Bioorganic & Medicinal Chemistry 20 (2012) 455–466

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Bivalent molecular probes for dopamine D_2-like receptors

Daniela Huber, Stefan Löber, Harald Hübner, Peter Gmeiner *

Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Schuhstrasse 19, D-91052 Erlangen, Germany

ARTICLE INFO

Article history:
Received 17 August 2011
Revised 14 October 2011
Accepted 19 October 2011
Available online 25 October 2011

Keywords:
GPCR
Subtype selectivity
Dopamine
Bivalent ligand
D_3 receptor

ABSTRACT

Merging two arylamidoalkyl substituted phenylpiperazines as prototypical recognition elements for dopamine D_2-like receptors by oligoethylene glycol linkers led to a series of bivalent ligands. These dimers were investigated in comparison to their monomeric analogues for their dopamine D_{2long}, D_{2short}, D_3 and D_4 receptor binding. Radioligand binding experiments revealed strong bivalent effects for some *para*-substituted benzamide derivatives. For the D_3 subtype, the target compounds **32**, **34** and **36** showed an up to 70-fold increase of affinity and a substantial enhancement of subtype selectivity when compared to the monovalent analogue **24**. Analysis of the binding curves displayed Hill slopes very close to one indicating that the bivalent ligands displace 1 equiv of radioligand. Obviously, the two pharmacophores occupy an orthosteric and an allosteric binding site rather than adopting a receptor-bridging binding mode.

1. Introduction

The dopamine D_2 receptor family consists of three particular subtypes named D_2, D_3 and D_4.[1] These aminergic GPCRs are associated with many central nervous system based diseases including schizophrenia, Parkinson's disease, drug addiction, and erectile dysfunction.[2] Numerous CNS-active drugs act through a stimulation or blockade of the dopamine receptor controlled signal transduction and a huge amount of investigations on structure–activity relationships, rationally based tuning of subtype selectivity and intrinsic activity as well as the propensity of the target proteins to form homo- and heteromeric complexes has been accumulated.[3–16]

Inspired by nature, hybrid molecules and bivalent ligands, which incorporate two pharmacophores connected by an appropriate linker, are currently evolving as a promising strategy in drug discovery.[17,18] Recently, design, synthesis and biological investigations of bivalent GPCR ligands have been described to target serotonin,[19] muscarinic,[20,21] opioid[22,23] and dopamine receptors.[24] At least two binding modes have been considered. Thus, the ligand can simultaneously interact with two primary (orthosteric) binding sites of two neighboring protomers. Alternatively, the bivalent ligand may address a primary binding site and a secondary (allosteric) binding site located in close proximity of an adjacent GPCR or at the identical protomer leading to a bitopic or dualsteric binding mode.[25] Bivalent ligands binding neighboring binding sites of two physically interacting GPCRs may serve as pharmacological tools to study the quaternary structure of receptor dimers and to gain insights into the role of GPCR oligomerization on biosynthesis, receptor function and internalization. On the other hand, bivalent ligands with a dualsteric binding mode facilitate the generation of subtype-selective agonists or antagonists because allosteric regions are frequently less conserved than the orthosteric binding pocket, which is usually very similar for all subtypes of a receptor family.

Very recently, we reported on the synthesis and biological investigations of bivalent ligands for the dopamine D_2 receptor

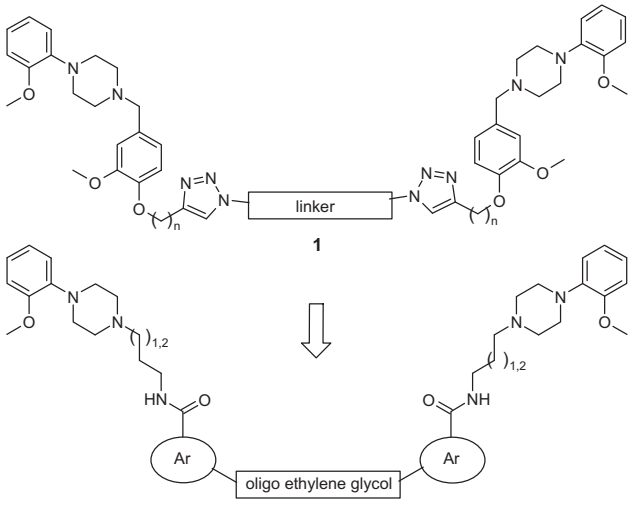


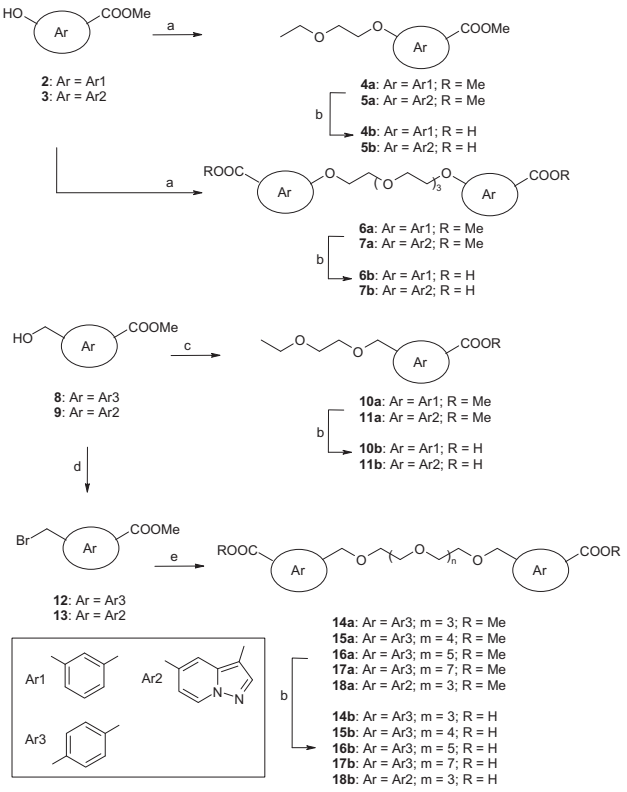
Chart 1. Bivalent dopamine D_2 receptor ligand **1** and novel arylcarboxamide based dimers.

* Corresponding author.
E-mail address: peter.gmeiner@medchem.uni-erlangen.de (P. Gmeiner).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.10.063

subtypes.[26] The test compounds incorporating the privileged structure of 1,4-disubstituted aromatic piperidines/piperazines (1,4-DAPs) of type **1** (Chart 1) differ in length and structure of the spacer unit that links two identical pharmacophores. Radioligand binding assays revealed that the bivalent ligands exhibit a distinct binding profile compared with monovalent analogues. Some of the bivalent ligands revealed a steepening of the competition curve for the D_2 receptors with Hill slopes close to two indicating the liberation of 2 equiv of radioligand. This behavior depended on the structure and the length of the linker arm. Whereas an octylene bridge between the triazole units led to a bivalent binding mode occupying two primary binding sites, Hill slopes close to one for oligoethylene glycol linked analogues indicated the displacement of 1 equiv of radioligand and thus, binding of a primary (orthosteric) and a secondary (allosteric) binding site. Because the oligoethylene glycol moiety seemed to be associated with high dopamine receptor binding affinity, we intended to explore oligoethylene glycol linked 1,4-DAPs beyond the phenylpiperazinylmethyl based pharmacophore.

As an extension of this study, we herein present the synthesis of a collection of bivalent amidopropyl- and amidobutylphenylpiperazine derivatives. The pharmacophore of this family of compounds was described to show high affinity to the receptors of the dopamine D_2 family when the amidobutylphenylpiperazines revealed substantial preference for the D_3 subtype.[2,27,28] We developed a focused library of bivalent target compounds and monovalent control agents incorporating a 2-methoxyphenylpiperazine head group, structurally different lipophilic appendages and oligoethylene glycol linkers of varying length. Employing radioligand binding studies including careful analysis of the competition curves, we envisioned to learn if the target products were able to adopt a bivalent binding mode addressing two adjacent binding sites of a dopamine receptor dimer or a dualsteric binding mode with subtype selective binding properties.



Scheme 1. Reagents and conditions: (a) $BrCH_2CH_2OEt$ (for **4a**, **5a**) or tetra(ethylene glycol)di-*p*-tosylate (for **6a**, **7a**), K_2CO_3, DMF, 70 °C, 16 h. (b) NaOH, H_2O, MeOH, reflux, 6 h. (c) $BrCH_2CH_2OEt$, KHMDS, toluene, DMF, 70 °C, 2 h. (d) for **9**: PBr_3, CH_2Cl_2, rt, 16 h. (e) tetra(ethylene glycol), KHMDS, toluene, THF, 0 °C to rt, 2 h.

2. Results and discussion

2.1. Synthesis

Our first series of target compounds was based on a benzoic acid amide moiety when the attachment point of the linker should be varied. As a spacer element, oligoethylene glycol units of different length were chosen. The mono- and bis-benzoic acid derivatives that should serve as precursors for the respective carboxamide based ligands were synthesized as outlined in Scheme 1.

Starting from methyl-3-hydroxybenzoate (**2**), reaction with bromoethyl ethyl ether or tetraethylene glycol bis-tosylate in the presence of a base (K_2CO_3) afforded the phenyl ethers **4a** and **6a**, respectively. Subsequent saponification led to the corresponding carboxylic acids **4b** and **6b**. An analogous procedure starting from methyl-4-hydroxymethylbenzoat (**8**) led to the methyl ester **10a** and the corresponding carboxylic acid **10b**. The respective bis-carboxylates were prepared starting from the benzyl bromide **12**. Reaction with tetra-, penta-, hexa- and octaethylene glycol afforded the diester derivatives **14a**, **15a**, **16a** and **17a**, respectively, which were converted into the corresponding carboxylic acids **14b**, **15b**, **16b** and **17b**.

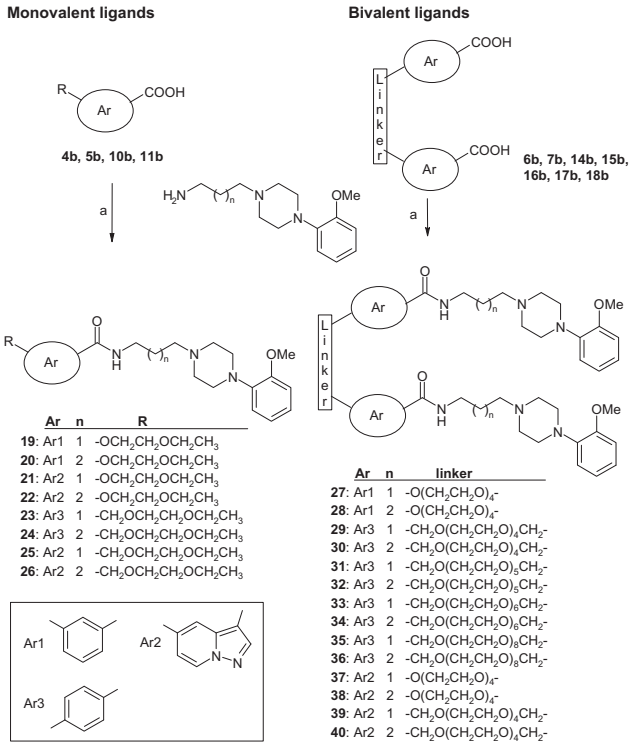
It has been shown that bicyclic heteroaromatic moieties can act as potent recognition elements for dopamine D_2-like receptor subtypes, when the pyrazolo[1,5-*a*]pyridine scaffold turned out to be an excellent functionality to control ligand affinity and selectivity.[29–31] Thus, we envisioned to incorporate a 3,5-disubstituted 7a-azaindole moiety as a heterocyclic bioisostere of the bis-substituted benzene. The syntheses of the mono- and bis-carboxylic acids started from methyl-5-hydroxypyrazolo[1,5-*a*]pyridine-3-carboxylate (**3**)[32] and the 5-bromomethyl derivative **13** which is available from **9**[31] by bromination with PBr_3. The reaction sequence was conducted as described for the above mentioned benzoic acid derivatives leading to the pyrazolopyridine-3-carboxylic acid **5b,11b** and the dimer **7b,18b**.

Finally, the newly prepared mono- and bis-carboxylic acids were converted into the carboxamides **19–40** by TBTU-mediated coupling with *N*-aminopropyl-*N*′-(2-methoxyphenyl)-piperazine and *N*-aminobutyl-*N*′-(2-methoxyphenyl)-piperazine[33] (Scheme 2).

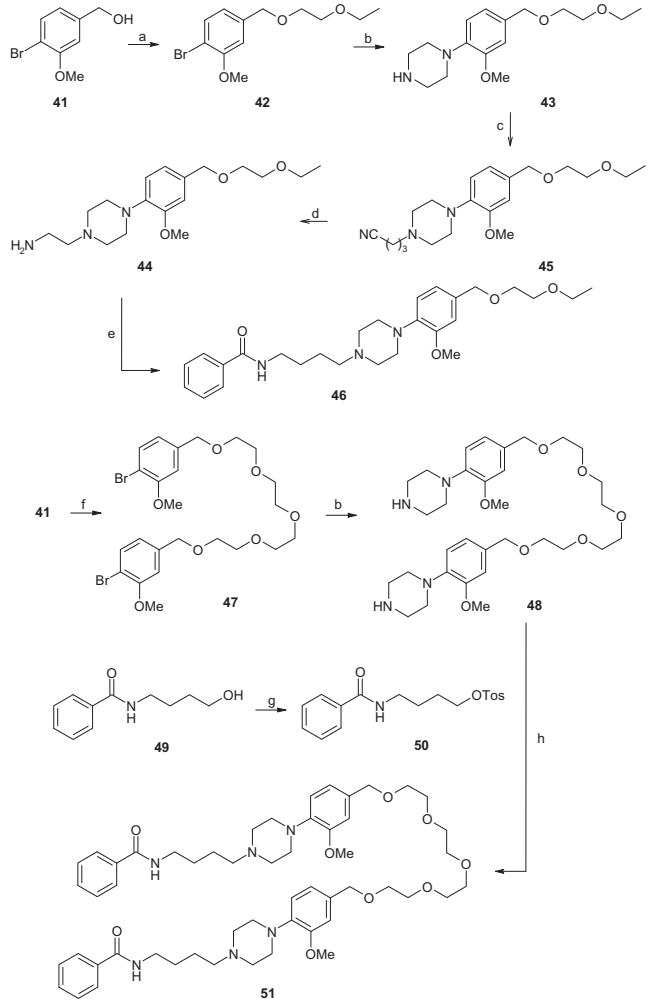
Intending to evaluate the importance of the chosen attachment position of the linker unit, we constructed an analogous derivative featuring an alternative mode of linking when the para-position of the phenylpiperazine moiety served as a point of attachment as well as a monovalent control ligand to allow the investigation of a putative bivalency effect. The synthesis started from 2-bromo-5-hydroxymethylanisole (**41**)[34], which was O-alkylated with 2-ethoxyethyl bromide to give the benzyl ether **42** (Scheme 3). Palladium assisted amination with piperazine followed by *N*-alkylation of the resulting intermediate **43** applying 4-bromobutyronitrile led to the *N*,*N*′-disubstituted piperazine derivative **45**. Finally, reduction of the cyano group gave the primary amine **44**, which was benzoylated yielding the desired carboxamide **46**. The synthesis of the bivalent ligand **51** started with cross-linking of the benzyl alcohol **41** with bis-tosylated tetraethylene glycol. Subsequent Buchwald–Hartwig reaction of the bis-benzylether **47** afforded the di-piperazine derivative **48**. Finally, dialkylation with the *N*-tosyloxybutyl benzamide **50**, which was derived from the respective butanol derivative **49**,[35] gave the final product **51**.

2.2. Receptor-ligand binding experiments

In vitro binding affinities of the molecular probes **27–40** and **51**, along with their monovalent control agents **19–26** and **46** were



Scheme 2. Reagents and conditions: (a) TBTU, DIPEA, CH_2Cl_2, DMF, 0 °C to rt, 4 h.



Scheme 3. Reagents and conditions: (a) $BrCH_2CH_2OEt$, KHMDS, toluene, THF, 0 °C to rt, 4 h; (b) piperazine, NaO*t*Bu, $Pd_2(dba)_3$, 2-(di-*t*-butylphosphino)biphenyl, toluene, 115 °C, 18 h; (c) $Br(CH_2)_3CN$, K_2CO_3, NaI, CH_3CN, 80 °C, 24 h; (d) $LiAlH_4$, Et_2O, −5 °C to rt, 3 h; (e) benzoyl chloride, Et_3N, $CHCl_3$, 0 °C to rt, 24 h; (f) tetra(ethylene glycol)di-*p*-tosylate, NaH, THF, 0 °C to rt, 4 h; (g) *p*-TosCl, Et_3N, THF, 40 °C, 16 h; (h) K_2CO_3, NaI, CH_3CN, 80 °C, 16 h.

measured by displacement of the radioligand [^{3}H]spiperone from human D_{2long}, D_{2short}, D_3, and $D_{4.4}$ receptors stably expressed in Chinese hamster ovary (CHO) cells (Table 1). Furthermore, binding data for the porcine dopamine D_1, serotonin 5-HT_{1A}, 5-HT_2 and the adrenergic α_1 receptor were determined applying striatal and cortical membranes and the radioligands [^{3}H]SCH23390, [^{3}H]WAY100635, [^{3}H]ketanserin and [^{3}H]prazosin, respectively (Supplementary data). The characterization of the test compounds involved receptor affinity and steepness of the competition curve when the data of the bivalent ligands was compared with that of the respective monovalent control agents.

The monovalent amidopropylphenylpiperazines (**19** and **23**) displayed moderate binding to the D_2-like receptors. Formal dimerization led to the bivalent analogues **27**, **29**, **31**, **33**, **35** indicating significantly higher affinities for D_{2long}, D_{2short} and D_3 whereas D_4 affinity was more or less unchanged. The most substantial increase of affinity was observed for the *meta*-disubstituted benzamide **27** with four oligoethylene glycol units displaying more than 20-fold gain of affinity for D_{2long} and D_{2short} and a factor of approximately 15 for D_3.

For the monovalent amidobutylphenylpiperazines **20** and **24**, a preference for D_3 binding could be determined. This is in good accordance to the unsubstituted *N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzamide[36] showing K_i values of 130, 90, 7.8 and 170 nM for D_{2long}, D_{2short}, D_3 and D_4, respectively. Again, dimerization led to an improvement of D_{2long}, D_{2short} and D_3 affinity. For some representatives of this group, the improvement of receptor recognition by formal dimerization was very strong. Thus, the para-disubstituted test compounds **32**, **34** and **36** incorporating five, six and eight oligoethylene glycol units, respectively, revealed an excellent gain of affinity for D_3 reaching a factor of approximately 70-fold (for **32**), 35-fold (for **34**) and 55-fold (for **36**), respectively. In contrast, only a 2- to 15-fold increase of affinity could be determined for D_{2long}, D_{2short}, D_4 and also for the related GPCRs D_1 (∼fivefold), 5-HT_{1A} (∼0.5-fold), 5-HT_2 (∼fivefold) and α_1 (2–4-fold), respectively, (Supplementary data). Moreover, these dimers exhibited substantial D_3 selectivity (>50-fold) over the dopamine receptor subtypes D_1, D_{2long}, D_{2short}, D_4 and the serotonin receptors 5-HT_{1A} and 5-HT_2. The behavior of the pyrazolo[1,5-*a*]pyridine based ligands **21**, **22**, **25**, **26** and **37–40** deviated from the benzamide derivatives. Single-digit nanomolar or even subnanomolar K_i values were determined at the D_{2long} and D_{2short} receptors for both mono- and bivalent azaindoles. Compared to the benzamide analogues, the dimer-specific increase of affinity was less pronounced. Interestingly, the 1,5-disubstituted bis-pyrazolo[1,5-*a*]pyridine **38** displayed excellent binding affinity with K_i values of 1.8, 0.59 and 0.34 nM for D_{2long}, D_{2short} and D_3 and strong selectivity over D_4 (K_i = 77 nM).

As expected, linkage of the pharmacophores via the phenylpiperazine head group resulted in dopamine receptor ligands with poor binding affinity. According to receptor–ligand models that are based on the recent D_3 crystal structure,[37] the arene system of phenylpiperazines directs to the transmembrane helix 5 in the lower part of the binding pocket. Large substituents or spacer elements are, thus, expected to induce repulsive interactions leading to a decrease of binding energy.

Table 1
Receptor binding data of compounds **19**–**40**, **46** and **51** at the human dopamine D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors

Compd	Valency/linker[c]	Ar	K_i values[a] [nM] (Hill slopes[b])			
			D_{2long}	D_{2short}	D_3	$D_{4.4}$
19	Mono	Ar1	270 (1.0)	140 (1.1)	170 (1.1)	140 (0.9)
20	Mono	Ar1	95 (0.9)	44 (0.9)	4.9 (1.0)	220 (0.9)
27	bi/4	Ar1	16 (0.9)	6.4 (1.1)	11 (1.2)	38 (1.0)
28	bi/4	Ar1	6.6 (0.9)	1.8 (0.8)	0.61 (1.1)	48 (0.8)
23	Mono	Ar3	780 (0.9)	320 (1.0)	330 (1.0)	240 (1.0)
24	Mono	Ar3	290 (0.9)	83 (0.9)	16 (0.9)	250 (1.0)
29	bi/4	Ar3	42 (0.9)	19 (0.9)	83 (0.9)	82 (0.9)
30	bi/4	Ar3	25 (0.9)	11 (0.9)	0.88 (0.8)	68 (0.8)
31	bi/5	Ar3	47 (0.8)	32 (0.8)	100 (0.9)	190 (0.9)
32	bi/5	Ar3	20 (0.7)	14 (0.8)	0.23 (0.7)	78 (0.9)
33	bi/6	Ar3	51 (1.2)	20 (1.0)	11 (0.7)	160 (0.9)
34	bi/6	Ar3	40 (0.9)	25 (0.9)	0.44 (0.8)	130 (1.0)
35	bi/8	Ar3	150 (0.9)	66 (1.0)	350 (1.1)	360 (1.0)
36	bi/8	Ar3	38 (0.8)	16 (1.0)	0.30 (0.7)	140 (0.9)
21	Mono	Ar2	29 (0.9)	6.4 (1.0)	78 (1.0)	70 (0.9)
22	Mono	Ar2	18 (0.9)	3.0 (0.9)	0.66 (0.9)	64 (0.9)
25	Mono	Ar2	67 (0.9)	32 (0.9)	160 (1.1)	45 (0.9)
26	Mono	Ar2	25 (0.9)	12 (0.9)	1.2 (1.1)	160 (0.8)
37	bi/4	Ar2	8.4 (1.0)	2.7 (1.1)	3.5 (1.1)	51 (1.1)
38	bi/4	Ar2	1.8 (1.0)	0.59 (0.8)	0.34 (0.9)	77 (1.0)
39	bi/4	Ar2	4.7 (0.9)	3.9 (0.8)	10 (1.0)	140 (0.9)
40	bi/4	Ar2	8.3 (0.9)	4.6 (0.8)	0.50 (0.9)	100 (0.9)
46	Mono		19000 (0.9)	9700 (0.9)	930 (1.0)	2400 (0.9)
51	bi/4		270 (1.1)	280 (1.2)	110 (1.1)	5400 (1.0)

[a] K_i values in nM are based on the means of 2–9 experiments each done in triplicate with SD or SEM values <35%
[b] Hill slopes are displayed as absolute values n_H which are derived from the same binding curves recorded for the determination of K_i values; the original n_H was negative but is displayed as absolute value.
[c] Number of ethylene glycol units.

Radioligand binding experiments normally lead to competitive binding curves that follow the law of mass action and, thus, show a Hill slope of one.[38] For particular bivalent ligands of type **1**, a steepening of the competition curves was observed, compared to the respective monomer, resulting in Hill slopes that reach the value of 2.0 for interactions with D_{2long} and D_{2short}. This is indicative of a positive cooperative binding.[9,39] Bivalent ligands addressing two adjacent binding sites of receptor dimers will induce such cooperativity because binding of the second pharamcophore is significantly accelerated due to the vicinity of the ligand and the, thus, facilitated enrichment of local concentration. In this case, bivalent binding leads to the liberation of two equivalents of radioligand and a substantial steepening of the competition curve. Careful investigation of the binding curves of our synthesized monomeric and dimeric target compounds revealed Hill slopes not significantly greater than 1 (absolute value) in most of the cases. Following our above mentioned reasoning, the arylcarboxamide linked ligands do not feature this bivalent binding mode with both recognition elements occupying the two binding pockets of the receptor dimer. SAR studies on our bivalent linkers indicate that the behavior depended on the structure and the length of the linker arm. Whereas an octylene bridge between two triazole units led to a bivalent binding mode occupying two primary binding sites, oligoethylene glycol linked analogues indicated the displacement of 1 equiv of radioligand. Obviously, the herein described bivalent ligands address a primary binding site and a secondary (allosteric) binding site located in close proximity of an adjacent GPCR or at the identical protomer.

3. Conclusion

A series of amidoalkylphenylpiperazine based dimers along with their monomeric analogues were synthesized. Radioligand binding studies showed an up to 70-fold increase of D_3 binding affinity and substantial enhancement of subtype selectivity for the target compounds **32**, **34** and **36** when compared to the monovalent control agent **24**. These dimeric ligands feature a linker incorporating 5–8 oligoethylene glycol units attached in para position to a benzamide scaffold in combination with an amidobutylphenylpiperazine derived pharmacophore. For the groups of *meta*-disubstituted benzamides and pyrazolo[1,5-*a*]pyridine derivatives, such strong effects could not be observed. Among the amidopropylphenylpiperazine based dimers, the *meta*-disubstituted test compound **27** with four oligoethylene glycol units displayed more than 20-fold gain of affinity for D_{2long} and D_{2short}. The subtype specific gain of affinity by exploitation of secondary binding elements (allosteric binding sites) shows that the bivalent ligand approach is a fruitful concept in drug discovery.

4. Experimental section

Dry solvents and reagents were of commercial quality and were used as purchased. MS were run on a JEOL JMS-GC Mate II spectrometer by EI (70 eV) with solid inlet or a Bruker Esquire 2000 by APC or ionization. HR-EIMS were run on a JEOL JMS-GC Mate II using Peak-Matching ($M/\Delta M > 5000$). NMR spectra were obtained on a Bruker Avance 360 or a Bruker Avance 600 spectrometer relative to TMS in the solvents indicated (J value in hertz). Melting points were determined with a MEL-TEMP II melting point apparatus (Laboratory Devices, USA) in open capillaries and are given uncorrected. IR spectra were performed on a Jasco FT/IR 410 spectrometer. Purification by flash chromatography was performed using Silica Gel 60; TLC analyses were performed using Merck 60 F254 aluminum sheets and analyzed by UV light (254 nm). Analytical HPLC was performed on Agilent 1100 HPLC systems employing a VWL detector. As column, a ZORBAX ECLIPSE XDB-C18 (4.6 × 150 mm, 5 μm) was used. HPLC purity was measured using following binary solvent systems: system A, eluent CH_3OH in 0.1% aqueous trifluoroacetic acid, 10% to 100% CH_3OH in 15 min, 100% for 3 min, flow rate 1.0 mL/min, λ 254 nm; system B, eluent CH_3CN in 0.1% aqueous trifluoroacetic acid, 10% CH_3CN for 2 min, then 10–50% CH_3CN in 13 min, then 50–100% CH_3CN in 9 min, then 100% for 1 min, flow rate 1.0 mL/min, λ 254 nm.

The purity of all test compounds and key intermediates was determined to be >95%. CHN elementary analyses were performed at the chair of Organic Chemistry of the Friedrich–Alexander University Erlangen–Nuernberg.

4.1. Methyl 3-(2-ethoxyethoxy)benzoate (4a)

A suspension of methyl 3-hydroxybenzoate (104 mg, 0.68 mmol), 2-bromoethyl ethyl ether (0.22 mL, 2.0 mmol) and K_2CO_3 (550 mg, 4.0 mmol) in DMF (13 mL) was stirred for 16 h at 70 °C. After addition of a saturated solution of $NaHCO_3$ the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (hexane/EtOAc 5:1) to give **4a** in 89% yield (136 mg). EI-MS: *m*/*z* 224 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.25 (t, J = 7.0 Hz, 3H), 3.61 (q, J = 7.0 Hz, 2H), 3.78–3.83 (m, 2H), 3.91 (s, 3H), 4.14–4.19 (m, 2H), 7.11–7.15 (m, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.58–7.60 (m, 1H), 7.61–7.65 (m, 1H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.1, 52.1, 66.9, 67.7, 68.9, 114.8, 120.2, 122.2, 129.4, 131.4, 158.9, 167.0; IR: (NaCl) ν (cm^{-1}): 2872, 1724, 1445, 1289, 1230, 1109, 756.

4.2. 3-(2-Ethoxyethoxy)benzoic acid (4b)

A mixture of methyl 3-(2-ethoxyethoxy)benzoate (**4a**) (104 mg, 0.47 mmol), aq. NaOH (1 mol/L, 8.7 mL) and MeOH (8.9 mL) was heated at reflux temperature for 6 h. After cooling to ambient temperature the solution was washed with Et_2O and subsequently acidified to pH 3 with aq. HCl. After extraction with CH_2Cl_2 the organic layer was dried over Na_2SO_4 evaporated under reduced pressure. The resulting solid (98 mg, 99%) was used without further purification. EI-MS: *m*/*z* 210 (M^+); ^{1}H NMR: (CD_3OD, 360 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 3.60 (q, J = 7.0 Hz, 2H), 3.77–3.83 (m, 2H), 4.12–4.19 (m, 2H), 7.14–7.20 (m, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.56–7.58 (m, 1H), 7.59–7.63 (m, 1H); IR: (NaCl) ν (cm^{-1}): 1684, 1454, 1307, 1247, 1119, 759.

4.3. Methyl 5-(2-ethoxyethoxy)pyrazolo[1,5-*a*]pyridine-3-carboxylate (5a)

Compound **5a** was synthesized from methyl 5-hydroxypyrazolo[1,5-*a*]pyridine-3-carboxylate[32] (50 mg) according to the procedure described for **4a** yielding 60 mg (88%) of **5a**. EI-MS: *m*/*z* 265 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.26 (t, J = 7.0 Hz, 3H), 3.62 (q, J = 7.0 Hz, 2H), 3.83–3.86 (m, 2H), 3.89 (s, 3H), 4.23–4.26 (m, 2H); 6.67 (dd, J^1 = 7.5 Hz, J^2 = 2.5 Hz, 1H), 7.42 (d, J = 3.0 Hz, 1H), 8.28 (s, 1H, H-2), 8.32 (d, J = 7.5 Hz, 1H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.1, 51.0, 66.9, 68.2, 68.4, 97.0, 102.5, 108.5, 130.0, 142.7, 145.3, 158.9, 164.1; IR: (NaCl) ν (cm^{-1}): 2870, 1697, 1648, 1539, 1275, 1248, 1213, 1119, 1056.

4.4. 5-(2-Ethoxyethoxy)pyrazolo[1,5-*a*]pyridine-3-carboxylic acid (5b)

Compound **5b** was synthesized from methyl 5-(2-ethoxyethoxy)pyrazolo[1,5-*a*]pyridine-3-carboxylate (**5a**, 50 mg)[31] according to the procedure described for **4b** yielding 43 mg (91%) of **5b**. EI-MS: *m*/*z* 250 (M^+); ^{1}H NMR: (DMSO-d_6, 600 MHz) δ (ppm): 1.34 (t, J = 7.0 Hz, 3H), 3.52 (q, J = 7.0 Hz, 2H), 3.73–3.78 (m, 2H), 4.21–4.25 (m, 2H), 6.81 (dd, J = 7.5 Hz, 3.0 Hz, 1H), 7.34 (d, J = 3.0 Hz, 1H), 8.27 (s, 1H), 8.69 (d, J = 7.5 Hz, 1H), 12.26 (s, 1H); ^{13}C NMR: (DMSO-d_6, 90 MHz) δ (ppm): 15.0, 65.7, 67.8, 68.0, 96.4, 102.4, 108.0, 130.9, 141.9, 145.1, 158.3, 164.1. IR: (NaCl) ν (cm^{-1}): 3435, 2877, 1662, 1531, 1281, 1218, 1121, 1056.

4.5. Tetraethylene glycole di(3-methoxycarbonyl phenyl) ether (6a)

Tetraethylene glycol di(*p*-toluenesulfonate) (0.1 mL, 0.25 mmol) was added dropwise to a solution of methyl 3-hydroxybenzoate (229 mg, 1.5 mmol) and K_2CO_3 (415 mg, 3.0 mmol) in DMF (4.6 mL). After being stirred at 70 °C for 16 h the mixture was treated with a saturated solution of $NaHCO_3$ and subsequently extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (hexane/EtOAc 1:1) to give **6a** in 90% yield (104 mg). EI-MS: *m*/*z* 463 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.67–3.75 (m, 8H), 3.84–3.89 (m, 4H), 3.90 (s, 6H), 4.14–4.19 (m, 4H,), 7.09–7.13 (m, 2H), 7.32 (t, J = 8.0 Hz, 2H,); 7.55–7.58 (m, 2H), 7.60–7.64 (m, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 52.1, 67.7, 69.7, 70.7, 70.9, 114.8, 120.1, 122.2, 129.4, 131.4, 158.8, 167.0; IR: (NaCl) ν (cm^{-1}): 2875, 1723, 1445, 1290, 1230, 1106, 756.

4.6. Tetraethylene glycole di(3-hydroxycarbonyl phenyl) ether (6b)

Compound **6b** was synthesized from **6a** (101 mg) according to the procedure described for **4b** yielding 88 mg (92%) of **6b**. ^{1}H NMR: (CD_3OD, 600 MHz) δ (ppm): 3.64–3.72 (m, 8H), 3.82–3.87 (m, 4H), 4.12–4.18 (m, 4H), 7.12–7.17 (m, 2H), 7.35 (t, J = 8.0 Hz, 2H), 7.53–7.56 (m, 2H,); 7.58–7.61 (m, 2H); ^{13}C NMR: (CD_3OD, 90 MHz) δ (ppm): 68.9, 70.8, 71.7, 71.8, 116.3, 120.7, 123.3, 130.6, 133.4, 160.4, 169.7; IR: (NaCl) ν (cm^{-1}): 2888, 1690, 1449, 1289 1243, 1102, 759.

4.7. Tetraethylene glycole di(3-methoxycarbonylpyrazolo[1,5-*a*]pyridine-5-yl) ether (7a)

Compound **7a** was synthesized from methyl 5-hydroxypyrazolo[1,5-*a*]pyridine-3-carboxylate[32] (70 mg) according to the procedure described for **6a** yielding 28 mg (86%) of **7a**. EI-MS: *m*/*z* 543 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 3.69–3.76 (m, 8H),3.88 (s, 6H), 3.90–3.93 (m, 4H), 4.22–4.26 (m, 4H), 6.64 (dd, J^1 = 7.5 Hz, J^2 = 3.0 Hz, 2H), 7.40 (d, J = 3.0 Hz, 2H), 8.26 (s, 2H), 8.31 (d, J = 7.5 Hz, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 51.0, 68.1, 69.2, 70.7, 70.9, 97.0, 102.5, 108.3, 130.0, 142.7, 145.3, 158.7, 164.0; IR: (NaCl) ν (cm^{-1}): 2876, 1698, 1649, 1540, 1277, 1248, 1215, 1109, 1056.

4.8. Tetraethylene glycole di(3-hydroxycarbonylpyrazolo[1,5-*a*]pyridine-5-yl) ether (7b)

Compound **7b** was synthesized from **7a** (34 mg) according to the procedure described for **4b** yielding 25 mg (78%) of **7b**. EI-MS: *m*/*z* 427 (M^+-2× COO); ^{1}H NMR: (DMSO-d_6, 600 MHz) δ (ppm): 3.54–3.63 (m, 8H); 3.77–3.83 (m, 4H), 4.19–4.25 (m, 4H), 6.79 (dd, J^1 = 7.5 Hz, J^2 = 3.0 Hz, 2H), 7.33 (d, J = 3.0 Hz, 2H), 8.26 (s, 2H), 8.67 (d, J = 7.5 Hz, 2H); 12.25 (s, 2H); ^{13}C NMR: (DMSO-d_6, 90 MHz) δ (ppm): 68.0, 68.5, 69.8, 69.9, 96.4, 102.5, 107.9, 130.9, 141.9, 145.1, 158.3, 164.2; IR: (NaCl) ν (cm^{-1}): 3433, 2892, 1663, 1537, 1284, 1227, 1110, 1057.

4.9. Methyl 4-(2-ethoxyethoxymethyl)benzoate (10a)

To a solution of methyl 4-(hydroxymethyl)benzoate (500 mg, 3.0 mmol) and 2-bromoethyl ethyl ether (2.0 mL, 18 mmol) in dry DMF (25 mL) was added dropwise KHMDS (18 mL, 0.5 M in toluene, 9.0 mmol). After being stirred at 70 °C was 2 h the mixture was treated with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried over $MgSO_4$. After

evaporation the crude product was purified by flash chromatography (hexane/EtOAc 5:1) to give **10a** in 49% yield (350 mg). EI-MS: *m*/*z* 238 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.23 (t, J = 7.0 Hz, 3H), 3.55 (q, J = 7.0 Hz, 2H), 3.61–3.67 (m, 4H), 3.91 (s, 3H), 4.64 (s, 2H), 7.42 (m, 2H), 8.01 (m, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.2, 52.0, 66.7, 69.9, 69.9, 72.6, 127.2, 129.3, 129.7, 143.7, 167.0; IR: (NaCl) ν (cm^{-1}): 2868, 1692, 1426, 1292, 1119, 764.

4.10. 4-(2-Ethoxyethoxymethyl)benzoic acid (10b)

2 N NaOH (15.4 ml) was added to a solution of **10a** (367 mg, 1.54 mmol) in MeOH (29.5 mL). After being stirred for 4 h at reflux temperature the solution was allowed to cool to room temperature. After washing with CH_2Cl_2, the mixture was acidified with 2 N HCl and extracted several times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Purification by flash chromatography (hexane/EtOAc 1:3 + 1% HCOOH) afforded **10b** in 70% yield (245 mg).). EI-MS: *m*/*z* 224 (M^+); ^{1}H NMR: (DMSO-d_6, 600 MHz) δ (ppm): 1.11 (t, J = 7.0 Hz, 3H), 3.44 (q, J = 7.0 Hz, 2H), 3.52–3.60 (m, 4H), 4.57 (s, 2H), 7.44 (m, 2H), 7.92 (m, 2H), 12.89 (s, 1H); ^{13}C NMR: (DMSO-d_6, 90 MHz) δ (ppm): 15.1, 65.5, 69.1, 69.4, 71.4, 127.1, 129.2, 129.7, 143.7, 167.1.

4.11. Methyl 5-(2-ethoxyethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-carboxylate (11a)

To a solution of **9** (62 mg, 0.3 mmol) and 2-bromoethyl ethyl ether (0.2 mL, 1.8 mmol) in dry THF (2.5 mL) was added dropwise KHMDS (1.8 mL, 0.5 M in toluene, 0.9 mmol) at 0 °C. After being stirred at ambient temperature for 2 h, the mixture was heated to 70 °C over night. After being cooled to room temperature the mixture was treated with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation the crude product was purified by flash chromatography (hexane/EtOAc 3:2) to give **11a** in 18% yield (15 mg). APCI-MS: *m*/*z* 279 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.24 (t, J = 7.0 Hz, 3H), 3.56 (q, J = 7.0 Hz, 2H), 3.64–3.67 (m, 2H), 3.68–3.71 (m, 2H), 3.91 (s, 3H), 4.67 (s, 2H), 7.01 (dd, J^1 = 7.0 Hz, J^2 = 2.0 Hz, 1H), 8.08–8.11 (m, 1H), 8.38 (s, 1H), 8.46–8.50 (m, 1H); ^{13}C NMR: (CDCl3, 90 MHz) δ (ppm): 15.2, 51.2, 66.8, 69.9, 70.2, 72.0, 103.5, 113.4, 116.6, 129.2, 139.1, 140.7, 145.1, 163.9.

4.12. 5-(2-Ethoxyethoxymethyl)pyrazolo[1,5-*a*]pyridin-3-carboxylic acid (11b)

1 N NaOH (0.5 mL) was added to a solution of **11a** (15 mg, 54 μmol) in MeOH (1 mL). After being stirred for 4 h at reflux temperature the solution was allowed to cool to room temperature. After washing with CH_2Cl_2, the mixture was acidified with 2 N HCl and extracted several times with CH_2Cl_2. the combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to give crude **11b** (16 mg, 112% yield), which was used for the next reaction without further purification. EI-MS: *m*/*z* 264 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.25 (t, J = 7.0 Hz, 3H), 3.58 (q, J = 7.0 Hz, 2H), 3.65–3.72 (m, 4H), 4.69 (s, 2H), 7.06 (dd, J^1 = 7.0 Hz, J^2 = 1.5 Hz, 1H), 8.13–8.16 (m, 1H), 8.46 (s, 1H), 8.51–8.55 (m, 1H).

4.13. Methyl 5-bromomethylpyrazolo[1,5-*a*]pyridin-3-carboxy late (13)

A solution of **9** (124 mg, 0.6 mmol) in CH_2Cl_2 (2 mL) was cooled to 0 °C. PBr_3 (0.11 mL, 1.2 mmol) was added dropwise and the mixture was stirred at room temperature over night. After evaporation of the solvent the crude product was treated with a saturated solution of $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. Purification by flash chromatography (hexane/EtOAc 3:1) yielded pure **13** (118 mg, 90%). EI-MS: *m*/*z* 269 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 3.96 (s, 3H), 4.56 (s, 2H), 7.02 (dd, J^1 = 7.0 Hz, J^2 = 2.0 Hz, 1H); 8.18 (dd, J^1 = 2.0 Hz, J^2 = 1.0 Hz, 1H), 8.43 (s, 1H), 8.52 (dd, J^1 = 7.0 Hz, J^2 = 1.0 Hz, 1H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 31.4, 51.3, 104.3, 114.7, 118.3, 129.5, 137.6, 140.4, 145.4, 163.6; IR: (NaCl) ν (cm^{-1}): 2359, 1691, 1643, 1536, 1367, 1274, 1243, 1049, 778.

4.14. Dimethyl 4,4′-(2,5,8,11,14-pentaoxapentadecan-1,15-diyl)dibenzoate (14a)

Compound **14a** was synthesized from commercially available methyl 4-bromomethylbenzoate (2.1 g, 9.2 mmol) according to the procedure described for **18a** yielding 0.91 g (50%) of **14a**. EI-MS: *m*/*z* 491 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 3.62–3.71 (m, 16H), 3.91 (s, 6H), 4.61 (s, 4H), 7.41 (m, 4H), 8.00 (m, 4H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 52.0, 69.9, 70.6, 70.6, 70.7, 72.6, 127.2, 129.3, 129.7, 144.7, 166.9; IR: (NaCl) ν (cm^{-1}): 2867, 1723, 1613, 1436, 1280, 1107, 756.

4.15. 4,4′-(2,5,8,11,14-Pentaoxapentadecan-1,15-diyl)dibenzoic acid (14b)

Compound **14b** was synthesized from **14a** (0.49 g) according to the procedure described for **18b** yielding 0.38 g (98%) of **14b**. ^{1}H NMR: (CD_3OD, 360 MHz) δ (ppm): 3.60–3.70 (m, 16H), 4.60 (s, 4H), 7.44 (m, 4H), 7.99 (m, 4H). ^{13}C NMR: (CD_3OD, 150 MHz) δ (ppm): 71.0, 71.6, 71.6, 71.6, 73.4, 128.4, 130.8, 131.2, 145.3, 169.7; IR: (NaCl) ν (cm^{-1}): 2874, 1695, 1613, 1427, 1295, 1100, 760.

4.16. Dimethyl 4,4′-(2,5,8,11,14,17-hexaoxaoctadecan-1,18-diyl)dibenzoate (15a)

Compound **15a** was synthesized from commercially available methyl 4-bromomethylbenzoate (2.1 g, 9.2 mmol) and pentaethylene glycol according to the procedure described for **18a** yielding 0.92 g (56%) of **15a**. APCI-MS: *m*/*z* 535 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.62–3.72 (m, 20H), 3.91 (s, 6H, 4.61 (s, 4H), 7.38–7.43 (m, 4H), 7.98–8.03 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 52.1, 69.9, 70.7, 70.7, 72.6, 127.2, 129.4, 129.7, 143.7, 167.0; IR: (NaCl) ν (cm^{-1}): 2867, 1721, 1280, 1107, 757.

4.17. 4,4′-(2,5,8,11,14,17-hexaoxaoctadecan-1,18-diyl)dibenzoic acid (15b)

Compound **15b** was synthesized from **15a** (775 mg) according to the procedure described for **18b** yielding 670 mg (91%) of **15b**. APCI-MS: *m*/*z* 507 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.63–3.73 (m, 20H), 4.63 (s, 4H), 7.41–7.47 (m, 4H), 8.02–8.08 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 69.9, 70.7, 70.7, 72.6, 127.3, 128.5, 130.3, 144.6, 170.9; IR: (NaCl) ν (cm^{-1}): 2870, 1715, 1248, 1103, 753.

4.18. Dimethyl 4,4′-(2,5,8,11,14,17,20-heptaoxahenicosan-1,21-diyl)dibenzoate (16a)

Compound **16a** was synthesized from commercially available methyl 4-bromomethylbenzoate (1.8 g, 7.8 mmol) and hexaethylene glycol according to the procedure described for **18a** yielding 0.59 g (38%) of **16a**. APCI-MS: *m*/*z* 579 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 3.59–3.72 (m, 24H), 3.91 (s, 6H), 4.62 (s, 4H), 7.39–7.44 (m, 4H), 7.98–8.04 (m, 4H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 52.1, 69.9, 70.6, 70.6, 70.6, 70.7, 72.6, 127.2, 129.3, 129.7, 143.7, 167.0; IR: (NaCl) ν (cm^{-1}): 2868, 1721, 1280, 1107, 757.

4.19. 4,4′-(2,5,8,11,14,17,20-heptaoxahenicosan-1,21-diyl) dibenzoic acid (16b)

Compound **16b** was synthesized from **16a** (0.57 g) according to the procedure described for **18b** yielding 0.39 g (71%) of **16b**. APCI-MS: *m*/*z* 551 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.60–3.75 (m, 24H), 4.63 (s, 4H), 7.39–7.46 (m, 4H), 8.00–8.08 (m, 4H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 69.9, 70.6, 70.6, 70.6, 70.7, 72.6, 127.3, 128.7, 130.3, 144.5, 171.2; IR: (NaCl) ν (cm^{-1}): 2871, 1717, 1692, 1247, 1105, 755.

4.20. Dimethyl 4,4′-(2,5,8,11,14,17,20,23,26-nonoxa heptacosan-1,27-diyl)dibenzoate (17a)

Compound **17a** was synthesized from commercially available methyl 4-bromomethylbenzoate (1.0 g, 4.5 mmol) and octaethylene glycol according to the procedure described for **18a** yielding 290 mg (28%) of **17a**. APCI-MS: *m*/*z* 668 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 3.57–3.72 (m, 32H), 3.91 (s, 6H), 4.62 (s, 4H), 7.36–7.44 (m, 4H), 7.95–8.04 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 52.1, 69.9, 70.6, 70.6, 70.7, 72.6, 127.2, 129.4, 129.7, 143.7, 167.0; IR: (NaCl) ν (cm^{-1}): 2869, 1720, 1281, 1108, 758.

4.21. 4,4′-(2,5,8,11,14,17,20,23,26-nonoxaheptacosan-1,27-diyl)dibenzoic acid (17b)

Compound **17b** was synthesized from **17a** (185 mg) according to the procedure described for **18b** yielding 170 mg (96%) of **17b**. APCI-MS: *m*/*z* 640 (M^++1); ^{1}H NMR: (CD_3OD, 600 MHz) δ (ppm): 3.55–3.71 (m, 32H), 4.62 (s, 4H), 7.42–7.49 (m, 4H), 7.97–8.02 (m, 4H); ^{13}C NMR: (CD_3OD, 90 MHz) δ (ppm): 71.1, 71.6, 71.6, 71.6, 71.6, 71.7, 73.5, 128.5, 130.9, 131.3, 145.3, 169.7; IR: (NaCl) ν (cm^{-1}): 2871, 1715, 1249, 1105, 756.

4.22. Dimethyl 5,5′-(2,5,8,11,14-pentaoxapentadecan-1,15-diyl)bispyrazolo[1,5-*a*]pyridin-3-carboxylate (18a)

A solution of tetraethylen glycol (400 μL, 2.31 mmol) in THF (30 mL) was cooled to 0 °C. After dropwise addition of KHMDS (0.5 M in toluene, 13.9 mL, 6.93 mmol) the mixture was stirred for 15 min. Subsequently, a solution of **13** (1.86 g, 6.93 mmol) in THF (8 mL) was added. After being stirred for 2 h at room temperature the mixture was treated with water and extracted with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. After evaporation the crude product was purified by flash chromatography (hexane/EtOAc 1:1) to give **18a** in 46% yield (620 mg). APCI-MS: *m*/*z* 571 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.64–3.74 (m, 16H), 3.91 (s, 6H), 4.65 (s, 4H), 7.00 (dd, J^1 = 7.0 Hz, J^2 = 2.0 Hz, 2H), 8.04–8.08 (m, 2H), 8.36 (s, 2H), 8.47 (dd, J^1 = 7.0 Hz, J^2 = 1.0 Hz, 2H); ^{13}C NMR:($CDCl_3$, 90 MHz) δ (ppm): 51.2, 70.1, 70.6, 70.7, 70.8, 71.9, 103.5, 113.4, 116.5, 129.1, 139.1, 140.7, 145.1, 163.8; IR: (NaCl) ν (cm^{-1}): 3510, 2924, 2858, 1703, 1649, 1533, 1371, 1238, 1049, 779.

4.23. 5,5′-(2,5,8,11,14-pentaoxapentadecan-1,15-diyl)bispyrazolo[1,5-*a*]pyridin-3-carboxylic acid (18b)

1 N NaOH (21 mL) was added to a solution of **18a** (0.66 g, 1.2 mol) in MeOH (22 mL). After being stirred at reflux temperature for 4 h, the solution was allowed to cool to room temperature. After washing with CH_2Cl_2, the mixture was acidified with 2 N HCl and extracted several times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (CH_2Cl_2/MeOH 98:2 + 1% HCOOH) gave **18b** in 99% yield (630 mg). APCI-MS: *m*/*z* 499 (M^++1–CO_2); ^{1}H NMR: ($CDCl_3$ + TFA, 360 MHz) δ (ppm): 3.71–3.79 (m, 16H), 4.69 (s, 4H), 6.96 (dd, J^1 = 7.0 Hz, J^2 = 1.5 Hz, 2H), 8.14–8.17 (m, 2H), 8.44 (s, 2H), 8.50–8.54 (m, 2H); ^{13}C NMR: ($CDCl_3$, + TFA, 150 MHz) δ (ppm): 70.1, 70.8, 70.9, 71.0, 71.6, 103.4, 113.3, 116.5, 129.1, 139.7, 140.2, 146.0, 168.9; IR: (NaCl) ν (cm^{-1}): 3510, 2924, 2858, 1703, 1649, 1533, 1371, 1238, 1049, 779.

4.24. 3-(2-Ethoxyethoxy)-*N*-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]benzamide (19)

A solution of **4b** (10 mg, 48 μmol) and DIPEA (30 μL, 0.18 mmol) in CH_2Cl_2 was cooled to 0 °C. After consecutive addition of solutions of TBTU (19 mg, 60 μmol) in DMF (1 mL) and 3-[4-(2-methoxyphenyl)piperazin-1-yl]propylamine (22 mg, 90 μmol) in CH_2Cl_2 (5 mL), the mixture was stirred for 4 h at room temperature. Addition of a saturated solution of $NaHCO_3$ was followed by extraction with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (CH_2Cl_2/MeOH 95:5) gave **19** in 94% yield (20 mg). EI-MS: *m*/*z* 442 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.21 (t, J = 7.5 Hz, 3H), 1.82 (br quint, J = 5.5 Hz, 2H), 2.65 (br t, J = 5.5 Hz, 2H), 2.69–2.75 (m, 4H), 3.07–3.13 (m, 4H), 3.51 (q, J = 7.0 Hz, 2H), 3.59 (br q, J = 5.5 Hz, 2H), 3.70 (t, J = 5.0 Hz, 2H), 3.86 (s, 3H), 4.13 (t, J = 5.0 Hz, 2H), 6.85–6.95 (m, 3H), 6.99–7.04 (m, 2H), 7.27 (t, J = 8.0 Hz, 1H), 7.35–7.39 (m, 1H), 7.43–7.46 (m, 1H), 8.30 (br s, 1H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.1, 24.3, 40.9, 50.6, 53.6, 55.4, 58.5, 66.8, 67.6, 68.8, 111.3, 113.5, 117.8, 118.1, 119.1, 121.0, 123.1, 129.3, 136.4, 141.1, 152.3, 159.1, 167.1; IR: (NaCl) ν (cm^{-1}): 3325, 2875, 1643, 1581, 1538, 1500, 1303, 1241, 1120, 750. HRMS ($C_{25}H_{35}N_3O_4$): calcd: 441.2628, found: 441.2627. HPLC (method A): t_R = 13.4 min; purity: 98.1%.

4.25. 3-(2-Ethoxyethoxy)-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]benzamide (20)

Compound **20** was synthesized from **4b** (10 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **19** yielding 20 mg (90%) of **20**. EI-MS: *m*/*z* 456 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.24 (t, J = 7.0 Hz, 3H), 1.61–1.75 (m, 4H), 2.47 (br t, J = 6.5 Hz, 2H), 2.63–2.67 (m, 4H), 3.05–3.08 (m, 4H), 3.48 (br q, J = 6.5 Hz, 2H), 3.59 (q, J = 7.0 Hz, 2H), 3.76–3.81 (m, 2H), 3.85 (s, 3H), 4.13–4.18 (m, 2H), 6.83–6.95 (m, 3H), 6.96–7.07 (m, 2H), 7.27–7.33 (m, 2H), 7.34–7.38 (m, 1H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 24.4, 27.4, 40.0, 50.5, 53.4, 55.3, 58.0, 66.9, 67.6, 68.8, 111.2, 113.2, 118.0, 118.2, 119.0, 121.0, 122.9, 129.5, 136.4, 141.2, 152.3, 159.0, 167.5; IR: (NaCl) ν (cm^{-1}): 3332, 2932, 1642, 1583, 1540, 1500, 1301, 1241, 1129, 751. HRMS ($C_{26}H_{37}N_3O_4$): calcd: 455.2784, found: 455.2784. HPLC (method B): t_R = 14.6 min; purity: 99.2.

4.26. 5-(2-Ethoxyethoxy)-*N*-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl] pyrazolo[1,5-*a*]pyridine-3-carboxamide (21)

Compound **21** was synthesized from **5b** (10 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **19** yielding 17 mg (89%) of **21**. EI-MS: *m*/*z* 482 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.25 (t, J = 7.0 Hz, 3H), 1.84 (br quint, J = 6.0 Hz, 2H), 2.63 (br t, J = 6.0 Hz, 2H), 2.72–2.75 (m, 4H), 3.14–3.17 (m, 4H), 3.56–3.67 (m, 4H), 3.80–3.84 (m, 2H), 3.86 (s, 3H), 4.21–4.26 (m, 2H), 6.63 (dd, J^1 = 7.5 Hz, J^2 = 3.0 Hz, 1H), 6.80–6.89 (m, 1H), 6.90–7.04 (m, 3H), 7.64 (d, J = 3.0 Hz, 1H), 8.10 (s, 1H), 8.26 (d, J = 7.5 Hz, 1H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.1, 24.4, 46.2, 49.1, 53.3, 55.4, 63.9, 66.8, 68.2, 68.4, 97.2, 105.4, 108.5, 111.3, 118.7, 121.2, 123.8, 129.6, 138.8, 141.9, 142.1, 152.2, 158.1, 164.3; IR: (NaCl) ν (cm $^{-1}$): 3309, 2928, 1650, 1540, 1502, 1453, 1280, 1240, 1119, 752;

HRMS ($C_{26}H_{35}N_5O_4$): calcd: 481.2689, found: 481.2689. HPLC (method A): t_R = 17.4 min; purity: 97.0.

4.27. 5-(2-Ethoxyethoxy)-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl] pyrazolo[1,5-*a*]pyridine-3-carboxamide (22)

Compound **22** was synthesized from **5b** (10 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **19** yielding 16 mg (82%) of **22**. EI-MS: *m/z* 496 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.25 (t, J = 7.0 Hz, 3H), 1.76 (br quint, J = 6.50 Hz, 2H), 1.98 (br quint, J = 7.5 Hz, 2H), 2.93–346 (m, 10H), 3.51 (br q, J = 6.0 Hz, 2H), 3.61 (q, J = 7.0 Hz, 2H). 3.81–3.84 (m, 2H), 3.86 (s, 3H), 4.21–4.26 (m, 2H), 6.64 (dd, J^1 = 7.5 Hz, J^2 = 2.5 Hz, 1H); 6.88 (d, J = 8.5 Hz, 1H), 6.93 (d, J = 4.0 Hz, 2H), 7.04–7.09 (m, 1H); 7.61 (d, J = 3.0 Hz, 1H), 8.24 (s, 1H), 8.32 (d, J = 7.5 Hz, 1H), 8.40 (s, 1H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.1, 21.1, 26.3, 37.9, 47.5, 52.3, 55.4, 56.7, 66.8, 68.1, 68.4, 97.3, 105.3, 108.6, 111.4, 118.8, 121.2, 124.5, 129.5, 138.9, 141.9, 142.3, 152.1, 158.2, 164.4; IR: (NaCl) ν (cm^{-1}): 3303, 2917, 1650, 1542, 1501, 1451, 1280, 1242, 1119, 753; HRMS ($C_{27}H_{37}N_5O_4$): calcd: 495.2846, found: 495.2846. HPLC (method A): t_R = 17.3 min; purity: 98.3.

4.28. 4-(2-Ethoxyethoxymethyl)-*N*-[3-[4-(2-methoxyphenyl)-piperazin-1-yl]propyl] benzamide (23)

Compound **23** was synthesized from **10b** (6.1 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **19** yielding 9.1 mg (74%) of **23**. EI-MS: *m/z* 496 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.21 (t, J = 7.0 Hz, 3H), 1.83 (br quint, J = 5.5 Hz, 2H), 2.65 (br t, J = 6.0 Hz, 2H), 2.70–2.74 (m, 4H), 3.07–3.11 (m, 4H), 3.52 (q, J = 7.0 Hz, 2H), 3.57–3.64 (m, 6H), 3.86 (s, 3H), 4.59 (s, 2H), 6.85–6.97 (m, 3H), 6.99–7.05 (m, 1H), 7.37 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H), 8.24 (s, 1H). ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 24.3, 40.9, 50.6, 53.6, 55.4, 58.5, 66.7, 69.7, 69.8, 72.6, 111.3, 118.1, 121.0, 123.1, 127.2, 127.4, 134.1, 141.1, 141.7, 152.3, 167.1; IR: (NaCl) ν (cm^{-1}): 3333, 2940, 2871, 1641, 1543, 1501, 1304, 1241, 1114, 750; HRMS ($C_{26}H_{37}N_3O_4$): calcd: 455.2784, found: 455.2785. HPLC (method A): t_R = 17.6 min; purity: 95.7.

4.29. 4-(2-Ethoxyethoxymethyl)-*N*-[4-[4-(2-methoxyphenyl)-piperazin-1-yl]butyl] benzamide (24)

Compound **24** was synthesized from **10b** (5.0 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **19** yielding 6.6 mg (64%) of **24**. EI-MS: *m/z* 496 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 1.62–1.76 (m, 4H), 2.49 (br t, J = 7.0 Hz, 2H), 2.65–2.69 (m, 4H), 3.06–3.10 (m, 4H), 3.49 (br q, J = 6.5 Hz, 2H), 3.54 (q, J = 7.0 Hz, 2H), 3.59–3.64 (m, 4H), 3.85 (s, 3H), 4.61 (s, 2H), 6.83–6.94 (m, 3H), 6.96–7.03 (m, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 24.4, 27.4, 39.9, 50.4, 53.4, 55.4, 58.0, 66.7, 69.8, 69.9, 72.6, 111.3, 118.2, 121.0, 123.0, 127.0, 127.5, 134.2, 141.2, 141.9, 152.3, 167.5; IR: (NaCl) ν (cm^{-1}): 3335, 2937, 2867, 1637, 1543, 1501, 1303, 1241, 1116, 750; HRMS ($C_{27}H_{39}N_3O_4$): calcd: 469.2941, found: 469.2941. HPLC (method A): t_R = 17.4 min; purity: 97.1.

4.30. 5-(2-Ethoxyethoxymethyl)-*N*-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl] pyrazolo[1,5-a]pyridine-3-carboxamide (25)

Compound **25** was synthesized from **11b** (4.2 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **19** yielding 5.9 mg (75%) of **25**. APCI-MS: *m/z* 496 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 2.02–2.13 (m, 2H), 2.87–3.14 (m, 6H), 3.23–3.37 (m, 4H), 3.54 (q, 2H), 3.60–3.69 (m, 6H), 3.86 (s, 3H), 4.64 (s, 2H), 6.86–6.89 (m, 1H), 6.91–6.96 (m, 2H), 6.99 (dd, J^1 = 7.0 Hz, J^2 = 1.5 Hz, 1H), 7.02–7.07 (m, 1H), 7.73–7.81 (m, 1H), 8.23–8.26 (m, 2H), 8.43–8.46 (m, 1H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 24.3, 37.9, 49.2, 53.3, 55.4, 56.7, 66.7, 69.8, 69.9, 72.1, 106.6, 111.3, 113.2, 117.3, 118.7, 121.2, 123.9, 128.8, 138.0, 139.9, 140.3, 141.5, 152.2, 164.0; IR: (NaCl) ν (cm^{-1}): 3413, 2925, 1647, 1554, 1502, 1454, 1246, 1117, 793, 752; HRMS ($C_{27}H_{37}N_5O_4$): calcd: 495.2846, found: 495.2844. HPLC (method B): t_R = 13.2 min; purity: >99.5.

4.31. 5-(2-Ethoxyethoxymethyl)-*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl] pyrazolo[1,5-*a*]pyridine-3-carboxamide (26)

Compound **26** was synthesized from **11b** (7.4 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **26** yielding 11 mg (79%) of **26**. APCI-MS: *m/z* 510 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.23 (t, J = 7.0 Hz, 3H), 1.66-1.79 (m, 4H), 2.64 (br t, J = 7.0 Hz, 2H), 2.77–2.91 (m, 4H), 3.10–3.24 (m, 4H), 3.48–3.56 (m, 4H), 3.61–3.67 (m, 4H), 3.86 (s, 3H), 4.63 (s, 2H), 6.45–6.51 (m, 1H), 6.85–6.88 (m, 1H), 6.89–6.94 (m, 2H), 6.97–7.04 (m, 2H), 8.20 (s, 1H), 8.21–8.23 (m, 1H), 8.42–8.45 (m, 1H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 23.6, 27.4, 38.9, 49.9, 53.4, 55.4, 57.9, 66.7, 69.8, 69.9, 72.0, 106.8, 111.2, 113.3, 117.3, 118.4, 121.1, 123.3, 128.7, 137.9, 140.3, 140.7, 140.8, 152.2, 163.5; IR: (NaCl) ν (cm^{-1}): 3323, 2933, 1647, 1554, 1502, 1454, 1242, 1117, 750; HRMS ($C_{28}H_{39}N_5O_4$): calcd: 509.3002, found: 509.3002. HPLC (method A): t_R = 14.4 min; purity: 95.2.

4.32. Tetraethylene glycol di[[3-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]phenyl] ether (27)

A solution of **6b** (10 mg, 0.023 mmol) and DIPEA (0.03 mL, 0.18 mmol) in CH_2Cl_2 (2 ml) was cooled to 0 °C. TBTU (19 mg, 0.06 mmol) in DMF (1 ml) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine (34 mg, 0.14 mmol) in CH_2Cl_2 (4.6 ml) were added and the solution was stirred for 4 h at room temperature. The reaction was treated with a saturated aqueous solution of $NaHCO_3$ followed by extraction with CH_2Cl_2. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) yielding 14 mg (68%) of **27**. EI-MS: *m/z* 898 (M^+); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.84 (br quint, J = 6.0 Hz, 4H), 2.66 (br t, J = 5.0 Hz, 4H), 2.71–2.77 (m, 8 H), 3.08–3.15 (m, 8H), 3.58 (br q, J = 5.5 Hz, 4H), 3.63–3.68 (m, 8H), 3.74–3.78 (m, 4H), 3.86 (s, 6H), 4.10–4.13 (m, 4H), 6.84–6.95 (m, 6H), 6.99–7.03 (m, 4H), 7.26 (t, J = 8.0 Hz, 2H), 7.36–7.40 (m, 2H), 7.42–7.45 (m, 2H), 8.30 (s, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.3, 40.6, 50.4, 53.6, 55.4, 58.2, 67.6, 69.6, 70.6, 70.8, 111.3, 113.4, 117.8, 118.2, 119.2, 121.0, 123.1, 129.3, 136.3, 141.0, 152.3, 159.00, 167.1; IR: (NaCl) ν (cm^{-1}): 3306, 2819, 1644, 1582, 1537, 1500, 1303, 1241, 1117, 751; HRMS ($C_{50}H_{68}N_6O_9$): calcd: 896.5048, found: 896.5046.

4.33. Tetraethylene glycol di[[3-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]phenyl] ether (28)

Compound **28** was synthesized from **6b** (10 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 24 mg (95%) of **28**. EI-MS: *m/z* 926

(M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.61–1.77 (m, 8H), 2.54 (br t, J = 7.0 Hz, 4H), 2.71–2.75 (m, 8H), 3.09–3.12 (m, 8H), 3.47 (br q, J = 6.5 Hz, 4H), 3.64–3.72 (m, 8H), 3.80–3.84 (m, 4H), 3.85 (s, 6H), 4.10–4.14 (m, 4H), 6.83–6.94 (m, 6H), 6.97–7.04 (m, 4H), 7.25–7.30 (t, J = 7.5 Hz, 2H), 7.31–7.34 (m, 2H), 7.34–7.37 (m, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.0, 27.3, 39.8, 50.1, 53.4, 55.4, 57.9, 67.6, 69.6, 70.7, 70.8, 111.3, 113.1, 118.1, 118.3, 119.2, 121.0, 123.1, 129.5, 136.3, 140.9, 152.3, 159.0, 167.5; IR: (NaCl) ν (cm^{-1}): 3320, 2939, 1643, 1582, 1537, 1501, 1301, 1242, 1118, 753; HRMS ($C_{52}H_{72}N_6O_9$): calcd: 924.5361, found: 924.5361. HPLC (method A): t_R = 17.0 min; purity: 95.6.

4.34. Tetraethylene glycol di[[4-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]benzyl] ether (29)

Compound **29** was synthesized from **14b** (9 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 15 mg (85%) of **29**. EI-MS: *m/z* 926 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.83 (br quint, J = 6.0 Hz, 4H), 2.64 (br t, J = 6.0 Hz, 4H), 2.70–2.74 (m, 8H), 3.07–3.11 (m, 8H), 3.56–3.68 (m, 20H), 3.86 (s, 6H), 4.56 (s, 4H), 6.84–6.96 (m, 6H), 6.98–7.04 (m, 2H), 7.36 (d, J = 8.5 Hz, 4H), 7.8 (d, J = 8.5 Hz, 4H), 8.22 (s, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.4, 40.8, 50.6, 53.6, 55.4, 58.4, 69.7, 70.6, 70.6, 70.7, 72.6, 111.3, 118.1, 121.1, 123.1, 127.2, 127.4, 134.1, 141.1, 141.7, 152.3, 167.1; IR: (NaCl) ν (cm^{-1}): 3333, 2940, 2819, 1643, 1542, 1501, 1304, 1241, 1113, 1028, 751; HPLC (method A): t_R = 16.0 min; purity: 97.4%. HPLC (method A): t_R = 17.0 min; purity: 97.4.

4.35. Tetraethylene glycol di[[4-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]benzyl] ether (30)

Compound **30** was synthesized from **14b** (8 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 16 mg (95%) of **30**. EI-MS: *m/z* 954 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.63–1.76 (m, 8H), 2.53 (br t, J = 7.0 Hz, 4H), 2.70–2.73 (m, 8H), 3.08–3.11 (m, 8H), 3.48 (br q; J = 6.5 Hz, 4H), 3.59–3.70 (m, 16H), 3.85 (s, 6H), 4.58 (s, 4H), 6.83–6.94 (m, 6H), 6.97–7.03 (m, 2H), 7.37 (d, J = 8.5 Hz, 4H), 7.74 (d, J = 8.5 Hz, 4H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.1, 27.3, 39.8, 50.2, 53.4, 55.4, 58.0, 69.7, 70.6, 70.6, 70.7, 72.6, 111.3, 118.3, 121.0, 123.1, 127.1, 127.5, 134.1, 141.0, 141.8, 152.3, 167.5; IR: (NaCl) ν (cm^{-1}): 3345, 2938, 1643, 1543, 1500, 1302, 1241, 1096, 1028, 751; HPLC (method A): t_R = 16.9 min; purity: 98.0.

4.36. Pentaethylene glycol di[[4-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]benzyl] ether (31)

Compound **31** was synthesized from **15b** (20 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 35 mg (95%) of **31**. APCI-MS: *m/z* 970 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.79–1.89 (m, 4H), 2.66 (br t, J = 5.5 Hz, 4H), 2.69–2.84 (m, 8H), 2.87–3.27 (m, 8H), 3.56–3.68 (m, 24H), 3.86 (s, 6H), 4.57 (s, 4H), 6.85–6.91 (m, 4H), 6.92–6.96 (m, 2H), 6.99–7.05 (m, 2H), 7.33–7.40 (m, 4H), 7.79–7.85 (m, 4H), 8.22–8.28 (m, 2H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 24.4, 40.7, 50.6, 53.4, 55.4, 58.3, 69.7, 70.6, 70.7, 72.6, 111.4, 118.1, 121.1, 123.2, 127.2, 127.5, 134.1, 141.0, 141.8, 152.3, 167.1; IR: (NaCl) ν (cm^{-1}): 3326, 2937, 2818, 1639, 1542, 1500, 1451, 1304, 1241, 1114, 733; HPLC (method B): t_R = 15.5 min; purity: 99%. HPLC (method B): t_R = 15.5 min; purity: 95.6.

4.37. Pentaethylene glycol di[[4-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]benzyl] ether (32)

Compound **32** was synthesized from **15b** (20 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 36 mg (97%) of **32**. APCI-MS: *m/z* 998 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.61–1.78 (m, 8H), 2.49 (br t, J = 6.5 Hz, 4H), 2.61–2.77 (m, 8H), 3.00–3.16 (m, 8H), 3.48 (dt, J^1 = 6.5 Hz, J^2 = 6.0 Hz, 4H), 3.57–3.74 (m, 20H), 3.85 (s, 6H), 4.58 (s, 4H), 6.74–6.82 (m, 2H), 6.83–7.03 (m, 8H), 7.35–7.41 (m, 4H), 7.71–7.78 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.3, 27.4, 39.9, 50.4, 53.4, 55.4, 58.1, 69.7, 70.6, 70.7, 72.6, 111.3, 118.2, 121.0, 123.0, 127.1, 127.5, 134.2, 141.2, 141.8, 152.3, 167.5; IR: (NaCl) ν (cm^{-1}): 3334, 2936, 2817, 1642, 1542, 1501, 1452, 1303, 1241, 1115, 751; HPLC (method B): t_R = 15.4 min; purity: 96.5.

4.38. Hexaethylene glycol di[[4-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]benzyl] ether (33)

Compound **33** was synthesized from **16b** (20 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 31 mg (84%) of **33**. APCI-MS: *m/z* 1014 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.86–2.05 (m, 4H), 2.80 (t, J = 5.5 Hz, 4H), 2.85–3.01 (m, 8H), 3.14–3.30 (m, 8H), 3.57–3.70 (m, 28H), 3.86 (s, 6H), 4.58 (s, 4H), 6.85–6.96 (m, 6H), 7.01–7.06 (m, 2H), 7.35–7.40 (m, 4H), 7.83–7.90 (m, 4H), 8.22–8.27 (m, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.1, 39.6, 49.7, 53.3, 55.4, 57.3, 69.8, 70.6, 70.7, 72.7, 111.4, 118.4, 121.1, 123.6, 127.3, 127.5, 133.7, 140.4, 141.9, 152.3, 167.3; IR: (NaCl) ν (cm^{-1}): 3584, 2925, 2871, 1652, 1541, 1501, 1453, 1303, 1242, 1114, 1026, 752; HPLC (method B): t_R = 15.5 min; purity: 99.4.

4.39. Hexaethylene glycol di[[4-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]benzyl] ether (34)

Compound **34** was synthesized from **16b** (20 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 31 mg (82%) of **34**. APCI-MS: *m/z* 1042 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.58–1.77 (m, 8H), 2.50 (br t, J = 6.0 Hz, 4H), 2.61–2.82 (m, 8H), 2.95–3.19 (m, 8H), 3.48 (dt, J^1 = 6.0 Hz, J^2 = 6.0 Hz, 4H), 3.57–3.76 (m, 24H), 3.85 (s, 6H), 4.58 (s, 4H), 6.71–6.79 (m, 2H), 6.83–6.94 (m, 6H), 6.95–7.03 (m, 2H), 7.35–7.42 (m, 4H), 7.71–7.78 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.3, 27.5, 39.9, 50.4, 53.4, 55.4, 58.1, 69.7, 70.6, 70.7, 72.6, 111.3, 118.3, 121.0, 123.0, 127.1, 127.5, 134.2, 141.2, 141.9, 152.3, 167.5; IR: (NaCl) ν (cm^{-1}): 3334, 2934, 2817, 1642, 1542, 1501, 1452, 1302, 1241, 1116, 771; HPLC (method B): t_R = 15.4 min; purity: 98.7.

4.40. Octaethylene glycol di[[4-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]benzyl] ether (35)

Compound **35** was synthesized from **17b** (20.5 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 33 mg (94%) of **35**. APCI-MS: *m/z* 1102 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.78–1.87 (m, 4H), 2.63 (br t, J = 6.0 Hz, 4H), 2.67–2.78 (m, 8H), 3.00–3.15 (m, 8H), 3.56–3.69 (m, 36H), 3.86 (s, 6H), 4.57 (s, 4H), 6.85–6.96 (m, 6H), 6.99–7.05 (m, 2H), 7.33–7.40 (m, 4H), 7.79–7.85 (m, 4H), 8.18–8.25 (m, 2H); ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 24.4, 40.9, 50.7, 53.6, 55.4, 58.5, 69.7, 70.6, 70.6, 70.6, 72.6, 111.3, 118.1, 121.1, 123.2, 127.2, 127.4, 134.1, 141.1, 141.7, 152.4, 167.1; IR: (NaCl) ν (cm^{-1}): 3341, 2874, 2820, 1643, 1542, 1501, 1453, 1304, 1241, 1113, 791, 753; HPLC (method B): t_R = 15.1 min; purity: 97.3.

4.41. Octaethylene glycol di[[4-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]benzyl] ether (36)

Compound **36** was synthesized from **17b** (20 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the

procedure described for **27** yielding 32 mg (89%) of **36**. APCI-MS: *m/z* 1130 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.60–1.75 (m, 8H), 2.47 (br t, J = 7.0 Hz, 4H), 2.58–2.71 (m, 8H), 2.98–3.13 (m, 8H), 3.48 (dt, J^1 = 6.0 Hz, J^2 = 6.0 Hz, 4H), 3.56–3.72 (m, 32H), 3.85 (s, 6H), 4.59 (s, 4H), 6.67–6.77 (m, 2H), 6.83–6.94 (m, 6H), 6.95–7.02 (m, 2H), 7.35–7.42 (m, 4H), 7.71–7.78 (m, 4H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.3, 27.5, 39.9, 50.4, 53.4, 55.4, 58.1, 69.8, 70.6, 70.6, 70.6, 70.7, 72.6, 111.3, 118.3, 121.0, 123.0, 127.1, 127.5, 134.2, 141.2, 141.9, 152.3, 167.5; IR: (NaCl) ν (cm^{-1}): 3565, 2872, 1642, 1548, 1501, 1454, 1303, 1242, 1115, 752; HPLC (method B): t_R = 15.4 min; purity: 97.6.

4.42. Tetraethylene glycol di[[3-*N*-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propyl]aminocarbonyl]pyrazolo[1,5-*a*]pyridine-5-yl] ether (37)

Compound **37** was synthesized from **7b** (10 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 16 mg (86%) of **37**. EI-MS: *m/z* 978 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.95 (br quint, J = 6.0 Hz, 4H), 2.79 (br t, J = 6.0 Hz, 4H), 2.87–2.92 (m, 8H), 3.22–3.25 (m, 8H), 3.59 (br q, J = 6.0 Hz, 4H), 3.65–3.74 (m, 8H), 3.86 (s, 6H), 3.85–3.89 (m, 4H), 4.17–4.23 (m, 4H), 6.60 (dd, J^1 = 7.5 Hz, J^2 = 2.77 Hz, 2H), 6.84–6.97 (m, 6H), 6.99–7.05 (m, 2H), 7.59 (d, J = 2.5 Hz, 2H), 7.64 (s, 2H), 8.23 (s, 2H), 8.25 (dd, J^1 = 7.5 Hz, J^2 = 0.5 Hz, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.7, 38.6, 49.8, 53.4, 55.4, 57.3, 68.0, 69.3, 70.7, 70.8, 97.2, 105.6, 108.4, 111.3, 118.3, 121.2, 123.5, 129.5, 140.4, 141.5, 142.1, 152.2, 157.9, 164.0; IR: (NaCl) ν (cm^{-1}): 3308, 2941, 1650, 1541, 1501, 1453, 1281, 1240, 1118, 769. HPLC (method A): t_R = 16.9 min; purity: 96.2.

4.43. Tetraethylene glycol di[[3-*N*-[4-[4-(2-methoxyphenyl) piperazin-1-yl]butyl]aminocarbonyl]pyrazolo[1,5-*a*]pyridine-5-yl] ether (38)

Compound **38** was synthesized from **7b** (10 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 18 mg (97%) of **38**. EI-MS: *m/z* 1006 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.66–1.82 (m, 8H), 2.61 (br t, J = 6.5 Hz, 4H), 2.79–2.83 (m, 8H), 3.17–3.20 (m, 8H), 3.49 (br q, J = 6.0 Hz, 4H), 3.65–3.72 (m, 8H), 3.85 (s, 6H), 3.83–3.89 (m, 4H), 4.15–4.20 (m, 4H), 6.60 (dd, J^1 = 7.5 Hz, J^2 = 2.5 Hz, 2H), 6.84–7.05 (m, 8H), 7.58 (d, J = 2.5 Hz, 2H), 8.19 (s, 2H), 8.25 (dd, J^1 = 7.5 Hz, J^2 = 0.5 Hz, 2H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 23.6, 27.5, 38.8, 49.9, 53.2, 55.4, 57.8, 68.0, 69.3, 70.7, 70.8, 97.2, 105.6, 108.5, 111.3, 118.5, 121.1, 123.3, 129.5, 140.7, 141.3, 142.2, 152.2, 157.9, 163.8; IR: (NaCl) ν (cm^{-1}): 3305, 2938, 1650, 1541, 1500, 1450, 1280, 1240, 1117, 751. HPLC (method A): t_R = 17.1 min; purity: 96.3.

4.44. 5,5′-(2,5,8,11,14-Pentaoxapentadecan-1,15-diyl)bis[*N*-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl]pyrazolo[1,5-*a*]pyridin-3-carboxamide] (39)

Compound **39** was synthesized from **18b** (23.5 mg) and 3-[4-(2-methoxyphenyl)-piperazin-1-yl]propylamine according to the procedure described for **27** yielding 38.5 mg (88%) of **39**. APCI-MS: *m/z* 1006 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.82–1.91 (m, 4H), 2.66 (br t, J = 6.1, 4H), 2.70–2.82 (m, 8H), 3.07–3.20 (m, 8H), 3.55–3.70 (m, 20H), 3.86 (s, 6H), 4.59 (s, 4H), 6.82–6.88 (m, 2H), 6.91–7.05 (m, 8H), 7.58–7.65 (m, 2H), 8.21–8.25 (m, 4H), 8.41 (dd, J^1 = 7.0 Hz, J^2 = 0.5 Hz, 2H); ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 25.0, 39.6, 50.6, 53.7, 55.4, 58.1, 69.9, 70.6, 70.7, 70.7, 72.0, 107.1, 111.3, 113.2, 117.4, 118.4, 121.2, 123.2, 128.7, 137.7, 140.3, 140.8, 140.9, 152.3, 163; IR: (NaCl) ν (cm^{-1}): 3324, 2938, 2819, 1648, 1552, 1500, 1453, 1241, 1115, 792, 752; HPLC (method A): t_R = 9.4 min; purity: 98.1.

4.45. 5,5′-(2,5,8,11,14-Pentaoxapentadecan-1,15-diyl)bis[*N*-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl]}pyrazolo[1,5-*a*]pyridin-3-carboxamide] (40)

Compound **40** was synthesized from **18b** (7.3 mg) and 4-[4-(2-methoxyphenyl)-piperazin-1-yl]butylamine according to the procedure described for **27** yielding 13 mg (93%) of **40**. APCI-MS: *m/z* 1034 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.61–1.75 (m, 8H), 2.50 (br t, J = 6.5 Hz, 4H), 2.65–2.77 (m, 8H), 3.03–3.17 (m, 8H), 3.49 (dt, J^1 = 6.0 Hz, J^2 = 6.0 Hz, 4H), 3.58–3.75 (m, 16H), 3.85 (s, 6H), 4.59 (s, 4H), 6.40–6.54 (m, 2H), 6.83–7.04 (m, 10H), 8.17 (s, 2H), 8.19–8.23 (m, 2H), 8.41 (dd, J^1 = 7.0 Hz, J^2 = 0.5 Hz, 2H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.3, 27.8, 39.3, 50.4, 53.4, 55.4, 58.1, 69.9, 70.6, 70.6, 70.7, 72.0, 106.9, 111.3, 113.3, 117.2, 118.3, 121.0, 123.0, 128.7, 137.9, 140.3, 140.7, 141.2, 152.3, 163.4; IR: (NaCl) ν (cm^{-1}): 3313, 2935, 1647, 1554, 1502, 1452, 1242, 1115, 793, 750; HPLC (method A): t_R = 9.5 min; purity: 94.7.

4.46. 1-Bromo-4-(2-ethoxyethoxy)methyl-2-methoxybenzene (42)

Compound **42** was synthesized from **41** (200 mg) according to the procedure described for **11a** yielding 97 mg (36%) of **42**. EI-MS: *m/z* 288 (M^+), 288 (M^++2); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.23 (t, J = 7.0 Hz, 3H), 3.56 (q, J = 7.0 Hz, 2H), 3.63–3.68 (m, 2H), 3.69–3.74 (m, 2H), 3.80 (s, 3H), 4.60 (s, 2H), 6.69 (dd, J^1 = 9.0 Hz, J^2 = 3.0 Hz, 1H), 7.11 (d, J = 3.0 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.2, 55.5, 66.7, 69.9, 70.2, 72.3, 112.6, 114.2, 114.8, 133.0, 138.8, 159.2; IR: (NaCl) ν (cm^{-1}): 2974, 2866, 1595, 1574, 1471, 1296, 1273, 1240, 1119, 1055, 1018, 806.

4.47. 1-[4-(2-Ethoxyethoxymethyl)-2-methoxyphenyl] piperazine (43)

A solution of **42** (391 mg, 1,35 mmol), piperazine (256 mg, 3.0 mmol), NaO*t*Bu (156 mg, 1.62 mmol), $Pd_2(dba)_3$ (12.4 mg, 0.01 mol %) and 2-(di-*tert*-butyl-phosphino)biphenyl (16 mg (4 mol %) in toluene (4.6 mL) was heated for 18 h at 115 °C. After being cooled to room temperature the mixture was filtered through a pad of Celite© and the filtrate was evaporated. The residue was purified by flash-chromatography (CH_2Cl_2/MeOH/Me_2EtN 9:1:0.1) to give **43** in 56% yield (225 mg). APCI-MS: *m/z* 295 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 2.18–2.25 (m, 1H), 2.76–2.90 (m, 4H), 2.97–3.11 (m, 4H), 3.54 (q, J = 7.0 Hz, 2H), 3.60–3.64 (m, 2H), 3.65–3.69 (m, 2H), 3.79 (s, 3H), 4.65 (s, 2H), 6.80 (dd, J^1 = 8.5 Hz, J^2 = 3.0 Hz, 1H), 7.01–7.08 (m, 2H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.2, 46.6, 54.3, 55.5, 66.7, 68.8, 69.9, 70.0, 113.6, 114.3, 121.0, 134.9, 145.1, 156.2; IR: (NaCl) ν (cm^{-1}): 3431, 2929, 1610, 1500, 1458, 1298, 1219, 1119, 1049, 910, 818.

4.48. 4-[4-[4-(2-Ethoxyethoxymethyl)-2-methoxyphenyl] piperazin-1-yl]butyronitrile (45)

A mixture of **43** (86 mg, 0.29 mmol), K_2CO_3 (89 mg, 0.65 mmol), 4-bromo butyronitrile (59 μL, 0.59 mmol), NaI (23 mg, 0.16 mmol) and CH_3CN (5 mL) was stirred for 24 h at reflux temperature. After being cooled to room temperature the mixture was filtered through a pad of Celite© followed by washing with acetone. After evaporation the residue was purified by flash-chromatography (EtOAc) to give **44** in 68% yield (73 mg). APCI-MS: *m/z* 362 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 1.83–1.89 (m, 2H), 2.46 (t, J = 7.0 Hz, 2H), 2.52 (t, J = 7.0 Hz, 2H), 2.54–2.64 (m, 4H), 3.87 (br t, J = 4.5 Hz, 4H), 3.54 (q, J = 7.0

Hz, 2H), 3.60–3.68 (m, 4H), 3.79 (s, 3H), 4.64 (s, 2H), 6.79 (dd, J^1 = 8.5 Hz, J^2 = 3.0 Hz, 1H), 7.02–7.07 (m, 2H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 14.9, 15.2, 22.8, 53.1, 53.8, 55.5, 56.4, 66.7, 68.8, 69.9, 70.0, 113.5, 114.3, 119.8, 120.9, 134.8, 144.7, 156.2; IR: (NaCl) ν (cm^{-1}): 3514, 2871, 2247, 1500, 1458, 1300, 1215, 1132, 1028, 816, 752, 715.

4.49. 4-[4-[4-(2-Ethoxyethoxymethyl)-2-methoxyphenyl] piperazin-1-yl]butylamine (44)

A solution of **45** (39 mg, 0.11 mmol) in Et_2O (2 mL) was cooled to −5 °C. Addition of $LiAlH_4$ (270 μL, 1 M in Et_2O, 0.27 mmol) was followed by stirring for 3 h at room temperature. After addition of one drop of $NaHCO_3$ (sat. aqueous solution), the mixture was filtered through a layered pad of Celite©/$MgSO_4$/ Celite©. Washing with Et2O was followed by evaporation and purification by flash-chromatography (CH_2Cl_2/MeOH/Me_2EtN 95:5:0.1) to give **45** in 36% yield (14.4 mg). APCI-MS: *m/z* 366 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.22 (t, J = 7.0 Hz, 3H), 1.49–1.64 (m, 4H), 2.42 (t, J = 7.0 Hz, 2H), 2.48–2.67 (m, 6H), 2.76 (t, J = 6.5 Hz, 2H), 2.88 (br t, J = 5.0 Hz, 4H), 3.54 (q, J = 7.0 Hz, 2H), 3.60–3.69 (m, 4H), 3.79 (s, 3H), 4.63 (s, 2H), 6.78 (dd, J^1 = 8.5 Hz, J^2 = 3.0 Hz, 1H), 7.02–7.09 (m, 2H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 15.2, 24.5, 31.2, 41.8, 53.0, 53.9, 55.5, 58.5, 66.7, 68.8, 69.8, 70.0, 113.5, 114.3, 121.0, 134.9, 144.7, 156.2; IR: (NaCl) ν (cm^{-1}): 3361, 2868, 1500, 1462, 1298, 1215, 1124, 816, 717.

4.50. *N*-[4-[4-[4-(2-Ethoxyethoxymethyl)-2-methoxyphenyl] piperazin-1-yl]butyl] benzamide (46)

A solution of **44** (8.5 mg, 23 μmol) and Et_3N (10 μL, 72 μmol) in $CHCl_3$ (1 mL) was cooled to 0 °C. After 15 min benzoyl chloride (4 μL, 35 μmol) was added. After being stirred for 24 h at room temperature, the reaction was treated with a saturated aqueous solution of $NaHCO_3$ followed by extraction with $CHCl_3$. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. The crude product was purified by preparative HPLC (ZORBAX ECLIPSE XDB-C8, 0.1% aqueous TFA/MeOH, gradient) yielding 6.8 mg (60%) of **46**. APCI-MS: *m/z* 470 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.21 (t, J = 6.9 Hz, 3H), 1.60–1.77 (m, 4H), 2.49 (br t, J = 6.0 Hz, 2H), 2.54–2.71 (m, 4H), 2.86 (br t, J = 4.5 Hz, 4H), 3.46–3.57 (m, 4H), 3.59–3.68 (m, 4H), 3.78 (s, 3H), 4.62 (s, 2H), 6.68–6.74 (m, 1H), 6.78 (dd, J^1 = 8.5 Hz, J^2 = 3.0 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 3.0 Hz, 1H), 7.38–7.57 (m, 3H), 7.74–7.85 (m, 2H). ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 15.2, 23.8, 27.4, 39.8, 52.7, 53.7, 55.5, 57.9, 66.7, 68.9, 69.8, 70.0, 113.6, 114.5, 121.0, 127.0, 128.5, 131.3, 134.8, 135.0, 144.5, 156.3, 167.8; IR: (NaCl) ν (cm^{-1}): 3313, 2927, 1645, 1539, 1498, 1296, 1093, 802, 710; HRMS ($C_{27}H_{39}N_3O_4$): calcd: 469.2941, found: 469.2941; HPLC (method B): t_R = 14.1 min; purity: 95.3.

4.51. 1,15-Bis(4-bromo-3-methoxyphenyl)-2,5,8,11,14-pentaoxapentadecane (47)

A suspension of NaH (1.8 g, 60% in paraffin, 45 mmol) in THF (50 mL) was cooled to 0 °C. Addition of a cooled solution of **41** (3.66 g, 17 mmol) in THF (30 mL) was followed by stirring for 15 min. After adding tetraethylene glycol di-*p*-tosylate (1.12 mL, 2.8 mmol) the mixture was warmed to room temperature and stirred for 4 h. Addition of water was followed by extraction with EtOAc, drying (Na_2SO_4) and evaporation of the solvent. The residue was purified by flash-chromatography (hexane/EtOAc 6:1 to 1:1) to give **47** in 74% yield (1.24 mg). EI-MS: *m/z* 592 (M^+); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 3.67–3.69 (m, 8H), 3.70–3.73 (m, 8H), 3.79 (s, 6H), 4.58 (s, 4H), 6.69 (dd, J^1 = 8.5 Hz, J^2 = 3.0 Hz, 1H), 7.08 (d, J = 3.0 Hz, 1H), 7.38 (d, J = 9.0 Hz, 1H). ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 55.5, 70.2, 70.6, 70.7, 70.8, 72.3, 112.6, 114.3, 114.7, 133.0, 138.7, 159.1; IR: (NaCl) ν (cm^{-1}): 2868, 1595, 1469, 1354, 1296, 1111, 876, 810, 754.

4.52. 1,1′-[2,5,8,11,14-Pentaoxapentadecan-1,15-diylbis(2-methoxy-4,1-phenylene)]dipiperazine (48)

Compound **48** was synthesized from **47** (1.24 g, 2.1 mmol)) and piperazine (0.79 g, 9.2 mmol) according to the procedure described for **43** yielding 0.50 g (40%) of **48**. APCI-MS: *m/z* 603 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 2.11–2.28 (m, 2H), 2.77–2.89 (m, 8H), 2.96–3.06 (m, 8H), 3.62–3.73 (m, 16H), 3.78 (s, 6H), 4.63 (s, 4H), 6.79 (dd, J^1 = 9.0 Hz, J^2 = 3.0 Hz, 2H), 7.00–7.07 (m, 4H). ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 46.6, 54.3, 55.5, 68.8, 69.8, 70.6, 70.6, 113.5, 114.4, 121.0, 134.8, 145.2, 156.2; IR: (NaCl) ν (cm^{-1}): 2924, 2854, 1500, 1462, 1265, 1211, 1095, 814, 756.

4.53. 4-(Benzamido)butyl *p*-toluene sulfonate (50)

To a cooled solution (0 °C) of **49** (382 mg, 1.98 mmol) in THF (3.2 mL) was added Et_3N (385 μL, 2.77 mmol). After stirring for 15 min a solution of TosCl (566 mg, 2.97 mmol) in THF (5 mL) was added dropwise and the mixture was stirred at 40 °C for 16 h. Addition of ice-water was followed by extraction with CH_2Cl_2, drying (Na_2SO_4) and evaporation of the solvent. The residue was purified by flash-chromatography (hexane/EtOAc 4:1 to 1:1) to give **50** in 53% yield (370 mg). APCI-MS: *m/z* 348 (M^++1); ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm): 1.62–1.80 (m, 4H), 2.43 (s, 3H), 3.43 (dt, J^1 = 6.5 Hz, J^2 = 6.5 Hz, 2H), 4.07 (t, J = 6.0 Hz, 2H), 6.23–6.39 (m, 1H), 7.30–7.36 (m, 2H), 7.37–7.45 (m, 2H), 7.46–7.52 (m, 1H), 7.71–7.81 (m, 4H). ^{13}C NMR: ($CDCl_3$, 150 MHz) δ (ppm): 21.6, 25.8, 26.4, 39.2, 70.2, 126.9, 127.9, 128.5, 129.9, 131.5, 132.9, 134.5, 144.9, 167.7; IR: (NaCl) ν (cm^{-1}): 3321, 2927, 1643, 1539, 1358, 1176, 941, 818, 710, 663. HPLC (method A): t_R = 13.4 min; purity: 96.

4.54. *N*,*N*′-[2,5,8,11,14-Pentaoxapentadecan-1,15-diylbis[(2-methoxy-4,1-phenylene)piperazin-4,1-diylbutan-4,1-diyl]] dibenzamide (51)

A suspension of **48** (147 mg, 0.24 mmol), **50** (73 mg, 0.48 mg), K_2CO_3 (339 mg, 0.98 mmol) and NaI (73 mg, 0.49 mmol) ind CH_3CN (15 mL) was stirred for 16 h at reflux temperature. After being cooled to room temperature, the solvent was removed under reduced pressure and the residue was treated with a saturated solution of $NaHCO_3$. Extraction with CH_2Cl_2, drying (Na_2SO_4), evaporation of the solvent and subsequent purification by preparative HPLC (ZORBAX ECLIPSE XDB-C8, 0.1% aqueous TFA/MeOH, gradient) gave **51** in 12% yield (28 mg). APCI-MS: *m/z* 954 (M^++1); ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm): 1.62–1.73 (m, 8H), 2.47 (br t, J = 7.0 Hz, 4H), 2.52–2.74 (m, 8H), 2.84 (br t, J = 4.5 Hz, 8H), 3.44–3.51 (m, 4H), 3.60–3.69 (m, 16H), 3.77 (s, 6H), 4.60 (s, 4H), 6.77 (dd, J^1 = 8.5 Hz, J^2 = 3.5 Hz, 2H), 6.80–6.84 (m, 2H), 6.97 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 3.0 Hz, 2H), 7.36–7.51 (m, 6H), 7.75–7.81 (m, 4H). ^{13}C NMR: ($CDCl_3$, 90 MHz) δ (ppm): 24.4, 27.5, 39.9, 52.9, 53.8, 55.5, 58.1, 68.8, 69.8, 70.6, 70.6, 113.4, 114.4, 120.9, 127.0, 128.5, 131.3, 134.8, 135.1, 144.5, 156.2, 167.8; IR: (NaCl) ν (cm^{-1}): 3321, 2935, 1643, 1539, 1496, 1296, 1126, 802, 714. HPLC (method B): t_R = 14.6 min; purity: 95.7.

4.55. Radioligand binding studies

Receptor binding studies were carried out as described previously.[40] In brief, competition binding experiments with the human D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors were run on membrane preparations from CHO cells stably expressing the corresponding receptor. Assays were run with membranes at protein concentrations per well

of 1–12 μg/mL and [^{3}H]spiperone (specific activity = 84 Ci/mmol, PerkinElmer, Rodgau, Germany) at final concentrations of 0.1–0.3 nM according to the individual K_D values in binding buffer (50 mM Tris, 1.0 mM EDTA, 5.0 mM $MgCl_2$, 100 μg/ml bacitracin and 5 μg/ml soybean trypsin inhibitor at pH 7.4). The K_D values were 0.050–0.26, 0.030–0.22, 0.090–0.20, and 0.14–0.35 nM for the D_{2long}, D_{2short}, D_3 and D_4 receptor, respectively. The corresponding B_{max} values were in the range of 615–7500 fmol/mg for D_{2long}, 675–2980 fmol/mg for D_{2short}, 1525–11000 fmol/mg for D_3, and 340–1895 fmol/mg for D_4 receptor, respectively. Nonspecific binding was determined in the presence of 10 μM haloperidol. Specific binding represented about 85% of the total binding. Protein concentration was established by the method of Lowry using bovine serum albumin as a standard.[41]

4.56. Data analysis

The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM 5.0 (GraphPad Software, San Diego, CA). Competition curves were fitted to a sigmoid curve by nonlinear regression analysis in which the log EC_{50} value and the Hill coefficient were free parameters. EC_{50} values were transformed to K_i values according to the equation of Cheng and Prusoff.[42]

Acknowledgment

This research was funded by a Grant of the Deutsche Forschungsgemeinschaft (DFG: Gm 13/8).

Supplementary data

Supplementary data (supplementary data including HPLC chromatograms and ^{13}C NMR spectra of the target compounds and receptor binding data for the porcine dopamine D1, serotonin 5-HT1A, 5-HT2 and the drenergic alpha1 receptor) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.10.063.

References and notes

1. Schwartz, J. C.; Giros, B.; Martres, M.-P.; Sokoloff, P. *Semin. Neurosci.* **1992**, *4*, 99.
2. Löber, S.; Hübner, H.; Tschammer, N.; Gmeiner, P. *Trends Pharmacol. Sci.* **2011**, *32*, 148.
3. Enguehard-Gueiffier, C.; Gueiffier, A. *Curr. Med. Chem.* **2006**, *13*, 2981.
4. Zhang, A.; Neumeyer, J. L.; Baldessarini, R. *J. Chem. Rev.* **2007**, *107*, 274.
5. Ehrlich, K.; Götz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Härterich, S.; Hübner, H.; Lanig, H.; Gmeiner, P. *J. Med. Chem.* **2009**, *52*, 4923.
6. Heidbreder, C. A.; Newman, A. H. *Ann. N.Y. Acad. Sci.* **2010**, *1187*, 4.
7. George, S. R.; O'Dowd, B. F.; Lee, S. P. *Nat. Rev. Drug Disc.* **2002**, *1*, 808.
8. Milligan, G. *Mol. Pharmacol.* **2004**, *66*, 1.
9. Smith, N. J.; Milligan, G. *Pharmacol. Rev.* **2010**, *62*, 701.
10. Vivo, M.; Lin, H.; Strange, P. G. *Mol. Pharmacol.* **2006**, *69*, 226.
11. Ng, G. Y. N.; ÓDowd, B.; Lee, S. P.; Chung, H. T.; Brann, M. R.; Seeman, P.; George, S. R. *Biochem. Biophys. Res. Commun.* **1996**, *227*, 200.
12. Guo, W.; Shi, L.; Filizola, M.; Weinstein, H.; Javitch, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17495.
13. Guo, W.; Urizar, E.; Kralikova, M.; Mobarec, J. C.; Shi, L.; Filizola, M.; Javitch, J. A. *EMBO J.* **2008**, *27*, 2293.
14. Rocheville, M.; Lange, D. C.; Kumar, U.; Patel, S. C.; Patel, R. C.; Patel, Y. C. *Science* **2000**, *288*, 154.
15. Koschatzky, S.; Tschammer, N.; Gmeiner, P. A. C. S. *Chem. Neurosci.* **2011**, *2*, 308.
16. Wang, M.; Pei, L.; Fletcher, P. J.; Kapur, S.; Seeman, P.; Liu, F. *Mol. Brain* **2010**, *3*, 25.
17. Horwedel, C.; Tsogoeva, S. B.; Wei, S.-W.; Efferth, T. *J. Med. Chem.* **2010**, *53*, 4842.
18. Dollinger, S.; Löber, S.; Klingenstein, R.; Korth, C.; Gmeiner, P. *J. Med. Chem.* **2006**, *49*, 6591.
19. Soulier, J.-L.; Russo, O.; Giner, M.; Rivail, L.; Berthouze, M.; Ongeri, S.; Maigret, B.; Fischmeister, R.; Lezoualc'h, F.; Sicsic, S.; Berque-Bestel, I. *J. Med. Chem.* **2005**, *48*, 6220.
20. Fejada, F. R.; Nagy, P. I.; Xu, M.; Wu, C.; Katz, T.; Dorsey, J.; Rieman, M.; Lawlor, E.; Warrier, M.; Messer, W. S., Jr. *J. Med. Chem.* **2006**, *49*, 7518.
21. Christopoulos, A.; Grant, M. K. O.; Ayoubzadeh, N.; Kim, O. N.; Sauerberg, P.; Jeppesen, L.; El-Fakahany, E. E. *J. Pharm. Exp. Ther.* **2001**, *298*, 1260.
22. Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. *J. Med. Chem.* **2006**, *49*, 256.
23. Daniels, D. J.; Lenard, N. R.; Etienne, C. L.; Law, P.-Y.; Roerig, S. C.; Portoghese, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 19208.
24. Decker, M.; Lehmann, J. *Curr. Top. Med. Chem.* **2007**, *7*, 347.
25. Mohr, K.; Tränkle, C.; Kostenis, E.; Barocelli, E.; De Amici, M.; Holzgrabe, U. *Br. J. Pharmacol.* **2010**, *159*, 997.
26. Kühhorn, J.; Hübner, H.; Gmeiner, P. *J. Med. Chem.* **2011**, *54*, 4896.
27. Boeckler, F.; Gmeiner, P. *Pharmacol. Ther.* **2006**, *112*, 281.
28. Heidler, P.; Zohrabi-Kalantari, V.; Calmels, T.; Capet, M.; Berrebi-Bertrand, I.; Schwartz, J.-C.; Stark, H.; Link, A. *Bioorg. Med. Chem.* **2005**, *13*, 2009.
29. Löber, S.; Aboul-Fadl, T.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 633.
30. Löber, S.; Hübner, H.; Utz, W.; Gmeiner, P. *J. Med. Chem.* **2001**, *44*, 2691.
31. Bettinetti, L.; Schlotter, K.; Hübner, H.; Gmeiner, P. *J. Med. Chem.* **2002**, *45*, 4594.
32. Hübner, H.; Skultety, M.; Gmeiner, P. WO 2008113559, 2008; *Chem. Abstr.* **2008**, *149*, 378773.
33. Skultety, M.; Hübner, H.; Löber, S.; Gmeiner, P. *J. Med. Chem.* **2010**, *53*, 7219.
34. Speicher, A.; Backes, T.; Grosse, S. *Tetrahedron* **2005**, *61*, 11692.
35. Petricci, E.; Mugnaini, C.; Radi, M.; Corelli, F.; Botta, M. *J. Org. Chem.* **2004**, *69*, 7880.
36. Leopoldo, M.; Lacivita, E.; Colabufo, N. A.; Contino, M.; Berardi, F.; Perrone, R. *J. Med. Chem.* **2005**, *48*, 7919.
37. Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. *Science* **2010**, *330*, 1091.
38. Urban, J. D.; Clarke, W. P.; von Zastrow, M.; Nichols, D. E.; Kobilka, B.; Weinstein, H.; Javitch, J. A.; Roth, B. L.; Christopoulos, A.; Sexton, P. M.; Miller, K. J.; Spedding, M.; Mailman, R. B. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 1.
39. Albizu, L.; Balestre, M. N.; Breton, C.; Pin, J. P.; Manning, M.; Mouillac, B.; Barberis, C.; Durroux, T. *Mol. Pharmacol.* **2006**, *70*, 1783.
40. Hübner, H.; Haubmann, C.; Utz, W.; Gmeiner, P. *J. Med. Chem.* **2000**, *43*, 756.
41. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
42. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.