DEVELOPMENT OF PHOTOCLEAVABLE LINKER GROUPS FOR APPLICATION TO
PHOTOCLEAVAGE OF LIPOSOMES AND OF CAGING ALCOHOLS AND CARBOXYLIC
ACIDS

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ABSTRACT

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The experimental work described in this dissertation addresses two problems: the design of a novel photocleavable analog of natural lipids and the development of a new photolabile protecting group for alcohols and carboxylic acids.

Several designs of a photocleavable lipid have been explored. The most promising design contained a 3',5'-dialkoxybenzoin chromophore (photolabile linker), substituted with a pentacosan-13-oxy group (hydrophobic tails), and aspartic acid (polar “head”). Photochemical studies of this de novo designed photolabile lipid showed that upon irradiation with 350 nm light it, in fact, undergoes cleavage, forming benzofuran derivatives and releasing aspartic acid.

In our search for a new photolabile protecting (caging) group for alcohols, we have explored the photochemical properties of two chromophores. 3-Hydroxy-2-naphthalenemethanol ethers and esters and 4,4'-dimethoxytrityl ethers were synthesized and their ability to release the protected substrate upon irradiation was examined. 3-Hydroxy-2-naphthalenemethanol derivatives were found to be the more suitable for this purpose. Alkoxy and carboxy derivatives of 3-hydroxy-2-naphthalenemethanol release model substrates, i.e., benzoic acid and benzyl alcohol quantitatively with good quantum yields. The photodeprotection reaction is assumed to proceed via concerted excited state proton transfer, thus avoiding C-O bond heterolysis and the formation of ion pairs. The commonly known problem of lowering the photoefficiency of deprotection reaction due to ion pair recombination is therefore circumvented. Surprisingly, in some cases the chemical yield of substrate release was far from quantitative. This is probably caused by a side reaction of the substrate with a quinone methide intermediate.
5-O’-(4,4’-Dimethoxytrityl) derivatives of a number of DNA nucleosides were shown to regenerate the free nucleoside upon irradiation with 254 nm light with good to excellent chemical yields. Since the 4,4’-dimethoxytrityl group is widely employed in oligonucleotide synthesis, this photoreaction provides a useful orthogonal alternative for deprotection of the 5’ position despite the poor quantum yields.
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TABLE OF CONTENTS

CHAPTER I. INTRODUCTION AND LITERATURE REVIEW

| Introduction | 1 |
| Design of Photosensitive Liposomes Based on Synthetic Photocleavable Lipid | 2 |

References | 17 |

Photolabile Protecting Groups | 18 |

1. Benzyloxycarbonyl protection | 19 |
2. o-Nitrobenzyl alcohol derivatives | 20 |
3. Benzoin group | 29 |
4. p-Hydroxyphenacyl group | 35 |
5. Coumarinyl group | 38 |
6. Other photoremovable protecting groups using benzyl carbocation formation as a driving force | 41 |
7. Silyl (tris(trimethylsilyl)-silyl) protecting group | 44 |
8. Photoremovable protecting groups based on the electron transfer chemistry | 45 |
9. Conclusion | 50 |

References | 52 |

CHAPTER II. RESULTS DISCUSSION AND EXPERIMENTAL PART

Design and Synthesis of a New Photocleavable Lipid Analog | 55 |

Experimental part | 68 |

Design of a New Photoremovable Protecting (Caging) Group | 80 |
Photoremovable protecting group based on the 2-oxymethylnaphthalene-3-ol chromophore

Design of a new photoremovable protecting group based on 4,4'-dimethoxytrityl chromophore

Experimental part

SUPPORTING MATERIAL. Characterization data on compounds synthesized

Part I. Design and synthesis of a new photocleavable lipid analog

Part II. Design of a new photoremovable protecting (caging) group
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Figure 2.1. Steady state photolysis of 10 monitored by UV spectrophotometry</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>Figure 2.2. Measurement of rate constant of dark hydrolysis of 10</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>Figure 2.3. Steady state photolysis of 25 monitored by UV spectrophotometry</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>Figure 2.4. UV absorption spectra of 13.</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>Figure 2.5. Photolysis of 15a and 15b monitored by $^1$H NMR</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>Figure 2.6. Photodeprotection of benzoic acid and benzyl alcohol monitored by HPLC</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>Figure 2.7. Quenching of quinone methide 6 by oxygen</td>
<td>94</td>
</tr>
<tr>
<td>8</td>
<td>Figure 2.8 Quenching of quinone methide 6 by 1,3-cyclooctadiene</td>
<td>95</td>
</tr>
<tr>
<td>9</td>
<td>Figure 2.9 UV absorption spectra of 52-57</td>
<td>99</td>
</tr>
<tr>
<td>10</td>
<td>Figure 2.10 Quantum yield for release of thymidine as a function of the concentration of the electrolyte (sodium perchlorate)</td>
<td>100</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 1.1</td>
</tr>
<tr>
<td>2</td>
<td>Table 1.2</td>
</tr>
<tr>
<td>3</td>
<td>Table 2.1</td>
</tr>
<tr>
<td>4</td>
<td>Table 2.2</td>
</tr>
</tbody>
</table>
CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

Introduction

The main strategic goal of the work described in this dissertation is the design and development of photolabile liposomes by means of incorporation of a photocleavable lipid analog into the liposome bilayer. Possible applications of photolabile liposomes lie in such areas as imaging, sensing and, most importantly, therapeutics\(^1\).

The strategy for design of the photocleavable lipid is introduction of a photolabile linker connecting polar and nonpolar parts of the lipid molecule. Upon irradiation of this molecule the connection between polar and nonpolar parts of the lipid is destroyed and the molecule loses its amphiphilic properties, thus, disturbing the bilayer, and increasing bilayer permeability.

The experimental work was performed pursuing two goals: 1) design and synthesis of the photocleavable lipid analog based on the known photolabile linkers and 2) design of a new photolabile linker (or protecting group), that can be used for photolabile lipid design.

The dissertation consists of two main parts: 1) introduction and literature review (literature review covers published data on research on photolabile liposomes and photoremovable protecting groups) and 2) results and discussions (this part describes the progress in experimental work towards design of a new photocleavable lipid analog and a new photoremovable protecting group).
Design of Photosensitive Liposomes Based on Synthetic Photo cleavable Lipids

Over the last few decades, design of photosensitive liposomes has become a rapidly developing area of photochemical sciences. Interaction with light can cause structural reorganization of the bilayer of a photosensitive liposome which will lead to release of its contents. The use of light allows for spatial and temporal control of liposome cleavage. Possible applications of this process lie in such areas as imaging, sensing and, most importantly, therapeutics\(^{1}\).

The strategies used for design of photosensitive liposomes include photoinduced change in the association of polyelectrolytes with liposomes, photoinduced polymerization of some or all of the bilayer components and photochemical changes in the structure of individual lipids forming the bilayer. The latter is the focus of this brief review.

The first attempt to affect the membrane permeability using photosensitive lipid was performed by Kano \textit{et al.}\(^{2}\) in 1981. The authors introduced amphiphilic compound 1 (Scheme 1.1) into the bilayer membrane of dipalmitoylphosphatidylcholine liposomes. Cis-isomer of 1 was experimentally shown to better maintain the bilayer structure than trans-1. Upon irradiation the azobenzene moiety undergoes trans-cis isomerization which, thus, disturbs the bilayer.

Liposomes with bromothymol blue dye entrapped within the bilayer were studied. This dye is very hydrophobic in its acidic (closed ring) form and can be entrapped within the bilayer. Upon basification bromothymol blue becomes negatively charged and is released into aqueous media. Liposomes containing 1 were shown to release bromothymol blue dye upon basification more rapidly after irradiation with 366 nm light.
Osmotic shrinkage experiments also showed that irradiation of liposomes containing 1 makes them more permeable to water. Overall this first investigation demonstrated the feasibility of using synthetic photosensitive amphiphiles to regulate membrane permeability using light.

Scheme 1.1

In later work by Morgan et al. a phospholipid analog of dipalmitoylphosphatidylcholine was used. The azobenzene moiety was introduced into one of the chains of the phospholipid to form Azo-PC lipid (7) (Scheme 1.2). Synthesis of 7 started with nitroso compound 3, obtained by oxidation of the amine 2, and amino acid 5, formed upon reduction of the nitrobenzene 4. Compounds 5 and 3 were condensed to form azobenzene derivative 5, which was then transformed to Azo-PC by mixed anhydride acylation of 2-palmitoylglycerophosphatidylcholine.

Irradiation of compound 7 causes trans-cis isomerization of azobenzene chromophore (Scheme 1.2). Having linear hydrophobic chains, trans-7 is more compatible with other components of the membrane and capable of better maintaining bilayer structure. Upon irradiation, trans-7 is isomerized to cis-7, which perturbs the membrane, thus causing its destabilization and increasing its permeability.

Azo-PC, thus synthesized, was mixed with dipalmitoyl-phosphatidylcholine (5 molar % of Azo-PC) and sonicated to form liposomes. The studies of liposomes obtained showed that upon irradiation permeability of the membrane increased insignificantly. Thus, light scattering
versus temperature profiles showed only a slight decrease of phase transition temperature for irradiated liposomes.

Scheme 1.2

Experiments with a fluorescent marker gave no evidence of increased permeability of the membrane upon irradiation. On the other hand, osmotic shrinkage experiments showed increased permeability to water after irradiation.
In summary, liposomes containing Azo-PC showed changes in their physical properties, such as phase transition temperature and membrane permeability for neutral molecules upon irradiation. However, the effect of photoisomerization on the permeability was insignificant.

Scheme 1.3.

In consequent work by the same authors\textsuperscript{4,5} a new phosphatidylcholine analog was prepared. Bis Azo-PC (8) contains an azobenzene chromophore in each hydrophobic chain. Irradiation of this molecule should lead to \textit{trans-cis} isomerization of both azobenzene fragments, which should cause much more disturbance in the bilayer, than observed with Azo-PC.

Bis Azo-PC (8) was synthesized in very much the same manner as previously described for compound 7 (Scheme 1.4). Azobenzene derivative 6 was converted to mixed anhydride with pivaloyl chloride and than was condensed with glycerophosphatidilcholine to give bis Azo-PC (8).
Large unilammelar liposomes were prepared from dipalmitoyl-L-α-phosphatidylcholine containing 6 molar % of bis Azo-PC and their behavior upon irradiation was studied. Experiments with liposomes having a fluorescent marker entrapped within the membrane showed a fast dramatic increase in fluorescence in the solution upon irradiation. These results suggest that bis Azo-PC containing liposomes are capable of releasing their contents upon irradiation.

Scheme 1.4

Interesting results were also obtained from electron microscopy. It was found that the size of the liposomes increased from ~25 to ~50 nm after irradiation. These results along with fluorescence polarization versus temperature profiles were explained as photoinduced fusion of the liposomes.

Later publications by the same authors describes an interesting dependence of behavior of bis Azo-PC containing liposomes on the concentration of cholesterol in the bilayer. Thus, it was
shown that Azo-PC containing liposomes can be exposed to a certain number of low intensity laser pulses without significant leakage of their contents; however, rapid release was achieved if the light intensity above certain threshold was achieved, or if the sufficient number of pulses were applied. This light intensity threshold was shown to be dependent on the concentration of the cholesterol in the bilayer. High concentrations of cholesterol was shown to decrease this threshold.

Another interesting feature of the liposomes rich in cholesterol is the character of their behavior after the irradiation. Cholesterol free liposomes release their contents in a rapid “burst” during irradiation and than continue to leak the trapped reagent for about two hours in the dark. Liposomes containing 20% cholesterol in the membrane only release their contents during the period of light exposure, remaining stable in the dark. The contents of cholesterol rich liposomes can be released “stepwise” in the series of pulses, whereas cholesterol free liposomes tend to lose all their contents after a single pulse.

Cholesterol was also shown to influence on the effectiveness of different wavelength light used for irradiation. Two populations of liposomes were prepared having two different fluorescent markers each entrapped in liposomes containing different concentrations of cholesterol in their membranes (0 mol % and 20 mol %). The liposomes were mixed together and exposed to blue (470 nm) light. Irradiation led to leakage of only one fluorescent marker, the one that was entrapped in cholesterol rich liposomes, whereas cholesterol free liposomes remained intact. A subsequent laser pulse at 355 nm released the fluorescent marker from cholesterol free liposomes. Thus, the possibility of wavelength controlled solute release from photosensitive liposomes was established.
These effects of cholesterol concentration were explained by the cholesterol-induced phase separation in the bilayer\textsuperscript{8}. Cholesterol can cause formation of lipid domains within the membranes, thus forming areas with higher local concentration of the photosensitive component of the bilayer. These areas can be much more sensitive to the irradiation than the rest of the membrane.

Scheme 1.5

1-palmitoylglycerophosphocholine

\[\begin{array}{c}
\text{1-palmitoylglycerophosphocholine} \\
\text{glycerophosphocholine}
\end{array}\]

\[\begin{array}{c}
\text{1-palmitoylglycerophosphocholine} \\
\text{glycerophosphocholine}
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\[\begin{array}{c}
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\[\begin{array}{c}
\text{1-palmitoylglycerophosphocholine} \\
\text{glycerophosphocholine}
\end{array}\]
One more approach to the design of photosensitive liposomes based on photoinduced cis-trans isomerization was proposed by Pidgeon et al. Retinoic acid was introduced into phospholipid molecules to serve as hydrophobic chain. Two different phospholipids were prepared according to this strategy: 1-palmitoyl-2-retionyl-glycero-3-phosphocholine (10) and 1,2-diretinonyl-glycero-3-phosphocholine (11) (Scheme 1.5). The lipids were prepared according to the procedure developed by Warner and Benson, in which glycerophosphocholine was coupled with the imidazolide of retinoic acid in the presence of sodium methylsulfinylmethide.

Retinoic acid is known to undergo cis-trans isomerization upon irradiation with 360 nm light and such structural changes in the bilayer components should disturb the bilayer causing the leakage of the liposome contents.

The liposomes were prepared from compounds 10 and 11 without addition of natural phospholipids. Experiments with fluorescent marker showed that liposomes prepared from both 10 and 11 released their contents rapidly and quantitatively upon irradiation. Liposomes prepared using lipid 10 showed somewhat higher photostability. Thus, for quantitative release of the fluorescent marker they required approximately three times longer period of irradiation than liposomes prepared with 11.

In 1989 Kusumi et al. published their data on photodesintegratable liposomes. Their strategy included a photoinduced formation of a polar moiety within the hydrophobic chains of the lipid. The authors synthesized phospholipids having 2-nitrobenzyl esters on the terminal carbons of both hydrophobic chains (Scheme 1.6). Upon irradiation the 2-nitrobenzyl esters were cleaved, thus forming two carboxylate ions on the terminal carbons of the hydrocarbon chains, which eventually led to the disintegration of the bilayer.
Phospholipid analog 12 was synthesized according to Scheme 1.7. Ortho-nitrobenzyl alcohol was reacted with dodecanoyl dichloride to afford ester 16, which was then coupled with glycerophosphocholine in presence of dicyclohexylcarbodiimide to afford final product 12.

Scheme 1.6.

Liposomes were obtained from pure 12 without addition of natural phospholipids. Electron microscopy showed complete disintegration of liposomes upon prolonged irradiation. Experiments with ovalbumin (protein with molecular weight of about 43 kilodalton) entrapped within the liposomes showed that after a brief period of irradiation 75% of protein were released. Interestingly, only 5% of phospholipid underwent the photochemical decomposition within this period. These results suggest that decomposition of even a small fraction of lipid can completely disintegrate the liposome.

Introduction of photolabile linker connecting the polar and nonpolar parts of the lipid molecule is a simple and straightforward strategy for the design of photocleavable lipids. However, this approach did not receive much attention until recently. The first attempt to use this strategy for photosensitive liposome design was reported by the Kutateladze group12. In 2002 Wan et al.
reported the successful synthesis of a photocleavable lipid based on a dithiane photolabile linker and the properties of liposomes prepared with this lipid.

Scheme 1.7.

In a subsequent publication the authors reported synthesis of a number of photocleavable amphiphiles based on a dithiane moiety. Synthesis of some of these phospholipids analogs is depicted in scheme 1.8. Generally synthesis of these compounds starts with coupling of an appropriate aldehyde with the lithium salt of the dithio compound. Free phenolic hydroxyl is then esterified with phosphocholine by treatment with cyclic chlorophospholane followed by trimethylamine. For lipid analog 27a 5-nitropyridine moiety is introduced to serve as internal sensitizer.

Phospholipid analogs 19a,b, 22 and 27a were used for preparation of photosensitive liposomes using mixtures of palmitoyl-oleoyl phosphatidylecholine, cholesterol and the photocleavable lipid. All these amphiphiles were shown to form stable liposomes, showing virtually no leakage of their contents in the dark.
Scheme 1.8

1) BuLi, OHC- \( \rightarrow \) BuLi, OHC-

2) NMe3

R = a) C\(_{12}\)H\(_{25}\); b) C\(_{14}\)H\(_{29}\)

OC\(_{18}\)H\(_{35}\) = \( \text{O} \_\text{O} \_\text{O} \)
In the presence of sensitizer dithio derivatives of carbonyl compounds undergo photoinduced oxidative cleavage releasing the carbonyl compound (this photochemistry is described in details in the next chapter). Thus, upon irradiation of compounds 19a,b, 22 and 27a their polar and nonpolar parts are disintegrated and the molecule, having lost its amphiphilic properties, can no longer support the bilayer structure.

To induce the photocleavage of molecules such as 19a,b and 22, the presence of a light absorbing molecule (sensitizer) is required. Water soluble sensitizers were shown to be ineffective. Apparently, being dissolved in the bulk solution, they simply can not reach the dithio moiety hidden in the hydrophobic layer of the membrane. In order to prepare photosensitive liposomes containing lipids 19a,b and 22 a sensitizer of hydrophobic nature had to be incorporated into the bilayer. Lipid analog 27a, on the other hand, has sensitizer “built in” to the molecule and, therefore, can be used for liposome preparation without addition of sensitizer.

Photoinduced leakage of contents of the liposomes was studied using a pulse field gradients (PFG) NMR technique for determining the self-diffusion coefficient of probe molecules entrapped within the liposomes. When the probe molecule is entrapped in the liposome its diffusion coefficient is equal to that of the carrier liposome, however, as soon as the molecule is released to the bulk solution its diffusion coefficient changes by orders of magnitude.

Using the PFG NMR technique it was shown that liposomes prepared with photosensitive phospholipids analogs 19a,b, 22 and 27a release their contents upon irradiation effectively. The new approach to design of photosensitive liposomes developed in Kutateladze group seems, despite of certain drawbacks, to be a promising direction in this area of photoscience.

In 2002 Nagasaki et al. attempted to use photocleavable lipid analogs for preparation of liposomes that could serve as gene delivery systems. Their approach to design of the
A photocleavable lipid was to introduce photolabile ortho-nitrobenzyl moiety between the polar and nonpolar parts of the lipid molecule.

Scheme 1.9

Nonpolar chains of this lipid analog are represented by two dodecyl hydrocarbon chains, whereas the polar head of the lipid is made of basic amino acids lysine or arginine, instead of the traditionally used phosphocholine moiety. Synthesis of lipid analog 31 started with amino acid 28. Compound 28 was nitrated and amino functionality was protected with tert-butoxycarbonyl derivative to give 29. Carboxylic acid 29 was than coupled with didodecyl amine and the tert-
butyloxy carbonyl protection was cleaved. The product thus obtained was coupled with lysine (a) or arginine (b). Final deprotection then lead to the desired products 32a and 32b.

Upon irradiation of amphiphiles 32a,b, the amino acids were released as amides and the whole molecule lost its amphiphilic properties. The transfection efficiency of liposomes prepared with compounds 32a,b were shown to be much greater with UV irradiation than that of liposomes prepared with lipofectin (commercially available mixture of lipids for liposome preparation).

A similar approach to photocleavable lipid design was used by Chandra et al.\textsuperscript{15} In their study a photocleavable lipid with only one hydrophobic “tail” was used. The polar part of the lipid was represented with acidic amino acids asparagine and glutamine. The synthesis and photochemistry of this lipid analog is depicted in scheme 1.10.

The course of synthesis of lipids 34a,b very much resembles that of compounds 32a,b. Lipids 34a was obtained in 53 % yield and lipid 34b in 64 % yield.

Scheme 1.10.
Liposomes were prepared with 95% (by weight) 1,2-distearoyl-glycero-3-phosphocholine and 5% photocleavable lipids 34a,b. The experiments with liposomes loaded with fluorescent marker showed efficient release of liposomal contents upon irradiation, whereas in the dark virtually no leakage was detected.

The studies of photosensitive liposomes, described above, have demonstrated that light can be successfully used for selective temporal and spatial release of water soluble agents encapsulated within the liposome. The next step in development of pharmaceutical application of these systems as drug delivery systems should be research focused on the design of photocleavable lipids that can be used in in vivo experiments. In particular, the photocleavable lipids and the products of their photodecomposition should be nontoxic. Another logical direction of photosensitive liposomes research is the use of two photon absorption phenomena. Near IR light is capable of penetrating the living tissues without significant absorption and scattering and, therefore, may represent a promising new way for in vivo photoinduced release of therapeutic agents.
References

Photolabile Protecting Groups

Over the last few decades photoremovable protecting groups have found many applications in organic synthesis. Since they do not require any specific reagent for cleavage, many photoremovable protecting groups have proved to be useful in development of new orthogonal protection strategies\(^1\).

The possibility that control of the release of biologically active compounds can be achieved within the living tissue using caged (photoactivatable) bioagents has provided a powerful new tool for studying the consequences of events followed by chemical signaling in cell biology and neuroscience\(^2,3\).

A good photoremovable protecting group should comply with the following requirements:

1) The photodeprotection reaction should be clean and have high quantum yield.
2) The chemical yield of photodeprotection should be close to quantitative.
3) The protected form of substrate should be stable in the dark.
4) The chromophore used for design of photoremovable protecting group should have high extinction coefficient at a wavelength where no other group would be damaged by the irradiation.
5) The byproducts of the photoreaction should be inert towards the substrate and have low extinction coefficient at wavelength of irradiation, so that they do not act as a photofilter. For biological application these byproducts must be nontoxic as well.
6) For time-resolved biological experiments the rate of release of the substrate should be higher than the rate of the response of the system.
None of the photoremovable protecting group developed to now meets all these requirements; however, some have been extensively used in organic synthesis and biological experiments with caged bioagents.

1. Benzyloxycarbonyl Protection

The first report of a photoremovable protecting group appeared in the literature in 1962. Barltrop and Schofield\textsuperscript{4,5} studied the photochemical behavior of glycine with the amino group protected with variously substituted benzyloxycarbonyl groups. They found that these derivatives of glycine can be photochemically cleaved with chemical yields up to 75\% (scheme 1.11). The quantum yield of this transformation was found to be dependent on the substitution pattern in the benzene ring, the highest quantum yield (0.28) was detected in the case of meta-methoxybenzyloxycarbonylglycine (Table 1). Later, in 1966, it was found by Chamberlin\textsuperscript{6} that the introduction of two methoxy substituents in the meta positions of the benzene ring of the benzyloxycarbonyl protecting group significantly increases its photoreactivity. The chemical yield for photorelease of several substituted amino acids varied in the range between 48 and 85\%.

\textbf{Scheme 1.11}

\begin{align*}
\text{O} & \quad \text{N} & \quad \text{OH} \\
\text{R} & \quad \text{OH} & \quad \text{H}_2\text{N} & \quad \text{CO}_2 \\
\text{254 nm, H}_2\text{O} & \quad \text{OH} & \quad \text{H}_2\text{N} & \quad \text{CO}_2
\end{align*}

Initially the mechanism of these transformations was assumed to involve heterolysis of the C-O bond with formation of benzyl carbocation. This assumption was mainly based on the three facts: 1) benzyl alcohol was found to be the main byproduct of the photodeprotection
reaction; 2) higher quantum yields were obtained for derivatives with electron donating substituents in the benzene ring (the meta-methoxy group is believed to have electron donating effect in the excited state); 3) quantum yields increased with an increase in the percentage of water in the media. The mechanism of this reaction was studied in detail by Pincock et al. in 1997. Their results suggest the homolytical cleavage of the C-O bond with formation of a radical pair followed by electron transfer to form an ion pair, whose participation in the mechanism was previously proposed.

Table 1.1

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<th>R</th>
<th>Quantum yield of photorelease of glycine.</th>
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<tbody>
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<td>H</td>
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<tr>
<td>p-NO₂</td>
<td>0.0055</td>
</tr>
<tr>
<td>m-OMe</td>
<td>0.28</td>
</tr>
<tr>
<td>p-OMe</td>
<td>0.17</td>
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2. o-Nitrobenzyl alcohol derivatives

The most popular and well studied type of photoremovable protecting group is derivatives of ortho-nitrobenzyl alcohol. The first attempt to use ortho-nitrobenzyl alcohol as a protecting group for carboxylic acids appeared in 1966. Barltrop et al. demonstrated that a number of carboxylic acids can be quantitatively released upon irradiation of their corresponding
ortho-nitrodiphenylmethyl esters (Scheme 1.12). The authors proposed a mechanism that includes formation of intermediate 5 by transfer of oxygen from an excited nitro group.

The phenyl group at the benzylic position of compound 4 was introduced in order to avoid formation of azobenzene-2,2'-dicarboxylic acid which acted as a light filter, thus significantly decreasing the yield of deprotection. The applicability of ortho-nitrodiphenylmethyl group in amine functionality protection as N-(o-nitrodiphenylmethyloxycarbonyl) derivatives has been demonstrated as well.

![Scheme 1.12.](image)

Various photoremovable protecting groups based on the ortho-nitrobenzyl alcohol chromophore have been designed over the last few decades. Some examples of their use are provided in Scheme 1.13. Introduction of a second ortho-nitrobenzyl radical at the α-position of ortho-nitrobenzyl alcohol was suggested by Patchornik *et al.* in 1970⁹. Such a symmetrical substitution pattern prevented the formation of diastereomers in the case of protection of optically active compounds. This protecting group has been tested on various carboxylic and amino acids (8a-c), giving photodeprotection yields close to quantitative.
The first example of a biologically active compound inactivated by a photosensitive protecting group (caged bioagent) was developed by McGray et al.\textsuperscript{10} In 1980 they presented
synthesis of adenosine triphosphate with a terminal phosphate esterified with ortho-nitrophenethyl alcohol (8f). Active ATP could be quantitatively released upon irradiation of this compound. Caged ATP has since been used by many groups to study the kinetics of the response of living cells and tissues on the appearance of active ATP.

In order to extend the wavelength of irradiation above 320 nm two methoxy groups were introduced into the benzene ring, thus forming the 4,5-dimethoxy-2-nitrobenzyl protecting group\textsuperscript{11}. It has proven to be useful for protection of hydroxyl functionalities of some carbohydrates (8h)\textsuperscript{12}, nucleosides (8i)\textsuperscript{13} and amino functionalities of various amino acids. The yields for photorelease were found to be close to quantitative even for the most light sensitive amino acid, tryptophan (8j)\textsuperscript{11}. In these studies the formation of undesired azobenzene byproducts was avoided using “aldehyde trapping” reagents such as hydrazine, hydroxylamine hydrochloride or semicarbazide hydrochloride.

The quantum yield of photocleavage of ortho-nitrobenzyl esters usually varies from 26 to 65% depending on the substitution pattern in the benzene ring of the protecting group and nature of substrate. In 1991 Frechet \textit{et al.} attempted to investigate the relationship between the efficiency of the photoreaction and the structure of ortho-nitrobenzyl alcohol\textsuperscript{14}. Several cyclohexyl amines protected with differently substituted orthonitrobenzyl carbamates were synthesized and the quantum yields of cyclohexylamine photorelease were measured. The highest quantum yield (62%) was detected for N-[(2,6-dinitrobenzyl)-oxy]carbonyl cyclohexyl amine. Overall the authors concluded that the quantum yield of this photoreaction is affected by a complex combination of steric and electronic factors.
Scheme 4.
One of the most impressive applications of ortho-nitrobenzyl protection in synthesis was reported by the Nicolaou group. Unsubstituted orthonitrobenzyl alcohol was used to protect the anomeric center of a carbohydrate in the course of synthesis of the antibiotic calicheamicine $\gamma_1$ (Scheme 1.14)$^{15}$. Ortho-nitrobenzyl protection was introduced in an early step of the synthesis and was shown to survive such harsh conditions as treatment with methoxide, bromine and diisobutylaluminium hydride. The selective removal of orthonitrobenzyl alcohol (85%) allowed for liberation of anomeric hydroxyl, to which the enediyne fragment was attached.

Ortho-nitrobenzyl caged ATP (8f on the Scheme 1.15) has also served as a model compound for studies of the mechanism of the photodeprotection reaction. Walker et al.$^{16}$ utilized laser flash photolysis techniques to study this reaction. Their findings suggested a two step mechanism at neutral pH: the first is release of the proton immediately after excitation (monitored using indicator dye); in the second, slower step ATP and 2-nitrosoacetophenone are concomitantly released. The mechanism consistent with their results is depicted in scheme 5. The excited state of caged ATP forms so-called aci-intermediate 10, which quickly ionizes to form 11 (decay of aci-form was monitored by LFP). Intermediate 11 exists in fast equilibrium
with its cyclic form 12. The rate limiting step of the reaction is the formation of 2-nitrosoacetophenone with concomitant release of ATP and decay of aci-form 10 (TRIR). At pH close to neutral the reaction was accelerated by the presence of acid. This fact was attributed to protonation of the phosphate, which favors the ATP leaving process.

However, the mechanism described was not general and contradicted some experimental findings, such as acid catalysis for non-basic leaving groups, and ab initio calculations that predict much higher potential barrier for cyclization of ionized aci-form 11, than that for aci-form 10\textsuperscript{17}. In 2003 a revised mechanism was proposed by Il’ichev et al.\textsuperscript{18} This mechanism was based on the study of reaction of 2-nitrobenzyl alcohol methyl ether in non-aqueous and aqueous solvents at different pH. The new revised mechanism is depicted in scheme 1.16.

Scheme 1.16.
The authors proposed that the aci-form 16 plays the role of both acid and base, thus producing intermediates 15 and 17; the introduction of intermediate 17 explains the acid catalysis of disappearance of 16 in aprotic solvents where ionization of 16 does not occur. Cyclization of intermediate 16 and hydration of intermediate 17 is assumed to proceed irreversibly. The consequent formation of hemiacetal 21 was proven to be the rate limiting step in this reaction.

The ortho-nitrobenzyl protecting group and its numerous derivatives have been proven to be very applicable in organic synthesis; however, for its use in cell biology to study the response kinetics of a living cell to rapid release of a biologically active compound, the applicability of this group is limited. The photodeprotection reaction proceeds through hemiacetal or hemiketal intermediate such as 21. Under physiological conditions the lifetimes of these intermediates were found to be in the range of seconds\textsuperscript{18}, which apparently makes the ortho-nitrobenzyl group inapplicable for most time resolved experiments.

Scheme 1.17.
In 1997 Hasan et al. discovered yet another photoremoveable protecting group utilizing the ortho-nitrobenzyl chromophore. In this case the substrate is attached to the \(\beta\)-position (scheme 1.17). The new protecting group was tested for photolabile protection of the 5'-hydroxyl group of thymidine. The chemical yields of photodeprotection reaction were found to be close to quantitative, whereas quantum yields varied depending on substitution pattern in the benzene ring (3-35%).

Scheme 1.18

One more useful application of ortho-nitrobenzyl chromophore is as a cage for \(\text{Ca}^{2+}\) cations. In 1988 two different series of photosensitive \(\text{Ca}^{2+}\) chelating agents were developed. They were designed on the base of known BAPTA (1,2-bis(2-aminophenoxy)ethane-
N,N,N',N'-tetraacetic acid) and EDTA\textsuperscript{21} (ethylenediamine tetraacetic acid) chelators. In the "nitr" series of compounds (26) the carbonyl moiety formed upon irradiation decreases the electron density on the carboxyl oxygens, thus, decreasing by 40-fold the affinity of the chelate molecule to calcium cation. The second group of compounds, based on DM-nitrophen (28) is simply cleaved upon irradiation loosing its ability to chelate Ca\textsuperscript{2+} (Scheme 1.18).

The main advantage of the "nitr"-cages over DM-nitrophen is their remarkable selectivity for calcium ion, whereas DM-nitrophen demonstrates much higher affinity change upon photolysis and reacts with a higher quantum yield.

The photosensitive calcium chelators described above, as well as their later modifications have been used extensively over the last two decades. They have found many applications in the study of such physiological processes as muscle contraction and neurotransmitter release\textsuperscript{22}.

3. Benzoin group.

Scheme 1.19.

\begin{center}
\[
\begin{array}{c}
\text{O} \\
\text{X} \\
\text{O} \\
\end{array}
\xrightarrow{h\nu}
\begin{array}{c}
\text{O} \\
\text{X} \\
\text{O} \\
\end{array}
\]
\end{center}

\(X = \text{OTos, Cl, OAc}\)

In 1964 Sheehan \textit{et al.}\textsuperscript{23} reported an interesting behavior of benzoin esters and other desyl systems upon irradiation. The authors detected formation of 2-phenylbenzofurane 31 upon irradiation of benzoin chloride, tosylate and acetate (Scheme 1.19). The yield of benzofuran
product was very much dependent on the substitution pattern and nature of the media and for most of the cases did not exceed 40%.

Scheme 1.20.

In a later publication they found a substitution pattern favoring benzofuran formation and proposed to use 3',5'-dimethoxybenzoin as a photoremovable protecting group (Scheme 1.20). Upon irradiation of 3',5'-dimethoxybenzoin acetate and 3',5'-dimethoxybenzoin phthaloyl glycinate, 2-phenyl-5,7-dimethoxybenzofurane was formed in 95% yield, and phthaloylglycine was isolated in 87% yield.

Over the last decade 3',5'-dimethoxybenzoin and unsubstituted benzoin became commonly used photoremovable protecting groups. Some examples of their use are collected on the scheme 1.21. In 1992 benzoin protected adenosine cyclic 3',5'-monophosphate was synthesized by Givens et al. This compound was shown to release cAMP quantitatively upon irradiation with 350 nm light with a quantum yield 36%.
A series of experiments with caged neurotransmitters were conducted by Gee et al. in 1996. This authors have shown that glutamic and γ-aminobutyric acid can be released from
their caged analogues 35 and 36 with quantum yield 14%. Another part of their study is evaluation of the biological response of pyramidal neurons preincubated with benzoin glutamate 35 following irradiation with 351-364 nm light. It was shown that 8 µs laser pulses caused an action potential from direct stimulation of glutamate receptors of neurons.

A new interesting application of photoremovable protecting groups was proposed by Bochet’s group in 200028,29. It was shown that two photoremovable protecting groups, 3',5'-Dimethoxybenzoinyl and 4,5-dimethoxy-2-nitrobenzyl could be removed orthogonally. Thus, upon irradiation with 420 nm light (3’5’-dimethoxybenzoinyl does not absorb at this wavelength) compound 37 selectively releases one of its carboxy groups to form 38, whereas upon irradiation with shorter 254 nm wavelength only the benzoin protection is cleaved to form compound 39.

Scheme 1.22.

The rate of the release of the substrate from the benzoin protected derivatives was measured to be 2.9e8 s\(^{-1}\) for caged cAMP\(^{25,26}\) 34 and 1.2e-7 s\(^{-1}\) for glutamate 35\(^{27}\). The first
mechanistic view of benzoin esters photocleavage was proposed in 1971 by Sheehan et al. (Scheme 1.22)\textsuperscript{24}. In their mechanism the excited singlet state of starting benzoin reacts via the Paterno-Büchi pathway to form housane intermediate 44. Subsequent ring opening and loss of acetate give rise to final products of the reaction. More recent modifications of this mechanism include formation of ketocation 45 either by heterolysis of C-O bond or by homolysis followed by electron transfer. Cation 44 then reacts to give cation 46, which, upon deprotonation, forms final benzofuran 47\textsuperscript{30,31}.

Scheme 1.23.

In 1997 Shi et al. investigated photolysis of various 3',5'-dimethoxysubstituted benzoin esters using nanosecond laser flash photolysis technique\textsuperscript{32}. Their findings suggested the mechanism shown in scheme 1.23. The electron deficient n-\pi\textsuperscript{*} excited state of the acetophenone chromophore of the starting benzoin forms an intramolecular exiplex with the electron rich 3',5'-dimethoxybenzene ring. The exiplex can then return to the starting benzoin ester or react to form
carbocation 50 (transient was monitored at 330 and 420 nm, $k_d = 10^6 \text{ s}^{-1}$), releasing the anion.

Cation 50 then undergoes deprotonation to form benzofuran 51.

Scheme 1.24.

The mechanism described above, however, only explains the photochemical behavior of 3',5'-dimethoxysubstituted benzoin esters; the first report on the mechanistic study of photochemical transformations of unsubstituted benzoin esters appeared in 2001. Rajesh et al. investigated photolysis of benzoin diethyl phosphate using nanosecond and picosecond laser flash photolysis\textsuperscript{33}. According to their study, the reactive state in this photoreaction is a triplet and
it proceeds via two competing pathways (scheme 1.24). In most of the solvents (aqueous acetonitrile, methanol, water etc.) the excited triplet state (which decays too fast at room temperature, but can be monitored at -100ºC) cyclizes to form byradical 55, which eliminates diethyl phosphoric acid so fast that it can not be observed as an intermediate.

In fluorinated solvents such as trifluoromethyl alcohol and hexafluoroisopropanol, the reaction takes a different pathway that leads to the formation of the product of solvolysis 58. The authors proposed a different mechanism for these conditions. The first step is formation of triplet cation 54 that has absorbance at 570 and 660 nm with a lifetime about 500 ns. The rate of decay of 54 is determined by the rate of triplet to singlet intersystem crossing. Once the singlet cation 55 is formed it’s immediately quenched by a solvent molecule to form the final product 58.

Overall, the benzoin chromophore has many advantages as a photoremovable protecting group: the release of substrate is fast, efficient and in most cases quantitative. However, this protecting group also has certain disadvantages. Benzoins can only be used as protecting group for acids, since attempts to use benzoin carbonate derivatives to protect alcohols and amines failed34. Another serious problem, which has been encountered by some authors, is poor stability of benzoin esters toward hydrolysis35; this is a serious drawback for use of benzoins as caging groups for biologically active compounds.

4. para-Hydroxyphenacetyl group

In 1996 Givens et al. reported a new photoremovable protecting group based on the para-hydroxyphenacetyl chromophore36. It was shown that para-hydroxyphenacetyl derivatives of many biologically active acids can be efficiently released upon irradiation. In more recent works by the same authors para-hydroxyphenacetyl protected adenosinetriphosphate36,37, dipeptide AlaAla38,39, γ-aminobutyric acid38 and the nonapeptide hormone Bradykinin39 were all shown to release their
substrates quantitatively upon photolysis, with quantum yields varying depending on the substrate (Scheme 1.25). Other products of photolysis include para-hydroxybenzoic acid, para-hydroxyacetophenone and 1,4-di-(parahydroxyphenyl)butanedione-1,4.

Scheme 1.25.

\[ \text{HOOCArgProSerPheGlyProProArgNH}_2 \xrightarrow{h\nu} \text{HOOCArgProSerPheGlyProProArgNH}_2 \phi = 20\% \]

The first mechanism for this reaction was proposed by Givens et al.\textsuperscript{37} Their findings suggested that reactive excited state in this reaction is triplet (quenching studies with piperylene and oxygen) and the rate of release of ATP was found to be $5.5 \times 10^5 \text{ s}^{-1}$. According to this
mechanism, the release of the substrate is concomitant with triplet decay. The biradical 64 formed after release of substrate then forms cyclic intermediate 66 (attempts to observe this intermediate by TRIR, however, failed) which upon solvolysis produces the final product (Scheme 1.26).

Scheme 1.26.

Among the advantages of the the parahydroxyphenacyl group are:

1) Solubility of its derivatives in aqueous media and hydrolytic stability.

2) The main photobyproduct, parahydroxyphenylacetic acid, has a blueshifted absorbance compared to the starting phenacyl derivative, and therefore it cannot act as a light filter.

3) Release of substrate is fast.

The main disadvantage of this group is low absorbance at wavelengths above 320 nm. Attempts to prolong the wavelength of irradiation were made introducing two methoxy groups into the benzene ring. That, however, reduced the quantum yield dramatically\textsuperscript{40}. Another feature of this group that must be counted as a disadvantage is the dependence of the quantum yield on the nature of the substrate, thus, $\gamma$-parahydroxyphenacyl glutamate is released upon irradiation
with a quantum yield of only 8%\textsuperscript{38}, whereas for other substrates such as ATP or AlaAla the quantum yield of photorelease was found to be around 30%.

5. Coumarinyl group

The possibility of using a coumarin chromophore for the design of photoremovable protecting groups had been discovered only relatively recently. In 1998 Furuta \textit{et al.} suggested using 7-hydroxycoumaryl-4-ylmethyl group as photoremovable protection for carboxyl and amino functionalities\textsuperscript{41} (Scheme 1.27).

Scheme 1.27.

Compounds 67 and 70 were shown to release glutamic acid quantitatively upon excitation with 365 nm light. The quantum yield of this reaction was found to be modest (only 1.9%), however, their high two photon absorption cross section makes these compounds unique, due to the possibility of using long wavelength (640, 800 nm) for deprotection. The uncaging action cross section (product of two photon absorption cross section and quantum yield of deprotection) for these to compounds was found to be in the range of 0.9 GM (1GM = 10^{-50} cm^4 s/photon) for
740 nm and 0.4 GM for 800 nm, which is significantly higher that the values found for other photoremovable protection groups.

The discovery of 7-hydroxycoumaryl-4-ylmethyl group awoke the interest among photoscientists to the coumarin chromophore. In 2002 Eckardt et al. published their results on cAMP caged with differently substituted coumarins\textsuperscript{42}. They found that cAMP can be quantitatively released from its caged derivatives \textbf{71a} and \textbf{71b} with quantum yields 22-27\% (scheme 1.28).

Scheme 1.28.

\begin{center}
\includegraphics[width=0.5\textwidth]{scheme1.png}
\end{center}

\textit{a: R = Me; b: R = Et}

The special advantage of this chromophore is that the photobyproducts \textbf{72a} and \textbf{72b} have strong fluorescence which can be used to monitor the progress of the photodeprotection reaction. Later in 2003 the same chromophore was used to prepare caged ATP, ADP and AMP\textsuperscript{43}. These compounds were shown to liberate their substrates quantitatively; however the quantum yields of these reactions were found to be substantially lower than that of caged cAMP \textbf{71}. Schönleber et al. in 2002 used the same chromophore to synthesize caged cytosinediphosphate (CDP)\textsuperscript{44}. This compound was shown to release CDP under irradiation with 436 nm light with the rate constant 2e8 s\textsuperscript{-1}. However, the quantum yield again was rather poor: only 2.9\%. 
Scheme 1.29.

\[
\begin{align*}
\text{(72)} & \quad \text{X} = \text{C}_6\text{H}_{13}\text{COO} \\
\text{(a)} & \quad \text{MeO} \\
\text{(b)} & \quad \text{CN} \\
\text{(c)} & \quad \text{MeSO}_3 \\
\text{(d)} & \quad \text{OP(0)(0Et)}_2 \\
\end{align*}
\]

\[
\begin{align*}
\text{(73)} & \quad \overset{1^*}{\text{MeO}} \\
\text{(74)} & \quad \overset{\text{MeO}}{\text{OHX}} \\
\text{(75)} & \quad \overset{\text{MeO}}{\text{OX}} \\
\text{(76)} & \quad \overset{\text{MeO}}{\text{HOX}} \\
\end{align*}
\]
The first studies on the mechanism of coumaryl ester photocleavage were published by Schade et al. in 1999\textsuperscript{45}. The authors studied several derivatives of 7-methoxy-4-methylcoumarin (Scheme 1.29). The studies of photochemistry of 7\textsuperscript{2e} in \textsuperscript{18}O labeled water showed no incorporation of label into the diethylphosphoric acid released. The quantum yield of photoreaction was shown to increase as the polarity of the media and leaving group ability increased. These data suggested an ionic mechanism. The authors, therefore, proposed a photoinduced S\textsubscript{N}1 cleavage of starting material with formation of ion pair 74. The ion pair either recombined to go back to starting compound or escaped the solvent cage and after reaction with water formed the products of photoreaction.

Overall the coumaryl group is very promising in terms of biological applications, in particular, because of the possibility of using two photon absorption. Near IR light is capable of penetrating the living tissue without being absorbed or scattered. Another advantage of two photon excitation is that uncaging can be performed in a precise spatial manner: the probability of simultaneous absorption of the two photons depends quadratically on the light intensity, and, therefore, the uncaging reaction will only take place in the focal volume.

The main disadvantage of this group is quantum yield. It is generally low and very much dependent on the leaving group and the nature of the media; this makes the coumaryl group inapplicable for use in organic synthesis.

6. Other photoremovable protecting groups using benzyl carbocation formation as a driving force

In 1998 Misetic et al. discovered that 9-phenylxanthen-9-yl (pixyl) group, used previously to protect the 5'-position in nucleosides could also be removed photochemically
(Scheme 1.30). These authors showed that a number of pixyl protected alcohols, including 2-deoxythymidine can be released photochemically under irradiation at 254 or 300 nm. The chemical yields were found to be in the range between 78 and 97%, but quantum yields were not reported.

In subsequent work a number of substituted S-pixyl analogs have been studied using 2′-deoxythymidine as the protected substrate. As a result of this study compounds 79a-e (Scheme 1.30 and Table 1.2) were found to be promising photoremovable protecting groups for 5′-hydroxyl in nucleosides. The efficiency of the photodeprotection reaction with substituted S-pixyl group was shown to be significantly higher than that for the pixyl group. But again authors did not measure the quantum yield of the reaction; instead they used t_{1/2} (time for 50% recovery of substrate), apparently always using the same Rayonet setup.

Scheme 1.30

![Scheme 1.30](image)

Another advantage of the S-pixyl analogs over the “regular” pixyl group is that the wavelength of irradiation could be moved up to 350 nm, thus, compounds 78d,e showed good chemical yields of deprotection and good t_{1/2} values upon irradiation with 350 nm light.
Scheme 1.31.

Table 1.2.

<table>
<thead>
<tr>
<th>compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>t₁/₂ (min)</th>
<th>Yield of deprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td>78a</td>
<td>MeO</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4.8</td>
<td>93 %</td>
</tr>
<tr>
<td>78b</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4.3</td>
<td>95 %</td>
</tr>
<tr>
<td>78c</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>4.2</td>
<td>92 %</td>
</tr>
<tr>
<td>78d</td>
<td>H</td>
<td>H</td>
<td>MeO</td>
<td>H</td>
<td>7.3 *</td>
<td>99 %</td>
</tr>
<tr>
<td>78e</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>H</td>
<td>11.2 *</td>
<td>97 %</td>
</tr>
</tbody>
</table>

(*) irradiation using 350 nm wavelength.

The mechanism of the photoreaction presumably includes heterolysis of the C-O bond with formation of pixyl carbocation. This assumption is supported by the fact that efficiency and chemical yield of photodeprotection reaction is strongly decreased when the reaction is conducted in less polar media.
Another photoremovable protecting group for alcohols was designed by Furuta et al. in 2001. Anthraquinon-2-ylmethoxycarbonyl (Aqmoc) protected galactose derivative 80a and adenosine 80b were synthesized and their photochemistry studied. The Aqmoc protected substrates were released upon irradiation with satisfactory (68-91%) chemical yields, and quantum yield of disappearance of starting material 80a were found to be 10% (scheme 1.32).

7. Sisyl (tris(trimethylsilyl)-silyl) protecting group

In 1997 Brook et al. discovered that sisyl protection can be photolytically removed to release free alcohols in 62-95% yield. Despite the absence of any π-systems in the molecule some sisylated derivatives have significant absorption at wavelength as long as 250-300 nm (due to σ-conjugation).
8. Photoremovable protecting groups based on electron transfer chemistry

The possibility of utilizing the photoinduced electron transfer reaction in design of photoremovable protecting groups has been recognized recently\(^{51}\). The general mechanism is depicted on the scheme 1.33.

Scheme 1.33

\[
\begin{align*}
S^* + PC &\xrightarrow{hv} \text{ET} \quad S^+ PC^+ \\
S^* + PC &\xrightarrow{RET} S^+ PC^- \\
\text{rxn} &\quad \text{CH}
\end{align*}
\]

The sensitizer (S) absorbs light forming the excited state (S\(^*\)), which reacts with the protected substrate (PC) to form an ion radical pair via electron transfer. The ion radical pair then either undergoes reversed electron transfer or reacts to form products of deprotection.

The important advantage of this strategy is the possibility to decouple the photon absorption and bond dissociation step.

1. Sulfonates

The first report on the electron transfer based photoremovable protection appeared in 1985. Hamada and coworkers discovered that tosylamides can release free amine upon irradiation in the presence of an electron rich aromatic compound and secondary reductant (sodium borohydride or ascorbic acid)\(^{52}\).

A number of various tosylamides and sensitizers were tested showing that the chemical yield of amine released can be as high as 88\%. The proposed mechanism suggests that the sulfonamide radical anion created by photoinduced electron transfer decomposes to produce
amine, hydroxide anion and sulfanyl radical. The secondary reductant then captures the sulfanyl
radical and sensitizer radical cation to prevent undesired radical reactions (scheme 1.34).

Scheme 1.34

\[
\text{ArH} \xrightarrow{\text{hv}} \text{ArH}^+ \\
\text{ArH}^+ + \text{ArH} \xrightarrow{\text{ET}} \text{ArH}^+ + \text{ArH} \\
\text{ArH}^+ + \text{H}_2\text{O} \xrightarrow{\text{NaBH}_4 \text{ or ascorbic acid}} \text{ArH} \\
\text{ArH}^+ + \text{OH}^- \xrightarrow{\text{H}_2\text{O}} \text{ArH} \\
\text{ArH} = \begin{array}{c}
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\text{OMe} \\
\end{array}
\]

\( R_1 = \text{H; Me} \)
\( R_2 = \text{PhCH}_2\text{CH}_2 ; \text{PhCH}_2 ; \text{Ph} \)

In subsequent studies, an attempt to incorporate the electron rich photosensitizer into the
sulfanyl group was made\(^5\). Thus, 4-(4,8-dimethylxynaphthylmethyl) benzenesulfonyl (DNMBS)
protecting group was synthesized. It showed relatively good chemical yield for release of some simple amines (77-91%), whereas the quantum yield was found to be 65% (scheme 1.35).

Scheme 1.35

One of the examples of the successful application of this protecting strategy was discovered by Brunco et al. in the synthesis of tryptophan derivatives such as 83 (Scheme 1.36)^54.

Scheme 1.36

The attempt to release amino acids from their DNMBS derivatives revealed the main disadvantage of this protecting strategy. DNMBS protected glycine could only be released with very poor chemical yield; this was attributed to secondary oxidation of carboxyl with consequent decarboxylation, by one of the radicals generated in electron transfer process^55.
2. Dithianes.

In a series of articles Kutateladze et al. investigated the possibility of photorelease of ketones and aldehydes from their dithiane derivatives\textsuperscript{56-58}. The photorelease is achieved using the triplet state oxidant benzophenone. The mechanism of the reaction (studied by means of LFP) is depicted in scheme 1.37\textsuperscript{57}. Benzophenone triplet exited state abstracts an electron from the substrate molecule forming an ion radical pair. The substrate radical cation then loses a proton to the benzophenone radical anion to form the zwitterion radical 87, which undergoes intramolecular electron transfer followed by fragmentation to form either the products of deprotection 90 or decomposition 91. The latter process becomes major for some $\alpha$-branched aliphatic aldehydes\textsuperscript{59}.

Scheme 1.37

\[ \text{Ph}_2\text{CO} \xrightarrow{\text{hv}} \text{Ph}_2\text{CO}^- \]

\[ \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \xrightarrow{\text{R}_1\text{R}_2\text{S}} \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \]

\[ \xrightarrow{\text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}}} \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \]

\[ \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \xrightarrow{\text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}}} \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \]

\[ \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \xrightarrow{\text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}}} \text{R}_1\text{R}_2\text{S} \overset{\text{h}^+}{\text{OH}} + \text{Ph}_2\text{CO}^- \]

\[ \text{R}_2 = \text{H} \]

\[ \text{R}_1 + \text{S} \text{CHO} \]

\[ \text{R}_1 \]

(84) (85) (86)

(87) (88)

(89)

(90) (91)
The 1,3-dithioprotection was tested photolytically on a number of ketones and aldehydes generally giving high chemical yields of photodeprotection (92-97%). Lower yields (35-38%) were obtained for aliphatic aldehydes\textsuperscript{56,57}. The photochemistry associated with photoinduced electron transfer in dithianes was also successfully applied in the design of photocleavable liposomes\textsuperscript{58}.

It was also discovered that the phenacyl group is also capable of being cleaved under photoinduced electron transfer conditions. In the work of Banerjee \textit{et al.} it was shown that sensitized photolysis using electron rich aromatic compounds (for instance N,N,N’,N’-tetramethylbenzidine) phenacyl protected carboxylic acids can be released with chemical yields varying from 70 to 100\%\textsuperscript{61}. A serious drawback for this technique was also discovered. Substrates with easily reduced functionalities (bromides, nitrates, $\alpha,\beta$-unsaturated esters) could not be deprotected using this protocol, presumably, because they compete for electrons from the sensitizer, and thus undergo sidereactions when reduced.

The mechanism suggested by Banerjee \textit{et al.} is depicted in scheme 1.\textsuperscript{38,61}. The excited state of sensitizer and the protected substrate undergo an electron transfer reaction to produce a phenacyl ester radical anion. Consequent elimination of carboxylate anion produces phenacyl radical. The latter abstracts hydrogen from the sensitizer radical cation to form acetophenone and cation 96, which then hydrolyzes to form N-methylaniline.

The proposed mechanism was supported by the facts that N-methylaniline indeed formed as a byproduct and the yield of the reaction decreased when a sensitizer incapable of H atom transfer was used.
Conclusions.

Photoremovable protecting groups is a fast growing field of photochemical sciences. Initially photoremovable protections were to be used in organic synthesis. This application requires most of all high chemical yields in the protection and deprotection steps and high quantum yield of deprotection, whereas the rate of photorelease and reactivity and toxicity of sideproducts are not considered to be matters of such importance. Derivatives of ortho-nitrobenzyl alcohol, para-hydroxyphenacyl and the dithiane moiety are the most ideal candidates for use in organic synthesis.

In contrast, the use of caged bioagents in biological research is a relatively new idea. The requirements for the caging group to be used in biological applications involve a high rate of photorelease of the biologically active compound, low toxicity of sideproducts (especially for in
vivo experiments) and long wavelength of irradiation. Among the caging groups developed by now the benzoin, phenacyl and coumaryl groups are very promising in terms of release of carboxylic acids or phosphates; however, for other functionalities such as amines and alcohols these groups are not as applicable. Using these groups for caging alcohols or amines requires connection via carboxylate or carbamate and, therefore, the rate of photorelease becomes dependent on the rate of decomposition of carbonate derivative formed, which often is not fast enough.

Another promising direction in the development of new caging groups is the use of two photon absorption. This technique allows the use of long wavelength light (near IR) which is capable of penetrating living tissues, and the uncaging process can be localized in a very precise spatial manner.

Thus, the future perspectives of photoremovable protecting (caging) groups research lies in the development of new groups that can be used for direct protection of alcohols, amines and phenols as well as the design of protecing groups capable of two photon absorption.
References


CHAPTER II
RESULTS, DISCUSSION AND EXPERIMENTAL PART

Design and Synthesis of a New Photocleavable Lipid Analog

The structural design of a photocleavable lipid includes three structural components: a polar “head” (e.g. phosphocholine moiety, blue in Scheme 2.1) and two nonpolar “tails” (hydrocarbon chains, black on Scheme 2.1), connected by a photocleavable linker (red on Scheme 2.1). Excitation of the photolabile linker will lead to disassembly of polar and nonpolar parts of the molecule and, therefore, loss of the amphiphilic properties.

The photolabile linker to be used in the design of the photocleavable lipid has to meet the following requirements: 1) it has to have appropriate functional groups to attach all the necessary fragments; 2) it has to provide a connection between the polar and nonpolar parts that is hydrolytically stable; 3) the quantum and chemical yields of cleavage of the linker should be high; 4) the excitation wavelength must be such that lipid parts other than the linker would not be affected by the light.

Among the known photolabile linkers and protecting groups benzoin esters seem to fit these requirements better than others\(^1\). All necessary fragments can be attached to the benzoin moiety via ether or ester bonds (Scheme 2.1). Quantum yield of release of different kinds of phosphates from its benzoin esters were shown to be in the range of 30-35%, and chemical yields were found to be close to quantitative\(^2\). An excitation wavelength of 350 nm can be used for its cleavage. At this wavelength light is unlikely to affect any other structural fragments of the lipid molecule.

The photochemistry of benzoin esters depends strongly on the character of substitution in the aromatic rings: thus, 3’,5’-dimethoxy and 2’,3’-dimethoxysubstituted as well as unsubstituted
benzoin esters are known to undergo photocyclization with formation of benzofuran. However, introduction of other substituents such as a nitro group or halogen leads to formation of Norrish type 1 photocleavage products upon irradiation.

For our goals we needed the substitution pattern to be 3’,4’-, in order to make both polar tails of the lipid molecule be oriented in a parallel direction (Scheme 2.1).

Scheme 2.1.

Naturally occurring lipid phosphatidyl choline (1) and photocleavable lipid designed based on 3’,4’-disubstituted benzoin moiety (2).

Since the photochemistry of 3’,4’-disubstituted benzoins was not known, we had to prepare a model compound in order to study its chemical and photochemical properties. We have selected 3’,4’-dimethoxybenzoin diethylphosphate to be this model compound. It was synthesized starting with 2-phenyl-[1,3]dithiane (4) (Scheme 2.2). Compound 4 was coupled with 3,4-dimethoxybenzaldehyde (3) to produce a protected benzoin precursor 5. Attempt to cleave dithioacetal group oxidatively using [bis(trifluoroacetoxy)iodo]benzene led to the formation of a mixture of desired benzoin 6 and the overoxidation product 7. In order to avoid the undesirable oxidation of hydroxyl it was protected as an acetate. The dithioacetal group was
then cleaved with [bis(trifluoroacetoxy)iodo]benzene to give benzoin acetate 9 in 93% yield. The acetate protecting group was cleaved under mild basic conditions (diluted potassium carbonate in aqueous methanol) to again give a mixture of (6) and (7). In order to avoid the exposure of benzoin (6) to air (which, apparently, causes its oxidation) it was taken to the next step without isolation*.

Scheme 2.2

*A control experiment was conducted to show that benzoin 6 can be oxidized to diketone 7 by oxygen from the air. A mixture of compounds 6 and 7 was exposed to oxygen gas, which lead to disappearance of 6.
Final product was obtained by treating the hydrolysis mixture directly with diethylchlorophosphate in pyridine as phosphorilating agent in 52% yield over the two steps.

A study of the photochemistry of 3’-4’-dimethoxybenzoin diethyl phosphate included: 1) steady state photolysis monitored by UV spectrophotometry; 2) photolysis monitored by following the pH changes in the reaction mixture; 3) study of the photoproduct composition by means of GC/MS and 4) measurements of quantum yield of disappearance of starting material (10).

Figure 2.1. Steady state photolysis of 10 monitored by UV spectrophotometry.

As it was previously discussed in the literature review section the benzoin esters undergo photocyclization reaction to form a benzofuran derivative and release the acid\textsuperscript{3}. For our model compound the photoreaction was expected to produce benzofurans 11 and 12 with release of diethyl phosphoric acid 13 (Scheme 2.3). Steady state photolysis (irradiation at 350 nm) monitored by UV spectrophotometry showed appearance of a new absorbing band around 300 nm, which is characteristic for benzofuran chromophore (Figure 2.1). However, other features of
the UV spectra of photoproducts such as an absorption band above 300 nm did not match the data reported in the literature\textsuperscript{4}.

Monitoring the pH of the photoreaction mixture showed a significant decrease of the pH as the irradiation proceeded. This was attributed to the appearance of diethylphosphoric acid. At the same time, a control experiment showed appearance of the acid in a solution kept in darkness. The rate of the dark hydrolysis of compound 10 was measured by following change in the UV spectra. The rate constant was found to be 1.01e-3 s\textsuperscript{-1} (Figure 2.2).

Figure 2.2. Measurement of rate constant of dark hydrolysis of 10.

The chemical yield for release of the diethyl phosphoric acid upon irradiation of compound 10 was found to be 56\% by acid/base titration of the reaction mixture after irradiation. GC/MS analysis of the reaction mixture after irradiation showed the presence of two peaks with mass spectra corresponding to compounds 11 and 12, but also a number of peaks corresponding to products of Norrish type I reaction (scheme 2.3).
Scheme 2.3.

\[
\begin{array}{c}
\text{MeO} \quad \text{OMe} \\
\text{Ph} \quad \text{O} \\
\text{EtO} \quad \text{Et} \\
\end{array}
\xrightarrow{350\text{nm}}
\begin{array}{c}
\text{MeO} \quad \text{OMe} \\
\text{Ph} \quad \text{O} \\
\text{C}_6\text{H}_6 \quad \text{OH} \\
\end{array}
\]

\[+ \quad \text{Norrish type 1 products}\]

The quantum yield of the disappearance of starting material 10 upon irradiation with 350 nm light was measured by following the changes in UV absorbance and was found to be 0.13%.

Based on these results (low quantum and chemical yields, poor hydrolytic stability) we concluded that 3’,4’-disubstituted benzoin can not play the role of photolabile linker in the design of a photocleavable lipid.

Scheme 2.4.

Naturally occuring lipid phosphatidyl choline (1) and photocleavable lipid designed based on 3’,5’-disubstituted benzoin moiety (14).

As was mentioned in the literature review, 3’,5’-dimethoxybenzoin esters are known to undergo photocyclization accompanied by release of the acid. This process is known to be
efficient in terms of both quantum and chemical yields\textsuperscript{3}. Design of the photocleavable lipid based on the 3’,5’-disubstituted benzoin chromophore still requires parallel alignment of hydrocarbon tails, therefore they both have to be placed on the same substituent (Scheme 2.4, compound 14). In this case we did not have to prepare and study model compounds, because the photochemistry of the 3’,5’-disubstituted benzoin chromophore is well known.

Synthesis of compound 14 starts with 3,5-dimethoxybenzaldehyde (Scheme 2.5). One of the methoxy groups was hydrolyzed by sodium ethanethiolate and than protected with a tert-butyldimethylsilyl group. Benzaldehyde thus obtained was coupled with 2-phenyl-[1,3]dithiane lithium salt, followed by acetylation to give benzoin precursor 15. The tert-butyldimethylsilyl group was cleaved with tetrabutylammonium fluoride to provide 16.

Hydrophobic tails of the lipid analog are represented by two 12-C hydrocarbon chains attached to one of the phenol hydroxyls of the benzoin chromophore. Pentacosan-13-ol 18 was obtained by Grignard reaction between dodecyl magnesium bromide (17) and ethyl formate. Coupling of pentacosan-13-ol with 16 was achieved via the Mitsunobu reaction\textsuperscript{5} in 57% yield.

Initially the dithioacetal group of 19 was attempted to be cleaved using [bis(trifluoroacetoxy)iodo]benzene. However, the reaction produced desired benzoin acetate (20) in only 54 % yield. This problem was solved using a different reagent - mercury (II) perchlorate. Stirring a solution of 19 in the presence of this agent produced the desired product 20 in 96 % yield. Consequent removal of acetate protection using a mild basic condition (diluted solution of potassium carbonate in aqueous methanol) gave benzoin 21 in 89 % yield. 3’,5’-Disubstituted benzoin 21 turned out to be sensitive towards oxidation just as 3’4’-disubstituted benzoin 6; oxidation product 22 was isolated from the reaction mixture after acetate protection removal in 5 % yield.
In order to achieve the synthetic goal (compound 14) the hydroxyl group of benzoin 21 had to be esterified with phosphocholine. The most common procedure for this kind of transformation is
depicted in scheme 2.6. The hydroxyl group is first esterified with cyclic oxochlorophospholane (23) to produce the unstable intermediate 24. Consequent nucleophilic opening of the cycle with trimethylamine should lead to the desired phosphocholine derivative 14.

Scheme 2.6.
The first attempt to obtain intermediate 24 using triethylamine in benzene as a base failed, no reaction progress was detected by TLC even after prolonged stirring. Changing reaction conditions to dichloroethane as solvent and diisopropylethyl amine as base improved the results: TLC analysis of the reaction mixture after 24 hours of stirring at 0°C showed complete disappearance of starting material and appearance of a spot, that could be assigned to compound 24 based on its anticipated Rf. Compound 24 was carried into the next step without isolation.

Incubating the reaction mixture under high pressure and elevated temperature for three days led to the formation of a very polar mixture of unidentified products.

Another well known protocol for preparation of phosphocholine derivatives utilizes chlorophospholane 25\(^7\). Phosphorus (III) chlorides are known to be more active in nucleophilic substitution reactions than the corresponding derivatives of pentavalent phosphorus. According to this protocol, reaction of benzoin 21 with phospholane 25 is supposed to lead to the formation of cyclic intermediate 26, which upon treatment with bromine compound 26 is oxidized and the ring opened to form dibromide 27. Treatment of compound 27 with an aqueous solution of trimethyl amine should then yield the desired product 14.

Attempts to apply this strategy to our benzoin 21 failed. A complicated mixture of products that resulted from this reaction that could not be resolved by means of chromatography on silica gel.

The second approach we investigated to obtain compound 14 involved formation of acyclic chlorophosphate, which was than reacted with choline tosylate to form desired product 14\(^8\). The starting benzoin 21 did react with phosphorus oxychloride, however, subsequent addition of choline tosylate to the mixture again led to formation of some extremely polar products, none of which could be isolated (Scheme 2.7).
The next strategy we followed to obtain final product 14 included attachment of the whole phosphocholine fragment in one step. The original procedure for this transformation was developed by Knopik et al.\textsuperscript{9} We tested these reactions using the model compound benzoin bromide 22 (Scheme 2.8).

Reaction of compound 22 with the potassium salt of phosphocholine in the presence of crown ether at elevated temperature lead to decomposition of 22 within 2 hours. Compound 23
was not found in the reaction mixture. Alternative routes to compound 23 included attempted reaction of 22 with the tetrabutylammonium salt of phosphocholine in acetonitrile. Reaction was conducted for 48 hours and no disappearance of starting material 22 was detected.

The failure to obtain compound 14 using conventional methods of introduction of phosphocholine required a change of strategy in our approach to the design of a photocleavable lipid. In 2005 Chandra et al. reported synthesis of a photocleavable lipid using amino acids asparagine and glutamine as the lipids polar “head”. These amino acids possess zwitterionic properties at pH close to neutral and therefore can be used instead of a phosphocholine moiety. A synthetic route leading to photocleavable lipid based on the benzoin chromophore and aspartic acid is depicted in Scheme 2.9.

Scheme 2.9.
Benzoin 21 was coupled with Boc-L-aspartic acid-4-tert-butyl ester using the conventional dicyclohexylcarbodiimide procedure to afford 24 in 96 % yield. Removal of Boc and t-butyl ester protections using trifluoroacetic acid in dichloromethane gave final product 25 in 97 % yield as mixture of two diastereomers. Photochemistry of compound 25 was studied using steady state photolysis monitored by UV spectrophotometry. Results are depicted in Figure 2.3.

Figure 2.3. Steady state photolysis of 25 monitored by UV spectrophotometry.

![Absorption vs Wavelength](image)

An absorption band appearing around 300 nm is characteristic for the benzofuran chromophore which forms in this photoreaction. Decreasing absorption at 340-380 nm, on the other hand, is evidence for the disappearance of benzoin chromophore. This figure suggests that the photoreaction proceeds in the expected direction, forming a mixture of benzofurans 26 and 27 and releasing aspartic acid (Scheme 2.10).

Further attempts to study the photochemistry of compound 25 (investigation of photoproducts composition, quantum and chemical yields measurements) proved to be
impossible because of its poor thermal stability. As shown by $^1$H NMR, compound 25 partially decomposes within 24 hours at room temperature or even at 4°C.

Scheme 2.10

\[
\begin{align*}
\text{MeO} & \quad \text{O} \\
\text{Ph} & \quad \text{NH}_3 \\
\text{O} & \quad \text{O} \\
\text{C}_{12}\text{H}_{25} & \quad \text{C}_{12}\text{H}_{25} \\
350 \text{ nm, MeCN} & \\
\end{align*}
\]

Overall, 3’,4’- and 3’,5’-dialkoxybenzoin chromophores were concluded to be inappropriate for use in the design of photocleavable lipid analog. Phospholipid analog 14 was shown to be synthetically inaccessible, whereas an attempt to use different zwitterionic moiety to mimic phosphocholine led to a thermally unstable compound.

Experimental part

Synthesis.

2-Phenyl-[1,3]dithiane (4). A solution of 13.8 ml (12.55 g, 0.16 mol) of acetyl chloride in 23 ml of methanol was stirred for 5 minutes at 0°C. The ice bath was then removed and benzaldehyde (1.8 ml, 1.16g, 16 mmol) and 1,3-propanedithiol (2 ml, 1.86 g, 17 mmol) were added. The reaction mixture was stirred for 30 minutes at room temperature and solvents were
removed in vacuo. The residue obtained was recrystallized from methanol to afford 3.06 g of 4 (96 %). Characterization data were consistent with those reported in literature\textsuperscript{4}.

M. p. 71-72ºC (lit. m.p 72-73ºC). $^1$H NMR $\delta$ 7.46 (m, 2H), 7.39-7.26 (m, 3H), 5.17 (s, 1H), 3.06 (m, 2H), 2.91 (m, 2H), 2.17 (m, 1H), 1.94 (m, 1H). MS $m/z$ 198 (M$^+$ + 2, 9), 197 (M$^+$ + 1, 12), 196 (M$^+$, 91), 155 (11), 131 (41), 122 (100), 121 (98), 105 (35).

(3,4-Dimethoxy-phenyl)-(2-phenyl-[1,3]dithian-2-yl)-methanol (5). A solution of 500 mg (2.55 mmol) of 4 in 10 ml of dry THF was cooled to 0ºC and butyllithium (1.12 ml of 2.5 M solution in hexane, 2.85 mmol) was added to the solution under vigorous stirring. Reaction mixture was stirred for 30 minutes at 0ºC and then solution of 500 mg (3 mmol) of 3,4-dimethoxybenzadehyde in 2ml of THF was added dropwise. The reaction mixture was stirred for 30 minutes at 0ºC and then for one hour at room temperature and was quenched by dropwise addition of a saturated solution of ammonium chloride at 0ºC. The mixture obtained was extracted with ethyl acetate, extracts were washed with brine and dried over sodium sulphate. Solvents were removed in vacuo and the residue was recrystallized from diethyl ether to afford 610 mg of 5 (66%).

M. p. 141-143ºC. $^{13}$C NMR $\delta$ 149.01, 147.82, 137.73, 131.13, 129.92, 128.47, 127.80, 120.96, 111.32, 109.68, 81.05, 66.83, 56.03, 55.67, 27.65, 27.37, 25.15. MS $m/z$ 197 (9), 196 (33), 195 (100), 167 (34), 165 (8), 139 (29), 122 (8), 121 (57), 108 (7).

Attempted preparation of 3',4'-dimethoxybenzoin (6). A solution of 200 mg (0.55 mmol) of 5 and 356 mg (0.83 mmol) of [bis(trifluoroacetoxy)iodo]benzene in 6 ml of 10% aqueous methanol were stirred at room temperature for 10 minutes. The reaction mixture was then poured into 100 ml of saturated solution of sodium bicarbonate. The mixture obtained was extracted with ethyl acetate, extracts were washed with brine and dried over sodium sulphate.
The solvents were removed and the residue was subjected to silica gel column chromatography.
The only product, that could be isolated from the mixture was identified as 7 (50 mg, 34 %).

*Acetic acid (3,4-dimethoxy-phenyl)-(2-phenyl-[1,3]dithian-2-yl)-methyl ester (8)*. To a solution of 362 mg (1 mmol) of 5 in 2 ml of dichloromethane were added 0.1 ml (102 mg, 1.3 mmol) of pyridine and 94μl (86 mg, 1.1 mmol) of acetyl chloride. The reaction mixture was stirred for 40 minutes at room temperature and then was diluted with 100 ml of dichloromethane. The solution obtained was washed with 10% hydrochloric acid solution, saturated solution of sodium bicarbonate and brine and dried over sodium sulphate. Solvents were removed and the residue crystallized from methanol to afford 323 mg of 8 (80 %).

M. p. 114-116ºC. $^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.36, 148.98, 147.53, 137.01, 131.07, 127.96, 127.49, 127.26, 121.43, 109.45, 80.03, 63.50, 55.65, 55.36, 27.23, 27.12, 24.63, 20.92.

*3’,4’-Dimethoxybenzoin acetate (9)*. A solution of 645 mg (1.5 mmol) of [bis(trifluoroacetoxy)iodo]benzene in 3 ml of 15% aqueous acetonitrile was added dropwise to a solution of 404 mg (1 mmol) of 8 in 3 ml of 15% aqueous acetonitrile. The reaction mixture was stirred at room temperature for 40 minutes and was diluted with 20 ml of ethyl acetate. Solution obtained was washed with saturated solution of sodium bicarbonate and brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to silica gel column chromatography (Ethyl acetate / Hexane : 1/3) to afford 304 mg of 9 (95%) as colorless oil.

*3’,4’-Dimethoxybenzoin diethyl phosphate (10)*. A solution of 200 mg (0.64 mmol) of 9 in 50 ml of 50% aqueous methanol saturated with argon was mixed with 90 mg (0.66 mmol) of potassium carbonate. The reaction mixture was stirred at room temperature for 15 minutes and then methanol was removed in vacuo. The resulting mixture was extracted with dichloromethane.
saturated with argon and extracts were washed with water and dried over magnesium sulfate. Solvents were removed and the residue was dissolved in 5 ml of pyridine and 0.12 ml (143 mg, 0.82 mmol) of diethylchlorophosphate were added. The reaction mixture was stirred at room temperature overnight and then poured into 50 ml of saturated solution of sodium bicarbonate. The mixture obtained was extracted with ethyl acetate and extracts were washed with 10% hydrochloric acid, saturated solution of sodium bicarbonate, brine and dried over sodium sulfate. The solvent was then removed and the residue was subjected to silica gel column chromatography (Ethyl acetate / Hexane : 3/1) to afford 141 mg of 10 (54%) as colorless oil.

^1H NMR (200MHz, CDCl₃) δ 7.93 (m, 1H), 7.51 (m, 1H), 7.39 (m, 3H), 6.83 (m, 2H), 6.62 (m,1H), 6.57 (m,1H), 4.20 (m,2H), 3.85 (m, 8H), 1.33 (t, J = 6.1 Hz, 3H), 1.17 (t, J = 6.1 Hz, 3H). ^13C NMR (100 MHz, CDCl₃) δ 193.52, 193.47, 149.81, 149.33, 134.33, 133.39, 128.79, 128.52, 127.12, 127.07, 121.30, 111.15, 110.65, 79.82, 79.78, 64.20, 64.14, 63.84, 63.78, 55.90, 55.91, 55.76, 15.97, 15.90, 15.81, 15.74. MS m/z 408 (M⁺, 3), 362 (3), 304 (15), 303 (83), 275 (14), 256 (4), 247 (18), 167 (23), 165 (100), 151 (37), 140 (15), 105 (48).

Oxidation of 3',4'-Dimethoxybenzoin acetate with oxygen gas. A solution of 100 mg (0.32 mmol) of 9 in 25 ml of 50% aqueous methanol saturated with argon was mixed with 45 mg (0.33 mmol) of potassium carbonate. Oxygen gas was bubbled through reaction mixture for 1.5 hours under vigorous stirring. Complete disappearance of 6 was detected by TLC after this period, and methanol was evaporated in vacuo. Resulting mixture was extracted with dichloromethane and extracts were washed with water and dried over magnesium sulfate. The resulting solution was analyzed by means of GC/MS.

3-(tert-Butyldimethylsilyloxy)-5-methoxybenzaldehyde. A suspension of 720 mg of sodium hydride (18 mmol, 60% suspension in mineral oil, washed with hexane) in 26 ml of dry
dimethylformamide was cooled to 0°C. Ethyl thiolate (3.6 ml, 4.86 g, 80 mmol) was slowly added to the mixture. The reaction mixture was stirred at 0°C for 15 minutes, refluxed for 1 hour and solution of 3,5-dimethoxybenzaldehyde (15) (890 mg, 5.36 mmol in 25 ml of DMF) was added dropwise. The reaction mixture was refluxed for another hour and then quenched by consequent addition of: 1) 360 ml of saturated solution of NaCl; 2) 36 ml of formalin and 3) 68 ml of glacial acetic acid. The mixture obtained was extracted with dichloromethane, extracts were washed with 10% HCl, water and dried over Na₂SO₄. Solvent was removed under reduced pressure, and residue obtained was subjected to silica gel column chromatography (Dichloromethane / Methanol = 50:1) to give 740 mg of brown solid, which was used in the next step without further purification.

A solution of 740 mg (4.86 mmol) of 3-hydroxy-5-methoxybenzaldehyde and 735 mg (4.9 mmol) of tert-butyldimethylsilyl chloride in 23 ml of dry tetrahydrofuran was cooled to 0°C and 0.68 ml (490 mg, 4.9 mmol) of triethylamine were added dropwise. The reaction mixture was stirred at room temperature for 3 hours and then THF was removed under reduced pressure. The residue obtained was dissolved in dichloromethane and filtered through silica gel. After removal of solvent 925 mg of yellow oil were obtained (64 % over two steps).

$$\text{H NMR} \ \delta \ 9.89 \ (s, \ 1H), \ 7.26 \ (s, \ 1H), \ 7.03 \ (dd, \ J = 2.4, \ 1.2 \ Hz, \ 1H), \ 6.94 \ (dd, \ J = 2.1, \ 1.2 \ Hz, \ 1H), \ 6.65 \ (t, \ J = 2.4 \ Hz, \ 1H), \ 3.84 \ (s, \ 3H), \ 0.99 \ (s, \ 9H), \ 0.23 \ (s, \ 6H).$$

$$\text{C NMR} \ \delta \ 191.93, \ 161.35, \ 157.50, \ 138.56, \ 114.62, \ 113.18, \ 106.76, \ 55.70, \ 25.79, \ 18.32, \ -4.31. \ MS \ m/z \ 266 (M^+, \ 18), \ 210 \ (26), \ 209 \ (100), \ 182 \ (8), \ 181 \ (47), \ 166 \ (8), \ 165 \ (16), \ 151 \ (10), \ 135 \ (5), \ 104 \ (4).$$

1-Acetoxy-[3-(tert-butyldimethylsilyloxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (15). A solution of 515 mg (2.63 mmol) of 2-phenyl-1,3-dithiane in 4 ml of dry THF was cooled to 0°C under flow of argon and 1.26 ml of BuLi (2.5M solution in hexane) was
slowly added. The reaction mixture was stirred at 0°C under flow of argon for 30 minutes and then solution of 700 mg of 3-(tert-butyldimethylsilyloxy)-5-methoxybenzaldehyde (4) (2.63 mmol) in 4.5 ml of THF was added. Reaction mixture was stirred under flow of argon at room temperature for one hour and then 30 ml of saturated solution of ammonium chloride was added dropwise. Resulting mixture was extracted with dichloromethane, extracts were washed with water and dried over sodium sulfate. Evaporation of solvent gave 1.21 g of yellow oil (99%), which was used in the next step without further purification.

To a solution of 1.21 g (2.61 mmol) of 1-hydroxy-[3-(tert-butyldimethylsilyloxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane in 5.6 ml of dichloromethane were added 0.28 ml (270 mg, 3.47 mmol) of pyridine and 0.24 ml (264 mg, 3.34 mmol) of acetyl chloride. The reaction mixture was stirred for 40 minutes at room temperature and then poured into 30 ml of water. The suspension obtained was extracted with dichloromethane, and extracts were washed with 10% hydrochloric acid, saturated solution of sodium bicarbonate, water and dried over sodium sulfate. Solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography (Dichloromethane / Petroleum ether : 3/1) to give 1.039 mg of (15) as a yellow oil (73%).

\[^{1}\text{H NMR}\ δ 7.77 (m, 2H), 7.29 (m, 3H), 7.25 (t, J = 2.25 Hz, 1H), 6.11 (m, 1H), 6.07 (s, 1H), 6.92 (m, 1H), 3.54 (s, 3H), 2.68 (m, 4H), 2.11 (s, 3H), 1.90 (m, 2H), 0.94 (s, 9H), 0.12 (s, 6H).\]^{13}\text{C NMR} δ169.25, 159.34, 155.54, 137.21, 136.82, 130.88, 127.99, 127.54, 113.17, 106.98, 106.73, 80.15, 63.38, 55.05, 27.34, 27.21, 25.68, 24.67, 20.89, 18.14, -4.37. MS m/z 444 (5), 313 (4), 281 (5), 267 (5), 197 (33), 196 (45), 195 (100), 181 (11), 165 (9), 121 (54).

1-Acetoxy-[3-hydroxy-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (16). A solution of 1 g (1.98 mmol) of 1-Acetoxy-[3-(tert-butyldimethylsilyloxy)-5-methoxyphenyl]-2-
phenyl-2-(1,3-dithian-2-yl)ethane (15) in 4.6 ml of THF was cooled to 0°C. Solution of 790 mg (2.5 mmol) of tetrabutylammonium fluoride in 2.5 ml of THF was added. The reaction mixture was stirred at room temperature for 1 hour and then poured into 100 ml of water. The resulting mixture was stirred for 20 minutes, and extracted with ethyl acetate. The extracts were dried over sodium sulfate, after evaporation of solvent 650 mg of yellow oil was obtained. Product was recrystallized from toluene to give 613 mg of white powder (79%).

\[ \delta 7.78 (m, 2H), 7.33 (m, 3H), 6.28 (t, J = 2.3 Hz, 1H), 6.09 (m, 1H), 6.05 (s, 1H), 5.90 (m, 1H), 5.1 (br. s, 1H), 3.56 (s, 3H), 2.69 (m, 4H), 2.11 (s, 3H), 1.92 (m, 2H). \]

\[ \delta 169.57, 159.65, 155.55, 137.17, 137.09, 130.91, 128.00, 127.62, 108.47, 106.53, 102.01, 80.23, 63.30, 55.12, 53.40, 27.33, 27.19, 24.60, 20.90. MS m/z 330 (2), 256 (3), 195 (100), 181 (3), 165 (4), 152 (5), 121 (32). \]

Pentacosane-13-ol (18). A solution (5 ml, 1M) of dodecyl magnesium bromide in diethyl ether were cooled to 0°C under atmosphere of argon and 0.2 ml (184 mg, 2.5 mmol) of ethyl formate was added to the solution slowly. Reaction mixture was refluxed for 1 hour and than cooled to 0°C. 1.9 ml of 1M sulfuric acid was added and reaction mixture was poured into 100 ml of diethyl ether. Mixture obtained was washed with 10% HCl, water and dried over sodium sulfate. Solvent was removed under reduced pressure and crystalline residue was recrystallized from acetone to give 437 mg of 18 (95%).

M. p. 66-68°C \[ \delta 3.56 (br. s 1H), 1.50-1.18 (m, 44H), 0.88 (t, J = 6.59 Hz, 6H). \] 13C NMR \[ \delta 72.06, 37.54, 31.94, 29.74, 29.90-29.65 (several signals), 29.37, 25.67, 22.70, 14.11. MS m/z 350 (2), 199 (40), 139 (4), 125 (27), 111 (62), 97 (100), 85 (20), 83 (88), 71 (33), 69 (81), 57 (80), 55 (60). \]
1-Acetoxy-[3-(pentacosyl-13-oxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (19). A solution of 0.35ml (284 mg, 1.4 mmol) of tri-n-butylphophine and 336 mg (1.33 mmol) of 1,1’-(azodicarbonyl)-dipiperidine in 4.5 ml of dry toluene was stirred at 0°C for ~1 hour (until orange color disappears) under flow of argon. A solution of 188mg (0.51 mmol) of pentacosane-13-ol (12) and 200 mg (0.51 mmol) of 1-Acetoxy-(-3-hydroxy-5-methoxyphenyl)-2-phenyl-2-(1,3-dithian-2-yl)ethane (6) in 6 ml of toluene / THF mixture (5:1) was prepared in the separate vessel and was added to the reaction mixture dropwise over the period of 15 minutes. The reaction mixture was stirred at 0°C for one hour and incubated without stirring at -20°C for 20 hours. After that 30 ml of pentane were added, the mixture was stirred for 1 hour at 0°C and then subsequently filtered. Solvent was removed under reduced pressure and resultant was subjected to silica gel column chromatography (Chloroform : Petroleum ether = 3:1) to give 244 mg of 19 (64 %).

$^1$H NMR $\delta$ 7.77 (m, 2H), 7.38-7.21 (m, 3H), 6.29 (t, $J = 2.3$ Hz, 1H), 6.11 (s, 1H), 6.00 (m, 2H), 3.90 (p, $J = 5.7$ Hz, 1H), 3.58 (s, 3H), 2.67 (m, 4H), 2.10 (s, 3H), 1.98 (m, 2H), 1.64-1.12 (m, 44H), 0.88 (t, $J = 6.39$ Hz, 6H). $^{13}$C NMR $\delta$ 169.08, 159.40, 158.45, 137.16, 136.70, 130.88, 127.82, 127.38, 80.14, 77.97, 63.84, 54.94, 33.91, 33.77, 31.83, 29.80-29.40 (several signals), 29.26, 27.25, 27.12, 25.32, 25.25, 24.60, 22.58, 20.74, 14.00. MS (MALDI) 681.25 (M$^+$ - OAc).

3’-(1-dodecyl-tridecyloxy)-5’-methoxybenzoin acetate (20). (protocol 1) To A suspension of 200 mg (0.27 mmol) of 1-Acetoxy-[3-(pentacosyl-13-oxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (19) in mixture of 1 ml of acetonitrile and 70 μl of water were added 147 mg (0.34 mmol) of [bis(trifluoroacetoxy)iodo]benzene in 0.3 ml of acetonitrile. The reaction mixture was stirred at room temperature for 3 hours and 50 ml of ethyl acetate were
added. The solution obtained was washed with saturated solution of sodium bicarbonate and brine and dried over sodium sulfate. Solvent was removed under reduced pressure and residue was subjected to silica gel column chromatography (10% EtOAc in hexane) to give 94 mg of \( \text{20} \) as colorless oil (54%).

\[ \text{\( ^1\)H NMR} \delta 7.94 (m, 2H), 7.51 (m, 1H), 7.39 (m, 2H), 6.74 (s, 1H), 6.55 (m, 2H), 6.39 (t, \( J = 2.2 \) Hz, 1H), 4.15 (qin, \( J = 5.7 \) Hz, 1H), 3.75 (s, 3H), 2.21 (s, 3H), 1.69-1.19 (m, 44H), 0.88 (t, \( J = 6.4 \) Hz, 6H). \( ^{13}\)C NMR \( \delta 193.55, 170.39, 61.24, 160.30, 135.39, 134.73, 133.38, 128.77, 128.57, 108.58, 106.17, 102.89, 78.19, 77.75, 55.54, 33.84, 33.81, 31.92, 29.78-29.45 (several signals), 29.34, 25.32, 22.68, 20.77, 14.09. MS (MALDI) 673.82 (M\(^+\) + Na). \]

(Protocol 2) To a solution of 318 mg (0.43 mmol) of 1-Acetoxy-[3-(pentacosyl-13-oxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (7) in 60 ml of acetonitrile and 1.1 ml of water were added 327 mg (0.72 mmol) of mercury perchlorate triydrate. The reaction mixture was stirred at room temperature for 10 minutes and then filtered through layer of silica gel. 100 ml of water were added to the resulting solution and mixture was extracted with ethyl acetate. The extracts were washed with saturated sodium bicarbonate solution and water. Removal of solvent gave 270 mg of \( \text{20} \) (96%).

\( 3'-(1\text{-dodecyltridecyloxy})\)-5\( '\)-methoxylbenzoin (21). To a solution of 94 mg (0.14 mmol) of acetic acid 1-[3-(1-dodecyl-tridecyloxy)-5-methoxyphenyl]-2-oxo-2-phenylethyl ether (19) in 30 ml of methanol and 3 ml of water were added 20 mg (0.14 mmol) of potassium carbonate. The reaction mixture was stirred at room temperature for 40 minutes and than 100 ml of water was added. The resulting emulsion was extracted with chloroform, extract were washed with water and dried over sodium sulfate. The solvents were removed in vacuo and the residue was
subjected to silica gel column chromatography to afford 79 mg of \( \mathbf{21} \) as colorless oil (89%) and few mg of oxidation product \( \mathbf{22} \).

\(^1\)H NMR \( \delta \) 7.93 (m, 2H), 7.52 (m, 1H), 7.39 (m, 2H), 6.43 (m, 2H), 6.33 (t, \( J = 2.3 \) Hz, 1H), 5.83 (d, \( J = 6.2 \) Hz, 1H), 4.49 (d, \( J = 6.2 \) Hz, 1H), 4.12 (q, \( J = 5.6 \) Hz, 1H), 3.72 (s, 3H), 1.64-1.53 (m, 44H), 0.88 (t, \( J = 6.6 \) Hz, 6H). \(^{13}\)C NMR \( \delta \) 198.76, 161.27, 160.34, 140.88, 133.81, 133.61, 129.07, 128.60, 107.77, 105.34, 102.25, 78.15, 76.21, 55.26, 33.82, 33.79, 31.90, 29.68-29.53 (several signals), 29.33, 25.29, 22.66, 14.07. MS \( m/z \) 608 (M\(^+\), 9), 503 (12), 502 (28), 258 (14), 241 (7), 153 (100), 151 (59), 125 (17), 123 (10), 105 (22).

Compound \( \mathbf{22} \), \(^1\)H NMR \( \delta \) 7.96 (m, 2H), 7.64 (tt, \( J = 7.4, 1.3 \) Hz, 1H), 7.5 (m, 2H), 7.05 (d, \( J = 2.2 \) Hz, 2H), 6.7 (t, \( J = 2.2 \) Hz, 1H), 4.22 (qin, \( J = 5.5 \) Hz, 1H), 3.81 (s, 3H), 1.8-1.15 (m, 62H), 0.88 (t, \( J = 6.6 \) Hz).

\( 3'-(1\text{-dodecyldodecyloxy})-5'-\text{methoxylbenzoin tert-butyloxycarbonyl-4-tert-butyl ester aspartate (24)} \). A solution of 125 mg (0.21 mmol) of \( \mathbf{21} \), 65 mg (0.22 mmol) of Boc-aspartic acid 4-tert-butyl ester, 100 mg (0.48 mmol) of dicyclohexylcarbodiimide and catalytic amount (~ 3 mg) of 4-dimethylaminopyridine in 1.5 ml of dichloromethane was stirred at room temperature overnight and then was filtered. The solvents were removed and the residue was purified by silica gel column chromatography (Ethyl acetate / Hexanes : 1/9) to afford 177 mg of \( \mathbf{24} \) as mixture of two diastereomers (96%).

\(^1\)H NMR \( \delta \) 7.91 (m, 2H), 7.55-7.30 (m, 3H), 6.77 (d, \( J = 3.6 \) Hz, 1H), 6.53 (m, 2H), 6.37 (m, 1H), 5.53 (m, 1H), 4.70 (m, 1H), 4.13 (qin, \( J = 5.5 \) Hz, 1H), 3.74 (s, 1.5H), 3.73 (s, 1.5Hz), 2.88 (m, 2H), 1.65-1.07 (m, 62H), 0.88 (t, \( J = 6.4 \) Hz). \(^{13}\)C NMR \( \delta \) 192.88, 192.57, 170.85, 170.75, 169.71, 161.21, 161.19, 160.23, 160.17, 155.40, 135.06, 135.01, 133.41, 133.37, 128.78, 128.75, 128.57, 128.55, 108.67, 108.60, 106.17, 106.03, 102.82, 96.12, 81.59, 81.47, 79.88,
78.44, 78.34, 78.17, 78.10, 55.32, 55.31, 50.16, 37.84, 33.79, 33.75, 31.91, 29.8-29.5 (several signals), 29.34, 28.27, 28.03, 27.93, 25.31, 22.67, 14.09. MS (MALDI) 918 (M⁺ + K), 902 (M⁺ + Na).

3’-(1-dodecyltridecyloxy)-5’-methoxylbenzoin 1-aspartate (25). Compound 24 (50 mg, 0.057 mmol) was dissolved in 0.7 ml of dichloromethane and 0.34 ml of trifluoroacetic acid were added. The reaction mixture was stirred at room temperature overnight, and then the solvents were removed in vacuo. The residue was dried under reduced pressure in presence of phosphorus pentoxide to afford 41 mg of 25 (97%).

1H NMR (CD3OD) δ 8.01 (m, 2H), 7.51 (m, 1H), 7.49 (m, 2H), 6.62 (m, 2H), 6.43 (t, J = 2.1 Hz, 1H), 4.55 (m, 1H), 4.22 (m, 1H), 3.74 (s, 3H), 3.11 (m, 2H), 1.57-1.10 (m, 46H), 0.89 (t, J = 6.9 Hz, 6H). MS (MALDI) 830, 832 (M⁺ + Ag).

Photochemistry.

Photolysis of 10 monitored following changes in UV absorption. 5e-5 M solution of 10 in argon saturated acetonitrile was irradiated in quartz cell with 350 nm light. UV spectra of the reaction mixture were taken after 2, 4, 6, 8, 10, 12, 14, 16 and 18 minutes of irradiation.

Photolysis of 10 monitored following changes in pH. 1e-3 M solution of 10 in argon saturated water was irradiated with 350 nm light in pyrex tube. The pH of the reaction mixture was measured after 4, 8, 12, 16 and 20 minutes of irradiation. In control experiment pH of the same solution kept in the darkness was monitored.

Studies of photoproduct composition after photolysis of 10. 1e-3 M solution of 10 in argon saturated acetonitrile was irradiated with 350 nm light in pyrex tube for 30 minutes. Reaction mixture was analyzed by means of GC/MS.
**Photolysis of 10. Measurements of chemical yield of the photorelease of diethyl phosphoric acid.** 5 ml of 1e-3 M solution of 10 in argon saturated water were irradiated with 350 nm light in pyrex tube for 30 minutes. Reaction mixture was than titrated with 1e-3 M sodium hydroxide solution, following changes in pH. The yield of diethylphosphoric acid was found to be 56 %.

**Photolysis of 10. Measurements of quantum yield of the disappearance of 10.** 3 ml of 5e-4 M solution of 10 in argon saturated acetonitrile was irradiated in quartz cell in Rayonet photoreactor with 350 nm light for 6 minutes (within 10% conversion of starting material). After periods of 2, 3, 4, 5 and 6 minutes of irradiation 100 μl aliquots were taken and diluted 10 times for analysis of its UV spectra. Absorbance at 246 nm was measured. Light intensity was measured using ferroxylate actinometry. Quantum yield of disappearance of 10 upon irradiation was found to be 0.13 +/- 0.0195 %.

**Measurements of the rate of dark hydrolysis of 10.** 4e-5 M solution of 10 was kept in the quartz tube in the darkness for 2 hours. Absorbance of the solution at 265 nm was measured every 15 seconds. Fitting of the data obtained gave rate constants $k_{obs} = 9.44e-4 +/- 1.84e-5 \text{ s}^{-1}; 1.15e-3 +/- 1.52e-5 \text{ s}^{-1}; 9.3e-4 +/- 1.91e-5 \text{ s}^{-1}$. 
Design of a new photoremovable protecting (caging) group

Photoremovable protecting group based on the 2-oxymethylnaphthalene-3-ol chromophore

Ortho-hydroxy benzyl alcohol derivatives are known to form quinone methide (3) intermediates upon photoexcitation (Scheme 2.11)\textsuperscript{11, 12, 13}. Having dual electrophilic and nucleophilic properties\textsuperscript{14}, the ortho-quinone methides are considered to be valuable synthetic intermediates. They also posses interesting biological properties such as DNA alkylating ability\textsuperscript{15}.

Scheme 2.11.

\[
\begin{align*}
\text{(1)} & \quad \text{OH} & \quad \text{O} & \quad \text{O} & \quad \text{(3)} & \quad \text{AcOH} \\
\text{(2)} & \quad \text{OH} & \quad \text{O} & \quad \text{CN} & \quad \text{(3)} & \quad \text{(4)} \\
& & \quad \text{hv} & & & \\
& & \quad \text{hv} & & & 
\end{align*}
\]

Although, chemical and physical properties of ortho-quinone methides as well as methods of generation of these species have been studied extensively over the last few decades\textsuperscript{14, 15}, the kinetic and efficiency aspects of photorelease of agents esterified (or etherified) with ortho-hydroxybenzyl alcohol have not received much attention.

Ortho-hydroxybenzyl alcohol has an absorption maximum at 274 nm, which is too energetic for this chromophore to be used in the design of a photoremovable protecting group. In order to extend the wavelength we selected naphthol chromophores 5, 7, and 9 (Scheme 2.12) as cores for the new photoremovable protecting group design. Upon formation of naphthoquinone
methide 6 aromaticity of both rings is lost, whereas naphthoquinone methides 8 and 10 keep one of the ring aromatic. This should make photoreactions of 7 and 9 somewhat more efficient.

Scheme 2.12

A good photoremovable protecting group should comply with the following requirements: 1) the photodeprotection reaction should be clean and have high quantum yield; 2) the chemical yield of photodeprotection should be close to quantitative; 3) a synthetic method of introduction of the protecting group has to be developed.

Scheme 2.13
In order to evaluate the general applicability of the concept of using quinine methide formation photochemistry in the design of a new photoremovable protecting group, two model compounds, 13 and 14, have been synthesized (Scheme 2.13). The synthesis starts with commercially available methyl 3-hydroxy-2-naphthoate (11). Compound 11 was treated with lithium aluminum hydride to form diol 12, which than was reacted with ethyl alcohol in acidic condition and acetic acid to produce model compounds 13 and 14 respectively. UV absorption spectra of compounds 13 and 14 have maxima at 230 nm, 266 nm and 325nm (Figure 2.4) and no absorption above 340 nm.

Figure 2.4. UV absorption spectra of 13.

Photochemistry of compounds 13 and 14 was studied using ¹H NMR. Solutions of compounds 13 and 14 (5e-2 M in deuterated methanol) were irradiated with 300 nm light in a Rayonet photoreactor. The appearance of acetic acid was monitored at 1.95 ppm (methyl group, singlet), and appearance of ethanol was monitored at 1.27 ppm (methyl group, triplet). Along with the appearance of ethanol and acetic acid released, NMR showed the disappearance of starting material (methylene singlet) and the appearance of diol 12 (Figure 2.5).
Figure 2.5. Photolysis of 15a and 15b monitored by \(^1\)H NMR.

In order to measure the quantum efficiency and chemical yield of this photodeprotection reaction, protected derivatives of easily detectable compounds had to be synthesized. Compounds 15-17a-b were selected for these experiments (Scheme 2.14), since benzyl alcohol and benzoic acid can be efficiently detected by means of HPLC.

Scheme 2.14

\[
\begin{align*}
(15) & \quad X = \text{a) PhCOO, b) PhCH}_2\text{O} \\
(16) & \\
(17) &
\end{align*}
\]

Our first attempt to prepare compound 15b via direct alkylation of diol 12 failed. The reaction produced a mixture of substituted naphthol 18 as a major component along with diether 19. The next synthetic strategy we employed involved protection of the naphthol hydroxyl of naphthoate 11 followed by reduction of the ester moiety with formation of alcohol 21. Compound 21 is then either alkylated to form compounds 22. Final deprotection, gives final product 15b (Scheme 2.15).
The first protection group used in this reaction sequence was tert-butyldimethylsilyl protection. Compound 20a was obtained in 95% yield using a conventional procedure for protection of phenols. However, attempts to reduce the ester functionality of compound 20a failed. Lithium aluminum hydride not only caused reduction of ester, but also led to hydrolysis of the tert-butyldimethylsilyl protection, thus forming diol 12. Attempts using milder reducing agent, diisobutylaluminum hydride lead to formation of a mixture of starting material and desilylated compound 11.

The next group we investigated for use in this synthetic sequence was 2-methoxyethoxymethyl protection. Compound 20b was obtained in nearly quantitative yield using
a conventional procedure for introduction of MEM protection. Reduction of the ester functionality of \(20b\) with lithium aluminum hydride produced alcohol \(21b\) in 80% yield. Alkylation of compound \(21b\) was achieved using sodium hydride and benzyl bromide in THF, producing ether \(22b\) in 93% yield. The regular procedure for MEM protection removal requires treatment of the protected derivative with trifluoroacetic acid in dichloromethane. Our attempt to use this procedure to obtain compound \(15b\) from \(22b\) failed. The reaction resulted in formation of a complicated mixture of products that could not be resolved using chromatography on silica gel.

Another efficient method for removal of MEM protection from phenols requires the presence of a heteroatom containing substituent in an ortho-position to protected phenol. Upon treatment with Montmorillonite clay, such phenol derivatives can be efficiently deprotected at room temperature in a short period of time\(^{16}\). The heteroatomic ortho-substituent with heteroatom, apparently, is required to provide a chelation site for Montmorillonite clay. Treatment of compound \(22b\) with Montmorillonite K-10 in benzene at room temperature led to no changes, and attempts to accelerate the reaction by elevating the temperature led to the formation of a complicated mixture of degradation products.

Another common method for MEM protection removal includes treatment of protected substrate with trimethylsilyl iodide in acetonitrile\(^{17}\). However, treatment of solutions of compound \(22b\) in acetonitrile with trimethylsilyl chloride and sodium iodide at -20°C or at room temperature only resulted in formation of complex mixture of degradation products.

Carbon tetrabromide in isopropyl alcohol has been reported as an efficient agent for the release of alcohols and phenols from MEM protected forms\(^{18}\). Application of this procedure for deprotection of compound \(22b\), however, failed. Refluxing a solution of \(22b\) in isopropyl alcohol
in the presence of carbon tetrabromide overnight resulted in formation of black polymer-like material which could not be purified or characterized.

The last protection we tried in this synthetic strategy was the methylthiomethyl group (MTM). Compound 20c was obtained using sodium hydride in HMPA as a base and methylthiomethyl chloride with 90% yield. Reduction with lithium aluminum hydride resulted in formation of alcohol 21c in nearly quantitative yield. Compound 21c was then etherified using benzyl bromide and sodium hydride as base to give ether 22c in 89% yield. Regular procedure for removal of MTM protection includes treatment of protected substrate with mercury (II) chloride in aqueous acetonitrile in presence of calcium carbonate (to avoid acidic reaction of the media). Refluxing compound 22c in this media for prolonged period of time resulted in no changes, starting material was recovered with 65% yield. Attempt to conduct this reaction without addition of calcium hydride lead to formation of complex mixture of very polar products, which could not be isolated and identified.

Failure of the synthetic sequence depicted in Scheme 2.15 was, apparently, caused by very high stability of the products of phenol hydroxyl protection. On the other hand, less stable protections, such as tert-butyldimethylsilyl were not stable enough to survive the course of the synthesis. However, the tert-butyldimethylsilyl group still could be used if reactions that require basic conditions, such as treatment with lithium aluminum hydride could be avoided. A synthetic scheme for synthesis of compounds 15a and 15b using TBDMS protection is depicted in Scheme 2.16.

Synthesis starts with diol 12, selective protection of primary hydroxyl with a dimethoxytrityl protecting group led to formation of intermediate 23, which was not isolated because of its poor hydrolytic stability, (probably caused by neighboring phenolic hydroxyl) and
was taken to the next step without purification. Introduction of TBDMS protection produced compound 24 in 82% yield over the two steps. Removal of dimethoxytrityl protection was achieved oxidatively using cerium (IV) triflate in aqueous methanol producing compound 25 in 85% yield. Benzylation of alcohol 25 using a regular procedure (benzoyl chloride in pyridine) lead to formation of ester 27 in 90% yield. Removal of TBDMS protection produced the final product 15a in 83% yield.

Scheme 2.16
In order to obtain compound 15b alcohol 25 was alkylated in 91% yield using benzyl bromide and sodium hydride as base. Removal of TBDMS protection following the alkylation reaction revealed that the TBDMS group had migrated to the primary hydroxyl during the alkylation step. Apparently, unlike lithium aluminum hydride, which caused hydrolysis of TBDM protection, sodium hydride only caused migration of silyl to the stronger base ArCH₂O⁻.

According to these results, compound 25 can only be alkylated using mild conditions and avoiding strong bases. The following synthetic route leading to final product 15b was proposed and realized. Alcohol 25 was first transformed to bromide 29 using carbon tetrabromide in the presence of triphenyl phosphine. The bromide obtained was etherified with benzyl alcohol in presence of silver triflate. This reaction produced a rather complex mixture of products, but chromatography on silica gel allowed isolation of impure 30 which upon treatment with tetrabutylammonium fluoride was transformed to desired product 15b in 49% yield over the two steps.

In order to examine chromophores 7 and 9, compounds 31 and 32 had to be synthesized (Scheme 2.17). The synthetic sequence used for preparation of compounds 15a,b was employed. Diol 34 was obtained by reduction of naphthoate 33. Because of the poor thermal stability of compound 34 it was taken to the next step without isolation or purification. Introduction of dimethoxytrityl and tert-butyldimethylsilyl protecting groups produced compound 35 in 86% yield over the three steps. Removal of dimethoxytrityl protection followed by esterification of alcohol obtained produced compound 36 in 78% yield over the two steps. Attempts to remove tert-butyldimethoxysilyl protection using conventional procedure (tetrabutylammonium fluoride in THF) failed. The reaction produced only mixtures of very polar products of decomposition of 36. Attempts to use alternative procedure (hydrofluoric acid in acetonitrile) were unsuccessful as
well. In this case after prolonged stirring at room temperature, 73% of starting material was recovered from the reaction mixture.

Scheme 2.17

Diol 39 was obtained by formilation of naphthol 38 in basic media in 85% yield. Introduction of dimethoxytrityl and tert-butyldimethylsilyl protecting groups produced compound 40 in 90% yield over the two steps. Removal of dimethoxytrityl protection followed by esterification of the alcohol obtained produced compound 42 in 75% yield over the two steps.
Attempts to remove tert-butyldimethylsilyl protection in compound 42 led to results very similar to those obtained in the case of compound 37. Upon the reaction with tetrabutylammonium fluoride compound 42 produced a polar mixture of decomposition products, while reaction with hydrofluoric acid left the starting material intact.

Because of synthetic inaccessibility of derivatives such as 16a and 17a only the 2-oxymethylnaphthalene-3-ol (15) chromophore seems to be promising in terms of developing a new photoremovable protection.

Using compounds 15a, b the efficiency of 2-oxymethylnaphthalene-3-ol chromophore as a new photoremovable protecting group for alcohols and carboxylic acids was studied. Chemical yields of photorelease of benzyl alcohol and benzoic acid were found to be close to quantitative (97 and 96% respectively) (Figure 2.6).

Figure 2.6. Photodeprotection of benzoic acid and benzyl alcohol monitored by HPLC.

Quantum yields for photorelease of benzoic acid and benzyl alcohol from compounds 15a and 15b were found to be 9.25 and 9.4%. The finding of independency of quantum yields of photorelease on the nature of the leaving group is in the perfect agreement with the mechanism
of quinine methide formation reported by Webb et al\textsuperscript{10}. According to this mechanism formation of quinone methide proceeds via excited state intramolecular proton transfer, and, therefore, the rate of formation of quinine methide depends on the pKa of phenol hydroxide in the excited state and pKb of benzylic oxygen.

Scheme 2.18.

To demonstrate the general applicability of this group as photoremovable protection we have synthesized several derivatives with functionalities protected with the
2-oxymethylnaphthalene-3-ol (MN) group and studied their photochemistry (Scheme 2.18). To examine applicability of an MN group to protect phenols, two compounds were prepared. Compound 46 is estrone with C-3 hydroxyl protected with an MN group. Attempts to obtain this compound via alkylation of estrone cesium oxide with bromide 29 failed. The only product isolated was identified as 3-tert-butyldimethylsilyloxyestrone. Apparently the basicity of the solution of cesium carbonate in dimethylformamide caused migration of tert-butyldimethylsilyl protection from 29 to estrone. In order to avoid basic conditions Mitsunobu coupling was employed. Estrone was coupled with alcohol 25 in the presence of tributylphosphine and 1,1’-(azodicarbonyl)dipiperidine. Subsequent removal of tert-butyldimethylsilyl protection gave desired product 46 in 43 % yield.

tert-Butyloxycarbonyltyrosine methyl ester (47) was selected as a second phenol substrate for protection with the MN group. Compound 48 was obtained according to the procedure used for compound 46 in 56 % yield.

21-Hydroxyprogesterone (49) was selected as an alcohol substrate for protection with the MN group. The hydroxyl at C-21 was etherified with the MN group, using silver-assisted coupling of 49 with bromide 29 followed by removal of tert-butyldimethylsilyl protection. Compound 50 was obtained in 45 % yield.

Photochemical properties of compounds 44, 46, 48 and 50 were studied in order to examine the chemical yields of photorelease of corresponding substrates. The data obtained is summarized in Table 2.1.
Table 2.1.

<table>
<thead>
<tr>
<th>Structure of substrate</th>
<th>Conditions</th>
<th>Chemical yield of photorelease</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
<td>300 nm light, 40 % aqueous methanol</td>
<td>96 %</td>
</tr>
<tr>
<td><img src="image2" alt="Structure 2" /></td>
<td>300 nm light, 40 % aqueous acetonitrile</td>
<td>27 %</td>
</tr>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td>300 nm light, 40 % aqueous acetonitrile</td>
<td>25 %</td>
</tr>
<tr>
<td><img src="image4" alt="Structure 4" /></td>
<td>300 nm light, 100 % methanol</td>
<td>20 %</td>
</tr>
<tr>
<td><img src="image5" alt="Structure 5" /></td>
<td>300 nm light, 100 % water</td>
<td>23 %</td>
</tr>
<tr>
<td><img src="image6" alt="Structure 6" /></td>
<td>300 nm light, 40 % aqueous methanol</td>
<td>23 %</td>
</tr>
<tr>
<td><img src="image7" alt="Structure 7" /></td>
<td>300 nm light, 100 % methanol</td>
<td>15 %</td>
</tr>
<tr>
<td><img src="image8" alt="Structure 8" /></td>
<td>300 nm light, 40 % aqueous acetonitrile</td>
<td>29 %</td>
</tr>
<tr>
<td><img src="image9" alt="PhCH₂OH" /></td>
<td>300 nm light, 40 % aqueous acetonitrile</td>
<td>97 %</td>
</tr>
<tr>
<td><img src="image10" alt="PhCOOH" /></td>
<td>300 nm light, 40 % aqueous acetonitrile</td>
<td>96 %</td>
</tr>
</tbody>
</table>
Upon irradiation of compound 44 with 300 nm light in aqueous acetonitrile, N-tert-butyloxy carbonyl phenylalanine was released in 96 % yield. The chemical yield for photorelease of estrone from 46 was found to be rather low. Only 27 % of estrone was released from 46 upon irradiation in aqueous acetonitrile. Photochemical behavior of compound 48 was studied in greater detail. It was irradiated in different solvents and the chemical yield of photorelease of tert-butyloxy carbonyl tyrosine methyl ester was found to vary between 20 and 25 % depending on the nature of solvent. Similar results were obtained for compound 50: the chemical yield of photorelease of 21-hydroxyprogesterone was found to vary between 15 and 29% depending on the nature of solvent.

The low chemical yield of photoregeneration of compounds 45, 47 and 49 can be rationalized by a side reaction of naphthoquinone methide with the released substrate. This problem, apparently, requires further investigation. Chemical yields should be improved by introduction of an efficient trapping reagent for quinone methides.

Figure 2.7. Quenching of quinone methide 6 by oxygen.

![Quenching plot showing the increase in the decay rate of the transient at 430 nm with increase of concentration of oxygen in water. \( k_q = 1.7 \times 10^6 \text{s}^{-1}\text{M}^{-1} \).]
In order to prove formation of quinone methide as an intermediate, LFP experiments were performed for compound 6. A transient with maximum absorbance at 430 nm was observed. This transient has lifetime of 19.2 μs in argon saturated acetonitrile and 30.1 μs in argon saturated water. Quenching studies have shown that this transient is sensitive to the presence of oxygen (Figure 2.7).

Quenching studies were also performed using the triplet quencher 1,3-cyclooctadiene. The transient at 430 nm turned out to be sensitive to this agent as well (Figure 2.8).

Figure 2.8. Quenching of quinone methide 6 by 1,3-cyclooctadiene.

Quenching plot showing the increase in the decay rate of the transient at 430 nm with increase of concentration of 1,3-cyclooctadiene in acetonitrile. $k_q = 3.4 \times 10^5 \text{s}^{-1} \text{M}^{-1}$.

Sensitivity towards triplet quenchers has never been reported for quinone methides. The fact that transient of naphthoquinone methide 25 is quenched by 1,3-cyclooctadiene can be explained by a Diels-Alder reaction between 25 and 1,3-cyclooctadiene. There are, indeed,
numerous reports in the literature that quinone methides can play the role of dienophile in cycloaddition reactions\textsuperscript{11}.

Overall, 2-oxymethylnaphthalene-3-ol ethers and esters were shown to release corresponding acids and alcohols efficiently. The only drawback in applying this chromophore as photoremovable protecting or caging group is low the chemical yield of photorelease for certain substrates.

**Design of a New Photoremovable Protecting Group Based on 4,4’-Dimethoxytrityl Chromophore**

One of the common cleavage mechanisms of photoremovable protecting groups is photoinduced heterolysis of the C-O bond with formation of an ion pair, which then undergoes media assisted separation to form unprotected substrate. This mechanism is characteristic for such photoremovable protecting groups as coumaryl\textsuperscript{21}, O-pixyl\textsuperscript{22} and anthraquinon-2-ylmethoxycarbonyl\textsuperscript{23}.

In 1989 McClelland et al. reported a flash photolysis study of differently substituted trityl carbocations\textsuperscript{24}. The cations were generated upon irradiation of aqueous acetonitrile solutions of the corresponding triarylacetonitriles and triaryl acetates. The results obtained by these authors suggest that differently substituted trityl ethers and esters can undergo heterolysis upon excitation and therefore along with formation of trityl carbocation should also release cyanic or acetic acid. Therefore, this photoreaction could be used for design of a new photoremovable protecting group.

The 4,4’-dimethoxytrityl group is a common protecting group for primary alcohols\textsuperscript{25} which is often used in DNA and RNA synthesis. As model compounds for studies of
applicability of 4,4′-dimethoxytrityl (DMTr) group as a photoremovable protecting group
DMTr-protected DNA nuleosides were selected. The dimethoxytrityl chromophore has no
absorbance above 295 nm, therefore 254 nm wavelength light was selected for irradiation.
Preliminary studies of photochemical behavior of 5′-(4,4′-dimethoxytrityl)-thymidine in 60%
aqueous methanol showed that this compound releases free thymidine upon irradiation with 254
nm light. Photorelease was found somewhat more efficient in the presence of added electrolyte
(sodium perchlorate). The chemical yield for photorelease of thymidine was found to be 89%.

Scheme 2.19.
Table 2.2.

<table>
<thead>
<tr>
<th>Nucleoside released</th>
<th>Yield of photorelease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxythymidine (52). MeOH/H₂O : 2/3</td>
<td>89%</td>
</tr>
<tr>
<td><img src="image" alt="Deoxythymidine" /></td>
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</tr>
<tr>
<td>Deoxyadenosine (53). MeOH/H₂O : 2/3</td>
<td>97%</td>
</tr>
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<td><img src="image" alt="Deoxyadenosine" /></td>
<td></td>
</tr>
<tr>
<td>N²-isobutyroyldeoxyguanosine (54). MeOH/H₂O : 1/1</td>
<td>98%</td>
</tr>
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<td></td>
</tr>
<tr>
<td>N³-benzoyldeoxycytidine (55). MeOH/H₂O : 1/1</td>
<td>0%</td>
</tr>
<tr>
<td><img src="image" alt="N³-benzoyldeoxycytidine" /></td>
<td></td>
</tr>
<tr>
<td>N³-propionyldeoxycytidine (56). MeOH/H₂O : 2/3</td>
<td>26%</td>
</tr>
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</tr>
<tr>
<td>Deoxycytidine (57). MeOH/H₂O : 2/3</td>
<td>85%</td>
</tr>
<tr>
<td><img src="image" alt="Deoxycytidine" /></td>
<td></td>
</tr>
</tbody>
</table>
Chemical yields of photorelease of a number of DNA nucleosides upon irradiation of their 5’-(4,4’-dimethoxytrityl) protected forms have been measured by means of HPLC. Results are summarized in Table 2.2. 5’-(4,4’-Dimethoxytrityl) protected thymidine, cytidine, N4- benzoylcytidine, adenosine and N2-isobutyrolguanosine were purchased from Aldrich, whereas, cytidine (48) and N4-propionylcytidine (49) had to be synthesized.

Synthesis of N-propionylcytidine followed the procedure developed by the Mitsuo Sekine group26 and is depicted on the Scheme 2.19. Synthesis of cytidine was performed using 2-(azidomethyl)-benzoyl protection, according to a procedure developed by Wada et al.27 Final product 48 was obtained in 64 % yield and 5’-dimethoxytrityl cytidine (50) was obtained in 56 % yield; products were characterized by means of 1H NMR.

Figure 2.9. UV absorption spectra of 52-57.

According to UV absorption spectra of these nucleosides (Figure 2.9), they all have significant absorption at 254 nm. Therefore, in order to be released quantitatively upon irradiation, the nucleoside molecules have to be photochemically stable. The results of study on
the chemical yields of photorelease of various nucleosides showed that purine nucleosides have photodeprotection yields close to quantitative.

On the other hand, pyrimidine nucleosides were deprotected in less than 90%. Thus upon irradiation of N\textsuperscript{4}-benzoyl-5’O-(4,4’-dimethoxytrityl)-deoxycytidine release of the nucleoside was not detected. N\textsuperscript{4}-Propionyl-5’O-(4,4’-dimethoxytrityl)-deoxycytidine, with aliphatic amide protection at N\textsuperscript{4} gives only 26% yield of photorelease of nucleoside upon irradiation. N- Unprotected cytidine and thimidine not having any amide protection gave reasonably good yields of photodeprotection (around 85%).

The difference in electronic structure of nucleosides with amino group protected with amide is clearly seen on Figure 2.9: nucleosides 54, 55 and 56 have additional absorption peaks or shoulders above 300 nm. We believe that the poor yields for photodeprotection of 55 and 56 are explained by their poor photochemical stability and by photochemistry associated with amide protection.

Figure 2.10. Quantum yield for release of thymidine as a function of the concentration of the electrolyte (sodium perchlorate).
The quantum yield of photodeprotection of 5’-O-(4’,4-dimethoxytrityl) protected DNA nucleosides was studied using thymidine as a model substrate. The quantum yield of photorelease of thymidine from its protected form in aqueous methanol was found to be only 0.01%. As was mentioned above, presence of an electrolyte improves the efficiency of this photoreaction. A possible explanation for this phenomenon is that electrolyte suppresses the ion pair recombination process, thus increasing the probability of heterolysis reaction to finally form the unprotected substrate.

The quantum yield for release of thymidine as a function of the concentration of the electrolyte (sodium perchlorate) is shown in Figure 2.10. Results obtained suggest that electrolyte does not have much effect on the quantum yield of release of thymidine. Quantum yield achieves maximum (0.018%) at ~0.4 M sodium perchlorate and decreases as the concentration of electrolyte increases further.

Overall, the 4,4’-dimethoxytrityl group was shown to be capable of functioning as a photoremovable protecting group for primary alcohols. High chemical yields of photorelease for photostable substrates have been demonstrated. However, the short wavelength of irradiation (254 nm required) and the low quantum yield for photorelease appear to be serious drawbacks in potential applications of this group as a general photoremovable protecting or caging group.
**Experimental part**

*Synthesis.*

**3-Hydroxymethyl-naphthalen-2-ol (12).** LiAlH₄ (1.14 g, 15 mmol) was suspended in 48 ml of dry THF and solution of 3 g (14.8 mmol) of methyl-3-hydroxy-2-naphtolate in 80 ml of THF was added to the suspension at room temperature over the period of 20 min. The reaction mixture was stirred at room temperature for 3 hours and then poured into wet diethyl ether. The mixture obtained was acidified with 10% HCl solution. Organic layer was separated and washed with 10% NaHCO₃ solution and water and dried over sodium sulphate. Solvents were removed under reduced pressure and the residue was recrystallized from ethanol-water mixture. 2.52 g of product obtained (85%). Characterization data were consistent with that reported in literature²⁸.

M.p. 187-189°C (lit. m. p. 186°C). ¹H NMR (DMSO-d₆) δ 9.81 (s, 1H), 7.92 (s, 1H), 7.75 (d, \( J = 7.8 \text{ Hz}, 1\text{H} \)), 7.63 (d, \( J = 8.1 \text{ Hz}, 1\text{H} \)), 7.33 (m, 1H), 7.23 (m, 1H), 7.08 (s, 1H), 5.16 (t, \( J = 5.7 \text{ Hz}, 1\text{H} \)), 4.62 (d, \( J = 5.7 \text{ Hz}, 2\text{H} \)).

**3-Ethoxymethyl-naphthalen-2-ol (13).** 3-Hydroxymethyl-naphthalen-2-ol (12) (300 mg, 1.72 mmol) was dissolved in 60 ml of ethanol and 15 ml of concentrated hydrochloric acid were added. The reaction mixture was refluxed for 3 hours and then poured into 400 ml of ice. The mixture obtained was extracted with chloroform, extracts were washed with 10% NaHCO₃ solution, water and dried over sodium sulphate. Solvents were removed and the residue subjected to chromatography on silica gel (DCM / Hexane = 3/1) to give 250 mg (63%) of product as colorless solid. Characterization data were consistent with that reported in literature²⁹.

M. p. 79-80°C (lit. m. p. 81°C). ¹H NMR (acetone-d₆) δ 7.87 (d, \( J = 8.1 \text{ Hz}, 1\text{H} \)), 7.83 (s, 1H), 7.79 (d, \( J = 8.1 \text{ Hz}, 1\text{H} \)), 7.53 (s, 1H), 7.51 (m, 1H), 7.41 (m, 1H), 4.81 (d, \( J = 0.9 \text{ Hz}, 2\text{H} \)), 3.73 (q, \( J = 7.2 \text{ Hz}, 2\text{H} \)), 1.35 (t, \( J = 7.2 \text{ Hz}, 3\text{H} \)).
**Acetic acid 3-hydroxy-naphthalen-2-ylmethyl ester (14).** 3-Hydroxymethyl-naphthalen-2-ol (12) (300 mg, 1.72 mmol) was dissolved in 15 ml of glacial acetic acid and the reaction mixture was stirred at 70ºC for 48 hours. Reaction mixture was then poured into 60 ml of chloroform, and the mixture obtained was washed with 10% NaHCO₃ and water and dried over sodium sulfate. Solvents were removed and the residue subjected to chromatography on silica gel (DCM / Et₂O = 10/1) to give 267 mg of product (65%) as colorless solid. Characterization data were consistent with that reported in literature²⁹.

M. p. 94-96ºC (lit. m. p. 96ºC). ¹H NMR (acetone-d₆) δ 7.85 (m, 2H), 7.76 (dd, ə = 8.4, 0.9 Hz, 1H), 7.50 (m, 1H), 7.39 (m, 1H), 5.32 (s, 2H), 3.52 (s, 3H).

**3-Benzylxy-naphthalen-2-yl)-methanol (18).** 3-Hydroxymethyl-naphthalen-2-ol (12) (200 mg, 1.15 mmol) dissolved in 3 ml of dry HMPA and 138 mg of sodium hydride (60% suspension in mineral oil, 3.46 mmol) were added. Solution was stirred at room temperature for 5 minutes and 165 μl (236 mg, 1.38 mmol) of benzyl bromide were added. The reaction mixture was stirred for 5 minutes at room temperature and then 0.1 ml of methanol was added. The mixture obtained was poured into100 ml of saturated solution of sodium bicarbonate, and the resulting suspension was extracted with diethyl ether. Extracts were washed with brine and dried over sodium sulfate. Solvent was removed and the products were isolated using preparative TLC. 232 mg (80%) of yellowish oil was obtained; product was identified as compound 18.

M. p. 77-79ºC. ¹H NMR δ 7.69 (m, 3H), 7.54-7.30 (m, 7H), 7.12 (s, 1H), 5.10 (s, 2H), 4.81 (s, 2H), 2.59, (br. s, 1H). ¹³C NMR δ 154.97, 136.56, 134.01, 130.74, 128.80, 128.73, 128.15, 127.66, 127.51, 127.34, 126.48, 126.31, 124.04, 106.45, 70.04, 62.40.

Less polar product (19) (75 mg, 18%) was isolated as colorless oil.
$^1$H NMR $\delta$ 7.91 (s, 1H), 7.72 (d, $J = 7.92$ Hz, 1H), 7.69 (d, $J = 8.1$ Hz, 1H), 7.22-7.50 (m, 12H), 7.17 (s, 1H), 5.17 (s, 2H), 4.81 (d, $J = 0.75$ Hz, 2H), 4.68 (s, 2H). MS $m/z$ 354 (M+, 23), 248 (10), 246 (25), 181 (19), 158 (8), 157 (60), 156 (100), 129 (20), 128 (91), 127 (15), 115 (17).

3-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-2-carboxylic acid methyl ester (20a).

Methyl-3-hydroxy-2-naphtolate (1 g, 4.95 mmol) was dissolved in 16 ml of dry DMF and 710 mg (10.4 mmol) of imidazole and 122 mg (1 mmol) of DMAP were added. After addition of 1.34 g (8.93 mmol) of tert-Butyl-dimethyl-silyl chloride in three portions, the reaction mixture was stirred at room temperature overnight. The reaction was quenched by addition of 100 ml of water. The mixture obtained was extracted with chloroform, extracts were washed with saturated sodium bicarbonate solution, water and dried over sodium sulfate. Solvent was removed in vacuo and the residue was subjected to chromatography on silica gel (DCM:Hexane = 1:2) to give 1.48 g (95%) of 20a as colorless oil.

$^1$H NMR $\delta$ 8.31 (s, 1H), 7.82 (d, $J = 8.28$ Hz, 1H), 7.69 (d, $J = 8.28$ Hz, 1H), 7.49 (m, 1H), 7.38 (m, 1H), 7.23 (s, 1H), 3.49 (s, 3H), 1.07 (s 9H), 0.29 (s, 6H). 13C NMR $\delta$ 167.26, 151.34, 135.95, 132.49, 128.57, 128.05, 128.01, 126.25, 124.45, 115.93, 51.00, 25.70, 18.27, -4.40. MS $m/z$ 45 (5), 57 (5), 59 (15), 114 (7), 115 (7), 141 (5), 185 (23), 186 (5), 211 (11), 229 (19), 244 (7), 259 (100), 260 (20), 261 (6).

Attempted preparation of [3(tert-Butyl-dimethylsilanyloxy)-naphtalene-2-yl] methanol (21a). LiAlH$_4$ (380 mg, 10 mmol) were suspended in 16 ml of THF and solution of 1.48 g (4.68 mmol) of 20a in 27 ml of THF was added dropwise. The reaction mixture was stirred for three hours at room temperature and then poured into 200 ml of wet cold diethyl ether. A portion of 30 ml of 10 % HCl were added and the mixture obtained was extracted with diethyl ether. Extracts were washed with saturated sodium bicarbonate solution, water and dried over
sodium sulfate. Solvent was removed under reduced pressure and the residue was recrystallized from EtOAc / Hexane mixture. 620 mg of crystalline material was obtained. Analysis of its $^1$H NMR showed that the product is identical to compound 11. Desired [3(tert-Butyl-dimethylsilanyloxy)-naphtalene-2-yl] methanol (19a) was not found in the reaction mixture.

3-(2-Methoxy-ethoxymethoxy)-naphtalene-2-carboxylic acid methyl ester (20b).

Methyl-3-hydroxy-2-naphtolate (1g, 4.95 mmol) was dissolved in 10 ml of dry dichloromethane, and 0.88 ml (960 mg, 7.68 mmol) of 1-Chloromethoxy-2-methoxy-ethane and 1.3 ml (960 mg, 7.46 mmol) of diisopropylethyl amine were added. The reaction mixture was stirred at room temperature for 48 hours and than was diluted with diethyl ether. Solution obtained was washed with water and dried over sodium sulfate. Product was purified using silica gel column chromatography (EtOAc: Hexane = 1:3). 1.053 g of 20b and 264 mg of 11 were obtained. Yield: 99%; conversion: 73%.

$^1$H NMR δ 8.30 (s, 1H), 7.81 (d, $J = 8.1$ Hz, 1H), 7.75 (d, $J = 8.1$ Hz, 1H), 7.56 (s, 1H), 7.51 (m, 1H), 7.39 (m, 1H), 5.46 (s, 2H), 3.93 (m, 5H), 3.59 (m, 2H), 3.38 (s, 3H). $^{13}$C NMR δ 166.56, 155.54, 136.57, 128.92, 128.35, 127.55, 126.50, 126.41, 124.19, 123.82, 119.15, 100.73, 71.65, 69.58, 58.00, 52.15. MS m/z 290 (M$^+$, 10), 215 (5), 202 (5), 171 (5), 170 (36), 143 (5), 142 (19), 127 (8), 115 (8), 114 (15), 89 (100), 59 (97).

[3-(2-Methoxy-ethoxymethoxy)-naphtalene-2-yl]-methanol (21b). LiAlH₄ (173 mg, 4.55 mmol) were suspended in 7.5 ml of dry THF, and solution of 753 mg (2.87 mmol) of 20b in 12 ml of THF was added dropwise over a period of 15 minutes. The reaction mixture was stirred at room temperature for 2 hours. Reaction was quenched by addition of 100 ml of wet cold diethyl ether and 15 ml of water consequently. The mixture obtained was filtered, and solution
was washed with brine and dried over sodium sulfate. Product was purified using chromatography on silica gel (CHCl₃: Et₂O = 1:1). 542 mg of 21b was obtained (80%).

1H NMR δ 7.70 (m, 3H), 7.46-7.26 (m, 3H), 5.37 (m, 2H), 4.79 (d, J = 0.36 Hz, 2H), 3.80 (s, 2H), 3.51 (s, 3H), 3.32 (m, 2H), 2.97 (m, 2H). 13C NMR δ 153.03, 133.79, 130.83, 129.07, 127.45, 127.27, 126.63, 126.08, 124.12, 108.75, 93.34, 71.49, 67.96, 61.63, 58.83.

2-benzoxymethyl-3-(2-methoxy-ethoxymethoxy)-naphthalene (22b). Compound 21b (200 mg, 0.76 mmol) of and 3.8 ml (5.47 g 32 mmol) of benzyl bromide were dissolved in 7.6 ml of dry THF and ~25 mg of dry sodium hydride were added. Reaction mixture was refluxed overnight and then quenched by addition of 20 μl of methanol. Mixture obtained was filtered, solvents were removed under reduced pressure and the residue was subjected to chromatography on silica gel (EtOAc: Hexane = 1:3) to afford 249 mg of 22b (93%).

1H NMR δ 7.81 (s, 1H), 7.75 (m, 2H), 7.50-7.24 (m, 8H), 5.40 (s, 2H), 4.75 (d, = 0.75 Hz, 2H), 4.67 (s, 2H), 3.82 (m, 2H), 3.53 (m, 2H), 3.36 (s, 3H). 13C NMR δ 151.23, 134.78, 130.38, 128.07, 127.77, 127.63, 126.14, 126.11, 124.85, 122.06, 98.96, 71.54, 69.30, 60.34, 58.96. MS m/z 352 (M⁺, 9), 247 (7), 246 (16), 231 (5), 186 (12), 171 (5), 167 (5), 158 (6), 157 (25), 156 (100), 150 (5), 149 (18), 141 (6), 129 (13), 128 (44), 127 (9), 126 (5), 115 (10), 113 (5), 105 (6).

Attempted preparation of 3-Benzoxymethyl-naphtalene-2-ol (15b). Protocol 1. Compound 22b (100 mg, 0.28 mmol) was dissolved in 2 ml of benzene and 86 mg of montmorillonite K10 were added. The reaction mixture was stirred for 1 hour at room temperature, no sign of reaction proceeding appeared (monitored by TLC) after that period. The reaction mixture was heated to 50ºC and stirred for another hour, which lead to formation of complicated mixture of degradation products (indicated by TLC).
Protocol 2. Compound 22b (100 mg, 0.28 mg) was dissolved in 5 ml of dichloromethane and 3.4 ml of trifluoroacetic acid were added to the solution. The reaction mixture was stirred for 30 minutes at room temperature, after this period TLC showed partial consumption of starting material and formation of number of long-tailed overlapped spots.

Protocol 3. Compound 22b (50 mg, 0.14 mg) was dissolved in 1.5 ml of dry acetonitrile and 18 μl (15 mg, 0.14 mmol) of trimethylsilyl chloride and 21 mg (0.14 mmol) of sodium iodide were added consequently at -20°C. The reaction mixture was stirred at -20°C for 20 minutes and then one more portion of the same quantity of each: trimethylsilyl chloride and sodium iodide were added. Reaction mixture was stirred for two hours at -20°C and then was poured into 50 ml of saturated solution of sodium bicarbonate. Mixture obtained was extracted with chloroform; extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to preparative TLC to afford 20 mg of yellowish oil. 1H NMR analysis of the product revealed that it was a complex mixture.

Protocol 3. Compound 22b (50 mg, 0.14 mmol) was dissolved in 0.7 ml of isopropanol and approximately 5 mg (0.015 mmol) of carbon tetrabromide were added. The reaction mixture was refluxed for 2 hours, and no sign of reaction was detected by TLC.

3-Methylsulfanylmethoxynaphtalene-2-carboxylic acid methyl ester (20c). Methyl-3-hydroxy-2-naphtolate (1g, 4.95 mmol) of was dissolved in 25 ml of dry HMPA, 130 mg (5.41 mmol) of sodium hydride were added to the solution and the reaction mixture was stirred at room temperature for 30 minutes. After this period 0.45 ml (530 mg, 5.52 mmol) of chloromethyl methyl sulfide were added dropwise. The reaction mixture was stirred overnight at room temperature and then was poured into 200 ml of saturated solution of sodium bicarbonate. The mixture obtained was extracted with diethyl ether; extracts were washed with brine and dried
over sodium sulfate. Solvent was removed and the product was purified using chromatography on silica gel (EtOAc / Hexane = 1/4) to afford 1.17 g of colorless oil (90%).

$^1$H NMR δ 8.32 (s, 1H), 7.82 (dd, $J = 8.07$, 1.3 Hz, 1H), 7.74 (dd, $J = 8.1$, 0.57 Hz, 1H), 7.52 (m, 1H), 7.39 (m, 1H), 7.29 (s, 1H), 5.32 (s, 2H), 3.92 (s, 3H), 2.32 (s, 3H). $^{13}$C NMR δ 166.47, 152.49, 135.62, 132.83, 128.62, 128.39, 128.14, 126.89, 124.88, 122.79, 110.81, 73.22, 52.16, 14.57. MS m/z 263 (M$^{+}$+1, 1.21), 262 (M$, 1.39), 215 (12), 183 (5), 170 (6), 142 (7), 126 (5), 115 (7), 114 (14).

(3-Methylsulfanylmethoxynaphtalene-2-yl)-methanol (21c). Compound 21c was synthesized according to the procedure used for compounds 21b. Yield: 97%.

$^1$H NMR δ 7.73 (m, 3H), 7.13 (m, 1H), 7.32 (m, 1H), 7.15 (s, 1H), 5.26 (s, 2H), 4.83 (s, 2H), 2.16 (br. s 1H), 2.27 (s, 3H). $^{13}$C NMR δ 153.31, 133.70, 131.10, 129.10, 127.46, 127.71, 127.62, 126.62, 126.35, 124.31, 107.99, 72.65, 61.97, 14.93.

2-Benzoxymethyl-3-methylsulfanylmethoxy-naphtalene (22c). (3-Methylsulfanylmethoxynaphtalene-2-yl)-methanol (21c) (650 mg, 2.78 mmol) was dissolved in 28 ml of THF. 1 ml of HMPA and 92 mg of sodium hydride were added to the solution and it was stirred for 30 minutes at room temperature. 5 ml (7.15g, 42 mmol) of benzyl bromide were added to the solution and reaction mixture was refluxed for 2 hours. Reaction was quenched by addition of 2 ml of methanol. The mixture obtained was poured into 200 ml of saturated solution of sodium bicarbonate, and the resulting suspension was extracted with diethyl ether. Extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to chromatography on silica gel (EtOAc / Hexane :1/5) to afford 820 mg of 22c as a colorless oil (91%).
\[ ^1H \text{ NMR} \delta 7.90 \text{ (s, 1H)}, 7.77 \text{ (d, } J = 7.89 \text{ Hz, 1H)}, 7.71 \text{ (d, } J = 8.1 \text{ Hz, 1H)}, 7.45-7.23 \text{ (m, 8H)}, 7.16 \text{ (s, 1H)}, 5.24 \text{ (s, 2H)}, 4.75 \text{ (d, } J = 3.93 \text{ Hz, 2H)}, 4.68 \text{ (s, 2H)}, 2.22 \text{ (s, 3H)}. \]

**Attempted preparation of 3-Benzoxymethyl-naphthalene-2-ol (15b). Protocol 1.**

Compound **22c** (100 mg, 0.31 mmol) was dissolved in 3.3 ml of 25% aqueous acetonitrile. Calcium carbonate (45 mg, 0.45 mmol) and 81 mg (0.31 mmol) of HgCl\(_2\) were added and the reaction mixture was refluxed for 8 hours. Within this period no sign of reaction proceeding appeared (TLC), 65 mg of starting material was recovered.

**Protocol 2.** Compound **22c** (100 mg, 0.31 mmol) was dissolved in 3.3 ml of 25% aqueous acetonitrile. 81 mg (0.31 mmol) of HgCl\(_2\) were added and the reaction mixture was refluxed for 4 hours. Within this period partial decomposition of starting material was detected by TLC with formation of very polar mixture of products, none of which could be isolated using chromatography on silica gel.

**{3-[4,4’-Dimethoxytrityl]-naphthalene-2-yloxy}-tert-butyldimethylsilane (24).**

Compound **12** (300 mg, 1.72 mmol) was dissolved in 5.1 ml of dry pyridine and 570 mg (1.68 mmol) of dimethoxytrityl chloride and 42 mg (0.34 mmol) of DMAP were added. The reaction mixture was stirred at room temperature overnight and then pyridine was evaporated. The residue was dissolved in dichloromethane and the solution was washed with brine and dried over sodium sulfate. Solvent was removed and the residue was taken to the next step without further purification.

The mixture obtained was dissolved in 9 ml of dry DMF. 257 mg (3.78 mmol) of imidazole, 42 mg (0.34 mmol) of DMAP and 516 mg (3.44 mmol) of TBDMSCl were added. The reaction mixture was stirred at room temperature overnight and then was poured into 200 ml of saturated solution of sodium bicarbonate, and the resulting suspension was extracted with
ethyl acetate. Extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to chromatography on silica gel (EtOAc / Hexane : 1/4) to afford 764 mg of 24 as white solid (82% over two steps).

M.p. 139-141°C. $^1$H NMR $\delta$ 8.30 (s, 1H), 7.91 (m, 1H), 7.66 (m, 1H), 7.56 (dt, $J = 6.96$, 1.5 Hz, 2H), 7.49-7.16 (m, 9H), 7.04 (s, 1H), 6.84 (dt, $J = 9.03$, 3.03 Hz, 4H), 4.30 (d, $J = 1.14$ Hz, 2H), 3.78 (s, 6H), 0.82 (s, 9H), 0.13 (s, 6H). $^{13}$C NMR $\delta$ 158.53, 151.07, 145.24, 136.54, 133.34, 132.00, 130.02, 129.26, 128.20, 127.88, 126.77, 126.22, 125.71, 125.58, 123.73, 113.24, 112.69, 96.15, 86.67, 62.19, 55.18, 25.70, 18.07, -4.37. MS m/z 590 (M$^+$, 2), 305 (5), 304 (30), 303 (100), 273 (7), 229 (9), 227 (5), 215 (10), 195 (7), 165 (5), 152 (6), 141 (5). HRMS M$^+$ 590.2847 calcd for C$_{38}$H$_{42}$O$_4$Si 590.2852.

$[3$-(tert-Butyldimethylsilyloxy)-naphthalene-2-yl]-methanol (25). Compound 24 (200 mg, 0.34 mmol) of 24 was dissolved in 4 ml of wet acetonitrile and 40 mg (0.053 mmol) of cerium triflate were added. The reaction mixture was stirred at room temperature for 3 hours and then was poured into 100 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed and the residue was subjected to silica gel column chromatography (CHCl$_3$) to afford 83 mg of 25 (85%).

$^1$H NMR $\delta$ 7.79 (m, 2H), 7.71 (d, $J = 7.53$ Hz, 1H), 7.45 (m, 1H), 7.37 (m, 1H), 7.17 (s, 1H), 4.86 (s, 2H), 2.21 (br. s 1H), 1.09 (s, 9H), 0.37 (s, 6H). $^{13}$C NMR $\delta$ 151.84, 133.96, 132.83, 129.08, 127.63, 127.42, 126.30, 126.09, 124.03, 113.33, 62.40, 25.79, 18.24, -4.17. MS m/z 288 (M$^+$, 5), 233 (7), 232 (27), 231 (100), 229 (6), 215 (17), 214 (18), 213 (80), 212 (5), 210 (18), 199 (6), 198 (7), 197 (8), 185 (26), 157 (8), 156 (6), 155 (19), 153 (6), 152 (7), 141 (9), 139 (5), 129 (6), 128 (20), 127 (10), 115 (5). HRMS M$^+$ 288.1541 calcd for C$_{17}$H$_{24}$O$_2$Si 288.1546.
**Benzoic acid 3-(tert-butyl-dimethylsilyl-oxy)-naphthalene-2-yl methyl ester (27).**

Compound 25 (100 mg, 0.35 mmol) was dissolved in 0.7 ml of dry pyridine and 60 μl (49 mg, 0.35 mmol) of benzoyl chloride were added. The reaction mixture was stirred at room temperature for two hours and then was poured into 50 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to chromatography on silica gel (EtOAc / Hexane = 1/5) to afford 120 mg of 27 (90 %).

$^1$H NMR δ 8.13 (m, 2H), 7.91 (s, 1H), 7.79 (d, $J = 8.67$ Hz, 1H), 7.71 (d, $J = 7.56$ Hz, 1H), 7.57 (m, 1H), 7.50-7.40 (m, 3H), 7.35 (m, 1H), 7.21 (s, 1H), 5.57 (s, 2H), 1.07 (s, 9H), 0.36 (s, 6H). $^{13}$C NMR δ 166.47, 151.99, 134.42, 132.89, 130.39, 126.69, 129.27, 128.83, 128.33, 128.19, 127.76, 126.41, 126.30, 124.00, 113.34, 63.01, 25.75, 18.29, -4.17. MS m/z 394 (M$^+$ + 4, 2), 392 (M$^+$, 5), 337 (8), 336 (23), 335 (100), 255 (5), 230 (12), 229 (16), 216 (5), 215 (58), 200 (10), 199 (42), 185 (19), 179 (56), 155 (9), 153 (10), 142 (16), 139 (17), 128 (42). HRMS M$^+$ 392.1808 calcd for C$_{18}$H$_{28}$O$_3$Si 392.1808.

**Benzoic acid 3-hydroxynaphthalene-2-ylmethyl ester (15a).** Compound 27 (120 mg, 0.31 mmol) was dissolved in 0.5 ml of dry THF and 0.33 ml of 1 M solution of terabutylammonium fluoride in THF was added dropwise. The reaction mixture was stirred at room temperature for 5 minutes and then was poured into 50 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed and the residue was dissolved in dichloromethane and passed through short silica gel column to afford 75 mg of 15b (87%).

$^1$H NMR δ 8.08 (m, 2H), 7.90 (s, 1H), 7.77 (d, $J = 8.1$ Hz, 1H), 7.69 (d, $J = 8.1$ Hz, 1H), 7.63 (s, 1H), 7.57 (m, 1H), 7.50-7.38 (m, 3H), 7.36-7.28 (m, 2H), 5.56 (s, 2H). $^{13}$C NMR δ
168.41, 152.81, 135.42, 133.61, 132.01, 129.97, 129.36, 128.59, 128.49, 127.80, 127.01, 126.30, 124.14, 123.88, 112.19, 63.81. MS m/z 265 (11), 264 (44), 171 (5), 168 (7), 158 (13), 157 (23), 156 (97), 139 (5), 129 (20), 128 (92), 127 (17), 115 (23), 105 (6), 102 (5), 92 (10), 91 (100). HRMS M+ 278.0939 calcd for C_{18}H_{14}O_{3} 278.0943.

**3-(Benzyloxy-methyl-naphthalene-2-yloxy)-tert-butyl-dimethylsilane (26).** Compound 25 (200 mg, 0.69 mmol) was dissolved in 3 ml of dry HMPA and 30 mg of 60% suspension of sodium hydride in mineral oil (0.75 mmol) were added. Solution was stirred at room temperature for 5 minutes and 91 μl (131 mg, 0.77 mmol) of benzyl bromide were added. The reaction mixture was stirred at room temperature for another period of 5 minutes and then was poured into 100 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with diethyl ether; extracts were washed with brine and dried over sodium sulfate. Solvent was removed and the residue was subjected to silica gel column chromatography (DCM / Hexane: 1/5) to afford 223 mg of 26 as colorless oil (85%).

\[ ^1H \text{ NMR} \delta 8.07 (s, 1H), 7.93 (d, J = 7.74 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.62-7.41 (m, 7H), 7.26 (s, 1H), 5.30 (s, 2H), 5.11(d, J = 1.32 Hz, 2H), 1.15 (s, 9H), 0.29 (s, 6H). MS m/z 378 (M+, 4), 322 (16), 321 (53), 244 (10), 243 (37), 231 (11), 230 (31), 229 (100), 216 (18), 215 (72), 213 (12), 211 (12), 199 (20), 185 (23), 142 (12), 141 (52), 139 (11), 128 (16). \]

**3-(Benzyloxy-naphthalene-2-yl)-methanol (28).** Compound 26 (50 mg, 0.13 mmol) was dissolved in 0.3 ml of THF and solution of 47 mg (0.15 mmol) of tetrabutylammonium fluoride in 0.15 ml of THF was added dropwise. The reaction mixture was stirred at room temperature for 1 hour and was poured into 50 ml of water. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed
and the residue was dissolved in dichloromethane and passed through a short column of silica gel to afford 29 mg of 28 (83%).

**(3-Bromomethyl-naphthalen-2-yloxy)-tert-butyl-dimethyl-silane (29).** Compound 25 (200 mg, 0.69 mmol) was dissolved in 2 ml of dry THF and 195 mg (0.74 mmol) of triphenylphosphine were added to the solution. The solution was stirred at room temperature for 5 minutes and 256 mg (0.74 mmol) of carbon tetrabromide were added in three portions. The reaction mixture was stirred at room temperature for two hours. The precipitate formed was filtered off and the residue was dried and subjected to chromatography on silica gel (3% ethyl acetate in hexane) to afford 165 mg of 29 as white solid (67%).

M. p. 57-59°C. $^{1}$H NMR $\delta$ 7.86 (s, 1H), 7.76 (d, $J = 8.1$ Hz, 1H), 7.69 (d, $J = 8.28$ Hz, 1H), 7.45 (s, 1H), 7.36 (s, 1H), 7.18 (s, 1H), 4.71 (s, 2H), 1.15 (s, 9H), 0.41 (s, 6H). $^{13}$C NMR $\delta$ 151.72, 134.73, 130.58, 129.92, 128.80, 127.65, 126.70, 126.30, 125.49, 124.09, 113.39, 29.57, 25.89, 18.35, -4.09. MS $m/z$ 352 ($M^+ + 2$, 4), 350 ($M^+$, 4), 297 (9), 296 (43), 295 (100), 294 (44), 293 (92), 279 (13), 277 (11), 272 (8), 271 (20), 216 (10), 215 (51), 214 (16), 213 (24), 211 (11), 205 (12), 199 (38), 197 (12), 185 (19), 155 (23), 153 (15), 152 (13), 141 (25), 140 (10), 139 (24). HRMS $M^+$ 350.0710 calcd for C$_{17}$H$_{23}$BrOSi 350.0702.

**3-Benzylxoxymethyl-naphthalen-2-ol (15b).** Benzyl alcohol (52 mg, 50 μl, 0.48 mmol) was dissolved in 0.65 ml of dichloromethane. 164 mg (193 μl, 0.86 mmol) of di-tert-butylpyridine and 162 mg (0.63 mmol) of silver triflate were added to the solution. The solution was cooled to 0°C and solution of 200 mg (0.53 mmol) of 29 in 0.45 ml of dichloromethane was added dropwise under vigorous stirring. The reaction mixture was stirred for 1 hour and then was diluted with 50 ml of dichloromethane. Solution obtained was washed with 10% hydrochloric acid, saturated solution of sodium bicarbonate, brine and dried over sodium sulfate. Solvent was
removed under reduced pressure and the residue was subjected to chromatography on silica gel (3% EtOAc in hexane) to afford 148 mg of impure (3-Benzylxymethyl-naphthalen-2-yloxy)-tert-butyl-dimethyl-silane (30).

Compound 30, thus obtained, was dissolved in 2 ml of THF and 0.44 ml of 1M solution of tetrabutylammonium fluoride in THF were added dropwise at 0°C. The reaction mixture was stirred at 0°C for 5 minutes and then was poured into 50 ml of brine. Mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography to afford 51 mg (48%) of 15b as white solid.

M. p. 102-104°C. $^1$H NMR $\delta$ 7.69 (m, 2H), 7.53 (m, 2H), 7.45-7.20 (m, 7H), 4.87 (s, 2H), 4.61 (2H). $^{13}$C NMR $\delta$ 154.08, 136.73, 134.79, 128.65, 128.29, 128.23, 128.13, 127.82, 127.51, 126.44, 126.35, 124.70, 123.63, 111.11, 72.40, 71.41. 264 (M+, 19), 172 (5), 168 (5), 156 (45), 141 (6), 128 (20), 127 (8), 115 (15), 91 (100), 77 (12), 71 (7). HRMS M+ 264.1153 calcd for C$_{18}$H$_{16}$O$_2$ 264.1150.

{2-[4,4'-Dimethoxytrityl]-naphthalen-1-yloxy}-tert-butyl-dimethyl-silane (35).

Compound (1.5 g, 7.42 mmol) 33 in 40 ml of dry THF was added dropwise to the suspension of 570 mg of LiAlH$_4$ in 17 ml of dry THF over a period of 15 minutes. Reaction mixture was stirred at room temperature for 2 hours and then quenched by addition of 500 ml of wet cold diethyl ether and 15 ml of water consequently. The mixture obtained was filtered, and solution was washed with brine and dried over sodium sulfate. The solvents were removed and the residue was used in the next step without further purification.

The product, thus obtained, was dissolved in 25 ml of dry pyridine and 2.85 g (8.43 mmol) and 210 mg (1.72 mmol) of 4-dimethylaminopyridine were added. The reaction mixture
was stirred at room temperature for 3 hours and then was poured into 200 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was taken to the next step without further purification.

The mixture obtained was dissolved in 30 ml of dry DMF and 2.1 g (31 mmol) of imidazole, 362 mg (3 mmol) of 4-dimethylaminopyridine and 4g (26.7 mmol) of tert-butyldimethylsilyl chloride were added. The reaction mixture was stirred overnight and then poured into 200 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to chromatography on silica gel (DCM / Hexane : 2/1) to afford 3.76 g of 35 (86%).

$^1$H NMR δ 8.07 (m, 1H), 7.99 (d, $J = 8.46$ Hz, 1H), 7.86 (m, 1H), 7.65 (d, $J = 8.49$ Hz), 7.60 (m, 2H), 7.53-7.42 (m, 6H), 7.36 (m, 2H), 7.31-7.23 (m, 1H), 6.90 (m, 4H), 4.41 (s, 2H), 3.83 (s, 6H), 0.98 (s, 9H), -0.03(s, 6H). $^{13}$C NMR δ 158.49, 147.34, 145.26, 136.56, 134.13, 130.12, 128.31, 127.83, 127.77, 127.61, 126.71, 126.14, 125.50, 124.83, 124.68, 122.95, 121.63, 113.18, 96.16, 86.57, 61.16, 55.14, 25.96, 18.49, -3.48. MS m/z 590 (M$^+$, 3), 305 (4), 304 (26), 303 (100), 273 (5), 229 (6), 215 (5), 195 (5).

$^{[1]}$-(tert-Butyl-dimethyl-silanyloxy)-naphthalen-2-yI-methanol (36). Compound 35(200 mg, 0.34 mmol) was dissolved in 4 ml of 5% aqueous acetonitrile and 40 mg of cerium triflate were added. The reaction mixture was stirred for 1 hour at room temperature and then poured into 50 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvents were
removed and the residue was subjected to chromatography on silica gel (EtOAc / Hexane : 4/1) to afford 78 mg of 36 (80%) as white solid.

M. p. 78-79ºC. $^1$H NMR $\delta$ 8.11 (m, 1H), 7.81 (m, 1H), 7.54 (s, 2H), 7.47 (m, 2H), 4.88 (s, 2H), 1.17 (s, 9H), 0.21 (s, 6H). $^{13}$C NMR $\delta$ 148.17, 134.57, 127.98, 127.67, 126.62, 125.93, 125.01, 123.11, 122.07, 60.09, 26.07, 18.69, -3.45. MS $m/z$ 288 (2), 233 (8), 232 (9), 231 (63), 230 (6), 225 (5), 215 (15), 213 (7), 203 (5), 201 (6), 193 (6), 185 (10), 156 (15), 155 (7), 128 (8), 127 (5), 75 (100).

**Benzoic acid 1-(tert-butyl-dimethyl-silanyloxy)-naphthalen-2-ylmethyl ester (37).**

Compound 36 (200 mg, 0.69 mmol) was dissolved in 1.4 ml of dry pyridine and 120 μl (145 mg, 1. mmol) of benzoyl chloride were added. The reaction mixture was stirred for 1 hour at room temperature and than poured into 50 ml of saturated solution of sodium bicarbonate. The resulting suspension was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvents were removed and the residue was subjected to chromatography on silica gel (EtOAc / Hexane: 1/20) to afford 270 mg of 37 (98%) as colorless oil.

$^1$H NMR $\delta$ 8.16-8.05 (m, 3H), 7.79 (m, 1H) 7.57-7.38 (m, 7H), 5.55 (s, 2H), 1.14 (s, 9H), 0.21 (s, 6H). $^{13}$C NMR $\delta$ 166.65, 149.30, 134.86, 132.92, 130.27, 129.71, 128.34, 128.00, 127.69, 127.14, 126.33, 125.14, 123.34 121.93, 121.15, 62.32, 26.06, 18.72, -3.48. MS $m/z$ 392 (1), 181 (5), 180 (16), 179 (100), 156 (5), 135 (13), 105 (40), 77 (19), 73 (17).

**Attempted synthesis of benzoic acid 1-hydroxy-naphthalen-2-ylmethyl ester (16a).**

*Procedure 1.* Compound 37 (200 mg, 0.51 mmol) was dissolved 1.2 ml of THF and solution of 148 mg (0.58 mmol) of tetrabutylammonium fluoride was added dropwise. The reaction mixture was stirred at room temperature for 5 minutes. After this period TLC analysis shows complete
consumption of starting material accompanied by formation of number of polar products including diol 34.

Procedure 2. Compound 37 (262 mg, 0.67 mmol) was dissolved in 15 ml of acetonitrile and 26 µl (30 mg, 1.5 mmol) of 48% of hydrofluoric acid were added. The reaction mixture was stirred at room temperature for 72 hours. After this period TLC analysis shows complete consumption of starting material accompanied by formation of number of polar products including diol 34.

1-Hydroxymethyl-naphthalen-2-ol (39). 2-naphthol (37) (2.5 g, 17.4 mmol) was dissolved in 18.4 ml of 1M solution of sodium hydroxide at 0ºC. 1.86 ml of 37% solution of formaldehyde in water was added and the reaction mixture was stirred for 1.5 hours at 0ºC. After this period reaction mixture was neutralized with 1M solution of hydrochloric acid and the product was filtered off and recristallized from ethanol / water mixture to give 5.5 g of 39 as yellowish solid (85%). Characterization data were consistent with that reported in literature30.

1H NMR (DMSO-d6) δ 9.5 (br. s, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.44 (m, 1H), 7.27 (m, 1H), 7.15 (d, J = 9 Hz, 1H), 4.92 (s, 2H).

{1-[4,4’-Dimethoxytrityl]-naphthalen-2-yloxy}-tert-butyl-dimethyl-silane (40). Compound 40 was obtained using the same procedure as for compound 24. Compound 40 was obtained as colorless oil (yield: 90%).

1H NMR δ 7.91 (d, J = 8.1 Hz, 1H), 7.81-7.77 (m, 2H), 7.55 (m, 2H), 7.51 -7.19 (m, 10H), 7.12 (d, J = 8.9 Hz, 1H), 6.87 (d, J = 8.9 Hz, 4H), 4.58 (s, 2H), 3.82 (s, 6H), 0.91 (s, 9H), 0.11 (s, 6H). 13C NMR δ 158.41, 150.96, 145.40136.75, 134.27, 130.29, 129.60, 129.16, 128.47, 127.87, 127.72, 126.58, 125.99, 125.35, 123.50, 121.89, 121.05, 113.06, 86.40, 57.89, 55.19,
25.76, 18.29, -4.15. MS m/z 590 (M⁺, 1), 305 (5), 304 (26), 303 (100), 273 (5), 231 (5), 229 (5), 195 (5).

[2-(tert-Butyl-dimethyl-silanyloxy)-naphthalen-1-yl]-methanol (41). Compound 41 was obtained using the same procedure as for compound 25. Compound 40 was obtained as colorless oil (yield: 75%).

1H NMR δ 8.11 (d, J = 8.4 Hz, 1H), 7.77(d, J = 8.1 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.50 (m, 1H), 7.35 (m, 1H), 5.14 (d, J = 3 Hz, 2H), 1.84 (br. s 1H), 1.06 (s, 9H), 0.27 (s, 6H). 13C NMR δ 151.3, 133.29, 129.66, 129.58, 128.35, 126.78, 123.73, 123.34, 123.33, 120.57, 56.24, 25.78, 18.30, -4.08.

Benzoic acid 2-(tert-butyl-dimethyl-silanyloxy)-naphthalen-1-ylmethyl ester (42). Compound 42 was obtained using the same procedure as for compound 27. Compound 42 was obtained as colorless oil (yield: 91%).

1H NMR δ 7.99 (m, 3H), 7.78 (m, 2H), 7.48 (m, 2H), 7.35 (m, 3H), 7.13 (d, J = 8.9 Hz, 1H), 5.86 (s, 2H), 1.03 (s, 9H), 0.26 (s, 6H). 13C NMR δ 166.81, 152.72, 133.99, 132.74, 130.60, 130.32, 129.66, 129.39, 128.37, 128.22, 127.02, 123.79, 123.03, 120.66, 118.36, 58.49, 25.71, 18.30, -4.11.

Attempted preparation of benzoic acid 2-hydroxy-naphthalen-1-ylmethyl ester(31).

Procedure 1. Compound 42 (200 mg, 0.51 mmol) was dissolved 1.2 ml of THF and solution of 148 mg (0.58 mmol) of tetrabutylammonium fluoride was added dropwise. The reaction mixture was stirred at room temperature for 5 minutes. After this period TLC analysis shows complete consumption of starting material accompanied by formation of number of polar products including diol 39.
**Procedure 2.** Compound 42 (262 mg, 0.67 mmol) dissolved in 15 ml of acetonitrile and 26 µl (30 mg, 1.5 mmol) of 48% of hydrofluoric acid were added. The reaction mixture was stirred at room temperature for 72 hours. After this period TLC analysis shows complete consumption of starting material accompanied by formation of number of polar products including diol 39.

**BocPheMN (44).** BocPheOH (265 mg, 1mmol) of and 334 mg (1.2 mmol) of 25 were dissolved in 7 ml of dichloromethane and 14 mg (0.11 mmol) of 4-dimethylaminopyridine and 480 mg (2.33 mmol) of dicyclohexylcarbodiimide were added. The reaction was stirred overnight at room temperature and the precipitate formed was then filtered off. The solution obtained was washed with brine and dried over sodium sulfate. Solvent was then removed and the residue subjected to chromatography on silica gel (10 % ethyl acetate in hexane) to afford 524 mg of phenylalanine 3-(tert-butyl-dimethylsilyl-oxy)-naphtalene-2-yl methyl ester (98%) as colorless oil.

$^1$H NMR δ 7.74 (m, 2H), 7.67 (d, $J = 7.7$ Hz, 1H), 7.43 (m, 1H), 7.34 (m, 1H), 7.22-6.95 (m, 6H), 5.34 (s, 2H), 5.01 (d, $J = 8.3$ Hz, 1H), 4. 66 (dt, $J = 8.3$ Hz, 6.0 Hz, 5.8 Hz, 1H), 3.1 (m, 2H), 1.41 (s, 9H), 1.05 (s, 9H), 0.33 (s, 6H). $^{13}$C NMR δ 171.67, 154.99, 151.84, 135.88, 134.45, 129.86, 129.34, 128.69, 128.40, 127.82, 127.33, 126.89, 126.55, 126.52, 124.03, 113.21, 69.11, 63.09, 54.46, 38.38, 28.29, 25.74, 18.29, -4.17, -4.21. MS m/z 422 (15), 404 (10), 378 (29), 350 (20), 333 (10), 332 (31), 271 (11), 246 (12), 232 (18), 231 (79), 229 (13), 220(20), 216 (20), 215 (100), 214 (17), 213 (21), 201 (13), 200 (10), 199 (39), 185 (16), 164 (13), 158 (25), 157 (38), 146 (15), 141 (38), 129 (14), 128 (53), 120 (80), 115 (10), 100 (20). HRMS M$^+$ 535.2762 calcd for C$_{31}$H$_{41}$NO$_3$Si 535.2754.
A product obtained (426 mg, 0.98 mmol) was dissolved in 4 ml of dry THF and 1 ml of 1 M solution of tetrabutylammonium fluoride was added to the solution dropwise. The reaction mixture was stirred at room temperature for 10 minutes and then poured into saturated solution of ammonium chloride. The mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. The solvent was removed and the residue was passed through a short silica gel column to afford 404 mg of 44 as yellowish oil (98%).

$^1$H NMR $\delta$ 7.70 (m, 2H), 7.64 (d, $J = 8.1$ Hz, 1H), 7.41 (m, 1H), 7.31 (m, 1H), 7.23 (s, 1H), 7.19-6.89 (m, 5H), 5.34 (dd, $J = 33.7$, 12.4 Hz, 2H), 5.07 (d, $J = 7.9$ Hz, 1H), 4.66 (m, 1H), 3.05 (d, $J = 5.64$ Hz, 2H), 1.42 (s, 9H). $^{13}$C NMR 172.95, 155.34, 152.70, 135.40, 135.11, 131.15, 129.19, 128.44, 127.82, 126.96, 126.84, 123.73, 123.69, 111.19, 80.35, 63.77, 54.47, 38.11, 28.24, 24.63. MS m/z 395 (5), 351 (6), 264 (5), 263 (19), 178 (10), 158 (18), 157 (100), 156 (10), 129 (10), 128 (18), 127 (5), 107 (22). HRMS M$^+$ 421.1888 calcd for C$_{25}$H$_{27}$NO$_5$ 421.1889.

**Attempted preparation of estrone 2-hydroxy-naphthalen-1-ylmethyl ether (46).** Estrone (60 mg, 0.22 mmol) was dissolved in 1 ml of dry dimethylformamide and 80 mg (0.24 mmol) of cesium carbonate were added. The reaction mixture was stirred for five minutes at room temperature and 91 mg (0.26 mmol) of 25 were added. The reaction mixture was stirred for two hours at room temperature and then poured into saturated solution of ammonium chloride. The mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. The solution obtained turned out to be a rather complicated mixture of products, one of which could be isolated by means of preparative TLC. According to $^1$H NMR this product was identified as tert-butyldimethylsilylestrone (12 mg, 14%).
1H NMR δ 7.12 (d, J = 8.3 Hz, 1H), 6.62 (dd, J = 2.6, 8.3 Hz, 1H), 6.57 (d, J = 2.6 Hz, 1H), 2.85 (m, 2H), 2.58-1.32 (m, 13 H), 0.98 (s, 9H), 0.88 (s, 3H), 0.19 (s, 6H).

Estrone 2-hydroxy-naphthalen-1-ylmethyl ether (46). Tributylphosphine (0.44 ml, 360 mg, 1.78 mmol) was dissolved in 6 ml of dry toluene and 448 mg (1.78 mmol) of 1,1’-(azodicarbonyl)dipiperedine were added at 0ºC. The reaction mixture was stirred at 0ºC for one hour (until orange color faded away) and the solution of 324 mg (1.2 mmol) of estrone and 389 mg (1.35 mmol) of 25 in 8 ml of mixture of toluene and THF (1:1) was added dropwise over the period of 15 minutes. The reaction mixture was stirred at 0ºC for one hour and than incubated at -20ºC for 48 hours. The reaction mixture was then poured into 80 ml of pentane and stirred for one hour at 0ºC. The precipitate formed was consequently filtered and the solvent was removed in vacuo. The residue was purified using chromatography on silica gel to afford 510 mg of impure estrone 3-(tert-butyl-dimethyl-silanyloxy)-naphthalen-2-ylmethyl ether.

The product was dissolved in 2 ml of dry THF and 1 ml of 1M solution of tetrabutylammonium fluoride was added dropwise. The reaction mixture was stirred at 0ºC for 5 minutes and then was poured into 50 ml of brine. Mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed under reduced pressure and the residue was subjected to chromatography on silica gel (Ethyl acetate / Hexane : 1/3) to afford 220 mg of 46 (43 %).

1H NMR δ 7.74 (m, 2H), 7.68 (dd, J = 8.1, 0.6 Hz, 1H), 7.41 (m, 1H), 7.31 (m, 1H), 7.25 (s, 1H), 7.21 (d, J = 8.7 Hz, 1H), 6.86 (dd, J = 8.7, 2.8 Hz, 1H), 6.80 (d, J = 2.8 Hz, 1H), 5.3 (s, 2H), 2.89 (m, 2H), 2.60-1.86 (m, 8H), 1.72-1.30 (m, 6H), 0.90 (s, 3H). 13C NMR δ 155.87, 152.98, 138.00, 134.57, 133.25, 128.49, 128.01, 127.66, 126.50, 126.19, 124.80, 123.72, 115.28,
tert-Butyloxycarbonyltyrosine methyl ester 2-hydroxy-naphthalen-1-ylmethyl ether (48). Tributylphosphine (253 μl, 205 mg, 1 mmol) of was dissolved in 3.3 ml of dry toluene and 252 mg (1 mmol) of 1,1'- (azodicarbonyl)dipiperedine were added at 0°C. The reaction mixture was stirred at 0°C for one hour (until orange color faded away) and the solution of 200 mg (0.67 mmol) of tert-Butyloxycarbonyltyrosine methyl ester and 215 mg (1.14 mmol) of 25 in 2 ml of toluene were added dropwise over the period of 15 minutes. The reaction mixture was stirred at 0°C for one hour and then incubated at -20°C for 48 hours. The mixture was then poured into 80 ml of pentane and stirred for one hour at 0°C. The precipitate formed was consequently filtered and the solvent was removed in vacuo. The residue was purified using chromatography on silica gel to afford 228 mg of tert-Butyloxycarbonyltyrosine methyl ester 3-(tert-butyl-dimethyl-silanyloxy)-naphthalen-2-ylmethyl ether (60%).

$^1$H NMR δ 7.89 (s, 1H), 7.75 (d, $J = 7.9$ Hz, 1H), 7.68 (d, $J = 8.1$ Hz, 1H), 7.40 (m, 1H), 7.32 (m, 1H), 7.17 (s, 1H), 7.05 (d, $J = 8.6$ Hz, 2H), 6.93 (d, $J = 8.6$ Hz, 2H), 5.20 (s, 2H), 4.98 (d, $J = 8.1$ Hz, 1H), 4.55 (m, 1H), 3.96 (s, 3H), 3.05 (m, 2H), 1.42 (s, 9H), 1.03 (s, 9H), 0.32 (s, 6H). $^{13}$C NMR δ 172.45, 158.08, 155.14, 151.41, 134.05, 130.34, 129.21, 129.03, 128.15, 127.85, 127.77, 126.30, 126.17, 125.61, 123.97, 114.87, 113.31, 79.88, 66.05, 54.59, 52.14, 37.55, 28.33, 25.81, 18.30, -4.14. HRMS M$^+$ 565.2855 caled for C$_{32}$H$_{43}$NO$_6$Si 565.2860.

The product was dissolved in 3 ml of dry THF and 0.5 ml of 1M solution of tetrabutylammonium fluoride was added dropwise. The reaction mixture was stirred at 0°C for 5 minutes and then was poured into 50 ml of brine. Mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed
under reduced pressure and the residue was subjected to chromatography on silica gel to afford 169 mg of 48 as colorless oil (56%).

$^1$H NMR $\delta$ 7.74 (m, 2H), 7.65 (d, $J = 8.1$ Hz, 1H), 7.40 (m, 1H), 7.30 (m, 1H), 7.22 (s, 1H), 6.97 (m, 5H), 5.27 (s, 2H), 5.05 (d, $J = 7.8$ Hz, 1H), 4.56 (q, $J = 7.5$ Hz, 1H), 3.67 (s, 3H), 3.01 (t, $J = 7.2$ Hz, 2H), 1.42 (s, 9H). $^{13}$C NMR $\delta$ 172.40, 157.23, 155.24, 152.76, 134.49, 130.36, 130.29, 128.88, 128.47, 127.98, 127.69, 126.44, 126.09, 124.94, 123.64, 115.19, 110.53, 96.08, 80.14, 67.90, 54.53, 52.18, 37.49, 28.26. MS $m/z$ 447 (6), 446 (17), 395 (5), 351 (6), 264 (5), 263 (19), 178 (10), 158 (18), 157 (100), 156 (10), 129 (10), 128 (18), 127 (5), 107 (22), 91 (8). HRMS M$^+$ 451.1993 calcd for C$_{26}$H$_{29}$NO$_6$ 451.1995.

21-(2-hydroxy-naphthalen-1-ylmethyloxy)-progesterone (50). 21-hydroxyprogesterone (49) (50 mg, 0.15 mmol) was dissolved in 1 ml of dichloromethane and 97 $\mu$l (82 mg, 0.43 mmol) of 2,6 di-tertbutylpyridine and 80 mg (0.31 mmol) of silver triflate were added to the solution at 0°C. The reaction mixture was stirred for 15 minutes at 0°C and solution of 100 mg (0.26 mmol) of 29 in 0.5 ml of dichloromethane was added. The reaction mixture was stirred at room temperature for 1.5 hours and then diluted with 15 ml of dichloromethane and filtered. Solvents were then removed and the residue was subjected to chromatography on silica gel to afford 45 mg of impure 21-(2-tert-butyl-dimethyl-silanyloxy-naphthalen-1-ylmethyloxy)-progesterone.

The product was dissolved in 0.5 ml of THF and 0.1 ml of 1M solution of tetrabutylammonium fluoride was added. The reaction mixture was stirred at 0°C for 5 minutes and then was poured into 50 ml of brine. Mixture obtained was extracted with ethyl acetate; extracts were washed with brine and dried over sodium sulfate. Solvent was removed under
reduced pressure and the residue was subjected to chromatography on silica gel (Ethyl acetate / Hexane : 2/1) to afford 33 mg of 50 (45%) as colorless oil.

$^1$H NMR $\delta$ 8.72 (br. s 1H), 7.72 (m, 2H), 7.60 (s, 1H), 7.41 (m, 1H), 7.29 (m, 2H), 5.74 (s, 1H), 4.69 (m, 2H), 4.26 (m, 2H), 2.62-2.12 (m, 6H), 2.10-0.53 (m, 17 H), 0.73 (s, 3H). $^{13}$C NMR $\delta$ 210.19, 199.32, 170.56, 154.34, 135.36, 128.94, 127.99, 127.58, 126.53, 126.34, 124.54, 124.01, 123.36, 111.37, 75.60, 72.08, 58.97, 56.18, 53.53, 44.82, 38.58, 38.53, 35.71, 35.53, 33.91, 32.69, 31.85, 24.49, 22.99, 20.95, 17.34, 13.55. MS m/z 446 (8), 259 (5), 215 (5), 158 (7), 157 (6), 153 (9), 152 (100), 149 (11), 135 (35), 122 (5), 115 (5), 111 (5), 107 (19), 105 (12).

HRMS M$^+$ 486.2761 calcd for C$_{32}$H$_{38}$O$_4$ 486.2770.

5'-O-(4,4'-dimethoxytrityl)-N$^4$-propionyl-2'-deoxycytidine (52). 2'-deoxycytidine (268 mg, 1 mmol) was coevaporated with three portions of 10 ml of dry dimethylformamide and then was dissolved in 10 ml dry dimethylformamide. Triethylamine (0.14 ml, 1 mmol) and propionic anhydride (0.15 ml, 1.2 mmol) were added to the solution and the reaction mixture was stirred at 60°C overnight. The solvent was removed in vacuo and the residue was dissolved in dichloromethane. Solution was washed with saturated solution of sodium bicarbonate, brine and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was dissolved in 5 ml of dry pyridine. 400 mg (1.2 mmol) of 4',4-dimethoxytrityl chloride and ~5 mg of 4-dimethylaminopyridine were added to the solution and the reaction mixture was stirred at room temperature for 24 hours. The solvent was then removed under reduced pressure and the residue was dissolved in dichloromethane. The solution was washed with saturated solution of sodium bicarbonate, brine and dried over sodium sulfate. The solvent was removed in vacuo and the residue was subjected to chromatography on silica gel (5% methanol in dichloromethane) to
afford 375 mg of 52 (64%). Characterization data were consistent with that reported in literature\(^\text{26}\).

\(^1\)H NMR \(\delta\) 8.85 (br. s, 1H), 8.23 (d, \(J = 7.8\) Hz, 1H), 7.39 (m, 2H), 7.36-7.20 (m, 8H), 7.21 (m, 1H), 6.85 (m, 4H), 6.31 (t, \(J = 6\) Hz, 1H), 4.52 (q, \(J = 5.1\) Hz, 1H), 4.16 (m, 1H), 3.79 (s, 6H), 3.44 (m, 3H), 2.81 (m, 1H), 2.43 (q, \(J = 7.5\) Hz, 2H), 2.22 (m, 1H), 1.15 (t, \(J = 7.5\) Hz, 3H).

**5'-O-(4,4'-dimethoxytrityl)-N\(^4\)-\(\text{2'-azidomethylbenzoyl}\)-2'-deoxycytidine (53).** 2'-deoxycytidine (100 mg, 0.44 mmol) was coevaporated with pyridine three times and then suspended in 2.2 ml of pyridine. 0.28 ml (2.2 mmol) of trimethylsilyl chloride was added and reaction mixture was stirred for 15 minutes at room temperature. After this period 0.32 ml (2.2 mmol) of 2-azidomethylbenzoyl chloride was added and reaction mixture was stirred for two hours at room temperature. The reaction mixture was then cooled to 0°C and TMS protection was cleaved and reaction was quenched by subsequent addition of 0.5 ml of 29% ammonium hydroxide solution and 1 ml of water. The reaction mixture was stirred for 15 minutes at room temperature and solvents were then removed under reduced pressure. The residue was dissolved in chloroform/ methanol mixture (10/1) and filtered through short layer of silica gel. The mixture obtained was purified using chromatography on silica gel (chloroform / methanol: 9/1) to give 121 mg of N\(^4\)-2-azidomethylbenzoyl-2’-doxycytidine (71%). The product obtained was dissolved in 1.7 ml of pyridine. ~2 mg of dimethylaminopyridine and 115 mg (0.34 mmol) of dimethoxytrityl chloride were added to the solution. The reaction mixture was stirred for 12 hours and then poured into 50 ml of methanol and stirred for 10 minutes at room temperature. Resulting mixture was than mixed with 100 ml of water and extracted with chloroform, extracts were washed with solution of sodium bicarbonate, water and dried over sodium sulfate. Solvents
were removed under reduced pressure and the residue was subjected to silica gel column chromatography to give 144 mg of desired product (68%). Characterization data were consistent with that reported in literature27.

\(^1\)H NMR (CD\(_3\)OD / CDCl\(_3\)) δ 8.43(d, J = 7.5 Hz, 1H), 7.68 (m, 1H), 7.61-7.45 (m, 3H), 4.41 (m, 2H), 7.37-7.20 (m, 8H), 6.89 (m, 4H), 6.18 (m, 1H), 4.62 (s, 2H), 4.54 (m, 1H), 4.11 (m, 1H), 3.78 (d, J = 1.8 Hz, 6H), 4.46 (d, J = 3.3 Hz, 1H), 2.61 (m, 1H), 2.33 (m, 1H).

5'-O-(4,4'-dimethoxytrityl)- 2'-deoxycytidine (54). Compound 54 (144 mg, 0.21 mmol) was dissolved in 2 ml of aqueous dioxane (9/1) and 84 μl (0.42 mmol) of diphenylmethylphosphine were added. The reaction mixture was stirred for 20 minutes and then poured into 30 ml of saturated sodium bicarbonate solution. The mixture obtained was extracted with chloroform; extracts were washed with water and dried over sodium sulphate. Solvent was removed under reduced pressure and the residue was subjected to chromatography on silica gel (chloroform/methanol: 10/1) to afford 106 mg (85%) of 54. Characterization data were consistent with that reported in literature27.

\(^1\)H NMR δ 7.81 (d, J = 7.2 Hz, 1H), 7.51-7.25 (m, 10H), 6.81 (m, 4H), 6.35 (m, 1H), 5.46 (d, J = 7.2 Hz, 1H), 4.46 (m, 1H), 4.09 (m, 1H), 3.75 (s, 6H), 3.79 (m, 2H), 3.43 (m, 2H), 2.63 (m, 1H).

**Photochemistry**

**Photolysis of compounds 13 and 14 monitored by \(^1\)H NMR.** 0.05 M deairated solution of compounds 13 in 40 % aqueous (deuturated) acetonitrile-d was prepared. Solution was irradiated in the quartz cells with 300 nm light using Rayonett apparatus. \(^1\)H NMR analysis of reaction mixture showed appearance of peaks characteristic for ethanol (triplet at 1.18 ppm).
0.05 M deaerated solutions of compounds 14 in deuterated methanol was prepared and irradiated in the quartz cells with 300 nm light using Rayonet apparatus. $^1$H NMR analysis of reaction mixture showed appearance of peaks characteristic for acetic acid (singlet at 1.92 ppm).

**Photolysis of compound 15b. Quantum yield of photorelease of benzyl alcohol measurements.** 3 ml of 5e-3 M solution of 15b was irradiated in quartz cell in Rayonet apparatus with 300 nm light for 6 minutes (within 10% conversion of starting material). After periods of 2, 3, 4, 5 and 6 minutes of irradiation 100 μl aliquots were taken and diluted 10 times for HPLC analysis. Light intensity was measured using ferroxylate actinometry. Quantum yield of photorelease of benzyl alcohol upon irradiation of compound 9 was found to be 9.25%.

**Photolysis of compound 15b. Chemical yield of photorelease of benzyl alcohol measurements.** 5e-4 M solution of 15b in 40% aqueous acetonitrile was irradiated with 300 nm light in Rayonet apparatus for 135 minutes. 100 μl aliquots were taken for HPLC analysis after periods of 5, 15, 30, 50, 75, and 135 minutes. Chemical yield of photorelease of benzyl alcohol was found to be 97%.

**Photolysis of compound 15a. Quantum yield of photorelease of benzoic acid measurements.** 3 ml of 5e-3 M solution of 15a was irradiated in quartz cell in Rayonet apparatus with 300 nm light for 6 minutes (within 10% conversion of starting material). After periods of 2, 3, 4, 5 and 6 minutes of irradiation 100 μl aliquots were taken and diluted 10 times for HPLC analysis. Light intensity was measured using ferroxylate actinometry. Quantum yield of photorelease of benzoic acid upon irradiation of compound 10 was found to be 9.4%.

**Photolysis of compound 15a. Chemical yield of photorelease of benzoic acid measurements.** 5e-4 M solution of 15a in deaerated 40% aqueous acetonitrile was irradiated with 300 nm light in Rayonet apparatus for 105 minutes. 100 μl aliquots were taken for HPLC
analysis after periods of 5, 15, 30, 50, 75 and 105 minutes. Chemical yield of photorelease of benzyl alcohol was found to be 96%.

**Photolysis of compound 44. Chemical yield of photorelease of N-tert-butyloxy carbonylphenylalanine measurements.** 5e-4 M solution of 44 in deairated 40% aqueous methanol was irradiated with 300 nm light in Rayonett apparatus for 135 minutes. 100 μl aliquots were taken for HPLC analysis after periods of 5, 10, 20, 40, 70 and 100 minutes. Chemical yield of photorelease of benzyl alcohol was found to be 96%.

**Photolysis of compound 46. Chemical yield of photorelease of estrone measurements.** 1e-4 M solution of compound 46 in deairated 40% aqueous acetonitrile was irradiated with 300 nm light in Rayonett apparatus for 60 minutes. 100 μl aliquots were taken for HPLC analysis after periods of 5, 10, 20, 30, 50 and 60 minutes. The chemical yield of estrone photorelease achieved maximum after 30 minutes of irradiation (32 %). Prolonged irradiation caused the chemical yield to decrease to 27 % after 60 minutes period.

**Photolysis of compound 448. Chemical yield of photorelease of tert-butyloxy carbonyl tyrosine methyl ester measurements.** 1e-4 M solution of compound 48 in deairated 40% aqueous acetonitrile was irradiated with 300 nm light in Rayonett apparatus for 60 minutes. 100 μl aliquots were taken for HPLC analysis after periods of 5, 10, 20, 30 and 40 minutes. The chemical yield of BocTyrOMe photorelease achieved maximum after 30 minutes of irradiation (25 %). Prolonged irradiation caused the chemical yield to decrease to 19 % after 40 minutes period.

Change of solvents for the photoreaction changed the chemical yield of BocTyrOMe in the following manner:

40 % aqueous methanol: 25 % (30 minutes of irradiation);
100 % methanol: 20 % (40 minutes of irradiation);
100 % water: 30 % (50 minutes of irradiation).

**Photolysis of compound 50. Chemical yield of photorelease of 21-hydroxyprogesterone**

**measurements.** 1e-4 M solution of compound 50 in deairated 40% aqueous methanol was irradiated with 300 nm light in Rayonett apparatus for 50 minutes. 100 μl aliquots were taken for HPLC analysis after periods of 5, 10, 20, 30 and 40 minutes. The chemical yield of 21-hydroxyprogesterone photorelease achieved maximum after 30 minutes of irradiation (23 %). Prolonged irradiation caused the chemical yield to decrease to 17 % after 50 minutes period.

Change of solvents for the photoreaction changed the chemical yield of 21-hydroxyprogesterone in the following manner:

100 % methanol: 15 % (30 minutes of irradiation);
100 % acetonitrile: 29 % (35 minutes of irradiation).

**Photolysis of 5'-O-(4,4'-dimethoxytrityl) DNA nucleosides. Chemical yields of photorelease of compounds 51, 55-59.** 3 ml of 5e-4 M solution of 5'-O-(4,4'-dimethoxytrityl) DNA nucleoside in argon saturated aqueous methanol were irradiated in quartz cell with 254 nm light. Concentration of nucleoside released was measured by means of HPLC.

**Photolysis of 5'-O-(4,4'-dimethoxytrityl)-2'-deoxythymidine. Quantum yields of photorelease of thymidine in presence of sodium perchlorate at various concentrations.** 3 ml of 1e-3 M solution of 5'-O-(4,4'-dimethoxytrityl)-2'-deoxythymidine in 40% aqueous methanol in presence of sodium perchlorate at various concentrations was irradiated in quartz cell in Rayonett apparatus with 254 nm light for 60 minutes (within 10% conversion of starting material). After periods of 10, 20, 30, 50 and 60 minutes of irradiation 100 μl aliquots were taken for HPLC analysis. Light intensity was measured using ferroxylate actinometry.
**Laser flash photolysis experiments.** Rate measurements were conducted using nanosecond ($\lambda_{\text{exc}} = 266$ nm) flash photolysis system that has already been described\textsuperscript{31}. Initial concentration of naphthol 12 was c. a. 3e-4M for all the experiments. Reactions were monitored by following the decay of quinone methide absorbance at 430 nm and observed first–order rate constants were obtained by least–squares fitting of an exponential function.
References


SUPPORTING MATERIAL.

Characterization data on compounds synthesized.
Part I. Design and Synthesis of a New Photocleavable Lipid Analog.

2-Phenyl-[1,3]dithiane (4)
(3,4-Dimethoxy-phenyl)-(2-phenyl-[1,3]dithian-2-yl)-methanol (5)
Acetic acid (3,4-dimethoxy-phenyl)-(2-phenyl-[1,3]dithian-2-yl)-methyl ester (8)
3',4'-Dimethoxybenzoin diethyl phosphate (10).
MeCN; c = 3e-5M

![Absorbance vs Wavelength Graph](image-url)
3-(tert-Butyldimethylsilyloxy)-5-methoxybenzaldehyde

![Chemical Structure]

[Chemical Structure Image]

[1H NMR Spectrum]

[13C NMR Spectrum]
1-Acetoxy-[3-(tert-butyldimethylsilyloxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (15)
%Transmittance

Wavenumbers (cm⁻¹)

3500  2000  2500  1500  1000

1760.88
1694.40
1481.90 1437.18
1299.47
1282.85
1234.76
1192.50
1154.79
1120.37
1082.82
1034.28
983.82
883.67

81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99  100
1-Acetoxy(-3-hydroxy-5-methoxyphenyl)-2-phenyl-2-(1,3-dithian-2-yl)ethane (16)
Pentacosane-13-ol (18)

\[
\text{OH}
\]
1-Acetoxy-[3-(pentacosyl-13-oxy)-5-methoxyphenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (19)
3’-(1-dodecyl-tridecyloxy)-5’-methoxybenzoin acetate. (20)
3’-(1-dodecyldodecyloxy)-5’-methoxylbenzoin (21).
1-[3-(1-Dodecyl-tridecyloxy)-5-methoxy-phenyl]-2-phenyl-ethane-1,2-dione (22).
3’-(1-dodecyldodecyloxy)-5’-methoxybenzoin tert-butyloxycarbonyl-4-tert-butyl ester aspartate

(24)
3’-(1-dodecyltridecyloxy)-5’-methoxybenzoin 1-aspartate (25).
Part II. Design of a new photoremovable protecting (caging) group.

3-Hydroxymethyl-naphthalen-2-ol (12)

\[
\text{\begin{tikzpicture}
  \node (A) at (0,0) {OH};
  \node (B) at (0.5,0) {OH};
  \node (C) at (1,0) {OH};
\end{tikzpicture}}
\]
3-Ethoxymethyl-naphthalen-2-ol (13)

MeCN; c = 1e-4M
Acetic acid 3-hydroxy-naphthalen-2-ylmethyl ester (14)
3-Benzoxymethyl-naphtalene-2-ol (15b)
MeCN; c = 1e-4M

Absorption vs. Wavelength

% Transmission vs. Wavelength
Benzoic acid 3-hydroxynaphtalene-2-ylmethyl ester (15a)
MeCN; $c = 1 \times 10^{-4}$ M

Absorption

Wavelength (cm$^{-1}$)
(3-Benzylxy-naphthalen-2-yl)-methanol (18)
2-Benzylxoy-3-benzylxomethyl-naphthalene (19)

\[
\begin{align*}
\text{Ph} & \quad \text{O} \\
\text{Ph} & \quad \text{O}
\end{align*}
\]
3-(tert-Butyl-dimethyl-silanyloxy)-naphthalene-2-carboxylic acid methyl ester (20a)
3-(2-Methoxy-ethoxymethoxy)-naphthalene-2-carboxylic acid methyl ester (20b).
3-Methylsulfanylmethoxynaphtalene-2-carboxylic acid methyl ester (20c)
[3-(2-Methoxy-ethoxymethoxy)-naphtalene-2-yl]-methanol (21b)
(3-Methylsulfanylmethoxynaphtalene-2-yl)-methanol (21c)
2-benzoxy methyl-3-(2-methoxy-ethoxymethoxy)-naphthalene (22b)
2-Benzoxymethyl-3-methylsulfanylmethoxy-naphtalene (22c)
\{3-[4,4’-Dimethoxytrityl]-naphtalene-2-yloxy\}-tert-butyldimethylsilane (24)
[3-(tert-Butyldimethylsilyloxy)-naphtalene-2-yl]-methanol (25)

\[
\begin{array}{c}
\text{OH} \\
\text{OTBDMS}
\end{array}
\]
3-(Benzyloxymethyl-naphtalene-2-ylxyo)-tert-butyl-dimethylsilane (26)

\[
\begin{align*}
\text{OTBDMS} & \\
\text{O} & \text{Ph}
\end{align*}
\]
Benzoic acid 3-(tert-butyl-dimethylsilyl-oxy)-naphtalene-2-yl methyl ester (27)
(3-Bromomethyl-naphthalen-2-yloxy)-tert-butyl-dimethyl-silane (29)
{2-[4,4'-Dimethoxytrityl]-naphthalen-1-yloxy}-tert-butyl-dimethyl-silane (35)
[1-(tert-Butyl-dimethyl-silanyloxy)-naphthalen-2-yl]-methanol (36)
Benzoic acid 1-(tert-butyl-dimethyl-silanyloxy)-naphthalen-2-ylmethyl ester (37)
1-Hydroxymethyl-naphthalen-2-ol (39)
{1-[4,4'-Dimethoxytrityl]-naphthalen-2-yl oxy}-tert-butyl-dimethyl-silane (40)
[2-(tert-Butyl-dimethyl-silanyloxy)-naphthalen-1-yl]-methanol (41)
Benzoic acid 2-(tert-butyl-dimethyl-silanyloxy)-naphthalen-1-ylmethyl ester (42)
tert-Butyloxycarbonyl-phenylalanine 3-(tert-butyl-dimethylsilyl-oxy)-naphtalene-2-yl methyl ester
BocPheMN (44)

\[
\begin{align*}
\text{NHBoc} & \quad \text{O} \\
\text{O} & \quad \text{OH}
\end{align*}
\]
Estrone 2-hydroxy-naphthalen-1-ylmethyl ether (46)
tert-Butyloxycarbonyltyrosine methyl ester 3-(tert-butyl-dimethyl-silanyloxy)-naphthalen-2-ylmethyl ether

\[
\text{NHBoc}
\]

\[
\text{OTBDMS}
\]
tert-Butyloxycarbonyltyrosine methyl ester 2-hydroxy-naphthalen-1-ylmethyl ether (48)
21-(2-hydroxy-naphthalen-1-ylmethyloxy)-progesterone (50)