INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

SYNTHESIS OF DTT ANALOGUES AND APPLICATIONS OF DTT AND IMMOBILIZED DTT

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Master of Science

in the Department of Chemistry

Faculty of Science

University of Prince Edward Island

Krista Affleck

Charlottetown, P. E. I.

January, 2002

SIGNATURE PAGE(S)

Not numbered in thesis

REMOVED

ABSTRACT

The work involved in my research project is sponsored by an Industrial NSERC Scholarship and so is centred around the interests of the sponsoring company, BioVectra™ dcl. As dithiothreitol, or DTT, is one of BioVectra™ dcl's major products, they were interested in new methods of synthesis and new applications for DTT analogues. The aim of the work was to find more economical methods of synthesis of DTT isomers than those currently used by BioVectra™ dcl, as well as to find new applications for it.

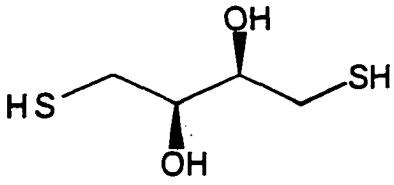


Figure 1 Structure of Dithiothreitol

The first part of the project was to find new ways to make dithiothreitol. Two different methods were tried, both from new starting materials. The challenge was the synthesis of these starting materials. The first starting material was *trans*-butene-1,4-diol which can be prepared from 2-butyne-1,4-diol by reduction with chromium.

Figure 2 Reduction of 2-butyne-1,4-diol to *trans*-2-butene-1,4-diol

This reaction was successful but the yields obtained were not good enough to

compensate for the high cost of the chromium reagent that would be used.

The second part of the project was the synthesis of another possible starting material, butadiene diepoxide. It would be prepared by the oxidation of butadiene.

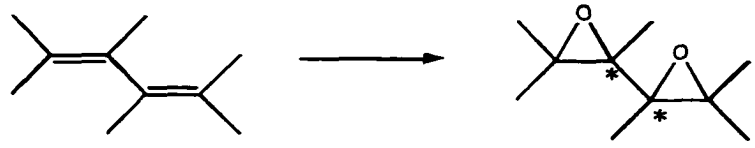


Figure 3 Oxidation of 1,3-Butadiene to Butadiene Diepoxide

It was determined that this reaction was also successful; however, under the reaction conditions, the product was being further oxidized and broken down into other compounds. As well, an excess of one conformation over another was not being obtained (at the *'s), as was desired. For these reasons, this method was abandoned.

The focus of the project was then changed to finding new applications for DTT and applications for new products being made by BioVectra dcl. These new products were all polymer-supported versions of DTT. In these, DTT is attached to polymer supports which make it insoluble in solution. Traditionally, DTT is used primarily as a biochemical reagent. It is useful in protein denaturation because of its ability to reduce disulfide bonds and stabilize monothiols in the reduced state. It is also used in protein extraction. Because it is a small molecule, it can pass through cell membranes and extract proteins from cells. It can be also used as a protecting group for thiols in organic synthesis. Despite its reducing abilities, its potential as a reducing agent for functional groups other than disulfides has not been extensively studied. It was believed that DTT and the polymer-supported DTTs could be useful in the reduction of various organic functional groups, namely nitro groups and azides.

DTT was found to be a useful reagent in the reduction of nitro groups under conditions milder than current methods. Several nitro compounds were successfully reduced and the reaction conditions needed were studied and determined.

ACKNOWLEDGEMENTS

There are many people who have helped me to reach this goal. These words do not begin to express my genuine gratitude and thankfulness. I especially give thanks

to my supervisor, Dr. Nola Etkin, for her support through the years, her vast knowledge and sound advice, and for her time, effort, patience and understanding which are all greatly appreciated;

to my supervisors at BioVectra™ dcl, Drs. Gary Reid, Tibor Breining and Richard Bethell for their suggestions and wisdom; to Dr. Wayne Goodwin for running GC samples and to Mike Hines for some HPLC work; to everyone at BioVectra™ dcl who made me feel welcome during my time there;

to NSERC for financial support;

to BioVectra™ dcl for financial support, the experience was wonderful and your contribution greatly appreciated;

to members of my supervisory and examination committees, Drs. Kathy Gottschall-Pass, Barry Linkletter, Kevin Smith, and Jeffrey Banks;

to my fellow Netkins, Monica Gill, Amanda Gallant, Liping Song, Lori Connolly, and especially to Tricia Adams for creating an enjoyable work environment; to former Netkin Melissa Wood who did some preliminary work on this project;

to the UPEI Chemistry Department for support, especially Dawna Lund for everything she does to keep the facilities in good condition;

to the Graduate Studies Committee;

to Ron Skinner at the PEI Food Technology Centre for some GC-MS work;

to CFI, ACOA, The Levesque Foundation and UPEI for their financial donations without which the graduate program would not exist;

to my friends and family, especially to my parents for their enduring support, patience and love throughout the past years;

and to my son, Tyler, for inspiring me.

TABLE OF CONTENTS

LIST OF SCHEMES

Chapter 3 New Synthetic Applications of Dithiothreitol & Immobilized DTT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

DTT	dithiothreitol
DTE	dithioerythritol
dcl	Diagnostic Chemicals Limited
TMSCl	trimethylsilyl chloride
NMR	nuclear magnetic resonance
TLC	thin layer chromatography
MHz	megahertz
THF	tetrahydrofuran
MCPBA	*meta*-chloroperoxybenzoic acid
e.e.	enantiomeric excess
ppm	parts per million
GC-MS	gas chromatography - mass spectrometry
AZT	3'-azidothymidine
DMSO	dimethyl sulfoxide

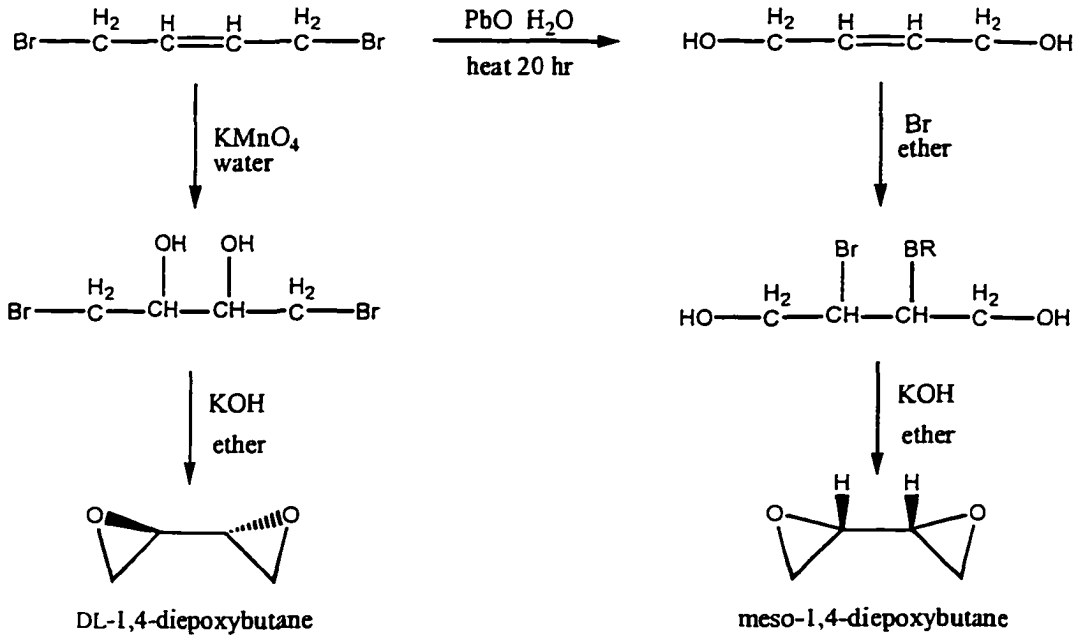
General Introduction

0.1 GENERAL INTRODUCTION

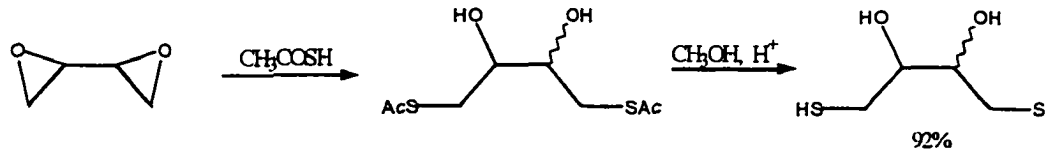
The work discussed in this thesis describes research sponsored by an Industrial NSERC Scholarship, and so, is centred around the interests of the sponsoring company, BioVectra™ dcl. As BioVectra™ dcl is the world's leading supplier of dithiothreitol (DTT), they were interested in investigating new methods of synthesis of DTT analogues and new applications. The aim of this work was to establish more economical methods of synthesis of DTT isomers than those currently used by BioVectra™ dcl, as well as to investigate new applications.

The main uses of DTT and DTE, dithioerythritol, are for biochemical applications. Both are useful in the reduction of disulfide bonds. Since they are water-soluble, they can permeate cell membranes for isolation and extraction of proteins[1]. They are also useful in organic synthesis as protecting groups for thiols.

Beech[2] published a synthesis for 1,3-diepoxybutane from 1,4-dibromo-2-butene (Scheme 1) which has 2-butene-1,4-diol as an intermediate and provides routes to the meso- and DL-isomers. Whitesides, Lilburn and Szajewski[3] reported the synthesis of a diastereomeric mixture of 1,4-dimercapto-2,3-butanediols from 1,3-diepoxybutane (Scheme 2). The union of these two schemes leads to the synthesis of DTT from *cis*-2-butene-1,4-diol. BioVectra dcl™ currently makes DTT from *cis*-2-butene-1,4-diol (Scheme 4). A corresponding route for DTE would start with *trans*-2-butene-1,4-diol. However, this



Scheme 1 Synthesis of 1,3-Diepoxybutane



Scheme 2 Synthesis of 1,4-Dimercapto-2,3-butanediols from 1,3-Diepoxybutane

starting material is very expensive in comparison with its *cis*-somer. An inexpensive synthesis for *trans*-2-butene-1,4-diol would be viable for the industrial production of DTE.

Chapter 1 describes the attempted synthesis of DTE from 2-butyne-1,4-diol using chromium as the reducing agent. Reduction with both stoichiometric and catalytic amounts of chromium were accomplished.

When reduction to *trans*-2-butene-1,4-diol did not appear to be an economically viable route, attention was given to 1,3-butadiene diepoxide as a possible starting material. If this could be made at low cost, then the initial steps shown above could be eliminated. If the right diastereoisomer of this starting material could be made stereospecifically, then DTT could be made exclusively. Chapter 2 discusses the attempted synthesis of 1,3-butadiene diepoxide from 1,3-butadiene using Jacobsen's catalyst and NaOCl. Several other conjugated dienes were also epoxidized.

Chapter 3 reports the new applications found for DTT and polymer-supported DTT. Efforts focused on DTT as an organic reducing reagent. DTT is not commonly known as an organic reducing agent though it has been shown to have the potential to be a useful and efficient one. The use of polymer-supported DTT was also investigated.

0.2 REFERENCES

1. Hart, R.A.; Lester, P.M.; Reifsnyfer, D.H.; Ogez, J.R. and Builder, S.E. *Bio/Technology*, **1994**, *12*, 1113-1117.

2. Beech, W. F. *J. Chem. Soc.* **1951**, 2483-7.

3. Whitesides, G. M.; Lilburn, J. E. and Szajewski, R.P. *J. Org. Chem.* **1977**, *42*, 332-338.

Chapter 1

Reduction of 2-Butyne-1,4-diol

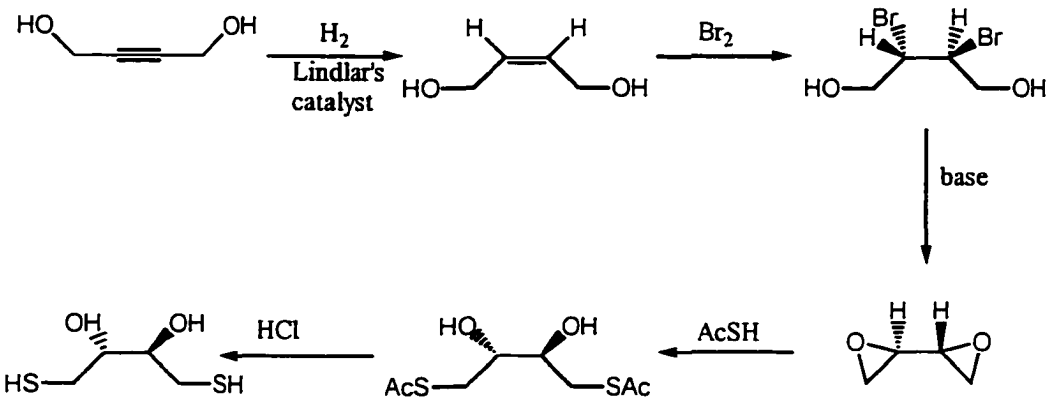
1.1 INTRODUCTION

General

Often in organic synthesis, there are few methods known for the formation of a desired intermediate. One such example is the reduction of an alkyne to a *trans*-alkene. More specifically, the reduction of 2-butyne-1,4-diol to *trans*-butene-1,4-diol, a compound of interest to the sponsoring company BioVectra dcl for the production of DTT.

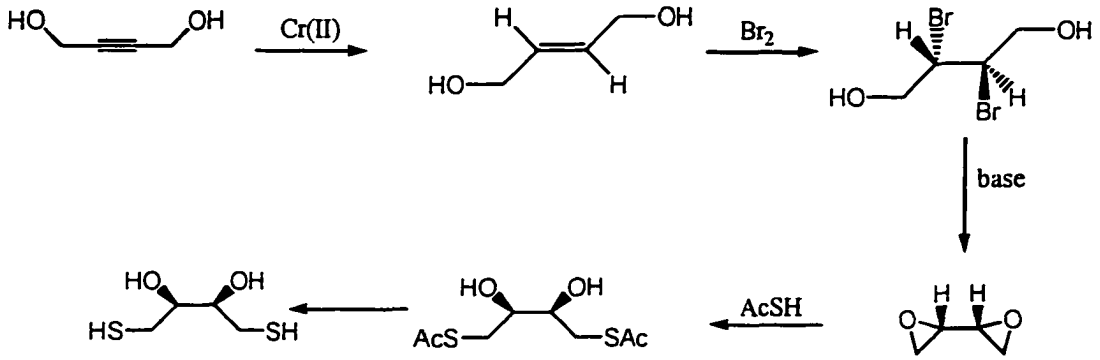
Scheme 3 Reduction of 2-Butyne-1,4-diol.

Cis-butene-1,4-diol is used by BioVectra™ dcl as an intermediate in the synthesis of dithiothreitol, DTT, one of their major products. Scheme 4 shows one route used for the synthesis of DTT. It is expected that DTE can be synthesized from *trans*-butene-1,4-diol via a similar route (Scheme 5). Successful reduction of 2-butyne-1,4-diol to *trans*-2-butene-1,4-



Scheme 4 Synthesis of Dithiothreitol

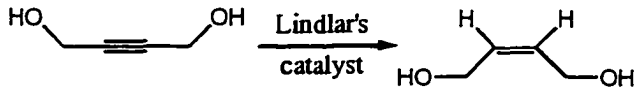
diol, followed by a similar reaction process, would lead to DTE.



Scheme 5 Proposed Synthesis of Dithioerythritol

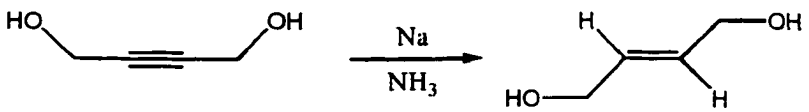
Reduction of Alkynes

Several methods are known for the reduction of alkynes. Catalytic hydrogenation is one of the most common because it is inexpensive and usually occurs with good yields. It,



Scheme 6 Catalytic Hydrogenation of an Alkyne

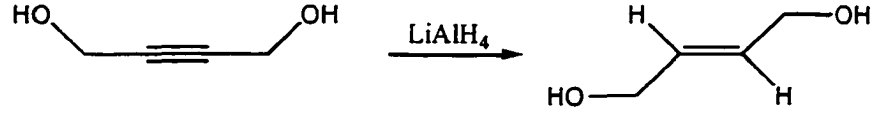
however, gives the *cis*-isomer of the alkene. The *trans*-isomer cannot be formed as easily. The known methods for the reduction of alkynes to *trans*-alkenes have several limitations, especially for industrial use. Dissolving metal reduction will successfully convert alkynes to



Scheme 7 Metal-Ammonia Reduction of an Alkyne

the corresponding *trans*-alkenes. This method involves dissolving Group I metals, most commonly sodium, in liquid ammonia to produce the active reducing agent. These reagents would not be practical in scale-up quantities because it is not safe to use liquid ammonia in a production plant.

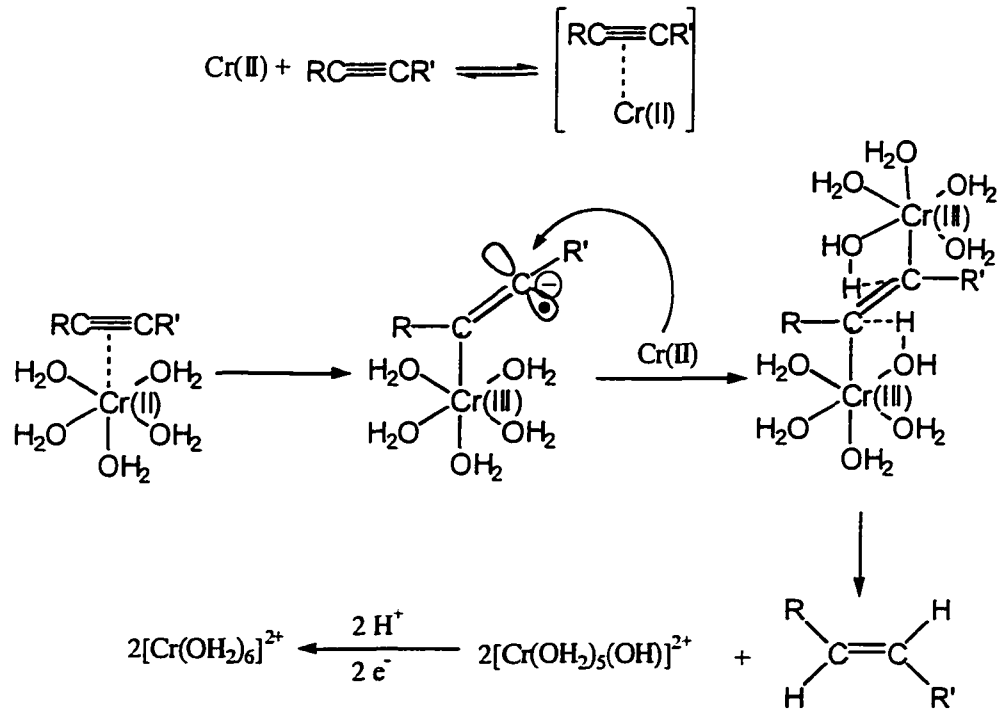
Hydridic reducing agents such as lithium aluminum hydride would also accomplish the task. However, these are expensive and would not be useful in industrial production.



Scheme 8 Reduction of an Alkyne with a Hydridic Reducing Agent

Chromium(II) has been shown[1] to be a useful reagent for the reduction of alkynes to *trans*-alkenes. Castro and Stephens reported the reduction of alkynes to *trans*-alkenes with Cr(II) that had been generated by mixing chromium sulfate with zinc metal under an atmosphere of nitrogen.

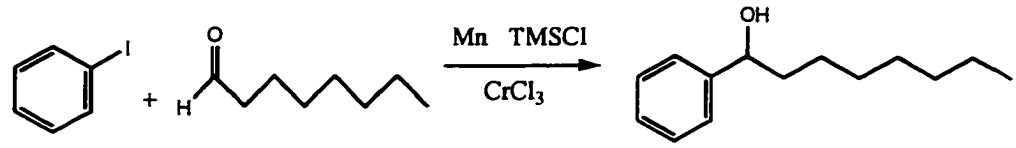
A less expensive method would be to prepare the chromium salt *in situ* from the reaction of chromium metal with sulfuric acid. Preparation of chromium sulfate by this method was reported by Lux and Illman[2]. The combination of these methods would make a less expensive process for the preparation of *trans*-alkenes.



Scheme 9 Mechanism for the Chromium Reduction of Alkynes.

Catalytic Reduction

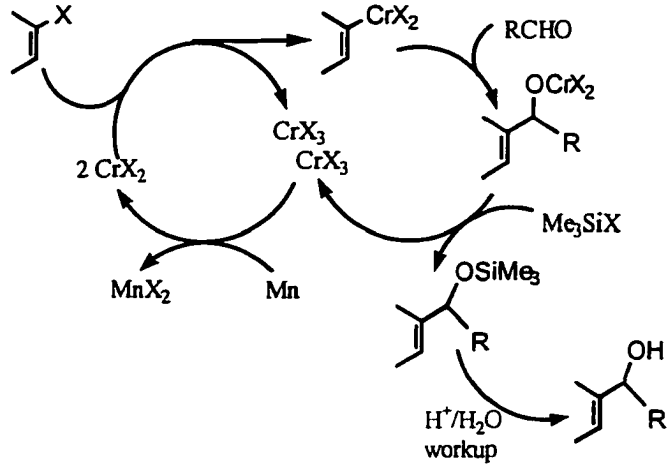
Chromium, however, is a toxic metal so its use in the quantities needed for the process described above would be environmentally undesired. Such quantities would also be very expensive. Its use could be minimized greatly by using catalytic quantities. If a catalytic amount of chromium could be paired with a stoichiometric amount of a less expensive and harmful reducing agent that can regenerate the Cr(II) *in situ*, then this method could be useful for industrial purposes. Such a co-reducing agent would have to be inert to reactions with the alkyne. If such a reducing agent could be found, both the cost and the production of



Scheme 10 Example of a Nozaki-Hiyama-Kishi Reaction

toxins could be minimized and the process be rendered useful.

Another possibility is the use of manganese and trimethylsilylchloride (TMSCl) to regenerate Cr(II). This combination was first reported[3] by Fürstner and Shi for Nozaki-Hiyama-Kishi reactions. These reactions involved the addition of organic halides to aldehydes. Catalytic amounts of $CrCl_3$ or $CrCl_2$ were paired with stoichiometric amounts of manganese powder and TMSCl to successfully complete the reactions. There was no apparent difference in starting with Cr(II) or Cr(III) as both gave successful results. The advantage is with Cr(III) as it is much easier to handle than Cr(II). The suspected catalytic cycle, as postulated



Scheme 11 Proposed Catalytic Cycle

by Furstner and Shi for the Nozaki-Hiyama-Kishi reactions is shown in Scheme 11. As can be seen from this diagram, the cycle can begin with either $CrCl_2$ or $CrCl_3$. The use of this method and its application to alkyne reduction will be discussed.

1.2 RESULTS & DISCUSSION

The common purpose throughout this chapter is the reduction of Cr(III) to Cr(II), which is then used to reduce 2-butyne-1,4-diol to *trans*-2-butene-1,4-diol. Several variations of this were attempted. Each procedure and its results will be described.

Stoichiometric Reduction

Two methods were used for the reduction of Cr(III) to Cr(II). The first was the reduction of the chromic ions in $Cr_2(SO_4)_3$ to chromous ions by zinc metal. This was a successful reaction as the solution, under an inert atmosphere, turned from green to blue, indicating the desired reaction had occurred. From here, reaction with the alkyne was carried out. The proton source for the reduction was assumed to be the solvent.

The second was the addition of sulfuric acid to chromium metal. A very exothermic reaction followed as the very blue Cr(II) was formed. Both were successful and gave comparable yields, but the latter method is preferred because chromium metal is less expensive than chromium sulfate.

In earlier attempts, the chromic solutions were transferred to the butyne via canulla, and in one case was even filtered first, all for the purpose of removing the zinc from the reaction mixture. In later attempts, however, the alkyne was added directly to the Cr(II) solution containing the zinc. There was no evidence to suggest that this affected the results in any way. In all cases, care was taken to ensure that no oxygen was admitted to the reaction

flask as this would destroy the reagent.

As the 2-butyne-1,4-diol was mixed with the aqueous Cr(II) solutions, the solution turned green as Cr(II) was oxidized to Cr(III). Basic work-up produced $CrO_3 \cdot xH_2O$ which is insoluble in water. The resulting precipitate was filtered off, removing traces of chromium from the product still in solution. The filtrate was evaporated to give a white solid residue, presumably *trans*-2-butene-1,4-diol and excess sodium hydroxide. Extraction of the product with ether from this residue proved to be troublesome. Approximately 25 extractions were needed in order to extract all of the product. To save both solvent and working time, the rest of the extractions were carried out using a Soxhlet apparatus. Yields using extraction were 7.5, 21, 23, and 87 % whereas using a Soxhlet apparatus gave yields of 56, 69, 77, 79, and 92 %. Yields from manual extraction were quite varied and most were fairly low. Use of the Soxhlet apparatus for the extraction greatly increased yields overall. No matter the extraction method, *trans*-2-butene-1,4-diol was always obtained quite cleanly.

No effect was noticed when the amount of solvent was reduced to only 30 mL from 275 mL. The yield using 30 mL of water was 77%, matching the yields obtained with more dilute solutions.

In each case though, the product was always pure *trans*-2-butene-1,4-diol. No signs of the *cis*-isomer were found. The ^1H NMR spectrum for *trans*-2-butene-1,4-diol shows two signals. A signal at 5.8 ppm corresponds to the two hydrogen atoms on the double bond. Another at 4.1 ppm corresponds to the two hydrogen atoms on the hydroxyl groups. The ^{13}C NMR spectrum shows two peaks, one near 62 ppm and another at 130 ppm. The peak at 62

ppm represents the two sp^3 carbons and the peak at 130, the two sp^2 carbons.

A major concern with this process is the chromium waste that is produced. The waste is obtained as a solid. It is filtered through Celite™ from the reaction solution. The water is then removed from this solution. Analysis shows that there are not excessive levels of chromium in the removed water. This is as expected since the water was removed on a rotary evaporator. Disposal, then, only comprises the chromium solid obtained from filtration.

Catalytic Reduction

Another member of the research group tried the same process using only a catalytic amount of the chromium reagent. Zinc was used to try to regenerate the Cr(II) species. Unfortunately, the reaction did not proceed as hoped as only 10% conversion was obtained according to the ^1H NMR spectrum of the product. This suggests that the zinc was not a sufficient reducing agent to convert Cr(III) to Cr(II) in the reaction mixture. And so, the chromium reagent was not regenerated and only a stoichiometric amount of 2-butyne-1,4-diol was reduced.

The second method attempted for catalytic reduction was that used for the Nozaki-Hiyama-Kishi reaction catalytic in chromium.[3] The Cr(II) species was generated from $CrCl_3$ by reduction with manganese under an atmosphere of nitrogen using THF as the solvent. As can be seen in Table 1, Mn is a better reducing agent for chromium as its reduction potential is more negative than that of Zn.

Table 1 Standard Reduction Potentials

Reaction	Standard Reduction Potential
Cr(III)(aq) + e- → Cr(II)	-0.424V
Zn(II)(aq) + 2e- → Zn	-0.76V
Mn(II)(aq) + 2e- → Mn	-1.18V

As the reaction was done in THF under anhydrous conditions, the protons were added upon aqueous workup. The conditions of the reaction were varied by changing the order in which the butyne and the TMSCl were added and by changing the molar equivalents of TMSCl and Mn. In all cases, the reaction was exothermic. 1M NaOH was added to precipitate the chromium as $Cr(OH)_3$. The chromium waste was filtered off, leaving a clear filtrate. When the water was removed on a rotary evaporator, a white solid remained. Soxhlet extraction was used to remove the product from the residue. The results obtained for each method are shown in Table 2.

Table 2 Variation of Conditions for Catalytic Chromium Reduction of 2-Butyne-1,4-Diol

Trial	molar equivalent of Cr	molar ratio of TMSCl and Mn to butyne	added first	yield	ratio butyne: butene	major product	ratio *cis:trans*
1	0.06	2.5:1	TMSCl	23%	12:1	*cis*	100:0
2	0.06	2.5:1	butyne	49%	2:3	*trans*	1:7.3
3	0.03	1:1	TMSCl	10%	3:2	*cis*	1.2:1
4	0.03	1:1	butyne	46%	19:1	*trans*	1:5

Unlike the stoichiometric chromium reduction where only the *trans*-2-butene-1,4-diol was obtained, the catalytic chromium reduction gave a mixture of *cis*- and *trans*-2-butene-1,4-diol. The ratio of *cis*:*trans* varied with the reaction conditions and were determined from NMR integration. When the TMSCl was added before the 2-butyne-1,4-diol, the *cis* isomer was obtained in excess. When the 2-butyne-1,4-diol was added first, the *trans* isomer was obtained in excess.

These results lead to suggestions for further work with this reaction. If adding the TMSCl after the substrate in the reaction leads to an excess of the *trans*-isomer, then slow dropwise addition of the TMSCl may produce an even larger excess. Another possibility would be to try the reaction without adding the TMSCl. Further work will also aid in explaining the mechanism of the reaction.

1.3 CONCLUSION

The reduction of 2-butyne-1,4-diol to *trans*-2-butene-1,4-diol was successful using stoichiometric amounts Cr(II) as the reducing agent. Several procedures and variations of these were used. In all cases, *trans*-2-butene-1,4-diol was produced exclusively. Yields from both the $Cr(SO_4)_2$ and Cr/H_2SO_4 reactions were comparable and respectable; however, they were less than satisfactory for industrial use. The yields would not compensate for the cost of the process as chromium is an expensive reagent.

The reduction of 2-butyne-1,4-diol was also successful using catalytic amounts chromium(II) as the reducing agent with Mn and TMSCl as co-reductants. However, both *cis*- and *trans*-2-butene-1,4-diol were produced. The ratio of the two and the major product produced could be controlled by changing the order in which the reagents were added.

1.4 EXPERIMENTAL

General

All reagents are available commercially and were not purified before use. Commercial silica TLC plates purchased from Aldrich Chemical Co. were used. An Innovative Technologies inert atmosphere glove box equipped with a -40°C freezer was used to prepare the catalytic reactions under an atmosphere of nitrogen. THF was dried using an Innovative Technologies solvent purification system.

NMR spectra were obtained on a Bruker (300 MHz) Avance 300 spectrometer using the specified deuterated solvent with TMS as internal standard, unless otherwise indicated. Continuous extractions were performed using a Soxhlet extractor.

trans-2-butene-1,4-diol

a) *Excess Amount of Chromium*

i) using $Cr_2(SO_4)_3$ and zinc to generate Cr(II)

$$Cr_2(SO_4)_3 + Zn \xrightarrow{H_2O} 2\,Cr(II) + Zn(II)$$

1) $Cr_2(SO_4)_3$ (25.5 g, 0.065 mol) and zinc powder (6.81 g, 0.104 mol, 1.6 mol eq) were combined with deionized water (120 mL) in a 500 mL Schlenk flask. The flask was purged with nitrogen and the mixture was left to stir overnight. The resulting blue solution was filtered. 2-butyne-1,4-diol (2.25 g, 0.02613 mol) was added to a 500 mL Schlenk flask. The flask was purged with nitrogen. The Cr(II) solution was transferred to this flask by syringe. The reaction mixture was left stirring overnight. 1 M NaOH (200 mL) was added to the reaction flask. The basicity of the solution was confirmed using pH paper. The precipitate formed was allowed to settle and then was filtered off by vacuum filtration. The water was removed from the filtrate on a rotary evaporator. The residue was extracted with diethyl ether until spotting on TLC silica plates using permanganate visualization solution[4] showed no more product being extracted. The organic layers were dried over $MgSO_4$, filtered by gravity and evaporated. A clear liquid remained (2.00 g, 87 %). ^1H NMR (D_2O, 300 MHz): δ 4.092-4.097 (dd, 4H), 5.833-5.837 (m, 2H); ^{13}C NMR (D_2O, 75 MHz): δ 62.007, 130.345.

2) $Cr_2(SO_4)_3$ (25.77 g, 0.0657 mol) and zinc powder (6.80 g, 0.1039 mol, 1.6 mol eq) were combined with deionized water (275 mL) in a 500 mL Schlenk flask. The flask was purged with nitrogen and the mixture was left to stir overnight. 2-butyne-1,4-diol (2.8 g,

0.03252 mol) was added to a 500 mL Schlenk flask. The flask was purged with nitrogen. The blue Cr(II) solution was transferred to this flask by canulla. The reaction mixture was left stirring for 2 hours. 1 M NaOH (200 mL) was added to the reaction flask. The basicity of the solution was confirmed using pH paper. The precipitate formed was allowed to settle overnight and was then filtered through Celite™. The water was removed from the filtrate on a rotary evaporator. The residue was extracted with diethyl ether until spotting on TLC silica plates using permanganate visualization solution showed no more product being extracted. The organic layers were dried over $MgSO_4$, filtered by gravity and evaporated to leave a residue. (0.65 g, 23 %). NMR data as in 1.

3) $Cr_2(SO_4)_3$ (25.63 g, 0.06535 mol) and zinc powder (6.51 g, 0.09956 mol, 1.5 mol eq) were combined with deionized water (275 mL) in a 500 mL Schlenk flask. The flask was purged with nitrogen and the mixture was left to stir overnight. 2-butyne-1,4-diol (2.81 g, 0.03264 mol) was added to a 500 mL Schlenk flask. The flask was purged with nitrogen. The blue Cr(II) solution was transferred to this flask by canulla. The reaction mixture was left stirring for 3 hours. 1 M NaOH (200 mL) was added to the reaction flask. The basicity of the solution was confirmed using pH paper. The precipitate formed was allowed to settle overnight and was then filtered through Celite™. The water was removed from the colorless filtrate on a rotary evaporator. A continuous extraction was set up using diethyl ether. It was continued for 4 days. The ether was evaporated leaving a liquid residue and a white solid. The residue was dissolved in water and extracted with hexane to give a colorless oil. (2.64

g, 92 %). NMR data as in 1.

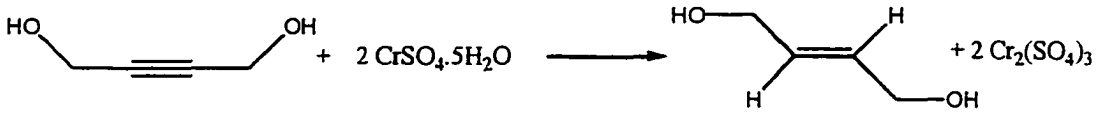
4) $Cr_2(SO_4)_3$ (25.01 g, 0.06377 mol) and zinc powder (6.63 g, 0.1013, 1.6 mol eq) were combined with deionized water (30 mL) in a 100 mL Schlenk flask. The flask was purged with nitrogen and the mixture was left to stir overnight. 2-butyne-1,4-diol (2.74 g, 0.03188 mol) was added to a 500 mL Schlenk flask. The flask was purged with nitrogen. The blue Cr(II) solution was transferred to this flask by canulla. The reaction mixture was left stirring for 2 hours. 1 M NaOH (200 mL) was added to the reaction flask. The basicity of the solution was confirmed using pH paper. The precipitate formed was allowed to settle overnight and was then filtered through Celite™. The water was removed from the colorless filtrate on a rotary evaporator. A continuous extraction was set up using diethyl ether. It was continued for 2 days. The ether was evaporated leaving a liquid residue. (1.88 g, 77 %). NMR data as in 1.

5) $Cr_2(SO_4)_3$ (25.59 g, 0.06525 mol) and zinc powder (6.63 g, 0.1013, 1.5 mol eq) were combined with deionized water (60 mL) in a 100 mL Schlenk flask. The flask was purged with nitrogen and the mixture was left to stir overnight. With a good flow of nitrogen, 2-butyne-1,4-diol (2.81 g, 0.03264 mol) was added to the flask. The reaction mixture was left stirring for 3 hours. The solution was mixed with 1 M NaOH (200 mL). The basicity of the solution was confirmed using pH paper. The precipitate formed was allowed to settle overnight and was then filtered through Celite™. The water was removed

from the colorless filtrate on a rotary evaporator. A continuous extraction was set up using diethyl ether and left for 4 days. The ether was evaporated leaving a liquid residue. (2.53 g, 88 %). NMR data as in 1.

ii) using chromium metal and sulfuric acid to generate Cr(II)

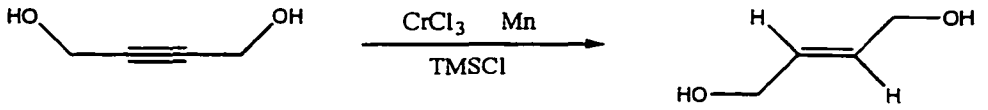
$$Cr + H_2SO_4 \longrightarrow CrSO_4.5H_2O$$



Cr powder (100 mesh, 20.0 g, 0.385 mol) and deionized H_2O (150 mL) were placed in a 500 mL Schlenk flask. H_2SO_4 (25 mL)was added to a dropping funnel which was fitted to the Schlenk flask. The apparatus was purged with nitrogen. The Cr/H_2O was cooled to 10°C in an ice-water bath. The ice-water bath was removed and replaced with a 20°C water bath. The H_2SO_4 was dripped in. The temperature of the water bath was kept between 20 - 30°C. When addition of H_2SO_4 was complete, the dropping funnel was replaced with a stopper and stirring continued overnight, under a flow of N_2. 2-butyne-1,4-diol (12.0 g, 0.1394 mol) was added to a Schlenk flask. Deionized H_2O (25 mL) was added to make a solution 4 M in alkyne. The flask was purged with nitrogen. The Cr(II) solution was cooled to 5°C in an ice-water bath. The ice-water bath was replaced with a water bath at 20°C. The stopper on the Cr(II) flask was replaced with a septum. A 60 mL syringe was purged with

nitrogen. The alkyne was added dropwise to the Cr(II) solution via syringe. The temperature of the water bath was kept at 20°C ± 5°C. After addition was complete, the mixture was left to stir for 5 hours. The reaction mixture and 1 L of 1 M NaOH were both cooled to 0 - 5°C. With stirring, the NaOH solution was slowly added to the reaction flask. A green precipitate formed. The basicity of the solution was tested with pH paper. The precipitate was left to settle overnight and then the mixture was filtered through Celite™. The filter cake, dark green solid, was washed three times with 300 mL portions of deionized H_2O. H_2O was removed from the colorless filtrate on a rotary evaporator. A white solid residue remained. This residue was divided in two and transferred into two extraction thimbles. Continuous extractions were carried out using diethyl ether. Extractions were continued for 6 days. Diethyl ether was removed from the extractions using a rotary evaporator. A faint yellow oil, *trans*-2-butene-1,4-diol was obtained (10.7 g, 87% yield). ^{1}H NMR (D_2O, 300 MHz): δ 4.038-4.073 (dd, 2H), 5.814 (m, 2H); ^{13}C NMR (D_2O, 75 MHz): δ 61.947, 130.276.

b) *Catalytic Amount of Chromium*



1.) $CrCl_3$ (0.2894g, 0.0001827 mol) was added to a 50 mL Schlenk flask. This was taken into the drybox. Manganese powder (3.4 g, 0.06188 mol) and THF (20 mL) were added. The flask was capped and taken out of the drybox. As TMSCl (0.75 mL, 0.006387 mol) was added via syringe, the mixture thickened and turned grey. With a good flow of

nitrogen, 2-butyne-1,4-diol (2.1753 g, 0.02526 mol) was added to the flask. The mixture bubbled vigorously and became hot. It was left to stir overnight. 1M NaOH (30 mL) was added and the mixture was stirred for 3 hours before being filtered under reduced pressure. The solvent was evaporated from the filtrate on a rotary evaporator. The white solid residue was transferred to a thimble and a continuous extraction was set up using diethyl ether. The extraction was continued for 4 days. The ether was evaporated from the extract to give *cis*-2-butene-1,4-diol (0.52g, 23%). ^{1}H NMR (D_2O, 300 MHz): δ 4.210-4.227 (dd, J_1 =1.5 Hz, J_2=4.5 Hz, 4H), 5.769 (m, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 57.364, 130.650. Also contained 2-butyne-1,4-diol ^{1}H NMR (D_2O, 300 MHz): δ 4.304 (s, 4H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 49.930.

2.) $CrCl_3$ (0.2790 g, 0.00176 mol) was added to a 50 mL Schlenk flask. This was taken into the drybox. Manganese powder (3.5136 g, 0.06395 mol) and THF (20 mL) were added. The flask was capped and taken out of the drybox. With a good flow of nitrogen, 2-butyne-1,4-diol (2.1852 g, 0.02538 mol) was added to the green solution. Heat was given off and the solution turned grey as TMSCl (7.5 mL, 0.0638 mol) was added dropwise via syringe. The mixture thickened and was left to stir overnight. 1M NaOH (100 mL) was added and the mixture was stirred for 3 hours before being filtered through Celite™ under reduced pressure. The solvent was evaporated from the filtrate on a rotary evaporator. The white solid residue was transferred to a thimble and a continuous extraction was set up using diethyl ether. The extraction was continued for 4 days. The ether was evaporated from the

extract to give *trans*-2-butene-1,4-diol (1.10g, 49% yield). ^{1}H NMR (D_2O, 300 MHz): δ 4.134-4.147 (dd, J=1.5 Hz, 4H), 5.875-5.894 (m, J=1.5 Hz, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 62.023, 130.390. Also contained small amounts of 2-butyne-1,4-diol ^{1}H NMR (D_2O, 300 MHz): δ 4.298 (s, 4H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 49.924, and *cis*-2-butene-1,4-diol ^{1}H NMR (D_2O, 300 MHz): δ 4.203-4.221 (dd, J_1=1.5 Hz, J_2=4.5 Hz, 4H), 5.7 (m, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 57.363, 130.640.

3.) $CrCl_3$ (0.1032 g, 0.0006516 mol) was added to a 50 mL Schlenk flask. This was taken into the drybox. Manganese powder (1.0218 g, 0.01859 mol) and THF (20 mL) were added. The flask was capped and taken out of the drybox. Heat was given off as TMSCl (7.5 mL, 0.0638 mol) was added dropwise via syringe. With a good flow of nitrogen, 2-butyne-1,4-diol (1.8096 g, 0.02101 mol) was added to the solution. The mixture was left to stir overnight. 1M NaOH (100 mL) was added and the mixture was stirred for 3 hours before being filtered through Celite™ under reduced pressure. The solvent was evaporated from the filtrate on a rotary evaporator. The white solid residue was transferred to a thimble and a continuous extraction was set up using diethyl ether. The extraction was continued for 4 days. The ether was evaporated from the extract to give *cis*-2-butene-1,4-diol (0.18g, 10%). ^{1}H NMR (D_2O, 300 MHz): δ 4.162-4.178 (dd, J=4.8 Hz, 4H), 5.709-5.737 (m, J=4.5 Hz, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 61.998, 130.426. Also contained 2-butyne-1,4-diol ^{1}H NMR (D_2O, 300 MHz): δ 4.243 (s, 4H); ^{13}C NMR (D_2O,

CH_3COCH_3 as external standard, 75 MHz): δ 49.911, and *trans*-2-butene-1,4-diol ^1H NMR (D_2O, 300 MHz): δ 4.094-4.102 (dd, *J*=1.5 Hz, 4H), 5.846 (m, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 57.321, 130.674.

4.) $CrCl_3$ (0.1019g, 0.000643 mol) was added to a 50 mL Schlenk flask. This was taken into the drybox. Manganese powder (1.0813 g, 0.01968 mol) and THF (20 mL) were added. The flask was capped and taken out of the drybox. With a good flow of nitrogen, 2-butyne-1,4-diol (1.6129 g, 0.01873 mol) was added to the flask. TMSCl (2.5 mL, 0.02129 mol) was added via syringe. The mixture was left to stir overnight. 1M NaOH (100 mL) was added and the mixture was stirred for 3 hours before being filtered under reduced pressure. The solvent was evaporated from the filtrate on a rotary evaporator. The white solid residue was transferred to a thimble and a continuous extraction was set up using diethyl ether. The extraction was continued for 4 days. The ether was evaporated from the extract to give *trans*-2-butene-1,4-diol (0.75g, 46%). ^1H NMR (D_2O, 300 MHz): δ 4.134 (dd, *J*=1.5 Hz, 4H), 5.879 (m, *J*=1.5 Hz, 2H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 62.027, 130.648. Also contained 2-butyne-1,4-diol ^1H NMR (D_2O, 300 MHz): δ 4.294 (s, 4H); ^{13}C NMR (D_2O, CH_3COCH_3 as external standard, 75 MHz): δ 49.925.

1.5 REFERENCES

1.Castro, C.E.; Stephens, R.D. *J. Am. Chem. Soc.* **1964**, *86*, 4358-4363.

2.Lux, H.; Illmann, G. *Ber.* **1958**, *81*, 2143-2150.

3.Furstner, A. and Shi, N. *J. Am. Chem. Soc.* **1996**, *118*, 12349-12357.

4.Casey, M.; Leonard, J.; Lygo, B. and Proctor, G. Advanced Practical Organic Chemistry. Glasgow: Blackie Academic & Professional, 1990, p. 114.

Chapter 2

Epoxidation of Conjugated Dienes

2.1 INTRODUCTION

General

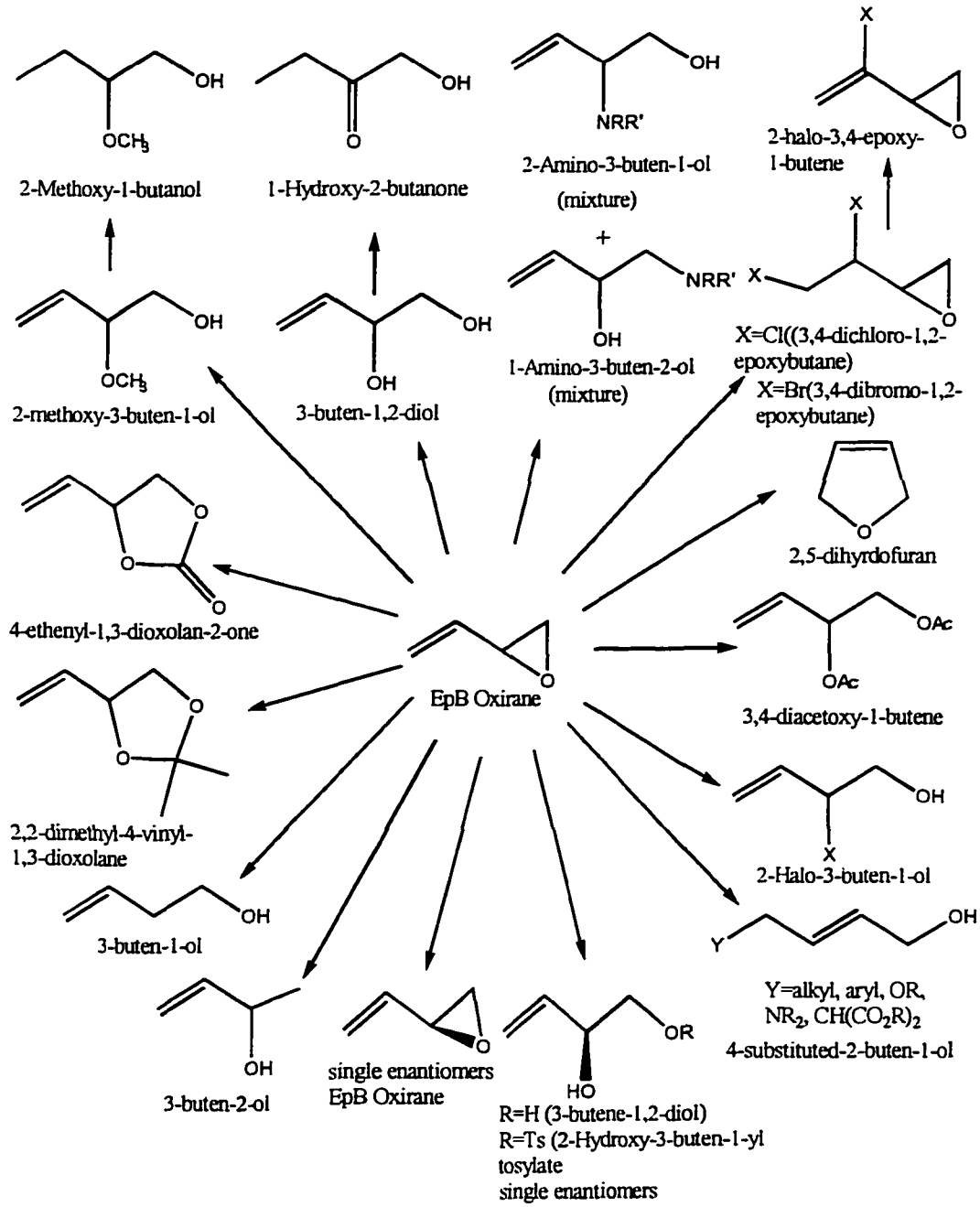
Another potential method of synthesis of DTT and DTE is from 1,3-butadiene diepoxide. This chapter describes the efforts made to synthesize this and other diepoxides, though most attention was directed towards the production of 1,3-butadiene diepoxide. A satisfactory process would be suitable for process scale-up and use in an industrial setting. To date, there have been no reports of diepoxidation of unfunctionalized conjugated dienes.

Scheme 12 Diepoxidation of 1,3-Butadiene

These diepoxides would have even more potential if they could be made diastereoselectively. If a method of preparation could be found that would preferentially produce one diastereoisomer then this method could have great applicability.

Epoxidation

Epoxides are common intermediates in organic synthesis. They can undergo a wide

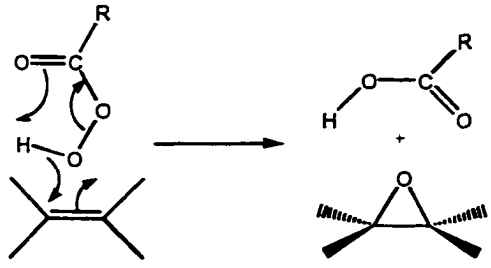


Scheme 13 Possible Derivatives of 3,4-Epoxy-1-butene

variety of reactions, such as reduction, nucleophilic substitution, and hydrolysis to name a few. The large number of reactions possible with epoxides leads to the derivation of numerous products. For this reason, epoxides are used as precursors for many organic compounds. Scheme 13[1] shows a number of the reactions that have been reported using epoxides as intermediates.

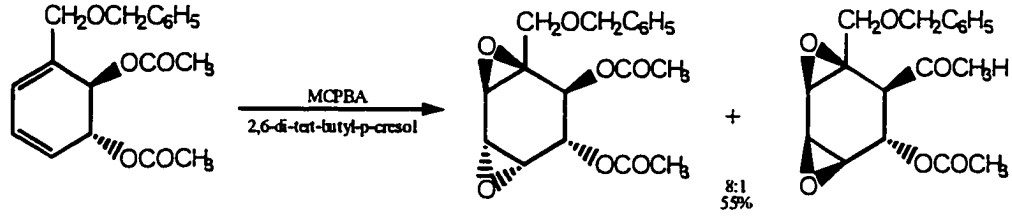
Alkenes are a common precursor for epoxides. Epoxidation of alkenes can be done by a variety of methods, as will be discussed. Most examples in the literature describe the epoxidation of unconjugated double bonds. There has been very little published regarding the epoxidation of conjugated double bonds. Such a reaction would produce 1,3-diepoxides which would have a large potential in organic synthesis, especially if the diepoxides could be made distereoselectively. However, this reaction is more difficult than simple alkene epoxidation as it proceeds much slower.[2] Reagents such as H_2O_2/NaOH and *t*-butylhydroperoxide/NaOH are not effective with conjugated dienes.

There are a wide variety of methods reported for the epoxidation of olefins. Some of these have achieved greater utility than others. One of the these is the *Prilezhaev* reaction which uses peracids, *m*-chloroperbenzoic acid being the most commonly used.[2] The mechanism is believed to be as shown in Scheme 14.



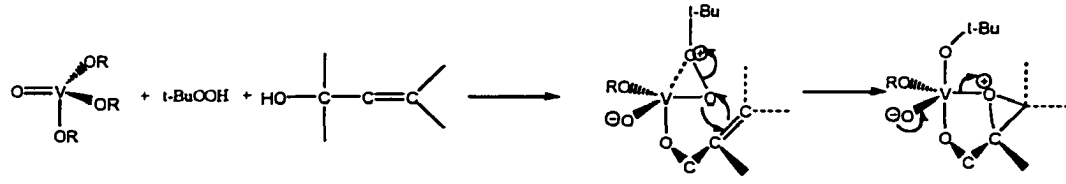
Scheme 14 An Example of the Prilezhaev Reaction

This reaction has been studied using a cyclic conjugated diene.[3] The diene used is fully substituted in all allylic positions as both double bonds are adjacent to acetate functionalities. So although epoxidation of a conjugated diene was achieved, it needed the allylic oxygens. Forcing conditions (MCPBA and 1,2-dichloroethane with 2,6-di-*tert*-butyl-*p*-cresol at 90°C for 2 hours) were needed in order for the second epoxidation to occur. A ratio of 8:1 trans diepoxide to cis diepoxide was obtained by this method. Though this method is selective, peracids are undesirable because of their instability which makes them very dangerous in industrial quantities.



Scheme 15 Diepoxidation of a Cyclic Conjugated Diene Using MCPBA.

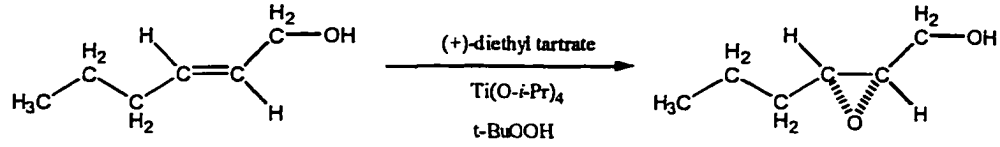
Also common is epoxidation using transition metals in combination with oxidizing agents. The most common transition metals used are vanadium and molybdenum, but cobalt, titanium, and osmium can also be used. The mechanism for the epoxidation is shown in Scheme 16.



Scheme 16 An Example of Epoxidation with Transition Metals

Another common method is the *Sharpless asymmetric epoxidation*[4] (Scheme 17). This method has gained widespread use in organic synthesis. It is highly enantioselective, but is only applicable to allylic alcohols. The neighbouring oxygen is needed to coordinate the reactant to the titanium which also coordinates to the *t*-butyl hydroperoxide, the oxidizing agent as can be seen in the transition state complex shown in Figure 1[5]. The coordination of the tartrate ester to the titanium creates a chiral environment and promotes stereoselective synthesis. If the (+)-tartrate is used, then the (-) product is obtained, and vice versa. Due to

the lack of an oxygen, this method would not be applicable to unfunctionalized olefins.



Scheme 17 Sharpless Asymmetric Epoxidation

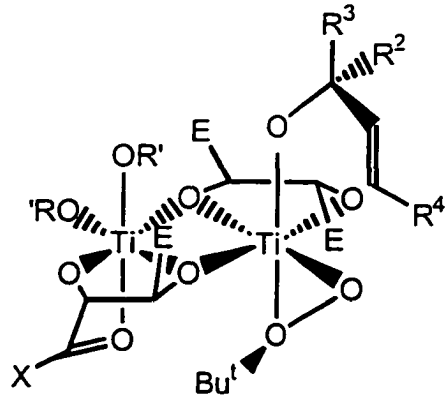


Figure 1 Sharpless Asymmetric Epoxidation Transition Complex

A stereoselective catalyst that is useful for unfunctionalized olefins is Jacobsen's catalyst, N,N'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III)chloride[6] (Scheme 19). The catalyst is a salen-based ligand coordinated through two nitrogens and two oxygens to a manganese centre. The *t*-butyl groups that are *ortho* and *para* to the oxygens prevent substrates from approaching the Mn

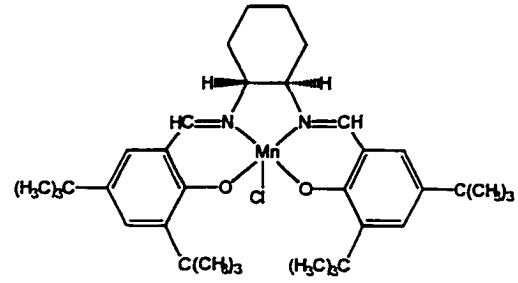
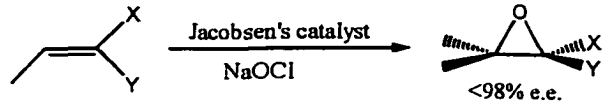


Figure 2 Jacobsen's Catalyst

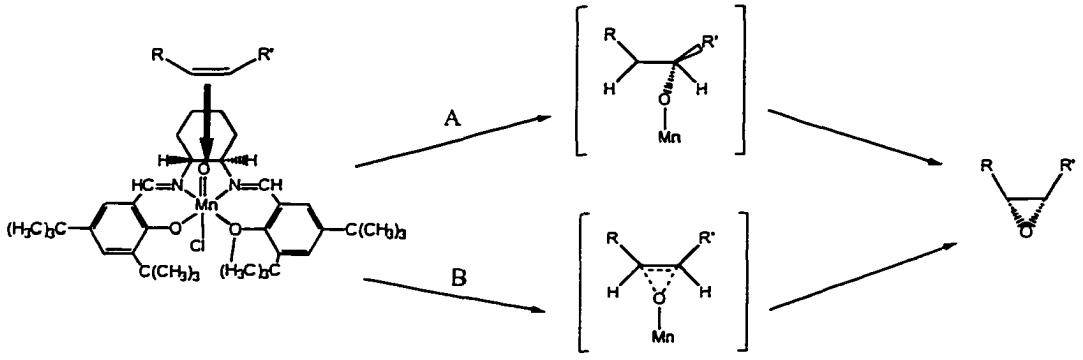
centre so they must approach over the cyclohexyl group. The chiral environment created by the hydrogens on the two chiral carbons direct the approach of the substrate. This causes the stereoselectivity. Enantiomeric excess of up to 98% has been achieved with unfunctionalized alkenes using Jacobsen's catalyst.



Scheme 18 Enantioselectivity of Jacobsen's Catalyst

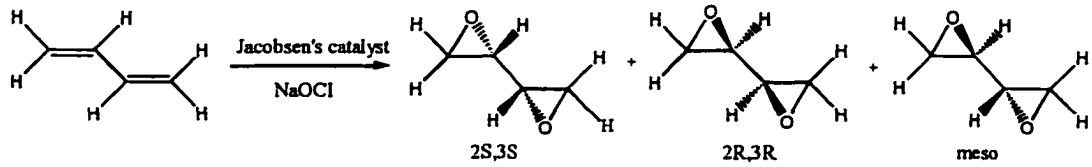
The oxygen source for this epoxidation is bleach, NaOCl. Iodosylbenzene is another commonly used oxidant but it is more expensive. For economical reasons, sodium hypochlorite was chosen. The epoxidation process is known to occur in two steps, the transfer of the oxygen to the metal centre, followed by the transfer of the oxygen from the metal centre to the alkene. The second step may proceed through a number of mechanisms.[7]

Oxygen transfer to an aryl substituted olefin occurs through the attack of an oxygen radical intermediate on the double bond, process A. Oxygen transfer to alkyl substituted olefins occurs through a concerted addition, process B.



Scheme 19 Possible Mechanisms of Epoxidation

If Jacobsen's catalyst would catalyze the epoxidation of unfunctionalized conjugated dienes, would the product be chiral? If a chiral product is formed, would the epoxidation be enantioselective? Since Jacobsen's catalyst selectively epoxidizes alkenes, it stands to reason that it should do the same for conjugated dienes. One stereoisomer should be formed preferentially. And since both enantiomers of Jacobsen's catalyst are available, each should be give the corresponding enantiomer of the diepoxide.



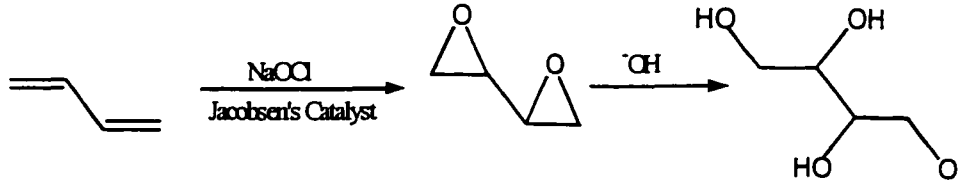
Scheme 20 Possible Diastereoisomers of Butadiene Diepoxide

This chapter will describe the efforts made to stereoselectively form diepoxides from various dienes using Jacobsen's catalyst and NaOCl.

2.2 RESULTS & DISCUSSION

Epoxidation of Butadiene

Efforts were made to synthesize several unfunctionalized diepoxides, with the majority of the effort being placed on the epoxidation of butadiene as it is an intermediate in the synthesis of DTE. The progress of the reaction was followed by watching the formation and loss of the monoepoxide signals in the NMR spectra of the organic layer of the reaction mixture. Spectra were obtained on aliquots of both the organic and aqueous layers. Monoepoxide was seen in the organic layer, however, diepoxide did not show up in the NMR spectra. When the organic layers were evaporated, there was no sign of any diepoxide. The diepoxide would have shown up as two signals near 2.6-2.8 ppm in the ^{1}H NMR spectrum. Accordingly, it was assumed that the diepoxide had gone into the water layer. The water layer was evaporated, giving a white solid. The NMR spectra showed evidence that other reactions were occurring after formation of the diepoxide. The ^{1}H NMR spectrum of the aqueous layer residue contained a large multiplet between 3 and 4 ppm. This corresponds to



Scheme 21 Oxidation of Butadiene

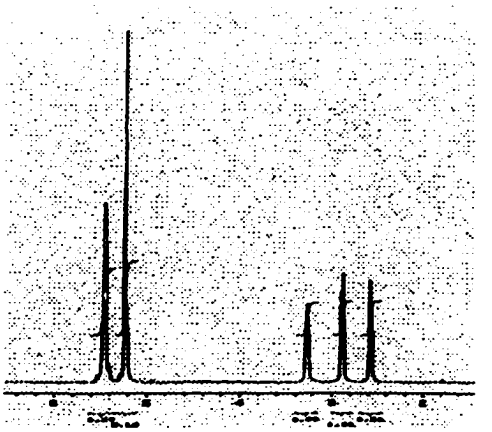


Figure 3 NMR Spectrum of 1,3-butadiene monoepoxide in organic layer

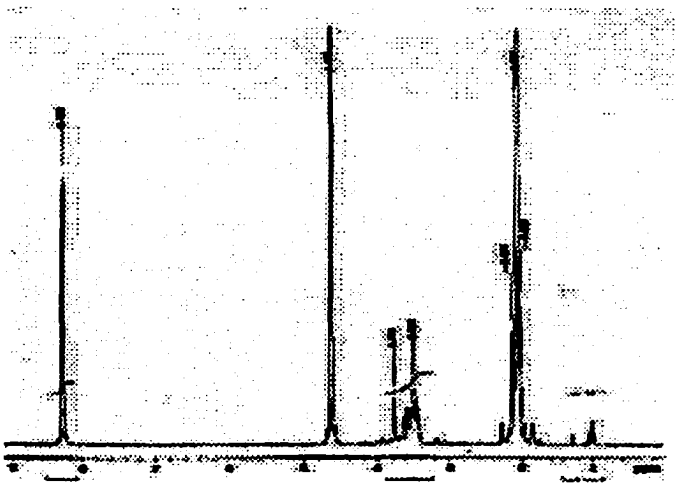


Figure 4 NMR spectrum of tetraol and formate in aqueous layer.

the spectrum of threitol. The total absence of any diepoxide signals suggested that it was instantly being converted to threitol as it was being formed which is understandable under the

basic conditions at which the reaction was carried out. The sodium hypochlorite solution used had a pH of 11.7. Purification of the threitol was attempted from ethanol. However, not all of the threitol could be removed from the other water-soluble residues. This, as well as loss of butadiene throughout the course of the reaction could account for the 31% yield.

Another possible reason for the low yield is that the threitol was being broken down. Signals at approximately 8.4 in the ^{1}H NMR spectrum and 170 ppm in the ^{13}C NMR spectrum of the threitol were puzzling. It was concluded that these signals corresponded to sodium formate. The threitol was being oxidized to formic acid, which under basic conditions was complexing to the sodium ions present in the solution to form sodium formate. The presence of sodium formate was confirmed by mixing a sample of the solid obtained in the reaction

Scheme 22 Oxidation of Threitol

with stock sodium formate. The presence of a single signal confirmed that the two were the same. The ^{1}H NMR spectrum showed a singlet at 8.48 ppm corresponding to the only hydrogen bonded to the carbon. The ^{13}C NMR spectrum showed a signal at 170 ppm corresponding to the only carbon in the molecule. Both spectra were run in D_2O with acetone added as an internal reference for the ^{13}C NMR spectrum.

The NMR spectra of the tetraol showed that more than one diastereomer was being formed. At this point it was decided to stop the epoxidation at the monoepoxide to determine if any enantioselectivity was achieved in the first epoxidation. If there was selectivity in the first step and it was lost in the second, then another method could be used for the second epoxidation. The first epoxide could be opened up, forming an allylic alcohol for which there are many selective epoxidation methods. If there was no selectivity in the first step, then hope of Jacobsen's catalyst being an asymmetric epoxidation catalyst for conjugated dienes would be lost. If this step was indeed enantioselective, and a chiral product was formed, then this would be very beneficial in itself as all of the reactions shown in Scheme 13 could be done enantioselectively. As both the (R,R) and (S,S) diastereomers of Jacobsen's catalyst are available, it is reasonable to assume that the two enantiomers of 3,4-epoxy-1-butene could be made separately.

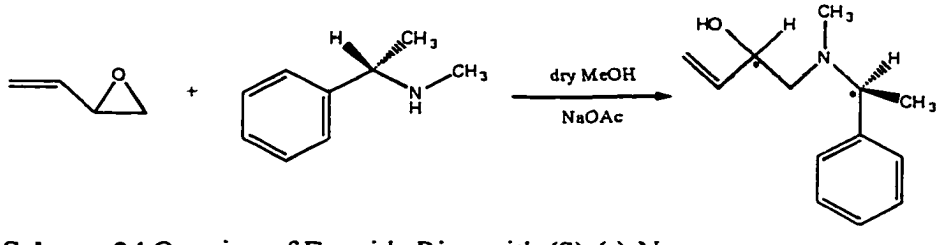
Scheme 23 Enantioselective Formation of 3,4-Epoxy-1-butene

Epoxybutene was formed by adding only one equivalent of NaOCl and stopping the reaction sooner than for the diepoxidation. Again, the progress of the reaction was followed by NMR spectroscopy. After separation of the layers, the organic layer was distilled to obtain 3,4-epoxy-1-butene. Yields ranged from 17 - 49%. The difference in the rates of the first and

second epoxidations was not appreciable for the monoepoxidation to be completed before the diepoxidation started. Therefore, the reactions had to be stopped at an in between point, when it was judged that the maximum possible amount of monoepoxide was present. At maximal monoepoxide concentrations, yield of monoepoxide would be greatest. This was a tough judgement, thus the variation in yields. Low yields are a result of some butadiene not being reacted and some monoepoxide having been further oxidized to the diepoxide (and onto threitol).

The optical rotation of the monoepoxide was measured using both manual and automatic polarimeters. Measurements suggested low enantiomeric excess. The results of this are inconclusive however because literature values found for 3,4-epoxy-1-butene are inconsistent.[8,9]

To determine if there was any enantiomeric excess in the monoepoxide, it was reacted with (S)-(-)-N,α-dimethylbenzylamine. This is a secondary amine which would open up the epoxide by nucleophilic attack and form a compound with two chiral centres. The product was examined by GC-MS to determine diastereomeric excess. The results, however, were

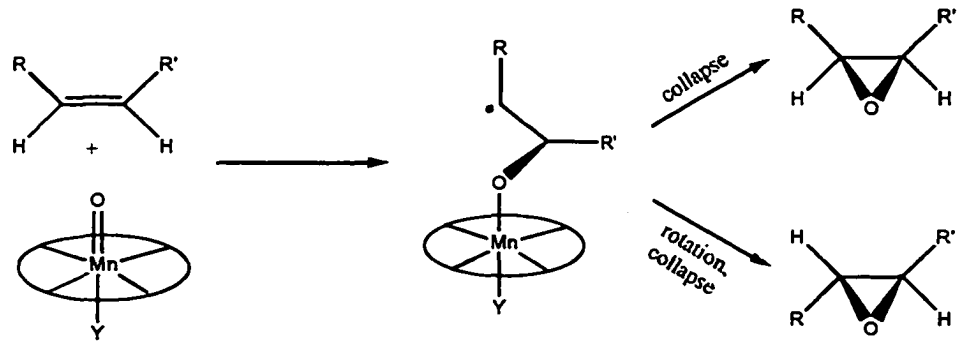


Scheme 24 Opening of Epoxide Ring with (S)-(-)-N,α-Dimethylbenzylamine

inconclusive. NMR spectra suggested the presence of two diastereoisomers. GC-MS, however suggested three isomers. As Scheme 13 shows, it is possible for secondary amines to open the epoxide two ways, giving a mixture of products.

It was, at this time, discovered that another group had tried the monoepoxidation of butadiene. Jørgensen *et al.* [10] reported a 50% yield of 3,4-epoxy-1-butene from 1,3-butadiene using Jacobsen's catalyst and sodium hypochlorite. They did not report an enantiomeric excess, but did give e.e.'s for the same epoxidation reaction using other chiral manganese salen complexes in a subsequent paper[11]. The yields for these reactions ranged from 61 - 72% with enantiomeric excesses of 10 to 17. Neither publication gave any report of diepoxidation.

The mechanism of the epoxidation reaction with Jacobsen's catalyst has been the focus of much study, however, no definite answer has been established. A possible reason for the low enantioselectivities obtained in this epoxidation is that the intermediate may involve a radical intermediate.[12-14]



Scheme 25 Radical Epoxidation of an Alkene by Jacobsen's Catalyst

If such a mechanism does occur, as evidence shows it does, then this method would not be suitable for the epoxidation of butadiene. Enantiomeric excess would not occur, at least to a large enough extent, for practical use.

Epoxidation of other dienes

Other substituted dienes were also epoxidized. The electron donating properties of alkyl substituents make double bonds more electron-rich and, presumably, also more reactive. 5,5-dimethyl-1,3-hexadiene and 2,5-dimethyl-2,4-hexadiene were both reacted with sodium hypochlorite in the presence of Jacobsen's catalyst. These reactions were easier than those with butadiene because all reagents were liquid, unlike butadiene, which is a gas. The reaction components were merely mixed together in a flask and left stirring.

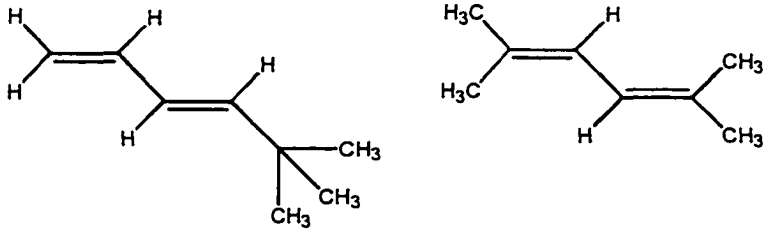


Figure 5 Epoxidized Dienes

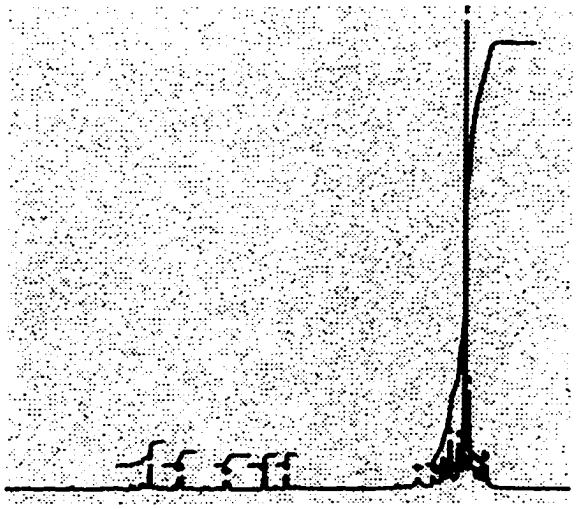


Figure 6 NMR spectrum of 1,3-epoxy-5,5-dimethylhexane

5,5-dimethyl-1,3-hexadiene was successfully epoxidized to 1,3-diepoxy-5,5-dimethylhexane with Jacobsen's catalyst and sodium hypochloride. Column chromatography purified the diepoxide. The resulting ^{1}H NMR spectrum was shifted upfield from that of the starting material. This was as expected, as there were double bonds in the starting material which appear further downfield than those of an epoxide.

2,5-dimethyl-2,4-hexadiene was successfully epoxidized to 2,4-diepoxy-2,5-hexane; two isomers were obtained, as shown in the NMR spectra. Column chromatography partially separated these isomers, but there were fractions in which the two were mixed. Since both the starting materials and the products are symmetrical, only three signals will be seen for the

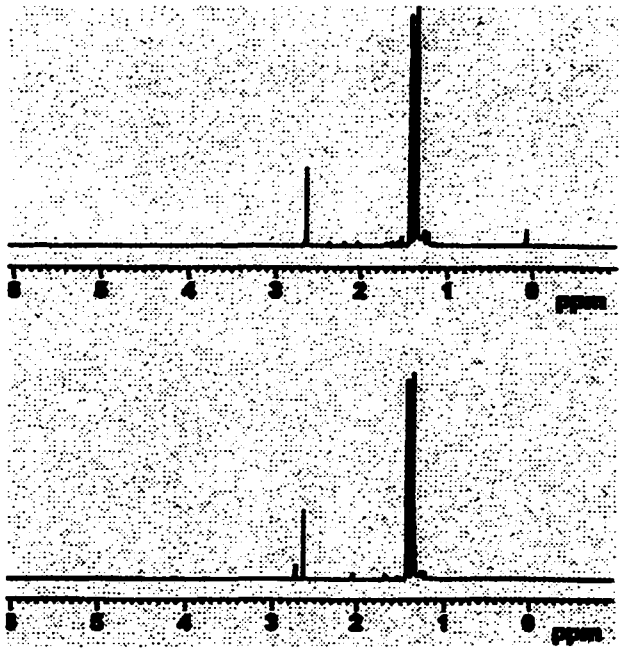


Figure 7 top: NMR spectrum of 2,4-Diepoxy-2,5-dimethylhexane. Bottom: NMR spectrum of the isomers of 2,4-Diepoxy-2,5-dimethylhexane.

different environments of the protons and four for the carbons; one each for the carbons on the corresponding ends of the double bonds, and one each for the corresponding methyl groups. The hydrogens of the methyl groups show up as two signals, one at 1.36 and the other at 1.42 ppm. The internal hydrogens are represented by a signal at 2.63 or 2.74 ppm, depending on the isomer. The diene was represented by similar looking signals that show at 1.77, 1.82, and 6.01ppm. The same is true for the ^{13}C NMR spectra. The signals of the diene appear at 18.36 and 26.61 ppm for the carbons of the methyl groups, 121.7 ppm for the internal carbons of the double bonds, and 132.4 ppm for the external carbons of the double

bonds. The signals of the diepoxide are at 19.57 and 24.75 ppm for the sp^3 carbons, and 58.86 and 60.63 ppm for the sp^2 carbons. In the diepoxide spectrum, the internal carbons of the epoxide rings appear more upfield than the external carbons. As with butadiene, the monoepoxide was seen when the reaction was stopped before completion.

For the substituted dienes, there was no evidence to suggest that the diepoxides were being oxidized as was butadiene diepoxide. This implies that the substituted diepoxides are more stable than butadiene diepoxide. The electron donating properties of the alkyl substituents must stabilize the epoxides and make them less susceptible to nucleophilic attack.

2.3 CONCLUSION

The diepoxidation of several conjugated dienes was carried out using Jacobsen's catalyst. 1,3-Butadiene, 5,5-dimethyl-1,3-hexadiene, 2,5-dimethyl-2,4-hexadiene were all successfully epoxidized to the corresponding diepoxides. From evidence obtained from the epoxidation of 1,3-butadiene, it appears that the epoxidation was not selective. This is in contrast to the results achieved by Jorgensen *et al.* Success was still achieved as this is the first report of epoxidation of unfunctionalized conjugated dienes. Also, under the reaction conditions used, 1,3-butadiene diepoxide was further oxidized to the tetraol and broken down to sodium formate. For these reasons, this synthesis would not be a practical method of synthesizing the desired precursor for dithiothreitol.

The monoepoxidation of 1,3-butadiene was studied to determine where the selectivity was lost, in the first or second step. As low e.e.'s were also obtained for the monoepoxidation, it was determined that there was little selectivity in the first step.

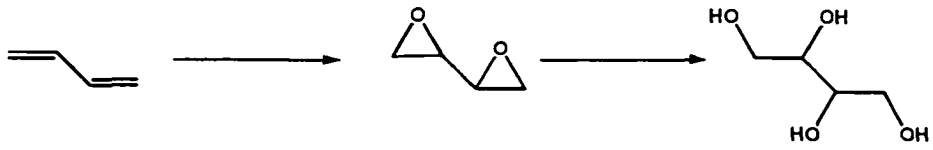
2.4 EXPERIMENTAL

General

NaOCl was obtained as household bleach and diluted to literature specifications. 12% NaOCl was obtained from BioVectra dcl and used without dilution. The 0.55 M NaOCl solution was prepared by diluting 78.5 mL of 5.25% NaOCl to 100 mL with 0.05 M Na_2HPO_4. The pH was adjusted to 11.34 with 10% HCl.

NMR spectra were obtained on either a Bruker (300 MHz) or Varian Gemini-300BB (300 MHz) spectrometer using the specified deuterated solvent with TMS as internal standard, unless otherwise indicated.

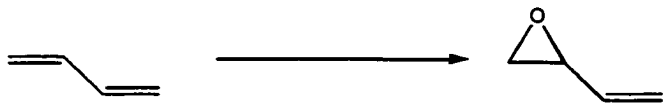
1,3-Butadiene diepoxide



N,N'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III)chloride (0.270 g, 0.000426 mol) was added to CH_2Cl_2 (35 mL) in a Fischer-Porter bottle. The bottle was assembled. As this solution was stirred, butadiene (3.58 g, 0.06618 mol) was added through a septum on the bottle via a needle

attached to a regulator on the pre-weighed butadiene canister. The butadiene was allowed to flow into the Fischer-Porter bottle for 15 minutes. The butadiene canister was weighed to find the mass of butadiene that had been added. NaOCl (150 mL, 0.2417 mol) was added as 0.55 M solution. The two-layers were stirred vigorously for 6 days. The reaction was monitored by NMR spectroscopy. The layers were separated. The aqueous layer was dried by rotary evaporation. The white solid residue was recrystallized from ethanol to give a white solid (3.78 g, 47%). ^{1}H NMR ($CDCl_3$, 300MHz) δ 3.5-3.7 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 62.136, 71.440. Also contained sodium formate ^{1}H NMR ($CDCl_3$, 300MHz) δ 8.290 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.492.

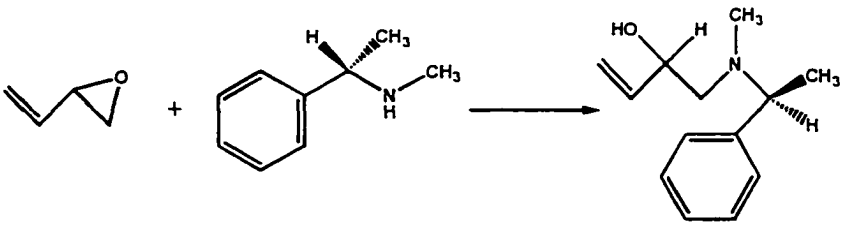
3,4-epoxy-1-butene



N,N'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III)chloride (0.300 g, 0.000472 mol) was added to CH_2Cl_2 (30 mL) in a Fischer-Porter bottle. The bottle was assembled. As this solution was stirred, butadiene was added through a septum on the bottle through a needle attached to a regulator on the pre-weighed butadiene canister. Butadiene (9.93 g, 0.184 mol) was allowed to flow into the Fischer-Porter bottle. The butadiene canister was weighed after addition to find the mass of butadiene that had been added. 12% NaOCl solution (100 mL) was added. The

two-layers were stirred vigorously for 3 days. The two layers were separated and the organic layer was distilled to produce 3,4-epoxy-1-butene (4.01 g, 0.0572 mol, 31 %), a colorless oil (b.p. 62°C - 66°C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.646-2.663 (dd, J_1=2.4 Hz, J_2=2.7Hz 1H), 2.947-2.964 (dd, J=1.5 Hz, 1H), 3.343 (m,1H), 5.3 (m, 1H), and 5.529 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 48.295, 52.132, 119.125, and 135.817.

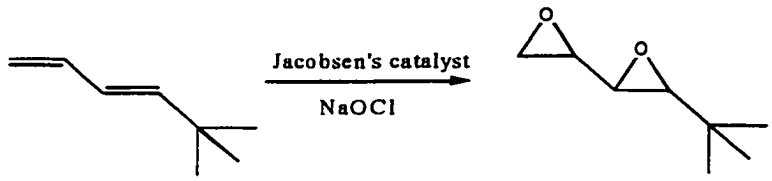
Monoepoxide + Chiral Amine



NaOAc (16 mg, 0.000195 mol) was added to dry methanol (10 mL) in a 25 mL round-bottomed flask. 3,4-epoxy-1-butene (0.3 mL, 0.00372 mol) was added. This mixture was cooled in an ice-water bath. (S)-(-)-N,α-Dimethylbenzylamine (0.25 mL, 0.00172 mol) was added and the reaction mixture was left to stir. The reaction was monitored by TLC with hexane/ethyl acetate (9:1). The solvent was evaporated and the product was extracted with ether and was washed with brine. The organic layer was dried over $MgSO_4$, filtered and the solvent evaporated on a rotary evaporator. The residue obtained (0.165 g, 47% crude yield) was purified by column chromatography over silica gel using 9:1 hexanes-ethyl acetate (0.0088g, 25% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.2 (t, J=7.5 Hz, 3H), 2.1 (s, 3 H),

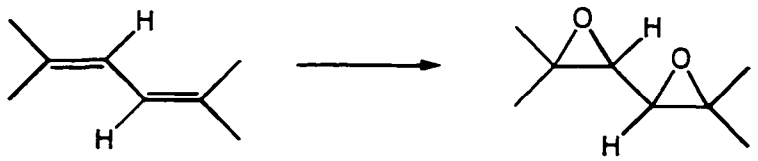
3.2-3.6(m, 1H), 3.6-3.8 (m, 1H), 4.0-4.2(m, 1H), 5.036-5.071 (d, J=10.5 Hz, 1H), 5.193-5.254 (dd, J_1=1.2 Hz, J_2=17 Hz, 1H), 5.6-5.8 (m, 1H), 7.2-7.4 (m, 5H).

5,5-dimethyl-1,3-hexadiene diepoxide



N,N'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III)chloride (0.0501 g) was dissolved in CH_2Cl_2. 5,5-dimethyl-1,3-hexadiene (0.4773g, 0.0043 mol) and 0.55 M NaOCl (35 mL) were added. The two layers were stirred vigorously for 3 days. The layers were separated, and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography over silica gel (19:1 petroleum ether-ethyl acetate). ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.11 (s, 9H), 2.835-2.859 (m, 1H), 3.048-3.062 (t, J=4 Hz, 1H), 3.45 (m, 1H), 3.836-3.864(dd, J_1=4 Hz, J_2=8 Hz, 1H), 4.143-4.156 (d, J= 4 Hz, 1H).

1,3-diepoxy-2,5-dimethylhexane



N,N'-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminomanganese(III)chloride (0.0502g, 0.0000790 mol) was added to CH_2Cl_2 (15 mL). 2,5-dimethyl-2,4-hexadiene (0.3 mL, 0.0021 mol) and 0.55 M NaOCl solution (16 mL, 0.0088 mol) were added. The two layers were left stirring overnight. The layers were separated. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layers were dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography over silica gel (9:1 petroleum ether-ethyl acetate) to give two isomers of 1,3-diepoxy-2,5-dimethylhexane. ^1H NMR ($CDCl_3$, 300MHz) δ 1.269 (s, 6H), 1.356 (s, 6H), 2.645 (s, 1H) or 2.638 (s, 1H); ^{13}C NMR ($CDCl_3$, 60MHz) δ 19.39, 24.58, 58.86, 60.45.

2.5 REFERENCES

1. Eastman Chemical Company, www.eastman.com/online_publications/p264a/p26404.htm October 19, 2001.

2. March, J. *Advanced Organic Chemistry*, **1985**, John Wiley & Sons, Inc., New York, p 735.

3. Demuth, M.R.; Garrett P. E.; White J. D. *J. Am. Chem. Soc.* 1976, **98**(2), 634.

4. Katsuki, T. and Sharpless, K.B.; *J. Am. Chem. Soc.* **1980**, *102*, 5976-5978.

5. E. N. Jacobsen in *Comprehensive Organometallic Chemistry II*, Vol. 12 (Eds.: G. Wilkinson, F. G. A. Stone, E. W. Abel, L. S. Hegedus), Permagon, New York, **1995**, chap. 11.1.

6. Jacobsen E. N.; Zhang, W.; Muci, A. R.; Ecker, J.R and Deng, L. *J. Am. Chem. Soc.* **1991**, *113*, 7063-7064.

7. Fu, H.; Look, G.C.; Zhang, W.; Jacobsen, E.N., Wong, C.J. *Org. Chem.* **1991**, 56, 6497.

8. Neagu, C. and Hase, T. *Tetrahedron Lett.*, **1993**, *34*(10), 1629-1630.

9. Crawford, R. J.; Lutener, S. B. and Cockcroft, R. D. *Can. J. Chem.*, **1976**, *54*, 3364-3376.

10. Thomsen, D.S.; Schiøtt, B. and Jørgensen, K.A. *J. Chem. Soc. Chem. Commun.* **1992**, 1072-1074.

11. Rasmussen, K.G.; Thomsen, D.S. and Jørgensen, K.A. *J. Chem. Soc. Perkin Trans. I* **1995**, 2009-2015.

12. Palucki, M.; Finney, N.S.; Pospisil, P. J.; Güler, M. L.; Ishida, T. and Jacobsen, E. N. *J. Am. Chem. Soc.* **1998**, *120*, 948-954.

13. Linde, C.; Åkermark, B., Norrby, P. and Svensson, M. *J. Am. Chem. Soc.* **1999**, *121*, 5083-5084.

14. Jacobsen, H. and Cavallo, L. *Andew. Chem. Int. Ed.* **2000**, *39*(3), 589-592.

Chapter 3

New Synthetic Applications of

Dithiothreitol & Immobilized DTT

3.1 INTRODUCTION

General

As mentioned, one of the major products of the sponsoring company is dithiothreitol

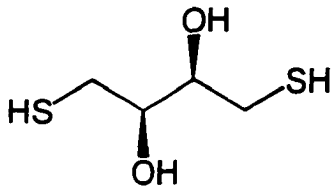
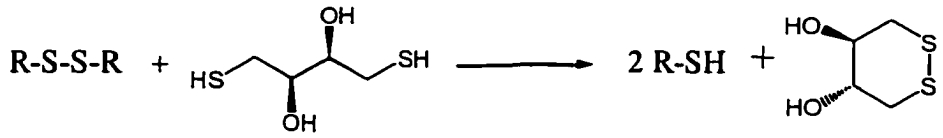


Figure 8 Dithiothreitol

(DTT), also known as *Cleland's reagent*[1]. It is used primarily as a biochemical reagent for protein denaturation because of its ability to reduce disulfide bonds and stabilize monothiols



Scheme 26 Reduction of Disulfide Bond

in the reduced state. It is also used in protein extraction.[2] Because DTT is a small, water-soluble molecule, it can pass through cell membranes and extract proteins from cells. It can also be used as a protecting group for thiols in organic synthesis. Despite its reducing abilities, its potential as a reducing agent for functional groups other than disulfides has not been extensively studied. This chapter will discuss the application of DTT to the reduction of various organic functional groups, namely nitro groups and azides.

If DTT were found to be an efficient reagent for such reductions, it would be

beneficial for at least two reasons. First, it would show that DTT does have other applications besides those listed above, leading to increased usage. Its newfound role in organic synthesis would be beneficial to industry because more product would be sold. Secondly, it would be beneficial to researchers because it would be a milder and safer alternative to the methods currently used for many reactions.

The use of immobilized DTT will also be discussed. This is DTT which is bonded to a polymer support. It is insoluble in the reaction medium; thus, when the reaction reaches completion, it can be filtered off. This is advantageous as it eliminates a step in the isolation and purification of the final products. Three different immobilized reagents will be examined.

$$R\text{-}NO_2 \xrightarrow{LiAlH_4} R\text{-}NH_2$$

Scheme 27 Nitro Reduction

Reduction of Nitro Groups

The reports of nitro reductions are as numerous as they are varied in the literature. A select few, though, stand out as being the most commonly used. The hydridic reagent $LiAlH_4$ is very efficient for this reduction, giving quantitative yields. However, amines are produced only from aliphatic nitro groups as aromatic nitro groups produce azo compounds.[3] Catalytic hydrogenation is also frequently used, but neither it, nor $LiAlH_4$ are selective as both will reduce other functional groups. For this reason they are not suitable for all substrates and alternate methods are required.

Another common way of reducing nitro groups is by combining metals and acid. The most common combination being sodium or lithium with sulfuric acid. The conditions needed for this reaction to be successful are also extreme in that concentrated acid is needed. These two methods mentioned could be replaced by one that gives similar results using milder conditions.

$$R\text{-}NO_2 \xrightarrow[H_2SO_4]{Na} R\text{-}NH_2$$

Scheme 30 Nitro Reduction

The reduction of nitro groups with DTT has been reported[4] in combination with heme-containing proteins and heme-related compounds. Balabanli *et al.* reported that using DTT with hemoglobin, myoglobin, cytochrome C, and hemin, all quantitatively reduced nitrotyrosine to aminotyrosine. Protoporphyrin IX itself did not, but with the addition of Fe^{2+}, the reaction was successful. However the authors reported that ferrous and ferric ions alone were not adequate catalysts for nitrotyrosine reduction in combination with DTT as no reduction was detected. It will be shown here that in our hands, the combination of DTT with ferric ions does indeed reduce nitro functionalities quantitatively.

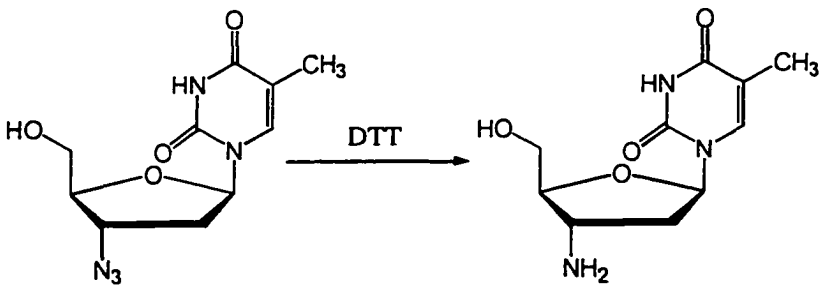
Reduction of Azides

A common method for reducing azides to amines is treatment with $LiAlH_4$. Another is catalytic hydrogenation. Both of these are powerful reducing agents, and as such do not

give good selectivity. DTT is a mild reducing agent and should be able to selectively reduce azides to their corresponding amines.

Scheme 29 Common Methods of Reducing Azides to Amines

It has previously been shown that DTT is a useful reagent for the reduction of azides. Handlon and Oppenheimer used DTT to reduce 3'-azidothymidine (AZT) to 3'-aminothymidine.[5] Quantitative yield was obtained by stirring AZT and DTT in buffer at pH 7.2 for 1 hr. Staros *et al.* used DTT to reduce several aryl azides.[6] They found the reduction to be pH dependent. All reactions were successful when carried out in aqueous buffer



Scheme 30 Reduction of AZT

solutions at room temperature. Cartwright *et al.* reduced 8-azidoadenosine nucleotides to their corresponding amines with DTT.[7] This group also had successful reductions in aqueous solutions at room temperature but reported reductions using methanol and buffer/methanol

mixtures as solvent. Meinjohanns *et al.* also reported azide reduction by DTT in organic solvent.[8] They reduced azides on solid-supported glycopeptides using CH_2Cl_2 as solvent. They also used a base catalyst, diisopropyleneamine, during the reduction. DTT reduction was found to be advantageous because the mild conditions were not harmful to the solid support. As shown by these references, azide reduction can be carried out in both aqueous and organic solvents. The reduction of azides in various aqueous and organic solvents will be further studied and reported.

The reduction of azides by DTT reported here are only novel in that the substrates and some of the solvents are new, not the process. These reactions were basically done to confirm that DTT does indeed reduce them, and that they would be useful for studies with immobilized DTT.

Reductions

There are a number of functional groups that could possibly have been chosen for reduction with DTT. Carbonyls and nitroso groups as well as nitro groups and azides all are potential substrates for reduction. It has been shown that DTT reduces azides but they were chosen for use in this work to verify that they were reactive to DTT so they could be used with the immobilized reagents. Nitro groups were chosen because if it could be shown that they can be reduced by DTT in the absence of heme, then such reaction conditions would have great advantages over current methods.

d Reagents

The use of immobilized, or solid-supported reagents has long been known to scientists, but the Merrifield synthesis[9], reported in 1963 for the synthesis of peptides probably sparked the most interest in this area. Still, their use has not been greatly exploited until more recently. A variety of polymer-supported reagents are now available for use in several disciplines. Those useful in organic chemistry include catalysts, reducing and oxidizing agents, scavenger resins and protecting groups.

Supports are usually a polymer that is either linear or cross-linked. The linear supports are meant to be soluble in the solvent used. Cross-linked supports, or resins, are designed to be insoluble and offer the advantage of easy removal. The immobilized reagents used in the work reported here were all insoluble resins used for easy purification of products. BioVectra™ dcl has recently started manufacturing three different immobilized DTT species. The differences between the three being the polymer support used and the way that DTT is attached. The usefulness of these reagents with substrates will be discussed.

Solid-supported reagents have many advantages over traditional reagents. Firstly, most are easily removed from the reaction mixture. Insoluble reagents, usually bound to a polymer, can be filtered off and recycled for reuse. This is beneficial because it eliminates the need for chromatography to separate product from reagent. Though column chromatography is commonplace in the organic chemistry laboratory, it is time consuming and can be costly. Alternate methods are a benefit. Secondly, unlike traditional solution synthesis, excess

reagent can easily be used to drive reactions to completion. Unused material can be recovered by filtration, whereas in solution synthesis recovery of excess reagent is difficult. Another advantage is that harmful or dangerous compounds in a solid-supported form can be handled and used more easily and safely.

There are also disadvantages to the use of immobilized reagents. There are the added steps of preparation of the support but this is outweighed if the reagent is recyclable, as this step only has to be done once and the reagent could be used many times. Also, since the polymer is cross-linked, reaction times will be longer with immobilized reagents because the cross-linking prevents solvation and access to the reagent. Therefore it will take longer for all of the substrate to 'see' the reagent. Another limitation in using immobilized reagents is that not all supports can withstand some reaction conditions. High temperatures and harsh reagents may break apart some functionalized polymers, and thus, pollute the reaction mixture and kill the reagent.

3.2 RESULTS & DISCUSSION

Azide Reduction

Though the reduction of azides with DTT had been reported in the literature, the reduction of several other azides was attempted. As well, new aspects of azide reduction with DTT were also studied. Several experiments were carried out to examine solvent effects in the reduction. The azide reductions that had previously been reported were in aqueous buffer solutions, methanol or a mixture of these, and dichloromethane. Other solvents were used to determine if the reaction is viable in non-buffered aqueous solution or in other organic solvents. If so, then the reduction of azides would be much more applicable as many azides, being organic compounds, are not soluble in aqueous solution.

Other solvents used were H_2O at pH 7.5, H_2O at pH 5, THF, CH_2Cl_2, MeCN and MeOH (Table 3). 4-carboxybenzenesulfonazide was used as the substrate for these reactions. The use of water at pH 7.5 is favorable over the use of buffered solutions because it eliminates the presence of salts that could later hinder purification.

Table 3 Solvent Effects for the Reduction of 4-Carboxybenzenesulfonazide

Solvent	Conversion
buffer, pH 7.5	100%
H_20, pH 7.5	100%
H_20, pH 5	20%
CH_2Cl_2	100%
THF	78%
MeOH	46%

The results for the reduction in water at pH 5 and pH 7.5 follow those of Cartwright *et al.* and Staros *et al.* in that the azide reduction is pH dependent. Conversion at pH 5 was not nearly as efficient as at pH 7.5. Cartwright *et al.* used a base-catalysed reaction for the azide reduction. Staros *et al.* proposed that the pH dependence is due to fully protonated thiols being less reactive.

The conditions needed for azide reduction with DTT are very mild. Azide reductions required only one equivalent of DTT at room temperature and were usually left to stir overnight. These conditions are much milder than those needed for the traditional methods of reduction.

DTT reduction of other azides was also accomplished with complete conversion. 4-Azido-1-fluoro-2-nitrobenzene was successfully reduced to the corresponding 4-fluoro-3-nitroaniline. The ^1H NMR spectrum of the product corresponds to that of 4-fluoro-3-nitroaniline in the Aldrich library.

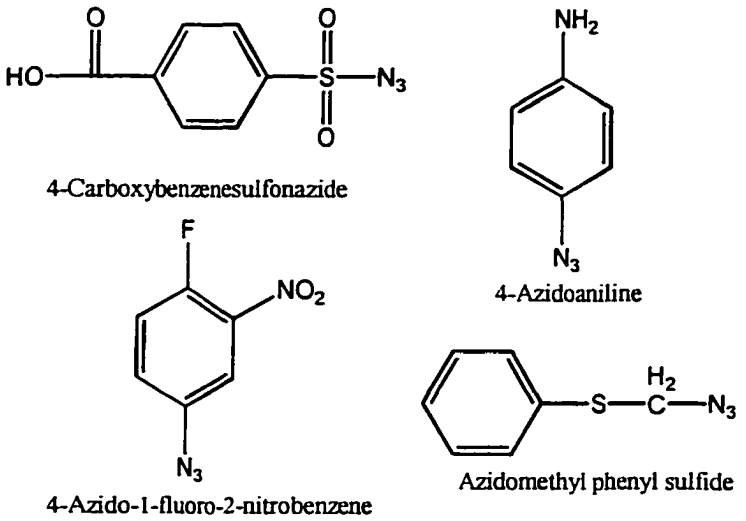
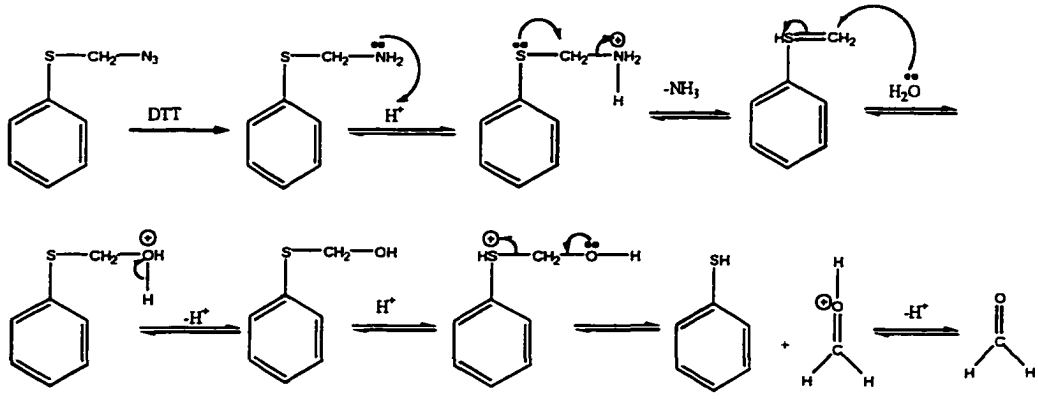


Figure 9 Azides reduced by DTT

Azidomethyl phenyl sulfide was also successfully reduced, however the results were not as expected. It was concluded that the corresponding amine decomposed to thiophenol through a reaction similar to acetal hydrolysis. In fact, the ^{1}H and ^{13}C NMR spectra of the product did match those of thiophenol. Gas chromatography was used to confirm that the product was indeed thiophenol. A spike test was used for confirmation. The suspected reaction scheme is shown in Scheme 31.



Scheme 31 Suspected method of formation of thiophenol.

Azidoaniline hydrochloride was also reacted with DTT. The results were as expected, 1,4-phenylenediamine was formed. A quantitative yield was calculated using the integration of the peaks in the ^{1}H NMR spectrum. It was not possible to calculate an accurate yield due to contamination of the 1,4-phenylenediamine with 1,2-dithiane-4,5-diol.

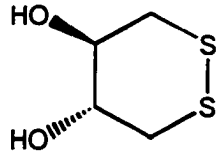
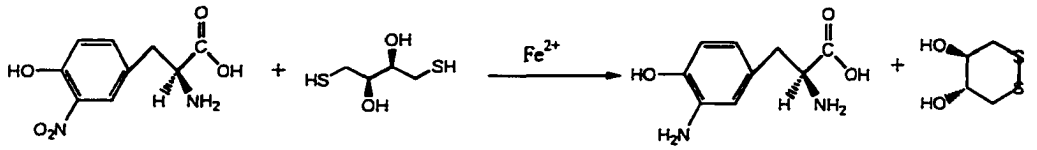


Figure 10 1,2-Dithiane-4,5-diol

Nitro Reduction

In their paper[2], Balabanli *et al.* reported a 0% conversion of 3-nitro-L-tyrosine to the corresponding aniline with both Fe^{2+} and Fe^{3+} with DTT in buffered solutions at pH 7. In our hands, these same reaction conditions gave 100% conversion. Balabanli *et* al. did, however,

get 100% conversion when hemoglobin and other heme-related compounds were used. Presumably, this was due to a complexation between the heme and the iron which solubilized the iron. Fe^{2+} and Fe^{3+} alone would be much more advantageous than these due to both availability and cost.



Scheme 32 Reduction of 3-nitro-L -tyrosine with DTT and Fe^{2+}

The nitro compounds used here were all aromatic derivatives of nitrobenzene, and nitrobenzene itself. The procedure used for their reduction was that of Balabanli *et al.* The substrate, DTT, $FeCl_3$ and solvent were combined and refluxed. A variation from the Balabanli procedure was that a small amount of charcoal was used in most reactions.

Nitrobenzene, being the simplest aromatic nitro compound, was used to study the conditions needed for nitro reduction with DTT. The reaction was performed numerous times under varying conditions, to determine the requirements necessary for the reaction to be successful. Table 4 shows the results of these reactions.

Table 4 Variation of Conditions for Nitro Reduction

	mol eq DTT	Temp (°C)	$FeCl_3$	Charcoal	Solvent	Time	Conversion
1	10	100	yes	yes	buffer, pH 7.5	10 min	100%
2	1	100	yes	yes	buffer, pH 7.5	10 min	34%
3	3	100	yes	yes	buffer, pH 7.5	1.5 hr	100%
4	3	100	yes	yes	buffer, pH 7.5	10 min	83%
5	3	r.t.	yes	yes	buffer, pH 7.5	4 days	0%
6	3	100	no	yes	buffer, pH 7.5	10 min	0%
7	3	100	yes	no	buffer, pH 7.5	10 min	76%
8	3	100	yes	no	buffer, pH 7.5	140 min	100%
9	3	100	yes	yes	H_2O pH 5	2 hr	0%
10	3	100	yes	yes	H_2O pH 7.5	5 hr	0%
11	3	67	yes	yes	THF	5hr	0%

It was found that the reduction of one nitro group required only 3 mole equivalents of DTT, not the 10 used by Balabanli *et al.* for the reduction of 3-nitro-L-tyrosine. One equivalent produced only a 34% conversion, one-third of the substrate was converted. Thus, it was assumed that 3 mole equivalents would give complete conversion as it did. Heat was also required as no reaction occurred when the reaction mixture was left stirring for 4 days at room temperature. The $FeCl_3$ was also required, though only a catalytic amount was used. It is suspected that the reaction mechanism may involve the reduction of the Fe^{3+} to Fe^{2+} by the DTT, and it is the Fe^{2+} that actually reduces the nitro group.

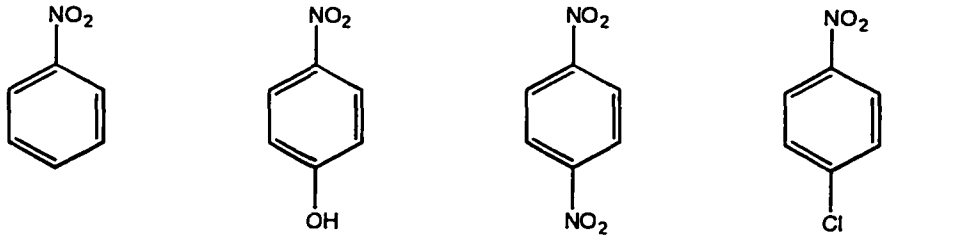
The charcoal was not necessary for a successful reaction, but a catalytic amount was found to speed up the reaction rate. It is suspected that the charcoal acts like a phase transfer agent. The nitro compounds used are not soluble in the aqueous reaction media. The molecules stick to the surface of the charcoal and react easier with the reducing agent than if they are in a micelle-like drop.

The reduction only occurred in the buffered aqueous solution. It did not occur in deionized water, or in water whose pH had been adjusted to 7.5 with NH_4OH. The reason for this is not known, but it was observable before reflux whether or not the reaction would be successful in the chosen solvent. Upon the addition of the $FeCl_3$ to the DTT and buffer, the solution turned to peach. This was quickly followed by darkening to green and then to black. Presumably, this was due to the change in the oxidation state of the Fe. In the non-buffered aqueous and the organic solutions, this color change did not occur and these reactions were unsuccessful, implying that the buffer was needed for the reduction of Fe^{3+} by DTT.

The conditions needed for complete nitrobenzene reduction with dithiothreitol include refluxing in buffer with 3 mole equivalents of DTT and catalytic amounts of $FeCl_3$ and charcoal for 1.5 hours. ^1H and ^{13}C NMR spectra of the product obtained from this process model those of aniline in the Aldrich Library.

The same process was used for the reduction of several other aromatic nitro compounds.The only variation from the nitrobenzene reduction was the reaction time. 1,4-

Dinitrobenzene, 4-nitrophenol, 4-chloronitrobenzene, and 3-nitro-L-tyrosine were all successfully reduced to their corresponding amines.



nitrobenzene 4-nitrophenol 1,4-dinitrobenzene 4-chloronitrobenzene

Figure 11 Nitro compounds reduced with DTT and Fe^{2+}

1,4-dinitrobenzene was successfully reduced to 1,4-phenylenediamine using the same conditions as above, except 6 molar equivalents of DTT were needed because of the two nitro groups. The reaction was left stirring for 6 hours to ensure complete reaction. The solid obtained was a mixture of the desired product, 1,4-phenylenediamine, and oxidized DTT, 1,2-dithiane-4,5-diol. Column chromatography using hexane/ethyl acetate/methanol (3:1:1) did not successfully separate the product from the oxidized DTT. Since the product could not be isolated, an accurate yield could not be obtained.

4-nitrophenol was successfully reduced to 4-aminophenol. An 83% conversion was obtained. Column chromatography was successful in separating the starting material from the product; however, it was not successful in separating the 1,2-dithiane-4,5-diol from the product. The oxidized DTT came off of the column in the same fractions as the 4-aminophenol. Because of this contamination, an accurate percent yield could not be

calculated. The yield reported, 43%, was calculated from the integration of the peaks in the ^{1}H NMR spectrum and does not take into account the recovered starting material.

4-chloronitrobenzene was reduced to 4-chloroaniline with 95% conversion.

Purification

In most cases, purification of the amine product was difficult because of the presence of 1,2-dithiane-4,5-diol (Figure 5), the result of oxidation of DTT. 1,2-dithiane-4,5-diol is soluble in both organic and aqueous solvents so extraction was not a sufficient method of separation. Its polarity, as seen by TLC, was also close to some of the amines produced so column chromatography was difficult, and in some cases unsuccessful. Ion exchange chromatography was also unsuccessful as 1,2-dithiane-4,5-diol was obtained with the amines and separation was not achieved. This proved to be a major problem. However, it was hoped that the use of immobilized DTT could resolve this problem.

Polymer-Supported DTT

Some of the newest products being produced by BioVectra™ dcl are immobilized reducing agents. VectraSynth™, P-SH, and P-DTT are all variations of DTT attached to polymer supports. The structures of these reagents are proprietary and so cannot be shown here. All are insoluble resins. For this reason, they can be filtered off after reactions are completed. With the oxidized DTT, still attached to the support, purification of products is

made easier by filtering off the immobilized reagent. It can then be regenerated and reused.

These immobilized reagents differ in their physical properties. They were all comprised of DTT and a polymer support, but differ in the type of polymer and the way in which the DTT was attached to the support. VectraSynth™ is an immobilized reagent designed for use in both aqueous and organic solvents and for use at low temperatures (below 50°C). P-SH and P-DTT have the same polymer support but differ in the attachment of the DTT to the support. They are designed for use in organic solvent and can be used at higher temperatures (<200°C).

All three supports should be useful for the reduction of azides which only requires room temperature and can be done in either aqueous or organic solvents. None, however, would be useful for the reduction of nitro groups as they require both aqueous buffer as solvent and elevated temperatures. VectraSynth™ cannot be heated and P-SH and P-DTT require organic solvents.

VectraSynth

Several reactions were performed with the immobilized reagents. 4-Carboxybenzenesulfonazide, 4-azidoaniline hydrochloride and 4-azido-1-fluoro-2-nitrobenzene were all reduced with VectraSynth™. Total conversion of 4-carboxybenzenesulfonazide to 4-carboxybenzenesulfonamide was obtained in the 0.5 M

phosphate buffer. The peaks for the aromatic hydrogens of the amide are further upfield than those of the azide. Also, a new broad peak at 7.5 was determined to represent the hydrogen atoms on the amide.

4-Azidoaniline hydrochloride was also reduced with one equivalent of VectraSynth™ in 0.5 M phosphate buffer. Both the ^{1}H and ^{13}C NMR spectra of the product correspond to those of 1,4-phenylenediamine in the Aldrich Library. 4-azido-1-fluoro-2-nitrobenzene was reduced to the corresponding amine, 4-fluoro-3-nitroaniline.

An attempt was made to reduce nitrobenzene with VectraSynth™. The conditions needed for nitro reduction were used, including heating to 100°C. The mixture was left at reflux for 2 hours, more time than needed with DTT. As VectraSynth™ is a resin and is not soluble, it is presumed that reaction times will be longer.

Table 5 Summary of Reductions with VectraSynth™

azide	solvent	conversion [a]
4-carboxybenzenesulfonazide	0.5 M phosphate buffer	100
4-azidoaniline hydrochloride	0.5 M phosphate buffer	100
4-azido-1-fluoro-2-nitrobenzene	H_2O/THF	100
nitrobenzene	0.5 M phosphate buffer	0

a) percent conversion calculated from integration of amide and azide peaks in the ^{1}H NMR spectra

P-DTT

Reduction of each azide was also carried out using P-DTT. The reaction with 4-carboxybenzenesulfonazide was attempted in both MeOH and in THF. In methanol, only an

18 % conversion was achieved, but the same reaction in THF gave 90% conversion. Reduction of 4-azidoaniline hydrochloride in THF was completed with 58% conversion. This was determined from the integration values from the ^{1}H NMR spectrum. The spectrum shows peaks corresponding to the starting material, 4-azidoaniline hydrochloride and to both 1,4-phenylenediamine and 1,4-benzoquinone. The conversion was obtained using the integration of both products. Reduction of 4-azido-1-fluoro-2-nitrobenzene with P-DTT was unsuccessful as no evidence of any conversion to 4-fluoro-3-nitroaniline was seen. Nor was there any conversion of azidomethyl phenyl sulfide in THF.

Table 6 Summary of Reductions with P-DTT

azide	solvent	conversion [a]
4-carboxybenzenesulfonazide	THF	90
4-carboxybenzenesulfonazide	MeOH	18
4-azidoaniline hydrochloride	THF	58
4-azido-1-fluoro-2-nitrobenzene	THF	0
azidomethyl phenyl sulfide	THF	0

a) percent conversion calculated from integration of amide and azide peaks in the ^{1}H NMR spectra

Reduction of nitrobenzene with P-DTT was also attempted in phosphate buffer, though the P-DTT is designed for use in organic solvent. No evidence of aniline was seen so results for this are inconclusive.

P-SH

Several reductions were also attempted with P-SH, as it is designed for use in organic solvents, THF was tried first. 4-Carboxybenzenesulfonazide was reduced, with 39 % conversion to 4-carboxybenzenesulfonamide in THF. As this was not a great result, reduction in aqueous solvent was attempted. In water in which the pH had been adjusted to 7.5 with NH_4OH, 23% conversion of the azide to the amide was obtained. In a 1:1 mixture of MeOH and water, only 8% conversion was obtained. Better yields were obtained in organic solvent.

4-Azidoaniline hydrochloride was also mixed with P-SH in THF. No reduction was detected when 4-azido-1-fluoro-2-nitrobenzene was reacted with P-SH or with azidomethyl phenyl sulfide.

Table 7 Summary of Reductions with P-SH

azide	solvent	conversion[a]
4-carboxybenzenesulfonazide	THF	39
4-carboxybenzenesulfonazide	H_2O, pH 7.5	23
4-carboxybenzenesulfonazide	MeOH/H_2O (1:1)	8
4-azidoaniline hydrochloride	THF	0
4-azido-1-fluoro-2-nitrobenzene	THF	0
azidomethyl phenyl sulfide	THF	0

a) percent conversion calculated from integration of amide and azide peaks in the ^1H NMR spectra.

In almost all cases of reduction with immobilized DTT, the percent yields obtained were well over 100%. As the only compounds added to the reaction mixtures were the azide,

the immobilized DTT and the solvent. The solvent was always removed on a rotary evaporator, therefore, it is presumed that the high yields are due to some of the supported reagent being left behind. However, filtration was always efficient as there was never any insoluble particles seen passing through the filter. Presumably, there must be something leeching of the support and contaminating the products.

Reaction with Regenerated Reagents

Regeneration reactions were tried with all three immobilized reagents. Again, VectraSynth™ was the most successful. After reaction with an azide, the reagent was regenerated by stirring with reducing agent. VectraSynth™ was regenerated by stirring with sodium borohydride while P-DTT and P-SH were regenerated with sodium cyanoborohydride.

In those cases where the azide and amide are colored, such as with 4-azido-1-fluoro-2-nitrobenzene reduction, the immobilized reagents, after use and regeneration, were also colored. This is presumably from some substrate that was attached to the resin. Though regeneration did take away some of the color, in many cases the color was still present when the immobilized reagent was reused. Whether this affected the second reductions is not known.

VectraSynth™ gave 100% conversion for all initial reduction reactions. For 4-carboxybenzenesulfonazide, this declined to 59% upon regeneration and reduction. However,

a third reduction with regenerated reagent gave 85% conversion. The difference between the second and third reductions could be due to reaction time. The third reduction was left stirring significantly longer than the second. The yields obtained were opposite to the conversions obtained. Whereas the second reduction gave the lowest conversion, it also gave the highest yield.

4-azido-1-fluoro-2-nitrobenzene was also completely reduced to its corresponding azide with VectraSynth. Regeneration and a second reduction also achieved complete reduction but only 10% yield.

4-Azidoaniline was reduced with 100% conversion in a first and in a second reduction using regenerated reagent. The yield of the first reaction was only 19%. The second was the only VectraSynth reduction to produce more than 100% yield.

All reductions with P-DTT and most with P-SH gave higher than theoretical yields. The ^{1}H NMR spectra also showed unidentified peaks. It is suspected that these could be due to parts of the reagent leeching into the reaction mixture. These reaction mixtures were left stirring overnight. Perhaps this was excess agitation for the solid-supported reagents. Excess stirring could be a cause of leeching.[10]

4-Azidoaniline hydrochloride was 63% reacted with P-DTT in the initial reduction but regeneration of this reagent and a second reduction gave complete reaction. 4-Azido-1-fluoro-2-nitrobenzene did not react at all with either P-DTT or P-SH. 4-Carboxybenzenesulfonazide was reduced with only 30% yields, much unlike the results

obtained with VectraSynth.

P-DTT and P-SH are not nearly as useful as VectraSynth as lower yields and higher than theoretical yields are obtained. Of course the impurities present that cause the high yields could be separated from the product, but when the object of using the immobilized reagents is to eliminate the purification step, this would not be logical.

3.3 CONCLUSION

Azides and nitro groups were successfully reduced to their corresponding amines using dithiothreitol as the reducing agent. The conditions necessary for each reduction were determined. It was found that nitro reduction must be done in a buffered solution with three equivalents of DTT whereas azide reduction is successful at room temperature with one equivalent of DTT in both aqueous and organic solvents. The only problem with the reductions was the purification as 1,2-dithiane-4,5-diol, or oxidized DTT, was difficult to separate from the amines. Column chromatography was difficult as the polarities of the amines were similar to that of the dithiane.

Immobilized DTT solved this problem in the cases of the azides. Because it is removed by filtration after reaction, there is no contamination from 1,2-dithiane-4,5-diol. However, it was not useful in the cases of the nitro groups because the requirements for nitro reduction and the conditions available for immobilized DTT use did not coincide.

Of the three different supported reagents used, VectraSynth™ was the most successful. It was effective in both aqueous and organic solvents and gave the best yields. Also, regenerated VectraSynth™ was the most effective on second use.

Many aspects of this area still need to be researched. There are several functional groups that can still be examined for reduction by DTT and Fe^{2+}. If a polymer-supported reagent can be found that can be both heated and used in organic solvents, then it could be very useful for the reduction of nitro groups.

3.4 EXPERIMENTAL

General

DTT, VectraSynth, P-DTT, P-SH and Lewatit CNP80 H^+ resin were supplied by BioVectra™ dcl. All reagents used are available commercially and were not purified before use. Commercial silica TLC plates purchased from Aldrich Chemical Co. were used. Ultra pure silica gel (230-400 mesh) was used for column chromatography and was purchased from Silicycle.

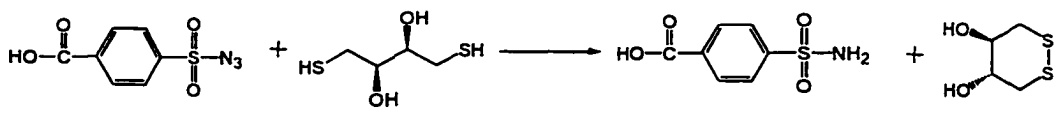
NMR spectra were obtained on either a Bruker (300 MHz) Avance 300 or Varian Gemini-300BB (300 MHz) spectrometer using the specified deuterated solvent with TMS as internal standard, unless otherwise indicated. GC analysis was done using a Phenomenex ZB-1 column (100% methylpolysiloxane) with a flame ionization detector using dichloromethane as the solvent.

0.1 M Phosphate Buffer

KH_2PO_4 (5.44 g, mol) was dissolved in 300 mL deionized H_2O. PH was adjusted to 7.5 by adding 50% KOH (w/w) dropwise. Volume was adjusted to 400 mL with deionized H_2O.

Azide Reduction

4-Carboxybenzene sulfonamide



a) Dithiothreitol (1.166 g, 0.0108 mol, 6 mol eq) was dissolved in 0.1 M phosphate buffer (25 mL). 4-Carboxybenzene sulfonazide (0.400 g, 0.00176 mol) was added with stirring. The mixture was left stirring overnight. Dilute HCl was added until the pH was 3 to pH paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was washed with 50 mL of deionized water, followed by 50 mL of 1 M NH_4OH. The ammonia wash was lyophilized to give a white solid. Column chromatography over silica using 8:1:1 hexane-ethyl acetate-methanol gave pure 4-Carboxybenzene sulfonamide as a white solid (0.239 g, 68%) ^1H NMR (DMSO-d6, 300 MHz) δ 7.3 (s, 2H), 7.7 (d, *J*=8 Hz, 2H), 8.0 (d, *J*=8 Hz, 2H) ^{13}C NMR (DMSO-d6, 75 MHz) δ 125.18, 129.70, 139.71, 145.60, 169.05.

b) Dithiothreitol (0.279 g, 0.00181 mol, 1 mol eq) was dissolved in 0.1 M phosphate buffer (25 mL). 4-Carboxybenzene sulfonazide (0.400 g, 0.00176 mol) was added with stirring. The flask was wrapped in foil and the mixture was left stirring overnight. Dilute HCl was added until the pH was 4 to pH paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was washed with 50 mL of deionized water, followed by 140 mL of 1 M NH_4OH. The ammonia wash was lyophilized to give a white solid (0.532 g, >100 %). ^1H and ^{13}C NMR data as in a.

c) Dithiothreitol (0.037 g, 0.00024 mol, 1 mol eq) was dissolved in 0.1 M phosphate buffer (25 mL). 4-Carboxybenzene sulfonazide (0.0517 g, 0.000227 mol) was added with

stirring. The mixture was left stirring overnight. Dilute HCl was added until the pH was 3 to pH paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was washed with deionized water, followed by 1 M NH_4OH to elute the amine. The ammonia wash was lyophilized to give a white solid. 4-Carboxybenzene sulfonamide was obtained as a white solid (0.0296 g, 67%) ^1H NMR data as in a.*

d) Dithiothreitol (0.0355 g, 0.00023 mol, 1 mol eq) was dissolved in deionized water (5 mL, pH 5). 4-Carboxybenzene sulfonazide (0.050 g, 0.00022 mol) was added with stirring. The pH of the resulting solution was 3. The flask was wrapped in foil and the mixture was left stirring overnight. Solid had not dissolved so enough THF was added to dissolve it. The solution was left stirring overnight. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was rinsed with deionozed water followed by 1 M NH_4OH to elute the amine. The ammonia wash was lyophilized to give a white solid (0.024 g, 54%) ^1H NMR data as in a.

e) Dithiothreitol (0.0812 g, 0.000526 mol, 1 mol eq) was dissolved in water (5 mL, pH 7.5). 4-Carboxybenzene sulfonazide (0.102 g, 0.000449 mol) was added with stirring. The flask was wrapped in foil and left stirring overnight. The water was removed on a rotary evaporator to give a beige solid (0.189 g, >100 % yield). ^1H and ^{13}C NMR data as in a.

f) Dithiothreitol (0.056 g, 0.000363 mol, 1 mol eq) was dissolved in methanol (20

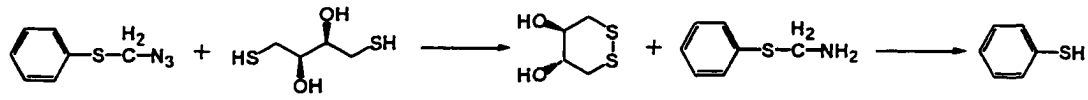
mL). 4- Carboxybenzene sulfonazide (0.0507 g, 0.000223 mol) was added with stirring. The mixture was left stirring overnight. The solvent was evaporated on a rotary evaporator. The residue was a mixture of 4-Carboxybenzenesulfonazide, 4-Carboxybenzenesulfonamide and 1,2-dithiane-4,5-diol (0.1089 g, >100% yield, 45% conversion). ^{1}H and ^{13}C NMR data as in a.*†

g) Dithiothreitol (0.022 g, 0.000143 mol, 1.4 mol eq) was dissolved in dichloromethane (10 mL). 4-Carboxybenzene sulfonazide (0.022 g, 0.0000968 mol) was added with stirring. The mixture was left stirring overnight. The solvent was evaporated on a rotary evaporator. The residue, a white solid was a mixture of 4-Carboxybenzenesulfonamide and 1,2-dithiane-4,5-diol (0.0436 g, >100 % yield, 100% conversion). ^{1}H and ^{13}C NMR data as in a.*

* also contains 1,2-dithiane-4,5-diol ^{1}H NMR (DMSO-d6, 300 MHz) δ 2.7-2.8 (2H), 3.0-3.1 (2H), 3.3-3.4 (2H), 5.2 (d, 2H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 72.56.

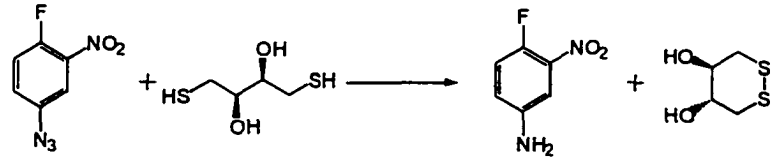
† contains 4-carboxybenzenesulfonazide ^{1}H NMR (DMSO-d6, 300 MHz) δ 8.1 (d, 2H), 8.2 (d, 2H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 127.721, 130.928, 136.699, 140.861, 165.783.

Thiophenol



Dithiothreitol (1.40 g, 0.00908 mol) was dissolved in 0.1 M phosphate buffer (25 mL). Azidomethyl phenyl sulfide (0.30 mL, 0.00212 mol) was added via syringe. The flask was wrapped in foil and the mixture was left stirring overnight. Azidomethyl phenyl sulfide had not dissolved in the aqueous solution so THF (15 mL) was added and the solution was again left to stir overnight. Dilute HCl was added until the pH was 3 to pH paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was washed with 50 mL of deionized water, followed by 60 mL of 1 M NH_4OH. The ammonia wash was lyophilized to give a white solid (0.564 g, 159% yield, 100% conversion). ^1H NMR (DMSO-d6, 300 MHz) δ 7.0-7.2 (m, 5H).

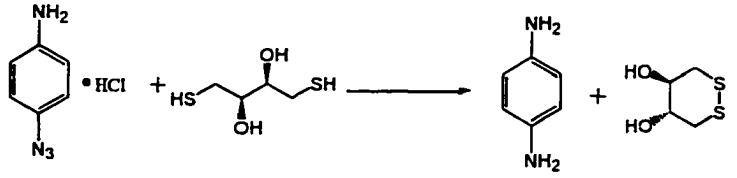
4-Fluoro–3-nitroaniline



4-azido-4-fluoro-2-nitrobenzene (0.050g, 0.000274 mol) and dithiothreitol (0.048 g, 0.000311 mol) were dissolved in 0.1 M phosphate buffer (5 mL) and left stirring overnight. An orange precipitate dissolved upon the addition of THF (4 mL). The pH was 8 to litmus paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+

resin. The column was washed with 50 mL of deionized water, followed by 60 mL of 1 M NH_4OH. The ammonia wash was lyophilized to give a yellow solid (0.0073 g, 17% yield, 100% conversion). ^1H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 6.9-7.1 (m, 2H), 7.3 (s, 1H); ^{13}C NMR (DMSO-d6 + $CDCl_3$, 75 MHz) δ 108.223, 118.235, 118.522, 120.478, 120.751, 145.881.

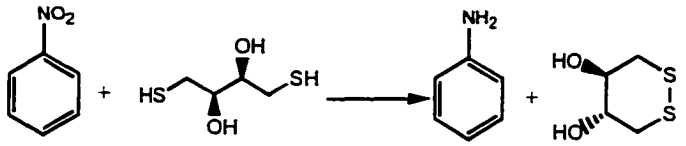
1,4-Phenylenediamine



Dithiothreitol (0.237 g, 0.00154 mol) was dissolved in 0.1 M phosphate buffer (25 mL). 4-Azidoaniline hydrochloride (0.050 g, 0.000293 mol) was added. The flask was wrapped in foil and the mixture was left stirring overnight. The pH was 8. Dilute HCl was added until the pH was 2 to pH paper. The reaction mixture was added to an ion exchange column of Lewatit CNP80 H^+ resin. The column was washed with 50 mL of deionized water, followed by 60 mL of 1 M NH_4OH. The ammonia wash was lyophilized to give a white solid (0.0409 g, 101% yield, 100% conversion). ^1H NMR (D_2O, 300 MHz) δ 6.6 (s, 4H).*

Nitro Reduction

Aniline



a) Dithiothreitol (1.500 g, 0.00972 mol, 10 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.0066 g, 0.0000244 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.00971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 10 min. The solution was allowed to cool to room temperature and the pH was measured to be 6. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated (1.235g, >100% yield, 100% conversion). ^1H NMR ($CDCl_3$, 300 MHz) δ 6.6-6.7 (dd, *J*=14 Hz, 2H), 6.7-6.8 (dd, *J*=13 Hz, 1H), 7.1 (d, *J*=14 Hz, 2H).*

b) Dithiothreitol (0.162 g, 0.00105 mol, 1 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.0086 g, 0.0000318 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.00971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 10 min. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and

evaporated to give a yellow solid (0.243 g, >100 % yield, 34% conversion). ^{1}H NMR data as in a.

c) Dithiothreitol (0.469 g, 0.00304 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 1 hour. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a mixture of white and yellow solids. (0.341 g, 377 % yield, 100% conversion) ^{1}H NMR data as in a; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 115.032, 118.458, 129.167, 146.215.

d) Dithiothreitol (1.950 g, 0.01264 mol, 3.1 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.019 g, 0.0000703 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.42 mL, 0.00408 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 5.5 hours. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a mixture of white and yellow solids. The residue was purified by column chromatography over silica gel using 3:2 hexane-ethyl acetate. A brown oil (0.121 g, 32 %) was obtained. NMR data as in c.

e) Dithiothreitol (0.462 g, 0.00299 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 13 min. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a mixture of white and yellow solids. (0.458 g, >100 % yield, 83 % conversion) ^1H NMR data as in a. Also contains nitrobenzene ^1H NMR ($CDCl_3$, 300 MHz) δ 7.5 (dd, *J*=13 Hz, 2 H), 7.6 (dd, *J*=11 Hz, 1 H), 8.2 (d, *J*=13 Hz, 2H).

f) Dithiothreitol (0.468 g, 0.00303 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) was added. A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was left stirring at room temperature for 4 days. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. 0% conversion. ^1H NMR data as in f.

g) Dithiothreitol (0.459 g, 0.00297 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). A very small amount of charcoal was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 10 min. The solution was allowed to cool to room

temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. 0% conversion. ^1H NMR data as in f.

h) Dithiothreitol (0.464 g, 0.00301 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was allowed to reflux for 10 min. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a mixture of yellow solid and oil (76% conversion). ^1H and ^{13}C NMR data as in c.[†]

i) Dithiothreitol (0.464 g, 0.00301 mol, 3 mol eq) was dissolved in 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and the solution was monitored by TLC (9:1 hexane/ethyl acetate). The reaction looked to be complete after 130 min of reflux. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a colorless oil (0.008 g, 9 %). ^1H NMR data as in a.

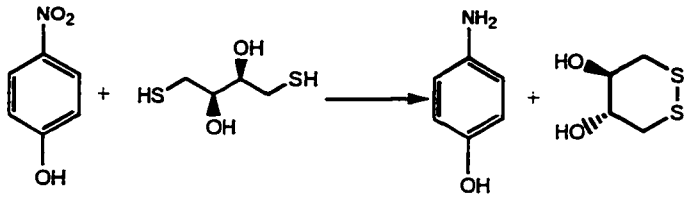
j) Dithiothreitol (0.474 g, 0.00307 mol, 3 mol eq) was dissolved in deionized H_2O (15

mL, pH 5). $FeCl_3$ (0.013 g, 0.0000481 mol) was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and refluxed for 2 hours. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a faint yellow oil. 0% conversion. ^1H and ^{13}C NMR data as in f.

k) Dithiothreitol (0.4608 g, 0.00298 mol, 3 mol eq) was dissolved in deionized H_2O (15 mL, pH 7.5). $FeCl_3$ (0.018 g, 0.0000666 mol) was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 100°C oil bath and refluxed for 5 hours. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a faint yellow oil. 0% conversion. ^1H and ^{13}C NMR data as in f.

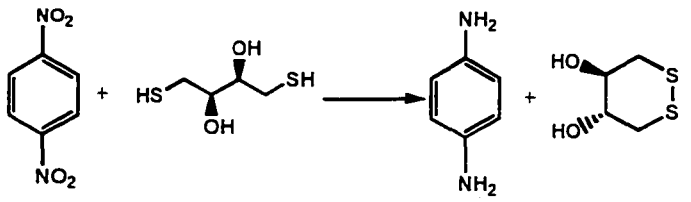
l) Dithiothreitol (0.4636 g, 0.00300 mol, 3 mol eq) was dissolved in THF (15 mL). $FeCl_3$ (0.019 g, 0.0000703 mol) was added. Nitrobenzene (0.1 mL, 0.000971 mol) was added via syringe. The reaction flask was immersed in a 67°C bath and refluxed for 5 hours. The solution was allowed to cool to room temperature. The solution was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was a faint yellow oil. 0% conversion. ^1H NMR data as in f.

4-Aminophenol



p-Nitrophenol (0.521 g, 0.00374 mol) and dithiothreitol (1.913 g, 0.0124 mol, 3.3 mol eq) were added to 0.1 M phosphate buffer (15 mL). $FeCl_3$ (0.0125 g, 0.0000462 mol) and a small amount of charcoal were added. The mixture was immersed in a 110°C oil bath and refluxed for 5 hours. The solution was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. 1.934 g of a bright yellow solid was obtained. Column chromatography over silica gel using 1:1 hexane-ethyl acetate gave a light yellow solid (0.177 g, 43%) ^1H NMR (DMSO-d6, 300 MHz) δ 6.4 (d, *J*=9 Hz, 4H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 73.42, 115.28, 115.56, 140.62, 148.25.

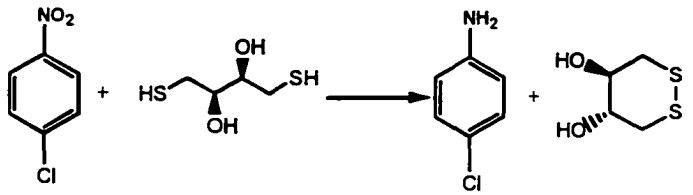
1,4-Phenylenediamine



Dinitrobenzene (0.503 g, 0.00299 mol) and dithiothreitol (2.862 g, 0.0185 mol, 6 mol eq) were added to 0.1 M phosphate buffer (25 mL). $FeCl_3$ (0.010 g, 0.0000369 mol) and a

small amount of charcoal were added. The mixture was immersed in a 100°C oil bath and refluxed for 6 hours. The solution was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and evaporated. A mixture of 1,4-phenylenediamine and 1,2-dithiane-4,5-diol (2.626 g) was obtained. Recrystallization from acetone separated some of the 1,2-dithiane-4,5-diol. The rest was removed by column chromatography (8:1:1 hexane/ethyl acetate/methanol) over silica gel, leaving a purple solid (0.0663 g, 16% yield). ^{1}H NMR (DMSO-d6, 300 MHz) δ 4.2 (s, 4H), 6.3 (s, 4H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 115.393, 138.911.

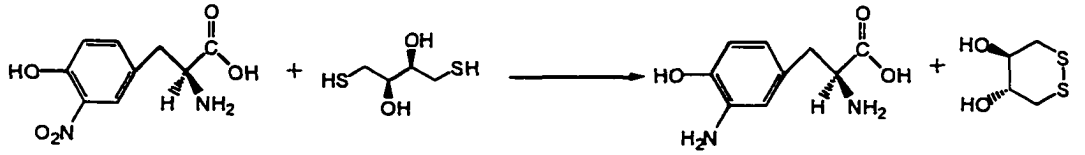
4-chloroaniline



4-chloronitrobenzene (0.1740 g, 0.001104 mol) and dithiothreitol (0.5380 g, 0.003488 mol) were added to 0.1M phosphate buffer (50 mL) in a round-bottom flask. $FeCl_3$ (0.011 g, 0.000017 mol) and a small amount of charcoal were added. The flask was immersed in a 100°C oil bath and refluxed for 2.5 hours. The solution was extracted three times with ethyl acetate. The organic layers were combined, dried over $MgSO_4$ and evaporated. 4-Chloroaniline was obtained as a yellow-brown residue. (0.6154 g, 437% yield, 94 % conversion) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.47 (s, 2H), 6.57-6.60 (d, *J*=9 Hz, 2H), 7.07-

7.10 (d, J=9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 116.18, 123.04, 129.02, 144.869. 1-Chloro-4-nitrobenzene was also present ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48-7.51 (d, J=9 Hz, 2H), 8.14-8.17 (d, J=9 Hz, 2H).

3-amino-L-tyrosine



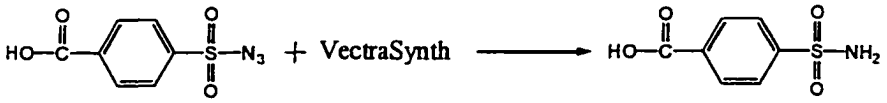
a.) 3-nitro-L-tyrosine (0.5065 g, 0.00223 mol) and dithiothreitol (1.1041g, 0.00715 mol, 3 mol eq) were added to 0.1 M phosphate buffer (35 mL). $FeCl_3$ (0.035 g, 0.000129 mol) and a small amount of charcoal were added. The mixture was immersed in a 100°C oil bath and refluxed for 7 hours. The solution was extracted three times with ethyl acetate. The water layer was evaporated to dryness on a rotary evaporator. The product was purified from the brown residue by column chromatography over silica gel using 4:1 methanol-ethyl acetate (0.2006g, 46%). ^{1}H NMR (D_2O, 300 MHz) δ 2.902-2.977 (dd, J=8 Hz, 1H), 3.094-3.157 (dd, J_1=4.5 Hz, J_2=14 Hz, 1H), 3.855-3.907 (dd, J=5 Hz, 1H), 6.626-6.652 (d, J=8 Hz, 1H), 6.754 (s, 1H), 6.804-6.830 (d, J=8 Hz, 1H); ^{13}C NMR (D_2O, 75 MHz) δ36.206, 56.453, 116.192, 118.261, 121.141, 127.859, 135.261, 144.239, 174.843.

b.) 3-nitro-L-tyrosine (0.5011 g, 0.00221 mol) and dithiothreitol (1.1237g, 0.00728 mol, 3 mol eq) were added to 0.1 M phosphate buffer (30 mL). $FeCl_3$ (0.026 g, 0.000096

mol) and a small amount of charcoal were added. The mixture was immersed in a 100°C oil bath and refluxed for 8 hours. The solution was extracted three times with ethyl acetate. The water layer was extracted three times with methanol. The methanol was evaporated to give a white solid residue (0.3265g, 75%). ^{1}H and ^{13}C NMR data as in a.

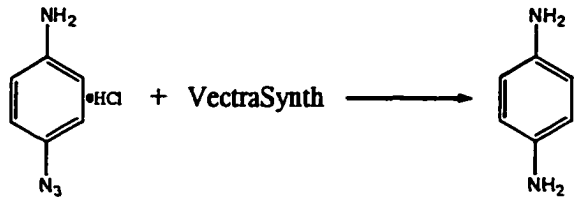
Reductions with VectraSynth™

4-Carboxybenzenesulfonamide



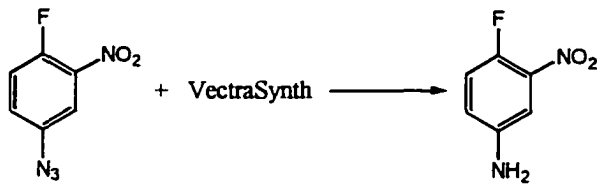
4-Carboxybenzene sulfonazide (0.051 g, 0.00022 mol) was dissolved in 0.5 M phosphate buffer (15 mL, pH 7.5). VectraSynth™ (2.27 g, 0.00022 mol, 1 mol eq) was added, but did not dissolve. The flask was wrapped in tinfoil and the reaction was left stirring for 24 hours. The solid was removed by vacuum filtration. The filtrate was lyophilized to give a white powder (0.209 g, >100 % yield, 100% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.2-7.6, (s, 2H), 7.7 (d, *J*=8 Hz, 2H), 7.9 (d, *J*=8 Hz, 2H); ^{13}C NMR (DMSO-d6 75 MHz) δ 124.67, 129.21, 143.89, 144.27, 167.80.

1,4-Phenylenediamine



4-azidoaniline hydrochloride (0.028 g, 0.000164 mol) was dissolved in 0.5 M phosphate buffer (15 mL, pH 7.5). It did not dissolve. Enough THF was added to dissolve it. VectraSynth™ (1.675 g, 0.000167 mol) was added. The flask was wrapped in foil and the mixture was left overnight to stir. The solution was filtered to separate the solid. Solvent was evaporated from the filtrate to give a dark solid (0.373g, >100% yield, 100% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 6.3 (s); ^{13}C NMR (DMSO-d6, 75 MHz) δ 115.42, 138.93.

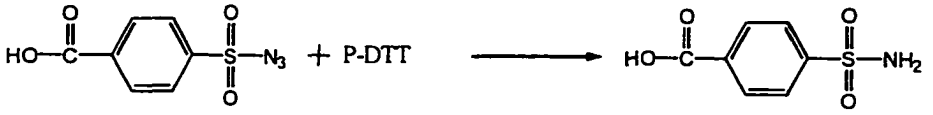
4-Fluoro-3-nitroaniline



4-Azido-1-fluoro-2-nitrobenzene (0.050g, 0.000275 mol) was added to deionized water. VectraSynth (4g, 0.0004 mol, 1.45 mol eq) was added. THF was added to dissolve the azide. The mixture was stirred overnight. The immobilized reagent was removed by filtration under reduced pressure. Solvent was removed from the filtrate on a rotary evaporator leaving a yellow solid (0.0058 g, 14 %). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 6.54 (m, 2H), 6.86 (s, 1H).

Reductions with P-DTT

4-Carboxybenzenesulfonamide

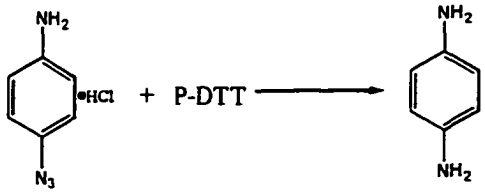


a) 4-Carboxybenzene sulfonazide (0.0388 g, 0.00017 mol) was dissolved in methanol (15 mL). P-DTT (0.1063 g, 0.00018 mol, 1 mol eq) was added, but did not dissolve. The reaction was left stirring overnight. The solid was removed by vacuum filtration. The filtrate was evaporated to give a white powder (0.037 g, >100 % yield, 18% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.54, (s, 2H), 7.92-7.95 (d, *J*=8 Hz, 2H), 8.11-8.13 (d, *J*=8 Hz, 2H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 129.249, 133.823, 147.017, 166.534.†

b) 4-Carboxybenzene sulfonazide (0.0129 g, 0.0000567 mol) was dissolved in THF (10 mL). P-DTT (0.40 g, 0.00022 mol, 12 mol eq) was added, but did not dissolve. The reaction was left stirring overnight. The solid was removed by vacuum filtration. The filtrate was evaporated to give a white powder (0.0602 g, >100 % yield). ^{1}H NMR data as in a.†

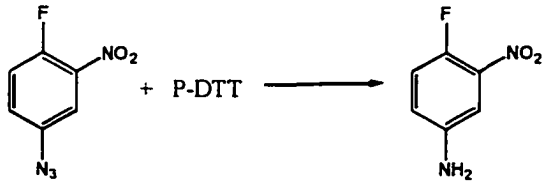
† contains 4-carboxybenzene sulfonazide: ^{1}H NMR (DMSO-d6, 300 MHz) δ 8.1 (d, *J*=8 Hz, 2H), 8.202-8.253 (d, *J*=8 Hz, 2H).

1,4-Phenylenediamine



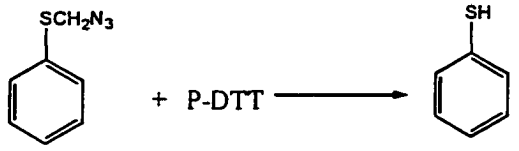
4-azidoaniline hydrochloride (0.0305 g, 0.000178 mol) was dissolved in THF (15 mL). P-DTT (0.0916 g, 0.000157 mol) was added. The mixture was left overnight to stir. The solution was filtered to separate the solid. Solvent was evaporated from the filtrate to give a brown solid (0% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.108-7.137 (d, *J*=9 Hz, 2H), 7.215-7.244 (d, *J*=9 Hz, 2H);

4-Fluoro-3-nitroaniline



4-Azido-1-fluoro-2-nitrobenzene (0.0206g, 0.000113 mol) was added to THF (10 mL). P-DTT (0.0699g, 0.000119 mol, 1 mol eq) was added. The mixture was stirred for 3 days. The immobilized reagent was removed by filtration under reduced pressure. Solvent was removed from the filtrate on a rotary evaporator leaving a yellow solid (0.023 g, >100 % recovery, 0% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.37-7.40 (m, *J*=1 Hz, 2H), 7.67-7.70 (m, *J*=1 Hz, 1H).

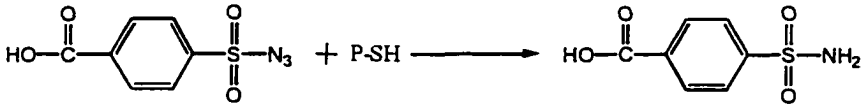
Thiophenol



P-DTT (0.0610 g, 0.000104 mol) was weighed into a round-bottom flask. THF (5 mL) and azidomethyl phenyl sulfide (14 μL, 0.0001 mol) were added. The flask was loosely capped and the reaction was left stirring for 3 days. The mixture was filtered to remove the immobilized reagent. Solvent was evaporated from the filtrate on a rotary evaporator, leaving a colorless residue (0.0219 g, >100 % recovery, 0% conversion). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.54 (s, 2H), 7.2-7.3 (m, *J*=2 Hz, 3H), 7.4-7.5 (d, *J*=2 Hz, 2H).

Reductions with P-SH

4-Carboxybenzenesulfonamide



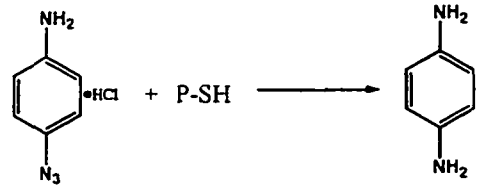
a) 4-Carboxybenzene sulfonazide (0.025 g, 0.00011 mol) was dissolved in THF (10 mL). P-SH (0.1303 g, 0.00024 mol, 2.2 mol eq) was added, but did not dissolve. The flask was left stirring overnight. The solid was removed by vacuum filtration. The filtrate was evaporated to give a white powder (0.022 g, >100 % yield, 34% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ, 7.5 (s, 2H), 7.9 (d, *J*=9 Hz, 2H), 8.0-8.1 (d, *J*=9 Hz, 2H); ^{13}C NMR (DMSO-d6, 75 MHz) δ 125.799, 129.083, 133.706, 147.725, 166.413.†

b) 4-Carboxybenzene sulfonazide (0.026 g, 0.00011 mol) was dissolved in MeOH-H_2O (1:1, 10 mL). P-SH (0.1397 g, 0.00024 mol, 2.4 mol eq) was added, but did not dissolve. The flask was left stirring overnight. The solid was removed by vacuum filtration. The filtrate was evaporated to give a white powder (0.0011 g, 50 % yield, 8% conversion). ^{1}H NMR data as in a.†

c) 4-Carboxybenzene sulfonazide (0.0104 g, 0.0000457 mol) was dissolved in H_2O (25 mL, pH 7.5). P-SH (0.5978 g, 0.00055 mol, 12 mol eq) was added, but did not dissolve. The flask was left stirring overnight. The solid was removed by vacuum filtration. The filtrate was evaporated to give a white powder (0.0136 g, >100 % yield, 23% conversion). ^{1}H NMRdata as in a.†

† contains 4-carboxybenzene sulfonazide.

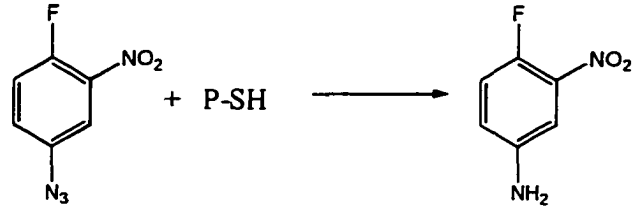
1,4-Phenylenediamine



4-azidoaniline hydrochloride (0.0224 g, 0.000131 mol) was dissolved in THF (15 mL). P-SH (0.1329 g, 0.000247 mol, 1.89 mol eq) was added. The mixture was loosely capped and left overnight to stir. The solution was filtered to separate the solid. Solvent was

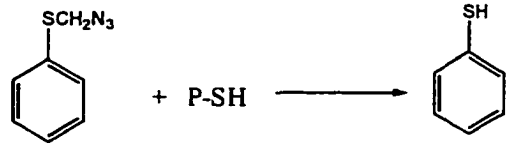
evaporated from the filtrate to give a brown solid (0.0195 g, >100 % yield). ^{1}H NMR (DMSO-d6, 300 MHz) δ 6.87 (s, 4H).

4-Fluoro-3-nitroaniline



4-Azido-1-fluoro-2-nitrobenzene (0.0203g, 0.000111 mol) was added to THF (10 mL). P-SH (0.1224g, 0.000227 mol, 2 mol eq) was added. The mixture was stirred for 3 days. The immobilized reagent was removed by filtration under reduced pressure. Solvent was removed from the filtrate on a rotary evaporator leaving a yellow solid (0.0365 g, >100 % recovery, 0% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.47-7.55 (m, 2H), 7.76-7.77 (m, 1H).

Thiophenol



P-SH (0.1118 g, 0.000207 mol, 2 mol eq) was added via syringe to a round-bottom flask containing THF (5 mL) and azidomethyl phenyl sulfide (14 μL, 0.0001 mol) were

added. The flask was loosely capped and the reaction was left stirring for 3 days. The mixture was filtered to remove the immobilized reagent. Solvent was evaporated from the filtrate on a rotary evaporator, leaving a colorless residue (0.0378 g, >100 % recovery, 0% conversion). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.53 (s, 2H), 7.2-7.3 (m, 3H), 7.4-7.5 (d *J*=7 Hz, 2H).

Regeneration of Immobilized DTT

VectraSynth™

A slurry of used VectraSynth™ was prepared in deionized H_2O. To this was added 200 mg $NaBH_4$ per gram VectraSynth™. The resulting mixture was left stirring overnight. 1 M acetic acid was added until the mixture was acidic. Regenerated VectraSynth™ was isolated by filtration under reduced pressure.

P-SH and P-DTT

A slurry of used P-SH or P-DTT was prepared in THF. To this was added 200 mg $NaCNBH_4$ per gram of used immobilized reagent. The mixture was left stirring overnight and then filtered to obtain regenerated P-SH or P-DTT.

Reactions with Regenerated Reagents

VectraSynth™

4-Carboxybenzenesulfonazide (0.025 g, 0.00011 mol) and VectraSynth™ (1.1240 g,

0.000 mol, 1.1 mol eq) were added to a round-bottom flask. MeOH (10 mL) was added. The mixture was left stirring overnight. The immoblized reagent was removed by vacuum filtration. The solvent was removed on a rotary evaporated leaving a white solid (0.0061 g, 28 % yield, 100 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.50 (s, 2H), 7.90-7.93 (d, *J*=6 Hz, 2H), 8.09-8.11 (d, *J*=6 Hz, 2H). The reagent was regenerated and added to 4-carboxybenzenesulfonazide (0.025 g, 0.00011 mol) in MeOH. This mixture was left stirring overnight, then filtered under reduced pressure to separate the resin. The filtrate was evaporated to give a white solid (0.0108 g, 49 % yield, 59 % conversion).† ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.52 (s, 2H), 7.90-7.93 (d, *J*=6 Hz, 2H), 8.08-8.11 (d, *J*=6 Hz, 2H). The VectraSynth was regenerated again. It was then added to 4-Carboxybenzenesulfonazide (0.0231 g, 0.000102 mol) in MeOH. The mixture was left stirring for 4 days. The immobilized reagent was removed by filtration under reduced pressure. The solvent was evaporated from the filtrate to leave a white solid (0.043 g, 21 % yield, 85% conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.51 (s, 2H), 7.90-7.93 (d, *J*=6 Hz, 2H), 8.08-8.11 (d, *J*=6 Hz, 2H).†

4-Fluoro-3-nitroaniline

4-Azido-1-fluoro-2-nitrobenzene (0.0213 g, 0.000116 mol) and VectraSynth™ (0.6247 g, 0.0000624 mol) were added to a round-bottom flask. Enough deionized water was added to make a slurry. The mixture was left stirring for 3 days. The immobilized reagent was removed by vacuum filtration and the solvent was evaporated from the filtrate. 4-Fluoro-

3-nitroaniline was obtained as a bright yellow solid with 100% conversion. ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 6.87-6.92 (m, 1 H), 7.09-7.22 (m, 1H). The VectraSynth™ was regenerated and added to 4-azido-1-fluoro-2-nitrobenzene (0.0206 g, 0.000113 mol). Deionized water was added to make a slurry. The mixture was left to stir for 3 days. It was then filtered under reduced pressure. The filtrate was evaporated to give a yellow solid. (0.0021 g, 10 % yield). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 6.85-6.89 (m, 1 H), 6.98-7.22 (m, 1H).

1,4-Phenylenediamine

4-Azidoaniline hydrochloride (0.0213 g, 0.000125 mol) and VectraSynth™ (1.1834 g, 0.000118 mol) were weighed into a round bottom flask. Enough deionized water was added to make a slurry. The mixture was left stirring overnight. The immobilized reagent was removed by vacuum filtration and the water was removed on a rotary evaporator. A brown solid remained (0.0167 g, 12% yield). ^{1}H NMR (DMSO-d6, 300 MHz) δ 6.74 (s, 4H). The VectraSynth™ was regenerated and then added to 4-azidoaniline hydrochloride (0.0209 g, 0.000122 mol). Enough deionized water was added to make a slurry. The mixture was left stirring overnight. The immobilized reagent was removed by vacuum filtration and the water was removed on a rotary evaporator to leave a dark colored solid (0.0201 g, 0.000186 mol, 152 % yield). ^{1}H NMR (DMSO-d6, 300 MHz) δ 6.63 (s, 4H).

P-DTT

4-Fluoro-2-nitroaniline

4-Azido-1-fluoro-2-nitrobenzene (0.0206g, 0.000113 mol) was added to THF (10 mL). P-DTT (0.0699g, 0.000119 mol, 1 mol eq) was added. The mixture was stirred for 3 days. The immobilized reagent was removed by filtration under reduced pressure. Solvent was removed from the filtrate on a rotary evaporator leaving a yellow solid (0.023 g, >100 % recovery, 0% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.37-7.40 (m, 2H), 7.67-7.70 (m, 1H). The P-DTT was regenerated and added to 4-azido-1-fluoro-2-nitrobenzene (0.0196 g, 0.000108 mol) in THF. The solution was stirred overnight and then filtered by vacuum filtration to remove the immobilized reagent. A yellow solid was obtained (0.029 g, >100 % yield, 0% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.402-7.424 (m, 2H), 7.684-7.697 (m, 1H).

1,4-Phenylenediamine

4-Azidoaniline hydrochloride (0.0200 g, 0.000117 mol) and P-DTT (0.0743 g, 0.000127 mol) were weighed into a round bottom flask. Enough THF was added to make a slurry. The mixture was left stirring for 3 days. The immobilized reagent was removed by vacuum filtration and the THF was evaporated on a rotary evaporator. A brown solid remained (0 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.1 (d, J=6 Hz, 2H), 7.1-7.2 (d, J=6 Hz, 2H). The P-DTT was regenerated and then added to 4-azidoaniline hydrochloride (0.0194 g, 0.000113 mol). Enough THF was added to make a slurry. The mixture was left

stirring overnight. The immobilized reagent was removed by vacuum filtration and the water was removed on a rotary evaporator to leave a brown residue. ^{1}H NMR (DMSO-d6, 300 MHz) δ 6.89-6.97 (dd, J_1=6 Hz, J_2=9 Hz, 4H).

P-SH

4-Carboxybenzenesulfonamide

a) 4-Carboxybenzenesulfonazide (0.0258 g, 0.000113 mol) and P-SH (0.1316 g, 0.000244 mol, 1 mol eq) were added to a round-bottom flask. Acetonitrile (5 mL) was added. The mixture was left stirring overnight. The immoblized reagent was removed by vacuum filtration. The solvent was removed on a rotary evaporated leaving a white solid (0.0248 g, 108 % yield, 29 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.53 (s, 2H), 7.91-7.94 (d, J=9 Hz, 2H), 8.09-8.12 (d, J=9 Hz, 2H).† The reagent was regenerated and added to 4-carboxybenzenesulfonazide (0.0246 g, 0.000108 mol) in acetonitrile. This mixture was left stirring overnight, then filtered under reduced pressure to separate the resin. The filtrate was evaporated to give a white solid (0.0217 g, 89 % yield, 9 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.55 (s, 2H), 7.92-7.95 (d, J=9 Hz, 2H), 8.13-8.16 (d, J=9 Hz, 2H).†

b) 4-Carboxybenzenesulfonazide (0.051 g, 0.000224 mol) and P-SH (0.2639 g, 0.000491 mol, 2.2 mol eq) were added to a round-bottom flask. Methanol (10 mL) was added. The mixture was left stirring overnight. The immoblized reagent was removed by

vacuum filtration. The solvent was removed on a rotary evaporated leaving a white solid (0.056 g, >100 % yield, 30 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.54 (s, 2H), 7.91-7.94 (d, *J*=9 Hz, 2H), 8.09-8.12 (d, *J*=9 Hz, 2H).† The reagent was regenerated and added to 4-carboxybenzenesulfonazide (0.0500 g, 0.00022 mol) in MeOH. This mixture was left stirring overnight, then filtered under reduced pressure to separate the resin. The filtrate was evaporated to give a white solid (0.0409 g, 92 % yield, 36 % conversion). ^{1}H NMR (DMSO-d6, 300 MHz) δ 7.47 (s, 2H), 7.69-7.79 (d, *J*=9 Hz, 2H), 7.87-7.99 (d, *J*=9 Hz, 2H).†

4-Fluoro-2-nitroaniline

4-azido-1-fluoro-2-nitroaniline (0.0345 g, 0.000189 mol) and P-SH (0.2038g, 0.000379 mol) were combined in a round bottom flask. MeOH (10 mL) was added as solvent. The mixture was stirred for 2 days. The immobilized reagent was removed by vacuum filtration. The solvent was removed on a rotary evaporator leaving a yellow solid residue (0.0391 g, >100 % recovery, 0% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.46-7.54 (m, 2H), 7.72-7.75 (m, 1H). The P-SH was regenerated and added to 4-azido-1-fluoro-2-nitroaniline (0.0340 g, 0.000187 mol). The mixture was left overnight to stir. It was then filtered to remove the immobilized reagent. The solvent was evaporated from the filtrate on a rotary evaporator, leaving a yellow solid residue (0.0265 g, 91 % recovery, 9% conversion). ^{1}H NMR (DMSO-d6 + $CDCl_3$, 300 MHz) δ 7.45-7.48 (m, 2H), 7.71-7.73 (m, 1H).

3.5 REFERENCES

1.Cleland, W.W. *Biochemistry*, **1964**, *3*, 480.

2. Hart, R.A.; Lester, P.M.; Reifsnyfer, D.H.; Ogez, J.R. and Builder, S.E. *Bio/Technology*, **1994**, *12*, 1113-1117.

3.March, J. Advanced Organic Chemistry 3rd Ed. New York: John Wiley & Sons, Inc., 1985, p1103.

4.Balabanli, B.; Kamisaki, Y.; Martin, E. and Murad, F. *Proc. Nat. Acad. Sci.* **1999**, *96*(23), 13136-13141.

5.Handlon, A.L. and Oppenheimer, N.J. *Pharm. Res.* **1988**, 297-299.

6.Staros, J.V.; Bayley, H.; Standring, D.N. and Knowles, J.R. *Biochem. Biophys. Res. Commun.*, **1978**, 568-572.

7.Cartwright, I.L.; Hutchinson, D.W. and Armstrong, V.W. *Nucleic Acids Res.*, **1976**, 2331-2339.

8. Meinjohanns, E.; Meldal. M.; Jensen. T.; Werdelin, O.; Galli-Stampino, L.; Mouritsen, S. and Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 871-884.

9. Merrifield, R.B. *J. Am. Chem. Soc.*, **1963**, *85*, 2149-2153.

10. Novabiochem Polymer Supported Reagents Handbook 2001, p12.