

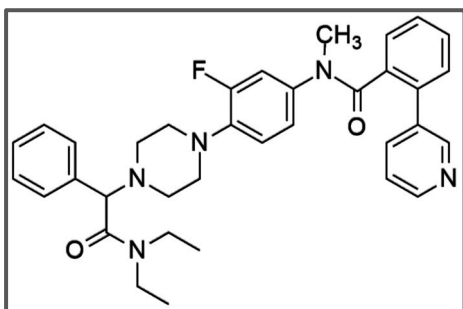
## Supporting information

### Improved chemical and radiochemical synthesis of Neuropeptide Y Y<sub>2</sub> receptor antagonist *N*-Methyl-JNJ-31020028 and preclinical PET studies

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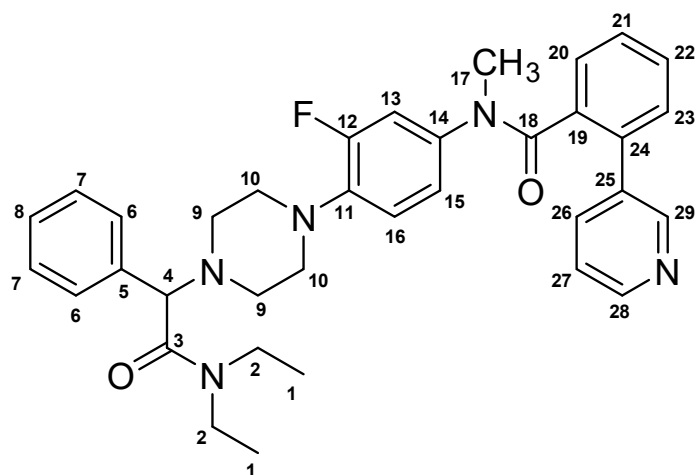
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## Characterisation of *N*-Me-JNJ-31020028



$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ /ppm: 8.51 (d,  $J = 4.0$  Hz, 1H), 8.15 (d,  $J = 1.4$  Hz, 1H), 7.5 (d,  $J = 7.2$  Hz, 1H), 7.41 (d,  $J = 7.8$  Hz, 1H), 7.31-7.23 (m, 5H), 7.21-7.18 (m, 1H), 7.02 (d,  $J = 7.2$  Hz, 1H), 6.38 (t,  $J = 9.0$  Hz, 1H), 5.84 (dd,  $J = 8.5, 2.4$  Hz, 1H), 5.80 (dd,  $J = 13.3, 2.4$  Hz, 1H), 4.15 (s, 1H), 3.39-3.30 (m, 2H), 3.13 (s, 3H), 3.23-3.08 (m, 2H), 2.92 (brs, 4H) 2.61-2.52 (m, 4H), 1.00 (t,  $J = 7.1$  Hz, 3H), 0.96 (t,  $J = 7.1$  Hz, 3H).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$ /ppm: 170.6, 169.8, 154.3 (d,  $J = 248.1$  Hz), 149.5, 148.2, 138.5 (d,  $J = 8.5$  Hz), 136.7 (d,  $J = 9.5$  Hz), 136.2, 135.8, 135.8, 135.0, 129.9, 129.5, 129.3, 129.2, 128.7, 128.7, 128.3, 123.0, 122.0 (d,  $J = 2.9$  Hz), 117.9 (d,  $J = 3.4$  Hz), 113.9 (d,  $J = 28.2$  Hz), 70.9, 51.2, 50.5, 41.6, 40.8, 37.1, 12.9, 12.4. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{35}\text{H}_{39}\text{FN}_5\text{O}_2^+$   $[\text{M}+\text{H}]^+$ : 580.3082, found: 580.3082.

### ■ $^1\text{H-NMR}$



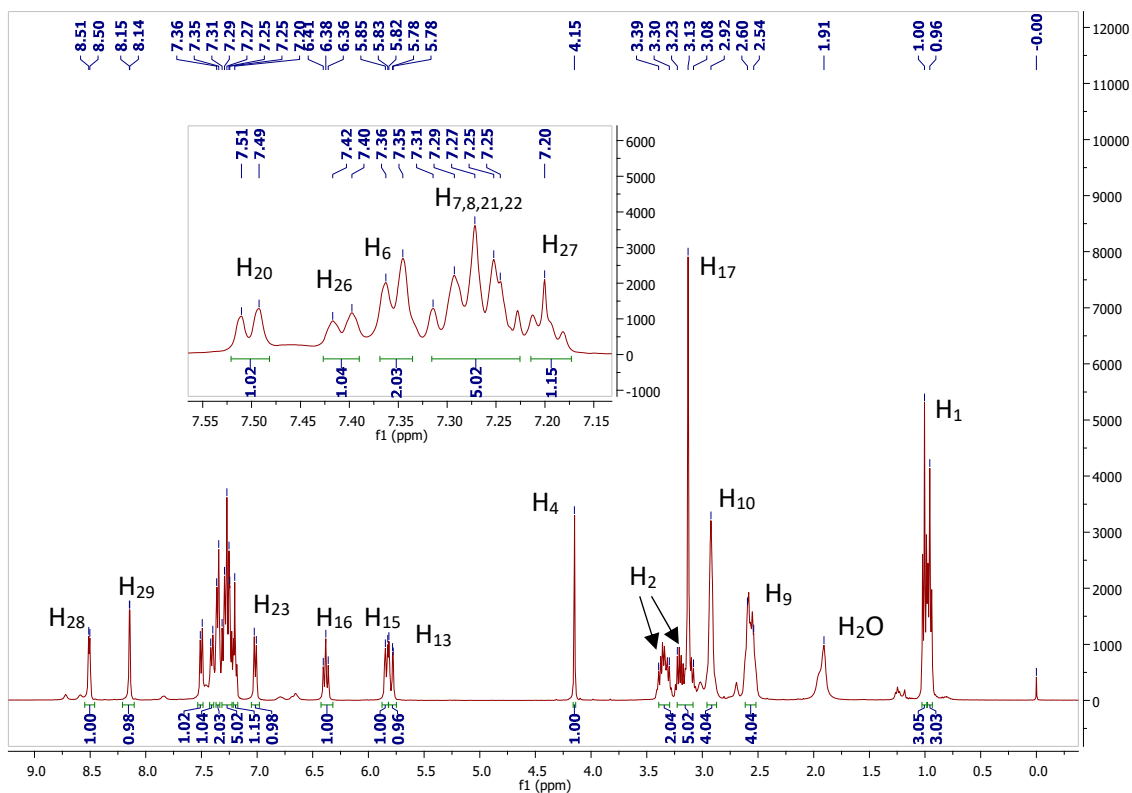


Figure S1-  $^1\text{H}$  NMR spectra of N-Me-JNJ-31020028 ang signal attribution.

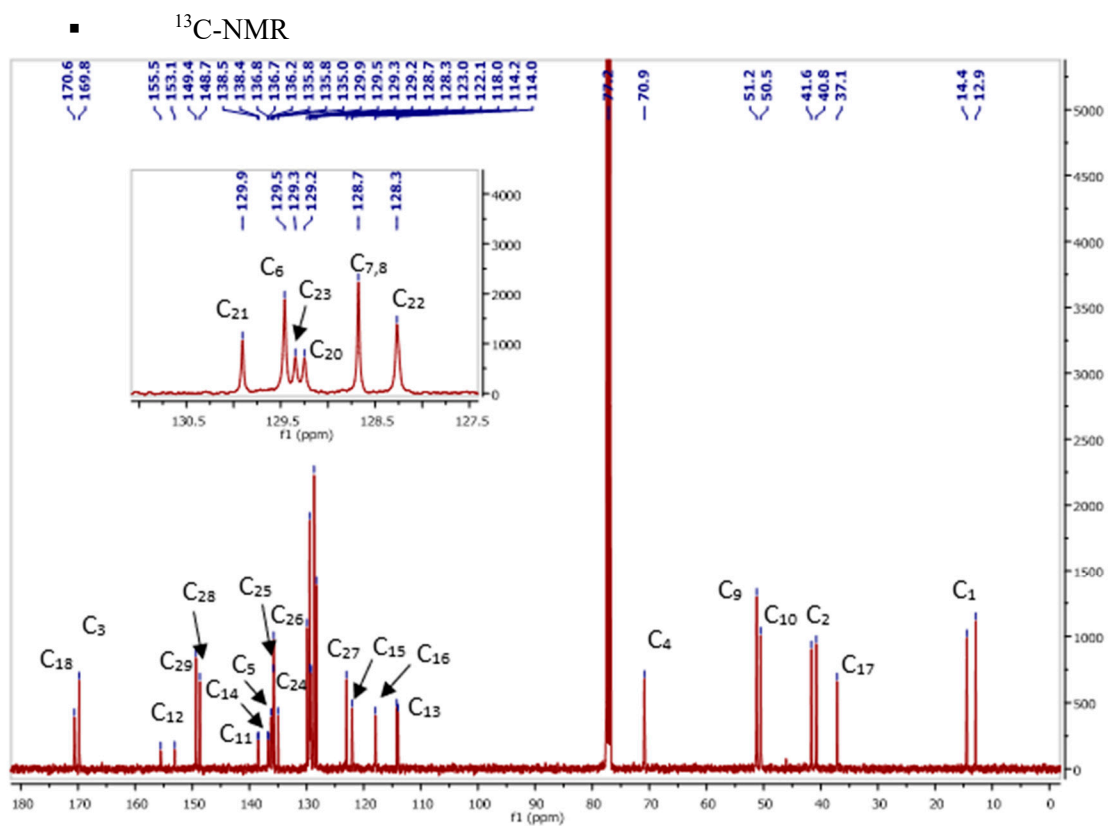
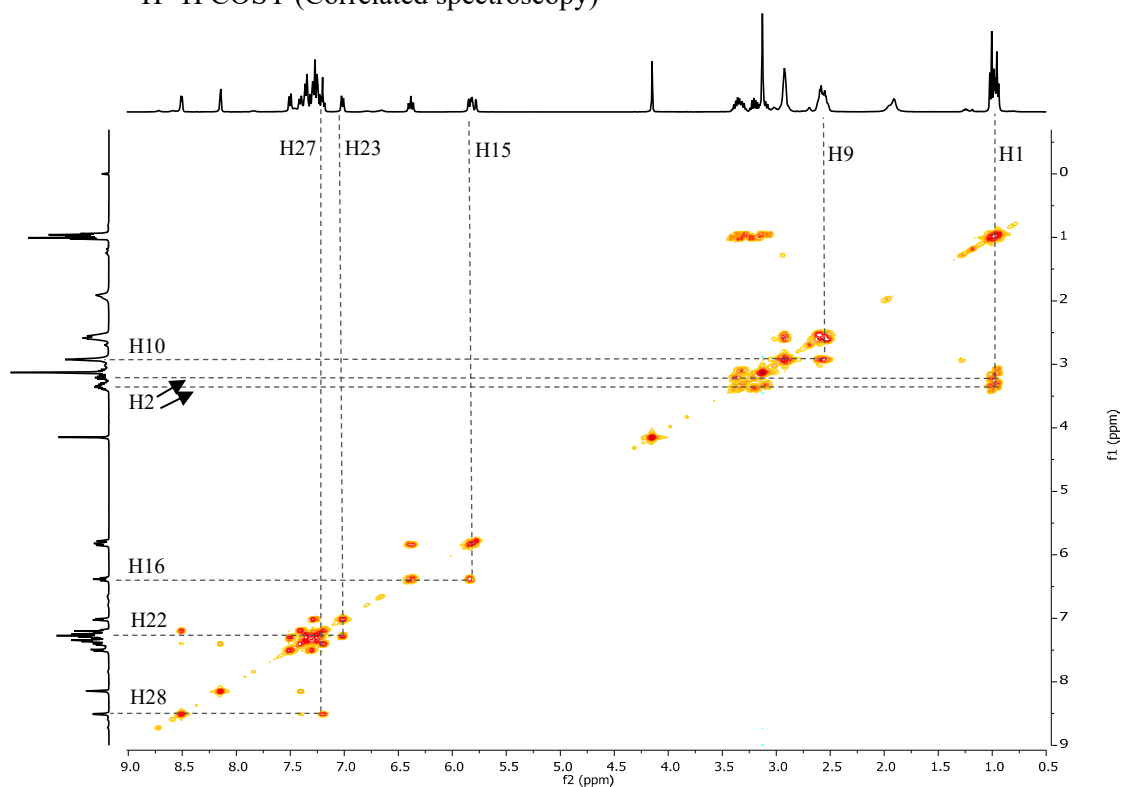


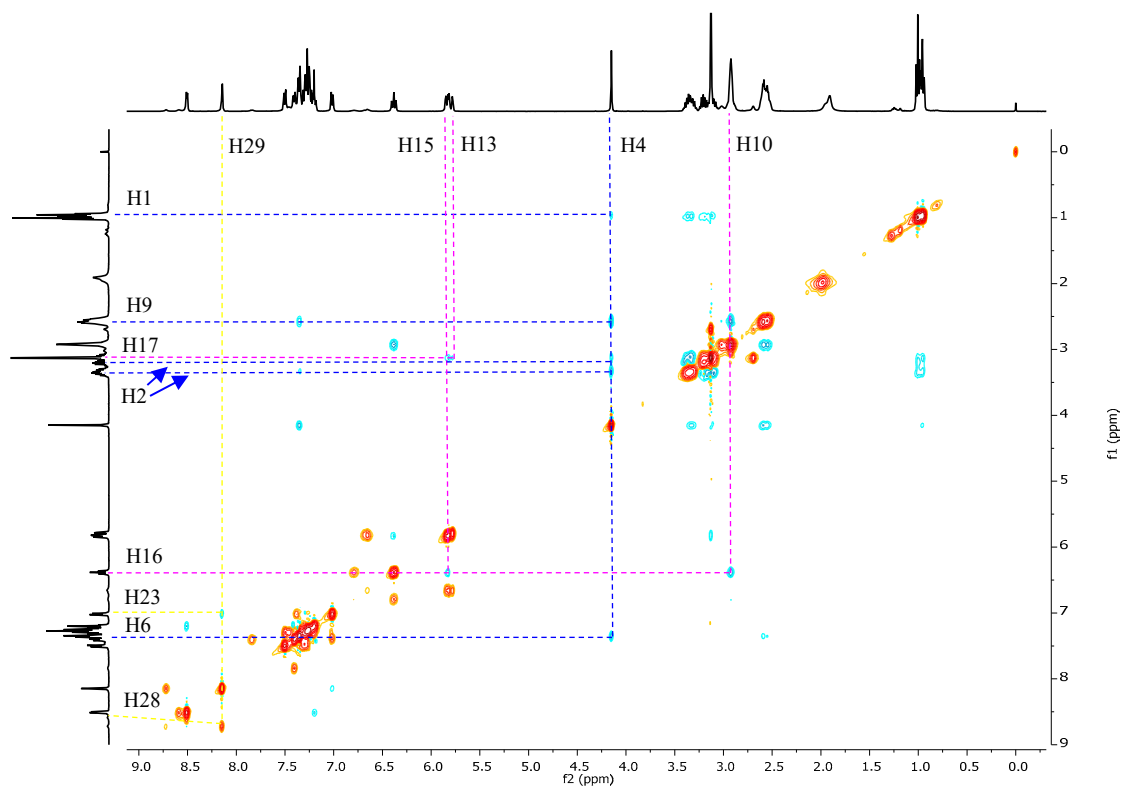
Figure S2-  $^{13}\text{C}$  NMR spectra of N-Me-JNJ-31020028 ang signal attribution.

▪  $^1\text{H}$ - $^1\text{H}$  COSY (Correlated spectroscopy)

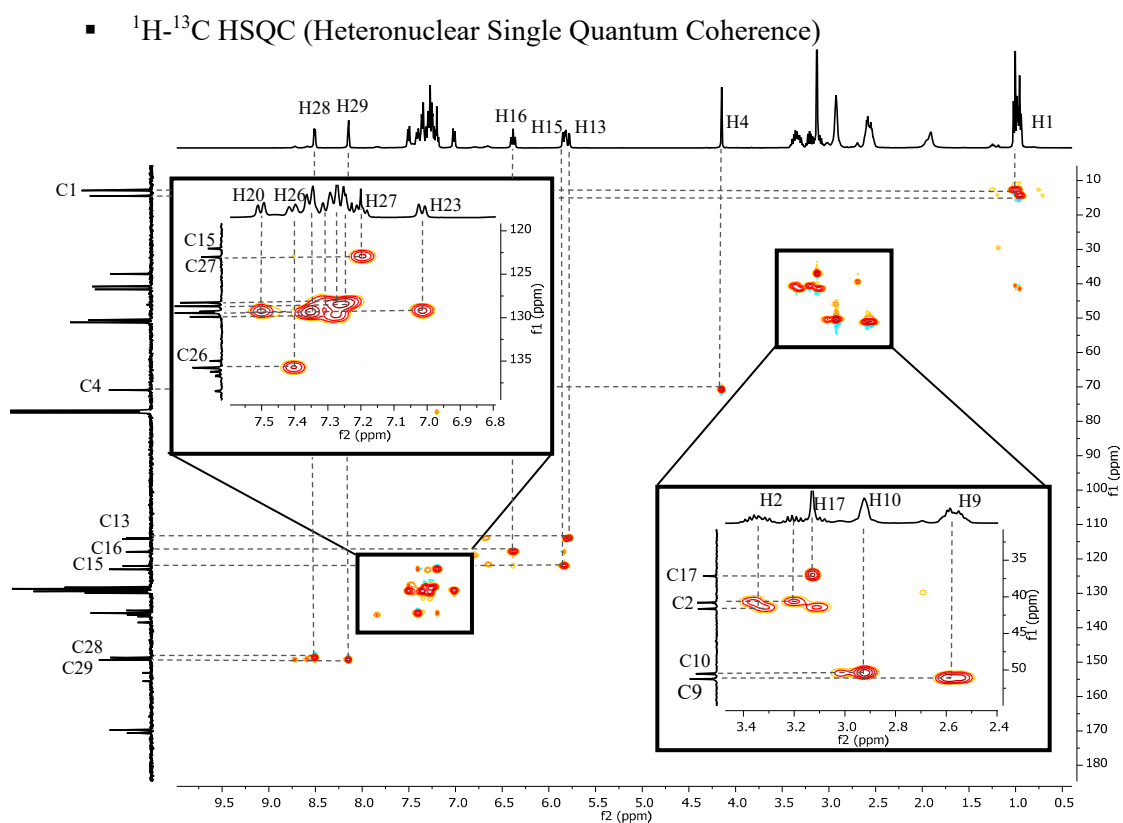


**Figure S3-**  $^1\text{H}$ - $^1\text{H}$  COSY spectra of *N*-Me-JNJ-31020028 and  $^1\text{H}$  signal attribution.

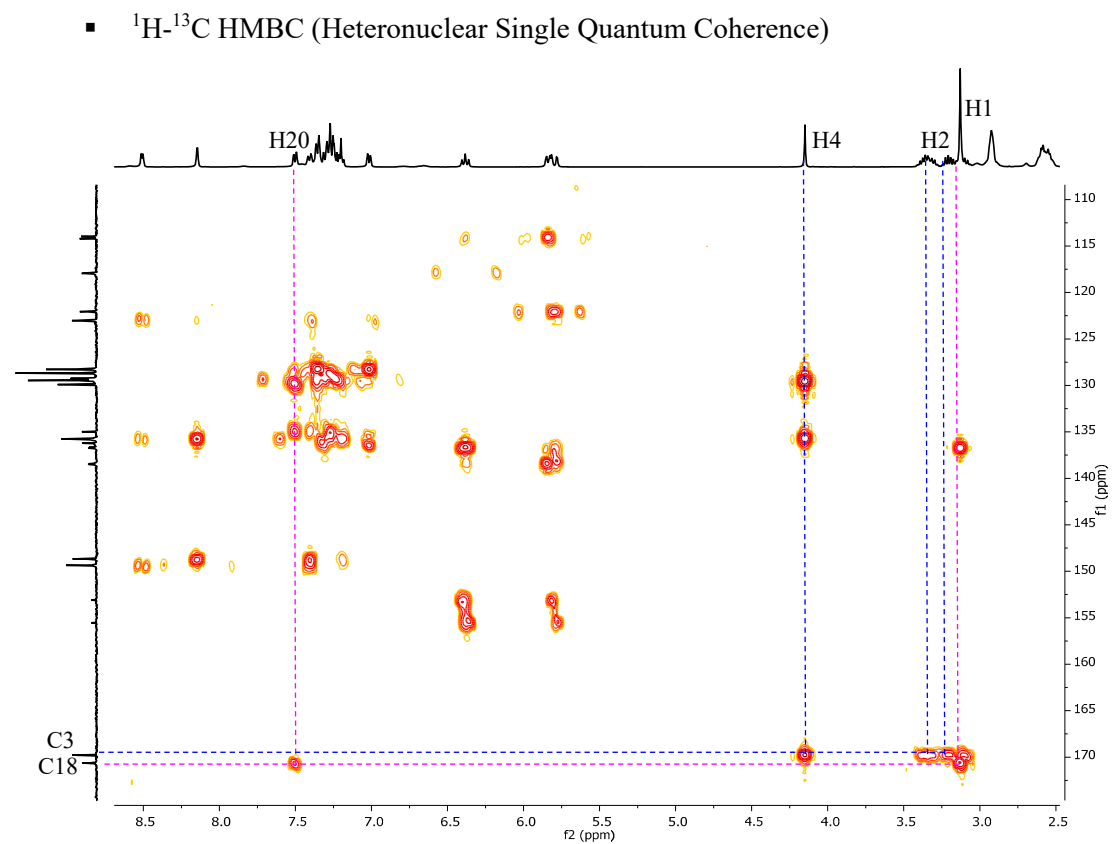
▪ NOESY Spectrum



**Figure S4-** NOESY spectrum of *N*-Me-JNJ-31020028. The most relevant correlations regarding the protons H4, H13, H15, H16 and H29 are presented.



**Figure S5-**  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of *N*-Me-JNJ-31020028 and  $^1\text{H}$  and  $^{13}\text{C}$  signal attribution. Inset: selected expansions of the  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum between  $\delta$  2.4 to 3.4 ppm and  $\delta$  6.8 to 7.6 ppm.



**Figure S6-** Selected expansion of  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of *N*-Me-JNJ-31020028. The most relevant correlations regarding the carbons C3 and C18 are presented.

■ HRMS

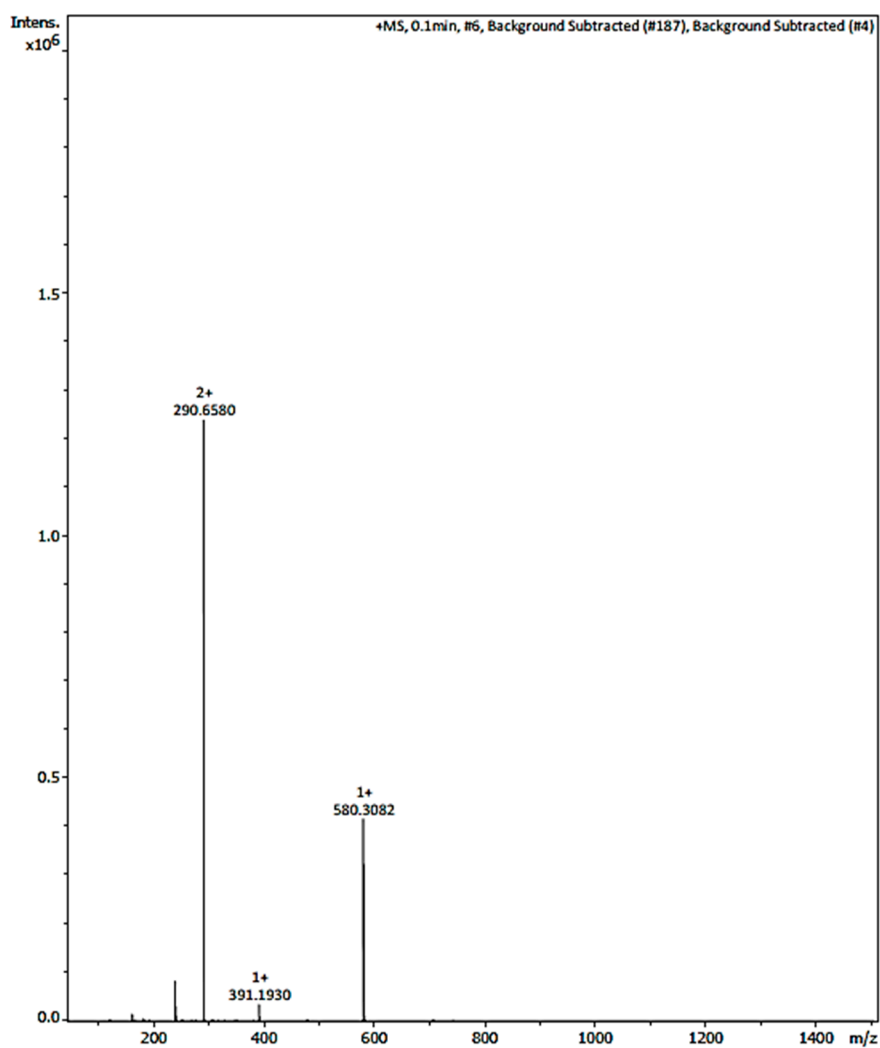


Figure S7- HRMS spectrum of *N*-Me-JNJ-31020028.

HPLCs

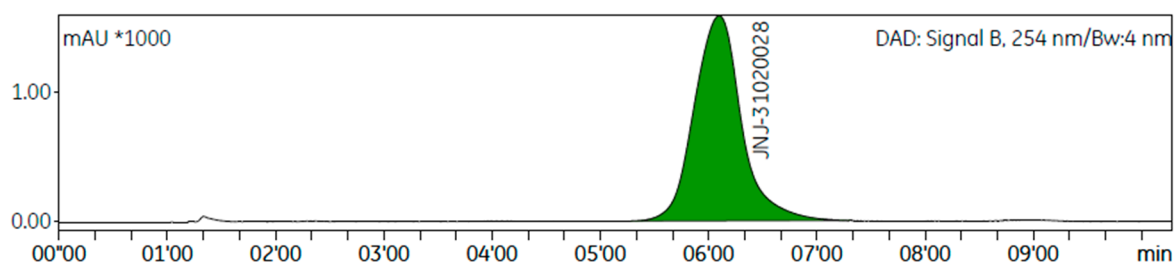
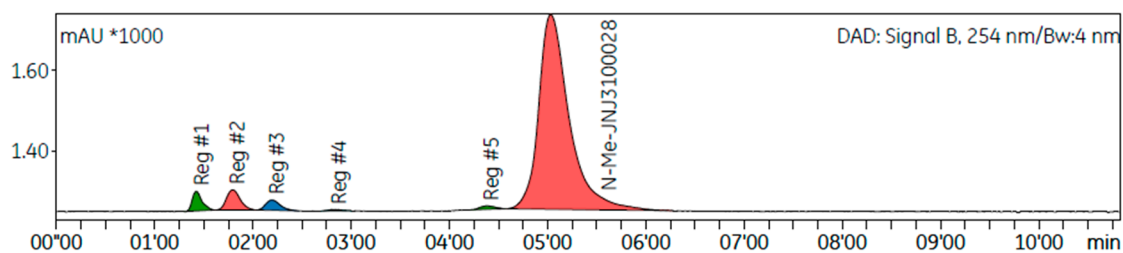


Figure S8- Analytical HPLC chromatogram of precursor JNJ-31020028 1. Rt: 6.06 min.



**Figure S9-** Representative analytical HPLC chromatogram of the reaction mixture obtained through continuous flow methodology. Retention times: Reg#1: 1.26 min; Reg#2: 1.48 min; Reg#3: 2.12 min; Reg#4: 2.50 min; Reg#5: 4.23 min; N-Me-JNJ-31020028: 5.02 min.

## Pre-clinical studies

**Table S1-** *In vivo* biodistribution. Values are expressed as percentage injected dose *per* gram (%ID/g) of body weight ( $n = 7$ ).

Region	Mean	SEM
Brain	0.5187	0.07698
Gut	12.57	1.217

**Table S2-** Whole body *ex vivo* biodistribution. Values are expressed as percentage injected dose *per* gram (%ID/g) of tissue ( $n = 5$ ).

Region	Mean	SEM
Blood	3.661	1.545
Urine	84.43	59.41
Liver	14.33	6.39
Spleen	1.504	0.5483
Lung	0.5568	0.04036
Heart	1.394	0.701
Kidney	1.986	0.4916
Stomach	3.082	0.7135
Small intestine	98.64	25.57
Large intestine	2.18	0.2095
Muscle	0.9318	0.193

Faeces	3.149	1.092
Bile	250.1	23.97
Brain	0.6362	0.06454

**Table S3-** Brain *ex vivo* biodistribution. Values are expressed as percentage injected dose *per* gram (%ID/g) of tissue (*n* = 5).

Region	Mean	SEM
Cerebellum	0.646	0.09664
Hypothalamus	1.03	0.1126
Amygdala	0.9318	0.08237
Striatum	1.013	0.1068
Pre-frontal cortex (PFC)	0.636	0.05916
Hippocampus	0.8976	0.09311