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ORIGINAL PAPER

Synthesis of BODIPY Derivatives Substituted with Various Bioconjugatable Linker Groups: A Construction Kit for Fluorescent Labeling of Receptor Ligands

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Abstract The goal of the present study was to design small, functionalized green-emitting BODIPY dyes, which can readily be coupled to target molecules such as receptor ligands, or even be integrated into their pharmacophores. A simple two-step one-pot procedure starting from 2,4-dimethylpyrrole and ω-bromoalkylcarboxylic acid chlorides was used to obtain new ω-bromoalkyl-substituted BODIPY fluorophores (**1a**–**1f**) connected via alkyl spacers of different length to the 8-position of the fluorescent dye. The addition of radical inhibitors reduced the amount of side products. The ω-bromoalkyl-substituted BODIPYs were further converted to introduce various functional groups: iodo-substituted dyes were obtained by Finkelstein reaction in excellent yields; microwave-assisted reaction with methanolic ammonia led to fast and clean conversion to the amino-substituted dyes; a hydroxyl-substituted derivative was prepared by reaction with sodium ethylate, and thiol-substituted BODIPYs were obtained by reaction of **1a**–**1f** with potassium thioacetate followed by alkaline cleavage of the thioesters. Water-soluble derivatives were prepared by introducing sulfonate groups into the 2- and 6-position of the BODIPY core. The synthesized BODIPY derivatives showed high fluorescent yields and appeared to be stable under basic, reducing and oxidative conditions. As a proof of concept, 2-thioadenosine was alkylated with bromoethyl-BODIPY **1b**. The resulting fluorescent 2-substituted adenosine derivative **15** displayed selectivity for the A_3 adenosine receptor (ARs) over the other AR subtypes, showed agonistic activity, and may thus become a useful tool for studying A_3ARs, or a lead structure for further optimization. The new functionalized dyes may be widely used for fluorescent labeling allowing the investigation of biological targets and processes.

Keywords BODIPY · Fluorescent dyes · Bioimaging · Adenosine receptor ligand

Abbreviations

AR	adenosine receptor
A_1AR	A_1 adenosine receptor
A_{2A}AR	A_{2A} adenosine receptor
A_{2B}AR	A_{2B} adenosine receptor
A_3AR	A_3 adenosine receptor
BHT	3,5-di-*tert*-butyl-4-hydroxytoluene
BODIPY	4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene
BSA	bovine serum albumin
CADO	2-chloroadenosine
CCPA	2-chloro-N^6-cyclopentyladenosine
CGS21680	4-[2-[[6-amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid
CHO-cells	Chinese hamster ovary cells
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DPCPX	8-cyclopentyl-1,3-dipropylxanthine
FCS	fetal bovine serum
$FWHM_{abs}$	full width at half maximum at the absorption
$FWHM_{em}$	full width at half maximum at the emission
h	human
HBSS	Hank's balanced salt solution

Fabian Heisig and Sabrina Gollos are contributed equally.

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HEK	human embryonic kidney cells
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
MW	microwave
NECA	5'-(*N*-ethylcarboxamido)adenosine
PBS	phosphate buffer saline
r	rat
RO-20-1724	4-(3-butoxy-4-methoxyphenyl)methyl-2-imidazolidone
R-PIA	(*R*)-N^6-(1-methyl-2-phenylethyl)adenosine
TRIS	tris(hydroxymethyl)aminomethan

Introduction

In recent years an increasing interest in the design of fluorophores and fluorescent-labeled compounds has been observed that can be used in numerous applications in biochemistry and molecular biology [1, 2]. Typically used fluorophores include fluorescein, rhodamine, coumarine, and borondipyrromethene (BODIPY) derivatives [1]. BODIPYs are well-known fluorescent probes, which were first described by Treibs and Kreuzer in 1968 [3]. Since that time many studies have appeared on the synthesis of a variety of BODIPY derivatives with different properties [2, 4–11]. BODIPY derivatives show a number of advantages: they are nonpolar and neutral, they exhibit photochemical stability, and they are characterized by exceptional spectral properties ($\varepsilon \sim 7 \times 10^4$ to 10^5 l mol^{-1} cm^{-1} at $\lambda_{max} \geq 500$ up to 630 nm^{-1}). Their high absorption coefficients and high fluorescence quantum yields ($\Phi > 0.5$) and the resulting high peak intensity makes them easily detectable fluorophores [12–16]. BODIPY derivatives have been used as fluorescent probes for studying interactions between ligands and their receptors [17–20], for DNA sequencing [21–23] and as fluorescent probes for proton [24–26] and metal ion detection [27–29]. BODIPYs have also been used for studying the structure, function, and dynamics of biological systems such as lipid membranes or proteins [30–33].

A number of BODIPY derivatives are commercially available in small quantities typically required for biochemical experiments. Our goal was to develop a simple, one-pot reaction procedure for new, small BODIPY derivatives, which allows structural modification, and in particular the introduction of different functional groups. These were to be attached via a spacer group of variable length to the BODIPY core and should allow for the coupling to and the fluorescent labeling of target molecules of interest.

Adenosine receptors (ARs) are a family of G protein-coupled receptors (GPCRs) subdivided into four distinct subtypes, A_1, A_{2A}, A_{2B}, and A_3. Fluorescent-labeled ligands, agonists and antagonists, for ARs have been previously described [17, 20, 34–50]. They have been shown to be useful tools for studying receptor physiology and pathophysiology at the molecular level [51]. In addition they have been utilized as tools for compound screening, and some of the flourescent assays were found to be complementary or even superior to radioligand-based ones [20, 39]. In the present study we utilized a newly prepared functionalized BODIPY derivative to synthesize a novel BODIPY-labeled adenosine derivative and investigated its receptor binding and functional properties.

Due to the high costs, the risk in handling, as well as public concerns regarding the use of radioactivity, the development of fluorescent dyes provides an alternative which has gained importance and may become the future method of choice for many studies.

Experimental

General Remarks

All reactions were performed in dry solvents, unless otherwise indicated. Dichloromethane (DCM) was freshly distilled over CaH_2 prior to use. Tetrahydrofurane (THF), ethanol and acetone was used as commercially obtained. All other reagents obtained from various providers (Acros, Aldrich, Fluka, Merck, Sigma) and used as obtained unless otherwise noted. Microwave reactions were carried out in a 50 mL sealed glass tube in a focused mono-mode microwave oven (Discover from CEM Corporation, Matthews, NC, USA). Maximum power levels, target temperatures and reaction times are given below. The reactions were monitored by thin layer chromatography (TLC) using silica gel coated aluminum sheets (0.2 mm layer, nano-silica gel 60 with fluorescence indicator UV_{254} (Merck, Darmstadt, Germany)). Column chromatography was performed on Merck silica gel 60 (mesh size 0.040-0.063 mm). NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer. The used solvents are given below. Mass spectra were determined on an API 2000 (Applied Biosystems, Darmstadt, Germany) mass spectrometer. HR-MS (EI) were determined on a MS 50 Kratos spectrometer and on a Thermoquest MAT 95XL instrument. Melting points are measured in open capillary tubes on a Wepa Apotec capillary melting point apparatus (Wepa, Höhr-Grenzhausen, Germany) and are uncorrected.

Determination of Fluorescent Quantum Yields

Fluorescent quantum yield measurements were performed on a fluorimeter (Cary Eclipse fluorescence spectrophotometer, Varian) and UV/Vis instrument (UV/Vis spectrometer lambda 25, Perkin Elmer instruments). The slit width was 5 nm for excitation and emission. Relative quantum yields were obtained by comparing the areas under the corrected emission

spectrum. The following equation was used to calculate the quantum yields.

$$\Phi_x = \Phi_{st} \cdot \frac{I_x}{I_{st}} \cdot \frac{A_{st}}{A_x} \cdot \frac{\eta_x^2}{\eta_{st}^2}$$

Where Φ_{st} is the reported quantum yield of the standard, I is the integrated emission spectrum, A is the absorbance at the excitation wavelength and η is the refractive index of the used solvents. The subscript x denotes unknown and st denotes standard. Rhodamin 6G (Φ=0.94 in ethanol) was used as a standard.

General Procedure for the Preparation of Bromoalkyl-BODIPY Derivatives 1a-1f

The appropriate bromoalkanoic acid chloride (n=1, 2, 3, 4, 5 and 10) (5 mmol) was dissolved in dry dichloromethane (50 mL) and the mixture was cooled to 0 °C. Within 30 min 0.95 g (10 mmol) of 2,4-dimethylpyrrole, dissolved in dry dichloromethane (20 mL), were added. After the reaction mixture was allowed to warm up to rt the solution was stirred for an additional 2.5 h (**1a**), 105 min (**1b**), 1.5 h (**1c**) or 3.5 h (**1e**, **1f**), respectively. The mixture was then cooled again to 0 °C and neutralized by the addition of dry triethylamine (2.8 mL, 20 mmol). After 30 min, boron trifluoride etherate (2.5 mL, 10 mmol) was added and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure and the residue was purified on a silica gel column using dichloromethane : petroleum ether (bp 60–80 °C) (1:1, for compounds **1a**–**c**; 2:1 for compounds **1e**, **1f**).

8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (1a)

Magenta-colored solid, (682 mg, 40 % yield); mp 220–221 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.51 (s, 6H), 2.52 (s, 6H), 4.65 (s, 2H), 6.06 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.7, 15.9, 24.5, 122.3, 131.0, 137.2, 140.9, 156.5; MS (ESI): *m/z*=342 $[M+H]^+$. HRMS (ESI) m/z calcd 363.0453 ($C_{14}H_{16}BBrF_2N_2Na$), found: 363.0451.

8-(2-Bromoethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (1b)

Red-colored solid (266 mg, 15 %); mp 157–159 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.42 (s, 6H), 2.50 (s, 6H), 3.45 (s, 4H), 6.07 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.9, 16.5, 30.1, 31.9, 122.6, 131.8, 140.3, 140.9, 155.6; MS (ESI): *m/z*=356 $[M+H]^+$. HRMS (ESI) m/z calcd 379.0588 ($C_{15}H_{18}BBrF_2N_2Na$), found: 379.0585.

8-(3-Bromopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1c)

Orange-colored solid (551 mg, 30 %); mp 153–155 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.15 (m, 2H), 2.43 (s, 6H), 2.50 (s, 6H), 3.11 (m, 2H), 3.54 (t, J=6.26 Hz, 2H), 6.04 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.5, 16.7, 27.2, 32.9, 34.0, 121.9, 131.0, 140.3, 144.2, 154.4; MS (ESI): *m/z*=368 $[M+H]^+$. HRMS (ESI) m/z calcd 391.0766 ($C_{16}H_{20}BBrF_2N_2Na$), found: 391.0764.

8-(5-Bromopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1e)

Orange-colored solid solid (1.211 g, 61 %); mp 134–136 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.61 (m, 4H), 1.91 (m, 2H), 2.40 (s, 6H), 2.49 (s, 6H), 2.94 (m, 2H), 3.41 (t, J=6.6 Hz, 2H), 6.03 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.3, 28.1, 28.5, 30.9, 32.2, 33.5, 121.6, 131.4, 140.2, 145.3, 153.9; MS (ESI): *m/z*=398 $[M+H]^+$. HRMS (ESI) m/z calcd 419.1079 ($C_{18}H_{24}BBrF_2N_2Na$), found: 419.1071.

8-(10-Bromodecanyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1f)

Orange-colored solid (280 mg, 12 %); mp 81–83 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.31–1.47 (m, 12H), 1.60 (m, 2H), 1.83 (m, 2H), 2.39 (s, 6H), 2.49 (s, 6H), 2.90 (m, 2H), 3.39 (t, J=6.9 Hz, 2H), 6.02 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.4, 28.1–32.8, 33.9, 121.5, 131.4, 140.3, 146.7, 153.7; MS (ESI): *m/z*=468 $[M+H]^+$, HRMS (ESI) m/z calcd 489.1863 ($C_{23}H_{34}BBrF_2N_2Na$), found: 489.1856.

8-(4-Bromobutyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (1d)

2,4-Dimethylpyrrole (0.95 g, 10 mmol) was dissolved in dry toluene (10 mL) in a sealed tube under an argon atmosphere. 5-Bromovaleroyl chloride (1.0 g, 5 mmol) dissolved in dry toluene (5 mL) was slowly added to the tube at rt. The mixture was heated for 2.5 h at 90 °C. After cooling to rt dry triethylamine (2.8 mL, 20 mmol) was added. The mixture was stirred for 30 min at rt, and boron trifluoride etherate (2.5 mL, 10 mmol) was added. The mixture was subsequently stirred overnight at 80 °C. The solvent was evaporated under reduced pressure and the residue was purified on a silica gel column using dichloromethane : petroleum ether (2:1). Orange colored solid (500 mg, 26 %); mp 164–166 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.78 (m, 2H), 2.04 (m, 2H), 2.40 (s, 6H), 2.49 (s, 6H), 2.95 (m, 2H), 3.43 (t, J=6.5 Hz, 2H), 6.04 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.5, 16.4, 27.5, 30.2, 32.7, 33.1, 121.8, 131.4, 140.2, 145.3, 154.1; MS (ESI):

m/z = 384 $[M+H]^+$. HRMS (ESI) m/z calcd 405.0923 ($C_{17}H_{22}BBrF_2N_2Na$), found: 405.0922.

General Procedure for the Synthesis of 8-(iodoalkyl)-4, 4-difluoro-1,4,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene Derivatives (2a–e)

The appropriate 8-(bromoalkyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene derivative (**1b**-**f**, 0.26 mmol) was dissolved in 10 mL of acetone. Then 0.78 g of sodium iodide (5.2 mmol) were added and the reaction mixture was stirred under reflux for 18 h. The solvent was removed under reduced pressure and the crude product was purified on a silica gel column using dichloromethane : petroleum ether (bp 60–80 °C) (1 : 1, for compounds **2a** and **2b**, 2 : 1 for compounds **2c**–**e**).

8-(2-Iodoethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (2a)

Red-colored solid (91 mg, 87 %); mp 168–169 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.42 (s, 6H), 2.50 (s, 6H), 3.45 (m, 4H), 6.07 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.9, 16.5, 30.1, 31.9, 122.6, 131.8, 140.3, 140.9, 155.6; MS (ESI): *m/z* = 403.3 $[M+H]^+$, HRMS (ESI) m/z calcd 425.0471 ($C_{15}H_{18}BIF_2N_2Na$), found: 425.0472.

8-(3-Iodopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (2b)

Orange-colored solid (98 mg, 91 %); mp 156–158 °C; ^{1}H-NMR ($CDCl_3$): δ=2.12 (m, 2H), 2.45 (s, 6H), 2.52 (s, 6H), 3.07 (t, 2H), 2.97 (t, J=6.62 Hz, 2H), 6.22 (s, 2H); ^{13}C-NMR ($CDCl_3$): δ=4.7, 14.8, 16.8, 29.4, 34.5, 121.8, 131.5, 140.3, 143.9, 154.4; MS (ESI): *m/z* = 417.0 $[M+H]^+$, HRMS (ESI) m/z calcd 439.0627 ($C_{16}H_{20}BIF_2N_2Na$), found: 439.0628.

8-(4-Iodobutyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (2c)

Orange-colored solid (104 mg, 93 %); mp 148–149 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.76 (m, 2H), 2.02 (m, 2H), 2.42 (s, 6H), 2.52 (s, 6H), 2.98 (t, J=8.5 Hz, 2H), 3.22 (t, J= 6.9 Hz, 2H), 6.06 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ= 5.3, 14.5, 16.5, 27.4, 33.9, 35.6, 121.8, 131.4, 140.2, 145.2, 154.1; MS (ESI): *m/z* = 431.0 $[M+H]^+$, HRMS (ESI) m/z calcd 453.0784 ($C_{17}H_{22}BIF_2N_2Na$), found: 453.0788.

8-(5-Iodopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (2d)

Orange-colored solid (111 mg, 96 %); mp 143–144 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.63 (m, 4H), 1.86 (quin, J= 6.9 Hz, 2H), 2.40 (s, 6H), 2.49 (s, 6H), 2.94 (t, J=7.9 Hz, 2H), 3.19 (t,, J=6.6 Hz, 2H), 6.04 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=6.5, 14.4, 16.4, 28.2, 30.7, 30.9, 32.8, 121.7, 131.4, 140.2, 145.9, 153.9; MS (ESI): *m/z* = 445.1 $[M+H]^+$, HRMS (ESI) m/z calcd 467.0941 ($C_{18}H_{24}BIF_2N_2Na$), found: 467.0944.

8-(10-Iododecanyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (2e)

Orange-colored solid (119 mg, 89 %); mp 102–104 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.28–1.40 (m, 10H), 1.86 (quin, J=6.9 Hz, 2H), 2.40 (s, 6H), 2.49 (s, 6H), 2.94 (t, J=7.9 Hz, 2H), 3.19 (t, J=6.6 Hz, 2H), 6.04 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=7.25, 14.4, 16.4, 28.5–31.9, 30.7, 33.5, 121.5, 131.4, 140.3, 146.6, 153.7; MS (ESI): 515.3 $[M+H]^+$, HRMS (ESI) *m/z* calcd 537.1724 ($C_{23}H_{34}BIF_2N_2Na$), found: 537.1727.

General Procedure for the Synthesis of 8-(aminoalkyl)-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene Derivatives 3a-3e

The appropriate 8-(bromoalkyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene derivative (**1b**-**f**, 0.4 mmol) was suspended in 7*N* NH_3 in methanol (12 mL). The reactions were performed under microwave irradiation (100 W, 100 °C, 10 bar, 20 min); for 8-(2-bromoethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene derivative (**1b**) at 100 W, 80 °C, 10 bar, for 20 min. The solvent was evaporated under reduced pressure and the crude product was dissolved in dichloromethane. The pure product was precipitated by dropwise addition of petroleum ether, and subsequent filtration yielded an orange-colored solid. The residue was washed with water to remove ammonium bromide.

8-(2-Aminoethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene (3a)

Red-colored solid solid (31 mg, 27 %); mp: 188–190 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.39 (s, 6H), 2.41 (s, 6H), 2.95 (t, J=7.5 Hz, 2H 3.00 (t, J=7.5 Hz, 2H), 6.07 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.9, 16.5 30.1, 31.9, 122.6, 131.8, 140.3, 140.9, 155.6; MS (ESI): *m/z* = 272 $[M+H]^+$, HRMS (ESI) m/z calcd 272.1732 ($C_{15}H_{20}BFN_3$), found: 272.1732.

8-(3-Aminopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3b)

Orange-colored solid (88 mg, 72 %); mp: 193–195 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.64 (m, 2H), 2.39 (s, 6H), 2.43 (s, 6H), 2.74 (t, J=7.5 Hz, 2H), 2.97 (m, 2H), 6.22 (s, 2H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.2, 16.1, 24.9, 34.5, 41.7, 121.8, 130.9, 141.0, 147.2, 153.1; MS (ESI): *m/z* =306 $[M+H]^+$, HRMS (ESI) m/z calcd 286.1888 ($C_{16}H_{22}BFN_3$), found: 286.1888.

8-(4-Aminobutyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3c)

Orange-colored solid (72 mg, 56 %); mp: 188–190 °C; ^{1}H-NMR (500 MHz, DMSO-d_6): δ=1.63 (m, 2H), 1.74 (m, 2H), 2.40 (s, 6H), 2.43 (s, 6H), 2.87 (m, 2H), 2.96 (m, 2H), 6.25 (s, 2H), 7.75 (br, 2 H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ= 13.9, 15.8, 27.2, 28.2, 38.5, 40.0, 121.6, 130.6, 140.8, 146.0, 153.4; MS (ESI): *m/z* =320 $[M+H]^+$, HRMS (ESI) m/z calcd 300.2045 ($C_{17}H_{24}BF_2N_3$), found 300.2047.

8-(5-Aminopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3d)

Orange-colored solid (121 mg, 91 %), mp: 212–212 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.64-1.69 (m, 6H), 1.95-1.97 (m, 2H), 2.38 (s, 6H), 2.49 (s, 6H), 2.95 (m, 2H), 6.03 (s, 2H), 8.09 (br, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.6, 27.0, 27.4, 27.9, 31.2, 39.6, 121.8, 131.4, 140.1, 145.3, 154.1; MS (ESI): *m/z* =333 $[M+H]^+$, HRMS (ESI) m/z calcd 314.2202 ($C_{18}H_{26}BFN_3$), found 314.2194.

8-(10-Aminodecyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3e)

Brown-orange solid (92 mg, 57 %); mp: 126–128 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.38-1.88 (m, 14H), 2.41 (s, 6H), 2.51 (s, 6H), 2.92 (t, J=7.2 Hz, 2H), 3.01 (m, 2H), 6.04 (s, 2H), 8.06 (br, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.4, 26.5-30.4, 31.9, 121.6, 131.4, 140.2, 146.6, 153.7; MS (ESI): *m/z* =404.4 $[M+H]^+$, HRMS (ESI) m/z calcd 384.2985 ($C_{23}H_{36}BFN_3$), found 384.2990.

8-(5-Hydroxypentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (4)

A solution of sodium ethanolate (7 g, 83.5 mmol) in aq. ethanol (45 mL) was treated with 8-(5-bromopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (**1e**) (120 mg, 0.3 mmol) and potassium iodide (10 mg) in ethanol (5 mL). After refluxing the mixture for 2 d the solvent was removed under reduced pressure followed by lyophilization. The crude product was suspended in 50 mL of dichloromethane and extracted with 50 mL of water. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified on a silica gel column, using dichloromethane, yielding 40 mg (40 %) of **4** as an orange-colored solid.

mp: 116–118 °C; ^{1}H-NMR (500 MHz, DMSO): δ = 1.5-1.6 (m, 6H), 2.39 (s, 6H), 2.40 (s, 6H), 2.92 (m, 2H), 3.40 (m, 2H) (m, 2H), 5.74 (s, 1H), 6.21 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 14.4, 15.9, 26.4, 28.0, 31.4, 32.1, 121.8, 130.9, 140.9, 147.0, 153.1; MS (ESI): *m/z* =334 $[M+H]^+$; m/z calcd 357.1923 ($C_{18}H_{25}BF_2N_2ONa$), found 357.1915.

General Procedure for the Synthesis of 8-[S-(alkyl)]thioacetate]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (5a-d)

The reaction was performed in analogy to the procedure described by Sheperd et al. [52] The appropriate 8-(bromoalkyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene derivative (**1b**-**d**, 1 mmol) was suspended in 30 mL of acetone, and 140 mg (1 mmol) potassium thioacetate were added. The solution was stirred under reflux for 3 h. The solvent was subsequently evaporated and the residue was dissolved in dichloromethane and washed three times with water. The organic phase was dried over anhydrous Na_2SO_4. After evaporating the solvent under vacuum the product could be obtained as a dark orange-colored residue. The product was used without further purification.

8-[S-(2-Ethyl)]thioacetate)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (5a)

Orange-colored solid (337 mg, 96 %); mp 154–156 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=2.37 (s, 3H), 2.47 (s, 6H), 2.52 (s, 6H), 3.08 (m, 2H), 3.25 (m, 2H), 6.07 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.5, 16.4, 28.3, 29.7, 122.0, 131.4, 140.7, 142.0, 154.8, 194.9; MS (ESI) 352.4 $[M+H]^+$; HRMS (ESI) *m/z* calcd 373.1331 ($C_{17}H_{21}BF_2N_2OSNa$), found 373.1332.

8-[S-(3-Propyl)]thioacetate]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-s-indacene (5b)

Orange-colored solid (349 mg, 96 %); mp.158–160 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.89 (m, 2H), 2.35 (s, 3H), 2.45 (s, 6H), 2.51 (s, 6H), 3.02 (m, 4H), 6.05 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.4, 27.4, 29.0, 30.6, 31.7, 121.7, 131.5, 140.3, 144.6, 154.2, 195.4; MS (ESI) 365.3 $[M+H]^+$; HRMS (ESI) *m/z* calcd 387.1488 ($C_{18}H_{23}BF_2N_2OSNa$), found 387.1485.

8-[S-(4-Butyl)]thioacetate]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (5c)

Orange-colored solid (337 mg, 89 %); 159–161 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.66–1.78 (m, 4H), 2.31 (s, 3H), 2.38 (s, 6H), 2.49 (s, 6H), 2.91 (m, 4H), 6.03 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=14.4, 16.4, 27.9, 28.7, 30.1, 30.6,

30.8, 121.7, 131.4, 140.2, 145.6, 154.0, 195.4; MS (ESI) 380.3 $[M+H]^+$; HRMS (ESI) *m/z* calcd 401.1644 ($C_{19}H_{25}BF_2N_2OSNa$), found 401.1641.

8-[S-(5-Pentyl)]thioacetate]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (5d)

Orange-colored solid (0.35 mg, 89 %); 146–148 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.63 (m, 6H), 2.33 (s, 3H), 2.40 (s, 6H), 2.51 (s, 6H), 2.88–2.95 (m, 4H), 6.05 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=14.4, 16.4, 28.2, 28.8, 29.3, 29.4, 30.6, 31.4, 121.6, 131.4, 140.2, 146.0, 153.9; MS (ESI) 394.6 $[M+H]^+$; HRMS (ESI) *m/z* calcd 415.1801 ($C_{20}H_{27}BF_2N_2OSNa$), found 415.1797.

General Procedure for the Synthesis of 8-thioalkyl-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (6a-c)

The reaction was performed in analogy to the procedure described by Sheperd et al. [52]. The appropriate 8-[S-(alkyl)]thioacetate)-4,4-difluor-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene derivative (**5b-d**; 1 mmol) was suspended in 30 mL of absolute ethanol and argon gas was bubbled through for 30 min in order to remove oxygen. After 30 min 166 mg (1.2 mmol) potassium carbonate were added and the solution was gently warmed to ~30 °C (heating above 40 °C has to be avoided since it leads to increased disulfide formation). The solution was stirred for 4 h under an argon atmosphere. The solution was poured into 30 mL of an aq. saturated ammonium chloride solution, extracted with dichloromethane, and dried over sodium sulfate. After evaporating the solvent under vacuum, the crude product was purified on a silica gel column using dichloromethane : petroleum ether (bp 60–80 °C) (2 : 1).

8-(3-Thiopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-*s*-indacene (6a)

Orange-colored solid (68 mg, 21 %); mp 116–118 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.88–1.94 (m, 2H), 2.42 (s, 6H), 2.50 (s, 6H), 2.69 (m, 2H), 3.03–3.06 (m, 2H), 6.04 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.7, 16.9, 25.9, 27.4, 35.6, 121.9, 131.6, 140.5, 145.3, 154.4; MS (ESI) 323.1 $[M+H]^+$; HRMS (ESI) *m/z* calcd 345.1382 ($C_{16}H_{21}BF_2N_2SNa$), found 345.1392.

8-(4-Thiobutyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-*s*-indacene (6b)

Orange-colored solid (94 mg, 28 %); mp 118–119 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.73–1.84 (m, 4H), 2.42 (s, 6H), 2.51 (s, 6H), 2.58 (m, 2H), 2.96 (m, 2H), 6.06 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=14.4, 16.4, 24.3, 27.9, 30.5, 34.4, 121.7, 131.4, 140.2, 145.7, 154.0; MS (ESI) 337.1 $[M+H]^+$; HRMS (ESI) *m/z* calcd 359.1538 ($C_{17}H_{23}BF_2N_2SNa$), found 359.1538.

8-(5-Thiopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-*s*-indacene (6c)

Orange-colored solid (144 mg, 41 %); mp 120–121 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.56 (br, 1H); 1.61–1.71 (m, 4H), 1.76 (m, 2H), 2.40 (s, 6H), 2.51 (s, 6H), 2.69 (t, J= 6.9 Hz, 2H), 2.95 (m, 2H), 6.05 (s, 2H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=14.4, 16.4, 28.3, 28.8, 28.9, 31.5, 38.5, 121.6, 131.4, 140.2, 146.1, 153.9; MS (ESI): *m/z*=351.6 $[M+H]^+$; HRMS (ESI) m/z calcd 373.1695 ($C_{18}H_{25}BF_2N_2SNa$), found 373.1688.

General Procedure for the Synthesis of 8-(bromoalkyl)-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene-2,6-disulfonate Derivatives (7a-7d)

8-(5-Bromoalkyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (**1b-1e**, 0.42 mmol) was dissolved in dichloromethane (4 mL). The solution was cooled to −20 °C and chlorosulfonic acid (97 mg, 0.84 mmol) was added. After stirring for 30 min at −20 °C the mixture was extracted three times with saturated aq. $(NH_4)_2CO_3$ solution. The water layers were combined and the solvent was removed by lyophilization. The crude product was purified on a silica gel column using dichloromethane : methanol (9 : 2) containing 2 % triethylamine.

8-(2-Bromoethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a, 4a-diaza-*s*-indacene-2,6-disulfonate (7a)

Orange solid (65 mg, 30 %); mp 197–199 °C; ^{1}H-NMR (500 MHz, D_2O): δ=2.72 (s, 6H), 2.78 (s, 6H), 3.65 (t, J=7.9 Hz, 2H), 3.78 (t, *J*=7.6 Hz, 2H); ^{13}C-NMR (125 MHz, D_2O): δ=16.4, 16.8, 33.1, 33.5, 134.0, 135.9, 144.6, 149.7, 157.35; MS (ESI): 512.3 $[M - H]^-$, HRMS (ESI): *m/z* calcd 513.9795 ($C_{15}H_{17}BBrF_2N_2O_6S_2$), found 513.9744.

8-(3-Bromopropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-2,6-disulfonate (7b)

Orange solid (82 mg, 37 %); mp 212–213 °C; ^{1}H-NMR (500 MHz, D_2O): δ=2.18 (m, 2H), 2.71 (s, 6H), 2.77 (s, 6H), 3.29 (m, 2H), 3.70 (t, 2H); ^{13}C-NMR (125 MHz, D_2O): δ=11.1, 17.1, 30.2, 35.9, 36.0, 133.8, 135.6, 144.4, 153.7, 156.7; MS (ESI): 551.0 $[M+Na]^+$, HRMS (ESI): *m/z* calcd 528.9915 ($C_{16}H_{19}BBrF_2N_2O_6S_2$), found 528.9883.

8-(5-Bromobutyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-2,6-disulfonate (7c)

Orange solid (71 mg, 31 %); mp 239–241 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ=1.65–1.72 (m, 2H), 1.97–2.03 (m, 2H), 2.60 (s, 6H), 2.67 (s, 6H), 3.07–3.10 (m, 2H), 3.62 (t, 7.6 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.5, 16.4, 27.5, 30.2, 32.7, 34.6, 121.8, 131.4, 140.2, 147.9 154.1; MS (ESI): *m/z* =543.0 $[M+H]^+$, HRMS (ESI) m/z calcd 542.0097 ($C_{17}H_{21}BBrF_2N_2O_6S_2$), found 542.0105.

8-(5-Bromopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-2,6-disulfonate (7d)

Orange solid (202 mg, 86 %); mp 243–245 °C; ^{1}H-NMR (DMSO-d_6): δ=1.85 (m, 2H), 1.96 (t, J=6.62 Hz, 2H), 2.27–2.36 (m, 2H), 2.68 (s, 6H), 2.74 (s, 6H), 3.14 (m, 2H), 3.64 (t, J=6.62 Hz, 2H); ^{13}C-NMR (DMSO-d_6): δ=13.6, 13.7, 27.5, 27.8, 30.1, 31.6, 34.8, 121.6, 137.5, 137.6, 148.4, 152.4; MS (ESI): *m/z* =557.3 $[M - H]^-$, HRMS (ESI) m/z calcd 555.0240 ($C_{18}H_{23}BBrF_2N_2O_6S_2$), found 555.0249.

8-(5-Aminopentyl),-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-2,6-di-sulfonate (8)

8-(5-Bromopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-2,6-disulfonate (**7c**, 300 mg, 0.54 mmol) was dissolved in 7 *N* NH_3 solution (24 mL) in methanol. The reaction was performed as described above for compounds **1**–**3**, **5**–**6** under microwave conditions. The solvent was evaporated under reduced pressure and the crude product was purified by preparative reversed-phase HPLC (gradient: methanol : water=10:90 to 50:50 over a period of 30 min) affording 90 mg (34 %) as a red solid. mp>300 °C.; ^{1}H-NMR (500 MHz, D_2O): δ =1.51 (m, 2H), 1.65 (m, 2H), 1.98 (s, 3H), 2.00 (s, 3H), 2.19 (m, 2H), 2.32 (s, 3H), 2.36 (s, 3H), 2.87 (t, J=7.51 Hz, 2H), 5,83 (t, J=7.5 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ=12.7, 13.0, 14.3, 14.4, 28.9, 30.9, 35.0, 41.9, 117.0, 117.5, 118.8, 122.4, 123.4, 126.0, 127.0, 130.1, 131.9, 132.3, 133.8; MS (EI): *m/z* =493.3 $[M+H]^+$.

4-Bromobutyric acid-3-[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacen)-8-yl]propylester (9)

Red-colored solid (239 mg, 10 %); mp 103–104 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.95 (m, 2H), 2.15 (q, J=6.5 Hz, 2H), 2.41 (s, 6H), 2.49 (s, 6H), 2.50 (m, 2H), 3.00 (m, 2H), 3.46 (t, J=6.5 Hz, 2H), 4.22 (t, J=6.1 Hz, 2H), 6.04 (s, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 14.4, 16.4, 25.1, 27.6, 30.7, 32.2, 32.6, 64.1, 121.8, 131.2, 140.2, 144.7, 154.3, 172.4; MS (ESI): 456.3 $[M+H]^+$; HRMS (ESI) *m/z* calcd 479.1112 ($C_{20}H_{26}BBrF_2N_2O_2Na$), found 479.1114.

1,3,5,7,8-Pentamethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (10)

Red-colored solid (26 mg, 2 %); mp 219–221 °C; [53] ^{1}H-NMR ($CDCl_3$): δ = 2.38 (s, 6H), 2.50 (s, 6H), 2.54 (s, 3H), 6.06 (s, 2H); ^{13}C-NMR ($CDCl_3$): δ = 14.4, 16.3, 17.4, 121.2, 132.1, 141.0, 141.4, 153.6; MS (ESI): 263.2 $[M+H]^+$; HRMS (ESI) *m/z* calcd 285.1346 $[M+Na]^+$; HRMS (ESI) m/z calcd 285.1348 ($C_{14}H_{17}BF_2N_2Na$), found 285.1346.

S-[2-(4,4-difluoro-11,3,5,7-tetra-methyl-4-bora-3a,4a-diaza-*s*-indacen-8-yl)ethyl]-2-thioadenosine (15)

2-Thioadenosine (**14**, 148 mg, 0.49 mmol) was dissolved in 5 mL DMF and sodium methanolate (28 mg, 0.49 mmol) was added. After stirring the solution for 5 min at room temperature, **1b** (281 mg, 0.49 mmol) was added and the reaction was stirred for 16 h at rt. After evaporating the solvent under vacuum, the crude product was purified on a silica gel column using dichloromethane : methanol (9 : 1) affording red solid (186 mg, 79 %); mp 182–184 °C; ^{1}H-NMR (500 MHz, $CDCl_3$): δ =2.47 (s, 6H), 2.52 (s, 6H), 3.16 (m, 1H), 3.28 (m, 2H), 3.44 (m, 1H), 3.73 (m, 2H), 4.12 (br, 1H), 4.29 (s, 1H), 4.36 (d, J=5.0 Hz, 1H), 4.39 (m, 1H), 5.72 (d, J=7.2 Hz, 1H), 5.78 (br, 2H), 5.96 (br, 1H), 6.07 (s, 2H), 7.12 (br, 1H), 7.69 (s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ=14.4, 16.6, 28.5, 32.0, 63.2, 72.4, 73.3, 87.2, 90.9, 118.3, 121.9, 131.5, 139.8, 140.8, 142.9, 149.5, 154.6, 154.9, 164.2; MS (EI): *m/z* =574.1 $[M+H]^+$; HRMS (ESI) *m/z* calcd 574.2218 $[M+H]^+$ ($C_{25}H_{30}BF_2N_7O_4SH$), found 574.2234.

Receptor-Radioligand Binding Studies

Rat brain cortical membrane preparations were used as a source for A_1ARs, and rat brain striatal membrane preparations as a source for A_{2A}ARs as previously described [54–56]. Membrane preparations of CHO cells recombinantly expressing the human A_1, A_{2B}, or A_3ARs, respectively, were used for assays at the human receptor subtypes. Membrane preparation of human embryonic kidney (HEK) cells expressing human A_{2A}AR were obtained from PerkinElmer (Product No.: RBHA2AM400UA) and used for assays at human A_{2A}ARs. Stock solutions of test compounds were prepared in dimethyl sulfoxide (DMSO); the final concentration of DMSO in the assays was 2.5 %. The radioligand concentrations and incubation times (incubation at rt) were as follows: [^{3}H]CCPA: 42.6 Ci/mmol, 1 nM (rat and human A_1), incubation for 90 min; [^{3}H]CGS21680: 41 Ci/mmol, 5 nM (rat and human A_{2A}), incubation for 60 min; [^{3}H]NECA: 15.3 Ci/mmol, 10 nM (human A_3), incubation for 180 min; [^{3}H]PSB-603: 73 Ci/mmol, 0.3 nM (human A_{2B}), incubation for 75 min. About 50–125 μg of protein/vial were used in the assays. Membranes were preincubated for 10–15 min with 0.12 IU/mL of

adenosine deaminase in order to remove endogenous adenosine. Binding assays were performed essentially as previously described. Binding assays at A_1, A_{2A} and A_3 receptors were carried out using polyethylene vials in a total volume of 400 μL assay buffer (50 mM Tris–HCl, pH 7.4) containing 100 μL of membrane protein suspension and 100 μL of radioligand solution in the presence of 10 μL of various concentrations of test compound. Nonspecific binding was determined in the presence of 10 μM 2-chloroadenosine in A_1AR assays, 50 μM 5'-N-ethylcarboxamido)adenosine (NECA) in A_{2A}AR assays, and 100 μM (*R*)-N^6-phenyl-isopropyladenosine (*R*-PIA) in A_3AR assays. Incubation was terminated by rapid filtration using a Brandel 48-channel cell harvester (Brandel, Gaithersburg, MD) through Whatman GF/B glass fiber filters. Filters were rinsed three times with 2 mL each of ice-cold Tris–HCl buffer (50 mM, pH 7.4) and subsequently incubated at least for 6 h with 2.5 mL of scintillation cocktail (Ready Safe™, Coulter) per vial before radioactivity was counted in a liquid scintillation counter (Tricarb 2900TR, Canberra Packard). A_{2B}AR binding assays were carried out in a total volume of 1,000 μL containing 25 μL of test compound dissolved in 775 μL Tris–HCl buffer (50 mM, pH 7.4), 100 μL radioligand solution, and 100 μL of membrane suspension. Nonspecific binding was determined in the presence of 10 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). The mixture was incubated for 75 min at rt followed by filtration through GF/B glass fiber filters using a 48-channel cell harvester. Filters were washed three times with ice-cold Tris–HCl buffer (50 mM, pH 7.4) containing 0.1 % bovine serum albumin (BSA), 2–3 mL each. Then filters were transferred to scintillation vials and incubated for 9 h with 2.5 mL of scintillation cocktail (Beckman Coulter). Radioactivity was counted in a liquid scintillation counter (Tricarb 2900TR, Canberra Packard) with a counting efficiency of 53 %. Curves were determined using 6–7 different concentrations of test compounds spanning 3 orders of magnitude. At least three independent experiments were performed, each in duplicate (human receptors) or triplicate (rat receptors). Data were analyzed with GraphPad Prism, Version 5.0 (GraphPAD, San Diego, CA, USA). For the calculation of K_i values by nonlinear regression analysis, the Cheng-Prusoff equation and K_D values of 0.2 nM (rat A_1AR) and 0.61 nM (human A_1AR) for [^{3}H]CCPA, 15.9 nM (rat A_{2A}AR) and 26.8 nM (human A_{2A}AR) for [^{3}H]CGS21680, 0.41 nM (human A_{2B}AR) for [^{3}H]PSB-603, and 6.2 nM (human A_3AR) for [^{3}H]NECA were used [57–60].

Functional Assays

Culture of CHO Cells

CHO cells stably expressing the human A_{2A} or the human A_3 receptor were grown adherently and maintained in DMEM F-12, supplemented with 10 % FCS, 100 IU/mL penicillin G, 100 μg/ml streptomycin, 1 mM glutamine and 200 μg/ml G 418, at 37 °C and 5 % CO_2. Prior to cAMP accumulation experiments cells were washed twice with PBS, trypsinized, resuspended in new medium and counted.

Measurement of cAMP Accumulation

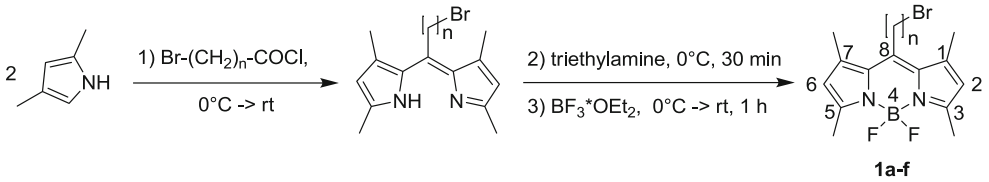
cAMP Accumulation in A_{2A}AR Expressing Cells

The cells were kept in 24-well plates (150,000–200,000) overnight in culture medium at 37 °C, 5 % CO_2. After removal of the culture medium, cells were washed with HBSS buffer (containing 20 mM HEPES; pH 7.3) and then incubated with HBSS buffer containing adenosine deaminase (1 IU/mL) for 120 min at 37 °C, 5 % CO_2. The cells were then preincubated with the phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone (Ro 20–1724, 40 μM) for 15 min. Test compounds were added at 37 °C. After incubation for 15 min the reaction was stopped by the removal of the reaction buffer followed by the addition of a hot lysis buffer (500 μL; 90 °C; 4 mM Na_2EDTA; 0.1‰ Triton X100). The multi-well plates were incubated at rt for 5 min and frozen at -20 °C. The plates were thawed on ice and the cells were homogenized. cAMP levels were quantified by incubation of 50 μL of each well with cAMP binding protein prepared from calf adrenal glands (75 μg/well) and [^{3}H]cAMP (final concentration 3 nM). The plates were incubated at 4 °C for 60 min and the samples were harvested by filtration through Whatman GF/B filters (Brandel 48-channel cell harvester). Each filter was rinsed three times with 1 mL of 50 mM TRIS–HCl, pH 7.4, the filters were punched out into scintillation vials and counted in a liquid scintillation counter with 2.5 mL Ultima Gold scintillation cocktail. The samples were counted after 6 h for 1 min. The amount of cAMP was determined using standard cAMP curves of three independent experiments each in triplicate [58, 61, 62].

cAMP Accumulation in Human A_3AR Expressing Cells

The cells were cultured on 24-well plate (150,000–200,000) overnight in the culture medium at 37 °C, 5 % CO_2. After removal of the culture medium, cells were washed with HBSS buffer (containing 20 mM HEPES; pH 7.3) and then incubated with HBSS buffer containing adenosine deaminase (1 IU/mL) for 120 min at 37 °C, 5 % CO_2. The cells were then preincubated with the phosphodiesterase inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidone (Ro 20–1724), 40 μM, for 10 min. Compounds were added (NECA, 10 μM final concentration; **15**, 100 μM final concentration) and incubation was continued for 5 min. at 37 °C. Then, 10 μM forskolin was added and the incubation was continued for 15 min at 37 °C. The reaction was stopped by removal of the reaction buffer followed by the addition of hot lysis buffer (500 μL; 90 °C; 4 mM

Table 1 Synthesis of ω-bromoalkyl-substituted BODIPY derivatives (**1a**-**1f**)[a]



entry	n	product	yield[b] (%)
1	1	**1a**	40
2	2	**1b**	12
3	3	**1c**	30
4	4	**1d**	33
5	5	**1e**	33
6	10	**1f**	11

[a] Standard reaction conditions: (1) 10 mmol of 2,4-dimethylpyrrole, 5 mmol of Br-$(CH_2)_n$-COCl (n=1–5, 10) in 20 mL CH_2Cl_2 at 0 °C for 30 min, then 90–210 min at rt. (2) 20 mmol of triethylamine, 0 °C, 30 min. (3) 10 mmol of BF_3*OEt_2, 0 °C, then 1 h rt. [b] Isolated yields after purification by silica gel chromatography

Na_2EDTA; 0.1‰ Triton X100). The multi-well plates were incubated at rt for 5 min and subsequently frozen at −20 °C. The plates were thawed on ice and the cells were homogenized. cAMP levels were quantified as described above.

Results and Discussion

Syntheses of Functionalized BODIPY Derivatives

A series of five bromoalkyl-substituted BODIPY derivatives with alkyl spacer lengths ranging from 1 to 5 carbon atoms, and one with ten carbon atoms (8-(ω-bromoalkyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene derivatives, **1a**-**1f**) were obtained by a two-step one-pot reaction procedure (Table 1).

2,4-Dimethylpyrrole was added to the appropriate ω-bromoalkylcarboxylic acid chlorides at low temperature (0 °C) under anhydrous conditions. The use of unsubstituted pyrrole instead of the 2,4-dimethyl derivative had not yielded the desired products, because the unsubstituted dipyrromethenes, which were formed as intermediates, decomposed easily [3]. After stirring the above reaction mixture for the indicated period of time, triethylamine was added at 0 °C followed by the addition of boron trifluoride etherate after 30 min, and the progress of the reaction could be observed by the developing fluorescence. The reaction was stopped after 1 h. The limited reaction time was important since a side product was formed upon prolonged reaction leading to a decrease in the yield of the products. In one case when the reaction time was extended from 1 h to 3 h side-product **9** (Fig. 1) could be isolated in 10 % yield. The formation of **9** can be explained by further reaction of product **1c** with remaining propionic acid chloride.

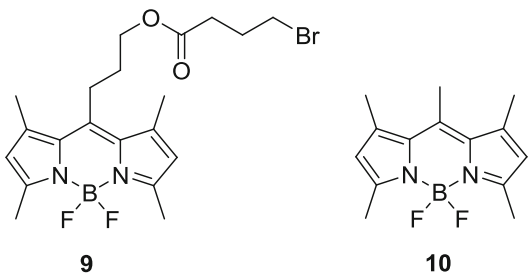


Fig. 1 Side-products **9** and **10** obtained during the synthesis of BODIPY derivatives **1** and **3**

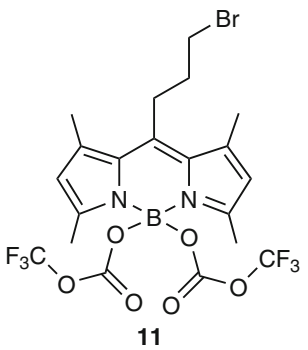
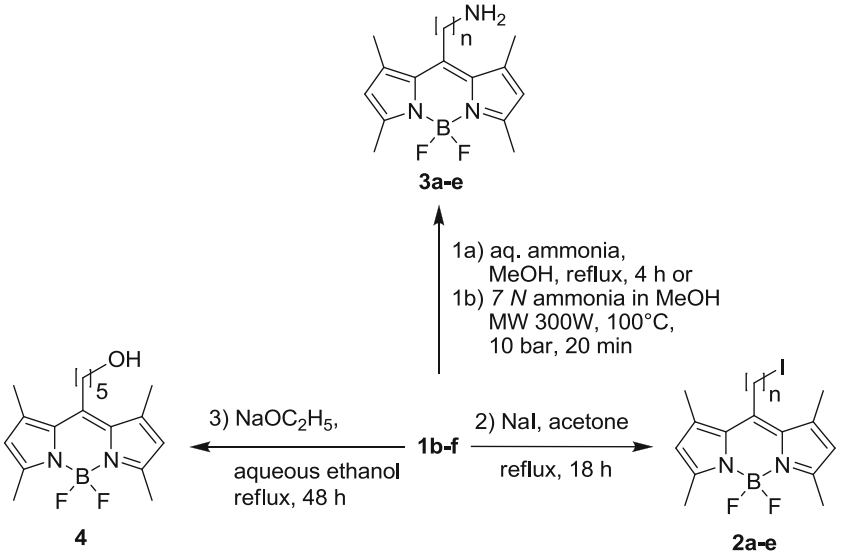


Fig. 2 Assumed product **11** built by treatment of **1c** with trifluoroacetic acid (5 %) in dichloromethane for 24 h at rt

The amount of **9** could be further increased by longer reaction times and the addition of a larger amount of propionic acid chloride. The structures **9** and **1e** were confirmed by crystal structure analysis and reported in preliminary publications [63, 64] (Table 1 and Fig. 1).

Because of this side-reaction it was not possible to increase the yield of the desired products by extending the reaction times. However, in one case, reaction in a pressure tube led to a higher yield of the product (**1d**), while in all other cases reactions under pressure did not give satisfactory yields. Interestingly, the synthesis of **1a** additionally led to the side-product **10** (Fig. 1), which was identified by NMR, LCMS and HRMS analyses. The amount of **10** formed was dependent on the quality of the used boron trifluoride etherate. Fresh boron trifluoride etherate decreased the amount of **10** (< 2 %), while aged boron trifluoride etherate led to an increase in the

Table 2 Synthesis of the aminoalkyl, iodoalkyl and hydroxylalkyl-substituted BODIPY dyes **2a-f**, **3a-e and 4**[a]



entry	n	product	yield[b] (%)
1	2	**2a**	98
2	3	**2b**	97
3	4	**2c**	98
4	5	**2d**	99
5	10	**2e**	97
6	2	**3a**	27
7	3	**3b**	72
8	4	**3c**	56
9	5	**3d**	91
10	10	**3e**	57
11	5	**4**	40

[a] Standard reaction conditions: (1a) compound **5** (2.5 mmol), 3.75 mmol aq. ammonia in MeOH at 70 °C. (1b) compound **1b-f** (0.4 mmol), 84 mmol 7*N* ammonia solution in methanol, MW 300 W, 10 bar at 100 °C, 20 min. (2) compound **1b-f** (0.18 mmol), 4 mmol NaI in acetone 60 °C, 18 h. (3) compound **5** (0,3 mmol), 85.5 mmol sodium ethylate, 80 °C, 48 h. [b] Isolated yields after purification by silica gel chromatography

formation of **10** (up to 30 % yield). Compound **10** could be removed by careful column chromatography on silica gel. The side product appears to be formed by a radical reaction mechanism caused by impurities in boron trifluoride etherate. The formation of this side-product was not observed when the reaction was performed in the presence of a radical inhibitor, such as iodine or 3,5-di-*tert*-butyl-4-toluene (BHT).

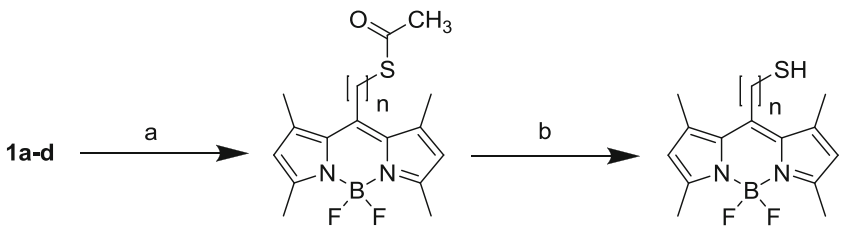
BODIPY derivatives have been reported to be chemically stable, but recent reports indicate instabilities of some derivatives [5, 6, 65]. However, detailed studies on the stability of BODIPY derivatives under different conditions are rare [66]. Recently Yang et al. studied the stability of *F*-, *C*-. and *O*-BODIPY derivatives under strong acidic and basic conditions [67]. They observed decomposition in the presence of a large excess of trichloroacetic acid. Therefore we investigated the stability of compound **1c** as an example in order to test whether the compounds could be used for fluorescent labelling under typical reaction conditions. We observed that compound **1c** was stable in a solution of sodium methanolate (5 %) in dichloromethane at rt for 24 h (see Supporting Information for LC-MS spectra). Furthermore **1c** was found to be stable in a solution of dichloromethane in the presence of Pd/C and H_2 at rt, as well as in the presence of hydrogen peroxide at rt for at least 24 h. Treatment of **1c** with trifluoroacetic acid (5 %) in dichloromethane at rt for 24 h led to a partial conversion (74 %) to a product of postulated structure **11** (M_r 556 g/mol, LCMS analysis) (Fig. 2). An analogous product had been described by Yang et. al., that was formed by the treatment of an *O*-substituted BODIPY derivative with a large excess of dichloroacetic acid.

The syntheses of the iodoalkyl-derivatives **2a-f** were achieved by reaction of the corresponding bromoalkyl derivatives **1b-f** with sodium iodide in acetone under reflux for 18 h. The products were obtained in high purity, and no formation of side products was observed (Table 2).

The synthesis of the amino derivatives **3a-3f** could be achieved in two different ways: the conventional method was to displace the bromide by treatment with saturated aq. ammonia solution. Harsh reaction conditions and long reaction times (4 h) were required to obtain product **3c** in 56 % yield after purification by column chromatography. Alternatively, the reaction was performed under microwave irradiation (100 °C, 10 bar, 20 min) resulting in a dramatic reduction of the reaction times. Side-reactions (e.g. formation of methoxy derivatives) were not observed. Therefore the microwave-assisted nucleophilic substitution procedure was advantageous to the standard method.

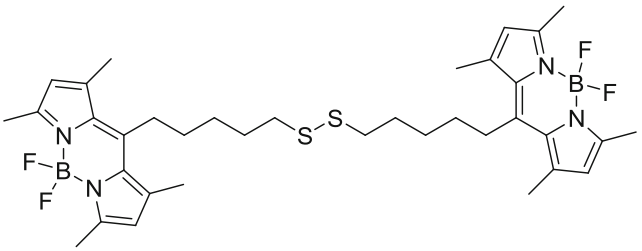
Purification of the aminoalkyl-substituted BODIPY derivatives **3a-c** was achieved by dissolving of the products in dichloromethane and recrystallization by dropwise addition of petroleum ether (bp 60–80 °C) leading to high purity of the products. An electropherogram confirming the purity of

Table 3 Synthesis of ω-thioalkyl-substituted BODIPY derivatives (**6a-e**)[a]



entry	n	product	yield[b] (%)
1	2	**5a**	96
2	3	**5b**	93
3	4	**5c**	89
4	5	**5d**	78
5	3	**6a**	21
6	4	**6b**	28
7	5	**6c**	41

[a] Standard reaction conditions: a) compound **1b-f** (1 mmol), 1.2 mmol potassium thioacetate in acetone under reflux for 3 h. b) compound **5b-e** (1 mmol), 1.2 mmol K_2CO_3 in ethanol at rt, 4 h. [b] Isolated yields after purification by silica gel chromatography



12

Fig. 3 Side product in the synthesis of 8-(5-thiopentyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (**12**)

compound **3e** obtained by capillary electrophoresis at two wavelengths, 220 and 495 nm, is shown in the Supporting Information as an example.

Hydroxy derivative **4** was obtained by treatment of bromoalkyl derivative **1e** with sodium ethylate in ethanol, while reaction with sodium hydroxide in methanol did not lead to the desired product, but to the methoxy derivative, instead.

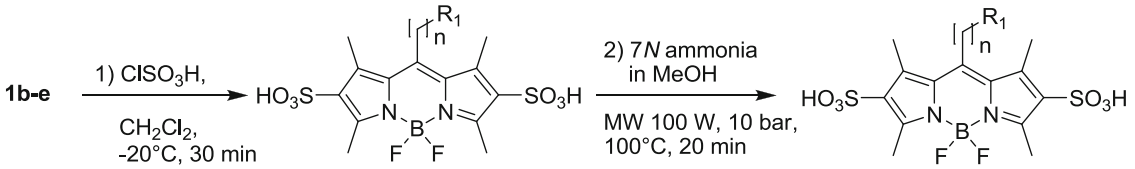
A series of thiol-substituted BODIPY derivatives (**6a-c**) was obtained applying a previously described method (Table 3) [52]. In a first step potassium thioacetate was added to the bromoalkyl-BODIPY derivatives to form the corresponding thioester derivatives **5a-d**. In the subsequent step the thioesters were cleaved by the addition of potassium carbonate at 30 °C under an argon atmosphere yielding thiol derivatives **6a-c**.

Despite the provision to exclude oxygen, side products were formed, which could be identified as the corresponding disulfide derivatives formed by oxidation. In one case the side product (**12**) was isolated and characterized (Fig. 3). At temperatures surmounting 30 °C, the amount of **12** formed was strongly increased to up to 63 %.

For biological investigations, sufficient water-solubility is an important prerequisite. Therefore, water-soluble BODIPY derivatives were synthesized by introduction of sulfonic acid functions [11, 14, 68, 69]. All positions in the BODIPY core, except for 2 and 6, bear a partial positive charge, and thus only these two positions will be susceptible to electrophilic substitution. For sulfonation reactions chlorosulfonic acid was used (Table 4). A similar reaction had previously been described by Worries et al. [14]. The polar, highly water-soluble derivatives were obtained in good yields by reaction of the bromoalkyl derivatives **1b-e** with two equivalents of chlorosulfonic acid at - 20 °C. After 30 min the organic layer was washed three times with saturated ammonium hydrogencarbonate solution, and the combined aq. layers were dried by lyophilization. In order to remove inorganic impurities the crude product was purified by column chromatography.

Substitution of the bromide in sulfonated derivative **7d** by ammonia was achieved under microwave irradiation according to the procedure described above. The reaction of **7d** with ammonia led to deprotonation of the exocyclic carbon atom (C1') of the alkyl chain resulting in the slightly fluorescent product **8a**. A similar reaction had previously been observed by Treibs and Kreuzer [3]. Under anhydrous or acidic conditions, this position will be protonated again yielding the desired product **8**, which is strongly fluorescent. Under

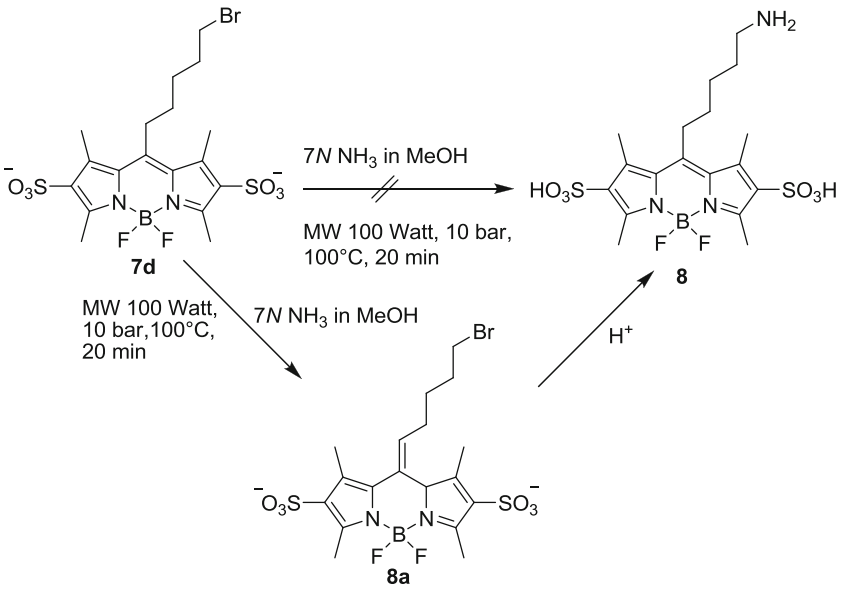
Table 4 Synthesis of water soluble-BODIPY derivatives **7a-d and 8**[a]



7a-d **8**

entry	n	product	R^1	yield[b] (%)
1	2	**7a**	Br	69
2	3	**7b**	Br	32
3	4	**7c**	Br	36
4	5	**7d**	Br	86
5	5	**8**	NH_2	34

[a] Standard reaction conditions (1) compound **1b-e** (0.42 mmol), 0.84 mmol chlorosulfonic acid in 20 mL CH_2Cl_2 at −20 °C for 30 min. (2) compound **7d** (0.54 mmol), 0.17 mol 7*N*-ammonia in MeOH, MW 100 W, 10 bar, 100 °C, 200 min. [b] Isolated yields after purification by silica gel chromatography

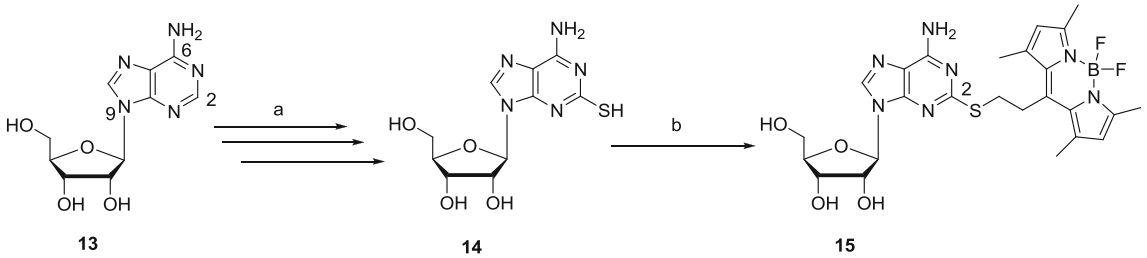


Scheme 1 Deprotonated form of compound **8**

basic conditions the deprotonated form of **8a** shows only weak fluorescence. Purification of the product by reverse phase HPLC with a gradient of methanol : water (1:9 (v/v)) over a period of 90 min led to the isolation of the pure neutral, fluorescent product **8** (Scheme 1).

Synthesis of Fluorescent-Labeled Adenosine Receptor Agonist

As a proof-of-principle study, we used one of the functionalized BODIPY derivatives to prepare a fluorescent-labeled adenosine derivative as an AR ligand. So far, several AR antagonists had been coupled via long linkers to different dyes to obtain non-selective or selective A_1-, A_{2A}- or A_3-antagonistic AR ligands [39, 41, 48, 49, 70]. Kecskes and coworkers developed fluorescent-labeled dendrimers as ligands at A_1-, A_{2A}- or A_3ARs [40, 42]. The AR agonists described so far that were fluorescent-labeled were either adenosine or NECA derivatives, which were substituted at the N^6- or the 2-position with different fluorophores via very long linkers. This has led to non-selective or A_1-, A_{2A}- and A_3-selective AR ligands [34–36, 46, 47, 49, 71–73]. These fluorescent ligands have been used, e.g., for the development of assays or for investigating the receptors with confocal microscopy [36, 41]. However, our approach was different: Since the developed BODIPY fluorophores are small they can be integrated into the pharmacophoric structure instead of just attaching a fluorophore via a spacer group in large distance from the pharmacophoric scaffold. It is well known that adenosine derivatives bearing large substituents in the 2-position may show A_{2A} and/or A_3AR subtype selectivity [74–76]. Some of the best A_{2A} and A_3AR agonists contain a heteroatom (NH, O, S) or a double or triple bond attached to the 2-position followed by an ethylene group with a terminally attached aromatic function. Therefore we designed compound **15**, which conforms with the pharmacophore for A_{2A} and A_3AR agonists, but in which the typically present phenyl ring was



Scheme 2 Synthesis of the fluorescent labeled adenosine derivative **15**[a]. [a]**Reagents**, **Conditions**: (**a**) three steps: 1. H_2O_2, CH_3COOH; 2. 5*N* aq. NaOH; 3. CS_2, MeOH, H_2O, 120 °C autoclave, 5 h; (**b**) **1b**, NaOMe, DMF, rt, 16 h

replaced by a BODIPY dye (see Scheme 2). The fluorophore was introduced into 2-thioadenosine (**14**) by alkylation with bromoethyl-substituted BODIPY **1b** in the presence of sodium methoxide in DMF. 2-Thioadenosine (**14**) was prepared from adenosine in a 3-step reaction sequence according to a published procedure (Scheme 1; for details, see the Electronic Supporting Information) [57, 77].

The desired product **15** was easily obtained in a high yield, while no formation of side products was observed. The synthesized fluorescent adenosine derivate **15** was subsequently investigated in radioligand binding studies at A_{2A} and A_3ARs (Table 5). In order to assess its receptor subtype selectivity, additional radioligand binding studies were performed at A_1 and A_{2B}ARs (see Table 5). For comparison, data for the parent 2-thioadenosine (**14**) and for two standard agonists, *N*-ethylcarboxamidoadenosine (NECA) and 2-[*p*-(2-carboxyethyl)phenethylamino]-5'-*N*-ethylcarboxyamidoadenosine (CGS21680) are provided.

NECA, a 2-unsubstituted, nonselective adenosine derivative, is similarly potent at A_1, A_{2A}, and A_3ARs, and less potent at A_{2B}ARs. The 2-substituted adenosine derivative CGS21680 shows highest affinity at A_{2A}, followed by A_3ARs. 2-Thioadenosine (**14**) was only potent at A_1, but not at other AR subtypes. The adenosine derivative **15** labeled with a BODIPY in the 2-position showed the highest affinity for A_3ARs with a K_i value of 662 nM (see Table 5 and Fig. 4). It was less potent at A_1 and A_{2A}ARs and inactive at A_{2B}ARs. Thus, **15** showed an at least about 10-fold selectivity for A_3 versus the other AR subtypes.

In functional studies compound **15** acted as an agonist at G_s protein-coupled A_{2A}ARs leading to an increase in cAMP levels, and also at G_i protein-coupled A_3ARs, where it showed a decrease in forskolin-induced cAMP levels (see Figs. 5 and 6), similar to the effects seen with the standard agonist NECA.

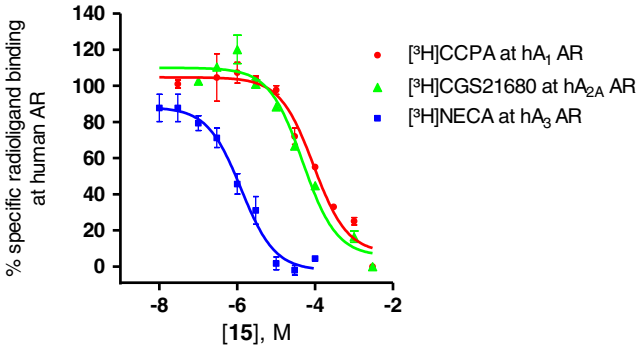


Fig. 4 Competition curves of BODIPY-labeled adenosine derivative **15** at human A_1-, A_{2A}- and A_3AR-expressing cell membranes. Data points represent means of three separate experiments performed in duplicates. A K_i value of 662±200 nM was determined at A_3ARs, while **15** showed an about 10-fold lower affinity for A_1 and A_{2A}ARs (n=3)

The fluorescent A_3AR agonist **15** may serve as a useful biological tool; it will also be used as a lead structure for further optimization with regard to affinity and selectivity. These results confirm that the developed tool kit is useful for the fluorescent labeling of ARs, and due to the small size of the fluorophore, it may even be integrated into the pharmacophore structure. The current results are likely to be of general significance, and may be extended to ligands for further receptors as well as other classes of target proteins.

Fluorimetric Characterization of the Compounds

The absorption maxima of all synthesized BODIPY derivatives were determined to be between 495 and

Table 5 Adenosine receptor affinities of BODIPY-labeled adenosine derivative **15** in comparison to unlabeled adenosine derivatives

K_i±SEM (nM) *(n=3)*

Compound	A_1 receptor [^{3}H]CCPA		A_{2A} receptor [^{3}H]CGS21680		A_{2B} receptor [^{3}H]PSB-603[a]	A_3 receptor [^{3}H]NECA
	rat brain cortex	human recombinant	rat brain cortex	human recombinant	human recombinant	human recombinant
CGS21680	1800 [74]	289 [74]	18 [78]	27 [74]	>10000 [59]	114[b] [79]
NECA	5.1 [74]	13.6 [80]	15 [78]	20 [74]	1890 [59]	6.2[c] [80]
14	80.3±14	n.d.[d]	>1000	n.d.[d]	>10000	>1000
15	6220±51	5230±2510[e]	>10000	7880±750	>10000	662±200

[a] antagonist radioligand was used, because an agonist radioligand for A_{2B}AR is not available

[b] [^{125}I]I-AB-MECA was used as a radioligand

[c] [^{3}PSB-11] was used as radioligand

n.d. not determined

[e] $n=2$

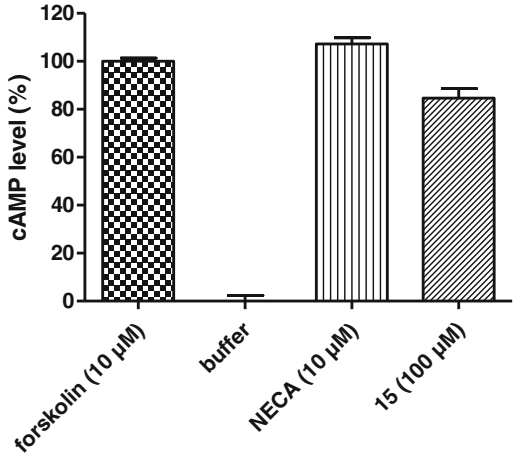


Fig. 5 Percent increase in cAMP levels induced by **15** (100 μM) in comparison to the effect of forskolin (10 μM, set at 100 %) and to the nonselective AR agonist NECA (10 μM) determined in CHO cells recombinantly expressing the G_s protein-coupled human A_{2A}AR. Data points represent means of three separate experiments performed in triplicates

506 nm (absorption and emission curves of the bromoalkyl BODIPY derivatives **1a**-**1f** are shown in the Supporting Information). The emission maxima were determined to be between 504–514 nm as expected for this type of BODIPY dyes.

The fluorescence quantum yields of the products were determined in ethanol with rhodamin 6G as a reference compound. They were found to be in a range between 0.62–0.99 (Table 6). Thus, the high quantum yields typically observed

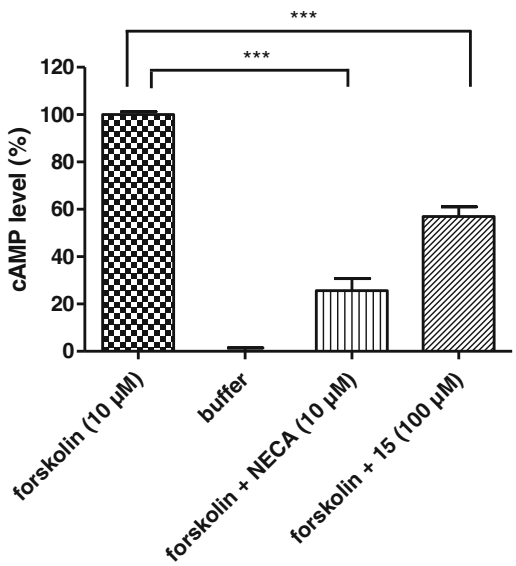


Fig. 6 Decrease in forskolin-induced cAMP levels by **15** (100 μM) and the nonselective AR agonist NECA (10 μM) determined in CHO cells recombinantly expressing the G_i protein-coupled human A_3AR. Data were analyzed using Student's *t*-test, Graphpad Prism version 5; significant differences are noted as follows: ***P<0.001. Data points represent means of three separate experiments performed in triplicates

Table 6 Spectroscopic data of synthesized BODIPY derivatives[a]

Compound	λ Abs [nm]	λ Emission [nm]	$FWHM_{ab}$ [nm]	$FWHM_{em}$ [nm]	ϕ^a	Stokes shift [nm]
1a	515	526	24	40	0.95	11
1b	504	512	22	30	0.85	8
1c	499	508	21	42	0.95	9
1d	498	506	19	34	0.94	8
1e	497	506	19	32	0.99	9
1f	497	504	18	32	0.77	7
2a	506	514	22	32	0.98	8
2b	499	508	18	42	0.95	9
2c	498	506	18	33	0.95	8
2d	497	506	17	34	0.91	9
2e	497	504	17	32	0.86	7
3a	504	509	23	36	0.68	5
3b	497	506	20	48	0.87	9
3c	497	506	20	34	0.93	9
3d	497	506	20	34	0.92	9
3e	497	504	19	35	0.70	7
4	497	507	22	36	0.81	10
5a	498	505	19	34	0.81	7
5b	498	506	20	35	0.79	8
5c	497	506	19	34	0.91	9
5d	497	508	19	32	0.83	11
5e	497	506	17	34	0.93	9
6a	498	508	21	45	0.89	10
6b	497	506	18	37	0.94	9
6c	498	506	19	34	0.91	9
6d	498	508	21	45	0.89	10
7a	504	542	19	34	0.65	38
7b	506	538	17	32	0.68	32
7c	505	540	18	37	0.62	35
7d	505	545	19	34	0.65	40
8	495	545	21	45	0.62	50
9	496	511	18	37	0.92	15
10	504	512	22	30	0.93	8
12	496	508	19	32	0.89	12
15	497	506	17	34	0.94	9

[a] The fluorescence quantum yields were determined in ethanol as solvent, and the optical density was kept below 0.05 to avoid inner filter effects. Rhodamine 6G was used as a reference (ϕ=0.94 in EtOH λ_{exc}=488 nm)

with BODIPY derivatives are also found in the functionalized derivatives synthesized in the present study.

Conclusion

In conclusion, we developed a simple and efficient route to obtain a variety of functionalized BODIPY derivatives. Due to

their high absorption and emission wavelengths they will not interfere with biological fluorophores. In contrast to many other fluorescent-labeling reagents [1, 11], the developed BODIPY derivatives were kept small in size in order not to interfere with biological processes. The new fluorescent dyes show excellent fluorescence characteristics including high fluorescence quantum yields and narrow absorption and emission curves. Furthermore the developed BODIPY derivatives are chemically stable under basic, reductive or oxidative conditions. As a proof of concept 2-thioadenosine was coupled with BODIPY derivative **1b** to obtain the fluorescent-labeled A_3AR **15** agonist by a straightforward alkylation procedure in high yield. It has been shown, that the developed dyes represent versatile tools and will be highly useful for the fluorescent labelling of small molecules and biological targets allowing the investigation of biological processes, including the establishment of assays for compound screening, by fluorimetric methods.

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