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Convergent synthesis of 2-thioether-substituted (*N*)-methanocarba-adenosines as purine receptor agonists†

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A linear route has been used to prepare (*N*)-methanocarba-nucleoside derivatives, which serve as purine receptor ligands having a pre-established, receptor-preferred conformation. To introduce this rigid ribose substitute, a Mitsunobu reaction of a [3.1.0]bicyclohexane 5′-trityl intermediate **3** with a nucleobase is typically followed by functional group modifications. We herein report an efficient scalable convergent synthesis for 2-substituted (*N*)-methanocarba-adenosines, which were demonstrated to bind to the A₃ adenosine receptor. The adenine moiety was pre-functionalized with 2-thioethers and other groups before coupling to the bicyclic precursor (**3**) as a key step to facilitate a high yield Mitsunobu product. This new approach provided the (*N*)-methanocarba-adenosines in moderate to good yield, which effectively increased the overall yield compared to a linear synthesis and conserved a key intermediate **3** (a product of nine sequential steps). The generality of this convergent synthesis, which is suitable as an optimized preclinical synthetic route, was demonstrated with various 2-thioether and 2-methoxy substituents.

Introduction

Nucleoside derivatives are used widely in a therapeutic capacity in cancer, infectious diseases and other conditions.[1,2] One approach to increase the specificity of action of nucleosides and nucleotides is to constrain the ribose ring in a preformed conformation that is complementary to the requirements at a target biopolymer, such as an enzyme or receptor protein. For example, the introduction of a North (*N*)-methanocarba ([3.1.0] bicyclohexane) ring system in place of the tetrahydrofuryl group of native ribose lowers the energy barrier in binding at a biological target resulting in increased affinity and selectivity,[3–6] *e.g.* of nucleosides at the A₃ adenosine receptor (AR) or of nucleotides at the P2Y₁ receptor (P2Y₁R). Substitution at the adenine C2 position with secondary amines, ethers, thioethers or alkynes is of particular interest in biological studies at purinergic receptors. For example, adenosine 2-thioethers in the native ribose series displayed enhanced AR affinity, and an adenine 2-methylthio group is a favored substitution in various P2 receptor ligands.[7,8] Also of note are 2-methylthio nucleotide derivatives that act as potent P2YR agonists, including selective P2Y₁R agonist MRS2365 **1** (K_i 0.4 nM), which is a (*N*)-methanocarba analogue of 2-methylthioadenosine 5′-diphosphate.[6] Similarly, among AR ligands the potent A₃AR agonist MRS3611 **2** (K_i 1.5 nM) is an (*N*)-methanocarba analogue having a 2-methylthio substitution (Chart 1).[5]

Although (*N*)-methanocarba nucleosides have broad applications as ligands for various G protein-coupled receptors (GPCRs) and enzymatic targets,[9,10] the conventional synthetic routes involve many linear steps and the overall final yield is typically <1% from readily available starting materials such as D-ribose.[9,11–14] Thus, it is of interest to identify more efficient synthetic approaches that might be adaptable to pharmaceutical development. Here, we compared a linear synthesis of 2-methylthio-(*N*)-methanocarba-adenosine (MRS4322, an A₃AR agonist with cerebroprotective efficacy)[15,16] with a convergent approach that is designed to increase overall yield and to optimally use the precious [3.1.0]bicyclohexane intermediate. We investigated the generality and scalability of the convergent route to the synthesis of (*N*)-methanocarba-adenosine derivatives having other C2 position substitution.

Results and discussion

Linear route

Typically, a linear route (Scheme 1, from key intermediate **3** *via* **5–7**) is used to prepare appropriately functionalized (*N*)-

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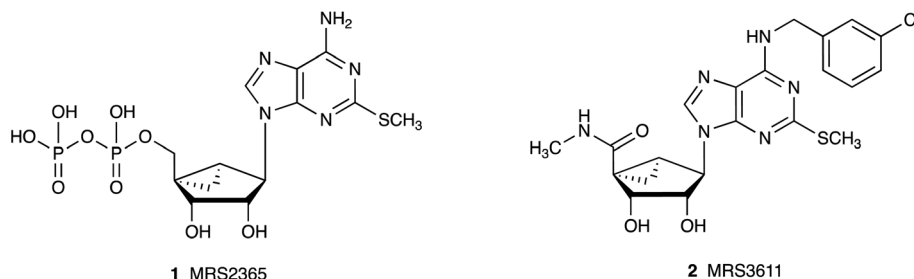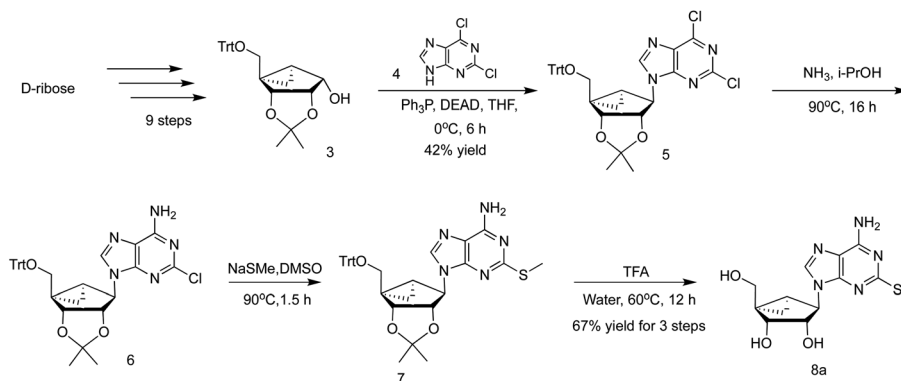
Chart 1 Structures of potent and selective purinergic agonists (**1**, P2Y$_1$R; **2**, A$_3$AR) that combine a (N)-methanocarba modification of ribose with an adenine 2-thioether.

Scheme 1 Linear route for preparation of 2-substituted (N)-methanocarba adenosine derivatives, shown here for 2-MeS-adenine analogue **8a**, similar to published synthetic schemes.[6,13] The intermediate **3** was prepared according to published procedures with minor modification.[12,14] More synthetic details concerning the synthesis of bicyclic intermediate **3** are found in ESI (Scheme S1†).

methanocarba-adenosine derivatives, including those containing a 2-alkylthio group, *e.g.* 2-methylthio derivative **8a**.[9,12,13] The final 2-alkylthioadenine nucleosides and related nucleotides are designed to activate purine receptors.[5,6] The synthesis features a protected bicyclic intermediate, shown here as a 5′-trityl protected derivative **3**, which is a precursor of the pseudoribose moiety. Most commonly, a *tert*-butyldimethylsilyl (TBDMS) ether protection of the 5′-hydroxyl group of the intermediate analogous to **3** has been used.[13] However, this precursor and subsequent nucleoside intermediates are sticky materials, which are difficult to handle for large scale production in process chemistry.[13] Here, we have used a 5′-trityl protecting group of the corresponding bicyclic intermediates, following the routes of Choi *et al.* and Michel and Strazewski,[12,14] which provided easily crystallizable intermediates leading to **3** in nine steps from D-ribose. As shown in Scheme 1, the nucleoside derivative **5** was obtained in a relatively low yield (42%) from the intermediate **3** by a Mitsunobu reaction. We initially prepared the AR agonist **8a** (MRS4322) on a scale of 137 g with an overall yield of 1.0% after thirteen steps starting from D-ribose (7.0 kg), including 28.1% for the last four steps (Scheme 1), using this linear route with 5′-trityl protection.

Moreover, the Mitsunobu reaction of 2,6-dichloropurine **4** with alcohols can lead to the undesired N^7-regioisomer.[17] There are a few reports that also mentioned the N^9-alkylation of 6-chloro-2-NH-Boc adenine derivatives in good yields, using

various alcohols *via* a Mitsunobu reaction.[18,19] Therefore, we explored new methods that could successfully transform the key precursor **3** into the desired nucleoside N^9-regioisomer with a 2-alkylthioadenine nucleobase.

Adenine pre-functionalization for alternative routes

As an alternative synthetic approach, rather than coupling a bicyclic intermediate (*e.g.* **3**), either in the ribo (2′-OH) or 2′-deoxy (N)-methanocarba series, to a reactive (6-chloro or 2,6-dichloro) purine nucleobase precursor (*e.g.* **4**),[5,6,11] we pre-functionalized the purine in a manner that would facilitate high yields in a subsequent Mitsunobu step. Selective protection of the exocyclic primary amine was necessary, because earlier attempts to couple various adenine derivatives containing a free 6-NH$_2$ through a Mitsunobu reaction failed.[11] It remains unclear if this was due to the formation of phosphazane complexes[20,21] with the betaine intermediate, or to the poor solubility of the 6-NH$_2$-purine.

The N^6-Boc protecting group was considered for 2-chloroadenine **9** (Scheme S2†). With the goal of improving the synthetic route to adenine 2-thioether derivatives, we first prepared N^6,N^6-di-Boc-2-chloro-adenine **11** in two steps *via* tri-Boc intermediate **10**, based on a literature report.[14] The di-Boc protected adenine derivative obtained was used in a Mitsunobu reaction with the alcohol (**3**). However, it was found that only ~10% of the coupling product **12** was observed by ^{1}H-NMR
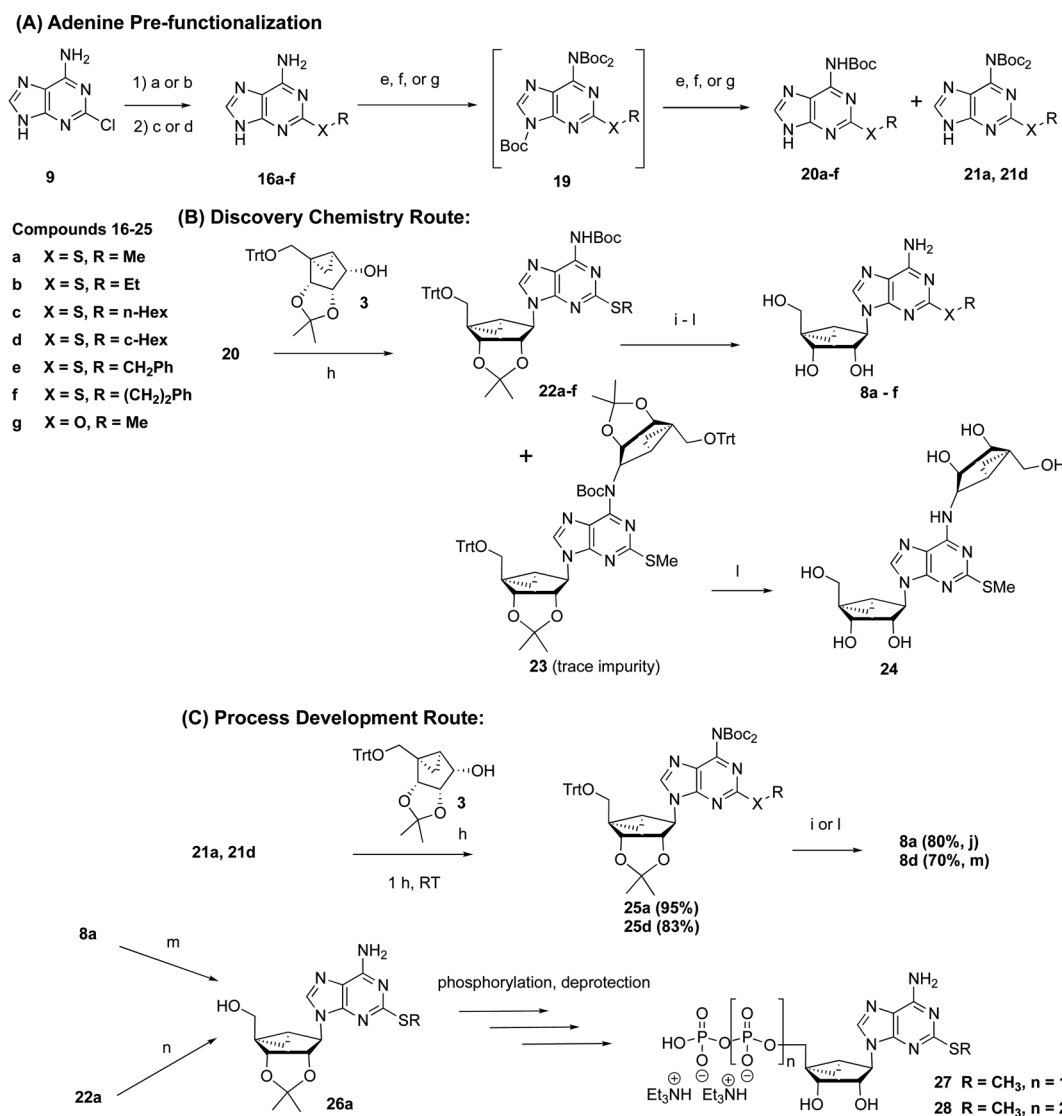
in contrast to the quantitative synthesis of similar kind of analog under identical conditions that was reported by Michel *et al.*[14] Other approaches to protection of the exocyclic amine of **9** were explored (*e.g.* **14**, **15**, Scheme S3, ESI†).[22]

Convergent route

The need for an efficient and scalable synthesis led us to seek a convergent route that envisaged the use of N^6-protected forms

of 2-substituted adenine derivatives **16** (Scheme 2A) as precursors for a subsequent Mitsunobu reaction to obtain the corresponding (*N*)-methanocarba nucleoside derivative **8a** and its *S*-alkyl homologues. In this context, we first synthesized 2-methylthioadenine **16a** in high yield (94%) by treating 2-Cl-adenine **9** with sodium methylthiolate at elevated temperature.

Consequently, we were curious to know the relative reactivity of N^6-Boc-protected 2-MeS-adenine **20a** with the alcohol **3** in

Scheme 2 General reaction scheme using the convergent route for preparation of 2-thioether-substituted (*N*)-methanocarba adenosine derivatives (**8a–g**) and nucleotides (**27** and **28**, Scheme S5†). The key differences from Scheme 1 are the pre-installation of a 2-thioether (A) and use of a Boc-protected exocyclic amine on adenine (B) prior to the Mitsunobu coupling with the bicyclic pseudoribose. Part (C) shows the scalable process route. An "a" designation after the compound numbers refers to R = Me. Other substituents are shown in Table S1.† Reaction conditions and yields: [a] (94%) (i) aq. NaSMe (3.0 equiv.), 140 °C, autoclave, 16 h; (ii) 6 N HCl to pH = 7–8; [b] (67%) (i) NaSMe (2.5 equiv.), DMF, 110 °C, 16 h; (ii) 6 N HCl, 60 °C, 2 h; (iii) 23% aqueous NH₄OH; [c] (40–99%) RSH (5 equiv.), Cs₂CO₃ (3.0–3.5 equiv.), DMF, 140 °C, 1 day; [d] (75%) NaOMe (20 equiv.), MeOH, 150 °C, 4 days; [e] (28–69%) (i) Boc₂O (4.0 equiv.), DMAP (0.2 equiv.), THF; (ii) aq. 10% NaOH, MeOH, 5–6 h; [f] (94%) (i) Boc₂O (4.0 equiv.), DMAP (0.2 equiv.), THF; (ii) aq. NH₄OH (23%), THF, 6 h; [g] (23–45%) (i) Boc₂O (4.0 equiv.), DMAP (0.2 equiv.), THF; (ii) sat. NaHCO₃, MeOH–H₂O (1 : 1), 60 °C, 5–16 h; [h] (67–94%) **20** or **21** (1.1–1.2 equiv.), alcohol **3** (1.0 equiv.) PPh₃ (1.5–2.0 equiv.), DIAD (1.5–2.0 equiv.), THF (~0.1 M), 1–2 h; [i] (59–70%) (i) aq. 4 N HCl or 4 N HCl (g)/MeOH, 35 °C, 16 h; (ii) Na₂CO₃, MeOH/H₂O. [j] (88%) 1 N HCl in H₂O, 50 °C, 18 h; (ii) Amberlite resin-93, MeOH, 16 h; [k] (46–75%) aq. 4 N HCl in MeOH or EtOH, 35 °C, 16 h; (ii) Amberlite resin-93, MeOH, 16 h; [l] (45%) aq. TFA in MeOH 50 °C, 17 h; (ii) Amberlite resin-93, MeOH, 16 h; [m] (variable yield, see ESI†) anhyd. acetone, 2,2-dimethoxypropane, *p*-TSA, room temperature, 18 h. [n] (58%) anhyd. acetone-TFA (1 : 2), room temperature, 3 h or anhyd. ZnBr₂, DCM, 10–20 min.

Table 1 Affinity at the human A₃AR of 2-alkylthio or 2-alkyloxy derivatives of (N)-methanocarba adenosine[a]

Compound	K_i (nM)
8a	1490 ± 410[b]
8b	970 ± 60
8c	548 ± 44
8d	1080 ± 90
8e	49.8 ± 5.5
8f	291 ± 89
8g	1140 ± 84

[a] Binding in membranes of HEK293 cells stably expressing hA₃AR,[16] using [^{125}I]N^6-(4-amino-3-iodobenzyl)-adenosine-5′-N-methyluronamide ([^{125}I]I-AB-MECA, 0.1 nM) as radioligand. [b] As reported in Liston et al.[16]

a Mitsunobu reaction (Scheme 2B). The 2-thioether **16a** was first Boc-protected with excess Boc-anhydride to yield a mixture of N-tert-butoxycarbonyladenine intermediates, **19a** (N^6,N^6,N^9-tri-tert-butoxycarbonyladenine) and **21a** (N^6,N^6-di-tert-butoxycarbonyladenine). The corresponding tri-Boc derivative **19a** formed during the reaction was largely cleaved in mild basic conditions to the N^6-mono-Boc derivative **20a**. The di-Boc intermediate **21a** was found to be less stable than mono-Boc **20a**, as it gradually decomposed, even upon long-term storage as a solid at room temperature, to mono-Boc **20a** as indicated by TLC. The phthaloyl group (e.g. **17**, Scheme S4, ESI†) was also considered for the amino-protection of **16a**, but Boc protection was more successful.[22,23]

The Mitsunobu reaction with either mono-Boc **20a** or di-Boc **21a** nucleobase (Schemes 2B and C) with a 5′-O-trityl bicyclic intermediate **3** proceeded in high yield (94%) to provide only N^9-regioisomers **22a** (Table S1†) or **25a**, respectively. Following acidic deprotection of **22a** or **25a**, nucleoside **8a** was obtained, and this step to remove three protecting groups simultaneously proceeded in high yield (88% at 50 °C). The isolated mono-Boc intermediate **20a** contained a small amount of unprotected **16a** as an impurity, which was problematic for the purity of subsequent steps leading to **8a**. The presence of a small amount of the di-Boc compound **21a** in the mono-Boc intermediate **20a** was not detrimental during the Mitsunobu reaction, because its Mitsunobu product (**25a**) was later deprotected to yield the same product **8a**. However, we preferred the di-Boc approach (Scheme 2C) for the scalable process development, as the mono-Boc route (Scheme 2B) produced a bis-alkylated impurity (**24**) via bis-adduct (**23**), which was inseparable from the desired product. The purging of **24** was unsuccessful by crystallization after global deprotection since the product (**8a**) and **24** have the same polarity characteristics [(**24**), analytical HPLC: retention time 6.89, 466 (m/z). Retention time for MRS4322 (**8a**): 6.64, 324 (m/z)].

For subsequent 5′-phosphorylation, compound **8a** could be reprotected with a 2′,3′-isopropylidene group to provide **26a**, which was then phosphorylated (and subsequently deprotected) to yield high potency P2Y₁R agonists, e.g. **27** and **28**.[6] Although our published method for the synthesis of **27** used benzoyl peroxide as oxidant in the phosphitylation reaction,[6] we found that use of H₂O₂ resulted in less undesired thioether oxidation.

Alternatively, the trityl group and Boc protection of **22a** were removed simultaneously using ZnBr₂ to yield **26a** directly.[24]

The overall yield of **8a** on a scale of 520 g from **3** using Scheme 2B was 60%. The outstanding advantage of the convergent route is that it spares the precious intermediate **3**, which itself required nine steps from D-ribose to prepare. We calculate that the linear synthesis required 540 g of **3** per 100 g of **8a** by the linear route compared to 230 g of **3** per 100 g of **8a** for the convergent route. Thus, the molar ratio of the key precursor **3** in the convergent route was reduced by 57% compared to the linear synthesis.

To test the applicability of this approach to other 2-thioether substituents, 2-Cl-adenine (**9**) was treated with various alkyl thiols, aryl–alkyl thiols, and the corresponding sodium salt to provide 2-thioethers of general formula **16b–16f**,[25] selected based upon the commercial availability of the thiols (Scheme 2). The 2-thioethers were then mono-Boc-protected (**20b–20f**), as with 2-methylthioadenine, and subjected to a Mitsunobu reaction with the alcohol (**3**) to produce the desired products **22b–22f** in excellent yields (Table S1†). For the selective deprotection of tri-Boc intermediate (**19a**), we screened several conditions for the scale-up process, and we found that basic conditions consisting of aq. sodium hydroxide (NaOH) in MeOH gave **20a** in good yield (62% from **16a**). The yields varied with this combination for other examples (**20f**, **20g** and **21d**). For example, the yield of **20f** with aq. NaOH in MeOH was 28%. However, we admit that these conditions were not optimized, as our principal objective was to obtain the corresponding product (**20**) in a reasonable or modest yield for examining the Mitsunobu reaction. Thus, only the conditions using sodium bicarbonate or aq. ammonia, which resulted in satisfactory yields, were shown in the Table S1.† In the case of cyclohexylthio derivative **8d**, the isolation of pure product was difficult from the corresponding mono-Boc adduct **22d** even by HPLC. However, we were able to obtain homogeneous **8d** via the di-Boc intermediate **25d** (Scheme 2C).

Additionally, to evaluate the generality of our methodology, we prepared 2-methoxyadenine (**16g**), which was subjected to the same reaction sequence with similar results as the 2-thioethers (Scheme 2). A Mitsunobu reaction of 2-MeO-adenine (**16g**) with the alcohol **3** proceeded well in moderate yield (67%). It is noteworthy that we achieved improved yields since the reactivity of N^9 of the N^6-mono-Boc-adenine derivatives **20** towards alkylation depends upon the substituent present at the C2 position. The 2-thioether derivatives typically resulted in 77–95% yield in the Mitsunobu reaction (Table S1†). The low reactivity of 2-halo-substituted adenine (e.g. Cl and I,[13] Scheme S2, ESI†) is probably due to decreased electron density of the purine ring. However, the convergent synthetic approach has fewer synthetic steps after the Mitsunobu reaction than the linear synthesis to produce **12** (Scheme S2, ESI†) and the corresponding deprotected product. Thus, this convergent approach is still sparing of the precious intermediate alcohol **3**. Activating/electron donating groups such as S-alkyl and O-alkyl likely increased the nucleophilic character of the purine nitrogen atoms to enhance yields. Thus, use of a 2-alkyloxy adenine precursor instead of 2-alkylthio is expected to be suitable for this synthetic approach.

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Pharmacological evaluation

The 2-substituted (*N*)-methanocarba-adenosine analogues **8a**–**8g** were evaluated in a standard radiolabeled A₃AR agonist binding assay (Table 1). All of the analogues exceeded the affinity of the parent **8a**, containing a 2-methylthio substituent. The highest affinity was observed for the *S*-benzyl derivative **8e**, with a K_i value of 49.8 nM, *i.e.* 30-fold higher affinity than **8a**. A₃AR agonists are under development for treatment of ischemic and inflammatory conditions.[16,26,27] We note that 2-alkylthio modifications of adenosine have already been well explored for receptor affinity in the ribose series,[30] but not in the (*N*)-methanocarba series. The receptor affinity of 2-alkyloxo-ether modified adenosine analogues was also studied in detail.[31,32]

Conclusions

We conclude that the most effective route is to install a 2-thioether or 2-ether group on the adenine precursor prior to N^6 protection and subsequent Mitsunobu coupling to the pseudoribose ((*N*)-methanocarba) moiety. Thus, we have identified a di-Boc-protected 2-methylthioadenine intermediate **21a** that facilitates an efficient convergent synthetic route to known purine receptor ligands and is applicable to exploration of novel SAR of (*N*)-methanocarba derivatives at adenosine and P2Y receptors. This convergent synthesis of 2-substituted (*N*)-methanocarba-adenosines effectively increased the overall yield of the sequence compared to the previously reported linear synthesis. In particular, the quantity of the precious intermediate alcohol **3** needed for the convergent reaction scheme was reduced (by 57% for **8a**) compared to the linear route. In order to optimize the convergent route, we compared various adenine amine protecting groups and found that N^6-di-Boc protection was the most versatile and provided the most easily purified intermediates. The thioether extended analogues prepared by the convergent route displayed enhanced receptor affinity. In summary, we have developed a facile convergent route, suitable for preclinical development, for the synthesis of (*N*)-methanocarba-nucleoside derivatives. This route allows for a variety of 2-substitutions to aid in structure activity studies. These bicyclic nucleoside derivatives serve as purine receptor ligands with a pre-established receptor-preferred conformation and display efficacy in various disease models.

Experimental section

Chemical synthesis

2-Chloroadenine (**9**) was purchased from Ark Pharm (Arlington Heights, IL, USA). All other reagents were from Sigma-Aldrich (St. Louis, MO, USA). ¹H-NMR spectra were obtained with a Bruker 400 MHz spectrometer in CDCl₃ (7.26 ppm), CD₃OD (HOD = 4.87 ppm) or in (CD₃)₂SO (¹H = 2.50 ppm and ¹³C = 39.52 ppm). The chemical shifts are expressed as ppm downfield and coupling constants (*J*) are given in Hz. TLC analysis was carried out on glass sheets precoated with silica gel F254 (0.2 mm) from Sigma-Aldrich. The purity of final nucleoside derivatives was checked using a Hewlett–Packard 1100 HPLC

equipped with an Eclipse 5 μm XDB-C18 analytical column (50 mm × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). Mobile phase: linear gradient solvent system, 10 mM TEAA (triethylammonium acetate) : CH₃CN from 95 : 5 to 0 : 100 in 20 min; the flow rate was 1.0 mL min⁻¹. Peaks were detected by UV absorption with a diode array detector at 230, 254, and 280 nm. All derivatives tested for biological activity showed >95% purity in the HPLC systems. Low-resolution mass spectrometry was performed with a JEOL SX102 spectrometer with 6 kV Xe atoms following desorption from a glycerol matrix or on an Agilent LC/MS 1100 MSD, with an Atlantis C18 column (Waters Corp., Milford, MA, USA). High resolution mass spectroscopic (HRMS) measurements were performed on a proteomics optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine, unless noted. Mass accuracies were observed.

General procedure for the synthesis of adenine 2-thioether derivatives (16a–16f)

Procedure A. To a 75 mL cylindrical sealed tube equipped with a stir bar was added 2-chloroadenine (1.0 equiv.), sodium thiomethoxide (6.0 equiv.) and anhydrous DMF (20 mL, ~0.1 M). The reaction mixture was stirred at 110 °C for 12–16 h. The reaction was monitored by mass spectrometry and continued until starting material disappeared. The solvent was removed under reduced pressure by rotary evaporation to obtain a solid which was dissolved in 6 N HCl (15 mL) and stirred at 60 °C for 2 h. The solution was cooled to 0 °C and slowly neutralized with aqueous ammonia (23%) until a pH of 8–9. A white solid appeared slowly and was filtered and dried under air to afford compound **16a**. Data matched with the literature report.[28]

¹H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 7.97 (s, 1H), 7.18 (s, 2H), 2.44 (s, 3H).

Yield: 67%; 3.80 g of **16a** from 5.30 g of 2-chloroadenine (**9**).

Large scale yield for step 1 leading to 520 g **8a**, using conditions (a) 94%; 500 g of **16a** from 500 g of 2-chloroadenine (**9**). See ESI.†

Procedure B.[25] In a 10 mL sealed tube, equipped with a stir bar was added 2-chloroadenine (1.0 equiv.), cesium carbonate (3.0–3.5 equiv.) and anhydrous DMF (~0.4 M). To this solution, the alkyl/aryl alkyl thiol (5.0–10.0 equiv.) was added, and the reaction mixture was stirred at 140–150 °C for one day. The reaction mixture was cooled to room temperature and diluted with water to get the product as a white ppt which was filtered off and dried under air. The product was used directly for the next step without any further purification.

Compounds **16b–16g**[c] were prepared by this method with slight modifications.

*2-(Ethylthio)-9H-purin-6-amine, **16b**.* Compound **16b** was prepared from 2-chloroadenine (510 mg, 3.0 mmol) (**9**) and 10.0 equiv. of ethanethiol in DMF (~0.4 M). Yield: 99%; 580 mg.

¹H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 7.94 (s, 1H), 7.17 (s, 2H), 3.04 (q, *J* = 7.3 Hz, 2H), 1.30 (t, *J* = 7.3 Hz, 3H).

¹³C NMR (100 MHz, DMSO-d_6) δ 164.38, 155.28, 152.65, 139.47, 115.54, 25.08, 15.43.

HRMS (ESI) *m*/*z*: [M + H]⁺ calculated for $C_7H_{10}N_5{}^{32}S$: 196.0657; found 196.0655.

2-(Hexylthio)-9H-purin-6-amine, **16c.** Compound **16c** was prepared from 2-chloroadenine (340 mg, 2.0 mmol) (**9**) and 5.0 equiv. of *n*-hexanethiol in DMF (~0.4 M). Yield: 40%; 200 mg.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H), 7.96 (s, 1H), 7.15 (s, 2H), 3.05 (t, J = 7.2 Hz, 2H), 1.63 (p, J = 7.3 Hz, 2H), 1.39 (t, J = 7.5 Hz, 2H), 1.33–1.13 (m, 4H), 0.92–0.76 (m, 3H).

^{13}C NMR (100 MHz, DMSO) δ 163.18, 154.87, 151.86, 138.22, 115.25, 30.85, 29.93, 29.07, 27.99, 22.00, 13.85.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{11}H_{17}N_5S$: 182.0930; found 182.0936.

2-(Cyclohexylthio)-9H-purin-6-amine, **16d.** Compound **16d** was prepared from 2-chloroadenine (340 mg, 2.0 mmol) (**9**) and 6.0 equiv. of *n*-hexanethiol in DMF (~0.4 M). Yield: 60%; 298 mg.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.13 (s, 2H), 3.70 (h, J = 4.4, 3.9 Hz, 1H), 2.05 (dd, J = 9.9, 4.7 Hz, 2H), 1.70 (dt, J = 10.0, 4.7 Hz, 2H), 1.58 (d, J = 12.4 Hz, 1H), 1.39 (q, J = 8.3, 6.4 Hz, 4H), 1.29–1.18 (m, 1H).

^{13}C NMR (100 MHz, DMSO) δ 162.94, 154.92, 151.96, 138.30, 115.33, 42.13, 32.84, 25.60, 25.29.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{11}H_{16}N_5{}^{32}S$: found 250.1128.

2-(Benzylthio)-9H-purin-6-amine, **16e.** Compound **16e** was prepared from 2-chloroadenine (510 mg, 3.0 mmol) (**9**) and 5.0 equiv. of benzyl mercaptan in DMF (~0.4 M). Yield: 80%; 620 mg.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 7.98 (s, 1H), 7.49–7.37 (m, 2H), 7.36–7.12 (m, 5H), 4.35 (s, 2H).

^{13}C NMR (100 MHz, DMSO) δ 162.69, 155.39, 150.91, 138.67, 137.91, 128.90, 128.27, 126.77, 116.37, 34.16.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{12}H_{12}N_5{}^{32}S$: 258.0813; found 258.0804.

2-(Phenethylthio)-9H-purin-6-amine, **16f.** Compound **16f** was prepared from 2-chloroadenine (510 mg, 3.0 mmol) (**9**) and 5.0 equiv. of 2-phenylethanethiol in (~0.4 M). Yield: 87%; 710 mg.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 1.9 Hz, 1H), 7.31 (d, J = 4.4 Hz, 4H), 7.21 (dt, J = 8.0, 4.0 Hz, 3H), 3.29 (dd, J = 9.2, 6.3 Hz, 2H), 2.96 (dd, J = 9.1, 6.4 Hz, 2H).

^{13}C NMR (100 MHz, DMSO) δ 162.79, 155.00, 152.10, 140.72, 138.50, 128.61, 128.30, 126.15, 115.45, 35.40, 31.50.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{13}H_{14}N_5{}^{32}S$: 272.0970; found 272.0968.

2-Methoxy-9H-purin-6-amine, **16g.** Compound **16g** was prepared according to a published report.[29] Yield: 75% (710 mg) from (510 mg, 3.0 mmol) (**9**) and 20 equiv. of NaOMe in MeOH (~0.3 M) for 4 days at 150 °C in a sealed tube.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.90 (s, 1H), 7.10 (s, 2H), 3.78 (s, 3H).

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_6H_8N_5O$: 166.0729; found 166.0728.

Synthesis of mono-Boc-2-MeS-adenine derivatives

***tert*-Butyl (2-(methylthio)-9H-purin-6-yl)carbamate, 20a using the conditions [g].** To a stirred solution of the 2-alkyl-thioadenine derivatives (**16a**) (1.0 g, 5.52 mmol, 1.0 equiv.) in THF (~0.1–0.2 M) were added Boc$_2$O (4.82 g, 22.1 mmol, 4.0 equiv.) and DMAP (135 mg, 20 mol%, 0.2 equiv.) and the mixture was

stirred overnight at room temperature. TLC showed a mixture of products [di-Boc-2-MeS-adenine (**21a**) and tri-Boc-2-MeS-adenine (**19**)]. The solvent (THF) was removed under reduced pressure by rotary evaporation and water (50 mL) was added. The crude was extracted with ethyl acetate (EtOAc, 2 × 120 mL) and the organic layer washed with brine (20 mL). The organic layer (EtOAc) was separated, dried on Na$_2$SO$_4$, filtered and concentrated to afford the crude product (**19** + **21a**), which was used directly for the next step without further purification. The obtained crude was dissolved in MeOH (30 mL) and saturated aq. NaHCO$_3$ was added (20 mL). The reaction mixture was stirred for 5 h at 60 °C. The reaction mixture was cooled to room temp. and neutralized with 4 N HCl or sat. sodium dihydrogen phosphate until pH becomes 7–7.5. Note: work-up was done although there was some starting material (**20a** + **21a**) left in the reaction mixture. MeOH was removed by rotary evaporation, and the aq. solution was extracted with EtOAc (3 × 100 mL), washed with brine (20 mL), separated, dried on Na$_2$SO$_4$, filtered, and concentrated to obtain the crude product. The product was purified by silica gel column chromatography to afford homogeneous mono-Boc-2-MeS-adenine (**20a**). Eluent: 30–50% EtOAc in hexane. Yield: 45%, 0.70 g.

^{1}H-NMR (400 MHz, chloroform-d) δ 11.31 (s, 1H), 8.19 (s, 1H), 7.75 (s, 1H), 2.62 (s, 3H), 1.54 (s, 9H).

^{13}C NMR (100 MHz, DMSO) δ 162.79, 155.00, 152.10, 140.72, 138.50, 128.61, 128.30, 126.15, 115.45, 35.40, 31.50.

ESMS calculated for $C_{22}H_{24}N_5O_7$: (M + H) 470.2, found 470.2.

Large scale yield for step 3 leading to 520 g **8a**, using conditions (e) 62%, 180 g of **20a** from 500 g of **19** using conditions [e]. See ESI.†

For compounds **20b–20g**, 0.560–1.17 mmol of the **16b–16g** was used; all other amounts were as given for the preparation of **8a**.

***tert*-Butyl (2-(ethylthio)-9H-purin-6-yl)carbamate, 20b.** Compounds **20b** was prepared from compound **16b** (1.0 mmol) using the conditions [e].

Typically, to a stirred solution of the compound **16b** (195 mg, 1.0 mmol, 1.0 equiv.) in THF (5 mL, ~0.2 M) were added Boc$_2$O (875 mg, 4.0 mmol, 4.0 equiv.) and DMAP (24 mg, 0.2 equiv.) and the mixture was stirred overnight at room temperature. The solvent (THF) was removed under reduced pressure by rotary evaporation and the crude was dissolved in ethyl acetate (EtOAc, 15 mL). The solution was washed with water (2 × 20 mL), separated the EtOAc layer, and concentrated to afford the crude oil, which was used directly for the next step without further purification. The obtained crude oil was dissolved in MeOH (3 mL) and added aq. 10% NaOH (3 mL). The reaction mixture was stirred for 5 h at room temperature. The reaction mixture was diluted with EtOAc (20 mL) and neutralized with 10% solution of sodium dihydrogen phosphate (NaH$_2$PO$_4$·H$_2$O) until pH becomes 7–7.5. The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The combined EtOAc layer was dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated by rotary evaporation to obtain an oily residue, which was purified by silica column to get a white solid. Eluent: 30–50% EtOAc in hexane. Yield: 58%; 170 mg.

^{1}H NMR (400 MHz, chloroform-d) δ 8.30 (s, 1H), 8.27 (s, 1H), 3.15 (q, J = 7.4 Hz, 2H), 1.46 (s, 9H), 1.32 (t, J = 7.3 Hz, 4H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 164.55, 162.42, 152.63, 144.34, 143.74, 109.95, 83.24, 28.03, 25.26, 14.56.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{12}H_{18}N_5O_2{}^{32}S$: 296.1185; found 296.1181.

***tert*-Butyl (2-(hexylthio)-9*H*-purin-6-yl)carbamate, 20c.** Compounds **20c** was prepared from compound **16c** (0.621 mmol) according to the same procedure as compound **20b**. Step (i) in THF (3 mL, 0.2 M), and (ii) aq. 10% NaOH in MeOH (1 : 1, ~0.2 M) under room temperature for 5 h. Yield: 60%; 130 mg.

^{1}H NMR (400 MHz, chloroform-*d*) δ 8.22 (s, 1H), 8.03 (s, 1H), 3.20 (t, *J* = 7.3 Hz, 2H), 1.70 (q, *J* = 7.4 Hz, 2H), 1.50 (s, 9H), 1.41 (t, *J* = 7.8 Hz, 2H), 1.31–1.19 (m, 4H), 0.84 (t, *J* = 8.0 Hz, 3H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 164.96, 163.26, 152.81, 143.70, 143.56, 109.35, 83.66, 31.51, 31.12, 29.25, 28.69, 28.13, 22.62, 14.09.

ESMS calculated for $C_{16}H_{25}N_5O_2S$: (M + H) 352.2; found 352.2.

***tert*-Butyl (*tert*-butoxycarbonyl)(2-(cyclohexylthio)-9*H*-purin-6-yl)carbamate, 21d.** Compound **21d** was prepared from **16d** (0.401 mmol) using the conditions [f].

To a stirred solution of the compound **16d** (100 mg, 0.401 mmol, 1.0 equiv.) in THF (2 mL, ~0.2 M) were added Boc$_2$O (350 mg, 4.0 mmol, 4.0 equiv.) and DMAP (10 mg, 0.2 equiv.). The reaction mixture was stirred overnight at room temperature. The solvent (THF) was removed under reduced pressure by rotary evaporation and the crude was dissolved in ethyl acetate (EtOAc, 15 mL). The solution was washed with water (2 × 20 mL), separated the EtOAc layer, and concentrated to afford the crude product, which was used directly for the next step without further purification. The obtained crude product was dissolved in THF (5 mL) and added 10% NH$_4$OH (5 mL). The reaction mixture was stirred for 40 h at room temperature. The reaction mixture was neutralized with 4.0 N HCl solution until pH 7.0–7.5. The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 20 mL). The combined EtOAc layer was dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated by rotary evaporation to obtain an oily residue, which was purified by silica column to get a white solid. Eluent: 30–40% EtOAc in hexane. Yield: 94%; 170 mg.

^{1}H NMR (400 MHz, chloroform-*d*) δ 11.33 (s, 1H), 8.37 (s, 1H), 3.95–3.75 (m, 1H), 2.13–2.08 (m, 2H), 1.75–1.70 (m, 2H), 1.59–1.50 (m, 1H), 1.50–1.34 (m, 22H), 1.28–1.21 (m, 1H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 164.79, 157.62, 150.07, 147.32, 143.27, 119.86, 84.42, 43.74, 32.90, 27.73, 25.92, 25.70.

ESMS calculated for $C_{21}H_{32}N_5O_4S$: (M + H) 450.2; found 450.3.

***tert*-Butyl (2-(benzylthio)-9*H*-purin-6-yl)carbamate, 20e.** Compound **20e** was prepared from **16e** according to the same procedure as compound **20b** using the conditions [e]. Step (i) in THF (6 mL, 0.2 M); yield of **21e**: 79%; 423 mg from **16e** (1.166 mmol). Step (ii) aq. 10% NaOH in MeOH (1 : 1, ~0.1 M) under room temperature for 6 h. Yield of **20e**: 69%; 107 mg from 200 mg of **21e**.

^{1}H NMR (400 MHz, chloroform-*d*) δ 8.35 (s, 1H), 8.11 (s, 1H), 7.48–7.27 (m, 5H), 4.66 (s, 2H), 1.68 (d, *J* = 1.3 Hz, 9H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 164.44, 161.95, 152.63, 144.20, 143.49, 137.71, 129.30, 128.57, 127.24, 109.67, 84.00, 35.65, 28.17.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{17}H_{20}N_5O_2S$: 358.1338; found 357.1340.

***tert*-Butyl (2-(phenethylthio)-9*H*-purin-6-yl)carbamate, 20f.** Compound **20f** was prepared from **16f** (0.560 mmol) according to the same procedure as compound **20b** using the conditions [e]. Step (i) in THF (3 mL, 0.2 M), and (ii) aq. 10% NaOH in MeOH (1 : 1, ~0.2 M) under room temperature for 5 h. Yield: 28%; 58 mg.

Using the conditions [g] on a (326 mg, 1.201 mmol) scale. Step (i) in THF (6 mL, 0.2 M), and step (ii) sat. NaHCO$_3$, MeOH : H$_2$O (6 mL, 1 : 1, 0.2 M), 60 °C, 12 h; yield of **20f**: 36%, 160 mg; yield of **21f**: 131 mg, 23%.

^{1}H NMR (400 MHz, chloroform-*d*) δ 8.23 (s, 1H), 7.34–7.25 (m, 4H), 7.21 (tt, *J* = 5.2, 3.4 Hz, 1H), 3.53–3.44 (m, 2H), 3.13–3.04 (m, 2H), 1.56 (s, 9H).

^{13}C NMR (100 MHz, chloroform-*d*) δ 164.44, 162.67, 152.71, 144.03, 143.57, 140.72, 128.89, 128.53, 126.42, 109.67, 83.91, 35.86, 32.55, 28.19.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{18}H_{22}N_5O_2{}^{32}S$: 372.1494; found 372.1490.

***tert*-Butyl (2-methoxy-9*H*-purin-6-yl)carbamate, 20g.** Compound **20g** was prepared from **16g** (1.211 mmol) using the conditions [g]. Step (i) in THF (6 mL, 0.2 M), and step (ii) sat. NaHCO$_3$, MeOH : H$_2$O (6 mL, 1 : 1, 0.2 M), 60 °C, 12 h. Yield: 41%; 132 mg.

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.89 (s, 1H), 10.57 (s, 1H), 8.24 (s, 1H), 3.87 (s, 3H), 1.51 (s, 9H).

^{13}C NMR (100 MHz, DMSO) δ 163.94, 161.94, 160.95, 152.76, 145.66, 108.73, 81.33, 54.25, 27.91.

HRMS (ESI) *m/z*: [M + H]$^+$ calculated for $C_{11}H_{15}N_5O_2H^+$: 266.1253; found 266.1252.

Mitsunobu reaction of 20a–20c, 20e–20g, and 21d with 5′-*O*-trityl bicyclic intermediate 3; typical procedure for 22a–22c, 22e–22g, and 25d

In a 25 mL round bottom flask, mono-Boc-2-MeS-adenine (**20a**) (300 mg, 1.07 mmol, 1.5 equiv.), alcohol **3** (315 mg, 0.711 mmol, 1.0 equiv.), and triphenylphosphine (PPh$_3$) [373 mg, 1.42 mmol, 2.0 equiv.] were added. The contents were co-evaporated with dry toluene (3 × 5 mL) and the residue dried under vacuum for 3 h. The mixture was dissolved in dry THF (10 mL) and DIAD (280 μL, 1.42 mmol, 2.0 equiv.) was added drop wisely *via* syringe at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 1–2 h at room temperature and monitored by TLC. The solvent (THF) was removed under reduced pressure by rotary evaporation, and the crude product was purified by silica gel column chromatography to afford the product (**22a**) along with di-(isopropyloxycarbonyl)hydrazine byproduct (DIAD-H$_2$) [UV inactive; ~13% based on the C–H proton (septet) integration of isopropoxy group by ^{1}H-NMR], which could be observed as a yellow spot after developing the TLC with *p*-anisaldehyde stain. Eluent: 15–30% EtOAc in hexane. TLC: R_f ~ 0.3 (30% EtOAc in hexane). Corrected yield by ^{1}H-NMR: 94%, 470 mg. Purity: 87% by ^{1}H-NMR.

Large scale yield for step 4 leading to 520 g **8a**: 1.8 kg, crude product was obtained as a foamy solid from **20a** (769 g,

2.73 mol, 1.10 eq.) and compound **3** (1.10 kg, 2.49 mol, 1.00 eq.). See ESI.†

¹H NMR (400 MHz, chloroform-*d*) δ 8.14 (s, 1H), 7.81–7.70 (m, 1H) 7.78 (s, 1H), 7.43–7.37 (m, 6H), 7.33–7.13 (m, 9H), 5.38–5.29 (m, 1H), 5.07 (d, *J* = 3.2 Hz, 1H), 4.63 (dd, *J* = 7.2, 1.6 Hz, 1H), 3.76 (d, *J* = 10.0 Hz, 1H), 3.04 (d, *J* = 10.0 Hz, 1H), 2.56 (s, 3H), 1.55 (s, 9H), 1.53 (s, 3H), 1.25 (s, 4H), 1.16 (t, *J* = 5.0 Hz, 1H), 0.94–0.85 (m, 1H).

¹³C NMR (100 MHz, chloroform-*d*) δ 166.67, 151.72, 149.75, 149.28, 143.84, 139.86, 128.76, 128.06, 127.29, 119.27, 112.49, 88.85, 87.01, 82.17, 81.81, 64.82, 59.02, 37.39, 30.71, 28.33, 27.91, 26.09, 24.47, 22.08, 21.97, 14.86, 13.19.

ESMS (ESI) *m/z*: [M + H]⁺ calculated for $C_{40}H_{44}N_5O_5S$: 706.3; found 706.3.

Compounds 22a–22c, 22e–22g, and 25d

***tert*-Butyl (9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-2-(ethylthio)-9*H*-purin-6-yl)carbamate, 22b.** Compounds **22b** was prepared from **20b** (0.190 mmol). Corrected yield: 84%; 119 mg.

¹H NMR (400 MHz, chloroform-*d*) δ 8.12 (s, 1H), 7.77 (s, 1H), 7.38 (m, 6H), 7.23 (m, 9H), 5.29 (d, *J* = 6.9 Hz, 1H), 5.04 (d, *J* = 3.6 Hz, 1H), 4.60 (d, *J* = 7.0 Hz, 1H), 3.73 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.14 (ddd, *J* = 14.0, 8.8, 5.2 Hz, 2H), 3.03 (dd, *J* = 10.2, 3.4 Hz, 1H), 1.53 (s, 9H), 1.50 (s, 3H), 1.36 (t, *J* = 7.3 Hz, 3H), 1.23 (m, 4H), 1.14 (m, 1H), 0.91–0.86 (m, 1H). Product was contaminated with hydrazine impurity (~9%).

¹³C NMR (100 MHz, chloroform-*d*) δ 166.27, 151.69, 149.68, 149.28, 143.81, 139.66, 128.71, 128.00, 127.24, 119.12, 112.42, 88.78, 86.98, 82.08, 81.75, 64.82, 59.00, 37.32, 30.54, 28.27, 26.04, 25.76, 24.40, 14.52, 13.15.

HRMS (ESI) *m/z*: [M + H]⁺ calculated for $C_{41}H_{46}N_5O_5{}^{32}S$: 720.3220; found 720.3212.

***tert*-Butyl (9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-2-(hexylthio)-9*H*-purin-6-yl)carbamate, 22c.** Compounds **22c** was prepared from **20c** (0.237 mmol). Corrected yield: 77%; 142 mg.

¹H NMR (400 MHz, chloroform-*d*) δ 8.16 (s, 1H), 7.88 (s, 1H), 7.44–7.42 (m, 6H), 7.35–7.21 (m, 9H), 5.35–5.33 (m, 1H), 5.08 (s, 1H), 4.63 (dd, *J* = 7.1, 1.5 Hz, 1H), 3.79 (d, *J* = 10.0 Hz, 1H), 3.25–3.12 (m, 2H), 3.06 (d, *J* = 10.0 Hz, 1H), 1.56 (s, 9H), 1.54 (s, 4H), 1.48–1.45 (m, 2H), 1.33 (q, *J* = 3.7 Hz, 4H), 1.28 (s, 2H), 1.26 (s, 3H), 1.18 (t, *J* = 5.0 Hz, 1H), 0.92–0.89 (m, 4H).

Product was contaminated with hydrazine impurity (~26%).

¹³C NMR (100 MHz, chloroform-*d*) δ 166.35, 151.68, 149.66, 149.26, 143.76, 139.62, 128.66, 127.96, 127.18, 119.23, 112.35, 88.74, 86.93, 81.92, 81.65, 64.78, 58.83, 37.26, 31.50, 31.44, 30.50, 29.36, 28.66, 28.22, 25.98, 24.36, 22.64, 21.98, 14.11, 13.08.

ESMS calculated for calculated for $C_{45}H_{53}N_5O_5S$: 775.4; found 775.4.

***tert*-Butyl (2-(cyclohexylthio)-9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-9*H*-purin-6-yl)carbamate, 22d.**

Compound **25d** was prepared from **21d** (0.293 mmol). Corrected yield: 83%; 212 mg.

¹H NMR (400 MHz, chloroform-*d*) δ 8.24 (s, 1H), 7.44–7.32 (m, 5H), 7.28–7.10 (m, 10H), 5.29 (d, *J* = 7.0 Hz, 1H), 5.04 (s, 1H), 4.60 (dd, *J* = 7.1, 1.5 Hz, 1H), 3.78 (dd, *J* = 10.1, 7.0 Hz, 2H), 3.04 (d, *J* = 10.0 Hz, 1H), 2.10–2.07 (m, 2H), 1.77–1.67 (m, 2H), 1.61 (m, 1H), 1.51 (s, 4H), 1.45 (s, 22H), 1.26–1.17 (m, 4H), 1.17–1.11 (m, 1H), 0.90–0.86 (m, 1H). Product was contaminated with hydrazine impurity (~10%).

¹³C NMR (100 MHz, chloroform-*d*) δ 165.35, 153.49, 150.56, 150.01, 143.77, 141.85, 128.65, 127.94, 127.15, 125.73, 112.32, 88.66, 86.97, 83.64, 81.73, 64.66, 59.11, 43.88, 37.34, 32.94, 32.88, 30.26, 27.86, 25.97, 24.33, 21.74, 14.14, 12.96, 10.93.

ESMS calculated for $C_{50}H_{60}N_5O_7S$: 874.4; found 874.5.

***tert*-Butyl (2-(benzylthio)-9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-9*H*-purin-6-yl)carbamate, 22e.** Compound **22e** was prepared from **20e** (0.280 mmol). Corrected yield: 95%; 172 mg.

¹H NMR (400 MHz, chloroform-*d*) δ 8.18 (s, 1H), 7.85 (s, 1H), 7.50–7.45 (m, 2H), 7.43–7.36 (m, 6H), 7.33–7.19 (m, 13H), 5.31 (d, *J* = 7.1 Hz, 1H), 5.10 (s, 1H), 4.60 (d, *J* = 7.1 Hz, 1H), 4.45 (s, 2H), 3.80 (d, *J* = 10.2 Hz, 1H), 3.04 (d, *J* = 10.1 Hz, 1H), 1.57 (s, 9H), 1.54 (s, 3H), 1.29–1.25 (m, 4H), 1.18 (t, *J* = 5.1 Hz, 1H), 0.94–0.87 (m, 1H). Product was contaminated with hydrazine impurity (~10%).

¹³C NMR (100 MHz, chloroform-*d*) δ 165.56, 151.61, 149.66, 149.28, 143.79, 139.76, 138.22, 129.36, 128.68, 128.32, 127.97, 127.91, 127.20, 126.95, 119.35, 112.39, 88.82, 86.95, 82.08, 81.73, 64.82, 59.04, 37.33, 35.85, 30.49, 28.23, 26.02, 24.41, 21.99, 13.11.

HRMS (ESI) *m/z*: [M + H]⁺ calculated for $C_{46}H_{48}N_5O_5{}^{32}S$: 782.3376; found 782.3384.

***tert*-Butyl (9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-2-(phenethylthio)-9*H*-purin-6-yl)carbamate, 22f.** Compound **22f** was prepared from **20f** (0.431 mmol). Corrected yield: 78%, 160 mg.

¹H NMR (400 MHz, chloroform-*d*) δ 8.19 (d, *J* = 1.4 Hz, 1H), 7.77 (s, 1H), 7.43–7.41 (m, 5H), 7.38–7.36 (m, 2H), 7.31–7.21 (m, 13H), 5.33 (d, *J* = 7.1 Hz, 1H), 5.14 (s, 1H), 4.61–4.59 (m, 1H), 3.83 (d, *J* = 10.1 Hz, 1H), 3.40 (qt, *J* = 13.6, 6.6 Hz, 2H), 3.07 (t, *J* = 7.9 Hz, 2H), 3.00 (d, *J* = 10.1 Hz, 1H), 1.57 (s, 9H), 1.54 (s, 3H), 1.33 (d, *J* = 6.3 Hz, 1H), 1.23 (s, 3H), 1.19 (t, *J* = 5.1 Hz, 1H), 0.89 (dd, *J* = 9.4, 5.7 Hz, 1H). Product was contaminated with hydrazine impurity (~38%).

¹³C NMR (100 MHz, chloroform-*d*) δ 165.90, 151.67, 149.57, 149.38, 143.77, 140.94, 139.54, 128.91, 128.67, 128.37, 127.97, 127.20, 126.24, 112.38, 88.82, 86.98, 81.94, 81.55, 64.85, 58.75, 37.32, 36.37, 32.92, 30.42, 28.22, 26.01, 24.37, 13.14.

HRMS (ESI) *m/z*: [M + H]⁺ calculated for $C_{47}H_{50}N_5O_5{}^{32}S$: 796.3533; found 796.3527.

***tert*-Butyl (9-((3a*R*,3b*R*,4a*S*,5*R*,5a*S*)-2,2-dimethyl-3b-((trityloxy)methyl)hexahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-yl)-2-methoxy-9*H*-purin-6-yl)carbamate, 22g.** Compound **22g** was prepared from **20g** (0.431 mmol). Corrected yield: 67%; 135 mg with 80% purity by ¹H-NMR.

^{1}H NMR (400 MHz, chloroform-d) δ 8.08 (s, 1H), 7.87 (s, 1H), 7.44–7.35 (m, 5H), 7.31–7.17 (m, 10H), 5.33 (d, J = 7.1 Hz, 1H), 5.02 (s, 1H), 4.66 (dd, J = 7.3, 1.5 Hz, 1H), 3.91 (s, 3H), 3.74 (d, J = 9.9 Hz, 1H), 3.07 (d, J = 9.9 Hz, 1H), 1.55 (s, 9H), 1.53 (s, 3H), 1.27 (s, 1H), 1.25 (s, 3H), 1.16 (t, J = 5.0 Hz, 1H), 0.96–0.90 (m, 1H). Product was contaminated with hydrazine impurity (~12%).

^{13}C NMR (100 MHz, chloroform-d) δ 162.29, 152.35, 150.88, 149.50, 143.80, 139.51, 128.69, 127.97, 127.23, 117.82, 112.40, 88.80, 86.91, 82.05, 81.91, 64.75, 59.16, 55.06, 37.34, 30.54, 28.23, 26.05, 24.40, 13.15.

HRMS (ESI) m/z: [M + H]$^+$ calculated for $C_{40}H_{44}N_5O_6$: 690.3292; found 690.3297.

Compounds 8a–8f

Conditions (j): 1.0 N HCl (5.0 mL) was added to a sealed tube containing the compound (22a) (70 mg, 0.1 mmol). The suspension was stirred at 50 °C for 18 h. The solvent was removed, and the crude was co-evaporated with toluene (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (~1.5 g, 1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 16 h. The MeOH solution was filtered, concentrated, and the crude was purified by silica gel column chromatography to get 8a. Eluent: 5% of 10% aq. NH$_4$OH (23%) in MeOH, then 5–15% MeOH in DCM. Yield: 32 mg, 88%.

Large scale yield for step 5 leading to 520 g 8a, using conditions (i).

Six individual runs (each with 315 g of 22a, 446 mmol, 1.00 eq.) were carried out to get a combined 520 g of 8a as a white solid, 1.61 mol, 60% yield for 2 steps. See ESI.†

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(methylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8a. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.44 (s, 1H), 4.77 (dd, J = 6.7, 1.6 Hz, 1H), 4.26 (d, J = 11.7 Hz, 1H), 3.93–3.85 (m, 1H), 3.35 (d, J = 9.5 Hz, 2H), 2.59 (s, 3H), 1.61 (dd, J = 8.6, 3.8 Hz, 1H), 1.54 (dd, J = 5.2, 3.9 Hz, 1H), 0.76 (ddd, J = 8.7, 5.3, 1.7 Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d_6). HRMS (ESI) m/z: [M + H]$^+$ calculated for $C_8H_{11}N_3O_2H^+$: 182.0930; found 182.0936.

Compounds 8b–8f were prepared using various acidic conditions.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(ethylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8b. Compound 8b was prepared from 22b (30 mg, 0.042 mmol) using the conditions [k].

Aq. 4.0 N HCl in MeOH (2.0 mL) was added to a 25 mL round-bottomed flask containing the compound (22b) (30 mg, 0.042 mmol). The solution was stirred at 35 °C for 16 h. The solvent was removed, and the crude was co-evaporated with ethanol (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 16 h. The MeOH solution was filtered, concentrated, and the crude was purified by silica gel column chromatography to get 8b. Eluent: 10–20% MeOH in DCM. Yield: 46%; 6.0 mg.

^{1}H NMR (600 MHz, methanol-d_4) δ 8.39 (s, 1H), 4.83 (s, 1H), 4.74 (dd, J = 6.8, 1.7 Hz, 1H), 4.24 (d, J = 11.6 Hz, 1H), 3.87 (dt, J = 6.7, 1.4 Hz, 1H), 3.31 (d, J = 11.7 Hz, 1H), 3.24–3.10 (m, 2H), 1.60 (ddd, J = 8.8, 3.9, 1.5 Hz, 1H), 1.53 (dd, J = 5.2, 4.0 Hz, 1H), 1.38 (t, J = 7.3 Hz, 3H), 0.74 (ddd, J = 8.7, 5.2, 1.7 Hz, 1H).

^{13}C NMR (151 MHz, methanol-d_4) δ 166.68, 156.78, 151.34, 139.72, 117.57, 77.75, 72.20, 64.43, 62.94, 37.82, 26.22, 24.54, 15.28, 12.30.

HRMS (ESI) m/z: [M + H]$^+$ calculated for $C_{14}H_{20}N_5O_3{}^{32}S$: 338.1287; found 338.1293.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(hexylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8c. Compound 8c was prepared from 22c (28 mg, 0.431 mmol) using the conditions [k].

Aq. 4.0 N HCl (1.0 mL) was added to a 25 mL cylindrical sealed tube containing the compound (22c) (28 mg, 0.431 mmol) in EtOH (3 mL). The solution was stirred at 35 °C for 16 h. The solvent was removed, and the crude was co-evaporated with ethanol (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 1 h. The MeOH solution was filtered, concentrated, and the crude was purified by preparative HPLC to get the pure 8c. Yield: 8.0 mg, 56%.

^{1}H NMR (400 MHz, methanol-d_4) δ 8.39 (s, 1H), 4.85 (s, 1H), 4.76 (d, J = 6.7 Hz, 1H), 4.25 (d, J = 11.6 Hz, 1H), 3.89 (d, J = 6.6 Hz, 1H), 3.36–3.28 (m, 1H), 3.25 (dt, J = 14.1, 7.3 Hz, 1H), 3.13 (dt, J = 13.6, 7.3 Hz, 1H), 1.75 (p, J = 7.2 Hz, 2H), 1.61 (dd, J = 9.0, 3.9 Hz, 1H), 1.54 (t, J = 4.7 Hz, 1H), 1.53–1.41 (m, 2H), 1.36 (h, J = 4.0 Hz, 4H), 0.97–0.89 (m, 3H), 0.79–0.71 (m, 1H). Compound was purified by HPLC and was contaminated with triethylammonium acetate buffer (~17% based on NCH$_2$ protons of the buffer at 3.03).

Prep. HPLC method: Phenomenex Luna 5 μm C18(2) 100 A, LC column (250 × 21.2 mm). Linear gradient solvent system: ACN : 10 mM TEAA from 40 : 80 to 80 : 20 in 40 minutes. Rt 43.32 min.

^{13}C NMR (100 MHz, methanol-d_4) δ 157.36, 147.25, 141.87, 130.23, 108.12, 68.35, 62.77, 54.98, 53.48, 28.39, 23.15, 22.49, 21.29, 20.20, 15.15, 14.19, 4.90, 2.83.

HRMS (ESI) m/z: [M + H]$^+$ calculated for $C_{18}H_{26}N_5O_3{}^{32}S$: 394.1913; found 394.1920.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(cyclohexylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8d. Compound 8d was prepared from 25d (96 mg, 0.1098 mmol) using the conditions [i].

Aq. 4.0 N HCl in MeOH (2.0 mL) was added to a 25 mL round-bottomed flask containing the compound (25d) (96 mg, 0.1098 mmol). The solution was stirred at 35 °C for 16 h. The solvent was removed, and the crude was co-evaporated with ethanol (2 × 5 mL). The residue was dissolved in MeOH (3.0 mL) and added aq. Na$_2$CO$_3$ (2.2 M) until the pH of the reaction mixture becomes ~8. The suspension was stirred at room temperature for 20 minutes. The solution was filtered and washed the precipitate with EtOH (2 × 5 mL). The combined solution was concentrated, and the crude was purified by silica gel column

chromatography to get **8d**. Eluent: 10% MeOH in DCM. Yield: 70%; 30.0 mg.

^{1}H NMR (400 MHz, methanol-d_4) δ 8.39 (s, 1H), 4.81 (s, 1H), 4.75 (dd, J = 6.7, 1.6 Hz, 1H), 4.25 (d, J = 11.6 Hz, 1H), 3.89 (d, J = 6.8 Hz, 2H), 3.36–3.27 (m, 1H), 2.16–2.10 (m, 2H), 1.81–1.78 (m, 2H), 1.68–1.58 (m, 2H), 1.54–1.47 (m, 5H), 1.36–1.27 (m, 2H), 0.75 (ddd, J = 8.8, 5.1, 1.7 Hz, 1H).

^{13}C NMR (100 MHz, methanol-d_4) δ 166.58, 156.78, 151.31, 139.72, 117.58, 77.79, 72.24, 64.47, 63.05, 44.73, 37.86, 34.50, 34.22, 27.16, 27.11, 26.97, 24.55, 12.32.

ESMS calculated for $C_{18}H_{26}N_5O_3S$: 392.2; found 392.2.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(benzylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8e. Compound **8e** was prepared from **22e** (89 mg, 0.1184 mmol) using the conditions [k]. Yield: 63%; 30.0 mg.

Aq. 4.0 N HCl in MeOH (2.0 mL) was added to a 25 mL round-bottomed flask containing the compound (**22e**) (89 mg, 0.1184 mmol). The solution was stirred at 35 °C for 16 h. The solvent was removed, and the crude was co-evaporated with ethanol (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 6 h. The MeOH solution was filtered, concentrated, and the crude was purified by silica gel column chromatography to get **8e**. Eluent: 5–10% MeOH in DCM. The product was crystallized on trituration with ethanol to get pure **8e**. Yield: 63%; 30.0 mg.

^{1}H NMR (600 MHz, methanol-d_4) δ 8.40 (s, 1H), 7.47–7.39 (m, 2H), 7.26 (t, J = 7.7 Hz, 2H), 7.19–7.14 (m, 1H), 4.74 (dd, J = 6.6, 1.7 Hz, 1H), 4.49–4.34 (m, 2H), 4.23 (d, J = 11.6 Hz, 1H), 3.88 (dt, J = 6.5, 1.4 Hz, 1H), 3.29 (d, J = 11.7 Hz, 1H), 1.59 (ddd, J = 8.8, 3.9, 1.5 Hz, 1H), 1.54 (dd, J = 5.2, 3.9 Hz, 1H), 0.74 (ddd, J = 8.7, 5.2, 1.7 Hz, 1H). One proton peak obscured by solvent peaks at 4.87.

^{13}C NMR (151 MHz, methanol-d_4) δ 166.14, 156.73, 151.27, 139.90, 139.75, 130.19, 129.35, 127.95, 117.67, 77.76, 72.18, 64.40, 62.89, 37.80, 36.33, 24.56, 12.33.

HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_8H_{11}N_3O_2H^+$: 182.0930; found 182.0936.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-(phenethylthio)-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8f. Compound **8f** was prepared from **22f** (92 mg, 0.116 mmol) using the conditions [k].

Aq. 4.0 N HCl in MeOH (2.0 mL) was added to a 25 mL round-bottomed flask containing the compound (**22f**) (92 mg, 0.116 mmol). The solution was stirred at 35 °C for 16 h. The solvent was removed, and the crude was co-evaporated with toluene (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 16 h. The MeOH solution was filtered, concentrated, and the crude was purified by silica gel column chromatography to get **8f**. Eluent: 5–10% MeOH in DCM. The product was crystallized on trituration with ethanol to get pure **8f**. Yield: 75%; 36.0 mg.

^{1}H NMR (500 MHz, methanol-d_4) δ 8.38 (s, 1H), 7.30–7.28 (m, 2H), 7.25–7.22 (m, 2H), 7.15–7.12 (m, 1H), 4.73 (dd, J = 6.6,

1.6 Hz, 1H), 4.23 (d, J = 11.6 Hz, 1H), 3.86 (d, J = 6.6 Hz, 1H), 3.39 (ddd, J = 13.3, 8.7, 7.0 Hz, 1H), 3.33–3.22 (m, 2H), 3.00 (t, J = 7.3 Hz, 2H), 1.58 (ddd, J = 8.7, 3.9, 1.4 Hz, 1H), 1.54–1.52 (m, 1H), 0.72 (ddd, J = 8.6, 5.1, 1.7 Hz, 1H). One proton peak obscured by solvent peaks at 4.87.

^{13}C NMR (126 MHz, methanol-d_4) δ 166.41, 156.74, 151.30, 142.11, 139.62, 129.85, 129.39, 127.22, 77.74, 72.18, 64.38, 62.81, 37.81, 37.38, 33.60, 24.64, 12.30.

HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{20}H_{24}N_5O_3{}^{32}S$: 414.1600; found 414.1607.

(1R,2R,3S,4R,5S)-4-(6-Amino-2-methoxy-9H-purin-9-yl)-1-(hydroxymethyl)bicyclo[3.1.0]hexane-2,3-diol, 8g. Compound **8g** was prepared from **22g** (25 mg, 0.0362 mmol) using the conditions [l]. Yield: 45%; 5.0 mg.

10% TFA in water (1.0 mL) was added to a 25 mL cylindrical sealed tube containing the compound (**22g**) (25 mg, 0.0362 mmol) in MeOH (5 mL). The solution was stirred at 50 °C for 17 h. The solvent was removed, and the crude was co-evaporated with ethanol (2 × 5 mL). The residue was dissolved in MeOH (5.0 mL) and treated with 1 mL of Amberlite resin-93 (1.2 mmol), which was previously washed with MeOH (3 × 3 mL). The reaction mixture was stirred for 1 h. The MeOH solution was filtered, concentrated, and the crude was the crude was purified by silica gel column chromatography to get **8e**. Eluent: 15–20% MeOH in DCM. Yield: 45%; 5.0 mg.

^{1}H NMR (400 MHz, chloroform-d) δ 8.32 (d, J = 5.8 Hz, 1H), 4.82–4.71 (m, 2H), 4.25 (dd, J = 11.8, 5.6 Hz, 1H), 3.96 (dd, J = 4.9, 2.7 Hz, 3H), 3.89 (d, J = 6.3 Hz, 1H), 3.30 (dd, J = 11.5, 5.5 Hz, 1H), 1.60 (p, J = 4.4 Hz, 1H), 1.51 (p, J = 4.7, 4.0 Hz, 1H), 0.80–0.68 (m, 1H).

^{13}C NMR (100 MHz, methanol-d_4) δ 163.78, 158.14, 152.21, 139.68, 116.31, 77.82, 72.24, 64.48, 63.28, 55.18, 37.93, 24.57, 12.22.

HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{13}H_{18}N_5O_4$: 308.1359; found 308.1353.

Conflicts of interest

T. E. L. and R. B. P. are employees of Astrocyte Pharmaceuticals Inc. The other authors have no competing interest to declare.

Acknowledgements

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