Synthesis, Crystal Structure, and Enzymatic Evaluation of a DNA-Photolesion Isostere

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Abstract: Nucleotide analogues are useful tools for the investigation of interactions between DNA-binding proteins and DNA at a molecular level. Herein we describe the synthesis of the DNA-lesion analogue **2**, which is required to determine the extent to which specific phosphodiesters in the DNA backbone contribute to the recognition of cyclobutane pyrimidine dimer DNA lesion by the dimer-specific repair enzymes DNA photolyases or T4-endonuclease V. The analogue **2** is a close structural mimic of cyclobutane pyrimidine dimers like **1**, which are the major lesions induced upon irradiation of cells with UV light. Instead of the negatively charged phosphate link in **1**, analogue **2** contains an uncharged but isosteric formacetal moiety. The analysis of this and other phosphodiester contacts is hoped to provide insight into the lesion recognition process, which is currently believed to require the flipping of the lesioned base out of the DNA double helix. The lesion analogue **2** is synthetically available in large quantities, which allowed us to establish a new, fast and sensitive DNA photolyase assay. A precise X-ray crystal structure analysis of the DNA-lesion analogue **2** is also presented. The structure underlines the isosteric character of **2** and reveals, in combination with the only other available X-ray crystal structure determined from a thymine-dimer triester analogue, interesting structural features of cyclobutane pyrimidine dimer lesions. We describe the incorporation of the lesion analogue **2** into oligonucleotides by using standard phosphoramidite chemistry. Initial enzymatic repair studies are reported with three different types of DNA photolyases. These studies show that the lesion analogue **2** is rapidly repaired by photolyases from *Anacystis nidulans*, *Neurospora crassa* and from the marsupial *Potorous tridactylis.* The enzymatic investigations indicate that all photolyases, including enzymes from higher organisms (*P. tridactylis*) accept the formacetal dimer as a lesion substrate and therefore could possess a similar DNA-lesion recognition process, in which the interaction with the central phosphate unit is only of limited importance.

Keywords: cyclobutane pyrimidine dimers · DNA cleavage · DNA repair · photolyases

Introduction

Cyclobutane pyrimidine dimers like **1** are intensively studied genotoxic DNA lesions that are formed upon irradiation of cells with UV light.[1] Their efficient removal from the genome is essential for all organisms in order to avoid cell death or cancerogenous cell growth.[2] Several specific DNA repair processes[3] including photoreactivation[4] are known to eliminate the dimer lesions from the genome. Since the discovery of the dimer specific repair enzyme DNA photolyase in *Escherichia coli* by Rupert and co-workers,[5] and of further photolyases in a variety of other organisms by Yasui, Eker, and others,[6] the light-dependent photorepair of pyrimidine dimer DNA lesions has attracted considerable interest. The successful cloning and overproduction[7] of the *E. coli* DNA photolyase has allowed detailed enzymatic,[8] spectroscopic,[9] mutagenic,[10] and non-DNA-substrate analogue studies.[11] These investigations and the determination of the three-dimensional structure of the *E. coli* photolyase by Deisenhofer, Sancar, and co-workers,[12] and of the *A. nidulans* enzyme by Miki, Yasui, Eker, and co-workers[12] illustrate the ongoing effort to unravel the mechanism and the dependencies of the repair process. Based on the current understanding of the repair process, we recently prepared the first flavin-containing model compounds, which are able to mimic the DNA repair process,[13] and flavin-containing oligopeptides that repair cyclobutane pyrimidine dimers in single-stranded DNA.[14]

The question of how DNA-repair enzymes achieve the selective recognition of DNA lesions in a sequence-independent, structure-specific DNA-binding mode, is currently

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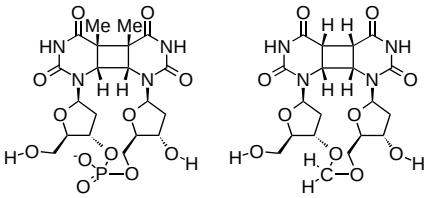
attracting considerable attention. It was recently discovered that some repair enzymes bind to damaged DNA with the lesioned base, or to a base in close vicinity of the DNA lesion, in an extra helical conformation.[15] Consequently, the molecular events that cause the bases to flip-out of the DNA double helix, and the pairing properties of DNA lesions within the DNA double helix are under intensive investigation. Previous studies of the DNA lesion recognition event performed with the photolyase enzymes included repair studies with small pyrimidine dimers[11] and various DNA-footprinting studies.[16,17] The results showed that photolyases contact five phosphodiesters in close proximity to the lesion and that these contacts contribute most of the free binding energy.[8] The enzyme shows intimate interactions with one phosphodiester at the 5′-side of the dimer, with three phosphodiesters at the 3′-side of the dimer, and with one phosphodiester opposite the dimer lesion through the minor groove in the complementary strand. The central intradimer phosphate group[16–18] was found to be only of minor importance for recognition, supporting the assumption that these enzymes flip the dimer lesion out of the DNA double helix. The T4-endonuclease V protein is another dimer specific repair enzyme, which also critically forms hydrogen bonds to a number of phosphodiesters in close proximity to the dimer lesion, including the central, intradimer phosphodiester.[15a] This enzyme flips the adenine base complementary to the 5′-side of the thymine dimer out of the DNA double helix. The cyclobutane thymine dimer stays inside the DNA double helix and the helix exhibits a sharp 60° inclination at the central thymine dimer.

In order to gain a deeper understanding of the lesion recognition step and of the base-flipping process, every single interaction between DNA repair enzymes and the phosphodiester backbone of the damaged DNA double helix needs to be investigated in detail. This is possible with oligonucleotides that contain nucleic acid analogues at the recognition site of the repair protein.[19,20] We reasoned that DNA-repair and DNA-binding studies with double-helical DNA in which the phosphodiesters are successively replaced by isosteric formacetals, as developed by Matteucci et al.[21] and van Boom et al.[22,23] may serve to elucidate the required recognition information. Herein we report the preparation of the first lesion analogue (**2**), in which the central phosphate is replaced by a formacetal linkage. Our initial goal was to confirm that the central phosphate is not required for the recognition by microbial DNA photolyases and to investigate whether or not this recognition motif extends to photolyases from higher organisms. We found that the formacetal-containing lesion isostere **2** is, in contrast to the natural cyclobutane thymine dimer **1**,[24,25] synthetically available in large quantities, which allowed crystallization experiments and the development of an HPLC-based DNA-repair assay.



1 **2**

We describe a precise X-ray crystal structure of the dimer **2**, and compare it to the X-ray structure obtained from a thymine dimer triester derivative. The comparison of the structures confirmed the isosteric character of the lesion analogue **2**. Investigations of the enzymatic repair with three different types of photolyases show that all enzyme types, including class II photolyases, are capable of repairing the artificial lesion **2**. We hope that compound **2** and further analogues will now enable a detailed analysis of the lesion recognition event and an investigation of the dynamics of dimer-lesion-containing DNA.[26]

Abstract in German: *Nucleotidanaloga sind sehr nützliche Werkzeuge zur Untersuchung der molekularen Wechselwirkungen zwischen DNA-bindenden Proteinen und DNA. Diese Publikation beschreibt die Synthese des DNA-Schadensanalogons **2**, mit dem untersucht werden kann, in welchem Ausmaß bestimmte Phosphordiestergruppen zur molekularen Erkennung von Pyrimidindimer-DNA-Schäden durch die dimerspezifischen DNA-Reparaturenzyme DNA-Photolyase oder T4-Endonuclease V beitragen. Das Analogon **2** imitiert strukturell Cyclobutanpyrimidin-Dimere, welche die wichtigsten durch Bestrahlung von Zellen mit UV-Licht hervorgerufenen DNA-Schäden sind. Statt der mittleren Phosphordiesterfunktionalität enthält **2** eine ungeladene, isosterische Formacetalgruppe. Die Analyse dieser und anderer Beziehungen zu Phosphordiestern soll Einblicke in den Schritt der Schadenserkennung liefern, welcher vermutlich das Herausklappen der geschädigten Stelle aus der DNA beinhaltet. Das Schadensanalogon **2** ist präparativ in größeren Mengen erhältlich, so daß ein neuer, sensitiver DNA-Photolyase-Assay entwickelt werden konnte. Eine sehr genaue Röntgenstrukturanalyse von **2** wird diskutiert. Die Struktur belegt den isostrukturellen Charakter des Analogons **2** und weist im Vergleich mit der einzig anderen verfügbaren Kristallstruktur eines Thymindimer-Triester-Derivates interessante Struktureigenschaften auf. Wir beschreiben den Einbau von **2** in Oligonucleotide mit der Phosphoramiditchemie. Erste Untersuchungen zur DNA-Reparatur mit den drei DNA-Photolyasen aus den Spezies A. nidulans und N. crassa sowie vom Beuteltier P. tridactylis ergaben nun, daß **2** durch diese Photolyasen effizient repariert wird. Diese enzymatischen Untersuchungen deuten an, daß alle Photolyasen, inklusive derjenigen aus höheren Organismen (P. tridactylis), das Formacetaldimer **2** als Substrat akzeptieren. Dementsprechend sollten die Schadenserkennungsprozesse sehr ähnlich sein, wobei die zentrale Phosphatgruppe nur von geringer Bedeutung ist.*

Results and Discussion

Synthesis and structure assignment: Our initial synthetic target was the formacetal deoxyuridine dimer **2**, because the four cyclobutane protons facilitate the structural assignment by comparison of the NMR spectra with that of a *cis-syn* uracil

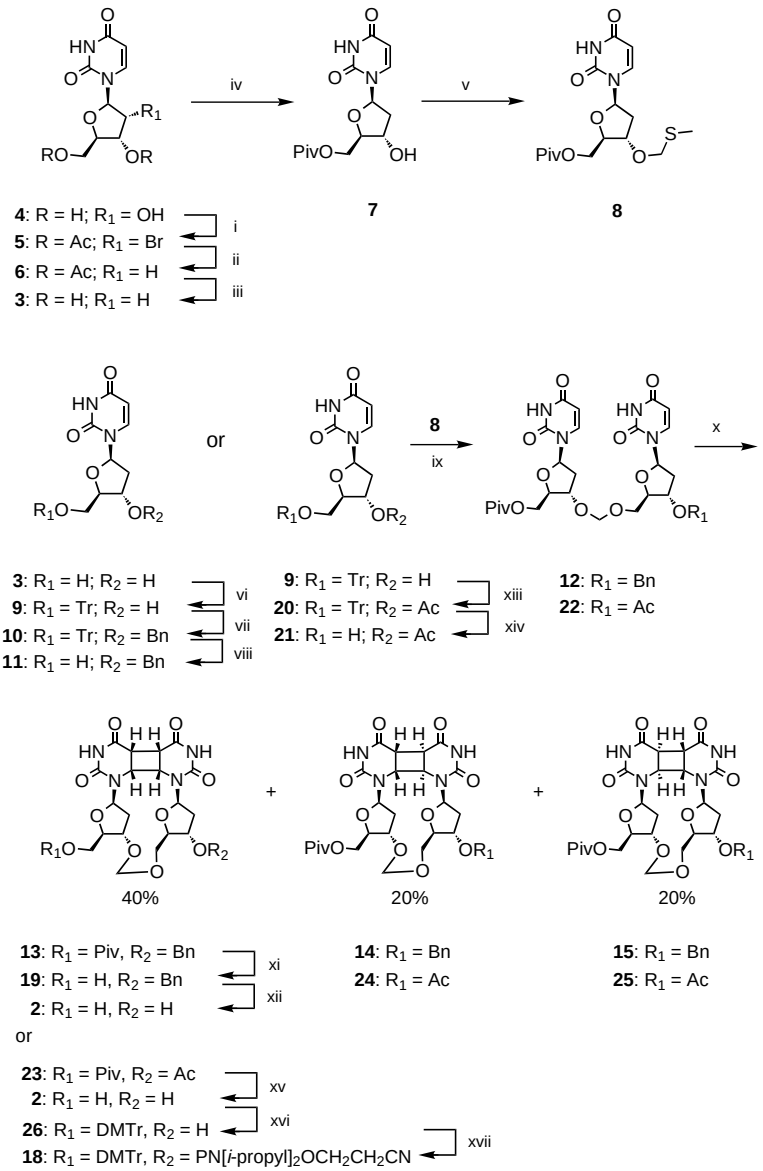
dimer derivative recently prepared in our laboratory.[27] In addition, results from enzymatic repair studies with the deoxyuridine isostere **2** are directly comparable with model studies performed with model compounds containing flavin and uracil dimers.[13]

For the synthesis of **2** in sufficient amounts, 10 gram quantities of the starting material deoxyuridine **3** were required. These were synthesized as described by Brokes et al. and Huang et al[28](Scheme 1) from uridine **4** in three steps, by the reaction of uridine **4** with acetyl bromide to produce **5**, followed by the reductive dehalogenation of **5** to **6** with Bu_3SnH. Cleavage of the acetyl groups in **6** with NH_3 saturated MeOH afforded the deoxyuridine starting material **3** as a white crystalline solid in an overall yield of 65%. One portion of **3** was then treated with pivaloyl chloride in pyridine to yield the 5′-pivaloyl-protected deoxyuridine derivative **7**,[29] which was treated with Ac_2O and AcOH in DMSO[30] to yield the 3′-methyl thiomethoxy deoxyuridine glycosyl donor **8**. A second batch of **3** was allowed to react with trityl chloride in pyridine to yield the 5′-trityl-deoxyuridine derivative **9**,[31] which was treated with benzylchloride and NaH to afford the 5′-trityl-3′-benzyl-deoxyuridine derivative **10**. Cleavage of the trityl group with trifluoroacetic acid (TFA) afforded the glycosyl-acceptor deoxyuridine **11**.[32]

Coupling of the two building blocks **8** and **11** to **12** was achieved after activation of the 3′-methyl thiomethoxy protection group with *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH) in THF.[22] The coupling product **12** is a white, crystalline solid and was obtained in gram quantities (78% yield). For the synthesis of the cyclobutane lesion analogue **2**, **12** was irradiated in 0.5 g portions in acetone in a standard photochemical reactor with a medium-pressure mercury lamp ($\lambda > 300$ nm). The irradiation leads to the formation of three compounds (**13**–**15**), which were subsequently separated by flash chromatography on silica gel using a $MeOH/CH_2Cl_2$ gradient. The first major fraction contained the photoproduct **13** (44% yield). The two minor photoproducts **14** and **15**, possessing *trans-syn* configuration, were obtained in 12% and 14% yield, respectively.

The formation of three photoproducts and the yields obtained can be rationalized as follows: Photodimerization of the deoxyuridine dinucleoside **12** with the two glycosidic bonds in the thermodynamically favorable *anti* conformation yields the desired *cis-syn* configured dimer **13** as the major photoproduct. Photodimerization of the deoxyuridine dinucleoside **12** with one of the two glycosidic bonds in the unfavorable *syn* conformation results, however, in the formation of the *trans-syn* configured photo products **14** and **15**. A fourth possible *cis-syn* isomer, formed upon photodimerization of the dinucleoside **12**, in which both glycosidic bonds are in *anti* configuration was, as expected, not isolated.

In order to confirm the structural assignment all three isolated product **13**–**15** were hydrolyzed with refluxing HCl (4N) (Scheme 2).[33] The sugar-free uracil dimers **16** and **17** were isolated and their NMR spectroscopic data were compared with data obtained recently from *cis-syn* and *trans-syn* configured uracil dimers in our laboratory.[27] The ^{1}H NMR spectrum (after H/D exchange) of the hydrolysis product of the major product **13** revealed two multiplets for the four cyclobutane protons. These signals are characteristic for a *cis-syn* structured cyclobutane ring.[27]



Scheme 1. i) AcBr, HBr in HOAc, CH_3CN, 50 °C;[28d] ii) Bu_3SnH; AIBN, toluene, 80 °C;[28] iii) NH_3, MeOH; 2 d, 65%; iv) PivCl, py, −20 °C, 3 h, 84%;[29] v) Ac_2O, AcOH, DMSO, 70 h, 58%; vi) TrCl, py, 40 h, 72%[31]; vii) NaH, BnBr, Bu_4NI, THF, −20 °C, 12 h, 80%; viii) TFA; *n*BuOH; 1 h, 74%; ix) NIS, TfOH; THF, 5 min, 78%; x) h$\tilde{\nu}$, acetone, 1.5 h, **13**: 44%, **14**: 12%, **15**: 14%; xi) 1N NaOH, Bu_4NBr, THF, 3 h, 91%; xii) H_2, Pd/C, MeOH, 40 °C, 10 h, 90% by TLC, not isolated; xiii) Ac_2O, py, DIEA, 1 h, 80%;[43] xiv) 80%, AcOH, 10 min, 100 °C, 89%; xv) NH_3 conc., 80 °C, 3 h, 57%; xvi) DMTr-Cl, py, DIEA, 15 h, 82%; xvii) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIEA, THF, 4 h, 51%.

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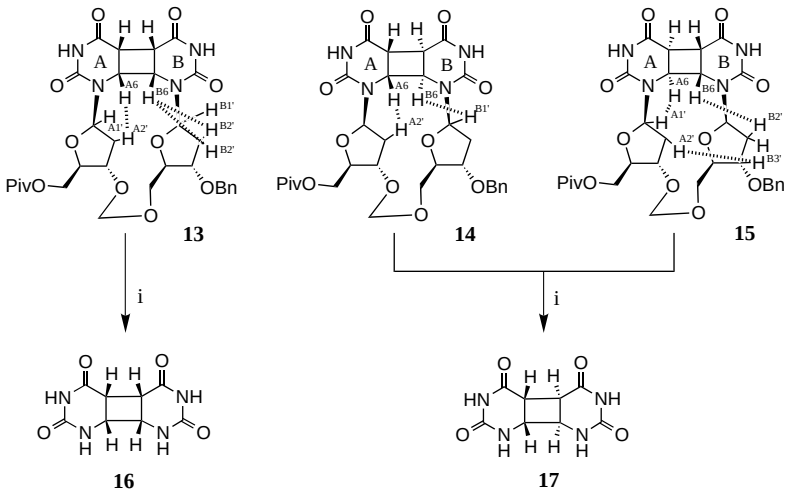


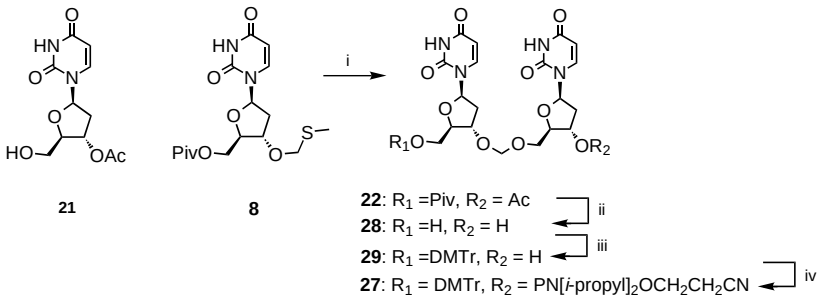
Scheme 2. Acid hydolysis of the three formacetal-linked uracil dimers **13**–**15**. i) 4N HCl, 30 min, 100 °C. The dotted lines indicate couplings observed in the NOE experiments.

Hydrolysis of **14** and **15** resulted in an identical product **17**, suggesting **14** and **15** to be the *trans-syn* isomers. After H/D exchange the ^{1}H NMR spectrum of **17** showed two signals, which are characteristic for *trans-syn* configuration of the cyclobutane uracil dimer.[27]

In order to assign the *trans-syn* isomers **14** and **15** A6-(*R*)/B6-(*R*) and A6-(*S*)/B6-(*S*) configuration (numbering see Scheme 2), and to secure the assignment of the *cis-syn* structure to **13**, ROESY spectra were measured for all three isomers. The experiment with the *cis-syn* compound **13** revealed strong NOEs between H-A6 and H-A2′ and between H-B6 and H-B2′ (see Scheme 2). This coupling, and the missing interactions between H-A1′ and H-A6 and between H-B1′ and H-B6 unambiguously secured the *cis-syn* structure of **13**. In the ROESY experiment, compound **14** exhibited a strong coupling between H-B1′ and the cyclobutane ring proton H-B6. In addition, a NOE between H-A2′ and H-A6 was detected, which is characteristic for the *trans-syn* structure **14** with A6-(*R*) and B6-(*R*) configuration of the cyclobutane moiety. The second, more polar, *trans-syn* isomer **15** featured a complex ^{1}H NMR spectrum. A strong NOE between H-A1′ and H-A6, together with a NOE between H-B2′ and H-B6 proved the *trans-syn* structure with A6-(*S*) and B6-(*S*) configuration of the cyclobutane ring in **15**. This is further confirmed by a strong NOE between H-A2′ and H-B3′.

The final steps were performed in order to convert the *cis-syn* isomer **13**, via **2**, into the phosphoramidite **18** required for the solid phase oligonucleotide synthesis (see Scheme 1). To this end, the 5′-pivaloyl group in **13** was removed under phase transfer conditions using 1N sodium hydroxide solution/THF/Bu_4NBr. The product **19** was subsequently debenzylated to **2** by catalytic hydrogenation with Pd/C (10%) in methanol. Compound **2**, however, could not be readily separated from the catalyst due to its low solubility in organic solvents, which made the two-step deprotection procedure inconvenient for the synthesis of the phosphoamidite **18** in larger quantities. In order to circumvent this problem, we replaced the benzyl group by the base-labile acetyl group. This required the acetylation of the 5′-trityl compound **9** with Ac_2O in pyridine to afford **20**, which was subsequently treated with AcOH to cleave the 5′-trityl group. The obtained 3′-acetyl protected nucleoside **21**[31] was coupled with **8**, after activation with *N*-iodo succinimide/TfOH. The product **22** was irradiated to yield the three 5′-pivaloyl-3′-acetyl protected photodimers **23**–**25**. Separation of the three dimers was achieved by flash chromatography on silica gel using an acetone/CH_2Cl_2 solvent gradient. Irradiation of **22** yielded approximately 41% of the *cis-syn* configured compound **23** and 16% and 17% of the two *trans-syn* configured isomers **24** and **25**, respectively. The 3′-acetyl protection allowed the removal of both protection groups in **23** to yield **2** in one step with aqueous NH_3 (25%). As a further advantage, the pivaloyl/acetyl protection strategy facilitated the separation of isomers **23**–**25**. Finally the formacetal dimer **2** was allowed to react with dimethoxytrityl chloride (DMTr-Cl) and diisopropylethylamine (DIEA) in pyridine to yield the DMTr-protected deoxyuridine dimer **26** (82% yield). Reaction of **26** with 2-cyanoethyl-diisopropyl-chlorophosporamidite and DIEA furnished the final phosphoramidite **18** in 51% yield as a white solid. The presented convergent synthesis with only seven to nine linear steps allows the preparation of the lesion isostere phosphoramidite **18** in gram quantities.

For comparison the formacetal-linked dimer phosphoramidite **27** was prepared (Scheme 3). To this end, the 5-pivaloyl-3′-acetyl protected dinucleotide **22** was not irradiated but directly deprotected with aqueous NH_3 (25%) at 80 °C. The unprotected dinucleotide product **28** was allowed to react with DMTr-Cl and DIEA in pyridine to yield the DMTr-protected nucleoside **29** in 82% yield. Reaction of **29** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite furnished the final phosphoramidite **27** as a white solid in 72% yield.



Scheme 3. i) NIS, TfOH; THF, 5 min, 63%; ii) conc. NH_3, 80 °C, 3 h, 63%; iii) DMTr-Cl, py, DIEA, 15 h, 82%; iv) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, DIEA, THF, 4 h, 72%.

X-ray crystal structure analysis of 2: For a comparison of the structures between the formacetal-bridged lesion **2** and the natural phosphate-bridged lesion **1**, crystals of the deoxyuridine dimer **2** were grown. The formacetal dimer **2** crystallized from H_2O as colorless platelets. The results of the X-ray crystal structure analysis, which was performed at 100 K (see Experimental Section; *R(F)* value = 0.026), are shown in Figure 1. Besides the cyclobutane dimer **2** the crystals contained one equivalent of H_2O.

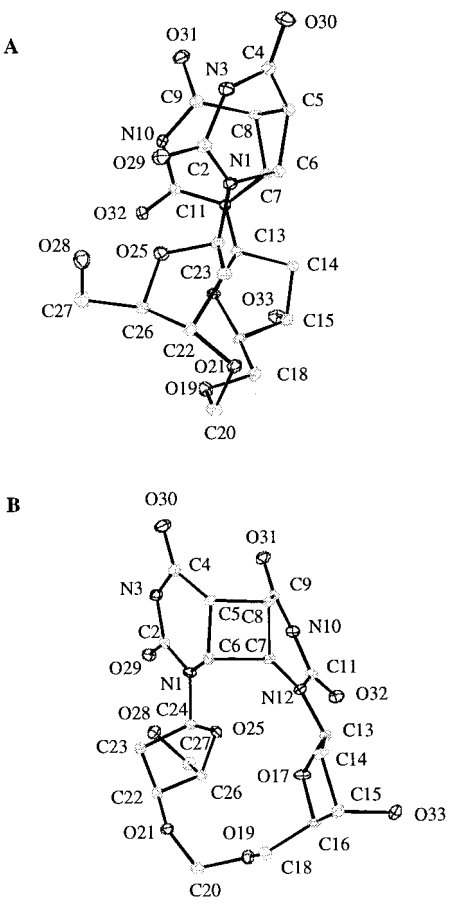


Figure 1. X-ray crystal structure of **2**. ORTEP plots of the molecular structure of **2** A: side view; B: front view. Arbitrary numbering. Displacement ellipsoids are shown at the 50% probability level.

Since an X-ray crystal structure of a phosphate-bridged thymidine **1** or a deoxyuridine analogue is not available, a direct comparison with the X-ray crystal structure of **2** is not possible. The X-ray crystal structure of a thymine dimer triester derivative determined by Hruska et al.[34] can, however, be considered as a good approximation of **1**. The major difference between the phosphotriester and the natural diester lesion is the P–O bond length, which is shortened by 0.05 Å in the triester derivative. The most striking feature of the phosphotriester structure is the *syn*-conformation $\chi(A) = 60.7^\circ$ of the glycosidic bond at the 5′-side of the dimer (ribose-ring A). The glycosidic bond of the 3′-ribose unit (B) is in *anti* conformation with $\chi(B) = -103.7^\circ$). The cyclobutane pyrimidine dimer features a -29°-CB$^-$ twist. The formacetal-linked deoxyuridine dimer **2** exhibits all three key structural characteristics. The glycosidic bond (O25–C24–N1–C2) of the 5′-sugar is *syn*-oriented with $\chi(A) = 69.3(3)^\circ$; the glycosidic bond (O17–C13–N12–C11) of the 3′-sugar features an *anti* conformation $\chi(B) = -72.7(3)^\circ$ and the cyclobutane ring exhibits a CB$^-$-twist of $-28.0(4)^\circ$. All bond lengths and bond angles of the cyclobutane moiety are very similar to those of the phosphotriester derivative. Superimposing the cyclobutane substructure of both structures reveals only minor differences, predominantly located in the backbone. The major difference was expected to be a shortening of the O21–O19 distance due to the expected C–O bond length of 1.421(2) Å, which is significantly shorter than a typical P–O-diester or a -triester bond of 1.60 Å and 1.55 Å, respectively. Such a bond shortening, however, can be readily compensated by small changes of the backbone torsional angles.[35] The O21–O19 distance is 10% shorter in the formacetal-linked deoxyuridine dimer than in the phosphotriester derivative. The descriptive torsion angles $\alpha - \varsigma$ differ only slightly in the two structures. Significant differences exist between the backbone torsional angles ε_A, ς_A, and δ_B, which change from -153°, -138°, and 81° in the phosphotriester structure to $-48.3(3)^\circ$, $87.6(3)^\circ$, and $143.8(3)^\circ$, respectively in the formacetal dimer **2**. The sugar pucker in the phosphotriester compound was determined to be $^2T_3$ for the 5′-sugar moiety (A) and $^3T_4$ for the 3′-sugar ring (B). In the formacetal dimer both sugar moieties possess approximately ^2E (2′-endo) DNA conformation. As NMR investigations of the natural thymine dimer phosphodiester reveal a rapid sugar pucker and CB$^+$/CB$^-$ twist interconversion for the thymine dimer in solution at room temperature,[36] we conclude that the key structural elements, such as the *syn* and *anti* conformations of the glycosidic bonds and the CB$^-$ twist of the cyclobutane moiety, are present in the formacetal structure. The observed structural differences are limited to three backbone angles and to the sugar pucker. The structural differences are located in rather flexible regions of the molecules, which supports the view that compound **2** is a close structural isostere of the DNA-photolesion **1**.

Incorporation of the formacetal dimer 2 into oligonucleotides: The formacetal dimer phosphoamidite **2** was incorporated into oligonucleotides like **30** (Figure 2). Standard

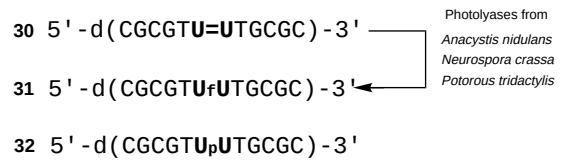


Figure 2. Depiction of the three prepared oligonucleotides **30**–**32** and of the repair reaction performed by the photolyase enzymes from *A. nidulans*, *N. crassa*, and *P. tridactylis*-GST fusion protein.

phosphoramidite oligonucleotide chemistry with a controlled pore glass bead support was employed by using a Pharmacia-Gene-Plus synthesizer. Due to the expected base sensitivity of the deoxyuridine dimer **2**, Pac-protected nucleotides,[37] which possess an enhanced base lability, were used for the synthesis. The oligonucleotides were prepared trityl-on in order to facilitate their purification. Initial deprotection experiments

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with the assembled oligonucleotides were performed in NH_3 solution at room temperature. During the deprotection, however, we observed the formation of two oligonucleotides, which were subsequently separated by preparative HPLC and analyzed by laser desorption mass spectrometry. The minor oligonucleotide featured the expected molecular weight for the oligonucleotide **30**. The second oligonucleotide exhibited a molecular weight of 16 mass units higher. We believe that this oligonucleotide is formed through a hydroxide induced cleavage of one dihydropyrimidine ring, a process which is well documented in the literature.[1] Systematic evaluation of various cleavage conditions finally revealed that deprotection of the dimer-containing oligonucleotides **30** can be achieved with DBU in anhydrous methanol.[38] Under these conditions, formation of the oligonucleotide by-product could be suppressed. In order to obtain the oligonucleotide **30** of highest possible quality we extended the deprotection time to four days and reduced the reaction temperature to 4 °C. The oligonucleotide **30** that contains dimer **2** was thus obtained in about 85–90% purity. The oligonucleotide **30** and the two reference oligonucleotides **31** and **32** were synthesized, deprotected and finally purified by reverse phase HPLC using a C18 reverse phase column and a Et_3NHOAc buffered H_2O/acetonitrile gradient. The oligonucleotides were subsequently detritylated with HCOOH and again purified by reverse phase HPLC. The purity of the final material was determined by analytical reverse phase HPLC and ion exchange chromatography. Laser desorption mass spectrometry confirmed the expected molecular weights.

Initial enzymatic evaluation of the formacetal, dimer-modified oligonucleotide 30 as a substrate for DNA photolyases: In order to investigate if the formacetal-linked dimer **2** is recognized as a lesion substrate by DNA photolyases, enzymatic studies were performed with three different types of enzymes. The photolyase from the cyanobacterium *A. nidulans*[39] was investigated as a member of microbial deazaflavin- and flavin-containing photolyases (class I/type II). The photolyase from the fungus *N. crassa*[40] was used as a representative member of the class I/type I, flavin/methenyltetrahydrofolate-containing photolyases. Both enzymes feature a strong sequence homology with the well studied *E. coli* enzyme.[8] As a member of the class II photolyases, isolated from higher organisms, the enzyme from the marsupial *P. tridactylis* was investigated.[6,41] This photolyase features a smaller sequence homology with the microbial enzymes and the structure of the second cofactor is currently not known. For our initial studies the *P. tridactylis* GST-fusion protein, which contains only the essential flavin cofactor, was used. Due to the lack of the second cofactor and to the presence of the GST-fusion protein, the activity of this photolyase was expected to be significantly lower.

Our interest in quantifying repair-enzyme DNA interactions on a molecular level has led us to developing a HPLC-based photolyase assay to conveniently quantify enzyme activity. Since the *E. coli* photolyase repairs single- and double-stranded DNA with equal efficiency,[42] we used the single-stranded oligonucleotide **30** as the substrate. For the assay, **30** (5.04 nmol) was dissolved in the photoreactivation buffer (200 μL: 5 mM DTT, 10 mM KH_2PO_4, pH 7.0, 0.1M NaCl). After the addition of the DNA-photolyase enzymes (25 pmol of *A. nidulans* and *N. crassa* enzyme and 250 pmol of the *P. tridactylis* GST-fusion protein) in the dark, the assay solution was transferred into a small cuvette and irradiated with monochromatic light. The wavelength of maximal photoreactivation activity was used in the experiments. The *A. nidulans* enzyme was assayed at $\lambda = 435$ nm. $\lambda = 405$ nm was used for photoreactivation experiments with the *N. crassa* photolyase and $\lambda = 380$ nm was employed in experiments performed with the *P. tridactylis* enzyme. After defined time intervals, samples were removed from the assay solution and concentrated acetic acid was added to stop the reaction. The prepared samples were analyzed by direct injection into the HPLC system in order to quantify the amount of repaired oligonucleotide formed. For the separation of the oligonucleotides **30** and **31**, an ion-exchange column with Nucleogel Sax 1000-8/46 proved to be optimal. Both oligonucleotides elute baseline separated with retention times of 12.3 min (**30**) and 14.1 min (**31**). Figure 3 depicts the series of HPLC chromatograms obtained in experiments performed with the *N. crassa* enzyme. In the presence of any of the three DNA photolyases irradiation caused the disappearance of the oligonucleotide **30** and the appearance of a new oligonucleotide **31**. No change in the concentration of **30** was observed in the absence of DNA photolyase. Co-injection of the independently synthesized repaired oligonucleotide **31** supports the assumption that the peak at 14.1 min corresponds to the repaired DNA strand. Figure 4 shows the time-dependent formation of the repaired oligonuclotide **31** for all three enzymes analyzed. Both microbial photolyases are able to completely repair the formacetal DNA-lesion isostere **2**. As expected, the activity of the *P. tridactylis* photolyase is significantly lower. Consequently, a higher enzyme concentration was required to observe the photoreactivation. Nevertheless, repair of DNA strand **30** to **31** is clearly detectable, which proves that even class II photolyases accept the synthetic lesion analogue **2** as a substrate. Our initial enzymatic results therefore demonstrate that the dimer **2** is a suitable lesion isostere for cyclobutane dimer DNA-photolesions such as **1**. Oligonucleotides containing **2** are suitable substrates for all three types of photolyases. The ability of all

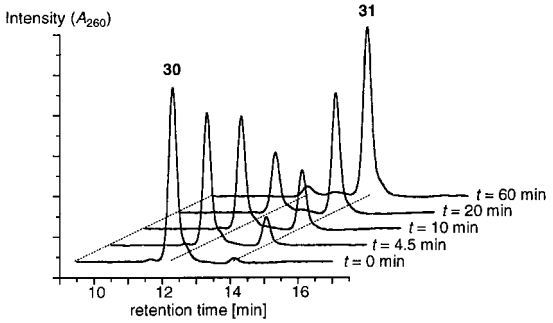

Figure 3. Photoreactivation of **30** by *Neurospora crassa* photolyase. Samples of the illuminated reaction mixture were analyzed by HPLC at various time intervals. **30:** Dimer-lesion **2**-containing oligonucleotide; **31:** repaired oligonucleotide. For conditions see Experimental Section.

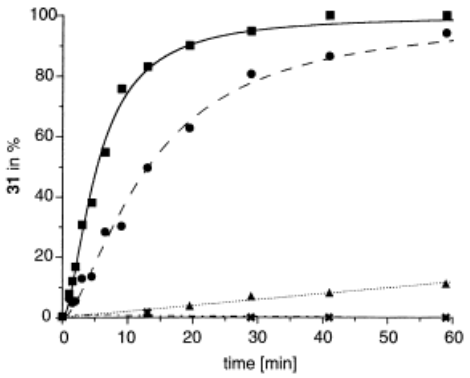

Figure 4. Time-dependent formation of the repaired oligonucleotide **31**. ●: *Neurospora crassa*; ■: *Anacystis nidulans*; ▲: *Potorous tridactylis*; ✖: Control experiment (no photolyase enzyme added). For conditions see Experimental Section.

three enzymes to repair the chemically altered lesion **2** strongly argues that the central intradimer phosphate is not crucial for the recognition of the lesion by all three studied DNA-photolyases. This point, however, needs further investigation, and a kinetic analysis of the complex formation step has been initiated.

Conclusions

The results presented show that a cyclobutane pyrimidine dimer lesion analogue like **2**, which contains an achiral and neutral formacetal group instead of the central phosphate unit is readily available in large quantities. The corresponding phosphoamidite **18** can be incorporated into oligonucleotides using machine-assisted oligonucleotide chemistry. Although the deprotection of the oligonucleotides containing dimer **2** requires special care, sufficient quantities of oligonucleotides containing the lesion analogue **2,** such as **30,** are available. For a detailed analysis of the interactions between the DNA backbone and the photolyase repair proteins, an enzyme assay was developed that allows quantification of the repair activity by using fast HPLC analysis. Using fluorescence tagged, oligonucleotides containing dimer **2**, this HPLC experiment can be employed as a convenient and highly sensitive DNA photolyase assay. A systematic evaluation of the enzymatic and quantum yield of **30** and of other formacetals oligonucleotide substrates, together with equilibrium binding studies are now in progress to determine the requirements for the efficient recognition of DNA lesions by photolyases and other repair proteins on a molecular level. Due to the availability of **2** in larger amounts this building block might facilitate the investigation of the structural distortions caused by cyclobutane pyrimidine dimer in double-helical DNA. In addition, analysis of the distance and sequence-dependence of electron transfer processes in DNA[44] might be possible using the dimer **2**.

Experimental Section

General: Reagents and solvents were reagent grade and used without further purification. Anhydrous $MgSO_4$ was used as the drying agent after aqueous workup. Evaporation and concentration in vacuo was done at H_2O-aspirator pressure. All reactions were performed in standard glassware. Degassing of solvents was accomplished by N_2 sparging for at least 45 min. Column chromatography (CC): Silica gel-*H* from Fluka. TLC: glass or aluminum sheets covered with silica gel 60 F_{254} from Merck; visualization by UV light. M.p.: Büchi SMP-20 apparatus; uncorrected. UV/VIS spectra: Varian Cary-5 spectrophotometer at room temperature; λ_{max} in nm (ε in $M^{-1}cm^{-1}$). Irradiation experiments were performed under nitrogen in a standard photochemical pyrex glass apparatus with ($\lambda > 300$ nm) with a TQ-150 (Haereus Nobelite) medium-pressure mercury lamp. IR spectra (cm^{-1}): Perkin Elmer-1600FTIR. Fluorescence spectra were measured on a Spex 1680, 0.22 m double Spectrometer, 450 Hg/Xe-lamp, in 1 cm quartz cuvettes at room temperature ^1H and ^{13}C NMR: Bruker-AMX-500, Varian Gemini-200 and -300 instruments at room temperature in $(CD_3)_2SO$; solvent peaks ($\delta = 2.49$ for ^1H; and $\delta = 39.7$ for ^{13}C NMR) as reference. MS (*m/z*): VG-ZAB-2SEQ instrument for FAB in a 3-nitrobenzyl alcohol matrix. Elemental analyses were performed by the Mikrolabor in the Laboratorium für Organische Chemie at ETH Zürich. Oligonucleotide synthesis was performed by using a Pharmacia-Gene-Plus synthesizer. Pac-amidites were purchased from Pharmacia. Controlled pore glass bead support was purchased from Sigma. Acetonitrile for the oligonucleotide synthesis was purchased from Roth. All solvents were stored for 12 h over 4 Å molecular sieves prior to oligonucleotide assembly. HPLC was performed with a Knaur HPLC system with a flow of 1 mL min^{-1} using a Nucleosil RP18 (240 mm × 4 mm, 100 Å/5 μm) column from Machery-Nagel. HPLC-grade solvents were purchased from Fluka. All oligonucleotides were detected at 260 nm. For analytical oligonucleotide HPLC-chromatography a linear gradient was used. Preparative HPLC was performed with a Vydac RP C18 column (250 mm × 21 mm, 100 Å/5 μm). Solvent system: A = 0.1 M NEt_3/HOAc in H_2O, B = 0.1 M NEt_3/HOAc in H_2O:MeOH (1:4). Ion exchange chromatography was performed on a Machery & Nagel Nucleogel Sax 1000-8/46 column with a NaCl gradient in 10 mM Na_2HPO_4, pH = 11.5: 0 min to 1 min: 0.18 M NaCl to 0.56 M NaCl; 5 min to 15 min: 0.56 M NaCl to 0.96 M NaCl.

X-Ray crystal structure data of 2: colorless platelets (H_2O); $C_{19}H_{24}N_4O_{10}\cdot H_2O$, $M_r = 486.4$; monoclinic, space group $P2_1$: $\rho_{calcd} = 1.54$ g cm^{-3}, $Z = 2$, $a = 9.398(2)$, $b = 9.886$ (1), $c = 11.647(1)$ Å, $\beta = 104.38(1)^\circ$, $V = 1048.2$ (2) Å^3, $Mo_{K\alpha}$ ($\lambda = 0.71073$ Å) radiation, $2\theta \leq 28^\circ$, 2659 unique reflections, $T = 100$ K. The crystal structure was solved by direct methods (SHELXTL PLUS) and refined by full-matrix least-squares analysis using experimental weights (heavy atoms anisotropic; H atoms riding model, fixed isotropic). Final ($R(F) = 0.026$, $wR(F) = 0.033$) for 346 variable and 2425 observed reflections ($F > 3.0\sigma(F)$). Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-100804. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

3′-*O*-Methylthiomethyl-5′-*O*-pivaloyl-2′-deoxyuridine (8): 5′-*O*-pivaloyl-2′-deoxyuridine **7**[29] (5.43 g, 17.4 mmol) was dissolved in DMSO (36 mL), acetic acid anhydride (26 mL), and acetic acid (8.25 mL). The solution was stirred for 70 H; then ice cold saturated sodium hydrogen carbonate solution (100 mL) was added to the solution and the mixture stirred for a further 1 h. The aqueous solution was extracted three times with chloroform (100 mL). The combined organic phases were washed four times with water (100 mL) and dried with $MgSO_4$. The solvent was evaporated in vacuo and the residual oil was purified by flash chromatography on silica gel (33–50% EtOAc/hexane). Compound **8** was obtained as yellowish oil (3.65 g, 10.1 mmol, 58%). R_F: 0.37 ($CHCl_3$/MeOH 10:1 (*v*:*v*)); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.23$ (s, 9H; $(CH_3)_3CCO$), 2.04–2.10 (m, 1H; 2′-H), 2.14 (s, 3H; SCH_3), 2.51–2.57 (m, 1H; H-2′), 4.22–4.27 (m, 3H; 3′-H; H-5′), 4.41–4.47 (m, 1H; H-4′), 4.60 (d, $^2J_{gem} = 11.8$ Hz, 1H; OCH_2S), 4.69 (d, $^2J_{gem} = 11.8$ Hz, 1H; OCH_2S), 5.75 (d, $^3J_{5-6} = 8.1$ Hz, 1H; H-5), 6.22 (dd, $^3J_{1'-2'a} = 7.0$ Hz, $^3J_{1'-2'b} = 6.2$ Hz, 1H; H-1′), 7.51 (d, $^3J_{5-6} = 8.1$ Hz, 1H; H-6), 9.39 (br s, 1H; NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.71$ (SCH_3), 27.13 ($(CH_3)_3CCO$), 37.77 (C-2′), 38.77 ($(CH_3)_3CCO$), 63.50 (C-5′), 73.82 (OCH_2S), 75.13 (C-3′), 82.23 (C-4′), 85.56 (C-1′), 102.54 (C-5), 139.07 (C-6), 150.22 (C-2), 163.37 (C-4), 177.94 ($(CH_3)_3CCO$); MS (FAB$^+$): *m/z* (%): 745 (27) [$M_2^+ + 1$], 373 (100) [$M^+ + 1$], 261 (30) [M^+ – uracil], 201 (21) [$M^+ + 1$ – uracil-CH_2SCH_3]; $C_{16}H_{25}N_2O_6S$ ([M+H]$^+$) calcd 373.1433; found 373.1426.

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3′-*O*-Benzyl-5′-*O*-trityl-2′-deoxyuridine (10): A solution of 5′-*O*-trityl-2′-deoxyuridine (**9**)[31] (5.0 g, 12.4 mmol) in THF (25 mL) was carefully added to a suspension containing Bu_4NI (50 mg, 0.13 mmol) and NaH (55% in mineral oil, 2.9 g, 62 mmol, 5.2 equiv) in THF (25 mL) at 0 °C. The mixture was stirred under argon for 1 H; then cooled to −20 °C, and benzyl bromide (1.5 mL, 15.4 mmol) was added. After stirring the reaction mixture overnight at room temperature, saturated $NaHCO_3$ solution (1 mL) was carefully added, and the reaction mixture was poured into CH_2Cl_2 (200 mL). The organic phase was separated and washed twice with saturated $NaHCO_3$ solution (50 mL) and once with water (50 mL). The organic phase was dried with $MgSO_4$ and the solvent was evaporated in vacuo. The residual material was dissolved in a minimum of CH_2Cl_2. This solution was diluted with Et_2O, and the product **10** was finally precipitated through dropwise addition of ice cold *n*-hexane. Compound **10** was obtained as a white powder (5.5 g, 9.8 mmol, 80%); R_F: 0.25 (EtOAc/hexane 3:4 (v:v));m.p. 88–90 °C. IR (KBr): $\tilde{\nu}$ = 3200, 3057, 2933, 2867, 1710, 1690 (C=O), 1489, 1448, 1377, 1272, 1194, 1074, 727, 699, 632; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.13–2.26 (m, 1H; H-2′), 2.53–2.63 (m, 1H; H-2′), 3.44–3.46 (m, 2H; H-5′), 4.17–4.22 (m, 1H; H-4′), 4.31–4.38 (m, 1H; H-3′), 4.49 (d, $^2J_{gem}$ = 11.8 Hz, 1H; $PhCH_2$), 4.59 (d, $^2J_{gem}$ = 11.8 Hz, 1H; $PhCH_2$), 5.37 (dd, $^3J_{5-6}$ = 7.9 Hz, $^4J_{3-5}$ = 2.1 Hz, 1H; H-5), 6.33 (t, ^3J = 6.4 Hz, 1H; H-1′), 7.29-7.41 (m, 20H; Tr-H and Ph-H), 7.79 (d, $^3J_{5-6}$ = 7.9 Hz, 1H; H-6), 8.44 (br s, 1H; NH); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 38.50 (C-2′), 63.30 (CH_2Ph), 71.67 (C-5′), 84.18 (C-4′), 85.32 (C-1′), 87.64 (CPh_3), 102.34 (C-5), 127.63 (Ar-CH), 127.88 (Ar-CH), 128.20 (Ar-CH), 128.84 (Ar-CH), 137.47 (Bn-C), 140.23 (Tr-C), 143.37 (C-6), 150.17 (C-2), 163.03 (C-4), (C-3′) covered by $CDCl_3$. MS (FAB$^+$): *m/z* (%): 583 (11)[M^++Na], 561 (23) [M^++1], 243 (100) [Ph_3C^+]; $C_{35}H_{32}N_2O_5$ (560.65): calcd C 74.98, H 5.75, N 5.00, O 14.27; found C 74.72, H 5.72, N 4.99.

3′-*O*-Benzyl-2′-deoxyuridine (11): 3′-*O*-Benzyl-5′-*O*-trityl-2′-deoxyuridine **10** (5.9 g, 10.5 mmol) was dissolved in *n*-butanol (36 mL). Then trifluoroacetic acid (12 mL) was added and the solution was stirred at room temperature for 1 h. A precipitate was collected and additional precipitate was recovered upon further addition of *n*-hexane to the filtrate. The combined precipitates were washed with diethyl ether and dried in vacuo at 45 °C yielding **11** (2.47 g, 7.8 mmol, 74%). R_F: 0.27 (EtOAc); M.p. 185–186 °C (ref. [45]: 186 °C); IR (KBr): $\tilde{\nu}$ = 3503, 3144, 3089, 3038, 2911, 2866, 1700 (C=O), 1684 (C=O), 1466, 1394, 1324, 1274, 1265, 1206, 1178, 1102, 1073, 1046, 860, 763, 704, 560, 531, 442 cm^{-1}; ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 2.03–2.17 (m, 1H; H-2′), 2.25–2.38 (m, 1H; H-2′), 3.52 (d, $^3J_{5'-4'}$ = 3.5 Hz, 2H; H-5′); 4.00–4.06 (m, 1H; H-4′); 4.12–4.18 (m, 1H; H-3′); 5.09 (br s, 1H; OH), 5.64 (dd, $^3J_{5-6}$ = 8.1 Hz, $^4J_{3-5}$ = 2.1 Hz, 1H; H-5); 6.12 (dd, $^3J_{1'-2'a}$ = 5.8 Hz, $^3J_{1'-2'a}$ = 7.9 Hz, 1H; H-1′); 7.27–7.39 (m, 5H; Ar-H), 7.84 (d, $^3J_{5-6}$ = 8.1 Hz, 1H; H-6); 11.32 (br s, 1H; NH); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 36.60 (C-2′); 61.55 (CH_2Ph); 70.09 (C-5′); 78.97 (C-3′), 84.31 (C-4′), 84.88 (C-1′), 101.95 (C-5), 127.60 (Ar-C), 127.66 (Ar-C), 128.39 (Ar-C), 138.27 (Bn-C), 140.52 (C-6), 150.58 (C-2), 163.21 (C-4); MS (FAB$^+$): *m/z* (%): 583 (11) [M^++Na], 319 (89) [M^++1], 207 (79) [M^+ − uracil].

5′A-*O*-Pivaloyl-3′B-benzyl-protected, formacetal-linked, deoxyuridine dimer (12): Compounds **11** (3.45 g, 9.5 mmol) and **8** (2.5 g, 7.8 mmol) were dissolved three times in THF (50 mL) and the solvent was evaporated to dryness to remove trace water. The oil obtained was dissolved in absolute THF (60 mL), and stirred for 1 h with activated molecular sieves (4 Å, 0.8 g) at 0 °C under argon. Then a solution of *N*-iodo succinimide (2.14 g, 9.5 mmol) and trifluoromethanesulfonic acid (50 μL) in absolute THF (28 mL) was added. The mixture was stirred for 5 min at room temperature and diluted with dichloromethane (200 mL). The solution was filtered, diluted with dichloromethane (200 mL), and washed once with 1M sodium thiosulfate solution (50 mL), once with $NaHCO_3$ solution, and finally once with water. The organic phase was dried with $MgSO_4$, filtered and evaporated in vacuo. Recrystallization of the residual material with EtOAc/hexane yielded **12** (3.91 g, 6.1 mmol, 78%)**.** For the elemental analysis **12** was recrystallized from EtOH. M.p. 118–119 °C; IR (KBr): $\tilde{\nu}$ = 3411, 3167, 2975, 2811, 2699, 1622, 1464, 1433, 1384, 1278, 1260, 1161, 1125, 1099, 1066, 894, 834, 702, 633, 556, 533 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.12 (s, 9H; $(CH_3)_3CCO$), 2.16–2.26 (m, 2H; H-A2′a, H-B2′a), 2.31–2.35 (m , 2H; H-A2′b, H-B2′b), 3.65–3.70 (m, 2H; H-B5′), 4.11–4.15 (m, 3H; H-B3′, H-A4′, H-B4′), 4.16–4.20 (m, 2H; H-A5′), 4.26–4.30 (m, 1H; H-A3′), 4.50 and 4.54 (2d, $^2J_{gem}$ = 11.9 Hz, 2H; OCH_2Ph), 4.74 and 4.72 (d, $^2J_{gem}$ = 7.1 Hz, 2H; OCH_2O), 5.63 and 5.64 (2 d, $^3J_{5-6}$ = 8.1 Hz, 2H; H-A5 and H-B5), 6.09 (t, 1H; H-1′), 6.13 (dd, ^3J = 6.0 Hz, ^3J = 8.2 Hz, 1H; H-1′), 7.25–7.35 (m, 5H; Ar-H), 7.58 (d, $^3J_{5-6}$ = 8.1 Hz, 1H; H-6), 7.66 (d, $^3J_{5-6}$ = 8.1 Hz, 1H; H-6), 11.34 (s, 2H; NH); ^{13}C NMR (125.8 MHz, DMSO): δ = 26.79 ($(CH_3)_3CCO$), 36.07 (C-2′), 36.57 (C-2′), 38.19 ($(CH_3)_3CCO$), 63.76 and 68.17 (C-5′), 70.25 (CH_2Ph), 76.78 and 79.02 (C-3′), 81.65 and 82.53 (C-4′), 84.37 and 84.60 (C-1′), 94.36 (OCH_2O), 101.96 and 102.02 (C-5), 127.51, 127.54, 128.24 and 137.96 (Ar-C), 140.19 (2xC-6), 150.30 and 150.38 (C-2), 162.97 and 162.99 (C-4), 177.18 ($(CH_3)_3CCO$). MS (FAB$^+$): *m/z* (%): 1928 (3) [M_3^++1], 1285 (30) [M_2^++1], 643 (100) [M^++1]; $C_{31}H_{38}N_4O_{11}$ (642.66): calcd C 57.94, H 5.96, N 8.72, O 27.39; found C 57.86, H 6.01, N 8.63.

Irradiation of the 5′A-*O*-pivaloyl-, 3′B-benzyl-protected, formacetal-linked, deoxyuridine dimer (12): The formacetal-linked dinucleoside **11** (2.5 g, 3.9 mmol, 1.0 equiv) was irradiated in five portions (0.5 g each) in acetone (350 mL). The compound **11** was dissolved in acetone, the solution was degassed with N_2 and irradiated for 1.5 h under an N_2 atmosphere in a pyrex irradiation apparatus (λ > 300 nm) with a TQ-150 medium-pressure mercury lamp. The five irradiation solutions were combined and the solvent was evaporated in vacuo. The residual oil was subjected to flash chromatography on silica gel-*H* (0.5–10% methanol/dichloromethane). Three products were isolated R_F: **13**: 0.37, **14**: 0.33, **15**: 0.26 ($CHCl_3$/MeOH 12:1 (v:v))

5A-(*R*),5B-(*R*),6A-(*R*),6B-(*R*)-5′A-*O*-Pivaloyl-, 3′B-benzyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (14): Yield: 310 mg (0.47 mmol, 12%); m.p. 150 °C, slow melting; IR (KBr): $\tilde{\nu}$ = 3422, 3222, 3100, 2963, 2867, 1707 (C=O), 1454, 1363, 1269, 1161, 1096, 972, 756, 699 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.14 (s, 9H; $(CH_3)_3CCO$), 2.17–2.23 (ddd, $^2J_{B2'a-B2'b}$ = 13.6 Hz, $^3J_{B1'-B2'a}$ = 8.1 Hz, $^3J_{B3'-B2'a}$ = 5.5 Hz, 1H; H-B2′a), 2.37–2.43 (m, 1H; H-A2′a), 2.60–2.66 (ddd, $^2J_{B2'a-B2'b}$ = 13.6 Hz, $^3J_{B3'-B2'b}$ = 7.4 Hz, $^3J_{B1'-B2'b}$ = 3.7 Hz, 1H; H-B2′a), 2.88–2.93 (ddd, $^2J_{A2'a-A2'b}$ = 13.8 Hz, $^3J_{A2'b-A3'}$ = 7.2 Hz, $^3J_{A1'-A2'b}$ = 5.0 Hz, 1H; H-A2′b), 3.23–3.25 (m, 1H; H-A5), 3.25–3.27 (m, 1H; H-B5), 3.62 (dd, $^2J_{B5'a-B5'b}$ = 11.0 Hz, $^3J_{B4'-B5'a}$ = 7.2 Hz, 1H; H-B5′a), 3.75–3.78 (m, 1H; H-A4′), 3.81–3.87(m, 2H; H-A3′, H-5′b), 4.01 (ddd, $^3J_{B4'-B5'a}$ = 7.2 Hz, $^3J_{B4'-B3'}$ = 7.0 Hz, $^3J_{B4'-B5'a}$ = 1.9 Hz, 1H; H-B4′), 4.05 (dd, $^2J_{A5'a-A5'b}$ = 12.0 Hz, $^3J_{B4'-B5'a}$ = 5.2 Hz, 1H; H-A5′a), 4.16 (dd, $^2J_{A5'a-A5'b}$ = 12.0 Hz, $^3J_{B4'-B5'b}$ = 3.8 Hz, 1H; H-A5′b), 4.22–4.26 (m, 1H; H-A6), 4.30–4.34 (m, 1H; H-B3′), 4.46 (d, $^2J_{gem}$ = 12.1 Hz, 2H; OCH_2Ph) overlap with 4.47 (d, $^2J_{gem}$ = 6.5 Hz, 1H; OCH_2O), 4.50–4.54 (m, 1H; H-B6), 4.95 (d, $^2J_{gem}$ = 6.5 Hz, 1H; OCH_2O), 5.32 (dd, $^3J_{B1'-B2'a}$ = 8.1 Hz, $^3J_{B1'-B2'b}$ = 3.7 Hz, 1H; HB1′), 6.00 (dd, $^3J_{A1'-A2'a}$ = 8.0 Hz, $^3J_{A1'-A2'b}$ = 5.0 Hz, 1H; H-A1′), 7.26–7.36 (m, 5H; Ar-H), 10.5 (s, 1H; NH), 10.7 (s, 1H; NH); ^{13}C NMR (125.8 MHz, [D$_6$]DMSO): δ = 26.86 ($(CH_3)_3CCO$), 36.71 (C-A2′), 37.48 (C-B2′), 38.18 (C-A5), 38.60 (C-B5), 39.76 ($(CH_3)_3CCO$), 53.63 (C-A6), 60.86 (C-B6), 62.99 (C-A5′), 70.23 (C-B5′), 70.73 (CH_2Ph), 77.97 (C-A3′), 79.14 (C-B3′), 79.27 (C-A4′), 82.15 (C-A1′), 84.00 (C-B4′), 91.39 (C-B1′), 97.07 (OCH_2O), 127.47 (Ar-C), 127.58 (Ar-C), 128.20 (Ar-C), 138.20 (Ar-C), 150.63 (C-2), 151.70 (C-2), 169.34 (C-4), 170.08 (C-4), 177.23 ($(CH_3)_3CCO$); MS (FAB$^+$): *m/z* (%): 1285 (9) [M_2^++1], 665 (14) [M^++Na], 643 (100) [M^++1], 537 (81) [$MH_2^+ - C_7H_7O$], 535 (50) [$M - C_7H_7O$]. $C_{31}H_{38}N_4O_{11}$+H_2O (660.68): Calcd C 56.36, H 6.10, N 8.48; found C 56.73, H 6.25, N 8.35.

5A-(*S*),5B-(*S*),6A-(*S*),6B-(*S*)-5′A-*O*-Pivaloyl-, 3′B-benzyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (15): Yield: 360 mg (0.54 mmol, 14%); m.p. 160 °C, slow melting; IR (KBr): $\tilde{\nu}$ = 3411, 3250, 3100, 2971, 1714 (C=O), 1455, 1400, 1361, 1279, 1217, 1160, 1094, 1061, 1028, 753, 694 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.13 (I, 9H; $(CH_3)_3CCO$), 1.93–1.95 (m, 2H; H-B2′), 2.54 (dd, $^2J_{A2'a-A2'b}$ = 13.5 Hz, $^3J_{A1'-A2'a}$ = 5.1 Hz, 1H; H-A2′a), 3.23 (ddd, $^2J_{A2'a-A2'b}$ = 13.5 Hz, $^3J_{A1'-A2'b}$ = 10.2 Hz, $^3J_{A2'b-A3'}$ = 4.7 Hz, 1H; H-A2′b), 3.42 (ddd, $^3J_{B5-B6}$ = 9.9 Hz, $^3J_{A5-B5}$ = 4.4 Hz, $^4J_{A6-B5}$ = 1.2 Hz, 1H; H-B5), 3.53 (ddd, $^3J_{A5-A6}$ = 10.5 Hz, $^3J_{A5-B5}$ = 4.4 Hz, $^4J_{A5-B6}$ = 1.3 Hz, 1H; H-A5), 3.77–3.87 (m, 3H; H-B4′, H-B5′a), 3.93 (dd, $^3J_{A4'-A5'b}$ = 7.4 Hz, $^3J_{A4'-A5'a}$ = 5.2 Hz, 1H; H-A4′), 3.98 (dd, $^2J_{A5'a-A5'b}$ = 11.2 Hz, $^3J_{A4'-A5'a}$ = 5.2 Hz, 1H; H-A5′), 4.11 (dd, $^2J_{A5'a-A5'b}$ = 11.2 Hz,$^3J_{A4'-A5'b}$ = 7.4 Hz, 1H; H-A5′b), 4.22 (d, 1H; $^3J_{A2'b-A3'}$ = 4.7 Hz, H-A3′), 4.26–4.29 (m, 1H; B-3′) overlap with 4.29 (ddd, $^3J_{A5-A6}$ = 10.5 Hz, $^3J_{A6-B6}$ = 6.4 Hz, $^4J_{A6-B5}$ = 1.2 Hz, 1H; H-A6), 4.43 (ddd, $^3J_{B5-B6}$ = 9.9 Hz, $^3J_{A6-B6}$ = 6.4 Hz, $^4J_{A5-B6}$ = 1.3 Hz, 1H; H-B6), 4.51 (d, $^2J_{gem}$ = 11.9 Hz, 2H; OCH_2Ph), 4.70 (d, $^2J_{gem}$ = 8.2 Hz, 1H; OCH_2O), 4.82 (d, $^2J_{gem}$ = 8.2 Hz, 1H; OCH_2O), 5.29 (dd, $^3J_{A1'-A2'b}$ = 10.2 Hz, $^3J_{A1'-A2'a}$ = 5.1 Hz, 1H; H-A1′), 5.61 (t, ^3J = 7.6 Hz, 1H; H-B1′), 7.27–7.37 (m, 5H; Ar-H), 10.45 (s, 1H; NH), 10.71 (s, 1H; NH); ^{13}C NMR (125.8 MHz, [D]$_6$DMSO): δ = 26.78 ($(CH_3)_3CCO$), 31.62 (C-A2′), 33.35 (C-B2′), 37.81 (C-A5), 38.16 (C-B5), 39.76 ($(CH_3)_3CCO$), 53.71 (C-B6), 60.38 (C-A6), 63.52 (C-A5′), 66.73 (C-

B5′), 70.53 (*C*H$_2$Ph), 75.21 (C-B3′), 81.31 (C-B4′), 81.36 (C-A4′), 82.36 (C-A3′), 83.20 (C-B1′), 90.01 (C-A1′), 94.76 (OCH$_2$O), 127.49 (Ar-C), 127.53 (Ar-C), 128.24 (Ar-C), 138.13 (Ar-C), 150.62 (C-2), 152.23 (C-2), 168.38 (C-4), 169.25 (C-4), 177.16 ((CH$_3$)$_3$*C*CO); MS (FAB$^+$): *m/z* (%): 1285 (7) [M_2^++1], 665 (70) [M^++Na], 643 (100) [M^++1], 641 (88) [M^+-1], 535 (43) [M^+-C$_7$H$_7$O]. C$_{31}$H$_{38}$N$_4$O$_{11}$ + H$_2$O (660.68): calcd C 56.34, H 6.11, N 8.48; found C 56.57, H 6.07, N 8.57.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*)-5′A-*O*-Pivaloyl-, 3′B-benzyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (**13**): Yield: 1.12 g (1.72 mmol, 44%). For the elemental analysis **13** was recrystallized from EtOH; m.p. 122 °C (decomp). IR (KBr): $\tilde{\nu}$ = 3444, 3238, 3089, 2972, 1712 (C=O), 1455, 1372, 1274, 1166, 1100, 1065, 1028, 900, 756, 700 cm^{-1}; ^{1}H NMR: (500 MHz, [D$_6$]DMSO): δ = 1.14 (s, 9H; (CH$_3$)$_3$CCO), 1.98–2.12 (m, 2H; H-B2′), 2.40–2.51 (m, 2H; H-A2′), 3.60–3.70 (m, 3H; H-A5, H-B5, H-B5′a), 3.80–3.90 (m, 3H; H-B5′b, H-A4′, H-B4′), 4.00–4.15 (m, 4H; H-A3′, H-B3′, H-A5′), 4.40–4.45 (m, 2H; H-A6, H-B6), 4.49 and 4.53 (2d, $^2J_{gem}$ = 11.9 Hz, 2H; OCH$_2$Ph), 4.70 and 4.75 (2d, $^2J_{gem}$ = 7.3 Hz, 2H; OCH$_2$O), 5.77–5.82 (m, 2H; H-A1′, H-B1′), 7.27-7.37 (m, 5H; Ar-H), 10.50 (s, 1H; H-B3 (NH)), 10.53 (s, 1H; H-A3 (NH)); ^{13}C NMR (125.8 MHz, DMSO): δ = 26.78 ((*C*H$_3$)$_3$CCO), 33.45 (C-B2′), 34.10 (C-A2′), 38.13 ((CH$_3$)$_3$*C*CO), 38.72 (C-B5), 40.63 (C-A5), 51.33 (C-B6), 53.17 (C-A6), 63.55 (C-A5′), 65.12 (C-B5′), 70.38 (CH$_2$Ph), 76.14 (C-B3′), 77.87 (C-A3′), 78.95 (C-A4′), 80.97 (C-B4′), 84.77 (C-A1′), 85.61 (C-B1′), 93.85 (OCH$_2$O), 127.45 (Ar-C), 127.63 (Ar-C), 128.20 (Ar-C), 138.00 (Ar-C), 152.74 (C-2), 153.38 (C-2), 166.95 (C-4), 167.26 (C-4), 177.15 ((CH$_3$)$_3$*C*CO); MS (FAB$^+$): *m/z* (%): 1285 (7) [M_2^++1], 665 (23) [M^++Na], 643 (100) [M^++1], 535 (70) [M^+-C$_7$H$_7$O]; C$_{31}$H$_{38}$N$_4$O$_{11}$ + 0.5 H$_2$O (651.68): calcd C 57.14, H 6.03, N 8.60; found C 57.09, H 6.08, N 8.58.

Hydrolysis of 13, 14, and 15: 50 mg of each of the three compounds **13–15** were dissolved in 4 N hydrochloride acid (10 mL) and heated for 30 min at 100 °C. The solvent was evaporated in vacuo and the residual material was suspended in methanol. The insoluble material was collected by filtration, washed with methanol and dried at room temperature in vacuo. Approximately 10 mg of **16** and **17** were obtained. **16**: ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.48 (2d , ^3J = 3.7 Hz, ^3J = 3.3 Hz, 2H; H-5), 3.83–3.90 (m, 2H; H-6), 7.57 (s, 2H; NH), 10.01 (s, 2H; NH); ^{1}H NMR (200 MHz, [D$_6$]DMSO + D$_2$O): δ = 3.51 (2d , $^3J_{5,6}$ = 3.3 Hz, $^3J_{5,6}$ = 3.7 Hz, 2H; H-5), 3.92 (2d , $^3J_{5,6}$ = 3.3 Hz, $^3J_{5,6}$ = 3.7 Hz, 2H; H-6); ^{13}C NMR (50.3 MHz, [D$_6$]DMSO): δ = 35.29 (C-5); 51.77 (C-6), 150.48 (C-2), 168.83 (C-4).

17: ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 3.22 (d, ^3J = 8.3 Hz, 2H; H-5), 3.82–3.89 (m, 2H; H-6), 8.02 (d, ^3J = 2.1 Hz, 2H; NH), 10.23 (s, 2H; NH); ^{1}H NMR (200 MHz, [D$_6$]DMSO + D$_2$O): δ = 3.22 (d, ^3J = 8.3 Hz, 2H; H-5), H-6 covered by HOD; ^{13}C NMR (50.3 MHz, [D$_6$]DMSO): δ = 35.29 (C-5), 51.77 (C-6), 150.48 (C-2), 168.80 (C-4).

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*) 3′B-Benzyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (**19**): Compound **13** (355 mg, 0.55 mmol) and Bu$_4$NBr (16 mg, 0.05 mmol) were dissolved in THF (9 mL) and 1 N NaOH (3 mL). After the mixture had been stirred vigorously for 3 h the solvent was removed in vacuo and the residual material was subjected to flash chromatography on silica gel (CHCl$_3$/MeOH 10:1). Compound **19** was obtained as a white foam (280 mg, 0.5 mmol, 91%). M.p. > 174 °C (decomp). IR (KBr): $\tilde{\nu}$ = 3443, 3222, 3078, 2933, 2878, 1739 (C=O), 1702 (C=O), 1455, 1372, 1274, 1189, 1094, 1024, 756, 733, 700 cm^{-1}. ^{1}H NMR: (500 MHz, [D$_6$]DMSO): δ = 1.96–2.10 (m, 2H; H-B2′), 2.39–2.41 (t, 2H; H-A2′), 3.40–3.46 (m, 2H; H-A5′), 3.61–3.65 (m, 4H; H-A5, H-B4′, H-B5, H-B5′a), 3.82–3.83 (m, 1H; H B-4′), 3.89 (d, $^2J_{gem}$ = 10.1 Hz, 1H; H-B5′b), 4.11–4.16 (m, 2H; H-A3′, H-B3′), 4.40–4.54 (m, 4H; OCH$_2$PH; H-A6, H-B6), 4.69 and 4.74 (2d, $^2J_{gem}$ = 8.0 Hz, 2H; OCH$_2$O), 4.81–4.83 (t, ^3J = 5.9 Hz, 1H; H-OA5′), 5.76–5.79 and 5.80–5.83 (m, 2H; H-A1′, H-B1′), 7.27–7.37 (m, 5H; Ar-H), 10.43 (s, 1H; H-B3 (NH)), 10.50 (s, 1H; H-A3 (NH)); ^{13}C NMR (125.8 MHz, DMSO): δ = 33.46 (C-2′), 34.22 (C-2′), 39.11 (C5, under DMSO), 40.47 (C-5), 51.31 (C-6), 52.89 (C-6), 60.75 (C-5′), 65.00 (C-5′), 70.42 (CH$_2$Ph), 76.04 (C-3′), 77.29 (C-3′), 80.95 (C-4′), 82.54 (C-4′), 84.51 (C-1′), 85.49 (C-1′), 93.74 (OCH$_2$O), 127.44 (Ar-C), 128.18 (Ar-C), 138.04 (Ar-C), 152.98 (C-2), 153.39 (C-2), 167.16 (C-4), 167.28 (C-4); MS (FAB$^+$): *m/z* (%): 1139 (17) [M_2^++Na], 581 (100) [M^++Na], 559 (44) [M^++1], 431 (14) [M^+-C$_7$H$_{13}$O$_4$+Na], C$_{26}$H$_{31}$N$_4$O$_{10}$ ([M+H]$^+$): calcd 559.2040; found 559.2050.

5′A-*O*-Pivaloyl-, 3′B-acetyl-protected, formacetal-linked, deoxyuridine dimer (**22**)**:** Compounds **8** (9.2 g, 24.7 mmol) and **21** (5.1 g, 19.0 mmol) were dissolved in absolute THF (200 mL) three times and the solvent was removed in vacuo in order to remove trace water. The oil obtained was dissolved in absolute THF (200 mL), and the mixture was stirred for 1 h under argon over activated molecular sieves (4 Å, 1 g). The reaction mixture was then cooled to 0 °C and a mixture *N*-iodo succinimide (5.6 g, 25 mmol), dissolved in absolute THF (100 mL), previously stirred for 1 h over activated molecular sieves (4 Å, 0.4 g), and trifluoromethanesulfonic acid (335 μL) was added. The stirring of the mixture was continued for an additional 5 min, then the mixture was diluted with CH$_2$Cl$_2$ (500 mL). The organic phase was separated and washed once with 1 M sodium thiosulfate solution (150 mL), once with saturated NaHCO$_3$ solution (150 mL) and once with water (150 mL). The organic phase was dried with MgSO$_4$ and the solvent was removed in vacuo. The residual material was recrystallized from acetone/methyl *tert*-butyl ether (1:1) to give **22** as a colorless powder (7.1 g, 12 mmol, 63%). For the elemental analysis **22** was recrystallized from acetone/cyclohexane. R_F: 0.38 (acetone/CHCl$_3$ 1:1 (v:v)); m.p. 149 °C; IR (KBr): $\tilde{\nu}$ = 3430, 3169, 3100, 2977, 2809, 1746 (C=O), 1729 (C=O), 1704 (C=O), 1618, 1470, 1436, 1397, 1380, 1284, 1263, 1162, 1124, 1098, 1050, 1004, 954, 890, 836, 766, 640, 558, 640, 558, 446 cm^{-1}. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 1.10 (s, 9H; (CH$_3$)$_3$CCO), 2.03 (s, 3H; CH$_3$CO), 2.22–2.31 (m, 4H; H-A2′, H-B2′), 3.70 (d, ^3J = 3.5 Hz, 2H; H-B5′), 4.05–4.20 (m, 4H; H-A4′, H-B4′, H-A5′), 4.22–4.30 (m, 1H; H-B3′), 4.72–4.78 (2d, $^2J_{gem}$ = 7.3 Hz, 2H; OCH$_2$O), 5.10–5.18 (m, 1H; H-A3′), 5.63 (t, 2H; H-A5, H-B5), 6.05–6.15 (m, 2H; H-1′), 7.57 (d, ^3J = 8.1 Hz, 1H; H-6), 7.65 (d, ^3J = 8.1 Hz, 1H; H-6), 11.35 (s, 2H; NH); ^{13}C NMR (125.8 MHz, DMSO): δ = 20.68 (CO*C*H3), 26.73 ((*C*H$_3$)$_3$CCO), 36.00 (C-2′), 36.50 (C-2′), 38.13 (CO*C*(CH$_3$)$_3$), 63.72 (A-5′), 67.77 (B-5′), 74.23 (B-3′), 76.65 (A-3′), 81.64 and 82.36 (C-4′), 84.12 and (C-1′), 94.23 (OCH$_2$O), 101.89 and 102.11 (C-5), 140.03 and 140.14 (C-6), 150.22 and 150.31 (C-2), 162.87 and 162.90 (C-4), 169.87 (*C*OCH$_3$), 177.11 (CH$_3$)$_3$*C*CO). MS (FAB$^+$): *m/z* (%): 1189.2 (35) [M_2^++1], 595 (100) [M^++1]; C$_{26}$H$_{34}$N$_4$O$_{12}$ (594.58): calcd. C 52.52, H 5.76, N 9.42; found: C 52.47, H 5.97, N 9.45.

Irradiation of the 5′A-*O*-Pivaloyl-, 3′B-acetyl-protected, formacetal-linked, deoxyuridine dimer (**22**)**:** Compound **22** (1.2 g, 2 mmol) was dissolved in acetone (350 mL) and N$_2$ was bubbled through the solution for 15 min. The solution was irradiated under N$_2$ for 3 h in a pyrex irradiation apparatus (λ > 300 nm) with a TQ-150 medium-pressure mercury lamp. The solvent was evaporated in vacuo, and the products were separated by flash chromatography on silica gel-*H* (CH$_2$Cl$_2$/acetone 4:1). Three main products were obtained: R_F: **23**: 0.53, **24**: 0.47, **25**: 0.28 (acetone/CHCl$_3$ 1:1 (v:v)). **5A-(*R*),5B-(*R*),6A-(*R*),6B-(*R*)-5′A-*O*-Pivaloyl-, 3′B-acetyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer** (**24**): Yield: 160 mg (0.26 mmol, 13%). M.p. 165 °C–178 °C, slow melting; IR (KBr): $\tilde{\nu}$ = 3511, 3244, 3100, 2967, 2878, 1706 (C=O), 1478, 1450, 1367, 1322, 1272, 1239, 1161, 1100, 1028, 983, 761 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.14 (s, 9H; (CH$_3$)$_3$CCO), 2.01 (s, 3H; CH$_3$CO), 2.21–2.27 (ddd, $^2J_{B2'a-B2'b}$ = 14.0 Hz, $^3J_{B1'-B2'a}$ = 8.1 Hz, $^3J_{B2'a-B3'}$ = 5.6 Hz, 1H; H-B2′a), 2.34–2.40 (ddd, $^2J_{A2'a-A2'b}$ = 13.9 Hz, $^3J_{A1'-A2'a}$ = 8.1 Hz, $^3J_{A2'a-A3'}$ = 7.5 Hz, 1H; H-A2′a), 2.70–2.76 (ddd, $^2J_{B2'a-B2'b}$ = 14.0 Hz, $^3J_{B2'b-B3'}$ = 7.5 Hz, $^3J_{B1'-B2'b}$ = 3.5 Hz, 1H; H-B2′b), 2.91–2.96 (ddd, $^2J_{A2'a-A2'b}$ = 13.9 Hz, $^3J_{A2'b-A3'}$ = 7.3 Hz, $^3J_{A1'-A2'b}$ = 4.8 Hz, 1H; H-A2′b), 3.24–3.27 (m, 2H; H-A5, H-B5), 3.67 (dd, $^2J_{B5'a-B5'b}$ = 10.7 Hz, $^3J_{B4'-B5'a}$ = 7.5 Hz, 1H; H-B5′a), 3.74–3.77 (m, 1H; H-A4′), 3.79–3.83 (m, 1H; H-A3′), 3.92 (dd, $^2J_{B5'a-B5'b}$ = 10.7 Hz, $^3J_{B4'-B5'b}$ = 1.8 Hz, 1H; H-B5′b), 4.03 (ddd, $^3J_{B4'-B5'a}$ = 7.5 Hz, $^3J_{B3'-B4'}$ = 4.6 Hz, $^3J_{B4'-B5'b}$ = 1.8 Hz, 1H; H-B4′), 4.06 (dd, $^2J_{A5'a-A5'b}$ = 12.0 Hz, $^3J_{A4'-A5'a}$ = 5.1 Hz, 1H; H-A5′), 4.17 (dd, $^2J_{A5'a-A5'b}$ = 12.0 Hz, $^3J_{A4'-A5'b}$ = 3.5 Hz, 1H; H-A5′b), 4.21–4.25 (m, 1H; H-A6), 4.47 (d, $^2J_{a-b}$ = 6.6 Hz, 1H; OCH$_2$O), 4.53–4.57 (m, 1H; H-B6), 4.97 (d, $^2J_{gem}$ = 6.6 Hz, 1H; OCH$_2$O), 5.26–5.31 (m, 1H; H-A3′), 5.36 (dd, $^3J_{B1'-B2'a}$ = 8.1 Hz, $^3J_{B1'-B2'b}$ = 3.5 Hz, 1H; H-B1′), 6.03 (dd, $^3J_{A1'-A2'a}$ = 8.1 Hz, $^3J_{A1'-A2'b}$ = 4.8 Hz, 1H; H-A1′), 10.58 (s, 1H; NH), 10.69 (s, 1H; NH); ^{13}C NMR (125.8 MHz, [D]$_6$DMSO): δ = 20.73 (*C*H$_3$CO), 26.87 ((*C*H$_3$)$_3$CCO), 36.75 (C-A2′), 37.08 (C-B2′), 38.18 ((CH$_3$)$_3$*C*CO), 38.56 (C-5), covered by DMSO (C-5), 53.62 (C-A6), 60.88 (C-B6), 62.93 (C-A5′), 70.08 (C-B5′), 74.79 (C-B3′), 77.85 (C-A3′), 79.03 (C-A4′), 81.94 (C-A1′), 83.81 (C-B4′), 91.28 (C-B1′), 97.12 (OCH$_2$O), 150.89 (C-2), 151.69 (C-2), 169.33 (C-4), 170.09 (C-4), 177.16 (CH$_3$CO), 177.25 ((CH$_3$)$_3$*C*CO). MS (FAB$^+$): *m/z* (%): 1783 (2) [M_3^++1], 1189 (16) [M_2^++1], 595 (100) [M^++1], 535 (39) [M^+-OCOCH$_3$]; C$_{26}$H$_{34}$N$_4$O$_{12}$ · H$_2$0 (614.74): calcd C 50.98, H 5.92, N 9.15, O 33.95; found C 50.91, H 5.98, N 9.19. **5A-(*S*),5B-(*S*),6A-(*S*),6B-(*S*)-5′A-*O*-Pivaloyl-, 3′B-acetyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer** (**25**): Yield: 170 mg (0.28 mmol, 14%); m.p. 183 °C, slow melting; IR (KBr): $\tilde{\nu}$ = 3346, 3100, 2971, 1712 (C=O), 1450, 1405, 1367, 1279, 1249, 1216, 1171, 1103, 1047, 755,

0947-6539/98/0404-0650 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 4

699, 593, 522, 412 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.13$ (s, 9H; (CH_3)$_3$CCO), 1.84–1.89 (dd, $^2J_{B2'a-B2'b} =$ 13.2 Hz, $^3J_{B1'-B2'a} = 4.0$ Hz, 1H; H-B2′a), 2.04 (s, 3H; CH_3CO), 2.09–2.16 (m, 1H; H-B2′b), 2.56 (dd, $^2J_{A2'a-A2'b} = 13.5$ Hz, $^3J_{A1'-A2'a} = 5.0$ Hz, 1H; H-A2′a), 3.24–3.31 (m, 1H; H-A2′b), 3.42 (dd, $^3J_{B5-B6} = 9.8$ Hz, $^3J_{A5-B5} = 4.4$ Hz, 1H; H-B5), 3.54 (dd, $^3J_{A5-A6} = 10.5$ Hz, $^3J_{A5-B5} = 4.4$ Hz, 1H; H-A5), 3.80–3.84 (m, 3H; H-B4′, H-B5′a, H-B5′b), 3.93 (dd, $^3J_{A4'-A5'b} = 7.4$ Hz, $^3J_{A4'-A5'a} = 5.3$ Hz, 1H; H-A4′), 3.99 (dd, $^2J_{A5'a-A5'b} = 11.2$ Hz, $^3J_{A4'-A5'a} =$ 5.3 Hz, 1H; H-A5′), 4.12 (dd, $^2J_{A5'a-A5'b} = 11.2$ Hz, $^3J_{A4'-A5'b} = 7.4$ Hz, 1H; H-A5′b), 4.24 (d, 1H; $^3J_{A2'b-A3'} =$ 4.6 Hz, H-A3′), 4.29 (dd, $^3J_{A5-A6} = 10.5$ Hz, $^3J_{A6-B6} = 6.5$ Hz, 1H; H-A6), 4.47 (dd, $^3J_{B5-B6} = 9.8$ Hz, $^3J_{A6-B6} = 6.5$ Hz, 1H; H-B6), 4.71 (d, $^2J_{gem} = 8.2$ Hz, 1H; OCH_2O), 4.85 (d, $^2J_{gem} = 8.2$ Hz, 1H; OCH_2O), 5.19 (m, 1H; H-A3′), 5.28 (dd, $^3J_{A1'-A2'b} = 10.0$ Hz, $^3J_{A1'-A2'a} = 5.0$ Hz, 1H; H-A1′), 5.58 (dd, $^3J_{B1'-B2'b} =$ 9.1 Hz, $^3J_{B1'-B2'a} = 4.0$ Hz, 1H; H-B1′). 10.46 (s, 1H; NH), 10.74 (s, 1H; NH); ^{13}C NMR (125.8 MHz, [D]$_6$DMSO): $\delta = 20.73$ (CH_3CO), 26.78 ((CH_3)$_3$CCO), 31.56 (C-B2′), 33.68 (C-A2′), 37.78 (C-A5), 38.00 (C-B5), 38.16 ((CH$_3$)$_3$C CO), 53.66 (C-B6), 60.35 (C-A6), 63.50 (C-A5′), 66.31 (C-B5′), 70.53 (C-B3′), 80.40 (C-B4′), 81.36 (C-A4′), 82.33 (C-A3′), 83.06 (C-B1′), 89.94 (C-A1′), 94.62 (OCH_2O), 150.65 (C-2), 152.27 (C-2), 168.32 (C-4), 169.26 (C-4), 170.21 (CH$_3$CO), 177.16 ((CH$_3$)$_3$CCO); MS (FAB$^+$): *m/z* (%): 1784 (3) [M_3^++1], 1189 (14) [M_2^++1], 617 (42) [M^++Na], 595 (86) [M^++1], 383 (100) [$M^+ - C_{11}H_{16}O_4$]; $C_{26}H_{34}N_4O_{12} \cdot H_20$ (614.74): calcd C 50.98, H 5.92, N 9.15, found: C 50.82, H 5.92, N 8.95.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*)-5′A-*O*-Pivaloyl-, 3′B-acetyl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (**23**): Yield: 410 mg (41%). For the elemental analysis **23** was recrystallized from EtOH. M.p. >210 °C (decomp); IR (KBr): $\tilde{\nu} = 3400$, 3214, 3085, 2956, 2877, 1720 (C=O), 1678 (C=O), 1449, 1384, 1277, 1250, 1164, 1086, 1050, 994, 950, 930, 896, 880, 766, 711, 591, 529, 426 cm^{-1}. ^{1}H NMR: (500 MHz, [D$_6$]DMSO): $\delta = 1.14$ (s, 9H; (CH_3)$_3$CCO), 1.89–1.94 (ddd, $^2J_{B2'a-B2'b} = 13.3$ Hz, $^3J = 4.7$ Hz, $^3J =$ 1.5 Hz, 1H; H-B2′a), 2.04 (s, 3H; COCH_3); 2.25–2.29 (m, 1H; H-B2′b), 2.40–2.48 (m, 2H; H-A2′), 3.60–3.68 (m, 3H; H-A5, H-B5, H-B5′a), 3.82–3.89 (m, 3H; H-B5′b, H-A4′, H-B4′), 4.02–4.12 (m, 3H; H-A3′, H-A5′a, H-A5′b), 4.42–4.48 (m, 2H; H-A6, H-B6), 4.71 (d, $^2J_{gem} = 7.4$ Hz, 1H; OCH_2O a), 4.78 (d, $^2J_{gem} = 7.4$ Hz, 1H; OCH_2O). 5.09–5.12 (m, 1H; H-B3′), 5.75–5.80 (m, 2H; H-A1′, H-B1′), 10.51 (s, 1H; H-B3 (NH)), 10.53 (s, 1H; H-A3 (NH)); ^{13}C NMR (125.8 MHz, [D]$_6$DMSO): $\delta = 20.67$ (COCH_3), 26.78 ((CH_3)$_3$CCO), 33.49 (C-B2′), 34.18 (C-A2′), 38.14 ((CH$_3$)$_3$CCO), 38.75 (C-B5), 40.58 (C-A5), 51.42 (C-B6), 53.09 (C-A6), 63.55 (C-A5′), 64.69 (C-B5′), 71.54 (C-B3′), 77.99 (C-A3′), 78.94 (C-A4′), 80.39 (C-B4′), 84.71 (C-A1′), 85.48 (C-B1′), 93.85 (OCH_2O), 152.73 (C-2), 153.48 (C-2), 166.95 (C-4), 167.22 (C-4), 170.08 (COCH_3), 177.15 ((CH$_3$)$_3$CCO); MS (FAB$^+$): *m/z* (%): 1784 (6) [M_3^++1], 1189 (16) [M_2^++1], 595 (100) [M^++1], 535 (39) [$M^+ -$ OCOCH_3]; $C_{26}H_{34}N_4O_{12}$ (594.58): calcd C 52.52, H 5.76, N 9.42; found: C 52.38, H 5.73, N 9.27.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*) Formacetal-linked, cyclobutane deoxyuridine dimer (**2**): Method A: Compound **23** (0.65 g, 1.09 mmol) was stirred with aqueous NH$_3$ solution (25%, 30 mL) for 3 h at 80 °C. The solvent was removed in vacuo. The residual material was taken up in aqueous AcOH (10%) and was extracted twice with CH$_2$Cl$_2$. The aqueous phase was evaporated to dryness and the residue was dissolved in water. Addition of ethanol caused crystallization of the product **2** (300 mg, 0.62 mmol, 57%) as a colorless solid. Method B: Compound **23** (570 mg, 0.96 mmol) was dissolved in THF (10 mL), ethanol (8 mL), and H$_2$O (2 mL). Then 1N KOH solution. (10 mL) was added. The reaction mixture was stirred for 15 min. After neutralization with NH$_4$Cl (600 mg, 11 mmol) the solvent was removed in vacuo. The residue was recrystallized from water (15 mL) to give the monhydrate of **2** (261 mg, 53 mmol, 56%); m.p. >230 °C (decomp); IR (KBr): $\tilde{\nu} = 3459$, 3259, 3061, 2954, 2875, 1708 (C=O), 1678 (C=O), 1479, 1446, 1410, 1372, 1281, 1256, 1192, 1134, 1092, 1070, 1018, 978, 948, 882, 854, 788, 774, 756, 636, 474, 420 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 1.74–1.79$ (m, 1H; H-B2′a), 2.03–2.09 (m, 1H; H-B2′b), 2.38–2.40 (t, $^3J_{A1'-A2'} = 5.9$ Hz, 2H; H-A2′), 3.38–3.47 (m, 2H; H-A5′), 3.56–3.58 (m, 1H; H-B4′), 3.59–3.64 (m, 4H; H-A4′, H-B5′a, H-A5, HB5), 3.87 (d, $^2J_{B5'a-B5'b} = 9.9$ Hz, 1H; H-B5′b), 4.13–4.17 (m, 2H; H-A3′, H-B3′), 4.34–4.39 (m, 1H; H-A6 or H-B6), 4.45–4.49 (m, 1H; H-A6 or H-B6), 4.70 (d, $^2J_{gem} = 7.4$ Hz, 1H; OCH_2O), 4.74 (d, $^2J_{gem} = 7.4$ Hz, 1H; OCH_2O), 4.82 (t, $^3J_{A5'-A5'-OH} = 5.4$ Hz, 1H; *H*-A5′OH), 5.21 (d, $^3J_{B3'-B3'-OH} = 4.8$ Hz, 1H; *H*-B3′OH), 5.77 (t, $^3J_{A1'-A2'} = 4.8$ Hz, 1H; H-A1′), 5.85 (dd, $^3J_{B1'-B2'b} = 8.4$ Hz, $^3J_{B1'-B2'a} = 6.0$ Hz, 1H; H-B1′), 10.43 (s, 2H; NH); ^{13}C NMR (125.8 MHz, [D]$_6$DMSO): $\delta = 34.13$ (CA2′), 36.72 (C-B2′), 38.93, 40.63 (C-A5, C-B5), 51.15, 53.15 (C-A6, C-B6), 60.85 (C-A5′), 64.98 (C-B5′), 67.80 (C-B3′), 77.26 (C-A3′), 82.72 (C-A4′), 83.10 (C-B4′), 84.73 (C-A1′), 85.09 (C-B1′), 93.84 (OCH_2O), 153.08, 153.27 (C-A2, C-B2), 167.23, 167.44 (C-A4, C-B4). Laser-desorption mass spectra (positive ions detected): *m/z* (%): 491.4 (100) [M^++Na]; $C_{19}H_{24}N_4O_{10} \cdot H_2O$ (486.43): calcd C 46.91, H 5.39, N 11.52; found: C 46.68, H 5.19, N 11.38.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*) 5′-*O*-Dimethoxytrityl-protected, formacetal-linked, cyclobutane deoxyuridine dimer (**26**): Compound **2** (150 mg, 0.32 mmol) was dissolved under argon in absolute pyridine (3 mL). Then DIEA (0.1 mL, 0.58 mmol) was added. DMTr-Cl (400 mg, 1.1 mmol) was added in portions over 15 h under argon. The pyridine was evaporated in vacuo and the remaining oil was dissolved in toluene and the solvent evaporated again. The product was subjected to flash chromatography on silica gel (2–10% methanol/CH$_2$Cl$_2$ with 1% NEt$_3$). The obtained oil was dissolved in a minimum of CH$_2$Cl$_2$ and added dropwise into ice-cold *n*-hexane. Compound **26** precipitated as an white powder, which contained traces of *n*-hexane and DIEA (207 mg, 0.26 mmol, 82%). R_F: 0.20 (CHCl$_3$/MeOH 1:1 (v:v)). M.p. 154–156 °C (decomp); IR(KBr): $\tilde{\nu} = 3434$, 3244, 3078, 2933, 1707 (C=O), 1605, 1509, 1467, 1447, 1378, 1300 , 1254, 1178, 1094, 1067, 1034, 828, 583 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]acetone): $\delta = 2.09$ (ddd, $^2J_{A2'a-A2'b} = 13.3$ Hz, $^3J_{A1'-A2'a} = 6.6$ Hz, $^3J_{A2'a-A3'} = 4.7$ Hz, 1H; H-A2′a), 2.30–2.41 (m, 2H; H-A2′b, H-B2′a), 2.94 (ddd, $^2J_{B2'a-B2'b} = 13.35$ Hz, $^3J_{B1'-B2'b} = 6.5$ Hz, $^3J_{B2'b-B3'} = 4.2$ Hz, 1H;H;B2′b), 3.17 (t, $^3J = 8.8$ Hz, 1H; H-A5 or H-B5), 3.20 (dd, $^2J_{B5'a-B5'b} = 10.5$ Hz, $^3J_{B4'-B5'a} = 3.6$ Hz, 1H; H-B5′a), 3.41 (dd, $^2J_{B5'a-B5'b} = 10.5$ Hz, $^3J_{B4'-B5'b} = 3.3$ Hz, 1H; H-B5′b), 3.64 (ddd, $^3J_{(A5-A6 \text{ or } B5-B6)} = 9.2$ Hz, $^3J_{A5-B5} = 6.5$ Hz, $^4J_{(A5-B6 \text{ or } B5-A6)} = 2.0$ Hz, 1H; H-A5 or H-B5), 3.70–3.72 (m, 1H; H-A4′), 3.76–3.80 (m, 9H; OH-B3′, H-B4′, H-A5′a, OCH$_3$), 4.00 (dd, $^2J_{A5'a-A5'b} = 11.4$ Hz, $^3J_{A4'-A5'b} = 1.8$ Hz, 1H; H-A5′b), 4.48–4.52 (m, 1H; H-A3′), 4.57–4.64 (m, 2H; HB3′, H-A6 or H-B6), 4.65 (d, $^2J_{gem} = 7.0$ Hz, 1H; OCH_2O), 4.67 (d, $^2J_{gem} = 7.0$ Hz, 1H; OCH_2O), 4.97 (ddd, $^3J_{(A5-A6 \text{ or } B5-B6)} = 8.8$ Hz, $^3J_{A6-B6} = 5.9$ Hz, $^4J_{(A5-B6 \text{ or } B5-A6)} = 2.0$ Hz, 1H; H-A6 or H-B6), 6.08 (dd, $^3J_{A1'-A2'b} = 7.4$ Hz, $^3J_{A1'-A2'a} =$ 6.6 Hz, 1H; H-A1′), 6.18 (dd, $^3J_{B1'-B2'a} = 7.4$ Hz, $^3J_{B1'-B2'b} = 4.1$ Hz, 1H; H-B1′), 6.89 (m, 4H; H-3DMTr), 7.25–7.35 (m, 7H; H2-DMTr, H2′-DMTr, H4′-DMTr), 7.46 (2d, $^3J = 8.4$ Hz, 2H; H3′-DMTr), 9.35 (s, 2H; H-A3, H-B3 (NH)); ^{13}C NMR (125.8 MHz, [D]$_6$acetone): $\delta = 35.89$ (C-B2′), 38.69 (C-A2′), 39.36, 43.77 (C-A5, C-B5), 51.99, 54.98 (C-A6, C-B6), 55.57 (OCH$_3$), 63.64 (C-B5′), 65.87 (C-A5′), 69.56, (C-B3′), 75.69 (C-A3′), 82.21 (C-A4′), 84.29 (C-B4′), 85.81 (C-A1′), 86.38 (C-B1′), 87.09 (C-DMTr), 94.97 (OCH_2O), 113.96 (C-3DMTr), 127.90 (C-4′DMTr), 128.68 (C-2′DMTr), 129.17 (C-3′DMTr), 131.02, 131.04 (C-2DMTr), 136.59, 136.64 (C1-DMTr), 146.01 (C1′-DMTr), 154.13, 154.38 (C-A2, C-B2), 159.81 (C-4DMTr), 166.71, 168.28 (C-A4, CB4); MS (FAB$^+$): *m/z* (%): 793 (10.6) [*M*+Na], 771 (13.9) [M^++1], 770 (17.4) [M^+], 303 (100) [DMTr$^+$]; $C_{40}H_{42}N_4O_{12} \cdot H_2O$ (788.82): calcd C 60.89, H 5.63, N 7.11; found: C 60.91, H 5.62, N 7.10.

5A-(*R*),5B-(*S*),6A-(*R*),6B-(*S*) 5′-*O*-Dimethoxytrityl-protected formacetal-linked, cyclobutane deoxyuridine dimer (2-cyanoethyl-*N,N*-diisoproyl)-phosphoramidite (**18**): Under argon, **26** (150 mg, 0.195 mmol) was dissolved in absolute THF (5 mL), and DIEA (67 µL, 0.39 mmol) was added. After the addition of 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (58 µL, 0.24 mmol), the reaction mixture was stirred for 4 h under argon. The solvent was evaporated in vacuo at 40 °C and the residual material was subjected to flash chromatography on silica gel (*n*-hexane/ethyl acetate 1:2, 1% triethylamine). The product fractions were collected, evaporated to dryness, and dissolved in a minimum of CH$_2$Cl$_2$. This solution was added dropwise into ice-cold *n*-hexane. The product **18** precipitated and was collected. Compound **18** was obtained as a colorless powder (95 mg, 0.098 mmol, 50%); R_F: 0.36 + 0.45 (EtOAc/Hex./NEt$_3$ 5:1:0.12 (v:v)); IR (KBr): $\tilde{\nu} = 3400$, 3233, 3067, 2966, 1714 (C=O), 1608, 1509, 1447, 1365, 1300, 1254, 1179, 1036, 980, 889, 830, 789, 756, 700, 585, 528 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 1.18–1.21$ (m, 12H; N[CH(CH_3)$_2$]$_2$); 2.12–2.43 (m, 3H; H-A2′, H-B2′a); 2.63 and 2.67 (2 t, $^3J = 6.2$ Hz, 2H; OCH$_2$CH_2CN); 2.75–2.88 (m, 1H; H-B2′b), 3.05–3.09 (m, 1H; H-A5), 3.23–3.43 (m, 2H; H-B5, H-A5′a), 3.53–3.65 (m, 3H; N[CH(CH$_3$)$_2$]$_2$, H-A5′b), 3.70–3.95 (m, 11H; OCH_3, OCH_2CH$_2$CN, H-A4′, H-B4′, H-B5′a), 4.08–4.12 (m, 1H; H-B5′b), 4.27–4.32 (m, 1H; H-B6), 4.56–4.69 (m, 4H; H-A3′, H-B3′, OCH_2O), 4.92–4.99 (m, 1H; H-A6), 6.09–6.12 (m, 1H; H-A1′), 6.18–6.27 (m, 1H; H-B1′), 6.82–6.85 (2 d, $^3J = 8.7$ Hz, 4H; H-3DMTr), 7.22–7.37 (m, 9H; H-2DMTr, H-2′DMTr, H-3′DMTr, H-4′DMTr), 8.91 (s, 2H; H3 (NH)). ^{13}C NMR (125.8 MHz, CDCl$_3$): $\delta = 20.39$, 20.54 (2d, $J_p = 7.4$ Hz, OCH$_2$CH_2CN), 24.51, 24.54, 24.57, 24.60, 24.62, 24.68 (N[CH(CH_3)$_2$]$_2$), 35.59, 35.83 (C-B2′), 37.41, 37.64 (C-A2′), 39.16, 39.72 (C-A5), 41.69, 42.27 (C-B5), 43.32 (d, $J_p = 12.6$ Hz, N[CH(CH$_3$)$_2$]$_2$), 51.34 and 51.52 (C-B6),

52.83, 53.29 (C-A6), 55.33 (OCH_3), 57.83 (d, $J_p = 19.7$ Hz, OCH_2CH_2CN), 58.03 (d; $J_p = 19.3$ Hz, OCH_2CH_2CN), 61.92 (A-5′), 64.24 (B-5′), 70.45 (d, $J_p = 14.8$ Hz, C-B3′), 70.89 (d; $J_p = 16.4$ Hz, C-B3′), 73.72 (C-A3′), 81.55, 81.61 (C-A4′), 82.34, 82.70 (C-B4′), 84.78 (C-B1′), 85.54, 85.57 (C-A1′), 86.55, 86.53 (C-DMTr), 94.23, 94.32 (OCH_2O), 113.20, 113.22 (C-3DMTr), 117.71, 118.00 (OCH_2CH_2CN), 127.37 (C-4′DMTr), 127.94, 127.95 (C-2′DMTr), 128.43, 128.47 (C-3′DMTr), 130.21, 130.25 (C-2DMTr), 135.29, 135.32, 135.37, 135.43 (C-1DMTr), 144.34 (C-1′DMTr), 152.67, 152.81, 153.57, 153.71 (C-A2 and C-B2), 158.79, 158.81, 158.84 (C-4DMTr), 166.09, 166.33, 167.26, 167.67 (C-A4 and C-B4). ^{31}P NMR (202.5 MHz, ^{1}H coupled, $CDCl_3$): 148.6, 149.5; MS (FAB$^+$): *m/z* (%): 971 (34.6) [M^++1], 303 (100) [DMTr$^+$].

Formacetal-linked, deoxyuridine dimer 28: Compound **22** (1.8 g, 3 mmol) was suspended in aqueous NH_3 solution (25%, 50 mL) and heated for 3 h at 80 °C. The aqueous phase was extracted three times with chloroform. The aqueous phase was evaporated in vacuo and the residual material was dissolved in ethanol. After the addition of silica gel (2.5 g) and evaporation of the solvent in vacuo, the powder was subjected to flash chromatography on silica gel (CH_2Cl_2/MeOH 10:1). The product **28** (0.9 g, 1.9 mmol, 63%) was obtained as a colorless powder after recrystallization from methanol. R_F: 0.27 ($CHCl_3$/MeOH 5:1 (v:v)); m.p. 140–142 °C; IR(KBr): $\tilde{\nu} = 3420$, 3267, 3100, 3056, 2935, 2867, 1690 (C=O), 1467, 1433, 1384, 1279, 1193, 1169, 1106, 1072, 1056, 1031, 944, 860, 816, 767, 558, 529, 421 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 2.08$-2.21 (m, 3H; H-A2′a, H-B2′a, H-B2′b), 2.26–2.33 (m, 1H; H-A2′b), 3.57 (d, $^3J_{A5'-A4'} = 3.6$ Hz, 2H; H-A5′), 3.63 (dd, 1H; $^2J_{B5'a-A5'b} = 10.9$ Hz, $^3J_{B5'a-B4'} = 5.0$ Hz, H-B5′a), 3.69 (dd, 1H; $^2J_{B5'a-A5'b} = 10.9$ Hz, $^3J_{B4'-B5'b} = 3.9$ Hz, H-B5′b), 3.89–3.92 (m, 1H; H-B4′), 3.95 (dd, 1H; $^3J_{A3'-A4'} = 6.3$ Hz, $^3J_{A4'-A5'} = 3.6$ Hz, H-A4′), 4.20–4.30 (m, 1H; H-B3′), 4.31–4.32 (m, 1H; H-A3′), 4.74 and 4.76 (2 d, $^2J_{gem} = 7.0$ Hz, 2H; OCH_2O), 5.11 (br s, 1H; A5′-OH*)*, 5.34 (br s, 1H; B3′-OH*)*, 5.64 and 5.65 (2 d, $^3J_{A5-A6} = 8.1$ Hz, $^3J_{B5-B6} = 8.1$ Hz, 2H; H-A5, H-B5), 6.12 (dd, $^3J_{A1'-A2'} = 8.0$ Hz, $^3J_{A1'-A2'} = 6.0$ Hz, 1H; H-A1′), 6.17 (t, $^3J_{B1'-B2'} = 6.8$ Hz, 1H; H-B1′), 7.67 and 7.84 (2 d, $^3J_{A5-A6} = 8.1$ Hz, $^3J_{B5-B6} = 8.1$ Hz, each 1H; H-A6, H-B6), 11.3 (br s, 2H; NH); ^{13}C NMR (125.8 MHz, [D$_6$]DMSO): $\delta = 38.92$ (C-A2′), 39.02 (C-B2′), 61.15 (C-A5′), 67.81 (C-B5′), 70.53 (C-B3′), 76.51 (C-A3′), 84.06 (C-A1′ and C-B1′), 84.99 and 85.04 (C-A4′ and C-B4′), 93.70 (OCH_2O); 101.81 and 101.86 (C-A5 and C-B5), 140.23 and 140.27 (C-A6 and C-B6), 150.32 (C-A2 and C-B2), 162.96 (C-A4 and C-B4); MS (FAB$^+$): *m/z* (%): 937 (10) [M_2^++1], 491 (13) [M^++Na], 469 (100) [M^++1]. $C_{19}H_{24}N_4O_{10} \cdot 0.5\,H_2O$ (468.42): calcd C 47.8, H 5.28, N 11.74; found: C 48.04, H 5.26, N 11.44.

5′A-*O*-Dimethoxytrityl-protected, formacetal-linked, deoxyuridine dimer (29): Compound **28** (650 mg, 1.4 mmol) was dissolved in dry pyridine (5 mL) under argon, then DIEA (0.3 mL, 1.74 mmol) was added. DMTrCl (750 mg, 2.1 mmol) was added to the reaction mixture in small portions. The reaction mixture was stirred under argon for 15 h. The pyridine was evaporated in vacuo at 40 °C and the residual material was twice dissolved in toluene and the solution evaporated to dryness. The residue was subjected to flash chromatography on silica gel (100–10% EtOAc/MeOH with 2% NEt_3). The product **29** was dissolved in a minimum amount of acetone, diluted with Et_2O and added dropwise into *n*-hexane at 0 °C. The resulting suspension was stored for 1 h at −20 °C. The yellowish precipitate was collected and dried in vacuo. Yield: 888 mg (1.2 mmol, 82%) **29** contained residual hexane. R_F: 0.30 ($CHCl_3$/MeOH 10:1 (v:v)); m.p. >110 °C, slow decomp; IR(KBr): $\tilde{\nu} = 3203$, 3059, 2945, 1691 (C=O), 1610, 1509, 1461, 1380, 1273, 1251, 1177, 1075, 1032, 828, 761, 703, 585, 555, 413 cm^{-1}; ^{1}H NMR (500 MHz, [D$_6$]acetone): $\delta = 2.16$–2.24 (m, 1H; H-A2′a or H-B2′a), 2.25–2.30 (ddd, $^2J_{2'a-2'b} = 13.5$ Hz, $^3J = 6.2$ Hz, $^3J = 3.3$ Hz, 1H; H-A2′b or H-B2′b), 2.35–2.41 (m, 1H; H-B2′a or H-A2′a), 2.48–2.52 (ddd, $^2J_{2'a-2'b} = 13.8$ Hz, $^3J = 6.1$ Hz, $^3J = 3.2$ Hz, 1H; H-B2′b or H-A2′b), 3.39 (dd, $^2J_{5'a-5'b} = 10.5$ Hz, $^3J_{4'-5'a} = 3.7$ Hz, 1H; H-A5′a or H-B5′a), 3.46 (dd, $^2J_{5'a-5'b} = 10.5$ Hz, $^3J_{4'-5'b} = 4.1$ Hz, 1H; H-A5′b or H-B5′b), 3.75–3.81 (m, 8H; OCH_3, H-A5′ or H-B5′), 4.06 (dd, $^3J = 7.2$ Hz, $^3J = 3.8$ Hz, 1H; H-A4′ or H-B4′), 4.18 (dd, $^3J = 7.2$ Hz, $^3J = 3.8$ Hz, 1H; H-A4′ or H-B4′), 4.19–4.41 (*q*, 1H; H-A3′ or H-B3′), 4.58–4.60 (*q*, 1H; H-A3′ or H-B3′), 4.83 (d, $^2J_{gem} = 7.0$ Hz, 1H; OCH_2O), 4.87 (d, $^2J_{a\text{-}b} = 7.0$ Hz, 1H; OCH_2O), 5.37 (d, $^3J_{(A5-A6\text{ or }B5-B6)} = 8.1$ Hz, 1H; H-A5 or H-B5), 5.56 (d, $^3J_{(A5-A6\text{ or }B5-B6)} = 8.1$ Hz, 1H; H-A5 or H-B5), 6.25 (dd, $^3 = 7.3$ Hz, $^3J = 6.3$ Hz, 1H; H-A1′ or H-B1′), 6.29 (dd, $^3 = 7.4$ Hz, $^3J = 6.3$ Hz, 1H; H-A1′ or H-B1′), 6.90–6.93 (m, 4H; H-3DMTr), 7.23–7.26 (m, 1H; H-4′DMTr), 7.32–7.36 (m, 6H; H-2DMTr, H-2′DMTr), 7.46–7.48 (m, 2H; H-3′DMTr), 7.71 (d,$^3J_{(A5-A6\text{ or }B5-B6)} = 8.1$ Hz, 1H; H-A6 or H-B6), 7.73 (d,$^3J_{(A5-A6\text{ or }B5-B6)} = 8.1$ Hz, 1H; H-A6 or H-B6), 9.96 (br s, 2H; H-3 (NH)): ^{13}C NMR (125.8 MHz, [D$_6$]acetone): $\delta = 38.88$ and 40.88 (C-A2′ and C-B2′), 55.56 (OCH_3), 64.42 and 69.32 (C-A5′ and C-B5′), 72.46 and 78.37 (C-A3′ and C-B3′), 85.03, 85.75 and 86.70 (C-A4′ and C-B4′ and C-A1′ and C-B1′ (two signals overlap at 85.75)), 87.57 (C-DMTr), 95.77 (OCH_2O), 102.63 and 102.72 (C-A5 and C-B5), 114.06 and 114.07 (C-3DMTr), 127.76 (C-4′DMTr), 128.75 (C-2′DMTr), 129.03 (C-3′DMTr), 131.01 and 131.04 (C-2DMTr), 136.44 and 136.55 (C-1DMTr), 140.87 and 140.92 (C-A6 and C-B6), 145.85 (C-1′DMTr), 151.24 (C-A2 and C-B2 overlap), 159.75 (C-4DMTr), 163.44 and 163.53 (C-A4 and C-B4). MS (FAB$^+$): *m/z* (%): 1541 (4.9) [M_2^++1]; 793 (8.6) [M^++Na], 771 (33.4) [M^++1], 303 (100) [DMTr$^+$].

5′A-*O*-Dimethoxytrityl-protected, formacetal-linked, deoxyuridine dimer-(2-cyanoethyl-*N,N*-diisoproyl)-2′B-deoxyuridine-phosphoramidite (27): Compound **29** (771 mg, 1 mmol) was dissolved in absolute CH_2Cl_2 (8 mL) under argon, then DIEA (0.38 mL, 2.2 mmol) was added. To this solution was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.25 mL, 1.1 mmol) and the mixture was stirred for 4 h under argon. After evaporation of the solvent in vacuo, the residue was subjected to flash chromatography on silica gel (33–90% EtOAc/*n*-hexane, 2% triethylamine). The product fractions were evaporated in vacuo and the residue was dissolved in a minimum of acetone. This solution was diluted with Et_2O and added dropwise into ice-cold *n*-hexane. Compound **27** was obtained as a colorless powder (700 mg, 0.72 mmol, 72%). R_F: 0.27 + 0.31 (EtOAc/Hex./NEt_3 5:1:0.12 (v:v)); m.p. 93–102 °C; IR(KBr): $\tilde{\nu} = 3211$, 3056, 2965, 2950, 1694 (C=O), 1606, 1509, 1461, 1379, 1272, 1251, 1179, 1067, 1032, 978, 878, 828, 806, 761, 726, 700, 585, 555, 522, 420 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.15$–1.22 (m, 12H; N[CH(CH_3)$_2$]$_2$), 2.12–2.25 (m, 2H; H-A2′a, H-B2′a), 2.44–2.53 (m, 2H; H-A2′b, H-B2′b), 2.58–2.63 (m, 2H; OCH_2CH_2CN), 3.37 (dd, $^2J_{A5'a-A5'b} = 10.5$ Hz, $^3J_{A4'-A5'a} = 3.1$ Hz, 1H; H-A5′a), 3.44 (dd, $^2J_{A5'a-A5'b} = 10.5$ Hz, $^3J_{A4'-A5'b} = 3.3$ Hz, 1H; H-A5′b), 3.56–3.87 (m, 12H; OCH_2CH_2CN (2H), N[CH(CH_3)$_2$]$_2$ (2H), H-B5′ (2H), 3.79 (s, 6H; OCH_3)), 4.12–4.21 (m, 2H; H-A4′, H-B4′), 4.36–4.40 (m, 1H; H-A3′), 4.45–4.50 (m, 1H; H-B3′), 4.75 (d, 2H; OCH_2O), 5.41 (2 d, $^3J_{(A5-A6)} = 8.1$ Hz, 1H; H-A5), 5.65 (2 d, $^3J_{(B5-B6)} = 8.1$ Hz, 1H; H-B5), 6.24–6.28 (m, 2H; H-A1′, H-B1′), 6.83–6.86 (m, 4H; H-3DMTr), 7.24–7.37 (m, 9H; H-2DMTr, H-2′DMTr, H-3′DMTr, H-4′DMTr), 7.46 and 7.48 (2 d, $^3J_{(B5-B6)} = 8.1$ Hz, 1H; H-B6), 7.72 (2 d, $^3J_{(A5-A6)} = 8.1$ Hz, 1H; H-A6), 9.07 (br s, 2H; H3 (NH)); ^{13}C NMR (125.8 MHz, $CDCl_3$): $\delta = 20.37$, 20.41, 20.43 and 20.47 (2 d, $^3J_p = 7.2$ Hz, OCH_2CH_2CN), 24.49, 24.56, 24.60, 24.63 and 24.65 (q, N[CH(CH_3)$_2$]$_2$), 39.01, 39.04, 39.63 and 39.67 (C-A2′ and C-B2′), 43.33 (d, $^2J_p = 12.3$ Hz, N[CH(CH_3)$_2$]$_2$), 55.29 (OCH_3), 57.95, 57.98, 58.10 and 58.13 (2 d, $^4J_p = 4.2$ Hz, C-B5′), 63.04 (C-A5′), 67.98 and 68.13 (d, $^2J_p = 18.6$ Hz, OCH_2CH_2CN), 73.22, 73.36 and 73.47 (2 d, $^2J_p = 16.8$ Hz, C-B3′), 77.85 and 78.00 (C-A3′), 84.29 and 84.33 (C-A4′), 84.43 (d, $^3J_p = 6.5$ Hz, C-B4′), 84.82 (d, $^3J_p = 3.5$ Hz, C-B4′), 85.15, 85.18, 85.36 and 85.43 (C-A1′ and C-B1′), 87.07 (C-DMTr), 95.45 and 95.56 (OCH_2O), 102.39 (C-A5), 102.56 and 102.62 (C-B5), 113.35 (C-3DMTr), 117.68 and 117.69 (OCH_2CH_2CN), 127.23 (C-4′DMTr), 128.05 and 128.12 (C-2′DMTr and C-3′DMTr), 130.08 (C-2DMTr), 135.13 and 135.28 (C-DMTr), 139.78 and 139.83 (C-B6), 139.97 (C-A6), 144.24 (C-1′DMTr), 150.15, 150.21, 150.27 and 150.29 (C-A2 and C-B2), 158.78 (C4DMTr), 163.08 and 163.19 (C-A4 and C-B4); ^{31}P NMR (202.5 MHz, ^{1}H-coupled, $CDCl_3$): 149.4, 149.5; MS (FAB$^+$): *m/z* (%): 971 (44.0) [M^++1], 303 (100) [DMTr$^+$]; $C_{49}H_{59}N_6O_{13}P$ (971.01): calcd C 60.61, H 6.12, N 8.65, P 3.19; found C 60.35, H 6.19 , N 8.52, P 3.18.

Oligonucleotide synthesis: Oligonucleoside synthesis was performed on a Pharmacia-Gene-Plus synthesizer, connected to a Olivetti-M 300 personal computer. Synthesis of the oligonucleotide **30** was performed by using a modified 10 μmol cycle and Pac-amidites. Syntheses of oligonucleotide **31** and **32** were performed by using a modified 1.3 μmol cycle and standard phosphoamidites. Solvents and solutions were made up according to the manufacturers protocol.[46] The phosphoramidite (0.1 M in MeCN) and 1*H*-tetrazole (0.5 M in MeCN) solutions were equal in concentrations to those used for the synthesis of natural oligodeoxynucleotides. Average coupling yields monitored by on-line trityl assay were generally in the range of 95–99%. All syntheses were run in the trityl-on mode, resulting in **DMTr-30**, **DMTr-31**, **DMTr-32**. For the deprotection and cleavage from the support of **DMTr-30**, the solid support was suspended in 10% DBU/MeOH (1 mL, dried over 3 Å molecular sieves) and kept at 4 °C with occasional shaking for four days. After addition of 1 M NEt_3/HOAc (2 mL) the support was filtered off and washed three times with 1 M NEt_3/HOAc (0.5 mL). The solution was concentrated to half of the volume and **DMTr-30** purified by HPLC (C_{18}-RP, 25–40% B in 40 min). After removal of the solvent in

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vacuo **DMTr-30** was detritylated by addition of 50% $HCOOH/H_2O$ (3 mL). After 5 min the formic acid was removed in vacuo and **30** purified by HPLC (C_{18}-RP, 5–10% B in 5 min, 10–25% B in 30 min). The isolated yield of **30** was 10%. For the deprotection and purification of **31** and **32**, the solid support was suspended in concentrated NH_3:MeOH = 3:1 (2 mL) and left for 15 h at 55 °C to effect deprotection and cleavage. The solvent was evaporated and the residue suspended in 0.3 M NEt_3/H_2CO_3 in H_2O and applied on a C18 Sep-Pak Cartridge. **DMTr-31** and **DMTr-32** were eluted by subsequent addition of 0.3 M NEt_3/H_2CO_3 in H_2O:MeCN (100:1 → 50:50). The fractions containing **DMTr-31 DMTr-32** were concentrated and detritylated by addition of 50% $HCOOH/H_2O$ (3 mL). After 5 min the formic acid was removed in vacuo and **31** and **32** were purified once more by the use of a C18 Sep-Pak Cartridge. The isolated yield of **31** was 35%, that of **32** 49%. Laser-desorption mass spectra (negative ions detected): calcd for **30**: 3548.6; found: 3549; calcd for **31**: 3548.6; found: 3548.

Enzymatic studies: 200 μL solutions containing **30** (5 nmol), 0.1 M NaCl, 10 mM KH_2PO_4, 5 mM DTT, and DNA photolyase (25 pmol in the case of *A. nidulans* and *N. crassa* and 250 pmol in the case of *P. tridactylis*) were prepared in pyrex glass analytical tubes. The enzymes were added in the dark. The assay solutions were mixed and irradiated at 435 nm (*A. nidulans*), 405 nm (*N. crassa*), 380 nm (*P. tridactylis*) (Spex 1680 Fluorolog, 0.22 m double Spectrometer, equipped with a 450 W Hg/Xe lamp and a double grating monochromator, measurements were performed at room temperature). At each time time interval 5 μL were removed and HOAc (5 μL) was added in order to stop the enzyme reaction. The samples were kept at −20 °C until analysis by ion-exchange chromatography was performed.

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