

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 January 2008 (03.01.2008)

PCT

(10) International Publication Number
WO 2008/000745 A2

(51) International Patent Classification:
C07H 19/16 (2006.01) A61P 25/04 (2006.01)
A61K 31/7076 (2006.01)

(74) Agent: DANIELSSON, Helena; Biovitrum AB,
S-SE-112 76 Stockholm (SE).

(21) International Application Number:
PCT/EP2007/056378

(22) International Filing Date: 26 June 2007 (26.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0601398-1 27 June 2006 (27.06.2006) SE
60/837,146 11 August 2006 (11.08.2006) US

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(71) Applicant (for all designated States except US): BIOVITRUM AB (publ) [SE/SE]; S-SE-112 76 Stockholm (SE).

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

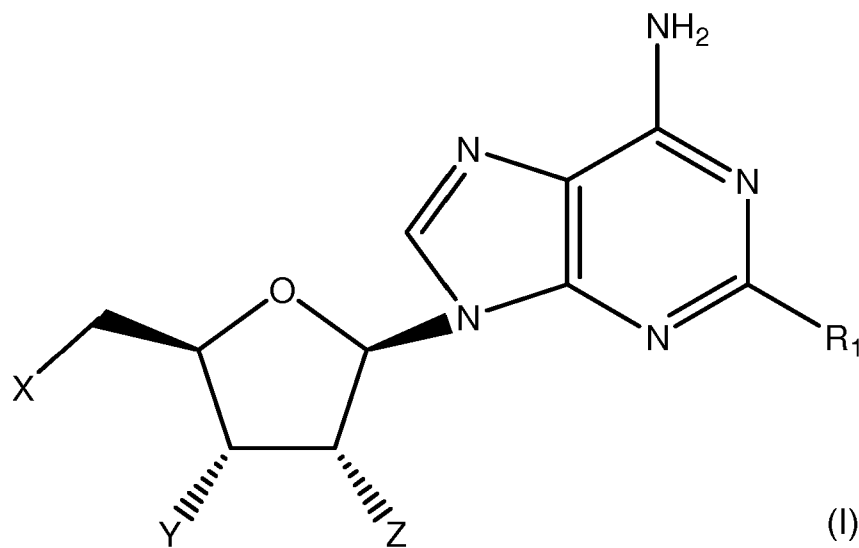
(75) Inventors/Applicants (for US only): HIGGINBOTTOM, Michael [GB/GB]; 6, Goose Cross, Highfields Caldecote, Cambridge Cambridgeshire CB3 7ZL (GB). SAVORY, Edward Daniel [GB/GB]; 23, Mayfield Way, Gt. Cambourne Cambridgeshire CB3 6JA (GB). BROWN, Giles Albert [GB/GB]; 9 Brothers Place, Cambridge Cambridgeshire CB1 8BN (GB). HORGAN, Viet-Anh Anne (nee Nguyen) [FR/GB]; 51 Welland Place, Ely Cambridgeshire CB6 2XA (GB). CHAPMAN, Emma Jane [GB/GB]; 26, York Street, Cambridge Cambridgeshire CB1 2PY (GB).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERAPEUTIC COMPOUNDS



(57) Abstract: Compounds of formula (I) below are disclosed. Their use as medicaments is described, in particular for the treatment of pain or inflammation. In said Formula, when X=Y=Z=OH, R₁ is OCH₂CF₂CF₃, phenoxy (substituted with 3-(4-trifluoromethylphenyl), 3,4-dichloro, (3-trifluoromethyl,4-fluoro), (3-trifluoromethyl,4-chloro), (3-chloro, 4-cyano), or 3,5-bis(trifluoromethyl)), 1-piperazinyl(4-(3,4-dichlorophenyl)), phenyl (substituted with 3,4-dichloro, 3,5-difluoro, 3,5-bis(trifluoromethyl) or 3,4,5-trifluoro) or 2-benzofuranyl; or when X=Y=OH and Z=OME,

R₁ is OCH₃, OCH₂CHF₂, OCH₂cyclopentyl, O-(2,5-difluorophenyl) or (S)-sec-butylamino; or when X=H and Y=Z=OH, R₁ is n-hexylamino or cyclopentylamino; or when (IV) X=Z=OH and Y=H, R₁ is cyclopentylamino; or a pharmaceutically acceptable salt thereof.

WO 2008/000745 A2

THERAPEUTIC COMPOUNDS

TECHNICAL FIELD

5 This invention relates to compounds that are adenosine receptor agonists, and to their use as medicaments, in particular as analgesic or anti-inflammatory compounds, and to methods of preventing, treating, or ameliorating pain or inflammation using these compounds.

10

BACKGROUND ART

Adenosine is a ubiquitous local hormone/neurotransmitter that acts on four known receptors, the adenosine A1, A2A, A2B and A3 receptors. Adenosine generally serves to
15 balance the supply and demand of energy in tissues. For example, in the heart released adenosine slows the heart by an A1 receptor mediated action in the nodes and atria, while simultaneously dilating the coronary artery to increase energy supply. Similarly, during inflammation adenosine serves to inhibit inflammatory activity, while in conditions of excessive nerve activity (such as epilepsy) adenosine inhibits nerve firing. This system, or
20 a variant on it, is present in all tissues.

Adenosine itself can be used to diagnose and treat supraventricular tachycardia. Adenosine A1 receptor agonists are known to act as powerful analgesics (Sawynok, J. Eur J Pharmacol. (1998) 347, 1-11; Giffin *et al*, (2003) 23, 4, 287-292). A2a agonists have
25 recently been shown to give significant pain relief in conditions of increased pain sensitivity (such as neuropathic and inflammatory hyperalgesia) (WO 2004/052377; WO 2004/078183; WO 2004/078184; WO 2005/084653) and are known to have anti-inflammatory activity (see, for example US 5,877,180; WO 99/34804; Linden *et al*, Expert Opin. Investig. Drugs (2005) 14, 7, 797-806; Sitkovsky *et al*, TRENDS in Immunology
30 (2005) 26, 6, 299-304; Linden *et al*, Journal of Immunology (2006) 117, 2765-2769; Cronstein *et al* (2004) 25, 1, 33-39). In experimental animals, A2A receptor agonists have been shown to be effective against a wide variety of conditions including sepsis (Linden *et al*, The Journal of Infectious Diseases (2004) 189, 1897-1904), arthritis (Cohen *et al*, J.

Orthop. Res. (2005) 23, 5, 1172-1178; Cohen *et al*, J. Orthop. Res. (2004) 22, 2, 427-435), and ischaemia/reperfusion injury arising from renal, coronary or cerebral artery occlusion (see, for example Day *et al*, J. Clin. Invest, (2003) 112, 883-891; Linden *et al*, Am. J. Physiol. Gastrointest. Liver Physiol. (2004) 286, G285-G293; Linden *et al*, Am J. Physiol. (1999) 277, F404-F412; Schlack *et al*, J. Cardiovasc. Pharmacol. (1993) 22, 89-96; Zu *et al*, J. Cardiovasc. Pharmacol. (2005) 46, 6, 794-802; Linden *et al*, Am J. Physiol. Heart Circ. Physiol. (2005) 288, 1851-1858; Kennedy *et al*, Current Opinion in Investigational Drugs (2006) 7, 3, 229-242). The common factor in these conditions is a reduction in the inflammatory response caused by the inhibitory effect of this receptor on most, if not all, inflammatory cells. A2a agonists are also known to promote wound healing (Montesinos, Am. J. Pathol. (2002) 160, 2009-2018).

However, the ubiquitous distribution of adenosine receptors means that administration of adenosine receptor agonists causes adverse side effects. This has generally precluded the development of adenosine-based therapies. Selective A1 receptor agonists cause bradycardia. A2A receptor agonists cause widespread vasodilation with consequent hypotension and tachycardia. The first selective A2A receptor agonist (2-[4-(2-carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine, or CGS21680), was tested in a Phase 2A clinical trial as a potential anti-hypertensive. However, administration of this compound caused a large fall in blood pressure and consequent increase in cardiac output. This has prevented use of CGS21680 as a medicament. Webb *et al*. (J. Pharmacol Exp Ther (1991) 259, 1203-1212), Casati *et al*. (J Pharmacol Exp Ther (1995) 275(2):914-919), and Bonnizone *et al*. (Hypertension. (1995) 25, 564-9) show that selective A2A adenosine receptor agonists cause hypotension and tachycardia. The degree of tachycardia induced is sufficient to preclude their use as medicaments. Alberti *et al*. (J Cardiovasc Pharmacol. 1997 Sep;30(3):320-4) discloses that selective A2A adenosine receptor agonists are potent vasodilators that reduce blood pressure and induce marked increments in heart rate and plasma renin activity. These side effects preclude their use as medicaments.

30

US 5,877,180 relates to agonists of A2A adenosine receptors which are stated to be effective for the treatment of inflammatory diseases. The preferred agonists, WRC0090 and SHA 211 (WRC0474), are disclosed to be more potent and selective than previously

reported adenosine analogs such as CGS21680 and CV1808. Administration of SHA 211 or WRC0090 is considered to reduce the possibility of side effects mediated by the binding of the analogs to other adenosine receptors. However, only *in vitro* data relating to the activity of SHA 211 is included. There is no demonstration that any of the compounds described could be therapeutically effective *in vivo* without causing serious side effects. Although side effects mediated by the binding of potent and selective adenosine A2A receptor agonists to other adenosine receptors is expected to be reduced by use of such agonists, the ubiquitous distribution of adenosine receptors means that these compounds would still be expected to activate adenosine A2A receptors in normal tissue and, therefore, cause serious side effects (such as hypotension and reflex tachycardia).

Ribeiro *et al.* (Progress in Neurobiology 68 (2003) 377-392) is a review of adenosine receptors in the nervous system. It is stated in the concluding remarks of this article (on page 387, right column, lines 4-10 of section 8) that “as noted a long time ago, activation of adenosine receptors at the periphery is associated with hypotension, bradycardia and hypothermia ... These side effects have so far significantly limited the clinical usefulness of adenosine receptor agonists”.

There is, therefore, a need to provide adenosine receptor agonists that can be administered with minimal side effects.

There is also a need to provide analgesics for the treatment of pain. Pain has two components, each involving activation of sensory neurons. The first component is the early or immediate phase when a sensory neuron is stimulated, for instance as the result of heat or pressure on the skin. The second component is the consequence of an increased sensitivity of the sensory mechanisms innervating tissue which has been previously damaged. This second component is referred to as hyperalgesia, and is involved in all forms of chronic pain arising from tissue damage, but not in the early or immediate phase of pain perception.

Thus, hyperalgesia is a condition of heightened pain perception caused by tissue damage. This condition is a natural response of the nervous system apparently designed to encourage protection of the damaged tissue by an injured individual, to give time for tissue

repair to occur. There are two known underlying causes of this condition, an increase in sensory neuron activity, and a change in neuronal processing of nociceptive information which occurs in the spinal cord. Hyperalgesia can be debilitating in conditions of chronic inflammation (e.g. rheumatoid arthritis), and when sensory nerve damage has occurred (i.e. neuropathic pain).

Two major classes of analgesics are known: (i) non steroidal anti-inflammatory drugs (NSAIDs) and the related COX-2 inhibitors; and (ii) opiates based on morphine. Analgesics of both classes are effective in controlling normal, immediate or nociceptive pain. However, they are less effective against some types of hyperalgesic pain, such as neuropathic pain. Many medical practitioners are reluctant to prescribe opiates at the high doses required to affect neuropathic pain because of the side effects caused by administration of these compounds (such as restlessness, nausea, and vomiting), and the possibility that patients may become addicted to them. NSAIDs are much less potent than opiates, so even higher doses of these compounds are required. However, this is undesirable because these compounds cause irritation of the gastro-intestinal tract.

There is, therefore, a need to provide analgesics, particularly anti-hyperalgesics, that are sufficiently potent to control pain perception in neuropathic and other hyperalgesic syndromes, and which do not have serious side effects or cause patients to become addicted to them.

Spongiosine (also known as 2-methoxyadenosine) is known to be a weak, non-selective adenosine receptor agonist (Ueeda et al J Med Chem (1991) 34, 1334-1339). This compound caused 25% inhibition of carageenan-induced inflammation in rats at 20 mg/kg po. However, reductions in mean blood pressure (41%), and in heart rate (25%) were also observed after administration of this compound at this dose (Bartlett *et al.* (J. Med. Chem. (1981) 24, 947-954)).

The applicant has previously found that spongiosine surprisingly is an effective analgesic at doses as much as one hundred times lower than would be expected to be required to have an analgesic effect based on the known affinity of this compound for adenosine receptors. At these doses, spongiosine does not cause the significant side effects associated with

higher doses of this compound, or other adenosine receptor agonists. Thus, the therapeutic effects of spongosine can be separated from its side effects. The activity of spongosine as an analgesic is the subject of International patent application no. PCT/GB03/05379, and the activity of compounds related to spongosine as analgesics is the subject of International patent application no. PCT/GB04/00935. Use of spongosine and related compounds to treat inflammation and other disorders is the subject of International patent application no. PCT/GB04/000952.

The Applicant has found that spongosine, and the related compounds described in PCT/GB04/00935 and PCT/GB04/000952, have increased affinity for adenosine receptors at pH below pH 7.4. It is believed that this property explains the surprising activity of these compounds at low doses.

The Applicant has found, however, that for some substituted adenosines which have increased affinity for adenosine receptors at pH below pH 7.4, closely related compounds do not retain this desired activity. This has made it extremely difficult to identify additional substituted adenosines that may be used as medicaments without causing serious side effects, since it has not been possible to predict which particular substituted adenosines will have increased affinity for adenosine receptors at reduced pH. By way of illustration of this unpredictability, the Table below gives the K_i (nM) values at rat adenosine A_{2a} receptors at pH 5.5 and 7.4 for compounds of a 2-aminoalkyl adenosine series and a 5'-amido adenosine series (these values were calculated using similar binding experiments to those described in relation to Example 1 below):

2-Aminoalkyl adenosines		
Structure	(K _i) nM	(K _i) nM
	(pH 5.5)	(pH 7.4)
NHCH ₃	24	1356
NHCH ₂ CH ₃	130	1200
NHCH ₂ CH ₂ CH ₃	1900	1900
NHCH ₂ CH ₂ CH ₂ CH ₃	47	17
NHCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	0.7	290

5'-Amido adenosines		
Structure	(Ki) nM	(Ki) nM
	(pH 5.5)	(pH 7.4)
CONH ₂	9.4	270
CONHCH ₃	11	55
CONHCH ₂ CH ₃	5.1	5.1
CONHcyclopropyl	4.3	4.3
CONHCH(CH ₃) ₂	4.6	1900
CONHCH ₂ CH ₂ CH ₃	35	35
CONHCH ₂ CH ₂ CH ₂ CH ₃	24	21

Only certain compounds in each series in the above table have increased affinity for adenosine receptors at reduced pH. In the 2-aminoalkyl series, as the alkyl chain length is increased to 3 or 4 carbons, the desired activity is lost, but recovered when the chain length is increased to 6 carbons. In the 5'-amido series, as the alkyl chain length is increased to 2 or more carbons, the desired activity is lost, with the unpredictable exception of the NHisopropyl amide which is more than 400 fold more active at pH 5.5 compared to pH 7.4.

10

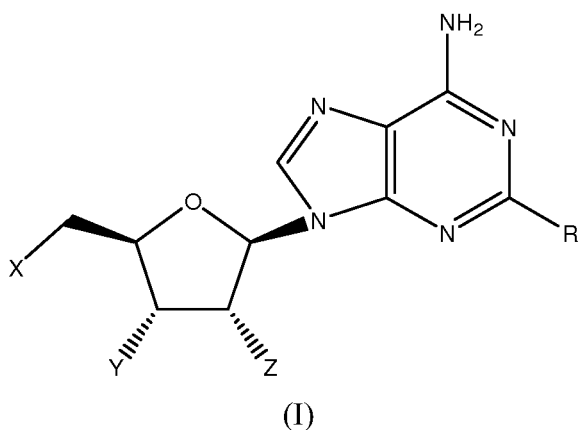
In spite of the difficulty in identifying additional substituted adenosines with increased affinity for adenosine receptors at reduced pH from the many millions of possible compounds, the Applicant has now identified certain other compounds that also have increased affinity for adenosine receptors at reduced pH. It is thought that these compounds can be used as medicaments without causing serious side effects.

15

DISCLOSURE OF THE INVENTION

According to the invention there are provided adenosine receptor agonists of the following formula:

5



wherein:

10

when $X=Y=Z=OH$, R_1 is $OCH_2CF_2CF_3$, phenoxy (substituted with 3-(4-trifluoromethylphenyl), 3,4-dichloro, (3-trifluoromethyl,4-fluoro), (3-trifluoromethyl,4-chloro), (3-chloro, 4-cyano), or 3,5-bis(trifluoromethyl)), 1-piperazinyl(4-(3,4-dichlorophenyl)), phenyl (substituted with 3,4-dichloro, 3,5-difluoro, 3,5-bis(trifluoromethyl) or 3,4,5-trifluoro) or 2-benzofuranyl; or

15

when $X=Y=OH$ and $Z=OMe$, R_1 is OCH_3 , OCH_2CHF_2 , OCH_2 cyclopentyl, O -(2,5-difluorophenyl) or (*S*)-sec-butylamino; or

when $X=H$ and $Y=Z=OH$, R_1 is n-hexylamino or cyclopentylamino; or

when $X=Z=OH$ and $Y=H$, R_1 is cyclopentylamino.

20

or a pharmaceutically acceptable salt thereof.

For the avoidance of doubt, reference to compounds of formula (I) below includes pharmaceutically acceptable salts of the compounds of formula (I).

25

Compounds of the invention are all believed to have increased affinity for adenosine receptors at pH below pH 7.4. In normal mammalian tissues extracellular pH is tightly

regulated between pH 7.35 and 7.45. Some tissues experience lower pH values, particularly the lumen of the stomach (pH between 2 and 3) and the surfaces of some epithelia (for example, the lung surface pH is approximately 6.8). In pathological tissues, for example during inflammation, ischaemia and other types of damage, a reduction in pH
5 occurs.

Because of the increased affinity of compounds of the invention for adenosine receptors at reduced pH, it is thought that the actions of these compounds can be targeted to regions of low pH, such as pathological tissues. Consequently, the doses of these compounds that are
10 required to give therapeutic effects are much lower than would be expected based on their affinity for adenosine receptors at normal extracellular physiological pH. Since only low doses of the compounds are required, the serious side effects associated with administration of adenosine receptor agonists are avoided or minimised. This has the surprising consequence (contrary to the teaching in the art, for example in US 5,877,180)
15 that some adenosine receptor agonists that are low affinity and/or non-selective agonists at physiological pH (such as spongosine) can be therapeutically effective without causing serious side effects.

It is believed that compounds of formula (I) can be administered at doses well below those
20 expected to be required based on their affinity for adenosine receptors at pH 7.4, and cause therapeutic effects at these doses without causing serious side effects.

Thus, according to the invention there is provided a compound of the invention for use as a
25 medicament.

Any pathological condition that can be prevented or improved by agonism of adenosine A2A receptors can be prevented, treated, or ameliorated by a compound of formula (I).

According to the invention there is provided use of a compound of formula (I) in the
30 manufacture of a medicament for the prevention, treatment, or amelioration of a pathological condition that can be improved or prevented by agonism of adenosine A2A receptors.

There is also provided according to the invention a method of prevention, treatment, or amelioration of a pathological condition that can be improved or prevented by agonism of adenosine A2A receptors, which comprises administering a compound of formula (I) to a subject in need of such prevention, treatment, or amelioration.

5

A person of ordinary skill in the art can readily test whether or not a pathological condition that is prevented, treated, or ameliorated by a compound of formula (I) is acting via adenosine A2A receptors. For example, this may be done by comparing the effect of the compound in an animal model of the pathological condition in the presence and absence of a selective antagonist of an adenosine A2A receptor. If the effect of the compound in the presence of the antagonist is reduced or absent compared with the effect of the compound in the absence of the antagonist, it is concluded that the compound is exerting its effect via an adenosine A2A receptor. Antagonists of adenosine A2A receptors are known to those of ordinary skill in the art (see for example Ongini *et al.*, *Farmacology* 2001 Jan-Feb;56(1-2):87-90).

15

Alternatively, an adenosine A2A receptor knockout mouse may be used (Ohta A and Sitkovsky M, *Nature* 2001; 414:916-20). For example, the effect of the compound on a mouse that has symptoms of the pathological condition is compared with its effect on an adenosine A2A knockout mouse that has corresponding symptoms. If the compound is only effective in the mouse that has adenosine A2A receptors it is concluded that the compound is exerting its effect via adenosine A2A receptors.

20

It is believed that compounds of formula (I) have analgesic and/or anti-inflammatory activity and can be administered with reduced probability and severity of side effects compared to other adenosine receptor agonists.

25

According to the invention there is provided use of a compound of formula (I) in the manufacture of a medicament for the prevention, treatment, or amelioration of pain, particularly hyperalgesia. There is also provided according to the invention a method of preventing, treating, or ameliorating pain (particularly hyperalgesia) which comprises administering a compound of formula (I) to a subject in need of such prevention, treatment, or amelioration.

30

Methods delineated herein include those wherein the subject is identified as in need of a particular stated treatment. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or
5 objective (e.g. measurable by a test or diagnostic method).

Compounds of formula (I) are believed to be effective in inhibiting pain perception in mammals suffering from pain, in particular neuropathic or inflammatory pain, even when administered at doses expected to give plasma concentrations well below those known to
10 activate adenosine receptors. Therefore, it is believed that compounds of formula (I) can treat pain (particularly neuropathic and inflammatory pain) without causing the significant side effects associated with administration of other adenosine receptor agonists.

As mentioned above hyperalgesia is a consequence in most instances of tissue damage,
15 either damage directly to a sensory nerve, or damage of the tissue innervated by a given sensory nerve. Consequently, there are many conditions in which pain perception includes a component of hyperalgesia.

According to the invention there is provided use of a compound of formula (I) as an
20 analgesic (particularly an anti-hyperalgesic) for the prevention, treatment, or amelioration of pain (particularly hyperalgesia) caused as a result of neuropathy, including Diabetic Neuropathy, Polyneuropathy, Cancer Pain, Fibromyalgia, Myofascial Pain Syndrome, Osteoarthritis, Pancreatic Pain, Pelvic/Perineal pain, Post Herpetic Neuralgia, Rheumatoid Arthritis, Sciatica/Lumbar Radiculopathy, Spinal Stenosis, Temporo-mandibular Joint
25 Disorder, HIV pain, Trigeminal Neuralgia, Chronic Neuropathic Pain, Lower Back Pain, Failed Back Surgery pain, back pain, post-operative pain, post physical trauma pain (including gunshot, road traffic accident, burns), Cardiac pain, Chest pain, Pelvic pain/PID, Joint pain (tendonitis, bursitis, acute arthritis), Neck Pain, Bowel Pain, Phantom Limb Pain, Obstetric Pain (labour/C-Section), Renal Colic, Acute Herpes Zoster Pain, Acute
30 Pancreatitis Breakthrough Pain (Cancer), Dysmenorrhoea/Endometriosis; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.

According to the invention there is also provided use of a compound of formula (I) as an analgesic (particularly and anti-hyperalgesic) for the prevention, treatment, or amelioration of pain (particularly hyperalgesia) caused as a result of inflammatory disease, or as a result of combined inflammatory, autoimmune and neuropathic tissue damage, including

5 rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis, and other arthritic conditions, cancer, HIV, Chronic Obstructive Pulmonary Disease (COPD), acute bronchitis, chronic bronchitis, emphysema, bronchiectasis, cystic fibrosis, pneumonia, pleurisy, acute asthma, chronic asthma, acute respiratory distress syndrome, adult respiratory distress syndrome (ARDS), infant respiratory distress syndrome (IRDS) acute

10 lung injury (ALI), laryngitis, pharyngitis, persistent asthma, chronic asthmatic bronchitis, interstitial lung disease, lung malignancies, alpha-anti-trypsin deficiency, bronchiolitis obliterans, sarcoidosis, pulmonary fibrosis, collagen vascular disorders, allergic rhinitis, nasal congestion, status asthmaticus, smoking related pulmonary disease, pulmonary hypertension, pulmonary oedema, pulmonary embolism, pleural effusion, pneumothorax,

15 haemothorax, lung cancer, allergies, pollinosis Hay fever), sneeze, vasomotor rhinitis, mucositis, sinusitis, exogenous irritant induced illness (SO₂, smog, pollution), airway hypersensitivity, milk product intolerance, Luffer's pneumonia, pneumoconiosis, collagen induced vascular disease, granulomatous disease, bronchial inflammation, chronic pulmonary inflammatory disease, bone resorption diseases, reperfusion injury (including

20 damage caused to organs as a consequence of reperfusion following ischaemic episodes e.g. myocardial infarcts, strokes), autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis) graft v. host rejection, allograft rejections, fever and myalgia due to infection, AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome,

25 osteoporosis, cerebral malaria and bacterial meningitis, bowel pain, cancer pain, back pain, fibromyalgia, post-operative pain; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.

According to the invention there is also provided use of a compound of formula (I) for the

30 prevention, treatment, or amelioration of ischaemic pain. The term "ischaemic pain" is used herein to mean pain associated with a reduction in blood supply to a part of the body. A reduced blood supply limits the supply of oxygen (hypoxia) and energy to that part of

the body. Ischaemia arises from poor blood perfusion of tissues and so ischaemic pain arises in coronary artery disease, peripheral artery disease, and conditions which are characterized by insufficient blood flow, usually secondary to atherosclerosis. Other vascular disorders can also result in ischaemic pain. These include: left ventricular hypertrophy, coronary artery disease, essential hypertension, acute hypertensive emergency, cardiomyopathy, heart insufficiency, exercise tolerance, chronic heart failure, arrhythmia, cardiac dysrhythmia, syncope, arteriosclerosis, mild chronic heart failure, angina pectoris, Prinzmetal's (variant) angina, stable angina, and exercise induced angina, cardiac bypass reocclusion, intermittent claudication (arteriosclerosis obliterans), arteritis, diastolic dysfunction and systolic dysfunction, atherosclerosis, post ischaemia/reperfusion injury, diabetes (both Types I and II), thromboembolisms. Haemorrhagic accidents can also result in ischaemic pain. In addition poor perfusion can result in neuropathic and inflammatory pain arising from hypoxia-induced nerve cell damage (e.g. in cardiac arrest or bypass operation, diabetes or neonatal distress); or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.

There is further provided according to the invention use of a compound of formula (I) for the manufacture of a medicament for the prevention, treatment, or amelioration of inflammation. There is further provided according to the invention a method of prevention, treatment, or amelioration of inflammation, which comprises administering a compound of formula (I) to a subject in need of such prevention, treatment, or amelioration.

In particular, it is believed that compounds of formula (I) can be used to prevent, treat, or ameliorate inflammation caused by, or associated with: cancer (such as leukemias, lymphomas, carcinomas, colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, hepatic, lung, breast, and prostate metastases, etc.); auto-immune disease (such as organ transplant rejection, lupus erythematosus, graft v. host rejection, allograft rejections, multiple sclerosis, rheumatoid arthritis, type I diabetes mellitus including the destruction of pancreatic islets leading to diabetes and the inflammatory consequences of diabetes); autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis); obesity; cardiovascular conditions associated with poor tissue perfusion and inflammation (such as atheromas, atherosclerosis, stroke, ischaemia-reperfusion injury, claudication, spinal cord injury,

congestive heart failure, vasculitis, haemorrhagic shock, vasospasm following subarachnoid haemorrhage, vasospasm following cerebrovascular accident, pleuritis, pericarditis, the cardiovascular complications of diabetes); ischaemia-reperfusion injury, ischaemia and associated inflammation, restenosis following angioplasty and inflammatory aneurysms; epilepsy, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp (particularly athletes' cramp), arthritis (such as rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis), fibrosis (for example of the lung, skin and liver), multiple sclerosis, sepsis, septic shock, encephalitis, infectious arthritis, Jarisch-Herxheimer reaction, shingles, toxic shock, cerebral malaria, Lyme's disease, endotoxic shock, gram negative shock, haemorrhagic shock, hepatitis (arising both from tissue damage or viral infection), deep vein thrombosis, gout; conditions associated with breathing difficulties (e.g. impeded and obstructed airways, bronchoconstriction, pulmonary vasoconstriction, impeded respiration, silicosis, pulmonary sarcosis, pulmonary hypertension, pulmonary vasoconstriction, bronchial allergy and vernal conjunctivitis); conditions associated with inflammation of the skin (including psoriasis, eczema, ulcers, contact dermatitis); conditions associated with inflammation of the bowel (including Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, inflammatory bowel disease); HIV (particularly HIV infection), cerebral malaria, bacterial meningitis, TNF-enhanced HIV replication, TNF inhibition of AZT and DDI activity, osteoporosis and other bone resorption diseases, osteoarthritis, rheumatoid arthritis, infertility from endometriosis, fever and myalgia due to infection, cachexia secondary to cancer, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, adverse effects from amphotericin B treatment, adverse effects from interleukin-2 treatment, adverse effects from OKT3 treatment, or adverse effects from GM-CSF treatment, and other conditions mediated by excessive anti-inflammatory cell (including neutrophil, eosinophil, macrophage and T- cell) activity; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.

30

Continuous low grade inflammation is known to be associated with obesity (in the presence or absence of insulin resistance and Type II diabetes) (Browning et al (2004) *Metabolism* 53, 899-903, Inflammatory markers elevated in blood of obese women;

Mangge *et al.* (2004) *Exp Clin Endocrinol Diabetes* 112, 378-382, Juvenile obesity correlates with serum inflammatory marker C-reactive protein; Maachi *et al.*, *Int J Obes Relat Metab Disord.* 2004 28, 993-997, Systemic low grade inflammation in obese people). A possible reason for this is that fat cells secrete TNF alpha and interleukins 1 and 6, which are pro-inflammatory.

Compounds of the invention that are selective agonists of adenosine A2A receptors are particularly preferred because it is believed that such compounds will have strong anti-inflammatory activity. By selective agonists of adenosine A2A receptors is meant agonists that activate adenosine A2A receptors at concentrations that are lower (preferably one thousandth to one fifth) than required to activate adenosine A1 receptors. Furthermore, A1 receptors have pro-inflammatory activity, so such effects are expected to be minimised for compounds that are selective for A2A receptors.

Compounds of formula (I) are believed to be much more effective at low doses than other adenosine receptor agonists. Thus, it is expected that compounds of the invention can be effectively administered at doses at which they have reduced probability and severity of side effects, or at which side effects are not observed. Such compounds provide significant advantages over other adenosine receptor agonists which only have anti-inflammatory effects at the same concentrations at which serious side effects are observed.

It is also believed that compounds of formula (I) may be effective as disease-modifying anti-rheumatic drugs (DMARDs), in particular for use in the prevention, treatment, or amelioration of rheumatoid arthritis, and possibly other arthropathies such as osteoarthritis.

Medications used to treat rheumatoid arthritis (RA) can be divided into two groups: those that help relieve RA symptoms; and those that help modify the disease. Drugs that help to relieve RA symptoms include nonsteroidal anti-inflammatory drugs (NSAIDs) that relieve pain and reduce inflammation in the affected joints, analgesics (such as acetaminophen and narcotic pain medications) that relieve pain but do not slow joint damage or reduce inflammation, and corticosteroids that are anti-inflammatory drugs.

DMARDs help to improve RA symptoms (such as joint swelling and tenderness), but also slow the progression of joint damage caused by RA. Thus, while there is no cure for RA, DMARDs help to slow the progression of RA. In the past DMARDs were usually used to treat RA after NSAID therapy failed. However, DMARDs are now beginning to be used
5 earlier in the course of RA because studies have suggested that early intervention with DMARDs offers important benefits. DMARDs and NSAIDs are often used in combination with each other.

Results from clinical studies have shown that known DMARDs slow the progression of RA. After 6 months of treatment, the rate of bone and cartilage damage had already started
10 to slow in patients' joints. After 1 year, patients showed very little progression of joint damage, and after 2 years X rays showed that few patients in the study had newly damaged joints during the second year of treatment.

Examples of known DMARDs include sulphasalazine, penicillamine, chloroquine, hydroxychloroquine, gold (by intramuscular injection or orally as auranofin), methotrexate,
15 cyclosporin, azathioprine, cyclophosphamide, leflunomide. More recently biological DMARDs have been developed which inhibit tumour necrosis factor alpha (TNF alpha). One example is Humira® which is indicated for reducing signs and symptoms and inhibiting the progression of structural damage in adults with moderately to severely active RA who have had an inadequate response to one or more DMARDs. Humira® is an anti-
20 TNF alpha antibody.

Many of the known DMARDs cause serious side effects. Consequently, it is desired to provide new DMARDs that can be administered with minimal side effects.

25 WO 2005/084653 shows the ability of spongiosine to reduce phorbol ester induced TNF alpha release in U937 human macrophage cells. On this basis, it is believed that spongiosine and related compounds of the invention also have DMARD activity.

According to the invention there is provided use of a compound of formula (I) in the
30 manufacture of a medicament for slowing the progression of arthropathy. There is also

provided according to the invention a method of slowing the progression of arthropathy, which comprises administering a compound of formula (I) to a subject in need thereof.

5 Preferably the progression of RA is slowed, and in particular the progression of joint damage caused by RA. A compound of the invention may be administered to the subject at any stage in the course of RA. A compound of the invention may be administered in combination with one or more NSAIDs or other DMARDs.

10 Compounds of the invention are believed to be effective as DMARDs even when administered at doses expected to give plasma concentrations well below those known to activate adenosine receptors. At these doses, it is believed that the compounds do not cause the significant side effects associated with administration of higher doses of spongiosine, or other adenosine receptor agonists.

15 A particular advantage of use of compounds of the invention as DMARDs is that it is believed that they will be orally active, in contrast to anti-TNF alpha antibodies which must be injected.

20 It has also been appreciated that compounds of formula (I) may be effective in preventing, treating, or ameliorating macro and micro vascular complications of type 1 or 2 diabetes (including retinopathy, nephropathy, autonomic neuropathy), or blood vessel damage caused by ischaemia (either diabetic or otherwise) or atherosclerosis (either diabetic or otherwise).

25 According to the invention, there is provided use of a compound of formula (I) in the manufacture of a medicament for the prevention, treatment, or amelioration of macro or micro vascular complications of type 1 or 2 diabetes, retinopathy, nephropathy, autonomic neuropathy, or blood vessel damage caused by ischaemia or atherosclerosis. According to the invention there is also provided a method of preventing, treating, or ameliorating macro
30 or micro vascular complications of type 1 or 2 diabetes, retinopathy, nephropathy, autonomic neuropathy, or blood vessel damage caused by ischaemia or atherosclerosis, in a subject in need of such prevention, treatment, or amelioration, which comprises administering a compound of formula (I) to the subject.

Compounds of formula (I) are believed to be effective in prevention, treatment, or amelioration of macro or micro vascular complications of type 1 and 2 diabetes, including retinopathy, nephropathy, autonomic neuropathy, or blood vessel damage caused by ischaemia or atherosclerosis (either diabetic or otherwise) even when administered at doses expected to give plasma concentrations well below those known to activate adenosine receptors. At these doses, it is believed that the compounds do not cause the significant side effects associated with administration of higher doses of spongiosine, or other adenosine receptor agonists.

5

Compounds of formula (I) are also believed to be effective in the promotion of wound healing. According to the invention there is provided use of a compound of formula (I) in the manufacture of a medicament for the promotion of wound healing. There is also provided according to the invention a method of promoting wound healing in a subject, which comprises administering a compound of formula (I) to the subject.

10

The amount of a compound of formula (I) that is administered to a subject is preferably an amount which gives rise to a peak plasma concentration of the compound that is less than the EC50 value of the compound at adenosine receptors (preferably at pH 7.4).

15

Thus, preferably the amount of a compound of the invention that is administered to a subject should be an amount which gives rise to a peak plasma concentration that is less than the EC50 value of the compound at adenosine receptors.

20

Preferably the peak plasma concentration of the compound is one ten thousandth to one half (or one ten thousandth to one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or one fiftieth to one third, or one fiftieth to one half, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the EC50 value.

25

30

Preferably the amount of a compound of the invention that is administered gives rise to a plasma concentration that is maintained for more than one hour at one ten thousandth to one half (or one ten thousandth to one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or one fiftieth to one half, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the EC50 value of the compound at adenosine receptors.

10 For the avoidance of doubt, the EC50 value of a compound is defined herein as the concentration of the compound that provokes a receptor response halfway between the baseline receptor response and the maximum receptor response (as determined, for example, using a dose-response curve).

15 The EC50 value should be determined under standard conditions (balanced salt solutions buffered to pH 7.4). For EC50 determinations using isolated membranes, cells and tissues this would be in buffered salt solution at pH 7.4 (e.g. cell culture medium), for example as in Daly *et al.*, Pharmacol. (1993) 46, 91-100), or preferably as in Tilburg *et al* (J. Med. Chem. (2002) 45, 91-100). The EC50 could also be determined *in vivo* by measuring
20 adenosine receptor mediated responses in a normal healthy animal, or even in a tissue perfused under normal conditions (i.e. oxygenated blood, or oxygenated isotonic media, also buffered at pH 7.4) in a normal healthy animal.

It will be appreciated that the EC50 value of the compound is likely to be different for
25 different adenosine receptors (i.e. the A1, A2A, A2B, A3 adenosine receptors). The amount of the compound that is to be administered should be calculated relative to the lowest EC50 value of the compound at the different receptors.

Alternatively, the amount of a compound of the invention that is administered may be an
30 amount that gives rise to a peak plasma concentration that is less than the lowest Kd value of the compound at adenosine receptors (i.e. less than the lowest Kd value of the compound at A1, A2A, A2B, and A3 adenosine receptors). Preferably the peak plasma concentration of the compound is one ten thousandth to one half (or one ten thousandth to

one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one third, or one thousandth to one fifth, or one thousandth to one twentieth, or one
5 fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or one fiftieth to one half, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the lowest Kd value.

Preferably the amount of the compound that is administered is an amount that gives rise to a plasma concentration that is maintained for more than one hour at one ten thousandth to
10 one half (or one ten thousandth to one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or
15 one fiftieth to one half, or one fiftieth to one fifth, or one fiftieth to one third, or one tenth to one half, or one tenth to one fifth) of the lowest Kd value of the compound at adenosine receptors.

The Kd value of the compound at each receptor should be determined under standard conditions using plasma membranes as a source of the adenosine receptors derived either
20 from tissues or cells endogenously expressing these receptors or from cells transfected with DNA vectors encoding the adenosine receptor genes. Alternatively whole cell preparations using cells expressing adenosine receptors can be used. Labelled ligands (e.g. radiolabelled) selective for the different receptors should be used in buffered (pH 7.4) salt solutions (see e.g. Tilburg et al, J. Med. Chem. (2002) 45, 420-429) to determine the
25 binding affinity and thus the Kd of the compound at each receptor.

Alternatively, the amount of a compound of the invention that is administered may be an amount that is one ten thousandth to one half (or one ten thousandth to one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth
30 to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or one fiftieth to one half, or one fiftieth to one third, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the minimum

amount of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered.

- 5 Preferably the amount administered gives rise to a plasma concentration that is maintained for more than one hour at one ten thousandth to one half (or one ten thousandth to one fifth, or one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth
- 10 to one half, or one hundredth to one fifth, or one fiftieth to one half, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the minimum plasma concentration of the compound that gives rise to the side effects.

The appropriate dosage of a compound of the invention will vary with the age, sex, weight, and condition of the subject being treated, the potency of the compound (such as its EC50

15 value for an adenosine receptor), its half life, its absorption by the body, and the route of administration, etc. However, the appropriate dosage can readily be determined by one skilled in the art.

- 20 A suitable way to determine the appropriate dosage is to assess cardiovascular changes (for example by ecg and blood pressure monitoring) at or around the EC50 value of the compound for an adenosine receptor (preferably the receptor for which it has highest affinity) to determine the maximum tolerated dose. The therapeutically effective dose is then expected to be one ten thousandth to one half (or one ten thousandth to one fifth, or
- 25 one ten thousandth to one twentieth, or one ten thousandth to one hundredth, or one ten thousandth to one thousandth, or one thousandth to one half, or one thousandth to one fifth, or one thousandth to one twentieth, or one fiftieth to one tenth, or one hundredth to one half, or one hundredth to one fifth, or one fiftieth to one half, or one fiftieth to one third, or one fiftieth to one fifth, or one tenth to one half, or one tenth to one fifth) of the maximum
- 30 tolerated dose.

Example 23 below shows how a suitable dosage range for compounds of the invention may be determined. Spongosine is the compound used in this example, but it will be appreciated

that similar methods may be used for compounds of the invention. The preferred dosage of spongosine was determined to be less than 28 mg in humans. This dosage gives rise to plasma concentrations between 0.5 and 0.9 μM (close to the K_d at adenosine A2A receptors at pH 7.4). Based on this result, a preferred dosage range for spongosine is 0.03
5 to 0.3 mg/kg.

The minimum plasma concentration of spongosine giving maximal analgesic relief in a rat adjuvant model of arthritis was 0.06 μM , considerably less than the EC_{50} of spongosine at the adenosine A2A receptor which is approximately 1 μM . Preferred dosing levels in
10 humans give maximum plasma concentrations between 0.005 and 0.5 μM which are significantly lower than those expected to provide an analgesic or an anti-inflammatory effect by an action on this receptor.

Alternatively, appropriate therapeutic concentrations of compounds of the invention are
15 expected to be approximately 10-20 times the K_i for an adenosine receptor (the receptor for which the compound has the highest affinity) at pH 5.5.

It is expected that the amount of a compound of the invention that is administered should be 0.001-15 mg/kg. The amount may be up to 10, 5, 2, 1, 0.5, 0.2, 0.1, or 0.01 mg/kg. The
20 amount may be at least 0.001, 0.01, 0.1, 0.2, 0.5, 1, 2, 5, or 10 mg/kg. Preferred ranges are 0.001-10, 0.001-5, 0.001-2, 0.001-1, 0.001-0.1, 0.001-0.01, 0.01-15, 0.01-10, 0.01-5, 0.01-2, 0.01-1, 0.1-10, 0.1-5, 0.1-2, 0.1-1, 0.1-0.5, 0.1-0.4, 0.2-15, 0.2-10, 0.2-5, 0.2-2, 0.2-1.2, 0.2-1, 0.6-1.2 mg/kg.

25 Preferred doses for a human subject (for example a 70kg subject) are less than 420mg, preferably less than 28mg, more preferably less than 21mg, and preferably at least 0.07, 0.1, 0.7, or 0.8 mg, more preferably at least 3.5 or 7mg. More preferably 7-70mg, 14-70mg, or 3.5-21mg.

30 It is believed that the dosage amounts specified above are significantly lower (up to approximately 1000 times lower) than would be expected to be required for an analgesic or

an anti-inflammatory effect based on the EC50 value of the compound at the adenosine A2A receptor.

5 Particularly preferred dosage amounts give rise to plasma concentrations that are approximately one hundredth to one half of the EC50 value of the compound at the adenosine receptor for which it has highest affinity.

10 A compound of the invention may be administered with or without other therapeutic agents, for example analgesics, anti-hyperalgesics (such as gabapentin, pregabalin, cannabinoids, sodium or calcium channel modulators, anti-epileptics or anti-depressants) anti-inflammatories (such as opiates, steroids, NSAIDs, cannabinoids, tachykinin modulators, or bradykinin modulators), DMARDs, or anti-pathogenic agents.

15 In general, a compound of the invention may be administered by known means, in any suitable formulation, by any suitable route. A compound of the invention is preferably administered orally, parenterally, sublingually, transdermally, intrathecally, or transmucosally. Other suitable routes include intravenous, intramuscular, subcutaneous, inhaled, and topical. The amount of drug administered will typically be higher when administered orally than when administered, say, intravenously.

20

It will be appreciated that a compound of the invention may be administered together with a physiologically acceptable carrier, excipient, or diluent.

25 To maintain therapeutically effective plasma concentrations for extended periods of time, compounds of the invention may be incorporated into slow release formulations.

30 Suitable compositions, for example for oral administration, include solid unit dose forms, and those containing liquid, e.g. for injection, such as tablets, capsules, vials and ampoules, in which the active agent is formulated, by known means, with a physiologically acceptable excipient, diluent or carrier. Suitable diluents and carriers are known, and include, for example, lactose and talc, together with appropriate binding agents etc.

A unit dosage of a compound of formula (I) typically comprises up to 500 mg (for example 1-500 mg, or 5-500 mg) of the active agent. Preferably the active agent is in the form of a pharmaceutical composition comprising the active agent and a physiologically acceptable carrier, excipient, or diluent. Preferred amounts of the active ingredient in a unit dose are

5 0.001-10, 0.001-5, 0.001-2, 0.001-1, 0.001-0.1, 0.001-0.01, 0.01-15, 0.01-10, 0.01-5, 0.01-2, 0.01-1, 0.1-10, 0.1-5, 0.1-2, 0.1-1, 0.1-0.5, 0.1-0.4, 0.2-15, 0.2-10, 0.2-5, 0.2-2, 0.2-1.2, 0.2-1, 0.5 to 1, 0.6-1.2, typically about 0.2 or 0.6 mg of the active agent per kg of the subject. Preferred amounts of the active agent are less than 420mg, preferably less than 28mg, more preferably less than 21mg, and preferably at least 0.07, 0.1, 0.7 or 0.8mg,

10 more preferably at least 3.5 or 7mg. More preferably 7 to 70mg, or 14 to 70mg, 3.5 to 21mg, 0.07-0.7mg, or 0.7-7mg. At these levels, it is believed that effective treatment can be achieved substantially without a concomitant fall (for example, no more than 10%) in blood pressure and/or increase in compensatory heart rate.

15 A unit dosage of a compound of the invention may further comprise one or more other therapeutic agents, for example analgesics, anti-hyperalgesics, anti-inflammatories, DMARDs, or anti-pathogenic agents.

Preferably a compound of the invention is administered once per day, although the

20 compound may be administered at a frequency of two or three times per day if desired.

Compounds of the invention can also serve as a basis for identifying more effective drugs, or drugs that have further reduced side effects.

25 Examples of pharmaceutically acceptable salts are organic addition salts formed with acids which form a physiologically acceptable anion, for example, tosylate, methanesulphonate, malate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulphate, nitrate, bicarbonate, and carbonate salts.

30

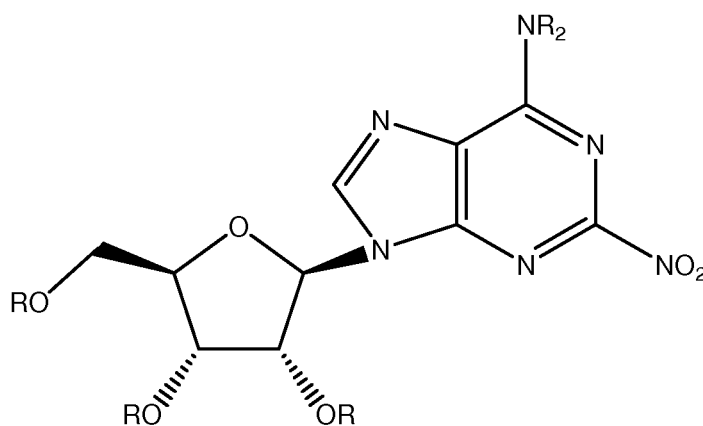
Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example,

sodium, potassium, or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The recitation of a listing of chemical groups in any definition of a variable herein includes
5 definitions of that variable as any single group or combination of listed groups. The
recitation of an embodiment for a variable herein includes that embodiment as any single
embodiment or in combination with any other embodiments or portions thereof.

There is also provided according to the invention methods of synthesis of compound
10 numbers 1-21 as defined in Example 1 below.

According to the invention there is provided a method of producing a compound of any of
formulae 1-7 as defined in Example 1, which comprises reacting a compound of the
following general formula (A) with a base and: (i) $\text{CF}_3\text{CF}_2\text{CH}_2\text{OH}$; (ii) 3-(4-
15 (trifluoromethyl)phenyl)phenol; (iii) 3,4-dichlorophenol; (iv) 3-trifluoromethyl, 4-
fluorophenol; (v) 3-trifluoromethyl, 4-chlorophenol; (vi) 3-chloro, 4-cyanophenol; or (vii)
3,5-bis(trifluoromethyl)phenol; and de-protecting the reaction product to produce the
compound of formula 1-7:



20

(A)

wherein R is a protecting group.

25

A preferred solvent is a non-alcohol solvent. Suitable examples are tetrahydrofuran (THF), dimethyl formamide (DMF) and acetonitrile. THF is preferred.

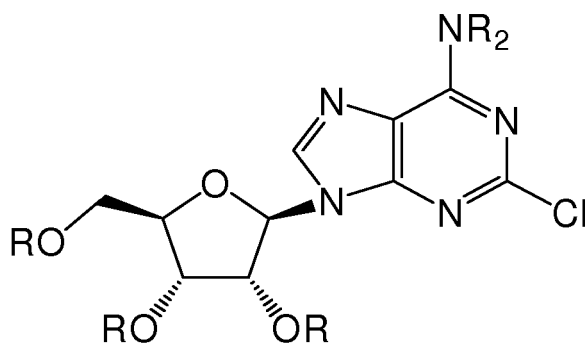
Suitable bases are sodium hydride, potassium tert-butoxide, butyllithium and lithium
5 hexamethyldisilazide (LHMDS). Sodium hydride is the preferred base for synthesising
compound 1 and potassium tert-butoxide is the preferred base for synthesising compounds
2-7.

Preferred protecting groups are carboxylic acid-derived acyl groups which may be
10 aliphatic, aromatic, heterocyclic, saturated or unsaturated. Examples of suitable protecting
groups are acetyl, propionyl, caproyl, palmitoyl, benzoyl, toluoyl, furoyl, sulfonyl,
isopropylidene, alkoxyalkylidene. Most preferably, the protecting groups are acetyl or
benzoyl. Alternatively, a silicon-based protecting group e.g. tert-butyldimethylsilyl
(TBDMS) may be used.

15

De-protection of the reaction product may be carried out using standard techniques, for
example, using sodium methoxide in methanol, or heating with ammonia. Where
isopropylidene or alkoxyalkylidene are used as protecting groups, an acid such as
trifluoroacetic acid (TFA) will be required for removal of the protecting groups. Silicon-
20 based protecting groups may be removed with tetrabutylammonium fluoride (TBAF), or an
acid such TFA.

There is also provided according to the invention a method of producing a compound of
formula 8 as defined in Example 1, which comprises reacting 2-chloro adenosine with 4-
25 (3,4-dichlorophenyl)piperazine to produce the compound of formula 8, or reacting a
compound of the following general formula with 4-(3,4-dichlorophenyl)piperazine, and de-
protecting the reaction product to produce the compound of formula 8:



wherein R is a protecting group as defined above.

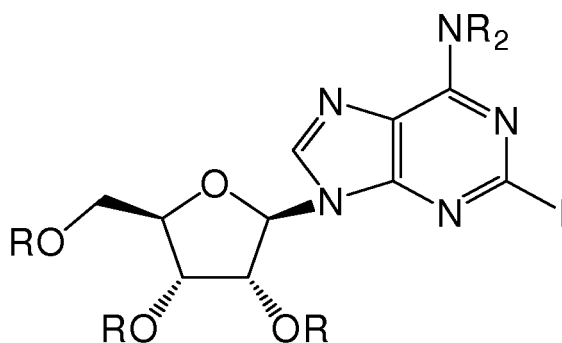
- 5 A preferred solvent is an organic solvent, such as DMF, methanol, or ethanol. The 4-(3,4-dichlorophenyl)piperazine may act as a solvent if desired. Alternatively, water may be used as solvent.

Preferably the reaction is heated to 100-200°C.

10

De-protection may be carried out as described above.

- There is also provided according to the invention a method of producing a compound of any of formulae 9-13 as defined in Example 1, which comprises reacting 2-iodo adenosine
- 15 with: (i) 3,4-dichlorophenylboronic acid; (ii) 3,5-difluorophenylboronic acid; (iii) 3,5-bis(trifluoromethyl)phenylboronic acid; (iv) 3,4,5-trifluorophenylboronic acid; or (v) 2-benzofuranylboronic acid, to produce the compound of formula 9-13, or reacting a compound of the following general formula (B) with: (i) 3,4-dichlorophenylboronic acid; (ii) 3,5-difluorophenylboronic acid; (iii) 3,5-bis(trifluoromethyl)phenylboronic acid; (iv)
- 20 3,4,5-trifluorophenylboronic acid; or (v) 2-benzofuranylboronic acid, and de-protecting the reaction product to produce the compound of formula 9-13:



(B)

wherein R is a protecting group as defined above.

5

Preferably the reaction is heated to 100-200°C.

A preferred solvent is an organic solvent, preferably an alcohol, toluene, ethyl acetate or DMF (or a combination thereof).

10

Preferably the reaction is carried out in the presence of a base, such as cesium carbonate, potassium carbonate, potassium hydroxide or sodium carbonate.

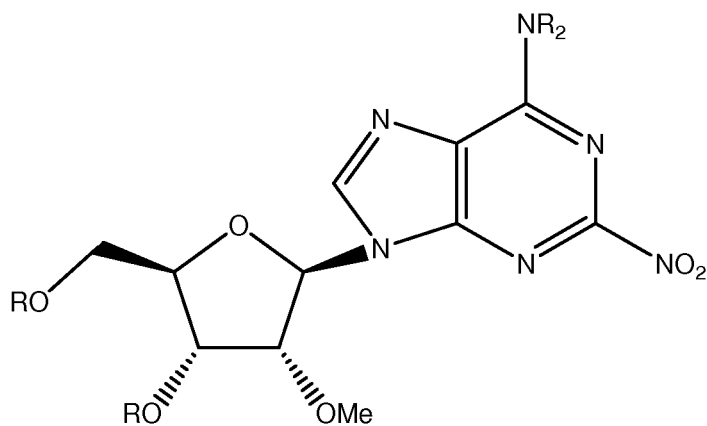
A palladium catalyst is preferably used, such as Pd(PPh₃)₄, Pd₂(dba)₂, Pd₂Cl₂(PPh₃)₂ or Pd(OAc)₂.

15

De-protection may be carried out as described above.

According to the invention there is also provided a method of producing a compound of formula 14 as defined in Example 1, which comprises de-protecting and methoxylating a compound of the following general formula (C) to produce the compound of formula 14:

20



(C)

5 wherein R is a protecting group.

Preferred protecting groups are carboxylic acid-derived acyl groups which may be aliphatic, aromatic, heterocyclic, saturated or unsaturated. Examples of suitable protecting groups are acetyl, propionyl, caproyl, palmitoyl, benzoyl, toluoyl, furoyl, or sulfonyl. Most preferably, the protecting groups are acetyl or benzoyl. Alternatively, a silicon-based protecting group may be used.

De-protection may be carried out as described above.

15 Methoxylation may be carried out using standard techniques, for example, using sodium methoxide in methanol. Alternatively, potassium methoxide may be used.

The preferred solvent is methanol.

20 It will be appreciated that methoxylation and de-protection may occur at substantially the same time. Alternatively, methoxylation could take place before de-protection.

According to the invention there is also provided a method of producing a compound of any of formulae 15-18 as defined in Example 1, which comprises reacting a compound of general formula (C) with a base and: (i) $\text{CHF}_2\text{CH}_2\text{OH}$; (ii) cyclopentanemethanol; (iii) 2,5-

difluorophenol; or (iv) (*S*)-*sec*-butylamine; and de-protecting the reaction product to produce the compound of formula 15-18.

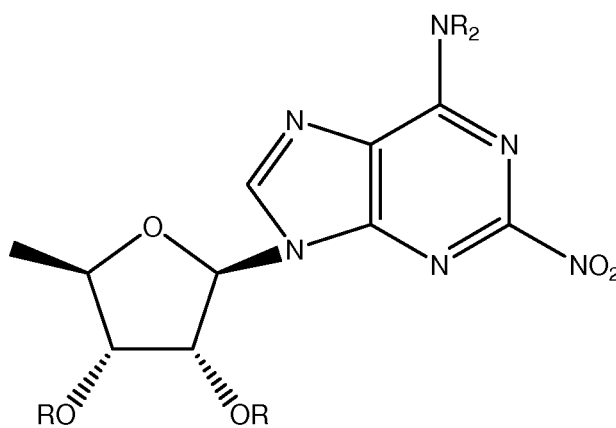
Preferred protecting groups are as described above for methods of producing a compound
5 of formula 14.

A preferred solvent is THF, although where (*S*)-*sec*-butylamine is a reactant this may act as the solvent.

10 Sodium hydride is the preferred base for synthesising compounds 15-16 and potassium *tert*-butoxide is the preferred base for synthesising compound 17.

De-protection may be carried out as described above.

15 There is also provided according to the invention a method of producing a compound of formula 19 or 20 as defined in Example 1, which comprises reacting a compound of the following general formula (D) with: (i) *n*-hexylamine; or (ii) cyclopentylamine; and de-protecting the reaction product to produce the compound of formula 19 or 20:



20

(D)

wherein R is a protecting group.

25 Preferred protecting groups are as described above for formula (A).

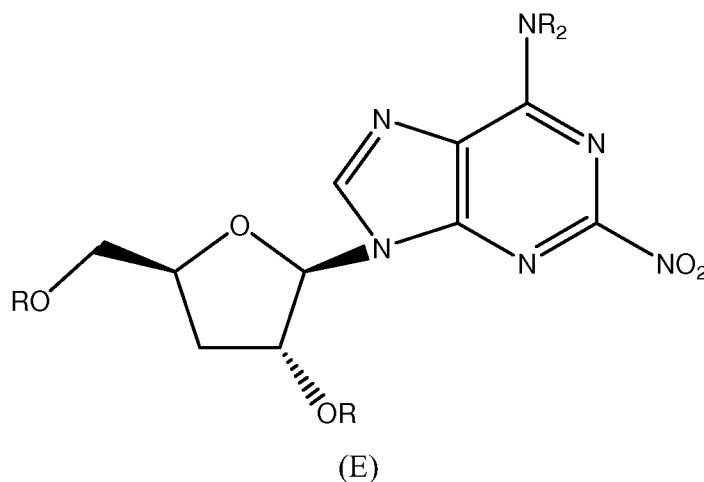
A preferred solvent is THF, although if desired the n-hexylamine or cyclopentylamine could act as a solvent.

De-protection may be carried out as described above.

5

According to the invention there is further provided a method of producing a compound of formula 21 as defined in Example 1, which comprises reacting a compound of the following general formula (E) with cyclopentylamine and de-protecting the reaction product to produce the compound of formula 21:

10



wherein R is a protecting group.

15

Preferred protecting groups are as described above for formula (C).

A preferred solvent is THF, although if desired the cyclopentylamine could act as a solvent.

20

De-protection may be carried out as described above.

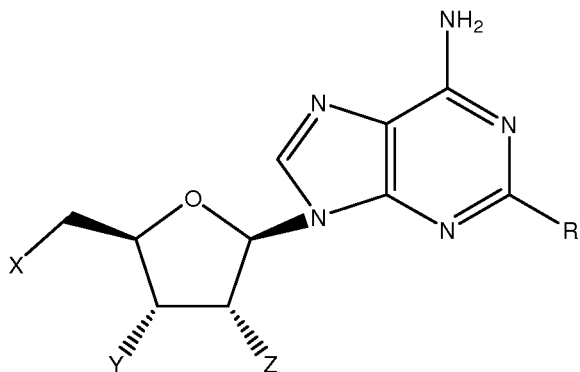
Preferably, methods of the invention for producing compounds of formulae 1-21 comprise the steps shown in Schemes 1-21 (found in Examples 2-22 below), respectively.

25

Particularly preferred methods of producing compounds of formulae 1-21 are as described in Examples 2-22 below.

Chemical structures of compounds of the invention are given in the Examples below. Binding experiments were carried out using rat A2a receptors. K_i values were determined for each compound at pH 5.5 and pH 7.4. To calculate this, rat striatal membranes were
5 incubated for 90 minutes at 22°C in the presence of 2nM [3H]-CGS21680, 1Unit/ml adenosine deaminase and increasing concentrations of the compound being studied, prior to filtration and liquid scintillation counting. The K_i values of the compounds were found to be in the range 0.96-220nM at pH 5.5, and in the range 47-25000nM at pH 7.4. $K_{i_{pH\ 7.4}}/K_{i_{pH\ 5.5}}$ for each compound is in the range 6-5400.

Example 1



When X=Y=Z=OH

Compound No.	Structure R ₁
1	OCH ₂ CF ₂ CF ₃
2	O-(3-((4-(CF ₃))Ph))Ph
3	O-(3,4-Cl ₂)Ph
4	O-(3-CF ₃ , 4-F)Ph
5	O-(3-CF ₃ , 4-Cl)Ph
6	O-(3-Cl, 4-CN)Ph
7	O-(3,5-(CF ₃) ₂)Ph
8	1-Piperazinyl(4-((3,4-Cl ₂)Ph))
9	(3,4-Cl ₂)Ph
10	(3,5-F ₂)Ph
11	(3,5-(CF ₃) ₂)Ph
12	(3,4,5-F ₃)Ph
13	(2-Benzofuranyl)

5 When X=Y=OH and Z=OMe

14	OCH ₃
15	OCH ₂ CHF ₂
16	OCH ₂ Cyclopentyl
17	O-(2,5-F ₂)Ph
18	NH-(S)-CH(CH ₃)CH ₂ CH ₃

When X=H and Y=Z=OH

19	NHCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃
20	NHCyclopentyl

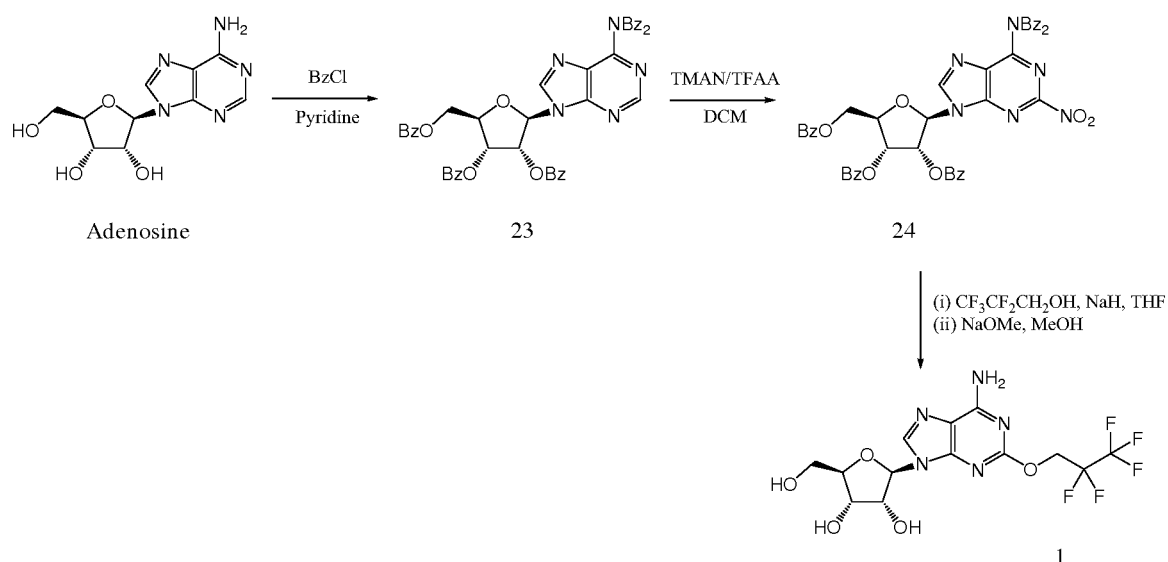
When X=Z=OH and Y=H

21	NHCyclopentyl
----	---------------

5

Example 2

Preparation of (2R,3R,4S,5R)-2-[6-amino-2-(2,2,3,3,3-pentafluoropropoxy)-9H-purin-9-yl]-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 1



10

Scheme 1

To a solution of adenosine (26.7g, 0.1mol) in pyridine (300mL) was added benzoyl chloride (81.2mL, 698mmol) and the resulting solution was refluxed at 80°C for 4h. The solvents were removed in vacuo and the residue dissolved in EtOAc and washed with aq. NaHCO₃, brine and water, and the organic phase dried over MgSO₄. Crystallisation from dichloromethane (DCM)/ethanol afforded 23 as a white crystalline solid in 2 batches (54g and 10g, 82% overall).

15

To a solution of tetramethylammonium nitrate (TMAN) (2.61g, 19.2mmol) in DCM (37mL) was added trifluoroacetic anhydride (TFAA) (2.67mL, 19.2mmol) and the resulting solution stirred for 1h. The mixture was cooled to 0°C and a solution of 23

20

(10.1g, 12.7mmol) in DCM (37mL) was added. The resulting solution was allowed to warm to rt over 4h. The solution was then washed with aq. NaHCO₃, brine and water(x3) and the organic phase dried over MgSO₄. Crystallisation from DCM/ethanol afforded 24 as a pale yellow solid (7.2g, 68%).

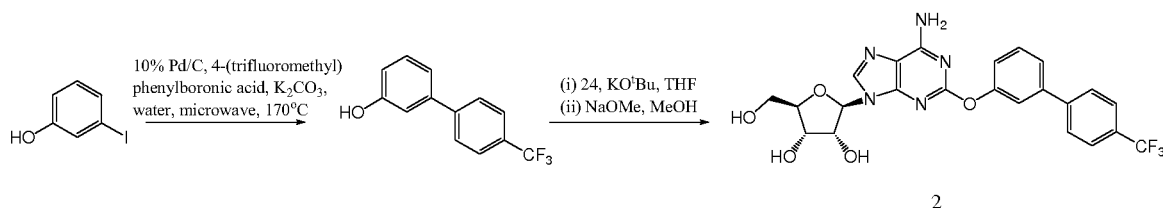
5 To a solution of CF₃CF₂CH₂OH (58μL, 0.58mmol) in tetrahydrofuran (THF) (10mL) was added NaH (23mg, 60% dispersion in mineral oil, 0.58mmol) and the resulting suspension stirred for 1h. 24 (400mg, 0.48mmol) was then added and the resulting solution stirred for 4d. A further 0.5eq of CF₃CF₂CH₂ONa in THF (5mL), prepared as above, was then added and stirring continued for 16h. The solvents were then removed in vacuo and the residue
10 dissolved in methanol before the addition of NaOMe (cat) and stirring of the resulting suspension for 16h. The solvents were removed in vacuo and the residue purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 60% methanol) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm,
15 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 25min, product eluted in 36% acetonitrile) to yield (2*R*,3*R*,4*S*,5*R*)-2-[6-amino-2-(2,2,3,3,3-pentafluoropropoxy)-9H-purin-9-yl]-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 1 as a white solid (26mg, 13%).

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
20 5-100% acetonitrile (+0.085% trifluoroacetic acid (TFA)) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.13min, 99.01%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
25 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.90min, 100%, ES⁺: 416.392 [MH]⁺.

Example 3

Preparation of (2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-{[4'-(trifluoromethyl)biphenyl-3-yl]oxy}-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 2



Scheme 2

To a mixture of 10% Pd/C (cat), 3-iodophenol (220mg, 1.00mmol) and 4-
 5 (trifluoromethyl)phenylboronic acid (284mg, 1.49mmol) was added a solution of K₂CO₃
 (415mg, 3.01mmol) in water (10mL) and the reaction mixture was heated in a Biotage
 microwave (170°C, absorption high, pre-stirring 10s) for 20min. The crude reaction
 mixture was then extracted into EtOAc (40mL x 3) and dried over MgSO₄ to yield 3-(4-
 (trifluoromethyl)phenyl)phenol as a yellow solid (212mg, 89%, 99% purity by HPLC)
 10 which was used without further purification.

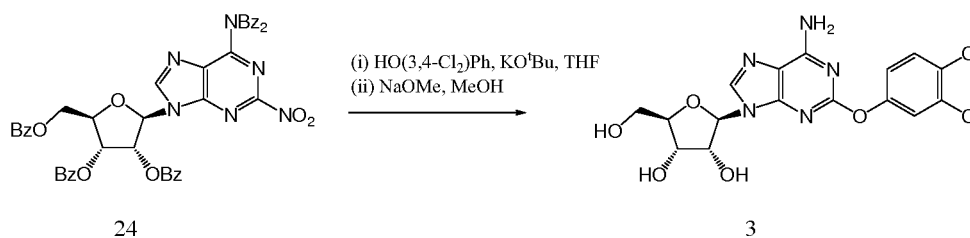
To a solution of 3-(4-(trifluoromethyl)phenyl)phenol (212mg, 0.89mmol) in THF was
 added KO^tBu (100mg, 0.89mmol) and the resulting suspension stirred for 30min before
 being added to a solution of 24 (400mg, 0.48mmol) in THF. Stirring was continued for 4d
 and the solvents were then removed in vacuo. The residue was dissolved in methanol
 15 (10mL), NaOMe (cat) was added and the resulting mixture was stirred for 16h. The
 solvents were removed in vacuo and the residue purified by flash column chromatography
 (normal phase, ICN silica, 18-32μ, gradient 5-15% ethanol in DCM, residue dry loaded)
 and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL
 20 acetonitrile) to yield (2*R*,3*R*,4*S*,5*R*)-2-((6-amino-2-([4'-(trifluoromethyl)biphenyl-3-
 yl]oxy)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 2 as a white solid
 (51mg, 21%).

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4μ, 1.5mL per min, 30°C, gradient
 25 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-
 300nm): Retention time 5.37min, 98.87%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.76min, 100%, ES⁺: 504.412 [MH]⁺.

5 Example 4

Preparation of (2R,3R,4S,5R)-2-[6-amino-2-(3,4-dichlorophenoxy)-9H-purin-9-yl]-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 3



10 Scheme 3

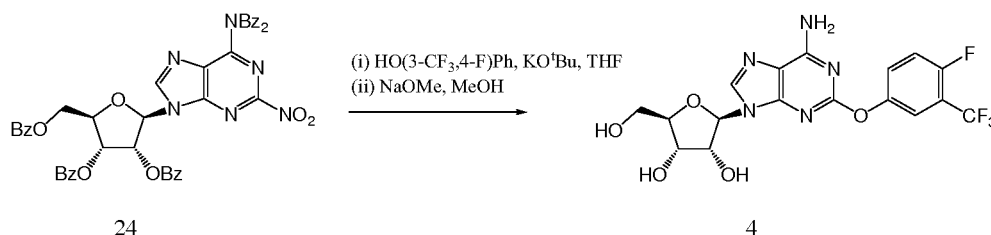
To a solution of 3,4-dichlorophenol (157mg, 0.96mmol) in THF (3mL) was added KO^tBu (108mg, 0.96mmol) and the resulting suspension stirred for 1.5h before being added to a solution of 24 (400mg, 0.48mmol) in THF. Stirring was continued for 2d and the solvents were then removed in vacuo. The residue was dissolved in methanol (15mL), NaOMe (cat) was added and the resulting mixture was stirred for 4d. The solvents were removed in vacuo and the residue purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 72% methanol) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 45% acetonitrile) to yield (2R,3R,4S,5R)-2-[6-amino-2-(3,4-dichlorophenoxy)-9H-purin-9-yl]-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 3 as a white solid (15mg, 7.6%).

25 HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.42min, 98.42%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.31min, 100%, ES⁺: 428.3 [MH]⁺.

5 Example 5

Preparation of (2R,3R,4S,5R)-2-{6-amino-2-[4-fluoro-3-(trifluoromethyl)phenoxy]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 4



Scheme 4

10

To a solution of 3-trifluoromethyl,4-fluorophenol (173mg, 0.96mmol) in THF (3mL) was added KO^tBu (108mg, 0.96mmol) and the resulting suspension stirred for 1.5h before being added to a solution of 24 (400mg, 0.48mmol) in THF (15mL). Stirring was continued for 20h and the solvents were then removed in vacuo. The residue was dissolved in methanol (15mL), NaOMe (cat) was added and the resulting mixture was stirred for 4d. The solvents were removed in vacuo and the residue purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 70% methanol), reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 50% acetonitrile) and recrystallisation from ethanol/heptane to yield (2R,3R,4S,5R)-2-{6-amino-2-[4-fluoro-3-(trifluoromethyl)phenoxy]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 44 as a white solid (23mg, 11%).

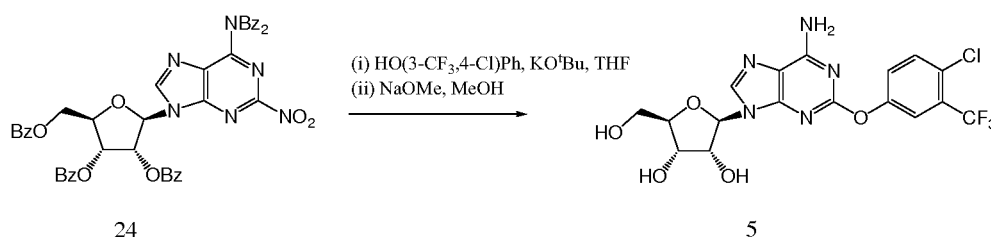
25

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.43min, 98.00%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.29min, 100%, ES⁺: 446.295 [MH]⁺.

5 Example 6

Preparation of (2R,3R,4S,5R)-2-{6-amino-2-[4-chloro-3-(trifluoromethyl)phenoxy]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 5



10

Scheme 5

To a solution of 3-trifluoromethyl,4-chlorophenol (582mg, 2.96mmol) in THF (10mL) was added KO^tBu (332mg, 2.96mmol) and the resulting suspension stirred for 2h before being added to a solution of 24 (1.66g, 2.00mmol) in THF (100mL). Stirring was continued for 2d and the solvents were then removed in vacuo. The residue was dissolved in methanol (40mL), NaOMe (170mg, 3.15mmol) was added and the resulting mixture was stirred for 16h. The solvents were removed in vacuo and the residue purified by flash column chromatography (normal phase, ICN silica, 18-32μ, gradient 5-20% ethanol in DCM, residue dry loaded) and twice by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 460 x 26mm (100g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 86% methanol) and (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 69% methanol) to yield (2R,3R,4S,5R)-2-{6-amino-2-[4-chloro-3-(trifluoromethyl)phenoxy]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 5 as a pale orange solid (188mg, 20%).

25

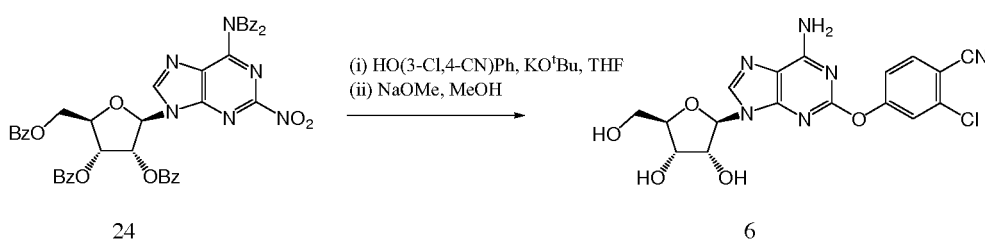
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.92min, 99.68%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.30min, 100% ES⁺: 461.859 [MH]⁺.

5

Example 7

Preparation of (2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-(3-chloro,4-cyanophenoxy)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 6



10

Scheme 6

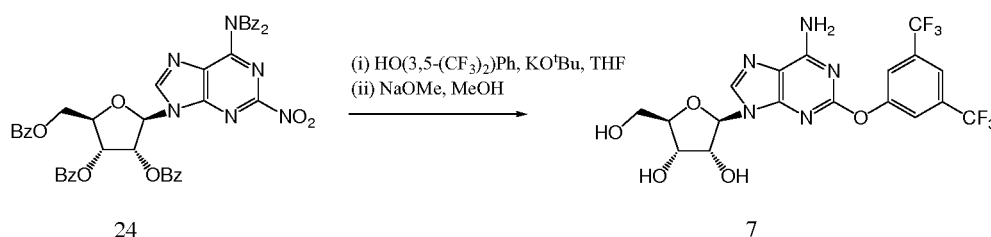
To a solution of 3-chloro,4-cyanophenol (147mg, 0.96mmol) in THF (5mL) was added KO^tBu (108mg, 0.96mmol) and the resulting suspension stirred for 30min before being
 15 added to a solution of 24 (400mg, 0.48mmol) in THF (20mL). Stirring was continued for 2d and the solvents were then removed in vacuo. The residue was dissolved in methanol (20mL), NaOMe (cat) was added and the resulting mixture was stirred for 16h. The solvents were removed in vacuo and the residue purified by flash column chromatography (normal phase, ICN silica, 18-32μ, gradient 5-20% ethanol in DCM, residue dry loaded)
 20 and by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 40% acetonitrile) to yield (2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-(3-chloro,4-cyanophenoxy)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 6 as a white solid (11mg, 5.5%).

25 HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.01min, 99.17%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.69min, 100%, ES⁺: 419.35 [MH]⁺.

5 Example 8

Preparation of (2R,3R,4S,5R)-2-(6-amino-2-[3,5-bis(trifluoromethyl)phenoxy]-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 7



Scheme 7

10

To a solution of 3,5-bis(trifluoromethyl)phenol (0.146mL, 0.96mmol) in THF (5mL) was added KO^tBu (108mg, 0.96mmol) and the resulting suspension stirred for 45min before being added to a solution of 24 (400mg, 0.48mmol) in THF (20mL). Stirring was continued for 47h and the solvents were then removed in vacuo. The residue was dissolved in methanol (15mL), NaOMe (cat) was added and the resulting mixture was stirred for 3d. The solvents were removed in vacuo and the residue purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 80% methanol) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 25min, product eluted in 55% acetonitrile) to yield (2R,3R,4S,5R)-2-(6-amino-2-[3,5-bis(trifluoromethyl)phenoxy]-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 7 as a white solid (28mg, 12%).

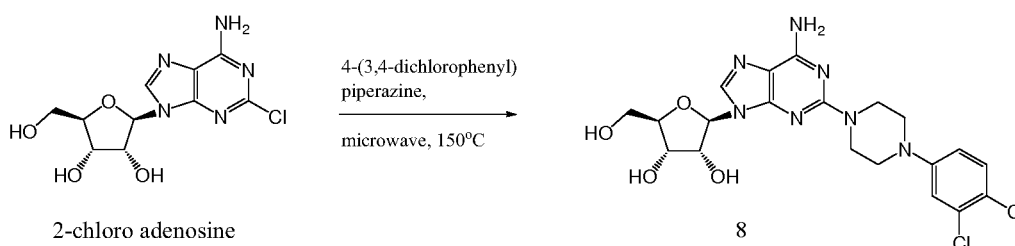
25

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.99min, 98.54%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 6.28min, 100%, ES⁺: 496.342 [MH]⁺.

5 Example 9

Preparation of (2R,3R,4S,5R)-2-(6-amino-2-(4-(3,4-dichlorophenyl)piperazin-1-yl)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 8



10

Scheme 8

To a solution of 2-chloro adenosine (80mg, 0.27mmol) in THF (4mL) was added 4-(3,4-dichlorophenyl)piperazine (122mg, 0.53mmol) and the resulting solution heated in a Biotage microwave (150°C, absorption high, pre-stirring 30s) for 30min. The solvents were then removed in vacuo and the residue purified twice by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 18min, product eluted in 80% acetonitrile) and (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 9min, product eluted in 55% acetonitrile) to yield (2R,3R,4S,5R)-2-(6-amino-2-(4-(3,4-dichlorophenyl)piperazin-1-yl)-9H-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 8 as a white solid (7.9mg, 6%)

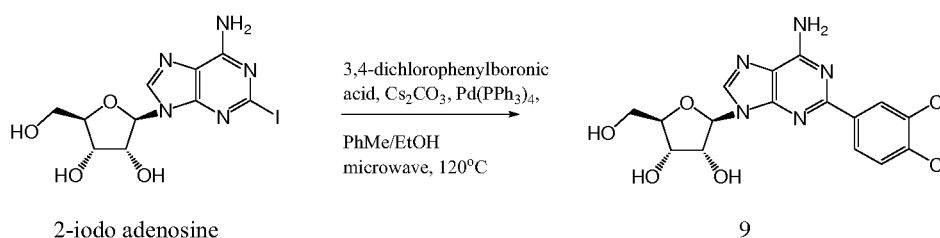
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.78min, 99.26%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.82min, 100%, ES⁺: 496.3 [MH]⁺.

Example 10

Preparation of (2*R*,3*R*,4*S*,5*R*)-2-{6-amino-2-(3,4-dichlorophenyl)-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9

5



Scheme 9

A solution of 2-iodo adenosine (200mg, 0.51mmol), 3,4-dichlorophenylboronic acid
 (116mg, 0.61mmol), cesium carbonate (364mg, 1.12mmol) and Pd(PPh₃)₄ (59mg,
 0.051mmol) in toluene (1.2mL) and ethanol (2.4mL) was heated in a Biotage microwave
 (120°C, absorption high, pre-stirring 30s) for 110min. The solvents were then removed in
 vacuo and the residue dissolved in EtOAc (30mL) and washed with sat. aq. NaHCO₃
 (20mL x2) and brine (20mL) and dried over MgSO₄. Purification by flash column
 chromatography (normal phase, ICN silica, 20g, 18-32μ, gradient 2.5-20% ethanol in
 DCM, residue dry loaded) and twice by reverse phase prep HPLC (Phenomenex Synergi,
 RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over
 10min, product eluted in 48% acetonitrile) and (Phenomenex Synergi, RP-Hydro 150 x
 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product
 eluted in 50% acetonitrile) afforded (2*R*,3*R*,4*S*,5*R*)-2-{6-amino-2-(3,4-dichlorophenyl)-9H-
 purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9 as a white solid (24.4mg, 11%).

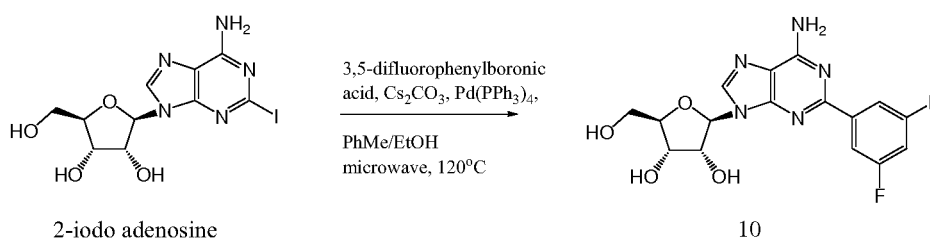
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-
 300nm): Retention time 4.77min, 100%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-
 300nm): Retention time 5.19min, 100%, ES⁺: 412.32 [MH]⁺.

Example 11

Preparation of (2R,3R,4S,5R)-2-{6-amino-2-(3,5-difluorophenyl)-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 10

5



Scheme 10

A solution of 2-iodo adenosine (200mg, 0.51mmol), 3,5-difluorophenylboronic acid (150mg, 0.95mmol), cesium carbonate (364mg, 1.12mmol) and Pd(PPh₃)₄ (59mg, 0.051mmol) in toluene (1.2mL) and ethanol (2.4mL) was heated in a Biotage microwave (120°C, absorption high, pre-stirring 15s) for 40min. The solvents were then removed in vacuo and the residue dissolved in EtOAc (20mL) and washed with sat. aq. NaHCO₃ (20mL x2) and brine (20mL) and dried over MgSO₄. Purification by reverse phase prep HPLC in 2 batches (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 35% acetonitrile) and (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 40% acetonitrile), combination of the purer fractions from both columns, concentration in vacuo to ~20mL and filtration of the resulting white precipitate gave (2R,3R,4S,5R)-2-{6-amino-2-(3,5-difluorophenyl)-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 10 as a white solid (16.8mg, 9%).

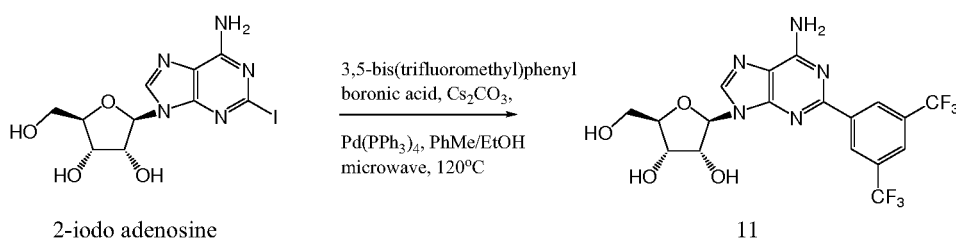
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.07min, 99.86%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 6.02min, 100%, ES⁺: 380.4 [MH]⁺.

Example 12

Preparation of (2R,3R,4S,5R)-2-{6-amino-2-[3,5-bis(trifluoromethyl)phenyl]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 11

5



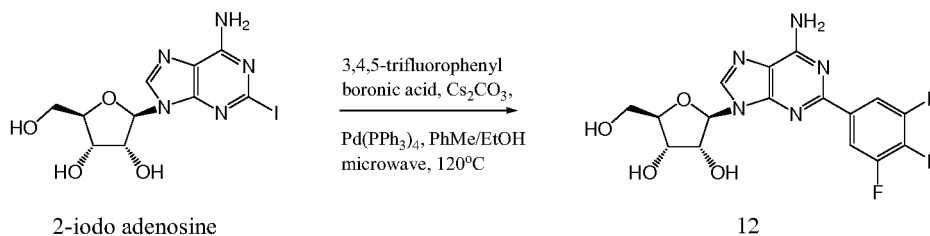
Scheme 11

A solution of 2-iodo adenosine (1.00g, 2.53mmol), 3,5-bis(trifluoromethyl)phenylboronic acid (784mg, 3.04mmol), cesium carbonate (1.81g, 6.08mmol) and Pd(PPh₃)₄ (293mg, 0.25mmol) in toluene (2.4mL) and ethanol (4.8mL) was heated in a Biotage microwave (120°C, absorption high, pre-stirring 30s) for 40min in 2 batches and the crude reaction mixtures were combined. The solvents were then removed in vacuo and the residue dissolved in EtOAc (200mL) and washed with sat. aq. NaHCO₃ (100mL) and brine (100mL). Purification by flash column chromatography (normal phase, ICN silica, 50g, 18-32μ, gradient 5-25% ethanol in DCM, residue dry loaded, product eluted in 25% ethanol) afforded (2R,3R,4S,5R)-2-{6-amino-2-[3,5-bis(trifluoromethyl)phenyl]-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 11 as an off-white glass (364mg, 30%).

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.46min, 99.86%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.89min, 100%, ES⁺: 479.886 [MH]⁺.

Example 13

Preparation of (2*R*,3*R*,4*S*,5*R*)-2-{6-amino-2-(3,4,5-trifluorophenyl)-9H-purin-9-yl}-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 12

Scheme 12

A solution of 2-iodo adenosine (200mg, 0.51mmol), 3,4,5-trifluorophenylboronic acid (107mg, 0.609mmol), cesium carbonate (364mg, 1.12mmol) and Pd(PPh₃)₄ (59mg, 0.051mmol) in toluene (1.2mL) and ethanol (2.4mL) was heated in a Biotage microwave (120°C, absorption high, pre-stirring 30s) for 40min. The solvents were then removed in vacuo and the residue dissolved in EtOAc (30mL) and washed with sat. aq. NaHCO₃ (20mL x2) and brine (20mL) and dried over MgSO₄. Purification by flash column chromatography (normal phase, ICN silica, 50g, 18-32μ, gradient 2.5-15% ethanol in DCM, residue dry loaded, product eluted in 15% ethanol) and by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 50% acetonitrile) afforded (2*R*,3*R*,4*S*,5*R*)-2-{6-amino-2-(3,4,5-trifluorophenyl)-9H-purin-9-yl}-5-(hydroxymethyl) tetrahydrofuran-3,4-diol 12 as a white solid (13.2mg, 7%).

20

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.49min, 100%.

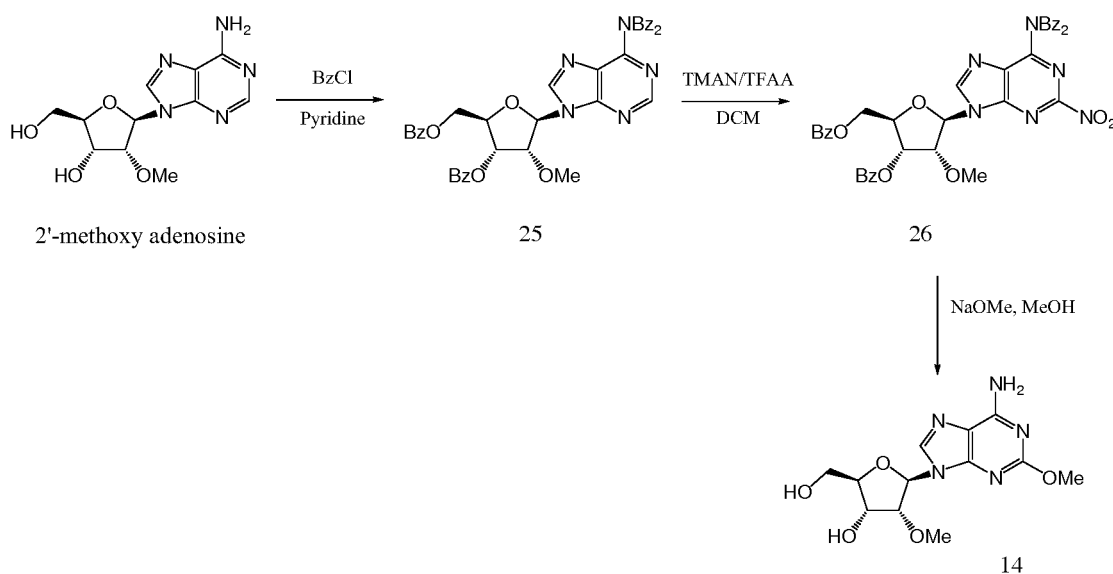
25

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.04min, 100%, ES⁺: 398.396 [MH]⁺.

Example 15

Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-2-methoxy-9*H*-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 14

5



Scheme 14

To a solution of 2'-methoxy adenosine (10g, 35.6mmol) in pyridine (75mL) was added
 10 benzoyl chloride (22.7mL, 196mmol) and the resulting solution was refluxed at 80°C for
 4h. The solvents were removed in vacuo and the residue dissolved in EtOAc and washed
 with aq. NaHCO₃, brine and water, and the organic phase dried over MgSO₄.
 Crystallisation from ethanol afforded 25 as a white solid in 2 batches (13.7g and 4.3g, 72%
 overall).

15 To a suspension of 25 (13.7g, 19.7mmol) and TMAN (3.48g, 25.6mmol) in DCM (100mL)
 was added drop-wise a solution of TFAA (3.77mL, 26.7mmol) in DCM (20mL) and the
 resulting solution stirred for 2h. The solution was then washed with aq. NaHCO₃ and water
 (x3) and the organic phase dried over MgSO₄. The residue was dissolved in ethanol
 (20mL), DCM (50mL) was added and the solvents were removed in vacuo to yield 26 as a
 20 pale yellow foam (14g, 96%, ~70% purity) which was used without further purification.

To a solution of 26 (3.7g, 5.31mmol) in methanol (70mL) was added NaOMe (1.15g,
 21.3mmol) and the resulting solution stirred at room temperature for 2d. Silica gel (15g)
 was then added and the solvents removed in vacuo. Purification by flash column

chromatography (normal phase, ICN silica, 18-32 μ , gradient 10% ethanol in DCM, residue dry loaded) and recrystallisation from hot water afforded (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-2-methoxy-9H-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 14 as a white crystalline solid (536mg, 32%).

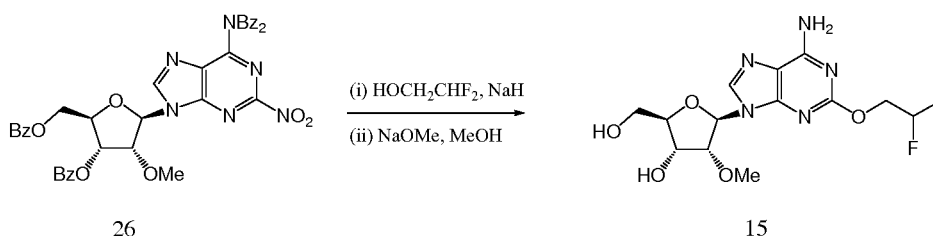
5

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 2.80-2.86min (split peak), 100%.

10 LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 3.42min, 100%, ES⁺: 312.063 [MH]⁺.

Example 16

15 Preparation of (2*R*,3*R*,4*R*,5*R*)-5-[6-amino-2-(2,2-difluoroethoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 15



Scheme 15

20

To a solution of CHF₂CH₂OH (0.165mL, 2.60mmol) in THF (10mL) was added NaH (104mg, 60% dispersion in mineral oil, 2.60mmol) and the resulting suspension stirred for 1h. A solution of 26 (956mg, 1.37mmol) in THF (10mL) was then added and the resulting solution stirred at room temperature for 2d. The solvents were then removed in vacuo and the residue dissolved in methanol (20mL) before the addition of NaOMe (cat) and stirring of the resulting suspension for 16h. The solvents were removed in vacuo and the residue purified by flash column chromatography (normal phase, ICN silica, 50g, 18-32 μ , gradient 2.5-15% ethanol in DCM, residue dry loaded, product eluted in 10-15% ethanol) and by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10 μ , 20mL per

25

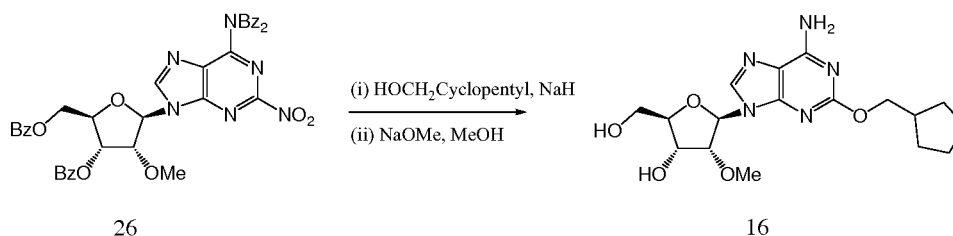
min, gradient 5-100% acetonitrile in water over 10min, product eluted in 25% acetonitrile) to yield (2*R*,3*R*,4*R*,5*R*)-5-[6-amino-2-(2,2-difluoroethoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 15 as a white solid (134mg, 27%).

5 HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 3.42min, 100%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 10 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 3.96min, 100%, ES⁺: 362.342 [MH]⁺.

Example 17

Preparation of (2*R*,3*R*,4*R*,5*R*)-5-[6-amino-2-(cyclopentylmethoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 16



Scheme 16

20 To a solution of cyclopentanemethanol (1.73mL, 19.1mmol) in THF (35mL) was added NaH (637mg, 60% dispersion in mineral oil, 15.9mmol) and the resulting suspension stirred for 1h. A solution of 26 (3.7g, 5.31mmol) in THF (35mL) was then added and the resulting solution stirred at room temperature for 2d. The solvents were then removed in vacuo and the residue dissolved in methanol (70mL) before the addition of NaOMe 25 (860mg, 15.9mmol) and stirring of the resulting suspension for 16h. Silica gel (15g) was then added and the solvents removed in vacuo. Purification by flash column chromatography (normal phase, ICN silica, 18-32μ, gradient 5-10% ethanol in DCM, residue dry loaded) and recrystallisation from methanol/water afforded (2*R*,3*R*,4*R*,5*R*)-5-

[6-amino-2-(cyclopentylmethoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 16 as a white crystalline solid (356mg, 18%).

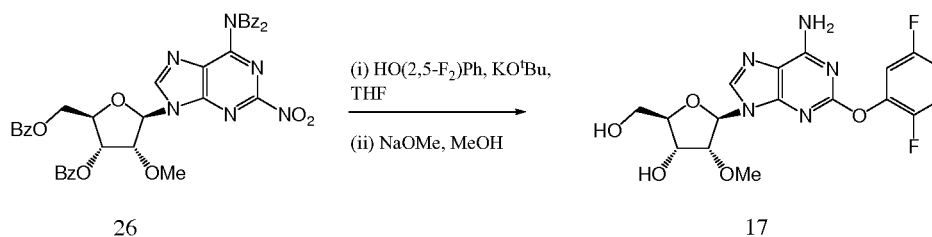
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
5 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.27min, 99.05%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
10 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.76min, 100%, ES⁺: 380.499 [MH]⁺.

Example 18

Preparation of (2R,3R,4R,5R)-5-[6-amino-2-(2,5-difluorophenoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 17

15



Scheme 17

To a solution of 2,5-difluorophenol (104mg, 0.80mmol) in THF (7mL) was added KO^tBu
20 (63mg, 0.56mmol) and the resulting suspension stirred for 30min before the addition of 26 (295mg, 0.42mmol). Stirring was continued for 6h and the solvents were then removed in vacuo. The residue was dissolved in methanol (7mL), NaOMe (86mg, 1.59mmol) was added and the resulting mixture was stirred for 16h, before being quenched with aq. citric acid. The solvents were removed in vacuo and the residue purified by reverse phase
25 column chromatography (LiChroprep RP-18, 40-63µm, 230 x 26mm (50g), 30mL per min, gradient 5-100% methanol in water over 45min, product eluted in 57% methanol) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10µ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 26% acetonitrile)

to yield (2*R*,3*R*,4*R*,5*R*)-5-[6-amino-2-(2,5-difluorophenoxy)-9H-purin-9-yl]-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 17 as a pale yellow solid (30.9mg, 18%).

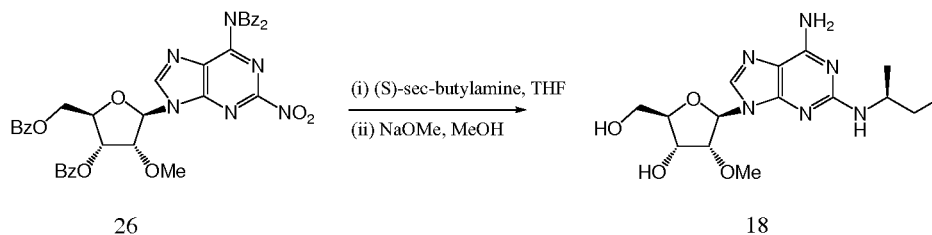
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
5 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.20min, 99.00%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
10 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.91min, 100%, ES⁺: 410.484 [MH]⁺.

Example 19

Preparation of (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-2-{(1*S*)-1-methylpropyl}amino)-9H-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol 18

15



Scheme 18

To a solution of 26 (445mg, 0.64mmol) in THF (8mL) was added (*S*)-sec-butylamine
20 (120μL, 1.18mmol) and further THF (8mL). Stirring was continued for 1wk and the solvents were then removed in vacuo. The residue was dissolved in methanol (8mL), NaOMe (cat) was added and the resulting mixture was stirred for 2d. Further NaOMe (cat) was added and the resulting mixture was stirred for 1d before being concentrated in vacuo. The residue was dissolved in acetonitrile/water (1:1, 3mL) and TFA/water (1:1, 0.5mL)
25 was added. This solution was purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26 (50g), 30mL per min, gradient 5-100% methanol in water over 30min) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 37% acetonitrile) in 2 batches to yield (2*R*,3*R*,4*R*,5*R*)-5-(6-amino-2-{(1*S*)-1-

methylpropyl]amino}-9H-purin-9-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-ol
18 as a white solid (6.5mg, 3%).

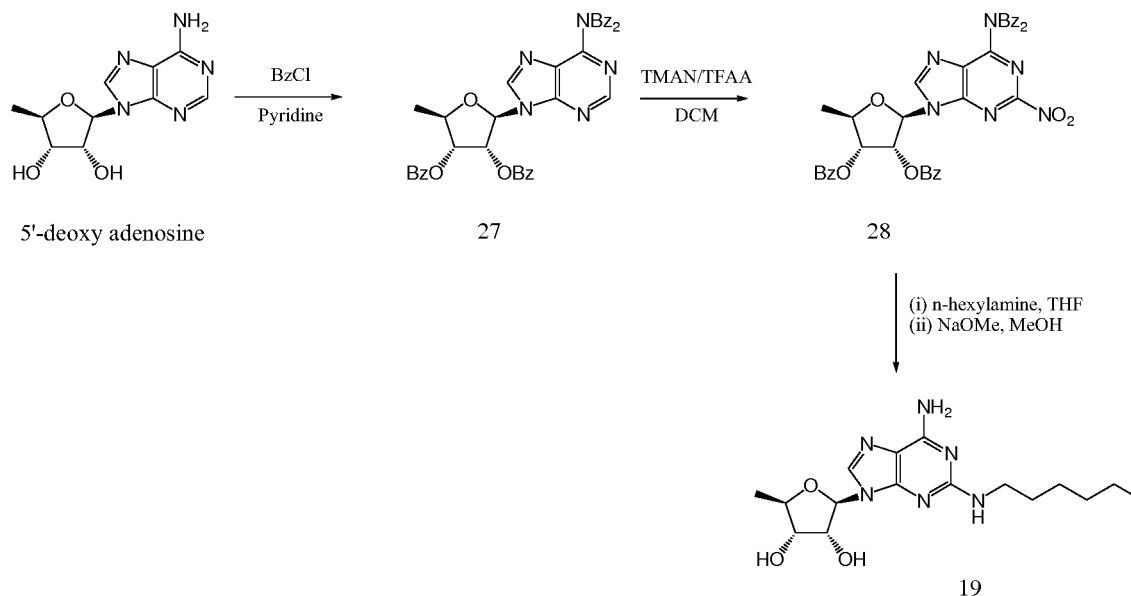
HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
5 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-
300nm): Retention time 3.54min, 99.23%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient
10 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-
300nm): Retention time 4.52min, 98.40%, ES⁺: 353.426 [MH]⁺.

Example 20

Preparation of (2R,3R,4S,5R)-2-[6-amino-2-(hexylamino)-9H-purin-9-yl]-5-methyltetrahydrofuran-3,4-diol 19

15



Scheme 19

To a solution of 5'-deoxy adenosine (600mg, 2.39mmol) in pyridine (15mL) was added
20 benzoyl chloride (1.8mL, 15.5mmol) and the reaction heated at 80°C for 5.5h. After
cooling to rt, the residue was concentrated in vacuo and dissolved in EtOAc (50mL). The
organic fraction was washed with 0.2M aq. HCl (25mL), aq. NaHCO₃ (25mL x2) and brine

(25mL) and dried over MgSO₄. Purification by flash column chromatography (normal phase, ICN silica, 18-32 μ , gradient 10-67% EtOAc in heptane, product eluted in 67% EtOAc) afforded 27 as a white foam (1.4g, 88%).

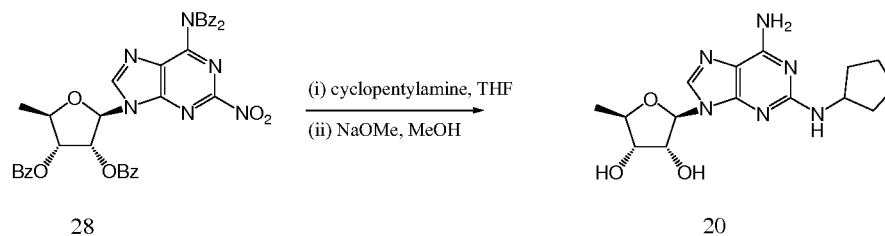
To a suspension of TMAN (0.64g, 5.35mmol) in DCM (25mL) was added TFAA (0.76mL, 5.35mmol) and the resulting solution was stirred for 3h, cooled to 0°C and a solution of 27 (2.38g, 3.56mmol) in DCM (25mL) was added. The resulting solution was allowed to warm to rt over 16h and the solvents were removed in vacuo. The residue was partitioned between EtOAc (100mL) and water (75mL) and the organic fraction was washed with brine (50mL) and dried over MgSO₄. Crystallisation from DCM/ethanol afforded 28 as a pale yellow solid in 2 batches (1.10g and 0.95g, 81% overall).

To a solution of 28 (300mg, 0.42mmol) in THF (15mL) was added n-hexylamine (111 μ L, 0.84mmol) and stirring was continued for 2d. The solvents were then removed in vacuo and the residue was dissolved in methanol (10mL). NaOMe (50mg, 0.93mmol) was added and the resulting mixture was stirred for 1d. The solvents were removed in vacuo and the residue was purified by reverse phase column chromatography (LiChroprep RP-18, 40-63 μ m, 230 x 26mm (50g), 30mL per min, gradient 5-100% methanol in water over 30min, product eluted in 70% methanol) and reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10 μ , 20mL per min, gradient 5-100% acetonitrile in water over 14min, product eluted in 37% acetonitrile) to yield (2*R*,3*R*,4*S*,5*R*)-2-[6-amino-2-(hexylamino)-9H-purin-9-yl]-5-methyltetrahydrofuran-3,4-diol 19 as a pale yellow solid (13.8mg, 9%).

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 4.46min, 99.24%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 6.12min, 100%, ES⁺: 351.535 [MH]⁺.

Example 21

Preparation of (2R,3R,4S,5R)-2-[6-amino-2-(cyclopentylamino)-9H-purin-9-yl]-5-methyltetrahydrofuran-3,4-diol 20

5

Scheme 20

To a solution of 28 (600mg, 0.84mmol) in THF (15mL) was added cyclopentylamine (166 μ L, 1.69mmol) and stirring was continued for 2d. The solvents were then removed in vacuo and the residue was dissolved in methanol (10mL). NaOMe (cat) was added and the resulting mixture was stirred for 16h. The solvents were removed in vacuo and the residue was purified by reverse phase column chromatography (LiChroprep RP-18, 40-63 μ m, 230 x 26mm (50g), 30mL per min, gradient 0-100% methanol in water over 45min, product eluted in 62% methanol) and twice by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10 μ , 20mL per min, gradient 5-100% acetonitrile in water over 25min, product eluted in 35% acetonitrile) to yield (2R,3R,4S,5R)-2-[6-amino-2-(cyclopentylamino)-9H-purin-9-yl]-5-methyltetrahydrofuran-3,4-diol 20 as a pale yellow solid (8mg, 3%).

20 HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30 $^{\circ}$ C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 3.70min, 99.51%.

25 LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4 μ , 1.5mL per min, 30 $^{\circ}$ C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.48min, 100%, ES $^{+}$: 335.404 [MH] $^{+}$.

Na₂SO₄ to afford 30 as a yellow solid (563mg, 79%, ~90% purity) which was used without further purification.

To a solution of 30 (320mg, 0.40mmol) in THF (7mL) was added cyclopentylamine (80μL, 0.81mmol) and stirring continued for 16h before further cyclopentylamine (80μL, 0.81mmol) was added. After 16h, the solvents were removed in vacuo and the residue was dissolved in methanol (7mL). NaOMe (cat) was added and the resulting mixture was stirred for 3d. The solvents were then removed in vacuo and the residue was dissolved in acetonitrile/water (1:1, 3mL) and TFA/water (1:1, 0.5mL) was added. This solution was purified by reverse phase column chromatography (LiChroprep RP-18, 40-63μm, 230 x 26mm (50g), 30mL per min, gradient 5-100% methanol in water over 30min, product eluted in 60% methanol), twice by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 30% acetonitrile) and again by reverse phase prep HPLC (Phenomenex Synergi, RP-Hydro 150 x 10mm, 10μ, 20mL per min, gradient 5-100% acetonitrile in water over 10min, product eluted in 30% acetonitrile) to yield (2*R*,3*R*,5*S*)-2-[6-amino-2-(cyclopentylamino)-9H-purin-9-yl]-5-(hydroxymethyl)tetrahydrofuran-3-ol as a white solid (1.6mg, 1%).

HPLC (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 3.56min, 99.39%.

LCMS (Phenomenex Synergi, RP-Hydro, 150 x 4.6mm, 4u, 1.5mL per min, 30°C, gradient 5-100% acetonitrile (+0.085% TFA) in water (+0.1% TFA) over 7min - held for 30s, 200-300nm): Retention time 5.37min, 97.87%, ES⁺: 335.404 [MH]⁺.

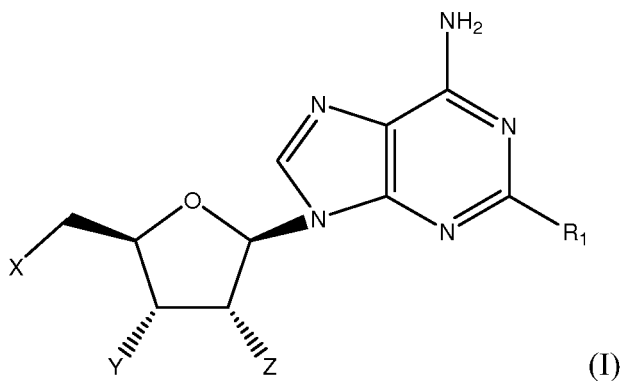
Example 23

Plasma concentrations of spongosine were determined after single oral dosing in 5 or 6 human volunteers. Tachycardia was determined using 12 lead ECGs. The minimum effective analgesic plasma concentration in the rat was 0.025 μM suggesting a minimum effective dose in the human would be approximately 0.8 mg which results in plasma concentrations greater than 0.025 μM for approximately 1.5h.

	<u>Dose</u>	<u>Plasma Cmax (μM)</u>	<u>Tachycardia side effect</u>
10	0.2 mg	0.01 \pm 0.005	No
	0.8 mg	0.04 \pm 0.02	No
	3.5 mg	0.13 \pm 0.04	No
	10.5 mg	0.3 \pm 0.04	No
15	21 mg	0.5 \pm 0.1	No
	28 mg	0.6 \pm 0.1	Yes

Claims

1. A compound of the following general formula:



5

wherein:

when $X=Y=Z=OH$, R_1 is $OCH_2CF_2CF_3$, phenoxy (substituted with 3-(4-trifluoromethylphenyl), 3,4-dichloro, (3-trifluoromethyl,4-fluoro), (3-trifluoromethyl,4-chloro), (3-chloro, 4-cyano), or 3,5-bis(trifluoromethyl)), 1-piperazinyl(4-(3,4-dichlorophenyl)), phenyl (substituted with 3,4-dichloro, 3,5-difluoro, 3,5-bis(trifluoromethyl) or 3,4,5-trifluoro) or 2-benzofuranyl; or

when $X=Y=OH$ and $Z=OMe$, R_1 is OCH_3 , OCH_2CHF_2 , OCH_2 cyclopentyl, O -(2,5-difluorophenyl) or (S)-sec-butylamino; or

when $X=H$ and $Y=Z=OH$, R_1 is n-hexylamino or cyclopentylamino; or

when $X=Z=OH$ and $Y=H$, R_1 is cyclopentylamino;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 for use as a medicament.

25

3. Use of a compound according to claim 1 in the manufacture of a medicament for the prevention, treatment, or amelioration of a medical condition that can be improved or prevented by agonism of adenosine A2A receptors.
- 5 4. The use according to claim 3 wherein the medical condition is pain.
5. The use according to claim 4, wherein the pain is hyperalgesia.
6. The use according to claim 4 or 5, wherein the pain is caused by neuropathy.
- 10 7. The use according to any of claims 4 to 6 wherein the pain is associated with Diabetic Neuropathy, Polyneuropathy, Sciatica/Lumbar Radiculopathy, Cancer Pain, Post Herpetic Neuralgia, Myofascial Pain Syndrome, Rheumatoid Arthritis, Fibromyalgia, Osteoarthritis, Pancreatic Pain, Pelvic/Perineal pain, Spinal Stenosis, 15 Temporo-mandibular Joint Disorder, HIV pain, Trigeminal Neuralgia, Chronic Neuropathic Pain, Lower Back Pain, Failed Back Surgery pain, back pain, post-operative pain, post physical trauma pain (including gunshot, road traffic accident, burns), Cardiac pain, Chest pain, Pelvic pain/PID, Neck Pain, Bowel Pain, Phantom Limb Pain, Obstetric Pain (labour/C-Section), Renal Colic, Acute Herpes Zoster Pain, Acute Pancreatitis Breakthrough Pain (Cancer), Dysmenorrhoea/ 20 Endometriosis; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.
8. The use according to claim 4 or 5 wherein the pain is caused by inflammatory 25 disease, or by combined inflammatory, autoimmune and neuropathic tissue damage.
9. The use according to any of claims 4, 5 or 8 wherein the pain is associated with Rheumatoid Arthritis, Osteoarthritis, Joint pain (tendonitis, bursitis, acute arthritis), 30 Lower Back Pain, Failed Back Surgery pain, back pain, post-operative pain, post physical trauma pain (including gunshot, road traffic accident, burns), Fibromyalgia, rheumatoid arthritis, spondylitis, gouty arthritis, and other arthritic conditions, cancer, HIV, Diabetic Neuropathy, Polyneuropathy, Sciatica/Lumbar

Radiculopathy, autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis) graft v. host rejection, allograft rejections, fever and myalgia due to infection, AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, osteoporosis, cerebral malaria and bacterial meningitis, bowel pain, cancer pain, back pain, fibromyalgia, post-operative pain; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition.

- 5
- 10 10. The use according to claim 4 wherein the pain is ischaemic pain.
11. The use according to claim 4 or 10 wherein the pain is associated with coronary artery disease, peripheral artery disease, conditions which are characterized by insufficient blood flow, left ventricular hypertrophy, essential hypertension, acute hypertensive emergency, cardiomyopathy, heart insufficiency, exercise tolerance, chronic heart failure, arrhythmia, cardiac dysrhythmia, syncope, arteriosclerosis, mild chronic heart failure, angina pectoris, Prinzmetal's (variant) angina, stable angina, and exercise induced angina, cardiac bypass reocclusion, intermittent claudication (arteriosclerosis obliterans), arteritis, diastolic dysfunction and systolic dysfunction, atherosclerosis, post ischaemia/reperfusion injury, diabetes (both Types I and II), thromboembolisms, and haemorrhagic accidents.
- 15
- 20
- 25 12. The use according to claim 3 wherein the said medical condition is associated with inflammation.
- 30 13. The use according to claim 12 wherein the inflammation is caused by or associated with cancer (such as leukemias, lymphomas, carcinomas, colon cancer, breast cancer, lung cancer, pancreatic cancer, hepatocellular carcinoma, kidney cancer, melanoma, hepatic, lung, breast, and prostate metastases, etc.); Chronic Obstructive Pulmonary Disease (COPD), acute bronchitis, chronic bronchitis, emphysema, bronchiectasis, cystic fibrosis, pneumonia, pleurisy, acute asthma, chronic asthma, acute respiratory distress syndrome, adult respiratory distress syndrome (ARDS), infant respiratory distress syndrome (IRDS) acute lung injury (ALI), laryngitis,

pharangitis, persistent asthma, chronic asthmatic bronchitis, interstitial lung disease, lung malignancies, alpha-anti-trypsin deficiency, bronchiolitis obliterans, sarcoidosis, pulmonary fibrosis, collagen vascular disorders, allergic rhinitis, nasal congestion, status asthmaticus, smoking related pulmonary disease, pulmonary hypertension, pulmonary oedema, pulmonary embolism, pleural effusion, pneumothorax, haemothorax, lung cancer, allergies, pollinosis Hay fever), sneeze, vasomotor rhinitis, mucositis, sinusitis, exogenous irritant induced illness (SO₂, smog, pollution), airway hypersensitivity, milk product intolerance, Luffer's pneumonia, pneumoconiosis, collagen induced vascular disease, granulomatous disease, bronchial inflammation, chronic pulmonary inflammatory disease, bone resorption diseases, reperfusion injury (including damage caused to organs as a consequence of reperfusion following ischaemic episodes e.g. myocardial infarcts, strokes), auto-immune disease (such as organ transplant rejection, lupus erythematosus, graft v. host rejection, allograft rejections, multiple sclerosis, rheumatoid arthritis, type I diabetes mellitus including the destruction of pancreatic islets leading to diabetes and the inflammatory consequences of diabetes); autoimmune damage (including multiple sclerosis, Guillam Barre Syndrome, myasthenia gravis); obesity; cardiovascular conditions associated with poor tissue perfusion and inflammation (such as atheromas, atherosclerosis, stroke, ischaemia-reperfusion injury, claudication, spinal cord injury, congestive heart failure, vasculitis, haemorrhagic shock, vasospasm following subarachnoid haemorrhage, vasospasm following cerebrovascular accident, pleuritis, pericarditis, the cardiovascular complications of diabetes); ischaemia-reperfusion injury, ischaemia and associated inflammation, restenosis following angioplasty and inflammatory aneurysms; epilepsy, neurodegeneration (including Alzheimer's Disease), muscle fatigue or muscle cramp (particularly athletes' cramp), arthritis (such as rheumatoid arthritis, osteoarthritis, rheumatoid spondylitis, gouty arthritis), fibrosis (for example of the lung, skin and liver), multiple sclerosis, sepsis, septic shock, encephalitis, infectious arthritis, Jarisch-Herxheimer reaction, shingles, toxic shock, cerebral malaria, Lyme's disease, endotoxic shock, gram negative shock, haemorrhagic shock, hepatitis (arising both from tissue damage or viral infection), deep vein thrombosis, gout; conditions associated with breathing difficulties (e.g.

impeded and obstructed airways, bronchoconstriction, pulmonary vasoconstriction, impeded respiration, silicosis, pulmonary sarcosis, pulmonary hypertension, pulmonary vasoconstriction, bronchial allergy and vernal conjunctivitis); conditions associated with inflammation of the skin (including psoriasis, eczema, ulcers, contact dermatitis); conditions associated with inflammation of the bowel (including Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, inflammatory bowel disease); HIV (particularly HIV infection), cerebral malaria, bacterial meningitis, TNF-enhanced HIV replication, TNF inhibition of AZT and DDI activity, osteoporosis and other bone resorption diseases, osteoarthritis, rheumatoid arthritis, infertility from endometriosis, fever and myalgia due to infection, cachexia secondary to cancer, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), AIDS related complex (ARC), keloid formation, scar tissue formation, adverse effects from amphotericin B treatment, adverse effects from interleukin-2 treatment, adverse effects from OKT3 treatment, or adverse effects from GM-CSF treatment, and other conditions mediated by excessive anti-inflammatory cell (including neutrophil, eosinophil, macrophage and T- cell) activity; or in any of the above pathological conditions where bacterial or viral infection is a cause or exacerbates the condition, macro or micro vascular complications of type 1 or 2 diabetes, retinopathy, nephropathy, autonomic neuropathy, or blood vessel damage caused by ischaemia or atherosclerosis.

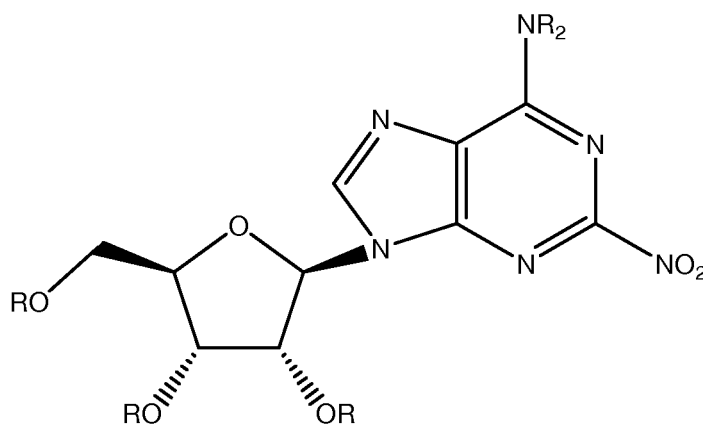
14. The use according to claim 3 wherein the said medical condition is associated with arthropathy.

15. The use according to claim 14 wherein the arthropathy is caused by or associated with rheumatoid arthritis, spondylitis, gouty arthritis, osteoarthritis, tendonitis, bursitis, acute arthritis, non-rheumatoid arthritis, or gout.

16. Use of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a disease-modifying antirheumatic drug (DMARD) for slowing the progression of arthropathy.
- 5 17. Use according to claim 16 in the manufacture of a DMARD for slowing the progression of rheumatoid arthritis.
18. Use of a compound according to claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the promotion of wound healing.
- 10 19. Use according to any of claims 3 to 18 at a dosage that, after administration to a subject, gives rise to a peak plasma concentration of the compound that is less than the EC50 value of the compound at adenosine receptors at pH 7.4.
- 15 20. Use according to any of claims 3 to 18 at a dosage that is one ten thousandth to one half of the minimum dosage of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the compound is to be administered.
- 20 21. Use according to any of claims 3 to 18 at a dosage that, after administration to a subject, gives rise to a plasma concentration of the compound that is maintained for more than one hour at one ten thousandth to one half of the minimum plasma concentration of the compound that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the
- 25 compound is to be administered.
22. Use according to any of claims 3 to 18 at a dosage of 0.001-15mg/kg.
23. A pharmaceutical composition comprising a compound according to claim 1, and a
- 30 physiologically acceptable carrier, excipient, or diluent.
24. A pharmaceutical composition according to claim 23, further comprising an NSAID or a DMARD.

25. A pharmaceutical composition according to claim 23, further comprising an anti-pathogenic agent.

5 26. A method of producing a compound of formula 1 as defined in Example 1, which comprises reacting a compound of the following general formula (A) with $\text{CF}_3\text{CF}_2\text{CH}_2\text{OH}$ and a base, and de-protecting the reaction product to produce the compound of formula 1:



10

(A)

wherein R is a protecting group.

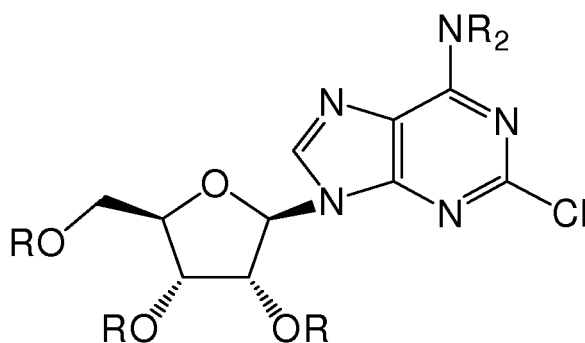
15 27. A method of producing a compound of formula 2 as defined in Example 1, which comprises reacting a compound of general formula (A) with 3-(4-(trifluoromethyl)phenyl)phenol and a base, and de-protecting the reaction product to produce the compound of formula 2.

20 28. A method of producing a compound of formula 3 as defined in Example 1, which comprises reacting a compound of general formula (A) with 3,4-dichlorophenol and a base, and de-protecting the reaction product to produce the compound of formula 3.

25 29. A method of producing a compound of formula 4 as defined in Example 1, which comprises reacting a compound of general formula (A) with 3-trifluoromethyl, 4-

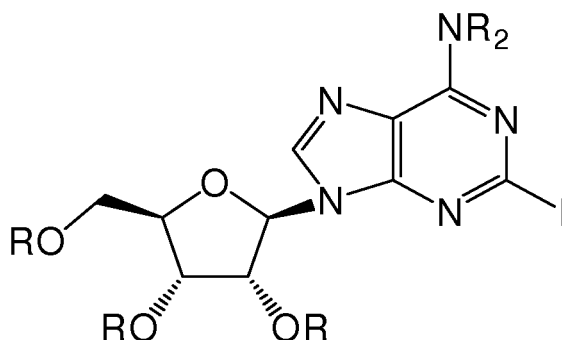
fluorophenol and a base, and de-protecting the reaction product to produce the compound of formula 4.

30. A method of producing a compound of formula 5 as defined in Example 1, which
5 comprises reacting a compound of general formula (A) with 3-trifluoromethyl, 4-chlorophenol and a base, and de-protecting the reaction product to produce the compound of formula 5.
31. A method of producing a compound of formula 6 as defined in Example 1, which
10 comprises reacting a compound of general formula (A) with 3-chloro, 4-cyanophenol and a base, and de-protecting the reaction product to produce the compound of formula 6.
32. A method of producing a compound of formula 7 as defined in Example 1, which
15 comprises reacting a compound of general formula (A) with 3,5-bis(trifluoromethyl)phenol and a base, and de-protecting the reaction product to produce the compound of formula 7.
33. A method of producing a compound of formula 8 as defined in Example 1, which
20 comprises reacting 2-chloro adenosine with 4-(3,4-dichlorophenyl)piperazine to produce the compound of formula 8, or reacting a compound of the following general formula with 4-(3,4-dichlorophenyl)piperazine, and de-protecting the reaction product to produce the compound of formula 8:



wherein R is a protecting group.

34. A method of producing a compound of formula 9 as defined in Example 1, which comprises reacting 2-iodo adenosine with 3,4-dichlorophenylboronic acid to produce the compound of formula 9, or reacting a compound of the following general formula (B) with 3,4-dichlorophenylboronic acid, and de-protecting the reaction product to produce the compound of formula 9:



(B)

wherein R is a protecting group.

35. A method of producing a compound of formula 10 as defined in Example 1, which comprises reacting 2-iodo adenosine with 3,5-difluorophenylboronic acid to produce the compound of formula 10, or reacting a compound of general formula (B) with 3,5-difluorophenylboronic acid, and de-protecting the reaction product to produce the compound of formula 10.
36. A method of producing a compound of formula 11 as defined in Example 1, which comprises reacting 2-iodo adenosine with 3,5-bis(trifluoromethyl)phenylboronic acid to produce the compound of formula 11, or reacting a compound of general formula (B) with 3,5-bis(trifluoromethyl)phenylboronic acid, and de-protecting the reaction product to produce the compound of formula 11.

37. A method of producing a compound of formula 12 as defined in Example 1, which comprises reacting 2-iodo adenosine with 3,4,5-trifluorophenylboronic acid to produce the compound of formula 12, or reacting a compound of general formula (B) with 3,4,5-trifluorophenylboronic acid, and de-protecting the reaction product to produce the compound of formula 12.

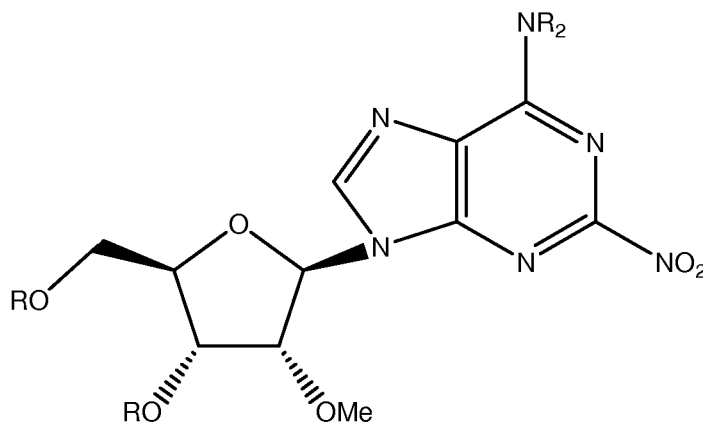
5

38. A method of producing a compound of formula 13 as defined in Example 1, which comprises reacting 2-iodo adenosine with 2-benzofuranylboronic acid to produce the compound of formula 13, or reacting a compound of general formula (B) with 2-benzofuranylboronic acid, and de-protecting the reaction product to produce the compound of formula 13.

10

39. A method of producing a compound of formula 14 as defined in Example 1, which comprises de-protecting and methoxylating a compound of the following general formula (C) to produce the compound of formula 14:

15



(C)

20

wherein R is a protecting group.

40. A method of producing a compound of formula 15 as defined in Example 1, which comprises reacting a compound of general formula (C) with CHF₂CH₂OH and a base, and de-protecting the reaction product to produce the compound of formula 15.

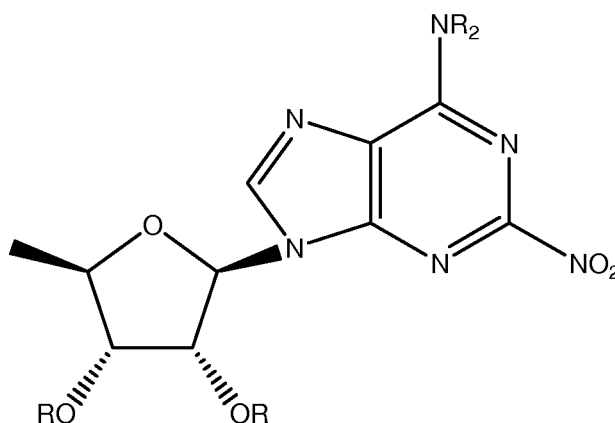
25

41. A method of producing a compound of formula 16 as defined in Example 1, which comprises reacting a compound of general formula (C) with cyclopentanemethanol and a base, and de-protecting the reaction product to produce the compound of formula 16.

42. A method of producing a compound of formula 17 as defined in Example 1, which comprises reacting a compound of general formula (C) with 2,5-difluorophenol and a base, and de-protecting the reaction product to produce the compound of formula 17.

43. A method of producing a compound of formula 18 as defined in Example 1, which comprises reacting a compound of general formula (C) with (S)-sec-butylamine and a base, and de-protecting the reaction product to produce the compound of formula 18.

44. A method of producing a compound of formula 19 as defined in Example 1, which comprises reacting a compound of the following general formula (D) with n-hexylamine and de-protecting the reaction product to produce the compound of formula 19:



(D)

25

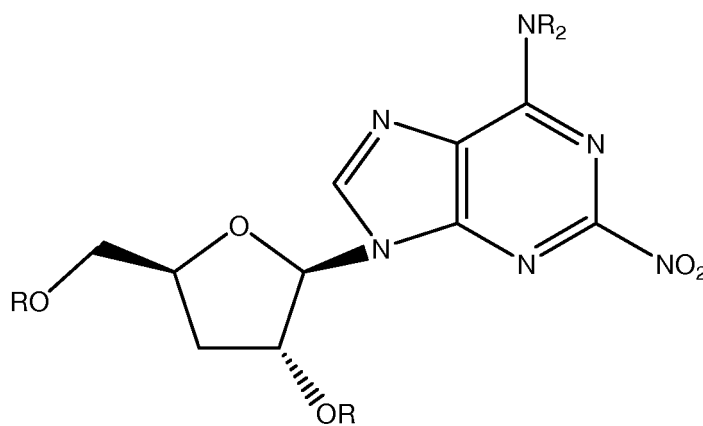
wherein R is a protecting group.

45. A method of producing a compound of formula 20 as defined in Example 1, which comprises reacting a compound of general formula (D) with cyclopentylamine and de-protecting the reaction product to produce the compound of formula 20.

5

46. A method of producing a compound of formula 21 as defined in Example 1, which comprises reacting a compound of the following general formula (E) with cyclopentylamine and de-protecting the reaction product to produce the compound of formula 21:

10



(E)

15

wherein R is a protecting group.