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CHIRAL IONOPHORES

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Abstract - It was shown by distribution experiments between water and lipophilic solvents that the desvalino-boromycin anion (a degradation product of the antibiotic boromycin [3]) is a highly enantiomer selective ionophore for phenyl glycinium-ion derivatives with preference for the compounds with (R)-configuration.
Two series of synthetic chiral crowns containing 9,9'-spiro-bifluorene (Figures 6 and 7) have been synthesized and tested for their ionophoric properties. It was found by electrochemical experiments that among these compounds the 9,9'-spiro-bifluorene-22-crown-5 discriminates best between either α-phenyl ethyl ammonuim or phenyl-glycinium-derivatives and inorganic ions such as Na^+, K^+ and NH_4^+.
It follows from distribution as well as from electrochemical investigations that the same ionophore is also moderately enantiomer selective for the chiral organic ammonium ions.
The selectivities of the investigated synthetic ionophores were discussed using models based on the X-ray structure of the 9,9'-spiro-bifluorene-22-crown-5,ammonium rhodanide complex and on the absolute configurations of cations and ionophores.

Ionophores or ion carriers are electrically charged or neutral ion receptors which form lipophilic complexes with hydrophilic ions such as Li^+, Na^+, K^+, NH_4^+ etc. and transport them thereby into lipophilic phases, for example natural or artificial lipophilic membranes.
It was found, in the early sixties, that some antibiotics such as valinomycin or macrotetrolides are selective ionophores with a high preference for K^+ vs. Na^+. This selectivity has many important consequences such as selective ion transport through biological and artificial lipid membranes[1].
In the course of our studies of the structure and properties of the unique boron containing antibiotic, boromycin[2,3], it was found that boromycin can be easily hydrolyzed by NaOH, KOH, RbOH or CsOH to D-valine and highly lipophilic salts of desvalino-boromycin anion. On the contrary the hydrolysis by tetramethylammonium hydroxide yields a salt which is not lipophilic. Its conversion with sodium, potassium, rubidium or caesium chlorides gives the corresponding lipophilic salt and tetramethylammonium chloride in quantitative yield. This unusual behaviour can be explained by the structure of the desvalino-boromycin anion as determined by J.D. Dunitz et al.[3] by an X-ray analysis of its rubidium salt (Figures 1,2). Desvalino-boromycin anion is a concave particle with a hydrophilic cavity containing 8 oxygens so distributed that they can complex strongly with cations of a suitable size. The rest of the anion's surface is covered with hydrogens and therefore is lipophilic. If the hydrophilic cavity is occupied with Na^+, K^+, Rb^+, Cs^+ or NH_4^+ the corresponding salt is lipophilic. Tetramethylammonium cation (r = 3,47 Å)[4] is too large to enter the cavity and complex with the oxygens. Thus its salt is hydrophilic and is less stable than the salts of ions which are able to complex. In boromycin itself the hydrophilic cavity is occupied by the ammonium ion of the D-valine which is bound as the ester to the remaining part of the molecule. The cavity in boromycin is covered with the lipophilic part of the α-amino acid. Boromycin is therefore a highly lipophilic almost spherical molecule, a droplet of fat.
The inspection of space-filling (Corey, Pauling, Koltun = CPK) models shows that D-valine fits the chiral cavity much better than its L-enantiomer. This suggested that desvalino-boromycin anion might be an enantiomer selective ionophore for suitable ammonium ions. It was not difficult to demonstrate by

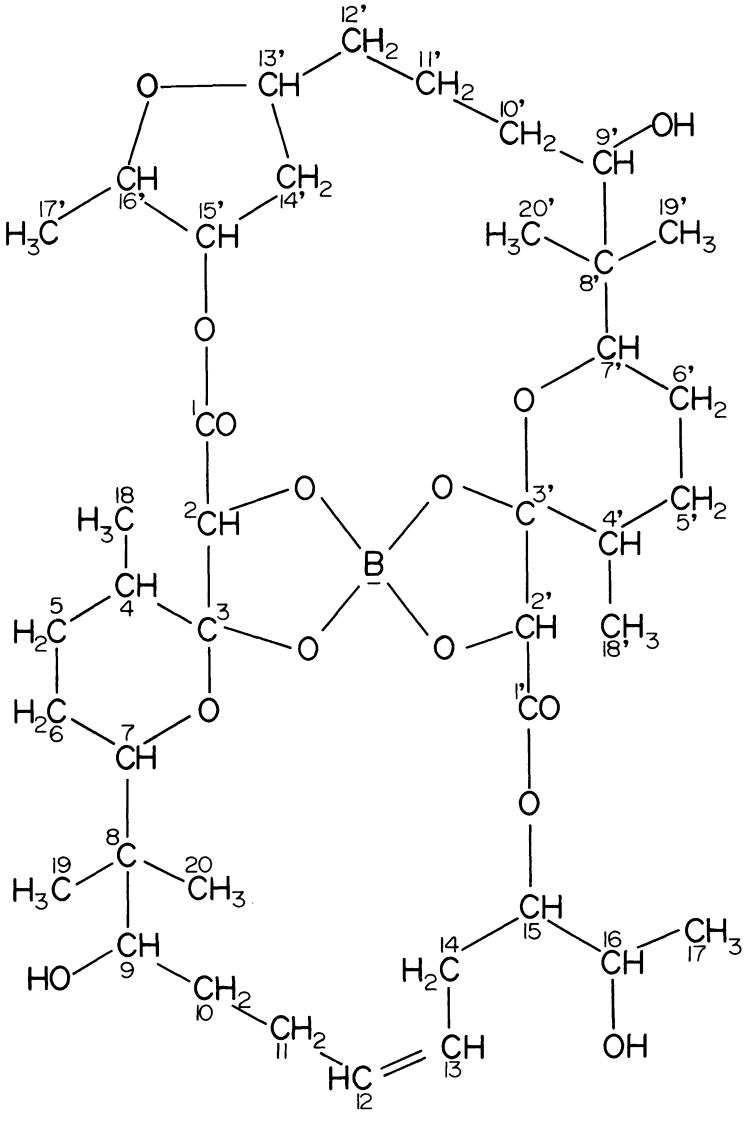


Figure 1. Constitution of the desvalino-boromycin anion.

partition experiments that this is in fact the case. The partition experiments for the determination of the enantiomer selectivity were carried out in our laboratory by the following standard procedure (Figure 3). To equal volumes of water and a lipophilic solvent (dichloroethane was the standard solvent) the chiral ligand and the racemic salt of the chiral cation are added and the mixture is shaken at 4°C. The concentration of the cations ($c_R^{aq} + c_S^{aq}$) in the aqueous phase was determined by optical absorption and its enantiomeric purity $p = \frac{c_R^{aq} - c_S^{aq}}{c_R^{aq} \quad c_S^{aq}}$ by circular dichroism. From these data and from initial concentrations of the ligand and racemic salt $^{\circ}c_L$, $^{\circ}c_R = {}^{\circ}c_S$ the concentrations of diastereomeric complexes, c_{LR} and c_{LS} and of cations c_R and c_S in the lipophilic phase can be calculated. That makes it possible to derive the ratio K_{LR} / K_{LS}, which characterizes the enantiomer selectivity. This ratio depends, among other less important parameters, on the temperature, the solvent and the anions present. These parameters must

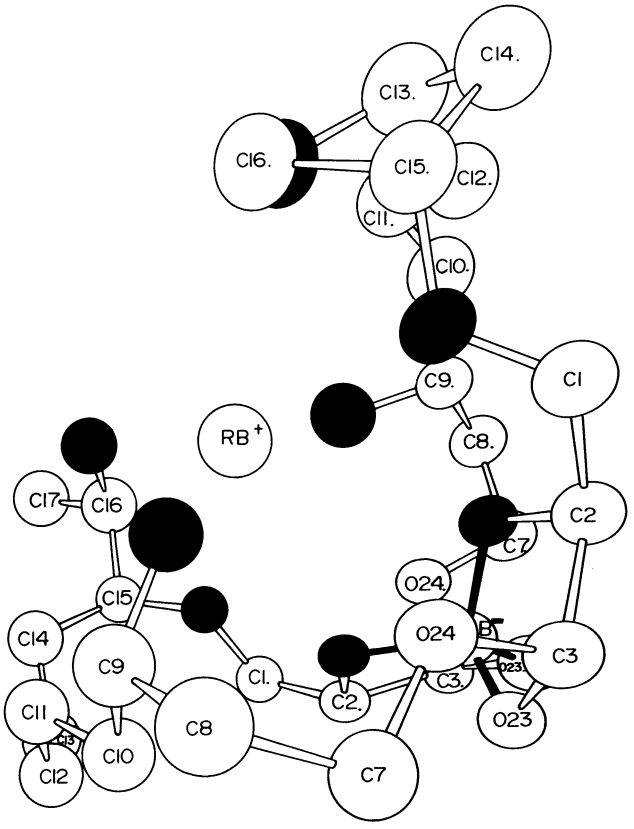


Figure 2. Perspective view of the main 28-membered ring in desvalinoboromycin Rb salt. Oxygens are shown, but other substituents were omitted for the sake of clarity (from Helv. Chim. Acta 57, 13 (1974)).

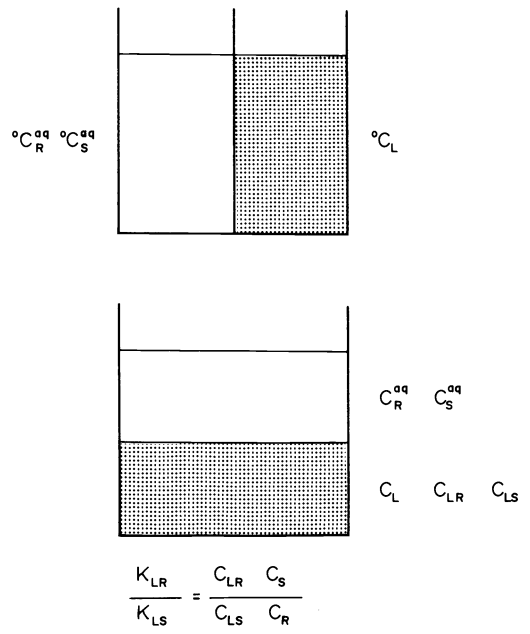
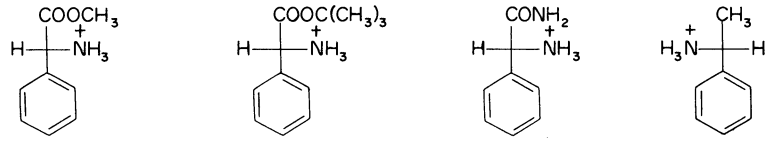


Figure 3. Determination of enantiomer selectivity by partition experiments. $^{o}c_{L}$, $^{o}c_{R}^{aq}$, $^{o}c_{S}^{aq}$ = initial concentrations; c_{R}^{aq}, c_{s}^{aq}, c_{L}, c_{LR} = concentrations after partition.

be defined and standardized to obtain reproducible and comparable results. For highest selectivities they must be optimized.
The K_{LR} /K_{LS} values obtained under standard conditions with desvalino-boromycin anion as ionophore and racemic phenylglycinium methylester (PGM), tert. butylester (PGB) amide (PGA) and α-phenylethyl ammonium (PEA) as cations are shown in Figure 4. They demonstrate that desvalino-boromycin-anion is a highly enantiomer selective negatively charged ionophore with a preference for (R)-enantiomers of the investigated cations.

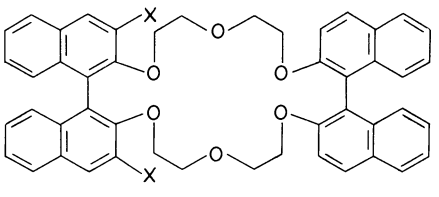


Tetramethylammonium desvalino – boromycin
Solvent $ClCH_2CH_2Cl$, t = 4°C

Cation	K_{LR}/K_{LS}	Δ(Δ G)cal
PGM	2.04	-392
PGB	2.27	-451
PGA	2.65	-536
PEA	1.00	0

Figure 4. Enantiomer selectivity of the desvalino-boromycin anion.

Soon after the discovery of natural ionophores selective for inorganic ions, a great number of synthetic ones became available, of which monomacrocyclic polyethers, the crowns[5], and polymacrocyclic polyethers, the cryptands[6], are best known and were most extensively studied. Many synthetic ionophores possess remarkable selectivity for different inorganic cations. The great variety of these relatively easily available compounds makes it possible to study extensively the relationship between their selectivity and structure[6]. The results of our experiments with desvalino-boromycin anion stimulated us to prepare some synthetic chiral crowns and to test them for their enantiomer selectivity. After we had started this work we learned from Professor D.J. Cram that he was working on a large similar project. In the meantime many notable results of this work have been published and reviewed[7,8,9]. From hundreds of compounds which have been prepared in his laboratory several possess a remarkable enantiomer selectivity. A few of the typical enantiomer selective ionophores prepared by Cram et al. are shown in Figure 5. One of the most enantiomer selective of these compounds is the dimethyl derivative of the bis-(α,α'-binaphthyl)-22-crown-6. With phenylglycinium methylester as cation and perchlorate as anion in chloroform and D_2O at 0°C, a K_{LR} / K_{LS} = 21 was found for this ionophore. This corresponds to a free energy difference (Δ(ΔG)) of 1650 cal for the diastereomeric complexes LR and LS in the lipophilic phase[10]. To be able to compare the results of our partition experiments with those of Cram et al., we determined under our standard



X = CH_3 X = CH_2Cl X = CH_2Br
X = CH_2OCH_2COOH X = $CH_2OCH_2COOCH_3$

Figure 5. Cram's substituted bis(α,α'-binaphthyl)-22-crowns-6.

conditions, using dichloroethane as solvent and perchlorate as anion, a K_{LR} / K_{LS} = 5.6 corresponding to $\Delta(\Delta G)$ = 950 cal. This illustrates how sensitive the enantiomer selectivity is to experimental conditions.
The starting material for our synthetic chiral ionophores was the 9,9'-spirobifluorene, which has a more rigid carbon skeleton than α,α'-binaphthyl. 9,9'-Spirobifluorene is easily available and can be smoothly substituted in the 2 and 2'positions by electrophylic reagents. With acetyl chloride and aluminium chloride it gives the chiral 2,2'-diacetyl-derivative (Figure 6),

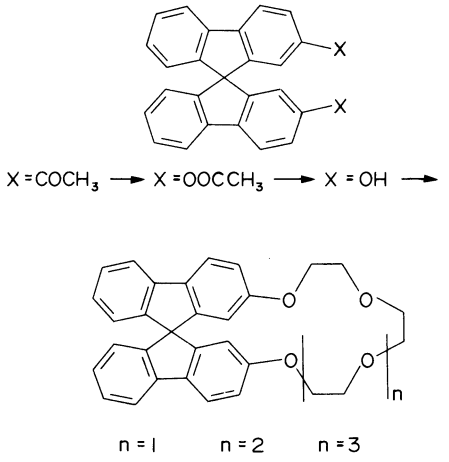


Figure 6. Synthesis of SBF-17-crown-4, SBF-20-crown-5, and SBF-23-crown-6.

which is converted into diacetyl-2,2'-dihydroxy-9,9'-spirobifluorene by the Baeyer-Villiger oxidation. The potassium salt of the free diphenol with the appropriate α,ω-dihalogeno-polyethers yields crowns with 17-, 20- and 23-membered rings and 4,5 and 6 ether-oxygens respectively. In this paper the abbreviations SBF-17-crown-4, SBF-20-crown-5 and SBF-23-crown-6 will be used for these compounds. A second series of chiral crowns was also prepared from 2,2'-diacetyl-9,9'-spirobifluorene (Figure 7) as starting material. This is oxidized by bromine and sodium hydroxide to 2,2'-dicarboxylic acid. The reduction of this acid by sodium-dihydro-(bis-methoxy-ethoxy)-aluminate gives the 2,2'-bis-(hydroxymethyl)-derivative, which is converted by hydrogen bromide in acetic acid into 2,2'-bis (bromo-methyl)-derivative. The latter reacts readily with appropriate α,ω-dihydroxy-polyethers in presence of

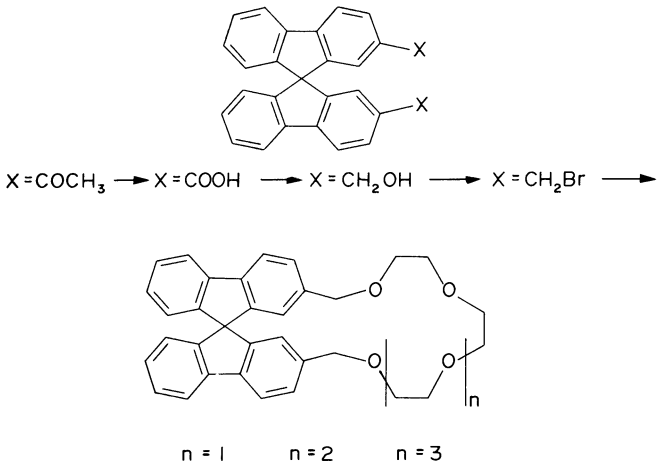
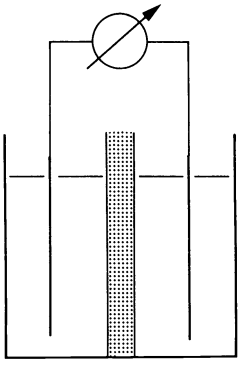


Figure 7. Synthesis of SBF-19-crown-4, SBF-22-crown-5, and SBF-25-crown-6.

potassium-tert-butoxide yielding crowns with 19-, 22- and 25-membered rings and 4,5 and 6 ether oxygens respectively, which shall be referred to as SBF-19-crown-4, SBF-22-crown-5 and SBF-25-crown-6.
The six prepared racemic chiral crowns were first investigated by an electrochemical method, which had been originally developed by Professor W. Simon et al.[11,12] in our laboratory for the determination of selectivity of ionophores for inorganic ions. The electrochemical cell used for such determinations is depicted schematically in Figure 8.



Membrane : 65oNPOE, 30PVC, 1-5L

$$EMF = E_o + \frac{RT}{F} \ln (a_X + K_{XY}^{pot} a_Y)$$

$$K_{XY}^{pot} = \frac{(U_{LY} + U_A)\ k_Y\ K_{LY}}{U_{LX} + U_A \quad k_X\ K_{LX}}$$

$$EMF = \frac{RT}{F} \ln \frac{K_{LR}}{K_{LS}}$$

Figure 8. Determination of the ion and enantiomer selectivity from EMF measurements. a = activities of the cations X,Y; u = mobilities of the complexes LX, LY and of the anion A in the lipophilic phase, k = partition coefficients between the lipophilic and aqueous phases.

The two compartments of the cell are separated by a polyvinylchloride (PVC) membrane containing a solution of the ionophore L in a water insoluble, non-volatile solvent (o-nitrophenyl-octylether, o-NPOE). One of the compartments contains an AgCl/Ag electrode immersed in a solution of a standard cation salt, e.g. 0.1 N (R,S)-α-phenylethylammonium (PEA) chloride. The other compartment contains a reference electrode in a solution of the chloride of the cation under investigation. If the membrane containing the ionophore is permeable for the latter cation a zero current potential E can be measured from which, using a Goldman-Hodgkin-Katz-type [13,14] equation

$$E = E_0 + \frac{RT}{F} = \ln(a_X + a_Y K_{XY}^{pot})$$

the K_{XY}^{pot} can be calculated. Under certain assumptions and experimental conditions discussed by W. Simon[15] K_{XY}^{pot} specifies the selectivity of the membrane for the cations X and Y. Potentials measured with membranes containing our crowns show that SBF-22-crown-5, in contrast to the other 5 investigated crowns, discriminates very well between PEA and inorganic cations. This is illustrated in Figure 9. The solvent o-NPOE alone, or with the SBF-20-crown-5 discriminate poorly, whereas the K_{XY}^{pot} of PEA measured with a membrane containing SBF-22-crown-5 is 10^2-10^3 times larger than that of inorganic cations. Such high selectivity is especially desirable when ionophores are used in biological liquids.
On account of this selectivity we decided to prepare enantiomeric SBF-22-crowns-5 and to test them for enantiomer selectivity. After several unsuccessful attempts to resolve 2,2'-dicarboxylic acid we were able to separate the diastereomeric camphanic acid esters[16] of the intermediate product, 2,2'-bis-(hydroxymethyl)-9,9'-spirobifluorene, by crystallization

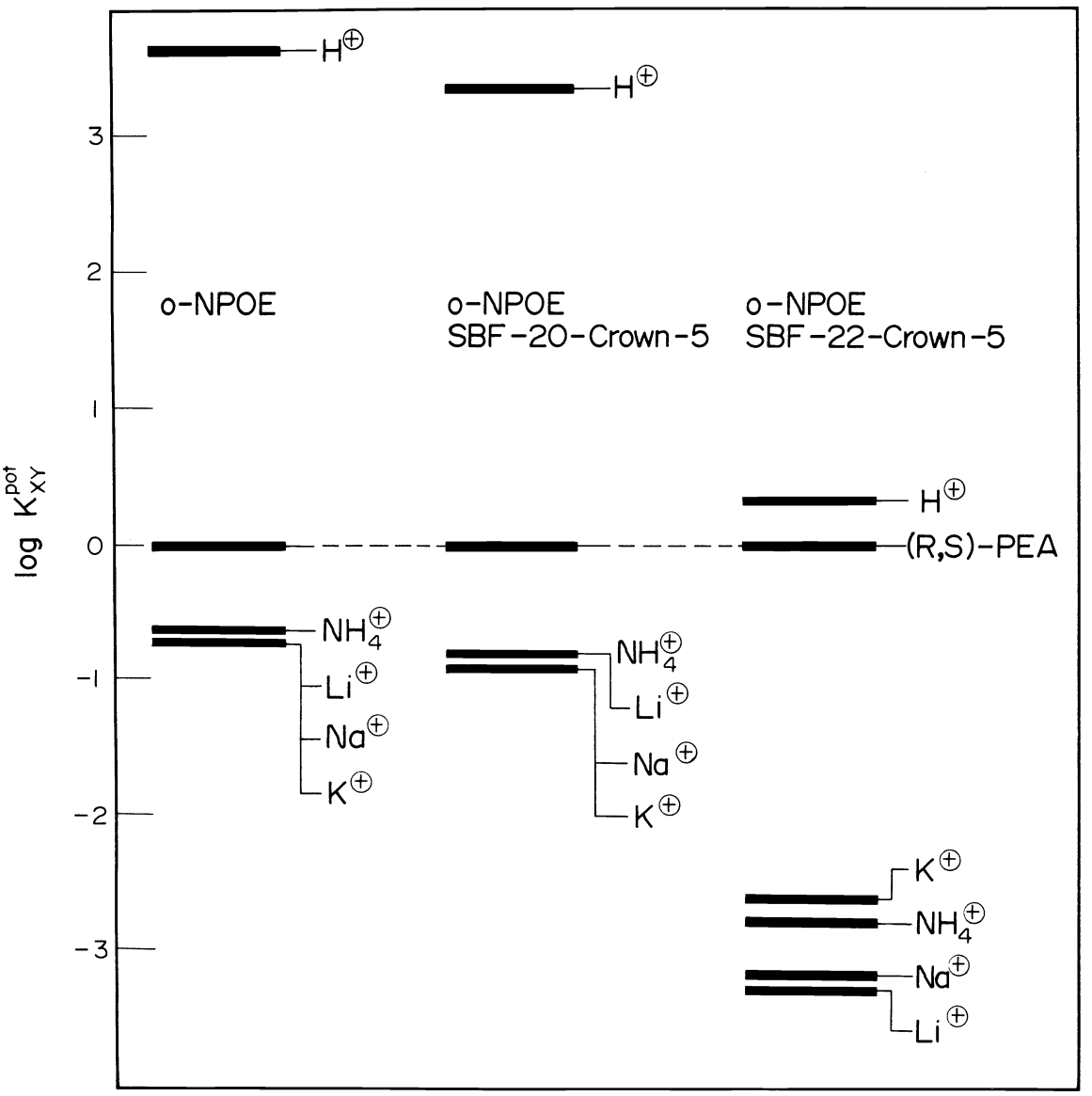


Figure 9. Discrimation of (R,S)-α-phenyl-ethyl-ammonium from inorganic cations by o-NPOE, o-NPOE + SBF-20-crown-5, and o-NPOE + SBF-22-crown-5.

from different solvents (Figure 10). The regenerated pure enantiomeric diols gave the enantiomeric SBF-19-crowns-4, SBF-22-crowns-5 and SBF-25-crowns-6 by the previously described method.
The absolute configurations of our enantiomeric crowns were determined by two independent methods: i. by comparison of the circular dichroism (CD) spectrum of the SBF-19-crown-4 with those of the hydrocarbons vespirenes (the absolute configurations of vespirenes were determined previously by analysis of their CD spectra using the exciton theory[17]), and ii. the absolute configuration of the enantiomeric 2,2'-bis(bromomethyl)-9,9'-spirobifluorenes, which are intermediates in our synthesis, was determined, on our request, by O.S. Mills and I.D. Hunt using the anomalous X-ray diffraction.
The CD spectra of the (R)-(-)-crowns with 19-, 22- and 25-membered rings are shown in Figure 11. The spectrum of the SBF-19-crown-4 differs from the others by containing a larger number of Cotton-effects. It follows from theoretical considerations as well as from previous experimental work on vespirenes[17], that only those 9,9'-spirobifluorene derivatives in which the two fluorene chromophores are non-orthogonal display the characteristic

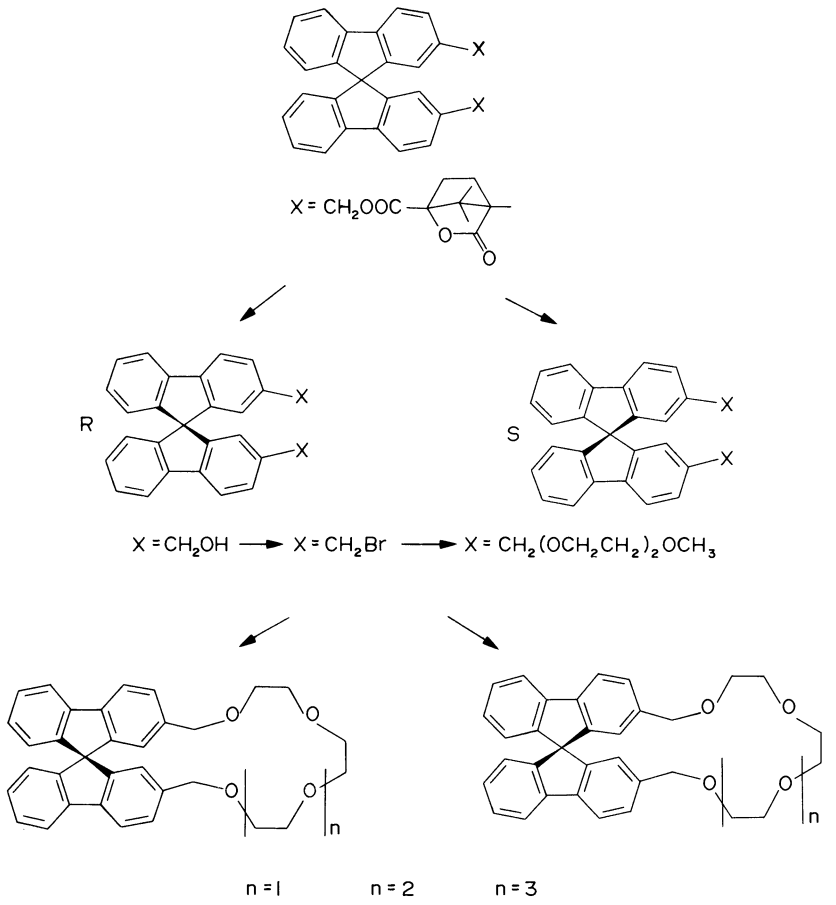


Figure 10. Synthesis of enantiomeric SBF-19-crowns-4, SBF-22-crowns-5, and SBF-25-crowns-6.

couplets in the CD spectrum. In vespirenes with 13-, 14- and 15-membered rings the fluorene chromophores are inclined because the polymethylene chains are too short to bridge the orthogonal fluorenes. The polyether chain in SBF-19-crown-4 is still to short, whereas in the crowns with larger, 22- and 25-membered rings the fluorenes are in an orthogonal position. The SBF-19-crown-4 possess therefore a CD spectrum comparable with those of vespirenes which had been used for determination of the absolute configuration.
The configurational assignments by chemical correlations of the compounds mentioned are summarized in Figure 12. Because of inherent difficulties in the determination of absolute configurations from CD[18,19], it is satisfying that in this rather transparent case both methods lead to the same result.
By the standard partition experiments mentioned earlier it can be shown that our enantiomeric crowns are moderately but distinctly enantiomer selective for several ammonium cations with a chiral substituent (Figure 13). $NaPF_6$ was added in these experiments to increase the solubility of the complexes in the lipophilic solvent. Of the three investigated crowns the SBF-22-crown-5 possesses the highest enantiomer selectivity.
The enantiomer selectivity of neutral crowns can also be detected and measured by the same electrochemical method[12,20], which was developed to measure the selectivity of ionophores for inorganic and racemic PEA ions. For that purpose we used membranes which contained enantiomeric crowns. Some typical results of such measurements are summarized in Figure 14. In spite of the difference of solvent, temperature, and anions present, the resulting K_{LR} / K_{LS} values obtained by partition and by electrochemical experiments are similar. The electrochemical method is therefore convenient for detection and determination of enantiomer selectivity with small quantities of ionophores. The similarity of the results obtained by partition and electrochemical experiments indicates that the enantiomer selectivity depends, at least in investigated cases, mainly on the interactions between the ionophore and cation. Such interactions can, in favourable cases, be

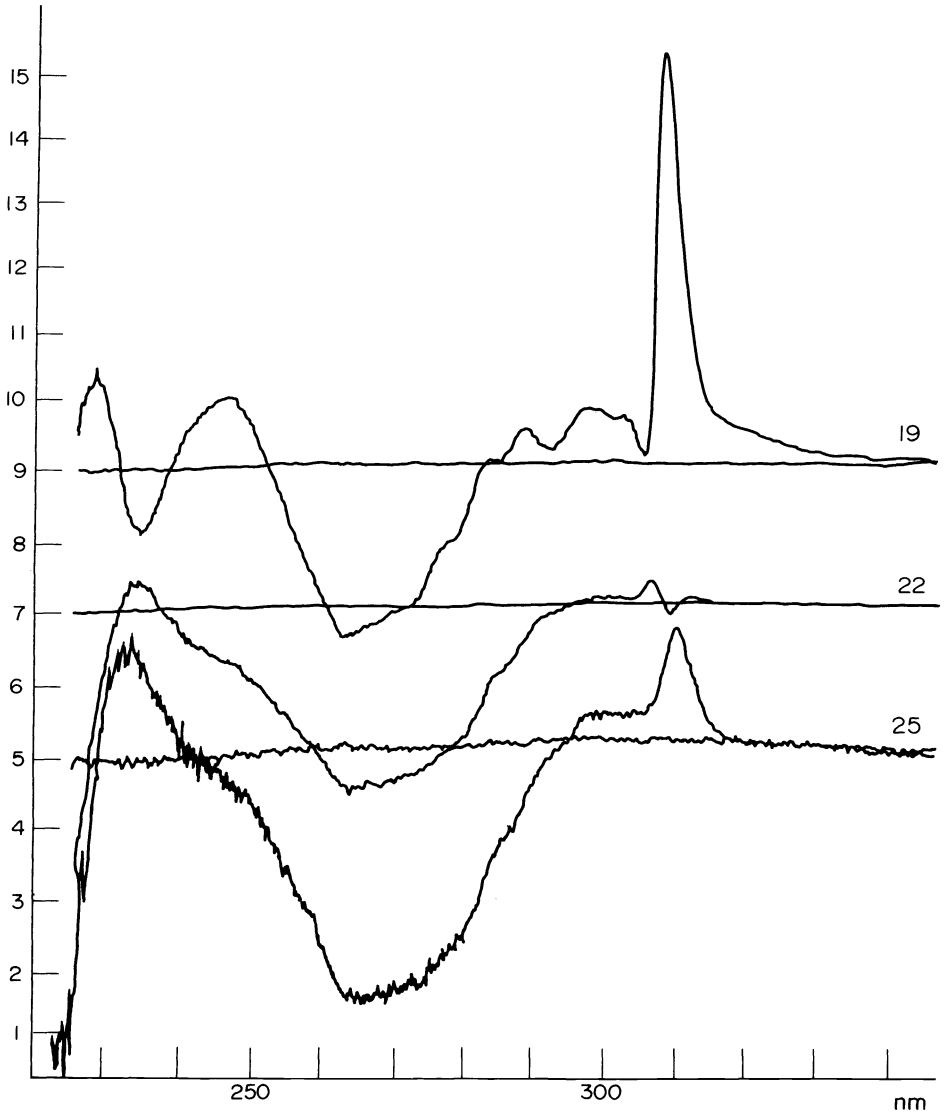


Figure 11. Circular dichroism spectra of (R)-(-)-SBF-crowns.

interpreted or even predicted by inspection of space-filling (CPK) molecular models, as shown several times by Cram et al.[7,8,9,21]. A solid base on which such models can be built are X-ray structural analyses of ionophores, cations or their complexes, if obtainable in the crystalline state.
Dr. M. Dobler has determined at our request the structure of the nicely crystalline complex of the (R)-SBF-22-crown-5 with ammonium rhodanide (Figure 15). The ammonium ion in the complex fits well into the cavity of the ionophore to which 3 of its 4 hydrogens are hydrogen bounded by the ether oxygens. Space-filling models (CPK) show that the cavity of the SBF-19-crown-4 is too small and that of the SBF-25-crown-6 is too large for complexing an ammonium ion efficiently which explains the superiority of the SBF-22-crown-5 as an ionophore for ammonium ion.
It is noteworthy that the investigated primary ammonium ions are more stable than the unsubstituted one. This implies that some positive interactions exist between the ionophore and the chiral substituent of the cation. Models can be proposed, such as the one schematically depicted in Figure 16, for the more stable complex between (R)-SBF-22-crown-5 and (R)-α-phenylethyl ammonium, which can be useful not only for explaining the observed facts but also for predicting the selectivity of an ionophore for analogous cations. Such heuristic models shouldn't be taken too seriously if they are not corroborated by structural analyses.

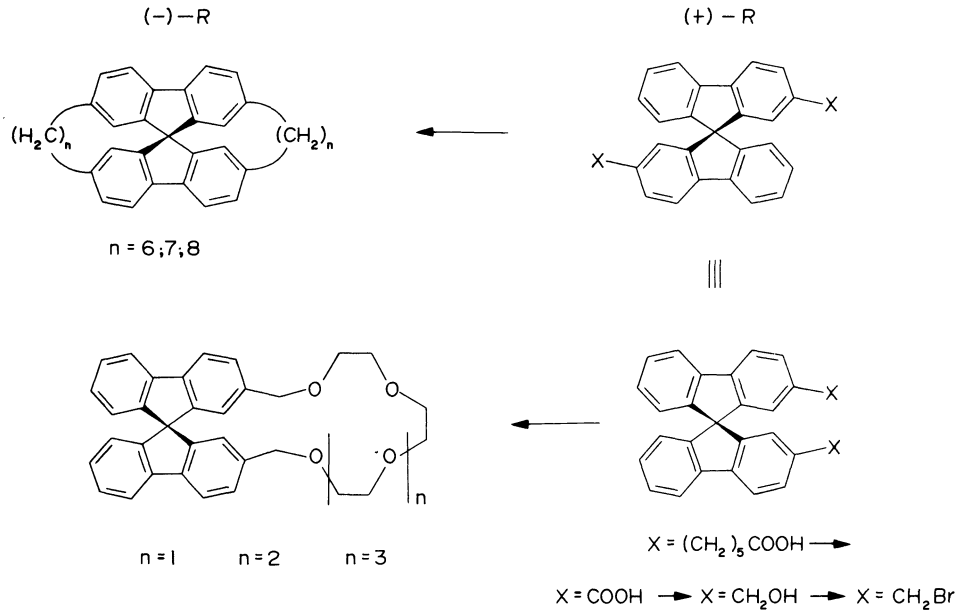


Figure 12. Determination of absolute configurations by chemical correlations.

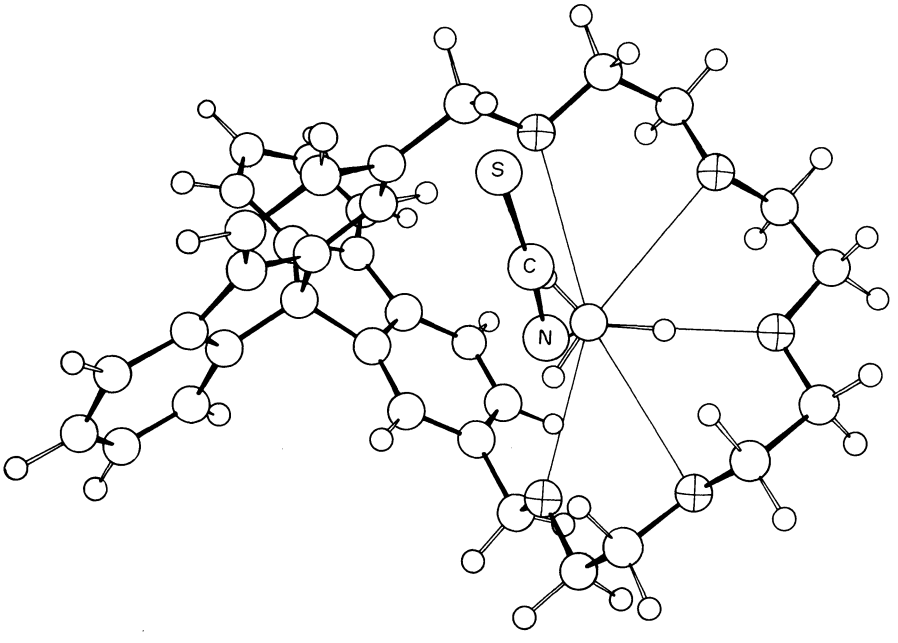


Figure 13. Enantiomer selectivity of the chiral crowns from partition experiments.

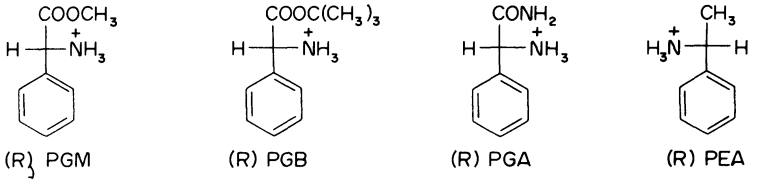


Figure 14. Enantiomer selectivity of the chiral crowns from EMF measurements.

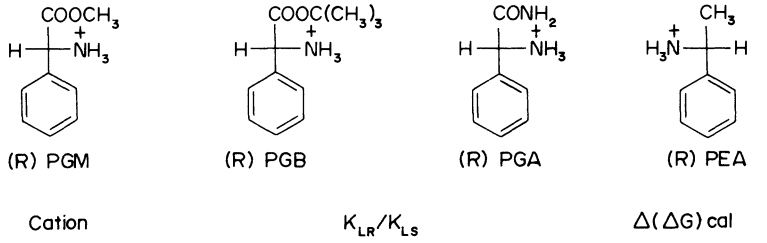


Figure 15. X-ray crystal suructure of the complex between (R)-SBF-22-crown-5 and ammonium rhodanide.

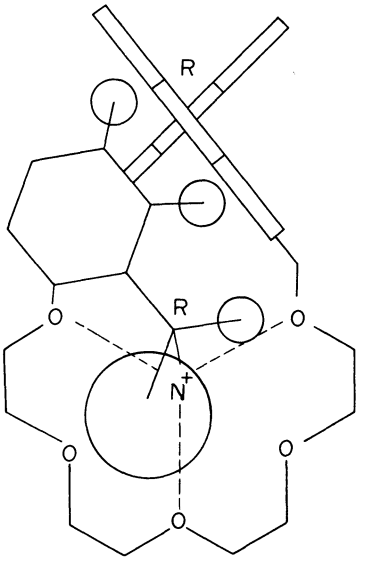


Figure 16. Model proposed for the structure of the complex between (R)-SBF-22-crown-5 and (R)-α-phenylethyl ammonium ion.

As already emphasized by Cram, ionophores resemble enzymes in several aspects. Ionophores possess a cavity - an active site - in which several small strategically distributed interactions between the ionophore and ion - the substrate - add together and thereby determine ion-selectivity and stereo-selectivity of the ionophore.
The fact that ion-selective and stereoselective ionophores exist among natural compounds produced by organisms which appeared at an early stage of evolution, stimulates speculations about their possible role in evolution.
It remains to thank my friends and younger colleagues with whom I have had the privilege to cooperate: Professor D.J.Cram, Los Angeles for the unidirectional exchange of information and samples, Professor W. Simon, Zürich, for electrochemical results, O.S. Mills, Manchester, and Dr. M. Dobler, Zürich, for X-ray structure determinations; the lion's share of the experimental work was done by Dr. D. Bedeković, A. Thoma, Mrs. A. Viviani and Dr. M. Žinić.

REFERENCES

1. Yu. A. Ovchinnikov, V.T. Ivanov, and A.M. Shkrob, Membrane Active Complexones, Elsevier, Amsterdam 1974.
2. R. Hütter, W. Keller-Schierlein, F. Knüsel, V. Prelog, G.C. Rodgers Jr., P. Suter, G. Vogel, W. Voser and H. Zähner, Helv. Chim. Acta 50, 1533 (1967).
3. J.D. Dunitz, D.M. Hawley, D. Mikloš, D.N.J. White, Yu. Berlin, R. Marušić and V. Prelog, Helv. Chim. Acta 54, 1709 (1971).
4. G. Anderegg, Helv. Chim. Acta 58, 1218 (1975).
5. C.J. Pedersen and H.K. Frensdorf, Angew. Chem., Int. Ed. 11, 16 (1972).
6. J.-M. Lehn, Structure and Bonding 16, 1 (1973).
7. D. J. Cram and Jane M. Cram, Science 183, 803 (1974).
8. D.J. Cram, R. Helgeson, L.R. Sousa, J.M. Timko, M. Newcomb, P. Moreau, F. de Jong, G.W. Gokel, D.H. Hoffman, L.A. Domeier, S.C. Peacock, K. Madan and L. Kaplan, Pure Appl. Chem. 43, 327 (1975).
9. D.J. Cram in Application of Biochemical Systems in Organic Chemistry, J.B. Jones, D. Perlman and C.J. Sih (Editors) Wiley-Interscience, New York, 1976.
10. S.C. Peacock and D.J. Cram, J.C.S. Chem. Commun. 1976, 282.
11. Z. Štefanac and W. Simon, Chimia 20, 436 (1966).
12. W. Simon, W.E. Morf and P.Ch. Meier, Structure and Bonding 16, 113 (1973).
13. D.E.Goldman, J. Gen. Physiol. 27, 37 (1943).
14. A.L. Hodgkin and B. Katz, J. Physiol.(London) 108, 37 (1949).
15. W. Simon, XVIth Solvay Conference on Chemistry, Brussels, November 22-26, 1976 (in press).
16. H. Gerlach, Helv. Chim. Acta 51, 1587 (1968).
17. G. Haas, P.B. Hulbert, W. Klyne, V. Prelog and G. Snatzke, Helv. Chim. Acta 54, 491 (1971).
18. S.F. Mason, J.C.S. Chem. Commun. 1973, 239.
19. A.M.F. Hezemans and M.P. Groenewege, Tetrahedron 29, 1223 (1973).
20. A.P. Thoma, Z. Cimerman, U. Fiedler, D. Bedeković, M. Güggi, P. Jordan, K. May, E. Pretsch, V. Prelog and W. Simon, Chimia 29, 344 (1975).
21. R.C. Helgeson, J.M. Timko, P. Moreau, S.C. Peacock, J.M. Mayer and D.J. Cram, J.Amer. Chem. Soc. 96, 6762 (1974).