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Discovery of (*R*)-*N*-(3-(7-methyl-1*H*-indazol-5-yl)-1-(4-(1-methylpiperidin-4-yl)-1-oxopropan-2-yl)-4-(2-oxo-1,2-dihydroquinolin-3-yl)-piperidine-1-carboxamide (BMS-742413): A potent human CGRP antagonist with superior safety profile for the treatment of migraine through intranasal delivery

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ABSTRACT

Calcitonin gene-related peptide (CGRP) receptor antagonists have been shown to be efficacious as abortive migraine therapeutics with the absence of cardiovascular liabilities that are associated with triptans. Herein, we report the discovery of a highly potent CGRP receptor antagonist, BMS-742413, with the potential to provide rapid onset of action through intranasal delivery. The compound displays excellent aqueous solubility, oxidative stability, and toxicological profile. BMS-742413 has good intranasal bioavailability in the rabbit and shows a robust, dose-dependent inhibition of CGRP-induced increases in marmoset facial blood flow.

Migraine is a severe headache that is often preceded or accompanied by sensory symptoms such as flashes of light, blind spots, tingling in the arms and legs, nausea, vomiting, and increased sensitivity to light and sound.[1] The current standard of care for the treatment of migraine is the class of 5-$HT_{1B/1D}$ receptor agonists known as the triptans.[2] Triptans are direct vasoconstrictors of blood vessels though activation of 5-HT_{1B} receptors and are contraindicated in patients with hypertension or ischemic heart disease.[3] Thus, a migraine drug that is devoid of cardiovascular liabilities is expected to have a significant advantage over existing agents.[4]

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide belonging to a family of bioactive peptides that includes calcitonin, adrenomedullin and amylin.[5] There are several potential roles for CGRP in migraine, including dilation of cranial blood vessels, degranulation of mast cells exacerbating neurogenic inflammation, and effects on central sensitization.[6] Studies have shown that CGRP levels are elevated during a migraine attack. Thus, antagonism of CGRP receptors should attenuate migraine symptoms, and in fact, four CGRP receptor antagonists have already demonstrated anti-migraine efficacy in Phase II clinical trials, This proof of concept in acute migraine has been demonstrated with the CGRP receptor antagonists BIBN-4096BS (olcegepant, **1**, Fig. 1), telcagepant (MK-0974, Fig. 1), MK-3207 (Fig. 1) and BI-44370 (not shown).[7] Efficacy of these agents was comparable to that of the current standard of care (triptans), but without the cardiovascular liabilities commonly seen with that class of agents.

Since rapid pain relief in a migraine is paramount to treatment success, new anti-migraine agents with a more rapid onset of action would also have a significant differentiation over the existing oral agents. Intranasally delivered anti-migraine agents should be able to deliver more rapid pain relief than oral agents. Thus, this concept, coupled with the clinical success of CGRP receptor antagonists prompted us to identify potent CGRP receptor antagonists that could be administered through intranasal (IN) administration[8] for rapid onset of action as an alternative to our efforts discovering orally active CGRP receptor antagonists.[9]

The IN route of administration is attractive as it affords noninvasive delivery with the likelihood for rapid onset of action.[10] In this context, we reported a high-affinity CGRP receptor antagonist **2** (BMS-694153, Fig. 1) that incorporated a novel 7-methylindazole

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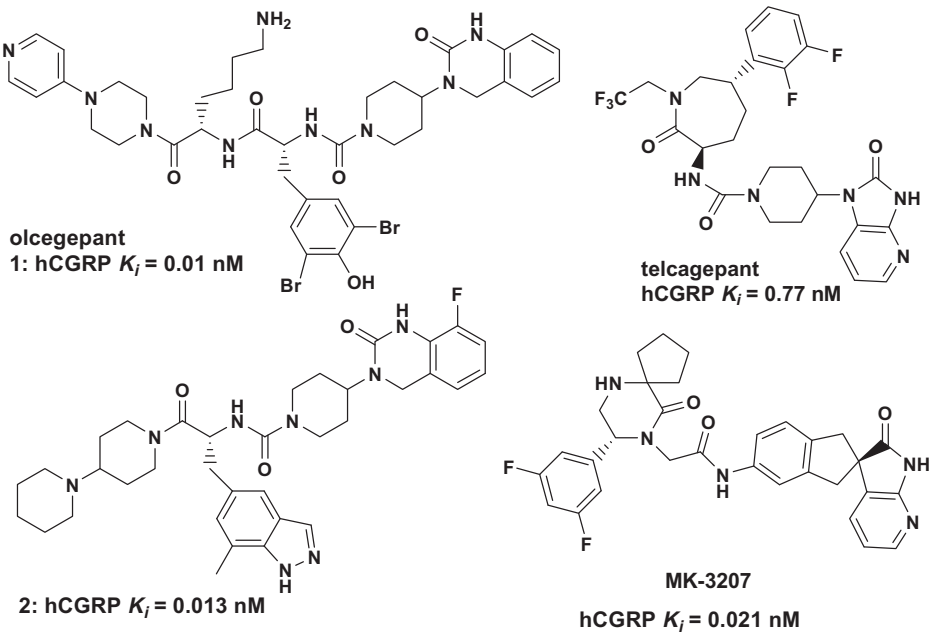
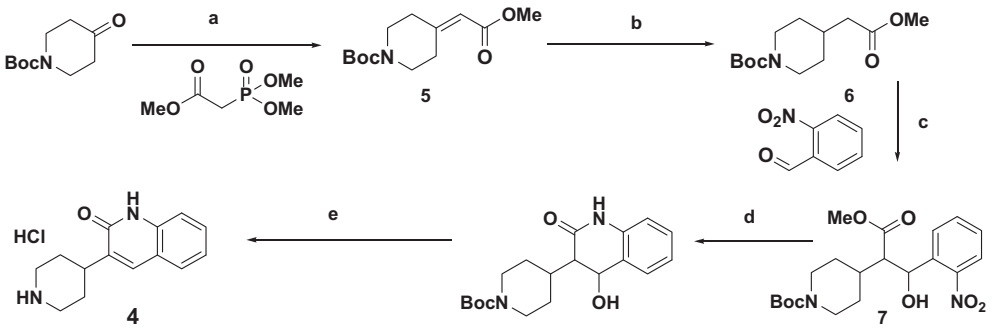


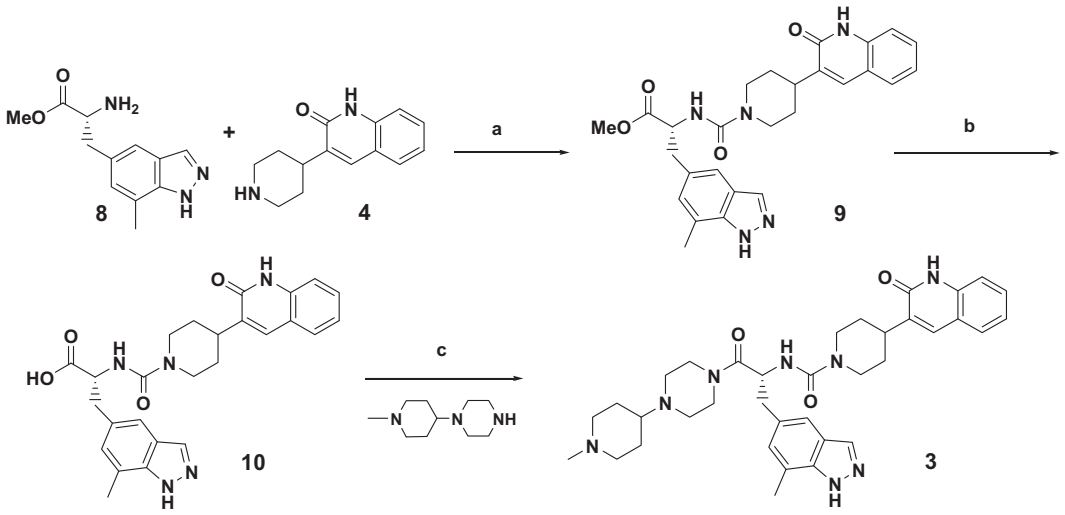
Figure 1. Known CGRP receptor antagonists.

amino acid as a key structural moiety and a dihydroquinazolinone as the key G-protein coupled receptor (GPCR) pharmacophore (Fig. 1).[8,11] The primary challenges that initially emerged in the development of **2** as an IN CGRP receptor antagonist were oxidative stability of the dihydroquinazolinone in aqueous solution that was accelerated by light, and nasal irritation and olfactory epithelial atrophy upon IN delivery seen in rodent toxicity studies (data not shown). In this Letter, we report the discovery of compound **3** (BMS-742413) as a CGRP receptor antagonist for IN administration with greatly improved oxidative stability with diminished nasal irritancy while retaining picomolar receptor binding potency and excellent aqueous solubility, both necessary because of the limitations of nasal delivery. Our initial efforts to modify **2** began by replacing the oxidatively susceptible benzylic methylene group of the dihydroquinazolinone in **2** with an electron-deficient sp^2-hybridized methine in **4**.[12] The synthesis of compound **4** starts with a Horner–Emmons reaction of N-Boc-4-piperidone with the ylide generated from trimethylphosphonoacetate to afford *tert*-butyl 4-(2-methoxy-2-oxoethylidene)piperidine-1-carboxylate **5** in excellent yield (Scheme 1). Catalytic hydrogenation mediated by palladium on carbon reduced the unsaturated double bond. Treatment of *tert*-butyl 4-(2-methoxy-2-oxoethyl)piperidine-1-carboxylate **6** with LDA generated the enolate which upon quenching with 2-nitrobenzadehyde provided the nitro alcohol **7** as a diastereomeric mixture. Reduction of the nitro group with iron in acetic acid, followed by treatment with hydrogen chloride in dioxane provided the desired quinolone **4**. Fragments **4** and **8**[8,11] were efficiently coupled with *N*,*N*′-disuccinimidyl carbonate to install the urea functionality in 78% yield (Scheme 2). Saponification of the methyl ester **9** with lithium hydroxide gave a nearly quantitative yield of carboxylic acid **10**. TBTU mediated coupling of acid with commercially available 1-(1-methylpiperidin-4-yl)piperazine provided compound **3** in excellent yield.

Compound **3** displayed concentration-dependent inhibition of [^{125}I]CGRP binding to the human CGRP receptor endogenously expressed in SK-N-MC cell membranes with a mean K_i of 23 ± 2 pM.[13] Saturation binding experiments involving concentration-dependent studies suggested a behavior consistent with competitive mechanism of receptor inhibition. Functional receptor antagonism by compound **3** was determined by measuring its ability to inhibit CGRP-stimulated formation of cyclic AMP in SK-N-MC cells.[13] The compound was found to be full, competitive antagonist (K_b = 22 pM). The selectivity of **3** for the CGRP receptor over other receptors in the calcitonin receptor family was assessed using



Scheme 1. Reagents and conditions: (a) NaH, DMF, 92%; (b) H_2, 10% Pd on carbon, MeOH, 94%; (c) LDA, THF, 94%; (d) Fe–HOAc, 77%; and (e) HCl in dioxane, EtOAc then crystallize from 5% water in isopropanol.



Scheme 2. Reagents and conditions: (a) succinimidylcarbonate, THF, 78%; (b) LiOH in THF/MeOH/H_2O, 96%; and (c) TBTU, Et_3N, DMF, 85%.

radioligand binding assays. Compound **3** displayed greater than 10,000 fold selectivity for CGRP over adrenomedullin receptors 1 and 2, calcitonin, and amylin receptors 1 and 3.[13]

Compound **3** was tested in two ex vivo assays measuring (i) reversal of CGRP-induced dilation (reversal protocol), or (ii) shift of CGRP-dose response (Schild analysis). The antagonist **3** showed potent and full reversal of CGRP-induced dilation of ex vivo human intracranial arteries (EC_{50} = 880 ± 50 pM), and produced a parallel rightward shift of the CGRP concentration–response curve in ex vivo human intracranial arteries (K_b = 91 pM).[13] These results compared favorably with the in vitro binding (K_i = 23 pM) and functional (K_b = 22 pM) assay results. Compound **3** did not demonstrate active contraction of ex vivo human coronary artery up to 10 μM. In contrast, sumatriptan (Imitrex™) showed progressive, concentration-dependent contraction with EC_{50} = 280 ± 50 nM (Fig. 2). The absence of active constriction by antagonist **3** is a reflection of the different mechanism of action for CGRP receptor antagonists: that of an anti-dilatory effect (returning dilated vessels to normal), and demonstrating that this class of agents (CGRP receptor antagonists) will likely be free from the cardiovascular liabilities seen with the triptans.

Compound **3** was tested in the novel non-invasive marmoset recovery model established in our laboratories for in vivo efficacy assessment of CGRP receptor antagonists.[8,9] In brief, marmosets were anesthetized and laser Doppler facial blood flow was increased by four separate intravenous (IV) administrations of hαCGRP (10 μg/kg) delivered at 45 min intervals (−30, 15, 60 and 105 min). The first delivery provided a baseline control response to hαCGRP, and the three subsequent deliveries reflected post-dose effects where the CGRP receptor antagonist was delivered subcutaneously (SC) at 0 min. When compared to pre-dose vehicle control (−30 min), Compound **3** (0.03 mg/kg, SC; 0 min) produced a strong 48% inhibition at 15 min post-dose. Robust 80% and 70% inhibitions were observed at 60 and 105 min post-dose, respectively, (Fig. 3). The total plasma levels during the 15–105 min post-dose time period ranged from 25 to 31 nM (Fig. 3).

In vitro safety profiling of **3** suggested a low potential for hepatic, cardiovascular or genotoxic liabilities. Compound **3** was well tolerated acutely in rats given ⩽60 mg/kg SC, providing very high exposure multiples (both C_{max} and AUC) of the projected efficacious human intranasal dose.[14] In a 10-day SC rat safety study, compound **3** was well tolerated with no evidence of systemic toxicity (0.3, 3, 10, and 30 mg/kg/day; F_{sc} = 82%). In a seven-day rat safety IN irritancy study, there were no findings when compound **3** was dosed at 75 and 175 mg/mL (12.5 μL/nostril in succinate buffer), the optimal allometrically scaled volume for rats. In contrast, our previous IN compound **2** showed significant irritation at both dose levels (Fig. 4). At higher concentrations, ⩾75 mg/mL, and higher volumes, ⩾25 μL/nostril, compound **3** caused dose-related

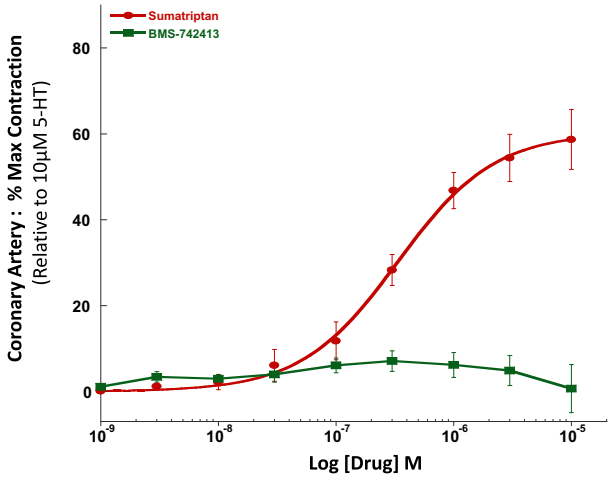


Figure 2. Effect of sumatriptan and compound **3** (BMS-742413) on ex vivo human coronary arteries.

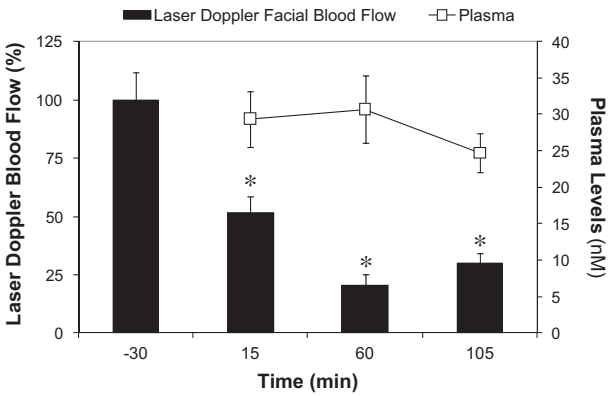


Figure 3. Efficacy in marmoset facial blood flow model.

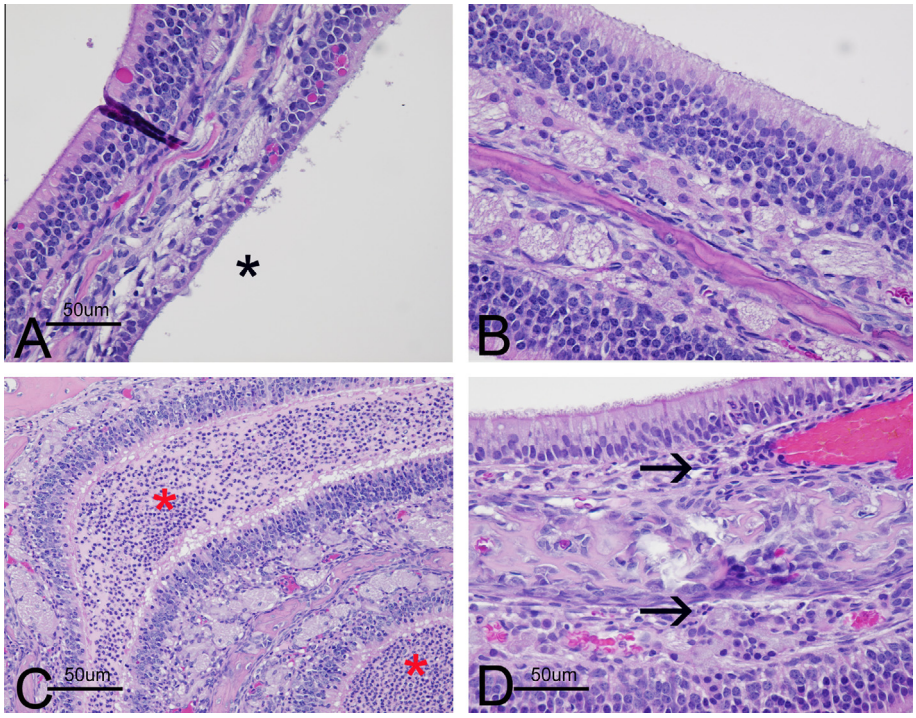


Figure 4. Nasal cavity and respiratory epithelium of rats dosed intranasally for 7 days with (A, C) compound **2**, or (B, D) compound **3**. In A and B, the rats were dosed at 75 mg/mL, 12.5 μL/nostril (total dose 1.875 mg), and in C and D, the rats were dosed at 175 mg/mL, 12.5 μL/nostril (total dose 4.375 mg). In A (compound **2**), the respiratory epithelium is atrophied (*). In B (compound **3**), there are no lesions. In C (compound **2**), the lumina are nearly occluded by exudate (red*), and many neutrophils infiltrate the lamina propria. In D (higher magnification than C; compound **3**), a few neutrophils infiltrate the lamina propria.

lesions in the nasal olfactory and respiratory epithelium, characterized primarily by epithelial atrophy. At 175 mg/mL (100 μL/nostril), compound **3** caused inflammation, erosion and ulceration, and single-cell necrosis of the olfactory epithelium and minimal to slight neutrophilic inflammation and single-cell necrosis of the respiratory epithelium. Notably however, at each dose above $\geqslant$75 mg/mL and volume above $\geqslant$25 μL/nostril, the incidence and severity of lesions was markedly less with compound **3** than with compound **2**.

Compound **3** possessed two protonatable nitrogens as part of the *N*-methylpiperidinyl-piperazine amide that resulted in a high polar surface area (116.18 Å^2). Consequently, binding of **3** to serum proteins in vitro was low over a range of species including human (f_u = 37%). A 40 mg/mL solution of **3** (succinate buffer pH 6, 40 °C) showed only 0.3% degradation after 3 months. In addition, the crystalline compound possessed phenomenal aqueous solubility easily sufficient to support nasal delivery (105 mg/ml at pH = 8.5 and >300 mg/mL at lower pH).

Compound **3** exhibited poor permeability in Caco-2 cells (16 nm/s (A–B); 16 nm/s (B–A)) and PAMPA assays, and showed minimal oral bioavailability in cynomolgus monkey (F = 0.3%) or mouse (F = 1.4%). In contrast, IN absorption of compound **3** in the rabbit was rapid and efficient. The time to reach peak concentrations (T_{max}) occurred within 15–20 min at all doses studied. The absolute bioavailability of **3** was found to be dependent on the concentration of the dosing solution with bioavailability increasing on going from a 10 mg/ml (13%) to a 100 mg/ml solution (30%), with C_{max} ranging from 0.12 to 2.0 μM. Plasma levels >10 nM were measured within 5 min. The antagonist was detected in plasma for at least 6 h post-dose and up to 24 h at the high dose.

Compound **3** demonstrated a low probability of drug–drug interactions involving CYP inhibition as measured by IC_{50}'s versus a variety of CYPs (CYP3A4 (BFC) >40 μM, (BzRes) 24 μM; CYP2D6 >40 μM; CYP2C9 >40 μM; CYP2C19 >40 μM; CYP1A2 >40 μM). Compound **3** showed no time-dependent CYP3A4 inhibition. The antagonist **3** did not prolong action potential duration in Purkinje fibers and had little effect on other action potential parameters up to 30 μM. The compound showed only weak activity in the hERG patch clamp assay (9.5% and 22% inhibition at 10 and 30 μM, respectively), suggesting a low potential for QT related electrocardiographic changes. Compound **3** at 10 μM showed no significant potential for off-target liabilities in a broad panel of receptor and ion channel binding and enzyme activity assays.

In summary, we have identified compound **3** (BMS-742413), a potent CGRP antagonist with much improved oxidative stability in comparison with our previous lead (**2**), by incorporating a quinolinone GPCR privileged moiety and 7-methylindazole D-amino acid as key structural motifs.[15] BMS-742413 (**3**) had outstanding aqueous solubility, suitable for IN delivery, and good intranasal bioavailability with peak concentrations occurring within 15–20 min. The compound reverses CGRP-induced dilation of ex vivo human intracranial arteries. The compound displayed excellent activity in a marmoset facial blood flow model, as well as favorable in vivo safety margins for both systemic and IN toxicity. BMS-742412 represents a significant clinical opportunity for a nasally delivered CGRP receptor antagonist. Additional reports on the progress of BMS-742413 will be made in due course.

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13. For experimental procedures used to measure [^{125}I]CGRP binding, CYP inhibition, functional antagonism (EC_{50}), ex vivo efficacy in intracranial arteries and pharmacokinetics properties, see the Supporting information in: (a) Degnan, A. P.; Chaturvedula, P. V.; Conway, C. M.; Cook, D. A.; Davis, C. D.; Denton, R.; Han, X.; Macci, R.; Mathias, N. R.; Moench, P.; Pin, S. S.; Ren, S. X.; Schartman, R.; Signor, L. J.; Thalody, G.; Widmann, K. A.; Xu, C.; Macor, J. E.; Dubowchik, G. M. *J. Med. Chem.* **2008**, *51*, 4858
(b) In this ex vivo assay, wire mounted human intracranial artery rings were pre-contracted with 10 mM KCl (to mimic endogenous tone), fully dilated with 1 nM CGRP. Concentration–response curves to CGRP antagonists were performed by the cumulative addition of CGRP antagonist on fully dilated vessels.
(b) The EC_{50} values (concentration of compounds that elicit 50% reversal) were estimated by the best fits of the data due to the 3-point assay used to test these antagonists ($n = 4$) vessels for each compound.
14. Our projection for the efficacious human dose was less than 20 mg total. The studies and calculations which went into this projection will be published separately in due course.
15. *Data for compound* ***3****:* Analytical HPLC showed >99.0% UV purity @ 230 nm. The enantiomeric excess (ee) was determined to be >99.9% (conditions: Chiralpak AD column, 4.6 × 250 mm, 10 μm; eluent: 70% (0.05% diethylamine)/heptane/30% ethanol; at 1.0 mL/min. for 45 min. The retention times were 18.7 min for *R* and 28.1 min for *S*). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 13.01 (s, 1H), 11.76 (s, 1H), 7.96 (s, 1H), 7.62 (d, J = 7.10 Hz, 1H), 7.60 (s, 1H), 7.42 (m, 1H), 7.36 (s, 1H), 7.26 (d, J = 8.25 Hz, 1H), 7.14 (m, 1H), 7.00 (s, 1H), 6.69 (d, J = 8.25 Hz, 1H), 4.78 (q, J = 7.79 Hz, 1H), 4.14 (d, J = 12.37 Hz, 2 H), 3.54 (dd, J = 9.16, 4.58 Hz, 1H), 3.24 (m, 1H), 3.11 (m, 1H), 2.97 (m, 1H), 2.89 (m, 2 H), 2.69 (m, 4 H), 2.32 (m, 1H), 2.21 (m, 1H), 2.07 (m, 4 H), 1.95 (t, J = 8.25 Hz, 1H), 1.87 (m, J = 11.28, 11.28, 3.55, 3.44 Hz, 1H), 1.76 (t, J = 12.03 Hz, 2 H), 1.68 (t, J = 11.11Hz, 2 H), 1.53 (t, J = 8.25 Hz, 1H), 1.32 (m, 4 H), 1.16 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 16.80, 27.30, 30.51, 30.51, 30.67, 35.50, 38.04, 41.74, 44.00, 44.16, 45.35, 45.78, 48.14, 48.39, 51.45, 54.76, 54.76, 60.61, 114.53, 117.79, 119.29, 119.34, 121.57, 122.78, 127.46, 127.79, 129.29, 129.79, 133.31, 133.72, 136.98, 137.41, 139.12, 156.50, 161.50, 170.42. Accurate mass analysis: m/z 639.3770, $[MH]^+$, $\delta = -0.2$ ppm. Optical rotation: $-27.36°$ @ 589 nm, concentration = 4.71 mg/mL in methanol.