

## Diverse modifications of the 4-methylphenyl moiety of TAK-779 by late-stage Suzuki–Miyaura cross-coupling†

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Chemokine receptor 5 (CCR5) antagonists provide a new therapeutic approach in the treatment of HIV-1 (AIDS). TAK-779 displays high affinity and selectivity for the CCR5 receptor and serves as a lead compound for the development of further antagonists. In order to increase the oral bioavailability replacement of the quaternary ammonium structure by a tertiary amine and modification of the 4-methylphenyl moiety were envisaged. Herein, a new synthetic strategy for the development of TAK-779 analogs by late stage diversification is reported. The Suzuki–Miyaura cross-coupling reactions allowed various modifications of the central amide building block **3** at the end of the synthesis leading to compounds **2f** and **2h** with a promising CCR5 binding affinity.

### 1. Introduction

The chemokine receptor 5 (CCR5) is a 7-transmembrane G-protein coupled receptor, which plays a crucial role as the second co-receptor for HIV-1 entry into target cells.<sup>1,2</sup> CCR5 antagonists represent a novel class of drugs for the treatment of infections with HIV-1 (AIDS).<sup>3,4</sup> Additionally the participation of the CCR5 receptor in the development and progression of certain inflammatory processes, *e.g.*, atherosclerosis,<sup>5</sup> rheumatoid arthritis<sup>6–8</sup> and asthma,<sup>9,10</sup> was observed. TAK-779 (**1**) developed by Takeda Inc. is among the most potent and selective CCR5 receptor antagonists reported so far and can serve as a lead compound for the development of novel antagonists (Fig. 1).<sup>11–13</sup> Shiraishi and coworkers explored different structural modifications of the lead

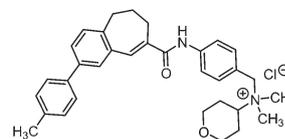


Fig. 1 TAK-779, **1**.

compound; however, variations of the 4-methylphenyl moiety were not performed due to the introduction of the 4-methylphenyl group at a very early stage of the synthesis, *i.e.* by arylation of benzo[7]annulen-5-one **6**.<sup>13</sup> TAK-779 (**1**) was not designed as a clinical candidate because of its poor oral bioavailability resulting from the quaternary ammonium structure (Fig. 1).<sup>14</sup> In order to develop TAK-779 analogs with increased oral bioavailability, the quaternary ammonium structure should be replaced by a tertiary amine. With the intention of increasing the CCR5 binding affinity the 4-methylphenyl moiety of the lead compound should be modified by the introduction of different substituents at the phenyl moiety, which are not accessible by the reported synthetic strategy. In this research program, we are highly interested in the development of TAK-779 analogs **2** with diverse and polar functional groups at the phenyl moiety. Compounds **2** with various aryl residues should be synthesized by a late-stage diversification strategy, *i.e.*, introduction of substituted aryl residues at the end of the synthesis.

Therefore, a general synthetic method, which allows the introduction of substituted aryl moieties, was required. The palladium-catalyzed Suzuki–Miyaura cross-coupling reaction is one of the most powerful and versatile methods for the formation of C–C bonds, which allows coupling of diverse aryl

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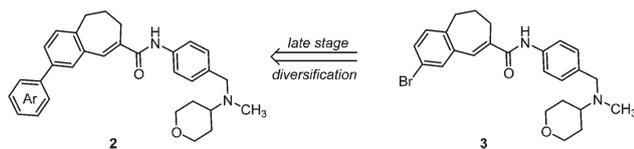


Fig. 2 Strategy for the synthesis of TAK-779 analogs 2.

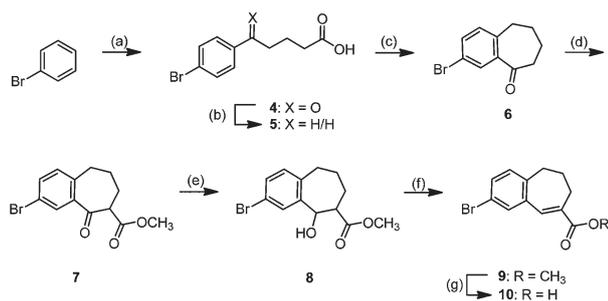
halides with different arylboronic acids and high functional group tolerance.<sup>15,16</sup> Amide **3** was chosen as the central building block for the synthesis of TAK-779 analogs **2**, because it allows the introduction of diverse aryl substituents at the final step (Fig. 2).

## 2. Results and discussion

### Synthesis

The synthesis of the building block **3** started with a Friedel-Crafts acylation of bromobenzene with glutaric acid monomethyl ester chloride, and a subsequent Wolff-Kishner reduction of the resulting  $\delta$ -keto acid **4** yielded acid **5**. An intramolecular Friedel-Crafts acylation of **5** afforded cyclic ketone **6** after a careful optimization (Scheme 1).

The ring closure of arylpentanoic acid **5** was performed in polyphosphoric acid (PPA) as a Brønsted acid at 100 °C. However, in contrast to the study of Shiraishi and coworkers who reported a yield of 67%<sup>13</sup> of ketone **6**, only 35% yield of ketone **6** resulted following the given reaction conditions. The yield even dropped to 25% after 23 h at 100 °C. Due to the early step of the planned synthesis route, an optimization of the intramolecular Friedel-Crafts acylation of **5** was required. Acid **5** was stirred with trifluoroacetic anhydride (TFAA) for 1 h and subsequently Lewis acids such as AlCl<sub>3</sub> or SnCl<sub>4</sub> were added at -30 °C. However, only with the weaker Lewis acid SnCl<sub>4</sub> the formation of a small amount of the desired product was observed. An elevation of the reaction temperature from -30 °C to +45 °C resulted in a total yield of 40%, which could not be further improved. Eaton's reagent (P<sub>4</sub>O<sub>10</sub> in MeSO<sub>3</sub>H)



**Scheme 1** Synthesis of brominated carboxylic acid **10**. Reagents and reaction conditions: (a) H<sub>3</sub>CO(CH<sub>2</sub>)<sub>3</sub>COCl, AlCl<sub>3</sub> (2.2 eq.), rt, overnight, then MeOH, 5 M NaOH, reflux, 3 h, 84%; (b) hydrazine monohydrate (5.5 eq.), KOH (4.4 eq.), triethylene glycol, 200 °C, overnight, 87%; (c) PPA, 90 °C, 1 h, 60%; (d) NaH (10 eq.), dimethyl carbonate, 80 °C, 1 h, 80%; (e) NaBH<sub>4</sub> (1.3 eq.), MeOH, CH<sub>2</sub>Cl<sub>2</sub>, -10 °C, 1 h, 75%; (f) MeSO<sub>2</sub>Cl (1.5 eq.), Et<sub>3</sub>N (3.0 eq.), CH<sub>2</sub>Cl<sub>2</sub>, rt, overnight, then DBU (4.0 eq.), CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h, 83%; (g) MeOH, 5 M NaOH, 94%.

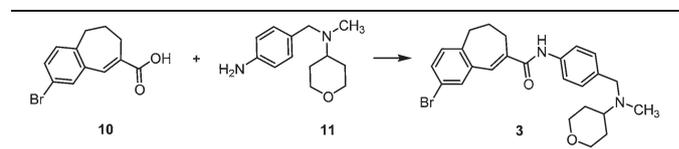
and Nafion®, a perfluorinated sulfonic acid resin, did not improve the reaction. The acid **5** was also transformed into an acid chloride by treatment with cyanuric chloride or oxalyl chloride and DMF and an intramolecular acylation was initiated by addition of AlCl<sub>3</sub>. This strategy led to 25% yield of benzosuberone **6**. Neither an increase of the reaction temperature nor extension of the reaction time did further increase the yield. Since all attempts to improve the yield by stronger conditions failed, a PPA promoted cyclization under milder conditions was performed: the temperature during the conversion was kept at 80–95 °C, the amount of PPA was 300 g per 0.1 mol (0.006 M) and the reaction was stopped after 30 min. This protocol led to the reproducible formation of benzosuberone **6** in 60% yield, even on a large scale (up to 20 g).

Claisen ester condensation of ketone **6** with dimethyl carbonate and NaH led to the formation of the  $\beta$ -keto ester **7** in 80% yield. Reduction of **7** was performed with NaBH<sub>4</sub> in MeOH at -20 °C to -10 °C<sup>13</sup> which afforded  $\beta$ -hydroxy ester **8** in 75% yield. Elimination of H<sub>2</sub>O from secondary alcohol **8** by reaction with methanesulfonyl chloride and subsequent treatment with DBU gave  $\alpha,\beta$ -unsaturated ester **9** in 83% yield, which was hydrolyzed to give acid **10**. Synthesis of the central building block **3** required a coupling of acid **10** with the aniline derivative **11**, which was synthesized by the reported reductive amination of *p*-nitrobenzylamine-HCl with tetrahydropyran-4-one, formaldehyde and NaBH(OAc)<sub>3</sub> and subsequent reduction of the aromatic nitro group with Zn<sup>0</sup> in concentrated HCl.<sup>13</sup>

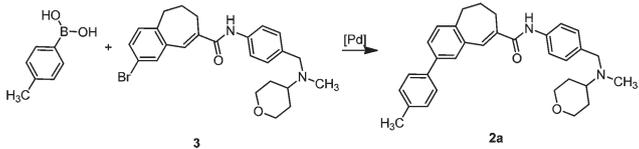
In order to optimize the amide formation various coupling reagents were tested (Table 1).<sup>17,18</sup> DCC and EDC normally promoting coupling reactions afforded only low yields (entries 1–3) and required high reaction temperatures and long reaction times (entry 4). HATU and COMU coupling reagents gave high yields (entries 5 and 6) under mild conditions and therefore they were employed for the synthesis of amide **3**.

The Suzuki-Miyaura cross-coupling reaction of aryl bromide **3** was carefully investigated using electron rich 4-methylphenylboronic acid (Table 2). Initially the coupling reaction was performed with 5 mol% of Pd(PPh<sub>3</sub>)<sub>4</sub> and 2 M aqueous K<sub>2</sub>CO<sub>3</sub> solution (5 eq.) in an ethanol-toluene mixture

**Table 1** Different coupling reagents for the synthesis of the key building block **3**



Entry	Coupling reagent	Temp.	Time	Yield [%] of <b>3</b>
1	DCC, DMAP	rt	10 h	10
2	EDC, HOBT, NMM	0 °C	15 h	33
3	EDC, HOBT, NMM	Reflux	6 h	42
4	EDC, HOBT, NMM	Reflux	3 d	58
5	HATU, Et <sub>3</sub> N	rt	12 h	72
6	COMU, NMM	rt	12 h	77

**Table 2** Optimization of the reaction conditions for the Suzuki–Miyaura coupling of aryl bromide **3** with 4-methylphenylboronic acid (1.5 eq.) and 5 mol% of the Pd catalyst


Entry	Catalyst (5 mol%)	Base	Solvent	Time, temp.	Yield [%] of <b>2a</b>
1	Pd(PPh <sub>3</sub> ) <sub>4</sub>	2 M K <sub>2</sub> CO <sub>3</sub> (5 eq.)	Ethanol-toluene = 1 : 3	12 h, 115 °C	19
2	Pd(PPh <sub>3</sub> ) <sub>4</sub>	KOH (10 eq.)	THF	12 h, 70 °C	60
3	Pd(PPh <sub>3</sub> ) <sub>4</sub>	KOH (5 eq.)	THF	20 h, 70 °C	73
4	PdCl <sub>2</sub> (dppf)	K <sub>2</sub> CO <sub>3</sub> (3 eq.)	DME	12 h, reflux	90
5	PdCl <sub>2</sub> (dppf)	KOAc (2 eq.)	DME	12 h, reflux	82
6	PdCl <sub>2</sub> (dppf)	NaOCH <sub>3</sub> (2 eq.)	DME	12 h, reflux	88

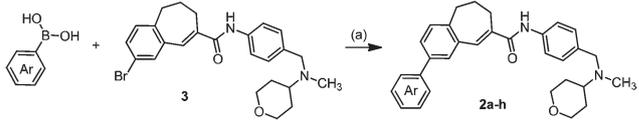
DME = dimethoxyethane.

(Table 2, entry 1). Biaryl product **2a** was isolated in 19% yield together with a large amount of starting material **3** (70%). In order to increase the conversion a stronger base (KOH) in THF was used. These conditions provided a yield of 60% (entry 2). However, despite prolongation of the reaction time the conversion was incomplete (entry 3). The use of DME as a solvent and solid K<sub>2</sub>CO<sub>3</sub>, known as Gronowitz conditions,<sup>19–21</sup> and the change of the catalyst to PdCl<sub>2</sub>(dppf), with the 1,1'-bis-(diphenylphosphino)ferrocene (dppf) ligand, one of the most efficient stabilizing agents for transition metal-catalyzed cross-coupling reactions, led finally to complete transformation of bromo derivative **3** to biaryl compound **2a** (entry 4). The use of various bases such as K<sub>2</sub>CO<sub>3</sub>, KOAc and NaOCH<sub>3</sub> led to comparable yields (entries 4–6).

Electron rich (e.g. **2a**, **2b**), electron deficient (e.g. **2c**, **2d**) as well as heterocyclic boronic acids (e.g. **2g**, **2h**) were employed for the arylation of **3** in order to generate a broad variety of test compounds. Moreover, these optimized conditions allowed the synthesis of compounds with different functional groups (e.g. phenol **2b**, aldehyde **2c** and ester **2d**), which are not accessible by other reported methods (see Table 3).

### CCR5 receptor binding affinity

The influence of structural modifications of the lead compound TAK-779 on the CCR5 receptor binding affinity was investigated. For this purpose the affinity of compounds **2a–h** to the CCR5 receptor was examined by competition receptor binding studies using [<sup>3</sup>H]TAK-779 as the radioligand (Table 4). In the *in vitro* assay an IC<sub>50</sub>-value of 8.8 nM was recorded for the unlabeled TAK-779. Replacement of the quaternary ammonium structure by a tertiary amine **2a** (IC<sub>50</sub> = 67 nM) resulted in an 8-fold decreased CCR5 affinity. Introduction of polar functional groups at the 4-position of the aryl substituent, e.g. 4-hydroxy (**2b**), 4-formyl (**2c**) and 4-acetyl-amino (**2e**), was not tolerated by the CCR5 receptor. However, the weakly basic dimethylamino derivative **2f** shows a moderate CCR5 receptor affinity (IC<sub>50</sub> = 138 nM). The bioisosteric replacement of the 4-methylphenyl moiety of **2a** by the

**Table 3** Reagents and reaction conditions: PdCl<sub>2</sub>(dppf) (5 mol%), base


Product	Ar	Yield [%]
TAK-779	—	—
<b>2a</b>		90 <sup>a</sup>
<b>2b</b>		81 <sup>b</sup>
<b>2c</b>		68 <sup>b</sup>
<b>2d</b>		73 <sup>b</sup>
<b>2e</b>		78 <sup>b</sup>
<b>2f</b>		93 <sup>b</sup>
<b>2g</b>		60 <sup>b</sup>
<b>2h</b>		94 <sup>c</sup>

<sup>a</sup> K<sub>2</sub>CO<sub>3</sub> (3 eq.). <sup>b</sup> KOAc (2 eq.). <sup>c</sup> NaOCH<sub>3</sub> (2 eq.), reflux, overnight, DME.

5-methylthienyl moiety (**2h**) was accepted by the CCR5 receptor, but the affinity was lower (IC<sub>50</sub> = 167 nM). The introduction of a 3-pyridinyl group of **2g** as an aryl moiety was not tolerated and led to a loss of affinity (IC<sub>50</sub> = 5900 nM).

## 3. Conclusion

In order to develop new TAK-779 analogs with increased oral bioavailability the quaternary ammonium structure was

Table 4 CCR5 binding affinities of compounds 2a–h

Product	IC <sub>50</sub> <sup>a</sup> [nM]
TAK-779	8.8 ± 1.7
2a	67 ± 37
2b	1500 <sup>b</sup>
2c	851 <sup>b</sup>
2d	395 <sup>b</sup>
2e	21% <sup>c</sup>
2f	138 <sup>b</sup>
2g	5900 <sup>b</sup>
2h	167 <sup>b</sup>

<sup>a</sup> [<sup>3</sup>H]TAK-779, *n* = 3. <sup>b</sup> *n* = 1. <sup>c</sup> % inhibition of [<sup>3</sup>H]TAK-779 binding at a test compound concentration of 1 μM.

replaced by the corresponding tertiary amine and the 4-methylphenyl moiety of the TAK-779 lead compound was modified. Suzuki–Miyaura cross-coupling of the key building block **3** with various arylboronic acids allowed the synthesis of a diverse set of test compounds, which were not accessible using previously reported synthetic strategies. Introduction of polar substituents turned out to be detrimental in terms of CCR5 receptor affinity. However the promising CCR5 affinity of the thiophene bioisostere **2h** and of the slightly basic dimethylamine derivative **2f** stimulated us to follow this approach in more detail.

## 4. Experimental

### Chemistry general

Unless otherwise noted, moisture-sensitive reactions were conducted under dry nitrogen. THF and 1,4-dioxane were dried with sodium/benzophenone and were freshly distilled before use. Flash column chromatography (fc): silica gel 60, 40–64 μm; parentheses include: diameter of the column, length of the column, fraction size, eluent, *R<sub>f</sub>* value. Melting point: melting point apparatus Stuart Scientific® SMP 3, uncorrected. IR: IR spectrophotometer FT-ATR-IR (Jasco®). <sup>1</sup>H NMR (400 MHz): Unity Mercury Plus 400 spectrometer (Varian®), AV400 (Bruker®), JEOL JNM-ECA-400. <sup>13</sup>C NMR (100 MHz): Unity Mercury plus 400 spectrometer (Varian®) JEOL JNM-ECA-400; δ in ppm relative to tetramethylsilane; coupling constants are given with 0.5 Hz resolution, the assignments of <sup>13</sup>C and <sup>1</sup>H NMR signals were supported by 2D NMR techniques; MS: APCI = atmospheric pressure chemical ionization, EI = electron impact, ESI = electro-spray ionization: MicroTof (Bruker Daltonics, Bremen), calibration with sodium formate clusters before measurement. The HPLC method for the determination of the product purity: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method: column: LiChrospher® 60 RP-select B (5 μm), 250 × 4 mm<sup>2</sup> column; flow rate: 1.00 mL min<sup>-1</sup>; injection volume: 5.0 μL; detection at λ = 210 nm; solvents: A: water with 0.05% (v/v) trifluoroacetic acid; B: acetonitrile with 0.05% (v/v) trifluoroacetic acid; gradient elution: (A%): 0–4 min: 90%,

4–29 min: gradient from 90% to 0%, 29–31 min: 0%, 31–31.5 min: gradient from 0% to 90%, 31.5–40 min: 90%.

## 5. Synthetic procedures

### 5-(4-Bromophenyl)-5-oxo-pentanoic acid (**4**)<sup>13</sup>

Anhydrous AlCl<sub>3</sub> (48 g, 0.36 mol, 2.2 eq.) was added portionwise to vigorously stirred bromobenzene (200 mL) at 0 °C. After stirring for 30 min, methyl 5-chloro-5-oxopentanoate (27 g, 0.16 mol) was added over a period of 30 min followed by stirring for 1 h at 0 °C and overnight at rt. Then crushed ice (200 g) and conc. HCl (150 mL) were added and the mixture was warmed until the suspended material was dissolved. The layers were separated; the aqueous layer was extracted with EtOAc (4 × 200 mL). The combined organic layers were washed thoroughly with water (400 mL) and brine (300 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo* to give an orange oil. The oil was dissolved in a mixture of MeOH (200 mL) and 2 M NaOH (200 mL), and the mixture was refluxed for 6 h. The mixture was concentrated *in vacuo*, the residue was acidified with conc. HCl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 200 mL). The combined organic layers were washed with water (300 mL) and brine (300 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give the crude product as a slightly yellow solid, which was finally recrystallized from an EtOAc–cyclohexane mixture to give 5-(4-bromophenyl)-5-oxo-pentanoic acid as a colorless solid. *R<sub>f</sub>* = 0.24, (MeOH–CH<sub>2</sub>Cl<sub>2</sub> = 5:95), mp 128–129 °C, yield 38 g (84%). C<sub>11</sub>H<sub>11</sub><sup>79</sup>BrO<sub>3</sub> (271.1 g mol<sup>-1</sup>). Purity (HPLC): 99%, *t<sub>R</sub>* = 17.45 min. Exact MS (APCI): *m/z* = calcd for C<sub>11</sub>H<sub>12</sub><sup>79</sup>BrO<sub>3</sub> [MH<sup>+</sup>] 270.9964, found 270.9959. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 2.07 (quint, *J* = 7.1 Hz, 2H, 3-CH<sub>2</sub>), 2.50 (t, *J* = 7.1 Hz, 2H, 2-CH<sub>2</sub>), 3.04 (t, *J* = 7.1 Hz, 2H, 4-CH<sub>2</sub>), 7.60 (d, *J* = 8.6 Hz, 2H, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>), 7.82 (d, *J* = 8.6 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2938 (C–H<sub>alkyl</sub>), 1705 (C=O).

### 5-(4-Bromophenyl)pentanoic acid (**5**)<sup>13</sup>

A solution of **4** (69.3 g, 0.25 mol), KOH (63 g, 1.12 mol, 4.4 eq.) and hydrazine monohydrate (49 g, 1.4 mol, 5.5 eq.) in triethylene glycol was heated to reflux (200 °C) in a Dean–Stark apparatus for 12 h. The mixture was poured into cold water and washed with Et<sub>2</sub>O. The aqueous layer was acidified with 6 M HCl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 200 mL). The combined organic layers were washed with water (2 × 200 mL) and brine (300 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated *in vacuo* to give the crude product as a yellow solid, which was recrystallized from EtOAc to give the acid **2** as a colorless solid. *R<sub>f</sub>* = 0.37 (MeOH–CH<sub>2</sub>Cl<sub>2</sub> = 5:95), mp 91–93 °C, yield 57 g (87%). C<sub>11</sub>H<sub>13</sub><sup>79</sup>BrO<sub>2</sub> (257.1 g mol<sup>-1</sup>). Purity (HPLC): 97%, *t<sub>R</sub>* = 19.37 min. Exact MS (APCI): *m/z* = calcd for C<sub>11</sub>H<sub>14</sub><sup>79</sup>BrO<sub>2</sub> [MH<sup>+</sup>] 257.0172, found 257.0165. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.61–1.72 (m, 4H, 3-CH<sub>2</sub>, 4-CH<sub>2</sub>), 2.38 (t, *J* = 7.0 Hz, 2H, 2-CH<sub>2</sub>), 2.58 (t, *J* = 7.0 Hz, 2H, 5-CH<sub>2</sub>), 7.03 (d, *J* = 8.4 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>), 7.40 (d, *J* = 8.4 Hz, 2H, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2917 (C–H<sub>alkyl</sub>), 2360 (COOH), 1698 (C=O).

### 3-Bromo-6,7,8,9-tetrahydrobenzo[7]annulen-5-one (6)<sup>13</sup>

Acid **5** (20 g, 77.8 mmol) was added portionwise to polyphosphoric acid (300 g) at 90 °C. The mixture was stirred for 1 h during which it turned brown. Next it was poured into ice (350 g) and the mixture was warmed until the suspended material was dissolved. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 × 200 mL). The combined organic layers were washed with water (300 mL) and brine (300 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo* to give the crude product as a dark brown oil, which was distilled to give the ketone **6** as a slightly yellow oil. *R*<sub>f</sub> = 0.16 (EtOAc-cyclohexane = 1 : 9), 185 °C (1.8 × 10<sup>-2</sup> mbar), yield 11 g (60%). C<sub>11</sub>H<sub>11</sub><sup>79</sup>BrO (239.1 g mol<sup>-1</sup>). Purity (HPLC): 98%, *t*<sub>R</sub> = 20.68 min. Exact MS (APCI): *m/z* = calcd for C<sub>11</sub>H<sub>12</sub><sup>79</sup>BrO [MH<sup>+</sup>] 239.0066, found 239.0043. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.75–1.98 (m, 4H, 7-CH<sub>2</sub>, 8-CH<sub>2</sub>), 2.72 (t, *J* = 6.0 Hz, 2H, 6-CH<sub>2</sub>), 2.88 (t, *J* = 6.1 Hz, 2H, 9-CH<sub>2</sub>), 7.08 (d, *J* = 8.1 Hz, 1H, 1-CH), 7.52 (dd, *J* = 8.1/2.2 Hz, 1H, 2-CH), 7.83 (d, *J* = 2.2 Hz, 1H, 4-CH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2938 (C-H), 1675 (C=O).

### Methyl 2-bromo-6,7-dihydro-5H-benzo[7]annulene-8-carboxylate (9)<sup>13</sup>

The suspension of NaH in mineral oil was washed three times with petroleum ether and filtered. NaH (3.3 g, 140 mmol, 10 eq.) was suspended in dimethyl carbonate (50 mL), and the mixture was heated to 80 °C for 30 min. Then a solution of **6** (3.34 g, 14 mmol) in dimethyl carbonate (50 mL) was added dropwise to the suspension. The mixture was refluxed for 3 h under a N<sub>2</sub> atmosphere. Next the reaction mixture was diluted with MeOH (100 mL) and poured into 1 M HCl (150 mL) under ice cooling. The aqueous mixture was concentrated *in vacuo* and extracted with EtOAc (2 × 200 mL). The combined organic layers were washed successively with water (150 mL) and brine (150 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give an orange oil which was purified by fc (petroleum ether-EtOAc = 8 : 2, Ø = 6 cm, *l* = 10 cm, *V* = 65 mL) to give the β-keto ester **7** in a yield of 3.33 g (80%) as light yellow prism-like crystals.

A solution of the β-keto ester **7** (3.33 g, 11.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was cooled down to -20 °C, and then NaBH<sub>4</sub> (560 mg, 14.5 mmol, 1.3 eq.) and abs. MeOH (2 mL) were added. The mixture was stirred at -10 °C for 1 h. The reaction mixture was poured into water and acidified with 1 M HCl. The organic layer was separated and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 100 mL). The combined organic layers were washed successively with water (100 mL) and brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give the crude product as a yellow oil, which was purified by fc (cyclohexane-EtOAc = 4 : 1 → 2 : 1, Ø = 4 cm, *l* = 14 cm, *V* = 65 mL) to give the β-hydroxy ester **8** in a yield of 2.48 g (75%) as a yellow oil.

Methanesulfonyl chloride (1.43 g, 12.5 mmol, 1.5 eq.) was added to a solution of β-hydroxy ester **8** (2.48 g, 8.3 mmol) and triethylamine (2.52 g, 25 mmol, 3 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) under ice cooling. The reaction mixture was stirred overnight at rt. Then DBU (5.05 g, 33.2 mmol, 4 eq.) was added dropwise

under ice cooling. The reaction mixture was stirred for 1 h at rt and then poured into water, 1 M HCl was added (pH 3) and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 100 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give the crude product, which was purified by fc (petroleum ether-EtOAc = 9 : 1, Ø = 4 cm, *l* = 12 cm, *V* = 65 mL) to give **9** as a colorless solid. *R*<sub>f</sub> = 0.90 (EtOAc-cyclohexane = 9 : 1), yield 1.94 g (83%). C<sub>13</sub>H<sub>13</sub><sup>79</sup>BrO<sub>2</sub> (281.1 g mol<sup>-1</sup>). Purity (HPLC): 98%, *t*<sub>R</sub> = 22.77 min. Exact MS (APCI): *m/z* = calcd for C<sub>13</sub>H<sub>14</sub><sup>79</sup>BrO<sub>2</sub> [MH<sup>+</sup>] 281.0172, found 281.0181. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.98–2.08 (m, 2H, 6-CH<sub>2</sub>), 2.78 (t, *J* = 6.0 Hz, 2H, 7-CH<sub>2</sub>), 2.71–2.79 (m, 2H, 5-CH<sub>2</sub>), 3.81 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 7.02 (d, *J* = 8.1 Hz, 1H, 4-CH), 7.32 (dd, *J* = 8.1/2.1 Hz, 1H, 3-CH), 7.44 (d, *J* = 2.1 Hz, 1H, 1-CH), 7.61 (s, 1H, 9-CH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2970, 2924 (C-H<sub>alkyl</sub>), 1705 (C=O).

### 2-Bromo-6,7-dihydro-5H-benzo[7]annulene-8-carboxylic acid (10)

In a round bottom flask the ester **9** (1.45 g, 5 mmol) was dissolved in MeOH (50 mL), and 5 M NaOH (50 mL) was added. The mixture was heated to reflux for 3 h. After cooling down to rt, the mixture was concentrated *in vacuo* and acidified with conc. HCl to give a precipitate. The mixture was cooled in an ice bath to complete the precipitation and the solid was filtered off, washed with a 1 M HCl solution and water and dried overnight to give the acid **10** as a colorless solid. *R*<sub>f</sub> = 0.70 (EtOAc-MeOH = 95 : 5), mp 211–213 °C (dec.), yield 1.25 g (94%). C<sub>12</sub>H<sub>11</sub><sup>79</sup>BrO<sub>2</sub> (267.1 g mol<sup>-1</sup>). Purity (HPLC): 99%, *t*<sub>R</sub> = 20.09 min. Exact MS (APCI): *m/z* = calcd for C<sub>12</sub>H<sub>12</sub><sup>79</sup>BrO<sub>2</sub> [MH<sup>+</sup>] 267.0015, found 267.0022. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 2.02–2.10 (m, 2H, 6-CH<sub>2</sub>), 2.65 (t, *J* = 6.0 Hz, 2H, 7-CH<sub>2</sub>), 2.76–2.80 (m, 2H, 5-CH<sub>2</sub>), 7.04 (d, *J* = 8.1 Hz, 1H, 4-CH), 7.35 (dd, *J* = 8.1/2.1 Hz, 1H, 3-CH), 7.47 (d, *J* = 2.0 Hz, 1H, 1-CH), 7.72 (s, 1H, 9-CH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2970, 2866 (C-H<sub>alkyl</sub>), 2534 (COOH), 1662 (C=O).

### 2-Bromo-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)-aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (3)

The amine **11** (815 mg, 3.7 mmol, 1 eq.) was added to a mixture of acid **10** (1.0 g, 3.7 mmol), *N*-methylmorpholine (750 mg, 7.4 mmol, 2 eq.) and COMU® (1.75 g, 4.1 mmol, 1.1 eq.) in acetonitrile (10 mL). The reaction mixture was stirred at rt overnight. Next, water (20 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo* to give the crude product as an orange oil, which was purified by fc (EtOAc-MeOH = 95 : 5, Ø = 4 cm, *l* = 14 cm, *V* = 30 mL) to give the product as a faint yellow oil. Addition of acetonitrile induced crystallization of the oil, which was then recrystallized from acetonitrile to give **3** as a colorless solid. *R*<sub>f</sub> = 0.11 (EtOAc-MeOH = 95 : 5), mp 147 °C, yield 1.33 g (77%). C<sub>25</sub>H<sub>29</sub><sup>79</sup>BrN<sub>2</sub>O<sub>2</sub> (469.4 g mol<sup>-1</sup>). Purity (HPLC): 97%, *t*<sub>R</sub> = 19.34 min. Exact MS (APCI): *m/z* = calcd for C<sub>25</sub>H<sub>30</sub><sup>79</sup>BrN<sub>2</sub>O<sub>2</sub>

[MH<sup>+</sup>] 469.1485, found 469.1495. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.60–1.83 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.06–2.18 (m, 2H, 6-CH<sub>2</sub>), 2.23 (s, 3H, N-CH<sub>3</sub>), 2.63–2.72 (m, 3H, 7-CH<sub>2</sub>, 4-H<sub>pyran</sub>), 2.72–2.82 (m, 2H, 5-CH<sub>2</sub>), 3.37 (td, *J* = 11.5/2.4 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.60 (s, 2H, Ph-CH<sub>2</sub>-N), 4.04 (dd, *J* = 10.7/3.7 Hz, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 7.04 (d, *J* = 5.1 Hz, 1H, 4-CH), 7.23 (s, 1H, 9-CH), 7.33 (m, 3H, 3-CH, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>), 7.43 (d, *J* = 2.0 Hz, 1H, 1-CH), 7.55 (d, *J* = 8.4 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>), 7.60 (s, 1H, N-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 28.1 (C-6), 29.3 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.4 (C-7), 34.4 (C-5), 37.6 (N-CH<sub>3</sub>), 57.4 (Ph-CH<sub>2</sub>-N), 59.7 (C-4<sub>pyran</sub>), 67.8 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 119.8 (C-2), 120.2 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 129.5 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 131.1 (C-3), 131.2 (C-4), 132.7 (C-9), 134.4 (C-1), 136.2 (C-4a), 136.6 (C-4<sub>phenyl</sub>), 136.8 (C-1<sub>phenyl</sub>), 139.5 (C-9a), 141.4 (C-8), 167.8 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3313 (N-H), 2966, 2920 (C-H<sub>alkyl</sub>), 1647 (C=O), 1512 (C=C).

### 2-(4-Methylphenyl)-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (2a)

A 50 mL Schlenk flask was equipped with a Dimroth condenser and a magnetic stirring bar and then closed. The flask was flame-dried under vacuum and filled with N<sub>2</sub>. Under a permanent flow of N<sub>2</sub>, amide 3 (200 mg, 0.42 mmol), PdCl<sub>2</sub>(dppf) (20 mg, 0.02 mmol, 5 mol%), K<sub>2</sub>CO<sub>3</sub> (174 mg, 1.26 mmol, 3 eq.) and 4-methylphenylboronic acid (86 mg, 0.63 mmol, 1.5 eq.) were suspended in dry dimethoxyethane (15 mL). The flask was sealed and heated to reflux for 12 h. After cooling to rt, the mixture was filtered through a short silica pad (EtOAc). The filtrate was concentrated *in vacuo* to give the crude product as a brown oil, which was purified by fc (EtOAc-MeOH = 95 : 5,  $\varnothing$  = 3 cm, *l* = 8 cm, *V* = 30 mL) to give the purified product as a colorless oil. Recrystallization from acetonitrile afforded the amide 2a as a colorless solid. *R*<sub>f</sub> = 0.28 (CH<sub>2</sub>Cl<sub>2</sub>-MeOH = 95 : 5), mp 160–162 °C, yield 74 mg (90%). C<sub>32</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub> (480.6 g mol<sup>-1</sup>). Purity (HPLC): 99%, *t*<sub>R</sub> = 21.31 min. Exact MS (APCI): *m/z* = calcd for C<sub>32</sub>H<sub>37</sub>N<sub>2</sub>O<sub>2</sub> [MH<sup>+</sup>] 481.2850, found 481.2835. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.52–1.79 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.03–2.11 (m, 2H, 6-CH<sub>2</sub>), 2.15 (s, 3H, N-CH<sub>3</sub>), 2.33 (s, 3H, CH<sub>3</sub>tolyl), 2.51–2.68 (m, 3H, 4-H<sub>pyran</sub>, 7-CH<sub>2</sub>), 2.74–2.89 (m, 2H, 5-CH<sub>2</sub>), 3.30 (td, *J* = 11.5/2.5 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.52 (s, 2H, N-CH<sub>2</sub>), 3.89–4.02 (m, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 7.13–7.18 (m, 3H, 4-CH, 3-H<sub>tolyl</sub>, 5-H<sub>tolyl</sub>), 7.25 (d, *J* = 8.4 Hz, 2H, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>), 7.31–7.39 (m, 2H, 3-CH, 9-CH), 7.41 (d, *J* = 8.1 Hz, 2H, 2-H<sub>tolyl</sub>, 6-H<sub>tolyl</sub>), 7.44 (d, *J* = 1.9 Hz, 1H, 1-CH), 7.50 (d, *J* = 8.5 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>), 7.65 (s, 1H, N-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 21.6 (CH<sub>3</sub>tolyl), 28.4 (C-6), 29.6 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.9 (C-7), 35.1 (C-5), 38.0 (N-CH<sub>3</sub>), 57.8 (Ph-CH<sub>2</sub>), 60.0 (C-4<sub>pyran</sub>), 68.2 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.5 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 127.2 (C-2<sub>tolyl</sub>, C-6<sub>tolyl</sub>), 127.3 (C-3), 129.8 (C-3<sub>tolyl</sub>, C-5<sub>tolyl</sub>), 130.0 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.3 (C-4), 130.3 (C-4<sub>tolyl</sub>), 131.1 (C-1), 134.7 (C-9), 135.1 (C-4a), 137.3 (C-4<sub>phenyl</sub>), 137.6 (C-1<sub>tolyl</sub>), 138.0 (C-2), 138.4 (C-1<sub>phenyl</sub>), 139.6 (C-9a), 141.9 (C-8), 168.5 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3298 (N-H), 2970, 2920 (C-H<sub>alkyl</sub>), 1644 (C=O), 1095, 1014 (C-O).

### 2-(4-Hydroxyphenyl)-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (2b)

As described for 2a, amide 3 (938 mg, 2 mmol), PdCl<sub>2</sub>(dppf) (75 mg, 0.1 mmol, 5 mol%), KOAc (400 mg, 0.4 mmol, 2 eq.) and 4-hydroxyphenylboronic acid (305 mg, 2.2 mmol, 1.1 eq.) in dry dimethoxyethane (20 mL) were heated to reflux for 12 h. After cooling to rt, the mixture was filtered through a short silica pad (EtOAc). Purification by fc (EtOAc-MeOH = 95 : 5,  $\varnothing$  = 5 cm, *l* = 10 cm, *V* = 30 mL) afforded a colorless oil, which was recrystallized from acetonitrile to give the amide 2b as a colorless solid. *R*<sub>f</sub> = 0.15 (CH<sub>2</sub>Cl<sub>2</sub>-MeOH = 95 : 5), mp 248 °C (dec.), yield 782 mg (81%). C<sub>31</sub>H<sub>34</sub>N<sub>2</sub>O<sub>3</sub> (482.6 g mol<sup>-1</sup>). Purity (HPLC): 99%, *t*<sub>R</sub> = 18.57 min. Exact MS (APCI): *m/z* = calcd for C<sub>31</sub>H<sub>35</sub>N<sub>2</sub>O<sub>3</sub> [MH<sup>+</sup>] 483.2642, found 483.2617. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.60–1.86 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.02–2.18 (m, 2H, 6-CH<sub>2</sub>), 2.23 (s, 3H, N-CH<sub>3</sub>), 2.61–2.77 (m, 3H, 4-CH<sub>pyran</sub>, 7-CH<sub>2</sub>), 2.79–2.91 (m, 2H, 5-CH<sub>2</sub>), 3.37 (td, *J* = 11.5/1.7 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.59 (s, 2H, Ph-CH<sub>2</sub>-N), 4.04 (dd, *J* = 11.1/3.8 Hz, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 6.87 (d, *J* = 8.6 Hz, 2H, 3-H<sub>hydroxyphen.</sub>, 5-H<sub>hydroxyphen.</sub>), 7.18 (d, *J* = 7.8 Hz, 1H, 4-CH), 7.32 (d, *J* = 8.2 Hz, 2H, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>), 7.36 (d, *J* = 1.9 Hz, 1H, 1-CH), 7.39 (s, 1H, 9-CH), 7.42 (m, 3H, 3-CH, 2-H<sub>hydroxyphen.</sub>, 6-H<sub>hydroxyphen.</sub>), 7.56 (d, *J* = 8.3 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>), 7.72 (s, 1H, N-H). The signal for the OH moiety is not seen in the spectrum. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 28.0 (C-6), 29.2 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.7 (C-7), 34.8 (C-5), 37.6 (N-CH<sub>3</sub>), 57.5 (Ph-CH<sub>2</sub>-N), 59.8 (C-4<sub>pyran</sub>), 67.8 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 116.1 (C-3<sub>hydroxyphen.</sub>, C-5<sub>hydroxyphen.</sub>), 120.4 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 126.8 (C-3), 128.3 (C-2<sub>hydroxyphen.</sub>, C-6<sub>hydroxyphen.</sub>), 129.9 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.1 (C-4), 130.7 (C-1), 132.9 (C-9a), 134.7 (C-4<sub>phenyl</sub>), 134.9 (C-9), 135.4 (C-4a), 137.2 (C-2), 137.7 (C-1<sub>hydroxyphen.</sub>), 139.1 (C-1<sub>phenyl</sub>), 141.1 (C-8), 155.8 (C-4<sub>hydroxyphen.</sub>), 168.5 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3351 (N-H), 2950 (C-H<sub>alkyl</sub>), 1647 (C=O), 1132 (C-O), 812, 652 (out of plane).

### 2-(4-Formylphenyl)-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (2c)

As described for 2a, amide 3 (100 mg, 0.21 mmol), PdCl<sub>2</sub>(dppf) (8 mg, 0.01 mmol, 5 mol%), KOAc (14 mg, 0.42 mmol, 2 eq.) and 4-formylphenylboronic acid (36 mg, 0.23 mmol, 1.1 eq.) in dry dimethoxyethane (5 mL) were heated to reflux for 12 h. Purification by fc (EtOAc-MeOH = 95 : 5,  $\varnothing$  = 2 cm, *l* = 7 cm, *V* = 10 mL) afforded a pale yellow oil, which was recrystallized from acetonitrile to give the amide 2c as a colorless solid. *R*<sub>f</sub> = 0.28 (CH<sub>2</sub>Cl<sub>2</sub>-MeOH = 95 : 5), mp 188–190 °C, yield 72 mg (68%). C<sub>32</sub>H<sub>34</sub>N<sub>2</sub>O<sub>3</sub> (494.6 g mol<sup>-1</sup>). Purity (HPLC): >99%, *t*<sub>R</sub> = 19.34 min. Exact MS (APCI): *m/z* = calcd for C<sub>32</sub>H<sub>35</sub>N<sub>2</sub>O<sub>3</sub> [MH<sup>+</sup>] 495.2642, found 495.2669. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.56–1.82 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.13–2.22 (m, 2H, 6-CH<sub>2</sub>), 2.21 (s, 3H, N-CH<sub>3</sub>), 2.56–2.71 (m, 1H, 4-CH<sub>pyran</sub>), 2.74 (t, *J* = 6.6 Hz, 2H, 7-CH<sub>2</sub>), 2.87–2.97 (m, 2H, 5-CH<sub>2</sub>), 3.37 (td, *J* = 11.6/2.3 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.57 (s, 2H, Ph-CH<sub>2</sub>-N),

4.04 (dd,  $J = 11.5/4.2$  Hz, 2H,  $\text{CH}_{2\text{equat.}}-\text{O}-\text{CH}_{2\text{equat.}}$ ), 7.29 (d,  $J = 8.1$  Hz, 1H, 4- $\text{CH}$ ), 7.32 (d,  $J = 8.6$  Hz, 2H, 3- $\text{H}_{\text{phenyl}}$ , 5- $\text{H}_{\text{phenyl}}$ ), 7.43 (s, 1H, 9- $\text{CH}$ ), 7.50 (dd,  $J = 7.8/1.9$  Hz, 1H, 3- $\text{CH}$ ), 7.53–7.59 (m, 3H, 1- $\text{CH}$ , 2- $\text{H}_{\text{phenyl}}$ , 6- $\text{H}_{\text{phenyl}}$ ), 7.64 (s, 1H, N- $\text{H}$ ), 7.75 (d,  $J = 8.3$  Hz, 2H, 2- $\text{H}_{\text{formylphen}}$ , 6- $\text{H}_{\text{formylphen}}$ ), 7.96 (d,  $J = 8.3$  Hz, 2H, 3- $\text{H}_{\text{formylphen}}$ , 5- $\text{H}_{\text{formylphen}}$ ), 10.06 (s, 1H,  $\text{CH}=\text{O}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 28.3 (C-6), 29.7 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.1 (C-7), 35.1 (C-5), 38.0 (N- $\text{CH}_3$ ), 57.7 (Ph- $\text{CH}_2$ -N), 60.0 (C-4<sub>pyran</sub>), 68.2 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.5 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 127.6 (C-3), 127.9 (C-3<sub>formylphen.</sub>, C-5<sub>formylphen</sub>), 129.9 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.6 (C-4), 130.8 (C-2<sub>formylphen.</sub>, C-6<sub>formylphen</sub>), 131.4 (C-1), 134.3 (C-9), 135.5 (C-9a), 135.6 (C-4<sub>phenyl</sub>), 138.2 (C-2), 138.9 (C-8), 140.7 (C-1<sub>phenyl</sub>), 143.3 (C-4<sub>formylphen</sub>), 146.9 (C-1<sub>formylphen</sub>), 168.2 (O=C-NH), 192.3 (CH=O). The signal for the carbon atom C-4a is not visible. FT-IR (neat):  $\tilde{\nu}$  ( $\text{cm}^{-1}$ ) = 3305 (N-H), 2966, 2947 (C-H<sub>alkyl</sub>), 1697 (HC=O), 1647 (C=O), 1099, 1010, (C-O).

#### Methyl 4-[8-(N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)-aminomethyl]phenyl}carbamoyl)-6,7-dihydro-5H-benzo[7]-annulen-2-yl]benzoate (2d)

As described for 2a, amide 3 (100 mg, 0.21 mmol),  $\text{PdCl}_2(\text{dppf})$  (8 mg, 0.01 mmol, 5 mol%), KOAc (14 mg, 0.42 mmol, 2 eq.) and 4-methoxycarbonylphenylboronic acid (43 mg, 0.23 mmol, 1.1 eq.) in dry dimethoxyethane (5 mL) were heated to reflux for 12 h. Purification by fc (EtOAc–MeOH = 95 : 5,  $\varnothing = 2$  cm,  $l = 8$  cm,  $V = 10$  mL) afforded a colorless oil, which was recrystallized from acetonitrile to give the amide 2d as a colorless solid.  $R_f = 0.17$  ( $\text{CH}_2\text{Cl}_2$ –MeOH = 95 : 5), mp 191 °C, yield 82 mg (73%).  $\text{C}_{33}\text{H}_{36}\text{N}_2\text{O}_4$  (524.6 g mol<sup>-1</sup>). Purity (HPLC): >97%,  $t_R = 22.19$  min. Exact MS (APCI):  $m/z = \text{calcd}$  for  $\text{C}_{33}\text{H}_{37}\text{N}_2\text{O}_4$  [ $\text{MH}^+$ ] 525.2748, found 525.2776.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 1.59–1.79 (m, 4H, 3- $\text{CH}_2$ <sub>pyran</sub>, 5- $\text{CH}_2$ <sub>pyran</sub>), 2.08–2.21 (m, 2H, 6- $\text{CH}_2$ ), 2.21 (s, 3H, N- $\text{CH}_3$ ), 2.64 (tt,  $J = 11.0/3.8$  Hz, 1H, 4- $\text{CH}_{\text{pyran}}$ ), 2.73 (t,  $J = 6.6$  Hz, 2H, 7- $\text{CH}_2$ ), 2.85–2.97 (m, 2H, 5- $\text{CH}_2$ ), 3.37 (td,  $J = 12.0/1.8$  Hz, 2H,  $\text{CH}_2$ <sub>axial</sub>–O- $\text{CH}_2$ <sub>axial</sub>), 3.57 (s, 2H, Ph- $\text{CH}_2$ -N), 3.94 (s, 3H,  $\text{CO}_2\text{CH}_3$ ), 4.04 (dd,  $J = 11.0/4.0$  Hz, 2H,  $\text{CH}_{2\text{equat.}}-\text{O}-\text{CH}_{2\text{equat.}}$ ), 7.27 (d,  $J = 8.9$  Hz, 1H, 4- $\text{CH}$ ), 7.31 (d,  $J = 7.4$  Hz, 2H, 3- $\text{H}_{\text{phenyl}}$ , 5- $\text{H}_{\text{phenyl}}$ ), 7.42 (s, 1H, 9- $\text{CH}$ ), 7.49 (dd,  $J = 7.9/1.6$  Hz, 1H, 3- $\text{CH}$ ), 7.50–7.60 (m, 3H, 1- $\text{CH}$ , 2- $\text{H}_{\text{phenyl}}$ , 6- $\text{H}_{\text{phenyl}}$ ), 7.62 (s, 1H, N- $\text{H}$ ), 7.65 (d,  $J = 8.5$  Hz, 2H, 2- $\text{H}_{\text{methylbenzoate}}$ , 6- $\text{H}_{\text{methylbenzoate}}$ ), 8.11 (d,  $J = 8.4$  Hz, 2H, 3- $\text{H}_{\text{methylbenzoate}}$ , 5- $\text{H}_{\text{methylbenzoate}}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 28.3 (C-6), 29.6 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.9 (C-7), 35.1 (C-5), 38.0 (N- $\text{CH}_3$ ), 52.6 ( $\text{CO}_2\text{CH}_3$ ), 57.7 (Ph- $\text{CH}_2$ -N), 60.0 (C-4<sub>pyran</sub>), 68.2 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.5 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 127.2 (C-3<sub>methylbenzoate.</sub>, C-5<sub>methylbenzoate.</sub>), 127.6 (C-3), 129.4 (C-1<sub>methylbenzoate.</sub>), 129.9 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.5 (C-4), 130.6 (C-2<sub>methylbenzoate.</sub>, C-6<sub>methylbenzoate.</sub>), 131.4 (C-1), 134.3 (C-9), 134.4 (C-9a), 135.4 (C-4a), 137.2 (C-4<sub>phenyl</sub>), 138.5 (C-2), 138.9 (C-8), 142.9 (C-1<sub>phenyl</sub>), 145.3 (C-4<sub>methylbenzoate.</sub>), 167.4 ( $\text{CO}_2\text{CH}_3$ ), 168.4 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  ( $\text{cm}^{-1}$ ) = 3286 (N-H), 2951, 2920 (C-H<sub>alkyl</sub>), 1701 (C=O<sub>ester</sub>), 1647 (C=O), 1076, 1014, (C-O).

#### 2-[4-(Acetamido)phenyl]-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]-annulene-8-carboxamide (2e)

As described for 2a, amide 3 (100 mg, 0.21 mmol),  $\text{PdCl}_2(\text{dppf})$  (8 mg, 0.01 mmol, 5 mol%), KOAc (14 mg, 0.42 mmol, 2 eq.) and 4-acetamidophenylboronic acid (43 mg, 0.24 mmol, 1.1 eq.) in dry dimethoxyethane (5 mL) were heated to reflux for 12 h. Purification by fc (EtOAc–MeOH = 95 : 5,  $\varnothing = 2$  cm,  $l = 6$  cm,  $V = 30$  mL) afforded a pale yellow oil, which was recrystallized from acetonitrile to give the amide 2e as a colorless solid.  $R_f = 0.10$  ( $\text{CH}_2\text{Cl}_2$ –MeOH = 95 : 5), mp 164–166 °C, yield 88 mg (78%).  $\text{C}_{33}\text{H}_{37}\text{N}_3\text{O}_3$  (523.6 g mol<sup>-1</sup>). Purity (HPLC): 99%,  $t_R = 18.41$  min. Exact MS (APCI):  $m/z = \text{calcd}$  for  $\text{C}_{33}\text{H}_{38}\text{N}_3\text{O}_3$  [ $\text{MH}^+$ ] 524.2908, found 524.2919.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 1.57–1.79 (m, 4H, 3- $\text{CH}_2$ <sub>pyran</sub>, 5- $\text{CH}_2$ <sub>pyran</sub>), 2.12–2.20 (m, 2H, 6- $\text{CH}_2$ ), 2.21 (s, 6H, N- $\text{CH}_3$ ,  $\text{COCH}_3$ ), 2.56–2.70 (m, 1H, 4- $\text{CH}_{\text{pyran}}$ ), 2.72 (t,  $J = 6.7$  Hz, 2H, 7- $\text{CH}_2$ ), 2.83–2.94 (m, 2H, 5- $\text{CH}_2$ ), 3.37 (td,  $J = 11.6/2.3$  Hz, 2H,  $\text{CH}_2$ <sub>axial</sub>–O- $\text{CH}_2$ <sub>axial</sub>), 3.57 (s, 2H, Ph- $\text{CH}_2$ -N), 4.04 (dd,  $J = 11.4/4.2$  Hz, 2H,  $\text{CH}_{2\text{equat.}}-\text{O}-\text{CH}_{2\text{equat.}}$ ), 7.23 (d,  $J = 7.8$  Hz, 1H, 4- $\text{CH}$ ), 7.31 (d,  $J = 8.5$  Hz, 3H, 3- $\text{H}_{\text{phenyl}}$ , 5- $\text{H}_{\text{phenyl}}$ , N- $\text{H}_{\text{acetamidophenyl}}$ ), 7.38–7.44 (m, 2H, 9- $\text{CH}$ , 3- $\text{CH}$ ), 7.48 (d,  $J = 2.0$  Hz, 1H, 1- $\text{CH}$ ), 7.51–7.62 (m, 6H, 2- $\text{H}_{\text{phenyl}}$ , 6- $\text{H}_{\text{phenyl}}$ , 2-, 3-, 5-, 6- $\text{H}_{\text{acetamidophenyl}}$ ), 7.66 (s, 1H, N- $\text{H}$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 25.1 ( $\text{COCH}_3$ ), 28.4 (C-6), 29.7 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.9 (C-7), 35.0 (C-5), 38.0 (N- $\text{CH}_3$ ), 57.7 (Ph- $\text{CH}_2$ -N), 60.0 (C-4<sub>pyran</sub>), 68.2 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.5 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 120.6 (C-3<sub>acetamidophenyl</sub>, C-5<sub>acetamidophenyl</sub>), 127.2 (C-3), 127.9 (C-2<sub>acetamidophenyl</sub>, C-6<sub>acetamidophenyl</sub>), 129.9 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.4 (C-4), 130.9 (C-1), 134.7 (C-9), 135.2 (C-9a), 137.3 (C-1<sub>acetamidophenyl</sub>), 137.7 (C-4<sub>phenyl</sub>), 138.5 (C-2), 138.9 (C-8), 141.9 (C-1<sub>phenyl</sub>), 146.3 (1C, C-4<sub>acetamidophen</sub>), 168.5 (O=C-NH), 170.4 ( $\text{COCH}_3$ ). The signal for the quaternary carbon atom C-4a is not visible. FT-IR (neat):  $\tilde{\nu}$  ( $\text{cm}^{-1}$ ) = 3305 (N-H), 2947, 2920 (C-H<sub>alkyl</sub>), 1670, 1697 (C=O), 1111, 1010, (C-O).

#### 2-[4-(Dimethylamino)phenyl]-N-{4-[N-methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-6,7-dihydro-5H-benzo[7]-annulene-8-carboxamide (2f)

As described for 2a, amide 3 (200 mg, 0.42 mmol),  $\text{PdCl}_2(\text{dppf})$  (16 mg, 0.02 mmol, 5 mol%), KOAc (84 mg, 0.84 mmol, 2 eq.) and 4-(dimethylamino)phenylboronic acid (106 mg, 0.63 mmol, 1.5 eq.) in dimethoxyethane (10 mL) were heated to reflux for 12 h. Purification by fc (EtOAc– $\text{CH}_2\text{Cl}_2$  + 5% MeOH = 1 : 2,  $\varnothing = 3$  cm,  $l = 8$  cm,  $V = 30$  mL) afforded a pale yellow solid, which was recrystallized from acetonitrile to give the amide 2f as a colorless solid.  $R_f = 0.19$  ( $\text{CH}_2\text{Cl}_2$ –MeOH = 95 : 5), mp 174–176 °C, yield 202 mg (93%).  $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_2$  (509.7 g mol<sup>-1</sup>). Purity (HPLC): 99%,  $t_R = 18.43$  min. Exact MS (ESI):  $m/z = \text{calcd}$  for  $\text{C}_{33}\text{H}_{40}\text{N}_3\text{O}_2$  [ $\text{MH}^+$ ] 510.3115, found 510.21.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  (ppm) = 1.61–1.80 (m, 4H, 3- $\text{CH}_2$ <sub>pyran</sub>, 5- $\text{CH}_2$ <sub>pyran</sub>), 2.10–2.21 (m, 2H, 6- $\text{CH}_2$ ), 2.21 (s, 3H, N- $\text{CH}_3$ ), 2.64 (tt,  $J = 11.4/4.2$  Hz, 1H, 4- $\text{CH}_{\text{pyran}}$ ), 2.71 (t,  $J = 6.0$  Hz, 2H, 7- $\text{CH}_2$ ), 2.82–2.92 (m, 2H, 5- $\text{CH}_2$ ), 3.00 (s, 6H, N( $\text{CH}_3$ )<sub>2</sub>), 3.37 (td,  $J = 11.6/2.4$  Hz, 2H,  $\text{CH}_2$ <sub>axial</sub>–O- $\text{CH}_2$ <sub>axial</sub>), 3.57 (s, 2H,

Ph-CH<sub>2</sub>-N), 3.96–4.09 (m, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 6.80 (d, *J* = 8.8 Hz, 2H, 3-*H*<sub>aminophenyl</sub>, 5-*H*<sub>aminophenyl</sub>), 7.20 (d, *J* = 7.8 Hz, 1H, 4-*CH*), 7.31 (d, *J* = 8.4 Hz, 2H, 3-*H*<sub>phenyl</sub>, 5-*H*<sub>phenyl</sub>), 7.38–7.45 (m, 2H, 9-*CH*, 3-*CH*), 7.46–7.52 (m, 3H, 1-*CH*, 2-*H*<sub>aminophenyl</sub>, 6-*H*<sub>aminophenyl</sub>), 7.56 (d, *J* = 8.4 Hz, 2H, 2-*H*<sub>phenyl</sub>, 6-*H*<sub>phenyl</sub>), 7.62 (s, 1H, N-*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 28.4 (C-6), 29.6 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.9 (C-7), 35.0 (C-5), 37.9 (N-CH<sub>3</sub>), 41.0 (N(CH<sub>3</sub>)<sub>2</sub>), 57.7 (Ph-CH<sub>2</sub>-N), 59.9 (C-4<sub>pyran</sub>), 68.2 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 113.2 (C-3<sub>aminophenyl</sub>, C-5<sub>aminophenyl</sub>), 120.4 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 126.7 (C-3), 127.9 (C-2<sub>aminophenyl</sub>, C-6<sub>aminophenyl</sub>), 128.8 (C-1<sub>aminophenyl</sub>), 129.8 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.3 (C-4), 130.5 (C-1), 134.9 (C-9a), 135.0 (C-4<sub>phenyl</sub>), 136.2 (C-9), 138.1 (C-2), 139.7 (C-8), 140.7 (C-1<sub>phenyl</sub>), 150.4 (C-4<sub>aminophenyl</sub>), 168.6 (O=C-NH). A signal for the quaternary carbon atom C-4a is not visible. FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3332 (N-H), 2785 (C-H<sub>alkyl</sub>), 1647 (C=O), 1199, 1138, (C-O).

#### N-{4-[N-Methyl-N-(tetrahydro-2H-pyran-4-yl)aminomethyl]-phenyl}-2-(pyridin-3-yl)-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (2g)

As described for 2a, amide 3 (100 mg, 0.21 mmol), PdCl<sub>2</sub>(dppf) (8 mg, 0.01 mmol, 5 mol%), KOAc (40 mg, 0.42 mmol, 2 eq.) and pyridine-3-ylboronic acid (30 mg, 0.23 mmol, 1.1 eq.) in dimethoxyethane (5 mL) were heated to reflux for 12 h. Purification by fc (EtOAc-MeOH = 95 : 5,  $\varnothing$  = 2 cm, *l* = 8 cm, *V* = 10 mL) afforded a colorless oil, which was recrystallized from acetonitrile to give the amide 2g as a colorless solid. *R*<sub>f</sub> = 0.06 (CH<sub>2</sub>Cl<sub>2</sub>-MeOH = 95 : 5), mp 153–154 °C, yield 59 mg (60%). C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>2</sub> (467.6 g mol<sup>-1</sup>). Purity (HPLC): >98%, *t*<sub>R</sub> = 14.97 min. Exact MS (APCI): *m/z* = calcd for C<sub>30</sub>H<sub>34</sub>N<sub>3</sub>O<sub>2</sub> [MH<sup>+</sup>] 468.2646, found 468.2654. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.60–1.87 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.12–2.23 (m, 2H, 6-CH<sub>2</sub>), 2.21 (s, 3H, N-CH<sub>3</sub>), 2.64 (tt, *J* = 10.7/4.4 Hz, 1H, 4-CH<sub>pyran</sub>), 2.74 (t, *J* = 6.5 Hz, 2H, 7-CH<sub>2</sub>), 2.85–3.01 (m, 2H, 5-CH<sub>2</sub>), 3.37 (td, *J* = 11.6/2.4 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.58 (s, 2H, Ph-CH<sub>2</sub>-N), 4.04 (dd, *J* = 11.5/3.7 Hz, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 7.26–7.28 (m, 1H, 4-*CH*), 7.32 (d, *J* = 8.4 Hz, 2H, 3-*H*<sub>phenyl</sub>, 5-*H*<sub>phenyl</sub>), 7.37 (ddd, *J* = 7.9/4.9/0.9 Hz, 1H, 5-*H*<sub>pyridine</sub>), 7.43 (s, 1H, 0.9-*CH*), 7.45 (dd, *J* = 7.8/2.0 Hz, 1H, 3-*CH*), 7.52 (d, *J* = 1.7 Hz, 1H, 1-*CH*), 7.56 (d, *J* = 8.4 Hz, 2H, 2-*H*<sub>phenyl</sub>, 6-*H*<sub>phenyl</sub>), 7.62 (s, 1H, N-*H*), 7.82–7.91 (m, 1H, 4-*H*<sub>pyridine</sub>), 8.60 (dd, *J* = 4.9/1.6 Hz, 1H, 6-*H*<sub>pyridine</sub>), 8.85 (dd, *J* = 2.4/0.9 Hz, 1H, 2-*H*<sub>pyridine</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 28.1 (C-6), 29.3 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.6 (C-7), 34.7 (C-5), 37.7 (N-CH<sub>3</sub>), 57.4 (Ph-CH<sub>2</sub>-N), 59.7 (C-4<sub>pyran</sub>), 67.9 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.2 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 123.7 (C-5<sub>pyridine</sub>), 127.1 (C-3), 129.6 (C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.4 (C-4), 130.8 (C-1), 133.9 (C-9), 134.3 (C-4<sub>pyridine</sub>), 135.3 (C-4a), 136.1 (C-4<sub>phenyl</sub>), 136.1 (C-2), 136.2 (C-3<sub>pyridine</sub>), 136.9 (C-1<sub>phenyl</sub>), 138.7 (C-9a), 142.6 (C-8), 148.3 (C-2<sub>pyridine</sub>), 148.7 (C-6<sub>pyridine</sub>), 168.3 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3302 (N-H), 2939, 2924 (C-H<sub>alkyl</sub>), 1643 (C=O), 1141, 1010 (C-O).

#### N-{4-[Methyl(tetrahydro-2H-pyran-4-yl)aminomethyl]phenyl}-2-(5-methylthiophen-2-yl)-6,7-dihydro-5H-benzo[7]annulene-8-carboxamide (2h)

As described for 2a, amide 3 (60 mg, 0.13 mmol), PdCl<sub>2</sub>(dppf) (10 mg, 0.01 mmol, 10 mol%), NaOCH<sub>3</sub> (14 mg, 0.25 mmol, 2 eq.) and 5-methylthiophen-2-ylboronic acid (20 mg, 0.14 mmol, 1.1 eq.) in dimethoxyethane (5 mL) were heated to reflux for 12 h. Purification by fc (EtOAc-MeOH = 95 : 5,  $\varnothing$  = 2 cm, *l* = 6 cm, *V* = 10 mL) afforded an orange oil, which was recrystallized from acetonitrile to give the amide 2h as an orange solid. *R*<sub>f</sub> = 0.28 (CH<sub>2</sub>Cl<sub>2</sub>-MeOH = 95 : 5), mp 161–163 °C, yield 59 mg (94%). C<sub>30</sub>H<sub>34</sub>N<sub>2</sub>O<sub>2</sub>S (486.6 g mol<sup>-1</sup>). Purity (HPLC): 96%, *t*<sub>R</sub> = 21.46 min. Exact MS (APCI): *m/z* = calcd for C<sub>30</sub>H<sub>35</sub>N<sub>2</sub>O<sub>2</sub>S [MH<sup>+</sup>] 487.2414, found 487.2437. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ (ppm) = 1.54–1.80 (m, 4H, 3-CH<sub>2</sub>pyran, 5-CH<sub>2</sub>pyran), 2.05–2.19 (m, 2H, 6-CH<sub>2</sub>), 2.22 (s, 3H, N-CH<sub>3</sub>), 2.61–2.78 (m, 3H, 4-CH<sub>pyran</sub>, 7-CH<sub>2</sub>), 2.79–2.89 (m, 2H, 5-CH<sub>2</sub>), 3.37 (td, *J* = 11.7/2.2 Hz, 2H, CH<sub>2</sub>axial-O-CH<sub>2</sub>axial), 3.63 (s, 2H, Ph-CH<sub>2</sub>-N), 4.05 (dd, *J* = 11.8/2.1 Hz, 2H, CH<sub>2</sub>equat.-O-CH<sub>2</sub>equat.), 3.59 (s, broad, 3H, CH<sub>3</sub>thiophene), 6.72 (dt, *J* = 3.5/1.1 Hz, 1H, 4-*H*<sub>thiophene</sub>), 7.08 (d, *J* = 3.5 Hz, 1H, 3-*H*<sub>thiophene</sub>), 7.15 (d, *J* = 7.9 Hz, 1H, 4-*CH*), 7.31 (s, 1H, 0.9-*CH*), 7.34 (d, *J* = 8.8 Hz, 2H, 3-*H*<sub>phenyl</sub>, 5-*H*<sub>phenyl</sub>), 7.39 (dd, *J* = 7.8/2.0 Hz, 1H, 3-*CH*), 7.47 (d, *J* = 2.0 Hz, 1H, 1-*CH*), 7.57 (d, *J* = 8.4 Hz, 2H, 2-*H*<sub>phenyl</sub>, 6-*H*<sub>phenyl</sub>), 7.64 (s, 1H, N-*H*). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ (ppm) = 16.0 (CH<sub>3</sub>thiophene), 28.4 (C-6), 29.6 (C-3<sub>pyran</sub>, C-5<sub>pyran</sub>), 30.9 (C-7), 35.1 (C-5), 37.9 (N-CH<sub>3</sub>), 57.7 (Ph-CH<sub>2</sub>-N), 60.0 (C-4<sub>pyran</sub>), 68.1 (C-2<sub>pyran</sub>, C-6<sub>pyran</sub>), 120.5 (C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 123.2 (C-3<sub>thioph.</sub>), 125.8 (C-2<sub>thioph.</sub>), 126.7 (C-3), 129.5 (C-5<sub>thioph.</sub>), 129.9 (C-4<sub>thiophene</sub>, C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 130.4 (C-4), 133.3 (C-1), 134.5 (C-9), 135.2 (C-4a), 137.4 (C-4<sub>phenyl</sub>), 138.6 (C-1<sub>phenyl</sub>), 139.2 (C-2), 141.7 (C-9a), 141.7 (C-8), 168.4 (O=C-NH). FT-IR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3294 (N-H), 2924, 2843 (C-H<sub>alkyl</sub>), 1643 (C=O), 1141, 1010 (C-O).

## 6. CCR5 assay

### Materials

The CCR5 receptor containing membrane homogenates was commercially available (MERCK, Millipore, Darmstadt, Germany). Homogenizers: Elvehjem Potter (B. Braun Biotech International, Melsungen, Germany) and Soniprep 150 (MSE, London, UK). Centrifuges: cooling centrifuge model Rotina 35R (Hettich, Tuttlingen, Germany) and high-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96-well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Vortexer: Vortex Genie 2 (Thermo Fisher Scientific, Langenselbold, Germany). Harvester: MicroBeta FilterMate-96 Harvester. Filter: Printed Filtermat Type A and B. Scintillator: Meltilex (Type A or B) solid state scintillator. Scintillation analyzer: MicroBeta Trilux (all from Perkin Elmer LAS, Rodgau-Jügesheim, Germany).

Chemicals and reagents were purchased from different commercial sources and were of analytical grade.

### General protocol for the CCR5 binding assay

The assay was performed with the radioligand [<sup>3</sup>H]TAK-779 (specific activity 79.1 Ci/mmol, custom synthesis by Perkin Elmer). The CCR5 receptor containing membrane fragments was used according to the instructions of the manufacturer and incubated with various concentrations of the test compound, 2 nM [<sup>3</sup>H]TAK-779, and the binding buffer (50 mM HEPES pH 7.4, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub> and 0.2% BSA) at room temperature. The filtermats were washed with a buffer solution (50 mM HEPES pH = 7.4, 500 mM NaCl and 0.1% BSA). The test compound solutions were prepared by dissolving approximately 10 μmol (usually 2–4 mg) of the test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicate in 96-well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by the addition of 50 μL of the respective assay buffer, 50 μL of test compound solution in various concentrations (10<sup>-5</sup>, 10<sup>-6</sup>, 10<sup>-7</sup>, 10<sup>-8</sup>, 10<sup>-9</sup> and 10<sup>-10</sup> mol L<sup>-1</sup>), 50 μL of the corresponding radioligand solution and 50 μL of the respective receptor preparation into each well of the multiplate (total volume 200 μL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500–600 rpm at the specified temperature. The assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 μL of water. Subsequently, the filtermats were dried at 95 °C. The solid scintillator was melted on the dried filtermats at a temperature of 95 °C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [<sup>3</sup>H]-counting protocol. The overall counting efficiency was 20%. The IC<sub>50</sub> values were calculated using the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis.

## Abbreviations

bp	Boiling point
COMU <sup>TM</sup>	(1-Cyano-2-ethoxy-2-oxoethylideneaminoxy)-dimethylamino-morpholino-carbenium hexafluorophosphate
dec.	Decomposed
DCC	Dicyclohexyl-carbodiimide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMAP	4-(Dimethylamino)-pyridine

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
HATU	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i> ]pyridinium-3-oxide hexafluorophosphate
HOBt	Hydroxybenzotriazole
IC <sub>50</sub>	Half maximal inhibitory concentration
NMM	<i>N</i> -Methylmorpholine
PPA	Polyphosphoric acid

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