Notes

Synthesis and Binding Properties of 2-Amino-5-phosphono-3-pentenoic Acid Photoaffinity Ligands as Probes for the Glutamate Recognition Site of the NMDA Receptor

Roland Heckendorn,* Hans Allgeier, Jaqueline Baud, Wilfried Gunzenhauser, and Christof Angst

Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland

Received April 30, 1993•

Four ω-benzoylated (*E*)-2,10-diamino-4-(phosphonomethyl)dec-3-enoic acids have been synthesized and tested *in vitro* for binding affinity to the glutamate recognition site of the NMDA (*N*-methyl-D-aspartate) receptor. The ω-4-azidosalicylamide derivative **24** was iodinated to give the photoaffinity ligand **25** (CGP 55802 A) which showed an IC_{50} value of 34 nM in the *in vitro* [^{3}H]CGP 39653 binding assay. This compound and its radioactive ^{125}I-form are the first photoaffinity ligands for the NMDA receptor with high affinity to the glutamate recognition site.

Introduction

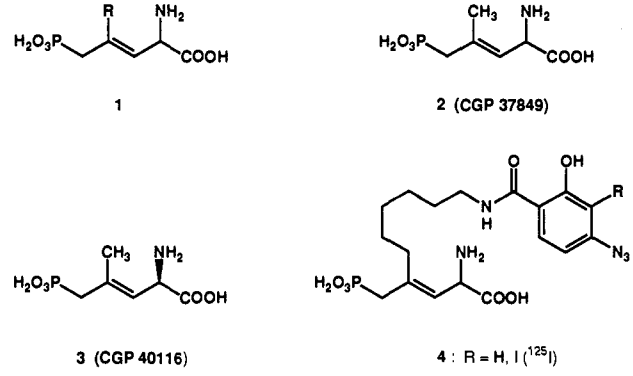
Excitatory amino acid receptors have been the object of intensive pharmacological investigations over the past few years.[1-4] A major part of this research focused on the *N*-methyl-D-aspartate (NMDA) type of these glutamate receptors which has been implicated in normal neuronal functioning including excitatory synaptic transmission, regulation of neuronal plasticity, and learning as well as in the pathologies of acute and chronic diseases of the central nervous system such as epilepsies, Parkinson's disease, and neurodegeneration following a stroke. Due to this important physiological role, the NMDA receptor complex has become one of the pharmacologically best characterized excitatory amino acid receptors.

Attempts to isolate and purify the receptor protein involving affinity chromatography[5] or photoaffinity labeling of the associated phencyclidine recognition site[6,7] have met with limited success. Recently, however, substantial progress in the molecular characterization of the NMDA receptor has been achieved. Several cDNA's coding for subunits of this receptor have been cloned, yet the native structural compositions are still unknown.[8-11]

In our laboratories we have synthesized a series of competitive NMDA antagonists[12] of type 1 which exhibit high *in vitro* affinity for the NMDA receptor in the [^{3}H]-CGP 39653 binding assay.[13] The (*E*)-2-amino-5-phosphono-3-pentenoic acids **2** (CGP 37849, Chart I) and its active *R*-enantiomer **3** (CGP 40116) are representatives of the most potent members of this series and also show pronounced anticonvulsant activity in mice and rats after oral administration[14] and neuroprotective activity in a stroke model in rats.[15] Thus, they are promising candidates for the treatment of epilepsies and brain ischemia.

Structure–activity relationship studies in this series of compounds have revealed a window for structural variations in the substituent R at the 4-position of the parent molecule 1. This observation offered the opportunity to design and synthesize a photoaffinity ligand for the glutamate recognition site of the NMDA receptor. Such a ligand, carrying a radioactive isotope, could be an important new tool to gain deeper insights into the molecular composition of the NMDA heterooligomer receptor complex *in situ*. Therefore, we decided to synthesize compounds of type **4** carrying a photoreactive aromatic azido group. A definite advantage of this well known type of photolabel[16] is the chance to introduce radioactive iodine at the ultimate step of the synthesis.

Chart I



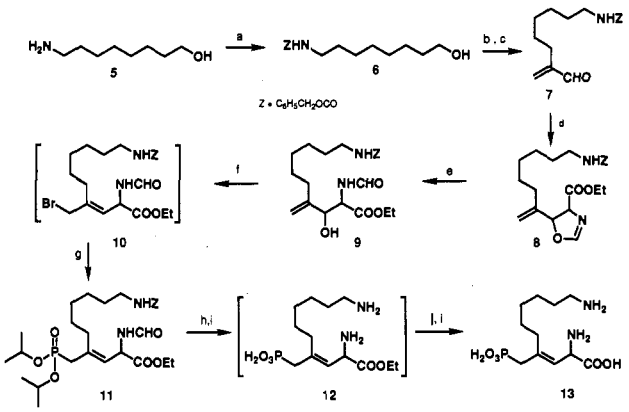
Chemistry

The primary synthetic goal for the envisaged ligands of type **4** was the preparation of the intermediate phosphono amino acid **13** (Scheme I). Starting with 8-aminooctan-1-ol (**5**) we prepared the *N*-benzyloxycarbonyl-protected alcohol **6**, which upon Swern-type oxidation and reaction with formaldehyde provided the labile α-methylene aldehyde **7**. On the basis of the fundamental work of Schöllkopf et al.[17] we developed a general methodology for the conversion of 2-alkyl-substituted α-methylene aldehydes to β,γ-unsaturated α-amino acids of type **1**.[18] The key step involves a copper(I)-catalyzed cycloaddition of the aldehyde with ethyl isocyanoacetate to form an oxazoline, a reaction which has been previously published by Ito et al.[19] When applied to aldehyde **7**, this procedure afforded a diastereomeric mixture of the oxazoline **8** in moderate yield. Mild hydrolysis gave the allylic alcohol **9**, which upon treatment with thionyl bromide stereoselectively rearranged to the unstable *E*-configurated primary allylic bromide **10**. In order to trap liberated

• Abstract published in *Advance ACS Abstracts*, October 1, 1993.

0022-2623/93/1836-3721$04.00/0

Scheme I[a]



[a]Reagents: (a) $C_6H_5CH_2OCOCl$, acetone, $NaHCO_3$; (b) $(COCl)_2$, DMSO, CH_2Cl_2, Hünig's base; (c) aqueous CH_2O, piperazine, H_2O, AcOH; (d) $CNCH_2COOEt$, Cu_2O, toluene; (e) H_2O, THF; (f) $SOBr_2$, $ClCH_2CH_2Cl$, 1,5-hexadiene; (g) triisopropyl phosphite; (h) $BrSi(CH_3)_3$, CH_2Cl_2; (i) EtOH, propylene oxide; (j) 2 N HCl.

hydrogen bromide, 4.8 equiv of 1,5-hexadiene was added to the reaction mixture. Treatment of **10** with triisopropyl phosphite afforded in an Arbusov-type reaction the phosphonate ester **11**. Simultaneous cleavage of the phosphonoester, the benzyloxycarbonyl, and the formyl groups with trimethylsilyl bromide provided the crude ester **12**, which was hydrolyzed with 2 N hydrochloric acid to the desired phosphono diamino carboxylic acid **13**. A strong nuclear Overhauser effect between H-C(2) and H-C(5) as well as between H-C(3) and the phosphonomethylene group corroborated the *E*-configuration of the double bond.

The next step required the selective benzoylation of the ω-amino group of diamino acid **13**. In order to evaluate the synthetic methodology and to elucidate the structure–activity relationship, we decided to synthesize also the ω-benzoylated model compounds **15** and **18** (Scheme II). Acylation experiments with benzoyl chloride under Schotten–Baumann conditions afforded a three component mixture of α- and ω-acylated and diacylated products. A similar result was obtained from the reaction of **11** with the *N*-hydroxy succinimide ester of 4-azidosalicylic acid[20] (NHS-ASA). However, resorting to the "Fujita-reagent" **14**, which was originally introduced for the chemoselective benzoylation of L-lysine,[21] we were able to preferentially prepare the ω-benzoylated derivative **15** (Scheme II) obtained in 55% yield as the monosodium salt after chromatography on reversed-phase silica gel. Similarly, we synthesized the corresponding *p*-azido reagent **17** which reacted with **13** to give the ω-acylated compound **18** in moderate yield. As a direct approach for the desired salicylamide **24** (Scheme IV) we tried to prepare the "Fujita-derivative" **20** (Scheme III). However, interaction of the hydroxy group with the electrophilic C=S function of the heterocycle prevented the isolation of **20** (the resulting side products are not shown here). Therefore, the novel, stable, and crystalline O-protected coupling reagent **23** was synthesized. Thereby, we took advantage of Wissner's procedure[22] for the generation of acid chlorides under neutral conditions. The O-silylated reagent **23** allowed the direct preparation of the desired ligand **24** (CGP 55425 A) in 28% yield (Scheme IV) as the protecting group was simultaneously cleaved under the reaction conditions (pH 10–11).

For the iodination of ligand **24**, the method of Ji et al.[23] was applied. In agreement with the reported reactivity of α-amino acids toward chloramine-T,[24] the reaction led to a complex mixture of products. Apart from unidentified compounds, it consisted of the two in the aromatic positions 3 and 5 monoiodinated and the 3,5-diiodinated derivatives. Chromatography on reversed-phase silica gel allowed the separation of the pure 3-iodinated compound **25** (CGP 55802 A) in 13% yield. The purity of the ligands **18**, **24**, and **25** was assessed by TLC (see Experimental Section), and the structures were verified by ^{1}H-NMR and FAB mass spectrometry. An important feature in the NMR spectra is the characteristic low-field shift of the ω-CH_2 group induced by the aroyl substituents.

The chemical stability of the azido compounds **18**, **24**, and **25**, all of them obtained as colorless solids upon lyophilization from water, was checked. Practically no change in purity as judged by TLC and ^{1}H-NMR analysis was noticed after storage of **18** and **24** in the dark for 6 months at room temperature and **25** at 5 °C.

The corresponding radioactive ^{125}I-labeled photoaffinity ligand **25** proved to be suitable for the photoaffinity labeling of the NMDA receptor. These studies will be reported in due course.[25]

Receptor Binding

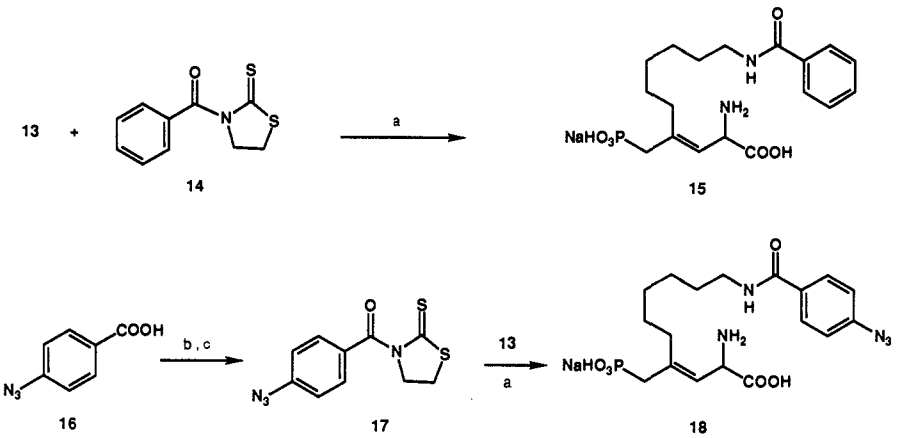
The radioligand [^{3}H]CGP 39653 was used for binding studies with compounds **13**, **15**, **18**, **24**, and **25**. As can be seen from Table I, all of them exhibit potent affinity with IC_{50} values ranging from 34 to 106 nM. Therefore, mainly the "backbone" of the phosphono amino acids seems to be responsible for the receptor binding which is only weakly affected by the presence of the ω-benzoyl residues compared to the diamino precursor **13**.

Experimental Section

Unless noted otherwise, reagents and solvents were obtained from commercial sources and used without further purification. TLC: precoated plates (Merck), silica gel 60 F_{254}. Reversed-phase TLC: precoated plates (Macherey-Nagel), silica gel SIL RP-18 W/UV254. Visualization with UV light, iodine vapor or ninhydrin (for amino acids). Organic extracts were generally dried with Na_2SO_4. Flash chromatography was done on Merck silica gel (230–400 mesh). Reversed-phase column chromatography was performed on silica gel 100 (Fluka) C_{18}-reversed-phase. Melting points were taken in open capillary tubes on a Tottoli apparatus (Büchi) and are uncorrected. IR spectra: Perkin-Elmer-983 spectrometer; principal absorption bands in cm^{-1}. ^{1}H NMR spectra: Varian-Gemini-200 or 300, Bruker-AM-360 or 400 and Varian-Unity-500 spectrometers; δ in ppm relative to internal Me_4Si or HDO signal, coupling constants *J* in hertz. FAB mass spectra: VG Analytical ZAB-HF spectrometer. The intermediate products **7**–**11** were characterized by ^{1}H-NMR spectroscopy and used directly without further purification. All organic azido compounds were protected from light. The experimental procedure for the binding test has been described previously.[13] Abbreviations: FC = flash chromatography; rt = ambient temperature.

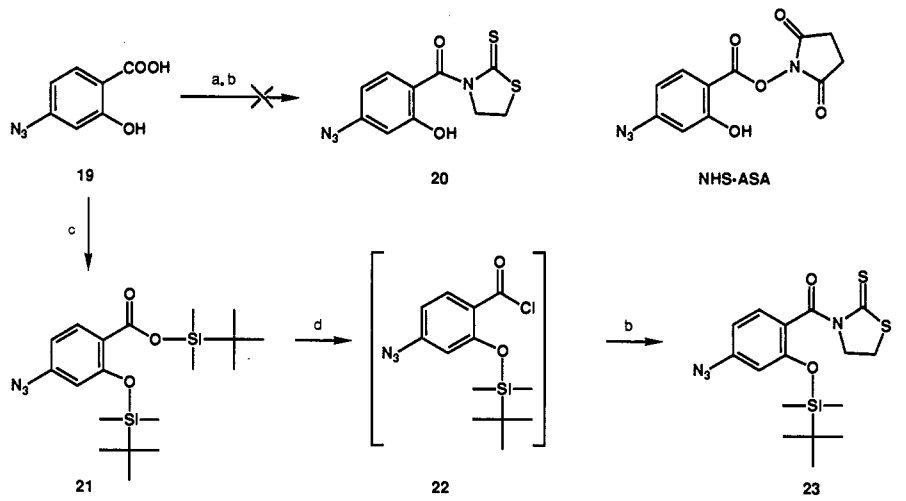
8-[(Benzyloxycarbonyl)amino]octan-1-ol (6). 8-Aminooctan-1-ol (**5**)[26] (30.94 g, 0.213 mol) was dissolved in acetone (3.11 L) and H_2O (1.55 L). $NaHCO_3$ (19.68 g, 0.234 mol) was added, and to the resulting stirred suspension was added dropwise benzyl chloroformate (33 mL, 0.234 mol) during 1 h. After stirring for 21 h at rt acetone was evaporated, and the crystalline precipitate was collected by filtration, washed with H_2O, and dried. Recrystallization from CH_2Cl_2–hexane gave **6** (53.16 g, 89%) as white needles: mp 86–88 °C; R_f (95:5 CH_2Cl_2–MeOH) 0.42; IR (CH_2Cl_2) 3580 (OH), 3410 (NH), 1700 (C=O), 1490, 1210; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.22–1.60 (m, 12H, CH_2-2,3,4,5,6,7), 3.18 (q, *J* = 7.5, 2H, CH_2-8), 3.62 (q, *J* = 5.5, 2H, CH_2-1), 4.72 (br, 1H, NH), 5.10 (s, 2H, $OCH_2C_6H_5$), 7.34 (m, 5H, C_6H_5). Anal. ($C_{16}H_{25}NO_3$) C, H, N.

Scheme II[a]



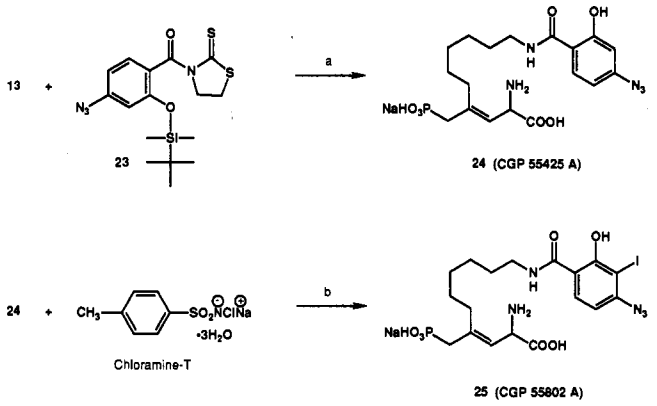
[a]Reagents: (a) 3 equiv of NaOH, H_2O, THF; (b) $SOCl_2$; (c) 1,3-thiazolidine-2-thione, NEt_3, THF.

Scheme III[a]



[a]Reagents: (a) $SOCl_2$; (b) 1,3-thiazolidine-2-thione, NEt_3, THF; (c) *tert*-butyldimethylsilyl chloride, NEt_3, CH_2Cl_2; (d) $(COCl)_2$, CH_2Cl_2, cat. DMF.

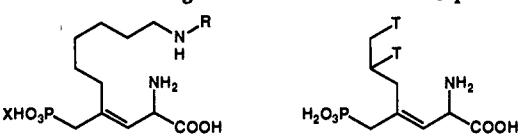
Scheme IV[a]

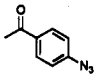
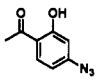
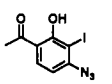


[a]Reagents: (a) 3 equiv of NaOH, H_2O, THF; (b) NaI, H_2O.

8-[(Benzyloxycarbonyl)amino]-2-methyleneoctanal (7). To a solution of oxalyl chloride (7.5 mL, 87.4 mmol) in CH_2Cl_2 (107 mL) was added under argon at −60 °C DMSO (14.1 mL, 199 mmol) in CH_2Cl_2 (20 mL). After 15 min a solution of alcohol **6** (22.21 g, 79.5 mmol) in CH_2Cl_2 (278 mL) and DMSO (5 mL) was added dropwise at −60 to −50 °C. Stirring was continued for 30 min, and then *N*-ethyl diisopropylamine (67.6 mL, 397 mmol) was dropped in at −50 °C. After having been stirred for 1 h at −50 °C, the reaction mixture was allowed to warm up to 0 °C and poured into H_2O (400 mL). The organic layer was washed sequentially with 1 N HCl, 1 N $NaHCO_3$, H_2O, and brine, dried, and evaporated to give the crude aldehyde [22.2 g, R_f (2:1 hexane–EtOAc) 0.50] as a yellowish oil. To this was added under argon a solution of piperazine (3.9 g, 45.3 mmol) in H_2O (50 mL) followed by AcOH (5.1 mL, 89 mmol) and aqueous (36%) formaldehyde

Table I. In Vitro Binding Affinities for NMDA Receptors[a]



compound	X	R	IC_{50}, nM[b]
13	H	H	42
15	Na		106
18	Na		38
24 (CGP 55425 A)	Na		36
25 (CGP 55802 A)	Na		34

[a] Rat forebrain membranes were prepared, and inhibition of [^{3}H]CGP 39653 binding was performed as detailed in ref 13. [b] Values given are the means of two to three determinations; the maximum variance (geometric mean) was 30%.

(6.67 mL, 87.4 mmol). The mixture was stirred and heated rapidly (preheated bath at 120 °C) to reflux for 1 h. The reaction mixture was poured into ice/H_2O and extracted with EtOAc (2 × 100 mL). The organic extracts were washed sequentially with 1 N $NaHCO_3$, H_2O, and brine, dried, and concentrated. The crude product (23.9 g) was purified by FC (2:1 hexane–EtOAc) to yield α-methylene aldehyde **7** (12.65 g, 55%) as a yellow oil: R_f (2:1 hexane–EtOAc) 0.55; IR (film): 3315 (NH), 2980, 1696 (C=O), 1550, 1250; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.15–1.45 (m, 8H, CH_2-4,5,6,7), 2.13 (t, J = 7, 2H, CH_2-3), 3.08 (q, J = 6, 2H, CH_2-8), 4.65 (br, 1H, NH), 5.00 (s, 2H, OCH_2), 5.90 (s, 1H, vinyl), 6.16 (s, 1H, vinyl), 7.25 (m, 5H, C_6H_5), 9.43 (s, 1H, CHO).

Ethyl 5-[8-[(Benzyloxycarbonyl)amino]oct-1-en-2-yl]oxazoline-4-carboxylate (8). To a suspension of Cu_2O (250 mg, catalytic) in toluene (63 mL) was added under argon within 30 min a solution of methylene aldehyde **7** (12.64 g, 43.7 mmol) and ethyl isocyanoacetate (5.54 mL, 43.7 mmol) in toluene (63 mL) at 40 °C. After the slightly exothermic reaction, the mixture was stirred for 2 h at 40 °C. Another portion of ethyl isocyanoacetate (0.55 mL, 5 mmol) was added, and stirring was continued for 1 h at 40 °C. The reaction mixture was cooled, and the insoluble parts were filtered off and washed with toluene. The clear red filtrate was subjected to FC. Elution with 2:1 hexane–EtOAc afforded oxazoline **8** (5.83 g, 33%) as a yellowish oil: R_f (1:1 EtOAc–hexane) 0.40 (tailing spot due to slight hydrolysis on the plate); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.25–1.38 (t and m, 7H, CH_3 and CH_2-5,6), 1.48 (m, 4H, CH_2-4,7), 2.00 (oct, 2H, CH_2-3), 3.18 (q, J = 6, 2H, CH_2-8), 4.25 (m, 2H, OCH_2CH_3), 4.40 (dd, J = 8.4 and 1.2, 1H, H-4), 4.75 (br, 1H, NH), 4.96 (s, 1H vinyl), 5.05–5.13 (s and m, 4H, $OCH_2C_6H_5$, vinyl and H-5), 6.98 (d, J = 1.2, 1H, H-2), 7.27–7.37 (m, 5H, C_6H_5).

Ethyl 10-[(Benzyloxycarbonyl)amino]-2-f(ormylamino)-3-hydroxy-4-methylenedecanoate (9). The oxazoline **8** (5.82 g, 14.5 mmol) was heated in THF (29 mL) and H_2O (14.5 mL) at reflux for 4 h. The reaction mixture was concentrated, and the residue was taken up in CH_2Cl_2. After drying the solvent was evaporated to give allylic alcohol **9** (6.30 g, ca. 100%) as a gum: R_f (2:1 EtOAc–hexane) 0.42 (homogeneous spot); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.21–1.38 (t and m, 7H, CH_3 and CH_2-7,8), 1.48 (m, 4H, CH_2-6,9), 1.93–2.13 (m, 2H, CH_2-5), 2.45 (br, 1H, OH), 3.17 (q, J = 6, 2H, CH_2-10), 4.22 (m, 2H, OCH_2CH_3), 4.62 (br, 1H, NHCOOR), 4.84 (m, 2H, H-2,3), 4.98 (s, 1H, vinyl), 5.07 (s, 2H, $OCH_2C_6H_5$), 5.15 (s, 1H, vinyl), 6.26 (d, J = 8, 1H, NH-formyl), 7.28–7.38 (m, 5H, C_6H_5), 8.19 (s, 1H, HCO). The preparation of **9** starting from **5** was repeated until 25 g were collected.

Ethyl (*E*)-10-[(Benzyloxycarbonyl)amino]-4-(bromomethyl)-2-(formylamino)dec-3-enoate (10) and Ethyl (*E*)-10-[(Benzyloxycarbonyl)amino]-4-[diisopropylphosphonomethyl]-2-(formylamino)dec-3-enoate (11). To a solution of allylic alcohol **9** (13.7 g, 32.6 mmol) in 1,2-dichloroethane was added under argon 1,5-hexadiene (18.5 mL, 156 mmol). The clear yellow solution was cooled to 10 °C, and $SOBr_2$ (6.10 mL, 78.2 mmol) was dropped in at that temperature within 15 min. Stirring was continued for 1 h at 10 °C and then for 1.5 h at rt. The reaction mixture was poured into ice/saturated aqueous $NaHCO_3$, whereby a pH of 8 resulted. The organic layer was separated, and the aqueous phase was extracted once more with CH_2Cl_2. The organic extracts were washed with brine, dried, and evaporated to give crude bromide **10** (27 g, contains volatile side products which were not removed due to instability of **10**): R_f (2:1 EtOAc–hexane) 0.55, side products at the start and 0.07, 0.6, 0.95. To this crude **10** was added triisopropyl phosphite (64.3 mL, 261 mmol), slight vacuum (ca. 0.1 bar) was applied, and the mixture was stirred at 80 °C (bath temperature). The isopropyl bromide formed was collected in a cooling trap (CO_2). After 17 h at 80 °C, excess phosphite was distilled off at 80 °C under high vacuum. The residue (23.4 g) was purified by FC (95:5 EtOAc–MeOH) to give phosphonate **11** (8.73 g, 47% from **9**) as a yellow gum: R_f (95:5 EtOAc–MeOH) 0.37; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.23–1.55 (m, 23 H, CH_3CH_2O, $(CH_3)_2CH$ and CH_2-6,7,8,9), 2.35 (m, 2H, CH_2-5), 2.54 (d, J = 20.5, 2H, CH_2-P), 3.18 (q, J = 6, 2H, CH_2-10), 4.19 (m, 2H, OCH_2CH_3), 4.65 (m, 2H, $HC(CH_3)_2$), 4.93 (br, 1H, NHCOOR), 5.09 (s, 2H, $OCH_2C_6H_5$), 5.30 (m, 2H, H-2, 3), 6.35 (d, J = 6.5, 1H, HNCHO), 7.36 (m, 5H, C_6H_5), 8.15 (s, 1H, HCO).

(*E*)-2,10-Diamino-4-(phosphonomethyl)dec-3-enoic Acid (13). To a solution of phosphonate **11** (8.63 g, 15.2 mmol) in CH_2Cl_2 (22 mL) was added dropwise under argon bromotrimethylsilane (9.8 mL, 75.8 mmol) during 1 h. The yellow solution was stirred at rt for 22 h. Then EtOH (22 mL) was dropped in slowly within 1 h while the temperature was kept at 22 °C. After having been stirred for 22 h at rt the reaction mixture was concentrated. The residue was repeatedly (4 ×) treated with toluene (25 mL) and evaporated to give a yellow foam which was dissolved in EtOH (152 mL). Upon slow addition of a solution of propylene oxide (7.5 mL, 107 mmol) in EtOH (7.5 mL), a crystalline precipitate appeared. After stirring for 16 h at rt the crystals were collected and washed with EtOH and Et_2O. After drying, the product was stirred with H_2O (46 mL) and insoluble parts were removed by filtration through a 1-cm layer of Hyflo. The filtrate was evaporated and repeatedly (3 ×) treated with toluene and evaporated. The residue (4.5 g) was suspended in EtOH (150 mL), and a solution of EtOH saturated with HCl was added until the pH was 2. To the resulting clear solution propylene oxide (7.4 mL) was added dropwise during 2 h. After 16 h at rt the precipitate was filtered and washed with EtOH and Et_2O. The resulting crude diamino-1-ethyl ester **12** (2.95 g) was hydrolyzed with 2 N HCl (50 mL) at reflux under argon during 17 h. The reaction mixture was evaporated, and the residue was treated repeatedly (6 ×) with 3:2 toluene–EtOH and evaporated to give a foam which was dissolved in EtOH (76 mL). Then a solution of propylene oxide (15 mL) in EtOH (15 mL) was added slowly until the pH reached 3. After 2 h at rt the crystals were filtered, washed with EtOH, and dried. The crude product (2.12 g) was purified by reversed-phase chromatography with H_2O as the eluent. The fractions showing the desired homogeneous and ninhydrin-positive spot on TLC were combined and lyophilized from the minimum amount of H_2O to give **13** (1.38 g, 28%) as a white hygroscopic foam: R_f (15:12:10:3 1-propanol–H_2O–pyridine–AcOH) 0.34; R_f (reversed-phase TLC, H_2O) 0.71; ^{1}H NMR (500 MHz, D_2O) δ 1.30–1.55 (m, 6H, CH_2-6,7,8), 1.62 (m, 2H, CH_2-9), 2.19–2.38 (m, 2H, CH_2-5), 2.53 (sextet, 2H, CH_2-P), 2.95 (t, J = 7.5, 2H, CH_2-10), 4.62 (d, J = 9.6, 1H, H-2), 5.32 (q, J = 4, 1H, H-3), strong NOE between H-2 and CH_2-5 as well as between H-3 and CH_2-P; FAB MS (matrix: thioglycerol) m/e 295 (M^+ + 1). Anal. ($C_{11}H_{23}N_2O_6P$·0.5HCl·0.7H_2O) H, Cl, N; C: calcd, 40.63, found, 39.7; P: calcd, 9.53; found, 10.6.

(*E*)-2-Amino-10-(benzoylamino-4-(phosphonomethyl)dec-3-enoic Acid Sodium Salt (15). To a solution of amino acid **13** (188 mg, 0.57 mmol) in H_2O (1.25 mL) and 1 N NaOH (1.25 mL, 1.25 mmol) was added dropwise a solution of 3-benzoyl-1,3-thiazolidine-2-thione (**14**)[21] (127 mg, 0.57 mmol) in THF (1.6 mL) at rt. After the mixture was stirred for 3 h (TLC indicated the presence of ca. 30% starting material) 1 N NaOH (0.53 mL) and reagent **14** (56 mg, 0.25 mmol) were added, and stirring was continued at rt for 3 h (TLC: only traces of **13** visible). The colorless reaction mixture was brought to pH 5 by the addition of 1 N HCl and evaporated until a volume of ca. 5 mL remained. Some insoluble material was removed by filtration, and the aqueous filtrate was purified by reversed-phase chromatography with H_2O as the eluent. The fractions containing the desired ninhydrin-positive product were combined, evaporated, and lyophilized from the minimum amount of H_2O to give **15** (141 mg, 55%) as a colorless foam: R_f (6:1:1 MeOH–H_2O–$CHCl_3$) 0.69; R_f (15:12:10:3 1-propanol–H_2O–pyridine–AcOH) 0.70; R_f (reversed-phase TLC, H_2O) 0.53; ^{1}H NMR (400 MHz, D_2O) δ 1.37–1.64 (m, 8H, CH_2-6,7,8,9), 2.32 (m, 2H, CH_2-5), 3.40 (t, J = 7.5, 2H, CH_2-10), 4.52 (d, J = 10, 1H, H-2), 5.31 (q, J = 5, 1H, H-3), 7.33 (m, 2H, meta to C=O), 7.62 (m, 1H, para to C=O), 7.76 (dd, J = 9 and 1, 2H, ortho to C=O); FAB MS (matrix: phenolsulfonic acid) m/e 399 (M^+ + 1, free acid), 421 (M^+ + 1, sodium salt). Anal. ($C_{16}H_{28}N_2NaO_6$·1.5 H_2O) C, H, N, Na; P: calcd, 6.92; found, 6.3.

3-(4-Azidobenzoyl)-1,3-thiazolidine-2-thione (17). 4-Azidobenzoic acid (**16**)[27] (1.87 g, 11.5 mmol) in $SOCl_2$ (9.4 mL) was stirred and refluxed in the dark under argon for 3 h. The red-brown solution was evaporated, and the oily residue was repeatedly (4 ×) treated with toluene (15 mL) and evaporated to give the crude acid chloride (2.08 g). This was dissolved in THF (93 mL) under argon, solid 1,3-thiazolidine-2-thione (1.37 g, 11.5 mmol) was added, and Et_3N (2.40 mL, 17.2 mmol) was dropped in at rt. The mixture was stirred at 60 °C for 1.5 h, poured into ice/H_2O, and extracted with CH_2Cl_2. The organic

extracts were washed sequentially with cold 1 N HCl, H_2O, and brine, dried, and evaporated to give the crude product. Purification by FC (CH_2Cl_2) and crystallization from Et_2O gave reagent **17** (2.04 g, 67%) as yellow needles: mp 106–109 °C; R_f (CH_2Cl_2) 0.70; IR (CH_2Cl_2) 2120 (N_3), 1690 (C=O), 1600, 1285 (C=S), 1230; ^1H NMR (200 MHz, $CDCl_3$) δ 3.46 (t, J = 7, 2H, CH_2S), 4.52 (t, J = 7, 2H, CH_2N), 7.02 (d, J = 8.5, 2H, ortho to N_3), 7.74 (d, J = 8.5, 2H, ortho to C=O). Anal. ($C_{10}H_8N_4OS_2$) C, H, N, S.

(*E*)-2-Amino-10-[(4-azidobenzoyl)amino]-4-(phosphonomethyl)dec-3-enoic Acid Sodium Salt (18). Amino acid **13** (854 mg, 2.90 mmol) was dissolved in H_2O (10.2 mL), 1 N NaOH (8.7 mL, 8.7 mmol), and THF (13 mL). The solution was stirred at rt, and solid azidobenzoyl reagent **17** (844 mg, 3.19 mmol) was added in one portion protected from light. After 10 min a clear solution resulted, and stirring was continued for 3 h. After addition of 1 N HCl (3.5 mL, pH reached 5) the reaction mixture was evaporated and dried at rt under high vacuum. The solid residue was digested and broken up with $CHCl_3$ (50 mL, removes most of the remaining 1,3-thiazolidine-2-thione). The mixture was filtered and washed copiously with $CHCl_3$. The colorless insoluble material was treated with H_2O (20 mL), stirred for 1 h at rt, and filtered through a pad of Hyflo. The filtrate was evaporated and purified by reversed-phase chromatography with H_2O as the eluent. The fractions containing the desired, ninhydrin-positive product (R_f see below) were combined and lyophilized from the minimum amount of H_2O to give the azidobenzoyl derivative **18** (427 mg, 29%, mixture of monosodium and disodium salt) as a white foam: R_f (6:1:1 MeOH–H_2O–$CHCl_3$) 0.75; R_f (15:12:10:3 1-propanol–H_2O–pyridine–AcOH) 0.79; R_f (reversed-phase TLC, H_2O) 0.26; IR (KBr) 3700–2600 (br, OH, NH as), 2115 (N_3), 1640 (C=O, amide, COO^-), 1605, 1500, 1290, 1050; ^1H NMR (300 MHz, D_2O) δ 1.28–1.68 (m, 8H, CH_2-6,7,8,9), 2.26 (m, 2H, CH_2-5), 2.46 (sextet, 2H, CH_2-P), 3.35 (t, J = 7.5, 2H, CH_2-10), 4.46 (d, J = 9.6, 1H, H-2), 5.37 (q, J = 4, 1H, H-3), 7.15 (d, J = 7.5, 2H ortho to N_3), 7.72 (d, J = 7.5, 2H, ortho to C=O); FAB MS (matrix: thioglycerol) m/e 462 (M^+ + 1, sodium salt). Anal. ($C_{16}H_{24.5}N_5Na_{1.5}O_6P\cdot 2H_2O$) H, N, P; C: calcd, 42.52; found, 42.04; Na: calcd, 6.78; found, 6.18.

***tert*-Butyldimethylsilyl 4-Azido-2-[(*tert*-butyldimethylsilyl)oxy]benzoate (21).** To a solution of 4-azidosalicylic acid (**19**)[20] (5.40 g, 30.1 mmol) in CH_2Cl_2 (120 mL) and Et_3N (16.8 mL, 120.6 mmol) protected from light was added a solution of *tert*-butylchlorodimethylsilane (9.99 g, 66.3 mmol) in CH_2Cl_2 (30 mL) at rt. After the mixture was stirred for 22 h, toluene (500 mL) was added, and the suspension was evaporated until the final volume was ca. 100 mL. The precipitated NEt_3·HCl was filtered off, and the filtrate was evaporated and dried to give the crude silylated intermediate **21** (12.32 g, >100%) as a brown oil: R_f (9:1 CH_2Cl_2–MeOH) 0.94 (tailing spot due to decomposition on the plate); IR (CH_2Cl_2) 2980, 2970, 2120 (N_3), 1705 (C=O), 1600, 1230, 1080, 840 (CH_3-Si); ^1H NMR (200 MHz, $CDCl_3$) δ 0.21 (s, 6H, $OSi(CH_3)_2$), 0.35 (s, 6H, O=$COSi(CH_3)_2$), 1.00 (2 overlapping s, 18H, $(CH_3)_3CSi$), 6.49 (d, J = 1.5, 1H, H-3), 6.65 (dd, J = 1.5 and 8, 1H, H-5), 7.78 (d, J = 8, 1H, H-6). This crude material was used directly in the next step.

3-[4-Azido-2-[(*tert*-butyldimethylsilyl)oxy]benzoyl]-1,3-thiazolidine-2-thione (23). To a solution of crude **21** (12.30 g, contains 30.1 mmol) in CH_2Cl_2 (50 mL) and DMF (5 drops), protected from light, was added dropwise at 0 °C oxalyl chloride (5.20 mL, 60.3 mmol). The resulting clear brown solution was stirred at 0 °C for 3 h then at rt for 17 h and evaporated. The oily residue was treated four times with toluene (20 mL) and concentrated to give the crude acid chloride **22** (9.48 g) as a brownish oil: IR (CH_2Cl_2) 2930, 2120 (N_3), 1750 (COCl), 1600, 1235, 840 (CH_3-Si). This was dissolved in THF (255 mL) under argon, and solid 1,3-thiazolidine-2-thione (3.59 g, 30.1 mmol) was added followed by Et_3N (6.30 mL, 45.2 mmol). The mixture was stirred at 60 °C for 3 h, then poured into ice/H_2O (a pH of 9 resulted), and extracted with Et_2O to give the crude product (13.0 g). Purification by FC (CH_2Cl_2) and two crystallizations from hexane gave reagent **23** (6.21 g, 52%) as yellow needles: mp 77–79 °C; R_f (CH_2Cl_2) 0.65; IR (CH_2Cl_2) 2960, 2930, 2120 (N_3), 1675 (C=O), 1600, 1310, 1230, 1150, 840 (CH_3-Si); ^1H NMR (300 MHz, $CDCl_3$) δ 0.25 (s, 6H, $Si(CH_3)_2$), 0.97 (s, 9H, $(CH_3)_3C$), 3.35 (t, J = 7, 2H, CH_2S), 4.62 (t, J = 7, 2H, CH_2N), 6.35 (d, J = 1.5, 1H, H-3), 6.68 (dd, J = 1.5 and 10, 1H, H-5), 7.29 (d, J = 10, 1H, H-6). Anal. ($C_{16}H_{22}N_4O_2S_2Si$) C, H, N, S, Si.

(*E*)-2-Amino-10-[(4-azido-2-hydroxybenzoyl)amino]-4-(phosphonomethyl)dec-3-enoic Acid Sodium Salt (24, CGP 55425 A). To a solution of the amino acid **13** (1.03 g, 3.5 mmol) in H_2O (10.5 mL) and 1 N NaOH (10.5 mL, 10.5 mmol) was added dropwise within 30 min a solution of reagent **23** (1.52 g, 3.85 mmol) in THF (15.7 mL) protected from light. After the mixture was stirred for 5 h at rt, 1 N NaOH (1.92 mL, 1.92 mmol) was added followed by solid **23** (0.76 g, 1.9 mmol), and stirring was continued in the dark for 18 h. Then 1 N HCl (ca. 6.2 mL) was added until the pH reached 5. Some insoluble material (mostly 1,3-thiazolidine-2-thione) was removed by filtration, and the clear filtrate was evaporated and dried at rt under high vacuum. The solid residue was treated with $CHCl_3$ (30 mL) for 30 min, and the mixture was filtered and washed with $CHCl_3$. The solid product was dissolved in H_2O (5 mL) and purified by reversed-phase chromatography with H_2O as the eluent. The fractions containing the desired, ninhydrin-positive product (R_f see below) were combined and lyophilized from the minimum amount of H_2O to give ligand **24** (528 mg, 28%) as an off-white foam: R_f (6:1:1 MeOH–H_2O–$CHCl_3$) 0.77; R_f (55:40:5:2 H_2O–2-propanol–NH_3 (conc)–AcOH) 0.73; R_f (reversed-phase TLC, H_2O) 0.16; IR (KBr) 3700–2600 (br, OH, NH assoc), 2115 (N_3), 1650–1600 (br, C=O, amide, COO^-), 1275, 1100, 1060, 970; ^1H NMR (400 MHz, D_2O) δ 1.35–1.62 (m, 8H, CH_2-6,7,8, 9), 2.28 (m, 2H, CH_2-5), 2.44 (sextet, 2H, CH_2P), 3.35 (t, J = 7.5, 2H, CH_2-10), 4.47 (d, J = 10, 1H, H-2), 5.27 (q, J = 4, 1H, H-3), 6.52 (m, 2H, ortho and para to OH), 7.73 (d, J = 9.5, 1H, ortho to C=O); FAB MS (matrix: phenolsulfonic acid) m/e 456 (M^+ + 1, free acid). Anal. ($C_{16}H_{25}N_5NaO_7P\cdot 3H_2O$) C, H, N.

(*E*)-2-Amino-10-[(4-azido-2-hydroxy-3-iodobenzoyl)amino]-4-(phosphonomethyl)dec-3-enoic Acid Sodium Salt (25, CGP 55802 A). A solution of ligand **24** (229 mg, 0.48 mmol) and NaI (79 mg, 0.53 mmol) in H_2O (7 mL), protected from light, was cooled to 0 °C, and chloramine-T trihydrate (149 mg, 0.53 mmol) was added. After being stirred for 1 h at 0 °C the reaction mixture was filtered in order to remove most of the formed *p*-toluenesulfonamide. The filtrate was brought to pH 6 by the addition of 0.1 N HCl, concentrated, and purified by reversed-phase chromatography (7.6 g of silica gel 100 C_{18} reversed-phase) with H_2O as the eluent. The first fractions containing some *p*-toluenesulfonamide were discarded. The next fractions showing a homogeneous, ninhydrin-positive spot (R_f see below) were combined and lyophilized from the minimum amount of H_2O to give ortho-iodinated ligand **25** (45 mg, 13%) as a yellowish powder: R_f (6:1:1 MeOH–H_2O–$CHCl_3$) 0.84; R_f (reversed-phase TLC, H_2O) 0.08; IR (KBr) 3700–2600 (br, OH, NH assoc), 2115 (N_3), 1650–1520 (br, C=O, amide, COO^-), 1400, 1305, 1100, 940; ^1H NMR (500 MHz, D_2O) δ 1.30–1.65 (m, 8H, CH_2-6,7,8,9), 2.27 (m, 2H, CH_2-5), 2.42 (d, J = 21.5, 2H, CH_2-P), 3.36 (t, J = 7.5, 2H, CH_2-10), 4.47 (d, J = 10, 1H, H-2), 5.26 (q, J = 4.5, 1H, H-3), 6.53 (d, J = 8.5, 1H, meta to C=O), 7.83 (d, J = 8.5, 1H, ortho to C=O); FAB MS (matrix: phenolsulfonic acid) m/e 582 (M^+ + 1, free acid), 604 (M^+ + 1, sodium salt). Anal. ($C_{16}H_{24}IN_5NaO_7P\cdot 6H_2O$) C, H; N: calcd, 9.84; found, 9.2. The following fractions proved to be mixtures of **25** together with the corresponding 3,5-diiodinated and 5-monoiodinated derivatives.

Acknowledgment. We thank the staff of our Physical and Analytical Department for spectral measurements and elemental analyses. Special thanks are due to Dr. T. Winkler for the NMR and W. Raschdorf for the MS spectra. We are also grateful to Mrs. A. Deville and Ms. M. P. Falcone for manuscript preparation.

References

(1) Kemp, J. A.; Leeson, P. D. The Glycine Site of the NMDA Receptor-Five Years on. *Trends Pharmacol. Sci.* **1993**, *14*, 20–25.
(2) Herrling, P. L. Synaptic Physiology of Excitatory Amino Acids. *Arzneim.-Forsch.* **1992**, *42*, 202–208.
(3) Rogawski, M. A. The NMDA Receptor, NMDA Antagonists and Epilepsy Therapy. *Drugs* **1992**, *44*, 279–292.
(4) Carter, A. J. Glycine Antagonists: Regulation of the NMDA Receptor-Channel Complex by the Strychnine-Insensitive Glycine Site. *Drugs Future* **1992**, *17*, 595–613.

(5) Ly, A. M.; Michaelis, E. K. Solubilization, Partial Purification, and Reconstitution of Glutamate- and N-Methyl-D-aspartate-Activated Cation Channels from Brain Synaptic Membranes. *Biochemistry* **1991**, *30*, 4307–4316.
(6) Ikin, A. F.; Kloog, Y.; Sokolovsky, M. N-Methyl-D-aspartate/Phencyclidine Receptor Complex of Rat Forebrain: Purification and Biochemical Characterization. *Biochemistry* **1990**, *29*, 2290–2295.
(7) Sonders, M. S.; Barmettler, P.; Lee, J. A.; Kitahara, Y.; Keana, J. F. W.; Weber, E. A Novel Photoaffinity Ligand for the Phencyclidine Site of the N-Methyl-D-aspartate Receptor Labels a M_r 120,000 Polypeptide. *J. Biol. Chem.* **1990**, *265*, 6776–6781.
(8) Moriyoshi, K.; Masu, M.; Ishii, T.; Shigemoto, R; Mizuno, N.; Nakanishi, S. Molecular Cloning and Characterization of the Rat NMDA Receptor. *Nature* **1991**, *354*, 31–37.
(9) Kutsuwada, T.; Kashiwabuchi, N.; Mori, H.; Sakimura, K.; Kushiya, E.; Araki, K.; Meguro, H.; Masaki, H.; Kumanishi, T.; Arakawa, M.; Mishina, M. Molecular Diversity of the NMDA Receptor Channel. *Nature* **1992**, *358*, 36–41.
(10) Monyer, H.; Sprengel, R.; Schoepfer, R.; Herb, A.; Higuchi, M.; Lomeli, H.; Burnashev, N.; Sakmann, B.; Seeburg, P. H. Heteromeric NMDA Receptors: Molecular and Functional Distinction of Subtypes. *Science* **1992**, *256*, 1217–1221.
(11) Meguro, H.; Mori, H.; Araki, K.; Kushiya, E.; Kutsuwada, T.; Yamazaki, M.; Kumanishi, T.; Arakawa, M.; Sakimura, K.; Mishina, M. Functional Characterization of a Heteromeric NMDA Receptor Channel Expressed from cloned cDNAs. *Nature* **1992**, *357*, 70–74.
(12) Angst, C.; Allgeier, H.; Heckendorn, R.; Wallach, D. Preparation of Phosphonyl Substituted 2-Aminoalk-3-enoic Acid Derivatives as Pharmaceutical Agents. Eur. Pat. Appl. EP 420806, 1991; *Chem. Abstr.* **1991**, *115*, 29632m.
(13) Sills, M. A.; Fagg, G.; Pozza, M. F.; Angst, C.; Brundish, D. E.; Hurt, S. D.; Wilusz, E. J.; Williams, M. [^{3}H]CGP 39653: A new N-Methyl-D-aspartate Antagonist Radioligand with low Nanomolar Affinity in Rat Brain. *Eur. J. Pharmacol.* **1991**, *192*, 19–24.
(14) Fagg, G. E.; Olpe, H.-R.; Pozza, M. F.; Baud, J.; Steinmann, M.; Schmutz, M.; Portet, C.; Baumann, P.; Thedinga, K.; Bittiger, H.; Allgeier, H.; Heckendorn, R.; Angst, C.; Brundish, D.; Dingwall, J. G. CGP 37849 and CGP 39551: Novel and Potent Competitive N-Methyl-D-aspartate Receptor Antagonists with Oral Activity. *Br. J. Pharmacol.* **1990**, *99*, 791–797.
(15) Sauer, D.; Allegrini, P. R.; Cosenti, A.; Pataki, A.; Amacker, H.; Fagg, G. E. Characterization of the Cerebroprotective Efficacy of the Competitive NMDA Receptor Antagonist CGP 40116 in a Rat Model of Focal Cerebral Ischemia: An in Vivo Magnetic Reconance Imaging Study. *J. Cereb. Blood Flow Metab.* **1993**, *13*, 595–602.
(16) Ji, T. H.; Ji, I. Macromolecular Photoaffinity Labeling with Radioactive Photoactivable Heterobifunctional Reagents. *Anal. Biochem.* **1982**, *121*, 286-289.
(17) Schöllkopf, U. Recent Applications of α-Metalized Isocyanides in Organic Synthesis. *Angew. Chem.* **1977**, *89*, 351–360.
(18) Allgeier, H.; Angst, C.; Bold, G.; Duthaler, R.; Heckendorn, R.; Togni, A. Preparation of Phosphino- and Phosphonopentenoates as NMDA Antagonists. Eur. Pat. Appl. EP 302826, 1989; *Chem. Abstr.* **1990**, *112*, 139560g.
(19) Ito, Y.; Matsuura, T.; Saegusa, T. $ZnCl_2$ and CuCl Promoted Aldol Reactions of Isocyanoacetate with α,β-Unsaturated Carbonyl Compounds. *Tetrahedron Lett.* **1985**, *26*, 5781–5784.
(20) Dupuis, G. An Asymmetrical Disulfide-containing Photoreactive Heterobifunctional Reagent Designed to Introduce Radioactive Labeling into Biological Receptors. *Can. J. Chem.* **1987**, *65*, 2450–2453.
(21) Nagao, Y.; Miyasaka, T.; Seno, K.; Fujita E.; Shibata, D; Doi, E. Peptide-bond Formation, Chemoselective Acylation of Amino Acids, and Crosslinking Reaction between Amino Acids Utilizing a Functional Five-membered Heterocycle, 1,3-Thiazolidine-2-thione. E. *J. Chem. Soc., Perkin Trans. 1*, **1984**, 2439–2446.
(22) Wissner, A.; Grudzinkas, C. V. Reaction of tert-Butyldimethylsilyl Esters with Oxalyl Chloride-Dimethylformamide: Preparation of Carboxylic Acid Chlorides under Neutral Conditions. *J. Org. Chem.* **1978**, *43*, 3972–3974.
(23) Kometani, T.; Watt, D. S.; Ji, T. Iodination of Phenols Using Chloramine T and Sodium Iodide. *Tetrahedron Lett.* **1985**, *26*, 2043–2046.
(24) The reaction of α-amino acids with chloramine-T in aqueous solution at ambient temperature has been described: Dakin, H.; Cohen, J. B.; Daufresne, M.; Kenyon, J. The Antiseptic Action of Substances of the Chloramine Group. *Proc. R. Soc. London Ser. B* **1916**, *89*, 232–251.
(25) Marti, T.; Benke, D.; Künzi, R.; Heckendorn, R.; Pozza, M. F.; Allgeier, H.; Angst, C.; Laurie, D.; Seeburg, P.; Möhler, H. Molecular Distinction of three NMDA-Receptor Types and Developmental Receptor Maturation Demonstrated with a Novel Photoaffinity Ligand. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(26) Schaeffer, H. J.; Schwender, C. F. Enzyme Inhibitors XXI. Hydrophobic and Hydroxylic Binding Sites of Adenosine Deaminase. *J. Pharm. Sci.* **1967**, *56*, 1586-1590.
(27) Shields, C. J.; Falvey, D. E.; Schuster, G. B.; Buchardt, O.; Nielsen, P. E. Competitive Singlet-Singlet Energy Transfer and Electron Transfer Acticvation of Aryl Azides: Application to Photo-Cross-Linking Experiments. *J. Org. Chem.* **1988**, *53*, 3501–3507.