In Vitro and in Vivo Characterization of 3-{2-[6-(2-*tert*-Butoxyethoxy)pyridin-3-yl]-1*H*-imidazol-4-yl}benzonitrile Hydrochloride Salt, a Potent and Selective NPY5 Receptor Antagonist

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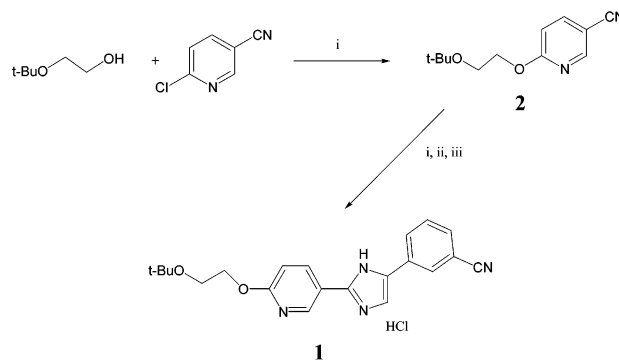
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Abstract: To investigate the anorectic potential of NPY5 receptor antagonists, we have profiled the in vitro and in vivo properties of 3-{2-[6-(2-*tert*-butoxyethoxy)pyridin-3-yl]-1*H*-imidazol-4-yl}benzonitrile hydrochloride salt (**1**). This compound was found to have excellent NPY5 receptor affinity and selectivity, potent functional antagonism, and good peripheral and central nervous system exposure in rats. This compound attenuated bovine pancreatic polypeptide induced food intake in rats but failed to demonstrate anorectic activity in rodent natural feeding models.

Introduction. Neuropeptide Y (NPY) is a 36 amino acid peptide with broad distribution within the central nervous system (CNS). On the basis of the potent orexigenic effects of NPY in vivo, many researchers have investigated the utility of NPY antagonists as potential anorectic agents. The pharmacological activity of NPY is mediated by at least six receptor subtypes: Y1−Y5 and y6 (in mouse and rabbit, a pseudogene in humans). Two of these NPY receptor subtypes, NPY1 and NPY5, have been implicated in mediating the orexigenic effects of NPY. Evidence supporting the role of the NPY5 receptor subtype in regulating food intake and body weight includes the finding that the orexigenic effects of peptide analogues of NPY correlate with affinity for the NPY5 receptor,[1] the anatomical location and physiological regulation of NPY5 receptors in areas of the CNS relevant to feeding,[2] and the determination that NPY5 antisense oligodeoxynulceotides have anorectic effects in rodents.[3] In addition, a number of papers have been published claiming anorectic effects with NPY5 receptor antagonists.[4] However, the relative importance and physiological roles of NPY1 and NPY5 receptors in mediating feeding behavior are still not fully defined,[5] and differences have been observed in the anorectic effects of NPY5 receptor antagonists on food intake induced by exogenous NPY5 agonists versus the effect observed in natural feeding models (vide infra).

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Scheme 1[a]

[a] Reagents: (i) lithium bistrimethylsilyamide/THF; (ii) H$_2$O/NaHCO$_3$/K$_2$CO$_3$, then 3-bromoacetylbenzonitrile; (iii) HCl.

Table 1. In Vitro Potency of Compound **1** at NPY Receptors

rat NPY5 K_i,[a,e] nM	hu NPY5 K_i,[b,e] nM	NPY1 IC$_{50}$,[c,e] μM	NPY2 IC$_{50}$,[c,e] μM	NPY5 Ca^{2+} mobil. IC$_{50}$,[d,e] nM
1.7 ± 0.3 (n = 4)	1.2 ± 0.4 (n = 4)	≫1 (n = 1)	≫1 (n = 1)	0.4 ± 0.3 (n = 2)

[a] [^{125}I][Leu31,Pro34]PYY binding in the whole rat brain in the presence of 1 μM BIBP3226 to block the NPY-1 receptors. [b] Cloned human chimeric receptors, using [^{125}I]PYY as the radioligand. [c] Cerep data (Cerep, Paris, France; values are from one experiment, performed in duplicate). [d] Ca^{2+} mobilization in NPY5 transfected human Bowes melanoma (HMCB) cell line. [e] All values are mean values ± SEM.

We originally identified a series of 2,4-diarylimidazoles[6] as NPY5 antagonists from a high-throughput screen and subsequently optimized the in vitro potency and PK properties of this series. We now report on our in vitro and in vivo findings with a key compound in this series, 3-{2-[6-(2-*tert*-butoxyethoxy)pyridin-3-yl]-1*H*-imidazol-4-yl}benzonitrile hydrochloride salt (**1**).[7]

Chemistry. Compound **1** was synthesized as outlined in Scheme 1. Condensation of the anion of 2-*tert*-butoxyethanol, generated using lithium bistrimethylsilylamide as the base, with 6-chloronicotinonitrile afforded the 2-alkoxy-5-cyanopyridine **2** in 87% yield. Construction of the imidazole ring of **1** relied on the condensation of the amidine, generated from the nitrile moiety of **2**, with an α-bromo ketone.

To circumvent difficulties in isolating and purifying the polar and often water-soluble amidines, we developed a convenient one-pot method to convert aryl- and heteroarylnitriles to amidines in situ, followed by further condensation with an α-bromoketone to obtain the corresponding aryl- or heteroarylimidazole. By use of this methodology, treatment of **2** with lithium bistrimethylsilylamide followed by addition of 3-bromoacetylbenzonitrile and conversion to the HCl salt afforded imidazole **1** in 23% yield from **2**.

Results and Discussion. As shown in Table 1, compound **1** has high affinity for the human and rat NPY5 receptors (human K_i = 1.2 nM; rat K_i = 1.7 nM) and low affinity (IC$_{50}$ ≫ 1 μM) for the NPY1 and NPY2 receptors. Compound **1** also potently antagonized NPY-induced Ca^{2+} mobilization in a stably transfected melanoma cell line expressing the human NPY5 receptor. In addition, compound **1** did not have any significant
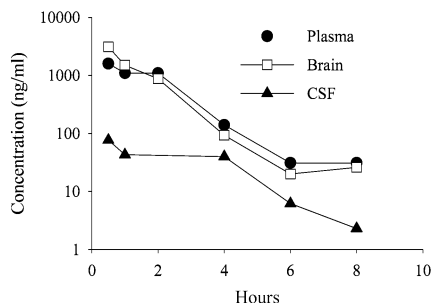
Table 2. Time-Course Study of Plasma, Whole Brain, and CSF Exposure of Compound **1** in Male Wistar Rats[a]

	C_{max} (μM)	t_{max} (h)	$t_{1/2}$ (h)
plasma	4.42	0.5	1.1
brain	8.55	0.5	1.1
CSF	0.213	0.5	1.6

[a] Male Wistar rats (implanted with a jugular vein cannula) were allowed free access to water and food. The oral dose of compound **1** (30 mg/kg, po, dosed in 30% w/v β-cyclodextrin) was administered via oral gavage. Data represent one animal at each time point.

activity at over 50 receptors, ion channels, and transporters ($\ll$50% inhibition at 1 μM).

To assess the exposure of compound **1** in rat, we performed a time-course study of plasma, whole brain, and cerebrospinal fluid (CSF) levels at a dose of 30 mg/kg, po. This study showed that **1** was well exposed in both the periphery and CNS (Table 2). The T_{max} for plasma, CSF, and brain occurs at 30 min, and the C_{max} was 4.42, 0.213, and 8.55 μM, respectively. Even after 6–8 h, significant levels of compound **1** in the plasma, brain, and CSF were observed. The essentially parallel time courses of exposure among plasma, brain, and CSF suggest that rapid equilibrium is achieved with the CNS. In vitro equilibrium dialysis studies indicate that compound **1** has unbound fractions of 0.013 ± 0.001 and 0.006 ± 0.0006 in rat plasma and brain tissue, respectively ($n = 6$). The ratio of unbound plasma to brain fractions (2.2) from the in vitro dialysis is entirely consistent with the brain-to-plasma ratio at t_{max} (e.g., 1.9 at 30 min). This concordance also indicates that compound **1** efficiently penetrates the brain such that equivalent free plasma and brain concentrations are achieved within the first 30 min after dose.[8] Overall, these studies demonstrate that **1** has sufficient oral exposure (peripheral and central) to be an excellent in vivo probe for investigating NPY5 mediated feeding effects in the rat.

Our initial objective was to verify that compound **1** could block the orexigenic effects of a selective NPY5 agonist. We performed a study in which male Sprague–Dawley (SD) rats were dosed orally (30 mg/kg) with compound **1**, and after 1 h, animals were treated with either the NPY5-selective agonist bovine pancreatic polypeptide[9] (bPP, 5 μg ICV (intracerebral ventricularly) or artificial CSF. Two hours later, food intake was measured. As shown in Figure 1, compound **1** produced a 53% percent inhibition of bPP-induced food intake.

We next explored the effect of **1** in a study measuring rat food intake for a 3 h period following 24 h of food deprivation. At a dose of 40 mg/kg, **1** produced no significant changes in food consumption in SD rats (see Figure 2). Compound **1** also did not cause any significant
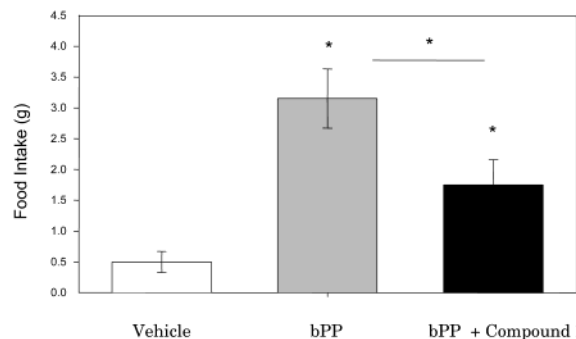

Figure 1. Effects of compound **1** (30 mg/kg, po, dosed in 0.5% aqueous methyl cellulose, dosed 1 h before bPP treatment) on bPP-elicited feeding (5 μg, icv) in male Sprague–Dawley rats. Values represent food intake after 2 h. (∗) $P \leq 0.05$ for comparisons against vehicle group unless otherwise noted. All values are mean values ± SEM ($n = 6$).
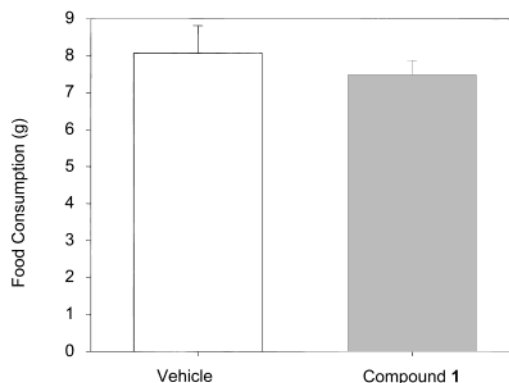

Figure 2. Feeding effects of compound **1** (40 mg/kg, po, dosed in 0.5% aqueous methyl cellulose) in Sprague–Dawley rats following 24 h of food deprivation. Food intake was measured following a 3-h test session. All values are mean values ± SEM ($n = 8$).
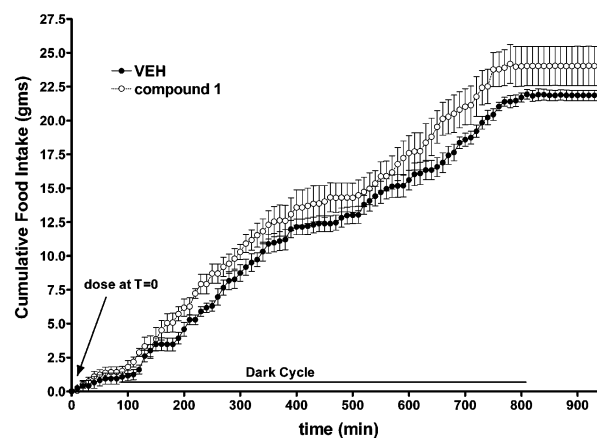

Figure 3. Effect of compound **1** (30 mg/kg, po, dosed in 30% w/v β-cyclodextrin) on spontaneous food intake in Wistar rats habituated to powdered chow. All values are mean values ± SEM ($n = 5$).

changes in spontaneous locomotor activity (data not shown), suggesting that the compound does not cause any overt behavioral effects. In a separate experiment designed to evaluate potential thermogenic effects of a selective NPY5 antagonist, **1** was found to cause no significant changes in oxygen consumption or respiratory quotient in male SD rats when dosed at 30 mg/kg, po (data not shown).

Compound **1** was further evaluated for anorectic effects in a natural feeding model using a continuous monitoring food intake system in which cumulative food intake was measured every 10 min throughout the dark phase. This system allows for a detailed analysis of feeding behavior throughout the night. As shown in Figure 3, compound **1**, at a dose of 30 mg/kg, po, failed to show any inhibition of spontaneous feeding.

Thus, we have found **1** to be a potent and selective NPY5 antagonist with good peripheral and central exposure in rats when dosed at 30 mg/kg, po. Compound **1** blocks bPP-induced feeding in the rat, demonstrating that this compound can antagonize a central NPY5-mediated effect in vivo. This result is consistent with the potency and CNS exposure of this compound, since CSF levels of **1** were >100 above the rat binding IC_{50} value. Thus, the question remains why **1** failed to demonstrate anorectic effects in the natural feeding models.

The demonstration that NPY5 antagonists have anorectic activity via NPY5 receptor blockade in natural feeding models is a key and obligatory criterion in establishing a physiological role for the NPY5 receptor in feeding. The early publications reporting NPY5 antagonists with anorectic activity added weight to the concept that the NPY5 receptor was the "feeding receptor". However, in some cases, the specificity of the anorectic effects has since been questioned,[10-12] and in other cases, the specificity of the anorectic action is not clearly determined. In addition, two recent publications[9,13] have reported findings similar to our own, i.e., that potent and selective NPY5 antagonists with good peripheral and CNS penetration block selective NPY5 receptor agonist induced feeding but have no effect in natural feeding models in rodents. That these findings hold true over a number of structurally distinct chemical series provides persuasive evidence that NPY5 receptor blockade does not produce anorexia in rodents under normal physiological conditions.

Thus, the physiological role of the NPY5 receptor remains unclear. It has been reported that NPY-induced food intake was significantly reduced in the NPY1 receptor knockout mouse but not significantly reduced in the NPY5 receptor knockout mouse.[14] Also, NPY5 receptor-null ob/ob mice, unlike the NPY-deficient mice, are equally obese compared to the ob/ob mice.[15] These collective findings using genetically modified rodents would suggest that endogenous NPY modulates feeding primarily, or at least to a large degree, through the NPY1 receptor. Recent publications[16,17] demonstrating that very selective and potent NPY1 antagonists blocks NPY-induced feeding and have anorectic effects in natural feeding models support this idea.

In summary, we have developed a potent and selective NPY5 antagonist that can block NPY-5 agonist induced feeding but has no anorectic effects at doses of 30–40 mg/kg, po, in natural feeding models. Further studies will be needed to fully understand the role NPY and its various receptors play in regulating food intake in animals and how the NPY-ergic system is integrated with other orexigenic and anorectic systems to control ingestive behavior and energy homeostasis. Compound **1** should be a useful tool to further probe the function of the NPY5 receptor.

Supporting Information Available: Experimental procedures including characterization data for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wyss, P.; Stricker-Krongrad, A.; Brunner, L.; Miller, J.; Crossthwaite, A.; Whitebread, S.; Criscione, L. The pharmacology of neuropeptide Y (NPY) receptor-mediated feeding in rats characterizes better Y5 than Y1, but not Y2 or Y4 subtypes. *Regul. Pept.* **1998**, *75–76*, 363–371. (b) Gerald, C.; Walker, M. W.; Criscione, L.; Gustafson, E. L.; Batzl-Hartmann, C.; Smith, K. E.; Vaysse, P.; Durkin, M. M.; Laz, T. M.; et al. A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature (London)* **1996**, *382* (6587), 168–171.

(2) Widdowson, P. S. Regionally-selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to the Y5 "feeding" receptor. *Brain Res.* **1997**, *758* (1, 2), 17–25.

(3) (a) Schaffhauser, A. O.; Stricker-Krongrad, A.; Brunner, L.; Cumin, F.; Gerald, C.; Whitebread, S.; Criscione, L.; Hofbauer, K. G. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. *Diabetes* **1997**, *46* (11), 1792–1798. (b) Tang-Christensen, M.; Kristensen, P.; Stidsen, C. E.; Brand, C. L.; Larsen, P. J. Central administration of Y5 receptor antisense decreases spontaneous food intake and attenuates feeding in response to exogenous neuropeptide Y. *J. Endocrinol.* **1998**, *159* (2), 307–312. (c) Flynn, M. C.; Turrin, N. P.; Plata-Salaman, C. R.; Ffrench-Mullen, J. M. Feeding response to neuropeptide Y-related compounds in rats treated with Y5 receptor antisense or sense phosphothio-oligodeoxynucleotide. *Physiol. Behav.* **1999**, *66* (5), 881–884.

(4) (a) Criscione, L.; Rigollier, P.; Batzl-Hartmann, C.; Rueger, H.; Stricker-Krongrad, A.; Wyss, P.; Brunner, L.; Whitebread, S.; Yamaguchi, Y.; Gerald, C.; Heurich, R. O.; Walker, M. W.; Chiesi, M.; Schilling, W.; Hofbauer, K. G.; Levens, N. Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. *J. Clin. Invest.* **1998**, *102* (12), 2136–2145. (b) Youngman, M. A.; McNally, J. J.; Lovenberg, T. W.; Reitz, A. B.; Willard, N. M.; Nepomuceno, D. H.; Wilson, S. J.; Crooke, J. J.; Rosenthal, D.; Vaidya, A. H.; Dax, S. L. α-Substituted *N*-(Sulfonamido)alkyl-β-aminotetralins: Potent and Selective Neuropeptide Y Y5 Receptor Antagonists. *J. Med. Chem.* **2000**, *43* (3), 346–350. (c) Daniels, A. J.; Grizzle, M. K.; Wiard, R. P.; Matthews, J. E.; Heyer, D. Food intake inhibition and reduction in body weight gain in lean and obese rodents treated with GW438014A, a potent and selective NPY-Y5 receptor antagonist. *Regul. Pept.* **2002**, *106* (1–3), 47–54. (d) Kordik, C. P.; Luo, C.; Zanoni, B. C.; Lovenberg, T. W.; Wilson, S. J.; et al. Pyrazolecarboxamide human neuropeptide Y5 receptor ligands with in vivo antifeedant activity. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2287–2290.

(5) (a) Chamorro, S.; Della-Zuana, O.; Fauchere, J. L.; Feletou, M.; Galizzi, J. P.; et al. Appetite suppression based on selective inhibition of NPY receptors. *Int. J. Obes.* **2002** *26*, 281–298. (b) Duhault, J.; Boulanger, M.; Chamorro, S.; Boutin, J. A.; Zuana, O. D.; et al. Food intake regulation in rodents: Y5 or Y1 NPY receptors or both? *Can. J. Physiol. Pharmacol.* **2000**, *78*, 173–185.

(6) Thurkauf, A.; Yuan, J.; Hutchison, A.; Blum, C. A.; Elliott, R. L.; Hammond, M. Preparation of diarylimidazoles as neuropeptide Y receptor ligands. PCT Int. Appl. WO 9901128, 1999; 46 pp.

(7) Elliott, R. L.; Oliver, R. M.; Hammond, M.; Patterson, T. A.; She, L.; Hargrove, D. M.; Martin, K. A.; Maurer, T. S.; Kalvass, J. C.; Morgan, B. M.; DaSilva-Jardine, P.; Stevenson, R. W.; Mack, C.; Cassella, J. The In Vitro and in Vivo Characterization of 3-{2-[6-(2-*tert*-butoxy-ethoxy)-pyridin-3-yl]-1*H*-imidazol-4-yl}-benzonitrile, (**I**), a Potent and Selective NPY-Y5 Antagonist. Presented at the 6th International NPY Conference, Sydney, Australia, April 22–26, 2001; Poster No. 6.

(8) Kalvass, J. C.; Maurer, T. S. Influence of Nonspecific Brain and Plasma Binding on CNS Exposure; Implications for Rational Drug Discovery. *Biopharm. Drug Dispos.* **2002**, *23*, 327–338.

(9) Kanatani, A.; Ishihara, A.; Iwaasa, H.; Nakamura, K.; Okamoto, O.; Hidaka, M.; Ito, J.; Fukuroda, T.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Ishii, Y.; Okabe, T.; Fukami, T.; Ihara, M.; L-152,-804: orally active and selective neuropeptide Y Y5 receptor antagonist. *Biochem. Biophys. Res. Commun.* **2000**, *272* (1), 169–173.

(10) Della Zuana, O.; Sadlo, M.; Germain, M.; Feletou, M.; Chamorro, S.; Tisserand, F.; de Montrion, C.; Boivin, J. F.; Duhault, J.; Boutin, J. A.; Levens, N. Reduced food intake in response to CGP 71683A may be due to mechanisms other than NPY Y5 receptor blockade. *Int. J. Obes.* **2001**, *25* (1), 84−94.

(11) Bannon, A. W.; Seda, J.; Carmouche, M.; Francis, J. M.; Norman, M. H.; Karbon, B.; McCaleb, M. L. Behavioral characterization of neuropeptide Y knockout mice. *Brain Res.* **2000**, *868* (1), 79−87.

(12) Criscione, L.; Schaffhauser, A. O.; Guerini, D.; Rigollier, P.; Schmid, T.; Buhlmayer, P. Effects of the Y5 Receptor Antagonist CGP71683A on Food Intake in Male Y5 Receptor Knock-Out Mice (Abstract). Presented at the 6th International NPY Conference, Sydney, Australia, April 22−26, 2001; Oral Session XII (New Compounds).

(13) Turnbull, A. V.; Ellershaw, L.; Masters, D. J.; Birtles, S.; Boyer, S.; et al. Selective Antagonism of the NPY Y5 Receptor Does Not Have a Major Effect on Feeding in Rats. *Diabetes* **2002**, *51*, 2441−2449.

(14) Kanatani, A.; Mashiko, S.; Murai, N.; Sugimoto, N.; Ito, J.; Fukuroda, T.; Fukami, T.; Morin, N.; MacNeil, D. J.; Van Der Ploeg, L. H. T.; Saga, Y.; Nishimura, S.; Ihara, M. Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice. *Endocrinology* **2000**, *141* (3), 1011−1016.

(15) Marsh, D. J.; Hollopeter, G.; Kafer, K. E.; Palmiter, R. D. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat. Med. (N. Y.)* **1998**, *4* (6), 718−721.

(16) Kanatani, A.; Hata, M.; Mashiko, S.; Ishihara, A.; Okamoto, O.; Haga, Y.; Ohe, T.; Kanno, T.; Murai, N.; Ishii, Y.; Fukuroda, T.; Fukami, T.; Ihara, M. A Typical Y1 Receptor Regulates Feeding Behaviors: Effects of a Potent and Selective Y1 Antagonist, J-115814. *Mol. Pharmacol.* **2001**, *59*, 501−505.

(17) Kanatani, A.; Kanno, T.; Ishihara, A.; Hata, M.; Sakuraba, A.; Tanaka, T.; Tsuchiya, Y.; Mase, T.; Fukuroda, T.; Fukami, T.; Ihara, M. The Novel Neuropeptide Y Y1 Receptor Antagonist J-104870: A Potent Feeding Suppressant with Oral Bioavailability. *Biochem. Biophys. Res. Commun.* **1999**, *266* (1), 88−91.

(18) Qian, S.; Chen, H.; Weingarth, D.; Trumbauer, M. E.; Novi, D. E.; Guan, X.; Yu, H.; Shen, Z.; Feng, Y.; Frazier, E.; Chen, A.; Camacho, R. E.; Shearman, L. P.; Gopal-Truter, S.; MacNeil, D. J.; Van der Ploeg, L. H. T.; Marsh, D. J. Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol. Cell. Biol.* **2002**, *22*, 5027−5035.