154. Synthesis and Structure of (Carboxymethyl)-Functionalized Cyclobuta-Fused Uracil Dimers

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Dedicated with best wishes to Professor *Dieter Seebach* on the occasion of his 60th birthday

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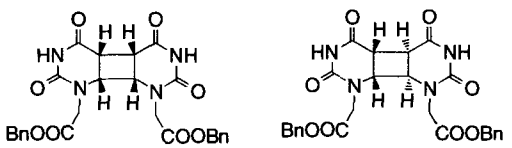
Herein, we report a convenient method for the preparation of four benzyl-ester-protected, (carboxymethyl)-functionalized cyclobuta-fused uracil dimers **1**–**4**, including the desired *cis-cisoid-cis,syn*-configured isomer **1**, a compound which is suitable for the preparation of catalytic antibodies, synthetic receptors, and model compounds, required for the investigation of the DNA-lesion, recognition step and DNA-repair mechanisms. A comprehensive structure determination of each isomer by ^{1}H-NMR, and in the case of the *cis-cisoid-cis,syn*- and the *cis-transoid-cis,syn*-dimers by X-ray crystal-structure analysis, is also provided.

1. Introduction. – On the exposure of cells to UV light, two pyrimidine bases, located above each other in the DNA double strand, can dimerize in a $[2\pi + 2\pi]$ cycloaddition to yield a cyclobuta-fused pyrimidine dimer [1]. The major photoproducts have *cis-cisoid-cis,syn*-configuration[1]) [2]. Upon irradiation of conformationally more flexible DNA, an additional isomer is formed which possesses *cis-transoid-cis,syn*-configuration[1]) [2]. Several detailed *in vitro* and *in vivo* studies have revealed the mutagenic potential of these photoproducts [3]. Both lesions were found to cause cell death or initiate tumor genesis. The *cis-cisoid-cis,syn*- and *cis-transoid-cis,syn*-dimers are responsible for the development of various skin cancers like basal cell and squamous cell tumors, and they are thought to be involved in the development of malignant melanoma. To counteract the harmful effect of UV irradiation, cells have developed defense systems, which are able to specifically detect and repair photolesions [4]. A deeper comprehension of the underlying processes which lead to efficient DNA-damage recognition and repair is currently desired in order to understand the prerequisites for this process *in vivo* [5].

To this end, several research groups started the preparation of synthetic receptors [6] [7] and catalytic antibodies [8] [9] aimed at mimicking the DNA-lesion recognition step. In addition, model compounds [10] [11] were prepared with the intention of investigating repair-mechanisms on a molecular level. For both approaches, easily functionalizable lesion-mimicking building blocks like the (carboxymethyl)-substituted pyrimidine dimers

[1]) The configurational descriptors *cis-cisoid-cis* and *cis-transoid-cis* refer to the relationships at the saturated bridgeheads, whereas *syn* and *anti* refer to the immediatly adjacent N-atoms being on the same or opposite side, respectively, of the plane perpendicular and bisecting all three rings of the cyclobutapyrimidine skeleton.

1–**4** are required [6–9] [11]. Synthetic efforts aimed at the preparation of the desired *cis-cisoid-cis,syn*-dimer **1** (see *Fig. 1*), however, yielded so far exclusively the biologically less abundant *cis-transoid-cis,syn*-dimers [8] [9]. We have recently reported on the synthesis of the four cyclobuta-fused uracil dimers **1**–**4**, including the desired *cis-cisoid-cis,syn*-isomer **1**, in addition to model compounds which mimic the DNA-repair process performed by light-dependent DNA-photolyase enzymes [11] [12a] (for the crystal structure of the *E. coli* photolyase, see [12b]). We now wish to give a full report of the synthetic procedures which afford the four benzyl-ester isomers **1**–**4**, together with a discussion of the NMR and UV spectra obtained. X-Ray crystal-structure analysis of the *cis-cisoid-cis,syn*- and the *cis-transoid-cis,syn*-isomers are presented. These structures reveal an interesting torsion angle difference between the isomers, which is important for the explanation of recently observed differences in repair rate [11].



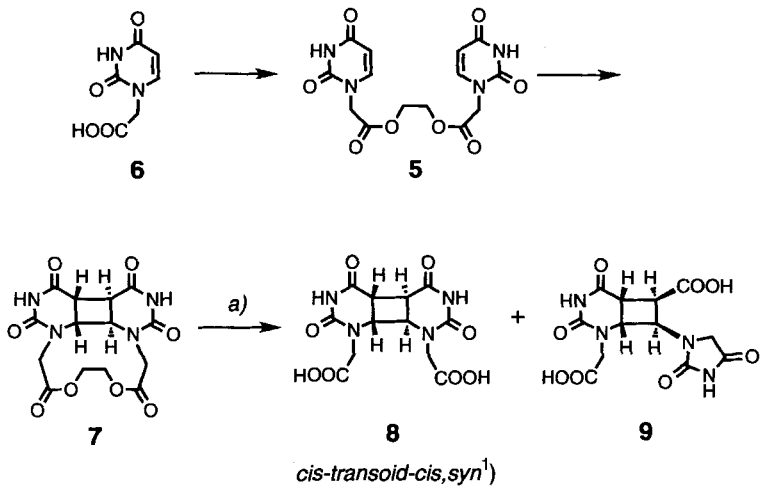
cis-cisoid-cis,syn[1]) *cis-transoid-cis,syn*[1])

Fig. 1. *Functionalized* cis-cisoid-cis,syn- *and* cis-transoid-cis,syn-*cyclobuta-fused uracil dimers* **1** *and* **2**, *respectively*

2. Results and Discussion. – 2.1. *Tether-Directed Syntheses Strategy.* The synthetic strategy recently reported by *Schultz* and coworkers [8] [9] for the preparation of (carboxymethyl)-substituted cyclobuta-fused uracil and thymine dimers is based upon the key step irradiation of the pyrimidine dimer precursor **5**, which is 1,1′-tethered by an ethylene glycol containing moiety and which was obtained from (carboxymethyl)-uracil **6** (see *Scheme 1*). In the photolysis step, the tether was intended to suppress the formation of *anti*-isomers[1]) and to cause predominant formation of the desired *cis-cisoid-cis,syn*- and *cis-transoid-cis,syn*-dimers. Irradiation of the tethered pyrimidine precursors, however, yielded only the biologically less abundant *cis-transoid-cis,syn*-compound **7**. Although steric considerations do not *a priori* exclude the formation of the corresponding *cis-cisoid-cis,syn*-dimer, *Schultz* and coworkers reported the detection of only a small amount of a potential second isomer by TLC. This is in accord with a report of *Gangamani et al.* [13], who observed only *cis-transoid-cis,syn*-products after irradiation of different poly(oxyethylene)-linked thymine dimers. Most surprisingly, cleavage of the ethylene-glycol-diester bridge in **7** under basic conditions yielded the bis(carboxymethyl)-functionalized cyclobuta-fused uracil dimer **8** in only low yields (20%) [9]. During our own synthetic efforts, using the tether-directed approach for the synthesis of **1**, we observed that the basic cleavage of the ethylene-glycol tether in **7** yielded, beside the known product **8**, a major unexpected product in over 70% yield. Investigation of this compound by mass spectrometry revealed, surprisingly, the correct molecular weight (M^+ 341) for another bis(carboxymethyl)-substituted cyclobuta-fused uracil dimer. This finding was supported by the elemental analysis, which showed the expected elemental composition for such a dimer. The ^{1}H- and ^{13}C-NMR spectra contained signals indica-

tive of a cyclobutane moiety. The total number of signals, however, was too high, giving evidence for an unsymmetrical structure. To investigate the configuration of the cyclobutane ring and to elucidate the structure of this new product, crystals suitable for X-ray structural analysis were grown. This analysis finally revealed the '*transoid*'-hydantoin (= imidazoline-2,4-dione) structure **9** of the product. We suppose that **9** is formed *via* base-induced opening of one dihydropyrimidine ring and subsequent reaction of the resulting urea function with the ethylene-glycol ester of the carboxymethyl group present on the same dihydropyrimidine ring. Formation of **9** reveals the high lability of the dihydropyrimidine ring within the strained cyclobuta-fused dimer system under basic conditions.

Scheme 1. *Synthesis of the* cis-transoid-cis,syn-*Cyclobuta-Fused Uracil Dimer* **8** *and of the Hydantoin Derivative* **9** *Using a Tether-Directed Approach*



a) LiOH (30 equiv.), H_2O, r.t., 20 h; 72%.

2.2. *Structural Analysis of the Hydantoin Derivative* **9**. The hydantoin **9** yielded colorless needles suitable for X-ray crystal-structure analysis, which was performed at 296 K (see *Exper. Part*). The crystals contained, besides the diprotonated hydantoin, 2 equiv. of H_2O. The molecular structure of **9** is depicted in *Fig. 2*. The '*cis-transoid-cis*'-structure of the cyclobutane moiety is clearly visible. The hydantoin part is connected *via* a single C–N bond *trans* to the dihydropyrimidine moiety, which is fused to the cyclobutane ring. The plane of the hydantoin ring is oriented almost perpendicular to the pyrimidine subunit, with the ring being sandwiched between the two carboxy groups.

2.3. *Synthesis of the Four Esters* **1**–**4** *of (Carboxymethyl)-Functionalized Cyclobuta-Fused Uracil Dimers*. Parallel to the irradiation of tethered uracil dimers, we pursued irradiation experiments with the untethered (carboxymethyl)-functionalized uracil derivative **10** (*Scheme 2*). Irradiation of **10** and subsequent separation of the four isomers finally proved successful for the synthesis of the *cis-cisoid-cis,syn*-dimer **1**[2]). Thus, ester-

[2]) The method of irradiating untethered uracil and thymine derivatives and separation of the resulting isomers was previously used for the preparation of other dimer derivatives [14].

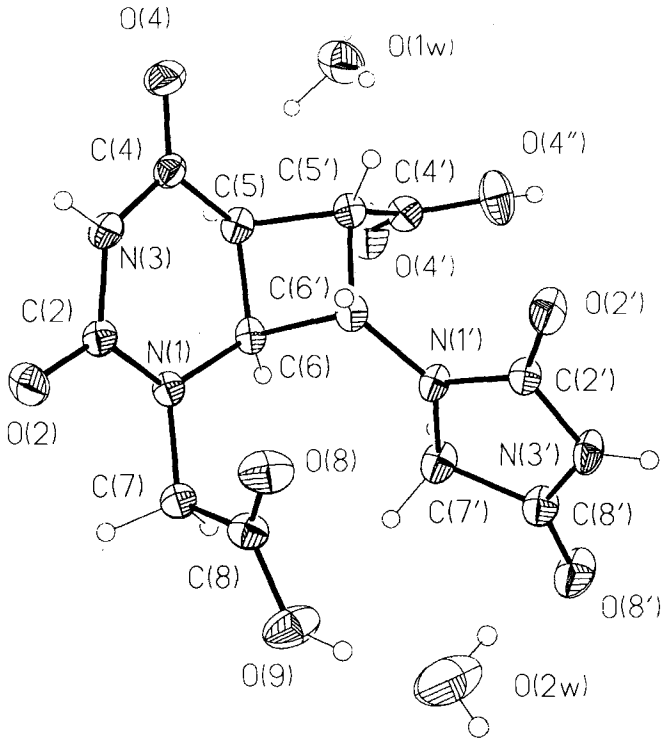
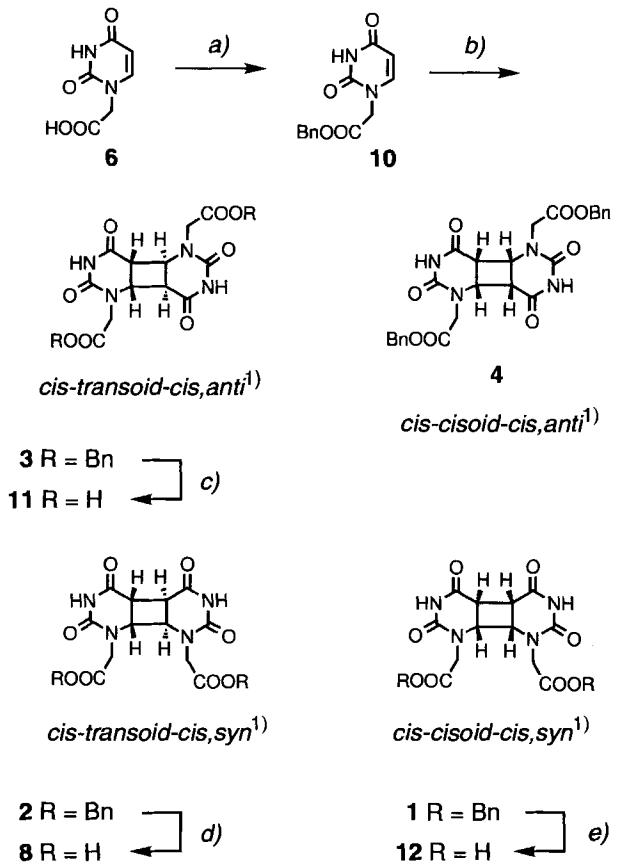


Fig. 2. *ORTEP Plot of the molecular structure of compound* **9**. Arbitrary numbering. Displacement ellipsoids are shown at the 50% probability level.

ification of **6** [15] with benzyl alcohol and 1,1'-carbonylbis[1*H*-imidazole] [16] gave the benzyl-ester-protected uracil derivative **10** in excellent yield. For the photodimerization, a suspension of **10** (4 g) was irradiated in acetone in a *Pyrex* vessel under N_2 with a medium-pressure Hg vapor lamp ($\lambda > 300$ nm). During the irradiation **10** slowly dissolved and one of the four isomers, the *cis-transoid-cis,anti*-diester **3**, which was completely insoluble in acetone, precipitated and was removed by filtration. From the filtrate, which contained starting material **10** and the remaining three isomers **1**, **2**, and **4**, about half of the *cis-transoid-cis,syn*-diester **2** was isolated as a solid, after evaporation and treatment of the residual oil with ice-cold $CHCl_3$. The remaining mixture was subjected to flash chromatography (silica gel *H*, $CHCl_3$/MeOH) yielding residual **2**, a small fraction of **4**, and the desired *cis-cisoid-cis,syn*-isomer **1**, still contaminated with small amounts of **10**. Pure **1** was obtained by recrystallization from acetone/$CHCl_3$. Irradiation of 12 g of the readily available ester **10** in three 4-g portions and collective workup yielded on average 5 g of *cis-transoid-cis,anti*-isomer **3**, 3.5 g of *cis-transoid-cis,syn*-isomer **2**, 100 mg of *cis-cisoid-cis,anti*-isomer **4**, and *ca.* 1.2 g of the *cis-cisoid-cis,syn*-isomer **1**, all within three to four working days starting from **6**.

The different solubility of the four isomers afforded different procedures for the cleavage of the benzyl-ester groups. The most insoluble but very stable *cis-transoid-cis,anti*-diester **3** was hydrolyzed with 1N aqueous NaOH solution to the corresponding

Scheme 2. *Synthesis of the Cyclobuta-Fused Uracil Dimers* **1**–**4**



a) 1,1'-Carbonylbis[1*H*-imidazol] (1.3 equiv.), BnOH (1.35 equiv.), DMF, r.t., 28 h; 95%. *b*) *hν* (> 300 nm), acetone, r.t., 3 h; **1**: 5%, **2**: 15%, **3**: 21%, **4**: 0.5%. *c*) 1N NaOH, r.t., 3.5 h; 89%. *d*) conc. HCl soln., reflux, 1 h; 51%. *e*) H_2, Pd/C, AcOH, r.t.; 91%.

diacid **11**. All other isomers were cleaved under nonbasic conditions to avoid the potential opening of one dihydropyrimidine unit: diester **2** was refluxed in conc. HCl solution to give the diacid **8**, and the better soluble isomer **1** allowed cleavage to **12** using mild catalytic hydrogenation. All compounds **8**, **11**, and **12** were obtained in fair (**8**) and excellent (**11**, and **12**) yields, and no hydantoin by-products were detected.

The structures of the four benzyl esters **1**–**4** were deduced by ^{1}H-NMR investigations (see *Sect. 2.5*) [17]. In addition, the isomers **1**–**3** yielded regular and transparent crystals, which in the case of **1** and **2** were of sufficient quality for X-ray analysis (see *Sect. 2.4*), but did not allow to obtain a high precision X-ray analysis.

2.4. *Structural Analysis of the* cis-cisoid-cis,syn- *and* cis-transoid-cis,syn-*Cyclobuta-Fused Uracil Dimers* **1** *and* **2**, *Respectively*. The *cis-transoid-cis,syn*-isomer **2** crystallized from $HCOOH/H_2O$ as colorless needles. In the crystal structure, **2** exhibits non-crystallographic C_2 symmetry, with the C_2-symmetry axis intersecting the cyclobutane ring

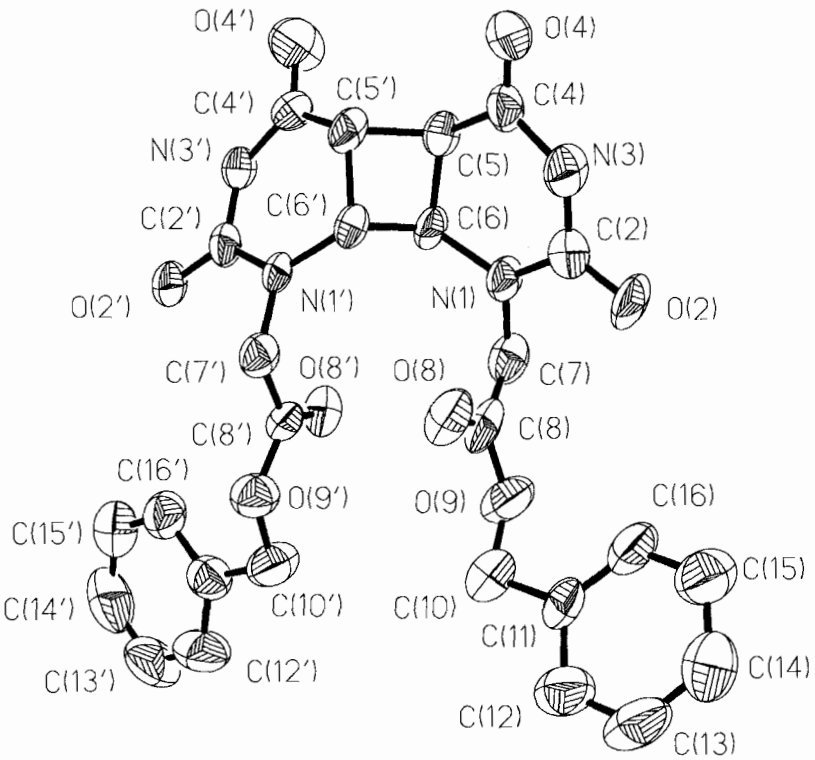


Fig. 3. *ORTEP Plot of the molecular structure of compound* **2.** Arbitrary numbering. Displacement ellipsoids are shown at the 50% probability level.

system parallel to the long axis of the molecule (*Fig. 3*). The cyclobutane ring is consequently symmetrically puckered. The planes of the ester groups are oriented *anti*-parallel to each other at a distance of *ca.* 3.22 Å. Both Ph groups and the four H-atoms of the CH_2 groups connected to the pyrimidine dimer moiety point in opposite directions.

The corresponding *cis-cisoid-cis,syn*-isomer **1** crystallized from EtOH/H_2O as colorless platelets. As depicted in *Fig. 4*, **1** has C_1 symmetry in the crystal and shows an edge-to-face orientation of the two Ph groups (distance C(13) $\cdots$ C(13') *ca.* 4.00(2) Å). The N(1)–N(1') distance is particularly short at only 3.07(1) Å. The C(7')–N(1') bond is almost in plane (4.5(5)°) with the dihydropyrimidine ring (C(6')–C(5')–C(4')–N(3')–C(2')). In addition, the C(7')–N(1') bond is almost parallel to the C(5')–C(6') bond yielding an angle between both bonds of only 6.5(5)°. The C(7)–N(1) bond on the other side of the dimer is, in contrast, significantly bent to the outside. This bond deviates markedly from the in-plane orientation with the dihydropyrimidine ring (C(6)–C(5)–C(4)–N(3)–C(2)) yielding an angle of 27.0(5)°. In addition, the C(7)–N(1) bond is not in line with the C(5)–C(6) bond but forms an angle of 40.0(5)°. This strong bending of the C(7)–N(1) bond away from the corresponding dihydropyrimidine ring minimizes the steric repulsion between the two CH_2 groups, connected to the highly strained *cis-cisoid-cis,syn*-cyclobutane ring system.

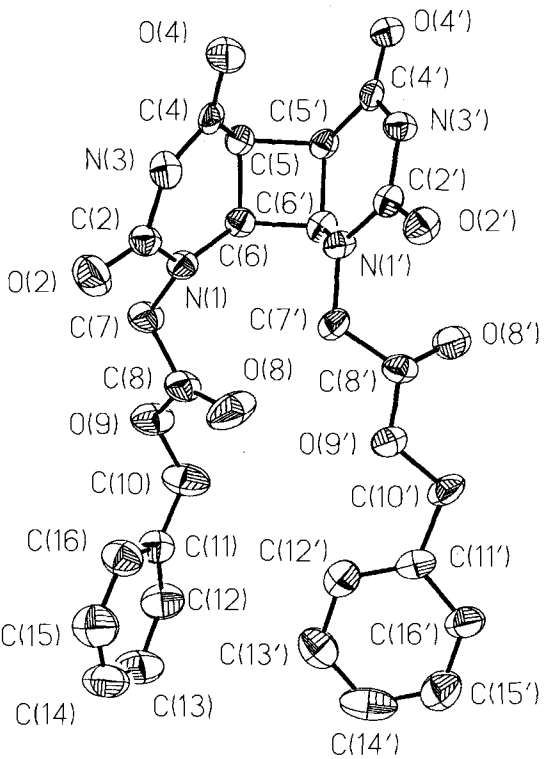


Fig. 4. *ORTEP Plot of the molecular structure of compound* **1**. Arbitrary numbering. Displacement ellipsoids are shown at the 50% probability level.

Both isomers **1** and **2** feature the expected puckered cyclobutane ring with bond lengths of *ca.* 1.53 Å for the C(5)–C(6) and C(5')–C(6') and *ca.* 1.56 Å for the C(5)–C(5') and C(6)–C(6') bonds. The torsion angle O(4)–C(4)–C(5)–C(5') is remarkably different in the two isomers. The angle differs by more than 20°, with −85.9(6)° for the *cis-cisoid-cis,syn*-isomer **1** and −62.5(7)° for the *cis-transoid-cis,syn*-isomer **2**. These torsion angles are of particular interest with respect to the electron-transfer-initiated cyclobutane ring cleavage, employed by the DNA-repair enzyme DNA-photolyase. This enzyme cleaves the cyclobutane ring system by transferring an electron to the dimer which then spontaneously forms the monomers. In the generally accepted splitting scenario, the electron initially occupies the π^* orbital of the C(4)–O(4) bond. Electron density is subsequently delocalized into the antibonding σ^* orbital of the C(5)–C(5') bond, which weakens this bond and causes the initial cleavage of the C(5)–C(5') bond [17]. The torsion angle O(4)–C(4)–C(5)–C(5') is particularly important, because it determines the overlap between these orbitals and, therefore, determines the efficiency of the electron delocalization.

2.5. *^{1}H-NMR Investigation of the Four Isomers* **1**–**4** [18]. In the ^{1}H-NMR spectra of the four isomers **1**–**4** (see *Fig. 5*), the coupling pattern allowed the assignment of the different configurations.

The four cyclobutane protons of **1**–**4** form a *AA'XX'* spin system. Two sets of ^{1}H-NMR signals corresponding to H–C(5)/H–C(5') and H–C(6)/H–C(6') are observed for the *cis-transoid-cis,syn*-isomer **2** at 3.41 ppm and 4.36 ppm (see *Fig. 3* for numbering). Both types of protons are isochronous and yield two *m*. In analogy, the ^{1}H-NMR spectrum of the C_s symmetric *cis-cisoid-cis,syn*-isomer **1** features two *m* for the isochronous protons H–C(5)/H–C(5') and H–C(6)/H–C(6') at 3.74 ppm and 4.21 ppm (see *Fig. 4* for numbering).

For the two remaining *anti*-isomers **3** and **4** rather different spectra were expected. The C_2-symmetric *cis-cisoid-cis,anti*-isomer **4** shows two signals at 3.79 ppm and 4.38 ppm, which correspond to the ring protons H–C(5)/H–C(5') and H–C(6)/H–C(6'). These protons are isochronous and in addition magnetically equivalent. As expected, they resonate as two *t*. The four cyclobutane protons H–C(5)/H–C(5') and H–C(6)/H–C(6') of the remaining C_i-symmetric *cis-transoid-cis,anti*-isomer **3** are again isochronous but not magnetically equivalent. They resonate consequently as two *m* at 3.76 ppm and 4.34 ppm. The *cis-cisoid-cis,anti*-isomer **4** and the *cis-transoid-cis,anti* isomer **3** isomer are, therefore, clearly distinguishable by the coupling pattern of the cyclobutane ring protons.

2.6. *UV/VIS Spectroscopic Investigation of the* cis-transoid-cis,syn-, cis-transoid-cis,anti-, *and the* cis-cisoid-cis,syn-*Isomers* **8**, **11**, *and* **12**, *Respectively*. The UV/VIS spectra of the (carboxymethyl)uracil **6** and of the three cyclobuta-fused uracil dimers **8**, **11**, and **12** (see *Fig. 6*) are in full agreement with reported UV/VIS spectra obtained from other uracil and cyclobuta-fused uracil derivatives [19]. The absorption of monomer **6** around 270 nm, which corresponds to the UV part of the sunlight, is clearly visible. Irradiation at this wavelength causes the formation of the cyclobuta-fused uracil dimers **8**, **11**, and **12**. Due to the negligible absorption of the cyclobuta-fused uracil dimers at these wavelengths, almost complete conversion of the uracil monomer **6** into the cyclobuta-fused dimers **8**, **11**, and **12** is observed. Light-induced monomerization is expected upon irradiation with light $\lambda < 210$ nm. Irradiation of cells at these wavelengths, however, is too harmful and, therefore, no alternative to the photorepair as performed by DNA-photolyases [12] with light $\lambda > 360$ nm.

3. Conclusion. – In the context of investigations of the molecular recognition of DNA-lesions and their repair mechanisms on a molecular level [1][3]), the *cis-cisoid-cis,syn*-cyclobuta-fused uracil dimer **1** was proposed to be an ideal building block for the preparation of catalytic antibodies [8] [9], synthetic receptors [6] [7], and model compounds [10] [11]. Unfortunately, the originally designed synthesis, based on the irradiation of uracil and thymine dimers, which are 1,1'-tethered by an ethylene-glycol-containing moiety such as **5**, was unsuccessful and yielded only the *cis-transoid-cis,syn*-isomer **8**, which is currently of limited biological interest.

We found that through direct irradiation of the uracil benzyl ester **10**, all four possible uracil dimers **1**–**4**, including the desired compound **1**, are readily available. Due to their different solubility characteristics, these dimers could be separated by a combination of selective precipitation and chromatography. The assignment of the *anti*[1]) structures is possible on the basis of their ^{1}H-NMR spectra. The *syn*[1]) isomers **1** and **2** were assigned by their X-ray crystal structures. Particularly interesting is the observation that the *cis-cisoid-cis,syn*-isomer **1** features an unusual O(4)–C(4)–C(5)–C(5') torsion angle (see *Fig. 4* for numbering). The magnitude of this angle is of importance in rationalizing the experimentally observed increased cleavage rate of the *cis-cisoid-cis,syn*-dimer in model compounds which mimic DNA-photolyases [11].

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[3]) About the possibility of blocking DNA repair as a potential goal in future cancer therapies, see [20].

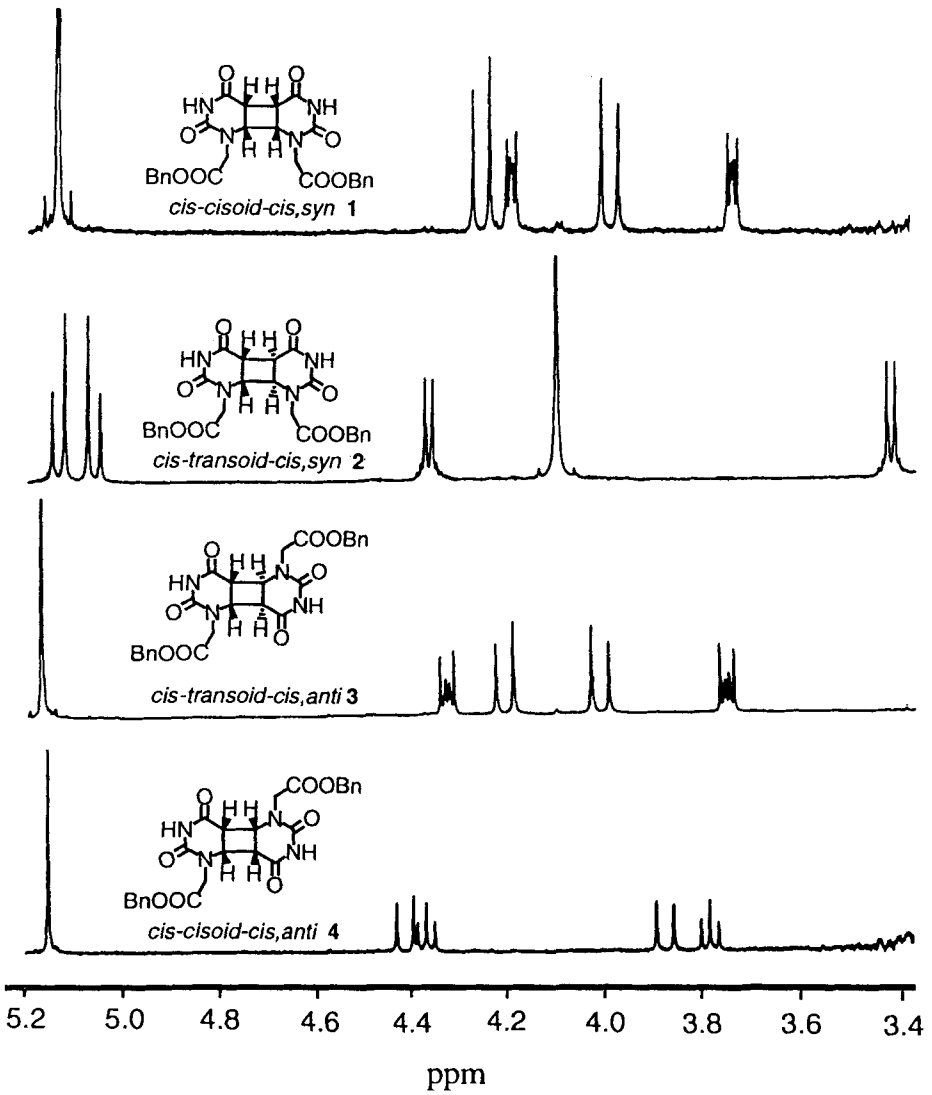

Fig. 5. *500-MHz ^{1}H-NMR Spectra* ((D$_6$)DMSO) *of all four cyclobuta-fused uracil dimers* **1**–**4**

The possibility of synthesizing the *cis-cisoid-cis,syn*-isomer **1** in large quantities has recently enabled the preparation of the first functionally active model compounds for the DNA-photolyase initiated DNA-repair process [11]. The ability to obtain compound **1** in gram quantities is now expected to allow the preparation of catalytic antibodies and of synthetic receptors, which are able to recognize and repair these functionalized dimers in solution and in native DNA.

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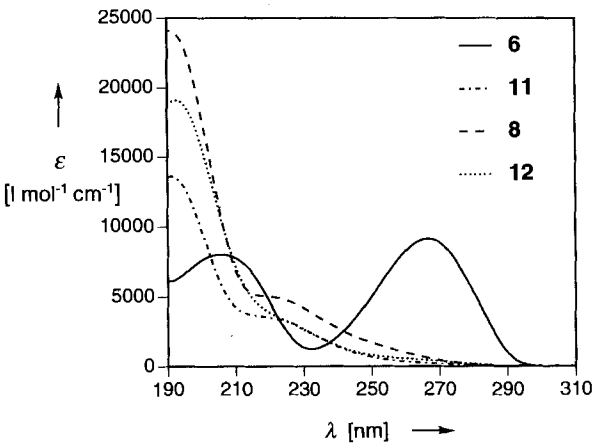

Fig. 6. *Electronic absorption spectra* (H_2O) *of* **6** *and of the three cyclobuta-fused uracil dimer isomers* **8**, **11**, *and* **12**

Experimental Part

General. Reagents and solvents were purchased reagent-grade and used without further purification. Anh. $MgSO_4$ was used as the drying agent after aq. workup. Evaporation and concentration *in vacuo* was done at water-aspirator pressure. All reactions were performed in standard glassware. Degassing of solvents was accomplished by vigorously bubbling N_2 through the soln. for at least 45 min. Irradiation experiments were performed under N_2 in a standard photochemical *Pyrex* glass apparatus with a cut off below 300 nm with a *TQ-150* (*Haereus Nobelite*) medium-pressure Hg lamp. Column chromatography (CC): silica gel *H* from *Fluka*. TLC: glass or aluminium 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 r.t.; λ_{max} in nm (ε in $M^{-1}cm^{-1}$). IR Spectra: *Perkin-Elmer-1600FTIR* in (cm^{-1}). ^1H- and ^{13}C-NMR Spectra: *Bruker-AMX-500*, *Varian-Gemini-200* and *-300* instruments, at r.t. in $(CD_3)_2SO$; δ in ppm, J in Hz, solvent peaks (2.49 ppm for ^1H and 39.7 ppm for ^{13}C) as ref. MS: *VG-ZAB-2SEQ* instrument for FAB in a 3-nitrobenzyl-alcohol matrix in *m/z* (rel. %). Elemental analysis were performed by the Mikrolabor in the Laboratorium für Organische Chemie at ETH-Zürich.

X-Ray Crystal-Structure Data of **9**. Colorless needles (H_2O); $C_{12}H_{12}N_4O_8 \cdot 2\,H_2O$ (M_r 376.29). Triclinic space group *P*1, $D_c = 1.573$ g cm^{-3}; $Z = 2$; $a = 5.477(4)$, $b = 10.748(7)$, $c = 13.714(12)$ Å; $\alpha = 91.86(17)$, $\beta = 97.9(6)$, $\gamma = 95.97(5)$; $V = 794.4(10)$ Å^3; MoK_α (λ 0.71073 Å) radiation, $3.0 \geq 2\Theta \leq 40°$, 1485 unique reflections, *T* 293 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 refined isotropically). Final $R(F) = 0.056$, $wR(F) = 0.072$ for 299 variables and 1329 observed reflections with $F > 4.0\sigma(F)$. Diffractometer: *Picker-Stoe*.

X-Ray Crystal-Structure Data of **1**. Colorless platelets ($EtOH/H_2O$); $C_{26}H_{24}N_4O_8$ (M_r 520.5). Monoclinic space group *C*2/*c*, $D_c = 1.471$ g cm^{-3}; $Z = 8$; $a = 52.69(9)$, $b = 7.809(13)$, $c = 11.516(16)$ Å; $\beta = 96.80(13)$; $V = 4702(13)$ Å^3; MoK_α (λ 0.71073 Å) radiation, $3.0 \geq 2\Theta \leq 40°$, 2086 unique reflections, *T* 293 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 $R(F) = 0.0468$, $wR(F) = 0.0645$ for 343 variables and 1356 observed reflections with $F > 4.0\sigma(F)$.

X-Ray Crystal-Structure Data of **2**. Colorless platelets ($HCOOH/H_2O$); $C_{26}H_{24}N_4O_8$ (M_r 520.5). Triclinic space group *P*1, $D_c = 1.321$ g cm^{-3}; $Z = 2$; $a = 9.042(16)$, $b = 11.342(16)$, $c = 13.66(3)$ Å; $\alpha = 91.99(17)$, $\beta = 109.28(12)$, $\gamma = 96.87(13)$; $V = 1309(4)$ Å^3; MoK_α (λ 0.71073 Å) radiation, $3.0 \geq 2\Theta \leq 40°$, 2431 unique reflections, *T* 293 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 $R(F) = 0.0576$, $wR(F) = 0.0772$ for 368 variables and 1304 observed reflections with $F > 4.0\sigma(F)$. Further details of the crystal-structure investigations are available on request from the Director of

the *Cambridge Crystallographic Data Centre*, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ (UK), on quoting the full journal citation.

(1RS,6RS,7RS,8RS)-7-Carboxy-8-(2,4-dioxoimidazolin-1-yl)-3,5-dioxo-2,4-diazobicyclo[4.2.0]octane-2-acetic Acid (**9**). The photodimer **7** [8] (2.0 g, 5.46 mmol) was suspended in 100 ml of aq. LiOH soln. (4.05 g, 169 mmol) and stirred at r.t. for 20 h. Then, conc. HCl soln. was added until a pH of 1 was reached. The soln. was stored at 4° for 3 d. The crystalline material was filtered and recrystallized from H_2O to yield **9** (1.35 g, 72%) as colorless needles which were dried over P_2O_5. M.p. 273–275°. IR (KBr): 3434, 3193, 3056, 2989, 1722, 1693, 1458, 1389, 1361, 1306, 1230, 1121, 817, 763, 750, 550, 432. ^{1}H-NMR (200 MHz, $(CD_3)_2SO$): 3.30 (*dd*, J = 2.5, 9.6, 1 H); 3.54 (*dd*, J = 2.5, 9.6, 1 H); 3.79 (*d*, J = 17.5, 1 H); 3.99 (*d*, J = 17.8, 1 H); 4.06 (*d*, J = 17.5, 1 H); 4.18 (*d*, J = 17.8, 1 H); 4.63 (*t*, J = 9.6, 1 H); 4.82 (*t*, J = 9.6, 1 H), 10.65 (*s*, NH), 10.98 (*s*, NH), 12.80 (br. *s*, 2 COOH). ^{13}C-NMR (50 MHz, $(CD_3)_2SO$): 35.52; 44.19; 47.20; 49.46; 53.70; 55.08; 151.33; 155.99; 169.99; 170.42; 170.80; 171.53. FAB-MS: 339 (75, $[M - H]^-$), 153 (100). Anal. calc. for $C_{12}H_{12}N_4O_8 \cdot H_2O$ (358.26): C 40.23, H 3.94, N 15.69; found: C 40.46, H 4.01, N 15.51.

Benzyl 1,2,3,4-Tetrahydro-2,4-dioxopyrimidine-1-acetate (**10**). *1,2,3,4-Tetrahydro-2,4-dioxopyrimidine-1-acetic acid* (**6**) (4.97 g, 29.2 mmol) and 1,1′-carbonylbis[1*H*-diimidazol] (6.00 g, 37.0 mmol) in DMF (50 ml) were stirred at r.t. After 10 min, $PhCH_2OH$ (4.20 g, 39.0 mmol) was added to the soln. and the mixture stirred at r.t. for 28 h. The mixture was evaporated, the solid residue suspended in H_2O (30 ml) and the solid material obtained filtered and dried over P_2O_5 *in vacuo*: **10** (7.19 g, 95%). Colorless needles. M.p. 187–188°. IR (KBr): 3422, 3156, 3111, 3031, 2878, 2827, 1733, 1717, 1682, 1468, 1428, 1384, 1347, 1254, 1231, 1205, 1103, 948, 886, 828, 806, 761, 710, 580, 550, 493, 428. ^{1}H-NMR (200 MHz, $(CD_3)_2SO$): 4.60 (*s*, 2 H); 5.20 (*s*, 2 H); 5.63 (*d*, J = 7.9, 1 H); 7.39 (*s*, 5 arom. H); 7.65 (*d*, J = 7.9, 1 H); 11.43 (*s*, NH). ^{13}C-NMR (50 MHz, $(CD_3)_2SO$); 48.80; 66.63; 101.36; 128.22; 128.49; 128.74; 135.79; 146.11; 151.28; 164.02; 169.12. EI-MS: 28 (8), 65 (10), 82 (52), 91 (100), 153 (21), 260 (4, M^+). Anal. calc. for $C_{13}H_{12}N_2O_4$ (260.25): C 60.00, H 4.65, N 10.76; found: C 59.93, H 4.92, N 10.50.

Irradiation of Benzyl Ester **10**. Ester **10** (4.00 g, 15.4 mmol) was suspended in acetone (200 ml) in a standard photochemical-reaction apparatus, equipped with a *TQ-150* medium-pressure Hg lamp, cooled with tap water. N_2 was bubbled through the soln. for 30 min. The suspension was then irradiated for 3 h under N_2 and filtered, giving the *cis-transoid-cis,anti*-isomer **3** (820 mg 21%) as a white solid. The filtrate was evaporated and the residual material suspended in Et_2O and sonicated for *ca.* 20 min. The soln. then was filtered and evaporated to give an oil. Addition of 60 ml of ice-cold $CHCl_3$ to the resulting oil caused precipitation of the *cis-cisoid-cis,syn*-isomer **2**, which was filtered off and recrystallized from $CHCl_3$. The filtrate was evaporated, the remaining material was treated with boiling toluene and the toluene was decanted to remove nonpolar impurities. The procedure was repeated once. The residual material (1.6 g) was dissolved in acetone (200 ml), silica gel (30 g) was added, and the slurry evaporated. Isolation of the remaining isomers was achieved by CC (silica gel *H*, $CHCl_3$/MeOH 15:1), which afforded the *cis-cisoid-cis,anti*-isomer **4** and the *cis-cisoid-cis,syn*-isomer **1**. Recrystallization of **1** from $CHCl_3$/acetone yielded **1** as a white powder.

Dibenzyl cis-*4a*-transoid-*4a,4b*-*cis*-*4b-Dodecahydro-2,4,6,8-tetraoxocyclobuta[1,2-d:3,4-d′]dipyrimidine-1,5-diacetate* (**3**): 1.64 g (21%). R_f ($CHCl_3$/MeOH 10:1) 0.42. M.p. 286°. IR (KBr): 3286, 1733, 1717, 1684, 1484, 1417, 1394, 1375, 1290, 1231, 1194, 987, 747, 696. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 3.76 (*m*, 2 H); 4.02 (*d*, J = 17.8, CH_2); 4.20 (*d*, J = 17.6, CH_2); 4.34 (*m*, 2 H); 5.17 (*s*, 2 CH_2); 7.36 (*s*, 10 arom. H); 10.69 (*s*, 2 NH). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): 43.0; 46.22; 52.89; 66.13; 127.77; 127.92; 128.34; 135.59; 151.13; 168.28; 168.37. FAB-MS: 543 (100, $[M + Na]^+$). Anal. calc. for $C_{16}H_{24}N_4O_8 \cdot 1/2\ H_2O$ (529.50): C 58.97, H 4.76, N 10.58; found: C 59.16, H 4.76, N 10.94.

Dibenzyl cis-*4a*-transoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,5,7-tetraoxocyclobuta[1,2-d:4,3-d′]dipyrimidine-1,8-diacetate* (**2**): 1.2 g (15%). R_f ($CHCl_3$/MeOH 10:1) 0.31. M.p. 217°. IR (KBr): 3199, 3067, 1689, 1477, 1406, 1365, 1317, 1276, 1217, 1194, 972, 741, 700. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 3.41 (*m*, 2 H); 4.09 (*s*, 2 CH_2); 4.36 (*m*, 2 H); 5.05 (*d*, J = 12.5, CH_2); 5.13 (*d*, J = 12.5, CH_2); 7.35 (*s*, 10 arom. H); 10.71 (*s*, 2 NH). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): one signal covered by $(CD_3)_2SO$; 48.42; 59.29; 66.00; 127.89; 128.04; 128.34; 135.48; 151.58; 169.22; 169.64. FAB-MS: 521 (71, MH^+), 261 (100). Anal. calc. for $C_{26}H_{24}N_4O_8 \cdot 1/2\ H_2O$ (529.50): C 58.97, H 4.76, N 10.58; found: C 58.85, H 4.76, N 10.88.

Dibenzyl cis-*4a*-cisoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,6,8-tetraoxocyclobuta[1,2-d:3,4-d′]dipyrimidine-1,5-diacetate* (**4**): 40 mg (0.5%). R_f ($CHCl_3$/MeOH 10:1) 0.42. M.p. 227–230°. IR (KBr): 1712, 1475, 1411, 1389, 1361, 1300, 1267, 1222, 1194, 1178, 994, 744, 694. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 3.79 (*t*, J = 8.7, 2 H); 3.89 (*d*, J = 17.9, CH_2); 4.38 (*t*, J = 8.7, 2 H); 4.43 (*d*, J = 17.9, CH_2); 5.17 (*s*, 2 $PhCH_2$); 7.37 (*s*, 10 arom. H); 10.66 (*s*, 2 NH). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): 44.06; 46.91; 48.16; 65.98; 127.73; 128.00; 128.34; 135.65; 151.45; 167.39; 168.62. FAB-MS: 521 (5, MH^+), 74 (100).

Dibenzyl cis-*4a*-cisoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,5,7-tetraoxocyclobuta[1,2*-d*:3,4*-d*']dipyrimidine-1,8-diacetate* (**1**): 400 mg (5%). R_f ($CHCl_3$/MeOH 10:1) 0.24. M.p. 180–183°. IR (KBr): 3267, 1744, 1696, 1475, 1389, 1361, 1278, 1218, 1200, 1000, 759, 694. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 3.74 (*m*, 2 H); 4.00 (*d*, *J* = 17.4, 2 H); 4.21 (*m*, 2 H); 4.24 (*d*, *J* = 17.4, 2 H); 5.14 (*s*, 2 CH_2); 7.36 (*s*, 10 arom. H); 10.58 (*s*, 2 NH). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): 38.11; 47.20; 54.97; 66.00; 127.75; 128.01; 128.33; 135.61; 152.63; 166.94; 168.56. FAB-MS: 521 (5, MH^+), 74 (100).

cis-*4a*-transoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,6,8-tetraoxocyclobuta[1,2*-d*:3,4*-d*']dipyrimidine-1,5-diacetic Acid* (**11**). Diester **3** (500 mg, 1 mmol) was suspended in 1N NaOH. The mixture was stirred at r.t. for 3.5 h, then conc. HCl soln. was added until pH 1 was reached. The soln. was allowed to stand for 24 h at 4°. The white precipitate was filtered, washed with H_2O several times, and dried over P_2O_5: 291 mg (86%) of **11**. M.p. > 300°. IR (KBr): 3433, 3237, 1733, 1698, 1485, 1433, 1384, 1301, 1237, 1206, 989, 801, 750, 656, 606, 566, 533. ^{1}H-NMR (200 MHz, $(CD_3)_2SO$): 3.76 (*m*, 2 H); 3.80 (*d*, *J* = 17.4, CH_2); 4.10 (*d*, *J* = 17.4, CH_2); 4.28 (*m*, 2 H); 10.61 (*s*, 2 NH). ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): 42.86; 45.91; 52.76; 151.03; 168.43; 169.74. FAB-MS: 341 (13, MH^+), 307 (100). Anal. calc. for $C_{12}H_{12}N_4O_8 \cdot 1\,H_2O$ (358.26): C 40.23, H 3.94, N 15.60; found: C 39.96, H 3.80, N 15.19.

cis-*4a*-transoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,5,7-tetraoxocyclobuta[1,2*-d*:3,4*-d*']dipyrimidine-1,8-diacetic Acid* (**8**) [8]. Diester **2** (1.05 g, 2.02 mmol) in 50 ml conc. HCl son. was refluxed for 1 h. The soln. was cooled and allowed to stand for 24 h at 4°. The white precipitate was filtered, washed with H_2O several times, and dried over P_2O_5: 350 mg (51%) of **8**. M.p. > 300°. IR (KBr): 3167, 3056, 2856, 1744, 1690, 1485, 1420, 1383, 1318, 1301, 1233, 1189, 977, 889, 850, 797, 750, 650, 600, 558, 522, 500, 458, 437. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 3.43 (*m*, 2 H); 3.90 (*d*, *J* = 17.5, 2 H, CH_2); 4.00 (*d*, *J* = 17.5, CH_2); 4.30 (*m*, 2 H); 10.64 (*s*, 2 NH); 12.80 (br. *s*, 2 COOH). ^{13}C-NMR (50 MHz, $(CD_3)_2SO$): one signal covered by $(CD_3)_2SO$; 48.14; 59.19; 151.17; 169.40; 170.33. FAB-MS: 341 (10, MH^+), 307 (100). Anal. calc. for $C_{12}H_{12}N_4O_8 \cdot 1/2\,H_2O$ (349.25): C 41.27, H 3.75, N 16.04; found: C 41.34, H 4.03, N 16.04.

cis-*4a*-cisoid-*4a,4b*-cis-*4b-Dodecahydro-2,4,5,7-tetraoxocyclobuta[1,2*-d*:4,3*-d*']dipyrimidine-1,8-diacetic Acid* (**12**). To the diester **1** (415 mg, 0.798 mmol) in AcOH (25 ml), a suspension of 10% Pd/C (10 mg), in AcOH (1 ml) was slowly added. The mixture was stirred for 2 h under H_2 and then filtered through *Celite*. The *Celite* was washed with hot AcOH, the combined AcOH solns. evaporated, and Et_2O added to the remaining oil to precipitate **12**. The precipitate was filtered off, washed with acetone (5 ml), and dried *in vacuo*: **12** (247 mg, 91%). White powder. M.p. 280–283°. IR (KBr): 3600–2800, 3422, 3189, 3067, 2844, 1710, 1690, 1483, 1394, 1372, 1278, 1189, 756, 533. ^{1}H-NMR (200 MHz, $(CD_3)_2SO$): 3.39 (*d*, *J* = 16.8, CH_2); 3.64 (*m*, 2 H); 4.10 (*d*, *J* = 16.8, CH_2); 4.13 (*m*, 2 NH); 10.28 (*s*, 2 H). ^{13}C-NMR (50 MHz, $(CD_3)_2SO$): 21.0; 47.4; 54.7; 152.5; 167.2, 170.1. FAB-MS (pos. mode): 341 (100, MH^+).

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