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An efficient synthesis of isotope-labeled PD0331179 and its labeled metabolite

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4-[1-Methyl-2,4-dioxo-6-(3-phenyl-prop-1-ynyl)-1,4-dihydro-2*H*-quinazolin-3-ylmethyl]-benzoic acid, PD0331179, was under investigation as a matrix metalloproteinase-13 inhibitor. ^{14}C-labeled and ^{2}H-labeled PD0331179 and its ^{2}H-labeled metabolite (PD0335699) were required to support its preclinical and clinical studies. [^{14}C] 3-phenyl-1-trimethyl/triphenylsilyl-propyne was efficiently prepared starting with [^{14}C] benzoic acid and used as a key-labeled reagent for the synthesis of [^{14}C] PD0331179. A one-pot coupling reaction between aryl iodide and trialkylsilyl propyne was developed to make this synthesis more efficient. The details of these syntheses are reported.

Keywords: isotope labeling; C-14; H-2; C–C cross-coupling; triphenylsilyl-propyne; MMP-13 inhibitor

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases that are key enzymes implicated in matrix degradation and reconstruction. The importance of MMPs in cancer and inflammation is well known as manifested by the proliferation of publications as well as the number of drug candidates in clinical trials.[1–4] One particular enzyme, MMP-13 (collagenase-3), is mainly expressed in articular cartilage during osteoarthritis (OA) and in the synovium of patients with rheumatoid arthritis and therefore an attractive drug discovery target.[5–8] PD0331179 was selected as a specific inhibitor of MMP-13 for the indication of OA.[9] Both radioisotope and stable isotope-labeled PD0331179 including its labeled metabolite were required to support its absorption, distribution, metabolism, and elimination, preclinic evaluation and bioanalytical mass spectrometry clinic studies as an internal standard, respectively.

In this paper, we report the synthesis of C-14 labeled PD0331179, H-2 labeled PD0331179, and H-2 labeled metabolite PD0335699 (Figure 1). [^{14}C] PD0331179 was synthesized in six steps starting with [^{14}C] benzoic acid, whereas [$^{2}H_7$]PD0331179 was prepared in four steps from [$^{2}H_7$] benzylbromide. An efficient synthesis for key-labeled intermediates, [^{14}C] and [$^{2}H_7$]3-phenyl-trialkylsilyl-1-propyne, was developed through a convenient two-step reaction sequence. In addition, one-pot C–C cross-coupling reaction between aryl iodide and labeled 1-triphenylsilylpropyne was achieved. The labeled metabolite [$^{2}H_7$]PD0335699 was prepared by convenient treatment of the [$^{2}H_7$]PD0331179 with an organic base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

Results and discussion

The synthesis of unlabeled PD0331179 involves a total of five steps from a commercially available starting material, 2-methylamino-benzoic acid **1** (Scheme 1). The iodo substitution of **1** with iodine in acetic acid and water gave 2-methylamino-5-iodobenzoic acid **2** in 56% yield. Reaction of methyl 4-(aminomethyl)benzoate **3** with **2** using coupling reagents, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, 1-hydroxybenzotriazole, and *N*-methylmorphine, in DMF provided the amide **4** in 95% yield. Cyclization of **4** with 1,1′-carbonyldiimidazole in THF afforded the quinazoline **5** in 75% yield, which was hydrolyzed with NaOAc to afford the acid **6**. The final C–C cross coupling between **6** and 2-phenyl-1-propyne catalyzed by $Pd(PPh_3)_4$ and CuI in diisopropylethylamine (DIPEA) furnished the final API, PD0331179, in 65%. The overall yield for the five-step synthesis was about 20%.

Because the C-14 labeling, the highlighted area of PD0331179, shown in Scheme 1, was preferred considering the metabolic stability, either starting material **1** or 2-phenyl-1-propyne could be chosen as a C-14 labeled chemical. Both labeled analogs were not commercially available. However, we selected 2-phenyl-1-propyne involved in the last step of the synthesis as our labeled target because it required less steps to prepare. Therefore, we focused on exploring an effective approach to the labeled 3-phenyl-1-propyne.

From our literature search, we found that p-chloro-substituted phenylpropyne **9a** was prepared by coupling p-cloro-benzylchloride with Grignard reagent.[10] By adapting this method, we can synthesize the labeled phenylpropyne **9b**. However, it was found that phenylpropyne was very volatile and not easy to isolate in a small scale (Scheme 2). Also, it will not be safe to use so volatile radiolabeled compound for the synthesis of [^{14}C]PD0331179.

Therefore, we proposed to synthesize a protected phenylpropyne that should not be so volatile and can be readily isolated and the protection group of which can be removed as needed later. In literatures, both bis(diisopropylamino)borane [11] and trimethylsilyl (TMS)[12,13] groups were utilized before as a protection group of an alkyne (Scheme 2). The former can be removed under aqueous

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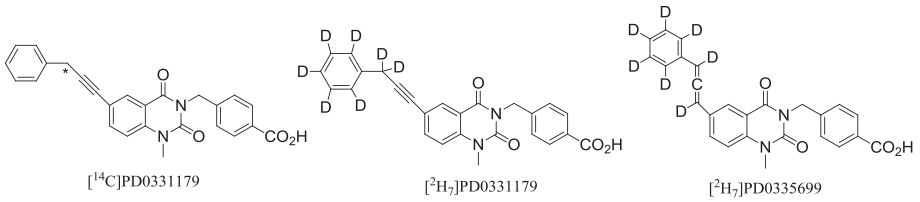
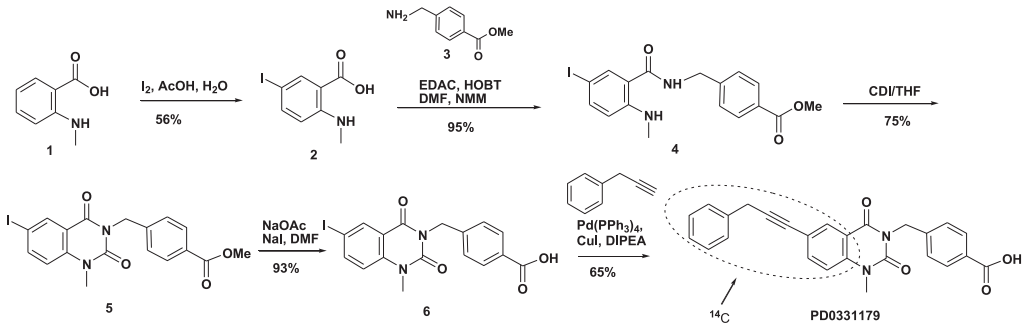
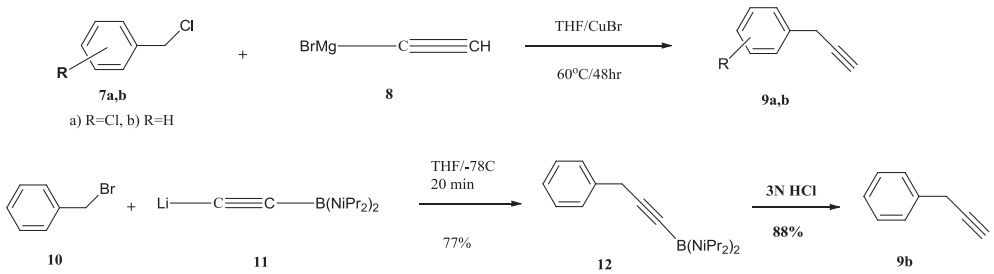


Figure 1. Structures of labeled compounds.



Scheme 1. Synthetic route of PD0331179.



Scheme 2. Known synthesis of 3-phenyl-1-propyne.

acidic condition, such as 3N HCl,[11] whereas the later can be performed in DMF under a basic condition such as KF.[12] However, the basic removal of TMS group is our better choice because KF could be a suitable base for the final C–C cross-coupling reaction of our synthesis. So, our proposal for a new approach to the labeled PD0331179 was investigated using stable isotope-labeled chemicals first (Scheme 3).

Several methods are reported in the literatures for the synthesis of 3-aryl-1-propynes.[14–16] However, the procedures are often cumbersome, unsafe to be performed, and give quite low yields. Therefore, a new general method to prepare 3-aryl-1-propynes in high yields from commercially available reagents was needed. We found that 3-phenyl-1-propyne can be prepared *in situ* very conveniently and in high overall yields. The synthesis started with 1-trimethylsilylethyne or triphenylsilylethyne. The treatment of trialkylsilylethyne **13 a**,**b** with *n*-BuLi in THF produced trialkylsilylethynyl lithium **14a**,**b**. Without isolation and purification of the lithium compound, it was immediately reacted with benzylbromide or [$^2H_7$]benzylbromide in the same pot in the presence of copper(I) bromide to give pure [$^2H_7$]3-phenyl 1-trialkylsilyl-1-propyne **15a** in 81% yield after purification. Then, **15a** was treated with an excess of potassium fluoride in DMF at 60 °C for 1 h to form the desired [$^2H_7$]3-phenyl-1-propyne **16**. Again, without isolation of the alkyne, it was coupled with the iodide **6** in the presence of $Pd(PPh_3)_4$, CuI, and DIPEA in the same pot to furnish the final target, [$^2H_7$]PD0331179, in a 78% isolated yield.

One of the metabolites of PD0331179 was allene derivative, PD0335699. It was known that isomerization between alkyne and allene took place under basic treatment. Normally, either *n*-BuLi or KOH with the help of a phase transfer catalyst promotes the conversion of alkyne to allene. However, in our case, both bases gave very poor yield (10–20%), and purification was very difficult. We found that DBU can promote the isomerization and afford a descent chemical yield (45%). It is interesting to point out that 91% deuterium remained at the highlighted position. That might further indicate that the isomerization between the alkyne and allene takes place through a hydrogen shift instead of a stepwise mechanism.

Having established conditions for the synthesis of PD0331179 and [$^2H_7$]PD0331179, it was readily adapted to synthesize the radiolabeled PD0331179 (Schemes 4 and 5). Our radiosynthesis started with a commercially available radioactive chemical, [^{14}C]benzoic acid. So, the labeled acid **17** was reduced by $LiAlH_4$ in THF to form [^{14}C]benzyl alcohol **18**. Without further purification, **18** was treated with PPh_3 and CBr_4 in methylene chloride to afford [^{14}C]benzyl bromide **19** in 82% yield after purification. Similarly, **19** was coupled with triphenylsilylethynyl lithium prepared *in situ* to give [^{14}C] 3-phenyl-1-triphenylsilylethyne **20** in 80% after purification. Because 3-phenyl triphenylsilylethyne has much higher boiling point and is nonvolatile compared with phenylpropyne and trimethylsilylethyne, the radio-safety issue was well addressed.

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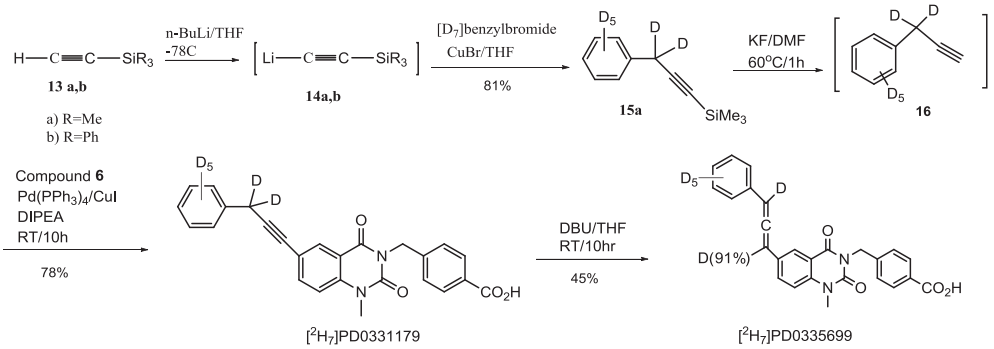
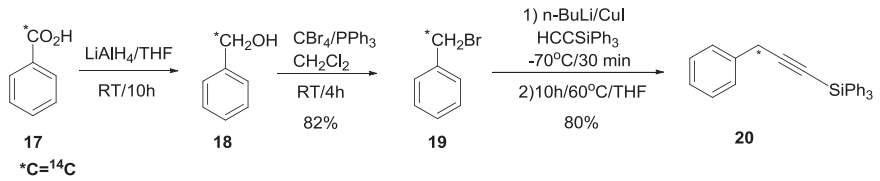
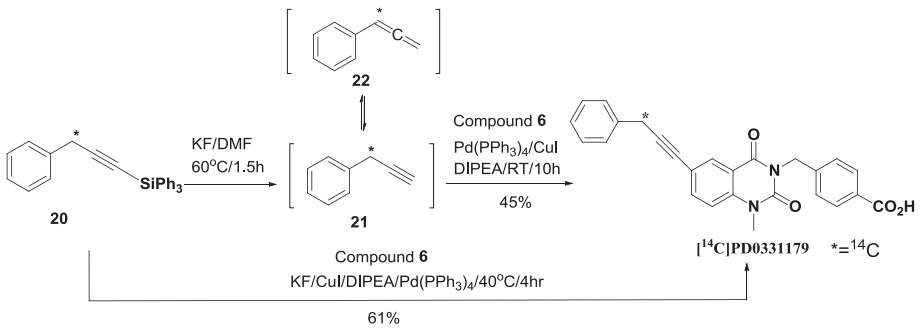


Scheme 3. Synthesis of [$^2H_7$]PD0331179 and its labeled metabolite [$^2H_7$]PD0335699.



Scheme 4. Synthesis of [3-^{14}C] 3-phenyl-1-triphenylsilylpropyne.



Scheme 5. Synthesis of [^{14}C]PD0331179.

[^{14}C] 3-Phenyl-1-triphenylsilylpropyne **20** was treated with KF in DMF at 60 °C for 1 h, and HPLC assay showed 100% of conversion of **20** to [^{14}C]3-phenyl-1-propyne **21**. However, the final cross-coupling reaction between **21** (prepared *in situ*) and the iodide **6** under the same condition as used in the preparation of [$^2H_7$]PD0331179 gave a lower yield (45% vs. 78%). One possible explanation for the lower yield is that under basic condition, the radiolabeled 3-phenyl-1-propyne **21** might be partially isomerized to allene derivative **22** that lost the reactivity of C–C cross-coupling reaction.

We came up to a solution to increase the yield by addition of all chemicals and reagents including reactants **20** and **6**, reagents KF and DIPEA, and catalysts $Pd(PPh_3)_4$ and CuI at the same time instead of stepwise addition and by reduction of the reaction temperature from 60 to 40 °C. In this way, [^{14}C]3-phenyl-1-propyne **21** produced *in situ* was coupled with the iodide **6** immediately before the isomerization took place. As a result, the new procedure for the removal of silyl protection group and cross-coupling reaction offered a better radiochemical yield (61% vs. 45%). The overall radiochemical yield from [1-^{14}C] benzoic acid **17** was 38% with a radiochemical purity of 99.4% and a specific activity of 53.8 mCi/mmol.

In conclusion, we have reported the efficient synthesis of ^{14}C-labeled and ^{2}H-labeled PD0331179 and its ^{2}H-labeled metabolite, PD00335699. A new approach to C-14 and deuterium-labeled key intermediate, 3-phenyl-1-propyne, was developed by using trimethylsilane or triphenylsilane as a protection group of the alkyne. This approach avoided potential loss of volatile radioactive material and simplified the purification process. It can be easily extended to synthesize various substituted alkynes. In addition, we discovered a one-pot C–C cross-coupling reaction of the aryl iodide with the trialkylsilyl-protected alkyne. Further study on the scope and limitation of this kind of C–C cross coupling is in progress.

Experimental

General methods

All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. LC-MS data were obtained on a Waters Micromass LCT mass spectrometer with flow injection analysis and electrospray ionization (ESI). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz instrument. Chemical purity of all compounds was determined by HPLC and LC-MS. Purifications were done by flash column chromatography on Biotage Flash 40 system. Quantitation of radioactivity of C-14 labelled compounds was performed using a Packard 2200CA liquid scintillation analyzer, with Scintiverse BD cocktail used throughout. Commercial reagents and solvents were purchased from

Sigma-Aldrich and used as-received unless otherwise noted. [^{14}C]benzoic acid (200 mCi, 54 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. [$^{2}H_7$]benzylbromide (>98% $^{2}H_7$ enrichment) was purchased from Sigma-Aldrich. Intermediate, 4-(6-iodo-1-methyl-2, 4-dioxo-1,4-dihydro-2 H-quinazolin-3-ylmethyl)-benzoic acid **6** and final API, PD0331179, were provided by Chemical R&D, Ann Arbor Lab, Pfizer Inc. All known compounds were identified by comparison of NMR spectra to those reported in the literature or the authentic samples.

4-[1-Methyl-2,4-dioxo-6-(3-[$^{2}H_5$]phenyl-[2-$^{2}H_2$]prop-1-ynyl)-1,4-dihydro-2 H-quinazolin-3-ylmethyl]-benzoic acid, [$^{2}H_7$]PD0331179

A mixture of [$^{2}H_7$] 1-trimethylsilyl-3-phenyl-1-propyne **15a** (0.80 g, 4.1 mmol), KF (0.476 g, 8.2 mmol) and DMF (15 mL) was stirred at 60 °C for 1.5 hr. After cooling to room temperature, compound **6** (1.65 g, 3.8 mmol), CuI (78 mg, 0.41 mmol) and DIPEA (2.0 ml) were added to the reaction mixture. After the mixture was purged with N_2 gas for 5 min, $Pd(PPh_3)_4$ (236 mg, 0.2 mmol) was added to the mixture at room temperature. The resulting mixture was stirred at room temperature for 10 hr and then quenched with water (80 mL). The suspension was stirred at room temperature for 30 min and then filtered to give the crude product as a brown solid. The solid was dissolved in THF (50 mL) and heated to reflux with charcoal (2.0 g) for 30 min. The charcoal was removed by filtration and washed with THF (2 x 5 mL). The filtrate was evaporated under vacuum to give a off-white solid. Further purification by flash chromatography (2% MeOH in CH_2Cl_2) gave the titled product (1.25 g, 78%) as a white powder. ^{1}H NMR (DMSO-d_6): δ 8.01 (d, J = 2.0 Hz, 1 H), 7.84 (d, J = 8.3 Hz, 2 H), 7.82 (dd, 1 H), 7.43 (d, J = 8.5, Hz, 1 H), 7.38 (d, J = 8.3 Hz, 2 H), 5.17 (s, 2 H), 3.51 (s, 3 H); ^{13}C NMR (DMSO-d_6): δ 168.1 (1C), 160.6 (1C), 150.9 (1C), 141.6 (1C), 139.2 (1C), 135.4 (1C), 132.1 (1C), 129.7 (2C), 128.8 (m, 1C), 128.6 (m, 2C), 128.2 (1C), 128.1 (m, 2C), 127.3 (2C), 117.6 (1C), 114.3 (1C), 113.3 (1C), 87.8 (1C), 81.8 (1C), 43.8 (1C), 29.8 (1C), 25. 4 (m, 1C); LC-MS: *m/z* 432 (M + H, $^{2}H_7$ enrichment >98%).

4-[1-Methyl-2,4-dioxo-6-(3-[$^{2}H_5$]phenyl-[$^{2}H_2$]propa-1,2-dieynl)-1,4-dihydro-2 H-quinazolin-3-ylmethyl]-benzoic acid, [$^{2}H_7$] PF-0335699

A solution of [$^{2}H_7$]PD0331179 (500 mg, 1.18 mmol) and DBU (450 mg, 1.0 mmol) in THF (8 mL) was stirred at room temperature for 8 hr. The reaction mixture was diluted with water (5 mL) and extracted with diethyl ether (2 x 5 mL). The aqueous layer was separated and acidified by 2 M HCl, then extracted with ethyl acetate (3 x 6 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over $MgSO_4$ and concentrated under vacuum to give the crude product. Purification by flash chromatography (5% MeOH in $CHCl_3$) afforded the titled product (225 mg, 45%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.53 (s, 3 H), 5.37 (s, 2 H), 7.15 (d, 1 H), 7.32 (t, 2 H), 7.35 (d, 1 H), 7.54 (2 H), 7.61 (dd, 1 H), 7.98 (d, 2 H), 8.22 (s, 1 H); ^{13}C NMR (DMSO-d_6): δ 203.6 (1C), 168.2 (1C), 162.3 (1C), 151.5 (1C), 142.6 (1C), 135.6 (1C), 130.7 (1C), 129.8 (2C), 128. 3 (m, 2C), 127.3 (2C), 126.7 (m, 2C), 123.3 (m, 1C), 120.5 (1C), 102.7 (m 1C), 98.3 (m, 1C), 44.5 (1C), 29.6 (1C); LC-MS: *m/z* 432 (M + H, $^{2}H_7$ enrichment 97.1%).

[^{14}C]1-Triphenylsilyl-3-phenyl-propyne (20)

To a solution of triphenylsilylacetylene (810 mg, 2.85 mmol) in dry THF (10 mL) was added n-BuLi (1.7, mL, 1.7 M in hexane) dropwise at −78 °C. The resulting mixture was stirred at −78 °C for 30 min and then at room temperature for 2 hr. The mixture was cooled at −78 °C again. To this cold mixture was added CuI (60 mg, 0.29 mmol) and then a solution of [^{14}C]benzylbromide **19** (500 mg, 2.8 mmol, 151 mCi) in THF (5 mL). The mixture was stirred for an additional 10 min at −78 °C and then at 60 °C for 20 hr. After cooling to room temperature, the reaction mixture was poured into a cold saturated NH_4Cl aqueous solution, stirred for 30 min, and extracted with diethyl ether (3 x 10 mL). The organic extract was washed with water (6 mL) and brine (6 mL), dried over $MgSO_4$ and concentrated under vacuum to give the crude product. Purification of the crude product by flash chromatography (EtOAc/hexane 10%) gave the titled product (0.838 g, 80%, 121 mCi) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.64 (d, 6 H), 7.42-7.25 (m, 14 H), 3.82 (s, 2 H); ^{13}C NMR ($CDCl_3$): δ 136.4 (1C), 135.3 (6C), 134.2 (3C), 130.7 (3C), 128.9 (1C), 128.4 (2C), 128.2 (2C), 128.0 (6C), 112.9 (1C), 84.2 (1C), 25.9 (1C); LCMS: *m/z* 375 (M + H)

4-[1-Methyl-2,4-dioxo-6-(3-phenyl-[3-^{14}C]prop-1-ynyl)-1,4-dihydro-2 H-quinazolin-3-ylmethyl]-benzoic acid, [^{14}C]PD0331179

A mixture of [^{14}C]1-triphenyllsilyl-3-phenyl-1-propyne **20** (0.4 g, 1.07 mmol, 57.8 mCi), KF (124 mg, 2.14 mmol), compound **6** (436 mg, 1.0 mmol), CuI (21 mg, 0.11 mmol) and DIPEA (0.5 mL) in DMF (5 mL) were purged with N_2 for 10 min. $Pd(PPh_3)_4$ (62 mg, 0.052 mmol) was added to the mixture at room temperature. The resulting mixture was stirred at 40 °C for 4 hr and then quenched with water (20 mL). The suspension was stirred at room temperature for 30 min and then filtered to give the crude product as a brown solid. The solid was dissolved in THF (15 mL) and heated to reflux with charcoal (0.5 g) for 30 min. The charcoal was removed by filtration and washed with THF (2 x 5 mL). The filtrate was evaporated under vacuum to give a off-white solid. Further purification by flash chromatography (2% MeOH in CH_2Cl_2) gave the product (263 mg, 62%, 53.8 mCi/mmol, 33.3 mCi) as a white solid. HPLC condition: column, YMC ODS-AQ, 5 μm, 250 x 4.6 mm; mobile phase, A = 0.1% TFA in H_2O, B = CH_3CN; 60% B linear gradient to 80% B over 15 min, hold A:B 20:80 to 45 min; flow rate, 1.0 mL/min; UV detection, 218 nm, retention time = 11.8 min; TLC: 6% MeOH in CH_2Cl_2, Rf = 0.45; ^{1}HNMR (DMSO-d_6): δ 12.78 (bs, 1 H), 8.04 (d, J = 2.0 Hz, 1 H), 7.83 (d, J = 8.3 Hz, 2 H), 7.82 (dd, 1 H), 7.21-7.53 (m, 8 H), 5.17 (s, 2 H), 3.54 (s, 2 H), 3.51 (s, 3 H); ^{13}C NMR (DMSO-d_6): δ 168.2 (1C), 160.8(1C), 150.9 (1C), 141.6 (1C), 139.2 (1C), 135.5 (1C), 132.2 (1C), 129.9 (2C), 128.9 (1C), 128.6 (2C), 128.2 (1C), 128.1 (2C), 127.3 (2C), 117.6 (1C), 114.3(1C), 113.3 (1C), 87.8(1C), 81.8 (1C), 43.9 (1C), 29.8 (1C), 25. 5 (1C); LC-MS: *m/z* 425 (M + H).

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Conflict of Interest

The authors did not report any conflict of interest.

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