Synthesis of Bivalent β_2-Adrenergic and Adenosine A_1 Receptor Ligands

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Research in the area of simutaneously targeting more than one G protein-coupled receptor (GPCR) has increased in recent times. By exploiting the cross talk between the β_2-adrenergic (β_2AR) and adenosine A_1 receptors (A_1AR) on adenylate cyclase activity, we synthesized a series of bivalent agonists for both GPCRs to generate responses from more than one receptor. We have demonstrated a relationship between the various β_2-adrenergic and A_1 adenosine bivalent parameters of linker and bifunctionality by using data that are drawn from in vitro assays. The hexyl-linked **12e** (K_i, 311 nM) and butyl-linked **12c** (K_i, 863 nM) bivalent compounds displayed reasonable binding affinities for the β_2AR when compared with the control (−)isoproterenol (K_i, 136 nM), and both compounds also exhibited a persuasive bifunctional trend for both receptors at various drug concentrations. The bivalent compound **12e** was also found to have significant EC_{50} potency (6 nM) at the β_2AR in DDT cells.

Introduction

A bivalent ligand is a single chemical entity that is composed of two covalently linked pharmacophores. There are two general types of bivalent ligands: homobivalent, where the two pharmacophores are the same, and heterobivalent, where the two pharmacophores are different.[1−3] Heterobivalent ligands can have pharmacophores that bind to the same or to different molecular targets.[2−4] Bivalent compounds can have enhanced receptor subtype selectivity,[1,2,5] and in the case where target cross-linking has been implicated, they have an enhanced affinity and can be used to estimate the distance between targets or their spatial distribution.[1,3,6,7] Furthermore, bivalent ligands have been shown to increase biological activity through the activation of different receptors that mediate the same effect and, in the case of an agonist/antagonist bivalent ligand for the same receptor, the ability to produce partial agonism.[2,4] From a therapeutic perspective, bivalent ligands may have pharmacokinetic and efficacy advantages compared with multiple drug regimens.

The cross talk between different G protein-coupled receptors (GPCRs)[a] allows the regulation of cellular responses from several extracellular mediators. A classic example of receptor cross talk is the bidirectional effects on adenylate cyclase activity. There are a number of GPCRs that either stimulate or inhibit adenylate cyclase through the activation of G proteins G_s or G_i, respectively, and in some cases, the G_i coupled receptors can inhibit the ability of G_s coupled receptors to stimulate the enzyme.[8] In several cell types, the activation of β-adrenergic receptors (βAR) stimulates adenylate cyclase, which is attenuated by the simultaneous activation of adenosine A_1 receptors (A_1AR).[9−11] This cross talk provides fine control of cAMP levels and may have physiological consequences. For example, cardiac β-adrenergic responsiveness is decreased during aging, and part of this decrease appears to be due to enhanced adenosine formation and the activation of A_1ARs with a resulting attenuation in β responsiveness.[12,13] In addition, it has been shown that certain cardiac arrhythmias that are initiated at high concentrations of a full β-agonist can be blunted by the activation of the A_1AR without affecting the βAR-mediated increase in contractility.[14]

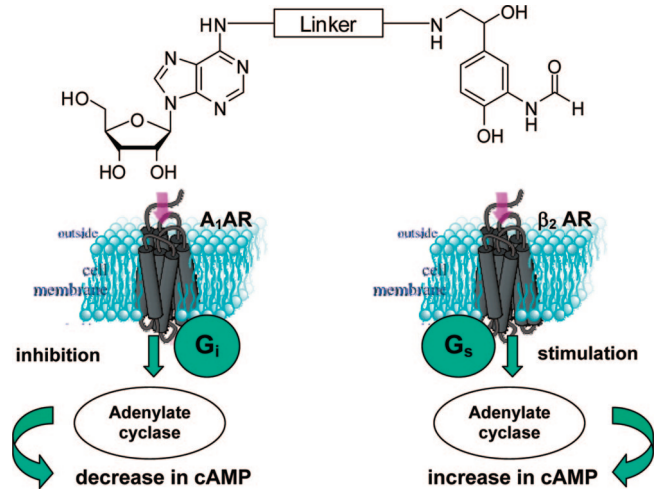

Figure 1. Bivalent ligands that target the β_2-adrenergic and adenosine A_1 receptors.

On the basis of the β_2AR/A_1AR cross talk, the objective of the present study is to synthesize a series of bivalent β_2AR/A_1AR agonists and to determine the effects of alkyl and ether spacers on their ability to activate both receptors and to produce an interactive response (Figure 1). The pharmacophores are based upon the well-known β_2-agonist formoterol and the endogenous A_1AR agonist adenosine. The spacer connection to each pharmacophore is important for the retention of affinity and activity. Therefore, the chosen linkage was between the side-chain amino of the β-agonist and the N^6 position of adenosine because large substituents in these positions have been shown to be well tolerated.[15,16] We characterized the bivalent

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[a] Abbreviations: A_1AR, adenosine A_1 receptor; β_2AR, β_2-adrenergic receptor; GPCR, G protein-coupled receptor; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; ScAMP-TME, 2′-O-monosuccinyladenosine 3′:5′-cyclic monophosphate tyrosyl methyl ester.

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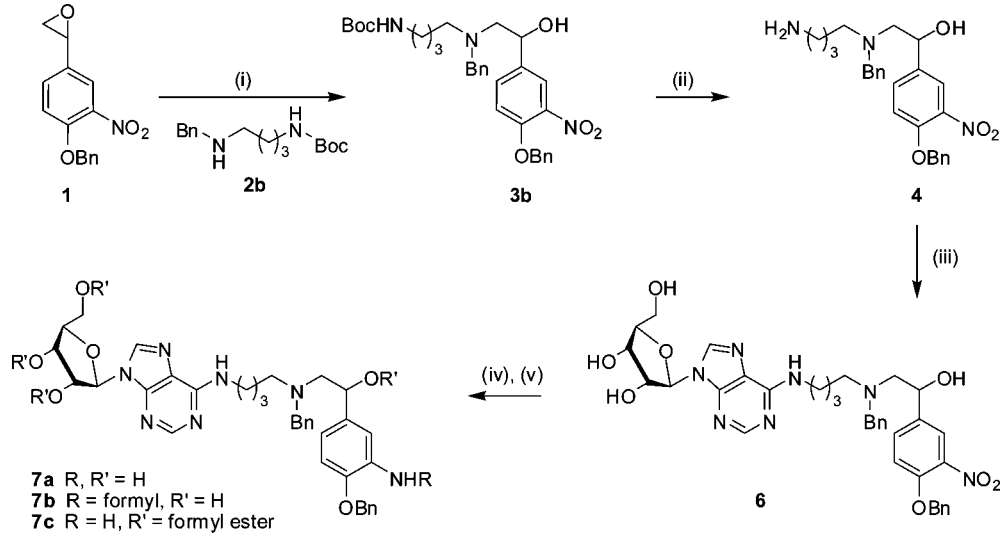
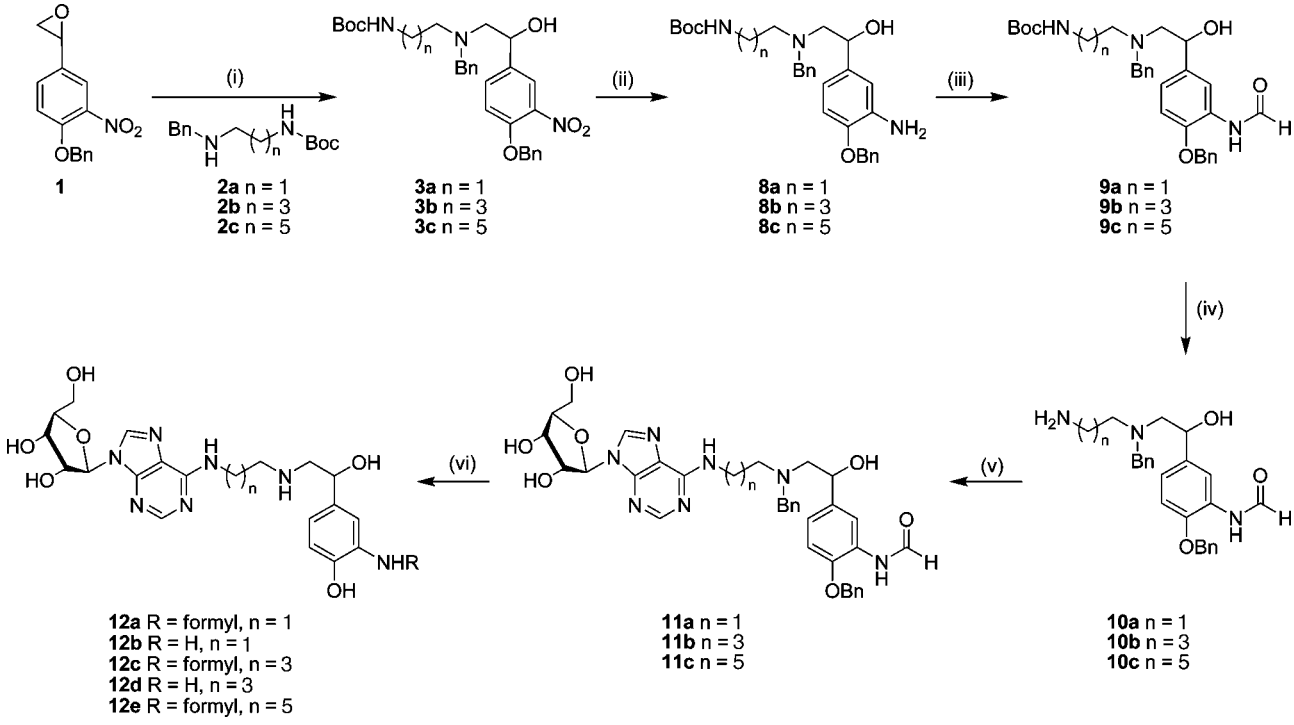
Scheme 1[a]



[a] Reagents and conditions: (i) **1** with **2b** in toluene/THF and $LiClO_4$, 24 h, 110 °C; (ii) TFA in CH_2Cl_2, 2 h, rt (**4** was isolated as the TFA salt); (iii) 6-chloropurine riboside (**5**), N(*i*-Pr)$_2$Et in *t*-BuOH, 80 °C, 24 h; (iv) PtO_2, H_2, (1 atm) in CH_3OH, rt, 9 h; and (v) Ac_2O, HCOOH, 12 h, rt.

Scheme 2[a]



[a] Reagents and conditions: (i) **1** with **2** in toluene/THF and $LiClO_4$, 24 h, 110 °C; (ii) PtO_2, H_2, 1 atm in CH_3OH; (iii) Ac_2O, HCOOH, 12 h, rt; (iv) TFA/CH_2Cl_2 (product isolated as the TFA salt); (v) 6-chloropurine riboside (**5**), N(*i*-Pr)$_2$Et in *t*-BuOH, reflux 24 h; (vi) 10% Pd/C, H_2, 50 psi in EtOH.

compounds by using DDT_1 MF-2 cells that express both receptor types and in which the activation of the A_1AR inhibits β_2AR-mediated increases in cAMP accumulation.[11,17]

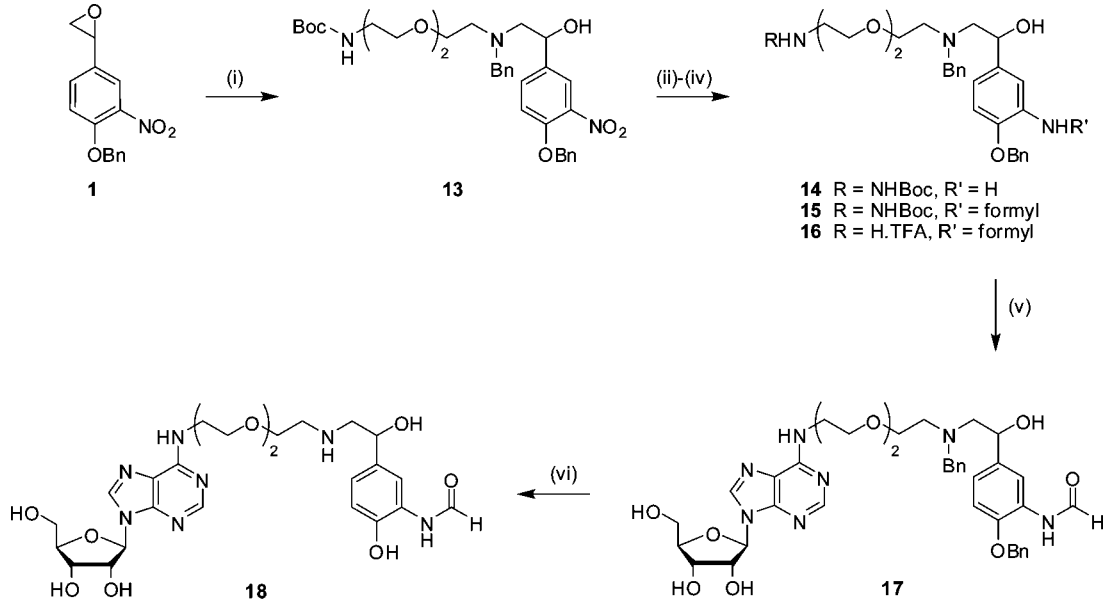
Results and Discussion

Chemistry. The desired starting material, 4-benzyloxy-3-nitrostyreneoxide (**1**), was successfully prepared on a multigram scale according to literature methodology.[18−20] Also, a series of diamine linkers that were both (mono-) Boc-protected and *N*-benzylated were synthesized via reductive alkylation of the corresponding Boc-protected diamines.[21] All linkers (compounds **2a**−**c**) were purified by column chromatography in yields that ranged from 51−71% and were utilized as nucleophiles, as shown in Schemes 1−3.

The reaction between *N*-benzyl amine **2b** and epoxide **1** initially proved to be quite problematic. Several conditions[19,20,22] were evaluated, and in all cases, these reactions returned unreacted starting material. However, when the epoxide precursor was treated with *N*-benzylamine **2b** in the presence of excess $LiClO_4$,[23] the desired ethanolamine **3b** was obtained in reasonable yield (Scheme 1).

The *t*-butoxycarbonyl (Boc) group of **3b** was cleaved by treatment with trifluoroacetic acid, and the desired product **4** was isolated as the TFA salt following the evaporation of the

Scheme 3[a]



[a] Reagents and conditions: (i) $BnNHCH_2CH_2(OCH_2CH_2)_2NHBoc$ in toluene/THF and $LiClO_4$, 24 h, 110 °C; (ii) PtO_2, H_2, 1 atm in CH_3OH; (iii) Ac_2O, HCOOH, rt; (iv) TFA/CH_2Cl_2 (product isolated as the TFA salt); (v) 6-chloropurine riboside (**5**), $N(i\text{-}Pr)_2Et$ in *t*-BuOH, 110 °C, 24 h; (vi) 10% Pd/C, H_2, 50 psi in EtOH.

excess trifluoroacetic acid. This amine was subsequently reacted with 6-chloropurine riboside (**5**) in the presence of Hünig's base to provide N^6-substituted adenosine **6**. The nitro group of **6** was reduced via catalytic hydrogenation (PtO_2 and hydrogen gas at 1 atm) to yield the corresponding aniline derivative **7a**. The formylation of this aniline was attempted by treatment with an aged mixture of formic acid and acetic anhydride.[25] Although characteristic formyl peaks appeared in the crude ^1H and ^{13}C NMR spectra, only starting material was isolated following column chromatography. This led us to believe that formyl ester derivative **7c** formed and was further hydrolyzed on the silica column to return the original starting material. Because the introduction and the purification of the formyl group in the presense of the adenosine component proved to be problematic, an alternative strategy was adopted in which the formyl group was introduced prior to the attachment of the adenosine component (Scheme 2).

This modified approach allowed us to synthesize our final targets in the same number of steps that was set out in our initial plan and to take advantage of a number of the synthetic steps that had already been optimized. Furthermore, the introduction of the adenosine component later in the sequence would also minimize the handling of polar products. A starting point for this approach involved the preparation of ethanolamine derivatives **3a**−**c** from epoxide **1** (Scheme 2). The nitro groups of compounds **3a**−**c** were subsequently reduced with H_2 and PtO_2 to afford the corresponding anilines **8a**−**c**, respectively. The formyl group was successfully introduced with an excess mixture of acetic acid and acetic anhydride (on a per aniline basis). ^1H and ^{13}C NMR spectroscopy showed peaks at 8.3 and 162 ppm, respectively, which is characterisitic of the formyl group for all analogs. The Boc protecting group of compounds **9a**−**c** was then cleaved by treatment with trifluoroacetic acid to afford the corresponding amines as TFA salts in quantitative yield. The subsequent incorporation of the adenosine component was achieved by the reaction of the amine moiety of compounds **10a**−**c** with 6-chloropurine riboside in the presence of Hünig's base in *t*-BuOH. In the final step of the synthesis, the benzyl protecting groups were concomitantly cleaved by the use of standard catalytic hydrogenation conditions (palladium on a carbon catalyst under an atmosphere of hydrogen gas at 50 psi). An attempt was made to purify the targets by preparatory TLC with various mixtures of MeOH/H_2O, but the targets proved to be difficult to isolate as a result of their high polarity. The purification of these polar compounds was subsequently achieved by preparatory HPLC. A reliable and reproducible HPLC method was developed, and compounds **12a**, **12c**, and **12e** were successfully purified, albeit in low yield. The corresponding deformylated compounds **12b** and **12d**, were also isolated in low yield as byproducts in the synthesis of **12a** and **12c**. No attempts were made to optimize these yields because sufficient material was obtained for the analysis and the pharmacological evaluation. The deformylated byproducts **12b** and **12d** possess a β_2AR component that is analogous to the known β_2AR agonist desformoterol[26] and were also evaluated as bivalent ligands.

The incorporation of polyethylene glycol units is a common approach for improving the water solubility of small molecules.[27] Accordingly, a bivalent ligand with a polyethylene glycol linker that joined the formoterol and adenosine components was also targeted. Compound **18** was prepared in seven steps from 4-benzyloxy-3-nitrostyreneoxide (**1**) according to the approach described above (Scheme 3).

Pharmacology. The bivalent compounds were subjected to several pharmacological assays whereby the affinities of the bivalent ligands were determined for the β_2AR and the A_1AR by displacement of specific [^{125}I]-(−)iodopindolol and [^{3}H]DPCPX binding, respectively, in DDT cell membranes. These assays included 10 μM 5′-guanylyl-imidodiphosphate to maintain the receptor in the agonist low affinity state. The compounds were also tested for their ability to stimulate (β_2AR effect) and inhibit (A_1AR effect) cAMP accumulation in DDT cells.[11,17] In these cells, the stimulation of cAMP accumulation that is mediated by β-agonists is attenuated by the activation of the A_1AR. Therefore, in the present experiments, the effects of the bivalents were determined alone and in the presence of the A_1AR antagonist DPCPX to block any inhibitory effects. The effects of the bivalent ligands were compared with

Table 1. K_i and EC_{50} Values for Bivalent Derivatives at the β_2-Adrenergic and Adenosine A_1 Receptors in DDT_1 MF-2 Cells

compd	K_i A_1AR[a] (nM)	K_i β_2AR[a] (nM)	EC_{50} β_2AR[b] (nM)
isoproterenol		136 ± 25(6)	20 ± 4(14)
CPA	9 ± 2(5)		
12a	1979 ± 256(6)	>10 000(3)[c]	ND[d]
12b	2749 ± 334(5)	>10 000(3)[c]	ND[d]
12c	1914 ± 190(5)	863 ± 196(4)	32 ± 4(5)
12d	1014 ± 67(4)	4314 ± 1359(4)	1087 ± 51(3)
12e	436 ± 63(5)	311 ± 62(4)	6 ± 2(4)
18	1907 ± 357(5)	2296 ± 355(4)	177 ± 37(3)

[a] K_i values were calculated from the concentration of the ligands that inhibited specific [^{3}H]DPCPX binding to the A_1AR or [^{125}I]-(−)iodopindolol binding to the βAR by 50%. [b] EC_{50} values are the concentration of ligands that gave half-maximal stimulation of cAMP accumulation in the presence of 1 μM DPCPX. [c] Less than 20% inhibition of radioligand binding at the highest concentration (10 μM) used. [d] ND is not determined. A maximal stimulation was not achieved with up to a 100 μM compound. Numbers in parentheses are the number of separate experiments.

those of the classical β-agonist (−)isoproterenol and the A_1AR agonist N^6-cyclopentyladenosine (CPA).

As shown in Table 1, the affinities of the ethyl-linked (**12a**) and butyl-linked (**12c**) bivalent ligands for A_1AR are similar and are in the low micromolar range. Increasing the linker length with the hexyl (**12e**) bivalent increased the affinity by about 4.5 times to 436 nM. The ether-linked bivalent (**18**) has an A_1AR affinity that is similar to that of the ethyl- and butyl-linked derivatives. The ethyl-linked (**12b**) and butyl-linked (**12d**) aniline bivalent compounds also had affinities that were in the low micromolar range. All of the bivalents had substantially lower affinites for the A_1AR as compared with the A_1AR agonist CPA. At the β_2AR, there is a linker-related effect on affinity. Therefore, the ethyl (**12a**) bivalent has an affinity that is greater than 10 μM, which is increased to 863 nM with the butyl (**12c**) derivative and is further increased to 311 nM with the hexyl (**12e**) derivative. Furthermore, the ethyl-linked aniline bivalent (**12b**) has an affinity that is greater than 10 μM, and the butyl-linked derivative (**12d**) has an increased affinity at 4.3 μM. The ether-linked bivalent (**18**) also has a low micromolar affinity for β_2AR.

The effects of the bivalent ligands on cAMP accumulation are shown in Figure 2. The ethyl-linked (**12a**) bivalent showed less than 15% stimulation of cAMP accumulation at the highest concentration that was employed (100 μM) as compared with (−)isoproterenol, and this stimulation was increased only slightly in the presence of DPCPX (Figure 2A). In contrast, the butyl (**12c**) and hexyl (**12e**) derivatives produced a biphasic cAMP accumulation response. A concentration-dependent stimulation of cAMP accumulation occurred in the range of 1−100 nM, after which there was a concentration-dependent inhibition of cAMP accumulation (Figure 2A,B). The peak cAMP accumulation effect for both compounds was comparable to that of (−)isoproterenol, and the inhibitory phase of cAMP accumulation was completely prevented by the inclusion of 1 μM DPCPX, which resulted in a stimulation plateau. This indicates that the inhibitory phase is mediated by activation of the A_1AR. The ether-linked bivalent (**18**) also showed a biphasic effect on cAMP accumulation (Figure 2C) with a maximal stimulation that was 70% the (−)isoproterenol maximum. In the presence of DPCPX, the inhibitory phase was abolished and the maximal stimulation was increased to that observed with (−)isoproterenol. As shown in Figure 2D, the ethyl-linked aniline bivalent (**12b**) produced less than 15% stimulation of cAMP accumulation at 100 μM as compared with (−)isoproterenol. In the presence of DPCPX, the stimulation by this derivative increased to over 50% of the (−)isoproterenol maximum. In contrast, the butyl-linked aniline bivalent (**12d**) produced a biphasic cAMP response with stimulation that occurred in concentrations up to 1 μM and inhibition that occurred at higher concentrations. The maximal stimulation was 50% of the (−)isoproterenol maximum. In the presence of DPCPX, the inhibitory phase was abolished, and the maximal stimulation was similar to that of (−)isoproterenol. The stimulation of cAMP accumulation that was produced by 0.1 μM **12c** and **12e** or 1 μM **12c** and **18** was blocked by the inclusion of 0.1 μM propranolol, which indicates that this response was mediated by the β_2AR (data not shown).

Table 1 shows the potencies (EC_{50}) of the bivalent ligands for the β_2AR stimulation of cAMP accumulation in the presence of DPCPX, and in general, they are greater than the corresponding affinities. The butyl-linked (**12c**) and hexyl-linked (**12e**) derivatives have potencies in the low nanomolar range, and the butyl derivative has a slightly lower and the hexyl derivative has a slightly higher potency than that of (−)isoproterenol (20 nM). In contrast, the ether-linked (**18**) and butyl-linked aniline (**12d**) derivatives have potencies that are 9 and 54 times lower than that of (−)isoproterenol, respectively. The EC_{50} for the ethyl-linked (**12a**) and ethyl-linked aniline (**12b**) bivalents could not be determined because a plateau for cAMP accumulation was not reached at the highest concentration that was used for each compound.

The data from the present study show that linking agonist pharmacophores for the β_2AR and the A_1AR into a single ligand can result in the retention of activity at both receptor types. Within the limited bivalent synthesized series, the affinity of the ligands for the A_1AR was relatively insensitive to the linker length. In contrast, for the β_2AR, there was a modest increase in affinity and potency with alkyl linker length. Interestingly, the ether-linked bivalent (**18**), which has the same number of methylene groups as **12e**, had a decrease in β_2AR affinity and potency, which suggests that the length, chemical nature, and conformational flexibility of the linker can also affect these parameters, as has been shown for other bivalent ligands.[1,6,28] The data show affinity and agonist activity of the bivalent compounds at each receptor type; however, the data do not directly address the notion of receptor cross-linking by a single bivalent molecule. The lack of major changes in the affinity of the bivalent ligands, especially with the A_1AR, suggests that receptor cross-linking is unlikely.

With the exception of the two bivalent compounds with relatively weak affinities for the β_2AR (**12a** and **12b**), all of the others produced the same maximal stimulation of cAMP accumulation as the classical β-agonist (−)isoproterenol when the inhibitory phase was blocked by DPCPX. This indicates that they are full β-agonists. Furthermore, the maximal inhibition of cAMP accumulation by these compounds is 75−85%, which has been shown to be the maximum produced by the classical A_1AR agonist CPA; this suggests that they are also full agonists at the A_1AR (Figure 2). In the absence of DPCPX, two of the bivalent compounds (**12b** and **18**) had reduced maximal responses compared with (−)isoproterenol. These suppressed maxima were due to the concurrent activation of the A_1AR, which resulted in an apparent (physiological) partial β-agonist response of the bivalent ligand. In DDT cells the A_1AR-mediated inhibition of (−)isoproterenol-stimulated cAMP accumulation is dominant because it occurs even when the β_2AR are saturated with agonist.[11,24] Therefore, the bivalent concentration-dependent relationship between the β_2AR-mediated stimulation of cAMP accumulation followed by the A_1AR-mediated inhibition phase is likely due to several factors including the differential affinity and the relationship between

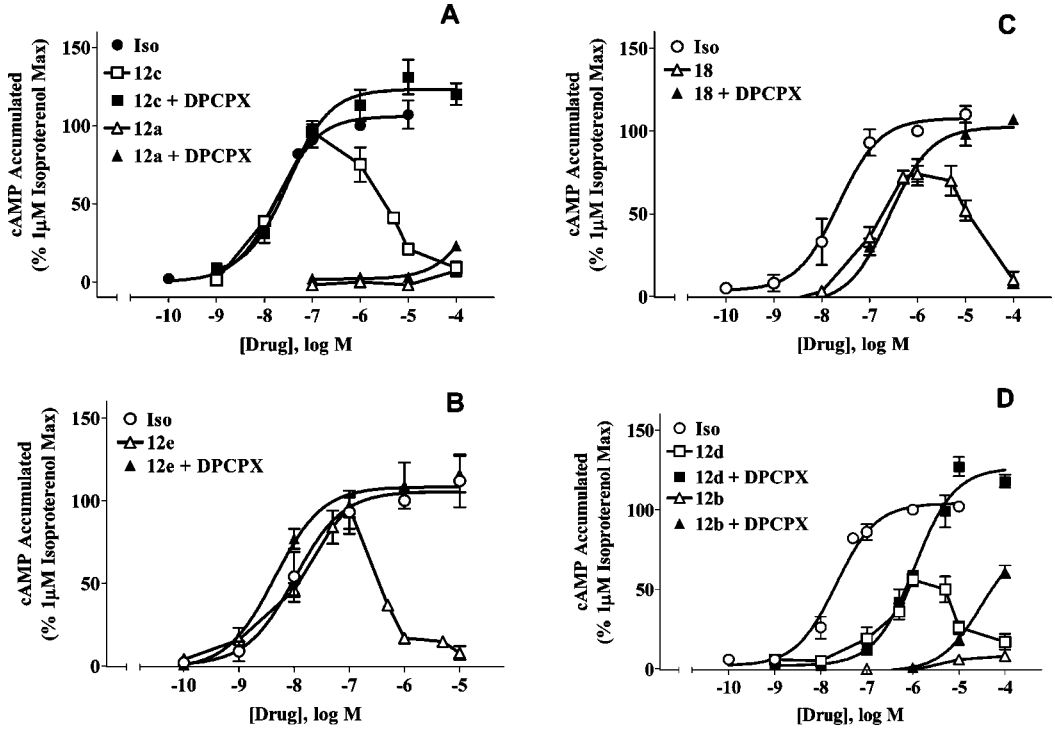


Figure 2. Effect of bivalent compounds and (−)isoproterenol on cAMP accumulation in DDT cells.

receptor occupancy and response (efficacy). Compounds **12c** and **12e** have 2.2- and 1.4-fold higher affinities for the β_2AR than for the A_1AR, respectively whereas **18** and **12d** have 1.8- and 4.4-fold higher affinities for the A_1AR than for the β_2AR, respectively. Therefore, the compounds with higher affinities for the β_2AR may produce higher maximum for cAMP accumulation compared with those that have slightly higher affinities for the A_1AR (Figure 2). In addition, agonists with higher efficacies will have nonlinear relationships (hyperbolic) between the receptor occupancy and response such that low receptor occupancy can achieve high levels of response. This effect will also shift the concentration response to the left of the occupancy curve, which will give the agonist a higher observed potency. The efficacy or shift in response from occupancy curves can be estimated by the K_i/EC_{50} ratio. According to the data from Table 1, the ratio for (−)isoproterenol is 6.8, whereas the ratios for **12c** and **12e** are 26.9 and 51.8, respectively. The relatively high ratios for **12c** and **12e** indicate that a relatively small fraction of occupied β_2ARs will produce a maximum response and may partially explain why these two compounds can produce a maximal response (compared with (−)isoproterenol) before the inhibitory phase occurs. In contrast, the K_i/EC_{50} ratio for **18** is 12.9 with a maximum response that is 70% of the (−)isoproterenol maximum, whereas the ratio for **12d** is 3.9 with a 50% maximum response. The reduced ratios for these compounds suggest that they will need to occupy a greater fraction of the β_2ARs to achieve a maximum response, which is suppressed because of the concurrent occupancy and activation of the A_1ARs. It should be pointed out that the efficacy for the bivalent-mediated A_1 inhibitory response will also affect the biphasic response relationship but the K_i/EC_{50} ratios for the A_1AR were not calculated because of the inability to determine an EC_{50} value accurately.

Conclusions

A series of bivalent β_2AR/A_1AR agonists were synthesized, and several were shown to produce a concentration-dependent biphasic cAMP response through receptor cross talk. The linker length and composition between the two receptor pharmacophores affected binding affinity more at the β_2AR than the A_1AR. Compound **12e**, which contains a hexyl spacer, possessed the highest affinity for both receptors and was the most potend β-agonist. The relationship between the β_2AR stimulatory and A_1AR inhibitory responses was likely dependent upon both receptor affinity and efficacy. Certain compounds (**12c** and **12e**) produced potent β_2AR-mediated stimulation of cAMP with the same maximum response as the full agonist (−)isoproterenol, whereas others (**18** and **12d**) had an A_1AR-mediated suppression of the maximal stimulatory response such that they appeared as partial β-agonists. The data suggest that bivalent agonists may be useful in the elucidation of the mechanisms that contribute to the modulation of cellular responses through receptor cross talk. Furthermore, bivalent ligands may hold promise in the development of physiological partial agonists by the differential activation of interacting receptors.

Experimental Section

Chemistry. 6-Chloropurine riboside was purchased from Toronto Research Chemicals. The mono Boc-protected amine linkers (structures not shown) were provided at a high degree of purity by Starpharma Pty. and were converted to secondary amine linkers (**2a**−**d**). Prepacked C_{18} columns (strata C18-E) were purchased from Phenomenex. Unless otherwise stated, the solvents were HPLC grade and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded in MeOD on a Bruker 300 UltraShield 300 MHz NMR instrument. Chemical shifts (δ) are recorded in parts per million (ppm) relative to MeOD, and coupling constants (J values) are in Hertz. HPLC/ES-MS was conducted on a Waters 2795 instrument with a 2996 diode array detector (chromatograms show total UV absorbance 200−300 nm) coupled to a Waters ZQ4000 instrument with an ESI probe and inlet flow split to give around 50 μL/min to the MS. The analytical chromatography column was a Waters Xterra C18 (hydrophilic) (0.3 × 100 mm^2), and unless otherwise stated, TFA method ACN/water (0.1% TFA) gradients at 0.4 mL/min were utilized. High-resolution electrospray mass spectra (HRMS) studies were conducted on a Bruker Bio-

Apex II FTMS instrument. High-resolution ES-MS data were obtained for all targeted compounds (**12a**−**e** and **18**). Preparatory HPLC was carried out on a Waters Xterra prep column (RP_{18}, 10 μm, 19 × 250 mm^2). We carried out thin layer chromatography by using 20 cm plates (Merck silica gel 60 F_{254}), and we conducted column chromatography by using Merck silica gel 60 (particle size 0.04−0.063 nm, 230−400 mesh).

***tert*-Butyl 2-(Benzyl(2-(4-(benzyloxy)-3-nitrophenyl)hydroxyethyl)amino)ethylcarbamate (3a).** To a stirred solution of 4-benzyloxy-3-nitrostyreneoxide (**1**) (343 mg, 1.27 mmol) in toluene/THF (30:15 mL) was added **2a** (396 mg, 1.58 mmol) in toluene (30 mL), followed by $LiClO_4$ (671 mg, 6.33 mmol). The reaction was stirred at 110 °C for 24 h under an atmosphere of nitrogen. The solvent was removed under reduced pressure, and the residue was purified by flash silica chromatography (eluent 3% MeOH/CH_2Cl_2) to produce the title compound as a yellow oil (310 mg, 47% yield). ^{1}H NMR (MeOD, δ): 1.42 (s, 9H, 3 × CH_3), 2.61 (t, 2H, J = 6.3, CH_2), 2.68 (dd, 2H, J = 4.2, J = 6.9, CH_2), 3.00−3.20 (m, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.68 (t, 1H, J = 6.6, CH), 5.26 (s, 2H, CH_2), 7.16−7.52 (m, 12H, ArH), 7.75 (d, 1H, J = 2.1, ArH). ^{13}C NMR (MeOD, δ): 28.1, 28.8, 39.4, 55.5, 60.6, 63.2, 71.5, 72.2, 80.1, 116.4, 124.2, 128.1, 128.3, 129.2, 129.3, 129.6, 130.2, 132.9, 137.6, 138.0, 140.4, 141.4, 152.1, 158.5. LCMS (hydrophilic): R_f (min) = 12.17, (ESI + ve) found 522 $[M + H]^+$ calcd for $C_{29}H_{36}N_3O_6$.

***tert*-Butyl 4-(Benzyl(2-(4-(benzyloxy)-3-nitrophenyl)hydroxyethyl)amino)butylcarbamate (3b).** The title compound was prepared using the same method that was described for **3a**. After purification by column chromatography (eluent 3% MeOH/CH_2Cl_2), the product was obtained as a yellow foam in 73% yield. ^{1}H NMR (MeOD, δ): 1.35−1.55 (m, 4H, 2 × CH_2), 1.41 (s, 9H, 3 × CH_3), 2.58 (t, 2H, J = 6.9, CH_2), 2.68 (d, 2H, J = 6.9, CH_2), 2.97 (t, 2H, J = 6.6, CH_2), 3.69 (s, 2H, CH_2), 4.69 (t, 1H, J = 6.6, CH), 5.22 (s, 2H, CH_2), 7.18−7.45 (m, 12H, ArH), 7.73 (d, 1H, J = 1.8, ArH). ^{13}C NMR (MeOD, δ): 25.3, 28.7, 28.9, 41.2, 55.4, 60.2, 62.9, 71.3, 72.2, 79.9, 116.3, 124.2, 128.1, 128.3, 129.1, 129.2, 129.6, 130.2, 133.0, 137.6, 138.1, 140.5, 141.3, 152.0, 158.5. LCMS (hydrophilic): R_f (min) = 9.05, (ESI + ve) found 550 $[M + H]^+$ calcd for $C_{31}H_{40}N_3O_6$.

***tert*-Butyl 6-(Benzyl(2-(4-(benzyloxy)-3-nitrophenyl)hydroxyethyl)amino)hexylcarbamate (3c).** The title compound was prepared using the same method that was described for **3a**. After purification by column chromatography (eluent 3% MeOH/CH_2Cl_2), the product was obtained as a yellow foam in 62% yield. ^{1}H NMR (MeOD, δ): 1.17 (br s, 4H, 2 × CH_2), 1.38 (br s, 4H, 2 × CH_2), 1.41 (s, 9H, 3 × CH_3), 2.44 (t, 2H, J = 7.1, CH_2), 2.60 (d, 2H, J = 6.6, CH_2), 2.97 (t, 2H, J = 7.1, CH_2), 3.56 (s, 2H, CH_2), 4.64 (t, 1H, J = 6.6, CH), 5.16 (s, 2H, CH_2), 7.12−7.45 (m, 12H, ArH), 7.74 (d, 1H, J = 2.1, ArH). ^{13}C NMR (MeOD, δ): 27.7, 28.0, 28.9, 30.9, 41.3, 55.5, 60.1, 62.9, 71.0, 72.1, 79.7, 116.1, 124.2, 128.0, 128.2, 129.1, 129.2, 129.6, 130.1, 133.0, 137.4, 137.9, 140.4, 141.1, 151.9, 158.3. LCMS (hydrophilic): R_f (min) = 19.04, (ESI + ve) found 578 $[M + H]^+$ calcd for $C_{33}H_{44}N_3O_6$.

2-((4-Aminobutyl)benzylamino)-1-(4-(benzyloxy)-3-nitrophenyl)ethanol: TFA Salt (4). A solution of trifluoroacetic acid/dichloromethane (1:1) (590 μL) was added dropwise to a stirred suspension of **3b** (210 mg, 0.38 mmol) in dichloromethane (4 mL). The mixture was stirred at room temperature for 2 h under argon. The solvent and excess TFA were removed under reduced pressure. The residue was redissolved in MeOH (40 mL), was concentrated in vacuo, and was freeze-dried to produce title compound **4** as the TFA salt (200 mg, 93% yield). ^{1}H NMR (MeOD, δ): 1.62−1.80 (m, 2H, CH_2), 1.83−2.09 (m, 2H, CH_2), 3.00 (t, 2H, J = 7.2, CH_2), 3.24−3.33 (m, 4H, 2 × CH_2), 4.42−4.60 (m, 2H, CH_2), 5.25 (s, 2H, CH_2), 7.26−7.64 (m, 12H, ArH), 7.83 (br s, 1H, ArH). ^{13}C NMR (MeOD, δ): 21.7, 25.6, 40.0, 56.4, 59.1, 59.4, 68.9, 72.2, 116.9, 123.9, 128.4, 129.3, 129.7, 130.6, 130.7, 131.4, 132.5, 132.6, 135.0, 137.3, 141.6, 152.7. LCMS (hydrophilic): R_f (min) = 7.32, (ESI + ve) found 450 $[M + H]^+$ calcd for $C_{26}H_{32}N_3O_4$.

2-(Benzyl(4-(N^6-adenosinyl)butyl)amino)-1-(4-(benzyloxy)-3-nitrophenyl)ethanol (6). To a suspension of 6-chloropurine riboside (**5**) (107 mg, 0.37 mmol) in *t*-BuOH (40 mL) was added **4** (175 mg, 0.31 mmol), followed by DIPEA (135 μL, 0.78 mmol). The reaction mixture was stirred for 24 h at 80 °C under nitrogen. Evaporation of the solvent and purification of the residue by flash silica chromatography (eluent 10% MeOH/CH_2Cl_2 + 1% NH_3) produced title compound **6** as a yellow oil (106 mg) in 49% yield. ^{1}H NMR (MeOD, δ): 1.49−1.63 (m, 4H, 2 × CH_2), 2.49−2.58 (m, 2H, CH_2), 2.60−2.69 (m, 2H, CH_2), 3.40−3.56 (m, 2H, CH_2), 3.56, 3.64 (d, 2H, J = 13.2, CH_2), 3.69−3.92 (m, 2H, CH_2), 4.14−4.20 (m, 1H, CH), 4.32 (dd, 1H, J = 2.7, J = 5.0, CH), 4.66 (t, 1H, J = 6.6, CH), 4.74 (t, 1H, J = 5.4, CH), 5.18 (s, 2H, CH_2), 5.94 (d, 1H, J = 6.6, CH), 7.10−7.48 (m, 12H, ArH), 7.71 (br s, 1H, ArH), 8.17 (s, 1H, CH−purine), 8.20 (s, 1H, CH−purine). ^{13}C NMR (MeOD, δ): 25.4, 28.2, 41.4, 55.3, 60.3, 62.9, 63.5, 71.3, 72.2, 71.7, 75.5, 88.2, 91.4, 116.2, 121.4, 124.3, 128.1, 128.3, 129.1, 129.2, 129.6, 130.2, 133.0, 137.6, 138.1, 140.3, 141.3, 141.4, 149.0, 152.0, 153.6, 156.3. LCMS (hydrophilic): R_f (min) = 14.87, (ESI + ve) found 700 $[M + H]^+$ calcd for $C_{36}H_{42}N_7O_8$.

2-(Benzyl(4-(N^6-adenosinyl)butyl)amino)-1-(4-(benzyloxy)-3-aminophenyl)ethanol (7a). To compound **6** (106 mg, 0.15 mmol) dissolved in methanol (6 mL) was added PtO_2 (10 mg), and the reaction mixture was stirred for 9 h under an atmosphere of hydrogen gas (at 1 atm). Filtration through a Millipore 0.45 μm filter and evaporation of the solvent afforded a residue that was purified by flash silica chromatography (eluent 5−10% MeOH/CH_2Cl_2). The title compound was obtained as a yellow gum (90 mg) in 89% yield. ^{1}H NMR (MeOD, δ): 1.40−1.65 (m, 4H, 2 × CH_2), 2.42−2.74 (m, 4H, 2 × CH_2), 3.40−3.93 (m, 6H, 3 × CH_2), 4.15−4.19 (m, 1H, CH), 4.32 (dd, 1H, J = 2.4, J = 4.8, CH), 4.59−4.66 (m, 1H, CH), 4.74 (t, 1H, J = 5.7, CH), 5.04 (s, 2H, CH_2), 5.94 (d, 1H, J = 6.3, CH), 6.70−6.90 (m, 2H, ArH), 7.10−7.50 (m, 11H, ArH), 8.18 (br s, 1H, CH), 8.21 (br s, 1H, CH). ^{13}C NMR (MeOD, δ): 25.2, 28.2, 41.4, 55.0, 60.1, 63.1, 63.6, 71.5, 72.2, 72.8, 75.5, 88.3, 91.4, 113.2, 115.0, 117.7, 121.5, 128.2, 128.6, 128.9, 129.3, 129.5, 130.4, 137.4, 137.8, 138.9, 140.0, 141.4, 147.6, 149.0, 153.6, 156.3. LCMS (hydrophilic): R_f (min) = 8.01, (ESI + ve) found 670 $[M + H]^+$ calcd for $C_{36}H_{44}N_7O_6$.

***tert*-Butyl 2-(Benzyl(2-(4-(benzyloxy)-3-aminophenyl)hydroxyethyl)amino)ethylcarbamate (8a).** To compound **3a** (280 mg, 0.54 mmol) dissolved in methanol (40 mL) was added PtO_2 (28 mg), and the reaction mixture was stirred for 2 h under an atmosphere of hydrogen gas (at 1 atm). Filtration through a Millipore 0.45 μm filter and evaporation of the solvent afforded a residue that was purified by flash silica chromatography (eluent 5−10% MeOH/CH_2Cl_2). The title compound was obtained as a yellow oil (240 mg) in 91% yield. ^{1}H NMR (MeOD, δ): 1.43 (s, 9H, 3 × CH_3), 2.45−2.75 (m, 4H, 2 × CH_2), 3.00−3.20 (m, 2H, CH_2), 3.58, 3.75 (d, 2H, J = 13.5, CH_2), 4.55 (dd, 1H, J = 4.5, J = 8.5, CH), 5.05 (s, 2H, CH_2), 6.61 (dd, 1H, J = 1.8, J = 8.2, ArH), 6.76 (d, 1H, J = 1.8, ArH), 6.81 (d, 1H, J = 8.4, ArH), 7.18−7.46 (m, 10H, ArH). ^{13}C NMR (MeOD, δ): 28.9, 39.4, 55.2, 60.4, 63.6, 71.5, 72.4, 80.0, 113.2, 114.8, 117.6, 128.1, 128.6, 128.9, 129.3, 129.5, 130.2, 137.3, 137.8, 138.8, 140.3, 147.5, 158.5. LCMS (hydrophilic): R_f (min) = 7.39, (ESI + ve) found 492 $[M + H]^+$ calcd for $C_{29}H_{38}N_3O_4$.

***tert*-Butyl 4-(Benzyl(2-(4-(benzyloxy)-3-aminophenyl)hydroxyethyl)amino)butylcarbamate (8b).** The title compound was prepared using the same method that was described for **8a**. Following flash chromatography (eluent 10% MeOH/CH_2Cl_2 + 1% NH_3), the product was isolated as a yellow oil in 47% yield. ^{1}H NMR (MeOD, δ): 1.30−1.50 (m, 4H, 2 × CH_2), 1.43 (s, 9H, 3 × CH_3), 2.38−2.70 (m, 4H, 2 × CH_2), 2.97 (t, 2H, J = 6.6, CH_2), 3.57, 3.70 (d, 2H, J = 13.5, CH_2), 4.56 (dd, 1H, J = 5.4, J = 7.5, CH), 5.07 (s, 2H, CH_2), 6.61 (dd, 1H, J = 2.1, J = 8.1, ArH), 6.75 (d, 1H, J = 1.8, ArH), 6.82 (d, 1H, J = 8.4, ArH), 7.18−7.48 (m, 10H, ArH). ^{13}C NMR (MeOD, δ): 25.3, 28.6, 28.9, 41.2, 55.0, 60.1, 63.2, 71.5, 72.2, 79.8, 113.2, 115.0, 117.7, 128.1, 128.6, 128.9, 129.3, 129.5, 130.3, 137.5, 137.7, 138.9, 140.5, 147.5, 158.5. LCMS (hydrophilic): R_f (min) = 7.59, (ESI + ve) found 520 $[M + H]^+$ calcd for $C_{31}H_{42}N_3O_4$.

***tert*-Butyl 6-(Benzyl(2-(4-(benzyloxy)-3-aminophenyl)hydroxyethyl)amino)hexylcarbamate (8c).** The title compound was prepared using the same method that was described for **8a**. Following flash chromatography (eluent 5−10% MeOH/CH_2Cl_2 + 1% NH_3), the product was isolated as a yellow oil in 61% yield. ^{1}H NMR (MeOD, δ): 1.19−1.26 (m, 4H, 2 × CH_2), 1.36−1.52 (m, 4H, 2 × CH_2), 1.43 (s, 9H, 3 × CH_3), 2.40−2.70 (m, 4H, 2 × CH_2), 2.99 (t, 2H, J = 7.1, CH_2), 3.58, 3.71 (d, 2H, J = 13.5, CH_2), 4.55 (dd, 1H, J = 5.4, J = 7.8, CH), 5.10 (s, 2H, CH_2), 6.61 (dd, 1H, J = 2.1, J = 8.4, ArH), 6.76 (d, 1H, J = 1.8, ArH), 6.83 (d, 1H, J = 8.4, ArH), 7.18−7.50 (m, 10H, ArH). ^{13}C NMR (MeOD, δ): 27.7, 27.9, 28.0, 28.9, 30.9, 41.3, 55.1, 60.0, 63.3, 71.4, 72.0, 79.7, 113.1, 114.7, 117.5, 128.0, 128.5, 128.8, 129.3, 129.5, 130.2, 137.3, 137.7, 138.8, 140.5, 147.4, 158.4. LCMS (hydrophilic): R_f (min) = 8.16, (ESI + ve) found 548 $[M + H]^+$ calcd for $C_{33}H_{46}N_3O_4$.

***tert*-Butyl 2-(Benzyl(2-(4-(benzyloxy)-3-formamidophenyl)hydroxyethyl)amino)ethylcarbamate (9a).** Acetic anhydride (1.02 mL, 10.8 mol) was added to formic acid (406 μL, 10.8 mol) at 0 °C, and the solution was stirred at 60 °C for 15 min. After cooling to 0 °C, a solution of **8a** (240 mg, 0.49 mmol) in CH_2Cl_2 (15 mL) was added, and the reaction was stirred at room temperature for 1 h under argon. The solvent was removed under reduced pressure, and the residue was redissolved in methanol (15 mL). To the solution was added 1 M NaOH (1.47 mL, 1.47 mmol), and the mixture was left to stir for 30 min. The solvent was removed, and the residue was redissolved in ethyl acetate, washed with water, and dried over $MgSO_4$ to yield the title compound (230 mg, 91% yield) as a yellow oil. ^{1}H NMR (MeOD, δ): 1.41 (s, 9H, 3 × CH_3), 2.49−2.75 (m, 4H, 2 × CH_2), 2.98−3.20 (m, 2H, CH_2), 3.60, 3.70 (d, 2H, J = 13.8, CH_2), 4.57−4.67 (m, 1H, CH), 5.19 (s, 2H, CH_2), 6.99−7.09 (m, 2H, ArH), 7.16−7.50 (m, 10H, ArH), 8.19 (s, 1H, ArH), 8.32 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 28.9, 39.4, 55.3, 60.4, 63.6, 71.8, 72.3, 79.9, 113.3, 120.8, 123.6, 127.8, 128.0, 128.8, 129.1, 129.3, 129.6, 130.3, 137.2, 138.1, 140.3, 148.7, 158.4, 162.0. LCMS (hydrophilic): R_f (min) = 8.14, (ESI + ve) found 520 $[M + H]^+$ calcd for $C_{30}H_{38}N_3O_5$.

***tert*-Butyl 4-(Benzyl(2-(4-(benzyloxy)-3-formamidophenyl)hydroxyethyl)amino)butylcarbamate (9b).** The title compound was prepared using the same method that was described for **9a**. The product was obtained as a yellow oil in 92% yield. ^{1}H NMR (MeOD, δ): 1.35−1.55 (m, 4H, 2 × CH_2), 1.42 (s, 9H, 3 × CH_3), 2.52−2.64 (m, 2H, CH_2), 2.68−2.77 (m, 2H, CH_2), 2.96 (t, 2H, J = 6.6, CH_2), 3.71, 3.78 (d, 2H, J = 13.5, CH_2), 4.64 (t, 1H, J = 7.1, CH), 5.20 (s, 2H, CH_2), 6.99−7.08 (m, 2H, ArH), 7.19−7.51 (m, 10H, ArH), 8.18 (s, 1H, ArH), 8.33 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 24.6, 28.5, 28.9, 41.0, 54.9, 59.7, 62.6, 71.3, 71.6, 79.7, 113.2, 120.6, 123.5, 127.8, 128.5, 128.7, 129.1, 129.4, 129.6, 130.5, 136.7, 138.0, 138.7, 148.6, 158.3, 161.9. LCMS (hydrophilic): R_f (min) = 8.54, (ESI + ve) found 548 $[M + H]^+$ calcd for $C_{32}H_{42}N_3O_5$.

***tert*-Butyl 6-(Benzyl(2-(4-(benzyloxy)-3-formamidophenyl)hydroxyethyl)amino)hexylcarbamate (9c).** The title compound was prepared using the same method that was described for **9a**. The product was obtained as a yellow oil in 89% yield. ^{1}H NMR (MeOD, δ): 1.14−1.25 (m, 4H, 2 × CH_2), 1.35−1.49 (m, 4H, 2 × CH_2), 1.42 (s, 9H, 3 × CH_3), 2.42−2.53 (m, 2H, CH_2), 2.62−2.71 (m, 2H, CH_2), 2.97 (t, 2H, J = 6.9, CH_2), 3.61, 3.68 (d, 2H, J = 13.5, CH_2), 4.61 (t, 1H, J = 6.6, CH), 5.12 (s, 2H, CH_2), 6.98−7.07 (m, 2H, ArH), 7.17−7.50 (m, 10H, ArH), 8.21 (s, 1H, ArH), 8.32 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 27.7, 27.9, 28.0, 29.0, 31.0, 41.3, 55.3, 60.2, 63.2, 71.8, 71.9, 79.8, 113.3, 120.8, 123.7, 128.1, 128.9, 129.2, 129.4, 129.7, 130.3, 137.2, 138.1, 140.3, 148.7, 158.4, 162.0. LCMS (hydrophilic): R_f (min) = 9.23, (ESI + ve) found 576 $[M + H]^+$ calcd for $C_{34}H_{46}N_3O_5$.

***N*-(5-(2-((2-aminoethyl)(benzyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide: TFA salt (10a).** A solution of trifluoroacetic acid/dichloromethane (1:1) (682 μL) was added dropwise to a stirred suspension of **9a** (230 mg, 0.44 mmol) in dichloromethane (3 mL), and the mixture was stirred at room temperature for 4 h under argon. After the evaporation of the solvent and excess TFA, the residue was then redissolved in MeOH (40 mL), concentrated in vacuo, and freeze-dried to produce the title compound as a yellow gum (236 mg, 100%). ^{1}H NMR (MeOD, δ): 3.28−3.40 (m, 2H, CH_2), 3.47−3.75 (m, 4H, 2 × CH_2), 4.56 (s, 2H, CH_2), 4.83 (t, 1H, J = 6.9, CH), 5.20 (s, 2H, CH_2), 6.97−7.12 (m, 2H, ArH), 7.25−7.67 (m, 10H, ArH), 8.19 (s, 1H, ArH), 8.35 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 35.6, 52.2, 60.4, 60.5, 69.3, 71.7, 113.7, 120.2, 123.5, 128.2, 128.8, 129.2, 129.6, 130.6, 130.7, 131.4, 132.5, 134.2, 137.9, 149.5, 162.2. LCMS (hydrophilic): R_f (min) = 6.27, (ESI + ve) found 420 $[M + H]^+$ calcd for $C_{25}H_{30}N_3O_3$.

***N*-(5-(2-((4-aminobutyl)(benzyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide: TFA salt (10b).** The title compound was prepared using the same method that was described for **10a**. The product was obtained as a brown gum in quantitative yield. ^{1}H NMR (MeOD, δ): 1.72 (m, 2H, CH_2), 1.95 (m, 2H, CH_2), 3.00 (t, 2H, J = 7.2, CH_2), 3.18−3.30 (m, 4H, 2 × CH_2), 4.42−4.58 (m, 2H, CH_2), 5.21 (s, 2H, CH_2), 7.00−7.14 (m, 2H, ArH), 7.20−7.65 (m, 10H, ArH), 8.22 (s, 1H, ArH), 8.35 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 21.8, 25.7, 40.0, 59.1, 59.5, 59.7, 68.8, 71.7, 113.7, 120.2, 123.4, 128.4, 128.8, 129.2, 129.6, 130.5, 130.7, 131.4, 132.5, 134.4, 137.4, 149.4, 162.2. LCMS (hydrophilic): R_f (min) = 9.23, (ESI + ve) found 448 $[M + H]^+$ calcd for $C_{27}H_{34}N_3O_3$.

***N*-(5-(2-((6-aminohexyl)(benzyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide: TFA salt (10c).** The title compound was prepared using the same method that was described for **10a**. The product was obtained as a yellow oil in quantitative yield. ^{1}H NMR (MeOD, δ): 1.43 (m, 4H, 2 × CH_2), 1.69 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 2.93 (t, 2H, J = 7.2, CH_2), 3.18−3.27 (m, 4H, 2 × CH_2), 4.42−4.60 (m, 2H, CH_2), 5.24 (s, 2H, CH_2), 7.01−7.64 (m, 12H, ArH), 8.22 (s, 1H, ArH), 8.35 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 24.3, 26.8, 27.0, 28.2, 40.4, 59.1, 59.5, 59.6, 68.8, 71.8, 113.7, 120.2, 123.5, 128.2, 128.7, 129.2, 129.6, 130.4, 130.8, 131.2, 132.4, 134.5, 137.4, 149.3, 162.3. LCMS (hydrophilic): R_f (min) = 9.23, (ESI + ve) found 476 $[M + H]^+$ calcd for $C_{29}H_{38}N_3O_3$.

***N*-(5-(2-(benzyl(2-(N^6-adenosinyl)ethyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide (11a).** To a suspension of 6-chloropurine riboside (381 mg, 1.33 mmol) in *t*-BuOH (20 mL) was added **10a** (236 mg, 0.44 mmol), followed by DIPEA (694 μL, 3.98 mmol), and the reaction mixture was stirred at 80 °C for 24 h under N_2. The solvent was evaporated under reduced pressure and the residue was purified by flash silica chromatography (eluent 10% MeOH/CH_2Cl_2 + 1% NH_3) to produce the title compound as a yellow oil (134 mg, 45% yield). ^{1}H NMR (MeOD, δ): 2.60−2.85 (m, 4H, 2 × CH_2), 3.45−3.95 (m, 6H, 3 × CH_2), 4.16−4.21 (m, 1H, CH), 4.31−4.36 (m, 1H, CH), 4.67 (t, 1H, J = 6.0, CH), 4.73−4.79 (m, 1H, CH), 5.10 (d, 2H, J = 1.8, CH), 5.96 (d, 1H, J = 6.3, CH), 6.60−7.45 (m, 12H, ArH), 8.10 (m, 1H, ArH), 8.19−8.26 (m, 2H, 2 × CH−purine), 8.29 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 39.7, 54.5, 60.7, 63.6, 63.8, 71.7, 72.6, 72.8, 75.5, 88.3, 91.4, 114.9, 120.7, 121.7, 123.8, 127.9, 128.0, 128.6, 128.8, 129.1, 129.6, 130.2, 137.1, 138.2, 140.1, 141.4, 148.8, 150.2, 153.3, 156.2, 162.0. LCMS (hydrophilic): R_f (min) = 6.54, (ESI + ve) found 670 $[M + H]^+$ calcd for $C_{35}H_{40}N_7O_7$.

***N*-(5-(2-(benzyl(4-(N^6-adenosinyl)butyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide (11b).** The title compound was prepared using the same method that was described for **11a**. After purification by flash chromatography (eluent 5−10% MeOH/CH_2Cl_2 + 1% NH_3), the product was obtained as a yellow oil in 38% yield. ^{1}H NMR (MeOD, δ): 1.50−1.63 (m, 4H, 2 × CH_2), 2.48−2.65 (m, 4H, 2 × CH_2), 3.40−3.55 (m, 2H, CH_2), 3.60−3.94 (m, 4H, 2 × CH_2), 4.15−4.19 (m, 1H, CH), 4.32 (dd, 1H, J = 2.4, J = 5.1, CH), 4.59−4.65 (m, 1H, CH), 4.74 (t, 1H, J = 5.4, CH), 5.15 (s, 2H, CH), 5.94 (d, 1H, J = 6.6, CH), 6.96−7.49 (m, 12H, ArH), 8.18 (m, 2H, ArH and CH−purine), 8.22 (s, 1H, CH−purine), 8.31 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 25.4, 28.1, 41.4, 55.0, 60.2, 63.3, 63.5, 71.8, 72.1, 72.7, 75.5, 88.2, 91.3, 113.2, 120.9, 121.4, 123.7, 127.8, 128.1, 128.8, 129.1, 129.2, 129.6, 130.3, 137.3, 138.1, 140.2, 141.4, 148.7, 150.3, 153.5, 156.2, 162.0. LCMS (hydrophilic): R_f (min) = 6.62, (ESI + ve) found 698 $[M + H]^+$ calcd for $C_{37}H_{44}N_7O_7$.

***N*-(5-(2-(benzyl(6-(N^6-adenosinyl)hexyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide (11c).** To a suspension of 6-chloropurine riboside (135 mg, 0.47 mmol) in *t*-BuOH (25 mL) was added **10c** (213 mg, 0.36 mmol), followed by DIPEA (158 μL, 0.9 mmol), and the reaction mixture was stirred for 24 h at 80 °C for 24 h under N_2. More 6-chloropurine riboside (135 mg, 0.47 mmol) and DIPEA (158 μL, 0.9 mmol) were added, and the reaction was stirred for an additional 24 h at 80 °C. The solvent was removed under reduced pressure, and the residue was purified by flash silica chromatography (eluent 5−10% MeOH/CH_2Cl_2 + 1% NH_3) to produce the title compound as a yellow oil (100 mg, 38% yield). ^{1}H NMR (MeOD, δ): 1.05−1.63 (m, 8H, 4 × CH_2), 2.33−2.48 (m, 2H, CH_2), 2.55−2.70 (m, 2H, CH_2), 3.40−3.62 (m, 4H, 2 × CH_2), 3.80 (dd, 2H, J = 2.7, J = 12.6, CH_2), 4.15−4.19 (m, 1H, CH), 4.33 (dd, 1H, J = 2.7, J = 5.0, CH), 4.56−4.63 (m, 1H, CH), 4.75 (t, 1H, J = 5.4, CH), 5.09 (s, 2H, CH), 5.94 (d, 1H, J = 6.6, CH), 6.93−7.42 (m, 12H, ArH), 8.14−8.21 (m, 2H, 2 × CH−purine), 8.22 (d, 1H, J = 1.5, ArH), 8.32 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 27.8, 27.9, 28.0, 30.4, 41.6, 55.2, 60.3, 63.1, 63.5, 71.8, 72.2, 72.7, 75.5, 88.2, 91.4, 113.1, 120.8, 121.4, 123.7, 127.9, 128.0, 128.8, 129.1, 129.2, 129.5, 130.3, 137.4, 138.0, 140.4, 141.3, 148.7, 150.2, 153.5, 156.2, 162.0. LCMS (hydrophilic): R_f (min) = 6.82, (ESI + ve) found 726 $[M + H]^+$ calcd for $C_{39}H_{48}N_7O_7$.

***N*-(2-hydroxy-2-(2-(N^6-adenosinyl) ethylamino)ethyl)phenyl)formamide (12a) and (12b).** Compound **11a** (130 mg, 1.35 mmol) was dissolved in ethanol, 10% Pd/C (173 mg) was added, and the reaction was shaken for 3 days under an atmosphere of hydrogen at 50 psi. The catalyst was removed by filtration, and the reaction vessel was recharged with 10% Pd/C (173 mg) and was left to shake for 3 days under the same catalytic hydrogenation conditions. The solution was filtered through a Millipore 0.45 μm filter, and the solvent was removed under reduced pressure. The oily residue was purified by preparatory HPLC on a C_{18} column with a gradient of 1−18% ACN/H_2O over 45 min to produce the desired compound **12a** as a pale-yellow solid (13.5 mg) in 14% yield. The deformylated byproduct **12b** was also isolated as a yellow-brown oil (8 mg) in 9% yield. **12a**: ^{1}H NMR (MeOD, δ): 3.20−3.49 (m, 4H, 2 × CH_2), 3.72−3.95 (m, 4H, 2 × CH_2), 4.18 (dd, 1H, J = 2.4, J = 5.1, CH), 4.33 (dd, 1H, J = 2.7, J = 5.1, CH), 4.74 (t, 1H, J = 5.7, CH), 5.99 (d, 1H, J = 6.3, CH), 6.87 (d, 1H, J = 8.1, ArH), 7.06 (dd, 1H, J = 2.1, J = 8.4, ArH), 8.13 (d, 1H, J = 1.8, ArH), 8.27 (s, 1H, CH−purine), 8.30 (s, 1H, CH−purine), 8.33 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 39.2, 49.9, 55.2, 63.5, 70.2, 72.7, 75.7, 88.2, 91.2, 116.2, 120.3, 121.8, 123.7, 127.0, 133.1, 142.3, 148.6, 149.8, 153.4, 156.6, 162.1. LCMS (hydrophilic): R_f (min) = 3.17, (ESI + ve) found 490 $[M + H]^+$ calcd for $C_{21}H_{28}N_7O_7$. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{21}H_{28}N_7O_7$, 490.2045; found, 490.2042. **12b**: ^{1}H NMR (MeOD, δ): 3.20−3.49 (m, 4H, 2 × CH_2), 3.72−3.95 (m, 4H, 2 × CH_2), 4.18 (dd, 1H, J = 2.4, J = 5.1, CH), 4.33 (dd, 1H, J = 2.7, J = 5.1, CH), 4.73 (t, 1H, J = 5.4, CH), 5.99 (d, 1H, J = 6.3, CH), 6.77 (d, 1H, J = 8.1, ArH), 6.83 (dd, 1H, J = 2.1, J = 8.3, ArH), 6.97 (d, 1H, J = 1.8, ArH), 8.27 (s, 1H, CH−purine), 8.33 (s, 1H, CH−purine). ^{13}C NMR (MeOD, δ): 39.1, 49.9, 55.2, 63.5, 70.0, 72.7, 75.7, 88.3, 91.2, 116.0, 116.8 120.6, 121.8, 132.3, 133.6, 142.3, 148.2, 149.8, 153.4, 156.6. LCMS (hydrophilic): R_f (min) = 1.74, (ESI + ve) found 462 $[M + H]^+$ calcd for $C_{20}H_{28}N_7O_6$. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{28}N_7O_6$, 462.2101; found, 462.2101.

***N*-(2-hydroxy-5-(1-hydroxy-2-(4-(N^6-adenosinyl) butylamino)-ethyl)phenyl)formamide (12c) and (12d).** Compound **11b** (110 mg, 0.16 mmol) was dissolved in ethanol (10 mL), 10% Pd/C (187 mg) was added, and the reaction was shaken for 2 days under an atmosphere of hydrogen at 50 psi. The solution was filtered through a Millipore 0.45 μm filter, and the solvent was removed under reduced pressure. The oily residue was purified by preparatory HPLC on a C_{18} column with a gradient of 1−18% ACN/H_2O over 45 min to produce the desired compound **12c** as an off-white solid (12 mg) in 15% yield. The deformylated byproduct **12d** was also isolated as a pale-yellow solid (3 mg) in 4% yield. **12c**: ^{1}H NMR (MeOD, δ): 1.74−1.84 (m, 4H, 2 × CH_2), 3.02−3.14 (m, 4H, 2 × CH_2), 3.62−3.72 (m, 2H, CH_2), 3.81 (dd, 2H, J = 2.4, J = 12.6, CH_2), 4.17 (dd, 1H, J = 2.4, J = 4.8, CH), 4.32 (dd, 1H, J = 2.4, J = 5.1, CH), 4.74 (dd, 1H, J = 5.3, J = 6.6, CH), 5.95 (d, 1H, J = 6.3, CH), 6.87 (d, 1H, J = 8.1, ArH), 7.04 (dd, 1H, J = 2.1, J = 8.4, ArH), 8.11 (d, 1H, J = 1.8, ArH), 8.22 (s, 1H, CH−purine), 8.26 (s, 1H, CH−purine), 8.31 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 25.0, 27.9, 40.9, 49.6, 55.6, 63.6, 70.6, 72.8, 75.5, 88.3, 91.3, 116.1, 120.4, 121.5, 123.7, 127.0, 133.6, 141.6, 148.5, 149.9, 153.6, 156.5, 162.1. LCMS (hydrophilic, formate method ACN/water (10 mM NH_4−formate)): R_f (min) = 3.33, (ESI + ve) found 518 $[M + H]^+$ calcd for $C_{23}H_{32}N_7O_7$. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{23}H_{32}N_7O_7$, 518.2358; found, 518.2370. **12d**: ^{1}H NMR (MeOD, δ): 1.74−1.86 (m, 4H, 2 × CH_2), 3.05−3.15 (m, 4H, 2 × CH_2), 3.62−3.73 (m, 2H, CH_2), 3.82 (dd, 2H, J = 2.7, J = 12.5, CH_2), 4.17 (dd, 1H, J = 2.4, J = 5.1, CH), 4.32 (dd, 1H, J = 2.4, J = 5.0, CH), 4.71−4.79 (m, 2H, 2 × CH), 5.95 (d, 1H, J = 6.6, CH), 6.62 (dd, 1H, J = 2.1, J = 8.1, ArH), 6.70 (d, 1H, J = 8.1, ArH), 6.78 (d, 1H, J = 1.8, ArH), 8.23 (s, 1H, CH−purine), 8.27 (s, 1H, CH−purine). ^{13}C NMR (MeOD, δ): 24.4, 27.8, 40.7, 49.7, 55.3, 63.6, 70.3, 72.7, 75.6, 88.3, 91.3, 114.7, 115.5, 117.5, 121.5, 133.6, 136.8, 141.7, 146.7, 149.5, 153.6, 156.5. LCMS (hydrophilic): R_f (min) = 1.88, (ESI + ve) found 490 $[M + H]^+$ calcd for $C_{22}H_{32}N_7O_6$. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{32}N_7O_6$, 490.2409; found, 490.2422.

***N*-(2-hydroxy-5-(1-hydroxy-2-(6-(N^6-adenosinyl)hexylamino)-ethyl)phenyl)formamide (12e).** Compound **11c** (100 mg, 0.14 mmol) was dissolved in methanol, 10% Pd/C (123 mg) was added, and the reaction was shaken for 2 days under an atmosphere of hydrogen at 50 psi. The solution was filtered through a Millipore 0.45 μm filter, and the solvent was removed under reduced pressure. The oily residue was purified by preparatory HPLC on a C_{18} column with a gradient of 1−18% ACN/H_2O over 50 min to produce the desired compound **12e** with traces of ammonium acetate. The compound was subjected to a prepacked C_{18} column to remove the ammonium acetate, and the title compound was produced as a light-yellow solid (3.5 mg) in 5% yield. ^{1}H NMR (MeOD, δ): 1.42−1.56 (m, 4H, 2 × CH_2), 1.66−1.80 (m, 4H, 2 × CH_2), 3.04 (t, 2H, J = 8.0, CH_2), 3.07−3.17 (m, 2H, CH_2), 3.61 (m, 2H, CH_2), 3.81 (dd, 2H, J = 2.1, J = 12.6, CH_2), 4.18 (m, 1H, CH), 4.32 (dd, 1H, J = 2.4, J = 5.0, CH), 4.74 (t, 1H, J = 5.7, CH), 5.95 (d, 1H, J = 6.3, CH), 6.88 (d, 1H, J = 8.1, CH), 7.05 (dd, 1H, J = 1.8, J = 8.3, ArH), 8.13 (br s, 1H, ArH), 8.21 (s, 1H, CH−purine), 8.25 (s, 1H, CH−purine), 8.31 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 26.9, 27.3, 27.4, 30.3, 41.4, 48.9, 55.1, 63.6, 70.0, 72.7, 75.5, 88.3, 91.3, 116.1, 120.3, 121.5, 123.7, 127.0, 133.2, 141.5, 148.6, 149.1, 153.5, 156.4, 162.1. LCMS (hydrophilic): R_f (min) = 4.53, (ESI + ve) found 546 $[M + H]^+$ calcd for $C_{25}H_{36}N_7O_7$. HR-ESMS (*m*/*z*): $[M + H]^+$ calcd for $C_{25}H_{36}N_7O_7$, 546.2671; found, 546.2673.

***tert*-Butyl 2-(2-(2-(Benzyl(2-(4-(benzyloxy)-3-nitrophenyl)hydroxyethyl)amino)ethoxy)ethoxy)ethylcarbamate (13).** The title compound was prepared using the same method that was described for **3a**. After purification by flash chromatography (eluent 5% MeOH/CH_2Cl_2 + 1% NH_3), the product was obtained as a yellow oil in 58% yield. ^{1}H NMR (MeOD, δ): 1.37 (s, 9H, 3 × CH_3), 2.64−2.88 (m, 4H, 2 × CH_2), 3.20 (t, 2H, J = 5.4, CH_2), 3.44−3.59 (m, 8H, 4 × CH_2), 3.70 (s, 2H, CH_2), 4.66 (t, 1H, J = 6.6, CH), 5.22 (s, 2H, CH_2), 7.16−7.49 (m, 12H, ArH), 7.77 (d, 1H, J = 2.1, ArH). ^{13}C NMR (MeOD, δ): 28.8, 41.4, 55.1, 61.1, 63.6, 70.6, 71.1, 71.2, 71.3, 71.4, 72.2, 80.1, 116.2, 124.1, 128.1, 128.3, 129.1, 129.3, 129.6, 130.2, 132.9, 137.5, 137.7, 140.5, 141.3, 151.9, 158.4. LCMS (hydrophilic): R_f (min) = 9.46, (ESI + ve) found 610 $[M + H]^+$ calcd for $C_{33}H_{44}N_3O_6$.

***tert*-Butyl 2-(2-(2-(Benzyl(2-(4-(benzyloxy)-3-aminophenyl)hydroxyethyl)amino)ethoxy)ethoxy)ethylcarbamate (14).** The title compound was prepared using the same method that was described for **8a**. After purification by flash chromatography (eluent 5−10% MeOH/CH_2Cl_2 + 1% NH_3), the product was obtained as a yellow oil in 45% yield. ^{1}H NMR (MeOD, δ): 1.39 (s, 9H, 3 × CH_3), 2.60−2.88 (m, 4H, 2 × CH_2), 3.21 (t, 2H, J = 5.4, CH_2), 3.43−3.59 (m, 8H, 4 × CH_2), 3.64, 3.78 (d, 2H, J = 13.5, CH_2), 4.54 (t, 1H, J = 6.6, CH), 5.03 (s, 2H, CH_2), 6.61 (dd, 1H, J = 2.1, J = 8.4,

ArH), 6.76 (d, 1H, J = 1.8, ArH), 6.80 (d, 1H, J = 8.4, ArH), 7.18−7.46 (m, 10H, ArH). ^{13}C NMR (MeOD, δ): 28.8, 41.3, 54.6, 60.8, 64.0, 70.3, 71.1, 71.2, 71.4, 72.3, 80.0, 113.1, 114.6, 117.4, 128.1, 128.6, 128.9, 129.3, 129.5, 130.2, 137.0, 137.8, 138.8, 140.5, 147.3, 158.3. LCMS (hydrophilic): R_f (min) = 7.74, (ESI + ve) found 580 $[M + H]^+$ calcd for $C_{33}H_{46}N_3O_6$.

***tert*-Butyl 2-(2-(2-(Benzyl(2-(4-(benzyloxy)-3-formamidophenyl)-hydroxyethyl)amino)ethoxy)ethoxy)ethylcarbamate (15).** The title compound was prepared using the same method that was described for **9a**. The product was obtained as a light-yellow oil in 99% yield. ^{1}H NMR (MeOD, δ): 1.39 (s, 9H, 3 × CH_3), 2.65−2.90 (m, 4H, 2 × CH_2), 3.20 (t, 2H, J = 5.4, CH_2), 3.44−3.63 (m, 8H, 4 × CH_2), 3.72, 3.81 (d, 2H, J = 13.5, CH_2), 4.61 (t, 1H, J = 6.6, CH), 5.17 (s, 2H, CH_2), 6.95−7.13 (m, 2H, ArH), 7.15−7.51 (m, 10H, ArH), 8.19 (s, 1H, ArH), 8.32 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 28.8, 41.3, 54.7, 60.7, 63.6, 70.0, 71.1, 71.2, 71.3 or (2C at 71.2), 71.7, 71.9, 80.0 113.2, 120.6, 123.5, 127.9, 128.3, 128.8, 129.1, 129.4, 129.6, 130.4, 136.6, 138.1, 139.7, 148.6, 158.3, 162.0. LCMS (hydrophilic): R_f (min) = 8.71, (ESI + ve) found 608 $[M + H]^+$ calcd for $C_{34}H_{46}N_3O_7$.

***N*-(5-(2-((2-(2-(2-aminoethoxy)ethoxy)ethyl)benzyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide: TFA salt (16).** The title compound was prepared using the same method that was described for **10a**. The product was obtained as a yellow gum in quantitative yield. ^{1}H NMR (MeOD, δ): 2.99−3.14 (m, 2H, CH_2), 3.32−3.41 (m, 2H, CH_2), 3.59 (m, 2H, CH_2), 3.60−3.75 (m, 6H, 3 × CH_2), 3.92 (m, 2H, CH_2), 4.52, 4.62 (d, 2H, J = 13.2, CH_2), 5.22 (s, 2H, CH_2), 7.12−7.64 (m, 12H, ArH), 8.24 (s, 1H, ArH), 8.35 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 40.5, 54.4, 59.7, 60.2, 66.0, 67.9, 68.6, 71.3, 71.4, 71.7, 113.7, 120.2, 123.5, 128.3, 128.6, 129.2, 129.6, 130.4, 130.9, 131.2, 132.5, 134.4, 137.9, 149.4, 162.3. LCMS (hydrophilic): R_f (min) = 8.10, (ESI + ve) found 508 $[M + H]^+$ calcd for $C_{29}H_{38}N_3O_5$.

***N*(5-(2-((2-(2-(*N*^{6}-adenosinylethoxy)ethoxy)ethyl)(benzyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)formamide (17).** The title compound was prepared using the same method that was described for **11a** (yield 50%, yellow oil, eluent 5−10% MeOH/CH_2Cl_2 + 1% NH_3). ^{1}H NMR (MeOD, δ): 2.55−2.85 (m, 4H, 2 × CH_2), 3.40−3.92 (m, 14H, 7 × CH_2), 4.17 (dd, 1H, J = 2.4, J = 4.7, CH), 4.32 (dd, 1H, J = 2.4, J = 5.0, CH), 4.55−4.63 (m, 1H, CH), 4.70−4.76 (m, 1H, CH), 5.09 (s, 2H, CH), 5.93 (d, 1H, J = 6.6, CH), 6.92−7.02 (m, 2H, ArH), 7.01−7.45 (m, 10H, ArH), 8.14−8.21 (m, 3H, ArH and 2 × CH−purine), 8.30 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 41.5, 54.7, 60.7, 63.5, 63.7, 70.4, 70.7, 71.3, 71.4, 71.7, 72.1, 72.7, 75.5, 88.2, 91.3, 113.1, 120.7, 121.5, 123.7, 127.8, 128.1, 128.8, 129.1, 129.2, 129.6, 130.2, 136.8, 138.1, 140.3, 141.5, 148.7, 150.2, 153.4, 156.2, 162.0. LCMS (hydrophilic): R_f (min) = 6.50, (ESI + ve) found 758 $[M + H]^+$ calcd for $C_{39}H_{48}N_7O_9$.

***N*-(5-(2-((2-(2-(*N*^{6}-adenosinylethoxy)ethoxy)ethyl)amino)-1-hydroxyethyl)-2-hydroxyphenyl)formamide (18).** We prepared the title compound by utilizing the same method that was described for **12e** (yield 16%, preparatory HPLC, C_{18} column with a gradient of 1−18% ACN/H_2O over 45 min. ^{1}H NMR (MeOD, δ): 2.72−2.90 (m, 4H, 2 × CH_2), 3.53−3.68 (m, 6H, 3 × CH_2), 3.69−3.93 (m, 6H, 3 × CH_2), 4.17 (dd, 1H, J = 2.4, J = 5.1, CH), 4.32 (dd, 1H, J = 2.4, J = 5.1, CH), 4.69 (dd, 1H, J = 4.8, J = 8.6, CH), 4.74 (dd, 1H, J = 5.4, J = 6.3, CH), 5.95 (d, 1H, J = 6.6, CH), 6.82 (d, 1H, J = 8.1, ArH), 6.99 (dd, 1H, J = 2.1, J = 8.4, ArH), 8.04 (d, 1H, J = 2.1, ArH), 8.20−8.25 (m, 2H, 2 × CH−purine), 8.29 (s, 1H, CHO). ^{13}C NMR (MeOD, δ): 41.6, 49.5, 57.5, 63.6, 70.5, 70.7, 71.4, 71.5, 72.8, 73.0, 75.5, 88.3, 91.3, 116.2, 120.6, 121.5, 124.0, 126.8, 135.3, 141.6, 148.3, 149.3, 153.5, 156.4, 162.0. LCMS (hydrophilic, formate method ACN/water (10 mM NH_4−formate)): R_f (min) = 3.75, (ESI + ve) found 578 $[M + H]^+$ calcd for $C_{25}H_{36}N_7O_9$. HR-ESMS (m/z): $[M + H]^+$ calcd for $C_{25}H_{36}N_7O_9$, 578.2569; found, 578.2566.

Cell Culture and cAMP Determination. DDT_1 MF-2 cells, derived from leiomyosarcoma of Syrian hamster vas deferens, were grown in 48-well plates by using Delbecco's modified Eagle's medium that contained streptomycin sulfate (0.1 mg/mL), amphotericin B (2.5 μg/mL), penicillin G (100 U/mL), and 5% fetal bovine serum at 37 °C. Cells were used in experiments at one day preconfluence. The culture medium was aspirated, and the cells were rinsed once with warm Hank's balanced salt solution (HBSS). Experiments were started by the addition of 1 mL of HBSS that contained adenosine deaminase (0.5 U/mL) and 20 μM rolipram and that was either with or without varying concentrations of test compounds. After a 6 min incubation at 37 °C, the medium was aspirated, and 0.5 mL of 50 mM HCl was added. The cAMP content in each well was determined by radioimmunoassay. Briefly, 5 μL from each plate was diluted with 100 μL of 50 mM HCl, and the cAMP was acetylated by the addition of 4.5 μL of a 3.5:1 mixture of triethylamine and acetic anhydride with vortex mixing. A 10 μL aliquot of [^{125}I]-ScAMP-TME that contained 20 000 cpm was added, followed by 100 μL of cAMP antibody dissolved in 50 mM Na−acetate buffer at pH 4.75 that contained 0.125% BSA. The samples were incubated at room temperature for 60 min, after which 50 μL of hydroxyapatite in a 1:1 suspension with water was added for an additional 10 min. The samples were diluted with 3 mL of ice-cold 10 mM Tris buffer at pH 7.0 and were then aspirated through Whatman GF/B glass fiber filters by the use of a Brandell cell harvester. The filters were washed with an additional 6 mL of ice-cold buffer, and we determined the retained radioactivity by using a Beckman gamma counter. The cAMP that was accumulated was determined from a standard curve. All assays were performed in quadruplicate.

Receptor Assays. DDT cell membranes and the displacement of specific [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine (2.5 nM) binding from the A_1AR was determined, as described previously.[29] The interaction of test compounds with the β_2AR was determined by the displacement of specific [^{125}I]-(−)iodopindolol binding. Briefly, cell membranes were incubated for 60 min at room temperature in a total volume of 0.25 mL that contained 50 mM Tris−HCl buffer at pH 7.4, 5 mM $MgCl_2$, 100 pM [^{125}I]-(−)iodopindolol, and 10 μM 5′-guanylyl-imidodiphosphate and that was either with or without varying concentrations of test compounds. Nonspecific binding was determined in parallel assays that contained 1 μM alprenolol. At the end of the incubation, each suspension was diluted with 3 mL of ice-cold incubation buffer, and the membranes were collected by retention on a Whatman GF/B glass fiber filter under reduced pressure. The filters were washed with an additional 6 mL of ice-cold buffer, and the radioactivity was determined in a gamma counter. All assays were performed in triplicate.

Data Analysis. The concentration of test compounds that stimulated cAMP accumulation by 50% the maximal response (EC_{50}) were determined by nonlinear regression analysis of the concentration response using the GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA). We determined the concentration of compounds that inhibited specific radioligand binding by 50% (IC_{50}) by nonlinear regression analysis using GraphPad Prism. We calculated the dissociation constants (K_i) for the compounds from the IC_{50} values by using the conversion described by Cheng and Prusoff.[30]

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and LCMS analyses for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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