

6-AMINO-2-{ [(1S)-1-METHYLBUTYL] OXY}-9-[5-(1-PIPERIDINYL)-7,9-DIHYDRO-8H-PURIN-8-ONE
MALEATEBackground of the Invention

5 The present invention relates to compounds, processes for their preparation, compositions containing them, to their use in the treatment of various disorders in particular allergic diseases and other inflammatory conditions for example allergic rhinitis and asthma, infectious diseases, cancer, and as vaccine adjuvants.

10 Vertebrates are constantly threatened by the invasion of microorganisms and have evolved mechanisms of immune defence to eliminate infective pathogens. In mammals, this immune system comprises two branches; innate immunity and acquired immunity. The first line of host defence is the innate immune system, which is mediated by macrophages and dendritic cells. Acquired immunity involves the elimination of pathogens at the late stages of infection and also
15 enables the generation of immunological memory. Acquired immunity is highly specific, due to the vast repertoire of lymphocytes with antigen-specific receptors that have undergone gene rearrangement.

The innate immune response was originally thought to be non-specific, but is now known to be able
20 to discriminate between self and a variety of pathogens. The innate immune system recognises microbes *via* a limited number of germline-encoded Pattern-Recognition Receptors (PRRs) which have a number of important characteristics.

Toll-like receptors (TLRs) are a family of ten Pattern Recognition Receptors described in man.
25 TLRs are expressed predominantly by innate immune cells where their rôle is to monitor the environment for signs of infection and, on activation, mobilise defence mechanisms aimed at the elimination of invading pathogens. The early innate immune-responses triggered by TLRs limit the spread of infection, while the pro-inflammatory cytokines and chemokines that they induce lead to recruitment and activation of antigen presenting cells, B cells, and T cells. The TLRs can modulate
30 the nature of the adaptive immune-responses to give appropriate protection *via* dendritic cell-activation and cytokine release (Akira S., et al, *Nat. Immunol.*, 2001: 2, 675-680). The profile of the response seen from different TLR agonists depends on the cell type activated.

TLR7 is a member of the subgroup of TLRs (TLRs 3, 7, 8, and 9), localised in the endosomal
35 compartment of cells which have become specialised to detect non-self nucleic acids. TLR7 plays a key rôle in anti-viral defence via the recognition of ssRNA (Diebold S.S., et al, *Science*, 2004: 303, 1529-1531; and Lund J. M., et al, *PNAS*, 2004: 101, 5598-5603). TLR7 has a restricted expression-profile in man and is expressed predominantly by B cells and plasmacytoid dendritic cells (pDC), and to a lesser extent by monocytes. Plasmacytoid DCs are a unique population of lymphoid-
40 derived dendritic cells (0.2-0.8% of Peripheral Blood Mononuclear Cells (PBMCs)) which are the primary type I interferon-producing cells secreting high levels of interferon-alpha (IFN α) and interferon-beta (IFN β) in response to viral infections (Liu Y-J, *Annu. Rev. Immunol.*, 2005: 23, 275-306).

Allergic diseases are associated with a Th2-biased immune-response to allergens. Th2 responses are associated with raised levels of IgE, which, *via* its effects on mast cells, promotes a hypersensitivity to allergens, resulting in the symptoms seen, for example, in allergic rhinitis. In healthy individuals the immune-response to allergens is more balanced with a mixed Th2/Th1 and regulatory T cell response. TLR7 ligands have been shown to reduce Th2 cytokine and enhance Th1 cytokine release *in vitro* and to ameliorate Th2-type inflammatory responses in allergic lung models *in vivo* (Fili L., et al, *J. All. Clin. Immunol.*, 2006: 118, 511-517; Moisan J., et al, *Am. J. Physiol. Lung Cell Mol. Physiol.*, 2006: 290, L987-995; Tao et al, *Chin. Med. J.*, 2006: 119, 640-648). Thus, TLR7 ligands have the potential to rebalance the immune-response seen in allergic individuals and lead to disease modification.

Central to the generation of an effective innate immune response in mammals are mechanisms which bring about the induction of interferons and other cytokines which act upon cells to induce a number of effects. These effects can include the activation of anti-infective gene expression, the activation of antigen presentation in cells to drive strong antigen-specific immunity and the promotion of phagocytosis in phagocytic cells.

Interferon was first described as a substance which could protect cells from viral infection (Isaacs & Lindemann, *J. Virus Interference. Proc. R. Soc. Lon. Ser. B. Biol. Sci.* 1957: 147, 258-267). In man, the type I interferons are a family of related proteins encoded by genes on chromosome 9 and encoding at least 13 isoforms of interferon alpha (IFN α) and one isoform of interferon beta (IFN β). Recombinant IFN α was the first approved biological therapeutic and has become an important therapy in viral infections and in cancer. As well as direct antiviral activity on cells, interferons are known to be potent modulators of the immune response, acting on cells of the immune system.

As a first-line therapy for hepatitis C virus (HCV) disease, interferon combinations can be highly effective at reducing viral load and in some subjects in eliminating viral replication. However, many patients fail to show a sustained viral response and in these patients viral load is not controlled. Additionally, therapy with injected interferon may be associated with a number of unwanted adverse effects which are shown to affect compliance (Dudley T., et al, *Gut.*, 2006: 55(9), 1362-3).

Administration of a small molecule compound which could stimulate the innate immune response, including the activation of type I interferons and other cytokines, could become an important strategy for the treatment or prevention of human diseases including viral infections. This type of immunomodulatory strategy has the potential to identify compounds which may be useful not only in infectious diseases but also in cancer (Krieg., *Curr. Oncol. Rep.*, 2004: 6(2), 88-95), allergic diseases (Moisan J., et al, *Am. J. Physiol. Lung Cell Mol. Physiol.*, 2006: 290, L987-995), other inflammatory conditions such as irritable bowel disease (Rakoff-Nahoum S., *Cell.*, 2004, 23, 118(2): 229-41), and as vaccine adjuvants (Persing et al., *Trends Microbiol.*, 2002: 10(10 Suppl), S32-7).

In animal models, imiquimod demonstrated adjuvant activities either topically (Adams S., et al, *J. Immunol.*, 2008, 181:776-84; Johnston D., et al, *Vaccine*, 2006, 24:1958-65), or systemically (Fransen F. et al, *Infect. Immun.*, 2007, 75:5939-46). Resiquimod and other related TLR7/8 agonists have also been shown to display adjuvant activity (Ma R. et al, *Biochem. Biophys. Res.*

Commun., 2007, 361:537-42; Wille-Reece U., *et al*, *Proc. Natl. Acad. Sci. USA*, 2005, 102:15190-4; Wille-Reece U., *et al*, US2006045885 A1).

5 Mechanisms which lead to induction of type I interferons are only partly understood. One mechanism which can lead to the induction of interferon in many cell types is the recognition of double-stranded viral RNA by the RNA helicases RIG-I and MDA5. This mechanism is thought to be the primary mechanism by which interferons are induced by Sendai virus infection of cells.

10 Further mechanisms for the induction of interferons are *via* TLR-dependent signalling events. In man, plasmacytoid dendritic cells (pDCs) are professional interferon-producing cells, able to make large amounts of interferons in response to, for example, viral infection. These pDCs are shown to preferentially express TLR7 and TLR9 and stimulation of these receptors with viral RNA or DNA respectively can induce expression of interferon alpha.

15 Oligonucleotide agonists of TLR7 and TLR9, and small molecule purine-based agonists of TLR7 have been described which can induce interferon alpha from these cell types in animals and in man (Takeda K. *et al*, *Annu. Rev. Immunol.*, 2003: 21, 335-76). TLR7 agonists include imidazoquinoline compounds such as imiquimod and resiquimod, oxoadenine analogues and also nucleoside analogues such as loxoribine and 7-thia-8-oxoguanosine which have long been known to induce
20 interferon alpha. International Patent Application publication number WO 2008/114008 (AstraZeneca AB/Dainippon Sumitomo Pharma Co. Ltd.) discloses 9-substituted-8-oxoadenine compounds as TLR7 modulators.

25 It remains unclear how small molecule purine-like compounds can induce type I interferons and other cytokines since the molecular targets of these known inducers have not been identified. However, an assay strategy has been developed to characterise small molecule inducers of human interferon IFN α (regardless of mechanism) which is based on stimulation of primary human donor cells with compounds, and is disclosed herein.

30 Brief Description of the Invention

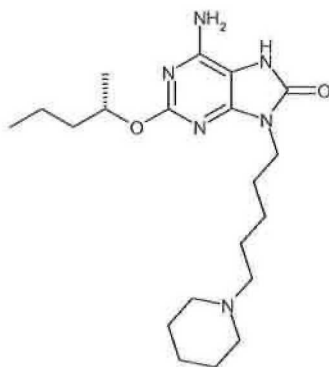
The compound 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, which is disclosed in International Application No. PCT/EP2009/060265, published as WO2010/018133, has been shown to be an inducer of human interferon and may possess an
35 improved profile with respect to known inducers of human interferon, for example enhanced potency, and may show enhanced selectivity for IFN α with respect to TNF α . It is expected that 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt will be more easily formulated and/or processed and/or handled. For example, the purity of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-
40 one may be improved via formation and/or recrystallisation of a maleate salt, and/or its stability of may be improved vis-à-vis the free base. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one which induces human interferon may be useful in the treatment of various disorders, for example the treatment of allergic diseases and other inflammatory conditions for example allergic rhinitis and asthma, the treatment of infectious
45 diseases and cancer, and may also be useful as a vaccine adjuvant. It is expected that 6-amino-2-

{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt will have similar pharmacological properties.

- 5 6-Amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one is a potent immunomodulator and accordingly, care should be exercised in its handling.

Summary of the Invention

- 10 In a first aspect, there is provided a compound which is 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one:



- 15 in the form of a maleate salt.

- Further, there is provided 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt, in which the ratio of maleate anion to 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one is 1:1.
- 20 There is thus provided, as a further aspect of the invention, a compound which is 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for use in therapy.
- 25 There is also therefore provided a compound which is 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for use in the treatment of allergic diseases and other inflammatory conditions, infectious diseases, and cancer.
- 30 There is also therefore provided a compound which is 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for use in the treatment of allergic rhinitis.
- 35 There is also therefore provided a compound which is 6-amino-2-{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for use in the treatment of asthma.

There is also therefore provided a vaccine adjuvant comprising a compound which is 6-amino-2-
{[(1*S*)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a
maleate salt.

- 5 There is further provided an immugenic composition comprising an antigen or antigen composition
and a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-(1-piperidinyl)pentyl]-7,9-
dihydro-8*H*-purin-8-one, in the form of a maleate salt.

- 10 There is further provided a vaccine composition comprising an antigen or antigen composition and
a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-
8*H*-purin-8-one, in the form of a maleate salt.

- 15 There is further provided a method of treating or preventing disease comprising the administration
to a human subject suffering from or susceptible to disease, an immugenic composition comprising
an antigen or antigen composition and a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-
[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt.

- 20 There is further provided a method of treating or preventing disease comprising the administration
to a human subject suffering from or susceptible to disease, a vaccine composition comprising an
antigen or antigen composition and a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt.

- 25 There is further provided the use of a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for the manufacture
of an immugenic composition comprising an antigen or antigen composition, for the treatment or
prevention of disease.

- 30 There is further provided the use of a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for the manufacture
of a vaccine composition comprising an antigen or antigen composition, for the treatment or
prevention of disease.

- 35 There is further provided the use of a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for the manufacture
of a medicament for the treatment of allergic diseases and other inflammatory conditions, infectious
diseases, and cancer.

- 40 There is further provided the use of a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for the manufacture
of a medicament for the treatment of allergic rhinitis.

- 45 There is further provided the use of a compound which is 6-amino-2- $\{[(1S)\text{-}1\text{-methylbutyl}]\text{oxy}\}$ -9-[5-
(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, for the manufacture
of a medicament for the treatment of asthma.

There is further provided a method of treatment of allergic diseases and other inflammatory conditions, infectious diseases, and cancer, which method comprises administering to a human subject in need thereof, a therapeutically effective amount of a compound which is 6-amino-2-
5 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt.

There is further provided a method of treatment of allergic rhinitis, which method comprises administering to a human subject in need thereof, a therapeutically effective amount of a compound which is 6-amino-2-
10 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt.

There is further provided a method of treatment of asthma, which method comprises administering to a human subject in need thereof, a therapeutically effective amount of a compound which is 6-amino-2-
15 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt.

The invention provides in a further aspect, a combination comprising a compound which is 6-amino-2-
20 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, together with at least one other therapeutically-active agent.

There is further provided a pharmaceutical composition comprising a compound which is 6-amino-2-
25 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, and one or more pharmaceutically acceptable diluents or carriers.

There is also provided a process for preparing a pharmaceutical composition which comprises admixing a compound which is 6-amino-2-
30 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, in the form of a maleate salt, with one or more pharmaceutically acceptable diluents or carriers.

6-Amino-2-
35 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, and salts thereof may be prepared by the methodology described in US Provisional Application Number 61/087777 and International Application No. PCT/EP2009/060265, published as WO2010/018133, incorporated herein by reference

35 Detailed Description of the Invention

It will be appreciated that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallised. These complexes are known as
40 "solvates". For example, a complex with water is known as a "hydrate". Solvents with high boiling points and/or solvents with a high propensity to form hydrogen bonds such as water, ethanol, *iso*-propyl alcohol, and *N*-methyl pyrrolidinone may be used to form solvates. Methods for the identification of solvates include, but are not limited to, NMR and microanalysis. Solvates of the 6-amino-2-
45 {[(1S)-1-methylbutyl]oxy}-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt are within the scope of the invention.

It will be appreciated that 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one will be present primarily in the form of the *S*-isomer, but may include small amounts, for example less than 5%, or less than 3%, and preferably less than 1%, or preferably less than 0.5% of the *R*-isomer. It will be appreciated that maleate salts of these mixtures are
5 considered within the scope of the present invention.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt may exist in tautomeric forms. It will be understood that the present invention encompasses all of the tautomers of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-
10 7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt, whether as individual tautomers or as mixtures thereof.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt may be in crystalline or amorphous form. Furthermore, some of the
15 crystalline forms of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt may exist as polymorphs, which are included within the scope of the present invention. The most thermodynamically stable polymorphic form or forms are of particular interest.

Polymorphic forms of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-
20 8*H*-purin-8-one in the form of a maleate salt may be characterised and differentiated using a number of conventional analytical techniques, including, but not limited to, X-ray powder diffraction (XRPD), infrared spectroscopy (IR), Raman spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and solid-state nuclear magnetic resonance (ssNMR).
25

It will be appreciated from the foregoing that included within the scope of the invention are solvates, hydrates, isomers and polymorphic forms of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt.

Examples of disease states in which 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-
30 7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt may have potentially beneficial effects include allergic diseases and other inflammatory conditions for example allergic rhinitis and asthma, infectious diseases, and cancer. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt is also of potential use
35 as a vaccine adjuvant.

As a modulator of the immune response, 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one in the form of a maleate salt may also be useful, as
40 stand-alone or in combination as an adjuvant, in the treatment and/or prevention of immune-mediated disorders, including but not limited to inflammatory or allergic diseases such as asthma, allergic rhinitis and rhinoconjunctivitis, food allergy, hypersensitivity lung diseases, eosinophilic pneumonitis, delayed-type hypersensitivity disorders, atherosclerosis, pancreatitis, gastritis, colitis, osteoarthritis, psoriasis, sarcoidosis, pulmonary fibrosis, respiratory distress syndrome, bronchiolitis, chronic obstructive pulmonary disease, sinusitis, cystic fibrosis, actinic keratosis, skin
45 dysplasia, chronic urticaria, eczema and all types of dermatitis.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt, may also be useful in the treatment and/or prevention of reactions against respiratory infections, including but not limited to airways viral exacerbations and tonsillitis. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be useful in the treatment and/or prevention of autoimmune diseases including but not limited to rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, Sjögrens disease, ankylosing spondylitis, scleroderma, dermatomyositis, diabetes, graft rejection, including graft-versus-host disease, inflammatory bowel diseases including, but not limited to, Crohn's disease and ulcerative colitis.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be useful in the treatment of infectious diseases including, but not limited to, those caused by hepatitis viruses (e.g. hepatitis B virus, hepatitis C virus), human immunodeficiency virus, papillomaviruses, herpesviruses, respiratory viruses (e.g. influenza viruses, respiratory syncytial virus, rhinovirus, metapneumovirus, parainfluenzavirus, SARS), and West Nile virus. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be useful in the treatment of microbial infections caused by, for example, bacteria, fungi, or protozoa. These include, but are not limited to, tuberculosis, bacterial pneumonia, aspergillosis, histoplasmosis, candidosis, pneumocystosis, leprosy, chlamydia, cryptococcal disease, cryptosporidiosis, toxoplasmosis, leishmania, malaria, and trypanosomiasis.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be useful in the treatment of various cancers, in particular the treatment of cancers that are known to be responsive to immunotherapy and including, but not limited to, renal cell carcinoma, lung cancer, breast cancer, colorectal cancer, bladder cancer, melanoma, leukaemia, lymphomas and ovarian cancer.

It will be appreciated by those skilled in the art that references herein to treatment or therapy may, depending on the condition, extend to prophylaxis as well as the treatment of established conditions.

As mentioned herein, 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be useful as therapeutic agent.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be formulated for administration in any convenient way.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may, for example, be formulated for oral, topical, inhaled, intranasal, buccal, parenteral (for example intravenous, subcutaneous, intradermal, or intramuscular) or rectal administration. In one aspect, 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt is formulated for oral administration. In a further aspect, 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-

8-one in the form of a maleate salt is formulated for topical administration, for example intranasal or inhaled administration.

5 Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch, cellulose or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycollate; or wetting agents such as sodium lauryl sulfate. The tablets may be
10 coated according to methods well known in the art.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such
15 as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or preservatives, for example, methyl or propyl *p*-
20 hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

Compositions for intranasal administration include aqueous compositions administered to the nose by drops or by pressurised pump. Suitable compositions contain water as the diluent or carrier for
25 this purpose. Compositions for administration to the lung or nose may contain one or more excipients, for example one or more suspending agents, one or more preservatives, one or more surfactants, one or more tonicity adjusting agents, one or more co-solvents, and may include components to control the pH of the composition, for example a buffer system. Further, the compositions may contain other excipients such as antioxidants, for example sodium metabisulfite,
30 and taste-masking agents. Compositions may also be administered to the nose or other regions of the respiratory tract by nebulisation. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may offer sufficient solubility and stability for presentation as an aqueous intranasal solution formulation.

35 Intranasal compositions may permit 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt to be delivered to all areas of the nasal cavities (the target tissue) and further, may permit the active compounds to remain in contact with the target tissue for longer periods of time. A suitable dosing regime for intranasal compositions would be for the patient to inhale slowly through the nose subsequent to the nasal cavity being
40 cleared. During inhalation the composition would be administered to one nostril while the other is manually compressed. This procedure would then be repeated for the other nostril. Typically, one or two sprays per nostril would be administered by the above procedure one, two, or three times each day, ideally once daily. Of particular interest are intranasal compositions suitable for once-daily administration.

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The suspending agent(s), if included, will typically be present in an amount of from 0.1 to 5% (w/w), such as from 1.5% to 2.4% (w/w), based on the total weight of the composition. Examples of pharmaceutically acceptable suspending agents include, but are not limited to, Avicel[®] (microcrystalline cellulose and carboxymethylcellulose sodium), carboxymethylcellulose sodium, 5 veegum, tragacanth, bentonite, methylcellulose, xanthan gum, carbopol and polyethylene glycols.

Compositions for administration to the lung or nose may contain one or more excipients may be protected from microbial or fungal contamination and growth by inclusion of one or more preservatives. Examples of pharmaceutically acceptable anti-microbial agents or preservatives 10 include, but are not limited to, quaternary ammonium compounds (for example benzalkonium chloride, benzethonium chloride, cetrimide, cetylpyridinium chloride, lauralkonium chloride and myristyl picolinium chloride), mercurial agents (for example phenylmercuric nitrate, phenylmercuric acetate and thimerosal), alcoholic agents (for example chlorobutanol, phenylethyl alcohol and benzyl alcohol), antibacterial esters (for example esters of para-hydroxybenzoic acid), chelating 15 agents such as disodium edetate (EDTA) and other anti-microbial agents such as chlorhexidine, chlorocresol, sorbic acid and its salts (such as potassium sorbate) and polymyxin. Examples of pharmaceutically acceptable anti-fungal agents or preservatives include, but are not limited to, sodium benzoate, sorbic acid, sodium propionate, methylparaben, ethylparaben, propylparaben and butylparaben. The preservative(s), if included, may be present in an amount of from 0.001 to 20 1% (w/w), such as from 0.015% to 0.5% (w/w) based on the total weight of the composition.

Compositions (for example wherein at least one compound is in suspension) may include one or more surfactants which functions to facilitate dissolution of the medicament particles in the aqueous phase of the composition. For example, the amount of surfactant used is an amount which will not 25 cause foaming during mixing. Examples of pharmaceutically acceptable surfactants include fatty alcohols, esters and ethers, such as polyoxyethylene (20) sorbitan monooleate (Polysorbate 80), macrogol ethers, and poloxamers. The surfactant may be present in an amount of between about 0.01 to 10% (w/w), such as from 0.01 to 0.75% (w/w), for example about 0.5% (w/w), based on the total weight of the composition.

30 One or more tonicity-adjusting agent(s) may be included to achieve tonicity with body fluids e.g. fluids of the nasal cavity, resulting in reduced levels of irritancy. Examples of pharmaceutically acceptable tonicity-adjusting agents include, but are not limited to, sodium chloride, dextrose, xylitol, calcium chloride, glucose, glycerine and sorbitol. A tonicity-adjusting agent, if present, may 35 be included in an amount of from 0.1 to 10% (w/w), such as from 4.5 to 5.5% (w/w), for example about 5.0% (w/w), based on the total weight of the composition.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be buffered by the addition of suitable buffering agents such as sodium 40 citrate, citric acid, trometamol, phosphates such as disodium phosphate (for example the dodecahydrate, heptahydrate, dihydrate and anhydrous forms), or sodium phosphate and mixtures thereof.

A buffering agent, if present, may be included in an amount of from 0.1 to 5% (w/w), for example 1 45 to 3% (w/w) based on the total weight of the composition.

Examples of taste-masking agents include sucralose, sucrose, saccharin or a salt thereof, fructose, dextrose, glycerol, corn syrup, aspartame, acesulfame-K, xylitol, sorbitol, erythritol, ammonium glycyrrhizinate, thaumatin, neotame, mannitol, menthol, eucalyptus oil, camphor, a natural
5 flavouring agent, an artificial flavouring agent, and combinations thereof.

One or more co-solvent(s) may be included to aid solubility of the medicament compound(s) and/or other excipients. Examples of pharmaceutically acceptable co-solvents include, but are not limited to, propylene glycol, dipropylene glycol, ethylene glycol, glycerol, ethanol, polyethylene glycols (for
10 example PEG300 or PEG400), and methanol. In one embodiment, the co-solvent is propylene glycol.

Co-solvent(s), if present, may be included in an amount of from 0.05 to 30% (w/w), such as from 1 to 25% (w/w), for example from 1 to 10% (w/w) based on the total weight of the composition.

15 Compositions for inhaled administration include aqueous, organic or aqueous/organic mixtures, dry powder or crystalline compositions administered to the respiratory tract by pressurised pump or inhaler, for example, reservoir dry powder inhalers, unit-dose dry powder inhalers, pre-metered multi-dose dry powder inhalers, nasal inhalers or pressurised aerosol inhalers, nebulisers or
20 insufflators. Suitable compositions contain water as the diluent or carrier for this purpose and may be provided with conventional excipients such as buffering agents, tonicity modifying agents and the like. Aqueous compositions may also be administered to the nose and other regions of the respiratory tract by nebulisation. Such compositions may be aqueous solutions or suspensions or aerosols delivered from pressurised packs, such as a metered dose inhaler, with the use of a
25 suitable liquefied propellant.

Compositions for administration topically to the nose (for example, for the treatment of rhinitis) or to the lung, include pressurised aerosol compositions and aqueous compositions delivered to the nasal cavities by pressurised pump. Compositions which are non-pressurised and are suitable for
30 administration topically to the nasal cavity are of particular interest. Suitable compositions contain water as the diluent or carrier for this purpose. Aqueous compositions for administration to the lung or nose may be provided with conventional excipients such as buffering agents, tonicity-modifying agents and the like. Aqueous compositions may also be administered to the nose by nebulisation.

35 A fluid dispenser may typically be used to deliver a fluid composition to the nasal cavities. The fluid composition may be aqueous or non-aqueous, but typically aqueous. Such a fluid dispenser may have a dispensing nozzle or dispensing orifice through which a metered dose of the fluid composition is dispensed upon the application of a user-applied force to a pump mechanism of the fluid dispenser. Such fluid dispensers are generally provided with a reservoir of multiple metered
40 doses of the fluid composition, the doses being dispensable upon sequential pump actuations. The dispensing nozzle or orifice may be configured for insertion into the nostrils of the user for spray dispensing of the fluid composition into the nasal cavity. A fluid dispenser of the aforementioned type is described and illustrated in International Patent Application publication number WO 2005/044354 (Glaxo Group Limited). The dispenser has a housing which houses a fluid-discharge
45 device having a compression pump mounted on a container for containing a fluid composition. The

housing has at least one finger-operable side lever which is movable inwardly with respect to the housing to move the container upwardly in the housing by means of a cam to cause the pump to compress and pump a metered dose of the composition out of a pump stem through a nasal nozzle of the housing. In one embodiment, the fluid dispenser is of the general type illustrated in Figures 30-40 of WO 2005/044354.

Aqueous compositions containing 6-amino-2-[[[1S]-1-methylbutyl]oxy]-9-[5-(1-piperidiny)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be delivered by a pump as disclosed in International Patent Application publication number WO2007/138084 (Glaxo Group Limited), for example as disclosed with reference to Figures 22-46 thereof, or as disclosed in United Kingdom patent application number GB0723418.0 (Glaxo Group Limited), for example as disclosed with reference to Figures 7-32 thereof. The pump may be actuated by an actuator as disclosed in Figures 1-6 of GB0723418.0.

Dry powder compositions for topical delivery to the lung by inhalation may, for example, be presented in capsules and cartridges of for example gelatine, or blisters of for example laminated aluminium foil, for use in an inhaler or insufflator. Powder blend compositions generally contain a powder mix for inhalation of a maleate salt of 6-amino-2-[[[1S]-1-methylbutyl]oxy]-9-[5-(1-piperidiny)pentyl]-7,9-dihydro-8H-purin-8-one and a suitable powder base (carrier/diluent/excipient substance) such as mono-, di-, or polysaccharides (for example lactose or starch). Dry powder compositions may also include, in addition to the drug and carrier, a further excipient (for example a ternary agent such as a sugar ester for example cellobiose octaacetate, calcium stearate, or magnesium stearate.

In one embodiment, a composition suitable for inhaled administration may be incorporated into a plurality of sealed dose containers provided on medicament pack(s) mounted inside a suitable inhalation device. The containers may be rupturable, peelable, or otherwise openable one-at-a-time and the doses of the dry powder composition administered by inhalation on a mouthpiece of the inhalation device, as known in the art. The medicament pack may take a number of different forms, for instance a disk-shape or an elongate strip. Representative inhalation devices are the DISKHALER™ and DISKUS™ devices, marketed by GlaxoSmithKline.

A dry powder inhalable composition may also be provided as a bulk reservoir in an inhalation device, the device then being provided with a metering mechanism for metering a dose of the composition from the reservoir to an inhalation channel where the metered dose is able to be inhaled by a patient inhaling at a mouthpiece of the device. Exemplary marketed devices of this type are TURBUHALER™ (AstraZeneca), TWISTHALER™ (Schering) and CLICKHALER™ (Innovata.)

A further delivery method for a dry powder inhalable composition is for metered doses of the composition to be provided in capsules (one dose per capsule) which are then loaded into an inhalation device, typically by the patient on demand. The device has means to rupture, pierce or otherwise open the capsule so that the dose is able to be entrained into the patient's lung when they inhale at the device mouthpiece. As marketed examples of such devices there may be mentioned ROTAHALER™ (GlaxoSmithKline) and HANDIHALER™ (Boehringer Ingelheim.)

Pressurised aerosol compositions suitable for inhalation can be either a suspension or a solution and may contain a maleate salt of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one and a suitable propellant such as a fluorocarbon or hydrogen-containing chlorofluorocarbon or mixtures thereof, particularly hydrofluoroalkanes, especially 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3,3-heptafluoro-n-propane or a mixture thereof. The aerosol composition may optionally contain additional composition excipients well known in the art such as surfactants e.g. oleic acid, lecithin or an oligolactic acid or derivative thereof e.g. as described in WO 94/21229 and WO 98/34596 (Minnesota Mining and Manufacturing Company) and co-solvents e.g. ethanol. Pressurised compositions will generally be retained in a canister (e.g. an aluminium canister) closed with a valve (e.g. a metering valve) and fitted into an actuator provided with a mouthpiece.

Ointments, creams and gels, may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agent and/or solvents. Such bases may thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil, or a solvent such as polyethylene glycol. Thickening agents and gelling agents which may be used according to the nature of the base include soft paraffin, aluminium stearate, cetostearyl alcohol, polyethylene glycols, wool-fat, beeswax, carboxypolymethylene and cellulose derivatives, and/or glyceryl monostearate and/or non-ionic emulsifying agents.

Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents or thickening agents.

Powders for external application may be formed with the aid of any suitable powder base, for example, talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents, suspending agents or preservatives.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may, for example, be formulated for transdermal delivery by composition into patches or other devices (e.g. pressurised gas devices) which deliver the active component into the skin.

For buccal administration the compositions may take the form of tablets or lozenges formulated in the conventional manner.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small

volume infusions or pre-filled syringes, or in multidose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or tonicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be formulated with vaccines as adjuvants to modulate their activity. Such compositions may contain antibody(ies) or antibody fragment(s) or an antigenic component including but not limited to protein, DNA, live or dead bacteria and/or viruses or virus-like particles, together with one or more components with adjuvant activity including but not limited to aluminium salts, oil and water emulsions, heat shock proteins, lipid A preparations and derivatives, glycolipids, other TLR agonists such as CpG DNA or similar agents, cytokines such as GM-CSF or IL-12 or similar agents.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be employed alone or in combination with other therapeutic agents. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt and the other pharmaceutically active agent(s) may be administered together or separately and, when administered separately, administration may occur simultaneously or sequentially, in any order. The amounts of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt and the other pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. The administration of a combination of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt with other treatment agents may be by administration concomitantly in a unitary pharmaceutical composition including both compounds, or in separate pharmaceutical compositions each including one of the compounds. Alternatively, the combination may be administered separately in a sequential manner wherein one treatment agent is administered first and the other second or *vice versa*. Such sequential administration may be close in time or remote in time.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be used in combination with one or more agents useful in the prevention or treatment of viral infections. Examples of such agents include, without limitation; polymerase inhibitors such as those disclosed in WO 2004/037818-A1, as well as those disclosed in WO 2004/037818 and WO 2006/045613; JTK-003, JTK-019, NM-283, HCV-796, R-803, R1728, R1626, as well as those disclosed in WO 2006/018725, WO 2004/074270, WO 2003/095441, US2005/0176701, WO 2006/020082, WO 2005/080388, WO 2004/064925, WO 2004/065367, WO 2003/007945, WO 02/04425, WO 2005/014543, WO 2003/000254, EP 1065213, WO 01/47883, WO 2002/057287, WO 2002/057245 and similar agents; replication inhibitors such as acyclovir, famciclovir, ganciclovir, cidofovir, lamivudine and similar agents; protease inhibitors such as the HIV protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, fosamprenavir,

brecanavir, atazanavir, tipranavir, palinavir, lasinavir, and the HCV protease inhibitors BILN2061, VX-950, SCH503034; and similar agents; nucleoside and nucleotide reverse transcriptase inhibitors such as zidovudine, didanosine, zalcitabine, abacavir, stavidine, adefovir, adefovir dipivoxil, fozivudine, todoxil, emtricitabine, alovudine, amdoxovir, elvucitabine, and similar agents;

5 non-nucleoside reverse transcriptase inhibitors (including an agent having anti-oxidation activity such as immunocal, oltipraz etc.) such as nevirapine, delavirdine, efavirenz, loviride, immunocal, oltipraz, capravirine, TMC-278, TMC-125, etravirine, and similar agents; entry inhibitors such as enfuvirtide (T-20), T-1249, PRO-542, PRO-140, TNX-355, BMS-806, 5-Helix and similar agents; integrase inhibitors such as L-870,180 and similar agents; budding inhibitors such as PA-344 and

10 PA-457, and similar agents; chemokine receptor inhibitors such as vicriviroc (Sch-C), Sch-D, TAK779, maraviroc (UK-427,857), TAK449, as well as those disclosed in WO 02/74769, WO 2004/054974, WO 2004/055012, WO 2004/055010, WO 2004/055016, WO 2004/055011, and WO 2004/054581, and similar agents; neuraminidase inhibitors such as CS-8958, zanamivir, oseltamivir, peramivir and similar agents; ion channel blockers such as amantadine or rimantadine

15 and similar agents; and interfering RNA and antisense oligonucleotides and such as ISIS-14803 and similar agents; antiviral agents of undetermined mechanism of action, for example those disclosed in WO 2005/105761, WO 2003/085375, WO 2006/122011, ribavirin, and similar agents. 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidiny)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be used in combination with one or more other agents which may

20 be useful in the prevention or treatment of viral infections for example immune therapies (e.g. interferon or other cytokines/chemokines, cytokine/chemokine receptor modulators, cytokine agonists or antagonists and similar agents); and therapeutic vaccines, antifibrotic agents, anti-inflammatory agents such as corticosteroids or NSAIDs (non-steroidal anti-inflammatory agents) and similar agents.

25 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidiny)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be used in combination with one or more other agents which may be useful in the prevention or treatment of allergic disease, inflammatory disease, autoimmune disease, for example; antigen immunotherapy, anti-histamines, steroids, NSAIDs, bronchodilators

30 (e.g. beta 2 agonists, adrenergic agonists, anticholinergic agents, theophylline), methotrexate, leukotriene modulators and similar agents; monoclonal antibody therapy such as anti-IgE, anti-TNF, anti-IL-5, anti-IL-6, anti-IL-12, anti-IL-1 and similar agents; receptor therapies e.g. entanercept and similar agents; antigen non-specific immunotherapies (e.g. interferon or other cytokines/chemokines, cytokine/chemokine receptor modulators, cytokine agonists or antagonists,

35 TLR agonists and similar agents).

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidiny)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may be used in combination with one or more other agents which may be useful in the prevention or treatment of cancer, for example chemotherapeutics such as alkylating

40 agents, topoisomerase inhibitors, antimetabolites, antimitotic agents, kinase inhibitors and similar agents; monoclonal antibody therapy such as trastuzumab, gemtuzumab and other similar agents; and hormone therapy such as tamoxifen, goserelin and similar agents.

The pharmaceutical compositions according to the invention may also be used alone or in

45 combination with at least one other therapeutic agent in other therapeutic areas, for example

gastrointestinal disease. The compositions according to the invention may also be used in combination with gene replacement therapy.

5 The invention includes in a further aspect a combination comprising 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, in the form of a maleate salt, together with at least one other therapeutically active agent.

10 The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical composition and thus pharmaceutical compositions comprising a combination as defined above together with at least one pharmaceutically acceptable diluent or carrier thereof represent a further aspect of the invention.

15 A therapeutically effective amount of a maleate salt of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one will depend upon a number of factors. For example, the species, age, and weight of the recipient, the precise condition requiring treatment and its severity, the nature of the composition, and the route of administration are all factors to be considered. The therapeutically effective amount ultimately should be at the discretion of the attendant physician. Regardless, an effective amount of a maleate salt of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one for the treatment of humans
20 suffering from frailty, generally, should be in the range of 0.0001 to 100 mg/kg body weight of recipient per day. More usually the effective amount should be in the range of 0.001 to 10 mg/kg body weight per day. Thus, for a 70 kg adult one example of an actual amount per day would usually be from 7 to 700 mg. For intranasal and inhaled routes of administration, typical doses for a 70 kg adult should be in the range of 1 microgramme to 1mg per day. This amount may be given in
25 a single dose per day or in a number (such as two, three, four, five, or more) of sub-doses per day such that the total daily dose is the same. Similar dosages should be appropriate for treatment of the other conditions referred to herein.

30 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be administered at any appropriate frequency e.g. 1-7 times per week. The precise dosing regimen will of course depend on factors such as the therapeutic indication, the age and condition of the patient, and the particular route of administration chosen.

35 Pharmaceutical compositions may be presented in unit-dose forms containing a predetermined amount of active ingredient per unit dose. Such a unit may contain, as a non-limiting example, 0.5 mg to 1 g of a maleate salt of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, depending on the condition being treated, the route of administration, and the age, weight, and condition of the patient. Preferred unit-dosage compositions are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of
40 an active ingredient. Such pharmaceutical compositions may be prepared by any of the methods well-known in the pharmacy art.

There is thus further provided a pharmaceutical composition comprising 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, in the form of a maleate

salt, and one or more pharmaceutically acceptable diluents or carriers. Optionally, the pharmaceutical composition may further comprise at least one other therapeutically active agent.

There is also provided a process for preparing such a pharmaceutical composition which comprises admixing 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, in the form of a maleate salt, with one or more pharmaceutically acceptable diluents or carriers.

There is also provided a process for preparing a maleate salt of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one comprising reacting 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one with a source of maleate anion (for example, maleic acid e.g. in a suitable solvent) to produce 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt. In one aspect, the process produces a 1:1 ratio of maleate anion:6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one.

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, in the form of a maleate salt may be prepared by the methodology described hereinafter.

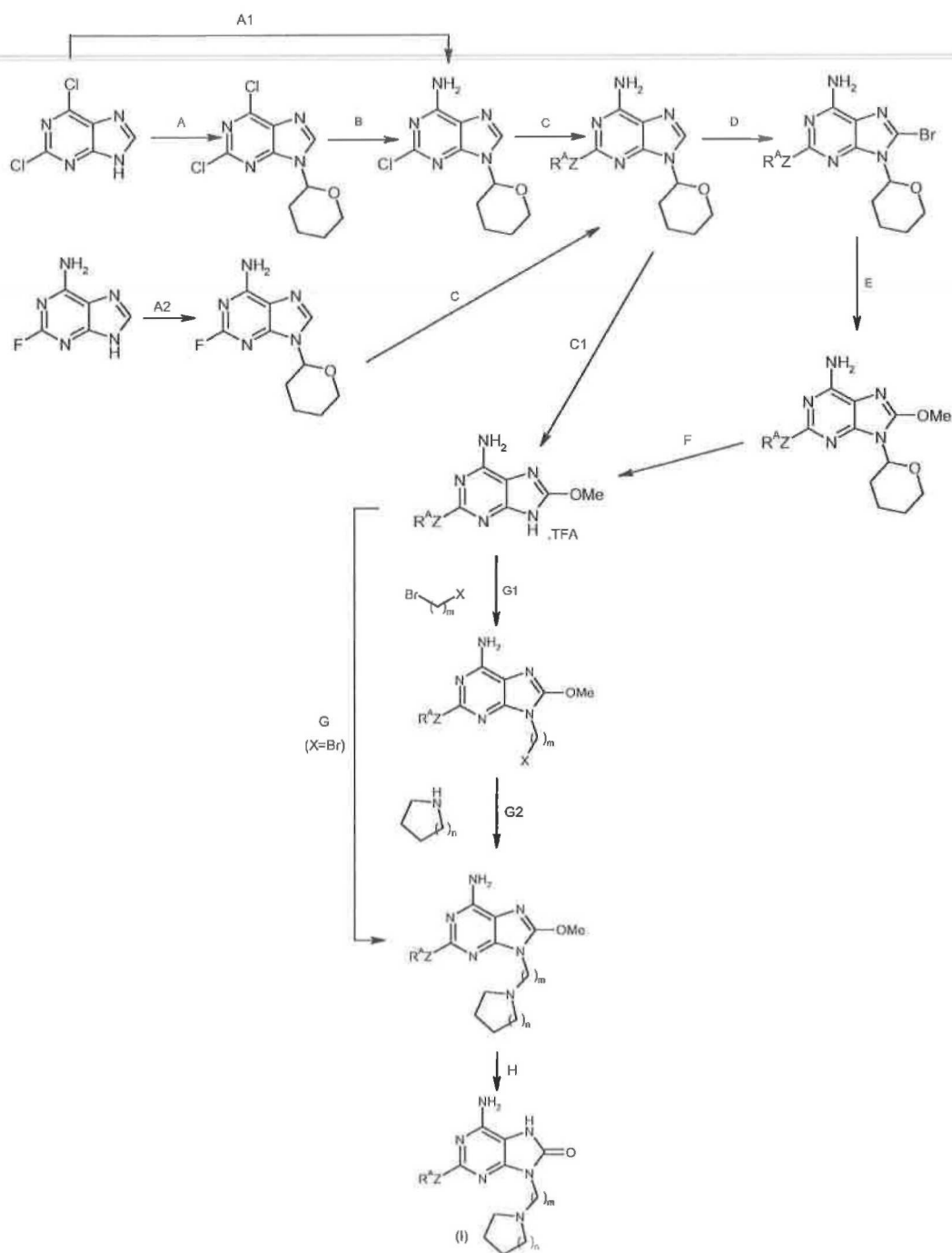
Abbreviations

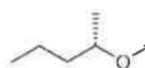
The following list provides definitions of certain abbreviations as used herein. It will be appreciated that the list is not exhaustive, but the meaning of those abbreviations not herein below defined will be readily apparent to those skilled in the art.

DMF	<i>N,N'</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
HPLC	High performance liquid chromatography
MDAP HPLC	Reverse phase HPLC on a C ₁₈ column using a
two-solvent gradient and analysis of the fractions by electrospray mass spectroscopy.	
SPE	Solid phase extraction
min	minutes
Stripped	Removal of solvent under reduced pressure
TFA	Trifluoroacetic acid
rt	room temperature
vol	volumes
BSA	<i>N,O</i> -bis(Trimethylsilyl)acetamide
CPME	Cyclopentyl methyl ether
TBME	tert-Butyl methyl ether
MeTHF	2-Methyl tetrahydrofuran
NMP	<i>N</i> -Methyl pyrrolidinone
DCM	Dichloromethane

The synthetic process to make 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, and maleate salts thereof are summarised in Scheme 1.

Scheme 1



in which $R^A Z =$ , $n = 2$, $m = 5$ and $X = Cl$.

5

Typical reaction conditions for each of the synthetic steps of Scheme 1 are provided below:

A: Dihydropyran/paratoluene sulfonic acid, e.g. 50 °C for 3-6 hours.

- A1: Dihydropyran/paratoluene sulfonic acid, e.g. 50 °C for 1 hour, then ammonia/isopropanol, e.g. 60 °C for 4 hours, then add water and cool to ambient temperature over 12-18 hours.
- 5 A2: BSA in acetonitrile, reflux, cool to 0 °C, then tetrahydropyran acetate in acetonitrile, warm to 10 °C, then aqueous sodium hydrogen carbonate.
- B: Ammonia/isopropanol, e.g. 50 °C for 5 hours, then ambient temperature for 12-18 hours, then 50 °C for 9 hours.
- 10 C: For Z = O, R^A = C₁₋₆alkyl: R^AONa/butanol/dimethoxyethane e.g. 93-110 °C for 12-18 hours.
- C1: N-Bromosuccinimide in dichloromethane e.g. 0-5 °C for 30 minutes then ambient temperature for 0.5-1 hour, then e.g. sodium methoxide/methanol under nitrogen/60-70 °C/12-18 hours, then TFA/methanol e.g. ambient temperature for 18-65 hours.
- 15 D: N-Bromosuccinimide in dichloromethane e.g. 0-5 °C for 30 minutes then ambient temperature for 36-48 hours, or N-bromosuccinimide in chloroform at <5 °C for 4-6 hours.
- E: Sodium methoxide/methanol e.g. reflux 4-6 hours.
- 20 F: TFA/methanol e.g. ambient temperature for 18-65 hours, or TFA/methanol e.g. ambient temperature for 70-74 hours.
- G: Potassium carbonate/DMF then 50 °C for 1-1.5 hours, then add (VI), stir 40 minutes, then add (IV)/triethylamine, then ambient temperature for 18 hours.
- 25 G1: Potassium carbonate/DMF, then 50 °C under nitrogen for 30 minutes, then ambient temperature, add (VI), stir for 20 hours.
- 30 G2: Solution in DMF with N,N'-diisopropylethylamine, then 50 °C for 48 hours, then more (IV) added then further 50 °C for 48 hours.
- H: Hydrogen chloride/methanol, then ambient temperature for 18 hours.
- 35 Compounds of formulae (IV), (VI), (XIA), (XII), (XIII), (XIV), and (XV), are either known in the literature or are commercially available, for example from *Sigma-Aldrich, UK*, or may be prepared by analogy with known procedures, for example those disclosed in standard reference texts of synthetic methodology such as *J. March, Advanced Organic Chemistry, 6th Edition (2007), WileyBlackwell*, or *Comprehensive Organic Synthesis (Trost B.M. and Fleming I., (Eds.), Pergamon Press, 1991)*, each incorporated herein by reference as it relates to such procedures.
- 40

6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one in the form of a maleate salt may also be prepared more generally by reacting 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one with a source of the maleate

anion in a suitable solvent e.g isopropyl alcohol. A suitable source of the maleate anion is maleic acid or maleic acid salts.

5 Examples of other protecting groups that may be employed in the synthetic routes described herein and the means for their removal can be found in *T. W. Greene 'Protective Groups in Organic Synthesis', 4th Edition, J. Wiley and Sons, 2006*, incorporated herein by reference as it relates to such procedures.

10 For any of the hereinbefore described reactions or processes, conventional methods of heating and cooling may be employed, for example temperature-regulated oil-baths or temperature-regulated hot-blocks, and ice/salt baths or dry ice/acetone baths respectively. Conventional methods of isolation, for example extraction from or into aqueous or non-aqueous solvents may be used. Conventional methods of drying organic solvents, solutions, or extracts, such as shaking with anhydrous magnesium sulfate, or anhydrous sodium sulfate, or passing through a hydrophobic frit,
15 may be employed. Conventional methods of purification, for example crystallisation and chromatography, for example silica chromatography or reverse-phase chromatography, may be used as required. Crystallisation may be performed using conventional solvents such as ethyl acetate, methanol, ethanol, or butanol, or aqueous mixtures thereof. It will be appreciated that specific reaction times temperatures may typically be determined by reaction-monitoring
20 techniques, for example thin-layer chromatography and LC-MS.

Where appropriate, individual isomeric forms 6-amino-2-([(1*S*)-1-methylbutyl]oxy)-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one may be prepared as individual isomers using conventional procedures such as the fractional crystallisation of diastereoisomeric derivatives or
25 chiral high performance liquid chromatography (chiral HPLC).

The absolute stereochemistry of compounds may be determined using conventional methods, such as X-ray crystallography.

30 General Experimental Details

Compounds were named using ACD/Name PRO 6.02 chemical naming software from Advanced Chemistry Developments Inc., Toronto, Ontario, M5H2L3, Canada.

35 Experimental details of LCMS systems B-D as referred to herein are as follows:

System B

Column: 30mm x 4.6mm ID, 3.5µm Sunfire C₁₈ column

Flow Rate: 3mL/min.

40 Temp: 30°C

UV detection range: 210 to 350nm

Mass spectrum: Recorded on a mass spectrometer using alternative-scan positive and negative mode electrospray ionisation

45 Solvents: A: 0.1% v/v solution of formic acid in water

B: 0.1% v/v solution of formic acid in acetonitrile

Gradient:	<u>Time (min.)</u>	<u>A%</u>	<u>B%</u>
5	0	97	3
	0.1	97	3
	4.2	0	100
	4.8	0	100
	4.9	97	3
	5.0	97	3

10

System C

Column: 50mm x 2.1mm ID, 1.7 μ m Acquity UPLC BEH C₁₈

Flow Rate: 1mL/min.

Temp: 40°C

15 UV detection range: 210 to 350nm

Mass spectrum: Recorded on a mass spectrometer using alternative-scan positive and negative mode electrospray ionisation

20 Solvents: A: 10mM ammonium bicarbonate in water adjusted to pH10 with ammonia solution
B: acetonitrile

Gradient:	<u>Time (min.)</u>	<u>A%</u>	<u>B%</u>
25	0	99	1
	1.5	3	97
	1.9	3	97
	2.0	0	100

System D

Column: 50mm x 4.6mm ID, 3.5 μ m XBridge C₁₈ column

30 Flow Rate: 3mL/min.

Temp: 30°C

UV detection range: 210 to 350nm

Mass spectrum: Recorded on a mass spectrometer using alternative-scan positive and negative mode electrospray ionisation

35

Solvents: A: 10mM ammonium bicarbonate in water adjusted to pH10 with ammonia solution
B: acetonitrile

Gradient:	<u>Time (min.)</u>	<u>A%</u>	<u>B%</u>
40	0	99	1
	0.1	99	1
	4.0	3	97
	5.0	3	97

Chromatographic purification was typically performed using pre-packed silica gel cartridges. The Flashmaster II is an automated multi-user flash chromatography system, available from Argonaut Technologies Ltd, which utilises disposable, normal phase, Solid Phase Extraction (SPE) cartridges (2 g to 100 g). It provides quaternary on-line solvent mixing to enable gradient methods to be run.

5 Samples are queued using the multi-functional open access software, which manages solvents, flow-rates, gradient profile and collection conditions. The system is equipped with a Knauer variable wavelength UV-detector and two Gilson FC204 fraction-collectors enabling automated peak cutting, collection and tracking.

10 Solvent removal using a stream of nitrogen was performed at 30-40°C on a GreenHouse Blowdown system available from Radleys Discovery Technologies Saffron Walden, Essex, CB11 3AZ, UK

¹H NMR spectra were recorded in either CDCl₃ or DMSO-*d*₆ on either a Bruker DPX 400 or Bruker Avance DRX or Varian Unity 400 spectrometer all working at 400 MHz. The internal standard used
15 was either tetramethylsilane or the residual protonated solvent at 7.25 ppm for CDCl₃ or 2.50 ppm for DMSO-*d*₆.

Mass directed autoperparative HPLC was undertaken under the conditions given below. The UV detection was an averaged signal from wavelength of 210nm to 350nm and mass spectra were
20 recorded on a mass spectrometer using alternate-scan positive and negative mode electrospray ionization.

Method A

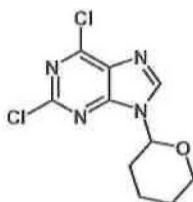
25 Method A was conducted on an XBridge C₁₈ column (typically 150mm x 19mm i.d. 5µm packing diameter) at ambient temperature. The solvents employed were:

A = 10 mM aqueous ammonium bicarbonate adjusted to pH 10 with ammonia solution.

B = acetonitrile.

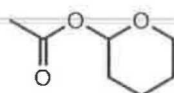
30 Intermediates

Intermediate 1: 2,6-Dichloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine

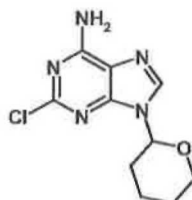


35 To 2,6-dichloropurine (25.0 g) (commercially available from, for example, Aldrich) was added ethyl acetate (260 mL), followed by *p*-toluenesulfonic acid (0.253 g). The mixture was heated to 50 °C and then 3,4-dihydro-2H-pyran (16.8g) (commercially available from, for example, Aldrich) was added. The reaction mixture was then heated at 50 °C for 4 hours. The reaction mixture was
40 evaporated *in vacuo* to give the *title compound* as a yellow solid (36.9 g).

¹H NMR (CDCl₃): 8.35 (1H, s), 5.77 (1H, dd), 4.20 (1H, m), 3.79 (1H, m), 2.20-1.65 (6H, m).

Intermediate 1A

5 Acetic acid (1.2 L, 1 eq) and pyridinium p-toluene sulfonate (530 g, 0.1 eq), were dissolved in dichloromethane (6 L). The solution was cooled to 0 °C. A solution of the dihydropyran (2.52 L, 1.35 eq) in dichloromethane (2.5 L) was charged cautiously over at least 15 mins. keeping the temperature below 5 °C. Once the addition was complete, the solution was warmed to 20 °C and stirred overnight. Water (5.0 L) was charged and the resultant biphasic was stirred vigorously before removing the aqueous layer. The organic phase was then washed with saturated sodium bicarbonate solution (5.0 L) and dried over magnesium sulfate. The dried organic phases were concentrated on the rotary evaporator, reducing the pressure to 20 mbar at 50 °C to ensure removal of DCM and excess dihydropyran. The product was afforded as a colourless to slightly yellow liquid (2.61 kg, 95% theoretical yield).

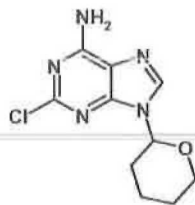
Intermediate 2: 2-Chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine

20 2,6-Dichloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (36.9 g) (for example, as prepared for Intermediate 1) was heated with 2M ammonia in isopropanol (250 mL) at 50 °C for 5 hours. After standing at ambient temperature overnight, a further quantity of 2M ammonia in isopropanol (100 mL) was added to break up the resultant cake and the reaction mixture was heated for a further 9 hours until the reaction was complete. To the reaction mixture was added water (70 mL) and the yellow solid filtered off. The solid was washed with isopropyl alcohol:water (5:1 (v/v), 60 mL) and then air-dried under suction to give a first crop. The filtrate was re-filtered after standing overnight to isolate precipitate and both solids were dried *in vacuo*. The first crop was pure with the second crop material showing a very minor impurity (isolated broad signal 3.5 ppm not seen in first crop) but was otherwise identical. Solid first crop (28.4 g), solid second crop (3.42 g).

30 ¹H NMR (CDCl₃): 8.01 (1H, s), 5.98 (2H, broad s), 5.70 (1H, dd), 4.16 (1H, m), 3.78 (1H, m), 2.15-1.60 (6H, overlapping m).

Intermediate 2 (alternative method): 2-Chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine

35



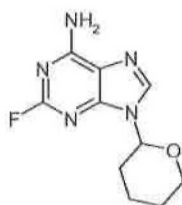
To a solution of 2,6-dichloropurine (25g) (commercially available from, for example, Aldrich) in dry ethyl acetate (200 mL) was added *p*-toluenesulfonic acid monohydrate (235mg) (commercially available from, for example, Aldrich). The reaction was heated to 50 °C and 3,4-dihydro-2*H*-pyran (18.1ml) (commercially available from, for example, Aldrich) was added in one go. The reaction was allowed to stir at 50 °C for 1 hour and the solvent was removed under reduced pressure. This afforded a yellow solid. A suspension of this solid (~36 g) in 2.0M ammonia in isopropanol (460 mL) was heated under nitrogen at 60 °C for 4 hours with an attached condenser. The reaction was poured into water (50 mL) and left to cool overnight. The precipitate was filtered and dried on a rotary evaporator (60 °C) for 30 minutes to afford the *title compound* as an off-white solid, 31 g (93%, 2 steps).

MS calcd for (C₁₀H₁₂ClN₅O)⁺ = 254, 256

MS found (electrospray): (M)⁺ = 254, 256 (3:1)

¹H NMR ((CD₃)₂SO): δ 8.43 (1H, s), 7.82 (2H, s), 5.55 (1H, dd), 4.00 (1H, m), 3.69 (1H, m), 2.21 (1H, m), 1.95 (2H, m), 1.74 (1H, m), 1.56 (2H, m).

Intermediate 3: 2-Fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-6-amine



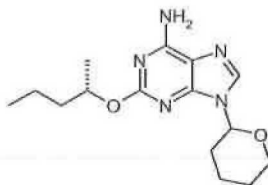
20

N,O-bis(trimethylsilyl)acetamide (975 mL, 3.988 mol) was added to a stirred suspension of 2-fluoro-1*H*-purin-6-amine (200 g, 1.306 mmol) (commercially available from, for example, AlliedSignal) in anhydrous acetonitrile (4 L) in a 10L controlled lab reactor and the resulting mixture heated to reflux and maintained at that temperature for 2 hours. The circulator was then re-programmed and the reaction mixture cooled to 0 °C. A solution of tetrahydropyranyl acetate (preparation described in *Tetrahedron Letters*, 2006, 47(27), 4741 and also described in Intermediate 1A) (282 g, 1.959 mol) in anhydrous acetonitrile (500 mL) was then added slowly *via* a dropping funnel followed by trimethylsilyl trifluoromethanesulfonate (283 mL, 1.567 mol) dropwise *via* a dropping funnel. No significant exotherm was observed. The circulator temperature was re-adjusted to 10 °C and stirring maintained for a further 1 hour. The mixture was then quenched by addition of 1M sodium carbonate (4 L). A solid precipitate was observed and the pH checked to be basic. Additional water was added to the suspension (1 L) and on standing the layers separated with the aqueous layer containing significant solid inorganics. The majority of the aqueous and inorganic solid was separated. The organic layer still contained significant solid and was cooled to 0 °C with stirring to encourage further precipitation. The solid was collected by filtration and the pad was washed

very well with water then dried *in vacuo* at 40 °C overnight to give the *title compound* as a cream coloured solid (152.8 g).

LCMS (System D): $t_{\text{RET}} = 1.71 \text{ min}$; $\text{MH}^+ = 238$

5 Intermediate 4: 2-[[[(1S)-1-Methylbutyl]oxy]-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine



Method A

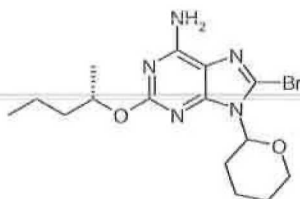
- 10 Sodium *tert*-butoxide (48.5 g, 505 mmol) was added portionwise to (S)-2-pentanol (185ml) (commercially available from, for example, Julich Chiral Solutions) at room temperature stirred until homogeneous (Note: reaction is exothermic). 2-Chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (32 g, 126 mmol) (for example, as prepared for Intermediate 2) was added and the reaction mixture heated at 70 °C for 72 hours. The reaction was cooled to room temperature and partitioned
- 15 between ethyl acetate (500 mL) and water (500 mL). The organic phase was washed with saturated sodium chloride solution (100 mL), dried (MgSO_4), filtered and evaporated. The residue was triturated with ether and the solid material filtered. The precipitate was re-washed with ether and the filtrates combined and evaporated. The crude material (approximately 30 g) was dissolved in DMSO:methanol (1:1) and purified by chromatography on a reverse phase (C_{18}) column (330 g)
- 20 using a gradient of 25-65% acetonitrile (+ 0.1%TFA)-water(+ 0.1%TFA) over 8 column volumes, the fractions were immediately neutralised with saturated aqueous sodium carbonate solution. Appropriate fractions were combined and partitioned between dichloromethane and saturated aqueous sodium hydrogen carbonate. The organic phase was dried by passage through a hydrophobic frit, filtered and evaporated to give the *title compound* as a pale cream foam (14.97 g).
- 25 LCMS (System B): $t_{\text{RET}} = 2.21 \text{ min}$; $\text{MH}^+ = 306$

Method B

- Sodium *tert*-butoxide (206 g, 2.144 mol) was added to (S)-2-pentanol (720 mL, 6.58 mol) (commercially available from, for example, Julich Chiral Solutions) in a 2L round bottomed flask.
- 30 The mixture was stirred at 50 °C until all the sodium *tert*-butoxide had dissolved. 2-Fluoro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (for example, as prepared for Intermediate 3) (130 g, 548 mmol) was then added in portions over 5 minutes. After 3 hours, LCMS analysis indicated complete consumption of the starting material and the mixture was poured into ice/water (3 L) and then extracted with methyl *tert*-butyl ether. This resulted in emulsion formation and the mixture was
- 35 filtered through Celite and the organic phase was separated. The aqueous layer was then treated with solid NaCl and then re-extracted with methyl *tert*-butyl ether. The organic extracts were combined and washed with brine, dried over magnesium sulfate, filtered and then evaporated to yield the title compound as a pale brown gum (158.59g).
- LCMS (System D): $t_{\text{RET}} = 2.65 \text{ min}$; $\text{MH}^+ = 306$

40

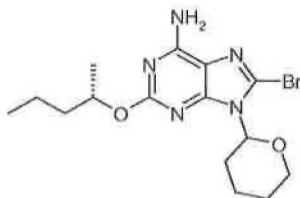
Intermediate 5: 8-Bromo-2-[[[(1S)-1-methylbutyl]oxy]-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine



- 5 N-Bromosuccinimide (12.16g, 68.3mmol) was added portionwise over 5 minutes to a stirred solution of 2-([(1S)-1-methylbutyl]oxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (14.9 g, 48.8 mmol) (for example, as prepared for Intermediate 4) in chloroform (80 mL) at <5 °C under an atmosphere of nitrogen. The reaction mixture was stirred at <5 °C for 5 hours then washed with saturated sodium hydrogen carbonate solution (80 mL) then water (80 mL). The foam was dissolved in dichloromethane (50 mL) and washed with water (50 mL) then brine (50 mL). The combined aqueous phases were washed with dichloromethane (50 mL). The combined organic layers were dried through a hydrophobic frit, and the solvent removed *in vacuo* to yield the *title compound* as an orange foam (18.5 g).

LCMS (System D): $t_{\text{RET}} = 3.06\text{min}$; MH^+ 384/386

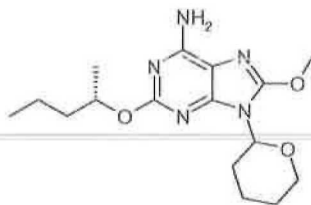
- 15 Intermediate 5 (alternative method): 8-Bromo-2-([(1S)-1-methylbutyl]oxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine



- 20 2-([(1S)-1-Methylbutyl]oxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (1050 g) was dissolved in DCM (10.5 L) to give a yellow/orange solution which was cooled to 0°C. N-Bromosuccinimide (922 g, 1.5 eq) was charged in 3 equal portions 20 mins. apart and the resultant reaction mixture was stirred at 0-5 °C for 4 hours. The reaction was then quenched by addition of a solution of 500g sodium thiosulfate pentahydrate in 5.0 L water. The resultant biphasic mixture was mixed thoroughly at 20 °C and then the phases were separated. The organics were washed again with a solution of 500g sodium thiosulfate pentahydrate in 5.0 L water then 500 g dipotassium phosphate in 5.0 L water and finally with 5.0 L water. The organic phase was dried over magnesium sulfate (822 g) and evaporated on a rotary evaporator until foaming became prohibitive. The mixture was then solvent-exchanged into methanol by repeated addition and removal of methanol until sufficient DCM had been removed (as confirmed by NMR). The product was afforded as a red/brown gum containing entrained solvent (1.28 kg corrected for solvent, 96% theoretical yield).

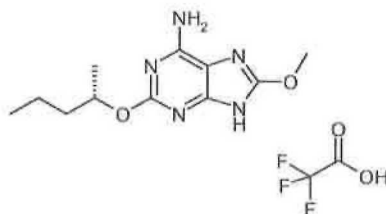
Intermediate 6: 2-([(1S)-1-Methylbutyl]oxy)-8-(methoxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine

35



8-Bromo-2-([(1S)-1-methylbutyl]oxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (for example, as prepared for Intermediate 5) (7.1 g, 18.48 mmol) was dissolved in anhydrous methanol (70 mL) and a solution of sodium methoxide (25%) in methanol (8 mL) was added dropwise under an atmosphere of nitrogen. The solution was heated to reflux at 90 °C for 4 hours under an atmosphere of nitrogen. Additional sodium methoxide in methanol (25% solution, 3 mL) was added and the reaction was stirred at 60 °C for a further 16 hours. An additional portion of sodium methoxide in methanol (25% solution, 5 mL) was added and the reaction was stirred at 90 °C for a further 7 hours. The solvent was removed on the rotary evaporator and the crude product was partitioned between ethyl acetate (75 mL) and saturated ammonium chloride solution (75 mL). The organic layer was washed with brine (75 mL). The solvent was removed on the rotary evaporator to yield the *title compound* as a pale orange foam (6 g).
LCMS (System C): $t_{\text{RET}} = 1.14$ min; MH^+ 336, 337

Intermediate 7: 2-([(1S)-1-Methylbutyl]oxy)-8-(methyloxy)-9H-purin-6-amine, trifluoroacetate salt



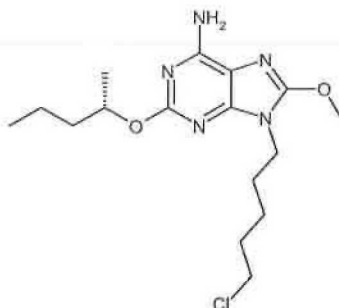
2-([(1S)-1-Methylbutyl]oxy)-8-(methyloxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (6 g, 17.89 mmol) (for example as prepared for Intermediate 6) was dissolved in methanol (50 mL). Trifluoroacetic acid (20.67 mL, 268 mmol) was added dropwise, and the mixture stirred at 20 °C for 72 hours under an atmosphere of nitrogen. The solvent was removed *in vacuo*, and the resulting solid was washed with ethyl acetate and filtered. The filtrate was stripped and the residue washed with ethyl acetate. The combined solid residues were dried in the vacuum oven for 2 hours to give the *title compound* as an off white solid (5.3 g).
LCMS (System C): $t_{\text{RET}} = 0.76$ min; MH^+ 252, 253

Intermediate 7 (alternative method): 2-([(1S)-1-Methylbutyl]oxy)-8-(methyloxy)-9H-purin-6-amine, trifluoroacetate salt

8-Bromo-2-([(1S)-1-methylbutyl]oxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (1.26 kg, corrected for residual solvent) was dissolved in anhydrous methyl tetrahydrofuran (MeTHF) (11.4 L) and 25% sodium methoxide in methanol (2.65 L, 3.5eq) was added. The resultant reaction mixture was heated to 65 +/- 5 °C for 3 hrs. The complete reaction was cooled to room temperature and washed with 20%w/v aqueous ammonium chloride solution (2 x 6.3 L) and brine (6.3 L). The organic phase was dried with MgSO_4 (1.8 kg) and filtered, washing through with MeTHF (6.3 L).

- The combined organic phases were evaporated to 6.3 L by vacuum distillation. MeOH (2.5 L) and TFA (1.26 L, 5eq) were added and the mixture heated to 60°C for 1.5 hours. Cyclopentyl methyl ether (CPME) (6.3 L) was added and the mixture reduced to 6.3 L by vacuum distillation. CPME (6.3 L) was again added and the reaction further concentrated to 6.3 L, when solids precipitated.
- 5 The slurry was cooled to 10°C then aged for 30 min. The product was filtered and washed with TBME (2 x 3.8 L) and dried *in vacuo* at 40°C to afford a white solid (886 g, 74% theoretical yield).

Intermediate 8: 9-(5-Chloropentyl)-2-[(1S)-1-methylbutyl]oxy}-8-(methyloxy)-9H-purin-6-amine



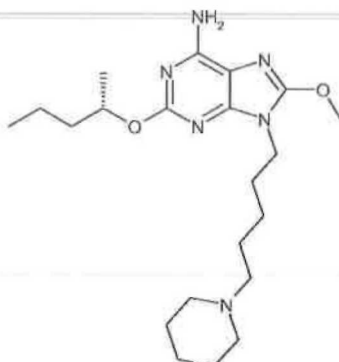
10

- 2-[(1S)-1-Methylbutyl]oxy}-8-(methyloxy)-9H-purin-6-amine, trifluoroacetate salt (600 mg, 1.642 mmol) (for example, as prepared for Intermediate 7) and potassium carbonate (567 mg, 4.11 mmol) were stirred at 60 °C in DMF (10 mL) for 1 hour under nitrogen. The reaction was cooled to room
- 15 temperature when 1-bromo-5-chloropentane (commercially available, for example, from Aldrich) (0.216 mL, 1.642 mmol) and triethylamine (0.343 mL, 2.464 mmol) were added and the mixture stirred at 20 °C under nitrogen for 16 hours. The mixture was then diluted with water (10 mL) and brine (10 mL) and extracted with dichloromethane (2 x 10 mL). The combined organic extracts were evaporated and the residue dissolved in dichloromethane and purified by column
- 20 chromatography using the Flashmaster II (70 g aminopropyl cartridge) with a 0-100% ethyl acetate in cyclohexane gradient over 40 minutes. The appropriate fractions were combined and evaporated *in vacuo* to give the *title compound* as a yellow gum (430 mg).
- LCMS (System D): $t_{\text{RET}} = 4.15\text{min}$; $\text{MH}^+ = 356, 358$

- 25 Intermediate 8 (alternative method as sulfuric acid salt): 9-(5-Chloropentyl)-2-[(1S)-1-methylbutyl]oxy}-8-(methyloxy)-9H-purin-6-amine, sulfuric acid salt

- Sodium hydroxide (2M, 2.52 L, 2.3 eq.) was added to a solution of 2-[(1S)-1-methylbutyl]oxy}-8-(methyloxy)-9H-purin-6-amine, trifluoroacetate salt (800 g, 1.0 eq.) in NMP (3.08 L). 1-Bromo-5-chloropentane (432 mL, 1.5 eq.) was added. The reaction mixture was heated to 50 °C for 6 h. The reaction mixture was cooled to 20-25 °C. Ethyl acetate (8.0 L) was added, followed by water (1.6 L). After stirring for 10 minutes, the phases were separated and the organic phase was then
- 30 washed with water (1.6 L). The ethyl acetate phase was further diluted with ethyl acetate (4.0 L) and heated to 50 °C. Sulfuric acid (117 mL, 1 eq.) was added dropwise. The reaction mixture was cooled to 10 °C over 1.5 hours and aged for half an hour. The product was isolated by filtration as a white solid, washed on the filter with ethyl acetate (2.4 L) and dried under reduced pressure at
- 35 40°C (570 g, 57% theoretical yield).

Intermediate 9: 2-[[[(1S)-1-Methylbutyl]oxy]-8-(methyloxy)-9-[5-(1-piperidinyl)pentyl]-9H-purin-6-amine



5

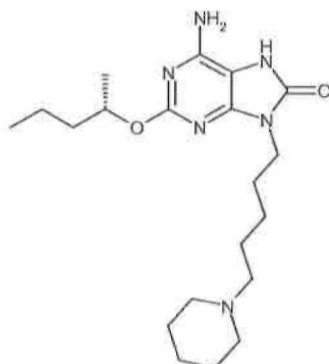
9-(5-Chloropentyl)-2-[[[(1S)-1-methylbutyl]oxy]-8-(methyloxy)-9H-purin-6-amine (for example, as prepared for Intermediate 8) (80 mg, 0.225 mmol), triethylamine (0.031 mL, 0.225 mmol) and piperidine (0.045 mL, 0.45 mmol) were suspended in DMF (3 mL) and the mixture heated to 70 °C for 18 hours. The solvent was removed and the residue partitioned between dichloromethane (4 mL) and saturated sodium bicarbonate (4 mL). The aqueous phase was re-extracted with further dichloromethane and the combined organic extracts were concentrated and the residue dissolved in 1:1 methanol:DMSO (1 mL) and purified by MDAP (Method A). The product-containing fractions were combined and evaporated under a stream of nitrogen to give the *title compound* (47.2 mg). LCMS (System D): t_{RET} = 3.11min; MH⁺ = 405

15

Examples

Reference Example 1: 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one

20



25

A solution of hydrogen chloride in dioxane (4M, 0.71 mL) was added to a solution of 2-[[[(1S)-1-methylbutyl]oxy]-8-(methyloxy)-9-[5-(1-piperidinyl)pentyl]-9H-purin-6-amine (for example, as prepared for Intermediate 9) (0.046 g, 0.126 mmol) in methanol (3 mL). The resultant mixture was allowed to stand overnight at room temperature and then blown down under nitrogen. The residue was dissolved in methanol and loaded onto a 2 g aminopropyl SPE cartridge (pre-conditioned with

methanol), eluted with methanol and the resultant solution blown down under nitrogen to give the *title compound* as a yellow solid (40.97 mg).

LCMS (System D): t_{RET} = 2.70min; MH⁺ = 391

- 5 A similarly prepared sample (1.7 g) was recrystallised from ethyl acetate (approximately 50 mL). The crystals were collected, washed with ice-cold ethyl acetate (15 mL) and dried *in vacuo* at 50 °C for 3 hours to give the *title compound* as a cream crystalline solid (1.33 g).
Melting point onset (DSC): 207.4 °C (see Fig. 2)
XRPD: (see Fig. 1 and Table 1)

10

Reference Example 1: (alternative method): 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one

- 9-(5-Chloropentyl)-2-[[[(1S)-1-methylbutyl]oxy]-8-(methoxy)-9H-purin-6-amine, sulfuric acid salt
15 (254 g, 1.0 eq) was dissolved in DMSO (1.27 L) and piperidine (280 mL, 5eq). The reaction mixture was heated to 70±3 °C for 15.5 h. The reaction mixture was cooled to 20±3 °C. Toluene (2.5 L) was added, followed by water (1.25 L). After stirring for 10 minutes, the phases were separated and the upper toluene phase was washed with water (0.5 L). A solution of hydrochloric acid (2.24 mol) in water (1.5 L) was added. The mixture was stirred for 10 minutes and then
20 allowed to separate with the lower (aqueous) phase retained. The aqueous solution was heated to 50±3 °C for 17 h and then cooled to 20±3 °C. Aqueous sodium hydroxide (2M, ca. 840 mL) was added dropwise until the solution had a pH of 10-11. The resulting suspension was cooled to 10±3 °C, aged for a further 30 min. then filtered. The cake was washed with water (7.6 L) and the product was dried under reduced pressure at 60 °C with a nitrogen bleed to constant weight (207 g,
25 95%th).

Polymorphism

- X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) were performed on 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one according
30 to the following methods.

XRPD

- XRPD data were acquired on a PANalytical X'Pert Pro powder diffractometer, equipped with an X'Celerator detector. The acquisition conditions were: radiation: Cu K α , generator tension: 40 kV,
35 generator current: 45 mA, start angle: 2.0° 2 θ , end angle: 40.0° 2 θ , step size: 0.0167° 2 θ . The time per step was 31.750 s. The sample was prepared by mounting a few milligrams of sample on a Si wafer (zero background) plate, resulting in a thin layer of powder.

- Characteristic peak positions and calculated d-spacings are summarised in Table 1. These were
40 calculated from the raw data using Highscore software. The experimental error in the peak positions is approximately ±0.1° 2 θ . Relative peak intensities will vary due to preferred orientation.

Table 1

Characteristic XRPD Peak Positions for Solid-state Form 1 of 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one

Form 1	
2 θ / °	d-spacing / Å
5.0	17.6
10.0	8.8
12.7	7.0
13.5	6.5
13.8	6.4
16.6	5.3
18.9	4.7
20.0	4.4
22.2	4.0
23.3	3.8
24.2	3.7
26.1	3.4

- 5 A representative XRPD diffractogram of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one is shown in Fig. 1.

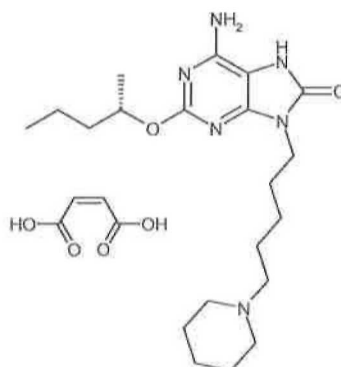
DSC

- 10 The DSC thermogram was obtained using a TA Instruments calorimeter. The sample was weighed into an aluminium pan, a pan lid placed on top and lightly crimped without sealing the pan. The experiment was conducted using a heating rate of 10 °C min⁻¹.

A representative DSC thermogram of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one is shown in Fig. 2.

15

Example 2: 6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, maleate salt



Preparation 1

6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one (for example, as prepared for Reference Example 1) (0.384 g, 0.98 mmol) was dissolved in isopropyl alcohol (4.6 mL, 12 vols) and heated to 40 °C. Maleic acid (0.114 g, 0.98 mmol) was added. A clear solution was obtained. During cooling to room temperature, precipitation occurred. The slurry was filtered, washing with isopropyl alcohol (5 mL) and dried under reduced pressure at 40 °C to constant weight. 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, maleate salt (0.305 g, 61%th) was obtained as a white solid.

¹H NMR confirms a 1:1 ratio of maleic acid: 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one. ¹H NMR (400 MHz, DMSO-d₆) δ ppm, 9.85 (1H, s, (CH₂)₃NHCO), 8.85 (1H, br s, NH⁺), 6.39 (2H, s, NH₂), 6.02 (2H, s, HO₂C(CH)₂), 5.00 (1H, m, J = 6.2 Hz, CH₃CH), 3.68 (2H, t, J = 6.8, Hz NCH₂), 3.40 (2H, m, NCH₂), 2.98 (2H, m, J = 8.1 Hz NCH₂), 2.82 (2H, br s, NCH₂), 1.85-1.24 (16H, m, 8 × CH₂), 1.21 (3H, d, J = 6.1 Hz, CHCH₃), 0.89 (3H, t, J = 7.3 Hz, CH₂CH₃), 2.5 (solvent (DMSO)).

Preparation 2

A solution of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one (for example, as prepared for Reference Example 1) (1.46 g, 3.74 mmol) in isopropyl alcohol (14.6 mL, 10 vols) was clarified (filtered at room temperature through a BondElut cartridge) and then heated to approximately 50 °C. A solution of maleic acid (0.434 g, 3.74 mmol) in isopropyl alcohol (2.9 mL, 2 vols) was added. The resulting solution was then seeded and cooled to 45 °C. Further seed was added. The resulting slurry was cooled to room temperature and held overnight (approximately 16 hours), then cooled in an ice/water bath for 30 minutes. The slurry was filtered, washing with isopropyl alcohol (4.5 mL, 3 vols and then 3 mL, 2 vols). The product was dried under reduced pressure at 40 °C to constant weight to give 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, maleate salt (1.305 g, 69%th).

Analysis by XRPD (figure 3) indicated this sample to be crystalline.

Biological Data

The compound of Reference Example 1 was tested for *in vitro* biological activity in accordance with the following assays, or similar assays:

Assay for the Induction of Interferon-α using Cryopreserved Human Peripheral Blood Mononuclear Cells (PBMCs)

Compound Preparation

The compound of Reference Example 1 was dissolved in DMSO. Serial 2-fold dilutions with DMSO were prepared and 0.25 μl dispensed into 384-well clear Greiner polypropylene plates.

Preparation of PBMCs

- Blood samples of up to 200 mL were obtained from healthy human donors. Whole blood in 25 mL volumes was overlaid onto 15 mL Ficoll gradients in Leucosep tubes, and centrifuged at 1000 g for 20 min. Cells in the band at the plasma/histopaque interface were carefully removed and washed twice with PBS (centrifuged at 400 g for 5 min to harvest). The final pellet was resuspended in
- 5 freezing medium (90% Heat-inactivated serum, 10% DMSO) to a cell concentration of 4×10^7 cells/mL. The resuspended cells were then cryopreserved (frozen) using a rate controlled freezer, and stored at -140°C for up to 4 months.

Incubation and Assay for Interferon- α

- 10 Immediately prior to assay, vials of cryopreserved (frozen) PBMCs were thawed rapidly in a water bath at 37°C . A 1:10 dilution of the cells in trypan blue was prepared and counted. The PBMCs were then diluted in growth media [RPMI 1640 containing 10% fetal calf serum (Invitrogen), Penicillin+Streptavidin (Gibco cat. # 25030-024, 1:50), L-Glutamine 2 mM, and 1000 units/mL recombinant human IFN-gamma (Preprotech catalogue #300-02)] to a density of 1×10^6 cells/mL,
- 15 and 50 μL /well dispensed to 384-well clear Greiner polypropylene plates containing 0.25 μL DMSO or test compound in 0.25 μL DMSO. Top final concentration of compound was typically 50 μM or 5 μM (to obtain curve fit for highly active compounds). Plates were incubated for 24 hours at 37°C in 5% CO_2 .
- 20 A multi-isoform immunoassay was used to quantify IFN- α in PBMC supernatants. Rabbit polyclonal antibody against human IFN- α (catalogue number 31101, Stratech Scientific) was diluted 1:10000 in assay buffer (RPMI 1640 containing 10% fetal calf serum, Invitrogen) and 20 μL was added to each well of an MSD (Meso-Scale Discovery) single small-spot 384-well GAR (goat anti-rabbit antibody coated) plate. The plate was incubated for 1 hour at room temperature with vigorous
- 25 shaking. Following three washes with PBS, 20 μL of cell supernatant were added to each well of the plate. The plate was then incubated for 1 hour at room temperature with vigorous shaking. A pair of monoclonal antibodies to IFN- α (catalogue numbers 21100 and 21112, Stratech Scientific) were labelled with sulfo-TAG (MSD), diluted 1:1000 in assay buffer and 20 μL added to each well of the plate. The plate was further incubated for 1 hour at room temperature with vigorous shaking.
- 30 Following three washes with PBS, 30 μL of $\times 2$ T buffer (MSD) was added to each well and the plate was read on an MSD Sector 6000 plate reader.

- Data were normalised to internal plate controls of 1 μM resiquimod ($n=16$) and DMSO ($n=16$). pEC_{50} values were derived by 4-parameter curve fit with IRLS in ActivityBase, from 11-point, two-
- 35 fold serial dilution of test compounds.

Results

Reference Example 1 had a mean pEC_{50} of >8.3

- 40 Assay for the Induction of Interferon- α and TNF- α using Fresh Human Peripheral Blood Mononuclear Cells (PBMCs)

Compound preparation

- The compound of Reference Example 1 was dissolved and serially diluted in DMSO to give $100\times$
- 45 the required concentration range using a Biomek 2000. 1 μL of test compound was transferred into

96-well tissue culture plates using a Biomek FX. Each compound was assayed in duplicate for each donor. Each plate contained a dilution series of the TLR7/8 agonist resiquimod as standard and Column 11 contained 1 μ L of 200 μ M resiquimod (giving a 2 μ M final concentration, used to define the approximate maximal response to resiquimod).

5

Preparation of PBMCs

Blood samples from two human donors were collected into sodium heparin (10 U/mL). 25 mL volumes of whole blood were overlaid onto 15 mL Histopaque in Leucosep tubes which were centrifuged at 800 g for 20 min and the band at the plasma/histopaque interface carefully removed.

10 The collected cells were centrifuged at 2500 rpm for 10 min and the pellet resuspended in 10 mL of media (RPMI 1640 (Low endotoxin) supplemented with 10% v/v foetal calf serum (FCS, low endotoxin) 100 U/mL penicillin G, 100 μ g/mL streptomycin, 10 mM L-glutamine and 1 $\times$ non-essential amino acids). A 1:20 dilution of the cells was prepared using trypan blue and the cells counted using a haemocytometer. The PBMCs were diluted to give a final concentration of 2 $\times$ 10⁶
15 per mL and 100 μ L of this cells suspension was added to wells containing 1 μ L of diluted test compound.

Incubation and Assays for Interferon- α and TNF- α

20 The cell preparations were incubated for 24 hr (37 °C, 95% air, 5% CO₂) after which a sample of the supernatant was removed using the Biomek FX and assayed for both IFN- α and TNF- α using the MSD (Mesoscale Discovery) electrochemiluminescence assay platform. The IFN- α assay was carried out similarly to that described above. The TNF- α assay was carried out as per kit instructions (Cat No K111BHB).

25 Cytokine released was expressed as a percentage of the 2 μ M resiquimod control (column 11). This percentage was plotted against compound concentration and the pEC₅₀ for the response determined by non-linear least squares curve fitting. For the IFN- α responses generally a 4 parameter logistic model was selected. For the TNF responses where a clear maximum response was obtained (i.e. a well defined plateau in the response was observed) then a 4 parameter model
30 was generally used. If the upper asymptote of the curve wasn't well defined then the curve fitting was generally constrained to a maximal response of 100% (i.e. to the response to 2 μ M resiquimod) or to the response of the highest concentration tested if this was greater than the resiquimod response. Some curves were bell shaped for one or both cytokines and the cytokine data on the down slope of the bell shaped response (i.e. concentrations above those giving the
35 maximal response) were generally excluded from the fit, usually with the exception of the concentration immediately above the peak response. Curve fitting thus concentrated on the up slope of the dose response curve.

Results

40 Reference Example 1 showed a mean pEC₅₀ for induction of IFN- α and TNF- α of ≥ 9 and ≤ 6.5 respectively.

Allergen-driven Cytokine Assay using Fresh Human Peripheral Blood Mononuclear Cells (PBMCs) from Atopic Volunteers

45

An assay based on co-culture of atopic human donor derived peripheral blood mononuclear cells (PBMCs) with allergen and test compounds was developed. After 5-6 days culture, cell supernatants were assayed for a range of cytokines.

5 Compound preparation

The compound of Reference Example 1 was dissolved in DMSO, then serially diluted in growth medium (RPMI 1640 medium supplemented with 100 U/mL penicillin G, 100 µg/mL streptomycin, 10 mM L-glutamine) to give 4× the required concentration range in the presence of 0.04%DMSO. Each compound was assayed in triplicate at all concentrations.

10

Preparation of PBMCs

Defibrinated human blood from volunteers known to be allergic to Timothy grass was centrifuged at 2500rpm for 15 minutes. The upper layer of serum was collected and heat-inactivated at 56 °C for 30 minutes (HI-autologous serum). The lower layer of cells was resuspended in 50 mL PBS (+Ca +Mg), 25 mL diluted blood were overlaid onto 20 mL Lymphoprep in 50ml tubes then centrifuged at 2500 rpm for 20 minutes at RT. The band at the serum/Lymphoprep interface was carefully removed. The collected cells were washed with PBS and re-suspended at 4×10^6 per mL in growth medium with HI-autologous serum. PBMCs were seeded at 0.4×10^6 cells per well in flat-bottomed 96 well plates in the presence of 10 µg/mL Timothy Grass antigen (Alk Abello) and test compounds at appropriate concentrations in a total volume of 200 µL.

20

Incubation and Cytokine assays

Plates were incubated at 37 °C in 5%CO₂ for up to 6 days. The cell medium from each well was harvested and stored at -20 °C prior to analysis. Cytokines and chemokines in supernatants were detected using Meso Scale Discovery 10 spot plates for Human TH1/Th2 cytokines.

25

In the above assay, data from separate studies with PBMCs from three allergic donors showed Reference Example 1 to reduce production of the Th2 cytokines IL-5 and IL-13 in a dose response manner with ≥50% reduction observed at 0.04 µM compared to the allergen control.

30

Reference Example 1 was also tested for *in vivo* biological activity in the following model:

Assay for the Induction of Interferon-α following intranasal dosing in the mouse

The compound of Reference Example 1 was dissolved in 0.2% Tween 80 in saline and administered intranasally (5 µL in total between the nostrils) to female BALB/c mice (n=6) under general anaesthesia. Animals were euthanased 2 hours after dosing and a terminal blood sample was taken and serum levels of Interferon-α measured using an ELISA assay.

35

In this model Reference Example 1 showed mean serum levels of Interferon-α of 21029 pg/mL. No Interferon-α was detected in vehicle treated controls.

40

Stability Testing

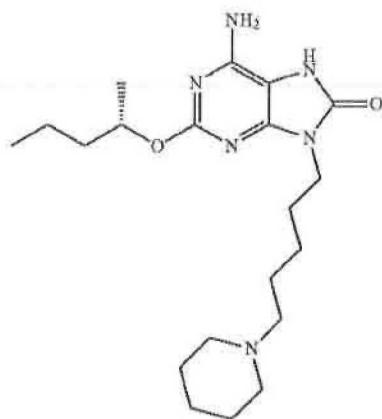
6-Amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one, maleate salt exhibited no significant degradation under conditions specified in Quality Guidelines Q1A(R2)

45

(Stability Testing of New Drug Substances and Products) and Q1B (Photostability Testing of New Drug Substances and Products) laid down by the International Conference for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

Claims

1. A compound which is 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one:



in the form of a maleate salt.

2. A vaccine adjuvant comprising a compound according to claim 1.
3. An immugenic composition comprising an antigen or antigen composition and a compound according to claim 1.
4. A vaccine composition comprising an antigen or antigen composition and a compound according to claim 1.
5. A pharmaceutical composition comprising a compound according to claim 1, and one or more pharmaceutically acceptable diluents or carriers.
6. A pharmaceutical composition according to claim 7 which is administered by intranasal or inhaled administration.

Fig. 1:

XRPD diffractogram of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one (Reference Example 1)

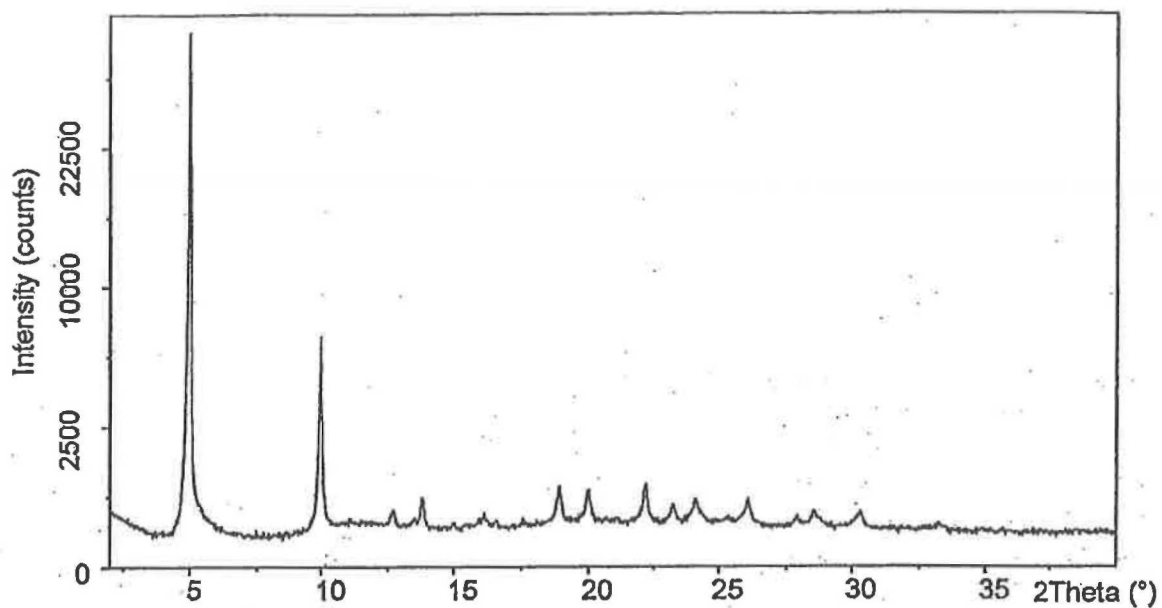


Fig. 2:

DSC thermogram of 6-amino-2-[[[(1S)-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8H-purin-8-one (Reference Example 1)

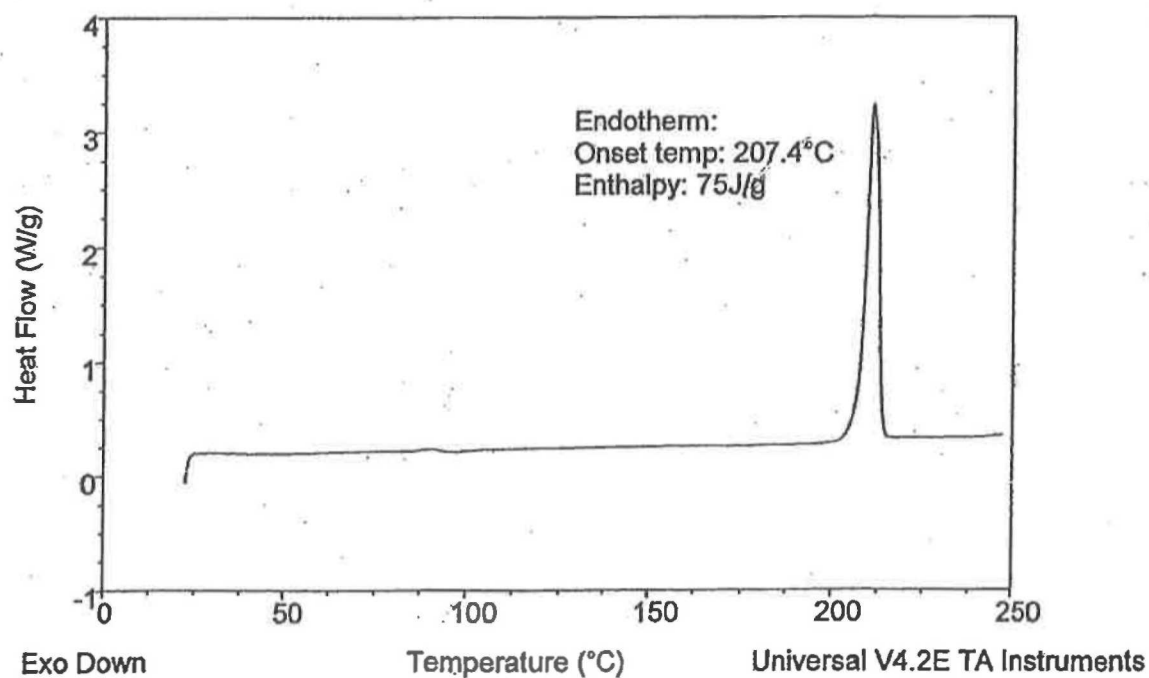


Fig. 3

XRPD diffractogram of 6-amino-2-[[1*S*]-1-methylbutyl]oxy]-9-[5-(1-piperidinyl)pentyl]-7,9-dihydro-8*H*-purin-8-one, maleate salt (Example 2, preparation 2)

