Side chain selective binding of *N*-acetyl-α-amino acid carboxylates by a 2-(guanidiniocarbonyl)pyrrole receptor in aqueous solvents

Carsten Schmuck

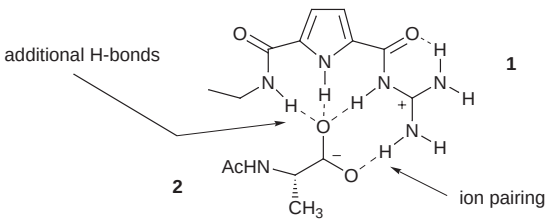
Institute of Organic Chemistry, University of Cologne, Greinstrasse 4, 50939 Cologne, Germany. E-mail: carsten.schmuck@uni-koeln.de

Received (in Liverpool, UK) 9th February 1999, Accepted 23rd March 1999

2-(Guanidiniocarbonyl)pyrrole 1 binds *N*-acetyl-α-amino acid carboxylates in 40% H_2O–DMSO with binding constants ranging from K = 360 to 1700 mol^{-1} depending on the structure of the amino acid side chain.

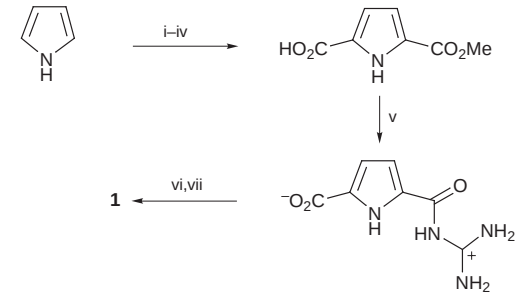
The development of artificial receptors for the selective molecular recognition of a given substrate is still a challenging task, especially in polar solvents.[1] A large number of host systems for hydrophobic solvents (mostly $CHCl_3$) have been described.[2] However, for the design of biosensors or the targeting of cellular molecules, like peptide hormones, neurotransmitters or carbohydrates, $CHCl_3$ is not the solvent of choice. There are only very few examples of artificial receptors that function in more 'natural' solvents like DMSO, MeOH or even water.[3] As the polarity of the surrounding solvent increases, the strength of hydrogen bonds and electrostatic interactions, mainly used for molecular recognition, decreases rapidly, due to the competitive solvation of donor and acceptor sites by the solvent.

Herein we describe a new class of receptor molecules for the binding of carboxylates in aqueous media. Our idea was to improve the binding affinity of guanidinium cations, well known for the complexation of oxo anions in organic solvents such as $CHCl_3$ or MeCN,[4] by adding additional binding sites. To serve this purpose, we chose substituted 2-(guanidiniocarbonyl)-1*H*-pyrroles. The pyrrole NH as well as suitable donor sites in the side chain should be able to hydrogen bond to the bound carboxylate in addition to ion pairing with the guanidinium unit. For the selective binding of *N*-acetyl-α-amino acid or peptide carboxylates[3,5] these primary interactions to the backbone can



provide the necessary binding energy even in polar solvents. Additonal interactions of the amino acid side chain with the receptor could then be used to achieve selectivity of the recognition process.[1b,6] By variation of this general theme (*e.g.* by combinatorial methods), a new class of receptor molecules should be accessible whose binding properties and selectivity can be tuned deliberately. We wish to demonstrate the usefulness of this general design by reporting the binding properties of a first example: the [5-(*N*-ethylcarbamoyl)-1*H*-pyrrol-2-ylcarbonyl]guanidinium cation **1**. The synthesis of **1** is shown in Scheme 1.

As anticipated, addition of **1** to a solution of *N*-acetyl-L-alanine carboxylate **2** in DMSO caused significant complexation induced shifts of the various protons of **2** in the ^1H NMR spectrum. Actually, at millimolar concentrations, the binding is so strong that a NMR titration in [$^2H_6$]DMSO just showed a linear increase of the shift changes until a molar ratio of 1 : 1 was



Scheme 1 *Reagents and conditions*: i, Cl_3CCOCl, Et_2O, reflux, 30 min, 85%; ii, NaOMe (0.1 equiv.), MeOH, room temp. 30 min, 63%; iii, $POCl_3$, DMF, CH_2Cl_2, 0 °C → reflux, 63%; iv, $KMnO_4$, acetone–H_2O (1 : 1), 40 °C, 30 min, 75%; v, guanidinium chloride (5 equiv.), NaOMe (5 equiv.), MeOH, reflux, 12 h, 72%; vi, $(COCl)_2$ (1.1 equiv.), DMF (cat.), CH_2Cl_2, reflux 2 h; vii, $EtNH_3Cl$, Et_3N, 68% over both steps.

reached, clearly proving the 1 : 1 binding stoichiometry.[7] Therefore NMR binding studies with various *N*-acetyl-α-amino acid carboxylates were performed in 40% water in DMSO (at higher concentrations of water the solubility of the receptor was too limited). The binding constants were calculated from the observed shift changes of the amide NH of the carboxylates (or of the α-CH in the case of acetate) using nonlinear least-squares fitting with a 1 : 1 association model (Fig. 1).[8]

As shown in Table 1, receptor **1** strongly binds carboxylates with binding constants up to $K \approx 2800$ mol^{-1}. These association constants are much larger than with the parent *N*-acetyl guanidinium cation, which, for example, binds **2** with K = 50 mol^{-1} (compared to K = 770 mol^{-1} for the binding of **2** by **1**). Obviously, as hoped for, the binding affinity of guanidinium cations for carboxylates can be significantly improved by additional hydrogen bonding donors in the receptor. Furthermore, the recognition process is selective for the amino acid side chain: phenylalanine is bound much stronger than alanine or lysine.

According to molecular modelling calculations† the general binding scheme for all carboxylates is the same: the guanidin-

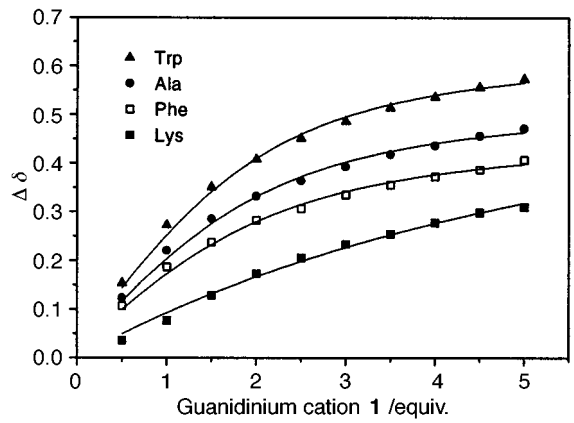


Fig. 1 NMR titration curves of **1** with various carboxylates in 40% water–[$^2H_6$]DMSO.

Table 1 Binding constants of **1** with various carboxylates[a]

Carboxylate	Solvent	K^b/mol^{-1}	$-\Delta G$/kJ mol^{-1}
Ac-L-Ala	DMSO	$>10^6$	
Ac-L-Ala	H_2O–DMSO	770	16.5
Ac-L-Phe	H_2O–DMSO	1700	18.4
Ac-L-Trp	H_2O–DMSO	810	16.6
Ac-L-Lys	H_2O–DMSO	360	14.6
Acetate	H_2O–DMSO	2790	19.7

[a] Measured by NMR titration, each one with 10 measurements at 25 °C; [carboxylate] = 1 mM in [$^2H_6$]DMSO or 40% H_2O–[$^2H_6$]DMSO. [b] Error limit in K < ± 5%.

ium cation forms an ion pair with the carboxylate which is simultaneously hydrogen bonded by both the pyrrole and the amide NH.[9] Acetate shows the highest binding constant with **1** because there are no unfavorable steric interactions with the receptor. The carboxylate group and the receptor binding sites are completely coplanar allowing maximum interaction. In the case of the amino acid carboxylates, the steric bulk of the *N*-acetyl group forces the carboxylate out of the plane of the receptor, thereby decreasing the binding affinity. The differences in complex stability among the various amino acids result from secondary interactions of the side chains with the receptor. The methyl group of alanine points away from the receptor molecule so that there are neither any stabilizing nor destabilizing interactions. In the case of phenylalanine the aromatic ring π-stacks with the acylguanidinium unit of **1** (Fig. 2). This cation–π interaction further stabilizes the complex.[10] Hence, the association constant for the binding of phenylalanine is more than two times larger than for the binding of alanine.

The indole ring of tryptophan is probably too large to effectively π-stack with the acylguanidinium unit. The positively charged ammonium group in lysine decreases the binding affinity relative to alanine due to unfavorable electrostatic interactions with the positively charged guanidinium group.

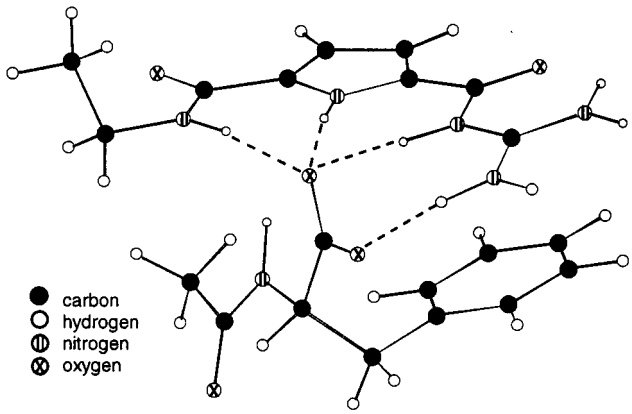


Fig. 2 Structure of **1** with *N*-acetylphenylalanine in water derived from molecular modelling. Intermolecular hydrogen bonds are shown as broken lines.

It is noteworthy that, even in 40% water–DMSO, this simple receptor **1** already shows a level of amino acid selectivity which is in the same range as described for other much more complex receptors in $CHCl_3$.[1b,2a,6] We hope that even greater selectivity including enantioselectivity can be achieved by variation of the substituent at the pyrrole ring.

Financial support for this work by the Fonds der Chemischen Industrie is gratefully acknowledged. The author thanks Professor Albrecht Berkessel (Cologne) for his generous support and helpful discussions.

Notes and references:

† Monte Carlo conformational searches with energy optimization using the Amber* force field and water GB/SA solvation as implemented in Macromodel ver. 6.0 (ref. 11).

1 (*a*) J.-M. Lehn, *Supramolecular Chemistry*; *Concepts and Perspectives*, VCH, Weinheim 1995; H. Dugas, *Bioorganic Chemistry, A Chemical Approach to Enzyme Action*, Springer Verlag, New York, 1996; see also (*b*) W. C. Still, *Acc. Chem. Res.*, 1996, **29**, 155.
2 (*a*) T. H. Webb and C. S. Wilcox, *Chem. Soc. Rev.*, 1993, 383; see also (*b*) *Chem. Rev.*, 1997, **97**, vol 5, special issue 'Molecular Recognition'.
3 For examples of peptide recognition in aqueous media see: A. Hossain, and H.-J. Schneider, *J. Am. Chem. Soc.*, 1998, **120**, 11 208; R. Breslow, Z. Yang, R. Ching, G. Trojandt and F. Odobel, *J. Am. Chem. Soc.* 1998, **120**, 3536; M. Davies, M. Bonnant, F. Guillier, J. D. Kilburn and M. Bradley, *J. Org. Chem.*, 1998, **63**, 8696; M. W. Peczuh, A. D. Hamilton, J. Sanchez-Qesada, J. deMendoza, T. Haak and E. Giralt, *J. Am. Chem. Soc.*, 1997, **119**, 9327.
4 Recent review articles: F. P. Schmidtchen, M. Berger, *Chem. Rev.*, 1997, **97**, 1609; A. Bianchi, K. Bowman-James and E. García-España, *Supramolecular Chemistry of Anions*, Wiley-VCH, New York, 1997; A. Galán and J. deMendoza, *Top. Curr. Chem.* 1995, **175**.
5 For a leading reference on general amino acid recognition, see: J. L. Sessler and A. Andrievsky, *Chem. Eur. J.*, 1998, **4**, 159 and references cited therein.
6 Side chain selective amino acid recognition in $CDCl_3$: J. Dowden, P. D. Edwards and J. D. Kilburn, *Tetrahedron Lett.*, 1997, **38**, 1095; C. P. Waymark, J. D. Kilburn and I. Gillies, *Tetrahedron Lett.*, 1995, **36**, 3051; S. S. Flack and J. D. Kilburn, *Tetrahedron Lett.*, 1995, **36**, 3409.
7 K. A. Connors, *Binding Constants*, Wiley, New York, 1987.
8 C. S. Wilcox, in *Frontiers in Supramolecular Chemistry and Photochemistry*, ed. H. J. Schneider and H. Dürr), VCH, Weinheim, 1990.
9 In accordance with this scheme, in the ^{1}H NMR spectrum one observes downfield shifts of all those receptor NHs, which participate in the proposed binding (up to 4 ppm in [$^2H_6$]DMSO). According to molecular dynamics calculations, the receptor conformation necessary for this type of binding is only 1 kJ mol^{-1} less stable than the energy minimum conformation in water (compared to 14 kJ mol^{-1} in $CHCl_3$).
10 T. H. Schrader, *Tetrahedron Lett.*, 1998, **39**, 517; D. A. Dougherty, *Science*, 1996, **271**, 163 and references cited therein.
11 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufiled, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.

Communication 9/01126I