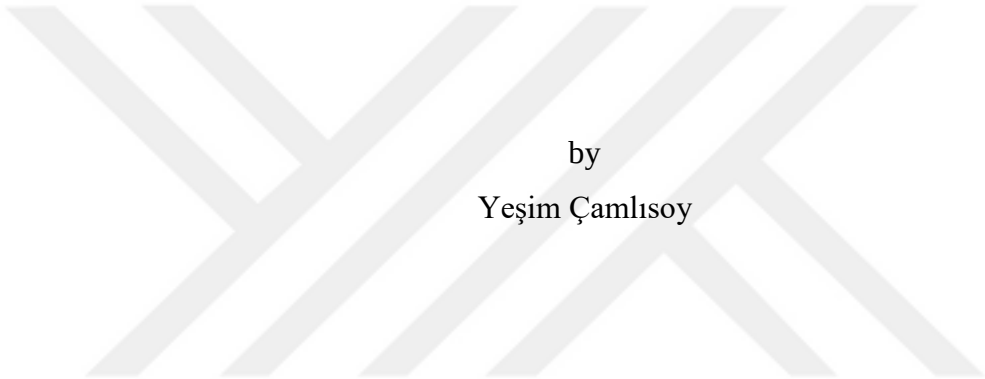


DEVELOPMENT OF CINCHONA BASED CATALYSTS GUIDED BY
COMPUTATIONAL INSIGHTS FOR ASYMMETRIC SYNTHESIS



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DEVELOPMENT OF CINCHONA BASED CATALYSTS GUIDED BY
COMPUTATIONAL INSIGHTS FOR ASYMMETRIC SYNTHESIS

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ABSTRACT

DEVELOPMENT OF CINCHONA BASED CATALYSTS GUIDED BY COMPUTATIONAL INSIGHTS FOR ASYMMETRIC SYNTHESIS

The reaction of 3-substituted 2-oxindoles with nitrosobenzene has an attractive product distribution. It is possible to obtain two different products with different biological activities in this reaction. However, controlling the regioselectivity and enantioselectivity of these products is challenging due to high reactivity of nitrosoarenes and the unknown factors influencing *O*- and *N*- selectivity. In this work, we employ for the first time a computational approach developed in our group, called CIPOC (Computational Identification of Potential (Organo)Catalysts) to gain insights into the factors that govern the activity and selectivity of the target reaction and guide experiments by uncovering novel catalytic structures not yet explored. CIPOC uses quantum mechanically optimized transition state models (theozymes) to determine optimal catalytic groups and their 3D arrangements around the substrates, and screens these catalytic elements in the conformer libraries of an organocatalyst database, consisting of 1586 candidates, using pharmacophore query tools commonly used for drug design.

Calculations identified hydroxyamination as the major reaction pathway that proceeds via a concerted addition-proton transfer mechanism. No catalysts in our database could be determined to steer the product distribution towards the aminoxylation products. A total of nine new catalysts, predicted to give promising efficiencies and selectivities, were proposed for experimental testing as efficient and selective catalysts for hydroxyamination reaction. Among the proposed compounds, four of them were synthesized and tested for activity for the first time for the target reaction. The effect of reaction time, temperature, reactant ratio, solvent were studied to optimize the reaction conditions. Characterization of the products pointed out to a facile decomposition pathway for the aminoxylation product, which was further justified by computations showing a significant weakening of the N-O bond upon protonation. The decomposition product is of particular interest to access C3-hydroxy-bearing stereogenic center, a motif common in therapeutic compounds. The highest enantioselectivity for the decomposition and accordingly the aminoxylation product was achieved as 98.4% by the urea-based cinchona catalyst at 0°C.

ÖZET

ASİMETRİK SENTEZ İÇİN HESAPLAMALI ANLAYIŞ İLE KINKONA TABANLI KATALİZÖRLER GELİŞTİRİLMESİ

3-substitüe 2-oksindollerin nitrosobenzen ile reaksiyonu çekici ürün dağılımına sahiptir. Bu reaksiyonda farklı biyolojik aktivitelere sahip iki farklı ürün elde etmek mümkündür. Ancak, nitrosoarenlerin yüksek reaktivitesi ve *O*- ve *N*-selektiviteyi etkileyen bilinmeyen faktörler nedeniyle bu ürünlerin regioselektivite ve enantioselektivitesini kontrol etmek zorlu bir süreçtir. Bu çalışmada, grubumuzda geliştirilen CIPOC (Potansiyel (Organo)Katalizörlerin Hesaplama Yoluyla Tanımlanması) adlı bir hesaplama yaklaşımını ilk kez kullanarak, hedef reaksiyonun aktivitesini ve seçiciliğini yönlendiren faktörler hakkında bilgi edinmeyi ve henüz keşfedilmemiş yeni katalitik yapıları ortaya çıkararak deneyleri yönlendirmeyi amaçladık. CIPOC, kuantum mekaniksel olarak optimize edilmiş geçiş konumu modelleri (teozimler) kullanarak optimal katalitik grupları ve bu grupların sübstrat etrafında üç boyutlu yerleşimlerini ve bu katalitik öğeleri, ilaç tasarımında yaygın olarak kullanılan farmakofor sorgu araçlarıyla organokatalizör veritabanının konformer kütüphanelerinde tarar. Hesaplamalar, hidroksiaminasyonun, tek adımlı bir eklenme-proton transfer mekanizmasıyla ilerleyen ana reaksiyon yolu olduğunu gösterdi. Veritabanımızdaki hiçbir katalizör, aminoksilasyon ürünlerinin dağılımını yönlendirmek için belirlenemedi. Hidroksiaminasyon reaksiyonu için ümit verici etkinlik ve seçicilik vadeden toplam 9 katalizör deneysel test edilmek üzere etkili ve seçici katalizörler olarak önerildi. Önerilen bileşikler arasında hedef reaksiyon için dört tanesi sentezlendi ve ilk kez aktivite için test edildiler. Reaksiyon şartlarını optimize etmek için reaksiyon süresi, sıcaklık, reaktan oranı ve çözücünün etkisi incelendi. Ürünlerin karakterizasyonu, protonlama ile N-O bağının önemli ölçüde zayıflamasını gösteren hesaplamalara ileveten doğrulanan, aminoksilasyon ürünü için basit bir ayrışma yoluna işaret etti. Ayrışma ürünleri, terapötik bileşiklerde yaygın olan C3-hidroksi taşıyan stereojenik merkeze erişim açısından özellikle ilgi çekicidir. Ayrışma ürünü ve buna bağlı olarak da aminoksilasyon ürünü için en yüksek enantioselektiviteye %98.4 olarak 0°C'de üre içeren kinkona katalizörü kullanılarak ulaşıldı.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	vi
ÖZET	vii
LIST OF FIGURES	xi
LIST OF TABLES.....	xvi
LIST OF SYMBOLS/ABBREVIATIONS.....	xviii
1. INTRODUCTION.....	1
2. THEORETICAL BACKGROUND AND LITERATURE	5
2.1. ORGANOCATALYSTS.....	5
2.2. CINCHONA ALKALOIDS.....	6
2.3. ASYMMETRIC SYNTHESIS AND ASYMMETRIC AMINOXYLATION/ HYDROXYAMINATION REACTIONS	8
2.4. COMPUTATIONAL CHEMISTRY	13
2.5. QUANTUM MECHANICS.....	14
2.6. AB INITIO METHODS.....	15
2.7. SEMI EMPRICAL METHODS.....	16
2.8. DENSITY FUNCTIONAL THEORY	16
2.9. BASIS SET	18
2.10. CONTINUUM SOLVATION MODEL	19
2.11. VIRTUAL SCREENING.....	19
3. METHODOLOGY	22
3.1. COMPUTATIONAL APPROACH.....	22
3.1.1. Generating Theozyme.....	22
3.1.2. Generating Conformer Library	23
3.1.3. Matching the Catalysts.....	24
3.1.4. Determination of Activity and Selectivity with QM	24
3.2. EXPERIMENTAL METHODOLOGY	25
3.2.1. Reaction of Oxindole with Nitrosobenzene.....	25

3.2.2.	Catalyst Synthesis	25
3.2.3.	Characterization Methods	27
3.2.3.1.	HPLC Methods	27
3.2.3.2.	NMR, NOESY, HETCOR Method	28
3.2.3.3.	LC-MS Method	28
3.2.3.4.	HR-MS Method	28
3.2.3.5.	FT-IR Method.....	28
4.	RESULTS AND DISCUSSION.....	29
4.1.	BINDING PREFERENCES OF SUBSTRATES	29
4.1.1.	Binding Mode 1	30
4.1.2.	Binding Mode 2	31
4.1.3.	Binding Mode 3	31
4.1.4.	Overall Mechanism.....	32
4.2.	SUBSTITUENT EFFECTS	33
4.3.	EFFECT OF DIFFERENT CATALYTIC GROUPS	34
4.4.	CATALYTIC ATOM MAPS (CAM) AND THE MATCHING RESULTS	36
4.5.	ACTIVITY AND SELECTIVITY OF THE CATALYSTS.....	44
4.6.	ASYMMETRIC SYNTHESIS OF CINCHONA ALKALOIDS	55
4.6.1.	Characterization of Catalysts	55
4.6.2.	Characterization of the Product	69
4.6.2.1.	Isocratic Elution.....	69
4.6.2.2.	Computational analysis for decomposition of the products	79
4.6.2.3.	Gradient Elution	80
4.6.3.	Optimization Study for the product	83
4.6.3.1.	Effect of reactant ratio	85
4.6.3.2.	Effect of reaction medium	86
4.6.3.3.	Effect of solvent	86
4.6.3.4.	Effect of reaction temperature	87
4.6.3.5.	Effect of reaction time and catalyst	88
4.6.3.6.	Effect of reaction temperature with the synthesized catalysts.....	89
4.6.3.7.	Enantioselectivity	90
5.	CONCLUSION AND FUTURE WORK.....	92

APPENDIX A..... 101

APPENDIX B..... 138



LIST OF FIGURES

Figure 1.1. The reaction of 3-substituted oxindole with nitrosobenzene.....	1
Figure 1.2. Computational Identification of Potential (Organo) Catalysts (CIPOC)	3
Figure 2.1. 9-epi-cinchona thiourea [6]	7
Figure 2.2. Structure of cinchona alkaloids	8
Figure 2.3. Two enantiomers of thalidomide [14]	9
Figure 2.4. Catalyst studied by Barbas and co-workers [30].....	10
Figure 2.5. Catalyst studied by Chen and co-workers [31]	11
Figure 2.6. Catalyst studied by Jia et. al. [32]	11
Figure 2.7. Catalyst studied by Companyó et. al. [33]	11
Figure 2.8. Proposed mechanism for aminoxylation product [33]	12
Figure 2.9. Hypothesis of Companyó et al. [33].....	12
Figure 3.1. Schematic representation of the theozyme (D= alcohol/amine/urea/thiourea) .	23
Figure 3.2.Catalyst library	24
Figure 3.3. Schematic representation of the catalyst synthesis.....	26
Figure 4.1. Enolization step and ion pair formation	30
Figure 4.2. Mechanism of binding mode 1	30
Figure 4.3. Mechanism of binding mode 2	31
Figure 4.4. Mechanism of binding mode 3	32
Figure 4.5. Unsubstituted TS and their relative Gibbs free energies for three binding modes	33

Figure 4.6. Lowest energy aminoxylation theozymes	35
Figure 4.7. Lowest energy hydroxyamination theozymes	36
Figure 4.8. Matching catalytic structure, CAT5, to ModelTS A3 for <i>O</i> -adduct	39
Figure 4.9. Matching catalytic structure, CAT8, to ModelTS H3 for <i>N</i> -adduct.....	40
Figure 4.10. Matching catalytic structure, CAT14, to ModelTS A3 for <i>O</i> -adduct	41
Figure 4.11. Matching catalytic structure, CAT19, to ModelTS H3 for <i>N</i> -adduct.....	41
Figure 4.12. Matching catalytic structure, CAT20, to ModelTS A3 for <i>O</i> -adduct	42
Figure 4.13. Matching catalytic structure, CAT27, to ModelTS H3 for <i>N</i> -adduct.....	43
Figure 4.14. Matching catalytic structure, CAT33, to ModelTS A3 for <i>O</i> -adduct	44
Figure 4.15. Matching catalytic structure, CAT38, to ModelTS H3 for <i>N</i> -adduct.....	44
Figure 4.16. Both aminoxylation and hydroxyamination TS and their enantioselectivity with cinchonidine (CD).....	45
Figure 4.17. Both aminoxylation and hydroxyamination TS and their enantioselectivity with quinine (QN)	46
Figure 4.18. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT5	48
Figure 4.19. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT14	49
Figure 4.20. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT20	50
Figure 4.21. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT36	51
Figure 4.22. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT38	52

Figure 4.23. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT39	53
Figure 4.24. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT38S	54
Figure 4.25. FT-IR spectrum of Catalyst 1 (CAT36)	56
Figure 4.26. LC-MSMS chromatogram of CAT36	57
Figure 4.27. Determining factor of Catalyst 1 (CAT36)	58
Figure 4.28. ^1H -NMR spectrum of Catalyst 1 (CAT36) (Solvent : CD_3OD)	59
Figure 4.29. ^{13}C -NMR spectrum of Catalyst 1 (CAT36) (Solvent : CD_3OD)	59
Figure 4.30. FT-IR spectrum of Catalyst 2 (CAT20)	61
Figure 4.31. LC-MSMS chromatogram of Catalyst 2 (CAT20)	62
Figure 4.32. The distinctive difference of Catalyst 2 (CAT20) when compared to free amine is the marked carbon on the structure	63
Figure 4.33. ^1H -NMR spectrum of Catalyst 2 (CAT20) (Solvent : CD_3OD)	64
Figure 4.34. ^{13}C -NMR spectrum of Catalyst 2 (CAT20) (Solvent : CD_3OD)	64
Figure 4.35. FT-IR spectrum of CAT38	65
Figure 4.36. LC-MSMS chromatogram of Catalyst 3 (CAT38)	66
Figure 4.37. ^1H -NMR spectrum of Catalyst 3 (CAT38) (Solvent : CD_3OD)	67
Figure 4.38. ^{13}C -NMR spectrum of Catalyst 3 (CAT38) (Solvent : CD_3OD)	67
Figure 4.39. ^1H -NMR spectrum of Catalyst 4 (CAT38S) (Solvent : CD_3OD)	68
Figure 4.40. ^{13}C -NMR spectrum of Catalyst 4 (CAT38S) (Solvent : CD_3OD)	69
Figure 4.41. The HPLC chromatogram of the reaction mixture (isocratic elution)	70

Figure 4.42. ^1H -NMR spectrum for the reaction mixture	71
Figure 4.43. Deuterium exchange analysis for the reaction mixture	72
Figure 4.44. ^1H -NMR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution	73
Figure 4.45. ^{13}C -NMR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution	74
Figure 4.46. NOESY spectrum of (a) 3-hydroxy-3-methyl-oxindole [70] and (b) the possible product the HPLC peak of which appears at 5.2 min in isocratic elution	75
Figure 4.47. NOESY spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution between 5-8 ppm.....	75
Figure 4.48. HETCOR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution	76
Figure 4.49. LC-MSMS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution	77
Figure 4.50. Fragment ions of 3-hydroxy-3-methyl-oxindole [69]	77
Figure 4.51. HR-MS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution at 164 g/mol	78
Figure 4.52. HR-MS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution at 254 g/mol	78
Figure 4.53. HR-MS analysis of reaction mixture at 254 g/mol.....	79
Figure 4.54. HR-MS analysis of reaction mixture at 164 g/mol.....	79
Figure 4.55. Relative energies vs distance graph for oxygen protonated for aminoxylation product	80
Figure 4.56. ^1H -NMR spectrum of the possible product the HPLC peak of which appears at 5.7 min in gradient elution.....	81

Figure 4.57. ^{13}C -NMR spectrum of the possible product the HPLC peak of which appears at 5.7 min in gradient elution.....82

Figure 4.58. HR-MS analysis of the possible product the HPLC peak of which appears at 5.7 min in gradient elution.....83



LIST OF TABLES

Table 2.1. The comparison of the literature results	13
Table 3.1. The amount of cyanate used in the synthesis of the selected catalysts.....	26
Table 3.2. The gradient elution condition.....	27
Table 4.1. The substituent effects relative to the parent oxindole (R=H, R'=H)	34
Table 4.2. The selected pharmacophore groups	37
Table 4.3. The representation of the selected pharmacophore groups and CAMs for ModelTS H3(thiourea)	38
Table 4.4. The matched catalyst with difunctional alcohol active side model	39
Table 4.5. The matched catalysts with di-functional amine active site model	40
Table 4.6. The matched catalyst with difunctional thiourea active side model.....	42
Table 4.7. The matched catalyst with difunctional urea active side model	43
Table 4.8. Gibbs free energies of the selected catalysts	46
Table 4.9. Structure of computationally selected catalysts.....	54
Table 4.10. IR Absorption peaks of Catalyst 1 (CAT36)	55
Table 4.11. Absorption peaks of Catalyst 2 (CAT20)	60
Table 4.12. Absorption peaks of CAT38	65
Table 4.13. The desired and the unpredicted products	70
Table 4.14. Reaction conditions (3M2O: 3-methyl-2-oxindole; N: nitrosobenzene; ratio: 3-methyl-2-oxindole: nitrosobenzene ratio; THF: tetrahydrofuran)	84
Table 4.15. The effect of the reactant ratio	85

Table 4.16. The effect of the reaction medium.....	86
Table 4.17. The solvent effect	87
Table 4.18. The effect of the reaction temperature.....	87
Table 4.19. The effect of the reaction time and the catalyst.....	88
Table 4.20. The effect of the reaction temperature with the synthesized catalysts	90
Table 4.21. Enantioselectivity achieved by the synthesized catalysts determined by HPLC data.....	91

LIST OF SYMBOLS/ABBREVIATIONS

∇^2	Laplacian operator
ΔG	Free energy of reaction (kcal/mol)
E	Energy of particle
ϵ	Dielectric constant
$\hat{f}_i$	Fock operator
$\hat{H}$	Hamiltonian operator
h	Planck constant
m	Mass of the particle
ψ	Wave function
V	Potential
Z	Atomic number
3M2O	3-methyl-2-oxindole
atm	Atmosphere
B3LYP	Becke's three-parameter hybrid functional
C-13 NMR	Carbon nuclear magnetic resonance
CADD	Computational aided drug design
CAM	Catalytic atom map
CD	Cinchonidine
CD ₃ OD	Deuterated methanol
CIPOC	Computational identification of potential organocatalysts
CN	Cinchonine
CVS	Consensus virtual screening
DCM	Dichloromethane
DEE	Diethyl ether
DFT	Density functional theory
DIAD	Diisopropyl azodicarboxylate
DMSO	Dimethyl sulfoxide
DPPA	Diphenylphosphoryl azide
Et ₂ O	Diethyl ether

FT-IR	Fourier transform infrared spectroscopy
GGA	Generalized gradient approximation
H-1 NMR	Proton nuclear magnetic resonance
HCl	Hydrochloric acid
HETCOR	Heteronuclear correlation
HPLC	High performance liquid chromatography
HR-MS	High-resolution mass spectroscopy
HTS	High-throughput screening
IRC	Intrinsic reaction coordinate
LBVS	Ligand-based virtual screening
LC-MS/MS	Liquid chromatography-mass spectroscopy
LDA	Local density approximation
LSDA	Local (Slater) density approximation
MOE	Molecular operating environment
MP	Moller plesset perturbation
N	Nitrosobenzene
NOESY	Nuclear overhauser effect spectroscopy
QD	Quinidine
QM	Quantum mechanics
QN	Quinine
QSAR	Quantitative structure-activity relationships
RMSD	Root mean square derivation
rt	Room temperature
SBVS	Structure-based virtual screening
SCF	Self-consistent field
SMD	Solvation model based on density
STO	Slatter type orbital
THF	Tetrahydrofuran
TOF	Time of flight
TS	Transition state
VS	Virtual screening

1. INTRODUCTION

Oxindoles, aromatic organic compounds, possess diverse biological effects such as antibacterial, antiviral, and anticancer activities due to their specific structural features [3]. Nitrosoarenes also serve as intriguing electrophiles in catalytic asymmetric nitroso aldol reactions. Oxindole skeleton contains a tetrasubstituted carbon center at its C3-position. It is a special motif that forms the core of many pharmaceuticals, and biologically active natural products. Therefore the development of new methods and obtaining new chiral catalysts for their asymmetric synthesis is one of the top topics studied recently. The reactions of 3-substituted oxindoles with nitrosobenzene are particularly attracting because of the diversity of the products related to the reaction selectivity. This reaction leads to two different products with different biological activities (Figure 1.1). Due to the high reactivity of nitrosoarenes and the unknown factors influencing *O*- and *N*-selectivity, controlling the regioselectivity and enantioselectivity of the resulting products proves to be challenging.

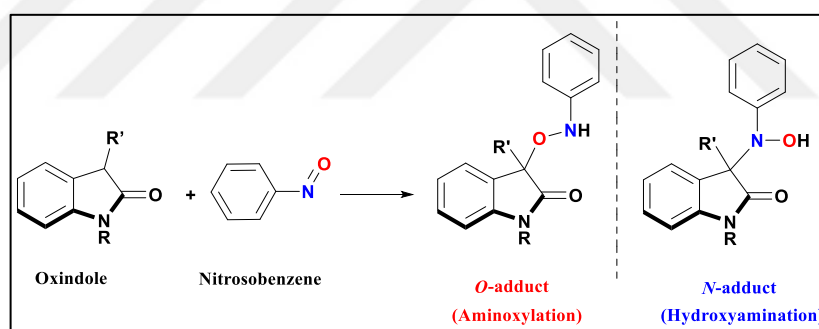


Figure 1.1. The reaction of 3-substituted oxindole with nitrosobenzene

The common use of small chiral organic molecules as organocatalysts in organic synthesis and the special interest of pharmaceutical industry in organocatalytic methods clearly demonstrate the impact of the prediction of active organocatalysts using computational methods in science and industry. Although quantum mechanical (QM) calculations can reveal reaction mechanisms successfully, due to the large number of conformers of commonly used multi-functional organocatalysts, prediction of effective catalyst is still a challenging task. The determination of effective and selective organocatalytic structures still largely relies on serendipity, and experimental trial and error. The purpose of this project is to allow easy and cost-effective determination of potential organocatalyst candidates for a

target reaction using a novel computational approach (Computational Identification of Potential (Organo) Catalysts (CIPOC)) (Figure 1.2) that combines the quantitative power of quantum mechanical calculations with drug design tools and determine the reliability of CIPOC by comparing the theoretical results with the results obtained from the experiments performed with the proposed organocatalysts. A similar "inside-out" approach (TUBITAK project, Project number : 114Z791), based on a theozyme model to provide maximum TS stabilization, has been successfully used to develop active catalytic proteins [1] and spirocyclic oligomers [2]. In this study, the "inside-out" approach will be used for the first time in combination with drug design tools to identify effective organocatalysts.

In this work, first of all, theozymes were generated with amine/alcohol/urea/thiourea functional groups, which are commonly involved in the structures of multifunctional organocatalysts. Three different binding preferences of the substrates and substituent effects of oxindole were studied. Free energy profiles for each active site model were calculated. The functional groups were selected as pharmacophore groups of potentially active organocatalysts. Cinchona as they were known as effective catalysts for the reactions of 3-substituted oxindoles with nitrosobenzene. Catalytic atom maps were generated using the arrangement of these pharmacophore groups in the active site models. The resulting catalytic atom maps were screened against a conformational library generated for cinchona alkaloid derivatives. Quantum mechanical (QM) calculations were used to determine the enantioselectivities of the matching organocatalysts and the catalyst-transition structure interactions were analyzed. Nine new catalyst candidates were proposed for experimental testing. In the experimental part, optimum reaction conditions like solvent effect, reaction medium, ratio of substrates, and temperature were studied with commercial chemicals and the product formation was investigated. The product determination and characterization were done by different techniques. Decomposition of aminoxylation product is determined and its formation is examined. Four catalysts, which were selected based on the theoretical results and used in the reaction for the first time, were synthesized and characterization studies were performed. Finally, the reaction was carried out in the presence of the synthesized catalysts and the product ratios with the enantioselectivity were determined.

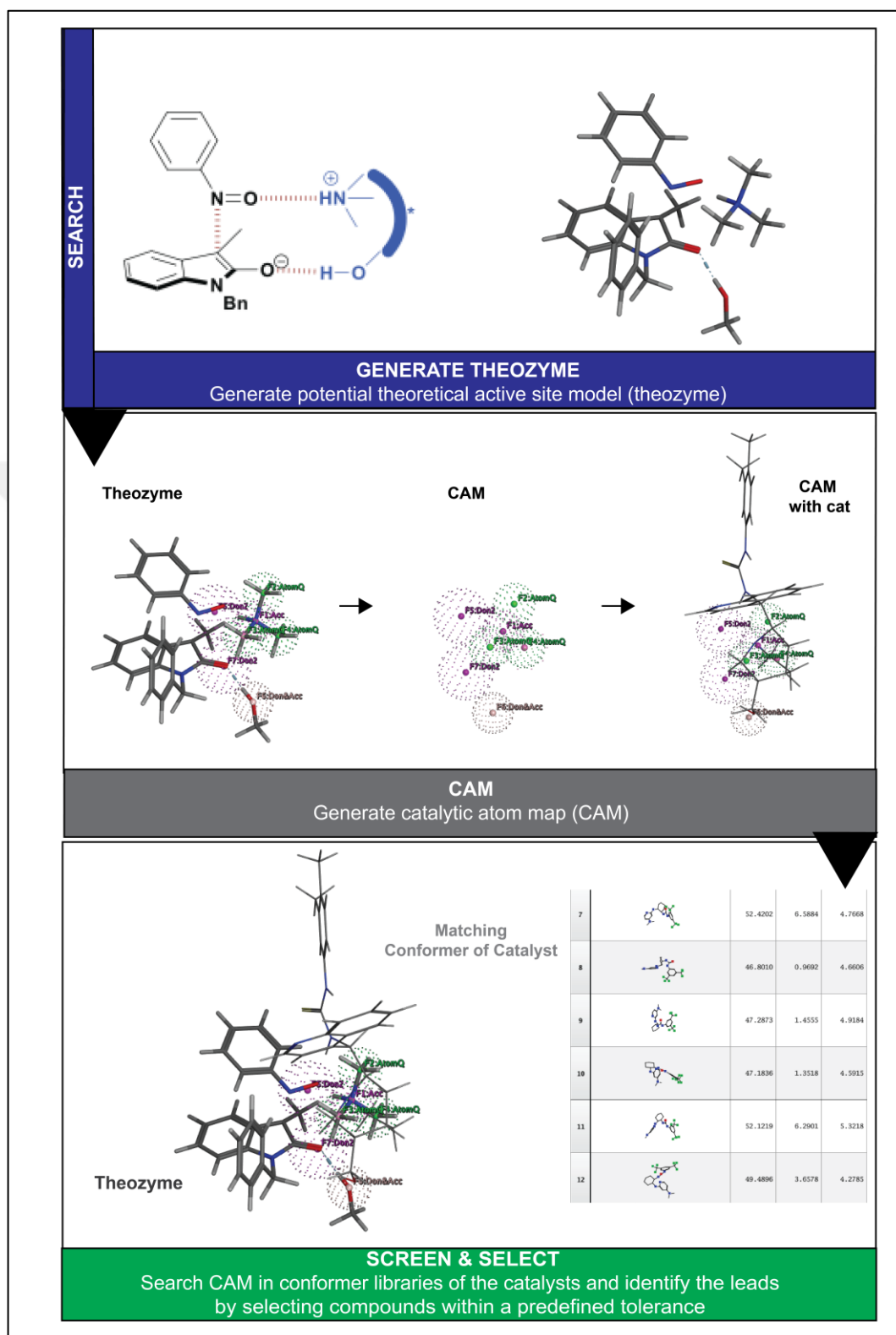


Figure 1.2. Computational Identification of Potential (Organo) Catalysts (CIPOC)

In the following chapter (Chapter 2), a foundational understanding of mathematics to support the advanced computational techniques utilized in this project, general information about the asymmetric synthesis and studies about the reaction of oxindole with nitrosobenzene are given. The next chapter (Chapter 3), explains both experimental methodology, characterization techniques and computational approach (CIPOC). In Chapter 4, both computational and experimental results are shown and discussed. Finally, all the conclusions are given in Chapter 5.



2. THEORETICAL BACKGROUND AND LITERATURE

Small organic molecules have taken their place among widely used catalysts in recent years, alongside enzymes and organometallic complexes [3]. The limited availability of effective substrates due to the high selectivity of enzymes and the potential heavy metal pollution caused by organometallic catalysts have led to an increasing interest in "green" organocatalysts that are easy to obtain, inexpensive, non-toxic, and resistant to air and moisture.

2.1. ORGANOCATALYSTS

Asymmetric organocatalysis involves using a chiral organic molecule to catalyze enantioselective reactions, in which one of the two enantiomers are produced selectively in higher amounts. These types of catalysts are commonly used in phase transfer catalysis (which is a distinct type of catalysis that can operate via either homogeneous or heterogeneous catalysis mechanisms, depending on the specific catalyst employed.), kinetic resolutions (which is a category of various reactions that exhibit preferential selection of one enantiomer over the other, with the aim of either synthesizing the desired molecule or producing intermediate products that can be easily separated), and other asymmetric synthesis methods. Many of these reactions can also be accomplished using organometallic or bioorganic catalysis. Unlike metal-ligand complexes, organocatalysts are not as sensitive to aerobic conditions and do not require rigorous water exclusion. They are also more versatile regarding substrate scope than enzymes and can be used in a wider range of organic solvents.

Compared to traditional metal catalysis, organocatalysis has various advantages. One of the significant benefits is the reduced toxicity associated with organocatalysis, although the toxicity of some organic catalysts has yet to be well-known. Additionally, organocatalysis is usually more tolerant of water and air, making it easier to perform reactions. These factors often need to be revised in metal-catalyzed reactions, simplifying organocatalysis operationally [4].

Chiral organic molecules that act as organocatalysts can be prepared synthetically or derived from naturally occurring chiral compounds. These catalysts can also be produced using solid-phase synthesis or high-throughput screening techniques. Some commonly used organocatalysts are better known as ligands rather than as asymmetric catalysts in their own right [5].

The most important aspect of synthetic organic chemistry is stereocontrol in organic reactions. Asymmetric catalysis is a popular strategy for achieving stereinduction in synthetic endeavors, with several natural product-derived chiral molecular frameworks being designed and developed as chiral organocatalysts. Over the last two decades, cinchona alkaloids have emerged as the most powerful class of compounds in asymmetric organocatalysis [6].

2.2. CINCHONA ALKALOIDS

The cinchona alkaloids are a significant source of various chiral organocatalysts. [7, 8] These alkaloids, once known for the antimalarial drug quinine, are abundant in nature, commercially available at reasonable prices, stable, and easy to handle in the laboratory. Many derivatives of cinchona alkaloids, containing hydroxyl groups, amines, ureas, and thiourea functionalities, have been used alone or in combination with an additional catalyst, such as a simple achiral compound or metal salt, in diverse types of enantioselective syntheses by asymmetric catalysis, a form of catalysis that involves a catalyst with chirality that guides the formation of a chiral compound, promoting the formation of a specific stereoisomer [9].

The convenient modification of cinchona alkaloids through simple reactions is one reason for their widespread use in asymmetric catalysis. [6]. These alkaloids and their simple derivatives have been widely used, but more complex derivatives, such as 9-*epi*-cinchona thioureas (Figure 2.1) have also been found to be efficient bifunctional organocatalysts and chiral selectors for chromatography. However, less attention has been given to other derivatives of cinchona alkaloids, such as amides, imides, imines, and sulfides that contain a substitution at the 9-position [10].

One of the most well-known reactions in which derivatives of quinine or quinidine are used is the asymmetric dihydroxylation of olefins, which produces diols in high enantiomeric excess. In addition, these alkaloids can also be used as chiral transmitters in phase transfer and base-catalyzed reactions with high enantioselectivity. [11]

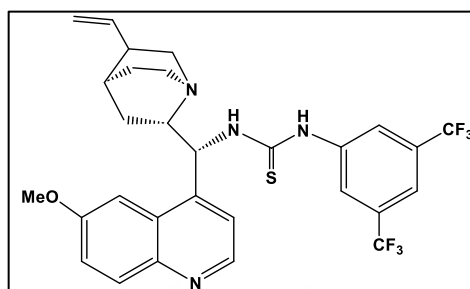


Figure 2.1. 9-epi-cinchona thiourea [6]

The cinchona alkaloids consist of two rigid rings, namely an aliphatic quinuclidine ring and an aromatic quinoline ring, connected by two carbon-carbon single bonds. This molecule possesses five stereocenters and occurs in pairs with different configurations at positions C8, C9, and N1. The eight main cinchona alkaloids found in the nature (Figure 2.2) are diastereomers and are commonly referred to as pseudo enantiomers because they yield enantiomeric products when employed as catalysts. The catalytic activity of cinchona alkaloids and their derivatives can be attributed to specific structural characteristics that impact the yields, diastereoselectivity, and enantioselectivity of the products. Numerous studies have demonstrated that cinchona compounds function as bifunctional catalysts, with the tertiary nitrogen atom on the quinuclidine ring serving as the catalyst's basic moiety. The hydroxyl group, urea, and thiourea located at the C9 position facilitate electrophile activation through hydrogen bonding. The relative orientation of the quinoline and quinuclidine rings creates a "chiral pocket" surrounding the reactive site, which governs the approach of substrates and leads to the formation of enantioselective products[6]

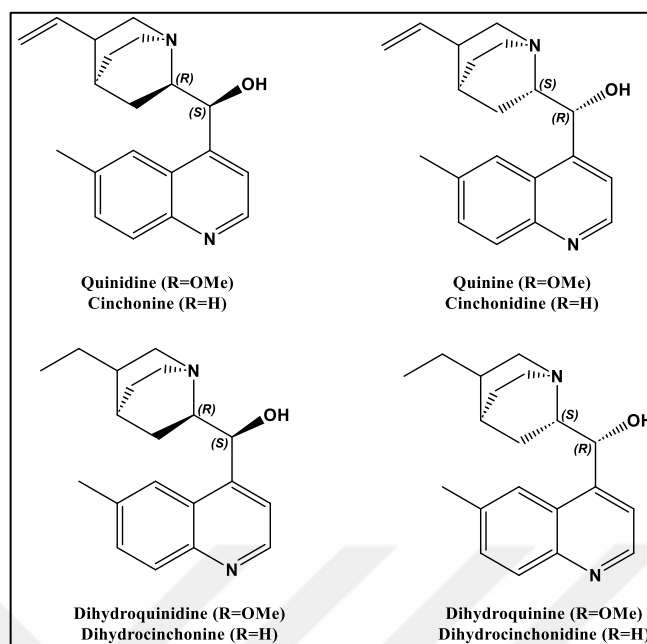


Figure 2.2. Structure of cinchona alkaloids

2.3. ASYMMETRIC SYNTHESIS AND ASYMMETRIC AMINOXYLATION/HYDROXYAMINATION REACTIONS

Four general approaches for producing enantiopure or enantioenriched compounds have evolved, including classical resolution (which is a stereochemical process that aims to separate racemic compounds into their individual enantiomers), chiral pool approach (which is a synthetic strategy that uses a chiral compound as a starting material to produce a desired molecule), enantioselective syntheses with covalently bound chiral auxiliaries (which refers to a stereogenic group or unit that is added to an organic molecule for a temporary period to control the stereochemical result of a synthesis), and enantioselective catalysis (also known as asymmetric catalysis is a method of catalysis where a chiral catalyst is employed to guide the formation of a chiral compound, increasing the likelihood of forming a specific stereoisomer). While classical resolution is still the most commonly used method, enantioselective catalysis is an elegant approach that has great potential for producing enantiopure compounds with high efficiency and selectivity. Biocatalysis is a promising area for enantioselective catalysis, but challenges such as finding an efficient biocatalyst and product isolation need to be addressed [12].

Enantioselective synthesis is becoming increasingly important in the pharmaceutical industry due to the demand for enantiopure or enantioenriched compounds [12]. The biological activity of each enantiomer could be different depending on the different interaction capacity of each enantiomer with an organism. For example, thalidomide is a medication and it was first introduced as a sedative and treatment in pregnant women for morning sickness in the 1950s. However, it was originally produced as a racemic mixture, meaning it contained equal amounts of two enantiomers, or mirror image molecules. It was withdrawn from the market, after discovered that thalidomide caused severe birth defects. Thalidomide is a chiral compound, meaning that it exists in two enantiomers (Figure 2.3). The *S*-enantiomer (responsible for counter clockwise rotation regarding the priority numbers of the substituents on the chiral center) is known for its sedative effects, while the *R*-enantiomer (responsible for clockwise rotation regarding the priority numbers of the substituents on the chiral center) is responsible for its teratogenic effects, and birth defects [13].

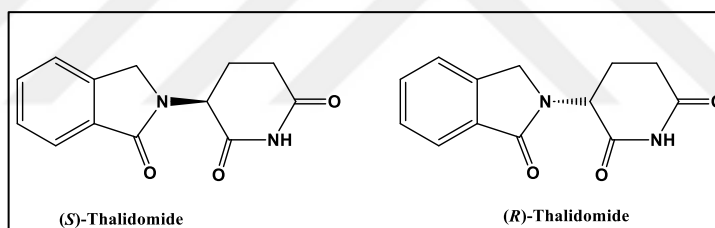


Figure 2.3. Two enantiomers of thalidomide [14]

Aromatic organic compounds known as oxindoles have various biological effects such as antibacterial, antiviral, and anticancer activities due to their skeletal structure [15]. Nitrosoarenes are also interesting electrophiles in catalytic asymmetric nitroso aldol reactions. The utilization of small chiral organic molecules as catalysts in asymmetric synthesis has remarkably increased in the past 20 years since the pioneering work of List, Lerner, and Barbas on the proline-catalyzed intermolecular aldol reaction [16] and MacMillan's discovery of the catalytic effects of amino acid-derived amines [17]. Organocatalysts with hydrogen bond donors such as hydroxyl or amide groups are used to control the regioselectivity in nitroso aldol reactions by forming hydrogen bonds with the oxygen or nitrogen atom of nitrosoarenes [18–27].

Their most common use is in the reaction of 3-substituted 2-oxindoles with nitrosobenzene. This is because the reaction is attractive in terms of both its products and selectivity. This reaction can produce two different products with different bioactivity (Figure 1.1). The 3-substituted-3-hydroxyoxindole obtained from aminooxylation is found in many natural products with broad-spectrum bioactivities, such as maremycin, convolutamydin, donaxaridin, diospyrin, and selogentin K, as well as pharmaceutical active products like SM-130686 and TMC-195A [28]. On the other hand, the hydroxyamination products, 3-substituted 3-amino oxindoles, are present in the structures of potential drug candidates for malaria and stress-related disorders, such as NITD609, SSR-149415, and AG-041R [29]. However, the high reactivity of nitrosoarenes and the unknown factors affecting *O*- and *N*-selectivity make it very difficult to control the regioselectivity and enantioselectivity of the adducts. In the nitrosobenzene reaction with 2-oxindoles selected as the target reaction and catalyzed by quinine alkaloid derivatives, it was observed that the main product varies depending on the catalyst.

Barbas and co-workers [30] synthesized a new dimeric quinidine catalyst (Figure 2.4) to produce aminooxylation product for the reaction of oxindole with nitrosobenzene (up to 87% yield and 96% ee). In their research, the oxindole is *N*-protected by benzyl group and they proposed a reaction mechanism for the formation of the aminooxylation product. In the proposed mechanism, the protonated catalyst interacts with the oxyanion and the alcohol functional group stabilizes the nitrosobenzene for the addition. Then, the addition of the enolate to activated nitrosobenzene to form intermediate is followed by the proton transfer from the protonated catalyst to the oxygen of the nitrosobenzene to form *O*-adduct.

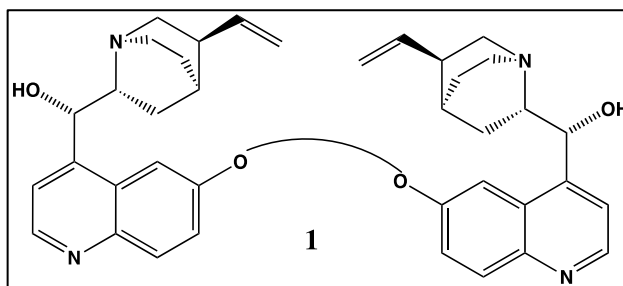


Figure 2.4. Catalyst studied by Barbas and co-workers [30]

Chen and co-workers [31] used cinchona alkaloid catalysts including dimmers with unprotected oxindole as substrate and reported the hydroxyamination products with the given catalyst in Figure 2.5 up to 90% yield and 54% ee.

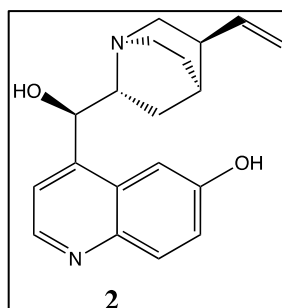


Figure 2.5. Catalyst studied by Chen and co-workers [31]

Jia et al. [32] studied different organocatalysts including the thiourea functional group (Figure 2.6). The reaction produced the hydroxyamination product up to 91% yield with up to 90% ee when benzyl protected oxindole was used as a reactant.

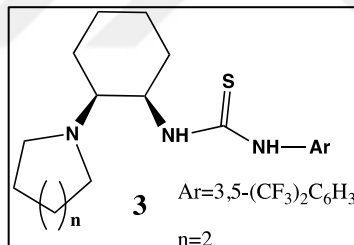


Figure 2.6. Catalyst studied by Jia et. al. [32]

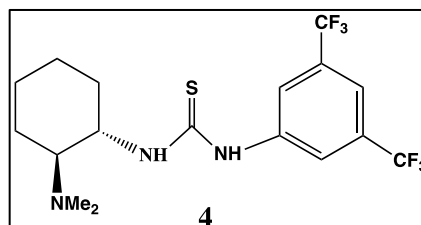


Figure 2.7. Catalyst studied by Companyó et. al. [33]

Lastly, Companyó et. al. [33] have studied nitroso-aldol reaction between *N*-unprotected 3-substituted-2-oxindoles and nitrosobenzene, catalyzed by tertiary amines. Especially when bifunctional amino-thioureas were used as the catalysts (Figure 2.7), hydroxyamination

product was obtained with high yield and enantioselectivity. However, in the presence of nitrogen-protecting group in the starting 3-substituted oxindole, after five days aminooxylation product, also a decomposition product, 3-hydroxy-3-methyl-oxindole, were produced. The proposed mechanism for the formation of the decomposition product is given in Figure 2.8.

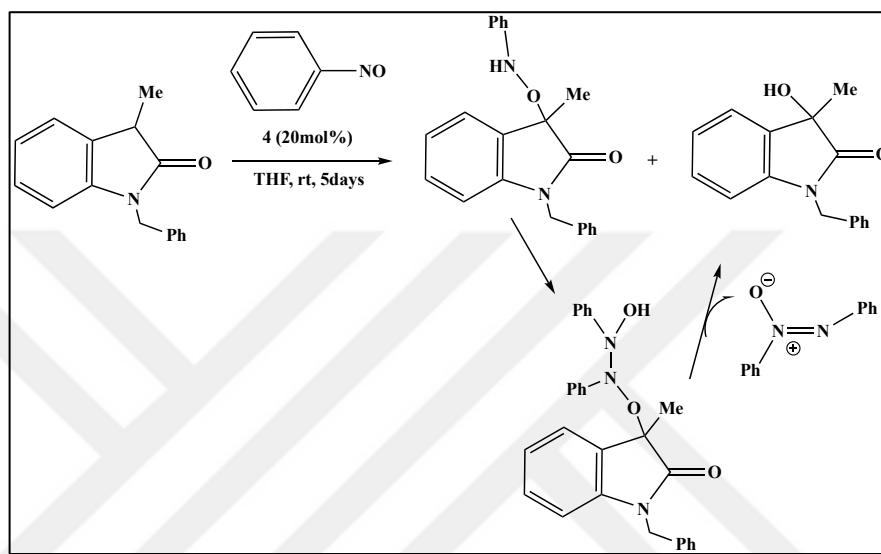


Figure 2.8. Proposed mechanism for aminooxylation product [33]

Companyó et al. [33] suggested that reversal in regioselectivity is caused by intermolecular hydrogen bonding involving the enolate oxygen in 3-substituted oxindoles that are not protected (Figure 2.9).

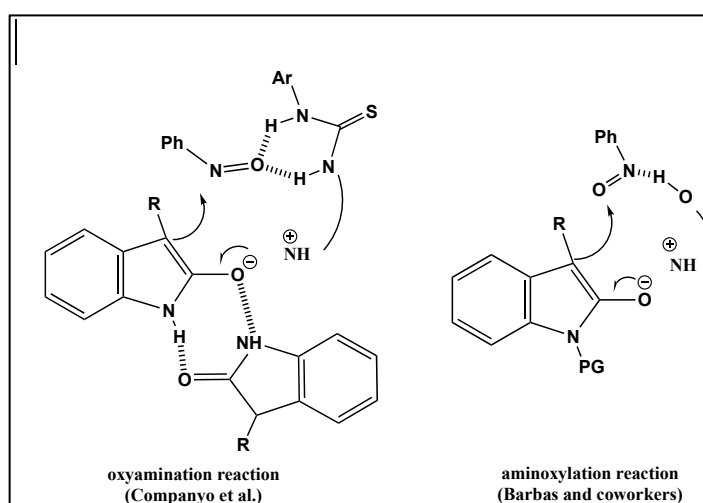


Figure 2.9. Hypothesis of Companyó et al. [33]

Table 2.1. The comparison of the literature results

Group	Catalyst	Oxindole*	Solvent	Reaction Time	Temp. (°C)	Product	Yield % (ee%)
Barbas	1	Protected (R=Bn, R'=Me)	THF	48h	-20	<i>O</i> -adduct	85 (92)
Chen	2	Unprotected (R=H, R'=Me)	DCM	6h	0	<i>N</i> -adduct	90 (54)
Wang	3	Protected (R=Bn, R'=Bn)	Toluene	6h	25	<i>N</i> -adduct	86 (83)
Rios	4	Unprotected (R=H, R'=Me)	Et ₂ O	2h	r.t	<i>N</i> -adduct	100 (70)
Rios	5	Protected (R=Bn, R'=Me)	THF	5 days	r.t	<i>O</i> -adduct	-

*R and R' shown in Figure 1.1.

The experimental findings on the organocatalyzed reactions of oxindoles with nitrosobenzene, summarized in Table 2.1, show that the product varies depending on the catalyst and the substituents. However, the mechanisms of the reaction as well as the substituent effects are still ambiguous and there are no computational studies to provide an understanding to the observed catalytic activities and outcomes. In order to clarify these results, understand the reaction mechanism, and identify effective organocatalysts, a computational study was carried out using the state-of-the-art computational techniques described in the following section.

2.4. COMPUTATIONAL CHEMISTRY

Computational chemistry uses computer simulation to solve chemical problems. The most common computational methods are based on either molecular or quantum mechanics. The classical methods that use molecular mechanical force fields to model molecular systems are Monte Carlo, and molecular dynamic simulations. They describe the interactions between atoms and molecules based on simple mathematical functions. These classical methods are often used for large systems and long-time scales. They have some limitations about the reactivity of molecules and electronic structure. However, quantum mechanical methods (QM) such as *ab initio*, semi-empirical and density functional theory (DFT) are the more accurate approaches that use QM to model the molecular system and different types of

approximations to solve the Schrödinger's equation.

2.5. QUANTUM MECHANICS

Quantum mechanics (QM) is a fundamental theory in physics. It describes the behavior of particles at an atomic and subatomic level. It calculates the electronic structure of molecules by solving the Schrödinger equation (equation 2.1).

The time-independent Schrödinger equation has the form [34]:

$$\left\{ -\frac{\hbar}{2m} \nabla^2 + V \right\} \Psi(r) = E \Psi(r) \quad (2.1)$$

In here, E is the energy of the particle and the abbreviation ∇^2 (pronounced 'delsquared') shown in equation 2.2 [34]:

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \quad (2.2)$$

It is usual to abbreviate the left-hand side of equation 2.3 to $\hat{H}\psi$, where $\hat{H}$ is the Hamiltonian operator [34],

$$\hat{H}\psi = E\psi \quad (2.3)$$

Solving Schrödinger equation to find analytical solutions is often difficult for complex systems. Therefore, computational methods solve the equation numerically and some approximations are made. Since, the atomic nucleus is significantly more massive than the electrons, its motion can be considered negligible in comparison. As a result, the nuclei can be treated as stationary or "frozen," with their kinetic energy assumed to be zero. The Born-Oppenheimer approximation proposes disregarding the attractive potential energy between the electrons and nuclei, treating the nuclear kinetic energy as independent of the electron behavior, and considering the nuclear-nuclear potential energy as a constant value and Schrödinger equation (equation 2.4) becomes;

$$(\hat{H}_{el} + \hat{V}_{nn})\varphi_{el} = E_{el}\varphi_{el} \quad (2.4)$$

These methods solve for the corresponding energy levels by approximating the wavefunction of the system. Density functional theory (DFT), Møller-Plesset Perturbation (MP) theory, and Hartree-Fock, are some alternative methods to provide approximate solution for many body solutions.

2.6. AB INITIO METHODS

Ab initio methods rely on quantum mechanical calculations that address the molecular Schrödinger equation linked to the molecular Hamiltonian. Due to the presence of electron-electron interaction terms, finding an analytical solution to the Schrödinger equation proves to be highly challenging. Consequently, a specific approximation is rigorously established based on fundamental principles of quantum theory, and the equation is subsequently solved using numerical iterative methods within a predetermined error range. Modern computational techniques offer highly precise and dependable numerical solutions for wavefunctions and energies, yielding detailed information about the system under investigation [35, 36].

The wavefunction still is not precisely known because we do not know the molecular orbital coefficients. However, a trial wavefunction can be created by making a guess. The optimization of the overall wavefunction is achieved in an iterative manner, varying the molecular orbital coefficients until no further changes in the overall wavefunction occur. The direction of the variation of molecular orbital coefficients is guided by the variational principle stating that the energy derived in this manner is an upper bound to the actual energy. The Hartree-Fock method serves as the fundamental approach for all *ab initio* quantum chemistry methodologies. The Hartree-Fock equation given as;

$$\hat{f}_i \chi_i = \varepsilon_i \chi_i \quad (2.5)$$

By solving for the optimal spin orbitals, the solution determines the configuration that minimizes the value of E . In Equation 2.5, $\hat{f}_i$ represents the Fock operator, χ_i represents an eigenfunction of $\hat{f}_i$, and ε_i represents the corresponding energy of the orbital. The Fock operator for each electron i is defined as (Equation 2.6);

$$\hat{f}_i = -\frac{1}{2}\nabla_i^2 - \sum_k^{nuclei} \frac{Z_k}{r_{ik}} + V_{HF}(i) \quad (2.6)$$

In the equation, the final term (i) corresponds to the Hartree-Fock potential, which represents the average repulsive potential experienced by each electron due to the presence of other electrons. (i) accounts for the interaction between each electron and the static field created by all the other electrons, including the exchange effects on the Coulomb repulsion.

The Hartree-Fock method, also known as the self-consistent field (SCF) method, gets its name from the iterative process used to reach the minimum energy value where the first derivative of the electronic energy with respect to the molecular orbital is attained. The SCF method begins with an initial estimation of molecular orbitals based on a given molecular geometry. χ_i is calculated and used to generate a new V_{HF} . This process is repeated until convergence is achieved within the iterative cycle.

2.7. SEMI EMPIRICAL METHODS

Semi empirical methods perform calculations by omitting or parameterizing certain integrals based on experimental data, such as ionization energies of atoms, or dipole moments of molecules. Therefore, this method is very fast and applicable for large molecules. This method may give accurate results when applied to molecules that are similar to the molecules used for parameterization. However, accuracy of semi-empirical methods is not reliable for many systems [26].

2.8. DENSITY FUNCTIONAL THEORY

Density functional theory (DFT) is used to calculate the electronic structure of molecules and materials in quantum chemistry. It is based on electron density instead of the wavefunction. Therefore, it is more efficient than other methods. The density functional theory deals with different situations, and these multi-component systems such as relativistic electrons, nuclei and time-dependent phenomena, electron-hole droplets, , and excited states, superconductors with electronic pairing mechanisms, bosons, molecular dynamics, and also spin-polarized systems [37]. Gradient-corrected consider both the values of the electron spin

densities and their gradients. In contrast, correlation and local exchange functionals only consider the values of the electron spin densities. The most commonly used gradient-corrected functional is the LYP functional of Lee, Yang, and Parr. Becke proposed a different gradient-corrected functional and combined with LYP to form the B-LYP method.

Another functional used in DFT is hybrid functionals. They are linear combinations of Hartree-Fock, gradient-corrected exchange, and local terms. The three-parameter formulation of Becke is the best-known hybrid functional known as B3LYP in Gaussian.

The variation among different methods of Density Functional Theory (DFT) lies in the selection of the functional expression used to calculate the exchange-correlation energy. It has been established that the exchange-correlation potential is a distinct functional applicable to all systems. However, finding a specific mathematical expression for this potential has been challenging, except in certain instances such as a uniform electron gas.

Local density approximation. The Local Density Approximation (LDA) assumes that the density in a given region can be treated as a uniform electron gas or, alternatively, that the density changes gradually over that region. The term "Slater" is frequently employed as a synonym for the L(S)DA exchange energy, which encompasses the electron density raised to the power of $4/3$.

Gradient-corrected methods. Generalized Gradient Approximation (GGA) methods incorporate the first derivative of the density as a variable and require that the Fermi and Coulomb holes integrate to specific values of -1 and 0 , respectively. Although GGA methods are sometimes referred to as non-local methods, this can be misleading since the functionals only rely on the density (and its derivative) at a particular point, unlike the Hartree-Fock exchange energy which depends on the density over a spatial volume. One of the earliest and most widely used GGA exchange functionals, proposed by A. D. Becke as the B or B88 functional, serves as a correction to the LSDA exchange energy. Several GGA functionals have been suggested for the correlation energy. Among them, the Lee, Yang, and Parr (LYP) functional is widely recognized and favored. However, it is worth noting that the LYP functional may appear complex or daunting to some due to its formulation.

Higher order gradient or meta-GGA methods. The natural progression of GGA methods involves enabling the exchange and correlation functionals to be influenced by higher order derivatives of the electron density. This includes considering the Laplacian ($\nabla^2\rho$) as the

second-order term. Alternatively, the functional can be designed to depend on the orbital kinetic energy density, denoted as " τ ." Incorporating either the Laplacian or orbital kinetic energy density as variables gives rise to meta-GGA functionals, and functionals that generally utilize orbital information can also fall into this category. Calculating the orbital kinetic energy density is typically more numerically stable compared to calculating the density's Laplacian.

Hybrid functionals. Several hybrid functionals, which combine Hartree-Fock exchange with exchange-correlation methods in DFT, are available. The one-parameter hybrid method, such as The B1B95 keyword is used to specify Becke's one-parameter hybrid functional, allows adjustment of only the proportion of Hartree-Fock (HF) exchange incorporated into the functional. Becke Three Parameter Hybrid Functionals devised by Becke in 1993. Multiple versions of this hybrid functional exist. B3LYP employs the non-local correlation from the LYP expression and the VWN functional III for local correlation. The LYP incorporates both local and non-local terms [34, 38].

2.9. BASIS SET

A basis set is the mathematical description of the orbitals within a system for the theoretical calculation. There are two types of basis functions, Slater-type and Gaussian type. Large basis sets may give more accurate results with larger flexibility, but they can be computationally expensive, and time-consuming. Therefore, a balance between the accuracy and computational cost is necessary.

There are numerous different Gaussian basis sets. STO-3G is the simplest basis set. This minimal basis set has only as many orbitals as are necessary to accommodate the electrons of the neutral atom [34].

The split-valence basis sets are developed by Pople. Double zeta basis have split core orbitals whereas split-valence basis set splits only the valence orbitals. Polarization basis sets in which d orbitals are added to all heavy atoms are used for larger flexibility.

Large-size versions of s- and p-type functions are diffuse functions that occupy a larger region of space. When electrons are far from the nucleus, like anions, and lone pairs, basis

sets with diffuse functions are important. The 6-31G (d) is an example for a basis set. When diffuse functions are added to heavy atoms, it becomes as 6-31+G (d) basis set [34].

2.10. CONTINUUM SOLVATION MODEL

The use of a solvent is common in many reactions; therefore, the role of the solvent in the reaction is crucial. The solvent can directly interact with the reactants in some cases, and these interactions need to be considered explicitly in the reaction mechanism. In other cases, the solvent may not directly participate in the reaction but can still impact the reaction outcome by affecting the environment around the reactants. In this scenario, the solvent can be considered as a "bulk medium." Despite not directly participating in the reaction, the solvent can still perturb the gas-phase behavior of the system by affecting the dielectric constant of the solvent, ultimately influencing the behavior of the reactants [34].

Continuum solvation models [39, 40], treat the solvent as a polarizable medium with a fixed dielectric constant, ϵ . In these models, the solvent is treated uniformly at a quantum mechanical level, and interactions between solvent molecules are limited to electrostatic terms. The presence of the solute within the solvent cavity leads to the polarization of the surrounding medium, resulting in the generation of an electric field known as the reaction field. This reaction field interacts with the charges of the solute, providing additional stabilization. To represent the effect of the reaction field, one can use a distributed arrangement of induced polarization charges on the surface of the dielectric [41].

2.11. VIRTUAL SCREENING

Over the past few decades, the experimental screening of large chemical libraries against a biological target, known as high-throughput screening (HTS), has been vital in discovering new lead compounds during the early stages of drug development. However, HTS is expensive, requiring costly equipment and facilities, and its effectiveness depends on the size of the compound library. Due to the high cost and low hit rate associated with HTS, *in silico* virtual screening (VS) has emerged as a promising alternative. Virtual screening has become a crucial step in early-stage drug discovery because it is drug target-relevant, cost-effective, and efficient, providing unique advantages over experimental HTS [42].

Virtual screening, also known as *in silico* screening, involves using computational methods to analyze extensive chemical databases to discover potential new drug candidates. This technique utilizes computer tools to evaluate large collections of compounds automatically. The virtual screening process involves identifying specific substructures, matching calculated molecular properties, and fitting putative ligands into the target receptor site. This method enables the design and optimization of different libraries using available compounds. As a result of its cost-effectiveness and increased accuracy, *in silico* screening has become an essential component of the drug discovery process [43]. Among the various *in silico* techniques used in drug design, structure-based virtual screening (SBVS) is particularly promising and effective. SBVS predicts the best mode of interaction between two molecules to form a stable complex, using scoring functions to evaluate the strength of non-covalent interactions between a ligand and the molecular target. The accuracy of SBVS software heavily depends on the scoring functions used. Various software programs are available for SBVS, and they use different algorithms, which can result in different outcomes from the same input. Recently, consensus virtual screening (CVS) has been used in some studies to improve the accuracy of SBVS and to reduce the number of false positives in the experiments [43].

Virtual screening methods are classified into two types: structure-based virtual screening (SBVS) and ligand-based virtual screening (LBVS). SBVS involves processing 3D structural information of the target protein of interest, either experimentally or through homology modeling, and docking small molecules to the target binding sites. The docked compounds are ranked based on predicted binding affinity and complementarity to the binding site, among other factors. Only a few top-ranked compounds are selected as candidates for further experimental assays [44–46].

Ligand-based virtual screening (LBVS) is a widely used strategy in drug discovery and lead optimization, particularly when the three-dimensional structures of potential drug targets are not accessible. This approach involves mining biological data to identify known active or inactive compounds, which are then utilized to retrieve other potentially active molecular scaffolds for experimental assessment. Various methods are employed in LBVS, such as similarity and substructure searching, quantitative structure-activity relationships (QSAR), pharmacophore mapping, and machine learning techniques [44–46].

The screening procedure comprises several steps, including target investigation, model construction, preparation of a library containing small molecule compounds, molecular docking/pharmacophore mapping, scoring/ranking, and the selection of compounds [44–46].

Pharmacophore modeling is a method that utilizes the relationship between the structure and activity of ligands to identify the three-dimensional characteristics that are necessary for a molecule to exhibit a specific biological activity. There are various techniques to determine pharmacophores, including ones that use the bioactive conformations of a group of molecules based on suggested corresponding points within them, those that consider both the matching points and relevant conformations, and ones that also take into account the potency of the molecules during the analysis[47].



3. METHODOLOGY

3.1. COMPUTATIONAL APPROACH

Cinchona alkaloids are multifunctional organocatalysts, and they have high conformational flexibility. Therefore, whether or not these functional groups can provide high catalytic activity and selectivity are not clearly identified. There still needs to be an approach to predict the catalytic placement of the functional groups in commonly used multifunctional organocatalysts. Therefore, a novel computational approach called CIPOC – Computational Identification of Potential (Organo)Catalysts (Figure 1.2) was developed to explore the catalytic performance of catalysts of interest and to propose a catalyst to synthesize in this project.

3.1.1. Generating Theozyme

Cinchona alkaloids have a tertiary amine functional group acting as a base involved in their structures. Therefore, all models include a trimethylamine functional group to deprotonate the C3 position of oxindole. The models (Figure 3.1) also include other functional groups of organocatalysts used in literature, such as alcohol, amine, urea, and thiourea as hydrogen bond donors. These models were optimized with quantum mechanical calculations using DFT. All DFT calculations were performed with Gaussian 16 [48]. Geometry optimizations were carried out at the B3LYP-D3/6-31G(d) level of theory [49–52]. This method is generally preferred for a large number of atoms as standard and it has also been confirmed that the quantum mechanical active site computed at this level successfully represented the arrangement of catalytic groups in the enzyme active site [53, 54]. Frequency calculations were carried out to ensure all stationary points as minima or saddle points and to calculate thermal corrections at 298.15 K. The models for the TSs were diversified around the forming C-X (X=O or X=N) bond. Also, single-point calculations were performed at the B3LYP-D3/6-311+G(d,p) level of theory [49–52]. This method is well supported in literature for organic reaction mechanisms [55]. Intrinsic reaction coordinate (IRC) calculations [56, 57] were performed to verify the reactants and products. Solvent effects were estimated through a SMD (solvation model based on density) model [58–60] using THF as the solvent to

represent the experimental conditions [31, 61]. To minimize inaccuracies in entropy estimation caused by treating low frequency vibrational modes as simple harmonic oscillations, Truhlar's quasi-harmonic correction was implemented. This correction involved adjusting all frequencies below 100 cm^{-1} taken as equal to 100 cm^{-1} . All energetics reported through the text are given in terms of quasi-harmonic energies in kcal/mol.

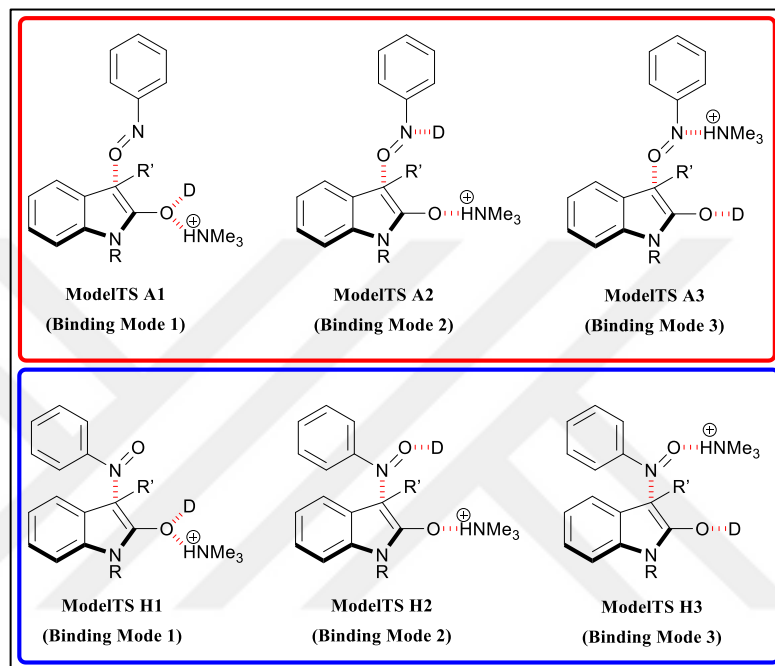


Figure 3.1. Schematic representation of the theozyme (D= alcohol/amine/urea/thiourea)

3.1.2. Generating Conformer Library

Conformational search was carried out for each explored catalyst to obtain an active conformer with a stochastic search method in the Molecular Operating Environment (MOE) software[62]. The MMFF94x force field was utilized during this process. The search was conducted with a threshold value of RMS Gradient 0.005. During the search, conformers with an RMSD value less than 0.25 were rejected as being equal. RMSD (root-mean-square deviation) measures the difference between two molecular structures. The rejection and iteration limit used was 1000, which implies that the search was allowed to run up to a maximum of 1000 iterations before terminating. The default parameters were used for all other aspects of the search.

The conformational library (Figure 3.2) consists of 1565 cinchona, and 21 β -isocupredine derivatives.

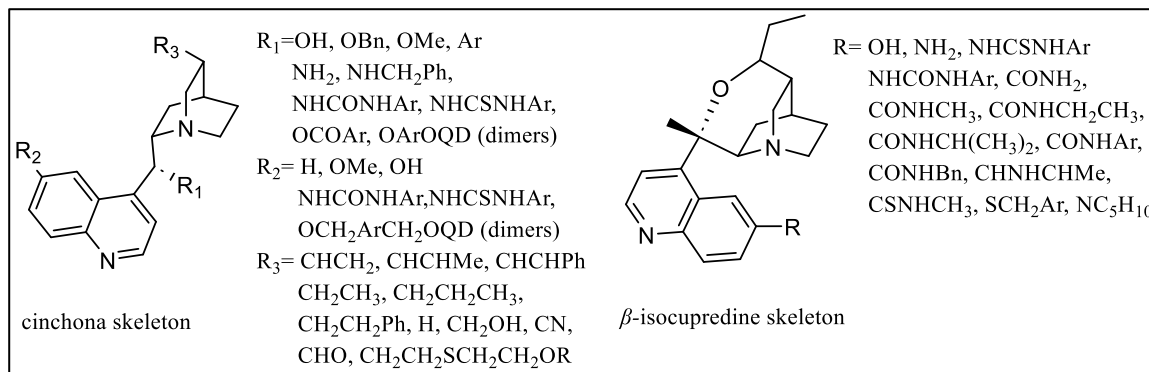


Figure 3.2. Catalyst library

3.1.3. Matching the Catalysts

Pharmacophores were constructed with the functional groups in the theozymes, and screened against the catalyst library. The hit catalysts were given with matching scores (RMSD) and sorted. The best five matches were selected, and by replacing the functional groups in the theozyme with the selected conformer of the catalyst, quantum mechanical calculations were performed, and activation energies were calculated.

Boltzman distribution of TSs were used to calculate enantiomeric excess with the following equation (3.1)[63].

$$ee \% = \frac{1 - e^{-\frac{\Delta G^\ddagger_{R/S}}{RT}}}{1 + e^{-\frac{\Delta G^\ddagger_{R/S}}{RT}}} \times 100 \quad (3.1)$$

3.1.4. Determination of Activity and Selectivity with QM

On the quantum mechanically optimized theozyme, replacing the model catalytic groups with the matching conformer of the catalyst gives the transition structure in the presence of the chiral bifunctional catalyst. The rate and selectivity of the leads were evaluated using

quantum mechanical calculations using DFT. All DFT calculations were performed as explained in the theozyme section (Section 3.1.1).

3.2. EXPERIMENTAL METHODOLOGY

At the beginning of the project, the reaction of oxindole with nitrosobenzene was studied and then, different catalysts were synthesized.

3.2.1. Reaction of Oxindole with Nitrosobenzene

Nitrosobenzene (0.2 mmol) was added to the mixture of 3-methyl-2-oxindole (0.6-0.2 mmol) and catalyst (0.04mmol) in 4mL of solvent [32]. The reaction was stirred at room temperature for different time intervals. Oxindole and catalyst were stirred one hour before starting the reaction. To finish the reaction, mixture was first diluted with 20 mL ethyl acetate and then quenched with 30 mL saturated aqueous ammonium chloride solution. The organic layer was separated and extracted three times with saturated aqueous ammonium chloride solution. The combined organic layers were dried over anhydrous magnesium sulfate and then filtrated. Using rotary evaporator, solvents were evaporated (40°C, 50 mbar) and the crude product was remained in the flask [61].

3.2.2. Catalyst Synthesis

Cinchonidine (3.1 mmol) and triphenylphosphine (3.7 mmol) was stirred in dry THF (10 mL) at 0 °C. Diisopropyl azodicarboxylate (DIAD, 3.7 mmol) was added to the solution with micropipette. Diphenylphosphoryl azide (DPPA, 3.7 mmol) in dry THF (5 mL) was added stirred at 0 °C around 5 minutes and then slowly added to the solution. After five minutes, the mixture was warmed to room temperature and stirred overnight. The solution was heated at 50 °C for two hours. Then, triphenylphosphine (1 g) was added and waited until gas evolution ceased (approx. three hours). The solution was cooled to room temperature and water (1 mL) was added. The solution was stirred for further four hours. The solvents were removed with rotary evaporator and the residue was mixed with DCM and 2.0 M HCl (1:1, 20 mL). The aqueous phase was extracted with DCM (3 x 10 mL). A saturated solution of

Na_2CO_3 was added to aqueous phase to make alkaline (pH=8-9) and extracted with DCM. The organic phases were collected and concentrated. The free amine was obtained as yellow residue without further purification [64].

Free amine (9-amino(9-deoxy) epi-cinchonidine, 1.36 mmol) in 4 mL dry THF and iso(thio)cyanate (1.50 mmol, Table 3.1) was stirred in 10 mL dry THF for about 10 minutes at 0 °C, separately. Then, free amine was added to cyanate slowly with micropipette. The solution was warmed to room temperature and stirred 24 hours. Schematic representation was given in Figure 3.3 [65].

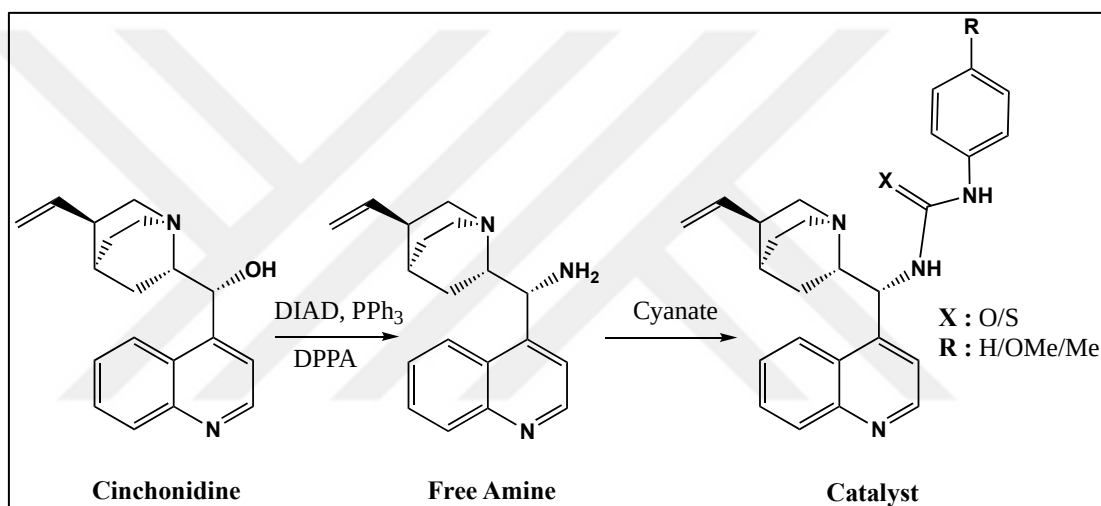


Figure 3.3. Schematic representation of the catalyst synthesis

Table 3.1. The amount of cyanate used in the synthesis of the selected catalysts

Catalyst Name*	Amount of Free amine (g)	Amount of cyanate (μL)
CAT20	1.008	450
CAT36	0.4	194
CAT38	0.4	163
CAT38S	0.4	203

*The structures given in Appendix, Table B.2.

3.2.3. Characterization Methods

3.2.3.1. HPLC Methods

Two different reverse phase HPLC methods were applied. The mobile phase and flow rate were changed in these two methods. 254 nm was chosen as the wavelength for the both methods.

Reverse Phase Method 1. 1.5 mg sample was dissolved in 10 mL methanol.

Flow rate: 0.5 mL/min

Mobile phase: Methanol:Deionized Water (isocratic-50:50)

Column: Inertsustain C18

Reverse Phase Method 2. 3 mg sample was dissolved in acetonitrile (500 μ L) and deionized water (400 μ L).

Flow rate: 1 mL/min

Mobile phase: Acetonitrile:Deionized Water (gradient, given in Table 3.2)

Column: Inertsustain C18

Table 3.2. The gradient elution condition

Time (min)	% Deionized Water	% Acetonitrile
0	60	40
2	20	80
15	60	40

Normal Phase Method. Sample was dissolved in ethanol.

Flow rate: 0.6 mL/min

Mobile phase: Hexane:Ethanol (90:10)

Column: Kramosil (chiral colum)

3.2.3.2. NMR, NOESY, HETCOR Method

NMR frequency is 400 MHz. Solvent was selected as CD₃OD for the catalyst synthesis, DMSO for the product determination.

3.2.3.3. LC-MS Method

Device: LCMS-8060 TripleQuadrupole

Positive Ionization (ES⁺)

Mobile Phase A: Deionized Water (%0.1FA +5mM Ammonium formate)

Mobile Phase B: Acetonitrile

Mass and product analysis was made between 50-1000 Da.

3.2.3.4. HR-MS Method

Device: ESI-TOF-MS

Positive Ionization (ES⁺)

Mass analysis of the product analysis was made between 50-1000 Da.

3.2.3.5. FT-IR Method

KBr pellet

4. RESULTS AND DISCUSSION

Experimental studies on the reaction of oxindole and nitrosobenzene showed a strong dependence of product distribution on the nature of the catalyst. While quinidine dimers gave aminoxylation products [61], thiourea derivatives yielded hydroxyamination products [32]. However, how catalytic functional groups present in the catalyst structures determine the regio- and enantioselectivity of the reaction is unclear. All of the alkaloids contain tertiary amine, along with groups such as alcohol, amine, urea, and thiourea, but their specific contributions to catalysis are not yet understood. Here, we investigate the energy profile of the reaction between oxindole and nitrosobenzene using different theozymes with the aim of determining the optimal catalytic functional groups, their three-dimensional arrangements for maximum TS stabilization, and their contributions to catalysis. These calculations provide valuable insights into the binding preferences of different functional groups to substrates, their effects on the activation energy and determine the mechanism of this reaction. The lowest energy theozymes were then used to generate the CAMs which were further screened in the catalyst library (Figure 3.2) to uncover novel catalyst candidates. Promising candidates were determined based on computed activation energies in the presence of full catalyst.

4.1. BINDING PREFERENCES OF SUBSTRATES

Three different mechanisms were investigated in this section. The hydrogen at the C3 position of oxindole is relatively acidic being alpha to the carbonyl group. In the first step of the reaction, the basic amine catalyst (trimethylamine) pulls off this proton forming an enolate intermediate, as shown in Figure 4.1. Therefore, oxindole becomes negatively charged, and the trimethylamine catalyst becomes positively charged. The protonated positively charged catalyst could then form an ion pair with the negatively charged enolate, as shown in Figure 4.1.

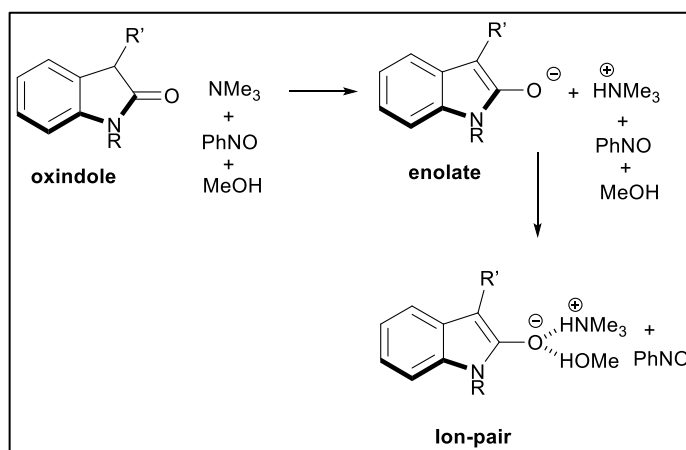


Figure 4.1. Enolization step and ion pair formation

4.1.1. Binding Mode 1

After the enolate and ion-pair formation, protonated catalyst and the alcohol functional group interact with the oxyanion, as shown in Figure 4.2. The enolate attacks the nitrogen of nitrosobenzene. The alcohol group interacts with the oxyanion. This step is the addition of the oxindole from the C3 position of nitrosobenzene.

When the double bond is broken, oxygen attached to nitrogen becomes negatively charged. The protonated catalyst rearranges to interact with this negatively charged oxygen, and the proton is transferred from the positively charged catalyst to the oxygen of the intermediate to form *N*- adduct (Figure 4.2). The formation of *O*-adduct occurs in a similar manner when oxygen of nitrosobenzene is added to the C3 position of oxindole.

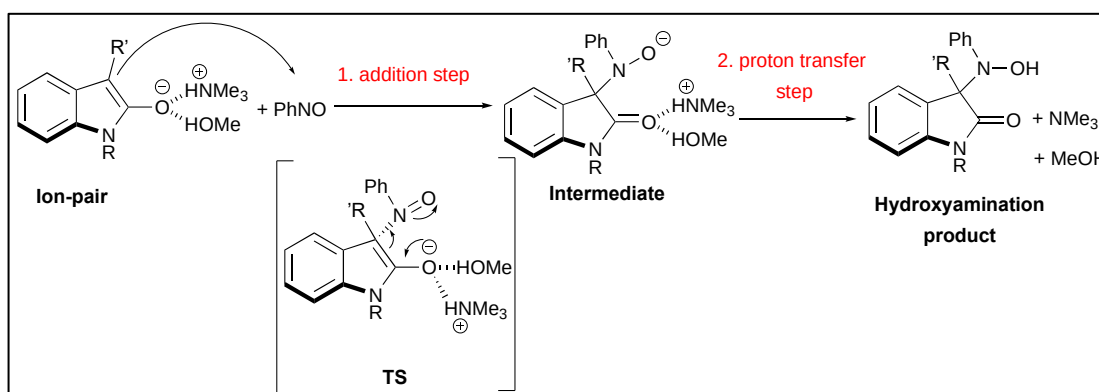


Figure 4.2. Mechanism of binding mode 1

4.1.2. Binding Mode 2

In this mode, after the formation of the enolate intermediate, the protonated catalyst interacts with the oxyanion (Figure 4.3), and the alcohol functional group stabilizes nitrosobenzene for addition. Then, the addition of the enolate to activated nitrosobenzene can take place.

After intermediate formation, the rearrangement of the catalytic protonated amine to form an ion-pair with the oxyanion leads to the proton transfer from the protonated catalyst to the oxygen of nitrosobenzene and *N*-adduct forms (Figure 4.3), and *O*-adduct forms similarly with the addition of oxygen of nitrosobenzene to oxindole from its C3 position.

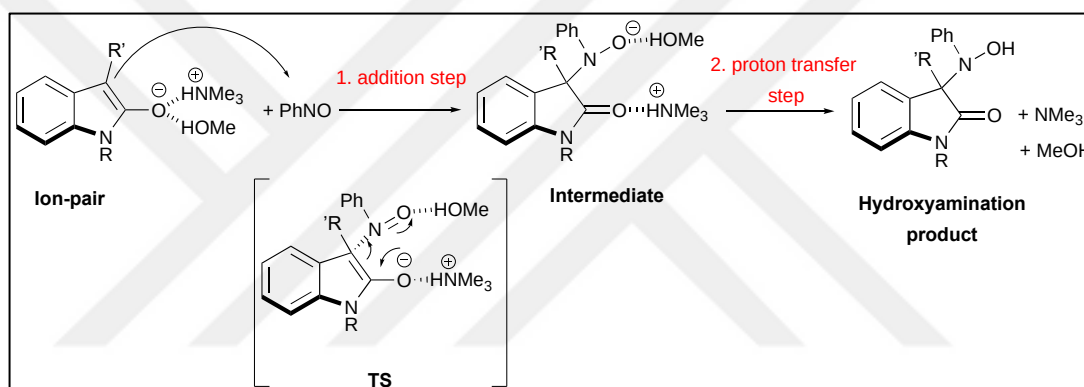


Figure 4.3. Mechanism of binding mode 2

4.1.3. Binding Mode 3

In this binding mode, the protonated catalyst interacts with nitrosobenzene, and the alcohol group stabilizes the oxyanion. The formation of the C-N bond occurs simultaneously with the proton transfer from the catalyst in a single TS. So, *N*-adduct forms concertedly from the enolate intermediate, as shown in Figure 4.4. When the oxygen of nitrosobenzene is added to C3 position of oxindole, *O*- adduct forms with a similar mechanism.

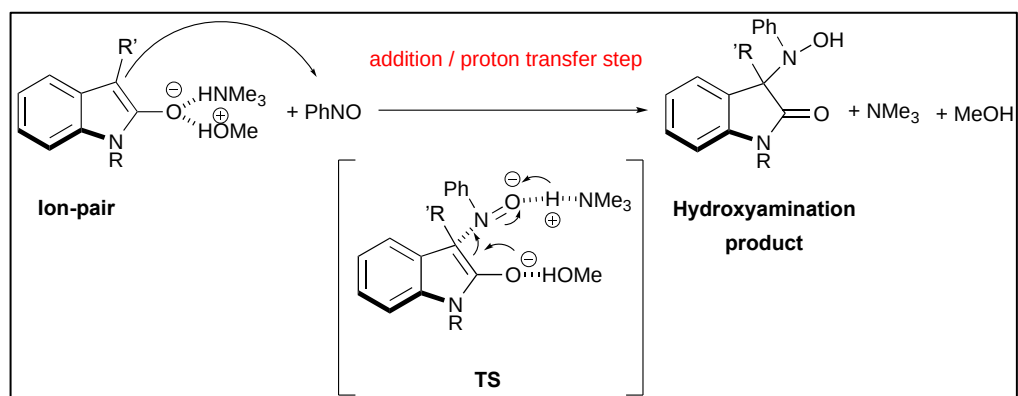


Figure 4.4. Mechanism of binding mode 3

4.1.4. Overall Mechanism

The located TS structures for the addition step leading to *N*- adduct (named as **ModelTS H**, hydroxyamination) and *O*-adduct (named as **ModelTS A**, aminoxylation) for all three mechanisms are given in Figure 4.5. Gibbs free energies are given with respect to the lowest energy TS (**ModelTS H3** $\Delta G^\ddagger = 26.9$ kcal/mol). In binding mode 1, **ModelTS H1** could not be located. All our attempts to locate **ModelTS H1** lead to **ModelTS H3**. When **ModelTS A1** is compared with the other TS, in this mechanism the activation energy is the highest. When all the binding modes compared, **ModelTS H** always has a lower activation barrier than **ModelTS A**. These results suggest that the concerted addition and proton transfer pathway is preferred over the others by at least 7.5 kcal/mol, and the reaction has a stronger tendency towards the hydroxyamination product.

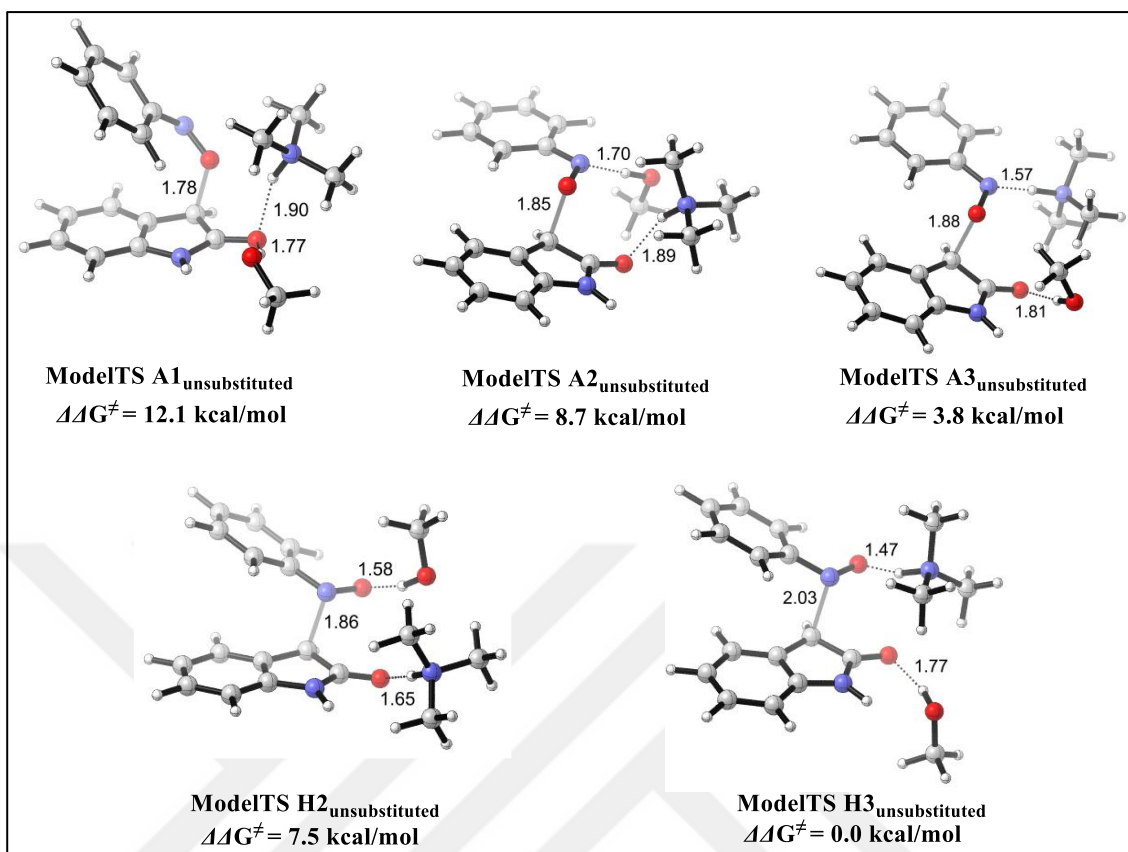


Figure 4.5. Unsubstituted TS and their relative Gibbs free energies for three binding modes

4.2. SUBSTITUENT EFFECTS

N-protected and *N*-unprotected oxindoles gave different products[31–33, 61]. To elucidate this, the effect of different substituents were examined in light of the mechanistic information found in the previous section. Parent theozyme ($R=H$, $R'=H$) is taken as the reference and energies of all the other models are given with respect to the parent theozyme. Methyl group ($R'=Me$) at the C3 position of oxindole as stereogenic center decreases the activation barrier by 5.1 kcal/mol for the hydroxyamination model (**ModelTS H3**). Methyl and benzyl groups ($R=Me/Bn$) were investigated as the protecting groups on nitrogen. Methyl ($R=Me$, $R'=Me$) increases the barrier by 0.9 kcal/mol when compared to unprotected C3-methyl substituted derivative ($R=H$, $R'=Me$), whereas the presence of benzyl protecting group lowers the activation energy by 1.5 kcal/mol for **ModelTS H3**. The effect of these groups have the same trend for the aminoxylation models (**ModelTS A3**). Since the lowest energy is $R=Bn$, $R'=Me$ as shown in Table 4.1 (**ModelTS H3**; $R=H$, $R'=H$; $\Delta G^\ddagger = 26.9$

kcal/mol), the rest of the calculations were performed with these derivatives.

Table 4.1. The substituent effects relative to the parent oxindole (R=H, R'=H)

Name	$\Delta\Delta G^\ddagger$ (kcal/mol)					
	*R=H, R'=H	R=H, R'=Me	R=Me, R'=H	R=Me, R'=Me	R=Bn, R'=H	R=Bn, R'=Me
TS A3	3.8	0.7	3.6	2.9	4.2	0.5
TS H3	0.0	-5.1	-1.8	-4.0	-0.1	-6.6

*The parent theozyme is shown in Figure 1.1.

4.3. EFFECT OF DIFFERENT CATALYTIC GROUPS

Different derivatives of cinchona alkaloids is catalyzed the reaction of oxindole with nitrosobenzene [31–33, 61] These alkaloids contain various functional groups, yet the manner in which these groups interact with substrates remains unknown. We studied theozymes with different catalytic groups (amine, alcohol, urea, thiourea) to understand their contribution to catalysis (Figure 4.6 and Figure 4.7). All models have tertiary amine as a basic group while the H-bond donors interacting with oxindole varies. For aminoxylation models (Figure 4.6), lowest activation barrier is obtained in the presence of urea and thiourea groups ($\Delta G^\ddagger=24.8$ and 25.0 kcal/mol, respectively). Having alcohol (**ModelTS A3(alcohol)**) or amine (**ModelTS A3(amine)**) groups to stabilize the oxindole, increases the barrier by 2.5 and 4.1 kcal/mol respectively with respect to **ModelTS A3(urea)**. Urea and thiourea groups have similar effects and have lowest activation barriers. This decrease in barrier by urea/thiourea can be explained by the bidentate hydrogen bonding and the acidic protons, which can stabilize the TS better than the other groups. The trend is similar for the hydroxyamination models (Figure 4.7).

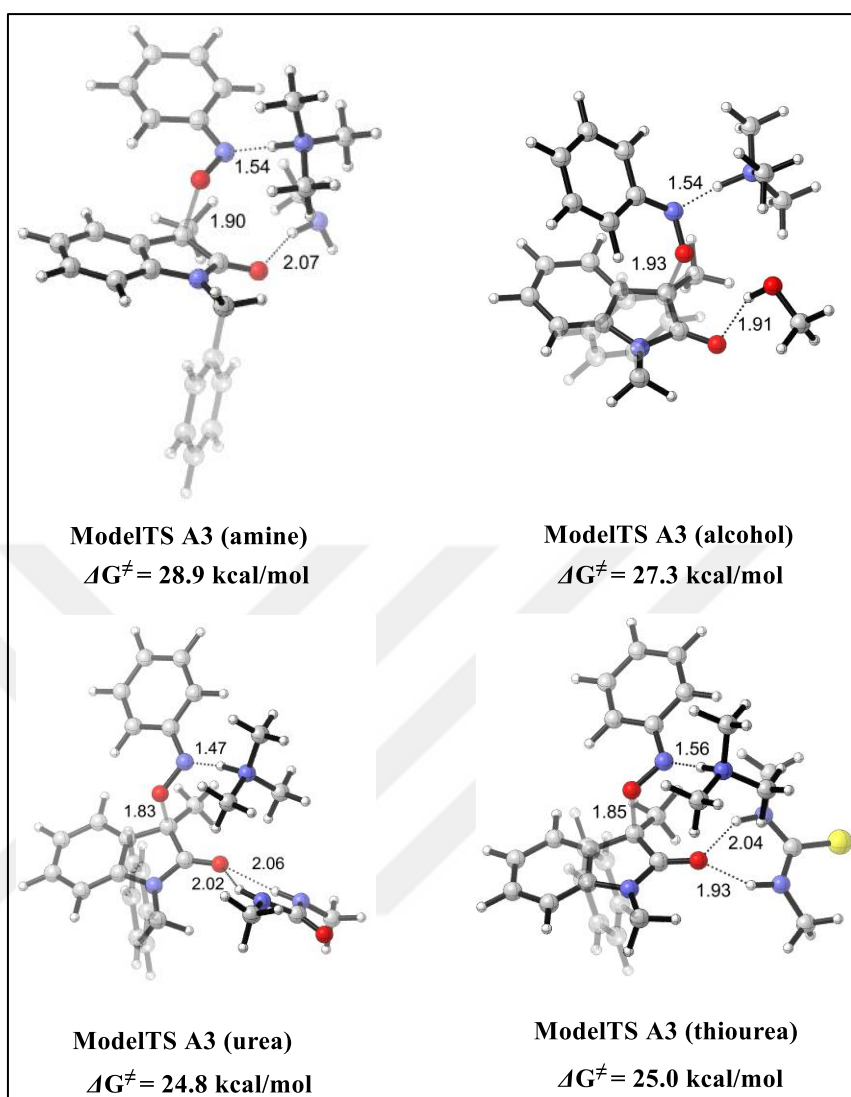


Figure 4.6. Lowest energy aminoxylation theozymes

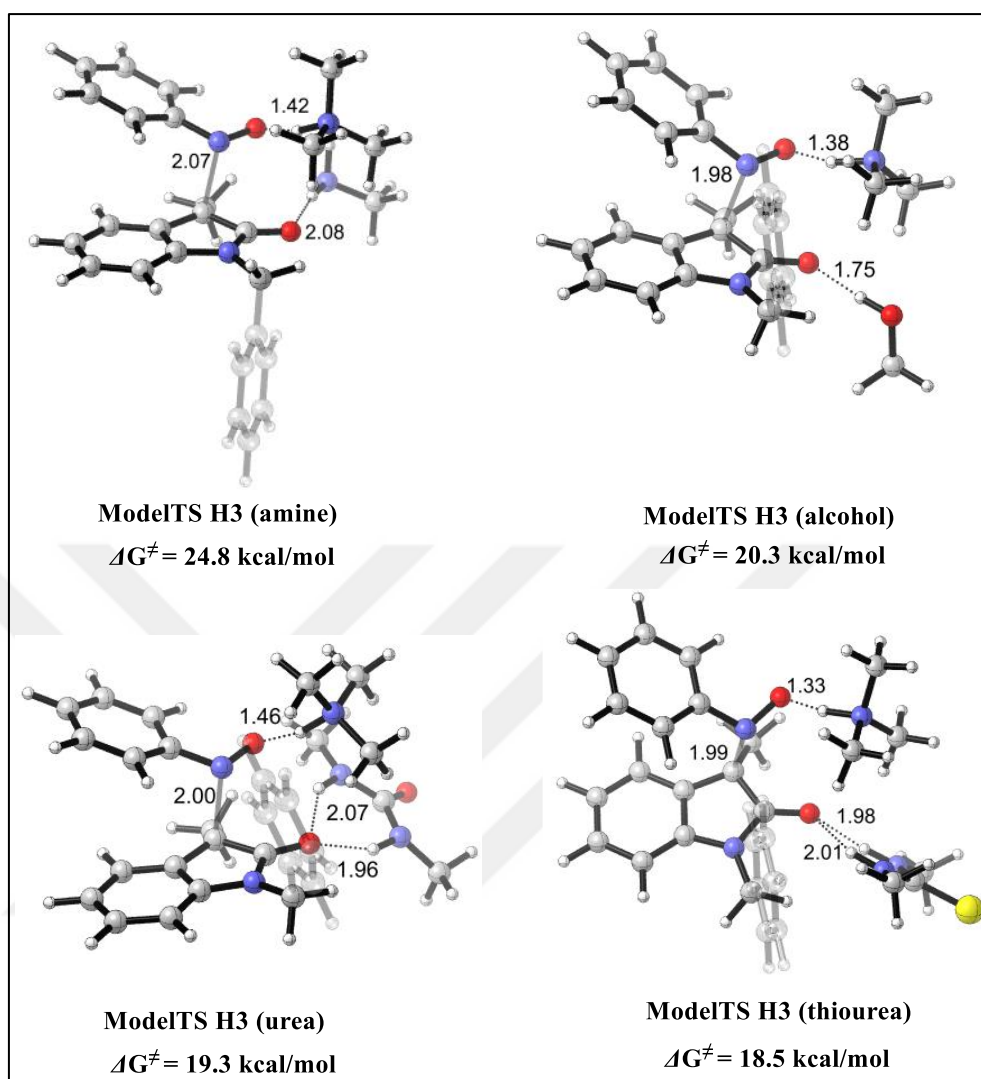


Figure 4.7. Lowest energy hydroxyamination theozymes

4.4. CATALYTIC ATOM MAPS (CAM) AND THE MATCHING RESULTS

Three types of catalytic atom maps (CAMs) were created using different features of the pharmacophore groups included in the lowest-energy theozyme (Table 4.2). These variants are shown in Table 4.3 on the lowest energy theozyme, **ModelTS H3(thiourea)**, (Figure 4.7) containing thiourea.

All the selected theozymes were screened in the catalyst library (Figure 3.2) using the pharmacophore tool in MOE. Number of conformations for some selected catalysts in the library are given in Appendix (Table B.1)

Table 4.2. The selected pharmacophore groups

	Protonated Amine Group	D*
CAM-1 (9 pharmacophore groups)	Acc : Nitrogen	Don : NH/OH
	AtomQ : Carbon	Don : NH/OH
	AtomQ : Carbon	Don2 : Projection vector
	AtomQ : Carbon	Don2 : Projection vector
	Don2 : Projection vector	
CAM-2 (7 pharmacophore groups)	Acc : Nitrogen	Don : NH/OH
	AtomQ : Carbon	Don : NH/OH
	AtomQ : Carbon	
	AtomQ : Carbon	
	Don2 : Projection vector	
CAM-3 (8 pharmacophore groups)	Acc : Nitrogen	Don : NH/OH
	AtomQ : Carbon	Don : NH/OH
	AtomQ : Carbon	Don2 : Projection vector
	AtomQ : Carbon	Don2 : Projection vector

*D = amine/alcohol/urea/thiourea

Three different CAMs were prepared. These maps are subset of each other. The CAM-1 includes the nitrogen of the basic tertiary amine (Acc), three atoms (AtomQ) bonded to this nitrogen (to identify the tertiary amine), projection of the nitrogen (Don2), two nitrogens on the thiourea as donor (Don) and their projection vectors (Don2). The projection of the nitrogens of thiourea are not included in CAM-2. The projection of the nitrogen of the basic tertiary amine is not present in CAM-3.

Table 4.3. The representation of the selected pharmacophore groups and CAMs for
ModelTS H3(thiourea)

	ModelTS H3 (thiourea)	Selected Pharmacophore Groups	CAM
CAM -1			
CAM -2			
CAM -3			

The best five matches based on visual inspection and the RMSD values of the matched catalysts for both aminoxylation and hydroxyamination pathways are given in Table 4.4-Table 4.7.

Alcohol functional group matches are listed in Table 4.4. Sample CAMs for **ModelTS A3(alcohol)** and **ModelTS H3(alcohol)** are shown in Figure 4.8 and Figure 4.9.

Table 4.4. The matched catalyst with difunctional alcohol active side model

Hit No. (O-adduct)	Catalyst Number	R ₁ ^a	R ₂ ^a	R ₃ ^a	RMSD
1	CAT1	OH	PhCH ₂ CH ₂ CH ₂ CH ₃	CHCH ₂	0.5057
2	CAT2	OH	Ph	CHCH ₂	0.5071
3	CAT3	OH	OCH ₂ PhCH ₂ OQD	CHCH ₂	0.4775
4	CAT4	OPhOQD	OH	CHCH ₂	0.5445
5	CAT5	NH ₂	OH	CH ₂ OH	0.6835
Hit No. (N-adduct)		R ₁	R ₂	R ₃	RMSD
1	CAT6	OH	OCH ₂ PhCH ₂ OQD	CH ₂ CH ₃	0.6289
2	CAT4	OPhOQD	OH	CHCH ₂	0.7499
3	CAT7	NHCSNHoxylyl	H	CH ₂ OH	0.4904
4	CAT8	NHCSNHPh	OH	CH ₂ OH	0.5010
5	CAT9	NHCONHBn	OCH ₃	CH ₂ OH	0.4095

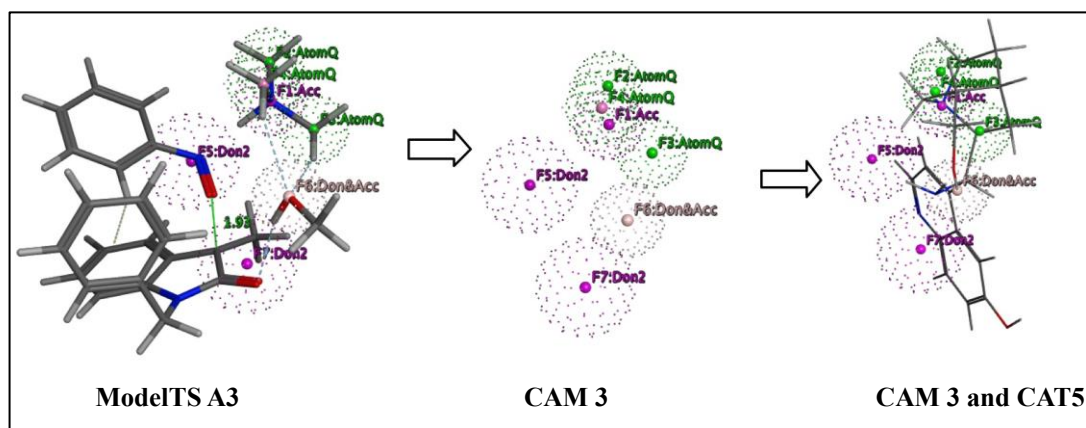
^aRefer to Figure 3.2 for catalyst structures

Figure 4.8. Matching catalytic structure, CAT5, to ModelTS A3 for O-adduct

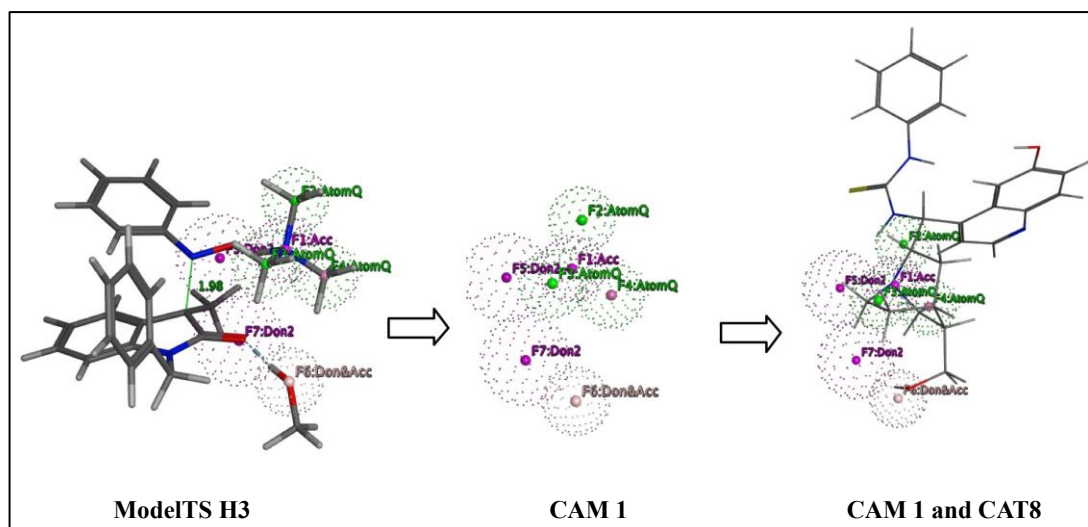


Figure 4.9. Matching catalytic structure, **CAT8**, to **ModelTS H3** for *N*-adduct

Amine functional group matches are shown in Table 4.5. The theozyme which includes amine functional group, and its sample CAM and matched catalysts are given in Figure 4.10 and Figure 4.11.

Table 4.5. The matched catalysts with di-functional amine active site model

Hit No. (<i>O</i> -adduct)	Catalyst Number	R ₁ ^a	R ₂	R ₃	RMSD
1	CAT10	NHCH ₂ Ph	OCH ₃	CH ₂ CH ₃	0.7876
2	CAT11	NH ₂	OCH ₃	CHCH ₂	0.7876
3	CAT12	NH ₂	OH	CHCH ₂	0.7877
4	CAT13	NH ₂	OCH ₃	CHCHPh	0.8699
5	CAT14	NHCH ₂ Ph	NHCONHBn	CH ₂ CH ₂ Ph	0.7105
Hit No. (<i>N</i> -adduct)	Catalyst Number	R ₁	R ₂	R ₃	RMSD
1	CAT15	NH ₂	OCH ₃	CH ₂ CH ₂ Ph	0.6993
2	CAT16	NH ₂	OCH ₃	CHCHPh	0.6993
3	CAT17	NH ₂	OH	CHCH ₂	0.7016
4	CAT18	NH ₂	OH	CH ₂ CH ₃	0.7018
5	CAT19	NH ₂	OCH ₃	CHCH ₂	0.7007

^aRefer to Figure 3.2 for catalyst structures

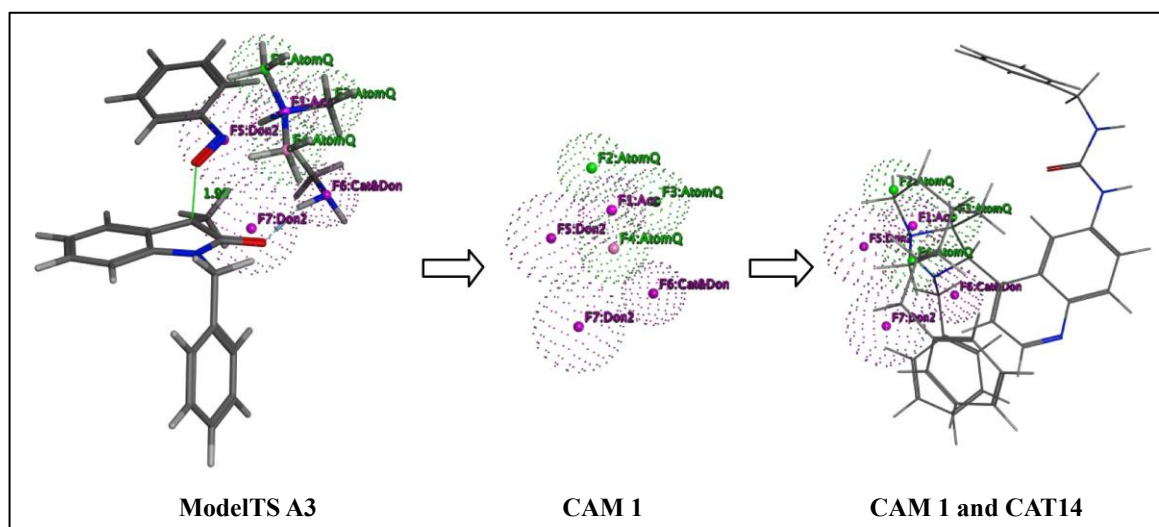


Figure 4.10. Matching catalytic structure, **CAT14**, to **ModelTS A3** for O-adduct

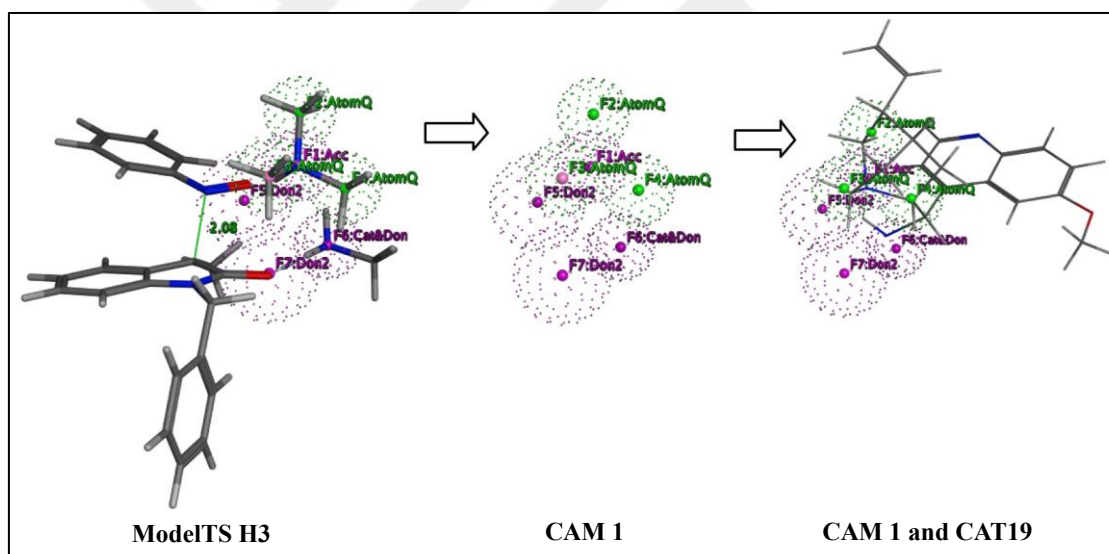
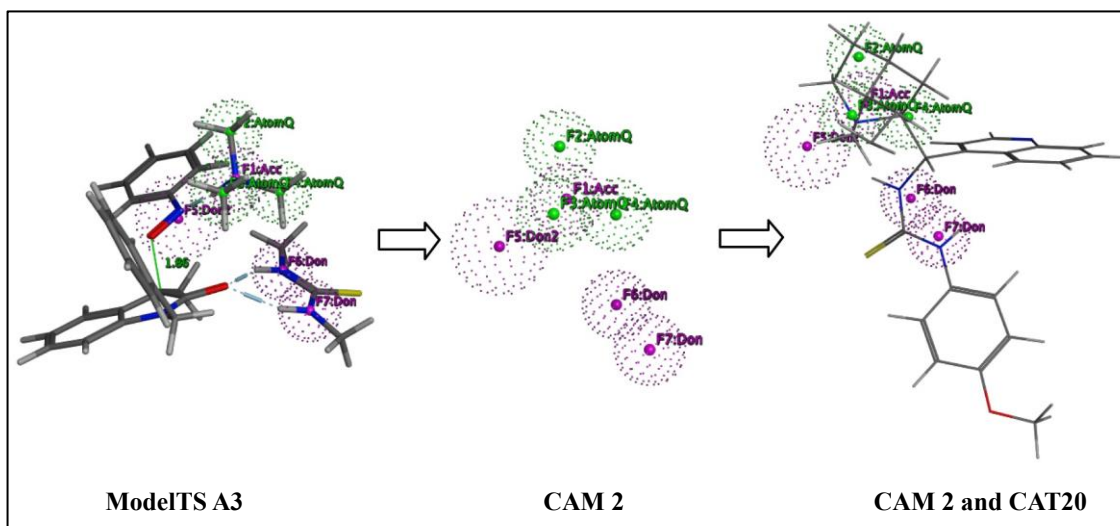


Figure 4.11. Matching catalytic structure, **CAT19**, to **ModelTS H3** for N-adduct

Thiourea functional group matches are listed in Table 4.6. Sample CAMs and matched catalysts are illustrated for the thiourea-based theozymes in Figure 4.12 and Figure 4.13.

Table 4.6. The matched catalyst with difunctional thiourea active side model

Hit No. (<i>O</i> - adduct)	Catalyst Number	R ₁ ^a	R ₂	R ₃	RMSD
1	CAT20	NHCSNHPhOCH ₃	H	CHCH ₂	0.8145
2	CAT21	NHCSNHPhOCH ₃	OCH ₃	CHCH ₂	0.8348
3	CAT22	NHCSNH3,5CF ₃	H	CHCH ₂	0.7658
4	CAT23	NHCSNH3,5CF ₃	OCH ₃	CHCH ₂	0.8123
5	CAT24	NHCSNHBn	NHCSNHPh	CHCH ₂	0.6646
Hit No. (<i>N</i> - adduct)	Catalyst Number	R ₁	R ₂	R ₃	RMSD
1	CAT25	NHCSNHBn	NHCSNHoxylyl	CHO	0.8076
2	CAT26	NHCSNHPh	OCH ₃	CH ₂ OH	0.8186
3	CAT27	NHCSNHPh	H	CH ₂ OH	0.8331
4	CAT28	NHCSNHBn	NHCONHBn	CH ₂ OH	0.8422
5	CAT29	NH ₂	NHCSNHoxylyl	CH ₂ OH	0.8890

^aRefer to Figure 3.2 for catalyst structuresFigure 4.12. Matching catalytic structure, CAT20, to ModelTS A3 for *O*-adduct

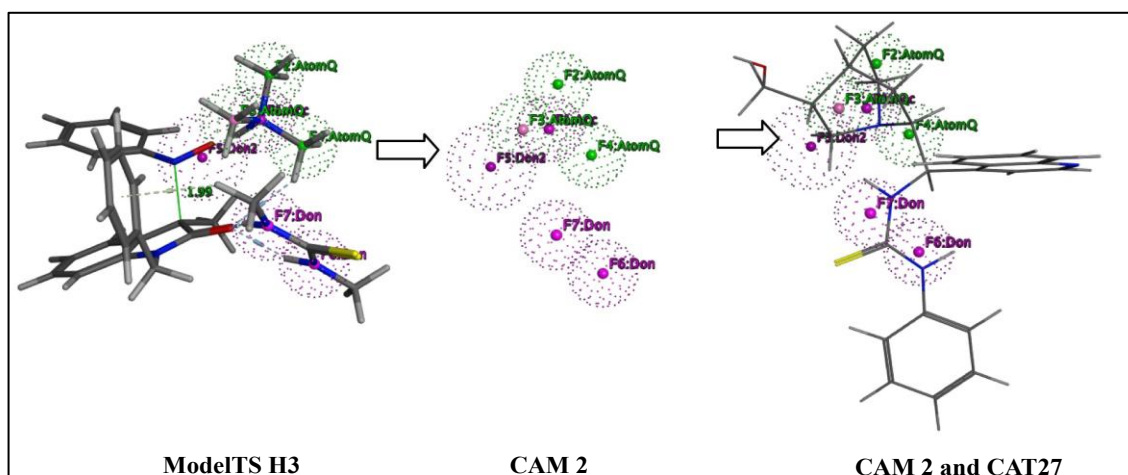


Figure 4.13. Matching catalytic structure, **CAT27**, to **ModelTS H3** for *N*-adduct

Urea functional group matches are summarized in Table 4.7. The theozyme which includes urea functional group, its sample CAM and matched catalysts are depicted in Figure 4.14 and Figure 4.15.

Table 4.7. The matched catalyst with difunctional urea active side model

Hit No. (<i>O</i> -adduct)	Catalyst Number	R ₁ ^a	R ₂	R ₃	RMSD
1	CAT30	NHCONHBn	H	CH ₂ OH	0.7007
2	CAT31	NHCONHBn	H	H	0.7058
3	CAT32	NHCONHBn	OH	CHCH ₂	0.7856
4	CAT33	NHCONHoxyl	NHCONHBn	CH ₂ CH ₃	0.7820
5	CAT34	NHCONHoxyl	NHCSNHPh	CN	0.7703
Hit No. (<i>N</i> -adduct)	Catalyst Number	R ₁	R ₂	R ₃	RMSD
1	CAT35	NHCONHPhCH ₃	OCH ₃	CHCH ₂	0.5302
2	CAT36	NHCONHPhOCH ₃	H	CHCH ₂	0.5488
3	CAT37	NHCONHPhCH ₃	H	CHCH ₂	0.5377
4	CAT38	NHCONHPh	H	CHCH ₂	0.5387
5	CAT39	NHCONHBn	NHCSNHBn	CH ₂ OH	0.4343

^aRefer to Figure 3.2 for catalyst structures

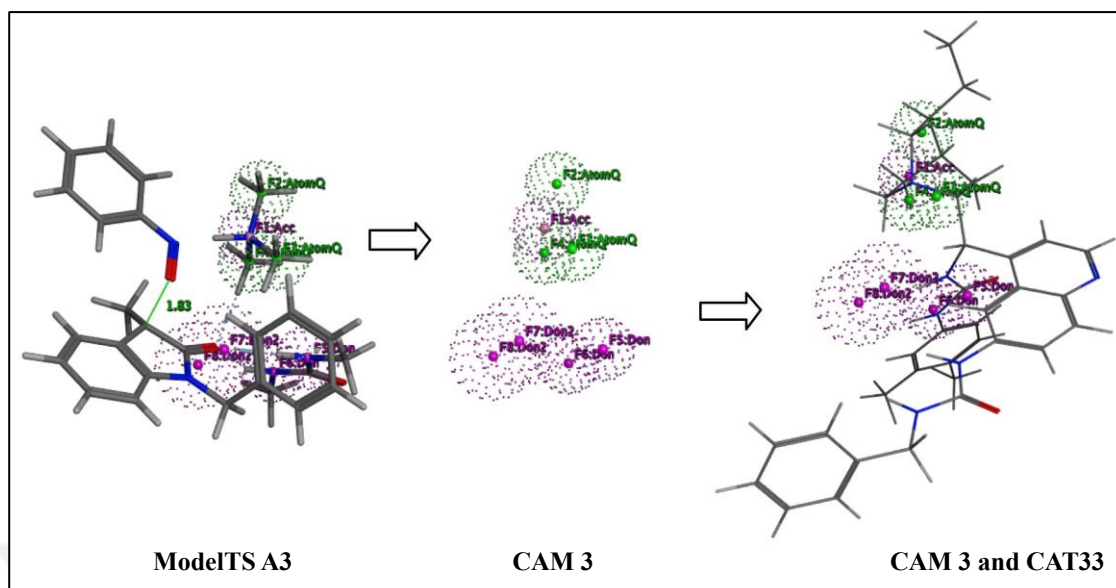


Figure 4.14. Matching catalytic structure, **CAT33**, to **ModelTS A3** for *O*-adduct

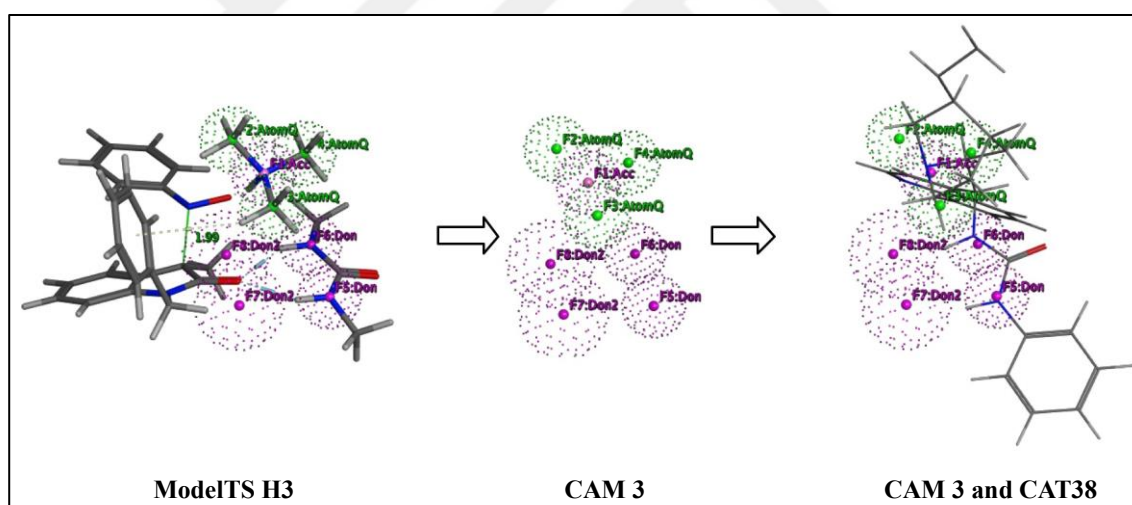


Figure 4.15. Matching catalytic structure, **CAT38**, to **ModelTS H3** for *N*-adduct

4.5. ACTIVITY AND SELECTIVITY OF THE CATALYSTS

QM calculations on the full catalytic systems were performed by replacing the functional groups in the theozyme with the selected conformer of the catalyst. The TS structures of parent cinchona alkaloids (cinchonidine, and quinine) are shown in Figure 4.16, Figure 4.17.

The lowest TS for cinchonidine catalyst is **(R)-TSH3_{CD}** with 11.3 kcal/mol activation barrier (Figure 4.16). The aminoxylation pathways are significantly higher in energy (**(R)-TSA3_{CD}**, $\Delta G^\ddagger=19.2$ kcal/mol, **(S)-TSA3_{CD}**, $\Delta G^\ddagger=15.8$ kcal/mol). Therefore, the hydroxyamination product is favored by 7.9 kcal/mol and in an *R*-selective fashion (51.5% ee). In quinine, the inclusion of methoxy group at the C6' position of the catalyst decreases the activation energy of the lowest energy hydroxyamination TS by 0.6 kcal/mol (Figure 4.17) compared to **(R)-TSH3_{CD}** and the activation energy of the *R*- and *S*-enantiomer are essentially the same, suggesting a racemic mixture. All the cinchona alkaloids (cinchonine, Figure B.6, cinchonidine, quinine, Figure B.7, and quinidine) favor the hydroxyamination product with a very low enantioselectivities which agree well with the experimental findings[61].

All the hit catalysts were studied with QM calculations at B3LYP-D3/6-311+G(d,p)//B3LYP/6-31G(d) level and the energies of the selected are given in Table 4.8.

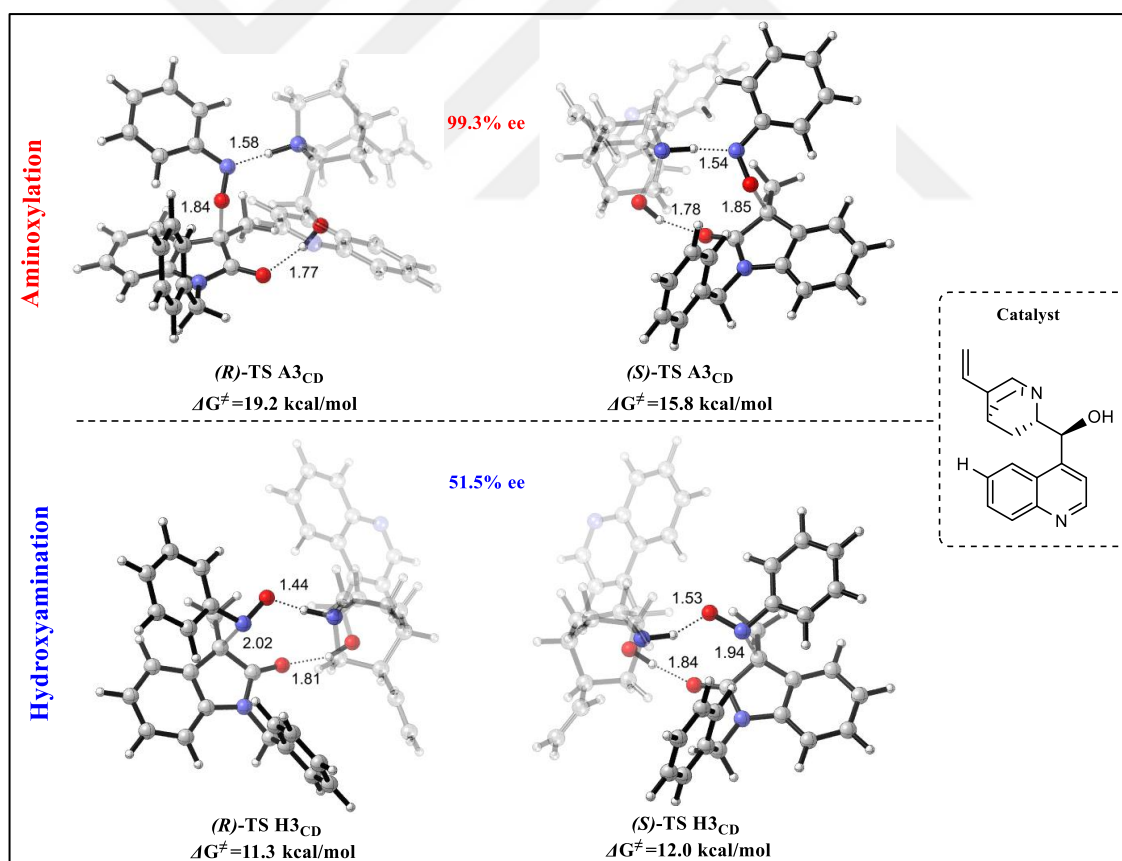


Figure 4.16. Both aminoxylation and hydroxyamination TS and their enantioselectivity with cinchonidine (CD)

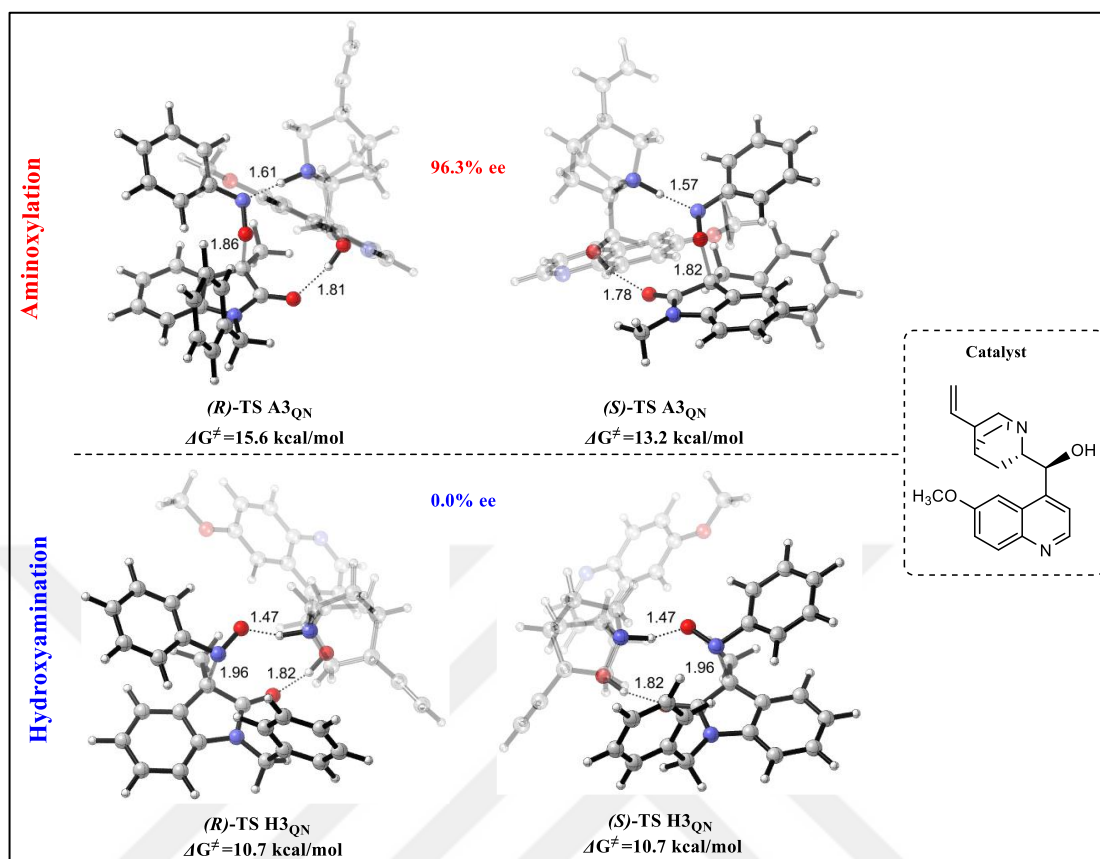


Figure 4.17. Both aminoxylation and hydroxyamination TS and their enantioselectivity with quinone (QN)

Table 4.8. Gibbs free energies of the selected catalysts

Catalyst name		Energies (kcal/mol)			
		(<i>R</i>)-TSA3	(<i>S</i>)-TSA3	(<i>R</i>)-TSH3	(<i>S</i>)-TSH3
<i>tert</i> -amine and alcohol	CAT5	18.5	19.3	17.5	17.7
	CAT8	21.4	21.1	21.9	21.0
	CAT9	24.4	24.0	17.3	17.7
<i>tert</i> -amine and amine	CAT14	22.4	15.0	21.1	14.7
	CAT15	27.9	28.2	23.0	20.7
	CAT18	28.3	28.2	26.0	23.4
<i>tert</i> -amine and thiourea	CAT20*	20.7	17.0	21.8	13.5
	CAT21	19.0	15.7	20.9	12.1
<i>tert</i> -amine and urea	CAT35	18.5	15.2	16.8	11.3
	CAT36*	19.5	16.3	18.8	12.1
	CAT37	19.5	16.0	17.6	12.0
	CAT38*	15.9	15.8	16.9	11.6
	CAT39	18.9	11.5	20.6	7.8

Table 4.8. Gibbs free energies of the selected catalysts (cont'd)

Catalyst name		Energies (kcal/mol)			
		(<i>R</i>)-TSA3	(<i>S</i>)-TSA3	(<i>R</i>)-TSH3	(<i>S</i>)-TSH3
<i>tert</i> -amine and urea	CAT21O	15.3	15.0	18.3	12.5
<i>tert</i> -amine and thiourea	CAT35S	19.5	16.3	21.5	14.2
	CAT37S	20.8	17.2	22.5	13.7
	CAT38S*	18.1	17.8	21.7	13.5

*Selected for experimental testing

CAT5, **CAT8** and **CAT9** are obtained from the matching of the structure containing *tert*-amine and alcohol functionalities.

The enantiomeric transition structures for both aminoxylation and hydroxyamination pathways for **CAT5** are shown in Figure 4.18. **CAT5** has three functional groups contains in its structure (two hydroxyl groups and one amine group at C9 position of cinchona skeleton). The activation of nitrosobenzene by two H-bonds (one from protonated amine, and one from C9-amine) favors (*R*)-TS **H3**_{CAT5} ($\Delta G^\ddagger=17.5$ kcal/mol). Due to the destabilizing effect of double H-bonding to oxindole, (*S*)-TS **H3**_{CAT5} is calculated to be 0.2 kcal/mol higher in energy leading to 15.4% ee. The aminoxylation pathway is found to be higher in energy ((*R*)-TS **A3**_{CAT5}, $\Delta G^\ddagger=18.5$ kcal/mol, (*S*)-TS **A3**_{CAT5}, $\Delta G^\ddagger=19.3$ kcal/mol) than the hydroxyamination pathway.

The located TS structures of other selected catalysts, **CAT8** and **CAT9**, which are found to be higher in energy, are given in Appendix, Figure B.8 and B.9, respectively.

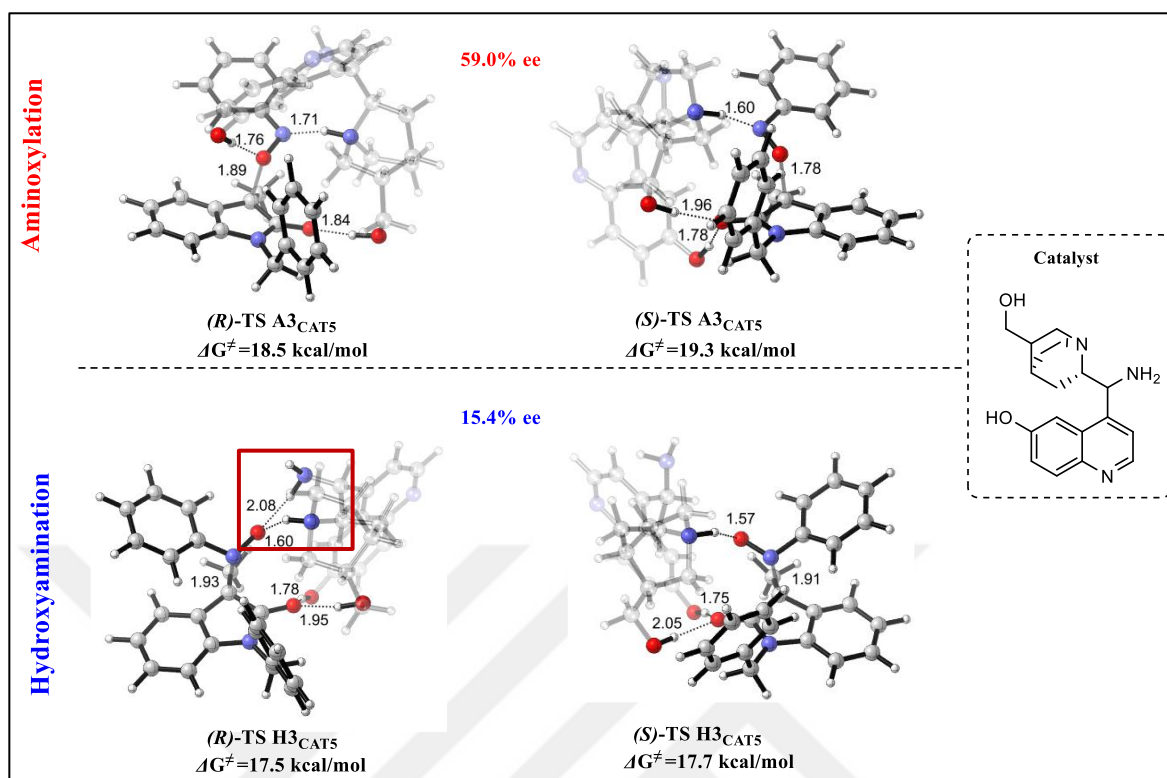


Figure 4.18. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT5**

For all selected catalysts including alcohol functional group favors *N*-adduct (hydroxyamination product). Low enantioselectivities were predicted. In addition, these derivatives have higher activation energies than parent catalysts. Therefore, these catalysts are not selected for synthesis.

CAT14, **CAT15** and **CAT15** are obtained from the matching of the structure containing *tert*-amine and amine functionalities.

CAT14 (Figure 4.19) strongly favors the *S*-enantiomer for both aminoxylation and hydroxyamination products ($\Delta G^\ddagger = 15.0$ kcal/mol, $\Delta G^\ddagger = 14.7$ kcal/mol, respectively). The lowest activation energy is 14.7 for **(S)-TS H3CAT14**. A π - π interaction between the phenyl of the R₃ and the phenyl of the R₁ (in the structure Figure 3.2) is expected to favorably contribute to the stabilization of **(S)-TS H3CAT14**. Even though the enantioselectivities are predicted to be 100 % for both pathways, the regioselectivity is low, **(S)-TS H3CAT14** being only 0.3 kcal/mol more stable than **(S)-TS A3CAT14**.

CAT15 (Figure B.10) and **CAT18** (Figure B.11) have higher activation energies than **CAT14** and in general, theozymes including amine functional group have the highest activation energies. For all selected catalysts including amine functional group favors *N*-adduct (hydroxyamination product). Because of higher barrier for **CAT15** and **CAT18** and lack of synthetic procedure for **CAT14**, these catalysts were not selected for synthesis.

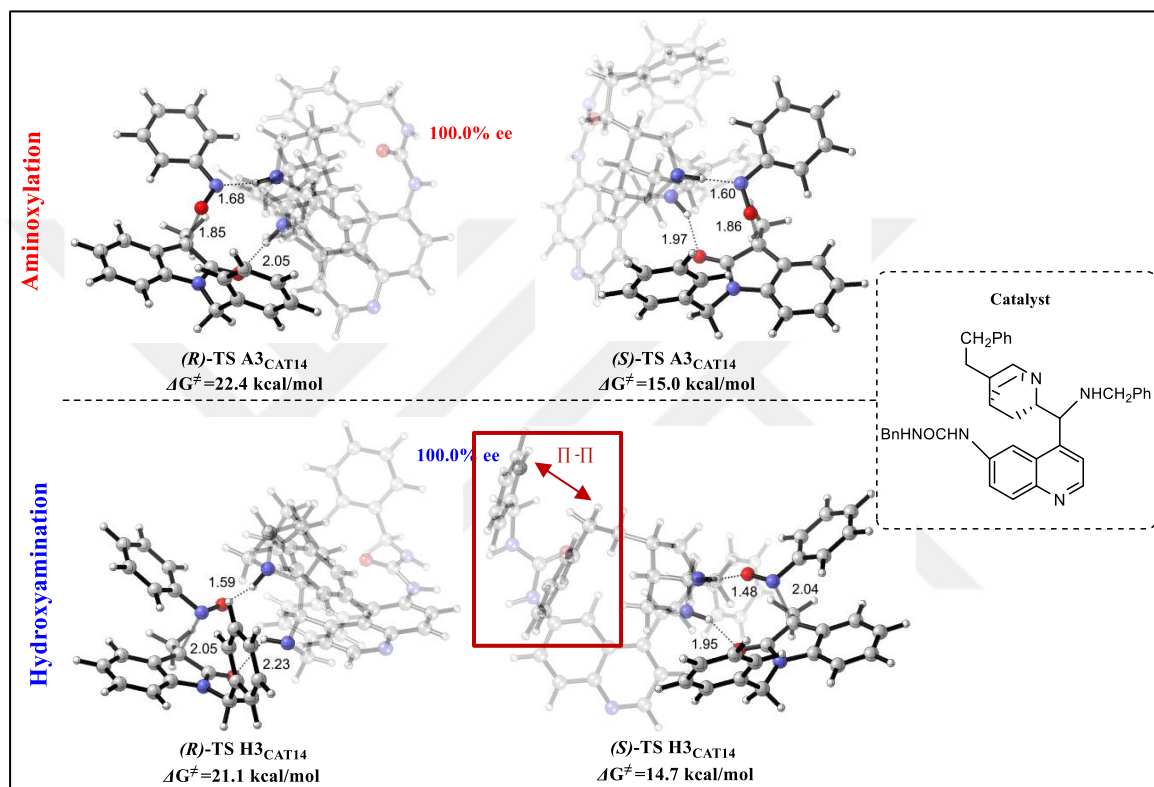


Figure 4.19. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT14**

CAT20 and **CAT21** are found from the matching of the structure containing *tert*-amine and thiourea functionalities.

CAT20 (Figure 4.20) that include the thiourea functional group in its structure is predicted to catalyze the reaction in an *S*-selective fashion for both reaction pathways. The lowest energy TS is **(S)-TS H3CAT20** with an activation barrier of 13.5 kcal/mol indicative of a strong preference for the *N*-adduct ($\Delta G^\ddagger$ for **(S)-TS H3CAT20** 13.5 kcal/mol, $\Delta G^\ddagger$ for **(S)-TS A3CAT20** 17.0 kcal/mol). Bidentate H-bonding of the thiourea group results in much lower activation energies compared to alcohol and amine containing catalyst candidates ($\Delta G^\ddagger$ for

thiourea (**S**)-TS **H3**_{CAT20} 13.5 kcal/mol, $\Delta G^\ddagger$ for amine (**S**)-TS **H3**_{CAT14} 14.7 kcal/mol, $\Delta G^\ddagger$ for alcohol (**S**)-TS **H3**_{CAT5} 17.7 kcal/mol)

Located TS **CAT21** are given in Figure B.12. Activation barrier for **CAT21** is found to be comparable with **CAT20**, strongly favoring the *S*-enantiomer of the *N*-adduct. Therefore, **CAT20** and **CAT21** are both selected for synthesis.

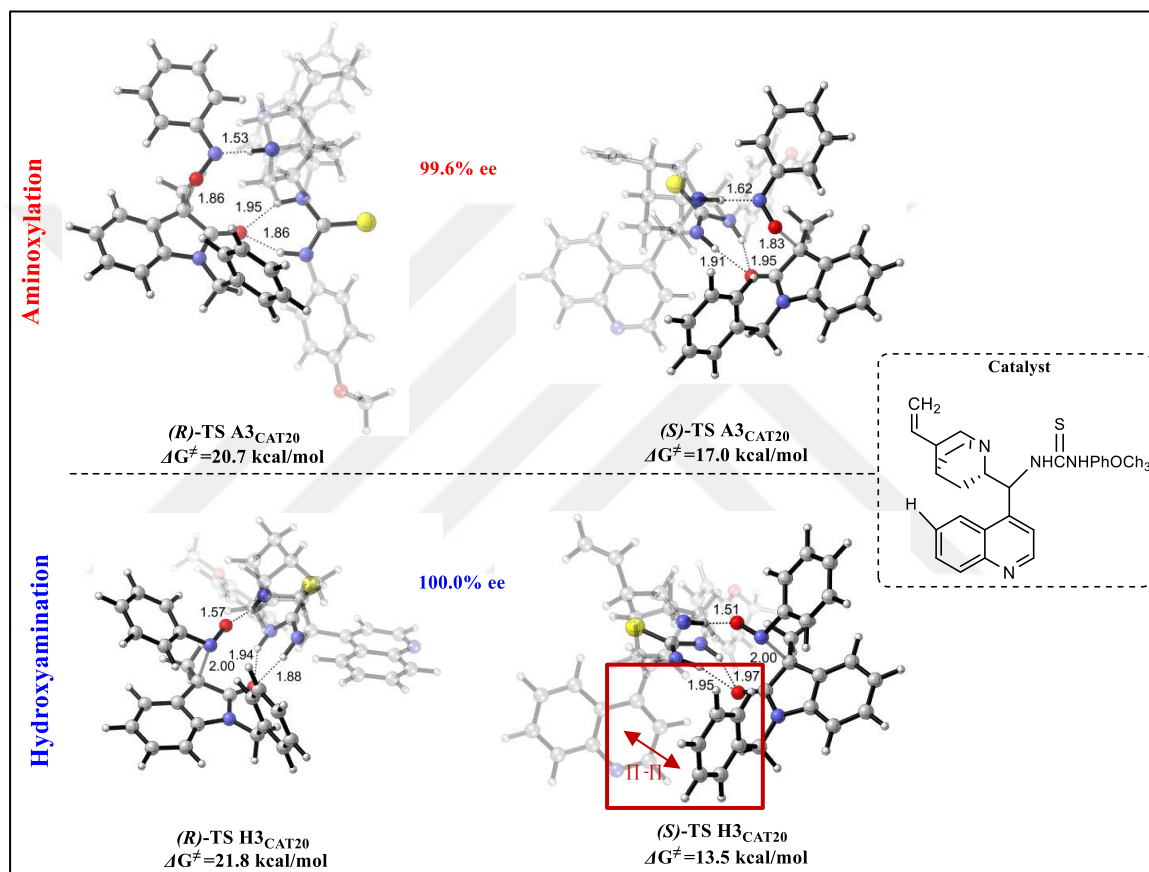


Figure 4.20. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT20**

CAT35 (Figure B.13), **CAT36**, **CAT37** (Figure B.14), **CAT38** and **CAT39** are obtained from the matching of the structure containing *tert*-amine and urea functionalities.

Located TSs for **CAT36** and **CAT38** that include the urea functional group are shown in Figure 4.21 and Figure 4.22, respectively. **CAT36** strongly favors the *S*-enantiomer with up to 100% ee for both adducts. As (**S**)-TS **H3**_{CAT36}, lowest energy TS ($\Delta G^\ddagger = 12.1$ kcal/mol), is stabilized by two addition π - π interactions compared to competing TSs: π - π interaction

between (1) benzyl protecting group of oxindole and quinoline ring of the catalyst (2) oxindole and nitrosobenzene.

The lowest activation barrier for **CAT38** is obtained for the hydroxyamination pathway leading to *S*-enantiomer of *N*-adduct ($\Delta G^\ddagger = 11.6$ kcal/mol). **(S)-TS H3_{CAT38}**, has a comparable activation energy with the parent catalysts with an advantage of 100% enantioselectivity.

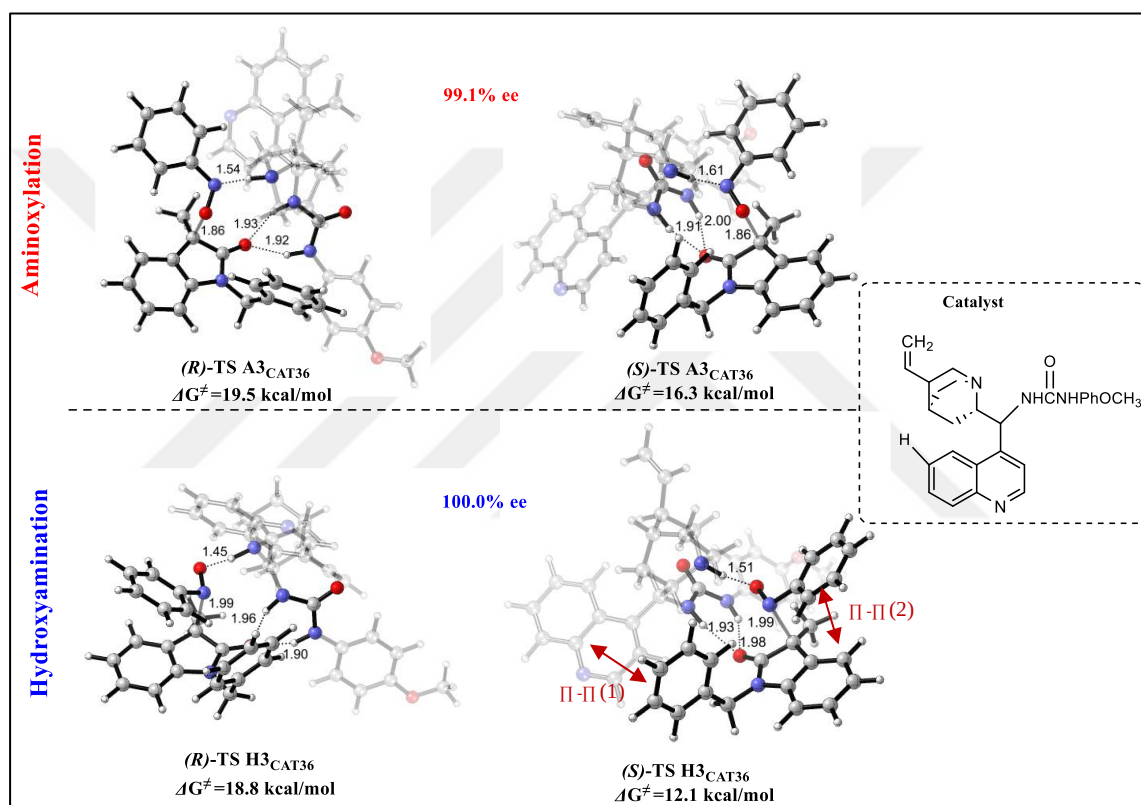


Figure 4.21. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT36**

CAT39 (Figure 4.23) has one urea at C9 position, one thiourea at C6' position groups and one hydroxyl group and it gives the lowest activation energy (**(S)-TS H3_{CAT39}** $\Delta G^\ddagger = 7.8$ kcal/mol) among all matching catalysts. The enantioselectivities are also 100% for both products.

As all the activation energies obtained **CAT36**, **CAT37**, and **CAT38** are promising, they are selected for experimental testing. Even though **CAT39** has the most promising catalyst predicted computationally, the cinchona alkaloids must be derived from both C9 and C6' of

the parent catalysts resulting in a more elaborate synthetic procedure which is planned as a future work.

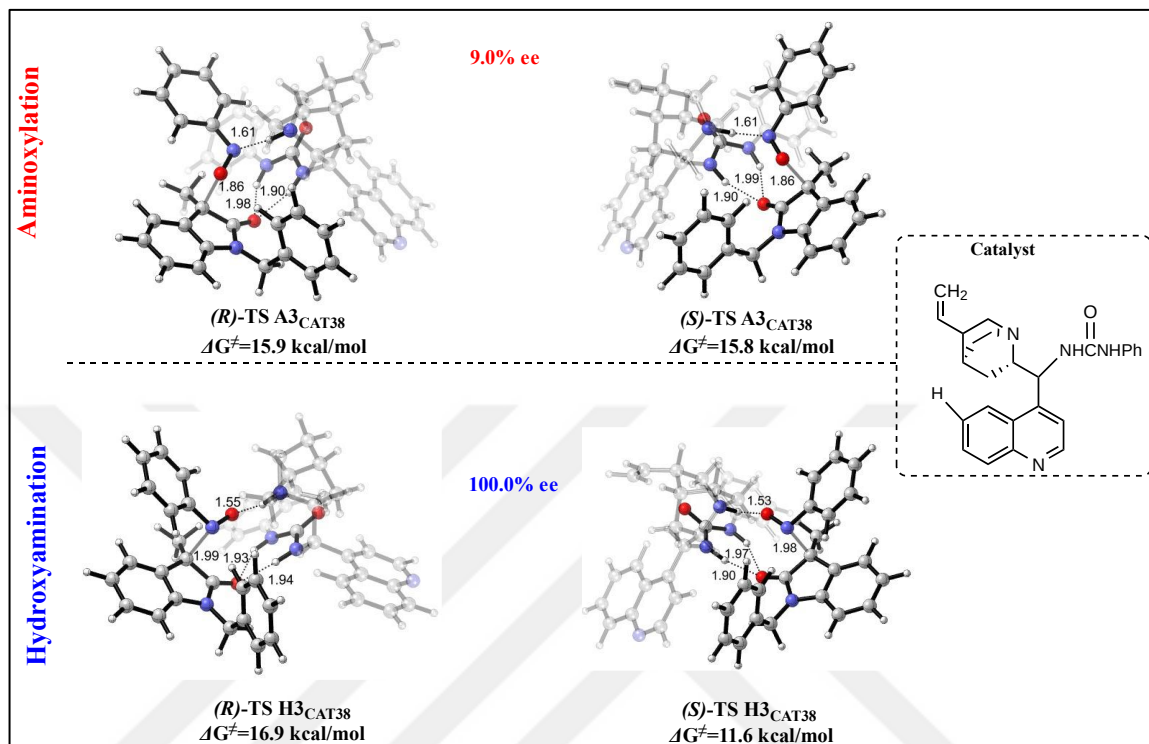


Figure 4.22. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT38**

To compare the effect of the urea and thiourea functional groups, all the urea/thiourea TS are converted to thiourea/urea in addition to the matched catalyst, and their activation barriers are also examined. **CAT38S** is given in Figure 4.56 and the other structures (**CAT21O**, Figure B.15, **CAT35S**, Figure B.16, and **CAT37S**, Figure B.17) are shown in Appendix B. If the catalysts are urea-based, it has “O” prefix at the end, and if the catalysts is thiourea-based, it has “S” prefix at the end.

CAT38S (Figure 4.24) gives *N*-adduct with 100% enantioselectivity. The reaction proceeds to give the *S*-enantiomer via **(S)-TS H3_{CAT38S}** with an activation energy of 13.5 kcal/mol. *O*-adduct disfavored compared to *N*-adduct by 4.3 kcal/mol.

Even though the thiourea and urea based theozymes are similar in activation energy, 1.9 kcal/mol energy difference between **CAT38** and **CAT38S** favors the urea based catalyst.

The other catalysts (**CAT21O**, **CAT35**, and **CAT37**) also showed a similar trend and the activation energies of urea based catalysts are lower than thiourea based catalysts.

All selected catalysts including the urea/thiourea functional group favors *N*-adduct (hydroxyamination product). Besides, selectivity was obtained higher for hydroxyamination reaction. Activation barriers were so close to each other.

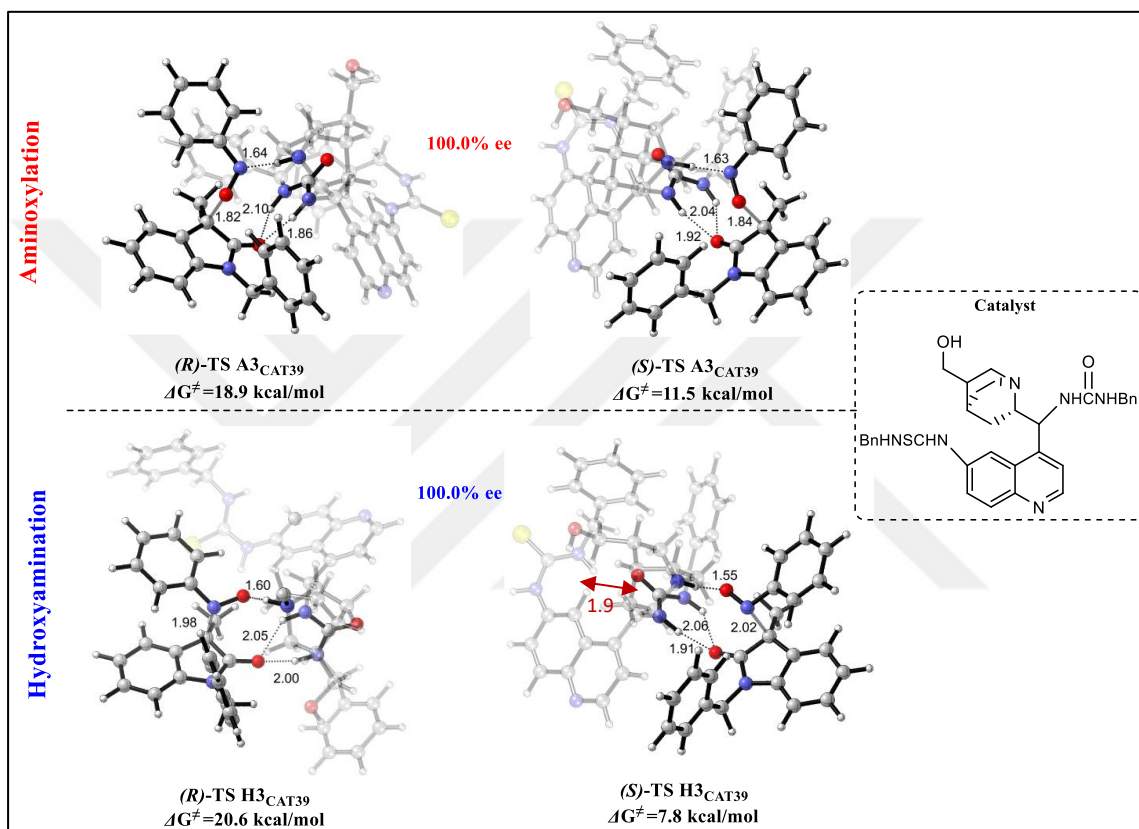


Figure 4.23. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT39**

When all these catalysts were searched for the ability to synthesize, nine catalysts were selected to synthesize experimentally. These are **CAT20**, **CAT21**, **CAT35**, **CAT35S**, **CAT36**, **CAT37**, **CAT37S**, **CAT38**, and **CAT38S** (Table 4.8).

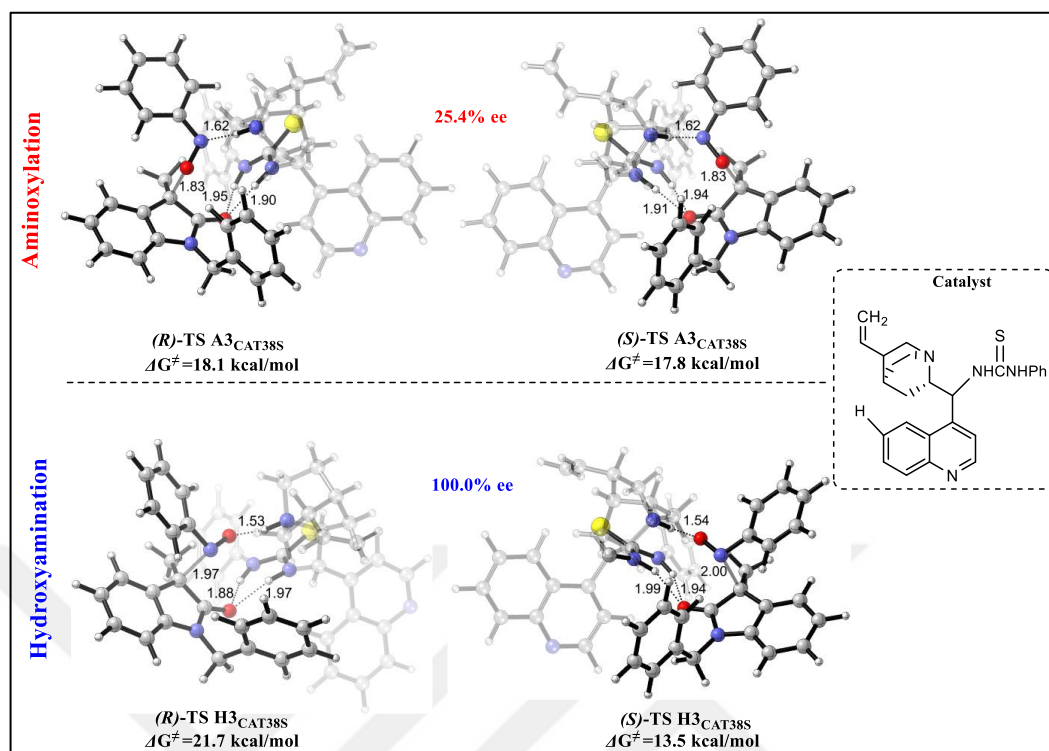


Figure 4.24. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT38S**

Table 4.9. Structure of computationally selected catalysts

Cinchonine Structure	Catalyst Number	R ₁	R ₂	R ₃
	CAT20	NHCSNHP _h OCH ₃	H	CHCH ₂
	CAT21	NHCSNHP _h OCH ₃	OCH ₃	CHCH ₂
	CAT35	NHCONHP _h CH ₃	OCH ₃	CHCH ₂
	CAT35S	NHCSNHP _h CH ₃	OCH ₃	CHCH ₂
	CAT36	NHCONHP _h OCH ₃	H	CHCH ₂
	CAT37	NHCONHP _h CH ₃	H	CHCH ₂
	CAT37S	NHCSNHP _h CH ₃	H	CHCH ₂
	CAT38	NHCONHP _h	H	CHCH ₂
	CAT38S	NHCSNHP _h	H	CHCH ₂

4.6. ASYMMETRIC SYNTHESIS OF CINCHONA ALKALOIDS

Asymmetric synthesis is studied in this project and for this purpose organocatalysts were selected for green chemistry. To be able to perform the target reaction with high yield and high enantioselectivity, the cinchona alkaloids selected according to theoretical results (Table 4.8) were aimed to be synthesized by the methods given in Chapter 3. Then, the reaction was performed with the synthesized catalysts (**CAT20**, **CAT36**, **CAT38S**).

4.6.1. Characterization of Catalysts

In computational part, nine different catalysts (Table 4.9) were proposed to synthesize. Only four of them (**CAT20**, **CAT36**, **CAT38**, **CAT38S**) could be obtained and the results are given below.

4.3.1.1. Catalyst 1 (CAT36). After the synthesis procedure, characterization analysis for the catalyst **1** was done.

FT-IR Analysis of Catalyst 1 (CAT36). When the FT-IR spectrum (Figure 4.25) of the catalyst was examined, the absorption peaks given in Table 4.10 were obtained. In this spectrum, the determining peaks are the peaks belonging to –NH, and carbonyl group of the bonded urea (Figure 4.27). The results are consistent with the literature [65].

Table 4.10. IR Absorption peaks of Catalyst **1** (**CAT36**)

Absorption Peak (cm ⁻¹)	Types of Vibration
3300.86 - 3258.99	N-H stretch (amide)
1688.92	C=O stretch (amide)
1553.19	N-H bend (amide)
1455.45	C-N bend (amide)
1634.46	C=C stretch (alkene)
1241.86 & 1171.35	C-O-C stretch (ether)

2935.23 – 2835.30	C-H stretch (alkane)
3053.02 – 2999.63	C-H stretch (aromatic)
1606.57 & 1509.41	C=C stretch (aromatic)
827.75	C-H bend (para-aromatic)

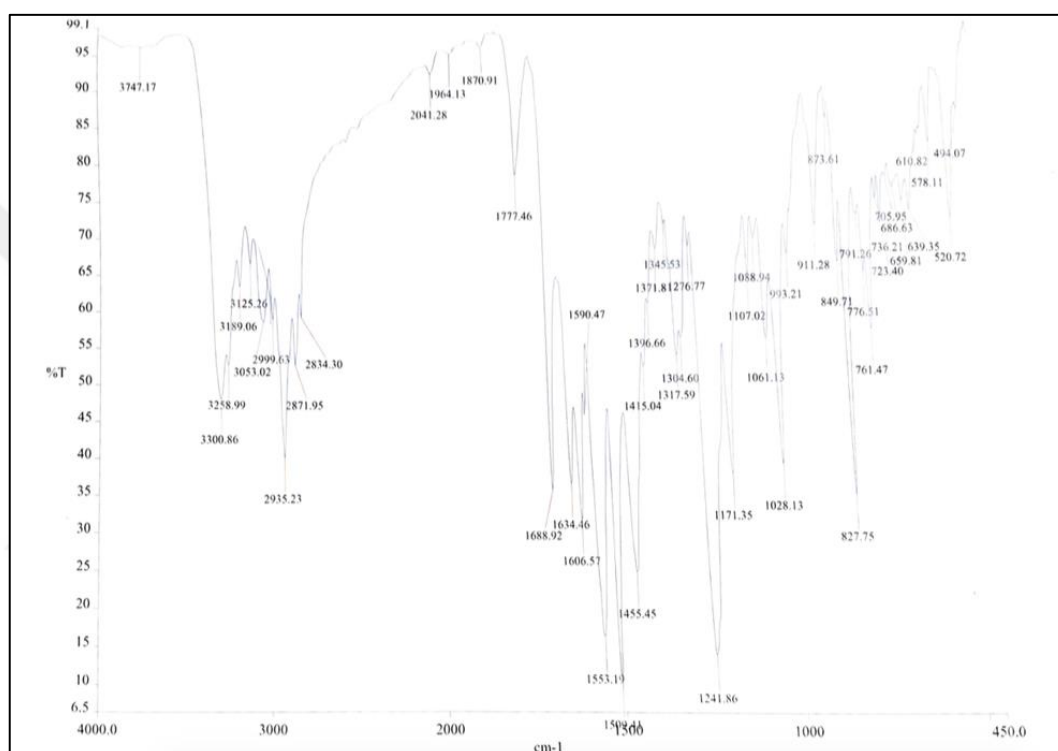


Figure 4.25. FT-IR spectrum of Catalyst 1 (CAT36)

LC-MSMS Analysis of Catalyst 1 (CAT36). Mass spectroscopy analysis was made for the catalyst and the result was given in Figure 4.26. The molecular ion peak of 443.419 [M+1] was observed by the MS detector connected to the LC instrument. Peaks of both molecular ion and the fragment ions (294, 277, 92, 80, 66) were found in this spectrum and the results are consistent with the literature[65]

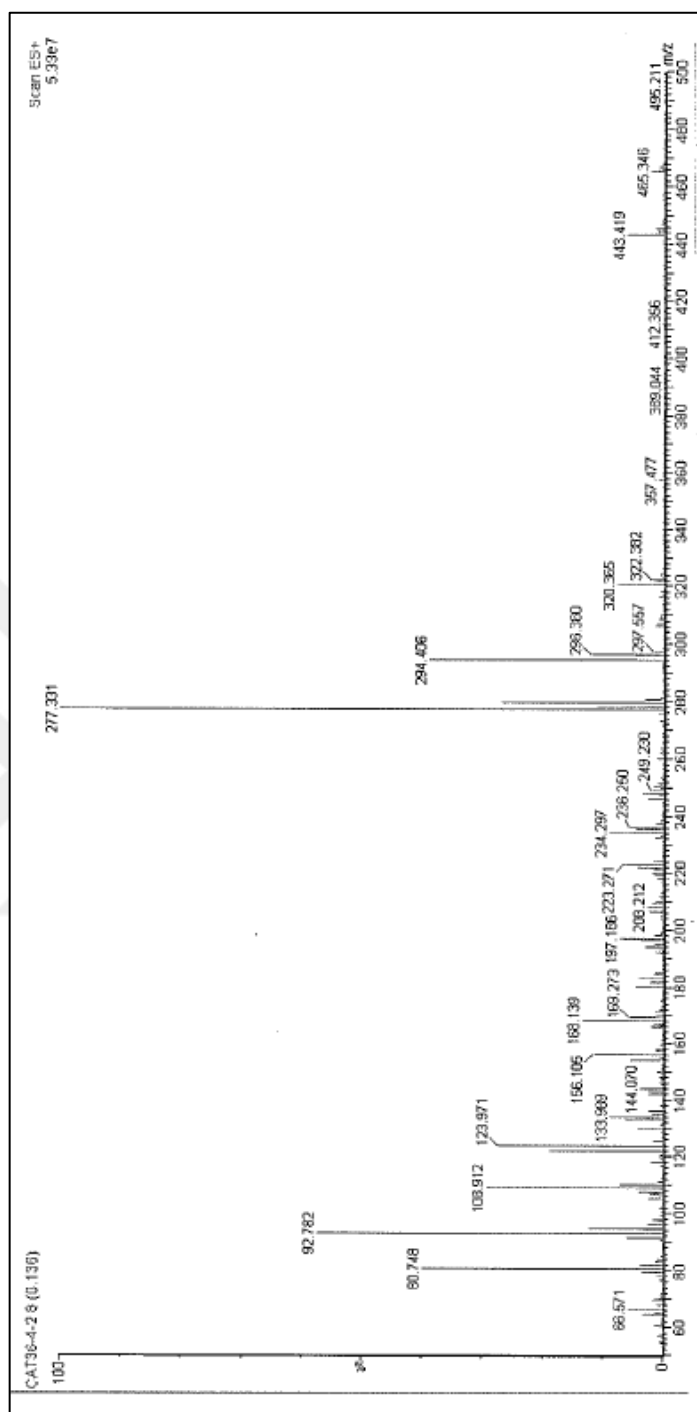


Figure 4.26. LC-MSMS chromatogram of **CAT36**

¹H-NMR Analysis of Catalyst 1 (CAT36). Proton NMR spectrum of Catalyst **1** (**CAT36**) was given in Figure 4.28. The determining factor for the occurrence of the synthesis is the proton of the carbon connected to isocyanate group. However, this carbon is connected to -OH in cinchonidine, to -NH₂ in free amine, and to -NH in **CAT36** and a proton of the carbon

(shown in Figure 4.27) is seen as doublet in all these compounds. The chemical shift values are very close. Therefore, characterization could not be made by ^1H -NMR spectrum.

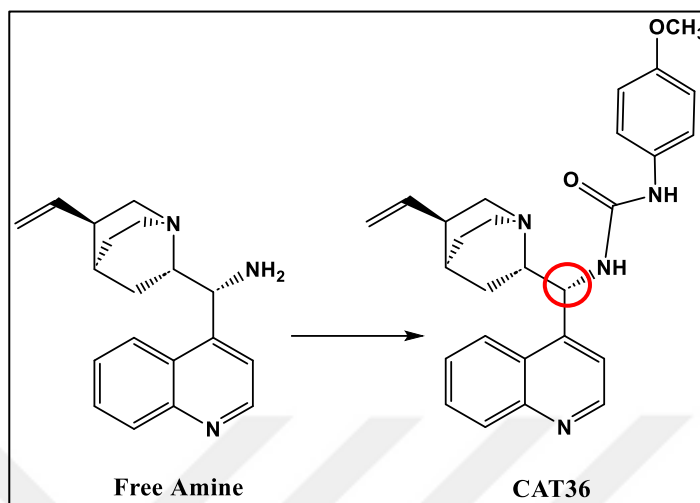


Figure 4.27. Determining factor of Catalyst 1 (CAT36)

^{13}C -NMR Analysis of Catalyst 1 (CAT36). ^{13}C NMR spectrum of Catalyst 1 (CAT36) was given in Figure 4.29. The peaks were determined as shown below. Number of peaks are the same as the number of carbons in the Catalyst 1 (CAT36), and also the results are consistent with the literature[65].

^{13}C -NMR (CD_3OD): δ 156.659, δ 155.836, δ 155.645, δ 149.569, δ 147.617, δ 139.818, δ 132.003, δ 131.942, δ 129.571, δ 128.595, δ 127.360, δ 126.811, δ 123.647, δ 121.406, δ 121.078, δ 114.102, δ 113.675, δ 113.599, δ 61.725, δ 59.994, δ 54.459, δ 54.406, δ 48.802, δ 38.784, δ 27.455, δ 25.594, δ 24.832.

When the spectrum of **CAT36** and the literature results were examined, the determining C-13 peak belonging to carbon connected to the isocyanate group (Figure 4.27) was seen at 61 ppm. Therefore, the presence of this peak proves that **CAT36** was synthesized.

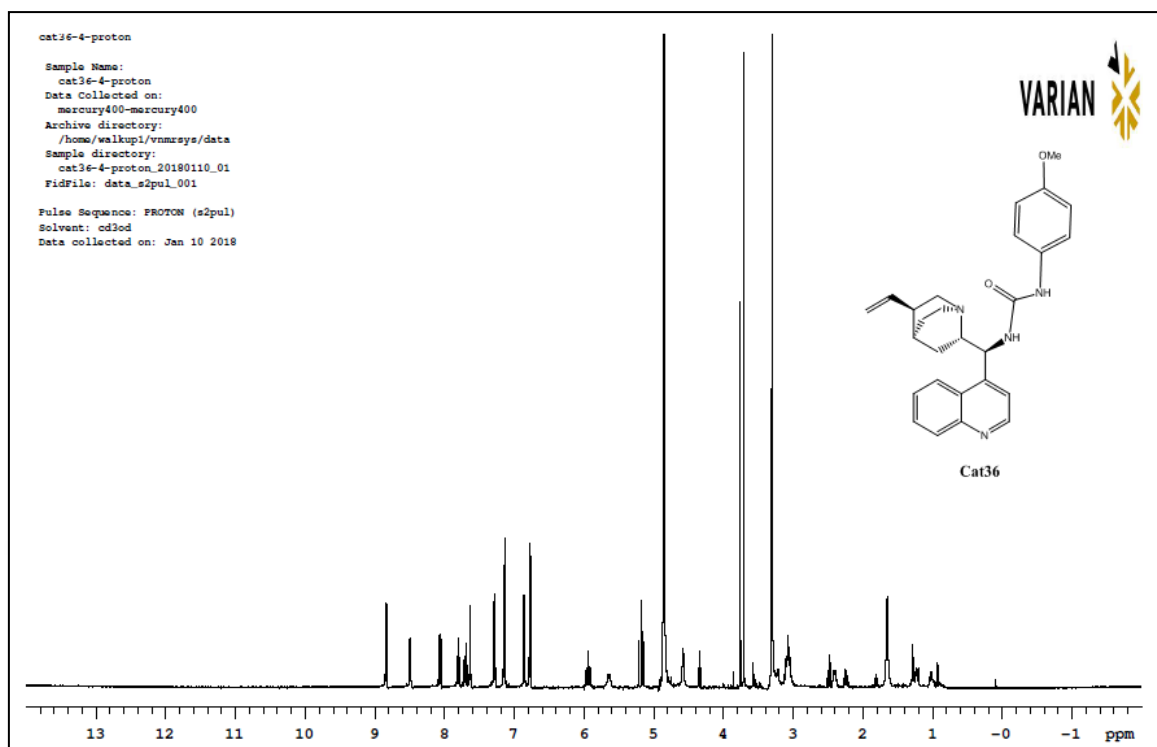


Figure 4.28. ^1H -NMR spectrum of Catalyst 1 (CAT36) (Solvent : CD_3OD)

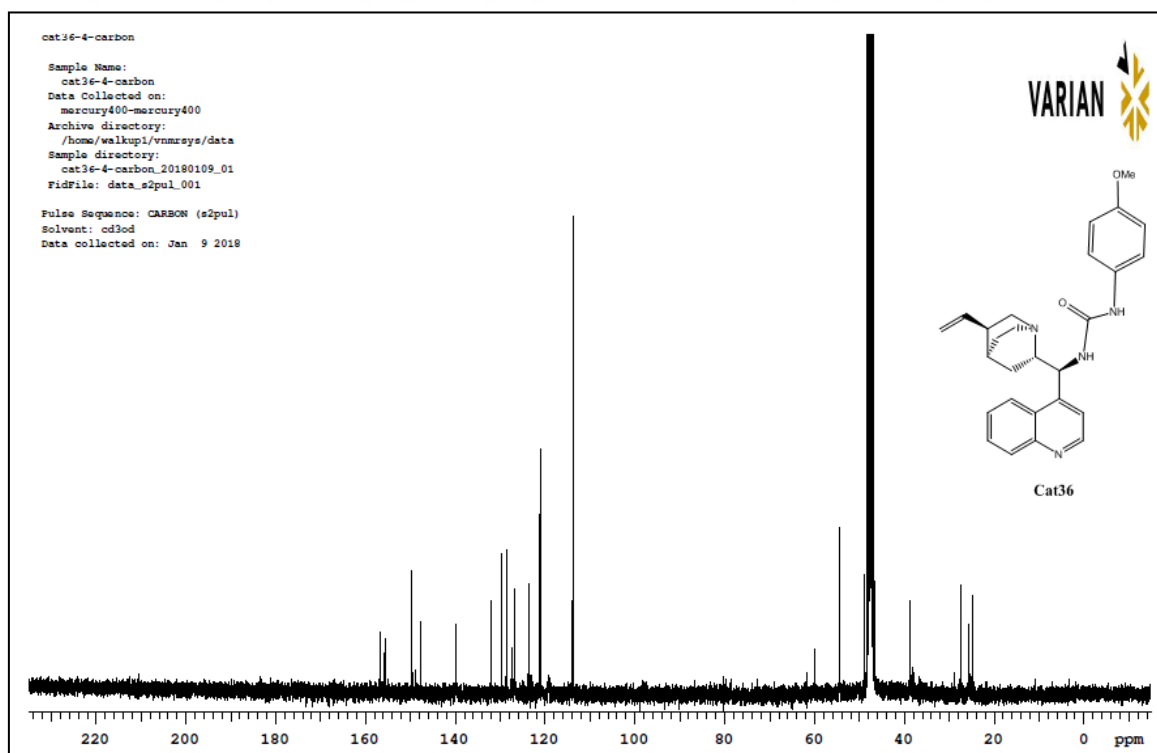


Figure 4.29. ^{13}C -NMR spectrum of Catalyst 1 (CAT36) (Solvent : CD_3OD)

4.3.1.2. Catalyst 2 (CAT20). After the synthesis procedure, characterization of the catalyst 2 was done by several techniques.

FT-IR Analysis of Catalyst 2 (CAT20). When the FT-IR spectrum (Figure 4.30) of the product was examined, the absorption peaks given in Table 4.11 were obtained. In this spectrum, the determining peaks are the peaks belonging to –NH, and carbonyl group of the bonded thiourea (Figure 4.32). The results are consistent with the literature[65].

Table 4.11. Absorption peaks of Catalyst 2 (CAT20)

AbsorptionPeak (cm ⁻¹)	Types of Vibration
3216.83	N-H stretch (amide)
3003.48	C-H stretch (aromatic)
2960.18 – 2835.21	C-H stretch (alkane)
2109.19	N=C=S stretch (isothiocyanates)
1603.39	C=C stretch (alkene)
1504.37 & 1463.99	C=C stretch (aromatic)
1246.29	C-O-C stretch (ether)
829.11	C-H bend (para-aromatic)

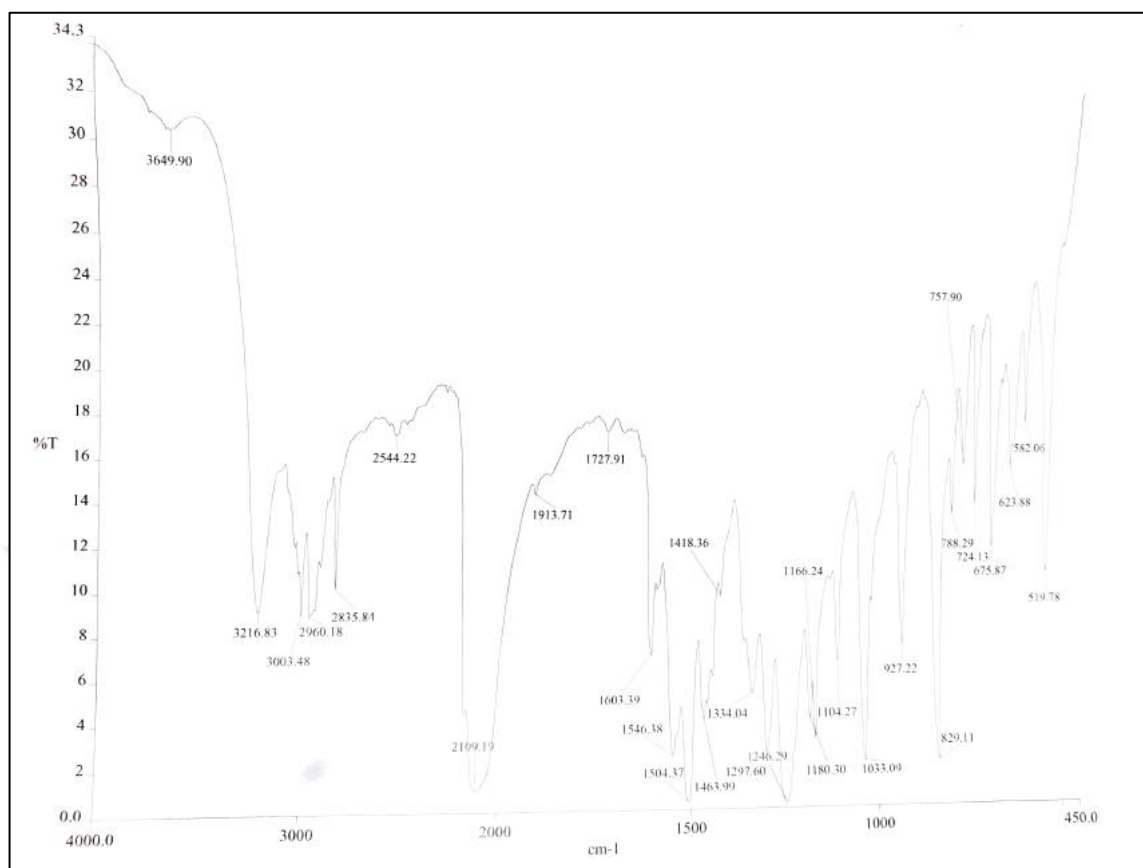


Figure 4.30. FT-IR spectrum of Catalyst 2 (CAT20)

LC-MSMS Analysis of Catalyst 2 (CAT20). Mass spectroscopy analysis was made for the catalyst and the result was given in Figure 4.31. The molecular ion peak of 459.423 [M+1] was observed by the MS detector connected to the LC instrument. Peaks of both molecular ion and the fragment ions, obtained by the loss of isothiocyanate and –NH groups, (294, and 277) were found in this spectrum and the results are consistent with the literature [65].

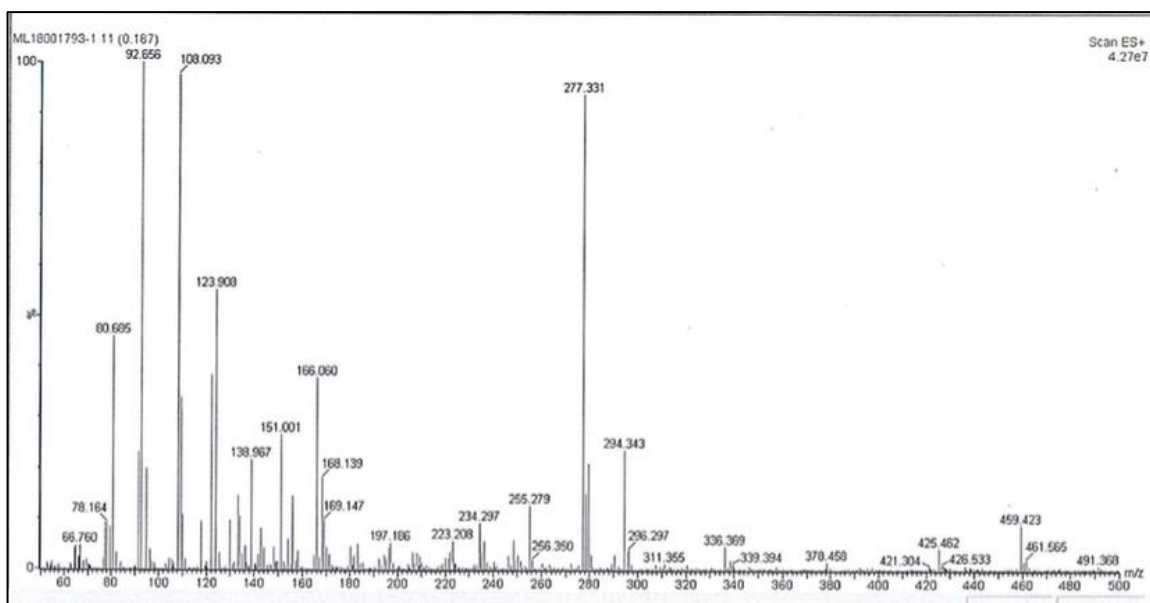


Figure 4.31. LC-MSMS chromatogram of Catalyst 2 (CAT20)

¹H-NMR Analysis of Catalyst 2 (CAT20). Proton NMR spectrum of Catalyst 2 (CAT20) was given in Figure 4.33. The presence of the proton which is the proof for the synthesis of **CAT20** is the proton on the carbon bonded to the isothiocyanate group. The starting chemical for the synthesis of Catalyst 2 (CAT20) was cinchonidine. The free amine was produced from it and then reacted with 4-methoxyphenyl isothiocyanate to give Catalyst 2 (CAT20). Afterall, free amine selected as the reactant to produce **CAT20**. However, the carbon bonded to the determining proton (shown in Figure 4.32) is connected to -OH in cinchonidine, -NH₂ in free amine, and -NH in **CAT20** and this proton was always seen as doublet in all these three compounds (cinchonidine, free amine and **CAT20**). Therefore, characterization could not be made by ¹H-NMR spectroscopy.

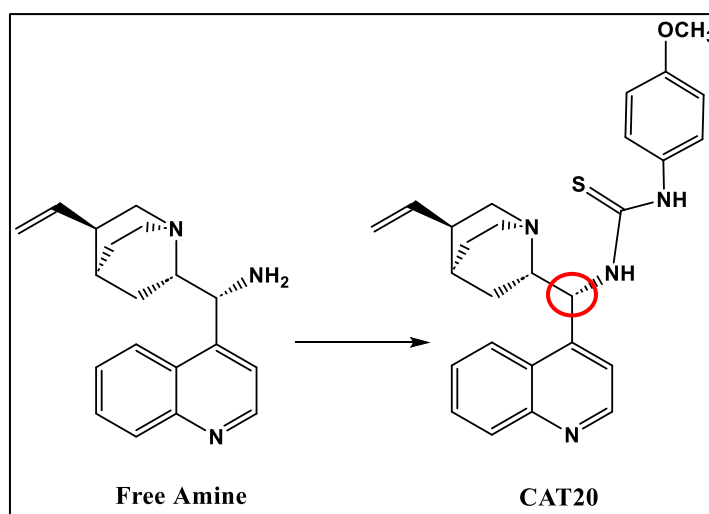


Figure 4.32. The distinctive difference of Catalyst 2 (**CAT20**) when compared to free amine is the marked carbon on the structure

^{13}C -NMR Analysis of Catalyst 2 (CAT20**).** ^{13}C NMR spectrum of Catalyst 2 (**CAT20**) was given in Figure 4.34. When the spectrum of Catalyst 2 (**CAT20**) and the literature results were examined, the determining C-13 peak for the proof of the synthesis of Catalyst 2 (**CAT20**) is the carbon bonded to the isothiocyanate group which is not found in free amine. This determining peak (Figure 4.32) was seen at 60 ppm. Therefore, by the presence of this peak it was proven that Catalyst 2 (**CAT20**) was synthesized.

^{13}C -NMR (CD_3OD): δ 181.414, δ 158.923, δ 157.787, δ 149.584, δ 147.548, δ 139.192, δ 129.647, δ 128.473, δ 126.933, δ 126.704, δ 126.491, δ 126.125, δ 124.775, δ 124.585, δ 123.472, δ 114.658, δ 114.582, δ 113.766, δ 60.398, δ 54.672, δ 54.512, δ 54.489, δ 48.642, δ 38.098, δ 27.073, δ 24.870, δ 24.298.

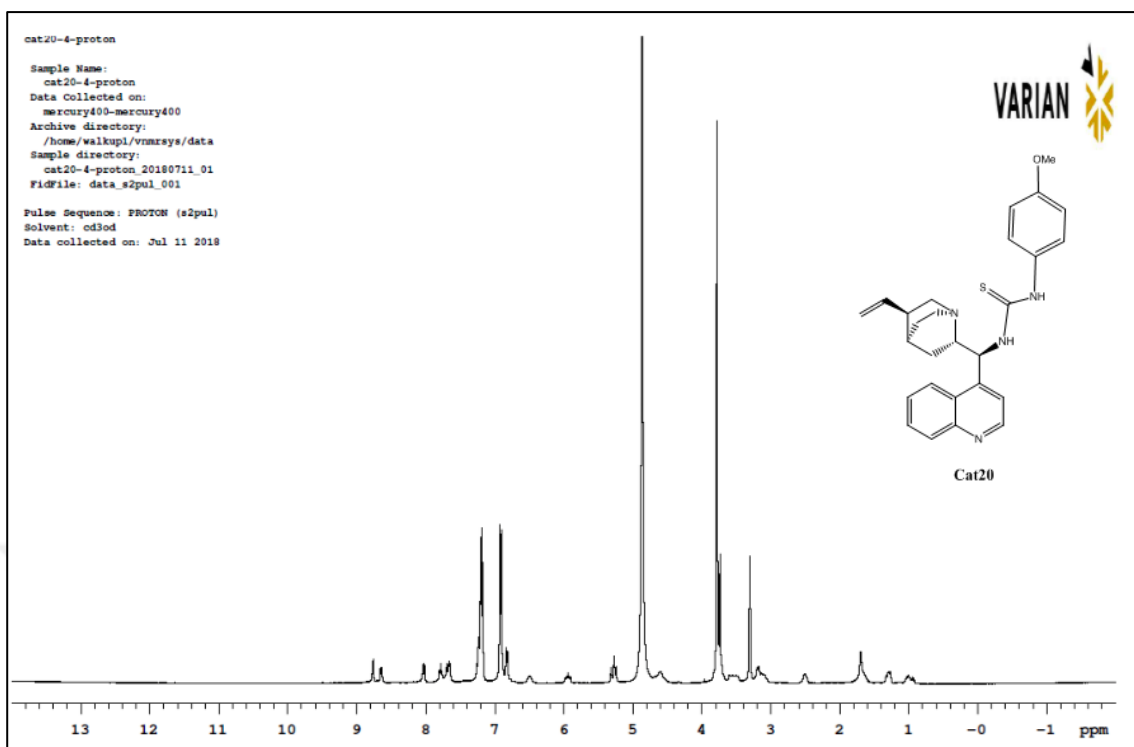


Figure 4.33. ^1H -NMR spectrum of Catalyst 2 (CAT20) (Solvent : CD_3OD)

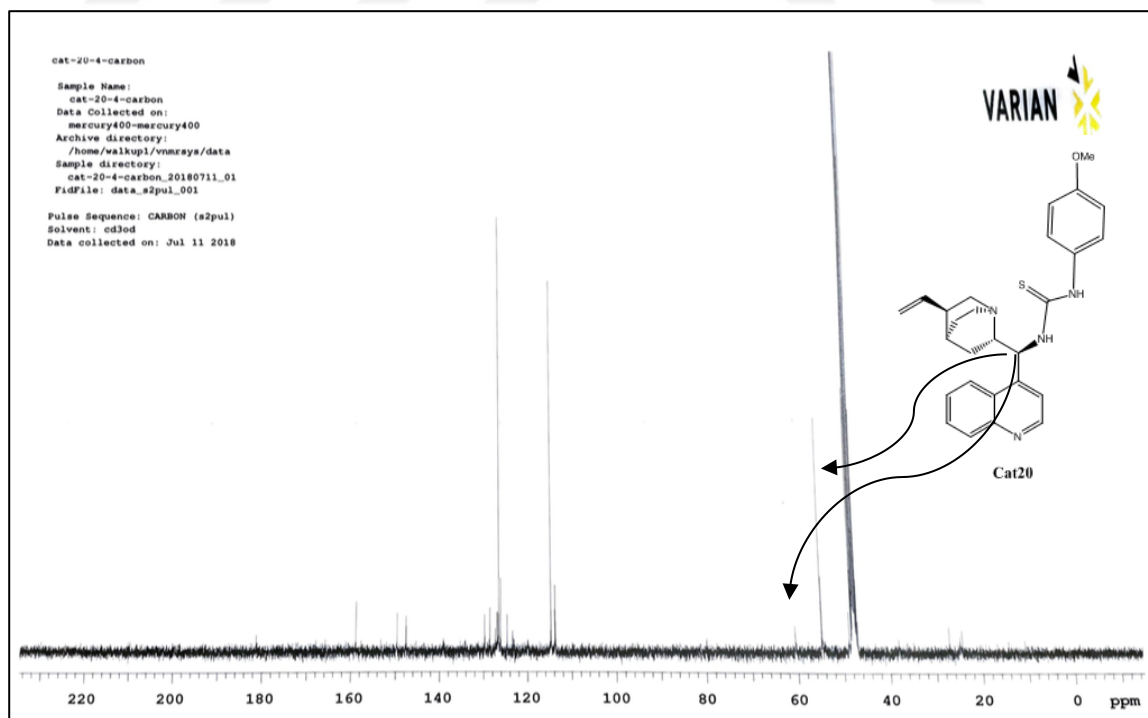


Figure 4.34. ^{13}C -NMR spectrum of Catalyst 2 (CAT20) (Solvent : CD_3OD)

4.3.1.3. Catalyst 3 (CAT38). After the synthesis procedure, characterization for the catalyst **3** was examined.

FT-IR Analysis of Catalyst 3 (CAT38). When the FT-IR spectrum (Figure 4.35) of the product was examined, the absorption peaks given in Table 4.12 were obtained. In this spectrum, the determining peaks are the peaks belonging to $-NH$, and carbonyl group of the bonded urea. The results are consistent with the literature. [65]

Table 4.12. Absorption peaks of **CAT38**

AbsorptionPeak (cm ⁻¹)	Types of Vibration
3133.56	C-H stretch (aromatic)
30.34.43 – 2980.31	C-H stretch (alkane)
1940.09	N=C=O stretch (isothiocyanates)
1649.56	C=C stretch (alkene)
1594.88 & 1439.23	C=C stretch (aromatic)

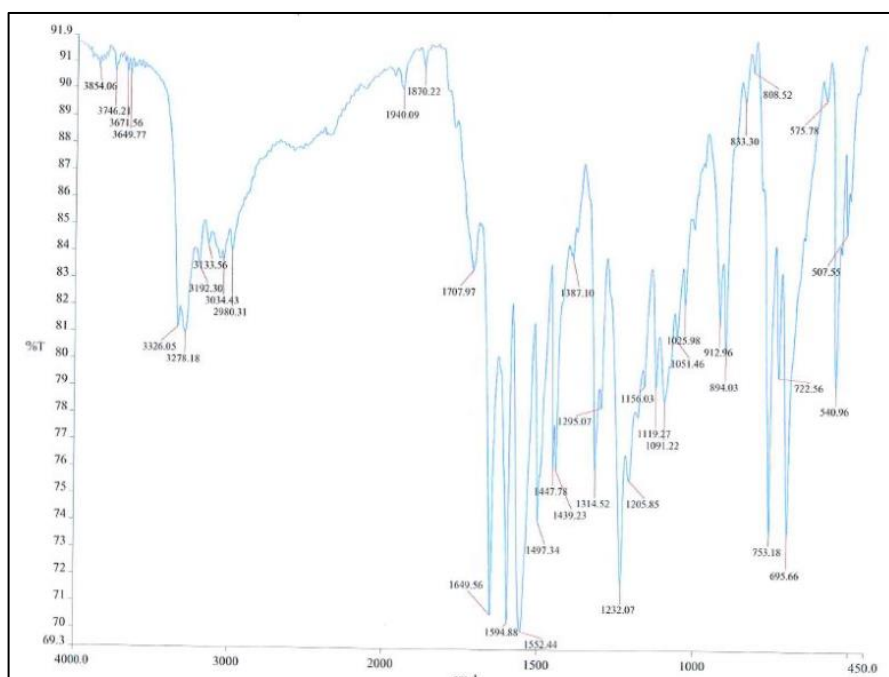


Figure 4.35. FT-IR spectrum of **CAT38**

LC-MSMS Analysis of Catalyst 3 (CAT38). Mass spectroscopy analysis was made for the catalyst and the result was given in Figure 4.36. The molecular ion peak of 413.082 [M+1] was observed by the MS detector connected to the LC instrument and the results are consistent with the literature[65].

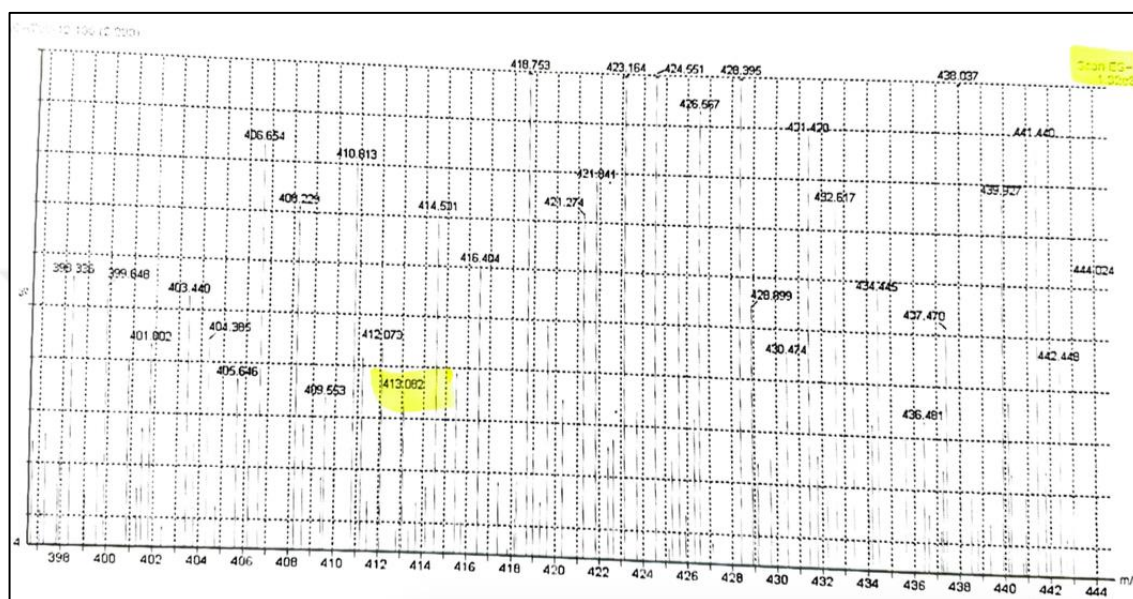


Figure 4.36. LC-MSMS chromatogram of Catalyst 3 (CAT38)

^1H -NMR Analysis of Catalyst 3 (CAT38). Proton NMR spectrum of Catalyst 3 (CAT38) was given in Figure 4.37. The presence of the proton which is the proof for the synthesis of Catalyst 3 (CAT38), is the proton on the carbon bonded to the isothiocyanate group. The starting chemical for the synthesis of Catalyst 3 (CAT38) was cinchonidine. The free amine was produced from it and then reacted with phenyl isocyanate to give Catalyst 3 (CAT38). However, the carbon bonded to the determining proton is connected to -OH in cinchonine, -NH₂ in free amine, and -NH in CAT38 and this proton is always seen as doublet in all these three compounds (cinchonidine, free amine and CAT38). Therefore, characterization could not be made by ^1H -NMR spectroscopy.

^{13}C -NMR Analysis of CAT38. ^{13}C NMR spectrum of Catalyst 3 (CAT38) was given in Figure 4.38. The determining C-13 peak for the proof of the synthesis of Catalyst 3 (CAT38) when compared to the C-13 spectrum of the free amine (starting chemical) is the carbon bonded to the isothiocyanate group. This determining peak was seen at 68 ppm. Therefore,

by the presence of this peak it was proven that Catalyst **3** (CAT38) was synthesized. Some carbon atoms were under the solvent peak around 45 ppm.

^{13}C -NMR (CD_3OD): δ 154.036, δ 152.573, δ 152.496, δ 139.078, δ 132.384, δ 131.797, δ 131.751, δ 131.713, δ 131.614, δ 130.707, δ 128.976, δ 128.633, δ 123.395, δ 122.435, δ 119.896, δ 119.019, δ 118.973, δ 115.863, δ 68.884, δ 36.977, δ 27.211, δ 20.860.

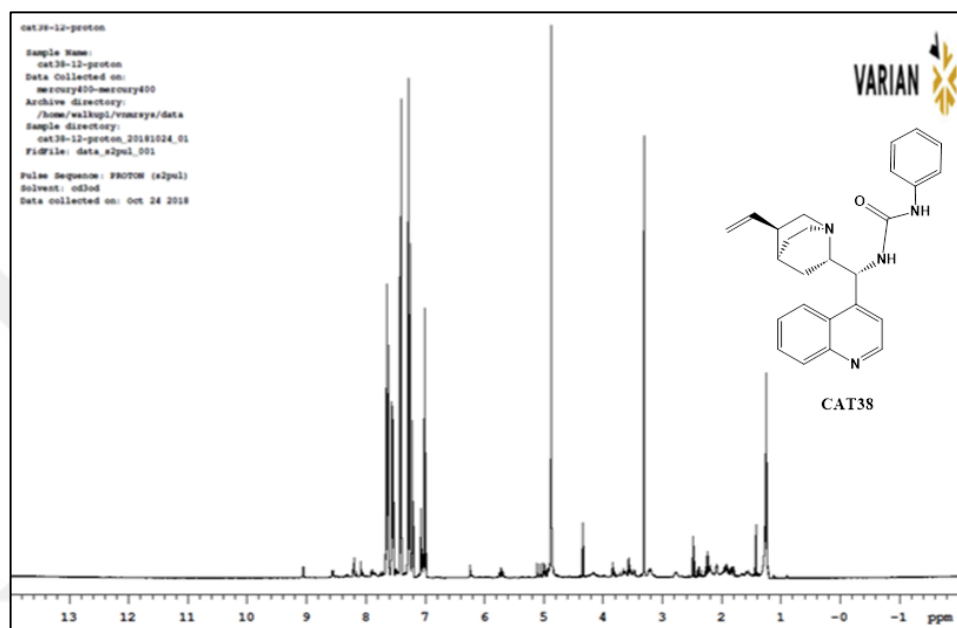


Figure 4.37. ^1H -NMR spectrum of Catalyst **3** (CAT38) (Solvent : CD_3OD)

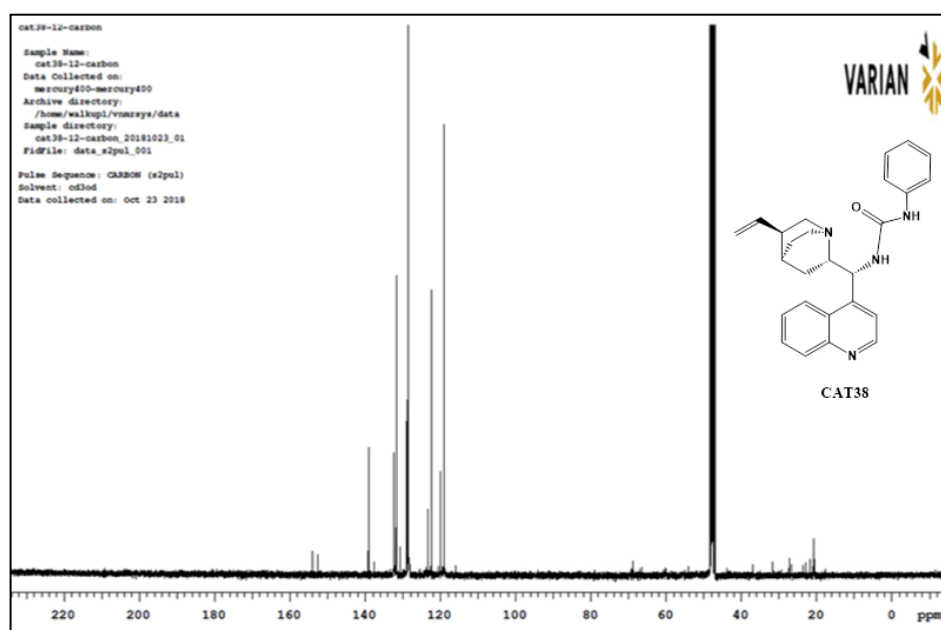


Figure 4.38. ^{13}C -NMR spectrum of Catalyst **3** (CAT38) (Solvent : CD_3OD)

4.3.1.4. Catalyst 4 (CAT38S). After the synthesis procedure, characterization for the catalyst 4 was examined.

^1H -NMR Analysis of Catalyst 4 (CAT38S). Proton NMR spectrum of Catalyst 4 (CAT38S) was given in Figure 4.39. As described before, characterization could not be made by ^1H -NMR spectroscopy.

^{13}C -NMR Analysis of Catalyst 4 (CAT38S). ^{13}C NMR spectrum of Catalyst 4 (CAT38S) was given in Figure 4.40. The determining C-13 peak for the proof of the synthesis of Catalyst 4 (CAT38S) when compared to the C-13 spectrum of the free amine (starting chemical) is the carbon bonded to the isothiocyanate group. This determining peak was seen at 67 ppm. Therefore, by the presence of this peak it is proven that Catalyst 4 (CAT38S) was synthesized. Some carbon atoms were under the solvent peak around 45 ppm. However, the catalyst is not pure, and there is more than 26 carbons.

^{13}C -NMR (CD_3OD): δ 180.808, δ 149.530, δ 147.555, δ 140.740, δ 138.819, δ 138.476, δ 131.699, δ 131.600, δ 129.503, δ 129.458, δ 128.619, δ 128.581, δ 128.497, δ 128.467, δ 127.506, δ 127.216, δ 126.660, δ 125.326, δ 125.196, δ 125.013, δ 124.602, δ 124.487, δ 123.626, δ 122.932, δ 118.640, δ 114.188, δ 113.807, δ 67.442, δ 60.482, δ 60.322, δ 54.887, δ 41.134, δ 39.191, δ 27.474, δ 27.413, δ 26.826, δ 25.080, δ 24.996.

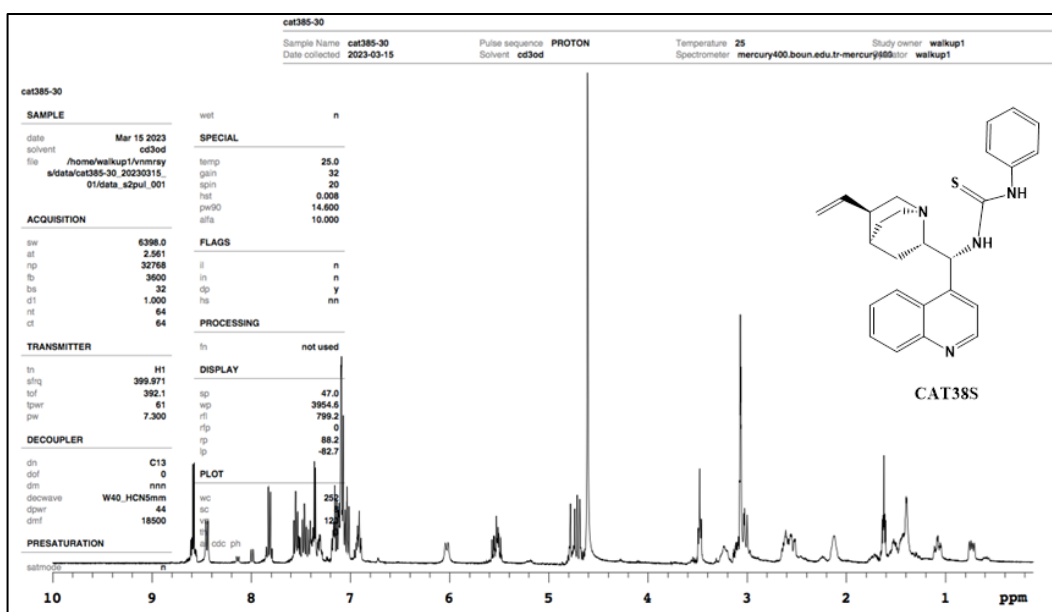


Figure 4.39. ^1H -NMR spectrum of Catalyst 4 (CAT38S) (Solvent : CD_3OD)

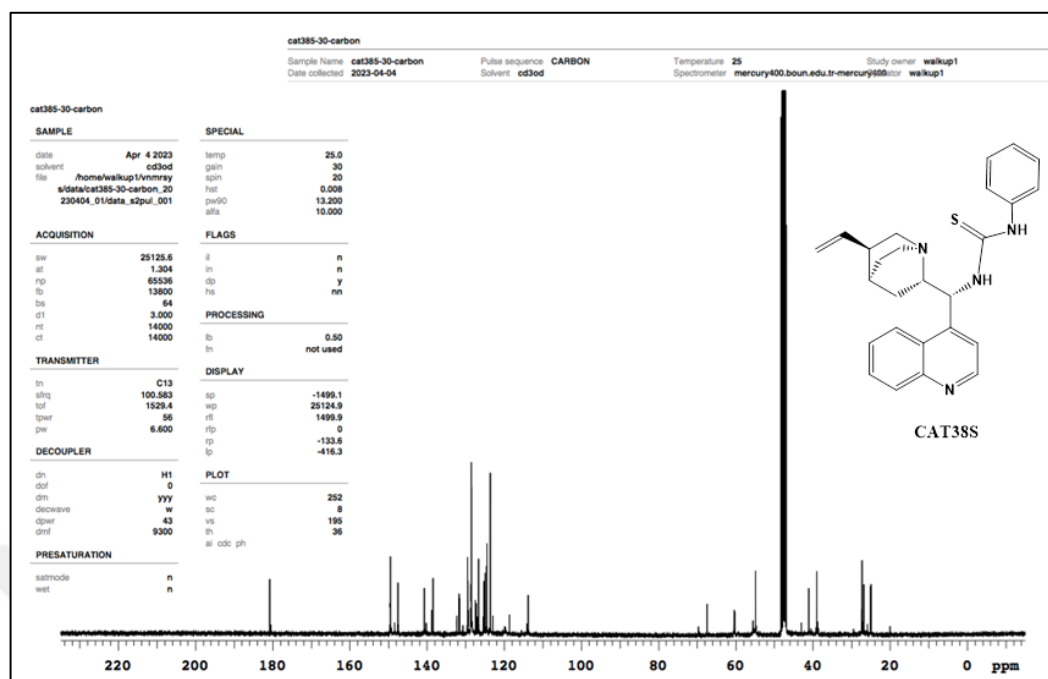


Figure 4.40. ¹³C-NMR spectrum of Catalyst 4 (CAT38S) (Solvent : CD₃OD)

4.6.2. Characterization of the Product

The reaction mixture was first examined by HPLC. At the beginning the retention time of all the reactants were determined, and then the new peaks were tried to be assigned to the possible compounds .

4.6.2.1. Isocratic Elution

This system was applied to the reaction mixture. The HPLC chromatograms of 3-methyl-2-oxindole (Figure A.1), and nitrosobenzene (Figure A.2) were given in Appendix The HPLC chromatogram of the reaction mixture was shown in Figure 4.41. According to the reaction stoichiometry, nitrosobenzene was the limiting reagent and was expected to be consumed completely, oxindole was in excess . The retention time of oxindole is 10 min, and two peaks were seen for the nitrosobenzene chromatogram, so the retention times of nitrosobenzene were assigned as 21 and 25 min in the given conditions. As seen in all chromatograms of the reaction mixtures, there was a new peak at 5 min. This could belong to the product. Therefore, it was purified with column chromatography (petroleum ether : ethyl acetate, 2:1). Characterization studies were carried out for the purified compound giving HPLC peak at 5.2 minutes in the specified conditions (Figure A.3).

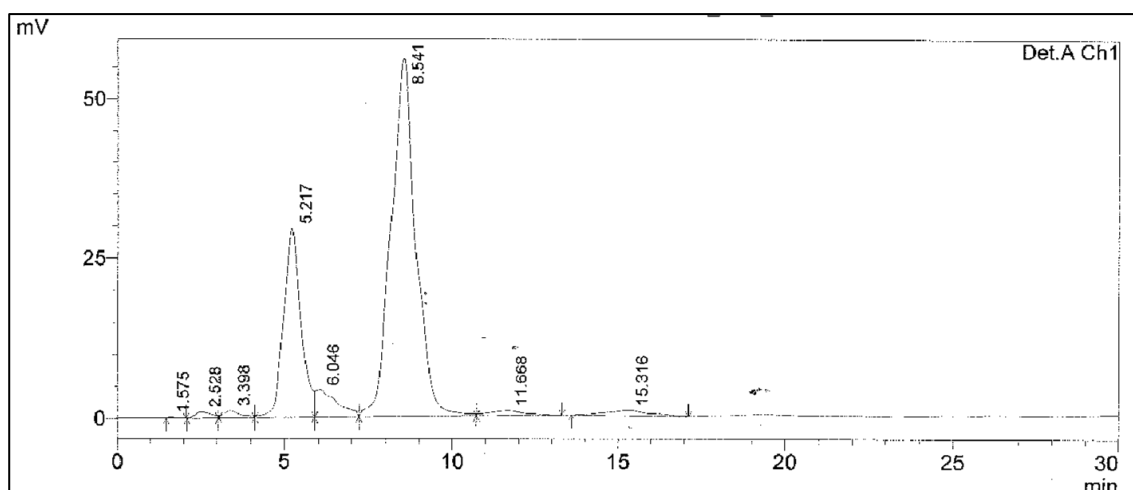


Figure 4.41. The HPLC chromatogram of the reaction mixture (isocratic elution)

In literature[31–33, 61, 66], the reaction of 3-methyl-2-oxindole with nitrosobenzene gives the product shown in Table 4.13-b-c. However, Companyó and co-workers also obtained a decomposition product (Table 4.13-a) with aminoxylation product after five days with aminoxylation product. They proposed that aminoxylation product is decomposed to 3-hydroxy-3-methyl-oxindole [33].

Table 4.13. The desired and the unpredicted products

Chemical Structure			
Chemical Name	3-hydroxy-3-methyl-oxindole (a)	3-(hydroxy(phenyl)amino)-3-methylindolin-2-one (b)	3-methyl-3-((phenylamino)oxy)indolin-2-one (c)
Molecular weight (g/mol)	164	254	254

¹H-NMR for the reaction mixture. The impure product was analyzed by ¹H-NMR spectroscopy, and the ¹H-NMR spectrum was obtained as shown in Figure 4.42. There are

mainly three peaks above 10 ppm and they could belong to the –OH (in hydroxyamination product, Table 4.13-b), –NH (in aminoxylation product, Table 4.12-c) found in the product and -NH of oxindole (Table 4.13-b-c). Companyó et al. produced hydroxyamination product and two peaks (belong to –NH of oxindole and –OH of the product) above 10 ppm was shown[33]. The deuterium exchange analysis (Figure 4.43) was carried out to understand if they belong to them. After this analysis, it was seen that all the peaks above 10 ppm and also the peak at around 5.8 ppm were disappeared. It can be concluded that the peak around 5.8 ppm must belong to an –OH or –NH group and also there are both hydroxyamination and aminoxylation products.

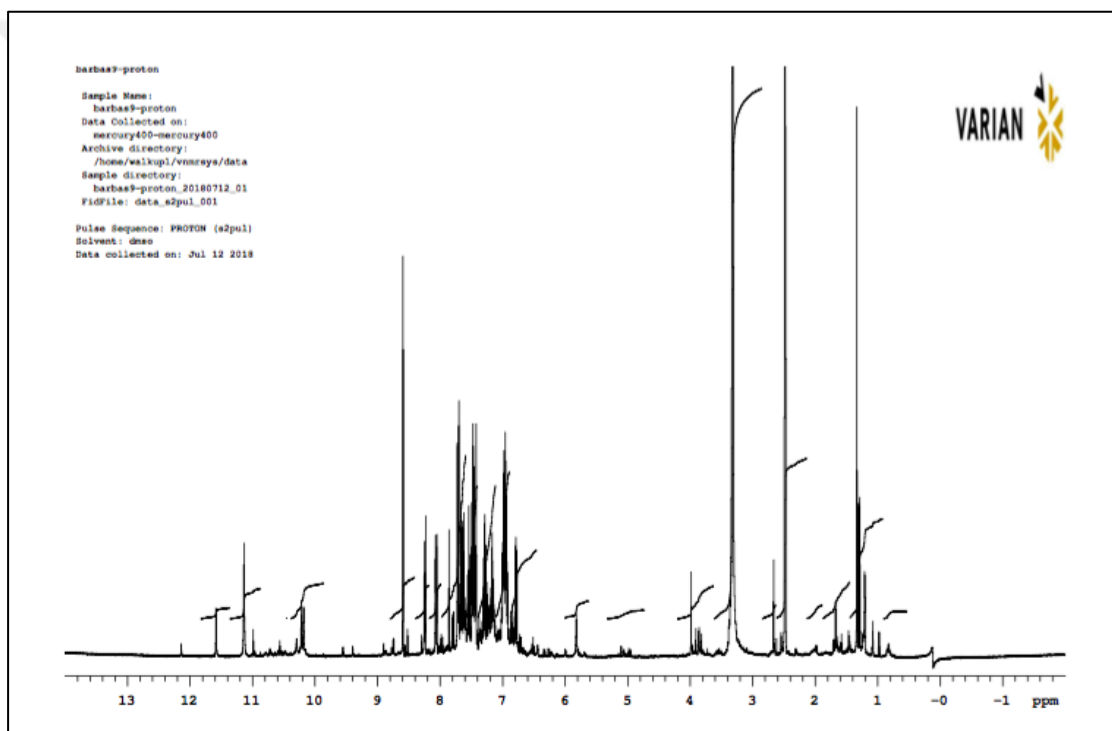


Figure 4.42. ^1H -NMR spectrum for the reaction mixture

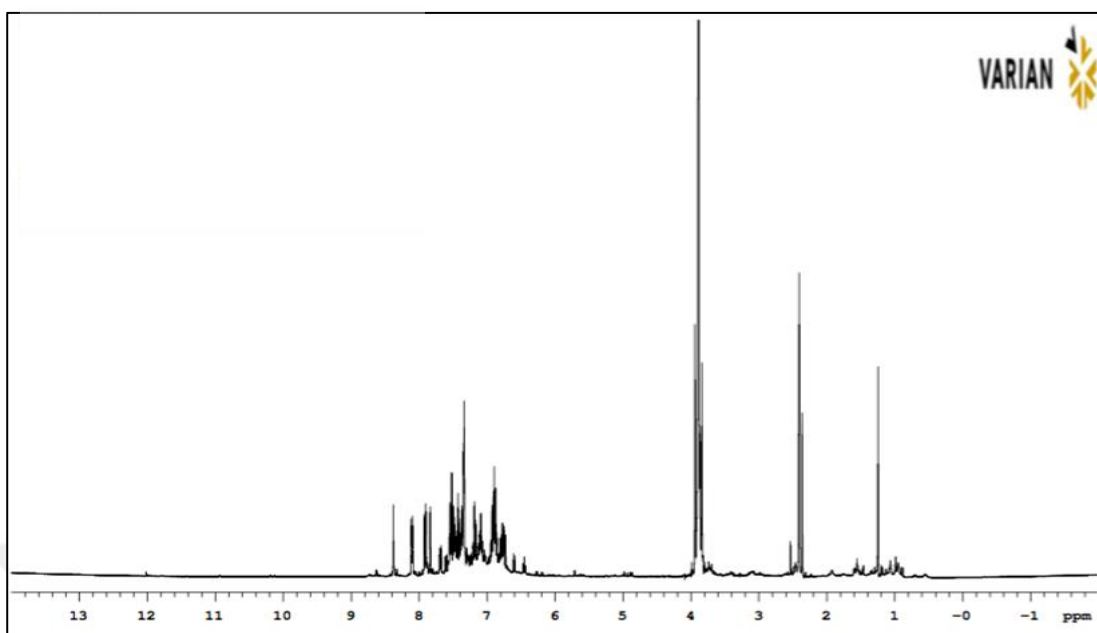


Figure 4.43. Deuterium exchange analysis for the reaction mixture

¹H-NMR for the purified product at 5.2 min. The distinctiveness between the desired product (Table 4.13-b-c) and the unpredicted product (Table 4.13-a) is the singlet proton peak at 5.80 ppm (Figure 4.44). There is an –OH or -NH peak at 5.8 ppm as described in the deuterium exchange analysis (Figure 4.43). This peak exists in the H-1 NMR spectrum of the unpredicted molecule in Table 4.13-a, but in the H-1 NMR spectrum of the desired product in Table 4.13-b-c this peak does not exist [31–33, 61, 66]. The singlet proton peak belongs to the proton of the hydroxyl group connected to the oxindole. In addition, the integral ratio of the aromatic protons shows that there are four protons belong to aromatics and the determined proton number from NMR result is the same as the proton number of the structure given in Table 4.13-a.

¹H NMR (400 MHz, DMSO): δ10.20 (s, 1H); δ7.28 (d, 2H); δ7.20 (t, 2H); δ5.80 (s, 1H); δ1.35 (s, 3H)

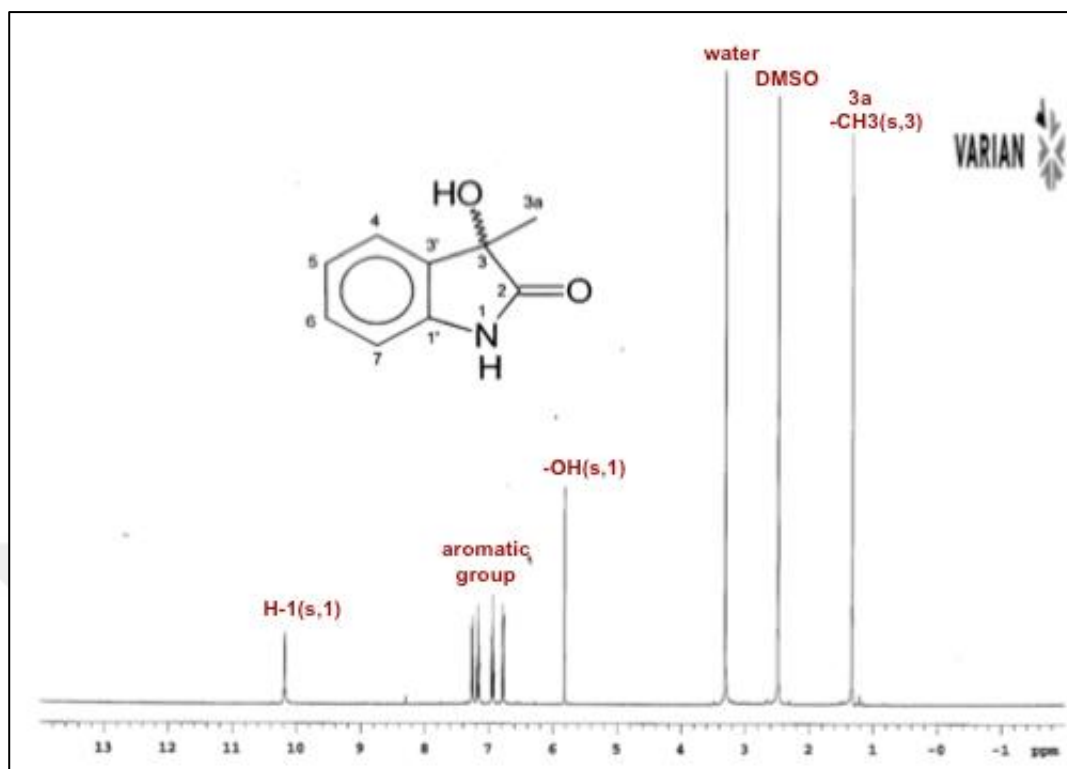


Figure 4.44. ^1H -NMR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution

^{13}C -NMR for the possible product at 5.2 min. The purified product was analyzed in C-13 NMR spectrum using DMSO as the solvent. The peaks for the carbons were given in Figure 4.45. Peak number is consistent with the structure given in Table 4.13-a [67–70].

^{13}C NMR (400 MHz, DMSO): d 180, 142, 134, 130, 122, 124, 110, 73, 25.

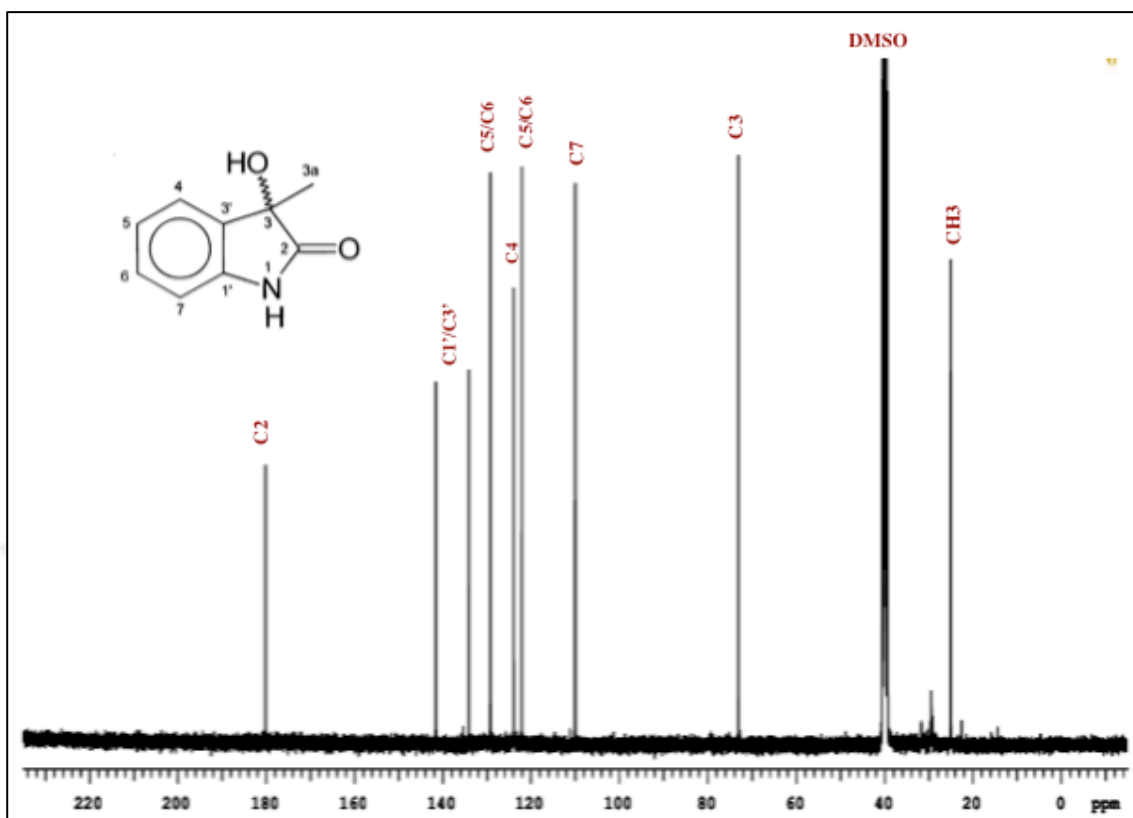


Figure 4.45. ^{13}C -NMR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution

NOESY for the possible product at 5.2 min. NOESY analysis is carried out to prove that the the possible product which gives a peak at 5.2 min in the HPLC chromatogram when isocratic elution was applied is the 3-hydroxy-3-methyl-oxindole. This spectrum must be symmetrical with respect to the diagonal (Figure 4.46). In addition, the neighbour hydrogens must be observed in this analysis. For instance, the hydrogen of C-6 must be neighbour with the hydrogens of C-5 and C-7 and when the hydrogen of C-6 investigated, there are two peaks refers to the hydrogens of C-5 and C-7 are under the diagonal as shown in Figure 4.47. Therefore, the results are convenient with the literature [69].

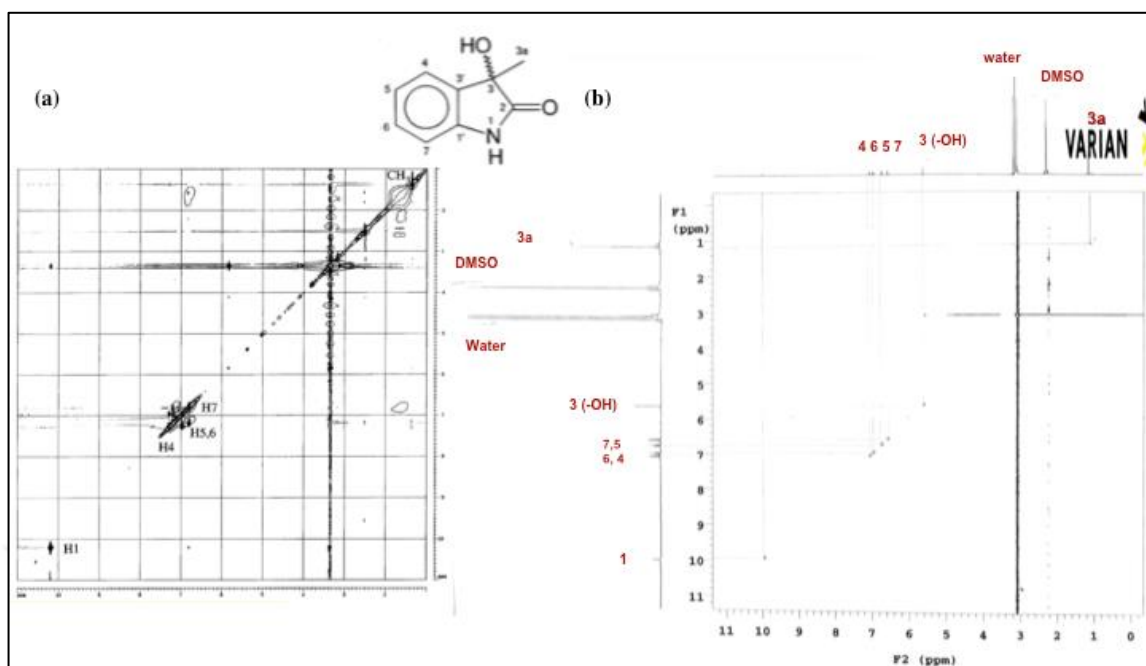


Figure 4.46. NOESY spectrum of (a) 3-hydroxy-3-methyl-oxindole [69] and (b) the possible product the HPLC peak of which appears at 5.2 min in isocratic elution

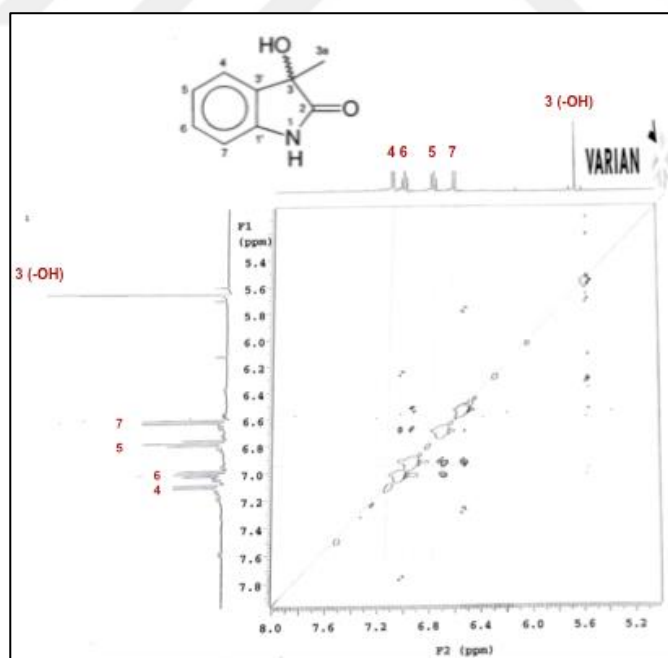


Figure 4.47. NOESY spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution between 5-8 ppm

HETCOR for the possible product at 5.2 min. HETCOR analysis is also carried out for the characterization. As shown in Figure 4.48, proton and bonded carbon groups are defined.

In the hydroxyamination and aminoxylation products (Table 4.13-b-c), there must be the aromatic protons and the carbons from the nitrosobenzene skeleton. However, all the protons at the aromatic region belongs to the carbons in the oxindole skeleton only in this structure and also the carbon at C-3 position is bonded to the –OH group. The result is convenient with the 3-hydroxy-3-methyl-oxindole product.

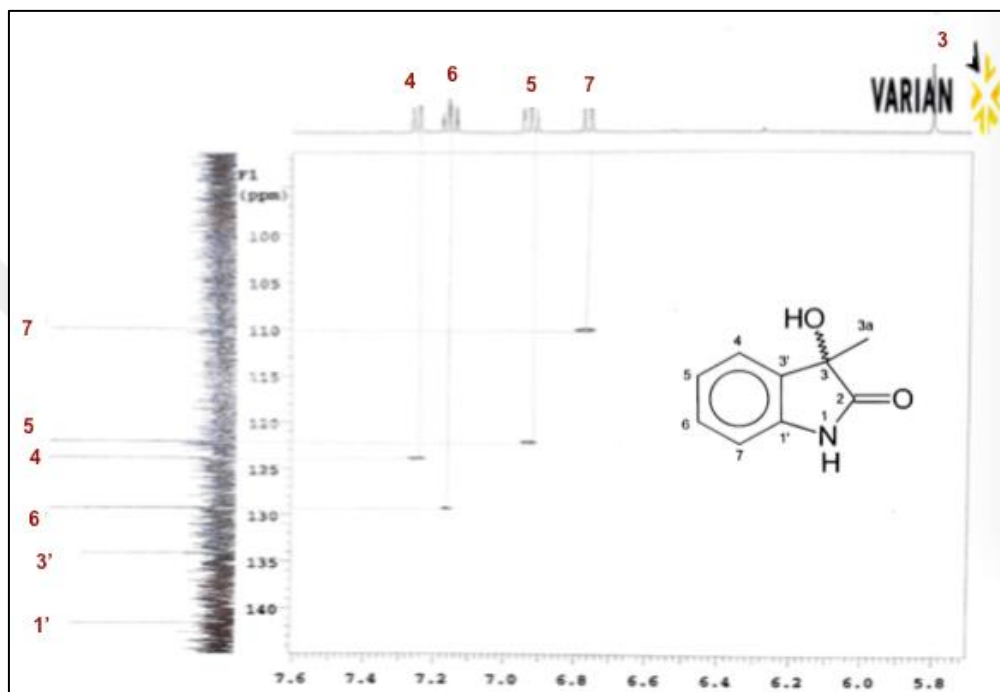


Figure 4.48. HETCOR spectrum of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution

LC-MS/MS analysis. The mass of unpredicted product was also determined (Figure 4.49). When the results were examined, instead of the desired product peak (Table 4.13-b-c), there was a peak at 164.2. Therefore, the obtained product was assigned to be 3-hydroxy-3-methyl-oxindole (Table 4.13-a). When the peaks examined, there were fragment peaks at 147 (-OH broken), 136 and 121 in the MS spectrum as in the literature (Figure 4.50) [68]. These results supports the suggestion that the product was 3-hydroxy-3-methyl-oxindole (Table 4.13-a) [67–73].

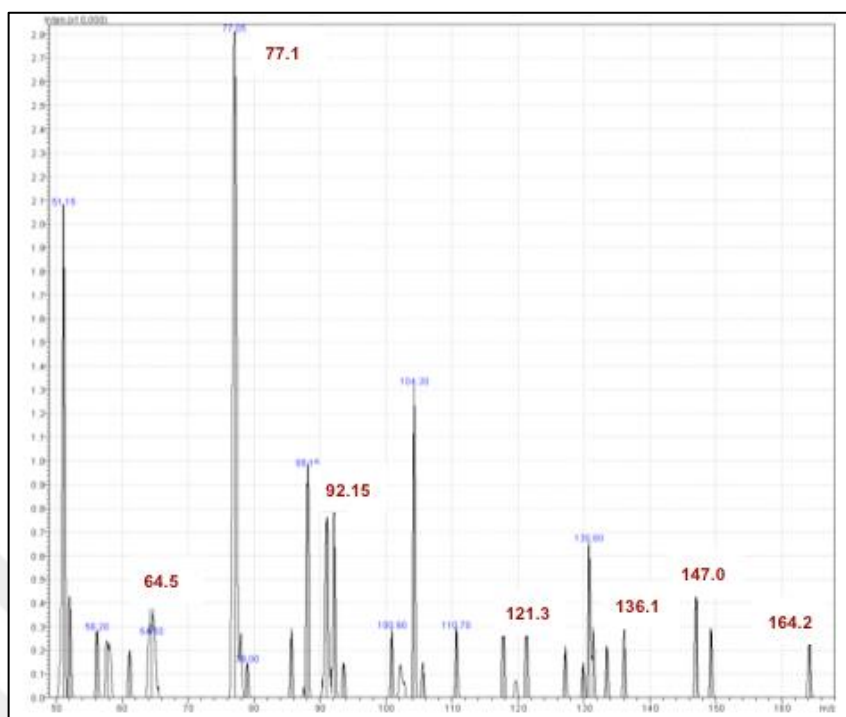


Figure 4.49. LC-MSMS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution

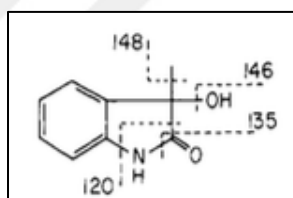


Figure 4.50. Fragment ions of 3-hydroxy-3-methyl-oxindole [68]

HR-MS analysis. The purified unpredicted product was screened in HR-MS for both 164g/mol (Figure 4.51) and 254 g/mol (Figure 4.52). It was positive ionization, therefore the masses were determined as +1. The calculated mass is the same with the 3-hydroxy-3-methyl-oxindole. However, when the results at 254 g/mol were investigated, it can be concluded that there is no desired product (Table 4.13-b-c) because the calculated mass is not the same with the exact mass. (PPM value is high)[74] .This purified sample is belong to the 3-hydroxy-3-methyl-oxindole (Table 4.13-a).

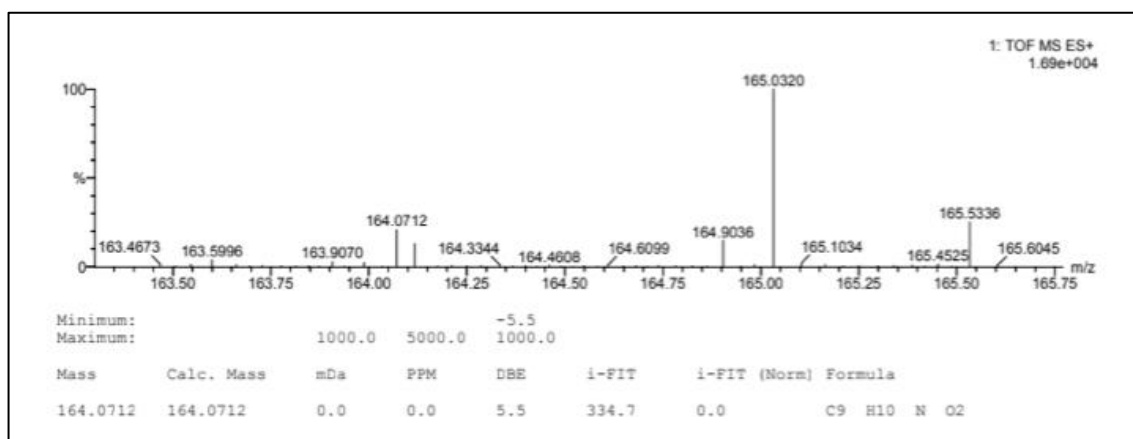


Figure 4.51. HR-MS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution at 164 g/mol

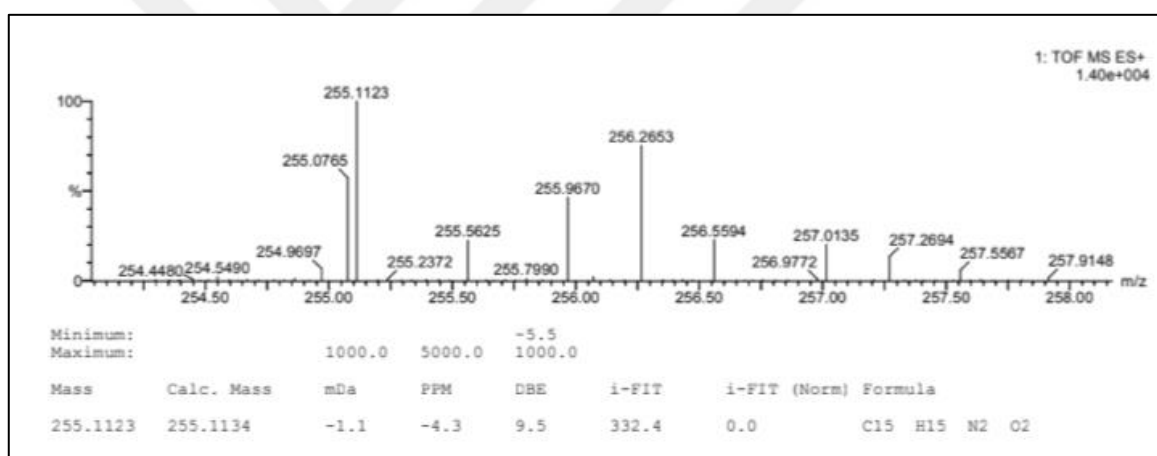


Figure 4.52. HR-MS analysis of the possible product the HPLC peak of which appears at 5.2 min in isocratic elution at 254 g/mol

To understand that if there is any evidence for the desired product (Table 4.13-b-c), HR-MS analysis were made for the reaction mixture. The results were given in Figure 4.53 for 254 g/mol and Figure 4.54 for 164 g/mol. It can be concluded that there are both the unpredicted and the desired product present in the reaction mixture. However, at isocratic elution in HPLC, there was no any other new peak except the peak of the unpredicted product (Table 4.13-a) at 5.2 min. Therefore, another HPLC method (gradient elution) was studied to determine the retention time of the desired product (Table 4.13-b-c).

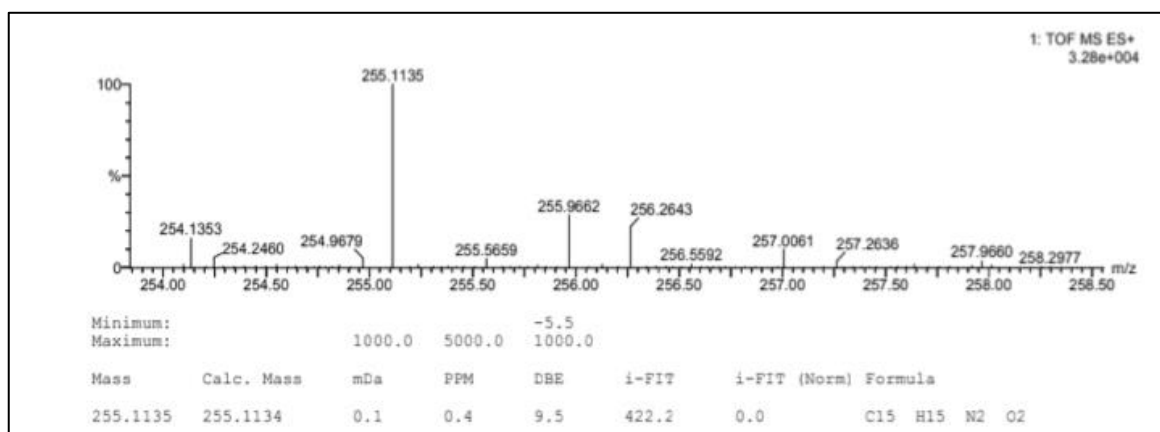


Figure 4.53. HR-MS analysis of reaction mixture at 254 g/mol

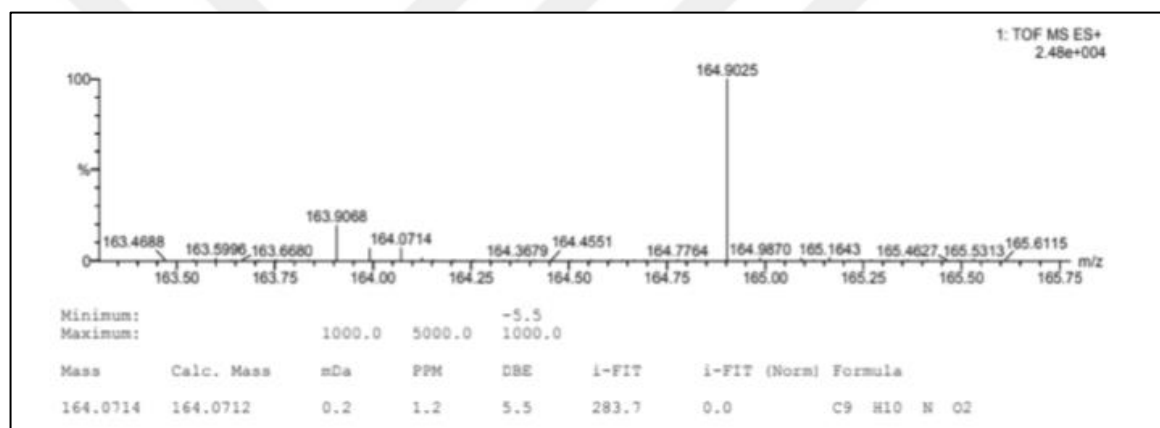


Figure 4.54. HR-MS analysis of reaction mixture at 164 g/mol

4.6.2.2. Computational analysis for decomposition of the products

The characterization studies explored that amination product is produced, but after this production, it is decomposed to 3-hydroxy-3-methyl-oxindole. Therefore, the decomposition was searched with quantum mechanics and hydroxyamination and amination products were protonated. The hydroxyamination product was protonated from;

- Oxygen of the nitrosobenzene (Figure B.1)
- Nitrogen of the nitrosobenzene (Figure B.2)
- Both the oxygen and nitrogen of the nitrosobenzene (Figure B.3). The distance between the nitrogen and the oxygen of the nitrosobenzene was increased, and the relative energies were calculated. The activation energy is high when the nitrogen of

the nitrosobenzene is protonated. However, when the oxygen is protonated, it becomes water and it breaks the bond with the nitrogen of the nitrosobenzene. Therefore, it can be concluded that the hydroxyamination product can also be decomposed after the the oxygen protonated.

The aminoxylation product was protonated from;

- Oxygen of the nitrosobenzene (Figure 4.55)
- Nitrogen of the nitrosobenzene (Figure B.4)
- Both the oxygen and nitrogen of the nitrosobenzene (Figure B.5).

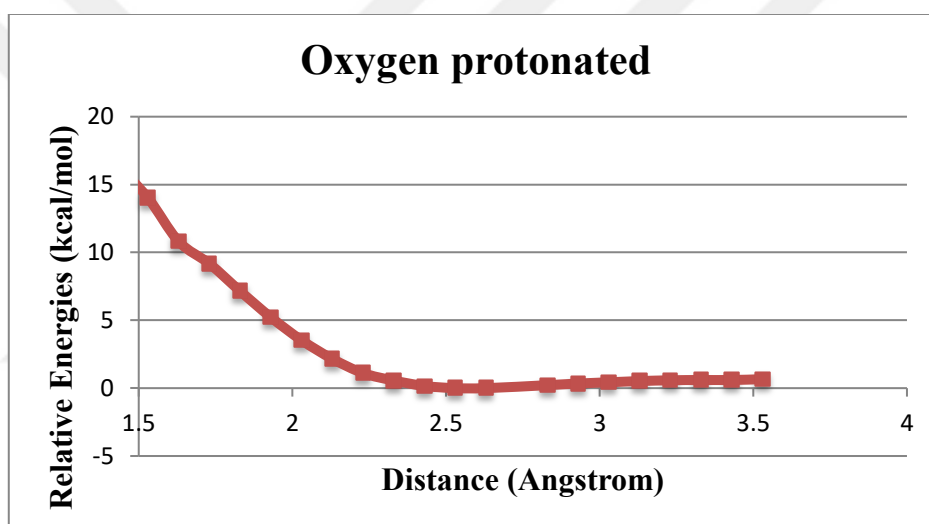


Figure 4.55. Relative energies vs distance graph for oxygen protonated for aminoxylation product

The distance between the nitrogen and the oxygen of the nitrosobenzene was increased, and the relative energies were calculated. There is no activation energy for the protonated oxygen which gives the 3-hydroxy-3-methyl-oxindole product as explained in the experimental part. However, the other protonated products have higher activation barriers. Therefore, it can be concluded that if the aminoxylation product find a proton during the reaction, it can decompose to the 3-hydroxy-3-methyl-oxindole as obtained.

4.6.2.3. Gradient Elution

The HPLC for the 3-methyl-2-oxindole (Figure A.4), nitrosobenzene (Figure A.5), the reaction mixture (Figure A.6) and the unpredicted product (Figure A.7) were given in Appendix. In the reaction mixture, nitrosobenzene was the limiting reagent and was

completely consumed, oxindole was the excess reagent. The retention time of oxindole is 3.5 min, but also there is a peak belonging to an impurity at 2.5 min (Figure A.8). The retention time of nitrosobenzene is 9.1 min. However, there are still, so many peaks to be determined. When literature was searched, it was observed that nitrosobenzene can undergo some series of reaction [75]. Therefore, degradation of nitrosobenzene was studied and it was left in a solvent a few days long, the HPLC results were given in Figure A.9. For this reason, the peaks at around 8-9 min and the peak at 15 min were considered to belong to nitrosobenzene and its decomposition products. Consequently, the possible peak for the product was considered as the new peak at 5.7 min. The purification was made with column chromatography (hexane : ethyl acetate ; 10-4:1). Characterization studies were carried out for the purified compound which gives peak at 5.7 minutes (Figure A.10).

¹H-NMR for the possible product giving HPLC peak at 5.7 min. As shown in Figure 4.56, there is only one peak at 9 ppm. The aromatic peaks were seen around 7 ppm. The methyl group at C-3 position was observed at 1.3 ppm. When the integrals were taken, there are only four hydrogens at aromatic region. Therefore, it can be concluded that, this purified peak at 5.7 min in HPLC did not belong to the desired product.

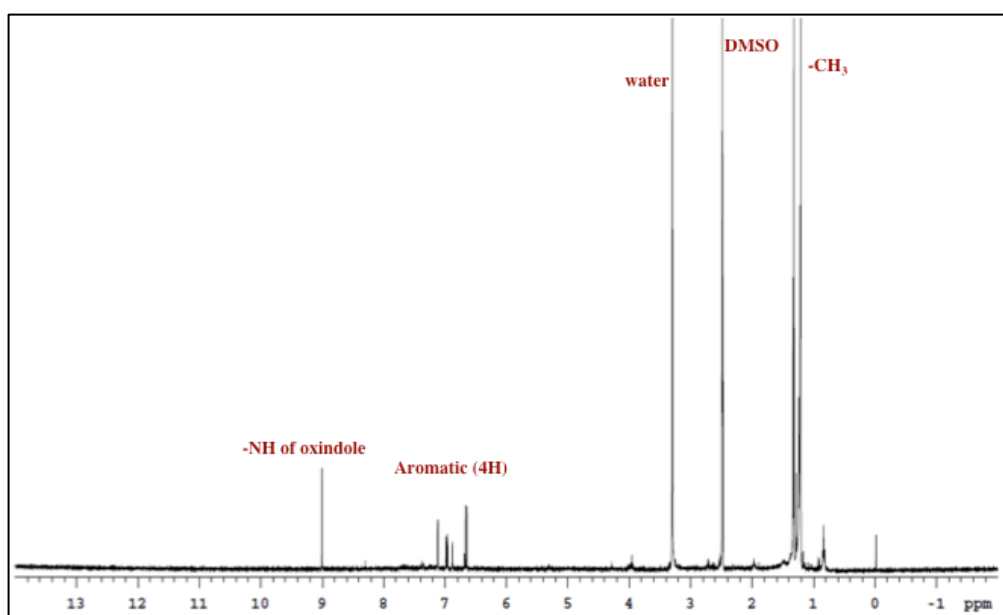


Figure 4.56. ¹H-NMR spectrum of the possible product the HPLC peak of which appears at 5.7 min in gradient elution

^{13}C -NMR for the possible product giving HPLC peak at 5.7 min. The NMR spectrum was given in Figure 4.57. It can be concluded When the results were compared with the literature [31–33, 61, 66], that there are many peaks around 30-40 (alkyl or alkene region) ppm, but the peaks around the aromatic region (around 120-140 ppm) is less than expected. Therefore, this peak at 5.7 min in HPLC could not belong to the desired product (Table 4.13-b-c).

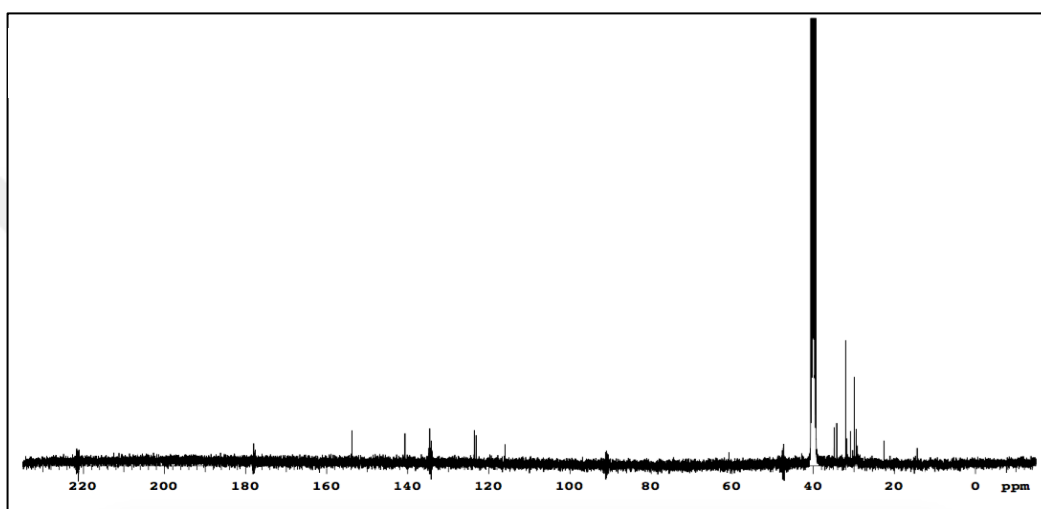


Figure 4.57. ^{13}C -NMR spectrum of the possible product the HPLC peak of which appears at 5.7 min in gradient elution

HR-MS analysis. To determine the peak at 5.7 min at gradient elution, HR-MS analysis (Figure 4.58) was also investigated. The possible molecular weight and the fractions of the desired product given in literature were searched, but they could not be located in the spectrum. Therefore, only the synthesis of 3-hydroxy-3-methyl-oxindole (unpredicted product) was studied in this work.

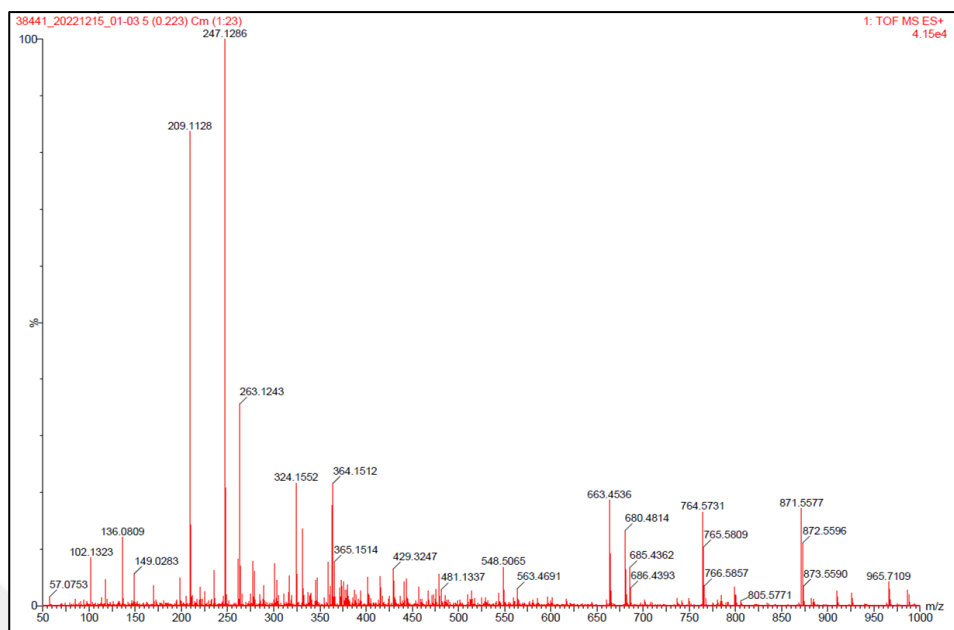


Figure 4.58. HR-MS analysis of the possible product the HPLC peak of which appears at 5.7 min in gradient elution

4.6.3. Optimization Study for the product

These optimization studies (Table 4.14) were considered for both isocratic and gradient elution in HPLC. In both HPLC studies, the only difference is the retention time of the oxindole and the product. The retention time of the product is 5.2 min at the isocratic elution for the given conditions, 2.1 min at the gradient elution for the given conditions. The retention time of the oxindole is 10 min at the isocratic elution, 3.5 min at the gradient elution.

From the results of the isocratic elution, it can be clearly seen that the peak of 3-hydroxy-3-methyl-oxindole is at 5.2 min for this method and the optimizations were made based on this unpredicted product. Factors effecting the product formation, and enantioselectivity were studied. These are:

1. Solvent
2. Reaction medium
3. Reactants ratio
4. Temperature

From the results of the gradient elution, it can be clearly seen that the peak of 3-hydroxy-3-methyl-oxindole is at 2.1 min by this method and the optimizations were made based on this unpredicted product. Factors effecting the product formation, and enantioselectivity were studied. These are:

1. Reaction time
2. Catalyst
3. Temperature (when the synthesized catalysts were used)

Table 4.14. Reaction conditions (3M2O: 3-methyl-2-oxindole; N: nitrosobenzene; ratio: 3-methyl-2-oxindole: nitrosobenzene ratio; THF: tetrahydrofuran)

Reaction Number	Reactant		Catalyst	Solvent	t (h)	T (°C)	Medium	Ratio
1	3M2O	N	Quinine (20%)	Toluene	48	25	atm	3:1
2	3M2O	N	Quinine (20%)	Toluene	48	25	atm	1:1
3	3M2O	N	Quinine (20%)	Toluene	48	25	atm	1:3
4	3M2O	N	Quinine (20%)	Toluene	72	25	atm	3:1
5	3M2O	N	Quinine (20%)	Toluene	72	25	Nitrogen	3:1
6	3M2O	N	Quinine (20%)	DEE	48	25	atm	3:1
7	3M2O	N	Quinine (20%)	THF	48	25	atm	3:1
8	3M2O	N	Quinine (30%)	Toluene	24	0	atm	3:1
9	3M2O	N	Quinine (30%)	Toluene	24	25	atm	3:1
10	3M2O	N	Quinine (30%)	Toluene	24	50	atm	3:1

11	3M2O	N	Cinchonidine (20%)	THF	96	25	atm	1:1
12	3M2O	N	CAT20	THF	96	25	atm	1:1
13	3M2O	N	CAT36	THF	96	25	atm	1:1
14	3M2O	N	CAT38S	THF	96	25	atm	1:1
15	3M2O	N	CAT20	THF	72	0	atm	1:1
16	3M2O	N	CAT36	THF	72	0	atm	1:1
17	3M2O	N	CAT38S	THF	72	0	atm	1:1

4.6.3.1. Effect of reactant ratio

For determining the effect of reactant ratio, all the conditions were kept the same except the reaction rate and different reactant ratios were used to determine its effect on the product formation. The reactant ratio was determined as the ratio of mole of oxindole to mole of nitrosobenzene. 3:1 ratio was used in Reaction 1 (Figure A.11), 1:1 ratio was used in Reaction 2 (Figure A.12) and 1:3 ratio was used in Reaction 3 (Figure A.13) as shown in Table 4.15. When the area under the curve was examined, 1:1 and 3:1 gave close results. The ratio was calculated as shown below.

$$\text{ratio} = \frac{\text{Area of the product peak at 5 min peak}}{\text{Area of (the product peak at 5 min peak + the excess reagent peak at 10 min peak)}}$$

Table 4.15. The effect of the reactant ratio

Comparison Conditions		Retention Time (min)	Area	Ratio
3:1	Reaction 1	5.409	1467490	0.567
		10.337	1118877	
1:1	Reaction 2	5.464	927890	0.599
		10.593	621774	
1:3	Reaction 3	5.463	958958	0.411
		10.588	1376050	

4.6.3.2. Effect of reaction medium

For determining the effect of reaction medium, all the conditions except the reaction medium were kept the same and medium was changed to determine its effect on the product formation. Atmospheric medium was used in Reaction 4 (Figure A.14), and nitrogen medium was used in Reaction 5 (Figure A.15) as shown in Table 4.16. When the area under the curve was examined, atmospheric medium gave higher ratio. Therefore, atmospheric medium was chosen as the reaction medium.

Table 4.16. The effect of the reaction medium

Comparison Conditions		Retention Time (min)	Area	Ratio
Atmospheric	Reaction 4	5.233	1121937	0.871
		8.545	165508	
Under Nitrogen	Reaction 5	5.171	1667283	0.436
		8.376	2160894	

4.6.3.3. Effect of solvent

For determining the solvent effect, all the conditions were kept same except the solvent and different solvents were used to compare their effects on the product formation. Toluene was used as the reaction solvent in Reaction 1 (Figure A.11), diethyl ether was used in Reaction 6 (Figure A.16) and tetrahydrofuran was used in Reaction 7 (Figure A.17) as shown in Table 4.17. When the area under the curve was examined, toluene was found as the best solvent, but THF is also can be used.

Table 4.17. The solvent effect

Comparison Conditions		Retention Time (min)	Area	Ratio
Toluene	Reaction 1	5.409	1467490	0.567
		10.337	1118877	
DEE	Reaction 6	5.456	1125324	0.349
		10.231	2095715	
THF	Reaction 7	5.460	1205076	0.554
		10.582	969324	

4.6.3.4. Effect of reaction temperature

For determining the effect of reaction temperature, all the conditions except the reaction temperature were kept the same and temperature was changed. 0°C was used in Reaction 8 (Figure A.18), 25°C was used in Reaction 9 (Figure A.19) and 50°C was used in Reaction 10 (Figure A.20) as shown in Table 4.18. When the areas under the curves were examined, the results from the reactions at 25°C and 50°C are very close. Therefore, room temperature was chosen as the reaction temperature.

Table 4.18. The effect of the reaction temperature

Comparison Conditions		Retention Time (min)	Area	Ratio
0°C	Reaction 8	5.262	1792691	0.737
		9.848	640200	
25°C	Reaction 9	5.313	2314990	0.824
		9.971	495025	
50°C	Reaction 10	5.254	2039761	0.849
		9.965	363308	

4.6.3.5. Effect of reaction time and catalyst

For determining the effect of time on the product formation, all the conditions except the reaction time were kept the same. The catalysts cannot be solved in toluene, therefore THF was used as solvent. **CAT38** could not be examined. This is because it was decomposed during the storage. Cinchonidine was used in Reaction **11** (Figure A.21-A.24), **CAT20** was used in Reaction **12** (Figure A.25-A.28), **CAT36** was used in Reaction **13** (Figure A.29-A.32), and **CAT38S** was used in Reaction **14** (Figure A.33-A.36) as shown in Table 4.19.

Table 4.19. The effect of the reaction time and the catalyst

Comparison Conditions		Retention Time (min)	Area	Ratio
Cinchonidine	Reaction 11 -24h	2.188	425373	0.332
		3.578	854324	
	Reaction 11 -48h	2.182	841094	0.689
		3.559	379976	
	Reaction 11 -72h	2.178	1072429	0.896
		3.548	124699	
	Reaction 11 -96h	2.179	853763	0.940
		3.545	54253	
CAT20	Reaction 12 -24h	2.163	217203	0.101
		3.490	1942680	
	Reaction 12 -48h	2.161	438782	0.277
		3.483	1144787	
	Reaction 12 -72h	2.163	574225	0.441
		3.490	727901	
	Reaction 12 -96h	2.161	186867	0.360
		3.488	331911	
CAT36	Reaction 13 -24h	2.173	382074	0.217
		3.534	1381533	
	Reaction 13 -48h	2.167	651787	0.480
		3.507	706728	

CAT36	Reaction 13 -72h	2.164	846780	0.717
		3.500	334693	
	Reaction 13 -96h	2.166	558503	0.770
		3.499	167265	
CAT38S	Reaction 14 -24h	2.163	244445	0.104
		3.490	2102547	
	Reaction 14 -48h	2.163	535377	0.304
		3.491	1223761	
	Reaction 14 -72h	2.162	670674	0.880
		3.492	914162	
	Reaction 14 -96h	2.157	242658	0.382
		3.487	393085	

For determining the effect of catalyst on the product formation, all the conditions except the catalyst were kept the same. In this analysis, gradient elution in HPLC was used. The ratio was calculated as shown below.

$$\text{ratio} = \frac{\text{Area of the product peak at 2.1 min peak}}{\text{Area of (the product peak at 2.1 min peak + the excess reagent peak at 3.5 min peak)}}$$

When the areas under the curves were examined, 72 hours can be considered as the optimum reaction time.

4.6.3.6. Effect of reaction temperature with the synthesized catalysts

For determining the effect of the reaction temperature, all the conditions except the reaction temperature were kept the same and temperature was changed. **CAT20** was used in Reaction **15** (Figure A.37), **CAT36** was used in Reaction **16** (Figure A.38) and **CAT38S** was used in Reaction **17** (Figure A.39) as shown in Table 4.20. When the areas under the curves were examined, the product obtained at 25°C has higher ratio than that obtained at 0°C. However, in literature, lower temperature increases enantioselectivity [61, 76, 77]. Therefore, these reactions were also examined for the enantioselectivity.

Table 4.20. The effect of the reaction temperature with the synthesized catalysts

Comparison Conditions		Retention Time (min)	Area	Ratio
CAT20	Reaction 12 -72h	2.163	574225	0.441
		3.490	727901	
CAT36	Reaction 13 -72h	2.164	846780	0.717
		3.500	334693	
CAT38S	Reaction 14 -72h	2.162	670674	0.880
		3.492	914162	
CAT20 - 0°C	Reaction 15	2.159	1112848	0.192
		3.493	4673139	
CAT36 - 0°C	Reaction 16	2.155	545788	0.105
		3.490	4641685	
CAT38S - 0°C	Reaction 17	2.157	1385150	0.153
		3.490	7646942	

4.6.3.7. Enantioselectivity

To study the change in the enantioselectivity of the reactions at different temperatures with the studied catalysts, the reactions including **CAT20**, **CAT36** and **CAT38S** were also studied under 0°C, since as known the enantioselectivity increases with decreasing temperature [61, 76, 77]. The enantioselectivity of each synthesized catalyst was determined by chiral HPLC. At the beginning, the 3-hydroxy-3-methyl-oxindole was analyzed by HPLC to observe the peaks of the unpredicted product (Figure A.40). Retention time of the 3-methyl-2-oxindole was found as 10 min in the given conditions (Figure A.41). All the samples were dissolved in ethanol, therefore pure ethanol was also analyzed by HPLC and retention time of ethanol was determined as 5 min (Figure A.42). In this method, normal phase HPLC with a chiral column was applied. **CAT20** was used in Reaction **15** (Figure A.43) at 0°C, **CAT36** was used in Reaction **16** (Figure A.44) at 0°C, and **CAT38S** was used in Reaction **17** (Figure A.45) at 0°C as shown in Table 4.14. In addition, the same catalysts were examined at room temperature. Cinchonidine was used in Reaction **11** (Figure A.46) at room temperature, **CAT20** was used in Reaction **12** (Figure A.47) at room temperature,

CAT36 was used in Reaction **13** (Figure A.48) at room temperature, and **CAT38S** was used in Reaction **14** (Figure A.49) at room temperature. The enantioselectivity of each synthesized catalyst for the given reactions were listed in Table 4.21. The enantioselectivities were calculated from the peaks of the two enantiomers at different temperatures.

Table 4.21. Enantioselectivity achieved by the synthesized catalysts determined by HPLC data

Comparison Conditions		Retention Time (min)	Area	ee%
CAT20-0°C	Reaction 15	14.250	13982857	95.4
		16.613	327711	
CAT36-0°C	Reaction 16	14.245	11604422	98.4
		16.566	93193	
CAT38S-0°C	Reaction 17	14.237	18370993	72.6
		16.793	2916515	
CAT20-rt	Reaction 12	13.906	9642048	62.2
		16.557	2244497	
CAT36-rt	Reaction 13	13.889	11746520	50.4
		16.544	3867378	
CAT38S-rt	Reaction 14	13.900	8094516	29.6
		16.699	4402099	
Cinchonidine	Reaction 11	13.895	1250408	28.8
		16.592	690416	

When the enantioselectivity capacities of the catalysts were compared (Reaction **11-14**), the synthesized catalysts (derivative of cinchonidine) cause higher enantioselectivity when compared to cinchonidine. In addition, decreasing the temperature increases the enantioselectivity as expected. The best catalyst among the studied catalysts can be considered as **CAT36**, the urea-based cinchona catalyst.

5. CONCLUSION AND FUTURE WORK

Regarding the theoretical calculations, in all TSs, it can be concluded that;

- Binding mode 3 has the lowest activation barrier. Therefore, addition and proton transfer occur in one step. For this concerted reaction mechanism, the formation of *N*-adduct is energetically more favorable than the *O*-adduct.
- In terms of regioselectivity, the reaction favors the hydroxyamination product with up to 99% for the urea and the thiourea-based catalysts. **CAT8**, the alcohol-based catalyst and **CAT14**, the amine-based catalyst can give both aminoxylation and hydroxyamination product.
- Urea-based catalysts have the lower activation energy than thiourea-based catalysts.
- The best catalyst is **CAT39**. However, the synthesis for derivation from both R₂ (C6') and R₃ (C9) groups (Figure 3.2) is more complicated. Therefore, other best catalysts were chosen to synthesize and this was suggested as a future work.
- All the cinchona alkaloids (cinchonine, cinchonidine, quinine, and quinidine) have a racemic mixture.
- All the catalysts that include urea and thiourea functional groups have 100% ee.
- Nine new catalyst candidates are proposed for experimental testing.

As a future work, decomposition pathway described by Companyó et al. can also be studied.

When all the experimental results were examined, it can be concluded that;

- Four different catalysts (**CAT20**, **CAT36**, **CAT38**, **CAT38S**) were synthesized from the selected catalysts. However, **CAT38** could not be used for the reaction, because it decomposed during storage, which indicates that it is not a stable compound. These catalysts were used in the reaction of oxindole with nitrosobenzene for the first time. Only the synthesis procedure of **CAT36** is known [65]. The others were synthesized accordingly.
- NMR studies of the reaction mixture revealed that the expected product was produced. For the purification of the product HPLC studies were carried out. The determination of the retention time of the product was studied by different reversed

HPLC methods. However, the peak of the expected product could not be determined in the chromatogram. Therefore purification, characterization and yield studies could not be performed. Except the NMR analysis, HR-MS analysis for the reaction mixture was also conducted to prove the formation of the product and its molecular weight was confirmed with this method.

- Analysis of the reaction mixture pointed out the formation of an unexpected decomposition product. It was proven to be 3-hydroxy-3-methyl-oxindole and be formed through decomposition of the aminoxylation product by different analytical and theoretical methods. All the further optimization studies were carried out for the decomposition product.
- As reaction solvents THF and toluene gave similar results, however only THF can solve the synthesized catalysts. Therefore, the optimal solvent was defined as THF.
- The ratio of oxindole to nitrosobenzene was also investigated for the maximum yield. 1:1 and 3:1 ratios gave close results. However, increasing the amount of the nitrosobenzene was not preferred.
- When comparing the results of the effects of the reaction medium, the best medium was assigned the atmospheric medium compared to the nitrogen medium.
- The results of the effect of the temperature pointed out that increasing the temperature also increase the product formation. Despite this fact, the room temperature was chosen for the most reactions since achieving a high enantioselectivity is also a significant for this project.
- Increasing the reaction time also increased the product formation. According to HPLC results, the optimum reaction time was selected as 72 hours.
- Among the synthesized catalysts, **CAT36** was determined as the best catalyst with the highest yield.
- For the enantioselectivity studies, the temperature was decreased to 0°C to be able to increase the enantioselectivity. However, this decrease in temperature also decreases the product formation.
- The enantioselectivities of the catalysts were determined by normal phase HPLC using a chiral column. The enantioselectivity increases with decreasing temperature which is consistent with literature [61, 76, 77]. In addition, **CAT36** has the highest enantioselectivity.

- The decomposition process studies demonstrated that when the aminoxylation product is protonated, it decomposes to 3-hydroxy-3-methyl-oxindole. Companyó et al. have a hypothesis for this decomposition (Figure 2.9) [33]. Therefore, the enantioselectivity results in experimental part were compared with those of the computational part of the aminoxylation product. All the urea and thiourea derivatives of the cinchona alkaloids have high enantioselectivity in the computational part. Consequently, the experimental results agree with the computational results.



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APPENDIX A: EXPERIMENTAL DATA

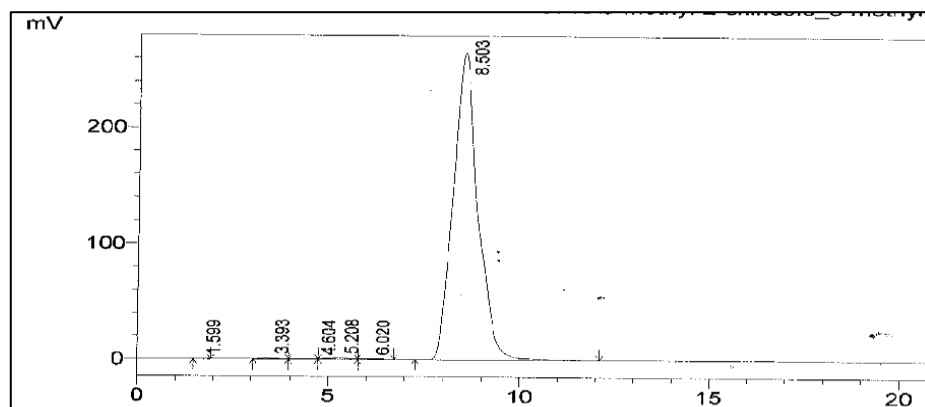


Figure A.1. The HPLC chromatogram of 3-methyl-2-oxindole (isocratic elution)

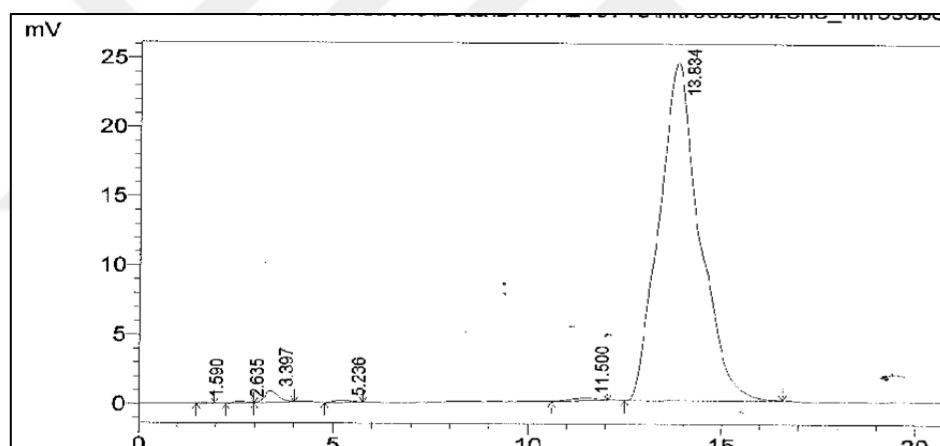


Figure A.2. The HPLC chromatogram of nitrosobenzene (isocratic elution)

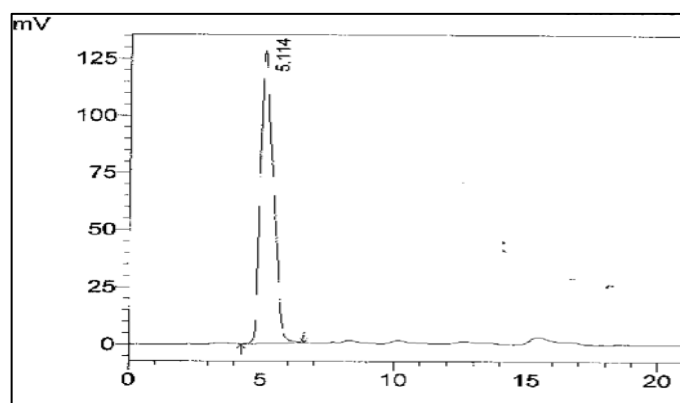


Figure A.3. The HPLC chromatogram of unpredicted product (isocratic elution)

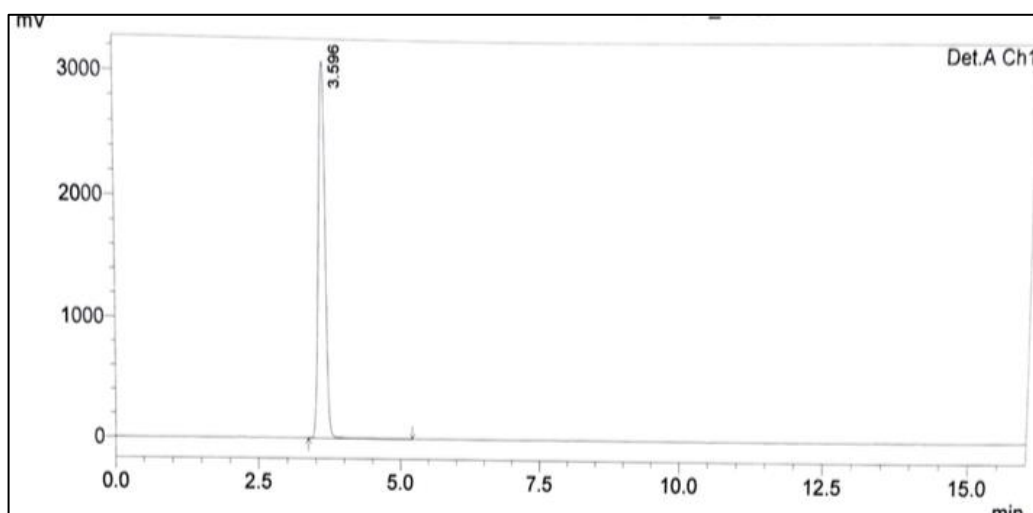


Figure A.4. The HPLC chromatogram of 3-methyl-2-oxindole (gradient elution)

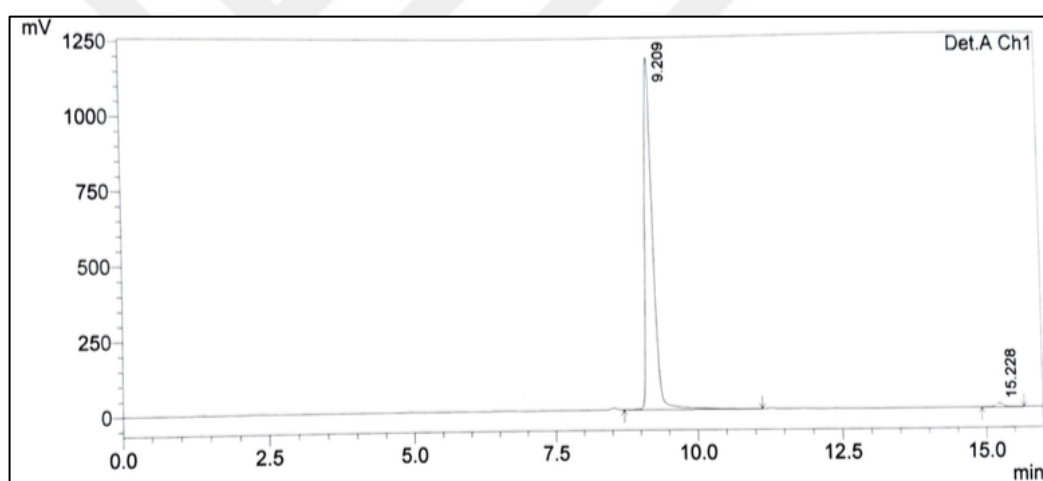


Figure A.5. The HPLC chromatogram of nitrosobenzene (gradient elution)

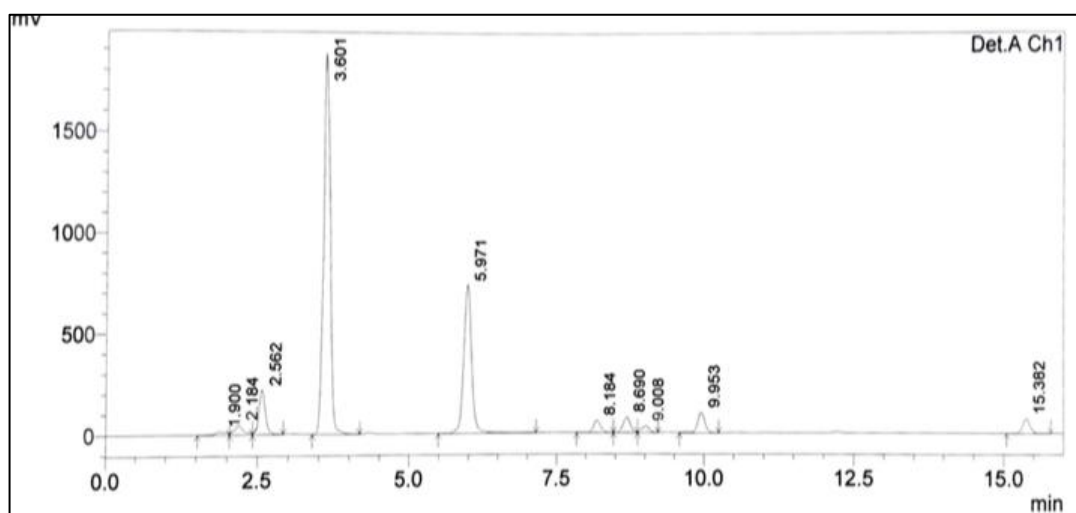


Figure A.6. The HPLC chromatogram of reaction mixture (gradient elution)

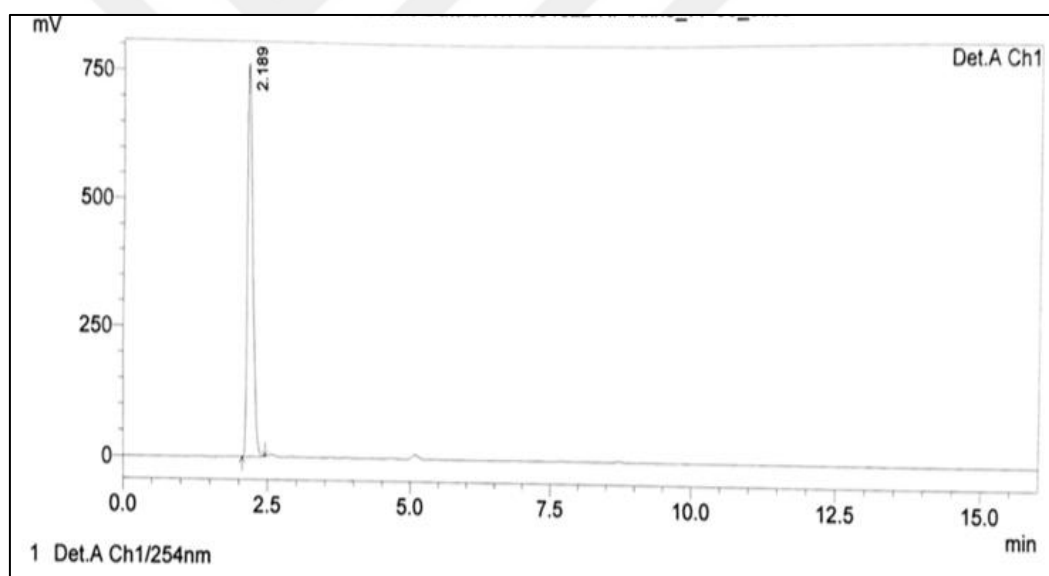


Figure A.7. The HPLC chromatogram of unpredicted product (gradient elution)

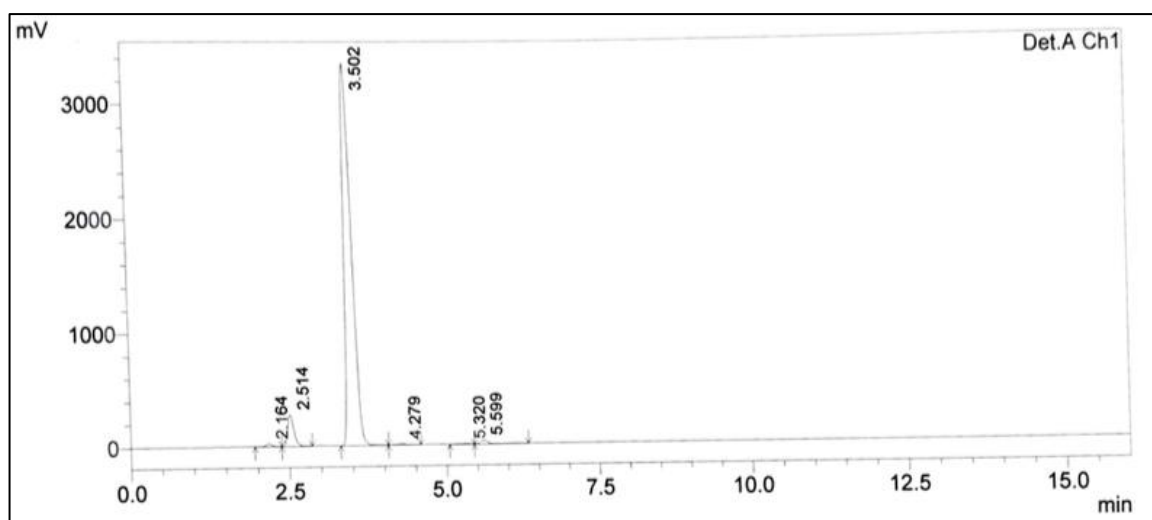


Figure A.8. Degredation of 3-methyl-2-oxindole (gradient elution)

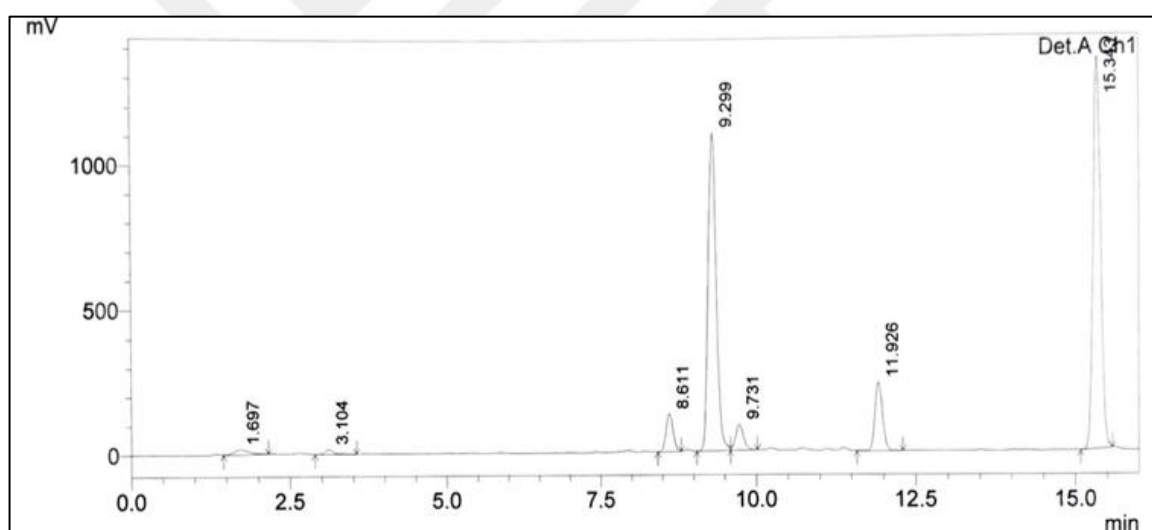


Figure A.9. Degredation of nitrosobenzene (gradient elution)

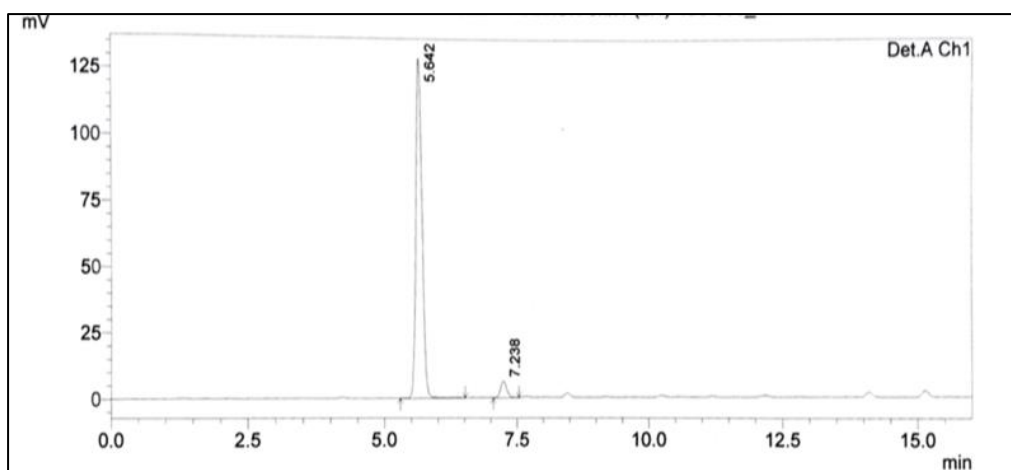


Figure A.10. The HPLC chromatogram of possible product (gradient elution)

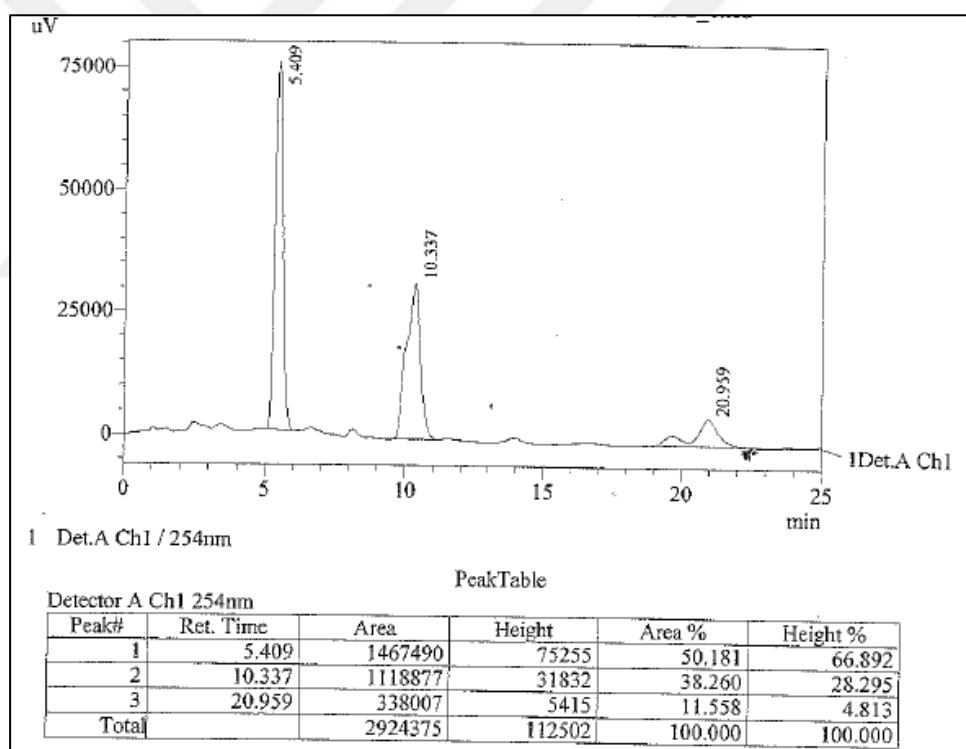


Figure A.11. The HPLC chromatogram of reaction mixture 1 (isocratic elution)
(Catalyst:QN)

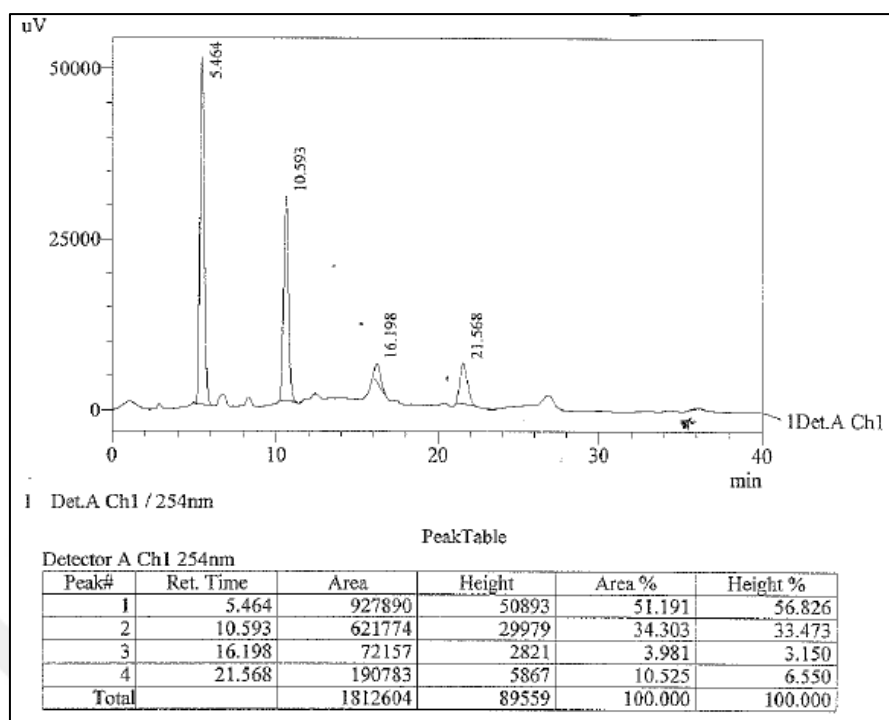


Figure A.12. The HPLC chromatogram of reaction mixture 2 (isocratic elution)
(Catalyst:QN)

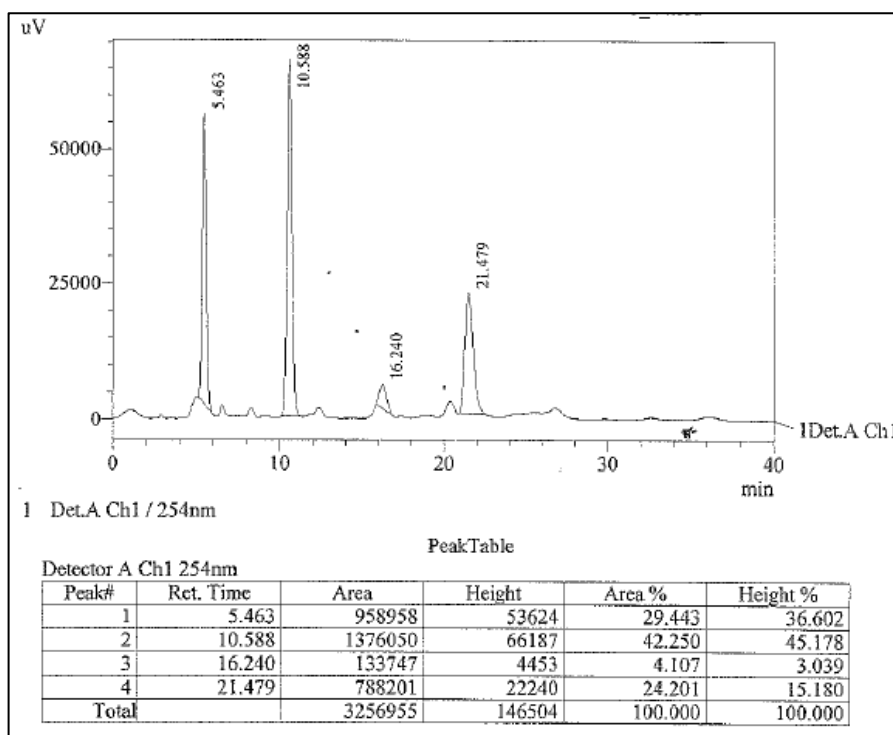


Figure A.13. The HPLC chromatogram of reaction mixture 3 (isocratic elution)
(Catalyst:QN)

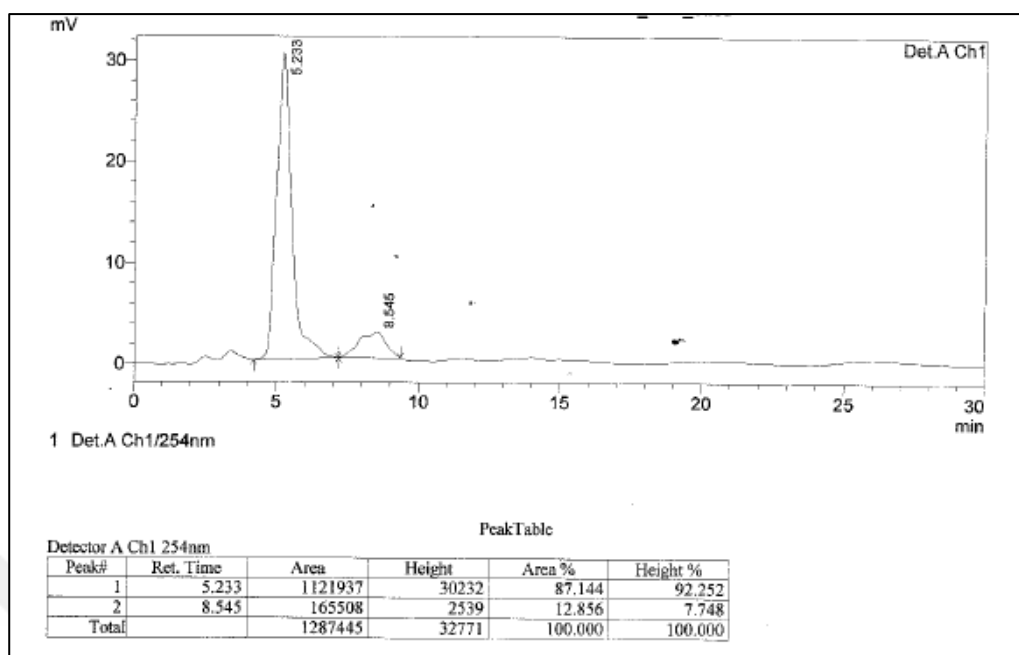


Figure A.14. The HPLC chromatogram of reaction mixture 4 (isocratic elution)
(Catalyst:QN)

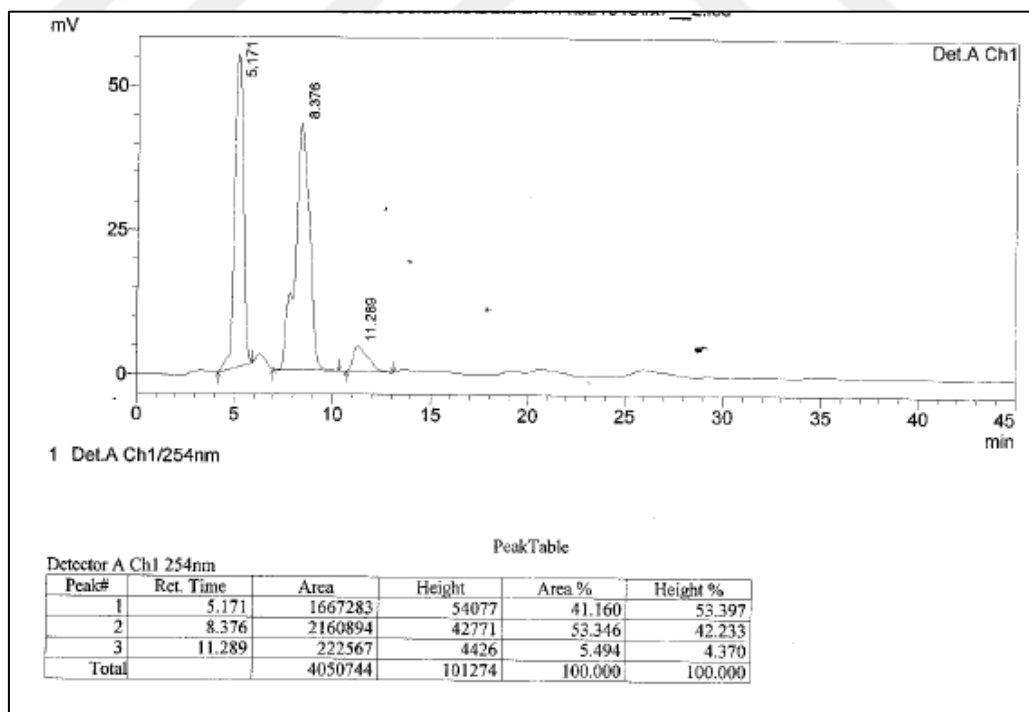


Figure A.15. The HPLC chromatogram of reaction mixture 5 (isocratic elution)
(Catalyst:QN)

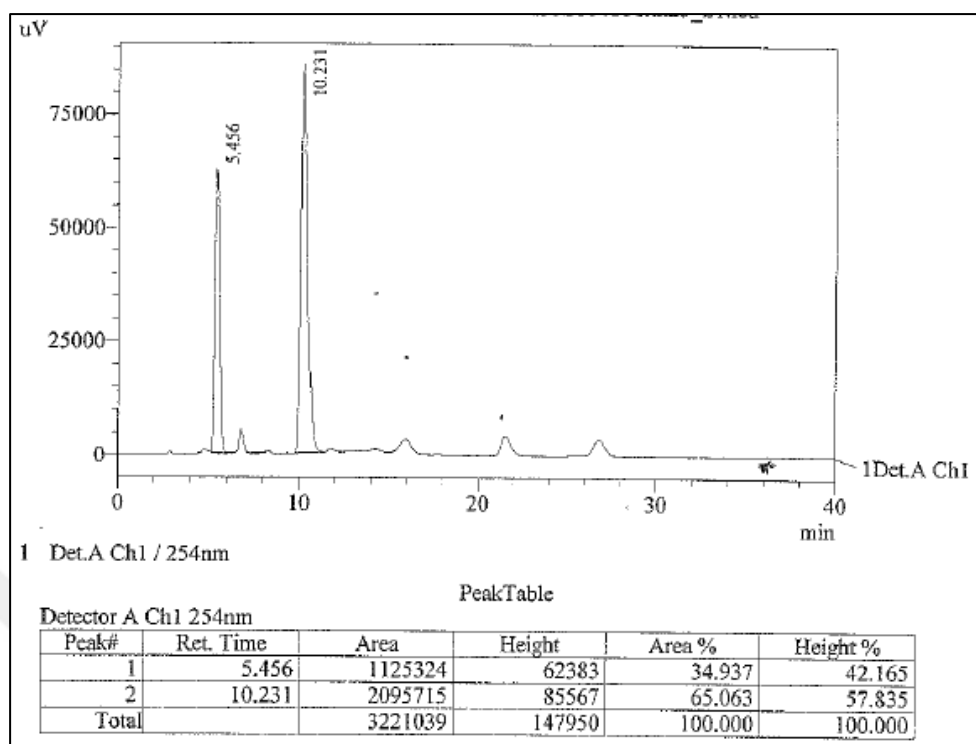


Figure A.16. The HPLC chromatogram of reaction mixture 6 (isocratic elution)
(Catalyst:QN)

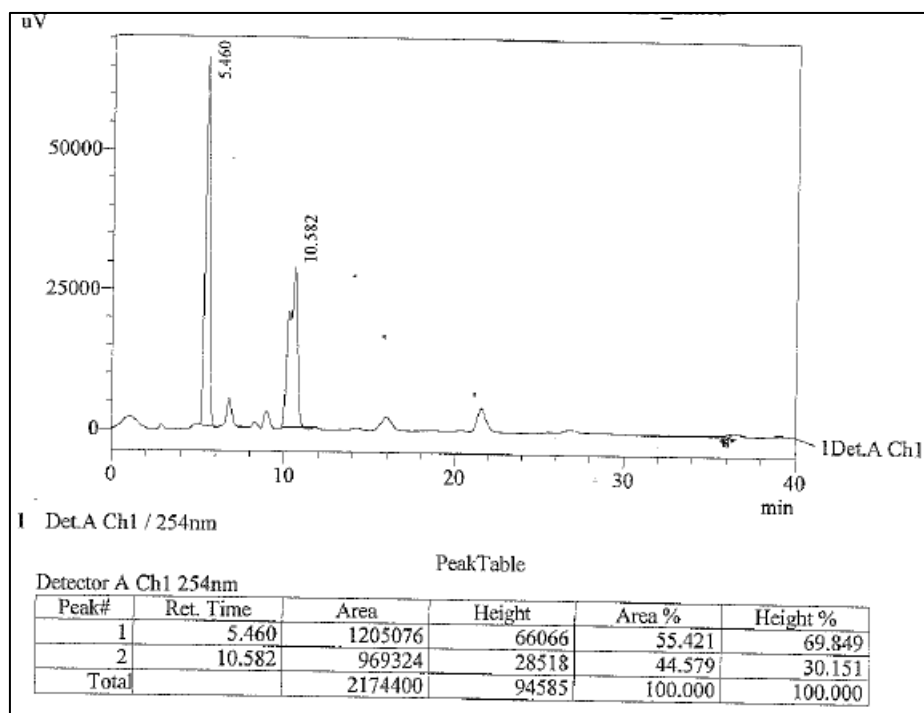


Figure A.17. The HPLC chromatogram of reaction mixture 7 (isocratic elution)
(Catalyst:QN)

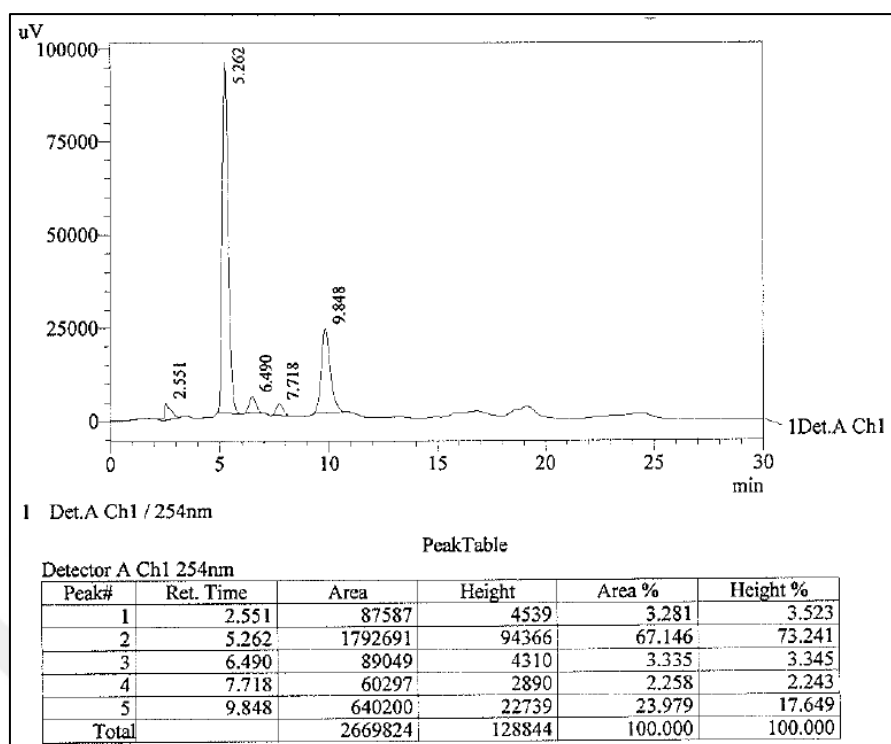


Figure A.18. The HPLC chromatogram of reaction mixture **8** (isocratic elution) (Catalyst:QN)

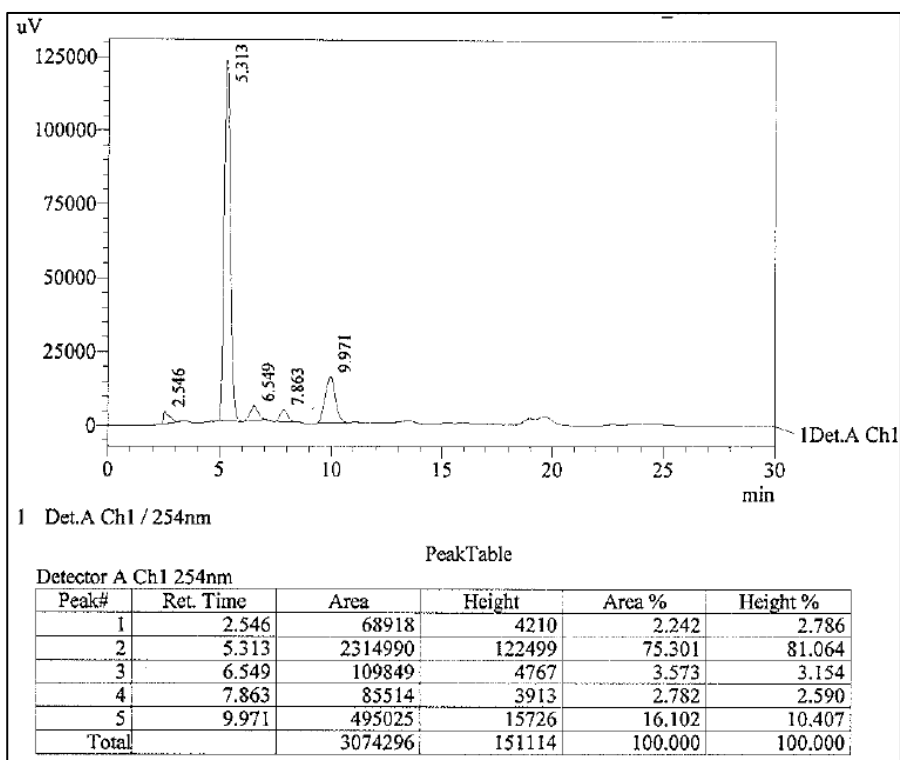


Figure A.19. The HPLC chromatogram of reaction **9** (isocratic elution) (Catalyst:QN)

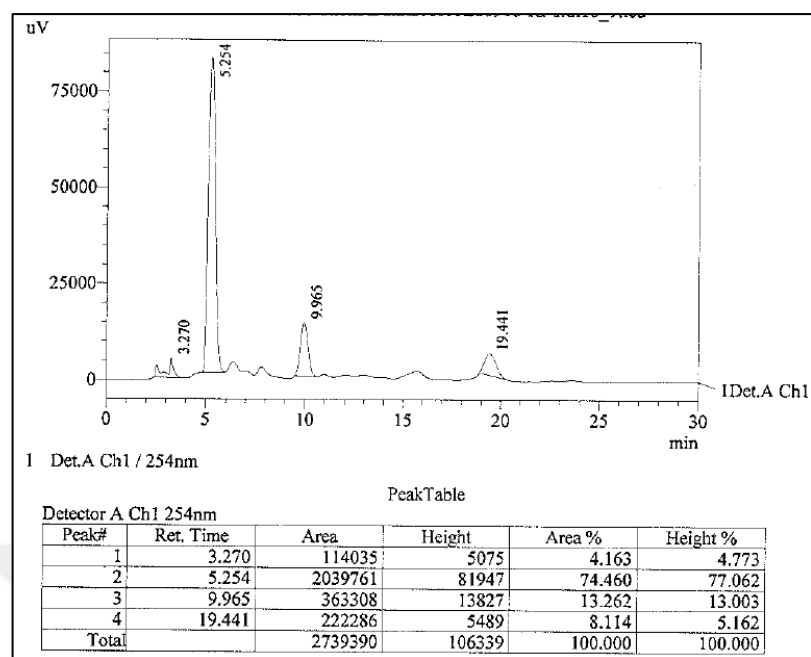


Figure A.20. The HPLC chromatogram of reaction mixture **10** (isocratic elution) (Catalyst:QN)

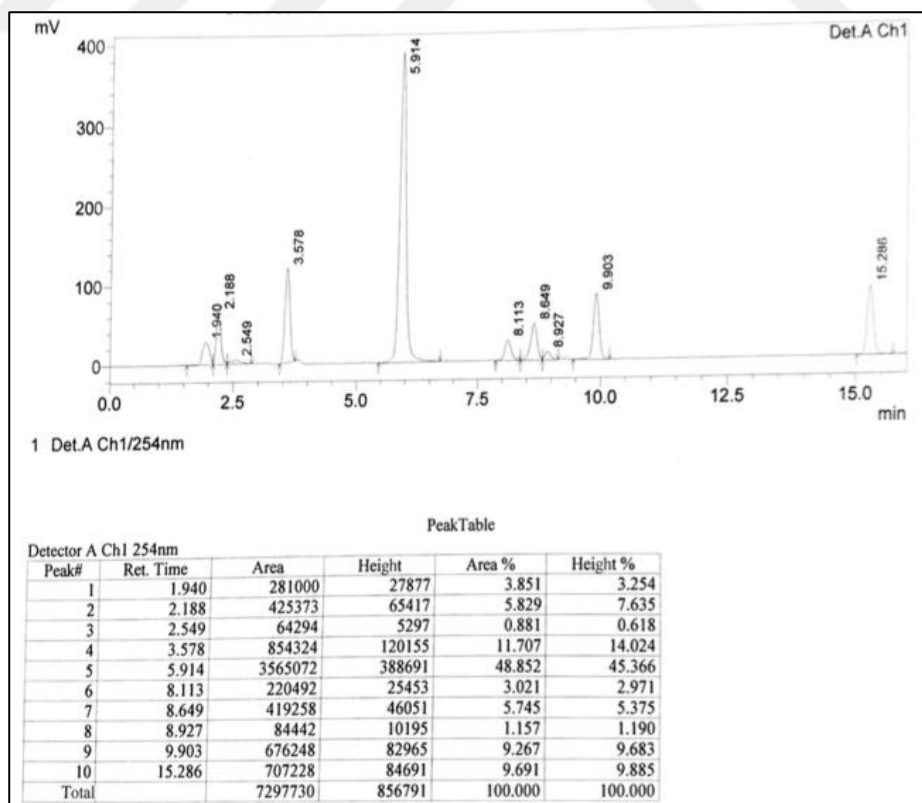


Figure A.21. The HPLC chromatogram of reaction mixture **11** at 24 hours (gradient elution) (Catalyst:CD)

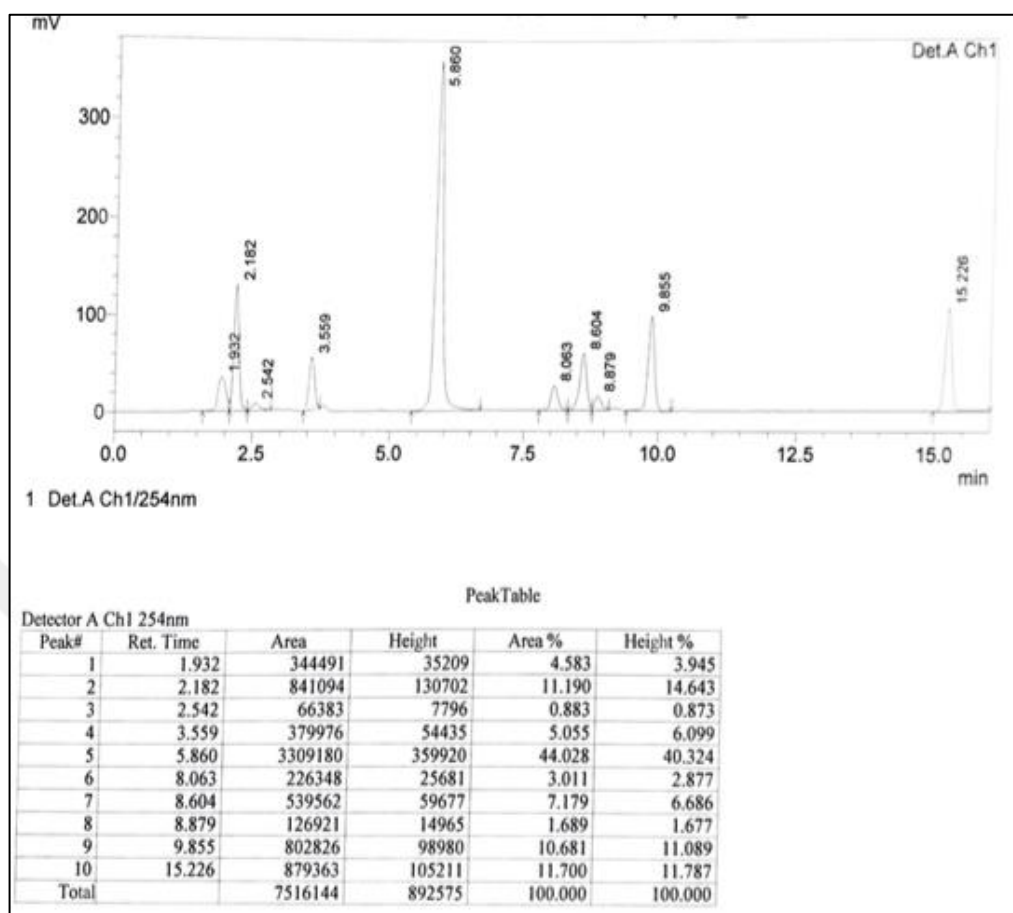


Figure A.22. The HPLC chromatogram of reaction mixture **11** at 48 hours (gradient elution) (Catalyst:CD)

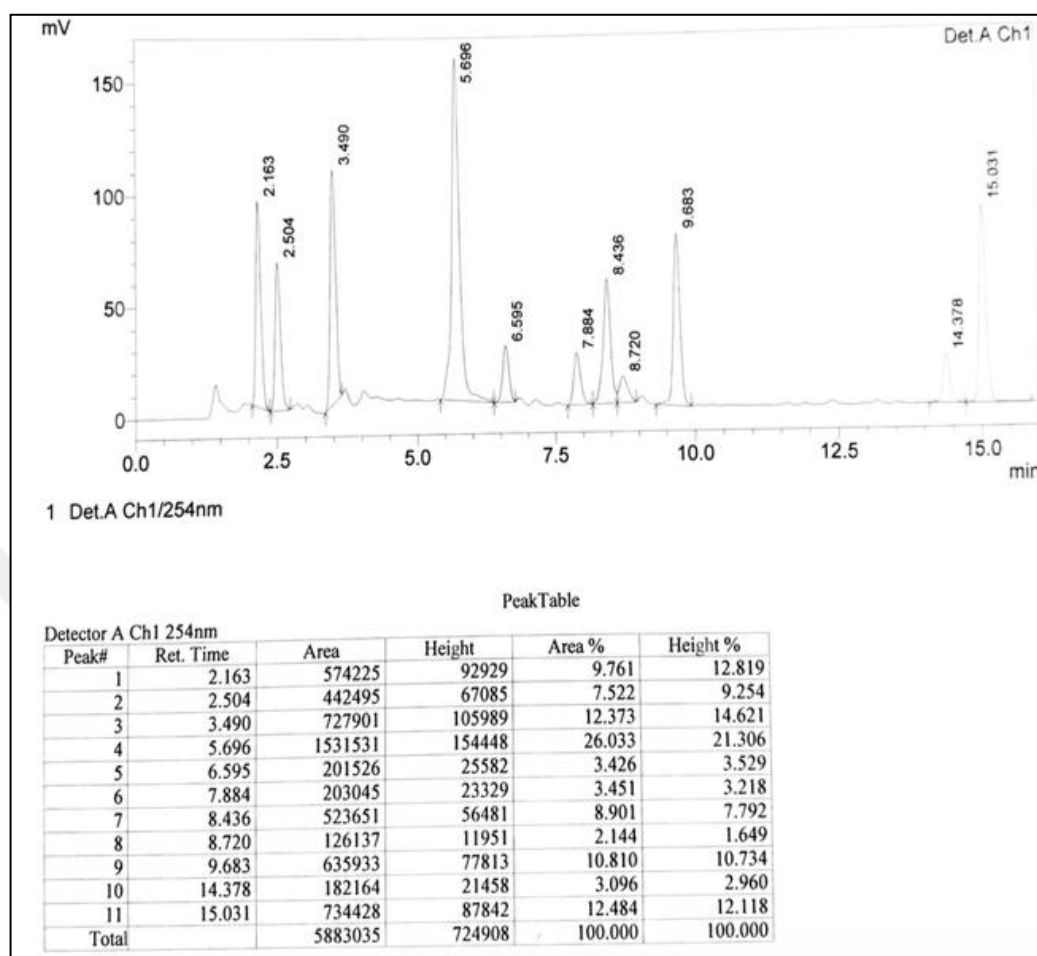


Figure A.23. The HPLC chromatogram of reaction mixture **11** at 72 hours (gradient elution) (Catalyst:CD)

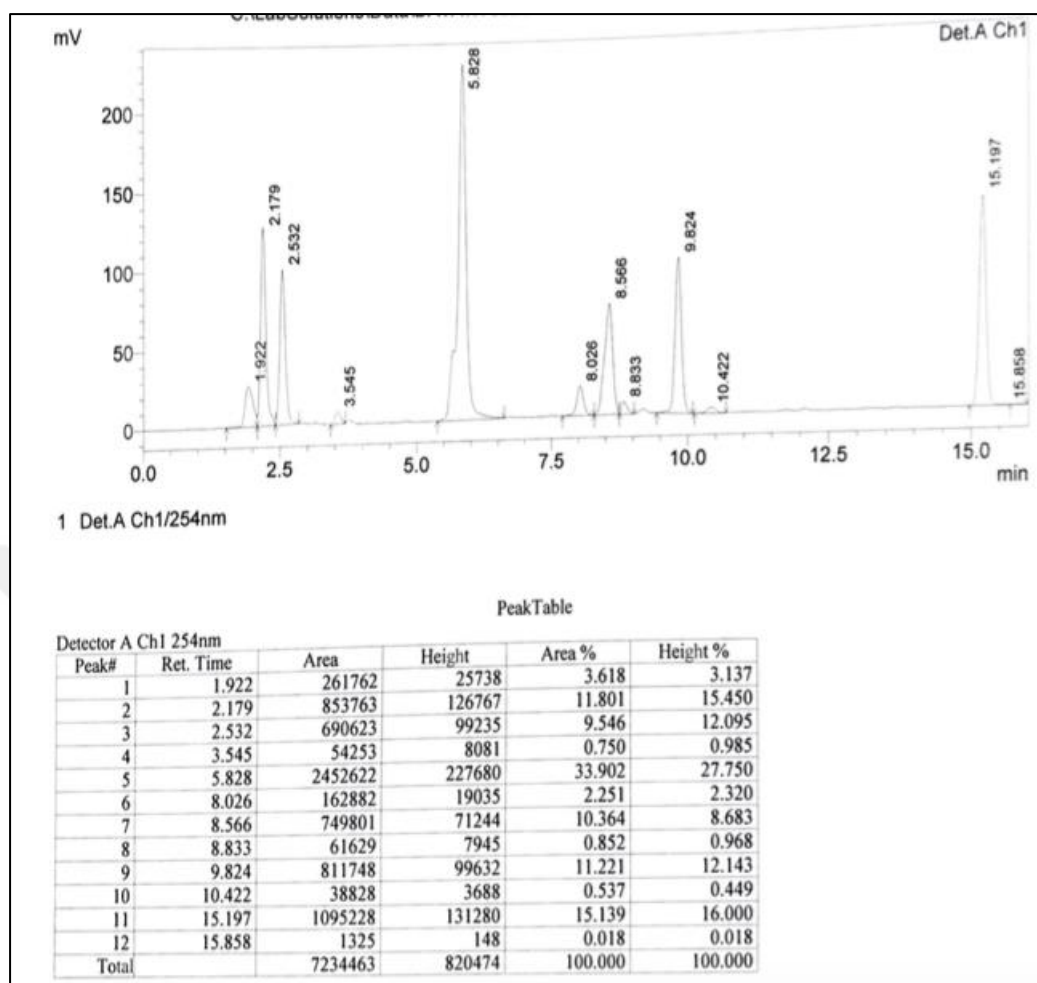


Figure A.24. The HPLC chromatogram of reaction mixture **11** at 96 hours (gradient elution) (Catalyst:CD)

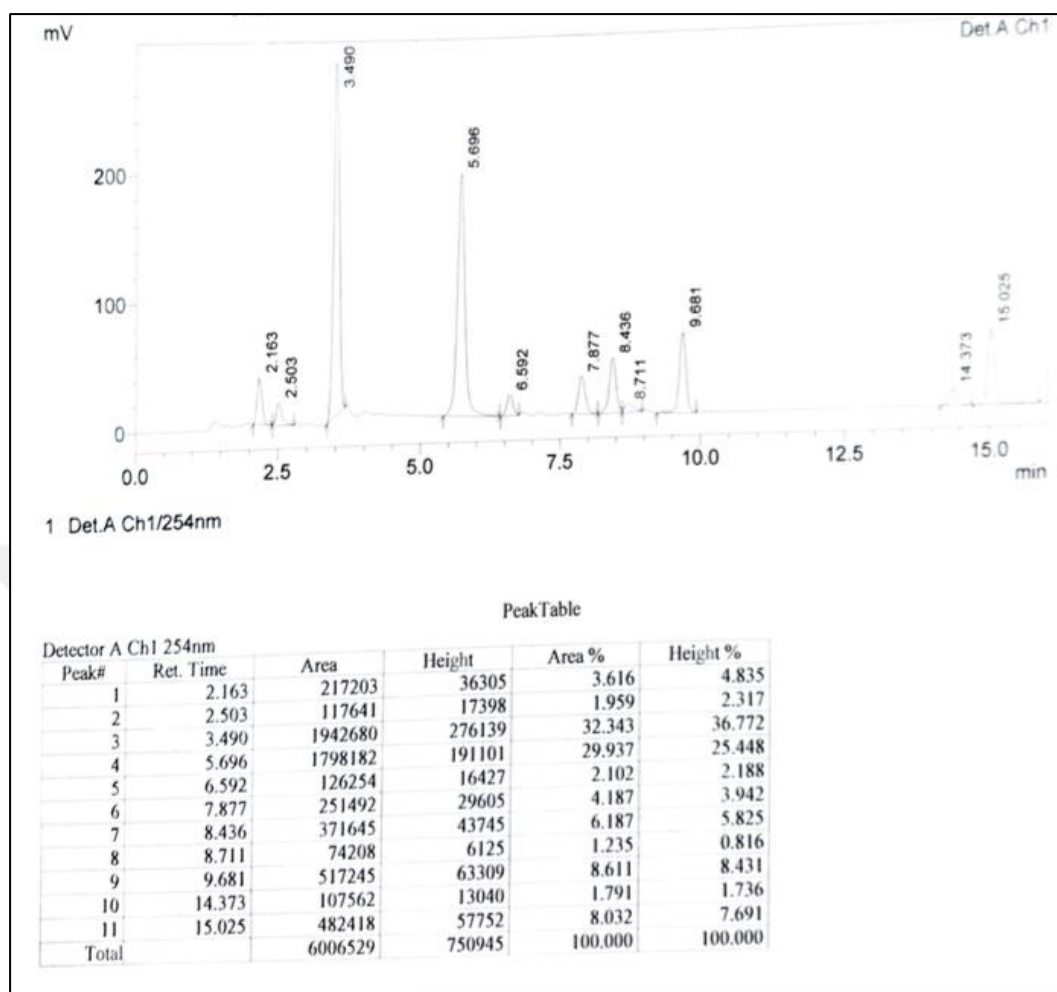


Figure A.25. The HPLC chromatogram of reaction mixture **12** at 24 hours (gradient elution) (Catalyst:CAT20)

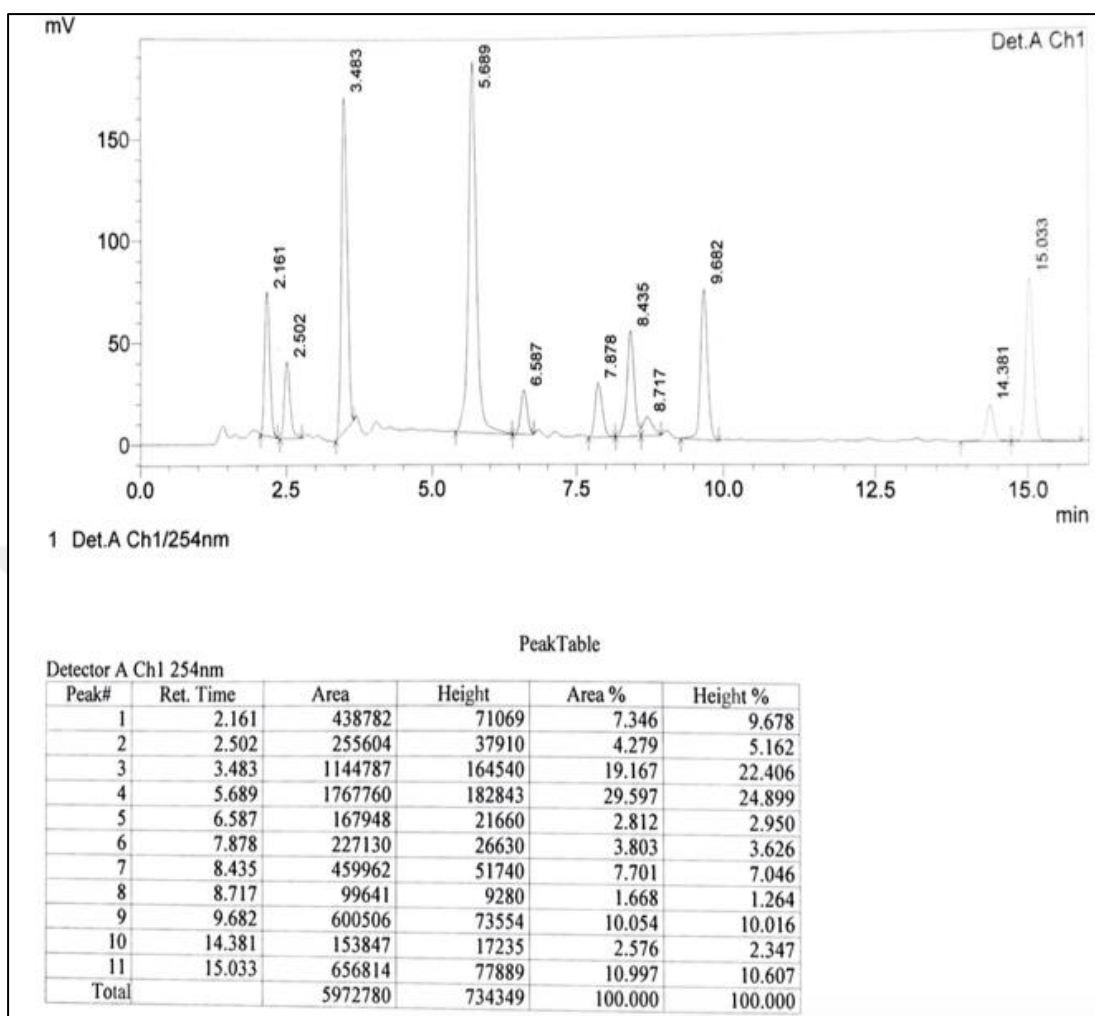


Figure A.26. The HPLC chromatogram of reaction mixture **12** at 48 hours (gradient elution) (Catalyst:CAT20)

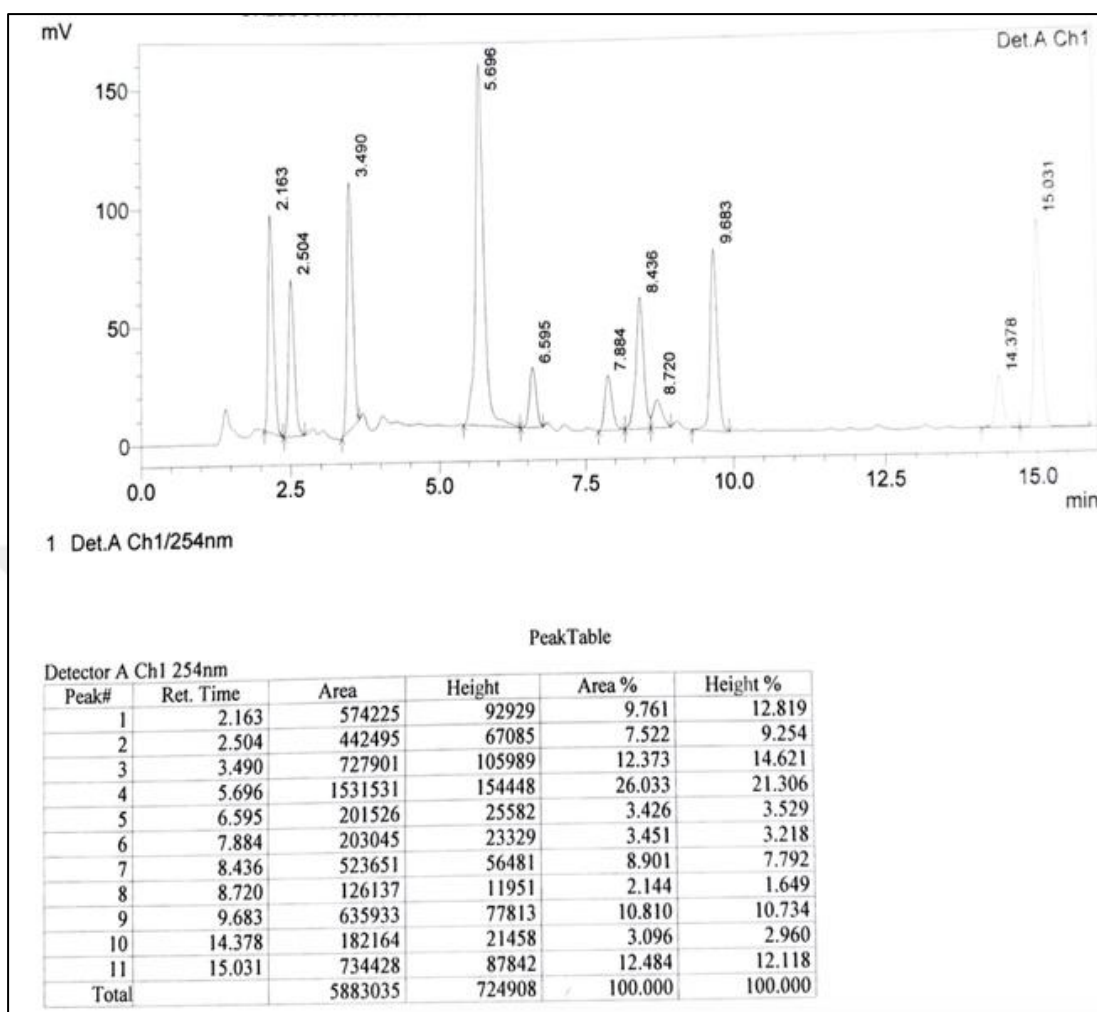


Figure A.27. The HPLC chromatogram of reaction mixture **12** at 72 hours (gradient elution) (Catalyst:CAT20)

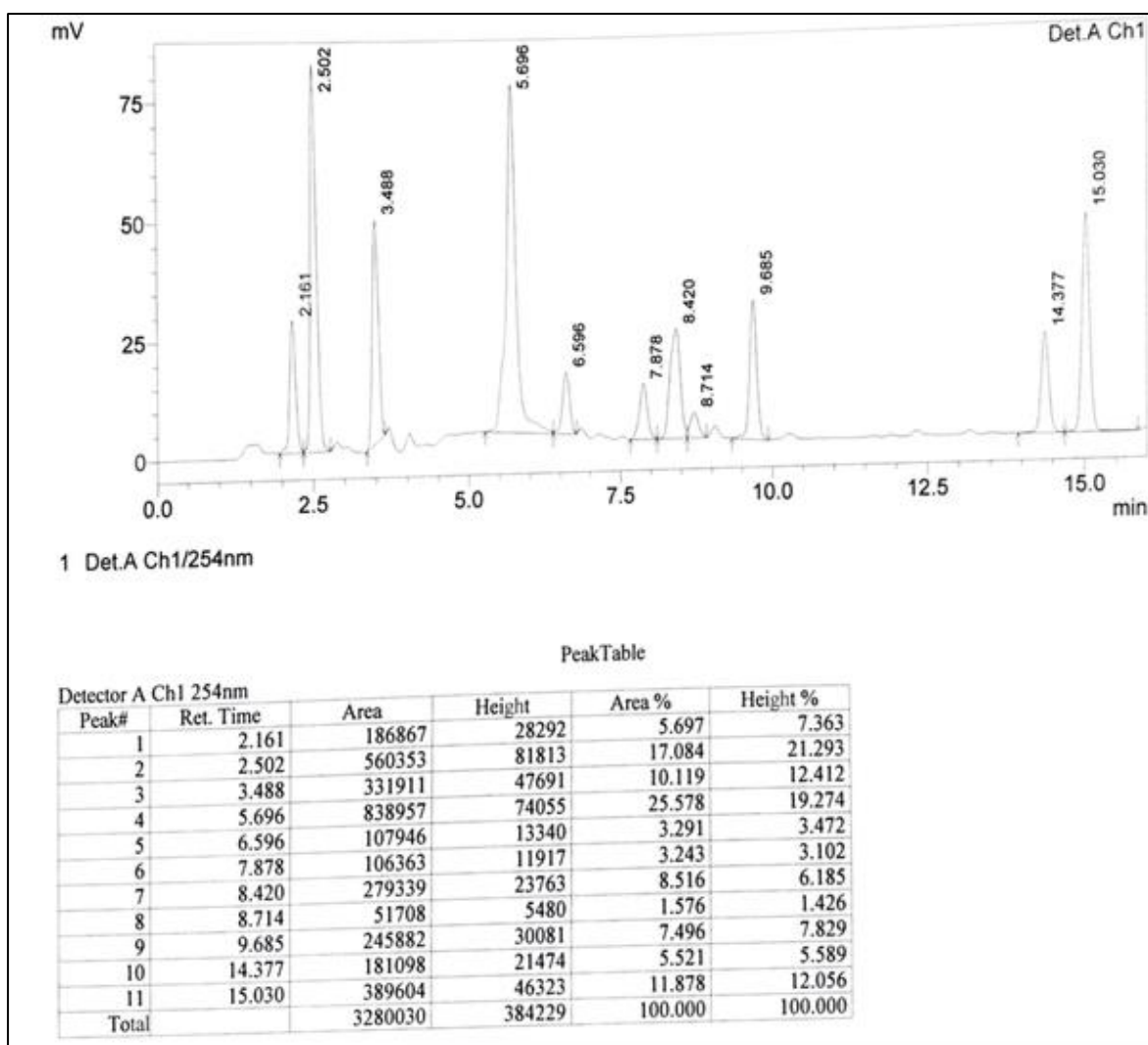


Figure A.28. The HPLC chromatogram of reaction mixture **12** at 96 hours (gradient elution) (Catalyst: **CAT20**)

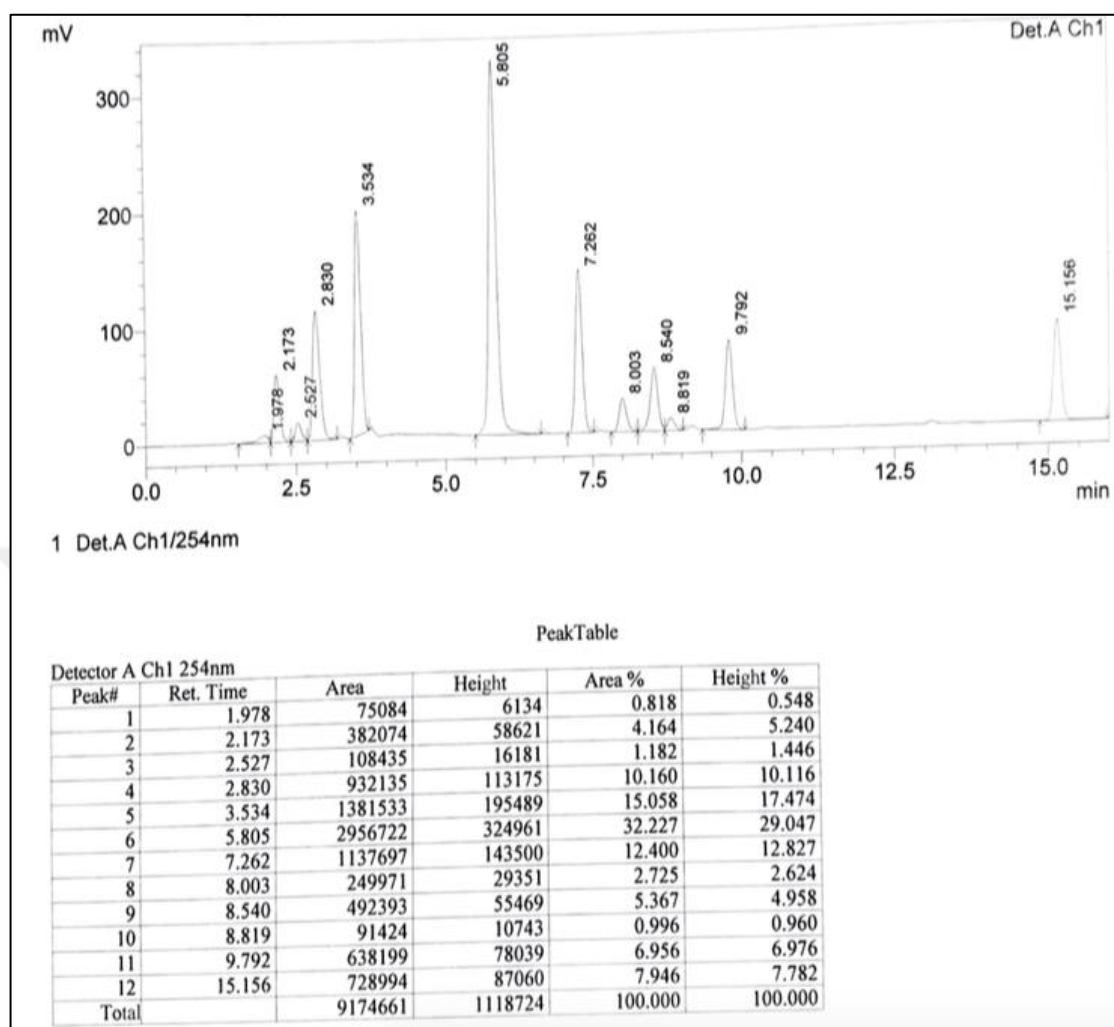


Figure A.29. The HPLC chromatogram of reaction mixture **13** at 24 hours (gradient elution) (Catalyst: **CAT36**)

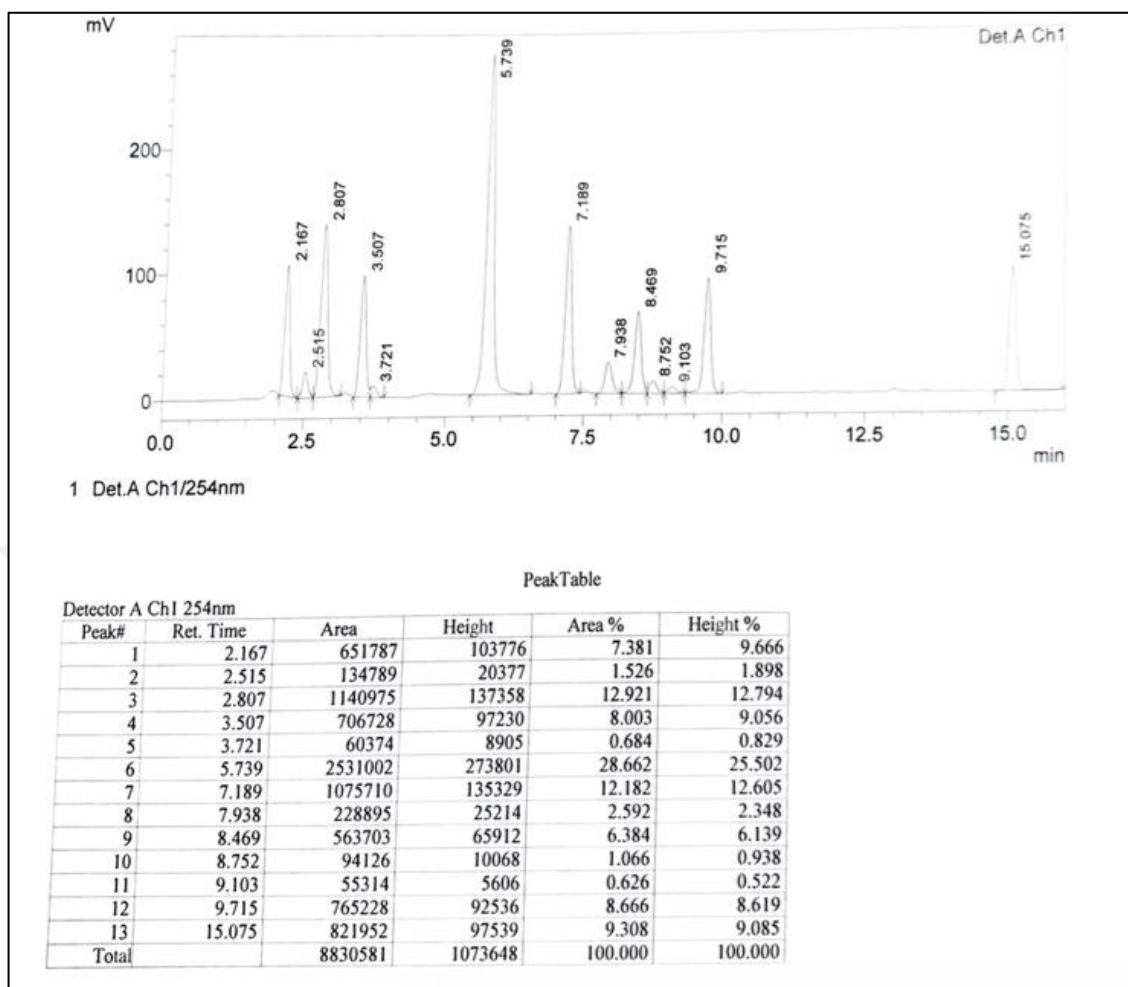


Figure A.30. The HPLC chromatogram of reaction mixture **13** at 48 hours (gradient elution) (Catalyst:CAT36)

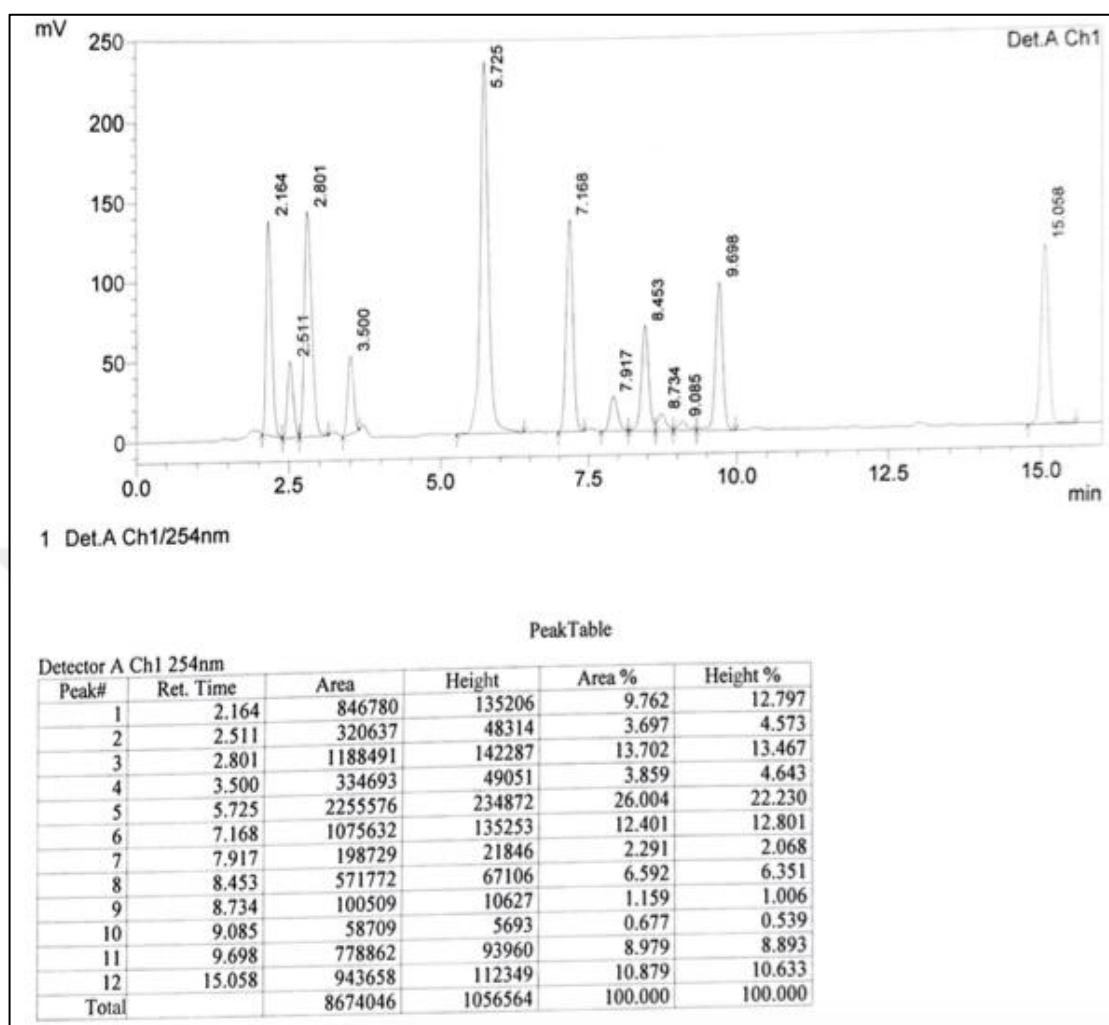


Figure A.31. The HPLC chromatogram of reaction mixture **13** at 72 hours (gradient elution) (Catalyst:CAT36)

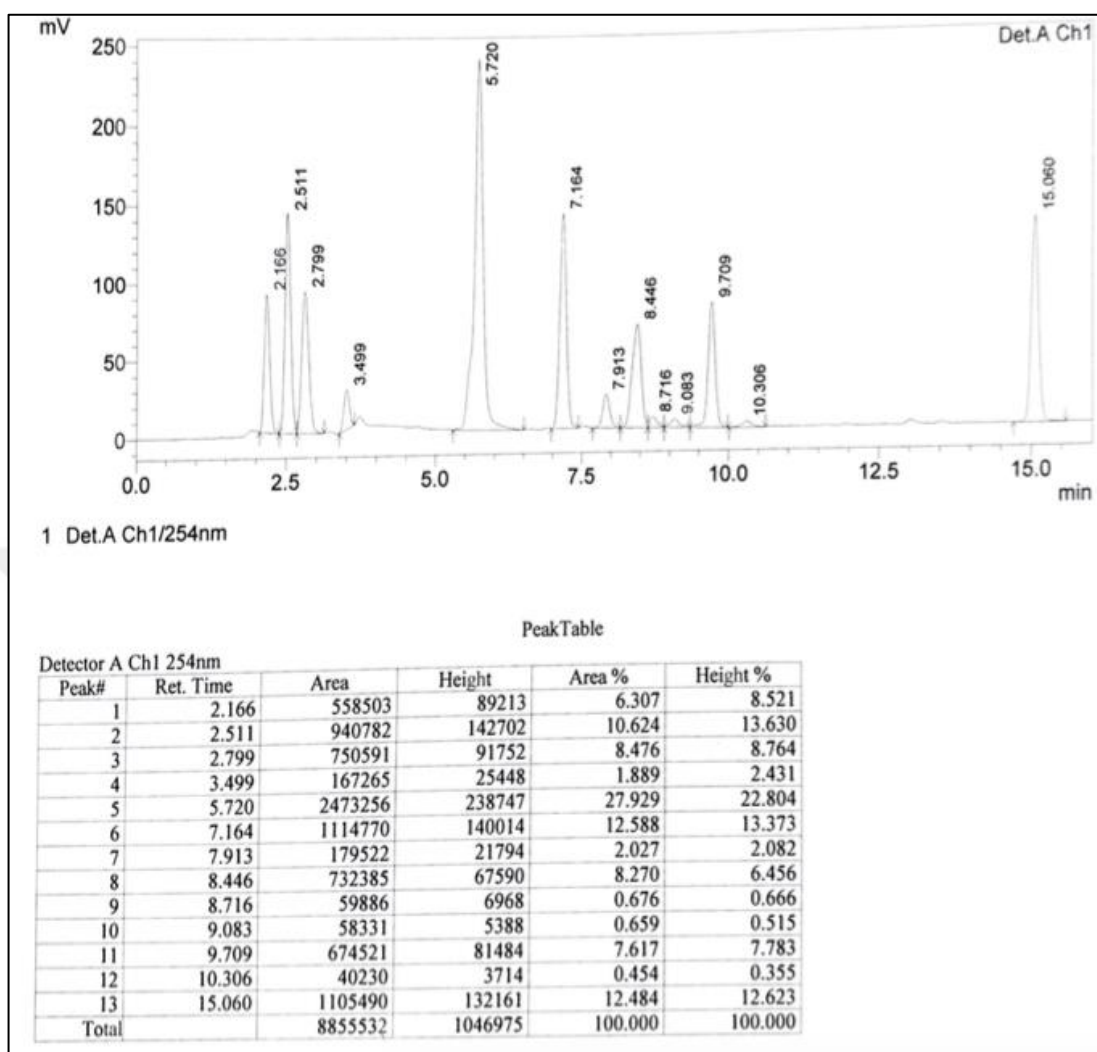


Figure A.32. The HPLC chromatogram of reaction mixture **13** at 96 hours (gradient elution) (Catalyst:CAT36)

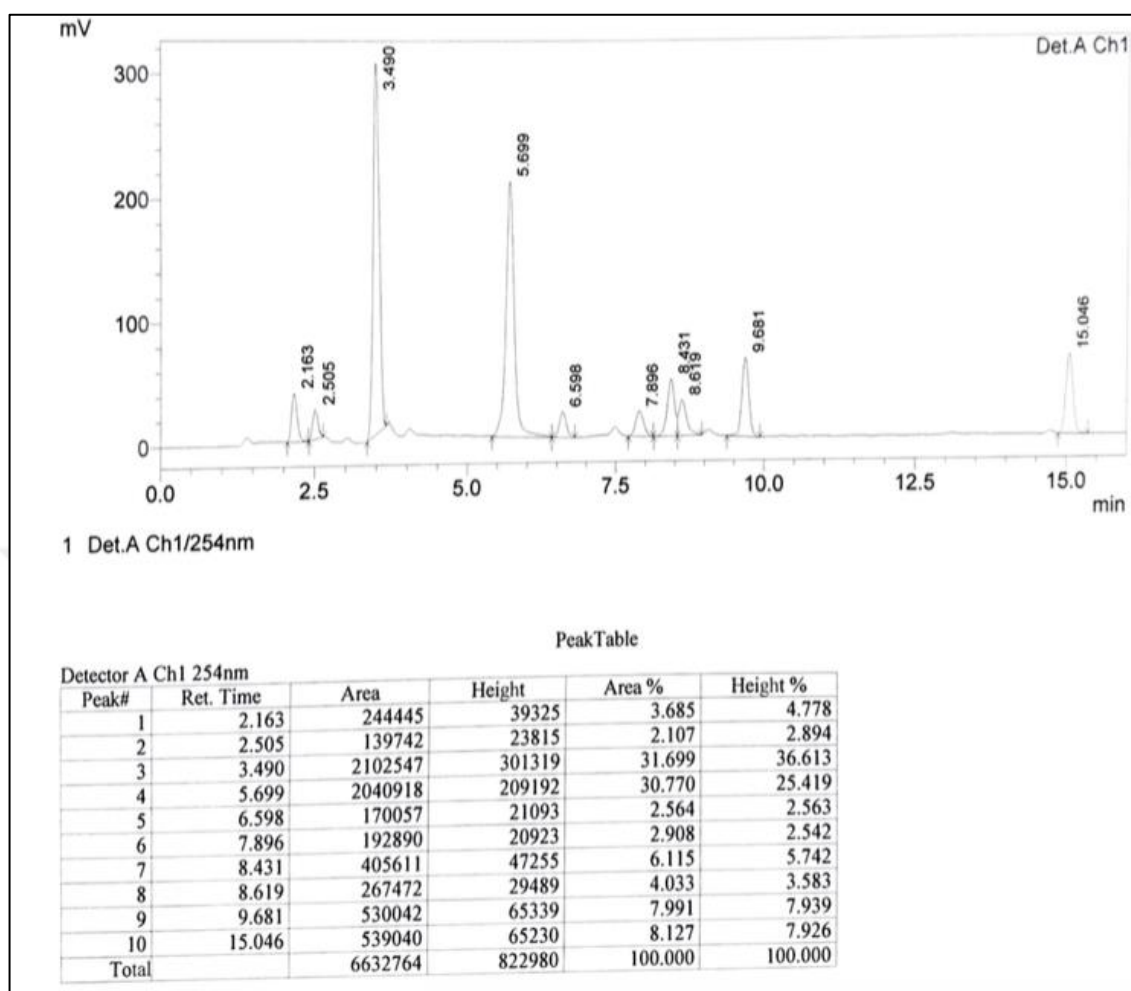


Figure A.33. The HPLC chromatogram of reaction mixture **14** at 24 hours (gradient elution) (Catalyst:CAT38S)

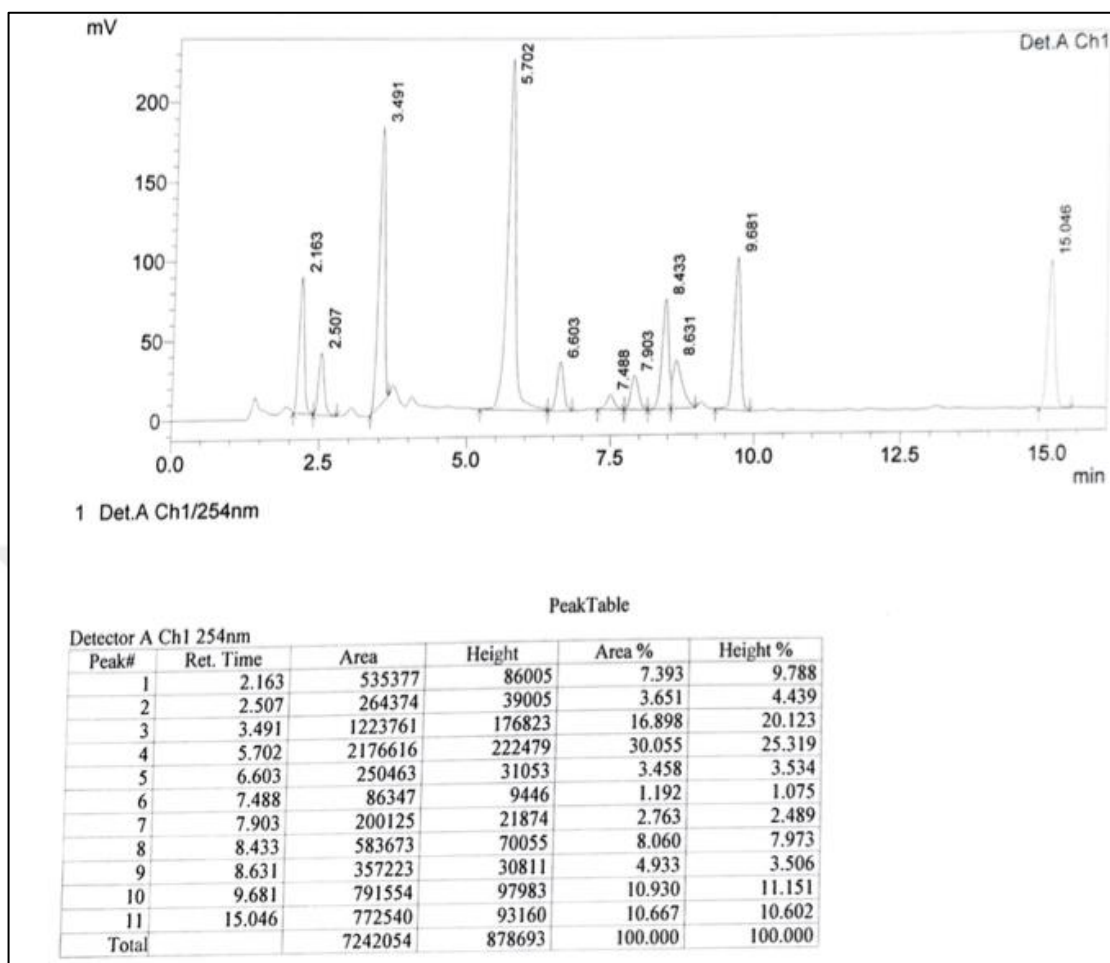


Figure A.34. The HPLC chromatogram of reaction mixture **14** at 48 hours (gradient elution) (Catalyst:CAT38S)

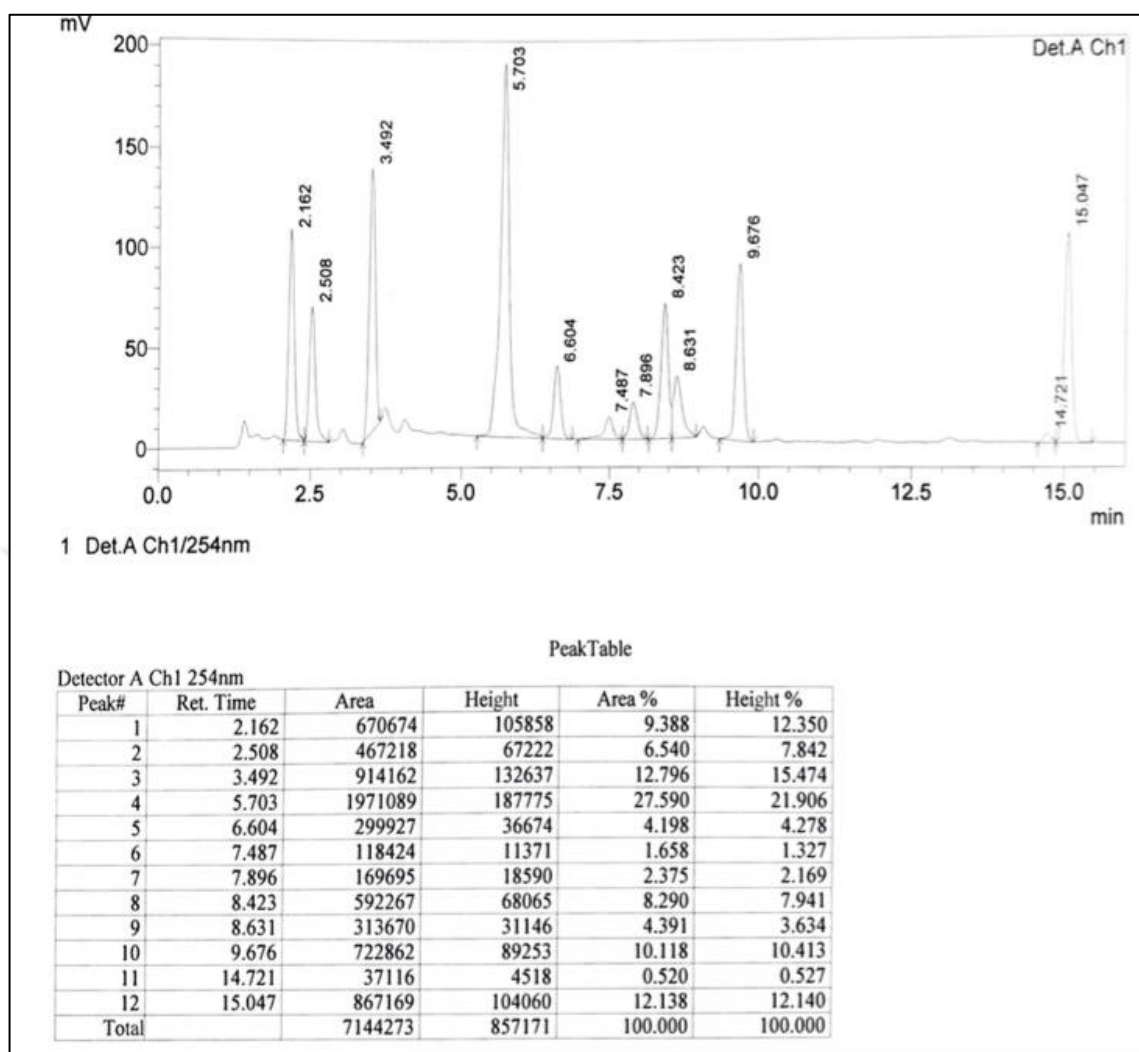


Figure A.35. The HPLC chromatogram of reaction mixture **14** at 72 hours (gradient elution) (Catalyst:CAT38S)

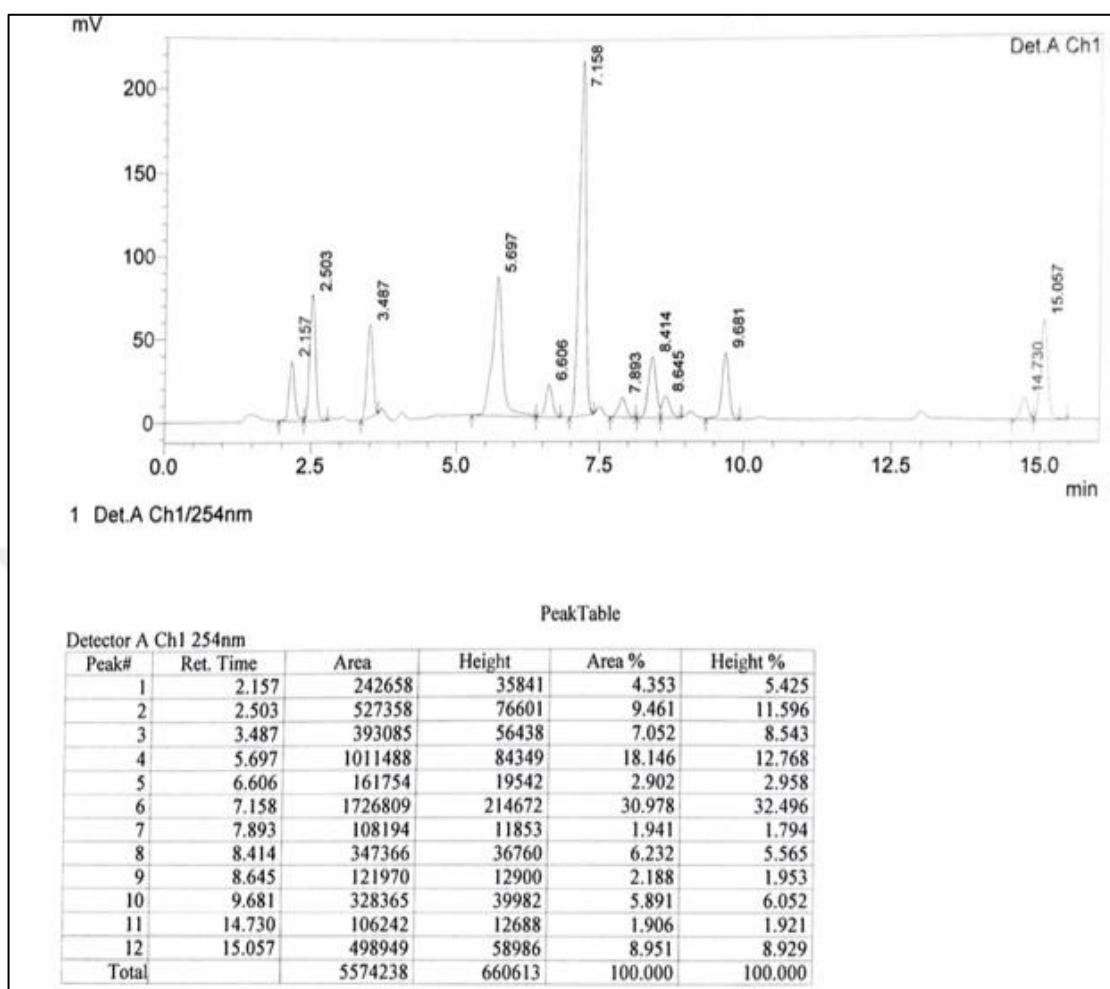


Figure A.36. The HPLC chromatogram of reaction mixture **14** at 96 hours (gradient elution) (Catalyst:CAT38S)

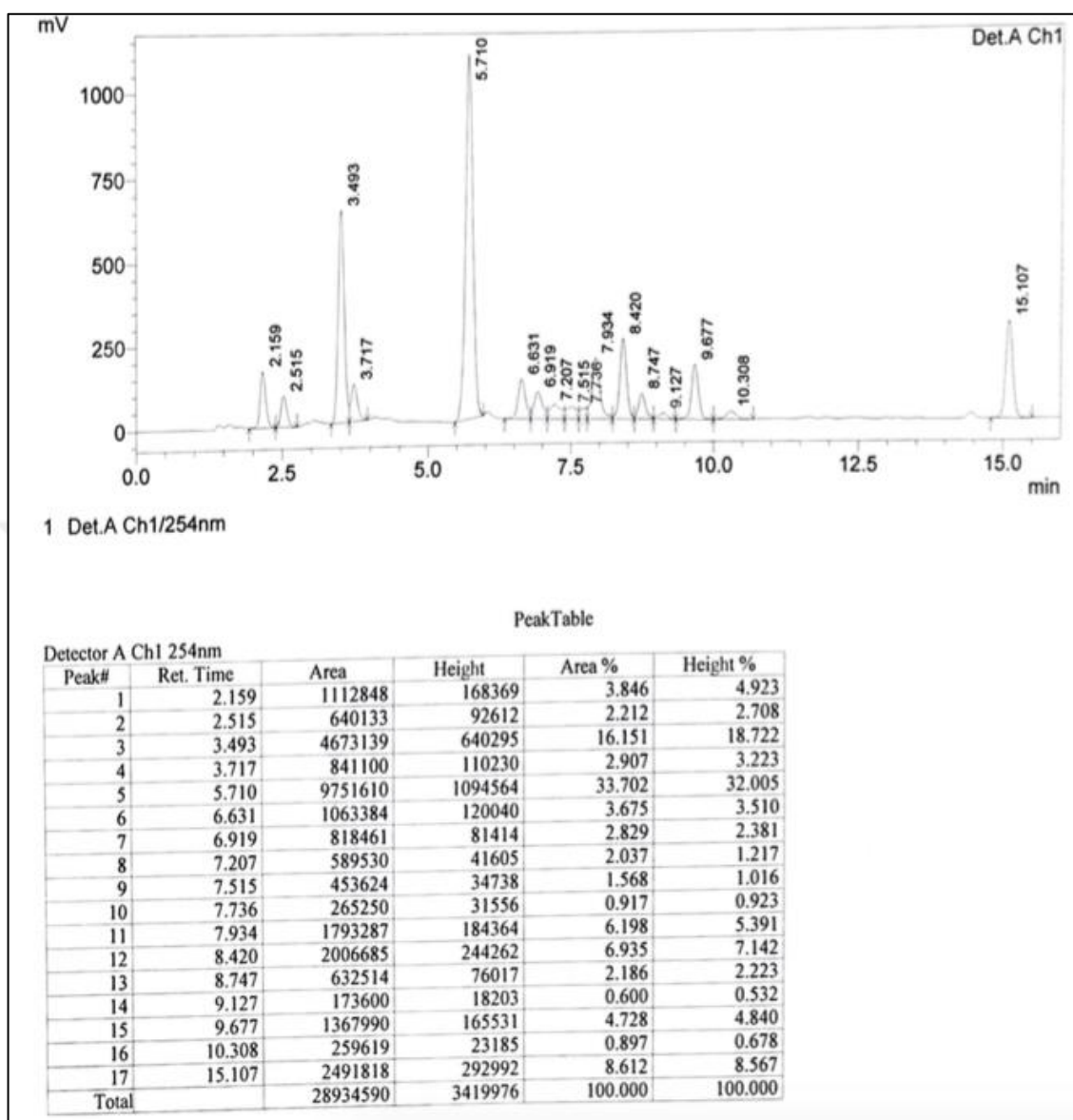


Figure A.37. The HPLC chromatogram of reaction mixture **15** (gradient elution)
(Catalyst:CAT20)

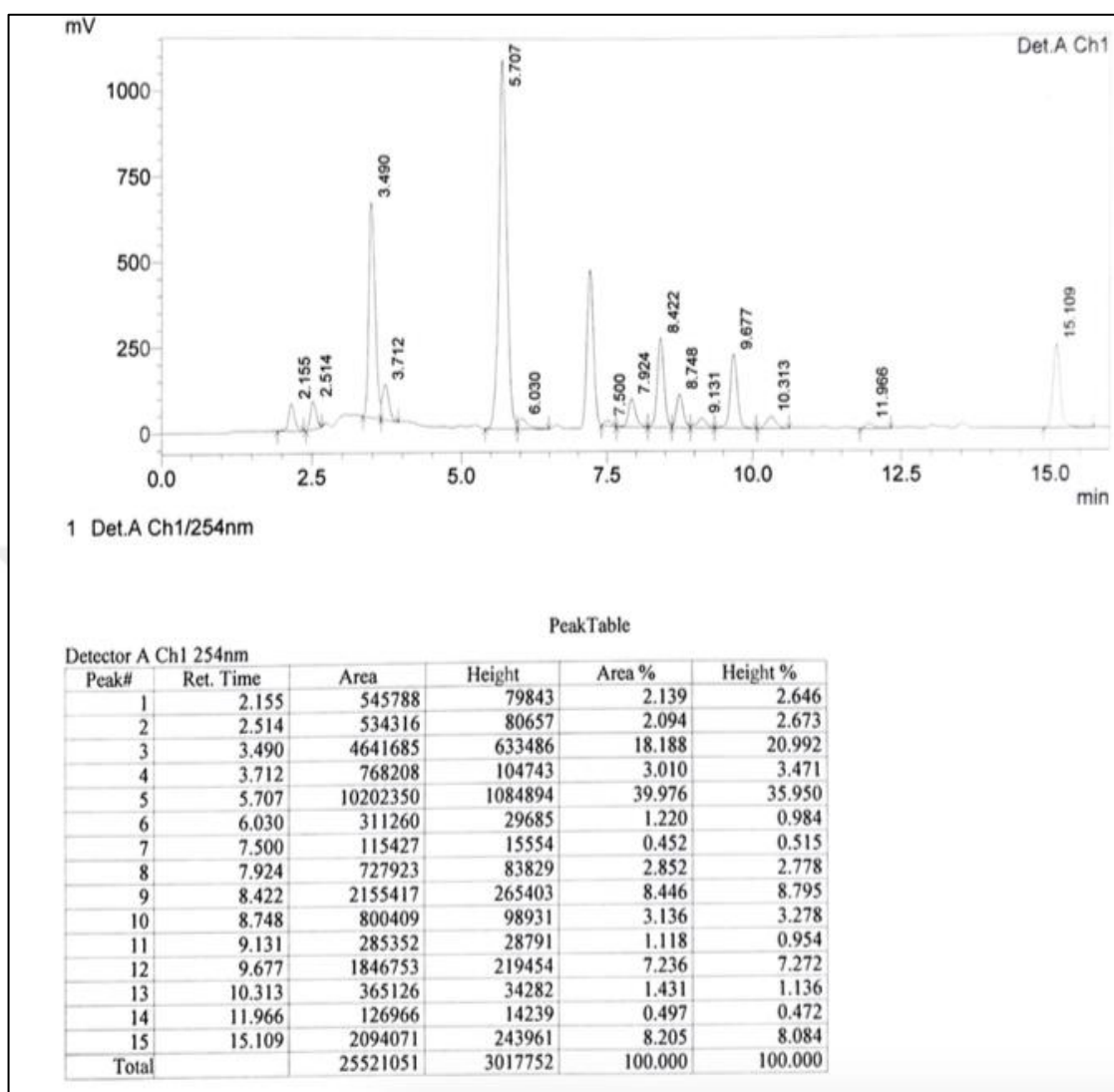


Figure A.38. The HPLC chromatogram of reaction mixture **16** (gradient elution)
(Catalyst:CAT36)

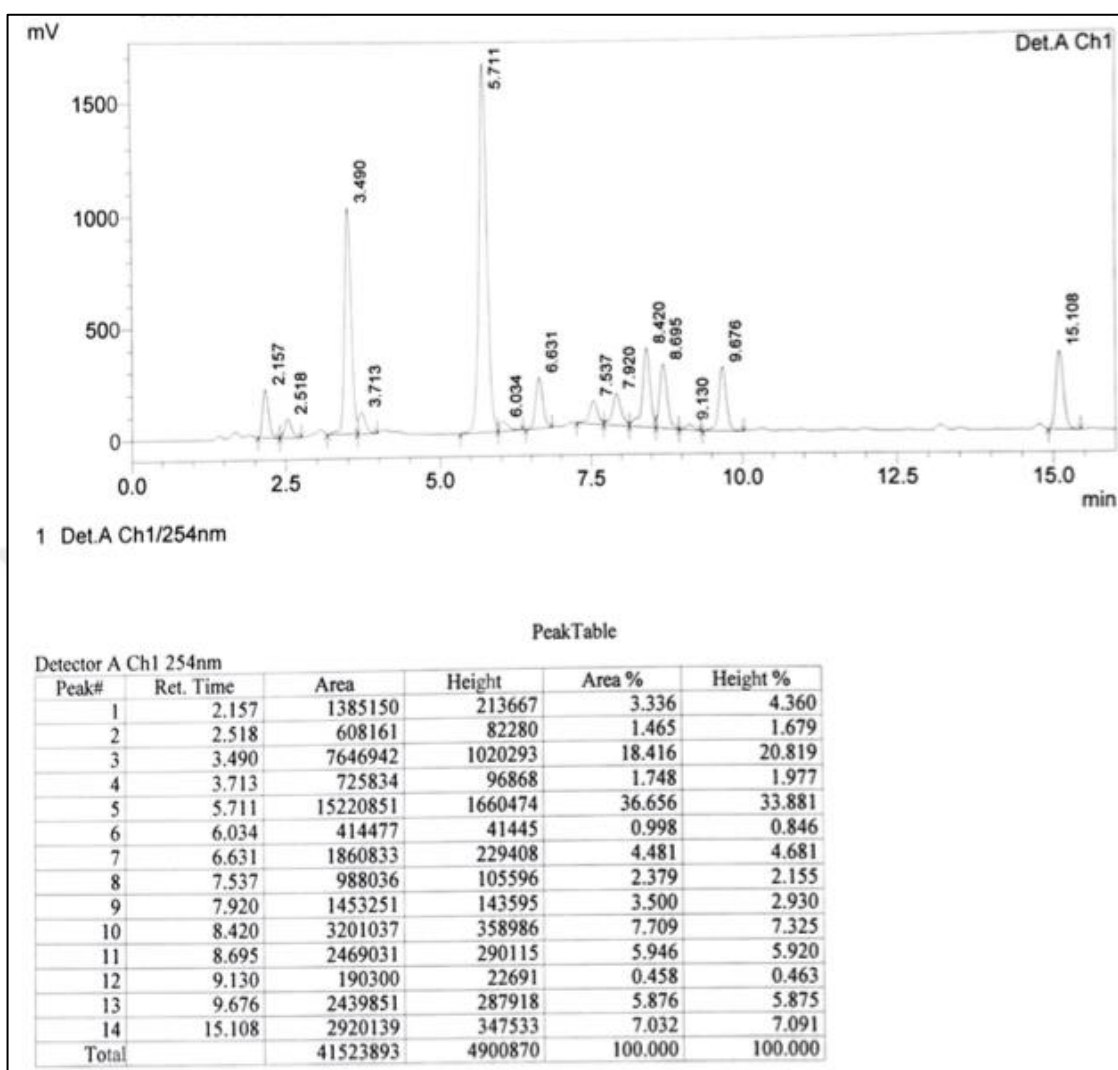


Figure A.39. The HPLC chromatogram of reaction mixture **17** (gradient elution)
(Catalyst: **CAT38S**)

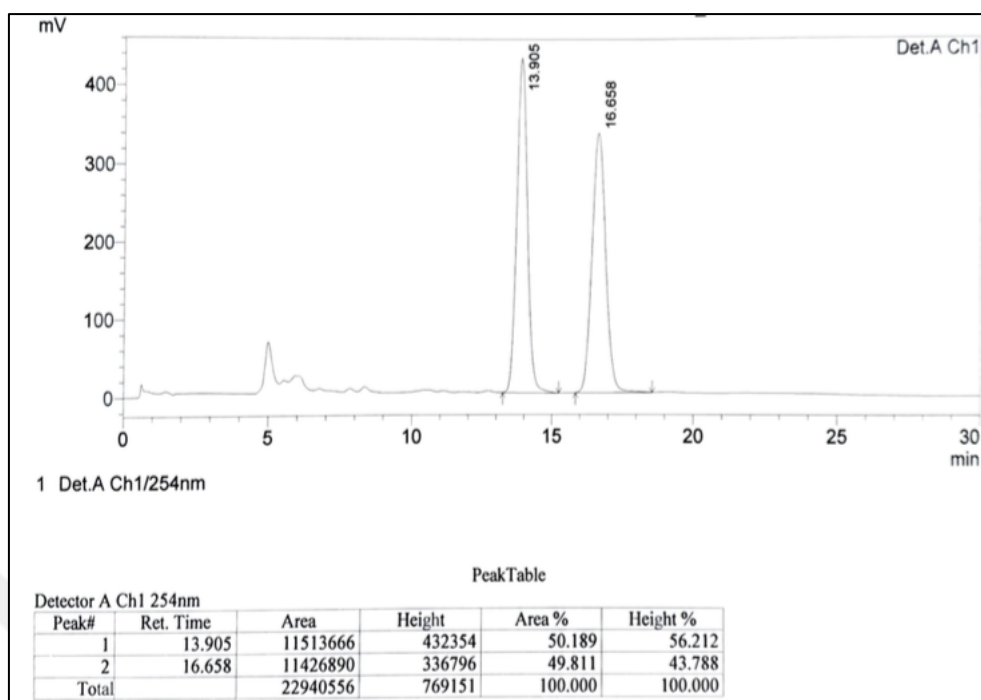


Figure A.40. The HPLC chromatogram of pure 3-hydroxy-3-methyl-oxindole (normal phase)

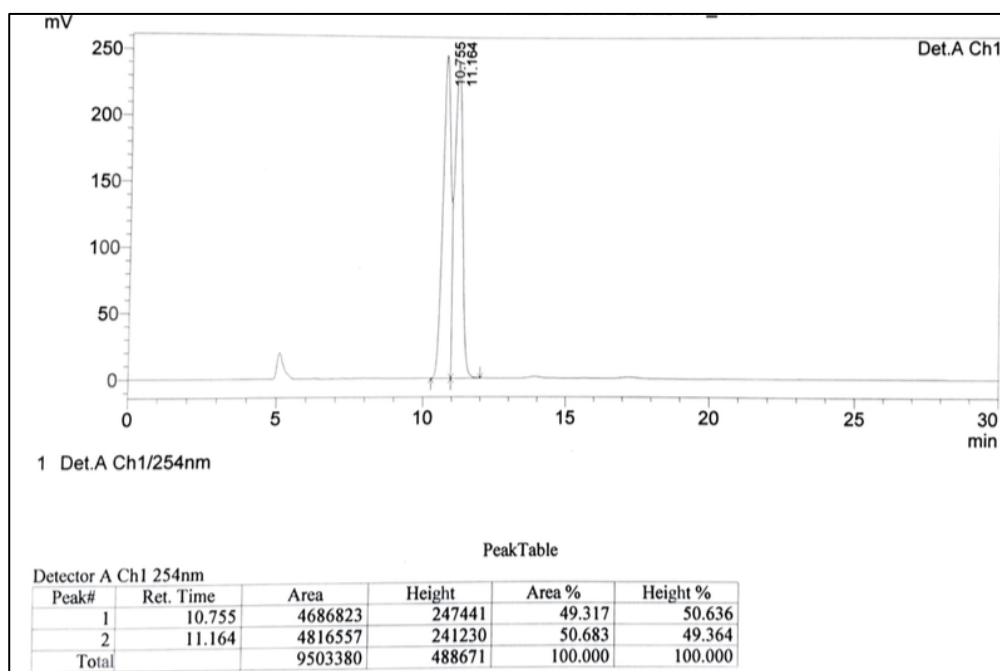


Figure A.41. The HPLC chromatogram of pure 3-methyl-2-oxindole (normal phase)

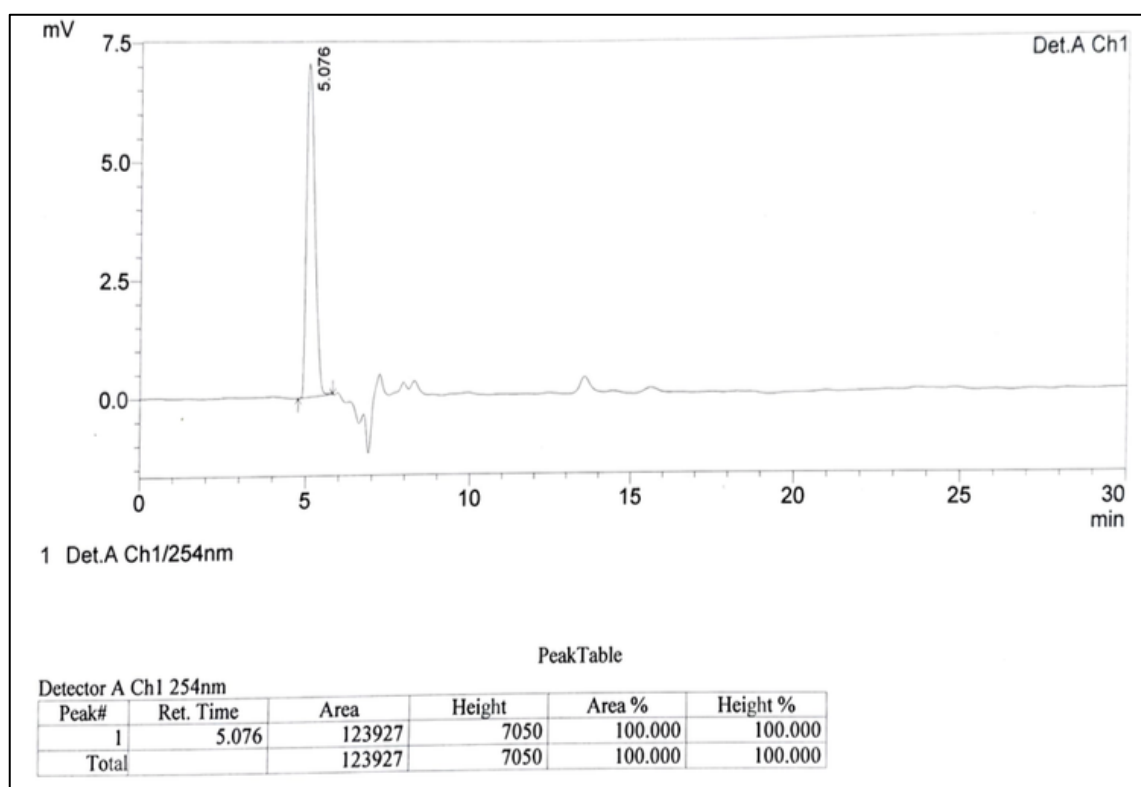


Figure A.42. The HPLC chromatogram of pure ethanol (normal phase)

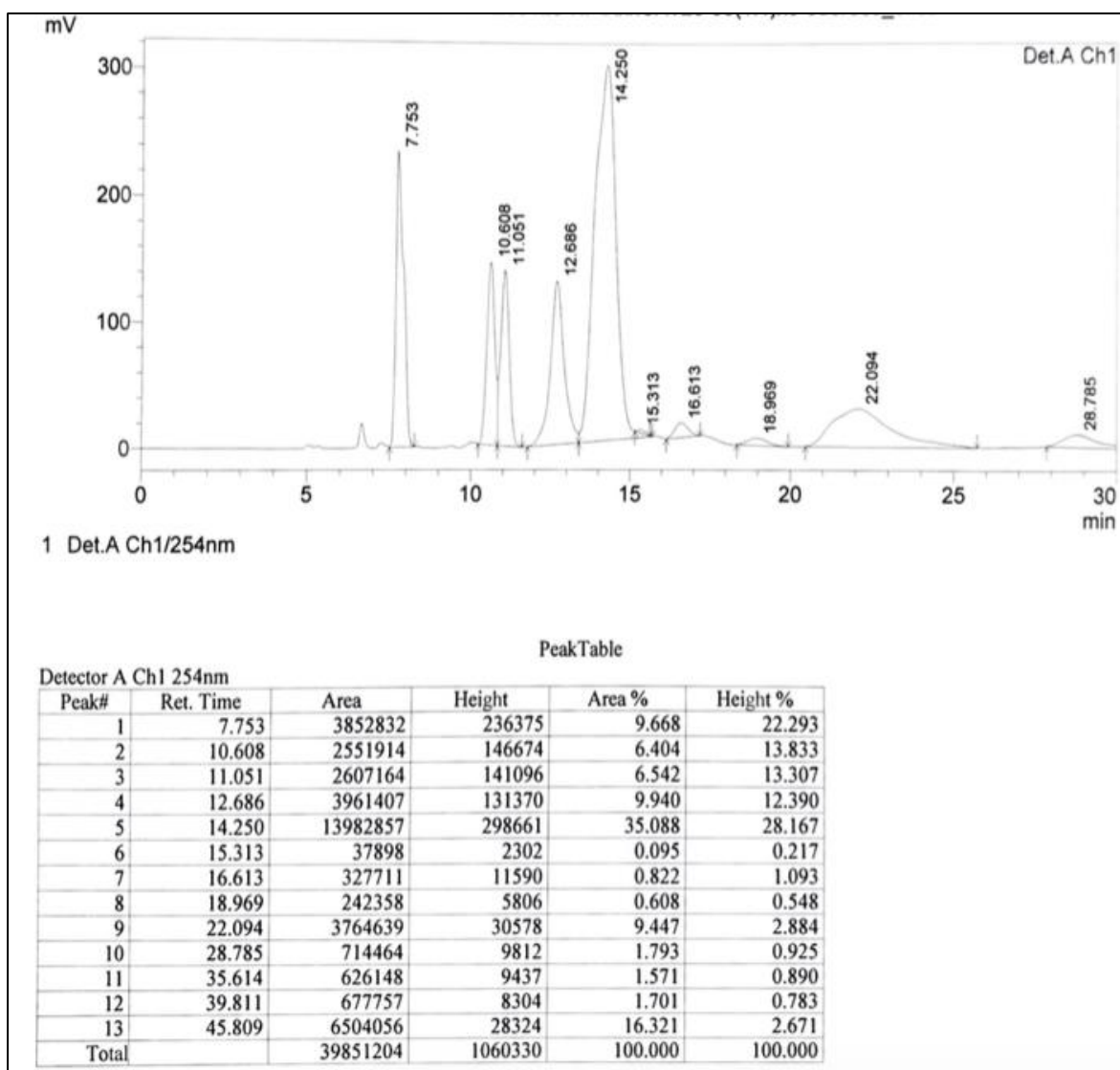


Figure A.43. The HPLC chromatogram of reaction mixture **15** (normal phase)
(Catalyst:CAT20)

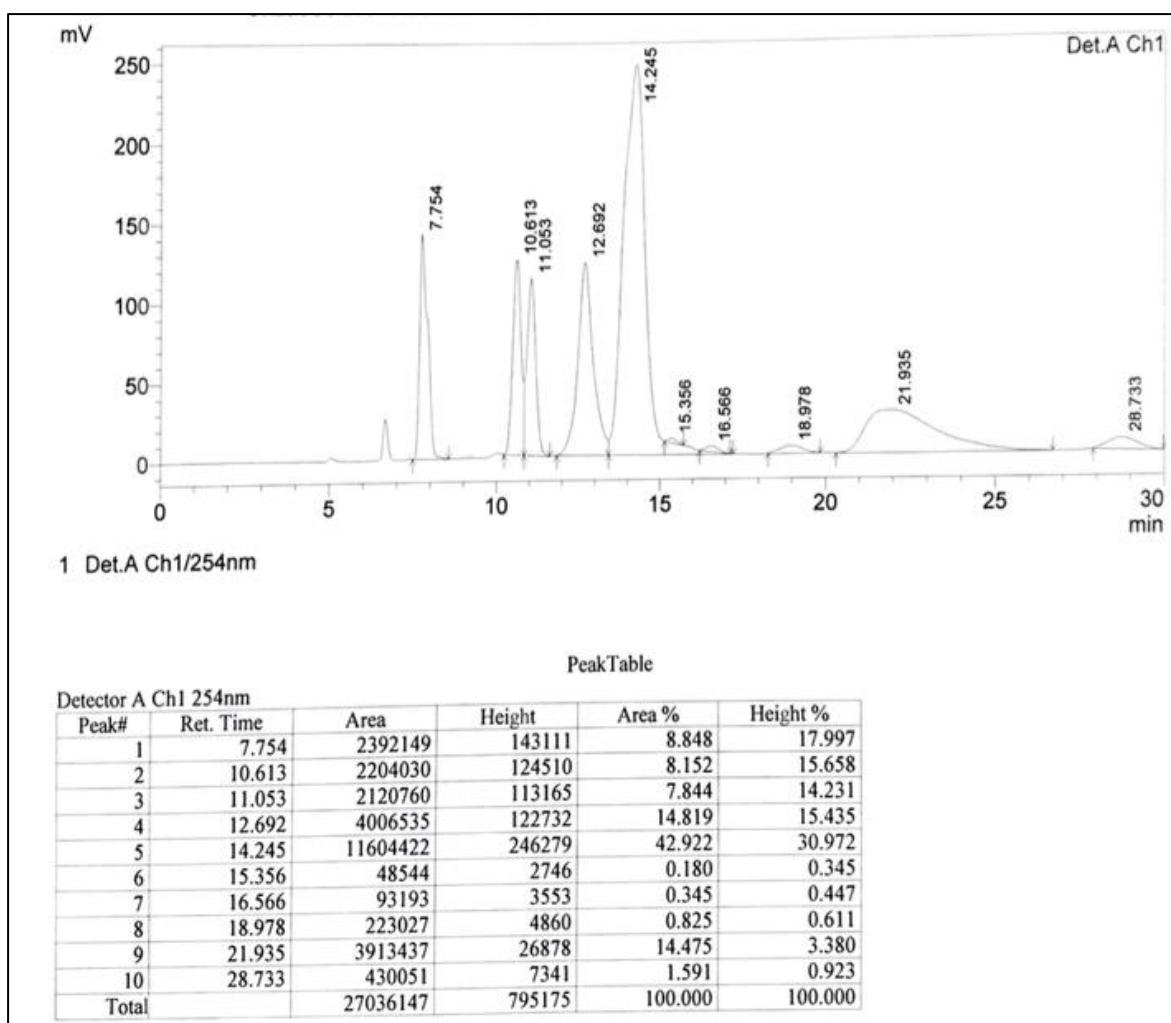


Figure A.44. The HPLC chromatogram of reaction mixture **16** (normal phase)
(Catalyst: **CAT36**)

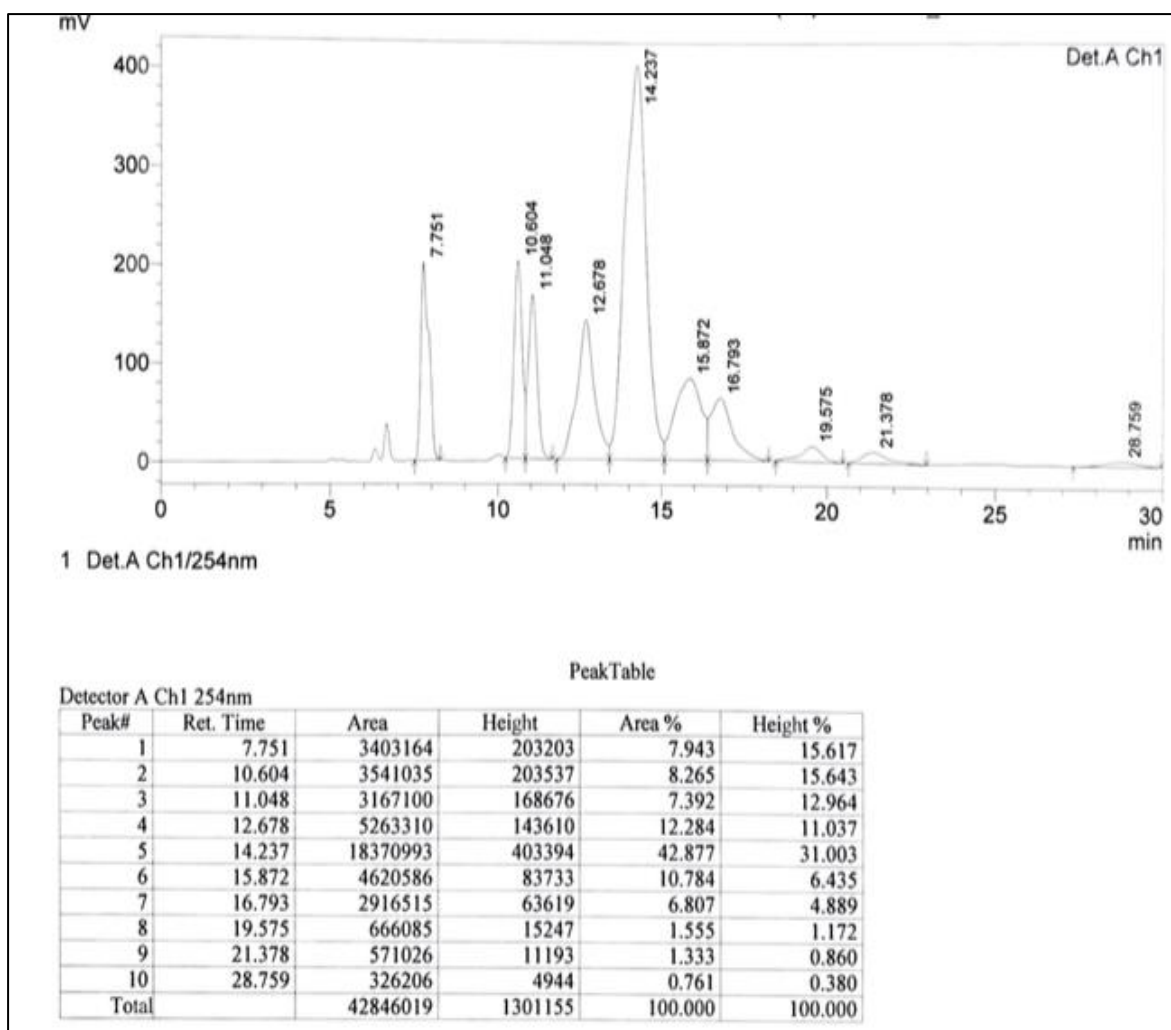


Figure A.45. The HPLC chromatogram of reaction mixture **17** (normal phase)
(Catalyst:CAT38S)

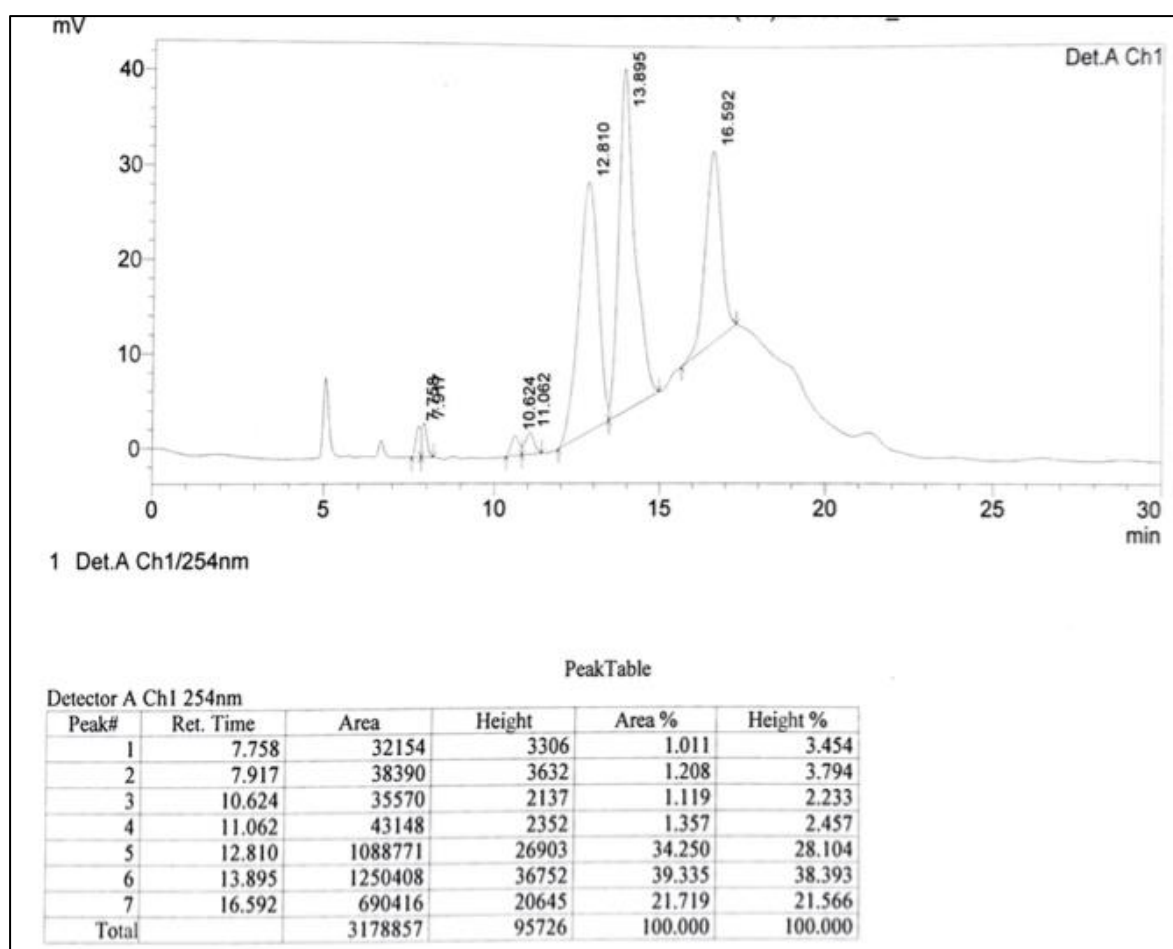


Figure A.46. The HPLC chromatogram of reaction mixture **11** (normal phase)
(Catalyst:CD)

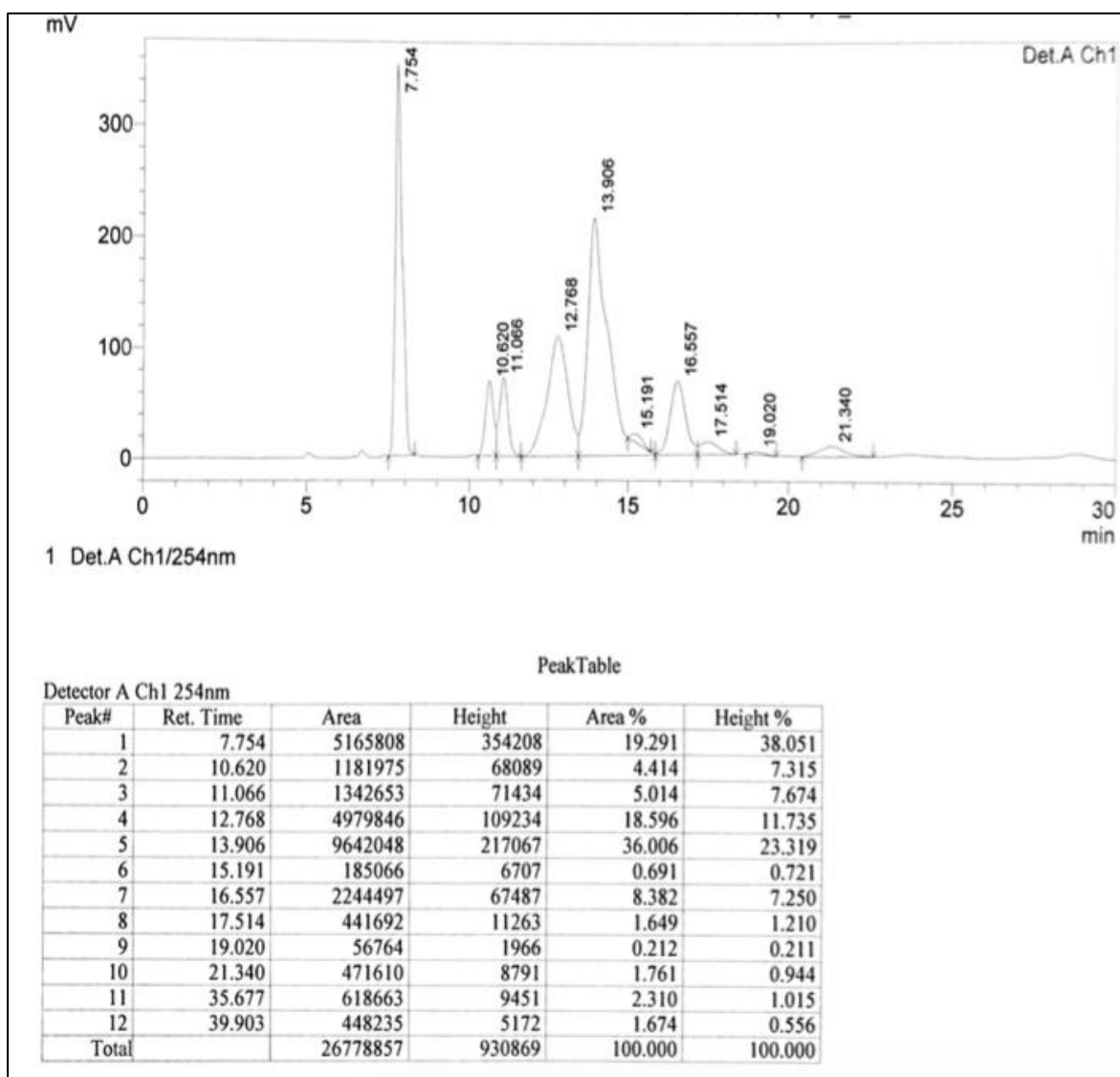


Figure A.47. The HPLC chromatogram of reaction mixture **12** (normal phase)
(Catalyst: **CAT20**)

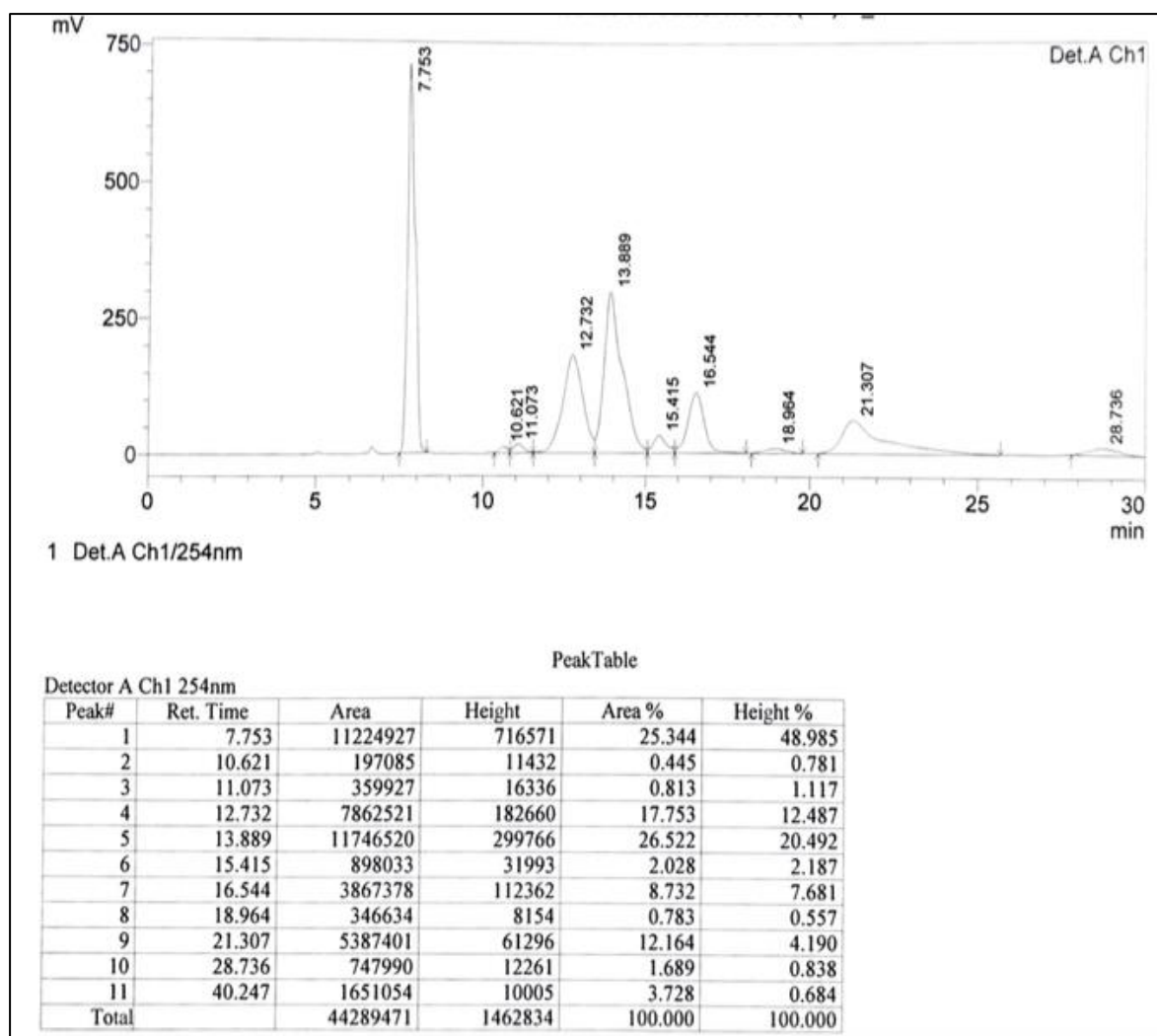


Figure A.48. The HPLC chromatogram of reaction mixture **13** (normal phase)
(Catalyst: **CAT36**)

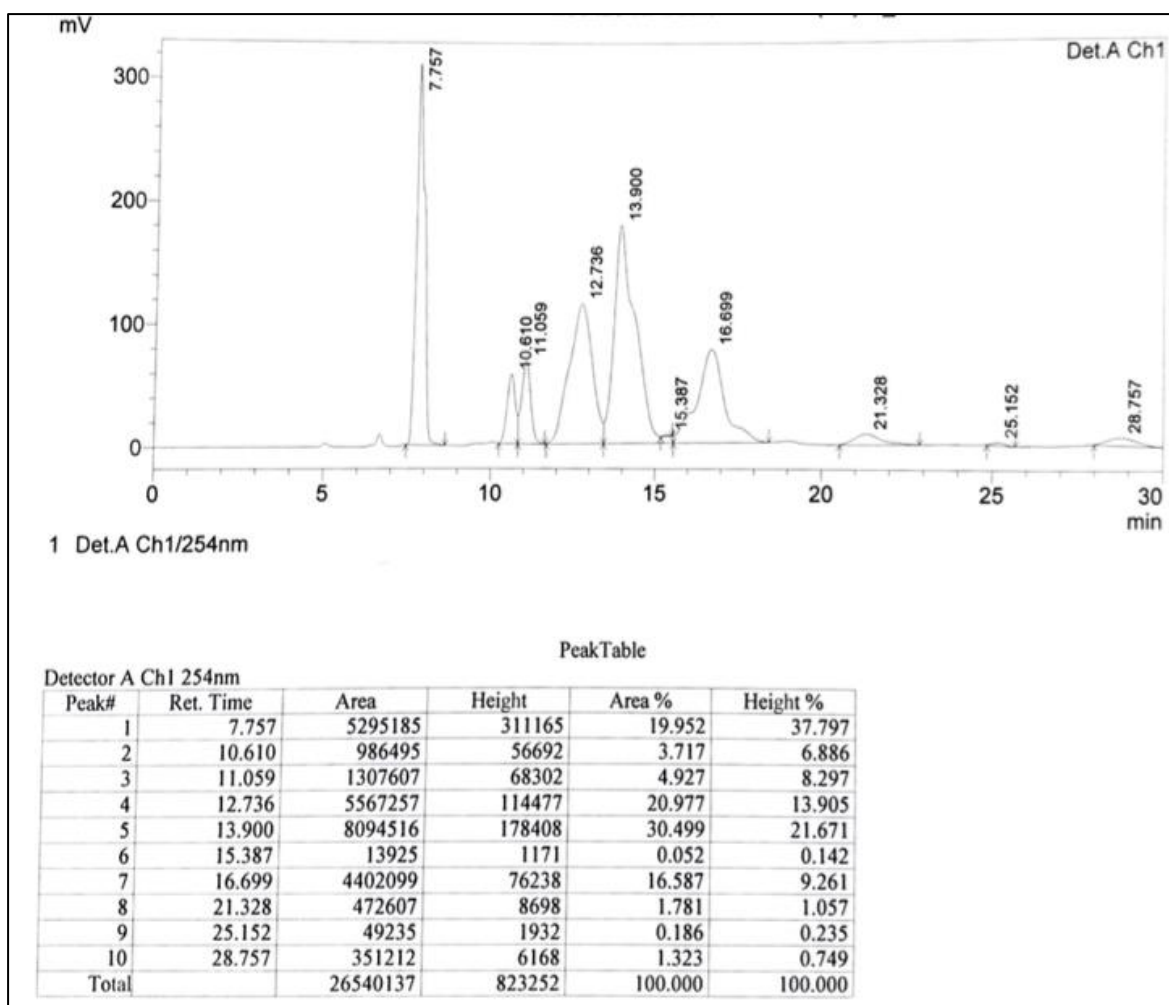


Figure A.49. The HPLC chromatogram of reaction mixture **14** (normal phase)
(Catalyst:CAT38S)

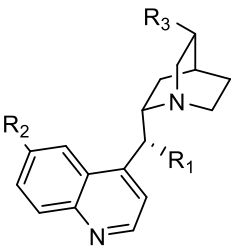
APPENDIX B: CONFORMER NUMBERS, ENERGIES AND COMPUTATIONAL DATA

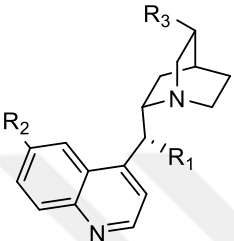
The conformer number of selected catalysts were given in Table B.1. The energies of the TS were given in Table B.3-Table B.8.

Table B.1. Conformer Numbers of selected catalysts

Catalyst Name	Conformer Number	Catalyst Name	Conformer Number
CAT5	158	CAT35	292
CAT8	296	CAT35S	339
CAT9	325	CAT36	274
CAT14	162	CAT37	262
CAT15	329	CAT37S	357
CAT18	142	CAT38	247
CAT20	302	CAT38S	306
CAT21	286	CAT39	226
CAT21O	404		

Table B.2. Catalyst names and structures

Cinchona skeleton	Catalyst name	R ₁	R ₂	R ₃
	CAT1	OH	PhCH ₂ CH ₂ CH ₂ CH ₃	CHCH ₂
	CAT2	OH	Ph	CHCH ₂
	CAT3	OH	OCH ₂ PhCH ₂ OQD	CHCH ₂
	CAT4	OPhOQD	OH	CHCH ₂
	CAT5	NH ₂	OH	CH ₂ OH
	CAT6	OH	OCH ₂ PhCH ₂ OQD	CHCH ₂
	CAT7	NHCSNHoxyl	H	CH ₂ OH
	CAT8	NHCSNHPh	OH	CH ₂ OH
	CAT9	NHCONHBn	OCH ₃	CH ₂ OH
	CAT10	NHCH ₂ Ph	OCH ₃	CH ₂ CH ₃
	CAT11	NH ₂	OCH ₃	CHCH ₂
	CAT12	NH ₂	OH	CHCH ₂
	CAT13	NH ₂	OCH ₃	CHCHPh



CAT14	NHCH ₂ Ph	NHCONHBn	CH ₂ CH ₂ Ph
CAT15	NH ₂	OCH ₃	CH ₂ CH ₂ Ph
CAT16	NH ₂	OCH ₃	CHCHPh
CAT17	NH ₂	OH	CHCH ₂
CAT18	NH ₂	OH	CH ₂ CH ₃
CAT19	NH ₂	OCH ₃	CHCH ₂
CAT20	NHCSNHPhOCH ₃	H	CHCH ₂
CAT21	NHCSNHPhOCH ₃	OCH ₃	CHCH ₂
CAT22	NHCSNH ₃ ,5CF ₃	H	CHCH ₂
CAT23	NHCSNH ₃ ,5CF ₃	OCH ₃	CHCH ₂
CAT24	NHCSNHBn	NHCSNHPh	CHCH ₂
CAT25	NHCSNHBn	NHCSNHoxyl	CHO
CAT26	NHCSNHPh	OCH ₃	CH ₂ OH
CAT27	NHCSNHPh	H	CH ₂ OH
CAT28	NHCSNHBn	NHCONHBn	CH ₂ OH
CAT29	NH ₂	NHCSNHoxyl	CH ₂ OH
CAT30	NHCONHBn	H	CH ₂ OH
CAT31	NHCONHBn	H	H
CAT32	NHCONHBn	OH	CHCH ₂
CAT33	NHCONHoxyl	NHCONHBn	CH ₂ CH ₃
CAT34	NHCONHoxyl	NHCSNHPh	CN
CAT35	NHCONHPhCH ₃	OCH ₃	CHCH ₂
CAT36	NHCONHPhOCH ₃	H	CHCH ₂
CAT37	NHCONHPhCH ₃	H	CHCH ₂
CAT38	NHCONHPh	H	CHCH ₂
CAT39	NHCONHBn	NHCSNHBn	CH ₂ OH

Table B.3. Energies of theozymes

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
ModelA2.1HH	6.1	6.2	34.9	39.0
ModelA2.2HH	2.7	-2.7	32.0	35.5
Model H2.2HH	1.2	1.0	30.8	34.4
ModelA2.3HH	-2.1	-2.2	27.5	30.7
ModelH2.3HH	-6.0	-6.1	23.3	26.9
ModelA2.3HMe	-5.5	-5.4	24.4	27.5
ModelH2.3HMe	-11.5	-11.5	18.2	21.8
ModelA2.3MeH	-2.4	-2.3	26.5	30.4
ModelH2.3MeH	-7.8	-7.6	20.9	25.1
ModelA2.3MeMe	-3.6	-3.6	26.8	29.8

ModelH2.3MeMe	-10.4	-10.4	19.1	22.9
ModelA2.3BnH	-2.5	-2.3	27.2	31.0
ModelH2.3BnH	-7.0	-6.9	22.9	26.8
ModelA2.3BnMe	-6.5	-6.3	24.6	27.3
ModelH2.3BnMe	-13.5	-13.4	17.4	20.3
ModelA2.3BnMe(amine)	-4.8	-4.5	24.9	28.9
ModelH2.3BnMe(amine)	-9.1	-8.8	21.4	24.8
ModelA2.3BnMe(urea)	-11.9	-12.0	22.5	24.8
ModelH2.3BnMe(urea)	-17.7	-17.9	17.1	19.2
ModelA2.3BnMe(thiourea)	-12.1	-12.0	20.8	25.0
ModelH2.3BnMe(thiourea)	-18.4	-18.2	15.0	18.5

Table B.4. Energies of selected catalysts including alcohol functional groups

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
(<i>R</i>)-TSA2.3 _{CAT5}	-14.4	-15.3	13.9	18.5
(<i>S</i>)-TSA2.3 _{CAT5}	-13.6	-14.3	12.9	19.3
(<i>R</i>)-TSH2.3 _{CAT5}	-15.4	-16.2	11.3	17.5
(<i>S</i>)-TSH2.3 _{CAT5}	-15.4	-16.3	11.5	17.7
(<i>R</i>)-TSA2.3 _{CAT8}	-7.2	-7.5	19.1	21.4
(<i>S</i>)-TSA2.3 _{CAT8}	-7.6	-8.1	20.2	21.1
(<i>R</i>)-TSH2.3 _{CAT8}	-6.5	-6.9	20.0	21.9
(<i>S</i>)-TSH2.3 _{CAT8}	-7.6	-8.0	18.8	21.0
(<i>R</i>)-TSA2.3 _{CAT9}	-7.2	-7.5	19.1	24.4
(<i>S</i>)-TSA2.3 _{CAT9}	-7.6	-8.1	20.2	24.0
(<i>R</i>)-TSH2.3 _{CAT9}	-6.5	-6.9	20.0	17.3
(<i>S</i>)-TSH2.3 _{CAT9}	-7.6	-8.0	18.8	17.7

Table B.5. Energies of selected catalysts including amine functional groups

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
(<i>R</i>)-TSA2.3 _{CAT14}	-5.9	-6.2	24.6	22.4
(<i>S</i>)-TSA2.3 _{CAT14}	-13.4	-13.8	18.6	15.0
(<i>R</i>)-TSH2.3 _{CAT14}	-7.5	-7.9	23.5	21.1
(<i>S</i>)-TSH2.3 _{CAT14}	-13.9	-14.4	17.0	14.7
(<i>R</i>)-TSA2.3 _{CAT15}	-0.1	-0.2	24.9	27.9
(<i>S</i>)-TSA2.3 _{CAT15}	0.3	0.4	25.1	28.2
(<i>R</i>)-TSH2.3 _{CAT15}	-5.1	-5.3	20.0	23.0
(<i>S</i>)-TSH2.3 _{CAT15}	-7.1	-7.1	17.1	20.7
(<i>R</i>)-TSA2.3 _{CAT18}	0.5	0.6	24.0	28.3
(<i>S</i>)-TSA2.3 _{CAT18}	0.4	0.5	24.0	28.2
(<i>R</i>)-TSH2.3 _{CAT18}	-2.0	-2.2	22.5	26.0
(<i>S</i>)-TSH2.3 _{CAT18}	-4.6	-4.7	19.8	23.4

Table B.6. Energies of selected catalysts including thiourea functional groups

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
(<i>R</i>)-TSA2.3 _{CAT20}	-7.7	-8.0	19.8	20.7
(<i>S</i>)-TSA2.3 _{CAT20}	-11.5	-11.8	15.6	17.0
(<i>R</i>)-TSH2.3 _{CAT20}	-6.7	-6.9	20.9	21.8
(<i>S</i>)-TSH2.3 _{CAT20}	-15.0	-15.2	11.0	13.5
(<i>R</i>)-TSA2.3 _{CAT21}	-9.6	-10.0	18.4	19.0
(<i>S</i>)-TSA2.3 _{CAT21}	-12.9	-13.2	13.5	15.7
(<i>R</i>)-TSH2.3 _{CAT21}	-7.8	-8.2	19.9	20.9
(<i>S</i>)-TSH2.3 _{CAT21}	-16.5	-16.7	9.7	12.1

Table B.7. Energies of selected catalysts including urea functional groups

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
(<i>R</i>)-TSA2.3 _{CAT35}	-9.8	-10.1	17.4	18.5
(<i>S</i>)-TSA2.3 _{CAT35}	-13.3	-13.7	14.5	15.2
(<i>R</i>)-TSH2.3 _{CAT35}	-11.8	-12.0	15.4	16.8
(<i>S</i>)-TSH2.3 _{CAT35}	-17.5	-17.9	10.0	11.3
(<i>R</i>)-TSA2.3 _{CAT36}	-12.1	-12.5	15.3	19.5
(<i>S</i>)-TSA2.3 _{CAT36}	-12.4	-12.8	14.8	16.3
(<i>R</i>)-TSH2.3 _{CAT36}	-10.0	-10.4	16.8	18.8
(<i>S</i>)-TSH2.3 _{CAT36}	-16.5	16.6	9.1	12.1
(<i>R</i>)-TSA2.3 _{CAT37}	-8.9	-9.1	17.5	19.5
(<i>S</i>)-TSA2.3 _{CAT37}	-12.5	-12.8	14.6	16.0
(<i>R</i>)-TSH2.3 _{CAT37}	-11.1	-11.4	15.6	17.6
(<i>S</i>)-TSH2.3 _{CAT37}	-16.5	-16.8	10.1	12.0
(<i>R</i>)-TSA2.3 _{CAT38}	-12.6	-12.9	14.2	15.9
(<i>S</i>)-TSA2.3 _{CAT38}	-12.7	-13.1	14.5	15.8
(<i>R</i>)-TSH2.3 _{CAT38}	-11.6	-11.9	15.3	16.9
(<i>S</i>)-TSH2.3 _{CAT38}	-16.9	-17.3	9.8	11.6
(<i>R</i>)-TSA2.3 _{CAT39}	-9.7	-9.8	16.5	18.9
(<i>S</i>)-TSA2.3 _{CAT39}	17.0	-17.3	11.4	11.5
(<i>R</i>)-TSH2.3 _{CAT39}	-8.3	-8.6	20.1	20.6
(<i>S</i>)-TSH2.3 _{CAT39}	-21.0	-21.6	8.0	7.8

Table B.8. Energies of selected catalysts including thiourea and urea functional groups

Name	$\Delta E^\ddagger$	$\Delta H^\ddagger$	$\Delta G^\ddagger$	$\Delta G^\ddagger_{\text{Quasi}}$
Reactants	0.0	0.0	0.0	0.0
(<i>R</i>)-TSA2.3 _{CAT21O}	-13.3	-13.7	14.5	15.3
(<i>S</i>)-TSA2.3 _{CAT21O}	-13.7	-14.2	14.3	15.0
(<i>R</i>)-TSH2.3 _{CAT21O}	-10.2	-10.5	17.2	18.3
(<i>S</i>)-TSH2.3 _{CAT21O}	-16.2	-16.6	11.3	12.5

(<i>R</i>)-TSA2.3 _{CAT35S}	-8.9	-9.1	18.0	19.5
(<i>S</i>)-TSA2.3 _{CAT35S}	-12.1	-12.4	15.0	16.3
(<i>R</i>)-TSH2.3 _{CAT35S}	-7.1	-7.3	20.0	21.5
(<i>S</i>)-TSH2.3 _{CAT35S}	-14.3	-14.2	10.1	14.2
(<i>R</i>)-TSA2.3 _{CAT37S}	-7.4	-7.7	19.5	20.8
(<i>S</i>)-TSA2.3 _{CAT37S}	-11.1	-11.3	15.5	17.2
(<i>R</i>)-TSH2.3 _{CAT37S}	-5.9	-6.2	21.7	22.5
(<i>S</i>)-TSH2.3 _{CAT37S}	-14.7	-14.9	11.7	13.7
(<i>R</i>)-TSA2.3 _{CAT38S}	-10.3	-10.6	16.7	18.1
(<i>S</i>)-TSA2.3 _{CAT38S}	-10.6	-10.9	16.3	17.8
(<i>R</i>)-TSH2.3 _{CAT38S}	-6.8	-7.2	20.7	21.7
(<i>S</i>)-TSH2.3 _{CAT38S}	-14.9	-15.1	11.0	13.5

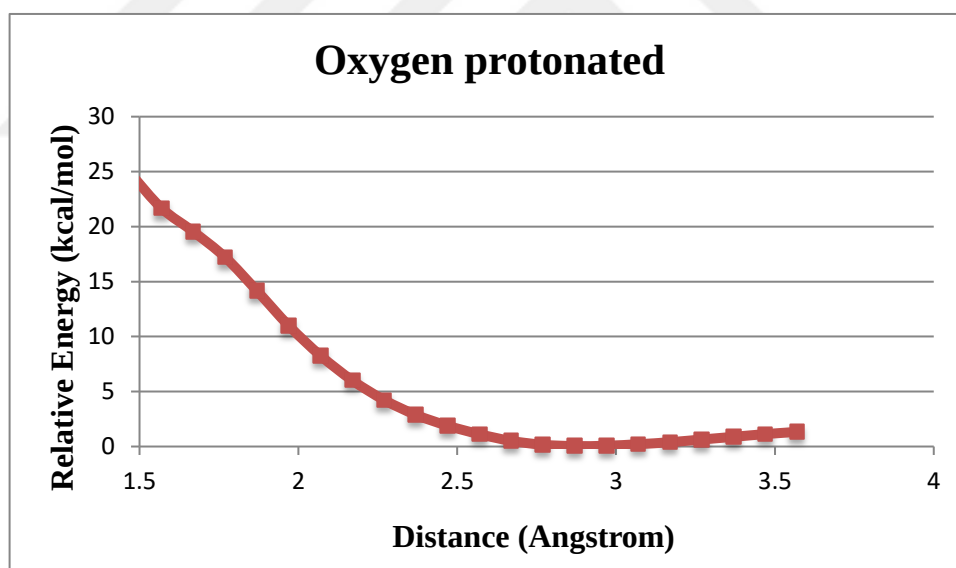


Figure B.1. Relative energies vs distance graph for oxygen protonated for hydroxyamination product

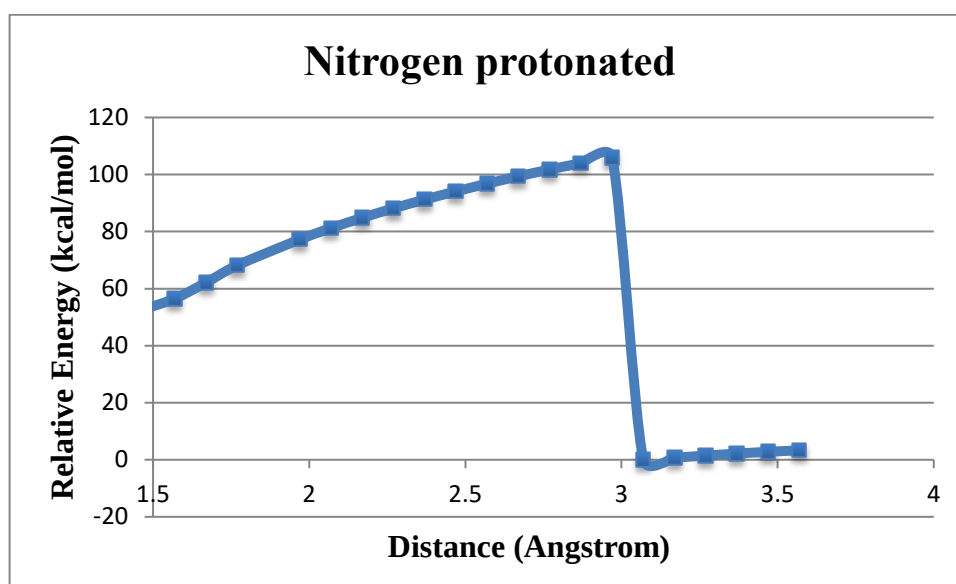


Figure B.2. Relative energies vs distance graph for nitrogen protonated for hydroxyamination product

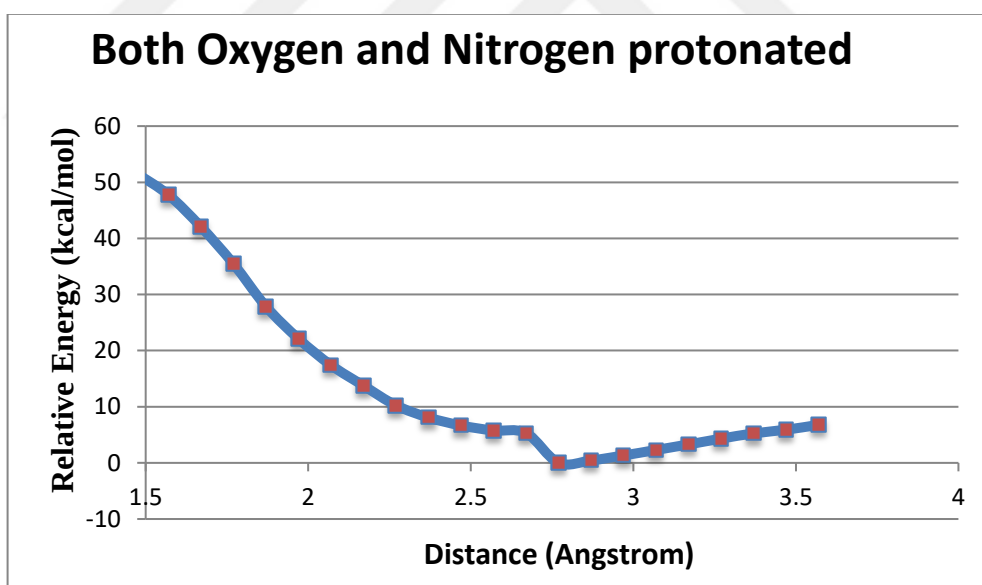


Figure B.3. Relative energies vs distance graph for both oxygen and nitrogen protonated for hydroxyamination product

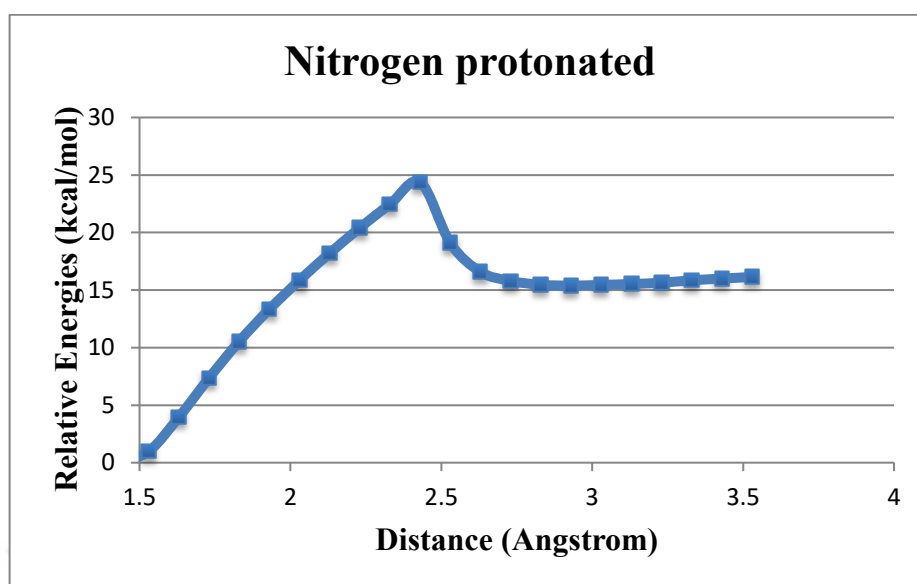


Figure B.4. Relative energies vs distance graph for nitrogen protonated for aminoxylation product

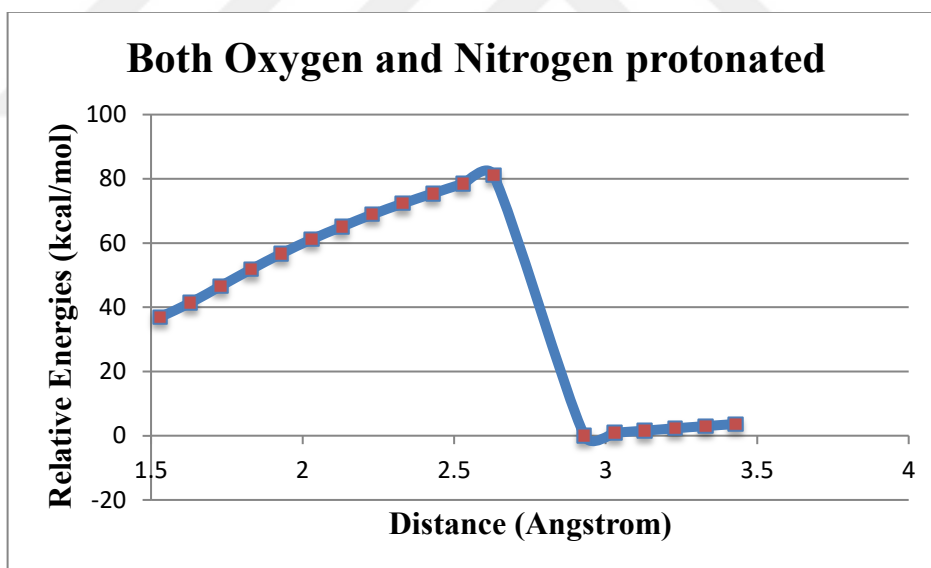


Figure B.5. Relative energies vs distance graph for both oxygen and nitrogen protonated for aminoxylation product

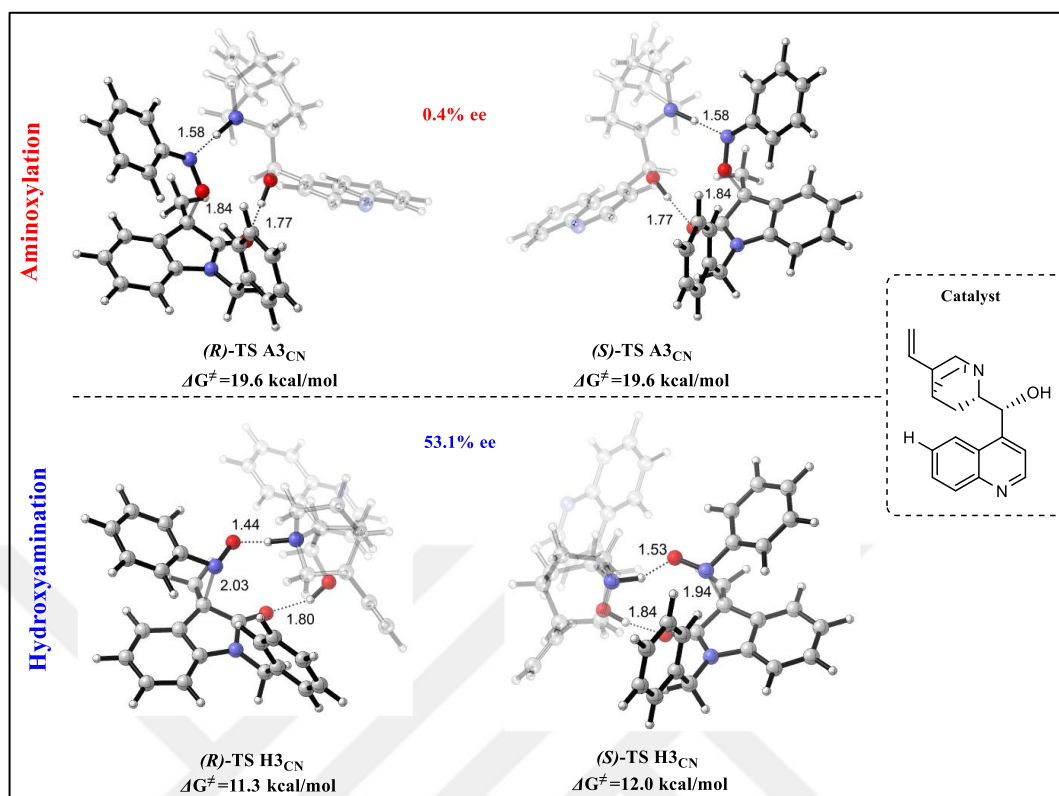


Figure B.6. Both aminoxylation and hydroxyamination TS and their enantioselectivity with cinchonine (CN)

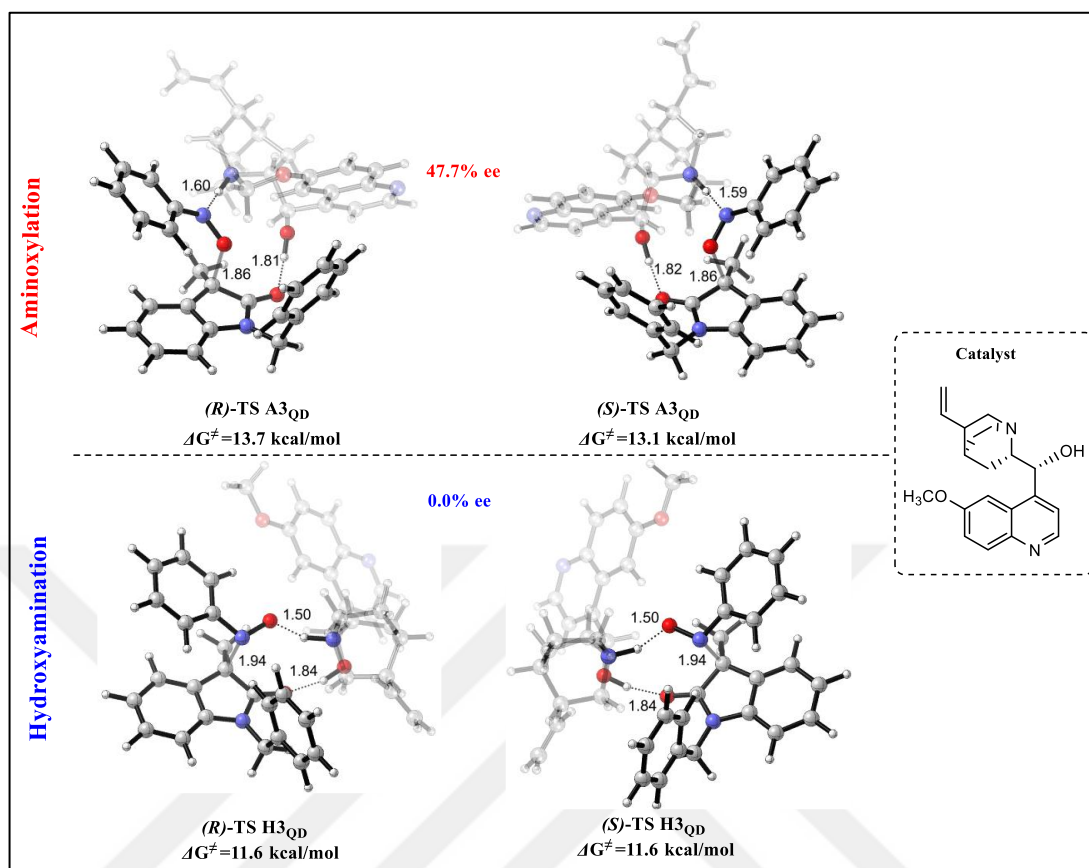


Figure B.7. Both aminoxylation and hydroxyamination TS and their enantioselectivity with quinidine (QD)

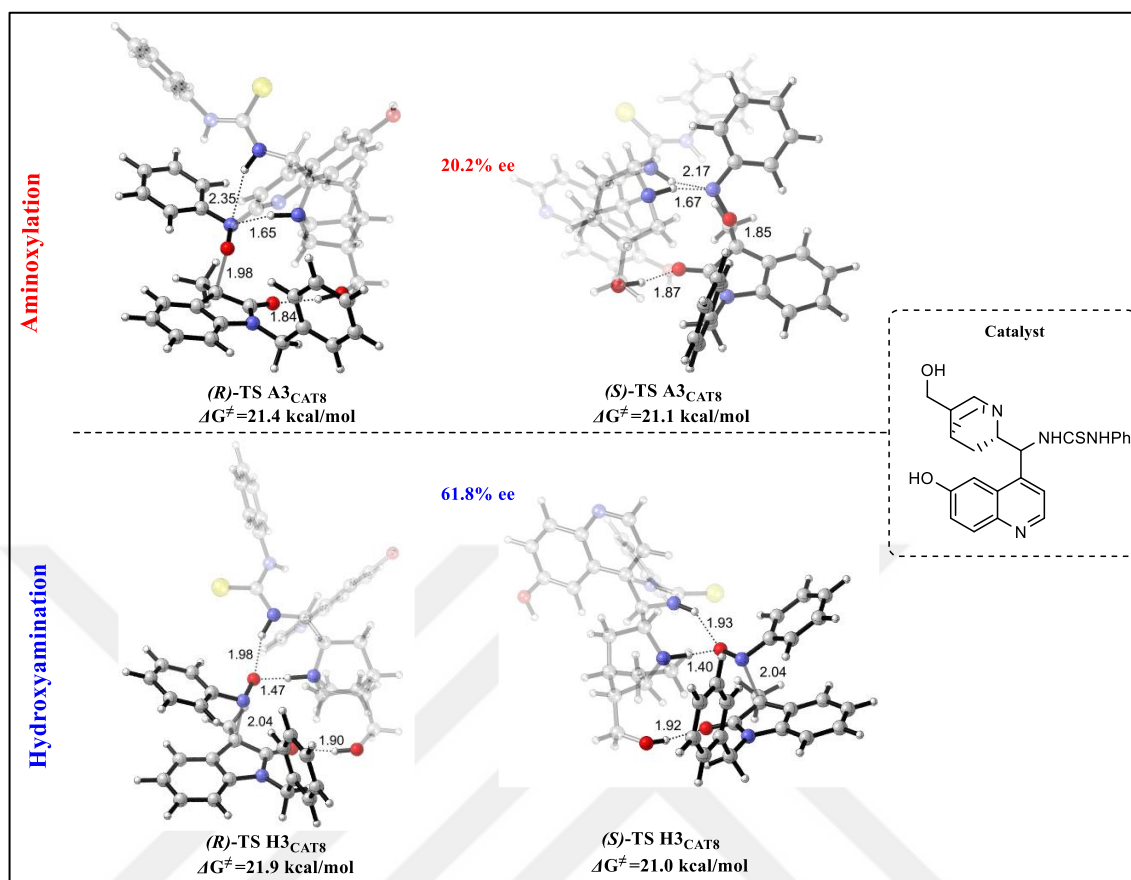


Figure B.8. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT8**

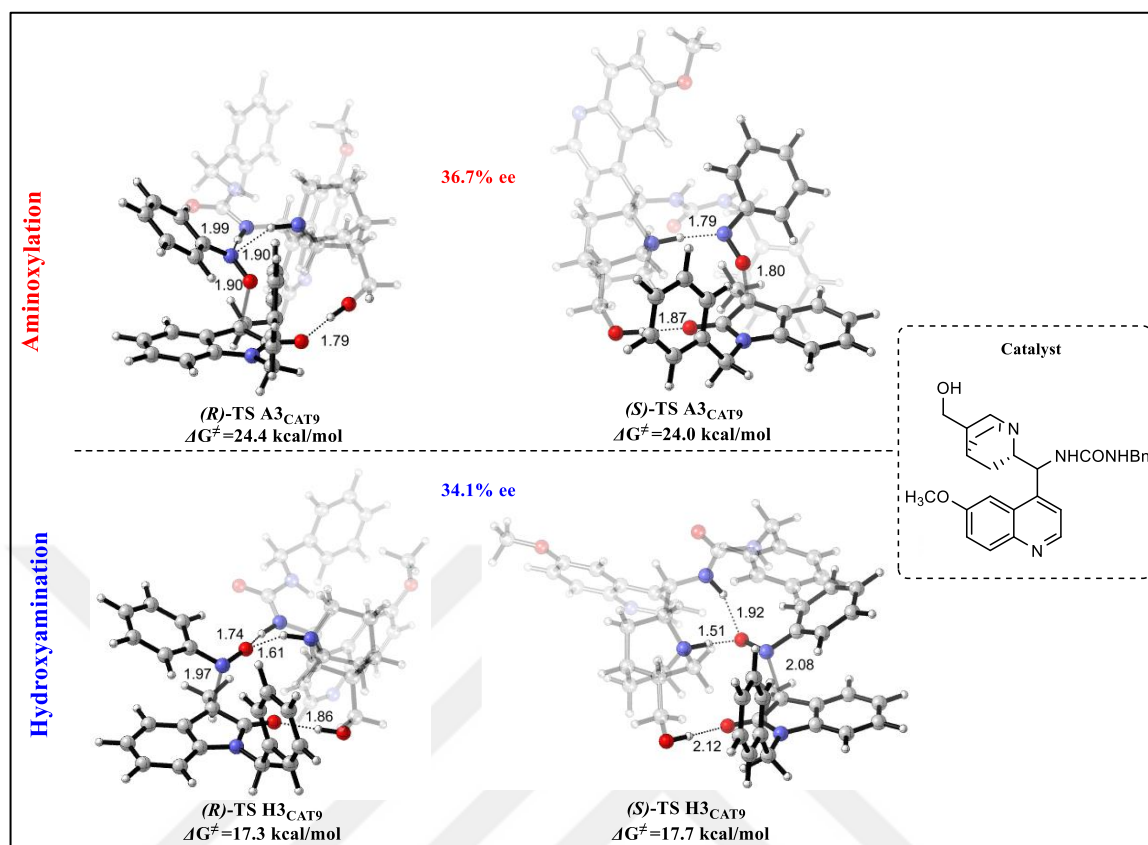


Figure B.9. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT9**

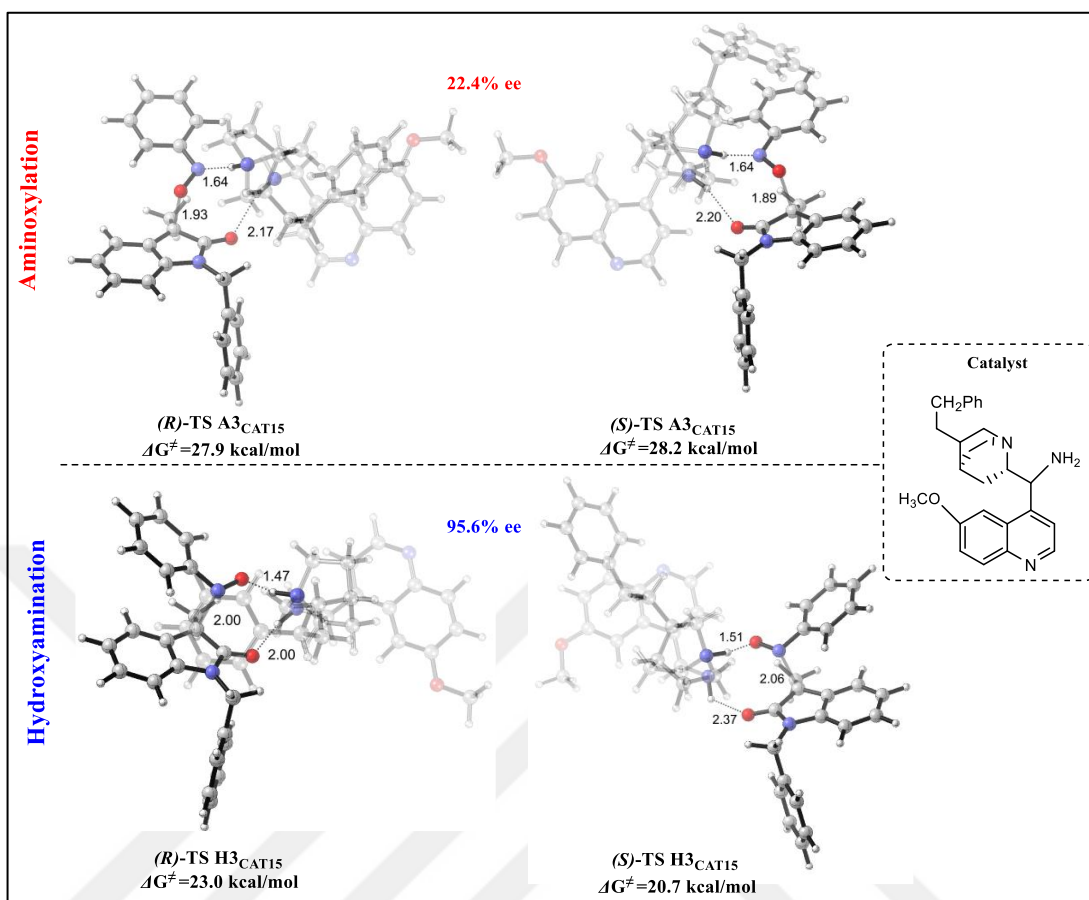


Figure B.10. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT15**

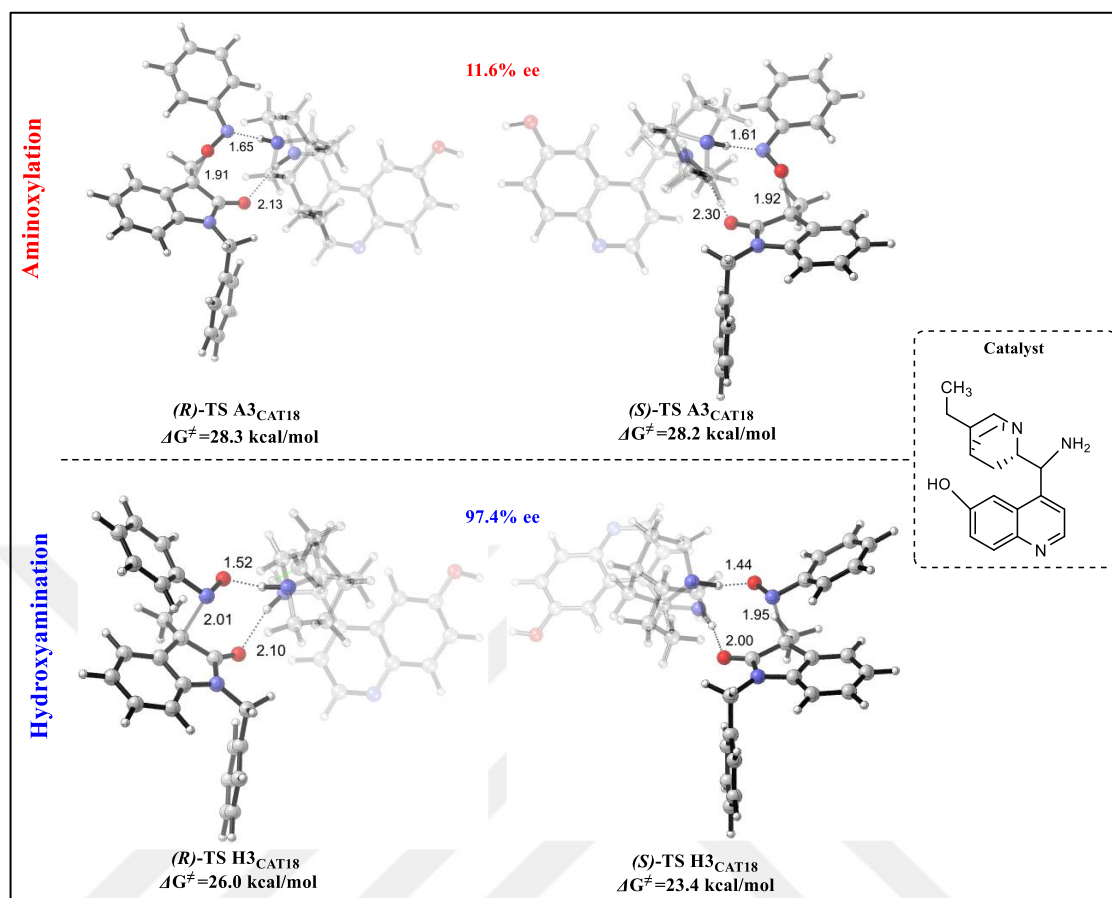


Figure B.11. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT18**

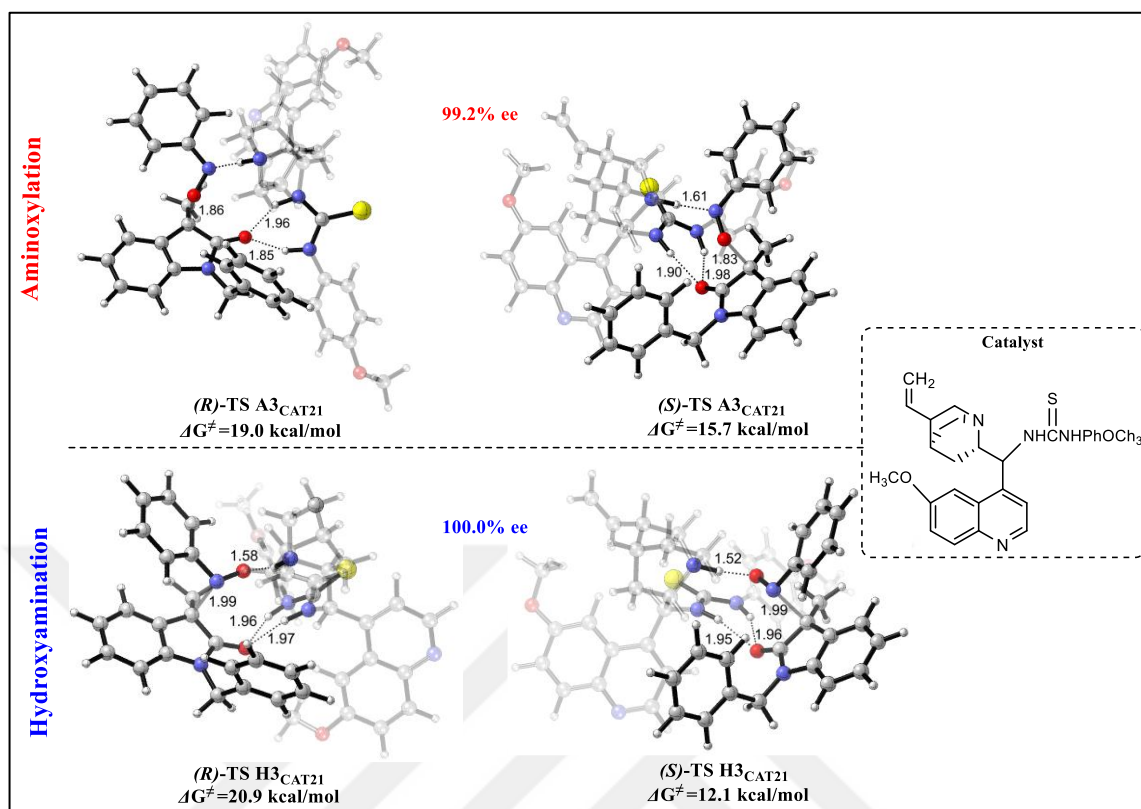


Figure B.12. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT21**

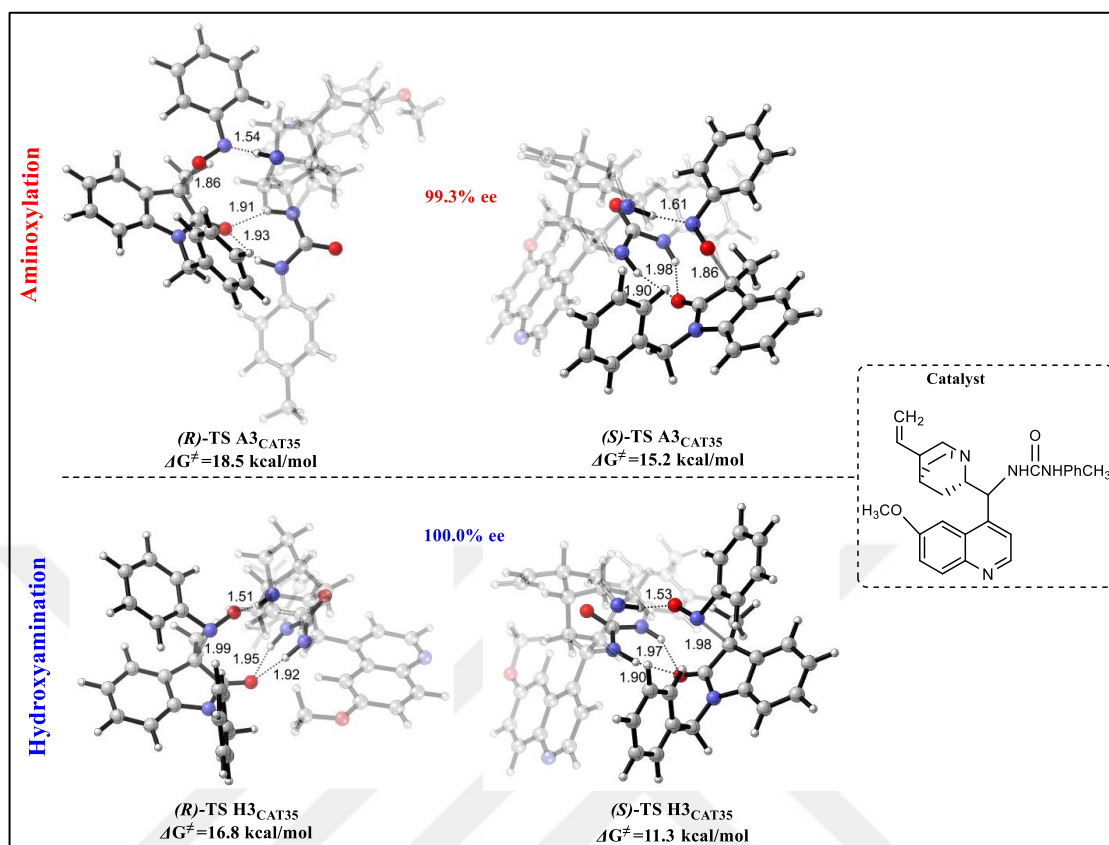


Figure B.13. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT35**

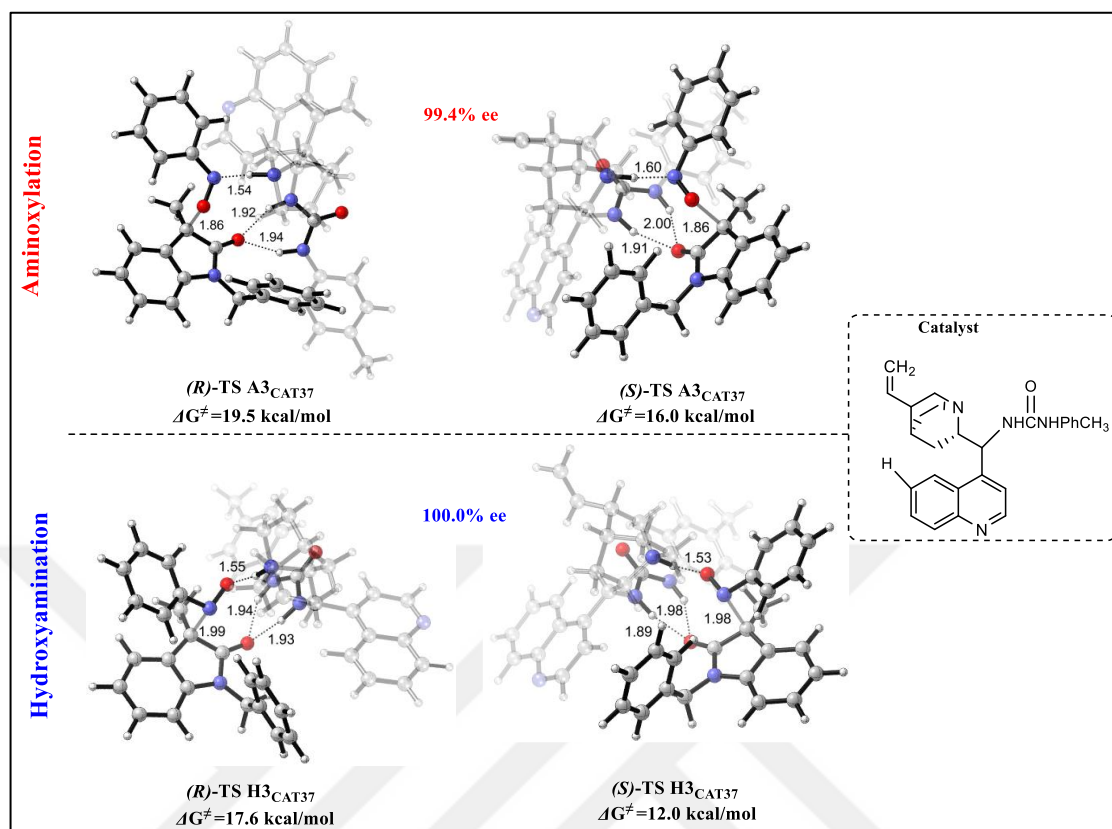


Figure B.14. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT37**

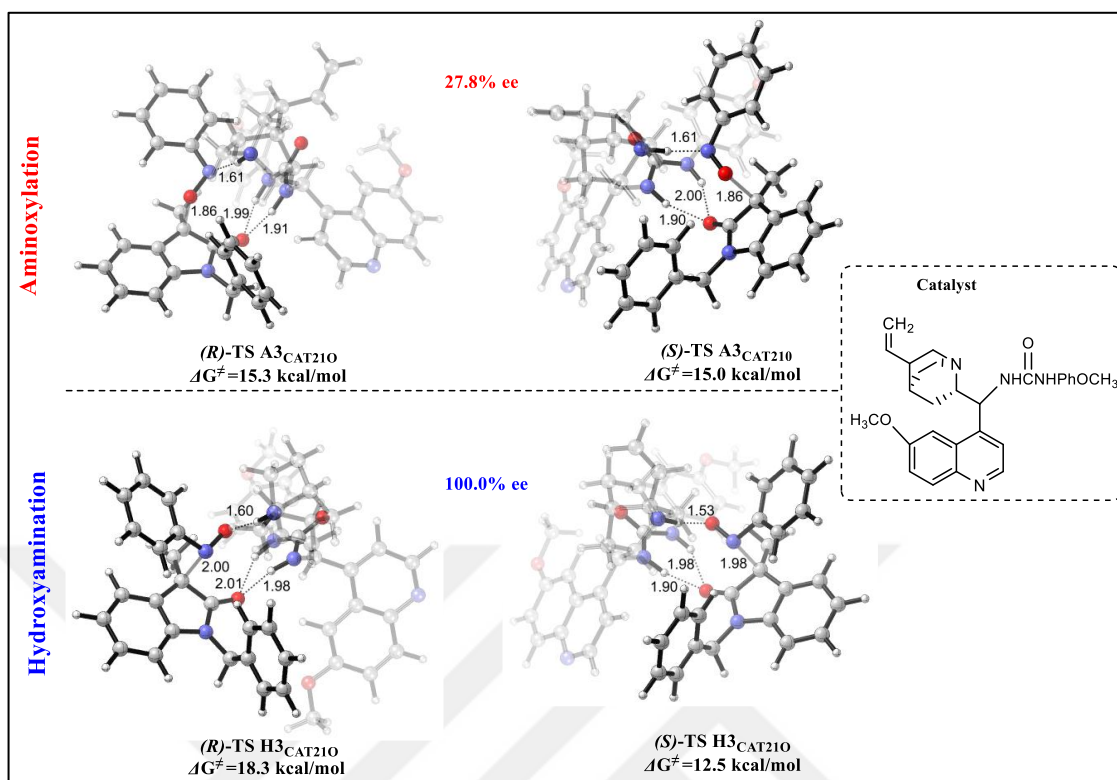


Figure B.15. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT210

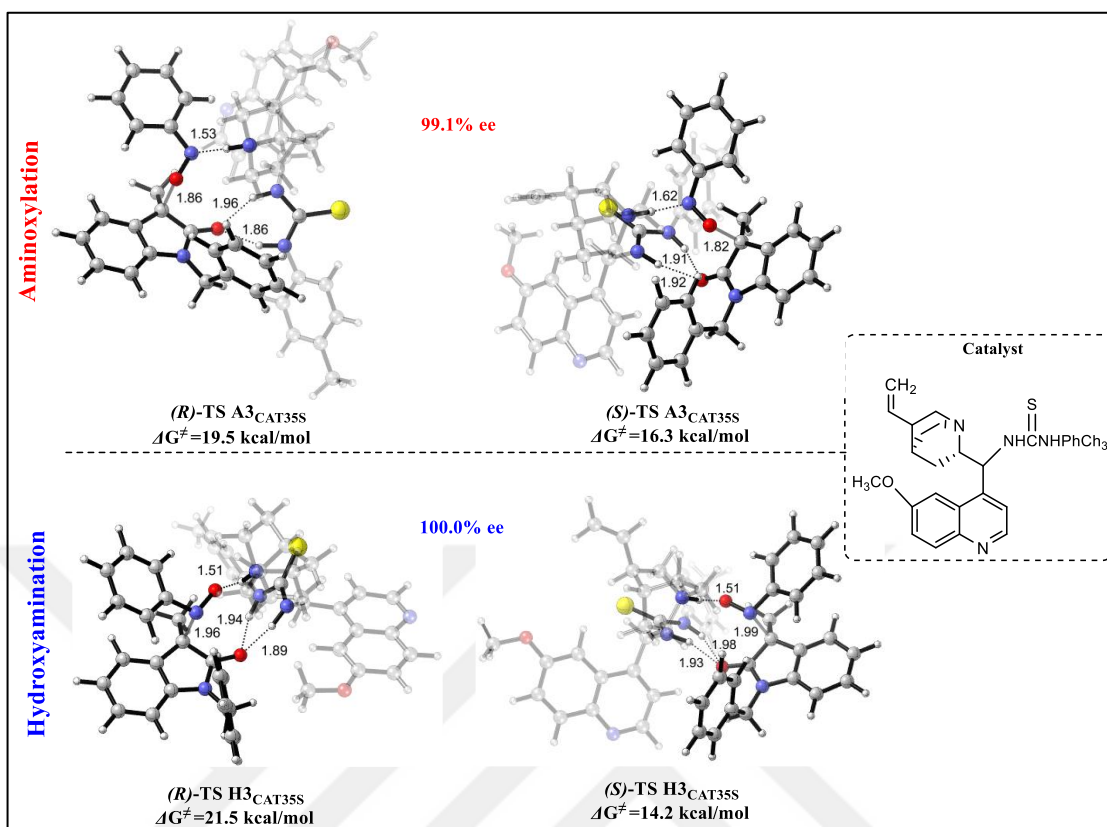


Figure B.16. Both aminoxylation and hydroxyamination TS and their enantioselectivity with CAT35S

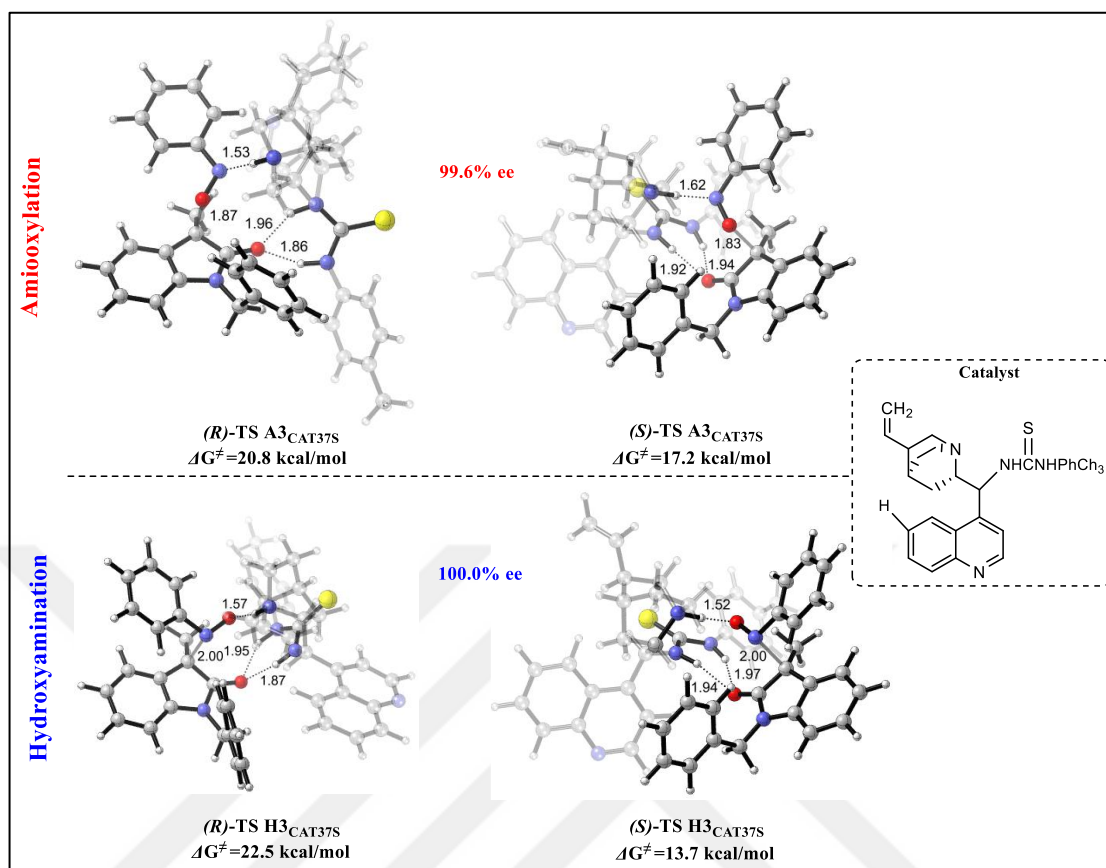


Figure B.17. Both aminoxylation and hydroxyamination TS and their enantioselectivity with **CAT37S**