Tetrahedron Vol. 49, No. 35, pp. 7627-7636, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Neutral Hosts for the Complexation of Creatinine

Philippe Bühlmann*,§ and Wilhelm Simon[1]

Department of Organic Chemistry, Swiss Federal Institute of Technology (ETH), 8092 Zürich, Switzerland

§Department of Chemistry, School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113, Japan

(*Received in Japan* 12 *April* 1993; *accepted* 1 *July* 1993)

Abstract: Three hydrogen bonds are formed between creatinine and derivatives of 2-amino-4(3*H*)pyrimidone. Changes in the tautomer ratio of the latter upon complexation can be observed by UV spectroscopy. The formation of a further hydrogen bond between creatinine and a 4-aminoacridin-5-yl-amino-substituted 4(3*H*)pyrimidone seems to be sterically prevented. The properties of three other, quite different compounds assumed to form three hydrogen bonds with creatinine have also been investigated. Dodecyl 4-octadecylallophanate and 2,6-bis(dodecylamino)-pyridine do not associate appreciably with creatinine, which can be explained by the formation of an intramolecular hydrogen bond in the former compound and the lack of tautomerization of the latter. With 6-(1-heptyloctylamino)-2(1*H*)-pyridone, however, creatinine forms a complex that is almost as stable as those with 2-amino-4(3*H*)-pyrimidone derivatives.

Creatinine (**1**) is one of the most important analytes in clinical chemistry. We have, therefore, been searching for hosts complexing this guest and have recently reported on the formation of a complex between 2-amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone (**2**) and the creatinine analogue 2-amino-1,5-dihydro-1-(1-heptyl-octyl)-4-*H*-imidazol-4-one (**3**), as evidenced by ^{13}C and ^{1}H NMR.[2] The presumed pattern of interactions between these two compounds (as shown in Figure 1) could now also be confirmed by UV spectroscopy.

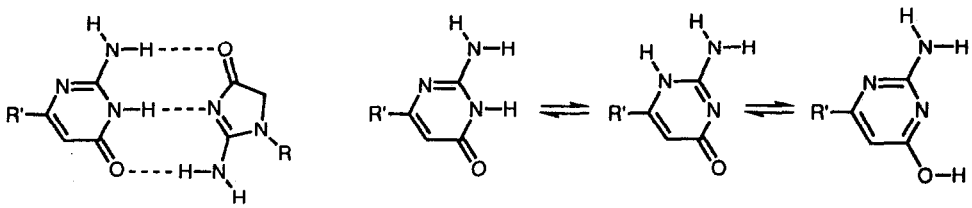


Figure 1. **1**: R = CH_3, **3**: R = $CH((CH_2)_6CH_3)_2$, **2**: R' = $CH((CH_2)_7CH_3)_2$, **4** (isocytosine): R' = H.

*2-Amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone (2)*

A 0.51 mM solution of the pyrimidone **2** in methylene chloride shows an absorption maximum at 284 nm with a molar absorption coefficient of $7.45 \cdot 10^3$ M^{-1} cm^{-1}. Addition of the creatinine analogue, **3**,

which does not absorb down to 280 nm, led to increases of up to 14% in the absorbance at that wavelength (Figure 2) and a slight bathochromic shift of the absorption maximum to 286 nm. In agreement with the interpretation of the NMR data,[2] these results can be explained by a change in the ratio of the tautomers **2a** and **2b** (cf. Figure 1) with increasing concentration of **3**. Upon addition of the latter, the concentration of **2a** increases, whereas that of **2b** decreases. As the alkyl substituent only slightly influences the chromophore of the pyrimidone, **2**, the spectrum of this compound ought to resemble that of isocytosine (**4**, cf. Figure 1).

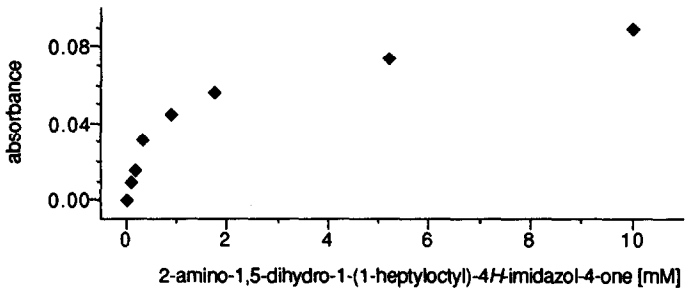


Figure 2. Absorbance of 2-amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone, **2**, at 284 nm in methylene chloride (0.51 mM) in function of the concentration of the creatinine analogue, **3** (1 mm sample cell; reference cell: 0.51 mM of **2** in methylene chloride).

Comparing the UV spectra of isocytosine and some of its derivatives in different solvents, it was concluded that in aprotic solvents, such as diethyl ether and acetonitrile, only tautomer **4a** occurred, whereas in aqueous solution, the ratio of **4a** and **4b** is close to 1:1.[3-5] Furthermore, in aqueous solution, tautomer **4a** has absorption maxima at 288 and 221 nm and tautomer **4b** at 259, 220 and 203 nm.[6] The magnitude of the dipole moments, which for **4a** is only about half that of **4b** (6.512 and 11.275 D,[6] or 4.5 and 10 D),[7] matches this interpretation.

Our data on pyrimidone **2** and the literature data for isocytosine thus agree very nicely. Tautomer **2a**, which was found to form a complex with creatinine, is analogous to the isocytosine tautomer **4a**. The absorption maxima of the two differ by only few nanometers. The relatively small increase of 14% in absorbance of pyrimidone **2** upon addition of the creatinine analogue, **3**, suggests that **2a** is the major tautomer in methylene chloride even in the absence of the creatinine analogue. This finding again matches well with the fact that **4a** in apolar solvents is the major tautomer of isocytosine. Under the assumption that tautomer **2b** does not show a significant absorption at 284 nm (- which seems reasonable when comparing its spectrum to that of the isocytosine tautomers -)[5] and that at a concentration of 10.0 mM of the creatinine analogue, **3**, only the host tautomer **2a** exists, the molar absorption coefficient of the latter is approximated as $9 \cdot 10^3$ M^{-1} cm^{-1}. This value corresponds well to that of the isocytosine tautomer **4a** given by Hélène and Douzou ($8.7 \cdot 10^3$ M^{-1} cm^{-1}; in water)[4] and thus confirms the consistency of the above argumentation. By extrapolating the absorbance of the pyrimidone, **2**, to very high concentrations of the creatinine analogue, **3**, a more precise result for the molar absorption coefficient should be obtained, but unfortunately this was not possible. A simplified characterisation of the system containing both compounds **2** and **3** would at least require the knowledge of the tautomerization constant, K_t, and the dimerization constant, K_d, of the host as well as the association constant, K_a, of host and guest (neglecting thereby further effects such as the dimerization of the guest or the formation of more than one type of dimers of the host). An attempt to determine K_t and K_d from ^{1}H NMR data of solutions of the pyrimidone, **2**, was made by expressing the chemical shifts of the signals of that compound as a function of its total concentration. However, as this function contains the product $K_t K_d$, the independent determination of

these two constants was not possible. Consequently, ^{1}H NMR data could not be used to confirm the 1:1 stoichiometry of the complex with creatinine, which we assume given all other spectroscopic evidence.[2]

So far, in most investigations on isocytosine, only the presence of the keto tautomers **4a** and **4b** has been considered. However, Blagoi and co-workers[8] presented IR spectroscopic data as well as semi-empirical quantum-mechanical calculations suggesting that the enol tautomers of isocytosine, particularly **4c** (cf. Figure 1), may be much more stable than thought previously. We must, therefore, point out that during this work no evidence for a tautomer analogous to **4c**, namely **2c**, was found. In particular, the IR spectra of **2** in chloroform (1.7 mM and higher concentrations) showed no signals above 3500 cm^{-1}, as would be expected for an OH stretching vibration. Instead, a sharp signal at 3479±3 cm^{-1}, presumably corresponding to an asymmetric NH_2 stretching vibration, was found. Lowering the sample concentration, the intensity of this signal increased relatively to the other signals, suggesting it to originate from a monomeric form of **2**.

2,6-Bis(dodecylamino)-pyridine (5)

A substantial disadvantage of the use of 6-amino-4(3*H*)-pyrimidone derivatives as creatinine hosts is that a substituent to which an additional hydrogen bond may be formed cannot be attached to the oxygen atom. Therefore, 2,6-bis(dodecylamino)-pyridine (**5**, Figure 3), with a nitrogen in the corresponding position, was synthesized. Though tautomer **5a** (cf. Figure 3), which dominates in pure solutions, does not exhibit the required positioning of the hydrogens, tautomer **5b** was assumed to be a possible host for creatinine. A change in the ratio of the tautomers upon complexation would be ideal for an optical detection of the analyte.

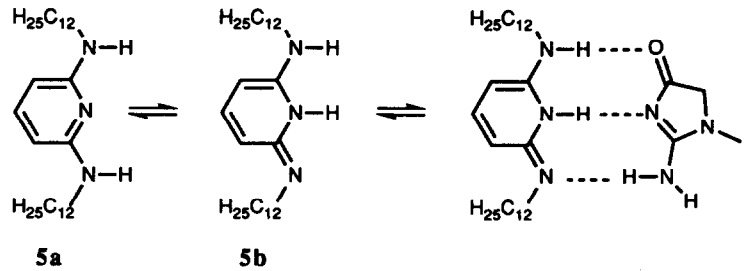


Figure 3. Hypothetical creatinine binding mechanism of 2,6-bis(dodecylamino)pyridine (**5**).

The ^{1}H NMR spectrum of 2,6-bis(dodecylamino)-pyridine (7.0 mM) in DMSO-d_6 gave evidence for the presence of tautomer **5a** only, the aromatic hydrogens forming an A_2B spin system (cf. Experimental) and only one signal each appearing for $N\underline{H}CH_2$ and $NHC\underline{H}_2$. After addition of creatinine in a 19.6-fold excess, the spectral properties of the diaminopyridine, **5**, did not change at all. The position of all signals due to the host, including that of N<u>H</u>, remained the same within 0.01 ppm and no new signals giving evidence for a second tautomer appeared. In contrast, a comparison of the chemical shifts of the nitrogen-bound hydrogens in solutions of 2-amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone (100 mM), **2**, and the creatinine analogue (100 mM), **3**, with those of a solution containing both at the same concentration revealed substantial differences. In the presence of the creatinine analogue, the signal of the amino hydrogens of the amino-pyrimidone was shifted from 6.5 to 6.6 ppm, while the signal of the hydrogen bound to the ring-nitrogen underwent a shift from 10.7 to 11.3 ppm.

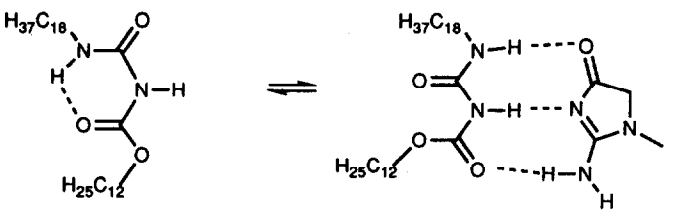
Equilibration of a 1.8 mM (0.12 wt %) solution of the diaminopyridine, **5**, in $CDCl_3$ with solid creatinine led to no observable increase in creatinine concentration as compared with a saturated solution of this guest alone in the same solvent. IR spectra of solutions of the diaminopyridine, the creatinine analogue, as well

as of both of them (in $CHCl_3$, 1 wt % each) gave no evidence for the formation of a complex of the two compounds, even though several broad bands between 2500 cm^{-1} and 3500 cm^{-1} indicated the dimerization of both of them.

It must be concluded that no tautomerization of the diaminopyridine occurred and, therefore, no complex bound by three hydrogen bonds was formed. A complex between tautomer **5a** and creatinine, under formation of only two hydrogen bonds, cannot be excluded, but the solubility experiment shows that interactions of such a type must be rather weak.

Dodecyl 4-octadecylallophanate (6)

In a further effort to find a substitute for the 2-amino-4(3*H*)-pyrimidone derivatives, the suitability of an allophanate ester as creatinine host was investigated. Because N-unsubstituted compounds of this class are on the whole poorly soluble even in water, the N-4 substituted dodecyl 4-octadecylallophanate (**6**, Figure 4) was chosen. It is worth mentioning that this compound was much less soluble in any solvent than was anticipated considering that the molecule consists of a polar central unit substituted with two long alkyl chains. The concentration of 1 wt % in chloroform used in the IR experiments was on the limit of solubility, and as low as 0.2 wt % in DMSO-d_6 was not soluble at all.



6

Figure 4. Hypothetical complex between dodecyl 4-octadecylallophanate (**6**) and creatinine.

The interaction of the allophanate derivative and the creatinine analogue was investigated by IR spectroscopy, as described above for the diaminopyridine. A notable shift was only observed for the NH stretching vibration band of the allophanate (3363 cm^{-1}, sharp), which shifted to 3356 cm^{-1} in the presence of the creatinine analogue (the second sharp NH stretching vibration band due to the allophanate was observed at 3430 cm^{-1}). The lack of larger changes indicates that the interaction between the two compounds is rather weak. ^1H NMR experiments confirmed this finding. The presence of the creatinine analogue (0.17 wt %), **3**, led to a shift of the signal of CONHCO (singlet, in $CDCl_3$ 0.17 wt %) from 6.81 to 7.00 ppm, whereas that of $NHCH_2$ (triplet) underwent an even smaller shift (7.70 to 7.72 ppm). When a 1.0 wt % solution of the allophanate in $CDCl_3$ was equilibrated with solid creatinine, no increase in creatinine concentration as compared with a saturated solution of this compound alone in the same solvent was observed.

The interaction between creatinine and the allophanate may be weak due to the ease of formation of an intramolecular hydrogen bond involving $NHCH_2$ in the latter compound (cf. Figure 4). The 6-membered ring thus obtained can be suspected to be very stable, and the minute influence of the creatinine analogue on the chemical shift of $NHCH_2$ would be easily understandable. Other compounds exhibiting similar intramolecular hydrogen bonds are known to occur, for example, in hydroxyacetophenones, malonic acid diamides, and the enol tautomers of β-diketones.[9] Without breaking the intramolecular hydrogen bond, only the formation of one or two hydrogen bridges between creatinine and the allophanate is possible. Just as in the case of the diaminopyridine, this can lead only to an insignificant complexation strength and, consequently, to no substantial extraction of creatinine into the organic phase.

6-(1-Heptyloctylamino)-2(1H)-pyridone

As shown earlier, 2-amino-4(3*H*)-pyrimidone derivatives may easily tautomerize and thus form dimers bound by three hydrogen bonds.[2] The suppression of this dimerization was expected to lead to an increase in the concentration of the free host and, consequently, to a greater ability of solutions of this compound to extract creatinine. Because a substitution of the heteroatoms in 2-amino-4(3*H*)-pyrimidones could not be used to prevent the formation of the 4(1*H*)-pyrimidone tautomer without abolishing its complexing ability, the nitrogen in the 3-position was replaced by a carbon. Therefore, 6-(1-heptyloctylamino)-2(1*H*)-pyridone (**7**) was synthesized, hoping that it would complex creatinine in a manner analogous to **2** (as shown in Figure 5).

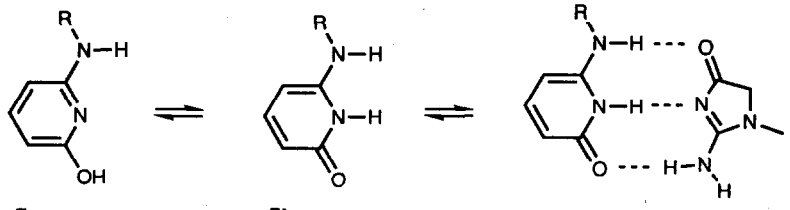


Figure 5. Two tautomers of the pyridone **7** and the presumed complex with creatinine. R: $CH[(CH_2)_6CH_3]_2$.

This compound may occur as tautomers **7a** and **7b** (cf. Figure 5), which represent a pyridinol-pyridone equilibrium, on which many investigations have been made.[10] An important factor influencing such equilibria is the substitution of these compounds. For some highly electronegative substituents at C-6 (e.g. chlorine), a dominance of the 2-pyridinol tautomer has been observed even in solution.[11] However, for several monoamino-substituted 2-pyridones investigated by Fujimoto and Inuzuka,[12] MINDO/3 calculations showed the 2(1*H*)-pyridone tautomers to be more stable than the corresponding 2-pyridinols, the energy difference between these two tautomers being smallest for the 6-amino compound. It was experimentally found by the same authors that the 6-amino-2(1*H*)-pyridone tautomer dominates in THF, but that upon addition of acetonitrile, the tautomer ratio becomes almost 1:1. The formation of dimers consisting of one tautomer **7a** and **7b** each seems possible, but ^{13}C NMR spectra of 6-ethylamino-2-pyridone in THF-d_8 at −120 °C revealed the presence of one tautomer only.[13] Furthermore, no evidence for the formation of a dimer of two tautomers analogous to **7a** and **7b** (cf. Figure 5) was found.

^1H NMR spectra of 6-(1-heptyloctylamino)-2(1*H*)-pyridone, **7**, and the creatinine analogue, **3**, showed that both NH of the pyridone are involved in hydrogen bonding and thus supported the assumption of a complex as shown in Figure 5. In the spectrum of a 7.2 mM solution of **7** in DMSO-d_6, the hydrogen bound to the ring nitrogen gave a very broad signal at low field (11.1 ppm), whereas the NHCH appeared as a sharp doublet. After adding creatinine (25.6 mM), the signal of the ring-NH moved downfield by 0.1 ppm, while the NHCH signal changed neither in position nor in shape. Of the signals for the aromatic hydrogens, two did not shift at all (7.13 and 5.39 ppm), whereas one doublet moved from 5.80 to 5.85 ppm. With $CDCl_3$ instead of DMSO-d_6 and **3** (103 mM) instead of creatinine, signals of **7** (8.3 mM) shifted from 5.61 to 6.68 ppm for the NHCH and from 5.64 to 5.50 ppm, 7.25 to 7.23 ppm and 5.30 to 5.31 ppm for the aromatic hydrogens. Using this solvent, the signal of the hydrogen bound to the ring nitrogen was too broad to be observed.

For a solubility test, solid creatinine was added to a 116 mM (2.5 wt %) solution of the pyridone in $CDCl_3$. After equilibration at room temperature, the creatinine concentration, quantified by ^1H NMR, was 7.3 mM, which is more than two orders of magnitude higher than that in $CDCl_3$ saturated with creatinine alone. The influence of water on the complex stability was found to be relatively small. A host solution of the

same concentration as above (116 mM, 2.5 wt %) was equilibrated with solid creatinine and D_2O. The creatinine concentration in the organic phase, determined as above, was 1.7 mM, which is 4.3 times smaller than in the first experiment. A similarly small effect of the presence of water has also been obtained by Adrian and Wilcox[14] for a complex between 2-aminopyrimidine and a host having two benzoic acid moieties, both forming two hydrogen bonds with the guest.

As the association constant of the complex of 2-amino-6-(1-octylnonyl)-4(3*H*)-pyrimidone, **2**, and creatinine could not be determined, as mentioned above, a comparison of the complexing abilities of the pyrimidone, **2**, and the pyridone, **7**, had to be made on the basis of the overall extraction, which in view of a creatinine sensor is also the most relevant criterion. When equilibrating a 90 mM solution of the pyrimidone in $CDCl_3$ with D_2O saturated with creatinine, a total creatinine concentration of 3.2 mM in the organic phase had been obtained. In the corresponding experiment with the pyridone (116 mM), the creatinine concentration in the organic phase was only 1.7 mM. Hence, the pyrimidone is a better host for creatinine than the pyridone, even though it has the manifest disadvantage of forming fairly stable dimers.

2-[5-(4-amino)-acridinylamino]-6-(1-octylnonadecyl)-4(3H)-pyrimidone (8)

Considering the above results, it seemed desirable to introduce a fourth hydrogen bond into the creatinine complex by using a 2-amino-4(3*H*)-pyrimidone derivative. One possibility of achieving this seemed to be given by the attachment of a substituent to the amino group. Such a substituent should be fairly rigid since a flexible one, upon complexation, would cause a substantial loss of entropy and thus contribute only very little to the stabilization of the desired host-guest complex. Thus, 2-[5-(4-amino)-acridinylamino]-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (**8**, Figure 6) was synthesized.

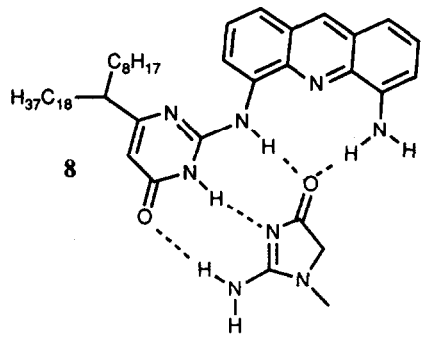


Figure 6. Hypothetical complex between 2-[5-(4-amino)-acridinylamino]-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (**8**) and creatinine.

The main disadvantage of this compound proved to be its chemical instability. UV-vis spectra of freshly prepared solutions of **8** in methylene chloride showed absorption maxima at 440, 378 and 276 nm with molar absorption coefficients of $9.8 \cdot 10^3$, $7.1 \cdot 10^3$ and $1.6 \cdot 10^5$ $M^{-1}cm^{-1}$, respectively. However, upon exposure to ambient light, these UV-vis spectral properties changed within minutes, even though the solvents had been purified by filtration through basic alumina just prior to spectroscopy. In decane, though, a somewhat greater stability was observed.

When adding the creatinine analogue (to obtain a 16.9 mM solution), **3**, to the acridinylamino-pyrimidone (0.47 mM), **8**, in methylene chloride, an increase of only a few per cent in the absorbance of the latter was observed between 500 and 310 nm. At shorter wavelengths, the optical density at this concentration was

too high for measurements. Due to the instability of the host, extraction experiments could not be made with methylene chloride, and hexane was taken instead. However, using a 0.1 mM solution of the acridinylamino-pyrimidone and 500 mM creatinine, no significant changes in absorbance were found that would have evidenced the extraction of creatinine from the aqueous into the organic phase.

Two different explanations, or the combination of both, may be given for these findings: Either the extraction of creatinine did take place but could not be detected optically as the strong chromophore acridine, uninfluenced by the guest, would dominate the spectrum and mask relevant changes, or the extraction was inhibited. The latter may be due to steric limitations. Short-range repulsive interactions between the non-bonding electron pair of the nitrogen in the 1-position of the 4(3*H*)-pyrimidone and a hydrogen of the other aromatic host moiety may occur. As a result of such a repulsion, rotation around the two exocyclic C-N axes may lead to a loss of coplanarity between the two aromatic rings. The NH_2 group supposed to be involved in the fourth hydrogen bond would be moved out of the 4(3*H*)-pyrimidone-guest plane and the expected formation of a fourth hydrogen bond could not occur. Simultaneously, the exocyclic NH may leave the pyrimidone-guest plane as well.

Evidence for such a repulsion was indeed found in crystal structures of compounds containing the phenyl-2-pyridylamine substructure (only ten such structures were found in the Cambridge Structural Database). Five of these showed the two aromatic rings to deviate appreciably from coplanarity. For the other five, almost complete coplanarity with angles of only a few degrees between the two ring planes was found. In the latter group, the bond angles at the exocyclic nitrogen and the adjacent carbon of the phenyl ring often significantly deviated from 120°. Both the deviations from coplanarity as well as the angle deformations thus strongly suggest the presence of a relevant repulsion. Occurring in the acridinylamino-pyrimidone, such a repulsion must greatly influence the distance between the hetero-atoms thought to be involved in hydrogen bonding and makes an accurate prediction of the host conformation difficult, even when using refined quantum-mechanical models.

Conclusions

The results with the allophanate and the diaminopyridine show that the formation of only two hydrogen bonds is insufficient for an appreciable complexation of creatinine. Under the formation of three hydrogen bonds, however, both aminopyrimidones and the aminopyridone allow for a substantial extraction of that guest into an apolar solvent. The difficulties with the optical detection of the creatinine extraction performed with the acridinylamino-pyrimidone as well as the complications with the accurate prediction of the conformation of that host let us conclude that 2-arylamino-4(3*H*)-pyrimidones are inappropriate for the introduction of a fourth hydrogen into the creatinine complex. A further attempt to replace the oxygen by another hydrogen bond acceptor is in progress.

EXPERIMENTAL

2,6-Bis(dodecylamino)pyridine (5).[15] To 2,6-dibromopyridine (4.74 g, 20 mmol, pract. ~95%) and 1-dodecylamine (14.8 g, 80 mmol, puriss.) in an argon atmosphere, a solution of $CuSO_4$ (1.3 mmol, 200 mg) in a few drops of water was added. The reaction mixture was heated to 220 °C, kept there for a few minutes and then stirred at 180 °C for 19 h. A brown suspension formed. It was dissolved in Et_2O and washed subsequently with 1 N NaOH, three times each with water and 0.25 N HCl and once with sat. aqueous NaCl. After drying (Na_2SO_4) and evaporating the solvent, a dark brown solid was obtained, which was purified by flash chromatography, first with toluene eluting 2-bromo-6-dodecylaminopyridine as major impurity, then with hexane/EtOAc (3:1). As the crystalline product eluted was still brown, it was further puri-

fied by three recrystallizations from EtOAc/EtOH. Each crystallization took about one week. A final flash chromatography with hexane/Et_2O (2:1) gave 2,6-bis(dodecylamino)pyridine as white crystals (250 mg, 3%): mp 45 °C; IR ($CHCl_3$) 3430w, 2928s, 2855s, 1593s, 1468s, 1370w, 1306w; ^1H NMR ($CDCl_3$) δ 7.23 (t, J = 8, 1 H, ar), 5.69 (d, J = 8, 2 H, ar), 4.19 (t, J = 5, 2 H, NH), 3.15 (q, J = 7, 4 H, CH_2NH), 1.58 (quint., J = 7, 4 H, CH_2CH_2NH), 1.5–1.1 (m, 36 H, $(CH_2)_9CH_3$), 0.88 (t, J = 7, 6 H, CH_3); ^1H NMR (DMSO-d_6) δ 6.96 (t, J = 8, 1 H, ar), 5.83 (t, J = 6, 2 H, NH), 5.53 (d, J = 8, 2 H, ar), 3.13 (q, J = 6, 4 H, CH_2NH), 1.48 (quint., J = 7, 4 H, CH_2CH_2NH), 1.4–1.1 (m, 36 H, $(CH_2)_9CH_3$), 0.85 (t, J = 7, 6 H, CH_3); EI-MS: 446 ($(M+1)^+$, 13), 445 (M^+, 42), 18 (100); Anal. Calc for $C_{29}H_{55}N_3$ (445.76): C, 78.14; H, 12.44; N, 9.43; Found: C, 78.29; H, 12.32; N, 9.36.

Dodecyl 4-Octadecylallophanate (6). *Dodecyl carbamate.* The synthesis of carbamic esters of lower alcohols is reported in literature.[16] Cyanogen bromide (12.9 g, 122 mmol, 97%) was dissolved in CH_2Cl_2 (12 mL, puriss. p.a.), the solution cooled to 0 °C and 1-dodecanol (42.4 g, 228 mmol, puriss.) in CH_2Cl_2 (50 mL) added. To start the reaction, acetyl chloride (0.23 g, 2.9 mmol, puriss p.a.) was added, producing a catalytic amount of HBr. After stirring at 0 °C overnight and at room temperature for 30 h, probes analysed by GPC revealed that the reaction still had not started. The reaction mixture was, therefore, refluxed for 2.5 h, cooled and washed three times with aqueous NaCl solution (1/10 sat.). After drying over Na_2SO_4, evaporation of the solvent and distilling off the unreacted 1-dodecanol at 0.3 torr/150 °C, a slightly yellow oil was obtained. It was purified by flash chromatography with hexane/EtOAc (2:1) as eluant, the fractions being controlled by TLC. To visualize the product, the TLC slabs were first sprayed with furfural (2% in acetone) and then with sulfuric acid (10% in acetone).[17] Heating made dodecyl carbamate appear as a dark blue spot. The product was isolated as a white solid (140 mg, 0.6%), mp 83 °C.

Dodecyl 4-Octadecylallophanate (6).[18] To a solution of dodecyl carbamate (140 mg, 0.61 mmol) in Et_2O (3 mL, puriss. p.a.) under a N_2 atmosphere, a solution of butyl lithium (0.64 mmol) in hexane was added dropwise. A white suspension formed within seconds. After 15 min, octadecyl isocyanate (0.18 g, 0.61 mmol) was added and the reaction suspension stirred at room temperature for 20 h. It was then diluted with Et_2O, washed with 0.1 N HCl at 0 °C and instantly dried (Na_2SO_4). The aqueous phase was extracted twice with Et_2O and the combined organic phases were again immediately dried (Na_2SO_4). The raw product obtained by evaporating the solvent was purified by flash chromatography with hexane/EtOAc (1:6) and two recrystallizations from hexane to yield white crystals (60 mg, 19%): mp 82 °C; IR ($CHCl_3$) 3430w, 3363w, 2927s, 2855s, 1728s, 1702s, 1552m, 1466m, 1378w; ^1H NMR ($CDCl_3$) δ 7.72 (t, J = 5, 1 H, $NHCH_2$), 6.98 (*s*, 1 H, CONHCO), 4.12 (t, J = 7, 2 H, OCH_2), 3.28 (q, J = 6, 2 H, $NHCH_2CH_2$), 1.64 (quint., J = 7, 2 H, OCH_2CH_2), 1.54 (quint., J = 7, 2 H, $NHCH_2CH_2$), 1.5–1.1 (m, 48 H, $OCH_2CH_2(CH_2)_9CH_3$ and $NHCH_2CH_2(CH_2)_{15}CH_3$), 0.88 (t, J = 7, 6 H, CH_3); EI-MS 526 ($(M+2)^+$, 7), 525 ($(M+1)^+$, 27), 524 (M^+, 56), 43 (100); Anal. Calc for $C_{32}H_{64}N_2O_3$ (524.87): C, 73.23; H, 12.29; N, 5.34; Found: C, 73.02; H, 12.23; N, 5.29.

6-(1-Heptyloctylamino)-2(1*H*)-pyridone (7). *6-Bromo-2(1H)-pyridone.* The compound was obtained from 6-bromo-2-pyridylamine[19] in 68% yield following the procedure described for the corresponding 6-chloro derivative.[20]

6-(1-Heptyloctylamino)-2(1H)-pyridone (7). 6-Bromo-2(1*H*)-pyridone (0.51 g, 0.29 mmol), 8-pentadecylamine (1.01 g, 0.44 mmol) and a spatula-tipful of $CuSO_4$ (dissolved in a drop of water) were heated to 190 °C in an argon atmosphere for 3 h. After cooling to room temperature, the reaction mixture was diluted with Et_2O and washed with sat. aqueous Na_2CO_3. The polar phase was extracted twice with Et_2O, all organic phases combined, dried over Na_2SO_4 and the solvent evaporated to yield 2.12 g of a brown oil. Purification by flash chromatography with EtOAc/EtOH (45:4) as eluant on a first column and with EtOAc alone on a second,

gave the product as a slightly brown oil (29 mg, 3%): IR ($CHCl_3$) 3277w, 3112w, 2929s, 2856s, 1623s, 1532w, 1438m, 1399m, 1158m; ^1H NMR ($CDCl_3$) δ 11.95 (*s*, br., 1 H, NH, ar), 7.24 (t, *J* = 8, 1 H, CH, ar), 6.12 (d, *J* = 8, 1 H, N*H*CH), 5.60 (d, *J* = 8, 1 H, CH, ar), 5.29 (d, *J* = 8, 1 H, CH, ar), 3.21 (m, 1 H, NHC*H*), 1.6–1.1 (m, 24 H, CH_2), 0.87 (t, *J* = 7, 6 H, CH_3); EI-MS 320 (M^+, 6), 221 (100). GPC (PL-Gel 100 column, length 60 cm, particle size 5 mm, pore size 10 nm, eluant THF) and TLC (silica gel 60, EtOAc/EtOH (45:4)) showed the compound to be pure. Due to the very limited amount of sample, no elemental analysis could be made.

2-[5-(4-amino)-acridinylamino]-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (8). *4,5-Diaminoacridine*. The compound was synthesized in 6 steps from 3-nitrophthalic acid and 2-nitroaniline according to literature procedures.[21]

3-Oxo-4-octyldocosanoic acid ethyl ester. The ester was prepared as described for *3-Oxo-4-octyldodecanoic acid ethyl ester*.[1]

*2-Thio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone*. As attempts to obtain 2-methylthio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone directly from 3-oxo-4-octyl-docosanoic acid ethyl ester and S-(methylthio)urea by refluxing in EtOH for 30 h failed (comp. Simon and Kovtunovskaya),[22] the following two-step synthesis was chosen:[23] 3-oxo-4-octyl-docosanoic acid ethyl ester (1.80 g, 3.64 mmol) and thiourea (0.56 g, 7.33 mmol, purum) were dissolved in EtOH. Sodium ethylate (7.52 mmol in EtOH) was added and the reaction solution refluxed for 24 h. After cooling to room temperature, it was diluted with Et_2O, washed with 0.05 N HCl and three times with aqueous NaCl solution (half sat.), dried (Na_2SO_4) and the solvent evaporated. Purification by flash chromatography with hexane/Et_2O (5:2) gave the 2-thio-4(3*H*)-pyrimidone as a weakly yellow oil (0.93 g, 52%).

*2-Ethylthio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone*.[22] To a solution of KOH (0.21 mg, 3.7 mmol) in EtOH (absoluted over Na, 20 mL) were added 2-thio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (0.93 g, 1.83 mmol) and ethyl iodide (0.58 g, 3.67 mmol, purum). After stirring at room temperature for 14 h, the reaction solution was diluted with Et_2O, washed with 1 N HCl and twice with sat. aqueous NaCl solution, dried (Na_2SO_4) and the solvent evaporated. Analysis by ^1H NMR and TLC with CH_2Cl_2/EtOAc (4:1) as eluant showed that further purification was unnecessary.

*2-[5-(4-amino)-acridinylamino]-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (8)*. The substitution of a 2-alkylthio group by an amine has been described for several 4-hydroxy-pyrimidines.[24] A first attempt, in which 2-ethylthio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone and 4,5-diaminoacridine were heated to 140 °C in xylene failed to give the desired product even after 3 d. So did a second attempt with $PbCl_2$ added. Finally, by heating 2-ethylthio-6-(1-octylnonadecyl)-4(3*H*)-pyrimidone (300 mg, 0.56 mmol) and 4,5-diaminoacridine (300 mg, 0.70 mmol) to 150 °C for 15 h, the reaction was successful. The resulting solid of unpleasant smell was extracted with Et_2O in a Soxhlet. From the solution thus obtained, the solvent was evaporated in a rotary evaporator with a small portion of *m*-chloroperbenzoic acid in the receiving flask to oxidize EtSH. The raw product was purified by flash chromatography with CH_2Cl_2/EtOAc (3:5) as eluant to yield the product (39 mg, 10%) as a red solid: mp 180 °C; IR ($CHCl_3$) 3359w, 3297w, 2927s, 2854s, 1667s, 1621s, 1621s, 1599m, 1568s, 1461s, 1405m; ^1H NMR ($CDCl_3$) δ 14.2 (br., 1 H, NH), 10.27 (*s*, 1 H, NH), 8.94 (d, *J* = 7, 1 H, CH ar), 8.70 (*s*, 1 H, CH ar), 7.7–7.3 (m, 4 H, CH ar), 7.03 (d, *J* = 7, 1 H, CH ar), 6.55 (*s*, 1 H, CH ar), 5.90 (br., 2 H, NH_2), 2.52 (m, 1 H, C*H*$(CH_2)_2$), 1.85 (m, 2 H, CH$(CH_2)_2$), 1.4–1.1 (m, 44 H, $CHCH_2(CH_2)_{16}$ and $CHCH_2(CH_2)_6$), 0.9-0.8 (m, 6 H, CH_3); FAB-MS (in 3-nitrobenzyl alcohol): 684 ($(M+3)^+$, 27), 683 ($(M+2)^+$, 77), 682.4 ($(M+1)^+$, 100), 681.3 (M^+, 36), 55 (100); Anal. Calc for $C_{44}H_{67}N_5O$ (682.05): C, 77.48; H, 9.90; N, 10.27; Found: C, 77.13; H, 9.50; N, 9.85.

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

REFERENCES AND NOTES

1. Deceased in November 1992.
2. Bühlmann, P.; Badertscher, M.; Simon, W. *Tetrahedron* **1993**, *49*, 595. Bühlmann, P. *Molecular Recognition of Creatinine, Ph. D. Thesis no. 10066*, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland 1993.
3. Kaito, A.; Hatano, M.; Ueda, T.; Shibuya, S. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 3073. Kwiatkowski, J. S.; Pullman, B. *Adv. Heterocycl. Chem.* **1975**, *18*, 199 and references cited therein.
4. Hélène, C.; Douzou, P. *C. R. Hebd. Séances Acad. Sci. (Paris)* **1964**, *259*, 4853.
5. Hélène, C.; Douzou, P. *C. R. Hebd. Séances Acad. Sci. (Paris)* **1964**, *259*, 4387.
6. Morita, H.; Nagakura, S. *Theoret. Chim. Acta (Berl.)* **1968**, *11*, 279.
7. Lés, A.; Ortega-Blake, I. *Int. J. Quantum Chem.* **1986**, *30*, 225.
8. Stepanian, S. G.; Radchenko, E. D.; Sheina, G. G.; Blagoi, Yu. P. *J. Mol. Struct.* **1990**, *216*, 77. Radchenko, Y. D.; Plokhotnichenko, A. M.; Ivanov, A. Y.; Sheina, G. G.; Blagoi, Y. P. *Biophysics* **1986**, *31*, 409. Sheina, G. G.; Stepanian, S. G.; Radchenko, E. D.; Blagoi, Y. P. *J. Mol. Struct.* **1987**, *158*, 275.
9. Colthup, N. B.; Daly, L. H.; Wiberley, S. E. *Introduction to Infrared and Raman Spectroscopy, 3rd ed.*; Academic Press: San Diego, 1990.
10. Millefiori, S.; Millefiori, A. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2981. Kuzuya, M.; Noguchi, A.; Okuda, T. *J. Chem. Soc. Perkin Trans. 2* **1985**, 1423. Kuzuya, M.; Noguchi, A.; Okuda, T. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3454. Beak, P.; Covington, J. B.; Smith, S. G.; White, J. M.; Ziegler, J. M. *J. Org. Chem.* **1980**, *45*, 1354. Beak, P. *Acc. Chem. Res.* **1977**, *10*, 186, and references cited therein.
11. De Kowalewski, D. G.; Contreras, R. H.; De Los Santos, D. *J. Mol. Struct.* **1989**, *213*, 201.
12. Fujimoto, A.; Inuzuka, K. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2292.
13. Gallant, M.; Phan Viet, M. T.; Wuest, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 721.
14. Adrian, J. C., Jr. ; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678.
15. Bernstein, J.; Stearns, B.; Shaw, E.; Lott, W. A. *J. Am. Chem. Soc.* **1947**, *69*, 1151.
16. Fuks, R., Hartemink, M. A. *Bull. Soc. Chim. Belg.* **1973**, *82*, 23. Petersen, U. Offenkettige Carbamidsäuren und deren Derivate. In *Methoden der organischen Chemie/ (Houben-Weyl), E4, Kohlensäurederivate*; Hagemann, H., Ed.; Georg Thieme Verlag: Stuttgart, New York, 1983; pp. 142, 156.
17. *Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie*; E. Merck: Darmstadt, 1984.
18. Blohm, H. W.; Becker, E. I. *Chem. Rev.* **1952**, *50*, 471. Piepenbrink, H.-F. Kohlensäurederivate. In *Methoden der organischen Chemie/(Houben-Weyl), 8, Sauerstoffverbindungen III*; Müller, E., Ed.; Georg Thieme Verlag: Stuttgart, 1952; pp. 75, 204.
19. den Hertog, H. J.; Wibaut, J. P. *Recl. Trav. Chim. Pays-Bas* **1936**, *55*, 122.
20. Cava, M. P.; Bhattacharyya, N. K. *J. Org. Chem.* **1958**, *23*, 1287.
21. Witmore, F. C.; Culhane, P. J. ; Neher, H. T. *Org. Synth. Coll.* **1941**, *1*, 56. Culhane, P. J. *Org. Synth. Coll.* **1941**, *1*, 125. Klein, E. R.; Lahey, F. N. *J. Chem. Soc.* **1947**, 1418. Goldberg, A. A.; Kelly, W. *J. Chem. Soc.* **1947**, 595.
22. Simon, I. B.; Kovtunovskaya, I. I. Ž. *Obšč. Chim.* **1951**, *21*, 760, engl. ed. 837.
23. Johnson, T. B. *J. Am. Chem. Soc.* **1914**, *36*, 1891.
24. Curd, F. H.; Graham, W.; Richardson, D. N.; Rose, F. L. *J. Chem. Soc.* **1947**, 1613. Brown, D. J. *The Chemistry of Heterocyclic Compounds 16, The Pyrimidines, Supplement II*; Wiley Interscience: New York, 1985; pp. 7.