protein. CPEP mutase was readily purified to homogeneity.[3,15] Using synthetic substrate and the coupled enzyme assay, carboxyphosphonoenolpyruvate has a K_m of 0.27 mM and a k_{cat} of 0.020 s^{-1} in the mutase reaction.[16] The low k_{cat} may derive from the fact that the carboxyphospho group transfer involved in the conversion of **3** to **4** is highly endergonic. In qualitative agreement with the observation of Hidaka et al.,[3] CPEP mutase is more than 10 times as active in the presence of Mn(II) as in the presence of Mg(II). We currently aim to establish whether the presumed rearrangement of **3** to **4** proceeds via a carboxyphospho-enzyme intermediate similar to that suggested for the interconversion of **1** and **2**,[17] and whether the decarboxylation of **4** to **5** is enzyme catalyzed.

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(15) CPEP mutase was purified by an ammonium sulfate precipitation, followed by chromatography on hydroxyapatite [eluting with potassium phosphate (10–200 mM), pH 6.5], and then Mono Q 10:10 [eluting with NaCl (0–800 mM) in 50 mM Tris·HCl buffer, pH 7.5].

(16) CPEP mutase (50 μg, 1.85×10^{-3} unit) was added to a solution containing 50 mM MES buffer, pH 6.5 (0.48 mL), 0.1 M $MnCl_2$ (10 μL), NADH (50 μg), malate dehydrogenase (14 μg), and carboxyphosphonoenolpyruvate (0.038–3.8 mM) at 30 °C. The consumption of NADH was monitored at 340 nm.

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New Carbohydrate-Based Materials for the Stabilization of Proteins

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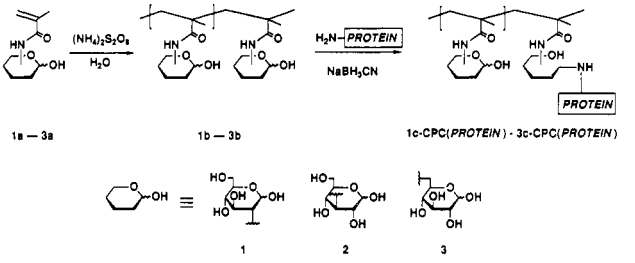
We report here the synthesis of a series of new carbohydrate-based materials and their use for the stabilization of proteins.[1] We prepared a series of aminoglucose-based monomers, **1a–3a**, by reaction of the appropriate amine with methacryloyl chloride in methanol. Treatment of **1a–3a** with ammonium persulfate in water at temperatures from 5 to 70 °C gave the

Scheme I



carbohydrate-based macromolecules **1b–3b** in yields of >80%. These water-soluble materials contain a high density of masked aldehyde functionality and have absolute molecular weights of $>4 \times 10^6$ daltons (Da) with polydispersities <1.4.[2,3] Incubation of macromolecules **1b–3b** with the desired protein and sodium cyanoborohydride in borate buffer (pH 8–9) at 37 °C gave carbohydrate–protein conjugates (CPC) of proteases [α-chymotrypsin [CPC(CT)], trypsin [CPC(Try)], and subtilisin BPN′ [CPC(BPN′)]], an endonuclease [CPC(*Eco*RI)], and an antibody that binds aldrin [CPC(M_{ab} 8H11)] (Scheme I).[4,5] Amino acid analysis of the CPC(proteases) found that approximately three to six lysines of each protein are conjugated to the carbohydrate-based macromolecule.[6] We found that the CPC(proteases) and the native enzymes have similar kinetic parameters (k_{cat} and K_m).[7-12]

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Table I. Peptide-Bond Formation Using Carbohydrate–Protein Conjugates of α-Chymotrypsin [**1c**-CPC(CT)], Subtilisin BPN′ [**1c**-CPC(BPN′)], and Thermolysin [**1c**-CPC(Th)] in Organic Solvents

entry	enzyme	solvent[a]	time, h	acceptor amino acid	donor amino acid[b]	product[c]	yield (%)
1	**1c**-CPC(CT)	THF	24	Ac-Phe-OEt	Ala-NH_2	Ac-PheAla-NH_2	98
2	**1c**-CPC(CT)	dioxane	24	Ac-Phe-OEt	Ala-NH_2	Ac-PheAla-NH_2	98
3	**1c**-CPC(CT)	CH_3CN	12	Ac-Phe-OEt	Ala-NH_2	Ac-PheAla-NH_2	100
4	**1c**-CPC(BPN′)	CH_3CN[d]	24	Cbz-LeuLeu-OMe	PheLeu-O′Bu	Cbz-LeuLeuPheLeu-O′Bu	95
5	**1c**-CPC(BPN′)	CH_3CN[d]	24	Cbz-ValLeu-OMe	PheLeu-O′Bu	Cbz-ValLeuPheLeu-O′Bu	90
6	**1c**-CPC(Th)	CH_3CN	48	Cbz-Phe-OH	Leu-OMe	Cbz-PheLeu-OMe	95
7	**1c**-CPC(Th)	CH_3CN	48	Cbz-Phe-OH	Leu-O′Bu	Cbz-PheLeu-O′Bu	90
8	**1c**-CPC(Th)	CH_3CN	48	Boc-MetLeuPhe-OMe	PheLeu-NH_2	Boc-MetLeuPhePheLeu-NH_2	70

[a] Reactions were carried out at 37 °C. Each solvent contains 5% (v/v) triethylamine and <5% (v/v) water. [b] Two equivalents of donor amino acid, relative to the acceptor amino acid, was used in each reaction. [c] All compounds were fully characterized by ^1H and ^{13}C NMR and high-resolution mass spectrometry. [d] The solvent was distilled from calcium hydride.

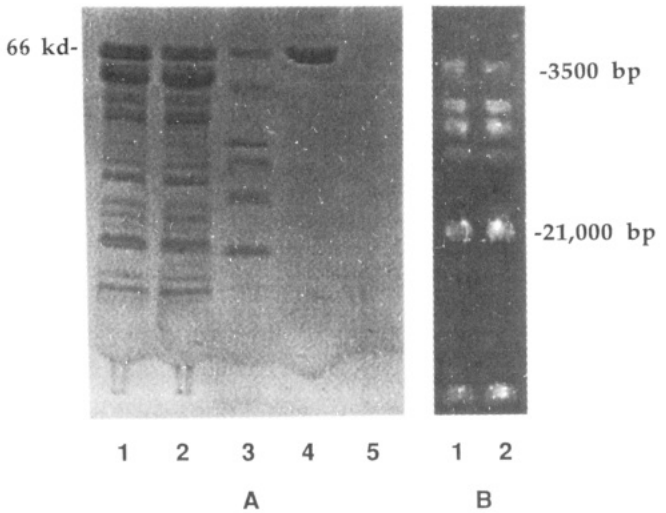

Figure 1. (A) SDS–PAGE gel of a digest of bovine serum albumin (BSA) by trypsin (lane 2) and **1c**-CPC(Try) (lane 1). BSA (50 mg/mL) in 100 mM Tris·HCl buffer pH 8.6 was digested by trypsin (0.05 mg/mL, 795 units/mL) or by **1c**-CPC(Try) (2.7 mg/mL, 795 units/mL) at 30 °C for 48 h. SDS–PAGE electrophoresis was performed using a Pharmacia PhastSystem with PhastGel homogeneous 20. Lanes 3–5 are molecular weight markers (66 000, 45 000, 36 000, 29 000, 24 000, 20 100 Da), BSA, and **1c**-CPC(Try). (B) Agarose gel of a digest of λ DNA by *Eco*RI (lane 1) and **1c**-CPC(*Eco*RI) (lane 2). λ DNA (0.25 μg) was digested by *Eco*RI or **1c**-CPC(*Eco*RI) for 1 h at 37 °C followed by heating at 90 °C for 3 min. DNA loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficoll in water) was added, and the solutions were electrophoresed on 0.8% agarose in TAE buffer and stained with ethidium bromide.

Table I contains the results of the use of CPC(proteases) for the catalytic synthesis of peptide bonds in organic solvents. Entries 1–3 of Table I show that **1c**-CPC(CT) gives greater than 95% yields of dipeptide in tetrahydrofuran (THF), dioxane, and acetonitrile (CH_3CN).[13] **1c**-CPC(Th) and **1c**-CPC(BPN′) also operate in acetonitrile and acetonitrile–water mixtures with high catalytic efficiency (Table I, entries 4–8). The V_{max} for the formation of peptide bonds in acetonitrile is approximately 0.1–1 mmol min^{-1} [mg of **1c**-CPC(CT)]$^{-1}$, which is of the same order of catalytic efficiency as that for the cleavage of peptide bonds in aqueous systems. In contrast to the high catalytic efficiency of the CPC(proteases), we could not detect any peptide coupling products when the reactions were run with the native enzymes under identical conditions.[14–17]

We have also found that the carbohydrate–protein conjugates of α-chymotrypsin [CPC(CT)], trypsin [CPC(Try)], and subtilisin BPN′ [CPC(BPN′)] exhibit enhanced stability at elevated temperatures and in distilled water solutions. α-Chymotrypsin, for example, suffers a complete loss of its activity within 30 min at 50 °C in buffered solution while **1c**-CPC(CT) retained greater than 60% of its activity after 18 h and 50% of its activity after 48 h under identical conditions.[18] We found that the α-chymotrypsin conjugate of **2b** had approximately the same degree

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of stabilization as the conjugate with **1b**, but the α-chymotrypsin conjugate with the greatest distance between the anomeric center and the polymer backbone, **3c**-CPC(CT), reproducibly had 10–15% higher thermal stability at 50 °C than the conjugates of **1b** and **2b**. The native proteases also lost their activity in distilled water at 45 °C within 1 h while the CPC analogues retained greater than 80% of their activity over a 24-h period under identical conditions. Circular dichroism studies of **1c**-CPC(CT) confirm that the protein's tertiary structure is retained at temperatures up to 55 °C.[19]

We have immobilized an antibody that binds the pesticide aldrin [**1c**-CPC(M_{ab} 8H11)] and examined its stability in methanol, acetonitrile, and 2-propanol with an enzyme-linked immunoassay. We chose to study M_{ab} 8H11 because the current method of detection of aldrin is limited by the presence of organic solvents in the ELISA.[20,21] We found that **1c**-CPC(M_{ab} 8H11) was competent for 5 h in acetonitrile, methanol, and 2-propanol with 96, 60, and 57% of the original binding, respectively, while the native antibody retained no binding ability under identical conditions.[22]

We have also examined the use of CPC(proteases) and CPC(endonucleases) in reactions involving cleavage of proteins and nucleic acids. Unlike other methodologies for protein stabilization, the CPC materials are soluble in aqueous solutions and are active on large molecules. **1c**-CPC(Try) was incubated with BSA, and the proteolytic cleavage was compared to that of the native enzyme by SDS page electrophoresis.[23] As shown in Figure 1A, we found that **1c**-CPC(Try) and native trypsin gave identical proteolytic cleavage patterns. **1c**-CPC(*Eco*RI) was incubated with λ DNA or plasmid pBR322, and the cleavage patterns were compared to that of native *Eco*RI by gel electrophoresis.[24,25] We found identical cleavage patterns for both the native and the stabilized enzymes (Figure 1B).

These new carbohydrate-based materials provide structural stability and a water-like microenvironment for the protein and do not significantly alter the active site of the enzymes or the binding site of antibodies. We are continuing to explore the generality of the use of these carbohydrate-based macromolecules for the stabilization of enzymes and other proteins, the preparation of new carbohydrate-based macromolecules, and their applications.

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Supplementary Material Available: IR, NMR, and mass spectral data for **1a–3a**, **1b–3b**, and the products listed in Table I (4 pages). Ordering information is given on any current masthead page.

Macrocyclic Lewis Acid Host–Halide Ion Guest Species. Complexes of Iodide Ion

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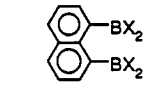
In contrast to the extraordinary achievements of cation complexation in host–guest chemistry,[1] only very recently has anion complexation by compounds containing electron-deficient atoms such as boron,[2] mercury,[3,4] tin,[5,6] and silicon[7] received attention, even though anion-inclusion complexes were reported as early as 1968.[8] Among the representative Lewis acid hosts, **1–3** are bidentate hosts that bind H^-,[2a] F^-,[2b,5] Cl^-,[2c,3-5] and Br^-.[3,4] We have recently reported the synthesis and structure of the very stable chloride ion complex of **4**.[9] Host **4** is the first member of a potential family of carborane-supported, cyclic and multidentate Lewis acids.



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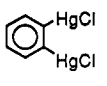

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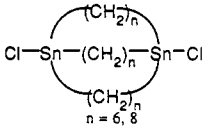
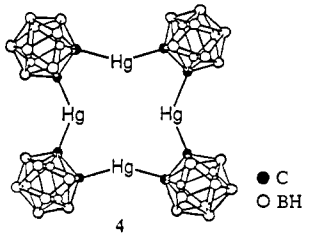


3



4

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