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Chiral synthetic pseudopeptidic derivatives as triplet excited state quenchers

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ABSTRACT

The behavior of 6 pseudopeptidic models, synthesized by connecting different protected amino acids (Trp, Tyr, Phe, and Lys) with various diamino spacers, as quenchers of the triplet excited state of tiaprofenic acid (and its methyl ester), has been investigated. A series of quenching constants have been determined, which depend on the nature of the quencher and on the stereochemistry of the excited drug. A significant degree of stereodifferentiation has been found for the peptidomimetic synthesized with Phe and Tyr linked by a piperazine bridge. The obtained results support the utility of laser flash photolysis (LFP) as a tool to investigate the interactions between photoexcited drugs and simple models of binding sites in proteins.

Synthetic models for the understanding of complex biological processes are attracting considerable interest. Among them, peptidomimetics are emerging as a prominent type of mimics.[1] In this regard, the interaction of drugs with transport proteins, such as serum albumins, has been modeled by means of abiotic conjugates between amino acids and non-steroidal anti-inflammatory drugs (NSAIDs),[2] with the aim of explaining the reported phototoxic and photoallergic side effects. From such studies, a more precise view of the primary events taking place when a photoexcited drug interacts with the binding pockets of human and bovine serum albumins (HSA and BSA, respectively) is emerging.[3] The laser flash photolysis technique is a valuable tool in such investigations.[2,4–6] In order to complete the picture, additional models are introduced here based on the *intermolecular* deactivation of excited NSAID derivatives by means of pseudopeptides. Two families of compounds have been used, bearing either tyrosine (Chart 1) or tryptophan (Chart 2), due to the role played by these amino acids in photochemical processes within proteins.[2–4]

The objective of this work is to evaluate the effect of the chemical structure of the pseudopeptide (nature of the amino acid and type of linker) on the rate constant for quenching of the triplet excited state of NSAIDs, using tiaprofenic acid and its methyl ester as photoexcitable species possessing intrinsic chirality (Chart 3).[7] Special attention has been paid to the stereochemistry of the quenching processes, where an important degree of stereodifferentiation has been found in some cases.

The synthesis of tyrosine derivatives **1** and **2** is carried out in two steps as depicted in Scheme 1. Thus, reaction between commercial *N*-Boc-phenylalanine and an excess of the corresponding diamine in DMF by using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate and hydroxybenzotriazole (PyBOP/HOBt methodology)[8] in the presence of *N,N*-diisopropyl-ethylamine (DIEA) provided the corresponding monoacylated products **7** and **8**. These compounds were subsequently coupled to *N*-Boc-tyrosine leading to pseudopeptidic models **1** and **2**, which were characterized by ^{1}H NMR, ^{13}C NMR, and ESI-MS.[9]

On the other hand, tryptophan derivatives **3**–**5** (Chart 2) will be described elsewhere.[10] Model **6** was synthesized for this study analogously to **4**, and its chemical characterization is given herein.[9]

Laser flash photolysis[11] of TPA and its derivatives in deoxygenated dioxane yielded a transient absorption spectrum in the 300–700 nm range, with maxima at 350 and 600 nm (lifetime ca.

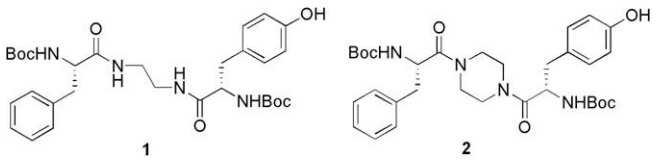

Chart 1.

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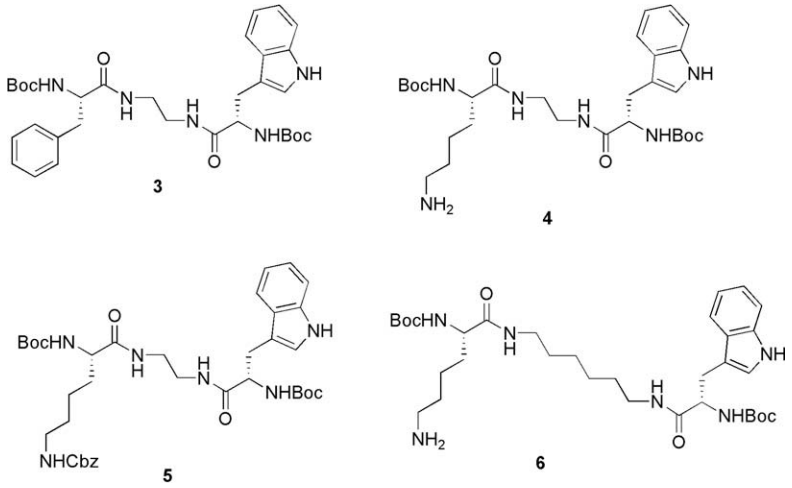
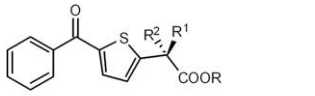
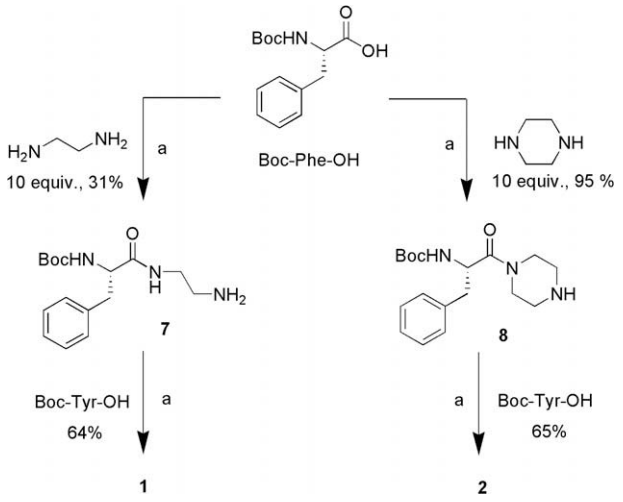


Chart 2.



R-(+)-TPA R^1=H, R^2=Me, R=H
S-(-)-TPA R^1=Me, R^2=H, R=H
R-(+)-TPA-Me R^1=H, R^2=Me, R=Me
S-(-)-TPA-Me R^1=Me, R^2=H, R=Me

Chart 3.



Scheme 1. Reagents and conditions: (a) PyBOP (1 equiv), HOBt (2 equiv), DIEA (2 equiv) in anhydrous DMF, 24 h.

4 μs), which matched that previously reported for the benzoylthiophene triplet.[12] A typical example is shown in Figure 1A for *S*(−)TPA-Me. Upon addition of **1**–**6**, the triplet–triplet absorption band decayed faster. In addition, a diminished transient absorption was observed beyond 625 nm, the maximum in the visible region shifted to 590 nm, and the ratio $\Delta A_{350}/\Delta A_{600}$ became higher (Fig. 1B and C). These changes are ascribed to the formation of ketyl and phenoxy or indolyl radicals, concomitantly with decay of the triplet excited state.[12]

The kinetics of the intermolecular interaction was analyzed by means of the Stern–Volmer relationship (Eq. 1), where the lifetime of the excited state in the absence (τ_0) and in the presence (τ) of a quencher (Q) is related to the concentration of quencher ([Q]), through the bimolecular quenching rate constant (k_q).[13]

$$\tau_0/\tau = 1 + k_q \times \tau_0[Q] \quad (1)$$

Noteworthy, triplet deactivation was clearly dependent on the nature of the protected amino acids used as building blocks, the type of spacer, and the stereochemistry of TPA or TPA-Me. Figure 2 shows two typical plots which illustrates the influence of the type of linker on the reactivity of the excited triplet state.

Table 1 contains a summary of all quenching constants and stereodifferentiation factors. From these data some conclusions can be drawn:

Rigid derivative **2** is more reactive than its flexible counterpart **1**, especially in the case of TPA-Me. Here, stereodifferentiation factor reaches 1.36, a fairly high value for the intermolecular quenching of a NSAID triplet state.[5] This can be probably attributed to the rigid nature of the linker, which allows for a better access of the quencher to the excited chromophore and enhances chiral discrimination between the two faces.

When comparing **1** with **3** as quenchers,[5,12] it becomes evident that the higher reactivity of the latter should be related to the lower oxidation potential of Trp as compared to Tyr,[12b] in accordance with the proton/electron transfer nature of the quenching process.[5,12]

As regards the comparisons within the series **3**–**6** a key factor appears to be the possible formation of a salt bridge between the carboxylic acid of TPA and the free amino group of the lysine residue. The fact that TPA-Me is not quenched to such extent seems to support this hypothesis.

This preferential binding for basic pseudopeptides is relevant in the context of transport proteins HSA and BSA, where drugs like tiaprofenic acid are known to bind polar regions in the binding sites. In fact, the role of basic polar residues at the entrance of binding pockets in HSA and BSA is well established, as anchors of acidic drugs through the formation of salt bridges.[14] A similar effect has been described for cyclooxygenases 1 and 2 (COX-1 and COX-2).[15] In this regard, compounds **4** and **6** could be seen as minimalistic models of binding sites in serum albumins and COX proteins, potentially useful in future bioorganic studies.[1]

In summary, quenching of the triplet excited state of tiaprofenic acid and its methyl ester by the six pseudopeptidic models **1**–**6** strongly depends on the quencher chemical structure (amino acid composition and nature of the linker) and (to some extent) on the

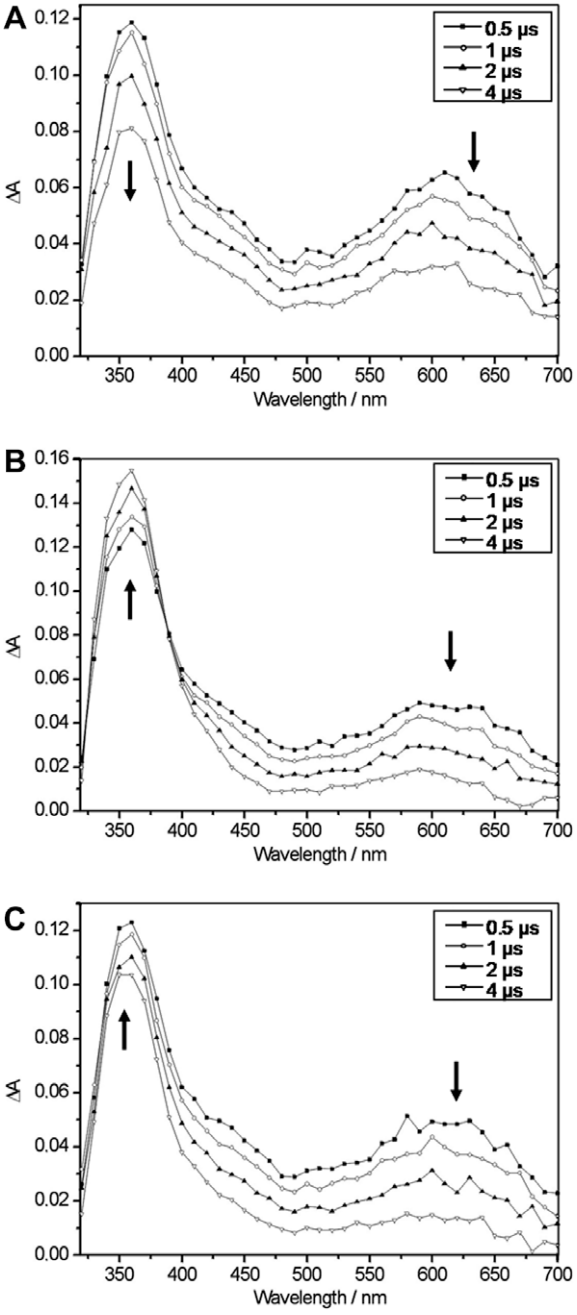


Figure 1. Representative example of laser flash photolysis measurements. Spectra recorded 0.5 (top trace), 1.0, 2.0, and 4.0 (bottom trace) µs after the laser pulse (355 nm). (A) Transient absorption spectra in deaerated dioxane of *S*-(−)-TPA-Me (0.7 mM); (B) idem, in the presence of compound **1** (2.4 mM); (C) idem, in the presence of compound **2** (0.5 mM). Note the analogous quenching by **1** and **2** but using markedly different quencher concentrations.

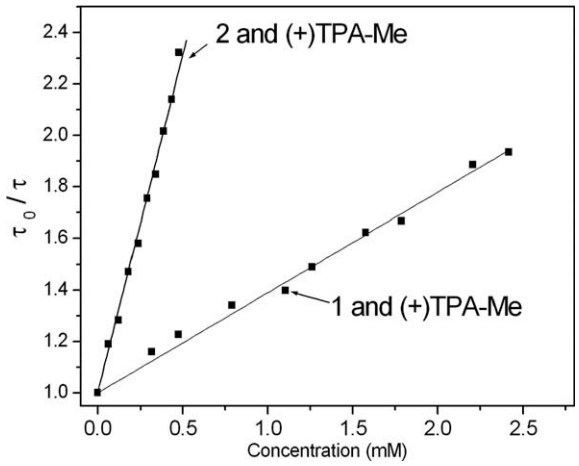


Figure 2. Stern–Volmer plots for quenching of (+)-TPA-Me triplet, generated after 355 nm laser flash photolysis in dioxane, by pseudopeptide **1** or **2**.

Table 1
Kinetic parameters for the quenching of photoexcited tiaprofenic acid (TPA) and its methyl ester (TPA-Me) by pseudopeptidic compounds **1**–**6** in dioxane

Quencher	Irradiated compound	k_q[a]		Stereodifferentiation[b]
		R(+)	*S*(−)	
1	**TPA**	2.0	1.6	1.25
	TPA-Me	1.1	1.1	1.00
2	**TPA**	3.9	4.5	0.87
	TPA-Me	7.5	5.5	1.36
3	**TPA**	3.9	4.1	0.94
	TPA-Me	4.3	3.6	1.19
4	**TPA**	6.4	6.7	0.95
	TPA-Me	3.2	3.6	0.89
5	**TPA**	3.1	3.8	0.79
	TPA-Me	3.7	4.3	0.87
6	**TPA**	6.4	5.3	1.21
	TPA-Me	3.7	3.2	1.16

[a] Units: $10^8\ M^{-1}\ s^{-1}$.
[b] Ratio = $k_qR(+)/k_qS(-)$.

stereochemistry of the excited drug. These results also support laser flash photolysis as a tool to investigate the interactions between photoexcited drugs and peptidomimetic models.

Acknowledgments

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9. *N-(tert-Butoxycarbonyl)-N-(2-{[N-(tert-butoxycarbonyl)-L-phenylalanyl]amino} ethyl)-L-tyrosinamide)* (**1**): ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.20 (br, 1H), 7.98 (br, 1H), 7.93 (br, 1H), 7.29–7.21 (m, 5H), 7.04 (d, 2H, *J* = 9 Hz), 6.90 (d, 1H,

J = 9 Hz), 6.79 (d, 1H, *J* = 9 Hz), 6.68 (d, 2H, *J* = 9 Hz), 4.14–4.04 (m, 2H), 3.11–2.66 (m, 8H), 1.34, 1.26 (2 × s, 18H) ^{13}C NMR (300 MHz; DMSO-d_6) δ 172.8, 172.6, 156.7, 156.1, 139.2, 131.0, 130.1, 129.1, 128.9, 127.1, 115.7, 78.9, 78.8, 57.0, 56.7, 38.6, 38.4, 37.9, 29.0, 28.8; ESIMS *m*/*z* 571.3 $[M+H]^+$; FAB(+) *m*/*z* 571.5 $[M+H]^+$; HRMS calcd for $C_{30}H_{43}N_4O_7$ $[M+H]^+$: 571.3132, found 571.3134; *N-(tert-butoxycarbonyl)-N-(2-{[N-(tert-butoxycarbonyl)-L-phenylalanyl] amino}piperazin-1-yl)-L-tyrosinamide)* (**2**): ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.24 (br, 1H), 7.28–7.03 (m, 9H), 6.71 (br, 2H), 4.61–4.49 (m, 2H), 3.40–3.16 (m, 4H), 2.88–2.70 (m, 4H), 1.37 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (300 MHz; DMSO-d_6) δ 171.1, 170.9, 156.8, 155.9, 138.5, 131.2, 130.4, 129.0, 128.5, 128.3, 127.3, 115.8, 79.1, 79.0, 52.3, 52.1 45.8, 45.5, 39.7, 37.3, 28.1; ESIMS *m*/*z* 597.4 $[M+H]^+$; FAB(+) *m*/*z* 597.5 $[M+H]^+$; HRMS calcd for $C_{32}H_{45}N_4O_7$ $[M+H]^+$: 597.3288, found 597.3291; *N-(tert-butoxycarbonyl)-N-(6-{[N^2-(tert-butoxycarbonyl)-L-lysyl]amino}hexyl)-L-(tryptophanamide)* (**6**): ^{1}H NMR (300 MHz; $CDCl_3$) δ 9.61 (br, 1H), 7.63 (d, 1H, *J* = 6 Hz), 7.33 (d,1H, *J* = 6 Hz), 7.15–7.03 (m, 3H), 6.29 (br, 1H), 5.55 (br, 2H), 4.45 (br, 1H), 4.12 (br, 1H), 3.28–3.07 (m, 6H), 2.62 (m, 2H), 2.40 (br, 2H), 1.77–1.02 (m, 14H), 1.43 (s, 9H), 1.40 (s, 9H); ^{13}C NMR (300 MHz; $CDCl_3$) δ 172.9, 172.3, 156.3, 155.9, 136.8, 127.8, 123.8, 122.1, 119.6, 119.1, 111.8, 110.6, 80.3, 80.2, 77.6, 55.7, 54.9, 41.7, 39.4, 39.2, 32.8, 29.2, 29.1, 28.7, 28.6, 26.2, 26.0, 23.1; ESIMS *m*/*z* 631.4 $[M+H]^+$; FAB(+) *m*/*z* 631.6 $[M+H]^+$; HRMS calcd for $C_{33}H_{55}N_6O_6$ $[M+H]^+$: 631.4183, found 631.4195.

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