Four Hydrogen Bonds − DDAA, DADA, DAAD and ADDA Hydrogen Bond Motifs[‡]

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Receptor molecules containing four hydrogen-bond acceptor or donor sites based on aminopyridines, aminonaphthyridines and urea subunits have been synthesized and their association has been investigated. DDAA (**13a**–**c**) and DADA (**18a**–**b**) arrays may form homodimers, while DAAD (**24a**–**d**) with ADDA (**25a**–**b**) may form heterodimers. While most parent heterocycles were only slightly soluble in standard organic solvents, substitution was able to enhance the solubility in most cases. The naphthyridine **24d**, bearing a substituent derived from lysine, possesses potential anchor groups for a covalent connection. Binding studies were carried out in chloroform and monitored by ^{1}H NMR, and the binding constants K_{ass} for the heterodimers DAAD·ADDA (**24·25**) were compared to the binding of smaller (ADD, **26**) or mismatching (DADD, **27**) counterparts, showing that the matching heterodimer is formed with a selectivity of > 50.
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Multiple hydrogen bonds can be found in many recognition processes, both in enzymes and in the genetic code.[1] Correct binding by two or three parallel[2] or antiparallel hydrogen bonds is the key feature of the genetic code, which uses substituted purines and pyrimidines for the recognition and the storage of genetic information.[3] The number of hydrogen bonds involved in a recognition event has two important influences on the quality of this process: (i) the more hydrogen bonds are involved in a recognition, the tighter the binding usually becomes,[4−7] and (ii) more hydrogen bonds can convey more information.

A hydrogen bond between a hydrogen-bond donor (D) and a hydrogen-bond acceptor (A) possesses a direction. Therefore, with two hydrogen bonds, two different pairs, formed from three different molecules, are possible: a heterodimer in which a DD unit binds AA, and a homodimer made of two DA molecules. Addition of one more hydrogen bond results in three possible dimers formed by six different units: DDD·AAA, DDA·AAD and DAD·ADA. With four hydrogen bonds, ten different molecules and six different dimers are possible (see Figure 1).

In addition to the stronger binding due to the four hydrogen bonds in these dimers, *more information* can be stored in each of the ten molecules **1**−**10**. While two hydrogen bonds may be oriented in two ways to give two different dimers originating from three different building blocks, three hydrogen bonds may give three dimers resulting from six different building blocks. Four hydrogen-donor and -acceptor sites give ten possible molecules **1**−**10**. For each of these, there is *only one* matching partner (out of ten) with which a strong dimer can be formed by the formation of four hydrogen bonds. However, the stability of the complexes with four hydrogen bonds will vary, depending on the number of secondary attractive or repulsive interactions.[6]

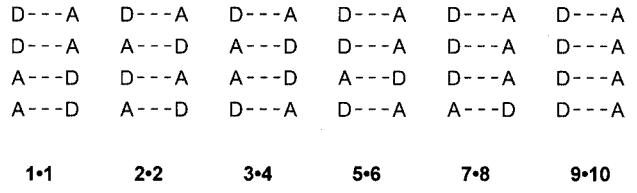

Figure 1. There are ten possible ways to arrange hydrogen-bond donors D and acceptors A for quadruple hydrogen-bond arrays (**1**−**10**); two of them form homodimers (**1·1**, **2·2**), the other eight (**3**−**10**) form heterodimers

Two arrays (**1** and **2**) are self-complementary[8−11] and have already been exploited for the construction of "intelligent" polymers.[12,13] In Meijer's systems, nitrogen and oxygen atoms are the acceptors (A), and N−H functions are the donors (D) (Figure 2).[14]

In this work we describe the syntheses of receptors **1**−**4** and present binding studies for *hetero*dimers consisting of different DAAD and ADDA molecules **3** and **4**.

[‡] Multiple hydrogen bonds, 3. Part 2 Ref.[33]

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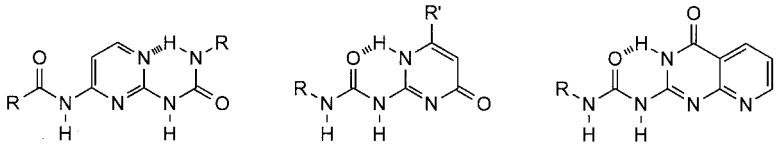
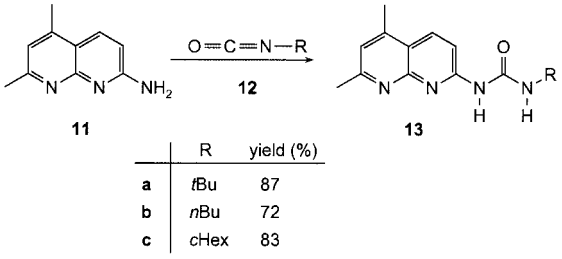


Figure 2. First[12] examples of self-complementary DADA[8] and DDAA[9,11] arrays

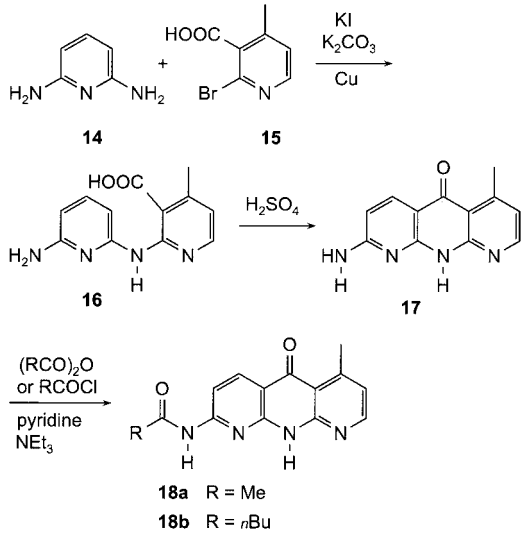
Synthesis of the Self-Complementary Sequences DDAA (1) and DADA (2)

Schemes 1 and 2 show self-complementary DDAA and DADA systems based on aminopyridine, aminonaphthyridine and urea units, which may form homodimers **1·1** and **2·2** connected exclusively by N····H−N hydrogen bonds. The syntheses of **13** and **18** are depicted in Schemes 1 and 2.



	R	yield (%)
a	*t*Bu	87
b	*n*Bu	72
c	*c*Hex	83

Scheme 1



Scheme 2

A very quick route to DDAA molecules containing two nitrogen atoms and two NH groups for hydrogen bonding is shown in Scheme 1. The aminonaphthyridine **11**[15] was synthesized easily in 84−90% yield by literature procedures. Addition to several isocyanates **12** formed the DDAA receptors **13** in 72−87% yield.[16]

From **13a**, single crystals have been obtained, and an X-ray analysis was carried out.[17] In this crystal, a dimer of **13a** was found but the hydrogen-bonding pattern was not as expected. As also known for *N*-(pyridin-2-yl)ureas[18,19] an (*E*,*Z*) conformer, stabilized by an intramolecular hydrogen bond, was found. On rotation of the NH−CO unit to form an (*E*) conformer, a new AD recognition site is formed, by which the molecules dimerize. The resulting homodimer also contains four hydrogen bonds but only two of them are intermolecular hydrogen bonds, while the other two are intramolecular hydrogen bonds, one within each urea molecule (Figure 3).

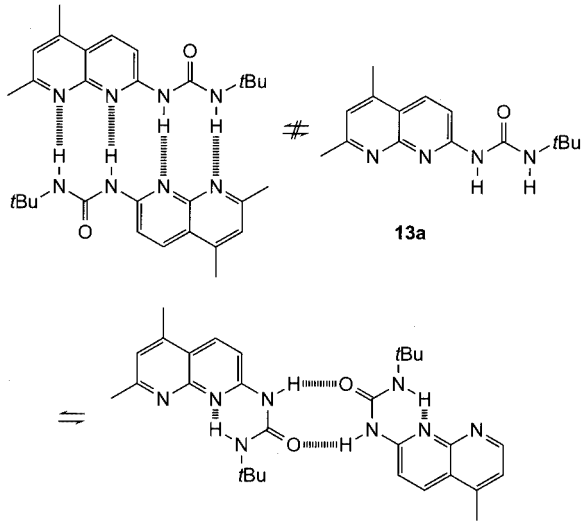


Figure 3. The DDAA molecule **13a** does not dimerize in the expected way, by establishing four parallel and antiparallel hydrogen bonds, but forms an $(AD)_2$ dimer containing two intramolecular hydrogen bonds[27]

Although not verified by X-ray analyses, it is very probable that the analogues **13b** and **13c** form the same AD homodimers containing these intramolecular hydrogen bonds.

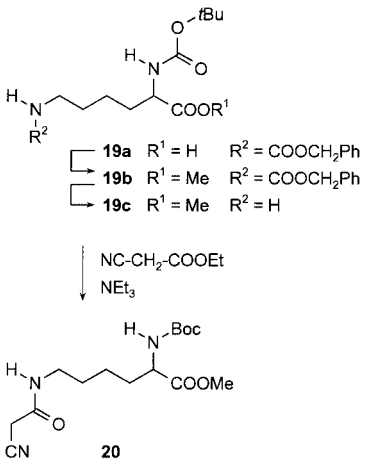
A DADA receptor based exclusively on N and NH units will result from an alternating arrangement of pyridine rings and NH units such as, for instance, in **17** or **18**. To avoid the rotational problems discussed above, the pyridine rings have to be connected in the 3-position. If this connection is performed through a carbonyl group the resulting molecule is an anthyridone **18**. Scheme 2 summarizes the synthesis.

The 2-bromo-3-pyridinecarboxylic acid **15** was synthesized by literature procedures[20] via its ethyl ester (56%) and subsequent saponification (66%). Compound **15** was then coupled with 2,6-diaminopyridine (**14**) under Ullmann conditions by first mixing the solids **14** and **15** with copper powder, copper iodide and potassium carbonate and then heating to 170 °C. The resulting (pyridylamino)pyridinecarboxylic acid **16** (24%) was then cyclized with hot concentrated sulfuric acid, giving 79% of the highly insoluble anthyridone **17**, which was only characterizable by IR. Subsequent treatment with acetic anhydride, however, gave the more soluble anthyridone **18a** in 50% yield. A valeric acid derivative **18b** was synthesized in analogous fashion, but both compounds were only soluble in very polar solvents, and association constants in chloroform could therefore not be determined. Attempts to synthesize other more lipophilic anthyridones were stopped by very low yields of the Ullmann coupling.

Syntheses of DAAD Molecules 3

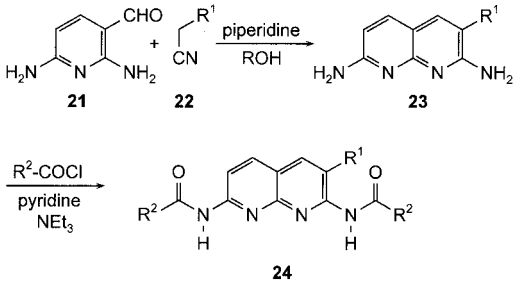
With the syntheses of the systems **13** and **18**, containing four hydrogen bonds, we have already encountered two important problems in the designing of multiple hydrogen-bond receptors: insolubility and "rearrangements" that may result in altered donor-acceptor sequences [e.g., rotations (see above), tautomerism[21]]. Therefore, for a DAAD receptor of type **3**, 2,7-diamino-1,8-naphthyridines in which all four nitrogen atoms are fixed in one plane were selected. In one case (**23d**), an amino acid residue was attached to the DAAD moiety on its remote side in order to allow covalent connection to other molecules. One possible reaction could be the incorporation of this four hydrogen bond forming unit into PNA.[22−24]

The naphthyridines **23** and **24** were synthesized by the Friedländer procedure.[15] The 2,6-diaminopyridine-3-carbaldehyde (**21**) starting material was prepared by literature procedures.[25] Condensation with cyanoacetamides gave the naphthyridines **23**.[26] The amino acid derived cyanoacetamide **20** was prepared from lysine as shown in Scheme 3.



Scheme 3

Many naphthyridines like **23** and related heterocycles show very low solubility (see also above) in most organic solvents, especially when the crystalline form is additionally stabilized by hydrogen bonds. Aliphatic substituents help to solubilize such compounds. The diaminonaphthyridines **23** were therefore acylated with valeryl or pivaloyl chloride to give the chloroform-soluble DAAD compounds **24**.[16,26] Only in the case of the amino acid derivative **23d** was such functionalization was unnecessary, as **23d** was already soluble in chloroform due to the amino acid component (Scheme 4).



	R^1	R^2
a	$CONH_2$	*t*Bu
b	CN	*t*Bu
c	CN	*n*Bu
d	$CONH(CH_2)_4CH(NHBoc)COOMe$	-

Scheme 4

Counterparts ADDA 4

The counterpart for a DAAD system **3** will be an ADDA unit **4**. A very convenient DD system is the urea molecule. In order to extend the DD system to produce an ADDA one, hydrogen-bond acceptors such as pyridine nitrogen atoms have to be attached to a urea moiety. Because ureas can easily be prepared from carbonic acid derivatives and amines, the reaction between 2-aminopyridines and carbonic acid derivatives was chosen for the construction of ADDA molecules **4**. One-step syntheses starting from phosgene are also conceivable, but the less toxic, non-gaseous ethyl chloroformate can be used as starting material too.[27−29] Two dipyridylureas **25** were synthesized in two steps from chloroformate, giving **25a** and its dimethyl derivative **25b**[18] in improved overall yields of 27 and 25%, respectively.

The dipyridylureas **25** are able to form dimers through two hydrogen bonds, as was determined by X-ray analysis.[18,19] Rotation about one bond between a pyridine ring and the urea allows an intramolecular hydrogen bond between one NH group and the non-adjacent pyridine nitro-

gen atom. The other NH group is oriented *cis* to the carbonyl group of the urea, thus forming a DA binding unit. In the crystal, two of these *cis*-amides form a dimer.[18,19] Because of the symmetry of this molecule, two degenerate conformations exist in rapid equilibrium at room temperature, resulting in broad NMR signals for 3-H in the pyridine rings (Figure 4).[16]

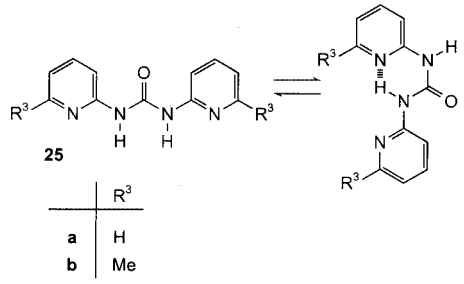


Figure 4. Rotation about one bond between one NH and the C=O group allows an intramolecular hydrogen bond between one NH group and the non-adjacent pyridine nitrogen atom; the other NH group is oriented *cis* to the carbonyl group of the urea, allowing the formation of DA·AD bound dimers as established by X-ray analysis[18,19] (see also Figure 3)

Binding Studies for DAAD·ADDA

Recognition between the DAAD receptors **23** and **24** and the complementary ADDA dipyridylureas **25** was investigated by NMR. Small volumes of **25** in $CDCl_3$ were added to $CDCl_3$ solutions of **23** or **24** and the chemical shifts were recorded. Figure 5 shows as an example the titration curve for the amide signals of **24c** during titration with **25a**. Both NH signals shift drastically to low field, indicating that both NH components take part in hydrogen bonds (Figure 6).[31]

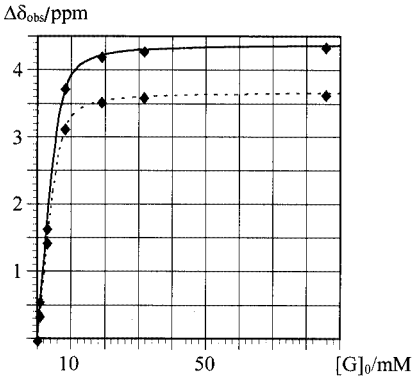


Figure 5. Titration of the DAAD receptor **24c** (6.4 mM) with the complementary ADDA moiety **25a**; the differences in chemical shifts $\Delta\delta_{obs}$ of the amide hydrogen atoms of **24c** are plotted against the total concentration $[G]_o$ of the dipyridylurea **25**

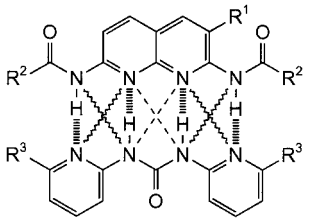
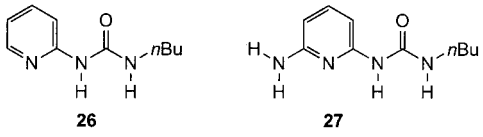


Figure 6. Heterodimer formation between DAAD naphthyridines **24** and ADDA dipyridylureas **25**; the hydrogen bonds are drawn with horizontally dashed lines (||||), the positive secondary interactions as longitudinally dashed lines (-----), the repulsive secondary interactions with zig-zag lines (/\/\/\) for an estimation of the binding constants according to the increments listed by Schneider et al.,[5,6] two positive and four negative secondary interactions have to be taken into account

From the titration curves (see Figure 5, for example), binding constants K_{ass} were determined[31] and the corresponding ΔG values calculated. Table 1 lists the results for the titration of four DAAD receptors **23** and **24** with two ADDA dipyridylureas **25** and two related compounds **26** (ADD) and **27** (DADD),[32,33] and compares them with ΔG_{calc} values obtained from the increments.[5] For the determination of the ΔG_{calc} values in addition to the primary and secondary interactions[6] of the hydrogen binding, the energy to cleave the intramolecular hydrogen bond in **25** has also been taken into account.



Surprisingly, the free enthalpy of binding (ΔG) calculated from the binding constant K_{ass} for the complex **24c·25a** matches the calculated value exactly.[34] Deviations were found for the other pairs, and can be explained by additional steric interactions. Thus, the change in the acyl residue R^2 in the DAAD receptors **24** from butyl (**24c**) to *tert*-butyl (**24b**) resulted in a decrease in the binding constants K_{ass} for both ADDA counterparts: from K_{ass} = 2000 to 47 (**25a**) and from K_{ass} = 160 to 16 (**25b**). However, the introduction of methyl groups into the dipyridyl urea **25** also diminishes the binding constants: from K_{ass} = 2000 to 160 (**24c**) and from K_{ass} = 47 to 16 (**24b**). If the ADDA counterpart **25a** is compared to those not complementary to the DAAD pattern [**26** (ADD) and **27** (DADD)], a decrease in K_{ass} of a factor of at least 50 is observed. The amino acid derivative **23d** showed a smaller than expected K_{ass} association constant. Presumably the less acidic N−H atoms of the amino groups in **23d** are the reason for the less tight binding relative to the *bis*(amido) compound **24c**. This difference shows that the good fit of the calculated and measured ΔG values for the couple **24c·25a** is not a given and argues for very careful use of empirical estimation of binding constants by the increment method.

Table 1. Binding constants K_{ass} and free enthalpies of binding ΔG of heterodimers as determined by titration of the DAAD receptors **23** or **24** with ADDA dipyridylureas **25** and related compounds **26** (ADD) and **27** (DADD)[32,33] at 295 K, and the corresponding calculated binding free enthalpies ΔG_{calc}

Complex		$\Delta\delta_{max}$ NH_a	$\Delta\delta_{max}$ NH_b	K_{ass} [M^{-1}]	ΔG [kJ mol^{-1}]	ΔG_{calc} [kJ mol^{-1}]	$\Delta\Delta G_{calc}$ [kJ mol^{-1}]
DAAD·ADDA	**24c·25a**	4.4	3.7	2000	−18.6	−17.9	−0.7
DAAD·ADDA	**24c·25b**	3.0	2.4	160	−12.5	−17.9	5.5
DAAD·ADD	**24c·26**	2.2	2.5	40	−9.0	−15.8	6.8
DAAD·DADD	**24a·27**	1.7	2.1	31	−8.4	−15.8	7.4
DAAD·ADDA	**24b·25a**	0.8	1.5	47	−9.4	−17.9	8.5
DAAD·ADDA	**24b·25b**	0.8	0.2	16	−6.8	−17.9	11.1
DAAD·ADDA	**23d·25a**	1.7	1.9	271	−13.7	−17.9	4.2

Experimental Section

General: For general information, see ref.[33]

***N*-Butyl-*N*′-(2,4-dimethyl-1,8-naphthyridin-7-yl)urea (13b):** A solution of 7-amino-2,4-dimethyl-1,8-naphthyridine[15] (**11**, 1.73 g, 10.0 mmol) in 50 mL of dry toluene was mixed under nitrogen with butyl isocyanate (**12b**, 990 mg, 10.0 mmol) and heated to reflux for 2 h. At room temp., the solid was filtered off, washed with toluene, dried and recrystallized first from ethanol and then from toluene, giving 1.97 g (72%) of a colourless solid, m.p. 204 °C. IR (KBr): $\tilde{\nu}$ = 3330, 3180, 3050 cm^{-1} (N−H), 2959, 2930, 2874 (aliph. C−H), 1684 (C=O), 1604, 1522 (arom.), 1560 (N−H), 1458 (C−H). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.85 (t, *J* = 7.3 Hz, 3 H, C*H*$_3$), 1.32 (m_c, 2 H, C*H*$_2$), 1.54 (m_c, 2 H, C*H*$_2$), 2.55 (s, 3 H, C*H*$_3$), 2.62 (s, 3 H, C*H*$_3$), 3.36 (q, *J* = 6.4 Hz, 2 H, C*H*$_2$), 6.99 (s, 1 H, Ar*H*), 7.51 (br. s, 1 H, Ar*H*), 8.11 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 9.52 (br. s, 1 H, N*H*), 10.51 (br. s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 272 (20), 229 (42), 200 (70), 173 (85), 71 (100). MS (CI/isobutane): *m*/*z* (%) = 273 (100). $C_{17}H_{22}N_4O$ (272.4): calcd. C 66.15, H 7.40, N 20.57, found C 65.99, H 7.38, N 20.48.

***N*-Cyclohexyl-*N*′-(2,4-dimethyl-1,8-naphthyridin-7-yl)urea (13c):** A solution of 7-amino-2,4-dimethyl-1,8-naphthyridine[15] (**11**, 1.73 mg, 10.0 mmol) and cyclohexyl isocyanate (**12c**, 2.48 g, 20.0 mmol) in 30 mL of dry toluene was heated to reflux under nitrogen for 2 h. After cooling to room temp., the colourless solid was filtered off, dried and recrystallized first from ethanol/toluene (1:1) and then from toluene, giving 2.46 g (83%) of a colourless solid, m.p. > 300 °C. IR (KBr): $\tilde{\nu}$ = 3446, 3181, 3034 cm^{-1} (N−H), 2933, 2851 (aliph. C−H), 1680 (C=O), 1600, 1523 (arom.), 1554 (N−H). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 1.3−1.9 (m, 10 H, C*H*$_2$), 2.57 (s, 6 H, C*H*$_3$), 3.66 (m_c, 1 H, C*H*), 7.18 (s, 1 H, Ar*H*), 7.28 (d, *J* = 9.0 Hz, 1 H, Ar*H*), 8.35 (d, *J* = 9.0 Hz, 1 H, Ar*H*), 9.61 (br. d, *J* = 7.2 Hz, 1 H, N*H*), 9.72 (s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 298 (21), 200 (42), 173 (100). MS (CI/isobutane): *m*/*z* (%) = 299 (100). $C_{17}H_{22}N_4O$ (298.4): calcd. C 68.43, H 7.43, N 18.78, found C 68.20, H 7.34, N 18.56.

2-[(6-Aminopyridin-2-yl)amino]-4-methyl-3-pyridinecarboxylic Acid (16): 2,6-diaminopyridine (**14**, 10.6 g, 97.2 mmol), 2-bromo-4-methyl-3-pyridinecarboxylic acid (**15**, 5.42 g, 25.1 mmol), dry potassium carbonate (5.84 g, 42.3 mmol), potassium iodide (ca. 100 mg) and powdered copper (ca. 100 mg) were ground and heated to 170 °C under nitrogen for 4 h. After cooling, the black solid was extracted with 70 mL of boiling ethyl acetate. After filtration of the hot solution, the solid residue was extracted with 70 mL of boiling water. After filtration of the hot solution, 15 g of potassium hydroxide was added to the black filtrate, which was allowed to stand for 15 h at 5 °C. The resulting solid was filtered off and was dissolved in 20 mL of conc. acetic acid. After dilution with 40 mL of water, the solution was allowed to stand for one week at 5 °C. Brown needles crystallized and were filtered off, dried and recrystallized twice [first from dimethyl sulfoxide/water (1:1), then from water], giving 1.50 g (24%) of colourless needles, m.p. 145−155 °C. IR (KBr): $\tilde{\nu}$ = 3422 cm^{-1} (COOH), 3095 (N−H), 1604 (C=O), 1560, 1492 (arom.). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.51 (s, 3 H, CH_3), 6.13 (d, *J* = 8.1 Hz, 1 H, ArH), 6.34 (br. s, 2 H, NH_2), 6.87 (d, *J* = 5.8 Hz, 1 H, ArH), 7.05 (br. s, 1 H, ArH), 7.43 (t, *J* = 8.0 Hz, 1 H, ArH), 8.13 (d, *J* = 5.6 Hz, 1 H, ArH) ppm. MS (EI, 70 eV): *m*/*z* (%) = 199 (100). MS (CI, isobutane): *m*/*z* (%) = 245 (100). HR-MS: $C_{12}H_{12}N_4O_2$: calcd. 244.0960, found 244.0960 (diff.: 0.1 ppm); $C_{11}{}^{13}CH_{12}N_4O_2$: calcd. 245.0994 found 245.0993 (diff.: 0.3 ppm).

2-Amino-6-methylpyrido[2,3-*b*]-1,8-naphthyridin-5(10*H*)-one (17): Conc. sulfuric acid (15 mL) was heated to 200 °C and 2-[(6-aminopyridin-2-yl)amino]-4-methyl-3-pyridinecarboxylic acid (**16**, 1.30 g, 5.33 mmol) was added in portions. After 10 min at 200 °C and subsequent cooling to room temp., the mixture was hydrolysed (100 mL of ice/water, 1:1) and, while cooling with ice, ca. 60 mL of conc. ammonia was added carefully until pH = 10 was reached. After 1.5 h, the solid was filtered off, washed with water and dried, resulting in 946 mg (79%) of a black solid, m.p. (dec.) > 260 °C. IR (KBr): $\tilde{\nu}$ = 3373, 3125 cm^{-1} (N−H), 1606 (C=O), 1560, 1490 (arom.).

***N*-(6-Methyl-5-oxo-5,10-dihydropyrido[2,3-*b*]-1,8-naphthyridin-2-yl)acetamide (18a):** Dry acetic anhydride (5 mL) was added under nitrogen to 2-amino-6-methylpyrido[2,3-*b*]-1,8-naphthyridin-5(10*H*)-one (**17**, 100 mg, 442 μmol). After having been heated at reflux for 5 h, the mixture was cooled to room temp. and was hydrolysed by addition of 20 mL of water and 2 N sodium hydroxide until pH = 10 was reached. The resulting solid was filtered off, dried and recrystallized from dimethyl sulfoxide, yielding 59.0 mg (50%) of a yellow solid, m.p. > 300 °C. IR (KBr): $\tilde{\nu}$ = 3414, 3244 cm^{-1} (NH), 2920 (aliph. C−H), 1696, 1620 (C=O), 1582 (arom.). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.20 (s, 3 H, C*H*$_3$), 7.12 (d, *J* = 4.8 Hz, 1 H, Ar*H*), 8.00 (d, *J* = 8.7 Hz, 1 H, Ar*H*), 8.46 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 8.55 (d, *J* = 4.7 Hz, 1 H, Ar*H*), 10.72 (br. s, 1 H, N*H*), 12.20 (br. s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 268 (61), 253 (8), 226 (100), 198 (13). MS (CI, isobutane): *m*/*z* (%) = 269 (100). HR-MS: $C_{14}H_{12}N_4O_2$: calcd. 268.0960, found 268.0959 (diff.: 0.5 ppm); $C_{13}{}^{13}CH_{12}N_4O_2$: calcd. 269.0994, found 269.0993 (diff.: 0.4 ppm).

***N*-(6-Methyl-5-oxo-5,10-dihydropyrido[2,3-*b*]-1,8-naphthyridin-2-yl)pentanamide (18b):** Valeryl chloride (1.28 g, 10.6 mmol) was slowly added under nitrogen to a mixture of 2-amino-6-methylpyrido[2,3-*b*]-1,8-naphthyridin-5(10*H*)-one (**17**, 800 mg, 3.34 mmol) in 40 mL of dry pyridine and triethylamine (1.07 g, 10.6 mmol). After having been heated at reflux for 2 h, the solvents were removed in vacuo and the residue was dissolved in 400 mL of dichloromethane and 160 mL of 0.5 N sodium carbonate solution. The layers were separated, and the aqueous layer was extracted three times with 100 mL of dichloromethane. The organic layer was filtered and the resulting solid was washed with ethanol and dried (crude yield: 687 mg). The dichloromethane and the ethanol layers were combined and the solvents were evaporated. Recrystallization from DMSO/H_2O (ca. 2:1) gave 531 mg (51%) of **18b**, m.p. > 260 °C. IR (KBr): $\tilde{\nu}$ = 3320 cm^{-1} (N−H), 2925 (aliphat. C-H), 1675, 1621 (C=O), 1516 (arom.). ^1H NMR (300 MHz, [D_6]DMSO): δ = 0.91 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$), 1.2−1.4 (m, 2 H, C*H*$_2$), 1.60 (m_c, 2 H, C*H*$_2$), 2.90 (s, 3 H, C*H*$_3$), 7.12 (d, *J* = 4.8 Hz, 1 H, Ar*H*), 8.04 (d, *J* = 8.8 Hz, 1 H, Ar*H*), 8.46 (d, *J* = 9.0 Hz, 1 H, Ar*H*), 8.55 (d, *J* = 4.8 Hz, 1 H, Ar*H*), 10.66 (s, 1 H, N*H*), 12.17 (s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 310 (41), 226 (100). MS (CI, isobutane): *m*/*z* (%) = 311 (100). HR-MS: $C_{17}H_{18}N_4O_2$: calcd. 310.1430, found 310.1430 (diff.: −0.1 ppm); $C_{16}{}^{13}CH_{18}N_4O_2$: calcd. 311.1463, found 311.1464 (diff.: −0.2 ppm). $C_{17}H_{18}N_4O_2$ (310.4): calcd. C 65.79 H 5.85 N 18.05, found C 65.22 H 5.64 N 17.71.

***N*$^{\varepsilon}$-Benzyloxycarbonyl-*N*$^{\alpha}$-*tert*-butoxycarbonyllysine Methyl Ester (19b):** A solution of diazomethane in diethyl ether was added to the doubly *N*-protected lysine **19a** (1.00 g, 2.63 mmol) in 25 mL of dry methanol until the mixture remained yellow. Gas was evolved, the mixture was stirred for an additional 1 h, and the degree of conversion was checked by TLC [CH_2Cl_2/EtOH (20:1): R_f(acid, **19a**) = 0.16; R_f(ester, **19b**) = 0.53]. Ca. 200 mg of silica gel was added to decompose excess diazomethane. After filtration, the solvent was evaporated in vacuo, giving 1.09 g of a colourless oil. IR (film): $\tilde{\nu}$ = 3337 cm^{-1} (N−H), 2950 (aliphat.), 1704 (C=O), 1530 (amide, arom.), 1455, 1366 (C−H), 1252 (C−O). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.2−1.9, 1.44 (m, C*H*, C*H*$_2$, s, *t*Bu, 16 H), 3.19 (m_c, 2 H, C*H*$_2$NH), 3.73 (s, 3 H, OC*H*$_3$), 4.28 (br. m, 1 H, N*H*), 4.82 (br. s, 1 H, N*H*), 5.09 (s, ca. 2 H, ArC*H*$_2$, traces of *H*$_2$O), 7.74 (m, 5 H, Ar*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 394 (< 1), 338 (6), 294 (12), 235 (8), 218 (21), 91 (100). MS (CI/isobutane): *m*/*z* (%) = 395 (7), 339 (19), 295 (100), 231 (17), 91 (32).

***N*$^{\alpha}$-*tert*-Butoxycarbonyllysine Methyl Ester (19c):** The doubly protected ester **19b** (1.075 g, 2.73 mmol) was placed under nitrogen in a round-bottomed flask, and methanol (5 mL), acetic acid (0.45 mL) and Pd/C (10% Pd, 200 mg) were then added. The flask was flushed with hydrogen and shaken for 4 h. After flushing with nitrogen, the catalyst was filtered off and washed with methanol. The combined methanol layers were concentrated in vacuo and purified by chromatography on silica gel [CH_2Cl_2/MeOH (10:3)]. Yield: 423 mg (62%) of a slightly yellow oil. IR (film): $\tilde{\nu}$ = 3303 cm^{-1} (N−H), 2932 (aliph.), 1710 (C=O), 1525 (amide, arom.), 1366 (C−H), 1250 (C−O). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.9−1.3, 1.34 (br. m, C*H*$_2$, C*H*, s, *t*Bu, 16 H), 2.9 (m, 2 H, C*H*$_2$N), 3.74 (s, 3 H, C*H*$_3$), 4.25 (br. s, 1 H, N*H*), 5.6 (br. m, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 260 (5), 204 (18), 187 (21), 160 (10), 143 (27), 142 (34), 116 (16), 87 (12), 84 (100), 82 (10), 72 (20). MS (CI/isobutane): *m*/*z* (%) = 261 (100), 205 (100), 61 (95).

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\varepsilon}$-(2-cyanoacetyl)lysine Methyl Ester (20):** Boc-protected lysine methyl ester **19c** was synthesized from the doubly protected ester **19b** (2.6 mmol) as described above, but no purification by chromatography was undertaken. The crude product was mixed with triethylamine (460 mg, 4.6 mmol) and ethyl cyanoacetate (880 mg, 7.8 mmol). The mixture was stirred, under nitrogen and protected from light, for 2 d at room temp. After addition of diethyl ether, some solid was filtered off and the solution was washed first with hydrochloric acid (pH = 2) until the aqueous layer remained acidic (three times), and then with water. After drying with $MgSO_4$, the solvent was removed and the remaining yellowish oil was purified by chromatography [150 mL of silica gel, 0.04−0.063 mm, first CH_2Cl_2 until all ethyl cyanoacetate had been removed, then CH_2Cl_2/EtOH (10:1), detection with anisaldehyde: red], giving 151 mg (0.46 mmol, 18% for 2 steps) of a brown oil. IR (film): $\tilde{\nu}$ = 3326 cm^{-1} (N−H), 2933 (aliph.), 2259 (CN), 1682 (several C=O), ca. 1538 (amide, arom.), 1367 (C−H). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.2−2.0, 1.45 (br. m, C*H*$_2$, C*H*, s, *t*Bu, 16 H), 3.3 (br. m, ca. 2 H, C*H*$_2$N), 3.38 (s, ca. 2 H, C*H*$_2$CN), 3.75 (s, 3 H, C*H*$_3$), 4.3 (br. s, 1 H, N*H*), 6.4 (br. s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 271 (3), 239 (2), 212 (16), 168 (13), 152 (9), 151 (100), 84 (11). MS (CI/isobutane): *m*/*z* (%) = 328 (4), 272 (24), 229 (11), 228 (100), 151 (11).

2,7-Diamino-1,8-naphthyridine-3-carboxamide (23a): 2,6-Diaminopyridine-3-carbaldehyde[25] (**21**, 1.57 g, 11.5 mmol), cyanoacetamide (**22a**, 19.3 g, 230 mmol) and piperidine (5 mL) were dissolved under nitrogen in dry ethanol (150 mL) and heated to reflux for 3.5 h. After removal of the solvent, the residue was partitioned between 150 mL of water and 150 mL of dichloromethane. The layers were separated and the yellow solid at the interface between the layers was filtered off. The aqueous layer was extracted three times with 50 mL of dichloromethane. The solid which formed at the interface was filtered off each time. After thorough washing with water and dichloromethane the residue was dried, giving 1.45 g (62%) of a yellow solid, m.p. 203−206 °C. IR (KBr): $\tilde{\nu}$ = 3399, 3350, 3197, 3076 cm^{-1} (N−H), 1651 (C=O), 1616, 1566, 1496 (arom.), 1547 (N−H). ^1H NMR (200 MHz, [D_6]DMSO): δ = 6.44 (d, *J* = 8.6 Hz, 1 H, Ar*H*), 6.69 (br. s, 2 H, N*H*$_2$), 7.32 (br. s, 4 H, N*H*$_2$), 7.59 (d, *J* = 8.6 Hz, 1 H, Ar*H*), 8.21 (s, 1 H, Ar*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 203 (100), 186 (14), 159 (41), 131 (24), 105 (20). MS (CI/isobutane): *m*/*z* (%) = 204 (100). HR-MS: $C_9H_9N_5O$: calcd. 203.0807, found 203.0799; $C_8{}^{13}CH_9N_5O$: calcd. 204.0841, found 204.0840.

***N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\varepsilon}$-[(2,7-diamino-1,8-naphthyridin-3-yl)carbonyl]lysine Methyl Ester (23d):** *N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\varepsilon}$-(2-cyanoacetyl)lysine methyl ester (**20**, 320 mg, 1.0 mmol) and 2,6-diaminopyridine-3-carbaldehyde[25] (**21**, 140 mg, 1.0 mmol) in 8 mL of dry methanol were mixed with piperidine (0.5 mL, ca. 5 mmol). The solution was heated at reflux under nitrogen for 18 h. After filtration, the organic layer was concentrated in vacuo to ca. 1 mL and was purified by chromatography [150 mL of silica gel, 0.06−0.04 mm, CH_2Cl_2/MeOH (10:3)] giving 260 mg (59%) of a yellow solid, which crystallized only slowly, m.p. 98−105 °C. IR (KBr): $\tilde{\nu}$ = 3346 cm^{-1} (NH), 2932 (aliph.), 1737, 1700 (C=O), 1551, 1493 (amid, arom.), 804 (arom.). ^1H NMR (500 MHz, $CDCl_3$): 1.2−2.0, 1.39 (br. m, C*H*$_2$, C*H*, s, *t*Bu, 16 H), 3.44 (br. t, *J* = 5.4 Hz, 2 H, C*H*$_2$N), 3.74 (s, 3 H, C*H*$_3$), 4.32 (br. s, 1 H, N*H*), 4.99 (br. s, 1.5 H, N*H*), 5.13 (br. s, 1 H, N*H*), 6.37 (br. s, 0.5 H, N*H*), 6.45 (br. d, *J* = 7.7 Hz, 1 H, Ar*H*), 6.55 (br. s, 1 H, N*H*), 7.63 (br. d, *J* = 7 Hz, 1 H, Ar*H*), 7.90 (br. s, 1 H, Ar*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 446 (16), 389 (7), 387 (8), 373 (7), 346 (6), 331 (9), 281 (6), 270 (11), 258 (13), 230 (8), 216 (27) 187 (100) 160 (51) 132 (29). MS (CI/isobutane): *m*/*z* (%) = 447 (84), 347 (29), 264 (34), 163 (84), 69 (100). HR-MS: $C_{21}H_{30}N_6O_5$ calcd. 446.22778, found 446.22770; $C_{20}{}^{13}CH_{30}N_6O$: calcd. 447.23111, found 447.23111.

2,7-Bis(2,2-dimethylpropanoylamino)-1,8-naphthyridine-3-carboxamide (24a): A suspension of 2,7-diamino-1,8-naphthyridine-3-carboxamide (**23a**, 100 mg, 493 μmol) in 5 mL of dry pyridine was treated under nitrogen with triethylamine (174 mg, 1.72 mmol). 2,2-Dimethylpropanoyl chloride (178 mg, 1.48 mmol) was then added carefully at 0 °C, and the mixture was heated at reflux for 1 h. After removal of the solvents, the residue was dissolved in 50 mL of dichloromethane. The organic layer was washed with 20 mL of 1 N sodium carbonate solution and extracted five times with 20 mL of dichloromethane. The combined organic layers were dried with sodium sulfate and the solvent was removed in vacuo. The remaining oil was purified by triple chromatography on silica gel: (i) 1 × 5 cm, acetone, R_f = 0.90; (ii) 2 g, dichloromethane/ethanol (10:1), R_f = 0.72 (nitrile **24b**), R_f = 0.65 (amide **24a**); (iii) 1 g, dichloromethane/ethanol (10:1). Nitrile **24b** was first recrystallized from toluene/ethanol (10:1) and then from toluene, giving 61.0 mg (35%) of a yellowish solid, m.p. 183−185 °C (analytical data see below). The amide **24a** was recrystallized from toluene/ethanol (20:1), giving 25 mg (14%) of colourless needles, m.p. 204 °C. IR (KBr): $\tilde{\nu}$ = 3382, 3183 cm^{-1} (N−H), 2970 (aliph. C−H), 1704, 1676 (C=O), 1607, 1582 (arom.), 1531 (N−H), 1383 (*tert*-butyl, C−H). ^1H NMR (300 MHz, [D$_6$]DMSO): δ = 1.26 [s, 9 H, C(CH_3)$_3$], 1.29 [s, 9 H, C(CH_3)$_3$], 7.96 (br. s, 1 H, N*H*), 8.31 (m$_c$, 2 H, Ar*H*), 8.52 (br. s, 1 H, N*H*), 10.56 (br. s, 1 H, N*H*), 11.95 (br. s, 1 H, N*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 371 (4), 353 (6), 314 (100), 296 (9), 269 (12), 213 (14), 187 (6). MS (CI/isobutane): *m*/*z* (%) = 372 (100). $C_{19}H_{23}N_5O_2$ (371.5): calcd. C 61.44, H 6.78, N 18.85, found C 61.21, H 6.71, N 18.69.

***N*-{3-Cyano-7-[(2,2-dimethylpropanoyl)amino]-1,8-naphthyridin-2-yl}-2,2-dimethylpropanamide (24b):** Triethylamine (435 mg, 4.31 mmol) and 2,2-dimethylpropanoyl chloride (830 mg, 6.88 mmol) were added dropwise under nitrogen to a suspension of 2,7-diamino-1,8-naphthyridine-3-carboxamide (**23a**, 350 mg, 1.72 mmol) in 20 mL of dry pyridine. After the mixture had been heated at reflux for 2 h, the solvents were removed in vacuo and the residue was dissolved in 50 mL of dichloromethane. The organic layer was washed with 20 mL of 2 N sodium carbonate solution and with 20 mL of water. The aqueous layer was extracted twice with 20 mL of dichloromethane. The combined organic layers were dried with sodium sulfate and the solvent was removed in vacuo, giving 541 mg of crude product which was purified by triple chromatography on silica gel: (i) 5 g, dichloromethane/ethanol (10:1), R_f = 0.72, (ii) 3 g, dichloromethane/ethanol (10:1), (iii) 1 g, dichloromethane/ethanol (10:1). The resulting solid was first recrystallized from toluene/ethanol (10:1) and then from toluene, giving 258 mg (43%) of a yellowish solid, m.p. 181−183 °C. IR (KBr): $\tilde{\nu}$ = 3438, 3221 cm^{-1} (N−H), 2969 (aliph. C−H), 2231 (C≡N), 1702, 1683 (C=O), 1608, 1515 (arom.), 1566 (N−H), 1478 (C−H). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.36 (s, 9 H, CH_3), 1.40 (s, 9 H, CH_3), 8.16 (br. s, 1 H, N*H*), 8.22 (d, *J* = 8.9 Hz, 1 H, Ar*H*), 8.47 (br. s, 1 H, N*H*), 8.52 (s, 1 H, Ar*H*), 8.62 (d, *J* = 9.0 Hz, 1 H, Ar*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 353 (32), 296 (100), 269 (85), 212 (32), 185 (78). MS (CI/isobutane): *m*/*z* (%) = 354 (100). $C_{19}H_{23}N_5O_2$ (353.4): calcd. C 64.57, H 6.56, N 19.82, found C 64.48, H 6.66, N 19.78.

***N*-{3-Cyano-7-(pentanoylamino)-1,8-naphthyridin-2-yl}pentanamide (24c):** 2,7-Diamino-1,8-naphthyridine-3-carboxamide (**23a**, 1.45 g, 7.14 mmol) was suspended under nitrogen in 30 mL of dry pyridine. First triethylamine (2.16 g, 21.4 mmol), and then valeryl chloride (3.46 g, 28.6 mmol), were added dropwise, and the suspension was heated to reflux for 2 h. After removal of the solvents in vacuo, the dark residue was dried and dissolved in 200 mL of a dichloromethane/water mixture (1:1). The layers were separated and the aqueous layer was extracted five times with 100 mL of dichloromethane. The aqueous layer was then continuously extracted with dichloromethane for 50 h. The combined organic layers were dried with sodium sulfate and the solvent was evaporated in vacuo. The remaining yellow solid (4.88 g) was purified by double chromatography on silica gel: (i) (90 g), dichloromethane/ethanol (10:1), R_f = 0.75, (1.79 g); (ii) (90 g), dichloromethane/ethanol (10:1). The resulting solid was recrystallized from toluene, giving 1.57 g (62%) of a colourless solid, m.p. 211 °C. IR (KBr): $\tilde{\nu}$ = 3368, 3331, 3256 cm^{-1} (N−H), 2957, 2929, 2871 (aliph. C−H), 2228 (C≡N), 1687 (C=O), 1605, 1570, 1504 (arom.), 1535 (N−H). ^1H NMR (500 MHz, $CDCl_3$): δ = 0.96 (t, *J* = 7.4 Hz, 3 H, CH_3), 0.97 (t, *J* = 7.4 Hz, 3 H, CH_3), 1.45 (sext, *J* = 7.5 Hz, 2 H, CH_2), 1.46 (sext, *J* = 7.5 Hz, 2 H, CH_2), 1.75 (quint, *J* = 7.4 Hz, 2 H, CH_2), 1.78 (quint, *J* = 7.4 Hz, 2 H, CH_2), 2.49 (t, *J* = 7.5 Hz, 2 H, CH_2), 2.69 (t, *J* = 7.5 Hz, 2 H, CH_2), 8.15 (br. s, 1 H, N*H*), 8.18 (d, *J* = 8.9 Hz, 1 H, Ar*H*), 8.35 (br. s, 1 H, N*H*), 8.49 (s, 1 H, Ar*H*), 8.59 (d, *J* = 8.9 Hz, 1 H, Ar*H*) ppm. MS (EI, 70 eV): *m*/*z* (%) = 353 (9), 324 (28), 296 (4), 269 (24), 240 (20), 212 (10), 185 (100). MS (CI/isobutane): *m*/*z* (%) = 354 (100). HR-MS: $C_{19}H_{23}N_5O_2$: calcd. 353.1852, found 353.1852; $C_{18}{}^{13}CH_{23}N_5O_2$: calcd. 354.1885, found 354.1885. $C_{19}H_{23}N_5O_2$ (353.4): calcd. C 64.57, H 6.56, N 19.82, found C 63.89 H 6.43 N 19.37.

***N*,*N*′-Dipyridin-2-ylurea (25a):** 2-Aminopyridine (50.0 g, 532 mmol) was dissolved under nitrogen in dry pyridine (150 mL), and ethyl chloroformate (75.0 g, 691 mmol) was added at 0 °C. After the mixture had been kept for 15 h at 5 °C, ice-cold water (250 mL) was added, and the solid was filtered off, washed thoroughly with water and dried. The yellow solid was recrystallized from ethanol, giving colourless crystals of ethyl pyridin-2-ylcarbamate (104.9 g, 67%, 70%[27]), m.p. 95 °C. IR (KBr): $\tilde{\nu}$ = 3183 cm^{-1} (N−H), 2987 (aliph. C−H), 1725 (C=O), 1586 (arom.), 1538 (N−H), 1440 (C−H), 1223, 1070 (C−O). ^1H NMR (90 MHz, $CDCl_3$): δ = 1.35 (t, *J* = 7.5 Hz, 3 H, CH_3), 4.27 (q, *J* = 7.5 Hz, 2 H, CH_2), 6.97 (m$_c$, 1 H, py-H), 7.68 (m$_c$, 1 H, py-H), 8.07 (d, *J* = 8.4 Hz, 1 H, py-H), 8.38 (m$_c$, 1 H, py-H), 10.33 (br. s, 1 H, NH) ppm. MS (EI, 70 eV): *m*/*z* (%) = 166 (74), 121 (23), 107 (27), 94 (100). MS (CI/isobutane): *m*/*z* (%) = 167 (100). The ethyl pyridin-2-ylcarbamate (16.6 g, 100 mmol) and 2-aminopyridine (14.1 g, 150 mmol) were dissolved under nitrogen in pyridine (200 mL) and heated at reflux for 6 d. After evaporation of the solvent, dichloromethane (500 mL) was added and the organic layer was washed with water (5 times 100 mL). After drying with magnesium sulfate, the solvent was evaporated and the residue was recrystallized first from ethanol/cyclohexane (1:2) and then from toluene, giving 8.62 g (40%) of **25a** (33%[28]), m.p. 169−170 °C. IR (KBr): $\tilde{\nu}$ = 3436, 3221, 3064 cm^{-1} (N−H), 2999 (aliph. C−H), 1700 (C=O), 1603, 1574, 1510 (arom.), 1554 (N−H). ^1H NMR (500 MHz, $CDCl_3$): δ = 6.99 (dd, *J* = 7.9, *J* = 5.1 Hz, 2 H, py-H), 6.9−8.4 (br. s, 2 H, py-H), 7.70 (dd, *J* = 7.8, *J* = 7.7 Hz, 2 H, py-H), 8.37 (d, *J* = 5.0 Hz, 2 H, py-H), 9.5−12.0 (br. s, 2 H, NH) ppm. MS (EI, 70 eV): *m*/*z* (%) = 214 (10), 121 (28), 94 (100), 78 (20). MS (CI/isobutane): *m*/*z* (%) = 215 (100).

NMR Titrations: ^1H NMR titrations were performed in $CDCl_3$ with the aid of a Bruker DRX 500, starting with ca. $5{\cdot}10^{-3}$ M of one component. For K_{ass} = 400, the choice of this concentration gives 50% complexation at 1:1 stoichiometry. The partner was added in small aliquots in such a way that several data points could be measured in the vicinity of 1:1 stoichiometry. Data points with very high or low ratios of the two components are of lower significance because at 1:1 stoichiometry the difference between an ob-

served complexation-induced chemical shift and the theoretical values for maximum complexation is largest.

Acknowledgments

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[1] See for example: L. Stryer, *Biochemistry*, W. H. Freeman and Company, New York, **1995**.
[2] When several hydrogen bonds are positioned next to each other, the hydrogen bond donor and acceptor sites may be located on the same or on opposite sides (e.g., DD·AA versus DA·DA). We use the expressions parallel and anti-parallel, respectively. In a parallel orientation, additional attractive secondary interactions exist, while the secondary interactions in an anti-parallel orientation are repulsive; see also ref.[4]
[3] See ref.[1], part V.
[4] This rule has exceptions. According to investigations by Schneider et al.,[5] each hydrogen bond contributes approx. 8 kJ/mol to the binding enthalpy. However, when hydrogen bonds are oriented next to each other, secondary Coulomb interactions[6,7] either favour or disfavour binding. Two parallel bonds additionally contribute approx. 3 kJ/mol while anti-parallel hydrogen bonds reduce the binding enthalpy by approx. 3 kJ/mol, due to electrostatic repulsions.
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