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Research Article

Synthesis of specifically deuterated adenosine A_1 antagonist: BG9928

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Abstract: BG9928, a high affinity adenosine A_1 antagonist, is currently in Phase II clinical trials for the treatment of congestive heart failure. A deuterium-labeled version of the molecule was synthesized and used as a standard for *in vivo* pharmacokinetic and *in vitro* metabolism studies. The labeled form of 3-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-propionic acid (BG9928) was obtained in a convergent manner by joining two major building blocks: the specifically labeled heterocycle 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione (**4**) and the hemiester 4-(2-methoxycarbonyl-ethyl)-bicyclo[2.2.2]octane-1-carboxylic acid (**10**). Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: adenosine A_1 receptor; A_1 antagonist; BG9928; uracil; bicyclo[2.2.2]octane

Introduction

Adenosine, an endogenous nucleoside and intercellular signaling molecule, binds to G-protein-coupled receptors and initiates a variety of cellular events including Ca^{2+} and potassium mobilization and cAMP regulation via adenylate cyclase.[1] Adenosine receptors (A_1, A_{2a}, A_{2b} and A_3), are comprised of seven transmembrane spanning domains which have generally high sequence homology between species and are expressed in many tissue types.[2] Adenosine has been implicated as a key mediator in physiological responses that contribute to a number of cardiovascular, renal and CNS disorders.[2]

Four adenosine receptor subtypes have been cloned and fully characterized, the adenosine A_1 receptor is expressed in the brain, heart, lung and kidney,[3] and has been a target of drug discovery efforts. Adenosine A_1 receptor antagonism has beneficial effects on renal blood flow, sodium excretion and pulmonary hemodynamics, and may be a promising new approach for the treatment of congestive heart failure.[4] Work in this laboratory led to the discovery of compound **12**, a potent and selective A_1 receptor antagonist BG9928.[5] This compound is currently being evaluated in Phase 2 human clinical trials for the treatment of congestive heart failure.

To support the *in vivo* pharmacokinetic and *in vitro* metabolism studies of BG9928, a stable isotopically labeled standard was needed. The installation of a specifically labeled deuterated *N*-3 propyl chain into BG9928 enabled the differentiation between the two propyl side chains of the dipropylxanthine core in metabolism studies and produced a mass spectroscopic standard that was seven mass units separated from the original drug substance.

Results and discussion

Synthesis of target compound **12** was accomplished in a convergent manner by joining two major building blocks: the specifically labeled heterocycle 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione (**4**) and the hemiester 4-(2-methoxycarbonyl-ethyl)-bicyclo[2.2.2]octane-1-carboxylic acid (**10**).

The deuterium-labeled uracil was constructed by a simple modification of Müller's procedure[6] (Scheme 1). The *N*-5 amine of 5,6-diamino-3-propyl-1*H*-pyrimidine-2,4-dione[7] was selectively protected with a *t*-BOC group.[8] The [$^2H_7$]-propyl chain was introduced at the *N*-1 position of the uracil by alkylation with [$^2H_7$]-1-iodopropane and K_2CO_3 in DMF at 80°C. Subsequent treatment of compound (**3**) with 4 N HCl removed the BOC group and gave the stable HCl salt (**4**).[9]

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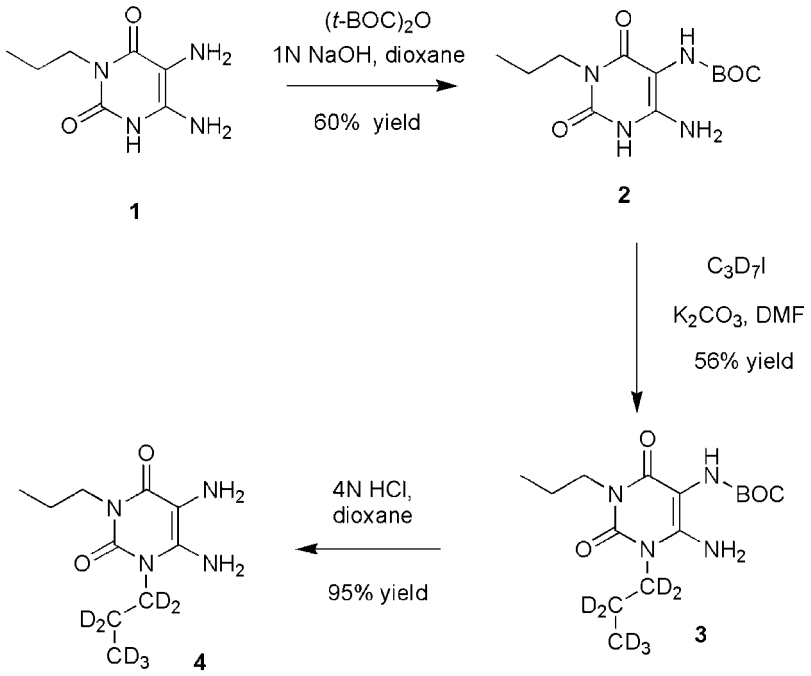

Scheme 1

The advanced hemiester intermediate (**10**) was prepared by simple homologation of bicyclo[2.2.2]octane-1,4-dicarboxylic acid monomethyl ester[10] (**5**) (Scheme 2). Careful attention was paid to the sequence of chemical transformations in order to maintain differentiation of the two carboxylic acids throughout the process. It was critical that the free carboxylic acid reside at the bridgehead position in the bicyclo[2,2,2] octane to couple with uracil (**4**). Selective reduction of the carboxylic acid with BH_3 in THF gave alcohol (**6**) in 62% yield. Subsequent oxidation of (**6**) with Dess–Martin periodinane in CH_2Cl_2 provided bridgehead aldehyde (**7**). Saponification of the ester in compound (**7**) with 2 N LiOH provided a means to differentiate the bridgehead positions on the bicyclooctane after subsequent transformations and gave compound (**8**) in 89% yield. Horner–Emmons–Wadsworth olefination of aldehyde (**8**) with the ylide derived from trimethylphosphonoacetate and KHMDS produced the vinylogous hemiester (**9**). The fully elaborated hemiester (**10**) was then obtained after catalytic hydrogenation of the double bond of compound (**9**) in EtOAc.

The synthesis of the target molecule was accomplished in two steps from the hemi-ester and uracil starting materials. The bridgehead acid function of compound (**10**) was coupled to the amine in position 5 of uracil (**4**) under the influence of O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) (Scheme 3).[11] Base-catalyzed cyclization and dehydration of (**11**) in boiling aqueous 2-propanol formed the xanthine ring system and continued heating produced the hydrolysis product. Recrystallization from hot acetonitrile twice gave the [$^2H_7$]-labeled xanthine (**12**) in 40% yield for the two final steps.

[$^2H_7$]-BG9928 was fully characterized by ^1H and ^{13}C NMR and mass spectrometric analysis. Proton NMR comparison of [$^2H_7$]-BG9928 to the non-labeled compound showed the loss of propyl peak absorptions of the *N-3* propyl side chain. The specific signals excised include the methylene at δ 4.05 ppm (triplet) adjacent to the *N-3* nitrogen, the methylene at 1.75 ppm (multiplet), and the methyl at 0.95 ppm (triplet). The decoupled ^{13}C NMR spectra were identical for the labeled and unlabeled compounds. Mass spectrometric analysis of the labeled compound showed an increase of 7 mass units. The isotopic purity of the material was calculated by comparison of the relative abundances of the labeled and unlabeled species in the mass spectrum. The observed isotopic purity of the final product was >99% which closely matched the isotopic purity of the starting material [$^2H_7$]-iodopropane (99.3% D).

The *N*-3 labeled form of 3-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-propionic acid was synthesized in a convergent synthetic manner with high isotopic and chemical purity. The use of this compound for *in vitro* and *in vivo* metabolism studies will be detailed in future publications.

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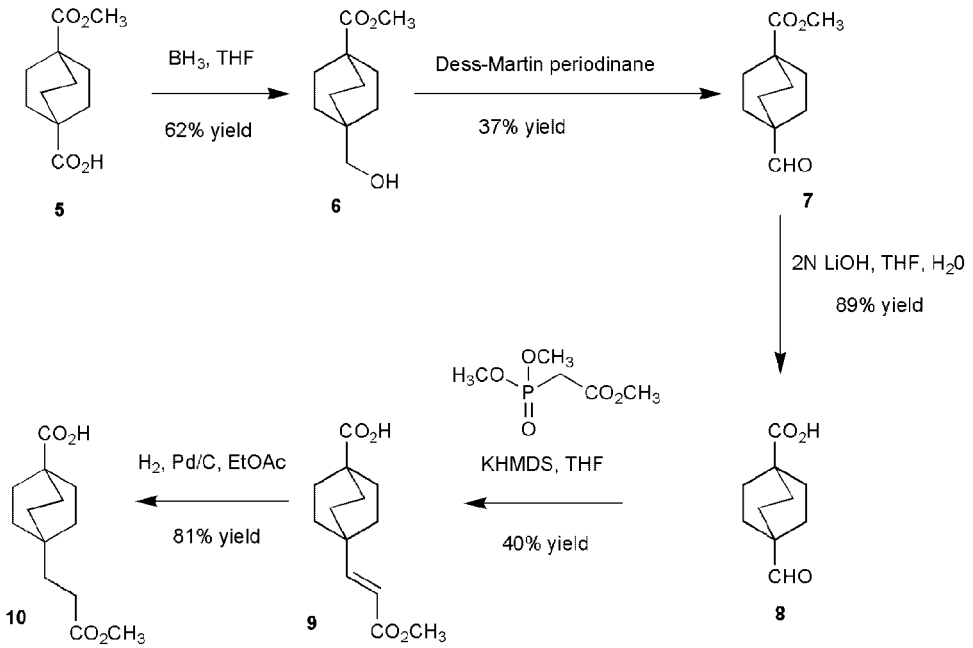
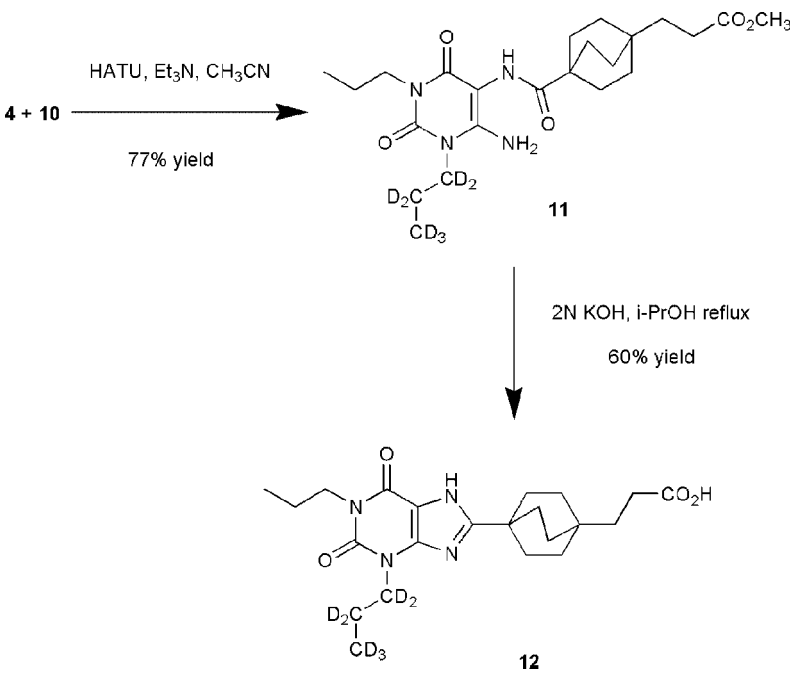


Scheme 2



Scheme 3

Experimental

General

Reaction courses and product mixtures were routinely monitored by HPLC (Hewlett Packard series 1100 HPLC system equipped with an YMC-ODS-AM C-18 column (5 μm, 100 × 4.6 mm) and a YMC-ODS-AM S-5 120A guard column using an 8 min gradient run from 20 to 100% acetonitrile in water (0.1% TFA buffer), UV absorbance monitored at 214 and 254 nm) or TLC on silica gel (precoated IB-F Baker-flex plates) and

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visualized with UV lamp or phosphomolybdic acid (PMA). Mass spectra were measured by electrospray pos. at 60 V with a Micromass VG Platform MS. ^{1}H and ^{13}C NMR spectra ($CDCl_3$ or DMSO-d_6) were measured with on a Bruker AVANCE 400 MHz spectrometer, peak positions are given in ppm (δ) downfield from TMS as an internal standard. Column chromatography was done with EM Science 230–400 mesh silica gel. 1-Iodopropane-d_7 was purchased from CDN Isotopes with isotopic purity of 99.3% D. Unless otherwise specified, reactions were run under a nitrogen atmosphere in oven-dried glassware.

(6-Amino-2,4-dioxo-3-propyl-1,2,3,4-tetrahydro-pyrimidin-5-yl)-carbamic acid tert-butyl ester, (2). A suspension of 5,6-diamino-3-propyl-1*H*-pyrimidine-2,4-dione hydrochloride[7] (25.0 g, 0.113 mol) in dioxane (225 mL) and water (110 mL) was treated with 3.2 N NaOH (35 mL, 0.113 mol). The resulting solution was cooled to 10°C and di-*t*-butyl dicarbonate (24.6 g, 0.124 mol) and 3.2 N NaOH (35 mL, 0.113 mol) was added and allowed to stir at rt for 18 h. The resulting precipitate was washed with cold water and toluene to give **2** (19.1 g, 60% yield) as a pink solid. ^{1}H NMR (DMSO-d_6) δ: 0.82 (t, 3H); 1.41 (s, 9H); 1.42–1.54 (sextet, 2H); 3.65 (t, 2H); 6.3 (b, 2H); 7.3 (s, 1H) ppm.

(6-Amino-2,4-dioxo-1-$^2H_7$-propyl-3-propyl-1,2,3,4-tetrahydro-pyrimidin-5-yl)-carbamic acid tert-butyl ester, (3). Compound **2** (7.5 g, 0.026 mol), anhydrous potassium carbonate (7.5 g, 0.054 mol) and 1-iodopropane-d_7 (5.0 g, 0.029 mol) were heated at 70°C for 18 h in anhydrous DMF (25 mL). The reaction mixture was concentrated under reduced pressure and the residue purified by flash chromatography (EtOAc–hexanes 1:1) to afford **3** as a yellow solid (4.7 g, 56% yield), $m/z = 334$ (MH^+).

5,6-Diamino-1-$^2H_7$-propyl-3-propyl-1H-pyrimidine-2,4-dione hydrochloride, (4). Compound **3** (550 mg, 1.65 mmol) was dissolved in 4 N HCl-dioxane (5 mL) and after 30 min the reaction mixture was concentrated *in vacuo* to afford the HCl salt as white solid (365 mg, 95% yield), $m/z = 234$ (MH^+).

4-Hydroxymethyl-bicyclo(2.2.2)octane-1-carboxylic acid methyl ester, (5). To a solution of bicyclo[2.2.2]octane-1,4-dicarboxylic acid monomethyl ester[10] (9.15 g, 0.043 mol) in anhyd. THF (100 mL) was added dropwise 1M BH_3–THF complex (86 mL, 0.086 mol). The solution was stirred for 16 h at rt and a 5% HCl in methanol solution (50 mL) was added. Concentration *in vacuo* gave a colorless oil which when purified by flash chromatography (EtOAc–hexanes 1:3) gave hydroxy ester **6** as a white solid (4.16 g, 49% yield). ^{1}H NMR ($CDCl_3$) δ: 1.28–1.33 (m, 6H); 1.60–1.69 (m, 6H); 3.16 (s, 2H); 3.51 (s, 3H) ppm.

4-Formyl-bicyclo(2.2.2)octane-1-carboxylic acid methyl ester, (7). Dess–Martin periodinane (10.6 g, 0.025 mol) was added to a stirred solution of **6** (4.16 g, 0.021 mol) in CH_2Cl_2 (100 mL) at rt After 2 h the reaction mixture was treated with 1 N sodium sulfite (50 mL) and stirred for 1 h. The mixture was diluted with 100 mL of CH_2Cl_2, washed twice with 1 N sodium sulfite (50 mL) and twice with saturated NaCl. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The resultant crude oil was purified by flash chromatography (EtOAc–hexanes 1:5) to afford aldehyde **7** as a white solid (1.53 g, 37% yield). ^{1}H ($CDCl_3$) δ: 1.62–1.68 (m, 6H); 1.79–1.84 (m, 6H); 3.63 (s, 3H); 9.38 (s, 1H) ppm.

4-Formyl-bicyclo(2.2.2)octane-1-carboxylic acid, (8). Lithium hydroxide (0.65 g, 0.027 mol) was added with stirring to a rt solution of **7** (1.5 g, 0.0077 mol) in THF (5 mL) and water (3 mL) After 16 h, the mixture was concentrated *in vacuo*, diluted with 15 mL of water and washed thrice with 15-mL portions of CH_2Cl_2. The aqueous phase was acidified with 2 N HCl (10 mL) and washed twice with CH_2Cl_2. The combined organic fractions were dried over Na_2SO_4 and concentrated *in vacuo* to give carboxylic acid **8** as a white solid (1.25 g, 89% yield) ^{1}H (DMSO d_6) δ: 1.68–1.73 (m, 12H); 9.42 (s, 1H) ppm.

4-(2-Methoxycarbonyl-vinyl)-bicyclo(2.2.2)octane-1-carboxylic acid, (9). To an ice cold solution of 0.5 M KHMDS in toluene (44 mL, 0.022mol) was added dropwise trimethylphosphonoacetate (2.35 mL, 0.014 mol) over 10 min. A THF (1 mL) solution of compound **8** (1.25 g, 0.0069 mol) was added over 30 min at 0°C. The reaction mixture was allowed to warm to rt, stirred an additional 16 h, and acidified with 2 N HCl (15 mL). After concentration under reduced pressure, the residue was taken up in EtOAc (50 mL), washed twice with 2 N HCl (20 mL) and twice with brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated to give a white solid. Purification by flash chromatography (EtOAc–hexanes 1:3) afforded **9** as a white solid (0.525 g, 40% yield). ^{1}H ($CDCl_3$) δ: 1.55–1.60 (m, 6H); 1.75–1.80 (m, 6H); 3.68 (s, 3H); 5.75 (d, 1H); 6.85 (d, 1H) ppm.

4-(2-Methoxycarbonyl-ethyl)-bicyclo(2.2.2)octane-1-carboxylic acid, (10). To a degassed solution of **9** (0.50 g, 0.0021 mol) in EtOAc (20 mL) was added 10% Pd/C (25 mg). The reaction mixture was charged with

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H_2, stirred for 16 h at rt. The catalyst was removed by filtration and concentrated to afford **10** as a white solid (0.41 g, 81% yield). ^{1}H ($CDCl_3$) δ: 1.29–1.35 (m, 6H); 1.46 (t, 2H); 1.73–1.80 (m, 6H); 2.16 (t, 2H); 3.58 (s, 3H) ppm.

3-(4-(6-Amino-2,4-dioxo-1-propyl-3-$^2H_7$-propyl-1,2,3,4-tetrahydro-pyrimidin-5-ylcarbamoyl)-bicyclo(2.2.2)oct-1-yl)-propionic acid methyl ester, (11). Compounds **4** (0.375 g, 0.0017 mol) and **10** (0.41 g, 0.0017 mol) were dissolved in CH_3CN (10 mL) and Et_3N (1mL, 0.0136 mol). HATU (0.68 g, 0.0018 mol) was added and the solution stirred at rt for 16 h. The reaction mixture was concentrated and the residue taken up in EtOAc (50 mL), washed with 10% citric acid (25 mL), saturated Na_2CO_3 (25 mL) and brine. The organic layer was dried over Na_2SO_4 and concentrated to a yellow oil **11** (0.58 g, 77% yield), $m/z = 456$ (MH^+).

3-(4-(2,6-Dioxo-1-propyl-3-$^2H_7$-propyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-bicyclo(2.2.2)oct-1-yl)-propionic acid, (12). A solution of amide **11** (0.58 g, 0.0013 mol) in 2-propanol (5 mL) and 2 N KOH (3.5 mL, 0.007 mol) was refluxed for 1 h. The reaction mixture was concentrated, taken up in water (25 mL) and the aqueous layer washed thrice with CH_2Cl_2 (20 mL). The aqueous fraction was acidified with concentrated HCl and washed thrice with EtOAc (15 mL). The combined organic fractions were then washed with brine, dried over Na_2SO_4, and concentrated to give a white solid. Twice recrystallized from hot CH_3CN gave **12** as a white solid (0.214 g, 40% yield), $m/z = 424$ (MH^+). ^{1}H ($CDCl_3$) δ: 0.94 (t, 3H); 1.45–1.55 (m, 8H); 1.65 (sextet, 2H); 1.9–1.98 (m, 6H); 2.25 (t, 2H); 3.95 (t, 2H); 11.95 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ 11.7, 21.7, 29.4, 30.8, 30.9, 34.2, 36.2, 39.8, 40.0, 40.2, 40.4, 40.6, 40.8, 41.0, 43.3, 107.1, 148.9, 151.6, 155.6, 161.9, 177.2 ppm.

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