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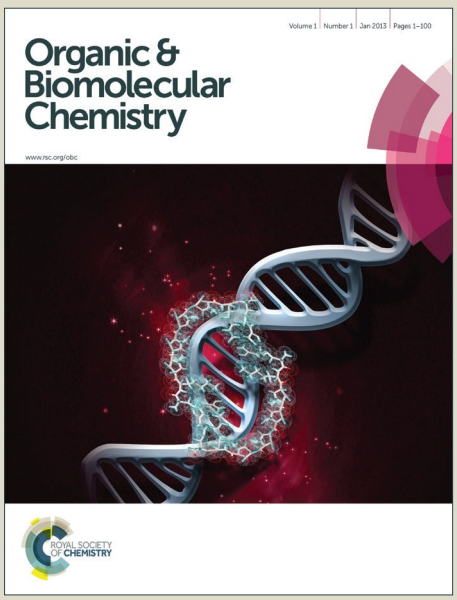

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A HIGHLY SELECTIVE RECEPTOR FOR ZWITTERIONIC PROLINE

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A chiral chromane receptor has been synthesized which mimics the oxyanion hole of the enzymes. In this receptor H-bonds and cation-π interactions team up to generate an apolar host-guest complex with zwitterionic proline. This complex allows the extraction of only proline to a chloroforom phase, while no other natural amino acids are extracted. Due to the chiral nature of the receptor, enantioselective extraction from the aqueous proline solution to a chlorform phase takes place. *L*-proline provided an easy way to resolve the receptor racemic mixture, while anisotropic effects, NOE and CD studies revealed the receptor absolute configuration. Modelling studies also support the proposed structures. The presence of an oxyanion-hole motif in this structure was corroborated by X-ray diffraction studies.

Introduction

Amino acids are essential in nature as a source for molecular recognition. They are also attractive guests, since they have a large functional group density, and therefore are able to experience many H-bonds. In particular, proline has been used both to prepare molecular receptors and chiral stationary phases and as a guest.[1] Furthermore, proline has many times been used as an organocatalyst in organic chemistry.[2] Association of the zwitterionic amino acid in apolar solvents is challenging due to its ionic structure, which provides water affinity but renders highly insoluble compounds in most organic solvents. Accordingly, like in molecular recognition of other amino acids, most of the proline enantioselective receptors found in the literature associate proline derivatives and not the natural zwitterionic amino acid[3] and therefore, receptors for the zwitterionic amino acid are scarce.[4]

Enzymes are particularly selective receptors, and in addition of reducing the activation energy of the reactions that catalyze,[5] they are able to bind selectively the substrate of these reactions. Mimicking the structures present in natural enzymes may inspire the development of new catalysts. The oxyanion hole moiety, which is present in many enzymes,[6] is a good candidate considering the high increase in the reaction rates that can be attributed to it. For example, in chymotrypsin the catalytic activity amounts to 10^{10} and as much as 10^4 of this hydrolytic power corresponds to the presence of the oxyanion hole; meanwhile, other factors, including the catalytic triad formed by serine, histidine and aspartic acid, amounts for the rest.[6h, 7]

Ureas and thioureas are probably the simplest oxyanion hole mimics,[8] but many other groups have also been used, as TADDOL,[9] BINOL,[9c, 10] oxazolines,[11] biphenylenol,[12] etc.[13] We have developed organocatalysts that resemble the oxyanion hole moiety employing amido groups connected by xanthones and chromenones scaffolds, which show a H-bond donor distance around 4.5 Å,[14] much closer than that of other organocatalysts to the distance between NH groups in natural oxyanion holes.[15] However, planar skeletons like xanthone and chromenone are not effective in the design of enantioselective receptors and catalysts, and they require the inclusion of an additional chiral group which may not be easily involved in the recognition of the catalytic event. The development of an intrinsically chiral scaffold which maintains the resemblance to the natural oxyanion hole could therefore lead to more enantioselective receptors or catalysts.

In this paper we present a chiral receptor based on a 8-amino-2-amido-2-phenylchromane scaffold which resembles the oxyanion hole structure that is able to associate enantioselectively proline in its zwitterionic form.

Results and Discussion

The 8-amino-2-carboxamide-2-phenylchromane scaffold of receptor **1** (Figure 1) was chosen because modelling studies showed that it places two H-bond donors, separated by 4.4 Å and with the correct orientation to resemble natural oxyanion holes. It also possesses,

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† Electronic Supplementary Information (ESI) available: ^{1}H NRM, ^{13}C NMR, HMQC, HMBC, COSY, ROESY, IR and HRM spectra of compound **1**; ^{1}H NMR, IR and HRM spectra of compounds **3**-**6**, modelling studies of Fig. 5; X-ray diffraction data of compound **1** and determination of the absolute configuration of (+)-**1** and (-)-**1** by ECD spectra. CCDC 1436301-1436302. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/x0xx00000x

an urea functional group which is known to associate carboxylates,[10] and a basic pyridine nitrogen which may form a strong H-bond with the guest.

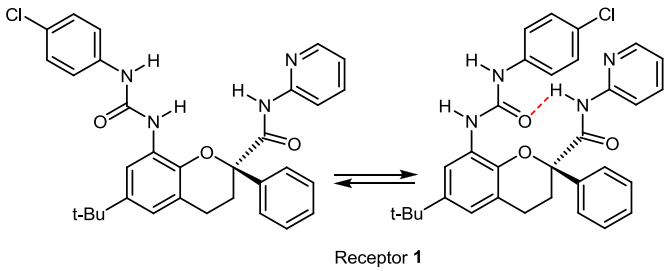


Figure 1: Chiral chromane receptor studied in this work, showing a possible conformation with an intramolecular H-bond (right).

Preparation of receptor **1** is shown in Figure 2. In the first step, hydroxyacetophenone **2** reacts with methyl benzoyl formate under basic conditions to yield the corresponding aldol compound and this intermediate alcohol cyclizes in sulfuric acid providing the chromane skeleton (compound **3**). Reduction of the nitro group to the amine by conventional procedures, followed by reaction with chlorophenyl isocyanate, generates the urea moiety (compound **5**) and the final reduction of the ketone and aminolysis of the methyl ester with the aminopyridine lithium anion yields the racemic receptor **1**.

Slow evaporation of a receptor **1** solution in ethyl acetate yielded crystals suitable for X-ray analysis. The obtained X-ray structure, shown in figure 3, exhibits an intramolecular H-bond between the urea carbonyl group and the amide NH, blocking the oxyanion hole structure. However, we expect that this intramolecular H-bond is relatively weak (the N-O distance is 3.011 Å) so it might not be present in solution. H-7 chemical shift (Figure 2) is very sensitive to the receptor conformational change, because the proximity of the urea carbonyl group strongly deshields this proton. The absorption of H-7 is similar in ester **6** (7.93 ppm) and in receptor **1**, in DMSO-*d6* (7.87 ppm) or deuterochloroform (8.22 ppm) which is consistent with a urea conformation in which the carbonyl oxygen atom lies in its proximity. Furthermore, the addition of tetrabutylammonium acetate to the NMR sample, which should break this intramolecular bond in order to form the host-guest complex, did not strongly change this H-7 chemical shift (7.86 ppm in the complex with the acetate). These facts showed that the intramolecular H-bond observed in the X-ray structure is already broken in solution even if the receptor is not involved in the formation of a complex.

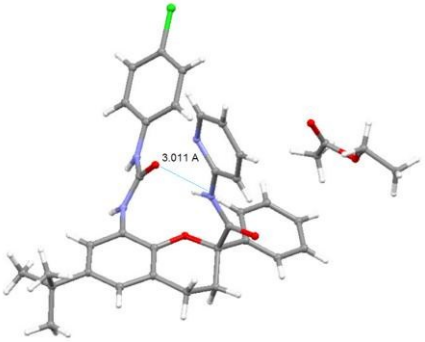


Figure 3: X ray structure of receptor **1** crystalized in the absence of water.

Additionally, crystallization of receptor **1** from a mixture of methanol and water also yielded appropriate crystals for X-ray analysis. In this case, the structure obtained (Figure 4) lacks the intramolecular H-bond in favor of a complex with a water molecule located in the receptor cleft. This structure confirms that the receptor cavity is a good oxyanion hole mimic, and therefore a guest carbonyl group should fit this cleft.

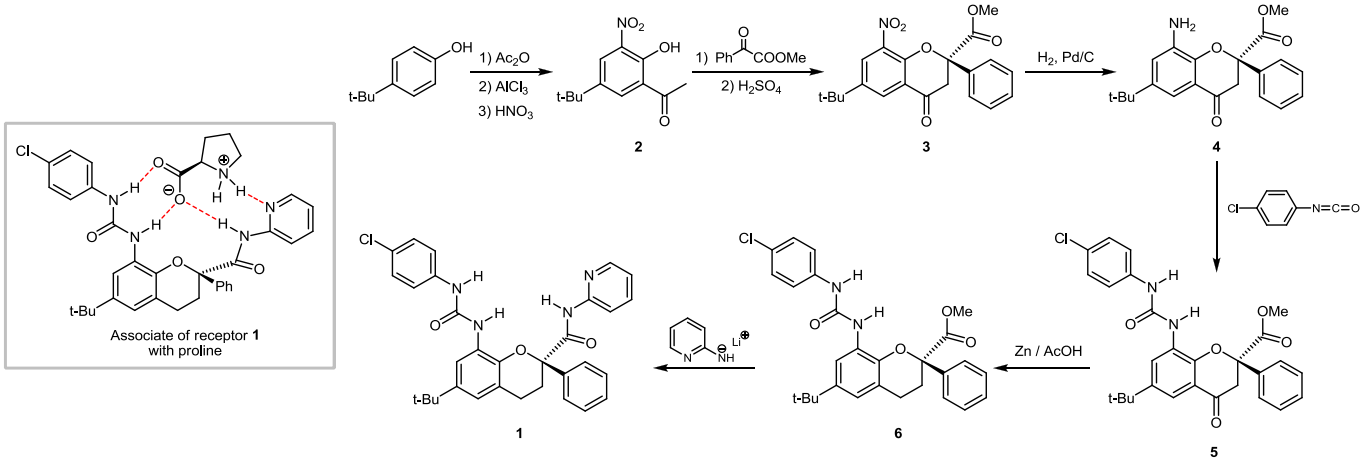


Figure 2: Preparation of the racemic receptor **1** and its complex with proline.

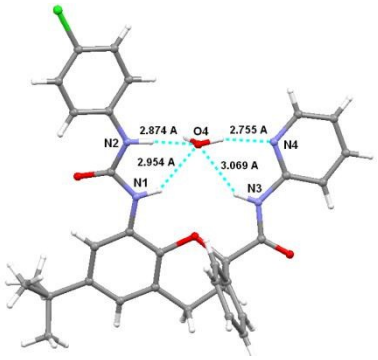


Figure 4: X ray structure of receptor **1** crystalized in the presence of water.

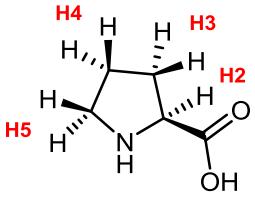
The presence in the receptor of both the urea group, which is suitable to associate carboxylates, and the pyridine, which is a good H-bond acceptor suggested amino acids as guests. Several amino acids were therefore tested; however even the most lipophilic natural amino acids as leucine, phenylalanine or valine were not extracted from aqueous solutions. Proline was the only natural amino acid which was easily extracted. In fact, an extraction experiment in which an aqueous saturated solution of several natural amino acids (see ESI) was treated with a receptor **1** chloroform solution yielded after HPLC analysis only proline, as the single amino acid in the organic phase. Therefore, receptor **1** can be useful in the separation of proline from mixtures with other amino acids.

The addition of proline to a chloroform solution of the receptor **1** racemic mixture split the receptor **1** ^{1}H-NMR signals showing the formation of diastereomeric complexes. In particular large splitting of the receptor **1** NHs took place, which resonate at 8.71 ppm in the free receptor and deshielded to 8.79 ppm and 9.43 ppm in the complexes. Graphical representation of the movement of the pyridine NHs after addition of portions of *L*-proline yielded a relative association constant of K_{rel} = 4.4, being the host-guest complex with the NH at 8.79 ppm the strongest one. To further assure this chiral recognition, an experiment was carried out with a solution of the racemic receptor **1** and a saturated aqueous solution of racemic proline. The NMR spectrum of the complex racemic mixture showed the amidopyridine proton at 8.85 ppm due to the average of both signals in the complexes. A similar experiment with the racemic receptor **1** and a saturated aqueous solution of *L*-proline yielded two different signals at 9.01 ppm and 8.81 ppm. From these data it is possible to deduce a value for the relative association constant of K_{rel} = 4.0 out of equation 1,[16] a value in good agreement with the previous one.

$$K_{rel} = \frac{[strong\ complex]}{[weak\ complex]} = \frac{\partial_x - \partial_{weak\ complex}}{\partial_{strong\ complex} - \partial_x} \qquad (1)$$

Considering the preference for one of the diastereomeric complexes, we attempted the resolution of the racemic mixture of receptor **1** using *L*-proline. Attempts to separate the enantiomers by preferential crystallization of one of the diastereomeric complexes with *L*-proline failed, but TLC chromatography yielded better results. Impregnation of SiO_2 preparative silica gel plates with the *L*-proline aqueous solution (10 g of *L*-proline in 100 mL of water) and drying at room temperature yielded, after elution, both diastereomeric host-guest complexes. Washing an ethyl acetate solution of the complexes with aqueous sodium hydroxide allowed us to obtain the enantiomerically pure receptors (rotatory powers of -36º, *C*=0.005 in $CHCl_3$, for the less polar one and +35º, *C*=0.008 in $CHCl_3$, for the most polar enantiomer), corresponding to the strongest complex the one with the smallest Rf.

Table 1: Chemical shifts of methylene groups in free *L*-proline and in *L*-proline forming the weak and strong complexes.



Signal	δ in free proline (ppm)	δ for proline in strong complex (ppm)	δ for proline in weak complex (ppm)
H-2	4.12	3.99	3.97
H-3	2.06; 2.34	2.05; 2.24	1.85; 2.17
H-4	1.99; 1.99	1.81; 1.94	1.56; 1.76
H-5	3.33; 3.41	3.29; 3.41	2.90; 3.07

NMR spectra of both complexes allowed us to deduce the geometry of these compounds (Table 1). In particular the less stable complex shows strong shielding of one of the H-5 proline protons, which moves from 3.41 ppm to only 2.93 ppm. This effect sets this proton in the anisotropic shielding cone of the receptor phenyl ring. H-4 protons are also close to the receptor aromatic ring, since they also shield strongly from 1.99 ppm to 1.76 ppm and 1.57 ppm. Therefore the configuration of the receptor in the weak host-guest complex with *L*-proline must be (*R,R*). On the other hand, the H-2 proline proton in the strong complex (*R/S*) showed a correlation in the ROESY spectrum with the receptor phenyl group (modelling studies placed this proton at 2.70 Å from the phenyl ring *meta* proton in this complex).

To understand the source of the enantioselective recognition, modeling studies of the host-guest complexes of *L*-proline with both enantiomers of receptor **1** were carried out (see details in the electronic supplementary information). For both enantiomers the phenyl group could be in either axial or equatorial position, but the more stable structures, which are shown in Figure 5, correspond to these latter conformation. The energy difference is in good agreement with experimental results (Maxwell-Boltzmann populations at 298 K afford a 2.5 relative association constant), and the most stable complex is formed between *L*-proline and *R* absolute configuration in the receptor. In all cases, the carboxylate group makes two H-bonds with the urea and an additional H-bond

with the amide, confirming the possibilities of the receptor as an oxyanion hole mimic. Proline ammonium group forms a strong H-bond with the basic pyridine in the receptor. Additionally, there is a cation-π interaction between the proline ammonium group and the receptor phenyl ring[17]

Comparison of both structures reveals that the main geometric difference is the different conformation of the proline, which in the weak complex places its methylene groups in the vicinity of the phenyl group H-atoms. In agreement with these modeling studies, proline methylene ^{1}H-NMR signals are significantly shielded in the weak complex (Table 1).

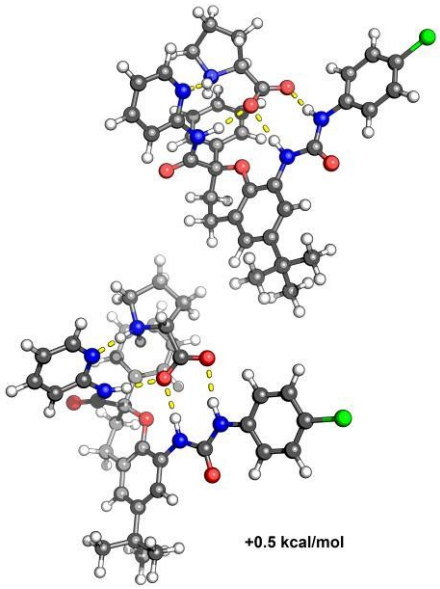


Figure 5: Modelling studies of both complexes of proline with receptor **1**.

Single point energy calculations on the proline structure present in the complex (obtained after deleting the receptor atoms) and on the receptor structure after deleting proline atoms, and comparison with the energy of the complexes, allowed us to calculate the energy of the interaction between these two fragments. In the strong complex both the proline and the receptor show more stable conformations ($\Delta\Delta G$= 1.0 kcal/mol and 0.2 kcal/mol, respectively), which is only partially compensated by a slightly higher interaction energy in the weak complex (0.5 kcal/mol). The proximity of the proline methylene protons and the receptor aromatic ring prevents the adoption of the more stable conformations in the weak host-guest complex.

Circular Dichroism measurements for the isolated receptor **1** enantiomers are also in agreement with the receptor absolute configuration in the strong complex. The receptor enantiomer forming the most stable complex with *L*-proline shows a positive Cotton effect at 250 nm. Quantum chemical simulation (see details in the electronic supplementary information) of the ECD spectra of the *R* enantiomer also showed this Cotton effect, which confirms that the stronger complex is formed between the receptor with *R* absolute configuration and *L*-proline.

Attempts to measure an absolute association constant for receptor **1** and *L*-proline in chloroform were not successful due to the poor proline solubility in this solvent. However, it was possible to carry out an enantioselective extraction of racemic proline to the chloroform phase with (*R*)-receptor **1**. When a $2x10^{-2}$ M solution of this compound in chloroform was treated with an aqueous saturated racemic proline solution, the ^{1}H-NMR spectrum showed the extraction of proline. From the integral at 4.00 ppm, it is possible to deduce that there is almost one equivalent of proline in the chloroform solution since the integral ratio between the receptor and proline protons is beyond 90%. Nevertheless, the relatively broad nature of the proline signals prevented an accurate assessment of the extraction enantioselectivity. Analysis of the extracted proline mixture was carried out by chiral HPLC analysis (ChiralPak Zwix(+) of 150x3 mm, 3 µm column) showing indeed an enantioselective extraction with a 1/3 ratio between the proline enantiomers.

Experimental

General experimental procedures

Solvents were purified by standard procedures and distilled before use. Reagents and starting materials obtained from commercial suppliers were used without further purification. IR specta were recorded as neat film or in nujol and frequencies are given in cm^{-1} Melting points are given in ºC. NMR spectra were recorded on 200 MHz and 400 MHz spectrometers. ^{1}H NMR chemical shifts are reported in ppm with tetramethylsilane (TMS) as internal standard. Data for ^{1}H are reported as follows: chemical shift (in ppm), number of hydrogen atoms, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br s = broad singlet), coupling constant (in Hz). Splitting patterns that could not be clearly distinguished are designated as multiplets (m). Data for ^{13}C NMR are reported in ppm and hydrogen multiplicity is included. High-resolution mass spectral analyses (HRMS) were measured using ESI ionization and a quadrupole TOF mass analyzer. Flash chromatography was performed on 70-200 mesh silica gel.

1-(5-*tert*-butyl-2-hydroxy-3-nitrophenyl)ethanone (2). In a flask provided with a magnetic stirring bar, acetic anhydride (850 mL, 9.0 mol), *t*-butylphenol (500 g, 3.3 mol) and concentrated sulfuric acid (2 mL) were added. The progress of the reaction was followed by TLC and upon completion, ice was added. The mixture was then extracted with ethyl acetate and washed with 4% aqueous Na_2CO_3 solution. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off under reduced pressure to isolate 4-*tert*-butylphenyl acetate (490 g, 77% yield). Spectroscopic data of this compound were consistent with those described in the literature.[18]

In a flask equipped with a condenser, stirring bar and thermometer, ethyl 4-*t*-butylphenol (490 g, 2.5 mol) was dissolved in nitrobenzene (600 mL) and $AlCl_3$ (185 g, 1.4 mol) was added, keeping the reaction temperature below 70°C during one hour. Then the mixture was

cooled down to room temperature and another portion of $AlCl_3$ (185 g, 1.4 mol) was added. The new reaction mixture was allowed to react at 70°C for 4 h. After that, the mixture was cooled, treated with concentrated HCl (250 mL) and kept overnight under stirring. The organic phase was then separated, washed with water and dried over anhydrous Na_2SO_4. The crude product was distilled under reduced pressure (20 mm), collecting the fraction distilling between 118 and 150°C [280 g, 57% yield of 1-(5-*tert*-butyl-2-hydroxyphenyl)ethanone]. Spectroscopic data for this compound coincide with those described in the literature.[19]

To a well stirred solution of 4-hydroxyacetophenone (250 g, 1.3 mol) in acetic acid (800 mL), a solution of fuming nitric acid (60 mL, 1.45 mol) in acetic acid (200 mL) was slowly added, keeping the temperature above 30°C. Once the addition was completed, the reaction mixture was poured onto a mixture of ice and water and filtered to recover the yellow-orange solid precipitate of compound **2** (162 g, 53% yield). Spectroscopic data of this compound were consistent with those described in the literature.[20]

6-*tert*-butyl-8-nitro-4-oxo-2-phenylchroman-2-methyl ester (3). Sodium (4 g, 170 mmol) was reacted with methanol (25 mL) in a round bottomed flask equipped with magnetic stirrer, thermometer and reflux condenser. Once all the sodium had reacted, the reaction was cooled down in an ice bath and the mixture was placed under argon. Then methyl benzoylformate (9 g, 51 mmol) and nitroacetophenone **2** (19 g, 80 mmol) were added, the ice bath was removed and the reaction mixture was allowed to react at room temperature with stirring during one hour. When the reaction was completed, the mixture was poured onto ice and aqueous concentrated HCl and the deep yellow precipitate. A solution of this latest compound (20 g) in CH_2Cl_2 (25 mL) was added dropwise at 0°C and with stirring to concentrated H_2SO_4 (100 mL). The progress of the reaction was followed by NMR and when it was complete, the reaction mixture was poured onto a mixture of ice and water. Compound **3** was isolated by vacuum filtration (14 g, 46% yield). Mp 122–124 °C; ^{1}H NMR (200 MHz, $CDCl_3$) d (ppm) 1.34 (9H, s), 3.32 (1H, d, *J* = 16 Hz), 3.63 (3H, s), 3.77 (1H, d, *J* = 16 Hz), 7.41–7.45 (3H, m), 7.66–7.71 (2H, m), 8,14 (1H, d, *J* = 2.6 Hz), 8.21 (1H, d, *J* = 2.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) d (ppm): 30.7 ($3CH_3$), 34.4 (C), 44.7 (CH_2), 53.4 (CH_3), 85.4 (C), 122.1 (C), 124.9 (2CH), 128.6 (CH), 128.8 (3CH), 129.1 (CH), 136.1 (C), 139.5 (C), 144.8 (C), 150.5 (C), 169.4 (C), 187.8 (C); IR (nujol) 3403, 1755, 1709, 1631, 1541, 1482, 1366, 1288 cm^{-1}; HRMS Calcd for $C_{21}H_{25}N_2O6$ 401.1713, found 401.1701.

8-Amino-6-*tert*-butyl-4-oxo-2-phenylchroman-2-methyl ester (4). Pd/C (5% weight, 1.9 g) was added to a solution of the nitro derivative **3** (9.65 g, 25.2 mmol) in MeOH (60 mL) and the resulting mixture was placed in a pressurized bottle (4 atm) under H_2 atmosphere. After one hour, the reaction mixture was filtered and the organic phase was evaporated. Compound **4** was obtained (8.73 g, 98% yield). Due to the instability of the amino group, this compound has to be kept refrigerated and under argon if it is not going to be reacted subsequently. ^{1}H NMR (200 MHz, $CDCl_3$) d (ppm) 1.25 (9H, s), 3.24 (1H, d, *J* = 16.0 Hz), 3.57 (1H, d, *J* = 16.0 Hz), 3.62 (3H, s), 7.01 (1H, d, *J* = 2.0 Hz), 7.26 (1H, d, *J* =2.0 Hz), 7.37–7.40 (5H, m), 7.57–7.62 (2H,m); ^{13}C NMR (50 MHz, $CDCl_3$) d (ppm): 31.0 ($3CH_3$), 34.0 (C), 45.4 (CH_2), 53.1 (CH_3), 84.3 (C), 111.8 (CH), 119.0 (CH), 119.9 (C), 124.7 (2CH), 128.6 (2CH), 128.7 (CH), 135.7 (C), 137.3 (C), 144.9 (C), 145.2 (C), 170.5 (C), 190.1 (C); IR (nujol) 3468, 3361, 2922, 2849, 1736, 1686, 1472, 1264, 1039, 736 cm^{-1}; HRMS Calcd for $C_{21}H_{24}NO_4$ 354.1699, found 354.1698.

6-*tert*-Butyl-8-(3-(4-chlorophenyl)ureido)-4-oxo-2-phenylchroman-2-methyl ester (5). *p*-Chloropheylisocyanate (0.45 g, 2.93 mmol) was added to a solution of the previous amino derivative (1.04 g 2.94 mmol) in dicloromethane (4 mL) and the resulting mixture was left under stirring for half an hour. After the completion of the reaction, the organic phase was evaporated. The crude product was dissolved in CH_2Cl_2 and purified by percolation over SiO_2, yielding compound **5** (1 g, 67% yield). Mp 145-150 °C; ^{1}H NMR (200 MHz, $CDCl_3$) d (ppm) 1.25 (9H, s), 3.44 (2H, s), 3.67 (3H, s), 7.14–7.46 (9H, m), 7.47 (1H, d, *J* = 2.6 Hz), 8.05 (1H, s), 8.07 (1H, s), 8.54 (1H, d, *J* =2.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) d (ppm): 30.8 ($3CH_3$), 34.4 (C), 44.4 (CH_2), 53.5 (CH_3), 84.4 (C), 115.9 (CH), 119.6 (C), 121.1 (CH), 124.2 (CH), 125.0 (2CH), 128.1 (C), 128.7 (3CH), 128.8 (2CH), 129.1 (CH), 135.9 (2C), 136.7 (C), 145.4 (C), 145.7 (C), 152.8 (C), 170.8 (C), 189.3 (C); IR (nujol) 3513, 3429, 3318, 2970, 1748, 1696, 1553 cm^{-1}; HRMS Calcd for $C_{28}H_{28}ClN_2O_5$ 507.1608, found 507.1686.

6-*tert*-Butyl-8-(3-(4-chlorophenyl)ureido)-2-phenylchroman-2-methyl ester (6). Zinc dust (20 g, 305.9 mmol) was added to a solution of the latest compound (2.9 g, 5.7 mmol) in AcOH (50 mL). The mixture was kept under stirring at 60°C for half an hour, following the progress of the reaction by NMR. When the reaction was complete, metallic Zn was filtered off and the liquid phase was added over a mixture of ice and water. Vacuum filtration provided a crude compound (2.5 g), which was then purified by chromatography over SiO_2 using CH_2Cl_2 as eluent. 1.7 g of the pure compound **6** (60% yield) were isolated. Mp 200-210 °C; ^{1}H NMR (200 MHz, $CDCl_3$) d (ppm) 1.26 (9H, s), 2.31–2.82 (4H, m), 3.69 (3H, s), 6.74 (1H, d, *J* = 2.1 Hz), 7.11–7.54 (10H, m), 7.67 (1H, s),7.89 (1H, d, J =2,14 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) d (ppm): 29.5 (CH_3), 31.1 (CH_2 + $3CH_3$), 34.0 (C), 52.9 (CH_2), 81.6 (C), 116.6 (CH), 119.8 (C), 120.4 (CH), 121.4 (2CH), 124.9 (2CH), 126.1 (C), 128.0 (C), 128.3 (CH), 128.5 (2CH), 128.6 (2CH), 136.9 (C), 138.1 (C), 140.3 (C), 143.9 (C), 153.2 (C), 172.0 (C); IR (nujol) 3325, 3234, 2975, 1748, 1670,1560 cm^{-1}; HRMS Calcd for $C_{28}H_{30}ClN_2O_4$ 493.1816, found 493.1890.

6-*tert*-Butyl-8-(3-(4-chlorophenyl)ureido)-2-phenyl-*N*-(pyridin-2-yl)chroman-2-carboxamide (receptor 1). In a round bottom flask, equipped with a magnetic stirrer and under argon atomosphere, aminopyridine (600mg) was dissolved in anhydrous THF (20 mL). A trace of bipyridyl was added and the reaction mixture was cooled to –70ºC. BuLi (2.5mL, 2 M in hexane) was slowly added to the previous cooled solution until bipyridyl change into red color. Compound **6** (500 mg, 1.02 mol) was then added and the reaction mixture was allowed to stand for 20 minutes at room temperature.

The reaction mixture was then poured over a 10% aqueous solution of formic acid (40 mL) and extracted with ethyl acetate (40 mL). The organic phase was washed with 4% aqueous sodium carbonate solution (20 mL) and dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography over SiO_2 using a 1:1 mixture of CH_2Cl_2 and EtOAc as eluent and 300 mg of the pure compound **1** (53% yield) were isolated. Mp 195-200 °C ^{1}H NMR (200 MHz, DMSO-d_6) d (ppm) 1.24 (9H, s), 2.31–2.86 (4H, m), 6.77 (1H, d, *J* = 1.8 Hz), 7.10 (1H, dt, *J* = 1.7, 5.4 Hz), 7.32–7.42 (6H, m), 7.63–7.72 (3H, m), 7.76 (1H, dt, *J* = 1.8, 8.0 Hz), 7.88 (1H, d, *J* =1.8 Hz), 8.05 (1H, d, *J* = 8.5 Hz), 8.33 (1H, d, *J* = 5.4 Hz), 8.72 (1H, s), 9.26 (1H, s), 10.19 (1H, s).; ^{13}C NMR (100 MHz, DMSO-d_6) d (ppm): 22.1 (CH_2), 29.5 (CH_2), 31.2 (3 CH_3), 33.9 (C), 82.4 (C), 114.3 (CH), 116.5 (CH), 120.2 (4CH), 120.7 (C), 125.3 (CH), 125.5 (C), 127.0 (C), 128,6 (7CH), 138.3 (CH), 138.8 (C), 139.6 (C), 140.3 (C), 143.0 (C), 148.0 (CH), 150.3 (C), 152.9 (C), 170.3 (C). IR (nujol) 3319, 2970, 1708, 1563, 1308 cm^{-1}; HRMS Calcd for $C_{32}H_{37}ClN_4O_3$ 555.2085, found 555.2155.

Conclusions

The racemic receptor **1** can be readily prepared from commercially available starting materials. It shows a suitable oxyanion hole observed in its X-ray structure and also presents a complementary surface with proline, due to H-bonds and cation-πinteractions, which allowed the formation of an apolar host-guest complex. Due to this preference for proline, this amino acid can be extracted exclusively from an aqueous solution in the presence of other natural amino acids. *L*-proline provided a way to resolve the receptor racemic mixture, since the diastereometic complexes which with *L*-proline present different stabilities. The structure of both diastereomeric complexes has been analysed by NMR, circular dichroism and modelling studies, showing steric hindrance as the main source for the receptor **1** chiral recognition.

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