Enantioselective amino acid recognition using acyclic thiourea receptors

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Received (in Cambridge, UK) 9th March 2001, Accepted 25th April 2001
First published as an Advance Article on the web 11th May 2001

A series of acyclic thiourea derivatives, designed to create a cleft with four hydrogen bond donors suitable for carboxylate recognition, have been prepared, and their ability to bind to *N*-protected amino acid carboxylate salts has been investigated. The crystal structure of one of the thioureas has been determined showing that it forms a hydrogen bonded centrosymmetric dimer in the solid-state, in a conformation appropriate for the desired binding of carboxylates. The thioureas show good discrimination between different amino acids and those thioureas incorporating chiral moieties show moderate enantioselectivity for a range of amino acid derivatives.

Introduction

Enantioselective recognition remains a major challenge for host–guest chemists.[1] The ability to discriminate between enantiomers, using synthetic receptors, would, for example, allow separation of racemates by selective transport across a membrane.[2] We, and others, have previously utilised thioureas as a binding site for the carboxylate functionality,[3] and incorporation of thioureas into macrocyclic structures has produced selective receptors for carboxylate derivatives such as amino acids.[4] Such receptors are, however, structurally rather complex and demanding to synthesise. In an effort to produce simpler enantioselective receptors for carboxylate derivatives we have now prepared a series of acyclic thioureas and have examined their ability to bind a range of amino acids.[5] In these novel receptors the thiourea functionality is linked to aryl amides to provide additional hydrogen bonds to the carboxylate moiety and it was anticipated that the formation of a well defined set of four hydrogen bonds around the carboxylate, binding to both the *syn* and *anti* lone pairs of the carboxylate,[6] should also serve to create a cleft which, when chiral building blocks are incorporated, might discriminate effectively between enantiomeric guests (Fig. 1). The use of pyridyl amides might further help to preorganise the chiral cleft since the desired U-shaped conformation may be stabilised by weak hydrogen bonding of the amide and thiourea hydrogens to the pyridyl nitrogen.[7] In order to test this hypothesis we prepared the achiral receptors **5** and **6**. We also prepared two chiral pyridyl receptors **7** and **15**, the former incorporating a primary amide, derived from phenylalanine, to provide further hydrogen bonding functionality, and the latter incorporating an electron deficient aromatic amide to provide aromatic interactions with guests with aromatic sidechains.

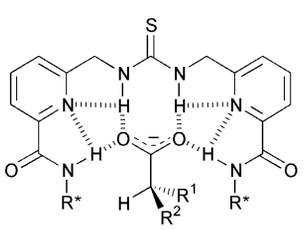
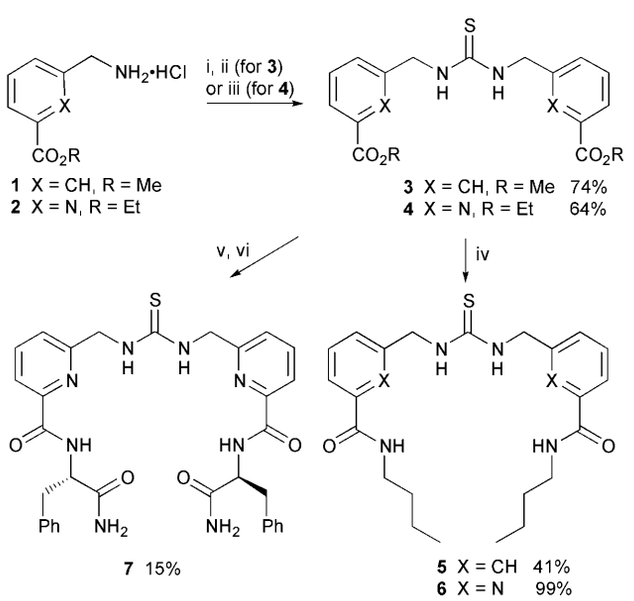


Fig. 1 Proposed binding of carboxylates by a bispyridylthiourea. R* represents a chiral group.

Results and discussion

The thiourea receptors **5**–**7** were readily prepared (Scheme 1)



Scheme 1 *Reagents and conditions*: (i) $CSCl_2$, K_2CO_3, H_2O, CH_2Cl_2; (ii) **1**, Et_3N; (iii) CS_2, DCC, DMAP; (iv) $nBuNH_2$; (v) LiOH, dioxane, H_2O; (vi) DPPA, (2*S*)-2-amino-3-phenylpropanamide.

by coupling of amino esters **1** or **2** to give the corresponding thiourea esters **3** and **4**, which could be converted directly to the butyl amides **5** and **6**, or, in the case of **4**, hydrolysed to the corresponding diacid, and coupled with (2*S*)-2-amino-3-phenylpropanamide to give **7**. Receptor **15** was prepared from the protected diamine **8**, derived from L-phenylalanine.[8] Treatment of **8** with hydrazine and coupling of the resulting amine with *p*-nitrobenzoyl chloride gave amide **9**. Removal of the Boc-protecting group, and coupling with acid chloride **12** gave **13**. Removal of the phthalimide group with hydrazine,

 DOI: 10.1039/b102298a

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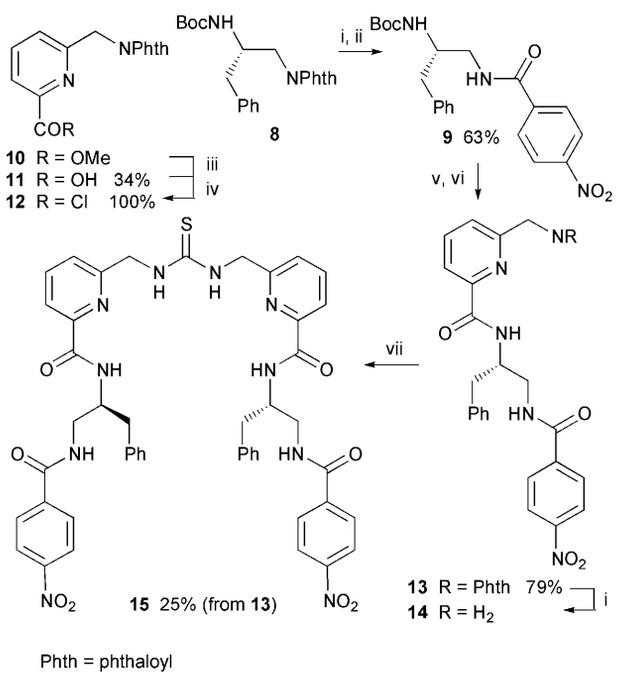
Table 1 Hydrogen bonds for thiourea **6** [Å and °]

D–H···A[a]	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠(DHA)
N(1)–H(1N)···O(2)#1	0.86(2)	2.24(2)	2.978(2)	144.3(18)
N(4)–HN4···O(2)#1	0.85(2)	2.09(2)	2.897(2)	157.5(19)
N(3)–HN3···O(2)#1	0.86(2)	2.34(2)	3.089(2)	145.9(19)

[a] Symmetry transformations used to generate equivalent atoms: #1 $-x+2, -y+1, -z+1$.

conversion of the resulting amine **14** to the isothiocyanate and coupling with a further equivalent of the amine, gave the desired thiourea **15** (Scheme 2).



Scheme 2 *Reagents and conditions*: (i) H_2NNH_2, EtOH; (ii) *p*-$NO_2C_6H_4COCl$, Et_3N, DMAP; (iii) TMSCl, NaI, CH_3CN; (iv) $SOCl_2$; (v) TFA, CH_2Cl_2; (vi) **12**, DMAP; (vii) CS_2, DCC, DMAP, then further addition of **14**.

We were able to obtain a crystal structure[9] of the achiral pyridyl receptor **6** which revealed that it forms a centrosymmetric dimer in the solid-state, with one amide carbonyl forming a triple hydrogen bond motif (Table 1) with both of the thiourea NH's and one amide NH of the other monomer unit (Fig. 2). The molecule adopts a folded conformation such that the three NH groups point inwards to an essentially planar cavity, into which the carbonyl of the second molecule can dock. Two of the pyridine rings experience intermolecular π–π stacking interactions with their centroids separated by 3.968 Å and the angle between the least squares planes being only 0.03°. Thus the crystal structure reveals, in part, the desired hydrogen bond arrangement required for carboxylate recognition depicted in Fig. 1. Dilution studies with NMR samples of all the thioureas, however, showed that the dimerisation observed in the solid state is not occurring to any significant extent in solution. Furthermore, in neat $CDCl_3$, the signals of the NH's for the pyridyl receptors **6** and **7** are considerably more downfield in comparison to those for receptor **5**, consistent with the notion that there are weak hydrogen bonds between the pyridyl nitrogen and the NH's in the former case (Fig. 1). Addition of 10% (by volume) CD_3SOCD_3 to these samples leads to significant downfield shifts for the signals for both the amide and thiourea NH's of receptor **5**, but only a small downfield shift for the amide NH of the pyridyl receptor **6**, and an upfield shift for the thiourea NH of this compound (Fig. 3).

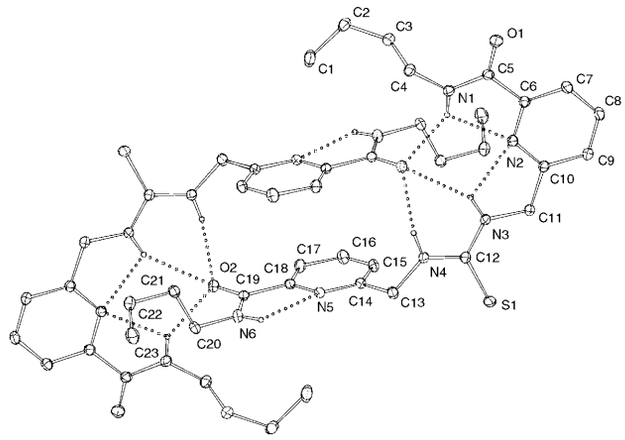


Fig. 2 Thermal ellipsoid plot (30% probability level) of **6** showing the hydrogen bonded centrosymmetric dimer.

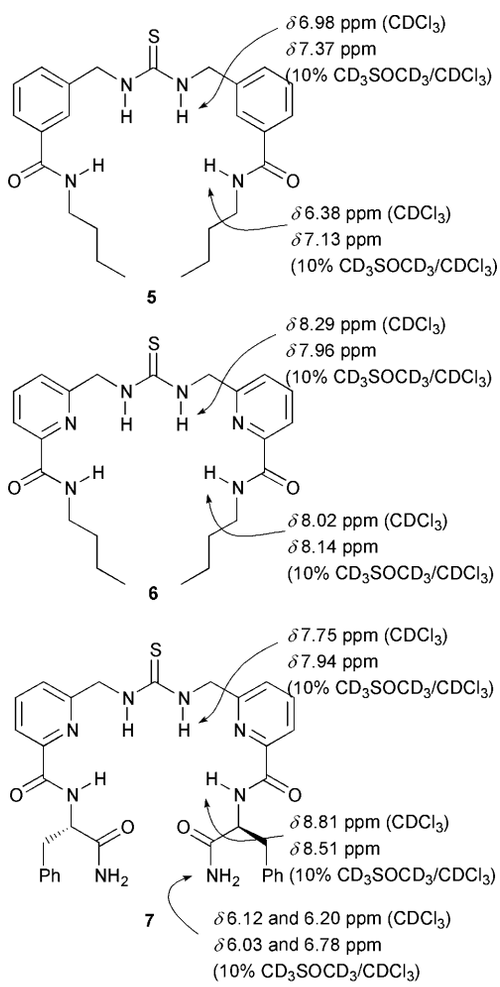


Fig. 3 Comparison of the NMR shifts for the NH signals of thioureas **5**–**7** in $CDCl_3$ and 10% CD_3SOCD_3–$CDCl_3$.

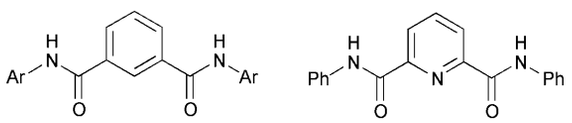
Similarly for the chiral pyridyl receptor **7**, addition of 10% (by volume) CD_3SOCD_3 to a sample in $CDCl_3$ gives just a small downfield shift for the thiourea NH signal and an upfield shift for the secondary amide NH signal, all of which is consistent with the notion that in the case of the pyridyl receptors **6** and **7** there is substantial intramolecular hydrogen bonding.

The benzo compound **5** proved to be rather insoluble in deuterochloroform and binding studies with this compound were carried out in 10% CD_3SOCD_3–$CDCl_3$ as solvent, whereas the pyridyl compounds were sufficiently soluble to allow studies also in neat $CDCl_3$. Initially we compared the

Table 2 Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1 : 1 complexes formed between thioureas **5** and **6** and tetrabutylammonium phenylacetate, in 10% CD_3SOCD_3–$CDCl_3$ at 20 °C

Receptor	Substrate	K_{ass}[a]/M^{-1}	$-\Delta G_{ass}$[b]/kJ mol^{-1}
5	$PhCH_2CO_2^-$ NBu_4^+	740	16.1
6	$PhCH_2CO_2^-$ NBu_4^+	420	14.7

[a] Errors were estimated as <10%. [b] Errors were estimated as <0.2 kJ mol^{-1}.



16 **17**

Fig. 4 Crabtree's anion receptors.

binding properties of the achiral receptors **5** and **6**. Using NMR titration experiments, monitoring the shift of both the NH and ArH signals of the receptors, we obtained binding constants for both **5** and **6** with the tetrabutylammonium salt of phenylacetic acid in 10% CD_3SOCD_3–$CDCl_3$ (Table 2).[10] For both receptors, binding with phenylacetic acid carboxylate leads to significant downfield shifts of the signals for both the urea NH (~2 ppm for **5**, >3 ppm for **6**) and amide NH (~1 ppm for **5**, ~0.5 ppm for **6**), indicating hydrogen bond formation, and smaller shifts (~0.2 ppm) of signals for the aromatic protons. The two binding constants are similar although binding with the pyridyl receptor **6** is slightly weaker. Thus the pyridyl receptor **6** may be more preorganised for binding as desired, but absolute binding of a carboxylate anion may be disfavoured by electrostatic repulsion between the anion and the pyridine nitrogen lone-pair—as suggested by Crabtree's studies on binding of various anions with related receptors **16** and **17** (Fig. 4).[11]

Binding studies with the chiral pyridyl receptor **7** and a range of amino acid derivatives, as the tetrabutylammonium salts, in neat $CDCl_3$, were carried out and the results are presented in Table 3. The receptor is strongly selective for different amino acids (*e.g.* >30 : 1 selectivity for *N*-Ac-L-Trp-CO_2^- over *N*-Ac-L-Ser-CO_2^-), but in general the receptor shows only moderate enantioselectivity with a general preference for L-amino acids and a highest enantioselectivity for *N*-Ac-Gln-CO_2^- (L : D ≈ 2 : 1). Binding constants were also highest for amino acids with sidechains incorporating hydrogen bonding functionality (*N*-Ac-Gln-CO_2^-) or an electron rich aromatic sidechain (*N*-Ac-Trp-CO_2^-). The corresponding *N*-Boc derivatives gave reduced binding constants, but gave better enantioselectivity in the case of *N*-Boc-Trp-CO_2^-. For all substrates tested, binding resulted in significant downfield shifts of the NH signals from the thiourea (1.6–2.1 ppm), the secondary amide (0.3–0.7 ppm) and for one of the primary amide protons (1.2–1.5 ppm), and an upfield shift (0.3–0.7 ppm) of the other primary amide proton. This suggests that binding of the amino acids involves hydrogen bonds to the thiourea, secondary amide and one of the primary amide NH's, but also involves breaking of an intramolecular hydrogen bond to the other primary amide NH. Conversely, binding of the tryptophan derivatives led to a significant upfield shift for the indole NH signal (>1 ppm) presumably reflecting the breaking of an intramolecular hydrogen bond between the indole NH and the carboxylate on binding, and similarly binding of the glutamine and asparagine derivatives led to upfield shifts for one of the primary amide NH's in each case.

As well as showing modest enantioselectivity in binding amino acid derivatives, the receptors can be used as chiral shift reagents. Thus, for example, we prepared a $CDCl_3$ solution of receptor **6** with the tetrabutylammonium salt of racemic *N*-Ac-Phe, which leads to two cleanly resolved signals for the $NCOCH_3$ group of the two resulting diastereomeric complexes at 1.86 and 1.88 ppm.

Table 3 Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1 : 1 complexes formed between thiourea **7** and tetrabutylammonium salts of various amino acid derivatives, in $CDCl_3$ at 20 °C

Entry	Substrate	K_{ass}[a]/M^{-1}	$-\Delta G_{ass}$[b]/kJ mol^{-1}
1	*N*-Ac-L-Ala-CO_2^- NBu_4^+	3450	19.8
2	*N*-Ac-D-Ala-CO_2^- NBu_4^+	2520	19.1
3	*N*-Ac-L-Phe-CO_2^- NBu_4^+	4770	20.6
4	*N*-Ac-D-Phe-CO_2^- NBu_4^+	2990	19.5
5	*N*-Ac-L-Asn-CO_2^- NBu_4^+	1690	18.1
6	*N*-Ac-D-Asn-CO_2^- NBu_4^+	800	16.3
7	*N*-Ac-L-Gln-CO_2^- NBu_4^+	9000	22.2
8	*N*-Ac-D-Gln-CO_2^- NBu_4^+	4520	20.5
9	*N*-Boc-L-Gln-CO_2^- NBu_4^+	1190	17.3
10	*N*-Boc-D-Gln-CO_2^- NBu_4^+	810	16.3
11	*N*-Ac-L-Ser-CO_2^- NBu_4^+	380	14.5
12	*N*-Ac-D-Ser-CO_2^- NBu_4^+	480	15.0
13	*N*-Ac-L-Trp-CO_2^- NBu_4^+	12400	23.0
14	*N*-Ac-D-Trp-CO_2^- NBu_4^+	14800	23.4
15	*N*-Boc-L-Trp-CO_2^- NBu_4^+	3140	19.6
16	*N*-Boc-D-Trp-CO_2^- NBu_4^+	2225	18.8

[a] Errors were estimated as <10%. [b] Errors were estimated as <0.2 kJ mol^{-1}.

Table 4 Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1 : 1 complexes formed between thiourea **15** and tetrabutylammonium salts of various amino acid derivatives, in $CDCl_3$ at 20 °C

Entry	Substrate	K_{ass}[a]/M^{-1}	$-\Delta G_{ass}$[b]/kJ mol^{-1}
1	*N*-Boc-L-Trp-CO_2^- NBu_4^+	1925	18.4
2	*N*-Boc-D-Trp-CO_2^- NBu_4^+	3785	20.1
3	(*R*)-Naproxen-CO_2^- NBu_4^+	1570	17.9
4	(*S*)-Naproxen-CO_2^- NBu_4^+	1870	18.4

[a] Errors were estimated as <10%. [b] Errors were estimated as <0.2 kJ mol^{-1}.

Thiourea **15** showed similar downfield shifts for the thiourea NH (~2 ppm), pyridyl amide NH (~0.5 ppm) and nitrobenzamide NH (~1.1 ppm) on titration with the enantiomers of *N*-Boc-Trp, and the receptor now showed a modest selectivity for the D-enantiomer (Table 4). A probable contribution to the binding from aromatic interactions is evidenced by downfield shifts (~0.2 ppm) of the signals for both aromatic protons from the *p*-nitrobenzamide on complexation. The receptor also bound the enantiomers of naproxen (as the tetrabutylammonium salts), with associated shifts (~0.2 ppm) for the signals for the protons from the *p*-nitrobenzamide, but was unable to discriminate effectively between the enantiomers.

In summary we have prepared a series of simple acyclic thiourea receptors which are both sidechain selective and moderately enantioselective for a range of amino acid derivatives. Despite the modest enantioselectivity observed the receptors are simple to prepare, and evidence from the NMR binding studies does suggest that the tweezer receptors do indeed wrap around the substrates as desired using a combination of several hydrogen bonding interactions. These results indicate that more selective receptors may result from the introduction of suitable functionality for more specific interactions with the sidechain functionality of the amino acid guests.

Experimental

General procedures

All reactions requiring anhydrous conditions were conducted in flame dried glassware under a static, inert atmosphere unless otherwise stated. CH_2Cl_2 was distilled from calcium hydride, and petrol was distilled and the fraction boiling between 40 and 60 °C was used. All other solvents were of commercial grade and were used without further purification. Thin layer chromatography was performed on plastic backed sheets (Camlab) coated with silica gel (SiO_2: 0.25 mm). Flash column chromatography was performed on Sorbil C_{60}, 40–60 mesh silica.

Infra-red spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. Proton NMR spectra were obtained at 270 MHz on a JEOL GX 270, at 300 MHz on a Bruker AC 300, and at 360 MHz on a Bruker Aspect 3000 spectrometer. Spectra were referenced with respect to the residual solvent peak for the deuterated solvent concerned. ^{13}C NMR spectra were obtained at 75 MHz on a Bruker AC 300 and at 100 MHz on a Bruker DPX 400. COSY spectra and ^1H–^{13}C correlation spectra were performed on a Bruker AC 300. Mass spectra were obtained on a VG analytical 70-250-SE normal geometry double focusing mass spectrometer or on a Micromass Platform quadrupole mass analyser with an electrospray ion source.

Methyl 3-{[({[3-(methoxycarbonyl)benzyl]amino}thiocarbonyl)-amino]methyl}benzoate 3

Based on a procedure originally reported by Anslyn,[12] a suspension of amine hydrochloride **1** (0.84 g, 4.17 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a vigorously stirred mixture of potassium carbonate (1.14 g, 8.34 mmol) in water (45 mL) and thiophosgene (0.73 mL, 9.52 mmol) in CH_2Cl_2 (45 mL). The mixture was stirred at rt for 1 hour then heated at reflux for 12 hours. After cooling to rt the organic layer was separated, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give crude isothiocyanate as an orange oil which was used in the next step without further purification. Amine **1** (0.65 g, 3.24 mmol) was added to a stirred solution of the isothiocyanate (0.67 g, 3.31 mmol) and triethylamine (1.32 mL, 9.72 mmol) in CH_2Cl_2 (30 mL). The mixture was heated at reflux for 12 hours and after allowing to cool to rt was washed with 2.0 M HCl (3 × 20 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give thiourea **3** as an orange viscous oil (1.15 g, 74%): R_f 0.47 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 3.83 (6H, s, CH_3), 4.70 (4H, d, *J* 5, $ArCH_2$), 6.74 (2H, br s, NH), 7.32 (2H, t, *J* 8, ArH), 7.45 (2H, d, *J* 8, ArH), 7.83 (2H, s, ArH), 7.84 (2H, d, *J* 8, ArH); δ_C (75.5 MHz, $CDCl_3$) 48.2, 52.4, 128.7, 129.0, 129.1, 130.6, 132.4, 137.9, 167.1, 182.9; *m*/*z* (ES^+) 373.5 $(M + H)^+$, 395.5 $(M - Na)^+$ HRMS: found 372.1223. $C_{19}H_{21}N_2O_4S$ $(M + H)^+$ requires 373.1222.

N-1-Butyl-3-({[({3-[(butylamino)carbonyl]benzyl}amino)-thiocarbonyl]amino}methyl)benzamide 5

Diester **3** (50 mg, 0.13 mmol) was dissolved in butylamine (5 mL) and heated at reflux for 12 hours. After cooling to rt the excess butylamine was removed *in vacuo* to yield a white solid which was purified by crystallisation (ethyl acetate–methanol) to give thiourea **5** as colourless crystals (25 mg, 41%): mp 88–90 °C (ethyl acetate–methanol); R_f 0.47 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 0.94 (6H, t, *J* 7, CH_3), 1.37 (4H, sextet, *J* 7, $CH_2CH_2CH_3$), 1.55 (4H, quintet, *J* 7, CH_2), 3.32 (4H, q, *J* 7, CH_2NHCO), 4.7 (4H, d, *J* 5, CH_2NHCS), 6.38 (2H, br s, NHCS), 6.98 (2H, br s, NHCO), 7.32 (2H, d, *J* 8, ArH), 7.40 (2H, d, *J* 8, ArH), 7.53 (2H, d, *J* 8, ArH), 7.54 (2H, s, ArH); δ_C {100 MHz, $[(CD_3)_2SO$–$CDCl_3$ 10 : 90 v/v]} 11.5, 18.1, 29.4, 37.8, 45.8, 124.0, 124.1, 126.7, 128.5, 133.0, 136.7, 166.8, 182.4; ν_{max}/cm^{-1} (neat) 3285, 3225, 3060, 2960, 2930, 2865, 1635, 1585 and 1535; *m*/*z* (ES^+) 455.4 $(M + H)^+$ (Found: C, 63.98; H, 7.59; N, 11.74. $C_{25}H_{34}N_4O_2S.MeOH$ requires C, 64.17; H, 7.87; N, 11.51%).

Ethyl 6-({[({[6-(ethoxycarbonyl)-2-pyridyl]methyl}amino)thio-carbonyl]amino}methyl)pyridine-2-carboxylate 4

Based on a procedure originally reported by Anslyn,[12] DMAP (0.57 g, 4.70 mmol) was added to a suspension of amine hydrochloride salt **2** (0.41 g, 1.88 mmol), DCC (0.19 g, 0.94 mmol) and carbon disulfide (0.40 mL, 6.58 mmol) in dry $CHCl_3$ (10 mL) at −10 °C. The solution was stirred at rt for 1 hour then heated at reflux for 12 hours. The solvent was removed *in vacuo* and the resultant orange oil was purified by flash column chromatography, eluting with ethyl acetate–petroleum ether (70 : 30 to 100 : 0 v/v) to give thiourea **4** as a yellow solid (0.25 g, 64%): mp 103–104 °C (ethyl acetate–petroleum ether); R_f 0.44 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 1.45 (6H, t, *J* 7, CH_3), 4.49 (4H, q, *J* 7, CH_2CH_3), 5.02 (4H, br s, CH_2N), 7.50 (2H, d, *J* 8, pyrH), 7.83 (2H, t, *J* 8, pyrH), 8.02 (2H, d, *J* 8, pyrH), 8.56 (2H, br s, NH); δ_C (75.5 MHz, $CDCl_3$) 14.4, 49.5, 62.2, 124.0, 125.7, 138.0, 147.2, 157.4, 165.1, 183.3; ν_{max}/cm^{-1} (neat) 3545, 3300, 1705, and 1530; *m*/*z* (ES^+) 403.0 $(M + H)^+$, 424.9 $(M + Na)^+$ (Found: C, 56.69; H, 5.68; N, 13.63. $C_{19}H_{22}N_4O_4S$ requires C, 56.70; H, 5.51; N, 13.92%).

N-1-Butyl-6-[({[({6-[(butylamino)carbonyl]-2-pyridyl}methyl)-amino]thiocarbonyl}amino)methyl]pyridine-2-carboxamide 6

Diester **4** (26 mg, 65 μmol) was dissolved in butylamine (4 mL) and the solution was heated at reflux for 12 hours. The excess butylamine was removed *in vacuo* and the resultant yellow solid was recrystallised from ethyl acetate–petroleum ether to give thiourea **6** as colourless crystals (32 mg, 100%): mp 95–96 °C (ethyl acetate–petroleum ether); R_f 0.31 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 0.80 (6H, t, *J* 7, CH_3), 1.21 (4H, sextet, *J* 7, CH_2CH_3), 1.44 (4H, quintet, *J* 7, $CH_2CH_2CH_3$), 3.26 (4H, q, *J* 7, CH_2CH_2N), 4.98 (4H, s, $CH_2NHC{=}S$), 7.48 (2H, d, *J* 8, pyrH), 7.77 (2H, t, *J* 8, pyrH), 7.96 (2H, d, *J* 8, pyrH), 8.02 (2H, br s, NH), 8.29 (2H, br s, NH); δ_C (75.5 MHz, $CDCl_3$) 13.8, 20.3, 31.8, 39.6, 49.5, 121.3, 125.2, 138.8, 149.0, 155.9, 164.1; ν_{max}/cm^{-1} (neat) 3360, 3300, 2950, 2860, 2360, 1670, 1640, 1570 and 1530; *m*/*z* (ES^+) 457.4 $(M + H)^+$, 479.5 $(M + Na)^+$; HRMS (FAB): found 457.2384. $C_{23}H_{33}N_6O_2S$ requires 457.2386. An X-ray crystal structure was obtained (see text).

N-[(1*S*)-2-Amino-1-benzyl-2-oxoethyl]-6-({[({[6-({[(1*S*)-2-amino-1-benzyl-2-oxoethyl]amino}carbonyl)-2-pyridyl]methyl}-amino)thiocarbonyl]amino}methyl)pyridine-2-carboxamide 7

LiOH (20.1 mL of a 1 M soln, 20.1 mmol) was added to diester **4** (0.81 g, 2.01 mmol) in 1,4-dioxane (23 mL) and the mixture was stirred for 1 hour. $KHSO_4$ (20.1 mL of a 1 M soln, 20.1 mmol) was added and the water was removed *in vacuo* and by azeotroping with acetonitrile (20 mL). Methanol (5 mL) was added to the resultant solid, the insoluble material was removed by filtration and the excess methanol was removed *in vacuo* to yield the diacid as a pale yellow solid (0.70 g, 100%) which was used in the next reaction without further purification. Diphenylphosphoryl azide (DPPA) (0.42 mL, 1.92 mmol) followed by triethylamine (0.53 mL, 3.83 mmol) were added to a stirred mixture of the diacid (0.30 g, 0.87 mmol) and (2*S*)-2-amino-3-phenylpropanamide (0.31 g, 1.92 mmol) in DMF (0.5 mL) at 0 °C. The mixture was stirred at 0 °C for 2 hours and at rt for 2 days. The solvent was removed *in vacuo* and the crude oil was suspended in CH_2Cl_2 (15 mL), washed with water (15 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give an oil which was purified by flash column chromatography eluting with methanol–CH_2Cl_2 (1 : 99 to 10 : 90 v/v) to give thiourea **7** as a white solid (84 mg, 15%): mp 126–128 °C (ethanol–water);

R_f 0.43 [methanol–CH_2Cl_2 (10 : 90 v/v)]; $[\alpha]_D^{20}$ −36.0° (*c* 1 in CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 3.12 (2H, dd, *J* 7, 14, CH_A-H_BPh), 3.17 (2H, dd, *J* 7, 14, CH_AH_BPh), 4.87 (2H, apparent q, *J* 7, CONHC*H*), 4.99 (4H, m, CSNHCH_2), 6.34 (2H, br s, NH_AH_B), 6.47 (2H, br s, NH_AH_B), 7.15–7.25 (10H, m, ArH), 7.43 (2H, d, *J* 8, ArH), 7.74 (2H, t, *J* 8, pyrH), 7.91 (2H, d, *J* 8, pyrH), 8.00 (2H, br s, CSNH), 8.80 (2H, d, *J* 8, CON*H*CH); δ_C (75.5 MHz, $CDCl_3$) 39.4, 50.0, 55.6, 121.5, 125.9, 127.8, 129.4, 130.2, 130.4, 138.0, 139.3, 149.6, 158.6, 165.8 and 175.69; ν_{max}/cm^{-1} 2395, 2360, 1660, 1650 and 1515; *m*/*z* (ES^+) 639.6 $(M + H)^+$, 661.6 $(M + Na)^+$, 677.5 $(M + K)^+$; HRMS (FAB): found 661.2311. $C_{33}H_{34}N_8O_4NaS$ requires 661.2321.

6-[(1,3-Dioxo-2,3-dihydro-1*H*-isoindol-2-yl)methyl]pyridine-2-carboxylic acid 11

Based on a procedure originally reported by Olah,[13] chlorotrimethylsilane (16.6 mL, 0.13 mol) was added to a solution of ester **10**[14] (10.1 g, 32.7 mmol) and sodium iodide (19.6 g, 0.13 mol) in acetonitrile (50 mL) at 0 °C. The reaction mixture was allowed to warm to rt and heated at reflux for 4 days. After allowing to cool to rt, water (200 mL) was added, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed successively with water (100 mL) followed by aqueous sodium thiosulfate (150 mL). The organic phase was extracted with saturated sodium hydrogen carbonate (50 mL) and the aqueous phase acidified to pH 3 using 2.0 M HCl. The acidic solution was extracted with methanol–CH_2Cl_2 [3 × 150 mL (5 : 95 v/v)], dried ($MgSO_4$) and the solvent removed *in vacuo* to give acid **11** as a white solid (3.12 g, 34%): mp 219–221 °C (from methanol–$CHCl_3$); R_f 0.21 [methanol–CH_2Cl_2 (10 : 90 v/v)]; δ_H {400 MHz, [$(CD_3)_2SO$–$CDCl_3$ 10 : 90 v/v]} 5.03 (2H, s, PhthNCH_2), 7.33 (1H, dd, *J* 1, 8, pyrH), 7.73 (2H, dd, *J* 3, 5, ArH), 7.77 (1H, t, *J* 8, pyrH), 7.82 (2H, dd, *J* 3, 5, ArH), 7.96 (1H, dd, *J* 1, 8, pyrH); δ_C (100 MHz, CD_3OD) 42.5, 123.1, 123.2, 123.7, 131.5, 134.0, 137.8, 147.6, 155.5, 165.7, 167.5; ν_{max}/cm^{-1} (neat) 3360, 1750, 1705, 1600 and 1420 (Found: C, 64.07; H, 3.55; N, 9.95. $C_{15}H_{10}N_2O_4$ requires C, 63.83; H, 3.57; N, 9.92%).

tert-Butyl-*N*-{(1*S*)-1-benzyl-2-[(4-nitrobenzoyl)amino]ethyl}-carbamate 9

Hydrazine hydrate (90 μL, 1.88 mmol) was added to a solution of phthalimide **8**[8] (0.48 g, 1.26 mmol) in ethanol (25 mL) and the mixture heated at reflux for 6 hours. After cooling to rt the insoluble material was removed by filtration and the excess solvent removed *in vacuo* to give the amine as a pale yellow solid, which was suspended in dry CH_2Cl_2 (20 mL). 4-Nitrobenzoyl chloride (0.52 g, 2.82 mmol) followed by triethylamine (0.39 mL, 2.82 mmol) and DMAP (50 mg, 0.41 mmol) were added. After stirring at rt for 12 hours solvent was removed *in vacuo* and the crude material was purified by flash column chromatography eluting with ethyl acetate–petroleum ether (30 : 70 v/v) to give amide **9** as a white solid (0.31 g, 63%): mp 184–186 °C (ethyl acetate); R_f 0.5 [ethyl acetate–petroleum ether (30 : 70 v/v)]; δ_H {300 MHz, [$(CD_3)_2SO$–$CDCl_3$ 10 : 90 v/v]} 1.29 (9H, s, $C(CH_3)_3$), 2.69 (1H, dd, *J* 9, 14, CH_AH$_B$Ph), 2.80 (1H, dd, *J* 6, 14, CH_AH_BPh), 3.38–3.33 (2H, m, CH_2N), 3.93 (1H, dt, *J* 6, 9, C*H*CH_2), 6.81 (1H, d, *J* 9, N*H*CH), 7.31–7.16 (5H, m, ArH), 8.07 (2H, d, *J* 9, ArH), 8.32 (2H, d, *J* 9, ArH), 8.80 (1H, t, *J* 5, N*H*CH_2); δ_C (100 MHz, DMSO-d_6) 26.5, 36.1, 42.0, 49.7, 75.8, 121.7, 124.2, 126.3, 127.1, 127.4, 137.2, 138.6, 147.2, 153.6, 163.2; ν_{max}/cm^{-1} (neat) 3350, 1690, 1644, 1550 and 1525; *m*/*z* (ES^+) 400.4 $(M + H)^+$ (Found: C, 63.28; H, 6.62; N, 10.44. $C_{21}H_{25}N_3O_5$ requires C, 63.15; H, 6.31; N, 10.52%).

N-{(1*S*)-1-Benzyl-2-[(4-nitrobenzoyl)amino]ethyl}-6-[(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)methyl]pyridine-2-carboxamide 13

TFA–CH_2Cl_2 [5 mL (50 : 50 v/v)] was added to a solution of protected amine **9** (0.21 g, 0.53 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the mixture was stirred for 12 hours at rt. The solvent was removed *in vacuo* and the resulting oil was triturated with ether (2 × 10 mL) to give the amine TFA salt as a pale yellow oil. Acid **11** (0.19 g, 0.68 mmol) was heated at reflux in thionyl chloride (2 mL) for 8 hours then cooled and stirred at rt for 12 hours. The excess thionyl chloride was removed *in vacuo* to give acid chloride **12** as a white solid, which was dissolved in dry DMF (5 mL) and added dropwise to a solution of amine TFA salt and DMAP (70 mg, 0.57 mmol) in DMF and the mixture was stirred at rt for 2 days. Solvent was removed *in vacuo* and the brown residue was purified by filtration through a pad of silica gel, eluting with ethyl acetate, to give amide **13** as a white solid (0.23 g, 79%): R_f 0.59 (ethyl acetate); mp 174–176 °C ($CHCl_3$–hexane); $[\alpha]_D^{20}$ −11.3° (*c* 1 in CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 8, 15, CH_AH$_B$Ph), 2.82 (1H, dd, *J* 7, 15, CH_AH_BPh), 3.38 (1H, ddd, *J* 3, 4, 14, NHCH_ACH$_B$), 3.63 (1H, ddd, *J* 5, 10, 14, NHCH_ACH_B), 4.37 (1H, m, NHC*H*CH_2), 4.97 (2H, s, CH2Phth), 7.11–7.25 (5H, m, ArH), 7.40 (2H, d, *J* 8, pyrH), 7.72 (2H, dd, *J* 3, 5, ArH), 7.76 (1H, t, *J* 8, pyrH), 7.84 (2H, dd, *J* 3, 5, ArH), 7.86–7.90 (3H, m, ArH and N*H*CH_2CH), 7.96 (1H, d, *J* 8, pyrH), 8.12 (1H, d, *J* 8, N*H*CHCH_2), 8.17 (2H, d, *J* 9, ArH); δ_C (100 MHz, $CDCl_3$) 39.3, 43.0, 47.3, 51.5, 121.6, 124.0, 124.1, 125.3, 127.5, 128.7, 129.5, 129.3, 132.4, 134.9, 136.9, 139.0, 140.1, 149.0, 149.9, 154.7, 165.7, 166.1, 168.4; ν_{max}/cm^{-1} (neat) 3350, 2360, 1685, 1644, 1600 and 1550; *m*/*z* (ES^+) 564.3$(M + H)^+$. HRMS: found 564.1879. $C_{31}H_{26}N_5O_6$ $(M + H)^+$ requires 564.1883.

N-2-{(1*S*)-1-Benzyl-2-[(4-nitrobenzoyl)amino]ethyl}-6-[({6-[({(1*S*)-1-benzyl-2-[(4-nitrobenzoyl)amino]ethyl}amino)-carbonyl]-2-pyridyl}methyl)amino]thiocarbonyl}amino)-methyl]-2-pyridinecarboxamide 15

Hydrazine hydrate (17 μL, 0.35 mmol) was added to a solution of phthalimide **13** (0.20 g, 0.35 mmol) in ethanol (5 mL) and the mixture heated at reflux for 12 hours. The insoluble material was removed by filtration and the filtrate concentrated *in vacuo* to give amine **14** as a yellow residue which was used in the next step without further purification. Carbon disulfide (74 μL, 1.21 mmol) was added to a solution of the amine (75 mg, 1.73 μmol), DCC (36 mg, 174 μmol) and DMAP (43 mg, 0.35 mmol) in dry CH_2Cl_2 (2 mL) at −10 °C and the mixture was stirred for 1 hour. After warming to rt the excess solvent and carbon disulfide were removed *in vacuo*. The resultant orange oil was dissolved in dry CH_2Cl_2 (2 mL) and further amine **14** (75 mg, 1.73 μmol) was added. After stirring at rt for 5 days the solvent was removed *in vacuo* and the brown residue was purified by flash column chromatography eluting with methanol–CH_2Cl_2 (1 : 99 to 10 : 90 v/v) to give thiourea **15** as a pale yellow solid (60 mg, 25%): R_f 0.59 (ethyl acetate); $[\alpha]_D^{20}$ + 36.0° (*c* 2 in CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 2.90 (1H, dd, *J* 8, 14, CH_AH$_B$Ph), 3.22 (1H, dd, *J* 6, 14, CH_AH_BPh), 3.58 (1H, dd, *J* 3, 14, CH_AH$_B$NH), 3.80 (1H, dd, *J* 10, 14, CH_AH_B-NH), 4.51 (2H, m, $CHCH_2$Ph), 5.02 (2H, d, *J* 17, CH_AH$_B$N-HCS), 5.10 (2H, d, *J* 17, CH_AH_BNHCS), 7.22–7.35 (10H, m, ArH), 7.56 (2H, d, *J* 8, pyrH), 7.73 (2H, br s, CH_2N*H*CO), 7.83 (4H, d, *J* 9, ArH), 7.89 (2H, t, *J* 8, pyrH), 8.07 (2H, d, *J* 8, pyrH), 8.11 (4H, d, *J* 8.5, ArH), 8.44 (2H, br m, NHCS), 8.89 (2H, d, *J* 5, N*H*CH); δ_C (100 MHz, $CDCl_3$) 37.3, 42.7, 47.2, 51.0, 120.0, 122.3, 123.9, 125.5, 126.7, 127.4, 127.7, 135.5, 137.7, 146.4, 148.1, 154.1, 162.6, 165.9, 182.3; ν_{max}/cm^{-1} (neat) 3285, 1650, 1595 and 1518; *m*/*z* (ES^+) 910.4 $(M + H)^+$ (Found: C, 62.38; H, 5.10; N, 15.46. $C_{47}H_{44}N_{10}O_8S$·MeOH requires C, 62.10; H, 4.88; N, 15.41%).

Acknowledgements

We thank the EPSRC for a studentship (GK) and the EC (TMR Network Grant "Enantioselective Separations" ERB FMRX-CT-98-0233) for financial support.

References

1 For a recent review on synthetic receptors, see: J. H. Hartley, T. D. James and C. J. Ward, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3155.

2 W. H. Pirkle and W. E. Bowen, *Tetrahedron: Asymmetry*, 1994, **5**, 773; J. T. F. Keurentjes, L. J. W. M. Nabuurs and E. A. Vegter, *J. Membrane Sci.*, 1996, **113**, 351.

3 For original use of thioureas for carboxylate recognition, see: E. Fan, S. A. Van Arman, S. Kincaid and A. D. Hamilton, *J. Am. Chem. Soc.*, 1993, **115**, 369; P. J. Smith, M. V. Reddington and C. S. Wilcox, *Tetrahedron Lett.*, 1992, **33**, 6085.

4 G. J. Pernia, J. D. Kilburn, J. W. Essex, R. J. Mortishire-Smith and M. Rowley, *J. Am. Chem. Soc.*, 1996, **118**, 10220.

5 Enantioselective recognition of amino acids: A. P. Davis and L. J. Lawless, *Chem. Commun.*, 1999, 9 and refs therein.

6 For related receptors, designed to bind to both the *syn* and *anti* lone pairs of a carboxylate, see: B. C. Hamann, N. R. Branda and J. Rebek Jr., *Tetrahedron Lett.*, 1993, **34**, 6837; J. S. Albert and A. D. Hamilton, *Tetrahedron Lett.*, 1993, **34**, 6483; C. Raposo, M. Crego, L. Mussons, C. Caballero and J. R. Moran, *Tetrahedron Lett.*, 1994, **35**, 3409; S. Nishizawa, P. Buhlmann, M. Iwao and Y. Umezawa, *Tetrahedron Lett.*, 1995, **36**, 6483.

7 The conformational preferences of pyridine-2,6-dicarboxamides and isophthalamides have been discussed in detail: C. A. Hunter and D. H. Purvis, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 792.

8 K. Burgess, J. Ibarzo and D. S. Linthicum, *J. Am. Chem. Soc.*, 1997, **119**, 1556.

9 Data were collected on a Nonius Kappa CCD area detector diffractometer with a rotating anode following standard procedures. Crystal data for **6**: $C_{23}H_{32}N_6O_2S$, $M_r = 456.61$, $T = 150(2)$ K, triclinic, space group $P\bar{1}$, $a = 9.4566(3)$, $b = 10.7149(4)$, $c = 13.2326(4)$ Å, $\alpha = 89.975(2)$, $\beta = 79.123(2)$, $\gamma = 65.1932(12)°$, $V = 1190.56(7)$ Å^3, $\rho_{calc} = 1.274$ g cm^{-3}, $\mu = 0.168$ mm^{-1}, $Z = 2$, reflections collected: 18090, independent reflections: 4737 ($R_{int} = 0.043$), final R indices [$I > 2\sigma I$]: $R_1 = 0.0429$, $wR_2 = 0.0976$, R indices (all data): $R_1 = 0.0529$. $wR_2 = 0.1023$. CCDC reference number 160088. See http://www.rsc.org/suppdata/p1/b1/b102298a/ for crystallographic files in .cif or other electronic format.

10 The binding constants were calculated by fitting the data to a 1 : 1 binding isotherm using *NMRTit HG* software, kindly provided by Professor C. A. Hunter, University of Sheffield. See: A. P. Bisson, C. A. Hunter, J. C. Morales and K. Young, *Chem. Eur. J.*, 1998, **4**, 845.

11 K. Kavallieratos, C. M. Bertao and R. H. Crabtree, *J. Org. Chem.*, 1999, **64**, 1675.

12 D. M. Kneeland, K. Ariga, V. M. Lynch, C.-Y. Huang and E. V. Anslyn, *J. Am. Chem. Soc.*, 1993, **115**, 10042.

13 G. A. Olah, S. C. Narang, B. Gupta and R. Malhotra, *J. Org. Chem.*, 1979, **44**, 1247.

14 P. Scrimin, U. Tonellato, D. Milani and R. Fornasier, *J. Chem. Soc., Perkin Trans. 2*, 1986, 233.