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Photodimerization in pyrimidine-substituted dipeptides

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Abstract: Ten N^{ε}-glycylornithineamide derivatives have been synthesized containing various N^{α}-linked pyrimidine-1-ylacetyl groups which can undergo $(2\pi + 2\pi)$ photodimerization on irradiation with UV light at 254 nm. The dimerization efficiency of the free and bound pyrimidine groups was compared in aqueous solution: it was dependent on the substitution of the pyrimidine ring. N^{α},$N^{\alpha'}$-bis-(uracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) and N^{α},$N^{\alpha'}$-bis-(5-bromouracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) were identified as possible candidates for optical data storage.

Keywords: pyrimidine; photodimerization; cycloaddition; dipeptide

INTRODUCTION

High capacity optical storage of information represents an important segment of information technology. With the availability of Blu-Ray disc technology, a digital storage capacity of 25 Gbytes can be achieved in a single 120 mm disc using blue light from a 405 nm diode laser. A further increase in the storage capacity can be achieved through the employment of even shorter wavelength lasers. Materials that change their property when irradiated with shorter wavelengths are thus of great interest. A suitable optical property for this type of recording can be based on photodimerization of chromophores in the ultraviolet range, e.g. 260 nm. Peptides substituted with azobenzene chromophores sensitive for the blue and green wavelengths for optical data storage have previously been investigated [1–3]. This paper describes chromophores attached to a peptide backbone, where the storage of data can be achieved through the principle of photodimerization of neighbouring chromophores undergoing $(2\pi + 2\pi)$ cycloaddition [4]. Peptides were chosen since they meet several important criteria as materials. They are amorphous, can be water soluble, transparent and it is possible to make thin smooth peptide films from these materials. The peptides are versatile, and variations in the backbone for similar systems have resulted in significantly improved properties with respect to recording speed [2]. Furthermore the peptides are environmentally friendly, and suitable for upgrade to large-scale production. The aim of this work was to find a reliable system, which possesses good dimerization efficiency, good recording speed and reversibility.

The system chosen (Figure 1) was tested by synthesizing a series of ornithine-based dipeptides containing chromophores of various pyrimidine analogues including thymine, which can undergo photoinduced $(2\pi + 2\pi)$ cycloaddition reactions on exposure to UV light at 254 nm. Thymine-substituted peptide nucleic acids (PNA) [5] with an ornithine-based backbone are known [6]. The peptides were synthesized by standard Merrifield solid-phase synthesis [7].

It is known [8] that one of the four bases in DNA, thymine, is dimerizable via $(2\pi + 2\pi)$ cycloaddition on exposure to UV-light, as shown in our system in Figure 1.

The two chromophores in the dipeptides studied here can be identical, e.g. thymine and thymine (homodipeptide) or they can be different, e.g. uracil and thymine (heterodipeptide), but should be able to form mutual cycloaddition reaction products (cycloadducts) as shown in Figure 1. The photodimerization process of pyrimidine bases is dependent on a number of factors. Two factors were investigated in this study. First, coupling the chromophores to a backbone should increase the probability of better alignment, and in all cases shorten the distance between two chromophores, giving a theoretical possibility of perfect alignment. Second, when the pyrimidines are in their excited states, the chemical properties of the atoms/groups in the C5–C6 bond will determine their capability for photodimerization, especially regarding dimerization efficiency.

Abbreviations: YAG-laser, yttrium- aluminium-garnet; kcps, kilo counts per second.

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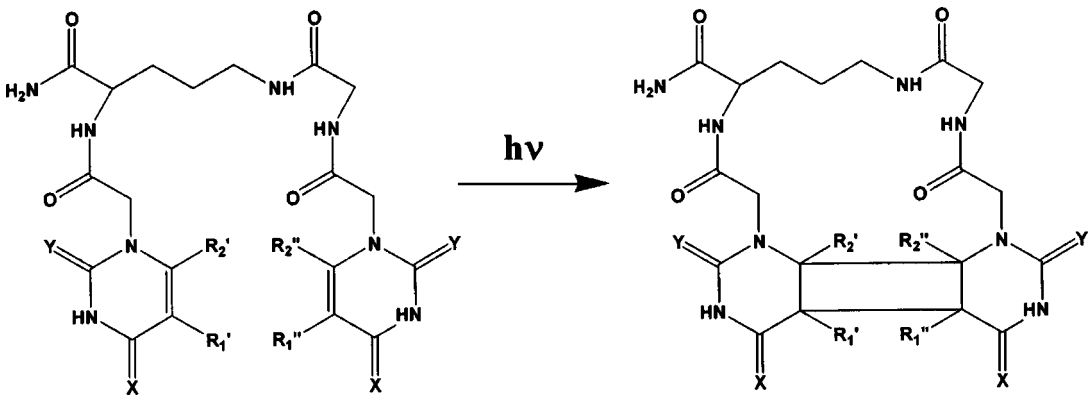

Figure 1 Photodimerization of neighbouring pyrimidine chromophores attached to an ornithine-based backbone.

MATERIALS AND METHODS

Melting point measurements were done on a Büchi SMP-20 apparatus. Peptide synthesis was carried out manually in a solid phase peptide synthesis reaction vessel. NMR spectra were recorded on a Bruker 250 MHz/52MM apparatus. Irradiation was done, using a UV-lamp, Spectroline® model ENF-260C/FE (CM-10), and spectra were recorded on a Shimadzu UV-1700 spectrophotometer. Also one irradiation experiment was performed using a 450 W xenon arc lamp, Xe-900 (Edinburgh Instruments Ltd), incorporated into a lamp-housing LAX 1450 (Müller Elektronik, Moosinning). MALDI-TOF mass spectra were recorded on a Bruker Reflex IV, using the following method: Reflectron MALDI-TOF with pulsed ion extraction (PIE™), positive ion analysis, where α-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid, were used as a matrix reagent. TLC was done in the solvent system ethyl acetate/methanol/acetic acid in a (75/20/5, v/v/v) ratio, using ALUGRAM® SIL G/UV_{254} 0.20 mm silica gel 60. Dynamic laser light scattering was measured on a Brookhaven Instrument, with a BI-ADP detector system, BI-200 SM Goniometer, BI-9000AT digital auto-correlator and a Melles Griot HeNe laser.

Peptide Synthesis

The dipeptides were assembled by a stepwise Merrifield method using three different monomer subunits. First, N^{α}-Fmoc-L-Orn (N^{δ}-Boc)-OH was coupled to 4-benzhydrylamine (BHA) resin. Then, Boc-Gly-OH was coupled via the δ-amino group of ornithine. The third monomer subunit was various pyrimidines all containing an *N*-1-acetic acid group for coupling to the N^{α}-amino groups. To obtain a *C*-terminal amide, the dipeptides were assembled on the BHA 1% DVB cross-linked polystyrene resin, (100–200 mesh) initially loaded with 0.42 mmol amino groups per gram resin. In most of the syntheses, activation by BOP allowed efficient incorporation of backbone and pyrimidine residues when the couplings were performed in DCM with a monomer concentration of 0.10 M and a BOP concentration of 0.10 M in combination with 0.20 M DIEA. Activation by HBTU allowed efficient incorporation of pyrimidine residues where BOP did not suffice. In that case, a 0.75 ml pyridine solution containing 0.20 M pyrimidine monomer and 0.40 M DIEA, and a 0.75 ml DMF solution containing 0.16 M HBTU, were mixed and added to the resin. After coupling of the monomer, the resin was washed sequentially with DMF (2 × 2 min) and DCM (2 × 2 min). The alternating deprotections of N^{α}-Fmoc and N^{δ}-Boc groups were accomplished with 20% (v/v) piperidine in DMF and 5% (v/v) *m*-cresol in TFA, respectively. The following protocol was used to deprotect the Fmoc group: 20% (v/v) piperidine in DMF (1 × 30 min), DMF (1 × 2 min), DCM (1 × 2 min), 10% (v/v) DIEA in DCM (1 × 2 min), DCM (1 × 2 min) and 50% DCM in DMF (1 × 2 min). The following protocol was used to deprotect the Boc group: 5% (v/v) *m*-cresol in TFA (2 × 2 min), DCM (3 × 2 min), 10% (v/v) DIEA in DCM (2 × 2 min) and DCM (2 × 2 min). The progress of the synthesis was monitored throughout by a ninhydrin reaction (Kaiser test) [9] which indicated that the single coupling of each backbone as well as each pyrimidine residue proceeded with an efficiency of >95%. Cleavage of a particular dipeptide from its resin was accomplished with neat anhydrous HF at 0 °C for 1 h. The compound was then extracted from the resin with 50% (v/v) TFA in DCM and was obtained after evaporation of the solvents.

Preparation of Pyrimidine Chromophores

Thymine 1-acetic acid (1). ^{1}H-NMR (DMSO-d_6), δ (ppm): 13.06 (H, NH), 1.74 (3H, CH_3), 7.47 (1H, CH), 4.40 (2H, CH_2), 11.31 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 152.5 (C-2), 164.4 (C-4), 109.7 (C-5), 134.8 (C-6), 55.1 (C-7), 176.3 (C-8), 16.1 (CH_3); $R_f = 0.51$, yield 52%, melting point 273°–277 °C; **1** was prepared using an N-alkylation method described specifically for 5-bromouracil [10], but the synthetic procedure worked satisfactorily for **1** and all the pyrimidine analogues synthesized as well.

Uracil 1-acetic acid (2). ^{1}H-NMR (DMSO-d_6), δ (ppm): 13.1 (H, NH), 5.57 (1H, CH), 7.58 (1H, CH), 4.39 (2H, CH_2), 11.31 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 151.9 (C-2), 165.9 (C-4), 100.3 (C-5), 142.2 (C-6), 54.8 (C-7), 177.1 (C-8); $R_f = 0.58$, yield 55%, melting point >300 °C; same synthetic procedure as for **1**.

5-Chlorouracil 1-acetic acid (3). ^{1}H-NMR (DMSO-d_6), δ (ppm): 13.1 (very broad) (H, NH), 8.13 (1H, CH), 4.38 (2H, CH_2), 11.90 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 153.2 (C-2), 165.1 (C-4), 107.1 (C-5), 136.1 (C-6), 54.3 (C-7), 174.7 (C-8); $R_f = 0.64$, yield 46%, melting point 283°–286 °C; same synthetic procedure as for **1**.

5-Bromouracil 1-acetic acid (4). ^{1}H-NMR (DMSO-d_6), δ (ppm): not visible (H, NH), 8.21 (1H, CH), 4.40 (2H, CH_2), 11.89 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 150.8 (C-2), 160.1 (C-4), 95.0 (C-5), 146.1 (C-6), 49.2 (C-7), 169.7 (C-8); $R_f = 0.53$, yield 48%, melting point 250°–251 °C; same synthetic procedure as for **1**.

5-Fluorouracil 1-acetic acid (5). ^{1}H-NMR (DMSO-d_6), δ (ppm): 13.2 (broad) (H, NH), 8.07 (1H, CH), 4.34 (2H, CH_2), 11.88 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 152.7 (C-2), 165.1 (C-4), 127.7 (C-5), 107.9 (C-6), 53.9 (C-7), 175.9 (C-8); $R_f = 0.45$, yield 52%, melting point 253°–256 °C; same synthetic procedure as for **1**.

5-Iodouracil 1-acetic acid (6). ^{1}H-NMR (DMSO-d_6), δ (ppm): 13.1 (broad) (H, NH), 8.19 (1H, CH), 4.39 (2H, CH_2), 11.65 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 153.6 (C-2), 167.3 (C-4), 59.2 (C-5), 151.5 (C-6), 53.5 (C-7), 177.2 (C-8); $R_f = 0.54$, yield 87%, melting point 193°–195 °C; **6** was synthesized by an iodination of uracil followed by N-alkylation [11].

2,4 Dithiouracil 1-acetic acid (7). ^{1}H-NMR (DMSO-d_6), δ (ppm): not visible (H, NH), 8.31 (1H, CH), 7.23 (1H, CH), 4.07 (2H, CH_2), 12.82 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 174.0 (C-2), 188.5 (C-4), 114.9 (C-5), 142.3 (C-6), 61.2 (C-7), 175.0 (C-8); $R_f = 0.68$, yield 92%, melting point >300 °C; **7** was synthesized according to ref. [12], and the thionation was done on 2-thiouracil.

6-Methyl 2-thiouracil 1-acetic acid (8). ^{1}H-NMR (DMSO-d_6), δ (ppm): not visible (H, NH), 5.98 (1H, CH), 2.12 (3H, CH_3), 3.91 (2H, CH_2), 12.64 (H, OH); ^{13}C-NMR (DMSO-d_6), δ (ppm): 184.2 (C-2), 168.9 (C-4), 94.9 (C-5), 161.4 (C-6), 57.7 (C-7), 179.0 (C-8), 21.2 (CH_3) $R_f = 0.57$, yield 96%, melting point >300 °C; same synthetic procedure as for **7**.

N^{α},$N^{\alpha'}$-bis-(thymine-1-ylacetyl)-(N^{ε}-glycylornithine amide) (9). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.10 (2H, Orn-NH_2), 8.05 (1H, Orn-NH-αC), 4.35 (1H, Orn-αCH), 1.73 (2H, Orn-βCH_2), 1.45 (2H, Orn-γCH_2), 3.87 (2H, Orn-δCH_2), 8.43 (1H, Orn-εNH), 3.51 (2H, Gly-αCH_2), 8.10 (1H, Gly-βNH); (thymine-1-ylacetyl substituent): 3.12 (2H, CH_2), 11.30 (1H, NH), 7.54 (1H, CH), 2.06 (3H, CH_3); m/z (MALDI-TOF MS) [Found: 543.34 $(C_{21}H_{28}N_8O_8 + Na)^+$ requires 543.49].

N^{α},$N^{\alpha'}$-bis-(uracil-1-ylacetyl)-(N^{ε}-glycylornithine amide) (10). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.10 (2H, Orn-NH_2), 8.08 (1H, Orn-NH-αC), 4.35 (1H, Orn-αCH), 1.68 (2H, Orn-βCH_2), 1.45 (2H, Orn-γCH_2), 3.70 (2H, Orn-δCH_2), 8.43 (1H, Orn-εNH), 4.09 (2H, Gly-αCH_2), 8.12 (1H, Gly-βNH); (uracil-1-ylacetyl substituent): 4.39 (2H, CH_2), 11.31 (1H, NH), 7.84 (1H, CH), 5.57 (1H, CH); m/z (MALDI-TOF MS) [Found: 515.40 $(C_{19}H_{24}N_8O_8 + Na)^+$ requires 515.43].

N^{α},$N^{\alpha'}$-bis-(chlorouracil-1-ylacetyl)-(N^{ε}-glycylorni thineamide) (11). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 5.97 (2H, Orn-NH_2), 8.04 (1H, Orn-NH-αC), 4.29 (1H, Orn-αCH), 1.69 (2H, Orn-βCH_2), 1.37 (2H, Orn-γCH_2), 3.17 (2H, Orn-δCH_2), 8.10 (1H, Orn-εNH), 4.11 (2H, Gly-αCH_2), 8.09 (1H, Gly-βNH); (5-chlorouracil-1-ylacetyl substituent): 4.45 (2H, CH_2), 13.0 (broad)(1H, NH), 8.27 (1H, CH); m/z (MALDI-TOF MS) [Found: 584.04 $(C_{19}H_{22}Cl_2N_8O_8 + Na)^+$ requires 584.32].

N^{α},$N^{\alpha'}$-bis-(bromouracil-1-ylacetyl)-(N^{ε}-glycylorni thineamide) (12). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.13 (2H, Orn-NH_2), 8.0 (1H, Orn-NH-αC), 4.35 (1H, Orn-αCH), 1.74 (2H, Orn-βCH_2), 1.49 (2H, Orn-γCH_2), 3.22 (2H, Orn-δCH_2), 8.05 (1H, Orn-εNH), 4.39 (2H, Gly-αCH_2), 8.0 (1H, Gly-βNH); (5-bromouracil substituent): 4.05 (2H, CH_2), 11.0 (1H, NH), 8.24 (1H, CH); m/z (MALDI-TOF MS) [Found: 673.3 $(C_{19}H_{22}Br_2N_8O_8 + Na)^+$ requires 673.23].

N^{α},$N^{\alpha'}$-bis-(fluorouracil-1-ylacetyl)-(N^{ε}-glycylorni thineamide) (13). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.10 (2H, Orn-NH_2), 8.16 (1H, Orn-NH-αC), 4.35 (1H, Orn-αCH), 1.73 (2H, Orn-βCH_2), 1.45 (2H, Orn-γCH_2), 3.20 (2H, Orn-δCH_2), 8.21 (1H, Orn-εNH), 4.09 (2H, Gly-αCH_2), 8.11 (1H, Gly-βNH); (5-fluorouracil-1-ylacetyl substituent): 4.25 (2H, CH_2), 11.75 (1H, NH), 8.09 (1H, CH); m/z (MALDI-TOF MS) [Found: 551.50 $(C_{19}H_{22}F_2N_8O_8 + Na)^+$ requires 551.41].

N^{α},$N^{\alpha'}$-bis-(iodouracil-1-ylacetyl)-(N^{ε}-glycylorni thineamide) (14). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.0 (2H, Orn-NH_2), 8.32 (1H, Orn-NH-αC), 4.31 (1H, Orn-αCH), 1.79 (2H, Orn-βCH_2), 1.50 (2H, Orn-γCH_2), 3.23 (2H, Orn-δCH_2), 8.14 (1H, Orn-εNH), 4.11 (2H, Gly-αCH_2), 8.25 (1H, Gly-βNH); (5-iodouracil-1-ylacetyl substituent): 4.05 (2H, CH_2), 12.87 (1H, NH), 8.10 (1H, CH); m/z (MALDI-TOF MS) [Found: 767.32 $(C_{19}H_{22}I_2N_8O_8 + Na)^+$ requires 767.23].

N^{α}-(uracil-1-ylacetyl), $N^{\alpha'}$-(bromouracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) (15). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.15 (2H, Orn-NH_2), 8.09 (1H, Orn-NH-αC), 4.37 (1H, Orn-αCH), 1.73 (2H, Orn-βCH_2), 1.45 (2H, Orn-γCH_2), 3.20 (2H, Orn-δCH_2), 8.20 (1H, Orn-εNH), 4.09 (2H, Gly-αCH_2), 8.12 (1H, Gly-βNH); (5-bromouracil-1-ylacetyl substituent): 4.45 (2H, CH_2), 11.0 (1H, NH), 8.23 (1H, CH); (uracil-1-ylacetyl substituent): 4.35 (2H, CH_2), 11.27 (1H, NH), 7.76 (1H, CH), 5.79 (1H, CH); m/z (MALDI-TOF MS) [Found: 594.04 $(C_{19}H_{23}BrN_8O_8 + Na)^+$ requires 594.33].

N^{α}-(fluorouracil-1-ylacetyl), $N^{\alpha'}$-(bromouracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) (16). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.20 (2H, Orn-NH_2), 7.97 (1H, Orn-NH-αC), 4.20 (1H, Orn-αCH), 1.42 (2H, Orn-βCH_2), 1.25 (2H, Orn-γCH_2), 3.11 (2H, Orn-δCH_2), 8.12 (1H, Orn-εNH), 4.38 (2H, Gly-αCH_2), 8.0 (1H, Gly-βNH); (5-bromouracil-1-ylacetyl substituent): 4.32 (2H, CH_2), 11.79 (1H, NH), 8.12 (1H, CH); (5-fluorouracil-1-ylacetyl substituent): 4.34 (2H, CH_2), 11.86 (1H, NH), 7.33 (1H, CH); m/z (MALDI-TOF MS) [Found: 612.12 $(C_{19}H_{22}BrFN_8O_8 + Na)^+$ requires 612.32].

N^{α},$N^{\alpha'}$-bis-(2,4-dithiouracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) (17). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 6.18 (2H, Orn-NH_2), 8.49 (broad) (1H, Orn-NH-αC), 4.34 (1H, Orn-αCH), 1.93 (2H, Orn-βCH_2), 1.45 (2H, Orn-γCH_2), 3.20 (2H, Orn-δCH_2), 8.71 (1H, Orn-εNH), 4.03 (2H, Gly-αCH_2), 8.49 (broad) (1H, Gly-βNH); (2,4-dithiouracil-1-ylacetyl substituent): 4.39 (2H, CH_2), not visible (1H, NH), 7.09 (1H, CH), 8.20 (1H, CH).

N^{α},$N^{\alpha'}$-bis-(6-methyl-2-thiouracil-1-ylacetyl)-(N^{ε}-glycylornithineamide) (18). ^{1}H-NMR (DMSO-d_6), δ (ppm): (Backbone): 5.27 (2H, Orn-NH_2), 8.60 (broad) (1H, Orn-NH-αC), 4.23 (1H, Orn-αCH), 2.12 (2H, Orn-βCH_2), 1.48 (2H,

Orn-γCH_2), 3.19 (2H, Orn-δCH_2), 8.60 (broad) (1H, Orn-εNH), 4.06 (2H, Gly-αCH_2), 8.60 (broad) (1H, Gly-βNH); (6-methyl-2-thiouracil-1-ylacetyl substituent): 3.91 (2H, CH_2), 10.79 (1H, NH), 5.99 (1H, CH), 1.98 (3H, CH_3); *m/z* (MALDI-TOF MS) [Found: 575.50 $(C_{21}H_{28}N_8O_6S_2 + Na)^+$ requires 575.62].

RESULTS AND DISCUSSION

The chemical structures of the pyrimidine chromophores and pyrimidine-substituted dipeptides prepared and investigated in this study are shown in Figures 2 and 3, respectively.

The synthesized dipeptides all have the general structure Gly-(N^{α}-pyrimidine)-L-Orn-(N^{α}-pyrimidine)-NH_2.

The synthesized peptides were not purified after cleavage from the resin. For all peptides synthesized the coupling and deprotection steps were checked by ninhydrin analysis. All steps were generally found to be close to quantitative and if not they were repeated. NMR showed that small amounts of impurities were present, but it was not found necessary to do a preparative HPLC. Earlier work [2] showed that similar compound structures (azobenzene-substituted peptides), when purified by preparative HPLC did not lead to any improvement of the recording properties, indicating that the behaviour is not affected significantly by the presence of small amounts of impurities. Nearly all synthesized compounds **1**–**18** studied had a concentration of approx. 200 μg in 1 ml H_2O, in order to reach an absorbance of 1, to make the various synthesized products comparable. Various solvents were tested (DMF, acetonitrile, NMP, water, ethanol, methanol and DCM). Water proved to be the best solvent, which practically dissolved all compounds tested without the need for heating. Furthermore, water's absorption does not

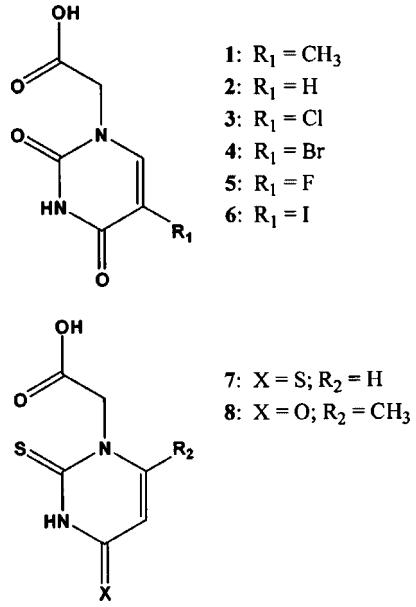

Figure 2 Chemical structures of pyrimidine chromophores **1**–**8**.

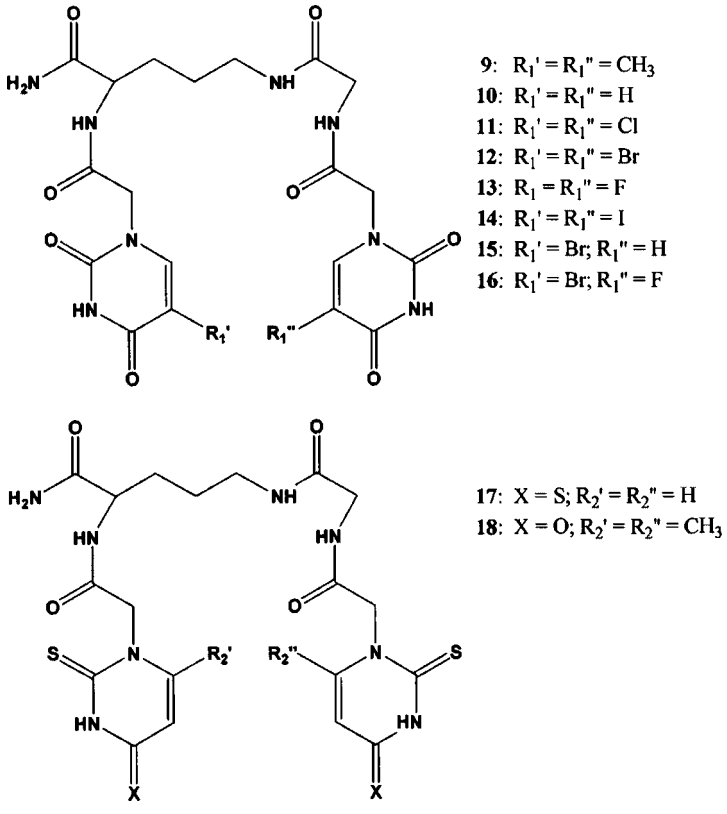

Figure 3 Chemical structures of pyrimidine-substituted dipeptides **9**–**18**.

interfere with the absorbance area of the pyrimidines. For the dimerization experiment, a quartz cuvette of the type 110-QS, with the light path of 1 mm, was used. The dimerization was done using a standard UV-lamp with 6 W and a wavelength of 254 nm, giving 420 $\mu W/cm^2$. The sample cuvette was placed 5 cm from the irradiating source, in a fixed position and irradiated for 15, 30 and 60 min, respectively. The sample was measured before irradiation and after each irradiation period in a UV-spectrophotometer giving an indication of the dimerization efficiency. The decrease in absorbance is an indication of how much of the compound in the solution has dimerized. The dimerization efficiency of the compounds **1**–**18** was investigated in this study. The significance of substituents was investigated by synthesizing various pyrimidine analogues. First the experiments were done on the free chromophores in solution, then on the chromophores attached to a peptide backbone. The absorption spectra of pyrimidines show a strong peak at ~260 nm, due to the 5,6-double bond. When two pyrimidines are converted to a dimer, the 5,6-bond is saturated and the absorption from the double bond disappears. Therefore, when a solution of pyrimidine is irradiated near or at λ_{max}, the chromophores are converted into dimers and the absorbance decreases.

Photodimerization of Pyrimidines and Corresponding Homodipeptides and Heterodipeptides

Only spectra for thymine 1-acetic acid (**1**) and 5-bromouracil 1-acetic acid (**4**) and their corresponding dipeptides **9** and **12** are shown here; their absorption values are shown in Figure 4.

The absorption values (λ_{max}) of all compounds tested are collected in Figure 5(a) for the chromophores and the dipeptides in Figure 5(b), and display the absorbance values plotted against time at 0, 15, 30 and 60 min, respectively. In Figure 5(a), **3** and **8** has been left out for clarity, due to the fact that these absorbance values resemble that of **7**.

Heterodipeptides **15** and **16** were synthesized in order to see if two different chromophores coupled to the same backbone, each having good dimerization efficiency, would lead to an even better dimerization efficiency. The results for **15** and **16** are shown in Figure 6.

A significant role in the photodimerization of pyrimidines is played by the electronic factors in the chromophores. The dimerization takes place through the C-5, C-6 bond of thymine and involves the formation of a cyclobutane ring [13] as shown for our system in Figure 1. When dealing with photodimerization of the free chromophores dissolved in water, the photodimerization is determined mainly by the electronic properties of the C5 and C6 atoms/groups, but also the bulkiness of the group(s) have a significant influence, e.g. methyl. The main hindrance for photodimerization arising in this case for solutions, especially for dilute solutions, is that the excited state lifetime of the bases is usually small compared with the diffusion processes in the solution [14]. Complete appreciation of the steric

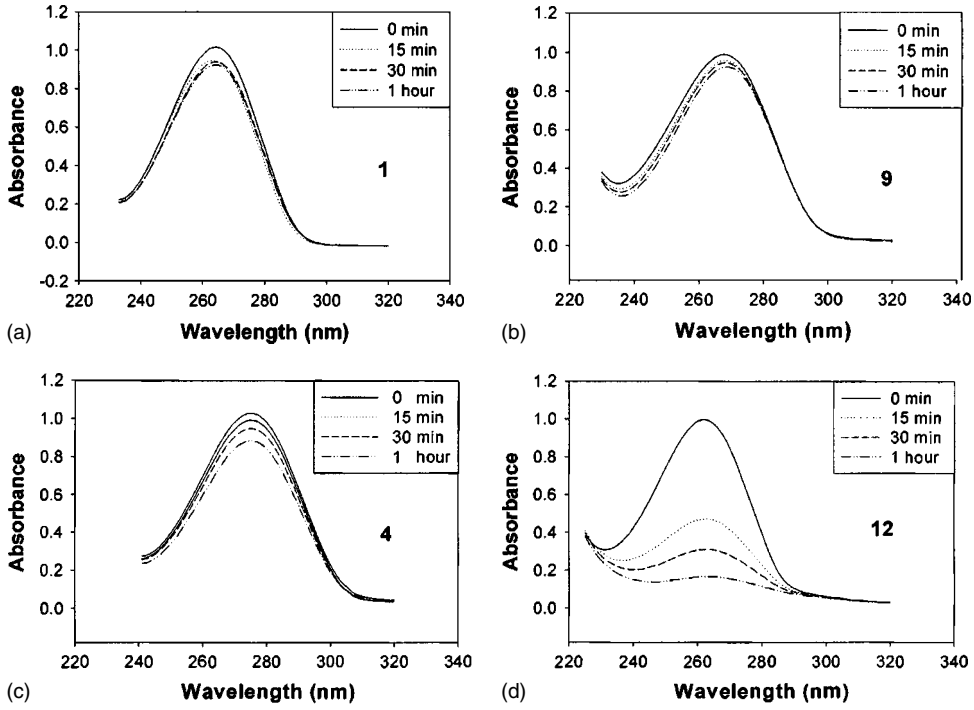

Figure 4 Absorption spectra of pyrimidines **1** (a) and **4** (c) compared with the corresponding pyrimidine-substituted dipeptides **9** (b) and **12** (d), all in water, for different irradiation times.

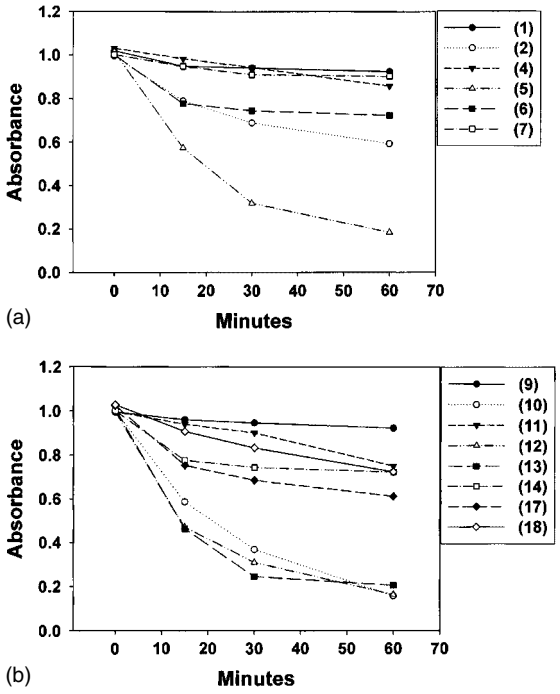

Figure 5 The absorbance values (λ_{max}) for pyrimidines **1**–**7** (a) and pyrimidine-substituted dipeptides **9**–**14**, **17**, **18** (b), all in water, for different irradiation times.

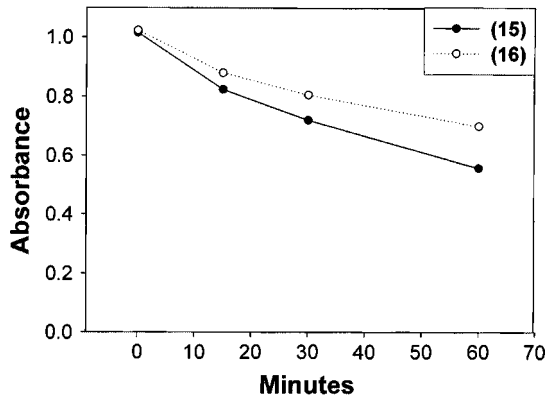

Figure 6 The absorbance values (λ_{max}) for pyrimidine-substituted dipeptides **17** and **18**, both in water, for different irradiation times.

and electronic complexity of the peptide-based compounds synthesized here requires an understanding of the chromophores behaviour, which is most conveniently tested on the free chromophores. Uracil has a greater photodimerization efficiency relative to thymine, and it has been reported that this is a result which primarily is due to the fact that singlet uracil undergoes intersystem crossing more efficiently than singlet thymine, and furthermore a smaller fraction of the meta stable uracil adducts fall apart [15]. Bringing the chromophores closer to each other increases the probability for cycloaddition, and this minimizes the diffusion effect. Looking at dimerization efficiency regarding atom radii, and steric hindrance, it would be expected that the larger the group in position C-5 or C-6, the more sterically hindered the cycloaddition will be. Therefore it would be expected that the chromophores containing H or F in position C-5 would be relatively unhindered and therefore possess good dimerization efficiency, which is also observed experimentally. Changing parameters such as solvent, concentration, temperature and pH (results not shown) did not have any significant effect on the dimerization efficiency, from the point of view of a final product. Overall there is an increase in dimerization efficiency when going from free chromophores to chromophores coupled to a peptide backbone. The reason for the poor dimerization efficiency of some compounds could be explained by the fact that most of the chromophores that absorb a photon revert from the excited state back into the ground state due to a life time of only $\sim 10^{-12}$ s, but a small amount manages to reach an long-lived triplet state [16].

By inspection of the spectra, some of the 5-halogen compounds have been found to shift their λ_{max} values. A plausible explanation for this observed shift in λ_{max} values is that a dehalogenation of the 5-halogen compounds takes place. In particular, the shifts in iodouracil as a free chromophore **6** and as a dipeptide **14**, as a function of irradiation time, are significant. Here a shift was observed from around 290 nm to 258 nm under irradiation, which is the λ_{max} for uracil. With chlorouracil both as a free chromophore **3** and as a dipeptide **11**, no dehalogenation was observed, and **3** and **11** both had poor dimerization efficiency.

5-Bromouracil shown in Figure 4(c) and (d), did not shift its λ_{max} either as a free chromophore **4** or as a dipeptide **12**, which is unexpected, since the dehalogenation for iodouracil as a free chromophore **6** and as a dipeptide **14** was significant and the fact that dehalogenation is more feasible in the peptide. With the fluorouracil both as a free chromophore **5** and as a dipeptide **13** a significant shift was observed going from 265 nm to 258 nm, indicating a dehalogenation. In order to verify the process of dehalogenation, compound **5** was first irradiated for 60 min to convert the free chromophores to dimers. This process was accompanied by a shift in wavelength. An experiment was performed on the dimerized compound **5** (cf. Figure 7), to see whether it would be possible to reverse the dimerization, thereby breaking the cyclobutane ring, and reforming free uracil chromophores. A strong indication of dehalogenation would be obtained if the broken dimers (free chromophores) and the same absorption maximum as that of unsubstituted uracil. A xenon lamp at 205 nm, was used for creating the free chromophores. After 20 h approx. 25% of uracil as a free chromophore **2** was observed as can be seen from Figure 7. Further experiments are in progress, using a more powerful lamp.

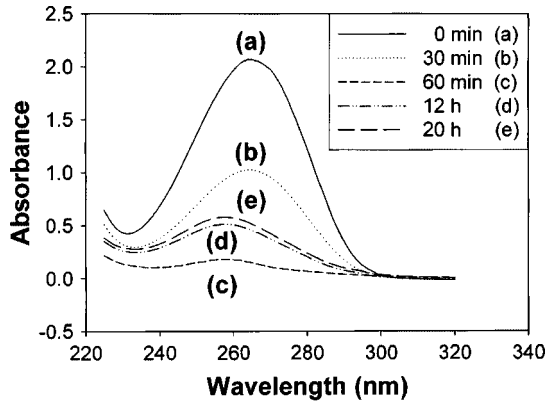

Figure 7 Absorption spectra of **5** in water after irradiation at 254 nm for 0 min (a), 30 min (b), and 60 min (c), respectively, and subsequent irradiation at 205 nm for 12 h (d) and 20 h (e), respectively.

A dehalogenation would lead to a radical in position C(5), and this would necessarily lead to a loss of the C(5), C(6) double bond, when the dimerization took place. Since the dimerization of some of the 5-halogenouracil derivatives takes place via a dehalogenation, these systems are not reversible. These results are consistent with experiments in which dehalogenation to uracil was observed in DNA [17,18]. It has been assumed that all cycloaddition reactions take place between two chromophores within the same molecule, i.e. intramolecularly. Dynamic light scattering experiments showed no increase in size (proportional to kcps) before and after irradiation, which would be expected if the dimerization also happened intermolecularly. The experiments all gave values in the range 55.7–56.1 kcps, before and after irradiation, indicating no increase in size. The effective diameter 1–2.3 nm measured, is a good approximation for the size of our system.

CONCLUSION

Overall there was an increase in dimerization efficiency when going from free chromophores to chromophores coupled to a peptide backbone. For fluorouracil the dimerization efficiency was good in both the free chromophore and the dipeptide. The drawback is that a significant shift in λ_{max} (believed to be due to dehalogenation) was observed, making the system irreversible, and therefore not suitable as a material for optical data storage. A significant increase in dimerization efficiency was observed for 5-bromouracil, and no dehalogenation was observable. For uracil a doubling of the dimerization efficiency was observed. Therefore, **10** and **12** are possible candidates as materials for optical data storage.

Acknowledgements

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