CLEAR: A Novel Family of Highly Cross-Linked Polymeric Supports for Solid-Phase Peptide Synthesis[1,2]

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Abstract: Cross-**L**inked **E**thoxylate **A**crylate **R**esin (CLEAR) supports were prepared by radical copolymerization, either in the bulk or suspension mode, of the branched cross-linker trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) with one or more of allylamine (**2**), 2-aminoethyl methacrylate·HCl (**3**), poly(ethylene glycol-400) dimethacrylate (**4**), poly(ethylene glycol) ethyl ether methacrylate (**5**), and trimethylolpropane trimethacrylate (**6**). The resultant highly cross-linked copolymers by the bulk procedures were ground and sieved to particles, whereas the suspension polymerization procedure gave highly cross-linked spherical beaded supports. CLEAR polymeric supports showed excellent swelling properties in an unusually broad range of solvents, including water, alcohols, tetrahydrofuran, dichloromethane, and *N*,*N*-dimethylformamide. To demonstrate their usefulness for peptide synthesis, CLEAR supports were derivatized with an "internal reference" amino acid [norleucine] and a handle [5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid] and were tested for both batchwise and continuous-flow solid-phase syntheses of challenging peptides such as acyl carrier protein (65-74), retro-acyl carrier protein (74-65), and the 17-peptide human gastrin-I. Comparisons to commercially available supports, *e.g.*, polystyrene, Pepsyn K, Polyhipe, PEG-PS, TentaGel, and PEGA were also carried out. CLEAR supports are entirely stable under standard conditions of peptide synthesis but are in some cases labile to certain strong bases.

Introduction

Since Merrifield's original report over three decades ago describing solid-phase synthesis of a simple tetrapeptide on low cross-linked polystyrene (PS) beads,[3] the approach has been improved and generalized to the synthesis of complicated peptides (including small proteins), long oligonucleotides, and a myriad of small organic molecules.[4] The success of such efforts is often affected by the choice of polymeric support, with regard to mechanical stability, swellability, compatibility with a range of hydrophilic and/or hydrophobic solvents, and applicability to both batchwise and continuous-flow reactors. The selection of possible supports introduced and tested over the years includes polyamides,[5] polyamide composites,[6] polystyrene−Kel-F,[7] polyethylene−polystyrene films,[8] cotton and other carbohydrates,[9] controlled-pore silica glass,[10] polyethylene glycol−polystyrene (PEG-PS and TentaGel) graft resins,[11−13] polyethylene glycol−polyacrylamide (PEGA) resins,[14] tetraethylene glycol diacrylate-cross-linked polystyrene,[15] and chemically modified polyolefin particles ("ASPECT").[16]

The conventional wisdom in the field has been that supports should have the minimal level of cross-linking consistent with stability, so that they can form the well-solvated gels within which solid-phase chemistry is known to take place.[17] Alternatively, porous but rigid supports with a high degree of cross-linking can be used.[18] The present paper introduces a unique

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(1) For preliminary accounts of portions of this work, see: (a) Kempe, M.; Barany, G. In *Peptides: Chemistry, Structure & Biology, Proceedings of the Fourteenth American Peptide Symposium*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, England, 1996; pp 865−866. (b) Kempe, M.; Barany, G. In *Innovations and Perspectives in Solid Phase Synthesis and Combinatorial Chemical Libraries, 1995*; Epton, R., Ed.; Mayflower Worldwide Ltd.: Kingswinford, England, 1996; in press.

(2) Abbreviations used for amino acids and the designation of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* **1972**, *247*, 977−983. The following additional abbreviations are used: AAA, amino acid analysis, AIBN, 2,2′-azobisisobutyronitrile; BOP, benzotriazolyl *N*-oxytris(dimethylamino)phosphonium hexafluorophosphate; CLEAR, title supports of this paper; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMF, *N*,*N*-dimethylformamide; EDT, 1,2-ethanedithiol, Fmoc, 9-fluorenylmethyloxycarbonyl; HPLC, high-performance liquid chromatography; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; IRAA, internal reference amino acid; NMM, *N*-methyl morpholine; PAL, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)-valeric acid; PEG, polyethylene glycol; PS, polystyrene; SEM, scanning electron microscopy; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid. All solvent ratios are volume/volume. All amino acids used were of the L-configuration.

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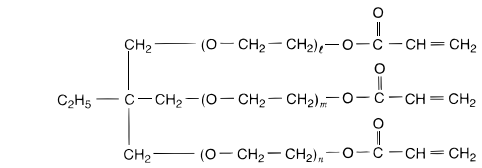
family of supports which are highly cross-linked (≥95% by weight of cross-linker), yet show excellent swelling properties and performance in batchwise and continuous-flow syntheses of several challenging peptides. These supports, prepared by radical copolymerization of the branched cross-linker trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) with other monomers, are termed CLEAR, an acronym for **C**ross-**L**inked **E**thoxylate **A**crylate **R**esin.

Results and Discussion

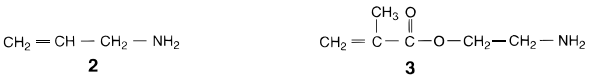
CLEAR Supports. The defining characteristic of the title materials is their preparation from the key trivalent cross-linker **1**. Previous polymeric supports for solid-phase peptide synthesis (SPPS) have been derived from monomers and cross-linkers containing, respectively, only one and two polymerizable groups, and using a low molar ratio of cross-linker with respect to monomer. The CLEAR supports differ in that they are prepared from a branched cross-linker used in a high molar ratio. In addition to the polymerizable vinyl endgroups, each branch of **1** contains a chain with on average four to five oxyethylene units. Thus, the resultant polymers have polyethylene glycol (PEG)-like character, even though individual oxyethylene chains are quite short compared to chains in previously reported PEG-containing supports such as PEG-PS,[11] TentaGel,[13] and PEGA.[14] The oxyethylene chains, together with the ester functionalities, confer a hydrophilic character on the resultant polymers. Compound **1** belongs to a family of cross-linkers which have previously been shown to give polymers with interesting properties for various applications.[19]

The CLEAR supports described here were prepared by copolymerizing **1** with various monomers and cross-linkers, *i.e.*,

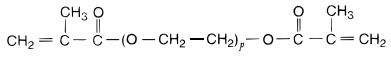
Chart 1



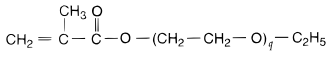
1 $\ell + m + n \sim 14$



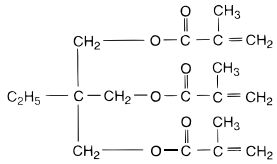
2 **3**



4 $p \sim 9$



5 $q \sim 3\text{-}5$



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one or more of **2**−**6**, in different ratios and under different conditions (Table 1). Amino-functionalized monomers, *e.g.*, **2** or **3**, were included in anticipation of the later need for starting points for synthesis. The fact that amino groups could be introduced *directly* rather than by transformation of another functional group, or by deprotection of a protected amino monomer, was an unanticipated yet advantageous discovery in the CLEAR family. Incorporation of amines into synthetic polymers has been reported to be difficult due to (i) addition of the amine to activated vinylic double bonds[20] and (ii) $O \rightarrow N$ acyl migration resulting in hydroxylated acrylamides when starting with amino acrylates.[21] For this work, the amine precursors were used in sufficiently high excess to achieve the required final substitution levels.

In one phase of this research, bulk polymerizations were carried out. For each of three representative formulations, termed CLEAR-I, -II, and -III (Table 1), the materials obtained after polymerization were ground and sieved to particles (106−125 μm). Upon visual inspection, the off-white ground particles of CLEAR-I and -III had a fluffy (slightly clumped) appearance, whereas CLEAR-II seemed more dense and powdery. Due to the grinding process, the particles had irregular shapes. Figure 1 shows a number of dry particles of CLEAR-II, as viewed by scanning electron microscopy (SEM), and is representative of images recorded on CLEAR-I, -II, and -III. The SEM of these bulk polymers did not show any macropores. Nitrogen adsorption/desorption studies on dry CLEAR materials reported putative surface areas and pore volumes that were below the limit of reliable measurement, leading to the conclusion that these polymers have no substantial microporous structure, either.

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Table 1. Preparation of CLEAR Supports[a]

	monomer or cross-linker (mmol)								loading[a] (mmol NH_2/g)	
support	**1**	**2**	**3**	**4**	**5**	**6**	solvent	mL	by AAA	by UV
CLEAR-I	9.0	9.0					cyclohexanol	17.5	0.26	0.29
CLEAR-II	3.0		3.6	7.0			cyclohexanol	16.0	0.30	0.25
CLEAR-III	8.0		4.4		3.0		cyclohexanol	20.0	0.30	0.27
CLEAR-IV	12.0	50.0				3.0	toluene[b]	12.7[b]	0.17	0.23
CLEAR-V	13.0	50.0					toluene[b]	12.8[b]	0.13	0.20

[a] The loadings varied slightly from batch to batch. Incorporation of components into the final polymers did not match the initial ratios; relevant elemental analysis data in Experimental Section. [b] Volume reported for these suspension polymerizations refers to organic solvent. The volume of the aqueous phase was ~10× that of organic; see Experimental Section for details. The amount of allylamine (**2**) used was high because it is soluble in both phases.

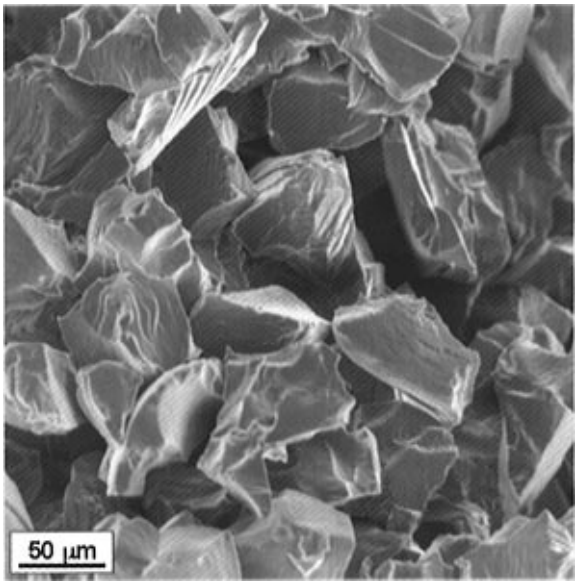

Figure 1. Scanning electron micrograph showing the shape and texture of CLEAR-II particles (ground and sieved). Working magnification: 350×; accelerating voltage: 10 kV.

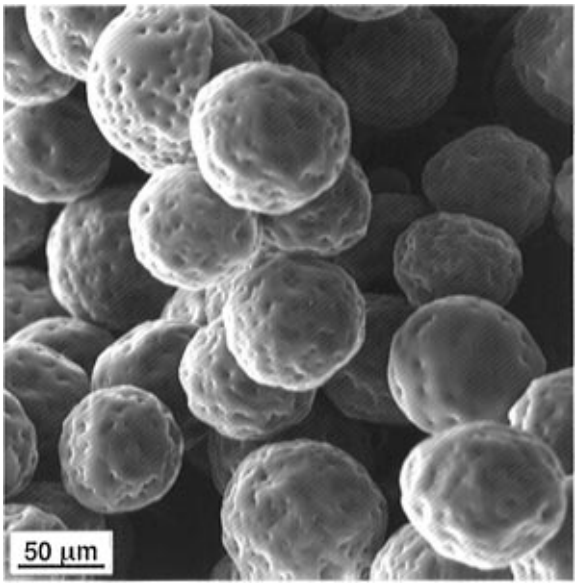

Figure 2. Scanning electron micrograph showing CLEAR-IV spherical beads prepared by suspension polymerization. Working magnification: 350×; accelerating voltage: 10 kV.

Suspension polymerizations were carried out successfully with a variety of formulations to provide spherically beaded materials; those termed CLEAR-IV and -V were chosen for further studies (Table 1). The beads were sieved, and the main fraction (106−125 μm) was collected. The scanning electron micrograph in Figure 2 shows the shape and the texture of CLEAR-IV. The

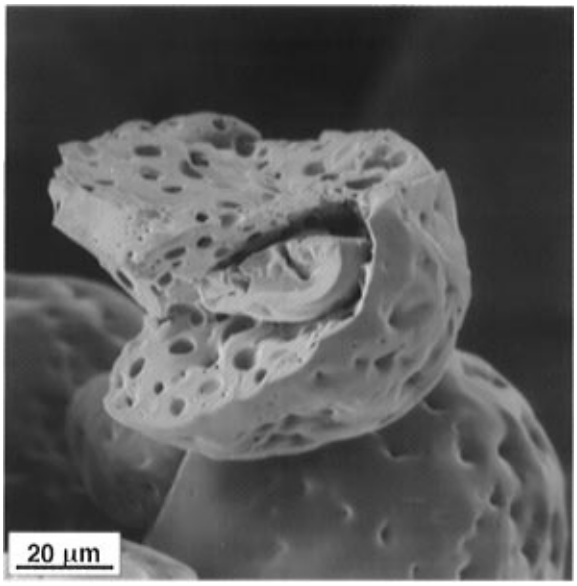

Figure 3. Scanning electron micrograph showing macropores visible on the inside of a broken CLEAR-V bead [*n.b.*, the vast majority of beads were intact spheres, but after considerable searching, an accidentally fractured bead was located in the SEM to make this point]. Working magnification: 1000×; accelerating voltage: 10 kV.

beads contain macropores, as shown in Figure 3. Surface area measurements by nitrogen adsorption/desorption on CLEAR-IV and -V beads gave results similar to those obtained on ground CLEAR-I, -II, and -III particles.

CLEAR supports swell in a wide range of solvents [Table 2; *n.b.*, that CLEAR-I swells somewhat better than the others in the family]. These results are interpreted to mean that *despite* its high degree of cross-linking, the polymeric network is highly dynamic. The fact that these materials swell in both hydrophilic and hydrophobic solvents is considerably advantageous, since it extends the range of chemistries that could be conducted on the supports, and it means that biological/biochemical assays of resin-bound compounds are possible.

The usefulness of CLEAR supports for SPPS (Scheme 1) was demonstrated in both batchwise and continuous-flow modes. The parent supports with free amino groups, formed by either bulk or suspension copolymerization, were acylated with an "internal reference" (IRAA) amino acid (Fmoc-Nle-OH). Deprotection and coupling of the handle 5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid (Fmoc-PAL-OH)[22] followed to provide Fmoc-PAL-Nle-CLEAR supports. These derivatized materials were then used for the synthesis of peptides in the $C \rightarrow N$ direction by repetitive cycles of "Fmoc chemistry", *i.e.*, deprotections, washings, and activation/couplings of suitably protected amino acid derivatives.

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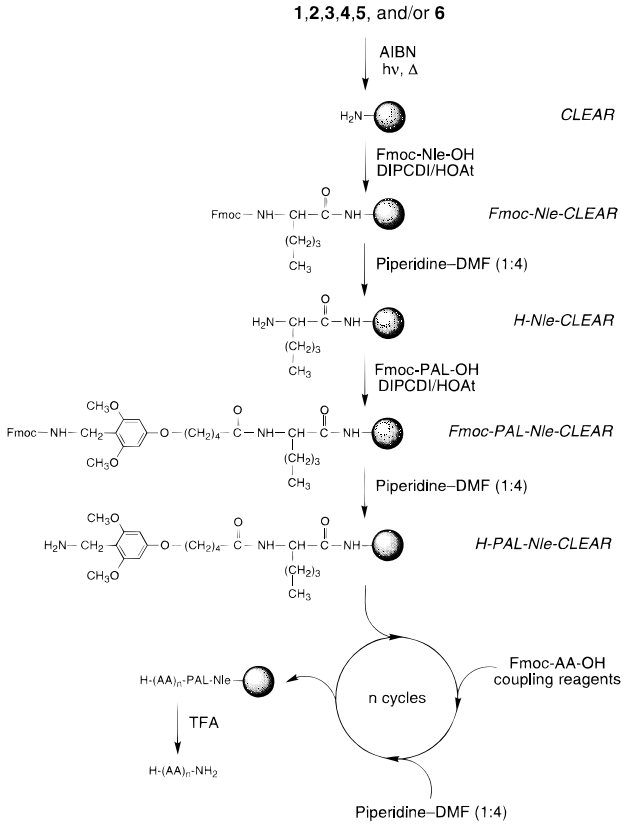
Table 2. Swelling of CLEAR Supports[a]

solvent	bed volume (mL) of 1 g polymer				
	CLEAR-I	CLEAR-II	CLEAR-III	CLEAR-IV	CLEAR-V
DMF	8	5	5	5.5	5
CH_2Cl_2	10	7	7	5.5	8
CH_3CN	6.5	4.5	5	5	5
THF	6.5	4.5	5	5	5
MeOH	7	5	5	5	5
H_2O	8	5	5	4	5
toluene	5	4	5	5	4
EtOAc	5	4	4	4.5	4.5
t-BuOMe	3.5	4	4	3	3
hexane	3	3	3	3	3
TFA	12	7	8	6.5	7.5

[a] For each set of swelling studies, the underivatized CLEAR support (0.1 g) was placed in a 3-mL syringe fitted at the bottom with a porous frit. The support materials were washed 10× with the indicated solvent, and then allowed to stand for 5 min in that same solvent. Excess solvent was removed by brief suction, and the bed volume of the fully solvated support was noted. The CLEAR supports were then washed 10× with CH_2Cl_2 and dried [air-drying for volatile solvents; *in vacuo* overnight for H_2O and DMF], and the procedure was repeated on the same resins with the next solvent. The swelling determination with TFA was carried out last. See refs 11d, 13d, 14b, 17b, 17c, and 29 for literature swelling data on a variety of commercially available supports.

Scheme 1. General Procedures for Solid-Phase Peptide Synthesis on CLEAR Supports



Continuous-Flow SPPS. The excellent mechanical stabilities of CLEAR supports facilitate their use in continuous-flow peptide synthesizers; no appreciable back-pressures were observed. Thus, CLEAR-II was used to prepare Leu-enkephalinamide (Figure 4) and the 17-peptide human gastrin-I (Figure 5). Couplings were mediated by *N*,*N*′-diisopropylcarbodiimide/1-hydroxybenzotriazole (DIPCDI/HOBt) and by benzotriazolyl *N*-oxytris(dimethylamino)phosphonium hexafluorophosphate/HOBt/*N*-methylmorpholine (BOP/HOBt/NMM) protocols, respectively. The product of the enkephalinamide synthesis was 96% pure, based on HPLC analysis directly after acidolytic cleavage of the PAL anchor. In the case of gastrin, the major component in the crude cleavage product was isolated by preparative HPLC and coincided with authentic gastrin [coelution with authentic standard on analytical HPLC, amino acid analysis, FABMS]; and other peaks are likely due to modifications of the acid-sensitive target structure. These experiments, which show that CLEAR supports are readily compatible with automated continuous-flow processes, are particularly significant since it is known that many of the commercially available supports for SPPS are unusable, or usable only with major difficulties, in this mode.

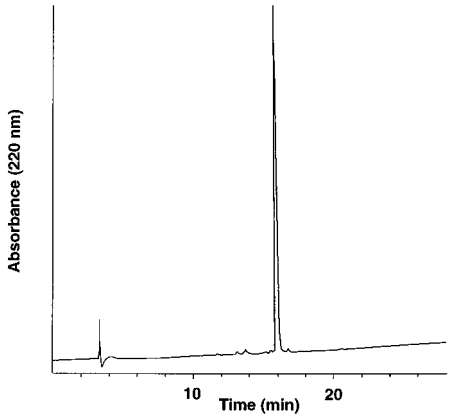


Figure 4. Analytical HPLC chromatogram of crude Leu-enkephalinamide, prepared on a PerSeptive Biosystems 9050 continuous-flow peptide synthesizer, starting with CLEAR-II (0.26 mmol/g). Elutions at 1 mL/min with a linear gradient over 20 min from 19:1 to 2:3 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile.

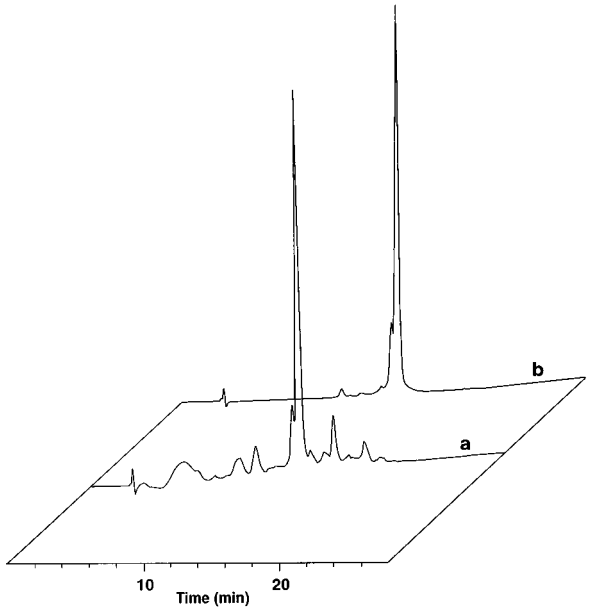


Figure 5. Analytical HPLC chromatogram of (a) crude human gastrin-I, prepared on a PerSeptive Biosystems 9050 continuous-flow peptide synthesizer, starting with CLEAR-II (0.26 mmol/g) and (b) human gastrin-I after purification by preparative HPLC. Elutions at 1 mL/min with a linear gradient over 28 min from 3:1 to 2:3 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile. Peptides were detected at 220 nm.

Batchwise Manual SPPS. The "difficult" sequence 65-74 of acyl carrier protein[17a] was synthesized manually on all three ground CLEAR supports (I, II, and III). In one protocol, couplings were mediated by DIPCDI/1-hydroxy-7-azabenzotriazole (DIPCDI/HOAt) in *N*,*N*-dimethylformamide (DMF). In a second protocol, couplings were mediated by DIPCDI alone, and couplings as well as washing steps were carried out in the nonconventional (for peptide synthesis) solvent acetonitrile. Both procedures gave good crude products, with peptide purities

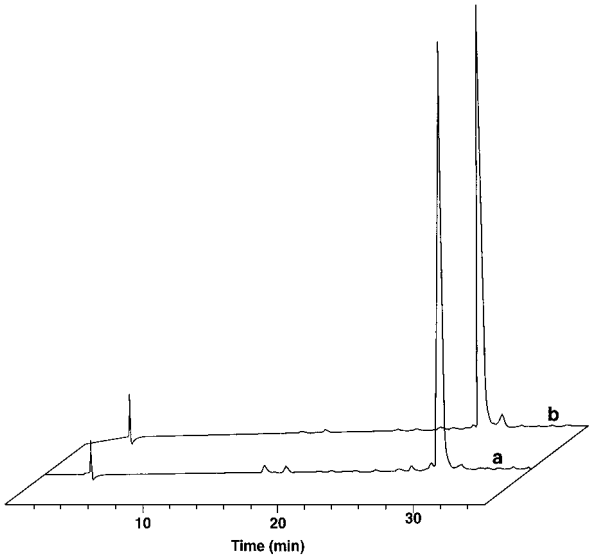


Figure 6. Analytical HPLC chromatogram of crude ACP (65-74) amide, prepared by batchwise manual synthesis on CLEAR-I (0.29 mmol/g). Elutions at 1 mL/min with a linear gradient over 30 min from 19:1 to 3:1 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile. Peptides were detected at 220 nm. (a) Couplings were mediated by DIPCDI/HOAt, and couplings and washings were performed with DMF as solvent. (b) Couplings were mediated by DIPCDI, and couplings and washings were performed with acetonitrile as solvent. Similar experiments were carried out on CLEAR-II and -III and are reported in supporting information: Figures 3 and 4.

slightly higher for the acetonitrile versus DMF syntheses [95% (CLEAR-I), 91% (CLEAR-II), and 91% (CLEAR-III) in acetonitrile; 91%, 82%, and 80%, respectively, in DMF; see Figure 6 for chromatographic data on material synthesized on CLEAR-I, and supporting information: Figures 3 and 4 for material synthesized on CLEAR-II and -III]. Similar results have been reported for PEG-PS supports,[11d] but most other commercially available materials are incompatible with acetonitrile as the solvent for stepwise incorporation of amino acids into peptides.

Many workers would prefer to use uniform, spherically beaded materials in solid-phase synthesis. The applicability of the beaded forms of CLEAR (IV and V) to batchwise SPPS were demonstrated first by applying these materials to the synthesis of Leu-enkephalinamide (for details, see Experimental Section and supporting information: Figures 11−13).

Comparative Batchwise SPPS. The exceedingly "difficult" retro-sequence 74-65 of acyl carrier protein[22] was prepared in side-by-side experiments using all five (both ground and beaded) CLEAR supports, along with a number of commercially available supports (Figure 7). Although each synthesis could likely be optimized further, the data reveal that performance characteristics on CLEAR were at least comparable, and in some cases better, to any found previously in our hands or in the literature [details in Figure 7 and in Experimental Section]. In particular, we noted difficulties in handling Pepsyn K (NovaSyn KR 100) and PEGA. With the former material, we experienced problems when draining off reagents and solvents because of excessive back pressure; after a few cycles, these simple operations were unacceptably time-consuming. PEGA was difficult to handle since, according to the manufacturer, the material is irreversibly destroyed when dried. Therefore, PEGA required handling in swollen form, which led to a number of difficulties, *e.g.*, quantitation of loading could not be done.

Chemical Stability of CLEAR. Our original intention was to develop CLEAR supports for SPPS. Although implicit in the excellent SPPS results reported above, we have also exposed

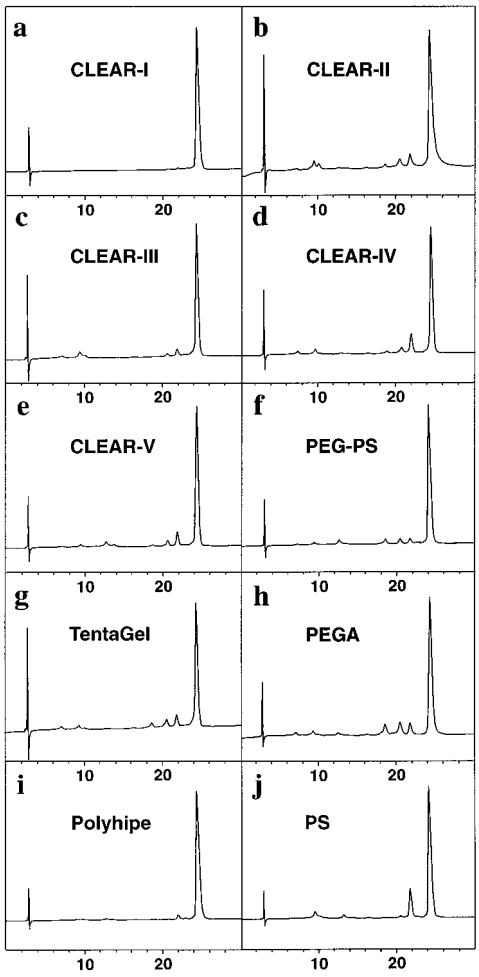


Figure 7. Analytical HPLC chromatogram of crude retro-ACP (74-65) amide, prepared by batchwise manual synthesis on (a) CLEAR-I; (b) CLEAR-II; (c) CLEAR-III; (d) CLEAR-IV; (e) CLEAR-V; (f) PEG-PS; (g) TentaGel; (h) PEGA; (i) Polyhipe; and (j) PS. Elutions at 1 mL/min with a linear gradient over 30 min from 17:3 to 3:1 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile. Peptides were detected at 220 nm (ordinates); time scale is in min (abscissa). The chromatogram shown in (b) was reproduced in the LC-MS mode, which indicated that the peaks preceding the major one were the des-Ile (9.4 min), des-Gln (20.5 min), and des-Asn (21.8 min) analogues of retro-ACP (74-65) amide.

our supports to *exaggerated* SPPS conditions in order to evaluate their stabilities and to establish whether or not any "leaking" of material (as has been described for some commercial supports) might occur.[23] Support samples were checked before and after treatment by gravimetry, elemental analyses, and Fourier transform infrared spectroscopy (FT-IR) [for elemental analysis data, see supporting information: Table 1; for FT-IR spectra, see Figures 8 and 9, and supporting information: Figures 14−16]. Treatment for 1 h with piperidine−DMF

(23) Throughout the Experimental Section, mass spectral data recorded on crude products directly after acidolytic cleavage of peptidyl-PAL-CLEAR supports are reported. We have examined such spectra carefully (see supporting information for the full spectra) and never found evidence for any non-peptidic material (*i.e.*, no "leaking" of materials from the supports into the cleaved solution). For example, breakdown of cross-linker **1** (as incorporated into the polymeric support) by hydrolysis or ammonolysis gives a set of peaks 44 amu apart, due to oxyethylene units. FT-MS ESI of material solubilized upon treatment of CLEAR-I, -IV, and -V with NH_4OH for 2 weeks shows such a pattern [for details, see Experimental section; mass spectra are in supporting information: Figures 17−19]. FT-MS ESI performed under the same conditions on crude ACP(65-74) amide *did not* show this pattern of signals separated by 44 amu but only the expected signals due to the peptides [for spectra see supporting information: Figure 20].

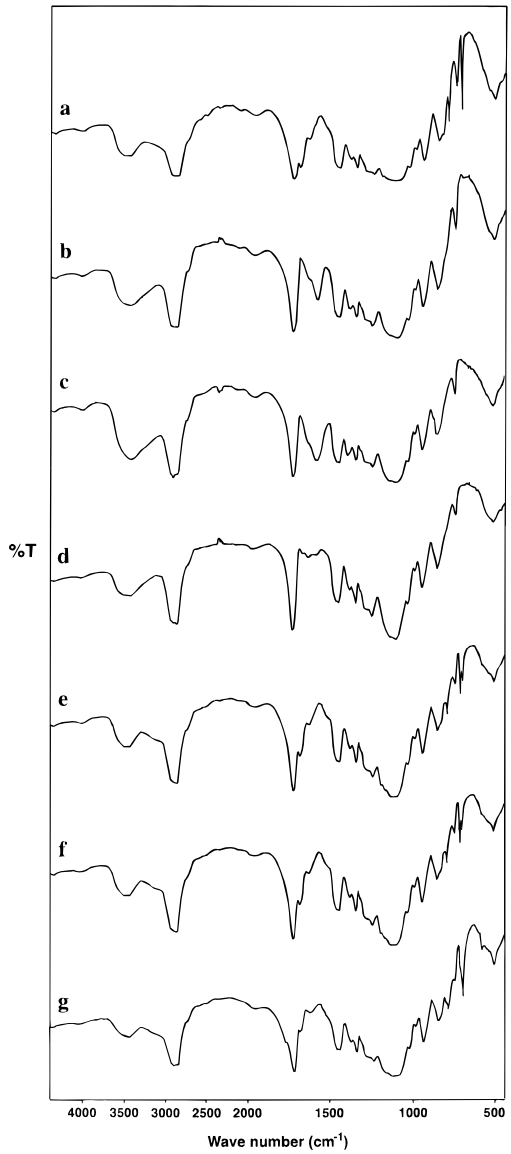


Figure 8. FT-IR spectra of CLEAR-II: (a) original; (b) after treatment with 2 M aqueous NaOH for 1 h; (c) after treatment with 2 M aqueous NaOH for 24 h; (d) after treatment with 2 M NH_4OH in aqueous MeOH for 2 weeks; (e) after treatment with piperidine−DMF (1:4) for 1 h; (f) after treatment with neat TFA for 2 h; and (g) after treatment with neat TFA for 48 h.

(1:4), the reagent used for Fmoc removal, revealed no nitrogen incorporation; however some subtle changes were seen in the carbonyl region of the FT-IR spectra [Figures 8 and 9, and supporting information: Figures 14−16]. To investigate the possibility that piperidine treatment might alter the supports and hence compromise their usefulness for peptide synthesis, we subjected Fmoc-PAL-Nle-CLEAR-I, -II, and -III to the deprotection reagent for the very prolonged period of 9 days, and followed this with SPPS of Leu-enkephalinamide on the materials so treated. Results of these syntheses were identical to those obtained on control (original untreated) CLEAR supports as well as on some commercially available supports manipulated in the same ways (Figure 10). When CLEAR supports (I, II, III, IV, and V) were treated with neat TFA for 48 h, no weight losses and no significant changes in the elemental compostions were observed, although the FT-IR spectra showed some changes in the carbonyl region [Figures 8 and 9, supporting information: Figures 14−16], perhaps due to adsorption of the acid.

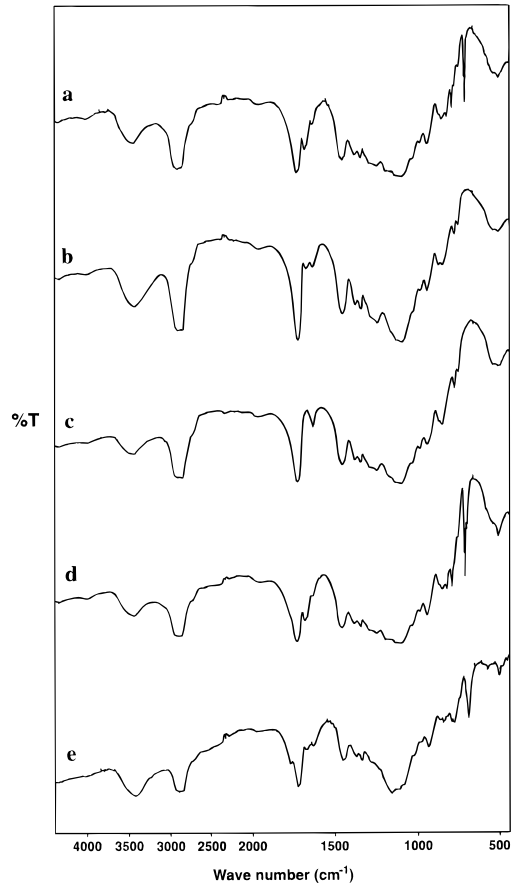


Figure 9. FT-IR spectra of CLEAR-IV: (a) original; (b) after treatment with 2 M NH_4OH in aqueous MeOH for 2 weeks; (c) after treatment with piperidine−DMF (1:4) for 1 h; (d) after treatment with neat TFA for 2 h; and (e) after treatment with neat TFA for 48 h.

The multiple ester groups in CLEAR were expected to be susceptible to nucleophilic cleavage by bases stronger than piperidine. To investigate this risk, the five CLEAR polymers were incubated in (i) 2 M aqueous NaOH and (ii) 2 M NH_4OH in aqueous MeOH. All CLEAR supports, except CLEAR-II, dissolved completely in NaOH after 24 h. CLEAR-II showed a weight loss of 22% after 1 h and 30% after 24 h treatment with NaOH. CLEAR-I and -V dissolved completely after two weeks of treatment with NH_4OH, whereas relatively little of the other CLEAR polymers dissolved under these conditions [8% of CLEAR-II, 10% of CLEAR-III, and 33% of CLEAR-IV were lost, as determined gravimetrically]. These experiments revealed that much of CLEAR-II, -III, and -IV resists base. To the extent that materials dissolve in the presence of NH_4OH, this reflects simple ammonolysis of all three ester linkages in cross-linker **1** (as integrated into the polymer) to provide among the products the expected trimethylolpropane (14/3 EO/OH) ethoxylate triol.

Conclusions

Each member of the CLEAR family of supports for solid-phase peptide synthesis is easily prepared by a single-step polymerization from commercially available monomers and cross-linkers. The CLEAR supports, both ground and beaded, possess adequate chemical and exceptional mechanical stability, and (unlike most commercially available supports) show good swelling in an unusally broad range of solvents. For solid-phase synthesis, the initial functional group is substituted with an appropriate handle. Several challenging peptide sequences have been assembled successfully on CLEAR by stepwise

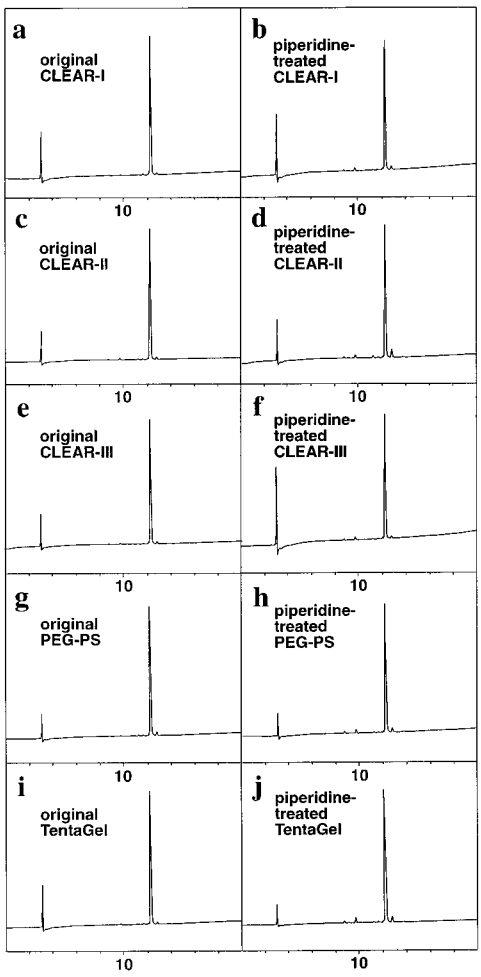


Figure 10. Analytical HPLC chromatogram of crude Leu-enkephalinamide, prepared by batchwise manual synthesis on (a) CLEAR-I (0.24 mmol/g); (b) CLEAR-I that had been treated with piperidine−DMF (1:4) for 9 days; (c) CLEAR-II (0.33 mmol/g); (d) CLEAR-II that had been treated with piperidine−DMF (1:4) for 9 days; (e) CLEAR-III (0.18 mmol/g); (f) CLEAR-III that had been treated with piperidine−DMF (1:4) for 9 days; (g) PEG-PS (0.18 mmol/g); (h) PEG-PS that had been treated with piperidine−DMF (1:4) for 9 days; (i) TentaGel (0.18 mmol/g); (j) TentaGel that had been treated with piperidine−DMF (1:4) for 9 days. Elutions at 0.8 mL/min with a linear gradient over 20 min from 19:1 to 2:3 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile. Peptides were detected at 220 nm (ordinates); time scale is in min (abscissa).

procedures, both in the batchwise and continuous-flow modes, and results were equal or superior to findings with alternative commercially available support materials. Within the CLEAR family, CLEAR-I (which swells the best) gives slightly better results for SPPS, whereas CLEAR-II has somewhat better handling properties and has the highest degree of resistance to strong base. Alternative CLEAR supports derived from monomers, bi- and trifunctional cross-linkers, and functionalized monomers different from but related to **1**−**6** are currently being designed. We anticipate that the described properties will translate well for future applications to solid-phase syntheses of other types of organic molecules and of combinatorial libraries.

Experimental Section

General Methods. Allylamine, poly(ethylene glycol-400) dimethacrylate, poly(ethylene glycol) ethyl ether methacrylate, trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate, trimethylolpropane trimethacrylate, polyvinyl alcohol (97% hydrolyzed, average M_w 50 000−85 000), KBr (FT-IR grade), 1,2-ethanedithiol (EDT), and lauric acid were obtained from Aldrich (Milwaukee, WI). 2-Aminoethyl methacrylate·HCl was from Eastman Fine Chemicals (Rochester, NY) and 2,2′-azobisisobutyronitrile (AIBN) was from Pfaltz & Bauer (Waterbury, CT). Fmoc-PAL-OH, HOAt, and Fmoc-PAL-PEG-PS were obtained from the Biosearch Division of PerSeptive Biosystems (Framingham, MA). DIPCDI and BOP were from Advanced ChemTech (Louisville, KY), HOBt from Chem-Impex International (Wood Dale, IL), and NMM from Fisher Biotech (Fair Lawn, NJ). All Fmoc-amino acids were either from Advanced ChemTech or Chem-Impex International. TentaGel S NH_2 was from Rapp Polymere (Tübingen, Germany), PL-PEGA Resin was from Polymer Laboratories (Amherst, MA), NovaSyn PR 500, NovaSyn P 500 "Polyhipe", NovaSyn KR 100, and NovaSyn KD 125 "Pepsyn K" were from Nova Biochem (La Jolla, CA), and Fmoc-amide Resin was from the Applied Biosystems Division of Perkin-Elmer (Foster City, CA).

The Kaiser qualitative ninhydrin test[24] was carried out specifically as described by Stewart and Young.[25] Analytical high-performance liquid chromatography (HPLC) was performed on a Beckman system comprising two Model 112 Solvent Delivery Modules, a Model 165 Variable Wavelength Detector, and a System Gold Analog Interface Module, controlled via an IBM PC by Beckman System Gold software. A Vydac C_{18} column (4.6 × 250 mm) was eluted at flow rates of 0.8 mL/min or 1 mL/min, and monitored at 220 nm. Amino acid analyses were carried out on a Beckman System 6300 high performance analyzer. Samples were hydrolyzed with propionic acid−12 N HCl (1:1) (peptide-resins and cleaved resins) or 6 N aqueous HCl (released peptides), containing 1 drop of phenol for Tyr-containing peptides, at 160 °C for 1 h. Reported cleavage yields are based on amino acid ratios with respect to norleucine (Nle) IRAA. The substitution levels of Fmoc-resins were determined by (i) amino acid analyses and (ii) spectrophotometric analyses [absorption at 301 nm of the fulvene-piperidine adduct formed upon deprotection of the amino groups] using a Beckman DU 650 spectrophotometer.

All FAB-MS experiments were carried out on a VG Analytical Ltd. 7070E-HF high resolution double-focusing mass spectrometer equipped with a VG 11/250 data system. The matrix was glycerol containing 1% TFA. Ions (at accelerating potentials of 5 kV) were generated from the impact on the target matrix of a neutral xenon atom beam derived from a Xe^+ ion beam at an accelerating potential of 8 kV. The FAB gun emission current was 1 mA. Average molecular weight data were recorded at low resolution (500 resolution) in continuum mode (uncentroided) scanning over the region of interest at 300 s/decade and signal-averaging the data. Unit resolution data, providing monoisotopic molecular weight data, were acquired at 200 resolution scanning 100 to 2000 amu at 10−20 s/decade. LC-MS was performed using a Beckman HPLC system comprising a Model 126 solvent delivery module, a Model 166 programmable detector module, and a Beckman Ultrasphere C_{18} column (2.0 × 150 mm). Elution was carried out at 0.2 mL/min with a linear gradient over 80 min from 9:1 to 3:1 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile; detection by UV absorbance at 220 nm. The HPLC system was connected to a Sciex API III triple quadropole mass spectrometer equipped with an ionspray interface [ionspray voltage: 5 kV; interface temperature: 60 °C; potential on first quadropole: 30 V; orifice voltage: 75 V]. Scanning was done, in increments of 0.5 amu, from 100 to 1 200 amu. FT-MS ESI was performed using an external electrospray ion source on a dual cell Finnigan 2001 FT-MS Fourier-transform ion cyclotron resonance mass spectrometer (Madison, WI) fitted with a 3.1 T magnet and an Odyssey Data System. A stainless-steel capillary at 120 °C desolvated ions in the electrospray source. A series of electrostatic lenses guided ions from the external source to the analyzer cell of the FT-MS, where all mass measurements were made. The base pressure in the cell was 3×10^{-9} Torr to collisionally trap ions. A potential of 6.5 V was applied to the trapping plates during ion accumulation (5 s) and then reduced to 0.35 V during ion excitation and detection. Ion excitation was by an rf excitation sweep (100 V peak-to-peak, 800 Hz/μs, low to high frequence) and 64 K data points were collected, summing 20−50 scans. Samples were sprayed from an aluminum coated fused silica

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

(25) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce: Rockford, IL, 1984; pp 105−106.

capillary (SGE Polysil tubing, SGE, Inc., Austin, TX) biased at 2.5−2.8 kV at flow rates of 1−2 μL/min using a Harvard Apparatus Model 22 syringe pump (South Natick, MA). Peptide samples were prepared in MeOH−H_2O (1:1) containing 1% TFA, and ammonolyzed polymer samples were prepared in NH_4OH−MeOH (1:4).

The textures of CLEAR materials were studied by scanning electron microscopy (SEM) using a JEOL JSM-840 scanning electron microscope (The Microscopy Facility at the Center for Interfacial Engineering, University of Minnesota). Polymer samples were mounted on SEM stubs and were coated with platinum. Images were recorded at accelerating voltages of 10 kV. Nitrogen adsorption/desorption isotherms[26] were recorded on a Micromeritics ASAP 2400 (Chemical Engineering, University of Lund, Sweden) using a 76-point pressure table and 45 s equilibration times after degassing under vacuum at 60 °C for 48 h. This technique gives the surface areas and the distribution of pores in the diameter range of 18 to 3000 Å. As described in the text, CLEAR particles did not show measurable surface areas and were concluded to lack microporous structure. Fourier transform infrared (FT-IR) spectra of resins (KBr pellets) were measured on a Perkin Elmer Model 1600 FT-IR spectrophotometer. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

CLEAR-I. Allylamine (**2**) (0.51 g, 9.0 mmol), trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) (8.21 g, 9.0 mmol), and AIBN (0.1 g, 0.6 mmol) were placed in a screw-cap culture tube, dissolved in cyclohexanol (17.5 mL), and purged with a stream of nitrogen for 5 min. The tube was sealed and irradiated overnight (350 nm) in a Rayonet Photochemical Reactor. The resulting polymer was ground in a mortar and wet-sieved with water (total ~ 6 L) through 106- and 125-μm sieves. The 106−125 μm fraction was collected and washed on a sintered glass filter funnel with acid water [deionized then acidified with TFA to pH 1−2, ~150 mL], deionized water (~300 mL), and MeOH (~150 mL). The fines were removed by repetitive (~10×) sedimentations (by gravity) and decantations using MeOH (~50 mL each time). The remaining particles were dried *in vacuo* overnight. Yield: 2.26 g. Anal. Found: C, 54.84; H, 8.01; N, 0.90, consistent with molar incorporation of **1**:**2** = 5:3 [approximately half of the **2** incorporated provides free amino groups]; note that the polymer is 96% by weight derived from **1**.

CLEAR-II. This polymer was made in the same way as CLEAR-I but starting with 2-aminoethyl methacrylate·HCl (**3**) (0.60 g, 3.6 mmol), poly(ethylene glycol-400) dimethacrylate (**4**) (3.88 g, 7 mmol), trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) (2.74 g, 3 mmol), and AIBN (0.1 g, 0.6 mmol) in cyclohexanol (16 mL). Yield: 2.65 g. Anal. Found: C, 54.68; H, 7.79; N, 0.70.

CLEAR-III. This polymer was made in the same way as CLEAR-I but starting with 2-aminoethyl methacrylate·HCl (**3**) (0.73 g, 4.4 mmol), poly(ethylene glycol) ethyl ether methacrylate (**5**) (0.74 g, 3 mmol), trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) (7.30 g, 8 mmol), and AIBN (0.1 g, 0.6 mmol) in cyclohexanol (20 mL). Yield: 1.84 g. Anal. Found: C, 54.86; H, 7.92; N, 0.73.

CLEAR-IV. Spherical beads were prepared by suspension polymerization using a reactor and an overhead stirrer following the designs described by Arshady and Ledwith.[27] An aqueous phase consisting of deionized water (120 mL; previously purged with a stream of argon for 5 min), 1% polyvinyl alcohol in water (6 mL), and ammonium laureate solution (5 mL of 1% lauric acid in water, adjusted to pH 10.3 with concentrated aqueous NH_4OH), and an organic phase (which had been purged with a stream of argon for 5 min) consisting of allylamine (**2**) (2.86 g, 50 mmol), trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) (10.94 g, 12 mmol), trimethylolpropane trimethacrylate (**6**) (1.02 g, 3.0 mmol), AIBN (0.5 g, 3.0 mmol), and toluene (17 mL) were stirred (400 rpm) under an argon atmosphere for 1 h at 70 °C. The beads were collected on a sintered glass filter funnel, washed with H_2O (~2 L) and MeOH (~300 mL). The beads were sieved, and the major fraction (106−125 μm) was suspended in MeOH and repetitively sedimented and decanted (~10×) to remove remaining smaller beads. The beads were finally dried *in vacuo* overnight. Yield: 2.38 g. Anal. Found: C, 55.05; H, 8.11; N, 0.86.

CLEAR-V. These beads were prepared in the same way as CLEAR-IV, but starting with an organic phase consisting of allylamine (**2**) (2.86 g, 50 mmol), trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate (**1**) (11.86 g, 13 mmol), AIBN (0.5 g, 3.0 mmol), and toluene (17 mL). Yield: 1.29 g. Anal. Found: C, 54.07; H, 8.15; N, 0.93, consistent with molar incorporation of **1**:**2** = 8:5 [approximately one-third of the **2** incorporated provides free amino groups]; note that the polymer is 96% by weight derived from **1**.

Fmoc-PAL-norleucyl ("Internal Reference")-Derivatized CLEAR's, TentaGel, and PEGA. Fmoc-Nle-OH (0.64 g, 1.8 mmol), dissolved in DMF (3 mL), was added to each of CLEAR-I, -II, -III, -IV, -V (loadings in Table 1; 1.0 g per experiment), TentaGel S NH_2 (0.28 mmol/g, 1.0 g), and PL-PEGA Resin (0.3−0.4 mmol/g, 3.3 g of 30% slurry in MeOH, washed extensively with DMF to remove MeOH). Coupling in each case was initiated by the addition of HOAt (0.23 g, 1.8 mmol) in DMF (3 mL) followed by DIPCDI (0.25 g, 1.8 mmol) in DMF (3 mL). The mixtures were shaken at 25 °C for 48 h and then filtered and washed with DMF (5 × 15 mL) and CH_2Cl_2 (5 × 15 mL). At this point, all CLEAR resins and TentaGel were negative to qualitative ninhydrin tests, whereas PEGA was slightly positive; nevertheless an acetylation step was carried out on all resins using acetic anydride (1 g) in CH_2Cl_2−pyridine (16 mL, 1:1) for 30 min, followed by washing with CH_2Cl_2 (5 × 15 mL) and DMF (5 × 15 mL). The Fmoc groups were removed by treatment with piperidine−DMF (1:4) (5 + 15 min), followed by washings with DMF (5 × 15 mL) and CH_2Cl_2 (5 × 15 mL). A solution of Fmoc-PAL-OH (0.89 g, 1.8 mmol) in DMF (3 mL) was added, and the next steps (activation, coupling, acetylation) followed the exact outline given just previously for introduction of Fmoc-Nle-OH.

Continuous-Flow SPPS of Leu-enkephalinamide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) on CLEAR-II. Chain assembly was carried out on a PerSeptive Biosystems 9050 continuous-flow peptide synthesizer, starting with Fmoc-PAL-Nle-CLEAR-II (0.5 g, 0.26 mmol/g), and using N^{α}-Fmoc-amino acids (0.65 mmol each, 5 equiv) with a DIPCDI/HOBt protocol as specified by the manufacturer (30-min couplings). The phenolic side-chain of tyrosine was protected as the *tert*-butyl ether. Fmoc-removal was achieved with piperidine−DBU−DMF (10:1:39) (6 min). The amino acid composition of the completed peptide-resin was Gly 2.06, Leu 0.92, Tyr 0.98, Phe 1.00, Nle 1.16. The peptide-resin was transferred to a syringe reactor. Cleavage of the peptide from the resin was achieved with TFA−CH_2Cl_2 (9:1) for 2 h at 25 °C. The filtrate was expressed from the vessel with positive nitrogen pressure, and the cleaved resin was washed (3 × 1.5 mL) with further TFA−CH_2Cl_2 (9:1). The combined filtrates were evaporated to dryness (including chasing 3× with CH_2Cl_2), suspended in water, and lyophilized. The cleavage yield was 74% based on amino acid analyses of peptide resins before and after cleavage. The crude peptide was shown to be 96% pure by HPLC (Figure 4). The amino acid composition of the crude peptide was Gly 2.03, Leu 1.01, Tyr 0.98, Phe 0.98. Leu-enkephalin has a calculated exact mass of 554.2853. FABMS, *m*/*z*, positive $[M + H]^+$: 555.3; negative $[M - H]^-$: 553.2; $[M - H + TFA]^-$: 667.2 (spectra in supporting information: Figure 1).

Continuous-Flow SPPS of Human Gastrin-I (pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2) on CLEAR-II. Chain assembly was carried out on a PerSeptive Biosystems 9050 continuous-flow peptide synthesizer, starting with Fmoc-PAL-Nle-CLEAR-II (0.3 g, 0.26 mmol/g), and using N^{α}-Fmoc-amino acids (0.63 mmol each, 8 equiv) with a BOP/HOBt/NMM protocol as specified by the manufacturer (1 h couplings). The β- and γ-carboxyl functions of aspartic and glutamic acids were protected as their *tert*-butyl esters and the phenolic side-chain of tyrosine as the *tert*-butyl ether. Fmoc removal was achieved with piperidine−DBU−DMF (10:1:39) (6 min). The amino acid composition of the completed peptide resin was Asp 1.11, Glu 4.51, Pro 1.09, Gly 1.89, Ala 1.41, Met 1.23, Leu 1.03, Tyr 1.30, Phe 1.43, Nle 1.70. The resin was transferred to a syringe reactor. Cleavage of the peptide from the resin was achieved in a reaction vessel with reagent R: TFA−thioanisole−

(26) (a) Dollimore, D.; Heal, G. R. *J. Appl. Chem.* **1964**, *14*, 109−114. (b) Emig, G.; Hoffmann, H. *J. Catal.* **1967**, *8*, 303−306.

(27) Arshady, R.; Ledwith, A. *React. Polym.* **1983**, *1*, 159−174.

(28) Commercial Fmoc-PAL-Nle-PEG-PS has a Nle IRAA *between* the PS and bifunctional PEG, which latter sometimes acts as a spacer and other times cross-links two Nle sites. Hence, ratios of incorporated amino acids to Nle of 2−3 represent quantitative yields past the PAL handle.

(29) Fields, G. B; Fields, C. G. In *Innovations and Perspectives in Solid Phase Synthesis: Peptides, Polypeptides and Oligonucleotides, 1992*; Epton, R., Ed.; Intercept Ltd.: Andover, England, 1992; pp 153−162.

EDT−anisole (90:5:3:2) (1.5 mL). The filtrate was expressed from the vessel with positive nitrogen pressure, and the cleaved resin was washed with further reagent R (3 × 1 mL). Ice-cold ethyl ether (8 mL) was then added to the combined filtrates to induce cloudiness. The mixture was maintained at 5 °C for 10 min and then centrifuged to collect a white precipitate, which was washed further with ether (3 × 5 mL), dissolved in water, and lyophilized. The cleavage yield was 60% based on amino acid analyses of peptide-resins before and after cleavage. The crude material was evaluated by analytical HPLC (Figure 5a) and amino acid analysis: Asp 1.37, Glu 4.68, Pro 1.04, Gly 1.96, Ala 1.31, Met 1.22, Leu 0.94, Tyr 1.24, Phe 1.24. A portion of the crude material (5 mg) was dissolved in 5 mL of 0.1% aqueous TFA and applied to an Alltech/Applied Science Econosil C_{18} (10 μ) preparative HPLC column (10 × 250 mm) and eluted at 4.5 mL/min with a linear gradient over 28 min from 7:3 to 2:3 of 0.1% aqueous TFA and 0.1% TFA in acetonitrile. The eluent of the major peak was collected, lyophilized and characterized by analytical HPLC (Figure 5b) and amino acid analysis: Asp 1.24, Glu 5.91, Pro 1.39, Gly 2.14, Ala 0.90, Met 0.80, Leu 0.79, Tyr 0.92, Phe 0.90. Human gastrin-I has a calculated average mass $[M + H]^+$ of 2099.25 and the observed average mass by FABMS was 2099.7 (spectrum in supporting information: Figure 2).

Batchwise Manual SPPS of Acyl Carrier Protein (65-74) Amide (H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-NH_2) on CLEAR-I, -II, and -III. A. The syntheses were carried out manually, in parallel. Fmoc-PAL-Nle-CLEAR resins I, II, and III (0.1 g, 0.25−0.29 mmol/g) were treated with piperidine−DMF (1:4) (5 + 15 min). For each cycle, N^{α}-Fmoc-amino acids (150 μmol) were each in turn dissolved in DMF (0.4 mL), and 2 h couplings were each initiated with 150 μmol of DIPCDI in DMF (0.2 mL) and 150 μmol of HOAt in DMF (0.2 mL). Solutions of amino acids, DIPCDI, and HOAt were made at triple the indicated scale and apportioned equally to the three parallel syntheses. The β-carboxyl function of aspartic acid and the phenolic side-chain of tyrosine were protected as their *tert*-butyl ester and ether, respectively. The β- and γ-carboxamide functions of asparagine and glutamine were protected as their trityl derivatives. Fmoc removal was achieved with piperidine−DMF (1:4) (5 + 25 min). All washings after couplings and deprotections were performed with DMF. Ninhydrin tests on peptide-resin aliquots upon completion of couplings were negative except after the formation of the Val-Gln bond on CLEAR-I. Amino acid compositions of the peptide-resins were Asx 2.11, Glx 1.00, Gly 0.99, Ala 2.07, Val 1.04, Ile 1.79, Tyr 1.00, Nle 1.04 from CLEAR-I; Asx 2.24, Glx 0.88, Gly 1.11, Ala 2.01, Val 0.90, Ile 1.82, Tyr 1.03, Nle 1.44 from CLEAR-II; Asx 2.26, Glx 0.89, Gly 1.15, Ala 2.03, Val 0.90, Ile 1.72, Tyr 1.04, Nle 1.44 from CLEAR-III. Cleavages of peptides from the CLEAR supports were achieved with TFA−CH_2Cl_2 (9:1) (2 mL) for 2.5 h. The solutions containing the cleaved peptides were expressed from the solid-phase reaction vessels with positive nitrogen pressure, and the cleaved resins were washed (3 × 1.5 mL) with further TFA−CH_2Cl_2 (9:1). The combined filtrates were evaporated to dryness (including chasing 3× with CH_2Cl_2), suspended in water, and lyophilized. Cleavage yields, calculated based on amino acid analyses of peptide resins before and after cleavage, were 88% from CLEAR-I, 91% from CLEAR-II, and 89% from CLEAR-III. The crude peptides were dissolved in aqueous 0.1% TFA−acetonitrile (1:1), and the purities were estimated by HPLC to be 91% (from CLEAR-I; Figure 6a), 82% (from CLEAR-II; chromatogram in supporting information: Figure 3a), and 80% (from CLEAR-III, chromatogram in supporting information: Figure 4a), respectively. The amino acid compositions of the crude peptides were Asx 2.15, Glx 0.98, Gly 1.09, Ala 1.99, Val 1.00, Ile 1.73, Tyr 1.05 from CLEAR-I; Asx 2.24, Glx 0.91, Gly 1.14, Ala 1.97, Val 0.89, Ile 1.77, Tyr 1.08 from CLEAR-II; Asx 2.24, Glx 0.89, Gly 1.15, Ala 1.97, Val 0.89, Ile 1.77, Tyr 1.09 from CLEAR-III. ACP (65-74) amide has a calculated exact mass of 1061.5506. FABMS on products from all syntheses, *m*/*z*, positive $[M + H]^+$: 1062.5; negative $[M - H]^-$: 1060.5 (spectra in supporting information: Figures 5−7). FT-MS ESI on the product from CLEAR-I, *m*/*z*, positive $[M + H]^+$: 1062.6; $[M + Na]^+$: 1084.6 (spectra in supporting information: Figure 20).

B. The syntheses were carried out manually in parallel with and following the previously described procedure for assembly of the ACP-(65-74) sequence in DMF, with the exception that the couplings were mediated by DIPCDI alone, and all couplings and washings were performed with acetonitrile instead of DMF [a few drops of DMF were added if needed to dissolve the amino acid derivatives]. Ninhydrin tests on peptide-resin aliquots upon completion of couplings were negative except after the formation of the Val-Gln bond on CLEAR-I and -II. Amino acid compositions of the peptide-resins were Asx 2.09, Glx 1.10, Gly 0.99, Ala 2.18, Val 1.05, Ile 1.65, Tyr 0.93, Nle 0.99 from CLEAR-I; Asx 2.21, Glx 1.04, Gly 1.09, Ala 2.13, Val 0.94, Ile 1.58, Tyr 1.01, Nle 1.36 from CLEAR-II; Asx 2.24, Glx 1.04, Gly 1.08, Ala 2.11, Val 0.95, Ile 1.56, Tyr 1.02, Nle 1.37 from CLEAR-III. The crude peptides were released from the supports as described previously, with cleavage yields of 86% (from CLEAR-I), 92% (from CLEAR-II), and 93% (from CLEAR-III), respectively. The purities (HPLC) of the peptides were 95% (CLEAR-I, Figure 6b), 91% (CLEAR-II; chromatogram in supporting information: Figure 3b), and 91% (CLEAR-III; chromatogram in supporting information: Figure 4b), respectively. The amino acid compositions of the crude peptides were Asx 2.11, Glx 1.01, Gly 1.05, Ala 2.05, Val 1.02, Ile 1.74, Tyr 1.01 from CLEAR-I; Asx 2.18, Glx 0.97, Gly 1.05, Ala 2.01, Val 0.95, Ile 1.77, Tyr 1.06 from CLEAR-II; Asx 2.16, Glx 0.99, Gly 1.04, Ala 2.02, Val 0.97, Ile 1.78, Tyr 1.05 from CLEAR-III. FABMS on products from all syntheses, *m*/*z*, positive $[M + H]^+$: 1062.5; negative $[M - H]^-$: 1060.5 (spectra in supporting information: Figures 8−10).

Batchwise Manual SPPS of Leu-Enkephalinamide (H-Tyr-Gly-Gly-Phe-Leu-NH_2) on CLEAR-IV and -V. The synthetic design was the same as for the earlier described continuous-flow synthesis. Syntheses started with Fmoc-PAL-Nle-CLEAR supports (0.1 g), and the solvent for reactions and all washings was DMF. At each cycle, Fmoc-removal was achieved with piperidine−DMF (1:4) (2 + 8 min). N^{α}-Fmoc-amino acids (100 μmol) were dissolved in DMF (0.4 mL), and 40-min couplings were each initiated with 100 μmol of DIPCDI in DMF (0.2 mL) and 100 μmol of HOAt in DMF (0.2 mL). Ninhydrin tests on peptide-resin aliquots upon completion of couplings were negative. The amino acid compositions of the completed peptide-resins were Gly 1.96, Leu 1.12, Tyr 0.91, Phe 1.00, Nle 0.96 (CLEAR-IV); Gly 2.05, Leu 1.13, Tyr 0.90, Phe 1.02, Nle 0.71 (CLEAR-V). Cleavage of the peptides from the resins were achieved with TFA−CH_2Cl_2 (9:1) for 2 h. The filtrates were expressed from the vessels with positive nitrogen pressure, and the cleaved resins were washed (3 × 1.5 mL) with further TFA−CH_2Cl_2 (9:1). The combined filtrates were evaporated to dryness (including chasing 3× with CH_2Cl_2), suspended in water, and lyophilized. The cleavage yields were 98% (CLEAR-IV) and 90% (CLEAR-V), based on amino acid analyses of peptide resins before and after cleavage. The crude peptides were estimated to be 86% (CLEAR-IV) and 89% (CLEAR-V) pure by HPLC [chromatograms in supporting information: Figure 11]. The amino acid compositions of the crude peptides were Gly 1.90, Leu 1.16, Tyr 0.88, Phe 1.06 (from CLEAR-IV) and Gly 1.95, Leu 1.22, Tyr 0.75, Phe 1.09 (from CLEAR-V). Leu-enkephalin has a calculated exact mass of 554.2853. FABMS, *m*/*z*, positive $[M + H]^+$: 555.4 [same for peptides from both resins; spectra in supporting information: Figures 12 and 13].

Batchwise Manual SPPS of Retro-Acyl Carrier Protein (74-65) amide (H-Gly-Asn-Ile-Tyr-Asp-Ile-Ala-Ala-Gln-Val-NH_2) on CLEAR-I, -II, -III, -IV, and -V; PEG-PS, TentaGel, PEGA, Pepsyn K, Polyhipe, and PS. The syntheses were carried out in parallel starting with Fmoc-PAL-Nle-CLEAR supports (0.1 g, 0.20−0.33 mmol/g), Fmoc-PAL-Nle-PEG-PS (0.1 g, 0.18 mmol/g), Fmoc-PAL-Nle-TentaGel (0.1 g, 0.18 mmol/g), Fmoc-PAL-Nle-PEGA [0.026 mmol, by spectrophotometric determination of the fulvene-piperidine adduct formed at the deprotection], NovaSyn PR 500 (Polyhipe with modified Rink linker) (0.1 g, 0.40 mmol/g), NovaSyn KR 100 (Pepsyn K with modified Rink linker) (0.1 g, 0.11 mmol/g), and Fmoc-amide Resin (PS with Knorr linker) (0.1 g, 0.63 mmol/g). At each cycle, N^{α}-Fmoc-amino acids (5 equiv, 0.25 M) were each in turn dissolved in DMF, and 1 h couplings were each initiated with DIPCDI (5 equiv, 0.5 M) in DMF and HOAt (5 equiv, 0.5 M) in DMF. Solutions of amino acids, DIPCDI and HOAt were made on a larger scale and apportioned equally to the 11 parallel syntheses. Amino acid side-chains were protected as described above in the synthesis of acyl carrier protein (65-74) amide. Fmoc-removal was achieved with piperidine−DMF (1:4) (5 + 10 min). All washings after couplings and deprotections

were performed with DMF. Ninhydrin tests on peptide-resin aliquots upon completion of couplings were negative except after the formation of the Gln-Val bond on PS, the Ala-Ala bond on PS, the Asp-Ile bond on PS, the Ile-Tyr bond on TentaGel and PS, and the Asn-Ile bond on CLEAR-II, PEGA, and PS. Draining off reagents and solvents from Pepsyn K was very slow already after the first cycle. After three cycles, the procedure could not be done in a timely fashion (>30 min for draining off 2 mL solvent), and Pepsyn K was taken out of the comparison study. During the last couplings, the same problem was seen with Polyhipe. Amino acid compositions of the peptide-resins were Asx 1.85, Glx 0.88, Gly 1.03, Ala 2.05, Val 1.14, Ile 2.04, Tyr 1.01, Nle 1.05 from CLEAR-I; Asx 1.96, Glx 1.01, Gly 1.06, Ala 2.16, Val 1.11, Ile 1.74, Tyr 0.95, Nle 1.19 from CLEAR-II; Asx 1.69, Glx 0.74, Gly 1.06, Ala 2.18, Val 1.29, Ile 2.05, Tyr 1.00, Nle 1.33 from CLEAR-III.; Asx 1.44, Glx 0.66, Gly 0.89, Ala 1.92, Val 1.20, Ile 2.26, Tyr 1.07, Nle 1.15 from CLEAR-IV; Asx 1.79, Glx 0.77, Gly 1.07, Ala 2.10, Val 1.08, Ile 2.15, Tyr 1.04, Nle 0.76 from CLEAR-V; Asx 1.90, Glx 0.77, Gly 1.04, Ala 2.08, Val 1.14, Ile 2.03, Tyr 1.04, Nle 2.86[28] from PEG-PS; Asx 1.93, Glx 0.96, Gly 1.02, Ala 2.15, Val 1.12, Ile 1.82, Tyr 0.98, Nle 1.23 from TentaGel; Asx 1.99, Glx 0.83, Gly 1.03, Ala 2.07, Val 1.14, Ile 1.91, Tyr 1.03, Nle 3.03 from PEGA; Asx 1.95, Glx 1.04, Gly 1.01, Ala 2.06, Val 1.05, Ile 1.85, Tyr 1.04, Nle 1.14 from Polyhipe; Asx 1.90, Glx 1.08, Gly 1.02, Ala 2.13, Val 1.09, Ile 1.80, Tyr 0.97 from PS. Cleavages of peptides from the CLEAR supports were achieved with TFA−EDT−H_2O (95:3:2) (2 mL) for 2 h. The solutions containing the cleaved peptides were expressed from the solid-phase reaction vessels with positive nitrogen pressure, and the cleaved resins were washed with further TFA (3 × 1.5 mL). The combined filtrates were evaporated to dryness. Ice-cold ethyl ether was then added to precipitate the peptides. The mixtures were centrifuged, the supernatants removed, and the pellets were suspended in water and lyophilized. Cleavage yields, calculated based on amino acid analyses of peptide resins before and after cleavage, were ≥98% from all the resins. The cleavage yield from PS could not be calculated, because it does not contain an IRAA. The crude peptides were dissolved in aqueous 0.1% TFA−acetonitrile (1:1), and the purities were shown to be ≥99% (CLEAR-I), 86% (CLEAR-II), 90% (CLEAR-III), 81% (CLEAR-IV), 86% (CLEAR-V), 89% (PEG-PS), 87% (TentaGel), 78% (PEGA), 97% (Polyhipe), and 78% (PS), respectively, by HPLC (Figure 7). The amino acid compositions of the crude peptides were Asx 2.09, Glx 0.93, Gly 1.05, Ala 2.15, Val 1.08, Ile 1.78, Tyr 0.90 from CLEAR-I; Asx 1.97, Glx 0.99, Gly 1.04, Ala 2.18, Val 1.13, Ile 1.74, Tyr 0.95 from CLEAR-II; Asx 2.01, Glx 1.05, Gly 0.99, Ala 2.18, Val 1.08, Ile 1.70, Tyr 0.99 from CLEAR-III; Asx 2.10, Glx 1.08, Gly 1.10, Ala 2.24, Val 1.11, Ile 1.68, Tyr 0.69 from CLEAR-IV; Asx 2.13, Glx 1.02, Gly 1.10, Ala 2.18, Val 1.00, Ile 1.72, Tyr 0.84 from CLEAR-V; Asx 2.19, Glx 1.07, Gly 1.06, Ala 2.16, Val 1.04, Ile 1.68, Tyr 0.81 from PEG-PS; Asx 2.12, Glx 1.02, Gly 1.05, Ala 2.19, Val 1.09, Ile 1.69, Tyr 0.84 from TentaGel; Asx 2.18, Glx 1.02, Gly 1.07, Ala 2.17, Val 1.07, Ile 1.65, Tyr 0.85 from PEGA; Asx 2.06, Glx 1.05, Gly 0.99, Ala 2.14, Val 1.01, Ile 1.79, Tyr 0.97 from Polyhipe; Asx 1.98, Glx 1.10, Gly 1.03, Ala 2.17, Val 1.10, Ile 1.76, Tyr 0.87 from PS. Retro-ACP (74-65) amide has a calculated exact mass of 1061.5506. LC-MS on the product from CLEAR-II, *m*/*z*, positive $[M + H]^+$: elution time 9.4 min: 949.5 [corresponds to the calculated $[M + H]^+$ of des-Ile-retro-ACP(74-65) amide]; elution time 20.5 min: 934.5 [corresponds to the calculated $[M + H]^+$ of des-Gln-retro-ACP(74-65) amide]; elution time 21.8 min: 948.5 [corresponds to the calculated $[M + H]^+$ of des-Asn-retro-ACP(74-65) amide]; and elution time 24.4 min (the main peak): 1062.5 [corresponds to the calculated $[M + H]^+$ of retro-ACP(74-65) amide].

Evaluation of Chemical Stability of Underivatized CLEAR Supports. In each experiment, underivatized CLEAR (100 mg) was placed in a 3-mL syringe fitted at the bottom with a porous frit and was treated with an appropriate reagent (2 mL, details and results below). After incubation for the given times, treatments were ended by appropriate washings [3−4 × 2 mL per solvent unless indicated otherwise; individual details below], and the resins were dried *in vacuo* overnight. The resultant treated resins, as well as the original, underivatized CLEAR supports, were analyzed by FT-IR (Figures 8 and 9; supporting information: Figures 14−16) and elemental analysis (supporting information: Table 1).

NaOH Treatment. The resins were incubated with 2 M NaOH in H_2O−MeOH (1:3). After 1 h, CLEAR-I and V dissolved completely; CLEAR-III and IV were unfilterable gels, and CLEAR-II had dissolved partially [22% mass loss; determined gravimetrically after washing with H_2O−MeOH (1:3); the mass loss after 24 h was 30%]. The elemental analyses of the solid-residues of NaOH-treated CLEAR-II were quite similar to the untreated control; the FT-IR indicated retention of the main carbonyl signal at 1737 cm^{-1} but abolition of the shoulder at 1695 cm^{-1}.

NH_4OH Treatment. After incubation with 2 M NH_4OH in H_2O−MeOH (1:3) for 2 weeks, CLEAR-I and V dissolved completely. The remaining resins were washed with H_2O. Mass losses (determined gravimetrically) were 8% from CLEAR-II, 10% from CLEAR-III, and 33% from CLEAR-IV. The elemental analyses of the solid-residue of NH_4OH-treated CLEAR-II, -III, and -IV were quite similar to the untreated controls (and in particular, *no increase in the N values*); the FT-IR indicated retention of the main carbonyl signal at 1737 cm^{-1}, but abolition of two shoulders at 1630−1695 cm^{-1}. For CLEAR-IV, which had considerable mass loss but did not dissolve completely, the combined filtrates were lyophilized; for CLEAR-I and V, the completely solubilized resins were lyophilized. From all three of these cases, "glue"-like solids were obtained which showed: C, 53.99; H, 8.36, N, 1.87 (CLEAR-I); C, 49.38; H, 7.71; N, 4.76 (CLEAR-IV); and C, 53.45; H, 8.17; N, 2.21 (CLEAR-V); furthermore, the FT-MS ESI showed peaks corresponding to $[M + Na]^+$ and $[M + NH_4]^+$ of trimethylolpropane (14/3 EO/OH) ethoxylate triol: $C_2H_5C(CH_2(OCH_2CH_2)_lOH)(CH_2(OCH_2CH_2)_mOH)(CH_2(OCH_2CH_2)_nOH]$, where $(l + m + n) = 7-20$, centered about $(l + m + n) = 12$ [calcd exact mass of M, 662.4; FT-MS ESI, *m*/*z* $[M + Na]^+$: 685.4; $[M + NH_4]^+$: 680.5 [spectra in supporting information: Figures 17−19].

Piperidine Treatment. After incubation with piperidine−DMF (1:4) for 1 h, the resins were washed with piperidine−DMF (1:4), DMF (10×), and CH_2Cl_2. Most of the resins were recovered from these experiments (92−94% recoveries, determined gravimetrically). The elemental analyses of piperidine-treated CLEARs were quite similar to untreated controls; the FT-IR indicated retention of the main carbonyl signal at 1737 cm^{-1} but abolition of the shoulders at 1630−1695 cm^{-1}.

TFA Treatment. After incubation with neat TFA (2 h or 48 h), the resins were washed with neat TFA, CH_2Cl_2 (5×), MeOH (15×), and CH_2Cl_2 (2×). No mass losses were observed, and the elemental analyses were unaltered. FT-IR indicated either no or relatively subtle changes [*e.g.*, for CLEAR-I and -V, appearance of a new shoulder at 1784 cm^{-1} after 48 h].

Batchwise Manual Syntheses of Leu-Enkephalinamide on Piperidine-Treated CLEAR-I, -II, -III, and on Piperidine-Treated PEG-PS and TentaGel. Fmoc-PAL-Nle-CLEAR-I, -II, and -III, Fmoc-PAL-(Nle)-PEG-PS, and Fmoc-PAL-Nle-TentaGel (0.2 g for each experiment) were placed in 12-mL syringes fitted at the bottoms with porous frits and were treated with 8 mL of piperidine−DMF (1:4) for 9 days. After completion of this incubation time, the resins were washed with piperidine−DMF (1:4) (4 × 2 mL), DMF (10 × 2 mL), and CH_2Cl_2 (3 × 2 mL) and dried *in vacuo* overnight. Within experimental error, and correcting for the (essentially immediate) loss of Fmoc, there had been no change in weight of any of these piperidine-treated PAL-supports. Using 0.1 g of treated resins, as well as 0.1 g of the original untreated resins as controls, Leu-enkephalinamide was synthesized in parallel by essentially the same manual batchwise procedure reported earlier in this paper, *i.e.*, DMF as solvent, 40-min DIPCDI/HOAt couplings, Fmoc-amino acids (5 equiv). The amino acid compositions of the completed peptide-resins were Gly 2.22, Leu 0.89, Tyr 1.00, Phe 0.88, Nle 0.84 (original CLEAR-I); Gly 2.08, Leu 1.02, Tyr 0.99, Phe 0.92, Nle 1.27 (piperidine-treated CLEAR-I); Gly 2.16, Leu 1.03, Tyr 0.94, Phe 0.88, Nle 1.07 (original CLEAR-II); Gly 2.05, Leu 1.07, Tyr 0.94, Phe 0.94, Nle 1.39 (piperidine-treated CLEAR-II); Gly 2.03, Leu 1.07, Tyr 0.99, Phe 0.90, Nle 1.20 (original CLEAR-III); Gly 2.05, Leu 1.09, Tyr 1.03, Phe 0.83, Nle 1.50 (piperidine-treated CLEAR-III); Gly 2.09, Leu 1.09, Tyr 0.98, Phe 0.83, Nle 2.71[28] (original PEG-PS); Gly 2.03, Leu 1.05, Tyr 1.00, Phe 0.91, Nle 3.20[28] (piperidine-treated PEG-PS); Gly 2.09, Leu 1.11, Tyr 1.01, Phe 0.79, Nle 1.17 (original TentaGel); Gly 2.05, Leu 1.03, Tyr 0.97, Phe 0.95, Nle 1.31 (piperidine-treated TentaGel). Cleavage of the

peptides was achieved with TFA−CH_2Cl_2 (9:1) for 2 h as described previously, with cleavage yields of 88% (original CLEAR-I), 82% (piperidine-treated CLEAR-I), 72% (original CLEAR-II), 73% (piperidine-treated CLEAR-II), 80% (original CLEAR-III), 75% (piperidine-treated CLEAR-III), 86% (original PEG-PS), 84% (piperidine-treated PEG-PS), 98% (original TentaGel), and 97% (piperidine-treated TentaGel) based on amino acid analyses of peptide resins before and after cleavage. The purities (HPLC) of the crude peptides were ≥99% (original CLEAR-I), 95% (piperidine-treated CLEAR-I), 97% (original CLEAR-II), 89% (piperidine-treated CLEAR-II), 98% (original CLEAR-III), ≥99% (piperidine-treated CLEAR-III), 97% (original PEG-PS), 94% (piperidine-treated PEG-PS), 99% (original CLEAR-TentaGel), and 90% (piperidine-treated TentaGel) (Figure 10). The amino acid compositions of the crude peptides were Gly 2.04, Leu 1.01, Tyr 1.01, Phe 0.94 (from original CLEAR-I); Gly 2.05, Leu 1.01, Tyr 1.01, Phe 0.93 (from piperidine-treated CLEAR-I); Gly 2.19, Leu 1.09, Tyr 0.84, Phe 0.88 (from original CLEAR-II); Gly 2.09, Leu 1.04, Tyr 0.99, Phe 0.88 (from piperidine-treated CLEAR-II); Gly 2.09, Leu 1.05, Tyr 1.01, Phe 0.86 (from original CLEAR-III); Gly 2.07, Leu 1.01, Tyr 1.01, Phe 0.91 (from piperidine-treated CLEAR-III); Gly 2.11, Leu 1.04, Tyr 0.99, Phe 0.86 (from original PEG-PS); Gly 2.13, Leu 1.04, Tyr 1.04, Phe 0.79 (from piperidine-treated PEG-PS); Gly 2.18, Leu 1.05, Tyr 1.04, Phe 0.73 (from original TentaGel); Gly 2.12, Leu 1.04, Tyr 1.00, Phe 0.84 (from piperidine-treated TentaGel).

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Supporting Information Available: Additional table listing elemental analysis data (Table 1); figures showing FABMS of peptides (Figures 1, 2, 5−10, 12, and 13); analytical HPLC chromatograms of peptides (Figures 3, 4, and 11); FT-IR spectra (Figures 14−16); FT-MS ESI of material released into solution and/or solubilized upon treatment of CLEAR supports with NH_4-OH (Figures 17−19); and FT-MS ESI of crude ACP(65-74) amide (Figure 20) (21 pages). See any current masthead page for ordering and Internet access instructions.

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