(COOH), 740 (C=C) cm^{-1}; ^1H NMR (270 MHz) δ 0.94 (6 H, d, J = 6.6 Hz, $CH_3 \times 2$), 1.41, 1.66 (each 2 H, quint, J = 7.5 Hz, $C_{3,4}$-H), 2.06 (2 H, dt, J = 7.3, 7.3, 5.9 Hz, C_5-H), 2.36 (2 H, t, J = 7.5 Hz, C_2-H), 2.58 (1 H, m, C_8-H), 5.16-5.23 (2 H, m, CH=CH); (100 MHz) δ 11.50 (1 H, br s, COOH); EI-MS m/z (relative intensity) 170 (M^+, 14), 152 ($M^+ - 18$, 13), 137 (19), 109 (13), 95 (28), 81 (22), 69 (100), 55 (77), 41 (85). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.54; H, 10.66. Found: C, 70.44; H, 10.66. This acid was esterified with CH_2N_2, and the E/Z ratio was found to be 1:11 [E isomer (t_R 10.6 min); Z-isomer (t_R 10.3 min)] by GLC analysis.

Isomerization of the (*Z*)-Acid 3b. 2 M $NaNO_2$ (3.2 mL) and 6 M HNO_3 (2.15 mL) were added to the (Z)-acid **3b** (7.7 g, 45.3 mmol) warmed at 70-75 °C under an atmosphere of N_2.[15] The mixture was then stirred vigorously for 0.5 h. The cooled reaction mixture was diluted with ether (50 mL), washed with water (50 mL) and saturated brine (30 mL × 3), dried over anhydrous Na_2SO_4, and evaporated. The oily residue was distilled under reduced pressure to give the (E)-acid **3a** (5.94 g, 77%): bp 117-120 °C (2.8 Torr) [lit.[9] bp 100-103 °C (3 Torr), lit.[10] bp 130-132 °C (12 Torr), lit.[12] bp 120-122 °C (5-6 Torr)]. GLC analysis revealed that E/Z ratio of **3a** was 8:1: IR (neat) 3300-2500 (COOH), 1710 (C=O), 970 (C=C) cm^{-1}; ^1H NMR (270 MHz) δ 0.96 (6 H, d, J = 6.6 Hz, $CH_3 \times 2$), 1.41, 1.64 (each 2 H, quint, J = 6.6 Hz, $C_{3,4}$-H), 2.00 (2 H, q, J = 6.6 Hz, C_5-H), 2.35 (2 H, t, J = 6.8 Hz, C_2-H), 2.17-2.30 (1 H, m, C_8-H), 5.32-5.38 (2 H, m, CH=CH); (100 MHz) δ 11.50 (1 H, br s, COOH); FI-MS m/z (relative intensity) 171 (MH^+, 19.9), 170 (M^+, 100); EI-MS m/z (relative intensity) 170 (M^+, 20), 152 (16), 137 (24), 109 (20), 95 (33), 81 (24), 69 (100), 55 (79), 41 (95). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.54; H, 10.66. Found: C, 70.69; H, 10.88.

Vanillylamine. A mixture of vanillin (15.2 g, 0.1 mol) and ammonium formate (20 g, 0.32 mol) was heated at 180 °C for 3 h[20] and, after cooling, evaporated until the odor of ammonia disappeared. To the residue was added concentrated HCl (12 mL). The mixture was refluxed for 1 h and then evaporated until the odor of HCl disappeared. The HCl salt was crystallized by adding EtOH (70 mL). Two recrystallizations from 95% EtOH yielded pure vanillylamine hydrochloride (8.99 g, 47.5%), mp 216-218 °C dec (lit.[3] mp 219-222 °C dec, lit.[10] mp 214 °C). IR and ^1H NMR data were identical with those reported in the literature.[3] Anal. Calcd for $C_8H_{12}NClO_2$: C, 50.67; H, 6.38; N, 7.39; Cl, 18.70. Found: C, 50.44; H, 6.40; N, 7.47; Cl, 18.90.

To a vigorously stirred solution of vanillylamine hydrochloride (3.66 g, 19.31 mmol) in water (50 mL) was added 2 M NaOH solution (9.38 mL, 18.76 mmol). The resulting white solid of free vanillylamine was collected by suction filtration, washed with water, dried over P_2O_5 in a vacuum desiccator, and amounted to 2.54 g (89%), mp 135-136 °C (lit.[9] mp 132 °C, lit.[19] mp 131-133 °C), which was used in the following steps without further purification.

(*E*)-*N*-(4-Hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide (Capsaicin) (1a). The (E)-acid **3a** (334 mg, 1.96 mmol) and thionyl chloride (720 mg, 5.88 mmol) were stirred at room temperature for 8 h and then heated at 100 °C for 0.5 h. The excess thionyl chloride was removed under reduced pressure. The resulting acid chloride **4a** [bp 100-102 °C (12 Torr)][24] was dissolved in dry ether (10 mL) and added to a stirred suspension of dry vanillylamine (600 mg, 3.92 mmol) in dry ether (25 mL) under an atmosphere of N_2. The mixture was kept at room temperature for 2 h and then gently refluxed for 2 h. After cooling, the precipitate was removed by suction filtration, and the filtrate was evaporated. The residue was purified by column chromatography on silica gel (Fuji-gel BW-200, 15 g, elution with 2:1 hexane-ethyl acetate). The oily product (542 mg, E/Z = 8:1,[25] 91%) was treated with 2:1 hexane-ether to give a crystalline solid (473 mg, E/Z = 12:1,[25] 79%), mp 60-63 °C. Two recrystallizations from the same solvents gave capsaicin (318 mg, 53%), mp 64-65 °C (lit.[3,8,12] mp 64-65 °C, lit.[9] mp 63.8 °C, lit.[10] mp 65 °C) as a white solid. IR, ^1H NMR, and mass spectral data were essentially identical with those reported in the literatures.[3,9] Anal. Calcd for $C_{18}H_{27}NO_3$: C, 70.79; H, 8.91; N, 4.59. Found: C, 70.69; H, 9.02, N, 4.49.

(*Z*)-*N*-(4-Hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide (*cis*-Capsaicin) (1b). The (Z)-acid **3b** (464 mg, 2.72 mmol) was treated with thionyl chloride (1.0 g, 8.17 mmol) in the same manner as noted above. The obtained acid chloride **4b** [bp 99-102 °C (13 Torr)][24] in dry ether (10 mL) was added to a suspension of dry vanillylamine (835 mg, 5.45 mmol) in dry ether (30 mL) under an atmosphere of N_2. The workup in the same manner as noted above gave the crude oily amide **1b** (745 mg, E/Z = 1:11,[25] 90%), which on crystallization from 2:1 hexane-ether afforded a crystalline solid (674 mg, 81%, E/Z = 1:13).[25] Two recrystallizations from the same solvents afforded *cis*-capsaicin (**1b**) (548 mg, 66%), mp 68.5-69.5 °C (lit.[23] mp 70 °C), as a white solid. IR, ^1H NMR, and mass spectral data were essentially identical with those reported by Gannett et al.[3] Anal. Calcd for $C_{18}H_{27}NO_3$: C, 70.79; H, 8.91; N, 4.59. Found: C, 70.78; H, 9.08; N, 4.61.

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(25) The E/Z ratio was determined on intensities of isopropyl signals by ^1H NMR analysis.

Nitrobenzophenone Oxime Based Resins for the Solid-Phase Synthesis of Protected Peptide Segments[1]

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This laboratory has reported the development of an oxime resin that allows the rapid synthesis and isolation of protected peptides.[3,4] This oxime support has been used successfully in the synthesis of an apolipoprotein model peptide[5] and a synthetic hemeprotein[6] and is now being applied in the syntheses of several small proteins.[7,8] During our efforts to synthesize peptides corresponding to partial and full sequences of our target proteins we have encountered difficulties in the use of our polystyrene-based oxime resin both in the synthesis of specific sequences of certain short peptides (<10 residues) and in the recoupling of smaller protected peptide segments on the oxime resin to assemble large peptides. Difficulties in the latter instance have necessitated the use of solution-phase couplings to couple larger protected peptides of about >15 residues. Nevertheless, we would still like to have a solid support as an effective option for use in the coupling of protected peptide segments. This paper describes our initial effort to explore alternative oxime solid-phase supports for the synthesis and assembly of protected peptides through the synthesis of a nitrobenzophenone oxime derivative and its attachment to a polyamide resin. We also report an improved procedure for the synthesis of our previously reported polystyrene-based oxime resin 1.[3,4]

Results and Discussion

Because the 4-nitrobenzophenone oxime (NBO) moiety has proved reliable in previous synthetic work, we decided to synthesize a molecule that would contain the NBO functionality and, in addition, a linker arm through which the oxime could be attached to a solid support. While the standard oxime resin is obtained by direct modification of polystyrene beads (Scheme I), this new approach offers

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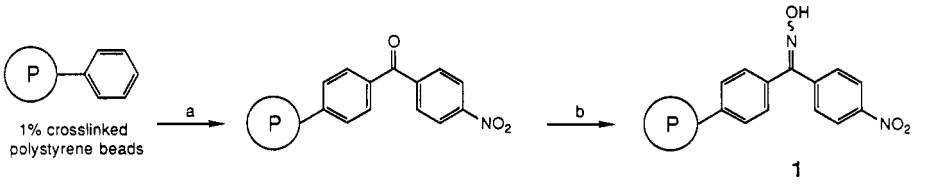
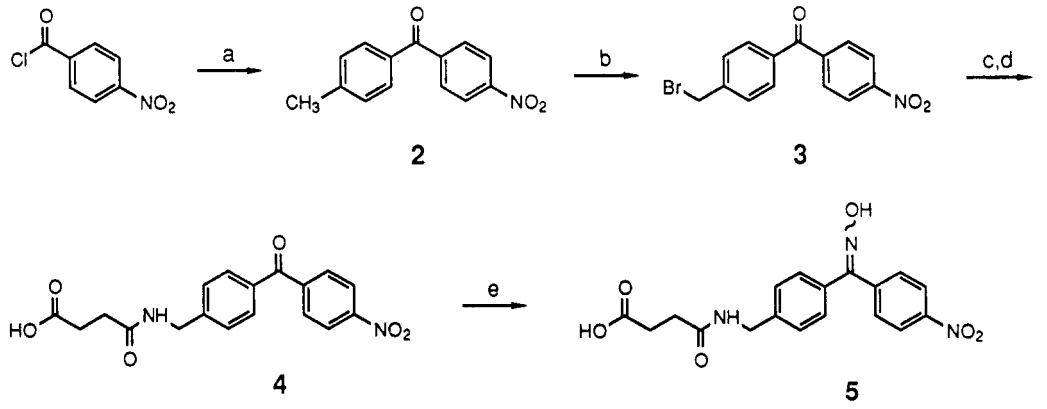
Scheme I. Synthesis of Polystyrene Oxime Resin[a]



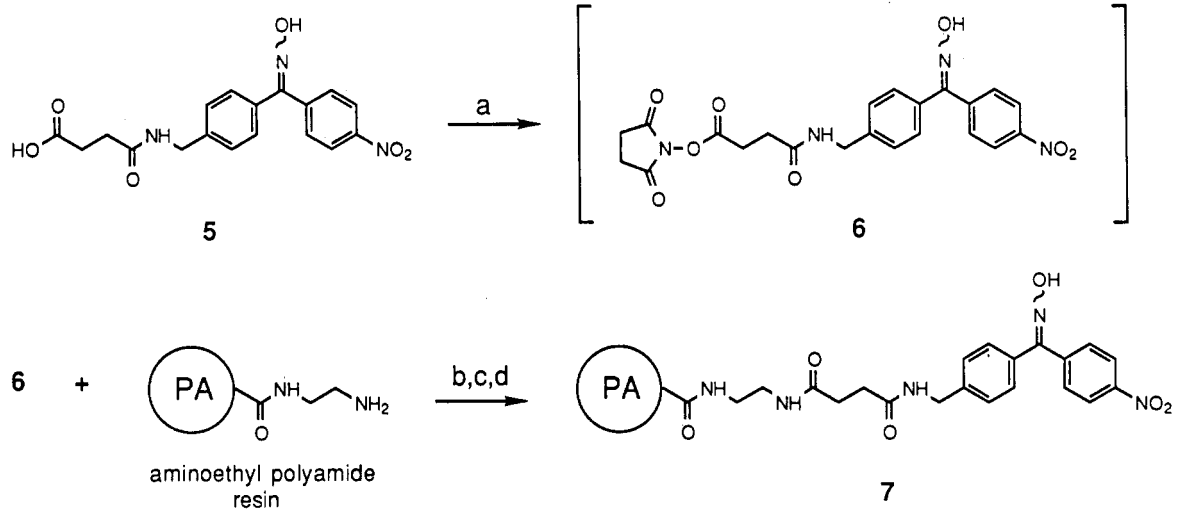
[a] (a) *p*-Nitrobenzoyl chloride, $AlCl_3$; (b) NH_2OH–HCl, EtOH, pyridine.

Scheme II. Synthesis of 4-(((Hydroxysuccinyl)amino)methyl)-4′-nitrobenzophenone Oxime[a]



[a] (a) $C_6H_5CH_3$, $AlCl_3$; (b) *N*-bromosuccinimide, CCl_4; (c) NH_3, tetrabutylammonium iodide, THF, MeOH; (d) succinic anhydride, NEt_3, THF; (e) NH_2OH–HCl, EtOH, pyridine.

Scheme III. Synthesis of Polyamide Oxime Resin[a]



[a] (a) *N*-Hydroxysuccinimide, DIC, THF, CH_2Cl_2; (b) DMF; (c) acetic anhydride, NMM, CH_2Cl_2, DMF; (d) *N*-hydroxypiperidine, CH_2Cl_2, DMF.

the flexibility of attaching the oxime to any material containing suitable functionality such as aminoalkyl groups. We have synthesized an appropriate benzophenone oxime as outlined in Scheme II.

This synthesis began with the Friedel–Crafts acylation of toluene with *p*-nitrobenzoyl chloride.[9] The resulting 4-methyl-4′-nitrobenzophenone (**2**) was then brominated at the methyl group to obtain the (bromomethyl)nitrobenzophenone (**3**). This compound was then combined with ammonia to obtain an (aminomethyl)nitrobenzophenone, which reacted directly with succinic anhydride to form 4-(((hydroxysuccinyl)amino)methyl)-4′-nitrobenzophenone (**4**), which was converted to the oxime **5** with hydroxylamine in ethanol/pyridine. This oxime was then

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(9) 4-Methyl-4′-nitrobenzophenone was also obtained by reaction of ditolyl cadmium (prepared from tolyl Grignard reagent and $CdCl_2$ at −78 °C for 1 h, followed by slow addition of the acid chloride) with *p*-nitrobenzoyl chloride, but this reaction proceeded in lower yield and was less convenient to do.

attached to a commercially available hydrophilic poly(dimethylacrylamide)-based resin functionalized with aminoethyl groups.[10]

We found that we could generate an active ester of the oxime carboxylic acid **5** using *N*-hydroxysuccinimide and DIC[11] (Scheme III). This HOSu active ester **6** thus obtained was stable in solution but appeared to react with itself if evaporated to dryness. The HOSu ester reacts cleanly with amino groups and allows the formation of the desired amide bond on addition of the ester to the amino polyamide resin. The resulting resin was acetylated to block any unreacted amino groups and then treated with *N*-hydroxypiperidine (HOPip) to deacetylate the oxime functionality. This polyamide oxime resin **7** (PAO resin) was then used for peptide synthesis in essentially the same manner as the polystyrene-based oxime resin.[3,4]

We have demonstrated the utility of the PAO resin as a support for the synthesis of protected peptides by synthesizing a peptide corresponding to a six-residue portion of the sequence of ribonuclease T_1, positions 83–88:[12] *t*-Boc-Asn-Asn-Gln-Leu-Ala-Gly-OH. The *t*-Boc-protected peptide was cleaved from the resin with HOPip to obtain the *N*-hydroxypiperidyl ester, which was reduced with zinc in acetic acid to obtain the peptide free acid. The purified peptide was identical with material previously synthesized on the polystyrene oxime resin and was obtained in 48% yield after purification by HPLC. This is about the same yield as obtained by using the polystyrene oxime resin (51%) and shows that the polyamide-based resin performs as well as the first resin for easily synthesized small peptides.[13]

The conditions for the synthesis of methylnitrobenzophenone **2** are mild in contrast with the published procedure for the *p*-nitrobenzoylation of polystyrene which uses refluxing dichloroethane with nitrobenzene as a cosolvent.[3,4] This observation lead us to reexamine the *p*-nitrobenzoylation of polystyrene, which we found to proceed in methylene chloride at room temperature. The resulting modified procedure for the preparation of polystyrene oxime resin, described in the Experimental Section, requires milder conditions for the acylation step and can be carried out more conveniently and on a larger scale under typical bench conditions. We also find this modified procedure to be more reproducible with respect to the substitution level of oxime in the resulting resin.

Conclusions

We have described a new strategy for the use of oxime resins for the synthesis of protected peptides. We have also described an improved procedure for the synthesis of our original polystyrene oxime resin. The polyamide oxime resin described can be used in the same manner as the original polystyrene oxime resin and may be more suitable for the synthesis of certain peptide sequences and for the condensation of protected peptide segments because of its more hydrophilic polymeric matrix.[10] In addition, the succinoyl linker arm ensures the separation of the growing peptide chain from the polymer backbone. While arguments have been made in favor of spacer linkages,[14] it is unclear if such a strategy improves the utility of a given resin.[15,16] In the current instance the linker is primarily of importance as the functional group through which the oxime **5** may be attached to a solid material which can be varied for the preparation of additional supports for the synthesis of protected peptides. Further, the synthesis of oxime **5** offers the potential for tuning the stability of the oxime linkage by introducing substituents into one or both of the phenyl rings of **5**. Such a strategy can only be used to a lesser extent when modifying a support in the manner used to prepare the polystyrene oxime **1**. The ultimate utility of these supports for protected peptide synthesis, in comparison with other supports for the same purpose,[17–20] will require further study.

Experimental Section

General. PepSyn gel resin with a substitution level of 1.0 mequiv/g of active ester was purchased from Milligen. Thin-layer chromatography (TLC) was on silica gel analytical plates from Merck. Melting points are uncorrected. Aluminum chloride and *p*-nitrobenzoyl chloride were from Aldrich. BioBeads S-X1 (1% cross-linked polystyrene) were purchased from BioRad. Protected amino acids were from Peninsula Laboratories. HCl/propionic acid for amino acid analysis was from Pierce. *N*-Hydroxypiperidine was prepared according to the literature.[21] Other chemicals were reagent grade. Elemental analyses were performed by the Microanalytical Service at The Rockefeller University.

4-Methyl-4′-nitrobenzophenone (2). In a 1-L round-bottomed flask *p*-nitrobenzoyl chloride (18.6 g, 0.10 mol) was dissolved with stirring in toluene (750 mL). Aluminum chloride (18.7 g, 0.14 mol) was added, and the resulting red solution was then stirred at room temperature for 1 h. Water (5 mL) was added dropwise, and the resulting mixture was stirred for 15 min and then extracted with water (100 mL) and 10% $NaHCO_3$ (2 × 100 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to a pale yellow solid (21.8 g). This material was recrystallized from chloroform/hexanes to obtain the product as a single isomer, as pale yellow needles, 18.9 g (78%). The filtrate afforded a mixture of isomers, 2.7 g (11%). An analytical sample was obtained by further recrystallization, mp 120–120.5 °C. TLC: methylene chloride/hexane (1:1), R_f = 0.34. IR: 1650, 1600, 1520, 1350 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.33 (d, J = 8.7 Hz, 2 H), 7.91 (d, J = 8.6 Hz, 2 H), 7.71 (d, J = 8.0 Hz, 2 H), 7.32 (d, J = 7.9 Hz, 2 H) (aromatic H's), 2.47 (s, 3 H, CH_3). Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.59; H, 4.52; N, 5.80.

4-(Bromomethyl)-4′-nitrobenzophenone (3). In CCl_4 (50 mL), 4-methyl-4′-nitrobenzophenone (965 mg, 4 mmol) was dissolved with stirring. *N*-Bromosuccinimide (712 mg, 4 mmol) and a catalytic amount of benzoyl peroxide were added.[22] The mixture was stirred under a bright lamp for 2 h, filtered, and evaporated to a pale brown solid. Chromatography on silica gel (methylene chloride/hexane, 1:1) yielded product, 849 mg (66%), mp 124–125 °C. TLC: methylene chloride/hexane (1:1), R_f = 0.33. IR: 1655, 1600, 1520, 1410, 1355 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.35 (d, J = 8.6 Hz, 2 H), 7.94 (d, J = 8.6 Hz, 2 H), 7.79 (d, J = 8.1 Hz, 2 H), 7.55 (d, J = 8.1 Hz, 2 H) (aromatic H's), 4.54 (s, 2 H, CH_2Br). Anal. Calcd for $C_{14}H_{10}NO_3Br$: C, 52.52; H, 3.15; N, 4.38. Found: C, 52.69; H, 3.16; N, 4.30.

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4-(((Hydroxysuccinyl)amino)methyl)-4'-nitrobenzophenone (4). 4-(Bromomethyl)-4'-nitrobenzophenone (2.91 g, 9.1 mmol) was dissolved in THF/MeOH (1:1, 100 mL), concentrated NH_3 (25 mL) and tetrabutylammonium iodide (10 mg) were added, and the resulting mixture was stirred for 3.5 h. Solvent was removed on a rotary evaporator, and the resulting solid was dried briefly in vacuo. THF (150 mL) and succinic anhydride (1.09 g, 11 mmol) were added to the dry solid and stirred. To the resulting slurry was added triethylamine (3.8 mL, 27 mmol), and the mixture was stirred for 10 min. The mixture was filtered and evaporated to a brown oil, which was chromatographed on silica gel ($CHCl_3$/MeOH/AcOH, 95:5:1), affording product, 1.96 g (61%). An analytical sample was obtained by recrystallization from THF, mp 180–180.5 °C. TLC: $CHCl_3$/MeOH/AcOH (18:2:1), R_f = 0.45. IR: 3320, 3100, 2920, 1680, 1660, 1600, 1545, 1510, 1410, 1350 cm^{-1}. ^1H NMR (CD_3SOCD_3): δ 8.51 (t, J = 5.8 Hz, 1 H, NH), 8.38 (d, J = 8.6 Hz, 2 H), 7.94 (d, J = 8.6 Hz, 2 H), 7.73 (d, J = 8.1 Hz, 2 H), 7.46 (d, J = 8.0 Hz, 2 H) (aromatic H's), 4.38 (d, J = 5.8 Hz, 2 H, CH_2-phenyl), 2.42 (br s, 4 H, CH_2CH_2). Anal. Calcd for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86. Found: C, 60.69; H, 4.52; N, 7.88.

4-(((Hydroxysuccinyl)amino)methyl)-4'-nitrobenzophenone Oxime (5). Benzophenone **4** (753 mg, 2.11 mmol) and hydroxylamine hydrochloride (734 mg, 10.6 mmol, 5 equiv) were dissolved in EtOH/pyridine (5:1, 18 mL) and heated at 85 °C for 1 h. Solvent was evaporated, and the resulting solid was triturated with water, filtered, and dried. The product was recrystallized from H_2O/MeOH/THF, mp 158–161 °C. Yield: 750 mg (96%) of a 65:35 mixture of isomeric oximes. IR: 3390, 3100, 2920, 1720, 1700, 1620, 1550, 1510, 1410, 1350 cm^{-1}. TLC: $CHCl_3$/MeOH/AcOH (18:2:1), R_f = 0.35. ^1H NMR (CD_3SOCD_3): δ 12.03 (br s, 1 H, CO_2H), 11.90 (s, 0.35 H), 11.65 (s, 0.65 H) (both NOH), 8.46 (t, J = 5.8 Hz, 0.35 H), 8.40 (t, J = 5.7 Hz, 0.65 H), 8.31 (d, J = 8.6 Hz, 1.3 H), 8.23 (d, J = 8.8 Hz, 0.7 H), 7.63 (d, J = 8.8 Hz, 0.7 H), 7.57 (d, J = 8.6 Hz, 1.3 H), 7.36 (d, J = 8.1 Hz, 0.7 H), 7.31 (d, J = 8.3 Hz, 1.3 H), 7.24–7.27 (m, 2 H) (aromatic H's), 4.34 (d, J = 5.5 Hz, 0.7 H), 4.27 (d, J = 5.4 Hz, 1.3 H) (CH_2-phenyl), 2.3–2.5 (m, 4 H, CH_2CH_2). Anal. Calcd for $C_{18}H_{17}N_3O_6$: C, 58.22; H, 4.61; N, 11.32. Found: C, 58.17; H, 4.63; N, 11.10.

***N*-2-Aminoethyl Amide Derivative of Polyamide Gel.**[10] PepSyn gen resin (2.0 g, 2.0 mmol active ester) was placed in a 60-mL polyethylene bottle. Ethylenediamine (50 mL) was added, and the resulting suspension was shaken gently overnight. The resulting modified resin was collected by filtration on a fritted funnel and washed several times with DMF and then with CH_2Cl_2. This amino resin was used directly in the next step. The substitution level of the resin based on picric acid titration of the amino groups of a small sample was 0.85 mmol/g.[23]

Coupling of Benzophenone Oxime 5 with Amino Resin. Benzophenone oxime **5** (743 mg, 2.0 mmol) and *N*-hydroxysuccinimide (230 mg, 2.0 mmol) were dissolved in CH_2Cl_2/THF (3:1, 20 mL), DIC (0.313 mL, 2.0 mmol) was added, and the resulting solution was stirred for 1 h. The formation of the HOSu active ester was observed by TLC: $CHCl_3$/MeOH/AcOH (18:2:1), R_f = 0.44. This solution was then added to a suspension in DMF (15 mL) of the amino resin from the preceding step, and the resulting mixture was then shaken gently at room temperature for 20 h.[24] The resulting resin was collected by filtration, washed with DMF and CH_2Cl_2, and dried in vacuo. Yield, 2.79 g of pale yellow beads. The resin was then acetylated in CH_2Cl_2/DMF (2:1, 30 mL) with acetic anhydride (200 μL, 2.1 mmol) and *N*-methylmorpholine (230 μL, 2.1 mmol) for 2 h. The resin was then filtered and washed with CH_2Cl_2. A Kaiser test on a small sample of resin indicated that no free amino groups remained.[25] The resin was then swollen in CH_2Cl_2/DMF (2:1, 30 mL) and treated with *N*-hydroxypiperidine (0.62 g, 6.1 mmol) for 24 h. The resulting benzophenone oxime polyamide resin **7** was collected by filtration, washed with CH_2Cl_2, and then used directly in the next step.

Coupling of First Protected Amino Acid to PAO Resin 7.[3-5] The resin from the preceding step was combined with *t*-Boc-glycine (526 mg, 3.0 mmol) and DCC (619 mg, 3.0 mmol) in CH_2Cl_2 (30 mL) and shaken gently for 24 h. The resulting protected aminoacyl resin was washed with CH_2Cl_2, DMF, and CH_2Cl_2 (2 × 30 mL each) and dried in vacuo. Amino acid analysis of a sample of this material showed a glycine substitution level of 0.45 mmol/g.

Solid-Phase Synthesis of *t*-Boc-Asn-Asn-Gln-Leu-Ala-Gly-OH. The synthesis was performed in a reaction vessel[26] previously silanized with 5% dichlorodimethylsilane in $CHCl_3$, rinsed thoroughly with water, and then dried in an oven. *t*-Boc-protected amino acids were coupled to 2.57 g of *t*-Boc-glycyl resin **7** in sequence after the resin was swollen with CH_2Cl_2 (2 × 30 mL). Each coupling cycle consisted of washing, deprotection, and coupling steps as follows (all wash volumes 30 mL): CH_2Cl_2 (2 × 1 min), 25% TFA in CH_2Cl_2 (1 × 1 min), 25% TFA in CH_2Cl_2 (1 × 30 min), CH_2Cl_2 (2 × 1 min), *i*PrOH (1 × 1 min), CH_2Cl_2 (2 × 1 min), *i*PrOH (1 × 1 min), CH_2Cl_2 (4 × 1 min), coupling of protected amino acid (see below), CH_2Cl_2 (4 × 1 min), CH_2Cl_2/DMF (2:1, 2 × 1 min), CH_2Cl_2 (2 × 1 min). Gentle shaking of the reaction vessel was maintained during the synthesis except when reagents and solvents were being added or removed. Alanine and leucine were coupled using the symmetrical anhydride method: *t*-Boc-amino acid (5.42 mmol, 6.2 equiv) was dissolved in CH_2Cl_2 (30 mL) and cooled on ice, DIC (410 μL, 3 equiv) was added, and the mixture was allowed to react for ca. 20 min and then added to the *t*-Boc-deprotected peptide-resin. The reaction was allowed to proceed for 45 min, the resin was washed, and completeness of coupling was confirmed with the Kaiser test.[25] Asparagine and glutamine were coupled using the carbodiimide/HOBt method: *t*-Boc-protected amino acid (3.94 mmol, 4 equiv) and HOBt (3.49 mmol, 4 equiv) were dissolved in CH_2Cl_2/DMF (1:1, 30 mL), and DIC (547 μL, 3.49 mmol, 4 equiv) was added to the ice-cooled solution, which was allowed to react for ca. 20 min. The solution was then added to the *t*-Boc-deprotected peptide resin, the reaction mixture was shaken gently for 2 h, the resin was washed, and completeness of coupling was confirmed.

Cleavage of *t*-Boc-Peptide from PAO-Resin. To the peptide resin from the preceding step, in CH_2Cl_2/DMF (1:1, 30 mL) was added HOPip (303 mg, 2.7 mmol) and the mixture was shaken overnight. The solvent was collected by filtration, and the resin was washed with DMF (2 × 20 mL). The combined filtrate and washes were evaporated in vacuo to a white solid, 0.93 g crude peptide-OPip ester.

Reduction of Peptide-OPip Ester. The crude peptide from the preceding step was dissolved in 90% acetic acid (20 mL), and zinc dust (1.71 g, 26.1 mmol) was added with vigorous stirring. After the mixture was stirred for 30 min, the supernatant was removed with a pipet and filtered through a Millex-HV 0.45 μm filter attached to a syringe. The remaining zinc dust was washed with acetic acid (2 × 5 mL), and the filtered washes were combined and evaporated to a white solid, 1.87 g.[27]

Purification of *t*-Boc-Asn-Asn-Gln-Leu-Ala-Gly-OH. The crude peptide from the zinc reduction was chromatographed on a reverse-phase HPLC column (Vydac C_4 prep) with 12% CH_3CN in H_2O containing 0.1% AcOH. Amino acid analysis, 2 h hydrolysis with HCl/propionic acid (1:1): Asx, 1.91; Glx, 1.01; Gly, 1.03; Ala, 1.00; Leu, 0.94. MS (^{252}Cf-fission fragment), exact mass calcd for $C_{29}H_{49}N_9O_{12}Na$ $(M + Na)^+$ 738.8, found 738.9. Yield, 0.30 g (48% based on starting substitution level of the resin).

Preparation of Polystyrene Oxime Resin (1). In a 5-L round-bottomed flask, 200 g of BioBeads S-X1 (1.92 mol as styrene) were swelled with 1.5 L of methylene chloride. Aluminum

(23) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984; p 107.

(24) Shaking of reaction vessels was achieved by using a wrist action shaker or an overhead stirrer fitted with a clamp and turned on its side to rotate the reaction vessel end over end at a moderate rate (ca. 1 rotation per s).

(25) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.

(26) Reaction vessels used were of the type described in ref 23, p 130. These vessels have a Teflon-lined screw cap at the top for adding reagents and a glass frit over a two-way stopcock at the bottom to allow the removal and addition of solvents under suction.

(27) Protected peptides often precipitate when the reduction mixture is added to water or 10% citric acid solution. This peptide did not precipitate well in water, and the reaction mixture was therefore directly evaporated. The high content of zinc salts in the crude peptide, however, does tend to result in lowered isolated yields after HPLC.

chloride (34 g, 0.255 mol) and *p*-nitrobenzoyl chloride (34 g, 0.183 mol) were dissolved in 2 L of CH_2Cl_2 and added with mixing to the resin mixture through a funnel fitted with a glasswool plug.[28] The resulting orange mixture was then allowed to stand at room temperature for 40 h with occasional stirring. The resin was then collected in a large coarse porosity fritted-glass funnel and washed with dioxane/4 N aqueous HCl (3:1, 6 L), dioxane/water (3:1), DMF, MeOH (4 L each), swollen with CH_2Cl_2 (2 L), washed with MeOH (2 L), and dried under suction and then vacuum. Yield, 221 g of pale yellow nitrobenzophenone resin. The above keto resin was converted to the oxime in pyridine/ethanol (1:5, 1.8 L) containing hydroxylamine hydrochloride (200 g). The mixture was heated at gentle reflux for 22 h and then collected in a fritted-glass funnel. The resin was washed with MeOH (2 × 1.5 L) and dried under vacuum. Yield, 223 g of oxime resin. A small sample of this resin was coupled with *t*-Boc-glycine, and the substitution level was determined by picric acid titration to be 0.60 mmol/g.[23,29]

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Registry No. **2**, 5350-47-0; **3**, 120712-34-7; **4**, 120712-35-8; (*E*)-**5**, 120712-37-0; (*Z*)-**5**, 120712-36-9; (*E*)-**6**, 120712-39-2; (*Z*)-**6**, 120712-38-1; 4-$(NO_2)C_6H_4COCl$, 122-04-3; PhMe, 108-88-3; $H_2NCH_2CH_2NH_2$, 107-15-3; BOC-Gly-OH, 4530-20-5; BOC-Ala-OH, 15761-38-3; BOC-Leu-OH, 13139-15-6; BOC-Gln-OH, 13726-85-7; BOC-Asn-OH, 7536-55-2; BOC-Asn-Asn-Gln-Leu-Ala-Gly-OH, 120712-40-5; BOC-Asn-Asn-Gln-Leu-Ala-Gly-OPip, 120712-41-6; succinic anhydride, 108-30-5; PepSyn gel resin, 120788-21-8; styrene-divinylbenzene copolymer, 9003-70-7.

(28) We thank Dr. Jeffrey Kelley for using this protocol to prepare a batch of oxime resin and sharing his results. In earlier experiments we did not filter the solution of $AlCl_3$ and nitrobenzoyl chloride on addition to the polystyrene beads. If filtration is omitted, the product resin may contain a small amount of dark granular impurity which does not interfere with the use of the resin.

(29) This procedure can also be used to prepare an oxime resin from macroporous polystyrene (Aldrich). After coupling of *t*-Boc-glycine, the substitution level of this material was determined by amino acid analysis to be 0.6 mmol/g and by picric acid titration[23] to be 0.1 mmol/g. These preliminary results suggest that diffusion of reagents through macroporous polystyrene is poor and not well suited to peptide synthesis (see also ref 20). These results were obtained by Dr. Tomikazu Sasaki.

Asymmetric Reduction of Ketones with Crystalline Cyclodextrin Complexes of Amine–Boranes

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One of the easist methods for the preparation of optically active secondary alcohols is the asymmetric reduction of prochiral ketones. Among the asymmetric reducing agents for ketones, chirally modified borane derivatives or complexes have been widely studied owing to their selectivity, stability, and solubility in a variety of solvents, and high asymmetric inductions have been reported in individual cases.[1-8] Significant asymmetric inductions have been also achieved by the use of borohydrides in a chiral environment such as in the presence of optically active catalysts under phase-transfer conditions[9,10] and in the chiral domain of D-glucofuranose,[11] bovine serum albumin,[12] and chiral crown ethers.[13,14]

Cyclodextrins provide also a chiral binding site[15] capable of including guest molecules and are known to induce asymmetric reductions of prochiral ketones dissolved[16] or suspended[17] in an alkaline aqueous solution of sodium borohydride to give the corresponding alcohols in low enantiomeric excess up to 36%. However, no amine–borane complexes included in cyclodextrins have been investigated as asymmetric reducing agents. In connection with our interest in solid state reactions, the use of microcrystals of cyclodextrin complexes has been investigated as rigid chiral matrices controlled by the crystalline lattice for asymmetric reaction,[18] and those studies mentioned above

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