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A new synthetic route to compounds of the AFDX-type with affinity to muscarinic M_2-receptor

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Abstract—[3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-propyl]dimethyl-{6-[(1-{2-[(6-oxo-5,6-dihydro-benzo[*e*]pyrido[3,2-*b*][1,4]diazepine-11-carbonyl)amino]ethyl}piperidin-2-yl-methyl)-propylamino]hexyl}ammonium bromide a hybride containing a fragment of the antagonist of muscarinic receptor AFDX-384 and a W84 moiety known as allosteric modulator of antagonist binding, was synthesized in a divergent synthesis starting from pipecolic acid, phthalic anhydride and 3-amino-2-chloropyridine. This new microwave assisted route is very convenient and allows to modify the piperidine ring, the benzodiazepine system, the phthalimide moiety and the chains connecting the ring systems. Yields and reproducibility were satisfying. © 2003 Elsevier Science Ltd. All rights reserved.

1. Introduction

Great efforts were made to develop selective agonists and antagonists of the subtypes of muscarinic receptors.[1] Ligands having a prevalence rather than a selectivity to one subtype are known to date. AFDX-384 is one of the compounds being a subtype-prevalent M_2-antagonist. On the other hand allosteric modulators of the muscarinic acetylcholine receptors[2,3] were developed which are able to influence the ligand binding to the receptor. They either enhance the equilibrium binding of the orthosteric agonist or antagonist, or diminish the corresponding to positive or negative cooperativity,[4] respectively. Recently, hexamethino-type compounds bearing lateral phthalimide moieties were reported to mediate positive or negative cooperative sensitively depending on the substitution pattern of the modulator. Moreover, the structure–activity-relationships are different for different orthosteric ligands of the receptor, e.g. for the antagonists *N*-methylscopolamine and the diazepine compound AFDX-384.[2]

Preliminary studies using W84 as a modulator and AFDX-384 as an orthosteric ligand revealed overlapping points of attachment on the receptor protein.[5] Thus, the purpose of this study was to develop a synthesis route to hybrides which are characterized by a part of the AFDX-384 molecule guiding the hybride to the M_2-receptor subtype and a W84-part binding to the allosteric site. This should open up the possibility to obtain a huge number of compounds of different substitution pattern.

Keywords: AFDX-384; W84; microwave assisted reaction; AFDX-384-W84 hybride.

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2. Results and discussion

The convergent synthesis of the hybride **1** was carried out by combining the AFDX-384 analogue **2** obtained from the diazepine carbonyl chloride **11** and the piperidine **9**, and a W84 fragment **3**. In order to improve the yield and purity of some intermediates microwave assisted reactions were extensively used. For the theory and the application of microwave assisted reactions see Ref. 6 (Scheme 1).

The multistep synthesis of compound **2** started from pipecolic acid **4**. The amino group was protected by using di*tert*butyl dicarbonate under common conditions. The amide **6** was obtained as described in Ref. 7 using *N*,*N*-benzylpropylamine, which was synthesized according to the reaction described by Hutchins et al.[8] The ^{13}C NMR spectra of **6** revealed the presence of rotamers. The rotations of the bulky Boc-group in position 1 and the benzyl-propylamide group in position 2 were sterically hindered resulting in a double set of signals. For the hydrochloride **7** and the nitrile **8** isomers were also observed. The Boc-group was cleaved with HCl in EtOH using a microwave oven. The advantage of this procedure was the time-saving (5 min instead of 5 d), higher yield and higher purity of product **7**, which can be used without further purification. The nitrile **8** was prepared using **7**, bromo acetonitrile and an auxiliary base by means of a microwave reaction. Again, the yields were better compared with the classical way of heating and stirring in an oil-bath (70% instead of 50%), the purity was higher and the reaction time was shortened (1 h versus 7 d). The nitrile **8** was reduced by means of $LiAlH_4$ and $AlCl_3$ using the method of Quast et al.[9] to obtain the piperidine derivative **9** (Scheme 2).

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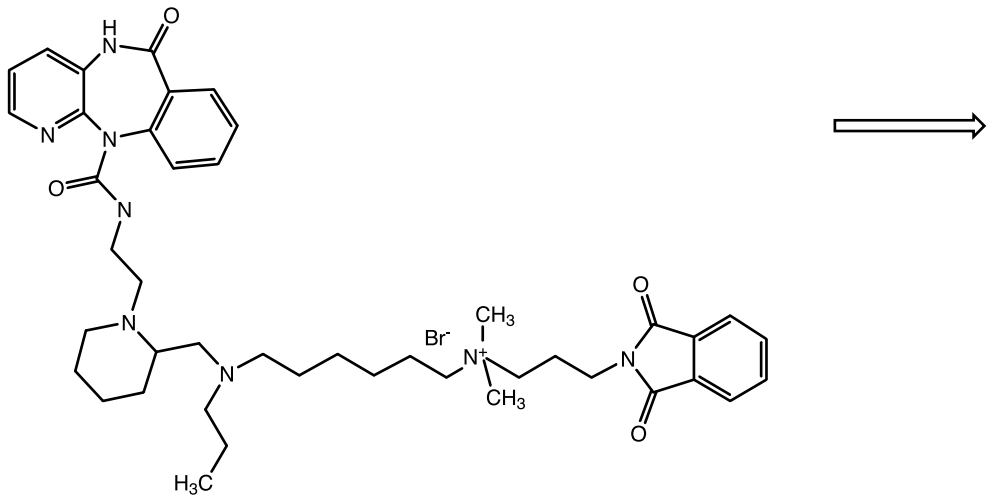

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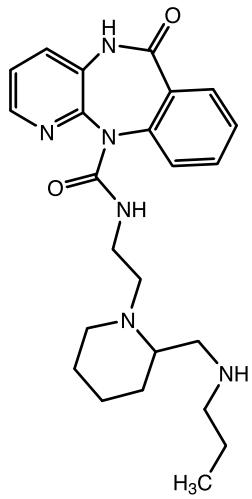

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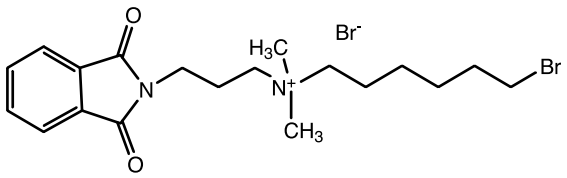


3

Scheme 1.

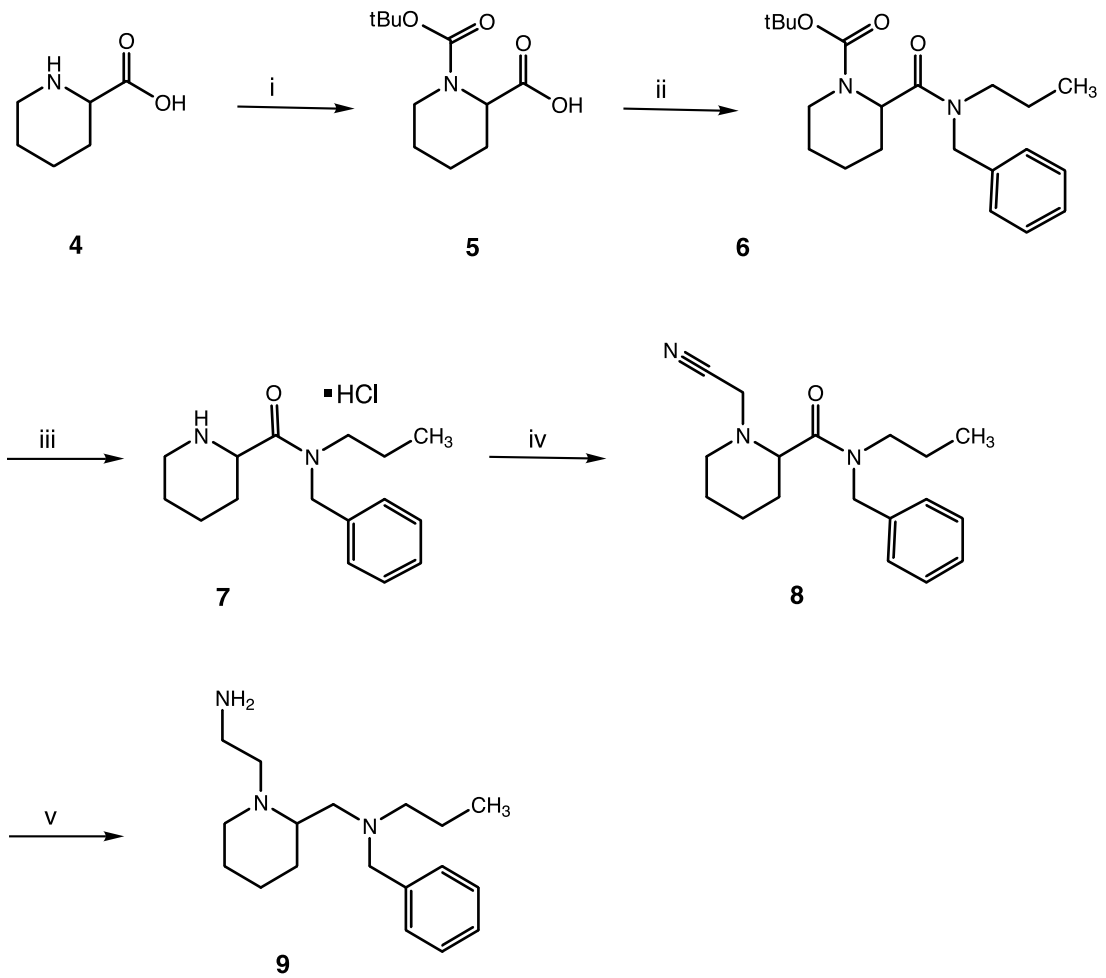
The synthesis of the benzodiazepine **10** by conversion of 3-amino-2-chloropyridine with ethyl 2-aminobenzoate was already described in Ref. 10. However, all mentioned procedures were hardly reproducible. We obtained either unidentified decomposition products or the working-up and cleaning procedure of the product was complicated, even when we carried out the reaction under the same conditions, the yields were ranging between 0 and 40%. In addition only ^{1}H NMR- and IR-data without any assignment could be found.[11,12] The new method utilizing microwaves resulted in 42% yield, which could be easily reproduced.

The diazepine **10** was transformed into the carbonyl chloride **11** by using phosgene.[10a] We used several auxiliary bases (Hünig's base, triethylamine) but we always isolate a mixture of compound **11** and its hydrochloride, which can be used in the next step without any further purification. However, the use of microwave was advantageous. We were able to obtain the pure product **11** without the hydrochloride and any other by-products (Scheme 3).

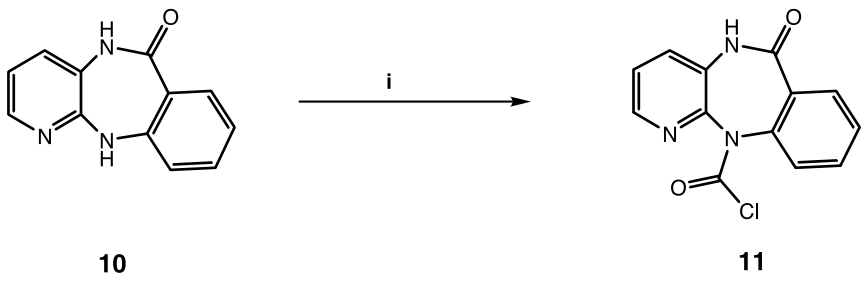
Compound **11** and the piperidine **9** were transformed in the microwave oven to obtain the AFDX-384 derivative **12**. The benzyl protecting group was cleaved by hydrogenation over palladium/charcoal to obtain compound **2** in a good yield and high purity (Scheme 4).

The W84 fragment **3**, obtained by reaction of *N*,*N*-dimethylaminopropylphthalimide with an excess of dibromohexane without any solvent,[13,14] and the AFDX derivative **2** were combined by using Hünig's base as an auxiliary. We obtained the hybride **1** as pale yellow crystals (Scheme 5).

The nearby developed synthesis presented here is highly appropriable to vary the substitution pattern in each part of the molecule, the diazepine ring system, the piperidine and the phthalimido moiety. This is important for optimisation of the lead structure in terms of allosteric potency, receptor subtype selectivity and positive cooperativity. Preliminary pharmacological studies have already demonstrated the allosteric potency of the hybride **1**.



Scheme 2. (i) Boc_2O, 16 h, 93%; (ii) (1) Boc_2O, DMAP, 30 min, (2) benzylpropylamine, 7 d, 67%; (iii) EtOH/HCl, microwave 5 min, 94%; (iv) $BrCH_2CN$, microwave, 2 h, 64%; (v) $LiAlH_4$, $AlCl_3$, 5 h reflux, 57%.



Scheme 3. (i) Phosgene, microwave, 2 h, 87%.

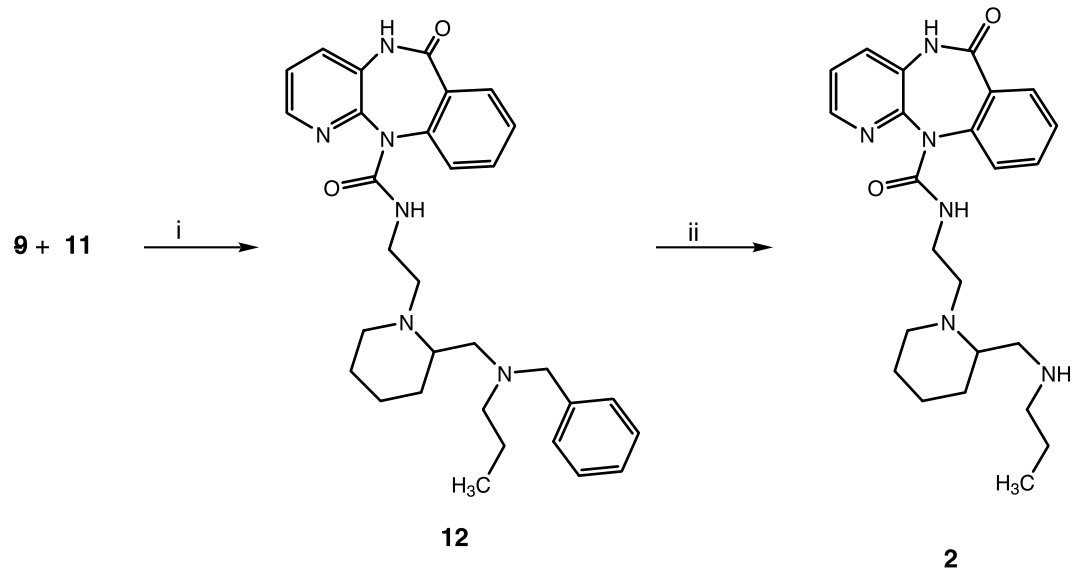
3. Experimental

3.1. General

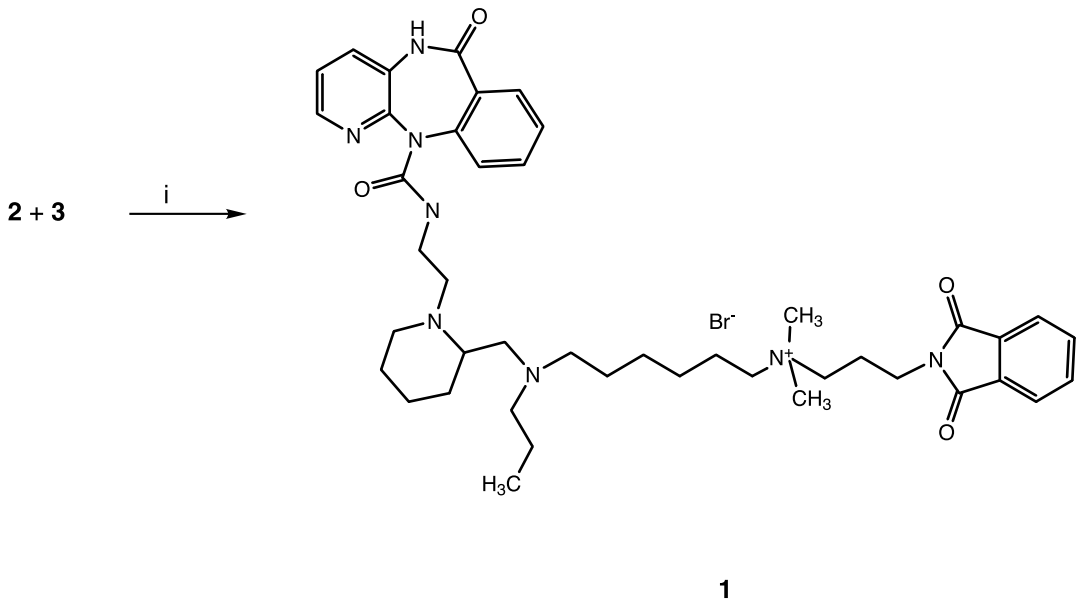
Melting points were determined using a Gallenkamp MPD350:BM3.5 apparatus and were uncorrected. ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Bruker 400 MHz spectrometer, FT-IR spectra on a Bio-Rad Pharmalyzir equipped with an ATR unit and mass spectra on a CH7 Varian-MAT. Microwave reactions were carried out in a Milestone MLS-Ethos 1600, using either a 250 ml 3-necked round-bottom flask (open system) or a quartz glass vessel (length 15 cm, diameter 2 cm) equipped with a 20 bar excess pressure valve (closed system) which were procured from Milestone. The temperature of the reaction was measured directly with a fibre optic sensor.

3.1.1. Piperidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 5. Compound **5** was synthesized according to Ref. 7.

3.1.2. 2-(Benzylpropylcarbamoyl)piperidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 6. The carboxylic acid **5** (5.73 g, 25.0 mmol) was dissolved in dry THF (100 ml). Dry pyridine (2.2 ml, 27.50 mmol), di*tert*butyl dicarbonate (5.73 g, 26.3 mmol) and DMAP (0.15 g, 1.25 mmol) were added and the mixture was stirred at 25°C for 30 min. Afterwards, the solution was treated with benzylpropylamine



Scheme 4. (i) K_2CO_3, THF, microwave, 10 min, 70%; (ii) Pd/C, H_2, 50 bar, 18 h, 99%.



Scheme 5. (i) $CHCl_3$, Hünig's base, 60°C, 24 h, 83%.

(4.10 g, 27.5 mmol) and stirred for 7 d at 25°C (monitoring by TLC, silica gel/ethyl acetate). The THF was evaporated in vacuo and the obtained mixture was diluted with ethyl acetate (300 ml). The organic layer was washed with 5% HCl (200 ml), brine (200 ml) and sat. $NaHCO_3$ (200 ml). The organic layer was dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting yellow oil was purified by column chromatography (silica gel/ethyl acetate) to obtain a colourless oil (yield: 6.01 g, 67%). R_f: 0.84. ^{1}H NMR ($CDCl_3$) δ: 7.26–7.12 (5H, m, aromat.), 5.02–4.36 (3H, m, 2-H, CH_2–Ph), 3.81 (1H, br, 6_a-H), 3.37-3.05 (3H, m, 6_b-H, N–CH_2–CH_2–CH_3), 1.83–1.12 (17H, m, 3-H, 4-H, 5-H, N–CH_2–CH_2–CH_3, *t*Bu), 0.82–0.75 ppm (3H, m, CH_3). ^{13}C NMR ($CDCl_3$) δ: 172.5, 172.1 (C═O amide, rotamers), 155.7 (C═O urethane, br), 137.9, 137.4 (Cquat, aromat., rotamers), 128.6, 128.4, 128.0, 127.6, 127.0, 126.7 (C-aromat., rotamers), 79.9, 79.6 (Cquat., *t*Bu, rotamers), 52.0 (CH_2–Ph), 51.7, (C-2, br), 50.7, 50.4 (C-6, rotamers), 48.7, 48.3 (N–CH_2–CH_2–CH_3, rotamers), 28.4, 28.0 (*t*Bu, rotamers), 27.0, 26.8, 25.1, 25.0, 21.8, 20.3, 19.8, 19.6 (C-3, C-4, C-5, N–CH_2–CH_2–CH_3, rotamers), 11.3 ppm (CH_3). IR (ATR): 2966, 2934, 2875 (CH), 1686 (C═O urethane), 1646 (C═O amide), 1450, 1391, 1364, 1159, 1142 cm^{-1}. MS (CI, NH_3), *m/z* (%): 361 [$(M+1)^+$, 100], 378 [$(M+NH_4)^+$, 54]; HRMS (CI/NH_3) 361.2492 [(MH^+) calcd for $C_{16}H_{24}N_2OH^+$ 361.2491].

3.1.3. Piperidine-2-carboxylic acid benzylpropylamide hydrochloride 7. The amide **6** (0.78 g, 2.2 mmol) was dissolved in EtOH (15 ml) and 0.7 ml conc. HCl. The solution was heated in a sealed glass tube in the microwave oven (gradient of heating: 3 min to 125°C; holding time: 5 min at 125°C). After cooling to 25°C the solvent was evaporated in vacuo and the residual oil was crystallized

with EtOH/Et_2O. Yield: 0.60 g (94%) colourless solid mp 171°C (dec). ^{1}H NMR (DMSO-d_6) δ: 9.28, 8.57 (2H, br, NH_2^+), 7.53–7.21 (5H, m, H-aromat.), 4.72–4.11 (3H, m, 2-H, CH_2–Ph), 3.23–2.81 (4H, m, 6-H, N–CH_2–CH$_2$–CH$_3$), 2.03–1.34 (8H, m, 3-H, 4-H, 5-H, N–CH$_2$–CH_2–CH$_3$), 0.91–0.75 ppm (3H, m, CH$_3$). ^{13}C NMR (DMSO-d_6) δ: 169.4, 169.2 (C=O amide, isomers), 137.6, 136.9 (Cquat, aromat., isomers), 129.0, 128.8, 127.9, 127.8, 127.6, 127.4 (C-aromat., isomers), 55.1, (C-2, br), 50.0, 49.9 (*C*H$_2$–Ph, isomers), 48.5, 48.2 (C-6, isomers), 47.9, 46.6 (N–*C*H$_2$–CH$_2$–CH$_3$, isomers), 27.1, 27.0, 21.7, 21.5, 21.4, 21.3, 19.9, 19.6 (C-3, C-4, C-5, N–CH$_2$–*C*H$_2$–CH$_3$, isomers), 11.4, 11.3 ppm (CH$_3$, isomers). IR (ATR): 3392–2417 (NH_2^+), 2928, 2783, 2706 (CH), 1646 (C=O amide), 1585 (C=C aromat.), 1443, 1398, 716, 694 cm^{-1}. MS (EI); *m/z* (%): 260 (M^+, 0.83, free base), 84 (100); HRMS (EI) *m/z* 260.1885 (M^+, calcd for $C_{16}H_{24}N_2O$ 260.1888).

3.1.4. 1-Cyanomethylpiperidine-2-carboxylic acid benzylpropylamide 8. The hydrochloride **7** (2.97 g, 10.0 mmol) and potassium carbonate (6.91 g, 50.0 mmol) were dissolved/suspended in EtOH (100 ml) and stirred for 30 min at 25°C. Bromoacetonitrile (3.5 ml, 50.0 mmol) was added and the reaction mixture was heated in the microwave oven (gradient of heating: 2 min to 78°C; holding time: 2 h at 78°C). The solution was filtered and evaporated after cooling to room temperature. The residue was diluted with sat. $NaHCO_3$ (100 ml) and dichloromethane (100 ml). The layers were separated and the aqueous layer was extracted with dichloromethane (3×100 ml). The combined organic layers were dried, filtered and evaporated in vacuo. The brown oil was purified by using column chromatography (silica gel/ethyl acetate) to obtain a pale yellow oil. Yield: 1.90 g (64%). R_f: 0.80. ^{1}H NMR ($CDCl_3$) δ: 7.29–7.12 (5H, m, H-aromat.), 4.76–4.34 (2H, m, CH_2–Ph, rotamers), 4.17–4.09 (1H, m, 2-H), 3.81–3.08 (5H, m, 2-H, CH_2–CN, N–CH_2–CH$_2$–CH$_3$), 2.84–2.77 (1H, m, H-6$_a$), 2.53–2.41 (1H, m, H-6$_b$), 1.78–1.19 (8H, m, 3-H, 4-H, 5-H, N–CH$_2$–CH_2–CH$_3$), 0.89–0.80 ppm (3H, m, CH$_3$, rotamers). ^{13}C NMR ($CDCl_3$) δ: 173.4, 173.2 (C=O amide, rotamers), 139.1, 138.4 (Cquat, aromat., rotamers), 129.3, 129.0, 128.6, 128.1, 127.7, 127.0 (C-aromat., rotamers), 116.9, 116.4 (nitrile, rotamers), 53.2, 52.2 (C-2, rotamers), 53.1 (*C*H$_2$–Ph), 49.0, 48.7 (C-6, rotamers), 44.2, 43.7 (N–*C*H$_2$–CH$_2$–CH$_3$, rotamers), 31.6, 31.5, 26.6, 26.5, 25.5, 24.3, 23.6, 22.1 (C-3, C-4, C-5, N–CH$_2$–*C*H$_2$–CH$_3$, rotamers), 13.0, 12.9 ppm (CH$_3$, rotamers). IR (KBr): 3064, 3031 (=CH), 2951, 2862, 2809, 2735 (CH), 2228 (nitrile), 1665 (C=O, amide), 1428, 1302, 1214, 731, 700 cm^{-1}. MS (EI); *m/z* (%): 299 (M^+, 1.1), 124 (8), 123 ($C_5H_9NCH_2CN^+$, 100), 96 (8), 91 ($PhCH_2^+$, 13); HRMS (EI) *m/z* 299.2006 (M^+, calcd for $C_{18}H_{25}N_3O$ 299.1998).

3.1.5. [1-(2-Aminoethyl)piperidin-2-yl-methyl]benzylpropylamine 9. $LiAlH_4$ (10.0 g, 263.5 mmol) was suspended in dry diethyl ether (250 ml) under Ar. $AlCl_3$ (16.1 g, 121.0 mmol) was carefully added to the suspension in small portions. The nitrile **8** (7.43 g, 24.8 mmol) was dissolved in dry THF (200 ml) and added dropwise to the slurry. When the addition was finished the mixture was refluxed for 5 h. The mixture was allowed to cool to room temperature and subsequently hydrolysed with ice water and stirred for 12 h. The mixture was filtered and the residue was washed twice with THF (150 ml). The filtrate and the washing liquids were evaporated in vacuo and diluted with sat. $NaHCO_3$ solution (100 ml) and dichloromethane (100 ml). The layers were separated and the aqueous layer was extracted with dichloromethane (3×100 ml). The combined organic layers were dried, filtered and evaporated. The resulting oil was distilled under reduced pressure. Yield: 4.06 g (57%), bp 81–85°C/10^{-3} mbar). ^{1}H NMR ($CDCl_3$) δ: 7.22–7.13 (5H, m, H-aromat.), 3.46 (1H, d, J=13.6 Hz, N–CH$_a$), 3.37 (1H, d, J=13.6 Hz, N–CH$_b$), 2.73–2.06 (11H, m, CH_2–Ph, H_2N–CH_2–CH_2–N, 2-H, 6-H, N–CH_2–CH$_2$–CH$_3$), 1.74–1.15 (8H, m, 3-H, 4-H, 5-H, N–CH$_2$–CH_2–CH$_3$), 0.78 ppm (3H, t, J=7.3 Hz, CH$_3$). ^{13}C NMR ($CDCl_3$) δ: 140.5 (Cquat, aromat.), 129.4, 128.4, 127.1 (C-aromat.), 60.1 (N–CH–CH_2–N) 59.2 (C-2), 57.3, 57.1, 56.9 (H_2N–*C*H$_2$–*C*H$_2$–N, *C*H$_2$–Ph), 52.1 (C-6), 40.0 (N–*C*H$_2$–CH$_2$–CH$_3$), 30.4, 25.7, 23.3, 20.7 (C-3, C-4, C-5, N–CH$_2$–*C*H$_2$–CH$_3$), 12.3 ppm (CH$_3$)). IR (KBr): 3358, 3297 (NH_2), 3028 (=CH), 2931, 2799, (CH), 1618 (C=C, aromat.), 1574, 1450, 736, 699 cm^{-1}. MS (CI, NH_3); *m/z* (%): 290 [(M+1)$^+$, 100]; HRMS (CI/NH_3) 290.2602 [(MH^+) calcd for $C_{18}H_{31}N_3^-H^+$ 290.2596].

3.1.6. 5,11-Dihydrobenzo[*e*]pyrido[3,2-*b*][1,4]diazepin-6-one 10. 3-Amino-2-chloropyridine (2.57 g, 20.0 mmol), ethyl 2-aminobenzoate (3.39 g, 20.5 mmol) and KO*t*Bu (7.29 g, 65.0 mmol) were suspended in dry 1,4-dioxane (100 ml) under Ar. The mixture was heated by microwaves (gradient of heating: 2 min to 60°C; holding time: 10 min at 60°C; gradient of heating: 3 min from 60 to 100°C; holding time: 2.5 h at 100°C). After cooling to 25°C the solution was treated with 1.0 M NaH_2PO_4 (60 ml) solution and stirred for 30 min. The dioxane was evaporated in vacuo and the residue was treated with 50 ml water. The solid was filtered, dried and recrystallised from methanol. Yield: 1.75 g (42%), pale yellow solid, mp 293°C (Ref. 10, 265–270°C). ^{1}H NMR (DMSO-d_6) δ: 9.86 (1H, s, 11-H), 8.49 (1H, s, 5-H), 7.87 (1H, dd, J=6.3, 1.5 Hz, 2-H), 7.70 (1H, dd, J=7.8, 1.8 Hz, 7-H), 7.27–7.37 (2H, m, 9-H, 4-H), 7.11 (1H, dd, J=8.1, 1.0 Hz), 6.89–6.95 ppm (2H, m, 3-H, 8-H), [Ref. 11, ($CDCl_3$) 9.90, (1H, br), 8.53 (1H, br), 7.87 (1H, dd, J=4.8, 1.6 Hz), 7.71 (1H, dd, J=7.9, 1.6 Hz), 7.32 (2H, m), 7.12 (1H, dd, J=8.1, 0.9 Hz), 6.91 ppm (2H, m)]. ^{13}C NMR (DMSO-d_6) δ: 167.3 (C-6), 151.1 (Cquat), 147.3 (Cquat), 142.7 (C-2), 133.5 (C-9), 132.1 (C-7), 128.6 (C-4), 124.2 (Cquat), 121.8 (Cquat), 121.0 (C-8), 119.6 (C-3), 118.6 ppm (C-10). IR (ATR): 3462, 3350 (2×NH), 3034 (CH), 1669 (C=O amide), 1629, 1594, 1520, 778, 755, 735, 692 cm^{-1} (Ref. 12 3260, 3180, 1670, 1603, 1470, 755, 740, 615 cm^{-1}. MS (EI); *m/z* (%): 211 (M^+, 100), 183 (M^+−CO, 15), 182 (21), 155 (11).

3.1.7. 6-Oxo-5,6-dihydrobenzo[*e*]pyrido[3,2-*b*][1,4]diazepine-11-carbonylchloride 11. The diazepine **10** (4.22 g, 20.0 mmol) and Hünig's base (7.0 ml, 40.0 mmol) were dissolved in dry 1,4-dioxane (150 ml) under Ar. A solution of 20% phosgene in toluene (18.5 ml, 35.0 mmol) was dropwise added over a period of 30 min. The solution was heated in a microwave oven (gradient of heating: 3 min to 85°C; holding time: 2 h at 85°C). After cooling to 25°C the mixture was quenched with 1.0 M NaH_2PO_4 (100 ml) and stirred for 1 h at room temperature. The dioxane was

evaporated and the solid was filtered by suction and dried over P_4O_{10} to obtain a pale yellow solid. The product can be recrystallised from ethyl acetate, but this is not necessary for the next step. Yield: 4.75 g (87%), mp 252°C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.02 (1H, br, 5-H), 8.51 (1H, br, 2-H), 7.86 (1H, d, J=7.6 Hz, 7-H), 7.77–7.53 ppm (5H, m, 3-H, 4-H, 8-H, 9-H, 10-H). ^{13}C NMR (DMSO-d_6) δ: 165.7 (C-6), 147.4 (C-11), 145.5 (C-2), 144.2 (Cquat.), 139.5 (Cquat.), 135.1 (Cquat.), 125.1 (Cquat.), 134.1, 131.7, 130.0, 127.2, 126.2, 126.1, 118.9 ppm (C-3, C-4, C-5, C-7, C-8, C-9, C-10). IR (ATR): 3183, 3075 (NH), 3045 (CH), 1730 (Cl–C═O), 1669 (N–C═O), 1594 (C═C, aromat), 1542, 1341, 779, 739 cm^{-1}. MS (EI); m/z (%): 273 (M^+, 15), 238 (M^+-Cl, 100), 211 (41), 210 (14), 183 (M^+-COCl, 11), 182 (13), 120 (14).

3.1.8. 6-Oxo-5,6-dihydrobenzo[*e*]pyrido[3,2-*b*][1,4]diazepine-11-carboxylic acid (2-{2-[(benzyl-propylamino)-methyl]piperidin-1-yl}ethyl]amide 12. The diazepine **11** (1.65 g, 6.0 mmol), Hünig's base (1.8 ml, 10.0 mmol) and the amine **9** (2.03 g, 7.0 mmol) were dissolved in dry THF in a sealed tube. The mixture was heated in a microwave oven (gradient of heating: 2.5 min to 110°C; holding time: 10 min at 110°C). The solvent was evaporated, the residue was diluted with 2 M K_2CO_3 solution (60 ml) and extracted with dichloromethane (3×60 ml). The combined organic layers were dried with Na_2SO_4, filtered and evaporated. The residue was recrystallised from ethyl acetate and petroleum ether to obtain a pale yellow solid (yield: 2.22 g, 70%), mp 153–154°C.). ^{1}H NMR ($CDCl_3$) δ: 9.64 (1H, br, NH–C═O), 8.27 (1H, d, J=6.3 Hz, 2-H), 7.86 (1H, dd, J=7.8, 1.2 Hz, 7-H), 7.55–7.50 (3H, m, 4-H, 9-H, 10-H), 7.37–7.16 (7H, m, 3-H, 8-H, phenyl), 6.63 (1H, br, N–CO–N*H*), 3.55–3.41 (4H, m, H_2C–NH–CO, CH_2–Ph), 3.19–2.30 (9H, m, HN–CH$_2$–CH_2–N, 2′-H, 6′-H, N–CH–CH_2–N, N–CH_2–CH$_2$–CH$_3$), 1.76–1.28 (8H, m, 3′-H, 4′-H, 5′-H, N–CH$_2$–CH_2–CH$_3$), 0.82 ppm (3H, t, J=7.4 Hz, CH$_3$). ^{13}C NMR ($CDCl_3$) δ: 167.9 (C═O, amide), 154.9 (C═O, urea), 146.5 (Cquat.), 145.4 (C-2), 141.8 (Cquat.), 139.4 (C-1″-phenyl), 133.3 (C-9), 131.2, 131.1 (C-7, C-4), 130.9 (Cquat.), 129.4 (Cquat.), 129.0 (C-3″-phenyl), 128.9 (C-8), 128.2 (C-2″-phenyl), 127.6 (C-4″-phenyl), 126.9 (C-10), 123.6 (C-3), 59.4 (C-2′), 56.9 (*C*H$_2$Ph), 55.1, 55.0 (C-6′, N–*C*H$_2$–CH$_2$–CH$_3$), 52.0, 51.8 (N–*C*H$_2$–CH–N, N–*C*H$_2$–CH$_2$–N–C═O), 37.3 (*C*H$_2$–N–C═O), 28.7 (N–CH$_2$–*C*H$_2$–CH$_3$), 23.7, 21.7, 20.1 (C-3′, C-4′, C-5′), 11.8 ppm (CH$_3$). IR (ATR): 3189, 3130 (NH), 3047, 2953, 2933, 2872, 2803 (CH), 1672 (C═O amide), 1655 (C═O urea), 1564 (C═C, aromat.), 1493, 1457, 1361, 779, 755, 734 cm^{-1}. MS (DCI, NH_3); m/z (%): 527 [(M+1)$^+$, 42]. Anal. calcd. for $C_{31}H_{38}N_6O_2$: C, 70.70; H, 7.27; N, 15.96; found C, 70.43; H, 7.07; N, 16.16.

3.1.9. 6-Oxo-5,6-dihydrobenzo[*e*]pyrido[3,2-*b*][1,4]diazepine-11-carboxylic acid [2-(2-propyl-aminomethyl-piperidin-1-yl)ethyl]amide 2. Compound **12** (3.40 g, 6.5 mmol) was dissolved in EtOH (150 ml), treated with 200 mg Pd/C (10%) and hydrogenated (50 bar, 60°C) for 18 h (monitoring by TLC, Al_2O_3 (bas.)/$CHCl_3$/MeOH, 20:1). The catalyst was filtered off and the solvent was evaporated in vacuo. The residue was recrystallised from ethyl acetate and diethyl ether. Yield: 2.80 g, 99%, pale yellow solid, mp 150°C. ^{1}H NMR ($CDCl_3$) δ: 11.0–10.4 – (1H, br, NH–C═O), 8.34 (1H, d, J=4.8, 1.8 Hz, 2-H), 7.92 (1H, dd, J=7.8, 1.5 Hz, 7-H), 7.60–7.51 (3H, m, 4-H, 9-H, 10-H), 7.37 (1H, td, J=7.8, 1.3 Hz, 8-H), 7.27 (1H, dd, J=8.1, 4.8 Hz, 3-H), 6.59 (1H, br, N–CO–N*H*), 3.38–3.29 (2H, m, H_2C–NH–CO), 2.84–2.79 (2H, m, N–CH–CH_2–N), 2.63–2.37 (6H, m, HN–CH$_2$–CH_2–N, 2′-H, 6$_a$′-H, N–CH_2–CH$_2$–CH$_3$), 2.18–2.14 (1H, m, 6$_b$′-H), 1.62–1.33 (9H, m, 3′-H, 4′-H, 5′-H, N*H*–CH$_2$–CH_2–CH$_3$), 0.87 ppm (3H, t, J=7.6 Hz, CH$_3$). ^{13}C NMR ($CDCl_3$) δ: 168.2 (C═O, amide), 154.9 (C═O, urea), 147.1 (Cquat.), 144.8 (C-2), 142.1 (Cquat.), 133.3 (C-9), 131.2, 131.1 (C-7, C-4), 130.9 (Cquat.), 129.2 (C-8), 128.9 (Cquat.), 127.5 (C-10), 123.6 (C-3), 60.0 (C-2′), 52.3 (N–*C*H$_2$–CH$_2$–CH$_3$), 52.1 (C-6′), 50.7, 50.4 (N–*C*H$_2$–CH–N, N–*C*H$_2$–CH$_2$–N–C═O), 37.8 (*C*H$_2$–N–C═O), 28.8 (N–CH$_2$–*C*H$_2$–CH$_3$), 24.7, 23.1, 22.9 (C-3′, C-4′, C-5′), 11.8 ppm (CH$_3$). IR (ATR): 3196, 3133, 3097 (NH), 3047, 2932, 2854, 2810, (CH), 1670 (C═O amide), 1657 (C═O urea), 1601, 1564 (C═C, aromat.), 1456, 1362, 1275, 778, 754 cm^{-1}, MS (DCI, NH_3); m/z (%): 437 [(M+1)$^+$, 100]; HRMS (CI/NH_3) 437.2655 [(MH^+) calcd for $C_{24}H_{32}N_6O_2^-H^+$ 437.2655].

3.1.10. [3-[1,3-Dioxo-1,3-dihydroisoindol-2-yl)-propyl]-dimethyl-{6-[(1-{2-[(6-oxo-5,6-dihydro-benzo[*e*]pyrido[3,2-*b*][1,4]diazepine-11-carbonyl)amino]ethyl}piperidin-2-ylmethyl)-propylamino]hexyl}ammonium bromide 1. The AFDX-384 analogue **2** (0.22 g, 0.5 mmol) was dissolved in 25 ml chloroform and treated with Hünig's base (0.9 ml, 5.0 mmol) and the bromide **3** (0.24 g, 0.5 mmol), which was prepared according to Refs. 13,14 in a sealed tube. The mixture was heated to 60°C for 24 h in an oil bath. A white solid precipitated and the TLC showed the absence of starting material. The solvent was evaporated and the oily residue was crystallized from diethyl ether to obtain a pale yellow solid. Yield: 0.35 g (83%), mp 209–211°C. ^{1}H NMR (DMSO-d_6) δ: 9.15 (1H, s, HN–CO), 8.41 (1H, d, J=6.6 Hz, 2-H), 8.33–8.30 (2H, d, J=7.3 Hz, 3-H, 6-H (phthalimide)), 7.95–7.82 (6H, m, 4-H, 5-H (phthalimide), 4-H, 7-H, 9-H, 10-H), 7.71 (1H, br, HN–CO–N), 7.54 (1H, t, J=7.4 Hz, 8-H), 7.03 (1H, t, J=7.3 Hz, 3-H), 3.64–2.52 (25H, m, 2′-H, 6′-H, $N^+(CH_3)_2$, N–CH–CH_2–N, N^+–CH_2–CH$_2$–CH_2–N(CO)$_2$, HN–CH_2–CH_2–N, N–CH_2–CH$_2$–CH$_3$, N^+–CH_2–(CH$_2$)$_4$–CH_2–N), 2.02–1.20 (18H, m, 3′-H, 4′-H, 5′-H, N–CH$_2$–CH_2–CH$_3$, N^+–CH$_2$–CH_2–CH$_2$–N(CO)$_2$, N^+–CH$_2$–(CH_2)$_4$–CH$_2$–N), 0.80 ppm (3H, t, J=7.3 Hz, CH$_3$). ^{13}C NMR (DMSO-d_6) δ: 168.2 (C═O, amide), 158.2 (C═O, imide), 154.9 (C═O, urea), 146.6 (Cquat.), 140.2 (Cquat.), 135.0 (C-9), 134.4 (C-3, C-4 phthalimide), 132.9 (C-1 phthalimide), 131.7 (Cquat.), 126.9, 126.5, 125.2 (C-4, C-8, C-10), 123.0 (C-2, C-5 phthalimide), 117.4 (C-2), 115.6 (Cquat.), 114.4 (C-7), 113.3 (C-3), 63.1 (N–*C*H$_2$–CH$_2$–N–C═O, br), 60.7, 60.4 (H$_2$*C*–N^+–*C*H$_2$), 54.6 (C-2′), 52.2 (C-6′), 49.9 (N–*C*H$_2$–CH$_2$–CH$_3$), 48.9 ($N^+(CH_3)_2$), 46.7 (N^+–CH$_2$–(CH$_2$)$_4$–*C*H$_2$–N), 46.5 (N–*C*H$_2$–CH–N), 37.2 (*C*H$_2$–N–C═O), 34.6 (*C*H$_2$–N(CO)$_2$), 32.1 (N^+–CH$_2$–*C*H$_2$–CH$_2$–N(CO)$_2$) 25.6, 25.0, 23.7 (C-3′, C-4′, C-5′), 18.8 (N–CH$_2$–*C*H$_2$–CH$_3$), 10.8 ppm (CH$_3$). IR (ATR): 3264 (NH), 3017, 2936 (CH), 1701 (C═O imide), 1679 (C═O amide), 1641 (C═O urea), 1609 (C═C aromat), 1510, 1454, 1373, 753, 721, 691 cm^{-1}. MS (FAB); m/z (%): 831, 83 (M^+, 0.4, 0.5), 751 (M^+-Br, 7.0), 437 (52), 238 (58), 185 (74), 93 (100), $C_{43}H_{59}BrN_8O_4$ requires 831.93.

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