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Kinetic studies on deprotection and depurination rates of protected nucleotide

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**KINETIC STUDIES ON DEPROTECTION AND DEPURINATION RATES
OF PROTECTED NUCLEOTIDE**

A Thesis

Presented to

The Faculty of the Department of Chemical and Materials Engineering

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Anjali Srivastava

December 2003

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ABSTRACT

KINETIC STUDIES ON DEPROTECTION AND DEPURINATION RATES OF PROTECTED NUCLEOTIDE

By: Anjali Srivastava

Under the acidic condition required for detritylation during oligonucleotide synthesis, significant depurination occurs. Despite past efforts, depurination remains a major cause of concern. In this study, the effect of various detritylating reagents on the rates of deprotection and depurination of benzoyl-protected deoxyadenosine both in free and immobilized form was studied. The rates of deprotection and depurination were determined by calculating the amounts of dimethoxytrityl cation and benzoyl-protected adenine in the reaction mixture using high performance liquid chromatography. These rates increased with increase in acid concentration, acid dissociation constant, and also with the polarity of the solvent. Furthermore, these rates were slower in the solid phase than in the solution phase. The yield for each reaction condition was also calculated to determine the selectivity of each reagent. It was found that 0.05M trichloroacetic acid and 0.3M dichloroacetic acid in dichloromethane and 0.3M MSA in methylisobutyrate were the most selective detritylating reagents with 98% yield.

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1.0 INTRODUCTION

1.1 DNA Microarray

Deoxyribonucleic acid (DNA) is the basic hereditary material in all cells and contains all the information necessary to make proteins by determining the sequence of amino acids. A segment of DNA, which is a double-stranded molecule, encodes the structure of an individual protein. Each strand of DNA, as shown in Figure 1, is a linear polymer that is made of nucleotide units. The nucleotide unit consists of a phosphate group, the sugar molecule (deoxyribose) and one of the four bases: adenine (A), cytosine (C), guanine (G) and thymidine (T). The phosphate and the deoxyribose form the backbone of the chain linked at the 3' and 5' positions of deoxyribose. On the other hand, the bases are side groups that are connected to the deoxyribose at the 1' position via an N-glycosidic linkage. Two complementary chains that are arranged in anti-parallel manner form a duplex of DNA. In DNA, adenine pairs only with thymine and cytosine pairs only with guanine [1].

Figure 1. Nucleotides bases of a DNA linked together by phosphodiester bond

A variety of recent technological breakthroughs in molecular biology (catalyzed by the work of Human Genome Project, fundamental advances in polymerase chain reaction (PCR) or other amplification techniques to make copies of a nucleic acid sample, the creation of gene sequencer, etc.) and steady improvements in nano- and microscale fabrication techniques have made possible the development of DNA chips. DNA chip technology is an exciting area in biological research today and has an immense potential for commercial success. It is an outstanding tool for the analysis of gene expression and hence for investigating the mechanism of many diseases and accelerating the drug development process that currently can require 15 years or more. This can lead to substantial cost savings by reducing pre-clinical drug development time.

The DNA chip utilizes the ability of DNA to bind to complementary strands. There can be more than 500,000 unique features (as the one produced by Affymetrix) on a single chip. This high feature density (approx. 10^6 sequences/cm²) facilitates multiple, independent measurements of gene activity, helping to ensure accurate assessment of gene expression levels [2,3]. A single DNA chip experiment can provide researchers with information for thousands of genes. The DNA microarray technology has applications in gene discovery, disease discovery, drug diagnosis (pharmacogenomics) and toxicological research (toxicogenomics) [4].

The terms DNA chip and DNA microarray are often used interchangeably. However, the DNA chips (also termed as DNA macroarrays) contain sample spot sizes of 300 microns or larger while DNA microarrays contain sample spot sizes of 200 microns or less leading to a higher density of probes (a probe is a nucleic acid strand with a known

sequence). Regardless of spot size, fragments of different commercially available identified DNA samples are tethered to a substrate using various techniques [5]. The immobilized DNA probes are then incubated in a solution of labeled nucleic acid targets, which may be messenger ribonucleic acid (mRNA), complementary DNA (cDNA) or genomic DNA. Bases in the probe bind (or hybridize) with complementary bases (as shown in Figure 2) in the target nucleic acid strand, and the amount of hybridization at each spot on the slide are analyzed using a fluorescence detection method.

Figure 2. DNA microarray experiment

1.2 Phosphoramidite Chemistry for Oligonucleotide Synthesis

Conventional phosphoramidite chemistry for oligonucleotide synthesis can be applied in DNA chip technology to synthesize oligonucleotide probes. This method provides high coupling efficiency. Each cycle is composed of detritylation, coupling, capping and oxidation steps with intervening wash steps [6].

Figure 3. Oligonucleotide synthesis using phosphoramidite chemistry

As shown in Figure 3, in the detritylation step, the 5'-protecting group is removed by acid treatment. In the coupling step, tetrazole is used to activate coupling of phosphoramidite to the 5'-hydroxyl groups by protonating the dialkylamino group of the function since the phosphoramidites cannot react directly with free 5'-hydroxyl group on a growing chain. During the coupling step, some molecules fail to react resulting in undesired and truncated species. If these truncated sequences were allowed to react further, the result would be near full-length oligos with internal deletions. This problem is

resolved by capping the free 5'-OH through acetylation. Hence, in the capping step, acetic anhydride and N-methylimidazole are used as acetylating reagents to cap the 5'-OH of the truncated species. In the oxidation step, iodine is used as a mild oxidant in basic aqueous tetrahydrofuran solution to oxidize the trivalent phosphate to pentavalent phosphate. This cycle is repeated until the desired oligodeoxyribonucleic acid (oDNA) has been synthesized. Finally the whole chain is cleaved from the support with the treatment of ammonium hydroxide.

In the synthesis of sequence-defined oligonucleotides via the phosphoramidite approach, two types of protecting groups are used: Permanent groups and temporary groups [7]. The permanent groups remain attached to the oligonucleotide throughout the synthesis and are removed at the end of the chain assembly. Base protecting groups are in this category. The most widely used base protecting groups are benzoyl for adenine and cytosine and isobutyryl for guanine. Usually, thymine is not protected because the actual number of side products is small in the short reaction times used. On the other hand temporary groups are used for transient protection. As shown in Figure 3, these are removed under acidic conditions after each coupling step. The dimethoxytrityl (DMT) group has been extensively used for the protection of 5'-hydroxyl functions in the synthesis of the oligodeoxyribonucleotides.

1.3 Methods of Fabrication

There are two general types of DNA microarrays: complementary DNA (cDNA) or RNA probes and oligodeoxyribonucleic acid (oDNA). Complementary DNA chips

contain presynthesized long DNA sequences that are immobilized on filters or glass slides using robotic spotting techniques [8]. Scientists from Stanford University pioneered this approach. On the other hand, oDNA chips contain shorter oligonucleotides (approximately 25 bases) that can be made either by conventional methods followed by on chip attachment or synthesized in-situ using photolithographic techniques. Affymetrix developed oDNA chips; and their photolithographically fabricated product is sold under the trademark of "Gene-chip."

To make the DNA chip using robotic techniques, a robotic instrument is used to print the DNA spots simply by dipping pins into wells containing the DNA and then striking these pins on the surface of the glass slides. This approach has the advantage of forming specific binding interactions with the solid surface due to use of longer probes and it can be installed and operated in any laboratory due to its ease of use, low cost and versatility. However, the main disadvantage of this technique is that it involves direct contact with substrates which can damage the surface of the substrate.

Microarrays prepared using a photolithographic approach have the advantage of low noise and better definition due to the single point attachment of the oligonucleotide probes. The main disadvantages of this approach are the limitation in the length of oligonucleotides, the coupling of nucleotides with lower specificity and the high cost of the photolithographic masks. Despite its disadvantages, the photolithographic method has the advantage of high resolution and high throughput due to its ability to synthesize high-density arrays using combinatorial masking strategies in a relatively small number of chemical steps. It has also the advantage of adapting the tools and techniques from the

semiconductor industry where they are fairly well characterized [2,8]. In this method, as shown in Figure 4, oligonucleotides are synthesized by directly removing the photo-labile 5'-protecting groups from the growing chain by exposure to ultraviolet light through a photolithographic mask.

Figure 4. Synthesis of oligonucleotide using photolithography method, picture taken from http://www.nature.com/ng/journal/v21/n1s/images/ng0199supp_20.gif.

Photoacid patterned array (PPA) fabrication is another photolithographic method of oligonucleotide synthesis utilizing the photoacid generators from the modern photoresists (photosensitive materials which after photoimaging and subsequent processing, resist action of certain chemicals in desired areas) to deprotect the 5'-protecting groups [3]. In this method, a substrate is coated with a polymer film containing photo-acid generator, which on exposure to ultraviolet light results in creation of photo-generated acid that activates the surface bound oligonucleotides by detritylation and allows attachment of next protected nucleotide.

1.4 Role of Solvent

During the detritylation step in oligonucleotide synthesis, acid in a solvent is used to remove the 5'-protecting group. The choice of solvent plays an important role in affecting the rate of reaction by affecting the acidity of an acid. Hence, the same acid behaves differently in various solvents. An acid on dissociation produces a H^+ ion and an anion, and the concentration of H^+ is a direct measure of its acidity. The parameter pK_a of an acid, as shown in Table 1, describes the acidity of an acid in water. Higher pK_a signifies lower acidity. Thus, the acidity decreases in the following order: MSA > TCA > DCA > MCA.

Table 1. Acidity Constant and pK_a of Acids

Acids	Acidity Constant	pK_a
MCA (monochloroacetic acid)	1.36×10^{-3}	2.87
DCA (dichloroacetic acid)	5.5×10^{-2}	1.25
TCA (trichloroacetic acid)	0.23	0.77
MSA (methanesulfonic acid)	3×10^2	~ -2.0

The dissociation of any acid by the solvent requires the separation of ions. The work required to separate these ions is inversely proportional to the dielectric constant of the solvent [9]. This energy is supplied by the solvation of the ion and also by the proton transfer from the acid to the solvent. Therefore, the ionization degree of an acid depends on the dielectric constant of the solvent. The dielectric constant describes the polarity of a solvent. As shown in Table 2, polar solvents have higher dielectric constant than non-

polar solvents. Thus, the polarity decreases in the following order: water < dichloromethane < methylisobutyrate. Water has a very high solvating ability and dielectric constant; therefore, it dissociates the acid and stabilizes the ions. If the dielectric constant and solvating ability of a solvent is lower than that of water, more energy will be required to separate the ions and hence, the extent of dissociation of acid will decrease.

Table 2. Dielectric Constant of Solvents

Solvent	Dielectric Constant
Water	78
Dichloromethane (DCM)	9.08
Methylisobutyrate (MIB)	5.6

1.5 Inherent Problem

During oligodeoxynucleotide synthesis, the stability of the growing deoxynucleotide to repetitive exposure to various reagents remains an inherent problem. The N-glycosidic bond between a purine base and its deoxyribose group in DNA is very susceptible to hydrolysis [10]. During the detritylation/deprotection step for each cycle, as shown in Figure 3, the growing oligonucleotide is subjected to acid conditions. Under these circumstances a small number of purine rings may be lost. This depurination, as shown in Figure 5, is a harmful side reaction in DNA synthesis since these bases are the key component in hybridization. Depurination occurs spontaneously with a relatively high

frequency under physiological conditions. In order to overcome this problem, efforts have been made to facilitate the removal of the DMT group by alternative reagents (acids in different solvents) and to explore new base protecting groups and the protecting groups for the 5'-OH function. Despite recent advances in the oligodeoxynucleotide synthesis,

depurination remains a major cause of concern.

Figure 5. Deprotection and depurination reactions during oligonucleotide synthesis

2.0 LITERATURE REVIEW

During the detritylation step for the synthesis of oligodeoxynucleotides using conventional phosphoramidite chemistry, the DMT group is removed by acid hydrolysis. This acid also causes the destabilization of N-glycosidic bond that results in depurination, thereby reducing the yield. For the automated synthesis of oligodeoxynucleotides, several effective methods have been developed, but the problem of minimizing depurination and enhancing deprotection is still receiving much attention from researchers. For the synthesis of the longest possible chains, rapid and complete removal of the 5'-protection with a minimum amount of side reaction must be carried out.

In order to reduce depurination, scientists have searched for various conditions to remove the dimethoxytrityl group selectively, developed various 5'-protecting groups that can be removed more selectively than the DMT group, and explored new base-protecting groups.

DMT is the most widely used 5'-protecting group since it can be removed rapidly under mild acidic conditions. An unsubstituted trityl group requires severe treatment for its removal that results in cleavage of N-glycosidic bond. A mono-substituted trityl group is also unsuitable for oligonucleotide synthesis using phosphoramidite chemistry, because deblockage of the 5'-protected group occurs with the addition of each base and the repeated acid exposure results in increased depurination. Therefore, scientists focused on developing new base-protecting groups and new detritylating reagents that can stabilize the glycosidic bond and therefore can reduce the rate of depurination.

2.1 Effect of Base Protecting Groups

Kume and his coworkers introduced the phthaloyl group into the amino group of deoxyadenosine and studied the depurination rate in solution phase. They observed that the addition of phthaloyl group resulted in considerable retardation on the rate of depurination. The half-life of depurination was 2 hours for phthaloyl-protected deoxyadenosine in comparison to 30 minutes in the case of benzoyl-protected base. Kume used 80% (v/v) acetic acid for detritylation [11].

Froehler and Matteucci used dialkylformidine as a depurination resistant protecting group for deoxyadenosine on a polymer support and 2% (v/v) dichloroacetic acid (DCA) in methylene chloride as the detritylating reagent. Experimental results indicated that the depurination rates were suppressed by 20-fold with formidines when compared to benzoyl as the protecting group. These groups were readily prepared, easily removed and were stable to reaction conditions. Scientists indicated that no depurination of formidine-protected deoxyadenosine was observed after 4 hours; on the other hand benzoyldeoxyadenosine was 75% depurinated after 4 hours [12].

McBride and coworkers investigated the effect of amidine-protecting group on depurination in solution phase. It was concluded that deoxyadenosine when protected with amidine was 20-fold more resistant to depurination than 6-benzoyldeoxyadenosine under the acidic conditions used for synthesizing DNA. With amidine-protecting group, no more than 5% depurination with trichloroacetic acid (TCA) was observed after 36 hours [13].

Mishra and his coworkers carried out depurination studies on monomer, dimer, trimer and tetramer DNA under the synthesis conditions in solution phase using both 3-methoxy-4-phenoxybenzoyl and phenoxyacetyl as the amino protecting groups and concluded that these groups impart stability to acid treatments. These groups have the advantage of reducing the protonation at N⁷ (the position of N in adenine that connects the base with the sugar group) by preventing the free rotation of glycosidic bond and inhibiting the approach of H₃O⁺ at N⁷ due to their hydrophobic nature. Scientists concluded that the half life of depurination with these groups was almost double than in the case of benzoyl as protecting group both with 10% (w/v) TCA in dichloromethane and with 80% acetic acid as detritylating reagents [14,15].

Nagaich and Misra used a α -phenyl cinnamoyl group for selective amine protection and observed that the half-life for depurination of deoxyadenosine in solution phase with α -phenyl cinnamoyl group was 14.5 hour in comparison to 4.5 hours in the case of benzoyl as the base-protecting group [16].

2.2 Effect of Detritylating Reagents

Some researchers studied a different method to reduce depurination. They used non-protic acids instead of protic acids. Waldmeier et al. used anhydrous zinc bromide in dichloromethane/2-propanol to remove the trityl protecting group from the nucleotide attached on a polymer support. It was observed that the deprotection was completed within 5 minutes with a significantly reduced rate of depurination. However, with prolonged treatment of this reagent, methanol formed a chelating intermediate that

reduced the yield to 30%. A saturated solution and a sufficient excess of reagents were used in order to avoid back reaction during neutralization. The back reaction was not well understood, and was postulated to be the main cause of unsatisfactory results obtained with zinc bromide [17].

Takaku and coworkers carried out the experiments with various acidic reagents in solid phase. They observed that TCA (3% w/v) in a (95:5) mixture of nitromethane and methanol was an effective acidic reagent for the detritylation step. Detritylation was completed within 5 minutes during which no depurination was observed. It was concluded that depurination rates with zinc bromide and 3% TCA were comparable, but detritylation rates were much slower in the case of zinc bromide [18].

Habus and Agrawal synthesized long oligonucleotides on a solid support that required a prolonged detritylation step. However, the prolonged exposure of an oligonucleotide to acid leads to an increased depurination rate. Hence, these scientists used a 2% (v/v) solution of dichloroacetic acid in methylene chloride in the presence of 0.1% anhydrous alcohol (methanol or ethanol) and concluded that this reagent was a highly effective non-depurinating detritylating agent for oligodeoxynucleotide synthesis on a solid support [19].

Patel et al. examined a number of commonly used detritylation conditions to obtain a reagent giving a minimum amount of depurination. They studied the effect of TCA (10% w/v) in chloroform, TCA (10% w/v) in dichloromethane, benzene sulphonic acid (BSA) (2% w/v) in dichloromethane/dimethylformamide (9:1 v/v), BSA (2% w/v) in dichloromethane/methanol (7:3 v/v), BSA (3% w/v) in dichloromethane/dimethylformamide (9:1

v/v). Patel and his coworkers concluded that a solution of BSA (3%) in a dimethylformamide and dichloromethane was an extremely effective reagent for the detritylation of deoxynucleotides attached to a solid support. The levels of depurination (2% in 5 minutes) with this reagent was lower than those observed with other reagents (10% in 5 minutes) [20].

Septak performed kinetic studies of depurination and detritylation of control pore glass (CPG) bound intermediates during oligonucleotide synthesis using different protic acids. It was concluded that detritylation was faster with 3% (w/v) TCA and 15% (v/v) DCA, but it resulted in an increased rate of depurination. In order to compensate for increased depurination with these acids, detritylation must be at least 3-fold faster than was obtained in the experiment. The depurination rate was reduced with 3% DCA, but in this case the deprotection rate was very slow. Septak reported that the use of 15% DCA resulted in 3-fold faster depurination while use of the stronger acid (TCA 3%) exhibited 4-fold faster depurination. He also concluded that the presence of a free or ion-paired DMT cation does not contribute to the depurination rate. Septak found that the half-life of depurination for 3% DCA was 1.29 hour, for 15% DCA was 0.42 hour and for TCA was 0.32 hour, which showed that 15% DCA was the most suitable detritylating reagent for minimizing the depurination rate and maximizing the deprotection rate [21].

Butkus and his coworkers studied and compared the effect of many haloacetic acids on the rate of detritylation and the rate of depurination both in solution and solid phase. They observed that in the case of protic acids, as their dissociation constant increased, the rate of reaction also increased. In addition, as the acid concentration increased, the rate of

reaction increased. It was also observed that the rate of detritylation and its selectivity depended on the nature of the solvent used. Butkus and his group used chlorinated hydrocarbons and acetonitrile as solvents in their study. They postulated that the use of dichloromethane as a solvent increased the rate of detritylation to a greater extent in comparison to acetonitrile. These researchers also indicated that the rate of detritylation is faster in the solution phase than in solid phase because in the solid phase additional time is required for the passage of reaction solutions through layers of particles and the penetration of molecules of the reagents within these particles [22].

Paul and Royappa indicated that during oligonucleotide synthesis the acid used in the detritylation step binds strongly to the oligonucleotide resulting in decreased concentration of the free acid required for detritylation. These scientists also concluded that the acid binds to the acetonitrile that is used for the washing step. Paul and his coworkers performed the experiments using DNA synthesized on CPG and DCA and TCA with a mixture of dichloromethane and acetonitrile and determined the binding capacity of acids. They also concluded that DCA was preferred to TCA as a deblock acid since DCA resulted in reduced depurination rate [23].

2.3 Evaluation of Previous Studies

The references cited above provide valuable information on rates of detritylation and depurination reactions, but since researchers used different reagents under dissimilar conditions, it is difficult to compare their results. In addition, for the solid phase synthesis of nucleotides, the operation of the reaction apparatus is not mentioned in research

papers. The reaction apparatus can affect the flow characteristics of the outlet species and hence the rates of measured reactions. In most of the investigations published so far scientists have indicated the acid strength in terms of either weight/volume percent or volume/volume percent instead of molarity, therefore it is difficult to compare the results since the weight percent can result in different acid strength under various conditions. Furthermore, researchers have calculated rates of detritylation from complete conversion, which is the disappearance of initial material in solution phase and vanishing of trityl color in solid phase. Any conversion reaching 99% or more can be considered as complete conversion. Scientists may have considered different percentage conversion to determine the complete conversion; hence comparing the time of complete conversion from many research papers may not be accurate. Therefore, further studies are required to investigate the effects of various detritylating reagents on the rates of deprotection and depurination.

3.0 RESEARCH HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis:

The concentration and dissociation constant of an acid are two key parameters affecting the rates of deprotection and depurination. Acids in higher concentration increase both the rate of deprotection and the rate of depurination but to different extents. Acids having higher dissociation constants also increase the rate of these reactions. The solvent also affects the acidity of an acid. More polar solvents increase the acidity of an acid and hence the rate of deprotection and depurination. There will be a condition in which the rate of deprotection will increase to a greater extent than the rate of depurination and at that condition the yield will be maximized for a limited period.

3.2 Objectives:

The purpose of this research is to study and compare the effect of various detritylating reagents on the rates of deprotection and depurination of benzoyl protected deoxyadenosine in the solution phase (homogenous phase) and on control pore glass (CPG) immobilized solid phase (heterogeneous phase).

4.0 MATERIALS AND EXPERIMENTAL PROCEDURE

4.1 Materials

Materials used are given in Appendix-A

4.2 Analytical Method

The study of the rate of deprotection and the rate of depurination was done using benzoyl-5'-O-4,4'-dimethoxytrityl-2'-deoxyadenosine (BzDMTdA) bound to control pore glass (CPG) in the solid phase experiments and free in solution phase experiments, as the starting materials. These starting materials on acidolysis were deprotected at the 5'-end and the products were dimethoxytrityl hydroxide (DMTOH), other DMT containing species (DMT*), benzoyl deoxyadenosine (BzdA, formed only in solution phase), and some amount of benzoyl adenine (BzA).

Solution Phase

Solid Phase

4.2.1 Analytical Equipment

In order to determine the rates of deprotection and depurination, the concentration of each species was determined using an Agilent 1100 series High Pressure Liquid Chromatography (HPLC) (refer to Appendix B for specifications). HPLC has the advantage of efficient component separation by using tightly packed columns and increased flow rate of the solvent. The solvent is forced through the column by a pump. A C-18 column was used for separation in which an 18-carbon alkane chain (octadecyl – $C_{18}H_{37}$) was attached to silica that made the stationary phase less polar. The temperature of the column was maintained at 25°C. The HPLC used a mixture of acetonitrile (solvent B) and 7.5mM phosphate buffer (solvent A, pH 3) at 0.5 ml/min for a gradient elution system. The composition and hence the polarity of the solvent changed from the beginning to the end of the chromatography. The composition of solvent B in solvent A changed from 10-100% in 14 minutes, remained at 100% for 3 minutes, changed to 10% in 3 minutes and remained at 10% for 5 minutes. The HPLC used a diode array detector to detect the absorption of ultraviolet or visible light by the solute present in the solvent. In this study 6 μ l was used as injection volume.

4.2.2 Determination of Retention Time of Various Species

The HPLC gave peaks of the different reaction products at their characteristic retention times. In order to determine the identity and retention times of the components used in this study, a 22 mM solution of each component (BzDMTdA, BzdA, BzA DMTOH and DMTCl) was prepared separately and analyzed by HPLC. Methanol was

used as the solvent for the case BzdA and BzA instead of dichloromethane, which was used for BzDMTdA, DMTOH, DMTCl, since these components do not dissolve in dichloromethane.

4.2.3 Sensitivity of HPLC

HPLC should be sensitive enough to detect as low as 1% depurination. Hence, a solution containing 1% of normal reaction concentration, 0.158 μM BzA in 2 ml methanol, was prepared and analyzed by HPLC. The solution was diluted further to the extent where HPLC failed to detect the BzA and that strength was considered as the sensitivity of HPLC. The sensitivity of the HPLC was determined to be 0.000111 μM BzA in the reaction mixture.

4.2.4 Determination of Response Factor (RF) of Various Species

In order to calculate the concentration during the reaction, the response factor for each component was determined. To facilitate that, solutions of different concentrations of each component (BzDMTdA, BzdA, BzA, DMTOH, DMTCl) were prepared separately. Methanol was used as the solvent for BzdA and BzA (since BzdA and BzA do not dissolve in dichloromethane) instead of the dichloromethane that was used as the solvent for BzDMTdA, DMTOH and DMTCl. A solution of 1,2-dimethoxybenzene (veratrole, which acts as an internal standard in the reaction mixture) was prepared in 5 ml of dichloromethane. An equal amount of this veratrole-containing solution was added to each sample. Each solution was then analyzed by HPLC to calculate its response factor. Plots of the response ratio (ratio of response of each component to the response of

veratrole) and the amount ratio (ratio of the concentration of each component to the concentration of veratrole) were then constructed and used as the calibration plots. These calibration plots are shown in Appendix C.

4.3 Solution Phase Experiments

4.3.1 Experimental Method

Solutions of the acids containing the concentration required to give the desired concentration in the reaction flask, as given in Table 3, were prepared in 3 ml of respective solvent. The starting material was a solution containing BzDMTdA, which was prepared separately in 3 ml of the same solvent. For each sample containing BzDMTdA, veratrole was added as an internal standard. A solution of 1.16 M triethylammonium acetate (TEAOAc) in methanol was also prepared to quench the reaction at the desired time.

Table 3. Experimental Matrix for Solution Phase

Acid	Concentration	Solvent
MCA (monochloro acetic acid)	0.2M, 0.3M, 1M, 2M, 3M	DCM (dichloromethane)
DCA (dichloro acetic acid)	0.05M, 0.1M, 0.2M	DCM
	0.1M, 0.2M	MIB(methyl isobutyrate)
TCA (trichloro acetic acid)	0.05M, 0.1M, 0.2M	DCM
	0.1M, 0.2M	MIB
MSA (methane sulfonic acid)	0.02M, 0.2M	DCM
	0.01M, 0.02M, 0.1M, 0.2M	MIB

A 0.1 ml aliquot of the original Bz-DMT-dA solution was reserved in a vial before adding an acid. This solution was analyzed as the zero reading, since it contained no acid. As shown in Figure 6, the remaining 2.9 ml of the reaction mixture was added to 2.9 ml of acid solution, thus acquiring the desired acid concentration. Then 0.1 ml aliquots of the mixed solution were removed to a 1 ml vial and quenched with 0.1 ml of TEAOAc at set intervals of 10 seconds, 30 seconds, 1 minute, 3 minutes, 5 minutes, 10 minute and 20 minutes as shown in Figure 6. Those solutions were then analyzed by the Agilent 1100 series HPLC.

Figure 6. Schematic diagram for solution phase experiment

4.3.2 Preparation of Buffer (Triethylammonium Acetate in Methanol)

Seven grams of TEA was placed in a bottle. Four ml of glacial acetic acid and 20 ml of methanol were added into the bottle. The pH of the solution was tested using pH strips. Depending upon acidity or basicity, TEA or acetic acid was added until reaching pH 7. Finally, methanol was added to dilute the solution to a final volume of 30 ml.

4.4 Solid Phase Experiments

As shown in Table 4, solutions of the acids to be studied at the desired concentrations were prepared in 10 ml of the respective solvents. Additionally, veratrole was added as an internal standard.

Table 4. Experimental Matrix for Solid Phase

Acid	Concentration	Solvent
DCA	0.1M, 0.2M, 0.3M	DCM
TCA	0.05M, 0.1M, 0.2M	DCM
MSA	0.05M, 0.1M, 0.2M	MIB
	0.05M	DCM

The solution was transferred to a 10 ml syringe. A Cole-Parmer syringe pump was used to hold the syringe containing the prepared acid solution. A column containing 1 μ mole 3'-CPG bound BzDMTdA was attached to the syringe (shown in Figure 7). The acid was then injected into the column at a rate of 200 ml/hr using the syringe pump. The fraction coming out of the column was collected continuously (without discarding any solution) in individual 1 ml vials at set intervals of 15 seconds, 30 seconds, 45 seconds, 1

minute, 1.25 minutes, 1.5 minutes, 1.75 minutes and 2 minutes. Those samples in vials were then injected to the HPLC for analysis. In order to completely remove the acid from the system before the system was used for another set of experiment, the reaction apparatus was washed thoroughly with acetonitrile, since acetonitrile binds and makes complexes with acids.

Figure 7. Schematic diagram for solid phase experiment

4.5 Error Analysis

In order to check for repeatability error, three experiments (a, b, c) were run consecutively with same solutions of acid (0.05M TCA in DCM for solution phase and

0.2M DCA in DCM for solid phase) prepared once. Concentration of the internal standard (veratrole) was compared at each time among these three experiments and the error was calculated using standard deviation for these veratrole concentrations.

For reproducibility error, another experiment (d) was run with fresh solution having the same acid condition and the concentration of veratrole was compared between the experiments b and d. The error was calculated by taking the average of the veratrole concentrations.

5.0 RESULTS AND DISCUSSION

Detritylation is the first step for oligonucleotide synthesis. In this step, acid removes the DMT (5'-protecting group) from the protected nucleotide (BzDMTdA) and forms the DMT cation (DMT^{cat}) which is a highly reactive species. It is very electrophilic; hence it can react with hydroxyl containing compounds (ROH or H_2O) present in the solution to form DMTOH or DMTOH. It can also react with nucleotides present in the medium and with the anion (A^-) of the acid to form other DMT containing species. Unreacted DMT^{cat} can also react with water and methanol in the HPLC solvent system to form DMTOH and DMTOCH_3 , respectively. Reaction of DMT^{cat} depends on the strength of the acid used, the properties of the solvent, and the concentration of water and alcohol present.

5.1 Determination of Retention Time

The HPLC retention times of the starting materials, the expected products and the DMT^{cat} products were identified using 1100 series HPLC. Table 5 shows the retention time for each component.

Table 5. Retention Time of Various Components

Compounds	Retention Time (minute)
Bz-DMT-dA	12.2
Bz-dA	5.4
Bz-A	6.1
DMTOH	12.8
DMT*	15.2
Veratrole	8.4

The DMT* compound observed at retention time 15.2 is probably a DMTOCH₃ species, which is the product of the DMT^{cat} and the buffered HPLC solvent system. The response factor of this component was determined by the analysis of DMTCl.

5.2 Determination of Response Factor

The response factor for each component was determined by analyzing a series of solutions with varying concentrations of each component with a constant internal standard (veratrole) concentration. The response factor was determined from the slope of the plot of response ratio vs. amount ratio defined as:

$$\text{Response ratio} = \frac{\text{Response of a component}}{\text{Response of veratrole}}$$

$$\text{Amount ratio} = \frac{\text{Concentration of a component}}{\text{Concentration of veratrole}}$$

The response of any component was taken as the percent area under the peak of that particular component. Plots for determination of response factors of these components are shown in Appendix C. Table 6 provides the value of the response factor of these components.

Table 6. Response Factor of Various Components

Compounds	Response Factor
Bz-DMT-dA	5.3
Bz-dA	2.3
Bz-A	1.9
DMTOH	3.0
DMT*	3.3

Using the response factor (RF), the concentration of each component was calculated from the HPLC profile using the percent area under the peak for each species.

$$\text{Concentration of a specific component} = \frac{(\text{Percent peak area of a component})}{(\text{Percent peak area of veratrole} * \text{RF})} * \text{Concentration of Veratrole}$$

5.3 Solution Phase

BzDMTdA, DMTOH, DMT*, BzdA and BzA are various components in the reaction mixture for solution phase experiments. For the different reaction conditions, concentration profiles of these components with respect to time were generated. It was observed that the concentration profiles of all the involved compounds followed the same trend under different reaction conditions. For example, as shown in Figure 8 for the case of the reaction with 0.1M TCA in MIB, as soon as the acid is added, deprotection begins, the concentration of BzDMTdA decreases with time whereas the concentration of BzdA increases. Simultaneously, depurination begins so the concentration of BzdA decreases and the concentration of BzA increases. The concentrations of DMTOH and DMT* increase with time and reach nearly a constant value.

Figure 8. Concentration profile of various species

5.3.1 Kinetics for Rate Constants and the Order of Reaction

In the solution phase, the following reaction takes place in the mixture

The above reaction, which is a series type of reaction, is homogenous (refer to Figure 8). The first reaction is the deprotection reaction and the second is the depurination reaction. The reaction is considered to take place in an isothermal, constant volume batch reactor. Using the kinetic data for the batch reactor, rate constants and the order of the reaction were calculated.

The rate expression for the first reaction in a constant volume batch reactor is

$$R_1 = -dC_a/dt = k_1 C_a^n \quad \text{Equation 1}$$

where R_1 = rate of disappearance of BzDMTdA

C_a = concentration of BzDMTdA at a particular time

k_1 = rate constant for first reaction

t = time

n = order of reaction

The rate expression for the second reaction is

$$R_2 = -dC_a/dt = k_2 C_a^n \quad \text{Equation 2}$$

where R_2 = rate of disappearance of BzdA after BzDMTdA is finished

C_a = concentration of BzdA at a particular time after BzDMTdA is finished

k_2 = rate constant for second reaction

The order and the rate constants of the reaction were determined from solution phase experiments, since in the solid phase experiments the starting materials (BzDMTdA for the first reaction and BzdA for the second reaction) were attached to the CPG and the concentration of these components could not be determined. Furthermore, in solution phase experiments, these constants were determined only from the reaction with weak acid in methyl isobutyrate (MIB), since reaction with strong acids in DCM/MIB or weak acids in DCM resulted in very fast reactions such that it was difficult to measure the accurate rate of reaction.

In order to calculate the rate constants and the order of the reaction, an experiment with 0.2M TCA in MIB was conducted. The experimental rate was calculated by the ratio of the concentration difference to the time difference, and the concentration was taken as the average of the two concentrations in the particular range of time. Data used in calculating the rate constants and the order of the reaction was analyzed using non-linear regression in Polymath software (version 5.1). The values of k_1 and k_2 were determined to be 3.0 and 0.007 respectively (refer to Appendix D for calculations). The rate constant values show that the first reaction is faster than the second reaction. Furthermore, the order of reaction for the two reactions was determined to be 1.1 and 0.9 respectively, which is nearly equal to one. For a first order reaction

$$(t_{1/2})_1 / (t_{1/2})_2 = k_2/k_1 \quad \text{Equation 3}$$

where $t_{1/2}$ is the half-life of a reaction (time required to reach 50% conversion), and

1 indicates the first reaction and 2 indicates the second reaction.

For the reaction with 0.2 M TCA in MIB

$$(t_{1/2})_1 / (t_{1/2})_2 = 0.4 \text{ (refer to Appendix E) and } k_2/k_1 = 0.002$$

The ratio of half-lives and the ratio of rate constants are not equal which shows that the reaction is not a first order reaction. In addition, the R-square value for the fit to the first reaction is very low, which shows that the model does not describe the data properly (in order to get the acceptable fits, models must show R-square values of 0.9 or greater). Hence, it cannot be confirmed that the reaction is a first order reaction.

5.3.2 Determination of %Deprotection and %Depurination

Once the concentration of each species was known, the rates of deprotection and depurination were calculated by the fraction of DMT containing products and the fraction of BzA respectively.

$$\% \text{ Deprotection} = \frac{\text{Concentration of product DMT}}{\text{Total concentration of DMT}} * 100$$

$$\% \text{ Depurination} = \frac{\text{Concentration of BzA}}{\text{Total concentration of adenine(A)}} * 100$$

where concentration of product DMT is the sum of the concentration of DMTOH and the concentration of DMT*.

The total concentration of DMT was calculated by adding the concentrations of all the DMT containing species (BzDMTdA, DMTOH, DMT*) at a particular time. Similarly, the total concentration of adenine was calculated by adding the concentration of all the

adenine containing species (BzDMTdA, BzdA, BzA) at a particular time (for example, refer to Appendix E for reaction with 0.1M TCA in DCM).

The rate of deprotection and the rate of depurination for solution phase experiments with different reaction conditions are given in Table 7 and Table 8, respectively. In this study complete conversion was defined as 99% deprotection.

Table 7. Percent Deprotection in Solution Phase

Time	0.05MDCA	0.1M DCA	0.2M DCA	0.1M DCA	0.2M DCA	0.05MTCA	0.1M TCA	0.2M TCA
(min)	in DCM	in DCM	in DCM	in MIB	in MIB	in DCM	in DCM	in DCM
0	0	0	0	0	0	0	0	0
0.167	81	96	99	3.8	4.5	100	100	100
0.5	96	100	100	3.9	4.9	100	100	100
1	99	100	100	4.4	6.5	100	100	100
3	99	100	100	5.6	11.0	100	100	98
5	100	100	100	6.5	14	100	100	100
10	100	100	100	9.0	22	100	100	100
20	100	100	100	14	34	100	100	100
Time	0.1M TCA	0.2M TCA	0.2M MCA	0.3M MCA	1M MCA	2M MCA	3M MCA	
(min)	in MIB	in MIB	in DCM	in DCM	in DCM	in DCM	in DCM	
0	0	0	0	0	0	0	0	
0.167	22	25	15	17	24	70	81	
0.5	39	41	25	32	50	97	99	
1	44	46	41	52	82	100	100	
3	46	58	78	89	99	100	100	
5	56	73	90	98	100	100	100	
10	71	91	99	100	100	100	100	
20	90	98	100	100	100	100	100	
Time	0.01M MSA	0.02M MSA	0.02MMSA	0.1M MSA	0.2M MSA	0.2M MSA		
(min)	in MIB	in MIB	in DCM	in MIB	in MIB	in DCM		
0	0	0	0	0	0	0		
0.167	89	90	100	100	100	100		
0.5	100	100	100	100	100	100		
1	100	100	100	100	100	100		
3	100	100	100	100	100	100		
5	100	100	100	100	100	100		
10	100	100	100	100	100	100		
20	100	100	100	100	100	100		

Table 8. Percent Depurination in Solution Phase

Time(min)	0.05M DCA	0.1M DCA	0.2M DCA	0.1M DCA	0.2M DCA	0.05MTCA	0.1M TCA	0.2M TCA
	in DCM	in DCM	in DCM	in MIB	in MIB	in DCM	in DCM	in DCM
0	0	0	0	0	0	0	0	0
0.167	2.4	5.1	4.0	0.8	1.0	7.7	14	19
0.5	4.0	7.3	8.1	1.0	1.1	18	30	38
1	7.2	12	15	1.2	1.4	31	48	59
3	17	22	31	1.4	2.0	63	71	89
5	27	45	54	1.8	3.0	79	84	98
10	45	63	78	1.8	4.7	96	95	99
20	68	86	92	2.7	8.9	99	98	99
Time(min)	0.1M TCA	0.2M TCA	0.2M MCA	0.3M MCA	1M MCA	2M MCA	3M MCA	
	in MIB	in MIB	in DCM	in DCM	in DCM	in DCM	in DCM	
0	0	0	0	0	0	0	0	0
0.167	0.18	1.0	0.9	1.2	2.0	2.8	5.3	
0.5	0.52	1.4	1.0	1.6	3.7	6.1	11	
1	1.9	2.5	2.2	2.6	7.0	10	19	
3	3.9	7	5.8	6.9	13	24	43	
5	6.3	11	11	13	19	37	50	
10	11	21	15	15	51	58	68	
20	25	35	24	29	58	77	86	
Time(min)	0.01M MSA	0.02M MSA	0.02M MSA	0.1M MSA	0.2M MSA	0.2M MSA		
	in MIB	in MIB	in DCM	in MIB	in MIB	in DCM		
0	0	0	0	0	0	0	0	
0.167	14	20	48	93	95	97		
0.5	55	69	80	97	97	98		
1	82	92	95	98	99	99		
3	98	98	99	99	99	100		
5	98	99	99	99	100	100		
10	99	99	100	100	100	100		
20	99	99	100	100	100	100		

5.3.3 Effect of Detritylating Conditions

It is seen from the study that for all the acids, the rate of deprotection is greater in DCM than in MIB. For example, as shown in Figure 9, with 0.1M DCA in DCM (weak acid in more polar solvent) the reaction achieves complete conversion within 30 seconds, while with the same acid in MIB (less polar solvent), only 3.9% deprotection is observed in 30 seconds. A similar solvent effect is also observed for TCA. However, for the case of 0.1M and 0.2M methanesulfonic acid (strong acid), as shown in Figure 10, the rate of reaction is so fast that the effect of solvent is not visible. But with 0.02M

methanesulfonic acid (MSA), the rate of reaction is faster in dichloromethane (DCM) than in methylisobutyrate (MIB).

Figure 9. Effect of solvent on %deprotection in solution phase

Figure 10. Effect of solvent on %depurination in solution phase

Thus, it can be confirmed that the solvent affects the rate of reaction. As shown in Table 2, the dielectric constants for DCM and MIB are 9.08 and 5.6 respectively. The larger polarity of DCM increases the acidity of the acid by dissociating and stabilizing the ions. As less energy is required to dissociate the acid into ions, the extent of dissociation of any acid is larger in DCM. Therefore, the rate of reaction is faster in DCM than in MIB.

Since the rate of deprotection with DCA and TCA in MIB (weak acids in less polar solvent) is very slow, these reaction conditions were not studied further. On the other hand, for the case of 0.1M and 0.2M MSA in DCM (strong acid in more polar solvent), with faster rate of deprotection, the rate of depurination (which is the undesirable reaction) is also very fast, limiting the yield of the desired product. Therefore, 0.1M and 0.2M MSA in DCM were also not studied further.

It is also observed from the study that as the concentration of the acid is increased, the extent of reaction increased. For example, as shown in Figure 11, with 0.1M DCA in MIB as reagent, 6.5% deprotection occurs in 5 minutes and with 0.2M DCA in MIB as reagent deprotection increases to 14%. Similar effects of acid concentration are observed with TCA and MSA.

Figure 11. Effect of concentration on %deprotection in solution phase

Figure 12. Effect of concentration on %depurination in solution phase

The rate of depurination increases as the acid concentration is increased. As shown in Figure 12, the amounts of depurination after 20 minutes are 2.7% and 8.9% for 0.1M DCA in MIB and for 0.2M DCA in MIB, respectively. When the acid dissociates, the resulting hydrogen ion reacts and increases both the rates of deprotection and depurination. Hence, as the acid concentration is reduced, the concentration of dissociated hydrogen ion is reduced, which reduces the rates of deprotection and depurination.

Furthermore, acids with high pK_a values result in lower rates of deprotection in comparison to acids with lower pK_a values. For example, as shown in Figure 13, reaction with 0.1M TCA in MIB achieves 44% conversion within 1 minute, however reaction

with 0.1M DCA in MIB achieves only 4.4% deprotection in the same time interval. Similarly, the rate of depurination also decreases as the pK_a of an acid is increased. As shown in Figure 14, reaction with 0.1M TCA in MIB achieves 25% depurination in 20 minutes, however reaction with 0.1M DCA in MIB achieves only 2.7% depurination. Acids with higher acidity (lower pK_a) dissociate easier, resulting in a higher concentration of H⁺ than those with lower acidity (higher pK_a). Hence, as acidity increases, the rates of deprotection and depurination increase. Among the acids used for the solution phase experiments, both the rates of deprotection and depurination increase in the order of DCA < TCA < MSA.

Figure 13. Effect of pK_a on %deprotection in solution phase

Figure 14. Effect of pK_a on %depurination in solution phase

5.3.4 Yield

In addition to the faster rate of deprotection, it can be seen that for all acid conditions the rate of depurination is also very fast (refer to Table 8); therefore, it is important to determine the yield of the desired product. Yield was calculated from the difference between the amount of deprotection and the amount of depurination (refer to Appendix

G). As shown in Figure 15, the yield increases with time, reaches a maximum and then decreases with time depending upon the concentration and acidity of the acid. Stronger acids or higher concentration of the same acid, cause the yield to decrease immediately after reaching maximum, but with weaker acids or with low concentration, yield decreases very slowly. This is because as the acid concentration or the acidity (due to the solvent) increases, both the rate of deprotection and the rate of depurination become very fast and that results in the lower yield in comparison to that for weak acids or for acids with low concentration.

Figure 15. Comparison of yield in solution phase

The reaction with MCA in DCM (weak acid in more polar solvent) was also studied in solution phase. In the case of 0.3M MCA, although the yield increased and reached near completion, the reaction took longer to achieve complete conversion. Therefore, MCA in DCM was not studied further.

The repeatability and reproducibility errors for all experiments in solution phase were determined to be 3.2 % and 6.6% respectively (refer to Appendix H for calculation).

5.4 Solid Phase

In the solid phase experiments, the starting material (BzDMTdA) is bound to control pore glass (CPG). On deprotection, only the 5'-protecting group products (DMTOH and DMT*) are removed in the eluting solution but benzoyl-deoxyadenosine (BzdA) remains attached to the CPG. Upon depurination, the BzA separates from the CPG and is also eluted with the DMT leaving the deoxyribose. Thus, the calculations were slightly different than in solution phase.

5.4.1 Determination of %Deprotection and %Depurination

The total DMT concentration was determined by adding up the product DMT for total time interval (details shown in Appendix F). Since, in solid phase, BzDMTdA and BzdA were attached to CPG, total adenine concentration could not be calculated independently. Therefore, total adenine concentration was assumed to be equal to the total DMT concentration. The rate of deprotection and the rate of depurination for different reaction conditions in solid phase are given in Table 9 and Table 10, respectively.

Table 9. Percent Deprotection in Solid Phase

Time(min)	0.1M DCA in DCM	0.2M DCA in DCM	0.3MDCA in DCM	0.05MTCA in DCM	0.1M TCA in DCM	0.2M TCA in DCM	0.05M MSA in DCM	0.05M MSA in MIB	0.1MMSA in MIB	0.2MMSA in MIB
0	0	0	0	0	0	0	0	0	0	0
0.25	58	84	97	92	93	95	54	40	75	95
0.5	87	97	100	100	100	100	86	75	98	99
0.75	96	99	100	100	100	100	96	91	100	100
1	98	100	100	100	100	100	99	98	100	100
1.25	99	100	100	100	100	100	100	99	100	100
1.5	100	100	100	100	100	100	100	100	100	100
1.75	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100	100	100

Table 10. Percent Depurination in Solid Phase

Time(min)	0.1M DCA in DCM	0.2M DCA in DCM	0.3M DCA in DCM	0.05MTCA in DCM	0.1M TCA in DCM	0.2M TCA in DCM	0.05M MSA in DCM	0.05M MSA in MIB	0.1MMSA in MIB	0.2MMSA in MIB
0	0	0	0	0	0	0	0	0	0	0
0.25	1.7	1.7	1.9	1.5	1.6	1.9	3.6	0.91	0.77	1.2
0.5	1.9	2.1	2.1	1.7	2.0	2.6	8.4	1.2	1.6	2.8
0.75	1.9	2.2	2.3	1.9	2.4	3.3	9.6	1.6	2.6	4.1
1	2.0	2.4	2.5	2.1	2.8	4.1	10	2.2	3.8	5.1
1.25	2.1	2.5	2.7	2.3	3.2	4.9	11	2.9	4.6	5.9
1.5	2.2	2.6	2.9	2.5	3.6	5.6	11	3.8	5.3	6.6
1.75	2.3	2.8	3.1	2.7	4.0	6.5	11	4.7	6.1	7.1
2	2.3	2.9	3.3	2.9	4.4	7.3	11	5.0	6.7	7.6

5.4.2 Effect of Detritylating Conditions

Similar to the solution phase, it is observed that as the acid concentration is reduced, it takes a longer to deprotect the DMT from the protected nucleotide bound to CPG. For example, as shown in Figure 16 and Table 8, reaction with 0.1M DCA in DCM requires 1.25 minutes for complete conversion. On the other hand, with 0.2M DCA and 0.3M DCA in DCM it requires 0.75 minutes and 0.5 minutes, respectively, for complete conversion. Similar effects of acid concentration can be observed with TCA and MSA. As shown in Figure 17, it is also observed that (similar to solution phase) as the acid concentration is increased, the rate of depurination is also increased (after 2 minutes, depurination is 2.3% for 0.1M DCA and 3% for 0.2M DCA).

Figure 16. Effect of concentration on %deprotection in solid phase

Figure 17. Effect of concentration on %depurination in solid phase

Furthermore, acids with high pK_a values show lower rate of deprotection in comparison to acids with lower pK_a values. For example, as shown in Figure 18, with 0.1M DCA in DCM, the reaction reaches complete conversion in 1.25 minutes, but with 0.1M TCA in DCM complete conversion occurs within 0.35 minutes. Similarly, as shown

in Figure 19, the rate of depurination also decreases as the pK_a of an acid is increased (after 2 minutes, depurination is 2.3% for 0.1M DCA and 4.4% for 0.1M TCA). Therefore, as in solution phase, among the acids used for the experiments, both the rate of deprotection and depurination increase in the order of $DCA < TCA < MSA$.

Figure 18. Effect of pK_a on %deprotection in solid phase

Figure 19. Effect of pK_a on %depurination in solid phase

5.4.3 Yield

Upon yield analysis for DCA reagent in the solid phase in Figure 20, it can be observed that the reaction with 0.1M DCA requires almost 1 minute to reach 96% yield, 0.2M DCA requires 30 seconds to reach 95% yield and 0.3M DCA requires 15 seconds to reach 95% yield. Thus, 0.3M DCA can be considered the most suitable reagent because it gives the fastest rate and the highest yield for a long period of time. But, in the case of oligonucleotide synthesis if the reaction is not stopped within 1 minute the yield decreases more sharply for 0.3M DCA than for 0.1M and 0.2M DCA. In this case, 0.1M DCA should be considered as the most suitable detritylating condition.

Figure 20: Percent yield with DCA in DCM in solid phase

Figure 21: Percent yield with MSA in MIB in solid phase

Likewise, as shown in Figure 21 for the case of MSA in MIB, the reaction requires 15 seconds and 30 seconds to achieve 95% yield for 0.2M and 0.1M acids, respectively. However, in both cases the yield decreases immediately after reaching the maximum. On the other hand, for 0.05M acid although the yield decreases slowly with time after reaching maximum, it takes almost 1 minute to achieve 95% yield (refer to Appendix G). If the reaction can be stopped within 1 minute, then 0.2M MSA should be considered the best detritylating reagent among the studied MSA concentrations. However, depending upon the process control (how fast the reaction is stopped after deprotection), lower acid concentrations may be preferred.

On comparing the percent yield among different acids, it is observed that 0.05M TCA and 0.3M DCA in DCM and 0.2M MSA in MIB give almost the same yield over time. For the case of 0.2M MSA in MIB, yield decreases more sharply after 45 seconds in

comparison to 0.05M TCA and 0.3M DCA. Therefore, for a single nucleotide synthesis, 0.05M TCA and 0.3M DCA can be considered the most suitable detritylating reagents.

The repeatability and reproducibility errors for all experiments in solid phase were determined to be 1% and 2%, respectively (refer to Appendix H).

5.5 Comparison of Solution Phase and Solid Phase Experiments

On comparing the results of solid phase experiments with solution phase experiments, it is observed that the rates of deprotection and depurination are faster in solution phase. From Figure 22, it can be seen that with 0.2M DCA in DCM in solution phase, the reaction achieves complete conversion within 10 seconds, but in solid phase the reaction achieves complete conversion in 45 seconds.

Likewise, with the same acid condition percent depurination in 30 seconds is 8.1% in solution phase but 2.1% in solid phase. This is due to the fact that, in the solution phase, the reaction begins immediately throughout the whole volume after the reagents have been mixed, and the reaction can be stopped instantly by quenching with TEAOAc. On the contrary, in the solid phase, the reaction time may be increased due to lower reactivity of the molecules fixed to CPG, the additional time required for the passage of reaction solutions through the particles, and the penetration of molecules of the reagents into these particles. Hence, diffusion may be the limiting factor for these fast reactions since the nucleotides within the pores are subjected to shorter reaction times and to lowered concentration of acid compared to the nucleotides at the surface.

Figure 22. Comparison of %deprotection in solution and solid phase

Figure 23. Comparison of yield in solution and solid phase

Comparing the yield of the reaction with the same acid (0.2M DCA in DCM) in solution phase and in solid phase, as shown in Figure 23, it is observed that yield increases and reaches a maximum in solid phase but decreases very slowly in comparison to solution phase experiments. This is due to the slow diffusion of molecules in the layers of CPG that results in lower reactivity of the molecules. Hence, the lower rates of deprotection and depurination result in higher yield in comparison to that for solution phase.

6.0 CONCLUSIONS

In this research work, the effect of various reaction conditions on the rate of deprotection and the rate of depurination of the protected deoxyadenosine was studied and compared in solution, the homogenous phase, and on CPG, the heterogeneous phase. Complete conversion was considered the point where the reaction achieved 99% deprotection. Reversed phase HPLC was used as an analytical tool to detect the amount of various components in the reaction mixture. The experimental kinetic data for the determination of the rate constant and the order of reaction did not fit the first order model accurately. Hence, the reaction was not confirmed as a pseudo first order reaction. In order to confirm the order of reaction, further study with more runs is required.

From this study, it was concluded that both the rate of deprotection and the rate of depurination increased as the acid strength and/or its dissociation constant were increased. It was also concluded that the rate of reaction increased with the polarity of the solvent. DCM was the suitable solvent for MCA, DCA and TCA. On the other hand, MIB was more suitable compared to DCM for MSA where the rate of depurination was very fast; hence the yield was very low.

Among the acids investigated, the rate of deprotection was minimum with MCA and maximum with MSA. Although efficient detritylation offered an advantage, the undesired depurination reaction reduced the overall yield; hence, the yield was determined for all reaction conditions to specify the selectivity of any reagent with minimum depurination. It was found that in the solution phase, with concentrated or stronger acids, depurination started immediately; hence, the yield started decreasing immediately after reaching a

maximum. However, with weaker acids such as MCA, yield increased slowly, remained at saturation for some time and then started decreasing slowly.

On CPG (heterogenous phase), the rate of reaction was slower in comparison to that in solution phase due to the additional time required for the penetration of reaction mixture between the particles. Therefore, in all conditions the yield reached the maximum slower than in solution phase and remained at saturation for a long time and then decreased slowly. Among the acids investigated in heterogeneous phase, 0.05M TCA, 0.3M DCA in DCM and 0.2M MSA in MIB gave the best yield of nearly 98% for the reaction with mononucleotide and remained at saturation over a range of time (for 1 minute). However, depending upon the level of process control (how fast the reaction can be stopped after deprotection) other acid reagents can be considered more advantageous than these.

These results improve our ability to properly balance the contradictory goals of obtaining maximum detritylation with minimum depurination in oligonucleotide synthesis. However, this study was based on the reaction mechanism with a mononucleotide. In order to understand the implication of detritylating conditions for oligonucleotide synthesis, further studies are required for fully protected CPG-immobilized dimer, trimer and oligomers. Furthermore, this study provides a basis for the use of photogenerated acids in solution and in solid polymer thin films to spatially direct the detritylation of substrate bound DMT-protected nucleotide (the PPA process) for DNA Chip Synthesis.

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Appendix A. List of Reagents and Equipments

LIST OF REAGENTS

Reagent	Purity	Supplier
BzDMTdA	99%	Aldrich Chemical Company Inc.
TCA	99%	Aldrich Chemical Company Inc.
DCA	97%	Aldrich Chemical Company Inc.
MCA	99%	Aldrich Chemical Company Inc.
MSA	98%	Aldrich Chemical Company Inc.
DCM	99.8%	JT Baker
MIB	99%	Aldrich Chemical Company Inc.
Veratrole	99%	Aldrich Chemical Company Inc.
BzdA	98%	Aldrich Chemical Company Inc.
DMTCI	95%	Aldrich Chemical Company Inc.
TEA	99.5%	EM Science
Acetonitrile	99.98%	EM Science
Methanol	99.9%	JT Baker
Glacial acetic acid	100%	JT Baker
Tetrahydrofuran	99.9%	Aldrich Chemical Company Inc.
Bezoyl Chloride	99.5%	Aldrich Chemical Company Inc.
Methylpyrrolidionne(NMP)	99.5%	Aldrich Chemical Company Inc.

BzA

The benzamide derivative of adenine, N-benzoyladenine is prepared by reaction of adenine with benzoyl chloride using N-methylpyrrolidinone (NMP) as a solvent and triethylamine as an acid scavenger and purified by crystallization from methanol/water.

DMTOH

4,4'- Dimethoxytrityl chloride (3.38g, 10mmol) in tetrahydrofuran and 0.1M aqueous sodium hydroxide (1:1, v/v; 40 ml) was stirred at room temperature for 30 min. The mixture was extracted into 80 ml of DCM dried Na₂SO₄. The concentrated extract was flash chromatographed on silica gel using methanol-dichloromethane (0:10 to 1:9) as the eluent to yield a clear oil that crystallized and was recrystallized from ether-light petroleum to give 4,4'- DMTOH as a white solid. (Ref : C. Bleasdale, S.B.Ellwood and B.T. Golding“ *4,4'-Dimethoxytrityl and 4-Monomethoxytrityl Tetrafluoro-borate: Convenient Reagents for the Protection of Primary Alcohols Including Sugars*, “J. Chem. Soc. Perkin Trans. 1, pp 803-804 (1990))

LIST OF EQUIPMENTS

1. Agilent 1100 series HPLC
2. Cole-Parmer Syringe Pump

Appendix B. HPLC Specifications

HPLC Specifications

Control flow	:	0.5 ml/min
Stop Time	:	25.00 min
Post Time	:	off
Solvent A	:	90.0 % (phosphate buffer)
Solvent B	:	10.0 % (acetonitrile)
Minimum Pressure	:	0 bar
Maximum Pressure	:	400 bar
Injection Volume	:	6.0 µl
Draw and Eject Speed	:	200 µl/min
Temperature	:	25 °C
Signal	:	254 and 230 nm
Band Width	:	100 nm
Store spectrum	:	Every 2 nd spectrum (range from 190 nm to 600 nm with a step of 2 nm and threshold of 1.00 mAU)
Lamps	:	UV/Vis

Appendix C. Response Factors

Plot of Response factors (RF)

Calculation for RF of DMT*

$$[\text{DMTCI}] = [\text{DMTOH}] + [\text{DMT}^*]$$

$$[\text{DMTOH}] = \frac{R_{\text{DMTOH}}}{R_{\text{veratrole}} * (\text{RF}_{\text{DMTOH}})} * [\text{veratrole}]$$

$$[\text{DMT}^*] = \frac{R_{\text{DMT}^*}}{R_{\text{veratrole}} * (\text{RF}_{\text{DMT}^*})} * [\text{veratrole}]$$

$$\text{RF}_{\text{DMT}^*} = \frac{R_{\text{DMT}^*} / R_{\text{veratrole}}}{([\text{DMTCI}] / [\text{Veratrole}] - (R_{\text{DMTOH}} / R_{\text{veratrole}} * (\text{RF}_{\text{DMTOH}})))}$$

[DMTCI]= 0.0038	
[veratrole]= 0.012	
RF of DMTOH = 3.0	
[DMTCI]	RF of DMT*
[veratrole]	
1.0	3.2
1.0	3.3
1.0	3.3
1.0	3.1
1.0	3.3

RF of DMT* =3.3

Data for calculation of Response Factors

RF of BzDMTdA

[BzDMTdA] =	0.0018
[veratrole]*vol	0.012
<u>[BzDMTdA]</u>	<u>%Area of BzDMTdA</u>
<u>[veratrole]</u>	<u>%Area of veratrole</u>
0.050	0.31
0.099	0.61
0.15	0.93
0.20	1.1
0.25	1.4
0.30	1.5

RF of BzdA

[BzdA]=	0.0016
[veratrole]=	0.015
<u>[BzdA]</u>	<u>%Area of BzdA</u>
<u>[veratrole]</u>	<u>%Area of Veratrole</u>
0.05	0.16
0.10	0.27
0.16	0.40
0.21	0.47
0.24	0.52
0.26	0.56

RF of DMTOH

[veratrole]*vol	0.0084
[DMTOH] =	0.0028
<u>[DMTOH]</u>	<u>%Area of DMTOH</u>
<u>[veratrole]</u>	<u>%Area of veratrole</u>
0.17	0.57
0.34	1.1
0.50	1.7
0.67	2.1
0.84	2.6
1.0	2.9

RF of BzA

[BzA]=	0.0023
[veratrole]=	0.025
<u>[BzA]</u>	<u>%Area of BzA</u>
<u>[veratrole]</u>	<u>%Area of BzA</u>
0.046	0.18
0.093	0.31
0.14	0.41
0.19	0.42
0.23	0.54
1.4	2.6

Appendix D. Rate Constants and Order of Reaction

Data for first reaction

<i>Rate</i>	<i>C ([BzDMTdA])</i>
0.004041	0.001295
0.000089	0.000779
0.000086	0.000746
0.000151	0.000649
0.000085	0.000531
0.000062	0.000457
0.000116	0.000368
0.000051	0.000259
0.000034	0.000156
0.12	0.000074

POLYMATH Results

Nonlinear regression (L-M)

Model: rate = $k_1 \cdot C^n$

<u>Variable</u>	<u>Ini guess</u>	<u>Value</u>	<u>95% confidence</u>
k1	0.02	3.0199409	0.0769915
n	1	1.1065318	0.0036104

Nonlinear regression settings
Max # iterations = 64

Precision

R² = 0.442037
R²adj = 0.3722917
Rmsd = 2.811E-04
Variance = 9.877E-07

General

Sample size = 10
Model vars = 2
Indep vars = 1
Iterations = 34

Data for second reaction

Rate	C ([BzDMTdA])
5.612E-06	5.148E-04
5.523E-06	3.899E-04
2.414E-06	2.346E-04
1.195E-06	9.050E-05
1.415E-07	1.456E-05
6.757E-08	7.272E-06

POLYMATH Results

Nonlinear regression (L-M)

Model: rate = $k_2 \cdot C^n$

Variable	Ini guess	Value	95% confidence
k2	0.005	0.0074995	0.0031098
n	1	0.9406617	0.0530929

Nonlinear regression settings

Max # iterations = 64

Precision

R² = 0.9633975

R²adj = 0.9542469

Rmsd = 1.804E-07

Variance = 2.929E-13

General

Sample size = 6

Model vars = 2

Indep vars = 1

Iterations = 6

Appendix E. Solution Phase Experiments

0.05M DCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0044 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.47	0.02	48.6	42	1.8	0.11
0.167	11	0.29	47.3	7.2	8.8	9.3
0.5	14	0.52	46.5	1.8	13	10
1	14	0.88	48.3	0.28	14	9.5
3	11	2.0	49.4	0.20	13	8.8
5	11	3.3	47.9	0.19	14	9.8
10	8.0	5.6	48.1	0.17	13	10
20	4.5	8.1	50.1	0.15	12	10

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0096	0.0005	0.86	0.037	0.0023
0.167	0.24	0.0062	0.15	0.19	0.198
0.5	0.31	0.011	0.038	0.29	0.22
1	0.28	0.018	0.0057	0.28	0.20
2	0.23	0.040	0.0041	0.26	0.18
5	0.23	0.069	0.0039	0.29	0.20
10	0.17	0.12	0.0035	0.28	0.21
20	0.090	0.16	0.0030	0.24	0.20

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA 5.3
 DMTOH 3.0
 DMT* 3.30
 [veratrole]= 0.014

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.00039	0.0014	4.44E-05	0.00083	0.0008	0.0016
0.5	9.7E-05	0.0019	8.02E-05	0.0013	0.0009	0.0022
1	1.5E-05	0.0017	0.000132	0.0013	0.0008	0.0021
3	1.1E-05	0.0014	0.000286	0.0012	0.0007	0.0019
5	1E-05	0.0014	0.000496	0.0013	0.0008	0.0021
10	8.9E-06	0.0010	0.000835	0.0012	0.0009	0.0021
20	7.7E-06	0.00054	0.0012	0.0011	0.0008	0.0019

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurina	yield
0.167	0.0020	0.0019	81	2.4	78
0.5	0.0023	0.0020	96	4.0	92
1	0.0021	0.0018	99	7.2	92
3	0.0019	0.0017	99	17	82
5	0.0021	0.0019	100	27	73
10	0.0021	0.0018	100	45	54
20	0.0019	0.0017	100	68	32

0.1M DCA in DCM in solution

Initial Concentration of Bz-DMT-dA = 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-d	DMTOH	DMT*
0	1.3	0.07	44	42	3.5	0.19
0.167	14	0.65	38	1.7	8.1	17
0.5	15	0.98	39	0.12	9.1	17
1	15	1.7	38	0	8.9	18
3	13	3.1	39	0	9.0	17
5	8.9	6.1	38	0	9.2	18
10	6.1	8.8	39	0	8.9	18
20	2.4	13	38	0	9.4	18

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.030	0.002	0.94	0.080	0.004
0.167	0.37	0.017	0.045	0.22	0.46
0.5	0.39	0.026	0.003	0.24	0.44
1	0.38	0.045	0.000	0.23	0.46
3	0.34	0.079	0.000	0.23	0.44
5	0.23	0.16	0.000	0.24	0.46
10	0.16	0.23	0.000	0.23	0.46
20	0.062	0.33	0.000	0.25	0.48

Response factor

BzdA =	2.3
BzA =	1.9
BzDMTdA	5.3
DMTOH =	3.0
DMT* =	3.3
[veratrole]=	0.010

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0	0.0036	0.0001	0.0000	0.0003	0.0000	0.0003
0.167	8.7E-05	0.0017	0.0001	0.0007	0.0014	0.0021
0.5	6.2E-06	0.0017	0.0001	0.0008	0.0014	0.0022
1	0	0.0017	0.0002	0.0008	0.0014	0.0022
3	0	0.0015	0.0004	0.0008	0.0013	0.0021
5	0	0.0010	0.0009	0.0008	0.0014	0.0022
10	0	0.0007	0.0012	0.0008	0.0014	0.0022
20	0	0.0003	0.0018	0.0008	0.0015	0.0023

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depur	yield
0	0	0	0	0	0
0.167	0.0022	0.0018	96	5.1	91
0.5	0.0022	0.0019	100	7.3	92
1	0.0022	0.0020	100	12	88
3	0.0021	0.0019	100	22	78
5	0.0022	0.0019	100	45	55
10	0.0022	0.0019	100	63	37
20	0.0023	0.0021	100	86	14

0.2M DCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA = 0.0035 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.062	0.028	42	48	1.8	0.0978
0.167	17	0.90	34	0.21	2.4	23
0.5	16	1.2	33	0.085	3.0	23
1	15	2.1	34	0	3.4	22
2	13	4.6	32	0	3.4	22
5	7.6	7.5	34	0	2.4	22
10	3.9	12	34	0	2.6	23
20	1.3	13	35	0	2.5	23

Normalized Data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0015	0.0007	1.1	0.043	0.0023
0.167	0.49	0.027	0.0061	0.070	0.67
0.5	0.49	0.036	0.0026	0.092	0.70
1	0.43	0.064	0	0.10	0.66
2	0.39	0.143	0	0.10	0.69
5	0.22	0.220	0	0.071	0.65
10	0.11	0.347	0	0.074	0.67
20	0.038	0.379	0	0.070	0.67

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA 5.3
 DMTOH 3.0
 DMT* = 3.3
 [veratrole]= 0.0082

Concentration

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product DMT
0.167	9.5E-06	0.0018	0.00012	0.00019	0.0017	0.0019
0.5	4.03E-06	0.0018	0.00016	0.00025	0.0018	0.0020
1	0	0.0016	0.00028	0.00027	0.0016	0.0019
3	0	0.0014	0.00062	0.00028	0.0017	0.0020
5	0	0.00082	0.00096	0.00019	0.0016	0.0018
10	0	0.00042	0.0015	0.00020	0.0017	0.0019
20	0	0.00014	0.0017	0.00019	0.0017	0.0018

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Depro	%Depuri	yield
0.167	0.0019	0.0019	99	6.0	93
0.5	0.0020	0.0019	100	8.1	92
1	0.0019	0.0019	100	15	85
3	0.0020	0.0020	100	31	69
5	0.0018	0.0018	100	54	46
10	0.0019	0.0019	100	78	22
20	0.0018	0.0018	100	92	7.7

0.1M DCA in MIB in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.01	0.036	30	18	0.89	0.11
0.167	0.061	0.052	21	18	0.38	0.026
0.5	0.074	0.067	21	19	0.40	0.030
1	0.099	0.078	21	18	0.44	0.042
2	0.16	0.068	21	18	0.56	0.060
5	0.17	0.11	21	17	0.62	0.074
10	0.28	0.11	20	17	0.86	0.12
20	0.58	0.18	21	17	1.3	0.21

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0003	0.0012	0.59	0.029	0.0035
0.167	0.0028	0.0024	0.84	0.018	0.0012
0.5	0.0035	0.0032	0.89	0.019	0.0014
1	0.0047	0.0037	0.87	0.021	0.0020
2	0.0077	0.0033	0.86	0.027	0.0028
5	0.0081	0.0055	0.83	0.030	0.0036
10	0.0140	0.0056	0.85	0.042	0.0061
20	0.0284	0.0086	0.82	0.064	0.010

Response factor

BzDMTdA= 5.3
 BzdA = 2.3
 BzA = 1.9
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole]= 0.016

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT+	Product
						DMT
0.167	0.0025	0.000020	0.000021	9.5E-05	6.0E-06	0.00010
0.5	0.0027	0.000025	0.000027	1.0E-04	6.9E-06	0.00011
1	0.0026	0.000034	0.000032	1.1E-04	9.9E-06	0.00012
3	0.0026	0.000055	0.000028	1.4E-04	1.4E-05	0.00016
5	0.0025	0.000057	0.000047	1.6E-04	1.7E-05	0.00018
10	0.0026	0.00010	0.000048	2.2E-04	3.0E-05	0.00025
20	0.0025	0.00020	0.000073	3.4E-04	5.0E-05	0.00039

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depuri	yield
0.167	0.0026	0.0026	3.8	0.8	3.0
0.5	0.0028	0.0027	3.9	1.0	2.9
1	0.0027	0.0027	4.4	1.2	3.3
3	0.0028	0.0027	5.6	1.0	4.6
5	0.0027	0.0026	6.5	1.8	4.7
10	0.0028	0.0027	9.0	1.8	7.3
20	0.0029	0.0027	14	2.7	11

0.2M DCA in MIB in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.18	0.036	34	18	0.82	0.031
0.25	0.074	0.062	24	17	0.41	0.045
0.5	0.085	0.065	24	17	0.44	0.054
1	0.22	0.081	23	16	0.54	0.093
3	0.26	0.11	23	15	0.87	0.17
5	0.36	0.16	23	14	1.2	0.22
10	0.41	0.23	23	13	1.8	0.36
20	0.84	0.45	23	12	2.9	0.60

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0054	0.0011	0.54	0.024	0.00092
0.25	0.0031	0.0026	0.69	0.017	0.0019
0.5	0.0035	0.0027	0.69	0.018	0.0022
1	0.0095	0.0035	0.68	0.023	0.0040
3	0.011	0.0048	0.63	0.038	0.0072
5	0.015	0.0070	0.62	0.050	0.0096
10	0.018	0.010	0.56	0.077	0.016
20	0.036	0.019	0.51	0.13	0.026

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] = 0.018

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.25	0.0023	2E-05	2.4E-05	9.79E-05	9.9E-06	0.00011
0.5	0.0023	2.1E-05	2.5E-05	0.000106	1.2E-05	0.00012
1	0.0023	2.7E-05	3.3E-05	0.000135	2.1E-05	0.00016
3	0.0021	3.7E-05	4.4E-05	0.000218	3.8E-05	0.00026
5	0.0021	5.5E-05	6.5E-05	0.000292	5.1E-05	0.00034
10	0.0019	7.9E-05	9.4E-05	0.000449	8.3E-05	0.00053
20	0.0017	0.00015	0.00018	0.000731	1.4E-04	0.00087

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depur
0.25	0.0024	0.0023	4.5	1.0
0.5	0.0024	0.0023	4.9	1.1
1	0.0024	0.0023	6.5	1.4
3	0.0023	0.0022	11	2.0
5	0.0024	0.0022	14	3.0
10	0.0024	0.0020	22	4.7
20	0.0026	0.0020	34	8.9

0.05M TCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA = 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	1.0	0.088	42	46	2.8	0.092
0.167	14	0.95	37	0.11	3.4	21
0.5	13	2.3	37	0.034	3.1	22
1	12	4.6	37	0.020	2.4	22
3	6.4	9.0	37	0.018	2.4	23
5	3.7	11	38	0	1.9	24
10	0.67	14	34	0	1.9	22
20	0.20	13	37	0	2.4	24

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.024	0.0021	1.1	0.067	0.0022
0.167	0.37	0.026	0.0031	0.092	0.58
0.5	0.35	0.063	0.000912	0.083	0.59
1	0.34	0.12	0.00055	0.066	0.59
3	0.17	0.24	0.000491	0.065	0.63
5	0.099	0.30	0	0.050	0.63
10	0.019	0.40	0	0.054	0.63
20	0.0055	0.36	0	0.066	0.66

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3
 DMT* = 3.3
 [veratrole] = 0.0087

Concentration

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	5.06E-06	0.0014	0.00012	0.0002681	0.0015	0.002
0.5	1.5E-06	0.0013	0.00029	0.0002424	0.0016	0.002
1	9.05E-07	0.0013	0.00057	0.0001917	0.0016	0.0017
3	8.07E-07	0.0007	0.0011	0.0001896	0.0017	0.0019
5	0	0.0004	0.0014	0.000145	0.0017	0.0018
10	0	7E-05	0.0018	0.0001575	0.0017	0.0018
20	0	2E-05	0.0017	0.000191	0.0017	0.0019

%Deprotection and %depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurination	yield
0.167	0.0018	0.0015	100	7.7	92
0.5	0.0018	0.0016	100	18	82
1	0.0017	0.0019	100	31	69
3	0.0019	0.0018	100	63	37
5	0.0018	0.0018	100	79	21
10	0.0018	0.0019	100	96	3.9
20	0.0019	0.0017	100	99	1.2

0.1M TCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0017 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.071	0.020	46	46	1.2	0.035
0.167	9.3	1.2	36	0.063	1.2	16
0.5	7.7	2.8	36	0.066	1.1	17
1	6.0	4.5	36	0.059	1.1	17
3	3.3	6.7	36	0.065	0.91	16
5	1.8	8.1	37	0.059	1.0	17
10	0.50	9.1	36	0.065	1.2	16
20	0.21	9.7	37	0.057	1.2	17

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0015	0.00043	1.0	0.027	0.00076
0.167	0.26	0.035	0.0018	0.035	0.45
0.5	0.21	0.076	0.0018	0.029	0.46
1	0.17	0.13	0.0016	0.031	0.47
3	0.091	0.18	0.0018	0.025	0.45
5	0.048	0.22	0.0016	0.028	0.46
10	0.014	0.25	0.0018	0.033	0.45
20	0.0056	0.26	0.0015	0.031	0.46

Response factors

BzdA = 2.3
 BzA = 1.9
 BzDMTdA= 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole]= 0.0045

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0	0.0017	6.08E-06	2.03E-06	7.91E-05	2.1E-06	8.1E-05
0.167	1.49E-06	0.000517	8.2E-05	5.09E-05	0.00062	0.00067
0.5	1.54E-06	0.000421	0.00018	4.28E-05	0.00062	0.00066
1	1.39E-06	0.000329	0.0003	4.58E-05	0.00063	0.00068
3	1.5E-06	0.000182	0.00044	3.69E-05	0.00061	0.00065
5	1.36E-06	9.61E-05	0.00052	4.17E-05	0.00062	0.00066
10	1.51E-06	2.71E-05	0.00059	4.94E-05	0.00061	0.00066
20	1.3E-06	1.12E-05	0.00062	4.56E-05	0.00063	0.00067

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprotection	%Depurination	Yield
0	0	0	0	0	0
0.167	0.00067	0.00060	100	14	86
0.5	0.00066	0.00060	100	30	70
1	0.00068	0.00063	100	48	52
3	0.00065	0.00062	100	71	29
5	0.00066	0.00062	100	84	15
10	0.00066	0.00062	100	95	4.4
20	0.00067	0.00063	100	98	1.8

0.2M TCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA = 0.0017 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.071	0.020	46	46	1.2	0.035
0.167	8.5	1.6	33	0.055	0.93	15
0.5	6.5	3.4	33	0.056	0.89	15
1	4.3	5.3	33	0.054	0.83	15
3	1.1	8.5	33	0.50	0.86	15
5	0.19	9.1	33	0.053	1.00	15
10	0.072	9.7	34	0.044	0.96	16
20	0.040	8.5	33	0.054	1.1	15

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0015	0.0004	1.01	0.027	0.0008
0.167	0.26	0.049	0.0017	0.028	0.45
0.5	0.20	0.10	0.0017	0.027	0.45
1	0.13	0.16	0.0016	0.025	0.45
3	0.032	0.25	0.0150	0.026	0.46
5	0.0056	0.27	0.0016	0.030	0.45
10	0.0021	0.29	0.0013	0.028	0.47
20	0.0012	0.26	0.0016	0.033	0.45

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA= 5.3
 DMTOH= 3.0
 DMT*= 3.3
 [veratrole]= 0.0045

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*
0.167	1.4E-06	0.00051	0.00012	4.2E-05	0.00061
0.5	1.4E-06	0.00039	0.00024	4.0E-05	0.00062
1	1.4E-06	0.00026	0.00038	3.7E-05	0.00061
3	1.3E-05	0.00006	0.00061	3.8E-05	0.00063
5	1.3E-06	0.000011	0.00065	4.4E-05	0.00062
10	1.1E-06	0.000004	0.00069	4.2E-05	0.00065
20	1.4E-06	0.000002	0.00061	4.9E-05	0.00061

%Deprotection and %Depurination

Time(min)	Product	Total DMT	Total A	%Deprot	%Depuri	yield
	DMT					
0.167	0.00066	0.00066	0.00063	100	19	81
0.5	0.00066	0.00066	0.00064	100	38	62
1	0.00065	0.00065	0.00064	100	59	41
3	0.00067	0.00068	0.00068	98	89	9.3
5	0.00066	0.00066	0.00066	100	98	1.7
10	0.00069	0.00069	0.00070	100	99	0.61
20	0.00066	0.00066	0.00062	100	99	0.41

0.1M TCA in MIB in Solution Phase

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0	0	18	22.9	0.12	2.5
0.25	0.13	0.039	8	10.4	0.50	2.2
0.5	0.13	0.020	10	10.2	1.3	2.7
1	0.32	0.07	9	10.2	2.0	2.4
2	0.54	0.10	9	8.6	1.5	2.6
3	0.79	0.14	9	8.0	1.8	2.3
4	1.2	0.22	11	8.8	2.7	2.8
5	1.2	0.24	9	7.2	2.9	2.5
10	1.9	0.40	9	4.6	4.1	2.4
15	2.3	0.58	10	2.9	5.1	2.4
30	2.5	0.99	10	0.92	6.8	2.6
60	2.3	1.5	8	0.61	7.3	2.1
120	1.2	2.8	9	0.051	7.9	2.5
180	0.79	3.2	9	0	7.9	2.3
240	0.49	3.7	9	0	8.3	2.6
300	0.25	3.6	8	0	6.7	2.3

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0	0	1.3	0.0065	0.14
0.25	0.015	0.0046	1.2	0.059	0.26
0.5	0.012	0.0020	1.0	0.13	0.27
1	0.034	0.007	1.1	0.21	0.25
2	0.060	0.012	0.96	0.17	0.29
3	0.089	0.016	0.91	0.20	0.26
4	0.11	0.021	0.83	0.25	0.26
5	0.13	0.025	0.77	0.31	0.26
10	0.22	0.046	0.52	0.47	0.27
15	0.24	0.061	0.30	0.54	0.26
30	0.25	0.098	0.091	0.67	0.25
60	0.28	0.18	0.075	0.90	0.25
120	0.13	0.31	0.0055	0.86	0.27
180	0.087	0.36	0	0.88	0.26
240	0.052	0.40	0	0.89	0.28
300	0.031	0.43	0	0.81	0.28

Response factor

BzdA =	2.3
BzA =	1.9
BzDMTdA =	5.3
DMTOH =	3.0
DMT* =	3.3

Initial Concentration of Bz-DMT-dA =
Concentration of veratrole =

0.0018 M
0.0037 M

Concentration

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product DMT
0	0.0018	0	0	1.6E-05	0.00031	0.00033
0.25	0.00085	2.5E-05	9.0E-06	7.2E-05	0.00029	0.00037
0.5	0.00071	2.0E-05	3.8E-06	1.6E-04	0.00030	0.00046
1	0.00069	5.6E-05	1.4E-05	2.6E-04	0.00029	0.00054
2	0.00067	9.8E-05	2.3E-05	2.1E-04	0.00032	0.00053
3	0.00064	1.5E-04	3.1E-05	2.4E-04	0.00029	0.00053
4	0.00058	1.9E-04	4.1E-05	3.1E-04	0.00029	0.00060
5	0.00054	2.1E-04	5.0E-05	3.7E-04	0.00030	0.00067
10	0.00037	3.6E-04	9.1E-05	5.7E-04	0.00031	0.00088
15	0.00021	3.9E-04	1.2E-04	6.5E-04	0.00029	0.00094
30	0.00006	4.1E-04	1.9E-04	8.2E-04	0.00028	0.0011
60	0.00005	4.6E-04	3.6E-04	1.1E-03	0.00029	0.0014
120	0	2.1E-04	6.1E-04	1.1E-03	0.00031	0.0014
180	0	1.4E-04	7.0E-04	1.1E-03	0.00029	0.0014
240	0	8.5E-05	7.8E-04	1.1E-03	0.00031	0.0014
300	0	5.0E-05	8.5E-04	9.8E-04	0.00031	0.0013

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depuri
0	0.0021	0.0018	0	0
0.25	0.0012	0.00089	30	0.22
0.5	0.0012	0.00074	39	0.52
1	0.0012	0.00076	44	1.9
2	0.0012	0.00079	44	2.8
3	0.0012	0.00081	46	3.9
4	0.0012	0.00081	51	5.0
5	0.0012	0.00080	56	6.3
10	0.0012	0.00081	71	11
15	0.0011	0.00072	82	17
30	0.0012	0.00066	95	29
60	0.0014	0.00087	96	42
120	0.0014	0.00083	100	74
180	0.0014	0.00084	100	83
240	0.0014	0.00086	100	90
300	0.0013	0.00090	100	94

0.2M TCA in MIB in Solution Phase

%Area under the Peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0	0	18	23	0.12	2.5
0.25	0.25	0.036	8.6	9.8	0.55	1.9
0.5	0.38	0.054	8.6	9.5	2.0	1.9
1	0.69	0.098	8.6	8.9	2.5	2.0
2	1.2	0.18	8.9	7.3	2.7	2.0
3	1.7	0.26	8.6	6.0	3.0	2.0
4	2.0	0.33	8.1	4.9	3.6	2.0
5	2.4	0.43	9.0	4.0	4.2	2.2
7	2.7	0.58	8.7	2.6	5.0	2.1
10	3.0	0.81	9.3	1.4	5.8	2.2
15	3.1	1.1	9.2	0.58	6.6	2.2
30	2.6	1.6	9.1	0.08	6.9	2.2
60	1.7	2.4	9.3	0.024	6.9	2.0
120	0.84	3.2	8.5	0	7.1	2.0
240	0.11	2.4	9.2	0	7.3	2.1
300	0.057	4.3	9.1	0	6.9	2.0
390	0.022	4.6	8.5	0	6.5	1.8

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0	0	1.3	0.0065	0.14
0.25	0.028	0.0042	1.1	0.064	0.22
0.5	0.044	0.0063	1.1	0.232	0.22
1	0.080	0.011	1.0	0.29	0.23
2	0.14	0.020	0.82	0.30	0.22
3	0.20	0.030	0.70	0.35	0.23
4	0.25	0.041	0.61	0.44	0.25
5	0.26	0.047	0.44	0.47	0.24
7	0.31	0.067	0.30	0.57	0.24
10	0.32	0.087	0.15	0.63	0.23
15	0.34	0.12	0.063	0.71	0.24
30	0.29	0.18	0.0088	0.76	0.24
60	0.19	0.25	0.0026	0.74	0.21
120	0.099	0.38	0	0.83	0.23
240	0.011	0.26	0	0.80	0.23
300	0.0063	0.47	0	0.76	0.21
390	0.0026	0.55	0	0.77	0.22

Response factor

BzdA =	2.3
BzA =	1.9
BzDMTdA =	5.3
DMTOH =	3.0
DMT* =	3.3

Initial Concentration of Bz-DMT-dA = 0.0018 M
Concentration of veratrole = 0.0037 M

Concentration

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0	0.0018	0	0	1.6E-05	0.00031	0.00033
0.25	0.00079	4.7E-05	8.2E-06	7.8E-05	0.00025	0.00033
0.5	0.00077	7.2E-05	1.2E-05	2.8E-04	0.00025	0.00053
1	0.00072	1.3E-04	2.2E-05	3.5E-04	0.00026	0.00061
2	0.00057	2.3E-04	3.9E-05	3.7E-04	0.00025	0.00062
3	0.00049	3.2E-04	5.9E-05	4.2E-04	0.00026	0.00069
4	0.00043	4.1E-04	8.0E-05	5.4E-04	0.00028	0.00082
5	0.00031	4.3E-04	9.3E-05	5.7E-04	0.00027	0.00084
7	0.00021	5.0E-04	1.3E-04	7.0E-04	0.00027	0.00097
10	0.00010	5.3E-04	1.7E-04	7.6E-04	0.00026	0.0010
15	4.4E-05	5.6E-04	2.4E-04	8.7E-04	0.00027	0.0011
30	6.2E-06	4.7E-04	3.5E-04	9.2E-04	0.00027	0.0012
60	1.8E-06	3.1E-04	5.0E-04	9.1E-04	0.00023	0.0011
120	0	1.6E-04	7.4E-04	1.0E-03	0.00026	0.0013
240	0	1.9E-05	5.2E-04	9.7E-04	0.00026	0.0012
300	0	1.0E-05	9.2E-04	9.3E-04	0.00024	0.0012
390	0	4.2E-06	1.1E-03	9.3E-04	0.00024	0.0012

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurination
0	0.0021	0.0018	0	0
0.25	0.0011	0.00084	29	0.97
0.5	0.0013	0.00085	41	1.4
1	0.0013	0.00088	46	2.5
2	0.0012	0.00084	52	4.6
3	0.0012	0.00087	58	6.8
4	0.0012	0.00092	66	8.8
5	0.0012	0.00083	73	11
7	0.0012	0.00084	82	16
10	0.0011	0.00081	91	21
15	0.0012	0.00084	96	28
30	0.0012	0.00083	99	42
60	0.0011	0.00080	100	62
120	0.0013	0.00090	100	82
240	0.0012	0.00054	100	96
300	0.0012	0.00093	100	99
390	0.0012	0.00108	100	100

0.2M MCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0037 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.77	0.074	46	44	2.8	0.11
0.167	2.1	0.12	42	33	2.2	1.2
0.5	3.9	0.15	43	30	4.9	1.2
1	6.3	0.32	42	26	9.3	1.2
3	12	0.8	42	9.7	18	1.3
5	14	1.5	42	4.4	22	1.3
10	14	2.1	42	0.59	25	1.3
20	13	3.6	42	0.18	25	1.3
40	14	7.8	39	0.28	29	0.87
60	9.6	8.9	40	0.24	29	0.81

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.017	0.002	0.96	0.062	0.002
0.5	0.050	0.003	0.80	0.054	0.029
0.75	0.092	0.004	0.71	0.11	0.027
1	0.15	0.008	0.62	0.22	0.030
3	0.28	0.019	0.23	0.43	0.030
5	0.33	0.037	0.10	0.52	0.032
10	0.35	0.051	0.014	0.59	0.031
20	0.32	0.086	0.004	0.60	0.032
40	0.35	0.20	0.007	0.75	0.022
60	0.24	0.22	0.006	0.71	0.020

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA : 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole]= 0.010

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.0015	0.00023	0.00002	0.00018	9.0E-05	0.00027
0.5	0.0014	0.00042	0.00002	0.00039	8.4E-05	0.00047
1	0.0012	0.00069	0.00004	0.00075	9.2E-05	0.00084
3	0.00045	0.0012	0.00010	0.0014	9.3E-05	0.0015
5	0.00020	0.0015	0.00020	0.0017	9.8E-05	0.0018
10	0.00003	0.0016	0.00028	0.0020	9.6E-05	0.0021
20	0.00001	0.0014	0.00046	0.00202	1.0E-04	0.0021

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurin	yield
0.167	0.0018	0.0018	15	0.9	14.1
0.5	0.0018	0.0018	25	1.0	24
1	0.0020	0.0019	41	2.2	39
3	0.0020	0.0018	78	5.8	72
5	0.0020	0.0019	90	11	80
10	0.0021	0.0019	99	15	84
20	0.0021	0.0019	100	24	75

0.3M MCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0030 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	1.6	0.03	40	46	3.9	0.13
0.167	1.6	0.16	33	34	2.8	1.5
0.5	4.6	0.24	34	31	6.9	1.5
1	7.1	0.36	34	22	12	1.5
3	13	0.93	33	5.4	23	1.6
5	7.6	1.0	31	0.98	24	1.6
10	13	2.0	31	0.13	23	1.4
20	10	3.3	33	0.055	26	1.6

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.040	0.00075	1.1	0.098	0.0033
0.5	0.050	0.0049	1.1	0.085	0.045
0.75	0.13	0.0070	0.90	0.20	0.044
1	0.21	0.011	0.65	0.36	0.045
3	0.38	0.028	0.16	0.69	0.048
5	0.25	0.033	0.032	0.78	0.051
10	0.43	0.064	0.0043	0.75	0.044
20	0.30	0.10	0.0017	0.80	0.050

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA= 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] = 0.0068

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product DMT
0.167	0.0014	0.00015	0.00002	0.00019	0.00009	0.00029
0.5	0.0012	0.00041	0.00003	0.00046	0.00009	0.00055
1	0.00083	0.00064	0.00004	0.00080	0.00009	0.00089
3	0.00021	0.00116	0.00010	0.0016	0.00010	0.0017
5	0.00004	0.00074	0.00012	0.0018	0.00011	0.0019
10	0.00001	0.0013	0.00023	0.0017	0.00009	0.0018
20	0.00000	0.00091	0.00036	0.0018	0.00010	0.0019

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprotection	%Depurination	yield
0.167	0.0016	0.0015	17	1.2	16
0.5	0.0017	0.0016	32	1.6	30
1	0.0017	0.0015	52	2.6	49
3	0.0019	0.0015	89	6.9	82
5	0.0019	0.0009	98	13	84
10	0.0018	0.0015	100	15	85
20	0.0019	0.0013	100	29	71

1M MCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA=

0.0029 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0	0.02	48	42	1.6	0.099
0.167	2.2	0.19	44	21	1.4	2.5
0.5	4.9	0.35	45	14	4.0	4.5
1	8.9	0.70	43	5.4	6.9	8.4
3	11	1.3	41	0.26	9.4	10.7
5	9.8	2.0	41	0.032	12	7.8
10	3.4	2.9	40	0.023	12	7.2
20	5.3	6.0	44	0	12	7.4

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0	0.00041	0.86	0.034	0.0021
0.167	0.049	0.0043	0.47	0.033	0.06
0.75	0.11	0.0078	0.32	0.090	0.10
1	0.21	0.0162	0.13	0.16	0.20
3	0.26	0.032	0.0063	0.23	0.26
5	0.24	0.048	0.0008	0.29	0.19
10	0.086	0.074	0.0006	0.29	0.18
20	0.12	0.14	0	0.27	0.17

Response factor

BzdA = 2.3
BzA = 1.9
BzDMTdA = 5.3
DMTOH = 3.0
DMT* = 3.3
[veratrole] = 0.0088

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.00079	0.00019	0.000020	0.00010	0.00015	0.00025
0.5	0.00054	0.00043	0.000037	0.00026	0.00027	0.00053
1	0.00021	0.00080	0.000076	0.00046	0.00052	0.00098
3	1.04E-05	0.00103	0.00015	0.00066	0.00069	0.0013
5	1.31E-06	0.00094	0.00023	0.00085	0.00051	0.00135
10	9.73E-07	0.00034	0.00035	0.00086	0.00049	0.00134
20	0	0.00047	0.00064	0.00080	0.00045	0.0012

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprotection	%Depurination	yield
0.167	0.0010	0.00100	24	2.0	22
0.5	0.0011	0.00100	50	3.7	46
1	0.0012	0.0011	82	7.0	75
3	0.0014	0.0012	99	13	87
5	0.0014	0.0012	100	19	80
10	0.0013	0.0007	100	51	49
20	0.0012	0.0011	100	58	42

2M MCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0029 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0	0.02	48	42	1.6	0.099
0.167	7.6	0.27	37	8.3	2.6	9.2
0.5	10	0.59	38	0.90	4.0	12
1	9.6	0.91	40	0.043	4.4	11
3	8.0	2.2	40	0.000	4.6	11
5	7.3	3.6	38	0.000	4.1	13
10	5.0	5.8	38	0.000	3.7	14
20	2.5	7.1	37	0.000	3.8	13

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0	0.00041	0.86	0.034	0.0021
0.5	0.21	0.0073	0.22	0.070	0.25
0.75	0.27	0.016	0.024	0.11	0.33
1	0.24	0.023	0.0011	0.11	0.29
3	0.20	0.055	0	0.12	0.28
5	0.19	0.094	0	0.11	0.33
10	0.13	0.15	0	0.098	0.36
20	0.068	0.19	0	0.10	0.35

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] = 0.0088

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.00037	0.00080	3.4E-05	0.00020	0.00066	0.00087
0.5	0.00004	0.00108	7.3E-05	0.00031	0.00088	0.0012
1	0.00000	0.00095	1.1E-04	0.00032	0.00077	0.0011
3	0	0.00079	2.6E-04	0.00034	0.00075	0.0011
5	0	0.00075	4.4E-04	0.00031	0.00090	0.0012
10	0	0.00052	7.2E-04	0.00029	0.0010	0.0012
20	0	0.00027	9.0E-04	0.0003	0.0009	0.0012

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprotec	%Depurina	yield
0.167	0.0012	0.0012	70	2.8	67
0.5	0.0012	0.0012	97	6.1	91
1	0.0011	0.0011	100	10	90
3	0.0011	0.0010	100	24	76
5	0.0012	0.0012	100	37	63
10	0.0012	0.0012	100	58	42
20	0.0012	0.0012	100	77	23

3M MCA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0029 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0	0.031	40	49	2.1	0.13
0.167	9.7	0.56	21	5.7	1.8	13
0.5	12	1.2	22	0.19	1.7	17
1	10	2.0	21	0	1.7	17
3	7.6	4.8	21	0	1.3	17
5	6.1	5.1	21	0	1.6	16
10	3.9	6.7	20	0	1.7	17
20	1.7	8.7	22	0	1.4	17

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0	0.00077	1.2	0.053	0.0034
0.5	0.45	0.026	0.26	0.082	0.62
0.75	0.53	0.056	0.0087	0.076	0.79
1	0.49	0.095	0	0.081	0.80
3	0.37	0.23	0	0.062	0.81
5	0.29	0.25	0	0.075	0.79
10	0.19	0.33	0	0.083	0.83
20	0.08	0.40	0	0.063	0.80

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA= 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] = 0.0062

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.00031	0.0012	8.6E-05	0.0001671	0.0012	0.0013
0.5	1E-05	0.0015	1.8E-04	0.0001544	0.0015	0.0016
1	0	0.0014	3.1E-04	0.0001656	0.0015	0.0017
3	0	0.0010	7.6E-04	0.0001257	0.0015	0.0016
5	0	0.00080	8.1E-04	0.0001526	0.0015	0.0016
10	0	0.00052	1.1E-03	0.0001692	0.0016	0.0017
20	0	0.00021	1.3E-03	0.0001282	0.0015	0.0016

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprotection	%Depurination	yield
0.167	0.0016	0.0016	81	5.3	76
0.5	0.0016	0.0017	99	11	88
1	0.0017	0.0017	100	19	81
3	0.0016	0.0018	100	43	57
5	0.0016	0.0016	100	50	50
10	0.0017	0.0016	100	68	32
20	0.0016	0.0015	100	86	14

0.01M MSA in MIB in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0031 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.035	0.02	37	16	0.58	0.0
0.0833	5.6	0.92	24	2.3	9.5	0.9
0.5	3.3	3.5	23	0	11	1.2
1	1.3	4.9	23	0	10	1.3
3	0.16	5.3	24	0	10	1.6
5	0.087	4.2	24	0	10	1.7
10	0.081	5.9	23	0	10	1.7
20	0.047	5.3	24	0	10	1.7

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.00095	0.00061	0.44	0.016	0.0011
0.0833	0.24	0.039	0.097	0.40	0.039
0.5	0.14	0.15	0	0.46	0.050
1	0.0570	0.21	0	0.45	0.055
3	0.0066	0.22	0	0.43	0.067
5	0.0036	0.17	0	0.43	0.072
10	0.0034	0.25	0	0.44	0.072
20	0.0020	0.22	0	0.44	0.073

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole]= 0.018

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.0833	0.00034	0.0019	0.000383	0.0024	0.00022	0.0027
0.5	0	0.0012	0.0015	0.0028	0.00028	0.0030
1	0	0.0005	0.0021	0.0027	0.00031	0.0030
3	0	0.00005	0.0022	0.0026	0.00038	0.0030
5	0	0.00003	0.0017	0.0026	0.0004	0.0030
10	0	0.00003	0.0025	0.0027	0.0004	0.0031
20	0	0.00002	0.0022	0.0027	0.00041	0.0031

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depuri	yield
0.0833	0.0030	0.0027	89	14	74
0.5	0.0030	0.0026	100	55	45
1	0.0030	0.0025	100	82	18
3	0.0030	0.0022	100	98	2.5
5	0.0030	0.0017	100	98	1.7
10	0.0031	0.0025	100	99	1.1
20	0.0031	0.0022	100	99	0.74

0.02M MSA in MIB in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.19	0.040	31	18	0.87	0.065
0.167	6.6	1.6	20	2.6	9.3	5.5
0.5	2.5	4.7	20	0.046	9.2	5.3
1	0.73	7.1	20	0.026	9.0	5.7
3	0.10	6.3	20	0.047	8.9	5.8
5	0.070	6.4	21	0.046	9.2	5.7
20	0.035	5.6	21	0.0058	9.5	5.9

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0060	0.0013	0.58	0.028	0.0021
0.167	0.33	0.080	0.13	0.46	0.27
0.5	0.12	0.234	0.0023	0.46	0.26
1	0.036	0.35	0.0013	0.44	0.28
3	0.0050	0.31	0.0023	0.44	0.29
5	0.0034	0.31	0.0022	0.45	0.28
20	0.0017	0.27	0.00027	0.45	0.28

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.1
 DMT* = 3.3
 [veratrole] = 0.016

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	0.00040	0.0024	0.00070	0.0025	0.00136	0.0038
0.5	7.1E-06	0.00091	0.00203	0.0024	0.0013	0.0037
1	3.9E-06	0.00026	0.0030	0.0024	0.0014	0.0038
3	7.1E-06	0.000036	0.0027	0.0023	0.0014	0.0038
5	6.9E-06	0.000025	0.0027	0.0024	0.0014	0.0038
20	8.5E-07	0.000012	0.0023	0.0024	0.0014	0.0038

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurina	yield
0.167	0.0042	0.0035	90	20	70
0.5	0.0038	0.0029	100	69	31
1	0.0038	0.0033	100	92	8.0
3	0.0038	0.0027	100	98	1.4
5	0.0038	0.0027	100	99	0.98
20	0.0038	0.0024	100	99	0.53

0.02M MSA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA= 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	1.6	0.036	43	42	4.4	0.19
0.1667	6.7	5.1	42	0.11	16	12
0.5	3.4	12	43	0	16	13
1	0.73	12	45	0	16	14
3	0.52	11	45	0	16	15
5	0.33	9.7	46	0	15	15
10	0	13	44	0	14	15

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.038	0.00083	0.96	0.10	0.0044
0.167	0.16	0.12	0.0026	0.38	0.28
0.5	0.081	0.27	0	0.37	0.31
1	0.016	0.26	0	0.35	0.31
3	0.011	0.25	0	0.35	0.34
5	0.0070	0.21	0	0.31	0.31
10	0	0.29	0	0.32	0.35

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.1
 DMT* = 3.3
 [veratrole] = 0.0099

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	4.84E-06	0.00071	0.000651	0.0012	0.0008	0.0021
0.5	0	0.00036	0.0014	0.0012	0.0009	0.0021
1	0	7.2E-05	0.0014	0.0011	0.0009	0.0021
3	0	5E-05	0.0013	0.0011	0.0010	0.0021
5	0	3.1E-05	0.0011	0.0010	0.0009	0.0020
10	0	0	0.0015	0.0010	0.0011	0.0021

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurina	yield
0.167	0.0021	0.0014	100	48	52
0.5	0.0021	0.0018	100	80	20
1	0.0021	0.0015	100	95	4.9
3	0.0021	0.0014	100	96	3.7
5	0.0020	0.0011	100	97	2.7
10	0.0021	0.0015	100	100	0

0.1M MSA in MIB in Solution phase

Initial Concentration of Bz-DMT-dA = 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.21	0.036	31	19	0.92	0.056
0.167	0.52	6.4	20	0.019	8.8	4.2
0.5	0.220	6.7	21	0	8.7	4.5
1	0.152	6.9	21	0	8.8	4.4
3	0.082	6.6	21	0.017	8.7	4.3
5	0.043	6.3	21	0	8.0	5.0
10	0.012	4.6	22	0.0058	8.7	4.6
20	0	2.2	22	0	7.9	5.1

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0067	0.0012	0.61	0.030	0.0018
0.167	0.026	0.31	0.00093	0.43	0.21
0.5	0.0103	0.31	0	0.41	0.21
1	0.0072	0.33	0	0.42	0.21
3	0.0039	0.32	0.00079	0.42	0.20
5	0.0020	0.30	0	0.38	0.24
10	0.00056	0.21	0.00026	0.40	0.21
20	0	0.10	0	0.36	0.23

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] = 0.016

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	2.7E-06	0.00018	0.0026	0.0022	0.00097	0.0032
0.5	0	7.1E-05	0.0026	0.0021	0.00099	0.0031
1	0	5.0E-05	0.0027	0.0022	0.00099	0.0031
3	2.3E-06	2.7E-05	0.0026	0.0022	0.00097	0.0031
5	0	1.4E-05	0.0025	0.0020	0.0011	0.0031
10	7.8E-07	3.9E-06	0.0018	0.0021	0.00099	0.0030
20	0	0	0.0008	0.0018	0.0011	0.0029

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurinat	yield
0.167	0.0032	0.0028	100	93	6.5
0.5	0.0031	0.0027	100	97	2.7
1	0.0031	0.0028	100	98	1.82
3	0.0031	0.0027	100	99	1.03
5	0.0031	0.0025	100	99	0.57
10	0.0030	0.0018	100	100	0.24
20	0.0029	0.0008	100	100	0

0.2M MSA in MIB in Solution Phase

Initial Concentration of Bz-DMT-dA = 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.18	0.036	34	18	0.82	0.031
0.167	0.48	7.5	25	0.095	3.4	10
0.5	0.220	7.6	25	0.052	3.5	10
1	0.026	7.7	26	0.051	3.1	10
3	0.032	7.1	25	0.053	3.6	9.7
5	0.013	6.8	25	0.062	2.8	10
10	0.013	4.6	26	0.11	3.3	9.8
20	0	1.8	27	0.061	2.7	10

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0054	0.0011	0.54	0.024	0.00092
0.167	0.019	0.30	0.0037	0.13	0.40
0.5	0.0087	0.30	0.0021	0.14	0.40
1	0.0010	0.30	0.0020	0.12	0.40
3	0.0013	0.28	0.0021	0.14	0.38
5	0.0005	0.27	0.0024	0.11	0.41
10	0.0005	0.18	0.0042	0.13	0.38
20	0	0.067	0.0023	0.10	0.37

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA = 5.3
 DMTOH = 3.0
 DMT* = 3.3
 [veratrole] 0.018

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	1.2E-05	1.5E-04	0.0028	0.00077	0.0021	0.0029
0.5	6.8E-06	6.8E-05	0.0028	0.00081	0.0021	0.0029
1	6.6E-06	7.8E-06	0.0028	0.00071	0.0021	0.0028
3	6.9E-06	9.9E-06	0.0026	0.00083	0.0021	0.0029
5	8.1E-06	4.1E-06	0.0025	0.00064	0.0022	0.0028
10	1.4E-05	4.0E-06	0.0017	0.00075	0.0021	0.0028
20	7.5E-06	0	0.00063	0.00058	0.002	0.0026

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurina	yield
0.167	0.0029	0.0029	100	95	5.0
0.5	0.0029	0.0029	100	97	2.36
1	0.0028	0.0028	100	99	0.28
3	0.0029	0.0026	100	99	0.40
5	0.0029	0.0025	100	100	0.20
10	0.0028	0.0017	100	100	0.00
20	0.0026	0.0006	100	100	0.00

0.2M MSA in DCM in Solution Phase

Initial Concentration of Bz-DMT-dA = 0.0036 M

%Area under peak at different retention time

Time(min)	Bz-dA	Bz-A	veratrole	Bz-DMT-dA	DMTOH	DMT*
0	0.19	0.057	44	44	2.2	0.15
0.1667	0.55	15	40	0.15	1.9	28
0.5	0.34	16	42	0.025	1.6	25
1	0.16	18	40	0.037	2.0	24
3	0.02	13	39	0.12	2.8	23
5	0	12	37	0.099	3.1	26
10	0	11	38	0.054	2.6	24
20	0	3.8	41	0.063	3.2	27

Normalized data with respect to standard

Time(min)	Bz-dA	Bz-A	Bz-DMT-dA	DMTOH	DMT*
0	0.0043	0.0013	1.01	0.050	0.0035
0.167	0.014	0.38	0.0037	0.047	0.71
0.5	0.008	0.39	0.0006	0.040	0.59
1	0.004	0.45	0.0009	0.050	0.61
3	0.0006	0.33	0.0031	0.072	0.59
5	0	0.33	0.0027	0.084	0.69
10	0	0.29	0.0014	0.069	0.62
20	0	0.094	0.0015	0.077	0.66

Response factor

BzdA = 2.3
 BzA = 1.9
 BzDMTdA= 5.3
 DMTOH= 3.0
 DMT* = 3.3
 [veratrole] 0.0094

Concentrations

Time(min)	BzDMTdA	BzdA	BzA	DMTOH	DMT*	Product
						DMT
0.167	6.7E-06	0.00006	0.0019	0.00015	0.00203	0.0022
0.5	1.1E-06	0.000035	0.0019	0.00012	0.0017	0.0018
1	1.7E-06	0.000017	0.0023	0.00015	0.0017	0.0019
3	5.6E-06	0.000003	0.0017	0.00022	0.0017	0.0019
5	4.7E-06	0	0.0017	0.00026	0.002	0.0022
10	2.5E-06	0	0.0014	0.00022	0.0018	0.0020
20	2.7E-06	0	0.0005	0.00024	0.0019	0.0021

%Deprotection and %Depurination

Time(min)	Total DMT	Total A	%Deprot	%Depurinat	yield
0.167	0.0022	0.0020	100	97	3.0
0.5	0.0018	0.0020	100	98	1.8
1	0.0019	0.0023	100	99	0.7
3	0.0019	0.0017	100	100	0.20
5	0.0022	0.0017	100	100	0.07
10	0.0020	0.0014	100	100	0.05
20	0.0021	0.0005	100	100	0.00

Appendix F. Solid Phase Experiments

0.1M DCA in DCM in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.52	46	23	6.9
0.5	0.065	53	12	4.5
0.75	0.036	61	4.3	1.7
1	0.033	64	1.3	0.47
1.25	0.034	64	0.57	0.13
1.5	0.032	65	0.35	0.047
1.75	0.034	64	0	0
2	0.032	65	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.0112	0.49	0.150
0.5	0.0012	0.23	0.085
0.75	0.0006	0.070	0.027
1	0.0005	0.020	0.007
1.25	0.0005	0.0088	0.0021
1.5	0.0005	0.0054	0.0007
1.75	0.0005	0	0
2	0.0005	0	0

Response factors

BzA 1.9
 DMTOH 3.0
 DMT* 3.3
 [veratrole] = 0.0065

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product
				DMT
0.25	3.9E-05	0.0010	0.00029	0.0013
0.5	4.2E-06	0.0005	0.00017	0.00067
0.75	2.0E-06	0.00015	5.4E-05	0.00020
1	1.8E-06	4.3E-05	1.4E-05	5.8E-05
1.25	1.8E-06	1.9E-05	4.1E-06	2.3E-05
1.5	1.7E-06	1.2E-05	1.4E-06	1.3E-05
1.75	1.8E-06	0	0	0
2	1.7E-06	0	0	0

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	58	58	1.7	1.7
0.5	29	87	0.18	1.9
0.75	8.8	96	0.089	1.9
1	2.5	98	0.078	2.0
1.25	1.00	99	0.078	2.1
1.5	0.56	100	0.074	2.2
1.75	0	100	0.078	2.3
2	0	100	0.074	2.3

Total DMT in reaction mixture = 0.0023

0.2M DCA in DCM in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.40	36	23	8.9
0.5	0.10	48	4.5	2.1
0.75	0.053	52	0.83	0.30
1	0.048	52	0.38	0.076
1.25	0.048	52	0	0.021
1.5	0.047	52	0	0
1.75	0.046	52	0	0
2	0.047	52	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.011	0.65	0.25
0.5	0.0021	0.093	0.043
0.75	0.001	0.016	0.0059
1	0.0009	0.0073	0.0015
1.25	0.0009	0	0.0004
1.5	0.0009	0	0
1.75	0.0009	0	0
2	0.0009	0	0

Response factors

BzA 1.9
 DMTOH 3.0
 DMT* 3.3
 [veratrole] = 0.0071

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product
				DMT
0.25	4.2E-05	0.0015	0.00053	0.0020
0.5	7.8E-06	0.00022	9.2E-05	0.00031
0.75	3.9E-06	3.8E-05	1.3E-05	5E-05
1	3.5E-06	1.7E-05	3.2E-06	2E-05
1.25	3.5E-06	0	8.8E-07	8.8E-07
1.5	3.4E-06	0	0	0
1.75	3.3E-06	0	0	0
2	3.4E-06	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depur	%Depuri
0.25	84	84	1.7	1.7
0.5	13	97	0.32	2.1
0.75	2.1	99	0.16	2.2
1	0.83	100	0.14	2.4
1.25	0.036	100	0.14	2.5
1.5	0	100	0.14	2.6
1.75	0	100	0.14	2.8
2	0	100	0.14	2.9

Total DMT in reaction mixture = 0.0024

0.3M DCA in DCM in solid Phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.35	33	19	10
0.5	0.049	45	0.64	0.35
0.75	0.047	46	0.13	0.041
1	0.048	46	0	0
1.25	0.048	46	0	0
1.5	0.048	46	0	0
1.75	0.048	46	0	0
2	0.049	46	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.011	0.58	0.31
0.5	0.0011	0.01	0.0077
0.75	0.001	0.0029	0.0009
1	0.0011	0	0
1.25	0.0011	0	0
1.5	0.001	0	0
1.75	0.001	0	0
2	0.0011	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3
[veratrole]=	0.0072

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product DMT
0.25	4E-05	0.0014	0.0007	0.0020
0.5	4E-06	3.3E-05	2E-05	5E-05
0.75	4E-06	6.9E-06	2E-06	8.9E-06
1	4E-06	0	0	0
1.25	4E-06	0	0	0
1.5	4E-06	0	0	0
1.75	4E-06	0	0	0
2	4E-06	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depur	%Depuri
0.25	97	97	1.9	1.9
0.5	2.4	100	0.20	2.1
0.75	0.42	100	0.19	2.3
1	0	100	0.19	2.5
1.25	0	100	0.19	2.7
1.5	0	100	0.19	2.9
1.75	0	100	0.19	3.1
2	0	100	0.19	3.3

Total DMT in reaction mixture = 0.0021

0.05M TCA in DCM in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.11	67	6.8	4.2
0.5	0.018	74	0.96	0.11
0.75	0.017	75	0	0
1	0.017	75	0	0
1.25	0.016	75	0	0
1.5	0.016	75	0	0
1.75	0.016	75	0	0
2	0.016	75	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.0016	0.10	0.063
0.5	0.00024	0.01294	0.0014
0.75	0.00022	0	0
1	0.00022	0	0
1.25	0.00022	0	0
1.5	0.00021	0	0
1.75	0.00022	0	0
2	0.00021	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3
[veratrole] =	0.012

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product DMT
0.25	1E-05	0.00041	0.000236	0.00065
0.5	1.6E-06	5.3E-05	5.32E-06	5.8E-05
0.75	1.5E-06	0	0	0
1	1.4E-06	0	0	0
1.25	1.4E-06	0	0	0
1.5	1.4E-06	0	0	0
1.75	1.4E-06	0	0	0
2	1.4E-06	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	92	92	1.5	1.5
0.5	8.2	100	0.22	1.7
0.75	0	100	0.21	1.9
1	0	100	0.21	2.1
1.25	0	100	0.20	2.3
1.5	0	100	0.20	2.5
1.75	0	100	0.20	2.7
2	0	100	0.19	2.9

Total DMT in reaction mixture = 0.00071

0.1M TCA in DCM in Solid Phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.083	60	5.4	2.8
0.5	0.025	62	0.58	0.086
0.75	0.022	62	0	0
1	0.023	64	0	0
1.25	0.022	64	0	0
1.5	0.023	63	0	0
1.75	0.023	64	0	0
2	0.022	64	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.00138	0.090	0.04574
0.5	0.0004	0.0092	0.00139
0.75	0.00036	0	0
1	0.00036	0	0
1.25	0.00035	0	0
1.5	0.00036	0	0
1.75	0.00036	0	0
2	0.00035	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3

Concentration of veratrole = 0.011

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product
				DMT
0.25	8E-06	0.00032	0.000151	0.00048
0.5	2.3E-06	3.3E-05	4.58E-06	3.8E-05
0.75	2.1E-06	0	0	0
1	2.1E-06	0	0	0
1.25	2E-06	0	0	0
1.5	2.1E-06	0	0	0
1.75	2.1E-06	0	0	0
2	2E-06	0	0	0

Total DMT in reaction mixture = 0.00051

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	93	93	1.6	1.6
0.5	7.4	100	0.45	2.0
0.75	0	100	0.41	2.4
1	0	100	0.41	2.8
1.25	0	100	0.40	3.2
1.5	0	100	0.41	3.6
1.75	0	100	0.40	4.0
2	0	100	0.40	4.4

0.2M TCA in DCM in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.096	44	4.9	3.2
0.5	0.039	48	0.38	0.045
0.75	0.042	48	0	0
1	0.043	48	0	0
1.25	0.043	48	0	0
1.5	0.044	48	0	0
1.75	0.046	48	0	0
2	0.047	48	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.0022	0.11	0.073
0.5	0.00082	0.0079	0.00094
0.75	0.00086	0	0
1	0.0009	0	0
1.25	0.00089	0	0
1.5	0.0009	0	0
1.75	0.00094	0	0
2	0.00099	0	0

Response factors

BzA 1.9
 DMTOH 3.0
 DMT* 3.3
 [veratrole] = 0.0097

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product DMT
0.25	1.1E-05	0.00035	0.00021	0.00057
0.5	4.2E-06	2.5E-05	2.8E-06	2.8E-05
0.75	4.4E-06	0	0	0
1	4.6E-06	0	0	0
1.25	4.6E-06	0	0	0
1.5	4.6E-06	0	0	0
1.75	4.8E-06	0	0	0
2	5.1E-06	0	0	0

%Deprotection and % depurination

Time(min)	Depro	%Depro	Depur	%Depur
0.25	95	95	1.9	1.9
0.5	4.7	100	0.71	2.6
0.75	0	100	0.74	3.3
1	0	100	0.77	4.1
1.25	0	100	0.77	4.9
1.5	0	100	0.78	5.6
1.75	0	100	0.82	6.5
2	0	100	0.85	7.3

Total DMT in reaction mixture = 0.00059

0.05M MSA in MIB in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.095	27	6.4	0.38
0.5	0.028	27	5.1	0.74
0.75	0.047	28	2.5	0.45
1	0.050	24	0.89	0
1.25	0.088	29	0.29	0
1.5	0.094	29	0.16	0
1.75	0.099	29	0	0
2	0.032	29	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.0035	0.24	0.014
0.5	0.0010	0.19	0.028
0.75	0.0017	0.089	0.016
1	0.0021	0.038	0
1.25	0.0031	0.010	0
1.5	0.0033	0.006	0
1.75	0.0034	0	0
2	0.0011	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3
[veratrole] =	0.035

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product
				DMT
0.25	6.5E-05	0.0027	0.00015	0.0029
0.5	1.9E-05	0.0022	0.00029	0.0025
0.75	3.1E-05	0.0010	0.00017	0.0012
1	3.9E-05	0.000432	0	0.00043
1.25	5.6E-05	0.000115	0	0.00012
1.5	6E-05	6.31E-05	0	6.3E-05
1.75	6.3E-05	0	0	0
2	2.1E-05	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depur
0.25	40	40	0.91	0.91
0.5	34	75	0.27	1.2
0.75	17	91	0.43	1.6
1	6.0	98	0.55	2.2
1.25	1.6	99	0.79	2.9
1.5	0.88	100	0.84	3.8
1.75	0	100	0.89	4.7
2	0	100	0.29	5.0

Total DMT in reaction mixture = 0.0072

0.05M MSA in DCM in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.94	67	19	4.1
0.5	1.4	74	12	3.1
0.75	0.37	83	4.3	1.6
1	0.27	87	1.1	0.45
1.25	0.17	88	0.41	0.043
1.5	0.11	88	0	0
1.75	0.065	88	0	0
2	0.048	89	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.014	0.28	0.060
0.5	0.019	0.16	0.041
0.75	0.00448	0.051	0.019
1	0.0031	0.013	0.0051
1.25	0.0019	0.0046	0.0005
1.5	0.0012	0	0
1.75	0.00074	0	0
2	0.00054	0	0

Response factors

BzA 1.9
 DMTOH 3.0
 DMT* 3.3
 [veratrole] = 0.026

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product DMT
0.25	0.00020	0.0025	0.00048	0.0029
0.5	0.00026	0.0014	0.00033	0.0017
0.75	6.2E-05	0.00044	0.00015	0.00059
1	4.4E-05	0.00011	4.1E-05	0.00015
1.25	2.6E-05	4.0E-05	3.9E-06	4.4E-05
1.5	1.7E-05	0	0	0
1.75	1.0E-05	0	0	0
2	7.6E-06	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	54	54	3.6	3.6
0.5	32	86	4.8	8.4
0.75	11	96	1.1	9.6
1	2.7	99	0.80	10
1.25	0.80	100	0.48	11
1.5	0	100	0.31	11
1.75	0	100	0.19	11
2	0	100	0.14	11

Total DMT in reaction mixture = 0.0055

0.1M MSA in MIB in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.063	31	8.5	1.5
0.5	0.070	34	2.3	0.97
0.75	0.098	35	0.32	0
1	0.10	35	0	0
1.25	0.073	35	0	0
1.5	0.067	35	0	0
1.75	0.066	35	0	0
2	0.060	35	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.002	0.271	0.049
0.5	0.0021	0.068	0.029
0.75	0.0028	0.0092	0
1	0.003	0	0
1.25	0.0021	0	0
1.5	0.0019	0	0
1.75	0.0019	0	0
2	0.0017	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3
[veratrole] =	0.070

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product DMT
0.25	7.4E-05	0.0062	0.00104	0.0072
0.5	7.7E-05	0.0016	0.00061	0.0022
0.75	1.0E-04	0.0002	0	0.00021
1	1.1E-04	0	0	0
1.25	7.7E-05	0	0	0
1.5	7.1E-05	0	0	0
1.75	7.0E-05	0	0	0
2	6.3E-05	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	75	75	0.77	0.77
0.5	23	98	0.80	1.6
0.75	2.19	100	1.1	2.6
1	0	100	1.1	3.8
1.25	0	100	0.80	4.6
1.5	0	100	0.74	5.3
1.75	0	100	0.73	6.1
2	0	100	0.66	6.7

Total DMT in reaction Mixture = 0.0096

0.2M MSA in MIB in solid phase

%Area under peak at different retention time

Time(min)	BzA	veratrole	DMTOH	DMT*
0.25	0.11	27	11	2.9
0.5	0.15	30	0.55	0
0.75	0.12	30	0.16	0
1	0.097	30	0	0
1.25	0.077	30	0	0
1.5	0.063	30	0	0
1.75	0.055	30	0	0
2	0.047	30	0	0

Normalized Data with respect to standard

Time(min)	BzA	DMTOH	DMT*
0.25	0.00402	0.40	0.11
0.5	0.00517	0.019	0
0.75	0.00414	0.0053	0
1	0.00321	0	0
1.25	0.00254	0	0
1.5	0.00211	0	0
1.75	0.00183	0	0
2	0.00157	0	0

Response factors

BzA	1.9
DMTOH	3.0
DMT*	3.3
[veratrole] =	0.012

Concentrations

Time(min)	BzA	DMTOH	DMT*	Product
				DMT
0.25	2.5E-05	0.0015	0.00039	0.001911
0.5	3.2E-05	7E-05	0	7.2E-05
0.75	2.6E-05	2E-05	0	2.0E-05
1	2.0E-05	0	0	0
1.25	1.6E-05	0	0	0
1.5	1.3E-05	0	0	0
1.75	1.1E-05	0	0	0
2	9.7E-06	0	0	0

%Deprotection and %Depurination

Time(min)	Depro	%Depro	Depuri	%Depuri
0.25	95	95	1.2	1.2
0.5	3.6	99	1.6	2.8
0.75	1.0	100	1.3	4.1
1	0	100	0.99	5.1
1.25	0	100	0.79	5.9
1.5	0	100	0.65	6.6
1.75	0	100	0.57	7.1
2	0	100	0.49	7.6

Total DMT in reaction mixture = 0.0020

Appendix G. Yield Calculations

%Yield in solution Phase

Time(min)	0.05M DCA	0.1M DCA	0.2M DCA	0.1M DCA	0.2M DCA	0.05M TCA	0.1M TCA	0.2M TCA
	in DCM	in DCM	in DCM	in MIB	in MIB	in DCM	in DCM	in DCM
0	0	0	0	0	0	0	0	0
0.167	78	91	95	3.0	3.4	92	86	81
0.5	92	92	92	2.9	3.8	82	70	62
1	92	88	85	3.3	5.1	69	52	41
3	82	78	69	4.2	8.9	37	29	11
5	73	55	46	4.7	11	21	15	2.0
10	54	37	22	7.3	18	4.0	5.0	1.0
20	32	14	8	11	25	1.0	2.0	1.0

Time(min)	0.1M TCA	0.2M TCA	0.2M MCA	0.3M MCA	1M MCA	2M MCA	3M MCA
	in MIB	in MIB	in DCM	in DCM	in DCM	in DCM	in DCM
0	0	0	0	0	0	0	0
0.167	22	24	14	16	22	67	76
0.5	39	40	24	30	46	91	88
1	42	43	39	49	75	90	81
3	42	52	72	82	87	76	57
5	49	62	80	84	80	63	50
10	59	70	84	85	49	42	32
20	65	63	75	71	42	23	14

Time(min)	0.01M MSA	0.02M MSA	0.02M MSA	0.1M MSA	0.2M MSA	0.2M MSA
	in MIB	in MIB	in DCM	in MIB	in MIB	in DCM
0	0	0	0	0	0	0
0.167	75	70	52	7.0	5.0	3.0
0.5	45	31	20	3.0	3.0	2.0
1	18	8.0	5.0	2.0	1.0	1.0
3	2.0	2.0	1.0	1.0	1.0	0
5	2.0	1.00	1.0	1.0	0	0
10	1.0	1.00	0	0	0	0
20	1.00	1.00	0	0	0	0

%Yield in Solid Phase

Time(min)	0.1MDCA in DCM	0.2MDCA in DCM	0.3MDCA in DCM	0.05MTCA in DCM	0.1MTCA in DCM	0.2MTCA in DCM	0.05MMSA in MIB	0.05MMSA in DCM	0.1MMSA in MIB	0.2MMSA in MIB
0	0	0	0	0	0	0	0	0	0	0
0.25	57	83	95	90	91	93	40	50	74	94
0.5	85	95	97	98	98	97	74	77	96	96
0.75	94	97	98	98	98	97	90	87	97	96
1	96	98	97	98	97	96	95	89	96	95
1.25	97	98	97	98	97	95	96	89	95	94
1.5	98	97	97	97	96	94	96	89	95	93
1.75	98	97	97	97	96	94	95	89	94	93
2	98	97	97	97	96	93	95	89	93	92

Appendix H. Error Calculations

Repeatability error in solution phase

Response of veratrole

time(min)	a	b	c
0.25	13200	13136	12959.3
0.5	13300	13596.2	13891.4
1	13700	13346.5	13129.7
3	12600	13159	13489.6
5	13600	13617.4	14075.1
10	12900	12442.3	13454.8

Mean= 13216.67 13216.23 13499.98

Mean of mean= 13310.9611

Deviation over a period of time

time(min)	Dev(a)	Dev(b)	Dev(c)	Devsq(a)	Devsq(b)	Devsq(c)
0.25	-17	-80	-541	278	6437	292338
0.5	83	380	391	6944	144375	153207
1	483	130	-370	233611	16969	137110
3	-617	-57	-10	380278	3276	108
5	383	401	575	146944	160935	330759
10	-317	-774	-45	100278	598973	2042

Sum of squares = 868333 930965 915564

Sdev = 425

Rsdev = 3.2

Rsdev = Sdev/mean

Repeatability error is 3.2%

Reproducibility error in solution phase

Response of veratrole

time(min)	c	d	Mean	Error
	15900	17400	16650	4.50
0.25	17300	18400	17850	3.08
0.5	15500	17000	16250	4.62
1	15400	17400	16400	6.10
3	15900	16400	16150	1.55
5	16100	18300	17200	6.40
10	16000	18100	17050	6.16

Reproducibility error is 6.1%

Repeatability error in solid phase

Response of veratrole

Time	a	b	c
0.25	21700	21600	22000
0.5	21800	21900	21700
0.75	21800	21900	22000
1	21900	22100	21900
1.25	21900	21900	22000
1.5	21900	21800	22000
1.75	21500	22300	22500
2	21800	21800	22000

Mean= 21787.5 21913 22013

Mean of mean= 21904.17

Deviation over the period of time

Time	Dev(a)	Dev(b)	Dev(c)	Devsq(a)	Devsq(b)	Devsq(c)
0.25	-88	-312.5	-13	7656	97656	156
0.5	13	-13	-313	156	156	97656
0.75	13	-13	-13	156	156	156
1	113	188	-113	12656	35156	12656
1.25	113	-13	-13	12656	156	156
1.5	113	-113	-13	12656	12656	156
1.75	-288	388	488	82656	150156	237656
2	13	-113	-13	156	12656	156

Sum of squares = 128750 308750 348750

Sdev = 193

Rsdev = Sdev/ mean

Repeatability error is 1%

Rsdev = 0.88

Reproducibility error in solid phase

Response of veratrole

time	a	d	Mean	Error
0.25	21700	21100	21400	1.4
0.5	21800	21000	21400	1.9
0.75	21800	21200	21500	1.4
1	21900	21200	21550	1.6
1.25	21900	21300	21600	1.4
1.5	21900	21400	21650	1.2
1.75	21500	21200	21350	0.70
2	21800	21500	21650	0.69

Reproducibility error = 2%