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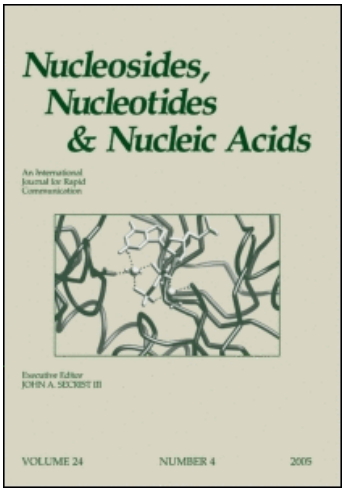

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New Photolabile Protecting Groups in Nucleoside and Nucleotide Chemistry—Synthesis, Cleavage Mechanisms and Applications

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NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1987-1996 (1998)

NEW PHOTOLABILE PROTECTING GROUPS IN NUCLEOSIDE AND NUCLEOTIDE CHEMISTRY - SYNTHESIS, CLEAVAGE MECHANISMS AND APPLICATIONS

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ABSTRACT. - New photolabile protecting groups have been found in the 2-(2-nitrophenyl)ethoxycarbonyl and the 2-(2-nitrophenyl)ethylsulfonyl group, respectively. The influence of substituents at the phenyl ring as well as the side-chain has been investigated regarding the photolysis rates on irradiation at 365 mn. ß-Branching in the side-chain leads to highly increased rates of photodeprotection. A new type of photocleavage mechanism consisting of a photoinduced ß-elimination process is proposed.

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INTRODUCTION. - Photolabile protecting groups of which the most commonly used representatives are derived from o-nitrobenzylalcohol[1] are widely used in synthetic organic chemistry[2] in order to extend normal blocking group strategies by a further orthogonal dimension. The o-nitrobenzyl group has already been used in nucleoside and nucleotide chemistry for OH-protection[3,4] and recently some derivatives thereof like the o-nitroveratryloxycarbonyl (NVOC) and the α-methyl-o-nitropiperonyloxycarbonyl (MeNPOC) functions have been considered as excellent candidates in the biochip production of oligonucleotide arrays by the photolithographic technique[5]. This method has already revolutionized microelectronics to a large extent and is now applied as another consequential technology in molecular biology to serve as analytic and diagnostic tools for DNA analyses[5-7]. Since the conventional DNA sequencing technology is a laborious procedure requiring electrophoretic size separation of labeled DNA fragments an alternative approach to *de novo* DNA sequencing, termed *sequencing by hybridization* (SBH) has been proposed[8-10] and applied with some success[11,12]. On the other hand, solid-phase chemistry, photolabile protecting groups, and photolithography have also been combined to achieve light-directed, spatially addressable parallel chemical synthesis to

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yield a highly diverse set of chemical products[13-15]. Besides the o-nitrobenzyl group and its modified derivatives new types of photolabile functions have been found recently in the benzoine residue[16,17] and the pyrenylmethyl group[18]. We have recently found that also the 2-(2-nitrophenyl)ethoxycarbonyl[19] and the 2-(2-nitrophenyl)ethylsulfonyl group are new interesting photolabile groups which differ in their photocleavage mechanisms completely from the o-nitrobenzyl counterpart and are therefore especially prone for application in nucleoside and nucleotide chemistry. A report on the first findings with these new blocking groups will be presented.

SYNTHESIS. - Based upon the findings that the 2-(2-nitrophenyl)ethoxycarbonyl group is a photolabile function we synthesized a series of substituted 2-(2-nitrophenyl)-ethanols starting from the corresponding subst. o-nitrotoluenes by base-catalysed aldol-type addition to formaldehyde. In a similar manner o-nitrophenylethane, o-nitrobenzyl-chloride and o-nitrobenzylmethylether have been converted into the corresponding 2-(2-nitrophenyl)ethanols carrying in the ß-position of the side-chain an additional substituent. The various ethanol derivatives have then been treated with diphosgene to give in good yields the corresponding chloroformates which have been used *in situ* for acylation of thymidine to form as the main reaction products the 5′-O-[2-(2-nitrophenyl)ethoxy-carbonyl]thymidines in yields between 60 and 80%. As minor side-products also small amounts of the 3′-isomers and the 3′,5′-disubstituted analogs have been separated and characterized.

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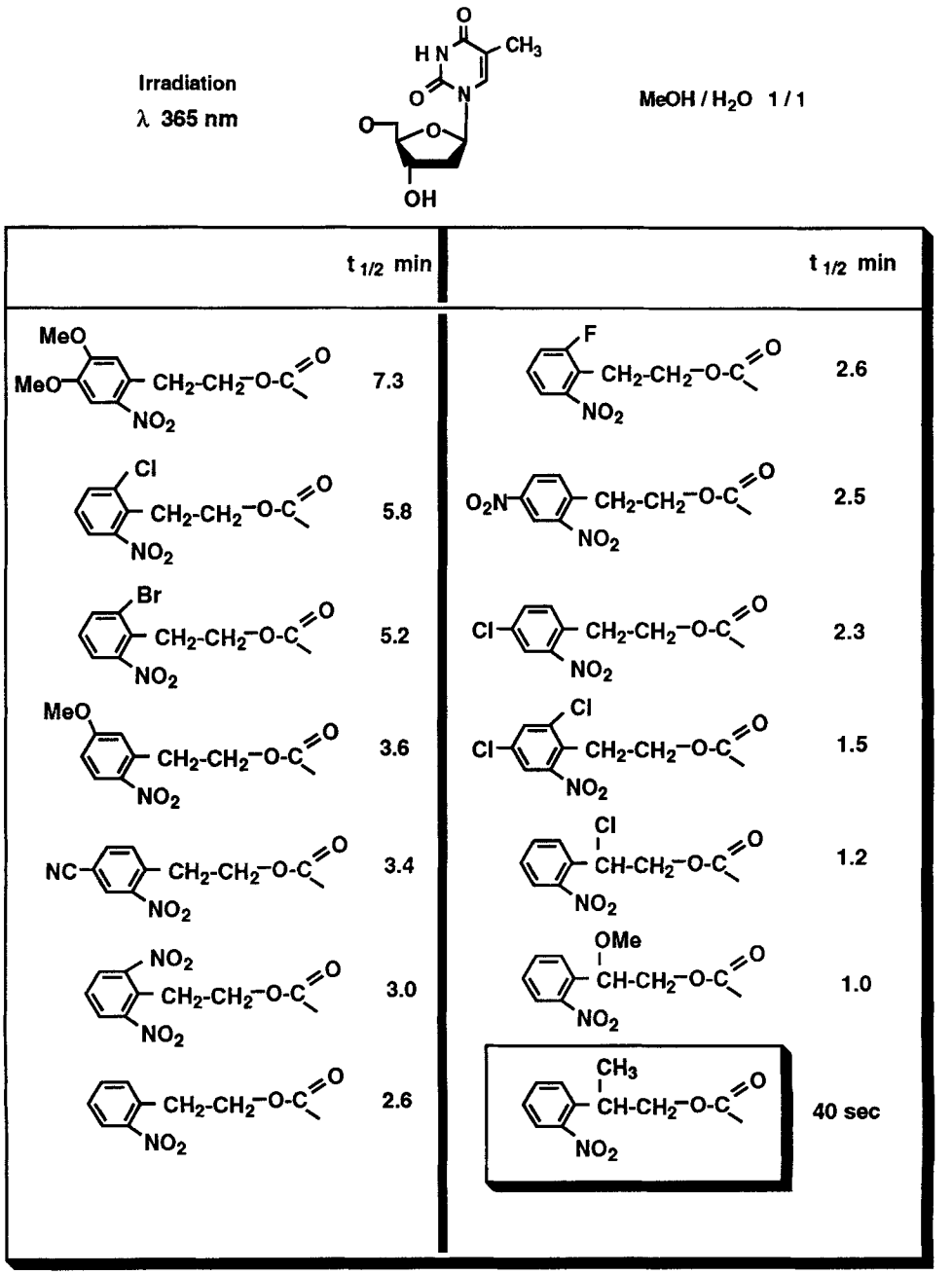
The newly synthesized thymidin-5′-yl-carbonates have then been investigated regarding their photolability under comparative studies applying the same photolysis conditions to a 0.1 mmolar solution in MeOH / H_2O 1:1 and a selected wavelength of 365 nm for irradiation. The relative half-lifes of the photocleavage reactions are listed in table 1 showing the influence of various substituents on the phenylring as well as in the side-chain. Sofar the 2-(2-nitrophenyl)propoxycarbonyl (NPPOC) group showed under the choosen standard conditions the fastest cleavage with a $t_{1/2}$ of only 40 sec. Comparisons with the o-nitrobenzyloxycarbonyl and the α-methyl-o-nitropiperonyloxycarbonyl (MeNPOC) group indicated that these groups have a $t_{1/2}$ of 2.5 min. The significant improvement of the photolability of the 2-(2-nitrophenyl)ethoxycarbonyl over the corresponding 2-nitrobenzyloxycarbonyl derivatives forced us to look into the cleavage mechanisms of these two series of compounds since it is expected from the different structures that the cleavage mode proceeds by different pathways. The photocleavage of the o-nitrobenzyl group leads in an intramolecular redox reaction to o-nitrosobenzaldehyde whereby this process is initiated by a hydrogen abstraction from the benzyl-side-chain, followed by the formation

TABLE 1. Comparisons of Photolabile 2-(2-Nitrophenyl)ethoxycarbonyl Protecting Groups



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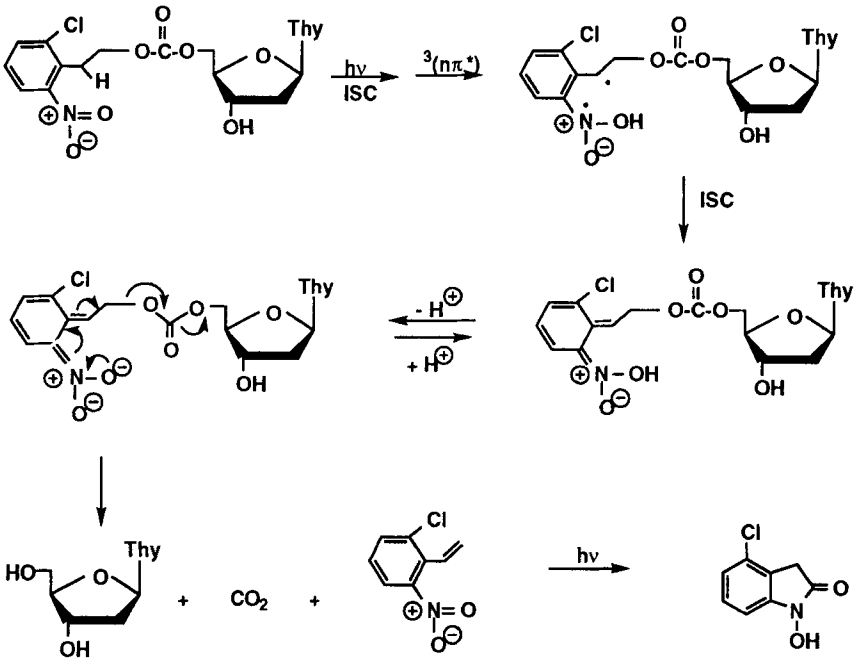

Scheme 1

of an intermediary carbo-cation, quenching by a nucleophile and finally dissoziation of the hemiacetal-type intermediate[20]. On the other hand, we propose for the photocleavage of the 2-(2-nitrophenyl)ethoxycarbonyl groups an entirely new mechanism consisting of a light-induced ß-elimination process. This mechanism is derived from the fact that on irradiation of 2-(6-chloro-2-nitrophenyl)ethoxycarbonylthymidine 6-chloro-2-nitrostyrene has been detected by HPLC and characterized by comparison with an authentic sample. 6-Chloro-2-nitrostyrene, however, is only seen as an intermediate since it is also photochemically unstable and further converted into 4-chloro-N-hydroxyoxindol[21] as the final product.

In the first step of this photoelimination a hydrogen abstraction from the ß-position in the sidechain is most likely, then intersystem crossing of the diradical takes place followed by ß-elimination forming thymidine, CO_2 and 6-chloro-2-nitrostyrene. Since this cleavage does not involve a chemical step like in the case of o-nitrobenzyl scission an accelerated breakdown of the molecule can be expected explaining the experimental findings in a rational manner.

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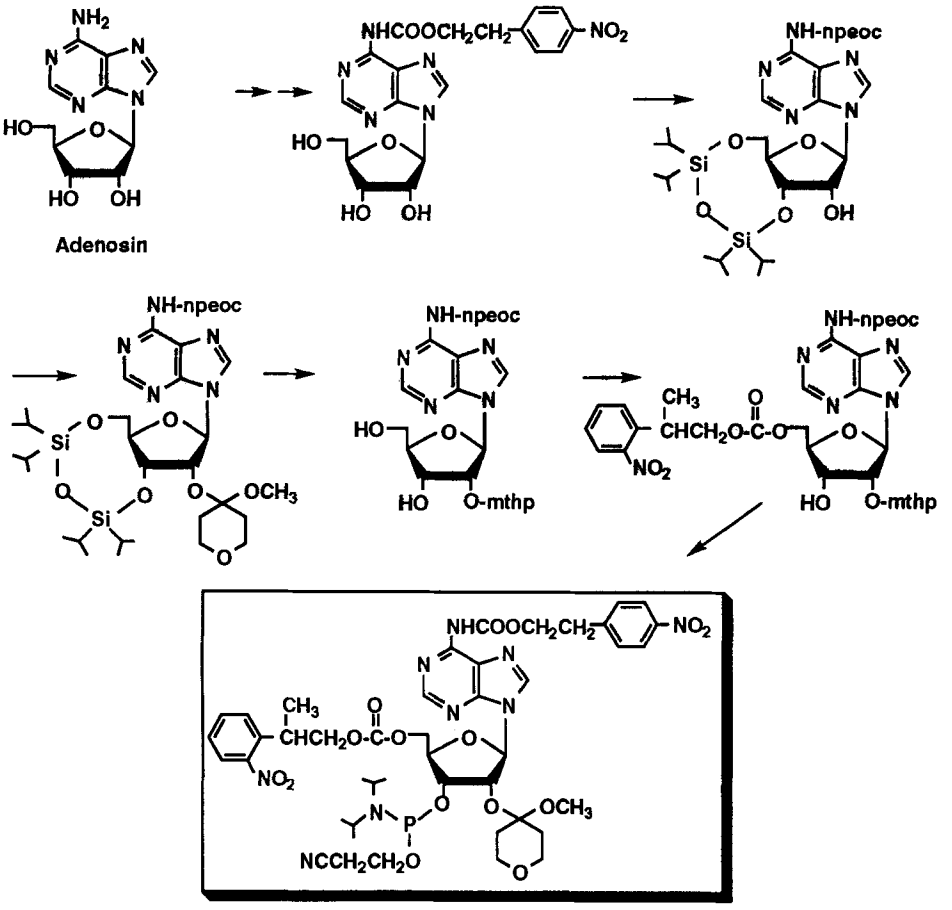

Scheme 2

The interesting photoactive properties of the NPPOC-group forced us to synthesize the the fully protected 3'-O-phosphoramidites of the four common 2'-deoxyribonucleosides dT, dC, dA and dG as well as the four ribonucleosides U, C, A and G. For base protection the 2-(4-nitrophenyl)ethoxycarbonyl was applied and the 2'-OH blocking in the ribo series was achieved by the 4-methoxytetrahydropyranyl group as an acid labile function. A typi-cal sequence of protection of adenosine leading to N^6-2-(4-nitrophenyl)ethoxy-carbonyl-5'-O-2-(2-nitrophenyl)propoxycarbonyl-2'-O-(4-methoxytetrahydropyranyl)-adenosine-3'-O-(ß-cyanoethyl, N-diisopropyl)phosphoramidite is exemplified in scheme 2.

The phosphoramidites have already been used for the built-up of oligonucleotide arrays on a solid-support showing very promising properties.

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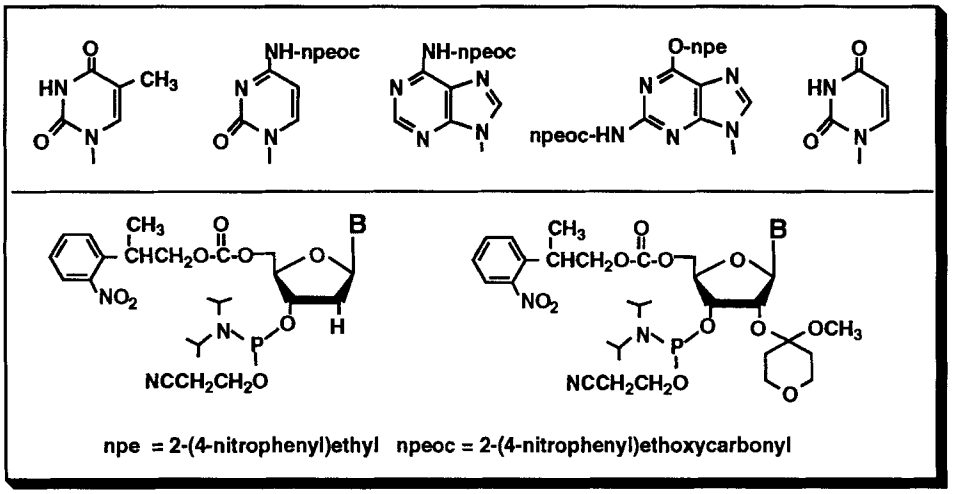

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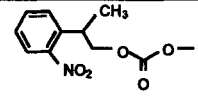
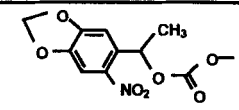
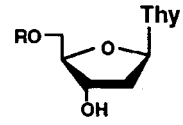
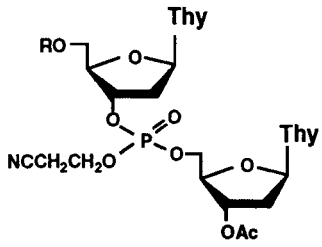
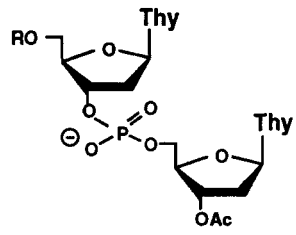
During the photocleavage reactions it was noticed that monitoring on the educt by HPLC does not give the correct answer regarding the generation of the anticipated 5'-OH component. We found a strong dependence on the solvent used in the photolyses and more striking was the amount of detectable 5'-deprotected product after complete disap-pearence of the educt. 5'-O-2-(-2-Nitrophenyl)propoxycarbonylthymidine showed in MeOH/H_2O 1:1 a $t_{1/2}$ of 40 sec and a detection of 72% of thymidine whereas the half-life in dioxane was twice as long and only 44% of thymidine could be found. A comparison with 5'-O-(α-methyl-2-nitropiperonyloxycarbonyl)thymidine revealed a slower cleavage rate in MeOH/H_2O but an improved $t_{1/2}$ in dioxane and the detectable amount of thymidine was 82% in both cases. Analogous studies have been performed with 5'-O-NPPOC- and 5'-O-MeNPOC-thymidylyl-(3'-OP-ß-cyanoethyl-5')-3'-O-acetylthymidine and the corresponding phospho-diesters showing similar results and no indications of a possible chain-cleavage reaction forming new products. Sofar the nature of the missing thymidine has not yet been elucidated but may be due to a secondary photoreaction leading to non-detectable side-products (Table 2).

Another new type of photolabile protecting groups have been found in the 2-(2-nitro-phenyl)ethylsulfonyl groups (NPPS). Their photolability was expected from analogy reasons since the 2-(4-nitrophenyl)ethylsulfonyl isomers are interesting protecting groups which can be cleaved by a ß-elimination process as shown already in nucleoside and nucleotide chemistry[22]. The 2-(2-nitrophenyl)ethylsulfonyl chlorides have been synthesized from the corresponding 2-(2-nitrophenyl)ethanols first by reaction with thionyl chloride to the

TABLE 2. Half-life times and detection yields of photolysis products

Irradiation: λ = 365 nm								
	MeOH / H_2O 1 / 1		Dioxane		MeOH / H_2O 1 / 1		Dioxane	
	$\tau_{1/2}$ sec	Yield %	$\tau_{1/2}$ sec	Yield %	$\tau_{1/2}$ sec	Yield %	$\tau_{1/2}$ sec	Yield %
	60	72	83	44	280	82	45	82
	70	66	80	32	180	58	75	79
	80	76	110	56	80	55	60	83

corresponding ethyl chlorides, then reaction with sodium thiosulfate to the Bunte salts and final oxidation with chlorine gas to the sulfonyl chlorides.

These reagents react preferentially with the 5'-OH group if the amino groups at the nucleobases have been blocked in the common manner. We have expected that from analogy reasons the 2-(2-nitrophenyl)ethylsulfonyl groups will reveal also interesting photolability due to the fact that the isomeric 2-(4-nitrophenyl)ethylsulfonyl group is a new type of protecting group which can chemically been cleaved by a ß-elimination process as shown recently in the nucleoside series[22]. We have sofar synthesized 5 different 5'-O-2-(2-nitrophenyl)-

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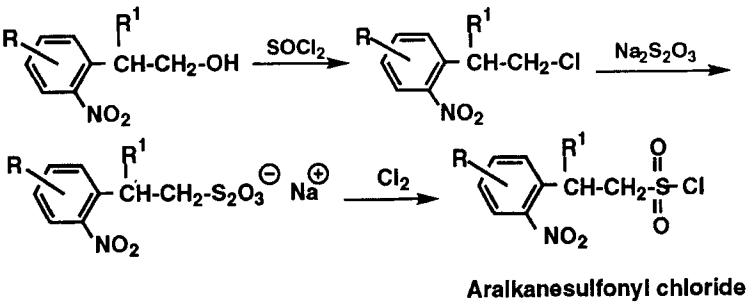
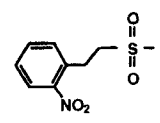
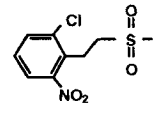
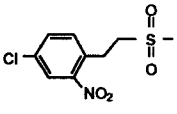
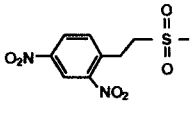
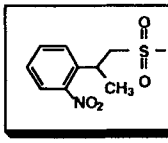


Scheme 3

TABLE 3. Half lifes times of 5'-O-2-(2-Nitrophenyl)ethylsulfonyl)-2'-deoxyribonucleosides

	T	dC NPEOC	dA NPEOC	dG NPE NPEOC
	11 min	19 min	21 min	20 min
	16 min	34 min	42 min	> 30 min
	11 min	16 min	18 min	16 min
	15 min	23 min	32 min	16 min
	50 sec	105 sec	66 sec	45 sec

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ethylsulfonyl derivatives of the 4 common base-protected 2'-deoxyribonucleosides and noticed that in the photolytic cleavage reactions the ß-branched 2-(2-nitrophenyl)-propylsulfonyl group is by far the most photolabile group in comparison to the subst.-phenyl derivatives (Table 3).

Regarding the photocleavage mechanism we propose in analogy to the 2-(2-nitrophenyl)-ethoxycarbonyl groups again a photolytic ß-elimination process since there seems to be some close relation between the chemical and the photochemical breakdown processes in these series of compounds. We will investigate the photolysis of the NPPS groups regarding the scission of this functionality and especially, in respect to solvent effects to decrease the cleavage rates even more pronounced. Furthermore sterically more restricted structural analogs to the 2-(2-nitrophenyl)ethoxycarbonyl and the 2-(2-nitrophenyl)ethylsulfonyl group will be evaluated as new types of photolabile protecting groups for nucleosides and nucleotides as well as monomeric building blocks for oligonucleotide syntheses.

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