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Effect of Linker Length and Composition on Heterobivalent Ligand-Mediated Receptor Cross-Talk between the A_1 Adenosine and β_2 Adrenergic Receptors

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Heterobivalent ligands that possess pharmacophores designed to interact with both the A_1 adenosine receptor (A_1AR) and the β_2 adrenergic receptor (β_2AR) were prepared. More specifically, these ligands contain an adenosine moiety that is linked via its N^6-position to the amino group of the saligenin-substituted ethanolamine moiety present in the well-known β_2AR agonist, salbutamol. The affinities of these ligands were determined at both receptors and found to vary with linker length and composition. With all compounds, affinity and functional potencies were found to have selectivity for the A_1AR over the β_2AR. In all cases, cAMP accumulation (a β_2AR-mediated response) was mainly observed when the A_1AR was blocked or its function decreased by pertussis toxin or chronic agonist treatment. This suggests that heterobivalent compounds for receptors that mediate opposite responses might be useful for elucidating the mechanisms of receptor cross-talk and how this interaction, in terms of responsiveness, may change under pathophysiological conditions.

Introduction

Activation of membrane-bound receptors, such as G protein-coupled receptors (GPCRs), ligand-gated ion channels and catalytic receptors, results in a diverse range of outcomes depending on the nature of the ligand, as well as the associated effector domains or protein signalling partners. All play a vital role in cell regulation and the coordination of intracellular events with broader biological function, and their ubiquitous nature has made them the targets for over 40% of marketed pharmaceuticals.[1,2] Endogenous ligands for these receptors include small molecules, peptides and metal ions.

The complex interplay between intracellular signalling cascades that can share downstream signalling partners allows for potential receptor cross-talk. In this manner, activation of one receptor might result in the altered signalling of others, allowing unique downstream signalling outcomes dependent on the cell environment and activating ligand. However, whilst simultaneous activation of more than one membrane receptor is a common endogenous event, drugs targeting membrane receptors are often designed to be as selective as possible, in order to minimise off-target effects.

More recently, heterobivalent ligands have been investigated as a means of providing novel pharmacological outcomes through an ability to bind concomitantly to two different target receptors.[3–8] Such ligands incorporate two pharmacophores joined via a linker or spacer moiety, and might be able to bind in a bridging or top–tail fashion, providing simultaneous binding in a manner that might not be possible by coadministration of two independent drugs. Simultaneous binding at two different receptors has the potential to result in unique intracellular signalling responses and consequently unique downstream effects through altered recruitment of shared intracellular signalling partners.

Our research focuses on targeting the β_2 adrenergic receptor (β_2AR) and the A_1 adenosine receptor (A_1AR). On activation, both of these GPCRs cause differential effects on the transmembrane enzyme, adenylyl cyclase, which is responsible for catalysing the conversion of ATP to the secondary messenger cAMP. Agonist binding at these receptors increases the rate of trimeric G-protein dissociation, with the $G\alpha$ subunit going on to interact with adenylyl cyclase. The nature of the particular $G\alpha$ subunit isoform determines the subsequent signalling cascade that is recruited. In the case of the β_2AR, signalling via the $G\alpha_s$ subunit causes activation of adenylyl cyclase, whereas the A_1AR signals via the $G\alpha_i$ subunit, which is inhibitory towards adenylyl cyclase. Heterobivalent ligands that can simultaneously deliver an agonist moiety at each receptor can potentially modify the opposing effects on adenylyl cyclase to achieve a desired response. For example, the specific targeting of the βAR/A_1AR system arises from the observation in the cardiovascular system, where enhanced A_1AR activation might overly attenuate normal β adrenergic responsiveness or prevent some adverse effects from exogenously administered catecholamines.[9,10]

Previous work reported by our group has generated heterobivalent β_2AR–A_1AR ligands with full agonist profiles at both receptors, compared with known classical ligands.[3] Such ligands comprised of an adenosine moiety linked through the

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N^6-position via a short alkyl linker of 2, 4 or 6 carbon atoms to the amino component of a catecholamine derivative (Figure 1).[3] A biphasic cAMP response was observed when

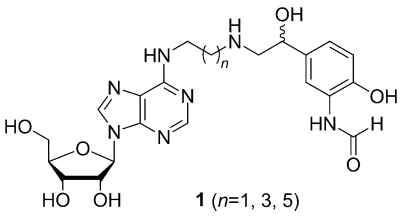


Figure 1. Heterobivalent ligands based on adenosine and fromoterol.

these compounds were presented to DDT_1 MF-2 cells expressing both receptors. At low ligand concentrations, β_2AR-mediated stimulation of cAMP accumulation dominated, due to the greater potency imparted by this pharmacophore. In contrast, A_1AR inhibition of cAMP accumulation dominated at higher ligand concentrations, due to higher population of A_1ARs and dominance of G_i over G_s.

The effect of linker lengths in bivalent ligands that allow bridging of two receptors has been discussed by numerous authors.[6,7,11–14] Reports by Portoghese and co-workers of an optimal linker length that allows bridging active sites in opioid dimers[4,5] led us to consider similar linker lengths in our own bivalent designs. Furthermore, the effect of linker type had not been explored in our previous work. The current investigation aims to look at the effect of linker-related structural considerations on potential β_2AR and A_1AR cross-talk.

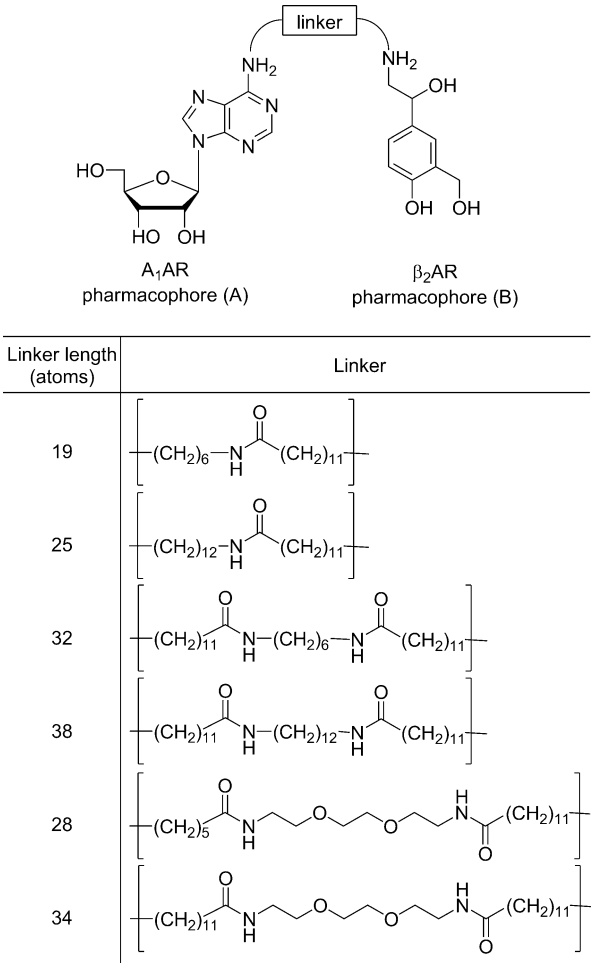


Figure 2. Heterobivalent target compounds with linked A_1R and β_2AR agonist pharmacophores.

Results and Discussion

Ligand design

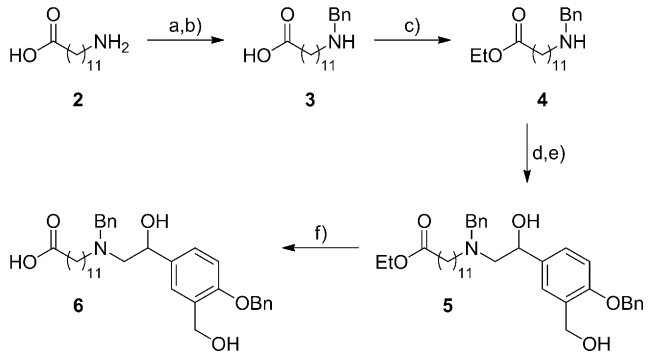
Bivalent ligands were designed to incorporate an A_1AR agonist pharmacophore and a β_2AR agonist pharmacophore, separated by a linker moiety (Figure 2). Adenosine (A) was chosen as the A_1AR agonist and appended to a linker moiety at the N^6-position. The incorporation of an alkyl group in the N^6-position is known to enhance A_1AR affinity and selectivity,[15] and alkyl linkers in this position have been used to append a wide range of moieties to adenosine.[16] The saligenin-based ethanolamine moiety (B) present in the well-known β_2AR agonist, salbutamol, was chosen as the β_2AR agonist pharmacophore and appended to the linker moiety through the side chain amine.

Linkers were designed to be between 19 and 38 atoms in chain length to provide for the possibility of cooperative binding. As diaminoalkanes are only commercially available in lengths of up to 12 methylene units, longer linker lengths were achieved by coupling diaminoalkanes to amino acids via amide bond formation. In our previous study,[3] we found that bivalent β_2AR–A_1AR ligands with lengthy alkyl linkers possessed poor solubility properties. Replacement of the alkyl chain with the corresponding polyethylene glycol (PEG) linker attached to N^6 atom of the adenosine pharmacophore (A) improved solubility, but also resulted in greatly decreased A_1AR affinity and potency.[3] Accordingly, in the current study linkers were designed with an alkyl chain attached to the N^6-position of the adenosine moiety (to maintain A_1AR potency), but also included a PEG component distal to the adenosine moiety to promote better solubility. A "monovalent control" comprised of the β_2AR pharmacophore joined to only linker was also prepared for pharmacology studies.

Chemistry

Bivalent ligands were prepared via a convergent approach where the β_2AR agonist moiety and A_1AR agonist moiety were prepared separately, then combined in the penultimate step of the synthesis.

The β_2AR agonist component of these ligands was prepared in six steps, starting with commercially available 1-aminododecanoic acid (**2**) (Scheme 1). Reductive alkylation of **2** with benzaldehyde under basic conditions afforded the benzylamine **3**. An ethanolic solution of **3** was then treated with oxalyl chloride to give the corresponding ethyl ester (**4**). Alkylation of the secondary amine moiety of **4** with 1-(4-(benzyloxy)-3-(hydroxy-



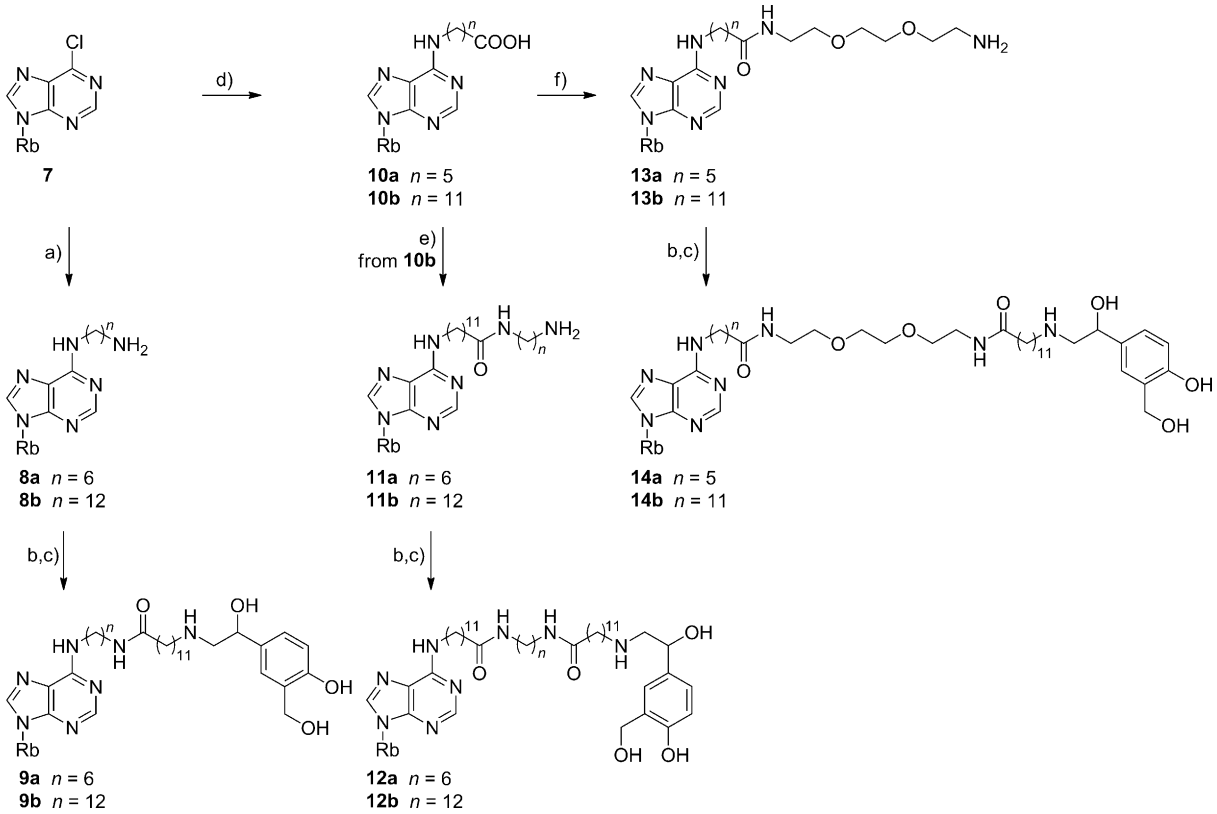
Scheme 1. *Reagents and conditions*: a) NaOH, benzaldehyde, MeOH, RT, 2 h; b) $NaBH_4$, EtOH, 0 °C→RT, 15 h; c) oxalyl chloride, EtOH, −77 °C, then DMF, RT, 15 h; d) 1-(4-(benzyloxy)-3-(hydroxymethyl)phenyl)-2-bromoethanone, DIPEA, $CHCl_3$, 15 h, RT; e) $NaBH_4$, EtOH, 0 °C→RT, 1 h; f) NaOH, MeOH, CH_2Cl_2, RT, 15 h.

methyl)phenyl)-2-bromoethanone[17] with direct sodium borohydride reduction of the resulting α-amino ketone provided amino alcohol **5**. Saponification of the ester moiety of **5** with sodium hydroxide in a solution of water, methanol and dichloromethane provided the desired β_2AR agonist moiety (**6**).

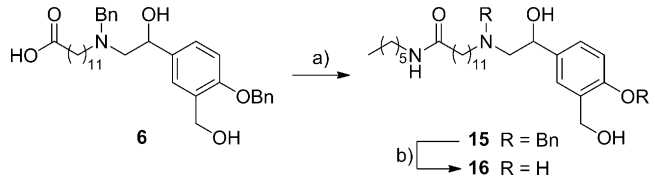
A_1AR agonist pharmacophores with a linker moiety containing a distal primary amine were prepared from commercially available 6-chloropurine riboside (**7**) (Scheme 2). Compounds **8a** and **8b** were prepared, using previously reported methodology, by treating **7** with the appropriate diaminoalkane.[18] Analogues with longer linkers were prepared by attachment of either 1-aminohexanoic acid or 12-aminododecanoic acid to the purine riboside core through an S_NAr displacement of the 6-chloro group to give **10a** and **10b**, respectively. Compound **10b** was extended via BOP-mediated coupling to either 1,6-aminohexane or 1,12-diaminododecane to provide **11a** and **11b**, respectively. Analogues with PEG chains were prepared similarly by BOP-mediated coupling of 2,2′-(ethylenedioxy)diethylamine to **10a** and **10b**, affording **13a** and **13b**.

The A_1AR agonist and β_2AR agonist components were then combined via a BOP-mediated amide coupling reaction. Subsequent cleavage of the *N*-benzyl protecting group by hydrogenolysis provided the desired dual pharmacophore compounds (Scheme 2). This reaction sequence was repeated, combining different A_1AR agonist units to the β_2AR agonist component (**6**) to provide the target compounds **9a**, **9b**, **12a**, **12b**, **14a** and **14b**.

A "monovalent" compound, comprised of β_2AR agonist component **6** and attached "linker" (but no adenosine moiety), was also prepared as a control. This compound (**16**) was obtained by BOP-mediated coupling of hexylamine with **6**, followed by N-debenzylation, as outlined in Scheme 3.



Scheme 2. *Reagents and conditions*: a) 1,6-diaminohexane or 1,12-diaminododecane, *t*BuOH, reflux, 15 h; b) **6**, BOP, Et_3N, DMF, RT, 15 h; c) H_2, $Pd(OH)_2$, NH_4OH, MeOH, RT, 15 h; d) 6-aminohexanoic acid or 12-aminododecanoic acid, DIPEA, *t*BuOH, reflux, 24 h; e) 1,6-diaminohexane or 1,12-diaminododecane, Et_3N, BOP, DMF, RT, 15 h; f) 2,2′-(ethylenedioxy)diethylamine, BOP, RT, 15 h.



Scheme 3. *Reagents and conditions*: a) hexylamine, BOP, Et_3N, DMF, RT, 15 h; b) H_2, $Pd(OH)_2$, NH_4OH, MeOH, RT, 15 h.

Pharmacology

Binding and cAMP functional assays were performed for both bivalent and monovalent control compounds. The affinity of each compound was determined by displacement binding assays using [^{125}I]-(−)iodopindolol for the β_2AR and [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine (DPCPX) for the A_1AR. The classical βAR agonist (−)-isoproterenol and A_1AR agonist N^6-cyclopentyladenosine (CPA) were used for comparison. As shown in Table 1, the monovalent β_2AR-only ligand control (**16**) exhibited a decreased affinity (6.3-fold) for the receptor as compared with (−)-isoproterenol. This decreased affinity might be attributed to the linker component, although a direct comparison of the *N*-alkyl substituent is not possible due to the different phenolic substituents. Bivalent compound **9a**, with a 19-atom linker, showed a 20.6-fold decrease in affinity compared with the monovalent control (**16**). Increasing the linker to 25 atoms (**9b**) resulted in an affinity similar to **16**, whereas bivalent compounds **12a** and **12b** with longer linkers of 32 and 38 atoms, respectively, had little or no interaction with the β_2AR at the highest concentration used (100 μM). A different linker effect on affinity was observed for the compounds that possessed a PEG component in their linker (**14a** and **14b**). Compound **14a**, which possessed a 28-atom linker, displayed mid-micromolar affinity, which was increased by 16.6-fold when the linker was extended to 34 atoms in compound **14b**.

At the A_1AR, the bivalent compounds with 19 and 25 linker atoms (**9a** and **9b**, respectively) showed a 1.6- and 1.8-fold decrease in affinity, as compared with CPA, whereas the bivalents with 32 and 37 linker atoms (**12a** and **12b**, respectively) showed only a very weak interaction with the receptor. In terms of the bivalent ligands with a PEG component in the linker (i.e., **14a** and **14b**), the compound with a 28-atom linker had a 27-fold lower affinity compared with CPA, whereas the compound with a 34-atom linker showed only a 3-fold decrease in affinity. All of the bivalent compounds, where determinations were made, showed a higher affinity for the A_1AR compared with the β_2AR. These ranged from a 2.9-fold higher affinity at the A_1AR for **9b** to 62-fold for **9a**.

The bivalent compounds were tested for their ability to inhibit and stimulate cAMP accumulation through the A_1AR and β_2AR, respectively. As indicated in Table 1, all of these compounds except **12b** inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner. In these assays, propranolol was added to prevent any ligand stimulation of cAMP accumulation through the β_2AR. The intrinsic activities of these compounds are equal to or greater than 92% of the maximal response produced by CPA indicating that they are approaching full agonist activity at the A_1AR. The bivalent compound with a 38-atom linker (**12b**) did not inhibit cAMP accumulation.

As shown in Figure 3, (−)-isoproterenol, salbutamol and monovalent control **16** stimulated cAMP accumulation in a concentration-dependent manner. All of the bivalent compounds alone produced little (<15% of the (−)-isoproterenol maximum) or no increase in cAMP accumulation. However, in the presence of DPCPX, added to block the A_1AR, a robust concentration-dependent stimulation of cAMP was observed except for **12b**, which was inactive either in the presence or absence of DPCPX (not shown on graph). The intrinsic activity of the monovalent control (**16**) and bivalent compounds **9a**, **9b**, **12a** and **14b** were less than that of (−)-isoproterenol (0.28–0.56)

Table 1. Intrinsic activities (IA), K_i and EC_{50} values of standard agonists and bivalent derivatives at the β_2 adrenergic (β_2A) and A_1 adenosine (A_1A) receptors.

Compd	*l*[a]	β_2A receptor			A_1A receptor		
		K_i [nM][b]	EC_{50} [nM][c]	IA[d]	K_i [nM][b]	EC_{50} [nM][c]	IA[d]
(−)-Isoproterenol		66 ± 8	12 ± 3	1.00	ND[e]	ND[e]	ND[e]
Salbutamol		ND[e]	93 ± 20	0.97 ± 0.09	ND[e]	ND[e]	ND[e]
CPA		ND[e]	ND[e]	ND[e]	85 ± 29	1.6 ± 0.1	1.00
16	–	413 ± 114	712 ± 89	0.56 ± 0.03	ND[e]	ND[e]	ND[e]
9a	19	8514 ± 1228	7789 ± 1713	0.43 ± 0.05	137 ± 39	168 ± 20	1.01 ± 0.01
9b	25	454 ± 171	2827 ± 620	0.47 ± 0.05	154 ± 39	624 ± 88	0.98 ± 0.02
12a	32	> 100 000[f]	7670 ± 1698	0.28 ± 0.03	> 100 000[f]	1885 ± 219	0.92 ± 0.02
12b	38	inactive	inactive	–	> 100 000[f]	inactive	–
14a	28	28 820 ± 9774	NC[g]	–	2323 ± 12	614 ± 89	0.98 ± 0.01
14b	34	1739 ± 226	4843 ± 1052	0.48 ± 0.07	256 ± 31	2226 ± 371	0.98 ± 0.03

[a] Linker length (*l*). [b] K_i values were calculated from the ligand concentration required to inhibit specific binding of [^{125}I]-(−)iodopindolol to the β_2AR or [^{3}H]-DPCPX to the A_1AR by 50%. Values are the mean ± SEM of n = 3–7 separate determinations. [c] β_2AR EC_{50} values are the ligand concentration required to stimulate cAMP accumulation by 50% in the presence of DPCPX (10 μM), while A_1AR EC_{50} values are the ligand concentration required to inhibit forskolin (1.0 μM)-stimulated cAMP accumulation by 50% in the presence of propranolol (1.0 μM). [d] Intrinsic activity (IA) is the maximal stimulation (β_2AR) or inhibition (A_1AR) of cAMP accumulation as compared with (−)-isoproterenol or N^6-cyclopentyladenosine (CPA), respectively, which are set at 1.00. Data are the mean ± SEM of n = 3–7 separate determinations. [e] ND: not determined. [f] Less than 50% inhibition of radioligand binding at concentrations up to 100 μM. [g] NC: EC_{50} could not be determined as the stimulation did not approach a plateau at the highest concentration used (100 μM).

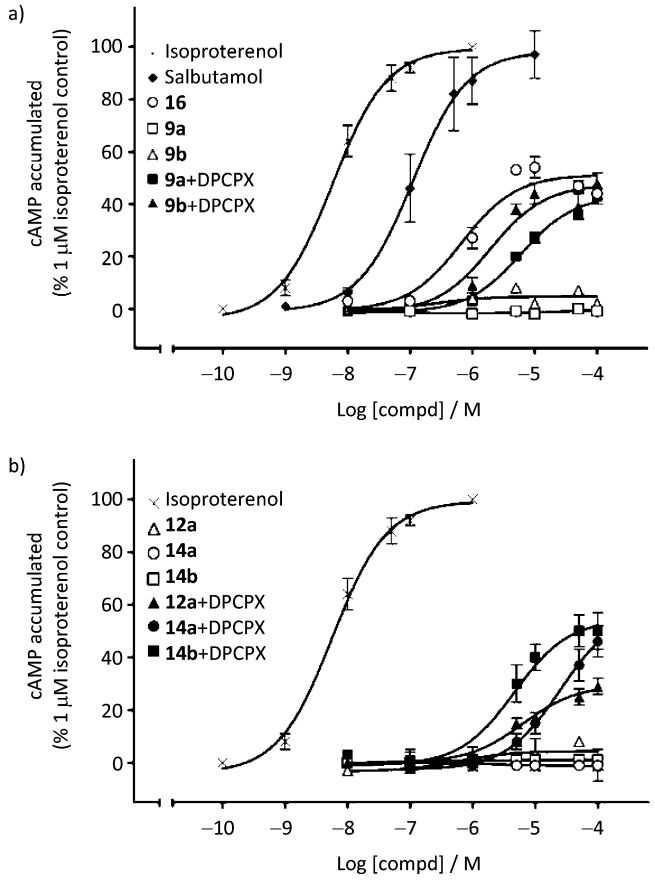


Figure 3. Effect of isoproterenol, salbutamol, compound **16** and the bivalent derivatives on cAMP accumulation in DDT_1 MF-2 cells. Cells were incubated with rolipram (20 μM) and the indicated concentration of test compound for 10 min at 36 °C. In parallel assays with the bivalent compounds, DPCPX (10 μM) was included. Each data point on the graphs is the mean ± SEM of $n = 3$–7 separate experiments. The isoproterenol data is the same on both graphs.

To further investigate a paradigm where bivalent ligand stimulation of cAMP accumulation can be unmasked, the effect of partial desensitization of the A_1AR was investigated. Compound **9b** was chosen as its functional potency for the two receptors was relatively close (4.5-fold), suggesting that changes in agonist potency for the A_1AR might unmask β_2AR stimulation. Pretreatment of DDT cells with 200 nM CPA for 18 h followed by six cell wash cycles to remove the CPA resulted in an 11.6-fold decrease in the EC_{50} value for CPA to inhibit forskolin-stimulated cAMP accumulation without a change in the maximal inhibition (control: $EC_{50} = 1.4 \pm 0.2$; CPA pretreated: $EC_{50} = 16.3 \pm 4.1$ nM, $n = 3$). As depicted in Figure 4, compound **9b** produced less than 20% stimulation of cAMP accumulation alone, and this stimulation was greatly increased in the presence of DPCPX. In CPA pretreated cells, **9b** alone initially stimulated cAMP accumulation to about 75% of the maximum stimulation in the presence of DPCPX followed by a decrease at higher **9b** concentrations. The stimulation of cAMP accumulation in the presence of DPCPX was the same in both control and CPA-pretreated cells. This data show that partial desensitization of the A_1AR can result in a substantial unmasking of a bivalent β_2AR response.

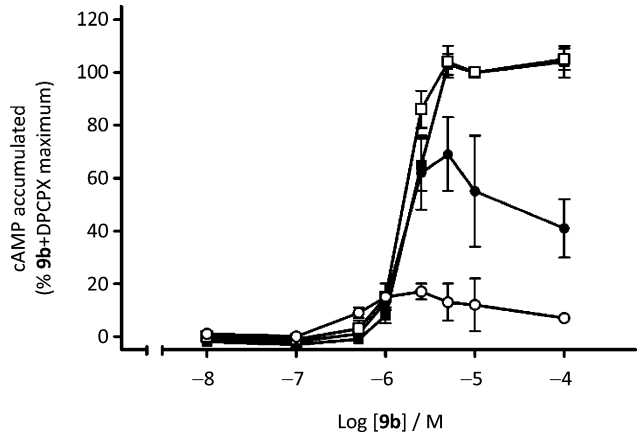


Figure 4. Effect of N^6-cyclopentyladenosine (CPA) pretreatment on **9b**-stimulated cAMP accumulation in DDT_1 MF-2 cells. Cells were incubated without (open symbols) or with (filled symbols) CPA (200 nM) for 18 h and then washed six times to remove the CPA. Cells were then incubated with rolipram (20 μM) and the indicated concentration of **9b** without or with DPCPX (1 μM) for 10 min at 36 °C. Each point on the graph is the mean ± SEM $n = 4$ separate experiments.

indicating that they are partial β_2AR agonists (Table 1). Although bivalent compound **14a** also showed stimulation of cAMP accumulation in the presence of DPCPX, no EC_{50} or intrinsic activity value could not be determined as the maximal stimulation was not reached at the highest concentration used (100 μM). The stimulation of cAMP accumulation by 50 μM of each of the active bivalent compounds in the presence of DPCPX was blocked by 10 μM propranolol, which is consistent with this activity being mediated by the β_2AR. In addition, pretreatment of DDT cells with pertussis toxin (100 ng mL^{-1} for 18 h) to uncouple the A_1AR from G_i and prevent the cAMP inhibitory effect of this receptor resulted in stimulation of cAMP accumulation by the bivalent compounds alone, similar to that observed in untreated cells in the presence of DPCPX (data not shown). This finding is consistent with the inhibitory effect on cAMP accumulation being mediated through the A_1AR. Similar to the affinity selectivity, the potency of the bivalent compounds (EC_{50} values) was greater for the A_1AR inhibition of cAMP accumulation compared with the β_2AR stimulatory response.

The current study extends our previous work showing that covalent linking of pharmacophores for the β_2AR and the A_1AR into a single compound can retain activity at both receptors.[3] Using a limited series of compounds, the effects of extended linker length and composition on affinity and agonist properties were investigated. In general, the bivalent compounds had decreased affinities and functional potencies for each receptor as compared with the classical βAR and A_1AR agonists, with greater decreases for the β_2AR. Varying the linker length had some interesting effects on the affinity of the bivalent compounds for the β_2AR. In terms of the bivalent ligands with link-

ers consisting predominantly of methylene units (i.e., **9a**, **9b**, **12a** and **12b**), the compound that contains a 25-atom linker (**9b**) showed optimal affinity, which was decreased when the linker was either shortened (19 atoms) or lengthened (32 and 38 atoms). A similar effect related to linker length was observed for functional potency at the β_2AR. A different pattern emerged with the A_1AR, where both affinity and potency of compounds **9a**, **9b**, **12a** and **12b** decreased with increasing linker length. Previous studies have provided evidence for simultaneous bridging across two receptors by a single bivalent molecule, partly based upon increases in ligand affinity for their targets.[19–21] In our present work, the observation that there was no parallel increase in affinity for the two receptors with the bivalents **9a**, **9b**, **12a** and **12b** suggests that simultaneous bridging across two receptors did not occur.

In the bivalent ligands that possessed a PEG component in the linker (**14a** and **14b**), a 9- to 16-fold increase in affinity for both receptors was observed when the linker length was increased from 28 to 34 atoms. This could be consistent with bridging across two receptors, though additional experimental approaches would be needed to further establish if this is actually the case. Although the affinity increased with increasing linker length, the potency for inhibiting cAMP accumulation was decreased. This difference between the changes in affinity and potency might be due to the composite nature of potency, which is dependent upon both affinity and efficacy (relationship between receptor occupancy and response) with efficacy perhaps being a more dominant factor in the potency change with increasing linker length. Ligand efficacy might also contribute to the observed differences between the affinity (K_i) and potency (EC_{50}) values for the other bivalent compounds tested (Table 1) and has been reported previously for another series of bivalent ligands for the β_2AR/A_1AR.[3] However, other factors might have contributed towards the observed differences in affinity and potency. For example, the assay conditions for measuring affinity (isolated membranes) and potency (intact cells) were different.

With one exception, the bivalent compounds alone had little or no effect on cAMP accumulation in DDT cells. However, when the A_1AR was blocked with an antagonist or uncoupled from its G_i protein with pertussis toxin, a robust stimulation of cAMP accumulation was observed with the majority of compounds. This stimulation appears to be mediated by activation of the β_2AR, as presence of the βAR antagonist, propranolol, prevented the effect. Previous studies have shown that A_1AR-mediated inhibition of cAMP accumulation in DDT cells is dominant over β_2AR stimulation.[3,22] As these bivalent compounds have higher affinity and functional potencies for the A_1AR over the β_2AR, the lack of β_2AR stimulation in the absence of DPCPX or with G protein-uncoupled A_1AR is likely due to their preferential binding to and activation of the inhibitory A_1AR. Interestingly, all of the active bivalent compounds were full agonists at the A_1AR, but partial agonists at the β_2AR. This partial agonist activity appears to involve the linker moiety, as salbutamol-derived monovalent control **16** was also found to be a partial agonist, whereas salbutamol is a full β-agonist.

GPCRs are under constant regulation, with their expression and sensitivity being altered under changing conditions through a variety of mechanisms. We sought to investigate if one potential alteration can affect the response profile of a bivalent compound. In order explore this possibility the A_1AR system was desensitized by chronic treatment with an A_1AR agonist and a bivalent response subsequently determined. As expected, desensitization not only decreased the potency with which CPA inhibits cAMP accumulation, but also led to an unmasking of the ability of a bivalent compound alone to stimulate cAMP accumulation. The unmasking of cAMP accumulation occurred at the lower bivalent concentrations and was followed by a decrease in activity at the higher concentrations likely due to A_1AR activation.

In a previous report, heterobivalent β_2AR and A_1AR agonists with much shorter linkers had higher affinities for the β_2AR or similar affinities for the two receptors.[3] These compounds alone produced biphasic cAMP responses with stimulation at lower concentrations followed by inhibition as the concentrations were increased. Although these compounds were full agonists at both receptors, for several, the A_1AR-mediated inhibitory component imparted partial β_2AR agonist properties. In the present work, using a different β_2AR pharmacophore and extending the linker length resulted in A_1AR selectivity with a predictable masking of β-stimulation unless the A_1AR was blocked, uncoupled from its G protein or partly desensitized. Furthermore, these bivalents were direct partial β_2AR agonists. Taken together, the data from these studies suggest that the potency and response characteristics of heterobivalent compounds for receptors producing opposite responses can be designed for selective effect by altering the spacing and/or linker composition between the two pharmacophore head groups. Furthermore, these compounds might be useful for investigative studies on how receptor cross-talk for opposing responses is regulated during health and disease.

Experimental Section

General procedures: Melting points (mp) were determined on a Mettler Toledo MP50 melting point system and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Ultrashield 400 Plus at 400 MHz and 101 MHz, respectively. Analytical HPLC was performed on a Waters Alliance 2690 fitted with a Waters 5996 photodiode array (PDA) detector and a Phenomenex Luna C_8 column (5 μm, 100 Å, 150×4.60 mm). Analyses were conducted using a gradient of 0→64% MeCN in H_2O over 10 min with 0.1% TFA throughout. All reagents were of >95% purity. Preparatory HPLC was performed on a Waters Prep LC 4000 system fitted with a Waters 486 Tuneable Absorbance Detector and either a Phenomenex Luna C_8 (10 μm, 100 Å, 250×30 mm) column or a Phenomenex Luna C_{18} (10 μm, 100 Å, 50×21.2 mm) column. Low-resolution mass spectrometry (LRMS) was performed on an Agilent 6120 single quadrapole LCMS system using electron spray ionization (ESI). High-resolution mass spectrometry (HRMS) was performed on a Waters Premier XE time-of-flight (ToF) mass spectrometer using ESI.

12-(Benzylamino)dodecanoic acid (3): A suspension of 12-aminododecanoic acid (**2**) (1 g, 4.6 mmol) in MeOH (25 mL) was treated

with freshly crushed NaOH (220 mg, 5.6 mmol). After the suspension dissolved, benzaldehyde (560 μL, 5.6 mmol) was added and the solution stirred for 2 h at RT. The reaction was then cooled on ice, and $NaBH_4$ (200 mg, 5.3 mmol) was added batchwise. The reaction was allowed to warm to RT and then stirred for an additional 15 h. The solution was adjusted to pH 6 with concd $HCl_{(aq)}$, and the solvent was removed in vacuo. Acetone (50 mL) was added and the suspension sonicated. The precipitate was isolated by filtration, washing with water (2.0 mL) then acetone (2.0 mL), to provide the title compound as a white solid (1.6 g, 94%): mp: 151–153 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.47–7.37 (m, 5H), 4.08 (s, 2H), 2.87 (t, J = 8.0 Hz, 2H), 2.21 (t, J = 7.3 Hz, 2H), 1.72–1.19 ppm (m, 18H); ^{13}C NMR (101 MHz, CD_3OD): δ = 179.9, 131.9, 130.3, 130.0, 129.7, 51.7, 47.8, 36.4, 29.4, 29.3, 29.3, 29.2, 29.1, 28.9, 26.7, 26.4, 26.1 ppm; LRMS (ESI+): m/z (%): 306 $[M+H]^+$ (100).

Ethyl 12-(benzylamino)dodecanoate (4): A solution of **3** (500 mg, 1.6 mmol) in abs EtOH at −77 °C was treated with oxalyl chloride (500 μL, 5.8 mmol). The solution was stirred and allowed to come to RT. DMF (1 drop) was added and the solution stirred at RT for a further 15 h. The solvent was removed in vacuo, saturated aq $NaHCO_3$ (50 mL) was added, and the solution extracted with CH_2Cl_2 (2×50 mL). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to provide the title product as a white solid (486 mg, 91%): mp: 85–91 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.28–7.13 (m, 5H), 4.04 (q, J = 7.1 Hz, 2H), 3.72 (br s, 2H), 2.55 (t, J = 7.2 Hz, 1H), 2.20 (t, J = 7.6 Hz, 1H), 2.10 (br s, 1H), 1.60–1.16 ppm (m, 13H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.9, 140.1, 128.4, 128.2, 127.0, 77.4, 77.1, 76.8, 60.1, 53.9, 49.4, 34.4, 29.9, 29.6, 29.5 (3C), 29.4, 29.3, 29.1, 27.3, 25.0, 14.3 ppm; LRMS (ESI+): m/z (%): 334.4 $[M+H]^+$ (100).

Ethyl 12-(benzyl(2-(4-(benzyloxy)-3-(hydroxymethyl)phenyl)-2-hydroxyethyl)amino)dodecanoate (5): A stirred solution of **4** (185 mg, 0.56 mmol) and DIPEA (300 μL, 1.7 mmol) in $CHCl_3$ (5 mL) was treated with 1-(4-(benzyloxy)-3-(hydroxymethyl)phenyl)-2-bromoethanone (185 mg, 0.25 mmol). The solution was stirred for 15 h at RT. The solvent was removed in vacuo at 25 °C, and the residue was redissolved in cold (0 °C) EtOH (10 mL). $NaBH_4$ (100 mg, 2.6 mmol) was added, and after the initial effervescence, the reaction was allowed to come to RT. The solution was stirred for 1 h, and the solvent was removed in vacuo at 25 °C. The residue was redissolved in $CHCl_3$ (50 mL), and the solution was washed with water (3×50 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography (EtOAc/petroleum spirits) to provide the title compound as a colourless oil (150 mg, 46%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.49–7.10 (m, 12H), 6.93 (d, J = 8.4 Hz, 1H), 5.10 (s, 2H), 4.73–4.62 (m, 3H), 4.10 (q, J = 7.1 Hz, 2H), 3.71 (d, J = 13.4 Hz, 1H), 3.60 (d, J = 13.4 Hz, 1H), 2.74–2.39 (m, 4H), 2.27 (t, J = 7.4 Hz, 2H), 1.66–1.13 ppm (m, 21H); ^{13}C NMR (101 MHz, CD_3OD): δ = 175.6, 156.7, 140.5, 138.8, 136.6, 130.9, 130.3, 129.5, 129.2, 128.8, 128.3, 128.1, 127.4, 127.2, 112.6, 72.1, 71.1, 63.3, 61.4, 60.5, 60.2, 55.3, 35.1, 30.7, 30.6 (3C), 30.5, 30.4, 30.2, 28.3, 27.9, 26.0, 14.6 ppm; LRMS (ESI+): m/z (%): 589.4 $[M+H]^+$ (100).

12-(Benzyl(2-(4-(benzyloxy)-3-(hydroxymethyl)phenyl)-2-hydroxyethyl)amino)dodecanoic acid (6): A solution of **5** (600 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was treated with 3 M methanolic NaOH (2.00 mL, 6.0 mmol). The solution was stirred for 15 h at RT, neutralized with 3 M methanolic HCl, and the solvent was removed in vacuo. The resulting crude product was purified by flash chromatography (CH_2Cl_2/MeOH + Et_3N, 1:4:0.005) to provide the title product as a colourless oil (350 mg, 61%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.50–7.13 (m, 12H), 6.94 (d, J = 8.4 Hz, 1H), 5.14–5.11 (m, 2H), 4.71–4.61 (m, 3H), 3.71 (d, J = 13.4 Hz, 1H), 3.61 (d, J = 13.4 Hz, 1H), 2.75–2.39 (m, 4H), 2.28 (t, J = 7.4 Hz, 2H), 1.65–1.52 (m, 2H), 1.50–1.38 (m, 2H), 1.33–1.12 ppm (m, 14H); ^{13}C NMR (101 MHz, CD_3OD): δ = 178.5, 184.8, 168.7, 167.0, 164.8, 159.0, 158.4, 157.7, 157.4, 157.0, 156.5, 156.2, 155.5, 155.4, 140.7, 100.2, 99.2, 91.4, 89.5, 88.6, 83.5, 63.3, 58.8 (2C), 58.7 (2C), 58.5, 58.3, 56.5, 56.1, 54.2 ppm; LRMS (ESI+): m/z (%): 562.5 $[M+H]^+$ (100).

6-Chloropurine riboside (**7**) was commercially available. N^6-(6-Aminohexyl)adenosine (**8a**) and N^6-(12-aminododecanyl)adenosine (**8b**) were prepared using a previously reported methodology, by treating **7** with the appropriate diaminoalkane.[18] Characterisation data were as reported in the literature.

***N*^{6}-(6-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)hexyl)adenosine (9a)**: A solution of **8a** (231 mg, 0.63 mmol) in DMF (1 mL) was treated with **6** (154 mg, 0.27 mmol), DIPEA (430 μL, 2.4 mmol) and BOP (280 mg, 0.63 mmol). The reaction was stirred for 15 h at RT. The solution was then diluted with EtOAc (100 mL) and washed with water (5× 50 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting crude was then purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to provide N^6-(6-(12-((2-hydroxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)benzylamino) dodecanamido)hexyl)adenosine as a colourless oil (66 mg, 26%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.22 (s, 1H), 8.19 (s, 1H), 7.46–7.26 (m, 11H), 7.16 (dd, J = 8.4, 2.2 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 5.94 (d, J = 6.5 Hz, 1H), 5.11 (s, 2H), 4.76–4.69 (m, 4H), 4.68 (s, 2H), 4.32 (dd, J = 5.1, 2.5 Hz, 1H), 4.22–4.11 (m, 1H), 3.92 (d, J = 13.4 Hz, 1H), 3.88 (dd, J = 12.6, 2.5 Hz, 1H), 3.81 (d, J = 13.4 Hz, 1H), 3.73 (dd, J = 12.6, 2.5 Hz, 1H), 3.57 (br s, 2H), 3.16 (t, J = 6.9 Hz, 2H), 2.86–2.59 (m, 4H), 2.15 (t, J = 7.4 Hz, 2H), 1.75–1.16 ppm (m, 26H); ^{13}C NMR (101 MHz, CD_3OD): δ = 176.3, 157.1, 156.8, 153.5, 149.7, 141.5, 138.8, 138.0, 135.9, 131.1, 130.9, 129.6, 129.5, 128.9, 128.8, 128.4, 127.3, 127.1, 121.3, 112.7, 91.3, 88.3, 75.5, 72.7, 71.2, 71.1, 63.6, 62.4, 60.4, 59.8, 55.3, 41.5, 40.3, 37.2, 30.6 (3C) 30.4 (3C) 30.2, 28.2, 27.7, 27.7, 27.1, 27.0 ppm; LRMS (ESI+): m/z (%): 910.5 $[M+H]^+$ (100).

A solution of N^6-(6-(12-((2-benzyloxy-2-(4-benzyloxy-3-hydroxymethyl)-phenyl)ethyl)amino)dodecanamido)hexyl)adenosine (55 mg, 0.061 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 2.1 mg, 0.003 mmol). This suspension was stirred for 15 h under a H_2 atmosphere at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size), and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over 40 min) to give the title compound as a white foam (17 mg, 39%); ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 8.21 (s, 1H), 8.07 (s, 1H), 7.21 (s, 1H), 7.09 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 5.87 (d, J = 6.7 Hz, 1H, 1H), 4.76–4.69 (m, 4H), 4.65 (s, 2H), 4.32 (dd, J = 5.1, 1.9 Hz, 1H), 4.21 (d, J = 2.0 Hz, 1H), 3.91 (dd, J = 12.7, 2.0 Hz, 1H), 3.73 (dd, J = 12.7, 2.0 Hz, 1H), 3.55 (br s, 1H), 3.14 (t, J = 7.0 Hz, 1H), 2.88–2.59 (m, 4H), 2.13 (t, J = 7.6 Hz, 2H), 1.91 (s, 3H), 1.74–1.19 ppm (m, 26H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 176.1, 156.1, 155.8, 153.4, 148.7, 141.2, 134.1, 128.5, 127.0, 126.9, 121.3, 115.8, 91.3, 88.1, 75.3, 72.6, 71.9, 63.4, 61.0, 56.9, 49.8, 41.4, 40.2, 37.1, 30.4 (3C), 30.3 (2C), 30.2, 30.1, 29.2, 27.9, 27.6, 27.5, 26.9 ppm; HPLC: 98% pure (t_R = 8.0 min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{37}H_{60}N_7O_8$: 730.4498, found: 730.4479.

***N*^{6}-(12-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)dodecyl)adenosine (9b)**: A solution of **8b** (285 mg, 0.63 mmol) in DMF (1 mL) was treated with **6**

(178 mg, 0.32 mmol), DIPEA (430 μL, 2.5 mmol) and BOP (280 mg, 0.63 mmol). The solution was stirred for 15 h at RT. The solution was then diluted with EtOAc (100 mL) and washed with water (5× 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting crude was purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to provide N^6-(12-(12-((2-hydroxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)benzylamino)dodecanamido)dodecyl)adenosine as a colourless oil (130 mg, 41%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (s, 1H), 8.16 (s, 1H), 7.43–7.18 (m, 11H), 7.14 (dd, J = 8.4, 2.2 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 5.91 (d, J = 6.5 Hz, 1H), 5.08 (s, 2H), 4.73 (dd, J = 6.5, 5.1 Hz, 1H), 4.69 (s, 2H), 4.67–4.62 (m, 1H), 4.32 (dd, J = 5.1, 2.3 Hz, 1H), 4.23–4.16 (m, 1H), 3.89 (dd, J = 12.7, 2.3 Hz, 1H), 3.78 (d, J = 13.4 Hz, 1H), 3.73 (dd, J = 12.7, 2.3 Hz, 1H), 3.60 (d, J = 13.4 Hz, 1H), 3.54 (s, 2H), 3.13 (t, J = 7.0 Hz, 2H), 2.73–2.43 (m, 4H), 2.14 (t, J = 7.4 Hz, 2H), 1.73–1.14 ppm (m, 38H); ^{13}C NMR (101 MHz, CD_3OD): δ = 175.9, 156.4, 156.0, 153.2, 148.5, 141.0, 139.4, 138.3, 135.8, 130.6, 130.1, 129.3, 129.1, 128.6, 128.1, 128.0, 127.0, 126.9, 121.2, 112.4, 91.3, 88.1, 75.2, 72.5, 71.3, 70.9, 63.3, 62.8, 60.4, 59.7, 55.0, 41.5, 40.2, 37.0, 30.4 (6C), 30.3, 30.2 (6C), 30.0, 28.1, 27.8, 27.7, 27.5, 26.9 ppm; LRMS (ESI+): m/z (%): 994.5 $[M+H]^+$ (100).

A solution of N^6-(12-(12-((2-benzyloxy-2-(4-hydroxy-3-(benzyloxymethyl)phenyl)ethyl) amino)dodecanamido)dodecyl)adenosine (85 mg, 0.091 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 3.2 mg, 0.006 mmol). The suspension was stirred for 15 h under a H_2 atmosphere at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size), and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over 40 min) to give the title compound as a white foam (52 mg, 70%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 8.21 (s, 1H), 8.06 (s, 1H), 7.21 (s, 1H), 7.09 (d, J = 8.3 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 5.87 (d, J = 6.7 Hz, 1H), 4.79–4.71 (m, 2H), 4.66 (s, 2H), 4.32 (dd, J = 5.1, 1.9 Hz, 1H), 4.21 (d, J = 2.0 Hz, 1H), 3.91 (dd, J = 12.7, 2.0 Hz, 1H), 3.72 (dd, J = 12.7, 2.0 Hz, 1H), 3.54 (br s, 2H), 3.13 (t, J = 7.2 Hz, 2H), 2.92–2.70 (m, 4H), 2.15–2.08 (m, 2H), 1.92 (s, 3H), 1.72–1.21 ppm (m, 38H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 176.2, 156.3, 156.2, 153.7, 148.7, 141.4, 134.1, 128.5, 127.4, 127.2, 121.7, 116.4, 91.9, 88.6, 75.5, 72.9, 71.8, 63.9, 61.9, 56.9, 50.0, 42.0, 40.7, 37.6, 30.8 (4C), 30.7, 30.6 (4C), 30.5, 29.2, 28.2 (3C), 28.2, 27.3 ppm; HPLC: 98% pure (t_R = 9.5 min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{43}H_{72}N_7O_8$: 814.5437, found: 814.5441.

N^6-(5-Carboxypentyl)adenosine (10a): A solution of **7** (500 mg, 1.8 mmol) and DIPEA (620 μL, 7.0 mmol) in *t*BuOH (50 mL) was treated with 6-aminohexanoic acid (660 mg, 3.5 mmol). The solution was heated at reflux for 24 h and then concentrated in vacuo. When an attempt was made to partition the residue between 1 M aq HCl (100 mL) and CH_2Cl_2 (100 mL), a precipitate formed. This precipitate was collected by suction filtration to provide the title compound as an amorphous solid (280 mg, 41%): mp: 151–155 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 8.21 (s, 1H), 8.01 (s, 1H), 5.84 (d, J = 6.8 Hz, 1H), 4.75 (dd, J = 6.8, 5.1 Hz, 1H), 4.31 (dd, J = 5.1, 1.7 Hz, 1H), 4.22 (dd, J = 2.0, 1.7 Hz, 1H), 3.91 (dd, J = 12.8, 2.0 Hz, 1H), 3.72 (dd, J = 12.8, 2.0 Hz, 1H), 3.56 (br s, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.80–1.41 ppm (m, 6H); ^{13}C NMR (101 MHz, CD_3OD): δ = 177.1, 155.4, 152.8, 147.8, 140.5, 120.9, 91.2, 87.8, 74.5, 72.1, 63.1, 40.9, 34.5, 29.4, 26.8, 25.1 ppm; LRMS (ESI+): m/z (%): 382.2 $[M+H]^+$ (100).

N^6-(11-Carboxyundecyl)adenosine (10b): A solution of **7** (500 mg, 1.8 mmol) and DIPEA (620 μL, 7.0 mmol) in *t*BuOH (50 mL) was treated with 12-aminododecanoic acid (750 mg, 3.5 mmol). The solution was heated at reflux for 24 h and then concentrated in vacuo. The residue was partitioned between 1 M HCl (100 mL) and CH_2Cl_2 (100 mL) and filtered. The precipitate was retained, and the organic portion of the filtrate was separated, washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated to dryness in vacuo. The resulting residue along with the retained precipitate were combined and purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to provide the title compound as a white amorphous solid (704 mg, 84%): mp: 145–149 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 8.22 (s, 1H), 8.07 (s, 1H), 5.86 (d, J = 6.7 Hz, 1H), 4.74 (dd, J = 6.7, 5.1 Hz, 1H), 4.31 (dd, J = 5.1, 1.9 Hz, 1H), 4.21 (dd, J = 2.1, 1.9 Hz, 1H), 3.91 (dd, J = 12.8, 2.1 Hz, 1H), 3.73 (dd, J = 12.8, 2.1 Hz, 1H), 3.55 (br s, 2H), 2.25 (t, J = 7.5 Hz, 2H), 1.73–1.21 ppm (m, 18H); ^{13}C NMR (101 MHz, CD_3OD): δ = 177.3, 155.4, 152.8, 147.7, 140.4, 120.9, 91.3, 87.8, 74.5, 72.1, 63.1, 41.1, 34.5, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 27.2, 25.3 ppm; LRMS (ESI+): m/z (%): 466.3 $[M+H]^+$ (100).

N^6-(11-((6-Aminohexylamino)formyl)undecyl)adenosine (11a): A solution of 1,6-diaminohexane (73 mg, 0.63 mmol) in DMF (500 μL) was treated with **10b** (98 mg, 0.21 mmol), Et_3N (88 μL, 0.63 mmol) and BOP (186 mg, 0.42 mmol). The solution was stirred for 15 h at RT then diluted with water (5 mL) and purified by preparatory HPLC on a C_{18} column (0–20% MeCN in H_2O with 0.1% TFA over 40 min) to provide the title compound as a colourless oil (30 mg, 25%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (s, 1H), 8.02 (s, 1H), 5.90 (d, J = 5.3 Hz, 1H), 4.58 (br s, 1H), 4.37–4.23 (m, 1H), 4.14 (br s, 1H), 3.84 (d, J = 12.4 Hz, 1H), 3.71 (d, J = 12.4 Hz, 1H), 3.50 (br s, 1H), 3.09 (t, J = 7.0 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.08 (t, J = 7.4 Hz, 2H), 1.81–1.10 ppm (m, 22H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 175.7, 158.1, 153.3, 148.5, 141.5, 120.5, 90.9, 87.5, 75.4, 71.8, 62.7, 42.0, 40.0, 39.5, 36.8, 30.0 (4C), 29.9, 29.8 (3C), 29.5, 27.7, 26.6, 26.5, 26.3 ppm; LRMS (ESI+): m/z (%): 664.3 $[M+H]^+$ (100).

N^6-(11-((12-Aminododecanylamino)formyl)undecyl)adenosine (11b): A solution of 1,12-diaminohexane (126 mg, 0.63 mmol) in DMF (500 μL) was treated with **10b** (98 mg, 0.21 mmol), Et_3N (88 μL, 0.63 mmol) and BOP (186 mg, 0.42 mmol). The solution was stirred for 15 h at RT then diluted with water (5 mL) and purified by preparatory HPLC on a C_{18} column (0–30% MeCN in H_2O with 0.1% TFA over 40 min) to provide the title compound as a colourless oil (45 mg, 32%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.25 (s, 1H), 8.00 (s, 1H), 5.81 (d, J = 6.9 Hz, 1H), 4.57 (br s, 1H), 4.20 (br s, 1H), 4.06 (s, 1H), 3.90 (d, J = 12.2 Hz, 1H), 3.76 (d, J = 12.2 Hz, 1H), 3.56 (br s, 2H), 3.13 (t, J = 7.2 Hz, 2H), 2.85 (t, J = 7.4 Hz, 2H), 2.13 (d, J = 7.6 Hz, 2H), 1.83–1.16 ppm (m, 38H); ^{13}C NMR (101 MHz, CD_3OD): δ = 175.4, 155.5, 153.3, 149.7, 141.3, 120.4, 90.9, 87.5, 75.2, 71.7, 62.6, 40.1, 39.9, 36.8, 29.9 (3C), 29.8, 29.7 (3C), 29.5, 27.9, 27.3, 26.8, 26.4 ppm; LRMS (ESI+): m/z (%): 648.5 $[M+H]^+$ (100).

N^6-(11-((6-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)hexylamino)formyl)undecyl)adenosine acetate (12a): A solution of **11a** (60 mg, 0.11 mmol) in DMF (1 mL) was treated with **6** (120 mg, 0.21 mmol), Et_3N (74 μL, 0.53 mmol) and BOP (49 mg, 0.11 mmol). The solution was stirred for 15 h at RT then diluted with EtOAc (100 mL) and washed with water (5×50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting crude was purified by flash chromatography (MeOH/CH_2Cl_2, 1:20) to provide N^6-(11-(1-(6-(12-((2-hydroxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)-benzylamino)dodecanamido)hexananamino)formyl)undecyl)adenosine as a colourless oil (70 mg, 59%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ = 8.21 (s, 1H), 7.97 (s, 1H), 7.43–7.23 (m, 11H), 7.12 (dd, J = 8.4, 2.1 Hz, 1H), 6.86 (d, J = 8.4 Hz, 1H), 5.82 (d, J = 6.8 Hz, 1H), 5.05 (s, 2H), 4.75 (dd, J = 6.8, 5.2 Hz, 1H), 4.68 (s, 2H), 4.65–4.57 (m, 2H),

4.24–4.21 (m, 1H), 3.91 (dd, J=12.8, 2.8 Hz, 1H), 3.89 (d, J=13.4 Hz, 1H), 3.71 (dd, J=12.8, 2.8 Hz, 1H), 3.60 (d, J=13.4 Hz, 1H), 3.53 (s, 2H), 3.12 (t, J=7.0 Hz, 4H), 2.46–2.70 (m, 4H), 2.12 (t, J=7.6 Hz, 4H), 1.74–1.17 ppm (m, 44H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ=176.0, 175.7, 157.0, 156.4, 153.8, 148.6, 141.3, 139.9, 138.3, 135.5, 131.0, 130.4, 129.9, 129.8, 129.3, 128.6, 128.6, 127.5, 127.3, 121.9, 112.9, 92.4, 88.8, 75.3, 73.1, 71.5, 70.8, 64.1, 63.5, 61.8, 59.9, 55.3, 42.0, 40.4, 37.9, 37.9, 36.8, 34.8, 30.9 (4C), 30.8 (4C) 30.7 (4C), 30.6 (4C), 30.4, 28.6 (2C), 28.2, 28.1, 27.5, 27.3, 26.6 ppm; LRMS (ESI+): m/z (%): 554.5 $[M+2H]^{2+}$/2 (100); 1107.9 $[M+H]^+$ (15).

A solution of N^6-(11-(1-(6-(12-((2-benzyloxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)hexylamino)formyl)undecyl)adenosine (75 mg, 0.068 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 2.4 mg, 0.003 mmol). The suspension was shaken in a Parr hydrogenator under a H_2 atmosphere (350 kPa) for 2 d at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size) and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over 40 min) to provide the title compound as a white foam (20 mg, 32%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ=8.21 (s, 1H), 8.14 (s, 1H), 7.26 (s, 1H), 7.12 (d, J=8.3 Hz, 1H), 6.77 (d, J=8.3 Hz, 1H), 5.90 (d, J=6.6 Hz, 1H), 4.83–4.79 (m, partially obscured by H_2O signal, 1H), 4.75–4.70 (m, partially obscured by H_2O signal, 1H), 4.66 (s, 2H), 4.32 (dd, J=5.1, 2.2 Hz, 1H), 4.20 (d, J=2.2 Hz, 1H), 3.90 (dd, J=12.7, 2.2 Hz, 1H), 3.74 (dd, J=12.7, 2.2 Hz, 1H), 3.55 (br s, 2H), 3.14 (t, J=7.0 Hz, 4H), 2.95 (d, J=7.3 Hz, 2H), 2.84 (t, J=7.6 Hz, 2H), 2.14 (t, J=7.5 Hz, 4H), 1.91 (s, 3H), 1.73–1.19 ppm (m, 48H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ=175.8 (2C), 155.9, 155.7, 153.1, 148.2, 140.9, 133.0, 128.2, 126.7, 126.5, 115.8, 91.3, 88.0, 75.0, 72.3, 70.7, 63.3, 61.0, 55.8, 41.4, 39.9, 37.0, 30.3, 30.2 (4C), 30.1 (3C), 30.0 (2C), 29.9 (2C), 27.9, 27.6, 27.5, 27.1, 26.7 (2C), 23.0 ppm; HPLC: 99% pure (t_R=9.3 min); HRMS (ESI+): m/z $[M+2H]^{2+}$/2 calcd for $C_{49}H_{84}N_8O_9$: 464.3175, found: 464.3167.

N^6-(11-((12-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)dodecanamino)formyl)undecyl)adenosine (12b): A solution of **11b** (200 mg, 0.31 mmol) in DMF (1 mL) was treated with **7** (200 mg, 0.36 mmol), Et_3N (215 μL, 1.5 mmol) and BOP (138 mg, 0.31 mmol). The solution was stirred for 15 h at RT then diluted with EtOAc (100 mL), washed with water (5×50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting crude was then purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:20) to provide N^6-(11-(1-(12-(12-((2-hydroxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)benzylamino)dodecanamido)dodecanamino)formyl)undecyl)adenosine as a colourless oil (150 mg, 70%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ=8.16 (s, 1H), 8.00 (s, 1H), 7.36–7.19 (m, 11H), 7.10 (dd, J=8.5, 2.1 Hz, 1H), 6.84 (d, J=8.5 Hz, 1H), 5.83 (d, J=6.7 Hz, 1H), 5.02 (s, 2H), 4.71 (dd, J=6.7, 5.0 Hz, 1H), 4.69–4.63 (m, 2H), 4.29 (dd, J=5.0, 1.9 Hz, 1H), 4.18 (dd, J=1.9, 12.7 Hz, 1H), 3.96 (d, J=13.4 Hz, 1H), 3.87 (dd, J=12.7, 1.9 Hz, 1H), 3.72 (d, J=13.3 Hz, 1H), 3.68 (dd, J=12.7, 1.9 Hz, 1H), 3.48 (br s, 2H), 3.09 (t, J=7.2 Hz, 4H), 2.79–2.51 (m, 4H), 2.09 (t, J=7.5 Hz, 4H), 1.67–1.10 ppm (m, 56H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ=175.3, 175.3, 156.1, 155.4, 152.8, 147.7, 140.5, 137.5, 136.2, 134.2, 130.3, 130.1, 129.1, 128.9, 128.5, 128.3, 127.6, 126.5, 126.1, 120.9, 112.0, 91.2, 87.7, 74.6, 72.1, 70.4, 69.6, 63.1, 61.5, 60.2, 58.8, 54.4, 41.1, 40.0, 39.9, 36.8, 36.8, 30.0, 29.9, 29.9, 29.9, 29.8, 29.7, 29.7, 29.6, 27.5, 27.3, 27.3, 26.4, 26.2 ppm; LRMS (ESI+): m/z (%): 596.5 $[M+2H]^{2+}$/2 (100).

A mixture of N^6-(11-(1-(12-(12-((2-hydroxy-2-(4-benzyloxy-3-(benzyloxymethyl)phenyl)ethyl)amino)dodecanamido)dodecanamino)formyl)undecyl)adenosine (150 mg, 0.13 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was heated at reflux until dissolution was observed. $Pd(OH)_2$ (20% on carbon, 5.4 mg, 0.006 mmol) was added, and the solution was further heated at reflux under a hydrogen atmosphere for 2 h. The solution was cooled and diluted with CH_2Cl_2 (5 mL) before being filtered through a nylon membrane filter (0.45 μm) and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over 40 min) to provide the title compound as a white foam (71 mg, 56%): ^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$, 1:1): δ=8.21 (s, 1H), 8.07 (s, 1H), 7.21 (s, 1H), 7.10 (d, J=8.3 Hz, 1H), 6.76 (d, J=8.3 Hz, 1H), 5.87 (d, J=6.7 Hz, 1H), 4.74 (dd, J=6.7, 5.2 Hz, 1H), 4.71–4.73 (m, partially obscured by H_2O signal, 1H), 4.66 (s, 2H), 4.32 (dd, J=5.2, 1.9 Hz, 1H), 4.21 (dd, J=2.0, 1.9 Hz, 1H), 3.91 (dd, J=12.7, 2.0 Hz, 1H), 3.73 (dd, J=12.7, 2.0 Hz, 1H), 3.54 (br s, 2H), 3.13 (t, J=7.2 Hz, 4H), 2.90–2.64 (m, 4H), 2.13 (t, J=7.5 Hz, 4H), 1.73–1.16 ppm (m, 56H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ=176.2, 156.4, 156.2, 153.7, 148.7, 141.4, 134.2, 128.4, 127.4, 127.2, 121.8, 116.5, 92.0, 88.6, 75.5, 72.9, 72.0, 63.9, 62.1, 57.1, 50.6, 42.0, 40.7, 37.6, 30.8 (11C), 30.6 (10C), 30.5, 29.4, 28.3, 28.2 (2C), 27.3 ppm (2C); HPLC: 99% pure (t_R=10.5 min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{55}H_{95}N_8O_9$: 1011.7217, found: 1011.7200.

N^6-(5-((2-(2-(2-Aminoethyloxy)ethoxy)ethylamino)formyl)pentyl)adenosine (13a): A solution of **10a** (130 mg, 0.34 mmol) in DMF (200 μL) was treated with 2,2′-(ethylenedioxy)diethylamine (219 mg, 0.36 mmol) and BOP (315 mg, 0.71 mmol). The solution was stirred for 15 h at RT then diluted with water (1 mL) and purified by preparatory HPLC on a C_8 column (0–25% MeCN in H_2O with 0.1% TFA over 40 min) to provide the title compound as a white foam (76 mg, 44%): ^{1}H NMR (400 MHz, CD_3OD): δ=8.21 (s, 1H), 8.16 (s, 1H), 5.91 (d, J=6.6 Hz, 1H), 4.74 (dd, J=6.6, 5.2 Hz, 1H), 4.33 (dd, J=5.2, 2.1 Hz, 1H), 4.20 (dd, J=2.1 Hz, 1H), 3.90 (dd, J=12.7, 2.3 Hz, 1H), 3.74 (dd, J=12.7, 2.3 Hz, 1H), 3.69–3.64 (m, 2H), 3.64–3.61 (m, J=1.0 Hz, 4H), 3.58 (br s, 2H), 3.53 (t, J=5.7 Hz, 2H), 3.35 (t, J=5.7 Hz, 2H), 3.05 (t, J=5.0 Hz, 2H), 2.21 (t, J=7.5 Hz, 2H), 1.77–1.59 (m, 4H), 1.52–1.37 ppm (m, 2H); ^{13}C NMR (101 MHz, CD_3OD): δ=175.8, 155.9, 153.1, 148.4, 141.0, 121.2, 91.2, 88.0, 75.1, 72.4, 71.0, 70.9, 70.4, 68.1, 63.3, 40.3, 40.2, 39.8, 36.7, 27.1, 26.3, 23.9 ppm; LRMS (ESI+): m/z (%): 512.3 $[M+H]^+$ (100).

N^6-(11-((2-(2-(2-Aminoethyloxy)ethoxy)ethylamino)formyl)undecyl)adenosine (13b): 2,2′-(Ethylenedioxy)diethylamine (1.6 mL, 11 mmol) at 0 °C was treated with **10b** (1.00 g, 2.1 mmol) followed by BOP (1.9 g, 4.3 mmol). The reaction was stirred for 15 h at RT. The solution was then diluted with water (5 mL) and purified by preparatory HPLC on a C_{18} column (0–40% MeCN in H_2O over 40 min with 0.1% NH_4OH) to provide the title compound as a white amorphous solid (1.2 g, 94%): mp: 147–150 °C; ^{1}H NMR (400 MHz, [D_6]DMSO): δ=8.33 (s, 1H), 8.19 (s, 1H), 7.87 (br s, 1H), 7.82 (d, J=5.7 Hz, 1H), 5.87 (d, J=6.2 Hz, 1H), 5.44 (br s, 2H), 5.18 (br s, 1H), 4.60 (dd, J=6.2, 4.6 Hz, 1H), 4.14 (dd, J=4.6, 3.1 Hz, 1H), 3.96 (dd, J=3.1, 3.4 Hz, 1H), 3.67 (dd, J=12.0, 3.4 Hz, 1H), 3.54 (d, J=12.0, 3.4 Hz, 1H), 3.51–3.41 (m, 8H), 3.38 (t, J=5.3 Hz, 2H), 3.34 (t, J=5.8 Hz, 2H), 3.17 (dt, J=5.7, 5.3 Hz, 2H), 2.62 (t, J=5.8 Hz, 1H), 2.03 (t, J=7.4 Hz, 1H), 1.64–1.19 ppm (m, 18H); ^{13}C NMR (101 MHz, CD_3OD/$CDCl_3$, 1:1): δ=175.5, 154.8, 152.0, 149.0, 141.7, 121.0, 90.4, 87.8, 75.6, 72.2, 71.2, 71.0, 70.5, 67.8, 63.1, 41.7, 40.3, 39.9, 36.9, 30.5 (6C), 30.3, 30.1, 27.8, 26.8 ppm; HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{28}H_{48}N_7O_7$: 596.3766, found: 596.3785.

N^6-(5-((2-(2-(2-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)-phenyl)ethyl)amino)dodecanamido)ethyloxy)ethoxy)ethylamino)formyl)pentyl)adenosine acetate (14a): A solution of **13a** (180 mg, 0.35 mmol) in DMF (1 mL) was treated with **7** (200 mg, 0.36 mmol), Et_3N (150 μL, 1.1 mmol) and BOP (315 mg, 0.71 mmol). The solution was stirred for 15 h at RT then diluted with water (1 mL) and purified directly by preparatory HPLC on a C_{18} column (0–30% MeCN in H_2O with 0.1% TFA over 40 min) to provide the title compound N^6-(5-((2-(2-(2-(12-((2-benzyloxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)ethyloxy)ethoxy)ethylamino)formyl)pentyl)adenosine as a white foam (96 mg, 26%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.24 (s, 1H), 8.20 (s, 1H), 7.47–7.22 (m, 12H), 7.18 (dd, J = 8.4, 1.9 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 5.98 (d, J = 6.5 Hz, 1H), 5.08 (s, 2H), 4.83–4.74 (m, 2H), 4.72 (s, 2H), 4.36 (dd, J = 5.0, 2.4 Hz, 1H), 4.20 (d, J = 2.3 Hz, 1H), 4.06 (d, J = 13.4 Hz, 1H), 3.96 (d, J = 13.3 Hz, 1H), 3.90 (dd, J = 12.5, 2.3 Hz, 1H), 3.75 (dd, J = 12.5, 2.3 Hz, 1H), 3.58 (s, 4H), 3.56–3.54 (br s, 2H), 3.54–3.50 (m, 4H), 3.38–3.32 (m, 4H), 2.99–2.70 (m, 4H), 2.25–2.13 (m, 4H), 1.74–1.19 ppm (m, 24H); ^{13}C NMR (101 MHz, CD_3OD): δ = 176.2, 176.0, 156.7, 156.1, 153.5, 148.8, 141.3, 138.6, 136.1, 135.4, 131.1, 129.7, 129.4, 129.4, 128.8, 128.2, 127.0, 126.8, 121.3, 112.6, 91.2, 88.1, 75.5, 72.6, 71.2, 70.9, 70.5, 63.4, 61.7, 60.3, 60.1, 59.4, 55.0, 41.4, 40.2, 37.0, 36.8, 30.5 (2C), 30.4 (2C), 30.3, 30.2, 30.1, 27.9, 27.4, 26.9, 26.6, 26.3 ppm; LRMS (ESI+): m/z (%): 528.3 $[M+2H]^{2+}$/2 (100); 1055.6 $[M+H]^+$ (10).

A solution of N^6-(5-((2-(2-(2-(12-((2-benzyloxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)ethyloxy)ethoxy)ethylamino)formyl)pentyl)adenosine (90 mg, 0.085 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 3.0 mg, 0.004 mmol). The suspension was stirred for 15 h under a H_2 atmosphere at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size) and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over 40 min) to give the title compound as a colourless oil (65 mg, 87%): ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1:1): δ = 8.20 (s, 1H), 8.11 (s, 1H), 7.25 (s, 1H), 7.11 (d, J = 8.2 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 5.90 (d, J = 6.6 Hz, 1H), 4.88–4.80 (m, 1H), 4.73–7.74 (m, 1H), 4.66 (s, 2H), 4.34 (dd, J = 5.0, 2.0 Hz, 1H), 4.21 (d, J = 2.0 Hz, 1H), 3.90 (dd, J = 12.7, 2.0 Hz, 1H), 3.73 (dd, J = 12.6, 2.0 Hz, 1H), 3.59 (br s, 6H), 3.52 (t, J = 5.4 Hz, 4H), 3.35 (t, J = 5.1 Hz, 4H), 3.12–2.85 (m, 4H), 2.25–2.11 (m, 4H), 1.92 (s, 3H), 1.74–1.21 ppm (m, 24H); ^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$, 1:1): δ = 179.7, 175.6 (2C), 175.4, 140.7, 132.2, 128.1, 126.6, 126.4, 121.0, 115.8, 91.1, 87.8, 78.7, 78.4, 78.0, 74.9, 72.2, 70.7, 70.2, 69.5, 63.1, 61.0, 54.8, 48.5, 41.0, 39.7, 36.8, 36.6, 30.0 (2C), 29.9 (2C), 29.8, 29.7, 27.2, 27.0, 26.7, 26.4, 26.1, 23.9 ppm; HPLC: 99% pure (t_R = 8.5 min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{43}H_{71}N_8O_{11}$: 875.5237, found: 875.5267.

N^6-(11-((2-(2-(2-(12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)-phenyl)ethyl)amino)dodecanamido)ethyloxy)ethoxy)ethylamino)formyl)undecyl)adenosine acetate (14b): A solution of **13b** (215 mg, 0.36 mmol) in DMF (500 μL) was treated with **6** (200 mg, 0.36 mmol), Et_3N (150 μL, 1.1 mmol) and BOP (31 mg, 0.71 mmol). The solution was stirred for 15 h at RT then diluted with water (1 mL) and purified directly by preparatory HPLC on a C_{18} column (0–30% MeCN in H_2O with 0.1% TFA over 40 min) to provide N^6-(11-((2-(2-(2-(12-((2-hydroxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)benzylamino)dodecanamido)ethyloxy)ethoxy)ethylamino)-formyl)undecyl)adenosine as a white foam (95 mg, 23%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (s, 1H), 8.08 (s, 1H), 7.62–7.21 (m, 12H), 7.16 (dd, J = 8.5, 2.1 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 5.88 (d, J = 6.8 Hz, 1H), 5.07 (s, 2H), 4.88 (br s, 1H), 4.74 (dd, J = 6.8, 5.2 Hz, 1H), 4.70 (s, 2H), 4.48 (d, J = 13.2 Hz, 1H), 4.37 (d, J = 13.2 Hz, 1H), 4.32 (dd, J = 5.2, 1.9 Hz, 1H), 4.21 (d, J = 1.9 Hz, 1H), 3.90 (dd, J = 12.7, 2.1 Hz, 1H), 3.73 (dd, J = 12.7, 2.1 Hz, 1H), 3.60 (br s, 2H), 3.59 (s, 4H), 3.52 (t, J = 5.5 Hz, 4H), 3.36 (d, J = 5.5 Hz, 4H), 3.24–3.07 (m, 4H), 2.26–2.10 (m, 4H), 1.85–1.14 ppm (m, 36H); ^{13}C NMR (101 MHz, CD_3OD): δ = 175.6, 156.6, 155.6, 152.9, 147.9, 140.7, 137.5, 132.8, 131.6, 130.9, 130.8, 130.0, 129.7, 129.0, 128.4, 127.7, 126.4, 125.8, 121.0, 112.3, 91.2, 87.8, 74.7, 72.2, 70.6, 70.5, 70.1, 68.0, 63.1, 60.0, 59.2, 58.3, 54.5, 41.2, 39.6, 36.8, 30.1 (3C), 30.0 (3C), 29.9, 29.8, 29.7, 29.5, 27.4, 27.0, 26.4, 26.3, 24.1 ppm; LRMS (ESI+): m/z (%): 554.5 $[M+2H]^{2+}$/2 (100); 1107.9 $[M+H]^+$ (20).

A solution of N^6-(11-(1-(2-(2-(2-(12-((2-benzyloxy-2-(4-benzyloxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamido)ethyloxy)ethoxy)ethylamino)formyl)undecyl)adenosine (85 mg, 0.075 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 2.6 mg, 0.003 mmol). The suspension was stirred for 15 h under a H_2 atmosphere at at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size) and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–20% MeCN in H_2O with 0.1% AcOH over 40 min) to provide the title compound as a colourless oil (39 mg, 55%): ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1:1): δ = 8.21 (s, 1H), 8.03 (s, 1H), 7.20 (s, 1H), 7.09 (d, J = 8.2 Hz, 1H), 6.76 (d, J = 8.2 Hz, 1H), 5.85 (d, J = 6.6 Hz, 1H), 4.82 (dd, J = 6.6, 4.5 Hz, 1H), 4.77–4.72 (m, 1H), 4.66 (s, 2H), 4.32 (dd, J = 4.5, 1.4 Hz, 1H), 4.22 (d, J = 1.4 Hz, 1H), 3.91 (dd, J = 12.7, 1.6 Hz, 1H), 3.72 (dd, J = 12.7, 1.6 Hz, 1H), 3.59 (br s, 6H), 3.52 (t, J = 5.3 Hz, 4H), 3.36 (t, J = 5.3 Hz, 4H), 3.02–2.78 (m, 4H), 2.15 (t, J = 7.6 Hz, 4H), 1.92 (s, 3H), 1.71–1.19 ppm (m, 36H); ^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$, 1:1): δ = 179.6, 175.4, 155.5, 152.9, 147.7, 140.5, 132.3, 127.6, 126.5, 126.2, 120.9, 115.7, 91.2, 87.8, 74.6, 72.1, 70.5, 70.2, 69.6, 63.1, 61.3, 55.0, 48.5, 41.1, 39.5, 36.7, 30.0 (3C), 29.9 (3C) 29.8 (3C), 29.7, 29.6, 27.3, 27.1, 26.9, 26.3, 26.2 (2C), 23.9 ppm; HPLC: 99% pure (t_R = 7.3 min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{49}H_{83}N_8O_{11}$: 959.6176, found: 959.6179.

N^6-Hexyl-12-((2-hydroxy-2-(4-benzoxy-3-(hydroxymethyl)phenyl)ethyl)benzylamino)dodecanamide (15): A solution of hexylamine (70 μg, 0.53 mmol) in DMF (500 μL) was treated with **6** (200 mg, 0.36 mmol), Et_3N (150 μL, 1.1 mmol) and BOP (115 mg, 0.71 mmol). The reaction was stirred for 15 h at RT. The solution was then diluted with EtOAc (100 mL) and washed with water (5×50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to provide the title compound as a colourless oil (120 mg, 52%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.39–7.07 (m, 12H), 6.81 (d, J = 8.4 Hz, 1H), 5.01 (s, 2H), 4.63 (s, 2H), 4.56–4.49 (m, 1H), 3.82 (d, J = 13.4 Hz, 1H), 3.41 (d, J = 13.4 Hz, 1H), 3.17–3.10 (m, 2H), 2.60–2.28 (m, 4H), 2.05 (d, J = 2.9 Hz, 2H), 1.67–1.08 (m, 26H), 0.80 ppm (t, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD): δ = 174.9, 157.2, 138.4, 137.3, 135.1, 131.2, 131.2, 130.0, 129.6, 129.6, 129.0, 128.5, 127.3, 127.2, 62.4, 61.0, 59.6, 55.3, 41.5, 40.3, 36.8, 30.8 (3C), 30.7 (3C), 28.4, 27.2, 26.9, 26.7, 23.1, 14.5 ppm; LRMS (ESI+): m/z (%): 545.5 $[M+H]^+$ (100).

N^6-Hexyl-12-((2-Hydroxy-2-(4-hydroxy-3-(hydroxymethyl)phenyl)ethyl)amino)dodecanamide (16): A solution of **15** (100 mg, 0.18 mmol) and 18 M aq NH_4OH (20 μL, 0.36 mmol) in MeOH (5 mL) was treated with $Pd(OH)_2$ (20% on carbon, 6.4 mg, 0.009 mmol). The suspension was stirred for 15 h under a H_2 atmosphere at RT. The mixture was then diluted with CH_2Cl_2 (5 mL), filtered through a nylon membrane filter (0.45 μm pore size) and concentrated in vacuo to yield a colourless oil. This oil was purified by preparatory HPLC on a C_{18} column (0–45% MeCN in H_2O with 0.1% AcOH over

40 min) to give the title compound as a white foam (17 mg, 39%): ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1:1): $\delta = 7.21$ (s, 1H), 7.09 (d, $J =$ 8.3 Hz, 1H), 6.75 (d, $J = 8.3$ Hz, 1H), 4.69–4.64 (m, 3H), 3.13 (t, $J =$ 7.1 Hz, 2H), 2.83–2.51 (m, 4H), 2.14 (t, $J = 7.5$ Hz, 2H), 1.64–1.21 (m, 26H), 0.87 ppm (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (101 MHz, 101 MHz, $CD_3OD/CDCl_3$, 1:1): $\delta = 175.6$, 155.4, 134.4, 127.7, 126.7, 126.6, 115.7, 72.4, 61.4, 57.4, 50.0, 40.0, 36.9, 32.1, 30.1, 30.1, 30.1 (3C), 30.0 (3C), 29.9, 29.8, 27.9, 27.2, 26.6, 23.2, 14.3 ppm; HPLC: 95% pure ($t_R = 9.9$ min); HRMS (ESI+): m/z $[M+H]^+$ calcd for $C_{27}H_{49}N_2O_4$: 465.3687, found: 465.3687.

Pharmacology

Cell culture and cAMP assay: DDT_1 MF-2 cells were grown in 150 mm plastic dishes using Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS), streptomycin (0.1 mg mL^{-1}), penicillin G (100 U mL^{-1}) and amphotericin B (2.5 μg mL^{-1}) in a humidified atmosphere of 95% air and 5% CO_2. Cells were subcultured twice weekly and used in experiments at one-day preconfluence. Experiments were started by aspirating the DMEM and rinsing the cells with warm Hank's balanced salt solution (HBSS). The cells were then gently detached by using HBSS containing EDTA (1 mM) and washed twice in HBSS by centrifugation (1000 rpm for 5 min) and gentle resuspension. The final cell pellet was resuspended in HBSS and diluted to 1×10^6 cells mL^{-1}. The cAMP content of the cells was determined using a HitHunter cAMP XS+ assay kit (EFC chemiluminescence; DiscovRx Corp., Fremont, USA) according to the manufacturer's instructions with slight modifications. HBSS containing rolipram (5 μM), adenosine deaminase (0.5 U mL^{-1}) and with and without varying concentrations of test compounds were added to 384-well plates followed by cells (5000/well) and cAMP antibody, and then the plates were incubated for 10 min at 37 °C. At the end of the incubation, lysis buffer and luminescence reagents were added according to the manufacturer's instructions, and the samples were incubated overnight at room temperature. The luminescence was determined using a BioTek Synergy 2 plate reader. The amount of cAMP per well was measured from a standard curve determined in parallel incubations containing known amounts of cAMP (1 nM to 10 μM). All test compounds were dissolved in DMSO with an equal volume of solvent added to control incubations. The final DSMO concentration was 5%.

Receptor binding assays: The preparation of DDT_1 MF-2 cell membranes and the ability of the test compounds to displace [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine from the A_1AR were determined as previously described.[22] The ability of the test compounds to displace [^{125}I]-(−)-iodopindolol from the β_2AR in the DDT cell membranes was determined as previously described.[3] For both receptor binding assays, 5′-guanylyl-imidodiphosphate (10 μM) was included to maintain the receptors in the agonist low-affinity state.

Data analysis: The concentration of test compounds that stimulated or inhibited cAMP accumulation (EC_{50}) was determined by nonlinear regression analysis of the concentration–response curves using the GraphPad Prism 3.0 program. The intrinsic activity (IA) of the compounds for stimulating or inhibiting cAMP accumulation was calculated as a fraction of the maximal stimulation produced by (−)-isoproterenol or maximal inhibition produced by CPA. The concentration of compounds that inhibited radioligand binding by 50% (IC_{50}) was also determined by the GraphPad program. The dissociation constant (K_i) of the compounds for each receptor was then calculated using the IC_{50} values as described by Cheng and Prusoff.[23]

Abbreviations

(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP); *N*,*N*-diisopropylethylamine (DIPEA); *N*,*N*-dimethylformamide (DMF); ethylenediaminetetraacetic acid (EDTA); trifluoroacetic acid (TFA).

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Keywords: A_1 adenosine receptors · β_2 adrenergic receptors · combined pharmacophores · heterobivalent ligands · structure–activity relationships

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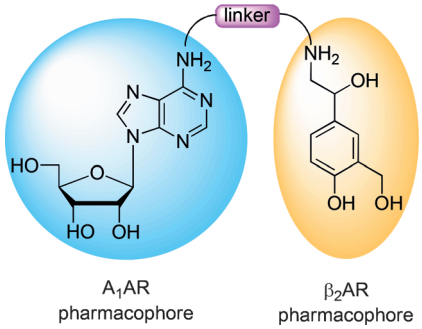
Effect of Linker Length and Composition on Heterobivalent Ligand-Mediated Receptor Cross-Talk between the A_1 Adenosine and β_2 Adrenergic Receptors



A molecular double date: Heterobivalent ligands containing pharmacophores designed to interact with both the A_1 adenosine receptor (A_1AR) and the β_2 adrenergic receptor (β_2AR) were prepared. The affinity and potency of these ligands at both receptors were found to be dependent upon the linker length and composition. The data suggest that heterobivalent ligands for receptors mediating opposite responses might be useful for investigating the regulation of receptor cross-talk in health and disease.