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Kinetic Control in the Regioselective Alkylation of Pterin Sensitizers: A Synthetic, Photochemical, and Theoretical Study†

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ABSTRACT

Alkylation patterns and excited-state properties of pterins were examined both experimentally and theoretically. 2D NMR spectroscopy was used to characterize the pterin derivatives, revealing undoubtedly that the decyl chains were coupled to either the O4 or N3 sites on the pterin. At a temperature of 70°C, the pterin alkylation regioselectively favored the O4 over the N3. The O4 was also favored when using solvents, in which the reactants had increased solubility, namely *N,N*-dimethylformamide and *N,N*-dimethylacetamide, rather than solvents in which the reactants had very low solubility (tetrahydrofuran and dichloromethane). Density functional theory (DFT) computed enthalpies correlate to regioselectivity being kinetically driven because the less stable O-isomer forms in higher yield than the more stable N-isomer. Once formed these compounds did not interconvert thermally or undergo a unimolecular "walk" rearrangement. Mechanistic rationale for the factors underlying the regioselective alkylation of pterins is suggested, where kinetic rather than thermodynamic factors are key in the higher yield of the *O*-isomer. Computations also predicted greater solubility and reduced triplet state energetics thereby improving the properties of the alkylated pterins as $^1O_2$ sensitizers. Insight on thermal and photostability of the alkylated pterins is also provided.

INTRODUCTION

Despite their low solubility in every solvent, a number of studies have been performed on the photochemistry of pterin sensitizers (1–5). A *N*-methyl pterin **2** (6) as well as other pterin derivatives (7) have been previously reported. We recently reported on alkyl-substituted pterins **3** and **4** which, due to the alkyl group [$CH_3(CH_2)_9$], were soluble in organic solvents (Fig. 1) (8).

Similarly, organic chemists doing mechanistic studies will often use substituents to make otherwise insoluble molecules solvate. For instance, *t*-butyldimethylsilyl substituents have been connected to the sugar of guanosine **6** to have the guanosine solvate (9–12). Various sensitizers such as porphyrins and chlorins have also had alkyl substituents or PEG substituents attached to enhance their solubility (13–16). Conjugated polymers with a ratiometric fluorescent response to singlet oxygen have also been solubilized with ethyl and hexyl substituents (17). There are other uses for alkyl-substituted sensitizers, for example, **5** (18), such as membrane binding (19–25), but little information exists in this vein for pterins.

Our preliminary synthetic and photochemical work with alkylated pterins **3** and **4** (8) was a first step. Substituted pterins had been previously reported. However, regioselectivity is yet to be achieved in nucleophilic substitutions with pterins. In contrast, regioselectivity has been achieved in nucleophilic substitutions with other sensitizers, such as porphyrins (26).

We hypothesized that both kinetic and thermodynamic principles would determine the stability of *O- vs N*-alkylated pterins. We also thought that alkylation of pterins would enable better control of their solubility and excited-state properties. To test these ideas, we examined pterins with methyl and decyl substitutions on the *O*- and *N*-sites of the amide group (Fig. 1). We aimed to determine: (1) whether pterins with decyl chain substituents could be further characterized by 2D NMR spectroscopy, (2) whether regioselectivity in the alkylation depends on temperature, solvent, or base, (3) whether computed enthalpies were related to the regioselectivity, and whether the attached alkyl group migrates around the pterin periphery via a "walk rearrangement," (4) whether computed solubilities predict lipophilic amplification in pterins **3** and **4** compared to pterins **1** and **2**, (5) whether pterin alkylation perturbs the excited-state energies and photostability, and (6) how the mechanism can be summarized. The results obtained here point to kinetics, not thermodynamics, in the regioselective formation of alkyl pterin derivatives, including properties that improve pterins as sensitizers for type II ($^1O_2$) photosensitized oxidation reactions.

MATERIALS AND METHODS

Materials. Pterin **1**, 1-iododecane, sodium hydroxide (NaOH), potassium carbonate (K_2CO_3), hydrochloric acid (HCl), pyridine, *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), tetrahydrofuran (THF), dichloromethane (DCM), dimethylsulfoxide (DMSO), and

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†This article is part of a Special Issue commemorating the XIII ELAFOT Conference (23–27 October 2017 - Villa Carlos Paz, Cordoba, Argentina).

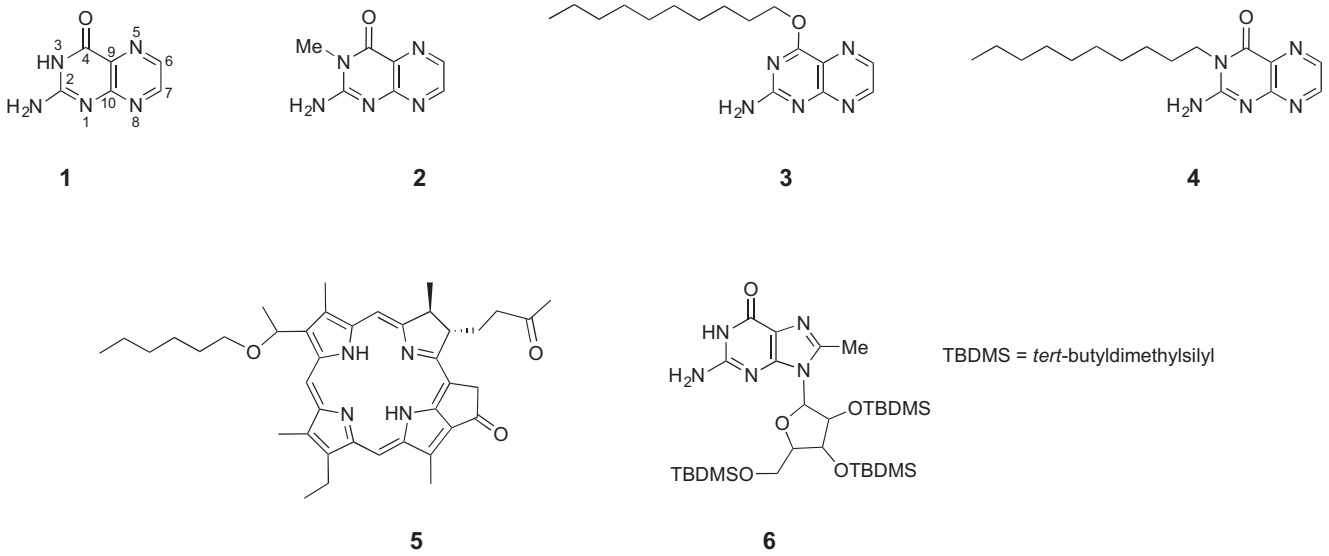


Figure 1. Parent pterin **1**, alkylated pterins **2**-**4**, an alkylated porphyrin **5**, and a TBDMS-substituted guanosine **6**. Atom numbering will not use subscripts to denote exocyclic atoms.

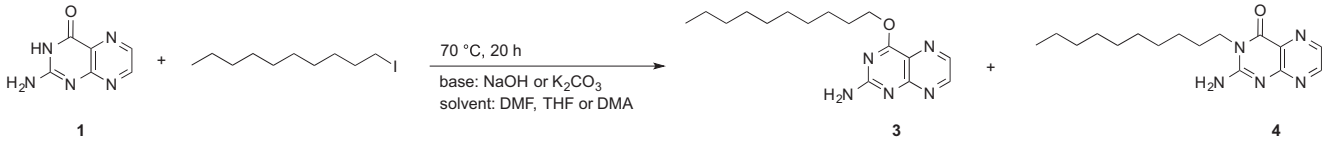


Figure 2. Synthesis reaction for pterins **3** and **4**.

DMSO-d_6, were obtained from Sigma and were used as received. Methanol and acetonitrile were from J. T. Baker (HPLC grade). Water was purified on a deionization system. For the purification of compounds, flash chromatography was used with silica of a 200–400 particle size.

Equipment. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker instrument at 400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR. High-performance liquid chromatography (HPLC) was carried out with a Prominence system from Shimadzu (solvent delivery module LC-20AT, online degasser DGU-20A5, communications bus module CBM-20, auto sampler SIL-20A HT, column oven CTO-10AS VP, photodiode array (PDA) detector SPD-M20A and fluorescence (FL) detector RF-20A) for monitoring of the photochemical reactions. A Synergi Polar-RP column (ether-linked phenyl phase with polar endcapping, 150 × 4.6 mm, 4 mm, Phenomenex) was used for product separation, with a flow of 0.3 mL min^{-1} and 90% of methanol and 10% of water were used as mobile phase.

Synthetic procedure. Pterins **3** and **4** were synthesized by a previously described method (8) with some modifications. Here, bases (NaOH or K_2CO_3) and solvents (DMF, THF, and DMA) were explored to expand on the previously studied conditions of K_2CO_3 in DMF (8). All reactions were performed under Ar and in anhydrous solvents. Base (NaOH or K_2CO_3, 1 equiv) was added to a solution of pterin **1** (1 equiv, 0.05–0.15 mmol) in anhydrous solvent (DMF, THF, or DMA, 10–12 mL). The mixture was sonicated and sparged with Ar for 20 min. Then, 1-iododecane (2 equiv) was added to the solution. The reaction mixture was placed into a water bath and was heated at 70°C with stirring for 20 h. The solution was cooled to room temperature and the solvent was evaporated to dryness under vacuum. The solid products were treated with NaCl (s.s.) (10 mL) then extracted with DCM (3 × 10 mL). The organic layers were separated, dried over Na_2SO_4, filtrated and the solvent was evaporated to dryness. The white solid residue obtained was separated by silica gel column chromatography (eluent: DCM 100% followed by DCM-methanol up to 10% methanol) (Fig. 2).

- 4-(Decyloxy)pteridin-2-amine (**3**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 2 Hz, 1H), 8.43 (d, J = 2 Hz, 1H), 7.28 (s, 2H), 4.46 (t, J = 7 Hz, 2H), 1.81 (m, 2H), 1.23 (m, 14H), 0.84 (t, J = 7 Hz, 3H) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 166.9, 161.6, 157.2, 150.9, 139.5, 123.4, 67.2, 31.3, 29.0, 28.9, 28.7, 28.6, 28.1, 25.4, 22.1, 14.0 ppm.

Table 1. ^{13}C and ^{1}H NMR data for pterins **3** and **4**.

	Pterin **3**			Pterin **4**	
Position	^{13}C signal/δ (ppm)	^{1}H signal/δ (ppm)	Position	^{13}C signal/δ (ppm)	^{1}H signal/δ (ppm)
b	166.9		b′	160.7	
a	161.6		f′	155.8	
f	157.2		a′	153.9	
d	150.9	8.78	d′	150.0	8.66
c	139.5	8.43	c′	139.1	8.36
e	123.4		e′	128.0	
O4-CH_2	67.2	4.46	N3-CH_2	41.7	3.95

Table 2. 2D HSQC correlations of ^{1}H protons with ^{13}C.

Pterin **3**		Pterin **4**	
^{13}C signal/δ (ppm)	HSQC cross signal	^{13}C signal/δ (ppm)	HSQC cross signal
166.9	–	160.7	–
161.6	–	155.8	–
157.2	–	153.9	–
150.9	8.78	150.0	8.66
139.5	8.43	139.1	8.36
123.4	–	128.0	–
67.2	4.46	41.7	3.95

Table 3. 2D HMBC correlations of ^{1}H protons with aromatic ^{13}C.

Pterin **3**		Pterin **4**	
^{1}H signal/δ (ppm)	^{13}C correlated peaks/δ (ppm)	^{1}H signal/δ (ppm)	^{13}C correlated peaks/δ (ppm)
8.78	139.5, 157.2	8.66	139.1, 155.8
8.43	123.4, 150.9	8.36	128.0, 150.0
4.46	166.9	3.95	152.9, 160.7

- 2-Amino-3-decylpteridin-4(3H)-one (**4**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 2 Hz, 1H), 8.36 (d, J = 2 Hz, 1H), 7.59 (s, 2H), 3.95 (t, J = 7 Hz, 2H), 1.56 (m, 2H), 1.23 (m, 14H), 0.85 (t, J = 7 Hz, 3H) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 160.7, 155.8, 153.9, 150.0, 139.1, 128.0, 41.7, 26.8, 31.3, 28.9, 28.8, 28.7, 26.0, 22.1, 14.0 ppm.

Steady-state photolysis. Continuous photolyzes were carried out by irradiating samples in quartz cells (0.4 cm optical path length). Two Rayonet RPR 3500 lamps (Southern N.E. Ultraviolet Co.) with emission centered at 350 nm [band width (fwhm) 20 nm] were employed as radiation source. Photolysis experiments were performed in air-equilibrated aqueous dispersions. Using aberchrome 540 (Aberchromics, Ltd.) as an actinometer, the quantum yields of pterin disappearance (Φ) was determined using the following equation:

$$\Phi = -(d[\text{compound}]/\text{d}t)_0/q_{n,p}^{0,V}$$

where $(d[\text{compound}]/\text{d}t)_0$ is the initial rate of compound consumption and $q_{n,p}^{0,V}$ is the incident photon flux density, which was found to be 2.5 ($\pm$0.2) $\times$ 10^{-5} Einstein L^{-1} s^{-1} at the excitation wavelength (27).

Computations. Density functional theory (DFT) calculations were carried out with the Gaussian 09 program package (28) and molecular structures were viewed with Gaussview 5 (29). The DFT functional used was B3LYP along with the D95** basis set (30). Frequency calculations established the type of stationary point obtained. Intrinsic reaction coordinate calculations demonstrated that saddle points connected minima. Thermal corrections for enthalpy were added at 298.15 K and 1 atm. Log P and log D values were computed with Marvin Sketch version 17.1.2 (ChemAxon Ltd. Budapest, Hungary) (31). For pterins **3** and **4**, the decyl group was optimized in a zigzag orientation of carbon atoms, that is, an all *anti* conformation. To facilitate the convergence of the pterin **3** and **4** geometries, an extra quadratic convergence for self-consistent field method (scf = xqc) and an ultrafine integral (int = grid = ultrafine) were used. Time-dependent density functional theory (TD-DFT) calculations of vertically excited singlet (S_1) and triplet states (T_1) were carried out with B3LYP/D95** using the optimized singlet ground state (S_0) structures. B3LYP/D95** has been shown to perform well in predicting the excited-state geometries and energetics of a series of heterocyclic and aromatic compounds (32).

RESULTS AND DISCUSSION

We present the results of a study of alkylated pterin sensitizers. First, we describe the synthesis and characterization of pterins **3** and **4**. Second, we examine the properties of alkyl pterins, including their excited-state energetics and photostability.

Synthesis and further characterization of pterins 3 and 4

Previously, we showed that the O4 and N3 of pterin **1** were the nucleophilic sites for alkylation with iododecane (8). In

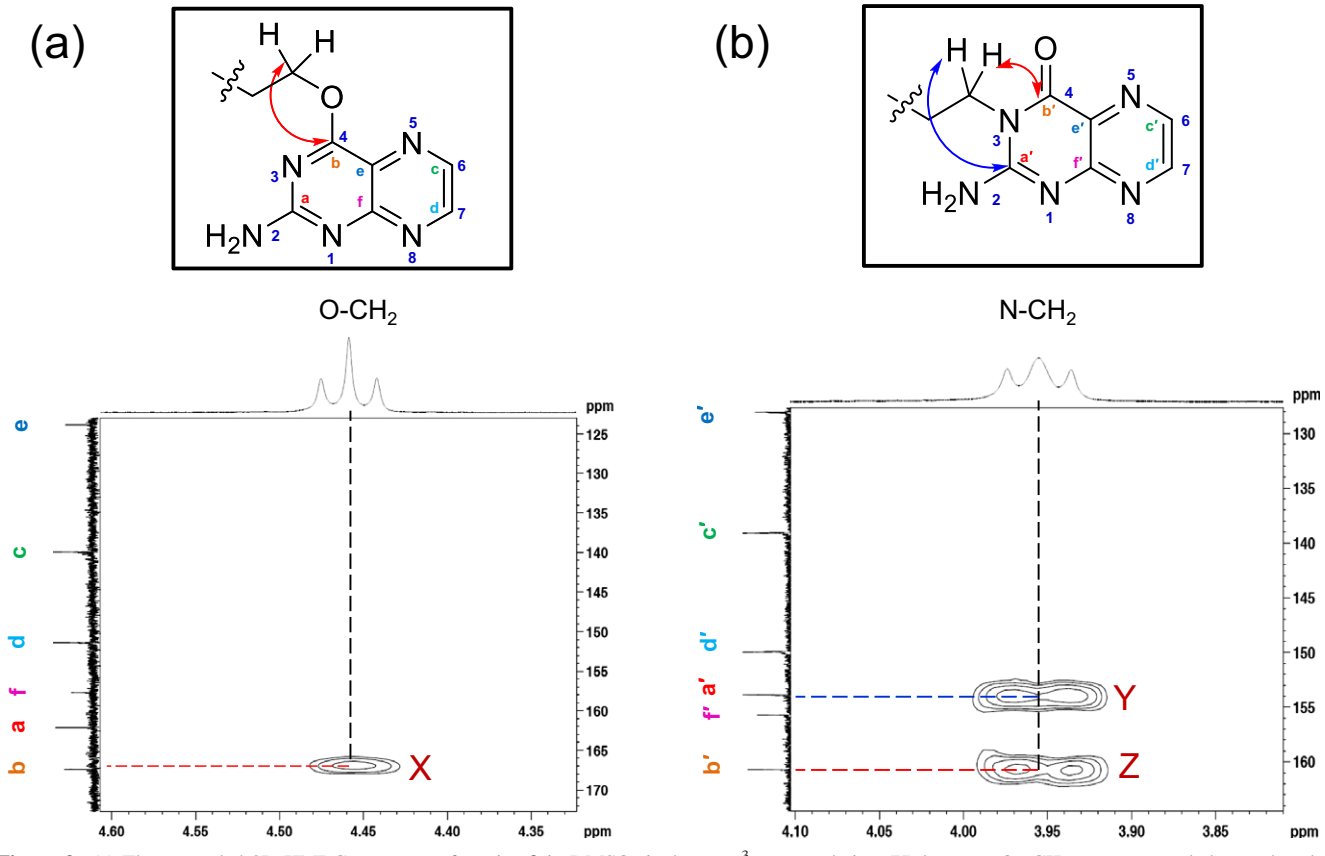


Figure 3. (a) The expanded 2D HMBC spectrum of pterins **3** in DMSO-d_6 shows a $^{3}J_{CH}$ correlation (X) between O$-$CH$_2$ protons coupled to carbon b suggesting *O4*-alkylation (b) The expanded 2D HMBC spectrum of pterin **4** in DMSO-d_6 shows two $^{3}J_{CH}$ correlations (Y,Z) between the N$-$CH$_2$ protons coupled to carbons a′ and b′ suggesting N3 alkylation.

Table 4. Synthesized percent yields of pterins **3** and **4** under different reaction conditions.

Entry	Solvent	Temperature (°C)	Base	Yields of products (%) **3**	**4**
1	DMF	25–40	K_2CO_3	0	0
2	DMF	60	K_2CO_3	35[a]	25[a]
3	DMF	70	K_2CO_3	37[a]	20[a]
4	DMF	90	K_2CO_3	0	0
5	DMF	70	NaOH	0	0
6	DMA	70	K_2CO_3	40[a]	25[a]
7	THF	70	K_2CO_3	~6[b]	~3[b]
8	DCM	40	K_2CO_3	0	0

[a]Isolated yields. [b]Detected in the reaction mixture by ^{1}H MMR spectroscopy.

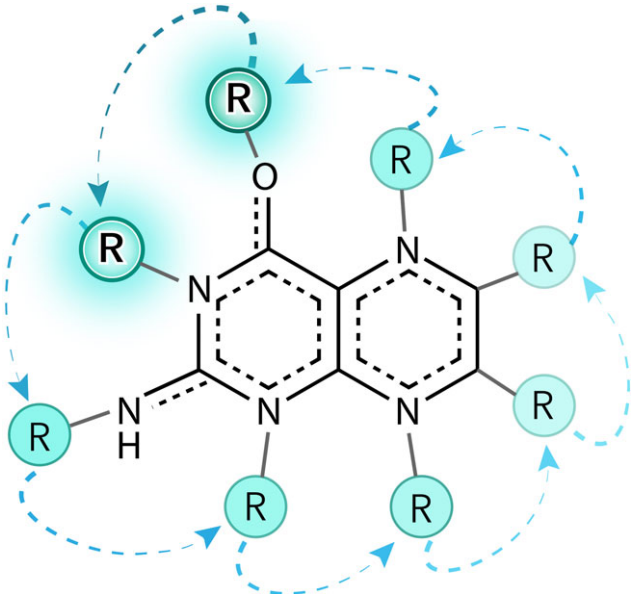


Figure 4. Schematic of the possible role of a walk rearrangement of the R group around the pterin periphery.

this study, we have collected 2D NMR spectra (HSQC and HMBC NMR) to complement the previously reported 1D NMR spectra (^{1}H and ^{13}C) (8) and provide additional spectroscopic evidence for pterin **3** and **4**. The carbon signals for **3** and **4** were definitively assigned through analysis of the ^{13}C, HSQC and HMBC NMR spectra (Tables 1–3, and Figures S1–S4, Supporting Information). Although, the key to the unambiguous assignment of the *O*- and *N*-alkylation were the HMBC NMR spectra.

Figure 3a is an expanded portion of HMBC spectrum for pterin **3,** which shows the O−CH_2 protons (4.46 ppm) in relation with the aromatic carbons. Only one cross peak is observed suggesting an exocyclic connection for the alkyl chain, and ruling out an endocyclic connection, limiting the alkyl chain's location to O4 or N2. Furthermore, cross peak X shows a connection between the O−CH_2 (4.46 ppm) and carbon b, which is consistent with O4 alkylation. Acidic and basic conditions do not change the UV spectra of the pterin **3** further supporting the O4 alkylation assignment. Figure 3b is an expanded portion of the HMBC spectrum for pterin **4**, in which two observable cross peaks (Y, Z) suggest the alkyl chain is connected to an endocyclic nitrogen. The N−CH_2 peak (3.95 ppm) does not show any correlation with the e′, f′, c′, or d′ carbons ruling out alkylation at N1, N5, and N8. However, the N−CH_2 peak does show correlation with the a′ and b′ carbons suggesting alkylation at N3.

Temperature, solvent, and base dependence yields of pterins 3 and 4

Table 4 shows the percent yields of pterin **3** and **4** in reactions of pterin **1** carried out in the presence of K_2CO_3 in DMF at temperatures ranging from 25 to 90°C. This study expands on the previously reported conditions of K_2CO_3 in DMF at 70°C (8). We observe that there was no reaction at 25°C up to 40°C (entry 1). The reaction yielded products **3** and **4** at 60°C in a 1.4:1 ratio, and a ratio of 1.85:1 at 70°C (which was the optimum temperature for regioselectivity for obtaining pterin **3**) (entries 2 and 3). However, at 90°C, there was no observed formation of products **3** and **4**, which is due to DMF condensation onto N2 site (entry 4).

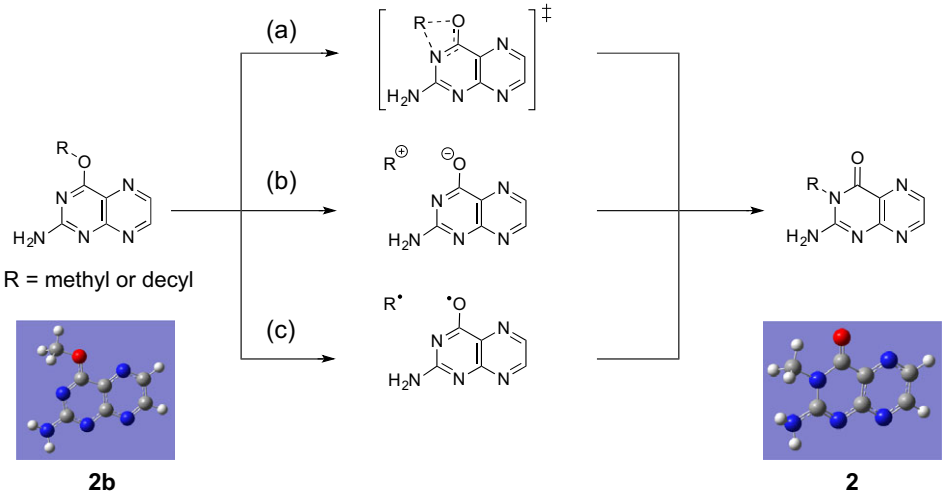


Figure 5. Possible mechanisms of alkyl pterin rearrangement: (a) concerted alkyl "walk" rearrangement, (b) a dissociative pathway through ions, and (c) a dissociative pathway through radical pair formation. The B3LYP/D95** optimized structures are shown for **2b** and **2**.

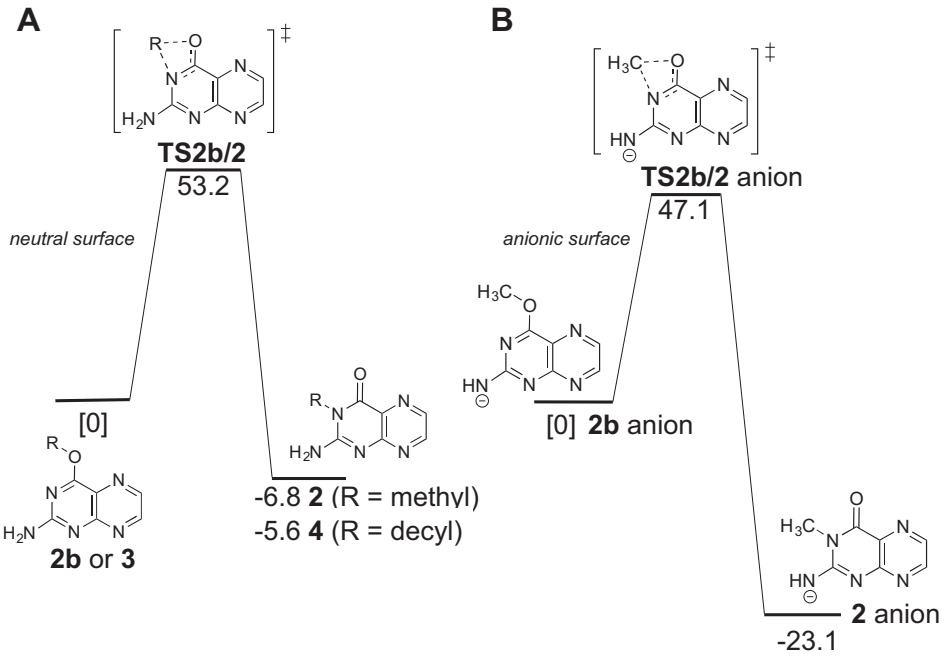


Figure 6. B3LYP/D95** potential energy surface of minimum energy structures and transition states (TS) for **2b** and **2** (R = methyl) (A), and **2b** anion and **2** anion (R = methyl) (B), and minimum energy structures for **3** and **4** (R = decyl). Further calculations on **2b** and **2** show that the formation of a radical pair or ion pair intermediate both require >60 kcal mol^{-1} energy. Relative enthalpies in kcal mol^{-1}.

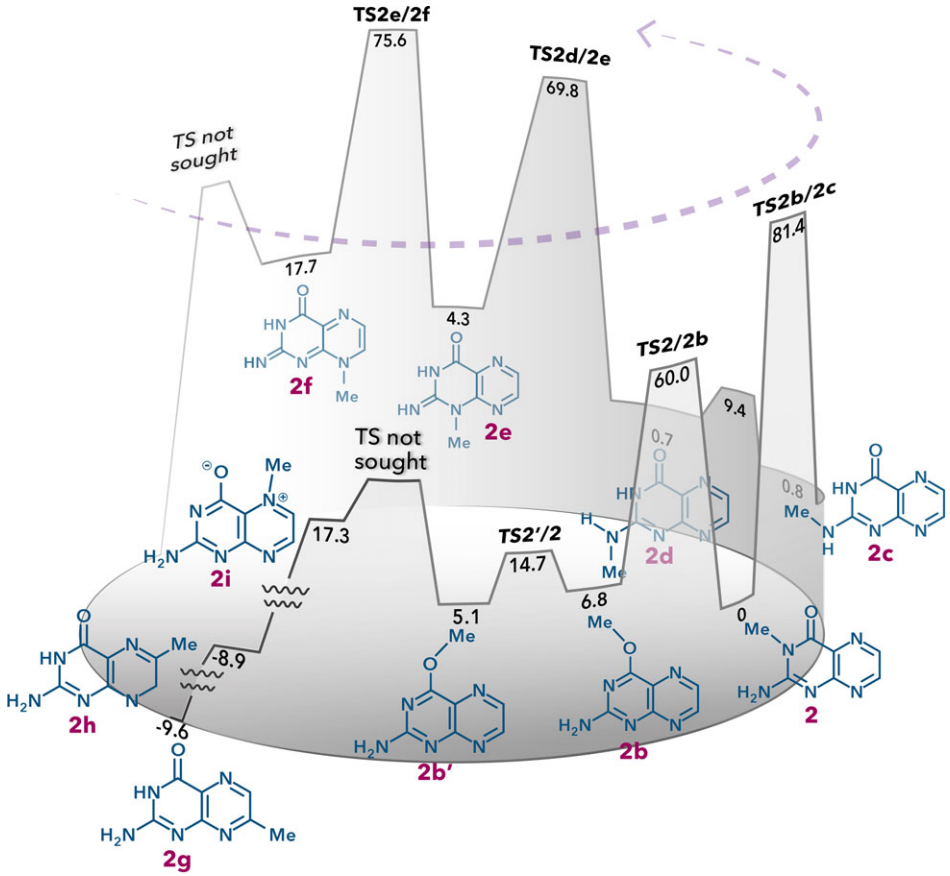


Figure 7. B3LYP/D95** computed potential energy surface (PES) for a walk rearrangement of the methyl group around the periphery of the pterin ring in a counter-clockwise direction. Compounds **2′**, **2**, and **2b**–**2i** optimized to minima, where species labeled "TS" are saddle points.

Table 5. Computed solubilities of pterin derivatives.

Pterin	Computed log P^a	Computed log D^a pH = 3	pH = 7	pH = 11	Reference
1		−0.96	−0.16	−1.96	This work
2	−0.89	−0.89	−0.89	−0.89	This work
3	3.53	3.52	3.53	3.53	8
4	2.81	2.81	2.81	2.81	8

[a]Computed with the MarvinSketch 17.1.2 (ChemAxon Ltd. Budapest, Hungary).

Table 4 also shows the percent yields of pterin **3** and **4** in reactions of pterin **1** carried out in the presence of bases (K_2CO_3 or NaOH) in various solvents (DMF, DMA, THF, and DCM). We tested NaOH and found that no products were obtained in DMF (entry 5). The reaction in DMA was found to produce regioselectivity of 1.6:1 for **3**:**4** which was the highest seen in our solvent series. Reactions in THF and DCM led to pterins **3** and **4** at very low yields (entries 7 and 8). Reactions in THF give yields less than 10% with pterins **3** and **4** as the only products, which appear to be due to the low solubility of pterin **1** in these solvents. At present, we have not examined H-bonding solvents such as methanol to probe effects on the regioselectivity due to their nucleophilic reactivity with 1-iododecane. Even DMSO proved to be impractical in the synthesis due to low pterin solubility.

Next, we wanted to investigate the stability of alkyl pterins, namely to see the relative energies of pterin isomers and determine whether pterins function as carriers of alkyl groups. Thus, we investigated this possibility with DFT.

DFT computed stabilities of alkyl pterin regioisomers

DFT computations provide a means of predicting the relative stability of isomeric alkylated pterins and possible mechanisms for the rearrangement of alkyl pterin. Calculations were performed on a methyl pterin derivative to understand the decyl group's impact on the "walk" rearrangement energetics (Fig. 4). In Fig. 5, three mechanisms can be envisioned for alkyl pterin rearrangement (neutral and anionic surfaces): (1) a concerted unimolecular through a alkyl "walk" rearrangement, (2) a dissociative pathway through an alkyl carbocation and pterin oxy anion formation, and (3) a dissociative pathway involving radical pair formation. We focused first on the concerted route (path A).

Figure 6A shows the relative enthalpies which were calculated for path A. The relative enthalpies for the *O*- and *N*-methyl pterins is 6.8 kcal mol^{-1} (**2b** and **2**, respectively), and for the *O*- and *N*-decyl pterins is 5.6 kcal mol^{-1} (**3** and **4**, respectively). In both cases, the *N*-alkylated pterin is computed to be more stable than the *O*-alkylated pterin. The relative enthalpies for anionic *O*- and *N*-methyl pterins were also carried out.

Figure 6B shows the calculated anionic surface, in which the *N*- and *O*-methyl pterin anions have a more pronounced stability

Table 6. Computed and experimental information on the parent and alkylated pterins.

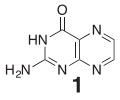
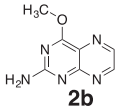
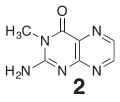
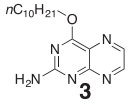
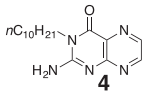
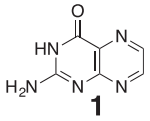
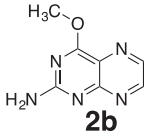
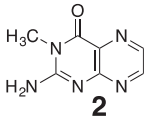
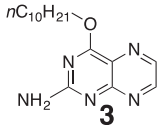
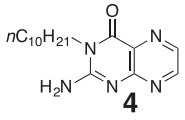
Compound	Computed S_0-S_1 kcal mol^{-1}	Computed S_0-T_1 kcal mol^{-1}	Experimental S_0-S_1 kcal mol^{-1}	Experimental S_0-T_1, kcal mol^{-1}	Fluorescence quantum yield $\Phi_F \pm$ SD	$^1O_2$ quantum yield $\Phi_\Delta \pm$ SD	Ref.
 **1**	81.3	68.0	71.4	58.1	0.33 ± 0.02	0.18 ± 0.02	(5,8)
 **2b**	75.4	64.4	–	–	–	–	(8)
 **2**	80.8	67.6	–	–	–	–	(8)
 **3**	76.0	64.3	–	–	0.012 ± 0.002	0.50 ± 0.02	(8)
 **4**	80.6	67.6	–	–	0.078 ± 0.008	0.37 ± 0.02	(8)

Table 7. TD-DFT computed electronic excitation properties for the parent and alkylated pterins.

Compound	Transition	Major contributions	Assignment
 **1**	S_0-T_1	HOMO-1→LUMO (91%)	n→π*
	S_0-S_1	HOMO-1→LUMO (98%)	n→π*
 **2b**	S_0-T_1	HOMO→LUMO (95%)	π→π*
	S_0-S_1	HOMO-1→LUMO (99%)	n→π*
 **2**	S_0-T_1	HOMO-1→LUMO (89%)	n→π*
	S_0-S_1	HOMO-1→LUMO (99%)	n→π*
 **3**	S_0-T_1	HOMO→LUMO (95%)	π→π*
	S_0-S_1	HOMO-1→LUMO (99%)	n→π*
 **4**	S_0-T_1	HOMO-1→LUMO (87%)	n→π*
	S_0-S_1	HOMO-1→LUMO (99%)	n→π*

difference of 23.1 kcal mol^{-1} than their neutral counterparts, with the *N*-methyl pterin remaining the more stable. The DFT calculations predict high activation enthalpies of 53.2 kcal mol^{-1} for the *O*- to *N*-rearrangement of methyl pterin through a concerted pathway. Further calculations on **2** and **2b** show that the formation of ion pair intermediates (path B) or radical pair (path C) both require greater than 60 kcal mol^{-1} of energy. The energy requirement for Path B is very high due to charge separation in the gas phase.

Figure 7 shows a computed energy diagram for a "walk" rearrangement of the methyl group around the periphery of the pterin ring in a counter-clockwise direction. Relative enthalpies in kcal mol^{-1} of the pterin isomers and transition states are shown. The [1,3]- and [1,4]-methyl shifts require very high activation energies. Thus, it is logical to conclude that the *O*- and *N*-alkyl pterins, for example, **3** and **4**, are non-interconverting species, unlike walk processes observed for molecular rearrangements (33–43), such as the walk rearrangement of bicyclo[2.1.0]pent-2-ene (44).

Computed solubilities

Table 5 lists the computed log *P* and log *D* values that were obtained with the ChemAxon algorithm. Computationally, we have found that the more lipophilic decylated pterins **3** and **4** show a three- to four-fold increase in their computed solubilities as compared to the parent pterin **1**. Experimentally, we have found this increase in solubility to be true for organic solvents. Similarly, the computed log *D* values are higher for the decylated pterins **3** and **4** than the methyl pterin **2** or parent pterin **1**. The computed log *D* results with parent pterin **1** are in-line with literature, sparing solubility in water and very low solubility in most organic solvents (8,45–47). As shown in the literature, covalently attaching an alkane chain, for example, from a -CH_2- to -$(CH_2)_8$- bridging, to a dye, for example, diketo-pyrrolo-pyrrole dye, increases the dye's solubility in organic solvents (48). One reference showed that the increase in solubility could be as high as by a factor of 60 (49). Computations were used not only to predict alkyl pterin lipophilicity but also excited-state energetics.

Computed excited-state energies and photostability

Next, theoretical calculations were carried out to examine whether the alkylation of pterins perturbs their excited-state energetics and photostability. Table 6 shows the computed vertical transition energies ($S_0 - S_1$ and $S_0 - T_1$) upon alkylation of pterin with methyl or decyl groups. The computed results show the proper ordering of the pterin excited-states. However, these calculations overestimate the singlet and triplet transition energies by ~10 kcal mol^{-1}, whether solvent is accounted for or not in the calculation. Time-dependent DFT enabled the computation of the triplet energies, and yield some insight about their potential as triplet sensitizers for the formation of $^1O_2$. Singlet oxygen forms mainly via energy transfer from the T_1 state of the sensitizer. That is, alkylation of pterin led to lower triplet energies, which is consistent with the previously reported enhanced $^1O_2$ quantum yield data (8). Pterin **1** has the highest-lying computed triplet (T_1 = 68.0 kcal mol^{-1}) of the series. Pterin alkylation at either O4 or N3 lowers T_1 by 0.4–3.6 kcal mol^{-1} compared to parent pterin **1**. An analysis between pterins **3** and **4** reveals further differences. Alkylation at O4 leads to a stabilizing effect and lowers the excited-state energies compared to alkylation at N3. That is, *O*-alkylated pterin **3** has a $S_0 - S_1$ transition of ~76 kcal mol^{-1} and a $S_0 - T_1$ transition of ~64 kcal mol^{-1}. In contrast, *N*-alkylated pterin **4** has a higher $S_0 - S_1$ transition of ~81 kcal mol^{-1} and a higher $S_0 - T_1$ transition of ~68 kcal mol^{-1}.

Table 7 and Figure 8 show further computed data on electronic excitations of parent pterin **1** and alkylated pterins **2**-**4**. We observe an intense $S_0 - T_1$ transition for pterins **1**, **2**, and **4** is the HOMO-1 → LUMO transition. The HOMO-1 possesses nonbonding character with involvement of the σ-bonding orbitals from the alkyl group, while the LUMO possesses π-antibonding character. Thus, the $S_0 - T_1$ transition for pterins **1**, **2**, and **4** is described as n → π* transition. On the other hand, the most intense $S_0 - T_1$ transition for pterins **2b** and **3** is the HOMO → LUMO transition, where the HOMO has π-bonding character, and the LUMO has π-antibonding character. Thus, $S_0 - T_1$ transition for pterins **2b** and **3** can be described as π → π* transition. The above results are consistent with literature (50), which had focused more on singlet excited-state properties than we do here. Notably in Fig. 8, the alkylated pterins show contributions of σ orbitals from alkyl of methyl or decyl groups to HOMO-1, HOMO, and LUMO orbitals and appear to provide stability compared to parent pterin **1**. Given the above-computed information, we wanted to assess whether pterin alkylation increased photostability, as we discuss next.

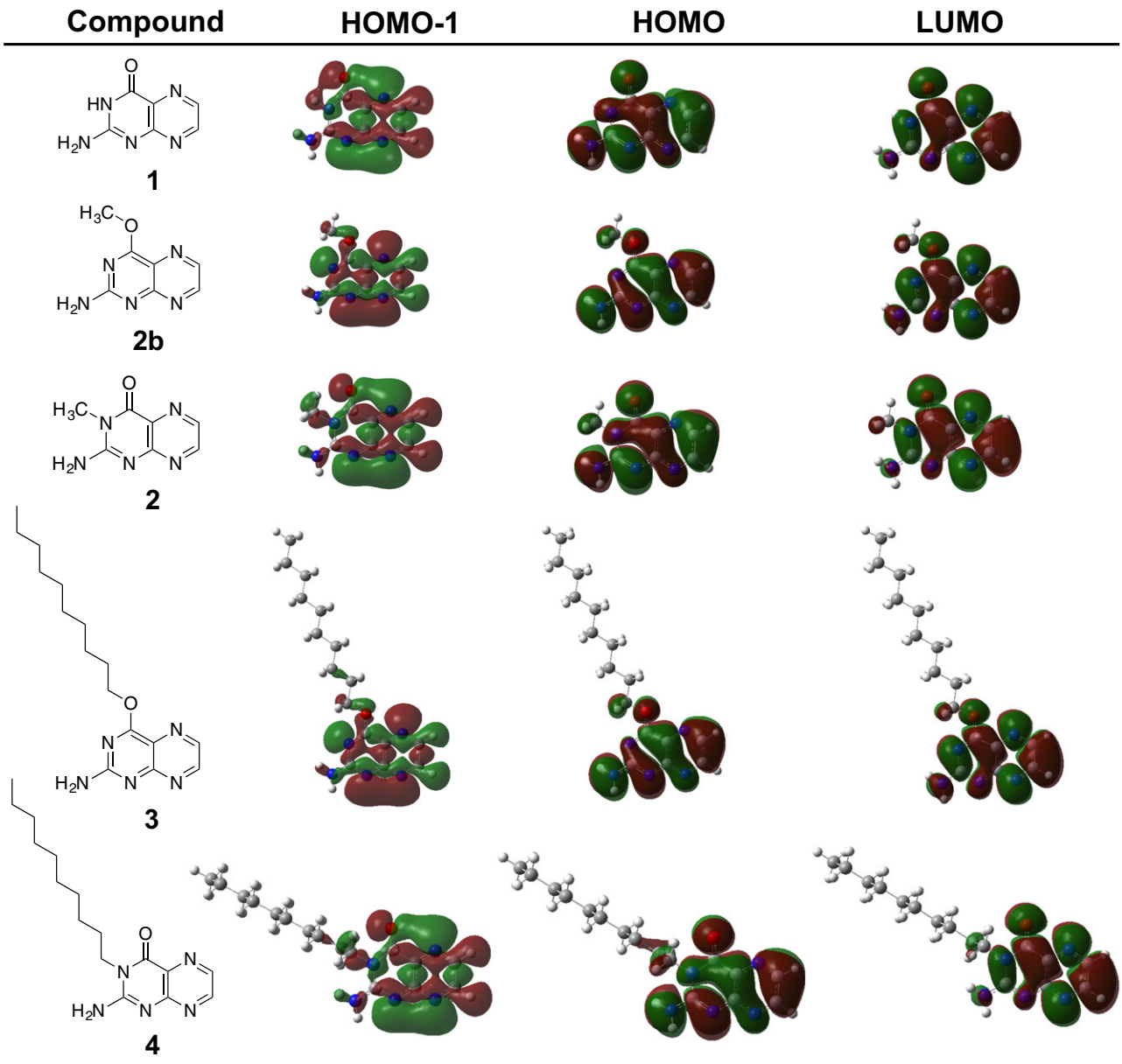


Figure 8. DFT computed molecular orbitals for the parent and alkylated pterins, in which the positive isovalues are in red, and the negative isovalues are in green. Isovalue = 0.01.

In terms of photostability, our previous report (8) showed that the decyl chain pterins **3** and **4** degrade under UVA illumination. In the current paper, we expand on this topic where Fig. 9 shows an HPLC trace of pterin **3** (retention time = 13.8 min) that decomposes upon UVA irradiation. While several photodegradation products appear with retention times ranging from 6–11 min, they have not been characterized. The inset shows a decrease in the concentration as a function of time, where the corresponding quantum yield of consumption (Φ_{consum}) pterin **3** was found to be 4.3 ($\pm$0.7) $\times$ 10^{-4}, which is slightly lower than the Φ_{consum} for pterin **1** (8.2 $\times$ 10^{-4}) and for pterin **1** anion (1.2 $\times$ 10^{-3}) (5). We believe that the photodegradation process is indicative of sensitizers bearing free amine groups, which are thus prone to type I photosensitized oxidation reactions (electron transfer and H atom abstraction) and decomposition (51–53). Relatedly, there are reports of pterins that upon irradiation form radicals (54, 55), including radicals from the $^3\pi\pi^*$ state in the presence of O_2 (56).

Mechanistic summary

Here, we postulate on pterin alkylation patterns, and thermal and photostability. Our 2D NMR data provided evidence that the decyl group attaches to pterin at the O4 or N3 position. There was no evidence for alkylation at any other position. The nucleophilicity at O4 and N3 are similar, this is apparent due to the ~1.6:1 ratios of both isomers. Solvents DMF and DMA are superior to THF and DCM as the reactant pterin **1** is more highly soluble in the former two solvents. One reason the solubility enhancement is so beneficial is that the reactions of pterins are

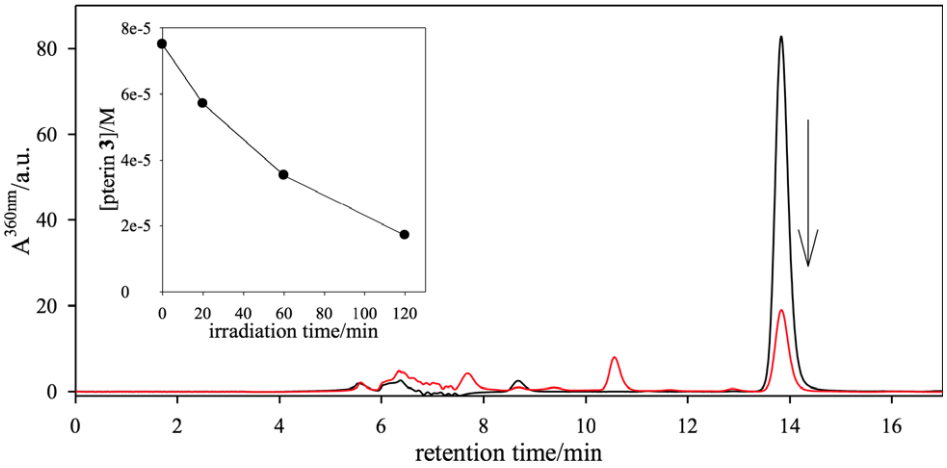


Figure 9. HPLC chromatogram recorded at 360 nm of a methanol/water (50/50) solution of pterin **3** (75 μM, retention time = 13.8 min) prior to (black line) and after (red line) UVA irradiation for 120 min. Inset: time-evolution of pterin **3** concentration upon UVA irradiation.

not amenable to common organic solvents and purification techniques. In addition to solubility enhancements, it is logical to suggest an increase in the pterins' resistance against forming of aggregates due to alkylation, similar instances of deaggregation of porphyrins and other aromatics due to alkylation in organic solvents (57–65). This decrease in aggregation has proven necessary to improve sensitizer performance (66–68). Both the computational and experimental work indicates that alkyl substitution in pterins lower the triplet energies and improve their performance as $^1O_2$ sensitizers. Indeed, the photostability increases slightly upon alkylation of pterin, which is also beneficial. Aside from solubility and stability issues, the alkylation enables pterins **3** and **4** to serve as sensitizers within membrane locales, halting pterins' tendency to traverse across membranes.

CONCLUSION

In this paper, we report on our study of the regioselective alkylation and excited-state properties of pterins. Our study has provided evidence for kinetics and nucleophilicity being the dominant factors, rather than thermodynamics and basicity in determining the regioselectivity of these reactions. This gives us insight into controlling the selective formation of pterin derivatives. One further benefit to our success in the regioselective alkylation of pterin is the potential to attach other substituents, such as $CH_2CH_2CF_2CF_2CF_2CF_3$, to solubilize pterins in fluorous phases for a possible amplification of type II at the expense of type I at fluorous solvent-water interfaces.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. HSQC NMR spectra of pterin **3** in DMSO-d_6.
Figure S2. HMBC NMR spectra of pterin **3** in DMSO-d_6.
Figure S3. HSQC NMR spectra of pterin **4** DMSO-d_6.
Figure S4. HMBC NMR spectra of pterin **4** DMSO-d_6.

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