The pH profiles of K_m are consistent with the complexation mode indicated by E where the anion of NCPA is bound to the macrocycle-containing PEIs mainly through interaction with the macrocyclic centers, instead of the ammonium cationic centers.

Since each macrocyclic metal center appears to represent the reaction site for NCPA,[25] the K_m values (listed in parentheses in Table I) calculated on the basis of $[Mac]_0$ concentrations correspond to K_d. The K_d values measured for HNB and NCPA in the presence of C indicate that HNB forms a considerably stronger complex than NCPA. Spectral titration (not shown) indicated the $pK_a = 6.3$ for the phenol group of HNB. The major portion of HNB is present as a dianion at pH 7-8, and, therefore, would manifest greater affinity toward the polymer compared with the monoanionic NCPA.

In summary, macrocycle-containing PEIs are newly prepared in the present study by the condensation of PEI with dicarbonyl compounds in the presence of transition-metal ions. As a consequence of the condensation reaction, the metal ions are very tightly bound to the polymer. The polycationic environment created by the metal ions attracts benzoate anions. When an anionic ester is used, the anionic portion is anchored by the macrocyclic metal center of the polymer and a nearby amine of the polymer makes a nucleophilic attack at the bound ester linkage.

To develop artificial metalloenzymes that are capable of both recognition of the substrate structure and highly efficient catalytic conversion, it is desirable to incorporate several catalytic groups in planned positions. Efficient metallosynzymes may be obtained by tailoring PEI through creation of metal centers on the polymer and introduction of additional functional groups in proximal positions. This may be achieved by acylation of the nearby amines by using the metal centers as anchors. The macrocycle-containing PEIs may be viewed as polymers of macrocyclic metal complexes. For catalytic processes in which cooperation among multiple metal centers is necessary, the macrocycle-containing PEIs might lead to effective catalysts.

(25) The number (n) of binding sites estimated according to eq 2 represents the number of molecules of L that can be bound to the polymer without affecting binding of another L. It is possible that more than n molecules of L can be bound with less efficient K_d values. The n value of NCPA might not differ considerably from that of HNB. Even after an NCPA molecule acetylates a nitrogen atom of the polymer, however, the nearby metal centers can still bind NCPA leading to deacylation of NCPA, although K_d might be affected. This is why the number (ca. 140) of reaction sites for NCPA is much greater than n (ca. 18) for HNB.

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Solvation Effects in Solid-Phase Peptide Synthesis

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Abstract: The success of solid-phase peptide synthesis is highly dependent on the accessibility of the growing resin-bound peptide chain to reagents. To maximize this accessibility, the relationship between solvent properties and peptide–resin solvation has been explored. The tridecapeptide [Lys_9]-α-conotoxin G I (from *Conus geographus*), which contains nine side-chain-protected residues, was used to study solvation effects of protected peptide–resins. Efficient solvation of (aminomethyl)copoly(styrene–1% DVB) and peptide–copoly(styrene–1% DVB) could be directly correlated to solvent Hildebrand and hydrogen-bonding solubility parameters (δ and δ_h, respectively). Solvation was also highly dependent on the side-chain protecting group strategy (benzyl (Bzl), *tert*-butyl (tBu), or *p*-methoxybenzyl (Mob)) utilized. The most efficient solvation by a single solvent occurred with NMP, regardless of side-chain protection, although the relative solvation is greater for the Bzl-based versus tBu-based side-chain-protected conotoxin–resin. Mixed-solvent systems with optimized δ and δ_h values, such as 45% THF/NMP and 20% TFE/DCM, offered greater solvation than single solvents for the Bzl and tBu side-chain-protected conotoxin–resins. Solvation results for Mob side-chain-protected conotoxin–resin suggested that replacement of the tBu side-chain protecting group by the Mob group improves solvation by single solvents, such as NMP, while still providing the weak acid lability desired for side-chain deprotection following solid-phase peptide synthesis utilizing Fmoc chemistry.

Introduction

Since its inception in the early 1960s,[1] solid-phase peptide synthesis (SPPS) has become one of the most important methodologies in bioorganic chemistry. The success of this technique is highly dependent upon the accessibility of the free amino termini of the resin-bound peptide chains,[2] as the diffusion of reagents to resin reactive sites is not rate-limiting.[3-7] Therefore, efficient SSPS requires a thorough understanding of the physicochemical properties of the peptide–resin. ^{13}C and ^1H nuclear magnetic resonance (NMR) spectra of $CDCl_3/CHCl_3$-solvated copoly(styrene–1% divinylbenzene (DVB)) resins[8-10] and pulsed-field-gradient spin-echo NMR experiments of toluene-solvated copoly(styrene–5.7, 10, 20, or 40% DVB) resins[11] have shown that the linear chains are as accessible as if free in solution. This accessibility depends on the solvent and degree of DVB cross-

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linking. Electron paramagnetic spectra of benzene-solvated, nitroxide-labeled chloromethylated resins showed the lowest rotational correlation times when the degree of cross-linking was 1% DVB, versus 2, 4, 8, or 12% DVB,[12] while faster coupling kinetics were seen for N^{α}-[(nitrophenyl)sulfenyl]phenylalanine *N*-hydroxysuccinimide ester to 1 versus 2% DVB-cross-linked, dichloromethane-solvated (DCM = dichloromethane) Gly-resin[3] and N^{α}-(*tert*-butyloxycarbonyl)phenylalanine (Boc = *tert*-butyloxycarbonyl) preformed symmetrical anhydride to 1 versus 2% DVB-cross-linked, DCM-solvated Phe-resin.[6]

Peptide–polystyrene resins are expected to have physicochemical properties that differ considerably from the initial polystyrene resin, due to the peptide backbone introducing a polar component to the resin. This additional component would require a polar solvent to ensure optimum solvation and, hence, accessibility.[13-16] Electron microscopy has shown increased solvent polarity (*N*,*N*-dimethylformamide (DMF) versus DCM) to result in increased peptide–resin solvation when no side-chain protecting groups were present.[17] Subsequently, it was demonstrated that increased solvent polarity (1-methyl-2-pyrrolidinone (NMP) or DMF versus DCM) was extremely beneficial for synthetic efficiency and peptide–resin solvation when benzyl (Bzl) group based side-chain protection was present.[18-23] Polar solvents not only offered increased solvation of the peptide backbone but also served to inhibit aggregation (β-sheet formation) resulting from interchain hydrogen bonds.[24-30] Due to the improved accessibility of resin-bound peptide chains, polar, hydrogen-bond-accepting solvents (such as NMP and DMF) were determined to be optimal for solvation of peptide–resins requiring Bzl-based or no side-chain protection.[18-21,31,32]

While polar solvents are generally thought to improve SPPS, a critical evaluation relating solvent properties to resin and peptide–resin solvation has not been undertaken. In addition, the solvation of peptide–resins bearing *tert*-butyl (tBu) based side-chain protecting groups have not been examined. The current popularity of N^{α}-[(9-fluorenylmethoxy)carbonyl] (Fmoc) chemistry,[33] which employs tBu-based side-chain protection for Asp, Cys, Glu, His, Lys, Ser, Thr, and Tyr, makes a study of tBu group/peptide–resin solvation imperative. Transfer free energies of amino acid side-chain groups from cyclohexane to water or 1-octanol[34,35] show branched alkyl side chains to be considerably more nonpolar than a Bzl side chain. During the course of a synthesis, the accumulation of nonpolar, tBu-based side chains has been reported to result in interchain aggregates that are not disrupted by polar solvents such as *N*,*N*-dimethylacetamide (DMA).[36,37] This suggests that optimum solvation for Fmoc/tBu chemistry requires an investigation not only of solvent polarity but also of additional properties of both homogeneous and mixed-solvent systems. The work presented here has thus established the relationship between specific solvent properties and solvation of copoly(styrene–1% DVB) resin and Bzl or tBu side-chain-protected *Conus geographus* α-conotoxin G I[38] tridecapeptide amide–copoly(styrene–1% DVB).

Experimental Section

All amino acids are of the L configuration (except for Gly). Fmoc–Pro, –Ala, –Gly, –Cys(tBu), –Tyr(tBu), –Glu(tBu), –Ser(tBu), and –Lys(Boc), free base (aminomethyl)copoly(styrene–1% DVB) (substitution level 1.07 mmol/g), piperidine, DCM, DMF, NMP, methanol (MeOH), *N*,*N*-diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), hydrogen fluoride (HF), trifluoromethanesulfonic acid (TFMSA), and 1-hydroxybenzotriazole (HOBt) were obtained from Applied Biosystems, Inc. Fmoc–Cys(Bzl) and Fmoc–Cys(Mob) (Mob = *p*-methoxybenzyl) were purchased from Bachem AG, Switzerland, and Fmoc–Cys(Mob), –Tyr(Bzl), –Ser(Bzl), –Glu(Bzl), –Lys(Cbz) (Cbz = benzyloxycarbonyl), and –His(π-Bom) (Bom = benzyloxymethyl) were purchased from Bachem, Torrance, CA. 4-(2′,4′-Dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-linked copoly(styrene-1% DVB) resin (Rink resin) (substitution level 0.57 mmol/g), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), and Fmoc–His(π-Bum) (Bum = *tert*-butyloxymethyl) were obtained from Calbiochem, Fmoc–Asn pentafluorophenyl ester (Fmoc–Asn-OPfp) was from Cambridge Research Biochemicals, and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was from Richelieu Biotechnologies, St.-Hyacinthe, Quebec. 1-Octanol, tetrahydrofuran (THF), DMA, 2,2,2-trifluoroethanol (TFE), dimethyl sulfoxide (DMSO), acetonitrile, chloroform, toluene, cyclohexane, thioanisole, *p*-thiocresol, anisole, and 1,2-ethanedithiol (EDT) were purchased from Aldrich.

The [Lys_9]-α-conotoxins were synthesized on an Applied Biosystems 431A peptide synthesizer by solid-phase protocols;[1,2] the scale of the syntheses was 0.25 mmol. Resin was deprotected by a 3 min, followed by a 15-min treatment of 10 mL of 20% piperidine/DMF or NMP, filtered, and rinsed six times with a total of 90 mL of DMF or NMP. All Fmoc–amino acids (except for Fmoc–Asn–OPfp) were coupled with TBTU as follows: (a) 1.0 mmol of derivatized amino acid was dissolved in 2.0 mL of DMF or NMP; (b) 1.0 mL of 0.5 M HOBt in DMF and 1.0 mL of 1.0 M DIEA in DMF were added to the amino acid solution; (c) the amino acid/HOBt/DIEA solution was transferred to the resin; (d) 1.0 mmol of TBTU was dissolved in 1.0 mL of 0.5 M HOBt in DMF and 1.0 mL of 1.0 M DIEA in DMF; and (e) the TBTU solution was transferred to the resin solution and reacted for 40 min at room temperature while mixing. For Fmoc–Asn-OPfp coupling, an empty cartridge was used for step d. All Fmoc–amino acids (except for Fmoc–Asn-OPfp) were coupled with HBTU as follows: (a) 1.0 mmol of derivatized amino acid was dissolved in 2.5 mL of NMP; (b) 2.0 mL of 0.45 M HBTU + 0.45 M HOBt in DMF was added to the amino acid solution; (c) the amino acid/HBTU/HOBt solution was mixed for 10 min and then transferred to the resin; (d) 2.0 mmol (0.35 mL) of DIEA was added to the resin solution and reacted for 30 min at room temperature while mixing. For Fmoc–Asn-OPfp coupling, 0.50 M HOBt (no HBTU) in DMF was used for step b. The resin was filtered and rinsed six times with a total of 90 mL of DMF or NMP and a 2.0-mg sample removed for quantitative ninhydrin (Ruhemann's Purple) testing.[39] Each

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Table I. Solvent Properties[40,42-44,a]

solvent	δ	D	ϵ	δ_h	γ_c	AN	DN
acetonitrile	11.9	3.92	37.5	3.0	6.3	19.3	14.1
chloroform	9.2	1.01	4.81	2.8	1.5	23.1	
cyclohexane	8.2	0.00	2.02	0.1	0.0		
DCM	9.7	1.60	9.08	3.0	1.5	20.4	
DMA	10.8	3.72	37.8	5.0	12.3	13.6	27.8
DMF	12.1	3.86	36.7	5.5	11.7	16.0	26.6
DMSO	12.9	3.90	46.6	5.0	7.7	19.3	29.8
MeOH	14.5	1.70	32.7	10.9	18.7	41.3	19.0
NMP	11.3	4.09	32.0	3.5		13.3	27.3
1-octanol	10.3	1.68	10.3	5.8	18.7		
TFE	11.9		26.5	8.3		70.0	
THF	9.1	1.75	7.58	3.9	5.3	8.0	20.0
toluene	8.9	0.45	2.57	1.0	4.5		

[a] δ and δ_h are in units of $(cal/cm^3)^{1/2}$, while 1 $D = 10^{-18}$ esu cm.

Fmoc–amino acid was then deprotected as above. Ruhemann's Purple concentrations were determined at 570 nm with a Bausch & Lomb Spectronic 1001 spectrophotometer.

Cleavage reactions were performed by stirring 30 mg of peptide–resin in 0.48 mL of 10% TFA/90% DCM at room temperature (for the (tBu)conotoxin–resin) or 8.9% TFMSA/11.7% thioanisole/4% EDT/75.4% TFA in an ice bath (for the (Bzl)conotoxin–resin) for 1 h; 89% HF/9% anisole/2% *p*-thiocresol (1.5 h, 0 °C) was used for the cleavage and side-chain deprotection of the (Mob + Bzl)conotoxin–resin. Following TFA cleavage, 4.0 mL of H_2O was added and the diluted cleavage mixture was extracted three times with 4.0 mL of methyl *tert*-butyl ether. The H_2O layer was filtered and the resin rinsed with 1.0 mL of 30% acetic acid (AcOH). The combined filtrate and washing was centrifuged under vacuum to dryness. Following TFMSA cleavage, the cleavage mixture was filtered directly into methyl *tert*-butyl ether and the precipitated crude product washed with methyl *tert*-butyl ether and dried overnight. Products from peptide–resin cleavages were characterized by ^{252}Cf plasma desorption time-of-flight mass spectrometry (PD-TOF-MS) on an Applied Biosystems BioIon 20 biopolymer mass analyzer.

Solvation studies were carried out with use of a glass-fritted buret. Volumes were determined following several mixes and rinses of 500 mg of resin or peptide–resin with solvent.

Results and Discussion

The effectiveness of solvents used in SPPS has traditionally been correlated to their polarities or dipole moments (D). Recently, theory incorporating solvent electron donor (DN) and acceptor (AN) numbers[40] has been used to create mixed-solvent systems that minimize intermolecular β-sheet formation.[28,30,41] For this investigation several additional solvent properties, such as Hildebrand solubility parameter (δ), hydrogen-bonding solubility parameter (δ_h), dielectric constant (ϵ), and hydrogen-bonding index (γ_c), have been considered for the purpose of correlating solvents to solvation efficiency (Table I).[42-44] δ was calculated for TFE by[43]

$$\delta = \left(\frac{-\sum z\Delta U}{\sum zV}\right)^{1/2} \quad (1)$$

where z is the number of each group type (e.g., OH or CH_2), ΔU is the molar vaporization energy, and V is the molar volume. δ_h was calculated for TFE by[42]

$$\delta_h = \left(\frac{5000N}{\sum zV}\right)^{1/2} \quad (2)$$

where N is the number of hydroxyl groups.

The maximum solvation of nonprotonated (aminomethyl)copoly(styrene–1% DVB) resin[2] (AM-R) occurred in DCM and THF, while toluene and NMP were only slightly less beneficial (Table II). A comparison of resin solvation to solvent δ values showed maximum solvation to occur with solvents of δ = 9.0–9.8, although the overall correlation was only fair. Since cross-linked polystyrene has a δ value of 9.1,[45] the maximum solvation of the cross-linked polymer occurred in solvents having solubility parameters similar to those of the polymer.[46] The resin solvation data were also considered in light of other solvent properties. Figure 1 is a contour solvation plot of δ_h versus δ, where maximum solvation occurred at δ_h = 1.0–4.0 and δ = 8.9–11.3. A similar contour plot can be constructed when γ_c is used in place of δ_h. The best correlation between solvent properties and resin solvation is seen for this contour plot, where both δ and either δ_h or γ_c values are considered. Previous studies of (Boc)Phe–copoly(styrene–2% DVB)[47] and (Boc)Gly–copoly(styrene–1% DVB)[48] solvation showed similar relationships between relative solvation and solvent δ and δ_h values as seen here for AM-R. The addition of a poly(ethylene glycol) spacer (MW 2000) to AM-R (substitution level 0.6 mmol/g) altered this relationship,[48] where solvents of δ =

Table II. Resin Solvation by Single Solvents

	solvation, mL/g resin			
solvent	AM-R	2	1	3
acetonitrile	2.40	2.65	2.85	2.47
chloroform	8.85	4.28	5.10	4.64
cyclohexane	2.25	2.65	2.70	3.40
DCM	8.92	3.83	4.20	3.86
DMA	6.33	5.16	4.50	5.72
DMF	6.17	5.31	4.65	5.72
DMSO	3.46	4.57	4.05	5.56
MeOH	3.18	2.95	2.70	2.32
NMP	8.23	5.60	5.10	6.65
1-octanol	2.62	3.10	3.30	2.16
TFE	4.61	2.65	3.00	3.09
THF	8.92	4.28	4.20	4.33
toluene	8.63	3.68	3.90	3.71

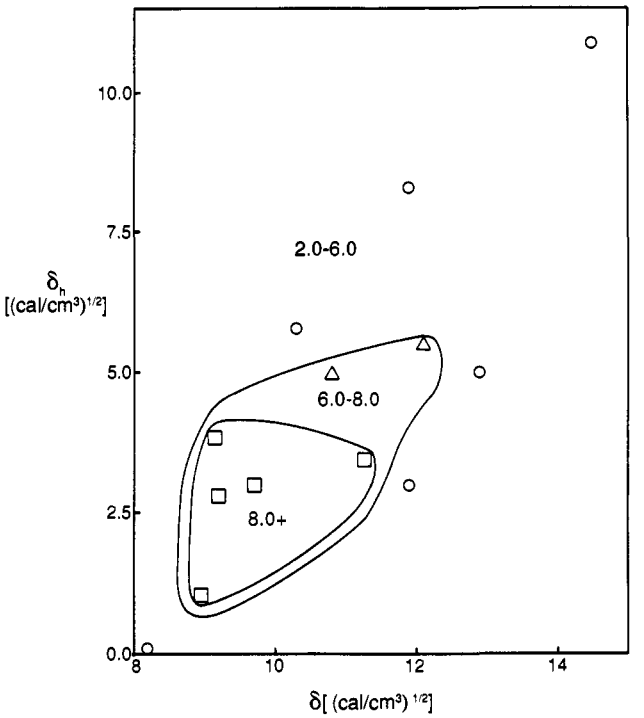

Figure 1. Contour solvation plot of AM-R as a function of δ_h and δ.

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11.9–12.1 and δ_h = 3.0–5.5 (acetonitrile, DMF) solvate the resin with equal or greater efficiency as a solvent of δ = 9.1 and δ_h = 3.5 (ethyl acetate). Poly(oxyethylene)-linked copoly(styrene–2% DVB) resin showed a comparable, altered solvation behavior[49] (MW of poly(oxyethylene) is 3300).

The *C. geographus* [Lys_9]-α-conotoxin G **I** was synthesized three times by the protocols described in the Experimental Section, where the side-chain protection was tBu-, Bzl-, or Mob + Bzl-based:

[1] Glu(OtBu)–Cys(tBu)–Cys(tBu)–Asn–Pro–Ala–Cys(tBu)–Gly–Lys(Boc)–His(π–Bum)–Tyr(tBu)–Ser(tBu)–Cys(tBu)–Rink Resin

[2] Glu(OBzl)–Cys(Bzl)–Cys(Bzl)–Asn–Pro–Ala–Cys(Bzl)–Gly–Lys(Cbz)–His(π–Bom)–Tyr(Bzl)–Ser(Bzl)–Cys(Bzl)–Rink Resin

[3] Glu(OBzl)–Cys(Mob)–Cys(Mob)–Asn–Pro–Ala–Cys(Mob)–Gly–Lys(Cbz)–His(π–Bom)–Tyr(Bzl)–Ser(Bzl)–Cys(Mob)–Rink Resin

Quantitative ninhydrin monitoring and crude peptide–resin weight gain data (Tables 1 and 2, supplementary material) showed the syntheses to be highly efficient. Following TFA cleavage of **1**, PD-TOF-MS (supplementary material) gave a primary $[M + H]^+$ molecular ion of m/z 1724.0, corresponding to [Cys-$(tBu)_{2,3,7,13}$,Lys_9,$His(\pi\text{-}Bum)_{10}$]-α-conotoxin (calculated m/z 1725). Following TFMSA cleavage of **2**, PD-TOF-MS (supplementary material) gave primary $[M + 2\,Na - H]^+$ molecular ions of m/z 1734.3, 1643.1, and 1553.0, corresponding to the [Lys_9]-α-conotoxin containing three Cys(Bzl) (calculated m/z 1730), two Cys(Bzl) (calculated m/z 1640), and one Cys(Bzl) (calculated m/z 1550), respectively. As previously reported, 1 M TFMSA–thioanisole/TFA cleavage only partially deprotects Cys(Bzl) residues.[50] PD-TOF-MS also gave primary $[M + Na + K - H]^+$ molecular ions of m/z 1750.6 (calculated m/z 1746), 1660.6 (calculated m/z 1656), and 1569.0 (calculated m/z 1566) corresponding to the same three partially deprotected peptides, respectively. Following HF cleavage of **3**, PD-TOF-MS (supplementary material) gave primary $[M + H]^+$ molecular ions of m/z 1411.0–1417.0, corresponding to [Lys_9]-α-conotoxin (calculated m/z 1414–1418, depending upon the Cys oxidation states), and m/z 1392.7, corresponding to [pyroGlu_1,Lys_9]-α-conotoxin (calculated m/z 1396), resulting from HF cleavage of Glu(Bzl).[51] The PD-TOF-MS analyses showed crude products of homogeneity (taking the partial deprotection of Cys(Bzl) into account) equal to or greater than that predicted from the ninhydrin and weight gain data.

NMP was the best single solvent for the solvation of both **1** and **2**, although the increased solvent polarity offered by NMP is more beneficial for **2** (Table II). In general, **1** is better solvated by relatively nonpolar (low-*D*) solvents (such as chloroform, DCM, and toluene), while **2** is better solvated by relatively polar (high-*D*) solvents (such as DMA, DMF, DMSO, and NMP). Neither **1** or **2** was solvated to any significant extent by acetonitrile, cyclohexane, MeOH, or 1-octanol. Contour solvation plots of δ versus δ_h for **2** (Figure 2) and **1** (Figure 3) show that the addition of a peptide chain, regardless of the side-chain protection, shifts the region of maximum solvation to higher δ and δ_h values compared to resin alone (Figure 1). A comparison of the contour plots shows the Bzl-based side-chain-protected conotoxin–resin (**2**) to be better solvated at higher δ_h value solvents, while the tBu-based side-chain-protected conotoxin–resin (**1**) is better solvated at lower δ and δ_h solvents (e.g., chloroform).

Since most SPPS incorporate both Bzl-based (Phe; Bzl side-chain protecting group) and branched alkyl-based (Ile, Leu, Val; tBu side-chain protecting group) side chains, the solvation effects

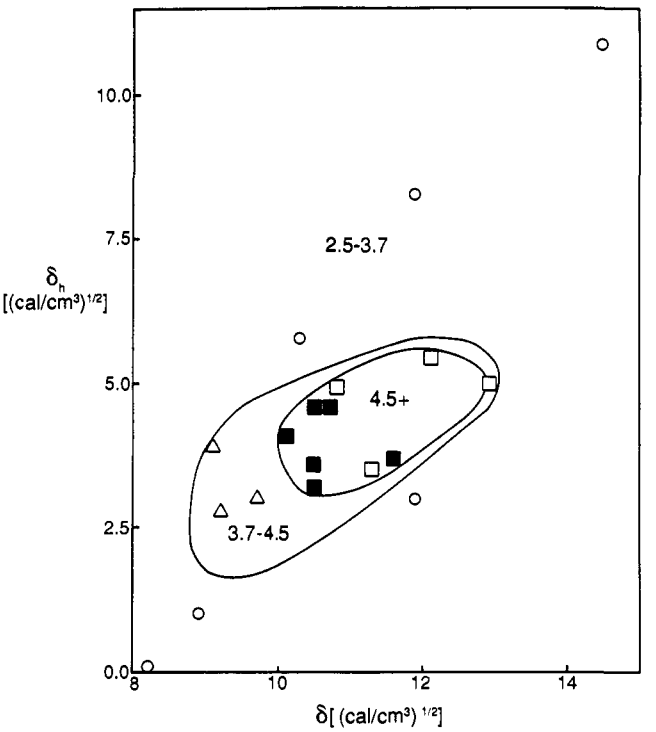

Figure 2. Contour solvation plot of **2** as a function of δ_h and δ. The open circles, triangles, and squares represent single solvents, and the closed squares represent mixed solvents.

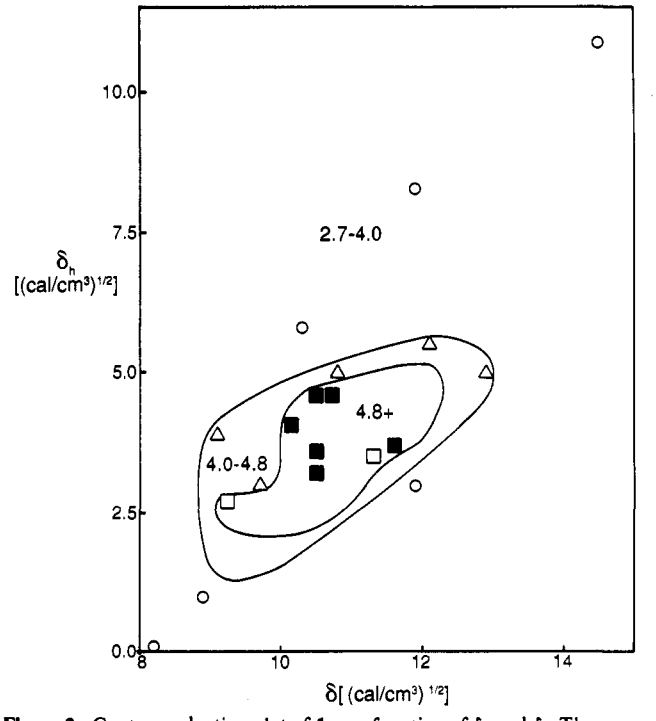

Figure 3. Contour solvation plot of **1** as a function of δ_h and δ. The open circles, triangles, and squares represent single solvents, and the closed squares represent mixed solvents.

of peptide–resins containing both side-chain types is of great interest. The solvation of [Ala_{15}]-growth releasing factor (GRF) 11–29-*p*-methylbenzhydrylamine–copoly(styrene–1% DVB), which contains eight Bzl-based side chains and six branched alkyl side chains, was previously examined.[23] The relative solvation of the GRF 11–29-resin by single solvents was NMP ≫ DMF > DMSO ≫ DCM, solvation behavior that is an approximate mixture of the solvation behaviors of the tBu-based and Bzl-based side-chain-protected conotoxin–resins discussed in the previous paragraph. The enhanced solvation by NMP may account for the recent solid-phase synthesis successes of long-chain (>35 residues)

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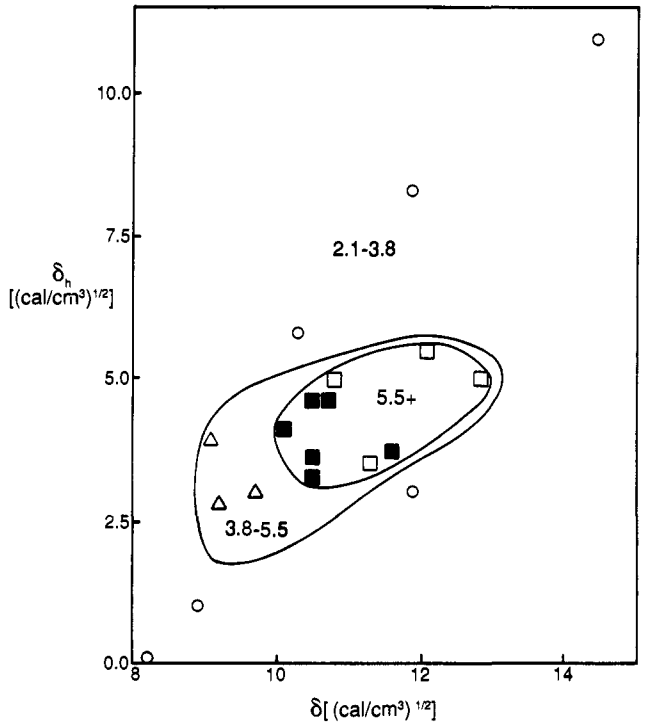

Figure 4. Contour solvation plot of **3** as a function of δ_h and δ. The open circles, triangles, and squares represent single solvents, and the closed squares represent mixed solvents.

peptides where NMP was a coupling solvent, such as rat transforming growth factor α (50 residues)[52,53] and human insulin-like growth factor I (70 residues)[23,54] by Boc chemistry and procalcitonin 1–57,[55] chicken antral peptide (36 residues),[56] human pancreastatin (52 residues),[57] [Cys(Acm)]cholecystokinin-releasing peptide (61 residues),[58] and yeast actin-binding protein 539–588[59] by Fmoc chemistry.

The conotoxin–resin studies clearly show that peptide–resin solvation by increasingly polar solvents is substantially improved with Bzl-based side-chain protection versus tBu-based side-chain protection. To improve solvation during Fmoc/tBu SPPS, there are two possible solutions: (a) the replacement of tBu with more polar side-chain protecting groups or (b) the introduction of a mixed-solvent system that solvates well both the polar peptide backbone and the nonpolar tBu side chains. A previous Fmoc SPPS of cytochrome *c* fragment 66–104 showed the replacement of apolar side chains (Lys(Boc), Met) by more polar side chains (Lys(Tfa), Met(O)) to be required for efficient synthesis.[36,37] This suggests that a general side-chain protecting group that is both weak-acid labile and more polar that the tBu group (e.g., Mob[60]) would improve solvation for Fmoc SPPS based on currently used single-solvent systems. The relative solvation of **3** (where four of the nine Bzl side-chain protecting groups from **2** have been replaced by Mob side-chain protecting groups) was very similar

Table III. Resin Solvation by Mixed Solvents

mixed solvent	solvation, mL/g resin		
	2	**1**	**3**
16% DMSO/NMP	5.60	5.10	6.34
20% TFE/DCM	5.75	6.30	6.65
20% MeOH/DCM	4.57	5.40	5.56
50% toluene/DMF	4.95	4.80	5.56
35% THF/NMP	6.22	5.70	6.34
45% DMF/THF	5.43	5.26	5.72

to that of **2** (Table II and Figure 4). Interestingly, the solvation of **3** by NMP and DMSO is far greater than the solvation of **2** by these two solvents. The similar nonsolvated volumes of **2** and **3** (in acetonitrile, for example) show that the enhanced solvation is due to the presence of the Mob groups and not to a generally larger volume of **3** in all solvents.

Replacement of the tBu side-chain protecting group by the Mob group should provide increased solvation by polar solvents during Fmoc SPPS. The use of Mob side-chain protection has some potential drawbacks, however. Cys(Mob) is subject to alkylation by TFA-liberated tBu groups.[61] The 2,4,6-trimethoxybenzyl (Tmob) group, used for side-chain protection of Fmoc–Asn and –Gln, forms a highly reactive carbonium ion upon TFA cleavage that readily modifies Trp residues.[59,62,63] TFA-liberated Mob groups may therefore be more difficult to scavenge than TFA-liberated tBu groups. However, the α-amino-*p*-methoxybenzyloxycarbonyl (Moz) group has not reported to modify Trp residues during TFA cleavage in the presence of 21–42% anisole.[64–66] SPPS utilizing Mob side-chain protection thus requires further investigation.

In considering mixed-solvent systems, there have been several combinations used successfully in SPPS, including 20% TFE/DCM[16,23,67] and 15–20% DMSO/NMP.[23,67] The solvation of all three conontoxin–resins has been examined in 16% DMSO/NMP, 20% TFE/DCM, and 20% MeOH/DCM (Table III). The 16% DMSO/NMP mixture offered no additional solvation compared to NMP alone for any of the conotoxin–resins, although it should be noted that the favorable solvation effects of added DMSO appear to be sequence, not side-chain protecting group, specific.[23,67] The 20% MeOH/DCM mixture solvated **2** and **3** reasonably well, although several single solvents (NMP, DMF, DMA) were better. However, the solvation of **1** by 20% MeOH/DCM was superior to that of any of the single solvents. The 20% TFE/DCM solvated all three conotoxin-resins best, with much larger relative solvation effects for **1**. To correlate the solvation effects of mixed-solvent systems to solvent properties, we have estimated the solubility parameters using[43]

$$\delta_{1+2} = \phi_1\delta_1 + \phi_2\delta_2 \qquad (3)$$

where ϕ is the volume fraction of the solvent. By eq 3, δ = 11.6 and δ_h = 3.7 for 16% DMSO/NMP, δ = 10.7 and δ_h = 4.6 for 20% MeOH/DCM, and δ = 10.1 and δ_h = 4.1 for 20% TFE/DCM. Comparison of the estimated mixed-solvent δ and δ_h values to the contour plots for **2**, **1**, and **3** (Figures 2–4) showed that these three mixed-solvent systems are located in the maximum solvation region for the resins. If these δ values truly correlate to relative solvation, it should be possible to design mixed-solvent systems

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for optimal solvation based on estimated δ and δ_h. Mixed-solvent systems such as 50% toluene/DMF (δ = 10.5, δ_h = 3.2), 35% THF/NMP (δ = 10.5, δ_h = 3.6), and 45% DMF/THF (δ = 10.5, δ_h = 4.6) would be predicted to solvate all three conotoxin–resins well on the basis of their δ and δ_h values. As shown in Table III, 50% toluene/DMF, 35% THF/NMP, and 45% DMF/THF indeed provided excellent peptide–resin solvation, regardless of side-chain protection. The 35% THF/NMP was the best solvent system for the solvation of **2** and the second best solvent system for the solvation of **1**.

The use of mixed-solvent systems for SPPS requires careful attention to acylation conditions.[68] A stable, preformed species (such as Fmoc amino acid symmetrical anhydrides or Pfp esters) could be used in any of the mixed-solvent systems explored here. However, if amino acid activation is required before use, a mixed-solvent system containing any of the aforementioned alcohols (MeOH, 1-octanol, TFE) may not be practical, as an alcohol could consume the activator. Activation can be performed in a single solvent, with the alcohol added once the activated species is obtained.[16,69] For in situ acylations, where activated species are not preformed, the TFE/DCM or MeOH/DCM solvent mixtures would be much less desirable than THF/NMP or DMF/THF. The presence of relatively nonpolar THF should also enhance in situ activation compared to polar solvents.[70,71] In addition, the THF/NMP and DMF/THF solvent mixtures are similar with respect to electron-accepting/donating potential,[28,41] as DMF, NMP, and THF are all electron-donating solvents.

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Summary

We have demonstrated that solvation of peptide–copoly(styrene–1% DVB) resins during solid-phase synthesis is dependent on both the growing peptide backbone and the nature of the side chains. Branched alkyl-based side chains do not enhance peptide–resin solvation by polar solvents as compared to Bzl-based side chains. This problem may be overcome by replacing branched alkyl side-chain protecting groups (e.g., tBu) with Bzl-based groups (e.g., Mob) or by using mixed-solvent systems that contain both a relatively polar and relatively nonpolar component (e.g., 20% TFE/DCM or 35% THF/NMP). The solvation of peptide–resins by single solvents and mixed-solvent systems can be correlated to the Hildebrand solubility (δ) and hydrogen-bonding solubility (δ_h) parameters.

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Registry No. [Cys(tBu)$_{2,3,7,13}$,Lys$_9$,His(π-Bum)$_{10}$]-α-conotoxin, 133399-52-7; [Lys$_9$]-α-conotoxin, 133374-41-1; [pyroGlu$_1$,Lys$_9$]-α-conotoxin, 133399-53-8.

Supplementary Material Available: Ninhydrin (Table 1), weight gain (Table 2), and PD-TOF-MS data for synthesized conotoxins and calculation of δ and δ_h for TFE (6 pages). Ordering information is given on any current masthead page.

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