Synthesis and Characterisation of Photolabile Protective Groups for DNA Synthesis

Final Project Thesis
ATEI Thessaloniki of Food Technology

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This Thesis was supervised by Prof. Dr. Dr. h.c. Pfleiderer from the Faculty of Chemistry of the University of Konstanz and Christos Ritzoulis MSc, PhD from the ATEI of Thessaloniki.

εν οίδα, ουδέν οίδα

en oida, ouden oida

true knowledge exists in knowing that you know nothing

Sokratis

The experimental research for the present thesis was accomplished from 01 October 2010 to 31 March 2011 in the laboratory of Prof. Dr. Dr. h.c. Pfleiderer in the Faculty of Chemistry of the University of Konstanz.

Herewith I declare that I wrote this Bachelor thesis independently under supervision and used no other sources and aids than those indicated.

_____ _____

Date Signature

My special thanks go to Prof. Wolfgang Pfleiderer for giving me such an interesting subject and his valuable support and guidance throughout the entire project.
Many thanks also go to Geeta for her very friendly work atmosphere, and her much appreciated advise and encouragement.
I am thankful to my Tutor Ch. Ritzoulis PhD, who made it possible for me to come to Konstanz and the opportunity to work on this project.

I am especially grateful to my family and Kostas for their patience, support and continuous encouragement.

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Glossary

ACN	Acetonitrile
br	broad signal
CC	Column Chromatography
DBU	1,8-Diazabicyvlo-[5.4.0]-undec-7-ene
d	Doublet
dd	Double of doublets
D_2O	Deuterium oxide 99.9
DMSO	Dimethylsulfoxide-d_6
DMF	N, N-Dimethylformamide
EtOAc	Ethyl acetate
HOAc	Acetic acid
HV	High pressure
m	Multiplet
MP	Melting Point
NEt_3	Triethylamine
NMR	Nuclear Magnetic Resonance
q	Quartet
R_f	Retention factor
r.t.	Room temperature
s	Singulet
t	Triplet
THF	Tetrahydrofurane
TLC	Thin Layer Chromatography
UV	Ultraviolet

1. Introduction

1.1 Protective groups

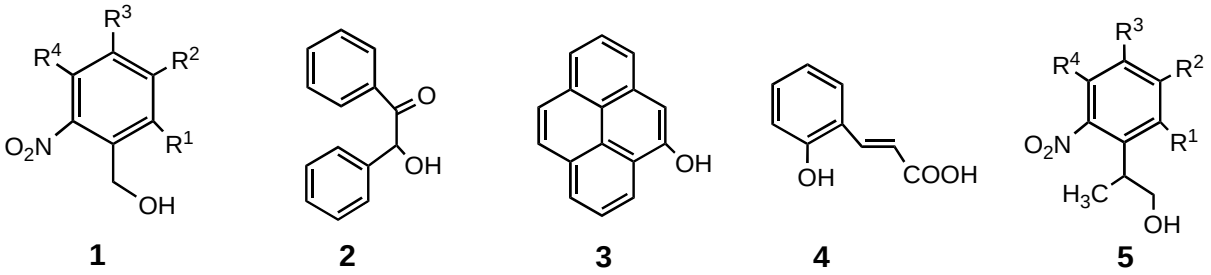
Protective groups are structures which protect particular functions of a molecule so that no undesired reactions with particular reagents take place but at the same time they can be cleaved off easily with appropriate conditions for example with acid or base. In the case of photolabile protective group irradiation with light at suitable wave length, the protective group is eliminated. The use of photolabile protective groups in nucleic acid[1], carbohydrate[2] and peptide[3] chemistry has been well established and plays an important role as extension of normal blocking-group stategies in synthetic organic chemistry. An important application of the photolabile protective group is the new technique of the light directed parallel synthesis. By using combination of solid phase synthesis and photolithography, it is possible to synthesize a big number of different sequences of biomolecules for e.g. of oligopeptides and oligonucleotides.

1.2 Protective groups of o-nitrobenzyl alcohol

The most common representatives featuring the anticipated photochemical lability are derived from o-nitrobenzyl alcohol and its derivatives[4] (**1**). It was shown that the quantum yield of photodeprotection is strongly influenced by substituents at the phenyl moiety[5] and the $CH_2(\alpha)$ group[6] which on substitution by a methyl group at $C(\alpha)$ revealed a strong increase in quantum yield of the photocleavage. The o-nitrobenzyl function has been used since its discovery in 1901[7] to protect hydroxy, amino, mercapto, carboxy and carbonyl functions. More recently, photolabile protection of the 5'-OH group of 2'-deoxyribo-nucleoside 3'-phosphoramidites[8] and its 3'-OH isomer[9] has been employed in the solid-phase synthesis of DNA probe arrays[10]. For this purpose the o-nitroveratryloxycarbonyl (NVOC) and the α-methyl-o-nitro Piperonyloxycarbonyl (MeNPOC) have been considered as excellent candidates in the chip production of oligonucleotide arrays by the photolithographic technique.

This method has already revolutionized the field of microelectronics to a large extent and is now applied as another consequential technology in molecular biology to serve as analytical and diagnostic tool for DNA analyses.

There are also other photolabile groups such as the benzoin residue[11] (**2**), the pyrenylmethyl group[12] (**3**) and cinnamyl esters[13](**4**).



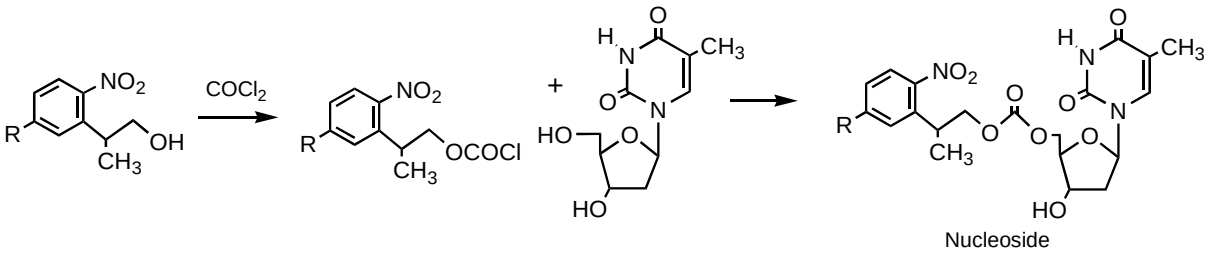
1 **2** **3** **4** **5**

1.3 Protective groups of 2-(2-nitrophenylethanol)

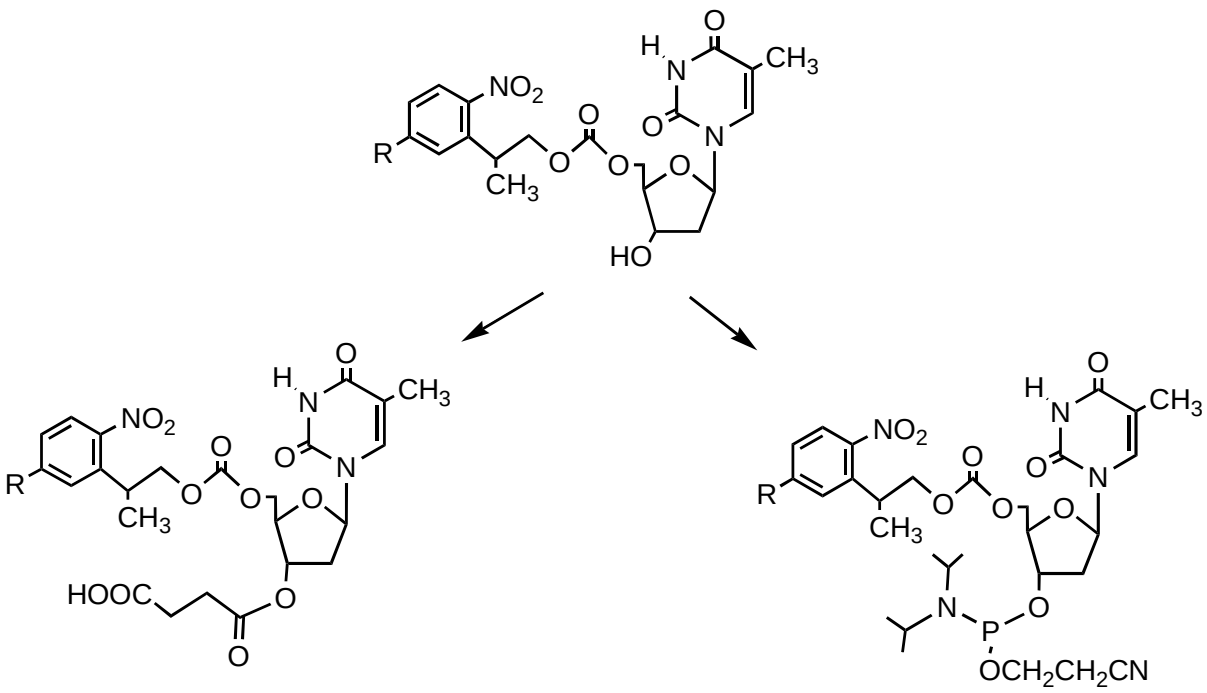
A tremendous improvement in the photolytic blocking-group strategy was achieved recently in the laboratory of Prof. W.Pfleiderer at the University of Konstanz by the introduction of the 2-(2-nitrophenylethanol (NPE)[14,15] and its derivatives[16] as entirely new types of photolabile functions with outstanding properties and especially prone for application in nucleoside and nucleotide chemistry. In contrast to the cleavage mechanism of the 2-nitrobenzyl group, which has been well studied[17], the photodecomposition of the NPE functions follows a new pathway established as a photoinduced ß-elimination process[18]. Further investigations have shown that branching in the α-position, preferentially by a methyl group increases the quantum yield of photocleavage by a factor of 10. This led to more detailed studies of modified 2-(2-nitrophenyl)-propanol (NPP) derivatives (**5**)[16] to further improve the photolytic features.

1.4 Solid support synthesis

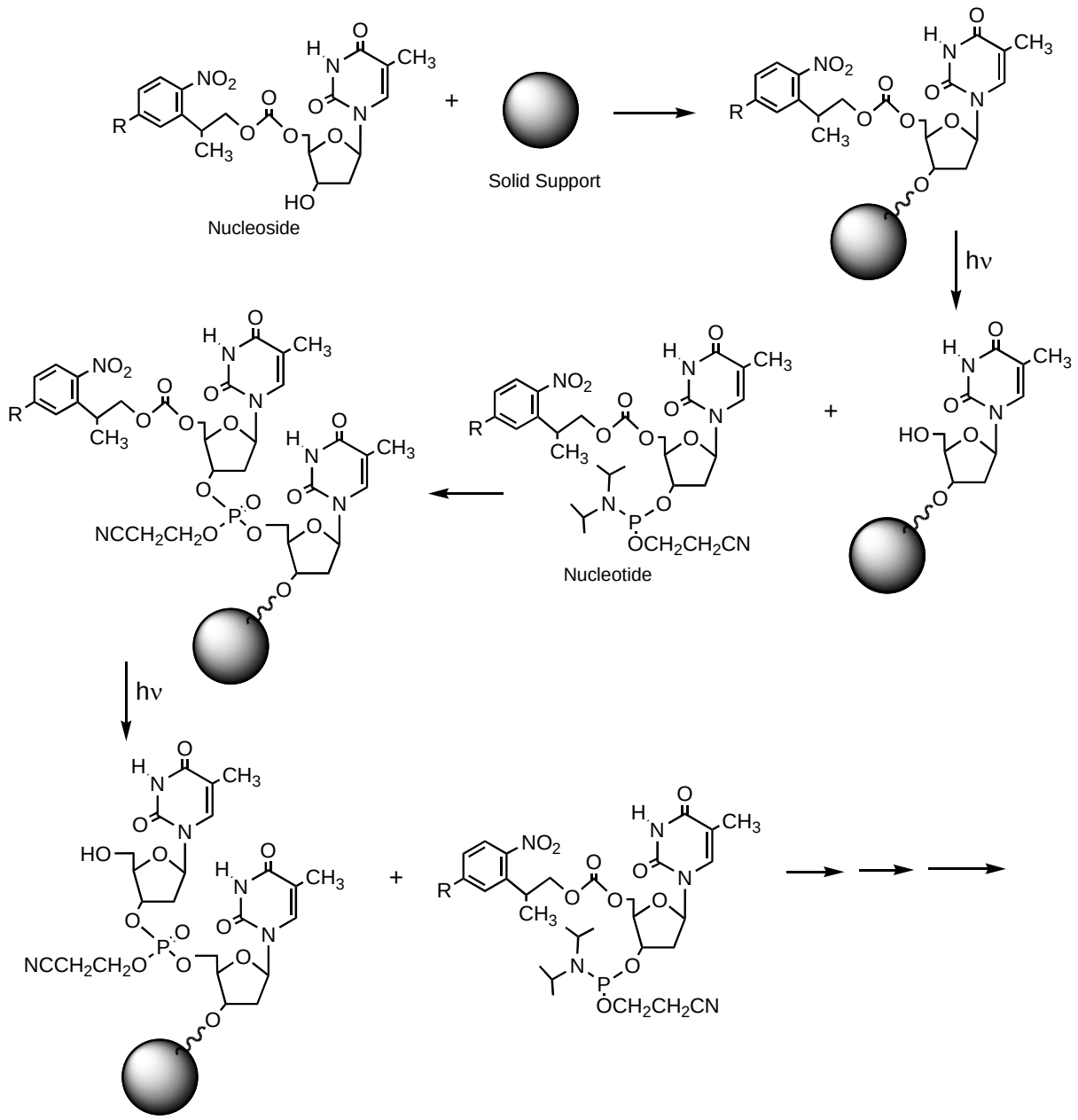
In order to demonstrate the anticipated project in general, the following schemes will illustrate the strategy to build-up a DNA-chip on a solid support. The synthesis will start with the preparation of the appropriate photolabile protecting group. This 2-(2-nitrophenyl)propanol derivative is then converted into its chloroformate with phosgen and subsequently coupled with the nucleoside thymidine to the corresponding 5'-carbonate.



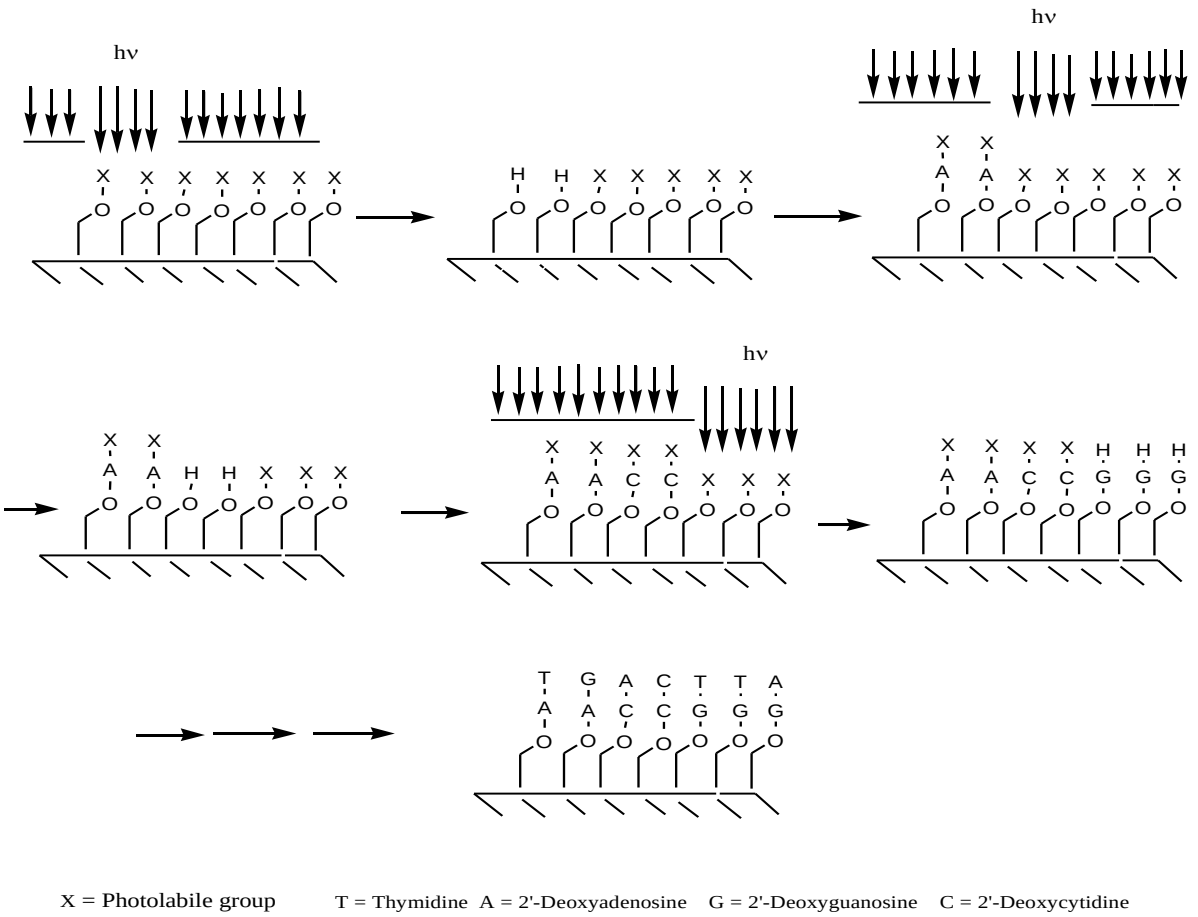
This building block is of general use to prepare with succinic anhydride the 3'-O-succinate which will then be attached to the surface of the solid-support to function as starting unit in the subsequent chain elongation to form an oligonucleotide. The second component is synthesized from the same building block by conversion into the corresponding phosphoramidite.



The assembly of these components to an oligonucleotide by the photolithographic technique can be visualized by the following scheme:

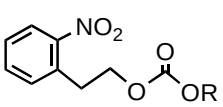


Analogous phosphoramidites have been prepared from the starting nucleosides 2'-deoxy-cytidine (dC), 2'-deoxyadenosine (dA) and 2'-deoxyguanosine (dG) which are then applied according to the anticipated oligonucleotide sequence. The following picture will show this process in a schematic manner:

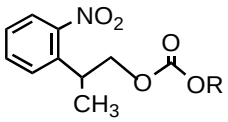


2. Research Proposal

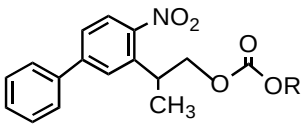
Recent investigations with the new type of photolabile protecting groups have shown that several structural features lead to improved properties which are directly dependent on the extinction at the appropriate wavelength of light irradiation. Since the pyrimidine and purine moiety in the nucleosides absorb light only below 300 nm the photolysis process should be achieved at longer wavelengths to avoid side-reactions. Since the mercury lamp offers a strong line of high intensity at 365 nm this light source is in common use. The project is based on the fact that the chromophore of the photolabile group should have an absorption band of high extinction in the area of 365 nm to guarantee a high efficent and fast cleavage on irradiation. It is known from recent results that the cleavage rate of 2-(2 nitrophenyl) ethyloxycarbonyl group (NPEOC) (**6**) can be improved by branching in α-position by a methyl group and by introduction of various substituent in position 4 and 5 of the benzene ring. Good canditates have so far been found in the series of 2-(2-nitrophenyl) propyloxycarbonyl (NPPOC) (**7**) and its 5-phenyl- (**8**) and 5-benzoyl derivatives (**9**).



NPEOC (**6**)



NPPOC (**7**)



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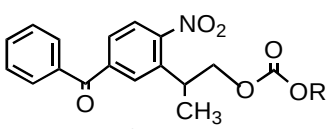

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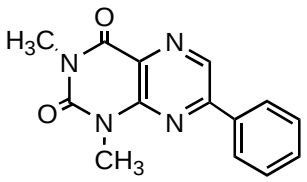
The new approach to be investigated in this bachelor thesis is based on special lumazine derivatives showing a strong absorption band at 360 nm. The basic molecule of this series is 1,3-dimethyl-7-phenyllumazine (**10**) which has been modified in its substituents (**11**) to fulfill the structural requirements for a photolabile protecting group.



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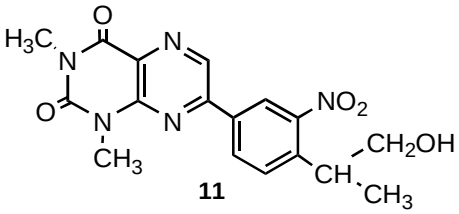
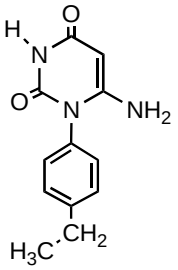


11

The o-nitrophenylpropanol substituent should be located at a different position preferentially at N-1. That means that the synthesis has to start with a Traube synthesis applying 4-ethylphenyl-urea and ethyl cyanoacetate to form in the first step 6-amino-1-(4-ethylphenyl) uracil (**12**). Alkylations can modify N-3 in different ways forming molecules (**13**) with good properties regarding solubilities and handling. The 5-amino group (**14**) will be introduced via its 5-nitroso derivative and subsequent condensation with 4-nitrophenylglyoxal should lead to 1-(4-ethylphenyl)-7-(4-nitrophenyl)-3-alkyllumazine (**15**).



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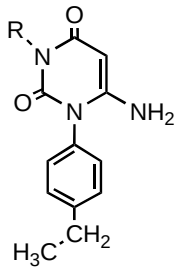

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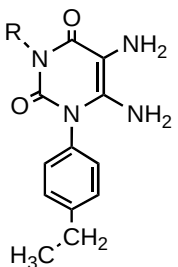

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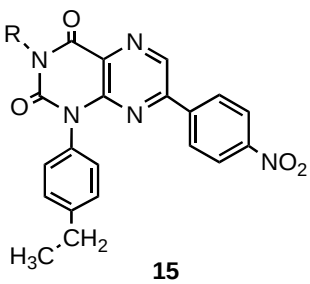
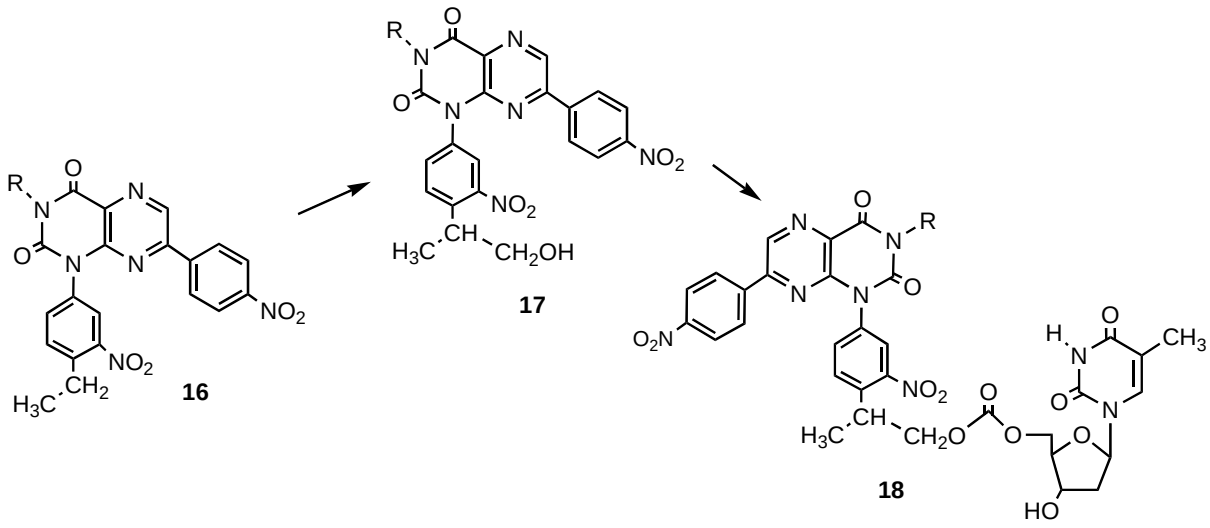


15

Nitration of **15** should proceed in ortho position of the ethyl group forming **16** and the subsequent reaction with paraformaldehyde will lead to 1-[4-(1-hydroxypropan-2-yl)phenyl]-

7-(4-nitrophenyl)-3-alkyllumazine (**17**). In order to attach **17** onto the nucleoside thymidine it has to be reacted with phosgene to get the corresponding chloroformate which will directly coupled with thymidine to the new photolabile building block (**18**). In photolysis studies the rate of photocleavage will be measured and compared with other lightsensitive molecules.

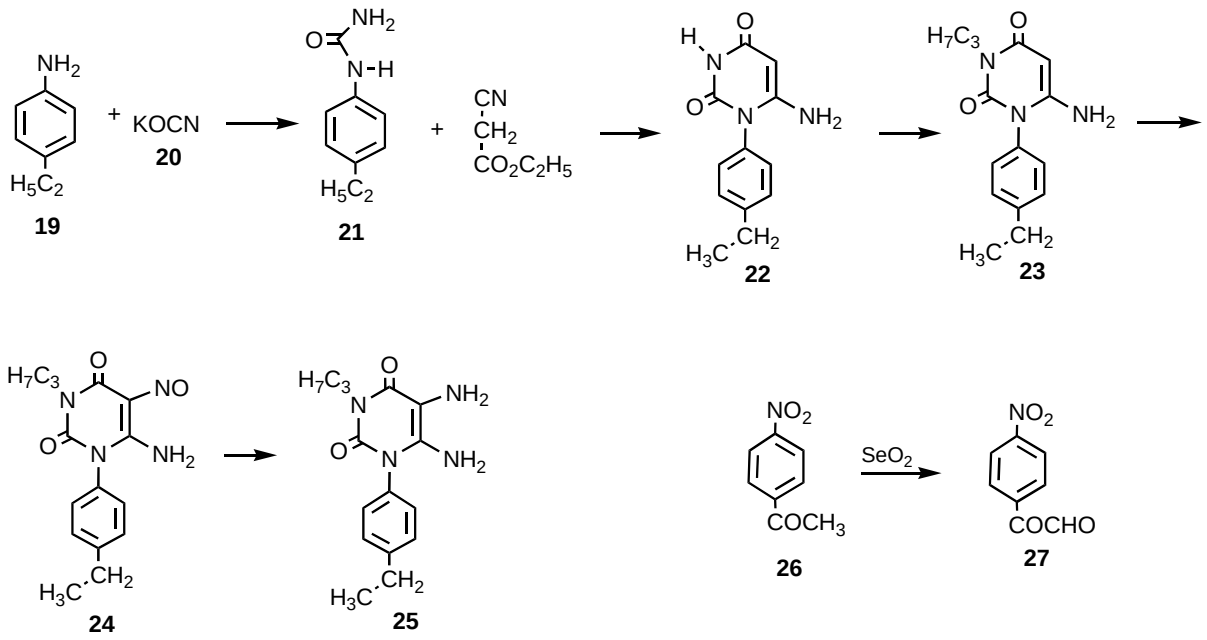


Depending on time available analogous reactions can be performed in order to increase the potential of the photolabile protecting groups.

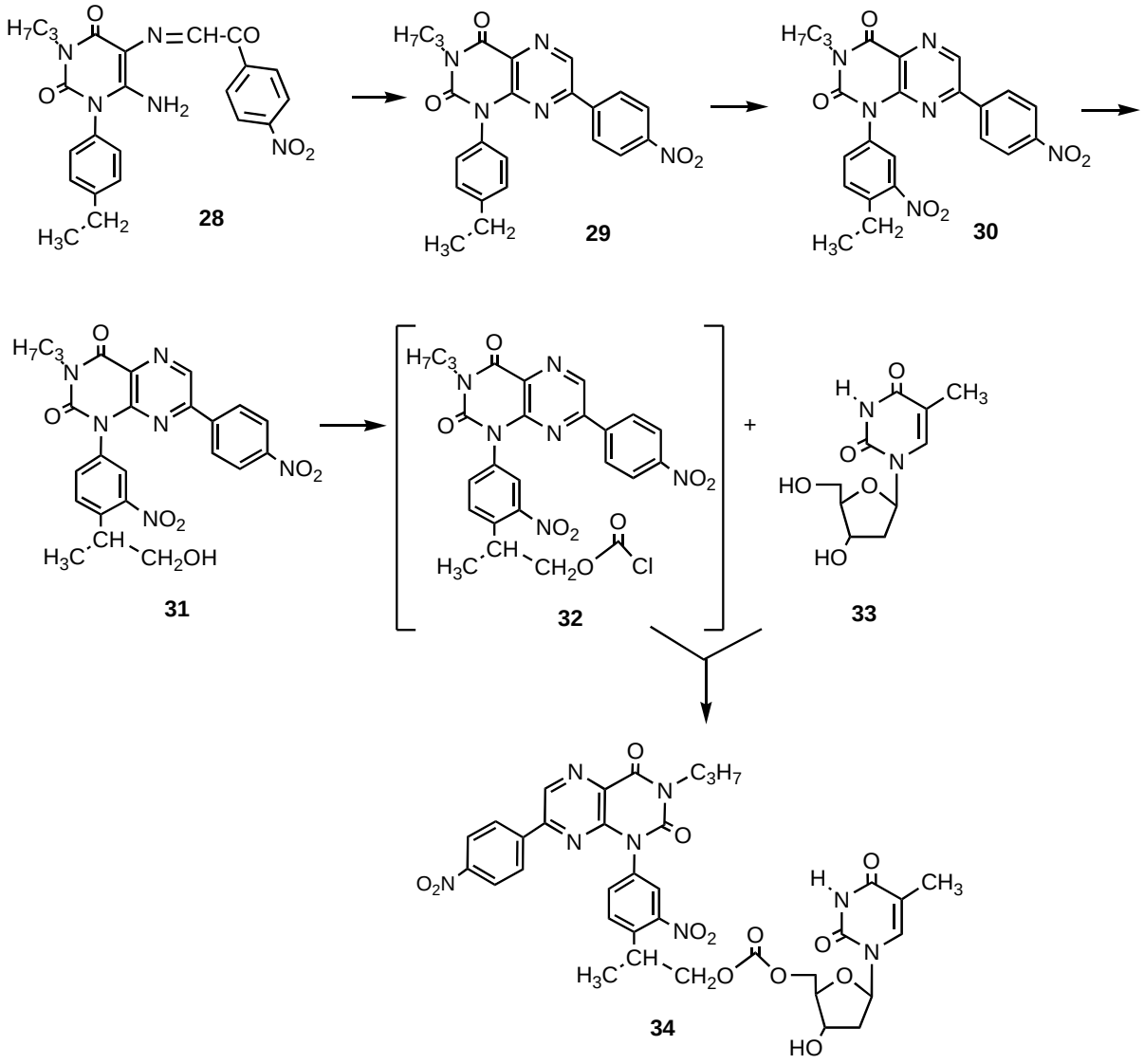
3. Results and Discussion

3.1 Synthesis

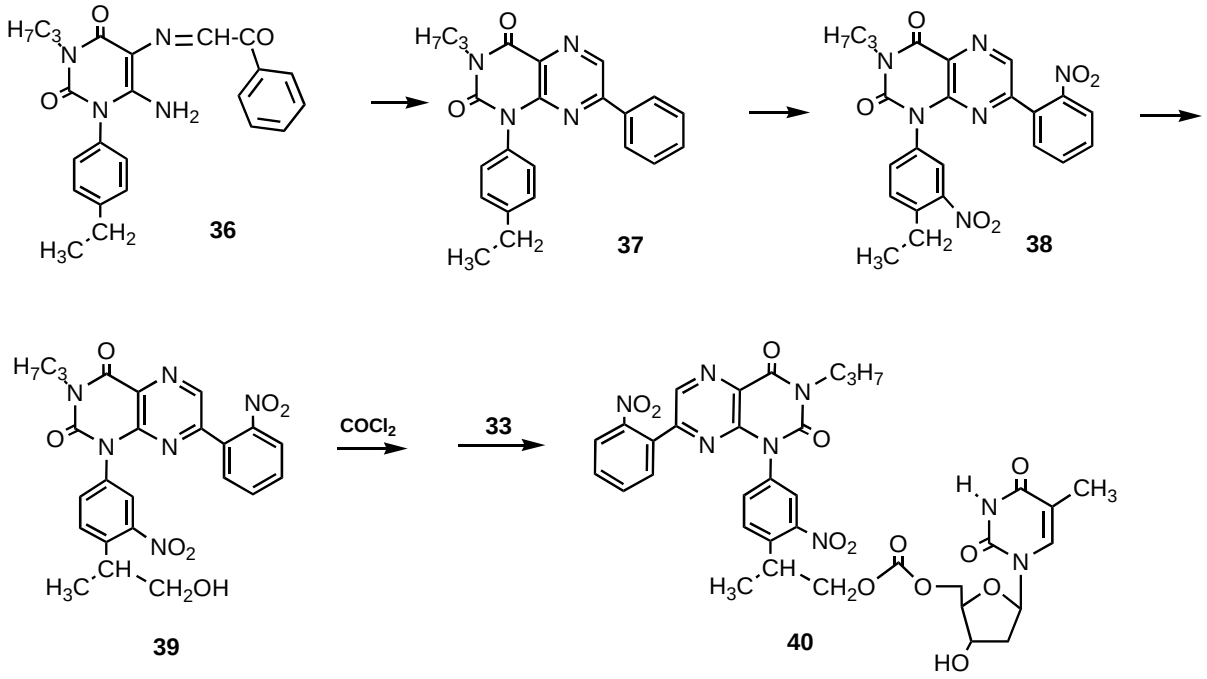
The experiments start with the preparation of 4-ethylphenylurea[19] (**21**) from 4-ethylaniline (**19**) and potassium cyanate (**20**) analogous to literature[20] in 92% yield. Compound **21** was then condensed in a classical Traube synthesis[21] with ethyl cyanoacetate and sodium methoxide to give the so far unknown 6-amino-1-(4-ethylphenyl)uracil (**22**) in 89% yield. The next step consisted of an alkylation with n-propyl iodide to give 6-amino-1-(4-ethylphenyl)-3-propyluracil (**23**). The subsequent nitrosation of **23** with sodium nitrite in dilute acidic medium proceeded well to the violett to red colored 6-amino-1-(4-ethylphenyl)-5-nitroso-3-propyluracil (**24**) in excellent yield. The conversion of the nitroso group into the amino function was then performed by reduction with ammonium sulfide at 90°C to give 80% yield of **25**. For the next step 4-nitrophenylglyoxal (**27**) was prepared from 4-nitroacetophenon (**26**) according to literature[22] using SeO_2 for the oxidation process.



The subsequent reaction between **25** and **27** under mild conditions leads in high yield to the corresponding Schiff's base **28**. Base catalyzed cyclisation in dilute ammonia resulted in the formation of 1-(4-ethylphenyl)-7-nitrophenyl-3-propyllumazine (**29**) a molecule with the anticipated pteridine nucleus. The next modification was achieved by nitration of **29** in a mixture of nitric acid and conc. sulfuric acid to proceed adjacent to the ethyl group yielding 1-(4-ethyl-3-nitrophenyl)-7-nitrophenyl-3-propyllumazine (**30**) in 83% yield. The following step was a very tricky reaction consisting of a hydroxymethylation of **30** by paraformaldehyde in DMSO and catalysed by DBU leading to **31**. The work-up of this reaction was complex and led finally only to a yield of 55% of 1-[4-(1-hydroxypropan-2-yl)phenyl]-7-(4-nitrophenyl)-3-propyllumazine (**31**). This new building block was then reacted in a one pot reaction first with phosgene to the corresponding chloroformate intermediate (**32**) which is so reactive that without isolation addition of the nucleoside thymidine (**33**) to the reaction solution led to a coupling in 5'-position of the sugar moiety of **33** forming 1-{4-[1-(5'-thymidinyloxycarbonyl oxypropan-2-yl)-3-nitrophenyl]-7-(4-nitrophenyl)-3-propyl}lumazine (**34**) in 25% yield.



A second series of reactions started from **25** which was converted into the Schiff's base **36** with phenylglyoxal (**35**)[23]. Cyclization in dilute base afforded 1-(4-ethylphenyl)-7-phenyl-3-propyllumazine (**37**). The subsequent nitration of **37** in a HNO_3/H_2SO_4 mixture led to a surprizing result since a dinitro derivative was obtained. From elemental analysis and ^{1}H-NMR-spectra data the structure of 1-(4-ethyl-3-nitrophenyl)-7-(2-nitrophenyl)-3-propyllumazine (**38**) was proven. The hydroxymethylation of **38** proceeded analogous to **31** and led to **39** with an isolated yield of 55%. The final coupling of **39** with thymidine (**33**) to the second photolabile building block **40** via a carbonate bridge could be achieved in the same manner like the preparation of **34** in a yield of 25%.

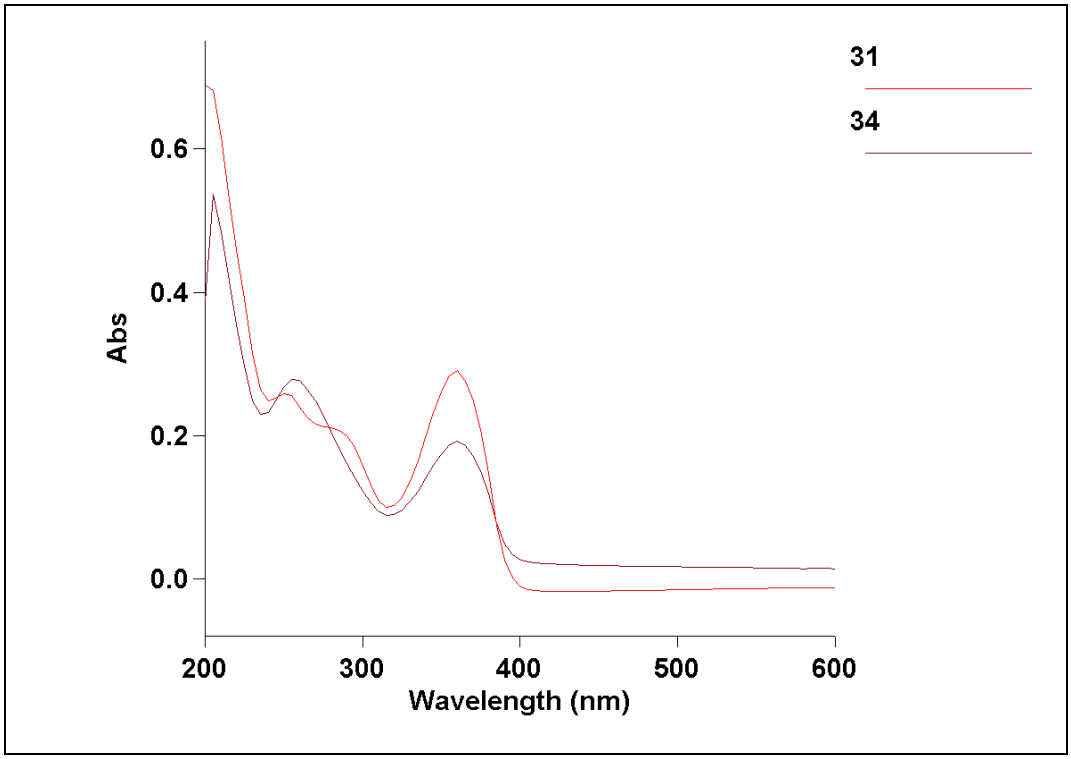


3.2 Physical Data

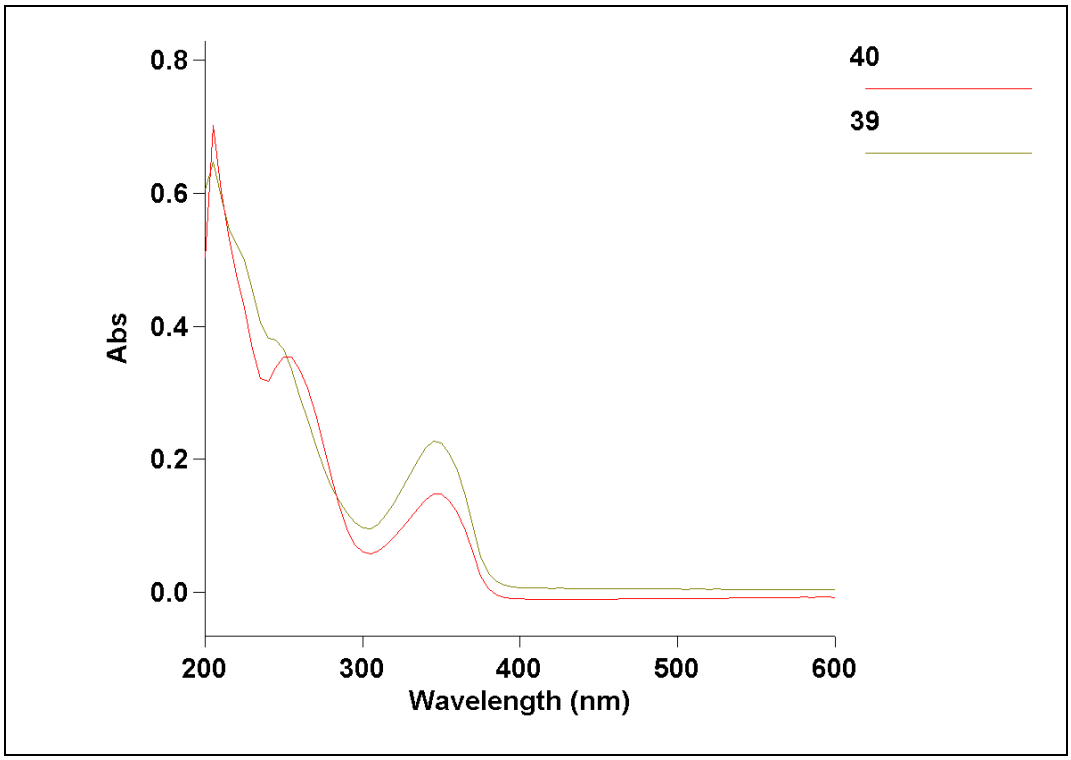
3.2.1 UV Spectra

UV-spectra are used to characterize chemical substances by their properties. Purity can be checked by the extinction coefficients. Compounds are usually measured in MeOH. Depending on the structure special regions are typical for the observed absorption bands. Our uracil derivatives show absorption maxima 265 nm. Nitrosation results in a bathochromic shift to 318 nm and very low band around 500 nm due to the red color of the substance. Reduction of the nitroso group to the amino group leads to an absorption maximum at 282 nm. Condensations to lumazine derivatives are associated again with a bathochromic shift to 345 nm and 357 nm, respectively. The different substitutions at the benzene nucleus at ortho- and para- position influence the location of the absorption bands as a characteristic property of the molecule. The UV-spectra of the 5'-O-protected thymidine nucleosides is the additive overlapping of thymidine (λ_{max} 267 nm) and the corresponding protective group.

Here are shown the UV-spectra of the compounds **31**, **34** and **39**, **40** from the last two steps of the synthesis with NO_2 groups at para and ortho positions respectively.



UV-spectra of compounds 31, 34 para NO_2-phenyl group



UV-spectra of compounds 39, 40 ortho NO_2-phenyl group

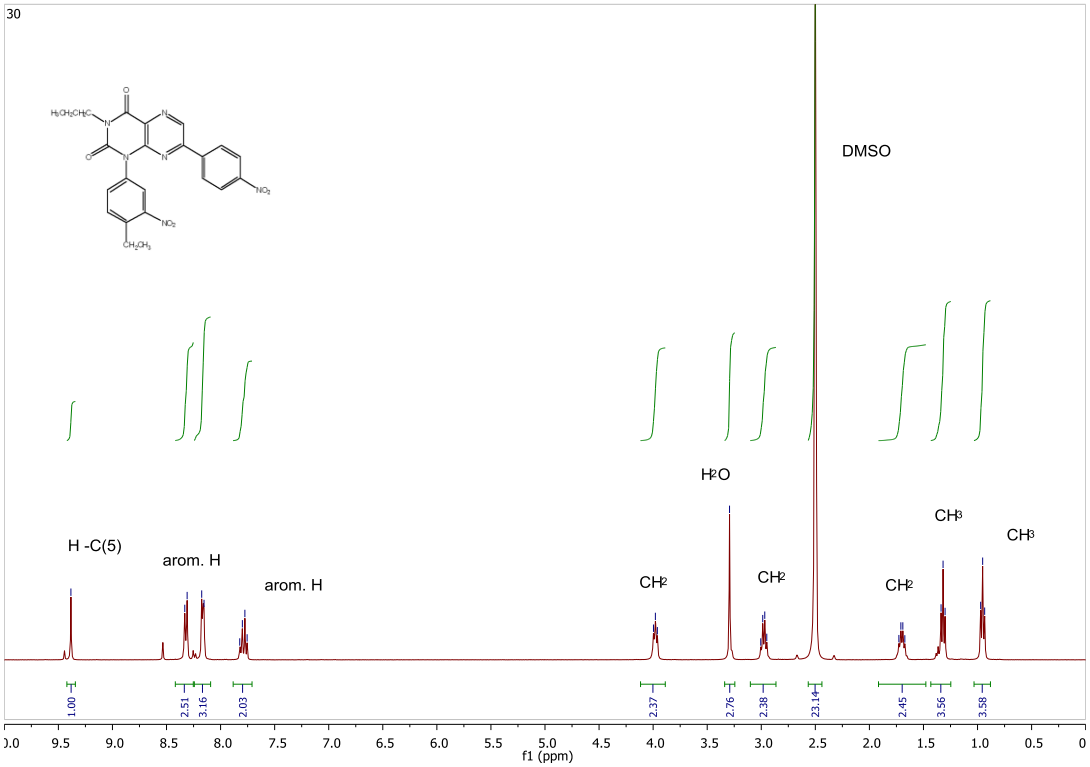
	λ max [nm] (log ε) [ ] = Shoulder MeOH		
21	[276 (2.89)] 238 (4.23) 203 (4.32)		
22	266 (4.38) [218 (4.00)] 203 (4.24)		
23	266 (4.26) [217 (3.96)] 204 (4.20)		
24	[544 (1.48)] 318 (4.13) 221 (4.29)		
25	282 (4.09) [216 (4.17)] 203 (4.35)		
28	420 (4.09) 270 (4.28) [214 (4.27)] [202 (4.46)]	36	405 (4.20) 274 (4.16) [217 (4.19)] [201 (4.45)]
29	358 (4.31) [288 (4.15)] [264 (4.22)] 254 (4.23) [219 (4.45)] 204 (4.57)	37	[364 (4.17)] 350 (4.24) [334 (4.08)] [273 (4.12)] [260 (4.17)] 228 (4.33) 204 (4.51)
30	[374 (4.15)] 356 (4.32) [286 (4.18)] 250 (4.30) 205 (4.58)	38	[358 (3.92)] 345 (4.03) [241 (4.23)] [223 (4.67)] 204 (4.36)
31	[372 (4.19)] 357 (4.30) [334 (4.11)] [284 (4.17)] [249 (4.28)] 204 (4.59)	39	[356 (4.02)] 345 (4.10) [247 (4.29)] [243 (4.30)] 223 (4.39) 205 (4.42)
34	360 (4.28) 255 (4.37) 205 (4.69)	40	346 (3.99) [262 (4.19)] 249 (4.24) [224 (4.26)] 204 (4.33)

Results from all UV data

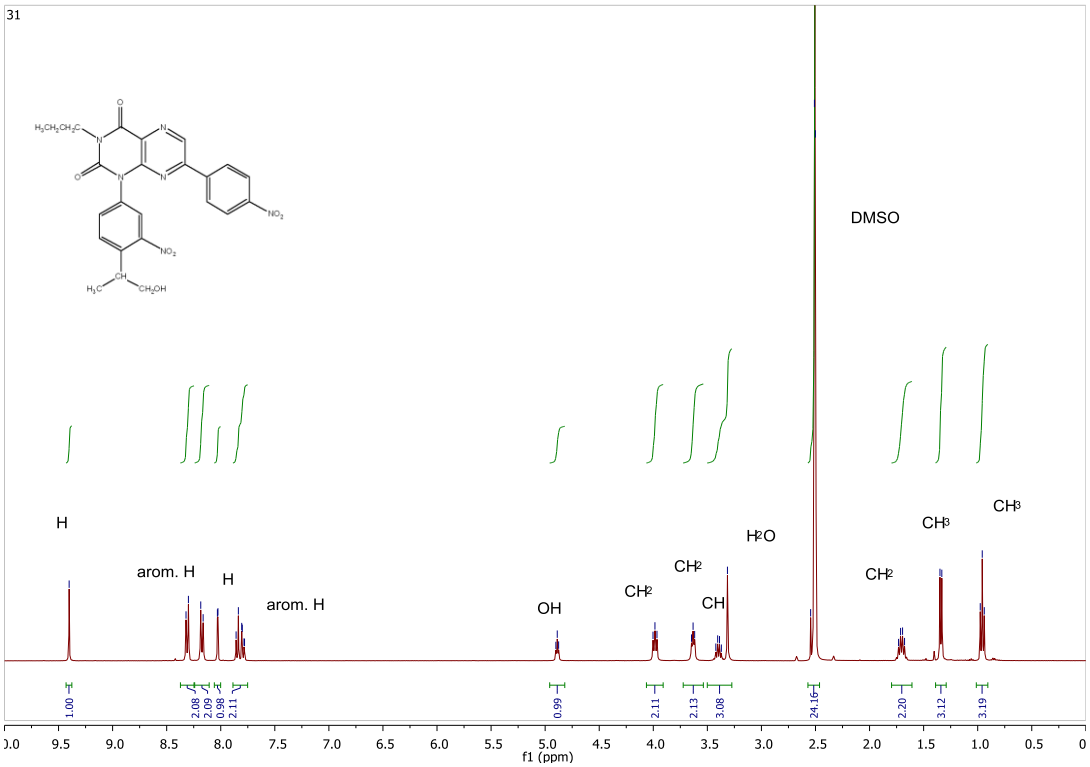
3.2.2 NMR Spectra

NMR-data are registered in detail in the experimental section and are measured either in $CDCl_3$ or DMSO-d_6. The aromatic protons of the substances appear at low field 7-8 ppm. From the splitting pattern of the aromatic protons can the structural assignments of the substituents be determined. A singlet peak signal at 9 ppm is characteristic for H-6 of the lumazine ring. In contrast to the aromatic proton signals resonate the alkyl protons adjacent to heteroatoms and the hydroxyl groups in the region 3-4 ppm. Methyl groups of ethyl and n-propyl substituents show up as triplets between 1-1.5 ppm due to coupling with adjacent CH_2 groups. Similarly such CH_2 groups appear as multiplets depending on the CH-neighborhood. The 5'-O-substituted thymidines are only little influenced by the photolabile protective group and show the normal pattern of nucleoside thymidine. A singlet at 8-9 ppm which can be exchanged by D_2O is due to the N-H proton and the H-C(1') sugar proton appears as pseudotriplet at 6.3 ppm. Between 4-4.5 ppm the sugar signals of H-(3'), H-(4') and H-C(5',5'') are detected and overlapped with the methylene protons. The H-C(2',2'') proton signals are shifted to higher field and appear as multiplets around 2-2.4 ppm. Finally the methyl group of the thymidine base appear as a sharp singlet at 1.8 ppm.

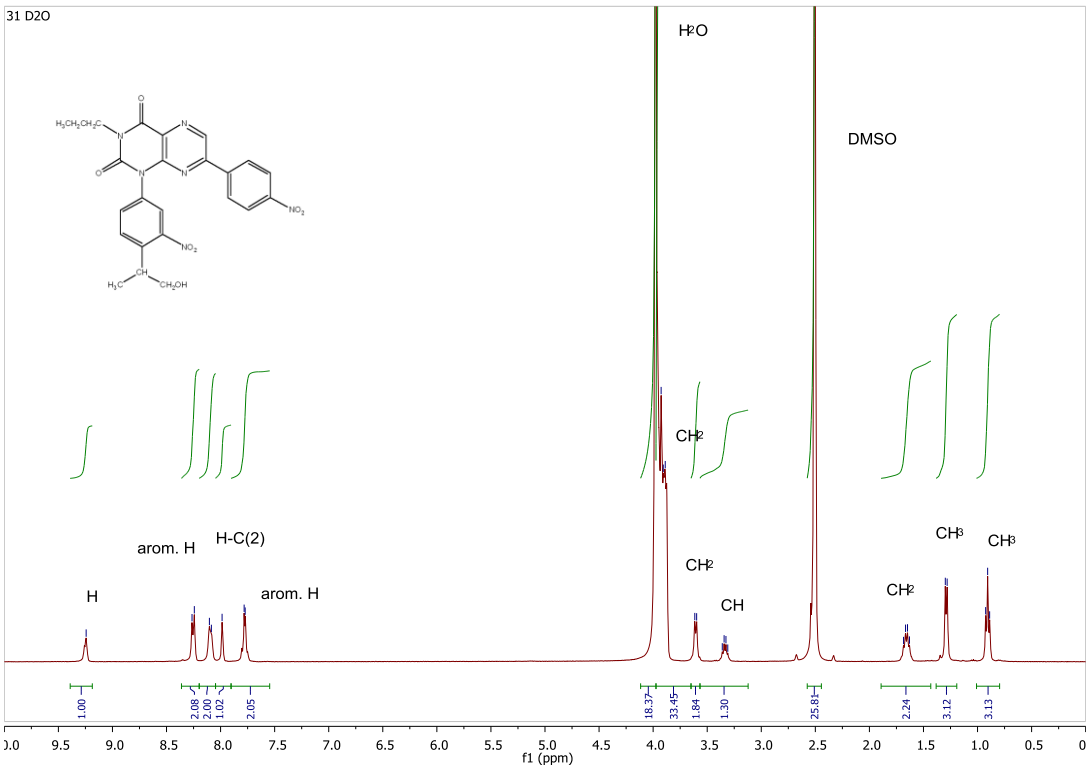
Here are shown the NMR spectra of the compounds **30**, **31**, **34**, **38**, **39**, **40** from the last three steps of the synthesis with NO_2-pheyl groups at para and ortho positions respectively.



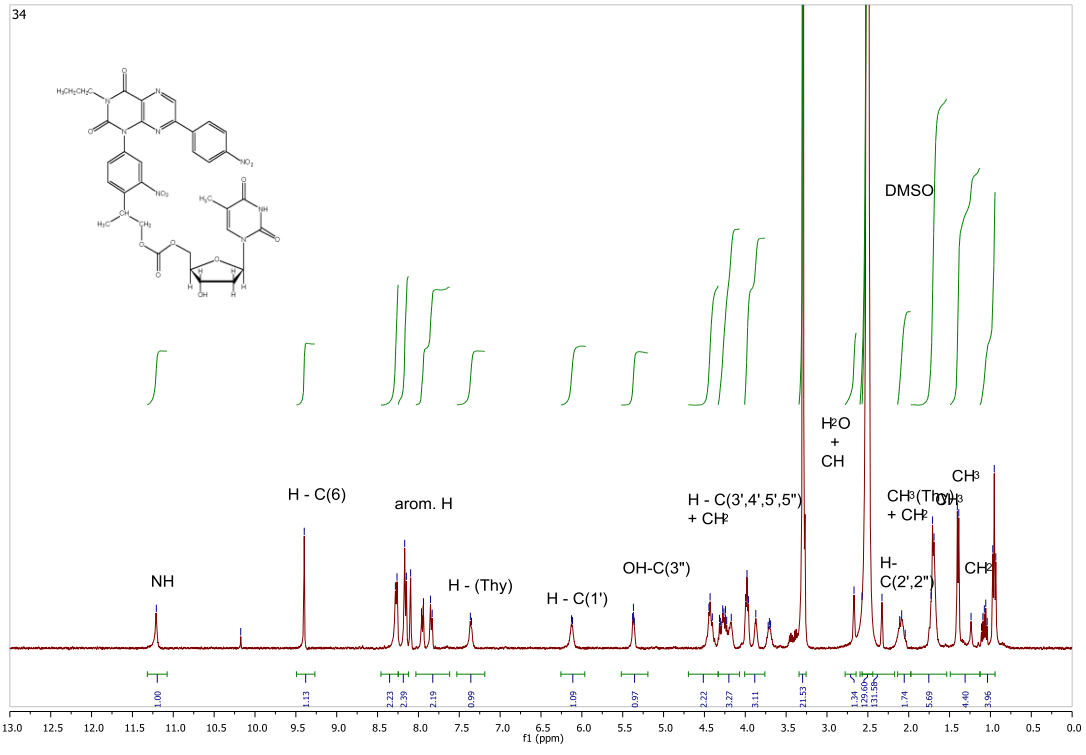
1-(4-Ethyl-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione ***(30)***



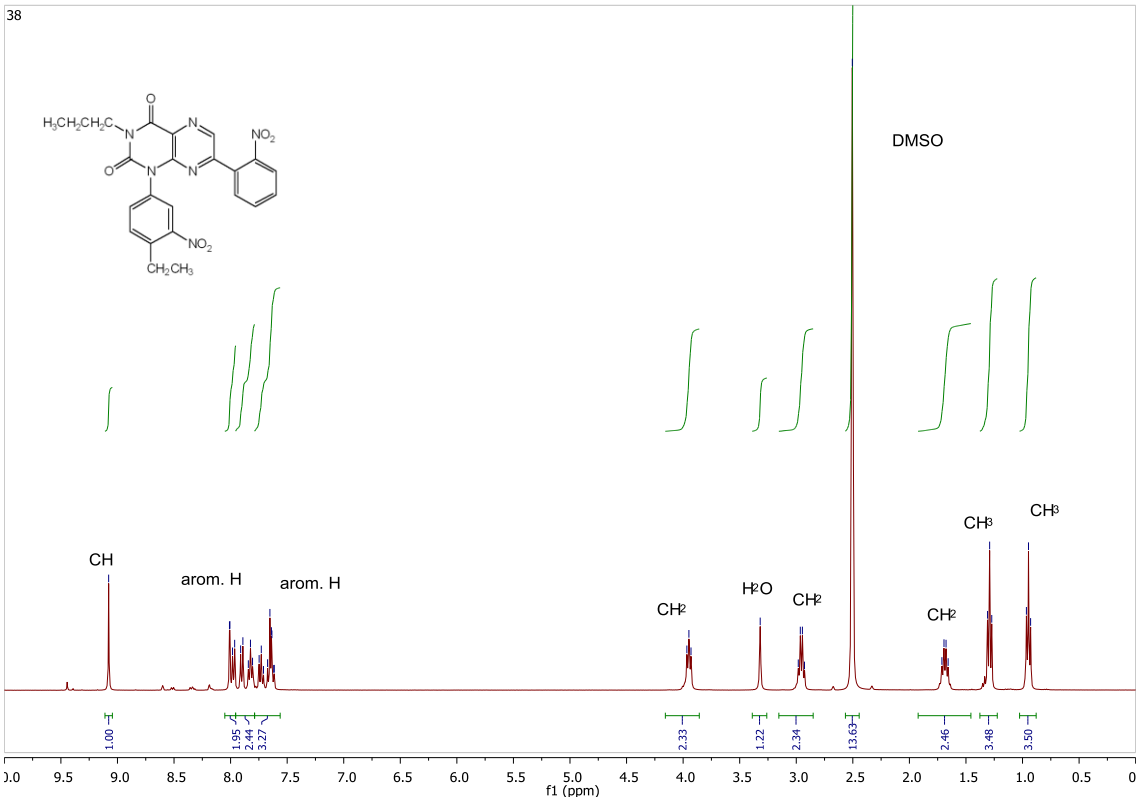
1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione ***(31)***



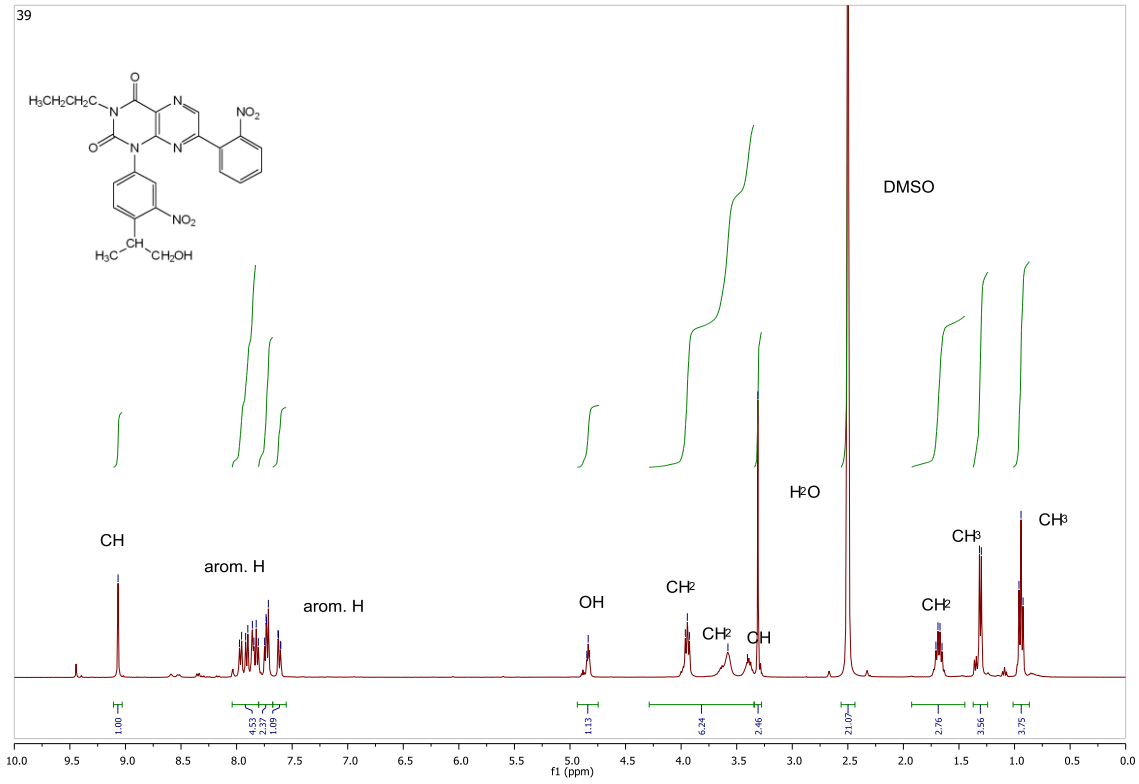
1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione ***(31)*** *in* D_2O



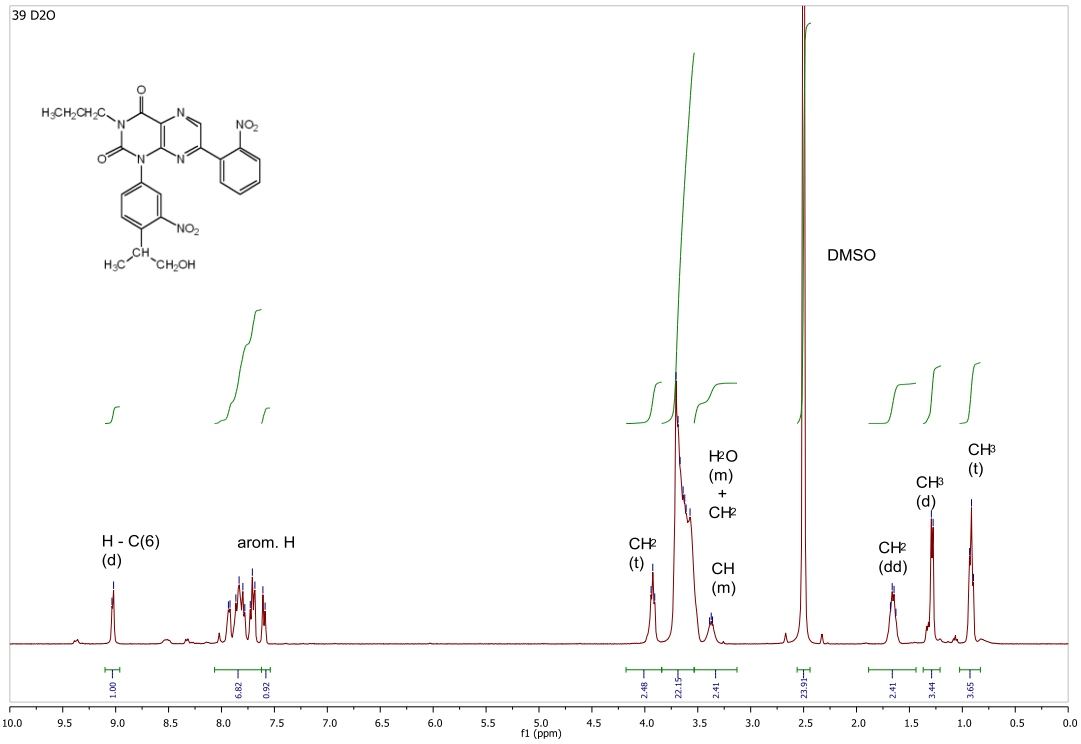
((2R,3R,5R)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-2-yl)methyl 2-(2-nitro-4-(7-(4-nitrophenyl)-2,4-dioxo-3-propyl-3,4-dihydropteridin-1(2H)-yl)phenyl) propyl carbonate ***(34)***



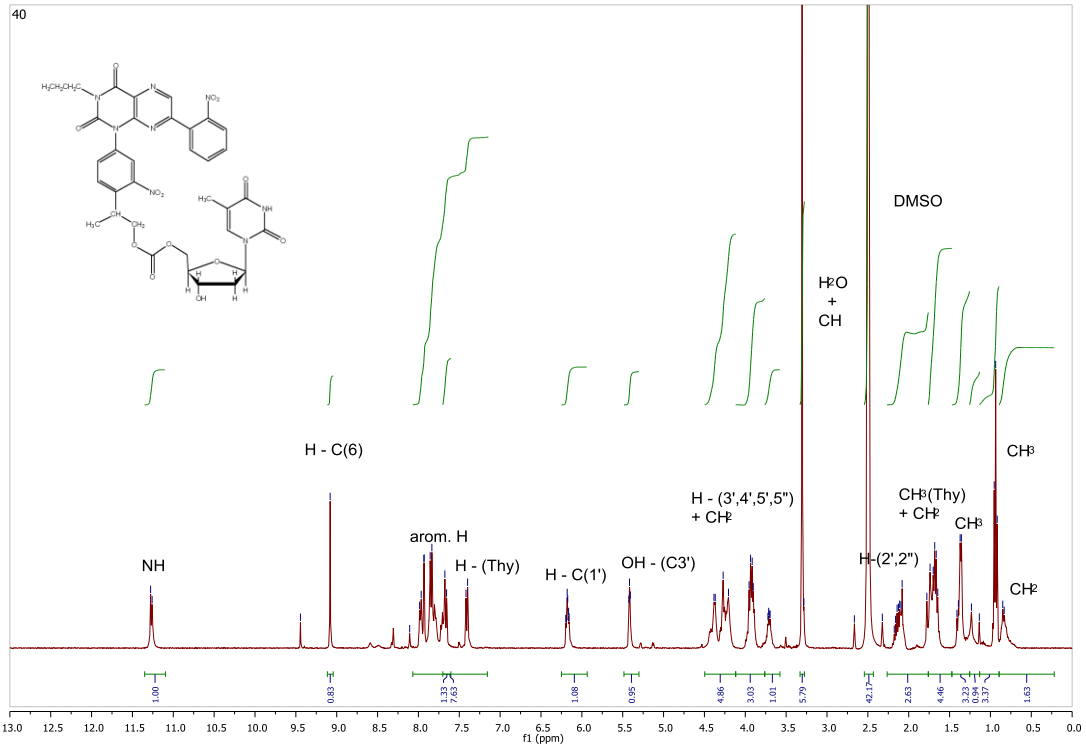
1-(4-Ethyl-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione ***(38)***



1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(2-nitrophenyl)-3-prophylpteridine-2,4(1H,3H)-dione ***(39)***



1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(2-nitrophenyl)-3-prophylpteridine-2,4(1H,3H)-dione ***(39)*** *in* D_2O

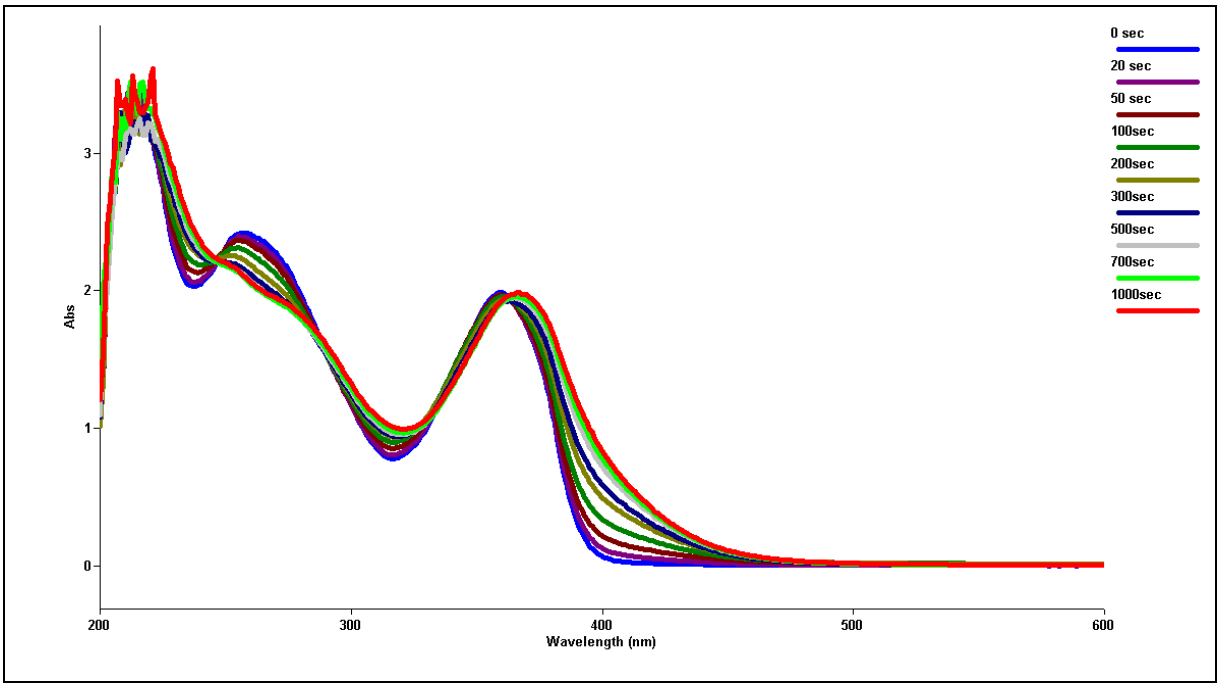


((2R,3R,5R)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydro-furan-2-yl)methyl 2-(2-nitro-4-(7-(2-nitrophenyl)-2,4-dioxo-3-propyl-3,4,dihydropteridin-1(2H)-yl)phenyl)propyl carbonate ***(40)***

3.2.3 Photolysis

The final compounds **34** and **40** were irradiated at 366 nm in time interval 0 sec, 20 sec, 50 sec, 100 sec, 200 sec, 300 sec, 500 sec, 700 sec, 1000 sec and 1200 sec to examine the photolytic result. Till 1200 sec. the solution were analyzed by HPLC and the half-life period of the photolytic cleavage was determined. For comparison the cleavage of the standard compound benzoyl-nppoc deoxythymidine was used.

UV spectra measured at different time intervals of irradiation. **(34)**

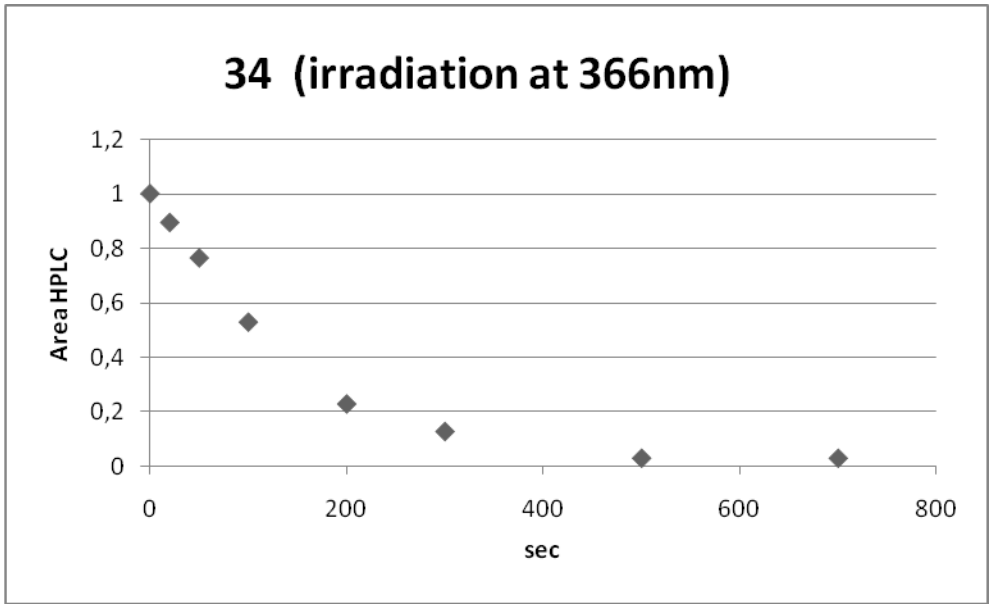


UV spectra of compound 34, para NO_2-phenyl group

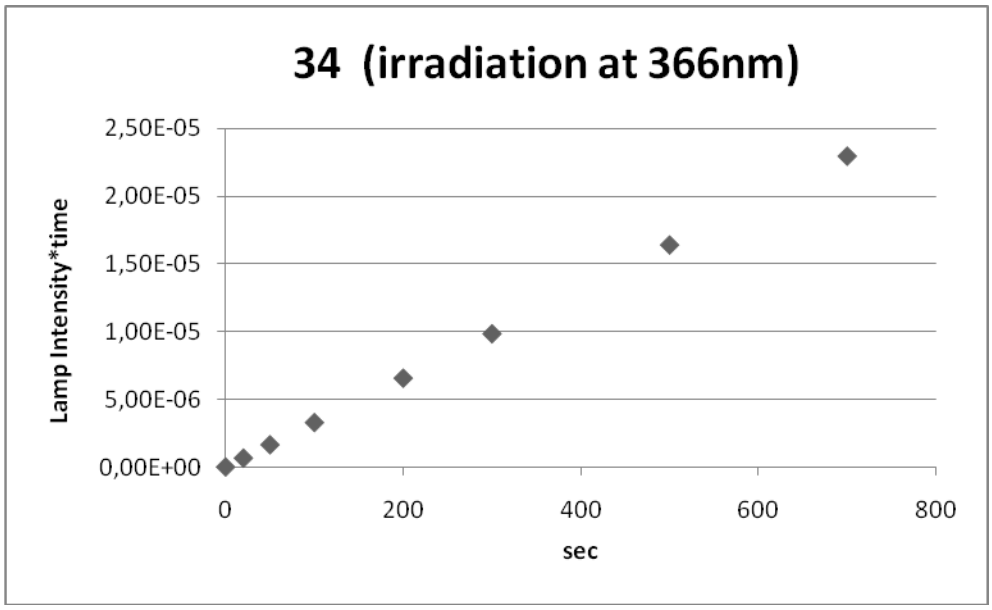
HPLC data measured at different time intervals of irradiation. **(34)**

time in sec.	area (HPLC)	area (HPLC)	time in sec.	Lamp. Intensity *time
0	862385	1	0	0,00E+00
20	771180	0,89424097	20	6,56E-07
50	659025	0,76418885	50	1,64E-06
100	455425	0,5280994	100	3,28E-06
200	196300	0,22762455	200	6,56E-06
300	109050	0,12645164	300	9,84E-06
500	24650	0,02858352	500	1,64E-05
700	24410	0,02830522	700	2,30E-05

Lamp intensity 3,28E-08

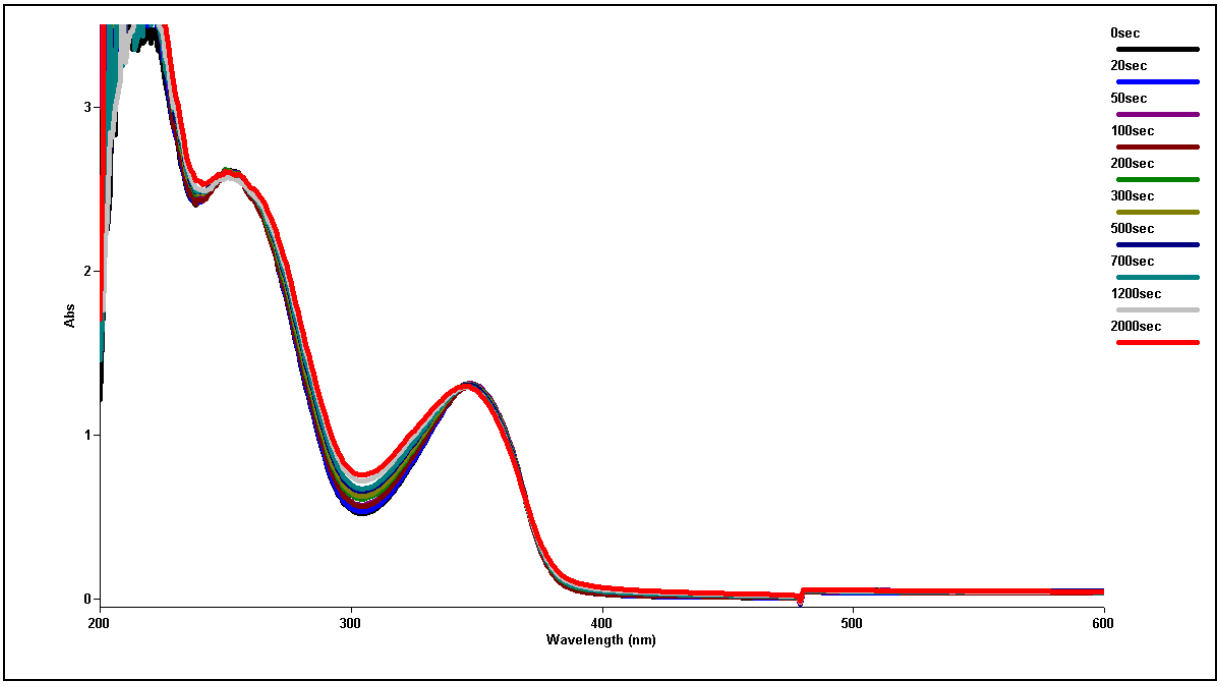


Photoreaction rate of thymidine derivative (34) with new protective group.



Stability of the irradiation lamp during the measurement.

UV spectra measured at different time intervals of irradiation **(40)**.

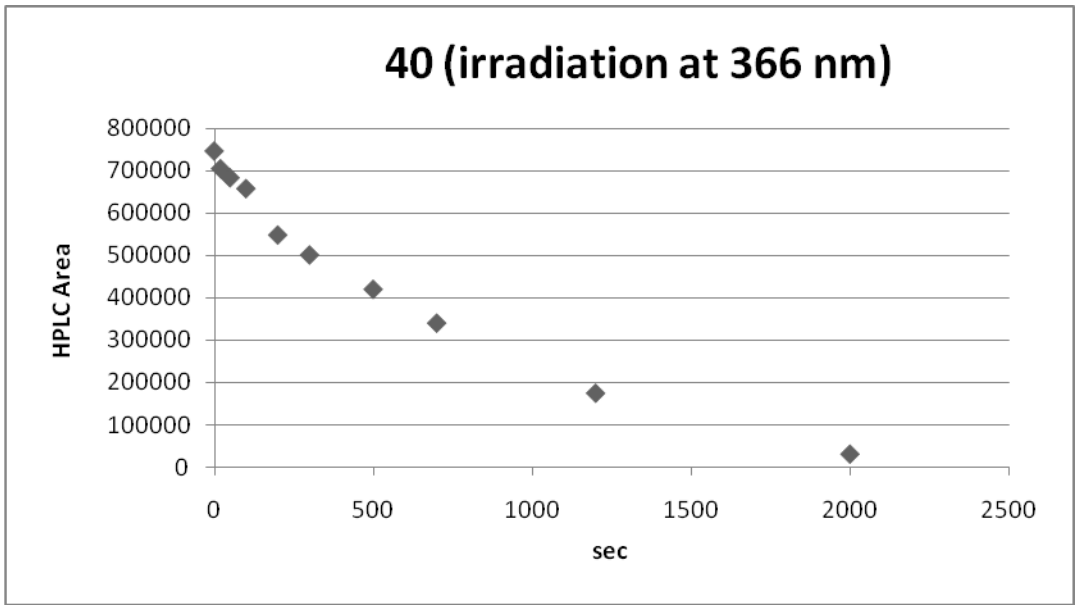


UV spectra of compound 40, ortho NO_2-phenyl group

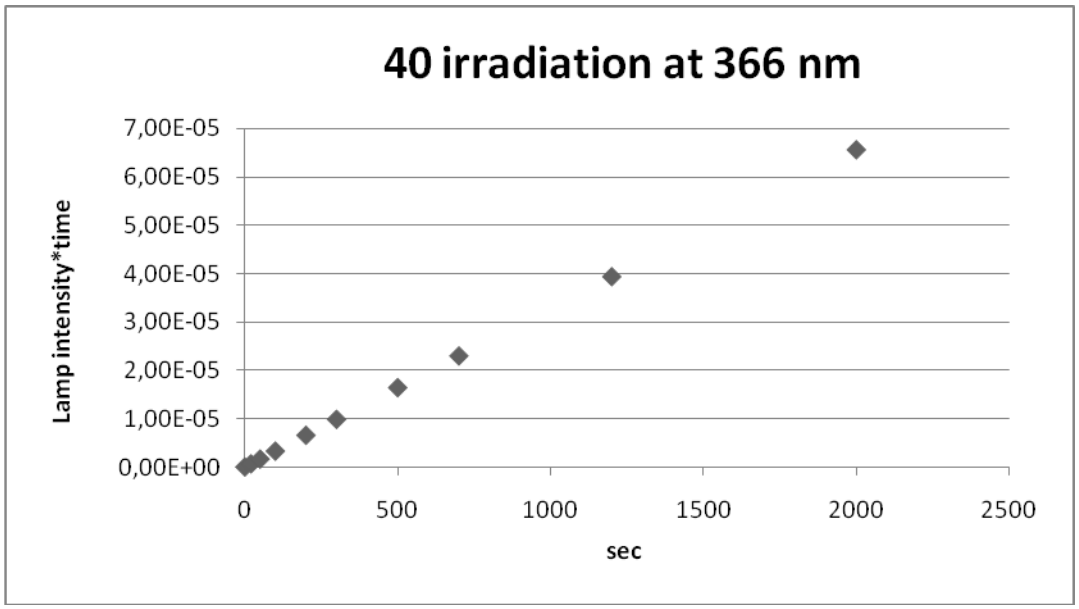
HPLC data measured at different time intervals of irradiation **(40)**.

time in sec	area HPLC**(40)**	area HPLC	time in sec	lamp intensity*time
0	747280	1	0	0,00E+00
20	705585	0,94420431	20	6,56E-07
50	684090	0,91544	50	1,64E-06
100	658610	0,881343	100	3,28E-06
200	548960	0,73461086	200	6,56E-06
300	501750	0,67143507	300	9,84E-06
500	420500	0,56270742	500	1,64E-05
700	340595	0,45577963	700	2,30E-05
1200	175090	0,23430307	1200	3,94E-05
2000	31210	0,0417648	2000	6,56E-05

Lamp intensity 3,28E-08



Photoreaction rate of thymidine derivative (40) with the new protective group.



Stability of the irradiation lamp during the measurement.

The results from the photolysis experiments show that compound **34** with the para NO_2-phenyl group has a half-life period of 120 sec. in comparison to the standard compound which has a half-life period of 150 sec. The compound **40** with the ortho NO_2-phenyl group is less reactive as seen from its half-life period of 620 sec.

4. Summary

The new type of photolabile protective groups have shown that several structural features lead to improved properties which are directly dependent on the extinction at the appropriate wavelength of light irradiation. The project is based on the fact that the chromophore of the photolabile group should have an absorption band of high extinction in the area of 365 nm to guarantee a high efficient and fast cleavage on irradiation.

The two photolabile protective groups were synthesized based on the lumazine nucleus which show a strong absorption band around 365 nm responsible for a very efficient cleavage by light. These photolabile lumazine nucleosides were prepared by chemical synthesies and characterized by different analytical methods. The lumanzine derivative was coupled in the last step via a carbonate linker to the nucleoside thymidine. The aims of the presented bachelor thesis have been achieved perfectly and described in all details. The synthetic part was performed very well and all newly prepared compounds have been characterized by elemental analysis, NMR- and UV- spectra and melting points.

The photolysis shows that the compound **34** is the most suitable new protective group due the high light sensitivity and good yield.

5. Experimental section

5.1 General

Chromatography

For analytical thin layer chromatography, silica gel 60 F_{254} from MERCK was used.
For preparative Column Chromatography, silica gel 60 (0.063-0.200 mm) from MERCK was used. For the preparative thin layer chromatography, silica gel 60 PF_{254} from MERCK was used.

UV-VIS

UV-VIS spectra were measured in MeOH as solvent (Uvasol, MERCK) using Perking-Elmer Lamda 15 and Varian 50 scan.

Melting Point

The melting points were measured with Büchi Melting Point apperatus B-545.

Elemental analysis

The elemental analyses were carried out in the micro analytical lab of the university of Konstanz.

Drying substances

All the synthesized substances were dried in a drying pistol (Büchi: TO–50) and some substances in the drying oven.

NMR

^{1}HNMR spectra were measured with 400.13 MHz spectrometer isa (model Avance III 400) from Bruker and with 399.79 MHz spectrometer ayta (model Avance III 400) from Bruker.
All the spectras were measured in DMSO-d_6: 2.5 ppm

Irradiation experiments in solution

Irradiation was done using an apparatus consisting of a 200W-Hg lamp and filter UV-DAD 8-1 (λ 365,6 nm) from the company Schott. The light intensity at 365 nm was calculated as I_0=3,28E-08. The irradiation was measured in MeOH solution and at specific time intervals.

HPLC Analysis

For HPLC experiments, Merck-Hitachi apparatus with gradient pump L-7100, UV detector L-7450A, interface D-7000, and autosampler L-7200 was used.

The peaks were detected at 262 nm.

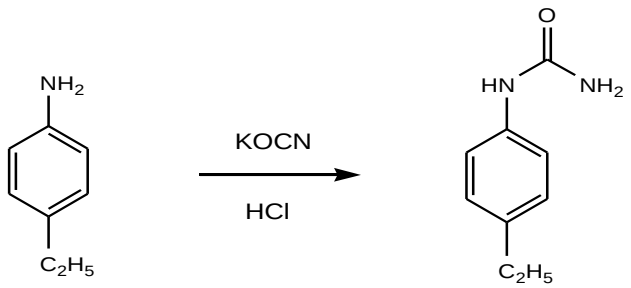
LiChroCART ® 125-4 RP – 18 (5 µm) column was used for elution.

Gradient 1

Time (min)	B (H_2O) %	C (H2O : AcN 1:1) %
0	100	0
3	100	0
10	0	100
15	0	100
22	100	0

5.2 Experimental part

1-(4-Ethylphenyl)urea (21)



19 4-Ethylbenzenamine

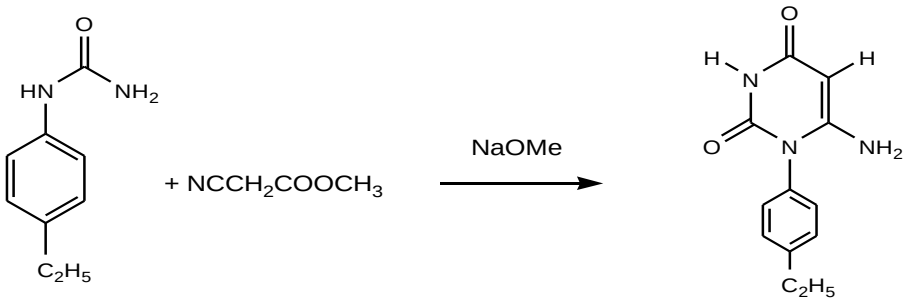
21 1-(4-Ethylphenyl)urea

4-Ethylaniline (**19)** (21 g, 0.1 mole) was added dropwise to 37% HCl soln. (10 ml).
A soln. of potassium cyanate (**20)** (8.1g, 0.1 mole) in H_2O (100 ml) was added at r.t. to the above mixture. After addition of H_2O (50 ml) the mixture was stirred for 3h at r.t.. The product was filtered off and dried at 70 °C to yield 14.8 g (92%) of **21.**
Chromatographically pure product, the compound **21** was crystallized from H_2O:EtOH (1:5).

Physical Data

MP: 129 – 130 °C
TLC: ($CHCl_3$:MeOH, 10:1) R_f = 0.55
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, NH), 7.28 (d, 2 arom. H), 7.04 (d, 2 arom. H), 5.73 (s, NH_2), 2.54 – 2.48 (m CH_2CH_3), 1.14 (t , CH_2CH_3).
UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [276 (2.89)] 238 (4.23) 203 (4.32)
Analysis $C_9H_{12}N_2O$ (164.2 g/mole): cal.: C 65.83, H 7.36, N 17.06; found: C 65.84, H 7.44, N 17.04

6-Amino-1-(4-ethylphenyl) pyrimidine-2,4(1H,3H)-dione (22)



21 **22**

1-(4-Ethylphenyl)urea

6-Amino-1-(4-ethylphenyl)pyrimidine-2,4(1*H*,3*H*)-dione

The compound **21** (32.8 g, 0.2 mole) was added in small portions to 3N sodium methoxide (300 ml). Methyl cyanoacetate (22 ml, 0.22 mole) was added dropwise to the mixture and boiled under reflux for 5h. After cooling H_2O (300 ml) was added to give a clear solution. This was neutralized with HOAc to give 41.19 g of compound **22** (89%) as precipitate which was filtered and dried at 60 °C and crystallizes from H_2O:EtOH (1:1).

Physical Data

MP: 307 – 308 °C

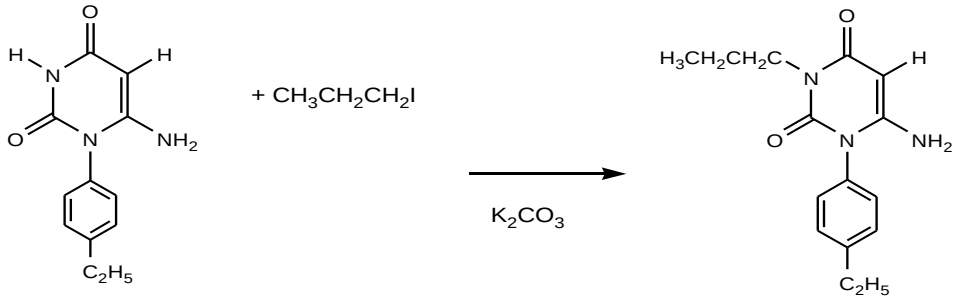
TLC: ($CHCl_3$:MeOH, 10:1) R_f = 0.27

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, NH), 7.35 (d, 2 arom. H), 7.20 (d, 2 arom. H), 6.04 (s, NH_2), 4.66 (s, H – C(5)), 2.68 (q, CH_2CH_3), 1.24 (t,CH_2CH_3).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 203 (4.24) [218 (4.00)] 266 (4.38)

Analysis $C_{12}H_{13}N_3O_2$ (231.25 g/mole): cal.: C 62.32, H 5.66, N 18.18; found: C 62.30, H 5.69, N 18.12

6 -Amino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1H,3H)-dione (23)



22

6-Amino-1-(4-ethylphenyl)pyrimidine-2,4(1*H*,3*H*)-dione

23

6-Amino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

The compound **22** (23.15 g 0.1 mole) was suspended in abs. DMF (250 ml) and heated to 60 °C. K_2CO_3 (60 g, 0.43 mole) and n- propyliodide (16 g, 0.1 mole) was added.
The reaction mixture was stirred at r.t. for 48h. DMF was removed and to the residue was added H_2O (500 ml). The purple precipitate obtained was separated by filtration and dried to give 21.9 g (80%) of crude product (**23)** which was crystallized from H_2O:EtOH (1: 2).

Physical Data

MP: 146 – 147 °C

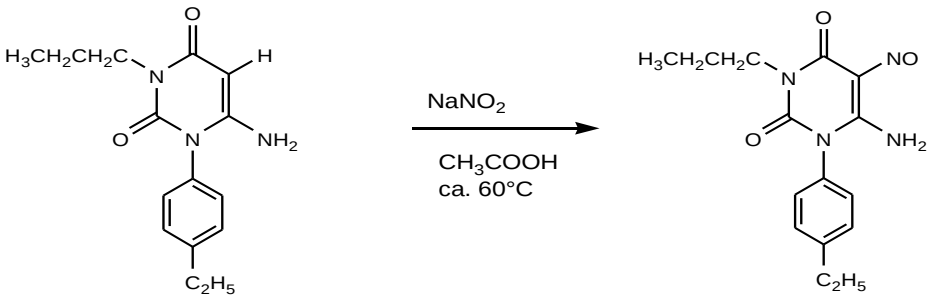
TLC: ($CHCl_3$:MeOH, 10:1) R_f = 0.49

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35 (d, 2 arom. H), 7.21 (d, 2 arom. H), 6.05 (s, NH_2), 4.79 (s, H – C(5)), 3.67 (t, $CH_3CH_2CH_2N$), 2.68 (q, CH_2CH_3), 1.50 (dd, $CH_3CH_2CH_2N$), 1.24 (t, CH_2CH_3), 0.82 (t, $CH_3CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 266 (4.26) [217 (3.96)] 204 (4.20)

Analysis $C_{15}H_{19}N_3O_2$ (273.4 g/mole): cal.: C 65.89, H 7.00, N 15.36; found: C 65.74, H 7.03, N 15.23

6-Amino-1-(4-ethylphenyl)-5-nitroso-3-propylpyrimidine-2,4(1H,3H)-dione (24)



23

6-Amino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

24

6-Amino-1-(4-ethylphenyl)-5-nitroso-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

The Compound **23** (27.3 g, 0.1 mole) was suspended in H_2O (250 ml) and EtOH (50 ml) and heated to 60 °C. $NaNO_2$ (10 g) was added to the mixture. Under stirring CH_3COOH (20 ml) was added dropwise and stirred for 2h at r.t. The crystallized precipitate was filtered off and dried in the drying oven to yield 25.8 g (85%) of **24**. Chromatographically pure product, and further purified by crystallization with H_2O:EtOH.

Physical Data

MP: 195 – 196 °C with decomposition

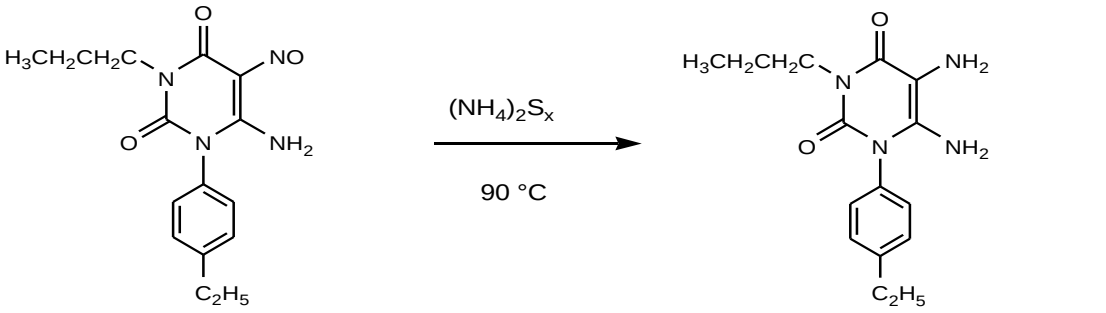
TLC: ($CHCl_3$: MeOH, 10:1) R_f = 0.47

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (s, NH), 7.92 (s, NH), 7.39 (dd, 4 arom. H), 3.87 (t, $CH_3CH_2CH_2N$), 2.71 (q, CH_2CH_3), 1.65 (dd, $CH_3CH_2CH_2N$), 1.26 (t, CH_2CH_3), 0.93 (t, $CH_3CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [544 (1.48)] 318 (4.13) 221 (4.29)

Analysis $C_{15}H_{18}N_4O_3$ (302.3 g/mole): cal.: C 59.6, H 6.00, N 18.87; found: C59.36, H 6.25, N 18.41

5,6-Diamino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1H,3H)-dione (25)



24

6-Amino-1-(4-ethylphenyl)-5-nitroso-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

25

5,6-Diamino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

The compound **24** (1.5 g, 0.5 mmole) was suspended in NH_4S_x (20%) and stirred at 80 – 90 °C for 1h. After bringing to r.t. the crystalline product **25** was filtered off and dried to give 1.2 g (80%) of pure product, which was crystallized from H_2O:EtOH.

Physical Data

MP: 204 – 205 °C

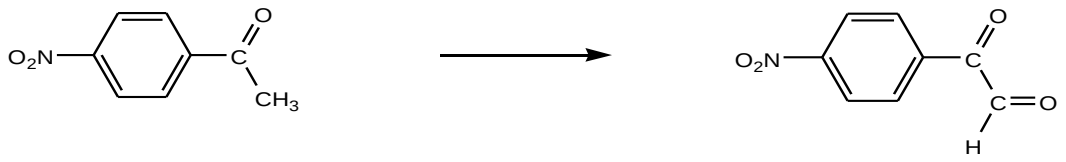
TLC: ($CHCl_3$: MeOH, 20:1) R_f = 0.36

^{1}HNMR (400 MHz, DMSO-d_6) δ 7.36 (d, 2 arom. H), 7.21 (d, 2 arom. H), 5.30 (s, NH_2), 3.73 (t, $CH_3CH_2CH_2N$), 3.01 (s, br., NH_2), 2.69 (q, CH_2CH_3) 1.53 (dd, $CH_3CH_2CH_2N$), 1.24 (t, CH_2CH_3), 0.83 (t, $CH_2CH_2CH_3$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 282 (4.09) [216 (4.17)] 203 (4.35)

Analysis $C_{15}H_{20}N_4O_2$ (288.35 g/mole): cal.: 62.25, H 6.99, N 19.43; found: C 62.35, H 6.98, N 19.38

2-(4-Nitrophenyl)-2-oxoacetaldehyde (27)

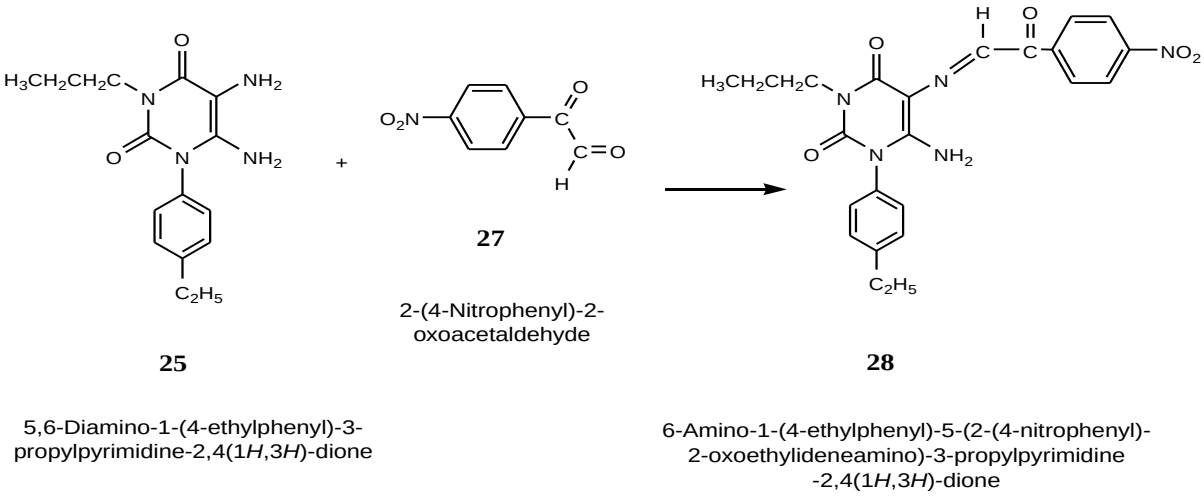


26 **27**

1-(4-Nitrophenyl)ethanone 2-(4-Nitrophenyl)-2-oxoacetaldehyde

A suspension of **26** (8.29 g, 0.02 mole) in dioxane was treated with SeO_2 (6 g, 0.05 mole) and refluxed for 10h. After cooling, the precipitate was filtered off. The clear filtrate which contains the required **27** was used for further reaction.

6-Amino-1-(4-ethylphenyl)-5-(2-(4-nitrophenyl)-2-oxoethylideneamino)-3-propylpyrimidine-2,4(1H,3H)-dione (28)



To a suspension of **25** (12.4 g, 0.05 mole) in H_2O (500ml) the above freshly prepared **27** was added dropwise at r.t. After stirring at r.t. for 1h, the dark orange colored precipitate was filtered and washed with H_2O and dried at 60 °C to give 18.1 g (80%) of **28**.

Physical Data

MP: 172 – 173 °C

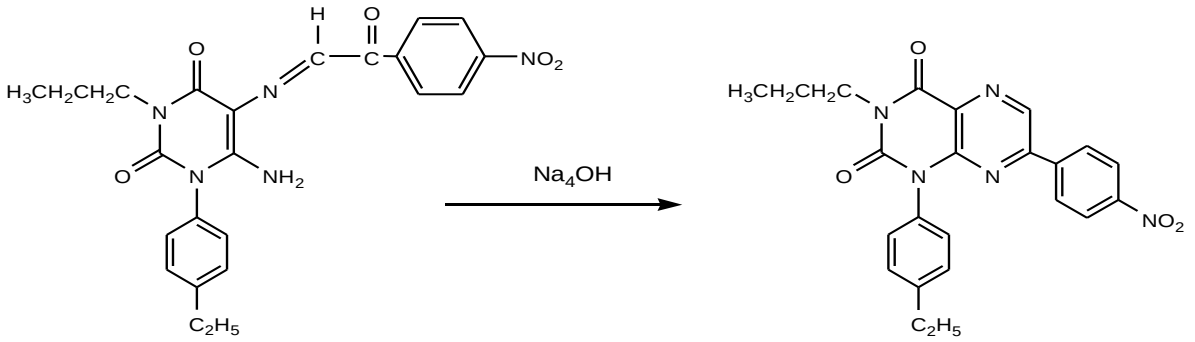
TLC: ($CHCl_3$: MeOH, 20:1) R_f = 0.80

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.41 (s, CH), 8.30 (d, 2 arom. H), 8.04 (d, 2 arom. H), 7.37 (dd, 4 arom. H) 6.53 (s, br., NH_2), 3.77 (t, $CH_3CH_2\mathit{CH_2}N$), 2.70 (q, $\mathit{CH_2}CH_3$), 1.59 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.25 (t, $CH_2\mathit{CH_3}$), 0.89 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 420 (4.09) 270 (4.28) [214 (4.27)] [202 (4.46)]

Analysis $C_{23}H_{23}N_5O_5$ x H_2O (467.45 g/mole): cal.: C 59.09, H 5.39, N 14.98; found: C 59.87, H 5.67, N 14.38

1-(4-Ethylphenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione (29)



28

6-Amino-1-(4-ethylphenyl)-5-(2-(4-nitrophenyl)-2-oxoethylideneamino)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

29

1-(4-Ethylphenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

The compound **28** (18 g, 0.045 mole) was suspended in 1N NH_4OH soln. (200 ml) and EtOH (100 ml). The mixture was stirred and heated at 100 °C for 1h. After cooling the precipitate was filtered off and dried at 100 °C to yield 15.1 g (83%) of **29**. Chromatographically pure product was crystallized from H_2O:EtOH.

Physical Data

MP: 260 °C with decomposition

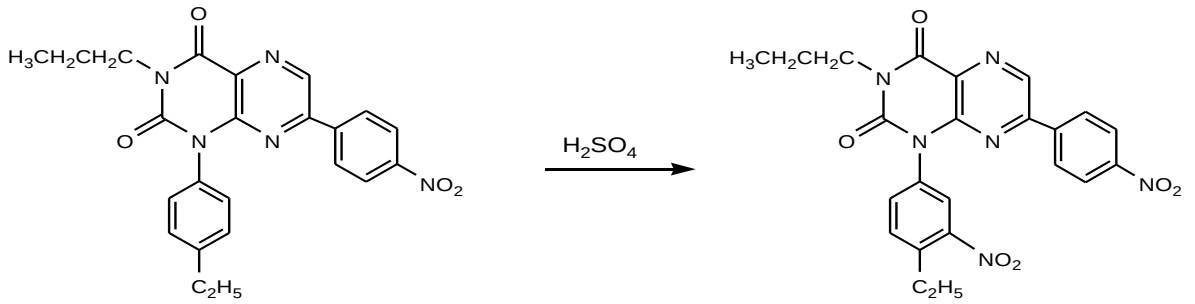
TLC: ($CHCl_3$: MeOH, 20:1) R_f = 0.75

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.33 (s, H – C(6)), 8.31 (d, 2 arom. H), 8.13 (d, 2 arom. H), 7.38 (dd, 4 arom. H), 3.97 (t, $CH_3CH_2\mathit{CH_2}N$), 2.74 (q, $\mathit{CH_2}CH_3$), 1.69 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.28 (t, $CH_2\mathit{CH_3}$), 0.94 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 358 (4.31) [288 (4.15)] [264 (4.22)] 254 (4.23) [219 (4.45)] 204 (4.57)

Analysis $C_{23}H_{21}N_4O_4$ (431.5 g/mole): cal.: C 64.40, H 4.90, N 16.23; found: C 63.98, H 4.83, N 16.11

1-(4-Ethyl-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione (30)



29

1-(4-Ethylphenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

30

1-(4-Ethyl-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

The compound **29** (4.31 g, 10 mmole) was dissolved under strong stirring in conc. H_2SO_4 (50 ml) at r.t. The soln. is cooled to 3 °C and 65% HNO_3 soln. (8 ml) was added dropwise, whereby the temp. was kept below 10 °C. After 1h stirring at this temp. the reaction mixture was brought to r.t. The clear soln. was poured slowly on ice and the resulting colorless precipitate was filtered off and dried at 80 °C (HV) to give chromatographically pure compound 3.7 g (86%) of **30**.

Physical Data

MP: 185 °C with decomposition

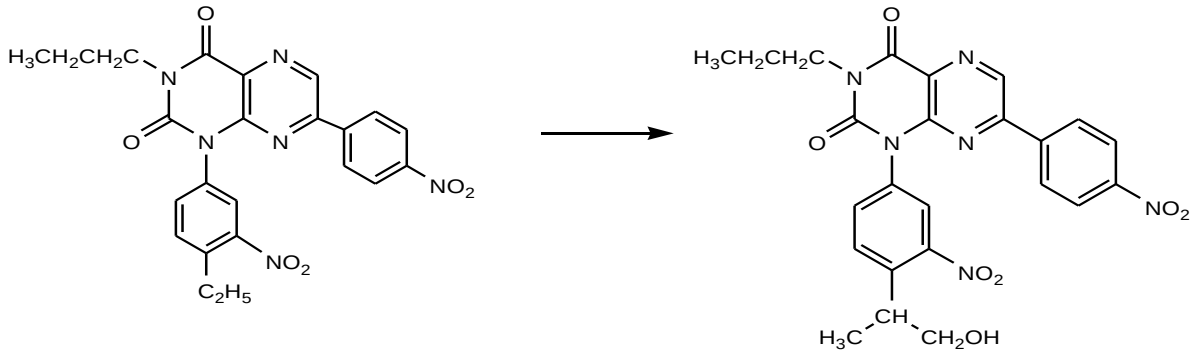
TLC: (pet.ether: ether, 1:3) R_f = 0.39

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.38 (s, H – C(6)), 8.32 (d, 2 arom. H), 8.16 (d, 2 arom. H), 7.79 (dd, 4 arom. H), 3.98 (t, $CH_3CH_2\mathit{CH_2}N$), 2.98 (dd, $\mathit{CH_2}CH_3$), 1.70 (dd, $CH_3\mathit{CH_2}CH_2N$) 1.32 (t, $CH_2\mathit{CH_3}$), 0.95 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [374 (4.15)] 356 (4.32) [286 (4.18)] 250(4.30) 209 (4.58)

Analysis $C_{23}H_{20}N_6O_6$ (476.5 g/mole): cal.; C 57.98, H 4.23, N 17.63; found: C 57.06, H 4.28, N 17.71

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione (31)



30

1-(4-Ethyl-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

31

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

3 mmole (1.43 g) of **30** was dissolved in DMSO (30 ml), then formaldehyde (0.27 g, 3 mmole) and DBU (0.456 g, 3 mmole) were added and heated at 70 °C for 3h. TLC shows still the presence of starting material, so the reaction mixture was stirred overnite at 70 °C. After diluting with EtOAc (50 ml) the reaction soln. was washed with sat. NaCl soln. (50 ml). The organic phase was separated, dried (Na_2SO_4) and evaporated to dryness. Purification was achieved by CC (20 x 6.5) using SiO_2 (150 g) in $CHCl_3$ and $CHCl_3$:MeOH (50:1). The product fraction was evaporated to yield 0.88 g (58%) of **31**.

Physical Data

MP: 115 – 127 with decomposition

TLC: ($CHCl_3$: MeOH, 50:1) R_f = 0.17

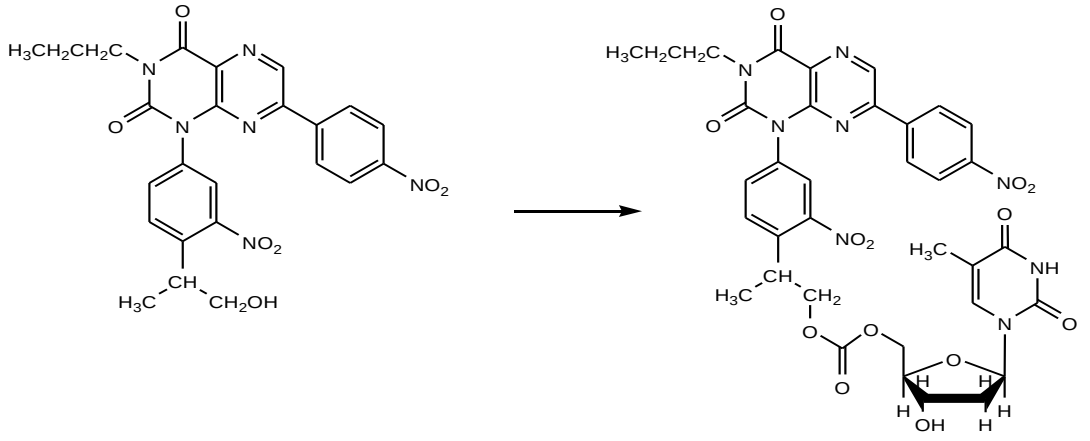
^{1}HNMR (400 MHz, DMSO-d_6) δ 9.40 (s, H – (6)), 8.30 (d, 2 arom. H), 8.17 (d, 2 arom. H), 8.02 (s, H – C(2)), 7.81 (dt, 2 arom. H), 4.88 (t, OH), 3.98 (t, $CH_3CH_2\mathit{CH_2}N$), 3.63 (t, $\mathit{CH_2}OH$), 3.39 (m, CH), 1.70 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.34 (d, $\mathit{CH_3}CH$), 0.96 (t, $\mathit{CH_3}CH_2CH_2N$).

^{1}HNMR(400 MHz, DMSO-d_6, D_2O) δ 9.24 (s, H – C(6)), 8.25 (d, 2 arom. H), 8.10 (d, 2 arom. H), 7.99 (s, H- C(2)), 7.78 (d, 2 arom. H), 3.98 (m, $CH_3CH_2\mathit{CH_2}N$) 3.60 (d, $\mathit{CH_2}OH$), 3.34 (dd, CH), 1.66 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.29 (d, $\mathit{CH_3}CH$), 0.91 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [372 (4.19)] 357 (4.30) [334 (4.11)] [284(4.17)] [250 (4.28)] 204 (4.59)

Analysis $C_{24}H_{22}N_6O_7$ (506.5 g/mole): cal.: C 56.91, H 4.37, N 16.59; found: C 56.54, H 4.32, N 16.41

((2R,3R,5R)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-2-yl)methyl 2-(2-nitro-4-(7-(4-nitrophenyl)-2,4-dioxo-3-propyl-3,4-dihydropteridin-1(2H)-yl)phenyl) propyl carbonate (34)



31

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(4-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

34

((2*R*,3*R*,5*R*)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-2-yl)methyl 2-(2-nitro-4-(7-(4-nitrophenyl)-2,4-dioxo-3-propyl-3,4-dihydropteridin-1(2*H*)-yl)phenyl) propyl carbonate

To an ice cold soln. of compound **31** (800 mg, 1.58 mmole) in THF (10 ml) and NEt_3 (253 mg, 2.5 mmole), diphosgene (320 mg, 1.6 mmole) in THF (10 ml) was added dropwise under N_2 and stirred for 30 min. to form **32** as intermediate. TLC showed still the presence of starting material, therefore again diphosgene (75 mg, 0.37 mmole) and NEt_3 (130 mg, 1.28 mmole) were added. After 15 min. stirring in ice-bath, the temp. was brought to r.t.

The resulting NEt_3:HCl was separated by filtration and washed with THF. The filtrate was evaporated to dryness, the residue was dissolved in CH_2Cl_2 (10 ml) and added slowly to an ice cold soln. of thymidine (**33)** (360 mg, 1.5 mmole) which was co-evaporated with pyridine (2x5 ml), and then dissolved in pyridine (5 ml). After stirring at r.t. overnite, MeOH (1ml) was added and taken up in CH_2Cl_2 (50 ml) and washed with 1N $NaHCO_3$ soln. (50 ml).

The organic layer was separated, dried over Na_2SO_4 and evaporated to give a yellow foam.

The crude product was purified by CC using SiO_2 (19 x 2.5 cm) and eluted with $CHCl_3$ and $CHCl_3$:MeOH (50:2).The product fraction was evaporated to give 0.11 g (10%) of **34** as a yellow foam.

Physical Data

MP: 156 °C with decomposition

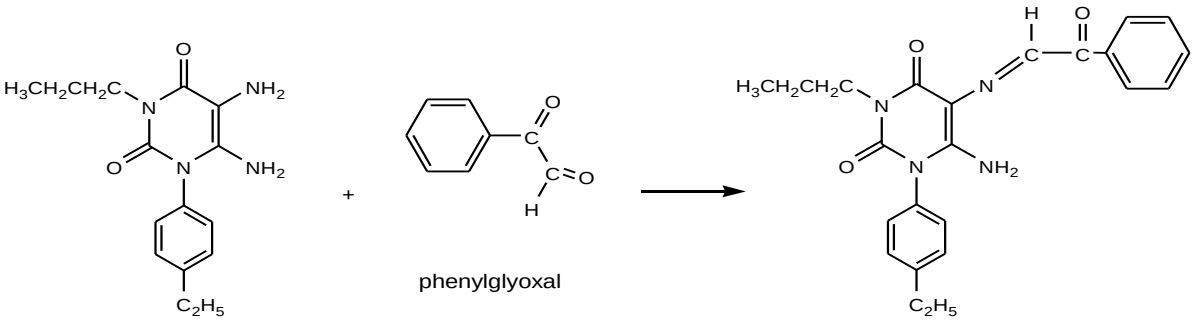
TLC: ($CHCl_3$: EtOAc, 1:1) R_f = 0.02

^{1}HNMR (400 MHz, DMSO-d_6) δ 11.21 (s, NH), 9.40 (s, H – C(6)), 8.21 (d, 2 arom. H), 8.10 (s, H – C(2)), 7.90 (dd, 4 arom. H), 7.36 (d, H – (Thy)), 6.12 (d, H – C(1')), 5.37 (t, OH – C(3')), 4.44 – 3.29 (m, H – C(3') – C(4') – C(5') – C(5") + $CH_3CH_2\mathit{CH_2}N$), 3.29 (m, $CH_3\mathit{CH}$), 2.10 (d, H – C(2') – C(2")), 1.71 (t, C – CH_3 (Thy)) + $CH_3\mathit{CH_2}CH_2N$), 1.39 (d, $\mathit{CH_3}CH$), 1.06 (m, $\mathit{CH_2}$ - CH), 0.95 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 360 (4.28) 255 (4.37) 205 (4.69)

Analysis $C_{35}H_{34}N_8O_{13}$ (774.7 g/mole): cal.: C 54.26, H 4.42, N 14.46; found: C 54.57, H 4.67, N 14.47

6-Amino-1-(4-ethylphenyl)-5-(2-oxo-2-phenylethylideneamino)-3-propylpyrimidine-2,4(1H,3H)-dione (36)



25

5,6-Diamino-1-(4-ethylphenyl)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

36

6-Amino-1-(4-ethylphenyl)-5-(2-oxo-2-phenylethylideneamino)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

To a suspension of **25** (28.8 g, 0.1 mole) in H_2O (500 ml), phenylglyoxal **(35)** (20 g) in EtOH (200 ml) was added dropwise at r.t. After stirring at r.t. for 1h, a dark orange colored precipitate was filtered off and dried in the drying oven to give 37 g (92%) of **36**.

Physical Data

MP: 210 - 212 °C

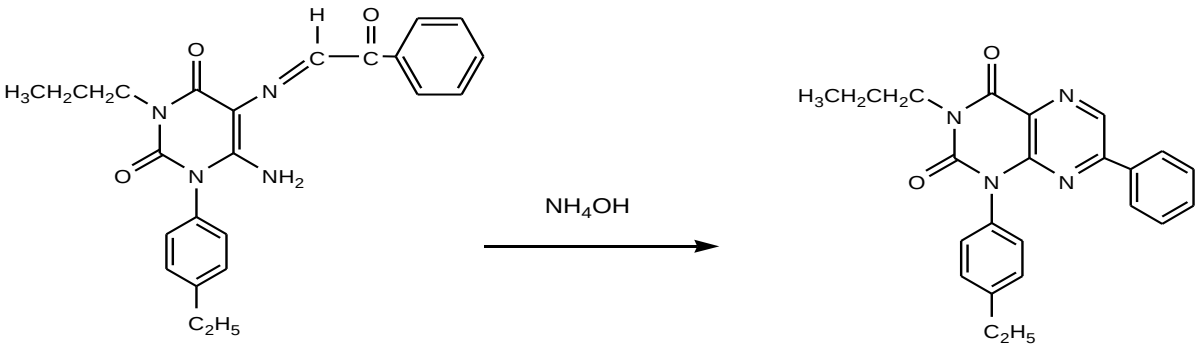
TLC: ($CHCl_3$: MeOH, 20:1) R_f = 0.74

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.49 (s, CH), 7.89 (d, 2 arom. H), 7.60 (t, 1 arom. H), 7.50 (t, 2 arom. H), 7.38 (dd, 4 arom. H), 6.55 (s, br., NH_2), 3.77 (t, $CH_3CH_2\mathit{CH_2}N$), 2.70 ($\mathit{CH_2}CH_3$), 1.58 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.24 (t, $CH_2\mathit{CH_3}$), 0.88 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 405 (4.20) 274 (4.16) [217 (4.19)] [201 (4.45)]

Analysis $C_{23}H_{24}N_4O_3$ (404.5 g/mole): cal.: C 68.29, H 5.73, N 13.85; found: C 68.15, H 5.80, N 13.83

1-(4-Ethylphenyl)-7-phenyl-3-propylperidine-2,4(1H,3H)-dione (37)



36

6-Amino-1-(4-ethylphenyl)-5-(2-oxo-2-phenylethylideneamino)-3-propylpyrimidine-2,4(1*H*,3*H*)-dione

37

1-(4-Ethylphenyl)-7-phenyl-3-propylpteridine-2,4(1*H*,3*H*)-dione

The compound **36** (20 g, 0.05 mole) was suspended in 1N NH_4OH soln. (200 ml) and EtOH (200 ml). The mixture was stirred and heated in the oil - bath at 90 – 95 °C for 1h.
After cooling the precipitate was filtered off and dried to give 18 g (93%) of **37**
Chromatographically pure product was crystallized from H_2O:EtOH.

Physical Data

MP: 222 - 223 °C with decomposition

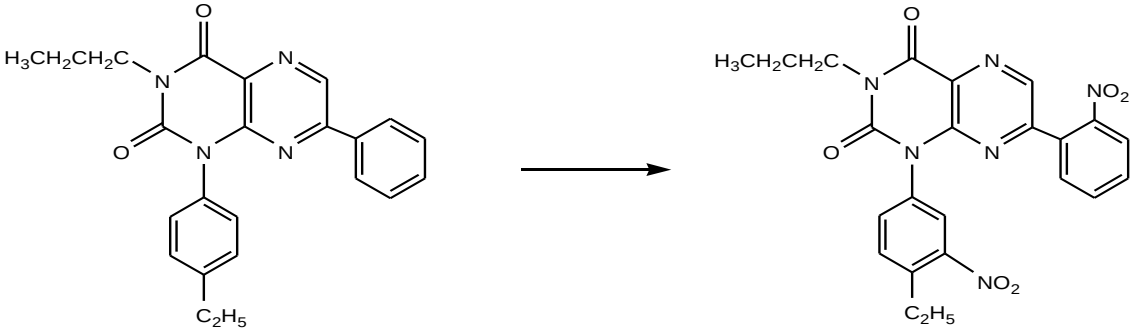
TLC: ($CHCl_3$: MeOH, 20:1) R_f = 0.76

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.22 (s, H – C(6)), 7.90 (d, 2 arom. H), 7.47 (dd, 3 arom. H), 7.37 (dd, 4 arom. H) 3.95 (t, $CH_3CH_2\mathit{CH_2}N$), 2.73 (q, $\mathit{CH_2}CH_3$), 1.68 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.26 (t, $CH_2\mathit{CH_3}$), 0.93 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [364 (4.17)] 350 (4.24) [334 (4.08)] [273 (4.12)] [260 (4.17)] 228 (4.33) 204 (4.51)

Analysis $C_{23}H_{22}N_4O_2$ (386.45 g/mole): cal.: C 71.48, H 5.74, N 14.49; found: C 71.48, H 5.83, N 14.46

1-(4-Ethyl-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1H,3H)-dione (38)



37

1-(4-Ethylphenyl)-7-phenyl-3-propylpteridine-2,4(1*H*,3*H*)-dione

38

1-(4-Ethyl-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

The compound **37** (3.86 g, 10 mmole) was dissolved under strong stirring in conc. H_2SO_4 (50 ml) at r.t. The soln. is cooled with ice at 3 - 4 °C, 65% HNO_3 (4 ml) was added dropwise, whereby the temp. was kept below 8 °C. After 1h stirring at this temp. the reaction mixture was brought to r.t. The clear soln. was poured slowly in ice, the resulting colorless precipitate was filtered off and dried at 80 °C (HV) to give chromatographically pure compound (3.54 g, 82%) of **38**.

Physical Data

MP: 222 - 224 °C with decomposition

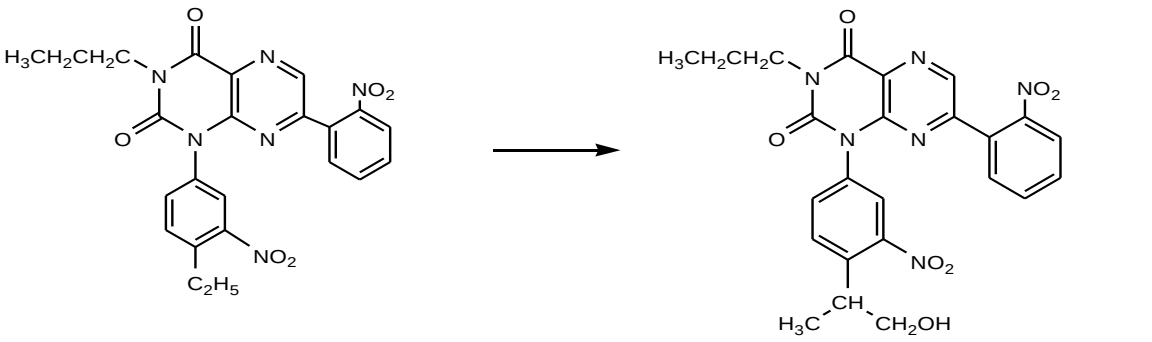
TLC: ($CHCl_3$: MeOH, 50:1) R_f = 0.73

^{1}HNMR (400 MHz, DMSO-d_6) δ 9.07 (s, H – C(6)), 8.00 – 7.61 (m, 7 arom. H), 3.94 (t, $CH_3CH_2CH_2N$), 2.95 (q, CH_2CH_3), 1.68 (dd, $CH_3CH_2CH_2N$), 1.29 (t, CH_2CH_3), 0.94 (t, $CH_3CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [358 (3.92)] 345 (4.03) [241 (4.23)] [223(4.67)] 204 (4,36)

Analysis $C_{23}H_{20}N_6O_2$ (476.45 g/mole): cal.: C 57.98, H 4.23, N 17.63; found: C 57.69, H 4.16, N 17.38

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(2-nitrophenyl)-3-prophylpteridine-2,4(1H,3H)-dione (39)



38

1-(4-Ethyl-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

39

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

2 mmole (0.953 g) of **38** were dissolved in DMSO (20 ml). Paraformaldehyde (18 g, 2 mmole) and DBU (0.304 g, 2 mmole) were added and heated at 120°C for 3h. TLC showed still the presence of starting material, therefore stirring at 120°C was continued overnite. After diluting with EtOAc (100 ml) the reaction soln. was washed with sat. NaCl soln. (50 ml). The organic phase was separated, dried (Na_2SO_4) and evaporated to dryness. Purification was achieved by CC (20 x 6.5) using SiO_2 (150 g) in $CHCl_3$ and $CHCl_3$:MeOH (50:1). The product fraction was evaporated to yield 0.55 (55%) of **39**.

Physical Data

MP: 228 °C with decomposition

TLC: ($CHCl_3$: MeOH, 50:1) R_f = 0.48

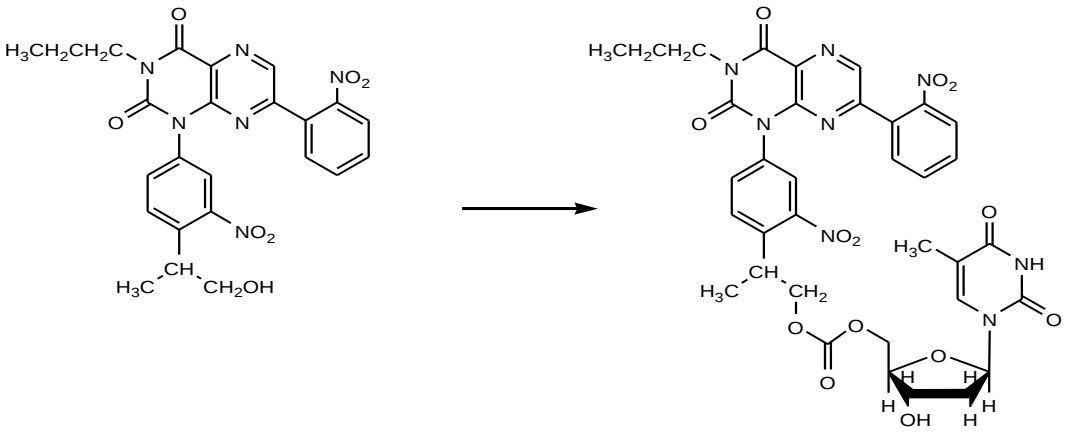
^{1}HNMR (400 MHz, DMSO-d_6) δ 9.07 (s, H – C(6)), 7.88 (ddd, 5 arom. H), 7.73 (dd, 2 arom. H), 4.84 (t, OH), 3.94 (t, $CH_3CH_2\mathit{CH_2}N$), 3.58 (s, br., $\mathit{CH_2}OH$) 3.40 (m, CH) 1.68 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.31 (d, $\mathit{CH_3}CH$), 0.94 (t, $\mathit{CH_3}CH_2CH_2N$).

^{1}H NMR (400 MHz, DMSO-d_6, D_2O) δ 9.03 (d, H – C(6)), 7.93 – 7.59 (m, 7 arom. H), 3.92 (t, $CH_3CH_2\mathit{CH_2}N$), 3.64 (m, $\mathit{CH_2}OH$), 3.37 (t, br., CH), 1.65 (dd, $CH_3\mathit{CH_2}CH_2N$), 1.28 (d, $\mathit{CH_3}CH$), 0.91 (t, $\mathit{CH_3}CH_2CH_2N$).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): [356 (4.02)] 345 (4.10) [247 (4.29)] [243 (4.30)] 223 (4.39) 205 (4.42)

Analysis $C_{24}H_{22}N_6O_7$ x H_2O (524.48 g/mole): cal.: C 54.95, H 4.58, N 16.00; found: C 55.01, H 4.45, N 15.74

((2R,3R,5R)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-2-yl)methyl 2-(2-nitro-4-(7-(2-nitrophenyl)-2,4-dioxo-3-propyl-3,4,dihydropteridin-1(2H)-yl)phenyl)propyl carbonate (40)



39

1-(4-(1-Hydroxypropan-2-yl)-3-nitrophenyl)-7-(2-nitrophenyl)-3-propylpteridine-2,4(1*H*,3*H*)-dione

40

((2*R*,3*R*,5*R*)-3-Hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-2-yl) methyl 2-(2-nitro-4-(7-(2-nitrophenyl)-2,4-dioxo-3-propyl-3,4-dihydropteridin-1(2*H*)-yl)phenyl) propyl carbonate

To an ice cold soln. of compound **39** (0.506 mg, 1 mmole) in THF (5 ml) and NEt_3 (202 mg, 2.2 mmole), diphosgene (0,2 g, 1mmole) in THF (5 ml) was added dropwise and stirred for 30 min. TLC showed still the presence of starting material, therefore again diphosgene (98 mg, 0.5 mmole) and NEt_3 (100 mg, 1 mmole) were added. After 15 min. stirring in ice bath, the temp. was brought to r.t. The resulting NEt_3:HCl was separated by filtration, washed with THF and the filtrate evaporated to dryness. The residue was dissolved in CH_2Cl_2 (5 ml) and added slowly to an ice cold soln. of thymidine (**33)** (0.36 g, 1.5 mmole) which was evaporated with pyridine (5 ml). After stirring at r.t. overnite, MeOH (1ml) was added and taken up in CH_2Cl_2 (50 ml) and washed with 1N $NaHCO_3$ soln. (50 ml).

The organic layer was separated, dried over Na_2SO_4 and evaporated to give a yellow foam. The crude product was purified by CC (27 x 4) using SiO_2 and eluted with $CHCl_3$ and $CHCl_3$:MeOH (50:2). The product fraction was evaporated to give 0.190 g (25%) of **40**, as a yellow foam.

Physical Data

MP: 148 - 154 °C with decomposition

TLC: ($CHCl_3$: EtOAc, 1:1) R_f = 0.01

^{1}HNMR (400 MHz, DMSO-d_6) δ 11.27 (d, NH), 9.44 (s, H – C(6)), 8.11 – 7.66 (m, 7 arom. H), 7.41 (d, H - (Thy)), 6.17 (td, H – C(1')), 5.42 (m, OH – C(3')), 4.38 – 3.70 (m, H – C(3') – C(4') – C(5') – C(5") + $CH_3CH_2CH_2N$), 3.30 (m, CH_3CH), 2.11 - 2.08 (m, H – C(2') – C(2")), 1.74 – 1.64 (m, C – CH_3 (Thy) + $CH_3CH_2CH_2N$), 1.38 (dd, CH_3CH), 0.94 (t, $CH_3CH_2CH_2N$), 0.84 (d, CH_2 - CH).

UV – Spectra: (MeOH), λ_{max} [nm] (log ε): 346 (3.99) [262 (4.19)] 249 (4.24) [224 (4.26)] 204 (4.33)

Analysis $C_{35}H_{34}N_8O_{13}$ (774.61 g/mole): cal.: C 54.27, H 4.42, N 14.46; found: C 54.12, H 4.96, N 14.34

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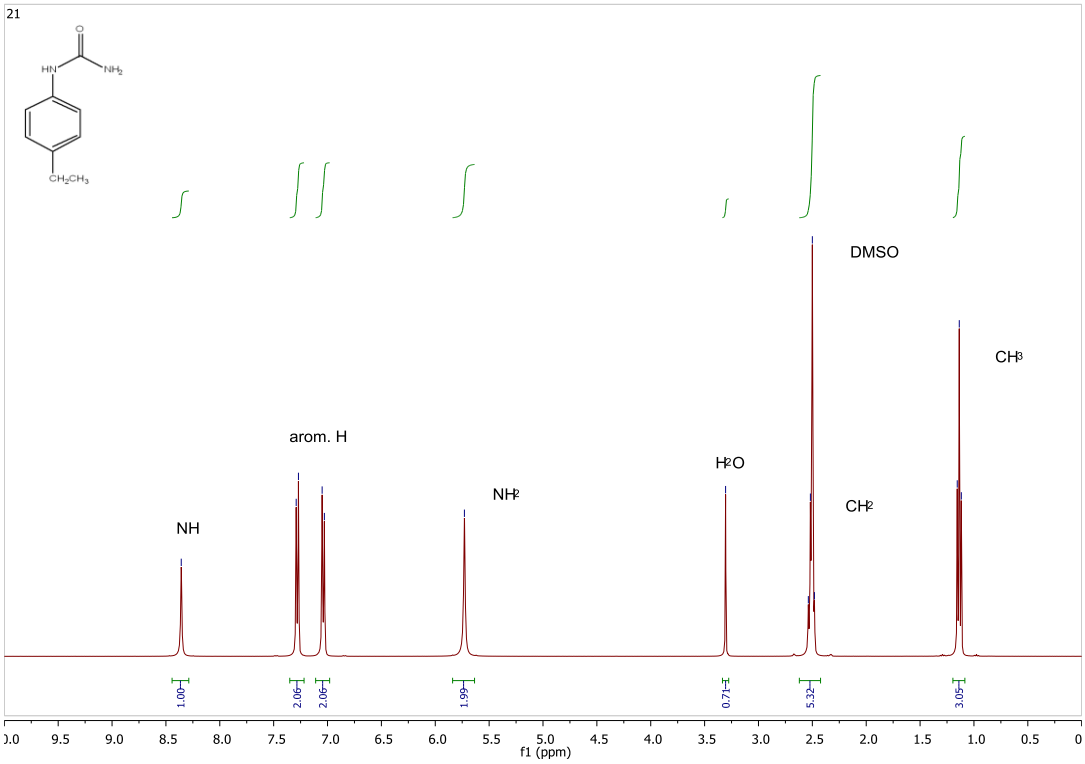
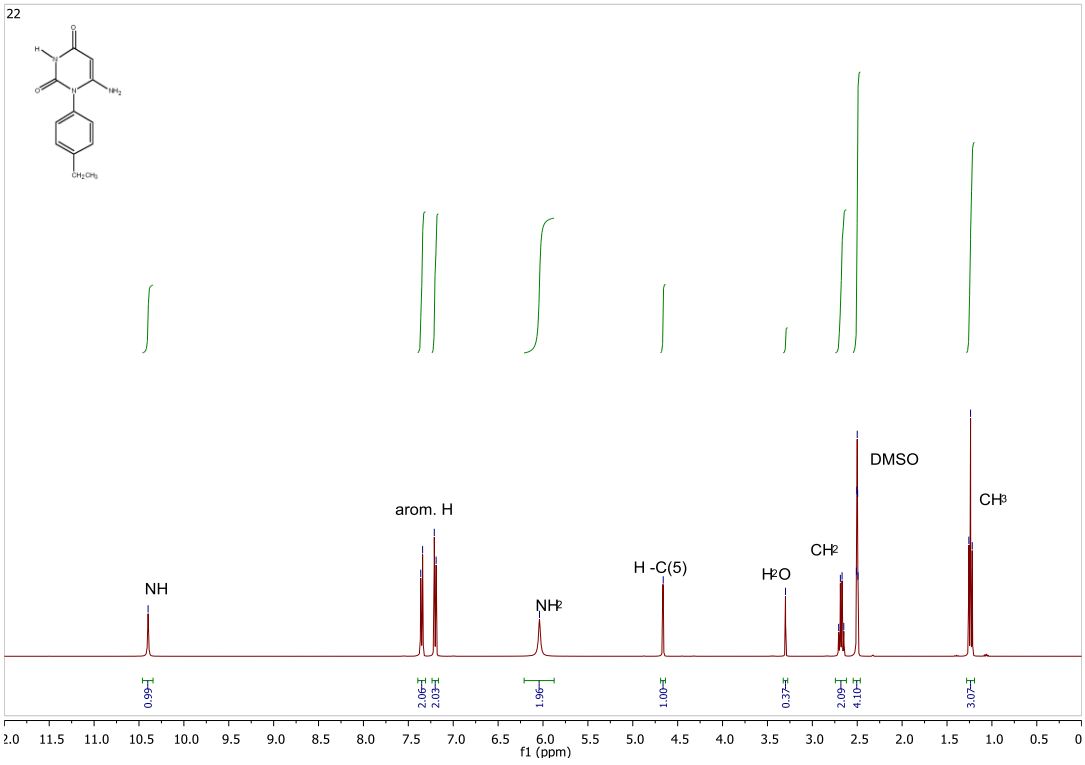
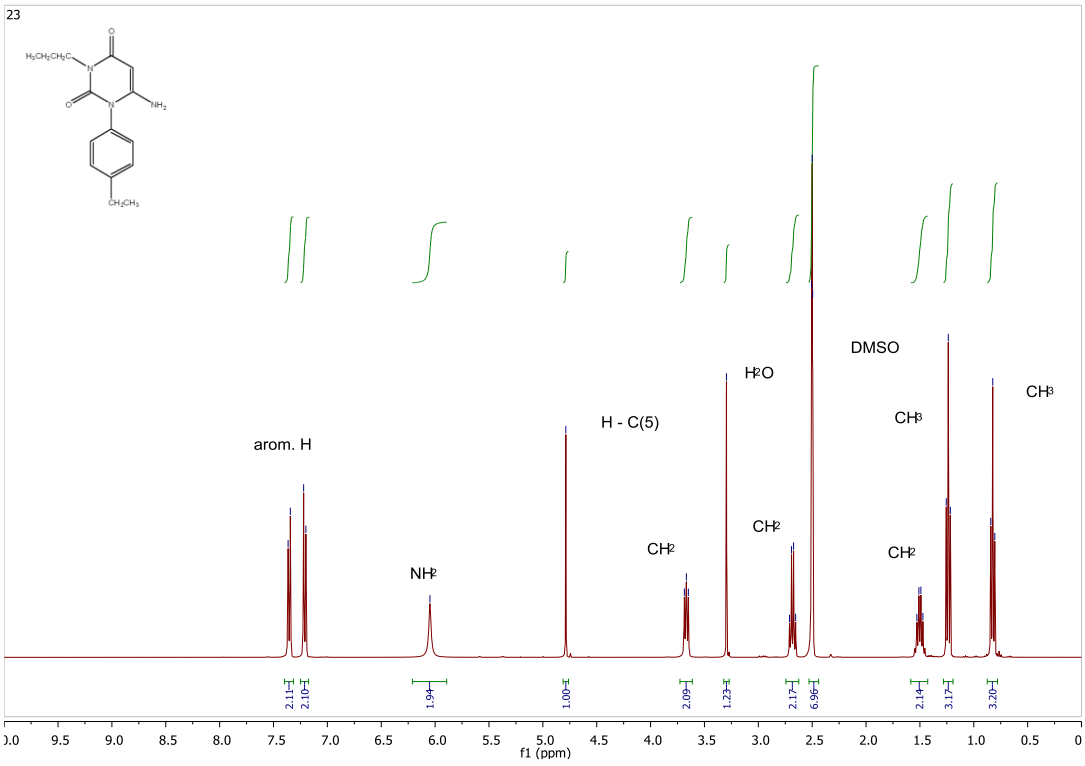
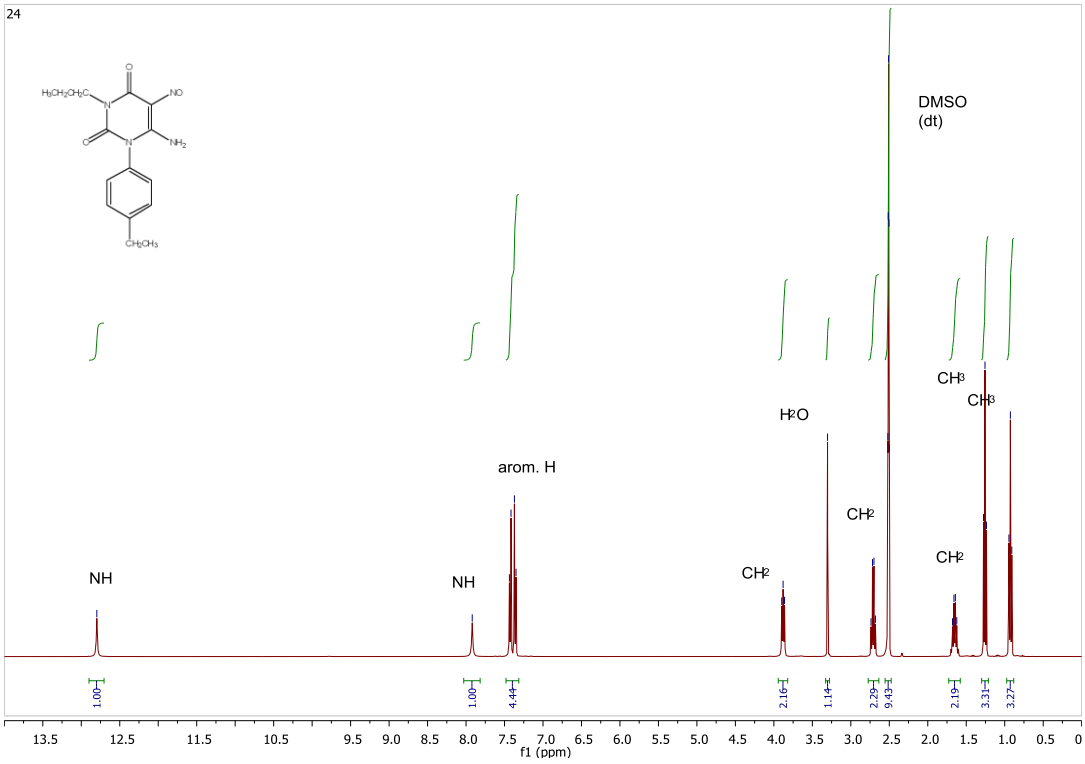
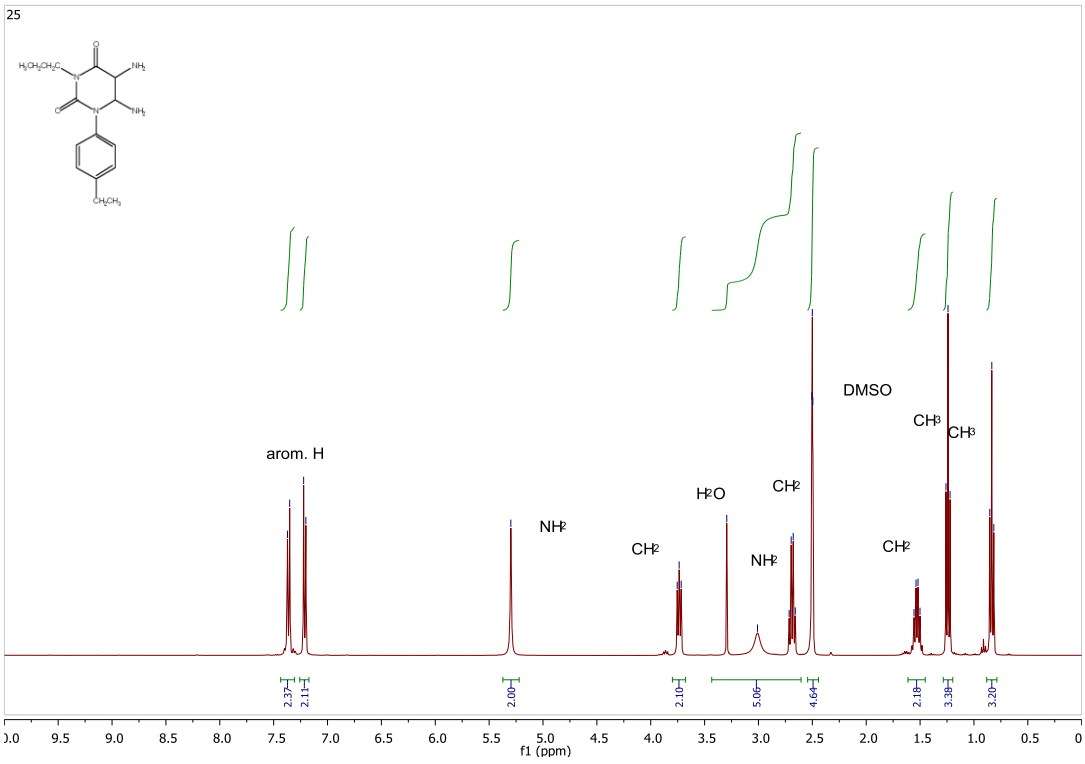
7. Attachment

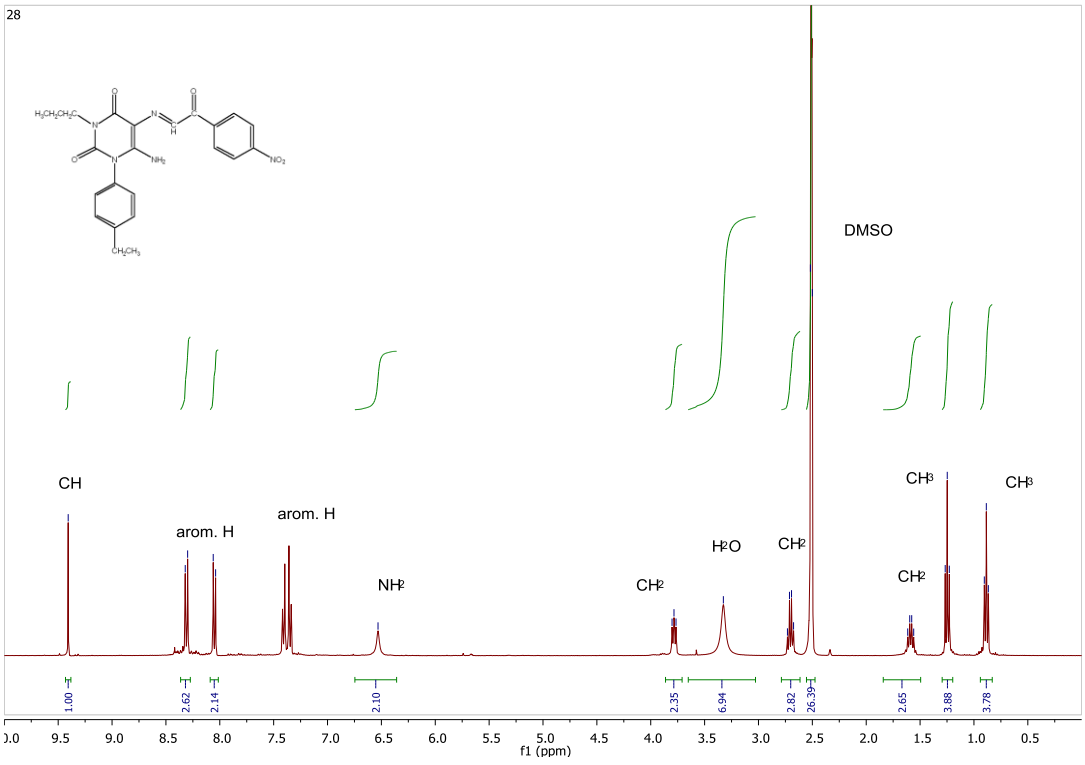


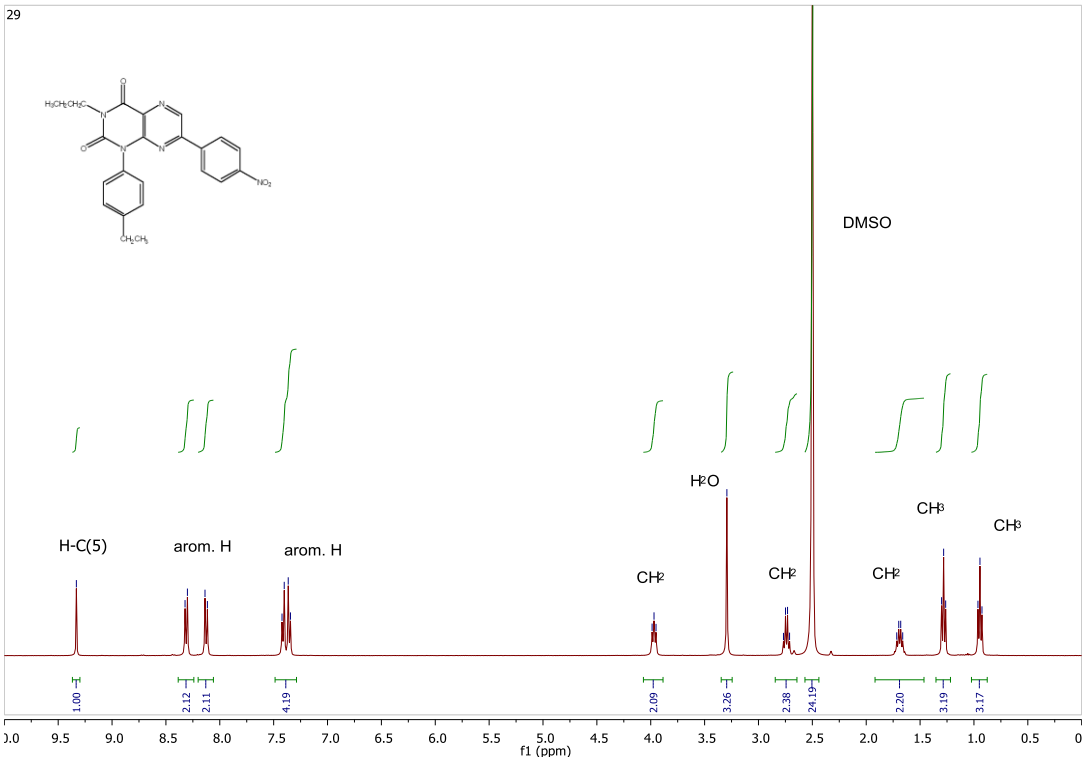


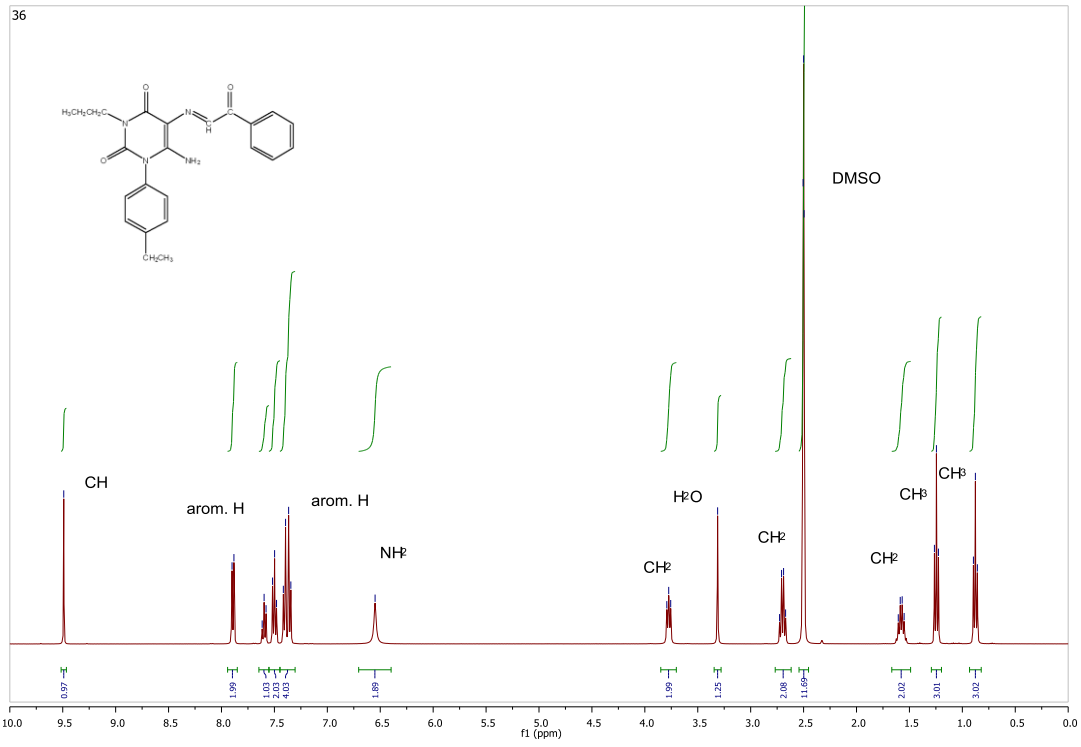

23
arom. H
NH2
H - C(5)
CH2
H2O
CH2
DMSO
CH2
CH3
CH3
f1 (ppm)

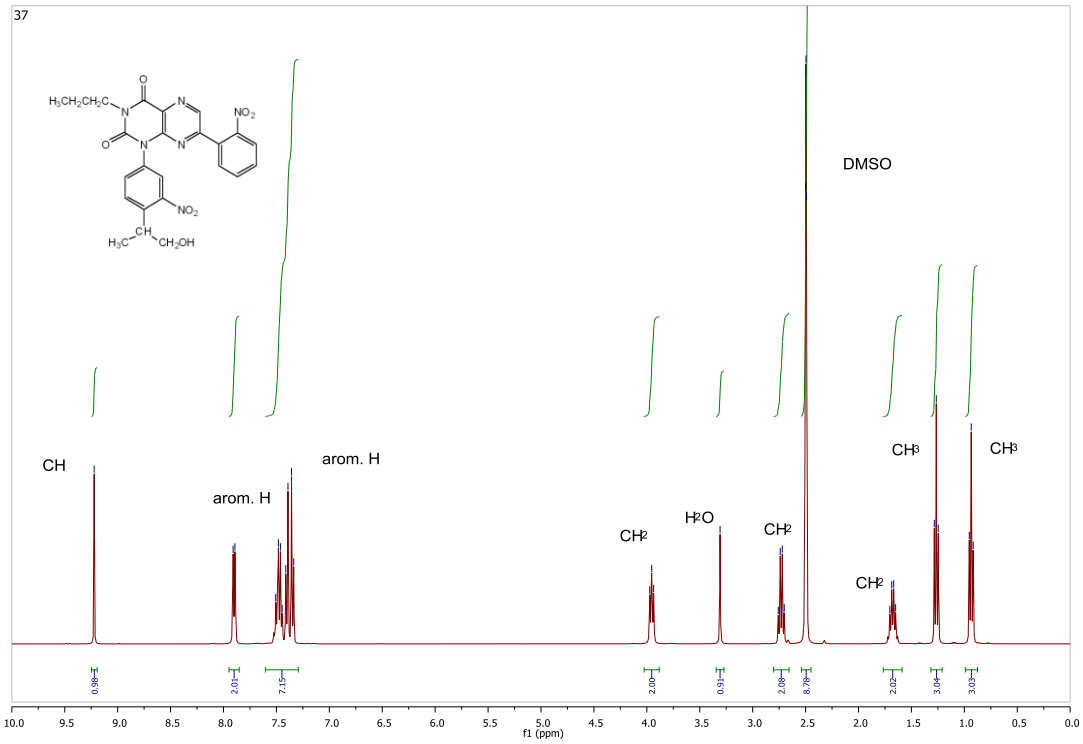

24
NH
NH
arom. H
CH2
H2O
CH2
DMSO
(dt)
CH2
CH3
CH3
f1 (ppm)


25
arom. H
NH2
CH2
H2O
NH2
CH2
DMSO
CH3
CH3
CH2
2.37
2.11
2.00
2.10
5.06
4.64
2.18
3.39
3.20
f1 (ppm)


28
CH
arom. H
arom. H
NH2
CH2
H2O
CH2
DMSO
CH3
CH3
CH2
1.00
2.62
2.14
2.10
2.35
6.94
2.82
26.39
2.65
3.88
3.78
f1 (ppm)


29
DMSO
H2O
CH3
CH3
H-C(5)
arom. H
arom. H
CH2
CH2
CH2
1.00
2.12
2.11
4.19
2.09
3.26
2.38
24.19
2.20
3.19
3.17
f1 (ppm)


36
DMSO
CH
arom. H
arom. H
NH2
H2O
CH2
CH2
CH2
CH3
CH3
0.97
1.99
1.03
2.03
4.03
1.89
1.99
1.25
2.08
11.69
2.02
3.01
3.02
f1 (ppm)


37
DMSO
CH
arom. H
arom. H
CH2
H2O
CH2
CH2
CH3
CH3
f1 (ppm)