Supplementary Information

Structural insights into probe-dependent positive allosterism of the GLP-1 receptor

Supplementary Table 1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Live cell assay using assay ready frozen cells.
	Target	Human GLP-1R (GenBank ID, NP_002053).
	Primary measurement	Cyclic 3'-5' adenosine monophosphate (cAMP) accumulation in the presence of IBMX.
	Key reagents	HEK293 cells stably expressing human GLP-1R, BETP[1], GLP-1(9-36)NH_2. (Bachem), cAMP HTRF assay (Cisbio), 384W Plates (Costar 3570).
	Assay protocol	1__5000 cells per well are plated in 15 µl
		2__100 nL compounds and controls are added
		3__5 µL GLP-1(9-36) (150 nM [final]) and IBMX (250 µM [final]) are added to all wells
		4__1-hour ambient incubation
		5__20 µL detection reagents added
		6__1-hour incubation, detect at 620 nm and 665 nm
		7__Interpolate, normalize data
Library	Library size	226,784
	Library composition	Synthetic small molecules biased for diversity and drug-likeness
	Source	Library comprised of molecules synthesized at Eli Lilly and Company and obtained from external vendors.
Screen	Format	384 well plate, cAMP detection with HTRF assay
	Concentration(s) tested	3 µM and 30 µM
	Plate controls	32 MIN controls (150 nM GLP-1(9-36)) and 32 MAX controls (150 nM GLP-1(9-36) + 150 nM BETP) in columns 1, 2, 23, 24.
	Reagent/ compound dispensing system	Compounds: Pintool with Mutimek (Beckman)
		Reagents and cells: Wellmate (Fisher)
	Detection instrument and software	Envision Plate Reader (Perkin Elmer)

	Assay validation/QC	3-day plate uniformity Z'>0.4 and test-retest MSR < 3.0 (according NIH Assay Guidance manual [2]).
	Correction factors	HTRF ratio was interpolated to [cAMP] using a logistic model [3].
	Normalization	% Stimulation using MAX and MIN controls [4]
Post-HTS analysis	Hit criteria	Statistically significant activity above controls (Median of MIN+3SD)
	Hit rate	0.45% at 3 µM (16% stimulation), 1.2% at 30 µM (23% stimulation).
	Additional assay(s)	Potency in concentration response format assay [5], GLP-1(9-36) shift assay [5], selectivity against ClassB GPCR subfamily members, GTPγS binding assay [6], non-reactivity with glutathione [5].
	Confirmation of hit purity and structure	HPLC-MS >95% purity and resynthesis.

1 Wootten, D. *et al.* Differential activation and modulation of the glucagon-like peptide-1 receptor by small molecule ligands. *Mol Pharmacol* **83**, 822-834, doi:10.1124/mol.112.084525 (2013).

2 Iversen, P. W. *et al.* in *Assay Guidance Manual* (eds G. S. Sittampalam *et al.*) (2004).

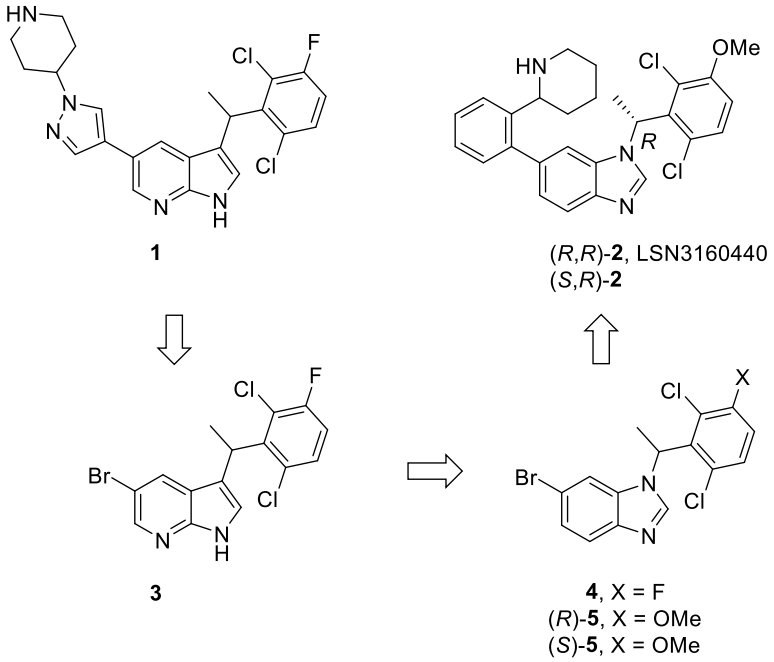
3 Cox, K. L. *et al.* in *Assay Guidance Manual* (eds G. S. Sittampalam *et al.*) (2004).

4 Campbell, R. M. *et al.* in *Assay Guidance Manual* (eds G. S. Sittampalam *et al.*) (2004).

5 Bueno, A. B. *et al.* Positive Allosteric Modulation of the Glucagon-like Peptide-1 Receptor by Diverse Electrophiles. *J Biol Chem* **291**, 10700-10715, doi:10.1074/jbc.M115.696039 (2016).

6 Willard, F. S. *et al.* Small molecule allosteric modulation of the glucagon-like Peptide-1 receptor enhances the insulinotropic effect of oxyntomodulin. *Mol Pharmacol* **82**, 1066-1073, doi:10.1124/mol.112.080432 (2012).

Supplementary Table 2. *In vitro* pharmacology of GLP-1(9-36) potentiators



Compound	Shift of GLP-1(9-36)			CRC of compound at EC_{20} of GLP-1(9-36)		MET binding
	[Compound] (μM)	GLP-1(9-36) log fold shift (SD)	E_{MAX} %, (SD)	EC_{50} nM, (SD)	E_{MAX} %, (SD)	Ki nM, (SD)
BETP	1	1.2 (0.4)	84 (10)	750 (300)	62 (5)	ND
1	10	1.9 (0.1)	59 (7)	>5000	99 (1)	20 (6)
3	10	2.1 (0.2)	98 (3)	1900 (700)	81 (9)	50 (90)
4	10	1.6 (0.2)	79 (12)	>5000	160 (1)	>50,000
(*R*)-**5**	10	2.6 (0.3)	89 (4)	900 (500)	88 (4)	>50,000
(*S*)-**5**	10	1.3 (0.1)	55 (5)	2700 (300)	17 (3)	>50,000
(*S*,*R*)-**2**	10	3.4 (0.2)	61 (5)	170 (30)	80 (10)	>50,000
(*R*,*R*)-**2**	1	3.2 (0.3)	93 (3)	24 (9)	98 (7)	>50,000

Shift Assay: Compound-induced shifts in the EC_{50} of GLP-1(9-36) were determined by measuring cAMP accumulation in GLP-1R-expressing HEK293 cells in the presence of fixed concentrations of test compound. A ratio of potencies was calculated (log fold shift) versus the EC_{50} of GLP-1(9-36) in the absence of compound. Assay: The EC_{50} of test compounds at a fixed EC_{20} concentration of GLP-1(9-36) was determined by measuring cAMP accumulation in GLP-1R-expressing HEK293 cells. Data for the shift and CRC

assays were normalized to the response of a maximally effective concentration of GLP-1(7-36). Compound efficacy values (E_{MAX}) are represented as arithmetic means, while potency values are represented as geometric means. Standard deviations (SD) are shown and n≥3 for all experiments. MET binding assay: Compound affinity for the kinase domain of MET was determined using an HTRF-binding assay. Potency values are geometric means, SD, n=3.

Supplementary Table 3. GPCR Profiling: LSN3160440 was profiled against 169 GPCRs in agonist mode at 10 μM using the β-arrestin path-hunter enzyme fragment complementation system at DiscoveRx (Eurofins, Fremont, CA)

GPCR ID	% Activity[1]	GPCR ID	% Activity[1]	GPCR ID	% Activity[1]
ADCYAP1R1	1%	DRD2L	0%	MC4R	8%
ADORA3	-3%	DRD2S	-4%	MC5R	-1%
ADRA1B	2%	DRD3	-8%	MCHR1	0%
ADRA2A	10%	DRD4	-12%	MCHR2	0%
ADRA2B	-3%	DRD5	3%	MLNR	7%
ADRA2C	3%	EBI2	-1%	MRGPRX1	5%
ADRB1	4%	EDG1	1%	MRGPRX2	16%
ADRB2	1%	EDG3	2%	MTNR1A	7%
AGTR1	-4%	EDG4	-3%	NMBR	-1%
AGTRL1	0%	EDG5	2%	NMU1R	12%
AVPR1A	2%	EDG6	2%	NPBWR1	2%
AVPR1B	1%	EDG7	0%	NPBWR2	2%
AVPR2	14%	EDG8	-6%	NPFFR1	-11%
BDKRB1	-1%	EDNRA	1%	NPSR1B	4%
BDKRB2	3%	EDNRB	2%	NPY1R	0%
BRS3	1%	F2R	-1%	NPY2R	4%
C3AR1	0%	F2RL1	3%	NTSR1	4%
C5AR1	-1%	F2RL3	-2%	OPRD1	-1%
C5L2	-12%	FFAR1	0%	OPRK1	1%
CALCR	0%	FPR1	11%	OPRL1	5%
CALCR-RAMP2	1%	FPRL1	1%	OPRM1	2%
CALCR-RAMP3	2%	FSHR	-5%	OXER1	-10%
CALCRL-RAMP1	-3%	GALR1	2%	OXTR	1%
CALCRL-RAMP2	-1%	GALR2	7%	P2RY1	3%
CALCRL-RAMP3	-5%	GCGR	-4%	P2RY11	0%
CCKAR	0%	GHSR1a	-7%	P2RY12	1%
CCKBR	-1%	GIPR	-3%	P2RY2	-1%
CCR10	-3%	GLP1R	0%	P2RY4	20%
CCR2	2%	GLP2R	1%	P2RY6	19%
CCR3	-18%	GPR1	-1%	PPYR1	6%
CCR4	1%	GPR103	-11%	PRLHR	4%
CCR5	2%	GPR109A	2%	PROKR1	0%
CCR6	1%	GPR109B	-5%	PROKR2	0%
CCR7	-3%	GPR119	-12%	PTAFR	-1%
CCR8	0%	GPR120	-5%	PTGER2	-1%
CCR9	4%	GPR35	6%	PTGER3	1%
CHRM1	-1%	GPR92	-3%	PTGER4	5%
CHRM2	0%	GRPR	0%	PTGFR	1%
CHRM3	-5%	HCRTR1	0%	PTGIR	-6%
CHRM4	-7%	HCRTR2	1%	PTHR1	1%
CHRM5	-4%	HRH1	-1%	PTHR2	0%
CMKLR1	-1%	HRH2	-2%	RXFP3	-1%
CNR1	1%	HRH3	-4%	SCTR	1%
CNR2	-13%	HRH4	-15%	SSTR1	1%
CRHR1	0%	HTR1A	11%	SSTR2	1%
CRHR2	0%	HTR1B	-7%	SSTR3	3%
CRTH2	-3%	HTR1E	3%	SSTR5	-2%
CX3CR1	0%	HTR1F	2%	TACR1	0%
CXCR1	1%	HTR2A	3%	TACR2	18%
CXCR2	2%	HTR2C	4%	TACR3	1%
CXCR3	5%	HTR5A	3%	TBXA2R	-1%
CXCR4	9%	KISS1R	-4%	TRHR	1%
CXCR5	27%	LHCGR	0%	TSHR(L)	-8%
CXCR6	-1%	LTB4R	1%	UTR2	-6%
CXCR7	42%	MC1R	3%	VIPR1	7%
DRD1	-3%	MC3R	-2%	VIPR2	2%

1. Data were normalized to percent stimulation with full agonist ligands

Supplementary Table 4. Orphan GPCR Profiling: LSN3160440 was profiled against 73 orphan GPCRs in agonist mode at 10 μM using the orphanMAX β-arrestin path-hunter enzyme fragment complementation system at DiscoveRx

GPCR ID	% Activity[1]	GPCR ID	% Activity[1]	GPCR ID	% Activity[1]
BAI1	14%	GPR161	-1%	GPR6	-18%
BAI2	5%	GPR162	-34%	GPR61	4%
BAI3	32%	GPR17	1%	GPR65	2%
CCRL2	36%	GPR171	70%	GPR75	-22%
DARC	8%	GPR173	-4%	GPR78	106%
GHSR1B	113%	GPR176	-4%	GPR79	101%
GPR101	95%	GPR18	41%	GPR83	15%
GPR107	-7%	GPR182	0%	GPR84	21%
GPR12	135%	GPR20	30%	GPR85	-26%
GPR123	-5%	GPR23	2%	GPR88	-6%
GPR132	42%	GPR25	93%	GPR91	81%
GPR135	10%	GPR26	103%	GPR97	7%
GPR137	-27%	GPR27	43%	LGR4	-24%
GPR139	-10%	GPR3	3%	LGR5	-3%
GPR141	1%	GPR30	13%	LGR6	-25%
GPR142	69%	GPR31	-13%	MRGPRD	108%
GPR143	2%	GPR32	27%	MRGPRE	-2%
GPR146	-7%	GPR37	-1%	MRGPRF	0%
GPR148	22%	GPR37L1	7%	MRGPRX4	0%
GPR149	1%	GPR39	10%	OPN5	17%
GPR15	-15%	GPR4	1%	OXGR1	46%
GPR150	28%	GPR45	2%	P2RY8	-2%
GPR151	64%	GPR50	5%	TAAR5	42%
GPR152	4%	GPR52	7%		
GPR157	-3%	GPR55	7%		

1. Data were normalized to % over basal

Supplementary Table 5. Kinase Profiling: In vitro biochemical protein kinase inhibitory activity of LSN3160440 was quantified using the TR-FRET assay format at Eurofins (Celle-Lévescault, France). 32 purified human protein kinases were tested

KINASE ID	IC50 (μM)
Abl	> 20
Akt1	> 20
Axl	> 20
Chk2	> 20
CK1	> 20
DRAK1	> 20
DRKY2	> 20
EPHB1	> 20
ERK2	> 20
FAK	> 20
FGFR3	> 20
FLT3	> 20
FMS	> 20
GSK3b	> 20
HIPK2	> 20
IKKa	> 20
IR	> 20
IRAK1	> 20
IRK	2
JAK2	> 20
JAK3	> 20
JNK3	> 20
KSR	> 20
KDR	> 20
MLK1	> 20
MNK2	> 20
p70S6K	> 20
PKCb2	> 20
SIK	> 20
SRC	> 20
STK33	> 20
TRKa	> 20

Supplementary Table 6. Cryo-EM data collection, refinement, and validation statistics

	GLP-1R/GLP-1(7-37)/(*R*,*R*)-**2**/G_s/Nb35 (EMDB-21147) (PDB 6VCB)
Data collection and processing	
Magnification	130,000
Voltage (kV)	300
Electron exposure (e–/Å2)	81.56
Defocus range (μm)	-1.0 to -2.0
Pixel size (Å)	1.04
Symmetry imposed	C1
Initial particle images (no.)	846,453
Final particle images (no.)	323,427
Map resolution (Å) FSC threshold	3.3 Å 0.143
Map resolution range (Å)	3.1 to 4.5

Refinement	
Initial model used (PDB code)	5VAI
Model resolution (Å) FSC threshold	3.6 Å 0.5
Map sharpening B factor (Å2)	-94.5
Model composition Non-hydrogen atoms Protein residues Ligands	 9074 9041 (1158 residues) 33 (1 polymer)
B factors (Å2) Protein Ligand	 83.0 82.6
R.m.s. deviations Bond lengths (Å) Bond angles (°)	 0.006 0.99
Validation MolProbity score Clashscore	 1.69 6.36

Poor rotamers (%)	0.85
Ramachandran plot	
Favored (%)	95.0
Allowed (%)	4.9
Disallowed (%)	0

Supplementary Note 1: Synthetic procedures

General procedures. All solvents and reagents were purchased from commercial sources and used as received, unless otherwise indicated. All solvents were ACS grade. Reactions were performed under air atmosphere unless otherwise indicated. ^{1}H NMR and ^{13}C NMR data for characterization of compounds were recorded on a Bruker AM-400 (400 MHz). NMR experiments were acquired at 25ºC in DMSO-d_6 referencing all the NMR spectra to the residual solvent signals at 2.50 and 39.5 ppm, for proton and carbon respectively. One-dimensional spectra were acquired using 16K data points and zero filled to 32K. Analytical HPLC was carried out on an Agilent 1260 liquid chromatography system equipped with a solvent degasser, quaternary pump, auto sampler, column compartment and a diode array detector (Agilent Technologies, Waldbronn, Germany). The UV wavelength was set at 214 nm. Electrospray mass spectrometry measurements were performed on a MSD quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) interfaced to the Agilent HPLC system. MS measurements were acquired simultaneously in both positive and negative ionization modes. Data acquisition and integration for LC-UV and MS detection were collected using Chemstation software (Agilent Technologies). Compounds **1** and **3** were synthesized as previously described [1]. Purity of compounds **4**, (*R*)-**5**, (*S*)-**5**, LSN3160440 and (*S*,*R*)-**2** was determined by two different analytical conditions: Method A: Column Gemini NX C18 (3 μm, 2 x 50 mm); UV:

[1] Chen, X. *et al.* Substituted pyrrolo[2,3-b]pyridines and pyrrolo[2,3-b]pyrazines as inhibitors of c-Met and RON tyrosine kinase receptor and their preparation, pharmaceutical compositions and use in the treatment of cancer. WO2010059771. (2010).

214 and 300 nm; MS ESI(+/-) 150-700. A: H_2O+0.1% formic acid; B: CH_3CN +0.1 % formic acid. Flow rate: 1.2 mL/min; column T 50ºC; gradient mode: from 5 to 95% B in 1.5 min; hold 0.5 min at 95% B. Method B: Column XBridge C18 (3.5 μm, 2.1 x 50 mm); UV: 214 and 300 nm; MS-ESI 100-800. A: 10mM ammonium bicarbonate pH 9.0; B: CH_3CN. Flow Rate: 1.2 mL/min; column T 50ºC; gradient mode: from 5 to 95% B in 1.5 min, hold 0.5 min at 95% B. All the final compounds had ≥95% purity by both methods. Optical rotations were measured with an Anton Paar MCP 500.

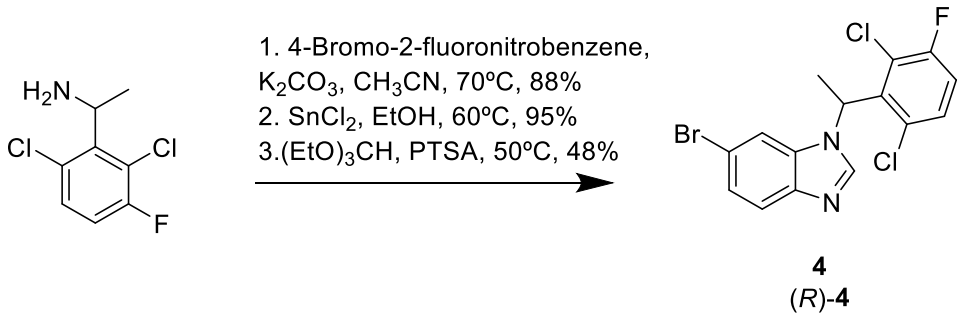
Abbreviations. Pd(dppf)Cl_2.DCM = (1,1'-bis(diphenylphosphino)ferrocene)palladium (II) dichloride dichloromethane complex, rt = room temperature, aq. = aqueous, EtOAc = ethyl acetate, DMF = dimethylformamide, THF = tetrahydrofuran, Boc = *tert*-butyloxycarbonyl, SCX = strong cation exchange, MeOH = methanol, DCM = dichloromethane, *p*-TsOH = *p*-toluenesulfonic acid, MTBE = methyl *tert*-butylether, SFC = supercritical fluid chromatography, DMSO = dimethylsulfoxide, UV = ultraviolet, DMF = dimethylformamide, VCD = vibrational circular dichroism.

Experimental Procedures and Characterization data

General method A for boronate formation and coupling with tert-butyl 2-(2-bromophenyl)piperidine-1-carboxylate. A 500 mL round bottom flask was charged with 6-bromobenzimidazole derivative (24.9 mmol), bis(pinacolato)diboron (12.9 g, 49.9 mmol), KOAc (4.9 g, 50.0 mmol), Pd(dppf)Cl_2.DCM (2.1 g, 2.5 mmol) and anhydrous 1,4-dioxane (250 mL). The mixture was purged with nitrogen for 5 minutes and heated at 90°C in a preheated metal block for 16 h. The reaction was cooled to rt and concentrated under

reduced pressure. EtOAc (200 mL) was added and the mixture was filtered through a pad of Celite®. The solid was washed with EtOAc (2x50 mL). The combined organic layers were washed with water (300 mL) and brine (200 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel eluting with hexane/EtOAc, from 20:80 to 0:100 to afford the corresponding dioxaborolanylbenzimidazole. This compound (2.2 mmol) and (±)-*tert*-butyl 2-(2-bromophenyl)piperidine-1-carboxylate (1.4 g, 4.1 mmol) were combined in DMF (6.7 mL). The mixture was purged with nitrogen for 10 minutes and Pd(dppf)Cl_2. DCM (0.13 g, 0.17 mmol) and 2M aq. Na_2CO_3 in water (5 mL) were added. The reaction mixture was stirred at 70ºC in a heating block for 20 h and then allowed to reach rt. It was filtered over a pad of Celite®, rinsing the solids with EtOAc and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc from 90:10 to 20:80 to afford a *N*-Boc-protected 1:1 mixture of stereomers at the newly-introduced chiral center.

General method B for N-Boc deprotection. N-Boc protected amine (0.17 mmol) was dissolved in THF (1.7 mL) and hydrogen chloride (4N in 1,4-dioxane) (0.86 mL, 3.44 mmol) was added dropwise. The mixture was stirred at rt for 16 hours and concentrated. The crude material was purified using a SCX cartridge, eluting with MeOH and 2N NH_3 in MeOH. Basic fractions were combined to afford the corresponding amine as a free base.

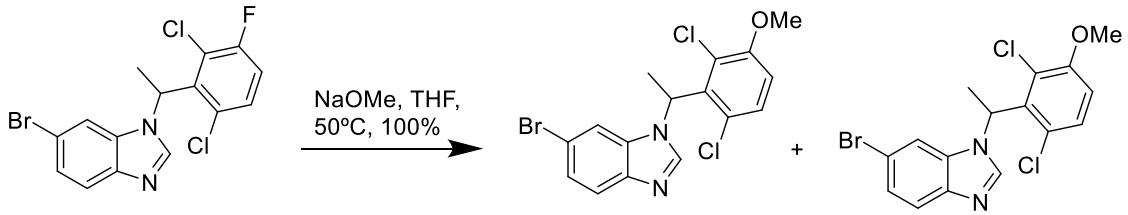


4
(*R*)-**4**

(±)-*6-Bromo-1-(1-(2,6-dichloro-3-fluorophenyl)ethyl)-1H-benzo[d]imidazole* (**4**). 4-Bromo-2-fluoro-1-nitrobenzene (37.6 g, 170.9 mmol) and K_2CO_3 (47.2 g, 341.8 mmol) were combined in CH_3CN (340 mL). 2,6-Dichloro-3-fluorophenylethanamine (35.6 g, 170.9 mmol) was added. The mixture was stirred in a 70 °C heating block under nitrogen for 8 h, then cooled to rt and poured into stirred ice-water (1 L). After 10 min the solid was collected by filtration, washed with 1:3 CH_3CN:water (300 mL) and water (300 mL, then dried in a vacuum desiccator to afford 63 g (88%) of (±)-5-bromo-*N*-1-(2,6-dichloro-3-fluoro-phenyl)ethyl-2-nitro-aniline as a yellow solid. This compound (4 g, 7.8 mmol) was taken up in ethanol (28 mL) with stirring. $SnCl_2$ (5.6 g, 29.4 mmol) was added and the resulting suspension was heated in a 60 °C heating block. The mixture cleared over 18 hours to an orange then brown solution. After 21 h, the mixture was cooled to rt, Celite® (18 g) was added, followed by saturated aq. $NaHCO_3$ (22 mL). Further solid $NaHCO_3$ (14 g) was added in portions to control gas evolution. 2 M aq. NaOH (13 mL) and DCM (88 mL) were added and the mixture stirred for 0.5 h. The upper layer was decanted, filtering through a paper filter, and the thick lower layer extracted with further DCM (2 x 60 mL). The organics were combined and concentrated, and the residue taken up in EtOAc and filtered through a short pad of silica, eluting with EtOAc. The eluate was concentrated to afford the crude reduction product (3.9 g, 95% yield) as a dark oil. The oil (1.4 g, 3.3

mmol) was taken up in trimethyl orthoformate (4.2 mL, 38.2 mmol) and *p*-TsOH.H_2O (64 mg, 0.3 mmol) was added. After stirring at 50ºC for 3 days, it was allowed to cool to rt, 3 volumes of MTBE were added and the solid was filtered. The mother liquor was concentrated, 50 mL of MTBE were added and the solid was filtered and combined with the first crop to provide 0.72 g of **4** (48%). MS(ESI) m/z: 389 $[M+H]^+$. For other spectroscopic data see (*R*)-**4**.

6-Bromo-1-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethyl]-1H-benzo[d]imidazole [(*R*)-**4**]. (*R*)-**4** was made essentially as **4**, starting from (*R*)-2,6-dichloro-3-fluorophenylethanamine (54% overall yield, 98% *ee*). Chiral reverse-phase HPLC method to determine *ee*: CHIRALPAK IA-3R column (3 μm, 4.6 x 50 mm), solvent A: NH_4HCO_3 10mM pH 9/solvent B: CH_3CN, gradient from 50 to 99 % B in 3.2 min, with a flow rate of 1.2 mL/min at 40ºC showed a retention time of 2.76 min for (*R*)-**4** and 3.14 min for (*S*)-**4**. (*R*)-**4**: MS(ESI) m/z: 389 $[M+H]^+$; $[\alpha]^{D}_{20}$ -108º (c=0.5, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 8.68 (s, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.63-7.60 (m, 1H), 7.53 (t, J = 8.8, 8.8 Hz, 1H), 7.33 (dd, J = 8.4, 2.0 Hz, 1H), 6.96 (d, J = 2.0 Hz, 1H), 6.30 (q, J = 7.2 Hz, 1H), 3.86 (s, 3H), 2.11 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 157.3 (d, $^1J_{CF}$ = 247 Hz), 145.0, 143.2, 136.1, 135.3, 131.4 (d, $^3J_{CF}$ = 8.1 Hz), 129.2 (d, $^3J_{CF}$ = 3.3 Hz), 125.3, 122.1, 121.6 (d, $^2J_{CF}$ = 19.4 Hz), 118.2 (d, $^2J_{CF}$ = 22.9 Hz), 115.3, 112.9, 52.8 (d, $^4J_{CF}$ = 1.5 Hz), 16.9.



4
(*R*)-**4**

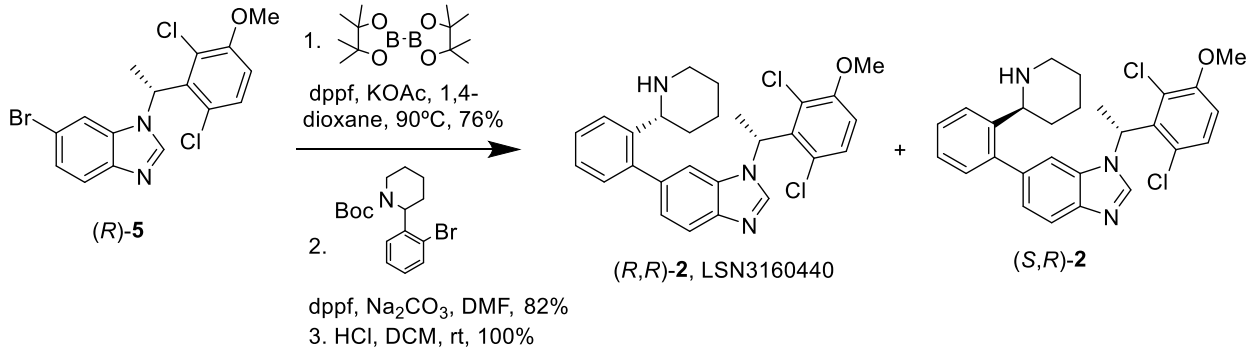
(*R*)-**5**
(*R*)-**5**

(*S*)-**5**

(±)-*6-Bromo-1-[1-(2,6-dichloro-3-methoxy-phenyl)ethyl]benzimidazole* (**5**). Compound **4** (51.0 g, 131.4 mol) was taken up in THF (100 mL) at 50 °C. A solution of 30% NaOMe in MeOH (500 mL, 2.67 mol) was added slowly, and the mixture stirred at 50 °C for 16 h. Water (600 mL) was added, and the resulting suspension left to cool with stirring. After 3 h, the solid was collected by filtration, washed with water and dried in a vacuum oven (40 °C, 10 mbar) to afford **5** as a grey solid (53.5 g, 100%). SFC chiral separation of 96 mg of **5** on a CHIRALPAK IC column (5 μm, 2x25 cm), mobile phase 35% (MeOH-0.2% dimethylethylamine) in CO_2, with a flow rate of 80 mL/min and using UV detection (220 nm) afforded (*R*)-**5** (0.86 min, 36 mg, 37%) and (*S*)-**5** (1.31 min, 34 mg, 34%). (*R*)-**5**: MS(ESI) m/z: 401 $[M+H]^+$; $[\alpha]_D^{20}$ -100º (c=0.5, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 8.65 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.49 (br d, *J* = 8.8 Hz, 1H), 7.31 (dd, *J* = 8.8, 1.6 Hz, 1H), 7.20 (d, *J* = 9.2 Hz, 1H), 6.97 (d, *J* = 1.6 Hz, 1H), 6.29 (q, *J* = 7.0 Hz, 1H), 3.86 (s, 3H), 2.09 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 154.9, 145.1, 143.2, 135.4, 135.0, 130.5, 125.2, 124.6, 122.5, 122.0, 115.1, 113.9, 113.0, 57.1, 53.0, 16.9. (*S*)-**5**: $[\alpha]_D^{20}$ +113° (c=0.24, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 8.65 (s, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.49 (br d, *J* = 8.8 Hz, 1H), 7.31 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.20 (d, *J* = 9.0 Hz, 1H), 6.97 (d, 1.9 Hz, 1H), 6.29 (q, *J* = 7.2 Hz, 1H), 3.86 (s, 3H), 2.09 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 154.9, 145.1, 143.2, 135.4, 135.0, 130.5, 125.2, 124.6, 122.5, 122.0, 115.1, 113.9, 113.0, 57.1, 53.0, 16.9.

6-Bromo-1-[(1R)-1-(2,6-dichloro-3-methoxy-phenyl)ethyl]benzimidazole [(*R*)-**5**)]. (*R*)-**5** was made essentially as **5**, starting from (*R*)-**4** (100%, 98% *ee*). Chiral SFC method for *ee* determination: CHIRALPAK IC column (5 μm, 4.6 x 100 mm), mobile phase 35% MeOH (0.2% isopropylamine), with a flow rate of 5 mL/min, at 100 bar of pressure and

40°C; retention time 0.87 min for (*R*)-**5**, 1.34 min for (*S*)-**5**. For spectral data of (*R*)-**5**, see section above.



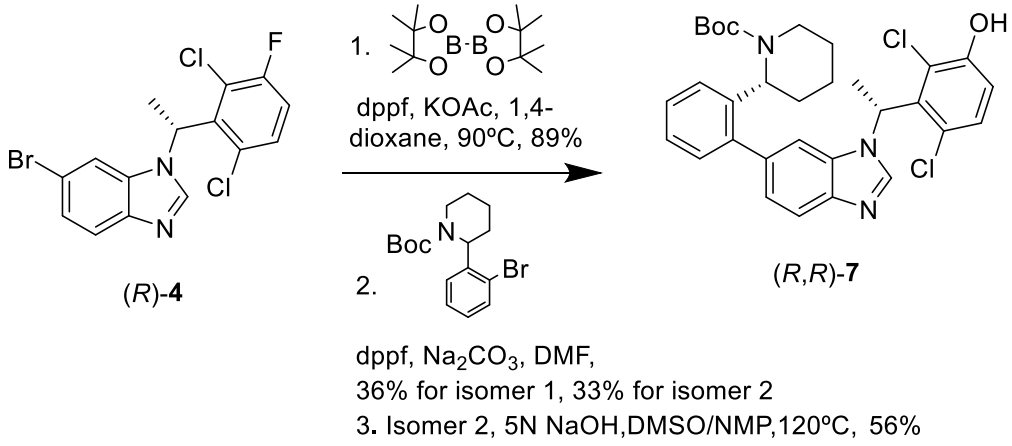
1-[(1R)-1-(2,6-Dichloro-3-methoxy-phenyl)ethyl]-6-[2-(RS)-(2-piperidyl)phenyl]benzimidazole [(*R*,*R*)-**2,** (*S*,*R*)-**2**]. 6-Bromo-1-[(1*R*)-1-(2,6-dichloro-3-methoxy-phenyl)ethyl] benzimidazole [(*R*)-**5**] was reacted using method A to afford a Boc-protected 1:1 mixture of (*R*,*R*)-**2** and (*S*,*R*)-**2** (59%) as a beige solid. MS(ESI) m/z: 580 $[M+H]^+$. Chiral separation in a CHIRALPAK IC column (5 μm, 100 x 4.6 mm); mobile phase: CO_2 (A)/MeOH (0.2% isopropylamine) (B), with isocratic elution (35% B) at 150 bar of pressure and 40ºC; flow rate: 5 mL/min; detection UV at 220 nm; afforded isomer 1 (1.52 min, 359 mg; 37%) and isomer 2 (1.73 min, 472 mg; 49%). Isomer 1 was deprotected using general method B to afford (*R*,*R*)-**2** (LSN3160440) (79.6 mg, 95%) as a white solid. MS(ESI) m/z: 480 $[M+H]^+$; $[\alpha]_D^{20}$ -89° (c=0.5, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 8.63 (s, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.59 (dd, J = 7.8, 1.0 Hz, 1H), 7.44-7.39 (m, 1H), 7.32 (td, J = 7.5, 1.2 Hz, 1H), 7.21 (td, J = 7.4, 1.3 Hz, 1H), 7.15 (d, J = 9.2 Hz, 1H), 7.09 (dd, J = 8.3, 1.7 Hz, 1H), 6.99 (d, J = 7.5 Hz, 1H), 6.76 (s, 1H), 6.31 (q, J = 7.1 Hz, 1H), 3.8 (s, 3H), 3.32 (m, 1H), 2.90 (d, J = 11.6 Hz, 1H), 2.34-2.24 (m, 1H), 2.10 (d, J = 7.2 Hz, 3H), 1.55-1.48 (m, 1H), 1.40-1.28 (m, 4H), 0.85-0.82 (m, 1H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 154.7, 144.4, 143.4, 143.1, 141.3, 135.8, 135.3,

134.0, 130.3, 130.0, 127.8, 127.2, 126.6, 124.5, 123.6, 122.4, 119.5, 113.6, 110.7, 57.7, 57.0, 52.8, 47.3, 34.6, 25.7, 25.4, 17.1.

The absolute configuration of LSN3160440, was determined at BioTools using VCD.

Isomer 2 was deprotected using general method B to afford (*S*,*R*)-**2** (62.7 mg, 95%) as a white solid. MS(ESI) m/z: 480 $[M+H]^+$; $[\alpha]_D^{20}$ -42° (c=0.5, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 8.63 (s, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.61 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.46-7.42 (m, 1H), 7.31 (td, *J* = 7.5, 1.2 Hz, 1H), 7.23-7.12 (m, 3H), 6.96 (d, *J* = 7.3 Hz, 1H), 6.77 (s, 1H), 6.32 (q, *J* = 7.1 Hz, 1H), 3.82 (s, 3H), 3.51-3.43 (m, 1H), 2.89 (d, *J* = 11.4 Hz, 1H), 2.30-2.19 (m, 1H), 2.11 (d, *J* = 7.2 Hz, 3H), 1.71-1.59 (m, 1H), 1.56-1.41 (m, 4H), 1.16-1.13 (m, 1H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 154.7, 144.5, 143.5, 143.1, 141.0, 135.7, 135.3, 134.0, 130.3 (2C), 127.7, 127.4, 126.7, 124.5, 124.1, 122.4, 119.6, 113.7, 110.4, 57.5, 57.0, 52.8, 47.4, 35.1, 25.9, 25.5, 17.1.

The absolute configuration of (*S*,*R*)-**2** was determined at BioTools using VCD.

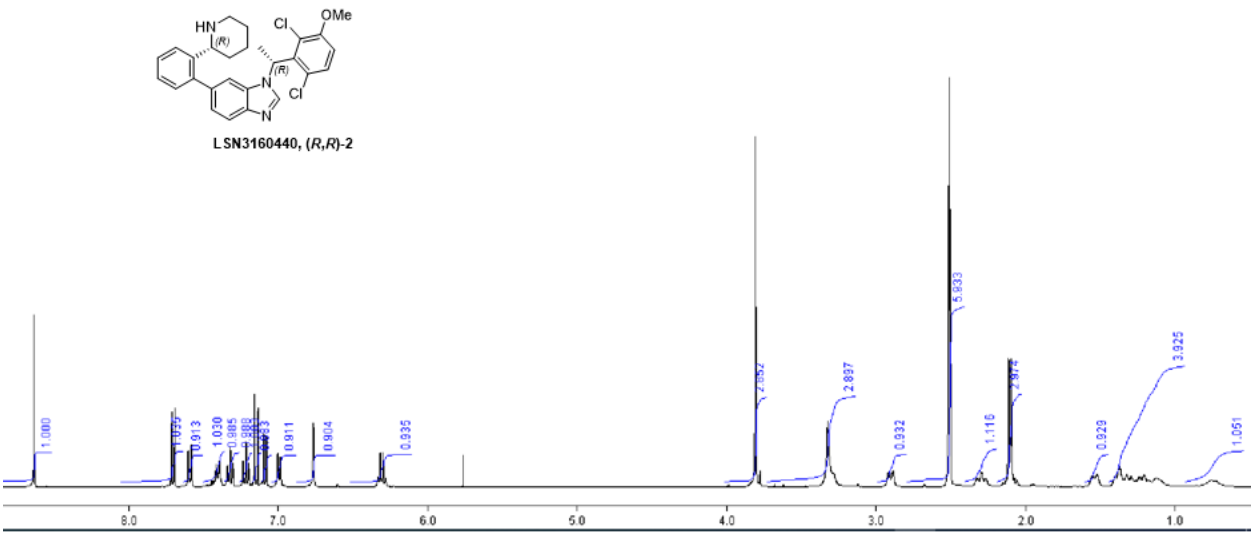


tert-Butyl 2-[2-[3-[(1R)-1-(2,6-dichloro-3-hydroxy-phenyl)ethyl]benzimidazol-5-yl]phenyl] piperidine-1-carboxylate [(*R*,*R*)-**6**], precursor of [^{3}H]LSN3160440. Bromide (*R*)-**4** was reacted using method A to afford a 1:1 mixture of N-Boc protected piperidines (46%) as a beige solid. Chiral separation in a Chiralpak AD column (5 μm, 100 x 4.6 mm);

mobile phase: CO_2 (A)/isopropanol (0.2% dimethylethylamine) (B), with isocratic elution (25% B) at 100 bar of pressure and 40ºC; flow rate: 2.5 mL/min; detection UV at 220 nm; afforded isomer 1 (1.84 min, 579 mg; 33%) and isomer 2 (3.14 min, 643 mg; 36%). MS(ESI) m/z: 568 $[M+H]^+$. A solution 5M aq NaOH (0.116 mL, 0.58 mmoles) was added to a solution of isomer 2 (110 mg, 0.19 mmoles) in a mixture of DMSO (0.4 mL) and NMP (0.2 mL) in a resealable tube. The tube was capped and the mixture was stirred at 120ºC in a preheated bath for 6h. Reaction was allowed to reach rt and a 5% aq citric acid was added to pH 5-6. The aqueous layer was extracted with EtOAc (2x). The organic layers were combined, washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by chromatography in silica gel eluting with DCM/EtOAc from 100:0 to 80:20 to 60:40 to obtain the corresponding phenol as a mixture of two diastereoisomers at the 1-position of the benzimidazole (82 mg, 75%). Chiral separation in a CHIRALPAK IC column (5 μm,100 x 4.6 mm); mobile phase: CO_2 (A)/MeOH (0.2% dimethylethylamine) (B), with isocratic elution (30% B) at 150 bar of pressure and 40ºC; flow rate: 5 mL/min; detection UV at 220 nm; afforded (*R*,*R*)-**6** (0.94 min, 34 mg; 31%) and an epimer formed during the reaction by isomerization of one chiral center (1.35 min, 27 mg; 25%). (*R*,*R*)-**6**: MS(ESI) m/z: 566 $[M+H]^+$; $[\alpha]_D^{20}$ -138º (c=0.5, MeOH); ^{1}H NMR (400.21 MHz, DMSO-d_6) δ: 10.55 (s, 1H), 8.61 (s, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.30 (td, J = 7.6, 1.4 Hz, 1H), 7.24-7.15 (m, 3H), 7.09 (dd, J = 8.2, 1.6 Hz, 1H), 7.02 (dd, J = 7.4, 1.4 Hz, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.73 (s, 1H), 6.26 (q, J = 7.1 Hz, 1H), 4.90 (t, J = 5.1 Hz, 1H), 3.84 (m, 1H), 3.20 (td, J = 12.8, 4.5 Hz, 1H), 2.09 (d, J = 7.2 Hz, 3H), 1.61-1.54 (m, 1H), 1.34-1.25 (m, 10H), 1.09-0.91 (m, 4H); ^{13}C NMR (100.63 MHz, DMSO-d_6) δ: 154.6, 153.1, 143.8, 142.5, 141.7, 140.6, 135.4, 134.7, 133.5, 130.4,

129.4, 127.1, 126.0, 125.0, 122.6, 122.3, 120.6, 119.0, 116.5, 110.4, 78.6, 52.4, 52.2, 40.9, 28.8, 28.0, 23.5, 17.6, 16.5.

1-[(1R)-1-(2,6-Dichloro-3-methoxy-phenyl)ethyl]-6-[2-(2-piperidyl)phenyl]benzimidazole ([^{3}H]LSN3160440). Radiolabeling of compound (*R*,*R*)-**6** was performed at Pharmaron. The proprietary methodology using [^{3}H]methyl nosylate generated [^{3}H]LSN3160440 with specific activity of 84 Ci/mmol and 99.8% radiochemical purity.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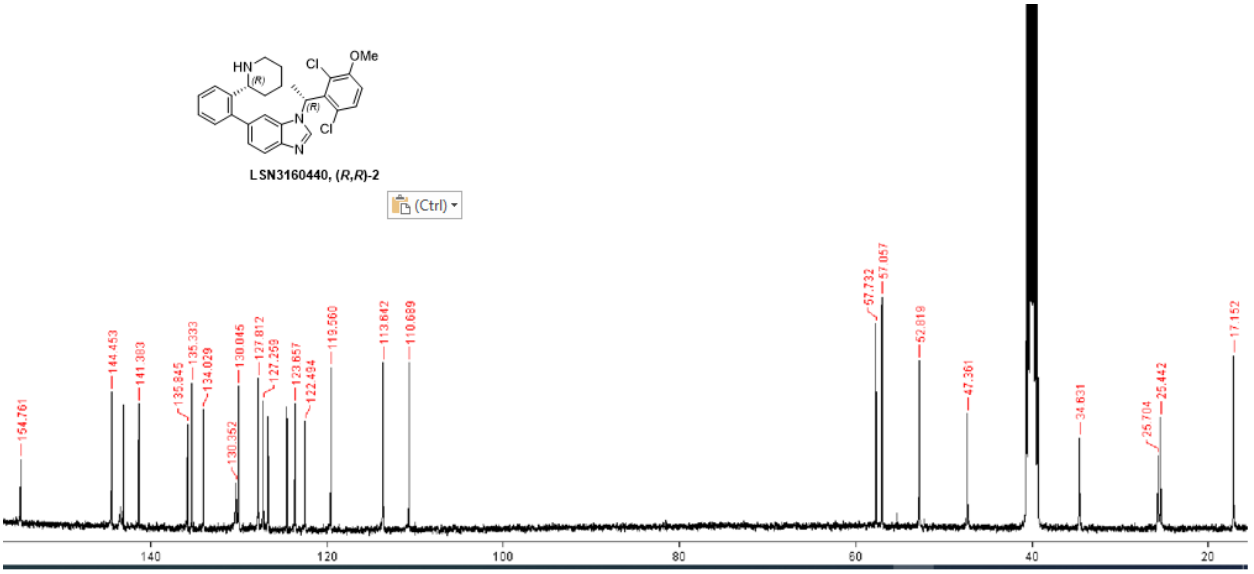
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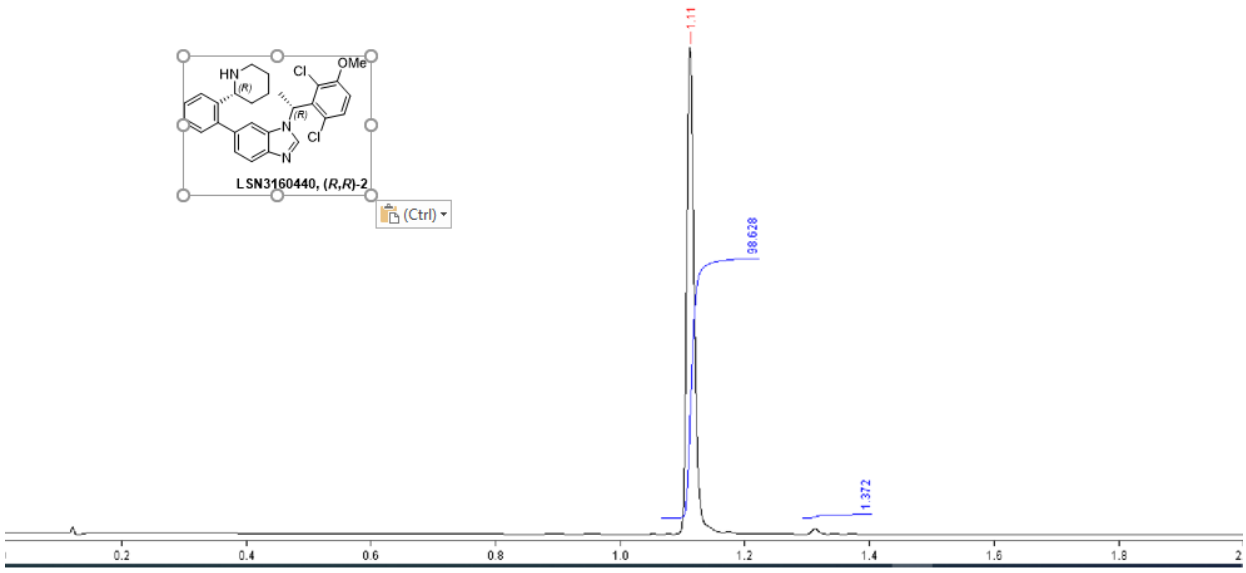
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