# A Reinvestigation of the Preparation, Properties, and Applications of Aminomethyl and 4-Methylbenzhydrylamine Polystyrene Resins[1]

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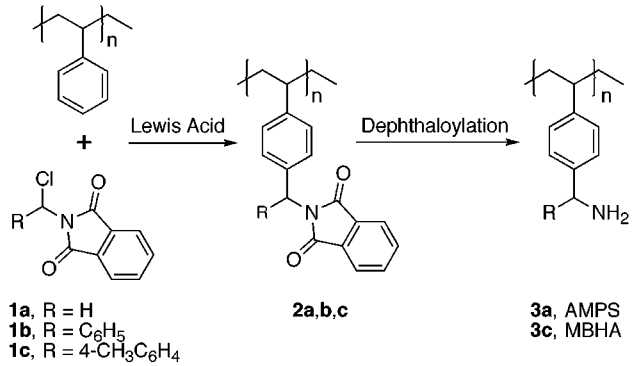
Mild, efficient conditions have been developed for the preparation of 4-methylbenzhydrylamine polystyrene (MBHA) and aminomethyl polystyrene (AMPS) resins by a two-step procedure with synthons **1a** and **1c**. The products possess excellent swelling characteristics and acylate readily with linkers yielding useful derivatives, which retain good swelling and reactivity. Comparative studies with these resins, and their poly(ethylene glycol) (PEG) derivatives, yield insights into the role of spacer arm and environment effects in synthesis facilitation.

## Introduction

Amino-functionalized polystyrenes provide indispensable platforms for attachment of handles and linkers,[3,4] spacer arms,[5] and other moieties [i.e., poly(ethylene glycol) (PEG)][6] for facilitation of solid-phase-mediated organic transformations (e.g., in peptide, DNA, and combinatorial synthesis applications). In this regard, 4-methylbenzhydrylamine polystyrene (MBHA)[7] and aminomethyl polystyrene (AMPS)[8] resins have been of lasting durability and usefulness. Nevertheless, there is a growing recognition that drastic processing conditions during resin modification and incompleteness in the multistep transformations involved can cause modification of cross-linking and other undesired side reactions. Standard procedures for the production of aminomethyl polystyrenes,[8] for example, use (hydroxymethyl)- or (chloromethyl)phthalimide in TFA−$CH_2Cl_2$ mixtures at elevated temperatures and trifluoromethanesulfonic acid as the strong acid catalyst, conditions that result in relatively inefficient incorporations and poorly swelling materials. Modified procedures to avoid some of these problems have been described recently.[9,10] Similar problems occur as a result of the Friedel−Crafts acylation with toluoyl chloride in the preparation of MBHA resin, and additional complications arise from the Leuckart reaction used to convert the resultant ketone to an amine (i.e., molten ammonium formate at 165 °C). Incompletion of the latter transformation, which leaves residual keto functionality, can give rise to other side reactions, for example, through Schiff's base formation with incoming amino groups.

**Scheme 1**




These problems highlight the need to develop additional mild, efficient means for the preparation of these and other important resins for solid-phase synthesis. Furthermore, much has been theorized and reported about the role played by spacer arms and environment effects in synthesis facilitation. For example, it is known that the addition of poly(ethylene glycol) spacer arms to solid-phase resins can often greatly improve synthetic results; however, not much is yet known about the exact causes of these observed improvements.

## Results

The route outlined in Scheme 1, which avoids use of the Leuckart amination reaction, was investigated as a means to access the desired resins from the related synthons **1a** and **1c**. The preparation of (chloromethyl)-phthalimide (**1a**), which is readily commercially avail-

(1) Portions of this work were reported in preliminary form: Fifth International Symposium on Solid-Phase Synthesis & Combinatorial Chemical Libraries, London, England, Sep 2−6, 1997. Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. In *Innovations and Perspectives in Solid-Phase Synthesis and Combinatorial Chemical Libraries, 1998: Collected Papers, Fifth International Symposium*; Epton, R., Ed.; Mayflower Scientific: Kingswinford, England; in press.

(2) Present address: Siddco, Inc., 9000 S. Rita Rd., Bldg. 40, Tucson, AZ 85747.

(3) For example, see: Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743 and references therein.

(4) Review: Songster, M. F.; Barany, G. *Methods Enzymol.* **1997**, *289*, 126−174.

(5) Cook, R. M.; Adams, J. H.; Hudson, D. *Tetrahedron Lett.* **1994**, *35*, 6777−6780.

(6) Review: Barany, G.; Albericio, F.; Kates, S. A.; Kemp, M. *Polym. Prepr., Am. Chem. Soc. Div. Polym. Chem.* **1997**, in press.

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(8) (a) Mitchell, A. R.; Kent, S. B. H.; Erickson, B. W.; Merrifield, R. B. *Tetrahedron Lett.* **1976**, *42*, 3795−3798. (b) Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43*, 2845−2852.

(9) Hider, R. C.; Goodwin, B. L. PCT Int. Patent 96/25440, 1996; *Chem. Abstr.* **1996**, *125*, 248887.

(10) Zikos, C. C.; Ferderigos, N. G. *Tetrahedron Lett.* **1995**, *36*, 3741−3744.

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**Scheme 2**

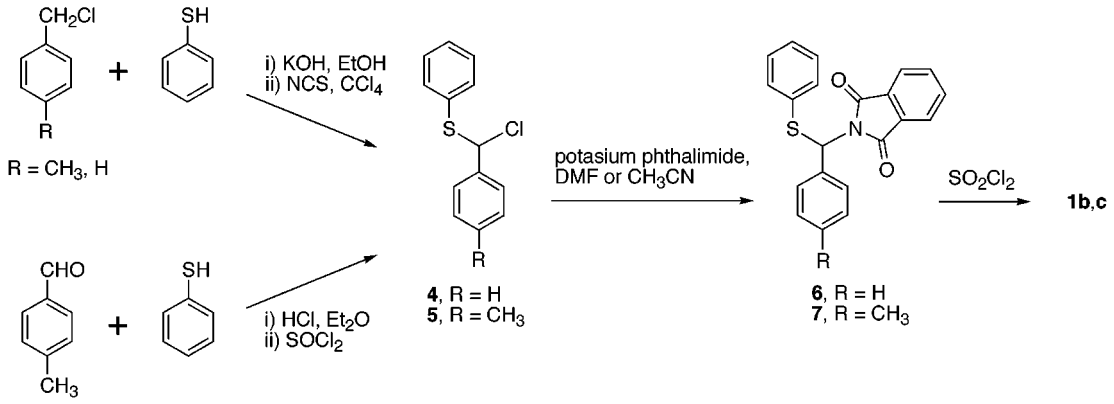


**Table 1. Optimization of Conditions for Dephthaloylation of Resins 2a and 2c**

| line | reagents and reaction condns | dephthaloylation efficiency (%) 2a → 3a | 2c → 3c |
|---|---|---|---|
| 1 | 10% hydrazine in ethanol, reflux, 6 h | 90 | 98 |
| 2 | 25% hydrazine in 1,4-dioxane, 70 °C, 18 h | 100 | 100 |
| 3 | 40% aqueous methylamine−$CH_2Cl_2$ (2:1), 25 °C, 72 h | 89 | n/d |
| 4 | 40% aqueous methylamine−$CH_2Cl_2$−1,4-dioxane (2:1:1), 25 °C, 72 h | 99 | 80 |
| 5 | 40% aqueous methylamine−1,4-dioxane (1:1), 25 °C, 72 h | 100 | 83 |
| 6 | 40% aqueous methylamine−1,4-dioxane (1:1), 55 °C, 72 h | 100 | 100 |
| 7 | 40% aqueous methylamine−1,4-dioxane (1:3), 55 °C, 72 h | 100 | 100 |

able, has been extensively studied;[11] however, no direct details are to be found in the literature concerning the preparation of synthon **1c**. A method was described by Worley[12] for the production of the related synthon **1b**,[13] which involved radical-induced chlorination of the adduct formed between thiophenol and benzyl chloride (Scheme 2, upper left). This procedure was satisfactory for the preparation of the methyl substituted variant, **1c**, starting with 4-methybenzyl chloride; however, it was considered impractical for large-scale synthesis. The alternative preparation (Scheme 2, lower left) from tolualdehyde is novel, although a similar method[14] has been applied in an analogous, poorly characterized synthesis.[15] In contrast to the literature reports, HCl gas was found to be an inefficient agent in the conversion of the initially formed hemithioacetal into chloride **3**. Addition of thionyl chloride drove the equilibrium involved to completion. The product obtained, without isolation, was then reacted with potassium phthalimide in acetonitrile, a superior solvent to the DMF previously used. Subsequent treatment of **5** with sulfuryl chloride gave **1c** in good overall yield and high purity.

Initial resin modification studies focused on preparation of aminomethyl polystyrene according to the methods of Mitchell,[8] but the transformations proceeded inefficiently and gave poorly swelling products. The variation of Zikos and Ferderigos,[10] which used **1a** with ferric chloride as the Lewis acid, followed by dephthaloylation with hydrazine in refluxing EtOH, was a considerable improvement, although dephthaloylation was incomplete (Table 1, line 1). Substitution of 1,4-dioxane for EtOH in combination with increased hydrazine excess provided quantitative removal (Table 1, line 2); however, removal of the highly insoluble byproduct bis(hydrazide) was still nearly impossible. The relatively mild methylamine-based reagent and conditions of Skorna et al.[16] (Table 1, line 3) formed two phases but was comparable in efficiency to hydrazine in EtOH and eliminated problems with byproduct removal. Further refinement resulted from the addition of 1,4-dioxane to give a single-phase reagent (Table 1, line 4), but 1,4-dioxane−methylamine (with no $CH_2Cl_2$) was an even superior reagent, giving excellent results at room temperature (Table 1, line 5) and forming a readily separable byproduct.

Ferric chloride proved a disappointing selection for the analogous preparation of MBHA resin **3c**, resulting in inefficient incorporations of **1c** and poorly swelling, discolored products. Alternative Lewis acids were investigated, and titanium tetrachloride gave the best substitution level, swelling properties, and product appearance; dichloroethane proved a better solvent for the reaction than $CH_2Cl_2$. In this preparation of resin **3c**, as well as with resin **3a**, complexes are formed between the polystyrene products and the Lewis acid employed, and careful washing protocols are necessary, as detailed in the Experimental Section, to ensure their complete elimination (as confirmed by negligible ash contents found during elemental analyses). Dephthaloylation conditions, again, required refinement. Hydrazine was a more effective reagent in this series (Table 1, lines 1 and 2), whereas methylamine cocktails at ambient temperature were less effective (Table 1, line 4). Although hydrazine in 1,4-dioxane at elevated temperature worked well, equivalent results were obtained with methylamine at 55 °C for 3 days (Table 1, line 6). The application of 1,4-dioxane−40% aqueous methylamine (3:1) in both series provided a more effective, safer, and more eco-

(11) (a) Sakellarios, E. J. *J. Am. Chem. Soc.* **1948**, *70*, 2822. (b) Truchlik, S.; Macko, J.; Mojik, I.; Bytricky, L.; Dulak, K.; Paldan, M.; Handlovsky, A. CS Patent 248 250, 1988; *Chem. Abstr.* **1988**, *110*, 75315.

(12) Worley, J. W. *J. Org. Chem.* **1979**, *44*, 1178−1180.

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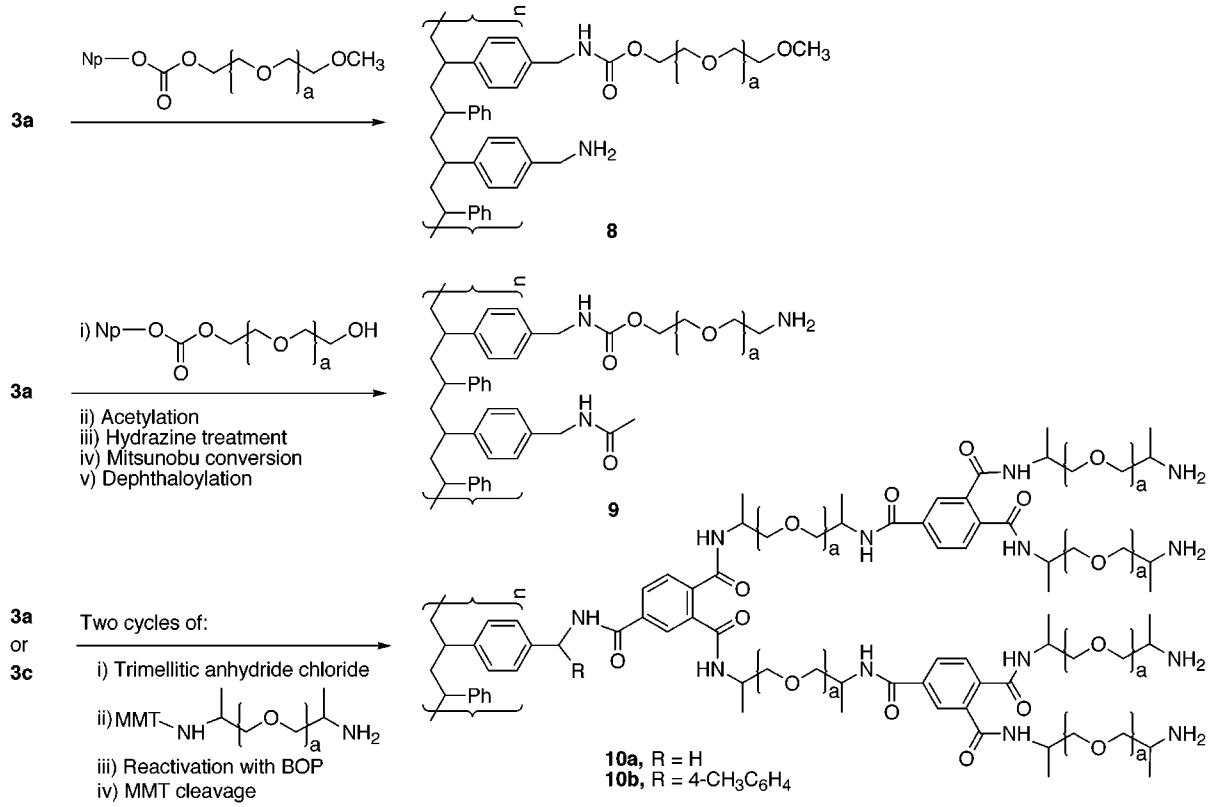
(14) Bordwell, F. G.; Pitt, B. M. *J. Am. Chem. Soc.* **1955**, *77*, 572.

(15) Tuleen, D. L.; Markham, V. C. *J. Org. Chem.* **1967**, *32*, 204.

(16) Skorna, G.; Stemmer, R.; Ugi, I. *Chem. Ber.* **1978**, *111*, 806−810.

**Scheme 3**




nomical reagent than hydrazine for dephthaloylation. A very close correlation has been found between values for nitrogen content determined by either a derivatization assay (i.e., Fmoc-Nle-OH loading) or elemental analysis for both **3a** and **3c**, indicating that the functionalization produced is accessible and reactive.

Three types of novel PEG graft copolymers have been prepared using amino-functionalized resins **3a** and **3c** (Scheme 3).[17] The most simple, **8**, was prepared by *p*-nitrophenyl (Np) chloroformate-mediated exact underloading of monomethoxy-PEG onto the amino groups of the 2 mmol/g AMPS resin (**3a**), producing a highly stable urethane bond. Adjustment of the molecular weight of the PEG, and ratio to the amount of resin, allows the production of resins of various compositions. The procedure described herein (see Experimental section) reproducibly gives close to 60% PEG content in the resulting copolymer with MeO−$PEG_{2000}$ and an amino substitution of 0.4 mmol/g. The chemistry involved has been modified further to permit attachment of PEG-diols; subsequent transformation of the pendant hydroxyl group to an amino functionality, via Mitsunobu chemistry, provides **9** (Scheme 3). An alternative multibranched variation, **10**, based on a low-loaded resin, was also prepared, using trimellitic anhydride chloride to establish a branching point for the bis-addition of PEG-diamines [partially protected at one terminus with a monomethoxytriphenylmethyl (MMT) group].[5]

With these materials at hand, we next sought to characterize their properties and relative synthetic utility. The qualitative observation of Zikos and Ferderigos[10] that a 2 mmol/g AMPS resin, produced by their similar approach using ethanolic hydrazine dephthaloylation, had excellent swelling in $CH_2Cl_2$ led us to measure the swelling of all our resins against a panel of six solvents ranging in polarity from THF to MeOH (Table 2).[18]

The synthetic usefulness of all of these resins has been assessed and directly compared in a variety of tests (Tables 3 and 4). The simultaneous comparison technique[19,20] is ideally suited as all reactions are performed under identical conditions. Surprising results were found in experiments designed to incorporate Fmoc-LinkerAm [4-[(*R*,*S*)-α-[1-(9*H*-fluoren-9-yl)methoxycarbonylamino]-2,4-dimethoxybenzyl]phenoxyacetic acid, also known as Rink linker] to a level of 0.2 mmol/g onto resins having widely ranging levels of amino functionalization (Table 3). These linker-substituted materials were desired to allow synthesis comparisons at similar loading levels. Very poor incorporation efficiencies were apparently obtained with the high loaded supports studied (lines 7, 9, and 11), with 2 mmol/g AMPS giving no detectable Fmoc incorporation (line 9). These findings are to be contrasted with the excellent loading that can be achieved when total substitution is desired (lines 2, 6, and 8). Presumably, premature removal of the Fmoc group occurred, after linker addition, as a result of the high concentration of unreacted primary amino groups. This conclusion is supported by the observation that the 2 mmol/g AMPS promoted rapid Fmoc removal from a variety of neutral derivatives and multifunctional scaffolds in neat DMF solution (e.g., cleavage of *N*-Fmoc-1-

(17) The PEG-PS graft copolymers have been assigned the following trade names: Champion I (**8**), Champion II (**9**), and Dendrogel (**10**).

(18) Solvation of peptide resin matrices has been studied in a wide range of solvents, see: (a) Fields, G. B.; Fields, C. G. *J. Am. Chem. Soc.* **1991**, *113*, 4202. (b) Cilli, E. M.; Oliveira, E.; Marchetto, R.; Nakaie, C. R. *J. Org. Chem.* **1996**, *61*, 8992−9000.

(19) Hudson, D. *J. Org. Chem.* **1988**, *53*, 617−624.

(20) Meister, S. M.; Kent, S. B. H. In *Peptides: Structure and Function: Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce: Rockford, IL, 1983; pp 103−106.

**Table 2. Swelling Characteristics of Selected Resins in Various Solvents**

| line | resin | notes | swollen volume (mL per g dry resin)[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | THF | $CHCl_3$[b] | DMF | DMSO | $CH_3CN$ | methanol |
| 1 | polystyrene | 1% DVB; native | 7.9 | 7.8 | 5.0 | 2.7 | 2.6 | 2.2 |
| 2 | **3c** (MBHA)[c] | 0.9 mmol/g | 9.0 | 8.8 | 6.2 | 2.6 | 2.5 | 2.3 |
| 3 | **3a** (AMPS)[c] | 1 mmol/g | 8.4 | 8.5 | 6.5 | 3.9 | 3.4 | 3.0 |
| 4 | **3a** (AMPS)[c] | 1.5 mmol/g | 8.6 | 8.6 | 8.1 | 6.0 | 4.0 | 3.0 |
| 5 | **3a** (AMPS)[c] | 2 mmol/g | 8.2 | 8.5 | 7.6 | 6.9 | 3.5 | 3.2 |
| 6 | **8** | 63% PEG; 0.39 mmol/g | 5.9 | 7.9 | 6.5 | 6.4 | 6.0 | 5.0 |
| 7 | **9** | 56% PEG; 0.27 mmol/g | 6.3 | 8.1 | 6.1 | 5.5 | 4.9 | 4.3 |
| 8 | **10b** | 61% PEG; 0.20 mmol/g | 5.8 | 5.9 | 5.5 | 4.8 | 4.4 | 3.7 |
| 9 | ArgoGel | PEG content unknown; 0.40 mmol/g | 6.4 | 8.8 | 6.8 | 6.5 | 6.5 | 6.0 |
| 10 | TentaGel | 75% PEG; 0.20 mmol/g | 4.0 | 4.5 | 4.0 | 3.7 | 3.7 | 3.5 |

[a] Values given were averaged from multiple determinations ($n \geq 3$, SD $\leq \pm 0.2$ mL). [b] Methylene chloride gives values similar to those from $CHCl_3$. [c] Corresponding resins obtained from other sources were also evaluated and found to swell to a lesser extent in THF, $CHCl_3$, DMF, and DMSO.

**Table 3. Addition of Fmoc-LinkerAm to Amino-Substituted Resins**

| line | resin | amino loading (mmol/g) | target loading (mmol/g) | obsd loading (mmol/g) | efficiency of linker addition (%) |
|---|---|---|---|---|---|
| 1 | ArgoGel | 0.40[a] | 0.20 | 0.11 | 55 |
| 2 | TentaGel | 0.21[a] | max | 0.18[b] | n/a |
| 3 | **10b** | 0.23 | 0.20 | 0.12 | 60 |
| 4 | **8** | 0.37 | 0.20 | 0.13 | 65 |
| 5 | **9** | 0.27 | 0.20 | 0.19 | 95 |
| 6 | **3a** (AMPS) | 1.0 | max | 0.60[b] | n/a |
| 7 | **3a** (AMPS) | 1.0 | 0.20 | 0.06 | 30 |
| 8 | **3a** (AMPS) | 2.0 | max | 0.95[b] | n/a |
| 9 | **3a** (AMPS) | 2.0 | 0.20 | 0 | 0 |
| 10 | **12**[c] | 2.0 | 0.26 | 0.24 | 92[d] |
| 11 | ArgoPore | 0.74[a] | 0.20 | 0.05 | 25 |

[a] Loadings for these resins are as reported by the respective manufacturers. [b] The observed loading was obtained after coupling with 1.1−1.5 equiv of Fmoc-LinkerAm (at which point the resin was completely ninhydrin negative). [c] Resin **12** was obtained from standard AMPS resin **3a** (2 mmol/g) by underloading as described in Scheme 4. [d] This combined value reflects the accuracy of stoichiometric underloading and linker addition.

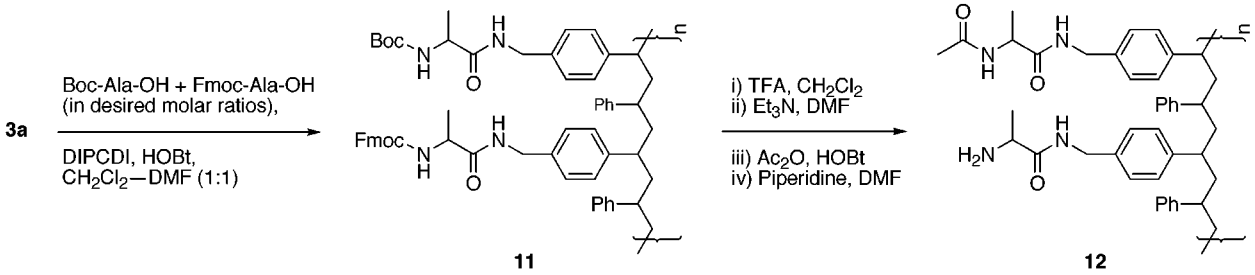
**Table 4. Evaluation of Resin Performance in Various Synthetic Procedures**

| line | resin[c] | synthesis of ACP 65-74 decapeptide test sequence[a] | | use of Aloc/OAl protection during peptide synthesis[b] | | stilbene synthesis | | α-CT digestion of Trp-Gly-derivatized resins |
|---|---|---|---|---|---|---|---|---|
| | | purity with 1−2 h couplings[d] (%) | purity with 30 min couplings[d] (%) | purity of allyl-protected peptide[e] (%) | allyl removal efficiency[e,f] (%) | yield[g] (%) | purity[h] (%) | amt cleaved (%) |
| 1 | ArgoGel | 90 | 90 | 90 | >95[i] | 80[i] | 40 | 1.5[i] |
| 2 | TentaGel | 85[j] | 90[j] | 95 | >95 | 300[j] | >90[j] | 6.0 |
| 3 | **10b**[k] | 85[j] | 90[j] | | | | | |
| 4 | **8** | 85 | 90 | 95 | >95 | 75 | >90 | 1.5 |
| 5 | **9** | 85 | 85 | 85 | >95 | 50 | >90 | 6.0[l] |
| 6 | **3a** (underloaded to 0.06 mmol/g)[m] | 85 | 85 | | | | | |
| 7 | **3a** (1 mmol/g) | | 75 | | | | | |
| 8 | **12**[n] | | 90 | 90 | >95 | 75 | >90 | <0.5 |
| 9 | ArgoPore | 75 | 75 | | | 55 | >90 | 4.5 |
| 10 | Macroreticular PS (300 Å) | | 70 | | | 50 | >90 | 5 |

[a] The sequence of the ACP 65-74 decapeptide is VQAAIDYING. [b] The pentapeptide sequence used for this test was Y(*t*-Bu)K-(Aloc)E(OAl)K(Boc)G. [c] The Fmoc-LinkerAm-resins used for the synthesis of the ACP 65-74 decapeptide were the products described in Table 3, except for entry 10, which was maximally loaded to 220 μmol/g; all other tests in this table were performed using maximally loaded resin samples. [d] Purities of the cleaved peptides were estimated by integration of the HPLC profiles of the crude products obtained directly from ether precipitation of the TFA cleavage cocktails. [e] Purities of the cleaved peptides were estimated by integration of the HPLC profiles of the crude products obtained directly from evaporation of the TFA−water cleavage cocktails. [f] Efficient allyl removal was found to be very dependent on the quality of the tetrakis(triphenylphosphine)Pd(0) complex. [g] Yields are based on the theoretical weight of product calculated from the amino substitution of the starting resin. [h] Purities were estimated by TLC of the crude products on silica gel $GF_{254}$ developed in toluene−1,4-dioxane−acetic acid (95:25:4); the *trans*-stilbene product obtained photodimerized quite readily giving a lower $R_f$ impurity, which was counted as "product" for these calculations. [i] The resin beads were observed to undergo agglomeration during the evaluation procedure. [j] The crude product also contained other byproducts resulting from PEG cleavage by TFA. These impurities are not included in HPLC or TLC estimates of purity but do contribute to the >100% mass yield in the stilbene synthesis test. [k] The formulation of resin **10b** used in these comparisons was based on a 0.45 mmol/g MBHA resin. [l] A value of 12% cleavage was observed for a 200−400 mesh version of resin **9** under similar conditions. [m] Entry 6 is the resin described in Table 3, line 7. [n] Resin **12** was obtained from standard AMPS resin **3a** (2 mmol/g) by underloading as described in Scheme 4.

amino-6-hexanol by the resin had $t_{1/2} = 4$ h). To avoid these problems, an alternative procedure was developed (Scheme 4) for underloading the 2 mmol/g AMPS resin, giving resin **12**. The method involved coupling a stoichiometrically known mixture of Boc- and Fmoc-amino acids and relied on the equal reactivity of the two derivatives. A homogeneous distribution of amino sites within beads is thus ensured, which may not necessarily result from the alternative underloading method (i.e., coupling with less than 1 equiv of linker followed by

**Scheme 4**




capping unreacted amino groups). Resin **12** also maintains the excellent swelling characteristics of the parent 2 mmol/g AMPS resin (data not presented). For all linker addition reactions to AMPS resins, the use of carbodiimide coupling in the presence of excess 1-hydroxybenzotriazole (HOBt) is recommended. Representative base-labile, acid-labile, and photolabile linkers have been attached to 1 and 2 mmol/g AMPS resins, with excellent incorporations observed using very modest excesses of reagents. For example, the substitution of 1 and 2 mmol/g AMPS with just a 10% excess of Fmoc-LinkerAm using DIPCDI, HOBt-mediated coupling resulted in quantitative addition and resins that gave negative ninhydrin tests (Table 3, lines 6 and 8). These linker-functionalized resins retain swelling profiles similar to those of the parent resins (data not presented).

Two sets of simultaneous syntheses (Table 4) were performed with the "notorious" ACP 65-74 decapeptide test sequence, reportedly difficult to synthesize because of the combined effects of interpeptide aggregation and steric hindrance.[21] Control supports, macroporous polystyrenes (lines 9 and 10) and a more highly loaded amino-functionalized polystyrene (line 7), gave inferior results; loading was clearly a critical issue. However, no significant difference could be distinguished between any of the PEG graft copolymers studied (lines 1 to 5), even with brief 30 min couplings for all residues using the modestly active (benzotriazol-1-yl-*N*-oxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP)/HOBt/*N*-methylmorpholine (NMM) procedure at suboptimal concentration. Even more remarkable, the underloaded AMPS resin **12** (line 8), prepared by the method shown in Scheme 4, synthesized the target in just as high yield and efficiency (as judged by HPLC and MS analyses).

In further tests (Table 4), a pentapeptide bearing both allyl[22] [i.e., allyloxycarbonyl (Aloc) for Lys and allyl ester (OAl) for Glu] and *tert*-butyl side-chain protecting groups was synthesized with good efficiency on several of these supports. Allyl removal was >95% complete on all resins tested. Although incomplete under the suboptimal conditions employed, the similarity in cleavage efficiencies showed that allyl removal proceeded at closely similar rates with all of the resins studied. Activation of the product resin-bound, partially protected peptides with BOP, to establish an amide bond between the deprotected side-chain Lys and Glu functional groups, gave similar product distributions upon cleavage. Intramolecular cyclization between the side chains predominated, but numerous oligomeric species were generated through interpeptide interactions. Additionally, Trp-Gly-functionalized versions were subjected to digestion with α-chymotrypsin (α-CT), a method that has been used to "shave" external regions of TentaGel beads.[23] Detectable cleavage occurred with the macroreticular PS resins (Table 4, lines 9 and 10), but none could be detected with **12** (line 8), which does not swell in water. In the PEG graft copolymer series, cleavage was less with ArgoGel and **8** (lines 1 and 4) than with TentaGel or **9** (lines 2 and 5).

Standard phosphoramidite-mediated DNA syntheses were performed using **8**, derivatized with DMT-T-succinate to a level of 50 $\mu$mol/g, comparing synthesis efficiency to results obtained with a panel of alternative standard support materials: controlled pore glass (CPG), macroreticular polystyrene, and polymethacrylate.[24] Average coupling efficiencies and product purities in the syntheses of test sequences were closely comparable for all these materials.

The usefulness of the subject resins for combinatorial chemistry was initially demonstrated with 2 mmol/g AMPS using a multistep protocol, which included a Horner−Emmons condensation, to form a stilbene analogue (Scheme 5).[25] The immobilization chemistry is a variant of a method described by Ellman et al.,[26] which involved the off-resin production of a corresponding substituted linker allyl ester. Noteworthy aspects of our synthesis include the completeness of the linker addition (**13a** → **14a**), subsequent bromination (giving **15a**), and displacement reactions (to **16a**). A good yield of column-purified stilbene **19** was obtained. Representative alternative supports (Table 4) were then taken through a

(21) The original publication describing synthetic difficulties with acyl carrier protein 65−74 was: Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem.* **1973**, *38*, 774−781. Improved protocols were later described on polyacrylamide based supports: Arshady, R.; Atherton, E.; Clive, D. L. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1981**, 529−537. Other studies showed that, for Boc chemistry, implementing coupling in DMF was the primary modification necessary: Live, D. H.; Kent, S. B. H. In *Peptides: Structure and Function: Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce: Rockford, IL, 1983; 65−68. This observation was later confirmed by detailed study with Fmoc chemistry on polystyrene (see ref 19). Nevertheless, this sequence still poses significant challenges; see, for example: Alewood, P.; Alewood, D.; Miranda, L.; Love, S.; Meutermans, W.; Wilson, D. *Methods Enzymol.* **1997**, *289*, 14−29 and other examples in this volume.

(22) Lyttle, M. H.; Hudson, D. In *Peptides: Chemistry and Biology: Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, The Netherlands, 1992; pp 583−584.

(23) Vágner, J.; Krchňák, V.; Sepetov, N. F.; Štrop, P.; Lam, K. S.; Barany, G.; Lebl, M. In *Innovations and Perspectives in Solid-Phase Synthesis: Peptides, Proteins and Nucleic Acids: Biological and Biomedical Applications, 1994: Collected Papers, Third International Symposium*; Epton, R., Ed.; Mayflower Worldwide: Birmingham, England, 1994; pp 347−352.

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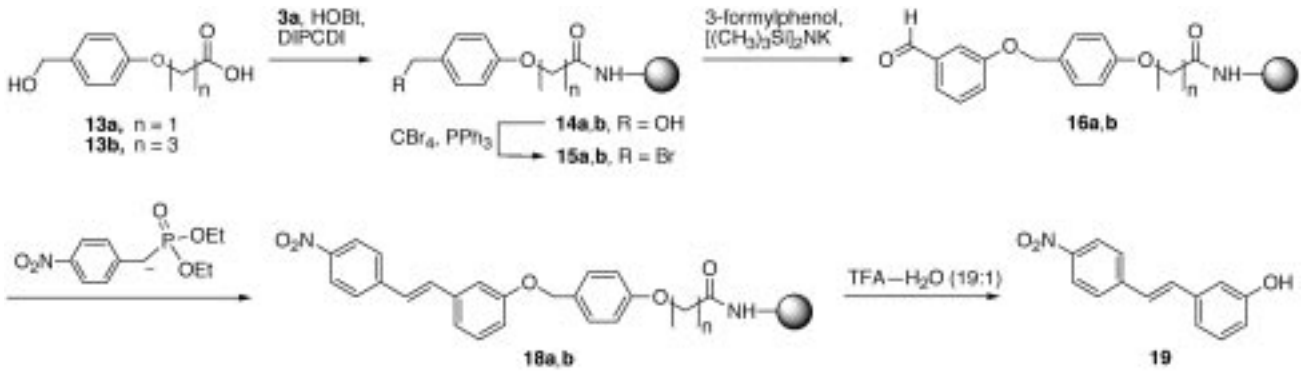
**Scheme 5**




similar five-step protocol, but using linker **13b** with abbreviated reaction times and no intermediate monitoring, in an attempt to determine differences in their synthetic performance. Indeed, significantly worse product purity was observed for the ArgoGel-mediated reaction (Table 4, line 1). In addition, a large proportion of PEG was cleaved from the TentaGel resin (Table 4, line 2). Of the unmodified 1% cross-linked polystyrenes, the underloaded 2 mmol/g AMPS was marginally superior to the fully loaded version. Both of the macroreticular PS resins (Table 4, lines 9 and 10) performed very well, but with lower overall yields.

## Discussion

Convenient, practical, and economical large-scale methods for the preparation of AMPS and MBHA resins are described. By careful optimization of resin modification chemistry, combining mild conditions with efficient conversions, products endowed with excellent swelling characteristics have been obtained. Dramatic changes in the swelling profiles were obvious with progressively higher loaded AMPS; swelling reached a plateau level at a substitution of 1.5 mmol/g for THF, $CHCl_3$, DMF, and DMSO (Table 2, line 4), with no significant improvement found for the 2 mmol/g loaded resin (Table 2, line 5), which represents a practical ceiling for the use of this resin and is a substitution level beyond which incorporations became progressively less efficient. DMSO, a solvent in which the parent PS swells negligibly (Table 2, line 1),[27] is particularly useful for promoting nucleophilic substitution reactions, and the improved swelling is expected to be beneficial. Similar swelling profiles are retained in acetylated, protected amino acid and handle substituted forms, suggesting that the phenomenon does not simply result from the presence of polar free amino groups and that good swelling should be maintained during small molecule assembly. This conclusion was substantiated by a stilbene synthesis involving the Horner−Emmons condensation reaction (Scheme 5). However, a note of caution regarding unexpected interresidue side reactions is raised by our observations of premature Fmoc removal mediated by pendant amino groups on the 1 and 2 mmol/g AMPS. Their use for high-load peptide synthesis by Fmoc chemistry, particularly when base-mediated "onium"-type coupling reactions are employed, is not generally recommended; premature Fmoc removal, as previously discussed,[28] will lead to numerous byproducts. Because benzylamine itself is not known to be an effective reagent, pendant amino groups appear to be able to act in consort; clearly "pseudo-dilution" or effective site-isolation effects are not operable. Carbodiimide-mediated coupling in the presence of excess HOBt is strongly recommended for linker addition and chain assembly on these highly substituted resins.

The syntheses of peptides and DNA by solid-phase methodology is well-known to be very dependent on the nature of the support. Recent reports describe similar variations for other chemical transformations.[29] Many practitioners of the art of performing organic transformations on resin-bound substrates have elected to use PEG-PS graft copolymers and describe consequent improvements in synthesis efficiency.[6,30] In addition to bearing what have been thought to be beneficial spacer arms, they are pressure stable, can advantageously be used in column reactors, and have been proven to be generally useful. Such graft copolymers can be classified into two types: those that incorporate PEG by in situ polymerization of ethylene oxide onto hydroxyl groups on the polystyrene backbone, such as TentaGel,[31] and the recently introduced branched variant ArgoGel,[32] and those that incorporate preformed, appropriately protected PEGs onto amino-modified resins, such as with PEG-PS.[33,34] Remarkably, the simple PEG-derivatized resin, **8** (Table 2, line 6) has a similar swelling profile to that of the more complex ArgoGel (Table 2, line 9). Deductions made from the swelling properties of products from different base resins are complicated by many considerations, for example, variation in particle size and particle size

(27) Reports have described improvement in peptide synthesis using DMSO on a highly loaded benzhydrylamine resin, even though the starting resin did not swell in this solvent; see: Marchetto, R.; de Oliveira, E.; Paiva, A. C. M.; Nakaie, C. R. In *Peptides 1990: Proceedings of the Twenty-First European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, The Netherlands, 1990; pp 122−124.

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distribution may confer different packing characteristics. Clearly, however, all three novel PEG graft copolymers possessed excellent swelling characteristics.

Study of the relative synthetic efficiency of the two closely related resins, **8** and **9**, for peptide synthesis was expected to provide an answer to the question of whether the PEG in such graft copolymers serves principally to modify the environment[35] or whether the spacer arm effect is additionally beneficial. Our results (Table 4) found no significant difference between **8** and **9**, which bear backbone and PEG terminal amino groups, respectively. These findings are in accord with qualitative studies by Barany et al.,[6] who obtained excellent synthetic results with a branched PEG-PS resin prepared as follows: (i) coupling Fmoc-Orn(Boc)-OH to 1 mmol/g AMPS; (ii) removal of the Fmoc group; (iii) attachment of a monomethoxy-PEG acid derivative; and (iv) peptide synthesis on the Orn side-chain following Boc deprotection. With resin **8**, synthesis is performed on aminomethyl groups distant from the site of PEGylation, not on amino groups proximal to a branch point. This formulation is simple and reproducible, and the PEG-urethane attachment linkage is highly stable to aggressive reagents (even if some leaching were to occur no loss of product would result).

Furthermore, the studies provide a valid comparison of both **8** and **9** with a similarly loaded amino-functionalized polystyrene, **12**, which is derived from the same parent AMPS resin but lacks PEG derivatization. This resin performed equivalently in all but the enzymatic hydrolysis comparisons. We conclude that for the chemically mediated transformations studied PEG does not play an active role in promoting synthesis efficiency, neither by its influence on the microenvironment nor by its spacer arm effect. Appropriate loading, the maintenance of good swelling characteristics, and the absence of extraneous cross-linking or other undesired functionality on the resin are the principal factors responsible for good synthesis efficiency. Another important influence is the poorly understood phenomenon of bead aggregation, which results in poor reaction efficiency and inefficient removal of reactants during washing. This undesirable effect was only significant for one of the supports studied (Table 4, line 1). Instability of the PEG attachment during synthesis and cleavage is also highly undesirable and was observed in two cases (Table 4, lines 2 and 3). Regarding peptide chain assembly, clearly the ACP 65-74 test decapeptide is not, intrinsically, difficult to synthesize. Because little difference was observed in product purity on the optimized supports with the different coupling times studied, the half-lives of the coupling reactions must, even for the sterically hindered residues incorporated, be very short indeed. Diminished coupling rates, resulting from steric effects or peptide aggregation mediated by hydrogen bonding or hydrophobic effects, were obvious at high loading with **3a** (Table 4, line 7) and the structurally dissimilar macroreticular resins (Table 4, lines 9 and 10). In addition, our results failed to confirm literature reports that allyl removal by Pd(0)-mediated transfer is facilitated on PEG-substituted polystyrenes over unmodified polystyrenes.[22,36] The similarity in product profiles from the side-chain cross-linking experiment on the partially protected peptide products resulting from allyl removal provided further confirmation of the comparable efficiency of the resins, and the extent of interpeptide reactions provided no evidence for site isolation.

The novel graft copolymer **8** gave excellent results in the phosphoramidite-mediated synthesis of a DNA target sequence. The application of this and other PEG-PS graft copolymers is truly beneficial for oligonucleotide assembly,[37] as compared to unmodified 1% cross-linked polystyrene, because rapid reactions are required in a range of solvents (e.g., acetonitrile, THF−water, and $CH_2Cl_2$). Any reaction requiring aqueous reagents will also be facilitated on PEG supports, as evidenced by a recent example involving a Suzuki cross-coupling reaction of an immobilized arylboronic acid with a variety of substituted aryl iodides.[38] Last, the ability of α-chymotrypsin to partially cleave a resin-immobilized peptide indicates PEG-PS copolymers are superior for bead-based assays because these resins allow reasonably efficient interactions between displayed ligands and their biological targets; in this application, PEG spacer arms do have a significant effect.

## Conclusions

Careful initial derivatization and subsequent modification provided improved resins possessing excellent swelling properties and performance characteristics. All PEG-derivatized polystyrenes tested performed similarly. Our results provide experimental confirmation of the well-established belief that resins that swell better provide better synthetic reactivity. In all probability, reported synthesis facilitation observed with PEG-polystyrenes results, simply, from the maintenance of good swelling during all stages of synthetic processes, rather than from spacer arm or environment effects. Of the novel PEG graft copolymers described, the simplest variant, **8**, proved both stable[39] and the most generally useful,[40] whereas the multibranched PEG-PS resins, ArgoGel and the novel dendromeric **10**, although for the most part excellent, had no obvious advantages.

## Experimental Section

**General Methods.** Reagents were supplied by Aldrich (Milwaukee, WI); all resins and linkers were supplied by Solid Phase Sciences (Novato, CA), except for ArgoGel and ArgoPore from Argonaut Technologies (San Carlos, CA), and TentaGel from Rapp Polymere (Tubingen, Germany); BOP and Fmoc-amino acids were supplied by Chem Impex (Wood Dale, IL). NMR spectra were provided by Acorn NMR (Fremont, CA), and MS analyses were performed on a Voyager MALDI-TOF spectrometer at the University of Michigan (Ann Arbor,

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(37) Resin **10b** has proved an efficient support for the assembly of a variety of complex peptide−DNA hybrids: Songster, M. F. Solid Phase Sciences, unpublished results.

(38) Ruhland, B.; Bombrun, A.; M. Gallop, M. A. *J. Org. Chem.* **1997**, *62*, 7820−7826.

(39) Negligible PEG loss was observed with either 2 h TFA treatment or concentrated $NH_3$ at 55 °C for 6 h.

(40) In addition to the detailed experiments presented, resin **8** has been used to synthesize many complex biomolecules, including a 68-residue peptide, and a variety of unmodified and fluorescently labeled DNA sequences.

MI). Analytical HPLC analyses were performed as reported previously,[19] but using a Waters 996 photodiode array detector, with data processed at 220 nm using the Waters Millennium system (integration values are quoted in increments of 5% and are significant to ±2.5%). Peptide syntheses were performed as described previously,[19] except 2 or 10 mL "syringe" reactors[41] were used in place of macro DNA synthesis columns. Elemental analyses were provided by Desert Analytics (Tuscon, AZ). Amino substitution levels were also determined by a derivatization procedure in which excess Fmoc-Nle-OH was coupled to the resin in a 2 mL syringe reactor for 2 h. The resin was thoroughly washed and dried, and then aliquots (ca. 5−10 mg) were accurately weighed into glass scintillation vials, treated with 30% piperidine in DMF (0.5 mL) for 10 min with vortex mixing, and diluted with MeOH (20 mL), and the absorption was determined at 301 nm. Quantitation was achieved by comparison with known standards, providing a calibration curve to correct for the weight increase resulting in derivatization and thereby giving original amino content. All amino acids used were of the L configuration; all solvent ratios are volume/volume.

***N*-(α-Chloro-4-methylbenzyl)phthalimide (1c).** *Cautionary note: the following procedure should be performed in a fume hood because of the toxic and irritant nature of the starting materials and byproducts.* Phthalimido derivative **7** (660 g, 1.8 mol) was stirred in $CH_2Cl_2$ (3 L) under argon, and sulfuryl chloride (150 mL, 250 g, 1.9 mol) was added dropwise over 3 h, turning the mixture bright red. The reaction was stirred overnight and then filtered and evaporated to about 1 L under reduced pressure. Petroleum ether (2 L) was added, and the mixture was chilled at −15 °C overnight. A voluminous solid was collected in a 3 L sintered glass funnel, washed with cold petroleum ether (2 L), and dried to a constant weight under high vacuum to give 395 g (85%) of a light yellow solid: mp 110−113 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.9 (dd, 2H), 7.75 (dd, 2H), 7.6 (d, 2H), 7.23 (s, 1H), 7.2 (d, 2H), 2.4 (s, 3H). Anal. Calcd for $C_{16}H_{12}NO_2Cl$: C, 67.26; H, 4.23; N, 4.90. Found: C, 67.40; H, 4.24; N, 5.17.

**(Aminomethyl)polystyrene (AMPS, 3a).** Different loadings, up to 2 mmol/g, are obtained by scaling the amount of (chloromethyl)phthalimide used; the following procedure, to obtain 1 mmol/g, is typical. Underivatized 1% cross-linked 100−200 mesh polystyrene (1.6 Kg) was placed in a 22 L reactor equipped with argon inlet, addition funnel, mechanical stirrer, reflux condenser, and heating mantle. Anhydrous $CH_2Cl_2$ (14 L), (chloromethyl)phthalimide (**1a**, 340 g, 1.8 mol), and anhydrous ferric chloride (68 g) were added, and the suspension was stirred under argon at reflux for 7 h, at which time all HCl evolution had ceased. The reaction was cooled overnight and the suspension filtered, and the beads were washed successively with $CH_2Cl_2$ (4 L), 1,4-dioxane (6 L), 1,4-dioxane−10% HCl (4:1, 6 L), 1,4-dioxane−water (4:1, 6 L), and MeOH (6 L). The resin was air-dried (giving 2.0 kg) and then returned to the reactor, resuspended in 1,4-dioxane (14 L), and stirred until fully swollen. Aqueous methylamine (40%, 4.5 L) was added and the reaction stirred at 25 °C for 3 days. The suspension was filtered, and the beads were washed with 1,4-dioxane−water (4:1, 6 L), 1,4-dioxane (6 L), DMF (6 L), $CH_2Cl_2$ (12 L), and MeOH (20 L). The resin was again air-dried, then passed through sieves to remove particles outside the 100−200 mesh range, and finally dried to constant weight in vacuo to provide 1600 g of AMPS resin: Elemental analysis found 1.5% N (corresponding to a loading of 1.07 mmol/g) and <0.05% Cl; Fmoc loading assay gave 1.00 mmol/g.

**4-Methylbenzhydrylamine Polystyrene (MBHA, 3c).** Underivatized 1% cross-linked 100−200 mesh polystyrene (40 g) was placed in a 1 L reactor equipped with argon inlet, addition funnel, mechanical stirrer, reflux condenser, and heating mantle. Anhydrous dichloroethane (400 mL), phthalimido derivative **1c** (16 g, 56 mmol), and titanium tetrachloride (1.6 mL, care must be observed when handling this reagent) were added, and the suspension was stirred under argon at reflux for 7 h, at which time all HCl evolution had ceased. The reaction was worked up analogously to the method described above for **3a**, except that dephthaloylation was performed with 1,4-dioxane−40% aqueous methylamine (3:1) at 55 °C for 3 days to give 44 g of product: Elemental analysis found 1.2% N (corresponding to a loading of 0.84 mmol/g) and 0.10% Cl; Fmoc loading assay gave 0.92 mmol/g. Higher loading levels can be achieved with a greater excess of **1c** but the reaction becomes progressively more inefficient.

***N*-[α-(Phenylthio)-4-methylbenzyl]phthalimide (7).** *Cautionary note: the following procedure should be performed in a fume hood because of the toxic and irritant nature of the starting materials and byproducts.* Thiophenol (420 mL, 460 g, 4.2 mol) was dissolved in $Et_2O$ (3 L) in a 5 L three-neck flask equipped with an HCl gas inlet bubbler, 500 mL dropping funnel, and argon inlet. The stirred solution was cooled in a dry ice−water bath for 1 h under argon, and HCl was bubbled in for 30 min before tolualdehyde (480 mL, 490 g, 4.1 mol) was finally added dropwise over 3 h; slow HCl addition was continued during aldehyde addition and for 2 h afterward. The reaction was stirred overnight and allowed to warm to 25 °C. Next, thionyl chloride (300 mL, 490 g, 4.1 mol) was added dropwise over 3 h and the solution stirred for 24 h, during which time the argon was turned off to allow the evolved gas ($SO_2$) to escape through the argon bubbler. The dark solution was poured cautiously into a 5 L round-bottom flask and the $Et_2O$ removed by vacuum transfer (in the hood). The resulting green oil, which contained intermediate **5**, was dried under high vacuum (with a NaOH trap) for 1 h. Acetonitrile (3 L) and potassium phthalimide (760 g, 4.1 mol) were then added, accompanied by evolution of heat and a color change from dark brown to pale yellow; after natural heat evolution subsided, the solution was heated to reflux overnight. The brown solution was cooled, most of the acetonitrile removed under reduced pressure, and the resulting residue taken up in ethyl acetate (4 L) and washed with water (6 L). The aqueous phase was back-extracted with $CH_2Cl_2$ (2 L), and the two organic phases were washed separately with brine (1 L), dried over $Na_2SO_4$, and concentrated to ca. 1 L. Petroleum ether (1.4 L) was then added to each flask, and they were chilled to −15 °C for 3 days. The crystalline products were isolated by filtration, washed with cold petroleum ether, and combined to give a tan solid that was dried to a constant weight (660 g, 45% yield). An analytical sample of the product was recrystallized from ethyl acetate and petroleum ether: mp 119−121 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.76 (dd, 2H), 7.7 (d, 2H), 7.64 (dd, 2H), 7.5−7.4 (m, 2H), 7.2 (m, 3H), 7.16 (d, 2H), 6.72 (s, 1H), 2.3 (s, 3H). Anal. Calcd for $C_{22}H_{17}NO_2S$: C, 73.51; H, 4.77; N, 3.90. Found: C, 72.95; H, 4.87; N, 4.39.

**Poly(ethylene glycol)-*graft*-polystyrene (8).** Poly(ethylene glycol) methyl ether (400 g, $M_n$ ca. 2000, 200 mmol) was dried overnight in vacuo, dissolved in warm anhydrous pyridine (2.3 L), and evaporated to a viscous state. Fresh pyridine (2 L) was added, the solution evaporated to ca. 1 L, and *p*-nitrophenyl chloroformate (40 g, 190 mmol) dissolved in $CH_2Cl_2$ (150 mL) was added dropwise to the stirred solution under argon. The reaction was stirred until all the initially formed white precipitate had dissolved (4 h) and for an additional 2 h afterward, whereupon the mixture was concentrated in vacuo to ca. 700 mL. Resin **3a** (200 g, 2 mmol/g, 400 mmol) was mechanically stirred in DMF−$CH_2Cl_2$ (2:1, 2.1 L) with HOBt (30 g, 220 mol) until fully swollen, and then the activated poly(ethylene glycol) solution was poured in as a constant stream with vigorous stirring. After being stirred overnight, the resin had swollen further, adsorbing all solvent, so $CH_2Cl_2$ was added to a total reaction volume of 4 L and stirring continued for another 24 h. The suspension was filtered, and the resin beads were washed successively with DMF (3×), $CH_2Cl_2$ (3×), MeOH (3×), $Et_2O$ (2×), and petroleum ether (2×) and then dried in vacuo to constant weight yielding 535 g (corresponding to 62% PEG content) of resin **8**: Final loading was 0.39 mmol/g as determined by Fmoc loading assay.

**Poly(ethylene glycol)-*graft*-polystyrene (9).** Poly(ethylene glycol) (50 g, $M_n$ ca. 2000, 25 mmol) was dried in vacuo

(41) Krchňák, V.; Weichsel, A. S.; Cabel, D.; Flegelová, Z.; Lebl, M. *Mol. Diversity* **1996**, *1*, 149.

and dissolved in $CH_2Cl_2$–pyridine (1:1, 200 mL), and the solution was evaporated. Fresh pyridine (300 mL) was added to the viscous mixture and the concentration repeated. *p*-Nitrophenyl chloroformate (2.5 g, 12 mmol) dissolved in $CH_2Cl_2$ (13 mL) was then added dropwise over 15 min to the vigorously stirred solution. A suspension of resin **3a** (6.25 g, 2 mmol/g, 12.5 mmol) and anhydrous HOBt (2.0 g, 2.7 mmol) in DMF–$CH_2Cl_2$ (1:1, 100 mL) was added to the activated PEG mixture, and the reaction was stirred overnight. The resin beads were isolated by filtration, washed with DMF (3×), capped with a solution of acetic anhydride (0.3 M) and HOBt (0.3 M) in DMF (200 mL) for 1 h, and washed with DMF (3×), at which point the resin was ninhydrin negative. The resin was then resuspended in DMF (200 mL) and treated with hydrazine hydrate (20 mL), and the mixture was vortexed gently for 2 h. The beads were filtered, washed successively with DMF (3×) and THF (3×), transferred to a round-bottom flask equipped with a pressure-equalizing dropping funnel, and stirred in a minimum volume of THF. Triphenylphosphine (9.0 g, 34 mmol) and phthalimide (4.5 g, 31 mmol) were added, followed by the dropwise addition of DEAD (5 mL, 32 mmol). The suspension was stirred for 2 days, and then the beads were filtered and washed successively with DMF (3×) and 1,4-dioxane (3×). The intensely yellow beads were resuspended in 1,4-dioxane–40% aqueous methylamine (3:1, 200 mL) and stirred for 2 days. After filtration and washing with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), the product was dried to constant weight to yield 14 g (corresponding to 56% PEG content) of resin **9** as pale yellow beads: Final loading was 0.27 mmol/g as determined by Fmoc loading assay.

**Poly(ethylene glycol)-*graft*-polystyrene (10b).** Poly(ethylene glycol) bis(2-aminopropyl) ether (400 g, $M_r$ ca. 2000, 200 mmol) was dissolved in pyridine (1.5 L) and evaporated to about one-half volume. The solution was vigorously stirred and MMT-Cl (64 g, 200 mmol) in $CH_2Cl_2$ (500 mL) added dropwise over 1 h from a pressure-equalizing dropping funnel. Triethylamine (29 mL, 210 mmol) was added to the now dark red solution, and stirring was continued overnight. The reaction was evaporated to dryness and the residue dissolved in ethyl acetate (500 mL) and refrigerated overnight. Precipitated triethylamine hydrochloride was removed by filtration and washed with cold $CH_2Cl_2$ (500 mL). The combined filtrates were evaporated and redissolved in $CH_2Cl_2$–DMF (1:1, 500 mL) containing *N*-methylimidazole (20 mL). MBHA resin (**3c**, 44.5 g, 0.45 mmol/g) was suspended in $CH_2Cl_2$–DMF (1:1, 500 mL), diisopropylethylamine (DIEA, 10 mL, 57 mmol) and pyridine (20 mL) were added, and the stirred mixture was placed in an ice bath. Trimellitic anhydride chloride (73 g, 350 mmol) dissolved in $CH_2Cl_2$ (100 mL) was added and the suspension stirred at 25 °C for 2 h. The beads were then isolated by filtration and washed with $CH_2Cl_2$–DMF (1:1, 3×). One-half of the mono-MMT-protected poly(ethylene glycol) solution obtained above was added to the beads with thorough mixing. The reaction was transferred to a 2 L glass screw-cap bottle, which was tightly sealed and shaken overnight. BOP (32 g, 71 mmol) and DIEA (15 mL, 86 mmol) were then added, and the reaction was shaken for another 24 h. The beads were isolated by filtration and thoroughly washed with DMF (3×) and $CH_2Cl_2$ (3×). The beads were repeatedly subjected to 5 min treatments with TFA–dimethyl phosphite–$CH_2Cl_2$ (5:5:90) until a colorless filtrate was obtained (1 h) and then washed with $CH_2Cl_2$ (3×), triethylamine–DMF (1:9, 2×), and DMF (3×). The resin was then retreated with trimellitic anhydride chloride, followed by the remaining one-half of the mono-MMT-protected poly(ethylene glycol) solution, exactly as described above, including MMT deprotection and washing with triethylamine. Last, the resin was washed with $CH_2Cl_2$ (3×) and MeOH (3×) and then dried in vacuo to constant weight, giving 113 g (corresponding to 61% PEG content) of dendromeric resin **10b** as off-white beads: Final loading was 0.20 mmol/g as determined by Fmoc loading assay.

**Underloading of (Aminomethyl)polystyrene Resins. Preparation of Resin 12.** DIPCDI (3.1 mL, 20 mmol) was added to a solution of Fmoc-alanine (0.78 g, 2.5 mmol), Boc-alanine (2.8 g, 15 mmol), and HOBt (3.1 g, 23 mmol) in $CH_2Cl_2$–DMF (1:1, 50 mL) and the solution mixed in a stoppered flask for 10 min. The activated amino acid mixture was then added to a suspension of resin **3a** (5 g, 2 mmol/g) in $CH_2Cl_2$–DMF (1:1) and shaken for 4 h. The resin beads were isolated by filtration and washed with DMF (3×) and $CH_2Cl_2$ (3×). The resin was resuspended in $CH_2Cl_2$, TFA was added (to 40% v/v), and the mixture was shaken for 30 min and then washed with $CH_2Cl_2$ (3×), DMF (3×), triethylamine–DMF (1:9, 2 × 5 min), and DMF (3×). The Boc-deprotected resin was capped with a solution of acetic anhydride (0.5 M) and HOBt (0.5 M) in DMF (100 mL) for 1 h, washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried to constant weight to give 6.2 g of white beads.

**Derivatization of 12 with Fmoc-LinkerAm.** Approximately half of this material (3 g) was treated with 30% piperidine in DMF, shaken for 15 min, and washed with DMF (5×). Fmoc-LinkerAm (0.86 g, 1.6 mmol), BOP (0.74 g, 1.6 mmol), and HOBt (0.2 g, 1.6 mmol) in DMF (10 mL) were treated with NMM (0.41 mL, 3.7 mmol) and added to the resin suspension. The mixture was shaken for 4 h, and then the beads were isolated by filtration, washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried to constant weight to give 3.4 g of white beads: Final loading was 0.24 mmol/g as determined by Fmoc analysis.

**Derivatization and Evaluation of Resin 8 for DNA Synthesis.** 5′-DMT-thymidine-3-succinate (0.37 g, 0.5 mmol), BOP (0.27 g, 0.6 mmol), and HOBt (75 mg, 0.55 mmol) in DMF (5 mL) were treated with NMM (0.14 mL, 1.3 mmol) and gently mixed by hand for 5 min. The activated solution was added to resin **8** (5 g, 0.39 mmol/g), preswollen in DMF, and the reaction was shaken for 6 h. The beads were isolated by filtration, washed with DMF (3×) and THF (3×), resuspended in acetic anhydride–pyridine–*N*-methylimidazole–THF (5:5:10:80), and mixed for 1 h. The beads were then washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried in vacuo to give 5.1 g of a ninhydrin-negative resin: Final loading was 96 $\mu$mol/g as determined by DMT analysis. Equally efficient incorporation was observed using twice the amount of **8** to provide a resin (50 $\mu$mol/g final loading) that was used for the comparative DNA synthesis evaluations. These experiments were performed on a Biosearch 8750 synthesizer using columns packed with 200 nmol of either resin **8** (derivatized as described here), polymethacrylate, CPG, or 1000 Å macroreticular polystyrene. Syntheses were performed in duplicate, and product yield and purity were determined by anion exchange HPLC using a Dionex DNAPac PA-1000 (4 mm × 25 cm) column.

**3-Hydroxy-4′-nitro-*trans*-stilbene (19). Preparation of (Hydroxymethyl)phenoxyacetic Acid (HMPA)-Derivatized AMPS Resin (14a).** 4-(Hydroxymethyl)phenoxyacetic acid (HMPA, **13a**, 0.73 g, 4.0 mmol) and HOBt (0.81 g, 4.4 mmol) in $CH_2Cl_2$–DMF (1:1, 20 mL) were treated with DIPCDI (0.7 mL, 4.4 mmol), stirred for 2 min, and added to AMPS resin **3a** (1.95 mmol/g, 1 g) preswollen in a minimum amount of $CH_2Cl_2$–DMF (1:1). This suspension was shaken overnight, and then the resin was isolated by filtration, washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried to give 1.5 g of ninhydrin negative HMPA–AMPS resin.

**Preparation of (Bromomethyl)phenoxyacetyl–AMPS Resin (15a).** Triphenylphosphine (0.31 g, 1.2 mmol) and carbon tetrabromide (0.39 g, 1.2 mmol) were added to a suspension of HMPA–AMPS resin **14a** (0.5 g, 0.65 mmol) in $CH_2Cl_2$ (10 mL) cooled to 0 °C. The resulting suspension was shaken overnight and filtered, and the beads were washed with $CH_2Cl_2$ (4×) and acetonitrile (2×) and dried in vacuo to give 0.55 g of resin **15a**: Final loading was 1.15 mmol/g as determined by Br analysis.

**Preparation of 3-Formylphenol Substituted Resin Intermediate 16a.** 3-Hydroxybenzaldehyde (0.37 g, 2.9 mmol) and potassium bis(trimethylsilyl)amide (0.58 g, 2.9 mmol) were shaken in DMF (5 mL) for 5 min, resin **15a** (0.5 g, 0.57 mmol) was then added, and the suspension was shaken overnight. The resin beads were isolated by filtration, washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried in vacuo to give 0.5 g of resin-bound aldehyde **16a**: residual Br

content <0.05 mmol/g as determined by Br analysis. Viability of immobilization and cleavage was confirmed when a portion of this resin (50 mg) was treated with TFA−water (19:1) for 2 h and the filtrate collected and concentrated to give 6 mg (~100%) of a product that was identical to the starting 3-hydroxybenzaldehyde.

**Preparation of Stilbene Intermediate 19 by On-Resin Horner−Emmons Condensation.** 4-Nitrobenzyl diethylphosphonate [0.27 g, 1 mmol, previously prepared by reaction of 4-nitrobenzyl bromide (5 g, 23 mmol) with triethyl phosphite (3.8 g, 23 mmol) at 100 °C for 3 h] was dissolved in DMF (5 mL), treated with sodium methoxide (81 mg, 1.5 mmol), and stirred for 15 min. The bright red solution was then added to the remaining portion of resin **16a** and the reaction shaken overnight. The resin beads were isolated by filtration and washed with water (5×), DMF−water (1:1, 5×), $CH_2Cl_2$ (5×), and MeOH (2×). After being dried in vacuo, the product stilbene was liberated by treatment with TFA−water (19:1) for 2 h. The filtrate was collected and evaporated and the crude product purified by flash chromatography (ethyl acetate−hexane 3:7−1:0) to give 68 mg (54% from **13a**) of pure title product, which was shown by $^1$H NMR, high-resolution MS (calcd for $C_{14}H_{11}NO_3$ 241.0739, found 241.0740), and thin-layer chromatography to be identical to an authentic sample of this stilbene.[25]

**Evaluation of Dephthaloylation Conditions (Table 1).** Resin samples (**2a** or **2c**, 50 mg) were placed in Pyrex culture tubes (Corning, 13 × 100 mm) equipped with Teflon lined screw-caps. Appropriate dephthaloylation cocktails (2 mL) were added (exact descriptions are given in Table 1) and the caps tightly sealed. The tubes were vortexed and then placed in heating blocks at the temperatures and for the times indicated in Table 1. Following this heating period, the contents of each vial were transferred to a standard peptide synthesis "syringe" reactor and the resins washed thoroughly with DMF, $CH_2Cl_2$, and MeOH. Dephthaloylation efficiencies were calculated from the amino content of the dried samples determined by Fmoc-Nle-OH loading as described above and are detailed fully in Table 1.

**Determination of Resin-Swelling Characteristics (Table 2).** The bottom frits from 20 mL disposable polypropylene columns (Bio-Rad, Hercules, CA) were replaced with porous polyethylene frits punched from 1/16th in. medium-porosity sheets (Porex Technology, Fairburn, GA). Each column was then mounted vertically on a ring stand and equipped with a two-way, luer-fitting, disposable stopcock (Cole-Parmer, Vernon Hills, IL). The resin sample (2 g) was placed in a 30 mL screw-capped polyethylene vial (VWR, So. Plainfield, NJ), THF (20 mL) was added, and the mixture was sonicated and vortexed until the resin was fully swollen (30 min). The suspension was transferred to the column and washed with THF until the bed volume was constant, at which point the measurement was recorded. The next solvent to be studied was then continuously eluted through the column until the refractive index of eluent was the same as the fresh solvent added and the bed volume was constant, at which point the next measurement was recorded. The solvent series was run in the order indicated in Table 2.

**Addition of Fmoc-LinkerAm to Amino-Substituted Resins (Table 3). Procedure for Underloading Resins (Target Loading = 0.20 mmol/g).** Fmoc-LinkerAm (2.7 g, 5.0 mmol), BOP (2.3 g, 5.1 mmol), and HOBt (0.7 g, 5.2 mmol) were dissolved in DMF and then treated with NMM (1.3 mL, 12 mmol). The solution was mixed for 3 min and then carefully diluted to 25 mL. Aliquots of the activated LinkerAm solution (1.0 mL, 0.4 mmol) were added to resin samples (2 g) preswollen in the minimum volume of $CH_2Cl_2$−DMF (1:1, ca. 15 mL) in 20 mL glass scintillation vials using screw-caps with conical polypropylene inserts (VWR), and the resultant suspensions shaken overnight. The beads were isolated by filtration, washed with DMF (3×), and capped with a solution of acetic anhydride (0.3 M) and HOBt (0.3 M) in DMF for 2 h. The beads were then washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (1×) and dried in vacuo to constant weight. All samples were found to be ninhydrin negative. Loading efficiency was determined by analysis of Fmoc as described above (see Table 3 for detailed results).

**Procedure for Maximally Loading Resins (Described Here for AMPS 3a).** Fmoc-LinkerAm (430 g, 0.80 mol) and HOBt (150 g, 1.1 mol) were dissolved in $CH_2Cl_2$−DMF (1:1, 2.25 L), and DIPCDI (140 mL, 0.90 mol) was slowly added with stirring over 10 min. The activated linker solution was rapidly added to resin **3a** [700 g, 0.95 mmol/g, 0.66 mol, preswollen in $CH_2Cl_2$−DMF (1:1, 4 L) for 30 min] and washed in with a small amount of additional solvent. The reaction was stirred overnight, and then the beads were isolated by filtration and washed with DMF (4 L), 1,4-dioxane (4 L), $CH_2Cl_2$ (8 L), and MeOH (4 × 4 L). The ninhydrin-negative resin was dried in vacuo to constant weight to yield 1.0 kg of Fmoc-LinkerAm-AMPS: Final loading was 0.63 mmol/g as determined by Fmoc analysis. Samples of the 2 mmol/g AMPS **3a** and TentaGel were also loaded by analogous procedures (see Table 3 for detailed results).

**Synthesis of ACP 65-74 Decapeptide, Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly (Table 4).** Peptide syntheses were performed in syringe reactors with 40 mg of the indicated Fmoc-LinkerAm-resin, underloaded as described above for Table 3 (except for the macroreticular polystyrene, which was maximally loaded). Side-chain protection for Asp and Tyr was provided by *t*-Bu; Asn and Gln side chains were protected with Trt. The basic synthesis cycle involved an initial DMF wash (2×), followed by Fmoc cleavage with piperidine−DMF (3:7, 1 min + 10 min), and then washing with DMF (5×), and coupling with the appropriate activated amino acid solution for the times indicated in Table 4. Fresh stock solutions of BOP/HOBt/NMM activated Fmoc-amino acids were prepared as follows: 2 mmol each of Fmoc-amino acid, BOP, and HOBt were activated with NMM (0.3 M in DMF, 15 mL), and the final volume was adjusted to 20 mL to give 0.1 M solutions. After final Fmoc removal, the resins were washed with $CH_2Cl_2$ (3×), and MeOH (1×) and dried. The resins were cleaved in the syringe reactors with TFA−triisopropylsilane−MeOH (38:1:1) for 2 h; the cleavage solutions were collected in glass scintillation vials and concentrated under nitrogen to a small volume. Anhydrous $Et_2O$ was added, and the mixtures were refrigerated for 2 h. The peptides were isolated by centrifugation, washed with $Et_2O$ (3×), and dried. The samples were then analyzed by analytical HPLC and MS; where baseline HPLC resolutions were not observed, forced droplines were used from slope inflection points or valleys, a method that may overestimate levels of impurities (see Table 4 for complete results).

**Evaluation of Allyl Protection during Peptide Synthesis (Table 4). Preparation, Deprotection, and On-Resin Cyclization of Pentapeptide Test Sequence: Tyr(*t*-Bu)-Lys(Aloc)-Glu(OAl)-Lys(Boc)-Gly.** Peptide syntheses were performed as described above for the ACP decapeptide, except 10 mL syringe reactors containing 250 mg of the indicated resin maximally loaded with Fmoc-LinkerAm were used. $N^{\alpha}$-Fmoc-protected Tyr(*t*-Bu), Lys(Aloc), Glu(OAl), Lys(Boc), and Gly were each coupled using the protocol described above, except that the Fmoc group on the N-terminal Tyr was left intact before the resins were dried. Following peptide chain assembly, the dry resins were divided into two portions. The first portion was placed in a 2 mL syringe reactor, the N-terminal Fmoc group removed, and the resin washed with DMF (3×), and then MeOH (3×), and the allyl-protected peptide was cleaved with TFA−water (19:1) for 2 h. The peptide was isolated by evaporation of the cleavage cocktail and the residue lyophilized from acetonitrile−water (1:1). The second portion of each resin was placed in Pyrex culture tubes (Corning, 13 × 100 mm) equipped with Teflon lined screw-caps. A stock solution of triphenylphosphine (1.7 g, 6.7 mmol) and morpholine (0.5 mL, 5.7 mmol) in THF (25 mL) was prepared and flushed with argon. Aliquots (4 mL) of this solution were added to each vial, followed by 5−10 mg of tetrakis(triphenylphosphine)Pd(0) complex that had been freshly opened under argon. The vials were tightly sealed and the suspensions shaken at 50 °C for 2 h. The contents of each vial were transferred to a syringe reactor and the beads

washed with DMF (1×), 0.4% concd HCl in DMF (2×), and DMF (1×). One-half of each suspension was then treated to remove the N-terminal Fmoc group, and, following TFA cleavage, the allyl-deprotected peptides were isolated. Solutions of BOP (45 mg, 0.10 mmol), HOBt (14 mg, 0.10 mmol), and DIEA (35 $\mu$L, 0.20 mmol) in DMF (1 mL) were added to each remaining peptide resin sample, and the suspensions were shaken overnight. The N-terminal Fmoc groups were then removed and the cyclized peptides isolated after TFA cleavage. The peptide samples were then analyzed by analytical HPLC and MS as described above (see Table 4 for complete results).

**Comparative Syntheses of Stilbene 19 (Table 4).** These experiments were performed essentially as described above for the basic synthesis of **19**, but with minimized reaction times and no monitoring of intermediates. Resin samples (200 mg) were placed in 10 mL syringe reactors and, after several washes, were left suspended in $CH_2Cl_2$−DMF (1:1). 4-(Hydroxymethyl)phenoxybutyric acid (HMPB, **13b**, 0.42 g, 2.0 mmol) and HOBt (0.45 g, 3.3 mmol) dissolved in $CH_2Cl_2$−DMF (1:1, 8 mL) were treated with DIPCDI (0.32 mL, 2.0 mmol) for 2 min. Aliquots (1 mL) of the activated linker solution were added to each syringe, which were then shaken for 2 h. The samples were then washed with $CH_2Cl_2$−DMF (1:1, 3×) and $CH_2Cl_2$ (3×). Triphenylphosphine (1.0 g, 3.8 mmol) and carbon tetrabromide (1.3 g, 3.8 mmol) were dissolved in $CH_2Cl_2$ (20 mL). Two milliliters of this stock solution was added to each syringe reactor, and the reactions were shaken for 2 h. The resins were washed with $CH_2Cl_2$ (4×) and acetonitrile (2×) and then treated for 2 h with 2 mL of a solution of 3-hydroxybenzaldehyde (0.5 g, 4.0 mmol) and potassium bis(trimethylsilyl)acetamide (0.88 g, 4.0 mmol) in DMF (20 mL). Next, the resins were washed with $CH_2Cl_2$−DMF (4×) and DMF (4×) and reacted with 2 mL of a solution of 4-nitrobenzyl diethylphosphonate (1.0 g, 3.7 mmol) and sodium methoxide (0.32 g, 5.9 mmol) in DMF (20 mL). The samples were shaken for 5 h, washed with DMF (5×), DMF−water (1:1, 5×), $CH_2Cl_2$ (3×), and MeOH (3×), and dried. Finally, the resins were treated with TFA−water (19:1) for 2 h, the filtrate was collected in tared scintillation vials, and the products were isolated by evaporation and thorough drying. All products were characterized as described above (see Table 4 for detailed results).

**α-Chymotrypsin Digestion of Trp-Gly Derivatized Resins (Table 4).** A standard solution of activated Boc-Trp-Gly-OH was prepared by dissolving the dipeptide (1.6 g, 4.5 mmol), BOP (2.0 g, 4.5 mmol), and HOBt (0.6 g, 4.5 mmol) in DMF (30 mL) and adding NMM (1.0 mL, 9.0 mmol). Samples of each support (50 mg) were placed in 2.5 mL syringe reactors, and the dipeptide solution (2.0 mL) was added. The reactors were shaken overnight, and then their contents were washed with DMF (4×). A capping solution of acetic anhydride (0.3 M) and HOBt (0.3 M) in DMF (2 mL) was then added, and the resins were shaken for 2 h. The resins were then washed with DMF (1×), 1,4-dioxane (1×), and the enzyme buffer (15% 1,4-dioxane in 0.1 M aqueous ammonium bicarbonate, 3×). A stock solution of α-chymotrypsin (50 mg) in buffer (50 mL) was prepared and each reactor incubated with aliquots (2 mL) for 48 h. The resins were washed with water (3×) and DMF (1×), and then 2 mL of an activated Fmoc-Nle solution [Fmoc-Nle-OH (2.1 g, 6.0 mmol) and HOBt (1.0 g, 7.4 mmol) dissolved in $CH_2Cl_2$−DMF (1:1, 30 mL) and treated with DIPCDI (0.95 mL, 6.0 mmol)] was added. The reactors were shaken for 2 h, and then the contents were washed with DMF (3×), $CH_2Cl_2$ (3×), and MeOH (3×). The resins were thoroughly dried and the loadings determined by UV analysis of Fmoc. The percent cleavage was then determined by comparison with Fmoc-loading values obtained by addition of Fmoc-Nle to the resins without prior dipeptide addition and enzyme incubation (see Table 4 for detailed results).

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