Desyl Esters of Amino Acid Neurotransmitters. Phototriggers for Biologically Active Neurotransmitters†

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The application of the desyl or 2-oxo-1,2-diphenylethyl moiety as a photolabile ligand for the release of phosphates such as cAMP and inorganic phosphate (P_i)[1,2] is extended to include selected excitatory amino acids. The synthesis and photochemical studies of *N*- and *O*-desyl-caged versions of the endogenous amino acid neurotransmitters glutamate and γ-aminobutyric acid (GABA) are reported. Photolysis at 350 nm of solutions of γ-*O*-desyl glutamate (**11**) and *O*-desyl GABA (**14**) in 1:1 H_2O−acetonitrile cleanly produced free glutamate and GABA, respectively, with rate constants of ca. 10^7 s^{-1}; 2-phenylbenzo[*b*]furan (**2**) was the only photobyproduct detected by HPLC. Photolysis quantum efficiencies for the disappearance of *O*-desyl amino acid esters were in the range of 0.29−0.31, and the appearance efficiencies of furan **2** (and the corresponding amino acid) were 0.14. The photolysis of **14** was efficiently quenched with sodium 2-naphthalenesulfonate, yielding a triplet lifetime of ca. 10 ns. Photolysis of **11** in mammalian brain tissue slices resulted in glutamate receptor activation, as indicated by whole cell electrophysiological measurements. Photolysis of the other desyl amino acids resulted in decomposition and produced several products but did not lead to the formation of furan **2**.

"Cages" or phototriggers for the rapid release of bioactive substrates to elicit biological responses are becoming increasingly important research tools for introduction of reagents with precise spatial and temporal control.[2,3] The most commonly used "cage" for nucleotides, neurotransmitters, and a variety of other important biochemical effectors is the *o*-nitrobenzyl functional group.[4] Critical features of an effective cage group include high efficiency for release of the substrate, rapid rate of release, and production of a biologically benign byproduct from the cage itself. Efforts to optimize photolysis quantum efficiencies and rates by substitution at the benzylic position or on the aromatic nucleus of the *o*-nitrobenzyl group have usually resulted in unpredictable and undesirable changes in the photochemistry and the bioavailability of the substrate.[3a] Moreover, the release rates tend to be highly sensitive to the nature of the leaving group, and the byproduct from the cage, *e.g.*, *o*-nitrosobenzaldehyde, is not biologically benign.[5] For these reasons, attention has been drawn to the design and study of new, potentially faster and less damaging cage groups. An emerging class that shows promise is the α-keto cage derivative, of which the desyl or 2-oxo-1,2-diphenylethyl group is a leading candidate for a phototrigger.[6]

Whereas the first reports of the photochemistry of desyl derivatives by Sheehan et al.,[7] were not encouraging because of the many and varied photoproducts obtained often in low yields, the more recent studies by our group,[8] Corrie and Trentham,[2,6] Baldwin et al.,[9] Pirrung and Shuey,[10] Futura et al.,[11] and Cameron et al.[12] have since independently demonstrated the utility of the desyl cage for the release of phosphates, nucleotides, and carbamates. Of particular importance is the demonstration that cAMP, as its caged desyl ester (**1**), was released by irradiation at 350 nm with a rate constant of 7.2×10^8 s^{-1}, and a quantum efficiency of 0.34 (eq 1).[1] This represented a remarkable increase by 3 orders of magnitude in the rate constant for a phototrigger while maintaining a reasonably high efficiency relative to the best nitrobenzyl analogues.[3b] The desyl ligand was expelled as 2-phenylbenzo[*b*]furan (**2**) concomitant with release of the cAMP and is a biochemically benign byproduct, in contrast to the release of the more reactive nitroso ketones and aldehydes from *o*-nitrobenzyl analogues.[3a,b]

† Dedicated to Professor Glen A. Russell on the occasion of his 70th birthday.

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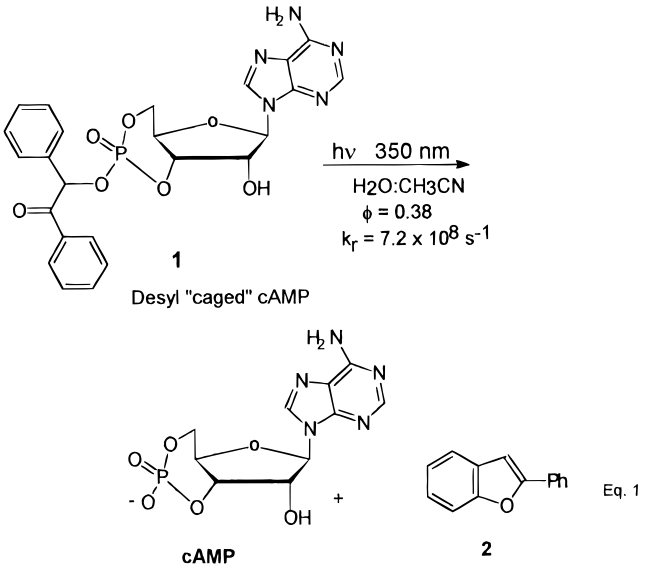


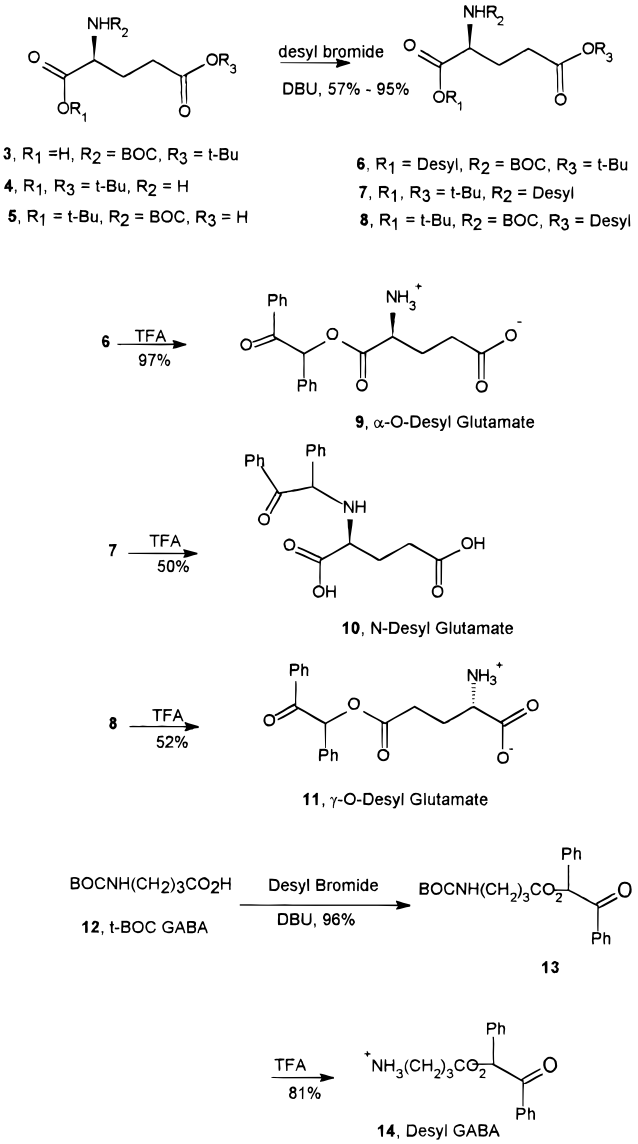
We report here the results of our investigations on a new class of phototriggers for the excitatory amino acid, glutamate, and for GABA, and contrast their photochemistry with the corresponding *o*-nitrobenzyl amino acids[13,14] which have proven to be very valuable for studies of receptor activation kinetics[14,15] and synaptic connection mapping.[15]

The desyl triggers for amino acids have not heretofore been extensively or thoroughly evaluated. Corrie and Trentham[3b] reported the synthesis of 3′,5′-dimethoxydesyl derivatives of GABA and alanine and noted that the esters were hydrolytically unstable, prompting the authors to abandon pursuit of the photolysis studies since the probes would not be useful in aqueous solution. In contrast, Sheehan's report[7c] of successful release of *t*-BOC-protected amino acids from the α-phenacyl esters, including the *N*-protected, *O*-desyl glycinates,[7b] encouraged us to investigate the feasibility of rapid photorelease of GABA and glutamate from the *N*- or *O*-desyl-protected precursors in more detail.[14] Another report extending the studies on the release of amines though the corresponding carbamates from *p*-methoxy-α-substituted phenacyl cage further illustrated the generality of this approach.[16] Two caveats to the use of α-keto phototriggers for release of amino acids in biological media, however, were the assertions by Sheehan et al.[7b,c] that these reactions proceed via a free radical mechanism and that even small amounts of water in the reaction media produce lower yields.

Results

Synthesis. *O*-Desyl amino acid esters and *N*-desyl-glutamate were synthesized starting with the commercially available, partially protected *O*-*tert*-butyl and

Scheme 1. General Synthesis of *N*- and *O*-Desyl Amino Acids



N-*tert*-butoxycarbonyl amino acids. Reaction of the unprotected nucleophilic site with desyl bromide, followed by treatment with trifluoroacetic acid (TFA) to remove the *tert*-butyl and *t*-BOC groups, provided desyl-caged **9**−**11** and **14** in reasonable yields and high purity (Scheme 1). Phototriggers **9**−**11** were isolated as mixtures of diastereomers. It was anticipated that the esters **11** and **14** would be more stable to hydrolysis because of the greater separation between the hydrolyzable ester function and the electron-withdrawing ammonium group in **9**.[13a]

Photochemistry. Photolysis of the desyl-caged amino acids gave mixed results. The photochemistry of **11** and **14** is shown in Scheme 2. Table 1 gives the quantum efficiencies measured for both the photolytic generation of the furan **2** and the disappearance of the starting desyl-caged amino acid. Photolysis of *O*-desyl GABA (**14**) was very efficient ($\Phi_{Dis} = 0.31$) and produced free GABA and **2**. No other products were observed by HPLC. Quantum efficiency measurements on the formation of **2** gave a photolysis efficiency of 0.13. Since the complementary products furan and GABA were the only two products detected (*vide infra*), it was assumed that the appearance efficiencies for both **2** and GABA would be

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Scheme 2. Photochemistry of *O*-Desyl-Caged GABA and Glutamate

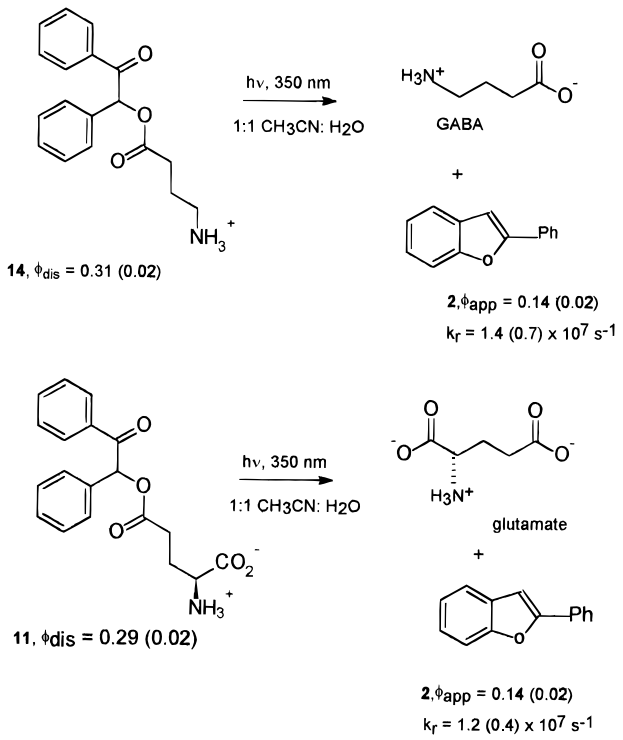

Table 1. Photolysis Quantum Efficiencies for Disappearance of 9−11 and 14 and the Appearance of 2

phototrigger	Φ_{furan}	$\Phi_{disappear}$
α-*O*-desyl glutamate (**9**)	*a*	*b*
N-desylglutamate (**10**)	*a*	*b*
γ-*O*-desyl glutamate (**11**)	0.14 ± 0.03	0.29 ± 0.02
O-desyl GABA (**14**)	0.14 ± 0.02	0.31 ± 0.02

[a] Benzil formed upon irradiation; no furan was detected from **10** and only traces were observed from **9**. [b] Not determined.

the same as had been the case in our previous studies for the desyl series of phosphates.[1] In preparative runs, the liberated GABA was isolated and compared with an authentic sample by analytical and preparative TLC.

In a separate series of experiments, it was shown that photolysis of **14** could be readily quenched by sodium 2-naphthalenesulfonate. The triplet lifetime of *ca.* 10 ns was derived from the Stern−Volmer slope from the quenching study, based on a rate constant for diffusion[1] of 1.0×10^{10} M^{-1} s^{-1} estimated for rate of diffusion in the binary solvent mixture of 1:1 aqueous buffer:dioxane employed. From these data, a rate constant for release of GABA was determined to be 1.4 (± 0.7) $\times$ 10^7 s^{-1}.

γ-*O*-Desyl glutamate (**11**) showed behavior similar to **14** upon photolysis, yielding a quantum efficiency for disappearance of 0.29, a rate constant of $k_r = 1.2$ (± 0.4) $\times$ 10^7 s^{-1}, and an appearance efficiency for the furan of 0.14, based again on linear Stern−Volmer quenching by 2-naphthalenesulfonate.

For the other two desyl-caged amino acids (α-*O*-desyl and *N*-desylglutamates (**9** and **10**)), the photochemistry diverges from pathways that lead to glutamate. The major byproduct detected by HPLC was benzil, possibly a product of the coupling of two benzoyl radicals formed in a type 1 photocleavage of the desyl derivatives. This was also a major pathway in one of the Sheehan desyl series.[7] Some furan **2** was also formed in the photolysis of **9**, indicative of photolysis to uncage the amino acid. In the case of the *N*-desylglutamate (**10**), however, no furan was produced upon photolysis. Similar results have been observed by Sheehan[7] in the photochemistry of other desyl caged glutamates.

In a preliminary study,[17] γ-*O*-desyl glutamate (**11**) was evaluated for its efficacy as a "caged" glutamate in the photogeneration of glutamate at mammalian neurons. Samples of hippocampal pyramidal neurons from ferrets were bathed in 1mM γ-*O*-desyl glutamate in ASCF bathing solution and then photolyzed with an 8 ms pulsed 50 mW UV argon laser. In a typical experiment, a 400 μm thick sample received 17 mW of radiation at 351−364 nm. Central neuron response was monitored by the whole cell patch clamp technique to determine the photostimulated action potentials and the inward ion currents. The responses of the glutamate receptors to released glutamate were determined by measuring the resulting inward ion currents at neurons synaptically connected to those being activated and were not optimized.[15] Figure 1 shows a series of traces obtained upon photolysis of **11**.

Discussion

Our photochemical studies of the desyl-caged amino acid neurotransmitters (Scheme 2) indicates that the desyl esters of γ-*O*-desyl glutamate (**11**) and GABA (**14**) may be useful as caged amino acid precursors for triggers of biochemical processes. The photolysis quantum efficiencies were competitive with those reported for the *o*-nitrobenzyl cages, and the rate of release of the free amino acid is considerably higher, fast enough to be useful for probing the initial stages of even the most rapid biological processes, *i.e.*, receptor activation and desensitization.[13]

The photolytic behaviors of **11** and **14** reflect a recurring pattern of reactivity in which the desyl moiety is converted to 2-phenylbenzo[*b*]furan (**2**).[1,3d,6,7a,b,8] The process is highly efficient, varying only slightly from a value of *ca.* 0.3. Bond cleavage occurs entirely through the desyl group's triplet state as established by linear Stern−Volmer quenching of the reaction by 2-naphthalenesulfonate and accords with our earlier observations of the photochemical behavior of several other desyl derivatives.[1,3d,8] While the mechanistic details are not complete at this stage of our studies, reasonable pathways would involve an electron transfer from the ketone triplet either before or following homolysis of the carboxyl group from the desyl protecting ligand to yield the carboxylate−desyl α-keto carbocation ion pair as depicted in Scheme 3.[8,18,19] Cyclization to the resulting oxacylcopentadienyl cation followed by loss of a proton yields **2**.

This scheme is in accord with the mechanistic picture previously developed from investigations of photolysis of benzylic phosphates.[3d,19] The recent studies by Pincock

(17) The absorbance of the desyl group is 150 M^{-1} cm^{-1} while that of the α-carboxy-2-nitrobenzyl group is 180 M^{-1} cm^{-1} at 360 nm.[13] Similar experiments had been performed in which photolysis of caged α-(4,5-dimethoxy-2-nitrobenzyl) glutamate (α-DMNB glutamate) generated free glutamate. The magnitude of the response for free **11** was approximately equal to that observed in experiments using α-CNB and much more efficacious than the α-DMNB caged glutamate.[15] A limiting factor in these determinations remains the low absorptivity of **11** at the concentrations employed for these experiments, however.[19]

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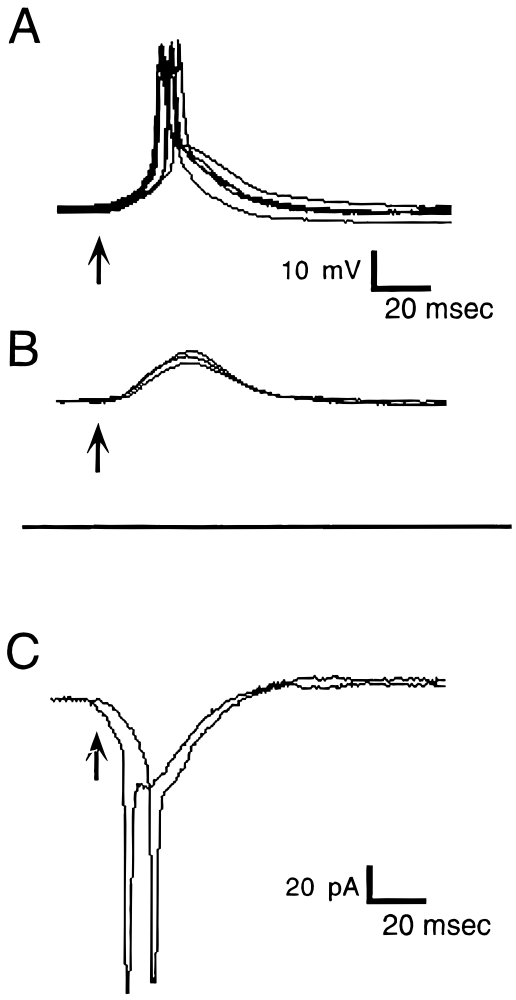
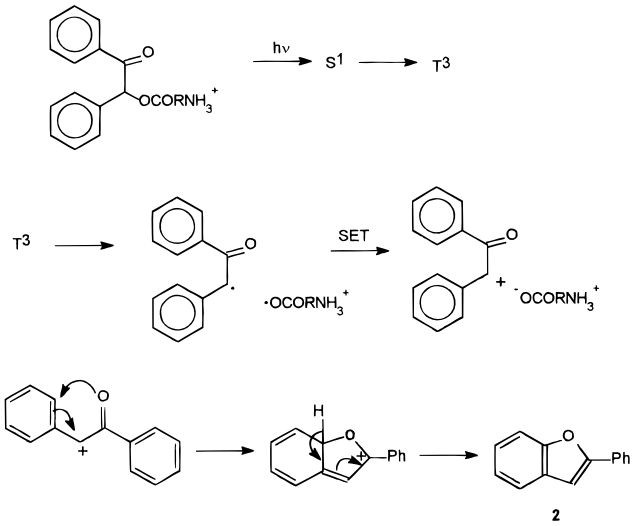


Figure 1. Scanning laser photostimulation of hippocampal pyramidal neurons with γ-desyl glutamate (**11**).[17] Arrows mark the beginning of the 8 ms uncaging laser flash. (A) In current clamp mode, uncaging desyl glutamate (1mM in ASCF bathing solution) in ten consecutive trials at the same location evokes an action potential from direct stimulation of the cell's glutamate receptors. The latency to peak is consistent at approximately 35 ms. (B) Stimulation of another location results in a subthreshold response. Three trials are shown. As in A, the latency to peak is also approximately 35 ms. Between cells latency to peak varied somewhat, ranging from 20 to 45 ms. (C) In voltage clamp mode, uncaging of the desyl glutamate near to the record neuron results in a large inward current, which causes the cells to escape clamp and fire an action potential. The response to uncaged glutamate begins as soon as the laser flash begins. Uncaging of γ-desyl glutamate results in large currents which can generate action potentials, but it also appears to have considerable intrinsic activity in its caged form. Addition of this compound results in a large (200−300%) increase of the holding current, and it was difficult to record for neurons for a long period of time in the presence of the caged compound.

et al.,[18] on the photosolvolysis of benzyl and naphthylmethyl carboxylic esters provide convincing evidence in favor of the pathway illustrated in Scheme 3 for photolysis reactions carried out in hydroxylic solvents. While the comparison of the triplet reactions of desyl esters with the results on benzylic esters reported by Pincock must account for the differences in the multiplicity and the relative oxidation potentials of the benzylic and α-ketobenzyl radicals, the striking similarity of the solvent effect on these two very similar reactions invites a parallel mechanistic interpretation. The almost immediate formation of benzil (**15**), either thermolytically or photolytically, in solutions of **9** and **10** at present lacks sufficient experimental explanation. The diketone is formed by the coupling of two benzoyl radicals, which could be generated from a photochemical type 1 cleavage, the most expedient explanation for the photochemical pathway.[7] No products resulting from the accompanying benzyl radicals were observed by HPLC upon photolysis of **9** or **10**. Conformational effects may also play a significant role in determining whether photosolvolysis or type I chemistry dominates in each individual case. Work is continuing on this aspect of the photochemistry.

Scheme 3. A Rational Mechanism for the Photorelease of "Caged" Amino Acids from Desyl Esters



Regardless of the mechanism, it is clear that the competing route to benzil renders the latter two probes unsuitable for the photorelease of free amino acid due to the competing absorption of the benzil, which is also a known triplet quencher. A further drawback is that **9** is hydrolytically unstable, rapidly degrading to free glutamate and benzoin in neutral aqueous solution in the absence of light.

The work described herein provides two examples of a new class of desyl-caged phototriggers which release biologically active amino acids very rapidly upon illumination at 350 nm. The concomitant formation of biologically inert 2-phenylbenzo[*b*]furan (**2**) is another salutary feature of this reaction when contrasted with the *o*-nitrobenzyl series. The results for *N*-desylglutamate (**10**) and α-*O*-desyl glutamate (**9**) point to a lack of generality of the desyl phototrigger series for amino acid photorelease, however, which cautions against developing strategies for other new desyl probes as phototriggers and encourages further pursuit of other cage chromophores. This work is in progress.

Experimental Section

Synthesis. General. All reactions were carried out under a dry argon atmosphere under subdued light. All reagents and solvents were used as received. Flash chromatography was performed according to the method of Still et al.[20] All melting points are uncorrected.

α-*O*-Desyl Glutamate (9). A solution of *N*-*t*-BOC-L-glutamic acid, γ-*tert*-butyl ester (**3**, 239 mg, 0.788 mmol), desyl

(20) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

bromide (204 mg, 0.741 mmol), and DBU (0.12 mL, 0.80 mmol) in benzene (17 mL) was heated at reflux for 1 h. The reaction solution was washed with water (1 × 25 mL) and brine (1 × 20 mL), dried (sodium sulfate), and concentrated to a pale brown oil. This oil was purified by flash chromatography (ethyl acetate/chloroform 0% to 10%) to give α-*O*-desyl γ-*tert*-butyl *N*-*tert*-butylglutamate (**6**) in a yield of 0.37 g (95%) as a colorless solid mixture of diastereomers: mp 87−90 °C; ^{1}H NMR ($CDCl_3$) 7.90 (m, 2H), 7.51 (m, 1H), 7.4 (m, 7H), 6.85 (two s, 1H), 5.13 (d, J = 8.1 Hz, 1H), 4.44 (m, 1H), 2.5−2.3 (m, 2H), 2.2−1.9 (m, 2H), 1.45 (s, 9H), 1.42 (s, 9H). Anal. Calcd for $C_{28}H_{35}NO_7$: C, 67.59; H, 7.09; N, 2.81. Found: C, 67.23; H, 7.05; N, 2.64.

A solution of **6** (0.17 g, 0.34 mmol) and TFA (2.0 mL, 26 mmol) in dichloromethane (4 mL) was stirred at room temperature for 3 h. The volatiles were removed in vacuo, and the residue was dissolved in water (15 mL). The resulting solution was frozen and lyophilized to give **9** as a mixture of diastereomers, in a yield of 151 mg (97%) as a cream-colored hygroscopic powder: mp 89−92 °C; ^{1}H NMR (D_2O) 7.92 (t, J = 8.7 Hz, 1H), 7.49 (m, 3H), 7.31 (m, 5H), 7.23 (two s, 1H), 4.40 (two t, J = 7.2 H, 1H), 2.81 (t, J = 7.3 Hz, 1H), 2.52 (m, 1H), 2.38 (q, J = 7.1 Hz, 1H), 2.27 (d, J = 7.0 Hz 1H); mass spectrum m/e (relative intensity) 342 ($M^+ - CF_3C)_2$, 100), 195 (52), 167 (40); exact mass calcd 342.1345, found 342.1340. Anal. Calcd for $C_{21}H_{20}NO_7F_3 \cdot H_2O$: C, 53.28; H, 4.68; N, 2.96. Found: C, 53.28; H, 4.69; N, 2.77. Attempted purification via reverse phase chromatography on Sephadex LH-20, with water as the mobile phase, resulted in extensive hydrolysis to free glutamic acid and benzoin, as determined by isolation and coelution with authentic samples on TLC.

***N*-Desylglutamic Acid, Trifluoroacetate Salt (10).** A solution of di-*tert*-butyl L-glutamate hydrochloride (**4**, 2.03 g, 6.86 mmol), desyl bromide (1.89 g, 6.86 mmol), and diisopropylethylamine (1.81 g, 14.0 mmol) in acetonitrile (30 mL) was stirred overnight at room temperature. Concentration gave a pale brown solid, which was purified by flash chromatography (ethyl acetate/chloroform 0% to 5%) to give a mixture of diastereomers of di-*tert*-butyl *N*-desylglutamate (**7**) in a yield of 1.77 g (57%) as a clear pale brown oil: ^{1}H NMR ($CDCl_3$) 8.10 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.8 (m, 2H), 7.45 (m, 6H), 4.05 (m, 1H), 2.56 (m, 1H), 2.3 (m, 2H), 2.2 (m, 2H), 1.50 (s, 9H), 1.37 (s, 9H).

A solution of **7** (1.75 g, 3.86 mmol) and TFA (20 mL) in dichloromethane (80 mL) was stirred at room temperature overnight. The volatiles were removed in vacuo, and benzene (2 × 20 mL) was evaporated to yield a residue as a pale brown solid foam. Half of this crude product was purified on Sephadex LH-20, using water as eluant, to give a mixture of diastereomers of *N*-desylglutamate (**10**) in a yield of 0.44 g (50%) as a hygroscopic colorless powder after lyophilization: mp 110−114 °C dec; ^{1}H NMR (D_2O) 7.98 (d, J = 7.8 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.51 (m, 7H), 6.29 (s, 1H), 3.94 (t, J = 6.4 Hz, 1H), 2.8−2.1 (m, 4H). Anal. Calcd for $C_{21}H_{20}NO_7F_3 \cdot H_2O$: C, 53.28; H, 4.68; N, 2.96. Found: C, 53.42; H, 4.66; N, 3.07.

γ-*O*-Desyl Glutamate (11). As in the procedure to form **6**, a solution of desyl bromide (605 mg, 2.2 mmol), *N*-*t*-BOC-glutamic acid, α-*tert*-butyl ester (672 mg, 2.2 mmol), and DBU (0.35 mL, 2.3 mmol) was heated at reflux in benzene (25 mL) and stirred overnight to give γ-*O*-desyl *tert*-butyl *N*-*t*-BOC-glutamate (**8**) in a yield of 1.06 g (96%) as a colorless oil: ^{1}H NMR ($CDCl_3$) 7.92 (d, J = 8.1 Hz, 2H), 7.51 (t, J = 7.4 Hz, 1H), 7.47−7.32 (m, 7H), 6.86 (s, 1H), 5.12 (dd, J = 13.6, 7.3 Hz, 1H), 4.5 (br s, 1H), 2.6 (m, 2H), 2.22 (m, 1H), 1.97 (m, 1H), 1.45 (s, 9H), 1.42 (two s, 9H).

As in the procedure for synthesis of **9**, γ-*O*-desyl *tert*-butyl *N*-*t*-BOC-glutamate (**8**) (1.06 g, 2.13 mmol) and TFA (2.0 mL) were stirred at room temperature in dichloromethane (5 mL) and gave, after LH-20 purification, γ-*O*-desyl glutamate (**11**) in a yield of 0.51 g (52%) as a hygroscopic colorless powder; the trifluoroacetic acid was removed from the product salt during lyophilization: mp 118−120 °C dec; ^{1}H NMR ($CDCl_3$) 2.18 (m, 2H), 2.71 (m, 2H), 3.76 (q, 1H), 7.13 (s, 1H), 7.39−7.49 (m, 7H), 7.62 (t, 1H, J = 7.33 Hz), 7.98 (d, 2H, J = 7.35 Hz); ^{13}C NMR (D_2O) 28.39, 32.654, 56.786, 79.04, 130.95, 131.61, 131.71, 131.81, 131.89, 132.02, 132.43, 132.92, 135.45, 136.35, 137.82, 176.52, 176.68; mass spectrum m/e (relative intensity) 342 ($M^+ + 1$, 95), 230 (45), 212 (95), 195 (64); exact mass calcd 341.1341, found 341.1346; UV λ_{max} (ϵ) 252 (9000), 300 (1000), 350 (200). Anal. Calcd for $C_{19}H_{19}NO_5 \cdot 0.5H_2O$: C, 65.13; H, 5.75; N, 4.00. Found: C, 64.97; H, 5.69; N, 3.88.

***O*-Desyl γ-Aminobutyric Acid, Trifluoroacetate Salt (14).** As for the synthesis of **6**, *N*-*t*-BOC-γ-aminobutyric acid (**12**, 500 mg, 2.46 mmol), desyl bromide (677 mg, 2.46 mmol), and DBU (396 mg, 2.60 mmol) were heated at reflux in benzene (25 mL) to give *O*-desyl *N*-*t*-BOC-γ-aminobutyrate (**13**) in a yield of 0.94 g (96%) as a clear colorless oil: ^{1}H NMR ($CDCl_3$) 7.92 (d, J = 7.8 Hz, 2H), 7.4 (m, 8H), 6.86 (s, 1H), 4.7 (br s, 1H), 3.20 (d, J = 5.9 Hz, 2H), 2.53 (m, 2H), 1.87 (dt, J = 6.9, 2.8 Hz, 2H), 1.4 (br s, 9H).

A solution of **13** (0.93 g, 2.3 mmol) and TFA (8.0 mL, 100 mmol) in chloroform (25 mL) was stirred overnight at room temperature. The volatiles were removed in vacuo, and benzene (1 × 15 mL) was evaporated from the residue, leaving a colorless oil. This oil was purified by chromatography on Sephadex LH-20, using water as eluant. The pure product fractions were combined and lyophilized to give *O*-desyl γ-aminobutyrate (*O*-desyl GABA, **14**) as 0.78 g (81%) of a colorless hygroscopic powder: mp 82−84 °C; ^{1}H NMR (D_2O) 1.82 (m, 2H), 2.38 (m, 2H), 2.84 (t, 2H, J = 7.92 Hz), 6.88 (s, 1H), 6.99 (m, 5H), 7.12 (t, 1H), 7.25 (d, 2H, J = 7.23), 7.69 (d, 2H, 7.23); ^{13}C NMR (D_2O) 24.6, 32.9, 41.1, 80.6, 131.1 d, 131.2 d, 131.3 d, 131.7 d, 132.0, 135.3, 136.1, 136.7, 175.9, 198.8. Anal. Calcd for $C_{20}H_{20}NO_5F_3$: C, 58.39; H, 4.90; N, 3.40. Found: C, 58.13; H, 4.75; N, 3.39.

Photochemistry. General. Dioxane and acetonitrile were used without further purification. All water was distilled and passed through a Nanopure deionizing system. Phosphate buffer for analytical HPLC was prepared using potassium hydrogen phosphate to afford a solution of 0.05 M KH_2PO_4 with an adjusted pH of 2.5. The HPLC system consisted of two HPLC pumps, a controller, an injector equipped with a 20 μL loop, a Econsosil C18 FU 25.0 × 4.6 cm column, a spectrophotometric absorbance detector set at 280 nm, and a recording integrator.

Photolysis of γ-*O*-Desyl Glutamate (11). In a 50 mL volumetric flask, 500 mg of **11** and 38 mg of *p*-anisic acid were dissolved in 50 mL of 50:50 acetonitrile:distilled water. Aliquots (5 mL) were placed in Pyrex phototubes. The solutions were degassed for 20 min at 0 °C. The solution was then photolyzed in a Rayonet reactor with four RPR 350 nm lamps for 32 min. Aliquots (0.05 mL) were removed at the following time intervals: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, 12.0, 17.0, 22.0, 27.0, 32.0 min. A 50 μL sample of each aliquot was diluted to 450 μL and then assayed by HPLC using the following conditions: 10 μL loop, mobile phase; 30% acetonitrile:70% 50 mM NH_4OAc pH 4.5 for 2 min to 75% acetonitrile at 20 min, held at 75% acetonitrile from 20−25 min and then to 30% acetonitrile for 30 min. The product was identified by coinjection with a known sample of 2-phenylbenzo[*b*]furan (**2**) by HPLC. Relative response factors for **11** and the product 2-phenylbenzo[*b*]furan were determined from standard solutions of known concentrations. *p*-Anisic acid was used as an internal standard. Another unidentified product formed as noted by HPLC. The yield of the minor product was estimated to be less than 10% of the furan. Light output was determined by the potassium ferrioxalate method.[21]

Photolysis of *O*-Desyl γ-Ammoniobutyrate, Trifluoroacetate Salt (14). In a 50 mL volumetric flask, 452 mg of **14** and 38 mg of *p*-anisic acid were dissolved in 50 mL of 50:50 acetonitrile:distilled water. Aliquots (5 mL) were taken and placed in Pyrex phototubes. The solutions were degassed for 20 min at 0 °C and were then photolyzed in a Rayonet reactor with four RPR 350 nm lamps for 32 min. Aliquots (0.05 mL) were removed at the following intervals: 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, 12.0, 17.0, 22.0, 27.0, 32.0 min. A 50 μL sample of each aliquot was diluted to 450 μL with acetonitrile. These samples were then assayed by HPLC using the following conditions: 10 μL loop, mobile phase 55% acetonitrile: 45% of a solution of 50 mM NH_4OAc pH 4.5 for 8 min to 75% acetonitrile at 15 min, held at 75% acetonitrile from 15 to 20

min and then to 55% acetonitrile at 25 min. Light output was determined by the potassium ferrioxalate method.[21] The product was identified by coinjection with known samples of 2-phenylbenzo[*b*]furan by HPLC. Relative response factors for **2** and **14** were determined from standard solutions of known concentrations. *p*-Anisic acid was used as an internal standard. The results are given in Table 1.

Quenching Studies of 11. In a 50 mL volumetric flask, 500 mg of **11** and 38 mg of *p*-anisic acid were dissolved in 50 mL of 1:1 acetonitrile:distilled water. Aliquots (3 × 5 mL) were placed in Pyrex phototubes. To two of the phototubes was added enough sodium 2-naphthalenesulfonate to make 0.021 and 0.061 M solutions of the quencher. The solutions were degassed for 20 min at 0 °C and then photolyzed in a Rayonet reactor with four RPR 350 nm lamps for 17 min. Aliquots of 0.05 mL were removed at the following times: 0, 12, and 17 min. A 50 μL sample of each aliquot was diluted to 450 μL which was assayed by HPLC using the following conditions: 10 μL loop, mobile phase 30% acetonitrile:70% of a solution of 50 mM NH_4OAc at pH 4.5 for 2 min to 75% acetonitrile at 20 min, held at 75% acetonitrile from 20 to 25 min and then to 30% acetonitrile for 30 min. A rate of disappearance of 1.4 ± 0.7 × 10^7 s^{-1} was determined from the slope of the Stern−Volmer concentration dependence of the efficiencies, assuming a diffusion-controlled rate constant for triplet quencher of 1.1 × 10^{10} M^{-1} s^{-1} in 1:1 CH_3CN/H_2O.[1] Relative response factors for **11** and 2-phenylbenzo[*b*]furan were determined from standard solutions of known concentrations. *p*-Anisic acid was used as the internal standard.

Quenching Studies of 14. In a 50 mL volumetric flask, 452 mg of **14** and 38 mg of *p*-anisic acid were dissolved in 50 mL of 1:1 acetonitrile:distilled water. Aliquots (3 × 5 mL) were placed in Pyrex phototubes. To two of the phototubes was added enough sodium 2-naphthalenesulfonate to make 0.02 and 0.058 M solutions of the quencher. The solutions were degassed for 20 min at 0 °C and then photolyzed in a Rayonet reactor with four RPR 350 nm lamps for 17 min. Aliquots of 0.05 mL were removed at the following times: 0, 12, and 17 min. A 50 μL sample of each aliquot was diluted to 450 μL which was assayed by HPLC using the following conditions: 10 μL loop, mobile phase 30% acetonitrile:70% of a solution of 50 mM NH_4OAc at pH 4.5 for 2 min to 75% acetonitrile at 20 min, held at 75% acetonitrile from 20 to 25 min and then to 30% acetonitrile for 30 min. A rate of disappearance of 1.2 ± 0.4 × 10^7 s^{-1} was determined from the slope of the Stern−Volmer concentration dependence, assuming a diffusion rate for triplet quenching of 1.1 × 10^{10} M^{-1} s^{-1} in 1:1 $CH_3CN:H_2O$.[1] Relative response factors for **14** and 2-phenylbenzo[*b*]furan were determined from standard solutions of known concentrations. *p*-Anisic acid was used as the internal standard.

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Supporting Information Available: ^1H NMR spectra of **7**, **8**, and **13** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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