Exploring ion-ion preferences through structure-property correlations: amino acid-derived, bis(guanidinium) disiloxane salts

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In a more synthetical approach to the study of ion-specific phenomena, four dipodal bis(guanidinium) siloxanes have been synthesized starting from glycine, β-alanine, γ-aminobutanoic acid, L-proline and 1,3-bis(3-aminopropyl)tetramethyldisiloxane. Together with their non-amide progenitor they were comparatively studied in regards to their interactions with nine different anions: sulphate, chromate, molybdate, benzoate, chloride, azide, nitrite, nitrate and thiocyanate. Their aqueous solubilities, form, \(^1\)H NMR and FT-IR spectra were examined while searching for anion-specific interactions falling in- or outside of the Hofmeister series. We show that although the “chao-“ and “kosmotropic” ions affect the properties of solutions in a predictable way, more selective cation-anion pairing is responsible for phase separation and crystallinity. As a prominent example, crystal structure of one of the benzoate salts was successfully obtained and reveals a synergy of hydrophobic packing, ionic and hydrogen bonding. Immobilized but still flexible siloxane bridges give rise to crystals described by \(P\,4_{2}/n\) space group and neatly segregated into hydro- and lipophilic sections.

The ionic bond is an interaction that is both elemental and peculiar. In stark contrast to covalently bound atoms (and despite comparable energies being involved) pairs of cations and anions can seldom be called a single molecule. Extremely dynamic, strictly charge-dependent and non-directional nature of that interaction has made it, traditionally, a poor choice for designing molecular receptors with high selectivity. However, one instance in which “ion-specific interactions” are referred to on a regular basis is the Hofmeister series, a classification based on their ability to salt in or salt out proteins1. But this usage of the term can sound somewhat contradictory to a supramolecular chemist - after all, can’t the interactions either be truly specific or always follow the same, well-established trend?

One can of course argue that this “trend” has eluded satisfactory theoretical explanation for well over a century2 – but recently the interplay between water structure, ions, macromolecular solutes and physicochemical properties of the solution is becoming, again, a widely debated subject3–5. Accordingly, the number of papers claiming to explain - at least in part - what is already known from experimental data has risen sharply6–8. With these attempts to establish an underlying cause came numerous exceptions to the rule - cases which a supramolecular chemist would be much more inclined to call “specific”. One interesting example is the “reverse Hofmeister”9, observed for some (less common) positively charged proteins, e.g. lysozyme10. This seems logical at first glance: anions with high charge density, usually restricted (in case of negatively charged polypeptides) to bulk water can now approach the cationic moieties and instead compete with native intramolecular interactions, promote the unfolding and “water-mixing” of the macromolecule. Conversely, however, research concerning the impact of anions on the salting in (or out) of ionic liquids did not show a reversed, but only a slightly altered (if at all) trend11–13. And as many other papers report inconsistent or downright conflicting findings14–16, perhaps it is time to move past treating Hofmeister as the universal reference, discern between solvation/structuring effect of ions and their truly selective interactions, and establish new experimental trends - aiding theoretical studies in breaking down influences from individual functional groups, their spacing and cooperation. It was our belief with the

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present study that valuable information could be obtained from comparative analysis of series of compounds: complicated enough to include more than one functionality, yet simple enough to allow for differentiation of their individual impact on bulk physicochemical properties.

Starting with the abovementioned lysozyme/ionic liquid behaviour discrepancy and wanting to also investigate the structuring effect of anions on the separating organic phase, we decided to synthesize amino acid-linked bis(guanidines), in which the polar moieties are separated by a siloxane bridge. This idea stemmed from a previous study, which revealed an abnormally low water solubility of the prepared 1,3-bis(3-guanidinopropyl)-t etramethyldisiloxane nitrate (parent chloride was fully water-miscible)\(^{17}\). Addition of amide bonds seemed very attractive, as they often co-exist with guanidine moieties in many polypeptides and are a key ingredient in their higher-than-primary structure. Guanidines, on the other hand, have well-established “chaotropic” properties in their own right\(^{18}\). Siloxane bridges, though fairly chemically and very thermally resistant, impart a strong tendency for disorganization and non-crystallinity on the molecule, which stems from the extremely low energy barrier for rotation around the Si-O bond\(^{19}\). This results in most siloxanes presenting as liquids, oils or waxy solids at room temperature (a point of connection with ionic liquids). The apparent crystallinity of the abovementioned nitrate was yet another reason for choosing that particular class of compounds.

Apart from the obvious advantage of easily comparable results, one of the drawbacks of articles referring to Hofmeister series as a common denominator is the limitation of studied anions to those with well-established influences on water solutions, we chose chromate and molybdate (same geometry and charge) instead. Azide, an ion very rarely studied in this context, was also included - due to its specific linear geometry (similar to thiocyanate) and significant electronic contribution from the characteristic $\text{N}^-$ moiety was considered. By choosing anions that could be arranged along the Hofmeister series, a common denominator for all compounds was achieved.

**Results and Discussion**

**Design and synthesis.** The intramolecular hydrogen bonding between amide moieties determines the $\alpha$-helical or $\beta$-sheet structure of a peptide chain; we theorized that in smaller molecules, amide-guanidino hydrogen bonds could also play an “organizing” role, and possibly even compete with anions for access to nitrogen-bound hydrogens. Therefore, apart from glycine, $\beta$-alanine and $\gamma$-aminobutanoic acid were chosen as linkers; despite not being proteinogenic, both play important roles in the human body. The final amino acid - proline - was chosen as it can fit both at the beginning (carbonyl-guanidine distance, strain) and the end (number of carbon atoms, lipophilicity) of the series. Being a secondary amine, with sterically-hindering ring, it shows severe restrictions in the Ramachandran plot - in contrast to glycine, the least restricted amino acid in terms of $\psi$ and $\phi$ angles\(^{20}\).

The syntheses utilized standard reagents and pathways to obtain symmetrical diamides with tetramethylidisiloxane backbone and guanidine terminal groups (Fig. 1). At later stages no salts other than chlorides were used to ensure anionic purity of samples (e.g. drying was performed using anhydrous ethanol). Non-amide bis(guanidinium) salt, 1,3-bis(3-guanidinopropyl)-tetramethyldisiloxane dichloride (GUA) was prepared as described before\(^{17}\). The residual solvent had to be removed by repeated lyophilisation; all compounds were obtained as pale yellow oils or semisolids and no crystallization was observed after 3 months in 4°C. All synthesized dichlorides were fully miscible with water at ambient temperature.

![Figure 1. Synthesis of five bis(guanidinium) disiloxane chloride salts. Please refer to Section S2 in the Supplementary Information for detailed procedures.](https://example.com/supplementary-figure)

- **1 (GUA)**
- **2 (GLY)**
- **3 (βALA)**
- **4 (GABA)**
- **5 (PRO)**
Anion-exchange and solubility studies. Preparation of different salts was combined with the study of their aqueous solubility and form. Solubility product constants were estimated on the basis of incremental addition of sodium salt solutions to samples containing 0.1 mmol of disiloxane. Molar concentrations of ions corresponding to the increments preceding and resulting in the permanent clouding of solution (or outright precipitation) were used to calculate the $K_w$ range (Fig. 2, for experimental details see Section S3 in Supplementary Information).

Apart from yellow chromates, all isolated salts were colourless liquids or solids. Salts containing monovalent anions were found to be very soluble in methanol, ethanol and dimethyl sulfoxide (DMSO), while sulfates, chromates and molybdates were only soluble in water, barely soluble in DMSO and insoluble in alcohols. No azides separated in the studied concentration range, and only the nitrite of GUA precipitated (solid, $K_w$ range: 5.26-5.42 x $10^{-3}$). This last dication was indeed the most prone to phase separation, in contrast with PRO, of which only thiocyanate, benzoate and chromate were obtained in such way. Based on those results, we theorized that there are two distinct mechanisms at play: pure lipophilic pairing, with no meaningful interactions, which was evident for thiocyanate, benzoate and chromate, but not for PRO (according to calculations, GUA is much more hydrophilic than PRO) and the aliphatic ring and lack of one guanidinium proton must have an additional, detrimental effect on hydration, thus promoting separation). The reverse trend for the rest of studied anions can only be explained by favourable cation-anion interactions, counteracting the dissociative effect of water. It is also possible that the anion has to compete for that interaction, the overall "polymeric salt" structure can effectively be less preferable. Furthermore, benzene is the only counterion for which the regular arrangement of diamides is retained – this observation and the notion that the carboxyl group

Comparison of $^1$H NMR spectra. Although not ideal, in order to study exchangeable protons, DMSO-d$_6$ was used in place of water in NMR experiments. It has been established that DMSO behaves similarly under the influence of different ions (i.e. the physicochemical properties of both solvents change in accordance with the same lyotropic series)\textsuperscript{22,23}. While most bisguanidines were studied as 0.05 M solutions, unfortunately only the sulphonates of GUA and GLY were sufficiently soluble to produce informative signals (Fig. 3) and spectra of their saturated solutions are presented instead. Details for all salts are summarized in Table 1.

While the previous experiment elucidated much about trends arising from the structure of the organic cation, anion-based trends become more visible in $^1$H NMR spectra. Signals from labile protons shift steadily upfield as the counterion is changed from sulphate to thiocyanate. While it was proved that the chemical shifts of solvent’s atoms change in the presence of ions in a manner not directly translatable to Hofmeister series rationale\textsuperscript{24}, the solvent-mediated impact on other solutes remains in agreement with the notion that poorly solvated anions render the polar solvent less aggressive\textsuperscript{25} (by actually allowing it to retain its typical, hydrogen-bonded structure or, in the case of DMSO, one based on other strong dipole-dipole interactions). It follows that if a sufficiently selective ion-ion interaction would be visible as a deviation from the uniform changes brought about by the dominant solvent-ion interactions. Anions derived from weak acids (azide, benzoate and - to a lesser extent - nitrite) produced some aberrations in the trend, and their relative arrangement sometimes changed, depending on the organic cation. However, a closer look at those anomalies (denoted A1-3 in Fig. 4) calls into question whether they can be fully ascribed to the basicity of the anion. Benzoate’s preference towards guanidinium hydrogens can
be easily seen by tracing the chemical shifts in GLY from chloride to sulphate: while the amide proton is actually shifted upfield in benzoate (compared to other two), guanidinium protons are shifted heavily downfield. Sulphate, on the other hand, doesn’t discriminate against amide hydrogens (A1). On the contrary, the chemical shift of guanidinium -N-H in GUA-SO4 is almost the same as in GUA-Bz (benzoate salt), but is much lower in GLY-SO4, where the alternative - amide - binding site is present (and the relevant amide proton signal is, in fact, shifted fairly downfield, corroborating the presence of, on average, stronger hydrogen bonds around this moiety). This is in accordance with our reasoning concerning the different trends seen in aqueous solubilities of salts. The second irregularity (A2) is more subtle, as it concerns only amide protons - far less affected by anion substitution, but also less prone to deviations in general. However, for GLY and βALA, the chemical shift of the amide proton in benzoate is notably smaller than in chloride salt, while for GABA and PRO the situation is reversed. This can be explained by strong interactions of the carboxylate anion interfering with internal structure (and, therefore, relative location of amide hydrogen), important in the former two cations, but less so in the longest molecule, GABA. Along this line, PRO can be presumed to be too constrained for the amide proton to be effectively dislocated - and

| Salt       | 1H shifts [ppm], multiplicity, integration | Salt       | 1H shifts [ppm], multiplicity, integration |
|------------|------------------------------------------|------------|------------------------------------------|
| GUA-Cl     | -                                       | GABA-Cl    | 8.01, t, 1H 7.78, t, 1H 7.46, br, 2H 7.03, br, 2H |
| GUA-NO2    | 7.86, br[b], 1H 7.30, br, 2H 7.06, br, 2H | GABA-NO2   | 7.96, t, 1H 7.90, t, 1H 7.46, br, 2H 7.09, br, 2H |
| GUA-NO3    | 7.46/7.42, t, 1H (5:1) 7.25, br, 2H 6.76, br, 2H | GABA-SCN   | 7.93, t[b], 1H 7.58, t[b], 1H 7.32, br, 2H 6.85, br, 2H |
| GUA-N3     | 7.60, br[b], 1H 7.31, br, 2H 6.83, br, 2H | GABA-Bz[d] | 8.11, t, 1H 8.89, br[b], 1H (8.4-7.9) 8.13, br, 4H |
| GUA-SO4[a] | 9.02, br[b], 1H 8.0-7.0) ~7.32, vbr, 4H | GLY-Cl     | 8.28, t, 1H 7.72, br[b], 1H 7.42, br, 4H GABA-N3 | 7.98, t, 1H (7.8-6.6) ~7.33, vbr[b], 5H |
| GUA-Bz[d]  | 8.20, t, 1H 7.68, br[b], 1H 7.39, br, 4H | GABA-NO3   | 7.40, t[b], 1H (7.0-7.6) 7.30, br[b], 5H |
| GUA-SCN    | 8.16, t, 1H 7.55, br[b], 1H 7.26, br, 4H | PRO-Cl     | 8.32, t, 1H 7.47, br, 4H |
| GUA-Bz[d]  | 8.19, t[b], 1H (7.0-7.6) ~7.30, br[b], 5H | GABA-SCN   | 7.91, t, 1H 7.47, t, 1H 7.30, br, 2H 6.78, br, 2H |
| GUA-Cl     | 8.21, t, 1H 8.73, br[b], 1H 8.26, br, 4H | PRO-NO3    | 8.07/8.02, t, 1H (3:1) 7.25, br, 4H |
| GUA-NO2    | 8.42, t, 1H 8.27, br[b], 1H 8.76, br, 2H | PRO-N3     | 8.10/8.06, t, 1H (3:1) 7.26, br, 4H |
| GUA-NO3    | 7.56, br, 4H | PRO-SCN    | 7.97, t, 1H 7.11, br, 4H |
| GUA-N3     | 8.07, t, 1H 7.65, br[b], 1H 7.59, br, 2H 7.03, br, 2H | PRO-NO3    | 8.48, t, 1H (9.3-7.6) ~7.40, vbr, 4H |

Table 1. 1H NMR signals of N-H protons in different salts of bis(guanidinium) disiloxanes (DMSO-d6, 400 MHz, 0.05 M). t - triplet, br - broad, vbr - very broad (>0.5 ppm). aDue to low solubility, 1H NMR spectra of sulphates were recorded for the highest possible concentration instead of standard 0.05 M. bPoorly resolved (t) or not visibly resolved (br) peak, but coupling evident in the neighboring -CH2- signal (presents as quartet). cNo related coupling observed in the neighbouring -CH2- signal (presents as triplet). d Benzoate salt.
that observation, together with extremely similar chemical shifts of guanidine -NH₃ protons in salts of GLY and PRO with poorly hydrogen-bonding anions, is a warning not to extrapolate a few similarities (in this case: merged -NH₃ signals, guanidine-carbonyl distance) to the entirety of intra- and intermolecular behaviour of a molecule.

The last registered, unmistakable anomaly was exclusive to the azide salts; in all cations with an amide linker, this countered caused the merging of all signals from guanidinium protons and the disappearance of related coupling in the neighbouring -CH₂- peak (A3). At first we presumed the basicity of N₃⁻ was high enough to greatly facilitate proton exchange throughout the guanidinium moiety. However, when we recorded the spectrum for GUA, despite the disappearance of coupling (indicating fast exchange), all three signals remained separate. Although we believe this proves that another truly ion-specific interaction is at play here (and underlines the importance of discerning between decoupling, fast exchange and merging of signals when studying ionic interactions by means of 'H NMR)²⁶,²⁷, we did not find a reliable explanation for why the presence of an amide bond would assist in equalization of guanidinium protons in the presence of azide. Possibly, N₃⁻ acts as a bridge between the two moieties, opening an additional pathway of proton transfer throughout the molecule. More specific research is probably called for, but was beyond the scope of presented work.

![Figure 4](Image 156x587 to 468x736)

**Figure 4.** Effect of counterion on the chemical shifts of nitrogen-bound hydrogens in bis(guanidinium) diamides. Anions were arranged as per standard Hofmeister series, with the exception of benzoate. Regions of the most interesting anomalies are indicated by grey brackets (A1-3).

FT-IR spectra of thin films. The siloxane salts separating from aqueous solution were rarely solid, let alone crystalline - as is the case with many precipitating proteins, polymers, surfactants and other flexible molecules of mixed polarity. Of the many possibilities for sample preparation for FT-IR spectroscopy, we therefore decided on thin films as best approximation of the partially disorganized state of the organic phase when it partitions from solution. A comparative spectrum of GUA-NO₃ obtained in KBr tablet validated this approach, and highlights how different degree of matter organization can immensely impact the observable interactions – even between fairly simple molecules or ions (Table 2).

The complementarity of FT-IR investigations is revealed by careful study of the differences between rather similar spectra of disiloxane salts. The most evident is the thiocyanate, with nearly universal disappearance of some individual bands in the N-H region and moving of rest to higher wavenumbers, indicating weaker hydrogen bonding. Particularly, the II band can only be pinpointed for βALA-SCN, another sign of noticeably stronger intramolecular bonding “substituting” for the anion due to favourable carbonyl-guanidinium distance. Furthermore, in GUA, where no intra- or intermolecular hydrogen bonding utilizing amide moieties can take place, a similar situation is also seen for the azide salt (Fig. 5). Much larger differences in band positions (and shapes) observed for salts of that cation further strengthen the notion that the amide group has a “buffering” effect on the molecule - or rather the whole organic phase. While in solution nitrate behaved very much according to its place in the Hofmeister series, in thin films it becomes evident that this particular anion also has some degree of affinity for guanidinium (albeit less so than the carboxylic group). This manifests through the frequencies in nitrates being on par or even lower than those in azides (bands I-III) or even chlorides (amide II band – however, it is worth noting that usually that band's frequency rises with stronger intermolecular hydrogen bonding²⁸; in our case, only with PRO this trend wasn't perfectly reversed). In solid GUA-NO₃ (in KBr) two more absorption bands could be discerned, and most other were, understandably, much sharper than in thin films. However, it was the inherent nitrate stretching absorption band that has changed most dramatically, prompting us to analyse that specific aspect of FT-IR spectra more closely. It is well known that changes in absorption intensity can be indicative of strong interactions (e.g. hydrogen bonding)²⁹, even if not accompanied by important frequency shifts. For a trigonal anion such as nitrate, only the asymmetric stretch is IR-active, and entails the shortening of one N-O bond, while other two become elongated. If we assume the nitrate-guanidinium ion pair forms analogously to carboxylate-guanidinium (Fig. 6), the difference in partial charge and distance between two oxygens involved in the bonding and the third, unused, would increase the change in dipole moment on passing from ground to excited state³⁰. That change is directly translatable to intensity of absorption, as was indeed found in our compounds (Table 3).

Whether we look at pure intensities or take the band widening into account, it is obvious that GABA interacts strongly with nitrate, next only to GUA. This highlights how important the spacing between different moieties...
Table 2. FT-IR data for five most common, discernible bands in spectra of bis(guanidinium) diamides (thin films obtained by evaporation of EtOH solutions). *Spectrum recorded in KBr tablet. †Additional band, not discernible in most cases. ‡Broad, jagged band, without a dominating maximum in denoted range. (sh) Appears only as a shoulder on the nearest band; wavenumber instead of brackets is given only when the maximum could be pinpointed with reasonable accuracy.

| Salt   | N-H ν [cm⁻¹] | C=O ν [cm⁻¹] | Amide II band [cm⁻¹] | Salt   | N-H ν [cm⁻¹] | C=O ν [cm⁻¹] | Amide II band [cm⁻¹] |
|--------|--------------|--------------|----------------------|--------|--------------|--------------|----------------------|
|        | I            | II           | III                  |        | I            | II           | III                  |
| GUA-Cl | 3332         | 3260         | 3164                 | GUA-N₂ | 3320h       | 3272         | 3168                 | 1651    | 1557                  |
| GUA-NO₃ | (sh)³⁴       | 3269         | 3177                 | GUA-SCN | (sh)       | 3269         | 3158                 | 1650    | 1551                  |
| GUA-NO₃ (sh) | 3380       | 3279         | 3202                 | GABA-Bz (sh) | (sh)       | 3269         | 3161                 | 1650    | 1550                  |
| GUA-Bz | 1510         | 3270         | 3159                 |        | 1678-1633   | (sh)         | 3269                 | 3161    | 1650                  |
| GUA-N₂ (sh) | 3358       | 3152         | 1661                 | GABA-Cl (sh) | (sh)       | 3269         | 3161                 | 1650    | 1550                  |
| GUA-SCN | 3320sh      | 3195         | 1645                 |        | 1661        | 1650         | 1550                 |
| GUA-Bz | 1510         | 3270         | 3159                 |        | 1678-1633   | (sh)         | 3269                 | 3161    | 1650                  |
| GLY-Cl | 3284sh       | 3263         | 3159                 | GABA-N₂ (sh) | (sh)       | 3269         | 3164                 | 1650    | 1555                  |
| GLY-NO₃ | 3375sh       | 3271         | 3174                 | GABA-NO₃ (sh) | (sh)       | 3273         | 3164                 | 1650    | 1550                  |
| GLY-Bz | 3339         | 3270         | 3159                 |        | 1678-1633   | (sh)         | 3269                 | 3164    | 1650                  |
| GLY-SCN | 3326         | 3174         | 1657                 |        | 1650        | 1550         | 1650                 |
| GLY-Cl | 3284sh       | 3263         | 3159                 | PRO-Cl | 3205       | 3145         | 1546                 |
| GUA-Cl | 1510         | 3270         | 3159                 |        | 1650        | 1613         | 1539                 |
| GUA-NO₃ | (sh)³⁴       | 3269         | 3164                 | PRO-SCN | 3410       | 3148         | 1650                 |
| GUA-Bz | 1510         | 3270         | 3159                 |        | 1650        | 1613         | 1539                 |
| GLY-Bz | 3326         | 3164         | 1663                 |        | 1650        | 1613         | 1539                 |
| βALA-Cl | 3320sh      | 3268         | 3159                 | PRO-N₃ | 3315       | 3151         | 1546                 |
|        | 1650         | 1557         | 1650                 |        | 1650        | 1613         | 1539                 |

Crystal structures. Ammonium siloxane compounds without sterically hindering groups are known for their reluctance to form single crystals. This feature can be connected to the absence of strong cohesion forces and excessive conformational flexibility of the Si-O-Si bridge of the molecular cation. However, we succeeded in obtaining single crystals of two salts of the series, which elucidated the characteristic structural and conformational features, as well as the aggregation capabilities of these compounds. Reiterated crystallizations from various solvents and using different methods (solvent evaporation, lowering temperature, diffusion) of precursor salt GUAN-NO₃ finally yielded best single crystals from 20% aqueous ethanol evaporated slowly at ambient temperature. Apart from the very small size of the crystals, allowing for only the strongest reflections to be measured reliably, these triclinic crystals (Table 4) turned out to be very strongly structurally disordered and this determined the quality of crystals and of the X-ray diffraction data was sufficient to allow for reliable structural model with H-sites consistent with the bond lengths of their carriers and with the H-bonding pattern has been obtained. While the dication is again located on a centre of inversion, the split positions of atoms

... in a molecule can be (even a very flexible one, such as siloxane) and again reinforces the given explanation for βALA salts being much less prone to precipitation. The reason for such a colossal difference in the presentation of nitrate absorption band (in thin films and in solid - recrystallized - sample in a KBr tablet) can be traced to the level of matter organization examined in both approaches. It is improbable that in the separating, liquid organic phase (approximated by thin films) the full potential of possible hydrogen networking is utilized - and it is just as unlikely that for such compounds as those studied, anything less could force them to adopt a crystalline form. Therefore the strongest, asymmetric bonding with only two nitrate oxygens dominates in non-crystalline, while in solid/crystalline phase all three oxygens must be fairly uniformly hydrogen bonded to guanidinium moieties (see Fig. 6 and subsequent section).
group’s name, are located inside helices constructed from those guanidinium and carboxylate moieties (Fig. 8a). Every such cluster of ionic groups is in turn surrounded by four apolar regions, containing aromatic rings and non-disordered siloxane methyl groups pointing inward. Symmetry viewed from the centre of those hydrophobic sections is described by a 4-fold rotoinversion axis. The inner crystal structure is therefore neatly segregated into hydrophilic and hydrophobic segments, with siloxane bridges and neighbouring alkyl chains acting as their boundaries (Fig. 8b). Together, these findings clarify that it was the cooperation between three major types of interactions: ionic bonds, hydrogen networking and hydrophobic effect that enabled the immobilization the inherently flexible siloxane molecules into a crystal lattice with long-range ordering and heightened symmetry. In the absence of such complementary forces - which can depend solely on the counterion present - crystallinity is unlikely. The apparently large contribution from hydrophobic packing (typically prominent in peptides and some macrocycles) in the obtained crystal structure underlines the importance of this factor for aqueous solubilities and other properties of smaller organic salts of mixed polarity.

The spatial arrangement of ions in GUA-NO₃ (although not definite due to the tentative determination) along with previous crystallographic data₃₃ corroborates the previously given explanation for large differences in FT-IR spectra of nitrates (i.e. crystalline state utilizing more symmetrical arrangement of ion-ion interactions, and thin films lacking in such uniformity). Furthermore, there are closely located voids, over 2.2 Å in diameter, in the GUA-Bz structure (see Figure S4.1.5 in Supplementary Information). Although too small to accommodate solvent molecules, they are located together with the benzoate rings in the same channels running down [z]. It appears that similarly arranged structures of GUA with other carboxylate anions could also be possible.
Four new, guanidinium-appended tetramethyldisiloxane diamides were synthesized using glycine, β-alanine, γ-aminobutanoic acid and L-proline. Those symmetrical molecules are characterized by mixed polarity and high flexibility of the central siloxane bridge, which result in their superficial resemblance to ionic liquids – despite their double positive charge that is easily accessible for counterions. The effect of those – namely: sulphate, chromate, molybdate, benzoate, nitrate, nitrite, azide, chloride and thiocyanate, on relationships between structure, aqueous solubilities, $^1$H NMR, FT-IR spectra and crystallinity – was studied in depth. It was established that – in contrast to typical ionic liquids, most polymers and peptides – the precipitation of studied salts, along with their non-amide precursor, was driven chiefly by ion-ion interactions, and not anion-solvent interactions (Hofmeister-type salting out/in phenomena). For such highly charged, small and flexible molecules, the separation from aqueous solution as an organic phase was found to follow two trends, depending predominantly either on the strength of bonds formed between ions, or pure lipophilicity of the formed salt. The latter mechanism dominated only for the very poorly hydrogen bonding thiocyanate, resulting in proline-derived bis(guanidine) separating most easily. For other anions, evidence of strong, preferential bonding points especially to benzoate, nitrate and chromate, with the first yielding even single crystals from aqueous ethanol. This level of organization turned out to be possible due to the synergy of salt bridges (ion-ion and hydrogen bonding) and very pronounced hydrophobic effect, which resulted in segregation of internal structure into polar and non-polar compartments.

**Figure 6.** Comparison between spatial arrangement of a guanidinium cation and a carboxylate anion in a “salt bridge” (motif combining ionic and hydrogen bonding) and an analogous arrangement with a nitrate ion. Note that in the latter, one nitrogen-oxygen bond in the anion becomes disymmetrized from the rest.

| Compound | N-O ν [cm$^{-1}$] | N-O/Si-O$^{\delta}$ ν Intensity ratio | N-O/Si-O ν I-HW$^{\delta\delta}$ ratio |
|----------|------------------|---------------------------------------|-----------------------------------|
| GUA (KBr) | 1384             | 2.28                                  | 69                                |
| GUA (thin film) | 1392           | 6.14                                  | 458                               |
| GLY      | 1372             | 2.10                                  | 256                               |
| βALA     | 1388             | 1.32                                  | 194                               |
| GABA     | 1361             | 3.52                                  | 297                               |
| PRO      | 1354             | 1.94                                  | 144                               |

**Table 3.** Nitrate-specific, out-of-phase stretching vibration in different bis(guanidine) samples studied by FT-IR. $^{\delta}$Si-O stretching band was chosen as a reference due to stability of its shape and minimal overlapping. $^{\delta\delta}$Intensity multiplied by band width at half height.

**Conclusions**

Four new, guanidinium-appended tetramethyldisiloxane diamides were synthesized using glycine, β-alanine, γ-aminobutanoic acid and L-proline. Those symmetrical molecules are characterized by mixed polarity and high flexibility of the central siloxane bridge, which result in their superficial resemblance to ionic liquids – despite their double positive charge that is easily accessible for counterions. The effect of those – namely: sulphate, chromate, molybdate, benzoate, nitrate, nitrite, azide, chloride and thiocyanate, on relationships between structure, aqueous solubilities, $^1$H NMR, FT-IR spectra and crystallinity – was studied in depth. It was established that – in contrast to typical ionic liquids, most polymers and peptides – the precipitation of studied salts, along with their non-amide precursor, was driven chiefly by ion-ion interactions, and not anion-solvent interactions (Hofmeister-type salting out/in phenomena). For such highly charged, small and flexible molecules, the separation from aqueous solution as an organic phase was found to follow two trends, depending predominantly either on the strength of bonds formed between ions, or pure lipophilicity of the formed salt. The latter mechanism dominated only for the very poorly hydrogen bonding thiocyanate, resulting in proline-derived bis(guanidine) separating most easily. For other anions, evidence of strong, preferential bonding points especially to benzoate, nitrate and chromate, with the first yielding even single crystals from aqueous ethanol. This level of organization turned out to be possible due to the synergy of salt bridges (ion-ion and hydrogen bonding) and very pronounced hydrophobic effect, which resulted in segregation of internal structure into polar and non-polar compartments.
The inclusion of an amide moiety results in a “buffering” effect, with the precursor bis(guanidine) disiloxane salt behaving more erratically, and showing much more pronounced changes in spectra on anion exchange. Presence of an amide group was also responsible for sharp decrease in solubilities of sulphates and molybdates.

| Salt       | GUA-NO₃ | GUA-Bz |
|------------|---------|--------|
| Formula    | C₁₂H₃₄N₈O₇Si₂ | C₂₆H₄₄N₆O₅Si₂ |
| Space group| P       | P 4/n  |
| Unit cell: |          |        |
| a (Å)      | 7.025(5) | 18.8363(2) |
| b (Å)      | 7.408(4) | 18.8363(2) |
| c (Å)      | 13.797(6)| 9.1296(2)  |
| α(°)       | 89.29(4)| 90     |
| β(°)       | 84.16(4)| 90     |
| γ(°)       | 61.91(6)| 90     |
| V(Å³)      | 629.6(5)| 3239.24(10)|
| Z/Z'      | 1/0.5  | 4/0.5  |
| Dₓ (g·cm⁻³)| 1.2148 | 1.1830 |

Table 4. Selected crystal data for salts GUA-NO₃ and GUA-Bz (detailed information are available in crystallographic information files, deposited with the Cambridge Crystallographic Database Centre, as Supplementary publications number CCDC 1886835-1886836; they are available free of charge from www.ccdc.cam.ac.uk).

Figure 7. Structures determined by X-ray diffraction of two symmetry-independent units of bis(guanidinium) salts. (a) GUA-NO₃ shown as a model of balls-and-sticks of arbitrary sizes; and (b) GUA-Bz with thermal ellipsoids shown at the 50% probability level and one set of disordered atoms O1 and C4 (in the second unit this disorder is ignored for clarity). Atomic labels of one asymmetric unit have been shown for non-H atoms.

Figure 8. Fragment of crystal structure of benzoate salt of GUA, showing neatly separated hydrophilic and hydrophobic sections. (a) View perpendicular to main 42 screw axis (grey), (b) view along the [z] axis. Cyan and red dashed lines show the extent of hydrogen bonding. Silicon atoms are coloured yellow, oxygen - red and nitrogen - pale violet.
other anions, especially the three mentioned before, show a marked selectivity towards the guanidinium moiety, with which they most probably form characteristic salt bridge-type connections.

We believe that the acquired body of data - especially the exceptions, noted specific guanidinium-anion interactions and the widespread non-adherence of studied compounds’ solubilities/precipitation trends to the Hofmeister series - reveals the persisting deficits in our understanding of ionic bonding as it affects small and middle-sized, charged organic molecules. Large literature gaps still exist in the spaces between simple inorganic salts, very poorly coordinating ionic liquids and macromolecules, including proteins.

Quite possibly, it is at those boundaries that truly ion-specific interactions can be sieved out from solvent structuring effects, dehydration of solute molecules and intramolecular interferences.

Data availability
All data generated or analyzed during this study are included in this published article (and its Supplementary Information files). Readers are encouraged to direct any further requests to the corresponding author.

Received: 20 March 2019; Accepted: 30 December 2019; Published online: 20 January 2020

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**Acknowledgements**

We would like to extend our gratitude to Professor Martin Chaplin, author of the website “Water Structure and Science” (http://www1.lsbu.ac.uk/water/). This extensive online resource offers a wide spectrum of current references pertaining to many subjects directly and indirectly linked with water structure and its properties. We recommend it to any scientist from neighbouring fields of research who would like to expand their knowledge.

**Author contributions**

Ł.T. and B.L. conceived and supervised the project. Ł.T. and Z.R. performed spectral measurements and analyzed the results. A.K. performed crystallographic determinations. Ł.T. and A.K. prepared the figures. All authors examined the results and reviewed the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41598-020-57539-0.

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