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Dimerization

Photosensitized Thymine Dimerization via Delocalized Triplet Excited States

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Abstract: A new mechanism of photosensitized formation of thymine (Thy) dimers is proposed, which involves generation of a delocalized triplet excited state as the key step. This is supported by chemical evidence obtained by combining one benzophenone and two Thy units with different degrees of freedom, whereby the photoreactivity is switched from a clean Paternò–Büchi reaction to a fully chemo-, regio-, and stereoselective [2+2] cycloaddition.

Introduction

Ultraviolet solar radiation reaching the Earth's surface has been widely reported as a potential mutagenic agent.[1] Nucleobases are able to absorb UVB light, which causes direct photoreactions from their singlet excited states, such as formation of cyclobutane thymine dimers (Thy<>Thy) or (6-4) photoproducts, which are among the most mutagenic alterations.[2] Nanosecond IR spectroscopy has revealed that intrinsically populated Thy triplets decay in DNA single strands via formation of biradical intermediates, which can result in cyclobutane dimers, although they predominantly decay through nonreactive pathways.[3] Although solar UVA light is less efficiently absorbed by nucleobases, the presence of endogenous or exogenous photosensitizers that can be excited in that region may greatly enhance UVA-mediated photochemical disorders. Moreover, CASPT2//CASSCF theoretical calculations on the photosensitized process indicated formation of a stabilized triplet excimer from a parallel-stacked triplet pair of nucleobases.[4]

In this context, benzophenone (BP) and drugs containing the BP chromophore have been widely reported as DNA photosensitizers.[5] It is assumed that BP-photosensitized DNA damage includes triplet–triplet energy transfer (TTET) from ^{3}BP* to Thy, followed by [2+2] cycloaddition yielding Thy<>Thy.[5,6] Formation of these dimers occurs along with the Paternò–Büchi reaction between ^{3}BP* and Thy resulting in the formation of oxetanes, which have been claimed as models to mimic the highly unstable intermediates involved in the repair of (6-4) photoproducts.[5b,7]

The rate constant for TTET should be related to the energy gap between ^{3}BP* and ^{3}Thy*.[8] Although it seems a slightly disfavored process in solution (triplet energies of 70 and 74 kcal mol^{-1} for BP and Thy, respectively),[5b] it is still feasible at room temperature through population of the upper vibrational states. In view of the thermodynamics, the process would be uphill and therefore expectedly slow, and this allows for alternative pathways such as the abovementioned Paternò–Büchi reaction to give oxetanes. This reaction requires a nπ* electronic configuration of the excited state, a requirement that is fulfilled in the case of BP.

In fact, previous studies have demonstrated that in solution ^{3}BP* is efficiently quenched by Thy derivatives (k_q in the range of 10^8–10^9 M^{-1} s^{-1}).[7a,9] Since the expected rate constant for TTET in solution is at least one order of magnitude lower (according to the Sandros equation), formation of oxetanes essentially accounts for the determined quenching rate constant. The intramolecular version of this reaction, in which one molecule of the chromophore is directly attached to the sugar moiety of thymidine, has also been investigated. Irradiation of the dyad leads to a mixture of photoproducts, in which the oxetanes are the major ones.[10]

A simplified mechanistic explanation of the reaction between ^{3}BP* and Thy is summarized in Equations (1)–(3):[11]

$$^3\text{BP}^* + \text{Thy} \rightarrow \text{oxetanes} \quad (1)$$

$$^3\text{BP}^* + \text{Thy} \rightarrow \text{BP} + {}^3\text{Thy}^* \quad (2)$$

$$^3\text{Thy}^* + \text{Thy} \rightarrow \text{Thy} <> \text{Thy} \quad (3)$$

Interestingly, irradiation of BP in the presence of Thy derivatives (2:1 ratio) gives mainly oxetanes, whereas dimers predominate in the presence of a large excess of Thy.[7a,9b] Although formation of dimers should be dependent on Thy concentration according to process (2), the observed changes in the oxetane-to-dimer ratio are clearly more marked than expected from kinetic analysis. Moreover, the unambiguous detection of cytosine dimers (Cyt<>Cyt) in photosensitized DNA (in spite of the high triplet energy of Cyt of ca. 77 kcal mol^{-1})[12] suggests that formation of locally excited pyrimidine

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triplet states may not be the only mechanism of dimerization and that cooperative excited states may play a significant role.[13] In this context, excited termolecular complexes (triplexes) have previously been proposed to explain the results obtained on photosensitization of some [4+2] and [2+2] cycloadditions.[14] Although the involvement of triplet triplexes or, in general, delocalized triplet excited states, has never been proven in the case of DNA, they could account for most of the experimental observations on the BP-photosensitized pyrimidine dimerization. This novel hypothesis is illustrated in Equations (4)–(7).

$$^3\mathrm{BP}^* + \mathrm{Thy} \rightarrow {}^3[\mathrm{BP}\cdots\mathrm{Thy}]^* \quad (4)$$

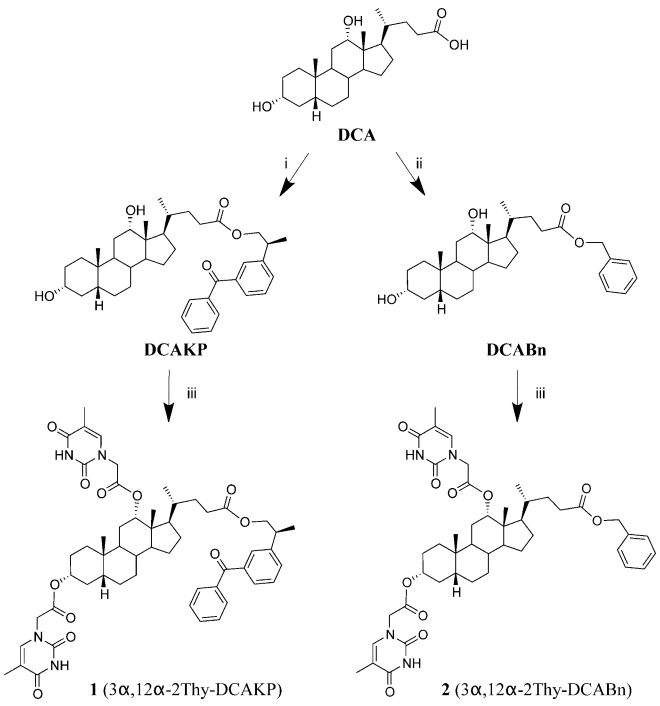
$$^3[\mathrm{BP}\cdots\mathrm{Thy}]^* \rightarrow \text{oxetanes} \quad (5)$$

$$^3[\mathrm{BP}\cdots\mathrm{Thy}]^* + \mathrm{Thy} \rightarrow {}^3[\mathrm{BP}\cdots\mathrm{Thy}\cdots\mathrm{Thy}]^* \quad (6)$$

$$^3[\mathrm{BP}\cdots\mathrm{Thy}\cdots\mathrm{Thy}]^* \rightarrow \mathrm{BP} + \mathrm{Thy} <> \mathrm{Thy} \quad (7)$$

Thus, quenching of ^{3}BP* by Thy would lead to oxetanes via triplet exciplexes, while interaction of the latter with a second Thy unit would afford triplet triplexes, the immediate precursors of Thy<>Thy.

To provide experimental evidence supporting the proposed mechanism, two Thy units have been now tethered to an appropriate bile acid scaffold. For this purpose, deoxycholic acid (**DCA**, Scheme 1) was selected as an appropriate skeleton, since it offers a rigid structure with two hydroxyl groups on the same face of the molecule allowing covalent attachment of the Thy bases. Linking of the carboxylic acid of the lateral chain with a BP moiety would lead to a fully intramolecular system in which binary BP/Thy and Thy/Thy interactions (required in the currently accepted mechanism) are possible; by contrast, ternary BP/Thy/Thy interactions (involved in the novel proposal) would be prevented due to geometrical strain. Conversely, increasing the degrees of freedom by replacement of the intramolecular with an intermolecular BP chromophore should allow assembly of a reactive triplet triplex providing experimental proof of the concept.



Scheme 1. Developed synthetic strategy to prepare DCA derivatives incorporating BP or Bn at the lateral chain and two Thy units. Reagents and conditions: i) **KP-OH**, 4-DMAP, EDC, pyridine; ii) benzyl bromide, DBU, DMF; iii) Thy-CH_2COOH, Et_3N, 2,4,6-trichlorobenzoyl chloride, 4-DMAP, THF.

Results and Discussion

Two analogues with **DCA** skeleton were synthesized (see Scheme 1). In one the two Thy units are covalently attached at positions 3α and 12α and the BP chromophore is esterified at the lateral chain, and in the other BP was replaced with a benzyl group, which does not absorb in the UVA region.

Briefly, the developed synthetic strategy outlined in Scheme 1 started with esterification of the carboxyl group of **DCA** with the *S* enantiomer of reduced ketoprofen (**KP-OH**) to give **DCAKP**. Then, in the presence of an excess of Thy-CH_2CO_2H, the two hydroxyl groups at the 3α and 12α positions were simultaneously esterified to provide 3α,12α-2Thy-DCAKP (**1**). To prepare the derivative without the BP unit, the carboxyl group at the lateral chain of **DCA** was treated with benzyl bromide to give **DCABn**. Subsequent treatment with Thy-CH_2CO_2H yielded 3α,12α-2Thy-DCABn (**2**).

Laser flash photolysis (LFP) was employed to monitor the triplet state obtained on 355 nm excitation of the BP chromophore in the presence of two Thy units in the following systems: 1) the fully intramolecular **1** and 2) the intermolecular 1:1 mixture of the parent BP and the non-absorbing **2**.

Thus, the triplet of **1** was monitored at 520 nm (Figure 1, top) and compared to that of **DCAKP**, as a reference compound with BP bound to the bile acid, but lacking the two Thy units. In this way, intramolecular hydrogen-atom abstraction, a known deactivation pathway for ^{3}BP* was also considered. Decays were fitted to a first-order exponential equation and their lifetimes τ were 0.08 and 1.01 μs, respectively. More accurate determination was achieved by means of energy transfer to naphthalene (NPH)[15] (Figure 1, top, inset), which led to a value of 0.097 μs. This indicates a fast intramolecular deactivation of the triplet, with a rate constant of $9.3\times10^6\ s^{-1}$ ($k = 1/\tau_{\mathbf{3\alpha,12\alpha\text{-}2Thy\text{-}DCAKP}} - 1/\tau_{\mathbf{DCAKP}}$). Then, the lifetime of ^{3}BP* was monitored on addition of increasing concentrations of **2** (Figure 1, bottom). Stern–Volmer plots of the reciprocal lifetimes obtained in each case allowed determination of the quenching rate constant, which was found to be $3.7\times10^8\ M^{-1}s^{-1}$. For comparison, quenching of ^{3}BP* by Thy occurred with $k = 1.4\times10^8\ M^{-1}s^{-1}$, which is reasonably similar when considering the two units of Thy per molecule in the case of **2**.

After completing the photophysical studies, the same systems were subjected to steady-state photolysis, in order to investigate their photoreactivity and the nature of the photoproducts. Thus, selective irradiation of the BP chromophore at $\lambda_{max} = 350$ nm was monitored through the changes in the UV/

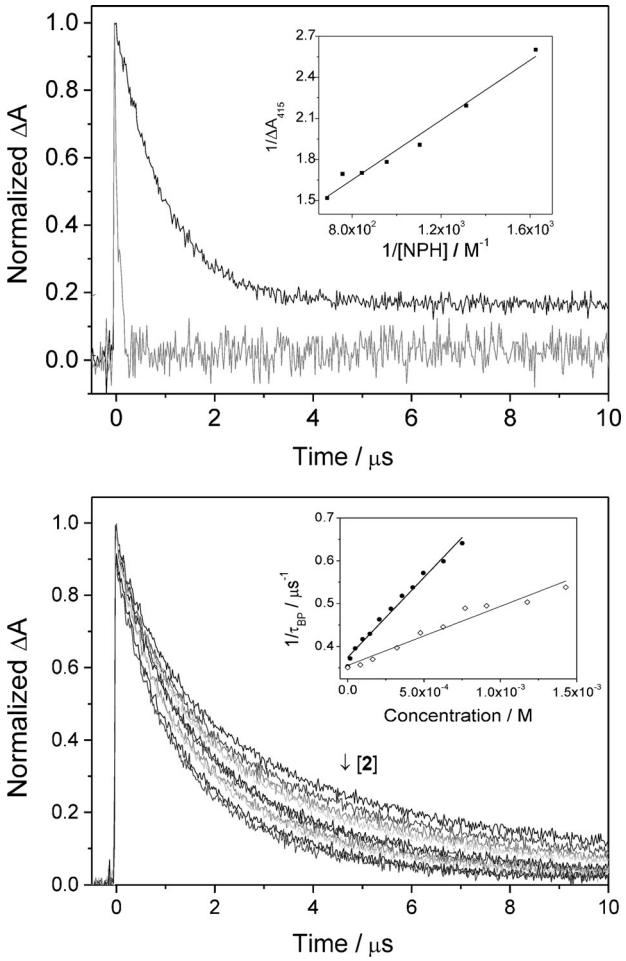


Figure 1. Top: LFP decays monitored at 520 nm of **DCAKP** (black) and **1** (gray) in CH_3CN:H_2O (4:1). Inset: double-reciprocal plot for energy transfer from **1** to NPH, revealed through the absorption of ^{3}NPH* at 415 nm immediately after the laser pulse. Bottom: LFP decays of BP monitored at 520 nm with increasing concentrations of **2**. Inset: Stern–Volmer plot for the quenching of BP by **2** (●) or by Thy (◇).

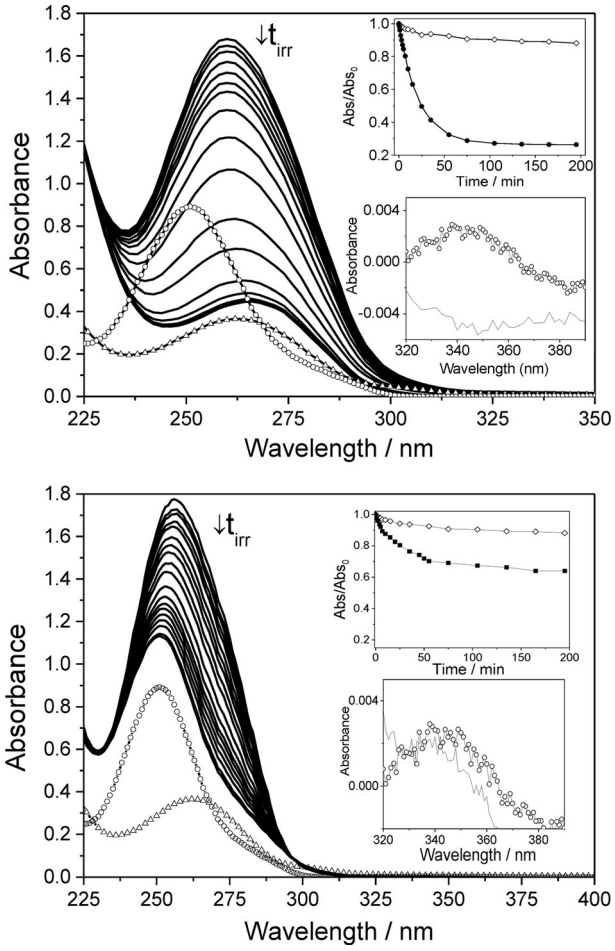


Figure 2. UV/Vis spectra of **1** (top) and BP**:2** (bottom) monitored at different irradiation times ($C_i = 4.4\times10^{-5}$ M in CH_3CN:H_2O (4:1), inert atmosphere, $\lambda_{max} = 350$ nm); UV spectra of BP (○)and Thy (△) recorded at 4.4×10^{-5} M. Upper insets: photoreaction kinetics of **1** (●) and BP:**2** (■) compared to BP:Thy (1:2) (◇). Lower insets: zoom of 320–390 nm region after prolonged irradiation times of **1** and BP:**2** compared to BP (○).

Vis spectra. The results for **1** are shown in Figure 2 (top), and those for the intermolecular mixture BP:**2** in Figure 2 (bottom).

A progressive decrease of the absorbance was observed in both systems, whereas the control BP:Thy mixture (1:2 ratio) was almost unreactive (Figure 2, insets). Furthermore, when the residual UV spectra of **1** and BP:**2** after completion of the reaction were compared to the hypothetical absorption due to unconsumed BP or Thy (Figure 2), a different reaction pattern was revealed. In fact, the UV spectrum of **1** at the end of the reaction was comparable to that of Thy and thus indicated formation of an oxetane; conversely, the UV spectrum of the BP:**2** system after irradiation resembled that of BP and thus indicated formation of Thy<>Thy (see also the difference on zooming the 300–400 nm region in the insets of Figure 2, top and bottom). From the initial rates in the linear region, at less than 10% conversion, the reaction quantum yields for Thy<>Thy formation in BP:**2** was about three times higher than that of BP:Thy (1:2). Taking into account the reported data for the latter system, an upper limit for the value in the former would be 0.03.[5c] Indeed, this was confirmed by analysis of the resulting photoproduct mixtures.

Thus, **1** was independently irradiated in acetonitrile under inert atmosphere, and the resulting crude product was purified by reverse-phase column chromatography. Only one photoproduct was obtained (Figure 3, left), structural characterization of which was achieved on the basis of ^{1}H and ^{13}C NMR spectroscopy, including ^{13}C DEPT-135, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HSQC experiments, and also on exact mass determination.[10,16] The photoproduct turned out to be a single oxetane derived from the Paternò–Büchi reaction between the KP moiety and the Thy unit at the 12α position. This was assessed by the appearance of a new singlet in the ^{1}H NMR spectrum at 4.72 ppm, with concomitant disappearance of one of the olefinic proton signals and with the upfield displacement of the aromatic signals. Furthermore, on formation of the new ring the ^{13}C NMR signal corresponding to the original carbonyl group at 196.9 ppm shifted to 91.7 ppm, in accordance with the values reported for analogous oxetanes. The regiochemical

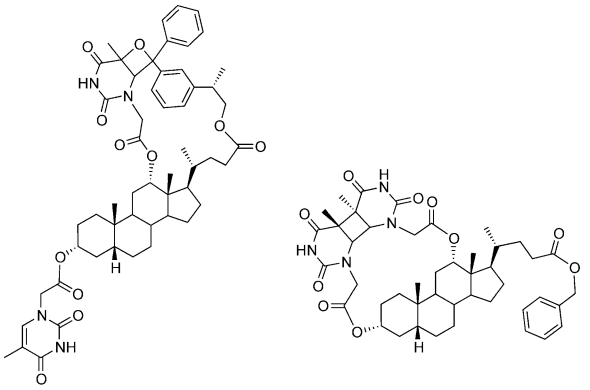


Figure 3. Photoproducts obtained on irradiation of **1** (left) and BP:**2** (right) in CH_3CN under N_2.

assignment depicted in Figure 3 was based on the comparison of the chemical shifts of the two carbon atoms formerly belonging to the Thy moiety with those found for related oxetanes.[6,9a] In fact, the obtained values of $\delta_C = 76.3$ ppm and $\delta_{CH} = 68.4$ ppm are in complete agreement with the expected values and far from those reported for the alternative regioisomers ($\delta_C = 52.7$–57.8 and $\delta_{CH} = 80.0$–90.0 ppm).

Next, the intermolecular system BP:**2** was irradiated in CH_3CN under nitrogen. The main photoproduct was isolated by column chromatography (80%) and was found to be a *trans–syn* Thy<>Thy (Figure 3, right), the structure of which was unambiguously established by crystal data (Figure 4).

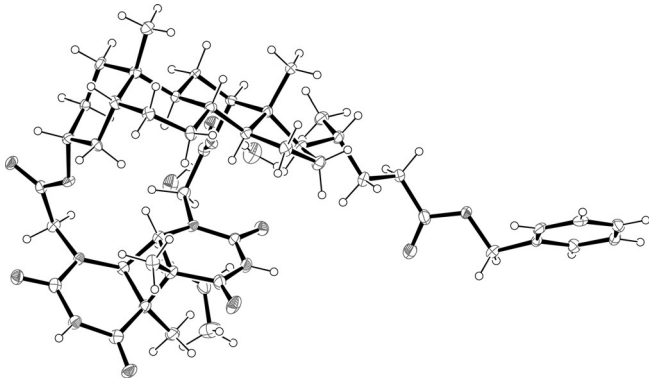


Figure 4. X-ray crystal structure of the Thy<>Thy **4** resulting from irradiation ($\lambda_{max} = 350$ nm) of BP:**2** in CH_3CN under N_2. CCDC 1031373 contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

The obtained results revealed that the photochemical behavior of the inter- and intramolecular systems is dramatically divergent. In principle, the arrangement of the two Thy bases and a BP unit covalently attached to the same scaffold was expected to disfavor formation of Thy<>Thy by the triplex mechanism; in fact, the predominant process was by far production of an oxetane. This is clearly related to the evolution of the exciplexes depending on the degrees of freedom. In **1** the geometry of the ^{3}[BP···Thy]* exciplex is appropriate for production of the oxetane but not for triplex formation. By contrast, in the BP:**2** mixture, the free arrangement of the ketone relative to the nucleobases allows assembling of the partners in the triplet triplex ^{3}[BP···Thy···Thy]* with the appropriate geometry to afford Thy dimers as the final products.

Conclusion

A new mechanistic pathway leading to photosensitized formation of cyclobutane pyrimidine dimers is proposed, in which the key step involves generation of a delocalized triplet excited state. The concept has been illustrated with systems combining one BP and two Thy units with different degrees of freedom, whereby the photoreactivity can be switched from a clean Paternò–Büchi to a fully chemo-, regio-, and stereoselective [2+2] dimerization. This finding underlines the importance of cooperative triplet excited states in DNA photodamage. Such delocalized chemical entities may predominate over locally excited triplet states when the thermodynamic requirement for energy transfer is not fulfilled.

Experimental Section

Additional experimental procedures, spectra of the new compounds, and details about steady-state and laser flash photolysis experiments are given in the Supporting Information.

Synthesis of (*S*)-KP-OH

BH_3·THF (6.600 mL of a 1 M solution, 6.60 mmol) was added dropwise on a stirred solution of (*S*)-ketoprofen (1.400 g, 5.50 mmol) in anhydrous THF (14 mL) at −20 °C, and the reaction mixture was allowed to warm overnight to room temperature. Then, the solution was cooled to 5 °C and treated with MeOH:H_2O (15:85, 17 mL). Afterwards, the solvents were concentrated in vacuum, the crude product was redissolved in CH_2Cl_2 and the solution was poured into brine, extracted with CH_2Cl_2, washed with $NaHCO_3$ (5%), dried over $MgSO_4$, and evaporated under reduced pressure. Purification by column chromatography (SiO_2, CH_2Cl_2:MeOH 95:5) gave the (*S*)-**KP-OH** as an oil (0.728 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.31$ (d, $J = 7.2$ Hz, 3H, CH_3), 1.49 (br s, 1H, OH), 3.04 (m, 1H, CH), 3.75 (m, 2H, CH_2), 7.40–7.84 ppm (m, 9H, arom); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 197.0$ (C), 144.5 (C), 137.7 (C), 137.6 (C), 132.5 (CH), 131.8 (CH), 130.1 (2×CH), 128.9 (CH), 128.5 (CH), 128.4 (CH), 128.3 (2×CH), 68.3 (CH_2), 42.3 (CH), 17.6 ppm (CH_3); MS: *m/z* found: 241.1219, calcd for $C_{16}H_{17}O_2$ $[M+H]^+$: 241.1229.

Synthesis of DCAKP

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; 0.348 mL, 1.97 mmol) was added dropwise to a stirred mixture of **DCA** (0.772 g, 1.97 mmol), **KP-OH** (0.394 g, 1.64 mmol), and 4-dimethylaminopyridine (4-DMAP; 0.200 g, 1.64 mmol) in anhydrous pyridine (8 mL) at 0 °C, and the reaction mixture was allowed to react for 1 h at 0 °C and then overnight at room temperature. Afterwards, the suspension was poured into 1 M HCl and extracted with CH_2Cl_2; the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography (SiO_2, AcOEt:hexane 60:40) gave **DCAKP** as a colorless solid (0.666 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.64$ (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.92 (d, $J = 6.3$ Hz,

3H, 21-CH_3), 1.33 (d, J=7.2 Hz, 3H, KP-CH_3), 3.17 (m, 1H, KP-CH), 3.61 (m, 1H, 3β-H), 3.95 (brs, 1H, 12β-H), 4.16 (dd, J=10.8 and 6.6 Hz, 1H, KP-CH_2), 4.21 (dd, J=10.8 and 7.2 Hz, 1H, KP-CH_2), 7.39–7.82 ppm (m, 9H, arom); ^{13}C NMR (75 MHz, $CDCl_3$): δ=196.9 (C), 174.2 (C), 143.8 (C), 137.9 (C), 137.7 (C), 132.6 (CH), 131.5 (CH), 130.2 (2×CH), 129.1 (CH), 128.8 (CH), 128.5 (CH), 128.4 (2×CH), 73.2 (CH), 72.0 (CH), 69.0 (CH_2), 48.4 (CH), 47.4 (CH), 46.6 (C), 42.2 (CH), 39.0 (CH), 36.6 (CH_2), 36.2 (CH), 35.3 (CH_2), 35.2 (CH), 34.2 (C), 33.8 (CH), 31.4 (CH_2), 31.0 (CH_2), 30.7 (CH_2), 28.8 (CH_2), 27.5 (CH_2), 27.3 (CH_2), 26.3 (CH_2), 23.8 (CH_2), 23.3 (CH_3), 18.1 (CH_3), 17.4 (CH_3), 12.9 ppm (CH_3); MS: m/z found: 615.4038, calcd for $C_{40}H_{55}O_5$ $[M+H]^+$: 615.4050.

Synthesis of 3α,12α-2Thy-DCAKP (1)

A stirred suspension of Thy-CH_2COOH (1.192 g, 6.47 mmol) in anhydrous THF (40 mL) was treated with Et_3N (1.810 mL, 12.99 mmol) and 2,4,6-trichlorobenzoyl chloride (1.230 mL, 7.85 mmol) and the resulting mixture was allowed to react for 1.5 h. Then, a solution of 4-DMAP (0.527 g, 1.32 mmol) and **DCAKP** (0.663 g, 1.08 mmol) in anhydrous THF (25 mL) was added and the resulting reaction mixture was stirred overnight. Afterwards, it was poured into $NaHCO_3$ (5%), extracted with CH_2Cl_2, and the combined extracts were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. Purification by column chromatography (SiO_2, CH_2Cl_2:MeOH 97:3, followed by Li Chroprep RP-18, CH_3CN:H_2O 80:20) gave **1** as a yellow solid (0.959 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.68 (s, 3H, CH_3), 0.74 (d, J=6.3 Hz, 3H, 21-CH_3), 0.88 (s, 3H, CH_3), 1.33 (d, J=6.9 Hz, 3H, KP-CH_3), 1.89 (brs, 3H, Thy-CH_3), 1.90 (brs, 3H, Thy-CH_3), 3.17 (m, 1H, KP-CH), 4.17 (d, J=6.9 Hz, 2H, KP-CH_2), 4.41 (d, J=17.1 Hz, 1H, Thy-CH_2), 4.45 (brs, 2H, Thy-CH_2), 4.58 (d, J=17.1 Hz, 1H, Thy-CH_2), 4.71 (brs, 1H, 3β-H), 5.12 (brs, 1H ,12β-H), 6.97 (brs, 1H, Thy-CH), 7.11 (brs, 1H, Thy-CH), 7.39–7.85 ppm (m, 9H, arom); ^{13}C NMR (100 MHz, $CDCl_3$): δ=196.9 (C), 174.1 (C), 167.2 (C), 166.8 (C), 164.7 (C), 164.3 (C), 151.6 (C), 150.9 (C), 143.8 (C), 140.9 (CH), 140.3 (CH), 137.8 (C), 137.7 (C), 132.7 (CH), 131.5 (CH), 130.2 (2×CH), 129.2 (CH), 128.9 (CH), 128.5 (CH), 128.4 (2×CH), 111.5 (C), 110.8 (C), 78.1 (CH), 76.9 (CH), 69.1 (CH_2), 49.7 (CH_2), 49.5 (CH), 49.4 (CH_2), 47.6 (CH), 45.3 (C), 41.9 (CH), 39.1 (CH), 35.7 (CH), 34.8 (CH), 34.7 (CH_2), 34.5 (CH), 34.2 (C), 32.1 (CH_2), 31.4 (CH_2), 31.0 (CH_2), 27.5 (CH_2), 27.0 (CH_2), 26.1 (CH_2), 25.9 (CH_2), 25.6 (CH_2), 23.6 (CH_2), 23.1 (CH_3), 18.1 (CH_3), 17.8 (CH_3), 12.5 (2×CH_3), 12.4 ppm (CH_3); MS: m/z found: 947.4810, calcd for $C_{54}H_{67}N_4O_{11}$ $[M+H]^+$: 947.4807.

Synthesis of DCABn

A stirred solution of **DCA** (2.510 g, 6.39 mmol) in anhydrous DMF (8 mL) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.060 mL, 7.19 mmol). Ten minutes later, benzyl bromide (0.850 mL, 7.18 mmol) was added dropwise and the solution was allowed to react overnight at room temperature. Then, the solvent was evaporated and the crude product was redissolved in AcOEt, washed with $NaHCO_3$ (5%), 1 M HCl, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by column chromatography (SiO_2, AcOEt:hexane 90:10) gave **DCABn** as a colorless solid (1.770 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.64 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95 (d, J=6.0 Hz, 3H, 21-CH_3), 3.60 (m, 1H, 3β-H), 3.95 (brs, 1H, 12β-H)), 5.08 (d, J=12.3 Hz, 1H, CH_2), 5.13 (d, J=12.3 Hz, 1H, CH_2), 7.38–7.28 ppm (m, 5H, arom); ^{13}C NMR (75 MHz, $CDCl_3$): δ=174.1 (C), 136.2 (C), 128.7 (2×CH), 128.4 (2×CH), 128.3 (CH), 73.2 (CH), 71.9 (CH), 66.2 (CH_2), 48.4 (CH), 47.4 (CH), 46.6 (C), 42.2 (CH), 36.6 (CH_2), 36.1 (CH), 35.3 (CH_2), 35.2 (CH), 34.2 (C), 33.8 (CH), 31.5 (CH_2), 31.0 (CH_2), 30.6 (CH_2), 28.8 (CH_2), 27.6 (CH_2), 27.3 (CH_2), 26.2 (CH_2), 23.8 (CH_2), 23.3 (CH_3), 17.4 (CH_3), 12.8 ppm (CH_3); MS: m/z found: 483.3480, calcd for $C_{31}H_{47}O_4$ $[M+H]^+$: 483.3474.

Synthesis of 3α,12α-2Thy-DCABn (2)

A stirred suspension of Thy-CH_2COOH (1.720 g, 9.36 mmol) in anhydrous THF (60 mL) was treated with Et_3N (2.620 mL, 18.80 mmol) and 2,4,6-trichlorobenzoyl chloride (1.750 mL, 11.23 mmol), and the resulting mixture was allowed to react for 1.5 h. Then a solution of 4-DMAP (0.232 g, 1.91 mmol) and **DCABn** (0.752 g, 1.56 mmol) in anhydrous THF (25 mL) was added and the mixture stirred, overnight. Afterwards, the reaction mixture was poured into $NaHCO_3$ (5%), extracted with CH_2Cl_2, and the combined extracts were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. Purification by column chromatography (SiO_2, CH_2Cl_2:MeOH 98:2) and Li Chroprep RP-18, CH_3CN:H_2O 80:20), gave **2** as a white solid (1.210 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.65 (s, 3H, CH_3), 0.77 (d, J=5.7 Hz, 3H, 21-CH_3), 0.86 (s, 3H, CH_3), 1.87 (brs, 3H, Thy-CH_3), 1.89 (brs, 3H, Thy-CH_3), 4.38 (d, J=17.4 Hz, 1H, Thy-CH_2), 4.40 (d, J=17.4 Hz, 1H, Thy-CH_2), 4.49 (d, J=17.4 Hz, 1H, Thy-CH_2), 4.57 (d, J=17.4 Hz, 1H, Thy-CH_2), 4.70 (m, 1H, 3β-H), 5.08 (brs, 2H, CH_2), 5.11 (brs, 1H, 12β-H), 6.98 (brs, 1H, Thy-CH), 7.05 (brs, 1H, Thy-CH), 7.26–7.37 (m, 5H, arom), 10.12 (s, 1H, Thy-NH), 10.21 ppm (s, 1H, Thy-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=174.0 (C), 167.3 (C), 166.7 (C), 164.7 (C), 164.6 (C), 151.5 (C), 150.9 (C), 140.8 (CH), 140.5 (CH), 136.0 (C), 128.6 (2×CH), 128.3 (3xCH), 111.2 (C), 110.7 (C), 77.9 (CH), 76.5 (CH), 66.2 (CH_2), 49.3 (CH+2×CH_2), 47.5 (CH), 45.2 (C), 41.7 (CH), 35.5 (CH), 34.7 (CH+CH_2), 34.2 (CH), 34.1 (C), 31.9 (CH_2), 31.3 (CH_2), 30.8 (CH_2), 27.3 (CH_2), 26.8 (CH_2), 26.0 (CH_2), 25.9 (CH_2), 25.3 (CH_2), 23.4 (CH_2), 22.9 (CH_3), 17.7 (CH_3), 12.4 (2×CH_3), 12.2 ppm (CH_3); MS: m/z found: 815.4200, calcd for $C_{45}H_{59}N_4O_{10}$ $[M+H]^+$: 815.4231.

Irradiation of 1

A solution of **1** (0.148 g, 0.16 mmol) in CH_3CN (250 mL) in a Pyrex round-bottom flask was purged with N_2 and irradiated in a photoreactor with eight lamps (λ_{max}=350 nm) for 2 h. Then, the solvent was concentrated under vacuum and the crude product was purified by column chromatography (SiO_2, CH_2Cl_2:MeOH 99:1) and (Li Chroprep RP-18, CH_3CN:H_2O, 80:20) to give **3** (3α-Thy-DCA-12α-Thy⟨O⟩KP; 0.095 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.75 (s, 3H, CH_3), 0.86 (m, 6H, CH_3+21-CH_3), 1.35 (d, J=7.2 Hz, 3H, KP-CH_3), 1.82 (brs, 3H, Thy-CH_3), 1.92 (brs, 3H, Thy-CH_3), 2.23 (m, 2H, CH_2), 3.10 (m, 1H, KP-CH), 3.57 (d, J=17.7 Hz, 1H, Thy-CH_2), 4.02 (t, J=10.5 Hz, 1H, KP-CH_2), 4.21 (d, J=17.1 Hz, 1H, Thy-CH_2), 4.51 (d, J=17.1 Hz, 1H, Thy-CH_2), 4.53 (dd, J=3.9 and 10.5 Hz, 1H, KP-CH_2), 4.61 (m, 1H, 3β-H), 4.62 (d, J=17.7 Hz, 1H, Thy-CH_2), 4.72 (brs, 1H, oxetane-CH), 5.27 (s, 1H, 12β-H), 6.91 (s, 1H, Thy-CH), 7.10–7.46 (m, 9H, arom), 8.96 ppm (s, 1H, Thy-NH), 9.38 (s, 1H, Thy-NH).^{13}C NMR (75 MHz, $CDCl_3$) δ=173.5 (C), 170.5 (C), 167.0 (C), 166.7 (C), 164.0 (C), 151.6 (C), 150.7 (C), 144.3 (C), 143.3 (C), 140.0 (CH), 138.2 (C), 129.6 (CH), 128.9 (2×CH), 128.8 (CH), 126.2 (CH), 126.1 (2×CH), 124.9 (CH), 124.6 (CH), 111.8 (C), 91.7 (C), 77.6 (CH), 77.2 (CH), 76.3 (C), 69.1 (CH_2), 68.4 (CH), 50.1 (CH), 50.0 (CH_2), 49.2 (CH_2), 47.7 (CH), 45.2 (C), 41.9 (CH), 39.8 (CH), 36.4 (CH), 35.7 (CH), 35.1 (CH), 34.7 (CH_2), 34.5 (C), 32.2 (CH_2), 31.7 (CH_2), 30.0 (CH_2), 28.0 (CH_2), 27.0 (CH_2), 26.2 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 24.5 (CH_3), 23.5 (CH_2), 23.3 (CH_3), 17.9 (CH_3), 17.8 (CH_3), 12.5 (CH_3), 12.4 ppm (CH_3); MS: m/z found: 947.4805, calcd for $C_{54}H_{67}N_4O_{11}$ $[M+H]^+$: 947.4807.

Irradiation of the mixture BP:2

A solution of **2** (0.102 g, 0.12 mmol) and BP (0.022 g, 0.12 mmol) in CH_3CN (150 mL) in a Pyrex round-bottom flask was purged with N_2 and irradiated in a photoreactor with eight lamps ($\lambda_{max}=350$ nm) for 6 h. Then, the solvent was concentrated under vacuum and the crude was purified by column chromatography (SiO_2, CH_2Cl_2:acetone 90:10) to give 3α,12α-Thy<>Thy-DCABn (**4**) (0.084 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.70$ (s, 3H, CH_3), 0.83 (d, $J=4.5$ Hz, 3H, 21-CH_3), 0.88 (s, 3H, CH_3), 1.59 (s, 3H, Thy-CH_3), 1.68 (s, 3H, Thy-CH_3), 3.40–3.56 (m, 2H, Thy-CH_2), 3.60 (br s, 1H, Thy<>Thy-CH), 4.16 (br s, 1H, Thy<>Thy-CH), 4.35–4.56 (m, 2H, Thy-CH_2), 4.92 (m, 1H, 3β-H), 5.12 (br s, 3H, CH_2+12β-H), 7.24–7.38 (m, 5H, arom), 8.40 (s, 1H, Thy-NH), 8.58 ppm (s, 1H, Thy-NH); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=174.3$ (C), 168.4 (C), 168.2 (C), 166.6 (2×C), 150.5 (C), 150.4 (C), 136.0 (C), 128.7 (2×CH), 128.5 (2×CH), 128.3 (CH), 79.0 (CH), 74.1 (CH), 66.5 (CH_2), 63.6 (CH), 52.5 (CH), 51.3 (CH_2), 49.4 (CH_2), 46.7 (CH), 45.3 (2×C), 45.0 (C), 39.9 (CH), 34.9 (CH), 34.5 (CH), 34.0 (CH_2), 33.2 (CH), 32.4 (C), 31.0 (CH), 30.6 (CH_2), 30.2 (CH_2), 29.6 (CH_2), 27.4 (CH_2), 25.7 (CH_2), 25.6 (CH_2), 25.1 (CH_2), 23.4 (CH_2), 23.1 (CH_2), 22.8 (CH_3), 22.4 (CH_3), 21.5 (CH_3), 17.1 (CH_3), 12.5 ppm (CH_3); MS: *m/z* found: 815.4266, calcd for $C_{45}H_{59}N_4O_{10}$ $[M+H]^+$: 815.4231.

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