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Molecular Recognition of Creatinine

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Abstract: A host-guest system involving derivatives of 2-amino-4(3*H*)-pyrimidone and creatinine was developed. ^{1}H NMR and ^{13}C NMR experiments give evidence of complex formation A geometry optimization with Gaussian 88 suggests a complex structure in which the 5-membered and the 6-membered heteroring are connected by one long and two shorter hydrogen bonds The hosts described strongly enhance the extraction of creatinine from its aqueous solution into CH_2Cl_2 and $CDCl_3$ As the creatinine concentration in the organic solvents may be determined by measuring the changes in the UV spectrum of the hosts upon complexation, derivatives of 2-amino-4(3*H*)-pyrimidone may eventually be used in optical creatinine sensors

Many host-guest systems in which hydrogen bonds play a crucial role in the recognition process have been described.[1] In an attempt to make use of such systems in analytical chemistry, host molecules for creatinine (**1**) have been developed Hydrogen bonds, which **1** can form with both hydrogen bond acceptors and donors, were the basis for the design of the hosts **3** and **4** (Figure 1)

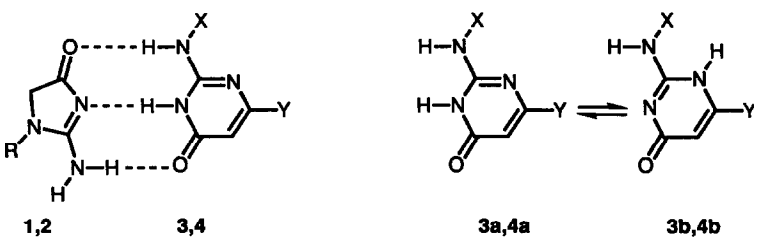

Figure 1. **1** R = CH_3, **2**. R = $CH((CH_2)_6CH_3)_2$, **3** Y = $CH((CH_2)_7CH_3)_2$, X = H, **4** Y = $CH_2CH_2CH_3$, X = 2-naphthyl

Compound **3** proved to be an efficient extractor for **1** After equilibrating a 90 mM solution of **3** in $CDCl_3$ with a saturated solution of **1** in D_2O, the concentration of **1** in the organic phase was 3 2 mM In the analogous experiment without **3**, the concentration of **1** in $CDCl_3$ was 37 times smaller (as determined by ^{1}H NMR)

The complex of **2** and **3** was investigated as a model system[2] for the complex of **1** and **3** In the ^{1}H NMR spectrum of **3** in $CDCl_3$ at room temperature two signals appeared at 13 3 and 11 1 ppm due to ring-NH, their intensities adding up to one proton. This suggests the presence of 2 predominant tautomers, **3a** and **3b** (see Figure 1), which may form a dimer with 3 hydrogen bonds [3] By cooling a solution of **3** to 233 K, the signal for the amino protons was split into 4 signals. Evidently, the slowdown in the rotation of the amino group around the C-NH_2 axis gave rise to distinct signals for the two amino group hydrogens of the two tautomers In the ^{13}C NMR spectrum at 233 K, 7 signals were observed for the aromatic carbons, as each tautomer led to four signals with two of them coinciding

Upon complexation with **2** the spectral properties of **3** changed drastically. In the ^1H NMR of an equimolar solution of **2** and **3** in $CDCl_3$ only one ring-NH signal appeared at 14 7 ppm at room temperature At 233 K the amino groups of **3** and **2** gave rise to 4 signals. In the ^{13}C NMR spectra of **3** with increasing concentration of **2**, the intensity of 3 signals due to **3b** decreased whereas that of 3 signals due to **3a** increased Hence, compound **2** forms a complex with **3a** while **3b** gradually disappears upon complex formation

CPK models as well as crystal structures of similar compounds show that the heteroatoms of **3** directly involved in the hydrogen bonds almost lie on a straight line In contrast, the heteroatoms of **1** involved in hydrogen bonding form a triangle due to the 5-ring structure of **1** In the complex of **3** with **1**, the three hydrogen bonds therefore cannot exhibit equal lengths Instead, two stable, planar complex structures are conceivable in which either of the two peripheral hydrogen bonds is clearly longer than the other two

Using the Gaussian 88 program, the geometry of a simplified host-guest complex was optimized at the HF/4-31G level to yield complex **5a** (Figure 2). A structure similar to **5b**, representing a second energy minimum, could not be found. An explanation for this finding was therefore sought.

Glycocyamidine and isocytosine, the two components of **5a** and **5b**, were optimized in their geometry and the corresponding dipole moments calculated In **5a** the dipoles were found to form an angle of 130°, whereas in **5b** they enclose an angle of 122° (Figure 2). As the interaction energy between two dipoles decreases with the increase of the angle they enclose, **5a** can be supposed to be more stable than **5b** This crude argument is supported by the fact that during the geometry optimization of **5b**, d_1 increased while d_3 decreased, the structure thus becoming more similar to **5a**

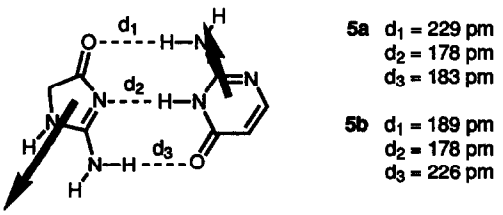

Figure 2. Simplified host-guest complex **5** with the calculated dipole moments (represented as arrows) of glycocyamidine (7 93 D) and isocytosine (4 71 D)

The extinction coefficient of **3** increased slightly upon complexation with creatinine To enhance this effect host **4** was synthesized Upon addition of **1**, the absorption maximum of **4** was shifted only slightly but the extinction coefficient rose substantially (Figure 3)

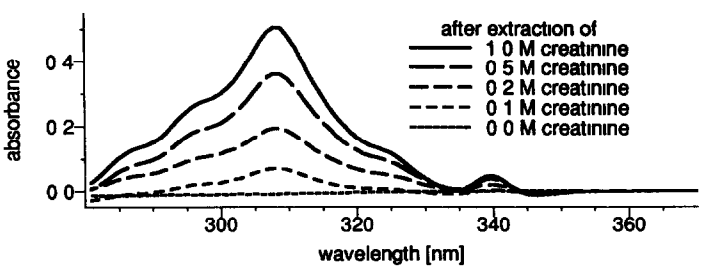

Figure 3. UV spectrum of **4**, 1 mM in CH_2Cl_2 Sample cell. equilibrated with different aqueous solutions of creatinine (**1**), reference cell equilibrated with water.

The extraction results and the optical properties of **4** are promising and compounds similar to **4** may eventually be used in an optical creatinine sensor An attempt is in progress to improve the stability of the host-creatinine complex by introducing another hydrogen bond

EXPERIMENTAL

2-Amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone (3). *2-Octyldecanoic acid*[4] 2,2-Dioctylmalonic acid diethyl ester[5] (10.0 g, 26 mmol) and 18-crown-6 (6.9 g, 26 mmol, purum > 97%) were dissolved in toluene (100 mL, puriss. p a) and a solution of KOH (1.6 g, 29 mmol) in EtOH was added. The EtOH was distilled off and the reaction solution stirred for 10 5 h at 100 °C After cooling to 80 °C, a solution of KOH (2 1 g, 37 mmol) in water (20 mL) was added and stirring continued for 7 h at 100 °C Cooled to room temperature, the solution was acidified to pH 1 with 1 N HCl Extraction with Et_2O, drying over $MgSO_4$ and solvent evaporation yielded the raw product as a slightly yellow oil (5 47 g, 74%)

2-Octyldecanoyl chloride Freshly distilled $SOCl_2$ (4.6 g, 38 mmol) was added to the above 2-octyldecanoic acid (5.45 g, 19 mmol). When gas evolution became weak, the temperature was slowly raised and reached 70 °C after 2 h, where it was kept for another hour. Surplus thionyl chloride was then distilled off

4-Octyl-3-ketododecanoic acid ethyl ester[6] A solution of monoethyl malonate[7] (5 27 g, 40 mmol) in THF (250 mL, absoluted over NaH) was cooled to -60 °C and a solution of butyl lithium in hexane (=0 80 mol, pract , ≈1 6 M) was added in portions of 10 mL. After letting the temperature rise to -20 °C, the solution was re-cooled to -60 °C and 2-octyldecanoyl chloride (5 47 g, 19 mmol) was added dropwise The reaction solution was stirred for 15 min at -60 °C and then for 2.5 h at room temperature After dilution with Et_2O the organic phase was washed with saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and the solvent evaporated The raw product was purified by flash chromatography (silica gel, particle size 0 043-0 060 mm, pressure 0 3 to 0 4 bar) with EtOAc/hexane (1:15) and triple distillation at 200 °C / 0.1 torr in a Kugelrohr apparatus Two impurities from 2-(2-octylnonanoyl)malonic acid diethyl and monoethyl ester were tolerated

*2-Amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone (3)*[8] The above reaction product (2 40 g) and guanidine carbonate (0 61 g, 3 4 mmol, purum > 99%) were refluxed in EtOH (25 mL, absoluted over Na) for 12 5 h Evaporation of the solvent yielded a yellow oil. After purification by flash chromatography (silica gel) with EtOAc the product was dissolved in THF and Et_2O, washed with water and the solvent evaporated The product was a slightly yellow, very viscous oil (1 03 g, 9% from diethyl 2,2-dioctyl-malonate) IR ($CHCl_3$, cm^{-1}) 3479w, 3325w, 3120w, 2928s, 2856s, 1656s, 1469m, ^1H NMR ($CDCl_3$, δ in ppm) δ 13 2 and 11 0 (2 s, br , together 1 H, NH of two tautomers), 6 5 (s, br , 2 H, NH_2), 5 58 (s, 1 H, aromatic), 2 27 (m, 1 H, $CH(CH_2)_2$), 1 6-1 4 (br , 4 H, $CHCH_2$), 1 4-1 1 (br , 28 H, $(CH_2)_7CH_3$), 0.86 (t, J = 7 Hz, 3 H, CH_3), MS *m/z* 349 (M^+, 2), 138 (100), Anal Calcd for $C_{21}H_{39}N_3O$ C, 72 16, H, 11 25, N, 12 02 Found C, 71 86, H, 11 34, N, 11 91

2-Amino-1,5-dihydro-1-(1-heptyloctyl)-4-*H*-imidazol-4-one (2). *8-Pentadecylcyanamide sodium salt*[9-11] A solution of BrCN (1.51 g, 14 3 mmol, Janssen Chimica 97%) in Et_2O (10 mL, puriss p a) was added dropwise within 15 min to a stirred solution of 8-pentadecylamine[12](6 50 g, 28 6 mmol) in EtOAc (30 mL, puriss p a) at 0 °C, upon which white crystals of 8-pentadecylammonium bromide began to precipitate After cooling to -10 °C and further precipitation, the crystals were filtered off and their weight was determined NaOEt (21 mmol) in EtOH (15 mL) was added to the reaction solution to neutralize unprecipitated 8-pentadecylammonium bromide and to convert 8-pentadecylcyanamide to its sodium salt. The solvent was then evaporated together with the unreacted BrCN

*2-Amino-1,5-dihydro-1-(1-heptyloctyl)-4-*H*-imidazol-4-one (2)* The 8-pentadecylcyanamide sodium salt obtained as described above was dissolved in acetone (45 mL, puriss. p a) and a solution of 2-chloroacetamide (1 34 g, 14 3 mmol) in acetone (15 mL) was added The solution immediately turned slightly pink After refluxing for 4 h the reaction mixture was allowed to stand for 20 h at room temperature The solvent was removed, the residue dissolved in Et_2O and washed subsequently with 0.1 N NaOH (with added NaCl to improve phase separation), 0 1 N HCl and twice with water After drying over anhydrous Na_2SO_4, the solvent was evaporated yielding a yellow oil (4 84 g) Purification by flash chromatography (silica gel) with EtOAc/EtOH (8 1) and recrystallization from hexane/EtOH gave 2 as a white powder (0 77 g, 17 %

calculated from 8-pentadecylamine) mp 149 °C; IR ($CHCl_3$) 3488w, 2929s, 2857s, 1693m, 1656s, 1558m, 1493s, 1305m; ^1H NMR ($CDCl_3$) δ 7 8 (s, br., 2 H, NH_2), 3.67 (s, 2 H, $(CO)CH_2$), 3.55-3 44 (m, CH), 1.6-1 1 (m, 24 H, $(CH_2)_6CH_3$), 0 87 (t, $J = 7$, 6 H, CH_3), MS *m/z* 311 ($(M+2)^+$, 10), 310 ($(M+1)^+$, 54), 309 (M^+, 61), 100 (100); Anal Calcd for $C_{18}H_{35}N_3O$· C, 69.58, H, 11.40; N, 13.58, Found· C, 69 87; H, 11.38; N, 13 47.

2-(2-Naphthylamino)-6-propyl-4(3*H*)-pyrimidone (4). *6-Propyl-2-methylthiouracil* [13] 4-Hydroxy-2-mercapto-6-propyl-pyrimidine (17 0 g, 100 mmol, purum) and NaOH (4.30 g, 0.108 mmol) were dissolved in water (20 mL) at 80 °C. The temperature was lowered and EtOH (40 mL, puriss. p a.) and MeI (14.4 g, 100 mmol) were added. After 30 min stirring at 45 °C and heating to 80 °C for a few minutes, the reaction solution was cooled to 10 °C. Crystals precipitated and were filtered off. More crystals precipitated upon addition of AcOH (450 mg, puriss. p.a.) to the mother liquor The combined product was washed with water and recrystallized from EtOH (90 mL) to yield white crystals (16.7 g, 91%), mp 155 °C

2-(2-Naphthylamino)-6-propyl-4(3H)-pyrimidone (4).[14] 6-Propyl-2-methylthiouracil (1.00 g, 5.4 mmol) and 2-naphthylamine (0.77 g, 5.4 mmol, purum, carcinogenic!) were dissolved in o-xylene (20 mL, puriss p a.) and heated to 140 °C for 90 h After distilling the solvent off the raw product was purified by flash chromatography (silica gel) with EtOAc/acetone (2:1). The fractions containing the product were combined and concentrated to a few mL, from which **4** crystallized. It was recrystallized twice from EtOH and once from toluene to yield off-white crystals (0.21 g, 14%) mp 194 °C; IR ($CHCl_3$) 3390w, 2960m, 2870m, 1665s, 1633s, 1587s, 1510m, 1455m, 1360m; ^1H NMR (DMSO) δ 8.37 (s, 1 H, CH aromatic), 7.9-7 3 (m, 6 H, CH aromatic), 5.76 (s, 1 H, CH(C=O)), 2.43 (t, $J = 7$, 2-H, $CH_2CH_2CH_3$), 1 70 (sext., $J = 7$, 2 H, $CH_2CH_2CH_3$), 0.95 (t, $J = 7$, 3 H, CH_3), ^1H NMR ($CDCl_3$) δ 10.8 (br , 1 H, NH), 9.1 (br , 1 H, NH), 8.45 (s, 1 H, CH aromatic), 7.9-7.2 (m, 6 H, CH aromatic), 5.82 and 5.79 (2 s, together 1 H, CH(C=O)), 2.53 (t, $J = 7$) and 2.2 (br.) (together 2 H, $CH_2CH_2CH_3$), 1 81 (sext , $J = 7$) and 1 6 (br) (together 2 H, $CH_2CH_2CH_3$), 1.03 (t, $J = 7$) and 1.0 (br.) (together 3 H, CH_3). Evidently, **4** in $CDCl_3$ occurs in two tautomeric forms, one of which is involved in a slow (conformational ?) equilibrium leading to very broad signals. MS *m/z* 280 ($(M+1)^+$, 11), 279 (M^+, 51), 28 (100); Anal. Calcd for $C_{17}H_{17}N_3O$. C, 73 10, H, 6 13, N, 15 04, Found· C, 73 01, H, 6.27; N, 14 82.

Acknowledgement Part of this work was supported by the Swiss National Science Foundation.

REFERENCES AND NOTES

(1) See e g Vögtle, F. *Supramolecular Chemistry*, J. Wiley & Sons. Chichester 1991, and ref. cited therein.
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