

BET isoform selectivity through diverse linkers for bivalent inhibitors: GSK785, a BRD2/4-selective bivalent BET inhibitor.

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Abstract

The inhibition of the Bromodomain and Extra Terminal (BET) family of proteins has been widely studied for over a decade for its potential therapeutic benefit in cancer and immunoinflammatory diseases. Selective inhibition within the four BET isoforms has been sought to facilitate the understanding of the individual role played by each family member, and to mitigate pharmacologically driven tolerability limitations observed in the clinic for pan-BET inhibitors. Herein, we present an investigation into the potential for isoform selectivity using bivalent inhibitors with constrained linker geometries. By employing a set of conformationally restricted diamines as linkers between two BET binding warheads, this work details the design and synthesis of two iterations of bivalent molecules. Whilst finding the BET isoforms to be highly accommodating of bivalent molecules with diverse linker geometries, we present the discovery of **9h** (GSK785), a bivalent inhibitor with an unprecedented BRD2/4-selective, BRD3 sparing profile.

Introduction

The first reports describing the efficacy of Bromodomain and Extra Terminal (BET) protein inhibitors in oncology and immune-mediated inflammatory diseases were published in 2010.^{1,2} This sparked over a decade of research within the scientific community to realise the therapeutic benefit of these molecules.³⁻⁹

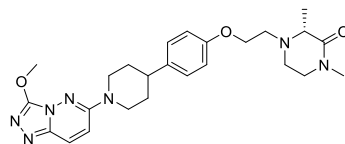
The BET family are epigenetic reader proteins which interact with acetylated lysine residues on histone tails. The family consists of four isoforms: BRD4, BRD3, BRD2, and the testes specific BRDT. Each isoform contains two bromodomain binding domains, BD1 (closest to the N-terminus) and BD2.¹⁰

The first reported BET inhibitors bound to both binding domains of all four isoforms (8 domains in total, referred to as pan-BET inhibition).^{1,11} Whilst these inhibitors showed potent anti-cancer and anti-inflammatory effects, they also showed pharmacologically-driven tolerability limitations in the clinic.¹²⁻¹⁴ To investigate opportunities to increase the therapeutic index of these molecules, selectivity within the 8 binding domains has been sought.

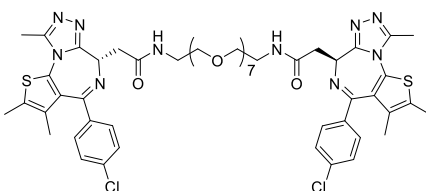
Whilst there is a high level of homology between the binding domains across the BET isoforms, there are larger residue differences between the BD1 and BD2 binding domains within the same isoform than there are for the BD1 and BD2 binding domains across the isoforms. “Second generation” BET inhibitors exploited these residue differences to yield selective BD1 and BD2 inhibitors, selectively inhibiting either domain across all four isoforms or for a single isoform.¹⁵⁻²³ These molecules have helped to tease apart the effects of inhibiting each of these domains in turn. For example, Gilan *et al.* reported that pan-BD1 inhibition phenocopied pan-BET inhibition, whilst pan-BD2 inhibition was more nuanced acting through alternative transcriptional mechanisms.²⁴

Achieving selectivity between the BET isoforms has proved challenging, particularly for monovalent inhibitors, due to sequence homology. Demonstrating robust progress towards isoform selectivity would provide a step forward in enabling the community to delineate the biological effects and therapeutic benefits of isoform-selective inhibition.

Since 2016, examples of a bivalent pan-BET inhibition modality have emerged in the literature.²⁵⁻²⁹ AZD5153 (**1**) and MT1 (**2**), shown in Figure 1, were found to bind both the BD1 and BD2 domains of the same BET protein simultaneously. Both exhibited a boosted affinity for the BET proteins compared to monovalent compounds, which resulted in unprecedented levels of efficacy in functional assays.



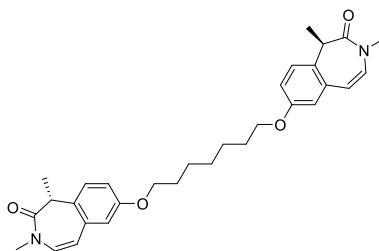
AZD5153 (1)



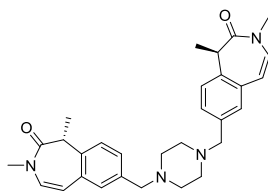
MT1 (2)

Figure 1. Structures of the first bivalent BET inhibitors, AZD5153 (1) and MT1 (2).

Our group have since published a series of bivalent BET inhibitors based on a highly ligand efficient benzoazepinone (BZP) warhead, for example compounds **3** and **4**, Figure 2.^{30,31}



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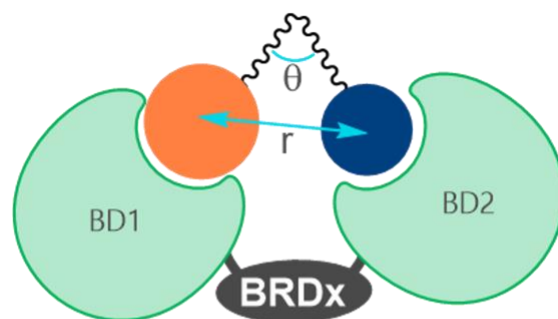
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Figure 2. Structures of compounds **3** and **4**.

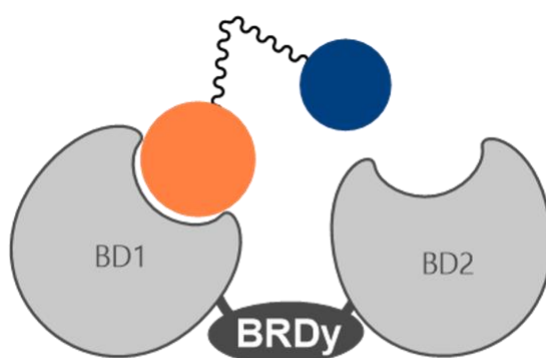
Whilst up to 12-fold selectivity for BRDT has been reported with bivalent inhibitors,³² we wanted to achieve a higher selectivity window between BET family members to further differentiate from pan-BET bivalent compounds. Our aim was therefore to investigate the

feasibility of obtaining greater than 100-fold selectivity amongst the BET isoforms starting from bivalent molecules constructed from the pan-BET BZP warhead exemplified in compounds **3** and **4**.

There are differences in the length and sequence between the BD1 and BD2 domains of each BET isoform; therefore, we postulated that the conformations available to each BET isoform to bind a molecule bivalently may also differ. Indeed, Waring *et al.* provide evidence that large-scale conformational changes occur in BRD4 with respect to the relative orientation of the BD1 and BD2 domains upon binding a bivalent inhibitor; so it is feasible that the other isoforms may have differing preferences. This is illustrated in Figure 3, where the shape of a bivalent inhibitor permits binding to both domains of BRD_x but not to BRD_y.



Angle and distance
matched to an isoform



Binding not possible with other isoforms
due to different interdomain distances & angles

Figure 3. Illustration showing how a bivalent molecule might obtain selectivity for one BET isoform (BRD_x) over another (BRD_y) by having a precise relative positioning (defined by illustrative parameters r and θ) of the warheads such that BRD_y is unable to place its two domains in the correct configuration to bind the molecule.

At the time this work was performed, there were no crystal structures available of bivalent BET inhibitors bound to full-length BET proteins. As a result, a structure-based drug design approach could not be employed. We therefore looked to adopt a diversity-oriented approach to design.

There were multiple variables to consider when contemplating the design strategy for bivalent inhibitors: the bromodomain binding motif (warhead), the exit vector from the warhead to achieve bivalency, and the linker. A diverse exploration of each of these variables would result in a

combinatorial explosion of potential compounds, which would be costly and time consuming to synthesise. We therefore adopted a design strategy to fix the warheads and exit vectors for bivalency, instead focussing solely on the linker connecting the two warheads.

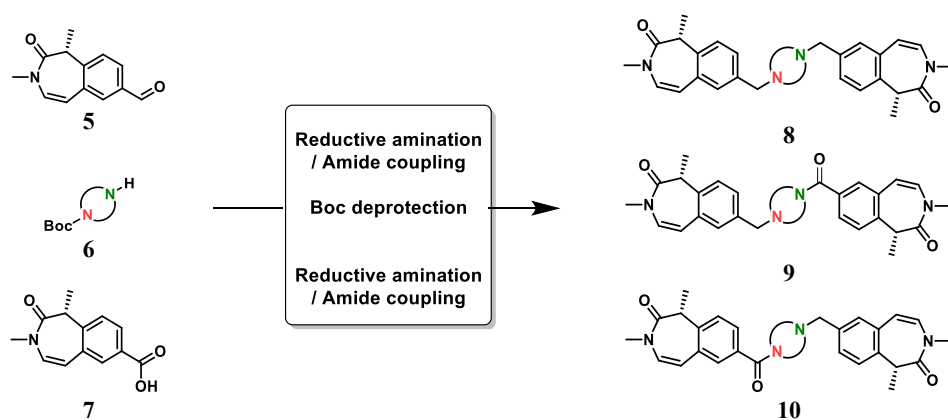
Our goal was thus to synthesise a diverse set of bivalent BET inhibitors, screening for isoform selectivity within the BET family. The ability of molecules to bind bivalently at BRD4 was tested using a cellular BRD4 BRET “BioSensor” (BS) assay, which produced a response when the BD1 and BD2 domains of a modified BRD4 protein were brought into proximity.²⁷ Selectivity amongst the isoforms was tested using a cellular BRET protein-protein interaction (PPI) assay set up for BRD2, BRD3, and BRD4.³³ This assay measured the displacement of a modified BET protein from chromatin upon binding of an inhibitor. Due to the modified nature of the proteins used in these assays, we used the results from the PPI assays as only an indicator of possible isoform selectivity. Key molecules were to be screened in a more costly, lower throughput, cellular thermal shift assay (CETSA). This assay measured the stabilisation of melt temperatures of the native BET proteins upon binding, thus giving a measurement of binding affinity that we deemed more physiologically accurate. Selectivity against BRDT was not evaluated in this work due to its localisation in the testes. Within this paper, we describe the discovery of GSK785, a BRD2/4 selective, BRD3-sparing bivalent BET inhibitor.

Results and Discussion

Our design strategy utilised the BZP binding motif and linkage vector in compounds **3** and **4**, whilst varying the linkers between the two warheads. A modular approach to synthesis was sought to enable an efficient synthetic execution. The chosen design utilised acid **5**, aldehyde **7** and protected diamine scaffolds **6**, shown in Scheme 1. Two iterations of synthesis were planned, the

first iteration producing bis-amine scaffold **8**, and a second iteration introducing an amide in the linker to yield scaffolds **9** and **10**.

Scheme 1. Design strategy for the synthesis of bivalent BET inhibitors using the BZP warhead. From the respective aldehydes, acids and Boc-protected diamines, a sequence of reductive amination or amide coupling, followed by Boc-deprotection and a second reductive amination or amide coupling could be employed to yield scaffolds **8**, **9**, and **10**.



The diversity in this design originates from the nature of the linkers, so we sought a source of diamine linkers with well-defined geometrical diversity. Grygorenko *et al.* published a study investigating the geometrical diversity of conformationally restricted diamines (CRDAs).³⁴ They enumerated all endocyclic, bicyclic diamines, where no unbridged ring was greater than six-membered, and no undesirable functionalities were present, such as hemiaminals, hydrazines, and aziridines. This process yielded 71 conformationally restricted diamines (CRDAs). Optimised 3D geometries for these molecules were calculated using DFT, followed by the measurement of four geometrical parameters, r , θ , φ_1 and φ_2 , illustrated in Figure 4. Here, the nitrogens are designated N^1 and N^2 . The vectors which bisect the C-N-C angles are denoted as n_1 and n_2 . The angle between vectors n_1 and n_2 is θ . The distance between N^1 and N^2 is r , and the angle between the vector formed by r and n_1 and n_2 is φ_1 and φ_2 respectively. The numbering is elected such that

$\varphi_1 > \varphi_2$. The distributions of these properties across the set of CRDAs are exemplified in Figure 5. At the intersection of two different parameters, a scatter plot of the respective properties is shown. At the intersection of the same parameters, a histogram showing the distribution of that parameter is shown, with colours representing the results of clustering.

The diamines published by Grygorenko *et al.*, along with the associated data, were deemed well-suited for our experiment and were therefore utilised in this work.

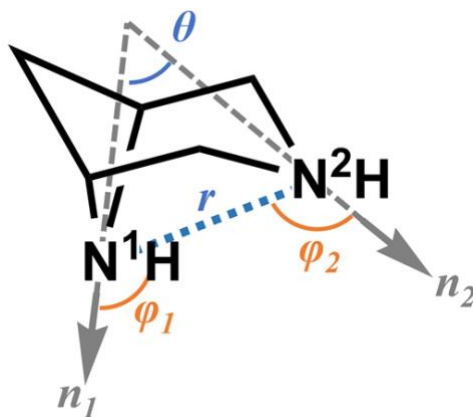


Figure 4. Illustration showing how the CRDA parameters are measured. Vectors n_1 and n_2 are designated to nitrogens N_1 and N_2 and bisect the respective C-N-C angles. The angle between the n_1 and n_2 vectors is θ . The distance between N^1 and N^2 is the length of r , and the angle between the vectors r and n_1 and n_2 are φ_1 and φ_2 respectively.

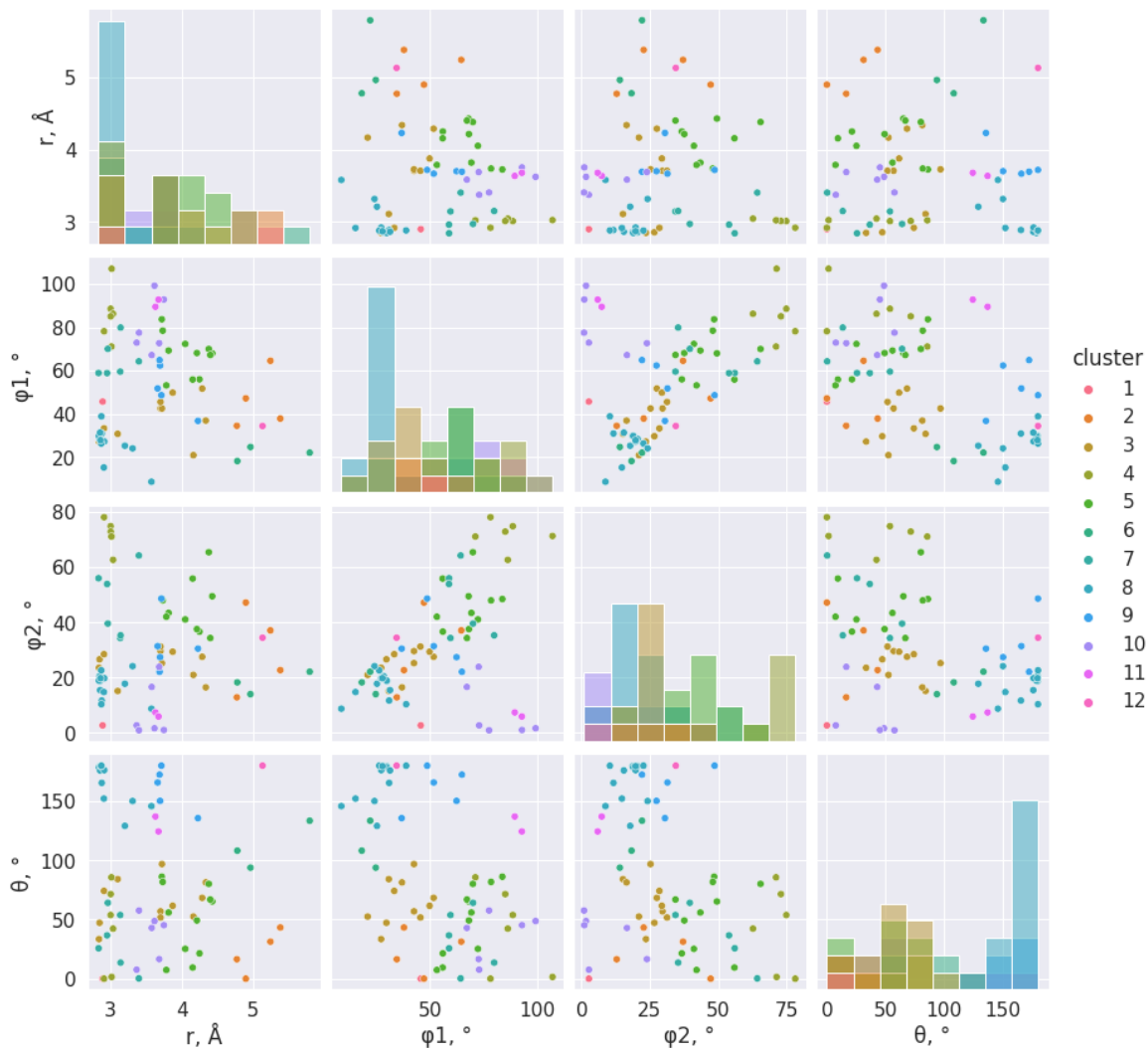


Figure 5. Plots showing the distributions for r , θ , ϕ_1 and ϕ_2 across the set of published diamines.

We then sought to select a representative set of these linkers to utilise for synthesis. To do this, the linkers were clustered to enable such a sampling. To perform the clustering, the unweighted pair group method with arithmetic mean (UPGMA) method was employed, producing 12 clusters from which a set of 18 linkers were chosen based on commercial availability.³⁵ Compounds with fewer stereoisomers were also prioritised to reduce the need for chiral purification, as most linkers were only available commercially as racemates at the time. The clusters are shown in Figure 5.

Figure 6 shows a plot of r vs θ , with selected CRDAs labelled. The numbered structures of the selected linkers are shown in Figure 7.

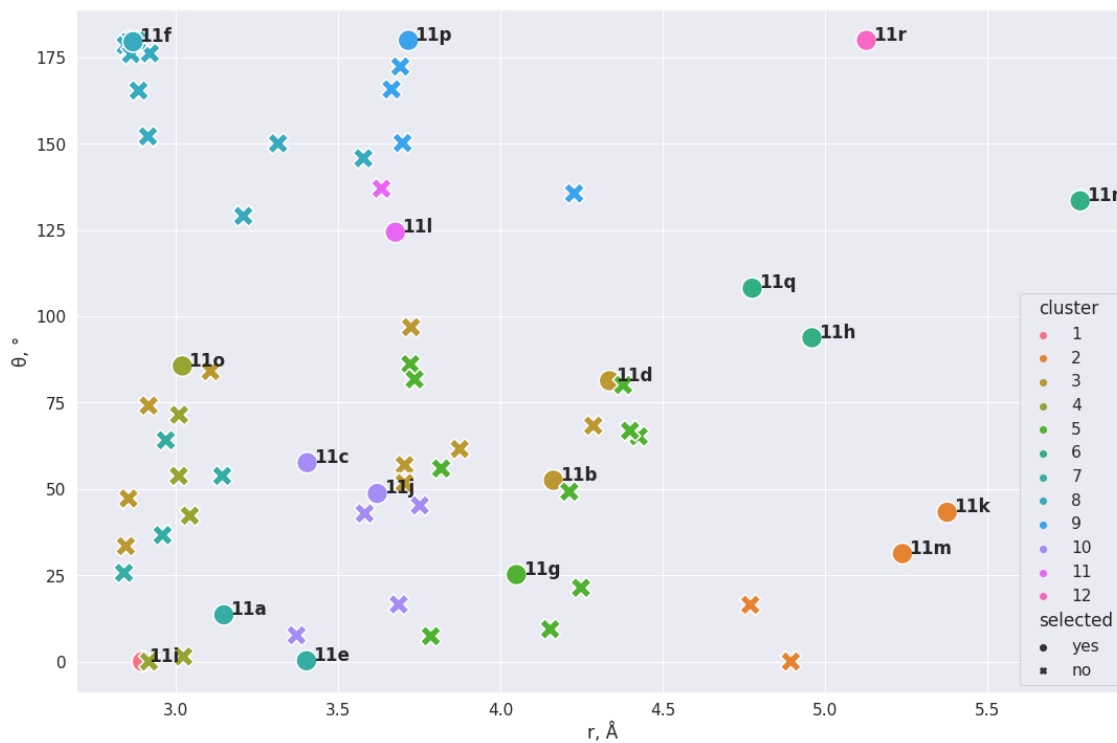


Figure 6. The library of CRDAs on a plot of r vs θ , coloured by cluster. Selected compounds are shown as circles and unselected compounds are shown as crosses. Selected compounds are annotated by their numbers.

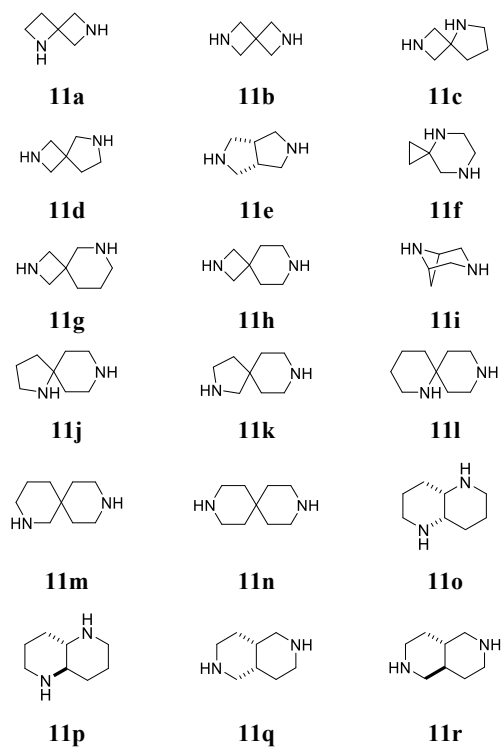
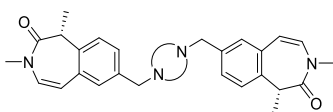


Figure 7. The structures of the selected CRDAs **11a-11r**

As discussed, the first iteration of synthesis aimed to prepare the set of bis-amine bivalent compounds, of chemotype **8**. The PPI and BRD4 BS data for this iteration is presented in Table 1.

Table 1. Biochemical data for **8a-q**.**8a-q**

Cmpd	Linker structure	BRD2 PPI [†]	BRD3 PPI [†]	BRD4 PPI [†]	BRD4 BS [†]	Cluster
8a		9.0 (5)	8.3 (4)	9.1 (3)	8.6 (4)	7
8b		9.1 (2)	7.8 (2)	8.4 (2)	7.9 (4)	3
8c		8.6 (5)	7.9 (3)	8.4 (4)	7.9 (6)	10
8d		9.5 (3)	8.9 (3)	9.1 (3)	8.4 (4)	3
8e		8.5 (4)	7.7 (3)	8.5 (3)	7.9 (5)	7
8f		9.0 (4)	8.4 (3)	9.0 (3)	8.3 (5)	8
8g		9.4 (6)	9.0 (4)	9.3 (3)	8.8 (4)	5
8h		9.0 (5)	8.4 (4)	8.9 (3)	8.4 (4)	6
8i		8.3 (4)	7.6 (2)	8.4 (3)	7.9 (3)	1
8j		9.1 (5)	8.7 (4)	9.0 (3)	8.4 (3)	10
8k		9.1 (3)	8.7 (3)	9.1 (3)	8.5 (3)	2
8l		6.9 (3)	6.6 (2)	7.5 (3)	7.4 (4)	11
8m		9.7 (5)	9.1 (5)	9.2 (3)	8.7 (4)	2
8n		8.8 (5)	8.3 (3)	9.0 (3)	8.5 (3)	6
8q1		8.8 (5)	8.4 (4)	8.7 (3)	8.2 (2)	6
8q2		9.3 (3)	8.8 (4)	9.3 (4)	8.7 (3)	6

[†]Average pIC₅₀ values are shown with the number of test occasions in the average shown in parenthesis.

The compounds for this set, **8a-q**, showed generally high potencies across the BET isoforms in the PPI assays. All the compounds from the iteration were found to be active in the BRD4 BS assay, suggesting that they were all binding bivalently to BRD4. Only **8b** showed notable selectivity from the set, with a 20-fold selectivity for BRD2 over BRD3. This was not further investigated as it did not meet the 100-fold selectivity threshold set out in our aims. **8l** was the only compound from the iteration which showed a potency below a pIC₅₀ of 7 for any of the isoforms.

There were no obvious relationships between the CRDA parameters and the potencies across the BET PPI, or BRD4 BS, assays (Figure 8). Unfortunately, synthesis with amine **11r** could not be performed as the purchased racemate contained only the *cis*-isomer **11q**. No other compounds from cluster 12 could be sourced commercially at the time, so this cluster remained unexplored. Furthermore, compounds **11o** and **11p** were not available at the time this iteration was performed, so these were also omitted. Due to the disappointing levels of selectivity observed across the set in general, these compounds were not revisited. In summary, the compounds from the first iteration showed generally high, non-selective binding to the BET isoforms, and did not yield any compounds of interest for further evaluation.

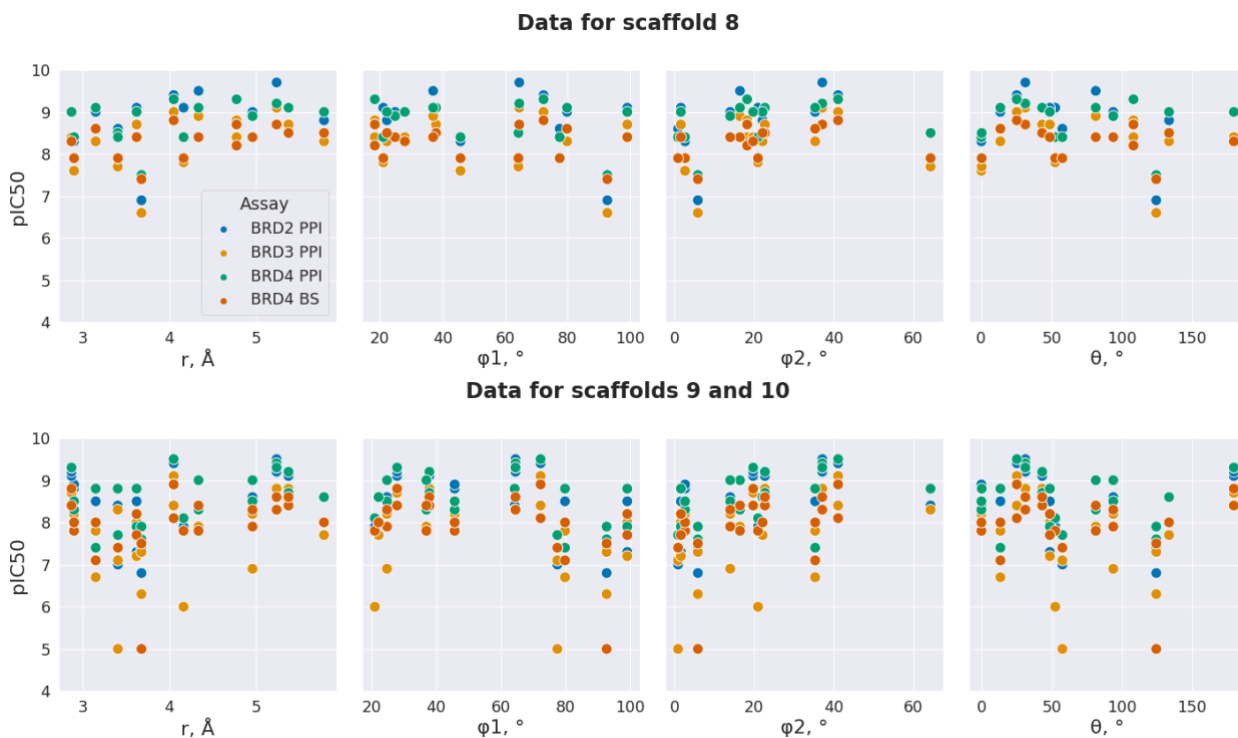
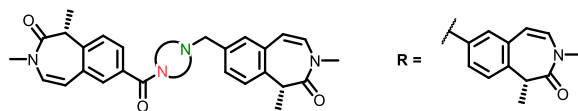


Figure 8. Assay potencies for both iterations of synthesis plotted against each of the CRDA parameters. The coloured markers represent the different assays, with blue for the BRD2 PPI assay, orange for BRD3 PPI assay, green for the BRD4 PPI assay and red for the BRD4 BS assay.

The methylene groups between the CRDA linkers and the warheads were thought to impart flexibility to the bivalent molecules, perhaps providing the ability for these molecules to access conformations which were tolerated by all BET isoforms examined. Therefore, synthesis progressed to the second iteration which incorporated amide linkers to gauge the effect of further conformational restriction on the bivalent molecules.

The next iteration of synthesis produced compounds of chemotypes **9** and **10**. The BET PPI and BRD4 BS results for these compounds are presented in Table 2. The potencies plotted against each of the CRDA parameters are also shown in Figure 8.

Table 2. Biochemical data for chemotypes **9** and **10**.



	Structure	BRD2 PPI [†]	BRD3 PPI [†]	BRD4 PPI [†]	BRD4 BS [†]	Cluster
9a		8.5 (1)	7.8 (3)	8.8 (1)	8.0 (1)	7
10a		7.4 (3)	6.7 (4)	7.4 (3)	7.1 (2)	7
9b		7.9 (4)	<6 (2)	8.1 (3)	7.8 (2)	3
9c		7.4 (3)	7.1 (3)	7.4 (3)	7.4 (2)	10
10c		7.0 (2)	<5 (2)	7.7 (2)	-*	10
9d		9.0 (3)	8.3 (3)	9.0 (4)	8.4 (3)	3
10d		8.3 (2)	7.9 (3)	8.3 (4)	7.8 (2)	3
9e		8.4 (2)	8.3 (3)	8.8 (2)	-*	7
9f		9.1 (3)	8.7 (2)	9.3 (2)	8.8 (4)	8
10f		9.2 (3)	8.4 (3)	9.3 (4)	8.4 (3)	8
9g		8.9 (3)	8.4 (3)	8.9 (3)	8.1 (3)	5
10g		9.4 (3)	9.1 (4)	9.5 (2)	8.9 (3)	5
9h (GSK785)		7.9 (9)	6.9 (2* ⁴)	8.5 (7)	7.9 (2)	6
10h		8.6 (2)	8.2 (1)	9.0 (1)	8.3 (1)	6
9i		8.8 (3)	7.8 (4)	8.3 (4)	7.8 (2)	1
10i		8.9 (3)	8.2 (3)	8.5 (3)	8.0 (3)	1
9j		7.3 (2* ¹)	7.2 (2)	7.9 (3)	7.7 (3)	10

	Structure	BRD2 PPI [†]	BRD3 PPI [†]	BRD4 PPI [†]	BRD4 BS [†]	Cluster
10j		8.5 (3)	8.0 (3)	8.8 (3)	8.2 (2)	10
9k		9.1 (2)	8.8 (2)	9.2 (2)	8.6 (2)	2
10k		8.7 (3)	8.4 (3)	8.7 (3)	8.4 (3)	2
9l		6.8 (1* ⁵)	6.3 (1* ⁵)	7.6 (3)	<5.0 (4)	11
10l		7.4 (3)	7.3 (2)	7.9 (2)	7.5 (3)	11
9m		9.2 (2)	8.8 (1)	9.4 (1)	8.3 (1)	2
10m		9.5 (3)	9.3 (3)	9.3 (3)	8.6 (4)	2
9n		8.0 (3)	7.7 (3)	8.6 (3)	8.0 (4)	6
9o1[‡]		9.0 (3)	8.3 (3)	9.0 (4)	8.6 (3)	6
9o2[‡]		9.7 (3)	9.0 (3)	9.5 (4)	9.0 (3)	6
9q1[‡]		9.7 (3)	9.2 (3)	9.3 (3)	8.5 (2)	4
9q2[‡]		9.4 (3)	8.9 (3)	9.0 (3)	8.4 (3)	4

[†]Average pIC₅₀ values are shown with the number of test occasions in the average shown in parenthesis. *^x Tested pIC₅₀ < 6 x times, which were not included in the average. [‡] Relative stereochemistry as shown. * Not obtained.

Notably, there was a greater range of potencies observed for the second iteration against each of the BET PPI assays compared to the first iteration, with more compounds showing potencies below a pIC₅₀ of 8. Compound **9l** did not produce a response in the BRD4 BS assay, which was notable as its analogue, **8l**, had the lowest potency from the first iteration, perhaps signalling that this linker geometry was not well tolerated for bivalent binding.

Figure 9 shows plots of BET isoform selectivities in the PPI assays against the CRDA parameters. Three compounds exhibited notable selectivity within the isoforms, **9b**, **9h** and **10c**, which will be discussed briefly.

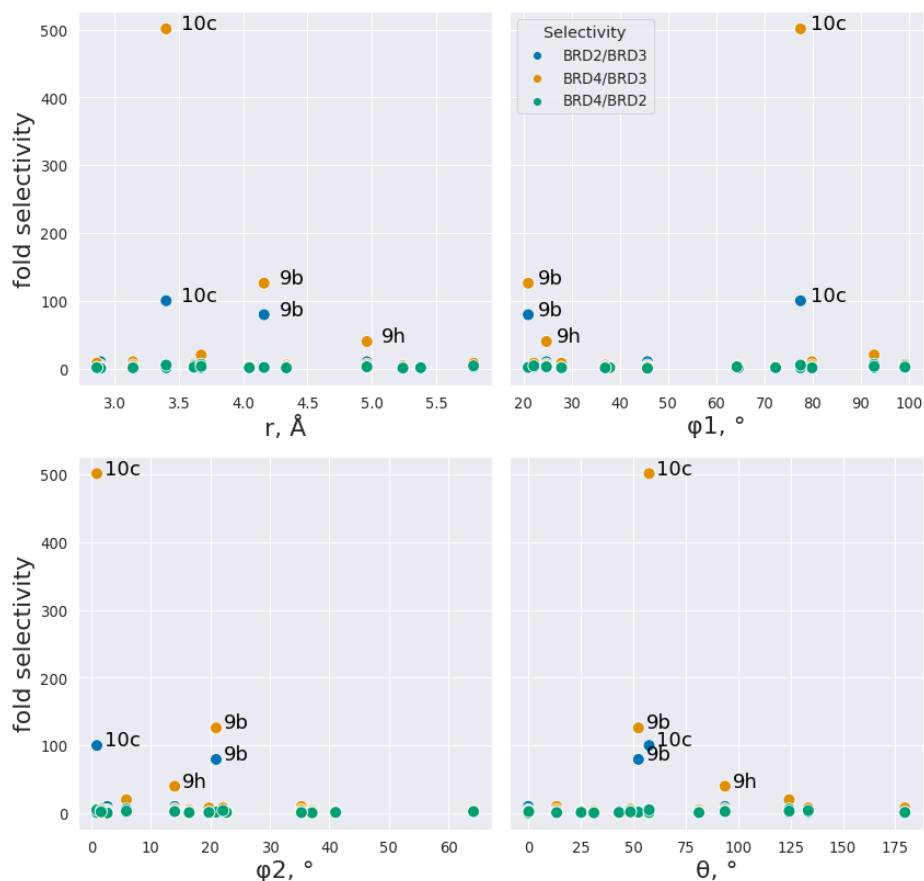


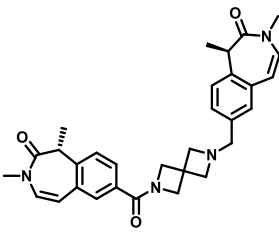
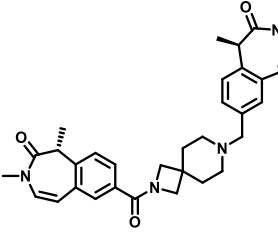
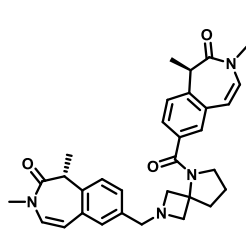
Figure 9. Isoform selectivities from the PPI assays plotted against CRDA parameters.

Overall, the data generated for the second iteration suggested that introduction of an amide into the linker used to construct the bivalent compounds resulted in a poorer ability of the resulting compounds to bind bivalently to the BET isoforms. It is possible the origin of this observation is due to the increased conformational flexibility of an amine when compared to an amide. However, this difference was not particularly pronounced, as the BRD4 BS data suggested that most other compounds were still able to bind bivalently at BRD4 and only three compounds appeared to show selectivity.

As with the data from the first iteration of compounds, there was not an obvious correlation between the CRDA parameters and activity, nor selectivity. At the time of synthesis, linkers **11r** and **11p** were not available from their respective racemates, therefore, their analogues were not prepared.

Of the compounds synthesised in the second iteration, **9b**, **9h** and **10c** showed notable selectivity in the PPI assays. To understand the phenotypic response for these compounds, their ability to inhibit the production of the MCP1 cytokine in human whole blood (hWB), a cytokine known to be inhibited by pan-BET, pan-BD1 or pan-BD2 inhibition as an indicator for an anti-inflammatory phenotype, was measured, which is shown along with the PPI assay results in Table 3.³⁶

Table 3. BET PPI potencies and MCP hWB potencies for **9b**, **9h** and **10c**.

	 9b	 9h (GSK785)	 10c
BRD2 PPI pIC ₅₀ (n)	7.9 (4)	7.9 (9)	7.0 (2)
BRD3 PPI pIC ₅₀ (n)	<6 (2)	6.9 (2* ⁴)	<5 (2)
BRD4 PPI pIC ₅₀ (n)	8.1 (3)	8.5 (7)	7.7 (2)
hWB (MCP-1) pIC ₅₀ (n)	7.3 (2)	7.4 (2)	6.8 (2)

*^x Tested pIC₅₀ < 6 x times, which were not included in the average.

Compounds **9b** and **9h** showed high pIC₅₀s in the hWB MCP1 assay, of 7.3 and 7.4 respectively whereas **10c** showed a pIC₅₀ of 6.8, akin to what would be expected for a monovalent BZP compound.³⁰ Therefore, **10c** was not thought to be binding bivalently to BRD4.

The distribution of individual test occasions in the BET PPI assays for **9b** and **9h** is presented in Figure 10 as box plots. Whilst the average potencies reported in Table 2 suggest **9b** to have a higher BRD4 selectivity compared to **9h**, the distributions show that the average BRD3 PPI pIC₅₀ for **9h** is skewed by two test occasions which reported pIC₅₀ values of 6.5 and 7.4, whereas the majority of test occasions measured an inactive response (pIC₅₀ < 6). The tabulated average pIC₅₀ values do not consider these inactive measurements, provided there are active measurements to include in the average. Our interpretation of these data, therefore, was that **9b** and **9h** had similar levels of BRD3 activity in the PPI assay when taking the inactive measurements of **9h** into account. Figure 10 also demonstrates that **9h** was consistently more active in the BRD4 PPI assay. Therefore, **9h** was chosen to progress to an orthogonal CETSA assay for further selectivity profiling.

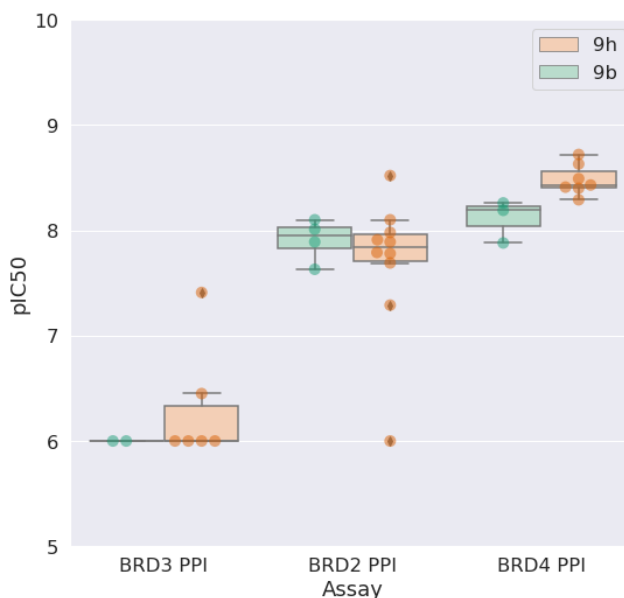
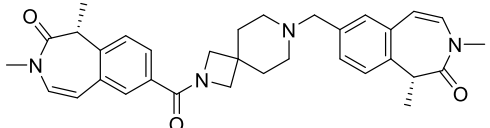


Figure 10. Box plots showing the distribution of BET PPI assay measurements for **9b** and **9h**. The individual test occasions are shown as circle markers and the bottom and top edges of the box plots represent the first and third quartile values respectively. The whiskers are drawn to the farthest value within $1.5 \times$ the interquartile range. Datapoints outside of the whiskers are considered outliers. Data for **9b** is shown in green on the left hand side of each plot, and the data for **9h** is shown in orange, on the right hand side of each plot.

The CETSA assay was considered a more physiologically relevant measurement of BET isoform selectivity due to the use of native cellular protein, as opposed to the modified proteins employed by the BRET technology. The results from the CETSA assay for **9h** are shown in Table 4 along with physicochemical measurements and hepatocyte clearance in human, dog, rat, and mouse. The corresponding figure for the CETSA assay can be found in Figure S1.

Table 4. Structure and data for **9h**.

 9h (GSK785)	
CETSA BRD2 pEC ₅₀ (n)	8.1 (4)
CETSA BRD3 pEC ₅₀ (n)	6.0 (2) [†]
CETSA BRD4 pEC ₅₀ (n)	7.0 (4)
FaSSIF, μg.mL ⁻¹	602
AMP, nm.s ⁻¹	415
Hepatocyte clearance mL.min ⁻¹ .g ⁻¹	14.64 / 18.83 / 35.64 / 93.96
Human / dog / rat / mouse	

[†]Tested <6.6 twice, which were not included in the average.

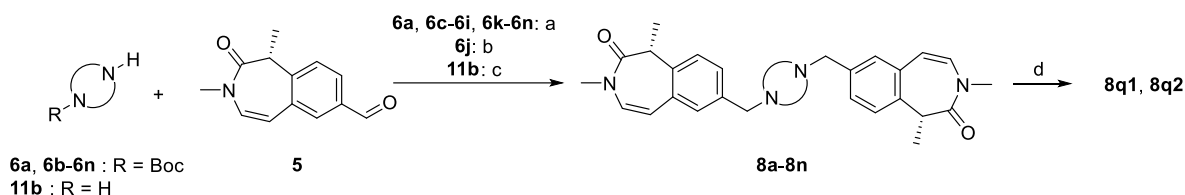
The CETSA measurements for compound **9h** showed an improved selectivity profile compared to the PPI assay, with a 125-fold selectivity for BRD2 over BRD3, 13-fold selectivity for BRD2 over BRD4, and 10-fold selectivity for BRD4 over BRD3. Compound **9h** also showed a high fasted state simulated intestinal fluid (FaSSIF) solubility of 602 μg.mL⁻¹, taken from an amorphous form, and a high artificial membrane permeability (AMP) of 415 nm.s⁻¹. Furthermore, reasonable levels of clearance in hepatocytes were measured from human, dog, rat and mouse of 14.64, 18.83, 35.64, and 93.96 mL.min⁻¹.g⁻¹, respectively. We thereby present **9h** (GSK785) as a BRD2/4-selective, BRD3-sparing bivalent BET inhibitor, with properties fit for use as an *in vitro* tool.

Chemistry

Compounds from the first iteration, chemotype **8**, (Scheme 2) were synthesised from aldehyde **5** and Boc-protected diamines **6a**, **6c** - **6n** in a three-step procedure following a sequence of: a sodium triacetoxyborohydride (STAB) mediated reductive amination, TFA or HCl-mediated Boc-deprotection, and STAB-mediated reductive amination sequence with aqueous workups performed at intermediate steps. **8b** was prepared in one-pot reductive amination from the unprotected

diamine **11b**, and **8j** was prepared with an intermediate cation-exchange purification. HPLC with a chiral stationary phase was used to separate the enantiomers of **8q** to give **8q1** and **8q2**. The synthesis of **5** has been previously described.³¹

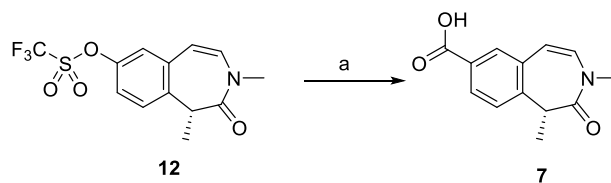
Scheme 2. Synthetic route for compounds of chemotype **8**



^a Reagents and conditions: Step 1 - STAB (3 equiv), DCM, rt, 16-20 h; Step 2 - HCl (4M in dioxane), rt, 3 h; Step 3 - Aldehyde **5**, STAB (3 equiv), DCM, rt, 16-20 h. ^b Reagents and conditions: Step 1 - Picoline borane (1.1 equiv), MeOH, AcOH, rt, 6 h; Step 2 – HCl (4M in dioxane) or TFA, rt, 2 h; Step 3 - Aldehyde **5** (1 equiv), picoline borane (1.1 equiv), MeOH, AcOH, rt, 3 h. ^c Reagents and conditions: Step 1 - Aldehyde **5** (2 equiv), triethylamine (4.05 equivalents), DCM, rt, 20 h. Step 2 – STAB (4 equiv), DCM, rt, 22 h. ^d Chiral separation.

Carboxylic acid **7** was synthesised from triflate **12** in a palladium-catalysed carbonylation reaction (**Scheme 3**). The synthesis of compound **12** has been previously described.³¹

Scheme 3. Synthesis of carboxylic acid **7**.

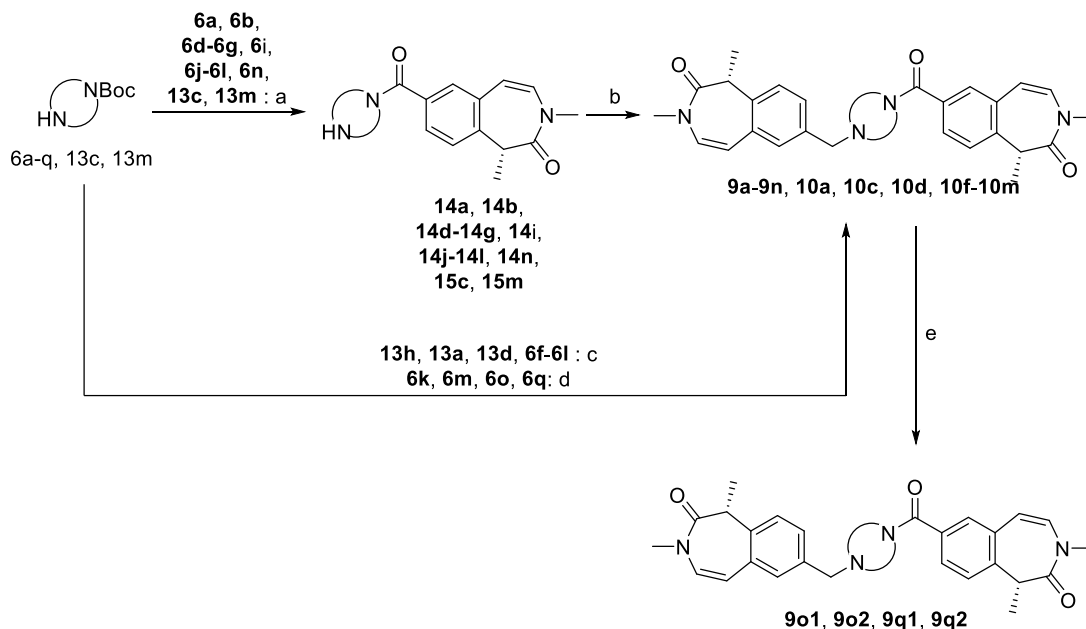


^a Reagents and conditions: PdOAc (0.05 equiv), Xantphos (0.1 equiv), triethylamine (3 equiv), carbon monoxide, DMF, 70 °C, 4 h.

Compounds **9a**, **9b**, **9d**, **9e**, **9f**, **9g**, **9i**, **9j**, **9l**, **9n**, **10c**, and **10m** were prepared in two parts. The first part was a two-step telescoped procedure following a STAB-mediated reductive amination between aldehyde **5** and the respective Boc-protected amines, followed by a TFA or HCl-mediated Boc-deprotection to give **14a**, **14b**, **14d**, **14e**, **14f**, **14g**, **14i**, **14j**, **14l**, **14n**, **15c**, and **15m**. These were then subjected to an amide coupling using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-

triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and diisopropyl ethylamine (DIPEA) with acid **7** to furnish the final products. Compounds **9h**, **10a**, **10d**, **10f**, **10g**, **10h**, **10i**, **10j**, **10k**, and **10l** were synthesised following a telescoped three-step procedure of a STAB-mediated reductive amination between the respective Boc-protected amines and aldehyde **5**, followed by a TFA or HCl-mediated Boc-deprotection and HATU-mediated amide coupling with acid **7** to furnish the final compounds. Compounds **9k**, **9m**, **9o1**, **9o2**, **9q1**, and **9q2** were prepared following a telescoped 3-step procedure of amide coupling between their respective Boc-protected amines and acid **7**, followed by a Boc-deprotection and reductive amination with aldehyde **5** to furnish the final compounds. Compounds **9q** and **9o** were purified using HPLC with a chiral stationary phase to yield compounds **9q1** and **9q2**, and **9o1** and **9o2**, respectively. These procedures enabled the use of the same Boc-protected amines to place the amide on either nitrogen in the cases where the linker was not symmetrical. Furthermore, the analogues of Boc-protected amines **6c** and **6m** were available (**13c** and **13m** respectively) and used for the synthesis of **10c** and **10m**. In all cases, synthesis yielded sufficient material for screening, and routes were not optimised. These routes are shown in **Scheme 4**.

Scheme 4. Syntheses of compounds 9a-9n, 10a, 10c, 10d, 10f-10m.



^a Reagents and conditions: Step 1: Acid **7** (0.91-1 equivalents), HATU (1.1-1.5 equivalents), DIPEA (1.1-3 equivalents), DCM, rt, 1-2 h. Step 2: TFA or 4M HCl in dioxane (17-25 equivalents), DCM, 0.3-3 h. ^b Reagents and conditions: Aldehyde **5** (1-2 equivalents), sodium triacetoxyborohydride (2.4-6 equivalents), DCM, THF or 2-Me-THF, rt, 21-76 h. ^c Reagents and conditions: Step 1: Aldehyde (1-1.05 equivalents), STAB (3 equivalents), DCM, rt, 3.5-21 h. Step 2: TFA (26-30 equivalents), DCM, rt, 0.5-4 h. Step 3: Acid **7** (1-1.05 equivalents), HATU (1-1.05 equivalents), DIPEA (2-3 equivalents), DCM, rt, 1 h. ^d Reagents and conditions: Step 1: Acid (0.91-1 equivalents), HATU (1.1 equivalents), DIPEA (3-4 equivalents), DCM, rt, 2-28 h. Step 2: TFA (17-25 equivalents), DCM, 1-1.5 h. Step 3: Aldehyde (1-1.5 equivalents), STAB (3 equivalents), DCM, rt, 3-21 h.

Following the preparation of the compounds for the second iteration, the batch of acid **7** used to prepare compounds **9a-9r**, **10c** and **10m** was found to have had an enantiomeric ratio of 80:20 in favour of the isomer drawn. The batch of acid **7** used to prepare compounds **10a**, **9d**, **9f-9l** was found to have an enantiomeric ratio of 70:30 in favour of the isomer drawn. Following this, compounds **9h**, **9k**, **9m**, **10f**, and **10h** were reprepared using an enantiomerically pure batch of acid **7** (see experimental) and the potencies in the BRD2, 3 and 4 PPI assays, and the BRD4 BS assay, were measured (Table S1). The potencies in these assays were not affected greatly by the diastereomeric purity in these cases; therefore, the data was deemed suitable for analysis. The

enantiomeric ratio of acid **7** was found to propagate to the diastereomeric ratio for compound **10i** (see experimental and Figure S2). Based on this finding, we report the diastereomeric purities of our compounds deduced from the enantiomeric purities of the batch of acid **7** used in their syntheses in the experimental section.

Conclusions

In summary, we present **9h** (GSK785), a selective bivalent BET inhibitor discovered through a parametrised, diversity-oriented synthetic approach over two iterations. Through this approach, we discovered that the BET isoforms were accommodating to bivalent compounds incorporating linkers with a range of geometrical properties. The introduction of amide groups in the linkers between the binding portions improved chances at achieving isoform selectivity, perhaps by further constraining the conformations available to bind to the BET isoforms. Thus, work to further increase rigidity may be a fruitful path to achieving higher levels of selectivity among the BET isoforms. Nonetheless, compounds **9b** and **9h** (GSK785) showed signs of selectivity in the BET PPI assays. Although we are currently unable to explain the apparent selectivity of these compounds, the BET PPI results for **9h** translated into an orthogonal CETSA assay, demonstrating a BRD2/4-selective, BRD3-sparing selectivity profile. This compound provides a tool to dissect the roles played by the BET proteins, and this design strategy can provide inspiration for other studies looking to obtain geometrical diversity using linkers in bivalent systems.

Experimental

General Experimental

Unless otherwise stated, all reactions were carried out under an atmosphere of nitrogen in heat or oven dried glassware and anhydrous solvent. Solvents and reagents were purchased from commercial suppliers and used as received. Reactions were monitored by thin layer chromatography (TLC) or liquid chromatography-mass spectrometry (LC-MS). TLC was carried out on glass or aluminium-backed 60 silica plates coated with UV₂₅₄ fluorescent indicator. Spots were visualized using UV light (254 or 365 nm) or alkaline KMnO₄ solution, followed by gentle heating. LCMS analysis was carried out on a Waters Acquity UPLC instrument equipped with a CSH C18 column (50 mm x 2.1 mm, 1.7 µm packing diameter) and Waters micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used: *Formic* – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) H₂O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. *High pH* – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile. Flash column chromatography was carried out using Biotage SP4 or Isolera One apparatus with SNAP silica cartridges. Mass directed automatic purification (MDAP) was carried out using a Waters ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used: *Formic* – Sunfire C18 column (100 mm x 19 mm, 5 µm packing diameter, 20 mL/min flow rate) or Sunfire C18 column (150 mm x 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) H₂O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile

containing 0.1% (v/v) formic acid. *High pH* – Xbridge C18 column (100 mm x 19 mm, 5 μ m packing diameter, 20 mL/min flow rate) or Xbridge C18 column (150 mm x 30 mm, 5 μ m packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile. NMR spectra were recorded at ambient temperature (unless otherwise stated) using standard pulse methods on any of the following spectrometers and signal frequencies: Bruker AV-400 (^1H = 400 MHz, ^{13}C = 101 MHz), Bruker AV-600 (^1H = 600 MHz, ^{13}C = 150 MHz), or Bruker AV4 700 MHz spectrometer (^1H = 700 MHz, ^{13}C = 176 MHz). Chemical shifts are referenced to trimethylsilane (TMS) or the residual solvent peak, and are reported in ppm. Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), δ (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), m (multiplet), br. (broad). Purity of synthesized compounds was determined by LCMS analysis. All compounds are >95% pure by HPLC analysis. As described in the text, some compounds were synthesised as mixtures of diastereomers. These have been indicated.

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (7)

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yltrifluoromethanesulfonateylate (**12**, 30g, 89 mmol), PdOAc₂ (1.004 g, 4.47 mmol), and Xantphos (5.18 g, 8.95 mmol) were added to a flask under N₂. DMF (200 mL) and triethylamine (37.4 mL, 268 mmol) were added and the mixture was purged with carbon monoxide from a balloon (1 L volume). A fresh balloon was fitted, and the mixture was heated at 70 °C for 2 h. Water (3 mL) was added, and the mixture stirred for a further 2 h, giving a dark, clear solution, which was then cooled, and water (100 mL) added. The resulting suspension was stirred for 2 h, then diluted with water (500 mL) and washed

with EtOAc (2 x 300ml). The combined organics were washed with a sodium bicarbonate solution (300 mL) and the combined aqueous layer acidified with acetic acid (20 mL), with addition of aqueous HCl (2M) to pH 4. The resulting suspension was stirred for 20 min, then filtered. The solid was washed with water and dried overnight in a vacuum oven to give (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 14.5g, 62.7 mmol, 70% yield) as a beige solid. ¹H NMR (MeOD-*d*₄, 400 MHz) δ 8.06 (1 H, dd, *J*=8.3, 1.5 Hz), 7.99 (1 H, d, *J*=1.5 Hz), 7.44 (1 H, d, *J*=8.3 Hz), 6.63 (1 H, d, *J*=9.1 Hz), 6.55 (1 H, d, *J*=9.1 Hz), 3.35 - 3.41 (1 H, m), 3.14 (3 H, s), 1.65 (3 H, d, *J*=5.4 Hz). LCMS (Formic, ES⁺) *t*_R = 0.76 min, [M+H]⁺ = 232.1. Chiral LC: 4.6 mm x 25 cm Chiralpak AD-H (5 μm), 3:1 Heptane:Ethanol (+0.1% isopropylamine) **7**: *t*_R = 9.61 min 100%. (*S*)-enantiomer expected at 7.06 min. (See Figure S2).

Method A example: (1*R*,1'*R*)-7,7'-((2,7-diazaspiro[3.5]nonane-2,7-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one), **8h**

A solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 28 mg, 0.13 mmol) in DCM (0.5 mL) was added to *tert*-butyl 2,7-diazaspiro[3.5]nonane-7-carboxylate hydrochloride (**6h**, 34 mg, 0.130 mmol). The reaction was stirred at rt for 16 h. STAB (83 mg, 0.390 mmol) was added, and the reaction stirred at rt for 4 h. The reaction mixture was then quenched by the addition saturated aq NaHCO₃ (1 mL) and extracted into DCM (1 mL). The organics were passed through a hydrophobic frit and concentrated under a positive pressure of N₂. HCl in dioxane (4 M, 1 mL, 4 mmol) and dioxane (1 mL) were added and the reaction stirred at rt for 3 h. The reaction was concentrated under a positive pressure of N₂ and DCM (1.5 mL) and saturated aq NaHCO₃ (1 mL) added. The mixture was vigorously stirred and passed through a hydrophobic frit, which was flushed with further DCM (1 mL) to afford a solution of the *N*-boc-deprotected intermediate. A solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-

7-carbaldehyde (**5**, 28 mg, 0.13 mmol) in 0.5 mL DCM was added and the reaction stirred at rt for overnight. STAB (83 mg, 0.390 mmol) was added, and the reaction stirred at rt for 5 h. The reaction was quenched by the addition of saturated aq NaHCO₃ (1 mL), vigorously stirred and passed through a hydrophobic frit, which was flushed with further DCM (1 mL). The organics were concentrated under a positive pressure of N₂. The residue was dissolved in DMSO / MeOH (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated under a positive pressure of N₂ to give (1*R*,1'*R*)-7,7'-((2,7-diazaspiro[3.5]nonane-2,7-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8h**, 18 mg, 0.034 mmol, 26% yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.27 - 7.33 (m, 2 H), 7.22 - 7.25 (m, 2 H), 7.16 - 7.20 (m, 2 H), 6.40 (app. d, *J*=8.8 Hz, 2 H), 6.28 (app. d, *J*=8.8 Hz, 2 H), 3.62 (s, 2 H), 3.42 (s, 2 H), 3.27 (br d, *J*=12.2 Hz, 2 H), 3.11 (d, *J*=1.5 Hz, 6 H), 3.01 (s, 4 H), 2.31 (br s, 4 H), 1.76 (br t, *J*=5.4 Hz, 4 H), 1.63 (app. br d, *J*=3.4 Hz, 6 H). LCMS (Formic, ES⁺) t_R = 0.42 min, [M+H]⁺ = 525.5.

(1*R*,1'*R*)-7,7'-((1,6-Diazaspiro[3.3]heptane-1,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8a**)**

Synthesised *via* Method A using *tert*-butyl 1,6-diazaspiro[3.3]heptane-6-carboxylate oxalic acid salt (**6a**, 73 mg, 0.15 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((1,6-diazaspiro[3.3]heptane-1,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8a**, 36 mg, 0.072 mmol, 48% yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.33 - 7.39 (m, 1 H), 7.24 - 7.31 (m, 4 H, obs.), 7.17 (s, 1 H), 6.43 (app. br dd, *J*=9.3, 5.4 Hz, 2 H), 6.31 (app. br dd, *J*=8.8, 1.5 Hz, 2 H), 3.72 (s, 2 H), 3.53 (s, 2 H), 3.44 (br d, *J*=8.3 Hz, 2 H), 3.22 - 3.37 (m, 4 H), 3.14 (m, 6 H), 3.09 (t, *J*=6.8 Hz, 2 H), 2.37 (t, *J*=6.8 Hz, 2 H), 1.66 (app. br s, 6 H). LCMS (Formic, ES⁺) t_R = 0.60 min, [M+H]⁺ = 497.4.

(1*R*,1'*R*)-7,7'-((2,6-diazaspiro[3.3]heptane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8b)

Triethylamine (0.52 mL, 3.73 mmol) was added to a solution of (R)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 397 mg, 1.844 mmol) and 2,6-diazaspiro[3.3]heptane dihydrochloride (**11b**, 158 mg, 0.922 mmol) in DCM (5 mL) in a 20 mL vial. The vial was capped, and the reaction was stirred overnight at room temperature. After 20 h, STAB (782 mg, 3.69 mmol) was added, and stirring was continued at rt for 22 h. A solution of saturated NaHCO₃ (5 mL) was added portion wise. The layers were separated, and the aqueous layer was extracted with DCM (2 x 5 mL). Isolute was added, and the mixture was concentrated under a stream of nitrogen at 50 °C. The residue was purified by flash silica chromatography eluting on a gradient of 0-20% MeOH (1% NH₃) / DCM. The desired fractions were concentrated under reduced pressure and dried under high vacuum, giving (1*R*,1'*R*)-7,7'-((2,6-diazaspiro[3.3]heptane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8b**, 255 mg, 0.860 mmol, 56% yield) as a white solid. ¹H NMR (MeOH-*d*₃, 400 MHz) δ 7.25 - 7.36 (m, 4 H), 7.23 (s, 2 H), 6.41 - 6.59 (m, 4 H), 3.63 (s, 4 H), 3.38 (s, 8 H), 3.18 - 3.20 (m, 1 H), 3.27 (br. s., 2 H), 3.10 (s, 6 H), 1.59 (br. s., 6 H). LCMS (TFA, ES⁺) t_R = 0.50 min, [M+H]⁺ = 497.2.

(1*R*,1'*R*)-7,7'-((2,5-Diazaspiro[3.4]octane-2,5-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8c)

Synthesised *via* Method A using *tert*-butyl 2,5-diazaspiro[3.4]octane-5-carboxylate oxalic acid salt (**6c**, 77 mg, 0.15 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((2,5-diazaspiro[3.4]octane-2,5-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8c**, 44 mg, 0.086

mmol, 57% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.17 - 7.36 (m, 6 H), 6.44 - 6.56 (m, 4 H), 3.85 (s, 2 H), 3.60 (br s, 2 H), 3.31 - 3.38 (m, 2 H), 3.13 - 3.24 (m, 2 H), 3.06 - 3.13 (m, 2 H), 3.03 (d, *J*=2.9 Hz, 6 H), 2.45 (t, *J*=7.1 Hz, 2 H), 1.98 - 2.04 (m, 2 H), 1.55 - 1.69 (m, 2 H), 1.42 - 1.55 (m, 6 H). ¹³C NMR (CDCl₃, 176 MHz) δ 170.1 (2C), 138.4, 135.0, 134.7 (2C), 134.2 (2C), 130.3, 130.2, 128.9, 128.8, 126.5, 124.4, 116.6 (2C), 116.5 (2C), 62.7, 62.3, 62.1, 62.0, 53.5, 51.5, 41.3 (2C), 38.2, 35.6 (2C), 21.0, 13.2, 13.1. LCMS (formic) *t*_R = 0.66 min, [M+H]⁺ = 511.5.

(1*R*,1'*R*)-7,7'-((2,6-Diazaspiro[3.4]octane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8d)

Synthesised *via* Method A using *tert*-butyl 2,6-diazaspiro[3.4]octane-6-carboxylate (**6d**, 27 mg, 0.12 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((2,6-diazaspiro[3.4]octane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8d**, 10 mg, 0.019 mmol, 16% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.15 - 7.35 (m, 6 H), 6.43 - 6.57 (m, 4 H), 3.52 (s, 2 H), 3.49 (s, 2 H), 3.12 - 3.23 (m, 2 H), 3.06 - 3.11 (m, 2 H), 2.99 - 3.05 (m, 8 H), 2.58 (s, 2 H), 2.40 - 2.47 (m, 2 H), 1.86 - 1.94 (m, 2 H), 1.43 - 1.54 (m, 6 H). LCMS (HpH, ES⁺) *t*_R = 1.14 min, [M+H]⁺ = 511.4.

1*R*,1'*R*)-7,7'-(((3*aS*,6*aS*)-Tetrahydropyrrolo[3,4-*c*]pyrrole-2,5(1*H*,3*H*)diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8e)

Synthesised *via* Method A using *tert*-butyl hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)carboxylate (**6e**, 32 mg, 0.15 mmol) as the amine to give (1*R*,1'*R*)-7,7'-(((3*aS*,6*aS*)-tetrahydropyrrolo[3,4-*c*]pyrrole-2,5(1*H*,3*H*)diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8e**, 61 mg, 0.119 mmol, 80% yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.39 (dd,

$J=8.0, 1.5$ Hz, 2 H), 7.29 (d, $J=5.0$ Hz, 2 H), 7.24 (d, $J=1.5$ Hz, 2 H), 6.44 (d, $J=9.3$ Hz, 2 H), 6.31 (d, $J=9.3$ Hz, 2 H), 3.62 (ABq, $J=19.6, 13.2$ Hz, 4 H), 3.21 - 3.40 (m, 2 H), 3.14 (s, 6 H), 2.60 - 2.76 (m, 6 H), 2.32 - 2.40 (m, 4 H), 1.67 (app. br d, $J=5.4$ Hz, 6 H). LCMS (Formic ES⁺) $t_R = 0.50$ min, $[M+H]^+ = 511.4$.

(1*R*,1'*R*)-7,7'-((4,7-Diazaspiro[2.5]octane-4,7-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8f)

Synthesised *via* Method A using *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (**6f**, 32 mg, 0.150 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((4,7-diazaspiro[2.5]octane-4,7-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8f**, 44 mg, 0.086 mmol, 57% yield) as a colourless gum. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.15 - 7.38 (m, 6 H), 6.42 - 6.56 (m, 4 H), 3.74 (br s, 2 H), 3.46 (dd, $J=16.1, 13.7$ Hz, 2 H), 3.17 (br dd, $J=8.6, 3.7$ Hz, 2 H), 3.02 (d, $J=3.9$ Hz, 6 H), 2.64 - 2.69 (m, 2 H), 2.39 (br t, $J=4.9$ Hz, 2 H), 2.17 - 2.33 (m, 2 H), 1.48 (br s, 6 H), 0.58 - 0.64 (m, 2 H), 0.38 - 0.44 (m, 2 H). LCMS (Formic, ES⁺): $t_R = 0.70$ min, $[M+H]^+ = 511.5$.

(1*R*,1'*R*)-7,7'-((2,6-Diazaspiro[3.5]nonane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8g)

Synthesised *via* Method A using *tert*-butyl 2,6-diazaspiro[3.5]nonane-2-carboxylate oxalic acid salt (**6g**, 81 mg, 0.15 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((2,6-diazaspiro[3.5]nonane-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8g**, 50 mg, 0.093 mmol, 62% yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.36 (dd, $J=8.3, 1.5$ Hz, 1 H), 7.25 - 7.33 (m, 3 H, obs.), 7.21 (d, $J=1.5$ Hz, 2 H), 6.42 (d, $J=8.8$ Hz, 2 H), 6.31 (d, $J=8.8$ Hz, 2 H), 3.66 (br s, 2 H), 3.50 (dd, $J=18.6, 13.2$ Hz, 2 H), 3.21 - 3.42 (m, 2 H), 3.15 (d, $J=4.9$ Hz, 8 H),

2.93 - 3.05 (m, 2 H), 2.42 - 2.57 (m, 2 H), 2.31 (br s, 2 H), 1.52 - 1.71 (m, 10 H). LCMS (Formic, ES⁺) t_R = 0.48 min, [M+H]⁺ = 539.4.

(1R)-7-(((6-(((S)-1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)methyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one (8i)

Synthesised *via* Method A using *tert*-butyl 3,6-diazabicyclo[3.1.1]heptane-3-carboxylate (**6i**, 55 mg, 0.28 mmol) as the amine to give (1R)-7-(((6-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)methyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one (**8i**, 84 mg, 0.161 mmol, 58% yield). ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.39 (dd, *J*=7.9, 1.6 Hz, 1 H), 7.24 - 7.32 (m, 3 H), 7.15 - 7.23 (m, 2 H), 6.40 - 6.51 (m, 4 H), 3.84 (s, 2 H), 3.58 (s, 2 H), 3.47 (br d, *J*=5.6 Hz, 2 H), 3.31 (app. dq, *J*=18.0, 6.9 Hz, 2 H), 3.10 (br d, *J*=2.4 Hz, 1 H), 3.06 (d, *J*=4.2 Hz, 7 H), 2.78 - 2.84 (m, 4 H), 1.53 (d, *J*=6.9 Hz, 3 H), 1.50 (app. d, *J*=6.9 Hz, 3 H). LCMS (Formic, ES⁺) t_R = 1.19 min, [M+H]⁺ = 479.4.

(1R,1'R)-7,7'-((1,8-Diazaspiro[4.5]decane-1,8-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one) (8j)

(R)-1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepine-7-carbaldehyde (**5**, 43 mg, 0.2 mmol) was dissolved in MeOH (0.9 mL) and AcOH (0.1 mL) and the solution added to *tert*-butyl 1,8-diazaspiro[4.5]decane-1-carboxylate hydrochloride (**6j**, 61 mg, 0.220 mmol). The mixture was stirred at rt for 10 min, after which a solution of picoline borane (24 mg, 0.220 mmol) in MeOH (0.45 mL) was added and the reaction stirred at rt for 6 h. The reaction was concentrated under a positive pressure of N₂, after which HCl in dioxane (4 M, 1 mL, 4 mmol) was added and the reaction stirred at rt for 2 h. The reaction was diluted with water and concentrated *in vacuo*. The

residue was dissolved in MeOH (2 mL) and passed through a Biotage silica-cation exchange (SCX, 500 mg) cartridge. The column was washed with MeOH (2 CV) and then flushed with methanolic ammonia (2 M). The first eluent was re-passed through an SCX (500 mg) column which was then flushed with methanolic ammonia (2 M). The methanolic ammonia eluents were combined and concentrated under a positive pressure of N₂. The residue was combined with (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 43 mg, 0.2 mmol) and a solution of picoline borane (24 mg, 0.220 mmol) in MeOH (1.35 mL) and AcOH (0.15 mL). The reaction was stirred at rt for 3 h and then concentrated under a positive pressure of N₂ and purified by MDAP (HpH method). The product failed to collect, therefore the waste was concentrated *in vacuo* and the residue purified by MDAP (formic method). The relevant product fractions were combined and concentrated *in vacuo*, taken up into DCM (10 mL) and washed with saturated aq NaHCO₃ (10 mL). The organics were passed through a hydrophobic frit and concentrated *in vacuo* to give (1*R*,1'*R*)-7,7'-((1,8-diazaspiro[4.5]decane-1,8-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8j**, 26 mg, 0.048 mmol, 24% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 6.49 - 6.64 (m, 2 H), 6.36 - 6.49 (m, 4 H), 5.62 - 5.74 (m, 2 H), 5.51 - 5.61 (m, 2 H), 2.86 (br s, 2 H), 2.72 (s, 2 H), 2.54 - 2.64 (m, 2 H), 2.23 - 2.36 (m, 6 H), 2.07 (br d, *J*=11.2 Hz, 2 H), 1.78 - 1.94 (m, 2 H), 1.35 (br t, *J*=12.0 Hz, 2 H), 1.02 - 1.20 (m, 2 H), 0.85 - 1.00 (m, 4 H), 0.71 - 0.82 (m, 6 H), 0.58 (br app. d, *J*=12.7 Hz, 2 H). Majority of peaks broad and overlapping. LCMS (Formic, ES⁺): t_R = 0.44 min, [M+H]⁺ = 539.4 (100% purity).

(1*R*,1'*R*)-7,7'-((2,8-Diazaspiro[4.5]decane-2,8-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8k**)**

Synthesised *via* Method A using *tert*-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate (**6k**, 74 mg, 0.307 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((2,8-diazaspiro[4.5]decane-2,8-

diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**8k**, 82 mg, 0.145 mmol, 47% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.29 - 7.35 (m, 2 H), 7.18 - 7.26 (m, 4 H), 6.47 (d, *J*=9.0 Hz, 2 H), 6.42 (d, *J*=9.1 Hz, 2 H), 3.59 (s, 2 H), 3.46 (s, 2 H), 3.31 (q, *J*=7.2 Hz, 2 H), 3.06 (s, 6 H), 2.57 (t, *J*=7.0 Hz, 2 H), 2.40 (s, 2 H), 2.36 (br t, *J*=6.8 Hz, 4 H), 1.48 - 1.63 (m, 12 H). LCMS (HpH, ES⁺): t_R = 1.33 min, [M+H]⁺ = 539.4.

(1*R*,1'*R*)-7,7'-((1,9-Diazaspiro[5.5]undecane-1,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8l**)**

Synthesised *via* Method A using *tert*-butyl 1,9-diazaspiro[5.5]undecane-9-carboxylate (**6l**, 38 mg, 0.150 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((1,9-diazaspiro[5.5]undecane-1,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8l**, 15 mg, 0.027 mmol, 18% yield) as a cream solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.33 (td, *J*=8.0, 1.5 Hz, 2 H), 7.15 - 7.26 (m, 4 H), 6.43 - 6.55 (m, 4 H), 3.60 (s, 2 H), 3.46 (s, 2 H), 3.10 - 3.25 (m, 2 H), 3.02 (s, 6 H), 2.61 (2 H), 2.42 (br t, *J*=5.4 Hz, 2 H), 2.13 - 2.23 (m, 2 H), 1.77 - 1.90 (m, 2 H), 1.40 - 1.59 (m, 12 H), 1.35 (br d, *J*=5.9 Hz, 2 H). LCMS (formic, ES⁺) t_R = 0.48 min, [M+H]⁺ = 553.6.

(1*R*,1'*R*)-7,7'-((2,9-Diazaspiro[5.5]undecane-2,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8m**)**

Synthesised *via* Method A using *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate hydrochloride (**6m**, 38 mg, 0.13 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((2,9-diazaspiro[5.5]undecane-2,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8m**, 18 mg, 0.033 mmol, 25% yield) as a white solid. ¹H NMR (CDCl₃, 400 MHz) δ 7.28 - 7.34 (m, 2 H), 7.21 - 7.25 (m, 2 H), 7.19 (s, 2 H), 6.39 (app. d, *J*=9.3 Hz, 2 H), 6.28 (app. dd, *J*=9.3, 3.4 Hz, 2 H), 3.41 (s, 4 H), 3.18 - 3.34 (m, 2 H), 3.13 (s, 3 H), 3.11 (s, 3 H),

2.23 - 2.40 (m, 6 H), 2.05 - 2.15 (m, 2 H), 1.50 - 1.68 (m, 14 H). LCMS (Formic, ES⁺) t_R = 0.45 min, [M+H]⁺ = 553.5.

(1*R*,1'*R*)-7,7'-((3,9-Diazaspiro[5.5]undecane-3,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8n)

Synthesised *via* Method A using tert-butyl 3,9-diazaspiro[5.5]undecane-3-carboxylate (**6n**, 31 mg, 0.12 mmol) as the amine to give (1*R*,1'*R*)-7,7'-((3,9-diazaspiro[5.5]undecane-3,9-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8n**, 20 mg, 0.036 mmol, 30% yield) as a white solid. ¹H NMR (MeOD-*d*₄, 400 MHz) δ 7.40 (dd, *J*=7.8, 1.5 Hz, 2 H), 7.27 - 7.31 (m, 4 H), 6.56 (d, *J*=9.3 Hz, 2 H), 6.48 (d, *J*=9.3 Hz, 2 H), 3.58 (s, 4 H), 3.23 - 3.32 (m, 2 H obs.) 3.12 (s, 6 H), 2.47 (app. br t, *J*=5.1 Hz, 8 H), 1.61 (br s, 6 H), 1.53 (app. br t, *J*=5.1 Hz, 8 H). LCMS (HpH, ES⁺) rt = 1.32 min, [M+H]⁺ = 553.8.

(1*R*,1'*R*)-7,7'-(((4*aS,8*aS**)-octahydro-2,6-naphthyridine-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8q1) and (1*R*,1'*R*)-7,7'-(((4*aR**,8*aR**)-octahydro-2,6-naphthyridine-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (8q2)**

Synthesised *via* Method A using (±)tert-butyl (4*aR*,8*aR*)-octahydro-2,6-naphthyridine-2(1*H*)-carboxylate (**6q**, 31 mg, 0.12 mmol) as the amine, with trifluoroacetic acid (1 mL) in place of HCl in dioxane. This procedure was repeated and the products of the two experiments were combined. The combined products were purified by chiral stationary phase chromatography (column: Chiralpak IG (250 x 30 mm, 5 μm), flow rate: 30 mL/min, detection wavelength: 215 nm, solvents: 75% EtOH(+0.2% isopropylamine)/ Heptane(+0.2% isopropylamine)) This gave two eluting compounds, the fractions for which were combined and concentrated *in vacuo* to give: First eluting

compound: (1*R*,1'*R*)-7,7'-(((4*aS**,8*aS**)-octahydro-2,6-naphthyridine-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8q1**, 101 mg, 0.177 mmol, 40 % yield) as a white solid. Chiral LC: 4.6 mm x 25 cm Chiralpak IG (5 μm), 3:1 EtOH (+0.2% isopropylamine): heptane **8q1**: $t_R = 20.9$ min. $^1\text{H NMR}$ (DMSO- d_6 , 400 MHz, 100 °C) δ 7.33 (2 H, dd, $J=8.0, 1.5$ Hz), 7.20 - 7.24 (4 H, m), 6.47 (2 H, d, $J=9.3$ Hz), 6.43 (2 H, d, $J=9.3$ Hz), 3.42 - 3.47 (2 H, m), 3.37 - 3.42 (2 H, m), 3.29 (2 H, q, $J=7.2$ Hz), 3.06 (6 H, s), 2.64 - 2.77 (2 H, m), 2.56 - 2.62 (2 H, m), 1.99 - 2.18 (6 H, m), 1.65 (2 H, br d, $J=7.3$ Hz), 1.51 (6 H, d, $J=7.2$ Hz), 1.26 - 1.41 (2 H, m). LCMS (HpH, ES $^+$) $rt = 1.51$ min, $[\text{M}+\text{H}]^+ = 539.4$. Second eluting

compound: (1*R*,1'*R*)-7,7'-(((4*aR**,8*aR**)-octahydro-2,6-naphthyridine-2,6-diyl)bis(methylene))bis(1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one) (**8q2**, 106 mg, 0.187 mmol, 42 % yield) as a white solid. $^1\text{H NMR}$ (DMSO- d_6 , 400 MHz, 100°C) δ 7.33 (2 H, dd, $J=8.0, 1.5$ Hz), 7.20 - 7.24 (4 H, m), 6.47 (2 H, d, $J=9.3$ Hz), 6.44 (2 H, d, $J=9.3$ Hz), 3.38 - 3.47 (4 H, m), 3.29 (2 H, q, $J=7.2$ Hz), 3.06 (6 H, s), 2.65 - 2.74 (2 H, m), 2.60 (2 H, dd, $J=11.2, 2.0$ Hz), 1.99 - 2.19 (6 H, m), 1.66 (2 H, br s), 1.51 (6 H, d, $J=7.2$ Hz), 1.26 - 1.40 (2 H, m). Chiral LC: 4.6 mm x 25 cm Chiralpak IG (5 μm), 3:1 EtOH (+0.2% isopropylamine): heptane **8q2**: $t_R = 24.0$ min 97%. LCMS (HpH, ES $^+$) $rt = 1.51$ min, $[\text{M}+\text{H}]^+ = 539.4$

Method B example: (*R*)-1,3-dimethyl-7-(1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14a)

(*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 347 mg, 1.500 mmol) and HATU (627 mg, 1.650 mmol) were dissolved in DCM (10 mL) and DIPEA (699 μL, 4 mmol) was added. The reaction was stirred at rt for 10 min and named stock solution A. *tert*-Butyl 1,6-diazaspiro[3.3]heptane-6-carboxylate oxalic acid salt (**6a**, 122 mg, 0.25 mmol) was added to a portion of stock solution A (1.78 mL) and the reaction stirred at rt for 1.5 h. The reaction

was diluted with DCM (5 mL) and washed with a 1:1 mixture of saturated aq NaHCO₃ and water (5 mL). The organics were passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was dissolved in DCM (2 mL) and TFA (0.5 mL, 6.49 mmol) added. The reaction was stirred at rt for 20 min, after which it was diluted with saturated aq NaHCO₃ (6 mL) and DCM (6 mL) and the reaction stirred at rt for 30 min. The reaction mixture was passed through a hydrophobic frit. The aqueous layer was extracted with 10% MeOH / DCM (2 x 10 ml) and the combined organics concentrated under a positive pressure of N₂. The residue was purified by MDAP (HpH method) to give (*R*)-1,3-dimethyl-7-(1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14a**, 26 mg, 0.079 mmol, 32% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.60 (dd, *J*=8.3, 1.5 Hz, 1 H), 7.57 (d, *J*=1.5 Hz, 1 H), 7.33 (d, *J*=8.3 Hz, 1 H), 6.60 (d, *J*=9.3 Hz, 1 H), 6.55 (d, *J*=9.3 Hz, 1 H), 4.45 (br s, 2 H), 4.11 (br s, 2 H), 3.34 (app. br d, *J*=8.3 Hz, 2 H), 3.18 - 3.26 (m, 1 H), 3.04 (s, 3 H), 2.46 (app. t, *J*=7.4 Hz, 2 H), 1.51 (br d, *J*=6.4 Hz, 3 H), 1.36 (s, 1 H). LCMS (HpH, ES⁺) t_R = 0.69 min, [M+H]⁺ = 312.3.

Method C example: 4-(*R*)-1,3-dimethyl-7-(2,5-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14c)

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 289 mg, 1.250 mmol) and HATU (523 mg, 1.375 mmol) was made up in DCM (10 mL) and DIPEA (0.655 ml, 3.75 mmol) was added. The solution was stirred at rt for 10 min. 2.1 mL of this solution was added to *tert*-butyl 2,5-diazaspiro[3.4]octane-5-carboxylate oxalic acid salt (**6c**, 98 mg, 0.250 mmol) and the reaction stirred at rt for 1 h. The reaction was diluted with DCM (5 mL) and washed with saturated aq NaHCO₃ (3 mL) and passed through a hydrophobic frit. TFA (0.5 ml, 6.49 mmol) was added to the eluent and the reaction stirred at rt for 3 h. MeOH (5 mL) was added and the reaction stirred at rt for 2 h. The reaction mixture was passed through an SCX ion

exchange cartridge (5 g) which was washed with MeOH (2 CV). The cartridge was eluted with methanolic ammonia (2 M) and the eluent concentrated under a positive pressure of N₂ and dried *in vacuo* to give (*R*)-1,3-dimethyl-7-(2,5-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14c**, 80 mg, 0.234 mmol, 93% yield) as a clear gum. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.62 (dd, *J*=1.5, 8.1 Hz, 1 H), 7.58 (d, *J*=1.5 Hz, 1 H), 7.34 (d, *J*=7.6 Hz, 1 H), 6.5-6.6 (m, 1 H), 6.5-6.5 (m, 1 H), 5.66 (s, 1 H), 4.0-4.1 (m, 4 H), 3.35 (q, *J*=6.8 Hz, 1 H), 3.08 (s, 3 H), 2.93 (br s, 2 H), 2.86 (t, *J*=6.8 Hz, 2 H), 1.7-1.8 (m, 2 H), 1.54 (d, *J*=6.8 Hz, 3 H). LCMS (HpH, ES⁺) t_R = 0.75 min, [M+H]⁺ = 326.4.

Method D example: (*R*)-1,3-dimethyl-7-(4,7-diazaspiro[2.5]octane-7-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14f**)**

(*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 119 mg, 0.256 mmol), HATU (209 mg, 0.275 mmol) and DIPEA (131 μL, 0.75 mmol) were stirred in DCM (2.5 mL) at rt for 10 min after which *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (53 mg, 0.25 mmol) was added and the reaction stirred at rt for 2 h. DCM (10 mL) was added and the organics washed with aq HCl (2M, 5 mL) and saturated aq NaHCO₃ (5 mL). The organic phase was passed through a hydrophobic frit and concentrated under a positive pressure of N₂. The residue was purified by flash silica chromatography eluting on a gradient of 0-5% MeOH / DCM. The relevant fractions were combined and concentrated under a positive pressure of N₂. HCl in dioxane (4 M, 2 mL) was added and the reaction stirred at rt for 2 h. The reaction was diluted with saturated aq NaHCO₃ (5 mL) and DCM (5 mL), stirred vigorously and passed through a hydrophobic frit. The eluent was concentrated *in vacuo* to give (*R*)-1,3-dimethyl-7-(4,7-diazaspiro[2.5]octane-7-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14f**, 79 mg, 0.243 mmol, 97% yield) as an orange gum. ¹H NMR (DMSO-*d*₆, 400 MHz,) δ 7.28 - 7.42 (m, 3 H), 6.56 - 6.62 (m, 1 H), 6.49 -

6.56 (m, 1 H), 3.57 (s, 2 H), 3.36 - 3.52 (m, 2 H), 3.09 - 3.31 (m, 3 H, obs.), 3.05 (s, 3 H), 2.73 (br s, 1 H), 1.51 (br d, $J=6.4$ Hz, 3 H), 0.23 - 0.60 (m, 4 H). LCMS (Formic, ES⁺) $t_R = 0.41$ min, $[M+H]^+ = 326.4$.

Method E example: (*R)-7-(1-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,6-diazaspiro[3.3]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10a)**

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 339 mg, 1.575 mmol) in DCM (12 mL) was prepared. 2 mL of this solution was added to a vial containing *tert*-butyl 1,6-diazaspiro[3.3]heptane-6-carboxylate oxalic acid salt (**6a**, 122 mg, 0.25 mmol) and DCM (3 mL) and the reaction stirred at rt for 3 h. STAB (159 mg, 0.75 mmol) was added and the reaction stirred at rt for 18 h. The reaction was diluted with DCM (10 mL) and washed with saturated aq NaHCO₃ (5 mL) and passed through a hydrophobic frit. The organics were concentrated under a positive pressure of N₂ and taken up into DCM (5 mL). TFA (0.5 mL, 6.49 mmol) was added, and the reaction stirred for 4 h, after which MeOH (10 mL) was added and the reaction mixture left to stand at rt overnight. The reaction mixture was bound to an SCX cartridge (5 g), which was washed with MeOH. The cartridge was then eluted with methanolic ammonia (2 M) and the eluents concentrated under a positive pressure of N₂ and dried *in vacuo*. A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 364 mg, 1.575 mmol), HATU (599 mg, 1.575 mmol) and DIPEA (524 μ L, 3.00 mmol) in DCM (15 mL) was stirred at rt for 10 min. 2.6 mL of this solution was added to the residue and the reaction stirred at rt for 1 h. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / MeOH (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to give (*R**)-7-(1-(((*R*)-1,3-dimethyl-2-oxo-

2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,6-diazaspiro[3.3]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10a**, 18 mg, 0.035 mmol, 14% yield) as a white solid. *70/30 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.49 (dd, *J*=8.1, 1.7 Hz, 1 H), 7.45 (d, *J*=1.7 Hz, 1 H), 7.32 (dd, *J*=7.8, 1.5 Hz, 1 H), 7.28 (d, *J*=8.3 Hz, 1 H), 7.23 (s, 1 H), 7.15 (d, *J*=8.3 Hz, 1 H), 6.50 (d, *J*=9.1 Hz, 1 H), 6.45 (d, *J*=9.1 Hz, 1 H), 6.41 (d, *J*=9.3 Hz, 1 H), 6.36 (d, *J*=9.3 Hz, 1 H), 4.27 - 4.39 (m, 2 H), 4.10 (br dd, *J*=10.3, 4.4 Hz, 2 H), 3.73 (s, 2 H), 3.34 (q, *J*=6.8 Hz, 1 H), 3.25 (q, *J*=6.8 Hz, 1 H), 3.00 - 3.10 (m, 8 H), 2.28 (t, *J*=6.6 Hz, 2 H), 1.51 (d, *J*=6.8 Hz, 3 H), 1.44 (d, *J*=6.8 Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) t_R = 0.97 min [M+H]⁺ = 511.3.

Method F example: (*R)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.3]heptane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9b**)**

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 65 mg, 0.3 mmol) in a mixture of 2:1 THF/DCM (2.0/1.0 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R**)-1,3-dimethyl-7-(2,6-diazaspiro[3.3]heptane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14b**, 19 mg, 0.06 mmol). STAB (38 mg, 0.18 mmol) was added and the mixture shaken and stood at rt for 18 h. Additional STAB (38 mg, 0.18 mmol) was added in THF (0.5 mL) and reaction stirred at rt for 3 h. The reaction was quenched with MeOH (1 mL) and stirred at rt for 10 min. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / DCM (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to give (*R**)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.3]heptane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9b**,

7.1 mg, 0.014 mmol, 21% yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 700 MHz) δ 7.63 (br d, *J*=7.6 Hz, 1 H), 7.59 (s, 1 H), 7.34 (br d, *J*=1.0 Hz, 1 H), 7.28 (br d, *J*=7.6 Hz, 1 H), 7.19 - 7.22 (m, 2 H), 6.61 (br d, *J*=8.9 Hz, 1 H), 6.56 (br d, *J*=1.0 Hz, 1 H), 6.53 (br d, *J*=8.9 Hz, 1 H), 6.47 (br d, *J*=1.0 Hz, 1 H), 4.41 (br d, *J*=8.1 Hz, 1 H), 4.37 (br d, *J*=1.0 Hz, 1 H), 4.11 (br s, 2 H), 3.50 (s, 2 H), 3.25 - 3.29 (m, 4 H), 3.18 (br s, 3 H), 3.05 (s, 3 H), 3.03 (s, 4 H), 1.52 (br s, 3 H), 1.49 (br s, 1 H). LCMS (Formic, ES⁺) t_R = 0.57 min [M+H]⁺ = 511.5.

Method G example: (*R*^{*})-7-((3*aR*,6*aS*)-5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)octahydropyrrolo[3,4-*c*]pyrrole-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9e)

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 65 mg, 0.3 mmol) in a mixture of 2:1 THF/DCM (2.0/1.0 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R*^{*})-1,3-dimethyl-7-((3*aR*,6*aS*)-octahydropyrrolo[3,4-*c*]pyrrole-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14e**, 20 mg, 0.06 mmol). STAB (38 mg, 0.18 mmol) was added, shaken to aid dispersement and the reaction stood at rt for 72 h. Additional STAB (38 mg, 0.18 mmol) was added, and reaction stirred at rt for 4 hr. The reaction was quenched with MeOH (1 mL). The reaction was concentrated under a positive pressure N₂, taken up into DMSO (0.6 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to (*R*^{*})-7-((3*aR*,6*aS*)-5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)octahydropyrrolo[3,4-*c*]pyrrole-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9e**, 3.2 mg, 0.006 mmol, 9% yield) *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.50 (d, *J*=8.4 Hz, 1 H), 7.46 (s, 1 H), 7.30 - 7.36 (m, 2 H), 7.19 - 7.26 (m, 2 H),

6.59 - 6.63 (m, 1 H), 6.54 (br d, $J=7.9$ Hz, 2 H), 6.43 - 6.50 (m, 1 H), 4.08 (br s, 1 H), 3.58 (br s, 2 H), 3.51 (br s, 1 H), 3.14 - 3.29 (m, 5 H), 3.05 (d, $J=8.4$ Hz, 7 H), 2.75 (br s, 2 H), 2.37 - 2.41 (m, 1 H), 2.31 - 2.37 (m, 1 H), 1.52 (br t, $J=8.1$ Hz, 6 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) $t_R = 0.57$ min $[M+H]^+ = 525.5$.

Method H example: (*R)-7-(9-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,9-diazaspiro[5.5]undecane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9l)**

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 323 mg, 1.5 mmol) in a mixture of 2:1 2-Me-THF/DCM (2 mL/1 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R**)-1,3-dimethyl-7-(1,9-diazaspiro[5.5]undecane-1-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14j**, 55 mg, 0.15 mmol). STAB (38 mg, 0.18 mmol) was added, shaken and the reaction stood at rt for 18 h. Additional STAB (38 mg, 0.18 mmol) in 2-Me-THF (0.5 mL) was added and reaction stirred at rt for 3 h. The reaction was quenched with MeOH (1 mL) and stirred for at rt for 10 min. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / DCM (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to give (*R**)-7-(9-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,9-diazaspiro[5.5]undecane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9l**, 11.9 mg, 0.021 mmol, 27 % yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.30 - 7.42 (m, 4 H), 7.25 (d, $J=1.5$ Hz, 1 H), 7.22 (d, $J=7.9$ Hz, 1 H), 6.58 - 6.62 (m, 1 H), 6.47 - 6.57 (m, 3 H), 3.46 (s, 2 H), 3.20 - 3.29 (m, 3 H), 3.18 (br s, 2 H), 3.05 (d, $J=8.4$ Hz, 6 H), 2.82 - 2.93 (m, 2 H), 2.41 - 2.46 (m, 1 H), 2.40 (br s,

2 H), 1.46 - 1.71 (m, 12 H), 1.42 (br d, $J=5.4$ Hz, 2 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) $t_R = 0.71$ min $[M+H]^+ = 567.6$.

Method I example: (*R*^{*})-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,9-diazaspiro[5.5]undecane-9-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10m)

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 258 mg, 1.2 mmol) in 2-Me-THF (2.4 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R*^{*})-1,3-dimethyl-7-(2,9-diazaspiro[5.5]undecane-9-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**55** mg, 0.15 mmol). STAB (38 mg, 0.18 mmol) in 2-Me-THF (0.8 mL) was prepared. An aliquot (0.2 mL) of this solution was added, shaken and the reaction stirred at rt for 18 h. Additional STAB (38 mg, 0.18 mmol) was added and reaction was stirred at rt for 3 hr. The reaction was quenched with MeOH (1 mL) and stirred for at rt for 10 min. The reaction was concentrated under a positive pressure of N₂ and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to (*R*)-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,9-diazaspiro[5.5]undecane-9-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10m**, 10.1 mg, 0.018 mmol, 27 % yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.29 - 7.38 (m, 4 H), 7.18 - 7.27 (m, 2 H), 6.57 - 6.62 (m, 1 H), 6.44 - 6.56 (m, 3 H), 4.08 (m, 1 H), 3.34 - 3.52 (m, 3 H), 3.13 - 3.28 (m, 6 H), 3.05 (d, $J=9.4$ Hz, 6 H), 2.25 - 2.41 (m, 2 H), 2.18 (br s, 2 H), 1.51 (br t, $J=7.4$ Hz, 10 H), 1.37 (br s, 2 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) $t_R = 0.61$ min $[M+H]^+ = 567.5$.

(*R)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9a)**

Synthesised *via* method H, using (*R**)-1,3-dimethyl-7-(1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14a**, 47 mg, 0.150 mmol) as the amine to give (*R**)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9a**, 2.7 mg, 0.005 mmol, 11% yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.54 - 7.66 (2 H, m), 7.28 - 7.40 (2 H, m), 7.17 - 7.28 (2 H, m), 6.46 - 6.67 (4 H, m), 4.14 (2 H, br s), 3.99 (2 H, br s), 3.71 (2 H, br s), 3.41 (2 H, br d, *J*=3.4 Hz), 3.30 (2 H, br s), 3.13 - 3.27 (2 H, m), 3.05 (3 H, s), 3.03 (3 H, s), 1.51 (6 H, br dd, *J*=10.8, 7.4 Hz). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) *t*_R = 0.62 min [M+H]⁺ = 511.5.

(*R*)-1,3-Dimethyl-7-(2,6-diazaspiro[3.3]heptane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14b)

Synthesised *via* Method B, using *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate oxalic acid salt (**6b**, 122 mg, 0.25 mmol) as the amine. The final aqueous was concentrated *in vacuo*, dissolved in MeOH, bound to an SCX ion exchange cartridge (5 g), which was washed with MeOH (20 ml). The cartridge was then eluted with methanolic ammonia (2 M), and this combined with the organics. No MDAP purification was performed. This gave (*R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.3]heptane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14b**, 46 mg, 0.140 mmol, 56% yield) as a clear gum. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.62 (dd, *J*=8.1, 1.7 Hz, 1 H), 7.57 (d, *J*=1.7 Hz, 1 H), 7.34 (d, *J*=8.1 Hz, 1 H), 6.54 (d, *J*=9.1 Hz, 1 H), 6.51 (d, *J*=9.1

Hz, 1 H), 4.23 (br s, 4 H), 3.63 (br s, 4 H), 3.38 (q, $J=7.2$ Hz, 1 H), 3.09 (s, 3 H), 1.55 (d, $J=7.2$ Hz, 3 H), 1.30 (s, 1 H). LCMS (HpH, ES⁺) $t_R = 0.64$ min, $[M+H]^+ = 312.2$.

(*R*^{*})-7-(5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,5-diazaspiro[3.4]octane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9c)

Synthesised *via* method G, using (*R*^{*})-1,3-dimethyl-7-(2,5-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14c**, 20 mg, 0.06 mmol) as the amine to give (*R*^{*})-7-(5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,5-diazaspiro[3.4]octane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one, (**9c**, 3.5 mg, 0.007 mmol, 10 % yield) *80/20 mixture of (*R*/*S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.68 (br d, $J=8.4$ Hz, 1 H), 7.65 (s, 1 H), 7.32 - 7.39 (m, 2 H), 7.28 (s, 1 H), 7.21 (br d, $J=8.4$ Hz, 1 H), 6.43 - 6.64 (m, 4 H), 4.40 - 4.56 (m, 1 H), 4.21 - 4.30 (m, 1 H), 4.04 - 4.18 (m, 2 H), 3.90 - 3.99 (m, 1 H), 3.82 (br s, 2 H), 3.18 (d, $J=4.4$ Hz, 1 H), 3.15 - 3.27 (m, 1 H), 3.05 (s, 3 H), 3.03 (s, 3 H), 2.07 (br t, $J=7.4$ Hz, 2 H), 1.59 - 1.72 (m, 3 H), 1.53 (br d, $J=6.9$ Hz, 3 H), 1.49 (br d, $J=5.9$ Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) $t_R = 0.64$ min, $[M+H]^+ = 525.5$

(*R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14d)

Synthesised *via* method B, using *tert*-butyl 2,6-diazaspiro[3.4]octane-6-carboxylate (**6d**, 53 mg, 0.25 mmol) as the amine. After the final extraction, the organic layer was retained. The final aqueous layer also contained product and was concentrated *in vacuo*, dissolved in MeOH, bound to an SCX ion exchange cartridge (5 g), which was washed with MeOH (20 mL). The cartridge was then eluted with methanolic ammonia (2 M) (2 CV), and this combined with the previously

retained organic phase and concentrated under a positive pressure of N₂. No MDAP purification was performed. This gave (*R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14d**, 61 mg, 0.167 mmol, 67 % yield) as an orange gum. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.59 (dd, *J*=8.1, 2.0 Hz, 1 H), 7.54 (d, *J*=2.0 Hz, 1 H), 7.31 (d, *J*=8.1 Hz, 1 H), 6.50 (d, *J*=9.3 Hz, 1 H), 6.47 (d, *J*=9.3 Hz, 1 H), 3.97 - 4.08 (m, 4 H), 3.35 (q, *J*=6.8 Hz, 1 H), 3.20 (s, 2 H), 3.05 (s, 3 H), 2.91 (s, 1 H), 2.72 - 2.84 (m, 2 H, obs.), 1.90 (t, *J*=7.1 Hz, 2 H), 1.51 (d, *J*=6.8 Hz, 3 H). LCMS (HpH, ES⁺) t_R = 0.69 min, [M+H]⁺ = 326.2.

(*R*^{*})-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.4]octane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9d**)**

Synthesised *via* method F, using (*R*^{*})-1,3-dimethyl-7-(2,6-diazaspiro[3.4]octane-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14d**, 20 mg, 0.06 mmol) as the amine to give (*R*^{*})-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.4]octane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one, (**9d**, 7.6 mg, 0.014 mmol, 22 % yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 700 MHz) δ 7.63 (br d, *J*=8.1 Hz, 1 H), 7.59 (s, 1 H), 7.33 (br d, *J*=8.5 Hz, 2 H), 7.24 (s, 1 H), 7.22 (d, *J*=8.1 Hz, 1 H), 6.60 (br d, *J*=8.9 Hz, 1 H), 6.56 (br d, *J*=9.3 Hz, 1 H), 6.53 (br d, *J*=8.9 Hz, 1 H), 6.48 (br d, *J*=8.9 Hz, 1 H), 4.17 - 4.26 (m, 2 H), 4.11 (br s, 1 H), 3.96 (br s, 2 H), 3.50 - 3.59 (m, 3 H), 3.18 (s, 3 H), 3.04 (s, 3 H), 3.03 (s, 2 H), 2.65 - 2.70 (m, 2 H), 2.01 - 2.05 (m, 2 H), 1.51 (br d, *J*=11.9 Hz, 6 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) t_R = 0.58 min, [M+H]⁺ = 525.5.

(*R*)-1,3-Dimethyl-7-((3*aR*,6*aS*)-octahydropyrrolo[3,4-*c*]pyrrole-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14e**)**

Synthesised *via* method C using *tert*-butyl (3*aR*,6*aS*)-hexahydropyrrolo[3,4-*c*]pyrrole-2(1*H*)carboxylate (**6e**, 53 mg, 0.25 mmol) as the amine. This was followed by addition of diethyl ether, sonication and removal of solvent under a positive pressure of N₂, followed by drying *in vacuo* to give (*R*)-1,3-dimethyl-7-((3*aR*,6*aS*)-octahydropyrrolo[3,4-*c*]pyrrole-2-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14e**, 82 mg, 0.238 mmol, 95% yield) as a yellow solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.4-7.5 (m, 2 H), 7.33 (d, *J*=8.3 Hz, 1 H), 6.4-6.6 (m, 2 H), 3.5-3.8 (m, 2 H), 3.3-3.5 (m, 4 H), 3.18 (s, 1 H), 3.08 (s, 3 H), 2.7-3.0 (m, 5 H), 1.54 (d, *J*=6.8 Hz, 3 H). LCMS (HpH, ES⁺) t_R = 0.77 min, [M+H]⁺ = 326.3.

(*R*^{*})-7-(4-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-4,7-diazaspiro[2.5]octane-7-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9f**)**

A stock solution of (*R*^{*})-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 258 mg, 1.2 mmol) in 2-Me-THF (2.4 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R*)-1,3-dimethyl-7-(4,7-diazaspiro[2.5]octane-7-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (49 mg, 0.15 mmol). A stock solution of STAB (381 mg, 1.8 mmol) in 2-Me-THF (0.8 mL) prepared. An aliquot (0.2 mL) of this solution was added, shaken to aid dispersment and the reaction stood at rt for 18 h. Additional STAB (95 mg, 0.45 mmol) in 2-Me-THF (0.5 mL) was added and reaction stirred at rt for 7 hr. The reaction was further stood at rt for 18 hr. The reaction was quenched with MeOH (1 mL) and stirred for at rt for 10 min. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / DCM (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to (*R*^{*})-7-(4-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-4,7-diazaspiro[2.5]octane-7-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9f**, 2.7 mg, 0.062 mmol, 62% yield). *80/20 mixture of (*R*/*S*) chirality based on the e.r. of

the starting material. ^1H NMR (DMSO- d_6 , 400 MHz) δ 7.27 - 7.43 (m, 4 H), 7.22 (br s, 1 H), 7.21 (br s, 1 H), 6.58 - 6.62 (m, 1 H), 6.49 - 6.56 (m, 2 H), 6.42 - 6.49 (m, 1 H), 4.05 - 4.12 (m, 1 H), 3.87 (s, 2 H), 3.58 (br s, 1 H), 3.20 - 3.27 (m, 2 H), 3.18 (s, 3 H), 2.96 - 3.11 (m, 7 H), 2.64 - 2.73 (m, 1 H), 1.51 (br d, $J=13.8$ Hz, 3 H), 1.49 (br d, $J=13.3$ Hz, 3 H), 0.63 (br s, 3 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES $^+$) $t_{\text{R}} = 0.76$ min $[\text{M}+\text{H}]^+ = 525.6$.

(*R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.5]nonane-6-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14g)

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 213 mg, 0.923 mmol) and HATU (368 mg, 0.967 mmol) were dissolved in DCM (7.5 mL) and DIPEA (393 μl , 2.250 mmol), and the reaction stirred at rt for 5 min. A third of the reaction mixture was added to tert-butyl 2,7-diazaspiro[4.5]decane-7-carboxylate trifluoroacetate (**6g**, 70 mg, 0.29 mmol) and the mixture stirred at rt for 1 h. The solvent was removed under a positive pressure of N_2 . The residue was dissolved in DCM (10 mL) and washed with aq HCl (1 M, 10 mL) and saturated aq NaHCO_3 (10 mL) after which the organic layer was passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by flash silica chromatography eluting on a gradient of 0-10% MeOH / DCM. The relevant fractions were combined and concentrated *in vacuo*. The residue was dissolved in HCl in dioxane (4 M, 1 mL, 4.00 mmol) and stirred at rt for 1 h. The solvent was removed under a positive pressure of N_2 . The residue was dissolved in DCM (1 mL) washed with saturated aq NaHCO_3 (1 mL) and passed through a hydrophobic frit. The solvent was removed under a positive pressure of N_2 to give (*R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.5]nonane-6-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14g**, 28 mg, 0.073 mmol, 25 % yield) as a yellow gum. ^1H NMR (DMSO- d_6 , 400 MHz, 120 $^\circ\text{C}$) δ 7.26 - 7.44 (3 H, m), 6.44 - 6.58 (2 H, m),

3.60 (2 H, s), 3.56 (2 H, br s), 3.30 - 3.44 (4 H, m), 3.04 - 3.12 (4 H, m), 2.74 (2 H, s), 1.80 - 1.94 (1 H, m), 1.45 - 1.58 (5 H, m). LCMS (Formic, ES⁺) t_R = 0.47 min, [M+H]⁺ = 340.5.

(R*)-7-(2-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-2,6-diazaspiro[3.5]nonane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one (9g)

Synthesised *via* method I, using (R*)-1,3-dimethyl-7-(2,6-diazaspiro[3.5]nonane-6-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (**14g**, 51 mg, 0.15 mmol) as the amine to give (R*)-7-(2-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-2,6-diazaspiro[3.5]nonane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one (**9g**, 4 mg, 0.007 mmol, 16% yield). *80/20 mixture of (R/S) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.34 (4 H, br d, *J*=10.3 Hz), 7.20 (2 H, br s), 6.42 - 6.70 (4 H, m), 3.66 - 3.79 (1 H, m), 3.41 - 3.64 (4 H, m), 3.18 (3 H, d, *J*=4.9 Hz), 3.01 - 3.09 (7 H, m), 2.78 - 2.93 (2 H, m), 2.62 - 2.73 (1 H, m), 1.71 (2 H, br s), 1.46 - 1.57 (6 H, m), 1.43 (2 H, br s). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) t_R = 0.61 min, [M+H]⁺ = 339.5.

(R)-7-(7-(((R)-1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-2,7-diazaspiro[3.5]nonane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one, 9h

(R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepine-7-carbaldehyde (**5**, 93 mg, 0.432 mmol) was stirred with tert-butyl 2,7-diazaspiro[3.5]nonane-2-carboxylate (**6h**, 98 mg, 0.432 mmol) in DCM (3 mL) for 1.5 h, after which STAB (275 mg, 1.297 mmol) was added and the reaction stirred at rt for 2 h, after which the reaction was diluted with DCM (2 mL) and washed with saturated aq NaHCO₃. (2 mL) The organics were passed through a hydrophobic frit. TFA (1 mL, 12.98 mmol) was added to the eluent, and the reaction stirred for 30 min, after which the

reaction was diluted with MeOH (10 ml) and passed through an aminopropyl ion exchange column (10 g). The eluent was concentrated under a positive pressure of N₂ to give a clear residue. A pre-stirred (10 min) solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 100 mg, 0.432 mmol), HATU (164 mg, 0.432 mmol) and DIPEA (0.227 mL, 1.297 mmol) in DCM (5 mL) was added to the aforementioned residue and the reaction stirred at rt for 1 h, after which the reaction was washed with saturated aq NaHCO₃ (10 ml), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by flash silica gel chromatography (40 g) eluting with a gradient of 0-10% methanolic ammonia (2 M) / DCM. The pure fractions were combined and concentrated under a positive pressure of N₂ to give (*R*)-7-(7-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,7-diazaspiro[3.5]nonane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9h**, 100 mg, 0.186 mmol, 43% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 100 °C) δ 7.63 (dd, *J*=8.3, 1.5 Hz, 1 H), 7.58 (d, *J*=1.5 Hz, 1 H), 7.30 - 7.36 (m, 2 H), 7.20 - 7.23 (m, 2 H), 6.55 (d, *J*=9.3 Hz, 1 H), 6.52 (d, *J*=9.3 Hz, 1 H), 6.48 (d, *J*=9.3 Hz, 1 H), 6.43 (d, *J*=9.3 Hz, 1 H), 3.86 (br s, 4 H), 3.45 (s, 2 H), 3.35 (q, *J*=6.8 Hz, 1 H), 3.29 (q, *J*=6.8 Hz, 1 H), 3.08 (s, 3 H), 3.06 (s, 3 H), 2.35 (t, *J*=5.4 Hz, 4 H), 1.74 (t, *J*=5.4 Hz, 4 H), 1.54 (d, *J*=6.8 Hz, 3 H), 1.51 (d, *J*=6.8 Hz, 3 H) (Figure S4). ¹³C NMR (DMSO-*d*₆, 151 MHz) δ 169.3, 169.0, 168.9, 138.1, 137.3, 134.6, 134.5, 134.4, 132.2, 131.9, 131.5, 129.4, 128.1, 127.5, 126.8, 124.6, 124.4, 115.8, 115.3, 63.1, 62.2 (2C), 58.8 (2C), 50.4, 40.6, 35.7 (2C), 35.6, 35.4, 34.0, 13.6, 13.6. m.p. 134.6-143.3 °C. LCMS (HpH, ES⁺) t_R = 1.07 min, [M+H]⁺ = 539.4 (100% purity) (Figure S3). HRMS (C₃₃H₃₈N₄O₃): [M+H]⁺ calculated 538.2944, found 539.3026. IR ν_{max} (cm⁻¹) 3418, 2935, 2869, 2798, 1661, 1632, 1370, 1062, 777.

(1*R*)-7-(3,6-Diazabicyclo[3.1.1]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14i)

Synthesised *via* method D, using *tert*-butyl 3,6-diazabicyclo[3.1.1]heptane-3-carboxylate (**6i**, 50 mg, 0.25 mmol) as the amine. MDAP purification was not performed. This gave (1*R*)-7-(3,6-diazabicyclo[3.1.1]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14i**, 48 mg, 0.119 mmol, 48% yield) as an orange gum. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.47 (dd, *J*=7.8, 1.5 Hz, 1 H), 7.40 (d, *J*=1.5 Hz, 1 H), 7.36 (d, *J*=7.8 Hz, 1 H), 6.54 (d, *J*=9.1 Hz, 1 H), 6.49 (d, *J*=9.1 Hz, 1 H), 3.70 - 3.84 (m, 6 H), 3.39 (q, *J*=7.0 Hz, 1 H obs.) 3.10 (s, 3 H), 2.62 - 2.68 (m, 1 H), 1.53 - 1.63 (m, 5 H). LCMS (HpH, ES⁺) *t*_R = 0.66 min, [M+H]⁺ = 312.3.

(1*R*^{*})-7-(3-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-3,6-diazabicyclo[3.1.1]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9i)

Synthesised *via* method H, using (1*R*^{*})-7-(3,6-diazabicyclo[3.1.1]heptane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14i**, 47 mg, 0.150 mmol) as the amine to give (*R*^{*})-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,6-diazaspiro[3.3]heptane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9a**, 3.4 mg, 0.007 mmol, 10% yield). *80/20 mixture of (*R*/*S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.54 (1 H, br d, *J*=7.9 Hz), 7.49 (1 H, s), 7.40 (1 H, br d, *J*=8.4 Hz), 7.35 (1 H, d, *J*=7.9 Hz), 7.32 (1 H, br s), 7.21 (1 H, d, *J*=7.9 Hz), 6.62 (1 H, d, *J*=9.4 Hz), 6.54 - 6.57 (1 H, m), 6.46 - 6.54 (2 H, m), 3.56 - 3.75 (6 H, m), 3.42 (1 H, br s), 3.09 - 3.27 (3 H, m), 3.06 (3 H, s), 3.03 (3 H, s), 2.41 - 2.48 (1 H, m), 1.92 - 2.08 (1 H, m), 1.46 - 1.56 (6 H,

m). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) t_R = 0.56 min, [M+H]⁺ = 511.5

(R)-1,3-dimethyl-7-(1,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (14j)

(R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepine-7-carboxylic acid (**7**, 97 mg, 0.419 mmol), HATU (167 mg, 0.440 mmol) were dissolved in DCM (4 ml) and DIPEA (77 μl, 0.440 mmol). The reaction mixture was stirred at rt for 5 mins after which *tert*-butyl 1,8-diazaspiro[4.5]decane-1-carboxylate (**6j**, 96 mg, 0.4 mmol) was added. The reaction was stirred at rt for 2 h. The solvent was removed under a positive pressure of N₂. The residue was dissolved in DCM (5 mL) and washed with water (5 mL). The aqueous layer was extracted with DCM (2 x 5 mL). The combined organics were evaporated under a positive pressure of N₂. The residue was purified by flash silica chromatography eluting on a gradient of 40-100% EtOAc / cyclohexane. The relevant fractions were combined and concentrated *in vacuo*. HCl in dioxane (4 M, 2.6 mL, 10.40 mmol) was added and the reaction mixture stirred at rt for 3 h. The solvent was removed under a positive pressure of N₂. The residue was then dissolved in DCM (2 mL) and washed with saturated aqueous NaCO₃ (2 mL) and passed through a hydrophobic frit. The solvent was removed under a positive pressure of N₂ to give (R)-1,3-dimethyl-7-(1,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (**14j**, 64 mg, 0.170 mmol, 42.6 % yield) as a yellow solid. ¹H NMR (MeOD-*d*₄, 400 MHz) δ 7.47 (1 H, dd, *J*=8.3, 1.5 Hz), 7.43 (1 H, d, *J*=8.3 Hz), 7.40 (1 H, d, *J*=1.5 Hz), 6.60 (1 H, d, *J*=9.3 Hz), 6.57 (1 H, d, *J*=9.3 Hz), 3.98 - 4.14 (1 H, m), 3.47 - 3.64 (2 H, m), 3.34 - 3.45 (2 H, m), 3.14 (3 H, s), 3.09 (2 H, br s), 1.89 - 2.01 (2 H, m), 1.71 - 1.87 (4 H, m), 1.57 - 1.71 (5 H, m). LCMS (HpH, ES⁺) t_R = 0.78 min, [M+H]⁺ = 354.6

(*R)-7-(1-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9j)**

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 172 mg, 0.8 mmol) in 2-Me-THF (2.4 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R**)-1,3-dimethyl-7-(1,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14j**, 35 mg, 0.1 mmol). A stock solution of STAB (254 mg, 1.2 mmol) in 2-Me-THF (0.8 mL) was prepared. An aliquot (0.2 mL) of this solution was added, shaken and the reaction stood at rt for 18 h. Additional STAB (64 mg, 0.3 mmol) was added and reaction was shaken and stood at rt for 18 h. The reaction was quenched with MeOH (1 mL) and stirred for at rt for 10 min. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / DCM (50%, 1 mL) and purified by MDAP (HpH method). The relevant fractions were combined and concentrated *in vacuo* to (*R**)-7-(1-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9j**, 16.9 mg, 0.031 mmol, 28 % yield) *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 700 MHz) δ 7.43 (br d, *J*=8.1 Hz, 1 H), 7.39 (s, 1 H), 7.31 - 7.35 (m, 2 H), 7.23 (s, 1 H), 7.20 (d, *J*=8.1 Hz, 1 H), 6.60 (br d, *J*=8.9 Hz, 1 H), 6.51 - 6.55 (m, 2 H), 6.46 - 6.49 (m, 1 H), 4.57 (br s, 1 H), 3.60 (br s, 3 H), 3.33 - 3.33 (m, 3 H), 3.11 - 3.24 (m, 2 H), 3.02 - 3.07 (m, 6 H), 2.80 (br s, 1 H), 2.54 - 2.57 (m, 2 H), 1.83 (br s, 1 H), 1.77 (br s, 1 H), 1.68 (br s, 4 H), 1.44 - 1.56 (m, 6 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) t_R = 0.60 min [M+H]⁺ = 553.5.

(*R*)-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9k)

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**5**, 60 mg, 0.259 mmol) and HATU (109 mg, 0.285 mmol) were dissolved in DCM (3 mL). DIPEA (0.136 mL, 0.778 mmol) was added, and the reaction stirred at rt for 15 min. *tert*-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate (**6k**, 68.6 mg, 0.285 mmol) was added and the reaction stirred at rt for 18 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous Na₂CO₃ (2 mL) and aqueous citric acid (1 M, 2 mL). The organic layer was passed through a hydrophobic frit and TFA (0.5 mL, 6.49 mmol) was added. The reaction was stirred for 2 h, after which MeOH (10 mL) was added and the reaction left to stand for 30 min. The reaction was bound to an SCX column (5 g), which was washed with MeOH. The column was then washed using methanolic ammonia (2 M) and the eluent concentrated under a positive pressure of N₂ and dried in a heat piston. A solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 55.8 mg, 0.259 mmol) in DCM (3 mL) was added to the residue and the reaction stirred at rt for 3 h. STAB (165 mg, 0.778 mmol) was added and the reaction stirred at rt for 2 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous NaHCO₃ (2 mL) and passed through a hydrophobic frit. The organic was concentrated under a positive pressure of N₂. The residue was purified by MDAP (HpH). Relevant fractions were combined and concentrated *in vacuo* to give (*R*)-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,8-diazaspiro[4.5]decane-8-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9k**, 104.7 mg, 0.178 mmol, 69 % yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 120°C) δ 7.28 - 7.40 (4 H, m), 7.17 - 7.28 (2 H, m), 6.53 (1 H, d, *J*=9.3 Hz), 6.40 - 6.50 (3 H, m), 3.63 (2 H, s), 3.28 - 3.54 (7 H, m), 3.19 - 3.27 (1 H, m), 3.09 (3 H, s), 3.06 (3 H, s), 2.63 (2 H, br t, *J*=6.7 Hz), 1.68 (2 H, br t, *J*=6.8 Hz), 1.48 - 1.61 (10 H, m). LCMS (Formic, ES⁺) t_R = 0.60 min [M+H]⁺ = 553.5.

(R)-1,3-Dimethyl-7-(1,9-diazaspiro[5.5]undecane-1-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (14l)

Synthesised *via* method D, using *tert*-butyl 1,9-diazaspiro[5.5]undecane-9-carboxylate (**6l**, 64 mg, 0.25 mmol) as the amine. MDAP purification was not required after isolation of the product. This gave (*R*)-1,3-dimethyl-7-(1,9-diazaspiro[5.5]undecane-1-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (**14l**, 67 mg, 0.155 mmol, 62% yield) as an orange gum. ¹H NMR (DMSO *d*₆, 400 MHz) δ 7.47 (dd, *J*=1.5, 8.3 Hz, 1 H), 7.43 (d, *J*=2.0 Hz, 1 H), 7.34 (d, *J*=8.3 Hz, 1 H), 6.61 (d, *J*=9.1 Hz, 1 H), 6.54 (d, *J*=9.1 Hz, 1 H), 3.2-3.4 (m, 3H, obs), 3.0-3.1 (m, 8 H, obs.), 1.7-1.8 (m, 2 H), 1.6-1.7 (m, 4 H), 1.5-1.6 (m, 5 H), 1.4-1.5 (m, 2 H). LCMS (HpH, ES⁺) *t*_R = 0.87 min, [M+H]⁺ = 368.4.

(R)-7-(9-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepin-7-yl)methyl)-2,9-diazaspiro[5.5]undecane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2H-benzo[d]azepin-2-one (9m)

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepine-7-carboxylic acid (**7**, 60 mg, 0.259 mmol) and HATU (109 mg, 0.285 mmol) were dissolved in DCM (3 mL). DIPEA (0.181 mL, 1.038 mmol) was added, and the reaction stirred at rt for 15 min. *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate hydrochloride (**6m**, 75 mg, 0.259 mmol) was added and the reaction stirred at rt for 2 h. The reaction was diluted with DCM (10 mL) and washed with saturated aq Na₂CO₃ (2 mL) and aq citric acid (1 M, 2 mL). The organic layer was passed through a hydrophobic frit. TFA (0.5 mL, 6.49 mmol) was added, and the reaction stirred for 1.5 h, after which MeOH (10 mL) was added, and the reaction left to stand for 16 h. The reaction was passed through an SCX column (5 g), which was washed with MeOH. The column was then washed with

methanolic ammonia (2 M), and the eluent concentrated under a positive pressure of N₂ and dried in a vacuum oven. A solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (55.8 mg, 0.259 mmol) in DCM (3 mL) was added to the resulting residue and the reaction stirred for 16 h at rt. STAB (165 mg, 0.778 mmol) was added and the reaction stirred at rt for 4 h. The reaction was diluted with DCM (10 mL) and washed with saturated aq NaHCO₃ (2 mL) and passed through a hydrophobic frit. The organic layer was concentrated under a positive pressure of N₂. The residue was purified by MDAP (HpH). Relevant fractions were combined and concentrated *in vacuo*, then transferred to a vial in DCM/MeOH and concentrated under a positive pressure of N₂ and *in vacuo* to give (*R*)-7-(9-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,9-diazaspiro[5.5]undecane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (77.1 mg, 0.129 mmol, 50% yield) as a white solid. LCMS (HpH, ES⁺) t_R = 0.77 min, [M+H]⁺ = 368.6. ¹H NMR (DMSO-*d*₆, 400 MHz, 120°C) δ 7.34 (2 H, s), 7.26 - 7.31 (2 H, m), 7.18 - 7.23 (2 H, m), 6.54 (1 H, d, *J*=9.0 Hz), 6.45 - 6.50 (2 H, m), 6.43 (1 H, d, *J*=9.3 Hz), 3.27 - 3.45 (10 H, m), 3.09 (3 H, s), 3.07 (3 H, s), 2.40 (4 H, m, *J*=10.9, 5.4, 5.4 Hz), 2.18 (2 H, br s), 1.56 (3 H, d, *J*=7.1 Hz), 1.51 (3 H, d, *J*=7.1 Hz), 1.43 (4 H, br d, *J*=5.1 Hz).

(*R*)-1,3-dimethyl-7-(3,9-diazaspiro[5.5]undecane-3-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (14n)

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (97 mg, 0.419 mmol), HATU (167 mg, 0.440 mmol) were dissolved in DCM (4 ml) and DIPEA (77 μl, 0.440 mmol). The reaction mixture was stirred at rt for 5 mins after which *tert*-butyl 1,8-diazaspiro[4.5]decane-1-carboxylate (**6n**, 96 mg, 0.4 mmol) was added. The reaction was stirred at rt for 2 h. The solvent was removed under a positive pressure of N₂. The residue was dissolved in DCM (5 mL) and washed with water (5 mL). The aqueous layer was extracted with DCM (2 x

5 mL). The combined organics were evaporated under a positive pressure of N₂. The residue was purified by flash silica chromatography eluting on a gradient of 40-100% EtOAc / cyclohexane. The relevant fractions were combined and concentrated *in vacuo*. HCl in dioxane (4 M, 2.6 mL, 10.40 mmol) was added and the reaction mixture stirred at rt for 3 h. The solvent was removed under a positive pressure of N₂. The residue was then dissolved in DCM (2 mL) and washed with saturated aqueous Na₂CO₃ (2 mL) and passed through a hydrophobic frit. The solvent was removed under a positive pressure of N₂ to give (*R*)-1,3-dimethyl-7-(3,9-diazaspiro[5.5]undecane-3-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14n**, 38 mg, 0.087 mmol, 21.71 % yield) as a yellow solid. ¹H NMR (MeOD-*d*₄, 400 MHz) δ 7.47 (1 H, br dd, *J*=8.3, 1.5 Hz), 7.43 (1 H, d, *J*=8.3 Hz), 7.39 (1 H, d, *J*=1.5 Hz), 6.60 (1 H, d, *J*=9.3 Hz), 6.57 (1 H, d, *J*=9.3 Hz), 3.77 (2 H, br s), 3.43 (2 H, br s), 3.10 - 3.17 (6 H, m), 2.83 (3 H, s), 1.76 (3 H, br s), 1.65 (5 H, br d, *J*=4.4 Hz), 1.52 - 1.59 (2 H, m). LCMS (HpH, ES⁺) t_R = 0.77 min, [M+H]⁺ = 368.6

(*R*^{*})-7-(9-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-3,9-diazaspiro[5.5]undecane-3-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9n)

A stock solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 129 mg, 0.6 mmol) in 2-Me-THF (1.2 mL) was prepared. An aliquot (0.6 mL) of this solution was added to a vial containing (*R*^{*})-1,3-dimethyl-7-(3,9-diazaspiro[5.5]undecane-3-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**14n**, 55 mg, 0.15 mmol). STAB (38 mg, 0.18 mmol) was added, shaken and the reaction stirred at rt for 18 h. Additional STAB (38 mg, 0.18 mmol) in 2Me-THF (0.5 mL) was added and reaction stirred at rt for 3 h. The reaction was quenched with MeOH (1 mL) and stirred for at rt for 10 min. The reaction was concentrated under a positive pressure N₂, taken up into 1:1 DMSO / DCM (50%, 1 mL) and purified by MDAP (HpH method). The relevant

fractions were combined and concentrated *in vacuo* (*R*^{*})-7-(9-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-3,9-diazaspiro[5.5]undecane-3-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9n**, 4.9 mg, 0.009 mmol, 21 % yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.28 - 7.42 (m, 4 H), 7.18 - 7.27 (m, 2 H), 6.57 - 6.62 (m, 1 H), 6.46 - 6.56 (m, 3 H), 4.08 (m, 2 H), 3.58 (br s, 2 H), 3.39 - 3.49 (m, 2 H), 3.18 (d, J=4.4 Hz, 4 H), 3.00 - 3.09 (m, 6 H), 2.53 - 2.56 (m, 1 H), 2.33 (br s, 3 H), 1.44 - 1.56 (m, 11 H), 1.24 (s, 1 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) t_R = 0.64 min [M+H]⁺ = 567 (94% purity).

(*R*)-7-((4*aS*,8*aS*)-6-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-2,6-naphthyridine-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9o1**) and (*R*)-7-((4*aR*,8*aR*)-6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-2,6-naphthyridine-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-on, (**9o2**)**

(*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 90 mg, 0.389 mmol) and HATU (163 mg, 0.428 mmol) were dissolved in DCM (4 mL). DIPEA (272 μL, 1.557 mmol) was added and the reaction stirred at rt for 15 min. *tert*-Butyl octahydro-2,6-naphthyridine-2(1*H*)-carboxylate (94 mg, 0.389 mmol) was added, and the reaction stirred at rt for 28 h. The reaction was dissolved in DCM (10 mL) and washed with saturated aqueous Na₂CO₃ (2 mL). The organic layer was passed through a hydrophobic frit. TFA (0.5 mL, 6.49 mmol) was added and the reaction stirred for 1.5 h, after which MeOH (10 mL) was added and the reaction left to stand for 16 h. The reaction was bound to an SCX cartridge (5 g), which was washed with MeOH. The compound was then eluted using methanolic ammonia (2 M), and the eluent concentrated under a positive pressure of N₂. A solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-

carbaldehyde (**5**, 84 mg, 0.389 mmol) in DCM (4 mL) was added to the residue and the reaction stirred for 2 h at rt. STAB (247 mg, 1.168 mmol) was added and the reaction stirred at rt for 3 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous NaHCO₃ (2 mL), passed through a hydrophobic frit and concentrated under a positive pressure of N₂. The residue was purified by MDAP (HpH method). Relevant fractions were combined and concentrated *in vacuo*. This was then purified by chiral stationary phase HPLC. (column: (R-R) Whelk O-1 (250 x 30 mm, 5 μm), flow rate: 30 mL/min, detection wavelength: 230 nm, solvents: 100% MeCN + 0.2% isopropylamine) This gave two compounds: First eluting compound: (*R*)-7-((4*aS*,8*aS*)-6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-2,6-naphthyridine-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9o1**, 82 mg, 0.139 mmol, 35% yield) as an off-white solid. (Absolute stereochemistry of ring junctions uncharacterised). ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.37 (dd, *J*=8.2, 1.5 Hz, 1 H), 7.30 - 7.35 (m, 3 H), 7.20 - 7.25 (m, 2 H), 6.53 (d, *J*=9.3 Hz, 1 H), 6.45 - 6.50 (m, 2 H), 6.43 (d, *J*=9.3 Hz, 1 H), 3.95 (d, *J*=13.2 Hz, 1 H), 3.82 (br d, *J*=13.7 Hz, 1 H), 3.48 (d, *J*=13.7 Hz, 1 H), 3.28 - 3.45 (m, 3 H), 3.17 (dd, *J*=13.2, 2.9 Hz, 1 H), 3.09 (s, 3 H), 3.07 (s, 3 H), 3.01 (ddd, *J*=13.2, 11.5, 3.2 Hz, 1 H), 2.71 - 2.78 (m, 1 H), 2.64 (br dd, *J*=11.2, 3.4 Hz, 1 H), 2.21 (dd, *J*=11.2, 3.4 Hz, 1 H), 1.99 - 2.12 (m, 2 H), 1.84 - 1.93 (m, 1 H), 1.68 - 1.81 (m, 2 H), 1.55 (d, *J*=6.8 Hz, 3 H), 1.52 (d, *J*=6.8 Hz, 3 H), 1.31 - 1.43 (m, 2 H). LCMS (HpH, ES⁺): t_R = 1.21 min, [M+H]⁺ = 553.5. Chiral LC: 4.6 mm x 25 cm (R-R) Whelk O-1 (5 μm), 3:2 CO₂ : 100% MeCN (+0.2% v/v isopropylamine) **9o1**: t_R = 6.211 min 100%. (*R*)-7-((4*aR*,8*aR*)-6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-2,6-naphthyridine-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9o2**, 70 mg, 0.120 mmol, 30% yield) as an off-white solid. (Absolute stereochemistry of ring junctions uncharacterised). ¹H NMR (DMSO-*d*₆, 400 MHz, 120

°C) δ 7.37 (dd, $J=8.2, 1.5$ Hz, 1 H), 7.29 - 7.35 (m, 3 H), 7.20 - 7.24 (m, 2 H), 6.53 (d, $J=9.3$ Hz, 1 H), 6.45 - 6.50 (m, 2 H), 6.43 (d, $J=9.3$ Hz, 1 H), 3.93 (d, $J=12.7$ Hz, 1 H), 3.82 (br d, $J=12.2$ Hz, 1 H), 3.40 - 3.49 (m, 2 H), 3.34 (dq, $J=21.0, 7.3$ Hz, 2 H), 3.17 (dd, $J=13.2, 2.9$ Hz, 1 H), 3.09 (s, 3 H), 3.06 (s, 3 H), 3.01 (ddd, $J=13.2, 11.2, 3.4$ Hz, 1 H), 2.70 - 2.77 (m, 1 H), 2.64 (dd, $J=11.5, 3.7$ Hz, 1 H), 2.21 (dd, $J=11.2, 3.4$ Hz, 1 H), 2.00 - 2.11 (m, 2 H), 1.89 (dt, $J=10.4, 3.9$ Hz, 1 H), 1.68 - 1.81 (m, 2 H), 1.54 (d, $J=6.8$ Hz, 3 H), 1.52 (d, $J=6.8$ Hz, 3 H), 1.29 - 1.42 (m, 2 H). LCMS (HpH, ES⁺): $t_R = 1.21$ min, $[M+H]^+ = 553.5$. Chiral LC: 4.6 mm x 25 cm (R-R) Whelk O-1 (5 μ m), 3:2 CO₂ : 100% MeCN (+0.2% v/v isopropylamine) **9o1**: $t_R = 10.172$ min 100%

(R)-7-((4a*S*,8a*S*)-5-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-1,5-naphthyridine-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9q1), and **(R)-7-((4a*R*,8a*R*)-5-(((R)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-1,5-naphthyridine-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (9q2)**

(*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carboxylic acid (**7**, 85 mg, 0.37 mmol) and HATU (154 mg, 0.40 mmol) were dissolved in DCM (3 mL). DIPEA (0.26 mL, 1.47 mmol) was added and the reaction stirred at rt for 15 min. *tert*-Butyl octahydro-1,5-naphthyridine-1(2*H*)-carboxylate (**6q**, 97 mg, 0.40 mmol) was added and the reaction stirred at rt for 2 h. The reaction was dissolved in DCM (10 mL) and washed with saturated aq Na₂CO₃ (2 mL) and 1 M aqueous citric acid (2 mL). The organic layer was passed through a hydrophobic frit. TFA (0.5 mL, 6.49 mmol) was added to the organic phase and the reaction stirred at rt for 1 h, after which MeOH (10 mL) was added and the reaction left to stand for 72 h. The reaction was passed through an SCX cartridge (5 g), which was washed with MeOH (2 CV). The compound was then eluted using methanolic ammonia (2 M) (2 CV) and the eluent concentrated under a positive pressure of N₂. A

solution of (*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepine-7-carbaldehyde (**5**, 79 mg, 0.368 mmol) in DCM (3 mL) was added to the resulting residue and the reaction stirred for 1 h at rt. STAB (234 mg, 1.103 mmol) was added and the reaction stirred at rt for 20 h. The reaction was diluted with DCM (10 mL) and washed with saturated aqueous NaHCO₃ (2 mL), passed through a hydrophobic frit and concentrated under a positive pressure of N₂. The residue was purified by MDAP (HpH method) and the relevant fractions were combined and concentrated *in vacuo*. This was then purified by chiral stationary phase SFC. (column: Chiralpak AS (250 x 20 mm, 5 μm), flow rate: 75 mL/min, detection wavelength: 230 nm, solvents: 40% propan-2-ol + 0.5% isopropylamine/CO₂) This gave two compounds: First eluting compound: (*R*)-7-((4*aS*,8*aS*)-5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-1,5-naphthyridine-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9q1**, 42 mg, 0.072 mmol, 20% yield) as a pale orange solid. Absolute stereochemistry undetermined. ¹H NMR (DMSO-*d*₆, 400 MHz, 120°C) δ 7.26 - 7.36 (m, 4 H), 7.19 - 7.24 (m, 2 H), 6.55 (d, *J*=9.0 Hz, 1 H), 6.48 (app. d, *J*=9.0 Hz, 2 H), 6.43 (d, *J*=9.3 Hz, 1 H), 4.25 - 4.46 (m, 1 H), 3.75 (br d, *J*=13.7 Hz, 2 H), 3.59 (br d, *J*=13.9 Hz, 1 H), 3.38 (q, *J*=7.2 Hz, 1 H), 3.32 (q, *J*=7.1 Hz, 1 H), 3.10 (s, 2 H), 3.07 (s, 3 H), 2.87 - 3.01 (m, 2 H), 2.43 (br d, *J*=3.2 Hz, 2 H), 1.85 - 2.04 (m, 2 H), 1.67 - 1.77 (m, 2 H), 1.59 - 1.67 (m, 1 H), 1.56 (d, *J*=6.8 Hz, 3 H), 1.52 (d, *J*=6.8 Hz, 5 H), 1.24 - 1.41 (m, 2 H). LCMS (HpH, ES⁺): t_R = 1.23 min, [M+H]⁺ = 553.4. Chiral LC: 4.6 mm x 25 cm Chiralpak AS (3 μm), 3:2 CO₂ : propan-2-ol (+ 0.1% v/v isopropylamine) **9q1**: t_R = 3.84 min 100%. Second eluting compound: (*R*)-7-((4*aR*,8*aR*)-5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)decahydro-1,5-naphthyridine-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**9q2**, 52 mg, 0.089 mmol, 24% yield) as a pale orange solid. Absolute stereochemistry undetermined. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.28 - 7.36 (m,

3 H), 7.27 (s, 1 H), 7.20 - 7.24 (m, 2 H), 6.54 (d, $J=9.0$ Hz, 1 H), 6.44 - 6.50 (m, 2 H), 6.42 (d, $J=9.3$ Hz, 1 H), 4.26 - 4.48 (m, 1 H), 3.67 - 3.81 (m, 2 H), 3.51 - 3.65 (m, 2 H), 3.38 (q, $J=6.6$ Hz, 1 H), 3.32 (q, $J=7.1$ Hz, 1 H), 3.09 (s, 3 H), 3.07 (s, 3 H), 2.90 - 3.02 (m, 2 H), 2.87 - 2.90 (m, 1 H, obs.), 2.38 - 2.46 (m, 2 H), 1.85 - 2.05 (m, 2 H), 1.73 (app. br d, $J=10.8$ Hz, 2 H), 1.63 (app. br d, $J=11.5$ Hz, 1 H), 1.54 (app. dd, $J=13.6, 7.0$ Hz, 7 H). LCMS (HpH, ES⁺): $t_R = 1.23$ min, $[M+H]^+ = 553.4$. Chiral LC: 4.6 mm x 25 cm Chiralpak AS (3 μ m), 3:2 CO₂ : propan-2-ol (+ 0.1% v/v isopropylamine) **9q2**: $t_R = 7.65$ min 100%.

(*R*)-1,3-Dimethyl-7-(2,5-diazaspiro[3.4]octane-5-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (15c)

Synthesised *via* method C, using *tert*-butyl 2,5-diazaspiro[3.4]octane-2-carboxylate (**13c**, 53 mg, 0.250 mmol) as the amine. This was followed by addition of diethyl ether, sonication and removal of solvent under a positive pressure of N₂, followed by drying *in vacuo* to give (*R*)-1,3-dimethyl-7-(2,5-diazaspiro[3.4]octane-5-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**15c**, 58 mg, 0.169 mmol, 67% yield) as a yellow solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.5-7.6 (m, 2 H), 7.3-7.4 (m, 1 H), 6.6-6.7 (m, 1 H), 6.5-6.6 (m, 1 H), 4.21 (br s, 1 H), 3.6-3.8 (m, 1 H), 3.1-3.5 (m, 6 H), 3.05 (s, 3 H), 2.28 (q, $J=7.2$ Hz, 2 H), 1.6-1.8 (m, 2 H), 1.53 (br d, $J=5.4$ Hz, 3 H). LCMS (HpH, ES⁺) $t_R = 0.75$ min, $[M+H]^+ = 326.4$.

(*R*^{*})-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,5-diazaspiro[3.4]octane-5-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10c)

Synthesised *via* method G, using (*R*^{*})-1,3-dimethyl-7-(2,5-diazaspiro[3.4]octane-5-carbonyl)-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**15c**, 20 mg, 0.06 mmol) as the amine to give (*R*^{*})-7-(5-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,5-

diazaspiro[3.4]octane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10c**, 4.1 mg, 0.008 mmol, 12 % yield). *80/20 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.44 - 7.56 (m, 2 H), 7.33 (m, 2 H), 7.22 (t, *J*=7.4 Hz, 2 H), 6.57 - 6.63 (m, 1 H), 6.45 - 6.56 (m, 3 H), 4.20 (br s, 2 H), 4.00 - 4.15 (m, 1 H), 3.81 (br s, 2 H), 3.12 - 3.20 (m, 4 H), 3.05 (m, 6 H), 2.53 - 2.56 (m, 1 H), 2.22 (t, *J*=6.6 Hz, 2 H), 1.58 - 1.74 (m, 2 H), 1.52 (br d, *J*=11.8 Hz, 3 H), 1.50 (br d, *J*=11.8 Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (Formic) t_R = 0.64 min, [M+H]⁺ = 525.5.

(*R)-7-(2-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.4]octane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10d)**

Synthesised *via* method E, using *tert*-butyl 2,6-diazaspiro[3.4]octane-6-carboxylate (**6d**, 53 mg, 0.250 mmol) as the amine to give (*R*)-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.4]octane-6-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10d**, 80 mg, 0.152 mmol, 61% yield). *70/30 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.49 (d, *J*=8.3 Hz, 1 H), 7.43 (s, 1 H), 7.33 (d, *J*=7.8 Hz, 1 H), 7.25 - 7.30 (m, 1 H), 7.17 - 7.22 (m, 2 H), 6.53 (d, *J*=9.3 Hz, 1 H), 6.44 - 6.51 (m, 2 H), 6.42 (d, *J*=9.3 Hz, 1 H), 3.58 (s, 4 H), 3.47 (br t, *J*=7.1 Hz, 2 H), 3.37 (br d, *J*=6.8 Hz, 1 H), 3.30 (br d, *J*=6.8 Hz, 1 H), 3.16 (s, 4 H), 3.09 (s, 3 H), 3.06 (s, 3 H), 2.05 (t, *J*=7.1 Hz, 2 H), 1.55 (d, *J*=6.8 Hz, 3 H), 1.50 (d, *J*=6.8 Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (HpH, ES+) t_R = 0.56 min, [M+H]⁺ = 525.3.

(*R*)-7-(7-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-4,7-diazaspiro[2.5]octane-4-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10f)

Synthesised *via* method E, using *tert*-butyl 4,7-diazaspiro[2.5]octane-4-carboxylate (**6f**, 53 mg, 0.250 mmol) as the amine to give (*R*)-7-(7-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-4,7-diazaspiro[2.5]octane-4-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10f**, 73 mg, 0.139 mmol, 56% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.41 (dd, *J*=8.3, 1.5 Hz, 1 H), 7.33 - 7.37 (m, 2 H), 7.31 (d, *J*=8.3 Hz, 1 H), 7.20 - 7.27 (m, 2 H), 6.52 (d, *J*=9.3 Hz, 1 H), 6.47 (app. d, *J*=9.3 Hz, 2 H), 6.43 (d, *J*=9.3 Hz, 1 H), 3.61 (br dd, *J*=3.7, 2.2 Hz, 2 H), 3.55 (s, 2 H), 3.28 - 3.41 (m, 2 H), 3.07 (d, *J*=7.3 Hz, 6 H), 2.48 - 2.53 (m, 2 H obs.) 2.44 (s, 2 H), 1.52 (app. dd, *J*=9.5, 6.8 Hz, 6 H), 0.66 - 0.81 (m, 4 H). ¹³C NMR (DMSO-*d*₆, 101 MHz, 100 °C) δ 169.8, 169.6, 169.3, 137.4, 135.9, 135.0, 134.5, 134.5, 132.0, 131.3, 129.3, 127.4, 126.2, 124.9, 124.9, 115.6, 115.0, 61.5, 60.2, 53.6, 47.1, 42.7, 42.4, 38.5, 35.6, 14.5, 13.4, 13.4. Two signals not observed due to signal broadening. LCMS (HpH, ES⁺) *t*_R = 0.59 min, [M+H]⁺ = 525.3.

(*R*)-7-(6-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.5]nonane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10g)

Synthesised *via* method E, using 1-*tert*-butyl 2,6-diazaspiro[3.5]nonane-2-carboxylate oxalic acid salt (**6g**, 136 mg, 0.25 mmol) as the amine, to give (*R*)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,6-diazaspiro[3.5]nonane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10g**, 32 mg, 0.059 mmol, 24% yield) as a white solid. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.56 (dd, *J*=8.1, 1.7 Hz, 1 H), 7.51 (d, *J*=1.7 Hz, 1 H), 7.27 - 7.32 (m, 2 H), 7.17 - 7.21 (m, 2 H), 6.50 (d, *J*=9.2 Hz, 1 H), 6.47 (d, *J*=9.2 Hz, 1 H), 6.41 (d, *J*=9.2 Hz, 1 H), 6.36 (d, *J*=9.2 Hz, 1 H), 3.79 (s, 4 H), 3.49 (s, 2 H), 3.35 (q, *J*=6.8 Hz, 1 H), 3.29 (q, *J*=6.8 Hz, 1 H), 3.05 (s, 3 H), 3.02 (s, 3 H), 2.46 - 2.48 (m, 2 H obs.) 2.31 (t, *J*=5.4 Hz, 2 H),

1.57 - 1.63 (m, 2 H), 1.50 (dd, $J=14.7, 6.8$ Hz, 8 H). LCMS (HpH, ES⁺) $t_R = 1.17$ min, $[M+H]^+ = 539.3$.

(*R)-7-(2-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,7-diazaspiro[3.5]nonane-7-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10h)**

Synthesised *via* method E, using *tert*-butyl 2,7-diazaspiro[3.5]nonane-7-carboxylate hydrochloride (**6h**, 66 mg, 0.250 mmol) as the amine to give (*R*)-7-(2-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,7-diazaspiro[3.5]nonane-7-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10h**, 78 mg, 0.145 mmol, 58% yield) as a white solid. *70/30 mixture of (*R/S*) chirality, based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.36 (dd, $J=8.3, 1.5$ Hz, 1 H), 7.27 - 7.34 (m, 3 H), 7.20 (d, $J=7.3$ Hz, 2 H), 6.53 (d, $J=9.3$ Hz, 1 H), 6.44 - 6.49 (m, 2 H), 6.42 (d, $J=9.3$ Hz, 1 H), 3.62 (s, 2 H), 3.34 - 3.45 (m, 5 H), 3.31 (q, $J=6.8$ Hz, 1 H), 3.09 (s, 3 H), 3.06 (s, 3 H), 3.05 (s, 4 H), 1.70 - 1.75 (m, 4 H), 1.54 (d, $J=6.8$ Hz, 3 H), 1.51 (d, $J=6.8$ Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) $t_R = 0.58$ min, $[M+H]^+ = 539.3$.

(1*R*)-7-(6-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-3,6-diazabicyclo[3.1.1]heptane-3-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10i)

Synthesised *via* method E, using *tert*-butyl 3,6-diazabicyclo[3.1.1]heptane-3-carboxylate (50 mg, 0.25 mmol) as the amine to give (1*R*)-7-(6-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-3,6-diazabicyclo[3.1.1]heptane-3-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10i**, 65 mg, 0.127 mmol, 51% yield) as a white solid. *70/30

mixture of (*R/S*) chirality, based on the e.r. of the starting material, was confirmed by chiral LC in favour of the diastereomer drawn. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.50 (d, *J*=8.3 Hz, 1 H), 7.44 (s, 1 H), 7.33 - 7.40 (m, 2 H), 7.28 (s, 1 H), 7.21 (d, *J*=8.3 Hz, 1 H), 6.54 (d, *J*=9.3 Hz, 1 H), 6.50 (d, *J*=9.3 Hz, 1 H), 6.46 (d, *J*=9.3 Hz, 1 H), 6.43 (d, *J*=9.3 Hz, 1 H), 3.74 (br dd, *J*=12.0, 5.6 Hz, 2 H), 3.68 (s, 2 H), 3.44 - 3.62 (m, 4 H), 3.38 (q, *J*=6.8 Hz, 1 H), 3.30 (q, *J*=6.8 Hz, 1 H), 3.09 (s, 3 H), 3.06 (s, 3 H), 2.55 (br q, *J*=7.3 Hz, 1 H), 1.53 - 1.59 (m, 4 H), 1.51 (d, *J*=6.8 Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) t_R = 0.56 min, [M+H]⁺ = 511.3. m.p. 139.8-145.7 °C. Chiral LC: 4.6 mm x 25 cm Chiralpak IB-N (5 μm), acetonitrile (0.1% v/v isopropylamine), **minor-10i**: t_R = 16.1 min 30%, **major-10i** (as drawn): t_R = 19.5 min 70 %.

(*R*^{*})-7-(8-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,8-diazaspiro[4.5]decane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (10j)

Synthesised *via* method E, using *tert*-butyl 1,8-diazaspiro[4.5]decane-1-carboxylate hydrochloride (**6j**, 69 mg, 0.25 mmol) as the amine to give (*R*^{*})-7-(8-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,8-diazaspiro[4.5]decane-1-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10j**, 44 mg, 0.080 mmol, 32% yield) as a white solid. *70/30 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.33 - 7.39 (m, 1 H), 7.29 - 7.32 (m, 2 H), 7.21 - 7.28 (m, 2 H), 6.52 (d, *J*=9.3 Hz, 1 H), 6.47 (app. d, *J*=9.3 Hz, 3 H), 3.52 (s, 2 H), 3.29 - 3.40 (m, 4 H), 3.08 (m, 7 H), 2.79 - 2.86 (m, 4 H obs.) 2.17 (tt, *J*=12.3, 2.7 Hz, 2 H), 1.96 (t, *J*=6.8 Hz, 2 H), 1.73 (app. quin, *J*=6.8 Hz, 2 H), 1.53 (app. dd, *J*=6.9, 6.2 Hz, 6 H), 1.36 (app. br d, *J*=12.3 Hz, 2 H). Individual diastereomer signals were not resolved. LCMS (HpH, ES⁺) t_R = 0.65 min, [M+H]⁺ = 553.3.

(*R)-7-(8-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one**
(10k)

Synthesised *via* method E, using *tert*-butyl 2,8-diazaspiro[4.5]decane-2-carboxylate (**6k**, 60 mg, 0.25 mmol) as the amine to give (*R**)-7-(8-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-2,8-diazaspiro[4.5]decane-2-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10k**, 24 mg, 0.043 mmol, 17% yield) as a white solid. *70/30 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.49 (dd, *J*=7.8, 1.5 Hz, 1 H), 7.43 (d, *J*=1.5 Hz, 1 H), 7.33 (app. d, *J*=7.8 Hz, 2 H), 7.20 - 7.26 (m, 2 H), 6.53 (d, *J*=9.3 Hz, 1 H), 6.45 - 6.50 (m, 2 H), 6.42 (d, *J*=9.3 Hz, 1 H), 3.46 - 3.61 (m, 4 H), 3.28 - 3.41 (m, 4 H), 3.09 (s, 3 H), 3.06 (s, 3 H), 2.46 - 2.52 (m, 2 H, obs.) 2.32 - 2.45 (m, 2 H), 1.77 (t, *J*=7.1 Hz, 2 H), 1.53 - 1.63 (m, 7 H), 1.51 (d, *J*=6.8 Hz, 3 H). Individual diastereomer signals were not resolved. LCMS (Formic, ES⁺) t_R = 0.60 min, [M+H]⁺ = 553.4.

(*R)-7-(1-(((*R*)-1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,9-diazaspiro[5.5]undecane-9-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one**
(10l)

Synthesised *via* method E, using *tert*-butyl 1,9-diazaspiro[5.5]undecane-9-carboxylate (**6l**, 64 mg, 0.25 mmol) as the amine to give (*R**)-7-(1-(((*R*)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]azepin-7-yl)methyl)-1,9-diazaspiro[5.5]undecane-9-carbonyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]azepin-2-one (**10l**) (15 mg, 0.026 mmol, 11% yield) as a white solid. *70/30 mixture of (*R/S*) chirality based on the e.r. of the starting material. ¹H NMR (DMSO-*d*₆, 400 MHz, 120 °C) δ 7.35 (app. td, *J*=7.9, 1.7 Hz, 2 H), 7.27 - 7.32 (m, 2 H), 7.24 (s, 1 H), 7.18 (d, *J*=8.3 Hz,

1 H), 6.49 (d, $J=9.3$ Hz, 1 H), 6.38 - 6.46 (m, 3 H), 3.69 - 3.78 (m, 2 H), 3.67 (s, 2 H), 3.33 (q, $J=6.8$ Hz, 1 H), 3.21 - 3.30 (m, 3 H), 3.05 (s, 3 H), 3.03 (s, 3 H), 2.51 - 2.56 (m, 2 H), 1.82 - 1.92 (m, 2 H), 1.60 - 1.66 (m, 2 H), 1.52 - 1.60 (m, 4 H), 1.51 (d, $J=6.8$ Hz, 3 H), 1.48 (d, $J=6.8$ Hz, 3 H), 1.38 - 1.46 (m, 2 H). Individual diastereomer signals were not resolved. LCMS (Formic) $t_R = 0.63$ min, $[M+H]^+ = 567.5$

(R)-1,3-dimethyl-7-(2,9-diazaspiro[5.5]undecane-2-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (15m)

(R)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]azepine-7-carboxylic acid (**7**, 93 mg, 0.4 mmol) and HATU (228 mg, 0.600 mmol) were dissolved in DCM (2.7 mL) and DIPEA (0.210 ml, 1.200 mmol) was added. The reaction was stirred for 5 min. *tert*-butyl 2,9-diazaspiro[5.5]undecane-9-carboxylate hydrochloride (**13m**, 128 mg, 0.440 mmol) was added and the reaction was stirred for 1 hr. The reaction mixture was extracted with DCM (2 x 5 mL) and aq HCl (2M, 5 mL). The combined organic layers were then washed with saturated aqueous NaHCO₃ (5 mL). The combined organic layers were concentrated *in vacuo*. The residue was purified by flash silica chromatography eluting on a gradient of 0-100% EtOAc/cyclohexane. The relevant fractions were combined and concentrated *in vacuo*. The residue was dissolved in HCl in dioxane (4 M, 2 ml, 8.0 mmol) and stirred at rt for 2 h. The reaction mixture was diluted with DCM (10 mL) and washed with saturated aqueous NaHCO₃ (2 x 5 mL). The organic layer was concentrated to give (R)-1,3-dimethyl-7-(2,9-diazaspiro[5.5]undecane-2-carbonyl)-1,3-dihydro-2H-benzo[d]azepin-2-one (**15m**, 68 mg, 0.185 mmol, 46 % yield) as white crystals. ¹H NMR (CDCl₃, 400 MHz) δ 7.33 - 7.42 (2 H, m), 7.31 (1 H, s), 6.40 - 6.46 (1 H, m), 6.33 - 6.38 (1 H, m), 3.58 - 3.69 (1 H, m), 3.28 - 3.39 (2 H, m), 3.16 (3 H, s), 2.84 - 3.07 (2 H, m), 2.64 - 2.78 (1 H, m), 2.42

- 2.61 (1 H, m), 1.79 (3 H, br s), 1.68 (4 H, br d, $J=5.4$ Hz), 1.57 (4 H, br s), 1.39 - 1.51 (2 H, m), 1.17 - 1.34 (1 H, m). LCMS (HpH, ES⁺) $t_R = 0.50$ min, $[M+H]^+ = 368.6$

Methods

Source of Biological Materials

Human biological samples used in the MCP-1 whole blood cytokine release assay were sourced ethically, and their research use was in accord with the terms of the informed consents under IRB/REC approved protocol number 07/H0311/103; IRAS project ID: 174597.

Hut-78 Cells: Catalogue Number: #8041901, Supplier Name: ECACC

HEK 293 WT Cells: Catalogue Number: CRL-1573, Supplier Name: ATCC

Cellular Thermal Shift Assay (CETSA)

Thermal stabilization was assessed for **9h** across six dilution points evenly spaced on a logarithmic scale between 5 μ M and 1.6 nM, with reference to the total stabilization control JQ1 (20 μ M). For each data point, approximately 50 million HuT 78 cells were treated with the corresponding amount of inhibitor for 2 hours under standard mammalian cell culture conditions for suspension cells. After treatment, the cells were collected, washed, and aliquoted at a density of 6 million cells into six wells of a PCR plate (two replicates at three different temperatures). Heat treatment was conducted for 3 minutes at 46, 47.5, and 49°C (approximate melting temperatures of BRD2, BRD4, and BRD3, respectively). Subsequently, the cells were incubated for an additional 3 minutes at room temperature before being transferred onto ice. The cells were lysed, and insoluble protein aggregates resulting from the heat treatment were removed using a 0.45 μ m filter plate, as previously described.^{1,2} The samples were then conditioned in sample buffer suitable for WES readout. BET protein levels were measured using a WES system (Protein Simple, cat. no. SM-W004-1, PS-ST 01, SM-W012-1, and PS-ST05-8) following the manufacturer's

specifications. The following antibodies were used: BRD4 (Bethyl, cat. no. A700-005), BRD2 (Abcam, cat. no. Ab139690), BRD3 (Bethyl, cat. no. A302-368A), and Tubulin (Cell Signaling Technology, cat. no. 2125) (Tubulin served as a reference for all BET proteins). Secondary antibodies included α -mouse (Protein Simple, cat. no. DM-002) and α -rabbit (Protein Simple, cat. no. DM-001). To normalize for differences in sample loading, the peak area of the BET protein of interest was normalized to the peak area of Tubulin. Finally, the estimated BET protein levels were normalized to the total stabilization control JQ1.

BRD2, BRD3, and BRD4 NanoBRET™ PPI Assays

Assay principles: The NanoBRET™ PPI assays are a bioluminescent resonance energy transfer assay where the BRD-NanoLuc® fusion protein acts as the donor at 480 nm generating the donor signal through the NanoBRET™ Nano-Glo® substrate. The H3.3-HaloTag acts as the acceptor when bound to the HaloTag® NanoBRET™ 618 ligand and gives a signal at 618nm. This assay provides a cellular target engagement PIC50 readout; when the ligand of interest is bound to the BRD-NanoLuc® fusion protein it is not able to bind with its native binding partner the H3.3-HaloTag® and the BRET signal is lost. Plasmid generation: All BRD-NanoLuc® fusion and H3.3-HaloTag® plasmids were provided by Promega (Madison, WI) and were amplified in TOP10 cells transformed using a heat shock transformation. Plasmid DNA was recovered using Maxiprep or Gigaprep kits (Qiagen, Hilden, Germany) as per manufacturer's instructions. Protocol for BRD2, BRD3 and BRD4 NanoBRET™ PPI Assays: Compounds were titrated 1:3 from a 1 mM stock solution in 100% DMSO, with 40 nL transferred per well to a white 384-well tissue culture (TC)-treated microplate (Nunc 164610; Thermo Fisher Scientific, Waltham, MA) using a Labcyte Echo 555 (Labcyte Inc., Sunnyvale, CA). HEK293-wt cells (ATCC CRL-1573) were seeded into T75 flasks (Thermo Fisher Scientific) at 4.8e6 cells/flask in a total volume of 12 mL and left to attach

for 4–6 h at 37 °C, 5% CO₂. The cells were then transfected with the following transfection mix (per T75 flask): 600 µL of OptiMEM, 0.12 µg of NanoLuc®-BRD DNA, 12 µg of Histone H3.3 HaloTag® DNA, and 48 µL of FuGENE HD. Following an overnight incubation at 37 °C, 5% CO₂, cells were washed with phosphate-buffered saline (PBS) and then detached by trypsinization using TrypLE Express. Cells were then centrifuged at 1500 rpm for 5 min, the supernatant discarded, and the cell pellet resuspended in OptiMEM + 4% USA fetal bovine serum (FBS) at 2e5 cells/mL (Invitrogen, Thermo Fisher Scientific, Waltham, MA). Promega 618 HaloTag® ligand was then added to the cell solution at a 1:1000 dilution. Cells were subsequently dispensed into compound plates and incubated for 18–24 h at 37 °C, 5% CO₂. NanoLuc® substrate solution was prepared (100× dilution of the NanoLuc® substrate in OptiMEM) with 10 µL of solution added per well and plates centrifuged at 1000 rpm for 1 min and shaken at 1000 rpm for 1 min, followed immediately by data analysis.

BRD4 Biosensor Assay

Assay principles: The NanoLuc®-BRD4 BD1BD2- HaloTag® Biosensor assay is based on a similar principle to the BRD PPI assays. However, the donor and acceptor are both on a BRD4 BD1BD2 truncate so conformational change of the BRD4 truncate caused by binding of bivalent compounds is instead measured, reporting a PEC₅₀. Plasmid generation: NanoLuc®-BRD4 BD1BD2- HaloTag® plasmid was provided by Promega (Madison, WI) and was amplified in TOP10 cells transformed using a heat shock transformation. Plasmid DNA was recovered using Maxiprep or Gigaprep kits (Qiagen, Hilden, Germany) as per manufacturer's instructions. Protocol for BRD4 Biosensor assay: Compounds were titrated 1:3 from a 1 mM stock solution in 100% DMSO, with 40 nL transferred per well to a white 384-well tissue culture (TC)-treated microplate (Nunc 164610; Thermo Fisher Scientific, Waltham, MA) using a Labcyte Echo 555 (Labcyte Inc.,

Sunnyvale, CA). HEK293-wt cells (ATCC CRL-1573) were seeded into a 6- well plate (Thermo Fisher Scientific) at 8^5 cells/well in a total volume of 2 mL and left to attach for 4 h at 37 °C, 5% CO₂. The cells were then transfected with the following transfection mix (per well): 100 µL of OptiMEM, 0.2 µg NanoLuc®-BRD4 BD1BD2- HaloTag® DNA, 1.2 µg of carrier DNA, and 8 µL of FuGENE HD. Following an overnight incubation at 37 °C, 5% CO₂, cells were washed with phosphate-buffered saline (PBS) and then detached by trypsinization using TrypLE Express. Cells were then centrifuged at 1500 rpm for 5 min, the supernatant discarded, and the cell pellet resuspended in OptiMEM at 2.2e5 cells/mL (Invitrogen, Thermo Fisher Scientific, Waltham, MA). Promega 618 HaloTag® ligand was then added to the cell solution at a 1:1000 dilution. Cells were subsequently dispensed into compound plates and incubated for 4 h at 37 °C, 5% CO₂. NanoLuc® substrate solution was prepared (100× dilution of the NanoLuc® substrate in OptiMEM) with 10 µL of solution added per well and plates centrifuged at 1000 rpm for 1 min and shaken at 1000 rpm for 1 min, followed immediately by data analysis.

MCP-1 Whole Blood Cytokine Release Assay

The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

Blood was collected in house by GSK in the blood donation unit, blood was collected into Sodium Heparin (1ml/100ml blood). Protocol for MCP-1 Whole Blood Cytokine Release Assay: Compounds were titrated 1:3 from a 3 mM stock solution in 100% DMSO, with 1 µL of compound transferred per well to a white 96-well TC-treated microplate (cat. 655088; Greiner Bio-One, Frickenhausen, Germany). 130 µL of human donor blood was added to each well and left for 30 min. 10 µL of lipopolysaccharide (LPS), dissolved in PBS, was then added per well (final assay concentration [FAC] of LPS, 200 ng/mL). Following overnight incubation of the assay microplates

at 37 °C, 5% CO₂, 140 µL of PBS was added per well followed by microplate centrifugation at 2000 rpm for 10 min. Supernatants were collected and further diluted 1:2 in PBS + 1% bovine serum albumin (BSA). 10 µL of diluted supernatant was then transferred to a V-bottom 96-well black microplate (cat. 651201; Greiner Bio-One) using a Biomek FX (Beckman Coulter, Brea, CA). For cytokine detection, MCP-1 BD cytometric bead array (CBA) beads (BD Biosciences, Franklin Lakes, NJ) were used. Capture bead solution was prepared by performing a 1:200 dilution of the MCP-1 capture bead stock solution in PBS + 1% BSA. 10 µL of this solution was added per well to the assay supernatant and microplates were incubated at room temperature on a microplate shaker for 2 h. Detection reagent was further prepared by performing a 1:400 dilution of the detection reagent stock solution. 10 µL of detection reagent was added per well and microplates were incubated at room temperature on a microplate shaker for 2 h. Data were acquired on an iQue Screener flow cytometer.

Intrinsic Clearance (CL_{int}) Measurements: Hepatocyte Intrinsic Clearance data was determined by Cyprotex UK. Test compound (0.5 µM) was incubated with cryopreserved hepatocytes in suspension. Samples were removed at 6 time points over the course of a 60 min (rat) or 120 min (dog & human) experiment and test compound analyzed by LC-MS/MS. Cryopreserved pooled hepatocytes were purchased from a reputable commercial supplier and stored in liquid nitrogen prior to use. Williams E media supplemented with 2 mM L-glutamine and 25 mM HEPES and test compound (final substrate concentration 0.5 µM; final DMSO concentration 0.25%) was pre-incubated at 37 °C prior to the addition of a suspension of cryopreserved hepatocytes (final cell density 0.5 x 10⁶ viable cells/mL in Williams E media supplemented with 2 mM L-glutamine and 25 mM HEPES) to initiate the reaction. The final incubation volume was 500 µL. The reactions were stopped by transferring 50 µL of incubate to

100 μL acetonitrile at the appropriate time points. The termination plates were centrifuged at 2500 rpm at 4 $^{\circ}\text{C}$ for 30 min to precipitate the protein. The remaining incubate (200 μL) was crashed with 400 μL acetonitrile at the end of the incubation. Following protein precipitation, the sample supernatants were combined in cassettes of up to 4 compounds and analyzed using Cyprotex generic LC-MS/MS conditions.

Intrinsic Clearance (CL_{int}) Data Analysis: From a plot of \ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line was determined. Subsequently, half-life ($t_{1/2}$) and intrinsic clearance (CL_{int}) were calculated using the equations below:

Elimination rate constant (k) = (- gradient)

$$\text{Half-life } (t_{1/2})(\text{min}) = \frac{0.693}{k}$$

$$\text{Intrinsic clearance } (\text{CL}_{\text{int}})(\mu\text{L}/\text{min}/\text{million cells}) = \frac{V \times 0.693}{t_{1/2}}$$

where V = Incubation volume (μL)/Number of cells

FaSSIF solubility

Compounds were dissolved in DMSO at 2.5 mg/mL and then diluted in Fast State Simulated Intestinal Fluid (FaSSIF pH 6.5) at 125 $\mu\text{g}/\text{mL}$ (final DMSO concentration is 5%). After 16 h of incubation at 25 $^{\circ}\text{C}$, the suspension was filtered. The concentration of the compound was determined by a fast HPLC gradient. The ratio of the peak areas obtained from the standards and the sample filtrate was used to calculate the solubility of the compound.

Artificial membrane permeability

Permeability across a lipid membrane was measured using the published protocol.³⁷

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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All authors were GlaxoSmithKline full-time employees when this study was performed. EPSRC Prosperity Partnership EP/S035990/1.

Conflict of Interest Disclosure

F.R, S.A, P.B, H.B, K.F, P.G, C.N, M.P, A.P, R.P and E.D were employees of GSK plc. at the time this work was performed.

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ABBREVIATIONS

AMP, Artificial membrane permeability; BD1, Bromodomain 1, First Bromodomain, C-terminal Bromodomain; BD2, Bromodomain 2, Second Bromodomain, N-terminal Bromodomain; BET, Bromodomain and Extra Terminal; BRET, Bioluminescence resonance energy transfer; BS, NanoBRET biosensor; BZP, Benzoazapinone; CETSA, Cellular thermal shift assay; CRDA, Conformationally restricted diamines; DIPEA, Diisopropyl ethylamide; FaSSIF, Fasted state simulated intestinal fluid; HATU, (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; HEK, Human embryonic kidney 293; hWB, Human whole blood; MCP1, Monocyte chemoattractant protein 1; MDAP, Mass directed automated purification; NanoBRET, NanoLuciferase BRET; pIC50, -log(Half maximal inhibitory concentration); PPI, Protein-Protein Interaction; STAB, Sodium triacetoxyborohydride; UPGMA, Unweighted pair group method with arithmetic mean.

SUPPORTING INFORMATION

- CETSA figure, assay details for diastereomeric and pure samples, HPLC traces, LCMS traces, and NMR Spectra (DOCX)
- Molecular formula strings (CSV)

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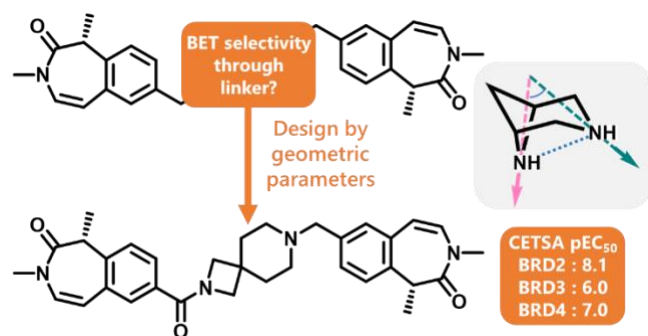
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Table of Contents Graphic



Supporting Information

BET isoform selectivity through diverse linkers for bivalent inhibitors: GSK785, a BRD2/4-selective bivalent BET inhibitor.

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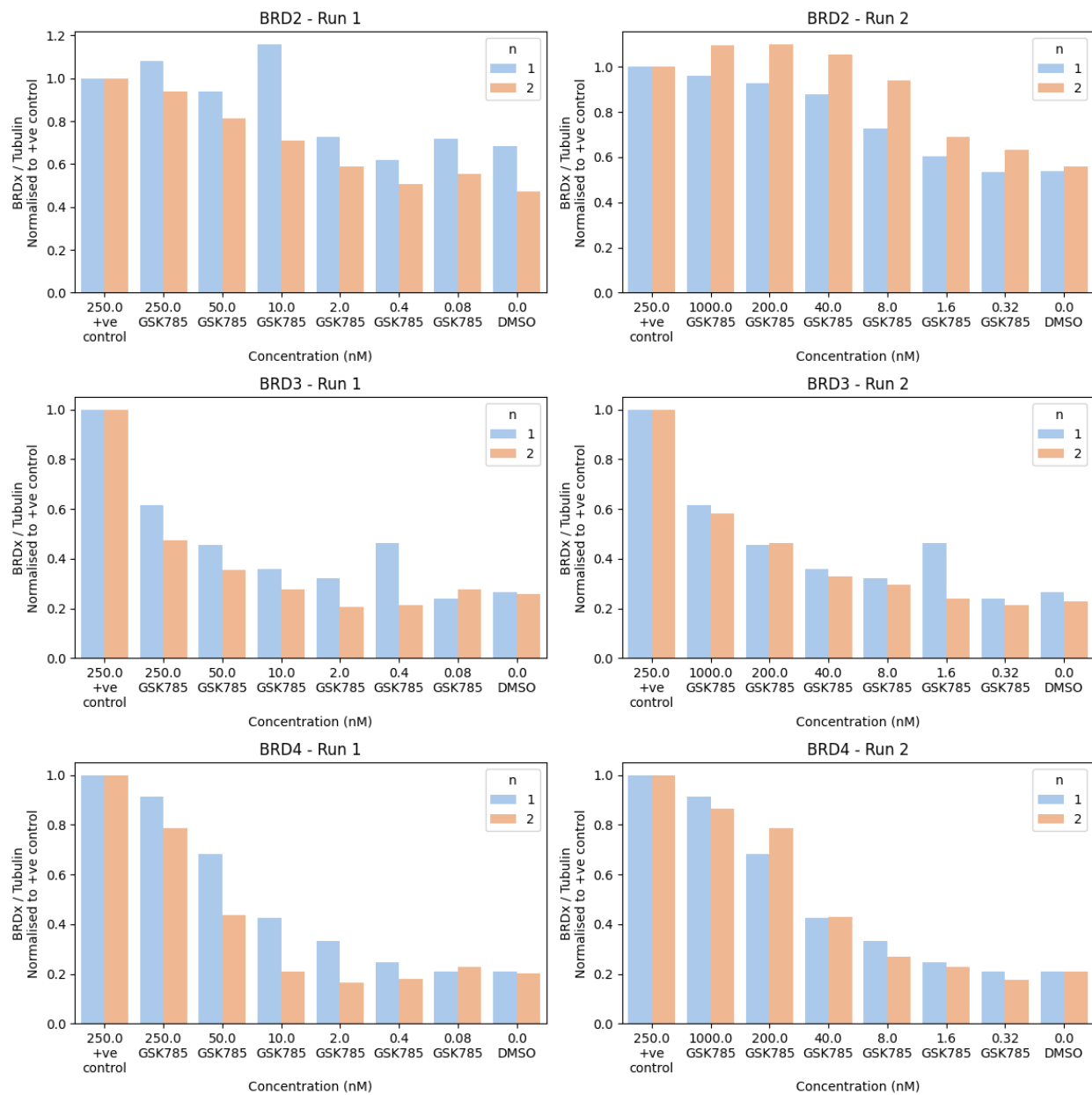


Figure S1. Processed data for the CETSA assay. Normalised BRDx / Tubulin peak areas at each tested concentration of compound **9h** (GSK785) are shown across each BET isoform, across each of the four test occasions.

Table S1. Comparison PPI and BRD4 BS data for diastereomeric mixtures of compounds compared to diastereomerically pure samples.

	Diastereomeric mixture			Diastereomerically pure (<i>R,R</i>)	
	(<i>R,R</i>):(<i>R,S</i>) <i>d.r</i>	BRD2, BRD3, BRD4 PPI [†]	BRD4 BS [†]	BRD2, BRD3, BRD4 PPI [†]	BRD4 BS [†]
9h	80:20	8.0 (1), 6.2* ¹ (1), 8.6 (1)	7.9 (1)	7.9 (9), 6.9 (2)* ⁴ , 8.5 (7)	7.9 (2)
10h	70:30	8.6 (2), 8.0 (2), 8.4 (2)	8.1 (2)	8.6 (2), 8.2 (1), 9.0 (1)	8.3(1)
10g	70:30	9.6 (2), 9.1 (3), 9.3 (1)	8.5 (2)	9.4 (3), 9.1 (4), 9.5 (2)	8.9 (3)
9k	80:20	9.1 (1), - [*] , 9.0 (1)	8.5 (1)	9.1 (2), 8.8 (2), 9.2 (2)	8.6 (2)
9m	80:20	9.4 (2), 8.8 (2), 9.2 (2)	8.7 (3)	9.2 (2), 8.8 (1), 9.4 (1)	8.3 (1)

[†]Average pIC₅₀ values are shown with the number of test occasions in the average shown in parenthesis. *^x Tested inactive x times. ^{*} Not obtained.

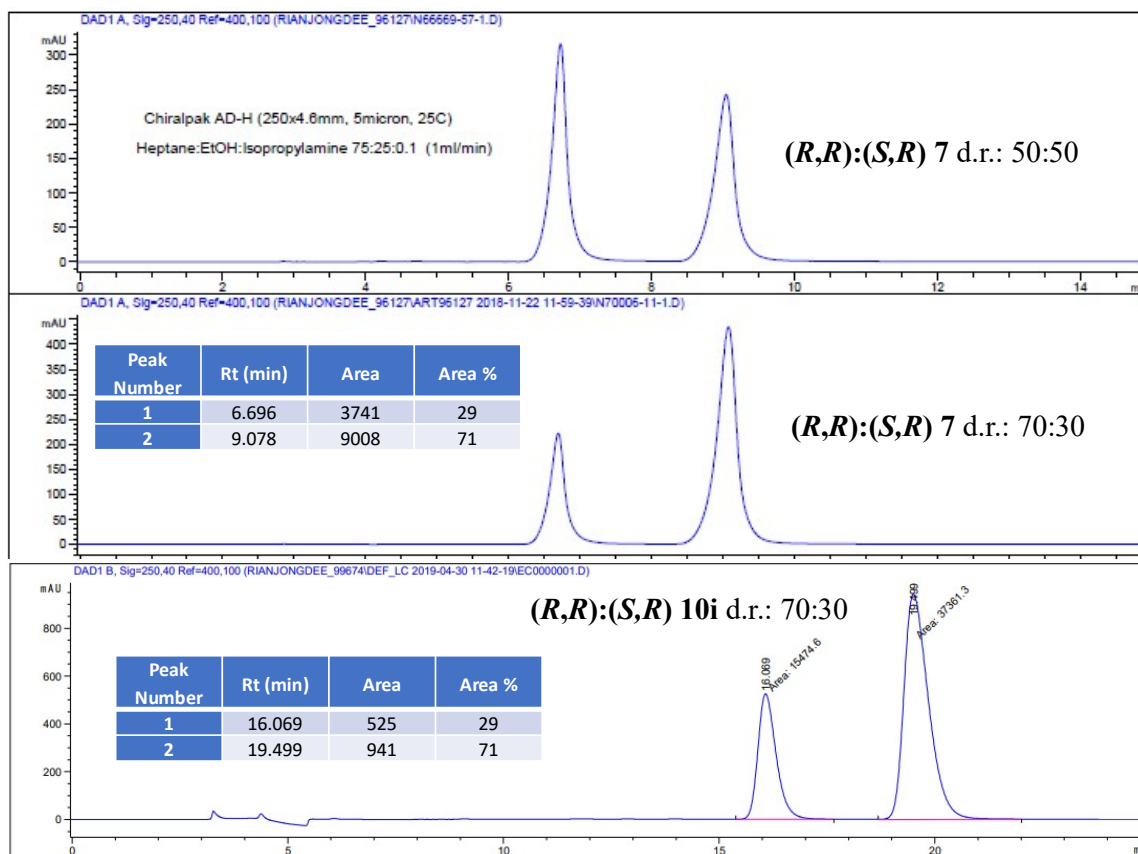


Figure S2. Chiral HPLC traces for the diastereomeric mixtures of **7** and **10i**

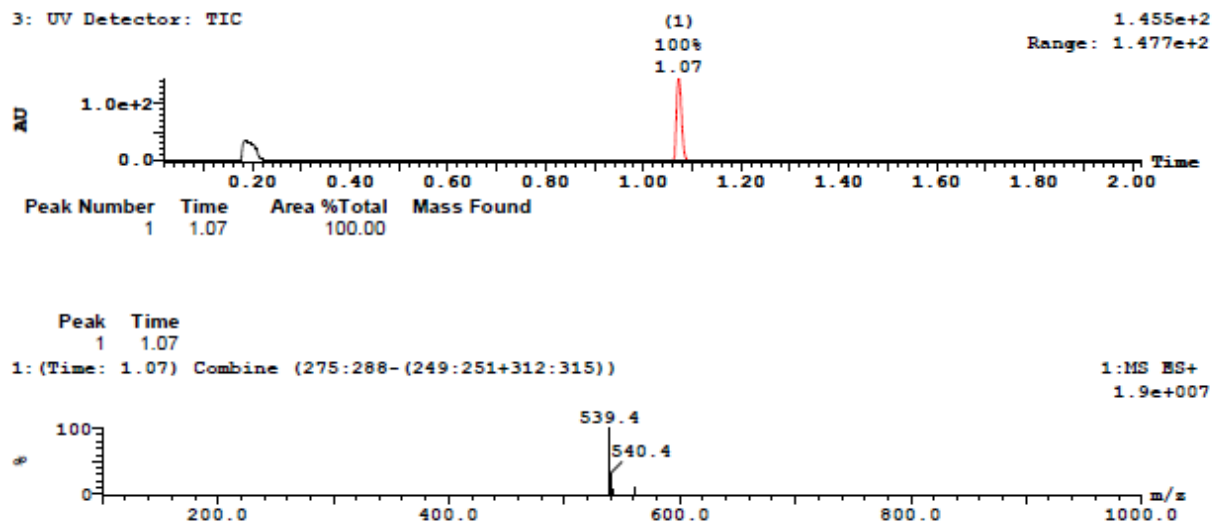


Figure S3. LCMS trace for 9h

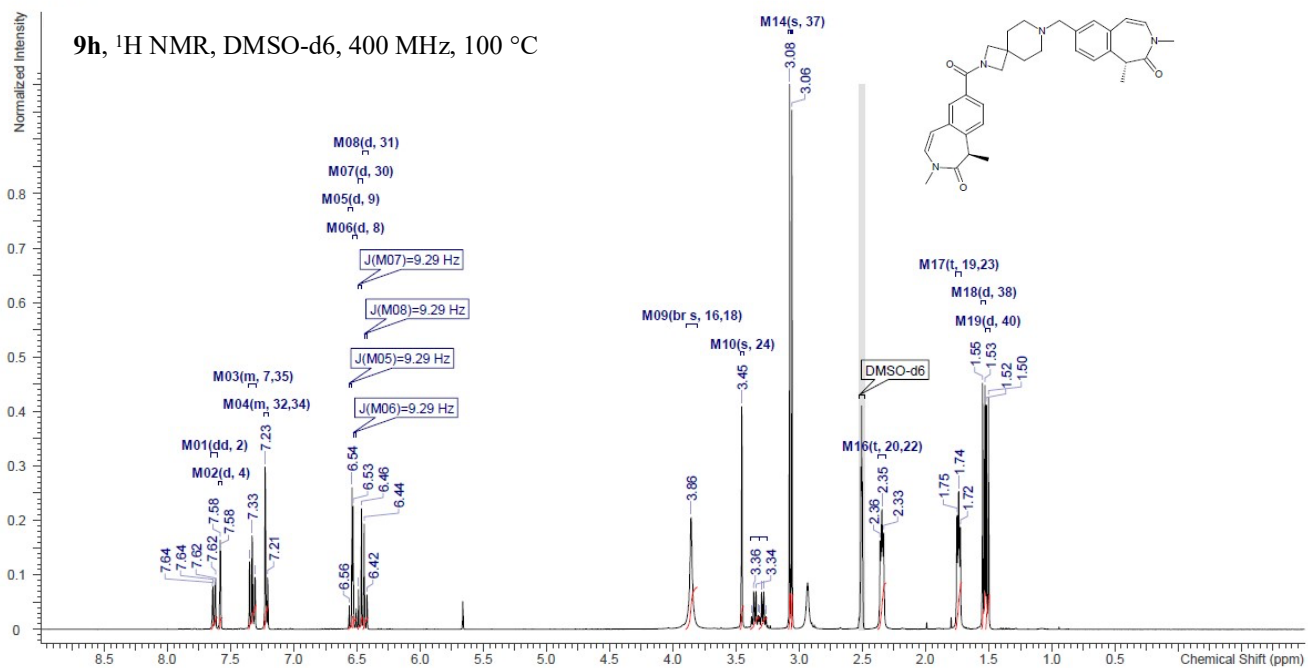


Figure S4. ^1H NMR spectrum of for 9h