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Research Article

Synthesis of ^{3}H and ^{14}C labeled (S)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one, MaxiPost™. An agent for post-stroke neuroprotection

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Summary

The syntheses of tritium labeled (*S*)-3-(5-chloro-2-[OC^3H_3]methoxyphenyl-1, 3-dihydro-3-fluoro-6-(trifluoromethyl)-1H-indol-2-one, and carbon-14 (*S*)-3-(5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-[2,3-$^{14}C_2$] indol-2-one are reported. The ^{3}H-labeled compound was prepared in a two-step synthesis from C^3H_3I. The final product was purified via chiral HPLC to yield the desired enantiomer in a 4% radiochemical yield and a specific activity of 60 Ci/mmol. The ^{14}C-labeled compound was prepared in a

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four-step synthesis from diethyl [carboxylate-$^{14}C_{1,2}$] oxalate. The final product was purified via chiral HPLC to yield the desired enantiomer in a 20% radiochemical yield and a specific activity of 28.4 μCi/mg.

Key Words: neuroprotection; stroke; maxipost^{TM}; tritium; carbon-14

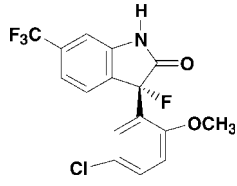
Introduction

Stroke represents one of the largest disease states for which there remains significant unmet medical need. In the United States alone, there are more than 700,000 new cases each year.[1] Stroke can be classified as being either hemorrhagic in origin, resulting from vessel rupture, or ischemic in origin resulting from vessel occlusion. Approximately 85% of all strokes are ischemic in origin. In acute ischemic stroke, a core area of severely damaged tissue located distal to an occluded vessel, is surrounded by a penumbra of tissue at risk because of proximity to the core and as a result of the low vascular perfusion to the tissue.[2] At the location of insult, the resulting hypoxia results in release of an extraordinary level of excitatory neurotransmitters resulting in pathologically high intracellular levels of calcium (Ca^{2+}). Our approach to neuroprotective therapy, has been to augment the opening of a particular type of potassium channel, the large-conductance maxi-K Ca^{2+}-dependent potassium channel. These channels constitute an intrinsic mechanism for controlling Ca^{2+} entry into the cell, and as such, are an attractive target for reducing potentially pathogenic levels of Ca^{2+} entry following stroke.[3] The ultimate goal of our approach is to rescue ischemically threatened but potential viable tissue in the penumbra which surrounds the core of infarcted tissue in acute stroke.

BMS-204352, **1a**, Maxipost^{TM} is a potent and selective opener of maxi-K channels and is a clinical candidate for post-stroke neuroprotection (Figure 1).[4,5] Herein, we report the synthesis of ^{14}C and ^{3}H-labeled BMS-204352 as radioligands which have been used in studying the pharmacokinetics and biodistribution of Maxipost^{TM}.

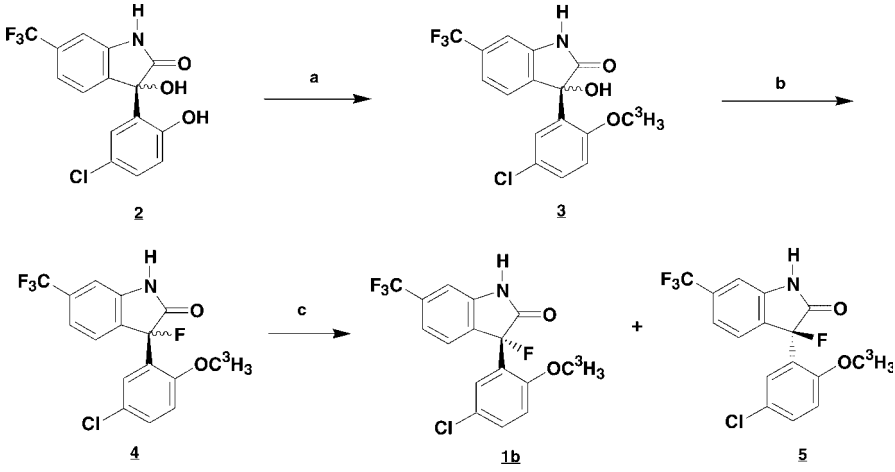
Results and discussion

Synthesis of **1b** was accomplished in a two-step synthesis starting from tritium labeled methyl iodide (Scheme 1). In the first reaction, the



1a

Figure 1. 1a, Maxipost™

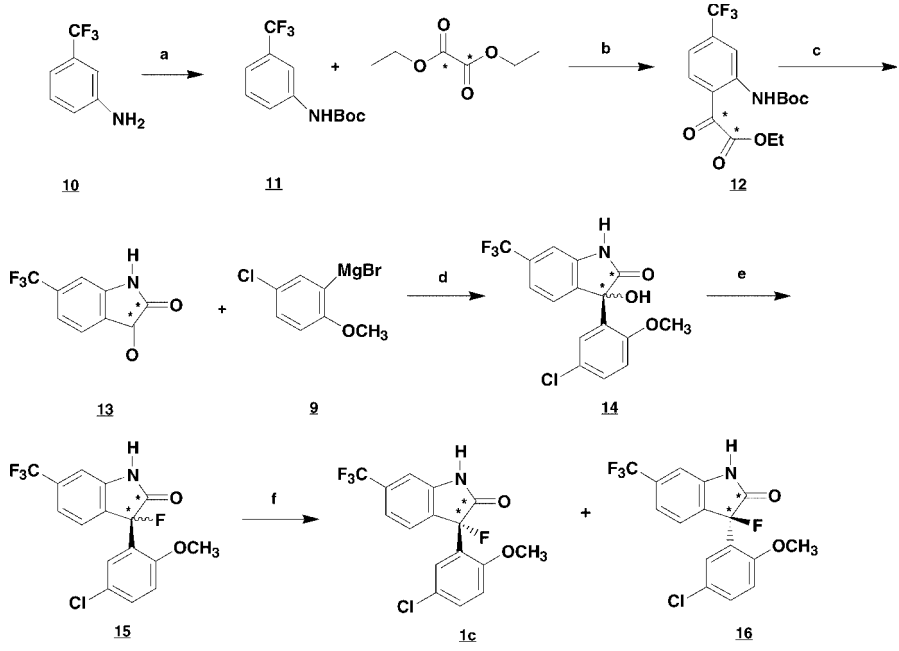


Scheme 1. Synthesis of (*S*)-3-(5-chloro-2-[OC^3H_3]methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one, 1b

phenolic hydroxyl of **2** was selectively methylated with tritium labeled methyl iodide. In order to optimize the yield of **3**, the methylation of **2** was carried out under a variety of conditions with non-radioactive material. The major issue to be addressed with this approach was the instability of **2** under the basic conditions necessary for the methylation. In the presence of base, **2** undergoes dehydration to the corresponding quinonemethide intermediate which subsequently decomposed to a complex mixture of products. We evaluated several base/solvent pairs for this methylation including NaH/DMF, K_2CO_3/acetone, Ag_2CO_3/acetone–H_2O, aqueous NaOH/phase-transfer catalyst/CH_2Cl_2, $Et_4NF \cdot xH_2O$/DME, K_2CO_3/DMF, and $CsCO_3$/DMF with K_2CO_3/DMF, or $CsCO_3$/DMF providing higher yields of **3**, with fewer side products. Yields of **3** were slightly better with $CsCO_3$/DMF

(53%) as compared to K_2CO_3/DMF (48%). Fluorination of **3** with DAST yielded the racemic material which was then purified via chiral HPLC.

Synthesis of the carbon-14 labeled Maxipost™ **1c**, was achieved in a four-step synthesis from diethyl [carboxylate-$^{14}C_{1,2}$] oxalate (Scheme 2). In this reaction, 3-(trifluoromethyl)aniline was protected as the *t*-Boc derivative and then reacted with diethyl [carboxylate-$^{14}C_{1,2}$] oxalate. Rigorous anhydrous conditions were essential for obtaining satisfactory yields of the intermediate keto-ester **12**. Deprotection of the keto-ester with 3 N HCl followed by reflux for 6 h yielded the 6-(trifluoromethyl)[2,3-$^{14}C_2$]isatin **13**. The sodium salt of **13** was then allowed to react with the Grignard reagent **9** prepared from 2-bromo-4-chloroanisole **8** to yield the penultimate product **14**, 3-(5-chloro-2-methoxyphenyl)-3-hydroxy-2,3-dihydro-6-(trifluoromethyl)-2H-[2,3-$^{14}C_2$] indol-2-one. Fluorination of **14** with DAST afforded the racemic product **15** which was purified on a semi-preparative Chiralpak OD column to yield the desired enantiomer **1c** in a 20% radiochemical yield and with a specific activity of 28.4 μCi/mg.



Scheme 2. Synthesis of carbon-14 labeled (S)-3-(5-chloro-2-methoxyphenyl)-1, 3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one[2,3-$^{14}C_2$], 1c

Materials and methods

All experimental conditions were optimized using non-labeled materials. All reagents were obtained from Aldrich and were either ACS grade or the highest quality material commercially available. The radiosynthesis of **3** was carried out at Vitrax. Carbon-14 labeled diethyl oxalate [carboxylate-$^{14}C_{1,2}$] was purchased from Vitrax. The identity of intermediates and final product was established by co-elution of the radiolabeled material with authentic unlabeled compound on HPLC.[4,6] Radioactive TLC analysis was obtained using a Bioscan QC Scan system. HPLC purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-1 detector and an *INUS* β-RAM radioactive flow through detector. Radiochemical purity was determined by HPLC. The specific activity of the tritiated samples were determined via HPLC. In this procedure, a radioactive sample was applied to the column, and the mass was determined by comparison of the UV absorbance to a standard curve and the total radioactivity was measured via liquid scintillation counting. Specific activity of the carbon-14 samples were determined gravimetrically with liquid scintillation counting.

High performance liquid chromatography

Method A: Samples were loaded on a Zorbax C18 column ($4.6 \times 250\,mm^2$) equilibrated with 50% CH_3CN/50% H_2O containing 0.1% TFA. The flow rate through the column was 1 ml/min and the sample was monitored by both UV (230 nm) and radioactivity.

Method B: Samples were loaded on a Chiralcel OD column ($4.6 \times 250\,mm^2$) equilibrated with 90% hexane/10% isopropanol. The flow rate through the column was 1 ml/min and the sample was monitored by both UV (220 nm) and radioactivity.

Method C: Samples were loaded on a YMC-Pack PVA-SIL NP column ($4.6 \times 250\,mm^2$) equilibrated with 99% CH_2Cl_2/1% CH_3OH. The flow rate through the column was 1 ml/min and the sample was monitored by both UV (245 nm) and radioactivity.

Method D: Samples were loaded on a Chiralcel OD column ($20 \times 250\,mm^2$, 10 μm) equilibrated with 90% hexane/10% isopropanol. The flow rate through the column was 6 ml/min and the sample was monitored by both UV (220 nm) and radioactivity.

Method E: Samples were loaded on a YMC-Pack ODS-A column, ($150 \times 6\,mm^2$) equilibrated with 70% CH_3CN/30% H_2O containing 0.1% TFA. The flow rate through the column was 1 ml/min and the sample was monitored by both UV (220 nm) and radioactivity.

Experimental

3-(5-chloro-2-[OC^{3}H$_3$]methoxyphenyl-3-hydroxy-1,3-dihydro-6-(trifluoromethyl)-2H-indol-2-one, **3**

3-(5-Chloro-2-hydroxyphenyl)-3-hydroxy-1,3-dihydro-6-(trifluoromethyl)-2H-indol-2-one[4], **2**, (3.5 mg, 0.01 mmol) and cesium carbonate (5.0 mg, 0.015 mmol) were combined in a 10 ml recovery flask. To this was added anhydrous DMF (100 µl) and the reaction vessel attached to a vacuum line bridge. A break seal ampoule containing H-3 labeled methyl iodide, (300 mCi, 0.004 mmol) was attached to the opposite side of the bridge. C^3H_3I was then vacuum transferred into the reaction flask at $-197°C$. The flask pressure was adjusted to 0.5 atm with N_2, isolated and allowed to stir at room temperature for 3 h. The volatiles were removed under full vacuum, trapped and quenched for disposal. The reaction residue was opened to the atmosphere and quenched with 0.5 N HCl (1.0 ml). The product was extracted Et_2O (5 ml, 3X), dried (Na_2SO_4), and filtered to yield crude **3** (155 mCi). The radiochemical purity of crude **3** was approximately 52% (method A). In this system, **2** has a retention time of approximately 5.6 min, while **3** has a retention time of approximately 9.3 min. The solvent was removed *in vacuo* and the residue was purified by HPLC (method A). The combined purified fractions were pooled, concentrated to dryness, extracted with absolute ethanol and filtered (0.22 µm) to yield **3** (80 mCi, 27%). The identity of the product was established by co-elution of the radiolabeled material with authentic unlabeled compounds[4,6] on HPLC. The radiochemical purity of **3** was $>98\%$ and the specific activity was found to be 68 Ci/mmol (method A). Analysis of **3** via chiral HPLC (method B) showed the two enantiomers with retention times of approximately 17.4 and 18.6 min).

(S-)-3-(5-Chloro-2-[OC^{3}H$_3$]methoxyphenyl-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one, **1b**

3 (8 mCi) was added to a 5 ml thick wall screw cap V-Vial and the sample was concentrated to dryness in a Savant speedvac. The residue

was dissolved in hexane (2 ml) concentrated to dryness in a Savant speedvac and then resuspended in hexane (2 ml) and ether (1 ml). The solution of **3** was passed through a $MgSO_4$ column ($0.5 \times 2\,cm^2$) and collected in a conical vessel (15 ml). The solvent removed (N_2) and the residue resuspended in ether (1 ml). The solution was placed in an ice-bath for 10 min, DAST, (diethylamino)sulfur trifluoride, (60 µl, 0.45 mmol) was added and the reaction allowed to stir at 0°C for 30 min after which time ether (10 ml) was added to the reaction vessel and the mixture washed with saturated $NaHCO_3$ (5 ml), H_2O (5 ml), saturated NaCl (5 ml), dried ($MgSO_4$), and filtered. The solvent was then removed in a Savant speedvac. The residue, **4**, was then dissolved in 90% hexane/10% isopropanol for purification by chiral HPLC (method B). In this system, **1b** had a retention time of approximately 8.7 min while the undesired enantiomer **5** had a retention time of approximately 12.0 min. The combined purifications were pooled, concentrated to dryness and redissolved in absolute ethanol to yield **1b** (1.2 mCi, 15%). The identity of the product was established by co-elution of the radiolabeled material with authentic unlabeled compound[4,6] on HPLC. The radiochemical purity of **1b** was $>99.5\%$ and the specific activity was found to be 68 Ci/mmol (method B).

2-Bromo-4-chloroanisole, 8

2-Bromo-4-chlorophenol (64.24 g, 0.30 mol) was added in portions to an ice-cold solution of 5 N NaOH (64 ml, 0.32 mol) under N_2 and stirred until dissolution was complete ($\sim$75 min). Dimethyl sulfate (43.7 g, 0.35 mol,) was slowly added to the stirred solution at 0–5°C. The mixture was allowed to warm to room temperature and then stirred for 16 h. The two-phase mixture was extracted with ether (2×100 ml) and the combined extracts were washed with 1 N NaOH (40 ml), water (40 ml), brine (40 ml), dried (Na_2SO_4) and filtered. The filtrate was concentrated *in vacuo* and the crude product **8** was distilled under vacuum to give 64.5 g (97%) of 2-bromo-4-chloroanisole, **8**, as a colorless oil, bp 78–81°C/ 4 torr. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 3 H), 6.81 (d, 1 H), 7.24 (dd, 1 H), 7.53 (s, 1 H); MS m/e 220 (M^+).

(5-Chloro-2-methoxyphenyl)magnesium bromide, 9

Magnesium turnings (0.335 g, 0.0138 mol) were suspended in anhydrous THF (10 ml) in a 50 ml two-neck round-bottom flask equipped with a

reflux condenser, stir bar, 10 ml addition funnel and nitrogen inlet. Dibromoethane (85 mg, 0.45 mmol) was added and the mixture stirred for 10 min, after which time a solution of **8** (2.03 g, 0.00917 mol) in anhydrous THF (7 ml) was added over 4 min. The mixture was heated at reflux for 6 h and stirred at ambient temperature for 16 h yielding a clear dark solution of **9** (and a small amount of unreacted magnesium), which was used directly in the next reaction.

N-(tert-Butoxycarbonyl)-3-(trifluoromethyl)aniline, **11**

A neat mixture of 3-(trifluoromethyl)aniline, **10** (33.00 g, 0.20 mol) and di-*tert*-butyl dicarbonate (49.20 g, 0.22 mol) was stirred at 80°C for 3 h and then allowed to cool to room temperature. The resulting *t*-BuOH formed in the reaction was removed on a rotary evaporator leaving the crude product as an amber oil which solidified on standing. The crude product was crystallized from hot hexane (~35 ml) to yield 45.61 g (85.3%) of **11** as white needles. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (s, 9 H), 6.56 (s br, 1 H) 7.28 (d, 1 H) 7.39 (*t*, 1 H), 7.49 (d, 1 H), 7.71 (s, 1 H); MS m/e 260 $(M\text{-}H)^-$.

6-(Trifluoromethyl) [2,3-$^{14}C_2$] isatin, **13**

Rigorous anhydrous conditions were followed in this procedure. All glassware, syringes, needles, pipettes and stir bar were oven dried at 120°C for 16 h prior to use. *N*-(*tert*-butoxycarbonyl)-3-(trifluoromethyl) aniline, **11**, (2.61 g, 0.010 mol) was dissolved in anhydrous THF (11 ml, added via syringe through the septum) in a 100 ml two-neck round-bottom flask, equipped with a stir bar, nitrogen inlet and rubber septum. The colorless solution was stirred and cooled with a dry ice/acetone bath to −78°C (bath temperature). After 10 min, *sec*-butyllithium (16.90 ml, 0.022 mol, 1.3 M solution in cyclohexane) was added dropwise over 9 min from a syringe inserted through the septum. The cooling bath was changed to a dry ice/methanol system and the yellow solution was allowed to stir at −45 to −35°C (bath temperature) for 2 h. The resulting yellow-brown mixture was cooled to −78°C and a solution of diethyl oxalate[carboxylate-$^{14}C_{1,2}$] (272 mg, 1.863 mmol, 51 mCi/mmol, 95 mCi) and non-labeled diethyl oxalate (1.266 g, 0.00866 mol) in anhydrous THF (1.5 ml) was added rapidly from a syringe. The resulting orange-brown solution was allowed to stir at −75°C for 1 h. The reaction mixture was diluted with ether (12 ml)

and quenched with 3 N HCl. The cooling bath removed and the mixture was allowed to reach ambient temperature. *(This mixture turns deep blue before turning yellow as it warms to room temperature.)* The organic layer was separated, and washed with water (10 ml), brine (10 ml) and dried (Na_2SO_4). The solvent was removed on a rotary evaporator to yield a dark yellow oil that was chromatographed on 65 g of silica gel. Elution with CH_2Cl_2 yielded the desired carbon-14 labeled keto-ester, **12**, as a bright yellow oil.

A solution of **12** in anhydrous THF (40 ml) and 3 N HCl (10 ml) was stirred under reflux for 6 h. The clear orange-red solution was cooled to 30°C, the solvent removed on a rotary evaporator and the resulting suspension cooled to 0°C. After 30 min, the resulting solid was filtered, rinsed with water and dried under vacuum for 64 h. Additional drying under vacuum for 12 h at 78°C yielded **13** (1.209 g, 57.7 mCi, 56.2%) as an orange–red solid. RadioTLC analysis of **13** showed R_f 0.32 (Analtech Silica Gel GHLF Uniplates, 250 μm, $10 \times 20\,cm^2$ scored, 95% CH_2Cl_2, 5% CH_3OH).

3-(5-Chloro-2-methoxyphenyl)-3-hydroxy-2,3-dihydro-6-(trifluoromethyl)-2H-[2,3-$^{14}C_2$] indol-2-one, **14**

A 100 ml round bottom flask, equipped with a stir bar and nitrogen inlet was charged with a suspension of oil-free sodium hydride (268 mg, 6.69 mmol) in anhydrous THF (11 ml). The isatin **13** (1.20 g, 0.0056 mol) was added in portions and the resulting purple solution was stirred for 30 min after which the slurry was cooled to 5°C and a solution of **9** was added via syringe inserted through a rubber septum. The resulting mud-brown solution was stirred at room temperature for 2 h. The mixture was diluted with ether (35 ml), cooled in an ice bath and acidified (pH paper) with 1 N HCl ($\sim$20 ml). The organic layer was separated, washed with 0.5 N NaOH (10 ml), 1 N HCl (10 ml), water (10 ml), brine (10 ml), and dried (Na_2SO_4). The solvent was removed on a rotary evaporator to yield a brown greasy solid, which was purified by flash chromatography (65 g silica gel, 50/50 ethyl acetate/hexane) to yield **14** (1.86 g, 53.0 mCi, 93.5%) as a yellow brown solid. RadioTLC analysis of **14** showed R_f 0.14 (Analtech Silica Gel GHLF Uniplates, 250 μm, 10×20 cm scored, 95% CH_2Cl_2, 5% CH_3OH). The radiochemical purity of **14** was $>95\%$ (HPLC method C). In this system, **14** has a retention time of approximately 11.0 min.

3-(5-Chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-[2,3-$^{14}C_2$]indol-2-one, **15**

Neat (diethylamino)sulfur trifluoride (DAST) (1.09 g, 0.0067 mol,) was added dropwise via a 1.0 ml plastic syringe to a cold suspension (−78°C) of **14** (1.86 g, 53 mCi, 0.0052 mol) in dry CH_2Cl_2 (17 ml) under nitrogen. The mixture was allowed to warm to 0–5°C and was maintained at that temperature for 45 min, after which time TLC analysis (Analtech Silica Gel GHLF Uniplates, 250 μm, CH_2Cl_2) showed that the reaction was complete. The solution was quenched by the slow addition of water (9 ml) at 0°C. The organic layer was washed with water (15 ml), brine (15 ml) and dried (Na_2SO_4). The solvent was concentrated on a rotary evaporator to yield an amber foam which was purified by flash chromatography (34 g silica gel) using a gradient elution of 0–0.5% CH_3OH/CH_2Cl_2. The desired fractions were combined and concentrated on a rotary evaporator to yield racemic **15** (1.48 g, 42.03 mCi, 79%) as a cream colored solid.

(S-)-3-(5-Chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-[2,3-$^{14}C_2$]indol-2-one, **1c**

Resolution of racemic **15** was obtained by chiral HPLC (HPLC method D). In this procedure, **15** (1.42 g) was dissolved in mobile phase (90% hexane/10% isopropanol, 84 ml) and the suspension was sonicated until a clear solution was obtained. Aliquots (0.95 ml) were injected onto the column and peaks corresponding to the (*S*)- and (*R*)-enantiomers were collected. Fractions containing the desired (*S*)-enantiomer were combined and concentrated on a rotary evaporator. The residue was dried under vacuum for 4 h to yield **1c** (652 mg, 28.4 μCi/mg, 18.52 mCi) as a yellow-tinted solid. The identity of the product was established by co-elution of the radiolabeled material with authentic unlabeled compounds[4,6] on HPLC. The radiochemical purity of (**1c**) was >99.5% (HPLC method E). In this system, **1c** has a retention time of approximately 8.6 min. The chiral purity of **1c** was >99.5% (HPLC method B). In this system, **1c** has a retention time of approximately 8.7 min, while the undesired enantiomer, **16**, has a retention time of approximately 12.0 min. ^{1}H NMR (**1c**) (500 MHz, $CDCl_3$) δ 3.55 (s, 3 H), 6.78 (d, 1 H), 7.16 (s, 1 H), 7.20 (d, 1 H), 7.28 (d, 1 H), 7.34 (dd, 1 H), 7.80 (d, 1 H), 8.02 (s br, 1 H).

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