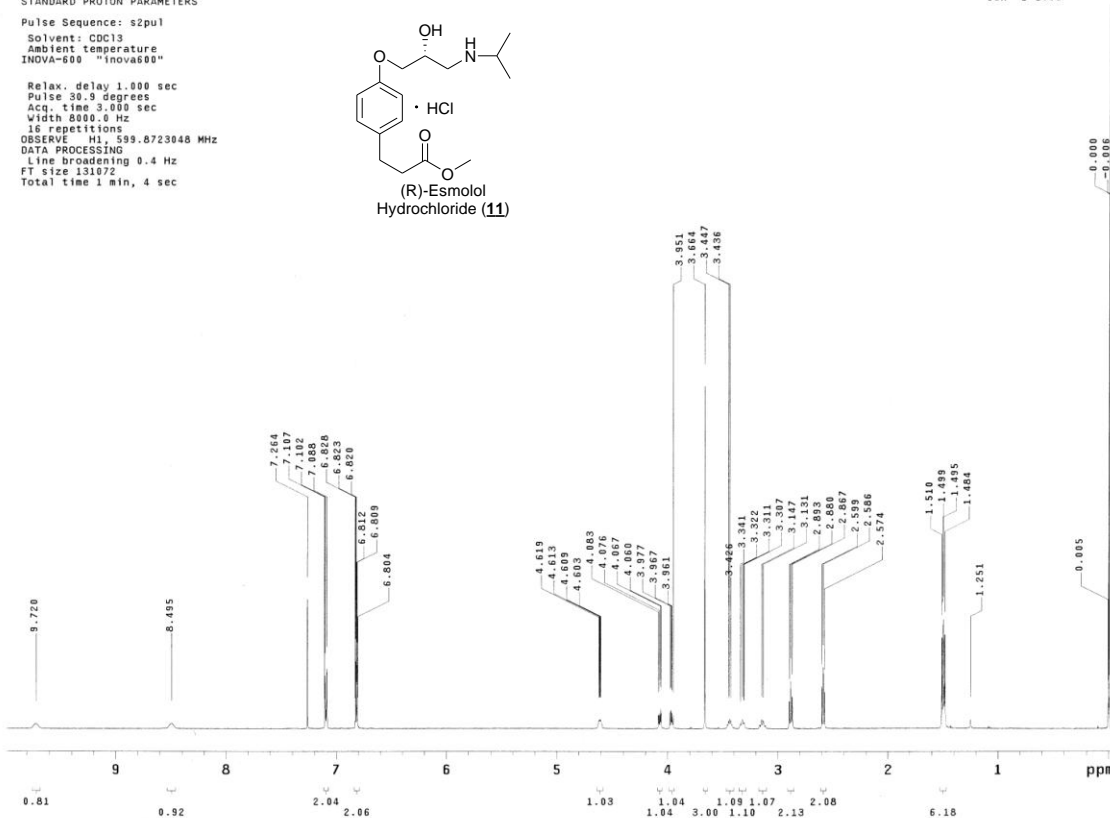
Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723048 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec



(R)-Esmolol
 Hydrochloride (11)

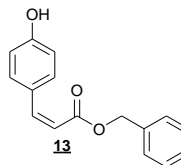
Jun 2 2008



p-Coumaric Acid Benzyl Ester

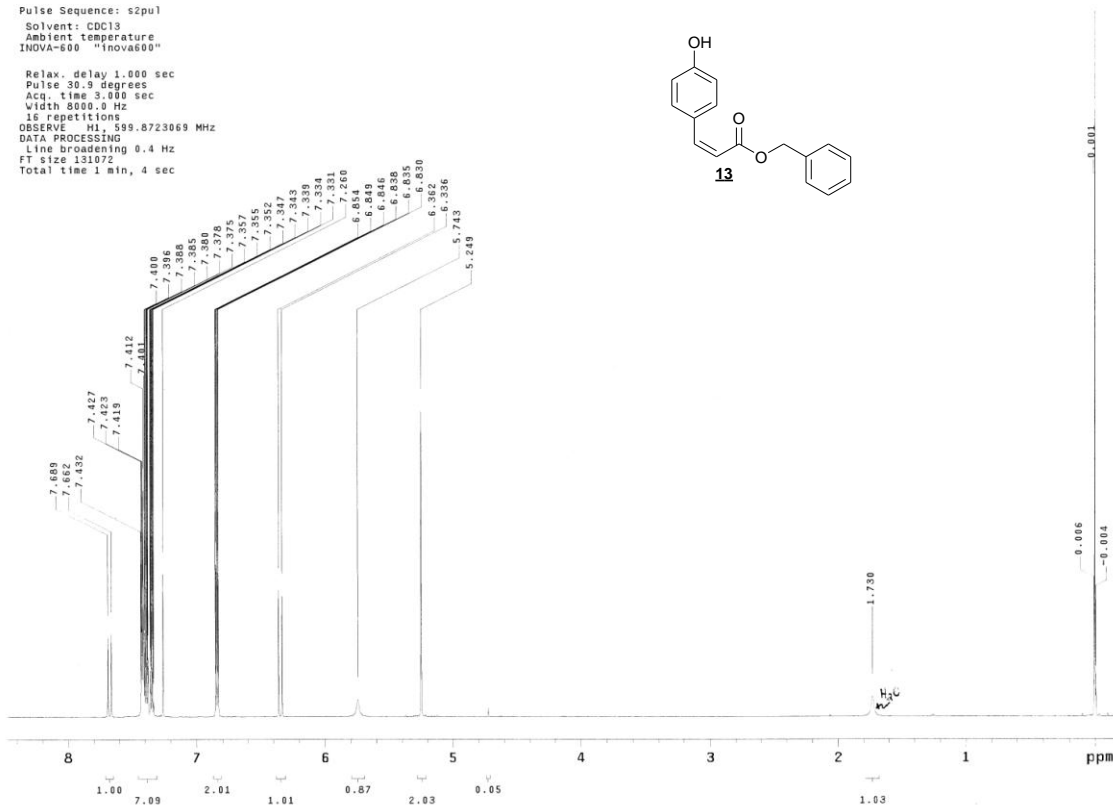
Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723069 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec



13

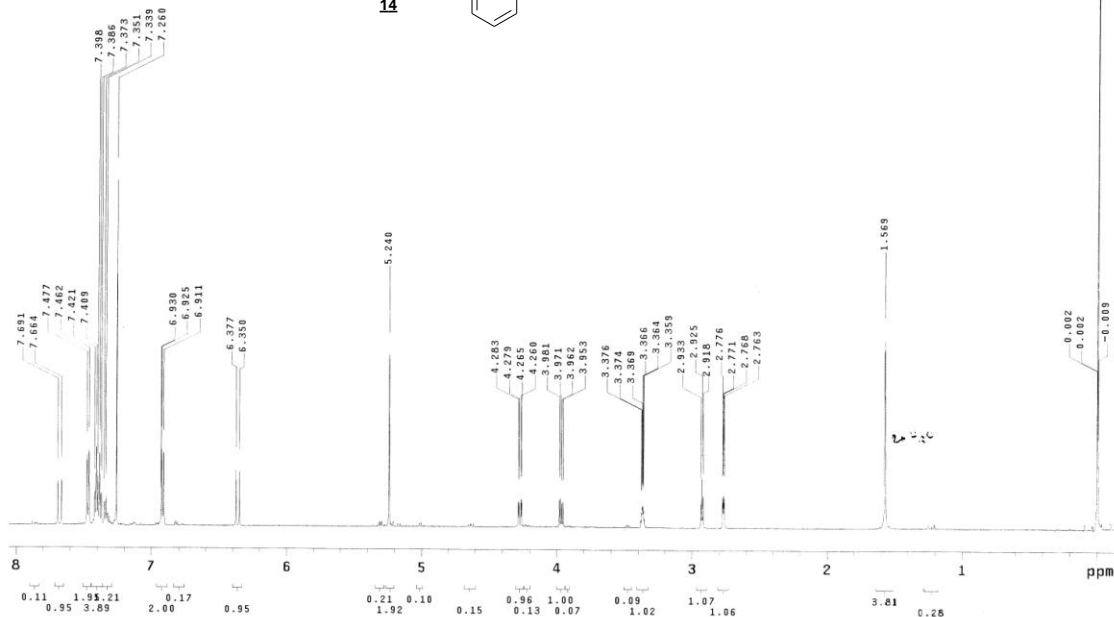
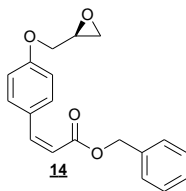
Aug 31 2009



p-Coumaric Acid R-Epichlorohydrin Rxn w/ Acetone
 Pulse Sequence: s2pu1
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Aug 4 2009

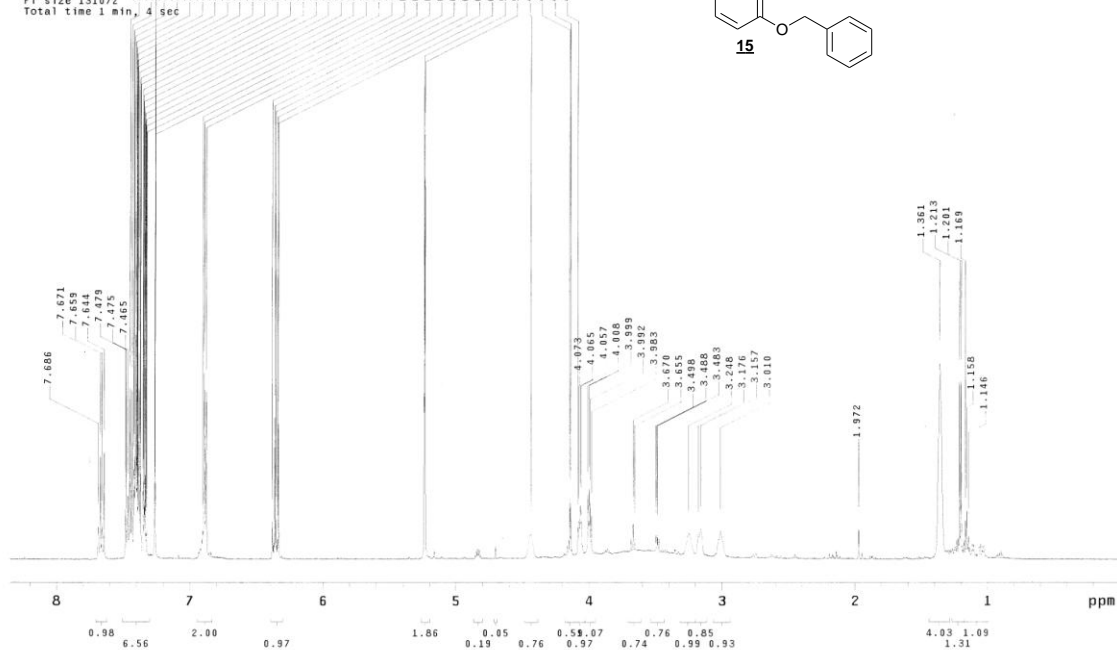
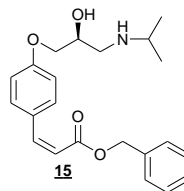
Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE HI 599.8723067 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec

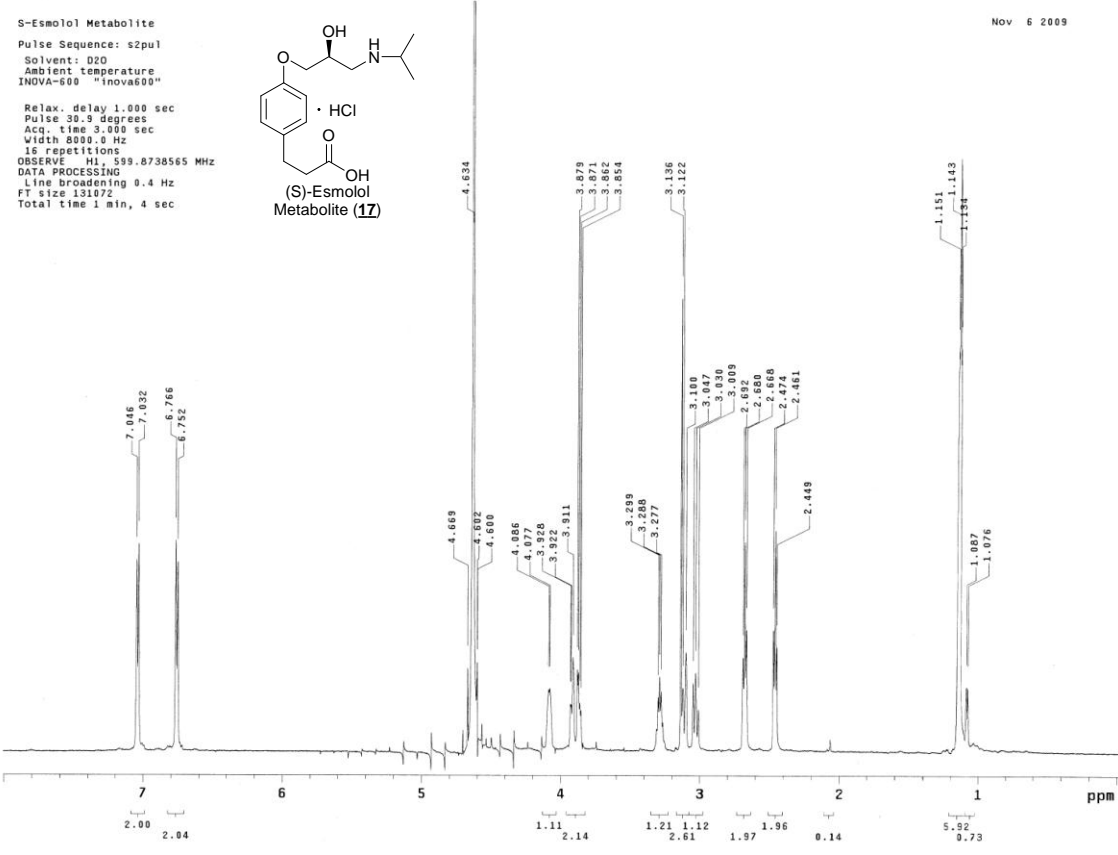
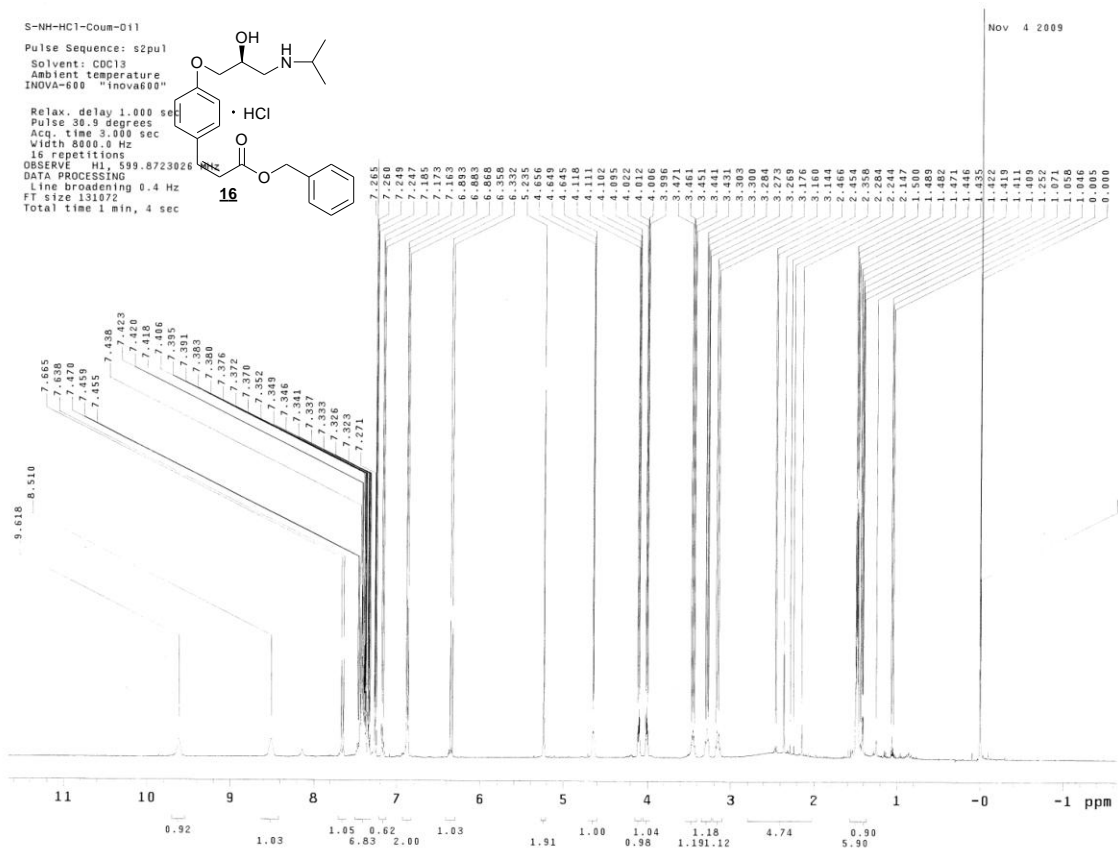


S-NH-Coum
 Pulse Sequence: s2pu1
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Nov 5 2009

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE HI 599.8723067 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec



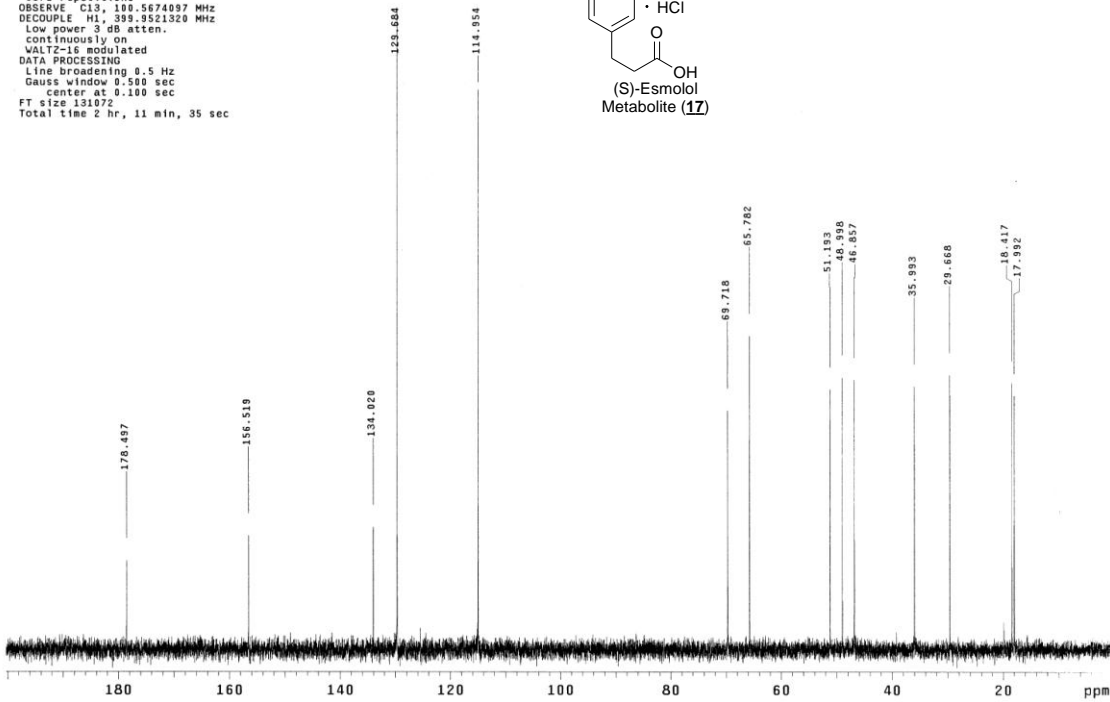
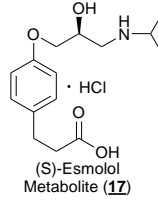


(S)-Esmolol Acid Metabolite

Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
UNITY-400 "vxrs400"

Relax. delay 0.100 sec
Pulse 60.6 degrees
Acq. time 1.285 sec
Width 27192.4 Hz
3872 repetitions
OBSERVE C13, 199.5674097 MHz
DECOUPLE H1, 399.9521320 MHz
Low power 3 dB atten.
continuously on
VALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
Gauss window 0.500 sec
center at 0.100 sec
FT size 131072
Total time 2 hr, 11 min, 35 sec

Dec 1 2009

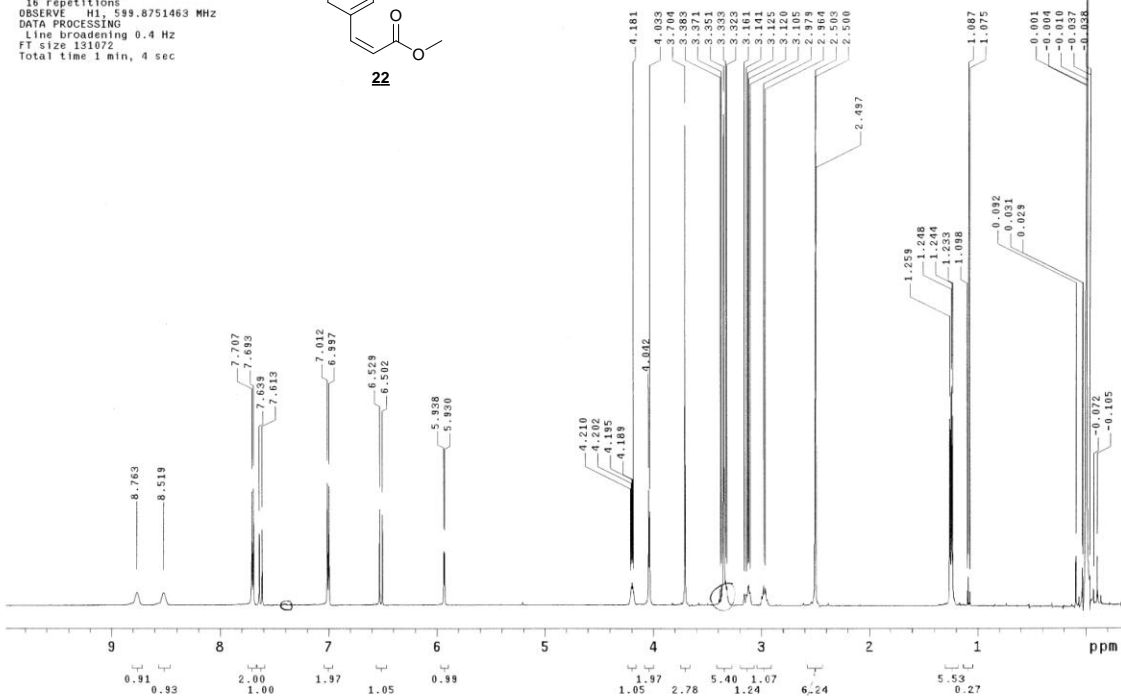
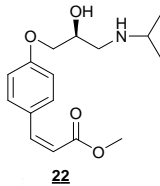


S-HCl Coumaric Acid

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
INOVA-600 "inova600"

Relax. delay 1.000 sec
Pulse 30.9 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 599.8751463 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec

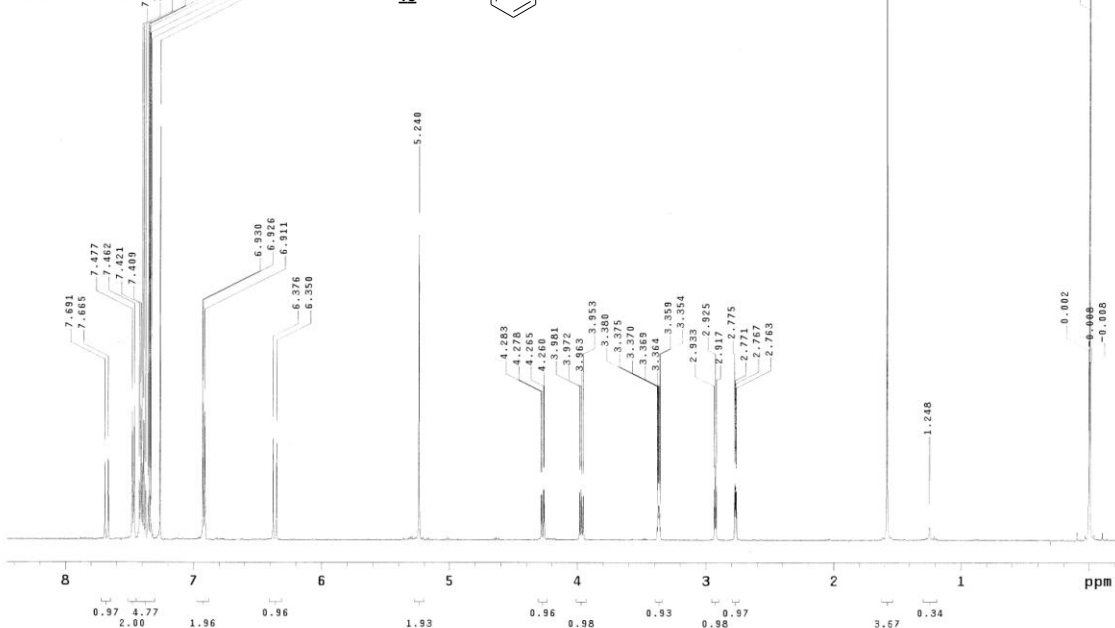
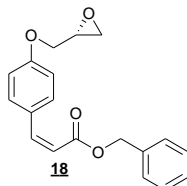
Aug 20 2009



R-epoxide coumaric acid benzyl ester
 Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Sep 3 2009

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE HI 599.8723061
 DATA PROCESSING
 Line broadening 0
 FT size 131072
 Total time 1 min,

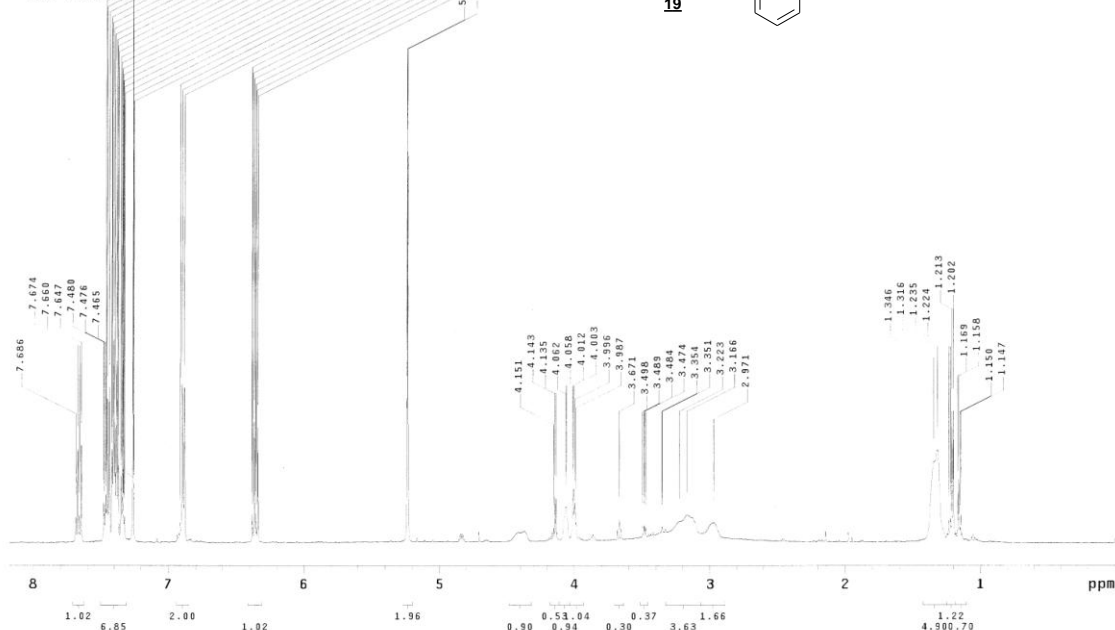
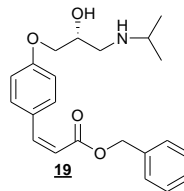


R-NH-Coum

Nov 5 2009

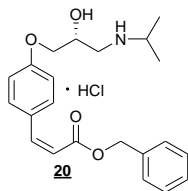
Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE HI 599.8723061
 DATA PROCESSING
 Line broadening 0
 FT size 131072
 Total time 1 min, 4 sec

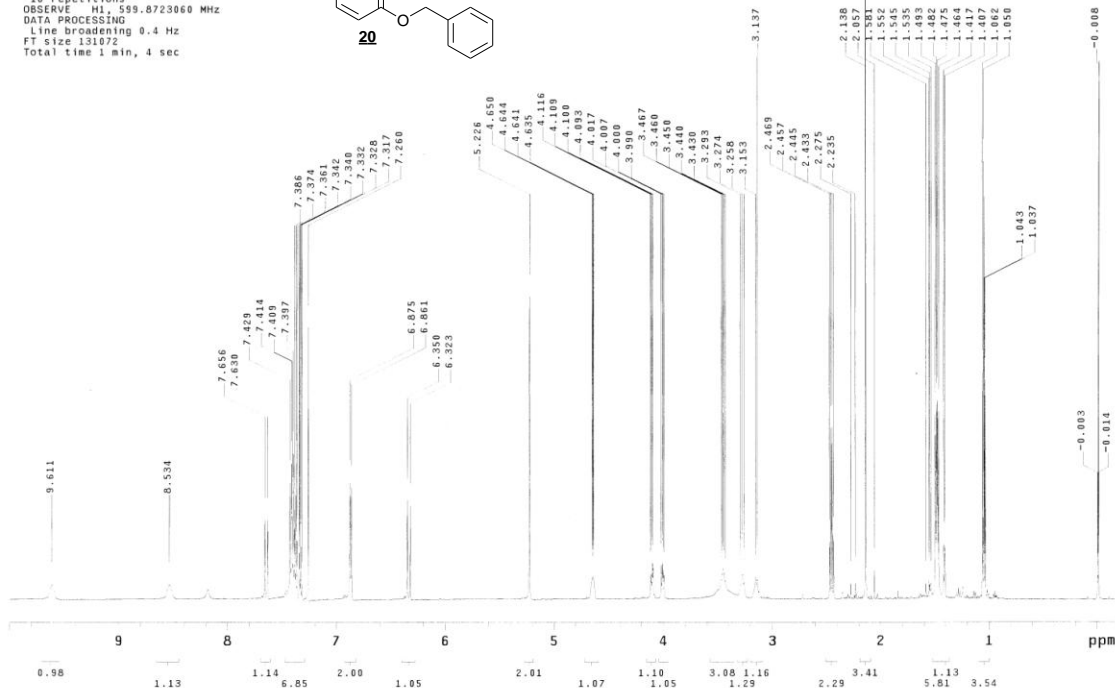


R-NH-HCl-Coum
 Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
 INOVA-600 "inova600"

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8723060 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec

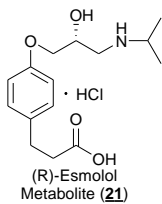


Nov 5 2009

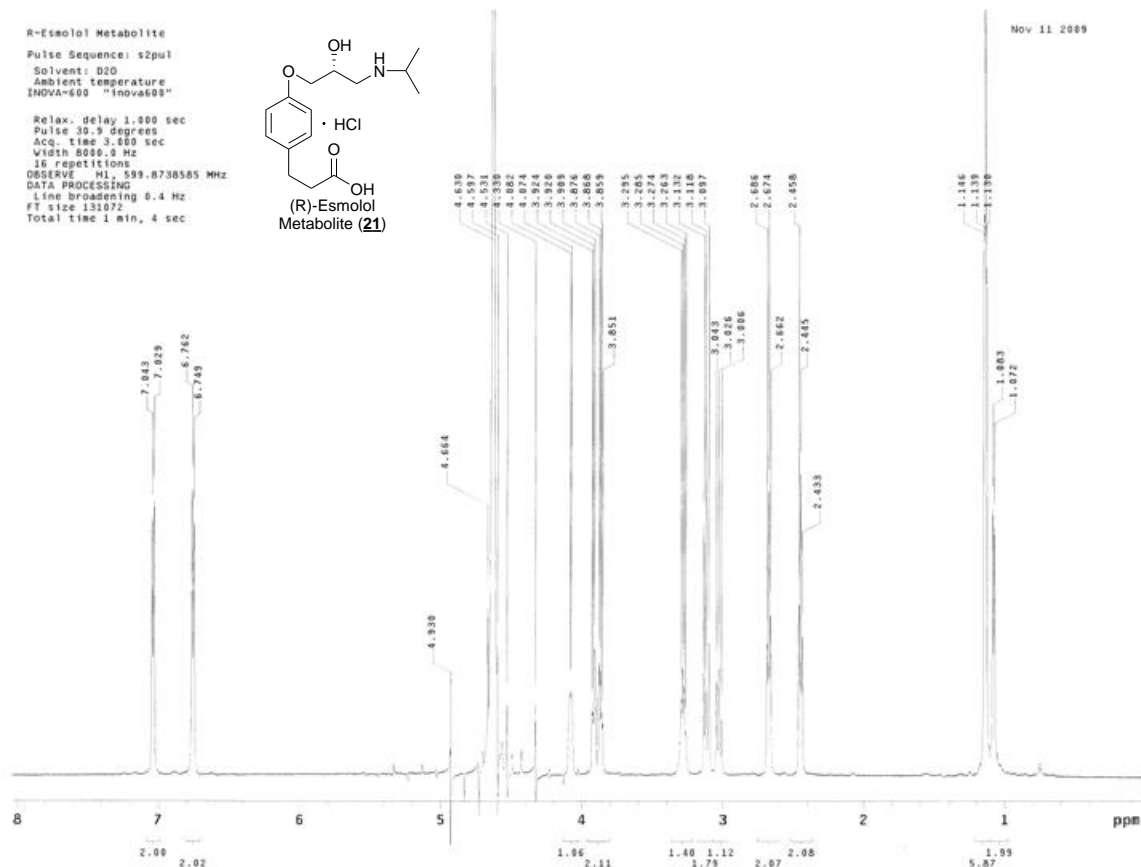


R-Esmolol Metabolite
 Pulse Sequence: s2pul
 Solvent: D2O
 Ambient temperature
 INOVA-600 "inova600"

Relax. delay 1.000 sec
 Pulse 30.9 degrees
 Acq. time 3.000 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE H1, 599.8738585 MHz
 DATA PROCESSING
 Line broadening 0.4 Hz
 FT size 131072
 Total time 1 min, 4 sec



Nov 11 2009

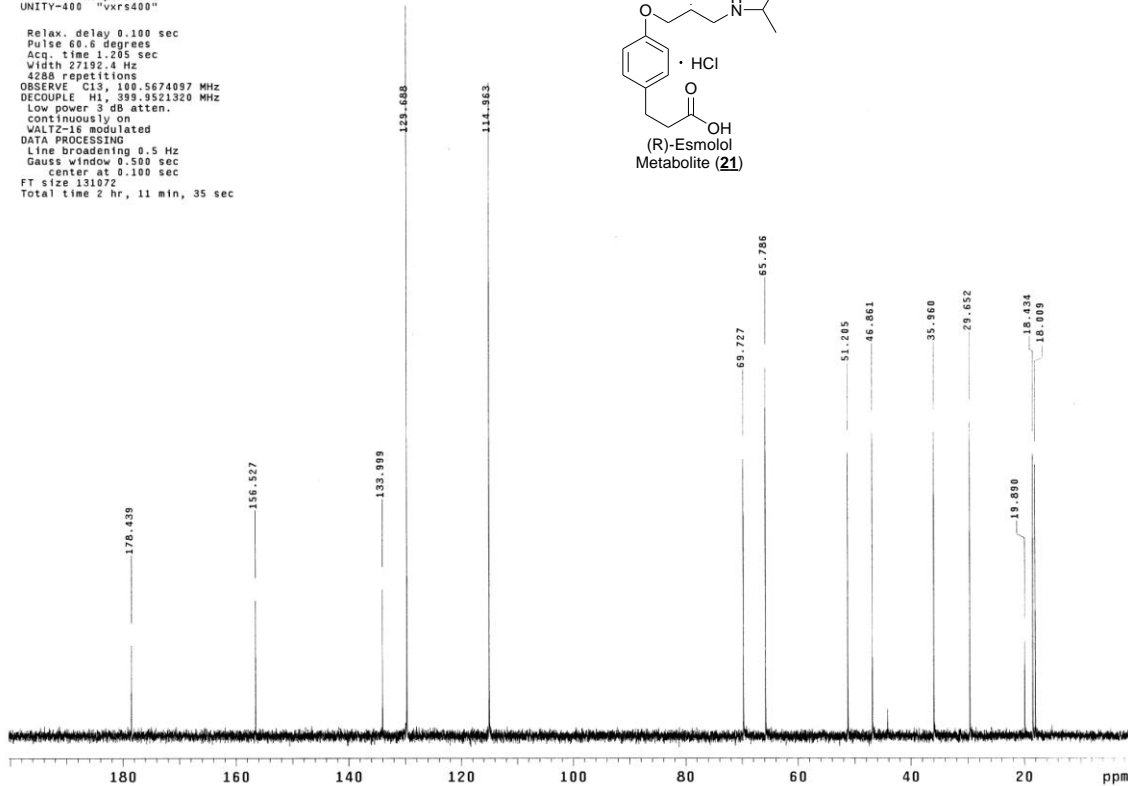
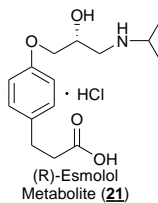


R-Esmolol Acid Metabolite

Nov 20 2009

Pulse Sequence: s2pul
Solvent: D2O
Ambient temperature
UNITY-400 "vrs400"

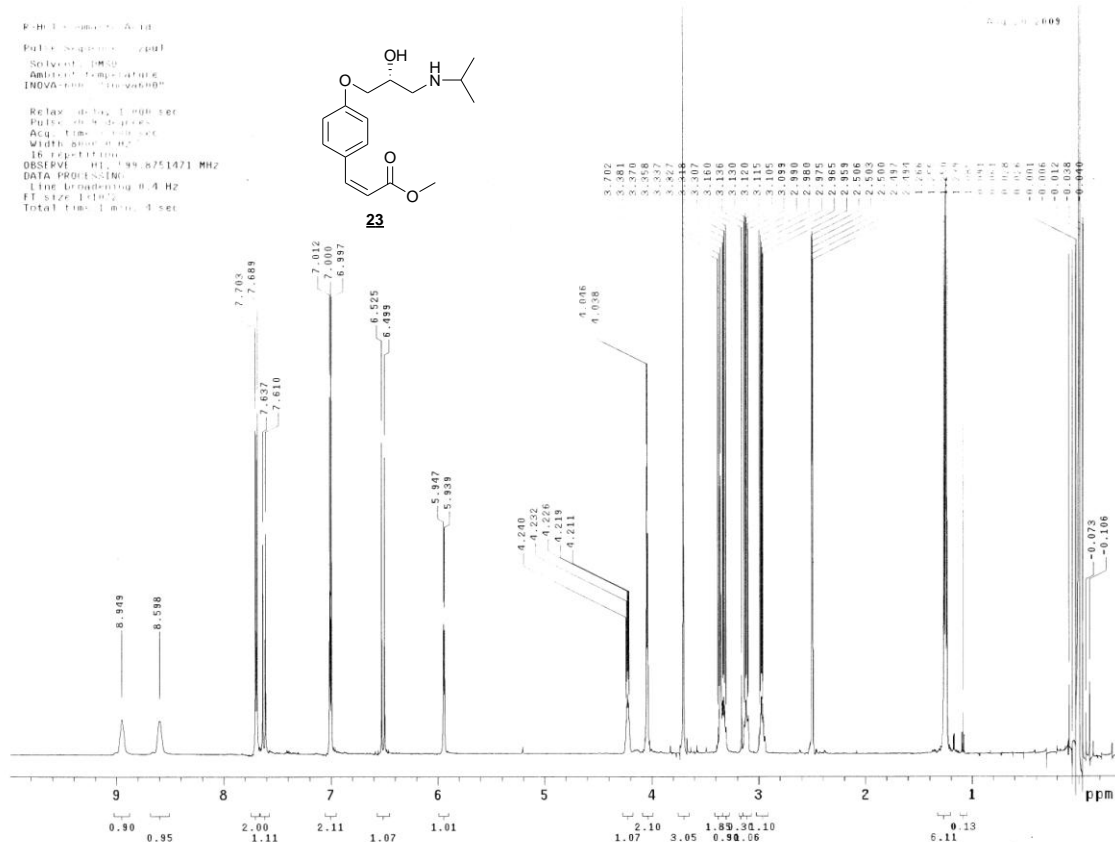
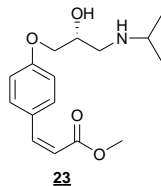
Relax. delay 0.100 sec
Pulse 60.6 degrees
Acq. time 1.265 sec
Width 27192.4 Hz
4288 repetitions
OBSERVE C13, 100.5674097 MHz
DECOUPLE H1, 399.9521320 MHz
Low power 3 dB atten.
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
Gauss window 0.500 sec
center at 0.100 sec
FT size 131072
Total time 2 hr, 11 min, 35 sec



Phthalate (23)

Pulse Sequence: s2pul
Solvent: DMF
Ambient temperature
INOVA-400 "vrs400"

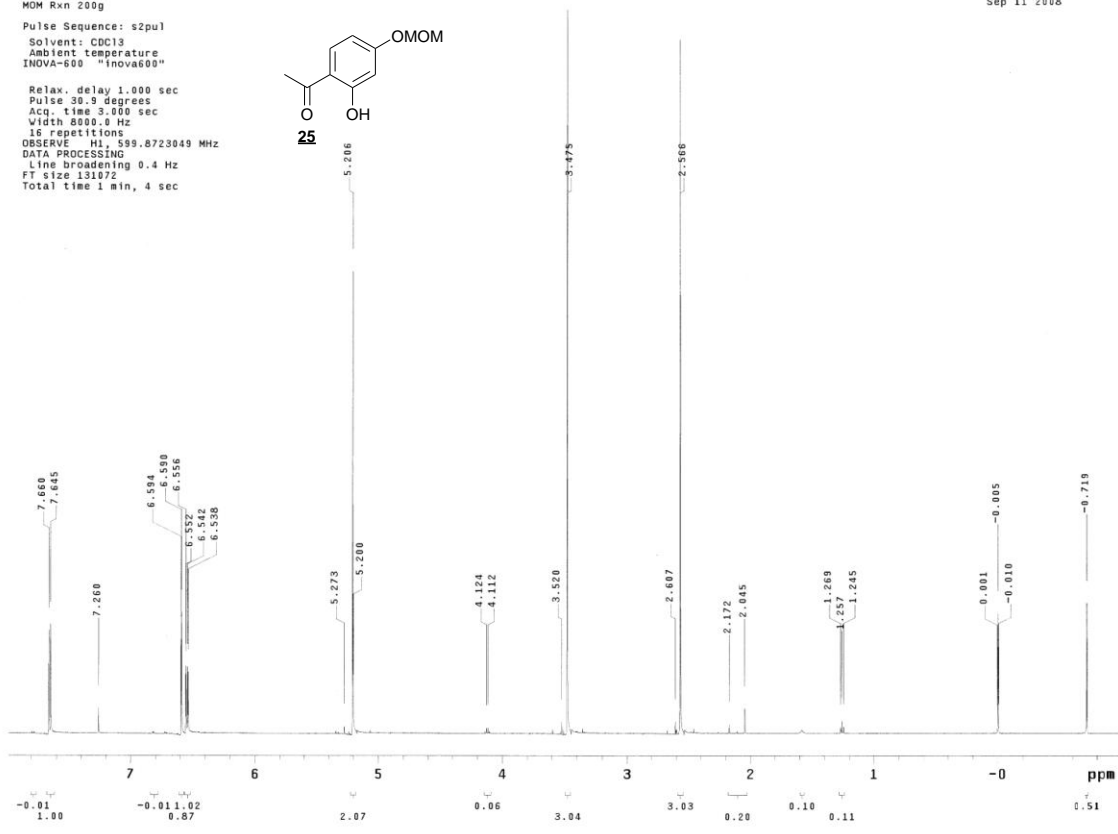
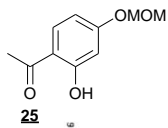
Relax. delay 1.000 sec
Pulse 60.6 degrees
Acq. time 1.000 sec
Width 20480.0 Hz
IR repetitions
OBSERVE H1, 499.8751471 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec



MOM Rxn 200g
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-600 "inova600"

Sep 11 2008

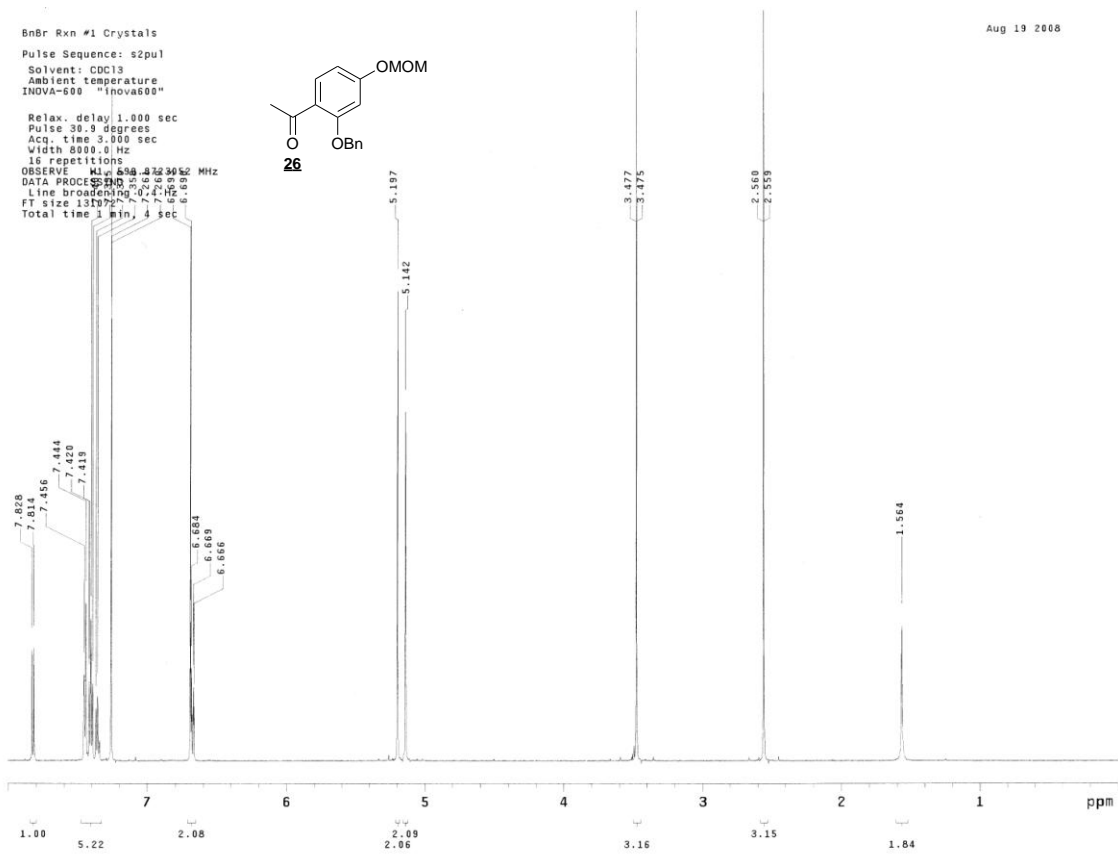
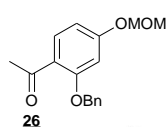
Relax. delay 1.000 sec
Pulse 30.9 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE HI: 599.8723049 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec

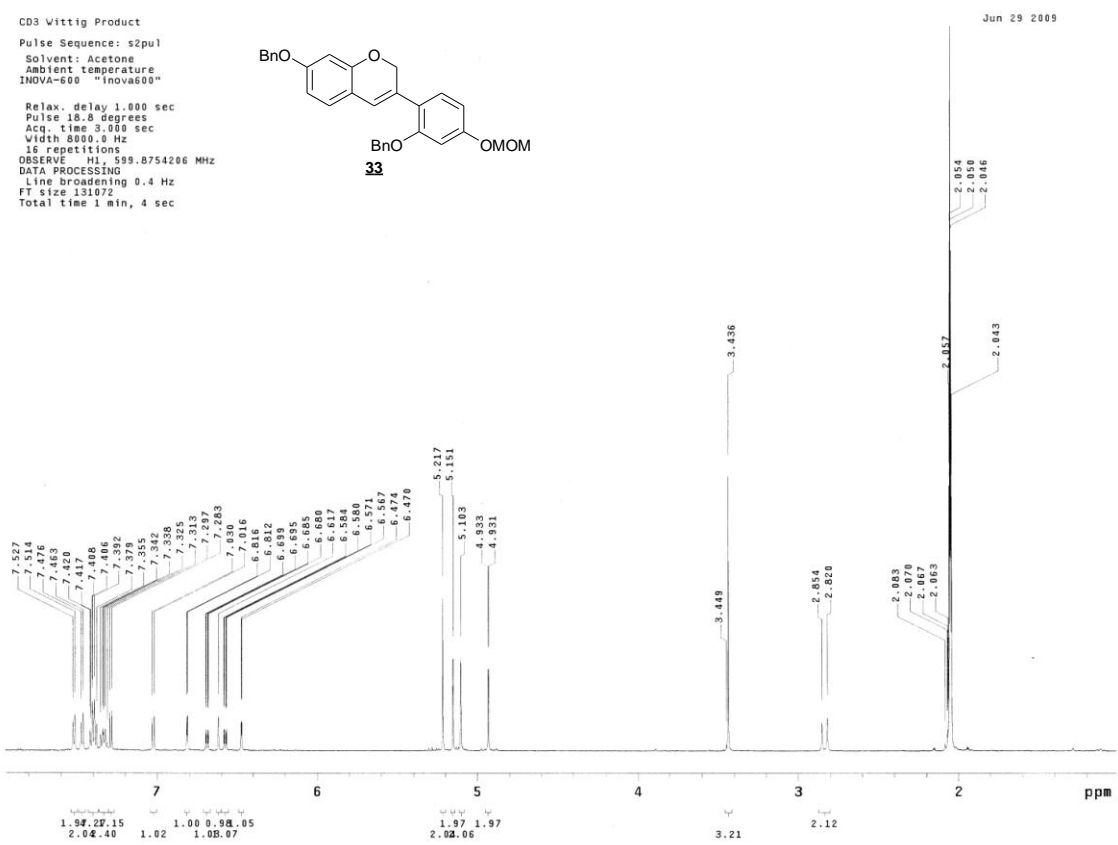
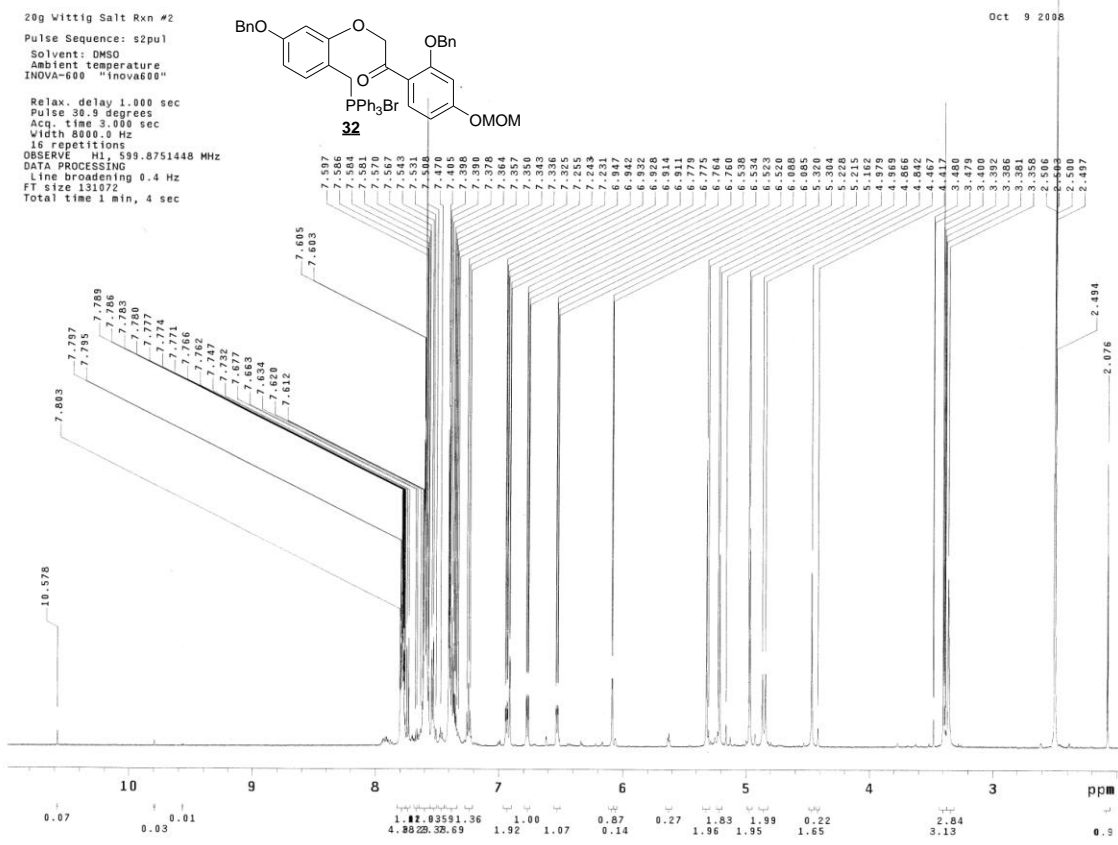


BnBr Rxn #1 Crystals
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
INOVA-600 "inova600"

Aug 19 2008

Relax. delay 1.000 sec
Pulse 30.9 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE HI: 599.8723049 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec

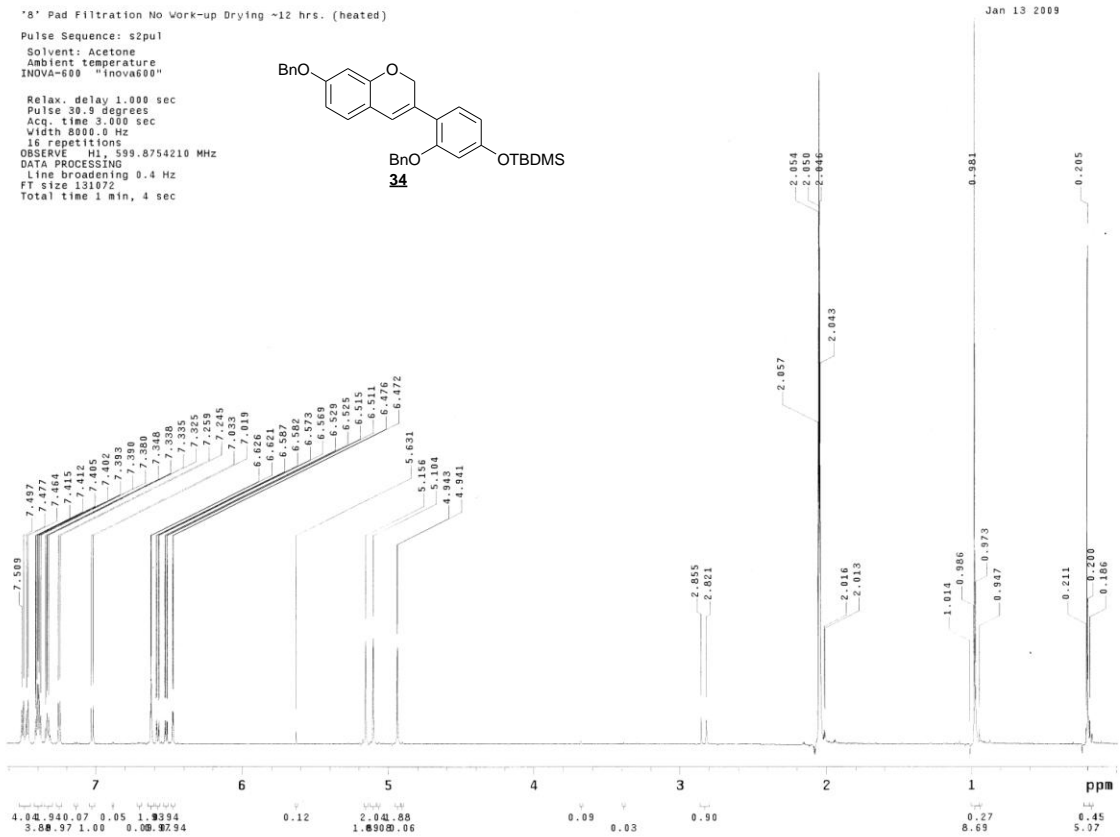
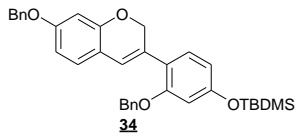




"8" Pad Filtration No Work-up Drying ~12 hrs. (heated)

Pulse Sequence: s2pu1
Solvent: Acetone
Ambient temperature
INOVA-600 "inova600"

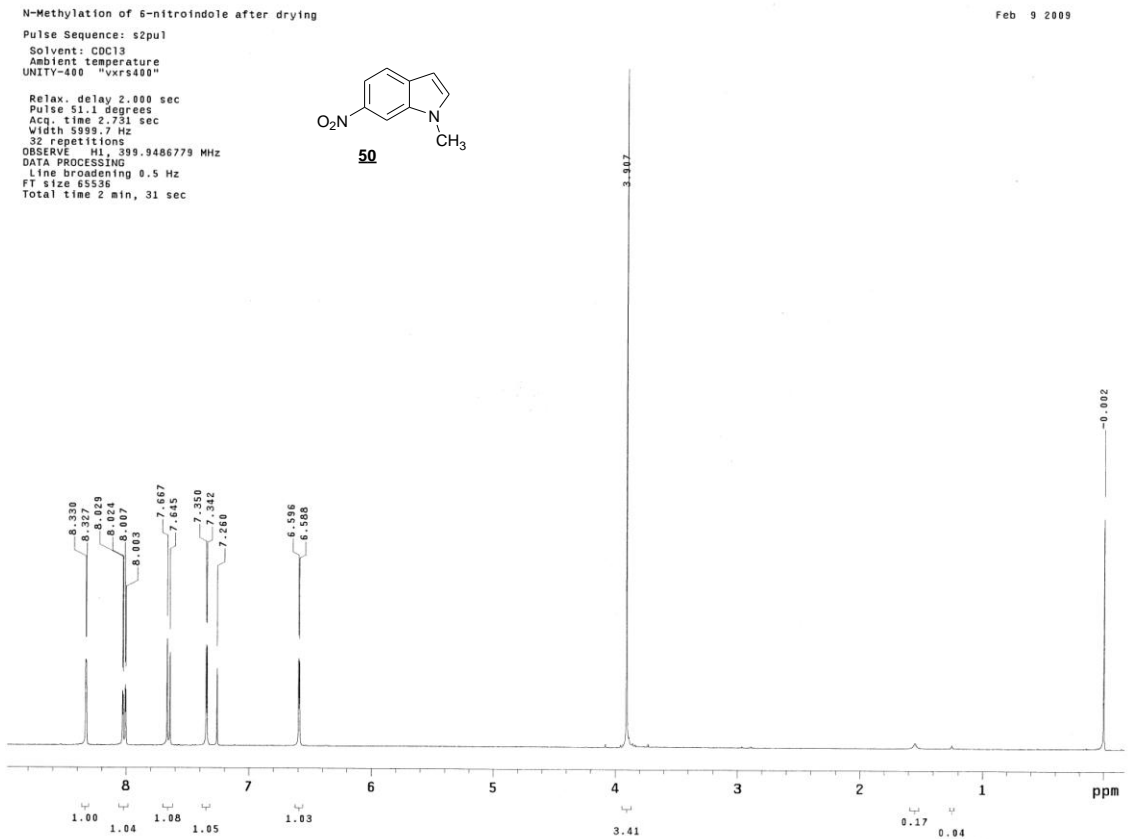
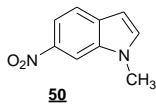
Relax. delay 1.000 sec
Pulse 30.9 degrees
Acq. time 3.000 sec
Width 8900.0 Hz
16 repetitions
OBSERVE H1, 599.8754210 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 131072
Total time 1 min, 4 sec



N-Methylation of 5-nitroindole after drying

Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
UNITY-400 "vrs400"

Relax. delay 2.000 sec
Pulse 51.1 degrees
Acq. time 2.751 sec
Width 5999.7 Hz
32 repetitions
OBSERVE H1, 399.9486779 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 min, 31 sec



¹³C N-Me Nitroindole

Nov 19 2009

Pulse Sequence: s2pul
Solvent: CDCl₃
Ambient temperature
UNITY-400 "vxs400"

Relax. delay 0.100 sec
Pulse 60.6 degrees
Acq. time 1.205 sec
Width 27192.4 Hz
2256 repetitions
OBSERVE C13, 100.5671563 MHz
DECOUPLE H1, 399.9511043 MHz
Low power 3 dB atten.
continuously on
VALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
Gauss window 0.500 sec
center at 0.100 sec
FT size 131072
Total time 1 hr, 49 min, 39 sec

