of which 300 were unobserved with $I < 2.58\sigma(I)$. The intensities were corrected for Lorentz and polarization effects and absorption correction was applied (μ(Mo Kα) = 38.4 cm^{-1}). The final agreement index was $R = 0.032$ for observed reflections.

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Registry No. 4, 32846-66-5; **5b**, 125848-32-0; **6b**, 125848-33-1; (±)-**8a**, 125848-35-3; (±)-**8b**, 125848-34-2; (±)-**9**, 125848-31-9; (±)-**10**, 125848-30-8.

Supplementary Material Available: ORTEP figures and tables of atomic coordinates, bond lengths, angles, and thermal parameters from the X-ray analysis of compounds **8b** and **9**; NMR spectra for compounds **5b**, **6b**, and **9** (19 pages). Ordering information is given on any current masthead page.

Solid-Phase Synthesis of *N*-Methyl- and *N*-Ethylamides of Peptides Using Photolytically Detachable ((3-Nitro-4-((alkylamino)methyl)benzamido)methyl)polystyrene Resin

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A convenient method for the solid-phase synthesis of C-terminal peptide *N*-alkylamides using a photolytically detachable ((3-nitro-4-((alkylamino)methyl)benzamido)methyl)polystyrene support is described. The method involves prior incorporation of an alkylamine moiety into the ((3-nitro-4-(bromomethyl)benzamido)methyl)-polystyrene resin, on which the peptides were assembled and subsequently cleaved in the form of the peptide *N*-alkylamides by photolysis. The *N*-alkylamino group acts as an anchoring function for the peptide as well as a latent reagent function for the C-terminal modification of the attached peptide. The method is particularly useful if the peptide contains Asp or Glu with a benzyl ester side chain protecting group. The synthetic applicability of the method is illustrated with the solid-phase synthesis of *N*-alkylamides of a few model peptides in 70–77% yields and analogues of the luteinizing hormone-releasing hormone in 48–56% yield.

C-Terminal peptide *N*-alkylamides are among the most important classes of biologically active peptides. C-Terminal modification of peptides has been observed to have significant influence on its biological properties as illustrated in the case of the naturally occurring luteinizing hormone-releasing hormone[1,2] (LH-RH). Several *N*-ethyl- and *N*-methylamides of LH-RH and its analogues are reported to be 200–300 times more active and have wide pharmaceutical applications. Peptide *N*-alkylamides are also used for structure-activity relationship studies and conformational studies.[3,4]

The different methods currently in use for the solid-phase synthesis of peptide *N*-alkylamides have several limitations. In the classical solid-phase method, a peptide–resin ester linkage can be cleaved with an alkylamine to get the corresponding *N*-alkylamides.[5,6] This method is not applicable to peptides containing Asp and/or Glu residues in which the additional carboxyl group is protected as the benzyl ester, which will also undergo aminolysis by the amine, resulting in the formation of Asn(*N*-alkylamide) and Glu(*N*-alkylamide). A solid-phase method for the synthesis of peptide *N*-alkylamides has been reported by Kornreich et al. using *N*-(alkylamino)-methyl resins.[7] Recently, a trifluoroacetic acid (TFA) labile anchoring group[8] and an oxime resin[9] have been reported for the solid-phase synthesis of peptide *N*-alkylamides.

The introduction of a photolytically cleavable anchoring linkage between the polymer and the growing peptide chain is one of the promising alternative methods to avoid the rigorous conditions used to obtain the peptides from the supports.[10,11] The photolabile *o*-nitrobenzyl anchoring group has been successfully used for the solid-phase synthesis of peptides.[12-18] In this paper we report a further

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(1) Koning, W.; Gieger, R.; Snadow, J. DOZ, 438 350, Hoechst AG, 1974.
(2) Coy, D. H.; Vilchez-Martinez, J. A.; Coy, E. J.; Schally, A. V. *J. Med. Chem.* **1976**, *19*, 423.
(3) Stimson, E. R.; Meinwald, Y. C.; Montelione, G. T.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1986**, *27*, 569.
(4) Mammi, S.; Goodman, M. *Int. J. Pept. Protein Res.* **1986**, *28*, 29.
(5) Coy, D. H.; Coy, E. J.; Schally, A. V.; Vilchez-Martinez, J. A.; Debeljuk; Carter, W. H.; Arimura, A. *Biochemistry* **1974**, *13*, 323.
(6) Takashima, H.; Fraefel, W.; du Vigneaud, V. *J. Am. Chem. Soc.* **1969**, *91*, 6182.
(7) Kornreich, W.; Anderson, H.; Porter, J.; Vale, W.; Rivier, J. *Int. J. Pept. Protein Res.* **1985**, *25*, 414.
(8) Breipohl, G.; Knolle, J.; Geiger, R. *Tetrahedron Lett.* **1987**, *28*, 5647.
(9) Lobl, T. J.; Maggiora, L. L. *J. Org. Chem.* **1988**, *53*, 1979.
(10) Pillai, V. N. R. *Synthesis* **1980**, 1.
(11) Pillai, V. N. R. *Organic Photochemistry*, Padwa, A., Ed.; Marcel Dekker: New York, 1987; Vol. 9, pp 225-312.
(12) Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 1575.
(13) Rich, D. H.; Gurwara, S. K. *Tetrahedron Lett.* **1975**, 301.
(14) Albericio, F.; Granier, C.; Labbe-Jullie, C.; Seager, M.; Couraud, F.; Van Rietschoten, J. *Tetrahedron* **1984**, *40*, 4313.
(15) Giralt, E.; Eritja, R.; Pedroso, E.; Granier, C.; Van Rietschoten, J. *Tetrahedron* **1986**, *42*, 691.
(16) Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936.
(17) Ajayaghosh, A.; Pillai, V. N. R. *Tetrahedron* **1988**, *44*, 6661.
(18) Ajayaghosh, A.; Pillai, V. N. R. *Indian J. Chem.* **1988**, *27B*, 1004.

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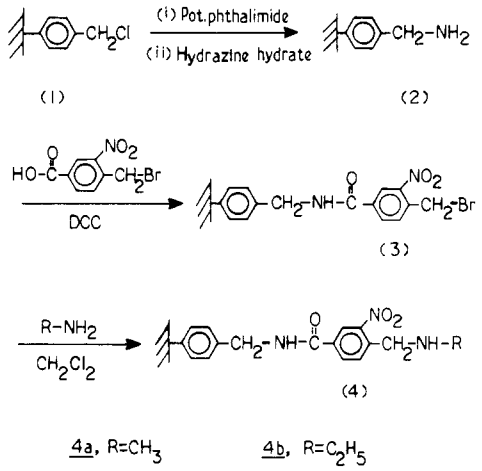
Scheme I. Preparation of ((3-Nitro-4-((alkylamino)methyl)benzamido)methyl)polystyrene Resin



application of *o*-nitrobenzyl anchoring groups for the solid-phase synthesis of peptide *N*-alkylamides.

Results and Discussion

Preparation of ((3-Nitro-4-((alkylamino)methyl)benzamido)methyl)polystyrene Resin. The anchoring group, 3-nitro-4-(bromomethyl)benzoic acid, was prepared from *p*-toluic acid by a two-step reaction.[12] (Aminomethyl)polystyrene resin was prepared from (chloromethyl)polystyrene resin by Gabriel's phthalimide synthesis.[19] The resin contained 0.68 mmol of NH_2/g as determined by the picric acid titration method.[20] Aminomethyl resin on coupling with the 3-nitro-4-(bromomethyl)benzoic acid in the presence of dicyclohexylcarbodiimide (DCC) gave the photolabile ((3-nitro-4-(bromomethyl)benzamido)methyl)polystyrene resin. This resin showed characteristic IR bands at 1350 and 1540 cm^{-1} of the NO_2 group and has a bromine content of 0.56 mmol/g of the resin. The resin (**3**) was suspended in CH_2Cl_2, dry methylamine or ethylamine gas was passed through the suspension at 0 °C, and the reaction mixture was then shaken at room temperature for 10 h. The resulting *N*-alkylamino resin (**4a,b**) showed characteristic IR absorptions at 1350 and 1540 cm^{-1} of the NO_2 group and at 3400 cm^{-1} (broad) of the NH group. The amine content of the resin was estimated by the picric acid method.[20] Formation of tertiary and quaternary ammonium salts during the reaction of resin **3** with the alkylamine was negligible since a large excess of the amine was used. This is evident from a comparison of the Br and NH capacities.

Preliminary Investigations on Resin 4: Preparation of *N*-Alkylamides of Boc-Amino Acids. The ability of the modified support **4** to release the attached carboxyl function into the corresponding *N*-alkylamides was investigated first. Boc-Amino acids were coupled with the resin **4** using the symmetric anhydride method. The acylated resins were suspended in anhydrous methanol or ethanol and irradiated at 350 nm for 12–18 h. The products were isolated from the photolysate and characterized by comparing with an authentic specimen prepared by other methods. The yields and melting points of the *N*-alkylamides are given in Table I.

Synthesis of the *N*-Alkylamides of Model Dipeptides and Tripeptides. The use of the modified supports **4a** and **4b** in peptide synthesis was demonstrated by the synthesis of a few dipeptide and tripeptide *N*-alkylamides. The peptides were assembled on the support by using Boc-amino acid anhydrides. The progress of the coupling was monitored by the semiquantitative ninhydrin test. After the synthesis, the peptides were removed from the supports by photolyzing the peptide resin suspended in mixture of solvents such as EtOH–CH_2Cl_2 or TFE–CH_2Cl_2. Boc-Asn-Gly-NHMe, Boc-Ile-Gly-NHMe, Boc-Leu-Ala-Val-NHMe, and Boc-Leu-Ala-Val-NHEt were obtained from the modified supports in 70–77% yield (Table II). The peptides were characterized by spectral, elemental, and amino acid analysis. The 270-MHz ^1H NMR spectra of Boc-Leu-Ala-Val-NHMe showed a broad singlet around δ 6.68, which is assigned for the NH proton of the CONHMe. This indicates that the attached peptide is released from the resin as its C-terminal *N*-methylamide.

Table I. Synthesis of *N*-Alkylamides of Boc-Amino Acids Using ((3-Nitro-4-((alkylamino)methyl)benzamido)-methyl)polystyrene Resin

no.	amide	yield, %	mp, °C
1	Boc-Ala-$NHCH_3$	74	25
2	Boc-Ala-NHC_2H_5	72	gum
3	Boc-Leu-$NHCH_3$	70	73
4	Boc-Leu-NHC_2H_5	70	gum

Table II. Photolytic Cleavage Yield and Melting Point of Some Boc-Dipeptide and Boc-Tripeptide *N*-Alkylamides

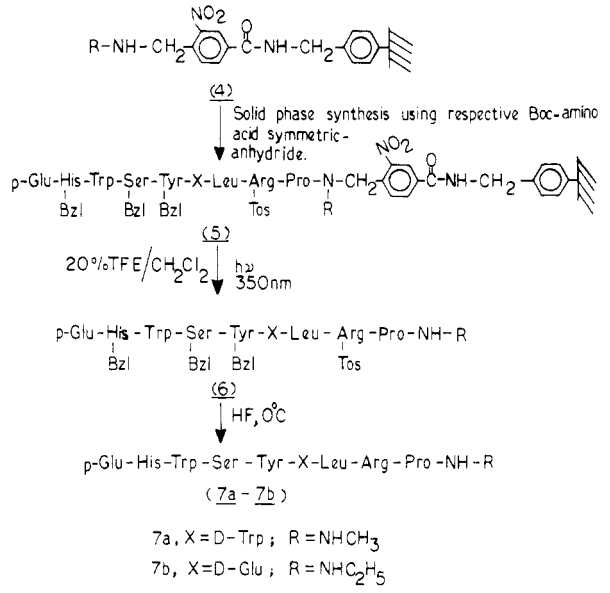
no.	peptide	yield, %	mp, °C
1	Boc-Asn-Gly-NHMe	76	170–172
2	Boc-Ile-Gly-NHMe	70	183–184
3	Boc-Met-Leu-Phe-NHMe	62	oil
4	Boc-Leu-Ala-Val-NHMe	78	188–190
5	Boc-Leu-Ala-Val-NHEt	77	179–180

Stability of the Support under the Peptide Synthetic Conditions. For the synthesis of long peptide sequences, the peptide–resin anchoring linkage should be stable under the various conditions encountered during the synthesis. This is particularly important during the Boc group deprotection step. The peptide–resin linkage should possess enough stability to withstand the repeated acid treatment for the removal of the Boc group. Since in the present study 4 N HCl–dioxane was used for the Boc removal, the stability of the resin was tested under the same acidic conditions, taking Val-N(CH_3)-resin and Val-N(C_2H_5)-resin as the test samples. Aliquots of the resins were separately treated with 4 N HCl–dioxane at definite intervals, and the amount of amino acid remaining on the resin was estimated. There was no loss of amino acid from the resin even after 24 h of treatment with 4 N HCl–dioxane, showing that the linkage between the resin and the first amino acid is stable enough for the repeated synthesis of peptides under the various conditions of the solid-phase method. The peptide–resin linkage was also found to be stable under the conditions of Boc removal using trifluoroacetic acid.

Synthesis of *N*-Alkylamides of LH–RH Analogues. The applicability of the modified support for the solid-phase synthesis of biologically active peptide analogues was illustrated by the photolytic removal of the fully protected nonapeptide *N*-alkylamide of LH–RH (Scheme II). Boc-Pro-N(CH_3)-polymer and Boc-Pro-N(C_2H_5)-polymer were prepared, and the loading was determined by amino group analysis. The remaining amino acid residues were incorporated by using the standard solid-phase procedures. N^{α}-Boc protection was used throughout the synthesis along with suitable selection of side-chain protecting groups. Boc-Amino acids were coupled by the symmetric anhydride method, and the progress of the coupling was mon-

(19) Mitchell, A. R.; Kent, S. B. H.; Erickson, B. W.; Merrifield, R. B. *Tetrahedron Lett.* **1976**, *42*, 3795.
(20) Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248.

Scheme II. Synthesis of *N*-Alkylamides of LH–RH Analogues on ((3-Nitro-4-((alkylamino)methyl)benzamido)methyl)polystyrene Resin



itored by the semiquantitative ninhydrin method. The postcoupling reaction with acetic anhydride was used to ensure complete acylation of the free amino groups. Removal of the Boc group was effected by 4 N HCl–dioxane.

Cleavage of the protected nonapeptide *N*-alkylamide was accomplished by irradiation of a suspension of the peptide–polymer in 20% solution of TFE in CH_2Cl_2 for 18–24 h. The fully protected peptide amides obtained after photolysis were treated with anhydrous HF at 0 °C in the presence of anisole to get the free peptide amides. The crude peptides were purified by chromatography on a Sephadex LH-20 column. This was further purified by HPLC using a Vydac C-18 column. Amino acid analysis of the purified peptides indicated the correct ratio of the amino acids.

The mechanism of the photolytic cleavage of *o*-nitrobenzyl and related systems is well established, and this involves a light-induced internal oxidation–reduction reaction of aromatic nitro compounds containing a carbon–hydrogen bond ortho to the nitro group.[21] This mechanism has already been established in the case of the low molecular weight compounds.[22] The mechanism of the photolytic removal of the peptide *N*-alkylamides from the modified resins is analogous to that of the low molecular weight *o*-nitrobenzyl systems.

The foregoing observations illustrate the applicability of the modified resins as photoremovable polymeric supports for the solid-phase synthesis of peptide *N*-alkylamides. The advantage of the approach is the possibility of obtaining the attached peptide in the C-terminal modified amide form from the support under mild, nondestructive photolytic conditions avoiding the direct interaction of the peptide and the alkylamine as encountered in the classical methods. The *N*-alkylamino group incorporated into the resin **3** prior to the peptide synthesis functions as a *latent reagent* for the conversion of the C-terminal group to the corresponding *N*-alkylamides. This method offers the possibility of obtaining C-terminal *N*-alkylamides of peptides containing Asp or Glu without any damage to the side-chain benzyl ester protection. Photosensitive amino acids such as Trp, His, etc. are not affected under the conditions of the photolytic removal of the peptides from the supports. The stability of such amino acids under the photolytic condition of cleavage of the 2-nitrobenzyl type resins has already been reported.[12] An added advantage of the method is that one is able to obtain fully protected peptide *N*-alkylamides, which can be subsequently used for further applications if needed. In conclusion, the method offers another useful application of ((3-nitro-4-(bromomethyl)benzamido)methyl)polystyrene resin, which is an established photoremovable polymeric support in solid-phase synthesis.

Experimental Section

All the solvents used were distilled and purified according to the literature procedure. Merrifield's chloromethylated polystyrene resin (2% DVB cross-linked, 200–400 mesh, 0.7 mmol of Cl/g) was purchased from Fluka. 3-Nitro-4-(bromomethyl)benzoic acid[12] and (aminomethyl)polystyrene resin were prepared by literature procedures.[18] Boc-Ala-OH, Boc-Leu-OH, Boc-Val-OH, and Boc-Pro-OH were prepared by Schnabel's procedure.[23] All other side-chain-protected Boc-amino acids were obtained from either Sigma or Fluka. Melting points were recorded on a hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on a Pye Unicam SP3-300 spectrometer. HPLC was done on a Waters Associates HPLC system. Photolyses were carried out in an immersion-type photochemical reactor equipped with a Philips HPK 125-W medium-pressure mercury lamp.

Preparation of ((3-Nitro-4-(bromomethyl)benzamido)methyl)polystyrene Resin. To the aminomethyl resin (15 g, 10.2 mmol of NH_2) swelled in DMF (100 mL) was added 3-nitro-4-(bromomethyl)benzoic acid (13.5 g, 52.5 mmol) followed by DCC (10.8 g, 52.5 mmol). The suspension was shaken at room temperature for 12 h. The resin was filtered and washed with MeOH and CH_2Cl_2. A second coupling was performed with 6.8 g of the nitro acid and 5.4 g of DCC in DMF (100 mL) for 3 h. The resin was collected by filtration and washed with DMF (60 mL × 2 × 3 min), MeOH (60 mL × 3 × 3 min), CH_2Cl_2 (100 mL × 3 × 3 min), and finally MeOH (60 mL × 2 × 3 min). The bromine content of the resin was found to be 0.56 mmol/g of the resin by Volhard's method. IR (KBr): 1650 (NHCO), 1340 and 1530 (NO_2) cm^{-1}.

Preparation of ((3-Nitro-4-((methylamino)methyl)benzamido)methyl)polystyrene Resin (4a). The bromomethyl resin **3** (10 g, 0.56 mmol of Br/g) was suspended in CH_2Cl_2 (100 mL) in a 250-mL stoppered bottle and cooled to 0–5 °C. Dry methylamine was bubbled through the suspension for 2 h. The reaction bottle was tightly stoppered and shaken for 12 h at room temperature. The resin was collected by filtration, washed with CH_2Cl_2 (50 mL × 3 × 3 min), THF (50 mL × 2 × 2 min), water (50 mL × 6 × 3 min), and MeOH (40 mL × 3 × 3 min), and dried in vacuo. The resin had a substitution of 0.6 mmol of NH/g as indicated by Gisin's picric acid method. IR (KBr): 1650 (NHCO), 1340 and 1530 (NO_2), 3400 (NH) cm^{-1}.

Preparation of ((3-Nitro-4-((ethylamino)methyl)benzamido)methyl)polystyrene Resin (4b). This resin was prepared from resin **3** by a procedure analogous to that described above using dry ethylamine. The capacity of the resin was found to be 0.54 mmol of NH/g based on picric acid titration. IR (KBr): 1650 (NHCO), 1340 and 1530 (NO_2), 3040 (NH) cm^{-1}.

General Procedure for the Solid-Phase Assembly of Peptides. Resin **4a** or **4b** (2 g) was placed in a silanized glass reaction vessel clamped to a manually operated mechanical shaker. In separate experiments symmetric anhydrides of Boc-amino acids were prepared by reacting 3 equiv of the respective Boc-amino acid in CH_2Cl_2 with 1.5 equiv of DCC for 1 h at 0 °C. The precipitated dicyclohexylurea (DCU) was removed by filtration, and the symmetric anhydride solution was added to the resin. After 3 h of shaking, the resin was collected by filtration and washed with CH_2Cl_2 (15 mL × 3 × 3 min) and MeOH (15 mL

(21) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333.

(22) Morrison, H. A. *The Chemistry of the Nitro and Nitroso Groups*; Feuer, H., Ed.; Wiley: New York, 1970; Part I, p 185.

(23) Schnabel, E. *Ann. Chem.* **1967**, *702*, 188.

× 3 × 3 min). Each coupling was carried out in duplicate to minimize error sequences. Each step of the coupling was thoroughly monitored by the semiquantitative ninhydrin method. Coupling of Boc-amino acid to the proline resin was monitored by the chloranil test.[24]

After each Boc-amino acid was incorporated, the resin was treated with 4 N HCl–dioxane (20 mL) for 30 min, filtered, and washed with dioxane (15 mL × 2 × 3 min) and CH_2Cl_2 (15 mL × 3 × 3 min). The deprotected resin was neutralized with 10% DIEA in CH_2Cl_2 (20 mL) by shaking for 10 min. The neutralized resin was washed with CH_2Cl_2 (15 mL × 3 × 3 min). The coupling, deprotection, and washings were repeated until the desired sequence was achieved.

Preparation of Boc-Leu-Ala-Val-NHMe. The tripeptide was assembled on resin **4a** (1 g, 0.54 mmol of NH/g) by the stepwise incorporation of the respective amino acid according to the general procedure of the solid-phase peptide synthesis. The peptide resin was suspended in TFE–CH_2Cl_2 (20% v/v, 100 mL) in an immersion-type photochemical reactor and was bubbled with dry N_2 for 1 h. The suspension was irradiated for 18 h at 350 nm. The crude peptide *N*-methylamide was obtained in 78% yield (103 mg) and purified by HPLC on a Bondapak C-18 column using chloroform–methanol (9:1): mp 188–190 °C; IR (KBr) 1650 (amide), 1710 (urethane) cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) δ 1.45 (s, 9 H, Boc), 0.95 (t, 6 H, $C_\delta H$, Leu), 1.37–1.42 (m, $C_\gamma H$ and $C_\beta H$, Leu), 1.25 (d, 3 H, $C_\beta H$, Ala), 2.2 (b, $C_\beta H$, Val), 2.8 (d, 3 H, CH_3, NHMe), 4.13 (b, 1 H, $C_\alpha H$, Ala), 4.3 (t, 1 H, $C_\alpha H$, Val), 4.4 (q, 1 H, $C_\alpha H$, Leu), 5.06 (d, 1 H, NH, Ala), 6.68 (br, 1 H, $NHCH_3$), 6.9 (d, 1 H, NH, Leu), 7.0 (d, 1 H, NH, Val). Anal. Calcd for $C_{20}H_{38}N_4O_5$: C, 57.95; H, 9.17; N, 13.52. Found: C, 57.50; H, 9.14; N, 13.97. Amino acid analysis: Leu, 1.09; Ala, 1.05; Val, 1.0.

pGlu-His(Bzl)-Trp-Ser(Bzl)-Tyr(Bzl)-D-Trp-Leu-Arg-(Tos)-Pro-N(CH_3)-Resin. The nonapeptide was assembled on resin **4a** (2 g, 0.54 mmol of NH/g) according to the general protocol of the solid-phase synthesis. Symmetric anhydrides of each Boc-amino acid were prepared as described in the general procedure and incorporated stepwise. The elongation of the peptide chain was terminated with the incorporation of pGlu, and the peptide resin was thoroughly washed with CH_2Cl_2 (15 mL × 3 × 3 min) and MeOH (15 mL × 2 × 2 min).

pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NHCH$_3$. The nonapeptide resin (1.8 g) was suspended in a mixture of TFE–CH_2Cl_2 (20%, 200 mL) and placed in an immersion-type photochemical reactor. The suspension was degased with dry N_2 for 1 h and irradiated with a Philips HPK 125-W medium-pressure mercury lamp at 350 nm for 24 h as described previously.[15] The crude protected peptide *N*-methylamide was obtained in 66% yield. This was then treated with liquid HF (10 mL) for 30 min at 0 °C in the presence of anisole (1 mL). The excess HF was blown off through a NaOH solution with dry nitrogen. The residue was dried in vacuo over KOH, and the free peptide was treated with ether for the removal of anisole. The solvent was removed to obtain the ccrude deblocked nonapeptide *N*-methylamide and purified on a Sephadex LH-20 column in 56% yield. [α_D] in 1% acetic acid, −58.6°. Amino acid analysis: Glu, 0.99; His, 1.0; Trp, 1.96; Ser, 0.99; Tyr, 1.01; Leu, 0.97; Arg, 0.98; Pro, 1.10.

pGlu-His(Bzl)-Trp-Ser(Bzl)-Tyr(Bzl)-D-Trp-Leu-Arg-(Tos)-Pro-N(C_2H_5)-Resin. The peptide was assembled on resin **4b** (2 g, 0.54 mmol of NH/g) by using the symmetric anhydrides of the respective Boc-amino acids following the general protocol of the solid-phase peptide synthesis. After the incorporation of the pGlu residue, the peptide resin was thoroughly washed with CH_2Cl_2 (15 mL × 3 × 3 min) and MeOH (15 mL × 3 × 3 min).

pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-NHC$_2$H$_5$. The peptide resin (1.8 g) was suspended in a mixture of TFE–CH_2Cl_2 (20%, 200 mL) and irradiated as described previously. After photolysis the peptide *N*-ethylamide was obtained in 68% yield. The crude peptide was taken up in liquid HF (10 mL) and stirred in the presence of anisole (1 mL) for 30 min at 0 °C to remove the protecting groups. The excess HF was blown off, and the residue was taken up in water and extracted with ether. After lyophilization, the deprotected peptide *N*-ethylamide was obtained in 61% yield. The crude deprotected peptide was then purified on a Sephadex LH-20 column, resulting in 48% yield of the pure peptide. [α_D] in 1% acetic acid, −56.9°. Amino acid analysis: Glu, 1.06; His, 0.96; Trp, 1.97; Ser, 0.99; Tyr, 1.03; Leu, 1.09; Arg, 0.99; Pro, 1.06.

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(24) Christensen, T. *Acta Chem. Scand.* **1979**, *33*, 763.

Chirospecific Syntheses of Nitrogen and Side-Chain Modified Anatoxin Analogues. Synthesis of (1*R*)-Anatoxinal and (1*R*)-Anatoxinic Acid Derivatives

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A straightforward and good yielding route to side-chain analogues of the potent neurotoxin and neurotransmitter (+)-anatoxin (**1**) has been developed. Peroxy acid oxidation of the (silyloxy)butadiene **43** derived from readily synthesized, optically pure (1*R*)-*t*-BOC-anatoxin (**42**) affords silyloxy ketone **44**. Fluorolysis of **44** followed by oxidative cleavage of the resultant α-hydroxy ketone **45** gives a mixture of α,β-unsaturated acid **46** and ester **41** in 57% combined yield. Other approaches to these compounds, based on literature precedent, failed. (1*R*)-*t*-BOC-anatoxinic acid (**46**) then serves as educt for the synthesis of a wide variety of anatoxin derivatives with modified side-chain functionality. These analogues, designed to serve as probes of the agonist binding site of the nicotinic acetylcholine receptor, include alcohol, aldehyde, amide, hydroxamate, and oxime ether functional groups.

Although the nicotinic acetylcholine receptor (nAChR) is the most well-characterized neurotransmitter receptor known,[1] there exists no high resolution X-ray crystal structure of this protein,[2] and the three-dimensional en-

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