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Letter

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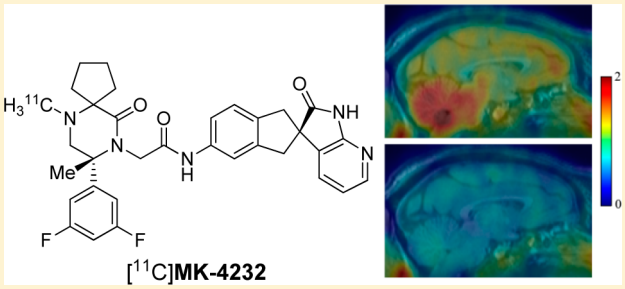
[^{11}C]MK-4232: The First Positron Emission Tomography Tracer for the Calcitonin Gene-Related Peptide Receptor

Ian M. Bell,*[,†] Steven N. Gallicchio,[†] Craig A. Stump,[†] Joseph G. Bruno,[∥] Hong Fan,[§] Liza T. Gantert,[§] Eric D. Hostetler,[§] Amanda L. Kemmerer,[∥] Melody McWherter,[†] Eric L. Moore,[‡] Scott D. Mosser,[∥] Mona L. Purcell,[§] Kerry Riffel,[§] Christopher A. Salvatore,[‡] Sandra Sanabria-Bohórquez,[§] Donnette D. Staas,[†] Rebecca B. White,[#] Mangay Williams,[§] C. Blair Zartman,[†] Jacquelynn J. Cook,[§] Richard J. Hargreaves,[⊥] Stefanie A. Kane,[‡] Samuel L. Graham,[†] and Harold G. Selnick[†]

Departments of [†]Medicinal Chemistry, [‡]Pain & Migraine, [§]Imaging, [∥]In Vitro Pharmacology, [⊥]Neuroscience & Ophthalmology, and [#]Pharmacokinetics Pharmacodynamics & Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania 19486, United States

Supporting Information

ABSTRACT: Rational modification of the potent calcitonin gene-related peptide (CGRP) receptor antagonist MK-3207 led to a series of analogues with enhanced CNS penetrance and a convenient chemical handle for introduction of a radiolabel. A number of ^{11}C-tracers were synthesized and evaluated in vivo, leading to the identification of [^{11}C]**8** ([^{11}C]MK-4232), the first positron emission tomography tracer for the CGRP receptor.



KEYWORDS: *MK-4232, calcitonin gene-related peptide receptor, positron emission tomography*

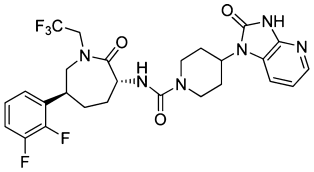
Migraine is a common neurovascular disorder, characterized by attacks of headache that are often accompanied by such symptoms as nausea, photophobia, and phonophobia.[1] It is a leading cause of disability and poses a significant economic burden.[1] Although the precise mechanisms underlying migraine pathogenesis are not fully understood, two theories have been most influential. The vascular theory attributes migraine headache to dilation of cerebral or meningeal arteries, while the neural theory holds that migraine is primarily a disorder of the central nervous system (CNS).[2]

The 37 amino acid neuropeptide calcitonin gene-related peptide (CGRP) is thought to play a key role in migraine pathogenesis.[3] It is distributed widely in the central and peripheral nervous system, and it is known to be a highly potent vasodilator.[3] More recently, significant evidence has pointed to an important role for CGRP as a neuromodulator in the brain.[2] The CGRP receptor (CGRP-R) is a G protein-coupled receptor (GPCR) and is heterodimeric in nature, consisting of the calcitonin receptor-like receptor (CLR) and receptor activity modifying protein 1 (RAMP1). The components of the CGRP receptor have been detected in the smooth muscle layer of intracranial blood vessels, as well as in the neurons in the trigeminal ganglion.[2]

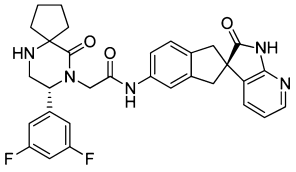
While a number of key experiments suggested that blocking CGRP function could have utility for migraine treatment, definitive evidence has been provided by clinical studies with the CGRP receptor antagonists olcegepant, telcagepant (**1**), MK-3207 (**2**), and BI-44370, which have all shown clinical efficacy as acute antimigraine therapies.[4−7] Although the clinical efficacy observed with these novel agents is not in dispute, the precise site of action of the compounds remains a topic for debate. It is certainly possible that a CGRP receptor antagonist (CGRP-RA) could achieve some antimigraine efficacy by blocking CGRP-induced activation of trigeminovascular pain pathways in the periphery, but the presence of CGRP and its receptor in parts of the brain that may be involved in migraine attacks suggests that a central site of action is also plausible.[8]

At clinically efficacious doses, both telcagepant (**1**)[9] and MK-3207 (**2**)[10] (Chart 1) have been found to effectively block the CGRP receptor in the periphery, based on results from the

Chart 1. Selected CGRP Receptor Antagonists



telcagepant, **1**
K_i = 0.77 nM



MK-3207, **2**
K_i = 0.021 nM

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capsaicin-induced dermal vasodilation pharmacodynamic model.[11,12] Nonetheless, other evidence, including the observation that dosing of telcagepant to rhesus monkeys led to measurable drug levels in their cerebrospinal fluid (CSF), has led to the suggestion that engagement of central CGRP receptors might be necessary for maximal antimigraine efficacy.[2]

In order to directly address the question of central CGRP receptor occupancy by antagonists such as telcagepant, we sought to identify a positron emission tomography (PET) tracer for the CGRP receptor that would be suitable for clinical use. Such a molecule should possess good membrane permeability and lack susceptibility for being actively transported out of the CNS, for example, by the P-glycoprotein (P-gp) efflux pump. Additionally, an ideal PET ligand should possess a good binding potential.[13] In terms of compound design, this translates to a good level of receptor affinity relative to the receptor abundance in the target tissue ($B_{max}/K_d > 10$), and a moderate lipophilicity to mitigate nonspecific binding ($logD_{7.0} < 3.5$).[14]

Overall, it seemed that MK-3207 (**2**) might be a reasonable starting point for this effort. While **2** was a substrate for the P-gp transporter (P-gp = 25, Table 1), it had acceptable passive permeability in LLC-PK1 cells ($P_{app} = 28 \times 10^{-6}$ cm/s) and lipophilicity ($logD_{7.0} = 2.35$), and excellent receptor affinity ($K_i = 21$ pM). Since the highest level of the CGRP-R in the brain of rhesus monkeys was found to be in the cerebellum ($B_{max} = 24$ nM),[15] and using K_i as a surrogate for K_d, the binding potential for a compound with this level of receptor affinity was impressive ($B_{max}/K_i > 1000$).

Table 1. Data for CGRP Receptor Antagonists

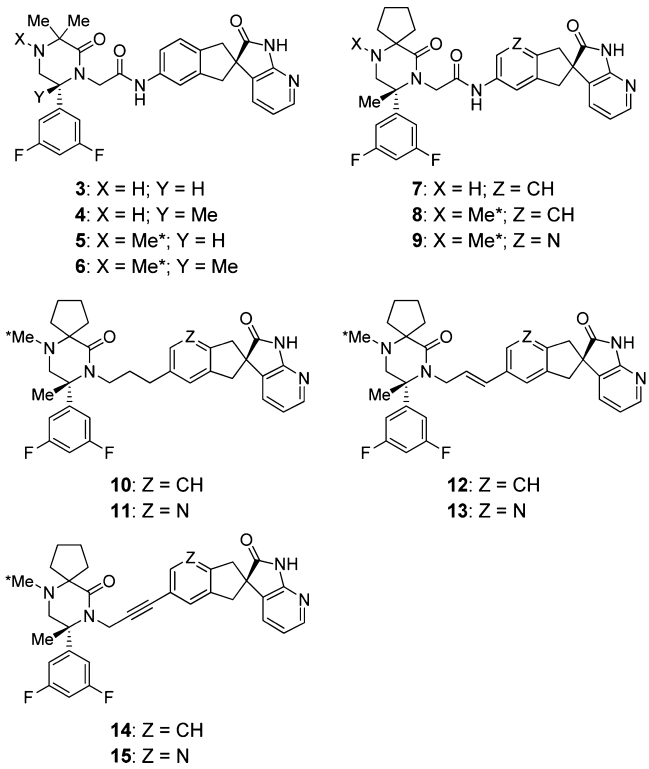
compd	CGRP K_i (pM)[a,b]	P-gp[c]	monkey f_u (%)[d]	CSF:PL (%)[e]
1	770 ± 70 (13)	24	3.8	1.3
2	21 ± 6 (14)	25	9.6	2.9
3	34 ± 11 (27)	33	42.9	4.35
4	110 (2)	20	33.3	ND
5	22 ± 1 (15)	8.9	20.5	ND
6	56 ± 20 (5)	2.8	11.7	4.79
7	40 ± 12 (8)	9.3	10.4	6.61
8	46 ± 15 (7)	1.7	4.3	3.12
9	17 (2)	14	ND	ND
10	500 (2)	NM[f]	0.2	ND
11	45 (2)	1.8	6.1	ND
12	94 ± 21 (5)	NM[f]	0.2	0.53
13	21 (2)	2.0	5.1	ND
14	110 ± 22 (6)	NM[f]	0.8	0.99
15	19 ± 7 (4)	1.5	3.1	2.64

[a]Mean value ± standard deviation, where appropriate; the number of replicates is in parentheses. [b]The K_i value for inhibition of ^{125}I-hCGRP binding was determined using membranes from HEK293 cells stably expressing human CLR/RAMP1.[11] [c]Human P-gp transport ratio BA/AB determined using LLC-MDR1 cells. [d]Unbound fraction determined in plasma using equilibrium dialysis methodology. [e]Ratio of CSF AUC to plasma AUC determined in cisterna magna-ported rhesus monkey.[12] [f]Accurate values could not be determined due to the physical properties of compound.

Both **1** and **2** were found to exhibit measurable levels in CSF following dosing in cisterna magna-ported rhesus monkeys (Table 1). For each compound, about one-third of the free plasma concentration was observed in CSF (for **2**, monkey plasma $f_u = 9.6\%$ and monkey CSF/plasma = 2.9%; Table 1), suggesting that there may be significant free drug in the CNS.[12] However, it should be noted that the CSF levels of P-gp substrates like **1** and **2** are unreliable indicators of free brain concentrations.[16]

Compound **3** (Chart 2), in which the piperazinone spirocyclopentyl moiety in **2** was replaced with geminal dimethyl substituents, retained excellent CGRP-R affinity and a high P-gp transport ratio. This modification also conferred a 4-fold increase in monkey plasma f_u (42.9% for **3** vs 9.6% for **2**; Table 1). In order to reduce the P-gp transport manifested in compounds **2** and **3**, we sought to explore modifications that would either add steric bulk close to polar functionality or replace polar groups with lipophilic alternatives.

Chart 2. Potential PET Tracers and Precursors



The addition of a benzylic methyl substituent to the piperazinone ring in **3** produced compound **4** (Chart 2), which had reduced CGRP receptor affinity but also had slightly attenuated susceptibility for P-gp transport (Table 1). Similarly, the addition of a methyl group to the piperazinone nitrogen of **3** afforded compound **5**, which also appeared to have a reduced level of P-gp transport (Table 1). Gratifyingly, combining these observations led to the tetramethylpiperazinone **6**, a potent CGRP-RA ($K_i = 56$ pM) with low susceptibility for active transport by P-gp (transport ratio = 2.8).

The reduction in P-gp transport achieved in modifying **3** (P-gp = 33) to give **6** (P-gp = 2.8) translated to a relatively modest increase in CSF levels relative to unbound plasma levels. For **3**, 10% of unbound drug was observed in CSF; whereas for **6**, 40% of unbound drug was observed in CSF (Table 1). These results suggested that **6** represented an improvement over **3** in terms of CNS penetrance, but that it was not optimal.

Application of the same strategy to **2** led to compounds **7** and **8** (Chart 2). Both **7** and **8** maintained high affinity for the CGRP-R (Table 1) but, while **7** was a moderate substrate for P-

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gp (P-gp = 9.3), **8** exhibited minimal susceptibility for this transporter (P-gp = 1.7). Moreover, compound **8** exhibited CSF levels that corresponded to more than 70% of unbound plasma levels, indicating an increased level of CNS penetrance with respect to **2**. The corresponding aza-analogue **9**, an *N*-methylated version of the previously reported MK-8825,[17] exhibited significantly increased P-gp susceptibility (P-gp = 14), perhaps because of the introduction of an additional hydrogen bond acceptor.

Importantly, the *N*-methyl substituent on the piperazinone ring of compounds like **6** and **8** offered a convenient approach to the synthesis of potential PET ligands: methylation of a suitable precursor (**4** or **7**) with [^{11}C]methyl triflate. Indeed, treatment of **7** with [^{11}C]methyl triflate in acetone followed by HPLC purification afforded [^{11}C]**8**, and the other potential PET tracers shown in Chart 2 were synthesized analogously.

Preliminary evaluations of the uptake kinetics of tracers [^{11}C]**5**, [^{11}C]**6**, and [^{11}C]**8** were conducted in anesthetized rhesus monkeys. Figure 1 illustrates the time-activity curves for [^{11}C]**5** and [^{11}C]**8** for both cerebellum (circles) and white matter (triangles).

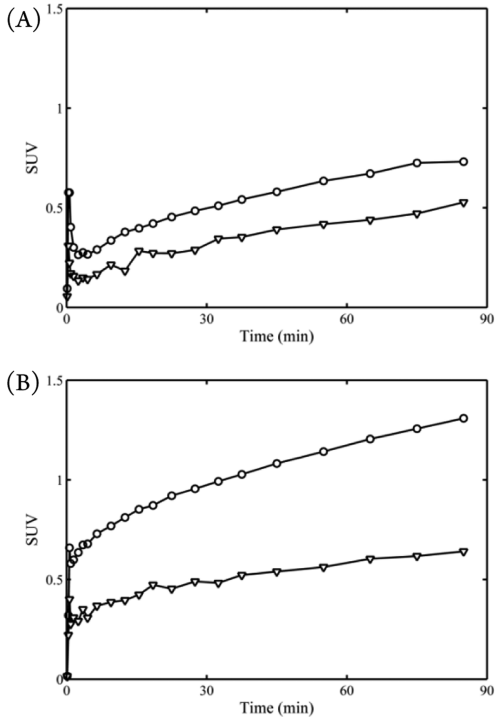

Figure 1. Decay-corrected time−activity curves showing SUV in cerebellum (circles) and white matter (triangles) for [^{11}C]**5** (A) and [^{11}C]**8** (B) in rhesus monkey.

Consistent with earlier autoradiography mapping studies, the highest level of binding activity in the brain was generally observed in the cerebellum.[12] The signal in white matter appeared to be indicative of nonspecific binding, and the ratio of the standardized uptake value (SUV) in cerebellum to SUV in white matter provided an estimate of the specific signal for the tracer. For [^{11}C]**5**, the apparent specific signal was modest (Figure 1A) and the uptake at 80 min postdose in cerebellum was only slightly higher than that in white matter ($SUV_{cerebellum}/SUV_{WM} \approx 1.4$; Table 2). In contrast, [^{11}C]**8** provided a significantly higher signal in cerebellum than in white matter ($SUV_{cerebellum}/SUV_{WM} \approx 2.0$). The results obtained with [^{11}C]**6** were intermediate between [^{11}C]**5** and [^{11}C]**8** (Table 2). Thus, the magnitude of the apparent specific signal for these tracers correlated inversely with their susceptibility for P-gp transport (Table 2).

In addition to increasing steric bulk around the piperazinone ring in order to attenuate P-gp transport, replacement of the polar central amide moiety with lipophilic groups was evaluated. These efforts led to the alkane, alkene, and alkyne analogues **10**−**15** (Chart 2), which were prepared as previously described.[18] In general, these compounds maintained excellent affinity for the CGRP-R, although replacement of the amide moiety with an ethylene spacer did lead to some reduction in receptor affinity (compare **10** with **8** and **11** with **9**; Table 1).

As expected, these amide replacements conferred increased lipophilicity, and the indanyl analogues **10**, **12**, and **14** had $logD_{7.0} > 4$, outside of the target range (Table 2). The high lipophilicity of compounds **10**, **12**, and **14** correlated with low unbound fraction in monkey plasma. Moreover, reliable values for permeability and P-gp transport could not be determined for these three compounds, apparently because their poor physical properties led to high levels of nonspecific binding. For the corresponding azaindanyl analogues (**11**, **13**, and **15**), the $logD_{7.0}$ values were more reasonable and the compounds exhibited good permeability and low susceptibility for P-gp transport in vitro (Table 2). Additionally, the CSF concentrations of **12**, **14**, and **15** following dosing in rhesus monkeys approximated the corresponding levels of unbound drug in plasma (Table 1), indicating that these compounds were indeed CNS penetrant. Overall, it appeared that this strategy of amide replacement generally afforded compounds with low P-gp susceptibility and good CNS penetrance.

On the basis of the overall profiles of compounds **10**−**15**, the [^{11}C]-tracers were prepared from the corresponding *des*-methyl analogues, using essentially identical methodology to that used to synthesize [^{11}C]**8**, and uptake kinetics were evaluated in anesthetized rhesus monkeys. The results of these studies are summarized in Table 2 in terms of SUV at 80 min postdose in cerebellum and white matter. On the basis of the ratio of $SUV_{cerebellum}/SUV_{WM}$ in these preliminary studies, it appeared that the highest specific signals were obtained with [^{11}C]**11**, [^{11}C]**13**, and [^{11}C]**15**; intermediate results were observed with [^{11}C]**12** and [^{11}C]**14**; and a low specific signal was found for [^{11}C]**10**. This rank-ordering of the tracers in terms of the magnitude of their specific signals correlates inversely with their CGRP K_i values (Table 2), consistent with the use of binding potential (B_{max}/K_d) as a guide to specific receptor binding. Overall, [^{11}C]**8** (MK-4232) had the best in vivo profile of the tested PET tracers and it was selected for further evaluation.

In radioligand binding assays, compound **8** had excellent affinity for CGRP receptors from both human (K_i = 46 pM) and rhesus monkey (K_i = 33 pM). In a cell-based functional assay, **8** was a potent CGRP receptor antagonist (cAMP IC_{50} = 0.097 nM) and its activity was right-shifted about 4-fold by the addition of 50% human serum (cAMP + HS IC_{50} = 0.39 nM). In terms of selectivity, **8** was >30,000-fold selective for the CGRP receptor versus a standard panel of more than 160 enzymes, receptors, and transporters. Thus, **8** is a highly potent and selective antagonist of primate CGRP receptors.

Baseline PET scans (Figure 1B) showed good brain uptake of [^{11}C]**8** in rhesus monkeys, with a good specific signal for CGRP receptors in the cerebellum. The summed baseline PET images (Figure 2A) revealed a regional distribution consistent with the known distribution of CGRP receptors in rhesus

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Table 2. Data for Potential PET Tracers

compd	CGRP K_i (pM)[a,b]	$logD_{7.0}$[c]	P-gp[d]	P_{app} (10^{-6}cm/s)[e]	approximate SUV @ 80 min[f] cerebellum	approximate SUV @ 80 min[f] white matter	$SUV_{cerebellum}/SUV_{WM}$[g]
5	22 ± 1 (4)	ND	8.9	27	0.7	0.5	1.4
6	56 ± 20 (5)	ND	2.8	29	1.2	0.7	1.7
8	46 ± 15 (7)	3.39	1.7	25	1.7	0.9	2.0
10	500 (2)	4.33	NM[h]	NM[h]	0.8	0.6	1.3
11	45 (2)	3.16	1.8	23	1.0	0.6	1.7
12	94 ± 21 (5)	4.15	NM[h]	NM[h]	1.0	0.7	1.4
13	21 (2)	3.36	2.0	21	1.2	0.7	1.7
14	110 ± 22 (6)	4.11	NM[h]	NM[h]	1.1	0.7	1.6
15	19 ± 7 (4)	3.41	1.5	17	1.3	0.7	1.9

[a]Mean value ± standard deviation, where appropriate; the number of replicates is in parentheses. [b]The K_i value for inhibition of ^{125}I-hCGRP binding was determined using membranes from HEK293 cells stably expressing human CLR/RAMP1.[11] [c]HPLC logD at pH 7.0. [d]Human P-gp transport ratio BA/AB determined at 5 μM test compound using LLC-MDR1 cells. [e]Permeability determined using control LLC-PK1 cells. [f]Specific uptake value observed in cerebellum or white matter of anesthetized rhesus monkeys 80 min after i.v. dosing of the [^{11}C]-tracer. [g]Ratio of specific uptake values in cerebellum and white matter at 80 min postdose. [h]Accurate values could not be determined due to the physical properties of compound.

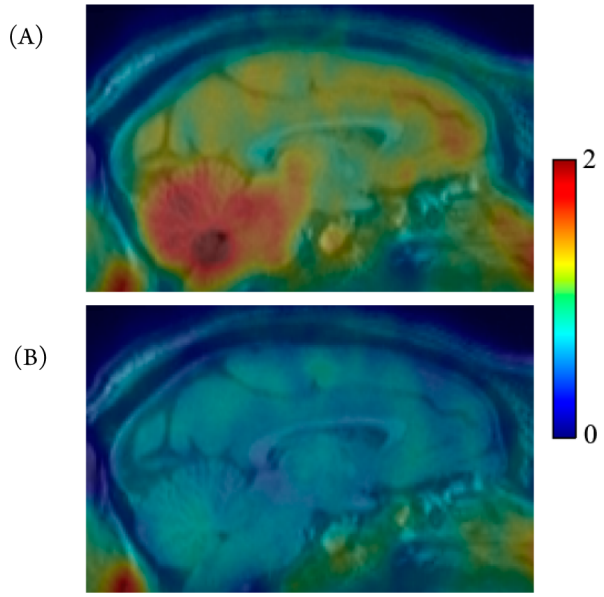
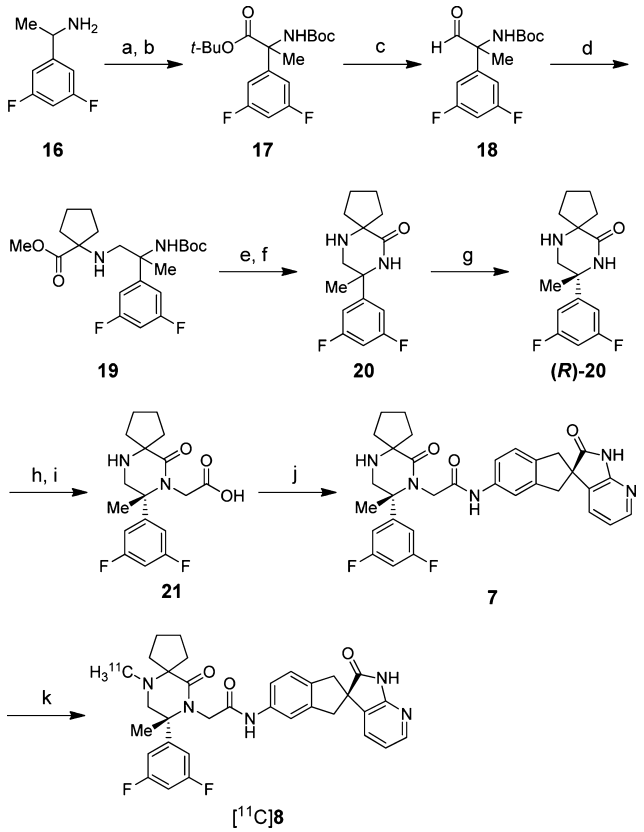


Figure 2. Overlay of MRI and summed [^{11}C]**8** PET images (50−120 min) in rhesus monkey brain: (A) baseline data; (B) data obtained in the presence of ∼10 μM plasma levels of MK-3207.

monkey brain, and a notably robust signal in the cerebellum. The images obtained in the presence of ∼10 μM MK-3207 (Figure 2B) illustrate a blockade of binding to the CGRP receptors in all regions. Taken together, these results demonstrate that [^{11}C]**8** should have utility for the study of central CGRP receptor occupancy.

The methodology used to synthesize compounds **3**−**15** has been described.[18,19] The synthesis of [^{11}C]**8** is outlined in Scheme 1. Following protection of [1-(3,5-difluorophenyl)-ethyl]amine (**16**) with di-*tert*-butyl dicarbonate, the bis-Boc-protected amine was allowed to rearrange under basic conditions to afford the protected amino acid **17**. Treatment of ester **17** with LAH at low temperature provided aldehyde **18**, and reductive amination with methyl 1-amino-cyclopentane-carboxylate hydrochloride led to amine **19**. Removal of the Boc protecting group from **19** followed by heating in the presence of acetic acid afforded the racemic piperazinone **20**, which was resolved by chiral HPLC. Alkylation of (***R***)-**20** with ethyl-bromoacetate and subsequent saponification provided carboxylic acid **21**. Standard amide coupling of **21** with (*R*)-5-amino-1,3-dihydrospiro[indene-2,3′-pyrrolo[2,3-*b*]pyridin]-2′-(1′*H*)-one[19,20] gave **7**. Lastly, treatment of **7** with [^{11}C]methyl triflate gave the PET tracer [^{11}C]**8**.

Scheme 1. Synthesis of [^{11}C]8[a]



[a]Conditions: (a) (1) Di-*tert*-butyl dicarbonate, CH_2Cl_2, 0 °C; (2) Di-*tert*-butyl dicarbonate, DMAP, 80 °C, 96%. (b) Potassium *tert*-butoxide, THF, −78 °C, 52%. (c) LAH, THF, −78 °C, 78%. (d) Methyl 1-aminocyclopentanecarboxylate, $NaCNBH_3$, AcOH, MeOH, 60%. (e) HCl, EtOAc, 0 °C, 99%. (f) AcOH, xylenes, 80 °C, 90%. (g) ChiralPak AD, EtOH/hexane/Et_2NH (60:40:0.1), (*R*)-**20** is the second major peak. (h) NaH, ethyl bromoacetate, THF, 0 °C, 87%. (i) LiOH, H_2O, THF, 95%. (j) (*R*)-5-Amino-1,3-dihydrospiro-[indene-2,3′-pyrrolo[2,3-*b*]pyridin]-2′(1′*H*)-one,[20] HATU, NMM, DMF, 68%. (k) [^{11}C]MeOTf, acetone, 32%.

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In conclusion, the identification of the first PET tracer for the CGRP receptor has been described. Compound **8** (MK-4232) is a highly potent, CNS-penetrant CGRP receptor antagonist. The PET tracer [^{11}C]**8** exhibited good rhesus monkey brain uptake in vivo and specific binding to central CGRP receptors. Further characterization of this tracer and its utility for investigating CGRP receptor occupancy in clinical studies will be reported elsewhere.

■ ASSOCIATED CONTENT

Supporting Information

Representative experimental procedures and NMR, MS, and HPLC data for all new test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(I.M.B.) Phone: +1-215-652-6455. Fax: +1-215-652-7310. E-mail: ian_bell@merck.com.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CGRP, calcitonin gene-related peptide; CGRP-R, calcitonin gene-related peptide receptor; CGRP-RA, calcitonin gene-related peptide receptor antagonist; CLR, calcitonin receptor-like receptor; CNS, central nervous system; CSF, cerebrospinal fluid; GPCR, G-protein coupled receptor; HPLC, high-performance liquid chromatography; LAH, lithium aluminum hydride; ND, not determined; PET, positron emission tomography; P-gp, P-glycoprotein; RAMP1, receptor activity modifying protein 1; SUV, standardized uptake value

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