Design and Synthesis of Photoaffinity-Labeling Ligands of the L-Prolyl-L-leucylglycinamide Binding Site Involved in the Allosteric Modulation of the Dopamine Receptor

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Pro-Leu-Gly-NH_2 (PLG), in addition to its endocrine effects, possesses the ability to modulate dopamine D_2 receptors within the central nervous system. However, the precise binding site of PLG is unknown. Potential photoaffinity-labeling ligands of the PLG binding site were designed as tools to be used in the identification of the macromolecule that possesses this binding site. Six different photoaffinity-labeling ligands were designed and synthesized on the basis of the γ-lactam PLG peptidomimetic **1**. The 4-azidobenzoyl and 4-azido-2-hydroxybenzoyl photoaffinity-labeling moieties were placed at opposite ends of PLG peptidomimetic **1** to generate a series of ligands that potentially could be used to map the PLG binding site. All of the compounds that were synthesized possessed activity comparable to or better than PLG in enhancing [^{3}H]-*N*-propylnorapomorphine agonist binding to dopamine receptors. Photoaffinity ligands that were cross-linked to the receptor preparation produced a modulatory effect that was either comparable to or greater than the increase in agonist binding produced by the respective ligands that were not cross-linked to the dopamine receptor. The results indicate that these photoaffinity-labeling agents are binding at the same allosteric site as PLG and PLG peptidomimetic **1**.

Introduction

L-Prolyl-L-leucylglycinamide (PLG) is a central nervous system neuropeptide that acts as a modulator of the dopamine receptor.[1] PLG has been shown to enhance the binding of agonists such as *N*-propylnorapomorphine (NPA)[2,3] and apomorphine[4] to the dopamine D_2 receptor. PLG has no effect on the binding of antagonists such as spiroperidol to dopamine receptors.[2] Studies have shown that PLG and PLG peptidomimetic **1** enhance [^{3}H]NPA and [^{3}H]quinpirole binding in a dose-dependent manner to the D_{2L}, D_{2S}, and D_4 dopamine receptor subtypes, but they do not enhance agonist binding to the D_1 and D_3 dopamine receptor.[5] Experiments have shown that PLG enhances the conversion of dopamine receptors from their low-affinity state to a high-affinity state for enhanced agonist binding.[2]

The precise mechanism of action behind PLG's ability to modulate dopamine receptors is unclear. Preliminary evidence suggests the existence of a putative PLG binding site.[6,7] However, it is not known whether the binding site is located on the dopamine receptor or whether it is part of another independent macromolecule that somehow is coupled to the dopamine receptor. Photoaffinity-labeling ligands were envisioned as one possible means of facilitating the identification the PLG binding site, thereby increasing our understanding of the mechanism by which PLG modulates the dopamine receptor. This paper describes the synthesis and preliminary pharmacological activity of such photoaffinity-labeling agents.

Results

Design Rationale. PLG peptidomimetic **1** was chosen as the scaffold on which the photoaffinity-labeling moieties would be attached because of its potent activity and the ease with which it can be synthesized. Peptidomimetic **1** is about 100 times more potent than PLG in potentiating apomorphine-induced rotational behavior in 6-OHDA-lesioned rats,[8] and it is more potent than PLG in protecting against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic degeneration.[9] Peptidomimetic **1** maintains the high affinity state of the D_2 receptor by increasing GTP hydrolysis.[10] It attenuates haloperidol-induced vacuous chewing movements in rats[11] and haloperidol-induced *c-fos* expression.[12]

We envisioned that placing a photoaffinity-labeling moiety at different positions on PLG peptidomimetic **1** would provide a way of potentially mapping out the PLG binding site. Initially compounds **2**−**4** were designed. These derivatives placed the photoaffinity moiety on either end of **1**. Although the introduction of a photoaffinity moiety onto **1** could introduce adverse steric interactions between the photoaffinity-labeling agent and the PLG binding site, previous SAR studies on PLG suggested that at the positions selected such steric issues might not be a significant problem. For example, several β-prolyl analogues of PLG that possessed γ-benzyl substitutions were found to be as active as PLG.[13] These results suggest that the PLG binding site would accommodate the photoaffinity moieties on **2** and **3**. In the case of **4**, the C-terminal 4-hydroxybenzylamide derivative of **1** was previously made in our laboratory[14] and in unpublished studies was shown to be active, thus suggesting that the PLG binding site could accommodate the photoaffinity-labeling moiety found in **4**.

Two photoaffinity-labeling moieties were chosen for incorporation into **1**; the 4-azidobenzoyl and 4-azido-2-hydroxybenzoyl groups. The 4-azidobenzoyl moiety was chosen because this group is reasonably stable and can withstand many reaction conditions except those that involve excessive heating, strong oxidizing, or strong reducing conditions.[15] Also, this group is stable at 37 °C and not that susceptible to intramolecular rearrangement after photolysis.[16] Finally, this moiety can be incorporated into organic molecules via the commercially available 4-azidobenzoic acid *N*-hydroxysuccinimide ester. The

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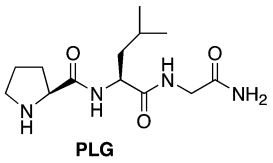
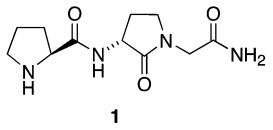


PLG



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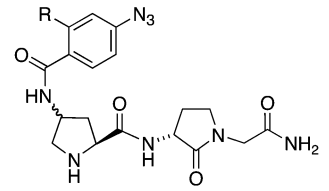
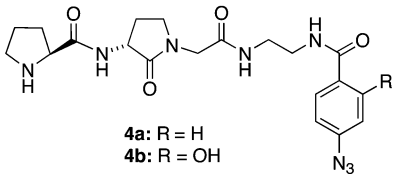


2a: *cis*, R = H **3a**: *trans*, R = H
2b: *cis*, R = OH **3b**: *trans*, R = OH



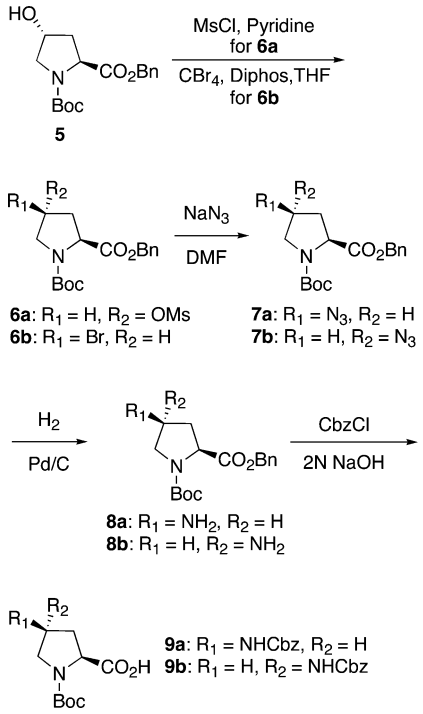
4a: R = H
4b: R = OH

4-azido-2-hydroxybenzoyl moiety provides a ring system that can be readily radioiodinated.[17] Like the 4-azidobenzoyl moiety, the 4-azido-2-hydroxybenzoyl moiety can be incorporated into organic molecules with the easily synthesized 4-azido-2-hydroxybenzoic acid *N*-hydroxysuccinimide ester.[18]

Syntheses. The synthesis of photoaffinity labels **2a**−**c** and **3a**−**c** required the 4-aminoproline derivatives **9a** and **b**, respectively. Their synthesis is depicted in Scheme 1. The protected hydroxyproline **5** was made from *trans*-hydroxy-L-proline following the method of Williams et al.[19] Compound **5** was converted to either the *cis*- or *trans*-aminoprolines **8a** and **8b**, respectively, through the procedures of Webb et al.[20] with only slight modifications. The protected *trans*-hydroxyproline **5** was converted to either the mesylate **6a** with retention of configuration or the bromide **6b** with inversion of configuration. During the formation of Boc-*cis*-4-bromoproline benzyl ester (**6b**), separation of the triphenylphosphine oxide from the product proved to be difficult. Therefore, this reaction was done with 1,2-bis(diphenylphosphino)ethane (diphos),[21] since approximately 70% of the diphos phosphine oxide byproduct could be filtered off before column purification. Conversion of both **6a** and **6b** to their corresponding azides **7a** and **7b** was accomplished with inversion of configuration in each case. Simultanous reduction of the azide and hydrogenolysis of the benzyl ester afforded the *cis*- and *trans*-4-aminoprolines **8a** and **8b**, respectively. The amino group of **8a** and **8b** was protected with the benzyloxycarbonyl group to give **9a** and **9b**, respectively.

The elaboration of **9a** and **9b** into the photoaffinity-labeling agents **2** and **3** is outlined in Scheme 2. Each of these 4-aminoproline derivatives was coupled to γ-lactam **10**, which was made from Boc-D-Met-Gly-OMe utilizing procedures previously established in our laboratory[22,23] and by Freidinger et al.[24] The coupling of **9a** and **9b** to **10** proved to be more difficult than initially expected. Initial attempts at coupling **9a** and **10** to give **11a** were successful in a moderate yield of 49%

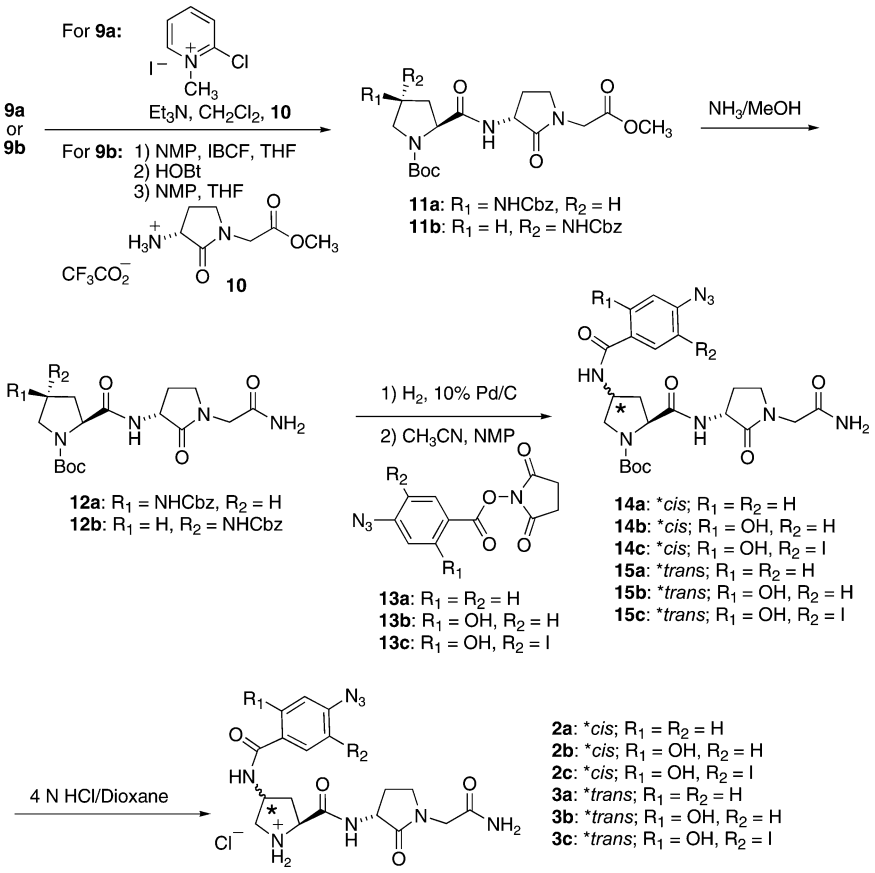
Scheme 1



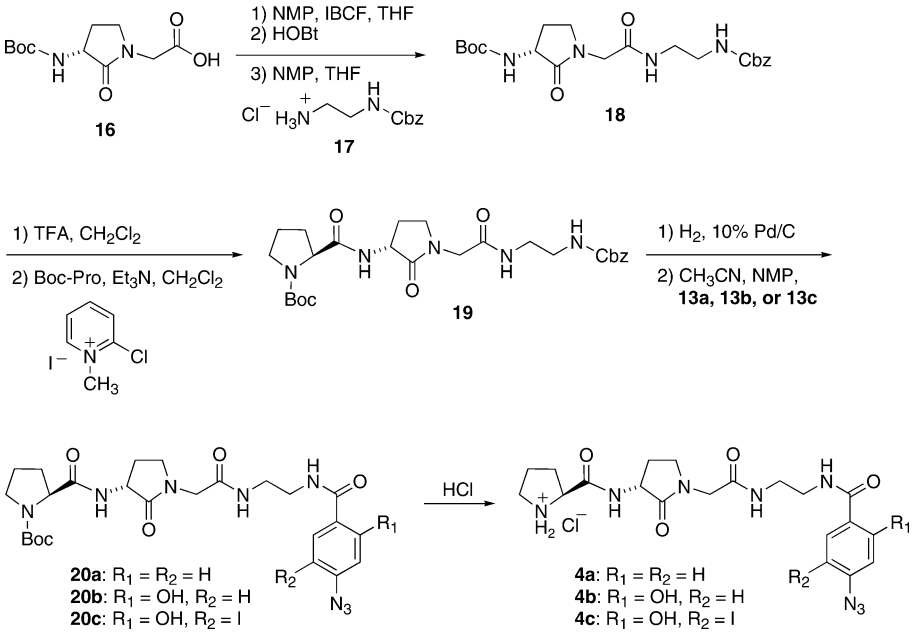
when using either EDC/HOBt or mixed anhydride coupling procedures. The yield of **11a** increased to 61%, when Mukaiyama's reagent,[25] 2-chloro-1-methylpyridinium iodide, was used as the coupling reagent. The coupling of **9b** and **10** to give **11b** in a 60% yield was successful when using the mixed anhydride coupling conditions of *N*-methylpiperidine[26] (NMP) and isobutylchloroformate (IBCF) followed by HOBt to suppress the side reaction found in the mixed anhydride coupling.[27] The methyl esters **11a** and **11b** were converted to their corresponding primary amides **12a** and **12b**, respectively, with methanolic ammonia. Deprotection by hydrogenolysis of the benzyloxycarbonyl group of **12a** and **12b** was followed in each case by coupling of the resulting free amine to either the commercially available 4-azidobenzoic acid *N*-hydroxysuccinimide ester (**13a**), 4-azido-2-hydroxybenzoic acid *N*-hydroxysuccinimide ester (**13b**),[18] or 5-iodo-4-azido-2-hydroxybenzoic acid *N*-hydroxysuccinimide ester (**13c**).[17] The *tert*-butoxycarbonyl-protected photoaffinity labels **14a**−**c** and **15a**−**c** were deprotected with HCl in dioxane to afford the photoaffinity-labeling ligands **2a**−**c** and **3a**−**c**.

The synthesis of photoaffinity labels **4a**−**c** is shown in Scheme 3. Freidinger's lactam **16**[22] was coupled to Cbz-ethylenediamine hydrochloride[28,29] (**17**) by initially using EDC/HOAt. An excellent yield of 83% was achieved. However, because of the cost of HOAt, other coupling reagents were explored. The EDC/HOBt coupling procedure was unsuccessful, while the NMP/IBCF mixed anhydride coupling procedure described above gave **18** in a 90% yield. The *tert*-butoxycarbonyl group of **18** was removed with TFA/CH_2Cl_2 and the resulting product was coupled to Boc-Pro-OH with Mukaiyama's reagent in a good yield (79%). This coupling reaction was initially attempted with EDC/HOBt, but this only afforded the product in a moderate yield (42%), while the NMP/IBCF mixed anhydride coupling procedure that worked well in the previous coupling step only afforded the desired product in a 10% yield. Use of the coupling reagent PyBroP was problematic, as separating **19** from the phosphine oxide byproduct was difficult. The benzyloxycarbonyl group of **19** was removed by hydrogenolysis and the deprotected product then coupled to either

Scheme 2



Scheme 3



13a, **13b**, or **13c** to give **20a**, **20b**, or **20c**, respectively. Removal of the *tert*-butoxycarbonyl group from **20a**−**c** afforded the photoaffinity-labeling agents **4a**−**c**.

Pharmacological Activity. The photoaffinity-labeling agents synthesized above were tested for their ability to increase the binding of the dopamine receptor agonist NPA to dopamine D_2 receptors from bovine striatal tissue as described by Srivastava et al.[2] These compounds were tested for their ability to increase [^{3}H]NPA binding at three different concentrations: 1, 10, and 100 nM. The data obtained for the 4-azidobenzoyl photoaffinity-labeling agents **2a**, **3a**, and **4a** are shown in Figure 1. The data for the 4-azido-2-hydroxybenzoyl photoaffinity-labeling agents **2b**, **3b**, and **4b** and the 4-azido-2-hydroxy-5-iodobenzoyl photoaffinity-labeling agents **2c** and **4c** are shown in Figure 2. Their activity was compared to that of the γ-lactam PLG peptidomimetic **1** (Figure 1) on which the photoaffinity-labeling agents were based.[23]

All of the photoaffinity-labeling agents showed significant activity in increasing the binding of [^{3}H]NPA to isolated dopamine receptors at one or more of the concentrations that

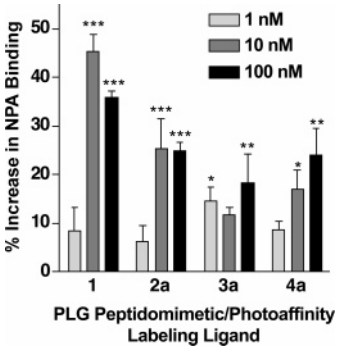


Figure 1. Stimulation of [^{3}H]NPA binding to dopamine D_2 receptors from bovine striatal membranes by PLG peptidomimetic **1** (data from ref 23) and the PLG peptidomimetic photoaffinity-labeling agents **2a**, **3a**, and **4a**. Data represent the percent increase in specific [^{3}H]NPA binding over the control value when the indicated concentration of compound was added directly to the assay buffer. Results are the means ± SEM of three or four separate experiments carried out in triplicate. Statistical comparisons were made using repeated measures of analysis of variance (ANOVA). Significantly different from control value: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

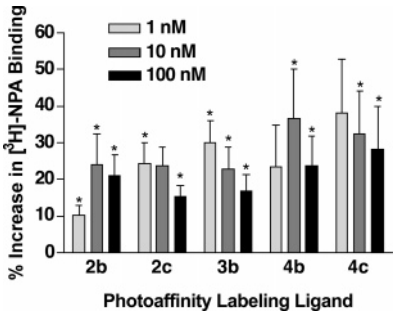


Figure 2. Stimulation of [^{3}H]NPA binding to dopamine D_2 receptors from bovine striatal membranes by the PLG peptidomimetic photoaffinity-labeling agents **2b**, **2c**, **3b**, **4b**, and **4c**. Data represent the percent increase in specific [^{3}H]NPA binding over the control value when the indicated concentration of compound was added directly to the assay buffer. Results are the means ± SEM of three or four separate experiments carried out in triplicate. Statistical comparisons were made using repeated measures of analysis of variance (ANOVA). *Significantly different ($p < 0.05$) from control value.

were tested. In general, however, they were less effective than the unsubstituted PLG peptidomimetic **1**. The agents for which the photoaffinity label was placed off of the carboxamide moiety of **1**, compounds **4a**−**c**, appeared to be the most effective of the photoaffinity-labeling agents that were made.

Experiments were carried out to determine if exposure to UV light affected the ability of dopamine receptor modulators to enhance the binding of [^{3}H]NPA. Thus, bovine striatal membranes were exposed to UV light, and the ability of PLG (1 μM) and **1** (10 nM) to enhance the binding of [^{3}H]NPA in this preparation was determined and compared with the ability of PLG and **1** to enhance the binding of [^{3}H]NPA in striatal membranes that had not been exposed to UV light. The respective percent increases in agonist binding induced by PLG (24.5 ± 5.2%) and **1** (51.1 ± 7.6%) were similar in both preparations, indicating that exposure to UV light under the photolysis conditions to be used in photoaffinity labeling did not affect the receptor modulatory process.

Two sets of experiments were carried out to determine if allosteric modulation of the dopamine D_2 receptor still occurs after the PLG binding site has been cross-linked to a photoaffinity-labeling agent. In the first set of experiments, the photoaffinity-labeling agents, **4b** and **4c**, were incubated with bovine striatal D_2 receptors and the preparations then were

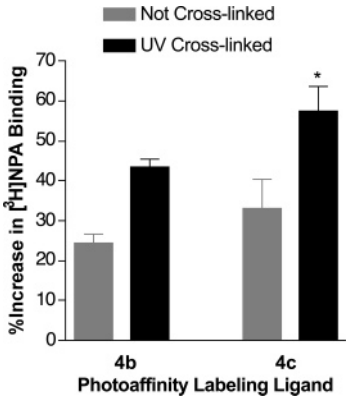


Figure 3. Stimulation of [^{3}H]NPA binding to dopamine D_2 receptors from bovine striatal membranes by photoaffinity-labeling agents **4b** and **4c** at a concentration of 100 nM with or without cross-linking to the membrane preparation. Results are the mean ± SEM of three separate experiments carried out in triplicate. Statistical comparisons were made using repeated measures of analysis of variance (ANOVA). Significantly different from the non-cross-linked value: $*p < 0.05$.

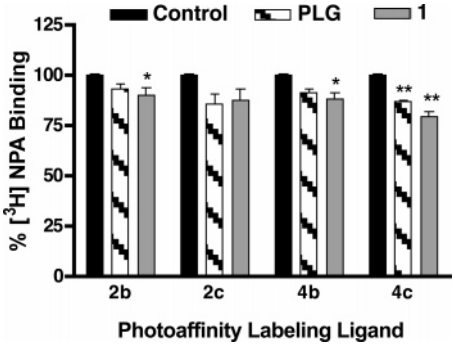


Figure 4. Stimulation of [^{3}H]NPA binding to dopamine D_2 receptors from bovine striatal membranes by PLG and **1** after the receptors have been photolyzed in the presence of 100 nM of the photoaffinity-labeling agents **2b**, **2c**, **4b**, and **4c**. Data represent the percent change in specific [^{3}H]NPA binding from the control value when either PLG (1 μM) or **1** (10 nM) was added directly to the assay buffer. Results are the means ± SEM of three or four separate experiments carried out in triplicate. Statistical comparisons were made using repeated measures of analysis of variance (ANOVA). Significantly different from control value: $*p < 0.05$, $**p < 0.001$.

subjected to photolysis. The ability of the two photoaffinity-labeling agents cross-linked to striatal membrane dopamine D_2 receptors to increase the binding of the agonist [^{3}H]NPA was determined, and the results were compared to the percent increase in [^{3}H]NPA binding produced by **4b** and **4c** on D_2 receptor preparations in which the two agents were not cross-linked to the dopamine receptor. The data obtained for these experiments are shown in Figure 3. They show that when **4b** and **4c** were cross-linked to the dopamine receptor preparation, they produced a modulatory effect that was either comparable to or greater than the increase in agonist binding produced by these two ligands when they were not cross-linked to the dopamine receptor.

In another experiment, the photoaffinity-labeling agents **2b**, **2c**, **4b**, and **4c** were incubated with the striatal membrane dopamine D_2 receptor preparation for 1 h and then the preparation was photolyzed for 5 min. After any unreacted photoaffinity-labeling agent was removed from the dopamine D_2 receptor preparation, the preparation was used in assays to determine if PLG (1 μM) and PLG peptidomimetic **1** (10 nM) were still able to enhance the binding of [^{3}H]NPA to dopamine D_2 receptors. The results of this experiment are shown in Figure 4. The results show that after exposure of the dopamine D_2

receptors to the photoaffinity-labeling agents **2b**, **2c**, **4b**, and **4c**, the allosteric modulators PLG and PLG peptidomimetic **1** were no longer able to enhance the binding of [^{3}H]NPA to the D_2 receptors. Rather, the binding of [^{3}H]NPA to the D_2 receptors was diminished slightly in several instances.

Discussion

The objective of the present study was to determine the feasibility of placing photoaffinity-labeling moieties at various positions about the structure of the potent PLG peptidomimetic γ-lactam **1**. The two initial positions on **1** that were examined were the fourth position of the N-terminal prolyl residue and the C-terminal carboxamide moiety. Use of the photoaffinity-labeling 4-azidobenzoyl moiety gave the photoaffinity-labeling agents **2a**, **3a**, and **4a** and use of the 4-azido-2-hydroxybenzoyl moiety gave the photoaffinity-labeling agents **2b**, **3b**, and **4b**. All of these analogues of PLG peptidomimetic **1** retained their ability to enhance the binding of the dopamine receptor agonist NPA to isolated dopamine D_2 receptors from bovine striatal tissue (Figures 1 and 2). These compounds were not quite as effective as the parent peptidomimetic from which they were designed, but they had activity comparable to or better than that of PLG in enhancing dopamine agonist binding to dopamine D_2 receptors. These results indicate that there is steric tolerance at the positions on PLG peptidomimetic **1** on which the photoaffinity moieties have been placed with respect to their interaction with the modulatory PLG binding site.

The photoaffinity-labeling agents **2c** and **4c**, which possessed the 4-azido-2-hydroxy-5-iodobenzoyl moiety, were made to examine the effect that iodination would have on the photoaffinity-labeling agents **2b** and **4b**, respectively. The fact that **2c** and **4c** retained significant dopamine receptor modulatory activity indicates that the corresponding radiolabeled derivatives will be useful probes in identifying the binding site at which PLG and its peptidomimetics act.

Further support of this potential was seen in the two sets of experiments that were carried out to determine if modulation of the dopamine D_2 receptor still occurs after the PLG binding site has been cross-linked with a photoaffinity-labeling agent. The cross-linking of **4b** to the receptor preparation produced a modulatory effect that was comparable to the increase in binding produced by **4b** when it was not cross-linked to the dopamine receptor. In the case of **4c**, cross-linking produced a modulatory effect that was significantly greater than the increase produced by **4c** on the receptor preparation when it was not cross-linked to the dopamine receptor. These results demonstrate that photoaffinity-labeling of the PLG binding site produces a receptor preparation that is being modulated by the covalently linked photoaffinity ligand. This provides strong support that the photoaffinity agents are labeling the modulatory binding site with which PLG and its peptidomimetics interact in modulating the dopamine D_2 receptor.

In a second set of experiments, the photoaffinity-labeling agents **2b**, **2c**, **4b**, and **4c** were first incubated with bovine striatal dopamine D_2 receptors and then the preparations were subjected to photolysis. It was observed that under these conditions PLG and PLG peptidomimetic **1** were unable to enhance the binding of [^{3}H]NPA to the dopamine D_2 receptors (Figure 4). Interestingly, the binding of [^{3}H]NPA to the D_2 receptors was actually diminished in several instances by PLG and **1** under these conditions. The reason for this is not clear but it may be related to the biphasic dose−response curve that is seen with PLG and its peptidomimetics when modulating dopamine receptor agonist binding. At high doses of PLG or **1** the binding of [^{3}H]NPA decreases. The labeling of the PLG binding site with the photoaffinity-labeling agents and the subsequent exposure of the labeled preparations with PLG and **1** may be simulating this situation. Thus, the photoaffinity-labeling agents may prove useful in delineating the biphasic dose−response curve seen with the dopamine receptor modulating agents. These results, we believe, also support the hypothesis that the photoaffinity-labeling agents are binding and subsequently labeling the site at which PLG and its peptidomimetic interact, since if these compounds were not binding at the same site as PLG or peptidomimetic **1**, we would have expected PLG and **1** to increase dopamine agonist binding.

In conclusion, six different photoaffinity-labeling ligands have been synthesized that all show significant activity in enhancing the binding of the dopamine D_2 receptor agonist [^{3}H]NPA. The 4-azido-2-hydroxybenzoyl-derived photoaffinity labels, **2b**, **3b**, and **4b** can all be easily radioiodinated for use in experiments that will be conducted to determine where PLG is binding. Thus, these photoaffinity-labeling agents should serve as useful probes in delineating the allosteric binding site on the dopamine D_2 receptor and in identifying the residues within the PLG binding site where the interactions take place.

Experimental Section

General Procedures. Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K and are uncorrected. Specific rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D-line) at 22 °C, unless otherwise stated. Elemental analyses were performed by M−H−W Laboratores, Phoenix, AZ. ^{1}H NMR were recorded on a 300-MHz Varian NMR spectrometer. ^{13}C NMR spectroscopy was performed on either a 300-MHz, a 500-MHz, or a 600-MHz Varian NMR spectrometer. 2D NMR experiments were performed on either a 300-MHz or a 600-MHz Varian NMR spectrometer. The chemical shifts are reported in parts per million (ppm) with the deuterated solvent serving as the internal standard. Coupling constants (*J*) are reported in Hertz (Hz). For compounds that displayed the presence of rotomers, resonances of both rotomers are given. HRMS and LRMS were obtained on a Bruker BioTOFII located in the Department of Chemistry. Column chromatography was performed on Baker analyzed reagent silica gel (60−200 mesh) and was carried out by either gravity or flash elution. Thin-layer chromatography (TLC) was carried out on Analtech 250 μm silica gel GF uniplates. Visulization was done with either UV, I_2, ninhydrin spray (amines) or indophenol spray (acids). Analytical and preparative HPLC purifications were performed on a Gilson HPLC system. 4-Azidobenzoic acid *N*-hydroxysuccinimide ester (**13a**) was purchased from Aldrich Chemical Co., Milwaukee, WI. For reactions requiring anhydrous conditions, the glassware was oven-dried, and the solvents were distilled.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(methylsulfonyl)oxy]proline Benzyl Ester (6a). Compound **5**[19] (3.5 g, 10.9 mmol) was dissolved in 35 mL of pyridine. This solution was then cooled to 0 °C. Methanesulfonyl chloride (2.07 g, 18.1 mmol) was added dropwise. The solution was allowed to warm to room temperature as it was stirred overnight. After 22 h the reaction was cooled back to 0 °C and 11 mL of 10% H_2O in pyridine was added dropwise. Once this solution was completely added, the reaction mixture was concentrated to an orange oil. The reaction mixture was then poured into ice-cold water. This was then extracted with EtOAc, and the combined organic layers were washed with 1 M $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give 4.35 g (93%) of **6a** as a dark oil: TLC R_f = 0.47 (hexanes/EtOAc, 1:1); $[\alpha]_D$ −43.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, rotamers present, COSY assignment) δ 1.29 and 1.40 (2s, 9H), 2.12−2.24 (m, 1H), 2.48−2.62 (m, 1H), 2.96 (s, 3H), 3.64−3.82 (m, 2H), 4.35 (m, 1H), 5.01−5.22 (m, 3H), 7.29 (s, 5H); ^{13}C NMR ($CDCl_3$, HMQC assignment, rotamers present) δ 28.4, 28.5, 28.6, 36.6, 37.8,

39.0, 52.5, 52.8, 57.6, 57.8, 67.1, 67.4, 67.6, 78.0, 78.3, 78.5, 128.3, 128.8, 135.3, 135.6, 153.5, 154.0, 171.8, 172.1.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-bromoproline Benzyl Ester (6b). Compound **5**[19] (3.22 g, 10.0 mmol) and carbon tetrabromide (3.43 g, 10.3 mmol) were dissolved in 30 mL of dry THF (0.33 M). Then, 1,2-bis(diphenylphosphino)ethane (diphos,[21] 2.40 g, 6.0 mmol) was added. The reaction was allowed to stir at room temperature for 20 h, at which time the suspension was filtered through a pad of Celite. The pad was rinsed with THF and the filtrate was evaporated to give a yellow oil. The crude product was purified on a silica gel column (4.5 × 50 cm) with elution initiated with hexanes/Et_2O (4:1) and then increasing to hexanes/Et_2O (1:1) to afford 3.14 g (82%) of **6b** as a clear oil: TLC $R_f = 0.16$ (hexanes/Et_2O, 4:1); $[\alpha]_D$ −41.3 (*c* 1.5 CH_2Cl_2); ^{1}H NMR ($CDCl_3$, COSY assignment, rotamers present) δ 1.20 and 1.32 (2s, 9H), 2.24−2.30 (m, 1H), 2.57−2.71 (m, 1H), 3.53−3.61 (m, 1 H), 3.82−3.93 (m, 1H), 4.11−4.34 (m, 2H), 4.91−5.16 (m, 2H), 7.19−7.22 (m, 5H); ^{13}C NMR ($CDCl_3$, HMQC assignment, rotamers present) δ 27.9, 28.3, 28.5, 40.2, 41.1, 41.8, 42.8, 55.5, 55.9, 58.2, 58.5, 67.3, 80.8, 128.4, 128.7, 128.8, 135.6, 135.8, 153.3, 153.8, 171.4, 171.7.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-azidoproline Benzyl Ester (7a). Compound **6a** (0.93 g, 2.35 mmol) and NaN_3 (0.70 g, 10.8 mmol) were suspended in 40 mL of dry DMF. This mixture was heated at 55 °C in an oil bath for 18.5 h. The mixture was allowed to cool to room temperature, whereupon it was poured into ice cold water. The mixture was extracted with EtOAc, and the combined organic layers were washed with H_2O and brine and then dried over $MgSO_4$, filtered, and concentrated to give 0.78 g (96%) of **7a** as a dark oil: TLC $R_f = 0.67$ (hexanes/EtOAc, 1:1); $[\alpha]_D$ −32.2 (*c* 1.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotamers present) δ 1.31 and 1.43 (2s, 9H), 2.11−2.21 (m, 1H), 2.33−2.48 (m, 1H), 3.39−3.50 (m, 1H), 3.60−3.72 (m, 1H), 4.00−4.12 (m, 1H), 4.31 and 4.34 (2d, 0.6H, $J = 3.6$ and 3.9 Hz), 4.44 and 4.47 (2d, 0.4H, $J = 3.0$ and 3.6 Hz), 5.04−5.26 (m, 2H), 7.32−7.33 (5H); ^{13}C NMR ($CDCl_3$, rotamers present, gHMQC assignment) δ 28.4, 28.7, 35.4, 36.4, 51.2, 51.6, 57.8, 58.1, 58.6, 59.6, 67.3, 80.7, 128.3, 128.4, 128.6, 128.7, 128.8, 135.6, 135.7, 153.6, 154.0, 171.4, 171.6; IR (neat): 2976, 2105 (N_3), 1751, 1700 cm^{-1}.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-azidoproline Benzyl Ester (7b). Compound **6b** (1.13 g, 2.94 mmol) was treated in a manner similar to that used to convert **6a** to **7a** to afford 1.01 g (99%) of **7b** as a yellow oil: TLC $R_f = 0.75$ (CH_2Cl_2/MeOH, 20:1); $[\alpha]_D$ −49.3 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotamers present) δ 1.26 and 1.39 (2s, 9 H), 2.01−2.10 (m, 1H), 2.16−2.29 (m, 1H), 3.36−3.61 (m, 2H), 4.04−4.08 (m, 1H), 4.28 (t, 0.6H, $J = 7.5$ Hz), 4.36−4.41 (apparent t, 0.4H, $J = 6.6$ and 8.1 Hz), 4.98−5.19 (m, 2H), 7.25−7.27 (m, 5H); ^{13}C NMR ($CDCl_3$, rotamers present) δ 28.2, 28.3, 28.5, 35.4, 36.4, 51.4, 51.6, 57.8, 58.0, 58.9, 59.4, 67.0, 80.6, 128.6, 135.6, 135.8, 153.5, 154.1, 172.1 172.3; IR (neat): 2978, 2105 (N_3), 1748, 1701 cm^{-1}.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-aminoproline (8a). Compound **7a** (1.0 g, 2.89 mmol) was dissolved in 50 mL of 10% H_2O/EtOH that contained 136 mg of 10% Pd/C. The reaction was hydrogenated at 75 psi for 28 h. The mixture was filtered through a pad of Celite and the pad was washed with a mixture of H_2O/EtOH (1:1). The filtrate was then concentrated to give a solid, which was triturated with absolute ethanol to yield 0.61 g (91%) of **8a** as a white solid: mp 225−228 °C (dec) [lit.[20] mp 225−227 °C (dec)]; $[\alpha]_D$ 21 (*c* 0.98, H_2O) [lit.[20] $[\alpha]_D$ 21 (*c* 0.24, H_2O)]; ^{1}H NMR (D_2O, rotamers present, gCOSY assignment) δ 1.24 and 1.28 (2s, 9H), 1.90−1.96 (m, 1H), 2.43−2.53 (m, 1H), 3.53−2.65 (m, 2H), 3.81−3.83 (m, 1H), 3.96−4.00 (m, 1H); ^{13}C NMR (D_2O, rotamers present) δ 27.7, 27.8, 33.9, 50.0, 49.8, 60.7, 82.5, 155.8, 180.4.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-aminoproline (8b). In procedures analogous to that used to make **8a**, **7b** (0.87 g, 2.51 mmol) yielded 0.57 g (95%) of **8b** as a white solid: mp 240−241 °C (dec) [lit.[20] 228−229 °C (dec)]; $[\alpha]_D$ −35.9 (*c* 1.0, H_2O) [lit.[20] −35 (*c* 0.17, H_2O)]; ^{1}H NMR (D_2O, rotamers present, COSY assignment) δ 1.22 and 1.27 (2s, 9H), 2.05−2.14 (m, 1H), 2.23−2.32 (m, 1H), 3.36−3.47 (m, 1H), 3.57−3.65 (m, 1H), 3.77−3.84 (m, 1H), 4.01−4.06 (apparent t, 1H, $J = 7.5$ and 8.1 Hz); ^{13}C NMR (D_2O, rotamers present, gHMQC assignment) δ 27.7, 28.9, 34.0, 34.6, 49.3, 49.7, 50.1, 59.4, 59.9, 82.2, 82.3, 155.6, 155.7, 170.1.

(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(N-benzyloxycarbonyl)aminoproline (9a). Compound **8a** (0.37 g, 1.61 mmol) was dissolved in H_2O (∼6 mL), and then a 2 N NaOH solution (0.065 g, 1.61 mmol in 0.75 mL of H_2O) was added. The reaction mixture was cooled to 0 °C and then CbzCl (0.30 g, 1.77 mmol) was added dropwise. One more equivalent of a 2 N NaOH solution was added to the reaction and the reaction was stirred at 0 °C for 1 h. The reaction mixture was stirred at room temperature overnight. The next day another 2 equiv of 2 N NaOH and 1.1 equiv of CbzCl at 0 °C were added. The reaction mixture was stirred overnight, where it eventually warmed to room temperature. The reaction mixture was stirred for two more days. The reaction mixture was washed with Et_2O to remove excess CbzCl. The water layer was acidified to pH ∼3 with solid citric acid and this solution then was extracted with EtOAc (3×). The organic layers were combined and washed with water and brine. They were then dried over $MgSO_4$, filtered, and concentrated to afford 0.47 g (80%) of a pale yellow glassy solid: mp 59−61 °C; TLC $R_f = 0.58$ (1-propanol/NH_4OH, 4:1); $[\alpha]_D$ −24.3 (*c* 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$, rotamers present, gCOSY assignment) δ 1.38 and 1.43 (2s, 9H), 1.9−2.12 (m, 1H), 2.30−2.46 (m, 1H), 3.42 (t, 1H, $J = 12.3$ Hz), 3.58−3.63 (m, 1H), 4.20−4.35 (m, 2H), 5.05−5.12 (m, 2H), 5.90 and 5.97 (2d, 0.75H, $J = 7.5$ and 8.4 Hz), 6.53 (br s, 0.25H, NH[30]), 7.26−7.30 (m, 5H), 9.33 (br s, 1H); ^{13}C NMR ($CDCl_3$, rotamers present, gHMQC assignment) δ 28.5, 28.7, 34.8, 36.9, 49.9, 50.8, 52.6, 53.8, 58.0, 58.2, 67.1, 81.3, 82.0, 128.3, 128.5, 128.7, 136.4, 154.0, 155.8, 156.2, 175.3, 176.9; HRFAB MS *m*/*z* 365.1720 $[M + H]^+$, $C_{18}H_{24}N_2O_6 + H^+$ requires 365.1712.

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(*N*-benzyloxycarbonyl)aminoproline (9b). The same procedures used above to make **9a** were used to convert **8b** (1.0 g, 4.34 mmol) to 1.0 g (63%) of **9b** as a white foamy solid: mp 69−71 °C; TLC $R_f = 0.64$ (1-propanol/NH_4OH, 4:1); $[\alpha]_D$ −21.7 (*c* 1.32, CH_3OH); ^{1}H NMR ($CDCl_3$, rotamers present, gCOSY assignment) δ 1.37 and 1.41 (2s, 9H) 1.84−2.24 (m, 2H), 3.29−3.70 (m, 2H), 4.26−4.36 (m, 2H), 5.04 (br s, 2H), 5.56 and 5.67 (2 br s, 0.7 H, NH), 6.56 (br s, 0.3 H, NH[30]), 7.29 (s, 5H), 9.76 (br s, 1H); ^{13}C NMR ($CDCl_3$, rotamers present, gHMQC assignment) δ 28.5, 28.7, 35.5, 37.0, 49.7, 50.2, 51.4, 52.3, 57.7, 58.0, 81.3, 128.2, 128.4, 128.7, 136.3, 154.2, 155.2, 156.2, 175.3, 176.7; FAB MS *m*/*z* 365.1 $[M + H]^+$.

Methyl 3(*R*)-[(4(*S*)-(4-Benzyloxycarbonylamino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetate (11a). Compound **9a** (0.35 g, 0.95 mmol), the TFA salt of 3(*R*)-2-oxo-1-pyrrolidineacetic acid methyl ester[22,23] (**10**, 0.27 g, 0.95 mmol), Et_3N (0.33 g, 3.4 mmol), and 2-chloro-1-methylpyridinium iodide (0.26 g, 1.03 mmol) were dissolved in 50 mL of CH_2Cl_2. The reaction mixture was heated at reflux for 2.5 days. The reaction mixture was allowed to cool and then it was washed with 10% citric acid, 1 M $NaHCO_3$, H_2O, and brine. The solution was dried over $MgSO_4$, filtered, and then concentrated to give a yellow oil. This oil was purified on a silica gel column (2.5 × 30 cm) by eluting initially with hexanes/EtOAc (1:1) and then increasing to EtOAc/hexanes (5:1). Crystallization from EtOAc/petroleum ether (bp 60−70 °C) afforded 0.30 g (61%) of **11a**: mp 92−95 °C; $[\alpha]_D$ −25.0 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment, rotamers present) δ 1.44 (s, 9H), 1.86−2.11 (m, 2H), 2.39−2.61 (m, 2H), 3.25−3.31 (m, 1H), 3.42−3.50 (m, 2H), 3.71−3.79 (m with s at 3.71, 4H), 3.99 (br d, 1H, $J = 18$ Hz), 4.15−4.25 (m, 3H), 4.43−4.49 (m, 1H), 5.01−5.10 (m, 2H), 7.33−7.34 (m, 5H); ^{13}C NMR (CD_3OD, gHMQC assignment, rotamers present) δ 25.8, 27.1, 27.5, 27.8, 35.3, 36.6, 44.2, 44.7, 49.3, 50.1, 51.0, 51.6, 52.1, 52.7, 59.4, 66.3, 80.5, 80.85, 127.7, 128.0, 128.3, 137.0, 154.6, 156.9, 169.4, 173.3, 174.4; ESI MS *m*/*z* 541.2 $[M + Na]^+$. Anal. ($C_{25}H_{34}N_4O_8$) C, H, N.

Methyl 3(*R*)-[(4(*R*)-(4-Benzyloxycarbonylamino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetate (11b). Compound **9b** (2.1 g, 5.76 mmol) was dissolved in 50 mL of dry THF. This solution was placed under Ar and then cooled to −23 °C. *N*-Methylpiperidine (0.57 g, 5.76

mmol) followed by isobutylchloroformate (0.79 g, 5.76 mmol) was added sequentially in a dropwise manner to the solution. The cloudy mixture was stirred at −23 °C for 10 min, HOBt (0.78 g, 5.76 mmol) was added, and the mixture was stirred at −23 °C for 20 min. A solution of **10** (1.65 g, 5.76 mmol) and *N*-methylpiperidine (0.57 g, 5.76 mmol) in THF that had been cooled to 0 °C was added. This mixture was stirred at −23 °C initially and then allowed to warm to room temperature, where it was stirred for 4 days. The THF was removed and the oil that was obtained was dissolved in CH_2Cl_2. This solution was washed with 1 M $NaHCO_3$, 10% citric acid, H_2O, and brine. The solution was dried over $MgSO_4$, filtered, and concentrated to give a golden oil that was crystallized from EtOAc/petroleum ether (bp 60−70 °C) to afford 1.78 g (60%) of **11b** as a white crystalline solid: mp 179−180 °C; $[\alpha]_D$ −15.2 (*c* 0.5 MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 1.44 (s, 9H), 1.98−2.11 (m, 1H), 2.20−2.26 (m, 2H), 2.42−2.51 (m, 1H), 3.30−3.39 (m, 1H), 3.42−3.53 (m, 2H), 3.70−3.74 (m with a s at 3.74, 4H), 4.03 (d, 1H, J = 17.4 Hz), 4.20 (d, 1H, J = 17.7 Hz), 4.26−4.33 (m, 2H), 5.07 (s, 2H), 7.34 (s, 5H); ^{13}C NMR (CD_3OD, gHMQC and gHMBC assignment) δ 25.9, 27.4, 36.7, 44.1, 44.6, 49.4, 50.8, 51.6, 51.8, 59.4, 66.2, 80.8, 127.7, 127.8, 128.3, 137.1, 154.7, 157.1, 169.4, 173.3, 174.2; HRFAB MS *m*/*z* 519.2436 [M + H]$^+$ and 541.2275 [M + Na]$^+$, $C_{25}H_{34}N_4O_8$ + H^+ requires 519.2455 and $C_{25}H_{34}N_4O_8$ + Na^+ requires 541.2275. Anal. ($C_{25}H_{34}N_4O_8$) C, H, N.

3(*R*)-[(4(*S*)-(4-Benzyloxycarbonylamino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (12a). Compound **11a** (0.70 g, 1.35 mmol) was dissolved in 20 mL of a 3 M NH_3/MeOH solution. The solution was capped tightly and it was stirred overnight (20 h) at room temperature. The solution was concentrated to give a foam that was purified by silica gel column chromatography (2.5 × 30 cm) with CH_2Cl_2/MeOH (20:1) to afford 0.60 g (88%) of **12a** as a white foam: $[\alpha]_D$ −14.9 (*c* 1.0, MeOH); mp 110−112 °C; ^{1}H NMR (CD_3OD, gCOSY assignment, rotamers present) δ 1.44 (s, 9H), 1.93−1.95 (m, 1H), 2.02−2.15 (m, 1H), 2.45−3.54 (m, 2H), 3.23−3.31 (m, 1H), 3.46 (m, 2H), 3.74−3.88 (m, 2H), 4.03 and 4.09 (2s, 1H), 4.15−4.22 (m,2H), 4.32−4.37(m, 1H), 5.02−5.11 (m, 2H), 7.31−7.35 (m, 5H); ^{13}C NMR (CD_3OD, gHMQC, rotamers present) δ 25.4, 27.3, 27.6, 28.0, 28.3, 35.4, 36.5, 45.1, 45.8, 49.3, 50.1, 51.3, 52.1, 52.7, 59.1, 59.3, 66.4, 80.5, 80.9, 127.8, 127.9, 129.4, 137.0, 154.5, 155.0, 156.9, 171.5, 173.2, 173.5, 174.0, 174.4; ESI MS *m*/*z* 526.2 [M + Na]$^+$. Anal. ($C_{24}H_{33}N_5O_7$) C, H, N.

3(*R*)-[(4(*R*)-(4-Benzyloxycarbonylamino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (12b). Compound **11b** (0.52 g, 1.0 mmol) was treated in the same manner as **11a** was above to afford 0.47 g (92%) of **12b** as a white solid: mp 185−187 °C; $[\alpha]_D$ −4.8 (*c* 1.0 MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 1.44 (s, 9H), 2.04−2.14 (m, 1H), 2.20−2.23 (m, 2H), 2.45−2.47 (m, 1H), 3.30−3.37 (m, 1H), 3.46−3.50 (m, 2H), 3.68−3.74 (m, 1H), 3.86 (dd, 1H, J = 7.8 and 17.1 Hz), 4.07 (d, 1H, J = 16.5 Hz), 4.21−4.41 (m, 3H), 5.07 (s, 2H), 7.33−7.34 (m, 5H); ^{13}C NMR (CD_3OD, gHMQC assignment) δ 25.5, 27.6, 36.7, 45.2, 45.7, 49.4, 51.2, 51.8, 59.4, 66.3, 80.8, 127.6, 127.8, 128.2, 137.0, 154.6, 157.0, 171.4, 173.2, 174.0; FAB MS *m*/*z* 504.2 [M + H]$^+$. Anal. ($C_{24}H_{33}N_5O_7$) C, H, N.

4-Azido-2-hydroxybenzoic Acid *N*-Hydroxysuccinimide Ester (13b). 4-Azido-2-hydroxybenzoic acid[18] (2.0 g, 11.2 mmol) and *N*-hydroxysuccinimde (1.4 g, 12.3 mmol), which had been recrystallized from EtOAc, were placed under an Ar atmosphere. The solids were dissolved in a solution of CH_2Cl_2/DMF (1:1, 20 mL). The reaction was cooled to −78 °C and 1-ethyl-3-(3-dimehylaminopropyl)carbodiimide hydrochloride (2.14 g, 11.17 mmol) was added. The reaction was allowed to warm to room temperature as it was stirred overnight. The reaction was stirred for 2 more days, after which time the reaction mixture was concentrated to remove CH_2Cl_2. The remaining mixture was poured into ice-cold water, and this mixture was extracted with EtOAc. The organic layers were combined and washed with 10% citric acid, 1 M $NaHCO_3$, H_2O, and brine. The organic layer was then dried over $MgSO_4$, filtered, and concentrated to a solid, which was crystallized from EtOAc/petroleum ether to afford 1.3 g (42%) of **13b** as a pale yellow crystalline solid: mp 160 °C (lit.[18] mp 160−161 °C); ^{1}H NMR ($CDCl_3$) δ 2.92 (s, 4H) 6.62 (dd, 1H, J = 1.8 and 6.6 Hz), 6.67 (d, 1H, J = 1.8 Hz), 7.96 (d, 1H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, gHMQC and gHMBC assignment) δ 26.0, 105.0, 107.8, 111.7, 132.1, 149.9, 163.6, 164.5, 169.0; IR(KBr) 2130 cm^{-1} (N_3).

4-Azido-2-hydroxy-5-iodobenzoic Acid *N*-Hydroxysuccinimide Ester (13c). Compound **13b** (1.60 g, 5.79 mmol) was suspended in CH_3CN (50 mL) under N_2. NaI (0.96 g, 6.37 mmol) was added to the suspension. After 5 min, the suspension became a solution and Chloramine T (1.45 g, 6.37 mmol) was added very slowly. The reaction was stirred for 1 h at room temperature. The product was diluted with water. A solution of 10% citric acid was added and the reaction mixture was extracted with EtOAc. The combined organic layers were washed with a 5% sodium thiosulfate solution and brine. The organic layer was dried with $MgSO_4$, filtered, and concentrated to a yellow solid. This crude product was crystallized from EtOAc to afford 250 mg (11%) of **13c** as pale yellow crystals: mp 189 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 4H), 6.81 (s, 1H), 8.37 (s, 1H), 9.64 (s, 1H); ^{13}H NMR ($CDCl_3$, gHMQC and gHMBC assignment) δ 26.0, 76.1, 107.1, 107.3, 141.3, 151.1, 163.3, 163.6, 168.8.

3(*R*)-[(4(*S*)-((4-Azidobenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (14a). In a Parr bottle, compound **12a** (0.21 g, 0.40 mmol) was dissolved in 20 mL of MeOH. This solution was purged under Ar and then 10% Pd/C (30 mg) was added. The Parr bottle was placed on a Parr shaker at 50 psi for 24 h. The reaction mixture was filtered through a pad of Celite and the pad then was rinsed with MeOH. The filtrate was concentrated to a white solid which was triturated with ether to afford 0.14 g (92%) of 3(*R*)-[(4(*S*)-(4-amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide as a colorless foam that was taken on without further purification. This material (0.141 g, 0.38 mmol) was placed under Ar and then dissolved in 20 mL of CH_3CN. *N*-Methylpiperidine (0.038 g, 0.38 mmol) was then added. This was followed by the addition of **13a** (0.10 g, 0.38 mmol). The reaction was heated at reflux for 18 h. After the reaction was cooled to room temperature, the CH_3CN was removed in vacuo. The residue that remained was dissolved in CH_2Cl_2 and this solution was washed with H_2O, dilute $NaHCO_3$, H_2O, and brine. The organic layer was then dried over $MgSO_4$, filtered, and concentrated to give a pale yellow foam that was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford **14a** (0.12 g, 61%) as a pale yellow foam: $[\alpha]_D$ −3.3 (*c* 1.35, CH_2Cl_2); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 1.45 (s, 9H), 2.07−2.20 (m, 2H), 2.41−2.51 (m, 1H), 2.62−2.72 (m, 1H) 3.43−3.50 (m, 3H), 3.78−3.91 (m, 2H), 4.04 (d, 1H, J = 17.1 Hz), 4.31−4.39 (m, 2H), 4.59−4.62 (m, 1H), 7.13 (d, 2H, J = 8.7 Hz), 7.85 (d, 2H, J = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 25.6, 27.6, 36.4, 45.1, 45.7, 51.6, 52.7, 59.4, 80.9, 118.8, 129.2 (two overlapping peaks), 130.4 (two overlapping peaks), 143.8, 154.5, 167.2, 171.3, 173.0, 174.6; IR (neat) 2124 cm^{-1} (N_3); HRESI MS *m*/*z* 537.2169 [M + Na]$^+$, $C_{23}H_{30}N_8O_6$ + Na^+ requires 537.2186. Anal. ($C_{23}H_{30}N_8O_6$) C, H, N.

3(*R*)-[(4(*S*)-((4-Azido-2-hydroxybenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (14b). Compound **12a** (0.12 g, 0.22 mmol) was treated in the same way as described above for the synthesis of **14a**, except no *N*-methylpiperidine was added to the reaction and **13b** (0.07 g, 0.24 mmol) was used as the acylating reagent. The crude product was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford **14b** (0.06 g, 51%) as a pale yellow glassy solid. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 3.82 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.07 min when eluted with CH_3CN/H_2O (99.5:0.5): $[\alpha]_D$ −5.5 (*c* 0.82 MeOH); ^{1}H NMR (CD_3OD) δ 1.45 (s, 9H), 2.09−2.19 (m, 2H), 2.43−2.69 (m, 2H),

3.42−3.50 (m, 3H), 3.76 (m, 2H), 4.05 (d, 1H, J = 17.1 Hz), 4.31−4.40 (m, 2H), 4.63−4.64 (m, 1H), 6.55 (dd, 1H, J = 2.3 and 6.8 Hz), 6.58 (d, 1H, J = 2.1 Hz), 7.70 (d, 1H, J = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 25.6, 27.6, 36.3, 45.1, 45.7, 51.6, 52.6, 53.2, 59.3, 80.9, 107.0, 109.8, 112.0, 129.2, 145.7, 154.5, 162.1, 168.9, 171.4, 173.0, 174.5; IR (Nujol mull) 2114 cm^{-1} (N_3); HRESI MS m/z 553.2160 [M + Na]$^+$, $C_{23}H_{30}N_8O_7$ + Na^+ requires 553.2135.

3(*R*)-[(4(*S*)-((4-Azido-2-hydroxy-5-iodobenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (14c). Compound **12a** (0.09 g, 0.18 mmol) was treated in the same way as described above for the synthesis of **14a**, except no *N*-methylpiperidine was added to the reaction and **13c** (0.08 g, 0.19 mmol) was used as the acylating reagent. The product **14c** was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford 60 mg (51%) of a pale yellow glassy solid. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 7.12 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.1 min when eluted with CH_3CN/H_2O (99.5:0.5): mp 175−178 °C (dec); $[\alpha]_{435}$ 6.0 (c 1.65, MeOH); ^{1}H NMR (CD_3OD, rotamers present) δ 1.46 (s, 9H). 2.05−2.21 (m, 2H), 2.41−2.68 (m, 2H), 3.48−3.52 (m, 3H), 3.80 (dd, 1H, J = 6 and 11.1 Hz), 3.89 (d, 1H, J = 16.8 Hz), 4.08 (d, 1H, J = 17.1 Hz), 4.34−4.37 (m, 2H), 4.67 (br m, 1H), 6.76 (d, 1H, J = 9 Hz), 7.34 (d, 1H, J = 4.2 Hz), 7.72 (d, 1H, J = 8.7 Hz); ^{13}C NMR (CD_3OD, rotamers present) δ 24.1, 26.1, 34.7, 43.6, 44.2, 47.6, 50.2, 51.2, 57.8, 77.6, 79.5, 107.2, 109.1, 126.1, 126.3, 126.6, 126.8, 145.1, 153.0, 160.8, 167.4, 169.8, 171.5, 173.1; IR (Nujol mull) 2115 cm^{-1} (N_3); HRESI MS m/z 679.1125 [M + Na]$^+$, $C_{23}H_{29}N_8O_7I$ + Na^+ requires 679.1102.

3(*R*)-[(4(*R*)-((4-Azidobenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (15a). Compound **12b** (0.19 g, 0.37 mmol) was treated the same way as described above for the synthesis of **14a** by using *N*-methylpiperidine (0.03 g, 0.35 mmol) and **13a** (0.09 g, 0.35 mmol). The crude product was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to give 0.09 g (51%) of **15a** as a white solid: mp 140−144 °C; $[\alpha]_D$ 14.3 (c 0.35, CH_2Cl_2); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 1.45 (s, 9H), 2.07−2.14 (m, 1H), 2.30−2.49 (m, 3H), 3.40−3.52 (m, 3H), 3.83−3.91 (m, 2H), 4.08 (d, 1H, J = 16.2 Hz), 4.34−4.44 (m, 2H), 4.59−4.65 (m, 1H), 7.11−7.14 (d, 2H, J = 8.7 Hz), 7.83−7.86 (d, 2H, J = 9 Hz); ^{13}C NMR (CD_3OD) δ 25.5, 27.6, 35.1, 36.3, 45.3, 45.8, 49.4, 51.2, 51.8, 59.4, 66.3, 80.8, 127.6, 127.8 (two overlapping peaks), 128.2 (two overlapping peaks), 137.0, 154.6, 157.0, 171.4, 173.2, 174.0; IR (neat) 2125 cm^{-1} (N_3); HRESI MS m/z 537.2168 [M + Na^+], $C_{23}H_{30}N_8O_6$ + Na^+ requires 537.2186. Anal. ($C_{23}H_{30}N_8O_6$) C, H, N.

3(*R*)-[(4(*R*)-((4-Azido-2-hydroxybenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (15b). Compound **12b** (0.08 g, 0.15 mmol) was treated in the same way as described above for the synthesis of **14a**, except no *N*-methylpiperidine was added to the reaction and **13b** (0.05 g, 0.16 mmol) was used as the acylating reagent. The crude product was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford **15b** (0.05 g, 62%) that was obtained as a white solid. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 3.89 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.05 min when eluted with CH_3CN/H_2O (99.5:0.5): mp 158−160 °C; $[\alpha]_D$ 3.4 (c 0.74, MeOH); ^{1}H NMR (CD_3OD) δ 1.45 (s, 9H), 2.07−2.17 (m, 1H), 2.28−2.49 (m, 3H), 3.39−3.51 (m, 3H), 3.83−3.91 (m, 2H), 4.08 (d, 1H, J = 17.1 Hz), 4.33−4.44 (m, 2H), 4.61−4.65 (m, 1H), 6.54 (dd, 1H, J = 2.1 and 6 Hz), 6.58 (d, 1H, J = 2.1 Hz), 7.78 (d, 1H, J = 8.4 Hz); ^{13}C NMR (CD_3OD) δ 25.5, 27.6, 36.3, 45.3, 45.8, 49.2, 51.2, 51.4, 59.4, 80.9, 107.0, 109.8, 112.5, 129.6, 145.6, 154.6, 161.5, 169.2, 171.4, 173.2, 174.0; IR (Nujol mull); 2114 cm^{-1} (N_3); HRESI MS m/z 553.2175 [M + Na]$^+$, $C_{23}H_{30}N_8O_7$ + Na^+ requires 553.2135.

3(*R*)-[(4(*R*)-((4-Azido-2-hydroxy-5-iodobenzoyl)amino)-1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide (15c). Compound **12b** (0.06 g, 0.11 mmol) was treated in the same way as described above for the synthesis of **14a**, except no *N*-methylpiperidine was added to the reaction and **13c** (0.05 g, 0.12 mmol) was used as the acylating reagent. The crude product was obtained as a pale yellow foam that was purified by silical gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford **15c** (0.03 g, 40%) as a white solid. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 3.72 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.22 min when eluted with CH_3CN/H_2O (99.5:0.5): mp 181 °C (dec); $[\alpha]_D$ 2.3 (c 1.45, MeOH); ^{1}H NMR (CD_3OD) δ 1.46 (s, 9H), 2.04−2.16 (m, 1H), 2.27−2.48 (m, 3H), 3.41−3.52 (m, 3H), 3.82−3.91 (m, 2H), 4.08 (d, 1H, J = 16.8 Hz), 4.35−4.41 (m, 2H), 4.60−4.64 (m, 1H), 6.75 (s, 1H), 7.21 (s, 1H); ^{13}C NMR (CD_3OD) δ 25.5, 27.6, 36.3, 45.3, 45.7, 49.2, 51.3, 50.1, 59.4, 73.8, 80.9, 106.9, 114.5, 138.9, 146.8, 154.6, 161.5, 168.0, 171.4, 173.2, 174.0; IR (Nujol mull) 2105 cm^{-1} (N_3); HRESI MS m/z 679.1161 [M + Na]$^+$, $C_{23}H_{29}N_8O_7I$ + Na^+ requires 679.1102.

3(*R*)-[(4(*S*)-(4-Azidobenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)-amino]-2-oxo-1-pyrrolidineacetamide·HCl (2a). Compound **14a** (0.06 g, 0.12 mmols) was dissolved in 2 mL of 4 N HCl in dioxane and the reaction was stirred at room temperature for 2 h. The reaction was concentrated and the residue azeotroped with CH_2Cl_2 to afford 0.053 g (86% yield) of a pale yellow hygroscopic solid. This product was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 6.14 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.37 min when eluted with CH_3CN/H_2O (3:2): $[\alpha]_D$ 14.7 (c 0.55, MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 2.01−2.15 (m, 1H), 2.26−2.35 (m, 1H), 2.40−2.48 (m, 1H), 2.81−2.91 (m, 1H), 3.47−3.57 (m, 3H), 3.67−3.74 (m, 1H), 3.93 (d, 2H, J = 4.2 Hz), 4.46−4.51 (apparent t, 1H, J = 7.2 and 7.8 Hz), 4.57−4.64 (apparent t, 1H, J = 9.3 and 9.6 Hz), 4.72−4.68 (m, 1H, 3-CH), 7.15 (d, 2H, J = 8.7 Hz), 7.87 (d, 2H, J = 8.7 Hz); ^{13}C NMR (CD_3OD, gHMQC assignment) δ 25.4, 34.8, 45.2, 45.6, 49.8, 50.3, 51.6, 59.2, 118.8, 129.3 (two overlapping peaks), 130.0 (two overlapping peaks), 144.1, 167.9, 168.6, 171.1, 173.2; HRESI MS m/z 415.1854 [M + H]$^+$, $C_{18}H_{23}N_8O_4Cl$ + H^+ requires 415.1842.

3(*R*)-[(4(*S*)-(4-Azido-2-hydroxybenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide·HCl (2b). The same procedure as that used to make **2a** was used on **14b** (0.02 g, 0.04 mmol) to give 0.02 g (97% yield) of product as a pale yellow hygroscopic solid. This product was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 3.6 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.2 min when eluted with CH_3CN/H_2O (4:1): $[\alpha]_D$ 2.0 (c 0.9, MeOH); ^{1}H NMR (CD_3OD) δ 2.01−2.12 (m, 1H), 2.24−2.33 (m, 1H), 2.39−2.48 (m, 1H), 2.79−2.89 (m, 1H), 3.46−3.74 (m, 4H), 4.00 (d, 2H, J = 3.6 Hz), 4.44 (t, 1H, J = 7.5 Hz), 4.62 (t, 1H, J = 9.3 Hz), 4.74 (t, 1H, J = 6 Hz), 6.59 (dd, 1H, J = 2.1 and 8.4 Hz), 6.63 (d, 1H, J = 2.1 Hz), 7.80 (d, 1H, J = 8.4 Hz); ^{13}C NMR (CD_3OD) δ 25.4, 34.6, 45.1, 45.5, 49.2, 50.3, 51.5, 59.3, 106.9, 110.0, 112.3, 130.0, 146.1, 161.5, 168.5, 169.2, 171.2, 173.3; HRESI MS m/z 431.1796 [M]$^+$, $C_{18}H_{23}N_8O_5$ requires 431.1791.

3(*R*)-[(4(*S*)-(4-Azido-2-hydroxy-5-iodobenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide·HCl (2c). The same procedure as that used to make **2a** was used on **14c** (0.02 g, 0.04 mmols) to give 14 mg (78% yield) of **2c** as a

pale yellow hygroscopic solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 3.48 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.08 min when eluted with CH_3CN/H_2O (3:2): $[\alpha]_D$ 2.0 (*c* 0.9, MeOH) ^{1}H NMR (CD_3OD) δ 2.00−2.14 (m, 1H), 2.30−2.48 (m, 2H), 2.79−2.89 (m, 1H), 3.47−3.56 (m, 3H), 3.69 (dd, 1H, *J* = 6.6 and 12 Hz), 3.96 (d, 1H, *J* = 17.1 Hz), 4.05 (d, 1H, *J* = 16.8 Hz), 4.44 (dd, 1H, *J* = 7.2 and 9 Hz), 4.60 (t, 1H, *J* = 9.6 Hz), 4.73 (t, 1H, *J* = 5.4 Hz), 6.83 (d, 1H, *J* = 8.7 Hz), 7.80 (d, 1H, *J* = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 25.3, 34.3, 45.0, 45.5, 49.5, 50.1, 51.5, 59.1, 79.0, 108.8, 110.4, 128.2, 148.2, 162.4, 168.6, 170.1, 171.1, 173.3; HRESI MS *m*/*z* 577.0777 $[M]^+$ and 579.0599 $[M+Na]^+$, $C_{18}H_{22}N_8O_5I^+$ requires 577.0752 and $C_{18}H_{22}N_8O_5I + Na^+$ requires 579.0641.

3(*R*)-[(4(*R*)-(4-Azidobenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)-amino]-2-oxo-1-pyrrolidineacetamide·HCl (3a). The same procedure as that used to make **2a** was used on **15a** (0.04 g, 0.07 mmol) to give 0.032 g (96% yield) of **3a** as a pale yellow hygroscopic solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 7.25 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.6 min when eluted with CH_3CN/H_2O (4:1): $[\alpha]_D$ 43.2 (*c* 1.6, MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 2.02−2.15 (m, 1H), 2.41−2.56 (m, 2H), 2.59−2.68 (m, 1H), 3.48−3.54 (m, 3H), 3.73−3.79 (m, 2H) 4.00 (d, *J* = 4.2 Hz), 4.59−4.67 (m, 3H), 7.15 (d, 2H, *J* = 8.4 Hz), 7.94 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR (CD_3OD) δ 25.5, 35.2, 45.2, 45.6, 50.0, 50.3, 51.4, 59.5, 118.8, 129.3 (two overlapping peaks), 130.0 (two overlapping peaks), 144.1, 168.0, 168.3, 171.1, 173.3; HRESI MS *m*/*z* 437.1650 $[M + Na]^+$, $C_{18}H_{23}N_8O_4Cl + Na^+$ requires 437.1662.

3(*R*)-[(4(*R*)-(4-Azido-2-hydroxybenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide·HCl (3b). The same procedure as that used to make **2a** was used on **15b** (0.02 g, 0.04 mmol) to give 0.02 g (85% yield) of **3b** as a pale yellow hygroscopic solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 3.23 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.07 min when eluted with CH_3CN/H_2O (4:1): $[\alpha]_D$ 46.1 (*c* 0.98, MeOH); ^{1}H NMR (CD_3OD) δ 2.01−2.14 (m, 1H), 2.42−2.68 (m, 3H), 3.47−3.79 (m, 4H), 4.00 (d, 2H, *J* = 3.3 Hz), 4.59−4.67 (m, 3H), 6.61 (dd, 1H, *J* = 2.3 and 8.9 Hz), 6.64 (d, 1H, *J* = 2.4 Hz), 7.88 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR (CD_3OD) δ 25.4, 35.2, 45.1, 45.6, 49.7, 50.2, 51.4, 59.6, 107.0, 110.0, 112.2, 130.0, 146.1, 161.7, 168.4, 169.6, 173.4 (two overlapping peaks); HRESI MS *m*/*z* 431.1838 $[M]^+$, $C_{18}H_{23}N_8O_5^+$ requires 431.1791.

3(*R*)-[(4(*R*)-(4-Azido-2-hydroxy-5-iodobenzoyl)amino-2(*S*)-pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide·HCl (3c). The same procedure as that used to make **2a** was used on **15c** (0.02 g, 0.04 mmol) to afford 14 mg (78% yield) of **3c** as a pale yellow hygroscopic solid. This material was shown to be pure by analytical HPLC analysis using Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column. The product was eluted with CH_3CN/H_2O (4:1) with t_R = 3.08 min. On a Supelcosil LSCi (4.6 mm × 25 cm) analytical column, the product had a t_R = 3.55 when eluted with $CHCl_3$/MeOH (4:1): $[\alpha]_D$ 46.3 (*c* 0.98, MeOH); ^{1}H NMR (CD_3OD) δ 2.01−2.15 (m, 1H), 2.42−2.68 (m, 3H), 3.48−3.56 (m, 3H), 3.76 (dd, 1H, *J* = 6.9 and 12 Hz), 4.01 (d, 2H, *J* = 3.3 Hz), 4.59−4.67 (m, 3H, 3-CH), 6.79 (s, 1H), 8.26 (s, 1H), 8.81 and 8.88 (2d, 1H, *J* = 5.7 and 7.8 Hz); ^{13}C NMR (CD_3OD) δ 25.5, 35.1, 45.2, 45.6, 49.8, 50.1, 51.4, 59.6, 74.0, 106.9, 114.5, 139.2, 147.2, 161.4, 168.2 (two overlapping peaks), 171.1, 173.3; HRESI MS *m*/*z* 557.0755 $[M]^+$ and 579.0587 $[M + Na]^+$, $C_{18}H_{22}N_8O_5I^+$ requires 557.0752 and $C_{18}H_{22}N_8O_5I + Na^+$ requires 579.0641.

***N*-[((2-Benzyloxycarbonylamino)ethylcarbamoyl)methyl]-3(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]pyrrolidin-2-one (18).** Compound **16**[22] (0.87 g, 3.37 mmol) was dissolved in dry THF and the solution was placed under Ar. The reaction mixture was cooled to −23 °C, and then *N*-methylpiperidine (0.39 g, 3.37 mmol) was added dropwise followed by isobutyl chloroformate (0.46 g, 3.37 mmol), upon which the solution became cloudy. After 10 min, HOBt was added and after another 10 min, a solution of **17**[28,29] (0.78 g, 3.37 mmol) and *N*-methylpiperidine (0.39 g, 3.37 mmol) in THF was added. The reaction was stirred at −23 °C initially and then allowed to warm to room temperature as stirring was continued overnight. The reaction mixture was concentrated and the residue that remained was partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was separated and washed with 10% citric acid, H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a white solid. This solid was dissolved in EtOAc and then precipitated with petroleum ether (bp 60−70 °C) to afford 1.31 g (90%) of **18** as a white solid: mp 157−159 °C; $[\alpha]_D$ 32.7 (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$, COSY assignment) δ 1.32 (s, 9H), 1.94−2.02 (m, 1H), 2.28−2.38 (m, 1H), 3.18−3.33 (m, 6H), 3.48 (d, 1H, *J* = 16.5 Hz), 3.96−4.04 (m, 1H), 4.21 (d, 1H, *J* = 16.5 Hz), 5.00 (s, 2H), 5.86 (br s, 1H), 5.99 (d, 1H, *J* = 6.9 Hz), 7.25 (s, 5H), 7.45 (br s, 1H); ^{13}C NMR ($CDCl_3$, gHMQC assignment) δ 26.0, 28.5, 40.3, 40.8, 45.2, 47.2, 52.0, 66.9, 128.3 (two overlapping peaks), 128.7 (two overlapping peaks), 136.7 (two overlapping peaks), 156.2, 157.3, 168.4, 173.3; ESI MS *m*/*z* 457.2 $[M + Na]^+$. Anal. ($C_{21}H_{30}N_4O_6$) C, H, N.

3(*R*)-[(2(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidinylcarbonyl)amino]-2-oxo-1-(*N*-(*N*′-benzyloxycarbonylamino)ethyl)pyrrolidineacetamide (19). Compound **18** (1.0 g, 2.30 mmol) was dissolved in 20 mL of dry CH_2Cl_2, and TFA (3.94 g, 34.5 mmol) was added dropwise to this solution. The solution was stirred at room temperature overnight. The reaction was concentrated and the concentrate was azeotroped with CH_2Cl_2 (3×) and Et_2O (1×) to afford a white solid that was taken on without any further purification.

The TFA salt of deprotected **18** (1.03 g, 2.30 mmol), Boc-Pro-OH (0.57 g, 2.30 mmol), Et_2N (0.80 g, 7.90 mmol), and 2-chloro-1-methyl pyridinium iodide (0.63 g, 2.48 mmol) were dissolved in 70 mL of dry CH_2Cl_2. This solution was then heated at reflux overnight. After 24 h, the reaction solution was washed with 10% citric acid, 1 M $NaHCO_3$, H_2O, and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to a pale pink solid. The crude material was purified by silica gel column chromatography (4.5 × 50 cm) eluting initially with $CHCl_3$ followed by $CHCl_3$/MeOH (20:1) to afford 0.90 g (79%) of **19** as a white foam: mp 68−70 °C; $[\alpha]_D$ −16.3 (*c* 0.9, MeOH); ^{1}H NMR (MeOH, gCOSY assignment, rotamers present) δ 1.41 (s, 9H), 1.76−2.15 (m, 5H), 2.39−2.40 (m, 1H), 3.24−3.45 (m, 8H), 3.76 (d, 1H, *J* = 16.2 Hz), 4.06 (d, 1H, *J* = 16.8 Hz), 4.12−4.17 (m, 1H), 4.31 (t, 1H, *J* = 9 Hz), 5.03−5.10 (m, 2H), 7.26−7.31 (m, 5H); ^{13}C NMR (MeOH, gHMQC assignment, rotamers present) δ 23.4, 24.1, 25.3, 26.8, 27.5, 28.3, 30.3, 31.2, 39.6, 40.0, 45.2, 46.1, 46.7. 47.1, 51.1, 51.3, 60.3, 60.6, 66.3, 80.1, 80.4, 127.8, 127.9, 128.3, 137.1, 154.8, 155.3, 157.7, 169.1, 173.4, 173.6, 174.4, 174.9; ESI MS *m*/*z* 554.3 $[M + Na]^+$. Anal. ($C_{26}H_{37}N_5O_7$) C, H, N.

3(*R*)-[(2(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azidobenzoyl)amino)-ethyl (20a). In a Parr bottle, compound **19** (0.20 g, 0.38 mmol) was dissolved in MeOH (20 mL). The Parr bottle was purged with Ar, and 10% Pd/C (30 mg) was added. The reaction vessel was placed on a Parr shaker at 50 psi for 24 h. The reaction mixture was filtered through a pad of Celite and the pad was rinsed with MeOH. The filtrate was concentrated to a white foam which was triturated with Et_2O to afford 0.15 g (100%) of 3(*R*)-[(2(*S*)-1-(*tert*-butoxycarbonyl)pyrrolidinylcarbonyl)amino]-2-oxo-1-(*N*-(*N*′-amino)ethyl)pyrrolidineacetamide as a white foam that was taken on without further purification. TLC R_f = 0.47 (*n*-butanol/H_2O/pyridine/AcOH, 4:1:1:1).

The above material (0.063 g, 0.16 mmol) was placed under Ar and then dissolved in 20 mL of CH_3CN. *N*-Methylpiperidine (0.016 g, 0.16 mmol) was added. This was followed by the addition of **13a** (0.04 g, 0.16 mmol). The reaction was heated at reflux for 18 h. The reaction was allowed to cool to room temperature, after

which time the CH_3CN was removed in vacuo. The remaining residue was dissolved in CH_2Cl_2 and this solution then washed with H_2O, dilute $NaHCO_3$, H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to yield a pale yellow foam that was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford 0.06 g (69%) of product as a pale yellow foam. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 3.77 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 3.05 min when eluted with CH_3CN/H_2O (99.5:0.5): $[\alpha]_D$ −20.9 (*c* 0.61, MeOH); ^{1}H NMR (CD_3OD, gCOSY assignment) δ 1.44 (s, 9H), 1.78−2.21 (m, 5H), 2.43−2.49 (m, 1H), 3.30−3.52 (m, 8H), 3.76−3.87 (m, 1H), 4,04−4.16 (m, 2H), 4.40 (t, 1H, *J* = 8.7 Hz), 7.13 (d, 2H, *J* = 8.7 Hz), 7.85 (d, 2H, *J* = 8.9 Hz); ^{13}C NMR (CD_3OD) δ 23.5, 25.5, 27.6, 31.3, 39.5, 45.3, 46.3, 46.8, 51.1, 60.7, 80.5, 118.8, 129.1 (two overlapping peaks), 130.9 (two overlapping peaks), 143.6, 154.8, 168.2, 169.2, 173.4, 174.7; IR (neat) 2123 cm^{-1} (N_3); HRESI MS *m*/*z* 565.2525 $[M + Na]^+$, $C_{25}H_{34}N_8O_6 + Na^+$ requires 565.2500.

3(*R*)-[(2(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azido-2-hydroxybenzoyl)amino)ethyl (20b). Compound **19** (0.07 g, 0.13 mmol) was treated in the same way as described above for the synthesis of **20a**, except no *N*-methylpiperidine was added to the reaction and **13b** (0.04 g, 0.14 mmol) was used as the acylating reagent. The crude product was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to give the product in a yield of 0.04 g (57%) as a pale yellow foam: $[\alpha]_D$ −14.6 (*c* 1.95 MeOH); ^{1}H NMR (CD_3OD) δ 1.43 (s, 9H), 1.80−2.24 (m, 5H), 2.38 (m, 1H), 3.35−3.55 (m, 8H), 3.75−3.85 (m, 1H), 4.05−4.16 (m, 2H), 4.32−4.37 (m, 1H), 6.54−6.57 (m, 1H), 6.59 (d, 1H, *J* = 2.4 Hz), 7.74 (d, 1H, *J* = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 23.6, 25.4, 27.6, 31.3, 38.8, 39.2, 45.3, 46.2, 46.8, 51.2, 60.6, 80.5, 107.0, 109.8, 112.5, 129.3, 145.5, 154.8, 161.7, 169.2, 169.5, 173.4, 174.6; IR (Nujol mull) 2113 cm^{-1} (N_3); HRESI MS *m*/*z* 581.2485 $[M + Na]^+$, $C_{25}H_{34}N_8O_7 + Na^+$ requires 581.2448. Anal. ($C_{25}H_{34}N_8O_7$) C, H, N.

3(*R*)-[(2(*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azido-2-hydroxy-5-iodobenzoyl)amino)ethyl (20c). Compound **19** (0.13 g, 0.25 mmol) was treated in the same way as described above for the synthesis of **20a**, except no *N*-methylpiperidine was added to the reaction and **13c** (0.10 g, 0.28 mmol) was used as the acylating reagent. The product was purified by silica gel column chromatography (1.5 × 51 cm) eluting initially with CH_2Cl_2/MeOH (20:1) followed by CH_2Cl_2/MeOH (10:1) to afford 0.10 g (59%) of **20c** as a pale yellow foam. This product was shown to be pure by analytical HPLC analysis with a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (99.9:0.1) with a t_R = 4.38 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 9.58 min when eluted with CH_3CN/H_2O (99.5:0.5): $[\alpha]_D$ −21.3 (*c* 3.2 MeOH); ^{1}H NMR (CD_3OD) 1.43 (s, 9H), 1.80−2.24 (m, 5H), 2.39−2.50 (m, 1H), 3.40−3.54 (m, 8H), 3.79−3.85 (m, 1H), 4.06−4.16 (m, 2H), 4.32−4.37 (m, 1H), 6.75 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (CD_3OD) δ 23.6, 25.4, 27.6, 31.4, 38.8, 39.2, 45.3, 46.2, 46.8, 51.2, 60.7, 73.5, 80.5, 107.0, 114.9, 138.9, 146.6, 154.8, 161.9, 168.2, 169.2, 173.3, 174.6; IR (Nujol mull) 2107 cm^{-1} (N_3); HRESI MS *m*/*z* 707.1496 $[M + Na]^+$, $C_{25}H_{33}N_8O_7I + Na^+$ requires 707.1415.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azidobenzoyl)aminoethyl·HCl (4a). The same procedure as that used to make **2a** was used on **20a** (0.06 g, 0.11 mmol) to afford 0.05 g (94%) of **4a** as a pale yellow solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with t_R = 7.04 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 11.22 min when eluted with CH_3CN/H_2O (3:2): $[\alpha]_D$ 23.7 (*c* 0.83, MeOH); ^{1}H NMR (CD_3OD) δ 2.04 (br m, 4H), 2.41−2.46 (m, 2H), 3.29−3.53 (m, 8H), 3.91 and 4.05 (2d, 2H, *J* = 16.8 and 17.1 Hz), 4.29 (br m, 1H), 4.54 (t, 1H, *J* = 9.5 Hz), 7.15 (d, 2H, *J* = 9 Hz), 7.86 (d, 2H, *J* = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 23.9, 25.3, 29.7,. 39.1, 39.3, 45.2, 46.1, 46.3, 51.4, 70.0, 118.9, 129.1, 130.9, 143.8, 168.4, 168.9, 169.0, 173.4; HRESI MS *m*/*z* 465.1972 $[M]^+$, $C_{20}H_{27}N_8O_4^+$ requires 465.1975.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azido-2-hydroxybenzoyl)aminoethyl·HCl (4b). The same procedure as that used to make **2a** was used on **20b** (0.02 g, 0.04 mmol) to afford 0.02 g (90%) of **4b** as a pale yellow solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with a t_R = 3.4 min. On a Hilbar-II LiChrosorb (4.6 × 260 mm) analytical column, the product had a t_R = 11.25 min when eluted with CH_3CN/H_2O (3:2): $[\alpha]_D$ 20.9 (*c* 1.23, MeOH); ^{1}H NMR (CD_3OD) δ 1.96−2.11 (m, 4H), 2.35−2.50 (m, 2H), 3.42−3.94 (m, 8H), 3.92 (d, 1H, *J* = 16.8 Hz), 3.92 (d, 1H, *J* = 16.8 Hz), 4.04 (dd, 1H, *J* = 6.5 and 8.3 Hz), 4.53 (t, 1H, *J* = 9.3 Hz), 6.57 (dd, 1H, *J* = 2.1 and 10.2 Hz), 6.61 (d, 1H, *J* = 2.4 Hz), 7.78 (d, 1H, *J* = 8.7 Hz); ^{13}C NMR (CD_3OD) δ 23.9, 25.3, 29.7, 38.8, 39.0, 45.1, 46.1, 46.4, 51.4, 60.0, 107.0, 109.9, 112.7, 129.6, 145.6, 161.6, 169.0, 169.1, 169.6, 173.4; HRESI MS *m*/*z* 459.2180 $[M]^+$, $C_{20}H_{27}N_8O_5^+$ requires 459.2104.

3(*R*)-[(2(*S*)-Pyrrolidinylcarbonyl)amino]-2-oxo-1-pyrrolidineacetamide *N*-(*N*′-(4-Azido-2-hydroxy-5-iodobenzoyl)aminoethyl·HCl (4c). The same procedure as that used to make **2a** was used on **20**c (0.03 g, 0.04 mmol) to give **4c** (23 mg, 92%) as a yellow solid. This material was shown to be pure by analytical HPLC analysis using a Supelcosil LSCi (4.6 mm × 25 cm) analytical column. The product was eluted with $CHCl_3$/MeOH (4:1) with a t_R = 3.37 min. On a Hilbar-II LiChrosor (4.6 × 260 mm) analytical column, the product had a t_R = 6.87 min when eluted with CH_3CN/H_2O (4:1): $[\alpha]_D$ 10.5 (*c* 1.15, MeOH); ^{1}H NMR (CD_3OD) δ 2.00−2.13 (m, 4H), 2.42−2.51 (m, 2H), 3.34−3.55 (m, 8H), 3.90−4.06 (m, 2H), 4.25−4.35 (m, 1H), 4.54 (t, 1H, *J* = 9.5 Hz), 6.77 (s, 1H), 8.18 (s, 1H); ^{13}C NMR (CD_3OD) δ 24.0, 25.4, 29.8, 38.9 (two overlapping peaks), 45.2, 46.1, 46.4, 51.5, 60.0, 73.8, 106.9, 114.8, 139.0, 146.7, 161.4, 168.2, 168.8, 168.9, 173.2; HRESI MS *m*/*z* 585.1092 $[M]^+$, $C_{20}H_{26}N_8O_5I^+$ requires 585.1065.

Pharmacological Assays. The photoaffinity-labeling agents were tested for their ability to increase the binding of the dopamine receptor agonist [^{3}H]-*N*-propylnorapomorphine ([^{3}H]NPA) to dopamine D_2 receptors from bovine striatal tissue as described by Srivastava et al.[2]

In the experiment in which the photoaffinity-labeling agents **2b**, **2c**, **4b**, and **4c** first were incubated with the bovine striatal membrane dopamine D_2 receptor preparation and this incubation mixture then subjected to photolysis, the following protocol was followed. Bovine striatal membranes (200 μg) were placed in glass test tubes in 1 mL of receptor binding buffer (50 mM Tris, 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl, 0.1 mM DTT, 0.1 mM PMSF, 100 μg/mL bacitracin, 5 μg/mL soybean trypsin inhibitor 1 at pH 7.4). The preparation was incubated in triplicate with 100 nM of photoaffinity-labeling agent at 25 °C for 1 h. The preparation was then exposed to UV light at a wavelength of 254 nm for 5 min. The contents were transferred from the glass test tube to 1-mL Eppindorf tubes which were then subjected to centrifugation for 30 min at a speed of 16.1 RCF (relative centrifugal field) The supernatant was removed and the pellet was resuspended in receptor binding assay buffer. Protein estimation was done using the Bradford assay reagent. The photoaffinity-labeled membranes (100 μg) were incubated in triplicates in the following groups: (A) control, (B) 1 mM dopamine to define nonspecific binding, (C) 1 μM PLG, and (D) 10 nM **1**. Each assay tube also contained 1 nM [^{3}H]NPA in a final volume of 1 mL. The samples were incubated for 1 h at 25 °C. Assays were terminated by rapid filtration through a Brandel cell harvester and the filters were placed in vials containing 5 mL of scintillation fluid, which were counted by liquid scintillation. The value for sample B was subtracted from the average of the triplicates of

samples A, C, and D to give the specific binding in all tubes. The following formula was used to calculate the percent change in [^{3}H]NPA binding: (A − C or D/A) × 100%. Statistical analysis was carried out with ANOVA and a post hoc Tukey test.

A direct comparison of the binding capability of the UV-cross-linked **4b** and **4c** and non-cross-linked **4b** and **4c** was carried out by incubating 200 μg of membranes with 100 nM of the photoaffinity-labeling ligands **4b** and **4c** in a total volume of 1 mL of receptor binding buffer for 1 h at room temperature. Control membranes were incubated under the same conditions, but without **4b** and **4c**. The membranes with **4b** and **4c** were placed on ice and exposed to UV light, while the control membranes were incubated on ice in the dark. The contents from control and analogue-treated membranes were subjected to centrifugation and protein estimation as described above. Subsequently, control membranes were incubated for 1 h with [^{3}H]NPA plus 100 nM of either **4b** or **4c**, while the analogue-treated membranes were incubated with just [^{3}H]-NPA. The reaction was terminated by rapid filtration and was worked up as described above. Once the membranes with the photoaffinity agents are irradiated and centrifuged, there is little possibility for free ligand interaction with the membranes; thus, during the second incubation, only [^{3}H]NPA is interacting with irradiated membrane samples.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(30) This NH is from the syn-rotamer that is stabilized by an intermolecular H-bond complex in solution. Support for this is seen from ^{1}H NMR experiments in which D_2O was added to the $CDCl_3$ sample and the peaks at 5.89−5.99 and 6.53 disappeared, indicating exchangeable protons. The ^{1}H NMR spectrum of the methyl ester did not possess the NH peak at 6.53, which is in agreement with the intermolecular H-bond complex, as it could not form with the methyl ester structure. For examples of this phenomenon, see: Marcovici-Mizrahi, D.; Gottlieb, H. E.; Marks, V.; Nudelman, A. On the Stabilization of the Syn-Rotamer of Amino Acid Carbamate Derivatives by Hydrogen Bonding. *J. Org. Chem.* **1996**, *61*, 8402−8406.

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