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Research Article

The syntheses of [^{14}C] and [$^{13}C_2$,$^{15}N_3$]aprepitant

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Summary
In support of a program to develop a treatment for chemotherapy-induced nausea and vomiting, two isotopically labeled forms of neurokinin-1 receptor antagonist aprepitant have been synthesized. A [^{14}C]-labeled version was synthesized for use in metabolism studies, while a [$^{13}C_2$,$^{15}N_3$]-labeled version was synthesized for use in a study to determine the bioavailability of the final market image. Both syntheses utilized labeled chloroacetonitrile which was synthesized in two steps from labeled potassium cyanide. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: substance P; hydroxyacetonitrile; chloroacetonitrile; NK1

Introduction
Neurokinin **1** (NK1) receptor is a G-coupled protein which has the highest binding affinity for substance P among the endogenous neurokinins.[1] The release of substance P has been linked to inflammation and transmission of pain.[2] As a potential drug target, inhibition of substance P binding to the NK1 receptor has received significant attention over the last decade as a possible treatment for pain, migraines, chemotherapy-induced nausea and vomiting (CINV), and depression.[3] Recently, Merck's lead NK1 antagonist, aprepitant (**1**),[4] was approved for treatment of CINV.

During the course of development, several isotopically labeled aprepitant tracers were prepared to address a range of drug metabolism and receptor pharmacology issues. The primary tracer used for metabolism and distribution studies contained the C-14 label in the morpholine ring, and it showed aprepitant to be extensively metabolized.[5] Since *N*-dealkylation of the triazolone ring is a major metabolic pathway in all animal models studied, a tracer with a C-14 label in the triazolone ring was desired to study the metabolic fate of this fragment. Additionally, a stable isotope labeled form of

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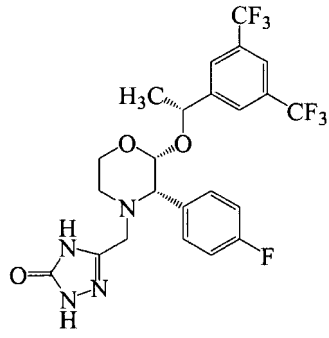
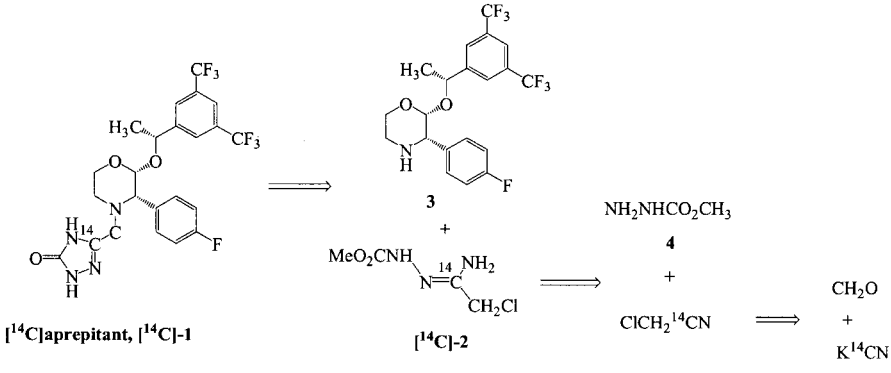


aprepitant, 1.

aprepitant with a minimum mass increase of 4 was required as a clinical tracer to determine the human bioavailability of the final market image (for an example of this type of application; see Reference[6]). Based on the synthesis of the above C-14-labeled tracer, incorporation of C-13 and N-15 could be readily accomplished to provide stable isotope labeled tracer with a mass increase of 5 amu.

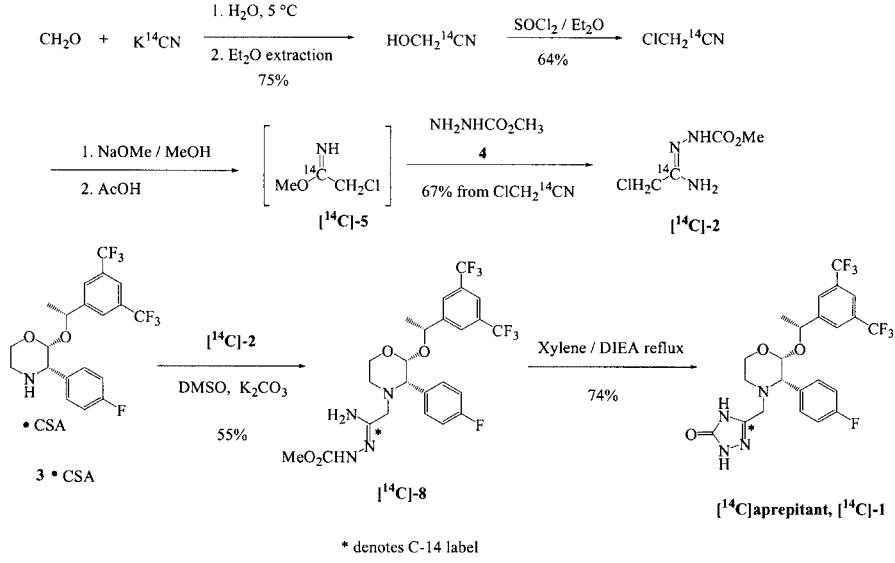
Results and discussion

A retrosynthetic analysis for the C-14-labeled version of NK1 antagonist tracer **[^{14}C]-1** based on the previously reported synthesis of aprepitant[7] led to the logical disconnection into amidrizone **[^{14}C]-2** and amine **3** (Scheme 1). Amine **3** was available internally[8] while **[^{14}C]-2** could be synthesized from [^{14}C]chloroacetonitrile by reaction with hydrazine **4**. Previous syntheses of labeled chloroacetonitrile have relied upon the dehydration of labeled chloroacetamide which necessitated harsh reaction conditions, the inconvenient and costly preparation of [^{14}C]chloroacetamide, and final purification by inefficient small-scale distillation.[9] We envisioned an alternative preparation of [^{14}C]chloroacetonitrile from [^{14}C]hydroxyacetonitrile which could be produced by the coupling of K^{14}CN and paraformaldehyde.[10]



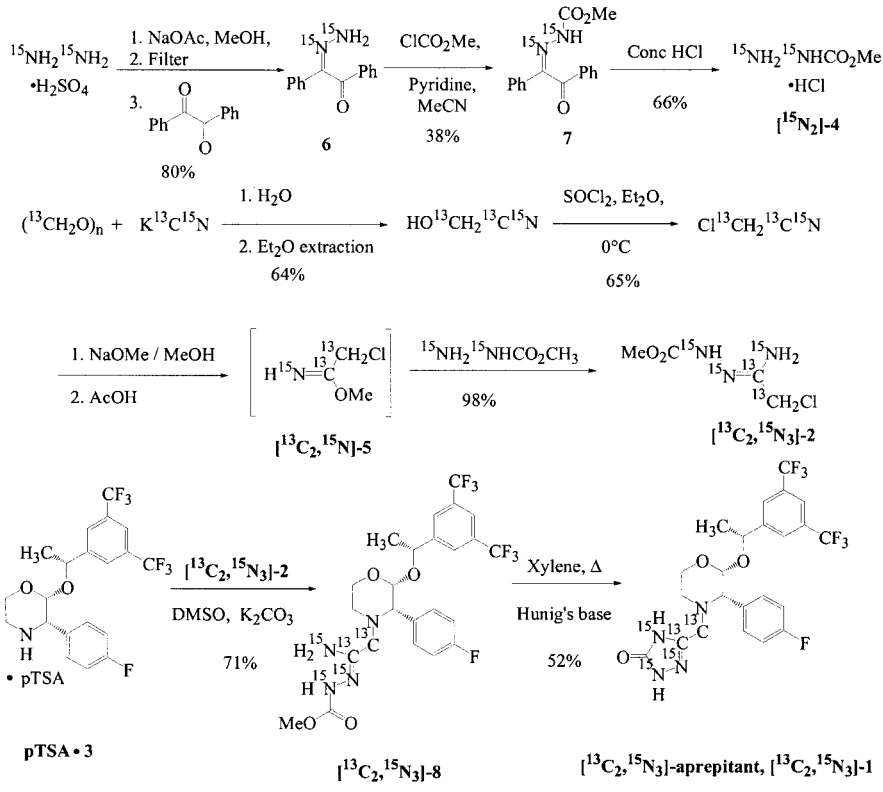
Scheme 1. Retrosynthetic analysis of [^{14}C]aprepitant

The reaction of $K^{14}CN$ and paraformaldehyde in water followed by continuous extraction for 12 h with Et_2O gave [^{14}C]hydroxyacetonitrile in 75% yield and high radiochemical purity (Scheme 2). The ethereal solution was reacted directly with $SOCl_2$ to afford [^{14}C]chloroacetonitrile in 64% yield. Maintaining the temperature at approximately 0°C was critical to minimize the by-product formation. The [^{14}C]chloroacetonitrile was then treated with NaOMe in MeOH followed by hydrazinocarboxylate **4** to give amidrizone **[^{14}C]-2** in a 67% radiochemical yield. The camphorsulfonic acid salt of morpholine **3** was coupled with **[^{14}C]-2** to give a 55% yield of **[^{14}C]-8** which was cyclized in hot xylene to give [^{14}C]aprepitant in 74% radiochemical yield (8.1 mCi, 94% radiochemical purity). Purification by preparative HPLC followed by crystallization gave 4.6 mCi of **[^{14}C]-1** with 99.8% radiochemical purity.



Scheme 2. Synthesis of [^{14}C]aprepitant, [^{14}C]-1

Our approach to [$^{13}C_2$,$^{15}N_3$]aprepitant was similar to that of the C-14 tracer except that the synthesis of **[$^{15}N_2$]-4** was also required. While the synthesis of **[$^{15}N_2$]-4** appeared trivial, attempts at mono-acylation of [$^{15}N_2$]hydrazine with methyl chloroformate using a variety of conditions gave substantial amounts of the bis-adduct unless a large excess of [$^{15}N_2$]hydrazine was utilized.[11] While the mono-adduct could be separated from the *bis*-adduct by column chromatography, other minor impurities were not separated and gave rise to impurities in subsequent reactions that were difficult to remove. As an alternative, a three-step procedure which provided **[$^{15}N_2$]-4** as a crystalline solid in high purity was used instead (Scheme 3). [$^{15}N_2$]Hydrazine was reacted with



Scheme 3. Synthesis of [$^{13}C_2$,$^{15}N_3$]aprepitant

benzil[12] to give the corresponding hydrazone, **6**, in 80% yield. Acylation of **6** with methyl chloroformate afforded **7** in 38% yield, and **7** was hydrolyzed in concentrated HCl to give carbamate **[$^{15}N_2$]-4** as the HCl salt in 66% yield and >95% purity.

The remaining steps in the synthesis of [$^{13}C_2$,^{15}N]aprepitant were accomplished as previously described for [^{14}C]aprepitant. Since both [$^{13}C_2$, ^{15}N]-hydroxyacetonitrile and [$^{13}C_2$,^{15}N]chloroacetonitrile are volatile, the quantity of compound present in the ethereal solutions was determined by ^1H NMR using naphthalene as an internal standard. Purification of the final compound was effected by preparative HPLC followed by crystallization to give 569 mg of [$^{13}C_2$, $^{15}N_3$]aprepitant with 99.7% UV purity (238 nm).

In summary, we have reported the syntheses of [^{14}C] and [$^{13}C_2$,$^{15}N_3$]aprepitant which served as important tracers for obtaining detailed late-stage drug metabolism information. Both syntheses benefitted from the use of an improved synthesis of labeled chloroacetonitrile from readily available labeled precursors.

Experimental

General

[$^{15}N_2$]hydrazine hydrogen sulfate, potassium [^{13}C,^{15}N]cyanide, and [^{13}C] paraformaldehyde were obtained from Cambridge Isotope Laboratories, and **3** was obtained from Merck Process Research. Anhydrous solvents were obtained from Aldrich and were dried over 4 Å molecular sieves for at least 24 h prior to use. Analytical HPLC was performed using a Shimadzu HPLC system with LC-10ATVP pumps, a SPD-10AVP UV detector, a CTO-10ASVP column oven heated to 30°C, and a SCL-10A controller. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian U-400 spectrometer and were referenced to the residual solvent peak (7.26 and 77.00 ppm for $CDCl_3$, 3.30 and 49.2 ppm for CD_3OD, and 2.49 and 39.5 ppm for $^2H_6$-DMSO). LC/MS analyses were performed on an HP MSD-100 using a XDB-C8 column, with 5–95% gradient over 15 min with MeCN-2 mM ammonium formate buffer (pH = 3.5) and electrospray ionization. The reaction products were identified by HPLC comparison with the commercially available materials or Merck Process Chemistry intermediates when available using either method A (40/60 to 60/40 MeCN/0.1% aqueous $HClO_4$ gradient elution over 30 min, Zorbax RX C-8); method B (35/65 to 80/20 MeCN/0.1% H_3PO_4 over 20 min, YMC ODS-AQ); method C (20% MeCN–0.1% H_3PO_4 for 30 min, Zorbax RX C-8); method D (0–5% MeCN–0.1% phosphoric acid over 30 min, Phenomenex Aqua C-18); or method E (45% MeCN/0.1% aqueous $HClO_4$ for 30 min, Zorbax RX C-8). All HPLC analyses were conducted using a flow rate of 1 ml/min on 4.6 mm × 250 mm columns heated to 30°C and concluded with a 10 min wash of 100% MeCN. Preparative HPLC conditions are described separately.

*Benzil [$^{15}N_2$]hydrazone (**[$^{15}N_2$]-6**)*

The procedure of Nenitzescu was modified as follows.[12] A solution of 15.5 g (117 mmol) of [$^{15}N_2$]hydrazine sulfate and 39.6 g (483 mmol) of NaOAc in 100 ml of water was heated for 30 min at 60°C under N_2. The solution was diluted with 80 ml of MeOH and was cooled to room temperature (rt). The resulting precipitate was removed by filtration, and 20.0 g (95.2 mmol) of benzil was added to the solution. The resulting slurry was heated at 60°C for 2 h with vigorous stirring. The solution was cooled to rt, and the precipitate removed by filtration to give 17.2 g (80%) of **[$^{15}N_2$]-6** as a yellow solid. LC/MS (M/Z (abundance)): 227.1 (100), 228.1 (15), 249.1(13). ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (m, 2H), 7.53 (m, 3H), 7.47 (m, 3H), 7.35 (m, 2H).

*N-(1-[^{15}N]-1-aza-3-oxo-2,3-diphenylprop-1-enyl)[^{15}N]methoxycarboxamide (**[$^{15}N_2$]-7**)*

A solution of 15.5 g (68.5 mmol) of hydrazone **[$^{15}N_2$]-6** in 400 ml of MeCN and 19 ml (240 mmol) of pyridine was stirred rapidly at rt as 21 ml (270 mmol) of methylchloroformate was added in 2 ml aliquots over 1h. The reaction mixture was stirred overnight at rt, and the precipitate was removed by filtration. The solution was concentrated to dryness at reduced pressure to give 23.1 g of a yellow solid which was purified by flash column chromatography on neutral alumina (100% hexane to 25% EtOAc in hexane). Product containing fractions were combined to give 7.40 g (26.1 mmol, 38%) of **[$^{15}N_2$]-7** as a yellow solid. LC/MS (M/Z (abundance)): 285.1 (100), 286.1 (18), 307 (15). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (t, $J = 80$ Hz, 1H), 8.16 (d, $J = 7.2$ Hz, 1H), 7.86 (d, $J = 7.1$ Hz, 1H), 7.60 (m, 2H), 7.45 (m, 3H), 7.33 (m, 3H), 3.81 (s, 3H).

*Methyl [$^{15}N_2$]hydrazinocarbamate hydrochloride (**[$^{15}N_2$]-4**)*

A biphasic solution of 3.33 g (11.7 mmol) of **[$^{15}N_2$]-7** in 30 ml of CH_2Cl_2 and 15 ml of conc. HCl was stirred and heated at 70°C under N_2, and the reaction followed by TLC (4:1 Hex:EtOAc) during which time a white precipitate formed. After 3 h, the solid was removed by filtration through a glass frit to give 998 mg (7.77 mmol, 66%) of **[$^{15}N_2$]-4**, as a white solid. ^{1}H NMR (400 MHz, D_6-DMSO) δ 3.66 (s). ^{13}C NMR (100 MHz, D_6-DMSO) δ 156.2 (br), 53.0.

[$^{13}C_2$,^{15}N]Hydroxyacetonitrile

A solution of 4.33 g (64.6 mmol) of K^{13}C^{15}N in 15 ml of water was cooled and stirred at 0°C as 1.96 g (42 mmol) of [^{13}C]paraformaldehyde was added. The solution was stirred for 35 min at which time the pH was adjusted to 2.5 with conc. H_2SO_4. The solution was transferred to a liquid–liquid continuous extraction apparatus and was extracted with 120 ml Et_2O for 48 h. Midway through the extraction an additional 50 ml of ether was added. ^{1}H NMR analysis of the ethereal solution using naphthalene as an internal standard showed a total of 2.50 g (64%) of [$^{13}C_2$,^{15}N]hydroxyacetonitrile to be present in 30.4 g (*ca.* 43 ml) of ether. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78 (br s, 1H), 4.24 (ddd, $J = 151.2$, 6.1, 1.1 Hz, 2H) spectrum also shows two 6 line multiplets at 7.44 (4.5 H), 7.80 (4.5H) for naphthalene and peaks at 3.44 (q, $J = 7.0$ Hz, 33.4H) and 1.17 (t, 50H, $J = 7.0$ Hz) for diethyl ether. ^{13}C NMR (100 MHz, $CDCl_3$) δ 118.0 (dd, $J = 60$, 16 Hz), 48.2 (d, $J = 58$ Hz); the spectrum also contains H^{13}C^{15}N at 109.1 (d, $J = 18$ Hz) and diethyl ether at 65.7,15.1.

[$^{13}C_2$,^{15}N]Chloroacetonitrile

A solution of *ca.* 1.39 g (23.1 mmol) [$^{13}C_2$,^{15}N]hydroxyacetonitrile in 33 ml of Et_2O and 4.1 ml (79 mmol) of pyridine was stirred and cooled at 0°C as 4.0 ml

(55 mmol) of $SOCl_2$ was added over 45 min. After complete addition, the reaction was stirred at 0°C for 1 h and at rt for 3 h, and 10 ml of a solution of saturated aq. NaCl was added. The aqueous layer was removed and the organic layer was extracted with 5 ml of saturated aq. NaCl. The organic layer was dried ($MgSO_4$) and filtered to give 23.7 g of an ethereal solution. ^{1}H NMR analysis using naphthalene as an internal standard showed the yield to be 1.20 g (14.9 mmol, 65%) of [$^{13}C_2$,^{15}N]chloroacetonitrile. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (ddd, J = 80, 7.8, 1.7 Hz). ^{13}C NMR(100 MHz, $CDCl_3$) δ 114.3 (dd, J = 66, 18 Hz), 24.5 (dd, J = 66, 2.5 Hz). The spectrum also shows peaks for naphthalene at 133.4, 127.8, 125.8, diethyl ether at 65.8 and 15.2, and a [$^{13}C_2$,^{15}N] containing by-product at 113 (dd) and 48.2 (d).

*N-((1Z)-2-[^{15}N]amino-1-[^{15}N]aza-3-chloro-[1-^{15}N,2,3-$^{13}C_2$]prop-1-enyl)-methoxycarboxamide (**[$^{13}C_2$, $^{15}N_3$]-2**)*

A solution of *ca.* 680 mg (8.51 mmol) of $Cl^{13}CH_2^{13}C^{15}N$ in 64 ml of ether was cooled to 0°C as 17 ml of a 0.33 M solution (5.64 mmol) of NaOMe in MeOH was added. The solution was warmed to rt and stirred for 1 h, and 0.50 ml of HOAc (8.74 mmol) and 984 mg (7.6 mmol) of methyl [$^{15}N_2$]hydrazinocarbamate hydrochloride were added sequentially. The reaction was stirred under N_2 overnight, and the solvent was removed under a stream of N_2 to give 1.43 g of **[$^{13}C_2$,$^{15}N_3$]-2** as a brown oil. ^{1}H NMR (D_6-DMSO, 400 MHz) δ 4.05 (d, J = 76 Hz, 2H), 3.58 (d, J = 4.3 Hz, 3H).

*N-[3-(2-{(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy}(3S,2R)-3-(4-fluorophenyl)morpholin-4-yl)(1Z)-2-[^{15}N]amino-1-[^{15}N,$^{13}C_2$]azaprop-1-enyl]-methoxy-carbox[^{15}N]amide (**[$^{13}C_2$, $^{15}N_3$]-8**)*

A slurry of 1.15 g (6.7 mmol) of chloroamidrizone **[$^{13}C_2$,$^{15}N_3$]-2**, 1.72 g (2.82 mmol) of *p*-toluenesulfonic acid salt **3**, and 2.14 g (15.4 mmol) of K_2CO_3 in 7 ml of DMSO was stirred at rt for 2 h at which time HPLC analysis (method A) showed the reaction to be complete. The reaction mixture was diluted with 100 ml of water and extracted with EtOAc (4 × 20 ml). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to dryness to give 4.7 g of a brown oil. The oil was purified by flash column chromatography on silica gel (20:1:2 $PhCH_3$:EtOAc:MeOH), and product containing fractions were combined and concentrated to give 2.7 g of a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.35 (m, 2H), 7.16 (s, 2H), 7.04 (t, J = 8.6 Hz, 2H), 4.89 (q, J = 6.5 Hz, 1H), 4.34 (d, J = 2.8 Hz, 1H), 4.24 (td, J = 11.7, 2.1 Hz, 1H), 3.77 (s, 3H), 3.66 (dd, J = 1.9, 11.2 Hz, 1H), 3.50 (m, 1H), 3.47 (s, 1H), 2.97 (d, J = 11.7 Hz, 1H), 2.92 (d, J = 17, 136 Hz, 1H), 2.51 (t, J = 11.8 Hz, 1H), 1.47(d, J = 6.6 Hz, 3H).

*[$^{13}C_2$,$^{15}N_3$]aprepitant (**[$^{13}C_2$,$^{15}N_3$]-1**)*

A solution of 2.37 g (4.20 mmol) of **[$^{13}C_2$,$^{15}N_3$]-8** in 50 ml of xylene and 15 ml of diisopropylethylamine was purged with N_2 and heated at 150°C for 3 h. HPLC analysis (method A) showed the reaction to be complete. The solvents were then removed at reduced pressure to afford 2.471 g of an orange oil. The compound was taken up in $PhCH_3$ and purified by flash column chromatography on silica gel (10:1:1 $PhCH_3$:MeOH:EtOAc). Product containing fractions were combined and concentrated to give 1.34 g of an off white solid, and HPLC analysis (method A) showed the compound to be 89% pure by UV analysis at 220 nm. The solid was dissolved in 5 ml of MeOH and 2 ml of 0.1% H_3PO_4 and was purified in four portions by preparative HPLC (Zorbax RX C-8, 50:50 MeCN:0.1% aq. H_3PO_4, 20 ml/min). The fractions were analyzed by HPLC (method A) and pure fractions were pooled. The combined fractions were concentrated at reduced pressure until H_2O began condensing, neutralized to pH > 8 with Na_2CO_3, and extracted with EtOAc (5×50 ml). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated, at reduced pressure to give 839 mg of a white solid. The solid was dissolved in 5 ml of MeOH and was heated at 30°C as 3 ml of water was added over 1 h by syringe pump. The slurry was heated at 50°C and was then cooled to rt. The solid was removed by filtration through a glass frit and was washed with 2 ml of water. The solid was dried under vacuum for 3 h to give 569 mg of [$^{13}C_2$,$^{15}N_3$]aprepitant. HPLC analysis (method B) showed the UV purity (220 nm) to be 99.6%. LC/MS (M/Z (abundance)): 534.1 (0.63) 535.0 (100), 536 (22.1), 537 (3.3), 538 (0.1). ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (s, 1H), 7.52 (t, $J = 6.5$ Hz, 2H), 7.32 (s, 2H), 7.05 (t, $J = 8.9$ Hz, 2H), 4.95 (q, $J = 6.6$ Hz, 1H), 4.35 (d, $J = 2.8$ Hz, 1H), 4.28 (td, $J = 11.7$, 2.4 Hz, 1H), 3.66 (dq, $J = 11.4$, 1.9 Hz, 1H), 3.56 (dm, $J = 140$ Hz, 1H), 3.49 (t, $J = 2.4$ Hz, 1H), 2.87 (d, $J = 11.9$ Hz, 1H), 2.85 (ddm, $J = 135$, 14.2 Hz, 1H), 2.49 (tt, $J = 11.5$, 2.7 Hz, 1H), 1.44 (d, $J = 6.7$ Hz, 3H).

[^{14}C]Hydroxyacetonitrile

The previously described route for [$^{13}C_2$,^{15}N]hydroxyacetonitrile was followed using 114 mg (1.75 mmol, 57 mCi/mmol) of K^{14}CN, 4 ml of water, and 56 mg (1.87 mmol) of paraformaldehyde. Liquid–liquid continuous extraction for 12 h gave 77 ml of an ethereal solution that contained 86 mCi of the desired product. ^{1}H NMR and HPLC (method C) analysis showed the compound to be of > 95% radiochemical purity.

[^{14}C]Chloroacetonitrile

The previously described route for [$^{13}C_2$,^{15}N]chloroacetonitrile was followed using 63 mCi (1.1 mmol, 57 mCi/mmol) of [^{14}C]hydroxyacetonitrile in 51 ml of

Et_2O, 140 mg (1.7 mmol) of pyridine, and 223 mg (1.9 mmol) of $SOCl_2$ to give 52 mCi in 23 ml of ether. HPLC analysis (method C) showed the product to have a purity of 82% with 18% [^{14}C]hydroxyacetonitrile present.

*N-((1Z)-2-amino-1-aza-3-chloro-[3-^{14}C]prop-1-enyl)methoxycarbox-amide (**[^{14}C]-2**)*

The previously described route for **[$^{13}C_2$,$^{15}N_3$]-2** was followed using 24.3 mCi (0.43 mmol, 82% purity, 57 mCi/mmol) of $ClCH_2$ ^{14}CN, 98.3 mg (1.3 mmol) of $ClCH_2CN$, 7 ml of MeOH, 89 mg (1.66 mmol) NaOMe, 100 mg (1.66 mmol) of HOAc, and 137 mg (1.52 mmol) of methyl hydrazinocarbamate hydrochloride to give 20 mCi of a methanol solution which contained 63% **[^{14}C]-2**, 16% [^{14}C]hydroxyacetonitrile, and 10% chloroacetonitrile by HPLC analysis (method D).

*[^{14}C]aprepitant, (**[^{14}C]-1**)*

The previously described route for **[$^{13}C_2$,$^{15}N_3$]-1** was followed using 20 mCi (1.83 mmol, 11 mCi/mmol, 63% radiochemical purity) of chloroamidrizone **[^{14}C]-2,** 1.4 g (2.0 mmol) of (*R*)-camphorsulfonic acid salt **3**, and 632 mg (5.6 mmol) of K_2CO_3 in 15 ml of DMSO. Product isolation was performed as described previously to give 11 mCi of **[^{14}C]-8**. The crude reaction extract was then refluxed in 30 ml Xylene overnight to give 10 mCi **[^{14}C]-1**. Prep-HPLC (Zorbax RX C-8, 45% MeCN–0.1% phosphoric acid, 20 ml/min) gave 5.1 mCi of **[^{14}C]-1** which was crystallized from 2.5 ml MeOH and 1 ml of H_2O as previously described to give 190 mg (4.6 mCi, 23% yield) of [^{14}C]aprepitant with a radiochemical purity of 99.8% (method E) and a specific activity of 13 mCi/mmol.

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References

1. Quartara L, Maggi CA. *Neuropeptides* 1998; **32**: 1–49.
2. Otsuka M, Yanagisawa M. *J Physiol* 1988; **395**: 255–270.
3. (a) Maggi CA, Patacchini R, Rovero P, Giachetti A. *J Auton Pharmacol* 1993; **13**: 23–93. (b) Navari RM, Reinhardt RR, Gralla RJ, Kris MG, Hesketh PJ, Khojasteh A, Kindler H, Grote TH, Pendergrass K, Grunberg SM, Carides AD, Gertz BJ. *N Engl J Med* 1999; **340**: 190–195. (c) Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hale JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR,

Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NMJ. *Science* 1998; **281**: 1640–1645.

4. Hale JJ, Mills SG, MacCoss M, Finke PE, Cascieri MA, Sadowski S, Ber E, Chicchi GG, Kurtz M, Metzger J, Eiermann G, Tsou NN, Tattersall FD, Rupniak NMJ, Williams AR, Rycroft W, Hargreaves R, MacIntyre DE. *J Med Chem* 1998; **41**: 4607–4614.
5. (a) Huskey S-EW, Dean BJ, Bakhtiar R, Sanchez RI, Tattersall FD, Rycroft W, Hargreaves R, Watt AP, Chicchi GG, Keohane C, Hora DF, Chiu S-HL. *Drug Metab Dispos* 2003; **31**: 785–791. (b) Huskey S-EW, Dean BJ, Doss GA, Wang Z, Hop CECA, Anari R, Finke PE, Robichaud AJ, Zhang M, Wang B, Strauss JR, Cunningham PK, Feeney WP, Franklin RB, Baillie TA, Chiu S-HL. *Drug Metab Dispos* 2004; **32**: 246–258.
6. Chavez-Eng CM, Constanzer ML, Matuszewski BK. *J Chrom B: Anal Tech Biomed Life Sci* 2002; **767**: 117–129.
7. Cowden CJ, Wilson RD, Bishop BC, Cottrell IF, Davies AJ, Dolling U-H. *Tetrahedron Lett* 2000; **41**: 8661–8664.
8. Brands KMJ, Payack JF, Rosen JD, Nelson TD, Candelario A, Huffman MA, Zhao MM, Li J, Craig B, Song ZJ, Tschaen DM, Hansen K, Devine PN, Pye PJ, Rossen K, Dormer PG, Reamer RA, Welch CJ, Mathre DJ, Tsou NN, McNamara JM, Reider P. *J Am Chem Soc* 2003; **125**: 2129–2135.
9. Hayashi N, Toga T, Murata T. *J Label Compd Radiopharm* 1974; **10**: 609–615.
10. (a) Castro CE, O'Shea SK, Wang W, Bartnicki EW. *Environ Sci Technol* 1996; **30**: 1185–1191. (b) Thirkettle JE, Baldwin JE, Griffin JP, Schofield CJ. *Chem Commun* 1997; 1025–1026.
11. Rutjes FPJT, Teerhuis NM, Hiemstra H, Speckamp WN. *Tetrahedron* 1993; **49**: 8605–8628.
12. Nenitzescu CD, Solomonica E. Phenylbenzoyldiazomethane. In *Organic Synthesis Collection*, vol. 2, Blatt AH (ed.). Wiley: New York, 1943; 496–497.

