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Conformational changes of functionalised indole receptors upon their interaction with anions

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The conformational analysis of four C2-amido and C7-ureido functionalised indole anion receptors was performed by a combination of heteronuclear NMR spectroscopy and *ab initio* quantum mechanical calculations. NOE experiments showed that *anti–anti* conformation across C2—C2α and C7—N7α bonds is predominant in acetone solution in the absence of anions. Upon anion binding to receptors, *syn–syn* conformation becomes predominant. The conformational changes upon anion binding are in good agreement with energetic preferences established by *ab initio* calculations. Chemical shift changes induced by interaction of anions suggest that binding of chloride and bromide anions occurs primarily to H1 and H7α protons. Nitrate anions favour interaction with H7α and H7γ ureido protons, whereas acetate anions interact strongly with all four available hydrogen bond donor groups.

Keywords: anion recognition; conformation analysis; host–guest systems; NMR spectroscopy

Introduction

The progress in the research of anion sensing and transport has been truly intense over the last two decades (*1*). Important developments in the area of supramolecular chemistry are stimulated by a wide range of anionic species that are involved in numerous biological processes. Anions are crucial in protein synthesis; they act as carriers of genetic information in the form of nucleic acids and serve as enzyme substrates or co-factors in enzymatic reactions. For illustration, chloride has an important physiological role in transport activity of anion-exchange proteins (*2*). Deficiency of bromide may influence ability of human eosinophils to produce antiparasitic compounds by the action of eosinophil peroxidase, an enzyme which preferentially uses bromide as a substrate to produce a brominating agent (*3*). Alternatively, anions can be found in noxious processes where excess nitrate or phosphate ions in aqueous systems can lead to algae blooms and even eutrophication (*4*). Biological functions have stimulated organic chemists to design, synthesise and examine the great variety of anionic hosts containing amides and thioamides, ureas and thioureas, pyrroles and indoles as well as charged ammonium, guanidinium and imidazolium moieties over the last few years (*5*). The stability of anion–receptor complexes is related to acidity of receptors' functionalities and to basicity of anions (*6*).

In the current study, we have focused on the indole scaffold-based receptors, which were shown to demonstrate good affinities and selectivities for anions (*7*). Study of the anion-binding properties of indole derivatives is stimulated by the fact that it is utilised by Nature in the sulphate-binding protein as hydrogen bond donor (*8*) as well as in the enzymatic active site of haloalkane dehalogenase where a chloride anion can be complexed (*9*). Indole-based anion-binding agents undergo interactions with anions through hydrogen bonding. Anion recognition and sensing can be effectively tuned by the choice of functional groups containing hydrogen bond donors. Besides acidic H1 proton, indole systems studied here bear C2-amido as well as C7-ureido functionalities, which may act as additional hydrogen bond donors. Their ability to participate in hydrogen bonds is further tuned by attaching alkyl chain or aromatic ring substituents (Figure 1). Furthermore, the substituents at positions C2 and C7 in receptors **1**–**4** allow conformational flexibility which may prove beneficial in the binding of anions. The syntheses of **1** and **4** were reported earlier together with X-ray crystal structure and anion-binding constants (*10*, *11*).

We have recently analysed conformational aspects of a few 2,7-bisphenyl-substituted indole receptors and established conformational changes upon anion binding (*12*). In the current work, the potential conformational preorganisation and conformational changes of indole anion receptors **1**–**4** upon interactions with two spherical halides and two oxoanions were studied by means of NMR spectroscopy. Ensemble averaged NMR parameters were confronted with energetic preferences established by *ab initio* calculations. We were stimulated by the results of

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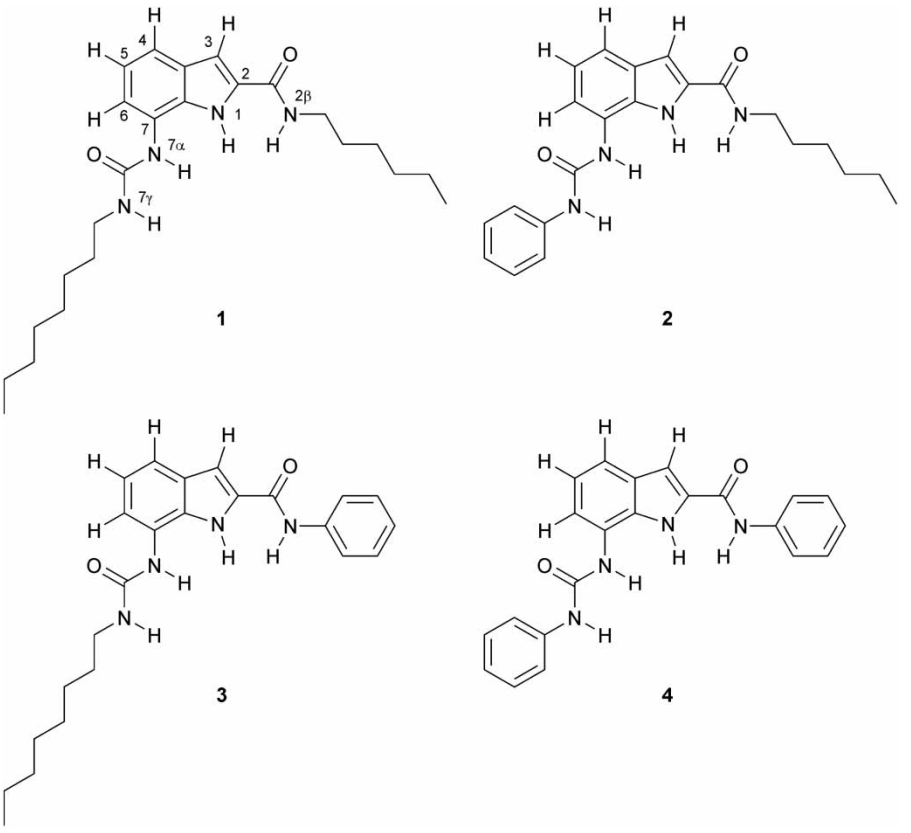

Figure 1. Anion receptors **1**–**4** and their atom numbering.

our recent study on receptor **1** which revealed different conformational preferences in the absence and in the presence of anions (*10*). The conformational properties of **1**–**4** and their complexes with anions were herein correlated with the electronic properties of C2 and C7 indole substituents with the goal to obtain new insights and thus provide fresh incentives in the design of anion-specific receptors.

Results and discussion

NMR signal assignment of receptors 1–4

The unambiguous assignment of ^{1}H, ^{13}C and ^{15}N NMR chemical shifts was achieved through assessment of their respective multiplicity and integrals in 1D spectra as well as through bond connectivities in homo- and hetero-nuclear 2D spectra. Selected ^{1}H and ^{15}N NMR chemical shifts of anion hosts **1**–**4** are given in Table 1. The complete ^{1}H and ^{13}C NMR chemical shifts are reported in the Supplementary Material, available online.

Four NH protons of **1** were well resolved over the wide chemical shift range between 6.0 and 11.2 ppm (Table 1). ^{1}H NMR spectrum of **2** revealed essentially the same characteristics as spectrum of **1**, except for the great downfield shift of H7γ ($\Delta\delta$ 2.26 ppm) and smaller for H6 protons ($\Delta\delta$ 0.10 ppm) caused by the substitution of alkyl chain by aromatic moiety attached to the ureido group at C7. Additionally, the considerable downfield shift of N7γ nitrogen atom ($\Delta\delta$ 20.3 ppm) in **2** with respect to **1** was in agreement with the electronic properties of aromatic vs. alkyl side chain substituent (Table 1). The major chemical shift differences between ^{1}H NMR chemical shifts of **1** and

Table 1. Selected ^{1}H and ^{15}N NMR chemical shifts for **1**–**4** (in ppm).

	H1	H2β	H7α	H7γ	H3	H6	N1	N2β	N7α	N7γ
1	11.16	7.78	8.32	6.01	7.03	7.16	137.8	113.0	104.3	90.1
2	10.72	7.85	8.44	8.27	7.08	7.26	135.7	113.0	106.0	110.4
3	11.42	9.64	8.35	6.06	7.29	7.16	138.1	128.3	104.4	90.2
4	11.07	9.61	8.59	8.35	7.33	7.39	135.4	128.5	106.8	112.0

Note: In acetone-d_6 at 298 K.

3 were the downfield shifts of H2β (Δδ 1.86 ppm) and H3 protons (Δδ 0.26 ppm) in the latter. N2β nitrogen is deshielded by Δδ of 15.3 ppm in **3** with respect to **1**. Substitution of both alkyl chains at C2 and C7 substituents in **1** by aromatic moieties in **4** resulted in downfield chemical shift changes of N2β (Δδ 15.5 ppm) and N7γ (Δδ 21.9 ppm) nitrogen atoms as well as in the corresponding protons (Table 1).

Chemical shift changes of 1–4 induced by interaction with anions

NMR chemical shift changes were used to localise interactions of chloride, bromide, nitrate and acetate anions with receptors **1**–**4** (Figures S1–S4, available online). The largest deshielding upon addition of chloride anions of 1.3 to 1.8 ppm was observed for H1 and H7α protons (Figure 2(a)). In this context, downfield chemical shifts reflect formation of hydrogen bond between receptor and anion. Minute changes in chemical shifts of H2β resonances in **1**–**4** suggest absence of interaction between the C2-amide group and chloride. Furthermore, variation of aliphatic and aromatic nature of N2β substituent does not influence interactions of H2β with chloride. H7γ chemical shift changes in **2** and **4** were up to 0.5 ppm larger with respect to Δδ values in **1** and **3** (Figure 2(a)). These observations suggest that electron-withdrawing effect of phenyl ring promotes interaction of H7γ proton with chloride anions. Considerable downfield shifts of H6 protons were observed upon addition of chloride, even though they were most likely not directly involved in chloride–receptor interactions. Observed Δδ values of H6 in **1**–**4** suggest apparent changes of their shielding environment due to rotation of vicinal ureido moiety. In addition, hydrogen bond formation between chloride anion and H7α generates polarisation of the aromatic C6—H6 bond via a through-space effect which is manifested through the deshielding of H6 resonance.

Bromide anion is related to chloride by shape but it exhibits different properties as a Lewis base. Perusal of Figure 2(b) shows major chemical shift changes for H1 and H7α protons in **1**–**4** in the presence of bromide anions. Larger downfield shifts of H7γ were observed in bromide complexes with **2** and **4** with respect to **1** and **3**, which can be attributed to substitution of octyl chain with electron-

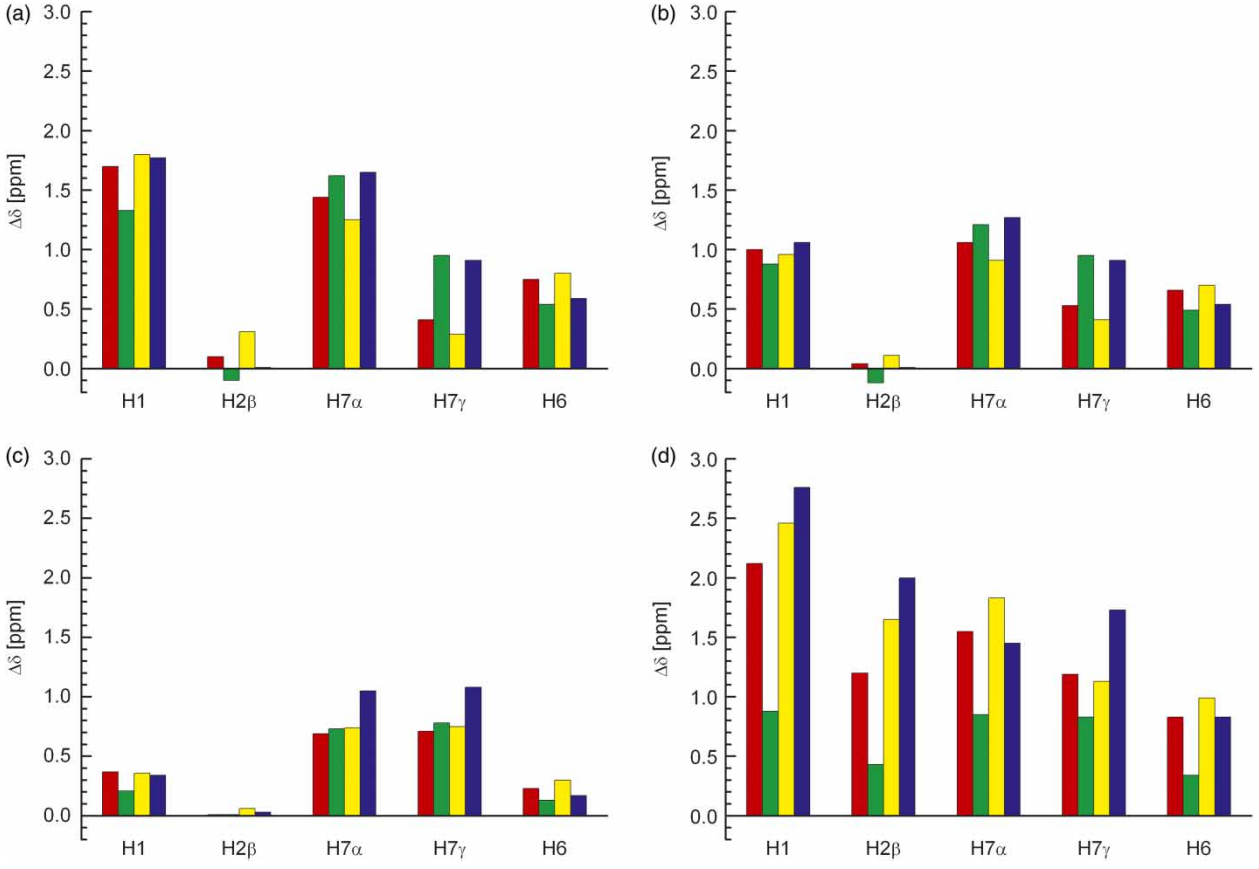

Figure 2. ^{1}H NMR chemical shift changes for receptors **1** (leftmost column), **2** (next-to-leftmost c.), **3** (next-to-rightmost c.) and **4** (rightmost c.) induced by addition of one equivalent of chloride (a), bromide (b), nitrate (c) and acetate anions (d); $\Delta\delta = \delta$(in the presence of anions) – δ(in the absence of anions).

withdrawing phenyl ring at N7γ. In comparison to chloride anions, smaller chemical shift changes were observed upon addition of bromide to **1**–**4** for H1 and H7α, which is in accordance with the lower basicity of bromide. Δδ values of H7γ in the presence of two halide anions were comparable (c.f. Figure 2(a) and (b)).

Chemical shift changes upon addition of nitrate were much smaller in comparison to halides (Figure 2(c)). The greatest Δδ values were observed for H7α and H7γ ureido protons in **1**–**4**, whereas H1 and H2β showed only minor downfield shifts. These results suggest that the key interactions between receptors **1**–**4** and nitrate occur at ureido NH protons. The largest Δδ values were observed for bisphenyl derivative **4** which indicated that electron-withdrawing groups attached to N2β and N7γ led to the strengthening of interactions between **4** and nitrate anions.

Addition of acetate to **1** resulted in large downfield shifts of all four NH protons (Figure 2(d)). Substantially smaller Δδ values for receptor **2** indicate unfavourable impact of phenyl ring at N7γ for the binding of acetate. Interaction between receptor and *Y*-shaped acetate is boosted in both N2β-phenyl-substituted receptors **3** and **4**. Appreciable differences in Δδ values between bisalkyl-substituted receptor **1** and bisphenyl-substituted receptor **4** are in excellent agreement with higher affinity of the latter receptor for acetate (stability constant for complex of acetate anions with **4** in DMSO/0.5% water was determined to be five times higher than with **1** in $CDCl_3$) (*10*, *11*). The size of anion and its (de)hydration energy are two important properties that affect selectivity and energetics of anion-binding receptors. Large anions will preferentially associate with large, hydrophobic cations. Smaller chloride has been shown to interact more strongly with receptors **1**–**4** than larger bromide. Trigonal planar nitrate and acetate can bind to receptors by two oxygen atoms. Addition of nitrate to **1**–**4** was shown to induce only medium-sized chemical shift changes, which are in addition limited to H7α and H7γ ureido protons. Our NMR data are in complete agreement with the lower basicity and weaker polarisation of nitrate. On the other hand, higher basicity and stronger polarisation of acetate promote its interaction with donor groups of receptors **1**–**4**. Overall comparison of ^{1}H chemical shift changes upon addition of one equivalent of anions showed that strength of anion–receptor interactions can be sorted in the following order: $NO_3^- < Br^- < Cl^- < CH_3CO_2^-$. This trend generally ensues predictions on the basis of (de)hydration energy (*1a*) which is in agreement with the binding constants of **1** with nitrate, bromide and chloride anions (*10*) as well as between **4** and chloride and acetate anions (*11*), albeit they were determined in $CDCl_3$ and DMSO/0.5% water, respectively.

Anion–receptor interactions based on proton chemical shift changes are supplemented by ^{15}N NMR data (Figure 3).

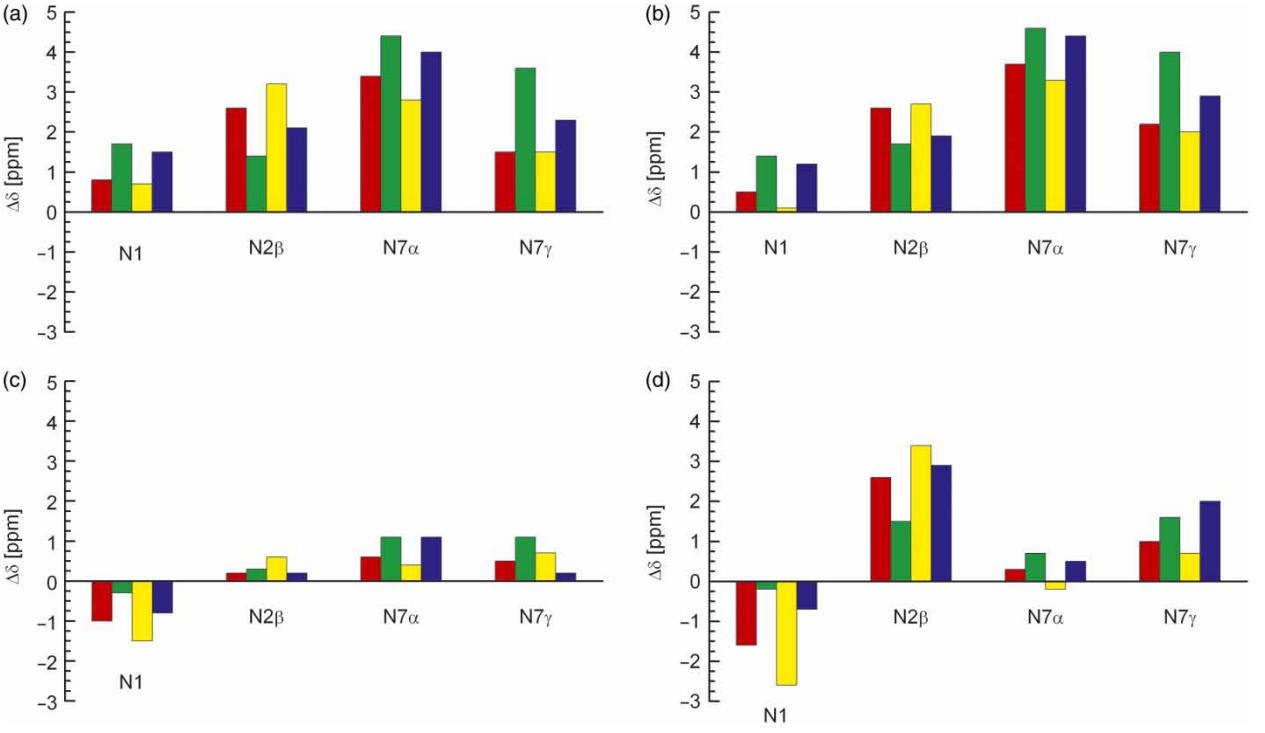

Figure 3. ^{15}N NMR chemical shift changes of receptors **1** (leftmost column), **2** (next-to-leftmost c.), **3** (next-to-rightmost c.) and **4** (rightmost c.) induced by addition of one equivalent of chloride (a), bromide (b), nitrate (c) and acetate anions (d); $\Delta\delta = \delta$(in the presence of anions) – δ(in the absence of anions).

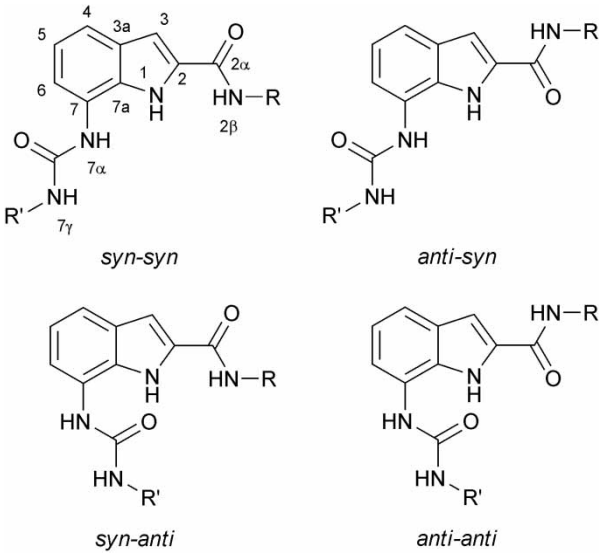

Figure 4. Major families of potential canonical conformers of the C2 and C7-substituted indole receptors. The first notation refers to the orientation across C2—C2α bond and the second across C7—N7α bond. R and R' stand for alkyl chain and/or phenyl ring in **1**–**4** as shown in Figure 1.

Interestingly, downfield shifts were observed not only for N1 and N7α but also for N2β and N7γ upon addition of chloride and bromide anions to **1**–**4** (Figure 3(a)–(b)). Upfield shifts of N1 were observed upon addition of *Y*-shaped nitrate and acetate anions to **1**–**4** (Figure 3(c)–(d)), which suggests that two competitive events must be involved that produce shielding and deshielding effects. Relatively minor nitrogen chemical shift changes upon addition of nitrate are in accordance with the observed proton chemical shift changes.

Conformational properties of 1–4 and their complexes with anions

2-Amido and 7-ureido substituents can adopt several conformations with respect to indole ring in receptors **1**–**4**. Among plethora of rotamers, four major energetically favoured conformations are likely to be preferred (Figure 4). The indole H1 proton with its central position in the receptor is enclosed by amide or ureido NH groups. The *syn–syn* orientation, where NH hydrogen bond donor groups are close together, appears to be predisposed for the most effective binding of anions. In contrast, it is estimated to be disfavoured in the absence of anions due to repulsion of hydrogen bond donor groups. The other three rotamers can be stabilised through NH—CO hydrogen bonds, which makes conformational studies even more interesting due to possible competitive effects. Conformational properties of anion receptors **1**–**4** were studied in acetone solution where intermolecular interactions were negligible as verified by the dilution experiments (Figure S5, available online). The potential π-stacking interactions or intermolecular association that could be reflected in chemical shift changes and NOE enhancements were greatly reduced and thus not observed in acetone.

Conformational properties of indole-based receptors in the absence and in the presence of anions were studied

Table 2. The key NOE enhancements observed for anion receptors **1**–**4** in the absence and in the presence of one equivalent of anions.

Saturated:		H1		H2β		H3	H6	H7α	
Enhanced:		H2β	H7α	H1	H3	H2β	H7α	H1	H6
No anion	**1**	0.8	2.6	1.0	13.6	3.4	1.6	4.3	9.1
	2	0.0	1.6	0.0	14.2	2.4	– [a]	2.3	7.1
	3	0.0	1.0	0.0	13.5	– [a]	– [a]	0.7	8.1
	4	0.0	4.0	0.0	14.2	– [a]	– [a]	1.9	7.8
Chloride	**1**	7.8	8.5	6.0	4.8	3.5	0.3	11.8	2.0
	2	3.5	4.8	– [a]	– [a]	– [a]	– [a]	5.3	4.2
	3	8.4	8.4	8.8	4.0	– [a]	– [a]	9.4	1.7
	4	11.2	11.6	12.4	6.0	– [a]	– [a]	12.5	3.1
Bromide	**1**	5.8	6.2	– [a]	– [a]	– [a]	– [a]	7.0	3.4
	2	4.2	5.6	– [a]	– [a]	– [a]	– [a]	7.4	4.3
	3	6.2	6.2	6.3	6.5	– [a]	0.4	6.7	3.8
	4	6.9	7.0	5.9	6.7	– [a]	0.7	8.5	3.3
Nitrate	**1**	2.4	3.1	2.9	11.5	2.2	1.5	4.4	9.4
	2	1.5	3.3	1.9	14.3	4.4	2.3	5.0	8.9
	3	3.4	3.4	2.9	12.6	– [a]	1.4	3.8	9.1
	4	– [a]	– [a]	– [a]	7.2	– [a]	1.4	– [a]	4.2
Acetate	**1**	8.5	4.8	5.4	3.6	0.2	0.2	3.8	2.2
	2	4.8	2.8	2.6	13.5	3.5	– [a]	– [a]	– [a]
	3	8.7	7.4	5.9	0.7	– [a]	– [a]	3.8	1.1
	4	8.0	5.8	5.4	2.0	– [a]	0.0	3.7	1.1

[a] ^{1}H signals were overlapped and pairwise NOE enhancements could not be quantified unequivocally.

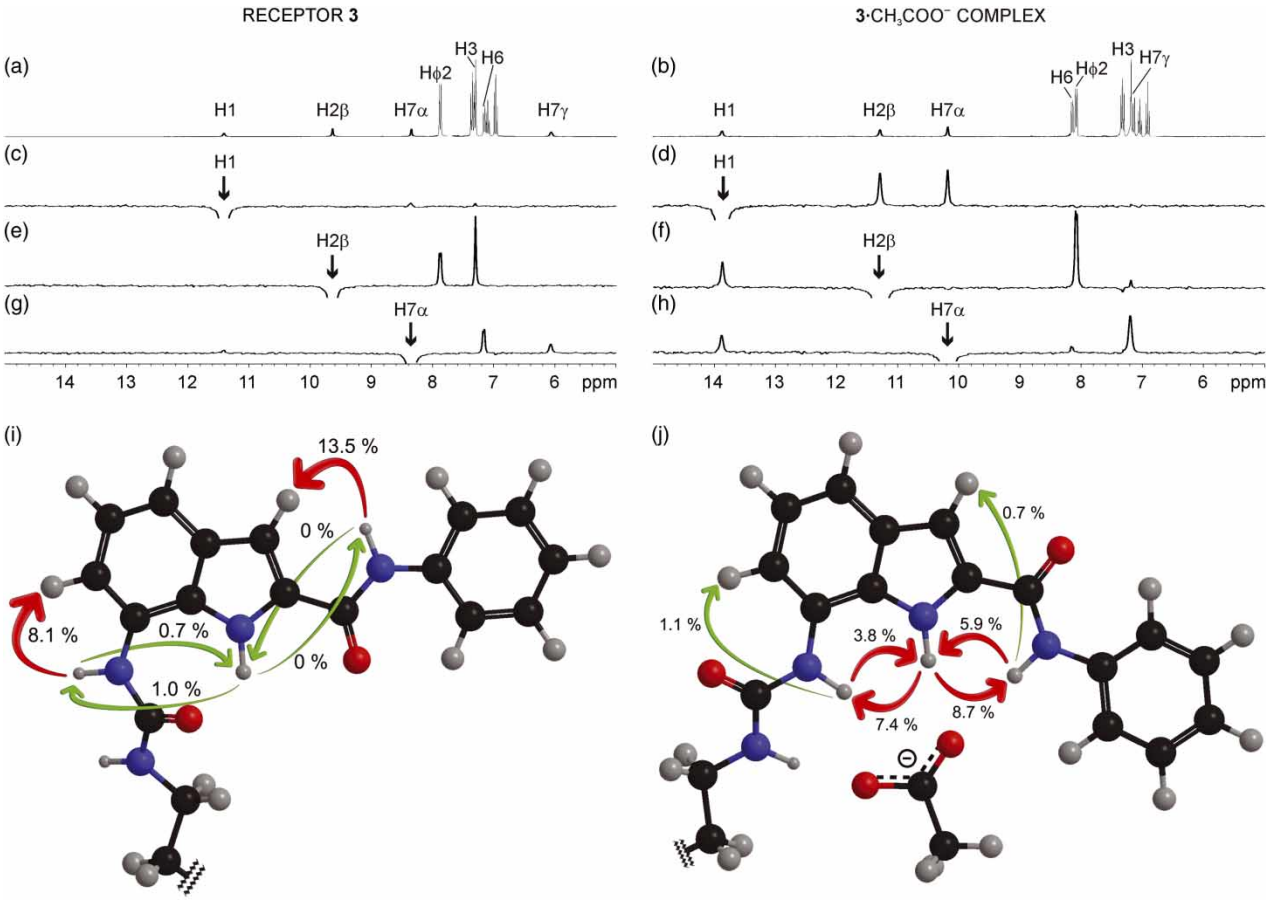

Figure 5. ^{1}H NMR spectra of **3** in the absence (a), upon addition of one equivalent of acetate anions (b), corresponding 1D difference NOE spectra upon saturation of H1 (c, d), H2β (e, f) and H7α protons (g, h). All spectra were acquired at 298 K. The predominant *anti–anti* conformation of **3** (i) and *syn–syn* conformation of **3** in the presence of acetate (j) as determined by NOEs, where arrows and numbers indicate NOE enhancements. Octyl chain attached to N7γ was truncated for simplicity.

with the use of 1D difference NOE experiments. As an example, NOE spectra for receptor **3** are shown in Figure 5 (left column). Well-resolved NH protons made it possible to quantify NOE enhancements. The orientation along C2—C2α bond was established through comparative analysis of NOEs between H2β and H1 vs. H3. The NOEs between H7α and H1 vs. H6 were used to determine the predominant conformation along C7—N7α bond in **1–4**. The saturation of H2β in **3** resulted in strong NOE at H3 (13.5%) and concurrent absence of NOE at H1 (Figure 5(e)). The saturation of H7 in **3** gave strong NOE of 8.1% at H6 and in accord weak NOE at H1 (0.7%, Figure 5(g)). The observed NOE enhancements suggest that *anti–anti* conformation of **3** is predominant in acetone in the absence of anions (Figure 5(i)).

Remarkable conformational changes were observed upon addition of acetate to receptor **3** (Figure 5, right column). The saturation of H2β resulted in small NOE at H3 (Figure 5(f)) and weak response at H6 upon the saturation of H7α (Figure 5(h)). In contrast, strong NOE enhancements were observed between H1, H2β and H7α protons, which suggest that all NH protons are spatially close together, and *syn–syn* conformer predominates in the **3**·$CH_3CO_2^-$ complex in acetone solution (Figure 5(j)).

The key NOE enhancements for conformational study of **1–4** are summarised in Table 2. Strong NOEs were observed at H3 upon saturation of H2β (13.5–14.2%), whereas negligible or no NOEs were present between H2β and H1 protons for **1–4** in the absence of anions. The saturation of H7α protons resulted in relatively strong NOE enhancements at H6 protons in **1–4** (7.1–9.1%). Weak NOE enhancements were observed between H7α and H1 protons for **1–4** in the absence of anions (Table 2). The analysis of NOE results suggests that the predominant conformation of all four indole anion receptors **1–4** in the absence of anions is *anti–anti*. In this orientation, C2-carboxamido and C7-ureido groups take up conformations in which all three substituent's NH protons point away from the indole H1, whereas C2α and C7β carbonyl groups are in favourable orientation to serve as hydrogen bond acceptors. Moderate NOE enhancements between

Table 3. Comparison of relative energies (kcal mol^{-1}) for the four major conformers of model receptor in the absence and in the presence of anions at B3LYP/6–311 + G(d,p) level.

	No anion		Chloride		Bromide		Nitrate		Acetate	
Conformer	*In vacuo*	In acetone	*In vacuo*	In acetone	*In vacuo*	In acetone	*In vacuo*	In acetone	*In vacuo*	In acetone
anti–anti	0.00	4.29	59.34	_[a]	34.44[b]	11.85	34.16[b]	20.30	39.75[b]	_[a]
anti–syn	2.70	0.00	10.06	0.00	9.78	0.00	8.46	3.77	8.39	0.00
syn–anti	1.40	10.15	30.73	_[a]	12.98[b]	_[a]	22.62[b]	14.26	26.16[b]	_[a]
syn–syn	7.49	_[a]	0.00	_[a]	0.00	_[a]	0.00	0.00	0.0	1.86

[a] Convergence was not achieved.
[b] Conformations along C2−C2α and C7−N7α bonds and position of anion with respect to receptor were restricted while all other degrees of freedom were optimised freely.

H1 and H7α in **1** and **4** are in accordance with some degree of conformational flexibility across C7—N7α bond.

Considerable changes of NOE enhancements were observed upon addition of chloride anions to **1**–**4**. Particularly outstanding are increased NOEs among H1, H2β and H7α (up to 12.5%) and decreased NOEs between H2β and H3 as well as H6 and H7α with respect to the receptors in the absence of anions. The variations of NOE enhancements in the absence and in the presence of chloride anions in **1**–**4** corroborate the interaction of chloride with H1, H7α and H7γ through hydrogen bond formation. Consequently, the predominant conformation of receptors **1**–**4** changes from *anti–anti* to *syn–syn* upon addition of chloride anions. Similar changes of NOE enhancements were observed upon addition of bromide to **1**–**4**, although they were much less pronounced (Table 2). Weaker receptor–bromide interactions are in agreement with chemical shift changes and could be attributed to bromide's lower basicity with respect to chloride anions. Interestingly, although chemical shift changes suggested interactions between receptors **1**–**4** and nitrate anions through hydrogen bond formation, only minor conformational changes were observed in receptor–nitrate complexes (Table 2). The saturation of H2β showed more intense NOE at H3 vs. H1, whereas the saturation of H7α resulted in stronger NOE enhancement at H6 vs. H1 which suggested minor if any conformational change upon addition of nitrate anions. Conformational changes along C2—C2α and C7—N7α bonds were observed upon addition of acetate anions to **1**, **3** and **4**. It is interesting to note that strong NOE at H3 was observed upon saturation of H2β in **2**·$CH_3CO_2^-$ complex, which suggests only minor conformational changes along C2—C2α bond upon interaction of **2** with acetate (Table 2). Unfortunately, spectral overlap for **2**·$CH_3CO_2^-$ complex thwarted more detailed conformational examination.

Conformational analysis by quantum mechanical calculations

The experimental observations on conformational equilibria in the absence and in the presence of anions were augmented by quantum mechanical calculations at B3LYP/6-311 + G(d,p) level using Gaussian 03 program (*13*). Structure calculations were performed on a model compound with methyl groups attached to the C2-amido and C7-ureido substituents ($R = R' =$ Me, Figure 4). Energy minimisations were performed without any constraints for the four major conformational families shown in Figure 4. The *in vacuo* relative energies are reported in Table 3. The *anti–anti* conformer of model receptor exhibits the lowest energy. The *syn–syn* conformer exhibits 7.5 kcal mol^{-1} higher energy. Relative energies of the four major conformers of the model receptor

were also computed for its complexes with chloride, bromide, nitrate and acetate anions. *Syn–syn* conformer was found to exhibit the lowest relative energy for all four anion–receptor complexes (Table 3). The *anti–anti* conformers exhibit remarkably higher energies between 34 and 59 kcal mol^{-1}. According to the calculated relative energies, *syn* orientation along C7—C7α bond is highly preferred in the anion–receptor complexes.

In order to evaluate the role of acetone, respective energies were calculated with the use of isodensity-polarised continuum model (Table 3). *Anti–syn* conformer exhibited the lowest energy in the absence of anions. *Anti–anti* conformer was characterised by 4.3 kcal mol^{-1} higher energy. Interestingly, *anti–syn* conformers exhibited the lowest energy for anion–receptor complexes (except for nitrate). Unfortunately, convergence could not be achieved for the other three conformers in the case of chloride–receptor complex. There is a small energy difference between *anti–syn* and *syn-syn* conformers of 1.9 kcal mol^{-1} for acetate–receptor complex. The reversed energetic preference of *syn–syn* over *anti–syn* conformers of 3.8 kcal mol^{-1} was observed for nitrate–receptor complex. In addition, there is a good agreement among relative preferences of conformers *in vacuo* and solvent model for nitrate-receptor complex (Table 3).

Conclusions

Indole-based anion receptors **1–4** with alkyl chain and/or phenyl ring attached at C2-amido and C7-ureido moieties were characterised by heteronuclear NMR experiments. NMR parameters reflect different propensities of receptors for interaction with anions. The conformational analysis of indole receptors was performed by a combination of NMR spectroscopy and quantum chemical calculations which showed good agreement. All four receptors exhibit conformational preorganisation in acetone solution, where *anti–anti* conformer is the most favoured in the absence of anions. In this orientation, NH protons are pointing away from the indole H1 proton, while C2α and C7β carbonyl groups serve as its hydrogen bond acceptors. Chemical shift changes induced by interaction with anions suggest that binding of chloride and bromide anions occurs primarily via H1 and H7α protons. Nitrate anions favour interaction with H7α and H7γ ureido protons, whereas acetate anions interact strongly with all four available hydrogen bond donor groups. Comparison of NOE enhancements in the absence and in the presence of anions revealed dramatic conformational changes of receptors **1–4** induced by complexation of chloride, bromide and acetate anions. Anion–receptor complexes predominantly adopt *syn–syn* conformation where all NH protons are spatially close and involved in interaction with anions. No conformational changes were observed by NOE experiments upon addition of nitrate anions to **1–4**, which also exhibit very weak interaction. Our study demonstrates that indole ring offers intriguing scaffold for the design of novel anion receptors in order to tune affinities and selectivities for anions.

Experimental

Receptors **1** (*10*) and **4** (*11*) as well as amine precursors were prepared as described in the literature.

General procedure for the preparation of receptors 2 and 3

The corresponding 7-amino-1H-indol-2-carboxylicacid amide (0.6 mmol) was dissolved in dichloromethane. Phenyl isocyanate and octyl isocyanate (0.9 mmol) were added and the mixture was refluxed for 24 h to obtain **2** and **3**, respectively. The residue was purified by column chromatography on silica gel (dichloromethane). Characterisation of **2** and **3** was done by NMR, IR, MS and elemental analyses. NMR spectra were recorded on a Varian Mercury 300 spectrometer. FT-IR spectra were recorded on a Perkin Elmer FT IR 1760 spectrometer (KBr). MS spectra were measured on a Varian MAT 212 spectrometer. Elemental analyses were obtained with a Heraeus CHN-O-Rapid analyser. Melting points were measured using Büchi B-540 (uncorrected).

Receptor **2**: 56%. m.p. 134°C; ^{1}H NMR ($CDCl_3$): $\delta = 10.93$ (s, 1H), 8.70 (s, 1H), 7.98 (s, 1H), 7.69 (d, $J = 7.1$ Hz, 1H), 7.59 (s, 1H), 7.48 (d, $J = 7.1$ Hz, 1H), 7.34 (t, $J = 8.2$ Hz, 1H), 7.18 (d, $J = 7.7$ Hz, 2H), 7.03 (d, $J = 7.7$ Hz, 2H), 6.95 (t, $J = 8.2$ Hz, 1H), 6.64 (s, 1H), 3.38 (m, 2H), 1.55 (m, 2H), 1.26 (m, 6H) and 0.88 (m, 3H). EI-MS (70 eV): m/z = 378 (M^+). IR (KBr): $\nu = 3316$, 2928, 2856, 2180, 1622, 1558, 1498, 1439, 1415, 1314, 1281, 1242, 1031, 825, 736 and 503. Calcd for $C_{22}H_{26}N_4O_2$: C 69.82, H 6.92, N 14.80; found: C 69.49, H 6.68, N 15.02.

Receptor **3**: 55%. m.p. 207°C; ^{1}H NMR ($CDCl_3$): $\delta = 11.13$ (s, 1H), 8.30 (dd, $J = 1.0$, 7.9 Hz, 1H), 8.03 (d, $J = 7.9$ Hz, 1H), 8.00 (s, 1H), 7.67 (m, $J = 1.0$, 7.9 Hz, 2H), 7.42 (m, $J = 7.9$ Hz, 2H), 7.29 (t, $J = 7.9$ Hz, 2H), 7.26 (s, 1H), 7.22 (s, 1H), 7.19 (s, 1H), 3.16 (m, 2H), 1.59 (m, 2H), 1.46 (m, 2H), 1.26 (m, 8H) and 0.87 (m, 3H). EI-MS (70 eV): m/z = 406 (M^+). IR (KBr): $\nu = 3956$, 3870, 3679, 3431, 3118, 2932, 2864, 2344, 1718, 1625, 1506, 1447, 1384, 1237, 1110, 1074, 608 and 539. Calcd for $C_{24}H_{30}N_4O_2$: C 65.08, H 7.68, N 12.65; found: C 65.48, H 7.52, N 12.30.

NMR experiments

^{1}H, ^{13}C and ^{15}N NMR spectra were acquired on Varian Unity Inova 300 MHz NMR spectrometer. All data were

recorded in acetone-d_6 at 298 K. Chemical shifts were referenced to the residual solvent signal of acetone-d_6 at δ 2.05 ppm for ^{1}H (297.801 MHz) and δ 29.92 ppm for ^{13}C (76.190 MHz), whereas ^{15}N (30.188 MHz) chemical shifts were referenced relative to external benzamide (δ 103.55 ppm). Individual resonances were assigned on the basis of their chemical shifts, signal intensities, multiplicity of resonances, H—H coupling constants as well as with the use of a series of 2D NMR experiments (COSY, gHSQC and gHMBC). Saturation delay in 1D difference NOE experiment was 5.0 s. All anions were added as TBA salts.

Ab initio *calculations*

Initial structures were generated by Chem3D Pro 10.0 software and energy minimisation at B3LYP/6-311 + G(d,p) level was performed without any constraints for four major conformational structures using Gaussian 03 (*13*). *Ab initio* calculations of model compound and their 1:1 Cl^-, Br^-, NO_3^- and $CH_3CO_2^-$ complexes were carried out. Anions in the anion–receptor complexes were placed initially at the expected equilibrium distance to H1 proton in the plane of indole ring. Their position was freely optimised except for the canonical forms indicated in Table 3, where in the absence of restraints, calculations converged to a global minimum. Tetrabutylammonium countercation was omitted in geometry optimisation of anion–receptor complexes. Frequency calculations verified that the optimised geometries were stable points on the potential energy surface. Relative energies in solution were calculated with the use of isodensity-polarised continuum model, where dielectric constant of acetone was used ($\varepsilon = 20.7$).

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