Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 11-28 (2000)

SYNTHESIS OF ISOTOPICALLY LABELLED VERSIONS OF ADENOSINE AGONIST GR79236

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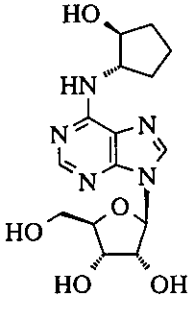
Summary

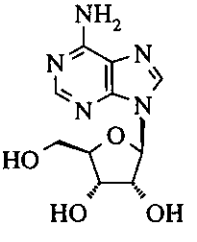
Versions of adenosine receptor agonist GR79236 (1), labelled either with carbon-14 (9) at C-8 of the purine ring or with tritium (15) in the cyclopentyl ring, were prepared in overall yields of 64% and 25% respectively. A mass labelled [M+4] version (24) containing carbon-13, nitrogen-15, and deuterium was also prepared in 3% yield.

Keywords: adenosine agonist, GR79236, N-nitration, mass label, radiolabel, recycling HPLC.

Introduction



GR79236 (1)



Adenosine (2)

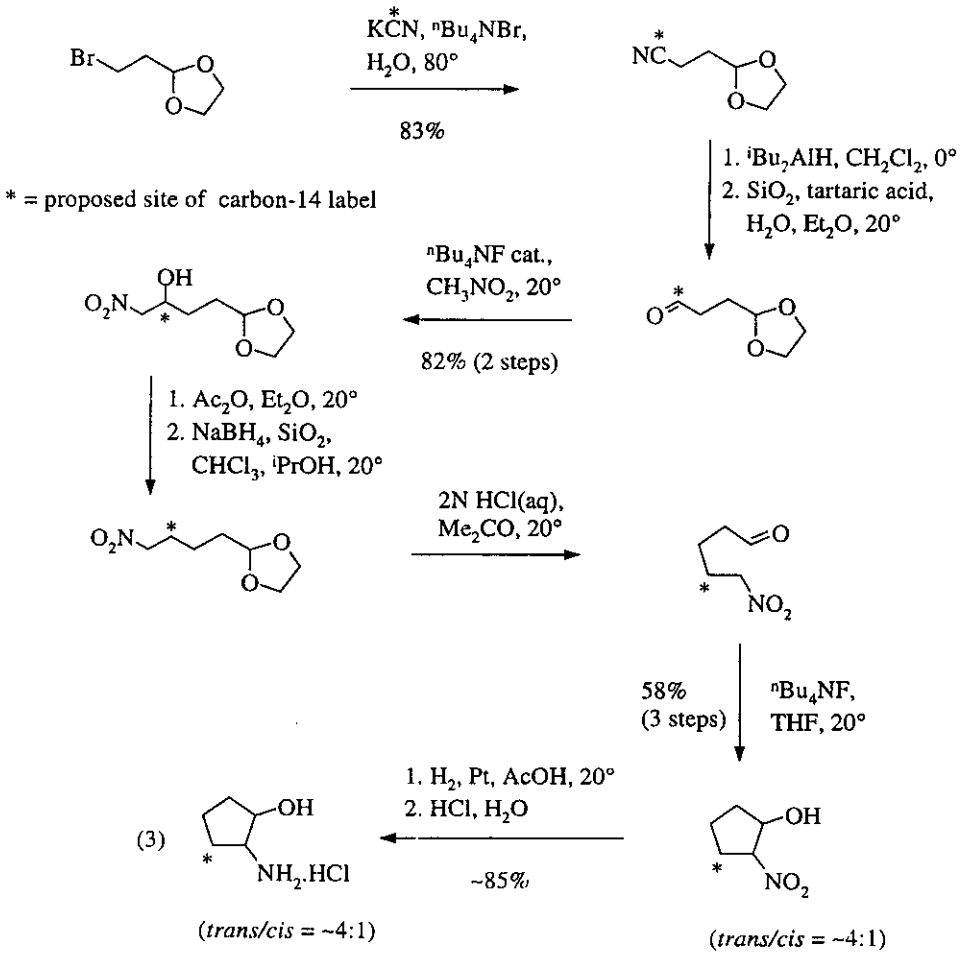
CCC 0362–4803/2000/010011–18$17.50

Received 4 June 1999
Accepted 24 June 1999

GR79236 (1) is an adenosine A1 receptor agonist. It was originally developed as a potential treatment for diabetes (1,2) because of its ability to reduce levels of non-esterified fatty acids by inhibition of lipolysis mediated through adenosine receptors. Subsequently (1) was reinvestigated as a selective agonist in regard to the observed cardioprotective effect of adenosine (2) during cardiac surgery. Studies of the absorption, distribution, metabolism, and excretion (ADME) of GR79236 (1) required the syntheses of labelled versions (3).

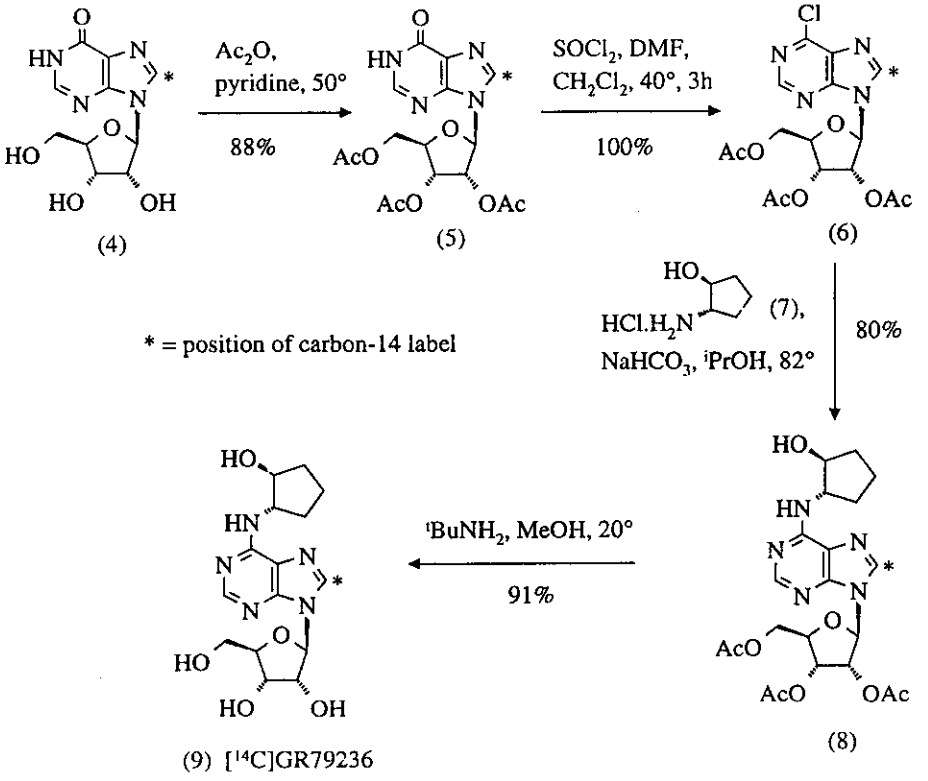
Results and Discussion

The preferred labelling for ADME work with GR79236 (1) was the incorporation of carbon-14 into the cyclopentyl ring. Tritium labelling was second choice because it



Scheme 1: Synthesis of 2-aminocyclopentanol

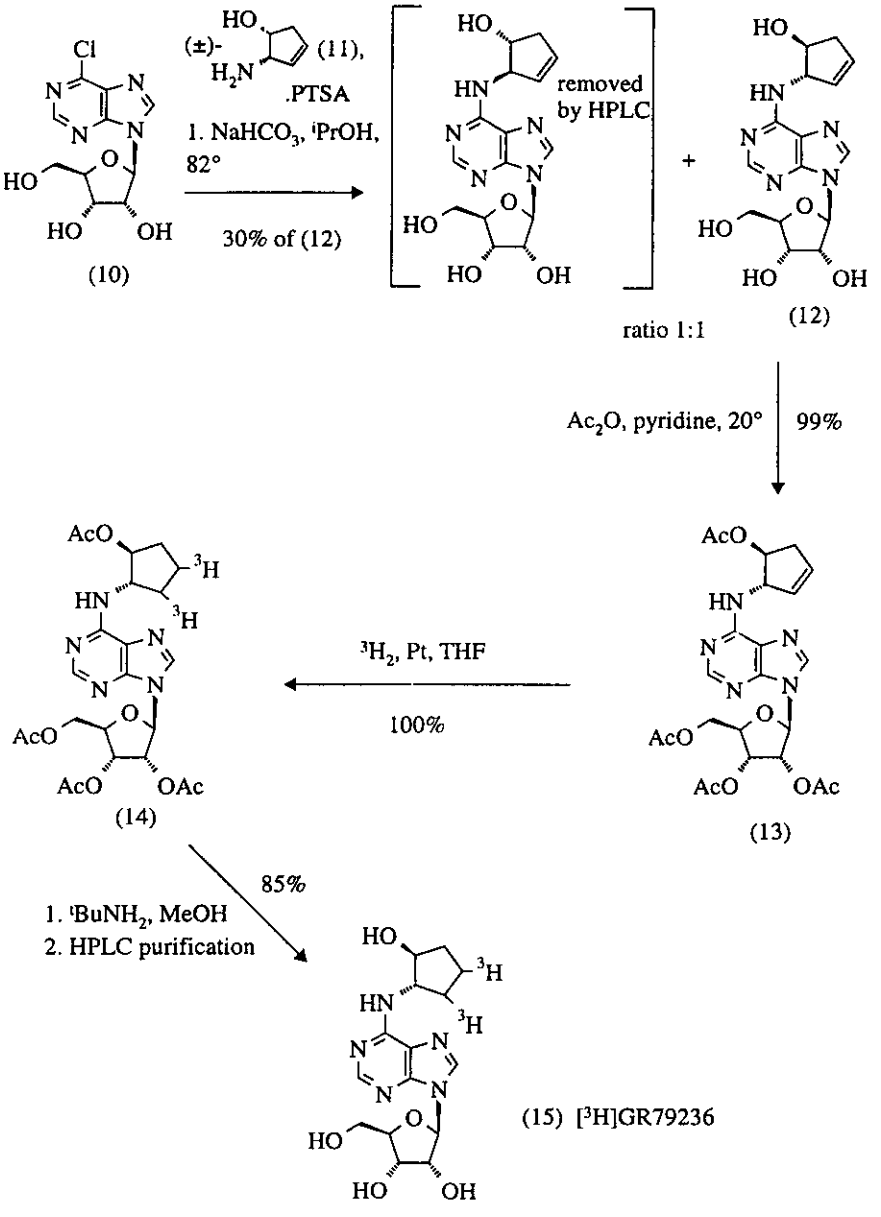
has a greater potential to be removed by metabolic oxidative processes. We envisaged that metabolic cleavage of the cyclopentyl group would generate either adenosine (2) or inosine (unlabelled (4)), both of which already have well documented metabolic profiles. However, initial investigations revealed that a seven-step synthesis would be necessary to generate 2-aminocyclopentanol (3) as a racemic *cis/trans* mixture, with incorporation of cyanide providing the source of the carbon-14 label (Scheme 1). In addition, coupling of amine (3) to a chloropurine derivative (*cf.* Scheme 2), followed by chromatographic separation of the resulting mixture of four diastereoisomers, would have been necessary to complete the synthesis of GR79236 in an estimated <10% yield from cyanide. Therefore, a shorter, convergent synthesis, *via* the patented route (1) to GR79236 (1), was employed for initial ADME work (Scheme 2).



Scheme 2: Synthesis of carbon-14 labelled GR79236

Commercially available 8-[^{14}C]inosine (4) was initially protected as the triacetate (5) in order to allow chromatographic purification at the penultimate stage of this small-scale synthesis. Silica gel chromatography of final product (1) was known to be inefficient. Treatment with thionyl chloride and dimethylformamide quantitatively converted (5) into chloropurine (6). Condensation of (6) with enantiomerically pure *trans*-aminocyclopentanol (7) (4) caused some loss of acetate groups in (8) owing to the mildly basic, protic conditions, (*cf.* mass labelled synthesis below). Nevertheless, both fractions from the chromatographic purification gave the same product (9) after deprotection. A final crystallisation gave [^{14}C]GR79236 (9) with radiochemical and chemical purities >98%, in an overall yield of 64% from [^{14}C]inosine (4).

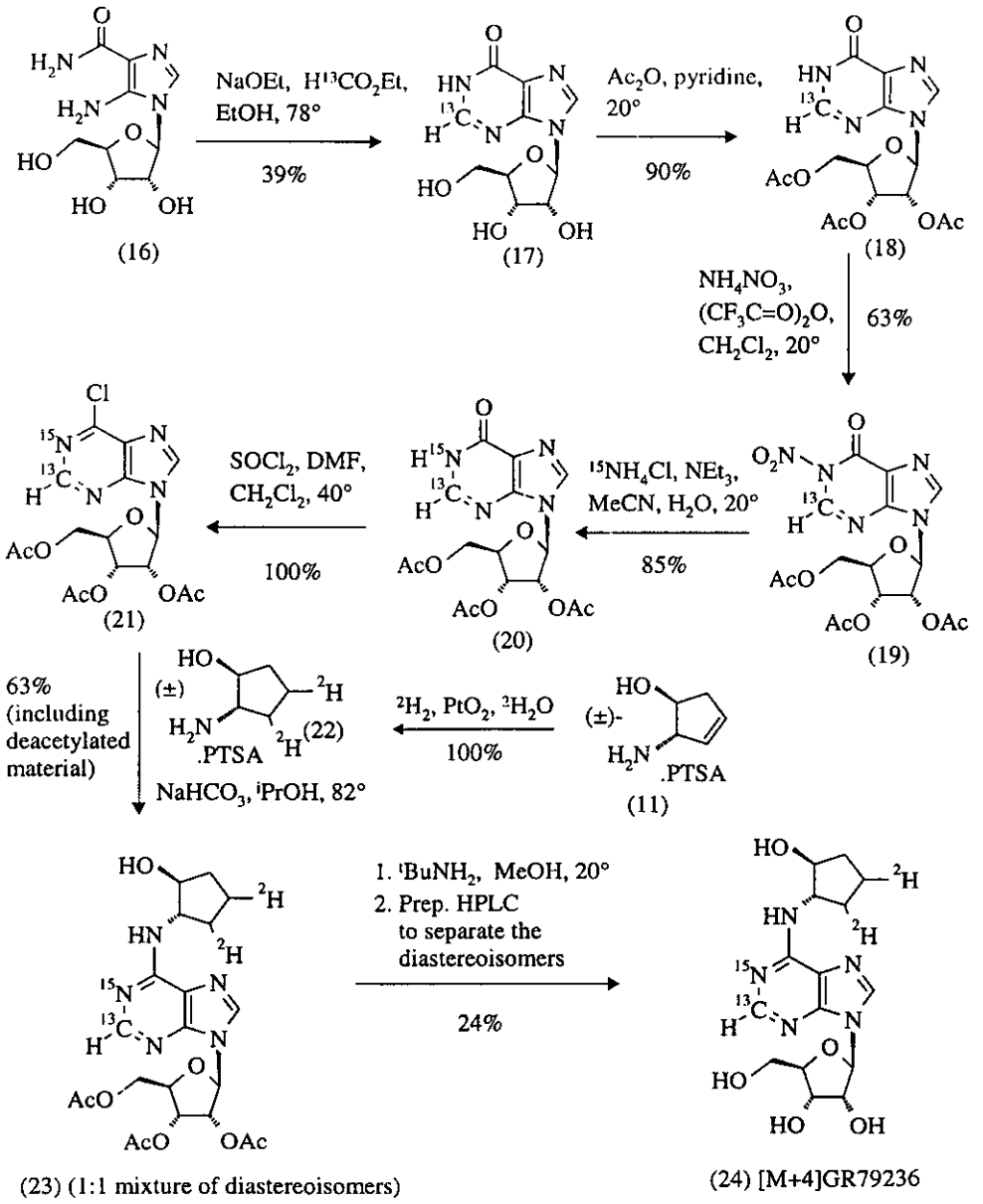
ADME work with (9) confirmed the need to establish the fate of the aminocyclopentanol moiety. Therefore, a version (15) labelled with tritium in the cyclopentyl ring was synthesised as shown in Scheme 3. Condensation of racemic 2-aminocyclopent-3-en-1-ol (11) (1,5) with (10) gave equal amounts of two diastereoisomeric products. Purification of this crude mixture to give the diastereoisomer (12) was carried out by preparative HPLC using a recycling modification (6). Recycling is most effectively used to enable the complete resolution of components that are incompletely separated on a single pass through the column. Here, with two easily separable major components, the key advantage of recycling was that the column could be overloaded, so that purification was effected with much fewer injections, i.e. economising on time, solvent consumption, and solvent waste (see also experimental for (17)). Diastereoisomer (12) was then peracetylated to give (13). This was a precaution to minimise the exchange of labile hydrogen atoms (-OH, -NH-) in (12) with tritium on the surface of the catalyst during the ensuing reduction. This would reduce the maximum specific activity attainable in the final product (15). [Note: Subsequent work with deuterium demonstrated high isotopic incorporations (1.78 [^{2}H]) both with and without the use of peracetylation.] After tritiation of olefin (13) over platinum black in tetrahydrofuran, the product (14) was estimated from uptake of activity (later confirmed in the isotopic dilution of (15) below) to contain ~0.5-0.55 [^{3}H] atoms per molecule. Deacetylation and preparative



Scheme 3: Synthesis of tritium labelled GR79236

HPLC gave [^{3}H]GR79236 (15). A portion of this material was diluted with unlabelled (1) to obtain a sample with a specific activity of 927mCi/mmol, determined by HPLC/UV assay. Tritium NMR spectroscopy of this product showed

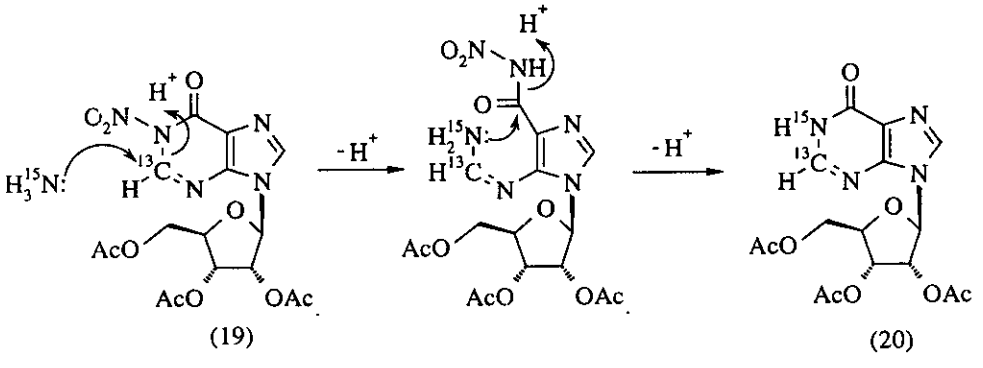
an isotopic incorporation similar to that found for deuterium sites in the mass labelled compound (24) below. COSY ^{1}H-NMR spectra of the products from the catalytic deuteration of olefins (11)-(13) showed that isotopic incorporation occurred only at



Scheme 4: Synthesis of mass labelled GR79236

at C-3" and C-4", not at C-5". This is a correction to the earlier assignment (3) of additional isotopic incorporation at the C-5" methylene group in compounds (14)-(15) and (22)-(24).

Synthesis of an [M+4] version of GR79236 (24) was required as an internal standard in order to develop a mass spectrometric assay (7,8) for (1). The extra four mass units in (24) gives a molecular ion quantitatively free of contributions derived from natural carbon-13 abundance in (1). The synthesis (Scheme 4) starting from commercially available AICA riboside (16) notably used a recently described, novel rearrangement (9) to incorporate the nitrogen-15 label. Base catalysed cyclization of AICA riboside (16) with ethyl [^{13}C]formate gave [^{13}C]inosine (17) in only 14% yield after crystallisation of crude product. However, recycling preparative HPLC of the mother liquor increased the total yield to 43%. After routine peracetylation, the [M+1]inosine derivative (18) was nitrated at N-1. The resulting N-nitroinosine (19) was rigorously purified from unreacted [M+1]triacetylinosine (18) to prevent isotopic dilution of [M+2] product (20). Displacement of nitramine from (19) *via* the rearrangement shown in Scheme 5 (attack of [^{15}N]ammonia at C-2 and not C-6 was demonstrated previously (9)) gave [M+2]triacetylinosine (20), routinely converted into the chloropurine (21).



Scheme 5: Proposed mechanism of incorporation of nitrogen-15 label

A further two extra mass units were incorporated from [M+2]-(±)-2-aminocyclopentanol (22), which was most conveniently prepared from the olefinic

tosylate salt (11). Deuteration of (11) over platinum black in deuterium oxide gave (22) with an incorporation of slightly greater than 2 [^{2}H], but solely at positions C-3" and C-4" according to NMR data. This, including the presence of the trideuterated species found in (24) below, is consistent with the Horiuti-Polanyi mechanism for catalytic reduction of olefins over platinum (10). Condensation of (22) with chloropurine (21) gave adduct (23). Deacetylation and preparative HPLC separation of the two resulting diastereoisomers gave the more polar isomer [M+4]GR79236 (24). Although MS analysis of (24) showed a spread of deuterium incorporation ($^2H_1$, $^2H_2$, and $^2H_3$ species), the [M+0] content was <<0.1%, i.e. suitable for use as the internal standard.

Conclusions

Syntheses of carbon-14 (9) and tritium labelled versions (15) were developed in order to enable ADME studies of GR79236 (1). A mass labelled [M+4] version (24) was prepared for use as an internal standard in a mass spectrometric assay of (1). Recycling preparative HPLC was demonstrated to be a very useful improvement over conventional HPLC for obtaining rapid and efficient separations on a gram scale.

Experimental

NMR spectra were recorded on Bruker AC250 or Varian Unity 400 spectrometers. Mass spectra were recorded by LC-MS on either Hewlett Packard 5989A (thermospray), Micromass Platform II (electrospray), or Perkin Elmer Sciex API 3$^+$ (ionspray) instruments. Microanalyses were performed using a Carlo Erba 1108 elemental analyser. Recycling preparative HPLC of (12) was carried out using a Gilson module system with single-piston pump, incorporating a custom made switching valve and pressure compensation reservoir as previously described (6). Recycling preparative HPLC of (17) was carried out using a Shimadzu LC-8A double-piston pump with a simple switching valve not requiring a pressure compensation reservoir. Thin layer chromatography was carried out using Merck Kieselgel 60 F_{254} glass plates. Radiochemical purities were determined by TLC using a Berthold Tracemaster 20 automatic TLC linear analyser, or by HPLC using an

online Canberra Packard Radiomatic Flo-One Beta detector. Chemical purities were determined by HPLC using Gilson UV detectors monitoring the wavelength quoted.

[8-^{14}C]Inosine, 2′,3′,5′-tri-O-acetate (5)

(-)-[8-^{14}C]Inosine (4) (313mg, 44.1mCi @ 53.0mCi/mmol, 0.79mmol, radiochemical purity 94.8% by HPLC) was treated with pyridine (7ml) and acetic anhydride (5.5ml). The stirred mixture was heated at 50° for 6h, cooled, and evaporated to dryness. The residue was further dried by addition and evaporation of toluene (5x10ml). The solid was triturated in boiling absolute ethanol (6ml) for 5min, and the resulting mixture left to cool in an ice -water bath for 30min. The crystallised solid was collected, washed with absolute ethanol (2x2ml), and dried by addition and evaporation of aliquots of toluene (2x10ml). The above trituration/crystallisation procedure was repeated with more absolute ethanol (5ml) to give the *title compound* (5) as an almost colourless solid (277mg, 37.1mCi, 88% radiochemical yield); TLC (ethyl acetate-methanol-water 40:10:1) radiochemical purity 99.5%.

6-Chloro-[8-^{14}C]purineriboside, 2′,3′,5′-tri-O-acetate (6)

A stirred mixture of [8-^{14}C]inosine-2',3',5'-tri-O-acetate (5) (276mg, 36.9mCi, 0.70mmol) and dimethylformamide (155µl, 2.0mmol) in dichloromethane (5ml) at 0-5° was treated with thionyl chloride (145µl, 2.0mmol). The resulting solution was heated at reflux for 3h, cooled, stirred with 8% aqueous sodium bicarbonate (10ml) for 10min, diluted with 8% aqueous sodium bicarbonate (50ml), and extracted with dichloromethane (1x50ml, 3x25ml). The combined extracts were dried ($MgSO_4$) and evaporated to dryness to give the *title compound* (6) as a pale straw coloured, viscous oil (36.9mCi, 100% radiochemical yield); TLC (ethyl acetate-methanol 9:1) radiochemical purity 99.1%.

N-[(1S, trans)-2-Hydroxycyclopentyl]-[8-^{14}C]adenosine, 2′,3′,5′-tri-O-acetate (8)

A mixture of chloropurine (6) (36.9mCi, 0.70mmol), chiral amine salt (7) (145mg, 1.05mmol), and sodium bicarbonate (177mg, 2.10mmol) in isopropanol (4ml) was heated at reflux for 4h. The mixture was filtered, washing the inorganic precipitate well with absolute ethanol. The filtrate was evaporated to dryness. The residue was purified by chromatography over silica gel (40g Merck 7754), eluting with ethyl

acetate-methanol (50:1) to give the *title compound* (8) and some deacetylated material in two batches, each as an almost colourless gum (total 35.1mCi, 93% radiochemical yield); TLC (ethyl acetate-methanol 9:1) R_f 0.29 (major) and R_f 0.21 (minor). Autoradiography showed no significant radiochemical impurities, the spot at R_f 0.21 appearing to be two extremely close components. Data for the corresponding unlabelled compound (R_f 0.29 only), prepared in a similar manner: δ_H(250MHz, $CDCl_3$) 8.35 (1H, s, 2-H), 7.95 (1H, s, 8-H), 6.18 (1H, d, 1'-H), 6.02 (1H, d, 2''-H), 5.92 (1H, t, 2'-H), 5.66 (1H, m, 3'-H), 4.5-4.0 (5H, 2m, 5'-CH_2, 4'-H, 1''-H, 6-NH), 2.45-1.60 (6H, m, 3''-CH_2, 4''-CH_2, 5''-CH_2), 2.16, 2.13, 2.08 (9H, 3s, 2'-OAc, 3'-OAc, 5'-OAc).

N-[(1S, trans)-2-Hydroxycyclopentyl]-[8-^{14}C]adenosine (9)

A solution of the triacetate (8) (30.3mCi, 0.56mmol, first batch with very little of the R_f 0.21 component) and *t*-butylamine (1ml) in methanol (5ml) was left to stand at 20° for 3 days, cooled, evaporated to dryness, and redissolved in methanol (15ml). The stirred solution was slowly distilled over 3h to ensure complete removal of *t*-butylamine, occasionally replacing methanol in the flask if the level became too low. The remaining solution was filtered and evaporated to dryness. The residual glass was dissolved in methanol (0.7ml), diluted gradually with ethyl acetate (10ml), seeded, and left to crystallise overnight [after 4h, more ethyl acetate (4ml) was added]. The crystalline product was collected, washed with ethyl acetate-methanol (97:3) followed by neat ethyl acetate, and dried under high vacuum to give the *title compound* (9) (181mg, 28.4mCi, 93% radiochemical yield); m.p. 160.5-163.6° (unlabelled reference sample m.p. 162-163.5°); TLC (*n*-butanol-water-formic acid 77:13:10) radiochemical purity 99.0%; HPLC (250x4.6mm 5µ Spherisorb C8 column eluted with 1:9 acetonitrile-0.01M pH4.5 aqueous triethylammonium phosphate at 1ml/min) radiochemical purity >99%, chemical purity >98% a/a at 266nm; isotope dilution assay 98.3%; specific activity 157µCi/mg (55.3mCi/mmol); δ_H (250MHz, D_2O) 8.23, 8.27 (2H, 2s, 2-H and 8-H), 5.95 (1H, d, 1'-H), 4.74 (1H, m, 2'-H), 4.40-4.05 (4H, m, 3'-H, 4'-H, 1''-H, and 2''-H), 3.95-3.70 (2H, m, 5'-CH_2), 2.35-1.55 (6H, m, 3''-CH_2, 4''-CH_2, and 5''-CH_2).

N-[(1S,trans)-2-Hydroxy-4-cyclopentenyl]adenosine (12)

A mixture of chloropurine (10) (3.0g, 10.5mmol), unsaturated amine tosylate (11) (2.9g, 10.6mmol), and sodium bicarbonate (3.6g, 42mmol) in isopropanol (100ml) was heated at reflux under nitrogen for 22h. The mixture was filtered, washing with isopropanol (200ml). The filtrate was evaporated to dryness to give a solid containing equal amounts of (12) and its diastereoisomer. The solid was kept dissolved in eluent at 60° and purified in aliquots (70mg/400µl optimum) using preparative HPLC with a recycling technique (6) (25x2cm 5µ Spherisorb ODS2 column eluted with water-ethanol 88:12 at 20ml/min, detection at 266nm). Product fractions containing only (12) were collected on the third cycle after each injection, combined, and evaporated to dryness. The residue was dissolved in hot ethanol (60ml) and filtered through a 1.0µm filter, washing with hot ethanol (60ml). The filtrate was evaporated to dryness to give a foam. Crystallisation from ethyl acetate (120ml) (initial solution being achieved using methanol as co-solvent, which was mostly removed by distillation) gave the *title compound* (12) as colourless crystals (1.085g, 30%); HPLC (25x0.46cm 5µ Spherisorb ODS2 eluted with water-ethanol 88:12 at 1.5ml/min) R_t 11.1min, chemical purity >99% a/a at 266nm; m/z (electrospray) 350 (MH^+, 100%); δ_H (400MHz, d_4-MeOD) 8.27 (1H, s, 8-H), 8.26 (1H, s, 2-H), 5.97 (1H, m, 3"-H), 5.95 (1H, d, J = 6.85Hz, 1'-H), 5.77 (1H, m, 4"-H), 5.04 (1H, broad m, 2"-H), 4.73 (1H, m, J = 4.9Hz, 2'-H), 4.32 (1H, m, 3'-H), 4.31 (1H, m, 1"-H), 4.16 (1H, m, 4'-H), 3.87 (1H, d of d, J = 2.45Hz, J = 12.72Hz, 5'-H), 3.73 (1H, d of d, J = 2.45Hz, J = 12.72Hz, 5'-H), 2.82 (1H, m, 5"-H), 2.33 (1H, m, 5"-H)..

N-[(1S,trans)-2-Acetoxy-4-cyclopentenyl]adenosine, 2',3',5'-tri-O-acetate (13)

A solution of tetraol (12) (100mg, 0.29mmol) in acetic anhydride (3.0ml) and pyridine (1.5ml) was stirred at 20° under nitrogen for 18h. The solvents were then removed, as their azeotropes with added toluene (2x20ml), by evaporation to dryness. The residue (181mg) was purified by flash chromatography over silica gel (25g Merck 9385) with ethyl acetate as eluent to give the *title compound* (13) (R_f 0.29) as a white foam (149mg, 99%); m/z (thermospray) 518 (MH^+, 100%); δ_H (400MHz,

$CDCl_3$) 8.40 (1H, 1s, 2-H), 7.90 (1H, s, 8-H), 6.18 (1H, d, 1'-H), 5.98, 5.84 (2H, 2m, 3''-H, and 4''-H), 5.91 (1H, t, 2'-H), 5.74 (1H, d, 6-NH), 5.67 (1H, m, 3'-H), 5.46 (1H, s, 5''-H), 5.26 (1H, m, 4'-H), 4.47-4.34 (3H, m, 5'-H and 1''-H), 3.01-2.92 and 2.42-2.34 (2H, m, 2''-CH_2), 2.15, 2.13, 2.09, and 2.07 (12H, 4s, 1''-OAc, 2'-OAc, 3'-OAc, and 5'-OAc).

N-[(1S,trans)-2-Acetoxy-3,4,-[$^3H_2$]cyclopentyl]adenosine, 2',3',5'-tri-O-acetate (14)

A mixture of the protected olefin (13) (25mg) and platinum black (10mg) in dry tetrahydrofuran (7.5ml) was stirred under an atmosphere of tritium gas at room temperature for 1h. The catalyst was removed by filtration through Hyflo. The filtrate was diluted with methanol and stripped of volatile tritiated residues. A total of 775mCi of product was produced. *Title compound* (14) was supplied as a solution in methanol (100mCi/25ml) with an estimated specific activity of ~16 Ci/mmol (based on an assumed quantitative recovery – *cf.* assay result for (15)); $\delta_{H\text{-}3}$ (426.6MHz, MeOH) 2.44 and 1.84 (m, 3''-^{3}H), 2.02 (m, 4''-^{3}H). This experiment was carried out as a routine Tritium Labelling Service by Amersham Pharmacia Biotech, Cardiff.

N-[(1S,trans)-2-Hydroxy-3,4,-[$^3H_2$]cyclopentyl]adenosine (15)

A sample (7.5ml, 30mCi) of the methanolic solution of compound (14) was diluted with methanol (15ml) and t-butylamine (5ml). The resulting homogeneous solution was stored under nitrogen at 20° for 16h and then evaporated to dryness. The residue was repeatedly (5x) treated with methanol (15ml) and evaporated to dryness under vacuum at 50°, in order to ensure complete removal of the t-butylamine. The final residue was purified by preparative HPLC (25x0.46cm 5μ Spherisorb ODS2 eluted with water-ethanol 88:12 at 1.0ml/min, detection at 266nm), collecting eluent in the range R_t 22-24min. The combined product fractions were evaporated to dryness and then dissolved in methanol (25ml, total activity 26.1mCi); TLC (*n*-butanol-water-formic acid 77:13:10) radiochemical purity 99%. The solution was isotopically diluted with unlabelled GR79236 (1) (9.02mg) and evaporated to dryness. The *title compound* (15) was supplied as a solution in ethanol-water 95:5 (10ml) (25.4mCi, 85% radiochemical yield); TLC (n-butanol-formic acid-water 77:10:13)

radiochemical purity >99%; HPLC (15x0.46cm 5μ Kromasil C8 eluted at 1.5ml/min with 19%B for 17min and then a gradient of 19-60%B from 17-25min; A = 0.01M aqueous trethylammonium phosphate pH3.6; B = methanol) R_t 17.5 min, radiochemical purity >99%, chemical purity 99.0% at 266nm; specific activity 927 mCi/mmol; assay by HPLC (25x0.46cm 5μ Spherisorb ODS2 eluted with water-ethanol 88:12 at 1.0ml/min, detection at 266nm) vs GR79236 (1) standard = 9.62mg (15), which gives an estimate of 14.8Ci/mmol for the input material (14); $\delta_{H\text{-}3}$ (426.6MHz, EtOH-H_2O 95:5) 2.20 and 1.58 (m, 3''-^{3}H), 1.71 (m, 4''-^{3}H).

[2-^{13}C]Inosine (17)

AICA riboside (16) (5.74g, 22.2mmol) was added in one portion to a stirred solution of sodium ethoxide, freshly prepared by dissolving metallic sodium (2.3g, 100mmol) in absolute ethanol (115ml). The resulting solution was stirred at reflux under nitrogen for 15min. [^{13}C]-Ethyl formate (*ex* Isotec Inc., 2.5g, 2.73ml, 33.3mmol, 99.3atom% ^{13}C) was then added dropwise and the resulting white suspension was stirred at reflux for 4h. Water (500ml) was added and the pH was adjusted to 7.0 by portionwise addition of Amberlite IR-120(H^+) resin. The resin was removed by filtration and thoroughly washed with water (2 x 50ml). The filtrate and washings were evaporated to dryness to provide a foam (7.04g). Crystallisation from water (16ml) provided the *title compound* (17) as a colourless solid (1.267g, 14% yield); HPLC (25x0.46cm 5μ Spherisorb ODS1 eluted with 40% methanol in water for 5min, 40-60% over 5min, and 60% for 10min, at 1.0ml/min) chemical purity >99% a/a at 266nm; m/z (thermospray) 270 (MH^+, 100%); δ_H (400MHz, D_2O) 8.21 (1H, d, 2-H, $J_{C\text{-}H}$=209.2 Hz), 8.33 (1H, s, 8-H), 6.08 (1H, d, 1'-H), 4.77 (1H, t, 2'-H), 4.45 (1H, d of d, 3'-H), 4.29 (1H, m, 4'-H), 3.95-3.83 (2H, m, 5'-CH_2); δ_C (100.6MHz, D_2O) 146.2 (s, 2-^{13}C). The liquors from the crystallisation were evaporated to dryness and the residue purified from uncyclised (16) by preparative HPLC (25x5cm 8μ BDS Hyperprep eluted with 5% methanol in water at 50ml/min, detection at 266nm, 250-300mg/ml injections, R_t(16) 10-12min, R_t(17) 14-16min) to provide a further batch of similarly pure *title compound* (17) (2.25g, 25% yield). This HPLC

system was used in recycle mode only to conserve eluent, as the product peak was collected cleanly after a single pass.

2-[^{13}C]Inosine, 2′,3′,5′-O-triacetate (18)

A mixture of [^{13}C]inosine (17) (3.5g, 13.0mmol), pyridine (21ml) and acetic anhydride (15.7ml) was stirred under nitrogen at 20°C for 24h and then evaporated to dryness. The residue was further dried by addition and evaporation of aliquots of toluene (2x 5ml) and ethanol (2 x 5ml). The resulting crude product was crystallised from ethanol (20ml) to provide the *title compound* (18) as a colourless solid (4.68g, 91%); HPLC (as for (17)) chemical purity >99% a/a at 266nm; m/z (thermospray) 396 (MH^+, 100%); δ_H (400MHz, $CDCl_3$) 12.94 (1H, s, 1-NH), 8.21 (1H, d, 2-H, $J_{C\text{-}H}$ = 207.2 Hz), 7.98 (1H, s, 8-H), 6.16 (1H, d, 1'-H), 5.87 (1H, t, 2'-H), 5.60 (1H, t, 3'-H), 4.46 (1H, m, 4'-H), 4.37 (2H, m, 5'-CH_2), 2.15, 2.14, and 2.10 (9H, 3s, 2'-OAc, 3'-OAc, and 5'-OAc); δ_C (100.6MHz, $CDCl_3$) 145.3 (s, 2-^{13}C).

1-Nitro-2-[^{13}C]inosine, 2′,3′,5′-O-triacetate (19)

Trifluoroacetic anhydride (8.96ml, 64mmol) was added dropwise to a stirred suspension of finely powdered ammonium nitrate (2.56g, 32mmol) in anhydrous dichloromethane (80ml) under nitrogen at 0°C. The vigorously stirred mixture was allowed to warm to 20°C until a clear solution was obtained (*ca.* 1.5h), when it was was cooled to -20°C. A solution of [^{13}C]-inosine triacetate (18) (1.58g, 4mmol) in anhydrous dichloromethane (10ml) was added and the mixture was stirred at -20°C under nitrogen for 2h. The mixture was then diluted with dichloromethane (40ml) and was thoroughly washed with pH 7.0 phosphate buffer (3 x 50ml) until the washings remained neutral. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was triturated with methanol (5ml) to give the *title compound* (19) as a pale yellow solid (686mg, 39%); HPLC (as for (17)) >97% chemical purity at 266nm; δ_H (400MHz, $CDCl_3$) 8.67 (1H, d, 2-H, $J_{C\text{-}H}$ =218.9 Hz), 8.02 (1H, s, 8-H), 6.11 (1H, d, 1'-H), 5.80 (1H, t, 2'-H), 5.53 (1H, t, 3'-H), 4.48 (1H, m, 4'-H), 4.40 (2H, m, 5'-CH_2), 2.16, 2.14, and 2.11 (9H, 3s, 2'-OAc, 3'-OAc, 5'-OAc); δ_C (100.6MHz, $CDCl_3$) 141.33 (s, 2-^{13}C). In a repeat reaction, the crude residue was crystallised from methanol (2ml), washed with methanol (1ml) followed by diethyl

ether (3ml), and dried under high vacuum to give the *title compound* (19) as a pale yellow solid (1.31g, 75%); HPLC (as for (17)) >96% a/a chemical purity at 266nm.

[2-^{13}C, 1-^{15}N]Inosine, 2′,3′,5′-O-triacetate (20)

A solution of 1-nitro-2-[^{13}C]-inosine triacetate (19) (630mg, 1.43mmol) in acetonitrile (22.5ml) was added to a stirred solution of [^{15}N]-ammonium chloride (90mg, 1.65mmol, >98atom% ^{15}N), potassium hydroxide (98mg, 1.5mmol) and triethylamine (203µl,1.5mmol) in water (7.5ml). The yellow solution was stirred at 20° for 3h and then evaporated to dryness. The solid residue was purified by flash chromatography over silica gel (Merck 9385, 100g), eluting with dichloromethane, followed by dichloromethane-methanol (95:5), to provide the *title compound* (20) as a colourless solid (460mg, 81%); HPLC (as for (17)) chemical purity >99% a/a at 266nm; m/z (thermospray) 397 (MH^+, 100%); δ_H (400MHz, d_4-MeOD) 8.08 (1H, d of d, 2-H, J_{C-H} =206.2 Hz, J_{N-H} =7.5Hz), 8.20 (1H, s, 8-H), 6.22 (1H, d, 1'-H), 5.97 (1H, t, 2'-H), 5.66 (1H, t, 3'-H), 4.48-4.33 (3H, m, 4'-H and 5'-CH_2), 2.16-2.04 (9H, 2s, 2'-OAc, 3'-OAc, and 5'-OAc); δ_C (100.6MHz, d_4-MeOD) 147.1 (d, 2-^{13}C, J_{C-N} = 8.7Hz).

6-Chloro-[2-^{13}C, 1-^{15}N]purineriboside, 2′,3′,5′-tri-O-acetate (21)

A stirred solution of 1-[^{15}N]-2-[^{13}C]-inosine triacetate (20) (1.56g, 3.94mmol) in anhydrous dichloromethane (28ml) was treated with thionyl chloride (820µl, 11.24mmol), followed by dimethylformamide (868µl, 11.21mmol). The resulting mixture was heated at reflux for 4.5h. After cooling to 20°, aqueous 8% w/w sodium bicarbonate (56ml) was added and the mixture was stirred for 10min. The mixture was then partitioned between dichloromethane (280ml) and aqueous 8% w/w sodium bicarbonate (280ml). The aqueous layer was separated and extracted with more dichloromethane (2 x 140ml). The combined extracts were dried (Na_2SO_4) and evaporated to dryness to give the *title compound* (21) as an oil (1.815g, 100%); HPLC (as for (17)) chemical purity >97% a/a at 266nm; m/z (thermospray) 415 (MH^+, 100%), 417 (MH^+, 48%); δ_H (400MHz, $CDCl_3$) 8.78 (1H, d of d, 2-H, J_{C-H} =210.4Hz, J_{N-H} =15.8Hz), 8.30 (1H, s, 8-H), 6.24 (1H, d, 1'-H), 5.95 (1H, t, 2'-H),

5.65 (1H, t, 3'-H), 4.52-4.36 (3H, m, 4'-H and 5'-CH_2), 2.16, 2.13, and 2.09 (9H, 3s, 2'-OAc, 3'-OAc, and 5'-OAc).

(±)-(1S,trans)-2-Amino-3,4-[$^2H_2$]cyclopentanol, 4-methylbenzenesulphonate (22)

A solution of the olefinic amine salt (11) (3.06g, 11.3mmol, labile protons first exchanged by evaporation to dryness of its solution in 10ml of deuterium oxide) in deuterium oxide (90ml) was deuterated over platinum black (500mg) at 20° and one atmosphere for 20min. After removal of catalyst by filtration through Hyflo, the solution was evaporated to dryness. The residue was redissolved in absolute ethanol (100ml), and evaporated again. Drying under high vacuum gave the *title compound* (22) as a colourless solid (4.09g, 100%); m/z (thermospray) 104 (MH^+, 100%); δ_H (400MHz, D_2O) 7.70 (2H, d, aromatic H), 7.38 (2H, d, aromatic-H), 4.19 (1H, q, 1"-H), 3.41 (1H, d of d, 2"-H), 2.41 (3H, s, aromatic CH_3), 2.17 (0.65H, t, 3"-H), 2.04 (1H, m, 5"-H), 1.78 (0.9H, m, 4"-H), 1.64 (1.3H, m, 5"-H and 3''-H).

N-[(1S,trans)-2-Hydroxy-3,4-[$^2H_2$]cyclopentyl]-[2-^{13}C, 1-^{15}N]adenosine, 2',3',5'-tri-O-acetate (23)

A stirred mixture of the chloropurine (21) (444mg, 1.07mmol), amine salt (22) (581mg, 1.60mmol) and sodium bicarbonate (354mg, 4.21mmol) in isopropanol (6ml) was heated at reflux under nitrogen. After 5h, more amine salt (22) (387mg, 1.07mmol) and sodium bicarbonate (236mg, 2.81mmol) were added. After a further 2h, the mixture was evaporated to dryness and the residue purified by flash chromatography over silica gel (Merck 9385, 100g). Elution with ethyl acetate-methanol 50:1 gave the *title compound* (23) as a colourless foam (177mg, 34%); m/z (thermospray) 482 (MH^+, 100%). Further elution with ethyl acetate-methanol 9:1, then 4:1, gave monodeacetylated material ['diacetate'] (137mg, 29%); m/z (thermospray) 440 (MH^+, 100%).

N-[(1S,trans)-2-Hydroxy-3,4-[$^2H_2$]cyclopentyl]-[2-^{13}C, 1-^{15}N]adenosine (24)

t-Butylamine (1ml) was added to a stirred solution of the triacetate (23) (168mg, 0.35mmol) in methanol (5ml) at 20°. After 3h, the mixture was evaporated to dryness to give a mixture of two diastereoisomers (~1:1) as a gum (121mg, 97%). A second batch (92mg) was obtained by similar deacetylation of the 'diacetate' (120mg,

0.27mmol) above. The combined batches were purified by preparative HPLC (25x2cm 5μ Spherisorb ODS2 column eluted with water-ethanol 88:12 at 20ml/min, detection at 266nm). Fractions containing the first eluted isomer were combined and evaporated to dryness. The resulting colourless solid (55mg) was crystallised from ethyl acetate (4ml) (initial solution being achieved using methanol as co-solvent, which was mostly removed by distillation) to give, after drying under high vacuum at 60°, the *title compound* (12) as colourless crystals (36mg, 16%); HPLC (as for (17)) chemical purity >99% a/a at 266nm; m/z (thermospray) 356 (MH^+, 100%); δ_H (400MHz, MeOD) 8.24 (1H, d of d, 2-H, $J_{C\text{-}H}$ =201.5Hz, $J_{N\text{-}H}$ =15.5Hz), 8.27 (1H, s, 8-H), 5.95 (1H, d, 1'-H), 4.73 (1H, m, 2'-H), 4.32 (2H, m, 2''-H and 3'-H), 4.16 (1H, m, 4'-H), 4.11 (1H, m, 1''-H), 3.92-3.70 (2H, m, 5'-CH_2), 2.28-1.60 (<4H, m, 5''-CH_2, 4''-H, and 3''-H); δ_C (100.6MHz, d_4-MeOD) 154.0 (s, 2-^{13}C). Evaporation of the mother liquors, followed by trituration of the residue with ethyl acetate and ether, and then drying as above, gave a second crop (17mg, 8%) with similar purity.

Acknowledgements

The authors thank Bob Boughtflower for advice and encouragement with recycling HPLC, Peter Moore and Nisha Mistry for NMR spectra, Mike Turner and Caroline Clegg for mass spectra, David Paul for microanalyses, and Steven Jackson for the HPLC purification of [M+4]GR79236.

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