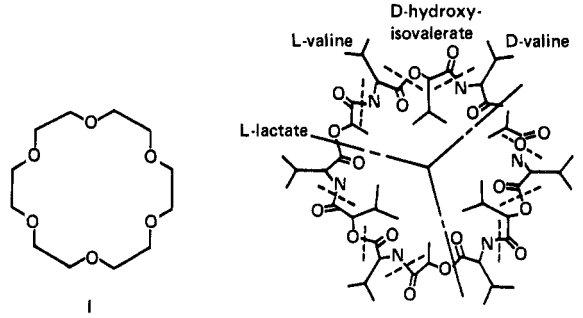
Synthesis of Two Novel Cyclic Polyether-Ester Compounds and Some Comparisons of Their Reactions with Na^+, K^+, and Ba^{2+} with Those of 18-Crown-6 and Valinomycin

Sir:

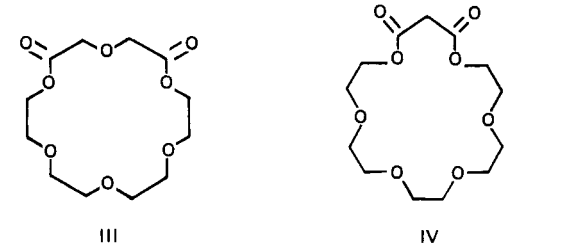
Macrocyclic polyether molecules such as 18-crown-6 (I) may be both compared to and contrasted with valinomycin (II) and other macrocyclic antibiotics in terms of chemical and physical properties and molecular structure. Both types of molecules are selective for certain alkali metal cations[1-5] and exhibit similar ion transport properties across biological membranes.[4] Valinomycin shows selectivity for K^+ over Ba^{2+} in methanol solution,[4] while by contrast, the opposite trend is true for 18-crown-6,[6] as illustrated in Table I. In terms of molecular structure, the 36-member ring of II must undergo a greater conformational change[4] upon complexation with K^+ than is required of the 18-member ring of the crown ligand (I).[7] Within the K^+ complex of II, the metal ion is surrounded by an octahedron of carbonyl oxygen atoms, whereas the six-coordination is nearly compressed into a plane of etherate oxygen atoms within the K^+ complex of I.[7]

We have synthesized two compounds analogous to I (III and IV) having carbonyl groups available for cation complexation as does valinomycin. Log K, ΔH, and $T\Delta S$ values for the reaction in CH_3OH solvent of III and IV with Na^+, K^+, and Ba^{2+} are given in Table I together with corresponding values, where available, for the reaction of these cations with I[6] and II.[4]



I
1,4,7,10,13,16-hexaoxacyclo-octadecane (18-crown-6)

II
valinomycin



III
1,4,7,10,13,16-hexaoxacyclo-octadecane-2,6-dione (2,6-dioxo-18-crown-6)

IV
1,4,7,10,13,16-hexaoxacyclo-nonadecane-17,19-dione (2,4-dioxo-19-crown-6)

Significant differences exist between the cation selectivity behavior of either III or IV and that of I as is shown by the log K values in Table I. The marked selectivity for Ba^{2+} over K^+ found for I in either aqueous[8] or CH_3OH[6] solvent and for either isomer of dicyclohexo-18-crown-6 in aqueous solution[2,8] is absent for both III and IV. In addition, the selectivity of K^+ over Na^+ shown by III and IV is considerably less than that found in the case of I. The decreased stabilities of the cation complexes of III and IV are seen to be a result primarily of less exothermic ΔH values, the $T\Delta S$ values favoring complexation of III and IV relative to I in all cases except that of Ba^{2+}-IV interaction.

Table I. Log K, ΔH, and $T\Delta S$ Values for the Reactions at 25 °C in CH_3OH Solvent of Na^+, K^+, and Ba^{2+} with Several Macrocyclic Molecules

Compound	M^{n+}	Log K^a	ΔH (kcal/mol)[a]	$T\Delta S$ (kcal/mol)
I[b]	Na^+	4.36	−8.4	−2.4
	K^+	6.05	−13.4	−5.2
	Ba^{2+}	7.0	−10.23	0.7
II[c]	Na^+	0.67		
	K^+	4.90	−4.54	2.15
	Ba^{2+}	3.34		
III	Na^+	2.5 ± 0.1	−2.27 ± 0.18	1.1
	K^+	2.79 ± 0.02	−5.87 ± 0.01	−2.06
	Ba^{2+}	3.1 ± 0.2	−0.46 ± 0.11	3.8
IV	Na^+	1.8 ± 0.18	−1.1 ± 0.2	1.4
	K^+	2.55 ± 0.03	−7.91 ± 0.06	−4.43
	Ba^{2+}	1.41 ± 0.11	−4.88 ± 0.13	−3.0

[a] Uncertainties are given as standard deviations based on three to six runs. [b] Values taken from ref 6. [c] Values for log K and ΔH (determined calorimetrically) taken from ref 4. The $T\Delta S$ values were calculated by us from these data.

Crystallographic structures of neither III and IV nor their cation complexes are yet available, therefore the donor atoms involved in cation binding are unknown. However, the effects of the carbonyl groups (in III and IV) and the additional $-CH_2-$ group (in IV) are to alter the cation complexation such that the log K values for the reactions of all three cations with III or IV compared to those with I are markedly lowered, and to significantly modify the selectivity pattern found for the reaction of I with these cations. The data in Table I show a considerably larger decrease in log K for formation of the Ba^{2+} than the Na^+ or K^+ complex in going from I to IV than from I to III resulting in a K^+, Ba^{2+} stability sequence for IV which is opposite to that found for I and III.

The log K values in Table I show the cation selectivity pattern for IV to be similar to that for II in that $K^+ > Ba^{2+}$. The significance of this finding is that it demonstrates the possibility of designing synthetic macrocyclic molecules containing oxygen donor atoms which will have cation selective properties similar to those of valinomycin and other naturally occurring cyclic antibiotics. Such synthetic molecules can then be used as models for the investigation of biological cation transport and selectivity processes.

The log K and ΔH values were determined by a calorimetric titration procedure which has been described.[8]

Compound III was prepared by slowly dripping diglycolyl dichloride (16.0 g, 0.094 mol) and tetraethylene glycol (18.2 g, 0.094 mol) in separate addition funnels to 1 L of rapidly stirring benzene at 50 °C. After stirring 48 h at 50 °C, the benzene was removed under reduced pressure and the product was distilled: 9.6 g (35%), bp 148 °C (0.16 Torr), mp 78.5–79.5 °C. In a second run using larger proportions, the product was extracted from the crude reaction mixture using hot hexane. After recrystallization from hexane, the yield and physical properties of the product isolated by this method were comparable to those of the distilled product. Compound III exhibited the following spectra: IR, 1735 cm^{-1} (C=O); NMR δ, 3.63 (s, 8 H, OCH_2CH_2O), 3.71 (m, 4 H, $COOCH_2CH_2O$), 4.28 (s, 4 H, $COCH_2O$), 4.35 (m, 4 H, $COOCH_2$).

Anal. Calcd for $C_{12}H_{20}O_8$: C, 49.31; H, 6.90; mol wt, 292.3. Found: C, 49.27; H, 6.87; mol wt, 297.

The preparation and characterization of compound IV were previously reported,[9] but we now present an improved method for its isolation. This compound was prepared by slowly dripping malonyl dichloride (45.5 g, 0.323 mol) and pentaethylene glycol (87.0 g, 0.323 mol) in separate addition funnels to 1 L of rapidly stirring benzene at 50 °C under a nitrogen atmo-

sphere. After stirring 72 h, the benzene was removed under reduced pressure and the product was extracted from the crude using hot hexane. This method gave 12.32 g (10.8%) of product: mp 65.3–66.0 °C; IR, 1740 cm^{-1} (C=O); NMR δ 3.48 (s, 2 H, $COOCH_2COO$), 3.70 (s, 12 H, $COCH_2CH_2O$), 3.79 (t, 4 H, $COOCH_2CH_2O$), 4.36 (t, 4 H, $COOCH_2$).

Anal. Calcd for $C_{13}H_{22}O_8$: C, 50.98; H, 7.24; mol wt, 306.3. Found: C, 50.90; H, 7.40; mcl wt, 306.

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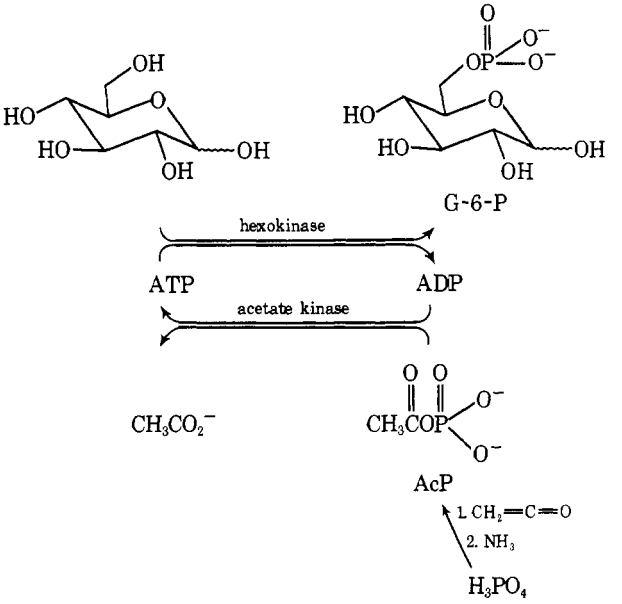
Large-Scale Enzymatic Synthesis with Cofactor Regeneration: Glucose 6-Phosphate[1]

Sir:

Many important reactions in enzyme-catalyzed biosynthesis consume cofactors in stoichiometric quantities. The cost[2] of the most commonly required cofactors has discouraged the use of these enzymatic reactions for the synthesis of organic compounds on any scale greater than a fraction of a mole.[3,4] We have previously proposed a scheme for the enzymatic regeneration of ATP from ADP or AMP, and outlined its possible use in large-scale cofactor-requiring synthesis.[5] Here we demonstrate the practicality of this scheme by the preparation of glucose 6-phosphate (G-6-P) from glucose on a mole scale.

A representative reaction was carried out in a 5-L flask modified to accept a pH electrode. The flask was charged with 1200 mL of solution (pH 6.6) containing glucose (1.4 mol), ATP (10 mmol), $MgCl_2$ (98 mmol), EDTA (4.8 mmol), and dithiothreitol (18 mmol). Polyacrylamide gel particles (20–50 μm in diameter) containing covalently immobilized hexokinase (ATP: D-hexose-6-phosphotransferase, E. C. 2.7.1.1, 1200 U.) and acetate kinase (ATP: acetate phosphotransferase, E. C. 2.7.2.1, 1100 U.) were suspended in this solution.[6] Diammonium acetyl phosphate (AcP, 0.7 M) was added continuously over 48 h at 40 mL/h to the magnetically stirred reaction mixture.[7] The solution was maintained between pH 6.6 and 6.9 by addition of 4 M potassium carbonate solution using an automatic pH controller.[8] The reaction was conducted at 25 °C, and the reaction mixture and reagent solutions were deoxygenated before use and maintained under argon. After



50 h of operation (1.36 mol of AcP added), enzymatic assay[9] indicated that 1.09 mol of G-6-P had been formed: its final concentration was 0.31 M. The polyacrylamide gel particles were allowed to settle, and the solution was decanted. Inorganic phosphate (0.27 mol, estimated by the difference between the AcP added and the G-6-P formed) was precipitated by addition of a stoichiometric quantity of $Ba(OH)_2$ and removed by filtration. G-6-P was then precipitated by addition of 1.2 mol of $Ba(OH)_2$: the resulting solid (502 g) contained 92% Ba G-6-P·$7H_2O$ (0.89 mol) by enzymatic assay.[9] This quantity corresponds to a 65% yield based on AcP added. The activities of hexokinase and acetate kinase were recovered in the gel in 93 and 75% yield, respectively. The turnover number for ATP during the reaction was >100; no effort was made to recover it.

Three points concerning experimental details deserve mention. First, the initial quantities of ATP and Mg(II) were chosen such that the concentration of MgATP and MgADP would be well above the Michaelis constants for the soluble enzymes,[10] even after dilution by the AcP solution. Second, the reaction proceeded satisfactorily with AcP having >80% purity. If the purity fell below 80%, complexation and precipitation of Mg(II) by the phosphate impurities made it difficult to maintain adequate concentrations of MgADP and MgATP in solution, and troublesome to isolate Ba G-6-P in high purity. Third, it was useful to carry out the reaction so that addition of AcP to the solution was overall rate-limiting and AcP was never present in the reaction mixture in high concentrations, to minimize spontaneous hydrolysis of AcP with concomitant release of phosphate.

Comparison of this preparation of G-6-P with existing chemical[11] or enzymatic[12] methods illustrates the potential of ATP-requiring enzymatic synthesis for the regioselective modification of unprotected, water-soluble, polyfunctional substrates. Since the hexokinases have broad substrate specificity,[13] this sequence should be directly applicable to the preparation of phosphates of a number of other sugars (e.g., fructose, mannose, deoxy-D-glucose, glucosamine). In broader terms, this conversion establishes that it is *practical* to couple enzymatic ATP regeneration with ATP-requiring enzymatic synthesis to achieve large-scale organic transformations. Reactions which require regeneration of ATP from AMP are also accessible using this reaction sequence, by adding adenylate kinase (AMP:ATP phosphotransferase, E. C. 2.7.4.3) to catalyze the conversion of AMP and ATP to ADP;[5] we will provide examples of this type of reaction sequence in the imme-