Synthesis and Photochemical Behavior of Peptide Nucleic Acid Dimers and Analogues Containing 4-Thiothymine: Unprecedented (5−4) Photoadduct Reversion

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Abstract: PNA dimers **1**−**5**, containing either 4-thiothymine or N^3-methyl-4-thiothymine, were prepared, and the crystal structure of compound **3** was established. With regard to their photochemistry, none of these PNA analogues were able to fully mimic the photochemical behavior observed in the dinucleotide series. Whereas **1** and **2** displayed a sequence dependent photochemistry to give mainly 4-(α-thyminyl) adducts, their rearranged isomers **3** and **4** yielded mostly (5−4) photoadducts. Photoproducts originating from **3** and **4** were shown to undergo spontaneous reversal to their dithyminyl parent derivatives under acid conditions. Moreover, as a result of this photochemical study, it can be suggested that in solution, even at the dimer stage, PNAs adopt a conformation reminiscent of A-type DNA.

Introduction

Peptide nucleic acids[1] (PNAs), which exhibit outstanding hybridization properties, have attracted considerable attention due to the potential use of these new molecules either in antisense or antigene strategies for modulating gene expression or as diagnostic and molecular biology tools.[2] PNAs are achiral and neutral oligonucleotide analogues in which the nucleobases are attached, via a methylenecarbonyl linker, to the secondary amine of a poly-*N*-(2-aminoethyl)glycine backbone (Figure 1).

These polyamide oligomers have demonstrated a remarkable ability to bind sequence−specifically to single-stranded DNA (ssDNA), RNA and double-stranded DNA (dsDNA).[2] Whereas binding to mixed ssDNA or RNA occurs via duplex formation, binding to homo ssDNA or RNA is achieved via $(PNA)_2$-DNA (or -RNA) triplex formation. Upon targeting dsDNA with pyrimidine PNA oligomers, a unique strand displacement mode[2] takes place forming a $(PNA)_2$-DNA triplex with a D loop that was recently found to be a part of a four-stranded PNA-DNA bundle.[3] All these properties cannot be fully rationalized since PNAs physicochemical behavior is not yet fully elucidated. Base pairing, stacking, and lack of a charged backbone are not sufficient enough to justify the high affinity of PNAs toward nucleic acids. In fact, the poorly understood backbone structuration, possibly mediated by interresidue hydrogen bonding[4] and suggested by the PNA preferred binding orientation when targeted to mixed ssDNA, RNA, and PNA[5] as well as the formation of helical duplexes,[5,6] might play a key role in these exceptional binding properties.

Some years ago, we demonstrated that the sequence-dependent photochemistry of dideoxynucleotides incorporating a 4-mercaptopyrimidine at the 3′- or 5′-end was controlled by the intrinsic 2-deoxyribose phosphate backbone conformation.[7] In particular, biologically important (6−4) pyrimidine−pyrimidone-type photoproducts[8a] could mainly be obtained when the modified base was introduced at the 3′-end of the dimer. Conversely, although no conformational modification was to be expected, dimers having the 4-mercaptopyrimidine base at the 5′-end yielded α-thymidinyl adducts upon irradiation. Recently, our results were fully validated at the ssDNA level.[8b]

To get further insight into the PNA backbone conformation, we are currently comparing the photochemical behavior of ssPNA and their analogues, incorporating a 4-mercaptopyrimidine at their C- or N-end, with regard to the one observed in the ssDNA series. In this respect, we have recently reported that 4-thiothymine-containing PNA dimers manifest a sequence dependent photochemistry that leads exclusively to α-thyminyl adducts[9] in contrast to what is observed in the dinucleotide phosphate series.[7] In a continuation of this study, we herein describe, the synthesis of **1** and **2** and the characterization of

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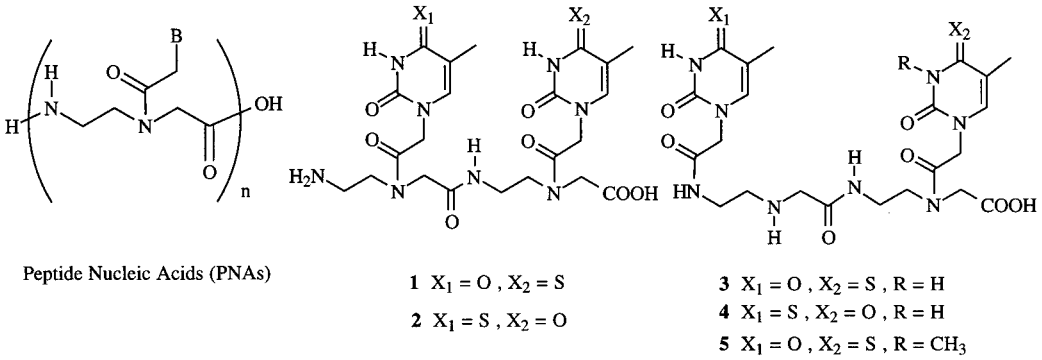

Figure 1.

their photoadducts together with the preparation and the photochemical behavior of three novel PNA analogues (**3**−**5**) (Figure 1) which were designed to elucidate the constraints applied, by the peptide backbone, on the PNA conformation.

Results and Discussion

Synthesis of Dimers 1 and 2. Dimers **1** and **2** (Scheme 1) were obtained in millimole preparative scale using liquid-phase synthesis employing *tert*-butyloxycarbonyl (Boc)-protected monomers and carbodiimide coupling activation as previously described.[10] Synthesis of **1** and **2** primarily required the preparation of two units, namely *N*-[2-(Boc-amino)ethyl]-*N*-(thymin-1-ylacetyl)glycine methyl ester **13** and its thiolated analogue [*N*-[2-(Boc-amino)ethyl]-*N*-(4-thiothymin-1-ylacetyl)-glycine methyl ester] **14**. Coupling N^1-carboxymethylated bases **6a** (thymin-1-ylacetic acid)[11] and **7b** (4-thiothymin-1-ylacetic acid) with **12** was accomplished by means of their pentafluorophenyl esters, which were prepared in situ, to afford **13** and **14**, respectively (86% yield). 4-Thiothymin-1-ylacetic acid was prepared in three steps from **6a**,[12] whereas **12** was obtained by methyl bromoacetate monoalkylation of *N*-Boc-1,2-aminoethane (**11**)[13] of ethylenediamine (**10**) (62% yield, two steps).

The carbamate function of **13** and **14** was cleaved using 20% trifluoroacetic acid (TFA) in anhydrous CH_2Cl_2 to give quantitatively trifluoroacetates **16** and **17**, respectively, whereas saponification of the ester function of **13** and **14** gave in quantitative yields, after acidification, **19** and **20**, respectively. After DCC/pentafluorophenol activation **19** and **20** were condensed with **17** and **16**, respectively, to yield dimers **21** and **22**. The latter were subsequently saponified, as described for the preparation of **19** and **20**, to yield the corresponding acids. Removal of the Boc protecting group of these carboxylic acid intermediates furnished **1** and **2**, respectively. After purification using reverse-phase HPLC, the overall yields (two-step deprotection) were 53% for **1** and 55% for **2**.

Interestingly, photolysis of **1** and **2** led to α-thyminyl photoproducts **24**−**27**, exclusively whose formation resulted from an hydrogen abstraction mechanism (Scheme 2).[9] Since (6−4) photoproducts could never be obtained, starting from compounds **1** and **2**, the latter appeared unable to give rise to the photochemistry which was observed with dideoxynucleotides.[7] This unambiguously indicated that both **1** and **2** are conformationally constrained in a form different from the B-DNA form that is necessary for (6−4) photoproduct formation.

It is noticeable that 5,6-dihydro-5-(α-thyminyl)thymine photoproduct, also known as spore photoproduct (SP), is the major adduct produced by irradiation of A-like form DNA.[14] Although SP and adducts **24**, **25**, and **27** are structurally distinct, they very likely derive from a similar mechanistic pathway which might originally involve hydrogen abstraction from the methyl of the adjacent thymine by a vicinal excited species. The proposed mechanism of formation of SP is outlined in Figure 2. In the case of **1** and **2**, radical binding, with the common methylene radical, occurred at either C-4 or C-6 positions of s^4T, depending of the structure of the final product, to give **24**, **25**, and **27**.

As in the dinucleotide series, sulfur substitution in **1** and **2** should have very little effect on their conformation compared to their parent molecules. Since, only α-thyminyl adducts could be obtained by irradiation of **1** and **2**, it might be concluded that in solution, both dimers exhibit a conformational state in which the nucleobase stacking is the one adopted by the A-like form of DNA observed in spores. The differences which were noticed, in terms of yields and of photoproduct structures, reflected the expected distance variations between the methyl group and atoms C-4 and C-6 of the thiolated base.

Interestingly, a P-form, where base stacking is reminiscent of the one found in A-form DNA duplexes, has recently been proposed to depict the conformation of a dsPNA in the solid state.[15a] Moreover, the P-form seems generally to be adopted by PNAs since it was also observed, in the solid state, for a PNA_2/DNA triplex[15b] and in solution for a PNA/DNA duplex.[15c] From our study, it can also be reasonably proposed that, even at the single strand and dimer level, PNAs adopt in solution a conformation with base stacking corresponding to the same A- (or related A-) form.

A number of chemical modifications of the PNA backbone[16] has been made in an effort to understand their binding properties. Among them, analogues having more than 11 atoms between the nucleobases have been proven to retain base pairing.[16a,b] We therefore took advantage of the known easy *N*-acyl migration reaction,[17] to prepare dimers **3** and **4** that contain 15 bonds between the nucleobases. Accordingly, we explored their

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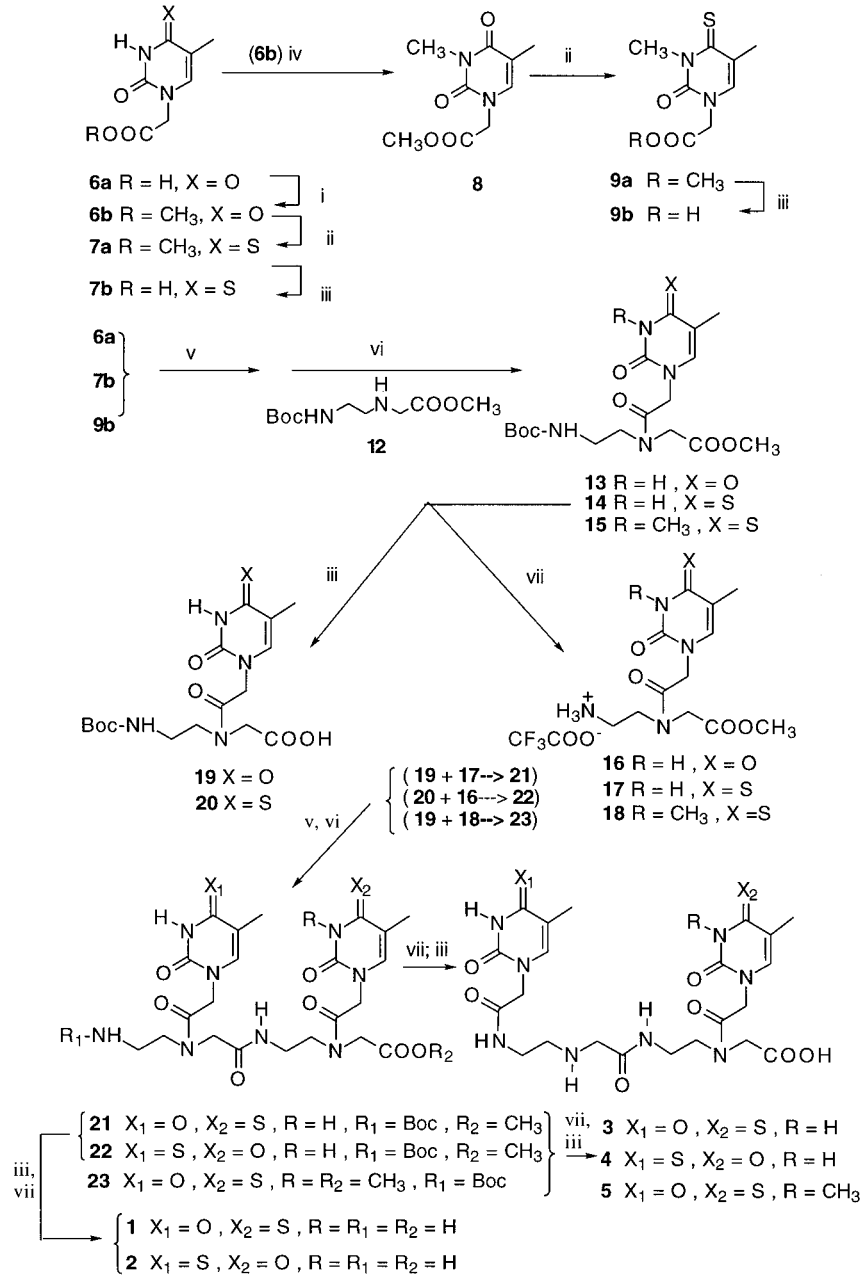
Scheme 1[a]



[a] (i) Thionyl Chloride, MeOH; (i) P_2S_5; (ii) 2 N aqueous NaOH, H_3O^+; (iv) MeI, CO_3K_2; (v) C_6F_5OH, DCC, DMF; (vi) TEA; (vii) TFA, CH_2Cl_2.

photochemical behavior in order to determine if they possibly could give rise to (6−4) photoproducts.

Synthesis of Dimers 3 and 4. These dimers were prepared from intermediates **21** and **22** (Scheme 1) and the synthetic route is almost identical with that followed for the preparation of **1** and **2**. It only differed by the inversion of the last two deprotection steps. Hence, reaction of **21** and **22** with 20% TFA in CH_2Cl_2 induced the cleavage of the carbamate while subsequent alkaline treatment concomitantly led to ester saponification and migration of the acyl group to the *N*-terminal position. Rearranged dimers **3** and **4** were finally purified by reverse-phase HPLC and isolated in 36.5% and 35% yield from **21** and **22**, respectively. The *N*-acyl rearrangement was ascertained from the simplification of the NMR spectra (^1H and ^{13}C) due to the removal of one tertiary amide. It was definitively established by X-ray crystallographic analysis of **3** (Figure 3).

The molecule shown in Figure 3 is in a zwitterionic form where an intramolecular hydrogen bond links the charged nitrogen N4′B−H to the peptidic oxygen O7′B. The peptide chain is folded to approach the bases at ∼4 Å. Moreover, by translation along the *b* axis, all bases are stacked in the crystal in a such way that the 4-thiothymine is sandwiched between two thymines. Two water molecules of crystallization were

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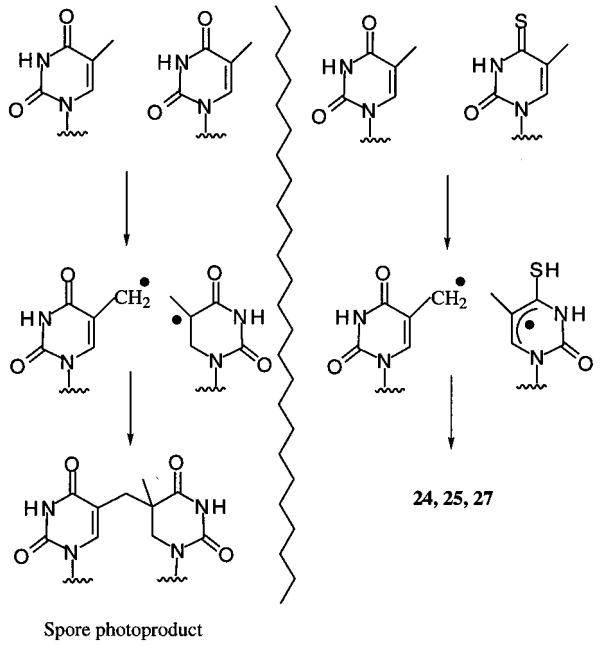

Figure 2.

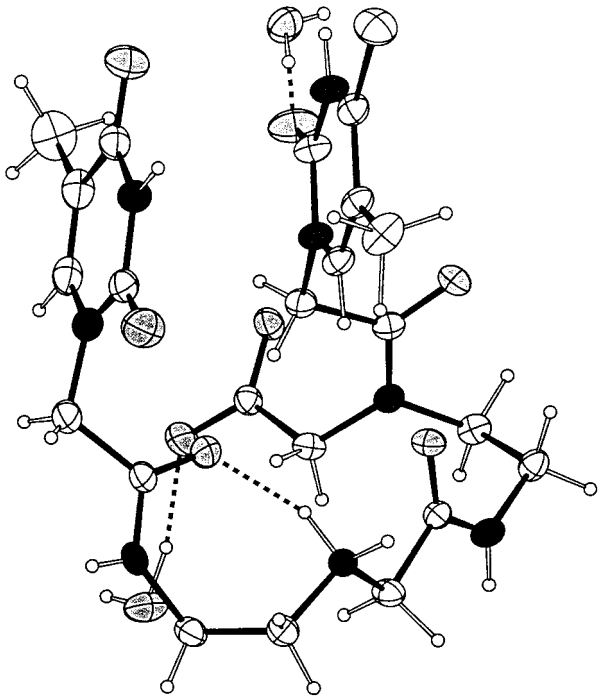

Figure 3. X-ray structure of compound **3**.

found in the asymmetric unit. All possible N−H or O−H hydrogen bonds are present in the crystal.[18]

Photolysis of 3 and 4. While photolysis of **1** and **2** was completed within 30 min, in the same irradiation conditions the photolysis of **3** was roughly 80% completed. In contrast, that of **4** was less than half completed after 6 h (estimated from the ^{1}H NMR spectra of the crude irradiation mixtures) of irradiation. Altogether this indicated a strong difference of reactivity within the two series. Irradiation of an aqueous solution of **3** led to the formation of a major (**28**) and a minor (**32**) photoproduct (Scheme 3) as indicated by the ^{1}H NMR spectrum of the crude irradiation mixture recorded immediately after photolysis.

Instability of compound **28** in aqueous solution was manifested by a slow broadening of its ^{1}H NMR spectrum signals which we tried to circumvent by transforming **28** in a more stable derivative. Presuming the presence of a thiol group, and in the light of previous observations on s^5(6−4) bipyrimidine chemistry,[7b] we attempted to obtain **29** by adding methyl methanethiosulfonate ($CH_3SO_2SCH_3$) to the crude irradiation mixture just after photolysis. Indeed, addition of this reagent to the solution of **28** induced instantaneously the apparition, in the ^{1}H NMR spectrum of the reaction mixture, of two new methyl signals corresponding to CH_3SO_2H and $SSCH_3$ (2.50 and 2.43 ppm), attesting to its transformation into **29** in agreement with the presence of a thiol group within **28**.

After the mixture stood at room temperature, **29** slowly gave rise to **30** that could finally be isolated in pure form by reverse-phase HPLC in 32% yield (from **3**). The UV spectrum of **30** (λ_{max} 318 nm) suggested the presence of either a (6−4) or (5−4) pyrimidinone chromophore.[19,20a] Its FAB mass spectrum (positive mode) exhibited a peak at *m*/*z* 551 $(M + H)^+$, suggesting the replacement of the sulfur atom by an oxygen atom. Inspection of the ^{1}H NMR spectrum (D_2O/TFA) revealed two singlets at 7.50 and 5.27 ppm, supporting a pyrimidinone and a 5,6-dihydrothymine structure, respectively. In addition to the carbon signals of the pyrimidinone, the ^{13}C NMR spectrum of **30** displayed a quaternary (55.7 ppm) and a methine (84.3 ppm) sp^3 carbon. This latter resonance was unambiguously attributed to C-6 of the saturated thymine from its ^3J correlations, in the HMBC spectrum, with N^1CH_2 and CH_3 protons within the same base. In the same experiment, the corresponding H-6 proton ^3J-correlated with carbons C-2, C-4, N^1CH_2, and CH_3. Chemical shifts of carbons C-5 and C-6 (55.7 and 84.3 ppm, respectively), being typical of (5−4) adducts[20] considering substituent differences, definitively ruled out a (6−4) structure[7b,20] for this photoadduct. Consequently, we proposed the mixed-disulfide structure for **29** and the s^6(5−4) bipyrimidine structure for **28** that should result from opening of a thietane intermediate generated by [2 + 2] photocycloaddition between the two bases in a respective antiparallel orientation as already suggested[20a,21] (Scheme 3). Difficulties encountered in isolating **28**, and obtention of **30** instead, are not surprising since spontaneous transformation of s^6(5−4) adducts into o^6(5−4) derivatives has already been observed in a bimolecular (Thy; s^{4}Urd) model reaction.[20a] It is noteworthy

(18) Salient features of the molecule. Torsion angles: χ_3: C2−N1−C8′−C7′= 87.8°, C2B−N1B−C8′B−C7′B = −84.8°; χ_2: N1−C8′−C7′−N4′ = 165.8°, N1B−C8′B−C7′B−N1′B = 159.6°; χ_1: C8′−C7′−N4′−C3′= −171.3°, C8′B−C7′B−N1′B−C2′B = 167.9°, C5′−N4′−C3′−C2′ = −111.1°, C7′−N4′−C3′−C2′ = 70.2; N4′−C3′−C2′−N1′ = 54.2°, C3′−C2′−N1′−C6′B = −88.4°, C2′−N1′−C6′B−C5′B = 174.9°, N1′−C6′B−C5′B−N4′B′ = 175.2°; C6′B−C5′B−N4′B−C3′B = 173.2°; C5′B−N4′B−C3′B−C2′B = −84.8°; N4′B−C3′B−C2′B−N1′B = −75.0°; C3′B−C2′B−N1′B−C7′B = 75.4°. Each molecule of compound **3** crystallizes with two molecules of water (W40 and W41), hydrogen bonded to **3**. Intramolecular hydrogen bonds: N4′B−Hx···O7′B, 2.733 (3); W40−H···O2, 2.721 (4); W41−H···O6″, 2.776 (4). Intermolecular hydrogen bonds: N3−H···W41, ($^1/_2 + X$, $-^1/_2 - Y$, $^1/_2 + Z$), 2.767 (5); N1′−H···O4B ($-^1/_2 + X$, $Y - ^1/_2$ *Z*), 2.803 (4); N4′B−Hy···O6′ ($-^1/_2 + X$, $-^1/_2 - Y$, $-^1/_2 + Z$) 2.679 (3); N1′B−H···O6′ ($^1/_2 + X$, $-^1/_2 - Y$, $-^1/_2 + Z$), 2.876 (3). N3B−H···W40 (X, $-Y$, $-^1/_2 + Z$) 2.799 (4) W40−H···S4 (X, $-Y$, $^1/_2 + Z$), 3.383 (3); W41−H···O2B ($^1/_2 + X$, $-^1/_2 - Y$, $^1/_2 + Z$), 2.891 (5).

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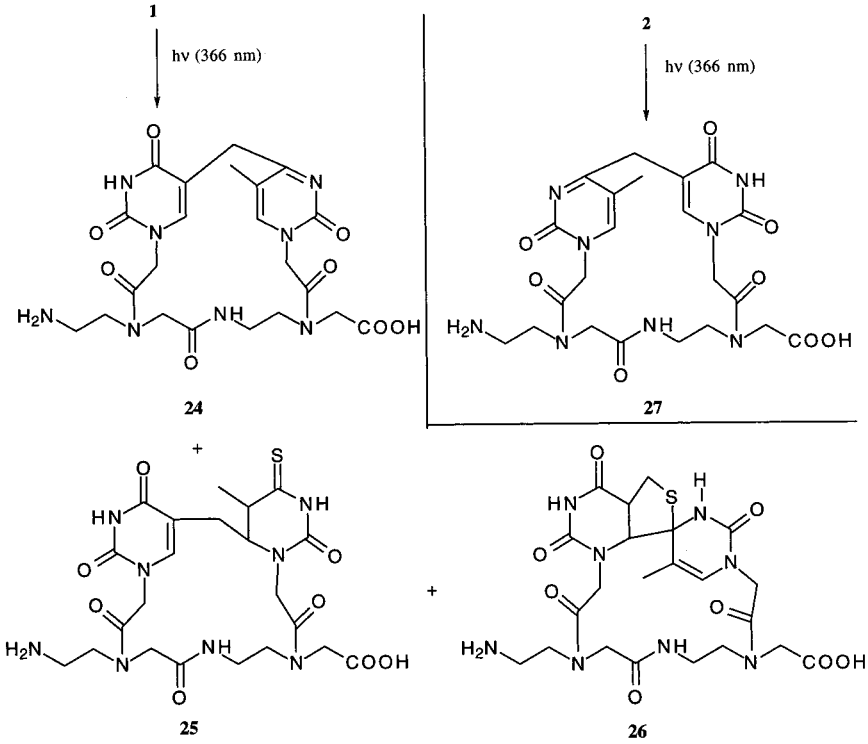
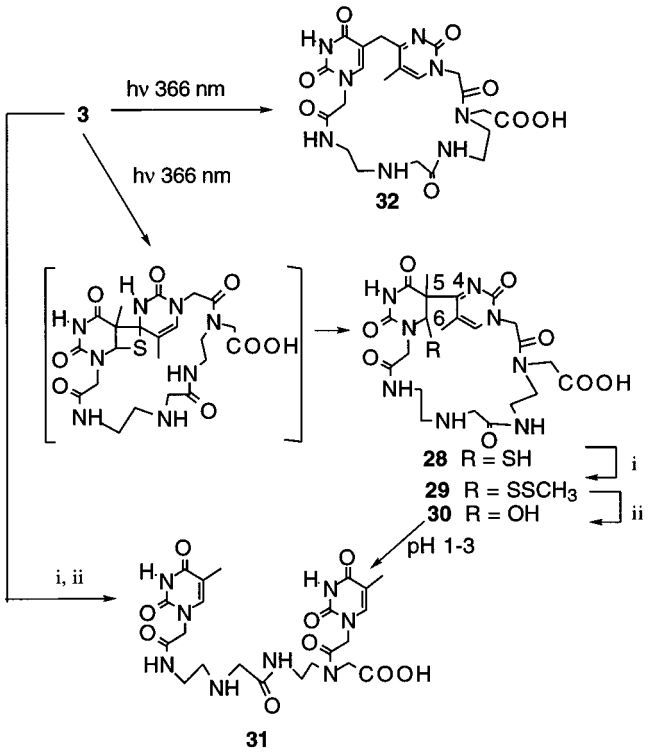
Scheme 2[9]

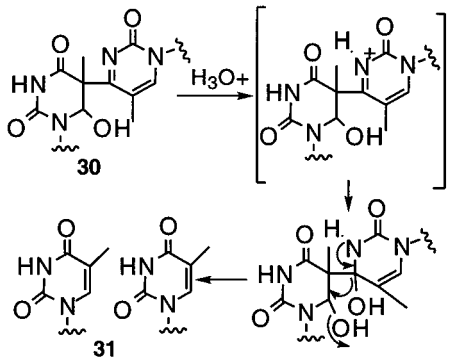


Scheme 3[a]



[a] (i) $MeSO_2SMe$; (ii) H_2O.

Scheme 4



that (5−4) adducts are relevant of nucleic acids photobiology[22,23] and that model (5−4) adducts have so far been obtained in bimolecular reactions only.[21,23] This is the first report of such a photochemical pathway occurring via an intramolecular photochemical pathway.

Under acid conditions (pH 1−3), compound **30** rearranged slowly into the two thymine-containing derivative **31** which could also be prepared in quantitative yield by treatment of **3** with $CH_3SO_2SCH_3$ (Scheme 3). To the best of our knowledge, such a rearrangement has never been observed and a plausible mechanism is suggested in Scheme 4.

Compound **32**, isolated in 4% yield, was easily identified as a 4-(α-thyminyl) adduct by comparison of its spectral data with those of similar photoproducts obtained in dinucleotide[7,24] and PNA[9] series. Its FAB mass spectrum (positive mode) exhibited a peak at m/z 533 $(M + 1)^+$ in accordance with H_2S elimination. Its UV spectrum (λ_{max} at 269 and 315 nm) was also typical of such an adduct. The high field part of its ^1H NMR spectrum displayed only one methyl group signal (s, 2.10 ppm). The ^{13}C NMR spectrum evidenced the appearance of a new methylene (40.5 ppm) and the replacement of the thiocarbonyl group (193.0 ppm) by a C-4 carbon (180.8 ppm) in accordance with a 4-(α-thyminyl) adduct.[7,9]

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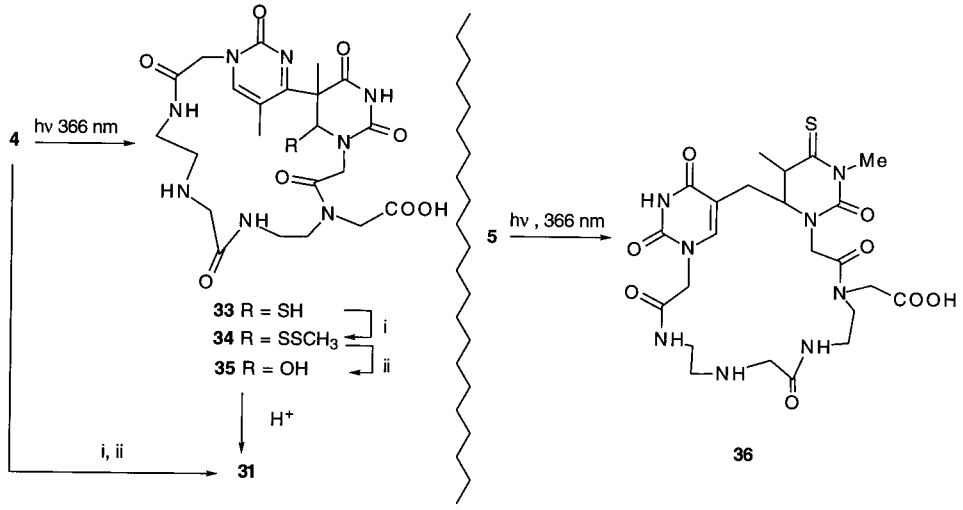
Scheme 5[a]



[a] (i) $MeSO_2SMe$; (ii) H_2O.

Photolysis of the other analogue **4** led, after the same reaction cascade as depicted for **3**, to the (5−4) adduct **35** (13.7%) which, under acidic conditions, was transformed into the dithymine dimer **31** (Scheme 5), confirming the generality of the spontaneous reversal of (5−4) photoadducts in acid conditions. No trace of the product corresponding to hydrogen abstraction pathway was observed in this case.

Since formation of (5−4) adducts is thought to proceed through a four-membered heterocyclic ring intermediate,[21,23] we attempted to isolate the putative corresponding thietane intermediate using the strategy successfully applied in the s[5](6−4) series.[25] Thus, we prepared the N^3-methyl-4-thiothymine-containing dimer **5** by closely following the route used in the cases of **3** and **4** (Scheme 1). The N^3-methyl-4-thiothymine derivative **9b** was prepared from methyl thymin-1-ylacetate **6b** that was regioselectively N^3-methylated (**8**) and thiolated to give methyl (N^3-methyl-4-thiothymin-1-yl)acetate **9a**. Subsequent alkaline saponification and acidification afforded N^3-methyl-4-thiothymin-1-ylacetic acid **9b**. The latter was condensed with **12**, providing **15**. Deprotection of the primary amine afforded **18** which was condensed with **19**, leading to the diprotected dimer **23**. Successive 20% TFA/CH_2Cl_2 and 2 N NaOH treatments provided **5** which after purification by HPLC was isolated in 27% yield (from **23**).

Photolysis of **5** did not afford the expected thietane but, instead, yielded one major 6-(α-thyminyl) photoadduct **36** (21%) resulting from an hydrogen abstraction pathway (Scheme 5) as deduced from (1) its FAB mass spectrum (positive mode) that displayed a peak at *m*/*z* 581 $(M + 1)^+$, (2) its UV spectrum (λ_{max} 277 nm),[7,24] and (3) its ^{1}H NMR spectrum that displayed signals for only one methyl group [doublet at δ, 1.35 ppm (major component) and 1.31 ppm (minor component)] in the high-field region and one olefinic H-6 proton in the downfield region [δ 7.41 ppm (minor component), 7.25 ppm (major component)]; these results were definitively confirmed by the ^{13}C NMR data which particularly showed the presence of a methylene at 25.6 ppm, two sp^3 methine carbons at 59.0 and 45.5 ppm (major component)[7,9,26] and finally a characteristic C═S at 210 ppm.[7,9] Interestingly, this is the first report of a 6-(α-thyminyl) adduct formation from a N^3-methyl-4-thiothymine although it was previously found to lead to 4-(α-thyminyl) adducts.[25]

Conclusion

In the present work we have established that incorporation of thio-substituted nucleobases in PNA requires no particular precaution. As it is well-known that such nucleobases exhibit remarkable photochemical properties which are currently exploited in photolabeling studies of systems containing nucleic acids,[27] the introduction of such modifications in PNA could receive many applications. For example, since oligonucleotides incorporating a 4-thiouridine at its 5′ end exhibit 100% cross-linking capacity with the residue located at the 3′ end of its complement,[28] thio-substituted nucleobase containing PNAs could be used to determine unambiguously the binding orientation of a stretch of PNA relative to its target.

Moreover, the comparison of the photochemistry of 4-thiothymine-containing PNA dimers **1** and **2** with that of their corresponding *N*-acyl rearranged analogues **3** and **4**, has shown a remarkable behavioral difference. Under irradiation, **1** and **2** undergo an hydrogen abstraction reaction that, compared to the dinucleotide series, is typical of the dimers in A-DNA form. In contrast, dimers **3** and **4** gave rise to a major [2 + 2] cycloaddition photoreaction as observed with dinucleotides containing the thionucleobase at their 3′ end. However, whereas in the dinucleotide series, photocycloaddition led to (6−4) adducts, in the latter case (5−4) adducts were formed. These adducts are analogues of those previously observed in tRNA and other model studies.[20−23] Interestingly, in contrast to that of **1** and **2**,[9] the photoreactivity of dimers **3** and **4** did not exhibit any sequence effects. The less constrained backbone structure of **3** and **4** could account for these observations. In this case, the antiparallel orientation of the bases, a prerequisite for (5−4) adduct formation, is permitted due to the presence of three supplementary atoms in the chain linking the two pyrimidines. Indeed, the structural constraints exercised in model dimers containing 11 atoms between the two bases (PNA or nucleotides) preclude the formation of these adducts.

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Finally, it is noteworthy that most of all biological photoadducts identified so far[29] (cyclobutane pyrimidine dimers, (6−4) photoproducts, and spore photoproduct) can be physiologically repaired by their respective specific (photo)lyases.[30−32] Excepted for these latter, studies with model compounds have demonstrated their possible reversion to their parent bases, under relatively common physical conditions.[8b,33,34] Interestingly, in this respect we have found that (5−4) adducts, which are naturally occurring nucleic acid photoproducts,[22,23] can also undergo a spontaneous repair process under mild acidic pH conditions. Since these adducts could be substrates for a photoreactivating enzyme,[23b] the biological significance of this reversal pathway remains to be assessed.

Experimental Section

General Remarks. Reagents and solvents were obtained from commercial sources and used without further purification unless indicated. CH_2Cl_2 and triethylamine were dried by heating, under reflux, with calcium hydride. Dry DMF was obtained by azeotropic distillation with toluene then redistilled over barium oxide. Amine (as their trifluoroacetate salts) and acids were dried at room temperature in a desiccator over P_2O_5 under high vacuum overnight prior to condensation.

Spectroscopic Measurements. UV spectra were recorded in reverse osmosed water (pH 6) on a Perkin-Elmer Lambda 5 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC250, AM300, or AC300P instruments. ^{1}H chemical shifts (δ) are reported in parts per million relative to residual solvent peak (HOD or HOD/TFA δ 4.80, DMSO δ 2.60, MeOD δ 3.30, $CHCl_3$ δ 7.27). ^{13}C chemical shifts are reported in parts per million relative to solvent peak ($CDCl_3$ δ 77.7, CD_3OD δ 49.0, DMSO δ 39.7). Spectra recorded in D_2O were calibrated relative to an external capillary standard of dioxane (δ 67.8). At room temperature in D_2O, compounds **1** and **2** were present as a mixture of four rotamers due to restricted rotation around tertiary amide bonds and consequently displayed a complex ^{1}H NMR pattern. ^{1}H NMR spectra of these compounds are given in the Supporting Information. Compounds **3**−**5** were present as mixtures of two rotamers (60:40, 55:45, and 60:40, respectively), consequently some signals in the ^{1}H NMR are described as major (ma.) and minor (mi.) component. For compounds **25**, **26**, **35**, and **36** only base moiety characteristic NMR signals are provided. EIMS were carried out using an AEI MS 50 spectrometer. FABMS and HRMS (EI or CI (CH_4)) were carried out using a Kratos MS 80 spectrometer. FABHRMS were performed by the Service Central d′Analyse du CNRS (Lyon, France). Melting points (mp) were determined with a Reichert apparatus and are uncorrected.

HPLC Purifications. Dimers **1**−**5** were purified on a Delta-Pak C18 (15 μm 100 Å) PrepPak Cartridge (47 mm × 30 cm) using a linear gradient composed of A (H_2O/CH_3CN/TFA 95/5/0.1) and B (H_2O/CH_3CN/TFA 50/50/0.1): time 0, 0%B; time 60 min, 80%B with a flow rate of 25 mL/mn and the effluent monitored at 260 nm; retention time (RT) **1**, 42 min; RT **2**, 44 min; RT **3**, 38 min; RT **4**, 45 min; RT **5**, 51 min. Products **24**−**27**, **30**−**32**, and **35** were purified on a Prep Nova-Pak HR C18 (6μm 60 Å) PrepPak cartridge (25 × 100 mm). A photodiode array detector (Waters 991) was used.

Photolyzed dimers **1** and **2** were dissolved in H_2O (20 mg/200 μL) and injected, then a 30 min, 8 mL/min linear gradient of 0−9.6% CH_3CN for **1** and 0−12% CH_3CN for **2** in 0.05 M aqueous ammonium acetate (pH 4.7) was used. Capacity factor (k'): k' **24** = 2.5; k' **25** = 6.3; k' **26** = 5.8; k' **27** = 1.4; k' **31** = 4.4.

Photolyzed dimers **3** and **4** were dissolved in H_2O (20 mg/200 μL) and injected. A 30 min, 8 mL/min linear gradient of 0−12% CH_3CN in 0.05 M aqueous ammonium acetate followed by a 10 min plateau was used. Capacity factor: k' **30** = 2.0; k' **31** = 4.4; k' **32** = 3.2; k' **35** = 3.1.

Photolyzed dimer **5** was dissolved in H_2O (10 mg/100 μL) and injected. A 20 min, 7 mL/min gradient (curve 4, System Controller Waters 600E) composed of solvents A (0.05 M aqueous ammonium acetate, pH 4.8) and B (0.05 M aqueous ammonium acetate (pH 4.8)/CH_3CN 88/12) 0−80% B, followed by a 30 min linear gradient of 80 to 100% B and a 10 min plateau, were used. Capacity factor: k' **36** = 7.9.

Fractions containing photoproducts were concentrated, desalted by RP-18 column chromatography with water used as eluent for 10 min then a linear gradient of methanol in water (50/50) 0−100% B in 20 min with a flow rate of 8 mL/min.

A. Synthesis. 1. General Procedures. Boc-Deprotection: Procedure A. Carbamate derivatives were stirred for 3 h at room temperature with 20% TFA in CH_2Cl_2. The solvent was removed under reduced pressure and the residue was repeatedly diluted with CH_2Cl_2 and concentrated until obtention of a foam that was rapidly used for the next step. **Procedure B.** Carbamate derivatives were stirred for 1 h at room temperature with 50% TFA in CH_2Cl_2. The solvent was removed under reduced pressure, and the residue was repeatedly diluted with CH_2Cl_2 and concentrated until obtention of a foam that was purified by HPLC.

Hydrolysis of the Methyl Ester Group: Procedure C. A suspension of methyl ester derivative in 2 N aqueous NaOH was stirred for 15 to 60 min at room temperature. The aqueous solution was cooled at 0 °C and the pH was adjusted to 2 by dropwise addition of 4 N HCl. The aqueous phase was either extracted three times with EtOAc and the joined organic phases were dried over sodium sulfate and concentrated to dryness (preparation of **19** and **20**) or carefully concentrated and either used for the next step (saponification of **21** and **22**) or purified by HPLC (preparation of **3**−**5**).

Coupling Reaction: Procedure D. To a solution of the free acid in dry DMF, pentafluorophenol (1.1 equiv) was added in a dry atmosphere. The solution was cooled to 0 °C and DCC (1 equiv) was added. The ice bath was removed after 1 h and the stirring was continued for 5 h at room temperature. The suspension was filtered. A freshly prepared solution of ammonium salt (1 equiv), and triethylamine (1 equiv) in dry DMF was poured dropwise to the filtrate. The solution was stirred for 20 h and then the solvent was removed in vacuo at room temperature. The residue was purified by silica gel chromatography.

2. Monomer Synthesis. Methyl (N^3-Methylthymin-1-yl)acetate (8). To a suspension of **6b** (1.48 g, 7.47 mM) and K_2CO_3 (2.43 g, 17.61 mM) in acetone (40 mL) was added CH_3I (2.4 mL, 38.28 mM). The reaction was stirred overnight at room temperature then filtrated. The filtrate was concentrated and chromatographed on silica gel using EtOAc/heptane 80:20 as eluent, affording **8** in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.94 (s, 1H), 4.45 (s, 2H), 3.78 (s, 3H), 3.35 (s, 3H); 1.94 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.8, 164.4, 152.2, 138.7, 110.8, 53.3, 50.2, 28.5, 13.5. HRMS (CI) $(M + H)^+$: calcd for $C_9H_{13}N_2O_4$ 213.0881, found 213.0881. Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 51.03; H, 5.78; N, 13.11.

Methyl (N^3-Methyl-4-thiothymin-1-yl)acetate (9a). To a boiling solution of **8** (2.35 g, 11.1 mM) in dioxane (65 mL) was added P_2S_5 (3.17 g, 14.28 mM). The solution was stirred for 2 h and then filtered on Celite, and the filtrate was concentrated to dryness. Purification of the residue by silica gel chromatography using a gradient of EtOAc in heptane (0→20%) afforded 2.53 g (90.7%) of **9a** as a yellow powder. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.86 (s, 1H), 4.73 (s, 2H), 3.81 (s,

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3H), 3.76 (s, 3H); 2.14 (s, 3H). ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 191.1, 168.3, 149.4, 136.7, 117.7, 52.7, 50.6, 35.4, 18.5. HRMS (CI) $(M + H)^+$: calcd for $C_9H_{13}N_2O_3S$ 229.0647, found 229.0645. Anal. Calcd for $C_9H_{12}N_2O_3S$: C, 47.37; H, 5.30; N, 12.28; S, 14.02. Found: C, 47.37; H, 5.19; N, 12.07; S, 14.13.

***N*^{3}-Methyl-4-thiothymin-1-ylacetic Acid (9b).** A suspension of **9a** (1.5 g, 6.58 mM) in 2 N NaOH (aqueous, 12 mL) was sonicated for 10 min. The reaction was cooled to 0 °C and acidified to pH 1 with concentrated HCl. The formed precipitate was filtrated, washed with cold water, and dried, yielding 1.3 g (93%) of **9b** as a slightly yellow powder. ^{1}H NMR (250 MHz, DMSO-d_6): δ 13.38 (br s, 1H), 7.86 (s, 1H), 4.62 (s, 2H), 3.76 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 191.1, 169.2, 149.5, 137.0, 117.6, 50.7, 35.5, 18.6. HRMS (CI) $(M + H)^+$: calcd for $C_8H_{11}N_2O_3S$ 215.0490, found 215.0473. Anal. Calcd for $C_8H_{10}N_2O_3S$: C, 44.86; H, 4.71; N, 13.08. Found: C, 45.24; H, 4.88; N, 13.11.

Methyl *N*-[2-(Boc-amino)ethyl]glycinate (12). To a solution of *N*-Boc-1,2-aminoethane (**11**, 2.2 g, 13.75 mM) and triethylamine (2.19 mL, 15.61 mM) in anhydrous CH_2Cl_2 (25 mL) was added dropwise methyl bromoacetate (1.27 mL, 13.70 mmol). The solution was stirred overnight in a dry atmosphere at room temperature, then diluted with CH_2Cl_2 (25 mL), and washed with brine. The organic phase was dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel using a gradient of EtOAc in heptane (25→50%), and **12** was isolated as an oil (2.19 g, 68.6%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.20 (br t, 1H), 3.66 (s, 3H); 3.35 (s, 2H), 3.14 (q, 1H, J = 5.8 Hz), 2.67 (t, 1H, J = 5.8 Hz), 1.89 (br s, 1H), 1.37 (s, 9H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 173.5, 156.6, 79.6, 52.3, 50.8, 49.3, 40.7, 28.9. MS (EI): m/z 232 $(M)^+$.

Methyl *N*-[2-(Boc-amino)ethyl]-*N*-(thymin-1-ylacetyl)glycinate (13). This compound was prepared, according to general procedure D, from **6a** (1.19 g, 6.46 mM) and using 22 mL of dry DMF. The alkaline solution was prepared using 10 mL of DMF. The residue was purified by silica gel chromatography using a gradient of methanol in CH_2Cl_2 (0→10%) to afford **13** as a white foam (2.2 g, 85.6%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.74 (br s, 1H), 7.03 (mi.) and 6.97 (ma.) (s, 1H), 5.72 (ma.) and 5.19 (mi.) (br t, 1H), 4.57 (ma.) and 4.42 (mi.) (s, 2H), 4.22 (mi.) and 4.05 (ma.) (s, 2H), 3.78 (mi.) and 3.72 (ma.) (s, 3H), 3.51 (m, 2H), 3.28 (m, 2H), 1.87 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 170.5, 168.3 (mi.) and 168.0 (ma.), 165.1, 156.6, 151.8, 141.6, 111.0, 80.2, 53.3 (mi.) and 52.8 (ma.), 50.6 (mi.) and 49.2 (ma.), 49.0, 48.3, 39.1, 28.8, 12.7. HRMS (CI) $(M + H)^+$: calcd for $C_{17}H_{27}N_4O_7$ 399.1879, found 399.1849. Anal. Calcd for $C_{17}H_{26}N_4O_7$: C, 51.25; H, 6.58; N, 14.06. Found: C, 50.75; H, 6.25; N, 13.96.

Methyl *N*-[2-(Boc-amino)ethyl]-*N*-[(4-thiothymin-1-yl)acetyl]-glycinate (14). Derivative **14** was prepared according to general procedure D, from **7b** (1.29 g, 6.45 mM) using 22 mL of dry DMF. The alkaline solution was prepared using 10 mL of DMF. The residue was purified by silica gel chromatography using a gradient of methanol in CH_2Cl_2 (0→1%) to afford **14** as a yellow foam (2.3 g, 86%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 10.98 (br s, 1H), 7.07 (mi.) and 7.03 (ma.) (s, 1H), 5.65 (ma.) and 5.18 (mi.) (br t, 1H), 4.60 (ma.) and 4.44 (mi.) (s, 2H), 4.19 (mi.) and 4.03 (ma.) (s, 2H), 3.74 (mi.) and 3.67 (ma.) (s, 3H), 3.47 (m, 2H), 3.23 (m, 2H), 1.99 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 191.6, 170.5, 167.8 (mi.) and 167.5 (ma.), 156.6, 149.4, 138.4, 119.7, 80.4, 53.4 (mi.) and 53.0 (ma.), 50.8 (mi.) and 49.4 (ma.), 49.0, 39.1, 28.8, 17.4. HRMS (EI) $(M)^+$: calcd for $C_{17}H_{26}N_4O_6S$ 414.1573, found 414.1573. Anal. Calcd for $C_{17}H_{26}N_4O_6S$: C, 49.27; H, 6.32; N, 13.52. Found: C, 49.55; H, 6.44; N, 12.65.

Methyl *N*-[2-(Boc-amino)ethyl]-*N*-[(*N*^{3}-methyl-4-thiothymin-1-yl)-acetyl]glycinate (15). Derivative **15** was prepared according to general procedure D, from **9b** (1.30 g, 6.07 mM) using 22 mL of dry DMF. The alkaline solution was prepared using 10 mL of DMF. The residue, purified by silica gel chromatography using a gradient of methanol in ether (0→1%) afforded **15** as a yellow foam (1.67 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05 (mi.) and 7.00 (ma.) (s, 1H), 5.55 (ma.) and 5.05 (mi.) (br t, 1H), 4.59 (ma.) and 4.42 (mi.) (s, 2H), 4.16 (mi.) and 4.00 (ma.) (s, 2H), 3.73 (mi.) and 3.66 (ma.) (s, 3H), 3.68 (s, 3H), 3.47 (m, 2H), 3.20 (m, 2H), 2.03 (s, 3H), 1.36 (ma.) and 1.34 (mi.) (s, 9H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 192.1, 170.4 (ma.) and 170.2 (mi.), 167.8 (mi.) and 167.4 (ma.), 156.5, 150.3, 135.1, 119.7, 80.3, 53.4 (mi.) and 52.9 (ma.), 50.6 (mi.) and 50.2 (ma.), 49.3, 49.1, 39.1 (ma.) and 38.9 (mi.), 36.1, 28.8, 19.4. HRMS (CI) $(M + H)^+$: calcd for $C_{18}H_{29}N_4O_6S$ 429.1819, found 429.1809. Anal. Calcd for $C_{18}H_{28}N_4O_6S$: C, 50.46; H, 6.59; N, 13.08; S, 7.47. Found: C, 50.18; H, 6.53; N, 12.66; S, 7.26.

3. Dimer Synthesis. Methyl *N*-[-2-[*N*′-[2-(Boc-amino)ethyl]-*N*′-(thymin-1-ylacetyl)glycyl]amino]ethyl]-*N*-[(4-thiothymin-1-yl)acetyl]-glycinate (21). The preparation of **17** was accomplished according to general procedure A from **14** (2.07 g, 5mM) using 12 mL of 20% TFA in CH_2Cl_2. The preparation of **19** was accomplished in 15 min according to general procedure C from **13** (1.99 g, 5 mM) using 20 mL of NaOH. The coupling reaction was accomplished according to general procedure D using 5 mM of **19** and **17** and purification was achieved through silica gel chromatography using a gradient of MeOH in CH_2Cl_2 (0→10%) affording **21** (2.2 g, 65%) as yellow foam. ^{1}H NMR (300 MHz, CD_3OD): δ 7.54 (ma.), 7.43 (mi.), 7.34 (mi.) and 7.23 (ma.) (s, 2H), 4.90−4.49 (s, 8H), 4.36 (mi.), 4.34 (mi.), 4.30 (mi.), 4.17 (mi.), 4.15 (ma.), 4.12 (ma.) and 3.99 (mi.) (s, 4H), 3.80 (mi.), 3.78 (mi.), 3.74 ma.), 3.71 (mi.) (s, 3H), 3.69−3.10 (m, 8H), 2.01 (mi.), 1.98 (mi.) and 1.92 (ma.) (s, 3H), 1.85 (mi.), 1.83 (mi.) and 1.78 (ma.) (s, 3H), 1.42 (s, 9H). ^{13}C NMR (75.5 MHz, CD_3OD) (several CH_2 were obscured under the solvent peak): δ 192.6, 172.1, 171.2, 171.1, 170.9, 169.7, 168.9, 166.4, 158.0, 152.7, 150.4, 143.4, 140.1 (ma.), 139.7 (mi.), 119.4 (ma.), 110.8, 80.2, 53.0, 52.8, 52.6, 51.4 (mi.) and 51.0 (ma.), 39.2 (ma.), 38.7 (mi.), 37.8 (mi.) and 37.5 (ma.), 28.4, 16.8, 12.0, 11.9. MS (FAB): m/z 703 $(M + Na)^+$.

Methyl *N*-2-[[*N*′-[2-(Boc-amino)ethyl]-*N*′-[(4-thiothymin-1-yl)-acetyl]glycyl]amino)ethyl]-*N*-(thymin-1-ylacetyl)glycinate (22). The preparation of **16** was accomplished according to general procedure A from **13** (1.98 g, 5 mM) using 12 mL of TFA/CH_2Cl_2. The preparation of **20** was accomplished in 15 min according to general procedure C from **14** (2.07 g, 5 mM) using 20 mL of NaOH. The coupling reaction was accomplished according to general procedure D using 5 mM of **20** and **16** and the purification was achieved through silica gel chromatography using a gradient of MeOH in CH_2Cl_2 (0→10%) affording **22** (2.18 g, 64%) as yellow foam. ^{1}H NMR (300 MHz, CD_3-OD): δ 7.47 (ma.) and 7.39 (mi.), 7.30 (mi.) and 7.28 (ma.) (s, 2H), 4.90−4.49 (s, 8H), 4.34 (mi.), 4.28 (mi.), 4.17 (mi.), 4.14 (ma.), 4.12 (ma.) and 3.98 (mi.) (s, 4H), 3.79 (mi.), 3.78 (mi.), 3.73 (ma.), 3.71 (mi.) (s, 3H), 3.66−3.12 (m, 8H), 2.01 (mi.), 2.00 (mi.) and 1.99 (ma.) (s, 3H), 1.84 (mi.), 1.80 (mi.) and 1.76 (ma.) (s, 3H), 1.42 (s, 9H). ^{13}C NMR (75.5 MHz, CD_3OD) (several CH_2 were obscured under the solvent peak): δ 192.5, 172.0, 171.1, 169.5, 169.2, 166.5, 158.0, 152.7, 150.4, 143.7, 143.5, 139.7, 139.6, 119.7, 119.6, 110.4, 80.2, 53.0, 52.7, 52.6, 51.4, 51.0, 39.2, 38.7, 37.9, 37.5, 37.2, 28.5, 16.8, 16.7, 12.0. MS (FAB): m/z 703 $(M + Na)^+$.

Methyl *N*-[2-[[*N*′-[2-(Boc-amino)ethyl]-*N*′-(thymin-1-ylacetyl)-glycyl]amino]ethyl]-*N*-[(*N*^{3}-methyl-4-thiothymin-1-yl)acetyl]glycinate (23). Preparation of **18** was accomplished according to general procedure A from **15** (1.57 g, 3.66 mM) using 12 mL of TFA/CH_2Cl_2. For the preparation of **19**, see synthesis of **21**. The coupling reaction was accomplished according to general procedure D using 3.47 mM of **19** and **18**. Product purification was achieved through silica gel chromatography using a gradient of MeOH in CH_2Cl_2 (0→10%) affording **23** (1.79 g, 74%) as yellow foam. ^{1}H NMR (300 MHz, CD_3-OD): δ 7.53 (ma.) and 7.44 (mi.) and 7.36 (mi.), 7.31 (mi.) and 7.25 (mi.) and 7.18 (ma.) (s, 2H), 4.86−4.43 (s, 7H), 4.37 (mi.), 4.30 (mi.), 4.18 (mi.), 4.15 (ma.), 4.13 (ma.) and 3.99 (mi.) (s, 4H), 3.81 (mi.), 3.79 (mi.), 3.74 (ma.), 3.73 (mi.), 3.72 (mi.), 3.71 (ma.), 3.68 (mi.) (s, 6H), 3.67−3.12 (m, 8H), 2.08 (mi.), 2.06 (mi.), 2.04 (mi.) and 1.98 (ma.) (s, 3H), 1.83 (mi.), 1.82 (mi.), 1.80 (mi.) and 1.74 (ma.) (s, 3H), 1.42 (s, 9H). ^{13}C NMR (75.5 MHz, CD_3OD) (several CH_2 were buried below the solvent peak): δ 192.5, 172.1, 171.0, 169.6, 169.0, 166.3, 156.0, 152.6, 151.2, 143.3, 137.1, 136.7, 119.9, 119.6, 110.8, 110.6, 80.2, 53.0, 52.8, 52.6, 51.4, 51.2, 51.0, 39.2, 38.7, 37.8, 37.5, 35.7, 28.4, 18.6, 12.0, 11.8. MS (FAB): m/z 595 $(M + H)^+$.

***N*-[2-[[*N*′-(2-Aminoethyl)-*N*′-(thymin-1-ylacetyl)glycyl]amino]ethyl]-*N*-[(4-thiothymin-1-yl)acetyl]glycine (1).** (1) Methyl ester saponification of **21** (1.146 g, 1.68 mM) was accomplished in 50 min

after dissolution in 10 mL of MeOH according to general procedure C using 10 mL of NaOH solution. (2) Boc deprotection was accomplished according to general procedure B using 10 mL of TFA/CH_2Cl_2. After HPLC purification, 690 mg of **1** (53%) was obtained as a yellow amorphous powder. UV (λ_{max} H_2O): 266, 338 nm. ^{13}C NMR (75.5 MHz, D_2O): δ 194.3, 194.1, 193.9, 175.7, 175.3, 174.5, 173.5, 173.4, 173.2, 172.0, 171.5, 169.5, 169.2, 155.1, 155.0, 152.6, 146.1, 145.8, 145.6, 143.4, 142.9, 142.5, 123.0, 122.6, 122.5, 122.3, 114.1, 114.0, 113.5, 53.2, 53.0, 52.9, 52.7, 52.5, 52.3, 51.3, 51.1, 50.9, 50.3, 50.2, 49.2, 48.9, 40.7, 40.3, 19.1, 19.0, 14.4. HRMS (FAB) $(M + H)^+$: calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.1985. Anal. Calcd for $C_{22}H_{30}N_8O_8S\cdot1.5CF_3COOH\cdot2H_2O$: C, 38.81; H, 4.62; N, 14.49; S, 4.13. Found: C, 38.15; H, 4.65; N, 14.16; S, 4.16.

***N*-[2-[[*N*′-(2-Aminoethyl)-*N*′-[(4-thiothymin-1-yl)acetyl]glycyl]-amino]ethyl]-*N*-(thymin-1-ylacetyl)glycine (2).** (1) Methyl ester saponification of **22** (1.079 g, 1.59 mM) was accomplished in 50 min after dissolution in 10 mL of MeOH according to general procedure C using 10 mL of NaOH solution. (2) Boc deprotection was accomplished according to general procedure B using 10 mL of TFA/CH_2Cl_2. After HPLC purification, 684 mg of **2** (55%) was obtained as a yellow amorphous powder. UV (λ_{max} H_2O): 267, 337 nm. ^{13}C NMR (75.5 MHz, D_2O): δ 194.9, 194.6, 176.3, 176.0, 175.1, 174.7, 173.8, 173.6, 173.5, 173.3, 173.1, 172.7, 172.0, 170.2, 170.0, 169.7, 155.5, 153.2, 147.0, 146.7, 146.5, 143.4, 143.2, 123.5, 123.1, 114.2, 114.1, 113.8, 53.9, 53.4, 52.7, 52.4, 52.0, 51.5, 50.8, 49.7, 41.3, 40.9, 40.7, 19.7, 15.0. HRMS (FAB) $(M + H)^+$: calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.1982. Anal. Calcd for $C_{22}H_{30}N_8O_8S\cdot1.5CF_3COOH\cdot2H_2O$: C, 38.81; H, 4.62; N, 14.49; S, 4.13. Found: C, 38.26; H, 4.71; N, 14.04; S, 3.95.

***N*-[2-[[[2-[(Thymin-1-ylacetyl)amino]ethyl]glycyl]amino]ethyl]-*N*-[(4-thiothymin-1-yl)acetyl]glycine (3).** (1) Boc deprotection was accomplished according to general procedure A from **21** (1.012 g, 1.49 mM) in 10 mL of TFA/CH_2Cl_2. (2) Methyl ester saponification and acyl migration were accomplished in 60 min according to general procedure C using 10 mL of NaOH solution. After HPLC purification, 401 mg of **3** (36.5%) was obtained as a yellow amorphous powder that crystallized from MeOH/H_2O (1:1). Mp: 195−198 °C. UV (λ_{max} H_2O) 266, 338 nm. ^{1}H NMR (300 MHz, D_2O): δ 7.41 (s, 1H), 7.36 (s, 1H), 4.76 (ma.) and 4.61 (mi) (s, 2H), 4.48 (mi) and (4.46) (ma.) (s, 2H), 4.28 (mi.) and 4.14 (ma.) (s, 2H), 3.98 (ma.) and 3.79 (mi.) (s, 2H), 3.68−3.20 (m, 8H), 2.01 (s, 3H), 1.81 (s, 3H). ^{13}C NMR (75.5 MHz, D_2O): δ 193.0, 174.1, 171.9, 170.4, 168.3, 153.8, 151.3, 144.7, 141.8, 141.6, 121.3, 112.6, 52.2, 51.4, 50.6, 49.8, 49.5, 48.6, 48.3, 38.4, 37.6, 17.6, 12.9. HRMS (FAB) $(M + H)^+$: calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.2018. Anal. Calcd for $C_{22}H_{30}N_8O_8S\cdot1.5CF_3COOH$: C, 40.71; H, 4.30; N, 15.19; S, 4.34. Found: C, 40.85; H, 4.54; N, 15.44; S, 4.61.

Crystal Structure of 3. Crystal data: $C_{22}H_{30}N_8O_8\cdot2H_2O$, M_w = 602.62, colorless crystal of 0.06 × 0.30 × 0.30 mm, monoclinic, space group *Cc*, $Z = 4$, $a = 7.634$ (2), $b = 22.258$ (4), $c = 17.021$ (3) Å, $\beta = 97.52$ (3), $V = 2867$ (1) Å^3, $d_{calc} = 1.40$ g cm^{-3}, $F(000) = 1272$, λ (Cu Kα) = 1.5418 Å, $\mu = 1.54$ mm^{-1}. Intensity data were measured on a Enraf-Nonius CAD-4 diffractometer using graphite-monochromated Cu Kα radiation and the ($\theta - 2\theta$) scan technique up to $\theta = 66°$. Of the 4835 collected reflections ($-8 \le h \le 8$, $-26 \le k \le 26$, $0 \le l \le 20$), 2534 were unique ($R_{int} = 0.057$) of which 2479 were considered as observed having $I \ge 3\sigma(I)$. Cell parameters were refined from 25 well-centered reflections with $13.1 \le \theta \le 20.6°$. The structure was solved by direct methods using SHELXS86[35] and refined by full-matrix least-squares with SHELX76,[36] minimizing the function $\sum w(F_o - |F_c|)^2$. Two water molecules were found on difference Fourier maps. The coordinates of the hydrogen atoms, located in difference Fourier maps, were refined. They were assigned an isotropic thermal factor equivalent to that of the bonded carbon atom, plus 10%. One hydrogen atom of the water molecules W40 was not located on difference map, it was set in theoretical position according to the probable hydrogen bond W40−H···O5. Convergence was reached at $R = 0.037$ and $R_w = 0.049$ (with $R_w = [\sum w(F_o - |F_c|)^2/\sum wF_o^2]^{1/2}$ and $w = 1/[\sigma^2(F_o) + 0.0002F_o^2]$, goodness of fit 0.95. The residual electron density in the final difference map was located between −0.35 and 0.22 e Å^3.

***N*-[2-[[[2-[[4-Thiothymin-1-yl)acetyl]amino]ethyl]glycyl]amino]-ethyl]-*N*-(thymin-1-ylacetyl)glycine (4).** (1) Boc deprotection was accomplished according to general procedure A from **22** (1.024 g, 1.50 mM) in 10 mL of TFA/CH_2Cl_2. (2) Methyl ester saponification and acyl migration were accomplished in 60 min according to general procedure C using 10 mL of NaOH. After HPLC purification, 386 mg of **4** (35%) was obtained as a yellow amorphous powder. UV (λ_{max} H_2O): 267, 338 nm. ^{1}H NMR (300 MHz, D_2O): δ 7.46 (s, 1H), 7.31 (s, 1H), 4.69 (ma.), 4.52 (mi) (s, 2H), 4.52 (mi) and (4.50) (ma.) (s, 2H), 4.19 (mi.), 4.10 (ma.) (s, 2H), 3.98 (ma.) and 3.79 (mi.) (s, 2H), 3.70−3.20 (m, 8H), 1.99 (s, 3H), 1.82 (s, 3H). ^{13}C NMR (75.5 MHz, D_2O): δ 193.0, 174.1, 171.4, 171.0, 168.4, 168.3, 167.8, 153.6, 151.5, 144.9, 144.7, 141.7, 121.6, 112.3, 52.9, 52.7, 50.8, 50.6, 49.7, 49.5, 48.8, 48.5, 38.5, 37.7, 17.7, 13.0. HRMS (FAB) $(M + H)^+$: calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.2010. Anal. Calcd for $C_{22}H_{30}N_8O_8S\cdot1.5\cdot CF_3COOH$: C, 40.71; H, 4.30; N, 15.19; S, 4.34. Found: C, 40.21; H, 4.76; N, 15.31; S, 4.53.

***N*-[2-[[[2-[(Thymin-1-ylacetyl)amino]ethyl]glycyl]amino]ethyl]-*N*-[(*N*^{3}-methyl-4-thiothymin-1-yl)acetyl]glycine (5).** (1) Boc deprotection was accomplished according to general procedure A from **23** (1.690 g, 2.43 mM) in 10 mL of TFA/CH_2Cl_2. (2) Methyl ester saponification and acyl migration were accomplished in 60 min according to general procedure C using 10 mL of NaOH. After HPLC purification, 447 mg of **5** (27%) was obtained as a yellow amorphous powder. UV (λ_{max} H_2O): 267, 331 nm. ^{1}H NMR (300 MHz, D_2O) [60:40 rotamer mixture]: δ 7.47 (s, 1H), 7.43 (mi.), 7.41 (ma.) (s, 1H), 4.80 (ma.), 4.71 (mi.) (s, 2H), 4.51 (mi), 4.49 (ma.) (s, 2H), 4.32 (mi.), 4.18 (ma.) (s, 2H), 4.08 (ma.), 3.87 (mi.) (s, 2H), 3.74 (mi.), 3.73 (ma.) (s, 3H), 3.70−3.20 (m, 8H), 2.12 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (75.5 MHz, D_2O): δ 194.9, 176.0, 173.4, 172.4, 172.0, 169.9, 169.4, 155.4, 153.8, 146.4, 140.3, 123.1, 114.2, 54.4, 53.8, 52.9, 51.5, 50.6, 40.3, 39.4, 39.1, 21.5, 14.8. HRMS (FAB) $(M + H)^+$: calcd for $C_{23}H_{33}N_8O_8S$ 581.2142, found 581.2133. Anal. Calcd for $C_{23}H_{32}N_8O_8S\cdot CF_3COOH\cdot 4H_2O$: C, 39.16; H, 5.39; N, 14.62; S, 4.17. Found: C, 38.71; H, 5.17; N, 15.05; S, 4.27.

B. General Photolysis Procedure. Irradiation experiments (20 mg/100 mL) were performed at pH 3−4, with an Original Hanau Quarzlampen Fluotest-Forte Ref. 5261 for 30 min (**1** and **2**), 6 h (**3** and **4**), and 5.5 h (**5**) (progress of the reaction was monitored by UV and ^{1}H NMR spectroscopy). Irradiations were performed under continuous nitrogen bubbling. See above for purification conditions.

Photoproduct 24: Yield, 17.9%; UV (λ_{max} H_2O) 267, 317 nm; ^{1}H NMR (300 MHz, D_2O) [50(a)/30(b)/20(c) rotamer mixture] δ 8.30(c), 8.26(a), 8.14(b) (1H, s), 7.15(a), 6.76(b), 6.34(c) (1H,s), 5.35−3.10 (18H), 2.09(a), 2.06(b), 2.05(c) (3H, s). ^{13}C NMR (75.5 MHz, D_2O) δ 176.1, 174.1, 173.2, 172.1, 172.0, 171.2, 168.1, 158.2, 155.4, 155.2, 154.9, 149.2, 145.8, 144.4, 119.3, 118.3, 113.7, 110.9, 55.3, 55.0, 54.4, 53.9, 53.1, 52.7, 52.3, 51.7, 50.9, 50.5, 49.9, 49.1, 48.5, 48.1, 42.6, 41.7, 40.4, 40.1, 16.0. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{29}N_8O_8$ 533.2108, found 533.2090.

Photoproduct 25: Yield 8.7%. UV (λ_{max} H_2O) 279 nm; ^{1}H NMR (300 MHz, D_2O) (80:20 isomer mixture) δ 7.57 (mi.) 7.46 (ma.) (1H, s), 1.42 (mi.) (d, $J = 7.3$ Hz), 1.37 (ma.) (d, $J = 6.7$ Hz) (3H). ^{13}C NMR (75.5 MHz, D_2O) δ 210.4, 175.9, 175.4, 173.6, 172.5, 168.8, 155.7, 154.2, 149.1, 148.6, 114.3, 112.9, 64.5, 63.5, 54.7, 54.2, 51.5, 51.3, 50.8, 49.0, 47.8, 40.9, 40.6, 39.6, 27.4, 25.4, 17.6. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.1971.

Photoproduct 26: Yield 4.3%; UV (λ_{max} H_2O) sh 255 nm; ^{1}H NMR (300 MHz, D_2O): δ 6.06 (1H, s), 1.64 (s). ^{13}C NMR (75.5 MHz, D_2O) δ 176.0, 175.5, 174.4, 174.2, 173.7, 155.8, 155.5, 132.4, 108.7, 83.0, 78.2, 53.7, 52.2, 51.4, 50.1, 49.8, 48.6, 47.2, 40.6, 40.2, 30.3, 19.3. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{31}N_8O_8S$ 567.1986, found 567.1952.

Photoproduct 27: Yield 44.2%; UV (λ_{max} H_2O) 268, 317 nm; ^{1}H NMR (300 MHz, D_2O) (50:50 rotamer mixture) δ 7.82, 7.78 (1H, s), 6.58, 6.18 (1H, s), 5.41 (d, $J = 16.1$ Hz), 5.01 (d, $J = 16.7$ Hz) (1H), 4.79 (d, $J = 16.1$ Hz), 4.66 (2H, m), 4.29 (d, $J = 16.7$ Hz), 4.15 (d, $J = 16.3$ Hz) (1H), 4.07−3.56 (8H, m), 3.47−3.20 (5H, m), 2.00 (3H,

(35) Sheldrick, G. M. SHELXS86. Program for the solution of crystal structures. University of Göttingen, Germany, 1986.

(36) Sheldrick, G. M. SHELX76. Program for crystal structure determination. University of Cambridge, England, 1976.

s). ^{13}C NMR (75.5 MHz, D_2O) δ 181.1, 181.0, 178.8, 178.4, 174.7, 174.6, 172.5, 171.7, 171.6, 168.5, 160.8, 160.4, 154.9, 154.8, 153.0, 152.8, 147.0, 146.7, 119.7, 118.7, 113.5, 113.0, 55.7, 55.1, 54.6, 54.5, 53.0, 52.0, 51.7, 51.5, 50.9, 49.2, 48.6, 40.8, 40.7, 39.3, 35.2, 34.3, 16.2, 16.1. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{29}N_8O_8$ 533.2108, found 533.2134.

Photoproduct 31: Yield 15.7% from **4** and 7.7% from **3**; UV (λ_{max} H_2O) 267 nm; ^{1}H NMR (300 MHz, D_2O) (60:40 rotamer mixture) δ 7.45 (1H, s), 7.40 (1H, s), 4.76 (mi.), 4.60 (ma.) (2H, s), 4.52 (2H, s), 4.06 (ma.), 3.95 (mi.) (2H, s), 3.87 (mi.) 3.72 (ma.) (2H, s), 3.58 (5H, br s), 3.45 (1H, m), 3.18 (2H, m), 1.88 (3H, s). HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{31}N_8O_9$ 551.2214, found 551.2231.

Photoproduct 32: Yield 4.0%. UV (λ_{max} H_2O) 269, 315 nm; ^{1}H NMR (250 MHz, D_2O) δ 7.89 (1H, s), 7.17 (1H, s), 4.78 (2H, s), 4.55 (2H, s), 4.08 (2H, s), 3.74 (2H, s), 3.69 (2H, s), 3.67 (2H, m), 3.54 (2H, m), 3.39 (2H, m), 3.16 (2H, m), 2.10 (3H, s). ^{13}C NMR (75.5 MHz, D_2O) δ 180.8, 179.1, 173.8, 173.2, 171.4, 169.7, 160.8, 156.2, 153.2, 146.8, 119.6, 114.8, 56.0, 54.4, 53.4, 52.6, 50.7, 49.8, 40.5, 17.0. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{29}N_8O_8$ 533.2108, found 533.2089.

Photoproduct 36: Yield 21.1%; UV (λ_{max} H_2O) 277 nm; ^{1}H NMR (300 MHz, D_2O/TFA) (80:20 isomer mixture) δ 7.41 (mi.), 7.25 (ma.) (1H, s), 2.40 (2H, m), 1.35 (ma.) (3H, d, $J = 6.4$ Hz), 1.31 (mi.) (3H, d, $J = 6.5$ Hz). ^{13}C NMR (62.9 MHz, D_2O/TFA) δ 210.7, 210.2, 173.7, 173.5, 172.0, 171.5, 170.8, 168.3, 167.8, 166.7, 166.4, 153.4, 153.2, 152.8, 146.5, 111.0, 110.4, 60.9, 59.0, 53.0, 51.2, 50.8, 50.5, 49.8, 49.0, 48.3, 47.6, 46.4, 45.5, 38.3, 37.8, 37.3, 37.1, 36.9, 25.6, 17.0, 16.7. HRMS (FAB) $(M + H)^+$ calcd for $C_{23}H_{33}N_8O_8S$ 581.2142, found 581.2145.

Preparation of Compounds 30 and 35. The irradiation mixture (20 mg) was lyophilized and the residue dissolved in D_2O (400 μL) then 40 μL of a 5% solution of $CH_3SO_2SCH_3$ in CD_3OD were added. Evolution of the reaction was controlled by ^{1}H NMR. After completion, the solution was freeze-dried then stored at −18 °C. This sequence was repeated 10 times, then lyophilisates were pooled and purified by HPLC.

Compound 30: Yield 32.2%; UV (λ_{max} H_2O) 318 nm; ^{1}H NMR (300 MHz, D_2O/TFA) δ 7.50 (1H, s), 5.27 (1H, s), 4.62 (1H, d, $J = 16.1$ Hz), 4.42 (1H, d, $J = 16.1$ Hz), 4.12 (1H, d, $J = 17.3$ Hz), 3.92−3.42 (7H, m), 3.35−2.80 (6H, m), 1.73 (3H, s), 1.20 (3H, s). ^{13}C NMR (75.5 MHz, D_2O/TFA) δ 175.1, 173.9, 173.6, 172.7, 170.0, 168.9, 158.1, 155.2, 153.7, 116.6, 85.6, 57.0, 53.6, 50.6, 50.0, 48.7, 48.3, 38.5, 37.7, 18.5, 16.0. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{31}N_8O_9$ 551.2214, found 551.2234.

Compound 35: Yield 13.7%. UV (λ_{max} H_2O) 318 nm; ^{1}H NMR (300 MHz, D_2O/TFA) (80:20 isomer mixture) δ 7.94 (1H, s), 5.63 (ma.), 5.55 (mi.) (1H, s), 2.22 (mi.), 2.12 (ma.) (3H, s), 1.61 (mi.), 1.57 (ma.), (3H, s). ^{13}C NMR (75.5 MHz, D_2O/TFA) δ 176.2, 174.5, 174.4, 172.2, 171.7, 167.7, 157.9, 154.4, 153.6, 153.3, 116.6, 86.5, 57.9, 55.4, 51.2, 50.3, 49.8, 49.3, 48.8, 39.3, 38.2, 18.3, 15.8. HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{31}N_8O_9$ 551.2214, found 551.2233.

Supporting Information Available: X-ray crystallographic analysis for **3** and ^{1}H NMR spectra (D_2O) of **1** and **2** (11 pages). See any current masthead page for ordering and Internet access instructions.

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