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Synthesis of [^{3}H](4-Fluorobutyl)propyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine: A Potent Radioligand for Corticotropin-Releasing Hormone Type 1 Receptor

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Summary

[^{3}H](4-Fluorobutyl)propyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine ([^{3}H]**LWH-154**), a novel potent radiolabelled analog of the nonpeptide corticotropin-releasing hormone type 1 receptor ($CRHR_1$) selective antagonist, butylethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (antalarmin), was prepared for the development of positron emission tomography radiotracers for $CRHR_1$ and evaluation as a nonpeptide radioligand for use in pharmacological studies. The precursor (4-fluorobutyl)prop-2-enyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (**6**) for tritiation was prepared in two steps from **3** in 76% total yield. Catalytic reduction of unsaturated fluoride **6** using tritium gas and palladium as catalyst gave [^{3}H]**LWH-154**. After HPLC purification, [^{3}H]**LWH-154** of high radiochemical purity was obtained with a specific activity of 69 Ci/mmol.

Key Words: corticotropin-releasing hormone, radioligand, tritium, antalarmin, positron emission tomography

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Introduction

Corticotropin-releasing hormone (CRH) coordinates the responses of the body to stress through the release of adrenocorticotropic hormone (ACTH) and regulates stress-induced autonomic, neuroendocrine, immune, and behavioral changes (1-4). Overproduction of CRH in the brain has been associated with mental disorders such as anxiety (5), depression (6), and substance abuse (7). Thus, it is essential to develop potent and subtype-selective radiolabelled corticotropin-releasing hormone receptor (CRHR) ligands for the diagnosis, monitoring, and ultimately, treatment of these disorders associated with chronic activation of CRHR. Peptide CRHR radioligands have been extensively used as research tools to study the physiological and pathological roles of CRH system (2,8). However, they can not penetrate through blood-brain barrier (BBB) and therefore, their usefulness is limited. Thus, a potent and selective nonpeptide CRHR radioligand would be valuable to further probe the pharmacology and distribution of CRHR in normal subjects and patients as well as, on a molecular level, further characterize specific membrane proteins which interact with CRH to produce biological actions. Additionally, nonpeptide CRHR radioligands would be also important and useful for the discovery and development of drugs for the treatment of CRH-related disorders.

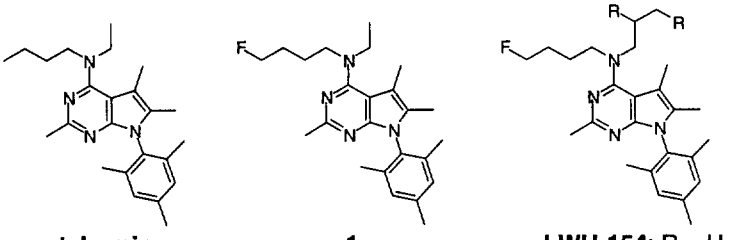


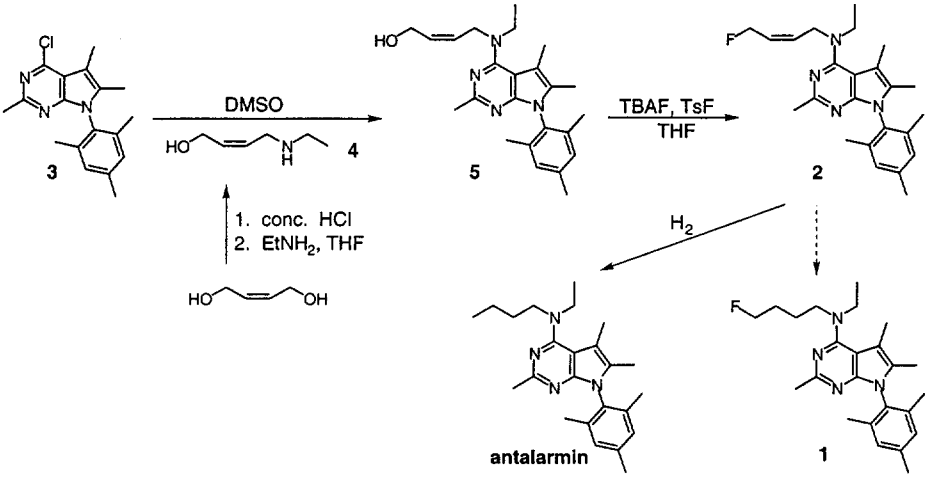
Figure 1. Structures of antalarmin and its fluorosubstituted derivatives

In our effort to develop potential positron emission tomography (PET) imaging agents for $CRHR_1$, two fluorosubstituted analogs of the selective nonpeptide $CRHR_1$ antagonist butylethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (antalarmin), ethyl(4-fluorobutyl)[2,5,6-trimethyl-7-(2,4,6-

trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (**1**) and (4-fluorobutyl)propyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (**LWH-154**), showed very high affinity for CRHR$_1$ (Ki = 3.5, 0.91 nM, respectively) (9) against [^{125}I]Tyr^0-sauvagine. To date, only a few pharmacokinetic studies (10-12) have focused on CRHR$_1$ antagonists in similar structure classes. Thus, to investigate the pharmacokinetic properties (e.g. ability to penetrate BBB, brain distribution, rate of equilibrium, ratio of specific binding to non-specific binding) before using ^{18}F-substituted analogs for PET studies, we attempted to label both fluorine-containing compounds with a long half-life isotope (i.e. tritium). Here we describe the design and preparation of a highly potent tritiated nonpeptide CRHR$_1$ ligand [^{3}H]**LWH-154**.

Results and Discussion

Initially, we attempted to introduce tritium atoms into compound **1** by direct catalytic tritiation of the unsaturated precursor **2** to afford [^{3}H]**1**. We anticipated that fluoride **2** would be accessible from intermediates 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidine (**3**) and amino alcohol **4**.

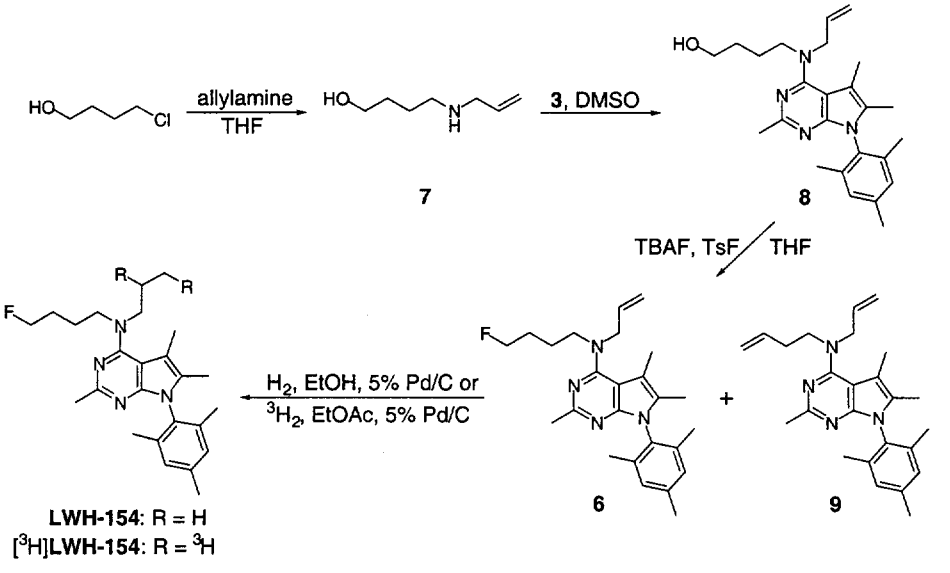


Scheme 1. Synthesis of precursor **2**

The synthesis of precursor **2** is shown in Scheme 1. Monochlorination of *cis*-2-butene-1,4-diol with concentrated hydrochloric acid followed by treatment with

ethylamine afforded *cis*-aminoalcohol **4**. The intermediate **3** was prepared from 2,4,6-trimethylaniline by modification of previously described methodology (9,13). Coupling **3** with **4** in dimethyl sulfoxide (DMSO) gave allylic alcohol **5**. Then, fluoride **2** was obtained by treatment of **5** with tetrabutylammonium fluoride (TBAF) and *p*-toluenesulfonyl fluoride (TsF) in the presence of 4Å molecular sieves (14). Unfortunately, attempts to evaluate the feasibility for the synthesis of [^{3}H]**1** by conversion of precursor **2** to saturated fluoride **1** using catalytic hydrogenation demonstrated that antalarmin was the only product formed and originated via reduction of both the double bond and hydrogenolysis of the fluorine functionality. It appears that the allylic fluoro-substituent was labile under the catalytic hydrogenation conditions used, since the reduction of the saturated fluoro-derivatives to **LWH-154** proceeded in 98% yield (see below).

Since **LWH-154** showed much higher affinity for $CRHR_1$ than antalarmin and **1** (9), [^{3}H]**LWH-154** was designed and synthesized as shown in Scheme 2. As the fluorine atom in precursor **6** is attached to a saturated hydrocarbon chain, it was expected to be stable to catalytic hydrogenation or tritiation in contrast to the allylic fluorine atom in **2**.



Scheme 2. Synthesis of [^{3}H]**LWH-154**.

The synthesis of [^{3}H]**LWH-154** is shown in Scheme 2. 4-Chloro-1-butanol was treated with allylamine to give aminoalcohol **7**. Coupling **3** with an excess amount of compound **7** followed by fluorination as in the preparation of fluoride **2** afforded unsaturated fluoride **6** in 79% yield. In contrast to the fluorination reaction in previous studies (8,14), a significant amount of the elimination product **9** was formed (11%). Hydrogenation of compound **6** using H_2 and 5% palladium on charcoal (Pd/C) successfully afforded **LWH-154** as the sole product with no evidence of fluorine hydrogenolysis. [^{3}H]**LWH-154** was prepared by tritiation of fluoride **6** using $^3H_2$ and Pd/C in ethyl acetate, and then purified by semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) to afford [^{3}H]**LWH-154** with high radiochemical purity (99.0%) and a specific activity of 69 Ci/mmol (2.55 TBq/mmol).

Conclusion

We have successfully developed an efficient synthetic route to provide [^{3}H]**LWH-154** in good chemical yield with a high radiochemical purity and specific activity. Given that **LWH-154** displayed subnanomolar affinity for $CRHR_1$, [^{3}H]**LWH-154** might prove to be useful to investigate pharmacokinetic properties of **LWH-154** for the development of PET tracers for $CRHR_1$. Moreover, as a potent nonpeptide radiolabelled $CRHR_1$ ligand, [^{3}H]**LWH-154** is a novel research tool which could be used for a variety of pharmacological purposes in addition to PET studies.

Experimental

Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. High-resolution mass measurements (HRMS) were obtained using a V. G. Micro Mass 7070F mass spectrometer. Mass spectroscopy for the determination of specific activity was carried out using the FAB positive technique with DTT:DTE (5:1) as the matrix. The mass spectrometer model MS25 was supplied by Kratos, Manchester UK. ^{1}H NMR spectra were recorded using a

Varian XL-300 spectrometer. Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a tetramethylsilane internal standard. Radioactivity was detected by homogeneous scintillation on a Berthold LB507A using Packard Flo-Scint 3 at 2 mL/min. Thin layer chromatography (TLC) was performed on 250 μm Analtech GHLF silica gel plates. Flash column chromatography was performed with Fluka (Item 60752) 40-63 μm silica gel 60.

(*Z*)-4-Ethylaminobut-2-en-1-ol (4). To a stirred solution of (*Z*)-2-butene-1,4-diol (50.0 g, 567 mmol) in CH_2Cl_2 (50 mL) was added dropwise conc. HCl (80 mL) and the mixture was stirred at room temperature for 20 h. The resulting solution was extracted with EtOAc and CH_2Cl_2 (1:4, 100 mL×5). The combined extracts were dried ($MgSO_4$), filtered, and evaporated. To the oily residue was added a solution of ethylamine (68 g, 1.5 mol) in THF (1000 mL) and then stirred at room temperature for 18 h. After evaporated and redissolved in ether (500 mL), the mixture was washed with 40 % aqueous NaOH (200 mL), dried ($MgSO_4$), filtered, and evaporated to afford **4** (18.1 g, 28%) as an amber-colored oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.79-5.87 (m, 1H), 5.64-5.72 (m, 1H), 4.17 (d, J=5.8 Hz, 2H), 3.29 (d, J=5.9 Hz, 2H), 2.68 (q, J=7.2 Hz, 2H), 1.13 (t, J=6.9 Hz, 3H); CIMS: *m/e* 116 (MH^+).

(*Z*)-4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]but-2-en-1-ol (5). A mixture of **3** (3.00 g, 9.56 mmol) and **4** (6.61 g, 57.4 mmol) in DMSO (22 mL) was heated at 130 °C for 6 h. After cooling to room temperature, water (300 mL) was added to the reaction mixture and then extracted with EtOAc (300 mL). The extract was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The crude product was purified via silica gel column chromatography (5% MeOH in CH_2Cl_2) to give **5** (3.69 g, 98%) as a light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 5.60-5.84 (m, 2H), 4.32 (d, J=5.8 Hz, 2H), 4.24 (d, J=6.8 Hz, 2H), 3.59 (q, J=7.2 Hz, 2H), 2.48 (s, 3H), 2.38 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H), 1.23 (t, J=6.9 Hz, 3H); HRMS: *m/e* calc'd for $C_{24}H_{32}N_4O^+$: 392.2576, found 392.2570; CIMS: 393 (MH^+).

((*Z*)-4-Fluorobut-2-enyl)ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (2). To a solution of tetrabutylammonium fluoride (3.40 g, 13.0 mmol) in THF (20 mL) in the presence of molecular sieves 4Å (13 g) was added a mixture of *p*-toluenesulfonyl fluoride (1.58 g, 9.07 mmol) and alcohol **5** (1.78 g, 4.53 mmol) in THF (10 mL). After stirring at room temperature for 16 h, the reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was evaporated and then chromatographed (silica gel, 20% EtOAc in *n*-hexane) to afford **2** (374 mg, 21%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 5.80-5.93 (m, 2H), 5.18 (d, J=5.8 Hz, 1H), 5.02 (d, J=5.8 Hz, 1H), 4.17-4.20 (m, 2H), 3.56 (q, J=6.8 Hz, 2H), 2.46 (s, 3H), 2.38 (s, 3H), 2.35 (s, 3H), 1.95 (s, 3H), 1.83 (s, 6H), 1.22 (t, J=6.9 Hz, 3H); HRMS: *m/e* calc'd for $C_{24}H_{31}FN_4^+$: 394.2533, found 394.2530; CIMS: 395 (MH^+).

4-(Prop-2-enylamino)butan-1-ol (7). A mixture of allylamine (53.5 g, 937 mmol) and 4-chlorobutan-1-ol (25.4 g, 234 mmol) in THF (30 mL) was refluxed for 22 h. After cooled and evaporated, the residue was dissolved in ether (400 mL) and washed with saturated aqueous K_2CO_3. The organic solution was dried ($MgSO_4$), filtered, and evaporated to afford **7** (24.4 g, 81%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83-5.96 (m, 1H), 5.18 (dd, J=15.6, 2.0 Hz, 1H), 5.11 (d, J=8.8 Hz, 1H), 3.56 (t, J=5.9 Hz, 2H), 3.24 (dd, J=4.9, 2.0 Hz, 2H), 2.63 (t, J=6.4 Hz, 2H), 1.58-1.63 (m, 4H); CIMS: *m/e* 130 (MH^+).

4-[Prop-2-enyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]butan-1-ol (8). A mixture of **3** (3.00 g, 9.56 mmol) and **7** (7.42 g, 57.4 mmol) in DMSO (22 mL) was heated at 130 °C for 6 h. After cooling to room temperature, water (300 mL) was added to the reaction mixture and then extracted with EtOAc (300 mL). The extract was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The crude product was purified via silica gel column chromatography (3% MeOH in CH_2Cl_2) to give **8** (3.75 g, 96%) as a colorless oil: mp 151-152 °C (HCl salt); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 5.92-6.05 (m, 1H), 5.22-5.35 (m, 2H), 4.14 (d, J=5.8 Hz, 2H), 3.68 (t, J=6.4 Hz, 2H), 3.52 (t, J=7.9 Hz, 2H), 2.48 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 1.94 (s, 3H), 1.83 (s,

6H), 1.70-1.80 (m, 2H), 1.53-1.62 (m, 2H); CIMS: *m/e* 407 (MH^+); Analysis calc'd for $C_{25}H_{34}N_4O \cdot HCl \cdot 0.25H_2O$: C, 67.09; H, 8.00; N, 12.51; found: C, 67.31; H, 7.99; N, 12.55.

(4-Fluorobutyl)prop-2-enyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (6) and but-3-enylprop-2-enyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (9). To a solution of tetrabutylammonium fluoride (4.44 g, 17.0 mmol) in THF (35 mL) in the presence of molecular sieves 4Å (17 g) was added a mixture of *p*-toluenesulfonyl fluoride (1.97 g, 11.3 mmol) and alcohol **8** (2.30 g, 5.66 mmol) in THF (20 mL) at room temperature. After stirring at reflux for 20 h, the reaction mixture was filtered through Celite and washed with EtOAc. The filtrate was evaporated and then chromatographed (silica gel, 20% EtOAc in *n*-hexane) to afford **6** (1.83 g, 79%) as a colorless oil and elimination-product **9** (246 mg, 11%) as a white crystalline solid. **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 5.91-6.04 (m, 1H), 5.30 (d, J=17.5 Hz, 1H), 5.21 (d, J=9.7 Hz, 1H), 4.53 (t, J=5.4 Hz, 1H), 4.37 (t, J=5.9 Hz, 1H), 4.14 (d, J=5.9 Hz, 2H), 3.54 (t, J=6.9 Hz, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.84 (s, 6H), 1.70-1.78 (m, 4H); HRMS: *m/e* calc'd for $C_{25}H_{33}FN_4^+$: 408.2689, found 408.2689; CIMS: 409 (MH^+); **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 5.77-6.05 (m, 2H), 5.20-5.34 (m, 2H), 4.98-5.09 (m, 2H), 4.16 (d, J=5.9 Hz, 2H), 3.57 (t, J=7.4 Hz, 2H), 2.47 (s, 3H), 2.39-2.45 (m, 2H), 2.37 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H); HRMS: *m/e* calc'd for $C_{25}H_{32}N_4^+$: 388.2627, found 388.2611; CIMS: 389 (MH^+).

(4-Fluorobutyl)propyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (LWH-154). A mixture of fluoride **6** (700 mg, 1.71 mmol) and Pd/C (5%, 200 mg) in EtOH (5 mL) was stirred under H_2 (1 atm) at room temperature for 16 h. The TLC (silica gel, 20% EtOAc in *n*-hexane) showed one spot to one spot conversion. The catalyst was removed via filtration through Celite, and the filtrate was evaporated to afford a nearly colorless oil. The residue was chromatographed (silica gel, 20% EtOAc in *n*-hexane) to afford **LWH-154** (690

mg, 98%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 4.52 (t, J=5.9 Hz, 1H), 4.36 (t, J=6.4 Hz, 1H), 3.59 (t, J=6.9 Hz, 2H), 3.49 (t, J=7.4 Hz, 2H), 2.45 (s, 3H), 2.37 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.84 (s, 6H), 1.63-1.76 (m, 6H), 0.88 (t, J=7.4 Hz, 3H); HRMS: *m/e* calc'd for $C_{25}H_{35}FN_4^+$: 410.2846, found 410.2857.

Radiosynthesis of [^{3}H](4-Fluorobutyl)propyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine ([^{3}H]LWH-154). The synthesis, HPLC purification, and determination of specific activity of **[^{3}H]LWH-154** were accomplished by Amersham Pharmacia Biotech Inc., Piscataway, New Jersey, as follow: In a 4 ml tritiation vessel were combined compound **6** (5 mg), ethyl acetate (2 ml), and Pd/C (5%, 12 mg) and the mixture stirred under tritium gas (10 Ci) at room temperature for 2.5 h. The solution was filtered and labile tritium removed by repeated rotary evaporations to dryness from methanol (3×5 ml). The crude material was purified by RP-HPLC (Gilson 305/306 pumps and Cecil CE2112 UV detector at 254 nM) on an Ultrasphere ODS (250×10 mm) column using a gradient system from water:methanol:triethylamine (80:20:0.1) to water:methanol: triethylamine (20:80:0.1). [^{3}H]**LWH-154** was collected, rotary evaporated to dryness, and had a radiochemical purity of 99% when analyzed by RP-HPLC (Spherisorb ODS-2, 250×4.6 mm; water:acetonitrile:triethylamine = 30:70:0.3; Hewlett Packard HP1100 series pumps and UV detector). Mass spectroscopy gave a spectrum that was consistent with the reference **LWH-154** and a specific activity of 69 Ci/mmol.

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