University of Southampton

Rationally Designed Receptors for Carboxylates

By

Richard J. Fitzmaurice

Doctor of Philosophy

Faculty of Engineering, Science and Mathematics

School of Chemistry

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ABSTRACT

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This thesis is principally concerned with the synthesis of a range of thiourea and guanidinium based receptors and their binding properties with carboxylates. Chapter 1 provides an introduction to the thesis and discusses, in the main part, the binding of monocarboxylates by synthetic receptors. Chapter 2 describes a range of thioureas and investigates in detail their binding with acetate *via* a range of techniques. A discussion of the enthalpic and entropic elements of their binding and the effect of preorganisation *via* intramolecular hydrogen bonding is also included. The addition of two amide-carboxylate hydrogen bonds results in a 50 fold increase in complex stability over 1,3-dimethylthiourea ($\Delta\Delta G \approx 9$ kJ mol^{-1}). Chapter 3 describes a family of guanidinium based receptors, analogous to the thiourea discussed in chapter 2. The binding of acetate in DMSO-d_6 is presented and evidence for significant increase in complex strength on preorganisation of the host *via* intramolecular hydrogen bonding to a pyridine nitrogen lone pair is reported ($\Delta\Delta G \approx 3.5$ kJ mol^{-1}). Chapter 4 describes work towards a rationally designed tweezer receptor based on the simple architectures described in chapter 3. Unfortunately, insolubility of the tweezers prevented evaluation of their binding properties with tripeptide guests.

For Gran,

Only seven and a half years in the end.

Contents

Preface

The research contained within this thesis was carried out under the supervision of Prof. J. D. Kilburn at the University of Southampton between October 2000 and December 2003. No part of this thesis has previously been submitted at this or any other University.

Acknowledgements

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Thanks all members of the Kilburn group past and present who have made the times in the lab more fun and for good quality regular tea breaks. Particular thanks must go to 'Fire' for getting me interested despite being half asleep most of the time, JBR&TWOS for entertainment, moderate views and all round safe driving, Dr Pament for high quality spelling and Sandra for making the lab nice and noisy even from some distance away.

In addition, I would like to thank Neil for short Fridays and his literally millennia of PhD experience, Weeman for being the general kicking post, Mr B. for an all too limited choice of weekend activities and Dave for putting up with me in the flat for three long years, particular sorry for the whinging.

Special thanks go to Mum, Dad, Gran and all the rest of the family for their help and support over my whole time in Southampton.

Finally I must thank Carole for making the last year and a bit of the PhD as good as it could possibly be.

Abbreviations

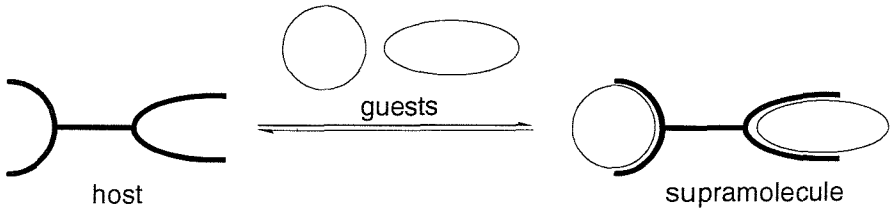
Ac	acetyl
Ala	alanine
Ar	aryl
Asn	asparagine
Boc	*tert*-butyloxycarbonyl
br	broad
Cbz	benzoyloxycarbonyl
CPK	Corey-Pauling-Kolten
d	doublet
DIPEA	N-ethyldiisopropylamine
DMAP	4-(N,N' dimethylamino)pyridine
DMF	N,N'-dimethylformamide
DMSO	dimethylsulphoxide
EDC	N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride
ES	electrospray
Et	ethyl
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanine monophosphate
His	histidine
IR	infrared
ITC	isothermal titration calorimetry
Leu	leucine
Lys	lysine
m	multiplet
Me	methyl
MP	melting point
Ms	methanesulfonyl
Ph	phenyl
Phe	Phenylalanine

pyr	pyridyl
q	quartet
quin	quintet
s	singlet
sat.	saturated
Ser	serine
t	triplet
TBA	tetrabutylammonium
^{t}Bu	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TPA	tripropylammonium
Trp	tryptophan
Trt	trityl
Tyr	tyrosine
Val	valine

1 Introduction

1.1 Supramoleuclar Chemistry

The phrase 'supramolecular chemistry' was coined by Lehn[1] in 1978 as he, Cram[2] and Pedersen[3] pioneered the field with work principally concerning the binding of small cationic metal centres with various polyethers. In essence the supramolecular chemist utilizes weak intermolecular forces to construct a supramolecule much as a synthetic chemist would use covalent bonds to construct a molecule (see Scheme 1). The subtle control of these weak intramolecular forces to provide effective, diverse and selective supramolecules, often discriminating between guests with only small changes in structure, is the main challenge in the field.



Scheme 1. Supramolecular chemistry.

The formation of a supramolecule relies on the combination and careful control of electrostatic forces, hydrogen bonding, π-π stacking, inductive forces and solvent effects.[4, 5] Generally, the strength of electrostatic interactions, up to 400 $kJmol^{-1}$, and the directionality of hydrogen bonding results in these being the most widely exploited interactions in synthetic systems.

Since its conception, the supramolecular chemistry has received much attention across a wide range of disciplines from biological to physical chemistry. Biology often supplies inspiration for the supramolecular chemist in terms of selectivity and diversity of enzymes; the high substrate specificity observed in natural systems is the pinnacle of supramolecular science. Biological systems also provide a large number of targets for the supramolecular chemist to complex with their synthetic host to form a supramolecule. The binding of biologically relevant species, such as metals, halides, carbohydrates, nucleotides and peptides, has received a great deal of

attention in the literature.[6-12] One such example by Hamilton[13] is ammonium **1** which binds a carboxylate anion using a combination of amide and ammonium binding functionality to create a mimic for the vancomycin carboxylate binding pocket **2**.[14] Pieters has also reported that the series of structurally related receptors bind Ac-D-Ala in $CDCl_3$.[15-18] Ungaro's 1,4,7-triazaheptane based receptor **3** displays the varied approaches often observed in supramolecular chemistry.[19] Whereas Hamilton's receptor attempts to closely mimic the vancomycin mode of binding, Ungaro's receptor **3** incorporates key elements, amines and aromatic surfaces, into a novel motif that showed *in vitro* antibacterial activity *cf.* vancomycin.

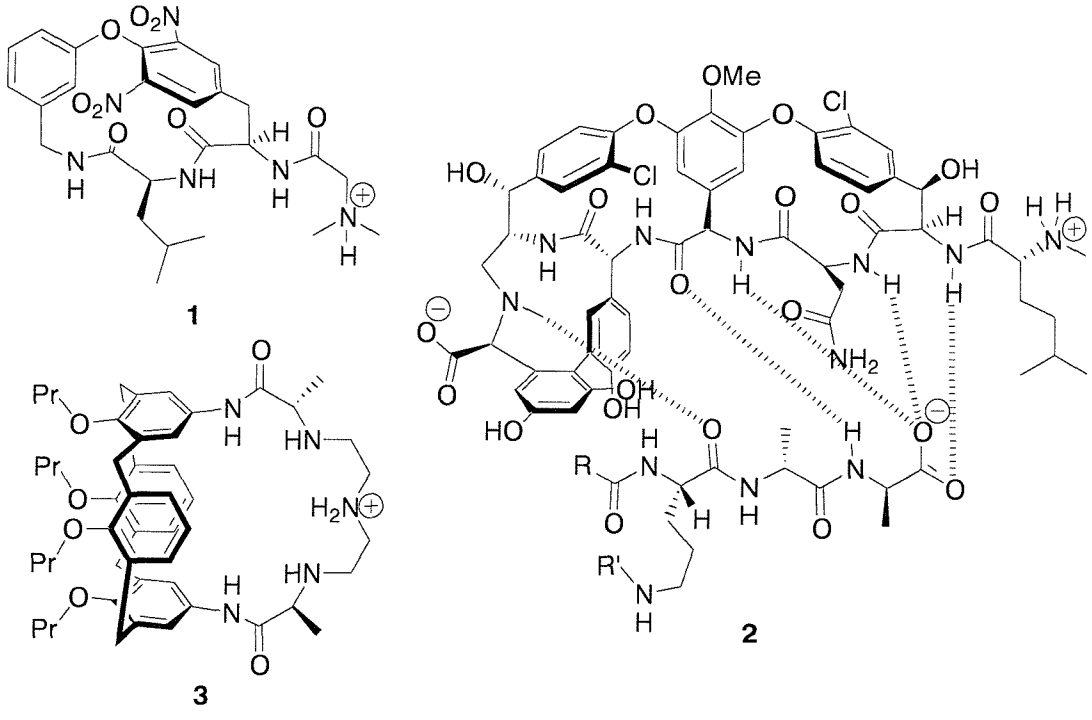


Figure 1. Hamilton's and Ungaro's vancomycin mimics.

The field of solution state supramolecular chemistry can be divided into five main sections: (i) cation binding *e.g.* metals, ammonium salts; (ii) anion binding[10, 20] *e.g.* halides and carboxylates; (iii) cation/anion binding *e.g.* zwitterions and salts;[8, 10] (iv) neutral guest binding *e.g.* carbohydrates[12] and amides; and (v) self aggregation.[5] This thesis is in principle concerned with the binding of anions, in particular carboxylates; hence these are discussed in more detail below.

1.2 Binding Anions

The binding of anions was the logical progression from the cation binding introduced by Lehn[1], Cram[2] and Pedersen.[3] A wide range of receptor designs have been employed and the applications of such systems to sensing, transport and extraction of a range of inorganic, organic and biologically relevant species have been reported. [5, 8, 10] The diversity and scope of this large field is beyond the reach of this thesis, hence only a representative range of publications are discussed below.

Ranganathan has reported a C_3-symmetric trisurea macrocycle containing di-cystine units.[21] Receptor **4** was found to bind chloride (K_a = 2.1 x 10^3 M^{-1}) and bromide (K_a = 201 M^{-1}) as their TBA salts in $CDCl_3$ but displayed no affinity for iodide. Receptor **4** also bound trigonal planar nitrate due to its C_3-symmetry with moderate efficacy (K_a = 520 M^{-1}) in $CDCl_3$.

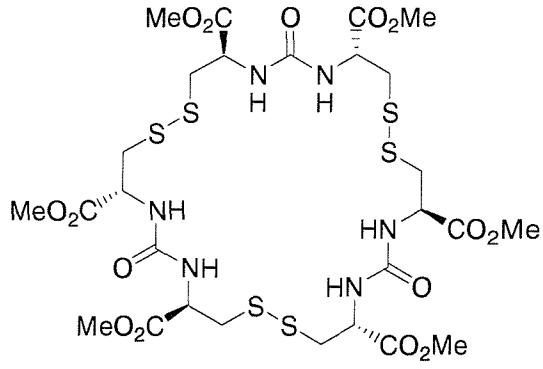


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Figure 2. Ranganathan's receptor for chloride.

Sessler has reported the binding of guanine monophosphate (GMP) by a functionalized sapphyrin as in **5**.[22] The free host sapphyrin binds GMP selectively due to the hydrogen bonding triad and transports the nucleic acid through a model membrane at pH = 7.

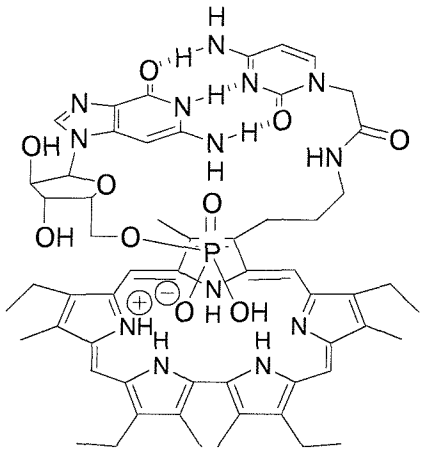

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Figure 3. Sessler's receptor for guanine monophosphate.

The use of metal centres for anion binding and transport has been explored *via* a range of strategies, one such example is de Jong and Reinhoudt's uranyl sal(oph)ene receptors for $H_2PO_4^-$.[23] Receptors **6-8** bound TPA dihydrogen phosphate ($K_a > 10^3$ M^{-1}) in DMSO with a considerably increased affinity when compared to chloride (>100 times) and receptor **8** was found to transport dihydrogen phosphate across an organic membrane, selectively even in the presence of an excess of chloride.

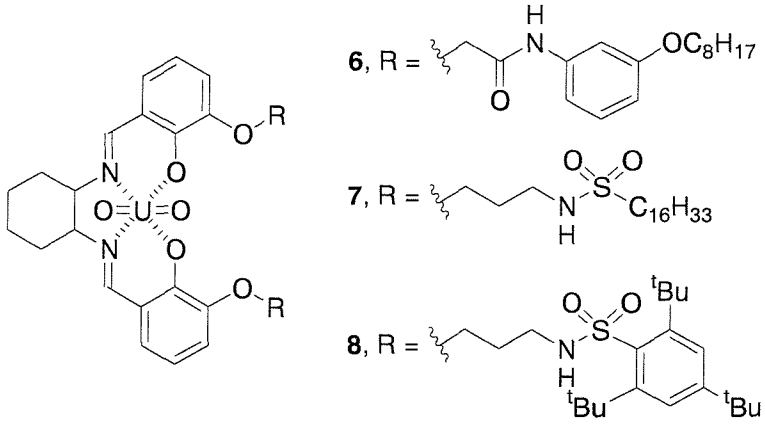


Figure 4. de Jong and Reinhoudt's neutral metal receptor for anions.

1.3 Binding Carboxylates and Carboxylic Acids

The binding of carboxylates has received a great deal of attention since the first research was published in the area by Lehn in 1979.[24] The binding of carboxylates is of significant interest due to their biological relevance such as binding the *C*-terminus of peptides *e.g.* mucopeptide precursor sequence L-Lys-D-Ala-D-Ala. A wide range of functional groups have been exploited for the binding of

carboxylates such as ammonium salts (see 1.3.1), amides (see 1.3.2), ureas and thioureas (see 1.3.3), guanidinium salts (see 1.3.4) and metals.[25]

1.3.1 Ammonium Salts

The interaction between a carboxylate and a protonated amine represents the simplest method, conceptually, for binding a carboxylate anion. Ammonium cations however form a non-directional charge-charge interaction with a carboxylate and hence the selectivity of such systems is generally determined by secondary interactions between host and guest. Kimura produced a series of macrocyclic pentamines **9-11** and hexamine **12**.[26] At neutral pH the macrocycles were all triply protonated and formed strong complexes with triscarboxylates (K_a = 55 – 1000 M^{-1}), such as citrate. The protonated macrocycles **10-12** also bound biscarboxylates that had little separation between the two carboxylates (e.g. succinate, maleate, and malonate) in a 1:1 binding stoichiometry. Biscarboxylates with a larger separation (e.g. fumarate, glutarate or aspartate) and monocarboxylates were not bound. In contrast, the protonated acyclic pentamine **13** was a poor receptor and only bound citrate ($K_a \approx 30$ M^{-1}).[26-29] Kimura also prepared bis(polyazacrowns) such as **14** which, when quadruply protonated, was presumed to form a sandwich complex with citrate ($K_a \approx 480$ M^{-1}).[30]

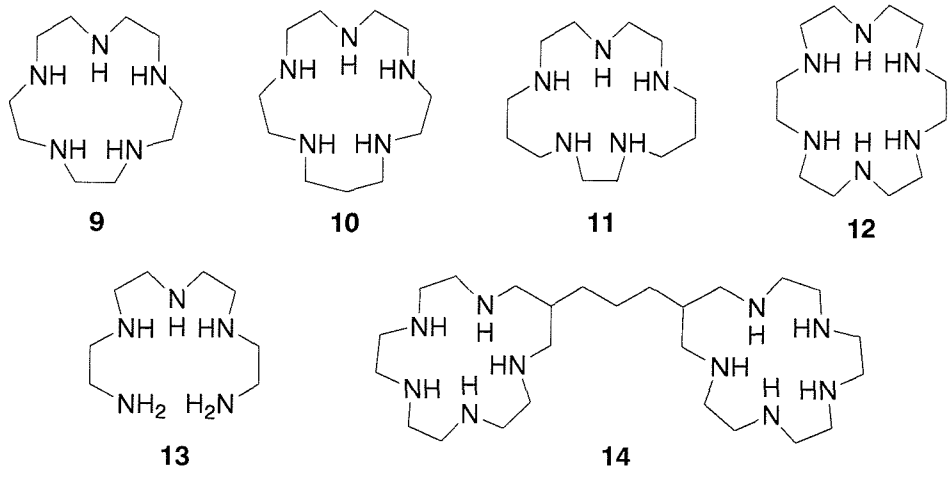


Figure 5. Kimura's polyaza macrocycles.

Studies with aza macrocycles by Bianchi revealed that compounds such as heptazacrown **15** can exhibit high selectivity for preorganised substrates *e.g.* for triacid **16** over its epimer **17**,[31] while more recently Gotor[32, 33] has reported that

incorporation of *trans*-cyclohexane-1,2-diamines into tetraaza macrocycles, such as **18**, gives receptors with enantioselective binding properties for tartrate, maleate and aspartate derivatives.[32]

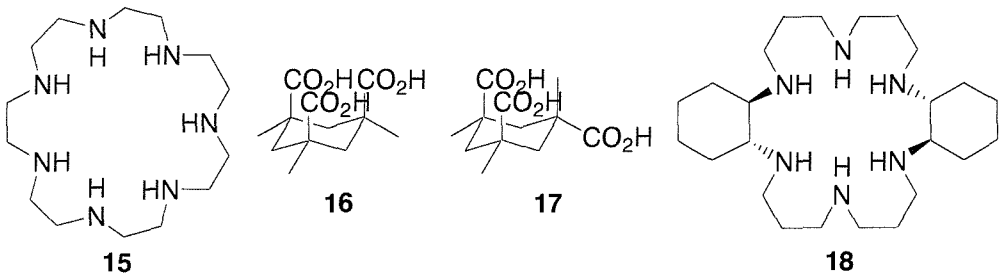


Figure 6. Bianchi's and Gotor's polyaza macrocycles.

Several groups have studied the complexation properties of aminated cyclodextrins.[34-40] Important early contributions came from Tabushi who prepared the relatively hydrophilic zwitterionic cyclodextrin derivative **19** and hydrophobic derivative **20**, which bound D-Trp ($K_a \approx 15\ M^{-1}$ and $K_a \approx 50\ M^{-1}$ respectively) at pH = 8.9. The stronger binding of D-Trp by **20** indicates that the hydrophobic environment created by this receptor enhances the coulombic interaction between ions in aqueous media.[41]

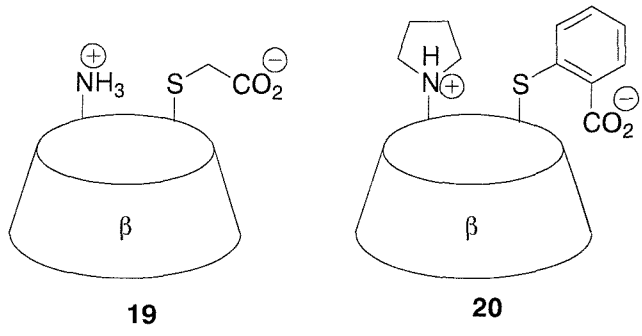
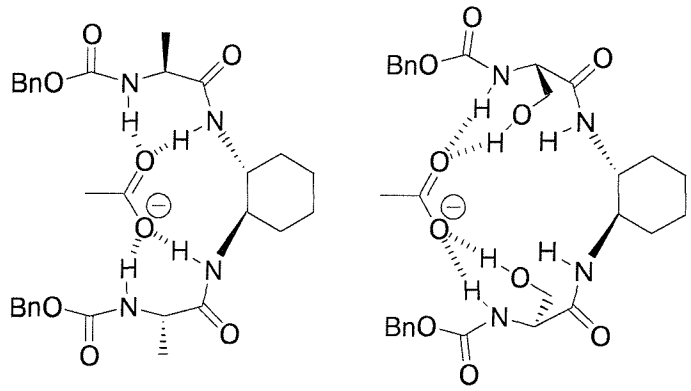


Figure 7. Tabushi's upper rim appended cyclodextrin.

1.3.2 Amides

Although amides lack the charge-charge component of an ammonium-carboxylate interaction the directionality of the amide H-bond donor affords the opportunity for a more selective receptor for carboxylates and carboxylic acids through prudent arrangement of a number of amides. Hamilton produced a family of structurally simple amide derivatives derived from cyclohexane diamine.[42] Tetra amide **21** bound TBA acetate ($K_a = 340\ M^{-1}$) in CD_3CN using a combination of four

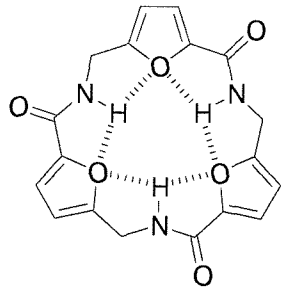
amide donor hydrogen bonds. The serine derivative **22** however bound TBA acetate significantly more strongly (K_a = 2.8 x 10^5 M^{-1}) in CD_3CN and it was concluded that in this case two of the amide NHs and the two serine OHs provide a tighter binding pocket for the carboxylate.



21 **22**

Figure 8. Hamilton's amide based receptors for acetate.

Incorporation of amides into a macrocyclic structure has been described by Chakraborty.[43] C_3-symmetric triamide **23** was found to bind TBA acetate *via* a 1:1 binding stoichiometry (K_a = 8.6 x 10^3 M^{-1}) in CD_3CN and the large change in the chemical shift ($\Delta\delta_{(saturation)}$ = 2.46 ppm) of the amide protons indicated a good binding fit. Molecular modelling suggests the presence of an array of intramolecular hydrogen bonds. Crabtree has described a series of related acyclic amide receptors which bind acetate in CD_2Cl_2.[44] Receptor **24** exhibits a high affinity for tetraphenylphosphonium acetate (K_a = 1.9 x 10^4 M^{-1}), however sulphonamide **25** binds acetate with similar efficiency (K_a = 2.1 x 10^4 M^{-1}) despite the increased acidity of the sulphonamide moiety, probably due to a greater degree of free rotation. Pyridyl receptor **26** shows limited affinity for acetate (K_a = 525 M^{-1}) presumably due to the cleft being too small, as exemplified by the high binding constant with the smaller fluoride (K_a = 2.4 x 10^4 M^{-1}). Szumna and Jurczak have described a macrocyclic receptor related to **26** which binds acetate selectively over other anions in DMSO-d_6.[45]



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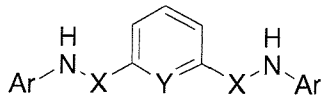

24, X = CO, Y = CH, Ar = p-(^{n}Bu)C_6H_4

25, X = SO_2, Y = CH, Ar = Ph

26, X = CO, Y = N, Ar = Ph

Figure 9. Chakraborty's macrocyclic triamde and Crabtree's acyclic amide receptors.

Gale has described the binding of simple carboxylates by pentapyrrolic calix[4]pyrrole **27**. An additional electron poor pyrrole in **27,** compared to the parent calix[4]pyrrole, results in a large increase in complex stability ($K_a > 10^4$ M^{-1} and K_a = 668 M^{-1} respectively) with TBA acetate in CD_2Cl_2.

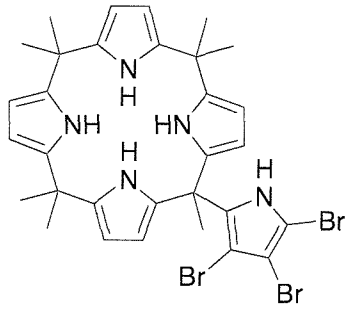

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Figure 10. Gale's calixpyrrole receptor.

Conformationally restricted (cyclic) *cis* amides and carbamates provide a complementary pair of hydrogen bond interactions for carboxylic acid recognition. Thus, Moran has described the chromenone derived receptor **28** which bound benzoic acid derivatives in $CDCl_3$ with large binding constants when the aromatic guest had a *para* electron donating substituent (*e.g.* K_a = 1.6 x 10^6 M^{-1} with 4-methylaminobenzoic acid).[46] The chiral receptor **29** also bound *N*-Cbz-amino acids in $CDCl_3$,[47] with the highest binding constant observed for *N*-Cbz-Gly (K_a = 1.3 x 10^4 M^{-1}). Using larger guest molecules (*e.g.* alanine and phenylalanine), steric repulsion lead to reduced binding strength. Modest enantioselectivity (~2:1) was observed for both *N*-Cbz-Ala and *N*-Cbz-Phe in favour of the L-enantiomer in each case. Recently Smith has described the changes in the *cis/trans* ratios of some secondary amides upon complexation of carboxylic acids.[48]

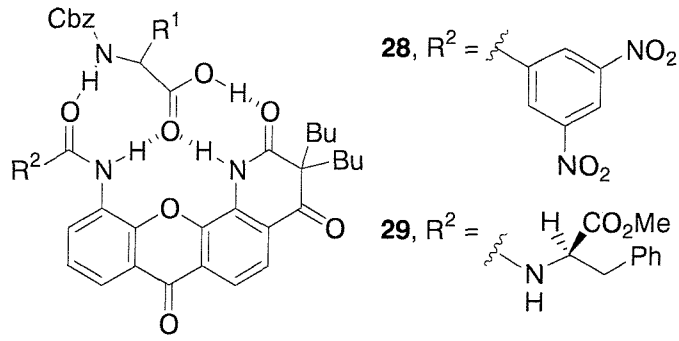

Figure 11. Moran's chromenone derived receptor for carboxylic acids.

Amidopyridines in principle provide an excellent structural motif for binding carboxylic acids with the ability to form two complementary hydrogen bonds from the carboxylic acid hydrogen to the pyridine nitrogen and the carboxylic acid carbonyl to the amide. However, in practice, unfavourable secondary interactions, particularly between the relatively electropositive carboxylic acid and amide protons, make amidopyridines a less potent binding site for carboxylic acids than ureas and thioureas are for carboxylates (see 1.3.3), and thus amidopyridines are generally only effective in relatively non-polar solvents. Helmchen has combined an amidopyridine with an additional hydrogen bond for the carbonyl oxygen *syn* lone pair, in molecular clefts such as **30**.[49] In Helmchen's studies a series of sterically similar, but electronically different, hosts were prepared in order to develop chiral solvating agents for carboxylic acids. When R = Ph or 1-napthyl, complexation of aromatic carboxylic acid guests (*e.g.* naproxen, phenylacetic acid and hydratropic acid) led to upfield shifts of the signals for the α-Hs of the guests in the ^{1}H NMR in $CDCl_3$ ($\Delta\delta \approx$ 0.28 ppm) suggestive of π-π stacking interactions. Receptor **30** (R = Ph) was also moderately enantioselective for the *S*-enantiomer of hydratropic acid (K_a = 1.1 x 10^3 M^{-1}) over the *R*-enantiomer (K_a = 700 M^{-1}) in $CDCl_3$.

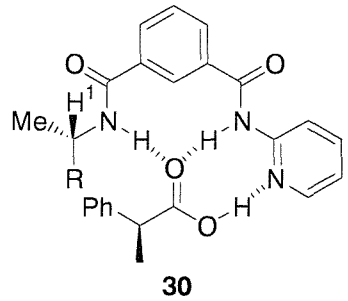

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Figure 12. Helmchen's amidopyridine receptor.

1.3.3 Ureas and Thioureas

Despite lacking the electrostatic complementarity offered by the guanidinium salt, ureas and thioureas have been shown to provide a strong binding site for carboxylates, using a bidentate hydrogen bonding motif. Wilcox was the first to utilise ureas and thioureas for carboxylate binding and reported that urea **31** bound benzoate in $CDCl_3$ (K_a = 2.7 x 10^4 M^{-1}).[50] Large downfield shifts of the signals for the NH protons were observed in ^1H NMR titration experiments, indicating strong hydrogen bonding between the urea hydrogens and carboxylate oxygens.

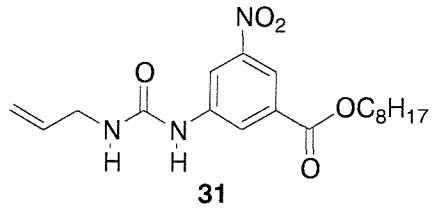

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Figure 13. Wilcox's urea receptor for benzoate.

Calixarenes are a popular scaffold for carboxylate receptors,[51-54] such as the biscalix[4]arene based urea receptor **32** described by Stibor. Host **32** aggregates in $CDCl_3$ but binding was observed with TBA benzoate (K_a = 730 M^{-1}) in 20% DMSO-d_6/$CDCl_3$ with selectivity over spherical anions *e.g.* chloride (K_a = 54 M^{-1}).[55]

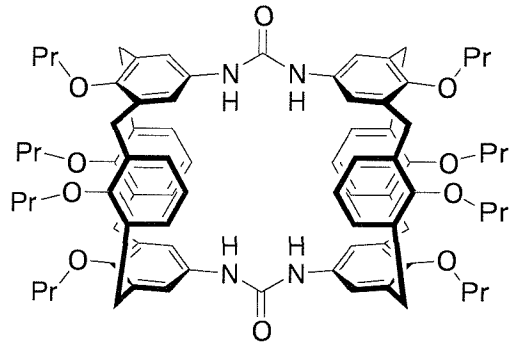

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Figure 14. Stibor's calixarene-urea receptor.

Moran has also described the spirobifluorene capped macrocyclic receptor **33** which forms complexes with *R*-mandelic acid with a 16:1 selectivity for the *R,R*-complex over the *R,S*-complex in DMSO-d_6.[56] The chromenone scaffold has been adapted to incorporate a fifth hydrogen bond from a hydroxamic acid moiety. Receptor **34** bound heteroaromatic triethylammonium carboxylates (*e.g.* 2-furoic acid and fusaric

acid) and triethylammonium α-keto carboxylates (*e.g.* pyruvic acid). The highest binding was observed for fusaric acid carboxylate (K_a = 4.3 x 10^5 M^{-1}) in 1% $CD_3OD/CDCl_3$ compared to TBA benzoate (K_a = 1.1 x 10^3 M^{-1}), indicating the importance of the fifth hydrogen bond.[57]

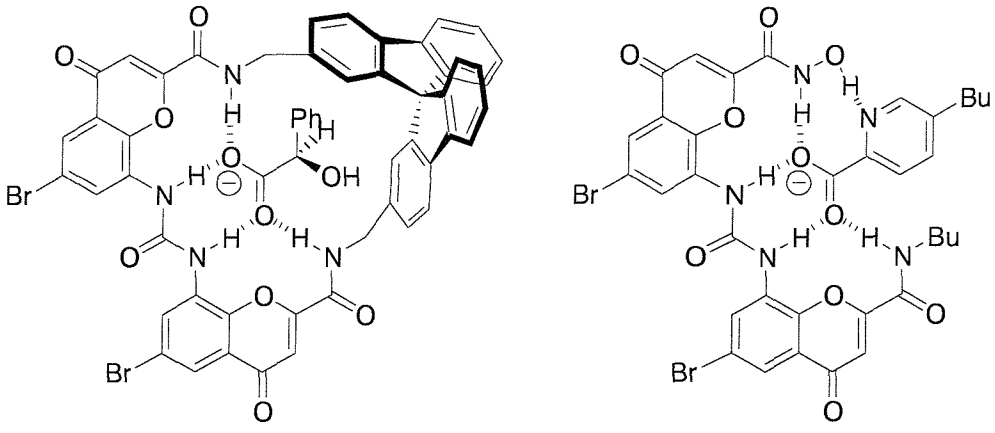


Figure 15. Moran's chromenone derived receptors.

Hamilton examined the binding of carboxylates by ureas and thioureas in polar solvents such as DMSO.[42] Addition of tetramethylammonium (TMA) acetate to a DMSO-d_6 solution of 1,3-dimethylurea gave large downfield shifts (> 1 ppm) of the urea NH resonance, which was again consistent with the formation of a bidentate hydrogen bonded complex as in **35** (K_a = 45 M^{-1}). Stronger binding was achieved when the acidity of the hydrogen bonding donor sites was increased by replacing the urea (pK_a = 26.9) with a thiourea (pK_a = 21.0). Thus 1,3-dimethylthiourea bound TMA acetate with approximately 8 fold increase in binding constant (K_a = 340 M^{-1}) compared to 1,3-dimethylurea.

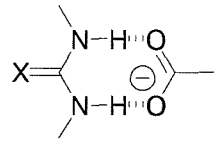
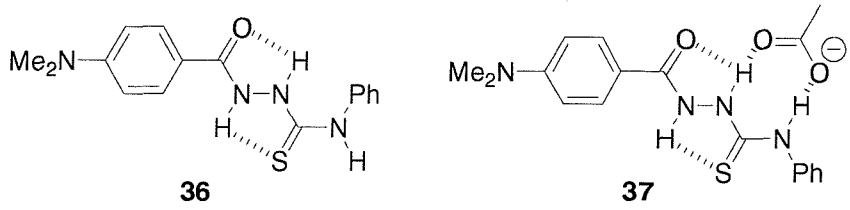


Figure 16. Urea/thiourea-carboxylate bidentate hydrogen bonding motif.

Recently Jiang has described the fluorescent sensing of acetate by a thiourea based receptor **36**.[58] The formation of a strong complex **37** upon addition of TBA acetate to the free thiourea **36** in CD_3CN (K_a = 4.8 x 10^5 M^{-1}) results is a significant perturbation in the fluorescent behaviour of the receptor due to conformational

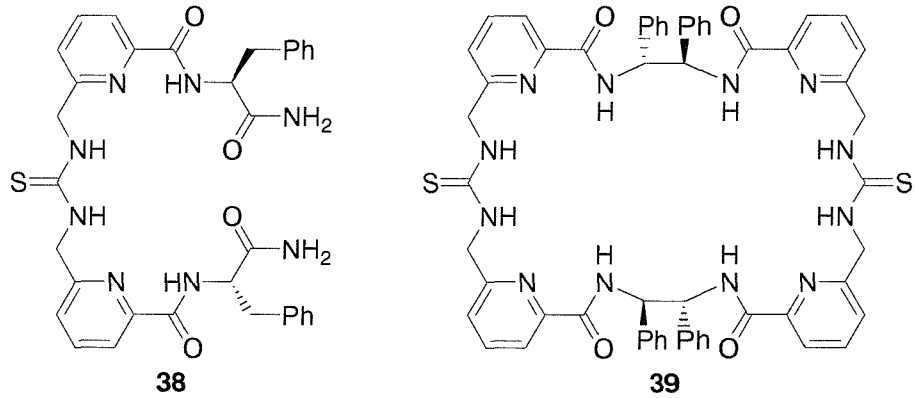
change. Other related fluorescent sensors for carboxylates have been reported recently by Teramae,[59] Davis[60] and Wu.[61]



36 **37**

Figure 17. Jiang's thiourea based fluorescent sensor.

Kilburn has described the enantioselective binding of *N*-protected amino acids by a pyridyl thiourea receptor.[62] Receptor **38** was titrated with a range of amino acid carboxylates (TBA salts) and exhibited some selectivity particularly for amino acids with electron rich aromatic side chains *e.g.* *N*-Ac-D-Trp (K_a = 1.5 x 10^4 M^{-1}) in $CDCl_3$. Receptor **38** was modestly enantioselective with a general preference for L-amino acids *e.g.* for *N*-Ac-Gln-O^- (K_a^L:K_a^D ~ 2:1). Recently Kilburn has extended this framework to macrocyclic receptor **39** which displays a broad NMR spectrum in $CDCl_3$ due to a wrapped conformation and hence does not bind in this solvent. Macrocycle **39** binds Boc-L-Glu as its bis TBA salt in DMSO-d_6 with a 1:1 stoichiometry (K_a(1:1) = 2.8 x 10^4 M^{-1}) however the D-enantiomer is bound in 1:2 (host:guest) stoichiometry (K_a(1:2) = 4.9 x 10^4 M^{-1}).[63]

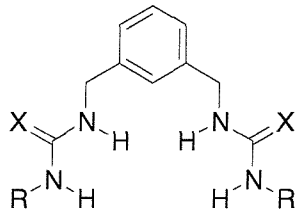


38 **39**

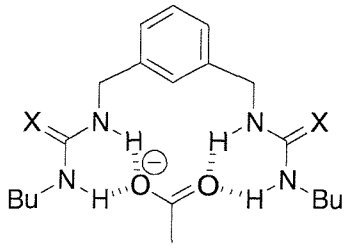
Figure 18. Kilburn's receptors for *N*-protected amino acids.

Although monoureas and monothioureas provide potent carboxylate binding sites using a bidentate array of hydrogen bonds, bisureas and bisthioureas can be used to provide even stronger binding using four hydrogen bonds. Umezawa used simple structures to show that bisthioureas, such as **40**, are stronger receptors than the

corresponding monoureas/thioureas.[64] Thus bisurea **41** bound TBA acetate (K_a = 43 M^{-1}) whereas bisthiourea **40** bound the same guest more strongly (K_a = 470 M^{-1}) in DMSO-d_6. Job plot analysis clearly indicated a 1:1 stoichiometry consistent with complex **42**, and large changes in chemical shifts were observed for the NH protons. The binding potency can in principle be increased by using thiouroniums. Thus bisthiouronium salt **43** bound TBA benzoate in DMSO-d_6 (K_a = 590 M^{-1}).[65] Recently Teramae has described fluorescent carboxylate sensors based on naphthalene and anthracene thiouronium salts.[66]



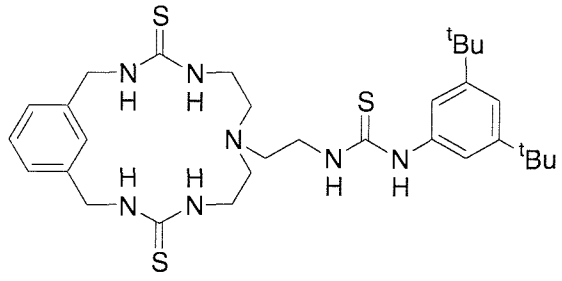
40, X = S, R = Bu
41, X = O, R = Bu
43, X = S^+MeBr^-, R = Bu



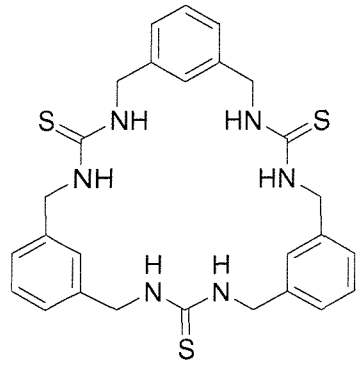
42

Figure 19. Umzawa's bisurea and bisthiourea receptors for monocarboxylates.

Tobe[67] and Lee[68] have described a tristhiourea receptors **44** and **45** for acetate which both display high affinities even in competitive media. Both receptors form tight complexes with acetate evinced by large downfield shifts in both thiourea and aromatic resonances in the ^1H NMR. Receptor **44** binds TBA acetate (K_a = 8.3 x 10^3 M^{-1}, DMSO-d_6, 60 °C) strongly in DMSO-d_6 as does **45** (K_a = 5.3 x 10^3 M^{-1}, DMSO-d_6). However, receptor **45** displays greater selectivity (>5:1) over other anions *e.g.* chloride and phosphate.



44



45

Figure 20. Tobe's thiourea cleft and Lee and Hong's tris-thiourea anion receptor.

1.3.4 Guanidinium Salts

1.3.4.1 Acyclic Guanidinium Salts

Guanidinium salts remain protonated over a much wider pH range than the ammonium salts (pK_a = 13.5 for guanidinium) and the binding of carboxylate salts combines electrostatic interactions with a bidentate hydrogen bonding array as in **46**.

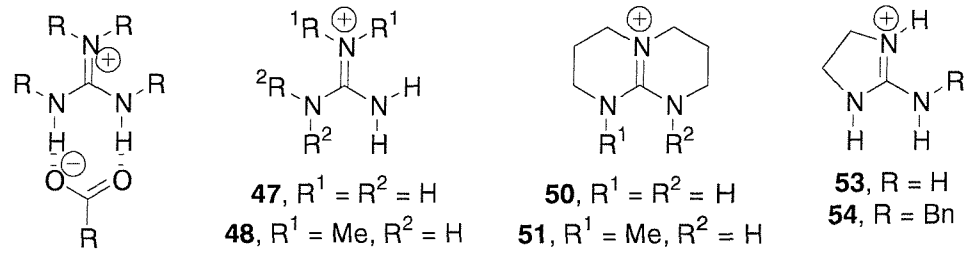
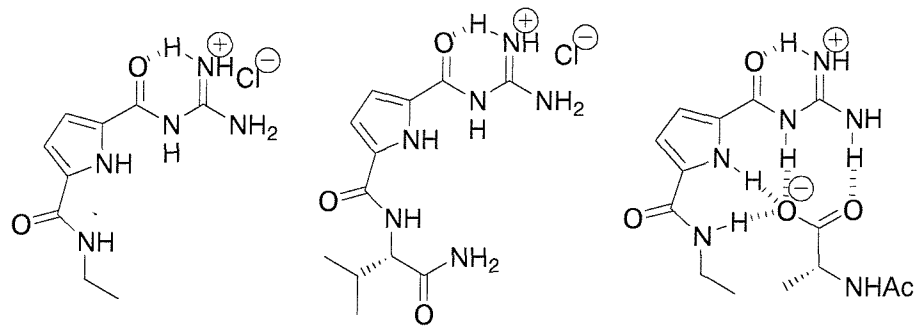


Figure 21. Guanidinium salts as carboxylate receptors.

Hamilton has used isothermal titration calorimetry to measure the association of a range of simple acyclic, monocyclic and bicyclic guanidiniums **47-54**, as their tetrafluoroborate salts, with TBA acetate in DMSO.[69] Sequential removal of hydrogen bonding sites results in a significant fall in the binding constants in the acyclic systems **47-49** (K_a = 7.9 x 10^3 M^{-1}, K_a = 3.4 x 10^3 M^{-1} and K_a = 110 M^{-1} respectively). Similarly bicyclic guanidinium salt **50** bound TBA acetate strongly, (K_a = 5.6 x 10^3 M^{-1}) but no binding was observed for the corresponding methylated compounds **51** and **52** - which was confirmed by ^{1}H NMR titration. Monocyclic guanidinium salts **53** and **54** also display high affinities for acetate, particularly receptor **54**, as the iodide salt (K_a = 7.2 x 10^3 M^{-1} in DMSO, as determined by isothermal titration calorimetry and K_a = 1.2 x 10^4 M^{-1} in DMSO-d_6, as determined by ^{1}H NMR titration[70]).

Binding of a simple guanidinium salt to a carboxylate can be enhanced by incorporation of additional hydrogen bonding functionality. Thus Schmuck has described guanidinocarbonyl pyrrole receptors such as **55** and **56** which bound carboxylates by ion pairing in combination with multiple hydrogen bonds from the guanidinium salt, pyrrole and amide as in **57**.[71, 72] Receptor **55** bound *N*-Ac-α-amino acid carboxylates in 40% H_2O/DMSO with association constants (K_a = 360 – 1.7 x 10^3 M^{-1}) dependent on the structure of the amino acid side chain. Similarly,

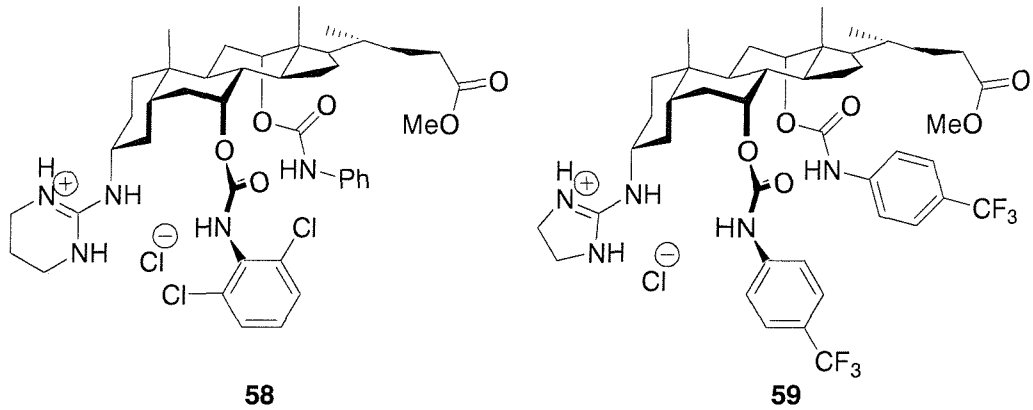
chiral receptor **56** bound *N*-Ac-α-amino acid carboxylates (K_a = 350 – 5.3 x 10^3 M^{-1}) in 40% H_2O/DMSO and showed some degree of enantioselectivity.[73]



55 **56** **57**

Figure 22. Schmuck's guanidinocarbonyl receptors.

Davis has described enantioselective carboxylate receptors created by attachment of a monocyclic guanidinium salt to a cholic acid scaffold. The secondary hydroxy groups of cholic acid could be modified to generate receptors in which three binding moieties were spaced to allow co-operative effects on the substrate with minimum interference from intramolecular interactions. Solutions of **58** and **59** were able to extract *N*-Ac-α-amino acids from neutral or basic aqueous solutions *via* exchange of chloride for carboxylate. The extraction efficiencies were moderate to good (52 - 87 mol%) for substrates with non-polar side-chains, although neither receptor was effective with the polar asparagine derivative. Receptor **58** showed generally higher extraction abilities (74 - 93 mol%) and was sensitive to side-chain structure with L:D selectivites between 5:2 and 9:1, the greatest selectivity being observed with phenylalanine and methionine side chains. Perhaps surprisingly, the substrate with the most sterically hindered asymmetric centre *N*-Ac-*tert*-Leu gave the lowest selectivity. ^{1}H NMR spectroscopy and molecular modelling both suggested plausible models for the binding geometries.[74-76]

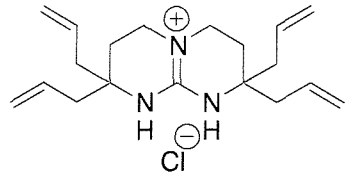


58 59

Figure 23. Davis' monocyclic guanidinium salt receptors.

1.3.4.2 Bicyclic Guanidinium Salts

Schmidtchen first reported the use of a bicyclic guanidinium salt for the formation of host-guest complexes with simple carboxylates.[77, 78] As a result of forming part of a bicyclodecane framework as in **60** the guanidinium salt becomes an almost perfect match for carboxylate anions with the two guanidinium salt protons aligned in the same direction. Titration of bicyclic guanidinium salt **60** with TBA *p*-nitrobenzoate in CD_3CN results in a shift of all host resonances in the ^1H NMR spectrum, with a pronounced shift in the guanidinium protons of more than 5 ppm. The shape of the titration curve fits the proposed 1:1 complex stoichiometry, and gave an estimation of the lower limit of complex stability ($K_a > 1.0 \times 10^4\ M^{-1}$) in CD_3CN.

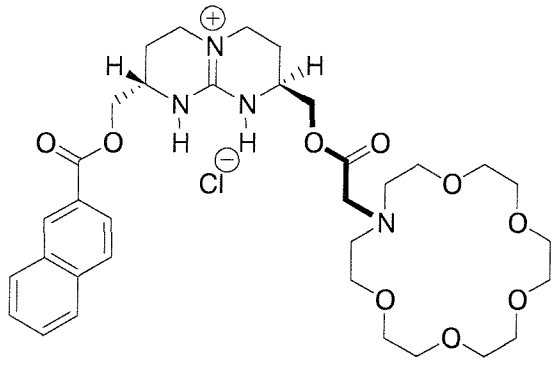


60

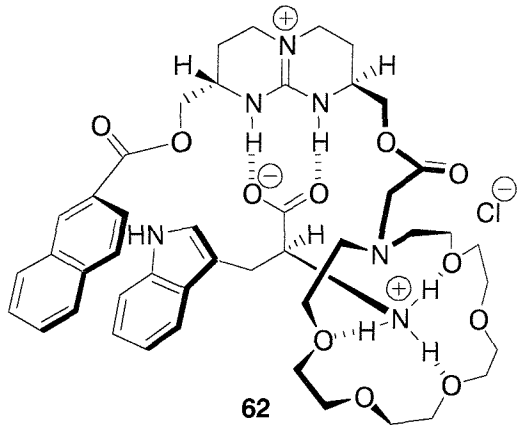
Figure 24. Schmidtchen's bicyclic guanidinium.

Receptors for amino acids have been developed by coupling the chiral bicyclic guanidinium salt to a crown ether. In de Mendoza's example of this approach an aromatic planar surface provides an additional π-stacking interaction, giving receptors with high selectivity for amino acids containing aromatic side chains.[79] The affinity of **61** towards amino acids was determined by liquid-liquid extraction experiments, in which an aqueous solution of Trp, Phe, or Val was extracted into a

CH_2Cl_2 solution of **61**. The extraction efficiencies *i.e.* the fraction of receptor molecules occupied by the substrate in the organic phase, as determined by NMR integration, were ~ 40% for Trp and Phe, but Val, without an aromatic side chain, was not detected. A competition experiment with a mixture of all three amino acids resulted in 100:97:6 Phe:Trp:Val selectivity. Chiral recognition was confirmed by the observation that the corresponding D-enantiomers were not extracted. A more precise account of the selectivity was achieved by HPLC analysis of diastereomeric dipeptides, prepared from extracts of racemic samples of Phe or Trp and a suitable optically pure L-Leu derivative. The amount of D-isomer in the extracts was lower than 0.5% for D-Trp, determined as L-Leu-D-Trp, and 2% or less for D-Phe, determined as L-Leu-D-Phe. This high degree of chiral recognition can be explained by the three simultaneous non-covalent interactions of the substrate with the flexible and foldable receptor as in **62**.



61

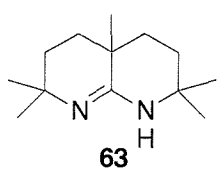


62

Figure 25. de Mendoza's receptor for aromatic amino acids.

1.3.4.3 Amidinium Salts

Davis has produced bicyclic amidine **63** analogous to the bicyclic guanidines described above (see Figure 24) An X-ray crystal structure of the naproxenate salt of achiral amidinium **63** confirms the expected carboxylate-amidinium interaction with two hydrogen bonds.[80, 81]



63

Figure 26. Davis' amidine receptor

Gale has described the use of calixarene derived bisamidinium salts as a template for the self-assembly of ditopic receptors.[82, 83] Thus bisamidinium salt **64** bound carboxylates, including crown ether and calixpyrrole derived carboxylates **65** and **66**, with a 1:2 stoichiometry in DMSO.

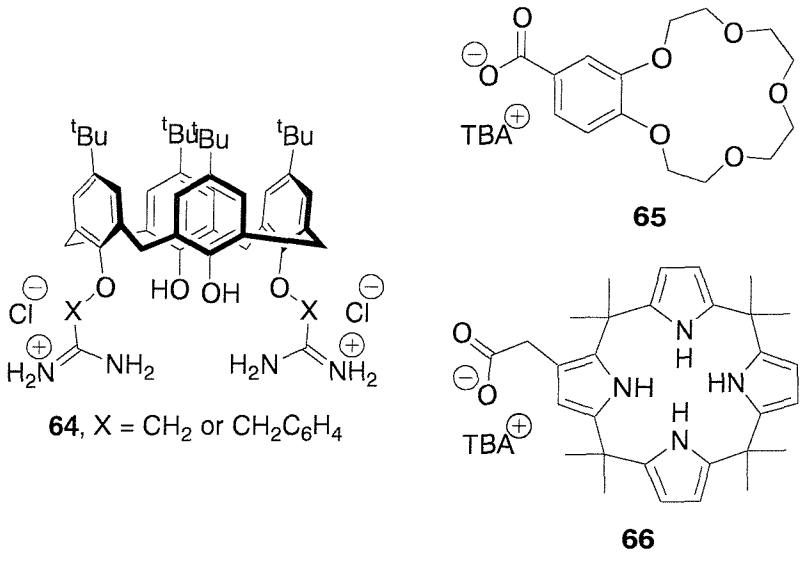


Figure 27. Gale's bisamidinium salt receptor.

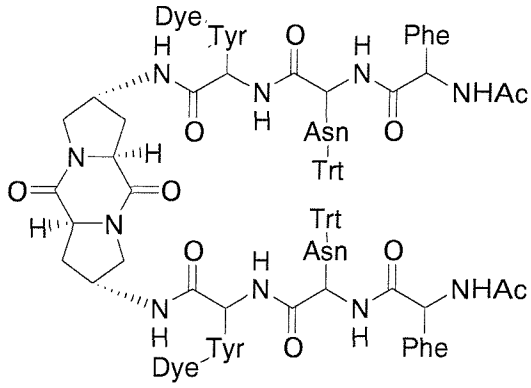
1.4 Peptide Receptors

The development of synthetic receptors for the recognition of peptides has been inspired by the strength and selectivity exhibited by naturally occurring peptide binding agents such as vancomycin.

1.4.1 Non-Specific Head Groups

Although a number of peptide receptors containing a carboxylate recognition site are discussed below, the binding of peptides purely through peptide-peptide interactions has also received some attention. Wennemers has described a dye labelled tweezer with a diketopiperazine head group which binds tripeptides in $CHCl_3$.[84] Receptor **67** was screened against a library of 24000 resin bound tripeptides and was found to bind Ac-D-Val-D-Val-D-His (K_a = 1.4 x 10^3 M^{-1}) selectively over is diastereomer Ac-D-Val-L-Val-D-His (K_a = 15 M^{-1}) as determined by an on bead binding assay. A systematic study indicated that both arms of the tweezer and a tripeptide scaffold are required for good binding. Still has described a related series of receptors with a steroidal hinge which is sequence selective for

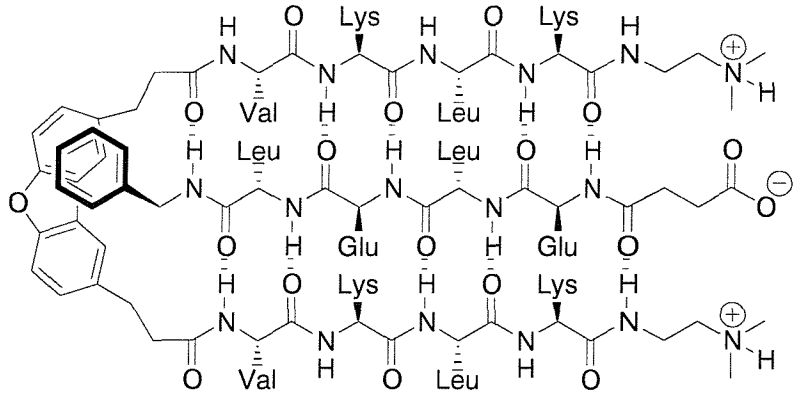
tetrapeptides.[85] A range of other tweezer receptors for tri-, tetra- and penta- peptides have been described with various hinge components elaborated with peptide side arms have been reported *e.g.* calix[4]arene hinge[86] and a steroidal hinge.[87]



67

Figure 28. Wennemers' tweezer for tripeptides.

Kelly has described peptide β-sheet mimic **68** that displays selectivity for anionic tetrapeptides with an amphiphilic periodicity of 2.[88] Hence the free host is highly sequence selective for Leu-Glu-Leu-Glu (K_a = 2 x 10^3 M^{-1}) over Leu-Ser-Leu-Ser (K_a = 357 M^{-1}) in H_2O (pH = 5.75).

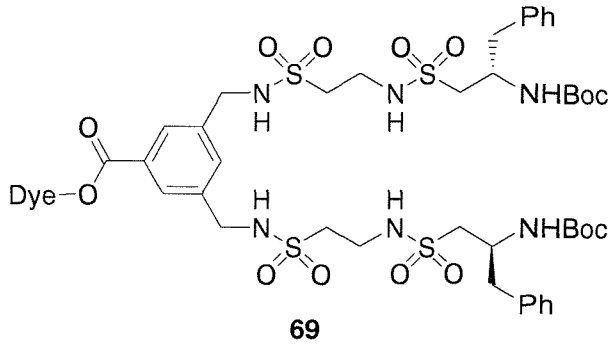


68

Figure 29. Kelly's peptide β–sheet mimic.

Liskamp has reported the binding of tripeptides by a series of dye labelled peptoids such as **69**.[89] Screening against a resin bound 24000 member library H-AA^3-AA^2-AA^1-resin resulted in a general preference for acidic residues and for a polar residue at AA^2. Receptor **69** was found to associate most strongly with the consensus

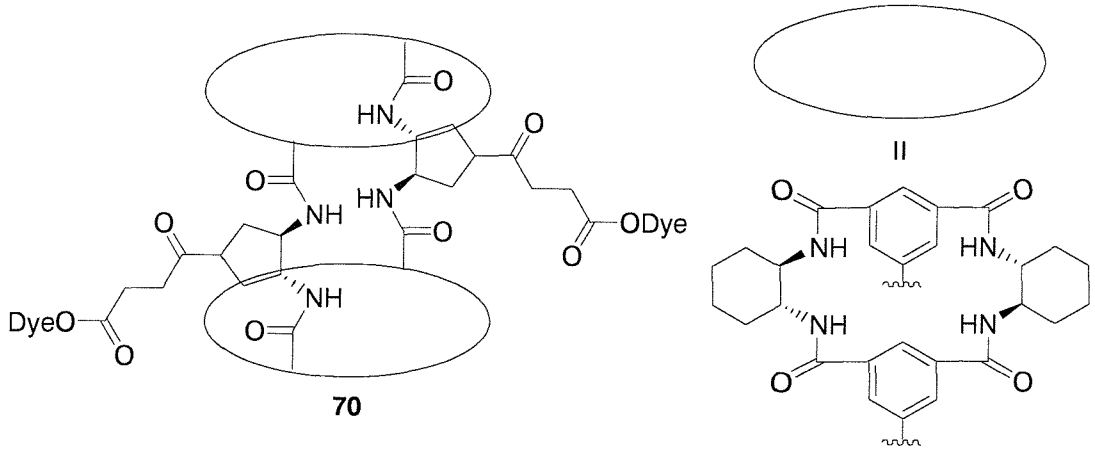
sequence D-Ala-L-Asp-D-Ser (K_a = 4.1 x 10^3 M^{-1}) in $CDCl_3$. Other tweezers in the series displayed some degree of diastereoselectivity.



69

Figure 30. Liskamp's sulphonamidopeptide receptor for tripeptides.

Still has developed a macrotricylic receptor which binds a range of tripeptides in $CHCl_3$.[90, 91] Receptor **70** was screened against a 50000 member resin bound library of tripeptides R-AA^1-AA^2-AA^3-resin and was found to preferentially bind valine rich tripeptides in particular with AA^1 = D-Asn/Gln and AA^2 = L-Val. Molecular modelling suggests that the macrocycle acts through an array of seven hydrogen bonds.



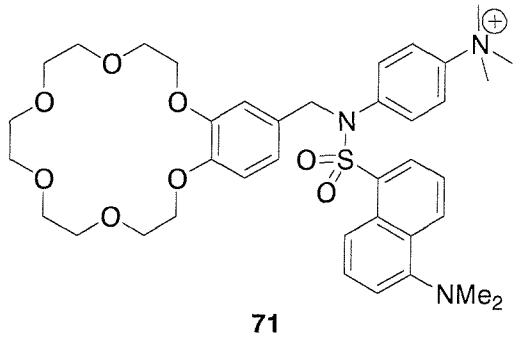
70

Figure 31. Still's macrotricyclic receptor for tripeptides.

1.4.2 Carboxylate Recognition

The development of receptors for the carboxy terminus of peptidic guests has received some study. Using a combination of β-sheet type interactions and the motifs described above for the binding of carboxylates, a range of receptors for amino acid carboxylates has been reported.

Schneider has described a receptor for peptide zwitterions incorporating a peralkyl ammonium salt and a crown ether as recognition sites.[92] Titration of receptor **71** with tripeptides in H_2O lead to changes in the fluorescence of the dansyl group consistent with a 1:1 binding stoichiometry as in **72**. As expected, receptor **71** shows the strongest association for tripeptides where the second amino acid contains an aromatic side chain to interact with the dansyl group. Titration of receptor **71** with the tripeptides Phe-Gly-Gly (K_a = 220 M^{-1}) and Gly-Gly-Phe (K_a = 215 M^{-1}) gave binding constants of similar magnitude to the tripeptide Gly-Gly-Gly (K_a = 210 M^{-1}) whereas tripeptides Gly-Phe-Gly (K_a = 1.7 x 10^3 M^{-1}) and Gly-Trp-Gly (K_a = 2.2 x 10^3 M^{-1}) are bound significantly more strongly. Schneider has also described related receptors built around a porphyrin skeleton which binds a range of di-, tri- and tetra-peptides.[93]



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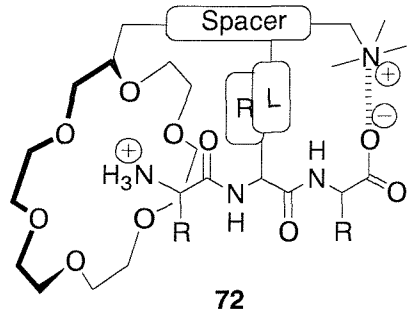
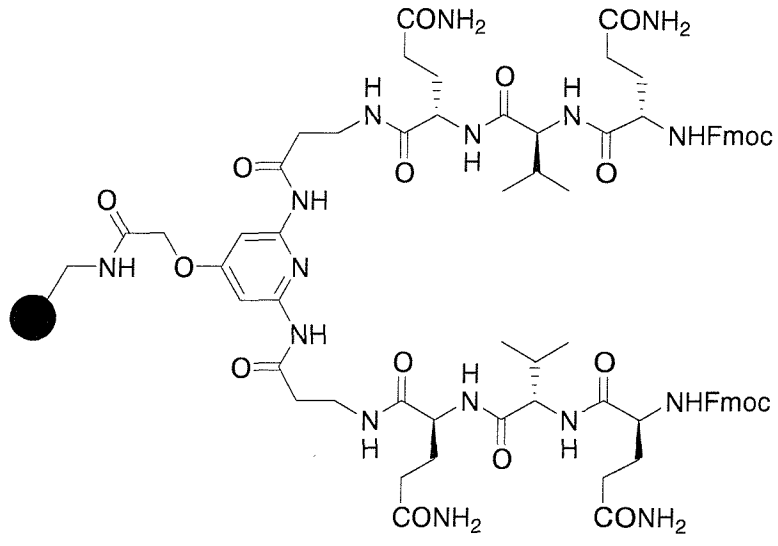


72

Figure 32. Schneider's peralkylammonium salt receptor for peptide zwitterions.

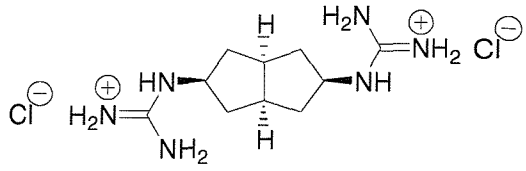
Kilburn has extended the amidopyridine motif described above (see Figure 12) with the introduction of a second H-bond donor to bind the carboxylate in a range of tweezer structures.[94] Receptor **73** was identified from the screening of a 1728 member library and was found to bind dye labelled Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH ($K_a \approx 3 \times 10^5$ M^{-1}) approximately 100 times more strongly than the side chain deprotected analogue Glu-Ser-Val-OH ($K_a \approx 1 \times 10^3$ M^{-1}) in $CDCl_3$. Tweezer **73** also displays approximately 30:1 selectivity for dye labelled-L-Glu(O^{t}Bu)-L-Ser(O^{t}Bu)-L-Val-OH ($K_a \approx 9 \times 10^3$ M^{-1}) over its enantiomer.[95] Gennari and Kilburn have also reported related tweezers with sulphonamidopeptide side arms which bind amino acids and peptides.[96]



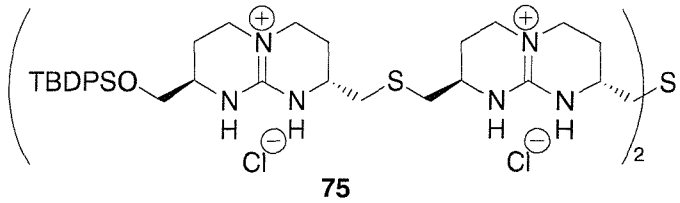
73

Figure 33. Kilburn's diamidopyrdine tweezer.

Hamilton has used bisguanidinium salts to recognise aspartate pairs in helical peptides. Thus, bisguanidinium salt **74** was added to 16mer peptides with two aspartate groups at different positions (i+3, i+4, i+11) in 10% H_2O/MeOH (K_a = 2.2 x 10^3 M^{-1} for i+3) and resulted in a 6-9 % increase in helicity of the peptides i+3 and i+4 but only a slight increase for peptide i+11, for which the helical conformation would place the two aspartates too far apart to form a 1:1 complex with the bisguanidinium salt.[97] Hamilton and de Mendoza have extended the bicyclic guanidinium salt motif to create synthetic receptors that similarly stabilise the α-helical conformation of glutamate and aspartate rich peptides.[98-100] The spacing of the guanidinium salt units in the tetraguanidinium salt **75** complements the carbon-carbon distances between the carboxylates of peptide **76** when in an α-helix. Addition of receptor **75** to the peptide resulted in strong binding (K_a > 1 x 10^5 M^{-1}) in 10% H_2O/MeOH and a significant increase in helical stability of the peptide. Titration of peptides **77** and **78** with asparagine acting as a neutral isostere for aspartate was carried out under the same conditions and results showed a correspondence between the number of aspartates and the degree of helix stabilization.



74



75

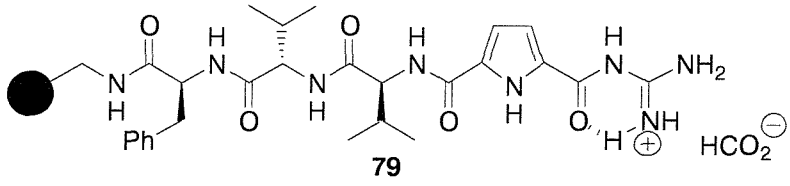
76, DDDD Ac-A-A-A-**D**-Q-L-**D**-A-L-**D**-A-Q-**D**-Q-Q-Y-NH_2

77, DDDN Ac-A-A-A-**D**-Q-L-**D**-A-L-**D**-A-Q-N-Q-Q-Y-NH_2

78, DDNN Ac-A-A-A-**D**-Q-L-**D**-A-L-N-A-Q-N-Q-Q-Y-NH_2

Figure 34. Hamilton's tetraguanidinium receptors for stabilization of α-helicies.

Schmuck has extended his guanidinocarbonyl pyrrole motif (see Figure 22) to identify a 'half tweezer' receptor for the *C*-terminus of the amyloid β-peptide.[101, 102] Receptor **79** was identified from a 125 member library *via* screening with dye labelled tetrapeptide Val-Val-Ile-Ala as its TMA salt in CD_3OD and the magnitude of binding was determined in CD_3OD by an on bead binding assay (K_a = 9.8 x 10^3 M^{-1}). Evidence for significant electrostatic and hydrophobic components in the binding was observed and molecular modelling suggested an antiparallel β–sheet array.

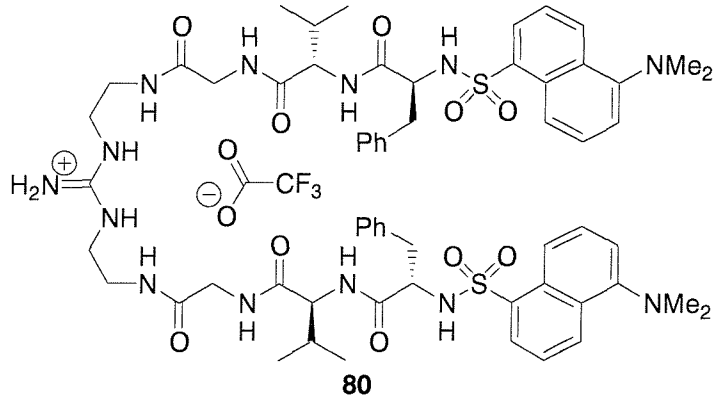


79

Figure 35. Schmuck's tetrapeptide receptor.

Kilburn has synthesised a 'tweezer' receptor for peptides with a carboxylate terminus using a guanidinium salt to provide the primary binding interaction for the carboxylate, and receptor arms with potential to form both hydrophobic and β–sheet like hydrogen bonding interactions with the backbone of the peptide substrate.[103] Solid phase synthesis of a library of tripeptides, attached to TentaGel resin *via* the amino terminus, was screened with tweezer **80** in an aqueous solvent system. Receptor **80** was found to bind to approximately 3% of the library members and following sequencing of 20 beads, showed 95% selectivity for valine at the carboxy

terminus of the tripeptides and 40% selectivity for Glu(O^{t}Bu) at the amino terminus. The binding constant (K_a = 4 x 10^5 M^{-1} in 17% DMSO/H_2O, pH = 9.2) for one of the peptides selected from the screening experiments (Cbz-Glu(O^{t}Bu)-Ser(O^{t}Bu)-Val-OH), with receptor **80** was measured using titration calorimetry. Recently Kilburn has described a related tweezer which also binds *N*-protected tripeptides and displays moderate enantioselectivity.[104]



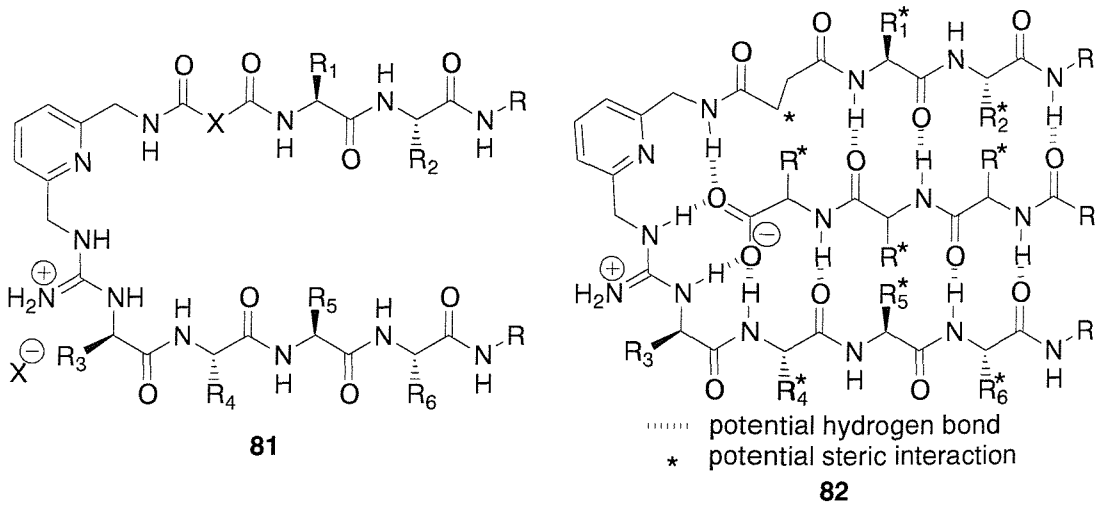
80

Figure 36. Kilburn's tweezer receptor.

1.5 Project Scope and Receptor Design

Recent progress in the binding of peptidic carboxylates by synthetic receptors has mostly been through the use of combinatorial chemistry, exemplified by the work of Liskamp,[89] Schmuck[101, 102] and Kilburn.[104] The empirical approach allows the rapid evaluation of a large number of potential receptors. However, it lacks the elegance of design seen in many receptors produced through a rational design approach such as de Mendoza's chiral bicyclic guanidinium salt receptor for amino acids (see Figure 25).[79]

This thesis proposes the development of an extended tweezer structure such as **81** using a rational design. The development of an extended tweezer structure such as Kilburn's guanidinium based receptor **80** (see Figure 36) using rational design has not yet been reported. It is believed that a tweezer based upon a combination of features from previously successful carboxylate receptors developed within the Kilburn group, and the work conducted by others, is a feasible concept.

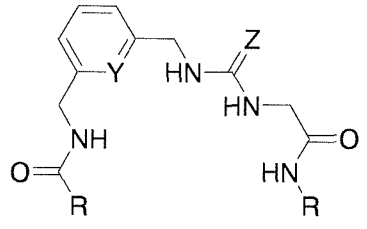


81 **82**

Figure 37. Tweezer receptor and its interaction with a tripeptide guest.

The biologically important tripeptide sequence L-Lys-D-Ala-D-Ala provides an interesting target as a guest molecule for a synthetic receptor. CPK modelling indicates that the hydrogen bonding network **82** describes a good fit between tweezer **81** and a general tripeptide of the form Ac-L-AA^1-L-AA^2-L-AA^3-O^-. Additional steric interactions can be controlled to provide a suitable enantioselective binding pocket and potentially the tailoring of this framework may provide a mimic for the antibiotic vancomycin.

A large component of the overall stability of the tweezer-peptide complex is derived from interaction between the host molecule and the carboxylate of the guest. A range of simple model carboxylate binding sites were conceived in order to maximise the key carboxylate binding interactions in the proposed tweezer **81**. Therefore carboxylate binding site **83** should provide an elementary binding site for these initial studies.



83, Y = CH or N, Z = S or NH_2

Figure 38. Model carboxylic acid binding site.

2 Thiourea Based Receptors

2.1 Introduction

Although thioureas provide a weaker interaction with carboxylates than the corresponding guanidinium salts, they present a well defined binding geometry and their simplicity of synthesis made them an ideal system for initial study. Hence, a range of thioureas were conceived to provide an insight into the efficacy of the carboxylate binding motif **84** described above (see 1.5). Subtle changes in the structure of the model carboxylate binding site, by variation in X, Y, R^1 and R^2, should provide both the optimum array of hydrogen bond donors and an evaluation of the effects of preorganisation through intramolecular hydrogen bonding (Y = N).

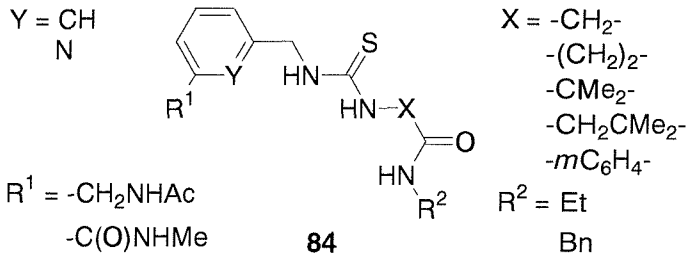
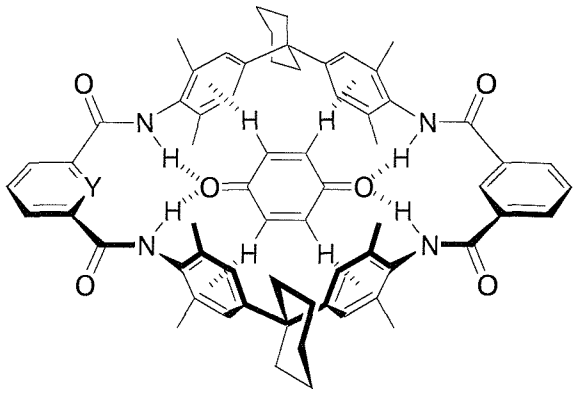


Figure 39. Thiourea model carboxylate binding site design.

The use of intramolecular hydrogen bonds to preorganise receptors has been exploited previously. Particularly pertinent examples include the use of the 2, 6-bis(aminomethyl)pyridine type motif described by Crabtree[44] in his simple receptors for anions (see Figure 9) and Hunter's[105] macrocyclic receptors for quinone (see Figure 40). Macrocycles **85** and **86** were found to complex *p*-benzoquinone with pyridyl receptor **86** exhibiting the greater affinity (K_a = 1.8 x 10^3 M^{-1}) than **85** (K_a = 1.2 x 10^3 M^{-1}) in $CDCl_3$. This was rationalised using molecular modelling which suggests that the preferred geometry of the pyridine containing macrocycle **86** is with both amides pointing into the cavity due to the presence of intramolecular hydrogen bonding with the lone pair of the pyridine nitrogen. The benzo analogue **85** however does not adopt a U-shaped cleft as its lowest energy conformation hence the binding of quinone is reduced.



85, Y = CH
86, Y = N

Figure 40. Hunter's macrocyclic receptor.

It should also be noted that, in addition to the desired preorganisational hydrogen bonding in the pyridine containing receptors, additional intramolecular hydrogen bonding is also possible due to the high proportion of donors and acceptors in the relatively flexible host molecules. Figure 41 describes a series of available intramolecular hydrogen bonding motifs for the thiourea model carboxylate binding sites. The presence of six, seven and eight member hydrogen bonding rings with the thiourea motif acting as both a hydrogen bond acceptor and donor are a feasible concept. In order to significantly participate in host-guest stabilisation, the intermolecular hydrogen bonds formed must overcome the enthalpic penalty for the breaking of any intramolecular hydrogen bonds present in the free host.

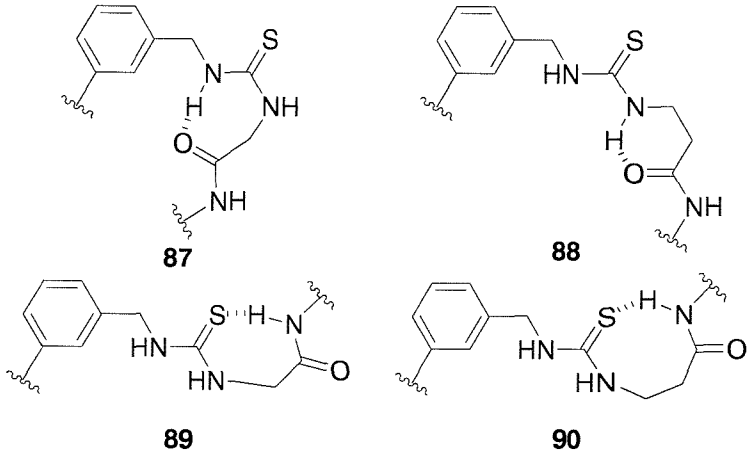


Figure 41. Proposed intramolecular hydrogen bonding motif for thiourea series.

2.2 Synthesis of Thioureas

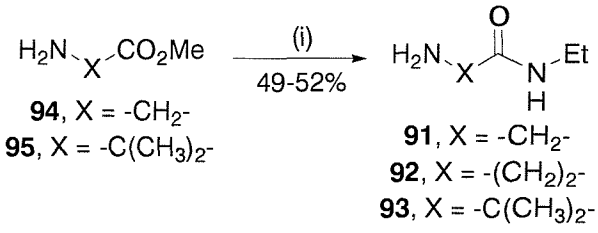
The synthesis of unsymmetrical thioureas is commonly achieved *via* an amine-isothiocyanate condensation with the isothiocyanate prepared from a different amine using an excess of a 'CS' equivalent.

2.2.1 Synthesis of Amine Precursors

To facilitate the synthesis of the required thioureas a range of amines were prepared containing the required functional variations.

2.2.1.1 Amino Acid Derived Amines

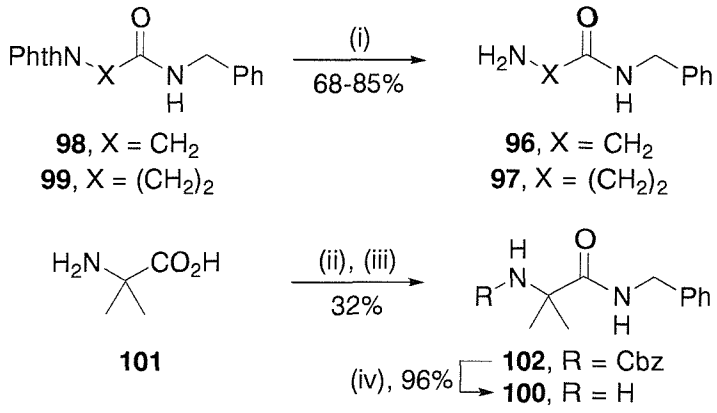
In order to derive the best mimic for the carboxylate binding site in the final desired tweezer receptor, the amino acid portion was initially terminated with a simple ethylamide as for amines **91-93** below. The aminolysis of esters **94** and **95** afforded the corresponding amino amides **91** and **93** as the free bases in approximately 50% yield. Amine **92** was prepared using the procedure of Stewart *et al.*[106]



Reagents and conditions. (i) 70%$EtNH_2/H_2O$.

Scheme 2. Synthesis of ethylacetamide containing amino acid amines.

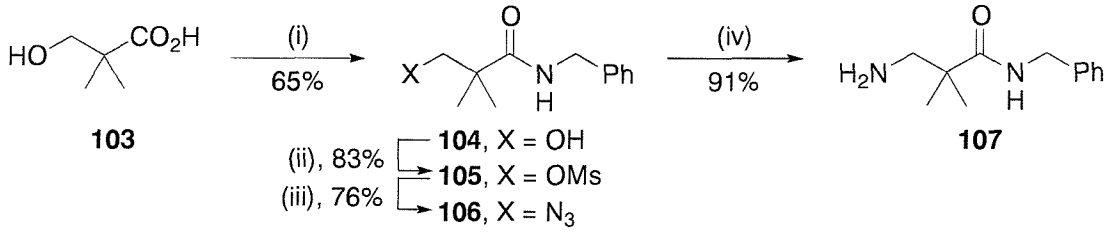
In order to investigate the effect of variation of the acetamide moiety on the efficacy of the model carboxylate binding site **84**, the corresponding benzylacetamides **96** and **97** were prepared. The synthesis of amines **96** and **97** was attempted using both Boc and phthalimide nitrogen protection. The phthalimide strategy afforded the best yields due to the simplicity of isolating the free bases **96** and **97**.



Reagents and conditions. (i) $(NH_2)_2.H_2O$, EtOH; (ii) CbzCl, 4 M NaOH; (iii) $BnNH_2$, EDC, HOBt, CH_2Cl_2; (iv) H_2/Pd/C, CH_3OH.

Scheme 3. Synthesis of amines **96**, **97** and **100**.

N-Phthalimide protected benzylacetamides **98** and **99** were synthesized using literature protocols[107] and deprotection under standard conditions yielded the corresponding amines **96** and **97** directly as their free bases without further purification in 68% and 85% yields respectively. Amine **100** was synthesized simply *via* Cbz protection of commercial 2-amino-2-methylpropanoic acid **101** in a 30% overall yield. The relatively low overall yield can be attributed to the steric congestion around the amine resulting in a low yield in the initial protection step.



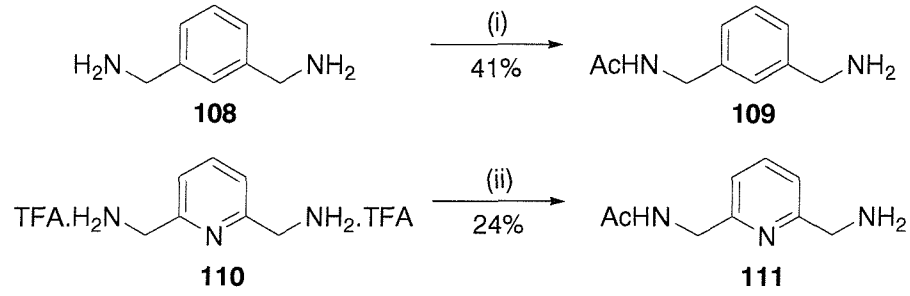
Reagents and conditions. (i) $BnNH_2$, EDC, CH_2Cl_2; (ii) MsCl, TEA, CH_2Cl_2; (iii) NaN_3, DMF; (iv) H_2/Pd/C, CH_3OH.

Scheme 4. Synthesis of extended *gem*-dimethyl amine.

The condensation of commercial acid **103** with benzylamine proceeded smoothly. Attempts to convert alcohol **104** directly to the corresponding protected amine using Mitsunobu[108, 109] chemistry with azide and phthalimide as nitrogen equivalents proved unsuccessful. Direct mesylation of alcohol **104** to give **105** followed by displacement afforded azide **106** in 63% overall yield. Simple hydrogenation gave the amine **107** directly as its free base in good yield.

2.2.1.2 Aromatic Derived Amines

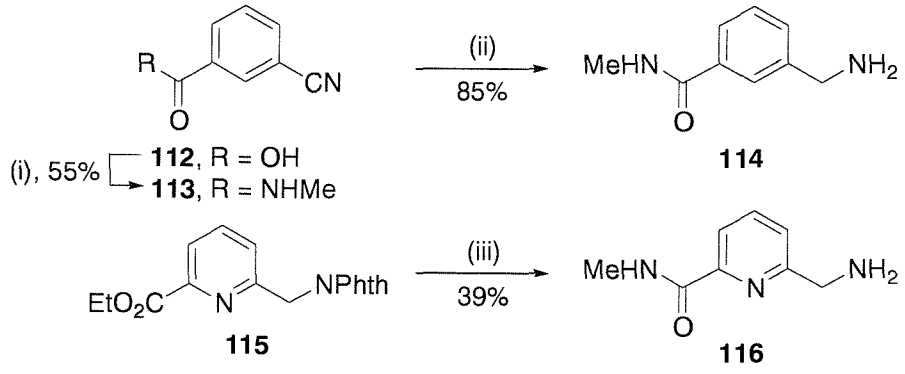
A range of aromatic amines were synthesized to provide diversity in the corresponding portion of the model carboxylate binding site **84**.



Reagents and conditions. (i) Ac_2O, TEA, $CHCl_3$; (ii) Ac_2O, TEA, CH_2Cl_2, DMF.

Scheme 5. Synthesis of benzylacetamide containing thioureas.

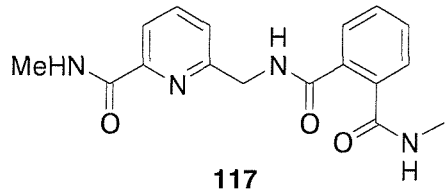
Statistical desymmetrisation of diamine **108** with acetic anhydride gave the related mono protected amine **109** in 41% yield. The synthesis of diamine **110** was achieved using the method of Miyahara in 44% over five steps from picolinic acid.[110] Statistical mono protection of diamine **110** was achieved using one equivalent of acetic anhydride, with a small amount of DMF to improve the solubility of the bis ammonium salt, and yielded amine **111** in a best yield of 24%. This reaction proved to be very capricious due to the insolubility of the starting salt and the difficulty of isolation of the amine product. The low yield of this step can be attributed to the limited solubility of the bis TFA **110** salt in the reaction media; however increasing the amount of DMF did not result in an increased yield.



Reagents and conditions. (i) $SOCl_2$, CH_3OH then 40% $MeNH_2/H_2O$, THF; (ii) H_2/Pd/C, CH_3OH, (iii) 40% $MeNH_2/H_2O$.

Scheme 6. Synthesis of amines **114** and **116**.

Conversion of acid **112** to amide **113** was achieved cleanly *via* aminolysis of the intermediate methyl ester in moderate yield. Hydrogenation of nitrile **113** to the corresponding amine **114** was slow but clean and proceeded in 85% yield in 72 h. Aminolysis of known ester[111] **115** to give the corresponding amide gave the free amine **116** directly *via* cleavage of the phthalimide group. Evidence for the presence of significant amounts of ring opened phthalimide **117** was seen in the ^{1}H NMR of the crude but all attempts at purification of this phthalate derivative for full characterisation were unsuccessful.



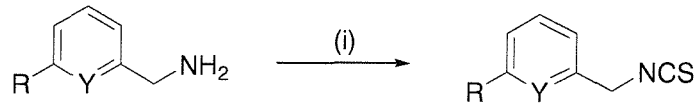
117

Figure 42. Proposed by-product from aminolysis of **115**.

Two routes were available for the synthesis of the required thioureas *via* formation of the isothiocyanate of each of the constituent amines prepared above. Thioureas were prepared *via* both routes and their synthesis is discussed below.

2.2.2 Thioureas via Benzyl/Pyridyl Isothiocyanates

In order to facilitate the synthesis of the desired thioureas a series of isothiocyanates were prepared.



109, Y = CH, R = -CH_2NHAc
114, Y = CH, R = -C(O)NHMe
111, Y = N, R = -CH_2NHAc
116, Y = N, R = -C(O)NHMe

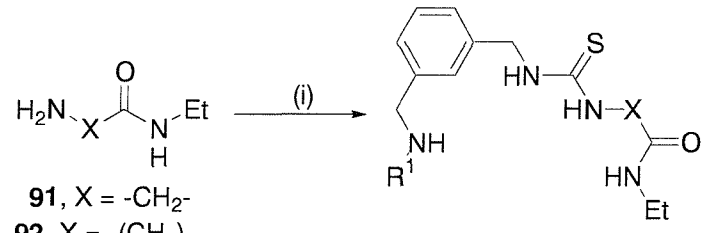
118, Y = CH, R = -CH_2NHAc
119, Y = CH, R = -CH_2NHBoc
120, Y = CH, R = -C(O)NHMe
121, Y = N, R = -CH_2NHAc
122, Y = N, R = -C(O)NHMe

Reagents and conditions. (i) $SCCl_2$, K_2CO_3, H_2O, CH_2Cl_2.

Scheme 7. Synthesis of benzylisothiocyanates.

Conversion of amine **109** to the corresponding isothiocyanate **118** was achieved with thiophosgene under basic biphasic conditions in 80% yield. Isothiocyanate **119** was prepared using an analogous procedure as described by Anslyn.[112] Attempts to

prepare isothiocyanates **118** and **119** using the DCC/CS_2 method described by Anslyn gave significantly lower yields.[113] The conversion of amine **114** to isothiocyanate **120** was achieved in a fair 67% yield. Attempts to isolate isothiocyanates **121** and **122** from the corresponding amines using either $SCCl_2$ or DCC/CS_2 were unsuccessful presumably due to the potential side reactions involving the pyridine nitrogen. The synthesis of thiourea **38** (see Figure 18) was achieved *via* use of an isothiocyanate analogous to **122** as a crude mixture and direct reaction with an amine. [62] However, attempts using comparable conditions were unsuccessful for both amines **111** and **116**.



91, X = $-CH_2-$
92, X = $-(CH_2)_2-$
93, X = $-C(CH_3)_2-$

123, R^1 = Boc, X = $-CH_2-$
124, R^1 = Ac, X = $-CH_2-$
125, R^1 = Ac, X = $-(CH_2)_2-$
126, R^1 = Ac, X = $-C(CH_3)_2-$

Reagents and conditions. (i) see Table 1.

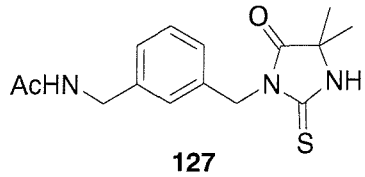
Scheme 8. Synthesis of ethylacetamide thiourea receptors.

RNCS	RNH_2	Conditions	Product	Yield
119	**91**	CH_2Cl_2, 24 h	**123**	80%
118	**91**	CH_2Cl_2, 24 h	**124**	78%
118	**92**	TEA, CH_2Cl_2, 24 h	**125**	85%
118	**93**	CH_2Cl_2,96 h	**126**	38%

Table 1. Yields for formation of initial thiourea receptors.

Reaction to produce thioureas **123** and **124** proceeded smoothly and in the case of **124** the clean thiourea precipitated from the reaction mixture. Condensation of amine **92** and isothiocyanate **118** in the absence of base displayed little or no reaction after 24 h. However, the addition of base resulted in clean reaction to the corresponding thiourea **125** in the same period. The reaction of isothiocyanate **118** with amine **93** to give **126** proceeded more slowly due to the tertiary centre adjacent to the reacting amine in **93**. In addition, a significant decrease in yield compared to thioureas **123**-

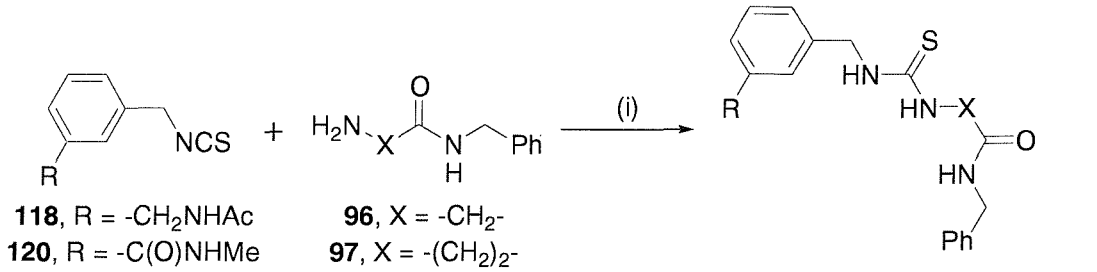
124 can be attributed to the formation of thiohydantoin **127** as a result of the displacement of ethylamine by the newly formed thiourea.



127

Figure 43. Thiohydantoin observed from the condensation of **93** and **118**.

In order to prevent difficulties with the purification of the thioureas as result of their insolubility, the ethylacetamide motif was switched to the corresponding more lipophilic benzylacetamide.



118, R = -CH_2NHAc
120, R = -C(O)NHMe

96, X = -CH_2-
97, X = -$(CH_2)_2$-
100, X = -$(CMe_2)_2$-
107, X = -CH_2CMe_2-
128, X = -*m*C_6H_4-

129, R = -CH_2NHAc, X = -CH_2-
130, R = -CH_2NHAc, X = -$(CH_2)_2$-
131, R = -CH_2NHAc, X = -$(CMe_2)_2$-
132, R = -CH_2NHAc, X = -CH_2CMe_2-
133, R = -CH_2NHAc, X = -*m*C_6H_4-
134, R = -C(O)NHMe, X = -CH_2-
135, R = -C(O)NHMe, X = -$(CH_2)_2$-

Reagents and conditions. (i) see Table 2.

Scheme 9. Synthesis of benzylthioureas **129-135**.

RNCS	RNH_2	Conditions	Product	Yield
118	**96**	CH_2Cl_2, 18 h	**129**	74%
118	**97**	TEA, CH_2Cl_2, 22 h	**130**	60%
118	**100**	CH_2Cl_2,24 h	**131**	-
118	**107**	CH_2Cl_2, 48 h	**132**	73%
118	**128**	CH_2Cl_2, 48 h	**133**	55%
120	**96**	TEA, CH_2Cl_2, 18 h	**134**	64%
120	**97**	TEA, CH_2Cl_2, 24 h	**135**	71%

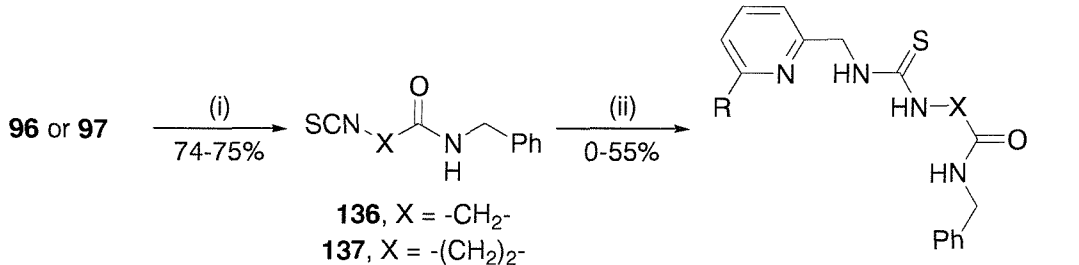
Table 2. Yields for formation of benzylthioureas **129-135**.

The condensation of isothiocyanates **118** and **120** with amines **96**, **97**, **100**, **107** and **128** to yield the corresponding thioureas **129-135** was achieved in fair to good yield

depending on the nature of the amine. As above with β–alanine derived amine **92** the presence of a base was required with amine **97** to decrease the reaction time. The reaction with amine **107** was slow but proceeded to completion in reasonable time without the presence of TEA. Reaction involving the less nucleophilic aniline **128**, prepared using the chemistry of Venuti,[114] required gentle reflux for the reaction to proceed albeit in an expected slightly reduced yield compared to aliphatic amines. The reaction of amine **100** did not produce the required thiourea **131** cleanly. Examination of the crude ^{1}H NMR indicated the presence of thiourea **131** and trace impurities. However, after purification the presence of another co-eluting product was observed. It could be that **131** decomposes to the corresponding thiohydantoin **127**, as observed above (see Figure 43), which could not be separated.

2.2.3 Thioureas via Amino Acid Isothiocyanates

The difficulty of synthesis of pyridine containing isothiocyanates **121** and **122** (see Scheme 7) was circumvented by the synthesis of the required thioureas using the isothiocyanatoacetamide **136** and isothiocyanatopropanamide **137**.



136, X = -CH_2-
137, X = -$(CH_2)_2$-

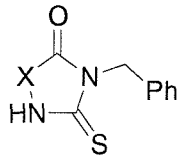
140, R = -CH_2NHAc, X = -CH_2-
141, R = -CH_2NHAc, X = -$(CH_2)_2$-
142, R = -C(O)NHMe, X = -CH_2-
143, R = -C(O)NHMe, X = -$(CH_2)_2$-

Reagents and conditions. (i) $SCCl_2$, $NaHCO_3$, CH_2Cl_2, H_2O; (ii) **111** or **116**, CH_2Cl_2.

Scheme 10. Synthesis of pyridyl thioureas.

Amines **96** and **97** were converted to the equivalent isothiocyanates **136** and **137** in 75% and 74% yield respectively when the reaction was carried out in the presence of $NaHCO_3$ at 4 °C. Reaction in the presence of K_2CO_3 or reaction at room temperature gave significant portions of the corresponding known thiohydantoins **138** and **139** (see Figure 44).[115, 116] Isothiocyanates **136** and **137** are stable for a few days at -20 °C or can be prepared and used directly in the following step. All attempts to

condense isothiocyanates **136** and **137** with amine **111** to yield thioureas **140** and **141** were unsuccessful. Reaction in EtOH, CH_3CN and CH_2Cl_2 at room temperature and at reflux failed to yield the required thioureas and the only products isolated had ^1H NMR's consistent with the formation of thiohydantoins **138** and **139**. The capricious nature of the synthesis of amine **111** and the difficulties with the following condensation lead to attempts to synthesize **140** and **141** being abandoned. However, thioureas **142** and **143** could be synthesized from amine **116** and isothiocyanates **136** and **137** in fair yields, 55% and 50% respectively. Therefore with pyridyl thioureas **142** and **143** in hand the effect of preorganisation *via* intramolecular hydrogen bonding in the model system could still be investigated.

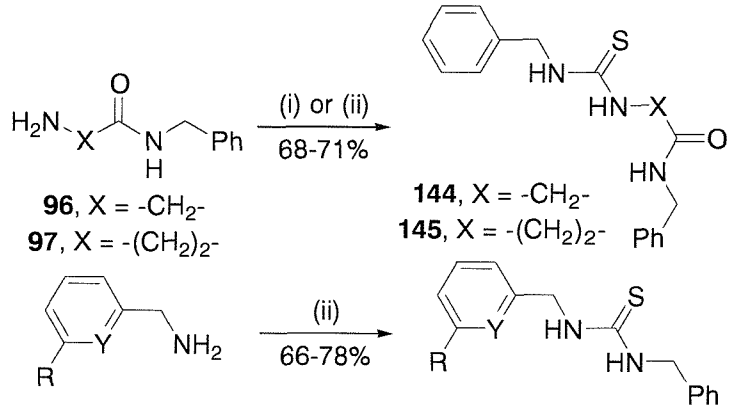


138, X = -CH_2-
139, X = -$(CH_2)_2$-

Figure 44. Thiohydantoin side product from formation of **136** and **137**.

2.2.4 Preparation of Control Compounds

In order to ascertain the importance and contribution of the amide components of the various receptor motifs synthesised above, a range of analogous thioureas were synthesised lacking one amide hydrogen bond donor in each case. In general the hydrogen bond donor omitted was replaced with a benzyl group to maintain the solubility of the thioureas.



96, X = -CH_2-
97, X = -$(CH_2)_2$-

144, X = -CH_2-
145, X = -$(CH_2)_2$-

109, Y = CH, R = -CH_2NHAc
114, Y = CH, R = -C(O)NHMe
116, Y = N, R = -C(O)NHMe

146, Y = CH, R = -CH_2NHAc
147, Y = CH, R = -C(O)NHMe
148, Y = N, R = -C(O)NHMe

Reagents and conditions. (i) BnNCS, CH_2Cl_2; (ii) BnNCS, TEA, CH_2Cl_2.

Scheme 11. Synthesis of control thioureas.

Thioureas **144-148** were synthesized from their corresponding amines **96, 97, 109, 114** and **116** using benzyl isothiocyanate in good yields. As observed above the rate of reaction of amine **97** was significantly increased in the presence of base.

2.3 Binding Studies with Thioureas

2.3.1 Host Conformational Analysis

Analysis of the chemical shifts of the free host in DMSO-d_6 provides some insight into the formation of intramolecular hydrogen bonds in the thiourea series synthesised above, in particular the pyridine containing receptors **142, 143** and **148**. In all cases the concentrations of the thioureas were of the order of 4-6 mM.

Thiourea	δ /ppm†			
	H-1	H-2	H-3	H-4
144	-	8.51	7.94	8.77
145	-	8.26	7.83	8.70
146	8.63	8.25	8.25	-
147	8.70	8.39	8.39	-
148	8.95	8.49	8.42	-
123	7.48	8.32	7.65	8.07
124	8.44	8.32	7.67	8.08
125	8.45	8.04	7.60	8.00
129	8.62	8.51	7.93	8.78
130	8.64	8.25	7.83	8.72
134	8.71	8.57	7.98	8.80
135	8.72	8.32	7.87	8.72
142	8.94	8.61	8.04	8.75
143	9.02	8.45	8.12	8.76
132	8.63	8.44	7.62	8.56
133	8.63	9.42	10.67	9.48
149	8.62	8.16	7.79	8.62
1,3-dimethylthiourea	-	7.70	7.70	-

† - Where protons are coincident they are reproduced in multiple columns for clarity.

Table 3. Host chemical shift analysis for thiourea series in DMSO-d6.

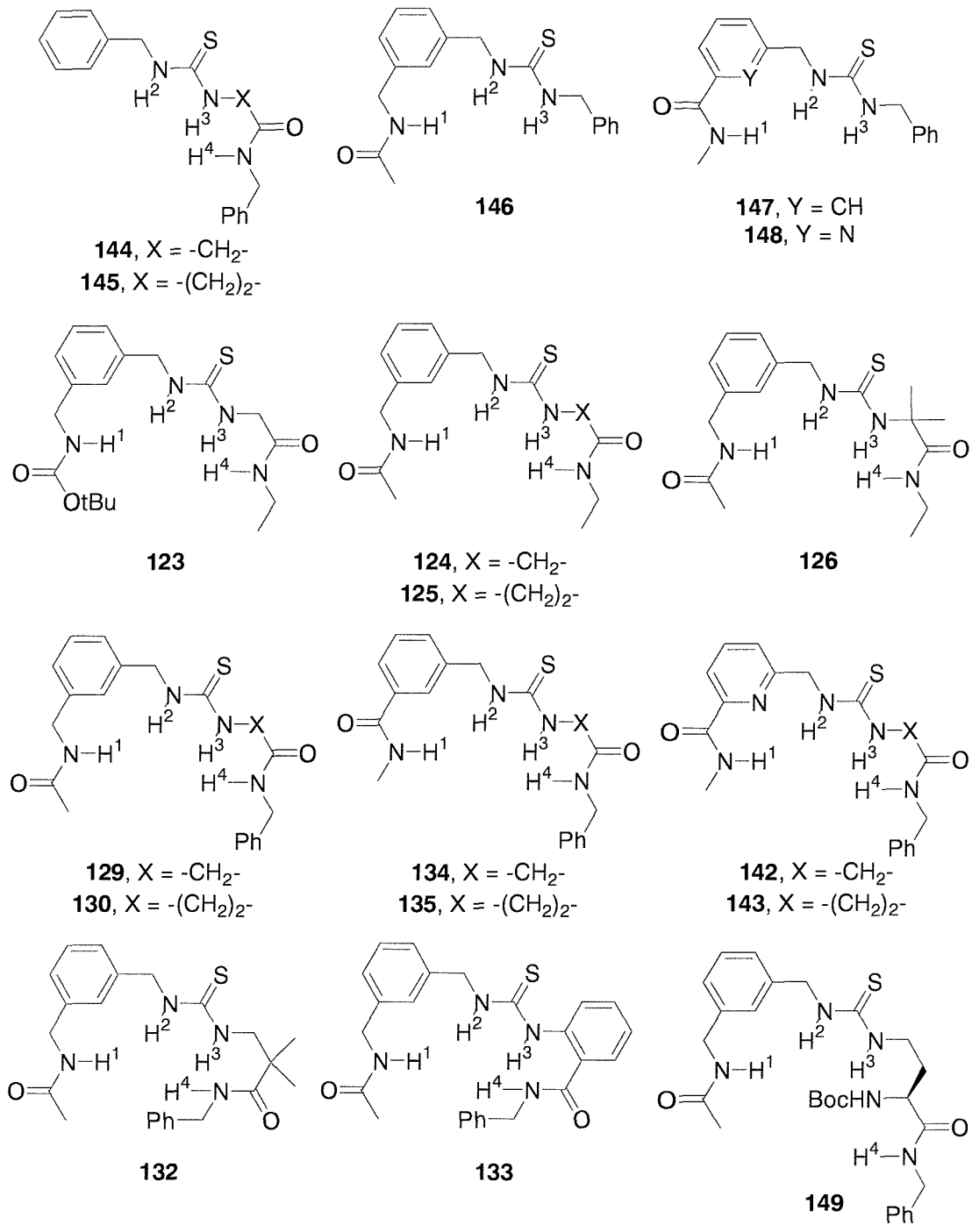


Figure 45. Model carboxylate binding site series.

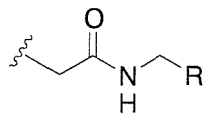
Some general trends were observed in the analysis of the chemical shifts of the free hosts. In all cases the chemical shift of amide H-4 in the thioureas derived from glycine **144**, **123**, **124**, **129**, **134** and **142** were shifted downfield approximately 0.25 ppm compared to their homologues derived from β-alanine. Presumably this was due to the formation of stronger intramolecular hydrogen bonds. In comparison with 1,3-

dimethylthiourea the H-2 signal in all thioureas is shifted significantly downfield presumably due to intramolecular hydrogen bonding.

Comparison of **148**, **142** and **143** with **147**, **134** and **135** indicates that in all cases the H-1 amide in the pyridine containing receptors was shifted significantly downfield with respect to their benzo analogues ($\Delta\delta$ = 0.25, 0.23 and 0.30 ppm respectively). This suggested the formation of the desired preorganised U-shape to provide a good fit for a carboxylate guest. However comparing H-2 in the same thioureas the pyridine containing receptors **148**, **142** and **143** do not show significant differences in chemical shift compared to benzo analogues **147**, **134** and **135**. In the cases of **134** and **135** this could be as a result of the observed intramolecular hydrogen bonding above (see Figure 41).

In thioureas **146**, **130**, **124**, **125**, **129**, **130**, **132** and **149** the shift of amide proton H-1 was consistent with that observed in the literature[107, 117] for a corresponding motif **150** (see Figure 46) indicating that no hydrogen bonding was observed to or from this functionality in these thioureas.

Amide proton H-4 in all receptors derived from glycine or β–alanine showed significant differences in chemical shift compared to literature values for analogous motif **150** and **151** (see Figure 46).[107, 117] In both the ethylacetamide and benzylacetamide containing receptors the shifts were downfield ($\Delta\delta \approx 0.2$ - 0.4 ppm) indicating significant hydrogen bonding either to the amide carbonyl or through the amide N-H. This was supported by the observed chemical shifts in thioureas **132** and **149**. In both cases thiourea H-2 and amide H-4 are less downfield than in the corresponding thiourea **130** due to steric demand and entropy respectively. Although the differences are only small this is corroborative evidence for the earlier arguments.

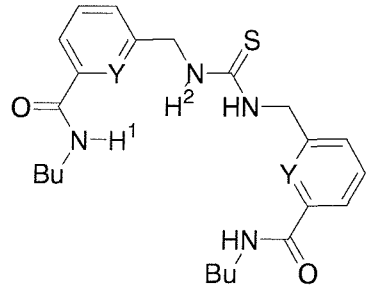


150, R = Ph, δ(amide) = 8.55 ppm
151, R = Me, δ(amide) = 7.64 ppm

Figure 46. Literature chemical shift value for amides in DMSO-d_6.

Therefore it could be surmised that the presence of the pyridine does result in some degree of preorganisation in the free host although only through hydrogen bonding to

the amide H-1 and little preorganisation to the thiourea H-2 is apparent. This is in accordance with Crabtree's[44] clefts (see Figure 9), Hunter's[105] macrocyclic receptors (see Figure 40) and Kilburn's[62] analogous receptors **152** and **153** in which significant preorganisation through intramolecular hydrogen bonding is observed on going from **152** to **153** in 10% DMSO-d_6/$CDCl_3$. However this is the first time this preorganisation effect has been observed in neat DMSO-d_6. Also significant hydrogen bonding is observed between either the amide carbonyl of H-4 and the thiourea proton H-2 or *vice versa* in all glycine and β–alanine derived receptors.



152, Y = CH
153, Y = N

Figure 47. Kilburn's receptor for benzoate.

2.3.2 Analysis of Chemical Shift Changes for Thioureas

The efficacy of model carboxylate binding sites was studied using TBA acetate as guest in both $CDCl_3$ and DMSO-d_6. Literature precedent[118] and previous experience within the Kilburn group[119] indicated the possibility for formation of dimers or higher order oligomers by thioureas in a non-polar solvent. Dilution experiments indicated that thioureas **123** and **124** only formed very weak dimers in $CDCl_3$ ($K_{dimer} < 10\ M^{-1}$), hence dimerisation of thioureas studied thereafter was ignored.

Addition of one equivalent of TBA acetate to thiourea **123** in $CDCl_3$ resulted in considerable complex induced shifts in the thiourea and amide protons in the host molecule in particular the thiourea protons H-2 ($\Delta\delta \approx 2.9$ ppm) and H-3 ($\Delta\delta \approx 3.2$ ppm) and the amide proton H-4 ($\Delta\delta \approx 1.3$ ppm). The magnitudes of these shifts were indicative of the formation of a strong host-guest complex in which the interactions between the thiourea/amide functionalities and the carboxylate of the acetate were key. The shift in H-4 ($\Delta\delta \approx 0.3$ ppm) was as expected, considerably smaller than for

the thiourea and amide protons due to the weak hydrogen bond donor ability of the carbamate functionality and the steric demand of the *tert*-butyl group. Overall thiourea **123** formed a stable complex with TBA acetate in $CDCl_3$ (K_a = 1.5 x 10^3 M^{-1}) In addition, the CH_2 protons derived from glycine exhibited a small complex induced shift and were resolved from a broad singlet to a doublet. Insolubility of all other thioureas in $CDCl_3$ in the concentration range of NMR titration experiments (~ 10 mM) prevented direct comparison in this media.

Attempts to ascertain the affinity of thiourea **126** for acetate were hampered by the instability of the motif in the basic conditions experienced in the titration. ^{1}H NMR indicated the decomposition of thiourea **126** to the corresponding thiohydantoin **127** (see Figure 43). Table 4 describes the changes in the chemical shift of the hydrogen bonding protons after one equivalent of guest was added in DMSO-d_6.

Thiourea	Δδ(1 eq.) /ppm†			
	H-1	H-2	H-3	H-4
123	0.00	0.66	0.84	-0.01
144	-	0.59	0.73	-0.02
145	-	0.62	0.68	0.07
146	0.04	1.41	1.41	-
129	0.01	0.50	0.66	-0.01
124	0.04	0.88	1.12	-0.03
130	0.02	0.55	0.63	0.06
125	0.01	0.57	0.64	0.05
147	0.10	0.97	0.97	-
134	0.10	0.54	0.82	0.00
135	0.05	0.59	0.70	0.05
148	0.45	1.24	1.46	-
142	0.24	0.57	0.70	0.00
143	0.41	0.97	1.31	0.10
132	0.02	0.35	0.39	0.03
133	0.00	0.26	0.28	0.02
149	0.01	1.08	1.17	0.54
1,3-dimethylthiourea	-	0.56	0.56	-

† - Where protons are coincident they are reproduced in multiple columns for clarity.

Table 4. ^{1}H NMR chemical shift data, 1 eq. of TBA-acetate added in DMSO -d_6.

In general throughout the thiourea series large shifts were observed for the thiourea protons H-2 and H-3 in accordance with the formation of the expected eight member bidentate hydrogen bonding motif normally observed in thiourea-carboxylate systems (see Figure 16). The magnitude of the thiourea shifts in H-2 and H-3 were suitably concurrent to indicate the formation of the symmetrical eight member

hydrogen bonding ring with minimal deviation from the expected linear thiourea-carboxylate geometry. The shifts in the amide protons H-1 and H-4 were in general small, especially for H-4 which shows little or no shift throughout the series (with the exception of thiourea **149**). Amide H-1 also does not shift significantly except in cases **148**, **142** and **143** which contain pyridine units although the glycine derived thiourea **142** displays a smaller shift than the β–alanyl or benzylamine derived analogues **143** and **148**.

2.3.3 Analysis of Binding Constants for Thiourea Series

NMR binding studies were conducted maintaining a constant concentration of host throughout and the binding curves were all fitted to a clean 1:1 binding mode. Binding studies with the thiourea series by isothermal titration calorimetry (ITC) were performed in DMSO at 25 °C with in a typical starting host concentration of 2 mM and titrating with a 78 mM guest solution. At these concentrations host-host and guest-guest interactions were assumed to be minimal and hence ignored.

Comparison of thiourea **123** with the simple 1,3-dimethylthiourea indicated a five fold increase in complex stability ($\Delta\Delta G$ = 4.2 kJ mol^{-1}) however small shifts in the carbamate, H-1, and amide, H-2, protons suggested a limited participation in the formation of the host-guest hydrogen bonding array. However, the exchange of intramolecular hydrogen bonds for intermolecular hydrogen bonds might result in little or no shift in the H-1 and H-4 NHs (see Table 4) but still account for the increase in complex stability.

Thiourea	NMR		ITC	
	K_a /M^{-1}	ΔG /kJmol^{-1}	K_a /M^{-1}	ΔG /kJmol^{-1}
123†	160±30	-12.6	-	-
144	230±20	-13.5	370±25	-14.7
145	230±20	-13.5	260±10	-13.8
146	240±50	-13.6	380±20	-14.7
147	260±30	-13.8	440±30	-15.1
148	370±30	-14.7	560±25	-15.7
129	260±20	-13.8	140±10	-12.3
124†	550±60	-15.6	-	-
130	610±70	-15.9	350±20	-14.5
125†	1.2 x 10^3±190	-17.6	-	-
132	180±10	-12.9	400±20	-14.9
133‡	200±10	-13.1	-	-
149	890±70	-16.8	960±50	-17.0
134	290±20	-14.0	230±10	-13.5
142	950±100	-17.0	980±90	-17.1
135	200±10	-13.1	270±10	-13.9
143	1.5 x 10^3±200	-18.1	2.2 x 10^3±320	-19.1
1,3-dimethylthiourea‡	30±10	-8.4	-	-

† - ITC data not obtained. ‡ - ITC output not sufficiently consistent to allow good fitting

Table 5. Binding constant data for thiourea series in DMSO by NMR and ITC.

The stability of the complexes of acetate and thioureas **144**, **145**, **146** and **147** were approximately equivalent as determined by NMR and the binding constants determined by ITC are in reasonable accordance Hence in these simple control systems missing one hydrogen bond donor H-4, in the cases of **144** and **145,** and H-1, in the cases of **146** and **147**, each amide-carboxylate hydrogen bond contributed a similar amount to the overall complex stability ($\Delta\Delta G \approx 5$ kJ mol^{-1}) compared to 1,3-dimethylthiourea. The effect of changing spacer length to the hydrogen bond donor H-4 from one methylene in **144** to two methylenes in **145** and the H-1 hydrogen bond donor from acetate in **146** to benzoate in **147** had no appreciable effect on the overall complex stability in these cases. Changing from benzo thiourea **147** to the corresponding pyrido thiourea **148** resulted in only a small increase in complex stability ($\Delta\Delta G = 0.7$ kJ mol^{-1}) despite a larger shift in the H-1 amide in **148** presumably due to electrostatic repulsion between the nitrogen lone pair and the carboxylate guest.

Receptors containing both H-1 and H-4 amides display significant variation in complex stability with acetate despite only small modifications in the molecular

structure. Replacement of a H-4 benzylacetamide in **129** and **130** with a corresponding ethylacetamide in **124** and **125** resulted in a two fold increase in complex stability ($\Delta\Delta G \approx 2$ kJ mol^{-1}), a similar degree of increase was observed on going from glycinyl, **129** and **124**, to a β–alanyl, **130** and **125**, derived receptor ($\Delta\Delta G \approx 2$ kJ mol^{-1}). The insolubility of the ethylacetamide based receptors however prevented further study of these higher binding receptors. However, the absolute values of the binding constants are not as important in this series as the relative effectiveness of the related structures.

Modification of thiourea **130** by constraining the flexible chain with either a gem-dimethyl in **132** or a rigid aromatic **133** did not effect an increase in the binding constant as determined by NMR or ITC. Presumably despite the inherent increase in entropy due to loss of fewer degrees of freedom in **132** and **133** the H-4 amide hydrogen bond donors are poorly arranged to associate with the carboxylate. Extension of the spacer for the H-4 amide by one methylene in **149** resulted in a two fold increase in binding constant with respect to **130** ($\Delta\Delta G \approx 1$ kJ mol^{-1}) and receptor **149** bound acetate with ~30 fold increase in binding constant with respect to 1,3-dimethylthiourea ($\Delta\Delta G \approx 9$ kJ mol^{-1}).

The effect of the introduction of pyridine to preorganise the system in **142** and **143** was marked in the case of receptors containing both amide hydrogen bond donor moieties in comparison to the benzo analogues **134** and **135**. The glycine derived thiourea **142** bound acetate with three times the affinity of the corresponding benzo compound **134** in DMSO ($\Delta\Delta G \approx 3$ kJ mol^{-1}) as determined by both NMR and ITC. The longer chained β-alanine derived pyrido thiourea **143** bound acetate with approximately eight fold increase in binding constant with respect to **135** ($\Delta\Delta G \approx 5$ kJ mol^{-1}). Therefore preorganisation through intramolecular hydrogen bonding to the pyridine lone pair is worth 3-5 kJ mol^{-1} in complex stability over the non pyrido analogues in this series. Overall the pyrido containing thioureas **142** and **143** bind acetate in DMSO with approximately 30 fold ($\Delta\Delta G \approx 9$ kJ mol^{-1}) and 50 fold ($\Delta\Delta G \approx 10$ kJ mol^{-1}) increase in binding constant respectively compared to 1,3-dimethylthiourea. This corresponds with the generally higher binding of the β-alanyl derived receptors compared to there gycinyl analogues observed above and the larger shift for the H-1 amide in **143** compared to respect to **142**. This increase in binding

affinity with the addition of preorganisation was contrary to that obtained with Kilburn's thioureas **152** and **153** above (see Figure 47).[62] The repulsion between the lone pair of the pyrido thiourea **153** and the carboxylate guest is such as to destabilise the host guest complex with respected to the benzo analogue **152** (K_a = 420 M^{-1} and K_a = 740 M^{-1} respectively, $\Delta\Delta G$ = 1.4 kJ mol^{-1}) in 10% DMSO-d_6/$CDCl_3$.

2.3.4 Analysis of Thermodynamic Data for Thiourea Series

ITC also allows derivation of the thermodynamic components of the binding event, Table 6 describes the associated data for the thioureas series.

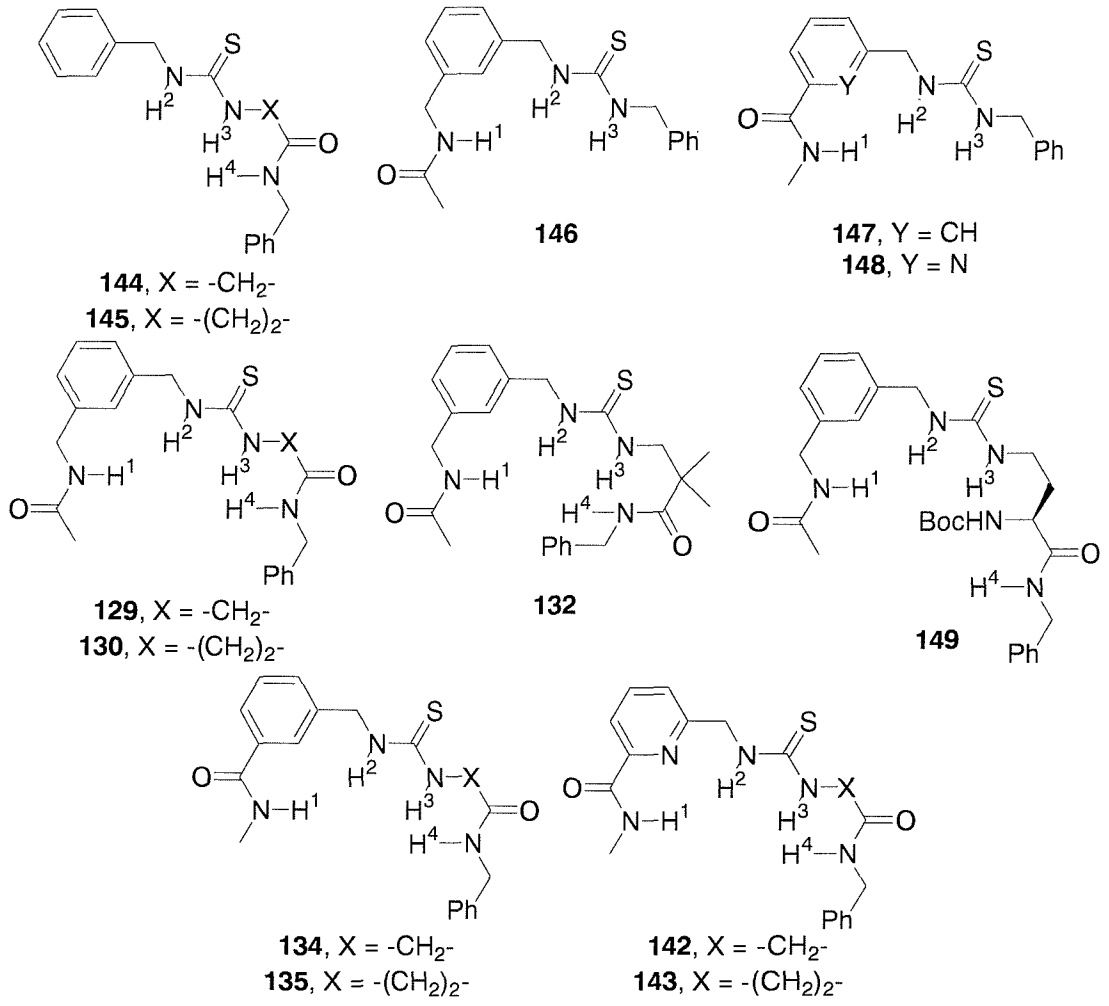


Figure 48. Thiourea series of carboxylate receptors.

Evaluation of the ITC data for thiourea **144** and **145** indicated as expected that acetate was bound by glycine derived receptor **144** with a larger entropic component to the binding of **145**. Conversely, the better arrangement of hydrogen bond donor H-4 in receptor **145** as evinced by the larger shift observed in the NMR studies results

in the binding of acetate being enthalpically more favoured than the shorter chained receptor **144**. However, the overall balance indicates that **144** and **145** were similarly effective receptors for acetate ($\Delta\Delta G < 1$ kJ mol^{-1}).

Thiourea	ΔH /kJmol^{-1}	$T\Delta S$ /kJmol^{-1}	ΔG /kJmol^{-1}
144	-4.8	9.9	-14.7
145	-6.6	7.1	-13.8
146	-9.4	5.3	-14.7
147	-8.6	6.5	-15.1
148	-12.4	3.3	-15.7
129	-4.4	7.9	-12.3
130	-4.7	9.8	-14.5
132	-2.8	12.1	-14.9
149	-12.5	4.5	-17.0
134	-6.3	7.2	-13.5
142	-3.8	13.3	-17.1
135	-8.7	5.2	-13.9
143	-4.3	14.8	-19.1

Table 6. Entropy and enthalpy contribution data for thiourea series in DMSO.

Thioureas **146** and **147** were found to bind acetate with similar efficacy and the relative entropic and enthalpic contributions to the free energy were very similar in each case indicating that a similar binding mode was observed in these two receptors. In **148** the expected higher binding constant compared to **146** and **147** was observed; however the binding was largely enthalpy dominated and relatively entropically disfavoured.

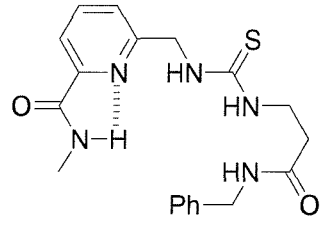
Thiourea **130** binds acetate driven by a large entropic component despite the relative flexibility of the receptor. The more constrained thiourea **132** was more entropically driven and the more rigid structure resulted in a less perfect hydrogen bond donor arrangement for H-4 and hence a lower enthalpy, thiourea **149** with a larger more flexible spacer between the thiourea and H-4 formed a better hydrogen bond donor arrangement and hence the binding of acetate was enthalpically driven with only a small entropic component to the overall binding strength due to the flexibility of the host molecule.

ITC of pyrido receptors **142** and **143** confirmed the findings of the NMR titrations in that preorganisation contributes significantly to the binding strength. Comparison of **142** and **134** showed that the binding of pyrido thiourea **142** was considerably more

entropically favoured due to less reorganization upon binding than **134**. However, the electron pair repulsion between the carboxylate anion and the pyridine lone pair resulted in an enthalpically less favoured binding event. The analogous β–alanine derived receptors **143** and **135** display similar changes in the relative entropic and enthalpic contributions in the binding of acetate.

2.4 Conclusions

The addition of extra hydrogen bond donor moieties in the form of amides resulted in significantly increased affinities of a range of simple thioureas for carboxylates, in the form of acetate. Appreciable evidence for the formation of intramolecular hydrogen bonds in the free host did not preclude increased stability of the host-guest complexes in DMSO. Thiourea **143** was identified as the optimum carboxylate binding site from this series. Increasing the flexibility of the receptor generally resulted in higher binding constants, presumably due to a better host-guest fit, despite the associated entropic penalties. The formation of an intramolecular hydrogen bond as in **143** significantly increases the overall complex stability complared to benzyl analogues due to preorganisation of the receptor molecule.



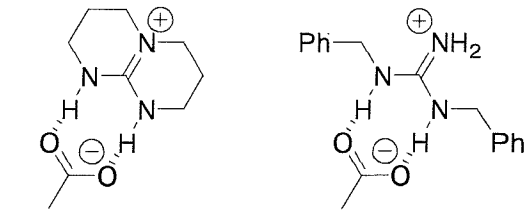
143

Figure 49. Strongest binding thiourea receptor for acetate

3 Guanidinium Salt Based Receptors

3.1 Introduction

Guanidinium **154**, as its tetraphenylborate salt, forms a very stable complex with TBA acetate in DMSO.[69] The analogous acyclic guanidinium **155**, as its bromide salt, forms a stable complex with TBA acetate even in highly competitive media.[24]

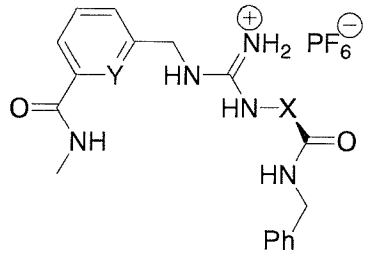


$K_a = 5.6 \times 10^3\ M^{-1}$ in DMSO **154**

$K_a = 25\ M^{-1}$ in 10% CH_3OH/H_2O **155**

Figure 50. Simple carboxylate receptors.

In tweezer architecture **81** (see Figure 37), the ideal case would be a receptor which binds carboxylates with good affinities in H_2O. In order for this to be feasible, the principle interaction with the carboxylate must be strong *i.e.* a guanidinium. In order to investigate the suitability of guanidinium salt containing receptor **156**, from both a synthesis and a supramolecular perspective, a range of receptors was conceived containing elements from those receptors discussed in Chapter 2.



X = CH_2, $-(CH_2)_2-$, Y = CH, N **156**

Figure 51. Proposed guanidinium based receptor for acetate.

Receptor **156** contains the same elements as the thioureas discussed above (see Chapter 2) with points of variation X and Y to provide some insight into the optimal

arrangement of hydrogen bond donors and intramolecular hydrogen bond derived preorganisation.

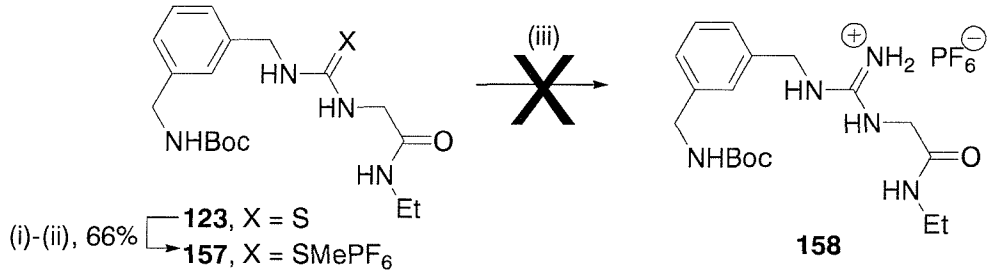
3.2 Guanidinium Salt Synthesis

A number of routes are available from the literature for the synthesis of guanidiniums.[120-126] With the thioureas from Chapter 2 in hand, activation of the sulphur followed by displacement with an amine was first investigated.

3.2.1 Guanidinium Salts via Thiourea Methylation

3.2.1.1 Acyclic Guanidinium Salts

Alkylation of the thiourea and subsequent treatment with an amine has been used to synthesise a range of substituted guanidiniums.[121, 123, 124] In principle, this is an ideal strategy as it requires relatively mild conditions and often does not require purification of the resultant guanidinium salts.

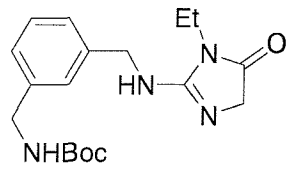


Reagents and conditions. (i) MeI, acetone; (ii) NH_4PF_6, CH_3OH, CH_2Cl_2; (iii) NH_3 sat. CH_3OH, 70°C, sealed tube.

Scheme 12. Guanidinium salt synthesis *via* S-methylation.

Methylation of thiourea **123** and subsequent counter ion exchange from iodide to hexafluorophosphate yielded thiouronium **157** in moderate yield. However all attempts to form the corresponding guanidinium salt **158** by displacement with NH_3 were unsuccessful and the only product isolated from multiple reactions was cyclic guanidine **159** albeit in very low 17% yield. None of the required guanidinium **158** could be isolated *via* extraction, column chromatography or precipitation. Whether the reaction was prone to decomposition or the isolation of the required product was

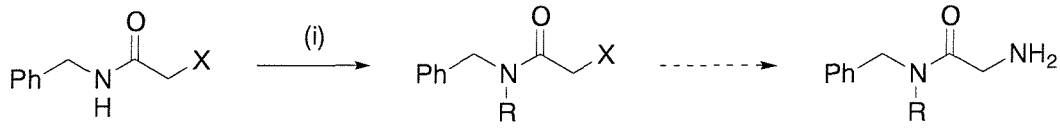
not successful could not be determined by observation of the ^{1}H NMR of the crude mixture.



159

Figure 52. Cyclic guanidine by-product.

As the standard S-methylation approach was not successful for the synthesis of these acyclic guanidiniums an alternate strategy was adopted. Protection of the glycine derived amide should prevent the above cyclisation problems and in addition simplify the purification of the polar thiouronium and guanidinium salt products.



160, X = NHBoc
162, X = NPhth
165, X = NHTrt

161, X = N(Cbz)Boc, R = Cbz
163, X = NPhth, R = Boc
166, X = NHTrt, R = Boc

164

Reagents and conditions. (i) CbzCl or Boc_2O, DMAP, CH_2Cl_2 or CH_3CN.

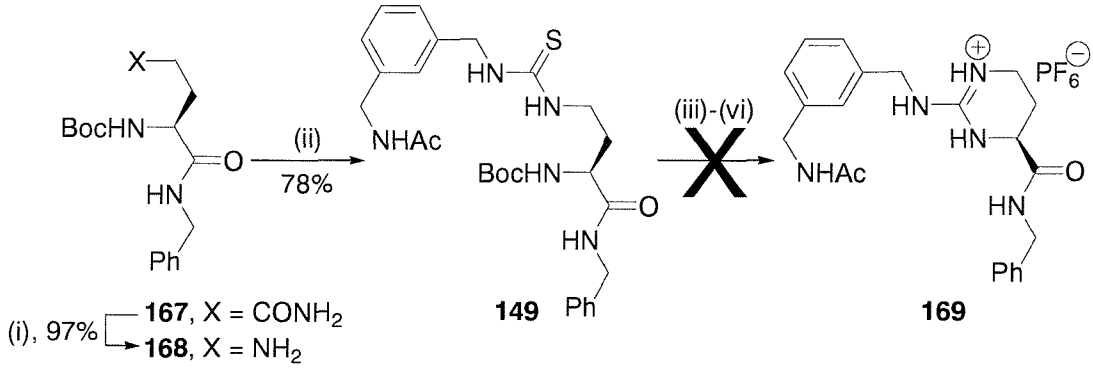
Scheme 13. Synthesis of amide protected glycine amides.

Attempts to monoprotect amide **160** with CbzCl were unsuccessful and only evidence of the bis protected compound **161** was observed by ESMS. Use of the phthalimide protecting group as in **162** allowed the synthesis of the appropriately Boc protected amide **163** in 93% yield. Attempts to deprotect the phthalimide **163** using standard $(NH_2)_2.H_2O$ procedure did not yield the desired amine **164** (R = Boc). The electronics of the system are such, that reaction at the Boc carbonyl and the amide carbonyl also occurs and both amide **162** and *N*-Boc benzylamine are isolated in significant proportions. In addition, the deprotected amide **164** (R = Boc) is not stable and decomposes to the corresponding amine protected carbamate **160** presumably *via* intramolecular Boc group transfer. Trityl protection of glycinyl amine as in **165** allowed simple conversion to Boc protected amide **166** in a good 89% yield. Deprotection of the trityl group in the presence of Boc was not successful with 1% TFA/CH_2Cl_2, 1% TFA/CH_2Cl_2 with Et_3SiH, 20% AcOH/EtOH, 0.1%

HCl/H_2O or hydrogenation and either the fully deprotected amide **164** (R = H) or amide **160** was isolated. The formation of amide **160** again indicated the decomposition of required deprotected amine **164** (R = Boc) *in situ*. All attempts to prepare Cbz or Fmoc protected amides of **162** and **165,** using the appropriate chloroformate, or tritylation of the secondary amide of **162** were unsuccessful, hence this synthetic approach was abandoned. However, it could be conceived that the application of the propensity for cyclisation could be used to generate a cyclic guanidinium similar to those reported by Davis (see page 16).

3.2.1.2 Cyclic Guanidinium Salts

Cyclic guanidinium motifs have been used to enhance efficacy and directionality of a guanidinium-carboxylate motif (see page 16). This, coupled with the other hydrogen bonding functionality in receptor **156**, should provide a good receptor for carboxylates. In addition, the chirality of the receptor may provide some enantioselective binding properties.



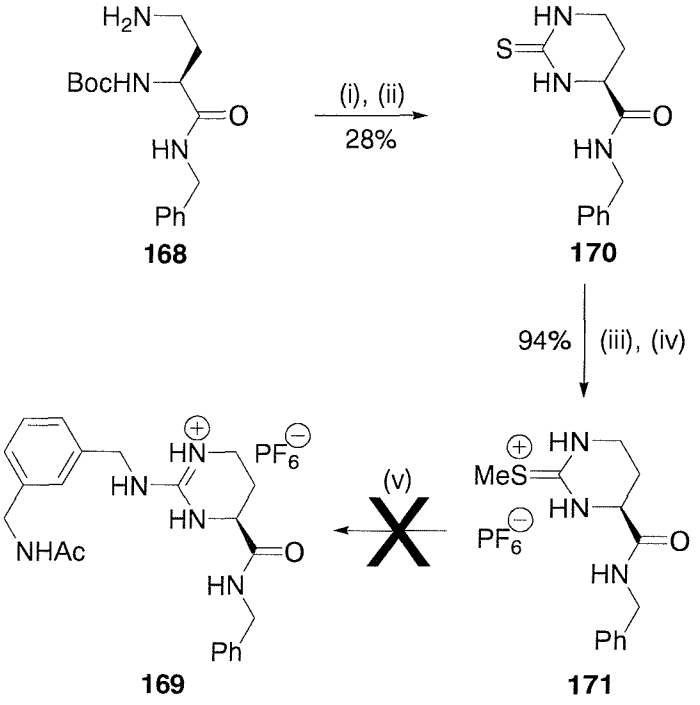
Reagents and conditions. (i) $PhI(OC(O)CF_3)_2$, DMF, H_2O; (ii) **118**, CH_2Cl_2; (iii) MeI, acetone; (iv) NH_4PF_6, CH_3OH, CH_2Cl_2; (v) 10% TFA/CH_2Cl_2; (vi) base, solvent.

Scheme 14. Cyclic guanidinium salt *via* intramolecular cyclisation.

Hofmann rearrangement of primary amide **167** to the corresponding amine **168** proceeded smoothly in excellent yield. Condensation of amine **168** with isothiocyanate **118** yielded the corresponding thiourea **149** in good yield. Anslyn[113] has reported the cyclisation of thiourea amines to form cyclic guanidines; however, all attempts to convert thiourea **149** to guanidinium salt **169** *via* a similar methodology were unsuccessful. Methylation and counter ion exchange proceeded

smoothly as evinced by ^{1}H NMR. However, after Boc deprotection all attempts to perform the desired cyclisation yielded no guanidinium salt **169**. NaOEt/EtOH as per Anslyn[113] proved too severe and TEA/CH_2Cl_2 and pyridine/CH_2Cl_2 gave inseparable mixtures of polar compounds. The isolation of the protonated guanidinium salt compared to free guanidine previously reported[113, 127] proved unsuccessful. This is in accordance with work published by Davis in which only poor yields <10% were obtained for an analogous procedure despite significant optimisation.[75] In this case, the apolar nature of Davis' substrates **58** and **59** (see Figure 23) resulted in a significantly simpler purification than in the case of guanidinium salt **169**.

An alternative methodology was described by Bonnet[128] involving the activation of a cyclic thiourea and subsequent addition of an amine to yield the desired cyclic guanidinium salt.



Reagents and conditions. (i) 20% TFA/CH_2Cl_2; (ii) CS_2, EDC, DIPEA, EtOH; (iii) MeI, acetone; (iv) NH_4PF_6, CH_3OH, CH_2Cl_2; (v) **109**, solvent.

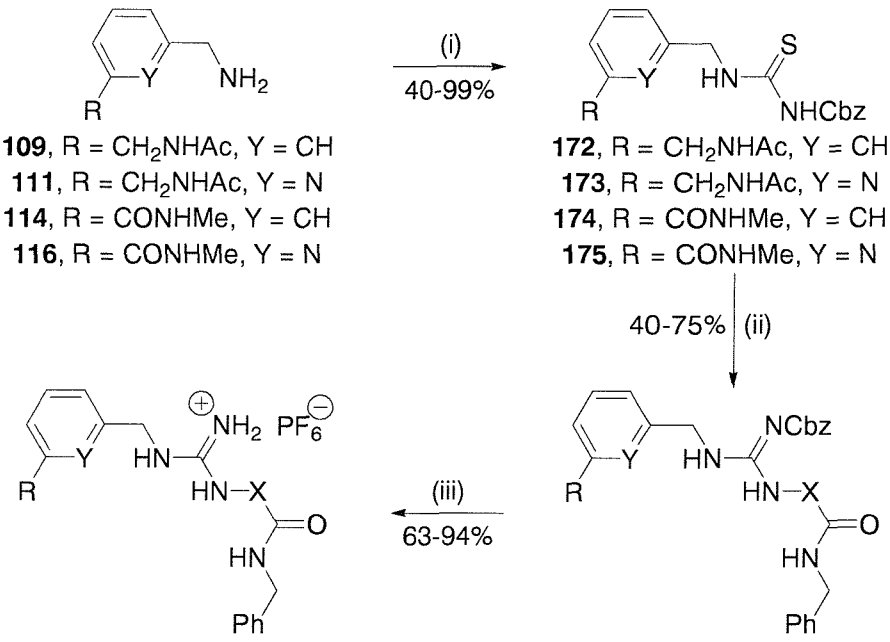
Scheme 15. Cyclic guanidinium salt *via* cyclisation/addition.

Conversion of amine **168** to cyclic thiourea **170** was best affected using CS_2 and EDC as opposed to $CSCl_2$. However, the low yield could be attributed to the

formation of what is essentially a *trans,trans* thiourea as opposed to the usually preferred *cis,cis* geometry for an acyclic thiourea. Methylation of thiourea **170** and subsequent counter ion exchange gave thiouronium **171** in good yield. All attempts to condense thiouronium **171** with amine **109** in EtOH, CH_3CN, THF and CH_2Cl_2 to yield guanidinium salt **169** were unsuccessful again, possibly due to poor reaction as observed by Davis[75] or difficulty with purification due to the polar functionalities in guanidinium salt **169**, compared to the examples described by Bonnet.[128] Hence the synthesis of cyclic guanidinium salts based on this carboxylate receptor motif was abandoned.

3.2.2 Acyclic Guanidinium Salts via Cbz Protection

In order to facilitate the synthesis of the required acyclic guanidiniums the method of Hamilton[120] was employed using the electron withdrawing nature of a carbamate to activate a thiourea to dehydration with EDC.



109, R = CH_2NHAc, Y = CH
111, R = CH_2NHAc, Y = N
114, R = CONHMe, Y = CH
116, R = CONHMe, Y = N

172, R = CH_2NHAc, Y = CH
173, R = CH_2NHAc, Y = N
174, R = CONHMe, Y = CH
175, R = CONHMe, Y = N

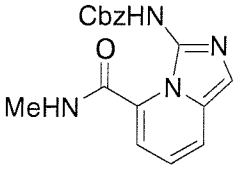
181, R = CH_2NHAc, Y = CH, X = $-CH_2-$
182, R = CH_2NHAc, Y = CH, X = $-(CH_2)_2-$
183, R = CONHMe, Y = CH, X = $-CH_2-$
184, R = CONHMe, Y = CH, X = $-(CH_2)_2-$

176, R = CH_2NHAc, Y = CH, X = $-CH_2-$
177, R = CH_2NHAc, Y = CH, X = $-(CH_2)_2-$
178, R = CONHMe, Y = CH, X = $-CH_2-$
179, R = CONHMe, Y = CH, X = $-(CH_2)_2-$

Reagents and conditions. (i) CbzNCS, CH_2Cl_2; (ii) **96** or **97**, EDC, DIPEA, CH_2Cl_2; (iii) H_2/Pd/C, CH_3OH then 60% HPF_6/H_2O.

Scheme 16. Synthesis of acyclic guanidinium salts.

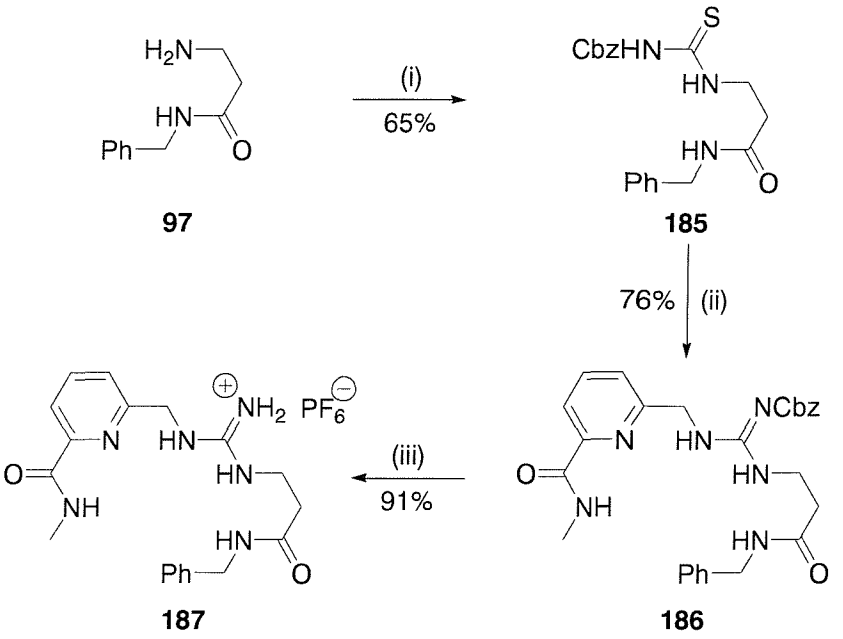
Amines **109**, **111**, **114** and **116** were condensed with CbzNCS, synthesised using the procedure of Hamilton,[120] to yield Cbz-thioureas **172-175** in fair to excellent yields. Reaction of thioureas **172** and **174** with EDC in the presence of the appropriate amines **96** and **97** gave the corresponding Cbz-protected guanidines **176-179** in moderate to good yields. Attempts to react thiourea **173** with amines in the presence of EDC did not produce the desired guanidine however the formation of an extended heteroaromatic was supported by ^{1}H NMR and ESMS. The reaction of thiourea **175** under the same conditions yielded sufficient material to be fully characterised and the structure determined as imidazo[1,5-a]pyridine **180** formed as result of attack of the pyridine nitrogen on the EDC activated thiourea. This is in accordance with work published by Bourdais[129] on the intramolecular cyclisation of pyridine with thioureas, mediated by carbodiimides. However, a significantly reduced reaction time was observed with thiourea **175**, presumably due to electron withdrawing effect of the Cbz group. Hydrogenation of guanidines **176-179**, addition of HPF_6 and extraction of the aqueous phase gave guanidinium salts **181-184** in good yields.



180

Figure 53. Product isolated from reaction of thiourea **175** with EDC.

The synthesis of the pyrido guanidinium salts *via* an inverted strategy similar to that used for the pyrido thioureas above (see 2.2.3) was the logical alternative strategy. As with the thiourea series above, the potential side reactions of the glycinyl derived thioureas may preclude successful synthesis of the corresponding guanidiniums *via* this route, hence only the β–alanine derived receptors were synthesised.

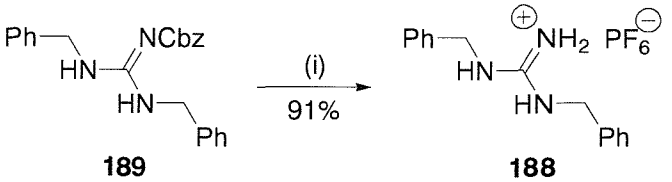


Reagents and conditions. (i) CbzNCS, CH_2Cl_2; (ii) **116**, EDC, DIPEA, CH_2Cl_2, DMF; (iii) H_2/Pd/C, CH_3OH then 60% HPF_6/H_2O.

Scheme 17. Synthesis of pyridyl guanidinium salt.

The condensation of amine **97** with CbzNCS gave the corresponding thiourea **185** in a fair yield. Reaction of thiourea **185** with the pyridyl amine **116** in the presence of EDC gave the corresponding guanidine **186** in good yield and deprotection proceeded smoothly to give the required guanidinium salt **187**, after addition of acid and extraction of the aqueous phase, in 44% overall yield.

In order to allow comparison of the importance of the amide hydrogen bonding functionality in guanidinium salts **181-184** and **187**, a simple control compound **188** was synthesised from the known Cbz-guanidine[120] **189** *via* the same procedure used above.



Reagents and conditions. (i) H_2/Pd/C, CH_3OH then 60% HPF_6/H_2O.

Scheme 18. Synthesis of dibenzyl guanidinium salt **188**.

3.3 Binding Studies with Acyclic Guanidinium Salts

The investigation of binding of guanidinium salts **181-184**, **187** and **188** was performed in DMSO-d_6 with TBA acetate as guest. Both NMR titration and isothermal titration calorimetry (ITC) were used to investigate the host-guest complexes formed and ITC was used to determine both binding constants and thermodynamic data.

3.3.1 Host Conformation Analysis

Analysis of the chemical shifts of the free host in DMSO-d_6 provides some insight into the formation of intramolecular hydrogen bonds in guanidinium salts **181-184** and **188**; in particular, pyridine containing receptor **187**.

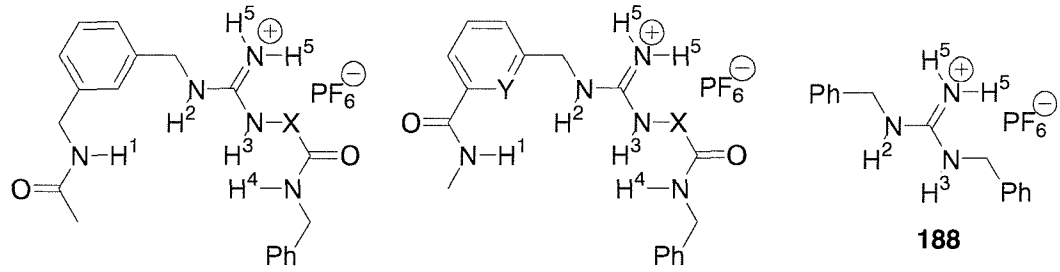


Figure 54. Acyclic guanidinium salt receptors.

Guanidinium salt	δ /ppm†				
	H-1	H-2	H-3	H-4	H-5
181	8.66	8.39	7.87	8.94	7.87
182	8.68	8.19	7.76	8.84	7.80
183	8.75	8.44	7.89	8.95	7.89
184	8.77	8.24	7.51	8.83	7.51
187	8.98	8.19	7.91	8.86	7.91
188	-	8.34	8.34	-	7.87

† - Where protons are coincident they are reproduced in multiple columns for clarity.

Table 7. Host chemical shift analysis for guanidinium salt series.

As with the thiourea series (see Chapter 2), the guanidiniums derived from glycine **181** and **183** displayed resonances for H-2, H-3 and H-4 more downfield compared to their one carbon homologues **182** and **184**; indicating a greater degree of intramolecular hydrogen bonding in **181** and **183**. The chemical shifts of the H-4 amide protons across the series are also significantly downfield from literature values

for simple amides ($\Delta\delta$ = 0.3 ppm), indicating hydrogen bonding to or from these amides throughout the series.[107] As with the thiourea series, pyridine containing guanidinium salt **187** displays a downfield shift in the H-1 resonance compared to the corresponding benzo analogue in ($\Delta\delta$ = 0.21 ppm), however the guanidinium proton H-2 does not show significant perturbation suggesting that there is no preorganisation to this proton as a result of intramolecular hydrogen bonding.

3.3.2 Analysis of Chemical Shift Changes for Guanidinium Salts

Binding studies were conducted in DMSO-d_6 and Table 8 describes the changes in the chemical shifts of the hydrogen bonding protons after one equivalent of guest was added.

Guanidinium salt	$\Delta\delta$(1 eq.) /ppm†				
	H-1	H-2	H-3	H-4	H-5
181	0.14	0.55	0.63	0.05	0.14
182	0.12	0.38	0.44	0.29	0.11
183	0.06	0.23	br	0.06	0.07
184	0.18	br	br	0.26	obs
187	0.54	br	br	0.02	obs
188	-	0.60	0.60	-	0.16

† - Where protons are coincident they are reproduced in multiple columns for clarity, br – proton broadens before one equivalent, obs – proton becomes obscured during experiment.

Table 8. Chemical shift data for guanidinium salt series.

Guanidinium salt **181** displays shift in the ^{1}H NMR for the guanidinium protons, H-2 and H-3, indicating the formation of the expected guanidinium-carboxylate hydrogen bonding interaction. The magnitudes of these shifts are commensurate with those observed in the simple control guanidinium salt **188**. Guanidinium salts **182** and **183** display smaller shifts in H-2 and H-3 possibly due to a weaker hydrogen bonding motif. The observed shift in the amide H-1 is relatively small across the series with the exception of the pyridine containing guanidinium salt **187** which displays a large shift ($\Delta\delta$ = 0.54 ppm) as per the thiourea series above (see Chapter 2). This indicates that the preorganisation of the amide functionality results in a stronger H-1 carboxylate hydrogen bond. In the case of guanidinium salts **181-184** the H-4 amide only shifts significantly in the β–alanine derived receptors **182** and **184** indicating that either breaking of intramolecular hydrogen bonding in the free hosts **181** and

183 results in no shift in H-4 or that **182** and **184** provide a considerably better fit with the carboxylate guest. However in the case of **187**, despite being the longer chained homologue, did not display a significant shift in H-4 indicating limited participation in the stabilisation of the host-guest complex.

3.3.3 Analysis of Binding Constants for Guanidinium Salts

NMR binding studies were conducted in DMSO-d_6 maintaining a constant concentration of host throughout. Isothermal titration calorimetry (ITC) was performed in DMSO at 25°C with a typical starting host concentration of 1 mM and titrating with a 48 mM guest solution. At these concentrations host-host and guest-guest interactions were assumed to be minimal and hence ignored.

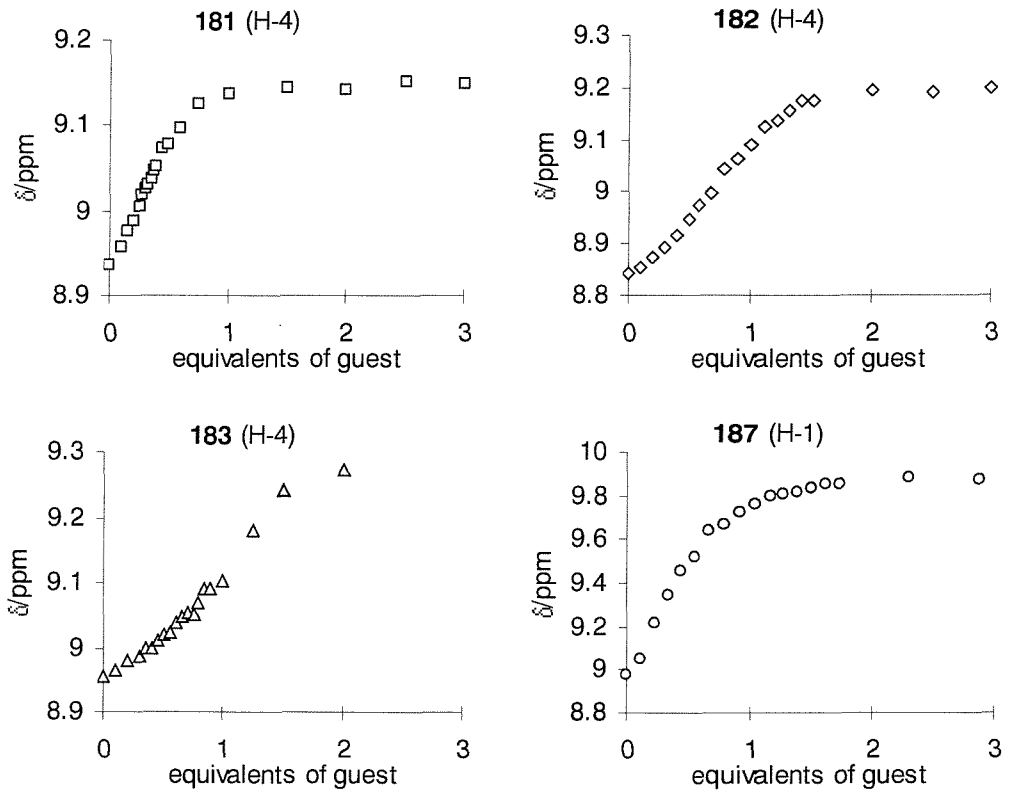
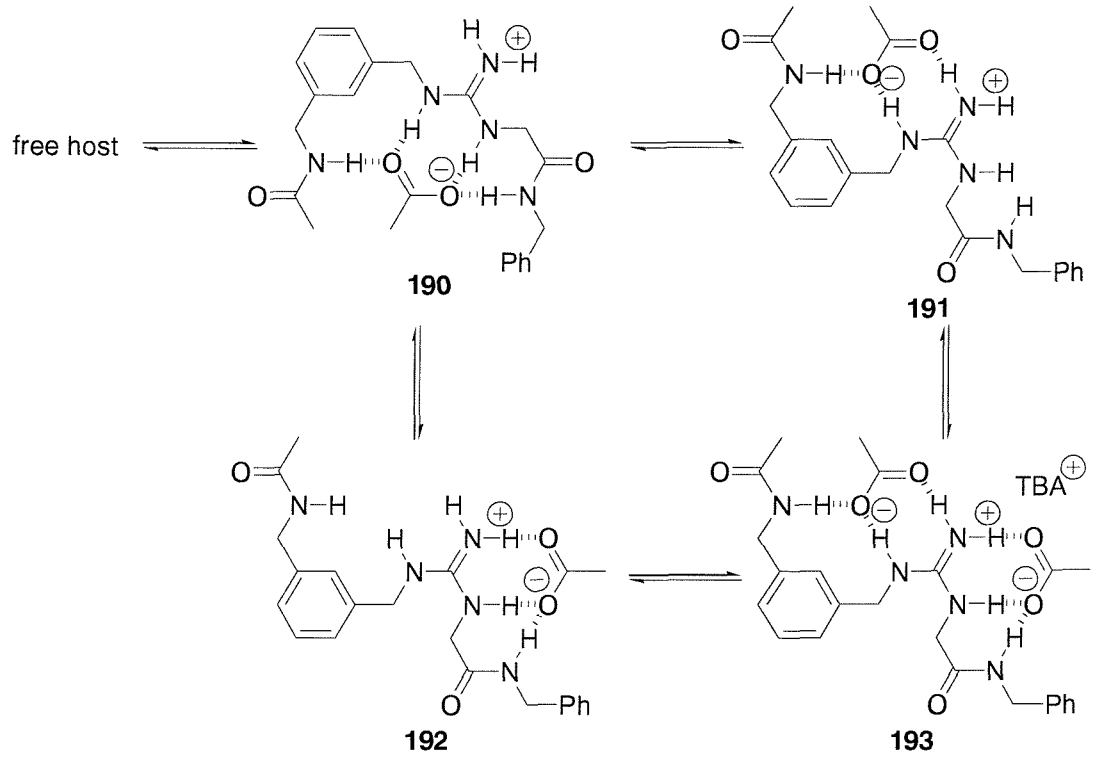


Figure 55. Sample NMR titration curves for guanidinium salt series.

Titration of guanidinium salt **181** with TBA acetate in DMSO-d_6 gave a curve which could be fitted to a 1:2 host:guest binding stoichiometry with a weak 1:2 component which, as expected, reached saturation at a 1:1 molar ratio indicative of tightly bound 1:1 complex. This was rationalised by the initial formation of the desired 1:1 complex **190** which is strongly bound principally due to the charge-charge

interaction between guanidinium and the carboxylate and additional stabilisation from the amide functionalities. Binding the second equivalent of acetate requires a significant change in host geometry, as in **191** and **192**, and the lack of charge-charge component results in weak 1:2 binding **193** in DMSO-d_6.



Scheme 19. Proposed binding of acetate by guanidinium salt complexes.

The NMR titration curves obtained for **182**, **183** and **187** could not be fitted using a 1:2 binding model. In all cases the curves do not reach saturation at an integer equivalent and results in poor curve fitting throughout the series despite the well resolved curve shape. The poor resolution and fitting of the binding of acetate by guanidiniums salts **182**, **183** and **187** could be attributed to the complex equilibrium available for binding: the formation of the desired hydrogen bonding motif **190** is presumably the preferred 1:1 binding mode. However, the relatively small participation of the one of the hydrogen bond donor amides, H-1 and H-4, in each case should allow the formation of the alternative 1:1 binding geometries **191** and **192** as intermediates for the formation of the expected 1:2 complex **193**. Conceivably this could complicate the shifts of the hydrogen bonding protons preventing good curve fitting.

Figure 56 describes the binding curves obtained for the ITC of the guanidinium salts studied. In general, the curves obtained were closely related and all were exothermic in their binding. Guanidinium salts **181**, **182**, **183**, **184** and **188** all displayed similar curves and similar heat outputs; **187** showed a marked reduction in the heat evolved on complexation.

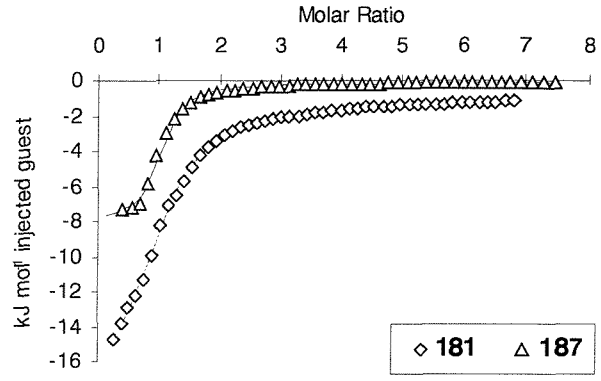


Figure 56. ITC data and fitted curves for **181** and **187**.

All curves could be fitted with a sequential binding model with two binding sites which is designed for systems in which more than one equilibrium is present but one binding mode is strongly favoured *i.e.* $K^{1:1} >> K^{1:2}$. Table 9 describes the 1:1 binding data for the guanidinium salts **181-184**, **187** and **188** in all cases the 1:2 (host:guest) binding stoichiometry was also observed but with very low binding constants ($K_a(1:2) \leq 120\ M^{-1}$) compared to the 1:1 component as determined by NMR and ITC curve fitting.

Guanidinium salt	NMR		ITC	
	K_a (1:1) /M^{-1}	ΔG /kJmol^{-1}	K_a (1:1) /M^{-1}	ΔG /kJmol^{-1}
181	$5.0 \times 10^3 \pm 660$	-21.1	$5.3 \times 10^3 \pm 240$	-21.2
182	-	-	$3.7 \times 10^3 \pm 210$	-20.4
183	-	-	$6.9 \times 10^3 \pm 600$	-21.9
184	-	-	$5.3 \times 10^3 \pm 320$	-21.3
187	-	-	$2.2 \times 10^4 \pm 3.7 \times 10^3$	-24.8
188	-	-	$3.1 \times 10^3 \pm 250$	-20.0

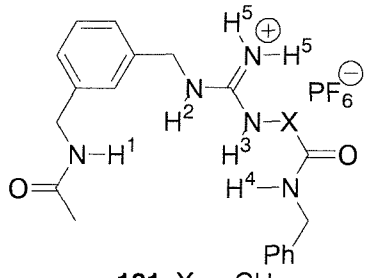
Table 9. Binding study data for guanidinium salt series.

For guanidinium salt **181** the NMR data and the ITC data are in good agreement with a large 1:1 component to the binding and a weak 1:2 (host:guest) element ($K_a \approx 40\ M^{-1}$). Evaluation of **181**, **182**, **183** and **184** indicates that in all cases only a small

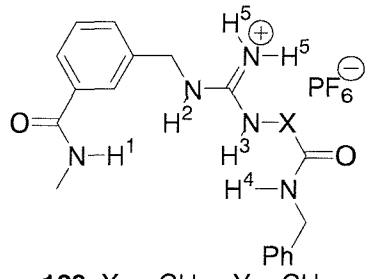
increase is observed in the binding constants with respect to the control receptor **188** and that no significant difference is observed in the binding strength in these receptors ($\Delta\Delta G$ < 1 kJ mol^{-1}). The pyridine containing receptor **187** forms a significantly stronger complex with acetate than its benzo analogue **184** ($\Delta\Delta G$ = 3.5 kJ mol^{-1}), indicating the importance of the preorganisation of the H-1 proton observed above (see page 56). This degree of increase in complex stability is similar in magnitude to that observed in the analogous thioureas series above (see page 41)

3.3.4 Analysis of Thermodynamic Data for Guanidinium Salts

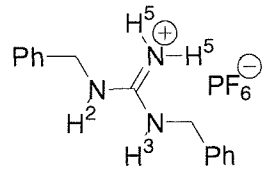
Table 10 describes the thermodynamic data obtained from the ITC of the guanidiniums **181-184**, **187** and **188** with TBA acetate in DMSO at 25°C.



181, X = $-CH_2-$
182, X = $-(CH_2)_2-$



183, X = $-CH_2-$, Y = CH
184, X = $-(CH_2)_2-$, Y = CH
187, X = $-(CH_2)_2-$, Y = N



188

Figure 54. Acyclic guanidinium salt receptors.

Guanidinium salt	ΔH /kJmol^{-1}	TΔS /kJmol^{-1}	ΔG /kJmol^{-1}
181	-17.6	3.7	-21.2
182	-23.1	-2.7	-20.4
183	-19.6	2.3	-21.9
184	-19.4	1.8	-21.3
187	-8.0	16.8	-24.8
188	-21.8	-1.8	-20.0

Table 10. Thermodynamic data for guanidinium salt series.

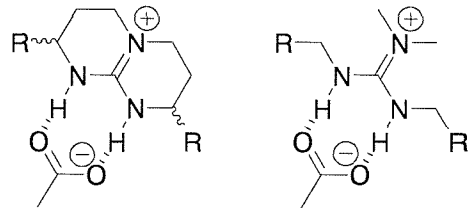
Analysis of the thermodynamic data for the binding of acetate shows that the formation of the complex is dominated by a large enthalpic component *i.e.* the receptor forms stronger hydrogen bonds with the guest than with the solvent in the cases of **181-184** and **188**. However, the binding event is only slightly affected by the entropic component of the binding. The binding of acetate by guanidinium salt **182** is

entropically more favoured than in **181** due to the better arrangement of the H-4 hydrogen bond donor supported by the larger chemical shift of this proton in **182** (see Table 8). However, the increased chain length in **182** results in an entropically disfavoured process, due to greater loss of degrees of freedom upon complexation resulting in a slightly reduced binding constant.

Comparison of **184** and the pyridyl analogue **187** indicated the expected shift in the binding from enthalpy driven to entropy driven. The preorganisation effect of the pyridyl nitrogen is such that the binding is strongly entropically favoured but the repulsion between the pyridyl nitrogen and the carboxylate anion results in a relatively small enthalpic element. The large entropic drive to the binding of acetate however leads to a large 1:1 binding constant in DMSO.

3.4 N,N-Dimethylguanidinium Salts

Although the *N,N'*-dialkyl guanidinium salts above are good receptors for carboxylates their binding is significantly complicated by the additional 1:2 component. In particular, analysis of the data by NMR was limited due to the poor fitting of the more complicated binding curves. The bicyclic guanidinium motifs described above (see 1.3.4.2) provide a good motif for the binding of acetate and the carbon scaffold is such as to restrict the binding only to a 1:1 host-guest complex as in **194**. However, the synthesis of the bicyclic systems is long and hence it was conceived that guanidinium salts such as **195** may provide a synthetically simpler alternative whilst still restricting the binding to 1:1 complex.

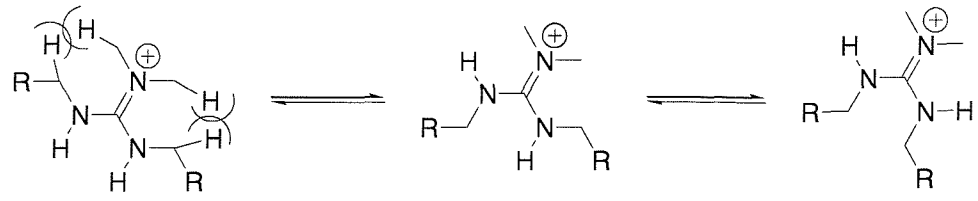


194 **195**

Figure 57. Proposed *N,N*-dimethylguanidinium receptor for acetate.

However one potential problem is that the conformation of the guanidinium itself may not be the desired *cis,cis* conformation **196** due to steric clash between the pendant alkyl chains, it could be proposed that **197** and **198** are likely conformations of the free guanidinium. Hence binding of a carboxylate *via* the usual hydrogen

bonding motif may require significant conformational change in the host molecule and an associated entropic penalty.

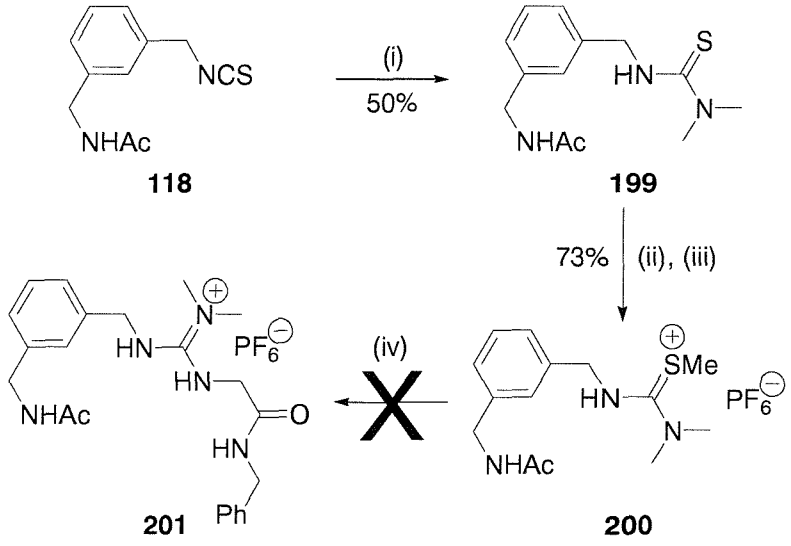


196 197 198

Scheme 20. Proposed conformations of *N,N*-dimethylguanidinium salts.

3.4.1 Synthesis of N,N-Dimethylguanidinium Salts

In order to circumvent the problems encountered with intramolecular cyclisation discussed above (see Scheme 12) an alternative strategy was conceived that using the glycine derived amine in the final step to form the guanidinium salt.



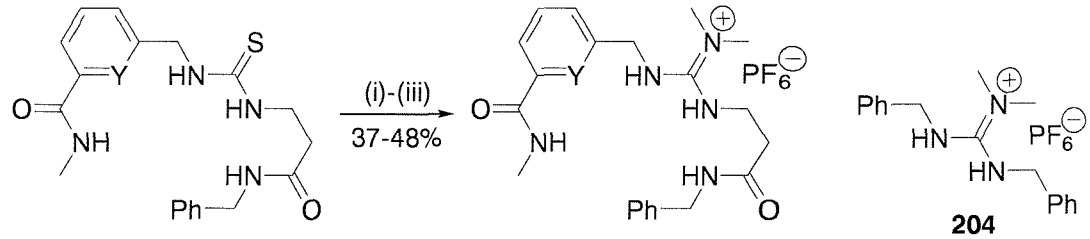
Reagents and conditions. (i) 40% Me_2NH/H_2O, CH_2Cl_2; (ii) MeI, CH_3OH, CH_2Cl_2; (iii) NH_4PF_6, CH_3OH, CH_2Cl_2; (iv) solvent.

Scheme 21. Synthesis of *N,N*-dimethylguanidinium salts.

Conversion of isothiocyanate **118** to the corresponding *N,N*-dimethylthiourea **199** was achieved in moderate yield under biphasic conditions, the slightly reduced yield presumably as a result of steric congestion. Methylation of **199** and counter ion exchange to the hexafluorophosphate salt to give **200** proceeded smoothly in good yield. Reaction of **200** with a suitable amine did not provide the corresponding

guanidinium **201**; however, the only product isolated from the reaction was thiourea **199**. Presumably, steric congestion around the reacting centre is too great and therefore thiouronium **200** acts as a methylating agent. Therefore this is a not a feasible route to the required motif.

The limited effect of glycinyl/β-alanyl chain length observed in the guanidinium salt series above (see 3.3.3) indicates that the same may be the case with the corresponding *N,N*-dimethyl compounds. Hence, switching to the β-alanyl derived amine and adopting the more conventional thiourea alkylation approach, provides a route to a representative range of *N,N*-dimethylguanidinium salts.



135, Y = CH
143, Y = N

202, Y = CH
203, Y = N

Reagents and conditions. (i) MeI, CH_3OH; (ii) NH_4PF_6, CH_3OH, CH_2Cl_2; (iii) 40% Me_2NH/H_2O, CH_3CN.

Scheme 22. Synthesis of dimethylguanidinium salts.

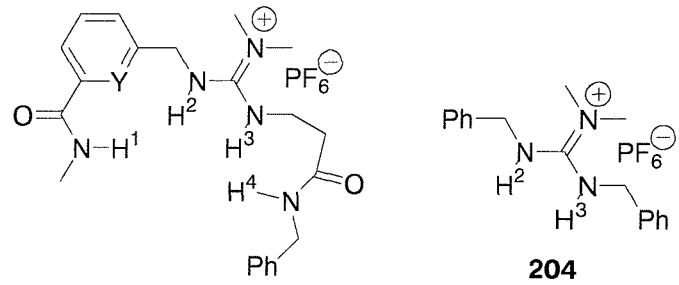
Methylation of thioureas **135** and **143** and counter ion exchange afforded the corresponding thiouronium salts which were converted directly to guanidinium salts **202** and **203** in 48% and 37% yield respectively. The control *N,N*-dimethylguanidinium **204** was synthesised in a similar fashion from *N,N'*-dibenzylthiourea in 37% yield.[130]

3.4.2 Binding Studies with N,N-Dimethylguanidinium Salts

The investigation of binding of *N,N*-dimethylguanidinium salts **202-204** was performed in DMSO with TBA acetate as the guest. Both NMR titration and ITC were used to obtain the binding data.

3.4.2.1 Host Conformational Analysis and Chemical Shift Changes for N,N-Dimethylguanidinium Salts

As with the thioureas (see Chapter 2) and the *N,N'*-dialkylguanidinium salts above (see page 55) analysis of the chemical shifts of the free host provides evidence for the formation of intramolecular hydrogen bonds.



202, Y = CH
203, Y = N

204

Figure 58. *N,N*-Dimethylguanidinium salt receptors for acetate.

Guanidinium salt	δ /ppm†			
	H-1	H-2	H-3	H-4
202	8.75	8.35	7.42	8.85
203	9.18	8.51	7.97	9.05
204	-	8.37	8.37	-

Table 11. Host chemical shift analysis for guanidinium salt series.

A significant change in the chemical shifts on going from **202** to **203** indicates appreciable differences in their conformations in DMSO-d_6. In particular, H-1 and H-2 are significantly downfield in the pyridine containing receptor **203** suggesting the presence of intramolecular hydrogen bonding to both moieties. However, protons H-3 and H-4 are also significantly shifted in **203** compared to the benzo analogue **202** in particular H-3 ($\Delta\delta$ = 0.55 ppm). As with the receptors discussed above (see Chapter 2), the amide H-4 is significantly downfield compared to literature examples indicating the presence of other intramolecular hydrogen bonds in **202** and **203**.[107]

3.4.2.2 Analysis of Binding Constants for N,N-Dimethylguanidinium Salts

Both NMR and ITC experiments were conducted under the same conditions as described for the *N,N'*-dialkylguanidinium salts above (see page 57).

Due to high degrees of signal broadening the shifts of the guanidinium protons H-2 and H-3 could not be determined after additon of one equivalent. As with the simple guanidinium salts (see Table 8), receptors derived from β-alanine displayed greater shifts in H-4 compared to H-1 upon addition of one equivalent of guest indicating greater participation of the H-4 amide in stabilisation the host-guest complex in these cases.

Guanidinium salt	Δδ(1 eq.) /ppm†			
	H-1	H-2	H-3	H-4
202	0.08	br	br	0.22
203	0.01	br	br	0.15
204	-	br	br	-

Table 12. Chemical shift changes for *N,N*-dimethylguanidinium salt series.

As expected NMR titration of *N,N*-dimethylguanidinium salts **202–204** in DMSO-d_6 gave curves consistent with a clean 1:1 binding stoichiometry. Figure 59 describes typical NMR and ITC binding curves observed for the binding of acetate by *N,N*-dimethylguanidinium salts in DMSO. The NMR binding curve did not reach saturation indicating that there is a poor association between host and guest supported by the relatively small shift in the amide protons observed above (see Table 12). However, the introduction of the dimethyl group in these guanidinium salts has successfully prevented the formation of the 1:2 (host:guest) complexes observed above (see Scheme 19).

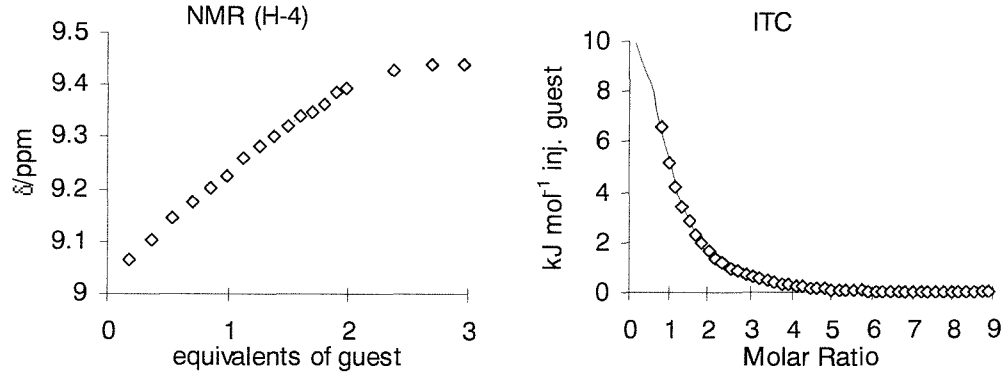
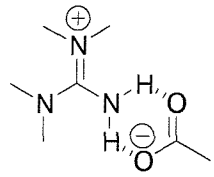


Figure 59. NMR and ITC data for **203**.

The ITC curves for the *N,N*-dimethylguanidinium salts **202-204** showed that contrary to the *N,N'*-dialkylguanidinium salts above (see Figure 56) the overall binding process is endothermic; indicating an enthalpically disfavoured binding event. This

endothermic binding of carboxylates by guanidinium salts has been observed previously by Hamilton.[69] Guanidinium **205**, as its tetraphenylborate, bound acetate weakly with an overall endothermic binding isotherm in DMSO. It was supposed that the poor arrangement of hydrogen bond donor results in weak bent hydrogen bonds and a low complex stability (K_a = 110 M^{-1}). It could be conceived that the adoption of a non binding geometry for the *N,N*-dimethylguanidiniums, as in **197** and **198** (see Scheme 20) could rationalise a similar poor hydrogen bonding association in the cases of **202-204**.



205

Figure 60. Binding of acetate by tetramethylguanidinium in DMSO.

Curve fitting of the NMR data provided binding constants in the case of **202** and **203**. However, broadening of guanidinium protons in **204** prevented the determination of a binding constant. ITC data for this series was fitted with a one site model. The absolute values of the binding constants determined by NMR and ITC are in poor agreement (see Table 13). However, the relative values determined for **202** and **203** are consistent.

Guanidinium salt	NMR		ITC	
	K_a /M^{-1}	ΔG /kJmol^{-1}	K_a /M^{-1}	ΔG /kJmol^{-1}
202	380	-14.7	5.3 x 10^3	-21.2
203	260	-13.8	3.9 x 10^3	-20.5
204	-	-	4.0 x 10^3	-20.6

Table 13. Binding constants data for *N,N* dimethylguanidinium salt series.

The low binding constants observed for **202** and **203** as determined by NMR support the argument that the guanidinium adopts a conformation in which the two protons are not in a suitable orientation for binding. Contrary to the guanidinium salts above (see Table 9); the binding is not significantly affected by the preorganisation of the receptor through intramolecular hydrogen bonding of H-1 and H-2 to the pyridine lone pair observed in **203**. ITC indicated that the *N,N*-dimethylguanidinium salts form similarly stable complexes with acetate in DMSO to their non-methylated

analogues discussed above (see Table 9) however the lack of agreement with the NMR derived binding constants prevents any firm conclusions being drawn. However, it can be stated that **202** and **203** are similarly effective receptors for acetate in DMSO ($\Delta\Delta G < 1$ kJ mol^{-1}).

3.4.2.3 Analysis of Thermodynamic Data for N,N-Dimethylguanidinium Salts

Table 14 describes the thermodynamic data obtained from the ITC of the guanidiniums **202–204** with TBA acetate in DMSO at 25°C.

Guanidinium salt	ΔH /kJmol^{-1}	$T\Delta S$ /kJmol^{-1}	ΔG /kJmol^{-1}
202	5.7	26.9	-21.2
203	12.9	33.3	-20.5
204	12.5	33.0	-20.6

Table 14. Thermodynamic data for *N,N*-dimethylguanidinium salts.

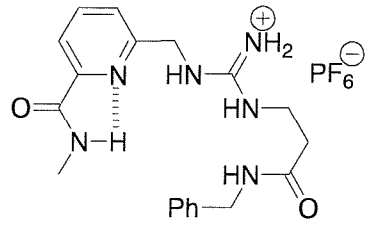
In all three cases the binding of acetate in DMSO were enthalpically disfavoured but strongly entropically driven. Guanidinium **202** was less enthalpically disfavoured than the pyrido analogue **203** and the control compound **204** presumably due to electron repulsion, in the case of **203** or no additional hydrogen bond donors, in the case of **204**. The binding with the preorganised pyrido receptor **203** was entropically more favoured than **202** due to the intramolecular hydrogen bonding. Receptor **204** was also more entropically favoured than **202** due loss of fewer degrees of freedom on binding. The unfavourable enthalpic component of the binding supports the suggestion that the free host is not in a suitable conformation to permit good hydrogen bonding between host and guest. The large positive entropy of binding and the unfavourable enthalpic component could be attributed to well solvated host and hence binding driven by release of solvent on complexation.

3.5 Conclusions

A range of model carboxylate binding sites based on *N,N'*-dialkylguanidinium salts have been synthesised and form stable complexes with carboxylates in DMSO largely *via* a 1:1 binding mode but with a small 1:2 component. The formation of the weak 1:2 complexes can be suppressed by replacement of guanidinium NHs with methyl groups, However, accurate determination of the effect on the binding

constants was not possible. The binding of acetate was found to be endothermic as opposed to the exothermic binding observed in the non-methylated series.

The binding of the carboxylate can be appreciably enhanced through the incorporation of a pyridine into the motif such as to provide preorganisation through intramolecular hydrogen bonds. The incorporation of the pyridine motif in both the guanidinium salts is fundamental in significantly improving complex stability. Hence guanidinium salt **187** was identified as the optimum receptor from this series. The modification of other features in the receptor motif, such as flexibility, did not appreciably affect the complex stability.



187

Figure 61. Optimum guanidinium salt receptor for carboxylates.

4 Towards Guanidinium Tweezers

4.1 Introduction

Analysis of a range of thiourea and guanidinium based carboxylate receptors allowed the determination of the optimum binding motif. The application of these optimised studies in a tweezer receptor for a tripeptide guest was the final goal of this project. Tweezer **206** was conceived in order to ascertain the feasibility of synthesis and the binding efficacy of the designed tweezer motif. It was hoped that use of a guanidinium based carboxylate binding site **207** would allow binding of the tripetide guest in H_2O. In the guanidinium series (see Chapter 3) it was determined that the the effect of changing spacer length to the hydrogen bond donor H-4 from one methylene to two methylenes and the H-1 hydrogen bond donor from acetate to benzoate had no appreciable effect on the overall complex stability. The presence of the pyridine, as in **207**, in the final tweezer **206** is highly desirable due the marked increase in binding observed in the model series. However, difficulty of synthesis of receptors containing this motif precluded its inclusion in the first generation of tweezers.

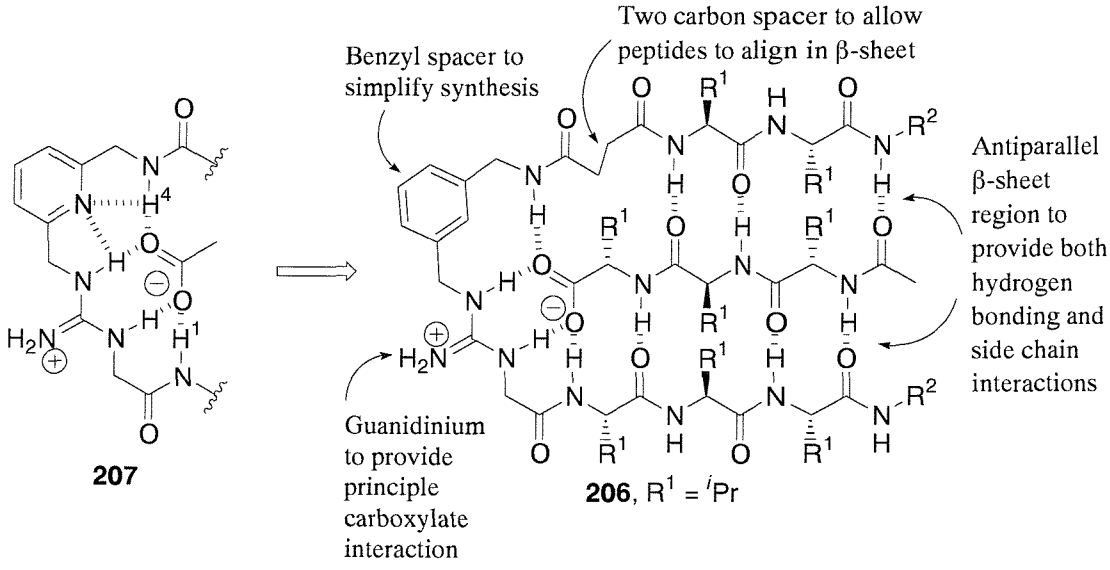


Figure 62. Target tweezer receptor.

CPK modelling indicated that the use of a glycine in the first position of the lower arm of the tweezer and a succinamide in the upper arm would provide the best

alignment for the following antiparallel β-sheet. The antiparallel β-sheet region of the tweezer should provide both additional complex stabilisation, through hydrogen bonding, and selectivity for a particular substrate, through side chain interactions. It was hoped the use of a hydrophobic all valine tweezer would maximise binding in polar media with an appropriately hydrophobic guest. In addition, the lack of amino acid side chain protection should simplify the synthesis, characterisation and binding studies of the designed tweezer motif. The use of valine in all positions should result in a relatively uncluttered ^{1}H NMR in the amide region to allow evaluation of the host-guest complex by this method.

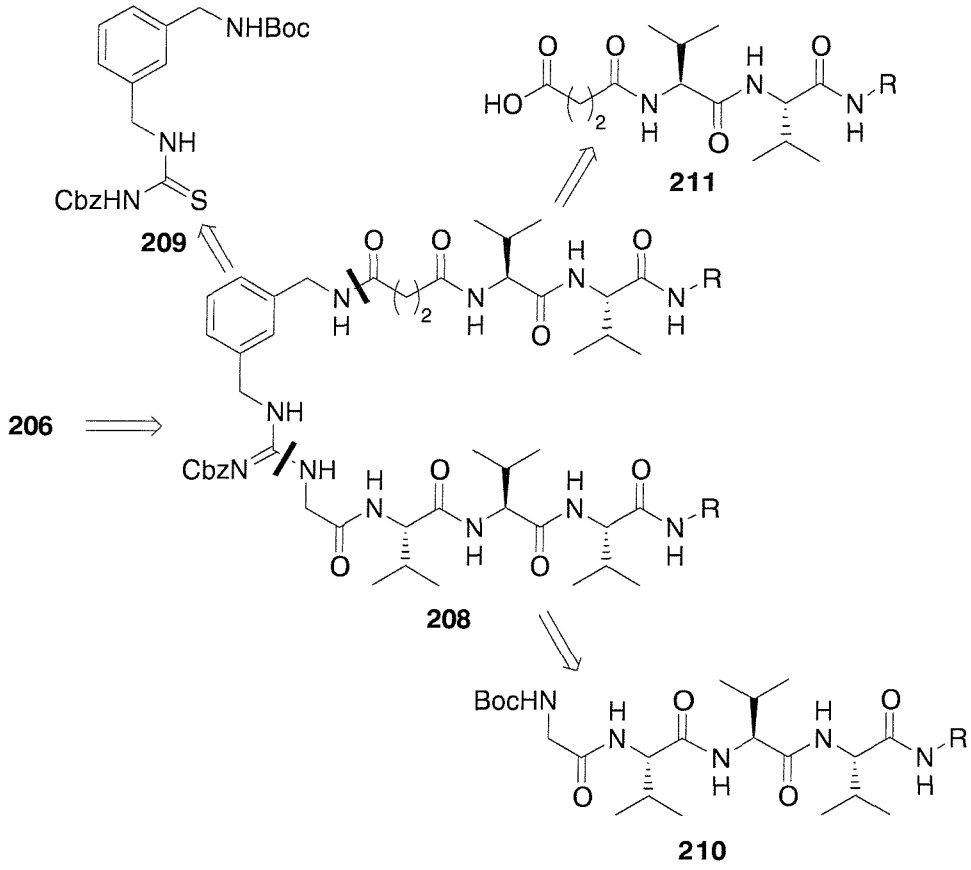


Figure 63. Retrosynthesis of tweezer **206**.

Tweezer **206** could be realised from the corresponding Cbz-protected guanidine **208** which in turn can be disconnected to thiourea **209**, protected amine **210** and acid **211** as shown in Figure 63. The two peptidic portions of the tweezer, **210** and **211,** are readily available *via* well established peptide chemistry and thiourea **209** from reaction of CbzNCS with an appropriate amine. The final steps of the synthesis proceeding first by condensation of thiourea **209** with **210**, using the procedure of

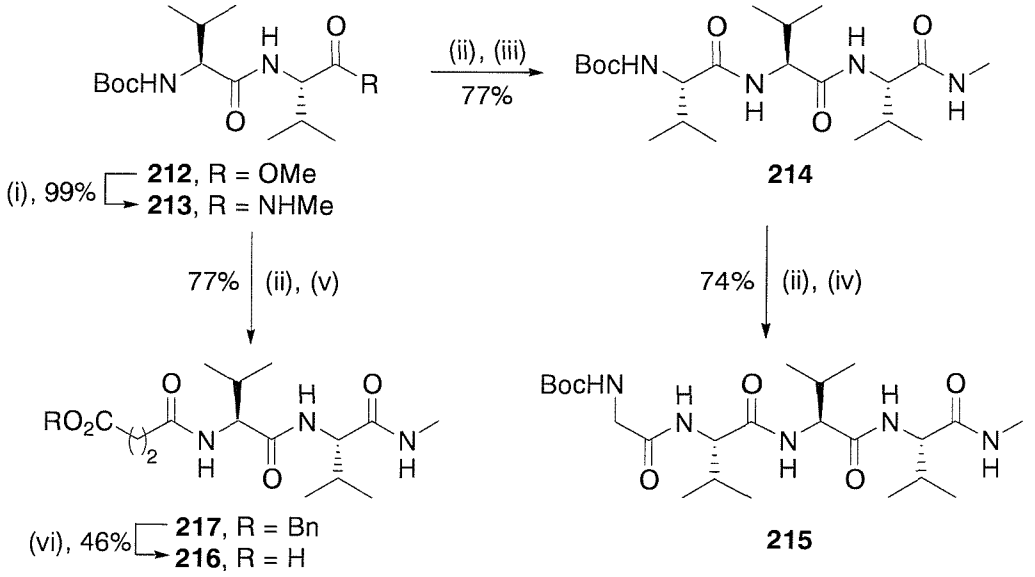
Hamilton,[120] followed by standard peptide coupling with acid **211** to obtain tweezer precursor **208**.

4.2 N-Methyl Terminating Tweezer

Use of a simple methyl group to terminate the tweezer **206** (R^2 = Me) should simplify analysis of the resultant host-guest complexes and allow simple determination of the participation of the last amide in hydrogen bonding. Therefore, tweezer **206** (R^2 = Me) was regarded as an ideal system for initial investigation.

4.2.1 Synthesis of N-Methyl Tweezer Arms

Aminolysis of methyl ester **212** with methylamine gave the corresponding amide **213** in excellent yield. Subsequent amino acid coupling using the standard Boc approach with *N*-Boc protected valine gave the tripeptide **214** in good yield which proved to be insoluble in H_2O, CH_3OH, CH_3CN and EtOAc despite the hydrophobic nature of the valine side chains. Hence, purification was achieved by washing repeatedly with these solvents. Another cycle of peptide coupling with *N*-Boc protected glycine gave the desired tetrapeptide **215** in good yield which again proved insoluble and was purified by repeated washing with the above solvents.



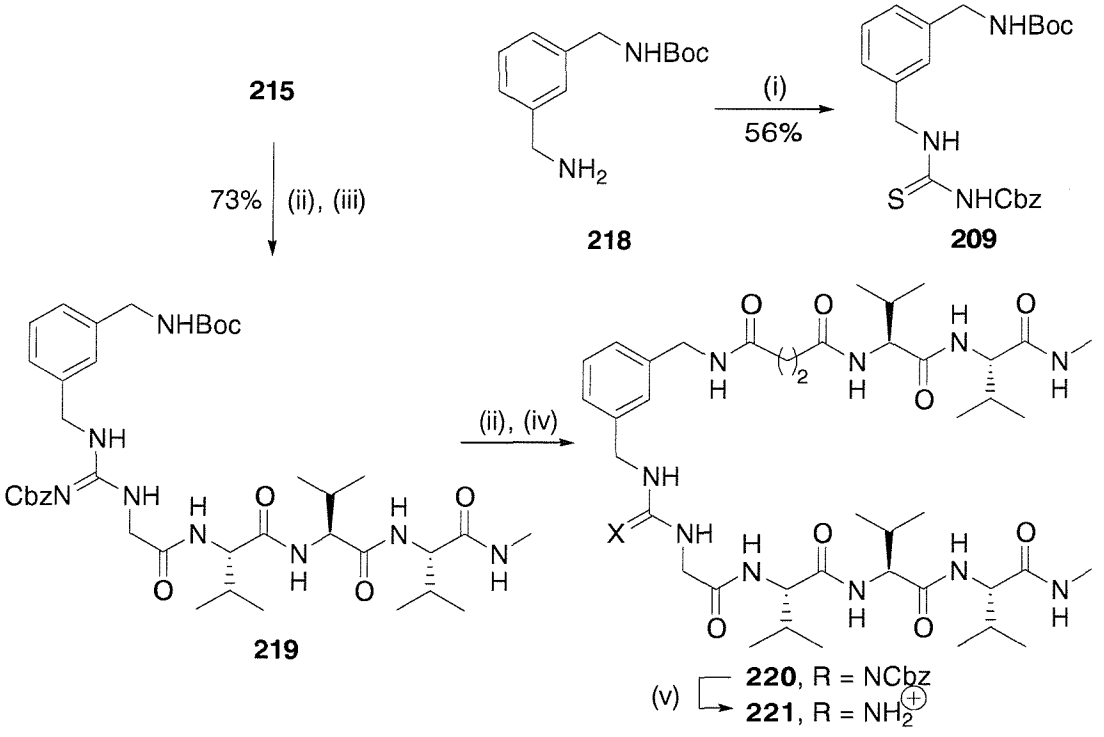
Reagents and conditions. (i) 40% $MeNH_2/H_2O$, THF; (ii) 20% TFA/CH_2Cl_2; (iii) Boc-Val-OH, EDC, HOBt, DIPEA, THF, DMF; (iv) Boc-Gly-OH, EDC, HOBt, DIPEA, DMF; (v) 4-(benzyloxy)-4-oxobutanoic acid, EDC, HOBt, DIPEA, THF, DMF; (vi) $H_2/Pd/C$, CH_3OH.

Scheme 23. Synthesis of *N*-methyl tweezer arms.

Deprotection of carbamate **213** and direct ring opening of succinic anhydride in the presence of base did not yield the required acid **216** directly due to difficulty of isolation of the polar product. Deprotection of carbamate **213** and coupling with 4-(benzyloxy)-4-oxobutanoic acid under standard conditions gave amide **217** in good yield. Hydrogenation of benzyl ester **217** gave acid **216** in a poor yield due to the insolubility of the product in CH_3OH and the extraction of the product from the solid supported palladium using DMF.

4.2.2 Synthesis of N-Methyl Tweezer

Reaction of amine **218**, synthesised using the procedure of Anslyn,[112] with CbzNCS gave the required thiourea **209** in moderate yield. Deprotection of carbamate **215** under standard conditions and reaction with thiourea **209** in the presence of EDC in CH_2Cl_2 with a few drops of DMF gave the desired Cbz protected guanidine **219** in good yield which was purified by washing the insoluble solid with 1% HCl, H_2O, CH_3OH, CH_3CN and Et_2O.



Reagents and conditions. (i) CbzNCS, CH_2Cl_2; (ii) 20% TFA/CH_2Cl_2; (iii) **209**, EDC, HOBt, DIPEA, DMF; (iv) **216**, EDC, HOBt, DIPEA, DMF; (v) H_2/Pd/C, DMF/H_2O.

Scheme 24. Synthesis of tweezer **221**.

Deprotection of carbamate **219** under standard conditions and attempted coupling with acid **216** to yield tweezer **220** gave a product which was only soluble in DMF and DMSO and attempted purification by washing using a range of solvents was unsuccessful due to the insolubility of the starting materials. ^{1}H NMR supported the presence of significant proportions of the required guanidine **220** and hence the mixture of compounds was hydrogenated under the conditions successful for the simple systems described above (see Chapter 3). Reaction in DMF at 40 °C over 48 h gave products in which no evidence of the Cbz group could be observed *via* ^{1}H NMR. However, the free guanidinium **221,** as either its chloride or hexafluorophosphate salt, also proved to be insoluble in all solvents except DMF and DMSO. Hence, separation of the guanidinium from equally insoluble residual starting materials was not possible.

Therefore, it was concluded that the N-methyl amide was too polar a functionality to permit simple synthesis of the required tweezer **221** evinced by the insolubility of the apparently lipophilic tripeptide **214** in non-polar media. Hence, it was hoped that switching to a more lipophillic amide would provide a greater degree of solubility in organic solvents.

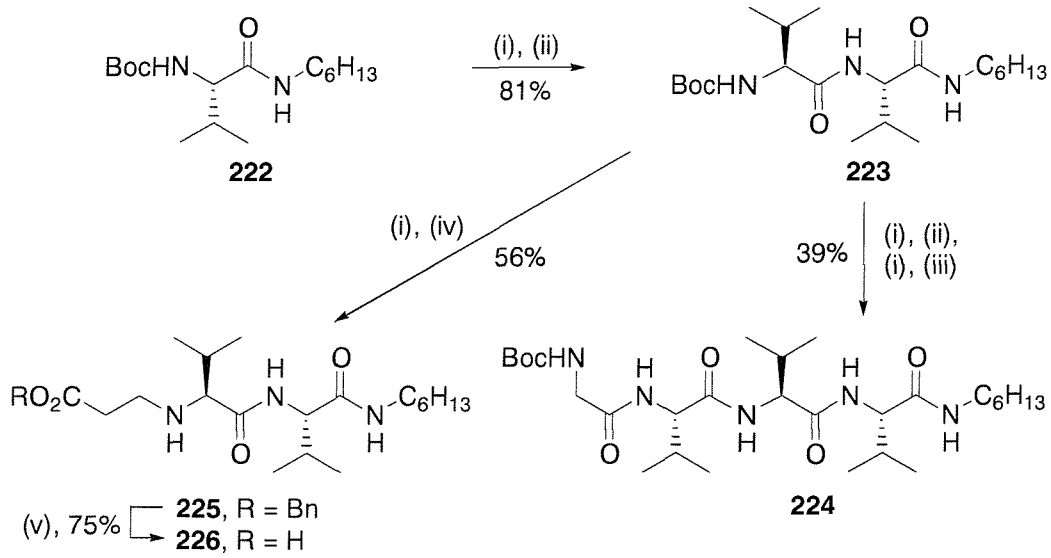
4.3 N-Hexyl Terminating Tweezers

Replacing the *N*-methyl amide with a long chain hydrocarbon should provide the required solubility. Hexylamine was determined as a suitable amine for the tweezer modification as the bulk of the NMR signals arising from would be in the region <2.5 ppm and hence not interfere with the amide and α-CH regions of interest during binding studies.

4.3.1 Synthesis of N-Hexyl Tweezer Arms

Synthesis of amide **222** was achieved by reaction *N*-Boc valine with n-hexylamine under standard peptide coupling conditions in 81% yield. Deprotection of carbamate **222** and subsequent reaction with *N*-Boc valine gave dipeptide **223** in good yield which was soluble in organic media and amenable to purification by column chromatography. Two further cycles of peptide coupling first with *N*-Boc

valine then *N*-Boc glycine gave tetrapeptide **224** in moderate yield. Although the intermediate tripeptide was soluble in organic media, the tetrapeptide **224** was only soluble in DMF and a CH_3OH/CH_2Cl_2 mixture. Tetrapeptide **224** was moderately soluble in DMSO but displayed gelation properties if left for 3 h.



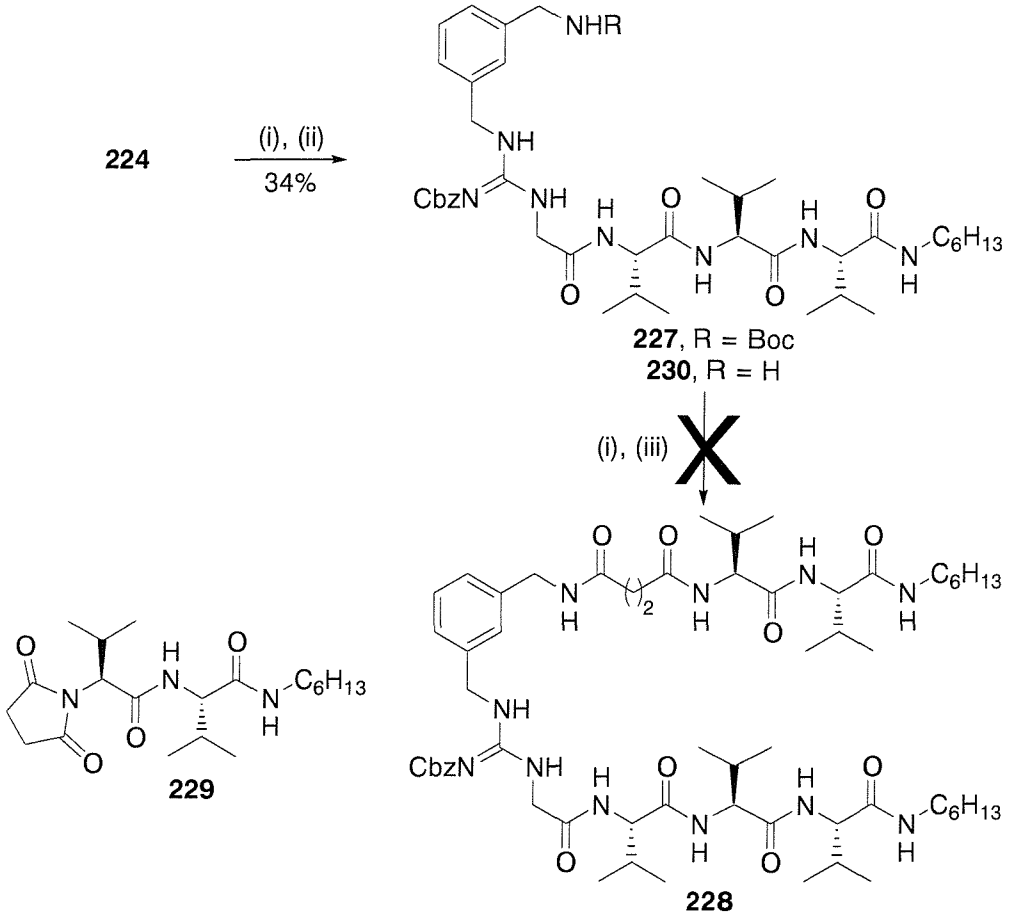
Reagents and conditions. (i) 20% TFA/CH_2Cl_2; (ii) Boc-Val-OH, EDC, HOBt, DIPEA, THF, DMF; (iii) Boc-Gly-OH, EDC, HOBt, DIPEA, THF, DMF; (iv) 4-(benzyloxy)-4-oxobutanoic acid, EDC, HOBt, DIPEA, CH_2Cl_2; (v) $H_2/Pd/C$, CH_3OH.

Scheme 25. Synthesis of N-hexyl tweezer arms.

Dipeptide **223** was deprotected and coupled with 4-(benzyloxy)-4-oxobutanoic acid to give amide **225** in moderate yield, again due to the insolubility of the product in non-polar media, Purification of **225** was achieved by washing the solid with H_2O, CH_3CN and Et_2O. Deprotection of ester **225** gave acid **226**, which was sufficiently soluble to allow purification by column chromatography, in moderate yield.

4.3.2 Synthesis of N-Hexyl Tweezer

Carbamate **224** was deprotected and condensed with **209** using the normal procedure in 30:1 CH_2Cl_2/DMF in acceptable yield and the insolubility of the resultant guanidine **227** allowed purification by washing with 1% HCl, H_2O, CH_3OH, CH_3CN and Et_2O. As with tetrapeptide **224**, guanidine **227** displayed gelation properties in DMSO upon standing for 2 h.



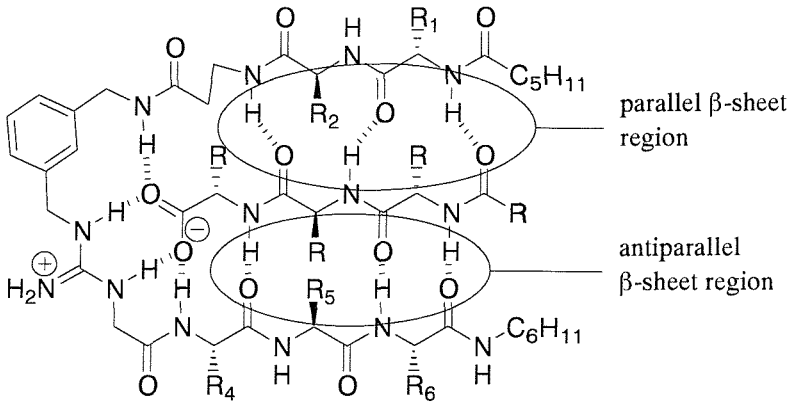
Reagent and conditions. (i) 20% TFA/CH_2Cl_2; (ii) **209**, EDC, DIPEA CH_2Cl_2, DMF; (iii) **226**, EDC, HOBt, DIPEA, DMF.

Scheme 26 Synthesis of *N*-hexyl tweezer **228**.

Deprotection of carbamate **227** and coupling with acid **226** under standard peptide coupling conditions did not give the required tweezer **228** and ESMS evidence suggested that succinamide **229** was the main product from the reaction and no evidence of guanidine **228** was observed by ^1H NMR or ESMS. The general poor solubility of the free amine **230** in DMF results in a large reaction volume for the peptide coupling and hence the intramolecular cyclisation of acid **226** to give succinamide **229** is preferred. Indeed upon purification by column chromatography in 20% NH_3 sat. CH_3OH/CH_2Cl_2 the free amine **230** was recovered in approaching quantitative yield. Hence this was not a feasible motif for the synthesis of a tweezer receptor due to unwanted side reactions.

4.4 Mixed Antiparallel-Parallel β-Sheet Tweezer

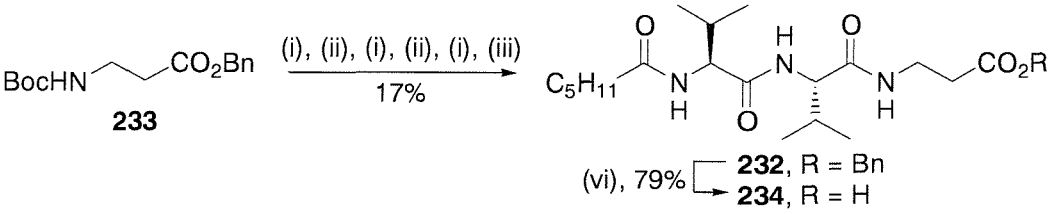
An alternative approach was available using amine **230** to derive a tweezer using which would complex a tripeptide with one antiparallel β-sheet region and one parallel region as in **231** (see Figure 64). Although this is, in principle, a weaker binding motif than the all antiparallel tweezer **206** it still provides a potentially interesting system for study. The replacement of the succinamide motif in tweezer **206** above with an aminopropanamide motif in **231** should reduce the possibility of side reactions in the final peptide coupling.



231

Figure 64. Mixed antiparallel-parallel β-sheet tweezer.

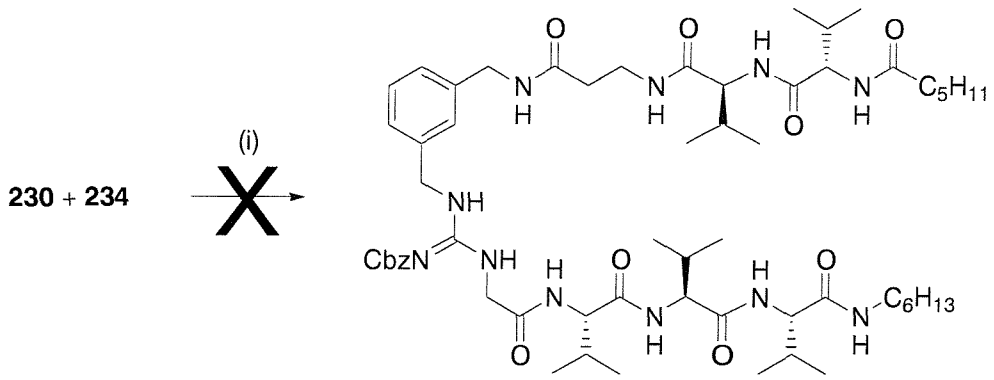
In order to facilitate the synthesis of the mixed β-sheet tweezer a new arm derived from β-alanine was synthesised. Again as with the tweezer above, a long alkyl chain was used to increase the solubility of the peptide constructs.



Reagents and conditions. (i) 20% TFA/DCM, (ii) Boc-Val-OH, EDC, HOBt, DIPEA, THF, DMF; (iii) hexanoic acid, EDC, HOBt, DIPEA, THF, DMF; (iv) H_2/Pd/C, CH_3OH.

Scheme 27. Synthesis of parallel β-sheet side arm.

Three amino acid coupling cycles yielded tripeptide **232** from carbamate **233** in moderate yield over six steps. Hydrogenation of ester **232** in CH_3OH yielded free acid **234** which could be purified by column chromatography. Tripeptide **232** displayed analogous gelation properties observed for **224** and **227**.



Reagents and conditions. (i) EDC, HOBt, DIPEA, DMF.

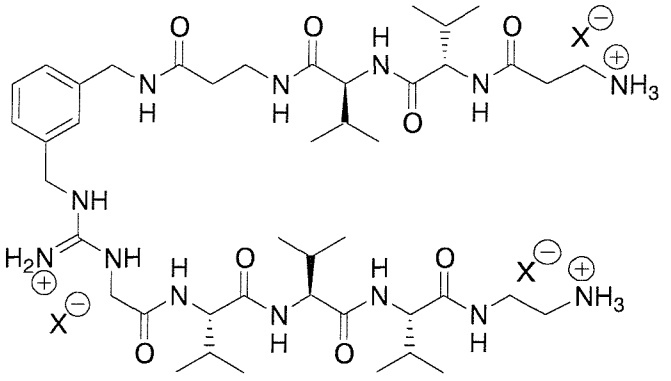
Scheme 28. Synthesis of mixed antiparallel-parallel β-sheet tweezer.

The coupling of amine **230** with acid **234** in DMF gave an insoluble white precipitate which dissolved only in DMSO after sonication and ^{1}H NMR suggests that this is principally required guanidine **235** however gelation of the DMSO after only a few minutes prevented good data being obtained. Attempts to improve the solubility by directly hydrogenating the crude reaction mixture gained from the coupling was only possible in DMSO at 50 °C due to insolubility and did not yield the required guanidinium salt **236**. Attempts to extract the product from the immobilised palladium using a range of polar solvents and solvent mixtures or under acid and basic conditions was unsuccessful. It could be assumed that either the product is completely insoluble in all media or the elevated temperature of the reaction resulted in decomposition, presumably through hydrogenation of the benzylic positions, of the required guanidinium **236**.

4.5 Conclusions

The insolubility of all three tweezer constructs prevented the completion of their synthesis. The isolation and purification of such tweezers might be improved by the use of more polar functionality on the terminal group or amino acid side chains to

provide better solubility in polar solvents. The combination of relatively hydrophilic amides and hydrophobic amino acid residues results in compounds which are not sufficiently soluble in polar or non-polar media to permit adequate purification. Use of ammoniums for the terminating groups, as in **237**, may increase the solubility of the final tweezer in polar solvents and therefore allow purification of the tweezer motif.



237

Figure 65. Water soluble tweezer receptor target.

Conclusions and Future Perspectives

Chapters 2 and 3 have described a series of thiourea and guanidinium salt based model carboxylate binding sites which bind acetate in DMSO. In both cases the binding can be significantly enhanced *via* preorganisation through hydrogen bonding from and amide to a pyridine nitrogen lone pair. Receptor **238** contains the arrangement of amide hydrogen bond donors which provides the highest binding constant with acetate in both the thiourea series (Chapter 2) and the guanidinium series (Chapter 3). As expected the guanidnium series gave overall higher binding contants but were generally less affected by small changes in receptor structure, with the exception of the addition of a pyridine.

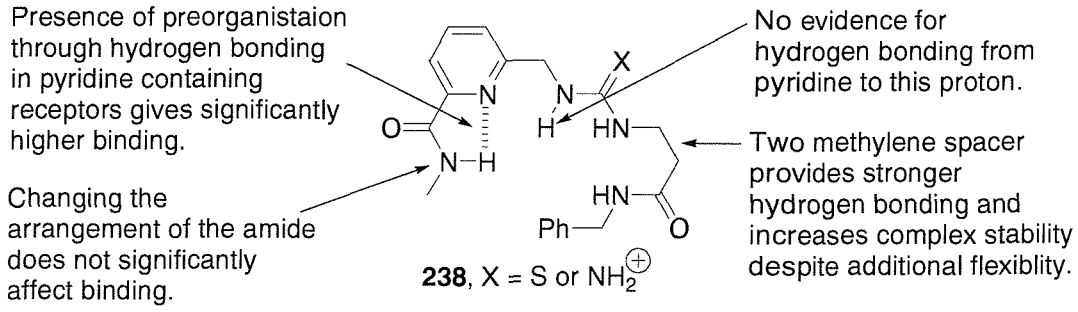


Figure 66. Optimum carboxylate binding site.

Application of the synthetic methodologies used for the synthesis of model carboxylate binding sites to a rationally designed tweezer has provided potential routes to a range of receptors for tripeptide carboxylate but their synthesis and subsequent binding studies have been hampered by insolubility of the peptide constructs.

The investigation of the binding of both the best thioureas and guanidinium salts in more competitive media would investigate the applicability in aqueous systems, especially for the guanidinium salts. The synthesis of a tweezer receptor with either different amino acid side chains or an end group with more tuneable solubility properties would allow investigation in to the design of the extended tweezer receptor and selectivity for particular biologically relevant guests.

Experimental

General Experimental

Reactions requiring a dry atmosphere were conducted in oven dried glassware under nitrogen. Where degassed solvents were used, a stream of nitrogen was passed through them immediately prior to use, unless otherwise stated. Solvents were of commercial grade and were used without further purification unless otherwise stated. DCM was distilled over calcium hydride, as was petroleum ether where the fraction boiling between 40 °C and 60 °C was used. TLC analysis was carried out using foil-backed sheets coated with silica gel (0.25 mm) and containing the fluorescent indicator UV_{254}. Flash column chromatography was performed, on Sorbsil C60, 40-60 mesh silica.

Instrumentation

Proton NMR spectra were obtained at 300 MHz on a Bruker AC 300 and at 400 MHz on a Bruker DPX 400 spectrometer. Carbon NMR spectra were recorded at 75 MHz on a Bruker AC 300 spectrometer and at 100 MHz on a Bruker DPX 400 spectrometer. Chemical shifts are reported in ppm on the δ scale relatively to the signal of the solvent used. Coupling constants (*J*) are given in Hz. Signal multiplicities were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique. In certain examples quaternary carbons are not oberseved due to long relaxation times these are stated in the appropriate sections.

Infra-red spectra were recorded on BIORAD Golden Gate FTS 135. All samples were run either as neat solids or as oils. Melting points were determined in open capillary tubes using a Gallenkamp Electrothermal melting point apparatus and are uncorrected.

Mass spectra were obtained on a VG analytical 70-250 SE normal geometry double focusing mass spectrometer. All electrospray (ES) spectra were recorded on a Micromass Platform quadrupole mass analyser with an electrospray ion source using acetonitrile as solvent. High resolution accurate mass measurements were carried out

at 10,000 resolution on a Bruker Apex III FT-ICR mass spectrometer. Microanalysis were performed by MEDAC Ltd., Surrey. Calorimetry experiments were performed on an Isothermal Titration Calorimeter from Microcal Inc., USA.

Experimental for NMR Binding Studies

Obtaining association constants by ^1H NMR titration experiments involves titration of a solution of host with a specific guest and recording a ^1H NMR spectrum after each addition. Upon complexation, protons in the host or guest may undergo a change in chemical shifts. In particular, protons involved in hydrogen bonding undergo a dramatic shift and therefore are used to determine association constants. After the data from the titration experiment have been acquired, curve fitting software is employed to determine the association constant. Free host and guest are in equilibrium with the host-guest complex. As association and dissociation is fast on the NMR time scale, only a time averaged spectrum of the host (or guest) and the host-guest complex is observed. Therefore, any observed chemical shift (δ_{obs}) is the mole fraction weighted average of the shifts observed in the free (δ_{free}) and complexed (δ_{bound}) molecule. During the curve fitting procedure, after an initial estimate for K_a and $\Delta\delta$, the theoretical δ_{obs} is obtained for each point. The theoretical values are then compared with the experimentally observed ones and the sum of the difference between each point is determined by the following equation:

$$\text{Sum of differences} = \Sigma\ (\delta_{obs\ (experimental)} - \delta_{obs(theoretical)})$$

If the sum of differences is positive (or negative), the K_a is increased (or decreased) and the value $\Delta\delta$ recalculated and the whole calculation repeated until the values converge. A detailed explanation of the theoretical basis to the above discussion has been published by Wilcox.[131] A more recent review on the determination of association constants from solution NMR data has been published by Fielding.[132]

Method Used for Obtaining Binding Constants

All ^1H NMR titration experiments were conducted on either a Brüker AM 300 spectrometer at 298 K, unless otherwise stated. $CDCl_3$ was passed over a pad of basic alumina prior to use and collected over molecular sieves (4Å). A sample of host was dissolved in the deuterated solvent. A portion of this solution was used as

the host NMR sample and the remainder used to dissolve a sample of the guest, so that the concentration of the host remained constant throughout the titration. Guest stock solutions were typically prepared such that 10μL of that solution contained 0.1 equivalents of guest with respect to host, unless otherwise stated. Successive aliquots of the guest solution were added to the host NMR sample and ^{1}H NMR sample recorded after each addition. The hydrogens monitored during binding studies were the thiourea or amide protons in the host molecule unless otherwise stated. The changes in chemical shifts of all the hosts signals as a function of guest concentration were analysed with purpose-written software, kindly provided by C.A. Hunter, where a 1:1 binding mode or a 1:2 mode was assumed. These programs fit the data to the appropriate binding model to yield the association constant, the bound chemical shift and the free chemical shift. For a greater degree of accuracy, association constants quoted an average of all the association constants obtained from each proton monitored in the host molecule.

Experimental for ITC Binding Studies

By directly measuring the heat evolved or absorbed as a function of time ITC can determine, in one stroke, all the thermodynamic parameters involved in a chemical process. In a single experiment the binding constant (K_a), the stoichiometry (n) and the enthalpy (ΔH) of the process investigated are determined. From the association constant, it is possible to determine the free energy and entropy of binding for the interaction. A typical ITC titration begins with a known concentration of macrocycle dissolved in solution to which controlled aliquots of the guest species under test are added through a syringe. As the complexation takes place, an endothermic or exothermic signal, depending on the nature of the complexation, is observed. As the guest concentration increases and the supply of macrocycle available for binding is exhausted, we reach a plateau in terms of the amount of heat evolved or absorbed on further addition of guest.

Method for Obtaining Calorimetric Data

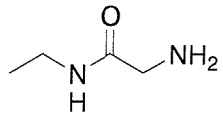
All binding experiments were performed on an isothermal titration calorimeter from Microcal Inc. (Northampton, MA). In a typical experiment a 1 - 2 mM receptor solution is added to the calorimetric cell. A 48 - 78 mM solution of

TBA acetate is introduced in 50 injections of 5 μL, for a total of 250μL of added guest. Such high concentrations of guests are important to generate sharp curves, necessary for acceptable curve fitting. The solution is continuously stirred to ensure rapid mixing and kept at 25°C, through the combination of an external cooling bath and an internal heater. Dilution effects are determined by performing a blank experiment by adding the same guest solution into the pure solvent and subtracting this from the raw titration to produce the final binding curve. Association parameters are found by applying either one-site or two-sites models, using the Origin software provided. These methods rely on standard nonlinear least-squares regression (Levenberg-Marquard method) to fit the curves, taking into account the change in volume that occurs during the calorimetric titration.

Experimental for Chapter 2

Synthesis

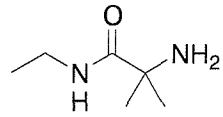
N-1-Ethyl-2-aminoacetamide[133] 91



91

Methyl 2-aminoacetate hydrochloride (5 g, 39.8 mmol) was stirred in 70% $EtNH_2/H_2O$ for 3 h. The solvents were removed *in vacuo*, the resultant viscous oil dissolved in water (30 mL) and freeze dried. The resultant white solid was dissolved in a minimum amount of sat. $NaHCO_3$ and extracted with $CHCl_3$ (15 x 10 mL). The organic phases were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **91** (2.12 g, 20.8 mmol, 52%) as a clear oil. IR (neat): 3303 (m), 3071 (w), 2972 (m), 2933 (m), 2876 (m), 1642 (m), 1527 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.24 (1 H, br, NH), 3.37-3.28 (4 H, m, 2 x CH_2), 1.17 (3 H, t, J = 7 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 172.6 (C), 44.7 (CH_2), 33.7 (CH_2), 14.8 (CH_3); ESMS: m/z (%): 103 ($(M+H)^+$, 100).

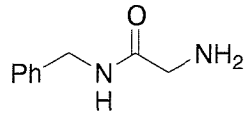
N-1-Ethyl-2-amino-2-methylpropanamide 93



93

Methyl 2-amino-2-methylpropanoate hydrochloride[134] (1.46g, 11.6 mmol) was stirred with 70% $EtNH_2/H_2O$ for 3 h. The solvent was removed *in vacuo*, partitioned between CH_2Cl_2 (20 mL) and H_2O (10 mL) and the aqueous phase washed with CH_2Cl_2 (3 x 20 mL). The organic phases were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **93** (810 mg, 6.23 mmol, 49%) as a clear oil. IR (neat): 3328 (m), 2970 (m), 2932 (w), 2874 (w), 1637 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.57 (1 H, br, EtN*H*), 3.25 (2 H, dq, J = 6, 7 Hz, CH_2), 1.35 (6 H, s, $C(CH_3)_2$), 1.14 (3 H, t, J = 7 Hz, CH_3CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): 187.3 (C), 54.7 (C), 34.0 (CH_2), 29.3 (CH_3), 14.8 (CH_3); ESMS: m/z (%): 131 ($(M+H)^+$, 100); HRMS (ES) for $C_6H_{15}N_2O$ $(M+H)^+$: calcd 131.1179, found 131.1180.

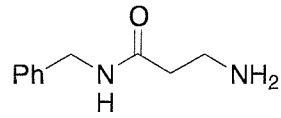
N-1-Benzyl-2-aminoacetamide[135] 96



96

N-1-Benzyl-2-(1,3-dioxo-2,3-dihydro-1*H*-2-isoindolyl)acetamide[107] **98** (2.6 g, 8.84 mmol) was stirred with $(NH_2)_2.H_2O$ (900 μL, 18.0 mmol) in EtOH (50 mL) at gentle reflux for 2 h. The solid was filtered off and the solvent removed *in vacuo* to yield the title compound **96** (994 mg, 6.06 mmol, 68%) as a hydroscopic solid. IR (solid): 3330 (w), 3268 (w), 3193 (w), 3062 (w), 2935 (w), 1681 (s), 1660 (s), 1622 (s), 1556 (s), 1536 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.60 (1 H, br, NH), 7.37-7.26 (5 H, m, Ar), 4.49 (2 H, d, J = 6 Hz, $ArCH_2$), 3.42 (2 H, s, CH_2NH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$): 172.8 (C), 138.4 (C), 128.8 (CH), 127.9 (CH), 127.6 (CH), 44.8 (CH_2), 43.21 (CH_2); ESMS: m/z (%): 165 ($(M+H)^+$, 100%); HRMS (ES) for $C_9H_{13}NO$ $(M+H)^+$: calcd 165.1022, found 165.1022. Data agrees with that reported by Royer.[135]

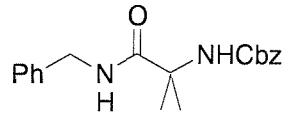
_N_-1-Benzyl-3-aminopropanamide 97



97

_N_-1-Benzyl-3-(1,3-dioxo-2,3-dihydro-1_H_-2-isoindolyl)propanamide[107] **99** (1.48 g, 4.81 mmol) was refluxed with $(NH_2)_2.H_2O$ (705 μL, 14.5 mmol) in EtOH (40 mL) for 3 h. The reaction was allowed to cool, the solid filtered off, washed with EtOH (50 mL) and the solvent removed *in vacuo* to yield a white solid. The solid was stirred in 10% CH_3OH/CH_2Cl_2 (50 mL) and the insoluble material filtered off. The solvent was removed *in vacuo* to yield the title compound **97** (731 mg, 4.11 mmol, 85%) as white solid. IR (solid): 3286 (m), 3063 (w), 3031 (w), 2932 (w), 1642 (s), 1548 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.53 (1 H, br, NH), 7.45-7.31 (5 H, m, Ar), 4.53 (2 H, d, J = 6 Hz, PhCH_2), 3.10 (2 H, br, CH_2NH_2), 2.44 (2 H, t, J = 6 Hz, C$H_2CH_2NH_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): 172.5 (C), 138.7 (C), 128.8 (CH), 127.8 (CH), 127.5 (CH), 43.4 (CH_2), 38.7 (CH_2), 38.3 (CH_2); ESMS: m/z (%): 179 ($(M+H)^+$, 100); HRMS (ES) for $C_{10}H_{15}N_2O$ $(M+H)^+$: calcd 179.1179, found 179.1178.

Benzyl _N_-[2-(benzylamino)-1,1-dimethyl-2-oxoethyl]carbamate 102

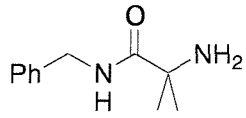


102

2-Amino-2-methylpropanoic acid **101** (500 mg, 4.85 mmol) was stirred in 4 M NaOH (1.2 mL) at 0 °C, CbzCl (5 x 150 μL, 5 34 mmol) and 4 M NaOH (5 x 300 μL) were added alternately over 20 min and the reaction stirred at 0 °C for 1.5 h. H_2O (4 mL) was added, washed with Et_2O (2 x 5 mL), acidified (c. HCl, pH ~ 2, CARE!) and extracted with EtOAc (3 x 10 mL). The EtOAc phases were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield a clear oil which was stirred with EDC (456 mg, 2.39 mmol) and HOBt (323 mg, 2.39 mmol) in CH_2Cl_2 (12 mL). Benzylamine (261 μL, 2.39 mmol) was added followed by DIPEA (917 μL, 5.26 mmol) and the reaction stirred for 48 h. CH_2Cl_2 (10 mL) was added, washed with sat. $NaHCO_3$ (2 x 5 mL), 1 M $KHSO_4$ (2 x 5mL) and brine (5 mL), dried

($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (CH_2Cl_2 – 5% CH_3OH/CH_2Cl_2) yielded the title compound **102** (515 mg, 1.57 mmol, 32%) as a white solid. MP = 122-125 °C; IR (solid): 3294 (m), 3265 (m), 3063 (w), 2935 (w), 1738 (w), 1701 (w), 1640 (s), 1551 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.29-7.15 (10 H, m, Ar), 6.53 (1 H, br, $PhCH_2NH$), 5.21 (1 H, br s, N*H*Cbz), 5.00 (2 H, s, OCH_2Ph), 4.36 (2 H, d, J = 5 Hz, $PhCH_2NH$); ^{13}C NMR (100 MHz, $CDCl_3$): 174.29 (C), 155.25 (C), 138.37 (C), 136.28 (C), 128.82 (CH), 128.72 (CH), 128.40 (CH), 128.28 (CH), 127.73 (CH), 127.57 (CH), 66.97 (CH_2), 57.19 (C), 43.90 (CH_2), 25.81 (CH_3); ESMS: m/z (%): 349 ($(M+Na)^+$, 100); HRMS (ES) for $C_{19}H_{22}N_2O_3Na$ $(M+Na)^+$: calcd 349.1522, found 349.1526.

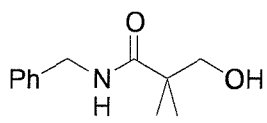
N-1-Benzyl-2-amino-2-methylpropanamide 100



100

Benzyl *N*-[2-(benzylamino)-1,1-dimethyl-2-oxoethyl]carbamate **102** (200 mg, 0.61 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 8 h. The solid was filtered off, washed with CH_3OH (20 mL) and the solvent removed *in vacuo* to yield the title compound **100** (112 mg, 0.58 mmol, 96%) as a clear oil. IR (solid): 3331 (m), 2968 (w), 2929 (w), 2873 (w), 1650 (s), 1518 (s) cm^{-1}; ^1H NMR (300 MHz, CD_3OD): 7.34-7.20 (5 H, m, Ar), 4.38 (2 H, s, CH_2), 1.33 (6 H, s, $C(CH_3)_2$); ^{13}C NMR (75.5 MHz, CD_3OD): 180.1 (C), 140.2 (C), 129.5 (CH), 128.4 (CH), 128.1 (CH), 55.7 (C), 44.1 (CH_2), 28.4 (CH_3); ESMS: m/z (%): 193 ($(M+H)^+$, 100).

N-1-Benzyl-3-hydroxy-2,2-dimethylpropanamide 104

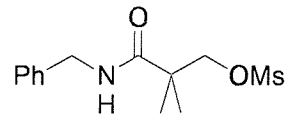


104

3-Hydroxy-2,2-dimethylpropanoic acid **103** (260 mg, 2.20 mmol) and EDC (420 mg, 2.20 mmol) were stirred in CH_2Cl_2 (10 mL) and benzylamine (219 μL, 2.00 mmol) then DIPEA (381 μL, 2.20 mmol) were added and the reaction stirred for 24 h. CH_2Cl_2 (10 mL) was added, washed with sat. $NaHCO_3$ (10 mL), 1 M $KHSO_4$ (10

mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (1-5% CH_3OH/CH_2Cl_2) yielded the title compound **104** (265 mg, 1.28 mmol, 65%) as a white solid. IR (solid): 3337 (br), 2957 (m), 2030 (m), 2859 (m), 1737 (w), 1639 (s), 1541 (s), 1506 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.27-7.17 (5 H, m, Ar), 6.30 (1 H, br, NH), 4.38 (2 H, d, J = 6 Hz, PhCH_2), 3.51 (2 H, s, CH_2OH), 1.14 (6 H, s, $C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): 178.2 (C), 138.7 (C), 129.2 (CH), 128.0 (CH), 128.0 (CH), 70.5 (C), 43.8 (CH_2), 43.4 (CH_2), 23.2 (CH_3); ESMS: m/z (%): 208 ($(M+H)^+$, 100); HRMS (ES) for $C_{12}H_{17}NO_2Na$ $(M+Na)^+$: calcd 230.1151, found 230.1152.

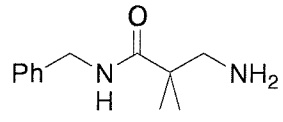
3-(Benzylamino)-2,2-dimethyl-3-oxopropyl methanesulfonate 105



105

N-1-Benzyl-3-hydroxy-2,2-dimethylpropanamide **104** (100 mg, 0.48 mmol) and dry TEA (101 μL, 0.72 mmol) were stirred in dry CH_2Cl_2 (3 mL) under N_2 at 0 °C. MsCl (41 μL, 0.52 mmol) was added and the reaction stirred at 0 °C for 30 min. CH_2Cl_2 (5 mL) was added, washed with 1% HCl (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **105** (113 mg, 0.40 mmol, 83%) as a clear oil. ^1H NMR (300 MHz, $CDCl_3$): 7.39-7.23 (5 H, m, Ar), 6.26 (1 H, br, NH), 4.45 (2 H, d, J = 5 Hz, PhCH_2), 4.22 (2 H, s, CH_2O), 2.91 (3 H, s, SO_2CH_3), 1.30 (6 H, s, $C(CH_3)_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$): 174.4 (C), 138.2 (C), 128.9 (CH), 127.6 (2 x CH), 76.2 (CH_2), 43.8 (CH_2), 42.8 (C), 36.9 (CH_3), 22.3 (CH_3).

***N*-1-Benzyl-3-amino-2,2-dimethylpropanamide 107**

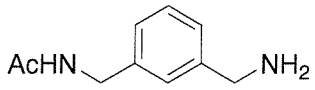


107

3-(Benzylamino)-2,2-dimethyl-3-oxopropyl methanesulfonate **105** (113 mg, 0.40 mmol) and NaN_3 (62 mg, 0.96 mmol) were stirred in DMF (3 mL) at 90 °C for 2 h. The solvent was removed *in vacuo* and EtOAc (10 mL) was added, washed with H_2O (5 mL) and the aqueous phase extracted with EtOAc (2 x 5 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and the solvent

removed *in vacuo* to yield azide **106** (106 mg) as a clear oil. Azide **106** (100 mg, 0.43 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 3 h. The solid was filtered off, washed with CH_3OH (10 mL) and the solvent removed *in vacuo* to yield the title compound **107** (61 mg, 0.30 mmol, 69%) as a yellow oil. **IR** (solid): 3311 (w), 3028 (w), 2964 (w), 2928 (w), 2871 (w), 1639 (s), 1541 (s) cm^{-1}; **^{1}H NMR** (300 MHz, CD_3OD): 7.33-7.20 (5 H, m, Ar), 4.38 (2 H, s, PhCH_2), 2.73 (2 H, s, CH_2NH_2), 1.20 (6 H, s, $C(CH_3)_2$); **^{13}C NMR** (75.5 MHz, CD_3OD): 179.6 (C), 140.5 (C), 129.5 (CH), 128.3 (CH), 128.0 (CH), 52.7 (CH_2), 44.2 (C), 44.0 (CH_2), 23.7 (CH_3); **ESMS**: m/z (%): 207 ($(M+H)^+$, 100); **HRMS** (ES) for $C_{12}H_{19}N_2O$ $(M+H)^+$: calcd 207.1492, found 207.1494.

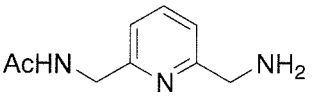
***N*-[3-(Aminomethyl)benzyl]acetamide 109**



109

Ac_2O (7 mL g, 74.1 mmol) in $CHCl_3$ (240 mL) was added dropwise to a stirred mixture of *m*-xylenediamine **108** (10.05 g, 73.9 mmol) and TEA (21.5 mL, 161 mmol) over 4 h and the reaction stirred for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (10% NH_3 sat. CH_3OH/CH_2Cl_2) yielded the title compound **109** (5.41 g, 33.3 mmol, 41%) as a yellow oil. **IR** (neat): 3276 (m), 3064 (w), 1649 (s), 1556 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 7.30-7.13 (4 H, m, Ar), 6.31 (1 H, br, NH), 4.38 (2 H, d, J = 7 Hz, NHCH_2), 3.81 (2 H, s, CH_2NH_2), 1.98 (3 H, s, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.2 (C), 143.5 (C), 138.6 (C), 128.9 (CH), 126.6 (CH), 126.3 (CH), 126.3 (CH), 46.3 (CH_2), 43.6 (CH_2), 23.2 (CH_3); ESMS: m/z (%): 179 ($(M+H)^+$, 100); HRMS (ES) for $C_{10}H_{15}N_2O$ $(M+H)^+$: calcd 179.1179, found 179.1179.

***N*-1-[6-(Aminomethyl)-2-pyridyl]methylacetamide 111**

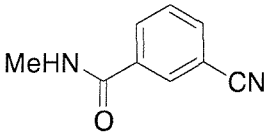


111

Ac_2O (281 μL, 2.99 mmol) in $CHCl_3$ (30 mL) was added dropwise to a stirred solution of [6-(ammoniomethyl)-2-pyridyl]methylammonium di(2,2,2-trifluoroacetate) **110** (1 g, 2.99 mmol), DMF (3 drops) and TEA (1.66 mL, 11.96

mmol) in $CHCl_3$ (4.5 mL) over 10 h and the reaction stirred for 24 h. The solvent was removed *in vacuo*, the resultant white solid partitioned between CH_2Cl_2 (10 mL) and sat. $NaHCO_3$ (10 mL) and the aqueous phase was extracted with CH_2Cl_2 (8 x 10 mL). The combined organic phases were dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (10% NH_3 sat. CH_3OH/DCM) yielded the title compound **111** (128 mg, 7.15 mmol, 24%) as a white solid. MP = 92-94 °C; IR (solid): 3274 (s), 3071 (m), 2927 (m), 1648 (s), 1594 (s), 1571 (s), 1561 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.56 (1 H, t, J = 7 Hz, pyr), 7.12-7.05 (3 H, m, pyr and NH), 4.46 (2 H, d, J = 5 Hz, AcNHCH_2), 3.89 (2 H, s, CH_2NH_2), 2.00 (3 H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.7 (C), 161.6 (C), 156.5 (C), 137.7 (CH), 120.5 (CH), 120.2 (CH), 47.9 (CH_2), 45.0 (CH_2), 23.5 (CH_3); ESMS: m/z (%): 180 ($(M+H)^+$, 100).

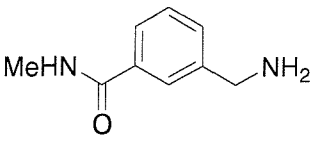
N-1-Methyl-3-cyanobenzamide[136] 113



113

3-Cyanobenzoic acid **112** (1.5 g, 10.2 mmol) was dissolved in CH_3OH (15 mL) and cooled to 4 °C, $SOCl_2$ (1.44 mL, 20.4 mmol) was added and the reaction stirred at reflux for 18 h. The solvent was removed *in vacuo* to yield a grey solid which was stirred in 40% $MeNH_2/H_2O$ (12 mL) and THF (6 mL) for 18 h. The solvent was removed *in vacuo*, dissolved in EtOAc (30 mL), washed with 1 M $KHSO_4$ (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (5% CH_3OH/CH_2Cl_2) yielded the title compound **113** (895 mg, 5.59 mmol, 55%) as a white solid. MP = 136-138 °C; IR (solid): 3288 (s), 3085 (w), 3066 (w), 2970 (w), 2943 (w), 2230 (m), 1742 (w), 1631 (s), 1553 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 8.06 (1 H, t, J = 1 Hz, Ar), 8.01 (1 H, dt, J = 8, 1 Hz, Ar), 7.77 (1 H, dt, J = 8, 1 Hz, Ar), 7.56 (1 H, t, J = 8 Hz, Ar), 6.51 (1 H, br, NH), 3.02 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 166.2 (C), 135.7 (C), 134.6 (CH), 131.3 (CH), 130.7 (CH), 129.6 (CH), 118.1 (C), 112.8 (C), 27.0 (CH_3); EIMS: m/z (%): 159 ($(M-H)^{+\bullet}$, 40) 130 (80), 102 (100).

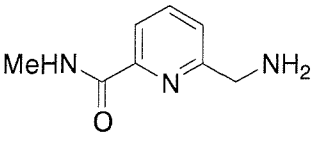
N-1-Methyl-3-(aminomethyl)benzamide 114



114

N-1-Methyl-3-cyanobenzamide **113** (756 mg, 4.73 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (10 mL) for 72 h. The solid was filtered off, the solvent removed *in vacuo* and purification by column chromatography (4% NH_3 sat. CH_3OH/CH_2Cl_2) yielded the title compound **114** (658 mg, 4.02 mmol, 85%) as a waxy solid. IR (solid): 3298 (w), 3072 (w), 2941 (w), 1629 (s), 1606 (s), 1580 (s), 1548 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 7.77 (1 H, s, Ar), 7.63 (1 H, d, J = 8 Hz, Ar), 7.48 (1 H, d, J = 8 Hz, Ar), 7.39 (1 H, t, J = 8 Hz, Ar), 7.14 (1 H, br, NH), 3.84 (2 H, s, CH_2), 2.87 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3CN): 167.1 (C), 144.1 (C), 134.5 (C), 129.3 (CH), 127.8 (CH), 125.1 (CH), 124.4 (CH), 45.2 (CH_2), 25.2 (CH_3); ESMS: m/z (%): 165 ($(M+H)^+$, 100); HRMS (ES) for $C_9H_{13}N_2O$ $(M+H)^+$: calcd 165.1022, found 165.1023.

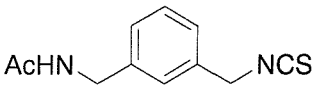
N-2-Methyl-6-(aminomethyl)-2-pyridinecarboxamide 116



116

Ethyl 6-[(1,3-dioxo-2,3-dihydro-1*H*-2-isoindolyl)methyl]-2-pyridinecarboxylate **115** (1.5 g, 4.84 mmol) was stirred in 40% $MeNH_2/H_2O$ (15 mL) for 18 h. The solvent was removed *in vacuo* and the yellow oil purified by column chromatography (2-10% NH_3 sat. CH_3OH/CH_2Cl_2) to yield the title compound **116** (304 mg, 1.84 mmol, 39%) as a waxy solid. MP = 105-107 °C; IR (solid): 3285 (w), 3063 (w), 2926 (w), 1650 (s), 1589 (s), 1533 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 8.08 (1 H, br, NH), 8.01 (1 H, d, J = 8 Hz, pyr), 7.75 (1 H, t, J = 8 Hz, pyr), 7.35 (1 H, d, J = 8 Hz, pyr), 3.96 (2 H, br, CH_2), 2.98 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 160.6 (C), 149.4 (C), 137.9 (C), 123.8 (CH), 121.1 (CH), 120.3 (CH), 47.5 (CH_2), 26.1 (CH_3); ESMS: m/z (%): 166 ($(M+H)^+$, 100); HRMS (ES) for $C_8H_{12}N_3O$ $(M+H)^+$: calcd 166.0975, found 166.0975.

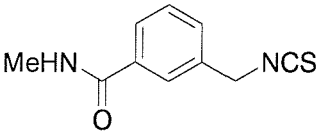
_N_-1-[3-(Isothiocyanatomethyl)benzyl]acetamide 109



118

_N_-[3-(Aminomethyl)benzyl]acetamide **109** (152 mg, 0.85 mmol) was stirred in CH_2Cl_2 (5 mL) and 0.5 M K_2CO_3 (5 mL). $SCCl_2$ (70 μL, 0.93 mmol) was added directly to the lower phase and reaction was stirred for 3 h. The phases were separated and the aqueous phase washed with CH_2Cl_2 (2 x 5 mL). The combined organic phases were washed with 2 M HCl (2 x 5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (CH_2Cl_2) yielded the title compound **118** (148 mg, 0.67 mmol, 80%) as a yellow solid. MP = 76-78 °C; IR (solid): 3290 (m), 2922 (w), 2208 (m), 2104 (m), 1644 (s), 1549 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.37 (1 H, m, Ar), 7.28-7.24 (3 H, m, Ar), 5.81 (1 H, br, AcN*H*), 4.71 (2 H, s, CH_2NCS), 4.47 (2 H, d, J = 6 Hz, AcNHC*H*$_2$), 2.07 (3 H, s, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 169.1 (C), 138.4 (C), 133.8 (C), 131.6 (C) 128.3 (CH), 126.8 (CH), 125.1 (CH), 125.0 (CH), 47.6 (CH_2), 42.4 (CH_2), 22.2 (CH_3); CIMS: m/z (%): 221 ($(M+H)^+$, 100), 162 (82).

_N_-1-Methyl-3-(isothiocyanatomethyl)benzamide 120

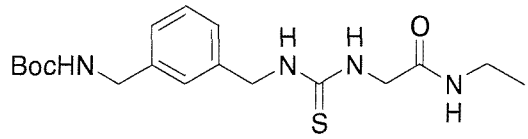


120

_N_-1-Methyl-3-(ammoniomethyl)benzamide chloride **114** (86 mg, 0.43 mmol) was stirred in CH_2Cl_2 (1 mL) and sat. K_2CO_3 (0.5 mL) at 4 °C. $SCCl_2$ (70 μL, 0.92 mmol) was added to the lower phase and the reaction stirred at room temperature for 3 h. CH_2Cl_2 (8 mL) and H_2O (4 mL) were added and the phases separated. The organic phase was washed with H_2O (5 mL), 2 M HCl (2 x 5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **120** (89 mg, 0.28 mmol, 67%) as a white solid. MP = 98-100 °C; IR (solid): 3307 (m), 3083 (w), 2946 (w), 2182 (m), 2103 (m), 1636 (s), 1546 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.74-7.71 (2 H, m, Ar), 7.45-7.41 (2 H, m, Ar), 6.67 (1 H, br, NH), 4.73 (2 H, s, CH_2), 3.00 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 167.7 (C), 135.6

(C), 135.0 (C), 133.4 (C), 129.8 (CH), 129.4 (CH), 126. 8 (CH), 125.7 (CH), 48.5 (CH_2), 27.0 (CH_3); CIMS: m/z (%): 207 ($(M+H)^{+\bullet}$, 24), 148 (100).

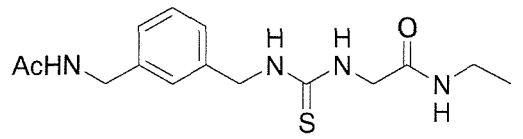
***tert*-Butyl *N*-(3-[([2-(ethylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzyl)carbamate 123**



123

tert-Butyl *N*-[3-(isothiocyanatomethyl)benzyl]carbamate[112] **119** (800 mg, 2.88 mmol) and *N*-1-ethyl-2-aminoacetamide[133] **91** (352 mg, 3.45 mmol) were stirred in CH_2Cl_2 (20 mL) for 24 h. The reaction was washed with sat K_2CO_3 (2 x 10 mL), 2 M HCl (2 x 10 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (50% EtOAc/petroleum ether-neat EtOAc) yielded the title compound **123** (880 mg, 2.32 mmol, 80%) as a white solid. MP = 64 °C (dec.); IR (solid): 3304 (m), 2969 (w), 2340 (w), 1678 (s), 1655 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.20 (1 H, br, $ArCH_2NHC(S)$), 7.94 (1 H, br, $NHCH_2CH_3$), 7.54 (1 H, t, J = 4.5 Hz, $NHCH_2C(O)$), 7.35 (1 H, br, BocN*H*), 7.27 (1 H, t, J = 7 Hz, Ar), 7.15-7.11 (3 H, m, Ar), 4.64 (2 H, br, $ArCH_2NHC(S)$), 4.10 (2 H, d, J = 6 Hz, $BocNHCH_2$), 4.06 (2 H, d, J = 4.5 Hz, $CH_2C(O)$), 3.10 (2 H, quin, J = 7 Hz, CH_2CH_3), 1.39 (9 H, s, $C(CH_3)_3$), 1.02 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 183.4 (C) 168.6 (C), 156.3 (C), 140.7 (C), 139.5 (C), 128.7 (CH), 126.4 (CH), 126.2 (CH), 125.9 (CH), 78.2 (C), 47.5 (CH_2), 43.8 (CH_2), 40.0 (CH_2), 33.9 (CH_2), 28.7 (CH_3), 15.1 (CH_3); ESMS: m/z (%): 403 ($(M+Na)^+$, 100); HRMS (ES) for $C_{18}H_{28}N_4O_3SNa$ $(M+Na)^+$: calcd 403.1774, found 403.1770.

***N*-1-Ethyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]aminoacetamide 124**

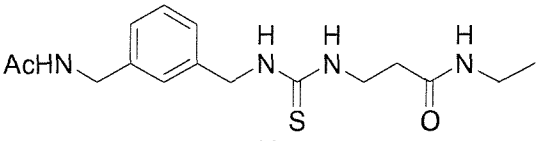


124

N-1-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (200 mg, 0.91 mmol) and *N*-1-ethyl-2-aminoacetamide[133] **91** (111 mg, 1.09 mmol) were stirred in dry CH_2Cl_2 (5 mL) under N_2 for 24 h. The resultant precipitate was filtered off and suspended in

H_2O (10 mL), filtration yielded the title compound **124** (227 mg, 0.71 mmol, 78%) as a white solid. MP = 162-164 °C; IR (solid): 3302 (m), 3262 (m), 1650 (s), 1552 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.41 (1 H, br, AcN*H*), 8.30 (1 H, br, $ArCH_2NHC(S)$), 8.05 (1 H, br, N*H*Et), 7.66 (1 H, br, $NHCH_2C(O)$), 7.37 (1 H, t, J = 7 Hz, Ar), 7.27-7.22 (3 H, m, Ar), 4.74, (2 H, s, $ArCH_2NHC(S)$), 4.33 (2 H, d, J = 6 Hz, $AcNHCH_2$), 4.16 (2H, d, J = 4 Hz, $NHCH_2C(O)$), 3.21 (2 H, m, CH_2CH_3), 1.97 (3 H, s, $COCH_3$), 1.12 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz; DMSO-d_6): 183.4 (C), 169.6 (C), 168.6 (C), 140.1 (C), 139.6 (C), 128.7 (CH), 126.7 (CH), 126.3 (CH), 126.2 (CH), 47.5 (CH_2), 42.5 (CH_2), 33.9 (CH_2), 23.0 (CH_3), 15.1 (CH_3), 1 x CH_2 obscured by solvent; ESMS: m/z (%): 345 ($(M+Na)^+$, 100); HRMS (ES) for $C_{15}H_{22}N_4O_2SNa$ $(M+Na)^+$: calcd 345.1355, found 345.1357.

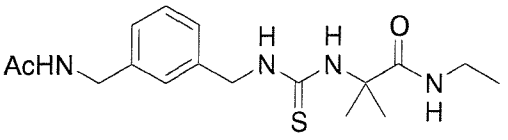
_N_-1-Ethyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino propanamide 125



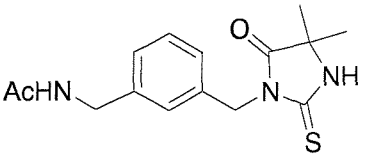
125

N-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (75 mg, 0.34 mmol) and *N*-1-ethyl-3-aminopropanamide[106] **92** (79 mg, 0.68 mmol) were stirred in dry CH_2Cl_2 for 24 h. Dry TEA (1 mL) was added and the reaction stirred for 2 h. The solvent was removed *in vacuo* and purification by column chromatography (10% CH_3OH/CH_2Cl_2) to yield the title compound **125** (97 mg, 0.29 mmol, 85%) as a white solid. MP = 136-139 °C; IR (solid): 3302 (m), 3262 (w), 1651 (s), 1553 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.43 (1 H, br, AcN*H*), 8.05 (1 H, br, $ArCH_2NHC(S)$), 7.97 (1 H, br, N*H*Et), 7.59 (1 H, br, $NHCH_2CH_2$), 7.38 (1 H, t, J = 8 Hz, Ar), 7.26-7.23 (3 H, m, Ar), 4.73 (2 H, br, $ArCH_2NHC(S)$), 4.34 (2 H, d, J = 6 Hz, $AcNHCH_2$), 3.71 (2 H, br, $NHCH_2CH_2$), 3.18 (2 H, m, CH_2CH_3), 2.44 (2 H, t, J = 7.5 Hz, $NHCH_2CH_2$), 1.98 (3 H, s, $CH_3C(O)$), 1.12 (3 H, t, J = 7.5 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 171.2 (C), 170.1 (C), 140.6 (C), 140.3 (C), 129.2 (CH), 127.2 (CH), 126.8 (CH), 126.7 (CH), 48.0 (CH_2), 43.1 (CH_2), 41.2 (CH_2), 40.2 (CH_2), 35.8 (CH_2), 34.3 (CH_2), 23.6 (CH_3), 15.7 (CH_3), 1 x C not observed; ESMS: m/z (%): 359 ($(M+Na)^+$, 100).

***N*-1-Ethyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino-2-methyl propanamide 126 and *N*-1-3-[(4,4-Dimethyl-5-oxo-2-thioxo-1-imidazolidinyl) methyl]benzylacetamide 127**



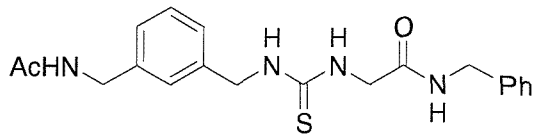
126



127

N-1-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (86 mg, 0.39 mmol) and *N*-1-ethyl-2-amino-2-methylpropanamide **93** (61 mg, 0.47 mmol) were stirred in dry CH_2Cl_2 (4 mL) under N_2 for 96 h. The solvent was removed *in vacuo* and purification by column chromatography (1% CH_3OH/CH_2Cl_2) yielded the thiourea **126** (54 mg, 0.15 mmol, 38%) as a white solid and thiohydantoin **127** (16 mg, 0.05 mmol, 12%) as a waxy solid. Data for **126**: MP = 144-148 °C; IR (solid): 3300 (br), 3081 (m), 2979 (m), 2932 (m), 2872 (w), 1642 (s), 1542 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.31 (1 H, br, AcN*H*), 7.27-7.11 (4 H, m, Ar), 6.89 (1 H, br, $CH_2NHC(S)$), 6.68 (1 H, br, EtN*H*), 6.39 (1 H, br, $NHC(CH_3)_2$), 4.66 (2 H, d, J = 4 Hz, $CH_2NHC(S)$), 4.25 (2 H, d, J = 6 Hz, $AcNHCH_2$), 3.19 (2 H, m, CH_2CH_3), 1.94 (3 H, s, $COCH_3$), 1.53 (6 H, s, $C(CH_3)_2$), 1.08 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 182.4 (C), 174.9 (C), 171.1 (C), 139.1 (C), 138.6 (C), 129.4 (CH), 127.3 (2 x CH), 127.3 (CH), 59.3 (CH_2), 49.2 (C), 43.0 (CH_2), 35.3 (CH_2), 26.5 (CH_3), 23.5 (CH_3), 14.9 (CH_3); ESMS: m/z (%): 373 ($(M+Na)^+$, 100). Data for **127**: MP = 192-194 °C; IR (solid): 3344 (m), 3093 (w), 2985 (w), 2923 (w), 2809 (w), 1721 (s), 1644 (s), 1537 (s), 1516 (s) cm^{-1}; ^1H NMR (300 MHz, 10% DMSO-d_6/$CDCl_3$): 10.20 (1 H, br, CNH), 7.73 (1 H, br, AcNH), 7.18-7.09 (4 H, m, Ar), 4.86 (2 H, s, CH_2N), 4.26 (2 H, d, J = 6 Hz, CH_2NH), 1.89 (3 H, s, $COCH_3$), 1.32 (6 H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6): 180.6 (C), 177.4 (C), 169.0 (C), 139.9 (C), 136.5 (C), 128.4 (CH), 126.2 (CH), 125.5 (CH), 125.3 (CH), 60.6 (C), 43.1 (CH_2), 41.9 (CH_2), 23.6 (CH_3), 22.5 (CH_3); ESMS: m/z (%): 323 ($(M+NH_4)^+$, 100); HRMS (ES) for $C_{15}H_{19}N_3O_2SNa$ $(M+Na)^+$: calcd 328.1090, found 328.1095; Anal. Calcd for $C_{15}H_{19}N_3O_2S$: C, 58.99; H, 6.27; N, 13.75. Found: C, 59.05; H, 6.27; N, 13.75.

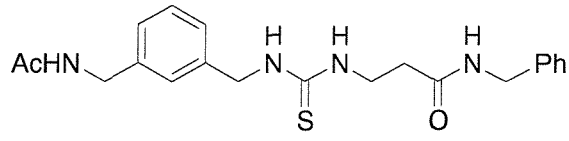
***N*-1-Benzyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino acetamide 129**



129

N-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (134 mg, 0.61 mmol) and *N*-1-benzyl-2-aminoacetamide **96** (97 mg, 0.59 mmol) were dissolved in dry CH_2Cl_2 (4 mL) under N_2 and stirred at room temperature for 18 h. The resultant precipitate was filtered off to yield the title compound **129** (169 mg, 0.44 mmol, 74%) as a pale solid. MP = 156-159 °C; IR (solid): 3267 (s), 3065 (m), 2926 (w), 1655 (s) , 1625 (m), 1561 (s), 1546 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.46 (1 H, br, N*H*CH_2Ph), 8.30 (1 H, br, AcN*H*), 8.19 (1 H, br, ArCH_2N*H*C(S)), 7.62 (1 H, br, N*H*CH_2C(O)), 7.33-7.21 (6 H, m, Ar), 7.19-7.11 (3 H, m, Ar), 4.64 (2 H, br, ArCH_2NHC(S)), 4.32 (2 H, d, J = 6 Hz, NHCH_2Ph), 4.22 (2 H, d, J = 6 Hz, AcNHCH_2), 4.17 (2 H, d, J = 5 Hz, CH_2C(O)), 1.94 (3 H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 169.6 (C), 169.3 (C), 166.4 (C), 140.1 (C), 139.7 (C) , 139.1 (C), 128.7 (CH), 128.6 (CH), 127.8 (CH), 127.6 (CH), 127.4 (CH), 126.7 (CH), 126.3 (CH), 47.2 (CH_2), 42.7 (CH_2), 42.5 (CH_2), 42.5 (CH_2), 22.5 (CH_3); ESMS: m/z (%): 385 ($(M+H)^+$, 100); HRMS (ES) for $C_{20}H_{24}N_4O_2SNa$ $(M+Na)^+$: calcd 407.1512, found 407.1513.

***N*-1-Benzyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino propanamide 130**

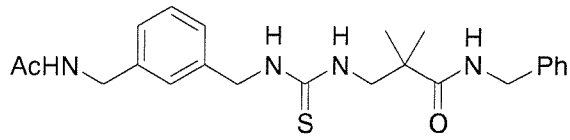


130

N-1-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (281 mg, 1.27 mmol), *N*-1-benzyl-3-aminopropanamide **97** (250 mg, 1.40 mmol) and TEA (360 μL, 2.56 mmol) were stirred in CH_2Cl_2 (10 mL) for 22 h. The solvent was removed *in vacuo* and purification by column chromatography (2-8% CH_3OH/CH_2Cl_2) yielded the title compound **130** (322 mg, 0.76 mmol, 60%) as a white foam. MP = 133-136 °C; IR (solid): 3283 (m), 3069 (w), 2932 (w), 1738 (w), 1639 (s), 1536 (s) cm^{-1}; ^1H NMR

(400 MHz, DMSO-d_6):, 8.40 (1 H, t, J = 6 Hz, N*H*CH_2Ph), 8.31 (1 H, br, AcNH), 7.94 (1 H, br, ArCH_2N*H*C(S)), 7.51 (1 H, br, N*H*CH_2CH_2), 7.33-7.22 (6 H, m, Ar), 7.16-7.10 (3 H, m, Ar), 4.63 (2 H, br, Ar*C*H_2NHC(S)), 4.27 (2 H, d, J = 6 Hz, NHCH_2Ph, 4.22 (2 H, d, J = 6 Hz, AcNHCH_2), 3.64 (2 H, br, NH CH_2CH_2), 2.44 (2 H, t, J = 5 Hz, NHCH$_2$CH_2), 1.86 (3 H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 171.7 (C), 169.6 (C), 140.1 (C), 139.9 (C), 139.8 (C), 128.7 (CH), 128.7 (CH), 127.7 (CH), 127.2 (CH), 126.7 (CH), 126.3 (CH), 126.2 (CH), 47.5 (CH_2), 47.4 (CH_2), 42.5 (CH_2), 42.5 (CH_2), 35.3 (CH_2), 23.0 (CH_3), 1 x C not observed; ESMS: m/z (%): 399 ((M+H)$^+$, 100); Anal. Calcd for $C_{21}H_{26}N_4O_2S$: C, 63.29; H, 6.58; N, 14.05. Found: C, 63.43; H, 6.60; N, 14.08.

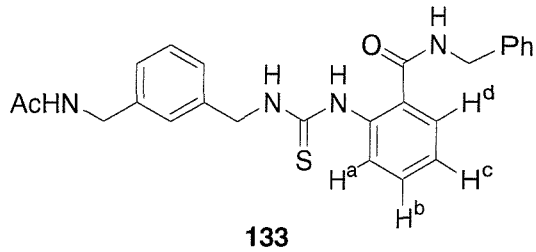
_N_-1-Benzyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino-2,2-dimethylpropanamide 132



132

N-1-Benzyl-3-amino-2,2-dimethylpropanamide **107** (20 mg, 0.10 mmol) and N-1-[3-(isothiocyanatomethyl)benzyl]acetamide **118** (22 mg, 0.10 mmol) were stirred in CH_2Cl_2 (1 mL) for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (2%-8% CH_3OH/CH_2Cl_2) yielded the title compound **132** (31 mg, 0.07 mmol, 73%) as a yellow foam. IR (solid): 3286 (m), 2967 (w), 1738 (s), 1632 (s), 1541 (s) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 8.35 (1 H, br, AcNH), 8.27 (1 H, br, N*H*CH_2Ph), 8.15 (1 H, br, ArCH_2N*H*C(S)), 7.34 (1 H, br, N*H*$CH_2C(CH_3)_2$), 7.32-7.7.10 (9H, m, Ar), 4.64 (2 H, br, Ar*C*H_2NHC(S)), 4.28 (2 H, d, J = 6 Hz, CH_2Ph), 4.21 (2 H, d, J = 6 Hz, AcNHCH_2), 3.66 (2 H, br, C$H_2C(CH_3)_2$), 1.85 (3 H, s, CH_3C(O)), 1.15 (6 H, s, $C(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 176.4 (C), 169.1 (2 x C), 139.9 (C), 139.7 (2 x C), 128.3 (CH), 128.3 (CH), 126.8 (CH), 126.6 (CH), 126.3 (CH), 125.9 (CH), 125.8 (CH), 47.2 (CH_2), 42.8 (C), 42.1 (CH_2), 23.5 (CH_3), 22.6 (CH_3), 2 x CH_2 obscured by solvent; ESMS: m/z (%): 449 ((M+Na)$^+$, 100); HRMS (ES) for $C_{23}H_{31}N_4O_2S$ (M+H)$^+$: calcd 427.2162, found 427.2168.

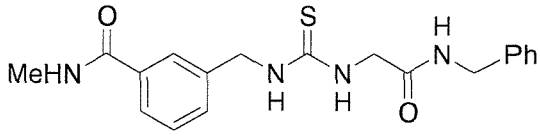
_N_-1-Benzyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino benzamide 133



133

_N_-1-Benzyl-2-aminobenzamide[114] **128** (66 mg, 0.26 mmol) and _N_-1-[3-(isothiocyanatomethyl)benzyl]acetamide **118** (58 mg, 0.26 mmol) were stirred in CH_2Cl_2 (1.5 mL) at gentle reflux for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (1-4% CH_3OH/CH_2Cl_2) yielded the title compound **133** (64 mg, 0.14 mmol, 55%) as a white solid. MP = 74 °C (dec.); IR (solid): 3264 (m), 3062 (w), 3030 (w), 2937 (w), 1739 (m), 1627 (m), 1600 (m), 1522 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 10.38 (1 H, br, N*H*Ar), 9.17 (1 H, t, J = 5.7 Hz, N*H*CH_2Ph), 9.11 (1 H, br, ArCH_2N*H*C(S)), 8.33 (1 H, t, J = 5.7 Hz, AcN*H*), 8.10 (1 H, br, Ar-H^a), 7.65 (1 H, dd, J = 8, 1 Hz, Ar-H^d), 7.45 (1 H, td, J = 8, 1 Hz, Ar-H^b), 7.34-7.14 (10 H, m, Ar), 4.71 (2 H, br, Ar*C*H_2NHC(S)), 4.47 (2 H, d, J = 5.7 Hz, *C*H_2Ph), 4.24 (2 H, d, J = 5.7 Hz, AcNHCH_2), 1.87 (3 H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 181.1 (C), 169.1 (C), 167.9 (C), 139.7 (C), 139.1 (C), 138.9 (C), 138.7 (C), 130.3 (CH), 128.3 (CH), 128.2 (CH), 127.7 (CH), 127.1 (CH), 126.8 (CH), 126.5 (CH), 126.0 (CH), 125.9 (CH), 125.8 (C), 125.4 (CH), 123.3 (CH), 47.3 (CH_2), 42.5 (CH_2), 42.1 (CH_2), 22.7 (CH_3); ESMS: m/z (%): 469 ($(M+Na)^+$, 100); HRMS (ES) for $C_{18}H_{17}N_3O_2SNa$ $(M-BnNH_2+Na)^+$: calcd 362.0934, found 362.0940; Anal. Calcd for $C_{25}H_{26}N_4O_2S$: C, 67.24; H, 5.87; N, 12.54. Found: C, 67.20; H, 5.92; N, 12.29.

_N_-1-Methyl-3-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzamide 134

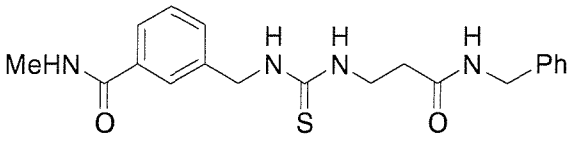


134

_N_-1-Methyl-3-(isothiocyanatomethyl)benzamide **120** (57 mg, 0.28 mmol), _N_-1-benzyl-2-aminoacetamide **96** (91 mg, 0.56 mmol) and TEA (10 μL, 0.14 mmol) were

stirred in CH_2Cl_2 (3 mL) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (2-10% CH_3OH/CH_2Cl_2) yielded the title compound **134** (68 mg, 0.18 mmol, 64%) as a white solid. MP = 168-171 °C; IR (solid): 3293 (m), 3064 (w), 2935 (w), 1727 (w), 1655 (s), 1638 (s), 1545 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.48 (1 H, br, N*H*CH_2Ph), 8.39 (1 H, q, *J* = 4 Hz, CH_3N*H*), 8.27 (1 H, br, ArCH_2N*H*C(S)), 7.75 (1 H, s, Ar), 7.71-7.67 (2 H, m, Ar and N*H*CH_2C(O)), 7.45-7.37 (3 H, m, Ar), 7.34-7.20 (5 H, m, Ar), 4.71 (2 H, br, Ar*C*H_2NHC(S)), 4.31 (2 H, d, *J* = 6 Hz, NHCH_2Ph), 4.17 (2 H, d, *J* = 6 Hz, *C*H_2C(O)), 2.77 (3 H, d, *J* = 4 Hz, *C*H_3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 167.3 (C), 165.1 (2 x C), 137.7 (C), 133.1 (2 x C), 128.4 (CH), 126.7 (CH), 126.7 (CH), 125.7 (CH), 125.3 (CH), 124.6 (CH), 123.9 (CH), 47.1 (CH_2), 45.5 (CH_2), 40.6 (CH_2), 24.7 (CH_3); ESMS: m/z (%): 393 ($(M+Na)^+$, 100); HRMS (ES) for $C_{19}H_{22}N_4O_2SNa$ $(M+Na)^+$: calcd 393.1355, found 39.1356; Anal. Calcd for $C_{19}H_{22}N_4O_2S$: C, 61.60; H, 5.99; N, 15.12. Found: C, 61.40; H, 6.04; N, 14.81.

N-1-Methyl-3-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl benzamide 135

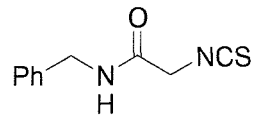


135

N-1-Benzyl-3-aminopropanamide **97** (61 mg, 0.34 mmol) and *N*-1-methyl-3-(isothiocyanatomethyl)benzamide **120** (64 mg, 0.31 mmol) were stirred in CH_2Cl_2 (4 mL) for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (5% CH_3OH/CH_2Cl_2) yielded the title compound **135** (86 mg, 0.22 mmol, 71%) as a white solid. MP = 147-150 °C; IR (solid): 3274 (m), 3074 (w), 2921 (w), 1638 (s), 1543 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.40 (1 H, br, N*H*CH_2Ph), 8.38 (1 H, br, CH_3N*H*), 8.01 (1 H, br, ArCH_2N*H*C(S)), 7.74 (1 H, s, Ar), 7.68 (1 H, d, *J* = 7 Hz, Ar), 7.56 (1 H, br, N*H*CH_2CH_2), 7.43-7.35 (2 H, m, Ar), 7.33-7.19 (5 H, m, Ar), 4.69 (2 H, br, Ar*C*H_2NHC(S)), 4.27 (2 H, d, *J* = 6 Hz, NHCH_2Ph), 3.65 (2 H, br, NHCH_2CH_2), 2.77 (3 H, d, *J* = 5 Hz, CH_3), 2.77 (2 H, t, *J* = 7 Hz, $NHCH_2CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 176.8 (C), 170.1 (C), 166.1 (C), 139.0 (C), 138.9 (C), 134.1 (C), 129.3 (CH), 127.7 (CH), 127.6 (CH), 126.7 (CH), 126.2 (CH), 125.7 (CH), 124.8 (CH), 41.5 (CH_2), 40.0 (CH_2), 34.3 (CH_2), 25.7

(CH_3), 1 x CH_2 obscured by solvent; ESMS: m/z (%): 407 ($(M+Na)^+$, 100), 385 (27); HRMS (ES) for $C_{20}H_{24}N_4O_2SNa$ $(M+Na)^+$: calcd 407.1512, found 407.1519; Anal. Calcd for $C_{20}H_{24}N_4O_2S$: C, 62.47; H, 6.29; N, 14.57. Found: C, 62.71; H, 6.18; N, 14.30.

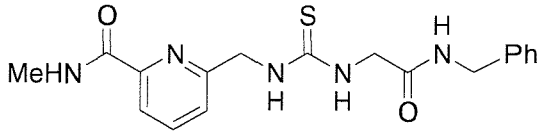
_N_-1-Benzyl-2-isothiocyanatoacetamide 136



136

_N_-1-Benzyl-2-aminoacetamide[135] **96** (100 mg, 0.61 mmol) was stirred in CH_2Cl_2 (5 mL) and sat. $NaHCO_3$ (2.5 mL) at 4 °C. $SCCl_2$ (93 μL), 1.21 mmol) was added to the lower phase and the reaction stirred at 4 °C for 10 min. CH_2Cl_2 (5 mL) was added, the phases separated, the organic phase washed with 2 M HCl (5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **136** (93 mg, 0.45 mmol, 74 %) as a brown solid. MP = 60-63 °C; IR (solid): 3290 (m), 3066 (w), 2923 (w), 2070 (s), 1664 (s), 1536 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.45-7.27 (5 H, m, Ar), 6.54 (1 H, br, NH), 4.51 (2 H, d, J = 6 Hz, PhCH_2NH), 4.36 (2 H, s, $CH_2NC(S)$); ^{13}C NMR (75.5 MHz, $CDCl_3$): 164.9 (C), 137.3 (C), 129.0 (CH), 128.1 (CH), 127.0 (CH), 48.4 (CH_2), 44.0 (CH_2); CIMS: m/z (%): 206 ($M^{+\bullet}$, 30), 91 (100).

_N_-2-Methyl-6-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl-2-pyridine carboxamide 142

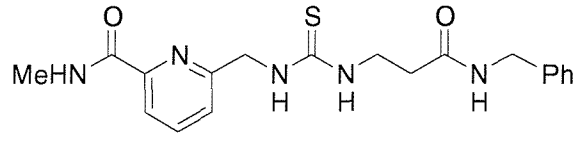


142

_N_-1-Benzyl-2-isothiocyanatoacetamide **136** (37 mg, 0.18 mmol) which was stirred in CH_2Cl_2 (3 mL) with _N_-2-methyl-6-(aminomethyl)-2-pyridinecarboxamide **116** (30 mg, 0.18 mmol) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (1-6% CH_3OH/CH_2Cl_2) yielded the title compound **142** (37 mg, 0.10 mmol, 55%) as a brown waxy solid. IR (solid): 3294 (br), 3086 (w), 2924 (w), 1656 (s), 1592 (w), 1543 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.76 (1 H, q, J = 4 Hz, CH_3NH), 8.58 (1 H, t, J = 6 Hz, $NHCH_2Ph$), 8.43 (1 H, br, pyrCH_2NH),

7.97-7.88 (3 H, m, pyr and N*H*CH_2C(O)), 7.51 (1 H, d, *J* = 8 Hz, pyr), 4.84 (2 H, br, pyrC*H*$_2$), 4.33 (2 H, d, *J* = 6 Hz, NHC*H*$_2$Ph), 4.20 (1 H, br, CH_2C(O)), 2.86 (3 H, d, *J* = 4 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 168.8 (C), 164.2 (C), 158.7 (C), 156.8 (C), 149.2 (C), 139.3 (CH), 138.3 (C), 128.3 (CH), 127.2 (CH), 126.8 (CH), 123.9 (CH), 120.0 (CH), 48.7 (CH_2), 47.1 (CH_2), 42.1 (CH_2), 26.0 (CH_3); ESMS: m/z (%): 372 ((M+H)$^+$, 100); HRMS (ES) for $C_{18}H_{21}N_5O_2SNa$ (M+H)$^+$: calcd 394.1308, found 394.1308.

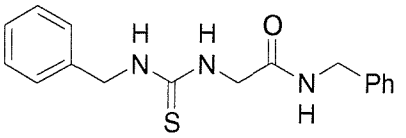
_N_-2-Methyl-6-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl-2-pyridinecarboxamide 143



143

N-1-Benzyl-2-aminopropanamide **97** (89 mg, 0.50 mmol) was stirred in CH_2Cl_2 (5 mL) and sat. $NaHCO_3$ (2.5 mL) at 0°C, $SCCl_2$ (76 μL, 1.00 mmol) was added and the reaction stirred for 20 min. CH_2Cl_2 (5 mL) was added, the phases separated, washed with 2M HCl (5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. *N*-2-methyl-6-(aminomethyl)-2-pyridinecarboxamide **116** (42 mg, 0.25 mmol) was added and the reaction stirred in CH_2Cl_2 (3 mL) for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (1-5% CH_3OH/CH_2Cl_2) yielded the title compound **143** (30 mg, 0.08 mmol, 31% overall) as a brown waxy solid. IR (solid): 3298 (br), 3087 (w), 2941 (w), 1648 (s), 1593 (w), 1536 (s) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 8.73 (1 H, q, *J* = 5 Hz, CH_3N*H*), 8.43 (1 H, br, N*H*CH_2Ph), 8.16 (1 H, br, pyrCH_2N*H*), 7.96-7.87 (2 H, m, pyr), 7.83 (1 H, br, N*H*CH_2CH_2), 7.47 (1 H, d, *J* = 7 Hz, pyr), 7.32-7.18 (5 H, m, Ph), 4.81 (2 H, br, pyrC*H*$_2$), 4.29 (2 H, d, *J* = 6 Hz, C*H*$_2$Ph), 3.70 (2 H, br, NHC*H*$_2$$CH_2$), 2.96 (3 H, d, *J* = 5 Hz, CH_3), 2.47 (2 H, t, *J* = 6 Hz, $NHCH_2$C*H*$_2$); ^{13}C NMR (100 MHz, CD_3OD): 173.8 (C), 167.1 (C), 158.0 (C), 150.3 (C), 139.9 (C), 139.4 (C), 129.5 (CH), 129.5 (CH), 128.4 (CH), 128.1 (CH), 125.6 (CH), 121.5 (CH), 54.8 (CH_2), 44.1 (CH_2), 41.6 (CH_2), 36.4 (CH_2), 26.4 (CH_3); ESMS: m/z (%): 386 ((M+H)$^+$, 100); HRMS (ES) for $C_{19}H_{23}N_5O_2SNa$ (M+Na)$^+$: calcd 408.1464, found 408.1467.

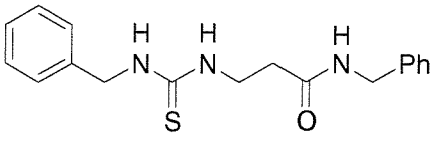
***N*-1-Benzyl-2-[(benzylamino)carbothioyl]aminoacetamide 144**



144

N-1-Benzyl-2-aminoacetamide **96** (75 mg, 0.42 mmol) and benzyl isothiocyanate (51 μL, 0.38 mmol) were stirred in CH_2Cl_2 (2 ml) for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (20% EtOAc/CH_2Cl_2 – neat EtOAc) to yield the title compound **144** (85 mg, 0.26 mmol, 68%) as a white solid. MP = 146-149 °C; IR (solid): 3286 (m), 3062 (w), 3036 (w), 2937 (w), 1738 (m), 1642 (s), 1537 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.45 (1 H, br, C(O)N*H*CH_2Ph), 8.21 (1 H, br, ArCH_2N*H*C(S)), 7.64 (1 H, br, N*H*CH_2C(O)), 7.33-7.22 (10 H, m, Ar), 4.66 (2 H, br, Ph*C*H_2NHC(S)), 4.32-4.29 (2 H, m, C(O)NHCH_2Ph), 4.18-4.15 (2 H, br, CH_2C(O)); ^{13}C NMR (100 MHz, 1:1 CD_3OD:$CDCl_3$): 170.9 (C), 138.6 (2 x C), 129.1 (CH), 129.1 (CH), 128.1 (CH), 128.0 (CH), 128.0 (CH), 127.8 (CH), 48.1 (CH_2), 43.8 (CH_2), 1 x CH_2 obscured by solvent, 1 x C not observed; ESMS: m/z (%): 336 ($(M+Na)^+$, 100); HRMS (ES) for $C_{17}H_{19}N_3OSNa$ $(M+Na)^+$: calcd 336.1141, found 336.1147.

***N*-1-Benzyl-3-[(benzylamino)carbothioyl]aminopropanamide 145**

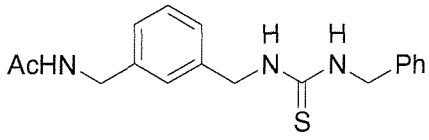


145

N-1-Benzyl-3-aminopropanamide **97** (35 mg, 0.21 mmol), benzyl isothiocyanate (35 μL, 0.27 mmol) and TEA (30 μL, 0.21 mmol) were stirred in CH_2Cl_2 (2 mL) for 24 h. CH_2Cl_2 (5 mL) was added, washed with H_2O (5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (2-6% CH_3OH/CH_2Cl_2) and recrystallisation (EtOAc/petroleum ether) yielded the title compound **145** (46 mg, 0.15 mmol, 71%) as a white solid. MP = 143-147 °C; IR (solid): 3289 (m), 3062 (w), 3034 (w), 2936 (w), 1739 (m), 1643 (s), 1522 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.41 (1 H, t, J = 6 Hz, C(O)N*H*CH_2Ph), 7.96 (1 H, br, PhCH_2N*H*C(S)), 7.52 (1 H, br, N*H*CH_2CH_2)), 7.34-7.22 (10 H, m, Ar), 4.64 (2 H, br, Ph*C*H_2NHC(S)), 4.27 (2 H, d, J = 6 Hz, C(O)NHCH_2Ph)), 3.64 (2 H, br,

NHCH_2CH$_2$), 2.43 (2 H, t, J = 7 Hz, NHCH$_2$CH_2); ^{13}C NMR (100 MHz, DMSO-d$_6$): 179.1 (C), 170.6 (C), 139.4 (2 x C), 128.2 (CH), 128.2 (CH), 127.3 (CH), 127.2 (CH), 126.8 (CH), 126.7 (CH), 42.0 (CH$_2$), 34.4 (CH$_2$), 2 x CH$_2$ obscured by solvent; ESMS: m/z (%): 328 ((M+H)$^+$, 100); HRMS (ES) for $C_{18}H_{21}N_3O_2SNa$ (M+Na)$^+$: calcd 350.1297, found 350.1294.

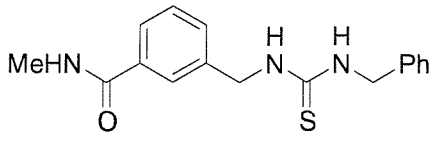
N-1-[3-([(Benzylamino)carbothioyl]aminomethyl)benzyl]acetamide 146



146

N-1-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (50 mg, 0.23 mmol) and benzylamine (55 μL, 0.50 mmol) were stirred in CH$_2$Cl$_2$ (2 mL) for 20 h. The solvent was removed *in vacuo* and purification by column chromatography (2-6% CH$_3$OH/CH$_2$Cl$_2$) yielded the title compound **146** (50 mg, 0.15 mmol, 66%) as a white solid. MP = 128-131 °C; ^{1}H NMR (400 MHz, DMSO-d$_6$): 8.31 (1 H, br, AcNH), 7.92 (2 H, br, 2 x thiourea-NH), 7.35-7.21 (6 H, m, Ar), 7.18-7.09 (3 H, m, Ar), 4.67 (4 H, br, CH_2NHC(S)), 4.23 (2 H, d, J = 6 Hz, AcNHCH_2), 1.87 (3 H, s, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d$_6$): 169.1 (C), 139.6 (2 x C), 139.2 (C), 128.2 (2 x CH), 127.2 (CH), 126.8 (CH), 126.1 (CH), 125.8 (CH), 125.7 (CH), 47.0 (CH$_2$), 42.0 (2 x CH$_2$), 22.6 (CH$_3$), 1 x C not observed; ESMS: m/z (%): 350 ((M+Na)$^+$, 100); HRMS (ES) for $C_{18}H_{21}N_3OSNa$ (M+Na)$^+$: calcd 350.1297, found 350.1297.

N-1-Methyl-3-([(benzylamino)carbothioyl]aminomethyl)benzamide 147

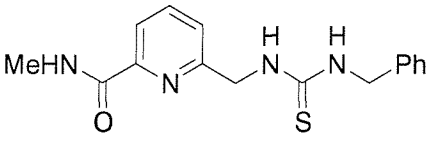


147

N-1-Methyl-3-(ammoniomethyl)benzamide chloride **114** (58 mg, 0.27 mmol), benzyl isothiocyanate (36 μL, 0.27 mmol) and TEA (46 μL, 0.33 mmol) were stirred in CH$_2$Cl$_2$ (2 mL) for 20 h. The solvent was removed *in vacuo* and purification by column chromatography (20-60% EtOAc/CH$_2$Cl$_2$) yielded the title compound **147** (66 mg, 0.21 mmol, 78%) as a white foam. MP = 78-81 °C; IR (solid): 3284 (m), 3062 (w), 3033 (w), 2935 (w), 1739 (m), 1642 (s), 1536 (s) cm^{-1}; ^{1}H NMR (400

MHz, DMSO-d_6): 8.40 (1 H, q, J = 4 Hz, CH_3NH), 8.00 (2 H, br, thiourea-NH), 7.77 (1 H, s, Ar), 7.69 (1 H, d, J = 7 Hz, Ar), 7.44-7.37 (2 H, m, Ar), 7.35-7.21 (5 H, m, Ar), 4.72 (2 H, br, CH_2), 4.69 (2 H, br, CH_2), 2.78 (3 H, d, J = 4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 184.3 (C), 170.2 (C), 140.0 (C), 139.0 (C), 135.8 (C), 132.1 (CH), 130.1 (CH), 130.0 (CH), 129.1 (CH), 128.9 (CH), 127.2 (CH), 126.9 (CH), 49.9 (CH_2), 49.4 (CH_2), 28.0 (CH_3); ESMS: m/z (%): 336 ($(M+Na)^+$, 100); HRMS (ES) for $C_{17}H_{19}N_3OS$ $(M+Na)^+$: calcd 336.1141, found 338.1147.

_N_-2-Methyl-6-([(benzylamino)carbothioyl]aminomethyl)-2-pyridine carboxamide 148

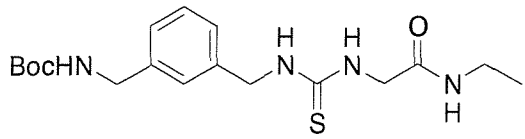


148

_N_-2-Methyl-6-(aminomethyl)-2-pyridinecarboxamide **116** (76 mg, 0.46 mmol), benzyl isothiocyanate (55 μL, 0.46 mmol) and TEA (71 μL, 0.51 mmol) were stirred in CH_2Cl_2 (2 mL) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (EtOAc) yielded the title compound **148** (96 mg, 0.30 mmol, 73%) as a white foam. IR (solid): 3288 (m), 3061 (w), 3031 (w), 2939 (w), 1739 (m), 1643 (s), 1537 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.65 (1 H, q, J = 5 Hz, CH_3NH), 8.21 (1 H, br, thiourea-NH), 8.13 (1 H, t, J = 5 Hz, thiourea-NH), 7.97-7.88 (2 H, m, Ar), 7.46 (1 H, br, pyr), 7.36-7.23 (5 H, m, Ar), 4.86 (2 H, d, J = 5 Hz, CH_2), 4.72 (2 H, br, CH_2), 2.85 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 183.0 (C), 165.2 (C), 155.8 (C), 148.7 (C), 138.3 (CH), 137.6 (C), 128.8 (CH), 127.7 (2 x CH), 124.9 (CH), 120.8 (CH), 49.4 (CH_2), 48.7 (CH_2), 26.10 (CH_3); ESMS: m/z (%): 315 (61), 337 ($(M+Na)^+$, 100); HRMS (ES) for $C_{16}H_{18}N_4OSNa$ $(M+Na)^+$: calcd 337.1093, found 337.1097.

Binding Studies

***tert*-Butyl *N*-(3-[([2-(ethylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzyl)carbamate 123 and TBA acetate in $CDCl_3$**



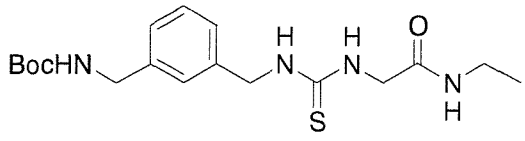
123

NMR data

[Host]/M	0.0087	Starting volume/μL	600
[Guest]/M	0.0526	K_a/M^{-1}	1.5×10^3

Volume added /μL	BocN*H*
0	5.1529
20	5.2629
40	5.3728
60	5.4282
80	5.4514
100	5.4621
140	5.4646
220	5.4811
260	5.4852
300	5.4900
400	5.4968

***tert*-Butyl *N*-(3-[([2-(ethylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzyl)carbamate 123 and TBA acetate in DMSO-d_6**



123

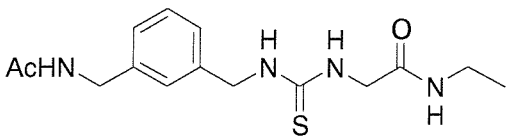
NMR data

[Host]/M	0.0088	Starting volume/μL	600
[Guest]/M	0.0526	K_a/M^{-1}	160

Volume added /μL	$ArCH_2NHC(S)$	$NHCH_2C(O)$
0	8.3171	7.6541

20	8.4957	7.8881
40	8.6246	8.0650
50	8.6180	8.0716
60	8.7610	8.2245
70	8.8214	8.3336
90	8.9197	8.4527
100	8.9751	8.4907
120	8.9900	8.5213
140	9.1719	8.7486
180	9.2876	8.9090
220	9.3240	9.0189
260	9.4554	9.1198
350	9.5042	9.1752
450	9.4876	9.1867

N-1-Ethyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino acetamide 124 and TBA acetate in DMSO-d_6



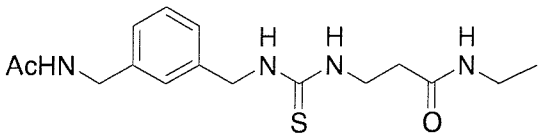
124

NMR data

[Host]/M	0.0088	Starting volume/μL	600
[Guest]/M	0.0526	K_a/M^{-1}	550

Volume added /μL	$ArCH_2NHC(S)$	$NHCH_2C(O)$
0	8.3163	7.6715
20	8.5130	7.9220
40	8.7081	8.1691
50	8.7966	8.2824
60	8.8710	8.3775
65	8.9305	8.4618
70	8.9478	8.4651
80	9.0322	8.5792
90	9.0818	8.6428
110	9.2008	8.7957
130	9.3116	8.9421
150	9.3711	9.0222
200	9.4571	9.1330
250	9.5902	9.2992
350	9.5935	9.3025
450	9.6042	9.3141

_N_-1-Ethyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino propanamide 125 and TBA acetate in DMSO-d_6



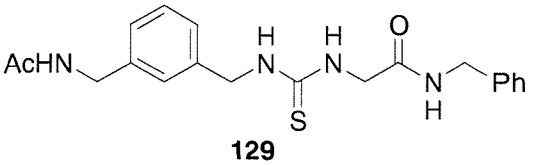
125

NMR data

[Host]/M	0.0099	Starting volume/μL	600
[Guest]/M	0.0523	K_a/M^{-1}	1.2×10^3

Volume added /μL	$ArCH_2NHC(S)$	$NHCH_2CH_2$
0	8.0426	7.6003
20	8.2319	7.7958
30	8.3683	7.9442
50	8.4418	8.0252
60	8.4625	-
70	8.5311	8.1182
80	8.5704	8.1794
90	8.6138	8.2372
100	8.6493	8.2823
120	8.7303	8.3736
150	8.7911	8.4741
200	8.9453	8.5911
250	8.9453	8.5956
300	8.9473	8.6311
400	8.9457	8.6187
500	8.9457	8.6171

_N_-1-Benzyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino acetamide 129 and TBA acetate in DMSO



129

NMR data

[Host]/M	0.0043	Starting volume/μL	600
[Guest]/M	0.0260	K_a/M^{-1}	260

Volume added	$ArCH_2NHC(S)$	$NHCH_2C(O)$

/μL		
0	8.5062	7.9319
20	8.6436	8.1074
40	8.7676	8.2663
50	8.8160	8.3322
60	8.8518	8.3783
70	8.8899	8.4315
80	8.9399	8.4911
90	8.9606	8.5324
100	9.0027	8.5968
120	9.0638	8.6428
150	9.1377	8.7636
200	9.2362	8.8756
300	9.3649	9.0503
400	9.4539	9.1647
500	9.4618	9.1782
580	9.4912	9.2021

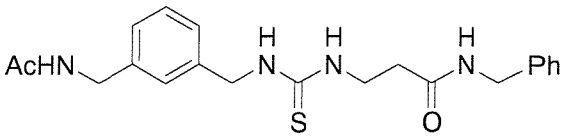
ITC data

[Host]/mM	1.95	ΔH/kJmol^{-1}	-4.4
[Guest]/mM	78.0	$T\Delta S$/kJmol^{-1}	7.9
K_a/M^{-1}	140	ΔG/kJmol^{-1}	-12.3

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	1008.88	3.72	478.95
0.27	1483.46	3.87	466.63
0.41	1330.44	4.02	452.21
0.55	1224.18	4.17	447.45
0.69	1135.37	4.32	431.35
0.83	1063.07	4.47	418.37
0.97	1001.05	4.62	406.58
1.11	944.98	4.77	395.26
1.25	897.84	4.92	388.10
1.39	852.07	5.08	379.56
1.53	813.04	5.23	370.26
1.68	780.48	5.38	361.20
1.82	746.29	5.54	352.66
1.96	717.68	5.69	345.71
2.11	688.04	5.85	338.06
2.25	661.04	6.00	330.70
2.39	636.96	6.16	324.55
2.54	612.76	6.32	316.10
2.68	593.27	6.47	310.41
2.83	573.44	6.63	303.03
2.98	555.23	6.79	297.37
3.12	537.55	6.95	291.53

3.27	519.54	7.11	286.61
3.42	504.21	7.27	280.94
3.57	491.55	7.43	276.19

_N_-1-Benzyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino propanamide 130 and TBA acetate in DMSO



130

NMR data

[Host]/M	0.0042	Starting volume/μL	600
[Guest]/M	0.0251	K_a/M^{-1}	610

Volume added /μL	N*H*CH_2Ph	AcN*H*	$ArCH_2NHC(S)$	N*H*CH_2CH_2
0	8.7231	8.6381	8.2528	7.8342
20	8.7421	8.6436	8.4276	8.0216
40	8.7548	8.6452	8.5467	8.1630
50	8.7604	8.6492	8.5968	8.2139
60	8.7676	8.6500	8.6500	8.2711
70	8.7707	8.6508	8.6722	8.3211
80	8.7739	8.6524	8.7350	8.3680
90	8.7779	8.6532	8.7779	8.4149
100	8.7850	8.6563	8.8033	8.4601
120	8.7898	8.6579	8.8589	8.5189
150	8.7977	8.6603	8.9495	8.6603
200	8.8073	8.6643	9.0440	-
300	8.8216	8.6659	9.1878	8.8804
400	-	8.6691	9.2021	8.8907
500	8.8287	8.6714	9.2132	8.9646
580	8.8263	8.6722	9.2267	8.9375

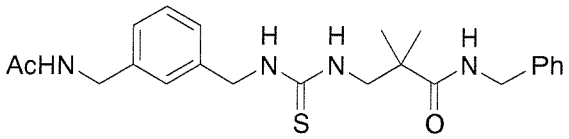
ITC data

[Host]/mM	1.96	$\Delta H/kJmol^{-1}$	-4.72
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	9.77
K_a/M^{-1}	350	$\Delta G/kJmol^{-1}$	-14.5

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	783.99	3.62	496.26
0.27	1368.73	3.77	484.27

0.40	1271.58	3.92	469.81
0.54	1185.35	4.06	458.29
0.67	1112.47	4.21	444.12
0.81	1048.42	4.36	437.64
0.95	993.55	4.50	423.80
1.08	946.24	4.65	413.45
1.22	900.12	4.80	401.58
1.36	860.56	4.95	393.40
1.50	824.94	5.10	384.75
1.63	789.31	5.25	377.05
1.77	756.68	5.40	367.95
1.91	726.68	5.55	362.52
2.05	698.51	5.70	354.75
2.19	676.04	5.85	346.52
2.33	651.98	6.01	339.03
2.48	628.11	6.16	333.24
2.62	608.33	6.31	327.86
2.76	589.39	6.47	319.79
2.90	571.80	6.62	316.17
3.05	559.45	6.78	308.75
3.19	539.23	6.93	302.87
3.33	526.00	7.09	296.36
3.48	508.46	7.24	290.80

_N_-1-Benzyl-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino-2,2-dimethylpropanamide 132 and TBA acetate in DMSO



132

NMR data

[Host]/M	0.0039	Starting volume/μL	600
[Guest]/M	0.0234	K_a/M^{-1}	180

Volume added /μL	AcN*H*	N*H*$CH_2C(CH_3)_2$
0	8.6333	7.6173
20	8.6391	7.7269
40	8.6430	7.8199
50	8.6492	7.8556
60	8.6524	7.8882
70	8.6532	7.9184
80	8.6555	7.9446
90	8.6548	7.9748

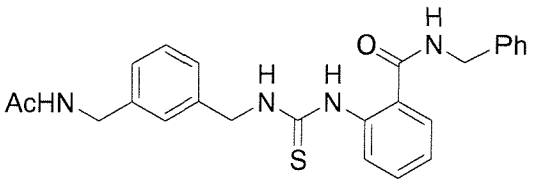
100	8.6571	8.0026
120	8.6611	8.0439
150	8.6643	8.1066
200	8.6698	8.1797
300	8.6754	8.2830
400	8.6833	8.3529
500	8.6849	8.4053
580	8.6865	8.6238

ITC data

[Host]/mM	1.99	$\Delta H/kJmol^{-1}$	-2.80
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	12.1
K_a/M^{-1}	400	$\Delta G/kJmol^{-1}$	-14.9

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.24	-195.86	6.59	-25.15
0.49	--	6.85	-22.76
0.73	-149.94	7.12	-21.58
0.98	-150.77	7.38	-22.15
1.22	-137.57	7.65	-23.00
1.47	-104.36	7.92	-19.95
1.72	-103.19	8.19	-18.27
1.97	-88.09	8.46	-19.44
2.22	-63.33	8.73	-16.39
2.47	-73.40	9.00	-16.14
2.72	-58.82	9.27	-14.40
2.97	-64.48	9.54	-12.66
3.22	-69.56	9.82	-14.09
3.48	-49.22	10.09	-14.74
3.73	-40.19	10.37	-18.30
3.99	-40.28	10.64	-13.02
4.24	-36.98	10.92	-8.55
4.50	-37.86	11.20	-9.50
4.76	-33.97	11.48	-12.41
5.02	-30.77	11.76	-13.66
5.28	-30.13	12.04	-10.23
5.54	-27.21	12.32	-9.27
5.80	-31.09	12.60	-5.16
6.06	-26.25	12.88	-3.85
6.32	-23.93	13.17	-6.98

_N_-1-Benzyl-2-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino benzamide 133 and TBA acetate in DMSO



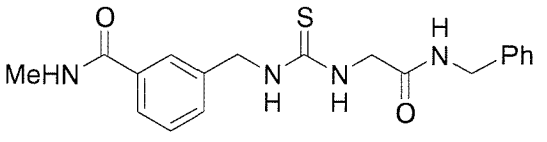
133

NMR data

[Host]/M	0.0037	Starting volume/μL	600
[Guest]/M	0.0224	K_a/M^{-1}	200

Volume added /μL	Ar
0	8.3942
20	8.3731
40	8.3735
50	8.3291
60	8.3172
70	8.3076
80	8.2949
90	8.2870
100	8.2770
120	8.2631
150	8.2433
200	8.2139
300	8.1809
400	8.1527
500	8.1448
600	8.1209

_N_-1-Methyl-3-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzamide 134 and TBA acetate in DMSO



134

NMR data

[Host]/M	0.0045	Starting volume/μL	600
[Guest]/M	0.0270	K_a/M^{-1}	290

Volume added /μL	N*H*CH_2Ph	ArCH_2N*H*C(S)
0	8.7977	8.5729
20	8.8033	8.7262
40	8.8271	8.8494
50	8.8104	8.9082
60	8.8097	8.9550
70	8.7850	9.0003
80	8.7914	9.0384
90	8.7977	9.0853
100	8.8025	9.1123
110	8.8065	9.1353
120	8.8120	9.1703
130	8.8192	9.2108
150	8.8192	9.2497
175	8.8184	9.3045
200	8.8263	-
250	8.8351	9.4197
300	8.8470	9.4706
400	8.8597	9.5564
500	8.8700	9.5945
600	8.8708	9.6032

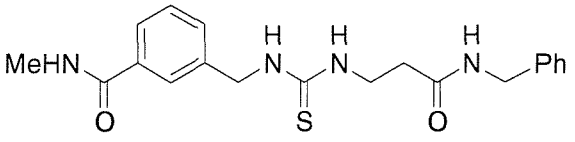
ITC data

[Host]/mM	1.96	ΔH/kJmol^{-1}	-2.92
[Guest]/mM	78.0	$T\Delta S$/kJmol^{-1}	12.2
K_a/M^{-1}	440	ΔG/kJmol^{-1}	-15.1

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	783.99	3.62	496.26
0.27	1368.73	3.77	484.27
0.40	1271.58	3.92	469.81
0.54	1185.35	4.06	458.29
0.67	1112.47	4.21	444.12
0.81	1048.42	4.36	437.64
0.95	993.55	4.50	423.80
1.08	946.24	4.65	413.45
1.22	900.12	4.80	401.58
1.36	860.56	4.95	393.40
1.50	824.94	5.10	384.75
1.63	789.31	5.25	377.05
1.77	756.68	5.40	367.95
1.91	726.68	5.55	362.52
2.05	698.51	5.70	354.75
2.19	676.04	5.85	346.52
2.33	651.98	6.01	339.03

2.48	628.11	6.16	333.24
2.62	608.33	6.31	327.86
2.76	589.39	6.47	319.79
2.90	571.80	6.62	316.17
3.05	559.45	6.78	308.75
3.19	539.23	6.93	302.87
3.33	526.00	7.09	296.36
3.48	508.46	7.24	290.80

_N_-1-Methyl-3-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl benzamide 135 and TBA acetate in DMSO



135

NMR data

[Host]/M	0.0043	Starting volume/μL	600
[Guest]/M	0.0260	K_a/M^{-1}	200

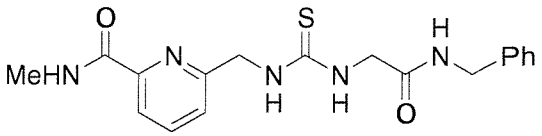
Volume added /μL	Amide-N*H*	$ArCH_2NHC(S)$	$NHCH_2CH_2$
0	8.7199	8.3195	7.8747
20	8.7350	8.4903	8.0669
40	8.7493	8.6222	8.2242
50	8.7533	8.6794	8.2886
60	8.7572	-	8.3457
70	8.7628	-	8.4196
80	8.7660	8.8200	8.4736
90	8.7699	8.8684	8.5253
100	8.7707	8.9074	8.5793
120	8.7771	8.9796	8.6770
150	8.7834	9.0607	8.7834
200	8.7961	9.1830	8.8780
300	8.8104	9.3276	9.0726
400	8.8152	9.4340	9.1852
500	8.8255	9.5103	9.2664
580	8.8295	9.5325	9.3204

ITC data

[Host]/mM	1.95	$\Delta H/kJmol^{-1}$	-8.67
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	5.19
K_a/M^{-1}	270	$\Delta G/kJmol^{-1}$	-13.9

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	--	3.72	-147.81
0.27	-692.88	3.87	-142.56
0.41	-619.70	4.02	-139.18
0.55	--	4.17	-131.95
0.69	-512.81	4.32	-122.69
0.83	-483.13	4.47	-117.56
0.97	-443.02	4.62	-112.44
1.11	-406.63	4.77	-106.98
1.25	-378.60	4.92	-99.11
1.39	-344.55	5.08	-98.09
1.53	-327.81	5.23	-97.72
1.68	-303.30	5.38	-93.99
1.82	-288.75	5.54	-89.19
1.96	-274.67	5.69	-87.64
2.11	-261.60	5.85	-86.69
2.25	-244.19	6.00	-81.74
2.39	-230.10	6.16	-76.64
2.54	-213.23	6.32	-77.73
2.68	-204.19	6.47	-70.25
2.83	-191.17	6.63	-67.17
2.98	-181.93	6.79	-64.72
3.12	-171.88	6.95	-59.26
3.27	-165.20	7.11	-57.58
3.42	-160.51	7.27	-55.85
3.57	-155.64	7.43	-55.24

_N_-2-Methyl-6-[([2-(benzylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl-2-pyridinecarboxamide 142 and TBA acetate in DMSO



142

NMR data

[Host]/M	0.0045	Starting volume/μL	600
[Guest]/M	0.0269	K_a/M^{-1}	950

Volume added /μL	MeN*H*
0	8.7429
20	8.7804
40	8.8398

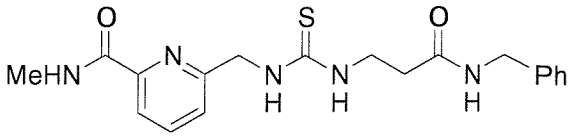
50	8.8620
60	8.8962
70	8.9033
80	8.9407
100	8.9804
120	9.0606
150	9.1321
200	9.2107
300	9.3418
400	9.3752
500	9.3799

ITC data

[Host]/mM	2.02	$\Delta H/kJmol^{-1}$	-3.77
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	13.3
K_a/M^{-1}	980	$\Delta G/kJmol^{-1}$	-17.1

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	715.51	3.61	491.36
0.27	962.89	3.75	479.13
0.40	989.12	3.90	467.45
0.53	961.24	4.04	456.29
0.67	929.41	4.19	445.55
0.80	903.81	4.33	432.68
0.94	877.00	4.48	424.38
1.08	848.19	4.63	418.69
1.21	823.15	4.78	408.64
1.35	793.00	4.93	401.80
1.49	766.93	5.07	393.35
1.63	742.84	5.22	386.07
1.76	717.38	5.37	374.40
1.90	692.76	5.52	365.85
2.04	668.80	5.67	360.15
2.18	650.01	5.83	353.46
2.32	629.50	5.98	345.24
2.46	610.25	6.13	338.64
2.60	592.91	6.28	334.02
2.75	579.55	6.43	323.90
2.89	562.17	6.59	321.10
3.03	545.05	6.74	311.73
3.17	531.93	6.90	306.41
3.32	514.74	7.05	301.32
3.46	503.28	7.21	296.49

_N_-2-Methyl-6-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl-2-pyridinecarboxamide 143 and TBA acetate in DMSO



143

NMR data

[Host]/M	0.0043	Starting volume/μL	600
[Guest]/M	0.0259	K_a/M^{-1}	1.5×10^3

Volume added /μL	MeN*H*	$ArCH_2NHC(S)$	$NHCH_2CH_2$
0	9.0158	8.4514	8.1154
20	9.1409	8.7866	8.5253
40	9.2521	9.0202	8.8120
50	9.2815	9.1266	8.9677
60	9.3180	9.2156	9.0909
70	9.3355	9.3355	9.1735
80	9.3760	9.3760	9.2672
90	9.3903	9.3903	9.3435
100	9.4245	9.4245	9.4245
120	9.4467	9.5230	9.5230
150	9.4841	9.6040	9.6040
200	9.5397	9.7311	9.7311
300	9.5778	9.8749	9.8749
400	9.6183	9.9551	9.9551
500	9.6191	9.9511	9.9511

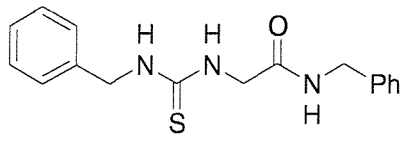
ITC data

[Host]/mM	1.95	$\Delta H/kJmol^{-1}$	-4.31
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	14.8
K_a/M^{-1}	2.2×10^3	$\Delta G/kJmol^{-1}$	-19.1

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	639.52	3.72	529.72
0.27	820.88	3.87	523.22
0.41	818.21	4.02	514.86
0.55	808.37	4.17	508.76
0.69	800.23	4.32	496.10
0.83	791.46	4.47	484.09
0.97	780.77	4.62	474.49

1.11	766.85	4.77	463.85
1.25	750.47	4.92	455.07
1.39	739.20	5.08	448.81
1.53	729.74	5.23	443.83
1.68	716.38	5.38	429.97
1.82	695.91	5.54	425.60
1.96	681.28	5.69	419.74
2.11	674.62	5.85	414.23
2.25	655.45	6.00	404.08
2.39	636.97	6.16	401.34
2.54	628.15	6.32	394.21
2.68	609.90	6.47	384.81
2.83	599.97	6.63	379.08
2.98	587.93	6.79	373.09
3.12	575.26	6.95	365.56
3.27	563.55	7.11	364.21
3.42	553.26	7.27	359.39
3.57	541.80	7.43	357.17

_N_-1-Benzyl-2-[(benzylamino)carbothioyl]aminoacetamide 144 and TBA acetate in DMSO



144

NMR data

[Host]/M	0.0037	Starting volume/μL	600
[Guest]/M	0.0224	K_a/M^{-1}	230

Volume added /μL	$PhCH_2NHC(S)$	$NHCH_2C(O)$
0	8.5110	7.9351
20	8.6826	8.1440
40	8.8240	8.3211
50	8.8859	8.3926
60	8.9360	8.4713
70	8.9900	8.5292
80	9.0265	8.5825
90	9.0742	8.6405
100	9.1052	8.6633
120	9.1774	8.7509
150	9.2577	8.8597
200	9.3689	9.0027
300	9.5150	9.1854

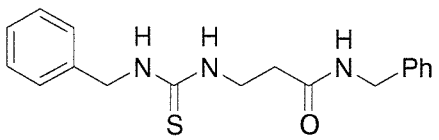
400	9.5421	9.2211
500	9.6549	9.3601
580	9.6787	9.3967

ITC data

[Host]/mM	1.20	$\Delta H/kJmol^{-1}$	-4.76
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	9.92
K_a/M^{-1}	373	$\Delta G/kJmol^{-1}$	-14.7

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.22	--	6.04	-44.59
0.45	-296.74	6.28	-39.47
0.67	--	6.53	-40.02
0.90	-252.58	6.77	-36.84
1.12	-230.79	7.01	-34.95
1.35	-199.77	7.26	-36.09
1.58	-184.97	7.51	-30.63
1.80	-169.56	7.75	-31.54
2.03	-146.85	8.00	-28.34
2.26	-135.95	8.25	-27.74
2.49	-126.48	8.50	-26.57
2.72	-117.78	8.75	-23.18
2.96	-108.83	9.00	-23.17
3.19	-92.78	9.25	-21.74
3.42	-78.58	9.50	-22.58
3.66	-76.18	9.76	-21.22
3.89	-70.73	10.01	-18.14
4.13	-69.66	10.27	-16.83
4.36	-62.97	10.52	-17.45
4.60	-62.04	10.78	-19.16
4.84	-57.20	11.03	-17.50
5.08	-54.20	11.29	-15.02
5.32	--	11.55	-13.74
5.56	-50.71	11.81	-15.05
5.80	-46.02	12.07	-12.55

_N_-1-Benzyl-3-[(benzylamino)carbothioyl]aminopropanamide 145 and TBA acetate in DMSO



145

NMR data

[Host]/M	0.0051	Starting volume/μL	600
[Guest]/M	0.0305	K_a/M^{-1}	230

Volume added /μL	C(O)N*H*CH_2Ph	$PhCH_2$N*H*C(S)	N*H*CH_2CH_2
0	8.7048	8.256	7.8262
20	8.7231	8.4331	8.0121
40	8.7413	8.5817	8.1734
50	8.7461	8.6428	8.2385
60	8.7501	8.7000	8.3036
70	8.7580	8.758	8.3553
80	8.7636	8.7636	8.4149
90	8.7683	8.8200	8.4641
100	8.7723	8.8724	8.5094
120	8.7819	8.9542	8.6031
150	8.7898	9.0273	8.7112
200	8.8025	9.1544	8.8025
300	8.8160	9.3030	8.9804
400	8.8247	9.3498	9.0511
500	8.8343	9.4634	9.1631
580	8.8375	9.4944	9.1957

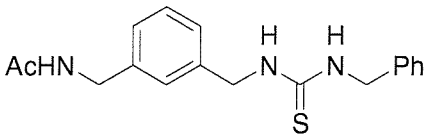
ITC data

[Host]/mM	1.99	$\Delta H/kJmol^{-1}$	-6.60
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	7.14
K_a/M^{-1}	260	$\Delta G/kJmol^{-1}$	-13.7

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	207.69	3.50	153.49
0.26	325.89	3.64	153.79
0.39	321.26	3.78	153.01
0.52	300.02	3.92	150.84
0.65	278.13	4.07	147.69
0.78	262.69	4.21	147.27
0.91	248.70	4.35	145.17
1.05	243.38	4.49	145.19
1.18	230.27	4.64	145.80
1.31	218.93	4.78	145.67
1.44	211.37	4.93	141.99
1.58	202.89	5.07	143.56
1.71	197.65	5.22	139.17
1.85	194.63	5.36	138.72
1.98	185.84	5.51	136.29
2.12	184.91	5.66	136.70

2.26	177.30	5.80	136.02
2.39	172.87	5.95	135.30
2.53	169.28	6.10	134.31
2.67	164.87	6.25	136.36
2.80	160.43	6.40	132.20
2.94	156.98	6.55	131.22
3.08	157.65	6.70	130.34
3.22	154.19	6.85	128.90
3.36	153.27	7.00	130.18

_N_-1-[3-([(Benzylamino)carbothioyl]aminomethyl)benzyl]acetamide 146 and TBA acetate in DMSO



146

NMR data

[Host]/M	0.0051	Starting volume/μL	600
[Guest]/M	0.0305	K_a/M^{-1}	240

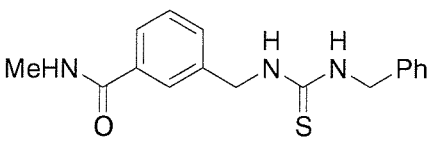
Volume added /μL	AcN*H*
0	8.6349
20	8.6436
40	8.6524
50	8.6579
60	8.6611
70	8.6643
80	8.6659
90	8.6675
100	8.6714
120	8.6738
150	8.6770
200	8.6794
300	8.6857
400	8.6889
500	8.6921
580	8.6929

ITC data

[Host]/mM	1.99	$\Delta H/kJmol^{-1}$	-9.40
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	5.28
K_a/M^{-1}	380	$\Delta G/kJmol^{-1}$	-14.7

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.62	-164.25
0.27	-931.32	3.77	-156.82
0.40	-869.83	3.92	-154.64
0.54	-756.58	4.06	-141.84
0.67	-682.36	4.21	-135.66
0.81	-606.31	4.36	-130.78
0.95	-559.83	4.50	-122.44
1.08	-503.55	4.65	-120.94
1.22	-467.64	4.80	-117.47
1.36	-428.27	4.95	-112.94
1.50	-397.56	5.10	-105.44
1.63	-366.15	5.25	-103.54
1.77	-339.40	5.40	-100.98
1.91	-311.52	5.55	-95.81
2.05	-282.12	5.70	-91.33
2.19	-263.92	5.85	-89.93
2.33	-249.50	6.01	-83.52
2.48	-241.16	6.16	-80.74
2.62	-224.77	6.31	-76.62
2.76	-216.45	6.47	-77.73
2.90	-202.60	6.62	-75.25
3.05	-208.35	6.78	-72.36
3.19	-194.28	6.93	-69.77
3.33	-181.70	7.09	-68.69
3.48	-175.18	7.24	-63.92

_N_-1-Methyl-3-([(benzylamino)carbothioyl]aminomethyl)benzamide 147 and TBA acetate in DMSO



147

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0319	K_a/M^{-1}	260

Volume added /μL	MeN*H*	Thiourea-N*H*
0	8.7008	8.3901
20	8.7278	8.5944
40	8.7556	8.8637
50	8.7668	8.9598
60	8.7715	9.0718

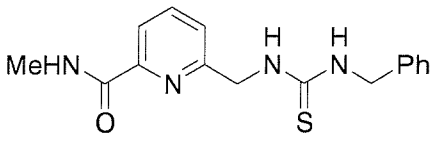
70	8.7826	9.1568
80	8.7922	9.2330
90	8.8009	9.2990
100	8.8048	9.3641
120	8.8120	9.4817
150	8.8192	9.6215
200	8.8343	9.7692
300	8.8541	9.9686
400	8.8645	10.0743
500	8.8788	10.1243
580	8.8748	10.1855

ITC data

[Host]/mM	1.95	$\Delta H/kJmol^{-1}$	-6.87
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	8.49
K_a/M^{-1}	480	$\Delta G/kJmol^{-1}$	-15.3

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.61	-122.75
0.27	-781.41	3.75	-113.94
0.40	-698.36	3.90	-106.49
0.53	-615.29	4.04	-94.75
0.67	-548.76	4.19	-88.91
0.80	-493.96	4.33	-86.30
0.94	-443.26	4.48	-85.77
1.08	-398.98	4.63	-83.09
1.21	-367.24	4.78	-77.36
1.35	-333.39	4.93	-70.92
1.49	-304.26	5.07	-67.20
1.63	-280.81	5.22	-65.93
1.76	-259.72	5.37	-63.66
1.90	-242.64	5.52	-61.58
2.04	-223.40	5.67	-57.44
2.18	-207.33	5.83	-56.98
2.32	-190.30	5.98	-48.93
2.46	-175.59	6.13	-49.32
2.60	-164.96	6.28	-49.68
2.75	-155.46	6.43	-47.52
2.89	-150.08	6.59	-45.78
3.03	-140.34	6.74	-48.63
3.17	-134.81	6.90	-48.32
3.32	-126.95	7.05	-43.65
3.46	-122.20	7.21	

_N_-2-Methyl-6-([(benzylamino)carbothioyl]aminomethyl)-2-pyridinecarboxamide 148 and TBA acetate in DMSO



148

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0318	K_a/M^{-1}	370

Volume added /μL	MeN*H*	Thiourea-N*H*	Thiourea-N*H*
0	8.9539	8.4887	8.4196
20	9.0789	8.8931	8.7580
40	9.1973	9.1973	9.0519
50	9.2378	9.4142	9.2378
60	9.2855	9.5317	9.2855
70	9.3196	9.6668	9.4094
80	9.3474	9.7502	9.4801
90	9.3864	9.8804	9.5770
100	9.4078	9.9448	9.6572
120	9.4491	10.0917	9.7700
150	9.4968	10.2514	9.8955
200	9.5603	10.4301	10.0433
300	9.6159	10.6168	10.2061
400	9.6564	10.7384	10.3110
500	9.6660	10.7630	10.3277
580	9.6795	10.8257	10.3817

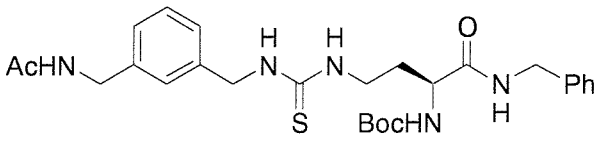
ITC data

[Host]/mM	2.06	$\Delta H/kJmol^{-1}$	-12.4
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	3.3
K_a/M^{-1}	560	$\Delta G/kJmol^{-1}$	-15.7

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.52	-206.03
0.26	-1545.13	3.66	-197.32
0.39	-1380.49	3.80	-185.37
0.52	-1229.05	3.94	-173.09
0.65	-1096.84	4.09	-159.95
0.79	-986.04	4.23	-150.42

0.92	-885.56	4.37	-146.53
1.05	-795.77	4.52	-140.22
1.18	-719.79	4.66	-137.36
1.32	-652.14	4.81	-126.47
1.45	-593.68	4.95	-121.05
1.59	-540.67	5.10	-113.71
1.72	-496.04	5.24	-108.87
1.86	-457.54	5.39	-108.21
1.99	-421.92	5.54	-103.99
2.13	-389.71	5.68	-97.09
2.27	-358.39	5.83	-93.47
2.40	-333.10	5.98	-86.09
2.54	-310.98	6.13	-85.65
2.68	-289.18	6.28	-84.89
2.82	-274.15	6.43	-83.36
2.96	-257.01	6.58	-76.68
3.10	-241.45	6.73	-79.98
3.24	-229.22	6.88	-80.51
3.38	-214.64	7.03	-74.52

tert-Butyl _N_-(1_S_)-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino-1-[(benzylamino)carbonyl]propylcarbamate 149 and TBA acetate in DMSO



149

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0190	K_a/M^{-1}	890

Volume added /μL	Amide-NH	$ArCH_2NHC(S)$
0	8.6182	8.1551
20	8.7247	8.3720
40	8.8565	8.6262
60	8.9741	8.8359
80	-	9.0837
100	-	-
120	9.2338	9.3951
140	9.2751	9.4737
160	9.3212	9.5325
180	9.3744	9.6429
200	9.4221	9.7383
220	9.4420	9.7764

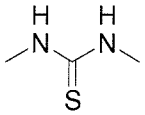
240	9.4737	9.8233
260	-	9.8852
280	-	9.8987
300	-	9.9654
400	9.5699	10.0473
500	9.5953	10.0925
600	9.6000	10.0584

ITC data

[Host]/mM	2.02	$\Delta H/kJmol^{-1}$	-12.53
[Guest]/mM	78.0	$T\Delta S/kJmol^{-1}$	4.50
K_a/M^{-1}	960	$\Delta G/kJmol^{-1}$	-17.03

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.62	-163.83
0.27	-1902.71	3.77	-152.13
0.40	-1718.36	3.92	-143.85
0.54	-1491.14	4.06	-134.22
0.67	-1312.49	4.21	-126.26
0.81	-1149.38	4.36	-119.89
0.95	-1010.55	4.50	-111.26
1.08	-889.81	4.65	-105.77
1.22	-785.34	4.80	-96.93
1.36	-693.11	4.95	-93.72
1.50	-624.87	5.10	-88.97
1.63	-558.15	5.25	-81.78
1.77	-499.27	5.40	-79.84
1.91	-444.85	5.55	-75.63
2.05	-396.16	5.70	-70.80
2.19	-365.61	5.85	-70.22
2.33	-331.77	6.01	-62.96
2.48	-304.86	6.16	-59.39
2.62	-278.14	6.31	-57.74
2.76	-251.37	6.47	-56.77
2.90	-240.70	6.62	-53.44
3.05	-219.16	6.78	-49.25
3.19	-204.83	6.93	-47.13
3.33	-192.70	7.09	-45.39
3.48	-174.95		

1,3-Dimethylthiourea and TBA acetate in DMSO



NMR data

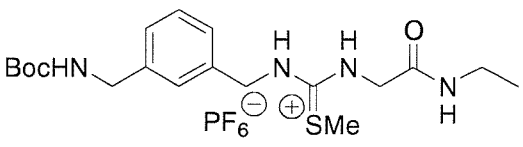
[Host]/M	0.0064	Starting volume/μL	600
[Guest]/M	0.0384	K_a/M^{-1}	30

Volume added /μL	NH
0	7.7023
20	7.8731
40	8.0034
50	8.0622
60	8.1130
70	8.1416
80	8.1750
90	8.2361
100	8.2671
120	8.2655
150	8.4149
200	8.4999
300	8.7326
400	8.8962
500	9.0090
600	9.1306

Experimental for Chapter 3

Synthesis

([(3-[(*tert*-Butoxycarbonyl)amino]methylbenzyl)amino][2-(ethylamino)-2-oxoethyl]aminomethylene)(methyl)sulfonium hexafluorophosphate 157



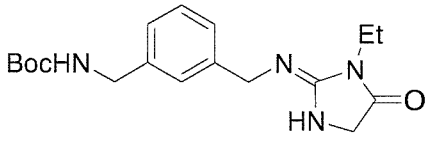
157

tert-Butyl *N*-(3-[([2-(ethylamino)-2-oxoethyl]aminocarbothioyl)amino]methyl benzyl)carbamate **123** (100 mg, 0.26 mmol) and MeI (18 μL, 0.29 mmol) were stirred in acetone (3 mL) for 3 h. The solvent was removed *in vacuo* to yield the

iodide salt (129 mg, 0.25 mmol, 94%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): 10.18 (1 H, s, $ArCH_2NHC(S)$), 8.46 (1 H, s, $HNCH_2C(O)$), 7.53 (1 H, s, $NHEt$), 7.27 (1 H, m, Ar) 7.25-7.18 (3 H, m, Ar), 4.98 (1 H, s, BocN*H*), 4.55 (2 H, d, *J* = 5 Hz, $ArCH_2NHC(S)$), 4.39 (2 H, d, J = 5 Hz, $CH_2C(O)$), 4.23 (2 H, s, $BocNHCH_2$), 3.24-3.18 (2 H, m, CH_2CH_3), 2.74 (3 H, s, SCH_3), 1.37 (9 H, s, $C(CH_3)_3$), 1.11 (3 H, t, *J* = 7 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 169.8 (C), 166.5 (C), 156.0 (C), 140.1 (C), 134.7 (C), 129.3 (CH), 127.5 (CH), 126.8 (CH), 126.6 (CH), 79.6 (C), 48.3 (CH_2), 48.2 (CH_2), 44.3 (CH_2), 35.1 (CH_2), 28.4 (CH_3), 15.6 (CH_3), 14.2 (CH_3); ESMS: m/z (%): 395 $(M-I)^+$, 100%).

Iodide salt (100 mg. 0.19 mmol) and NH_4PF_6 (62 mg, 0.38 mmol) were stirred in CH_3OH (1.5 mL) and CH_2Cl_2 (1.5 mL) for 24 h. The solvent was removed *in vacuo*, the resultant white solid was partitioned between CH_2Cl_2 (10 mL) and H_2O (10 mL) and the aqueous phase washed with CH_2Cl_2 (5 x 10 mL). The organic phases were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **157** (72 mg, 0.13 mmol, 70%) as a white foam. IR (solid): 3414 (m), 3284 (m), 2978 (m), 2932 (m), 1677 (s), 1612 (s), 1523 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 9.98 (1 H, br, $ArCH_2NHC(S)$), 7.60 (1 H, br, $NHCH_2C(O)$), 7.40-7.21 (4 H, m, Ar), 6.78 (1 H, br, $NHEt$), 5.01 (1 H, br, BocN*H*), 5.60 (2 H, d, *J* = 5 Hz, $ArCH_2NHC(S)$), 4.31 (2 H, d, *J* = 6 Hz, $CH_2C(O)$), 4.19 (2 H, d, *J* = 5 Hz, $BocNHCH_2$), 3.31 (2 H, m, CH_2CH_3), 2.69 (3 H, s, SCH_3), 1.46 (9 H, s, $C(CH_3)_3$), 1.18 (3 H, t, *J* = 7 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 173.9 (C), 166.5 (C), 156.2 (C), 140.3 (C), 134.4 (C), 127.7 (CH), 126.8 (CH), 126.7 (CH), 126.7 (CH), 79.7 (C), 48.5 (CH_2), 48.5 (CH_2), 44.3 (CH_2), 35.1 (CH_2), 28.4 (CH_3), 14.0 (CH_3), 13.6 (CH_3); ESMS: m/z (%): 395 $(M-PF_6)^+$, 100%).

tert-Butyl *N*-(3-[(1-ethyl-5-oxo-4,5-dihydro-1H-2-imidazolyl)amino]methyl benzyl)carbamate 159

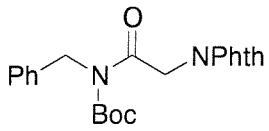


159

([(3-[(*tert*-Butoxycarbonyl) amino] methyl benzyl) amino][2-(ethylamino)-2-oxoethyl] amino methylene)(methyl)sulfonium hexafluorophosphate **157** (32 mg,

0.06 mmol) was stirred with NH_3 sat. CH_3OH (1.5 mL) in a sealed tube for 16 h. The solvent was removed *in vacuo* and purification by column chromatography (10% CH_3OH/CH_2Cl_2) yielded the title compound **159** (5 mg, 0.01 mmol, 17%) as a waxy solid. ^1H NMR (400 MHz, $CDCl_3$): 7.22-7.15 (4 H, m, Ar), 7.13 (1 H, br, guanidine-NH), 5.05 (1 H, br, BocN*H*), 4.24 (2 H, s, $COCH_2$), 4.21 (2 H, br d, J = 4 Hz, BocNHCH_2), 3.95 (2 H, s, ArCH_2NHC=N), 3.65 (2 H, q, J = 7 Hz, CH_2CH$_3$), 1.37 (9 H, s, $C(CH_3)_3$), 1.12 (3 H, t, J = 7 Hz, CH_2CH_3); ESMS: m/z (%): 347 ($(M+H)^+$, 100).

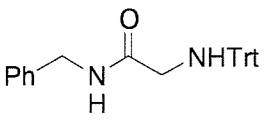
tert-Butyl _N_-benzyl-_N_-[2-(1,3-dioxo-2,3-dihydro-1H-2-isoindolyl)acetyl] carbamate 163



163

N-1-Benzyl-2-(1,3-dioxo-2,3-dihydro-1*H*-2-isoindolyl)acetamide[107] **98** (1.5 g, 5.1 mmol) and DMAP (1.24 g, 10.2 mmol) were stirred in CH_3CN (18 mL), Boc_2O (2.22 g, 10.2 mmol) was added and the reaction stirred for 20 min. The solvent was removed *in vacuo*, EtOAc (20 mL) was added and washed with 1 M $KHSO_4$ (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **163** (1.99 g, 4.76 mmol, 93%) as a brown solid. IR (solild): 2990 (w), 1772 (w), 1735 (s), 1700 (s), 1608 (w) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 7.81 (2 H, dd, J = 6, 3 Hz, Ar), 7.65 (2 H, dd, J = 6, 3 Hz, Ar), 7.23-7.15 (5 H, m, Ar), 4.97 (2 H, s, CH_2), 4.81 (2 H, s, CH_2), 1.38 (9 H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.5 (C), 169.0 (C), 153.7 (C), 138.4 (C), 135.1 (C), 133.3 (CH), 129.4 (CH), 128.7 (CH), 128.4 (CH), 124.5 (CH), 85.4 (C), 48.8 (CH_2), 44.8 (CH_2), 28.9 (CH_3); ESMS: m/z (%): 417 ($(M+Na)^+$, 100), 317 (51).

_N_-1-Benzyl-2-(tritylamino)acetamide[137] 165

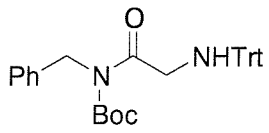


165

[2-(Benzylamino)-2-oxoethyl]ammonium 2,2,2-trifluoroacetate **96** (400 mg, 1.44 mmol) and dry TEA (510 μL, 3.6 mmol) were stirred in dry CH_2Cl_2 (5 mL) under

N_2, TrtCl (400 mg, 1.44 mmol) in dry CH_2Cl_2 (5 mL) was added and the reaction stirred for 30 min. H_2O (10 mL) was added and the phases separated. The organic phase was washed with brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **165** (584 mg, 1.44 mmol, 99%) as a white solid. MP = 154-156 °C (lit. [137] 157 °C); IR (solid): 3317 (w), 2059 (w), 3031 (w), 1649 (s), 1518 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.50-7.07 (20 H, m, Ar), 4.41 (2 H, d, J = 6 Hz, PhCH_2), 3.02 (2 H, br, CH_2NHTrt); ^{13}C NMR (100 MHz, $CDCl_3$): 171.5 (C), 145.2 (C), 138.6 (C), 128.9 (CH), 128.6 (2 x CH), 128.4 (2 x CH), 128.3 (CH), 128.1 (CH), 127.7 (CH), 127.7 (CH), 127.4 (CH), 127.0 (2 x CH), 71.3 (C), 48.5 (CH_2), 43.2 (CH_2); ESMS: m/z (%): 407 ($(M+H)^+$, 100). Data consistent with that reported by Losse.[137]

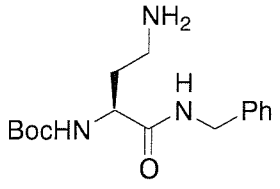
***tert*-Butyl *N*-benzyl-N-[2-(tritylamino)acetyl]carbamate 166**



166

N-1-Benzyl-2-(tritylamino)acetamide[137] **165** (200 mg, 0.49 mmol) was stirred with DMAP (76 mg, 0.62 mmol) in CH_3CN (5 mL), Boc_2O (148mg, 0.68 mmol) was added and the reaction stirred for 20 h. The solvent was removed *in vacuo* and purification by column chromatography (Et_2O) yielded the title compound **166** (220 mg, 0.43 mmol, 89%) as a yellow solid. MP = 124-126 °C; IR (solid): 3330 (w), 2968 (w), 1751 (s), 1735 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.41 (6 H, dd, J = 9, 1 Hz, Ar), 7.19-7.04 (14 H, m, Ar), 4.65 (2 H, s, PhCH_2N), 3.68 (2 H, s, CH_2NH), 1.20 (9 H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 175.4 (C), 152.8 (C), 146.5 (C), 138.5 (C), 129.4 (CH), 128.8 (CH), 128.4 (CH), 128.2 (CH), 127.7 (CH), 126.9 (CH), 84.1 (C), 71.7 (C), 50.8 (CH_2), 48.0 (CH_2), 28.5 (CH_3); ESMS: m/z (%): 507 ($(M+H)^+$, 100); HRMS (ES) for $C_{33}H_{35}N_2O_3$ $(M+H)^+$: calcd 507.2642, found 507.2546.

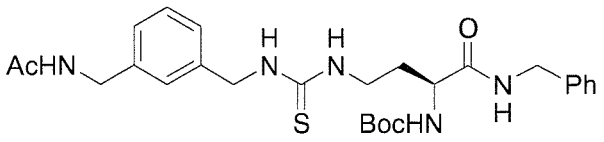
tert-Butyl _N_-(1S)-3-amino-1-[(benzylamino)carbonyl]propylcarbamate 168



168

tert-Butyl _N_-(1_S_)-4-amino-1-[(benzylamino)carbonyl]-4-oxobutylcarbamate **167** (500 mg, 1.49 mmol) was dissolved in DMF (8 mL) and H_2O (4 mL). Bis(trifluoroacetoxy)-iodosobenzene (770 mg, 2.98 mmol) was added and the reaction stirred at room temperature for 72 h. The solvent was removed *in vacuo*, sat. K_2CO_3 (4 mL) was added and extracted with $CHCl_3$ (4 x 5 mL). The combined organic phases were dried ($MgSO_4$), the solvent removed *in vacuo* and purification by column chromatography (5% CH_3OH/CH_2Cl_2) yielded the title compound **168** (268 mg, 0.87 mmol, 97%) as a yellow oil. $[\alpha]_D$ = -0.021 (*c* 0.24, CH_3OH, 24 °C); IR (film): 3294 (m), 3063 (w), 3031 (w), 2977 (m), 2931 (m), 1656 (s), 1523 (s) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): 8.42 (1 H, t, *J* = 6 Hz, N*H*CH_2Ph), 7.43-7.33 (5 H, m, Ar), 7.15 (1 H, d, *J* = 8 Hz, BocN*H*), 4.40-4.37 (2 H, br, C*H*$_2$Ph), 4.13 (1 H, m, CH), 2.70 (2 H, t, *J* = 6 Hz, C*H*$_2$$NH_2$), 1.87-1.70 (2 H, m, CHC*H*$_2$), 1.49 (9 H, s, $C(CH_3)_3$); ^{13}C NMR (75.5 MHz, CD_3OD): 174.4 (C), 157.8 (C), 139.7 (C), 129.5 (CH), 128.4 (CH), 128.2 (CH), 80.7 (C), 53.9 (CH), 43.9 (CH_2), 38.6 (CH_2), 34.6 (CH_2), 28.8 (CH_3); ESMS: m/z (%): 308 ($(M+H)^+$, 100); HRMS (ES) for $C_{16}H_{26}N_3O_3$ $(M+H)^+$: calcd 308.1968, found 308.1961.

tert-Butyl _N_-(1S)-3-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]amino-1-[(benzylamino)carbonyl]propylcarbamate 149

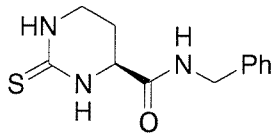


149

tert-Butyl _N_-(1_S_)-4-amino-1-[(benzylamino)carbonyl]-4-oxobutylcarbamate **168** (95 mg, 0.34 mmol) and _N_-1-[3-(isothiocyanatomethyl)benzyl]acetamide **118** (68 mg, 0.31 mmol) were stirred in CH_2Cl_2 (4 mL) for 20 h. The solvent was removed *in vacuo* and purification by column chromatography (50% EtOAc/petroleum ether-neat EtOAc) yielded the title compound **149** (126 mg, 0.24 mmol, 78%) as a yellow

solid. MP = 90-93 °C; $[\alpha]_D$ = -0.018 (*c* 0.18, CH_3OH, 24 °C); IR (solid): 3283 (m), 2976 (w), 2928 (w), 1654 (s), 1642 (s), 1542 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.38 (2 H, br, AcN*H* and N*H*CH_2Ph), 7.92 (1 H, br, ArCH_2N*H*C(S)), 7.54 (1 H, br, N*H*CH_2CH_2), 7.41-7.18 (9 H, m, Ar), 7.09 (1 H, br d, *J* = 6 Hz, BocN*H*), 4.63 (2 H, br, Ar*C*H_2NHC(S)), 4.48-4.37 (2 H, m, N*H*CH_2Ph), 4.32 (2 H, d, *J* = 6 Hz, AcNHCH_2), 4.02 (1 H, m, CH), 3.58-3.41 (2 H, m, NHCH_2CH_2), 1.99-1.88 (4 H, m, CH_3CO and CH_aH$_b$CH), 1.79 (1 H, m, CH$_a$$H_b$CH), 1.49 (9 H, s, C($CH_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 182.2 (C), 172.3 (C), 171.2 (C), 156.7 (C), 139.3 (C), 138.5 (C), 138.3 (C), 129.2 (CH), 129.0 (CH), 128.0 (CH), 127.9 (CH), 127.0 (CH), 127.0 (CH), 126.3 (CH), 80.8 (C), 52.2 (CH_2), 48.0 (CH), 43.9 (CH_2), 43.7 (CH_2), 41.4 (CH_2), 33.3 (CH_2), 28.7 (CH_3), 23.5 (CH_3); ESMS: m/z (%): 528 ($(M+H)^+$, 100); HRMS (ES) for $C_{27}H_{37}N_5O_4SNa$ $(M+Na)^+$: calcd 550.2458, found 550.2473.

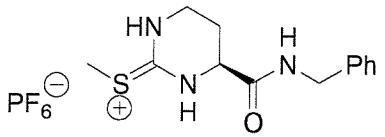
N4-Benzyl-(4S)-2-thioxohexahydro-4-pyrimidinecarboxamide 170



170

tert-Butyl *N*-(1*S*)-3-amino-1-[(benzylamino)carbonyl]propylcarbamate **168** (170 mg, 0.55 mmol) was stirred in 20% TFA/CH_2Cl_2 (5 mL) for 1 h, toluene (5 mL) was added and the solvent was removed *in vacuo* to yield a yellow oil which was stirred with CS_2 (67 μL, 1.1 mmol) in EtOH (3 mL). EDC (105 mg, 0.55 mmol) was added followed by DIPEA (1.65 mmol) and the reaction stirred for 4 h. The solvent was removed *in vacuo* and the solid digssolved in CH_2Cl_2 (10 mL). The CH_2Cl_2 was washed with 1M $KHSO_4$ (2 x 5 mL), H_2O (5 mL), sat K_2CO_3 (2 x 5 mL), H_2O (5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (5-10% CH_3OH/CH_2Cl_2) yielded the title compound **170** (38 mg, 0.15 mmol, 28%) as a white solid. ^1H NMR (400 MHz, CD_3OD): 7.30-7.18 (5 H, m, Ar), 4.37 (2 H, s, CH_2Ph), 4.00 (1 H, apparent t, *J* = 4.5 Hz, CH), 3.17 (1 H, m, NHCH_aH$_b$), 3.03 (1 H, ddd, *J* = 13, 10, 4.5 Hz, NHCH$_a$$H_b$), 2.07 (1 H, dq, *J* = 13, 4.5 Hz, CHCH_aH$_b$), 1.88 (1 H, m, CHCH$_a$$H_b$); ^{13}C NMR (100 MHz, CD_3OD): 177.72 (C), 173.57 (C), 140.18 (C), 129.90 (CH), 129.00 (CH), 128.78 (CH), 55.40 (CH), 44.71 (CH_2), 39.51 (CH_2), 23.90 (CH_2); ESMS: m/z (%): 250 ($(M+H)^+$, 100).

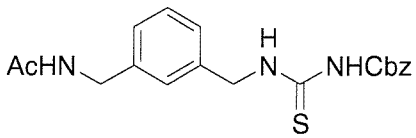
_N_4-Benzyl-(4S)-2-[(E)-1-methylsulfonio]hexahydro-4-pyrimidinecarboxamide hexafluorophosphate 171



171

_N_4-Benzyl-(4S)-2-thioxohexahydro-4-pyrimidinecarboxamide **170** (22 mg, 0.09 mmol) was stirred with MeI (9 μL, 0.13 mmol) in CH_3OH (1.5 mL) for 24 h. The solvent was removed *in vacuo* and the yellow solid was stirred with NH_4PF_6 (29 mg, 0.18 mmol) in CH_3OH (1.5 mL) for 8 h. The solvent was removed *in vacuo*, H_2O (2 mL) was added and extracted with EtOAc (2 x 4 mL). The organic phases were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **171** (37 mg, 0.09 mmol, 94%) as a waxy soild. $[\alpha]_D$ = +0.125 (*c* 0.2, CH_3OH, 24 °C); IR (solid): 3371 (w), 3231 (w), 2927 (w), 2855 (w), 2115 (s), 1664 (s), 1615 (s), 1598 (s), 1568 (s), 1530 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3OD):, 7.35-7.24 (5 H, m, Ar), 4.42 (2 H, m, NHCH_2Ph), 4.30 (1 H, t, *J* = 5 Hz, CH), 3.54 (1 H, m, NHCH_aH$_b$), 3.42 (1 H, ddd, *J* = 14, 9, 5 Hz, NHCH$_a$$H_b$), 2.64 (3 H, s, CH_3), 2.25-2.15 (2 H, m, CHCH_2); ^{13}C NMR (100 MHz, CD_3OD): 181.5 (C), 161.4 (C), 133.3 (C), 130.1 (CH), 129.1 (CH), 128.9 (CH), 54.8 (CH), 44.9 (CH_2), 39.8 (CH_2), 23.8 (CH_2), 13.9 (CH_3); ESMS: m/z (%): 246 ((M-PF_6)$^+$, 100).

Benzyl _N_-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]carbamate 172

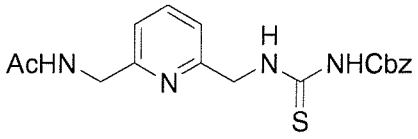


172

_N_-[3-(Aminomethyl)benzyl]acetamide **109** (461 mg, 2.59 mmol) in dry CH_2Cl_2 (4 mL) was added to (benzyloxy)methanoyl isothiocyanate[120] (500 mg, 2.59 mmol) in dry CH_2Cl_2 (8 mL) under N_2 and the reaction stirred for 18 h. Hexane (10 mL) was added and the resultant precipitate filtered off to yield the title compound **172** (662 mg, 1.78 mmol, 69 %) as a white solid. MP = 130-133 °C; IR (solid): 3289 (m), 3169 (w), 3030 (w), 1716 (s), 1680 (w), 1648 (s), 1609 (w), 1536 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 9.92 (1 H, br, ArCH$_2$N*H*C(S)), 8.15 (1 H, s, CbzN*H*), 7.38-7.23 (9 H, m, Ar), 5.85 (1 H, br, AcN*H*), 5.17 (2 H, s, CH_2Ph), 4.85 (2 H, d, *J* = 5 Hz,

AcNHCH_2), 4.43 (2 H, d, J = 6 Hz, ArCH_2NHC(S)), 2.04 (3 H, s, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 179.2 (C), 174.0 (C), 152.4 (C), 138.9 (C), 136.8 (C), 134.4 (C), 129.3 (CH), 129.0 (CH), 128.8 (CH), 128.4 (CH), 127.5 (CH), 127.3 (CH), 127.1 (CH), 68.3 (CH_2), 49.6 (CH_2), 43.6 (CH_2), 23.3 (CH_3); ESMS: m/z (%): 394 ((M+Na)$^+$, 100); HRMS (ES) for $C_{19}H_{21}N_3O_3SNa$ (M+Na)$^+$: calcd 394.1196, found 394.1191.

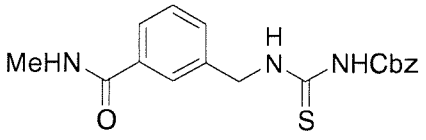
Benzyl *N*-[(6-[(acetylamino)methyl]-2-pyridylmethyl)amino]carbothioyl carbamate 173



173

N-1-[6-(Aminomethyl)-2-pyridyl]methylacetamide **111** (27 mg, 0.15 mmol) and DIPEA (54 μL, 0.30 mmol) were stirred in dry CH_2Cl_2 (2.5 mL) under N_2. (Benzyloxy)methanoyl isothiocyanate (31 mg, 0.16 mmol) was added and the reaction stirred for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (1-10% CH_3OH/CH_2Cl_2) yielded the title compound **173** (22 mg, 0.06 mmol, 40%) as a white solid. MP = 121-124 °C; IR (solid): 3289 (m), 3169 (w), 3030 (w), 1716 (s), 1648 (s), 1536 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 11.14 (1 H, br, ArCH$_2$N*H*C(S)), 8.62 (1 H, br, CbzN*H*), 7.69 (1 H, t, J = 8 Hz, Ar), 7.43-7.31 (6 H, m, Ar and AcN*H*), 7.20-7.16 (2 H, m, Ar), 5.23 (2 H, s, PhCH_2), 4.94 (2 H, d, J = 4 Hz, CH_2NHC(S)), 4.63 (2 H, d, J = 4 Hz, AcNHCH_2), 2.14 (3 H, s, CH_3); ESMS: m/z (%): 395 ((M+Na)$^+$, 100).

Benzyl *N*-[(3-[(methylamino)carbonyl]benzylamino)carbothioyl]carbamate 174

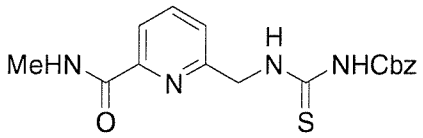


174

N-1-Methyl-3-(ammoniomethyl)benzamide chloride **114** (500 mg, 2.5 mmol) and DIPEA (1.36 mL, 7.5 mmol) were stirred in dry CH_3CN (15 mL), (benzyloxy)methanoyl isothiocyanate (480 μL, 2.5 mmol) was added and the reaction stirred for 18 h. The solvent was removed *in vacuo*, dissolved in EtOAc (60

mL), washed with 1% HCl (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **174** (884 mg, 2.5 mmol, 99%) as a yellow solid. MP = 147-149 °C; IR (solid): 3311 (w), 2969 (w), 1735 (s), 1647 (m), 1541 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 11.31 (1 H, s, CbzN*H*), 10.35 (1 H, t, *J* = 6 Hz, $ArCH_2NHC(S)$), 8.52 (1 H, q, *J* = 4 Hz, CH_3NH), 7.89 (1 H, s, Ar), 7.82 (1 H, d, *J* = 8 Hz, Ar), 7.59-7.44 (7 H, m, Ar), 5.30 (2 H, s, OCH_2Ph), 4.98 (2 H, d, *J* = 6 Hz, $ArCH_2NHC(S)$), 2.89 (3 H, d, *J* = 4 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 179.9 (C), 166.5 (C), 153.2 (C), 137.9 (C), 135.6 (C), 134.7 (C), 130.1 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.9 (CH), 126.4 (CH), 125.7 (CH), 66.8 (CH_2), 47.7 (CH_2), 26.2 (CH_3); ESMS: m/z (%): 380 ($(M+Na)^+$, 100); HRMS (ES) for $C_{18}H_{19}N_3O_3SNa$ $(M+Na)^+$: calcd 380.1039, found 380.1036.

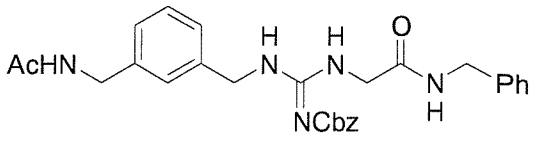
Benzyl *N*-[(6-[(methylamino)carbonyl]-2-pyridylmethyl)amino]carbothioyl carbamate 175



175

N-2-Methyl-6-(aminomethyl)-2-pyridinecarboxamide **116** (100 mg, 0.52 mmol) was dissolved in dry CH_2Cl_2 (1 mL) under N_2. (Benzyloxy)methanoyl isothiocyanate (85 mg, 0.52 mmol) in dry CH_2Cl_2 (7 mL) was added and the reaction stirred for 4 h. Hexane (10 mL) was added and the solid filtered off, purification by column chromatography (1-2% CH_3OH/CH_2Cl_2) yielded the title compound **175** (95 mg, 0.26 mmol, 51%) as a white solid. MP = 193-195 °C; IR (solid): 3368 (w), 3323 (w), 3273 (w), 1700 (s), 1657 (s), 1530 (s), 1504 (s) cm^{-1}; ^1H NMR (300 MHz, 1:1 $CD_3OD/CDCl_3$): 7.72 (1 H, d, *J* = 7.5 Hz, Ar), 7.61 (1 H, t, *J* = 7.5 Hz, Ar), 7.18 (1 H, d, *J* = 7.5 Hz, Ar), 4.95 (2 H, s, $PhCH_2$), 4.62 (2 H, s, $CH_2NHC(S)$), 2.70 (3 H, s, CH_3); ^{13}C NMR (100 MHz, DMSO): 179.5 (C), 164.1 (C), 154.6 (C), 154.0 (C), 148.9 (C), 138.9 (C), 135.9 (CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 124.4 (CH), 120.5 (CH), 67.2 (CH_2), 49.1 (CH_2), 26.1 (CH_3); ESMS: m/z (%): 381 ($(M+Na)^+$, 100); HRMS (ES) for $C_{17}H_{18}N_4O_3SNa$ $(M+Na)^+$: calcd 381.0992, found 381.0991.

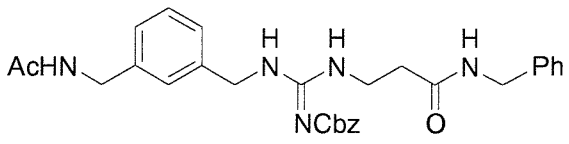
Benzyl *N*-((3-[(acetylamino)methyl]benzylamino)[2-(benzylamino)-2-oxoethyl] aminomethylene)carbamate 176



176

Benzyl *N*-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]carbamate **172** (160 mg, 0.43 mmol), 2-amino-N-benzylacetamide **96** (141 mg, 0.86 mmol) and DIPEA (150 μL, 0.86 mmol) were dissolved in dry CH_2Cl_2 (6 mL) and DMF (3 drops). The mixture was cooled to 4 °C, EDC (164 mg, 0.86 mmol) was added and the reaction stirred at 4 °C for 1 h and at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (10 mL), washed with 1% HCl (3 x 5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (1-5% CH_3OH/CH_2Cl_2) yielded the title compound **176** (119 mg, 0.17 mmol, 55%) as a white solid. MP = 129-132 °C; IR (solid): 3278 (m), 3067 (w), 3032 (w), 2929 (w), 1625 (s), 1591 (s), 1550 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 9.30 (1 H, br, NH), 7.22-7.06 (15 H, m, Ar and NH), 6.52 (1 H, br, NH), 5.86 (1 H, br, NH), 4.94 (2 H, s, CH_2Ph), 4.27 (2 H, s, CH_2), 4.22 (2 H, d, J = 6 Hz, CH_2), 4.20 (2 H, d, J = 6 Hz, CH_2), 3.75 (2 H, s, CH_2), 1.83 (3 H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 170.8 (C), 169.7 (C), 163.8 (C), 160.4 (C), 139.6 (C), 138.0 (2 x C), 137.4 (C), 129.8 (CH), 129.1 (CH), 128.8 (CH), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.8 (2 x CH), 127.1 (CH), 126.6 (CH), 67.2 (CH_2), 45.7 (CH_2), 43.9 (3 x CH_2), 23.4 (CH_3); ESMS: m/z (%): 502 ($(M+H)^+$, 100), 523 (62); Anal. Calcd for $C_{28}H_{31}N_5O_4$: C, 67.05; H, 6.23; N, 13.96. Found: C, 66.71; H, 6.18; N, 13.62.

Benzyl *N*-((3-[(acetylamino)methyl]benzylamino)[3-(benzylamino)-3-oxopropyl] aminomethylene)carbamate 177

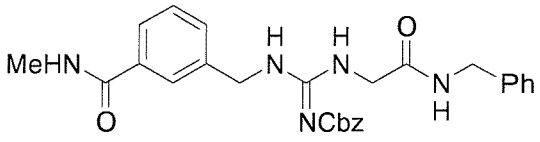


177

Benzyl *N*-[(3-[(acetylamino)methyl]benzylamino)carbothioyl]carbamate **172** (68 mg, 0.18 mmol) and *N*-1-benzyl-3-aminopropanamide **97** (65 mg, 0.36 mmol) were dissolved in dry CH_2Cl_2 (5 mL), DIPEA (31 μL, 0.18 mmol) was added and the reaction was cooled to 4 °C. EDC (70 mg, 0.36 mmol) was added and the reaction stirred at room temperature for 24 h. CH_2Cl_2 (5 mL) was added, washed with 1M

$KHSO_4$ (2 x 5 mL), H_2O (5 mL) and brine (5 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. Purification by column chromatography (2% CH_3OH/CH_2Cl_2) yielded the title compound **177** (37 mg, 0.07 mmol, 40%) as a waxy solid. MP = 136-139 °C; IR (solid): 3283 (m), 3065 (w), 3030 (w), 2934 (w), 1631 (s), 1596 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 9.16 (1 H, br, NH), 7.50 (1 H, br, NH), 7.34-7.11 (14 H, m, Ar), 6.64 (1 H, br, NH), 5.92 (1 H, br, NH), 5.01 (2 H, s, OCH_2Ph), 4.39 (2 H, br, CH_2), 4.29 (2 H, d, J = 6 Hz, CH_2), 4.25 (2 h, d, J = 6 Hz, CH_2), 3.48 (2 H, m, $NHCH_2CH_2$), 2.42 (2 H, br, $NHCH_2CH_2$), 1.86 (3 H, s, CH_3); ^{13}C NMR (100 MHz, CD_3OD): 173.5 (C), 173.0 (C), 165.0 (C), 161.5 (C), 140.4 (C), 139.8 (C), 138.9 (2 x C), 129.9 (CH), 129.5 (CH), 129.3 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.2 (CH), 127.6 (CH), 127.4 (CH), 127.2 (CH), 67.9 (CH_2), 45.6 (CH_2), 44.1 (CH_2), 38.6 (CH_2), 37.0 (CH_2), 22.6 (CH_3), 1 x CH_2 obscured by solvent; ESMS: m/z (%): 538 ($(M+Na)^+$, 100); HRMS (ES) for $C_{29}H_{34}N_5O_4$ $(M+H)^+$: calcd 516.2605, found 516.2607.

Benzyl *N*-[[2-(benzylamino)-2-oxoethyl]amino(3-[(methylamino)carbonyl]benzyl amino)methylene]carbamate 178

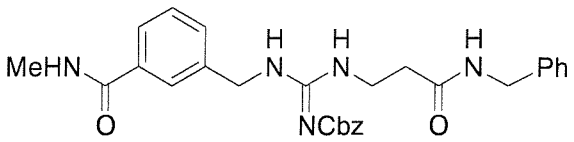


178

Benzyl *N*-[(3-[(methylamino)carbonyl]benzylamino)carbothioyl]carbamate **174** (70 mg, 0.20 mmol), *N*-1-benzyl-2-aminoacetamide **96** (64 mg, 0.39 mmol) and DIPEA (70 μL, 0.39 mmol) were stirred in dry CH_2Cl_2 (4 mL) and DMF (0.5 mL) under N_2 at 4 °C. EDC (74 mg, 0.39 mmol) was added and the reaction stirred at 4 °C for 1 h and at room temperature for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (1-4% CH_3OH/CH_2Cl_2) yielded the title compound **178** (71 mg, 0.15 mmol, 75%) as a white solid. MP = 173-176 °C; IR (solid): 3309 (w), 1635 (s), 1605 (s), 1556 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 9.25 (1 H, br, NH), 8.45 (1 H, br, NH), 8.40 (2 H, m, 2 x NH), 7.80 (1 H, s, Ar), 7.71 (1 H, d, J = 8 Hz, Ar), 7.48-7.38 (2 H, m, Ar), 7.38-7.18 (10 H, m, Ar), 4.96 (2 H, s, OCH_2Ph), 4.50 (2 H, d, J = 6 Hz, CH_2), 4.31 (2 H, d, J = 6 Hz, CH_2), 3.92 (2 H, br, CH_2), 2.76 (3 H, d, J = 5 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): 170.9 (C), 166.5 (C), 159.9 (C x 2), 142.1 (C), 137.8 (C), 134.5 (C), 129.7 (CH), 128.2 (CH), 128.2 (3 x

CH), 127.8 (CH), 127.5 (CH), 127.1 (CH), 126.7 (CH), 125.9 (CH), 65.6 (CH_2), 43.7 (CH_2), 42.1 (CH_2), 26.2 (CH_3), 1 x CH_2 obscured by solvent, 1 x C not observed; ESMS: m/z (%): 510 ($(M+Na)^+$, 100); HRMS (ES) for $C_{27}H_{29}N_5O_4$ $(M+H)^+$: calcd 488.2293, found 488.2295.

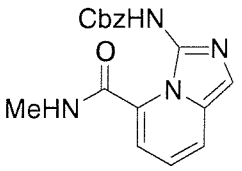
Benzyl *N*-[[3-(benzylamino)-3-oxopropyl]amino(3-[(methylamino)carbonyl] benzylamino)methylene]carbamate 179



179

Benzyl *N*-[(3-[(methylamino)carbonyl]benzylamino)carbothioyl]carbamate **174** (115 mg, 0.31 mmol), *N*-1-benzyl-3-aminopropanamide **97** (100 mg, 0.61 mmol) and dry TEA (83 μL, 0.61 mmol) were stirred in dry CH_2Cl_2 (5 mL). EDC (117 mg, 0.61 mmol) was added and the reaction stirred for 8 h. The solvent was removed *in vacuo* and purification by column chromatography (EtOAc – 10% CH_3OH/CH_2Cl_2) yielded the title compound **179** (115 mg, 0.21 mmol, 69%) as a white foam. MP = 162-166 °C; IR (solid): 3285 (m), 3064 (w), 2930 (w), 1738 (w), 1640 (s), 1537 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 9.01 (1 H, br, NH), 8.47 (1 H, t, J = 5 Hz, N*H*CH_2Ph), 8.40 (1 H, q, J = 4 Hz, CH_3N*H*), 7.76 (1 H, s, Ar), 7.69 (1 H, d, J = 8 Hz, Ar), 7.41-7.36 (2 H, m, Ar), 7.35-7.19 (11 H, m, Ar and NH), 4.96 (2 H, s, OCH_2Ph), 4.46 (2 H, d, J = 6 Hz, ArCH_2NHC(S)), 4.27 (2 H, d, J = 5 Hz, NHCH_2Ph), 3.47 (2 H, dt, J = 6, 6 Hz, NHCH_2CH_2), 2.77 (3 H, d, J = 4 Hz, CH_3), 2.45 (2 H, br, $NHCH_2CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 170.3 (C), 166.6 (2 x C), 139.3 (2 x C), 137.8 (C), 129.6 (CH), 128.2 (3 x CH), 127.7 (CH), 127.4 (CH), 127.2 (2 x CH), 126.7 (CH), 126.0 (CH), 118.0 (C), 65.5 (CH_2), 42.1 (CH_2), 37.3 (CH_2), 35.1 (CH_2), 26.2 (CH_3), 1 x CH_2 obscured by solvent, 1 x C not observed; ESMS: m/z (%): 524 ($(M+Na)^+$, 100); HRMS (ES) for $C_{28}H_{32}N_5O_4$ $(M+H)^+$: calcd 502.2449, found 502.2451; Anal. Calcd for $C_{28}H_{31}N_5O_4$: C, 67.05; H, 6.23; N, 13.96. Found: C, 66.98; H, 6.22; N, 13.71.

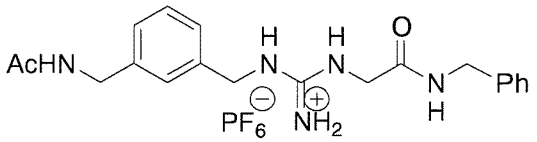
Benzyl *N*-5-[(methylamino)carbonyl]imidazo[1,5-a]pyridin-3-ylcarbamate 180



180

Benzyl *N*-[(6-[(methylamino)carbonyl]-2-pyridylmethyl)amino]carbothioyl carbamate **175** (74 mg, 0.21 mmol) and *N*-1-benzyl-2-aminoacetamide **96** (68 mg, 0.41 mmol) were stirred in dry CH_2Cl_2 (3 mL) and DMF (1 mL) at 4 °C. EDC (78 mg, 0.41 mmol) was added and the reaction stirred at room temperature for 8 h. The solvents were removed *in vacuo*, dissolved in $CHCl_3$ (10 mL) and extracted with 1 % HCl (3 x 5 mL). The aqueous phases were combined, basified (2 M NaOH, pH ~ 12) and extracted with EtOAc (3 x 10 mL). The combined EtOAc phases were dried ($MgSO_4$), the solvent removed *in vacuo* and purification by column chromatography (CH_2Cl_2 – 4% CH_3OH/CH_2Cl_2) yielded the title compound **180** (27 mg, 0.08 mmol, 40%) as a yellow foam. IR (solid): 3251 (w), 3048 (w), 2933 (w), 1732 (m), 1717 (m), 1652 (m), 1625 (m), 1542 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 9.42 (1 H, br, CbzN*H*), 8.77 (1 H, q, *J* = 3 Hz, CH_3NH), 7.68 (1 H, dd, *J* = 9, 1 Hz, Ar), 7.48 (1 H, s, NCH), 7.46-7.36 (5 H, m, Ph), 6.88-6.79 (2 H, m, Ar), 5.12 (2 H, s, OCH_2Ph), 2.78 (3 H, d, *J* = 3 Hz, CH_3NH); ^{13}C NMR (100 MHz, DMSO-d_6): 163.6 (C), 155.1 (C), 137.0 (C), 130.9 (CH), 130.5 (C), 130.4 (CH), 128.9 (CH), 128.5 (CH), 128.3 (CH), 120.2 (CH), 118.9 (C), 118.5 (CH), 114.2 (C), 66.7 (CH_2), 26.5 (CH_3); ESMS: m/z (%): 325 ($(M+H)^+$, 100); HRMS (ES) for $C_{17}H_{17}N_4O_3$ $(M+H)^+$: calcd 325.1295, found 325.1295.

((3-[(Acetylamino)methyl]benzylamino)[2-(benzylamino)-2-oxoethyl]amino methylene)ammonium hexafluorophosphate 181

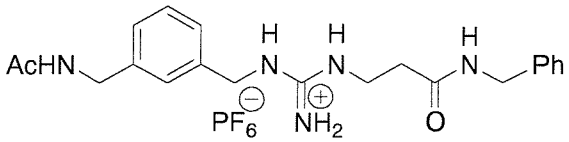


181

Benzyl *N*-((3-[(acetylamino)methyl]benzylamino)[2-(benzylamino)-2-oxoethyl] aminomethylene)carbamate **176** (70 mg, 0.14 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 4 h. The solid was filtered off, washed with CH_3OH (10 mL) and the solvent removed *in vacuo*. H_2O (2 mL) and 70% HPF_6/H_2O (6 drops) were added and extracted with EtOAc (2 x 5 mL) and the combined

organic phases were dried ($MgSO_4$). The solvent was removed *in vacuo* to yield the title compound **181** (45 mg, 0.09 mmol, 63%) as a white foam. IR (solid): 3414 (w), 3227 (w), 1632 (s), 1546 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 7.59 (1 H, br, NH), 7.30-7.14 (9 H, m, Ar), 6.92 (1 H, br, NH), 6.42 (1 H, br, NH), 6.28 (2 H, br, NH_2), 5.38 (1 H, br, NH), 4.29-4.27 (6 H, m, 3 x CH_2), 3.79 (2 H, d, J = 6 Hz, CH_2), 1.95 (3 H, s, CH_3); ^{13}C NMR (100 MHz, CD_3CN): 172.6 (C), 167.2 (C), 156.4 (C), 138.2 (C), 138.0 (C), 135.8 (C), 128.5 (CH), 128.3 (CH), 127.2 (2 x CH), 127.0 (CH), 126.9 (CH), 126.0 (CH), 44.6 (CH_2), 44.0 (CH_2), 43.1 (CH_2), 42.7 (CH_2), 20.9 (CH_3); ESMS: m/z (%): 368 ($(M-PF_6)^+$, 100); HRMS (ES) for $C_{20}H_{26}N_5O_2$ $(M-PF_6)^+$: calcd 368.2081 found 368.2082.

((3-[(Acetylamino)methyl]benzylamino)[3-(benzylamino)-3-oxopropyl]amino methylene)ammonium hexafluorophosphate 182

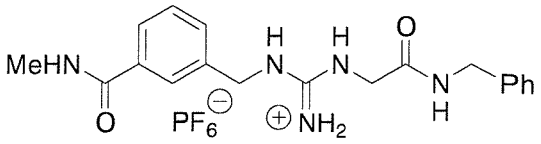


182

Benzyl *N*-((3-[(acetylamino)methyl]benzylamino)[3-(benzylamino)-3-oxopropyl] aminomethylene)carbamate **177** (34 mg, 0.07 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 48 h. The solid was filtered off, washed with CH_3OH (10 mL) and the solvent removed *in vacuo*. H_2O (3 mL) was added followed by 60% HPF_6/H_2O (5 drops) and the mixture was extracted with EtOAc (2 x 5 mL). The organic phase was washed with brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **182** (33 mg, 0.06 mmol, 94%) as a white foam. IR (solid): 3381 (w), 3008 (w), 2947 (w), 1738 (s), 1637 (s), 1559 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.46 (1 H, t, J = 6 Hz, N*H*CH_2Ph), 8.29 (1 H, t, J = 6 Hz, AcN*H*), 7.80 (1 H, br, ArCH_2N*H*C(N)), 7.42 (2 H, br, NH_2), 7.37 (1 H, br, N*H*CH_2CH_2), 7.27-7.07 (9 H, m, Ar), 4.29 (2 H, d, J = 6 Hz, ArCH_2NHC(N)), 4.22 (2 H, d, J = 6 Hz, NHCH_2Ph), 4.17 (2 H, d, J = 6 Hz, AcNHCH_2), 3.35 (2 H, dt, J = 6, 6 Hz, NHCH_2CH_2), 2.39 (2 H, t, J = 6 Hz, NHCH$_2$CH_2), 1.80 (3 H, s, CH_3); ^{13}C NMR (400 MHz, CD_3OD): 173.6 (C), 173.0 (C), 157.7 (C), 140.5 (C), 139.7 (C), 137.9 (C), 130.1 (CH), 129.6 (CH), 128.5 (CH), 128.3 (CH), 128.2 (CH), 127.5 (CH), 127.2 (CH), 45.9 (CH_2), 44.2 (CH_2), 44.2 (CH_2), 39.0 (CH_2), 35.9 (CH_2), 22.3

(CH_3); ESMS: m/z (%): 382 ($(M\text{-}PF_6)^+$, 100); HRMS (ES) for $C_{21}H_{28}N_5O_2$ $(M\text{-}PF_6)^+$: calcd 382.2238, found 382.2243.

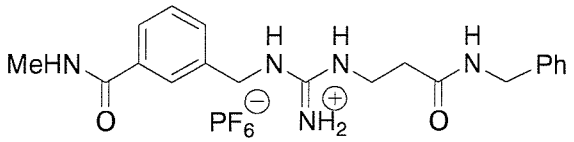
_N_-1-Methyl-3-[(ammonio[2-(benzylamino)-2-oxoethyl]aminomethyl)amino] methylbenzamide hexafluorophosphate 183



183

Benzyl *N*-[[2-(benzylamino)-2-oxoethyl]amino(3-[(methylamino)carbonyl]benzyl amino)methylene]carbamate **178** (71 mg, 0.15 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) and CH_2Cl_2 (1 mL) for 20 h. The solid was filtered off, washed with CH_3OH (10 mL) and the solvent was removed *in vacuo*. The residue was dissolved in H_2O (4 mL), 60% HPF_6/H_2O (10 drops) was added and extracted with EtOAc (3 x 5 mL). The combined organic phases were dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **183** (51 mg, 0.10 mmol, 70%) as a white foam. IR (solid): 3357 (w), 1737 (w), 1631 (s), 1556 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 8.39 (1 H, br, NH), 7.76 (1 H, s, Ar), 7.71 (1 H, d, J = 8 Hz, Ar), 7.61-7.52 (3 H, m, Ar and NH), 7.48-7.28 (5 H, m, Ar), 7.22 (1 H, br, NH), 6.69 (1 H, br, NH), 6.51 (2 H, br, NH_2), 4.51 (2 H, d, J = 5 Hz, CH_2), 4.43 (1 H, d, J = 6 Hz, CH_2), 4.01 (2 H, br, CH_2), 3.00 (3 H, s, CH_3); ^{13}C NMR (100 MHz, CD_3OD): 170.3 (C), 169.7 (C), 158.5 (C), 139.4 (C), 138.2 (C), 136.0 (C), 131.4 (CH), 130.2 (CH), 129.6 (CH), 128.6 (CH), 128.4 (CH), 127.6 (CH), 126.8 (CH), 45.7 (CH_2), 44.9 (CH_2), 44.3 (CH_2), 27.0 (CH_3); ESMS: m/z (%): 354 ($(M\text{-}PF_6)^+$, 100); HRMS (ES) for $C_{19}H_{24}N_5O_2$ $(M\text{-}PF_6)^+$: calcd 354.1934, found 352.1929.

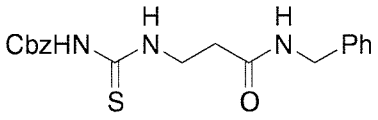
_N_-1-Methyl-3-[(ammonio[3-(benzylamino)-3-oxopropyl]aminomethyl)amino] methylbenzamide hexafluorophosphate 184



184

Benzyl *N*-[[3-(benzylamino)-3-oxopropyl]amino(3-[(methylamino)carbonyl]benzyl amino)methylene]carbamate **179** (52 mg, 0.10 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 2 h. The solid was filtered off, washed with CH_3OH (10 mL) and the solvent removed *in vacuo*. H_2O (5 mL) was added, followed by 60% HPF_6/H_2O (5 drops) and the mixture was extracted with EtOAc (2 x 5 mL). The organic phase was washed with brine (3 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **184** (44 mg, 0.09 mmol, 83%) as a white foam. IR (solid): 3275 (w), 2969 (w), 1738 (s), 1630 (s), 1543 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.52 (1 H, t, J = 6 Hz, N*H*CH_2Ph), 8.45 (1 H, q, J = 4 Hz, CH_3N*H*), 7.94 (1 H, br, ArCH_2N*H*C(N)), 7.79 (1 H, s, Ar), 7.75 (1 H, d, J = 7 Hz, Ar), 7.51 (3 H, br, NH_2 and N*H*CH_2CH_2), 7.46-7.41 (2 H, m, Ar), 7.34-7.21 (5 H, m, Ar), 4.43 (2 H, d, J = 6 Hz, ArCH_2NHC(N)), 4.29 (2 H, d, J = 6 Hz, NHCH_2Ph), 3.42 (2 H, q, J = 6 Hz, NHCH_2CH_2), 2.78 (3 H, d, J = 4 Hz, CH_3NH), 1 x CH_2 obscured by solvent; ^{13}C NMR (100 MHz, CD_3OD): 173.2 (C), 170.3 (C), 157.7 (C), 139.7 (C), 138.3 (C), 136.0 (C), 131.4 (CH), 130.1 (CH), 129.5 (CH), 128.5 (CH), 128.2 (CH), 127.5 (CH), 127.1 (CH), 45.6 (CH_2), 44.2 (CH_2), 39.0 (CH_2), 36.0 (CH_2), 27.0 (CH_3); ESMS: m/z (%): 368 ($(M-PF_6)^+$, 100); HRMS (ES) for $C_{20}H_{25}N_5O_2$ $(M-PF_6)^+$: calcd 368.2081, found 368.2079.

Benzyl {[3-(benzylamino)-3-oxopropyl]amino}carbonothioylcarbamate 185

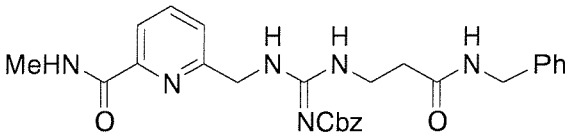


185

N-1-Benzyl-3-aminopropanamide **97** (500 mg, 1.69 mmol) and dry TEA (475 μL, 3.39 mmol) were stirred in dry CH_2Cl_2 (8 mL) and dry DMF (0.5 mL). (Benzyloxy)methanoyl isothiocyanate (300 μL, 1.55 mmol) in CH_2Cl_2 (2 mL) was added and the reaction stirred for 24 h. The solvent was removed *in vacuo* and purification by column chromatography (50% EtOAc/petroleum ether) yielded the title compound **185** (384 mg, 1.01 mmol, 65%) as a white solid. MP = 144-146 °C;

IR (solid): 3290 (w), 3168 (w), 3018 (w), 2969 (w), 1737 (s), 1707 (s), 1634 (s), 1520 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 10.06 (1 H, br, N*H*CH_2CH_2), 8.91 (1 H, br, CbzNH), 7.41-7.21 (10 H, m, Ar), 6.94 (1 H, br, N*H*CH_2Ph), 5.17 (2 H, s, CH_2O), 4.34 (2 H, d, J = 6 Hz, NHC*H*$_2$Ph), 3.88 (2 H, dt, J = 6, 6 Hz, NHC*H*$_2$$CH_2$), 2.55 (2 H, t, J = 6 Hz, $NHCH_2CH_2$); ^{13}C NMR (100 MHz, CD_3CN): 180.5 (C), 172.0 (C), 153.9 (C), 140.3 (C), 136.6 (C), 129.6 (CH), 129.4 (2 x CH), 129.0 (CH), 128.3 (CH), 128.0 (CH), 68.6 (CH_2), 43.6 (CH_2), 42.4 (CH_2), 34.6 (CH_2); ESMS: m/z (%): 394 ($(M+Na)^+$, 100); HRMS (ES) for $C_{19}H_{21}N_3O_3S$ $(M+Na)^+$: calcd 394.1196, found 394.1198; Anal. Calcd for $C_{19}H_{21}N_3O_3S$: C, 61.44; H, 5.78; N, 11.31. Found: C, 61.82; H, 5.77; N, 11.05.

Benzyl *N*-[3-(benzylamino)-3-oxopropyl]amino[(6-[(methylamino) carbonyl]-2-pyridylmethyl)amino]methylenecarbamate 186

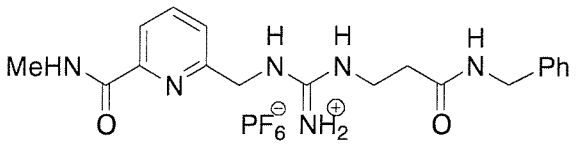


186

Benzyl *N*-([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)carbamate **185** (106 mg, 0.29 mmol), *N*-2-methyl-6-(aminomethyl)-2-pyridinecarboxamide **116** (81 mg, 0.49 mmol) and dry TEA (82 μL, 0.58 mmol) were stirred in dry CH_2Cl_2 (5 mL). EDC (111 mg, 0.58 mmol) was added and the reaction stirred for 5 h. The solvent was removed *in vacuo* and purification by column chromatography (5% CH_3OH/CH_2Cl_2) yielded the title compound **186** (111 mg, 0.22 mmol, 76%) as a white solid. **MP** = 118-120 °C; IR (solid): 3349 (w), 2945 (w), 1738 (m), 1667 (m), 1620 (s), 1597 (s), 1542 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 10.14 (1 H, br, guanidine-NH), 9.03 (1 H, br, guanidine-NH), 8.72 (1 H, br, CH_3N*H*), 8.50 (1 H, br, N*H*CH_2Ph), 8.00-7.84 (2 H, m, pyr), 7.48 (1 H, m, pyr), 7.36-7.16 (10 H, m, Ar), 4.97 (2 H, br, OC*H*$_2$Ph), 4.57 (2 H, d, J = 4 Hz, pyr-C*H*$_2$), 4.28 (2 H, d, J = 4 Hz, NHC*H*$_2$Ph), 3.52 (2 H, d, J = 6 Hz, NHC*H*$_2$$CH_2$), 2.83 (3 H, d, J = 3 Hz, C*H*$_3$NH), 1 x CH_2 obscured by solvent; ^{13}C NMR (100 MHz, CD_3OD): 173.8 (2 x C), 165.0 (C), 143.3 (CH), 139.9 (C), 139.7 (C), 139.0 (2 x C), 129.5 (CH), 129.4 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 125.6 (CH), 121.6 (CH), 67.8 (CH_2), 46.4 (CH_2), 44.2 (CH_2), 38.8 (CH_2), 37.3 (CH_2), 26.5 (CH_3), 1 x C not observed; ESMS: m/z (%): 503 ($(M+H)^+$, 100); HRMS (ES) for $C_{27}H_{31}N_6O_4$ $(M+H)^+$: calcd 503.2411,

found 503.2402; Anal. Calcd for $C_{27}H_{30}N_6O_4$: C, 64.53; H, 6.02; N, 16.71. Found: C, 64.58; H, 6.05; N, 16.45.

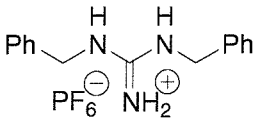
_N_-2-Methyl-6-[(ammonio[3-(benzylamino)-3-oxopropyl]aminomethyl) amino]methyl-2-pyridinecarboxamide hexafluorophosphate 187



187

Benzyl *N*-[3-(benzylamino)-3-oxopropyl]amino[(6-[(methylamino)carbonyl]-2-pyridylmethyl)amino]methylenecarbamate **186** (44 mg, 0.17 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 2 h. The solid was filtered off, washed with CH_3OH (5 mL) and the solvent removed *in vacuo*. H_2O (5 mL) was added followed by 60% HPF_6/H_2O (37 μL, 0.9 eq.) and the mixture was extracted with EtOAc (2 x 5 mL). The organic phase was washed with brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **187** (42 mg, 0.11 mmol, 91%) as a white foam. MP = 173 °C (dec.); IR (solid): 3468 (w), 3395 (w), 3336 (w), 3205 (w), 2970 (w), 1738 (w), 1641 (s), 1540 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.61 (1 H, q, J = 5 Hz, CH_3NH), 8.49 (1 H, t, J = 6 Hz, $NHCH_2Ph$), 7.99-7.90 (2 H, m, pyr), 7.84 (1 H, br, $pyrCH_2NH$), 7.55 (3 H, br, $NHCH_2CH_2$ and NH_2), 7.45 (1 H, d, J = 8 Hz, pyr), 7.28-7.15 (5 H, m, Ph), 4.51 (2 H, d, J = 6 Hz, $pyrCH_2$), 4.25 (2 H, d, J = 6 Hz, CH_2Ph), 3.40 (2 H, dt, J = 6, 6 Hz, $NHCH_2CH_2$), 2.81 (3 H, d, J = 5 Hz, CH_3), 1 x CH_2 obscured by solvent; ^{13}C NMR (100 MHz, CD_3OD): 173.3 (C), 166.9 (C), 158.1 (C), 155.6 (C), 150.7 (C), 140.0 (CH), 139.8 (C), 129.5 (CH), 128.5 (CH), 128.3 (CH), 125.7 (CH), 122.3 (CH), 46.9 (CH_2), 44.3 (CH_2), 39.2 (CH_2), 36.0 (CH_2), 26.4 (CH_3); ESMS: m/z (%): 369 ($(M-PF_6)^+$, 100); HRMS (ES) for $C_{19}H_{24}N_6O_2$ $(M-PF_6)^+$: calcd 369.2033, found 369.2031.

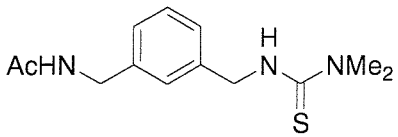
[Di(benzylamino)methylene]ammonium hexafluorophosphate 188



188

Benzyl *N*-di(benzylamino)methylenecarbamate **189** (44 mg, 0.17 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) for 2 h. The solid was filtered off, washed with CH_3OH (5 mL) and the solvent removed *in vacuo*. H_2O (5 mL) was added, followed by 60% HPF_6/H_2O (5 drops) and the mixture was extracted with EtOAc (2 x 5 mL). The organic phase was washed with brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **188** (42 mg, 0.11 mmol, 91%) as a white foam. IR (solid): 3352 (w), 2969 (w), 1738 (m), 1667 (m), 1631 (s), 1593 (s), 1542 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.00 (2 H, br, NH), 7.55 (2 H, br, NH_2), 7.39-7.24 (10 H, m, Ar), 4,43 (4 H, d, J = 6 Hz, CH_2); ^{13}C NMR (100 MHz, CD_3OD): 156.7 (C), 136.7 (C), 129.0 (CH), 128.1 (CH), 127.3 (CH), 45.1 (CH_2); ESMS: m/z (%): 240 ($(M-PF_6)^+$, 100); HRMS (ES) for $C_{18}H_{15}N_3O$ $(M-PF_6)^+$: calcd 240.1495, found 240.1498.

_N_-1-[3-([(Dimethylamino)carbothioyl]aminomethyl)benzyl]acetamide 199

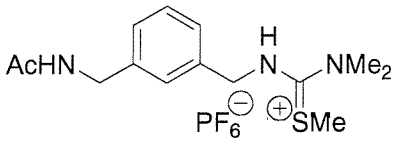


199

N-[3-(Isothiocyanatomethyl)benzyl]acetamide **118** (100 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (3 mL), 40% Me_2NH/H_2O (3 mL) was added and the reaction stirred at room temperature for 1 h. CH_2Cl_2 (5 mL) was added and the phases separated. The organic phase was washed with 1 M $KHSO_4$ (2 x 5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (10% CH_3OH/CH_2Cl_2) yielded the title compound **199** (60 mg, 0.23 mmol, 50%) as a waxy solid. IR (solid): 3284 (w), 3072 (w), 2967 (w), 2936 (w), 2877 (w), 1687 (w), 1638 (s), 1529 (s) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.28-7.06 (4 H, m, Ar), 6.51 (1 H, br, AcNH), 6.07 (1 H, br, NHC(S)), 4.75 (2 H, d, J = 5 Hz, AcNHCH_2), 4.27 (2 H, d, J = 5 Hz, CH_2NHC(S)), 3.21 (6 H, s, $N(CH_3)_2$), 1.93 (3 H, s, C(O)CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): 181.8 (C), 170.7 (C), 139.0 (C), 138.8 (C), 129.1 (CH), 127.0 (CH), 126.8 (CH), 126.8 (CH), 49.9 (CH_3), 43.5 (CH_2), 40.8

(CH_2), 23.3 (CH_3); ESMS: m/z (%): 266 ($(M+H)^+$, 100); HRMS (ES) for $C_{13}H_{19}N_3OSNa$ $(M+Na)^+$: calcd 288.1141, found 288.1410.

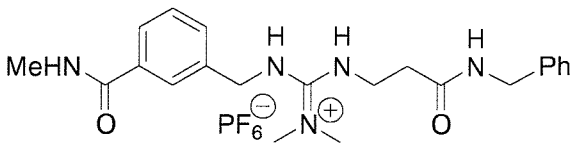
[(3-[(Acetylamino)methyl]benzylamino)(methylsulfanyl)methylene](dimethyl) ammonium hexafluorophosphate 200



200

N-1-[3-([(Dimethylamino)carbothioyl]aminomethyl)benzyl]acetamide **199** (300 mg, 1.13 mmol) was stirred with MeI (140 μL, 2.25 mmol) in acetone (3 mL) for 18 h. The solvent was removed in vacuo and the solid stirred with NH_4PF_6 (366 mg, 2.25 mmol) in CH_3OH (1.5 mL) and CH_2Cl_2 (1.5 mL) for 24 h. The solvent was removed *in vacuo*, the solid dissolved in H_2O (5 mL) and extracted with EtOAc (10 mL). The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the title compound **200** (3.52 mg, 0.83 mmol, 73%) as a waxy yellow solid. IR (solid): 3358 (w), 2968 (w), 1735 (w), 1647 (w), 1615 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 9.48 (1 H, br t, J = 5 Hz, NHC(S)), 7.54 (1 H, br, AcN*H*), 7.40 (1 H, s, Ar), 7.27-7.13 (3 H, m, Ar), 4.76 (2 H, d, J = 6 Hz, AcNHCH_2), 4.28 (2 H, d, J = 6 Hz, CH_2NHC(S)), 3.42 (6 H, s, $N(CH_3)_2$), 2.43 (3 H, s, SCH_3), 1.97 (3 H, s, $C(O)CH_3$); ^{13}C NMR (100 MHz, CD_3CN): 170.1 (C), 169.8 (C), 140.1 (C), 136.5 (C), 128.7 (CH), 126.6 (CH), 126.0 (CH), 125.7 (CH), 49.9 (CH_3), 42.1 (CH_2), 41.4 (CH_2), 21.8 (CH_3), 16.1 (CH_3); ESMS: m/z (%): 280 ($(M-PF_6)^+$, 100).

***N*-1-Methyl-3-([[3-(benzylamino)-3-oxopropyl]amino(1,1-dimethylammonio) methyl]aminomethyl)benzamide hexafluorophosphate 202**

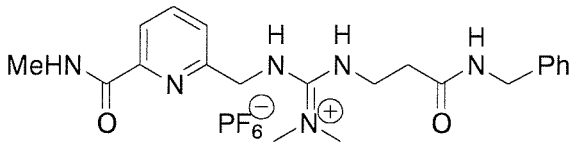


202

N-1-Methyl-3-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl benzamide **135** (40 mg, 0.10 mmol) was stirred with MeI (26 μL, 0.42 mmol) in CH_3OH (2 mL) for 24 h. The solvent was removed *in vacuo*, NH_4PF_6 (34 mg, 0.21 mmol) added and stirred in CH_3OH (2 mL) for 18 h. The solvent was removed *in*

vacuo, 40% $MeNH_2/H_2O$ (1 mL) added and stirred at gentle reflux in CH_3CN (1 mL) for 18 h. The solvent was removed *in vacuo* and purification by column chromatography (2-6% CH_3OH/CH_2Cl_2) yielded the title compound **202** (26 mg, 0.05 mmol, 48%) as a white foam. IR (solid): 3412 (w), 3275 (w), 2970 (w), 1738 (m), 1631 (s), 1586 (m), 1543 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.44 (1 H, t, J = 6 Hz, N*H*CH_2Ph), 8.33 (1 H, q, J = 5 Hz, CH_3N*H*), 7.95 (1 H, t, J = 6 Hz, ArCH_2N*H*C(N)), 7.75 (1 H, s, Ar), 7.68 (1 H, d, J = 8 Hz, Ar), 7.44-7.34 (3 H, m, Ar and N*H*CH_2CH_2), 7.24-7.12 (5 H, m, Ar), 4.32 (2 H, d, J = 6 Hz, Ar*C*H_3NHC(N)), 4.17 (2 H, d, J = 6 Hz, NHCH_2Ph), 3.34 (2 H, dt, J = 6, 6 Hz, NHCH_2CH_2), 2.81 (6 H, s, $N(CH_2)_2$), 2.69 (3 H, d, J = 5 Hz, CH_3NH), 1 x CH_2 obscured by solvent; ^{13}C NMR (100 MHz, CD_3OD): 173.0 (C), 170.2 (C), 161.0 (C), 139.8 (C), 138.9 (C), 136.3 (C), 131.8 (CH), 130.3 (CH), 129.6 (CH), 128.7 (CH), 128.3 (CH), 127.7 (CH), 127.5 (CH), 48.9 (CH_2), 44.2 (CH_2), 41.8 (CH_2), 39.7 (CH_3), 36.1 (CH_2), 27.0 (CH_3); ESMS: m/z (%): 396 ($(M-PF_6)^+$, 100); HRMS (ES) for $C_{22}H_{30}N_5O_2$ $(M-PF_6)^+$: calcd 396.2394, found 396.2399.

_N_-2-Methyl-6-([[3-(benzylamino)-3-oxopropyl]amino(1,1-dimethylammonio) methyl]aminomethyl)-2-pyridinecarboxamide hexafluorophosphate 203

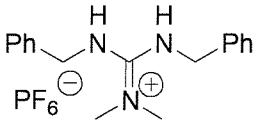


203

N-2-Methyl-6-[([3-(benzylamino)-3-oxopropyl]aminocarbothioyl)amino]methyl-2-pyridine carboxamide **143** (200 mg, 0.52 mmol) was stirred with MeI (100 μL, 1.61 mmol) in CH_3OH (3 mL) for 48 h. The solvent was removed *in vacuo*, NH_4PF_6 (170 mg, 1.04 mmol) added and stirred in CH_3OH (2 mL) and CH_2Cl_2 (2 mL) for 5 h. The solvent was removed *in vacuo*, 40% $MeNH_2/H_2O$ (2 mL) added and stirred at gentle reflux in CH_3CN (2 mL) for 48 h. The solvent was removed *in vacuo* and purification by column chromatography (2-4% CH_3OH/CH_2Cl_2) yielded the title compound **203** (104 mg, 0.19 mmol, 37%) as a white foam. MP = 89-92 °C; IR (solid): 3412 (w), 3286 (w), 2948 (w), 1738 (m), 1632 (s), 1594 (s), 1573 (s), 1543 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 8.84 (1 H, q, J = 4 Hz, CH_3N*H*), 8.72 (1 H, t, J = 5 Hz, N*H*CH_2Ph), 8.20 (1 H, t, J = 5 Hz, pyrCH_2N*H*), 8.04-7.94 (2 H, m, pyr), 7.66 (1 H, t, J = 5 Hz, N*H*CH_2CH_2), 7.58 (1 H, d, J = 7 Hz, pyr), 7.33-7.22 (5

H, m, Ph), 4.57 (2 H, d, J = 5 Hz, pyrCH_2), 4.31 (2 H, m, CH_2Ph), 3.53 (2 H, dt, J = 5, 6 Hz, NHCH_2CH_2), 2.95 (6 H, s, $N(CH_3)_2$), 2.77 (3 H, m, CH_3NH), 2.55 (2 H, t, J = 6 Hz, $NHCH_2CH_2$); ^{13}C NMR (100 MHz, CD_3OD): 173.8 (C), 166.9 (C), 161.3 (C), 156.3 (C), 150.6 (C), 139.9 (CH), 139.8 (C), 129.5 (CH), 128.5 (CH), 128.3 (CH), 125.8 (CH), 122.1 (CH), 49.4 (CH_2), 44.3 (CH_2), 41.6 (CH_2), 39.7 (CH_3), 36.3 (CH_2), 26.3 (CH_3); ESMS: m/z (%): 397 ($(M\text{-}PF_6)^+$, 100); Anal. Calcd for $C_{21}H_{29}F_6N_6O_2P$: C, 46.50; H, 5.39; N, 15.48. Found: C, 46.24; H, 5.51; N, 15.09.

[Di(benzylamino)methylene](dimethyl)ammonium hexafluorophosphate 204

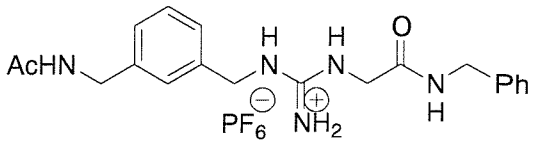


204

N,N'-Dibenzylthiourea[130] (82 mg, 0.32 mmol) was stirred with MeI (300 μL, 1.28 mmol) in CH_3OH (3 mL) for 20 h. The solvent was removed *in vacuo*, NH_4PF_6 (104 mg, 0.64 mmol) added and stirred in CH_3OH (1.5 mL) and CH_2Cl_2 (1.5 mL) for 18 h. The solvent was removed *in vacuo*, 40% $MeNH_2/H_2O$ (2 mL) added and stirred at gentle reflux in CH_3CN (2 mL) for 48 h. The solvent was removed *in vacuo*, H_2O (5 mL) added and extracted with EtOAc (2 x 5 mL). The organic phase was washed with brine (3 mL), dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by column chromatography (1% CH_3OH/CH_2Cl_2) yielded the title compound **204** (104 mg, 0.19 mmol, 37%) as a yellow foam. MP = 119-121 °C; IR (solid): 3419 (m), 3398 (m), 1738 (m), 1639 (s), 1576 (s) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 8.05 (2 H, t, J = 5.5 Hz, NH) , 7.38-7.22 (10 H, m, Ph), 4.40 (4 H, d, J = 5.5 Hz, CH_2), 2.97 (6 H, s, CH_3); ^{13}C NMR (100 MHz, CD_3OD): 160.7 (C), 138.2 (C), 130.0 (CH), 129.1 (CH), 128.6 (CH), 49.0 (CH_2), 39.8 (CH_3); ESMS: m/z (%): 268 ($(M\text{-}PF_6)^+$, 100); Anal. Calcd for $C_{17}H_{22}F_6N_3P$: C, 49.40; H, 5.36; N, 10.16. Found: C, 49.67; H, 5.36; N, 5.36.

Binding Studies

((3-[(Acetylamino)methyl]benzylamino)[2-(benzylamino)-2-oxoethyl]amino methylene)ammonium hexafluorophosphate 181 and TBA acetate in DMSO



181

NMR data

[Host]/M	0.0031	Starting volume/μL	600
[Guest]/M	0.0185	K_a/M^{-1}	4.4×10^3

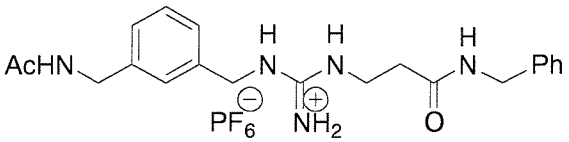
Volume added /μL	N*H*CH_2Ph
0	8.9360
20	8.9566
30	8.9757
40	8.9876
50	9.0043
55	9.0178
60	9.0241
65	9.0289
70	9.0368
75	9.0456
80	9.0503
90	9.0718
100	9.0766
120	9.0972
150	9.1250
200	9.1361
300	9.1425
400	9.1401
500	9.1496
600	9.1488

ITC data

[Host]/mM	1.30	[Guest]/mM	48.0
K_a^1/M^{-1}	5.3×10^3	K_a^2/M^{-1}	40
ΔH^1/kJmol^{-1}	-17.6	ΔH^2/kJmol^{-1}	-46.4
$T\Delta S^1$/kJmol^{-1}	3.7	$T\Delta S^2$/kJmol^{-1}	-37.4
ΔG^1/kJmol^{-1}	-21.2	ΔG^2/kJmol^{-1}	-9.0

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.43	-461.88
0.25	-3509.66	3.57	-441.45
0.38	-3301.98	3.71	-434.05
0.51	-3095.92	3.85	-414.97
0.64	-2918.50	3.98	-404.47
0.77	-2701.20	4.12	-377.63
0.89	-2366.75	4.26	-387.57
1.02	-1971.72	4.40	-367.49
1.15	-1689.36	4.54	-365.66
1.28	-1565.73	4.69	-360.24
1.42	-1374.91	4.83	-354.62
1.55	-1166.83	4.97	-334.40
1.68	-1013.23	5.11	-332.61
1.81	-903.97	5.26	-326.62
1.94	-814.94	5.40	-328.54
2.08	-735.98	5.54	-326.06
2.21	-688.24	5.69	-324.31
2.34	-636.51	5.83	-307.94
2.48	-605.63	5.98	-303.88
2.61	-579.44	6.12	-304.67
2.75	-551.49	6.27	-297.47
2.88	-524.10	6.41	-292.41
3.02	-502.96	6.56	-299.25
3.16	-490.29	6.71	-283.64
3.29	-477.77	6.86	-276.60

((3-[(Acetylamino)methyl]benzylamino)[3-(benzylamino)-3-oxopropyl]amino methylene)ammonium hexafluorophosphate 182 and TBA acetate in DMSO



182

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0190	K_a/M^{-1}	no fit

Volume added /μL	N*H*CH_2Ph	AcN*H*
0	8.8430	8.6762
20	8.8549	8.6833
40	8.8724	8.6921
60	8.8923	8.6984

80	8.9145	8.7072
100	8.9463	8.7207
120	8.9733	8.7302
140	8.9987	8.7469
160	9.0448	8.7620
180	9.0631	8.7691
200	9.0917	8.7763
220	9.1274	8.7938
240	9.1377	8.7993
260	9.1584	8.8089
280	9.1743	8.8128
300	9.1758	8.8112
400	9.1944	8.8168
500	9.1917	8.8216
600	9.1981	8.8152

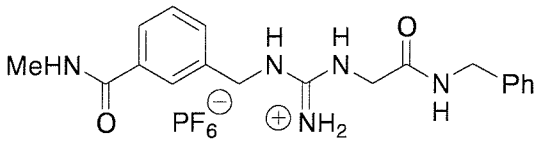
ITC data

[Host]/mM	1.30	[Guest]/mM	48.0
K_a^1/M^{-1}	3.7×10^3	K_a^2/ M^{-1}	40
$\Delta H^1/kJmol^{-1}$	-23.1	$\Delta H^2/kJmol^{-1}$	-87.1
$T\Delta S^1/kJmol^{-1}$	-2.7	$T\Delta S^2/kJmol^{-1}$	-78.2
$\Delta G^1/kJmol^{-1}$	-20.4	$\Delta G^2/kJmol^{-1}$	-8.9

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.43	-813.46
0.25	-4395.37	3.57	-785.61
0.38	-4197.58	3.71	-758.75
0.51	-3921.63	3.85	-724.32
0.64	-3568.98	3.98	-708.72
0.77	-3243.46	4.12	-691.08
0.89	-2920.71	4.26	-675.55
1.02	-2612.68	4.40	-658.78
1.15	-2316.97	4.54	-641.67
1.28	-2063.64	4.69	-629.25
1.42	-1835.85	4.83	-623.98
1.55	-1659.86	4.97	-608.81
1.68	-1524.27	5.11	-584.92
1.81	-1429.93	5.26	-578.32
1.94	-1353.16	5.40	-578.83
2.08	-1286.53	5.54	-573.27
2.21	-1244.69	5.69	-549.42
2.34	-1198.97	5.83	-517.92
2.48	-1112.26	5.98	-528.13
2.61	-1047.58	6.12	-528.41
2.75	-987.77	6.27	-514.09

2.88	-940.92	6.41	-514.24
3.02	-888.73	6.56	-505.10
3.16	-862.41	6.71	-499.91
3.29	-844.41	6.86	-484.73

_N_-1-Methyl-3-[(ammonio[2-(benzylamino)-2-oxoethyl]aminomethyl)amino] methylbenzamide hexafluorophosphate 183 and TBA acetate in DMSO



183

NMR data

[Host]/M	0.0036	Starting volume/μL	600
[Guest]/M	0.0216	K_a/M^{-1}	no fit

Volume added /μL	N*H*CH_2Ph	MeN*H*
0	8.9542	8.7465
20	8.9653	8.7553
40	8.9796	8.7648
60	8.9876	8.7779
70	8.9995	8.7826
80	8.9995	8.7898
90	9.0106	8.7914
100	9.0186	8.8017
110	9.0241	8.8089
120	9.0400	8.8128
130	9.0472	8.8184
140	9.0551	8.8168
150	9.0511	8.8240
160	9.0694	8.8446
170	9.0893	8.8573
180	9.0917	8.8692
200	9.1021	8.8875
250	9.1790	8.9344
200	9.2402	8.9781
250	9.2720	9.0059
300		9.0750
400		9.0726
500	8.9542	8.7465
600	8.9653	8.7553

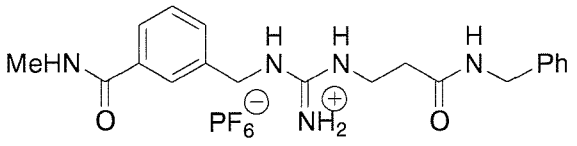
ITC data

[Host]/mM	1.00	[Guest]/mM	48.0

K_a^1/M^{-1}	6.9×10^3	K_a^2/ M^{-1}	130
$\Delta H^1/kJmol^{-1}$	-19.6	$\Delta H^2/kJmol^{-1}$	-51.8
$T\Delta S^1/kJmol^{-1}$	2.3	$T\Delta S^2/kJmol^{-1}$	-39.7
$\Delta G^1/kJmol^{-1}$	-21.9	$\Delta G^2/kJmol^{-1}$	-12.0

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.16	--	4.46	-714.24
0.33	-3803.86	4.64	-687.93
0.50	-3633.58	4.82	-663.37
0.66	-3405.39	5.00	-641.78
0.83	-3142.99	5.18	-629.93
1.00	-2835.03	5.36	-608.90
1.16	-2513.61	5.54	-593.49
1.33	-2190.81	5.73	-578.00
1.50	-1917.11	5.91	-566.14
1.67	-1701.98	6.09	-550.69
1.84	-1558.07	6.28	-533.86
2.01	-1456.14	6.46	-524.43
2.18	-1397.21	6.65	-512.88
2.35	-1312.05	6.83	-501.17
2.53	-1200.59	7.02	-493.70
2.70	-1105.44	7.21	-484.77
2.87	-1030.77	7.39	-472.75
3.05	-978.23	7.58	-459.66
3.22	-930.19	7.77	-453.58
3.40	-891.16	7.96	-441.09
3.57	-847.28	8.15	-438.13
3.75	-811.61	8.34	-432.19
3.93	-778.95	8.53	-424.01
4.10	-752.83	8.72	-418.32
4.28	-739.68	8.91	-408.89

_N_-1-Methyl-3-[(ammonio[3-(benzylamino)-3-oxopropyl]aminomethyl)amino] methylbenzamide hexafluorophosphate 184 and TBA acetate in DMSO



184

NMR data

[Host]/M	0.0016	Starting volume/μL	600
[Guest]/M	0.0195	K_a/M^{-1}	no fit

Volume added /μL	MeN*H*	Ar
0	8.7698	8.0860
20	8.7791	8.0939
40	8.8081	8.1035
50	8.8303	8.1098
60	8.8629	8.1225
70	8.8772	8.1297
80	8.9097	8.1416
90	8.9312	8.1472
100	8.9471	8.1567
120	8.9701	8.1630
130	8.9971	8.1734
140	9.0472	8.1815
150	9.0646	8.1940
170	9.0694	8.1924
200	9.0988	8.2044
300	9.1179	8.2147

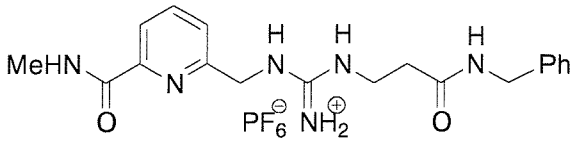
ITC data

[Host]/mM	1.25	[Guest]/mM	48.0
K_a^1/M^{-1}	5.3×10^3	K_a^2/ M^{-1}	70
$\Delta H^1/kJmol^{-1}$	-19.4	$\Delta H^2/kJmol^{-1}$	-43.8
$T\Delta S^1/kJmol^{-1}$	1.8	$T\Delta S^2/kJmol^{-1}$	-33.5
$\Delta G^1/kJmol^{-1}$	-21.3	$\Delta G^2/kJmol^{-1}$	-10.4

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.13	--	3.57	-626.64
0.26	-3833.02	3.71	-601.32
0.40	-3679.64	3.85	-579.90
0.53	-3468.25	4.00	-563.09
0.66	-3238.77	4.14	-549.27
0.80	-2939.42	4.29	-533.89
0.93	-2628.23	4.43	-520.15
1.07	-2287.00	4.58	-507.79
1.20	-1976.70	4.73	-494.01
1.34	-1722.09	4.87	-481.05
1.47	-1544.85	5.02	-470.80
1.61	-1436.82	5.17	-459.82
1.75	-1368.01	5.32	-453.51
1.88	-1232.94	5.47	-445.04
2.02	-1108.07	5.61	-434.55
2.16	-1009.39	5.76	-425.78
2.30	-937.17	5.91	-415.71
2.44	-881.18	6.06	-408.62

2.58	-829.86	6.22	-398.35
2.72	-790.22	6.37	-390.79
2.86	-751.86	6.52	-385.44
3.00	-718.03	6.67	-380.18
3.14	-685.65	6.82	-373.69
3.28	-663.54	6.98	-367.56
3.42	-648.30	7.13	-359.50

_N_-2-Methyl-6-[(ammonio[3-(benzylamino)-3-oxopropyl]aminomethyl)amino] methyl-2-pyridinecarboxamide hexafluorophosphate 187 and TBA acetate in DMSO



187

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0195	K_a/M^{-1}	2350

Volume added /μL	MeN*H*
0	8.9797
20	9.0488
40	9.2172
60	9.3451
80	9.4563
100	9.5230
120	9.6390
140	9.6707
160	9.7263
180	9.7645
200	9.7978
220	9.8105
240	9.8193
260	9.8320
280	9.8487
300	9.8495
400	9.8765
500	9.8709
600	9.8781

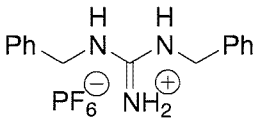
ITC data

[Host]/mM	1.17	[Guest]/mM	48.0

K_a^1/M^{-1}	2.2×10^4	K_a^2/M^{-1}	70
ΔH^1/kJmol^{-1}	-8.0	ΔH^2/kJmol^{-1}	-3.8
$T\Delta S^1$/kJmol^{-1}	16.8	$T\Delta S^2$/kJmol^{-1}	6.9
ΔG^1/kJmol^{-1}	-24.8	ΔG^2/kJmol^{-1}	-10.6

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	--	3.72	-57.71
0.27	--	3.87	-52.46
0.41	-1759.25	4.02	-49.37
0.55	-1726.12	4.17	-48.60
0.69	-1652.39	4.32	-47.69
0.83	-1389.76	4.47	-42.04
0.97	-1013.43	4.62	-41.44
1.11	-719.70	4.77	-36.06
1.25	-512.77	4.92	-36.09
1.39	-382.22	5.08	-37.50
1.53	-289.09	5.23	-35.82
1.68	-227.40	5.38	-34.40
1.82	-191.63	5.54	-28.81
1.96	-168.05	5.69	-35.26
2.11	-140.79	5.85	-28.43
2.25	-125.74	6.00	-32.19
2.39	-112.07	6.16	-31.92
2.54	-99.00	6.32	-27.99
2.68	-93.86	6.47	-23.07
2.83	-78.51	6.63	-27.37
2.98	-75.67	6.79	-28.21
3.12	-69.38	6.95	-25.68
3.27	-54.92	7.11	-26.57
3.42	-61.13	7.27	-25.86
3.57	-60.00	7.43	-23.99

[Di(benzylamino)methylene]ammonium hexafluorophosphate 188 and TBA acetate in DMSO



188

NMR data

[Host]/M	0.0040	Starting volume/μL	600
[Guest]/M	0.0239	K_a/M^{-1}	no fit

Volume added /μL	CH_2
0	4.7140
20	4.7104
40	4.7056
60	4.7016
80	4.6945
100	4.6890
120	4.6822
140	4.6726
160	4.6718
180	4.6663
200	4.6647
220	4.6615
240	4.6599
260	4.6552
280	4.6567
300	4.6544
400	4.6496
500	4.6472
600	4.6440

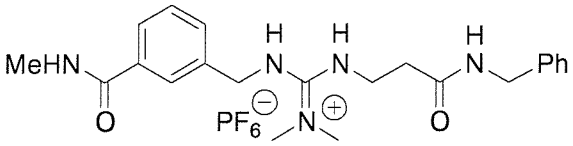
ITC data

[Host]/mM	1.00	[Guest]/mM	48.0
K_a^1/M^{-1}	3.1×10^3	K_a^2/ M^{-1}	30
$\Delta H^1/kJmol^{-1}$	-21.8	$\Delta H^2/kJmol^{-1}$	-46.4
$T\Delta S^1/kJmol^{-1}$	-1.8	$T\Delta S^2/kJmol^{-1}$	-37.6
$\Delta G^1/kJmol^{-1}$	-19.9	$\Delta G^2/kJmol^{-1}$	-8.9

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.16	--	4.46	-351.42
0.33	-3684.98	4.64	-338.65
0.50	-3285.25	4.82	-323.22
0.66	-2862.15	5.00	-315.15
0.83	-2450.29	5.18	-313.16
1.00	-2074.87	5.36	-295.30
1.16	-1777.55	5.54	-282.85
1.33	-1607.48	5.73	-270.24
1.50	-1480.68	5.91	-267.53
1.67	-1215.86	6.09	-260.13
1.84	-1034.12	6.28	-253.02
2.01	-901.01	6.46	-250.08
2.18	-804.05	6.65	-238.91
2.35	-728.03	6.83	-238.13
2.53	-667.13	7.02	-227.74

2.70	-612.91	7.21	-219.33
2.87	-564.68	7.39	-215.67
3.05	-524.42	7.58	-209.74
3.22	-494.68	7.77	-201.00
3.40	-461.81	7.96	-197.84
3.57	-439.18	8.15	-193.41
3.75	-418.27	8.34	-189.92
3.93	-385.92	8.53	-186.48
4.10	-383.46	8.72	-181.80
4.28	-363.72	8.91	-178.29

_N_-1-Methyl-3-([[3-(benzylamino)-3-oxopropyl]amino(1,1-dimethylammonio) methyl]aminomethyl)benzamide hexafluorophosphate 201 and TBA acetate in DMSO



201

NMR data

[Host]/M	0.0032	Starting volume/μL	600
[Guest]/M	0.0185	K_a/M^{-1}	380

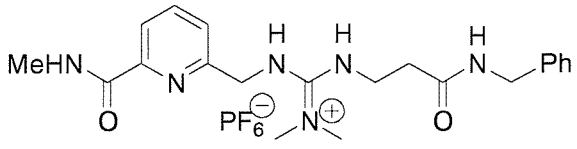
Volume added /μL	N*H*CH_2Ph	MeN*H*
0	8.8494	8.7477
20	8.8899	8.7561
40	8.9455	8.7720
60	8.9939	8.7887
80	9.0400	8.8073
100	9.0734	8.8255
120	9.1123	8.8327
140	9.1369	8.8403
160	9.1631	8.8486
180	9.1862	8.8601
200	9.2013	8.8653
220	9.2140	8.8721
240	9.2291	8.8780
260	9.2418	8.8816
280	9.2553	8.8856
300	9.2720	8.8915
400	9.3157	8.9110
500	9.3244	8.9157
600	9.3244	8.9145

ITC data

[Host]/mM	1.20	$\Delta H/kJmol^{-1}$	5.7
[Guest]/mM	48.0	$T\Delta S/kJmol^{-1}$	26.9
K_a/M^{-1}	5.3 x 10^30	$\Delta G/kJmol^{-1}$	-21.2

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	--	3.72	25.31
0.27	--	3.87	17.65
0.41	--	4.02	11.56
0.55	--	4.17	10.78
0.69	1016.65	4.32	8.97
0.83	778.95	4.47	10.61
0.97	609.83	4.62	11.43
1.11	487.49	4.77	8.63
1.25	388.54	4.92	5.43
1.39	319.90	5.08	8.82
1.53	264.70	5.23	5.49
1.68	219.13	5.38	5.69
1.82	180.89	5.54	4.91
1.96	151.46	5.69	8.07
2.11	128.27	5.85	3.02
2.25	102.88	6.00	3.12
2.39	88.14	6.16	5.98
2.54	76.72	6.32	3.17
2.68	65.70	6.47	4.60
2.83	54.96	6.63	5.29
2.98	45.36	6.79	6.49
3.12	37.92	6.95	5.61
3.27	31.59	7.11	0.51
3.42	28.31	7.27	5.70
3.57	23.33	7.43	9.69

_N_-2-Methyl-6-([[3-(benzylamino)-3-oxopropyl]amino(1,1-dimethylammonio) methyl]aminomethyl)-2-pyridinecarboxamide hexafluorophosphate 202 and TBA acetate in DMSO



202

NMR data

[Host]/M	0.0031	Starting volume/μL	600

[Guest]/M	0.0184	K_a/M^{-1}	260

Volume added /μL	N*H*CH_2Ph
0	9.0519
20	9.0638
40	9.1044
60	9.1433
80	9.1774
100	9.2013
120	9.2259
140	9.2577
160	9.2807
180	9.3014
200	9.3212
220	9.3395
240	9.3458
260	9.3641
280	9.3856
300	9.3943
400	9.4285
500	9.4388
600	9.4396

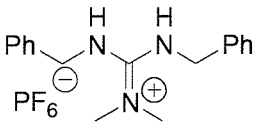
ITC data

[Host]/mM	1.00	$\Delta H/kJmol^{-1}$	12.8
[Guest]/mM	48.0	$T\Delta S/kJmol^{-1}$	33.3
K_a/M^{-1}	3.9×10^3	$\Delta G/kJmol^{-1}$	-20.5

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.16	--	4.46	--
0.33	--	4.64	32.93
0.50	--	4.82	29.41
0.66	--	5.00	22.46
0.83	1564.29	5.18	16.99
1.00	1241.52	5.36	17.22
1.16	1004.78	5.54	10.53
1.33	816.85	5.73	10.08
1.50	674.81	5.91	8.75
1.67	553.02	6.09	7.18
1.84	465.28	6.28	4.60
2.01	394.43	6.46	1.19
2.18	331.43	6.65	4.72
2.35	276.07	6.83	-1.07
2.53	233.19	7.02	-5.49
2.70	199.92	7.21	-3.52

2.87	170.38	7.39	-7.78
3.05	147.25	7.58	-7.99
3.22	127.30	7.77	-4.55
3.40	113.12	7.96	-10.36
3.57	93.39	8.15	-7.42
3.75	78.08	8.34	-6.40
3.93	78.44	8.53	-8.04
4.10	57.65	8.72	-8.55
4.28	52.74	8.91	-8.40

[Di(benzylamino)methylene](dimethyl)ammonium hexafluorophosphate 203 and TBA acetate in DMSO



203

NMR data

[Host]/M	0.0040	Starting volume/μL	600
[Guest]/M	0.0242	K_a/M^{-1}	no fit

Volume added /μL	CH_2	CH_3
0	4.6945	3.2595
20	4.6774	3.2285
40	4.6679	3.2102
60	4.6607	3.1959
80	4.6567	3.1864
100	4.652	3.1784
120	4.6496	3.1721
140	4.6472	3.1665
160	4.6459	3.1626
180	4.644	3.1586
200	4.6424	3.1554
220	4.6409	3.1522
240	4.6401	3.1498
260	4.6393	3.1483
280	4.6385	3.1467
300	4.6377	3.1443
400	4.6353	3.1387
500	4.6353	3.1387
600	4.6353	3.1379

ITC data

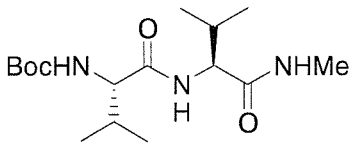
[Host]/mM	1.20	$\Delta H/kJmol^{-1}$	12.5

[Guest]/mM	48.0	$T\Delta S/kJmol^{-1}$	33.0
K_a/M^{-1}	4.0×10^3	$\Delta G/kJmol^{-1}$	-20.6

[H]/[G] after injection	cal/mol of injected G	[H]/[G] after injection	cal/mol of injected G
0.14	--	3.72	69.66
0.27	--	3.87	--
0.41	--	4.02	52.74
0.55	--	4.17	46.79
0.69	1866.16	4.32	39.91
0.83	1511.72	4.47	30.55
0.97	1235.69	4.62	28.49
1.11	1007.46	4.77	23.90
1.25	844.56	4.92	19.36
1.39	711.47	5.08	15.81
1.53	603.87	5.23	14.53
1.68	515.40	5.38	11.25
1.82	437.06	5.54	10.63
1.96	368.69	5.69	5.14
2.11	311.68	5.85	6.03
2.25	271.09	6.00	3.57
2.39	228.43	6.16	1.67
2.54	198.42	6.32	0.99
2.68	171.43	6.47	3.49
2.83	158.30	6.63	-0.15
2.98	132.87	6.79	-1.14
3.12	114.93	6.95	0.27
3.27	108.94	7.11	-5.20
3.42	89.37	7.27	-7.05
3.57	77.00	7.43	-4.51

Experimental for Chapter 4

tert-Butyl _N_-(1S)-2-methyl-1-[((1S)-2-methyl-1-[(methylamino)carbonyl] propyl amino)carbonyl]propylcarbamate 213

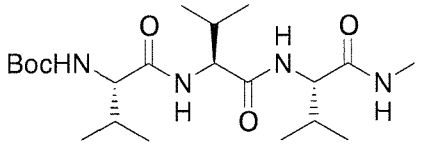


213

Methyl (2*S*)-2-((2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoylamino)-3-methylbutanoate[138] **212** (125 mg, 0.39 mmol) was dissolved in 40% $MeNH_2/H_2O$ (2 mL) and THF (2 mL) and the reaction stirred at room temperature for 48 h. The solvent was removed *in vacuo* to yield a solid which was recrystallised

(EtOAc/Et_2O) to yield the title compound **213** (125 mg, 0.39 mmol, 99%) as a white solid. MP = 190-193 °C; $[\alpha]_D$ = -0.12 (*c* 0.12, CH_3OH, 24 °C); IR (solid): 3285 (m), 3083 (w), 2965 (w), 2936 (w), 2875 (w), 1688 (w), 1638 (s), 1527 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.48 (1 H, br d, *J* = 8 Hz, CHC(O)N*H*CH), 6.31 (1 H, br, N*H*CH_3), 4.97 (1 H, br, BocN*H*), 4.23 (1 H, d, *J* = 7 Hz, C*H*C(O)NHCH$_3$), 3.91 (1 H, t, *J* = 6.53 Hz, BocNHC*H*), 2.81 (3 H, d, *J* = 5 Hz, NHC*H*$_3$), 2.30 (2 H, m, C*H*$(CH_3)_2$), 1.46 (9 H, s, C$(CH_3)_3$), 0.99 (3 H, d, *J* = 7 Hz, CHC*H*$_3$), 0.95 (3 H, d, *J* = 7 Hz, CHC*H*$_3$), 0.94 (3 H, d, *J* = 7 Hz, CHC*H*$_3$), 0.90 (3 H, d, *J* = 7 Hz, CHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 171.8 (C), 171.5 (C), 162.7 (C), 80.7 (C), 58.7 (CH), 51.0 (CH), 30.3 (CH), 30.1 (CH), 28.4 (CH_3), 26.3 (CH_3), 19.6 (CH_3), 19.5 (CH_3), 17.9 (CH_3), 17.7 (CH_3); ESMS: m/z (%): 352 ($(M+Na)^+$, 100); HRMS (ES) for $C_{16}H_{31}N_3O_4Na$ $(M+Na)^+$: calcd 352.2207, found 352.2210.

***tert*-Butyl *N*-(1S)-2-methyl-1-[((1S)-2-methyl-1-[((1S)-2-methyl-1-[(methyl amino)carbonyl]propylamino)carbonyl]propylamino)carbonyl]propyl carbamate 214**

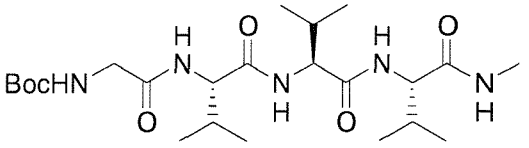


214

tert-Butyl *N*-(1*S*)-2-methyl-1-[((1*S*)-2-methyl-1-[(methylamino)carbonyl] propyl amino)carbonyl]propylcarbamate **213** (2.18 g, 6.64 mmol) was stirred in 20% TFA/CH_2Cl_2 (40 ml) for 2 h, toluene (20 mL) was added and the solvent removed *in vacuo* to yield a yellow waxy solid which dissolved with (2S)-2-[(tert-butoxycarbonyl)amino]-3-methylbutanoic acid (1.73 g, 7.97 mmol) and HOBt (1.08 g, 7.97 mmol) in THF (40 mL) and DMF (40 mL). EDC (1.52 g, 7.97 mmol) was added followed by DIPEA (2.32 mL, 13.28 mmol) and the reaction stirred for 20 h. The solvents were removed *in vacuo*, the residue mixed with EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL). The solid was filtered off and washed with sat. $NaHCO_3$ (20 mL), 1M $KHSO_4$ (2 x 20 mL), CH_3OH (20 mL), CH_3CN (20 mL) and Et_2O (2 x 20 mL) to yield the title compound **214** (2.20 g, 5.13 mmol, 77%) as a white solid. MP = 276 °C (dec.); IR (solid): 3274 (m), 2969 (w), 2936 (w), 1737 (m), 1690 (m), 1634 (s), 1524 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 7.81 (1 H, q, *J* = 4 Hz, N*H*CH_3), 7.75 (1 H, d, *J* = 8 Hz, amide-NH), 7.67 (1 H, d, *J* = 8 Hz, amide-NH), 6.81 (1 H, d, *J* = 8

Hz, BocN*H*), 4.24 (1 H, apparent t, *J* = 8 Hz, α-CH), 4.05 (1 H, apparent t, *J* = 8 Hz, α-CH), 3.79 (1 H, apparent t, *J* = 8 Hz, BocNHC*H*), 2.56 (3 H, d, *J* = 4 Hz, NHCH_3), 2.00-1.83 (3 H, m, C*H*(CH_3)$_2$), 1.37 (9 H, s, C(CH_3)$_3$), 0.92-0.71 (18 H, m, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 171.3 (C), 171.1 (C), 170.6 (C), 155.4 (C), 78.0 (C), 60.0 (CH), 57.8 (CH), 57.5 (CH), 30.5 (CH), 30.4 (CH), 30.0 (CH), 28.1 (CH_3), 25.3 (CH_3), 19.2 (2 x CH_3), 19.1 (CH_3), 18.2 (2 x CH_3), 18.0 (CH_3); ESMS: m/z (%): 451 ($(M+Na)^+$, 100); HRMS (ES) for $C_{21}H_{41}N_4O_5$ $(M+H)^+$: calcd 429.3072, found 429.3080.

***tert*-Butyl *N*-[2-((1S)-2-methyl-1-[((1S)-2-methyl-1-[((1S)-2-methyl-1-[(methyl amino)carbonyl]propylamino)carbonyl]propylamino)carbonyl]propylamino)-2-oxoethyl] carbamate 215**

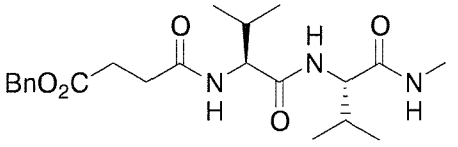


215

tert-Butyl *N*-(1*S*)-2-methyl-1-[((1*S*)-2-methyl-1-[((1*S*)-2-methyl-1-[(methylamino) carbonyl]propylamino)carbonyl]propylamino)carbonyl]propylcarbamate **214** (2.20 g, 5.13 mmol) was stirred in 20% TFA/CH_2Cl_2 (40 mL) for 2 h, toluene (20 mL) was added and the solvents removed *in vacuo* to yield a waxy solid which was dissolved with 2-[(tert-butoxycarbonyl)amino]acetic acid (908 mg, 5.64 mmol) and HOBt (761mg, 5.64 mmol) in DMF (40 mL). EDC (1.08 g, 5.64 mmol) was added followed by DIPEA (1.80 mL, 10.26 mmol) and the reaction stirred for 24 h. The solvents were removed *in vacuo* and the residue mixed with EtOAc (50 mL) and sat. $NaHCO_3$ (30 mL). The solid filtered off and washed with sat. $NaHCO_3$ (20 mL), 1 M $KHSO_4$ (2 x 20 mL), H_2O (20 mL), CH_3OH (20 mL), CH_3CN (20 mL) and Et_2O (2 x 20 mL) to yield the title compound **215** (2.34 g, 4.82 mmol, 74%) as a white solid. MP = 280 °C (dec.); IR (solid): 3286 (m), 3072 (w), 2969 (w), 2937 (w), 2875 (w), 1721 (w), 1688 (w), 1632 (s) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 7.95 (1 H, d, *J* = 8 Hz, amide-NH), 7.81 (1 H, q, *J* = 5.5 Hz, N*H*CH_3), 7.67 (1 H, d, *J* = 9 Hz, amide-NH), 7.57 (1 H, d, *J* = 8.5 Hz, amide-NH), 7.01 (1 H, apparent t, *J* = 6 Hz, BocN*H*), 4.27 (1 H, apparent t, *J* = 8.5 Hz, α-CH), 4.15 (1 H, apparent t, *J* = 8 Hz, α-CH), 4.04 (1 H, dd, *J* = 9, 7.5 Hz, α-CH), 3.55 (2 H, d, *J* = 6 Hz, BocNHCH_2), 2.56 (3 H, d, *J* = 5.5 Hz, NHCH_3), 1.99-1.84 (3 H, m, C*H*(CH_3)$_2$), 1.37 (9 H, s, C(CH_3)$_3$), 0.85-0.76

(18 H, m, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 181.0 (C), 170.2 (C), 169.8 (C), 169.7 (C), 169.2 (C), 77.2 (C), 57.1 (CH), 56.9 (CH), 56.2 (CH), 43.3 (CH_2), 29.1 (CH), 29.5 (CH), 29.2 (CH), 27.2 (CH_3), 24.4 (CH_3), 18.2 (3 x CH_3), 17.4 (CH_3), 17.3 (CH_3), 16.9 (CH_3).

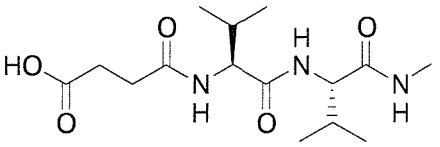
Benzyl 4-((1S)-2-methyl-1-[((1S)-2-methyl-1-[(methylamino)carbonyl] propyl amino)carbonyl]propylamino)-4-oxobutanoate 217



217

tert-Butyl *N*-(1*S*)-2-methyl-1-[((1*S*)-2-methyl-1-[(methylamino)carbonyl]propyl amino)carbonyl]propylcarbamate **213** (924 g, 2.81 mmol) was stirred in 20% TFA/CH_2Cl_2 (20 ml) for 2 h, toluene (10 mL) was added and the solvent removed *in vacuo* to yield a yellow waxy solid which dissolved with 4-(benzyloxy)-4-oxobutanoic acid[139] (640 mg, 3.08 mmol) and HOBt (455 mg, 3.08 mmol) in THF (20 mL) and DMF (10 mL). EDC (644 g, 3.08 mmol) was added followed by DIPEA (1.00 mL, 5.62 mmol) and the reaction stirred for 24 h. The solvents were removed *in vacuo* and the residue mixed with EtOAc (50 mL) and sat. $NaHCO_3$ (50 mL). The solid was filtered off and washed with sat. $NaHCO_3$ (20 mL), 1M $KHSO_4$ (2 x 20 mL), CH_3OH (20 mL), CH_3CN (20 mL) and Et_2O (2 x 20 mL) to yield the title compound **217** (995 mg, 2.37 mmol, 77%) as a white solid. MP = 249-252 °C; IR (solid): 3284 (m), 3074 (w), 2967 (w), 2940 (w), 2875 (w), 1737 (m), 1632 (s) cm^{-1}; ^{1}H NMR (100 MHz, DMSO-d_6): 7.94 (1 H, d, J = 8 Hz, CHN*H*), 7.80 (1 H, q, J = 5 Hz, N*H*CH_3), 7.62 (1 H, d, J = 8 Hz, CHN*H*), 7.39-7.30 (5 H, m, Ar), 5.07 (2 H, s, CH_2Ph), 4.18 (1 H, apparent t, J = 8 Hz, α-CH), 4.06 (1 H, apparent t, J = 8 Hz, α-CH), 2.56 (3 H, d, J = 5 Hz, NHCH_3), 2.54-2.41 (4 H, m, CH_2CH_2), 2.01-1.85 (2 H, m, C*H*(CH_3)$_2$), 0.84-0.80 (12 H, m, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 172.2 (C), 171.1 (C), 170.9 (C), 170.8 (C), 136.2 (C), 128.4 (CH), 127.9 (CH), 127.8 (CH), 65.4 (CH_2), 57.9 (CH), 57.8 (CH), 30.4 (CH), 30.2 (CH), 29.8 (CH_2), 29.1 (CH_2), 25.3 (CH_3), 19.2 (2 x CH_3), 18.3 (CH_3), 18.1 (CH_3); ESMS: m/z (%): 442 ((M+Na)$^+$, 100); HRMS (ES) for $C_{22}H_{34}N_3O_5$ (M+H)$^+$: calcd 420.2493, found 420.2501.

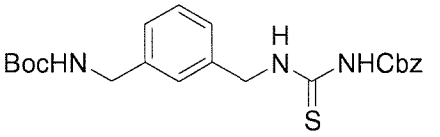
4-((1S)-2-Methyl-1-[((1S)-2-methyl-1-[(methylamino)carbonyl]propylamino) carbonyl]propylamino)-4-oxobutanoic acid 216



216

Benzyl 4-((1*S*)-2-methyl-1-[((1*S*)-2-methyl-1-[(methylamino)carbonyl]propylamino) carbonyl]propylamino)-4-oxobutanoate **217** (980 mg, 2.34 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (20 mL) for 48 h. The solid was filtered off, washed with DMF (100 mL) and the solvent removed *in vacuo* to yield a pale solid which was washed with CH_3CN (20 mL) and Et_2O (10 mL) to yield the title compound **216** (356 mg, 1.08 mmol, 46%) as a pale solid. MP = 197 °C (dec.); IR (solid): 3279 (m), 3085 (br), 2962 (w), 2934 (w), 2873 (w), 1715 (w), 1631 (s), 1545 (m) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 7.98 (1 H, d, J = 9 Hz, N*H*CH), 7.83 (1 H, q, J = 5 Hz, N*H*CH_3), 7.64 (1 H, d, J = 9 Hz, N*H*CH), 4.16 (1 H, dd, J = 9, 7 Hz, α-CH), 4.05 (1 H, dd, J = 9, 7 Hz, α-CH), 2.56 (3 H, d, J = 5 Hz, NHCH_3), 2.50-2.40 (4 H, m, CH_2CH_2), 2.01-1.88 (2 H, m, C*H*$(CH_3)_2$), 0.84-0.79 (12 H, m, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 173.3 (C), 171.6 (C), 171.4 (C), 171.3 (C), 58.5 (CH), 58.3 (CH), 30.9 (CH), 30.6 (CH), 30.3 (CH_2), 29.4 (CH_2), 25.8 (CH_3), 19.7 (2 x CH_3), 18.8 (CH_3), 18.6 (CH_3); ESMS: m/z (%): 442 ((M+TFA-H)$^-$, 100); HRMS (ES) for $C_{15}H_{27}N_3O_5Na$ $(M+Na)^+$: calcd 352.1843, found 352.1843.

***N*-[[[(3-[(*tert*-Butoxycarbonyl)amino]methylbenzyl)amino]carbothioyl]benzyl] carbamate 209**

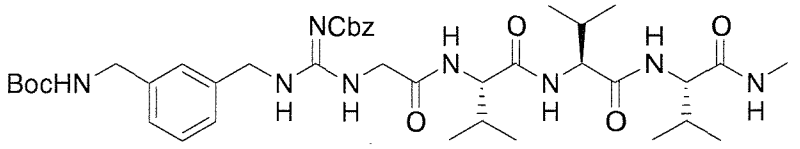


209

(Benzyloxy)methanoyl isothiocyanate (508 mg, 2.61 mmol) was dissolved in dry CH_2Cl_2 (9 mL) under N_2 and cooled to 4°C. *tert*-Butyl 3-(aminomethyl)benzylcarbamate[112] **218** (558 mg, 2.36 mmol) in dry CH_2Cl_2 (6 mL) was added to the reaction and stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the oil dissolved in Et_2O (50 mL), washed with 1 M $KHSO_4$ (3 x 10 mL), H_2O (10 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent removed

in vacuo to yield the title compound **209** (571 mg, 1.33 mmol, 56%) as a yellow oil which solidified on standing. MP = 99-101 °C; IR (solid): 3354 (m), 3179 (w), 2979 (w), 1716 (s), 1686 (s), 1522 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN): 10.11 (1 H, br, $ArCH_2NHC(S)$), 9.25 (1 H, br, CbzN*H*), 7.43-7.21 (9 H, m, Ar), 5.86 (1 H, br, BocN*H*), 5.19-5.18 (2 H, m, OCH_2Ph), 4.87 (2 H, d, J = 6 Hz, $ArCH_2NHC(S)$), 4.25 (2 H, d, J = 6 Hz, $BocNHCH_2$), 1.46 (9 H, s, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_3CN): 180.9 (C), 157.0 (C), 154.0 (C), 141.5 (C), 138.5 (C), 136.3 (C), 129.6 (CH), 129.5 (CH), 129.4 (CH), 129.0 (2 x CH), 127.1 (CH), 127.1 (CH), 79.4 (C), 68.6 (CH_2), 49.4 (CH_2), 44.6 (CH_2), 28.7 (CH_3); ESMS: m/z (%): 452 ($(M+Na)^+$, 100); HRMS (ES) for $C_{22}H_{27}N_3O_4SNa$ $(M+Na)^+$: calcd 452.1614, found 452.1615.

Benzyl *N*-((3-[(acetylamino)methyl]benzylamino)[2-((1S)-2-methyl-1-[((1S)-2-methyl-1-[((1S)-2-methyl-1-[(methylamino)carbonyl]propylamino)carbonyl] propylamino)carbonyl]propylamino)-2-oxoethyl]aminomethylene)carbamate 219

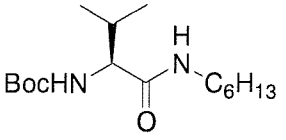


219

tert-Butyl *N*-[2-((1S)-2-methyl-1-[((1S)-2-methyl-1-[((1S)-2-methyl-1-[(methyl amino)carbonyl]propylamino)carbonyl]propylamino)carbonyl]propylamino)-2-oxoethyl]carbamate **215** (132 mg, 0.28 mmol) was stirred in 20% TFA/CH_2Cl_2 (4 mL) for 3 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with N-[[[(3-[(tert-butoxycarbonyl)amino]methyl benzyl)amino]carbothioyl] benzyl]carbamate **216** (140 mg, 0.32 mmol) and dry TEA (98 μL, 0.7 mmol) in dry CH_2Cl_2 (3 mL) at 4 °C. EDC (88 mg, 0.46 mmol) was added and the reaction stirred at room temperature for 20 h. The solvent was removed *in vacuo*, CH_3OH (10 mL) was added and the solid filtered off. The solid was washed with 1 M $KHSO_4$ (5 mL), CH_3OH (5 mL) and Et_2O (5 mL) to yield the title compound **219** (156 mg, 0.20 mmol, 73%) as a white solid. MP = 249 °C (dec.); IR (solid): 3283 (br), 3073 (w), 2969 (w), 2938 (w), 2875 (w), 1738 (w), 1633 (s), 1526 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6):, 9.20 (1 H, br, $NHCH_2C(O)$), 7.98 (1 H, d, J = 8 Hz, NHCH), 7.80 (1 H, q, J = 4 Hz, $NHCH_3$), 7.66 (1 H, d, J = 7 Hz, NHCH), 7.38-7.25 (8 H, m, Ar, NHCH and $ArCH_2NHC(N)$), 7.20-7.13 (4 H, m, Ar

and BocN*H*), 4.99 (2 H, br, OCH_2), 4.45 (2 H, br, BocNHCH_2), 4.29 (1 H, apparent t, J = 7 Hz, α-CH), 4.15 (1 H, apparent t, J = 8 Hz, α-CH), 4.11 (2 H, d, J = 7 Hz, ArCH_2NHC(N)), 4.04 (1 H, apparent t, J = 8 Hz, α-CH), 3.96 (2 H, br, $CH_2C(O)$), 2.56 (3 H, d, J = 4 Hz, NHCH_3), 1.98-1.83 (3 H, m, CH(CH_3)$_2$), 1.38 (9 H, s, $(CH_3)_3$), 0.85-0.77 (18 H, m, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, DMSO-d_6): 170.5 (C), 170.2 (C), 170.1 (C), 169.9 (C), 155.2 (C x 2), 140.7 (C), 136.7 (C), 136.7 (C), 136.4 (C), 127.7 (3 x CH), 127.3 (2 x CH), 125.2 (CH), 124.8 (CH), 77.2 (C), 64.4 (CH_2), 57.5 (CH), 57.3 (CH), 56.9 (CH), 45.2 (CH_2), 42.8 (CH_2), 30.3 (CH), 29.9 (CH), 29.5 (CH), 27.7 (CH_3), 24.8 (CH_3), 18.6 (3 x CH_3), 17.8 (CH_3), 17.7 (CH_3), 17.4 (CH_3), 1 x CH_2 obscured by solvent; ESMS: m/z (%): 781 ((M+H)$^+$, 100), 681 (81); HRMS (ES) for $C_{40}H_{61}N_8O_8$ (M+H)$^+$: calcd 781.4607, found 781.4618.

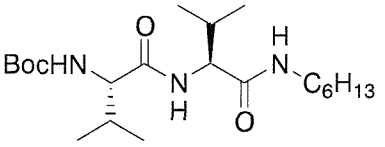
tert-Butyl _N_-(1S)-1-[(hexylamino)carbonyl]-2-methylpropylcarbamate 222



222

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-methylbutanoic acid (1.2 g, 5.53 mmol) and HOBt (747 mg, 5.53 mmol) were stirred in DMF (1 mL) and THF (14 mL). EDC (1.06 g, 5.53 mmol) was added followed by 1-hexanamine (689 μL, 5.25 mmol) then DIPEA (1.83 mL, 10.50 mmol) and the reaction stirred for 20 h. The solvents were removed *in vacuo*, Et_2O (80 mL) was added and washed with sat. $NaHCO_3$ (3 x 15 mL), 1% HCl (3 x 15 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield the title compound **222** (1.27 g, 4.23 mmol, 81%) as a white solid. MP = 74-77 °C; IR (solid): 3310 (br), 2962 (m), 2931 (m), 2873 (w), 1686 (m), 1651 (s), 1526 (m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 6.06 (1 H, br, N*H*CH_2), 5.10 (1 H, br d, J = 7 Hz, BocN*H*), 3.83 (1 H, dd, J = 9, 7 Hz, α-CH), 3.31-3.13 (2 H, m, NHCH_2), 2.09 (1 H, m, CH(CH_3)$_2$), 1.51-1.46 (2 H, m, $NHCH_2CH_2$), 1.43 (9 H, s, $C(CH_3)_3$), 1.33-1.22 (6 H, m, 3 x CH_2), 0.94 (3 H, d, J = 7 Hz, CHCH_3), 0.90 (3 H, d, J = 7 Hz, CHCH_3), 0.86 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 171.6 (C), 156.1 (C), 79.9 (C), 60.3 (CH), 39.6 (CH_2), 31.6 (CH_2), 30.9 (CH), 29.6 (CH_2), 28.4 (CH_3), 26.7 (CH_2), 22.7 (CH_2), 19.4 (CH_3), 18.1 (CH_3), 14.1 (CH_3); ESMS: m/z (%): 601 ((2M+H)$^+$, 100); HRMS (ES) for $C_{32}H_{64}N_4O_6Na$ (2M+Na)$^+$: calcd 623.4718, found 623.4733.

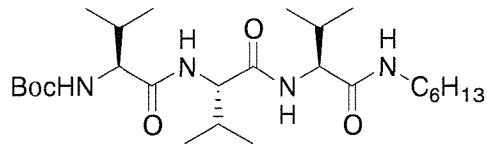
***tert*-Butyl *N*-(1S)-1-[((1S)-1-[(hexylamino)carbonyl]-2-methylpropylamino) carbonyl]-2-methylpropylcarbamate 223**



223

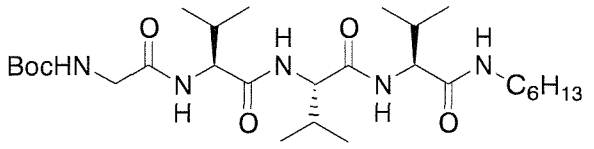
tert-Butyl *N*-(1*S*)-1-[(hexylamino)carbonyl]-2-methylpropylcarbamate **222** (1.16 g, 3.87 mmol) was stirred in 40% TFA/CH_2Cl_2 (30 mL) for 3 h, toluene (10 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoic acid (922 mg, 4.26 mmol) and HOBt (575 mg, 4.26 mmol) in DMF (1 mL) and THF (14 mL). EDC (814 mg, 4.26 mmol) was added followed by DIPEA (1.35 mL, 7.74 mmol) and the reaction stirred for 20 h. The solvents were removed *in vacuo*, Et_2O (50 mL) added, washed with sat. $NaHCO_3$ (3 x 20 mL), 1% HCl (3 x 20 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (Et_2O) and recrystallisation (CH_3OH/H_2O) yielded the title compound **223** (1.25 g, 3.13 mmol, 81%) as a white solid. MP = 161-164 °C; IR (solid): 3282 (w), 2961 (w), 2929 (w), 2873 (w), 1690 (m), 1640 (s), 1548 (m), 1518 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 6.59 (1 H, d, J = 7 Hz, NHC$H_2$$CH_2$), 6.31 (1 H, br, CHC(O)N*H*CH), 4.99 (1 H, d, J = 7 Hz, BocN*H*), 4.10 (1 H, apparent t, J = 7 Hz, C*H*C(O)NHC$_6H_{13}$), 3.83 (1 H, apparent t, J = 7 Hz, BocNHC*H*), 3.22-3.00 (2 H, m, NHCH_2), 1.42-1.36 (2 H, m, NHCH$_2$CH_2), 1.34 (9 H, s, C(CH_3)$_3$), 1.22-1.12 (6 H, m, 3 x CH_2), 0.85 (3 H, d, J = 8 Hz, CHCH_3), 0.81 (3 H, d, J = 8 Hz, CHCH_3), 0.76 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 171.9 (C), 170.9 (C), 156.2 (C), 80.4 (C), 60.7 (CH), 58.9 (CH), 39.7 (CH_2), 31.6 (CH_2), 30.5 (CH), 30.3 (CH), 29.5 (CH_2), 28.4 (CH_3), 26.7 (CH_2), 22.7 (CH_2), 19.5 (3 x CH_3), 17.9 (CH_3), 14.1 (CH_3); ESMS: m/z (%): 821 (33), 799 (18), 422 ($(M+Na)^+$, 100), 400 (34); HRMS (ES) for $C_{21}H_{41}N_3O_4Na$ $(M+Na)^+$: calcd 422.2995, found 422.2999.

***tert*-Butyl *N*-(1S)-1-[((1S)-1-[((1S)-1-[(hexylamino)carbonyl]-2-methylpropyl amino)carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylcarbamate**



tert-Butyl *N*-(1*S*)-1-[((1*S*)-1-[(hexylamino)carbonyl]-2-methylpropylamino) carbonyl]-2-methylpropylcarbamate **223** (670 mg, 1.68 mmol) was stirred in 20% TFA/CH_2Cl_2 (10 mL) for 2 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoic acid (401 mg, 1.85 mmol) and HOBt (250 mg, 1.85 mmol) in THF (10 mL). EDC (353 mg, 1.85 mmol) was added followed by DIPEA (585 μL, 3.36 mmol) and the reaction stirred for 6 h. The solvents were removed *in vacuo*, Et_2O (40 mL) added, washed with sat. $NaHCO_3$ (3 x 10 mL), 1% HCl (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (50% Et_2O/petroleum ether) yielded the title compound (578 mg, 1.16 mmol, 69%) as a white solid. MP = 246-250 °C; IR (solid): 3268 (br), 3073 (w), 2965 (w), 2936 (w), 2874 (w), 1741 (w), 1687 (w), 1632 (s), 1527 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 8.19 (1 H, br, NH), 7.69 (1 H, br, $NHCH_2$), 7.59 (1 H, br, NH), 5.74 (1 H, d, J = 8 Hz, BocN*H*), 4.57 (1 H, apparent t, J = 8 Hz, α-CH), 4.41 (1 H, apparent t, J = 9 Hz, α-CH), 4.34 (1 H, apparent t, J = 9 Hz, α-CH), 3.31-3.13 (2 H, m, NHC*H*$_2$), 2.13-2.01 (3 H, m, C*H*$(CH_3)_2$), 1.99-1.44 (11 H, m, $C(CH_3)_3$ and $NHCH_2CH_2$), 1.33-1.20 (6 H, m, $(CH_2)_3CH_3$), 0.95-0.87 (18 H, m, $CH(CH_3)_2$), 0.86 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 172.4 (C), 171.6 (C), 171.5 (C), 156.1 (C), 79.4 (C), 60.0 (CH), 59.1 (CH), 58.4 (CH), 39.6 (CH_2), 31.7 (CH_2), 31.6 (CH), 31.6 (CH), 30.3 (CH), 29.6 (CH_2), 28.6 (CH_3), 26.8 (CH_2), 22.7 (CH_2), 19.3 (CH_3), 19.3 (2 x CH_3), 18.7 (CH_3), 18.6 (CH_3), 18.5 (CH_3), 14.2 (CH_3); ESMS: m/z (%): 521 ($(M+Na)^+$, 100); HRMS (ES) for $C_{26}H_{50}N_4O_5Na$ $(M+Na)^+$: calcd 521.3673, found 521.3659.

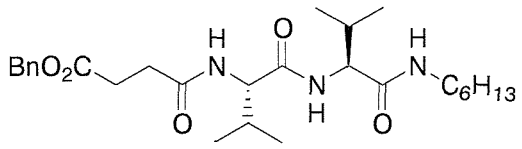
***tert*-Butyl *N*-[2-((1S)-1-[((1S)-1-[((1S)-1-[(hexylamino)carbonyl]-2-methyl propyl amino)carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylamino)-2-oxoethyl]carbamate 224**



224

tert-Butyl *N*-(1*S*)-1-[((1*S*)-1-[((1*S*)-1-[(hexylamino)carbonyl]-2-methylpropylamino) carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylcarbamate (663 mg, 1.27 mmol) was stirred in 20% TFA/CH_2Cl_2 (10 mL) for 3 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was triturated with Et_2O to yield a white solid. The solid was stirred with 2-[(tert-butoxycarbonyl)amino]acetic acid (319 mg, 1.83 mmol) and HOBt (247 mg, 1.83 mmol) in DMF (10 mL). EDC (350 mg, 1.83 mmol) was added followed by DIPEA (579 μL, 3.32 mmol) and the reaction stirred for 24 h. The solvents were removed *in vacuo*, EtOAc (20 mL) and sat. $NaHCO_3$ (10 mL) were added and the solid filtered off and washed with sat. $NaHCO_3$ (10 mL), 1% HCl (2 x 10 mL), H_2O (10 mL), CH_3CN (10 mL) and Et_2O (10 mL) to yield the title compound **224** (375 mg, 0.71 mmol, 56%) as a white solid. MP = >300 °C; IR (solid): 3275 (br), 3072 (w), 2964 (w), 2935 (w), 2875 (w), 1731 (w), 1631 (s), 1538 (s) cm^{-1}; ^1H NMR (400 MHz, 10% $CD_3OD/CDCl_3$): 4.54 (1 H, d, J = 8 Hz, α-CH), 4.43 (1 H, d, J = 8 Hz, α-CH), 4.17 (1 H, d, J = 9 Hz, α-CH), 3.81 (2 H, br, BocNHCH_2), 3.16-3.05 (2 H, m, NHC$H_2$$CH_2$), 1.99-1.88 (3 H, m, C$H$$(CH_3)_2$), 1.41-1.35 (11 H, m, $(CH_3)_3$ and $NHCH_2CH_2$), 1.33-1.12 (6 H, m, $(CH_2)_3CH_3$), 0.84-0.74 (21 H, m, $CH(CH_3)_2$ and CH_2CH_3); ^{13}C NMR (100 MHz, 10% $CD_3OD/CDCl_3$): 171.8 (C), 171.5 (C), 171.5 (C), 170.0 (C), 156.3 (C), 79.9 (C), 59.1 (CH), 58.5 (CH), 58.2 (CH), 39.6 (CH_2), 39.5 (CH_2), 32.0 (CH), 31.6 (CH_2), 31.3 (CH), 30.4 (CH), 29.4 (CH_2), 28.4 (CH_3), 26.7 (CH_2), 22.6 (CH_2), 19.2 (CH_3), 19.0 (2 x CH_3), 18.7 (CH_3), 18.6 (CH_3), 18.5 (CH_3), 14.0 (CH_3); ESMS: m/z (%): 578 ($(M+Na)^+$, 100); HRMS (ES) for $C_{28}H_{53}N_5O_6Na$ $(M+Na)^+$: calcd 578.3888, found 578.3894.

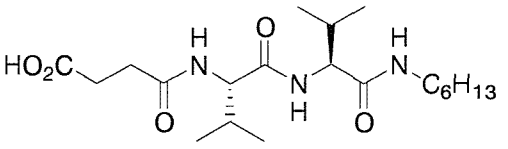
Benzyl 4-((1S)-1-[((1S)-1-[((1S)-1-[(hexylamino)carbonyl]-2-methylpropyl amino)carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylamino)-4-oxo butanoate 225



225

tert-Butyl *N*-(1*S*)-1-[((1*S*)-1-[(hexylamino)carbonyl]-2-methylpropylamino) carbonyl]-2-methylpropylcarbamate **223** (199 mg, 0.67 mmol) was stirred with 20% TFA/CH_2Cl_2 (5 mL) for 2 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid. The solid was stirred with 4-(benzyloxy)-4-oxobutanoic acid[139] (158 mg, 0.73 mmol) and HOBt (99 mg, 0.73 mmol) in CH_2Cl_2 (5 mL). EDC (139 mg, 0.73 mmol) was added followed by DIPEA (234 μL, 1.34 mmol) and the reaction was stirred for 6 h. The solvent was removed *in vacuo*, EtOAc (10 mL) and sat. $NaHCO_3$ (5 mL) were added and the solid filtered off and washed with sat. $NaHCO_3$ (5 mL), 1 % HCl (2 x 5 mL), H_2O (5 mL), CH_3CN (5 mL) and Et_2O (5 mL) to yield the title compound **225** (184 mg, 0.38 mmol, 56%) as a white solid. MP = 223-235 °C; IR (solid): 3277 (br), 3087 (w), 2961 (w), 2932 (w), 2872 (w), 1736 (m), 1631 (s), 1546 (s) cm^{-1}; ^1H NMR (400 MHz, 5% $CD_3OD/CDCl_3$): 7.27-7.23 (5 H, m, Ar), 5.06-4.99 (2 H, m, PhCH_2O), 4.15 (1 H, d, J = 7 Hz, α-CH), 4.00 (1 H, d, J = 7 Hz, α-CH), 3.19-3.02 (2 H, m, NHCH_2), 2.65 (2 H, t, J = 7 Hz, C(O)CH_2), 2.48 (2 H, m, C(O)CH_2), 2.08-1.98 (2 H, m, CH(CH_3)$_2$), 1.44-1.36 (2 H, m, NHCH_2CH_2), 1.26-1.17 (6 H, m, (CH_2)$_3$$CH_3$), 0.86-0.83 (12 H, m, CH(CH_3)$_2$), 0.80 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, 5% $CD_3OD/CDCl_3$): 173.0 (C), 172.7 (C), 172.4 (C), 172.2 (C), 135.7 (C), 128.6 (CH), 128.3 (CH), 128.2 (CH), 66.7 (CH_2), 59.0 (CH), 58.9 (CH), 39.5 (CH_2), 31.4 (CH_2), 30.8 (CH), 30.6 (CH_2), 30.2 (CH), 29.5 (CH_2), 29.2 (CH_2), 26.5 (CH_2), 22.5 (CH_2), 19.2 (CH_3), 19.1 (CH_3), 18.2 (CH_3), 18.0 (CH_3), 13.9 (CH_3); ESMS: m/z (%): 490 ($(M+H)^+$, 100); HRMS (ES) for $C_{27}H_{43}N_3O_5Na$ $(M+Na)^+$: calcd 512.3095, found 512.3087.

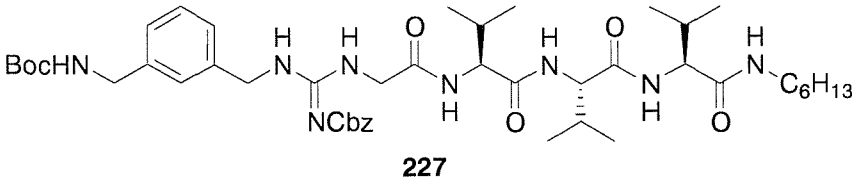
4-((1S)-1-[((1S)-1-[((1S)-1-[(Hexylamino)carbonyl]-2-methylpropylamino) carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylamino)-4-oxo butanoic acid 226



226

Benzyl 4-((1*S*)-1-[((1*S*)-1-[((1*S*)-1-[(hexylamino)carbonyl]-2-methylpropyl amino) carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylamino)-4-oxobutanoate **225** (40 mg, 0.08 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (3 mL) with c. HCl (15 μL) for 2 h. The solid was filtered off, washed with CH_3OH (5 mL) and the solvent was removed *in vacuo*. Purification by column chromatography (0.01% $CHCO_2H$/9.99% CH_3OH/CH_2Cl_2) yielded the title compound **226** (24 mg, 0.06 mmol, 75%) as a pale solid. MP = 188 °C (dec.); IR (solid): 3362 (br), 3292 (w), 2961 (w), 2932 (w), 2871 (w), 1701 (w), 1625 (s) cm^{-1}; ^1H NMR (400 MHz, 10% $CD_3OD/CDCl_3$): 3.77 (1 H, d, J = 6 Hz, α-CH), 3.68 (1 H, d, J = 7 Hz, α-CH), 2.83-2.65 (2 H, m, $NHCH_2CH_2$), 2.21-2.08 (4 H, m, HO_2CCH_2 and $CH(CH_3)_2$), 1.72-1.67 (2 H, m, $HO_2CCH_2CH_2$), 1.08-1.02 (2 H, m, $NHCH_2CH_2$), 0.92-0.85 (6 H, m, $(CH_2)_3CH_3$)), 0.54-0.48 (12 H, m, $CH(CH_3)_2$), 0.45 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, 10% $CD_3OD/CDCl_3$): 176.6 (C), 174.7 (C), 173.4 (C), 172.7 (C), 60.3 (CH), 60.1 (CH), 40.4 (CH_2), 32.4 (CH_2), 31.4 (CH), 31.3 (CH_2), 31.2 (CH_2), 30.4 (CH_2), 30.1 (CH), 27.5 (CH_2), 23.4 (CH_2), 20.0 (CH_3), 19.8 (CH_3), 18.9 (CH_3), 18.6 (CH_3), 14.6 (CH_3); ESMS: m/z (%): 512 ($(M+TFA-H)^1$, 100); HRMS (ES) for $C_{20}H_{37}N_3O_5Na$ $(M+Na)^+$: calcd 422.2625, found 422.2636.

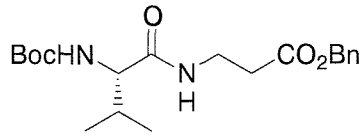
Benzyl *N*-((3-[(tert-butoxycarbonylamino)methyl]benzylamino)[2-((1S)-1-[((1S)-1-[((1S)-1-[(hexylamino)carbonyl]-2-methylpropylamino)carbonyl]-2-methyl propylamino)carbonyl]-2-methylpropylamino)-2-oxoethyl]aminomethylene) carbamate 227



227

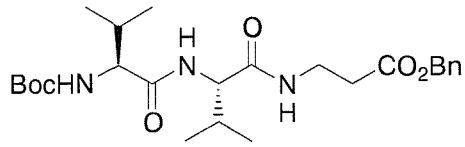
tert-Butyl *N*-[2-((1*S*)-1-[((1*S*)-1-[((1*S*)-1-[(hexylamino)carbonyl]-2-methylpropyl amino)carbonyl]-2-methylpropylamino)carbonyl]-2-methylpropylamino)-2-oxoethyl]carbamate **224** (70 mg, 0.13 mmol) was stirred in 20% TFA/CH_2Cl_2 (5 mL) for 2 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was triturated with Et_2O to yield a white solid. The TFA salt, *N*-[[[(3-[(*tert*-butoxycarbonyl)amino]methylbenzyl)amino]carbothioyl]benzyl]carbamate **209** (60 mg, 0.14 mmol) and DIPEA (44 μL, 0.25 mmol) were stirred in dry CH_2Cl_2 (3 mL) and DMF (100 μL) under N_2 at 4 °C. EDC (48 mg, 0.25 mmol) was added and the reaction stirred at room temperature for 24 h. The solvents were moved *in vacuo*, EtOAc (5 mL) and 1% HCl (5 mL) were added, the solid filtered off and washed with 1% HCl (5 mL), H_2O (5 mL), CH_3OH (5 mL), CH_3CN (5 mL) and Et_2O (5 mL) to yield the title compound **227** (38 mg, 0.04 mmol, 34%) as a white solid. MP = >300 °C (dec.); $[\alpha]_D$ = -0.17 (*c* 0.2, 10% CH_3OH/CH_2Cl_2, 23 °C); IR (solid): 3282 (br), 2962 (w), 2931 (w), 2873 (w), 1720 (w), 1692 (w), 1631 (s), 1554 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): 9.21 (1 H, br, $NHCH_2C(O)$), 7.95 (1 H, d, *J* = 8 Hz, N*H*CH), 7.81 (1 H, t, *J* = 5 Hz, N*H*CH_2CH_2), 7.65 (1 H, d, *J* = 8 Hz, N*H*CH), 7.38-7.12 (8 H, m, Ar, N*H*CH and $ArCH_2NHC(N)$), 7.20-7.12 (4 H, m, Ar and BocN*H*), 4.98 (2 H, s, OCH_2Ph), 4.42 (2 H, d, *J* = 6 Hz, BocNHCH_2), 4.29 (1 H, apparent t, *J* = 8 Hz, α-CH), 4.15 (1 H, apparent t, *J* = 8 Hz, α-CH), 4.11 (2 H, d, *J* = 6 Hz, ArCH_2NHC(N)), 4.07 (1 H, m, α-CH), 3.93-3.88 (2 H, m, $CH_2C(O)$), 3.10-2.94 (2 H, m, NHCH_2CH_2), 1.99-1.85 (3 H, m, C$H(CH_3)_2$), 1.40-1.33 (11 H, m, $(CH_3)_3$ and $NHCH_2CH_2$), 1.27-1.19 (6 H, m, $(CH_2)_3CH_3$), 0.87-0.76 (21 H, m, $C(CH_3)_2$ and CH_2CH_3); ^{13}C NMR (100 MHz, 10% $CD_3OD/CDCl_3$): 171.8 (C), 171.7 (C), 171.4 (C), 171.3 (C), 159.3 (C), 156.3 (C), 139.8 (C), 139.4 (C), 136.8 (C), 129.2 (CH), 128.4 (CH), 128.1 (CH), 127.9 (CH), 127.1 (CH), 126.5 (CH), 126.2 (CH), 79.6 (C), 66.9 (CH_2), 59.0 (CH), 58.7 (CH), 58.5 (CH), 45.2 (CH_2), 44.3 (CH_2), 39.5 (CH_2), 39.4 (CH_2), 31.5 (1 x CH, 1 x CH_2), 31.3 (CH), 30.3 (CH), 29.3 (CH_2), 28.4 (CH_3), 26.6 (CH_2), 22.6 (CH_2), 19.2 (CH_3), 19.0 (CH_3), 18.9 (CH_3), 18.6 (CH_3), 18.5 (CH_3), 18.4 (CH_3), 14.0 (CH_3), 1 x C not observed; ESMS: m/z (%): 851 ($(M+H)^+$, 100); HRMS (ES) for $C_{45}H_{71}N_8O_8$ $(M+H)^+$: calcd 851.5389, found 851.5373.

Benzyl 3-((2S)-2-[(tert-butoxycarbonyl)amino]-3-methylbutanoylamino) propanoate



Benzyl 3-[(*tert*-butoxycarbonyl)amino]propanoate[140] **223** (1.02 g, 3.66 mmol) was stirred in 20% TFA/CH_2Cl_2 (20 mL) for 1.5 h, toluene (10 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoic acid (929 mg, 4.02 mmol) and HOBt (543 mg, 4.02 mmol) in DMF (10 mL) and THF (10 mL). EDC (768 mg, 4.02 mmol) was added followed by DIPEA (1.28 mL, 7.32 mmol) and the reaction stirred for 3 h. The solvents were removed *in vacuo*, Et_2O (40 mL) was added, washed with sat. $NaHCO_3$ (3 x 10 mL), 1% HCl (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (80% Et_2O/petroleum ether) yielded the title compound (1.09 g, 2.88 mmol, 79%) as a white solid. MP = 90-93 °C; IR (solid): 3338 (m), 2991 (w), 2943 (w), 1731 (s), 1673 (s), 1654 (s), 1524 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.35-7.27 (5 H, m, Ar), 6.64 (1 H, t, *J* = 5 Hz, N*H*CH_2CH_2), 5.16 (1 H, d, *J* = 8 Hz, BocN*H*), 5.10 (2 H, s, OCH_2), 3.88 (1 H, apparent t, *J* = 8 Hz, α-CH), 3.61 (2 H, m, NHC*H*$_2CH_2$), 2,57 (2 H, t, *J* = 6 Hz, $NHCH_2CH_2$), 2.05 (1 H, m, C*H*$(CH_3)_2$), 1.41 (9 H, s, $(CH_3)_3$), 0.89 (3 H, d, *J* = 7 Hz, CHC*H*$_3$), 0.84 (3 H, d, *J* = 7 Hz, CHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 172.1 (C), 171.8 (C), 155.9 (C), 135.7 (C), 128.7 (CH), 128.4 (CH), 128.4 (CH), 79.8 (C), 66.6 (CH_2), 60.0 (CH), 35.0 (CH_2), 34.1 (CH_2), 31.0 (CH), 28.4 (CH_3), 19.3 (CH_3), 17.8 (CH_3); ESMS: m/z (%): 401 ($(M+Na)^+$, 100); HRMS (ES) for $C_{20}H_{30}N_2O_5Na$ $(M+Na)^+$: calcd 401.2047, found 401.2039.

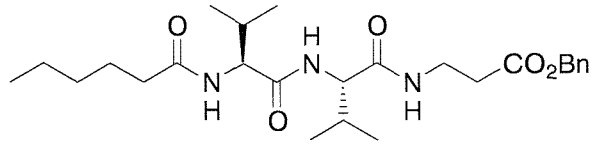
Benzyl 3-[(2S)-2-((2S)-2-[(tert-butoxycarbonyl)amino]-3-methylbutanoyl amino)-3-methylbutanoyl]aminopropanoate



Benzyl 3-((2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoylamino)propanoate (988 mg, 2.61 mmol) was stirred in 20% TFA/CH_2Cl_2 (5 mL) for 2 h, toluene (5 mL)

was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoic acid (663 mg, 2.87 mmol) and HOBt (387 mg, 2.87 mmol) in DMF (10 mL) and THF (10 mL). EDC (548 mg, 2.87 mmol) was added followed by DIPEA (910 μL, 5.22 mmol) and the reaction stirred for 3 h. The solvents were removed *in vacuo*, Et_2O (40 mL) added, washed with sat. $NaHCO_3$ (3 x 10 mL), 1% HCl (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (Et_2O) yielded the title compound (582 g, 1.22 mmol, 47%) as a white solid. MP = 136-139 °C; IR (solid): 3292 (br), 2966 (w), 2937 (w), 2875 (w), 1733 (m), 1690 (m), 1641 (s), 1523 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.35-7.28 (5 H, m, Ph), 6.97 (1 H, br, N*H*CH_2), 6.78 (1 H, d, *J* = 6 Hz, CHC(O)N*H*CH), 5.31 (1 H, d, *J* = 7 Hz, BocN*H*), 5.11 (2 H, s, OCH_2), 4.26 (1 H, apparent t, *J* = 8 Hz, C*H*C(O)NHCH$_2$CH$_2$), 3.93 (1 H, apparent t, *J* = 7 Hz, BocNHC*H*), 3.63-3.55 (1 H, m, NHCH_aH$_b$), 3.44 (1 H, m, NHCH$_a$$H_b$), 2.64-2.51 (2 H, m, NHCH$_2$CH_2), 2.14-2.04 (2 H, m, C*H*$(CH_3)_2$), 1.41 (9 H, s, $(CH_3)_3$), 0.92 (3 H, d, *J* = 6 Hz, CHCH_3), 0.91 (3 H, d, *J* = 6 Hz, CHCH_3), 0.88 (3 H, d, *J* = 6 Hz, CHCH_3), 0.87 (3 H, d, *J* = 6 Hz, CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 172.1 (C), 172.0 (C), 171.2 (C), 156.2 (C), 135.7 (C), 128.7 (CH), 128.5 (CH), 128.4 (CH), 80.0 (C), 66.7 (CH_2), 60.5 (CH), 58.6 (CH), 35.1 (CH_2), 34.1 (CH_2), 30.9 (CH), 30.7 (CH), 28.4 (CH_3), 19.4 (CH_3), 19.3 (CH_3), 18.1 (2 x CH_3); ESMS: m/z (%): 500 ($(M+Na)^+$, 100); HRMS (ES) for $C_{25}H_{39}N_3O_6Na$ $(M+Na)^+$: calcd 500.2731, found 500.2728.

Benzyl 3-[((2S)-2-[(2S)-2-(hexanoylamino)-3-methylbutanoyl]amino-3-methyl butanoyl)amino]propanoate 232

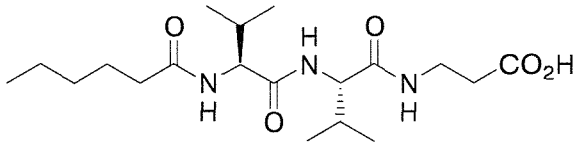


232

Benzyl 3-[(2*S*)-2-((2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methylbutanoylamino)-3-methyl butanoyl]aminopropanoate (540 mg, 1.13 mmol) was stirred in 20% TFA/CH_2Cl_2 (10 mL) for 1.5 h, toluene (5 mL) was added and the solvent removed *in vacuo* to yield a waxy solid which was stirred with hexanoic acid (155 μL, 1.24 mmol) and HOBt (167 mg, 1.24 mmol) in DMF (2.5 mL) and THF (5 mL). EDC (237 mg, 1.24 mmol) was added followed by DIPEA (394 μL, 2.26 mmol) and the

reaction stirred for 3 h. The solvents were removed *in vacuo*, the solid partitioned between EtOAc (30 mL) and sat. $NaHCO_3$ (10 mL) and the organic washed with sat. $NaHCO_3$ (2 x 10 mL), 1% HCl (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by column chromatography (50% EtOAc/petrol – EtOAc) and recrystallization (CH_3OH/H_2O) yielded the title compound **232** (242 g, 0.51 mmol, 45%) as a white solid. MP = 214-218 °C; IR (solid): 3280 (m), 3071 (w), 2962 (w), 2934 (w), 2873 (w), 1737 (m), 1632 (s), 1543 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 7.36-7.27 (6 H, m, Ph and $CH_2CH_2C(O)NH$), 7.21 (1 H, br, $NHCH_2CH_2$), 6.79 (1 H, d, J = 6 Hz, $CHC(O)NHCH$), 5.11 (2 H, s, OCH_2), 4.41 (1 H, apparent t, J = 7 Hz, $CHC(O)NHCH_2CH_2$), 4.21 (1 H, apparent t, J = 7 Hz, $CH_2CH_2C(O)NHCH$), 3.54 (1 H, m, $NHCH_aH_b$), 3.37 (1 H, m, $NHCH_aH_b$), 2.56-2.45 (2 H, m, $NHCH_2CH_2$), 2.29-2.12 (2 H, m, $CH_2CH_2CH_2C(O)$), 2.05-193 (2 H, m, $CH(CH_3)_2$), 1.61-1.48 (2 H, m, $CH_2CH_2CH_2C(O)$), 1.28-1.10 (4 H, m, $(CH_2)_2CH_3$), 0.92 (15 H, m, $CH(CH_3)_2$ and CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 173.8 (C), 172.3 (C), 172.2 (C), 171.6 (C), 136.0 (C), 129.0 (CH), 128.8 (CH), 128.6 (CH), 66.9 (CH_2), 59.0 (CH), 58.7 (CH), 36.9 (CH_2), 35.4 (CH_2), 34.4 (CH_2), 31.8 (CH_2), 31.8 (CH), 31.3 (CH), 25.9 (CH_2), 22.8 (CH_2), 19.5 (CH_3), 19.5 (CH_3), 19.0 (CH_3), 18.7 (CH_3), 14.3 (CH_3); ESMS: m/z (%): 498 ($(M+Na)^+$, 100); HRMS (ES) for $C_{26}H_{41}N_3O_5Na$ $(M+Na)^+$: calcd 498.2938, found 498.2935.

3-[((2S)-2-[(2S)-2-(Hexanoylamino)-3-methylbutanoyl]amino-3-methylbutanoyl) amino]propanoic acid 234



234

Benzyl 3-[((2*S*)-2-[(2*S*)-2-(hexanoylamino)-3-methylbutanoyl]amino-3-methyl butanoyl)amino]propanoate **232** (80 mg, 0.17 mmol) was hydrogenated at 1 atm over 10% Pd/C (cat.) in CH_3OH (5 mL) for 2 h. The solid was filtered off, washed with CH_3OH (5 mL) and the solvent was removed *in vacuo*. Purification by column chromatography (0.01% $CHCO_2H$/9.99% CH_3OH/CH_2Cl_2) yielded the title compound **234** (52 mg, 0.13 mmol, 79%) as a white solid. MP = 220 °C (dec); IR (solid): 3276 (w), 3094 (w), 2963 (w), 2935 (w), 2874 (w), 1711 (w), 1627 (s), 1544 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3OD): 4.19 (1 H, d, J = 8 Hz, α-CH), 4.12 (1 H, d,

J = 8 Hz, α-CH), 3.48 (1 H, m, NHCH_aH$_b$CH$_2$), 3.38 (1 H, m, NHCH$_a$$H_bCH_2$), 2.53-2.48 (2 H, m, NHCH$_2$CH_2), 2.28-2.23 (2 H, m, (CH$_2$)$_2$CH_2C(O)), 2.09-1.98 (2 H, m, CH(CH$_3$)$_2$), 1.61 (2 H, apparent quin, J = 8, 8 Hz, CH$_2$CH_2CH$_2$C(O)), 1.37-1.28 (4 H, m, (CH_2)$_2$CH$_3$), 0.96-0.91 (12 H, m, CH(CH_3)$_2$), 0.90 (3 H, t, J = 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, CD$_3$OD): 176.4 (C), 175.1 (C), 173.8 (C), 173.4 (C), 60.3 (CH), 60.1 (CH), 36.8 (CH$_2$), 36.3 (CH$_2$), 34.6 (CH$_2$), 32.5 (CH$_2$), 32.0 (CH), 31.6 (CH), 26.7 (CH$_2$), 23.4 (CH$_2$), 19.8 (CH$_3$), 19.7 (CH$_3$), 18.9 (CH$_3$), 18.8 (CH$_3$), 14.3 (CH$_3$); ESMS: m/z (%): 498 ((M+TFA-H)$^-$, 100); HRMS (ES) for C$_{19}$H$_{35}$N$_3$O$_5$Na (M+Na)$^+$: calcd 408.2469, found 408.2462.

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