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Synthesis and evaluation of homo-bivalent GnRHR ligands

Kimberly M. Bonger,[a] Richard J. B. H. N. van den Berg,[a] Laura H. Heitman,[b] Ad P. IJzerman,[b] Julia Oosterom,[c] Cornelis M. Timmers,[d] Herman S. Overkleeft[a,*] and Gijsbert A. van der Marel[a,*]

[a]*Department of Bio-organic Synthesis, Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands*
[b]*Division of Medicinal Chemistry, LACDR, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands*
[c]*Department of Molecular Pharmacology, N.V. Organon, PO Box 20, 5340 BH, Oss, The Netherlands*
[d]*Department of Medicinal Chemistry, N.V. Organon, PO Box 20, 5340 BH, Oss, The Netherlands*

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Abstract—G protein coupled receptors (GPCRs) are important drug targets in pharmaceutical research. Traditionally, most research efforts have been devoted towards the design of small molecule agonists and antagonists. An interesting, yet poorly investigated class of GPCR modulators comprise the bivalent ligands, in which two receptor pharmacophores are incorporated. Here, we set out to develop a general strategy for the synthesis of bivalent compounds that are projected to bind to the human gonadotropin-releasing hormone receptor (GnRHR). Our results on the dimerisation of a known GnRHR antagonist, with as key step the Huisgen 1,3-cycloaddition, and their ability to bind to and antagonize GnRH-induced GnRHR stimulation, are presented here.

1. Introduction

G protein coupled receptors (GPCRs) are structurally conserved membrane proteins that are characterized by a common seven helix transmembrane motif. GPCRs exert their primary role, signal transduction across the cellular membrane, under the influence of distinct types of (endogenous) ligands, including photons, ions, heterocyclic and peptidic molecules or even proteins. The crucial role GPCRs play in many biological processes and the availability of selective small molecule GPCR agonists and antagonists explain why GPCRs are among the most 'druggable' of all target classes.

In the last decades, increasing evidence has become available that GPCRs assemble in the cell membrane as either homodimers or heterodimers.[1] It is likely that, at least in specific examples, dimerisation is a prerequisite for cellular expression and activity of GPCRs.[1,2]

Several reports have appeared in the literature describing the design of bivalent ligands that target specific GPCRs,[3] including the serotonin,[4] muscarinic[5] and the extensively studied opioid receptors.[6] Often these bivalent ligands exhibit increased potency and selectivity when compared to their monovalent counterparts.

Several hypotheses have been postulated to rationalise the enhanced activity and selectivity observed with some bivalent GPCR ligands. These include the following (represented in Fig. 1): (a) The bivalent ligand interacts simultaneously with two neighbouring receptors. (b) The bivalent ligand occupies both its primary binding site and a secondary, low affinity binding site on the same receptor protein located in close proximity to the primary site. (c) Enhanced binding affinity proceeds through an univalently bound state, the unbound recognition unit being in the locus of neighbouring binding sites (this would be equivalent to a high local concentration of the free pharmacophore). (d) The bivalent ligand induces or stabilises receptor dimerisation.

The gonadotropin-releasing hormone receptor (GnRHR) is a well-studied GPCR, with the decapeptidic gonadotropin-releasing hormone (GnRH) acting as

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* Corresponding authors. Fax: +31 715274307 (H.S.O.); e-mail addresses: h.s.overkleeft@chem.leidenuniv.nl; marel_g@chem.leidenuniv.nl

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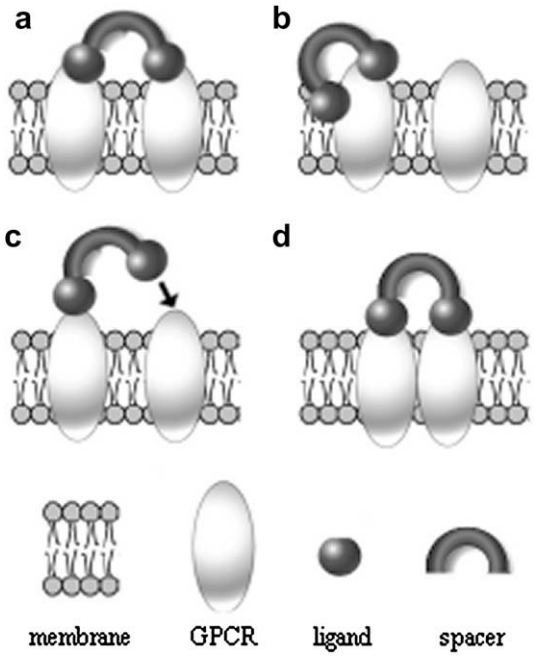


Figure 1. Schematic representation of bivalent ligand binding. (a) binding at neighbouring GPCRs, (b) binding at secondary binding site, (c) increased local concentration of free pharmacophore and (d) induction/stabilisation of GPCR dimerisation.

its endogenous ligand.[7] GnRH is secreted by the hypothalamus and operates as a key regulator in mammalian sexual maturation and reproductive functions. After binding of GnRH to the GnRHR, the release of gonadotropins (LH and FSH) in anterior pituitary gonadotropes is stimulated. The major signal transduction route for the GnRHR is via the Gαq protein which induces release of intracellular calcium. Several types of molecules have been reported as GnRHR modulators, covering peptide-based agonists and antagonists and non-peptidic antagonists.[8] Imidazopyrimidinone **1**[9] (Fig. 2) is a well-documented example of a low molecular weight antagonist with high affinity for the GnRHR. Interestingly, up to this date, no non-peptidic agonist for the GnRHR has been reported.

There is some literature evidence that dimerisation of the GnRHR plays a role in signal transduction.[10,11] Already more than two decades ago, several studies on monomeric and dimeric peptide-based GnRHR modulators were conducted.[10] In these studies, peptide antagonists were modified to allow binding to monoclonal antibodies, resulting in bivalent ligands in which the antibody acts as the spacer. It was observed that ligand dimerisation resulted in GnRHR agonism, which was rather unexpected since the monomeric peptides showed an antagonistic effect on GnRHR signalling. From these studies it was concluded that GnRHR dimerisation is a prerequisite for biological functioning. These observations were later corroborated by studies using genetically engineered cells expressing the GnRHR fused to both red fluorescent protein (GnRHR-RFP)[11] and green fluorescent protein (GnRHR-GFP). When treated with an effective agonist, the GnRHR was shown to aggregate, which was evidenced by enhanced red fluorescence that results from fluorescence resonance energy transfer (FRET) from GnRHR-GFP to GnRHR-RFP.

One of the major scientific challenges in the study of GnRHR dimerisation, as with all GPCRs, is the lack of reliable (bio)structural information. Although GnRHR homology models can be built based on the bovine rhodopsin X-ray structure, the spatial alignment of a membrane-anchored GnRHR dimer cannot be rationalised. In order to study the phenomenon of GnRHR dimerisation in more detail a ligand-based approach, in which two distinct GnRHR pharmacophores are connected by a spacer system of variable length and rigidity, emerges as an appealing option. In the context of a research programme aimed at understanding and modulating GnRHR-mediated signalling, we decided to design a set of well-defined bivalent ligands based on the earlier reported and potent antagonist **1**. With the exception of the antibody dimerised peptide agonists described above, no precedents on dimeric GnRHR ligands have appeared to date. We therefore decided to focus on a strategy towards bivalent ligands that is flexible both with respect to the nature and size of the linker entity and to the site to which the linker is attached to the monovalent ligand.

2. Design and synthesis

The design of our first compound library is outlined in Figure 2 and involves the introduction of a terminal acetylene onto the imidazopyrimidinone ligand **1**, followed by homodimerisation to a bis-functionalized azide spacer by means of the copper-catalyzed 1,3-dipolar Huisgen cycloaddition now commonly referred to as the Click reaction.[12] As spacer entities we have selected a set of ethylene glycols equipped with an azide at both termini for coupling to two acetylene-containing ligand moieties. Because of the lack of structural information on GnRHR dimers we aimed for bivalent molecules in which the pharmacophores are connected by either short (a minimum of 6 atoms) or long (up to 26 atoms) spacers. Bovine rhodopsin has a cylindrical diameter of about 35 Å, as can be determined from the X-ray diffraction structure, and we assume that the GnRHR will have similar geometrical properties. The compounds with short spacers should not be able to bridge to both members of a GnRHR dimer (mechanism A or D, Fig. 1), at least when we assume the most distinct dimer orientation, that is, side by side contact of two hypothetical cylinders to be the relevant dimeric structure. These compounds might provide enhanced pharmacophoric properties by following other mechanistic pathways such as B or C. The dimeric ligand with the longest spacer is estimated to span about 40 Å in its linear conformation which indicates that this compound in theory can bind to two GPCRs. However, it cannot be excluded that a mechanism of GnRHR dimerisation will occur in which the hypothetical 7-TM cylinders are more closely interacting, thus spanning a shorter distance in binding sites than 40 Å. In the latter case, also shorter spacers should allow simultaneous binding of the two pharmacophoric elements.

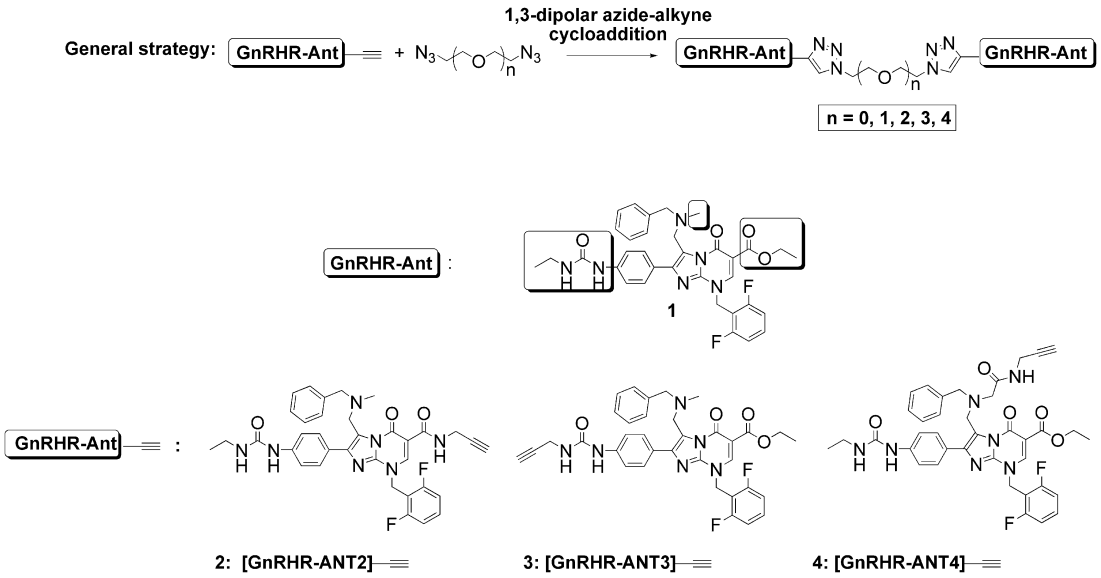


Figure 2. Design of the compound library.

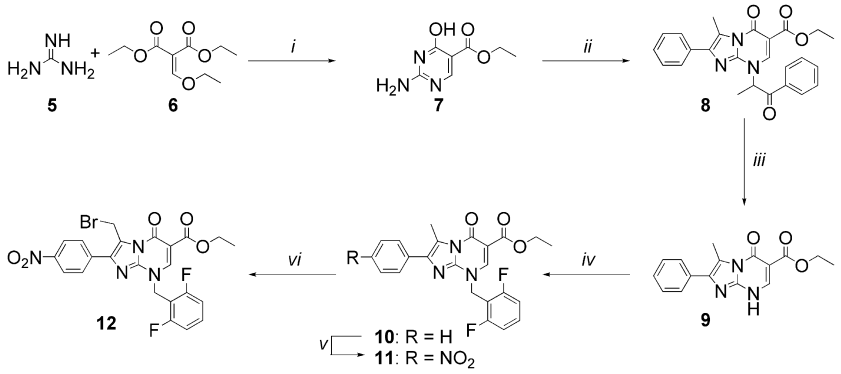
Screening of the literature information[9] on the structure–activity relationships around **1** has revealed three positions within ligand **1** that may be amenable for functionalization without completely compromising antagonistic activity. These are the tertiary, benzylic amine (as long as the pK_a is not largely affected), the urea moiety (of which the ethyl functionality may be substituted) and the ethyl ester position. Based on this information, we decided to synthesise acetylene derivatives **2**, **3** and **4**, and couple these to the bis-azides through the Click reaction. In order to evaluate a potential effect of bivalency, we also prepared the corresponding monovalent ligand-spacer derivatives. We here report the synthesis of this bivalent ligand library. Further, the propensity of our compound library to bind to the GnRHR, relative to lead compound **1**, and to antagonize GnRH-mediated GnRHR signalling, is presented.

The synthesis of acetylene derivatives **2**, **3** and **4** is based on the common key intermediate **12** (Scheme 1), which is in fact an advanced intermediate in the literature synthesis of ligand **1**.[9] Following this procedure, but with some adaptations, compound **12** was readily prepared as follows (Scheme 1). Guanidine **5** and diethyl 2-(ethoxymethylene)malonate **6** were condensed to form pyrimidine **7** in 79% yield. Reaction of **7** with α-bromopropiophenone gave a mixture of O- and N-alkylated products of which **8**, the major regioisomer, was isolated after crystallization in 39% yield. Cleavage of the phenacyl moiety in **8** with zinc in acetic acid gave phenylimidazopyrimidinone **9**. Subsequent alkylation with 2,6-difluorobenzyl bromide and subsequent aromatic substitution with sodium nitrate in concentrated sulfuric acid provided compound **11** which was further brominated to obtain key intermediate **12**.

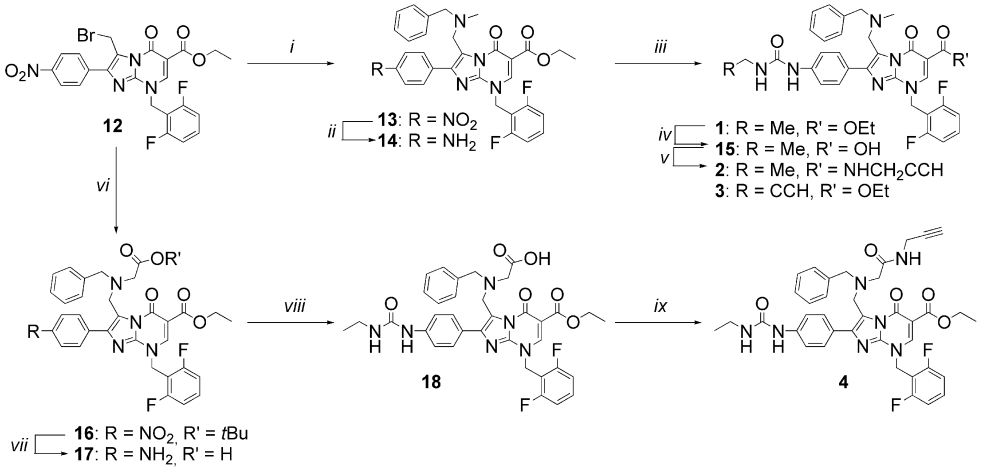
Compound **12** was then aminated with *N*-methyl-*N*-benzylamine after which the nitro functionality in **13** was reduced to the amine using iron in an acidic ethanol solution (Scheme 2). Subjecting the obtained amine **14** to ethyl isocyanate in pyridine resulted in GnRHR antagonist **1**. Saponification of the ethyl ester in **1** and subsequent coupling with propargyl amine under standard peptide condensation conditions gave acetylene derivative **2** in 63% yield over the two steps. Acetylene functionalized ligand **3** was obtained in one step from amine **14** by reaction with propargyl isocyanate, which was freshly prepared from propargyl amine and diphosgene (71% yield).

The synthesis of **4** was accomplished as follows. Nucleophilic displacement of the allylic bromide in **12** by *tert*-butyl 2-(benzylamino)acetate (DiPEA, THF) gave tertiary amine **16** almost quantitatively. At this stage, reduction of the nitro functionality using the conditions previously applied for the transformation of **13** to **14** (iron, acidic ethanol) proved abortive, since next to producing the desired aniline also transesterification of the *tert*-butyl ester to the corresponding ethyl ester was observed. Switching to the iron/dichloromethane/trifluoroacetic acid reagent system however gave simultaneous reduction of the nitrophenyl to the aniline and acidolysis of the *tert*-butyl ester, to produce zwitterionic compound **18** in excellent yield. Treatment of **18** with ethyl isocyanate in pyridine, followed by condensation of the carboxylic acid with propargylamine (BOP, DiPEA, DMF), gave target compound **4** in 38% over the two steps.

Having the acetylene functionalized ligands **2**, **3** and **4** in hand, we focused on the synthesis of bis-azides **21a–e** and the entailed Click reaction. The required bis-azide **21a** was prepared from dichloroethane and stored in solution.[13] The bis-azides **21b–e** were prepared from the corresponding ethyleneglycol derivatives **19b–e** by (1) transformation to bis(paratoluenesulfonate) esters **20b–e** and (2) displacement of the sulfonate esters by azide ion. The synthesis of the bivalent ligands and their monovalent ligand-spacer counterparts is presented in Scheme 3. As an example, acetylene **2** is reacted with



Scheme 1. Reagents and conditions: (i) NaOEt, EtOH, rt, 4 d, 79%; (ii) α-mromopropiophenone, KI, K_2CO_3, DMF, rt, 5 d, 39%; (iii) Zn, HOAc, 80 °C, 6 h, 88%; (iv) 2,6-difluorobenzyl bromide, KI, K_2CO_3, DMF, rt, 18 h, 70%; (v) $NaNO_3$, concd H_2SO_4, 0 °C, 4 h, 82%; (vi) NBS, AIBN, CCl_4, 80 °C, 2 h, 83%.

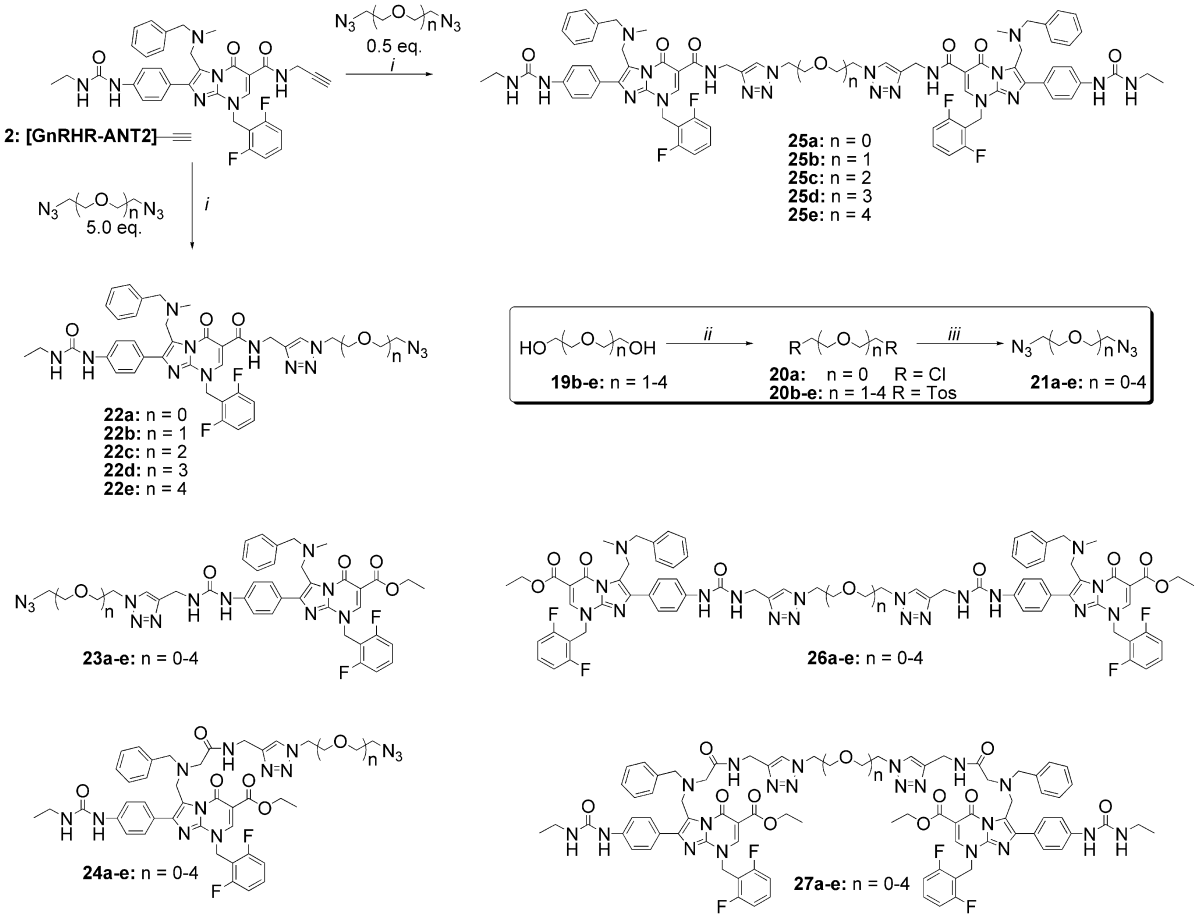


Scheme 2. Reagents and conditions: (i) *N*-Methyl-*N*-benzylamine, DiPEA, THF, rt, 18 h, 97%; (ii) Fe, concd HCl, EtOH, rt, 4 h, 85%; (iii) ethyl isocyanate, pyridine, rt, 18 h, 88% (R = Me) or propargylamine, diphosgene, EtOAc, reflux, 4 h, then **14** in pyridine, rt, 18 h, 71% (R = CCH); (iv) 1 M LiOH, H_2O/MeOH/THF (12:2:1 v/v/v), rt, 1 h, 83%; (v) propargylamine, BOP, DiPEA, DMF, rt, 18 h, 75%; (vi) *tert*-butyl 2-(benzylamino)acetate, DiPEA, THF, rt, 18 h, 97%; (vii) Fe, TFA/DCM (1:1 v/v), rt, 18 h, 97%; (viii) ethyl isocyanate, pyridine, rt 18 h, 71%; (ix) propargylamine, Bop, DiPEA, DMF, rt, 18 h, 54%.

each of the five bis-azides **21a**–**e**, with $n = 0$, 1, 2, 3 and 4, under the agency of a catalytic amount of copper sulfate and sodium ascorbate in a mixture of *tert*-butanol, acetonitrile and water. Employing a fivefold excess of the bis-azide **21a**–**e** showed complete conversion of **2** (RP-HPLC) and afforded the monosubstituted derivatives **22a**–**e** as their TFA salt in yields ranging from 30% to 47% after preparative HPLC purification. The bivalent ligands were obtained by applying essentially the same conditions, but with 0.5 equivalents of bis-azides **21a**–**e**, giving target compounds **25a**–**e** (13–36%). In the same way, and with comparable efficiency, monosubstituted compounds **23a**–**e** and **24a**–**e**, as well as bivalent compounds **26a**–**e** and **27a**–**e**, were prepared.

3. Biological evaluation

The bivalent ligands **25a**–**e**, **26a**–**e** and **27a**–**e** were tested on their ability to bind to the GnRH receptor and to antagonize its stimulation by GnRH. As reference compounds, known antagonist **1**, the three acetylene derivatives **2**, **3** and **4** and the mono-functionalized azides **22a**–**e**, **23a**–**e** and **24a**–**e** were included in the assays. The results are summarized in Table 1. For the functional assay we made use of Chinese hamster ovary (CHO) cells, stably transfected with the GnRHR and equipped with the calcium sensitive NFAT luciferase reporter gene. To measure antagonistic efficacy, the GnRHR cells were stimulated with a submaximal (EC_{80}) concentration of the agonist in the presence of several concentrations of the test compounds. Antagonistic activity is detected as a decrease of the luminescence signal upon addition of the luciferase substrate. The pIC_{50} ($-\log IC_{50}$) values of the compounds in the antagonistic assay are listed in column A of Table 1. The binding affinity of the compounds was monitored in a displacement assay on membrane fractions of GnRHR-expressing CHO cells with the radioactive GnRHR agonist [^{125}I]triptorelin. We first performed a single-point assay for all compounds, in which the percentage of [^{125}I]triptorelin displacement at 1 μM compound was determined (column B). Furthermore,



Scheme 3. Reagents and conditions: (i) $CuSO_4$, sodium ascorbate, *t*-BuOH/MeCN/H_2O (2:1:1 v/v/v), 60 °C, 3 h; (ii) KOH, TsCl, DCM, 0 °C, 3 h, >95%; (iii) NaN_3, TBAI, DMF, 80 °C, 18 h, >95%.

for a small number of ligands, including the reference compounds and a few bivalent ones, pK_i values were determined (column C). Introducing bivalency might affect the intrinsic activity of the compounds and thus change their profile from an antagonist into an agonist. Additional assays performed with all compounds in an agonistic set-up, that is, when tested alone in the luciferase reporter gene assay, did not provide any actives (data not shown).

The combined results of both antagonistic and radioligand assays indicate that lead compound **1** exhibits more antagonistic potency than all newly synthesised compounds. pIC_{50} values (column A in Table 1) are always lower than the available corresponding pK_i values (column C). This is not surprising since a high concentration of GnRH is also present in the functional assay. Interestingly, monomeric analogues **3** and **4** show a comparable binding affinity (pK_i) as ligand **1**, but they are less active in the functional assay. Replacement of the ethyl ester in **1** with a propargyl amide, as in compound **2**, strongly reduces antagonistic potency. None of the compounds incorporating this modification showed a significant antagonistic effect in our functional assay at concentrations up to 10 μM (pIC_{50} < 5.0). They do, however, displace radioligand binding to some extent at a concentration of 1 μM (Table 1, column B). The results of the monovalent and bivalent ligands based on acetylene derivatives **3** and **4** have higher affinities. In the series based on **3** the 10-fold increase in antagonistic potency going from monovalent ligand **23d** to bivalent ligand **26d** is noteworthy. The series based on acetylene derivative **4** suggests that the monovalent ligands are slightly more active than their bivalent counterparts. However, the difference in this series is too marginal to allow interpretation with respect to potential bivalent modes of binding.

The most obvious conclusion from the here presented results is that, without exception, all newly synthesised ligands, both monomeric and dimeric, are poor GnRHR antagonists compared to parent compound **1**. With some exceptions, this also holds true for their GnRHR affinities in our binding assay. The picture becomes less clear when we compare binding affinity and/or antagonistic ability of the three sets of ligands. Related studies on other GPCRs revealed that bivalent pharmacophores linked through both short spacers (leading to molecules that are unlikely to bind to two individual GPCRs) and long spacers (molecules that in theory can bind to two individual GPCRs) can lead to enhanced selectivity and potency with respect to subunit type.[5,6] Also, the intrinsic activity of the bivalent molecules may alter with different spacer lengths.[4,10] The data we have amassed

Table 1. Functional and binding properties to GnRHR for the mono- and bivalent ligands

Compound	Spacer length (*n*)	A pIC_{50} ± SEM[a]	B % displacement[b]	C pK_i ± SEM[b]
1		7.15 ± 0.16	99 ± 1	8.32 ± 0.05
2		5.15 ± 0.33	85 ± 1	6.79 ± 0.08
22a	0	<5.0	78 ± 2	n.d.
22b	1	<5.0	68 ± 2	n.d.
22c	2	<5.0	81 ± 3	n.d.
22d	3	<5.0	69 ± 1	n.d.
22e	4	<5.0	70 ± 2	n.d.
25a	0	<5.0	26 ± 2	n.d.
25b	1	<5.0	64 ± 2	n.d.
25c	2	<5.0	73 ± 5	n.d.
25d	3	<5.0	86 ± 1	n.d.
25e	4	<5.0	85 ± 2	n.d.
3		6.17 ± 0.18	98 ± 3	8.87 ± 0.15
23a	0	5.59 ± 0.19	76 ± 2	n.d.
23b	1	5.44 ± 0.34	85 ± 2	n.d.
23c	2	5.62 ± 0.23	84 ± 2	n.d.
23d	3	5.52 ± 0.16	84 ± 4	n.d.
23e	4	6.17 ± 0.03	90 ± 4	n.d.
26a	0	5.72 ± 0.26	68 ± 1	n.d.
26b	1	5.50 ± 0.47	88 ± 1	n.d.
26c	2	6.02 ± 0.39	94 ± 6	7.63 ± 0.09
26d	3	6.45 ± 0.07	90 ± 3	7.53 ± 0.20
26e	4	5.87 ± 0.24	89 ± 1	n.d.
4		6.00 ± 0.24	98 ± 0	8.28 ± 0.02
24a	0	6.14 ± 0.01	74 ± 3	n.d.
24b	1	6.28 ± 0.10	66 ± 2	n.d.
24c	2	6.33 ± 0.06	83 ± 2	n.d.
24d	3	6.14 ± 0.07	76 ± 3	n.d.
24e	4	6.08 ± 0.06	78 ± 4	n.d.
27a	0	5.72 ± 0.21	65 ± 9	n.d.
27b	1	5.81 ± 0.22	71 ± 4	n.d.
27c	2	6.08 ± 0.09	84 ± 8	7.23 ± 0.12
27d	3	5.88 ± 0.26	71 ± 4	7.16 ± 0.08
27e	4	6.51 ± 0.00	89 ± 0	6.71 ± 0.02

n.d., not determined.

[a] CHO cells that stably express the GnRHR were stimulated with a submaximal (EC_{80}) concentration of GnRH and were incubated with increasing concentrations of the compounds. The pIC_{50} value is the negative logarithm of the concentration of compound needed to inhibit the agonistic response by 50%.

[b] Membranes of GnRHR-expressing CHO cells were incubated with the radioactive GnRHR agonist [^{125}I]triptorelin. The percentage of [^{125}I]triptorelin displacement by incubating the fractions with a single concentration of 1 μM of test compound (column B); pK_i values were determined (column C) by incubating the membranes with increasing concentrations of test compound. The pK_i value is calculated based on the concentration of compound needed to displace 50% of radioligand [^{125}I]triptorelin.

with our set of bivalent compounds however do not allow us to speculate on a preferred mode of action in GPCR signalling. Changes in the attachment sites of the spacer and variations in the spacer moiety itself should provide more structure–activity information. Moreover, also hetero-bivalent ligands, incorporating two distinct GnRHR pharmacophores, are required. The synthetic strategy presented here, allowing sequential introduction of two ligands, seems to be suitable for the preparation of libraries with broader diversity, provided that the corresponding acetylene-modified ligands and azide-containing spacers of the desired nature are available. Furthermore, additional (bio)chemical experiments concerning compounds with significantly enhanced potency compared to their monomeric counterpart, such as **26d**, are vital in order to elaborate on the potential modes of binding to GnRHR as delineated in Figure 1. Research along these lines is now conducted in our laboratories.

4. Experimental

4.1. GnRHR luciferase reporter gene assay

Chinese hamster ovary, CHO-K1, cells with stable expression of the human gonadotropin-releasing hormone receptor (GnRH-R) and nuclear factor activated T-cell luciferase reporter gene were grown to 80–90% confluence in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% w/v foetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin and 400 μg/ml geniticin. On the day of the assay, cells were washed twice with phosphate-buffered saline and then harvested with cell dissociation solution. Cells were resuspended in assay medium consisting of DMEM supplemented with 1 mg/L insulin and 5 mg/L apo-transferrin and 3% v/v DMSO. Then, 10 μl cell suspension containing 7500 cells was added to each well of a 384-well white culture plate.

Thereafter, 10 μl of test compound was added at 10 concentrations ranging from final concentration of 10 μM to 0.3 nM with half log intervals. Compounds were allowed to pre-incubate with cells for 30 min followed by addition of 10 μl agonist GnRH at a final concentration of 3 nM which produces approximately 80% of the maximal effect (EC80) when given alone. After 4-h stimulation, 15 μl of luclite® was added to each well for detection of luciferase protein and plates were left at room temperature for 1 h in the dark. Finally, the luminescence signal was quantified on the TopCount® Microplate Scintillation and Luminescence Counter.

4.2. Radioligand binding assays

Ganirelix was provided by Organon (Oss, The Netherlands). [^{125}I]Triptorelin (specific activity 2200 Ci mmol^{-1}) was purchased from Perkin-Elmer Life Sciences B.V. (Groningen, The Netherlands). CHO cells stably expressing the human GnRH receptor were provided by Organon (Oss, The Netherlands). All other chemicals and cell culture materials were obtained from standard commercial sources.

CHO (Chinese hamster ovary) cells expressing the wild-type human GnRH receptor were grown in Ham's F12 medium containing 10% bovine calf serum, streptomycin (100 μg mL^{-1}), penicillin (100 IU mL^{-1}) and G418 (0.4 mg mL^{-1}) at 37 °C in 5% CO_2. The cells were subcultured twice weekly at a ratio of 1:20. For membrane preparation the cells were subcultured 1:10 and transferred to large 14-cm diameter plates. For membrane preparation the cells were detached from the plates by scraping them into 5 mL PBS, collected and centrifuged at 1400*g* (3000 rpm) for 5 min. Pellets derived from 30 plates were pooled and resuspended in 30 mL of ice-cold 50 mM Tris–HCl buffer supplemented with 2 mM $MgCl_2$, pH 7.4. An UltraThurrax was used to homogenize the cell suspension. Membranes and the cytosolic fraction were separated by centrifugation at 100,000*g* (31,000 rpm) at 4 °C for 20 min. The pellet was resuspended in 10 mL of the Tris buffer and the homogenization and centrifugation steps were repeated. Tris buffer (10 mL) was used to resuspend the pellet and the membranes were stored in 500 μL aliquots at −80 °C. Membrane protein concentrations were measured using the BCA (bicinchoninic acid) method.[14]

On the day of the assay membrane aliquots containing 20 μg protein were incubated in a total volume of 100 μL assay buffer (50 mM Tris–HCl, pH 7.4, supplemented with 2 mM $MgCl_2$ and 0.1% BSA) at 22 °C for 45 min. Displacement experiments were performed using five concentrations of competing ligand in the presence of 30,000 cpm [^{125}I]Triptorelin. Non-specific binding was determined in the presence of 1 μM Ganirelix and represented approximately 20% of the total binding. Total binding was determined in the presence of buffer and was set at 100% in all experiments, whereas non-specific binding was set at 0%. Incubations were terminated by dilution with ice-cold Tris–HCl buffer. Separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B filters presoaked with 0.25% PEI for 1 h using a Brandel harvester. Filters were subsequently washed three times with ice-cold wash buffer (50 mM Tris–HCl, pH 7.4, supplemented with 2 mM $MgCl_2$ and 0.05% BSA). Filter-bound radioactivity was determined in a γ-counter.

All data were analyzed using the non-linear regression curve-fitting program GraphPad Prism v. 4 (GraphPad Software Inc., San Diego, CA, USA). Inhibitory binding constants (K_i values) were derived from the IC_{50} values according to $K_i = IC_{50}/(1 + [C]/K_d)$ where [C] is the concentration of the radioligand and K_d its dissociation constant.[15] The K_d value (1.1 nM) of [^{125}I]Triptorelin was obtained by computer analysis of saturation curves (data not shown). All values obtained are means of at least three independent experiments performed in duplicate.

4.3. Chemical procedures

Reactions were executed at ambient temperatures unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere. All solvents were removed by evaporation under reduced pressure. Residual water was removed from starting compounds by repeated coevaporation with dioxane, toluene or dichloroethane. Reactions were monitored by TLC analysis using silica gel coated plates (0.2 mm thickness) and detection by 254 nm UV-light or by either spraying with a solution of $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (25 g/L) or $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C. Column chromatography was performed on silica gel (40–63 μm). The ^{1}H and ^{13}C-APT NMR spectra, ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMQC experiments were performed on a 200/50.1 MHz, 300/75.1 MHz, 400/100 MHz, 500/125 MHz or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants (J) are given in Hz. All presented ^{13}C-APT spectra are proton decoupled. IR spectra were recorded on an apparatus fitted with a single bounce diamond crystal ATR-element and are reported in cm^{-1}. For LC/MS analysis, a HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C_{18} column (4.6 mmD × 250 mmL, 5μm particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq TFA and coupled to a mass instrument with an electronspray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC-system equipped with a semi-preparative C_{18} column (5 μm C_{18}, 10 Å, 150 × 21.2 mm) was used. The applied buffers were A: 5% MeCN/H_2O + 0.1% TFA and B: MeCN. High resolution mass spectra were recorded on a mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10%, capillary temperature 275 °C) with resolution R = 100,000 at m/z 400. The high resolution mass spectrometer was calibrated prior to measurements with a calibration solution (caffeine, MRFA, Ultramark 1621).

4.3.1. Ethyl 2-amino-4-hydroxypyrimidine-5-carboxylate (7). To a solution of guanidine carbonate (18.0 g, 200 mmol) in ethanol (300 mL) were added sodium

ethoxide (13.6 g, 200 mmol) and diethyl ethoxymethylene malonate (40.0 mL, 200 mmol). After stirring for 4 d the volatiles were removed, the residue dissolved in water (500 mL) and neutralized with aqueous HCl (2 M). The titled compound was collected by filtration as a white powder (29.0 g, 158 mmol, 79%). ESI-MS (*m*/*z*): 183.9 $[M+H]^+$, 206.0 $[M+Na]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H,), 4.11 (q, 2H, J = 7.2 Hz), 1.21 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9 (C), 163.0 (CH), 158.7, 158.2, 103.6 (3× C), 58.9 (CH_2), 13.8 (CH_3).

4.3.2. Ethyl 5,8-dihydro-3-methyl-5-oxo-8-(1-oxo-1-phenylpropan-2-yl)-2- phenylimidazo[1,2-*a*]pyrimidine-6-carboxylate (8). To a solution of ethyl ester **7** (15.0 g, 81.9 mmol) in DMF (280 mL) were added potassium carbonate (28.3 g, 204.8 mmol), potassium iodide (13.6 g, 81.9 mmol) and 2-bromopropiophenone (30.6 mL, 204.8 mmol). After stirring for 5 d the volatiles were removed, the residue dissolved in $CHCl_3$ (500 mL) and washed with water (3× 200 mL). The organic phase was dried (Na_2SO_4) and concentrated. Flash column chromatography (EtOAc) of the residue afforded crystals which were recrystallized from EtOAc and petroleum light ether. Yield 136.0 g (31.7 mmol, 39%). TLC R_f = 0.7 (MeOH/DCM, 1:19, v/v), ESI-MS (*m*/*z*): 430.1 $[M+H]^+$, 452.1 $[M+Na]^+$. ^1H NMR (200 MHz, $CDCl_3$): δ 8.54 (s, 1H), 8.14 (d, 2H, J = 8.6 Hz), 7.71–7.50 (m, 5H), 7.45–7.28 (m, 3H), 6.83 (q, 1H, J = 7.3 Hz), 4.42 (q, 2H, J = 7.3 Hz), 2.89 (s, 3H), 1.82 (d, 3H, J = 7.3 Hz), 1.21 (t, 3H, J = 6.8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 195.6, 164.2, 156.6 (3× C), 144.5 (CH), 141.0, 137.6 (2× C), 134.4 (CH), 133.8, 133.3, 129.0, 128.7, 128.3, 128.0, 127.4 (11× CH), 122.0, 101.2 (2× C), 61.1 (CH_2), 57.0 (CH), 16.5, 14.3, 12.9 (3× CH_3).

4.3.3. Ethyl 5,8-dihydro-3-methyl-5-oxo-2-phenylimidazo[1,2-*a*]pyrimidine-6-carboxylate (9). To a solution of **8** (5.00 g, 11.6 mmol) in acetic acid (120 mL) was added activated zinc (12.1 g, 185 mmol). The mixture was stirred at 80 °C for 6 h, filtered through Celite and the filtrate concentrated. The residue was crystallized by the addition of water (300 mL). The crystals were collected and recrystallized ($CHCl_3$/Et_2O/petroleum light ether) to yield white powder (3.05 g, 10.3 mmol, 88%). TLC R_f = 0.45 (MeOH/DCM, 1:19 v/v). ESI-MS (*m*/*z*): 297.9 $[M+H]^+$, 595.2 $[2M+H]^+$. ^1H NMR (600 MHz, $CDCl_3$/CD_3OD): δ 8.68 (s, 1H), 7.54–7.47 (m, 5H), 4.34 (q, 2H, J = 7.3 Hz), 2.86 (s, 3H), 1.39 (t, 3H, J = 6.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$/CD_3OD): δ 165.0, 158.0 (2× C), 157.3 (CH), 147.3, 128.5, 128.3, 127.9, 127.3 (7× CH), 118.7, 101.3 (2× C), 59.7 (CH_2), 13.3, 10.8 (2× CH_3).

4.3.4. 8-(2,6-Difluorobenzyl)-5,8-dihydro-3-methyl-5-oxo-2-phenylimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (10). To a solution of **9** (6.00 g, 20.2 mmol) in DMF (220 mL) were added potassium carbonate (3.07 g, 22.2 mmol), potassium iodide (1.67 g, 10.0 mmol) and bromo-2,6-difluorotoluene (4.60 g, 22.2 mmol). The mixture was stirred for 18 h, after which the volatiles were removed. The residue was dissolved in a mixture of EtOAc and water (500 mL, 1:1 v/v). The water layer was extracted with EtOAc (2× 200 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. The residue was crystallized from $CHCl_3$ and Et_2O to yield 6.08 g (14.3 mmol, 70%) of off-white crystals. TLC R_f = 0.75 (toluene/EtOAc, 1:1 v/v). ESI-MS (*m*/*z*): 424.0 $[M+H]^+$, 847.5 $[2M+H]^+$. ^1H NMR (600 MHz, $CDCl_3$): δ 8.36 (s, 1H), 7.68 (d, 2H, J = 7.5 Hz), 7.43 (t, 2H, J = 7.5 Hz), 7.39–7.37 (m, 1H), 7.33 (t, 1H, J = 7.4 Hz), 6.99 (t, 2H, J = 7.9 Hz), 5.50 (s, 2H), 4.37 (q, 2H, J = 7.0 Hz), 2.90 (s, 3H), 1.38 (t, 3H, J = 7.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.2 (C), 161.7 (dd, 2× C, J_1 = 260.5 Hz, J_2 = 6.0 Hz), 156.9 (C), 146.2 (CH), 140.6, 138.0, 133.4 (3× C), 131.6 (t, 1× CH, J = 10.5 Hz), 128.4, 128.0, 127.4 (5× CH), 121.8 (C), 111.8 (dd, 2× CH, J_1 = 21.0 Hz, J_2 = 4.5 Hz), 109.6 (t, 1× C, J = 18.0 Hz), 100.9 (C), 61.11, 42.9 (2× CH_2), 14.3, 12.21 (2× CH_3).

4.3.5. 8-(2,6-Difluorobenzyl)-5,8-dihydro-3-methyl-2-(4-nitrophenyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (11). To a cooled (0 °C) solution of **10** (2.01 g, 4.74 mmol) in concentrated sulfuric acid (20 mL) was added over a period of 30 min sodium nitrate (0.40 g, 4.74 mmol). After 4 h, the reaction mixture was poured on ice (100 mL) and $CHCl_3$ was added (150 mL). The organic layer was separated and the water layer extracted with $CHCl_3$ (3× 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was crystallized from $CHCl_3$ and Et_2O to yield 1.83 g (3.91 mmol, 82%) of yellow crystals. TLC R_f = 0.8 (toluene/EtOAc, 1:1 v/v). ESI-MS (*m*/*z*): 469.2 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.32–7.85 (m, 4H), 7.48–6.95 (m, 3H), 5.51 (s, 2H), 4.38 (q, 2H, J = 7.0 Hz), 2.96 (s, 3H), 1.39 (t, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7 (C), 161.7 (dd, 2× C, J_1 = 250.0 Hz, J_2 = 7.0 Hz), 156.7 (C), 147.0 (CH), 146.6, 141.1, 140.1, 135.9 (4× C), 131.8 (t, 1× CH, J = 11.0 Hz), 128.3 (2× CH), 124.1 (C), 123.6 (2× CH), 112.2 (d, 2× CH, J = 15.0 Hz), 111.7 (t, 1× C, J = 18.0 Hz), 101.3 (C), 61.3, 43.2 (2× CH_2), 14.3, 12.5 (2× CH_3).

4.3.6. 3-Bromomethyl-8-(2,6-difluorobenzyl)-5,8-dihydro-2-(4-nitrophenyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (12). To a solution of pyrimidine derivative **11** (4.68 g, 10.0 mmol) in carbon tetrachloride (500 mL) were added *N*-bromosuccinimide (1.96 g, 11.0 mmol) and 2,2′-azobis(isobutyronitrile) (0.33 g, 2.0 mmol). The reaction mixture was stirred at 80 °C until a clear solution was observed (2–3 h). The volatiles were removed and the residue was dissolved in $CHCl_3$ (200 mL). The organic layer was washed with aqueous $NaHCO_3$ (200 mL) and the water layer was extracted with $CHCl_3$ (3× 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was crystallized from DCM and Et_2O to yield 4.55 g (83%) of brown crystals. TLC R_f = 0.4 (toluene/EtOAc, 1:1 v/v). ESI-MS (*m*/*z*): 547.2, 549.1 $[M+H]^+$, 569.1, 570.9 $[M+Na]^+$. ^1H NMR (500 MHz, MeOD/$CDCl_3$): δ 8.53 (s, 1H), 8.36 (d, 2H, J = 8.8 Hz), 8.05 (d, 2H, J = 8.8 Hz), 7.52–7.35 (m, 1H), 7.02 (t, 2H, J = 8.0 Hz), 5.54 (s, 2H), 5.30 (s, 2H), 4.40 (q, 2H, J = 6.6 Hz), 1.39 (t, 3H, J = 5.1 Hz). ^{13}C NMR (500

MHz, MeOD/$CDCl_3$): δ 163.1 (C), 161.4 (dd, 2× C, J_1 = 250.0 Hz, J_2 = 6.3 Hz), 156.0 (C), 147.2 (CH), 147.1, 141.6, 140.1, 138.7 (4× C), 131.6 (t, 1× CH, J = 10.0 Hz), 128.4, 123.5 (4× CH), 123.2 (C), 112.1 (d, 2× CH, J = 21.3 Hz), 109.3 (t, 1× C, J = 18.8 Hz), 101.1 (C), 62.4, 61.2, 43.4 (3× CH_2), 13.8 (CH_3).

4.3.7. 8-(2,6-Difluorobenzyl)-5,8-dihydro-3-(*N*-methyl-*N*-benzylaminomethyl)-2-(4-nitrophenyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (13). To a cooled (0 °C) solution of bromide **12** (2.74 g, 5.0 mmol) in THF (50 mL) were added benzylmethylamine (0.77 mL, 6.0 mmol) and DiPEA (8.5 mL, 50 mmol). The reaction mixture was stirred for 18 h at ambient temperature after which the volatiles were removed. The residue was dissolved in $CHCl_3$ (200 mL) and the organic layer washed with aqueous $NaHCO_3$ (10%, 200 mL). The water layer was extracted with $CHCl_3$ (3× 100 mL) and the combined organic layers dried (Na_2SO_4) and concentrated. The crude product was purified by silica gel column chromatography (20% to 50% EtOAc in toluene) to afford 2.84 g (4.83 mmol, 97%) as an off-white solid. TLC R_f = 0.7 (toluene/EtOAc, 1:1 v/v). ESI-MS (*m*/*z*): 588.3 $[M+H]^+$. ^1H NMR (600 MHz, $CDCl_3$): δ 8.50 (s, 1H), 8.32–8.27 (m, 4H), 7.43–7.38 (m, 1H), 7.26–7.21 (m, 5H), 7.02 (t, 2H, J = 8.4 Hz), 5.52 (s, 2H), 4.45–4.37 (m, 4H), 3.69 (s, 2H), 2.72 (s, 3H), 1.40 (t, 3H, J = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 163.8 (C), 161.8 (dd, 2× C, J_1 = 250.5 Hz, J_2 = 6.0 Hz), 156.5, 146.9 (2× C), 146.6 (CH), 141.5, 139.8, 139.2, 139.1 (4× C), 131.8 (t, 1× CH, J = 10.5 Hz), 129.1, 129.0, 128.9, 128.8, 128.1, 126.9 (8× CH), 126.0 (C), 123.7 (CH), 111.9 (dd, 2× CH, J_1 = 21.0 Hz, J_2 = 4.5 Hz), 109.5 (t, 1× C, J = 18.0 Hz), 101.6 (C), 61.3 (2× CH_2), 49.7, 43.6 (2× CH_2), 41.6, 14.4 (2× CH_3).

4.3.8. 2-(4-Aminophenyl)-8-(2,6-difluorobenzyl)-5,8-dihydro-3-(*N*-methyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (14). To a cooled (0 °C) solution of nitro derivative **13** (2.65 g, 4.5 mmol) in EtOH (50 mL) were added iron powder (1.26 g, 22.5 mmol) and hydrochloric acid (37%, 26.3 mL, 315 mmol). After 4 h, aqueous $NaHCO_3$ (10% 300 mL), $CHCl_3$ (300 mL) and Hyflo were added. The mixture was filtered, the organic layer was separated and the water layer extracted with $CHCl_3$ (3× 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Column chromatography of the residue (10–50% EtOAc in toluene) afforded red brown crystals (2.12 g, 3.8 mmol, 85%). TLC R_f = 0.5 (DCM/MeOH, 9:1, v/v). ESI-MS (*m*/*z*): 558.1 $[M+H]^+$. ^1H NMR (200 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.86 (d, 2H, J = 8.8 Hz), 7.46–7.11 (m, 6H), 6.99 (t, 2H, J = 8.0 Hz), 6.76 (d, 2H, J = 8.8 Hz), 5.50 (s, 2H), 4.38 (q, 2H, J = 7.3 Hz), 4.31 (s, 2H), 3.65 (s, 2H), 2.17 (s, 3H), 1.38 (t, 3H, J = 7.3 Hz). ^{13}C NMR (200 MHz, $CDCl_3$): δ 164.2, (C), 161.6 (d, 2× C, J = 257.8), 156.4, 146.2 (2× C), 145.4 (CH), 141.8, 141.1, 139.7, 138.9 (4× C), 131.4 (t, 1× CH, J = 10.6 Hz), 129.8, 129.0, 127.9, 126.5 (9× CH), 123.7, 122.0 (2× C), 114.9 (CH), 111.9 (t, 1 × CH, J = 10.5 Hz), 101.4 (C), 61.1, 50.4, 43.1 (4× CH_2), 41.1, 14.3 (2× CH_3).

4.3.9. 8-(2,6-Difluorobenzyl)-5,8-dihydro-2-(4-ethylaminocarbonylaminophenyl)-3-(*N*-methyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (1). To a solution of **14** (0.50 g, 0.89 mmol) in pyridine (16 mL) was added ethyl isocyanate (0.35 mL, 0.45 mmol). After 18 h the volatiles were removed and the residue was dissolved in DCM (50 mL). The solution was washed with aqueous $NaHCO_3$ (10%, 25 mL), dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (0–15% MeOH in DCM). Recrystallization of the solid (DCM, petroleum light ether) afforded the titled compound as an off-white solid (0.49 g, 0.78 mmol, 88%). TLC R_f = 0.6 (DCM/MeOH, 9:1, v/v). ESI-MS (*m*/*z*): 629.5 $[M+H]^+$, 1257.8 $[2M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 8.49 (s, 1H), 8.18 (s, 1H), 7.91 (d, 2H, J = 8.5 Hz), 7.49 (d, 2H, J = 8.5 Hz), 7.42–7.36 (m, 1H), 7.23–7.10 (m, 5H), 7.00 (t, 2H, J = 8.0 Hz), 5.90 (s, 1H), 5.50 (s, 2H), 4.36 (q, 2H, J = 7.5 Hz), 4.30 (s, 2H), 3.62 (s, 2H), 3.30–3.24 (m, 2H), 2.15 (s, 3H), 1.38 (t, 3H, J = 6.6 Hz), 1.16 (t, 3H, J = 7.3 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.2 (C), 161.5 (dd, 2× C, J_1 = 250.0 Hz, J_2 = 7.5 Hz), 156.3, 155.2 (2× C), 145.5 (CH), 140.7, 140.4, 139.5, 138.9 (4× C), 130.9 (t, 1× CH, J = 10.0 Hz), 128.4, 128.1, 127.2, 126.7, 125.9 (7× CH), 125.8, 121.7 (2× C), 117.2 (2× CH), 111.2 (dd, 2× CH, J_1 = 20.0 Hz, J_2 = 3.8 Hz), 109.3 (t, 1× C, J = 18.8 Hz), 100.4 (C), 60.4, 60.2, 49.7, 42.1 (4× CH_2), 40.5 (CH_3), 33.8 (CH_2), 14.9, 13.7 (2× CH_3). HRMS *m*/*z* calcd for $C_{34}H_{34}N_6O_4F_2+H^+$: 629.26824, obsd 629.26963.

4.3.10. 8-(2,6-Difluorobenzyl)-5,8-dihydro-2-(4-ethylaminocarbonylaminophenyl)-3-(*N*-methyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid (15). To a solution of ethyl acid **1** (1.11 g, 1.77 mmol) in THF (12 mL) were added MeOH (2 mL), water (1 mL) and aqueous LiOH (1 M, 2.66 mmol, 2.66 mL). After 1 h the reaction was complete and the reaction mixture was neutralized with 1 M HCl and the volatiles evaporated. The crude product was purified by column chromatography on silica gel (5:94:1 to 30:65:5 MeOH/DCM/H_2O) to yield an off-white solid (0.88 g, 1.5 mmol, 83%). TLC analysis (1:4 MeOH/DCM) R_f = 0.10. ESI-MS (*m*/*z*): 601.4 $[M+H]^+$, 1201.6 $[2M+H]^+$. ^1H NMR (200 MHz, $CDCl_3$): δ 8.91 (s, 1H), 7.97 (d, 2H, J = 8.8 Hz), 7.60 (d, 2H, J = 8.5 Hz), 7.06–7.53 (m, 8H), 5.79 (s, 2H), 4.31 (s, 2H), 3.63 (s, 2H), 3.22 (m, 2H), 2.18 (s, 3H), 1.11 (t, 3H, J = 7.3 Hz). ^{13}C (100 MHz, DMSO-d_6): δ 162.6 (C), 160.1 (dd, 2× C, J_1 = 249.5 Hz, J_2 = 6.6 Hz), 159.0, 153.7 (2× C), 146.1 (CH), 140.1, 139.4, 139.0 (4× C), 129.7 (t, 1× CH, J = 10.1 Hz), 127.1, 126.9, 126.4, 126.1, 125.9, 125.0 (7× CH), 124.4, 123.9 (2× C), 115.8 (2× CH), 110.0 (d, 2× CH, J = 24.2 Hz), 108.6 (t, 1× C, J = 18.8 Hz), 100.4 (C), 59.3, 48.4, 42.4 (3× CH_2), 39.5 (CH_3), 32.4 (CH_2), 13.8 (CH_3).

4.3.11. 8-(2,6-Difluorobenzyl)-5,8-dihydro-2-(4-ethylaminocarbonylaminophenyl)-3-(*N*-methyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid propargyl ester (2). Derivative **15** (0.60 g, 1.0 mmol) and propargylamine (89 μL, 1.3 mmol) were dissolved

in DMF (50 mL). BOP (0.59 g, 1.3 mmol) and DiPEA (0.5 mL, 3.0 mmol) were added and the mixture was allowed to stir for 18 h. The volatiles were evaporated and the residue redissolved in a DCM/MeOH mixture (200 mL, 9:1 v/v). The mixture was washed with $NaHSO_3$ (10%, 100 mL), $NaHCO_3$ (10%, 100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel column chromatography (50–100% EtOAc in toluene) to yield an off-white solid (0.48 g, 0.75 mmol, 75%). An analytical pure sample for biological evaluation was prepared by an additional purification on a semi-preparative RP-HPLC-system (linear gradient of 5.0 CV; 30–45%B). ESI-MS (*m*/*z*): 638.4 [M+H$^+$]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.07 (s, 1H), 9.04 (s, 1H), 8.86 (s, 1H), 7.83 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.4 Hz), 7.20–7.13 (m, 8H), 6.15 (t, 1H, J = 5.2 Hz), 5.67 (s, 2H), 4.23 (s, 2H), 4.15 (s, 1H), 3.57 (s, 2H), 3.14–3.10 (m, 3H), 2.10 (s, 3H), 1.06 (t, 3H, J = 6.8 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.3 (C), 161.1 (dd, 2× C, J_1 = 251.3, J_2 = 7.5 Hz), 159.7, 155.0 (2× C), 146.7 (CH), 141.0, 140.8, 140.2, 139.1 (4× C), 131.1 (t, 1× CH, J = 10.6 Hz), 128.6, 128.5, 128.1, 127.9, 126.7 (6× CH), 125.7, 121.0 (2× C), 117.2 (CH), 111.8 (d, 2× CH, J = 25.0 Hz), 110.7 (t, 1× C, J = 17.5 Hz), 101.3 (C), 81.1 (CH), 73.0 (C), 60.7, 49.7, 43.6 (3× CH_2), 41.0 (CH_3), 33.9, 28.2 (2× CH_2), 15.4 (CH_3). HRMS *m*/*z* calcd for $C_{35}H_{33}N_7O_3F_2$+H$^+$: 638.26857, obsd 638.26710.

4.3.12. 8-(2,6-Difluorobenzyl)-5,8-dihydro-2-(4-propargylaminocarbonylaminophenyl)-3-(*N*-methyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (3). Propargyl amine (685 μL, 20 mmol) was added to a cooled (ice) solution of diphosgene (970 mg, 50 mmol) in 50 mL EtOAc. The solution was refluxed for 4 h and the volatiles evaporated. The residue was redissolved in pyridine (50 mL) and amine **14** (2.19 g, 4.00 mmol) was added. The mixture was stirred overnight and evaporated. The crude solid was taken up in $CHCl_3$ (200 mL) and the mixture was washed with $NaHCO_3$ (3× 100 mL). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel column chromatography (EtOAc/toluene 2:1) to yield an off-white solid (1.80 g, 2.80 mmol, 71%). An analytical pure sample for biological evaluation was prepared by an additional purification on a semi-preparative RP-HPLC-system (linear gradient of 5.0 CV; 30–45%B). ESI-MS (*m*/*z*): 639.4 [M+H$^+$], 661.3 [M+Na$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.82 (s, 1H), 8.67 (s, 1H), 7.83 (d, 2H, J = 8.0 Hz), 7.46 (d, 2H, J = 8.4 Hz), 7.20–7.12 (m, 8H), 6.98 (br s, 1H), 5.60 (s, 2H), 4.26 (q, 2H, J = 6.4 Hz), 4.21 (s, 2H), 3.90–3.89 (s, 2H), 3.55 (s, 2H), 3.09 (s, 1H), 2.07 (s, 3H), 1.33 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.7 (C), 161.4 (dd, 2× C, J_1 = 248.4 Hz, J_2 = 7.7 Hz), 155.9, 154.6 (2× C), 147.9 (CH), 141.0, 139.9, 1139.8, 139.1 (4× C), 131.2 (t, 1× CH, J = 10.0 Hz), 128.5, 128.4, 127.9, 126.6, (7× CH), 124.2, 121.5 (2× C), 117.7 (2× CH), 111.8 (d, 2× CH, J = 22.7 Hz), 110.8 (t, 1× CH, J = 18.2 Hz), 100.0 (C), 82.0 (CH), 72.7 (C), 60.7, 60.2, 49.6, 43.7 (4× CH_2), 40.8 (CH_3), 28.7 (CH_2), 14.2 (CH_3). HRMS *m*/*z* calcd for $C_{35}H_{32}N_6O_4F_2$+H$^+$: 639.25259, obsd 639.25051.

4.3.13. 8-(2,6-Difluorobenzyl)-5,8-dihydro-3-(*N*-methyl-*N*-[*tert*-butyl-2-(benzylamino)acetate])-2-(4-nitrophenyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (16). To a cooled (0 °C) solution of **12** (2.74 g, 5.0 mmol) in THF (50 mL) were added *tert*-butyl 2-(benzylamino)acetate (1.33 g, 6.0 mmol) and DiPEA (8.5 mL, 50 mmol). The reaction mixture was stirred for 18 h at ambient temperature after which the volatiles were removed. The residue was dissolved in $CHCl_3$ (200 mL) and the organic layer washed with aqueous $NaHCO_3$ (10%, 200 mL). The water layer was extracted with $CHCl_3$ (3× 100 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was purified by silica gel column chromatography (20–33% EtOAc in toluene) to afford 3.40 g (4.9 mmol, 97%) as an off-white solid. TLC R_f = 0.8 (toluene/EtOAc, 1:1 v/v). ESI-MS (*m*/*z*): 688.4 [M+H$^+$], 710.6 [M+Na$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H), 8.23–8.19 (m, 4H), 7.43–6.99 (m, 8H), 5.49 (s, 2H), 4.61 (s, 2H), 4.40 (q, 2H, J = 7.3 Hz), 3.85 (s, 2H), 3.38 (s, 2H), 1.40 (t, 3H, J = 6.9 Hz), 1.37 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.3, 163.1 (2× C), 161.4 (dd, 2× C, J_1 = 250.0 Hz, J_2 = 7.0 Hz), 156.0, 146.6 (2× C), 146.5 (CH), 141.4, 139.2, 139.0, 138.5 (4× C), 131.4 (t, 1× CH, J = 10.0 Hz), 129.7, 129.3, 128.4, 127.9, 126.6 (7× CH), 124.7 (C), 123.2 (2× CH), 111.5 (d, 2× CH, J = 23.0 Hz), 109.5 (t, 1× C, J = 18.0 Hz), 101.2, 80.0 (2× C), 60.8, 57.9, 55.0, 47.2, 43.3 (5× CH_2), 27.7, 14.0 (4× CH_3). HRMS *m*/*z* calcd for $C_{36}H_{35}F_2N_5O_7$+H$^+$: 688.25773, obsd 688.25557.

4.3.14. 2-(*N*-((6-(Ethoxycarbonyl)-8-(2,6-difluorobenzyl)-2-(4-aminophenyl)-5,8-dihydro-5-oxoimidazo[1,2-*a*]pyrimidin-3-yl)methyl)- *N*-benzylamino)acetic acid (17). Iron powder (1.26 g, 22.5 mmol) was added to a cooled (0 °C) solution of **16** (3.09 g, 4.50 mmol) in DCM/TFA (150 mL, 1:1 v/v). After stirring for 18 h at ambient temperature, the solution was evaporated. Aqueous $NaHCO_3$ (10%, 300 mL), $CHCl_3$ (300 mL) and Celite were added. The mixture was filtered, the organic layer dispensed and the water layer extracted with $CHCl_3$ (3× 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Column chromatography of the residue (0–50% MeOH in EtOAc) afforded red brown crystals (2.61 g, 4.30 mmol, 97%). TLC R_f = 0.2 (DCM/MeOH, 9:1 v/v). ESI-MS (*m*/*z*): 602.5 [M+H]$^+$, 1203.3 [2M+H]$^+$. ^{1}H NMR (200 MHz, MeOD/TFA): δ 8.86 (s, 1H), 7.84 (d, 2H, J = 8.8 Hz), 6.58 (d, 2H, J = 8.0 Hz), 7.52–7.04 (m, 8H), 5.61 (s, 2H), 5.14 (s, 2H), 4.57 (s, 2H), 4.36 (q, 2H, J = 7.3Hz), 4.10 (s, 2H), 1.38 (t, 3H, J = 7.3 Hz). ^{13}C NMR (50 MHz, MeOD/TFA): δ 170.0 (C), 163.0 (d, 2× C, J = 250.0 Hz), 164.1 (Cq), 159.1, 150.4 (2× C), 149.3 (CH), 146.0, 143.1 (2× C), 132.9 (s, CH), 132.0 (C), 129.7, 129.3 (7× CH), 121.0 (C), 115.7 (2× CH), 113.7 (C), 112.8 (d, 2× CH, J_1 = 22.7 Hz), 111.2 (t, 1× C, J = 18.2 Hz), 101.9 (C), 62.1, 60.0, 56.9, 50.7, 44.8 (5× CH_2), 41.6 (CH_3).

4.3.15. 2-(*N*-((6-(Ethoxycarbonyl)-8-(2,6-difluorobenzyl)-2-(4-(3-ethylureido)phenyl)-5,8-dihydro-5-oxoimidazo[1,2-*a*]pyrimidin-3-yl)methyl)-*N*-benzylamino)acetic acid (18). To a solution of **17** (2.61 g, 4.30 mmol) in pyridine (30 mL) was added ethyl isocyanate (1.4 mL, 21.5 mmol). The mixture was stirred for 18 h and subsequently water (30 mL) was added. The mixture was stirred for 1 h and the volatiles were removed. The residue was redissolved in DCM (200 mL) and the solution washed with aqueous $NaHCO_3$ (10%, 100 mL), dried (Na_2SO_4) and concentrated. Column chromatography of the residue (0–50% MeOH in EtOAc) afforded off-white crystals (2.05 g, 3.1 mmol, 71%). ESI-MS (*m*/*z*): 673.5 $[M+H]^+$, 695.3 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$/TFA): δ 8.56 (s, 1H), 7.49–7.13 (m, 10H), 7.04 (t, 2H, $J = 8.0$ Hz) 5.51 (s, 2H), 5.04 (s, 2H), 4.48 (s, 2H), 4.33 (q, 2H, $J = 6.8$ Hz), 4.04 (s, 2H), 3.26 (q, 2H, $J = 7.2$ Hz), 1.36 (t, 3H, $J = 7.2$ Hz), 1.16 (t, 3H, $J = 7.6$ Hz). ^{13}C NMR (400 MHz, $CDCl_3$/TFA): δ 167.2, 162.8 (2× C), 161.3 (dd, 2× C, $J = 251.0$ Hz), 158.6, 156.3 (2× C), 147.8 (CH), 146.0, 142.4, 140.3 (4× C), 132.2, 129.8, 129.2, 128.9 (8× CH), 128.2 (2× C), 119.9 (2× CH),111.8 (d, 2× CH, $J = 23.0$ Hz), 108.8, 101.1 (2× C), 61.6, 59.5, 49.7, 43.7, 38.5, 34.9 (6× CH_2), 14.6, 13.8 (2× CH_3). HRMS *m*/*z* calcd for $C_{35}H_{34}N_6O_6F_2+H^+$: 673.25807, obsd 673.25730.

4.3.16. 8-(2,6-Difluorobenzyl)-5,8-dihydro-2-(4-ethylaminocarbonylaminophenyl)-3-(*N*-propargylamidomethyl-*N*-benzylaminomethyl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carboxylic acid ethyl ester (4). To a solution of **18** (1.0 g, 1.5 mmol) and propargylamine (134 μL, 1.95 mmol) in DMF (50 mL) were added BOP (0.88 g, 1.95 mmol) and DiPEA (0.76 mL, 4.5 mmol) and the mixture was allowed to stir for 18 h. The solution was evaporated and dissolved in DCM/MeOH mixture (200 mL, 9:1 v/v) and washed with $NaHSO_3$ (10%, 100 mL), $NaHCO_3$ (10%, 100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography (50–100% EtOAc in toluene) to yield an off-white solid which was additionally crystallized from MeOH/$CHCl_3$ and Et_2O to provide an off-white solid (573 mg, 0.81 mmol, 54%). An analytical pure sample for biological evaluation was prepared by an additional purification on a semi-preparative RP-HPLC-system (linear gradient of 5.0 CV; 30–45%B). ESI-MS (*m*/*z*): 710.6 $[M+H^+]$. ^1H NMR (500 MHz, DMSO-d_6/TFA): δ 9.03 (s, 1H), 8.95 (s, 1H), 8.71 (br s, 1H), 7.60–7.42 (m, 10H), 7.17 (t, 2H, $J = 8.0$ Hz), 6.65 (t, 1H), 6.25 (br s, 1H), 5.67 (s, 2H), 5.60 (s, 2H), 5.13–5.10 (m, 1H), 4.82–4.79 (m, 1H), 4.58–4.56 (m, 1H), 4.38–4.34 (m, 3H), 3.92–3.90 (m, 2H), 3.16–3.11 (m, 3H), 1.34 (t, 3H, $J = 7.2$ Hz), 1.08 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (500 MHz, DMSO/TFA): δ 163.9, 163.0 (2× C), 161.2 (dd, 2× C, $J_1 = 250.0$ Hz, $J_2 = 7.5$ Hz), 157.9, 155.4 (2× C), 149.7 (CH), 144.5, 142.7, 141.8 (3× C), 131.9 (t, 1× CH, $J = 10.0$ Hz), 131.0 (2× CH), 129.9 (C), 129.8, 129.3, 128.9 (5× CH), 124.0 (C), 117.9 (2× CH), 112.1 (d, 2× CH, $J = 20.0$ Hz), 112.1 (C), 110.8 (t, 1× C, $J = 18.8$ Hz), 101.1 (C), 79.7 (CH), 74.0 (C), 60.9, 59.0, 49.6, 44.2, 38.5, 34.2, 28.3 (7× CH_2), 15.5, 14.3 (2× CH_3). HRMS *m*/*z* calcd for $C_{38}H_{37}N_7O_5F_2+H^+$: 710.28970, obsd 710.28799.

4.4. General procedure for ditosylated polyethyleneglycols (20b–e)

Polyethylene glycol **19** (150 mmol) was dissolved in DCM (150 mL). *p*-Toluenesulfonyl chloride (2 equiv 300 mmol, 57.2 g) was added and the mixture was cooled to 0 °C with an ice bath. Powdered KOH (8 equiv 1.2 mol, 67.2 g) was carefully added in small portions so that the mixture was kept below 5 °C. After stirring for 3 h at 0 °C, DCM (150 mL) and ice-water (300 mL) were added. The organic layer was separated and the water layer was extracted with DCM (2× 150 mL). The combined organic layers were washed with water (1× 100 mL), dried ($MgSO_4$) and evaporated to yield ditosylated polyethyleneglycols quantitatively, which were used without further purification.

4.4.1. 1,5-Ditosyl-3-oxapentane (20b). 1H NMR (200 MHz, CDCl3): δ 7.78 (d, 4H, $J = 8.4$ Hz), 7.35 (d, 4H, $J = 7.7$ Hz), 4.11–4.07 (m, 4H), 3.63–3.58 (m 4 H), 2.45 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 144.9, 132.7 (4× C), 129.8, 127.8 (8× CH), 69.0, 68.6 (4× CH_2), 21.5 (2× CH_3).

4.4.2. 1,8-Ditosyl-3,6-dioxaoctane (20c). ^1H NMR (200 MHz, $CDCl_3$): δ 7.78 (d, 4H, $J = 8.0$ Hz), 7.34 (d, 4H, $J = 8.0$ Hz), 4.16–4.11 (m, 4H), 3.67–3.62 (m, 4H), 3.52 (s, 4H), 2.44 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 144.7, 132.7 (4× C), 129.8, 127.8 (8× CH), 70.5, 69.1, 68.4 (6× CH_2), 21.5 (2× CH_3).

4.4.3. 1,11-Ditosyl-3,6,9-trioxaundecane (20d). ^1H NMR (200 MHz, $CDCl_3$): δ 7.78 (d, 4H, $J = 8.0$ Hz), 7.34 (d, 4H, $J = 8.4$ Hz), 4.17–4.13 (m, 8H), 3.67–3.62 (m, 4H), 3.55 (s, 4H), 2.44 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 144.7, 132.7 (4× C), 129.7, 127.8 (8× CH), 70.5, 70.3, 69.1, 68.4 (8× CH_2), 21.5 (2× CH_3).

4.4.4. 1,14-Ditosyl-3,6,9,12-tetraoxatetradecane (20e). ^1H NMR (200 MHz, $CDCl_3$): δ 7.78 (d, 4H, $J = 8.0$ Hz), 7.34 (d, 4H, $J = 8.4$ Hz), 4.17–4.13 (m, 12H), 3.67–3.62 (m, 4H), 3.55 (s, 4H), 2.44 (s, 6H).^{13}C NMR (50 MHz, $CDCl_3$): δ 144.7, 132.7 (4× C), 129.7, 127.8 (8× CH), 70.6, 70.5, 70.3, 69.1, 68.4 (10× CH_2), 21.5 (2× CH_3).

4.4.5. 1,2-Diazidoethane (21a). *Caution: Because of the explosive nature of low molecular weight diazides, 1,2-diazidoethane was not isolated, but stored as a solution in DMF.* 1,2-dichloroethane (150 mmol, 15.2 g) was dissolved in DMF (225 mL). NaN_3 (600 mmol, 39.0 g) and TBAI (5 mol%, 7.5 mmol, 2.8 g) were added and the mixture was heated at 80 °C for 18 h. Et_2O was added and the resulting solids were filtered. The Et_2O was evaporated and the concentration of the final solution of 1,2-diazidoethane in DMF was determined by ^1H NMR and was used without further purification. ^1H NMR (500 MHz, $CDCl_3$): δ 3.53 (s, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 49.0 (2× CH_2). IR: 2100 (N_3).

4.5. General procedure for the synthesis of bis-azide ethyleneglycols 21

Ditosylated ethyleneglycol **20** (150 mmol) was dissolved in DMF (225 mL). NaN_3 (600 mmol, 39.0 g) and TBAI (5 mol%, 7.5 mmol, 2.8 g) were added and the mixture was heated at 80 °C for 18 h. The DMF was evaporated and the solid residue was suspended in Et_2O. The insoluble salts were filtered and the filtrate concentrated. This procedure was repeated twice until all the salts and DMF were removed. A slightly yellow liquid remained in quantitative yield.

4.5.1. 1,5-Diazido-3,6-oxapentane (21b). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.67 (t, 4H, J = 4.7 Hz), 3.40 (t, 4H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 69.4, 50.4 (4× CH_2). IR: 2089 (N_3), 1124 (C–O).

4.5.2. 1,8-Diazido-3,6-dioxaoctane (21c). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.64–3.56 (m, 8H), 3.31 (t, 4H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 70.4, 69.8, 50.4 (6× CH_2). IR: 2095 (N_3), 1115 (C–O).

4.5.3. 1,11-Diazido-3,6,9-trioxaundecane (21d). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.56–3.54 (m, 12H), 3.27 (t, 4H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 70.3, 69.6, 50.3 (8× CH_2). IR: 2091 (N_3), 1090 (C–O).

4.5.4. 1,14-Diazido-3,6,9,12-tetraoxatetradecane (21e). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.62–3.60 (m, 16H), 3.34 (t, 4H, J = 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 70.5, 69.9, 50.5 (10× CH_2). IR: 2095 (N_3), 1113 (C–O).

4.6. General method for the preparation of monovalent ligands 22a–e, 23a–e and 24a–e

To a solution of the desired acetylene functionalized ligand **2**, **3** or **4** (0.05 mmol) and bis-azide spacer **21a**, **21b**, **21c**, **21d** or **21e** (0.25 mmol) in a mixture of degassed *t*-BuOH/MeCN/H_2O (2:1:1 v/v/v, 900 μL) were added sodium ascorbate (1 equiv 50 μL of a 1 M solution in H_2O) and $CuSO_4$ (0.2 equiv 50 μL of a 0.2 M solution in H_2O). The reaction mixture was stirred and heated at 60 °C until RP-HPLC showed complete conversion of the starting material. The mixture was evaporated and redissolved in dioxane/H_2O/MeCN/TFA (10:1:1:1 v/v/v/v, 2 mL). The crude products were analyzed by LC/MS and purified by semi-preparative RP-HPLC (linear gradient of 5.0 CV; 30–45%B). Evaporation and lyophilization of the combined fractions furnished monovalent ligands **22a**–**e**, **23a**–**e** and **24a**–**e** as white amorphous powders.

4.7. General method for the preparation of bivalent ligands 25a–e, 26a–e and 27a–e

To a solution of the desired acetylene functionalized ligand **2**, **3** or **4** (0.10 mmol) and bis-azide spacer **21a**, **21b**, **21c**, **21d** or **21e** (0.05 mmol) in a mixture of degassed *t*-BuOH/MeCN/H_2O (2:1:1 v/v/v, 800 μL) were added sodium ascorbate (1 equiv 100 μL of a 1 M solution in H_2O) and $CuSO_4$ (0.2 equiv 100 μL of a 0.2 M solution in H_2O). The reaction mixture was stirred and heated at 60 °C until RP-HPLC showed complete conversion of the starting material. The mixture was evaporated and redissolved in dioxane/H_2O/MeCN/TFA (10:1:1:1 v/v/v/v, 2 mL). The crude products were analyzed by LC/MS and purified by semi-preparative RP-HPLC (linear gradient of 5.0 CV; 30–45% B). Evaporation and lyophilization of the combined fractions furnished bivalent ligands **25a**–**e**, **26a**–**e** and **27a**–**e** as white amorphous powders.

4.8. Compound 23a

Yield after RP-HPLC purification: 6.9 mg (8.0 μmol, 16%). LC/MS analysis: t_R 7.21 min (linear gradient 10–90%B in 13.5 min; *m/z*: 751.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.92 (s, 1H), 8.72 (br s, 1H) 8.01 (s, 1H), 7.53 (dd, 4H, J = 11.2 Hz, J = 9.5 Hz), 7.48–7.35 (m, 6H), 7.17 (t, 2H, J = 8.1 Hz), 6.81 (m, 1H), 5.65 (s, 2H), 5.11–5.08 (m, 1H), 4.80–4.76 (m, 1H), 4.56–4.51 (m, 1H), 4.54 (t, 2H, J = 5.2 Hz), 4.38–4.27 (m, 5H), 3.82 (t, 2H, J = 5.2 Hz), 2.48 (s, 3H), 1.13 (t, 3H, J = 7.1 Hz). HRMS *m/z* calcd for $C_{37}H_{36}N_{12}O_4F_2$+H$^+$: 751.30233, obsd 51.30002.

4.9. Compound 23b

Yield after RP-HPLC purification: 7.2 mg (7.9 μmol, 16%). LC/MS analysis: t_R 7.34 min (linear gradient 10–90% B in 13.5 min; *m/z*: 795.3 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.89 (s, 1H), 8.71 (br s, 1H) 7.96 (s, 1H), 7.55–7.40 (dd, 4H, J = 12.6 Hz, J = 9.1 Hz), 7.48–7.40 (m, 6H), 7.17 (t, 2H, J = 8.1 Hz), 6.78 (t, 1H, J = 5.5 Hz), 5.65 (s, 2H), 5.11–5.08 (m, 1H), 4.80–4.76 (m, 1H), 4.56–4.51 (m, 1H), 4.52 (t, 2H, J = 5.2 Hz), 4.38-4.27 (m, 5H), 3.83 (t, 2H, J = 5.2 Hz), 3.58 (t, 2H, J = 5.0 Hz), 3.36 (t, 2H, J = 4.8 Hz), 2.48 (s, 3H), 1.13 (t, 3H, J = 7.1 Hz). HRMS *m/z* calcd for $C_{39}H_{40}N_{12}O_5F_2$+H$^+$: 795.32855, obsd 795.32597.

4.10. Compound 23c

Yield after RP-HPLC purification: 14.7 mg (15.4 μmol, 31%). LC/MS analysis: t_R 7.38 min (linear gradient 10–90% B in 13.5 min; *m/z*: 839.3 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.89 (s, 1H), 8.71 (br s, 1H) 7.91 (s, 1H), 7.53–7.35 (m, 10H), 7.16 (t, 2H, J = 8.2 Hz), 6.75 (br s, 1H), 5.64 (s, 2H), 5.11–5.08 (m, 1H), 4.80–4.76 (m, 1H), 4.56–4.44 (m, 3H), 4.39–4.27 (m, 5H), 3.75 (t, 2H, J = 5.0 Hz), 3.58–3.47 (m, 10H), 3.38 (t, 2H, J = 5.1 Hz), 2.48 (s, 3H), 1.33 (t, 3H, J = 7.1 Hz). HRMS *m/z* calcd for $C_{41}H_{44}N_{12}O_6F_2$+H$^+$: 839.35476, obsd 839.35210.

4.11. Compound 23d

Yield after RP-HPLC purification: 14.7 mg (12.9 μmol, 26%). LC/MS analysis: t_R 5.90 min (linear gradient 10–90% B in 13.5 min; *m/z*: 883.3 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.89 (s, 1H), 8.71 (br s, 1H) 7.93 (s, 1H), 7.53 (dd, 4H, J = 13.0 Hz, J = 9.0 Hz), 7.48–7.40 (m, 6H), 7.17 (t, 2H,

J = 8.2 Hz), 6.76 (br s, 1H), 5.65 (s, 2H), 5.11–5.08 (m, 1H), 4.80–4.76 (m, 1H), 4.56–4.54 (m, 1H), 4.50 (t, 2H, J = 5.2 Hz), 4.39–4.27 (m, 5H), 3.80 (t, 2H, J = 5.0 Hz), 3.58–3.47 (m, 14H, J = 5.0 Hz), 3.35 (t, 2H, J = 4.8 Hz), 2.48 (s, 3H), 1.33 (t, 3H, J = 7.1 Hz). RMS *m/z* calcd for $C_{43}H_{48}N_{12}O_7F_2+H^+$: 883.38097, obsd 883.37797.

4.12. Compound 23e

Yield after RP-HPLC purification: 10.4 mg (10.0 μmol, 20%). LC/MS analysis: t_R 7.38 min (linear gradient 10–90%B in 13.5 min; *m/z*: 927.5 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.88 (s, 1H), 8.71 (br s, 1H) 7.93 (s, 1H), 7.53 (dd, 4H, J = 12.4 Hz, J = 9.2 Hz), 7.51–7.40 (m, 6H), 7.17 (t, 2H, J = 8.2 Hz), 6.78 (br s, 1H), 5.65 (s, 2H), 5.11–5.08 (m, 1H), 4.80–4.76 (m, 1H), 4.56–4.54 (m, 1H), 4.50 (t, 2H, J = 5.2 Hz), 4.38–4.27 (m, 5H), 3.80 (t, 2H, J = 5.0 Hz), 3.58–3.47 (m, 10H, J = 5.0 Hz), 3.35 (t, 2H, J = 4.8 Hz), 2.48 (s, 3H), 1.33 (t, 3H, J = 7.1 Hz). HRMS *m/z* calcd for $C_{45}H_{52}N_{12}O_8F_2+H^+$: 927.40719, obsd 927.40473.

4.13. Compound 26a

Yield after RP-HPLC purification: 27.9 mg (17.3 μmol, 35%). LC/MS analysis: t_R 7.50 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1389.5 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.01 (s, 2H), 8.98 (s, 2H), 8.73 (br s, 2H), 7.90 (s, 2H), 7.55–7.51 (m, 8H), 7.48–7.40 (m, 12H), 7.16 (t, 4H, J = 8.3 Hz), 6.85 (m, 2H), 5.65 (s, 4H), 5.11–5.08 (m, 2H), 4.87 (s, 4H) 4.81–4.77 (m, 2H), 4.56–4.51 (m, 2H), 4.37–4.29 (m, 10H), 2.48 (s, 6H), 1.13 (t, 6H, J = 7.1 Hz). HRMS *m/z* calcd for $C_{72}H_{68}N_{18}O_8F_4+H^+$: 1389.54764, obsd 1389.55646.

4.14. Compound 26b

Yield after RP-HPLC purification: 29.7 mg (17.9 μmol, 36%). LC/MS analysis: t_R 7.62 min (linear gradient 10–90% B in 13.5 min; *m/z*: 1433.7 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.00 (s, 2H), 8.98 (s, 2H), 8.70 (br s, 2H), 7.86 (s, 2H), 7.52 (dd, 8H, J = 11.4 Hz, J = 9.4 Hz), 7.48–7.34 (m, 12H), 7.16 (t, 4H, J = 8.3 Hz), 6.73 (t, 2H, J = 5.0 Hz), 5.64 (s, 4H,), 5.10–5.08 (m, 2H), 4.79–4.75 (m, 2H), 4.56–4.54 (m, 2H), 4.37–4.29 (m, 10H), 3.79 (t, 4H, J = 5.2 Hz), 2.48 (s, 6H), 1.33 (t, 6H, J = 7.3 Hz). HRMS *m/z* calcd for $C_{74}H_{72}N_{18}O_9F_4+H^+$: 1433.57385, obsd 1433.58036.

4.15. Compound 26c

Yield after RP-HPLC purification: 25.6 mg (15.0 μmol, 30%). LC/MS analysis: t_R 7.64 min (linear gradient 10–90% B in 13.5 min; *m/z*: 1477.7 [M+H$^+$]). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (s, 2H), 8.82 (s, 2H), 8.69 (br s, 2H), 7.90 (s, 2H), 7.80 (s, 2H), 7.55–7.32 (m, 12H,), 7.25–7.10 (m, 10H), 6.70 (br s, 2H), 5.62 (s, 4H), 5.10–5.05 (m, 2H), 4.79–4.70 (m, 2H) 4.56–4.45 (m, 6H), 4.37–4.10 (m, 10H), 3.73 (t, 4H, J = 4.9 Hz), 3.43 (s, 4H), 2.48 (s, 6H), 1.30 (s, 6H). HRMS *m/z* calcd for $C_{76}H_{76}N_{18}O_{10}F_4+H^+$: 1477.60007, obsd 1477.61412.

4.16. Compound 26d

Yield after RP-HPLC purification: 26.0 mg (14.9 μmol, 30%). LC/MS analysis: t_R 5.89 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1522.5 [M+H$^+$]). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 2H), 8.83 (s, 2H), 8.68 (br s, 2H), 7.91 (s, 2H), 7.82–7.78 (m, 2H), 7.55–7.35 (m, 12H), 7.25–7.10 (m, 10H), 6.70 (br s, 2H), 5.59 (s, 4H), 5.10–5.05 (m, 2H), 4.80–4.70 (m, 2H), 4.56–4.40 (m, 6H), 4.37–4.15 (m, 10H), 3.76 (t, 4H, J = 5.0 Hz), 3.55–3.45 (m, 8H), 2.48 (s, 6H), 1.30 (s, 6H). HRMS *m/z* calcd for $C_{78}H_{80}N_{18}O_{11}F_4+H^+$: 1521.62628, obsd 1521.64257.

4.17. Compound 26e

Yield after RP-HPLC purification: 31.1 mg (17.3 μmol, 35%). LC/MS analysis: t_R 5.91 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1565.6 [M+H$^+$]). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 2H), 8.83 (s, 2H), 8.69 (br s, 2H), 7.92 (s, 2H), 7.82–7.78 (m, 2H), 7.58–7.35 (m, 12H), 7.25–7.10 (m, 10H), 6.68 (br s, 2H), 5.59 (s, 4H), 5.10–5.05 (m, 2H), 4.80–4.70 (m, 2H), 4.56–4.40 (m, 6H), 4.37–4.15 (m, 10H), 3.77 (t, 4H, J = 5.1 Hz), 3.55–3.45 (m, 12H), 2.48 (s, 6H), 1.30 (s, 6H). HRMS *m/z* calcd for $C_{80}H_{84}N_{18}O_{12}F_4+H^+$: 1565.65250, obsd 1565.66724.

4.18. Compound 22a

Yield after RP-HPLC purification: 12.6 mg (14.7 μmol, 30%). LC/MS analysis: t_R 6.94 min (linear gradient 10–90%B in 13.5 min; *m/z*: 750.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (t, 1H, J = 5.0 Hz, CON*H*CH$_2$), 9.04 (s, 1H), 8.73 (s, 1H), 8.59 (br s, 1H), 8.13 (s, 1H), 7.54 (dd, 4H, J = 24.5 Hz, J = 9.0 Hz), 7.49–7.42 (m, 6H), 7.17 (t, 2H, J = 8.5 Hz), 6.28 (br s, 1H), 5.69 (s, 2H), 5.15–5.11 (m, 1H), 4.81–4.76 (m, 1H,), 4.71, 4.69 (2d, 1H, J = 5.5 Hz), 4.62–4.50 (m, 4H), 4.40–4.36 (m, 1H), 3.84 (t, 2H, J = 5.4 Hz), 3.11 (q, 2H, J = 5.2 Hz), 2.46 (s, 3H), 1.06 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{37}H_{37}N_{13}O_3F_2+H^+$: 750.31654, obsd 750.31831.

4.19. Compound 22b

Yield after RP-HPLC purification: 16.1 mg (17.7 μmol, 35%). LC/MS analysis: t_R 6.82 min (linear gradient 10–90%B in 13.5 min; *m/z*: 794.5 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (t, 1H, J = 5.0 Hz), 9.01 (s, 1H), 8.78 (s, 1H), 8.61 (br s, 1H), 8.04 (s, 1H), 7.55 (dd, 4H, J_1 = 14.5 Hz, J_2 = 8.7 Hz), 7.50–7.42 (m, 6H), 7.16 (t, 2H, J = 8.1 Hz), 6.33 (br s, 1H), 5.70 (s, 2H), 5.15–5.11 (m, 1H), 4.81–4.76 (m, 1H), 4.63 (ddd, 2H, J_1 = 32.9 Hz, J_2 = 15.2 Hz, J_3 = 5.7 Hz), 4.57 (t, 2H, J = 5.1 Hz), 4.54–4.53 (m, 1H), 4.46–4.40 (m, 1H), 3.86 (t, 2H, J = 5.2 Hz), 3.59 (t, 2H, J = 4.8 Hz), 3.36 (t, 2H, J = 5.0 Hz), 3.11 (q, 2H, J = 5.2 Hz), 2.46 (s, 3H), 1.05 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{39}H_{41}N_{13}O_4F_2+H^+$: 794.34453, obsd 794.34299.

4.20. Compound 22c

Yield after RP-HPLC purification: 22.3 mg (23.4 μmol, 47%). LC/MS analysis: t_R 7.25 min (linear gradient 10–90% B in 13.5 min; *m/z*: 838.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (t, 1H, J = 5.2 Hz), 9.01 (s, 1H), 8.84 (s, 1H), 8.63 (br s, 1H), 8.05 (s, 1H), 7.55 (dd, 4H, J_1 = 20.9 Hz, J_2 = 8.7 Hz), 7.50–7.41 (m, 6H), 7.16 (t, 2H, J = 8.2 Hz), 6.33 (br s, 1H), 5.70 (s, 2H), 5.15–5.11 (m, 1H), 4.82–4.76 (m, 1H), 4.71–4.63 (m, 1H), 4.61–4.47 (m, 2H), 4.55 (t, 2H, J = 5.1 Hz), 4.40–4.37 (m, 1H), 3.83 (t, 2H, J = 5.2 Hz,), 3.55–3.52 (m, 6H), 3.35 (t, 2H, J = 5.0 Hz), 3.11 (q, 2H, J = 7.1 Hz), 2.46 (s, 3H), 1.05 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{41}H_{45}N_{13}O_5F_2$+H$^+$: 838.37074, obsd 838.36890.

4.21. Compound 22d

Yield after RP-HPLC purification: 15.8 mg (15.8 μmol, 32%). LC/MS analysis: t_R 5.75 min (linear gradient 10–90%B in 13.5 min; *m/z*: 882.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (t, 1H, J = 5.2 Hz), 9.02 (s, 1H), 8.83 (s, 1H), 8.62 (br s, 1H), 8.05 (s, 1H), 7.55 (dd, 4H, J_1 = 21.8 Hz, J_2 = 8.7 Hz), 7.50–7.41 (m, 6H), 7.16 (t, 2H, J = 8.2 Hz), 6.33 (br s, 1H,), 5.70 (s, 2H), 5.15–5.11 (m, 1H), 4.82–4.76 (m, 1H), 4.69 (dd, 2H, J_1 = 15.1 Hz, J_2 = 5.8 Hz), 4.62–4.52 (m, 2H), 4.55 (t, 2H, J = 5.2 Hz), 4.40–4.37 (m, 1H), 3.83 (t, 2H, J = 5.2 Hz), 3.55–3.52 (m, 10H), 3.36 (t, 2H, J = 5.0 Hz), 3.11 (q, 2H, J = 6.9 Hz), 2.46 (s, 3H), 1.06 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{43}H_{49}N_{13}O_6F_2$+H$^+$: 882.39696, obsd 882.39498.

4.22. Compound 22e

Yield after RP-HPLC purification: 18.6 mg (17.9 μmol, 36%). LC/MS analysis: t_R 7.24 min (linear gradient 10–90%B in 13.5 min; *m/z*: 926.2 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (t, 1H, J = 5.2 Hz), 9.02 (s, 1H), 8.83 (s, 1H), 8.61 (br s, 1H), 8.07 (s, 1H), 7.55 (dd, 4H, J_1 = 17.8 Hz, J_2 = 8.7 Hz), 7.50–7.41 (m, 6H), 7.16 (t, 2H, J = 8.2 Hz), 6.33 (br s, 1H), 5.70 (s, 2H), 5.15–5.11 (m, 1H), 4.82–4.76 (m, 1H), 4.69 (dd, 2H, J_1 = 15.1 Hz, J_2 = 5.8 Hz), 4.62–4.52 (m, 2H), 4.55 (t, 2H, J = 5.2 Hz), 4.40–4.37 (m, 1H,), 3.82 (t, 2H, J = 5.2 Hz), 3.55–3.52 (m, 14H), 3.36 (t, 2H, J = 5.0 Hz), 3.11 (q, 2H, J = 7.1 Hz), 2.46 (s, 3H), 1.05 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{45}H_{53}N_{13}O_7F_2$+H$^+$: 926.42317, obsd 926.41971.

4.23. Compound 25a

Yield after RP-HPLC purification: 25.7 mg (15.9 μmol, 32%). LC/MS analysis: t_R 6.87 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1387.6 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.11 (t, 2H, J = 5.3 Hz), 8.99 (s, 2H), 8.71 (s, 2H), 8.59 (br s, 2H), 8.02 (s, 2H), 7.54 (dd, 8H, J_1 = 23.7 Hz, J_2 = 8.7 Hz), 7.49–7.40 (m, 12H), 7.16 (t, 4H, J = 8.2 Hz), 6.25 (t, 2H, J = 5.2 Hz), 5.69 (s, 4H), 5.13–5.10 (m, 2H), 4.92 (s, 4H), 4.79–4.70 (m, 2H), 4.68–4.59 (m, 2H), 4.57–4.50 (m, 4H), 4.40–4.36 (m, 2H), 3.14–3.09 (m, 4H), 2.46 (s, 6H), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{72}H_{70}N_{20}O_6F_4$+H$^+$: 1387.57961, obsd 1387.59127.

4.24. Compound 25b

Yield after RP-HPLC purification: 29.8 mg (18.0 μmol, 36%). LC/MS analysis: t_R 7.02 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1431.8 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (s, 2H), 9.02 (s, 2H), 8.78 (s, 2H), 8.62 (br s, 2H), 7.95 (s, 2H), 7.53 (dd, 8H, J_1 = 19.5 Hz, J_2 = 6.7 Hz), 7.49–7.40 (m, 12H), 7.15 (t, 4H, J = 8.2 Hz), 6.34 (br s, 2H), 5.69 (s, 4H), 5.13–5.10 (m, 2H), 4.79–4.70 (m, 2H), 4.68–4.64 (m, 2H), 4.57–4.46 (m, 8H), 4.40–4.36 (m, 2H), 3.83–3.77 (m, 4H), 3.11 (q, 4H, J = 7.2 Hz), 2.46 (s, 6H), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{74}H_{74}N_{20}O_7F_4$+H$^+$: 1431.60582, obsd 1431.61762.

4.25. Compound 25c

Yield after RP-HPLC purification: 11.1 mg (6.5 μmol, 13%). LC/MS analysis: t_R 7.20 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1475.8 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.14 (t, 2H, J = 5.3 Hz), 9.02 (s, 2H), 8.76 (s, 2H), 8.67–8.58 (m, 2H), 8.05 (s, 2H), 7.59–7.42 (m, 20H), 7.17 (t, 4H, J = 8.2 Hz), 6.38–6.42 (m, 2H), 5.70 (s, 4H), 5.13 (d, 2H, J = 13.5 Hz), 4.82–4.78 (m, 2H), 4.71–4.58 (ddd, 4H, J_1 = 5.1 Hz, J_2 = 15.1 Hz, J_3 = 42.5 Hz), 4.54–4.46 (m, 6H), 4.49–4.37 (m, 2H), 3.80 (t, 4H, J = 5.1 Hz), 3.52 (s, 4H), 3.14–3.10 (m, 4H), 2.48–2.46 (m, 6H), 1.07 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{76}H_{78}N_{20}O_8F_4$+H$^+$: 1475.63204, obsd 1475.64714.

4.26. Compound 25d

Yield after RP-HPLC purification: 14.1 mg (8.1 μmol, 16%). LC/MS analysis: t_R 7.25 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1519.7 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (br s, 2H), 9.00 (s, 2H), 8.82 (s, 2H), 8.67–8.58 (br s, 2H), 8.05 (s, 2H), 7.55 (dd, 8H, J_1 = 20.0 Hz, J_2 = 8.8 Hz), 7.50–7.38 (m, 12H), 7.17 (t, 4H, J = 8.2 Hz), 6.37 (br s, 2H), 5.69 (s, 4H), 5.13–5.11 (m, 2H), 4.81–4.78 (m, 2H), 4.71–4.61 (m, 2H), 4.59–4.42 (m, 8H), 4.40–4.34 (m, 2H), 3.81 (t, 4H, J = 5.4 Hz), 3.52–3.47 (m, 4H), 3.56–3.49 (m, 4H), 3.16–3.09 (m, 4H), 2.48 (br s, 6H), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{78}H_{82}N_{20}O_9F_4$+H$^+$: 1519.65825, obsd 1519.66753.

4.27. Compound 25e

Yield after RP-HPLC purification: 13.8 mg (7.7 μmol, 15%). LC/MS analysis: t_R 7.27 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1563.9 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (br s, 2H), 9.01 (s, 2H), 8.79 (s, 2H), 8.65–8.57 (br s, 2H), 8.05 (s, 2H), 7.55 (dd, 8H, J_1 = 21.9 Hz, J_2 = 8.7 Hz), 7.50–7.38 (m, 12H), 7.16 (t, 4H, J = 8.2 Hz), 6.34 (br s, 2H), 5.69 (s, 4H), 5.13–5.08 (m, 2H), 4.81–4.75 (m, 2H), 4.71–4.64 (m, 2H), 4.59–4.42 (m, 8H), 4.40–4.36 (m, 2H), 3.81 (t, 4H, J = 5.5 Hz), 3.57–3.43 (m, 12H), 3.14–3.09 (m, 4H), 2.46 (br s, 6H), 1.05 (t, 6H, J = 7.2 Hz). HRMS

m/z calcd for $C_{80}H_{86}N_{20}O_{10}F_4+H^+$: 1563.68447, obsd 1563.68651.

4.28. Compound 24a

Yield after RP-HPLC purification: 13.3 mg (14.2 μmol, 28%). LC/MS analysis: t_R 7.47 min (linear gradient 10–90%B in 13.5 min; *m/z*: 822.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H), 8.79 (br s, 2H), 7.90 (s, 1H), 7.51 (dd, 4H, J_1 = 13.8 Hz, J_2 = 8.7 Hz), 7.49–7.35 (m, 3H), 7.25–7.20 (m, 3H), 7.15 (t, 2H, J = 8.2 Hz), 6.35 (br s, 1H), 5.61 (s, 2H), 5.05–4.76 (br s, 2H), 4.57–4.52 (m, 2H), 4.51 (t, 2H, J = 5.6 Hz), 4.31 (q, 2H, J = 7.1 Hz), 4.06 (br s, 2H), 3.88–3.80 (m, 2H), 3.78 (t, 2H, J = 5.7 Hz), 3.16–3.11 (m, 2H), 1.34 (t, 3H, J = 7.2 Hz), 1.05 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{40}H_{41}N_{13}O_5F_2+H^+$: 822.33944, obsd 822.33927.

4.29. Compound 24b

Yield after RP-HPLC purification: 24.0 mg (24.5 μmol, 49%). LC/MS analysis: t_R 7.59 min (linear gradient 10–90%B in 13.5 min; *m/z*: 866.4 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H), 8.83 (br s, 2H), 7.79 (s, 1H), 7.51 (dd, 4H, J_1 = 20.4 Hz, J_2 = 8.7 Hz), 7.48–7.32 (m, 3H), 7.32–7.20 (m, 3H), 7.14 (t, 2H, J = 8.2 Hz), 6.39 (br s, 1H), 5.61 (s, 2H), 5.05–4.76 (br s, 2H), 4.57–4.42 (m, 2H), 4.49 (t, 2H, J = 5.2 Hz), 4.31 (q, 2H, J = 7.1 Hz), 4.06–4.01 (br s, 2H), 3.88–3.82 (m, 2H), 3.80 (t, 2H, J = 5.7 Hz), 3.59–3.52 (m, 2H), 3.32 (t, 2H, J = 5.1 Hz) 3.16–3.08 (m, 2H), 1.33 (t, 3H, J = 7.2 Hz), 1.06 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{42}H_{45}N_{13}O_6F_2+H^+$: 866.36566, obsd 866.36566.

4.30. Compound 24c

Yield after RP-HPLC purification: 23.1 mg (22.5 μmol, 45%). LC/MS analysis: t_R 7.71 min (linear gradient 10–90%B in 13.5 min; *m/z*: 910.6 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H), 8.86 (br s, 2H), 7.81 (s, 1H), 7.51 (dd, 4H, J_1 = 18.9 Hz, J_2 = 8.7 Hz), 7.48–7.32 (m, 3H), 7.32–7.20 (m, 3H), 7.16 (t, 2H, J = 8.3 Hz), 6.38 (br s, 1H), 5.61 (s, 2H), 5.08–4.76 (br s, 2H), 4.57–4.42 (m, 2H), 4.47 (t, 2H, J = 5.2 Hz), 4.31 (q, 2H, J = 7.1 Hz), 4.06–4.01 (br s, 2H), 3.92–3.80 (m, 2 H), 3.77 (t, 2H, J = 5.2 Hz), 3.55–3.49 (m, 6H), 3.33 (t, 2H, J = 5.0 Hz) 3.13–3.10 (m, 2H), 1.33 (t, 3H, J = 7.1 Hz), 1.06 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{44}H_{49}N_{13}O_7F_2+H^+$: 910.39187, obsd 910.39296.

4.31. Compound 24d

Yield after RP-HPLC purification: 10.6 mg (9.9 μmol, 20%). LC/MS analysis: t_R 7.73 min (linear gradient 10–90%B in 13.5 min; *m/z*: 954.7 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 1H), 8.75 (br s, 2H), 7.81 (s, 1H), 7.52–7.48 (m, 4H), 7.47–7.32 (m, 3H), 7.32–7.20 (m, 3H), 7.15 (t, 2H, J = 8.2 Hz), 6.31 (br s, 1H), 5.60 (s, 2H), 5.08–4.76 (br s, 2H), 4.57–4.42 (m, 2H), 4.46 (t, 2H, J = 5.2 Hz), 4.30 (q, 2H, J = 7.0 Hz), 4.06–4.01 (br s, 2H), 3.92–3.80 (m, 2H), 3.77 (t, 2H, J = 5.3 Hz), 3.58–3.49 (m, 10H), 3.35 (t, 2H, J = 5.1 Hz), 3.13–3.10 (m, 2H), 1.33 (t, 3H, J = 7.3 Hz), 1.06 (t, 3H, J = 7.2 Hz).

4.32. Compound 24e

Yield after RP-HPLC purification: 22.9 mg (20.6 μmol, 41%). LC/MS analysis: t_R 7.73 min (linear gradient 10–90%B in 13.5 min; *m/z*: 998.5 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.90–8.50 (m, 3H), 7.74 (s, 1H), 7.52–7.30 (m, 6H) 7.32–7.15 (m, 6H), 6.23 (br s, 1H), 5.53 (s, 2H), 5.08–4.76 (br s, 2H), 4.57–4.32 (m, 4H), 4.23 (q, 2H, J = 7.0 Hz), 4.06–4.95 (br s, 2H), 3.90–3.70 (m, 2H), 3.77–3.24 (m, 18H) 3.09–3.03 (m, 2H), 1.26 (t, 3H, J = 7.3 Hz), 1.00 (t, 3H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{48}H_{57}N_{13}O_9F_2+H^+$: 998.44430, obsd 998.44574.

4.33. Compound 27a

Yield after RP-HPLC purification: 15.3 mg (8.7 μmol, 17%). LC/MS analysis: t_R 7.94 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1531.7 [M+H$^+$]). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.97 (s, 2H), 8.82 (br s, 4H), 7.82 (s, 2H), 7.50 (dd, 8H, J_1 = 25.4 Hz, J_2 = 8.5 Hz,), 7.45–7.41 (m, 4H), 7.39–7.35 (m, 4H), 7.28–7.20 (m, 6H), 7.14 (t, 2H, J = 8.1 Hz), 6.38 (br s, 2H), 5.60 (s, 4H), 4.89 (br s, 4H), 4.80 (s, 4H), 4.50 (br s, 4H), 4.29 (q, 4H, J = 6.9 Hz,), 4.05 (br s, 4H), 3.88 (br s, 4H), 3.11–3.09 (m, 4H), 1.32 (t, 6H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{78}H_{78}N_{20}O_{10}F_4+H^+$: 1531.62187, obsd 1531.62231.

4.34. Compound 27b

Yield after RP-HPLC purification: 41.9 mg (23.2 μmol, 46%). LC/MS analysis: t_R 8.04 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1575.5 [M+H$^+$]). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.91 (s, 2H), 8.67 (br s, 4H), 7.71 (s, 2H), 7.47–7.11 (m, 20H), 6.21 (br s, 2H), 5.56 (s, 4H, 4.89 (br s, 4H), 4.50 (br s, 4H), 4.39 (t, 4H, J = 5.2 Hz), 4.25 (q, 4H, J = 6.9 Hz), 4.05 (br s, 4H,), 3.88 (br s, 4H), 3.70 (m, 4H), 3.09–3.07 (m, 4H), 1.31 (t, 6H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{80}H_{82}N_{20}O_{11}F_4+H^+$: 1575.64808, obsd 1575.67161.

4.35. Compound 27c

Yield after RP-HPLC purification: 13.0 mg (7.0 μmol, 14%). LC/MS analysis: t_R 7.86 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1619.8 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 2H), 8.81(br s, 4H), 7.79 (s, 2H), 7.50 (dd, 8H, J_1 = 21.1 Hz, J_2 = 8.7 Hz), 7.47–7.41 (m, 4H), 7.39–7.35 (m, 4H), 7.28–7.19 (m, 6H), 7.15 (t, 2H, J = 8.2 Hz), 6.36 (br s, 2H,), 5.60 (s, 4H), 4.89 (br s, 4H), 4.50 (br s, 4H), 4.42 (t, 4H, J = 5.2 Hz), 4.29 (q, 4H, J = 6.9 Hz), 4.05 (br s, 4H), 3.88 (br s, 4H), 3.70 (t, 4H, J = 5.2 Hz), 3.43 (s, 4H), 3.13–3.09 (m, 4H), 1.31 (t, 6H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{82}H_{86}N_{20}O_{12}F_4+H^+$: 1619.67430, obsd 1619.68708.

4.36. Compound 27d

Yield after RP-HPLC purification: 32.1 mg (17.0 μmol, 34%). LC/MS analysis: t_R 7.95 min (linear gradient 10–90%B in 13.5 min; *m/z*: 1663.9 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 2H), 8.83 (br s, 4H), 7.80 (s, 2H), 7.51–7.48 (m, 8H), 7.47–7.41 (m, 4H), 7.39–7.35 (m, 4H), 7.28–7.19 (m, 6H), 7.15 (t, 2H, J = 8.2 Hz), 6.39 (br s, 2H), 5.60 (s, 4H), 4.87 (br s, 4H), 4.50 (br s, 4H), 4.44 (t, 4H, J = 5.5 Hz), 4.30 (q, 4H, J = 6.9 Hz), 4.05 (br s, 4H), 3.88 (br s, 4H), 3.73 (t, 4H), 3.43 (s, 4H), 3.38 (s, 4H), 3.13–3.09 (m, 4H), 1.32 (t, 6H, J = 7.1 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{84}H_{90}N_{20}O_{13}F_4$+H$^+$: 1663.70051, obsd 1663.72310.

4.37. Compound 27e

Yield after RP-HPLC purification: 20.0 mg (10.3 μmol, 21%). LC/MS analysis: t_R 7.85 min (linear gradient 10–90% B in 13.5 min; *m/z*: 1707.6 [M+H$^+$]). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.96 (s, 2H), 8.83 (br s, 4H), 7.73 (s, 2H), 7.51–7.07 (m, 24H), 6.20 (br s, 2H, 5.56 (s, 4H, CH_2 $C_6H_4F_2$), 5.10–4.65 (br s, 4H), 4.55–4.43 (br s, 4H), 4.38 (t, 4H, J = 4.5 Hz), 4.22 (q, 4H, J = 6.9 Hz), 4.02 (br s, 4H), 3.88 (br s, 4H), 3.69 (t, 4H, J = 5.0 Hz), 3.43–3.32 (m, 12H), 3.08–3.03 (m, 4H), 1.25 (t, 6H, J = 7.1 Hz), 0.99 (t, 6H, J = 7.2 Hz). HRMS *m/z* calcd for $C_{86}H_{94}N_{20}O_{14}F_4$+H$^+$: 1707.72673, obsd 1707.74903.

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