Isolation, Structure, Synthesis and Cytotoxicity of an Unprecedented Flupirtine Dimer

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A previously unknown dimer of the well-established analgesic flupirtine has been found, and its structure was revealed by ESI-MS, NMR spectroscopy and an independent synthesis. Thus, starting from 2-amino-6-chloro-3-nitro-pyridine the target compound was obtained in a four-step synthesis. Key-step of this synthesis is a nickel-mediated aryl-aryl coupling. The dimer **4** did not show any cytotoxicity, and its IC_{50} values were $> 30\ \mu$m for all six human cancer cell lines and mouse fibroblasts used in this study.

Key words: Flupirtine, Dimerization, Aryl-Aryl Coupling

Introduction

Neurodegenerative diseases are the sixth-leading cause of death in Europe and North America. Worldwide up to 40 million people suffer from these diseases. Recently, neuroprotective properties have been credited to flupirtine (**1**), ethyl *N*-[2-amino-6-(4-fluorophenylmethyl-amino)pyridin-3-yl]carbamate (Fig. 1). Flupirtine has been used since 1984 as a centrally acting non-opiate analgesic [1]. In addition, it reduces muscle-tone, and it does not show the side effects of nonsteroidal anti-inflammatory drugs or of opiates.

Recently, flupirtine came twice in the focus of renewed scientific interest. First, it was discussed for treating memory impairment and sensorimotoric shortfalls usually associated with Gulf War Veteran's Illness [2]. This disease affected *ca.* 25% of the almost 700.000 veterans of the Persian Gulf War of 1990/1991. Second, its neuroprotective properties make **1** an interesting candidate for the treatment of neurodegenerative diseases, *e. g.* Alzheimer's and Parkinson's as well as Creutzfeld-Jacob disease [3, 4]. In addition, fibromyalgia [5] has been treated successfully applying flupirtine. During our own studies on neuroprotection we became interested in **1** and derivatives thereof.

Results and Discussion

Flupirtine has been in medicinal use in Germany for more than twenty years. Recently, the quantification of **1** and its related compounds (*e. g.* impurities resulting either from synthesis or by decomposition during storage) in pharmaceutical dosage forms by UPLC has been reported [6]. Several years ago, Bednarski *et al.* [7] re-examined some aspects of the metabolism of **1** and revealed the formation of flupirtine dimers **2** and **3** (Fig. 1); the structure of the dimers was deduced by interpretation of HRMS data. For these dimers quasi-molecular ions $m/z = 607.258937$ and $m/z = 607.259010$ matching $[M+H]^+$ with an empirical formula $M = C_{30}H_{33}F_2N_8O_4$ were detected [7].

To shorten our synthesis [8, 9] of flupirtine analogs we decided to use commercial **1** as a suitable starting material. Thus, commercial samples of **1** (as its maleate) were bought. Their analytical investigation showed the presence of a dimeric compound with $m/z = 607.2$ (ESI-MS, cation-sensitive mode) corre-

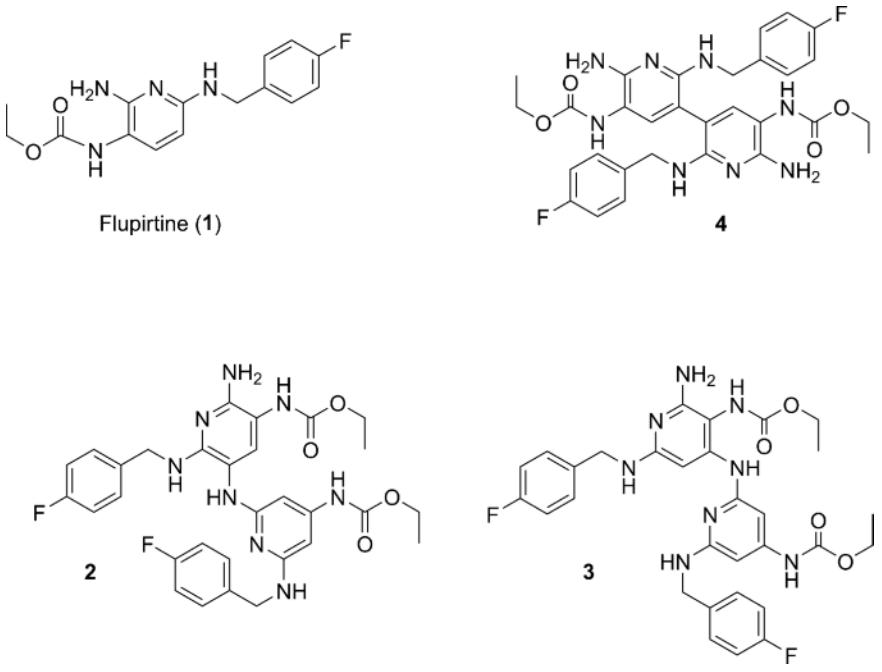
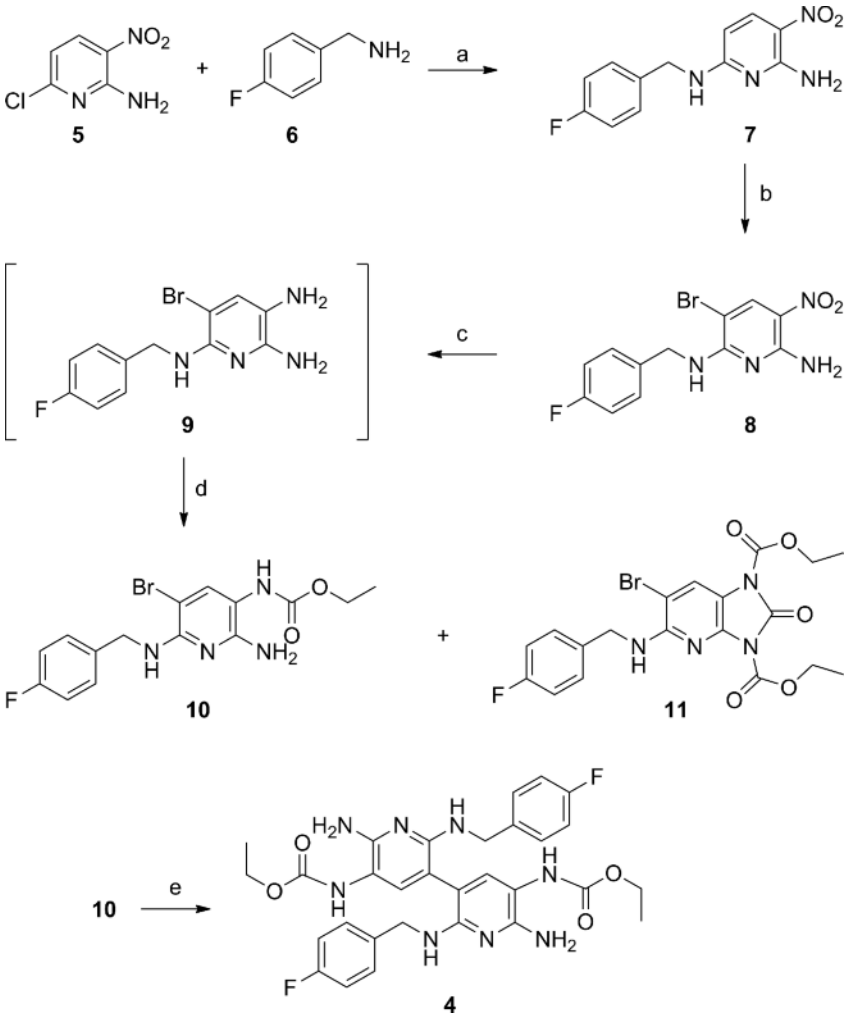


Fig. 1. Structure of flupirtine (**1**), of proposed *in vitro* dimers **2** and **3**, as well as of the novel dimer **4**.

sponding to $[M+H]^+$ with $M = C_{30}H_{32}F_2N_8O_4$; in addition a quasi-molecular ion $m/z = 304.1$ ($[M+2H]^+$) was detected. However, no dimers with an empirical formula $C_{30}H_{33}F_2N_8O_4$ [7] were detected in these samples. Isolation of this novel dimer by semi-preparative HPLC yielded enough material for its investigation by NMR. From the ^1H, ^{19}F and ^{13}C NMR spectra the dimer was assigned structure **4** (Fig. 1). Thus, compound **4** is characterized in its ^{19}F NMR spectrum by the presence of only one signal at $\delta = -120.1$ ppm. This suggests a symmetrical dimer. The signals for an ethyl carbamate group are found in the ^{13}C NMR spectrum at $\delta = 14.6$ and 60.1 ppm. The ^{13}C NMR spectrum also shows the presence of four quaternary carbons in the pyridine ring. Since there is still a primary amino group present (as indicated by the IR spectrum), the dimer has to possess a central C–C bond connecting the two pyridine rings. From the number of quaternary carbons in the pyridine ring and their chemical shifts in the NMR spectrum, the connecting bond between the two monomers has to be at position C-5 of the pyridine. To verify this proposed structure, we set out for an independent synthesis (Scheme 1).

Thus, reaction of 2-amino-6-chloro-3-nitro-pyridine (**5**) with 4-fluorobenzylamine (**6**) [10, 11] for 10 h under reflux gave the known compound **7** [8, 12] in excellent yield. Compound **7** is characterized in its ^{19}F NMR spectrum by the presence of a signal at $\delta = -115.9$ ppm showing $J_{F,H} = 8.9$ and 5.5 Hz, respectively. Bromination of **7** with NBS in the presence of ammonium acetate [13] provided the bromo compound **8** in almost quantitative yield. Compound **8** was reduced by Zn/NH_4Cl [14] to yield the diamine **9**. The reduction of **8** using Zn/NH_4Cl proceeds with better results than the well-established reduction using Raney-Nickel/H_2 at elevated temperatures and high pressures [8, 9]. Under these harsh conditions impure **9** is obtained that has to be re-crystallized several times. As an alternative, the use of Pd/H_2 was suggested [10]. The diamine **9** was transformed *in situ* [15, 16] into the corresponding N^3-ethylcarbamate **10** by reaction with ethyl chloroformate/triethylamine. As previously shown by Paradisi *et al.* [17, 18], the reaction of 2,3-diaminopyridines with ethyl chloroformate proceeds regioselectively at the *meta* position when the conditions are mild, and the temperature is kept low. No diacylation was observed.

From the reaction, however, a by-product **11** was isolated in 10% yield. The colorless solid showed in its ^1H NMR spectrum the presence of two ethyl carbamate moieties (*e. g.* $\delta = 1.40$ and 1.45 ppm for the methyl groups); the matching carbonyl groups were



Scheme 1. Synthesis of compound **4**. a) 2-Propanol, NEt_3, reflux, 10 h, 98.8 %; b) NBS, NH_4OAc, CH_3CN/THF, 0 °C, 3 h, 98.6 %; c) NH_4Cl, Zn, EtOAc, H_2O, 1 h, $T < 35$ °C, quant.; d) NEt_3, $ClCO_2Et$, 0 °C, 2 h, **10** (80.5 %), **11** (10 %); e) NEt_3, $NiCl_2$, PPh_3, Zn, THF, 60 °C, 2 d, 69 %.

detected in the ^{13}C NMR spectrum at $\delta = 149.9$ and 148.2 ppm. The ^{13}C NMR spectrum revealed also the presence of an extra carbonyl group at $\delta = 146.0$ ppm. There are four quaternary carbons in the pyridine ring, and C-2 of the pyridine ring shows a shifting of $|\Delta\delta| = 14$ ppm to higher field. From these data the structure of **11** was deduced. To corroborate this structure, suitable crystals were grown and subjected to a single-crystal X-ray analysis whose results affirmed the structure of **11** (Figs. 2 and 3) unambiguously [19]. As depicted in Fig. 3, two molecules of **11** are connected *via* a symmetrical bifurcated hydrogen bridge, thus resulting in a infinitive chain along the crystallographic glide plane [$\bar{1}$01]. This interaction can be considered as medium strong; no further intermolecular interaction is present. Interestingly enough in the formation of **11** no 6-bromo-5-[(4-fluorobenzyl)amino]-1,3-dihydro-2*H*-imidazo[4,5-*b*]pyridin-2-one [18] was formed other than the dicarboxylate **11**.

Aryl-aryl bond formation can be accomplished by many methods [20], Stille and Suzuki couplings being used quite often and very successfully. Nickel-catalyzed homo-coupling reactions have been studied since the early 1970s and shown to be very

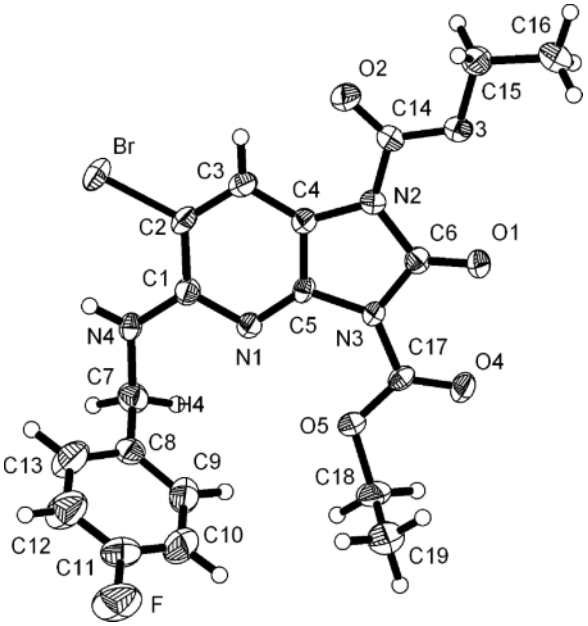


Fig. 2. Molecular structure of **11** in the crystal with atom labelling scheme (50 % probability ellipsoids; H atoms with arbitrary radii).

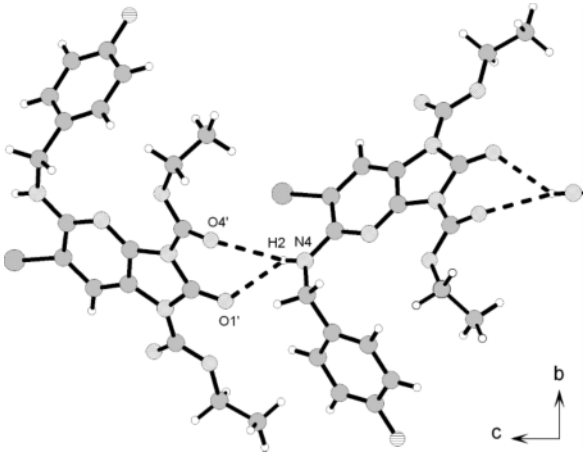


Fig. 3. The bifurcated intermolecular hydrogen bond in the crystal structure of **11**.

efficient for the synthesis of biaryls. Even though zero-valent nickel reagents are generally sensitive, tris(triphenylphosphine)nickel(0) can be generated *in situ* using the Tiecco/Testaferri [21] modification of Kende's procedure [22]. The dimerization reaction of **10** was crucial and failed in DMF [23, 24] or pyridine [25] as a solvent and gave only low yields of the dimer. In these solvents a fast de-bromination reaction took place; the main product was flupirtine (**1**). Coupling of **10** with $NiCl_2/PPh_3$ in the presence of triethylamine in THF, however, advanced nicely and gave dimeric **4** in 68.8 % isolated yield as an off-white solid. Compound **4** showed in its ESI-MS spectrum a cation with $m/z = 602.2$ corresponding to a quasi-molecular ion $[M+H]^+$ and a set of signals in its ^1H, ^{19}F and ^{13}C NMR spectra corresponding well with its dimeric structure. Compound **4** obtained by synthesis proved to be identical in every aspect to the material isolated from the commercial drug. Since **4** has been found as an impurity in commercial samples of the drug intended for human use (although in a rather low concentration of < 0.1 %) we became interested in its cytotoxicity. Testing of **4** in a colorimetric sulforhodamine assay [26] using six human cancer cell lines and mouse fibroblasts (NiH 3T3) gave $IC_{50} > 30 \mu M$ for each cell line.

Experimental Section

Cell lines and culture conditions

The human cancer cell lines 8505C, A2780, A549, MCF-7, 518A2, HT29 and mouse fibroblasts NiH 3T3 were included in this study. Cultures were maintained as monolayers in RPMI 1640 (PAA Laboratories, Pasching/Germany) supplemented with 10 % heat-inactivated fetal bovine serum (Sigma/Germany) and penicillin/streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5 % CO_2/95 % air.

Cytotoxicity assay [26]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0 – 100 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5 %. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was discarded, and the cells were fixed with 10 % TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done five times with water using alternate dispensing and aspiration procedures. Afterwards the plates were dyed with 100 μL of 0.4 % SRB (sulforhodamine B) for about 20 min. The plates were washed

with 1 % acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 μL of 10 mM Tris base solution were added to each well, and absorbance was measured at 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim/Germany). The IC_{50} values were calculated applying the two-parametric Hill slope equation.

Synthesis and analysis

Reagents were bought from commercial suppliers and used without any further purification. Melting points were measured with a Leica hot stage microscope and were not corrected. NMR spectra were recorded on Varian Gemini 200, Gemini 2000 or Unity 500 spectrometers at 27 °C with tetramethylsilane as an internal standard, δ values are given in ppm and J in Hz. Mass spectra were taken on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. Elemental analyses were measured on a Foss-Heraeus Vario EL unit. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 1000 and UV/Vis spectra on a Perkin-Elmer unit, Lambda 14. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried according to usual procedures.

*2-Amino-6-(4-fluorobenzylamino)-3-nitropyridine (**7**)*

To a suspension of 2-amino-6-chloro-3-nitro-pyridine (**5**, 47.00 g, 0.27 mol) in 2-propanol (240 mL), triethylamine (39.5 g, 0.39 mol) and 4-fluorobenzylamine (**6**, 36.2 g, 0.29 mol) were added, and the mixture was heated under reflux for 10 h. The mixture was cooled to 5 °C, water (750 mL) was added, and stirring was continued for another hour. The product was collected by filtration, washed with cold water (2 × 50 mL) and dried. Compound **7** (70.19 g, 98.8 %) was obtained as a pale-yellow solid; m. p. 180 – 181 °C (lit.: 171 – 174 °C [8]). – $R_f = 0.31$ (hexane-THF, 3 : 1). – IR (KBr): $\nu = 3413$s, 3368s, 3134m, 1636s, 1608s, 1509m, 1491m, 1405m, 1375m, 1281s, 1255s, 1172s, 1116m, 1014m, 774w cm^{-1}. – UV/Vis (MeOH): $\lambda_{max}(\log\varepsilon) = 220$ (3.72), 272 (3.49), 307 (3.25), 396 nm (3.97). – ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.97$ (d, $J = 9.3$ Hz, 1 H, 4-H), 7.42−7.36 (m, 2 H, 2 × 9-H), 7.18−7.12 (m, 2 H, 2 × 10-H), 6.00 (d, $J = 9.3$ Hz, 1 H, 5-H), 4.56 (d, $J = 5.4$ Hz, 2 H, 7-CH$_2$) ppm. – ^{13}C NMR (125 MHz, CDCl$_3$): $\delta = 161.3$ (d, $^1J_{C,F} = 242.6$ Hz, C-11), 160.4 (C-6), 155.7 (C-2), 135.2 (d, $^4J_{C,F} = 3.0$ Hz, C-8), 134.5 (C-4), 129.7 (d, $^3J_{C,F} = 7.8$ Hz, C-9), 117.6 (C-3), 115.1 (d, $^2J_{C,F} = 21.2$ Hz, C-10), 102.3 (C-5), 43.1 (C-7) ppm. – ^{19}F NMR (376 MHz, [D$_6$]DMSO): $\delta = -115.9$ (tt, $^3J_{F,H} = 8.9$ Hz, $^4J_{F,H} = 5.5$ Hz) ppm. – MS ((+)-ESI): m/z (%) = 262.1 (100) [M+H]$^+$, 285.1 (10) [M+Na]$^+$. – $C_{12}H_{11}FNO_4$ (262.24): calcd. C 54.96, H 4.23, N, 21.36; found C 54.72, H 4.29, N 21.31.

*2-Amino-6-(4-fluorobenzylamino)-3-bromo-5-nitropyridine (**8**)*

To a solution of **7** (20.00 g, 76.2 mmol) and ammonium acetate (0.58 g, 7.6 mmol) in a mixture of THF (150 mL) and acetonitrile (150 mL) NBS (13.70 g, 77.0 mmol) was added at 0 °C in several small portions, and stirring was continued at this temperature for 3 h. The solvent was removed under reduced pressure, and the residue subjected to chromatography (silica gel 60, chloroform-hexane-ethyl acetate, 9 : 5 : 1) to yield **8** (15.66 g, 98.6 %) as a yellow solid; m. p. 143 – 144 °C. – $R_f = 0.63$ (chloroform-hexane-ethyl acetate, 9 : 5 : 1). – IR (KBr): $\nu = 3490$m, 3322m, 3091w, 2948w, 1598s, 1553m, 1508m, 1474m, 1404m, 1273s, 1228s, 1124m, 1054w, 762m cm^{-1}. – UV/Vis (MeOH): $\lambda_{max}(\log\varepsilon) = 226$ (3.71), 281 (3.43), 403 nm (3.81). – ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 8.40$ (s, 1 H, 4-H), 7.32−7.27 (m, 2 H, 2 × 9-H), 7.07−7.02 (m, 2 H, 2 × 10-H), 4.66 (d, $J = 5.7$ Hz, 2 H, 7-CH$_2$) ppm. – ^{13}C NMR (100 MHz, CDCl$_3$): $\delta = 162.5$ (d, $^1J_{C,F} = 246.4$ Hz, C-11), 156.2 (C-6), 153.8 (C-2), 137.5 (C-4), 133.5 (d, $^4J_{C,F} = 3.4$ Hz, C-8), 129.5 (d, $^3J_{C,F} = 7.9$ Hz, C-9), 120.3 (C-3), 115.9 (d, $^2J_{C,F} = 21.5$ Hz, C-10), 93.7 (C-5), 45.3 (C-7) ppm. – ^{19}F NMR (376 MHz, [D$_6$]DMSO): $\delta = -115.9$ (tt, $^3J_{F,H} = 8.6$ Hz, $^4J_{F,H} = 5.4$ Hz) ppm. – MS ((+)-ESI): m/z (%) = 341.1 (100) [M+H]$^+$, 343.1 (92) [M+H]$^+$. – $C_{12}H_{10}BrFN_4O_2$ (341.14): calcd. C 42.25, H 2.95, N, 16.42; found C 42.17, H 3.06, N 16.32.

*Ethyl {2-amino-5-bromo-6-[(4-fluorobenzyl)amino]-pyridine-3-yl} carbamate (**10**)*

To a solution of **8** (10.00 g, 29.4 mmol) in EtOAc (150 mL) a solution of ammonium acetate (15.72 g, 294.0 mmol) in water (80 mL) was added. Zinc powder (11.54 g, 176.4 mmol) was added in small portions (keeping the temperature of the reaction below 35 °C). After stirring for an additional hour, the mixture was filtered through a small pad of Celite, washed with water (3 × 50 mL) and brine (2 × 50 mL) and dried (MgSO$_4$). The mixture was filtered, and dry triethylamine (4.16 g, 41.2 mmol) was added; the mixture was cooled to 0 °C, and ethyl chloroformate (3.82 g, 35.2 mmol) was slowly added. The mixture was stirred for 2 h, water (70 mL) was added, the phases were separated, and the organic phase was washed with water (3 × 50 mL) and brine (60 mL). The solvents were removed under diminished pressure, and the residue subjected to chromatography (silica gel 60, chloroform-ethyl acetate, 9 : 1) to afford **10** (9.04 g, 80.5 %) as a colorless solid; m. p. 146 – 147 °C. – $R_f = 0.30$ (chloroform-ethyl acetate, 9 : 1). – IR (KBr): $\nu = 3431$m, 3356m, 3293m, 2885w, 1679s, 1638m, 1608m, 1530s, 1508s, 1438m, 1267s, 1221m, 1153w, 1072m, 1011w, 816w, 500w cm^{-1}. – UV/Vis (MeOH): $\lambda_{max}(\log\varepsilon) = 208$ (3.94), 252 (3.59), 328 nm

(3.48). – ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.41-7.33$ (m, 3 H, 4-H, 2 × 9-H), 7.12−7.06 (m, 2 H, 2 × 10-H), 4.48 (d, $J = 6.1$ Hz, 2 H, 7-CH$_2$), 4.05 (q, $J = 7.1$ Hz, 2 H, 13-CH$_2$), 1.21 (t, $J = 6.9$ Hz, 3 H, 14-CH$_3$) ppm. – ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 161.0$ (d, $^1J_{C,F} = 241.6$ Hz, C-11), 155.0 (C-12, *C* =O), 152.1 (C-2), 150.7 (C-6), 137.2 (d, $^4J_{C,F} = 2.9$ Hz, C-8), 137.2 (C-4), 129.3 (d, $^3J_{C,F} = 8.0$ Hz, C-9), 114.7 (d, $^2J_{C,F} = 21.1$ Hz, C-10), 107.9 (C-3), 87.2 (C-5), 60.2 (C-13, CH$_2$), 43.4 (C-7), 14.6 (C-14, CH$_3$) ppm. – ^{19}F NMR (376 MHz, [D$_6$]DMSO): $\delta = -116.8$ (tt, $^3J_{F,H} = 8.9$ Hz, $^4J_{F,H} = 5.7$ Hz) ppm. – MS ((–)-ESI): *m/z* (%) = 380.9 (83) [M−H]$^-$, 383.0 (100) [M−H]$^-$, 335.2 (19) [M−EtOH]$^-$, 337.2 (19) [M−EtOH]$^-$. – $C_{15}H_{16}BrFN_4O_2$ (383.22): calcd. C 47.02, H 4.21, N, 14.62; found C 46.85, H 4.41, N 14.60.

*Diethyl 6-bromo-5-[(4-fluorobenzyl)amino]-2-oxo-1H-imidazo[4,5-b]pyridine-1,3(2H)-dicarboxylate (**11**)*

Compound **11** (1.42 g, 10 %) was obtained as a colorless solid; m. p. 162 °C. – $R_f = 0.63$ (chloroform-ethyl acetate, 9 : 1). – IR (KBr): $\nu = 3409$s, 2984w, 2938w, 1802s, 1726s, 1622s, 1510s, 1427s, 1372s, 1334s, 1221s, 1148s, 1072m, 1038m, 898w, 856m, 767m, 745m, 692m, 613w, 546w, 476w cm^{-1}. – UV/Vis (MeOH): $\lambda_{max}(\log \varepsilon) = 258$ (3.53), 337 nm (3.36). – ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 8.14$ (s, 1 H, 4-H), 7.38−7.33 (m, 2 H, 2 × 9-H), 7.02−7.96 (m, 2 H, 2 × 10-H), 4.63 (s, 2 H, 7-CH$_2$), 4.52−4.46 (m, 4 H, 14-CH$_2$, 7-CH$_2$), 1.45 (t, $J = 7.1$ Hz, 3 H, 18-CH$_3$), 1.40 (t, $J = 7.1$ Hz, 3 H, 15-C*H*$_3$) ppm. – ^{13}C NMR (100 MHz, CDCl$_3$): $\delta =$ 162.3 (d, $^1J_{C,F} = 245.4$ Hz, C-11), 151.3 (C-6), 149.9 (C-16, *C*=O), 148.2 (C-13, *C*=O), 146.0 (C-12, *C*=O), 138.1 (C-2), 135.0 (d, $^4J_{C,F} = 3.1$ Hz, C-8), 129.7 (d, $^3J_{C,F} = 8.0$ Hz, C-9), 126.8 (C-4), 115.5 (d, $^2J_{C,F} = 21.4$ Hz, C-10), 111.5 (C-3), 99.1 (C-5), 64.4 (C-17, CH$_2$), 64.3 (C-14, CH$_2$), 45.4 (C-7), 14.3 (C-18, CH$_3$), 14.2 (C-15, CH$_3$) ppm. – ^{19}F NMR (376 MHz, CDCl$_3$): $\delta = -120.1$ (tt, $^3J_{F,H} = 8.9$ Hz, $^4J_{F,H} = 5.7$ Hz) ppm. – MS ((+)-ESI): *m/z* (%) = 480.9 (8) [M+H]$^+$, 503.1 (100) [M+Na]$^+$, 984.8 (48) [2M+Na]$^+$. – $C_{19}H_{18}BrFN_4O_5$ (481.04): calcd. C 47.42, H 3.77, N, 11.64; found C 47.31, H 3.93, N 11.49.

*Diethyl {6,6′-diamino-2,2′-bis[(4-fluorobenzyl)amino]-3,3′-bipyridine-5,5′-diyl} biscarbamate (**4**)*

Under argon a solution of **10** (10.00 g, 26.1 mmol) and triethylamine (7.92 g, 78.3 mmol) in dry THF (100 mL) at 60 °C was added to a suspension of NiCl$_2$ (0.34 g, 2.6 mmol), triphenylphosphane (5.46 g, 20.8 mmol), powdered Zn (5.12 g, 78.3 mmol), and triethylamine (7.92 g, 78.3 mmol) in dry THF (100 mL). The mixture was stirred for 2 days at 60 °C, then the mixture was poured onto the top of a glass column containing silica gel. The product was eluted using a mixture of THF-diethyl ether (1 : 1). After dilution with the double volume of hexane, the precipitated product was collected by filtration and washed with a mixture of hexane-THF-diethyl ether (2 : 1 : 1) and finally dried overnight. Re-precipitation from THF with hexane gave **4** (5.46 g, 69.0 %) as a slightly off-white solid. – HPLC/DAD (LaChrom D-7000, Merck-Hitachi): Nucleosil 100 – 5 C18 – AB (Macherey-Nagel), 35 °C, 30 % CH$_3$CN/phosphate buffer (50 mM, pH = 2.8), 1 mL min^{-1}, retention time 12.5 min (retention time **1**: 4.5 min); m. p. 244 °C. – $R_f = 0.58$ (hexane-THF, 1 : 1). – IR (KBr): $\nu = 3392$m, 3324s, 2983w, 2850w, 1682s, 1638m, 1586m, 1528s, 1430s, 1384m, 1221s, 1157w, 1060m, 824w, 770w, 602w, 577w, 501w cm^{-1}. – UV/Vis (MeOH): λ_{max} (log ε) = 206 (3.83), 251 (3.41), 328 nm (3.37). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta =$ 7.34−7.30 (m, 4 H, 4 × 9-H), 7.04−6.99 (m, 4 H, 4 × 10-H), 6.99−6.95 (brs, 2 H, 2 × 4-H), 4.47−4.36 (m, 4 H, 2 × 7-CH$_2$), 4.05 (brq, $J = 7.0$ Hz, 4 H, 2 × 13-CH$_2$), 1.21 (brt, $J = 6.2$ Hz, 6 H, 2 × 14-CH$_3$) ppm. – ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta = 161.0$ (d, $^1J_{C,F} = 241.5$ Hz, C-11), 155.0 (C-12, *C* =O), 152.1 (C-6), 151.9 (C-2), 137.5 (d, $^4J_{C,F} = 2.8$ Hz, C-8), 136.6 (C-4), 129.4 (d, $^3J_{C,F} = 7.9$ Hz, C-9), 114.6 (d, $^2J_{C,F} = 21.0$ Hz, C-10), 107.5 (C-3), 104.8 (C-5), 60.1 (C-13, CH$_2$), 43.7 (C-7), 14.6 (C-14, CH$_3$) ppm. – ^{19}F NMR (376 MHz, [D$_6$]DMSO): $\delta = -117.0$ (tt, $^3J_{F,H} = 9.1$ Hz, $^4J_{F,H} = 5.7$ Hz) ppm. – MS ((+)-ESI): *m/z* (%) = 304.1 (22) [M+2H]$^{2+}$, 607.2 (100) [M+H]$^+$). – $C_{30}H_{32}F_2N_8O_4$ (606.52): calcd. C 59.40, H 5.32, N, 18.47; found C 59.32, H 5.47, N 18.36.

Table 1. Crystallographic data for compound **11**.

Identification code	i2t0902
Empirical formula	$C_{19}H_{18}BrFN_4O_5$
Formula weight	481.28
Crystal size, mm^3	0.23 × 0.10 × 0.09
Temperature T, K	200(2)
Wavelength λ, Å	0.71073
Crystal system; space group	monoclinic; $P2_1/n$
Unit cell dimensions	
a, Å	4.901(1)
b, Å	23.996(1)
c, Å	16.943(1)
β, deg	90.69(1)
Volume V, Å^3	1992.2(3)
Z; calculated density $D_{calcd.}$, g cm^{-3}	2; 1.61
Absorption coefficient μ(MoK_α), mm^{-1}	2.1
2θ range for data collection, deg	6.22−55.99
Limiting indices *hkl*	±6, −26 → 31, −19 → 22
Reflections collected / unique / R_{int}	9380 / 4644 / 0.0549
Data/ refined parameters	4644 / 271
Final indices $R1$ / $wR2$ [$I > 2\sigma(I)$]	0.0343 / 0.0740
Funal indices $R1$ / $wR2$ (all data)	0.0749 / 0.0991
Goodness of fit on F^2	0.804
Largest diff. peak / hole $\Delta\rho_{fin}$ (max / min), e Å^{-3}	0.41 / −0.43

Table 2. Selected bond lengths (Å) and bond angles (deg) for compound **11**.

C(1)–N(1)	1.355(4)	C(8)–C(13)	1.382(5)
C(1)–N(4)	1.344(4)	C(9)–C(10)	1.387(6)
C(1)–C(2)	1.423(4)	C(10)–C(11)	1.342(6)
C(2)–C(3)	1.381(5)	C(11)–C(12)	1.341(6)
C(2)–Br	1.892(3)	C(11)–F	1.376(5)
C(3)–C(4)	1.375(4)	C(12)–C(13)	1.380(6)
C(4)–C(5)	1.375(4)	C(14)–O(2)	1.202(3)
C(4)–N(2)	1.410(4)	C(14)–O(3)	1.312(4)
C(5)–N(1)	1.316(4)	C(14)–N(2)	1.398(4)
C(5)–N(3)	1.422(4)	C(15)–O(3)	1.466(4)
C(6)–O(1)	1.196(4)	C(15)–C(16)	1.508(5)
C(6)–N(3)	1.409(4)	C(18)–O(5)	1.466(4)
C(6)–N(2)	1.416(4)	C(18)–C(19)	1.478(6)
C(7)–N(4)	1.454(4)	N(3)–C(17)	1.414(3)
C(7)–C(8)	1.510(5)	C(17)–O(4)	1.200(3)
C(8)–C(9)	1.358(5)	C(17)–O(5)	1.306(4)
N(4)–C(1)–N(1)	118.1(3)	O(2)–C(14)–O(3)	126.2(3)
N(4)–C(1)–C(2)	121.7(3)	O(2)–C(14)–N(2)	120.7(3)
C(1)–C(2)–Br	118.9(3)	O(3)–C(14)–N(2)	113.1(2)
C(3)–C(2)–Br	119.5(2)	C(5)–N(1)–C(1)	116.7(3)
C(3)–C(4)–N(2)	132.4(3)	C(14)–N(2)–C(4)	122.9(2)
C(5)–C(4)–N(2)	108.2(2)	C(14)–N(2)–C(6)	126.7(3)
N(1)–C(5)–N(3)	126.8(3)	C(5)–N(3)–C(17)	128.8(3)
O(1)–C(6)–N(2)	127.7(3)	C(6)–N(3)–C(17)	121.1(2)
O(1)–C(6)–N(3)	127.5(3)	C(1)–N(4)–C(7)	123.0(3)
N(2)–C(6)–N(3)	104.8(2)	O(4)–C(17)–O(5)	126.5(3)
N(4)–C(7)–C(8)	112.6(3)	O(4)–C(17)–N(3)	122.5(3)
C(7)–C(8)–C(9)	121.6(3)	O(5)–C(17)–N(3)	110.9(2)
C(7)–C(8)–C(13)	120.5(3)	C(14)–O(3)–C(15)	113.7(2)
C(10)–C(11)–F	118.8(4)	C(17)–O(5)–C(18)	116.5(2)
C(12)–C(11)–F	118.4(4)		

Table 3. Hydrogen bonds for compound **11** (Å and deg)[a].

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(D–H···A)
N4–H2···O1^{i}	0.88	2.37	3.105(3)	141.6
N4–H2···O4^{i}	0.88	2.65	3.112(4)	113.8

[a] Symmetry operation: ^{i} $x-1/2, -y+1/2, z+1/2$.

Crystal structure determination

The data were collected with a Stoe IPDS diffractometer at 200 K using MoK_α radiation ($\lambda = 71.073$ pm). The structure was solved with Direct Methods using SHELXS-97 [19]. Anisotropic displacement parameters were used to refine all non-hydrogen atoms (SHELXL-97 [19]). The hydrogen atoms were placed in calculated positions. The graphical representation of the molecular structure was made with DIAMOND3 [27]. Crystallographic data for compound **11** are given in Table 1. Selected bond lengths and angles as well as the values of the hydrogen bond are found in Tables 2 and 3.

CCDC 903882 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

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