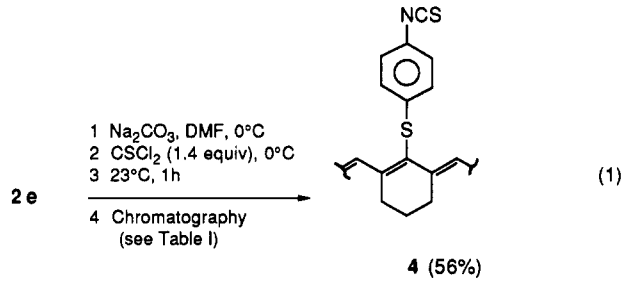

(1)

liminary results showed that for detection of proteins[10,11] **4** is vastly superior to fluoresceinisothiocyanate, a classical reagent for protein labeling with UV–vis chromophore.[12]

Finally, we wish to comment on possible mechanisms for the discussed reactions. The yields of **2b–e** were reduced in the presence of molecular oxygen, a free-radical scavenger, and nitrobenzene, an electron scavenger,[6] with a concomitant formation of a number of unidentified products that lacked NIR chromophore. When dry oxygen was bubbled through the mixtures in DMF/$PhNO_2$ (1:1), the yields of **2c** and **2d** from the fast reactions of **1** with PhONa and PhSNa, respectively, were decreased by 5%, the yields of **2d** and **2e** from the reactions with PhSH and 4-H_2NPhSH, respectively, were decreased by 15%, and product **2b** was not formed at all in an attempted reaction of **1** with $MeNH_2$ although **1** was consumed.[13] Under anaerobic conditions in the presence of $PhNO_2$ the yield of **2b** was only 60%, and the apparent rates for disappearance of **1** and formation of **2b** were both smaller by about 30% in comparison to that for the reaction conducted in the absence of $PhNO_2$. By contrast, molecular oxygen and $PhNO_2$ had no effect[13] on the slow reaction of **1** with MeONa.

These results are consistent with a unified mechanism (eqs 2–5) in which an $S_{RN}1$ pathway[14] leads to **2b–e**, and **3** is produced from an intermediate cation radical[14c] (eq 3). The suggested presence of radical intermediates was

$$(R\text{–}X)^+ + Y^- \rightarrow (R\text{–}X)^\bullet + Y^\bullet \quad (2)$$

$$(R\text{–}X)^\bullet \rightarrow R^{\bullet+} + X^- \quad (3)$$

$$R^{\bullet+} + Y^- \rightarrow (R\text{–}Y)^\bullet \quad (4)$$

$$(R\text{–}Y)^\bullet + (R\text{–}X)^+ \rightarrow (R\text{–}Y)^+ + (R\text{–}X)^\bullet \quad (5)$$

supported by observation of an EPR absorption for a mixture of **1** and PhSH in DMF.[15] Semiempirical calculations were also consistent with a high electron affinity of the cation **1**.[16] On the other hand, the reaction of **1** with MeONa to give **2a** may involve a direct addition of the nucleophile followed by elimination of chloride.[17]

We have shown straightforward and highly efficient chemistry for modification of the optical chromophore of **1**. This method should be applicable to the preparation of a broad spectrum of compounds[18,19] starting with similarly substituted analogs of **1**. A vast number of such dyes is readily available through classical syntheses.[2–5]

Acknowledgment. Support of this research by NIH (Grant AI28903) and NSF (Grants CHEM-8409599 and CHEM-890456) is gratefully acknowledged. We thank Dr. W. H. Nelson for EPR analyses and Dr. A. L. Baumstark for critical reading of the manuscript.

Supplementary Material Available: Analytical data, VIS-NIR, and ^1H NMR (400 MHz) spectra for **1–4** and analogs and a procedure for the preparation of **4** (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) Human angiotensin I (10^{-4} M in MeOH/H_2O (1:1)) was allowed to react with a 10-molar excess of fluoresceinisothiocyanate (FL-NCS) or **4** for 15 min at 23 °C followed by removal of the unbound dye on a short C18 cartridge (MeOH/H_2O (1:1)). Fluorescence of the labeled oligopeptide samples was observed on an SLM 8000 fluorometer (λ_{ex} = 499 nm, λ_{em} = 530 nm) for the labeling with FL-NCS and by using a 30-mW laser diode (λ_{ex} = 785 nm, λ_{em} = 820 nm) for the labeling with **4** to give the detection limits of 2×10^{-10} M and 4×10^{-14} M, respectively. A laser diode excitation source for fluorescein is not available.

(11) An attempted use of reactive dyes **1**, **2a**, or **2c** for direct labeling of proteins gave negative results at low concentration levels.

(12) *Luminescence Techniques in Chemical and Biochemical Analysis*; Baeyens, W. R. G., De Keukeleire, D., Korkidis, K., Eds.; Marcel Dekker: New York, 1991.

(13) Dye **1** itself is stable in the presence of oxygen under similar conditions of solvent, temperature and time.

(14) (a) Rossi, R. A.; de Rossi, R. H. *Aromatic Substitution by the $S_{RN}1$ Mechanism*; ACS Monograph 178; American Chemical Society: Washington, D.C., 1983. (b) Bunnett, J. F. *Acc. Chem. Res.* **1978**, *11*, 413. (c) Bard, A. J.; Ledwith, A.; Shine, H. J. In *Advances in Physical Organic Chemistry*; Gold, V., Bethell, D., Eds.; Academic: London, 1976; Vol. 13, p 155.

(15) A poorly resolved signal was observed, as expected for several radical intermediates. Neither DMF with **1** nor DMF with PhSH showed this absorption. This result does not rule out partial involvement of alternative mechanistic pathways in which (i) **2d** is formed by recombination of a radical pair (eq 2) followed by elimination of chloride, and (ii) hydrogen abstraction by the radical (R-SPh)• (eq 2) followed by elimination of benzenethiolate anion leads to **3**.

(16) AMPAC and MOPAC computations gave lower energy for the free radical derived from **1** over the cation **1** by 130 and 126 kcal/mol, respectively.

(17) Methoxide ion appears to be inert toward $S_{RN}1$ reactions (ref 14b). The slow substitution reaction of **1** with MeONa is not due to deprotonation of **1** at the methylene of N-Et to form an ylide, although reversible addition reactions at positions other than 4′ cannot be ruled out (see Scheme I for numbering). After the reaction was conducted in MeOD at 23 °C, the resultant product **2a** was devoid of deuterium. Heating of **2a** with MeOD/MeONa at 65 °C for 2 h (see Table I for concentrations) gave regioselective and complete deuteration at positions 1′ and 7′. With **1** the rate for formation of nondeuterated **2a** in the MeOD/MeONa system was four times greater at 65 °C than the apparent rate for hydrogen–deuterium exchange at this temperature. This unusual regioselective deuterium labeling can be rationalized in terms of two consecutive additions of methoxide anion at positions 2 and 2″ of the chromophore to form the most thermodynamically stable anion.

(18) Substitution of a 1,2-ethanediyl bridge for the 1,3-propanediyl bridge in the chromophore of **1** does not affect these efficient transformations.

(19) The same chemistries, albeit with lower yields (by ca. 5%), were observed for cation **1** with iodide counterion. The use of the iodide salt may be preferred for larger scale preparations due to a more facile chromatographic purification of the less polar iodides than perchlorates. Of interest is the reported lack of reactivity of iodide ion as a nucleophile with cation radicals (ref 14c).

Enantioselective Cation Binding with a Functionalized Podand Ionophore

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Summary: By adding an acetamido group to podand **1**, a new ionophore **2** is created which binds peptidic ammonium ions with high enantioselectivity.

The methylated podand ionophore **1** has been shown to bind simple chiral ammonium ions with modest enantioselectivity (up to 40% ee).[1] One likely reason for this

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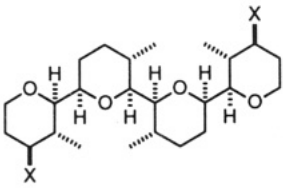
absence of high selectivity is that while the podand host itself is conformationally homogeneous, its complexes are not. The properties of the complexes likely reflect the contributions of several different orientations of the guest ammonium ion relative to the podand host, each with its own different selectivity. An attractive possibility for making the complexes less conformationally flexible is to provide "two-point binding" by adding functionality to the podand capable of new, specific interactions with the guest molecule.[2] The acetamido group of podand **2** was designed to provide such an interaction with α-amino acid esters and amides in the form of hydrogen bonding between the host amide NH and the guest carbonyl group. In this paper we report the synthesis and binding properties of **2** along with molecular modeling studies to support the proposed structure of its complexes.



1 X=H
2 X=NHAc
3 X=OAc
4 X=OH

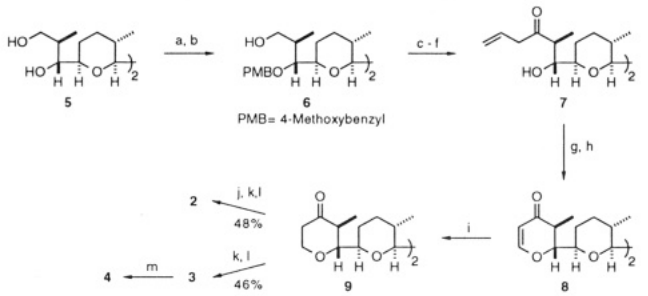
Podand **2** was prepared as outlined in Scheme I. The tetrol **5**, synthesized from (+)-diethyl tartrate as previously reported,[1b,d] was transformed to the diketone **9** using standard methodology. Stereoselective conversion to the diamide **2** was accomplished via dissolving metal reduction[3] of the oxime methyl ether of **9**. The large (11.1 Hz) vicinal ^{1}H NMR coupling constant observed between the proton adjacent to nitrogen and that adjacent to the ring methyl confirmed that the desired equatorial amide had been obtained. Direct reduction of **9** under similar conditions provided the equatorial diacetate **3** required for control experiments (vide infra). X-ray crystallography of the alcohol **4** derived from **3** confirmed the structure and stereochemistry assigned to these compounds (Figure 1).[4]

The binding properties of podands **2** and **3** were measured as previously described[1d,5] by extracting excess racemic organoammonium hexafluorophosphate guests from D_2O with $CDCl_3$ solutions of the podands. Integration of the resolved guest peaks in the ^{1}H NMR spectrum of the $CDCl_3$ phase allowed determination of binding enantioselectivity directly. Where the diastereomeric complexes were not sufficiently distinct by ^{1}H NMR, a Mosher's amide derivative of the extracted guest was prepared. The results are shown in Table I along with previously reported data for the unfunctionalized podand **1**.

It can be seen that the podand acetamide **2** displays markedly more selective binding with α-amino acid methyl esters (≈60–70% ee) than does the simple podand **1**. While we have been unable to obtain firm experimental evidence for the existence of the proposed host NH to guest carbonyl hydrogen bond, several control experiments are consistent with the importance of this interaction. First, a guest lacking a hydrogen-bond acceptor, α-phenylethylammonium ion, shows decreased enantioselectivity on going from **1** to **2**. Second, the podand acetate **3** which is also incapable of hydrogen bonding exhibits low enantioselectivity (27% ee) for phenylglycine methyl ester, a result comparable with that obtained for the unfunctionalized podand **1**. Parenthetically, acetate **3** is a poorer ionophore than either **1** or **2** and fails to extract most guest molecules at the concentrations employed here.

Also compatible with the proposed binding mode is the fact that guests having greater hydrogen bond acceptor ability are bound by **2** with still higher enantioselectivity. Thus, using α-amino acid amide guests instead of esters, we observed enantioselectivity as high as 83% ee (Table II). This level of enantioselection corresponds to a $\Delta\Delta G$ of ~1.4 kcal/mol.

Scheme I. Synthesis of Podand Receptors 2 and 3[a]



[a] Key: (a) *p*-$MeOC_6H_4CHO$, PPTS, PhH, Δ, 74%; (b) 10 equiv of DIBAL, CH_2Cl_2, −10 °C, 96%; (c) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, 96%; (d) CH_2=$CHCH_2MgBr$, THF, 0 °C; (e) $(COCl)_2$, DMSO, CH_2Cl_2, then Et_3N, 79%; (f) DDQ, CH_2Cl_2/H_2O, 82%; (g) O_3, $MeOH/CH_2Cl_2$, then Ph_3P; (h) MsCl, Et_3N, CH_2Cl_2, 60%; (i) H_2, $Pd(OH)_2/C$, MeOH, 72%; (j) NH_2OMe·HCl, EtOH/pyridine, 85%; (k) Li, NH_3/THF; (l) Ac_2O, Et_3N, CH_2Cl_2; (m) K_2CO_3, MeOH, 92%.

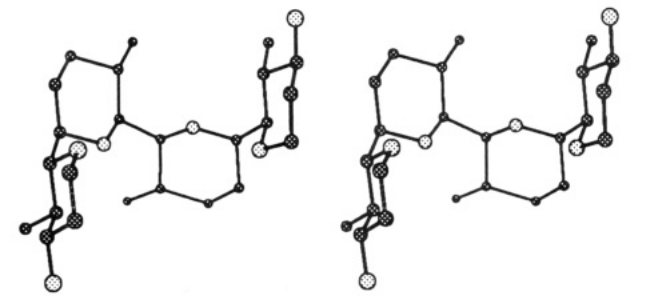


Figure 1. X-ray structure of alcohol **4**.

Table I. Enantioselectivity of Binding[a] of Podands 1–3 with α-Phenylethylammonium and α-Amino Acid Methyl Ester Hexafluorophosphates

	1 (% ee)	2 (% ee)	3 (% ee)
α-phenylethylammonium	42 (*S*)	20 (*S*)	–
Ala	40 (*S*)	63 (*S*)	no extraction
Met	36 (*S*)	72 (*S*)	no extraction
Phe	36 (*S*)	60 (*S*)	–
Val	34 (*S*)	57 (*S*)	–
phenylglycine	40 (*S*)	62 (*S*)	27 (*S*)

[a] Enantiomeric excess of indicated ammonium ion extracted from excess racemic ammonium PF_6^- (0.5 M) in D_2O by podand (0.02 M) in $CDCl_3$ at 25 °C. The absolute configuration of the major isomer extracted is given in parentheses.

(1) (a) Iimori, T.; Still, W. C.; Rheingold, A. L.; Staley, D. L. *J. Am. Chem. Soc.* **1989**, *111*, 3439. (b) Iimori, T.; Erickson, S. D.; Rheingold, A. L.; Still, W. C. *Tetrahedron Lett.* **1989**, *30*, 6947. (c) Erickson, S. D.; Still, W. C. *Tetrahedron Lett.* **1990**, *31*, 4253. (d) Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128.

(2) Li, G.; Still, W. C. *J. Org. Chem.* **1991**, *56*, 6964.

(3) Dissolving metal reduction of oximes has been reported previously: Terent'ev, A. P.; Gusar', N. I. *J. Gen. Chem. USSR* **1965**, *35*, 124.

(4) We thank Dr. J. C. Dewan (New York University) for this structure determination.

(5) (a) Kyba, E. P.; Timko, J. M.; Kaplan, L. J.; de Jong, F.; Gokel, G. W.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 4555. (b) Peacock, S. C.; Domeier, L. A.; Gaeta, F. C. A.; Helgeson, R. C.; Timko, J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 8190.

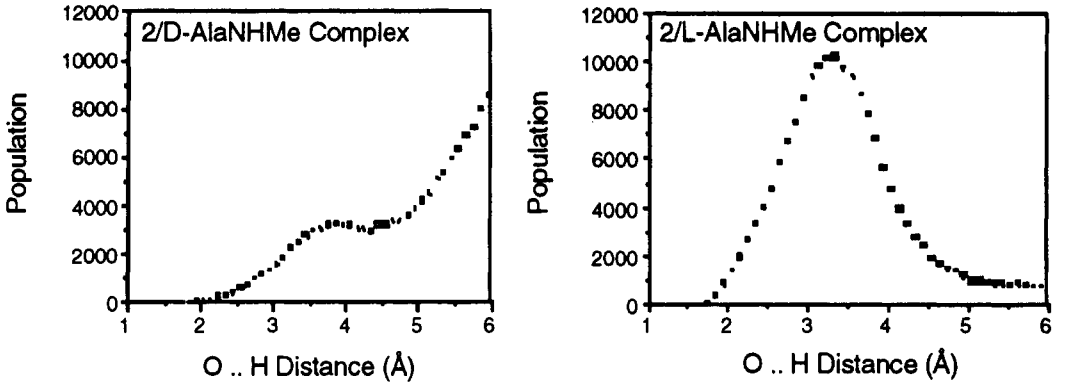
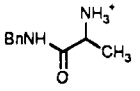
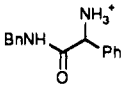
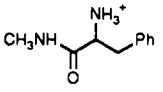


Figure 2. Theoretical populations of 2/D-AlaNHMe and 2/L-AlaNHMe vs (N)H/(C=)O distance.

Table II. Enantioselectivity of Binding[a] of Podand 2 with α-Amino Acid Amide Hexafluorophosphates

	73% ee (*S*)
	83% ee (*S*)
	81% ee (*S*)

[a] Enantiomeric excess of indicated ammonium ion extracted from excess racemic ammonium PF_6^- (0.5M) in D_2O by podand 2 (0.02 M) in $CDCl_3$ at 25 °C. Configuration of major isomer extracted is shown in parentheses.

While we have been unable to find direct evidence for the proposed hydrogen bond-like association between the host and guest, we did see it in a simulation of the 2/AlaNHMe·PF_6^- complex. Over the course of two 5000 ps, 300 K stochastic dynamics simulations[6] with D-Ala and L-Ala guests, we monitored the distance between the host acetamido (N)H and the guest amide (C=)O. Shown in Figure 2 are graphs of the populations of each complex having such H/O distances between 1.5 and 6 Å. In the less tightly bound complex of 2 with D-Ala, the amides spend the majority of their time widely separated. But in the L-Ala complex, there is a large population of structures with an H/O distance of ~3.2 Å. This peak occurs at too long a distance for a typical hydrogen bond (1.8–2.0 Å) but corresponds to a dipolar alignment of the host and guest amide groups. It is likely that this weak electrostatic association is responsible for stabilizing the L-Ala complex selectively.

In this study, the two-point host 2 is substantially more enantioselective than the one-point hosts 1 and 3, even though all hosts are conformationally homogeneous. It is noteworthy that the enhanced selectivity shows up with 2 even though the secondary amide/amide association appears to be weak and structurally ill-defined according to our molecular modeling. Though such flexible associations may favor binding entropically, we expect that a more highly structured complex would be associated generally with higher enantioselectivity. Experiments to test this hypothesis are underway.

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Supplementary Material Available: Preparative procedures and compound characterization data (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(6) AMBER* force field and a GB/SA continuum model for $CHCl_3$ using MACROMODEL V3.5X. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.*, **1990**, *11*, 440.