samples using a membrane with a MW cutoff of 10000 completely retained the material that inhibited hemagglutination. The activity also showed no fractionation after chromatography on a Sephadex G-10 column. We conclude that the molecular weights are high and that the inhibitory activity is not influenced by small variations in molecular weight. Treatment of poly(**1**-*co*-acrylamide) with neuraminidase (EC 3.2.1.18) abolished its activity. This control indicates that the α-sialoside groups in this polymer are substrates for this enzyme and demonstrates that the activity of the polymer in inhibiting hemagglutination is due to these groups.

Figure 1 summarizes the inhibition by these polymers of the hemagglutination of chicken erythrocytes by influenza X-31 virus.[8] *The values of K_i are calculated on the basis of the concentrations of sialic acid moieties in the solutions*, not of the polymer chains to which they are linked.[10] These values are thus directly comparable with one another and with values of monomeric and oligomeric derivatives of sialic acid. The most important of these data indicate that sialic acid groups incorporated into the polymer formed from **1** and acrylamide in ratios [**1**]/[acrylamide] $\simeq$ 0.2-2 are more effective than methyl α-sialoside in inhibiting hemagglutination by $\sim 10^4$-10^5 and are only approximately a factor of 10 less effective than the sialic acid groups in equine α_2-macroglobulin. *Polyvalent derivatives of sialic acids are thus much more effective than monomeric ones in inhibiting hemagglutination.*

We have prepared a substantial number of copolymers of **1** with N-substituted acrylamides and tested them for their ability to inhibit hemagglutination; a few examples are summarized in Table I. On the basis of these data, we infer that bulky groups and charged groups R (Scheme I) interfere with binding.

We rationalize the bell-shaped curve in Figure 1 using an argument based on a competition between entropy and efficiency of utilization of sialic acid groups. At low values of χ_{SA}, the distance between sialic acid groups is large and the entropic advantage of binding resulting from connecting them is small; at high values of χ_{SA}, only a few sialic acid groups can bind to the HA sites, and the rest, those between the bound groups, do not participate in binding. The value of χ_{SA} may also influence the conformation of the polymer and nonspecific interactions with the virus in ways important to its activity in binding.

This work demonstrates a new strategy for the design and synthesis of compounds that inhibit binding of influenza virus to the surface of cells.[11] This strategy is based on polyvalent polymeric inhibitors able to compete with the polyvalent virus-cell interaction.[12] It is possible, by basing the syntheses of these inhibitors on copolymerization reactions of relatively readily synthesized monomers, to prepare macromolecule structures easily, albeit with incomplete knowledge of the distribution of groups along the backbone of the polymer and in space. This strategy for the preparation of polyvalent binding agents should be generally applicable to the generation of a wide range of tight-binding agents directed toward receptors or ligands of which multiple copies are present on the surface of the target cell, microorganism, or virus.

Note Added in Proof. Results similar to those reported here have been obtained in independent work by Matrosovich et al.[13]

Acknowledgment. The influenza virus used in these studies was supplied by Dr. John Skehel. We thank Dr. Gary Glick for assistance with the hemagglutination inhibition assay.

(10) The hemagglutination inhibition constant K_i is defined as the lowest concentration of sialic acid residues that completely inhibits agglutination of erythrocytes by influenza virus at 4 °C under the assay conditions defined in ref 8.

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New Macrocyclic Pseudopeptides Containing Urethane Backbone Linkages

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The C-NH portion of the urethane bond of common N-terminus protecting groups (Z and Boc) has a high tendency to assume a cis conformation.[1,2] Since cis peptide bonds facilitate the formation of cyclic peptides,[3] we investigated whether the incorporation of urethane linkages into the backbone of a peptide may facilitate the formation of previously unknown macrocyclic pseudopeptides. Depending on their conformational rigidity, these compounds may find applications as cryptands,[4] as molecular hosts,[5] and as building blocks for molecular clefts.[6]

While most reaction schemes in pseudopeptide chemistry are based on the insertion of non-amide bonds between the C-terminus of one amino acid and the N-terminus of the neighboring amino acid,[7-9] we derived the urethane bond from the side-chain hydroxyl group of tyrosine. This approach gives rise to a nine-atom unit (the tyrosine-urethane unit) which at least in a formal sense can be regarded as a tripeptide surrogate (Figure 1). In order to test whether the tyrosine urethane unit could be used as a cyclization promoter, we attempted the synthesis of the 24-membered macrocyclic pseudopeptide (**1**) via Scheme I (see supplementary material for synthetic details). An advantage of macrocycle **1** over common cryptands such as crown ethers is the presence of pendent carboxylic acid groups (the C termini of tyrosine) that can be used for the attachment of additional molecules to the ring. We used this feature to increase the lipophilicity of the macrocycle by the attachment of hydrophobic hexyl chains to the ring.

The "tyrosine-urethane unit" was attached to alanine benzyl ester, and the resulting pseudodipeptide was cyclized by using conventional active esters.[10-12] The crude cyclization product consisted of 67% of **1** and 33% of a mixture of higher oligomers (mostly **2**), as determined by size exclusion chromatography (SEC). The two cyclic components (**1**, **2**) were separated by flash chromatography,[13] and their exact molecular weights were determined by FAB-MS (MH^+ of **1**, calcd 725, found 725; MH^+ of **2**, calcd 1087, found 1087). **1** was obtained in high purity in an isolated yield of 54%. The structure of **1** was confirmed by ^1H NMR (Figure 2 in supplementary material) and by elemental analysis (calcd for $C_{38}H_{52}N_4O_{10}$, C = 62.97, H = 7.23, N = 7.73; found, C = 63.00, H = 7.12, N = 7.69).

Both cyclic products combined accounted for over 80% of the total product. This high cyclization yield indicated that the "tyrosine-urethane unit" may find applications as a new type of

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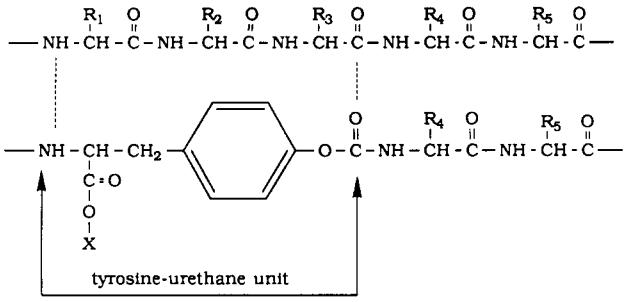

Figure 1. Overlap of the tyrosine–urethane unit with a regular tripeptide. The tyrosine–urethane unit is nine atoms long, corresponding (at least in a formal sense) to the length of a tripeptide segment.

Scheme I

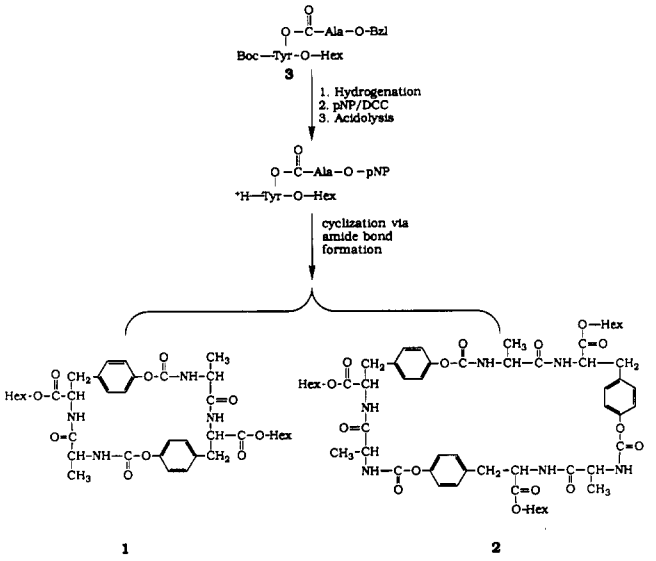

Table I. Metal Binding Studies[a]

peptide derivative	metal salt	picrate ions solubilized, mol/L	molar ratio of [metal]/[peptide][b]
none	Li picrate	~0	na[c]
none	Na picrate	~0	na[c]
none	K picrate	~0	na[c]
3	Li picrate	2.142×10^{-4}	0.026/1
3	Na picrate	1.075×10^{-4}	0.013/1
3	K picrate	3.055×10^{-5}	0.003/1
1	Li picrate	5.271×10^{-3}	0.636/1
1	Na picrate	2.100×10^{-4}	0.025/1
1	K picrate	5.666×10^{-5}	0.007/1

[a] 3 or 1 was dissolved in anhydrous chloroform, and an excess amount of either Li picrate, Na picrate, or K picrate was added. After stirring for 24 h, the excess of undissolved alkali picrate was removed and the amount of picrate ions present in the organic phase was determined by UV spectroscopy (see supplementary material for additional details). [b] Molar ratio of complexed metal ions to total peptide. [c] Not applicable.

cyclization promoter. Obviously, the Ala residue in **1** can be replaced by other amino acids.

The potential application of **1** as a cryptand was explored by examining the ion binding specificity of **1** toward alkali-metal ions (Li, Na, K) in a "phase-transfer" experiment[14] (Table I). Alkali picrates are virtually insoluble in chloroform. In the presence of peptides **3** or **1**, however, various amounts of metal ions were solubilized in the organic phase. The data reported in Table I were obtained after a uniform interval of 24 h, at which time the solubilization process had reached equilibrium.

When the linear pseudo*di*peptide **3** was used as the complexing agent, about 8 times more Li^+ ions than K^+ were solubilized, indicating a low degree of selectivity for Li^+ over K^+ ions. In absolute terms, however, only very small amounts of alkali ions were transferred into the chloroform phase, as indicated by the very low ratio of metal ions to peptide (see Table I). On the other hand, the macrocyclic pseudopeptide **1** solubilized about 0.6 molar equiv of Li^+ ions, which was 25 times higher than the amount of Na^+ and 91 times higher than the amount of K^+ ions. Thus, **1** is an effective and selective phase-transfer agent for Li^+ ions.

Since the structure of **1** can be readily modified by changing the C-terminus protecting groups or by replacing Ala by other L- or D-amino acids, our synthetic approach gives rise to a family of new macrocyclic pseudopeptides that may be used as synthetic building blocks in numerous bioorganic applications.

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Supplementary Material Available: Experimental details for the synthesis and characterization of **1** (6 pages). Ordering information is given on any current masthead page.

Physical Characterization of a Totally Synthetic 2[4Fe-4S] Clostridial Ferredoxin

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The 2[4Fe-4S] ferredoxins (Fd) serve as electron carriers in fermentative and photosynthetic pathways. In order to determine the influence of specific amino acids on the electron transferring properties of bacterial ferredoxins, Rabinowitz and co-workers have used semisynthetic procedures to substitute or delete specific amino acids near the N-terminus of native ferredoxins.[1-3] In this paper we report the first totally synthetic 2[4Fe-4S] ferredoxin, which, along with future synthetic variants, will be used to determine the effects of specific amino acid residues throughout the protein upon both its equilibrium and dynamic properties. The apoprotein was synthesized via standard tBOC procedures on polyvinyl (PAM) resins based on principles outlined by Merrifield.[4]

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