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Enantioselective Chromenone Benzoxazole Receptor for Glutamic Acid and Its Derivatives

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Abstract: Combination of a binaphthyl unit with chromenone benzoxazole fragments provided a chiral receptor that is enantioselective for glutamic acid and its derivatives. The receptor racemic mixture was resolved by TLCs impregnated with (*R*,*R*)-thiodilactic acid. High association constants were measured for dansylglutamic acid, using a fluorescent method. This receptor can be used for the resolution of the tosylglutamic acid racemic mixture.

Large-scale resolution of racemic mixtures is still a challenge.[1] Thus, the industrially important L-glutamic acid must be separated from the D form through acetylation and hydrolysis with a suitable microorganism.[2] Enantioselective transport, as suggested by Cram, is a very attractive procedure for the resolution of racemic mixtures and is especially suited for large-scale separations since chiral receptors are not consumed during the process.[3] In our opinion, the main drawback of this methodology is the lack of suitable receptors able to selectively transport one of the glutamic acids through the apolar membrane.[4] Several receptors have already been shown to associate glutaric acid.[5] We wished to explore the posibilities of receptor **1** since, from molecular models and modeling studies, chiral discrimination was expected.

The formation of the complex places the glutamic acid α carbon close to the binaphthyl aromatic sheet. In the best configuration (*R*,*S*), the α hydrogen is close to the aromatic system; otherwise the large α substituent (*S*,*S*-complex) collides with the naphthyl ring (Figure 1).

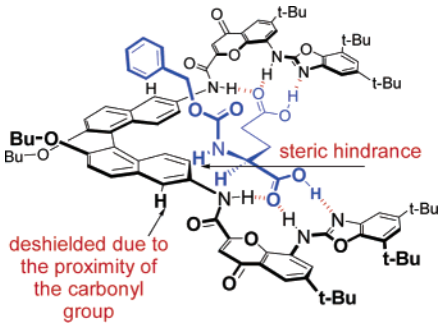

FIGURE 1. Proposed structure for the strong (*R*,*S*) complex between receptor **1** and the benzyloxycarbonyl derivative of (*S*)-glutamic acid. In this configuration, the hydrogen atom of the amino acid α carbon is placed close to the binaphthyl aromatic sheet, reducing the steric hindrance.

Preparation of receptor **1** can be accomplished from the known chromenone-2-carboxylic acid **2**[6] and the aminobinaphthyl **3**[7] (Scheme 1).

Cooperativity between both binding arms was initially tested by comparing several diacids with phenylthioacetic acid. Competitive ^{1}H NMR titrations were carried out by selecting a pair of acids and adding small portions of receptor **1** to their $CDCl_3$ solutions. Plotting the chemical shift of the selected signal of one of the acids with respect to the signal of the other guest provides a curve that can be evaluated with a homemade curve-fitting program, leading to the results shown in Figure 2. Good cooperativity can be deduced for the diacids tested.

Because the cleft matches glutaric-like diacids well, benzyloxycarbonyl L-glutamic acid was tested. The addition of small portions of the optically pure amino acid to a solution of the racemic receptor **1** in 95/5 chloroform/acetone mixture (the receptor signals were broadened during the titration in the absence of acetone) resulted in the splitting of most of the receptors signals. Especially significant was the strong deshielding of H-5 in the binaphthyl, which moves from 8.20 to 8.60 and 8.65 ppm in the diastereomeric complexes (Table 1).

CPK molecular models can account for this effect because complex formation fixes the carbonyl group of the chromenone 2 carboxamide close to this proton. However, graphic plotting of the movement of these protons during the titration, again making use of a homemade computer curve-fitting program, yielded a small K_{assoc} ratio of only 2.

In a search for substrates with a greater steric demand, phthaloyl and tosyl glutamic acids were tested. Both guests yielded the expected splitting of the binaphthyl protons of racemic host **1**. Graphic plotting afforded only small chiral recognition for the phthaloyl derivative but a promising K_{assoc} ratio of 5.1 for the sulfonyl amino acid. CPK molecular models show that the phthaloyl group collides with the receptor in both the weak and strong

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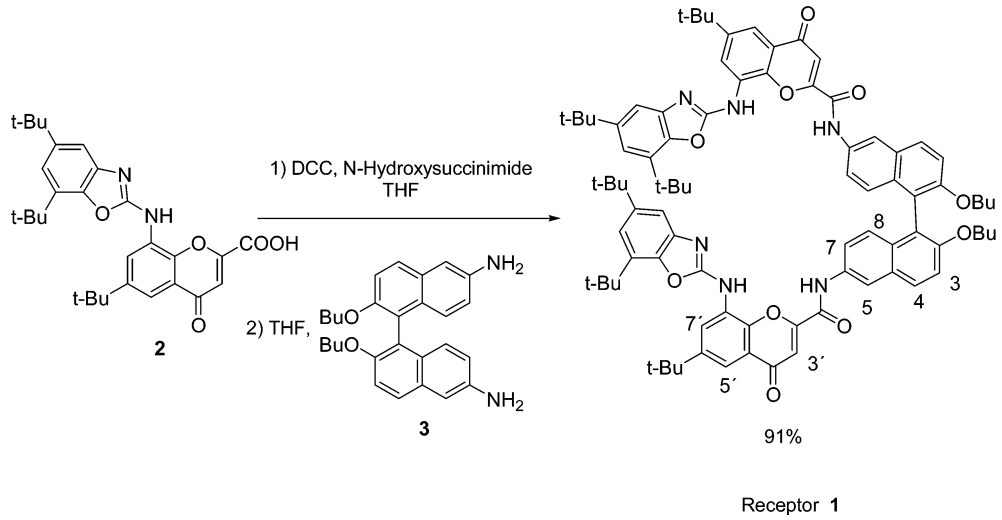
SCHEME 1. Synthesis of Receptor 1



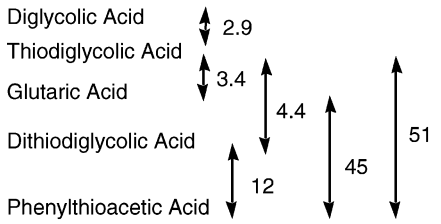


FIGURE 2. Relative association constants between phenylthioacetic acid, several diacids, and receptor **1** in $CDCl_3$ at 20 °C.

complexes, but the sulfonamide steric hindrance is predicted only in the weak associate, similar to Figure 1. As in previous cases we attempted to resolve the racemic mixture by taking advantage of its supramolecular properties.[8] Thus, TLC plates were impregnated with the optically pure amino acid derivatives, and the racemic receptor **1** was eluted with a 95/5 methylene chloride/ether mixture. Because this host is a yellow compound, separation of its enantiomers in TLC can be easily followed, although the R_f differences shown in Table 2 are not large. Better R_f differences can be obtained using thiodilactic acid.[9] The presence of two chiral centers in this guest provides better chiral recognition ($K_{rel} = 12$ in $CDCl_3$ at 20 °C), since steric effects in the weak complex are present now in both binaphthyl sheets.

Semiempirical geometry optimizations were carried out at the restricted Hartree−Fock (RHF) level, using the PM3 semiempirical SCF-MO method, including molecular mechanics correction for HCON linkages (keyword PM3MM), as implemented in the Gaussian 98W program.[10] These modeling studies showed that the presence of two chiral centers in this guest provide better chiral recognition. The calculated difference in energy between weak and strong complexes is 3.5 kcal/mol. This difference is much higher than expected from the experimental relative association constant, but it can be explained taking into account the low level of theory used in the calculation. One hundred milligrams of the racemic mixture of receptor **1** can be readily separated in preparative TLC (silica gel, 16 g) impregnated with (*R*,*R*)-thiodilactic acid.

Once the optically pure receptor **1** had become available, it was possible to measure its absolute association constants with the diacids. Initial experiments revealed that NMR methods were not suitable because of the large K_{assoc} of these guests. Therefore, a fluorescence method was tested. Since no suitable emission was detected for receptor **1**, the dansyl derivative of glutamic acid was chosen as the guest. Complex formation was readily followed since significant fluorescence quenching ($\lambda = 528$ nm) was observed both in the strong (85%) and weak associates (55%). Evaluation of the data revealed high association constants of $K_{assoc} = 3.7 \times 10^7\ M^{-1}$ and $K_{assoc} = 6.1 \times 10^6\ M^{-1}$. The ratio between both association constants (around 6) was similar to that obtained with the competitive method.

Although the complexes of receptor **1** with diglycolic and thiodiglycolic acids readily crystallize from methylene chloride/undecane solutions, we were unable to obtain suitable crystals for X-ray analysis. However, several aspects seem to support the proposed geometry for the complexes.

The absolute configuration of the enantiomerically pure receptor **1** that forms the strong complexes with (*S*)-glutamic acid derivatives was studied by comparing its CD spectrum with (*R*)-binaphthylphosphoric acid. To set the same conformation in both binaphthyl chromophores,

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TABLE 1. Chemical Shifts of Receptor 1 and Its Diastereomeric Complexes with Benzyloxycarbonyl Glutamic Acid in 95/5 Deuterochloroform/Acetone-d_6 at 20 °C

	proton									
	3′	**5′**	**7′**	**B4**	**B6**	**3**	**4**	**5**	**7**	**8**
receptor **1**	7.33	7.88	8.46	6.97	7.17	7.30	7.74	8.20	7.01	6.84
complexes	7.38/7.38	7.98/7.97	8.15/8.13	7.11/7.10	7.21/7.21	7.41/7.41	7.93/7.88	8.65/8.60	7.01/7.01	6.91/6.91
$\Delta\delta$	0.05/0.05	0.10/0.09	−0.31/−0.33	0.14/0.13	0.04/0.04	0.11/0.11	0.19/0.14	0.45/0.40	0.00/0.00	0.07/0.07

TABLE 2. R_f Values of Diastereomeric Complexes of Receptor 1 and Different Optically Pure Guests on a Silica Gel Support[a]

guest	strong complex (*R*,*S*)	weak complex (*S*,*S*)
Cbz-L-glutamic acid	0.55	0.43
phthaloyl-L-glutamic acid	0.20	0.15
tosyl-L-glutamic acid	0.36	0.17
thiodilactic acid	0.40	0.04

[a] Elution was carried out with 95/5 methylene chloride/ether.

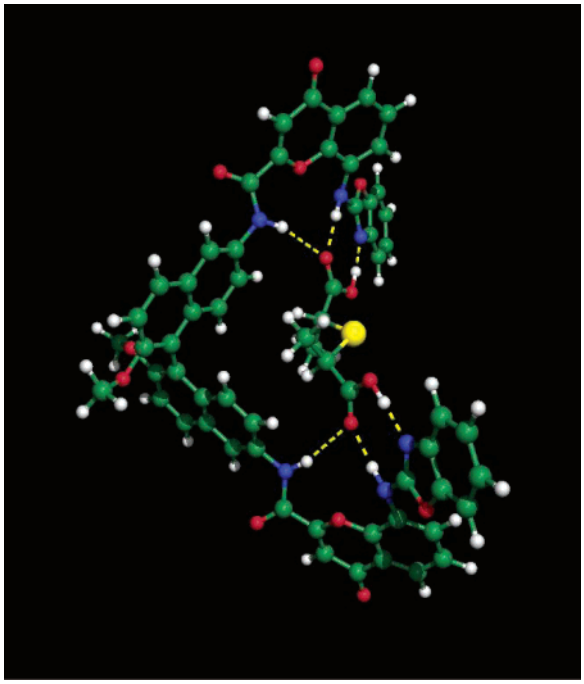

FIGURE 3. Modeling study of receptor **1** and thiodilactic acid.

the complex with diglycolic acid was used. A strong negative band at 225 nm (ϵ = 492) (9/1 hexane/CH_2Cl_2 as solvent) very similar to that of binaphthylphosphoric acid (228 nm, ϵ = 475) suggested that both compounds have the same (*R*) configuration.

ROESY of the strong (*R*,*S*)-complex revealed an expected nuclear Overhauser effect between proton H-7 in the binaphthyl and the tosylglutamic acid α proton (Figure 4), and in general, the anisotropic effects observed in the ^{1}H NMR spectra of the complexes were in good agreement with the proposed structure (Figure 4).

Since resolution of glutamic racemic mixtures was an attractive application of this receptor, we tested its possibilities. Tosyl glutamic acid was chosen as the substrate, owing to the easy ^{1}H NMR analysis of its enantiomers. In the complexes with receptor **1**, the tosyl methyl group was shielded at 1.97 and 2.02 ppm. In a

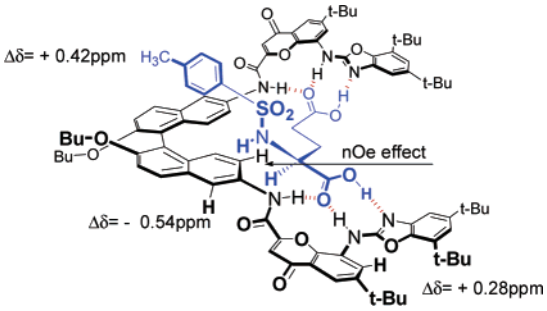

FIGURE 4. Proposed structure for the complex of receptor **1** and tosyl L-glutamic acid.

typical experiment, 5 mg of the optically pure receptor **1** was dissolved in $CDCl_3$ in an NMR tube, and slightly less than the stoichiometric amount of the tosyl glutamic acid racemic mixture was added. Integrals of the tosyl methyl groups revealed the expected 1/1 ratio in this step. Water and the sodium salt of the glutamic acid tosyl derivative were then added, and the solution was allowed to stand until equilibrium had been reached. A new integration of the methyl groups now revealed a ratio close to 6, in reasonably good agreement with the previously calculated relative association constant. Since both tosyl glutamic acid enantiomers show essencially the same stability in water, the system evolved toward the formation of the most stable complex in the organic phase. A single extraction provided 66% enantiomeric excess.

From an industrial point of view, the use of glutamic acid derivatives is not especially attractive. Nevertheless, neither natural glutamic acid nor its hydrochloride can be extracted at from the aqueous solution. In a search for a more lipophilic counterion, hexafluorophosphate was chosen, but this salt again preferred the aqueous phase, probably because it is only in this phase that the ammonium group can properly saturate its hydrogen bonds. The presence of a crown ether in the organic phase may provide the necessary hydrogen bonding acceptors. Therefore, an extraction experiment was carried out in a biphasic system with the racemic receptor **1** in $CDCl_3$ and L-glutamic acid as its hexafluorophosphate salt in the aqueous layer. ^{1}H NMR of this system revealed the spectrum of the free receptor **1**. However, the addition of small portions of the 18-crown-6 ether shifted the NMR signals and produced the expected splitting. Thus, the binaphthyl H-5 protons moved from 8.19 to 8.36 and 8.42 ppm. Graphic plotting of the movement of these protons showed a modest degree of chiral recognition, with a constant ratio of 1.8. TLC experiments with silica gel impregnated in a 1% methanol solution of the L-glutamic acid hexafluorophosphate salt and the crown ether split the racemic receptor **1** into two different spots with a large R_f difference (R_f = 0.13 and 0.36) when eluted with the previous solvent. We expect that small changes in

the structure of receptor **1** structure may further improve these results.

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Supporting Information Available: Experimental preparation of receptor **1**; circular dichroism of receptor **1**; ROESY of the strong complex between receptor **1** and tosylglutamic acid; atomic coordinates of resulted computed structures of the complexes betwen receptor **1** and thiodilactic acid enantiomers, supplied as in "protein data bank" (pdb) format. This material is available free of charge via the Internet at http://pubs.acs.org.

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