Supplementary Material

Cerebral Amyloid Angiopathy in a Mouse Model of Alzheimer's Disease Associates with Upregulated Angiopoietin and Downregulated Hypoxia-Inducible Factor

Synthesis of Methoxy-X04

General information

All reagents and solvents were purchased from Sigma Aldrich Co., were ≥95% pure and were used as received. Silica Gel 60 (particle size: 0.04-0.063 mm/230-400 mesh) was used for flash column chromatography. ^{1}H NMR data were recorded on Bruker Avance 200 (200.13) and Bruker Avance 400 (400.18) MHz spectrometers at 292-298 K and processed using the program MestReNova, version 14.2.0-26256, from Mestrelab Research S. L. The spectra were calibrated against residual $CHCl_3$ (δ_H = 7.26 ppm) or CHD_2SOCD_3 (δ_H = 2.50 ppm). Chemical shifts were determined using the weak coupling limit approximation. Coupling constants have been rounded off to the nearest whole number (in Hz) and are reported also for signals denoted as multiplets (m) if one coupling constant has been determined with a high degree of certainty.

Synthetic procedures

1,4-bis(bromomethyl)-2-methoxybenzene [1] **2** 2,5-dimethylanisole **1** (10.00 g, 73.43 mmol) was weighed out in a 500 mL round bottomed flask and dissolved in CCl_4 (300 mL). *N*-bromosuccinimide (27.44 g, 0.1542 mol) was added over 6 min at room temperature. Next, dibenzoyl peroxide (0.71 g, 2.9 mmol) was added in one portion and the reaction mixture stirred for 10 min at room temperature. The reaction flask was then placed in a silicone oil bath preheated to 85°C. After stirring for 20 min the temperature of the oil bath was adjusted to 95°C and stirring

continued for another 4 h. The oil bath was removed, and the reaction mixture allowed to cool to room temperature over a period of 1 h. The reaction mixture was then filtered under suction to remove precipitated succinimide. The solvent was evaporated under reduced pressure at 50°C over 1 h and most of the obtained solid (21.25 g of the 21.96 g crude yield) recrystallized from hexane (18 mL). Next, the recrystallized solid was washed with hexane (14 mL) at room temperature and redissolved in hexane (35 mL) under heating. The resulting solution was hot filtered, with hexane (5 mL) in the receiving Erlenmeyer flask, and cooled to room temperature. The precipitated solid was isolated by filtration under suction and washed with additional hexane (14 mL). Finally, the solid was dried under suction, affording the title compound (9.73 g, corresponding to 10.06 g from 21.96 g crude product, 47%). ^{1}H NMR (200 MHz, $CDCl_3$, 298.2 K) δ 7.29 (d, J = 8 Hz, 1H, Ar-*H* (*m* to OCH_3)), 6.95 (dd, J = 8 Hz, 2 Hz, 1H, Ar-*H* (*p* to OCH_3)), 6.91 (d, J = 2 Hz, 1H, Ar-*H* (*o* to OCH_3)), 4.54 (s, 2H, Ar-CH_2-Br), 4.47 (s, 2H, Ar-CH_2-Br), 3.91 (s, 3H, OCH_3)

Tetraethyl ((2-methoxy-1,4-phenylene)bis(methylene))bis(phosphonate) [1] **3** 1,4-bis(bromomethyl)-2-methoxybenzene **2** (8.02 g, 27.3 mmol) was weighed out in a 50 mL round bottomed flask and triethyl phosphite (13.60 g, 81.85 mmol) added under stirring over 5 min at room temperature. A condenser equipped with a septum and an empty balloon was mounted on the reaction flask and the flask placed in a silicone oil bath preheated to 165°C. A yellow solution formed immediately, and the mixture started to reflux. The pressure that developed inside the condenser resulted in the loss of some triethyl phosphite vapor. The temperature was subsequently lowered to 150°C and the reaction mixture stirred for an additional 5 h 40 min. The oil bath was removed, and the reaction mixture allowed to cool to room temperature under stirring. The crude product was purified by flash column chromatography (eluent: CH_2Cl_2/MeOH (19:1)), affording a pale yellow oil, which upon standing in a fridge overnight

formed a pale yellow-green solid (3.47 g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$, 291.9 K) δ 7.22 (dd, 1H, *J* = 8 Hz, 3 Hz, Ar-*H* (*m* to OCH_3)), 6.83 (br s, 1H, Ar-*H* (o to OCH_3)), 6.82-6.79 (m, 1H, *J* = 8 Hz, Ar-*H* (*p* to OCH_3)), 4.04-3.94 (m, 8H, OCH_2CH_3), 3.82 (s, 3H, OCH_3), 3.19 (d, 2H, $^{2}J_{PH}$ = 22 Hz, $CH_2PO(OEt)_2$), 3.10 (d, 2H, $^{2}J_{PH}$ = 22 Hz, $CH_2PO(OEt)_2$), 1.22 (q, *J* = 6 Hz, 12H, OCH_2CH_3) *Note*: The chemical shifts of the benzylic protons and the P-H coupling constants have not been unambiguously determined by phosphorous decoupling experiments.

4-(ethoxymethoxy)benzaldehyde [2] **5** 4-hydroxybenzaldehyde **4** (9.16 g, 75.0 mmol) was weighed out in a 500 mL round bottomed flask and CH_2Cl_2 (225 mL) and Adogen® (6.00 g) added. Next, an aqueous solution of NaOH (75 mL, 6.00 g NaOH, 0.150 mol) was added over 5 min at room temperature and the mixture stirred for another 10 min. Chloromethyl ethyl ether (95% pure) (10.64 g, corresponding to 10.11 g pure compound, 0.1069 mol) was then added dropwise over 5 min. The reaction flask was equipped with a septum and an empty balloon, and the reaction mixture vigorously stirred for 15 h at room temperature. Next, the organic phase was separated from the aqueous phase and successively washed with H_2O (75 mL), 1.2 M HCl (75 mL), a 3:1 (v/v) mixture of H_2O and saturated brine (100 mL), and saturated brine (75 mL). The solution was dried with anhydrous $MgSO_4$ and filtered. The $MgSO_4$ was washed with CH_2Cl_2 (50 mL) and the washings combined with the filtrate. The solvent was evaporated under reduced pressure at 50°C, affording a pale yellow oil (17.81 g). Finally, the crude product was purified by flash column chromatography (eluent: Et_2O/hexane (1:1)), affording the title compound as a clear liquid (12.64 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$, 293.1 K) δ 9.90/9.89 (s, 1H, C*H*O), 7.83/7.83 (m, *J* = 9 Hz, 2H, Ar-*H* (*o* to CHO)), 7.15/7.14 (m, *J* = 9 Hz, 2H, Ar-*H* (*m* to CHO)), 5.30/5.30 (s, 2H, OCH_2O), 3.74/3.73 (q, *J* = 7 Hz, 2H, CH_2CH_3), 1.22/1.22 (t, *J* = 7 Hz, 3H, CH_2CH_3) *Note*:

For reasons unclear to the authors all signals appeared doubled. As a result, two closely similar chemical shifts are reported for each signal.

4,4'-((1*E*,1'*E*)-(2-methoxy-1,4-phenylene)bis(ethene-2,1-diyl))bis((ethoxymethoxy)benzene) 6 Tetraethyl ((2-methoxy-1,4-phenylene)bis(methylene))bis(phosphonate) **3** (2.30 g, 5.63 mmol) was weighed out in a dried three-necked round bottomed flask and dissolved in dry THF (40 mL). Sodium hydride (60% (w/w) dispersion in mineral oil, 0.496 g, 0.298 g NaH, 12.4 mmol) was added in portions over 5 min. The reaction mixture was then heated to 75°C over 5 min and stirred for another 1 h. Next, 4-(ethoxymethoxy)benzaldehyde **5** (2.03 g, 11.3 mmol) was dissolved in dry THF (20 mL) and the solution added to the reaction mixture dropwise (by syringe) under stirring over 16 min. The reaction mixture was subsequently refluxed for another 2 h before the oil bath was removed and the mixture allowed to cool to room temperature over 20 min. Water (75 mL) was added to quench the reaction and the mixture stirred for 20 min. The resulting suspension was transferred to a 500 mL round bottomed flask together with THF/H_2O (1:1 (v/v)) (50 mL) used to clean the reaction flask. Next, the suspension was concentrated to dryness on a rotary evaporator (bath temperature: 50°C). Most of the residue (4.22 g out of 4.98 g) was partitioned between EtOAc (150 mL) and H_2O (75 mL). The aqueous phase was discarded, and the organic phase washed with H_2O (2 × 75 mL). The solution was dried with anhydrous $MgSO_4$ (6.10 g), filtered and the $MgSO_4$ washed with EtOAc (50 mL). The filtrate and washings were combined, and the solvent evaporated under reduced pressure, affording a yellow-green oil. Upon addition of hexane (35 mL) a solid precipitated. Decanting the supernatant and drying the precipitate afforded the title compound (1.36 g, corresponding to 1.60 g from 4.98 g residue, 62%). ^{1}H NMR (200 MHz, $CDCl_3$, 298.2 K) δ 7.56 (d, J = 8 Hz, 1H, Ar-*H* (*m* to OCH_3)), 7.47 (d, J = 9 Hz, 2H, Ar-*H* (*m* to OEOM)), 7.46 (d, J = 9 Hz, 2H, Ar-*H* (*m* to OEOM)), 7.36 (d, J = 17 Hz, 1H, C*H*=CH),

7.12-7.01 (m, 5H, Ar-*H* (*o* to OCH_3)/Ar-*H* (*p* to OCH_3)/C*H*=CH/CH=C*H*/CH=C*H*), 7.04 (d, *J* = 9 Hz, 2H, Ar-*H* (*o* to OEOM)), 7.03 (d, *J* = 9 Hz, 2H, Ar-*H* (*o* to OEOM)), 5.25 (s, 2H, OCH_2O), 5.24 (s, 2H, OCH_2O), 3.95 (s, 3H, OCH_3), 3.75 (q, *J* = 7 Hz, 4H, CH_2CH_3), 1.24 (t, *J* = 7 Hz, 6H, CH_2CH_3)

4,4'-((1*E*,1'*E*)-(2-methoxy-1,4-phenylene)bis(ethene-2,1-diyl))diphenol (Methoxy-X04)

[3] **7** 4,4'-((1E,1'E)-(2-methoxy-1,4-phenylene)bis(ethene-2,1-diyl))bis((ethoxymethoxy)benzene) **6** (0.126 g, 0.274 mmol) was weighed out in a 50 mL round bottomed flask and suspended in AcOH/H_2O (4:1 (v/v)) (25 mL). The suspension was stirred for 5 min at room temperature before being heated to 90°C and stirred for another 1 h 20 min. Next, the reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (100 mL). The organic phase was washed with H_2O (3 × 100 mL). The solution was dried with anhydrous $MgSO_4$ (2.49 g), filtered and the $MgSO_4$ washed with EtOAc (10 mL). The filtrate and washings were combined, and the solvent evaporated under reduced pressure, affording the title compound as a green solid (0.086 g, 91%). ^{1}H NMR (400 MHz, CD_3SOCD_3, 295.0 K) δ 9.60 (s, 1H, Ar-O*H*), 9.57 (s, 1H, Ar-O*H*), 7.58 (d, *J* = 8 Hz, 1H, Ar-*H* (*m* to OCH_3)), 7.45-7.41 (m, *J* = 9 Hz, 2H, Ar-*H* (*m* to OH)), 7.40-7.36 (m, *J* = 9 Hz, 2H, Ar-*H* (*m* to OH)), 7.20 (d, *J* = 17 Hz, 1H, C*H*=CH), 7.19 (d, *J* = 17 Hz, 1H, CH=C*H*), 7.18 (m, 1H, Ar-*H* (*o* to OCH_3)), 7.13 (d, *J* = 8 Hz, 1H, Ar-*H* (*p* to OCH_3)), 7.11 (d, *J* = 17 Hz, 1H, C*H*=CH), 7.01 (d, *J* = 16 Hz, 1H, CH=C*H*), 6.79-6.76 (m, *J* = 9 Hz, 2H, Ar-*H* (*o* to OH)), 6.77-6.75 (m, *J* = 9 Hz, 2H, Ar-*H* (*o* to OH)), 3.90 (s, 3H, OCH_3)

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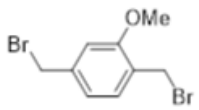
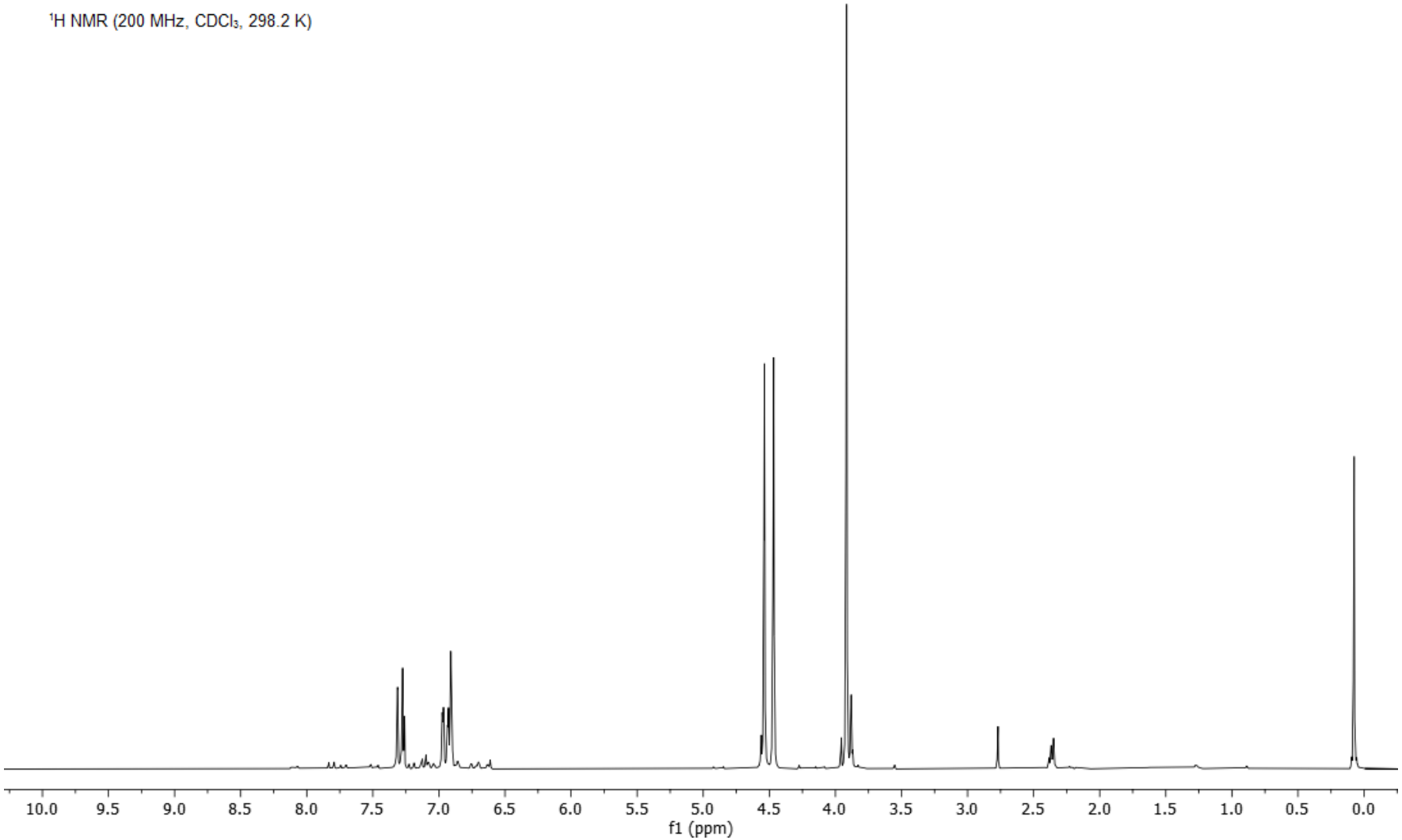
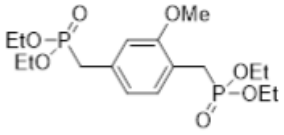


^{1}H NMR (200 MHz, $CDCl_3$, 298.2 K)





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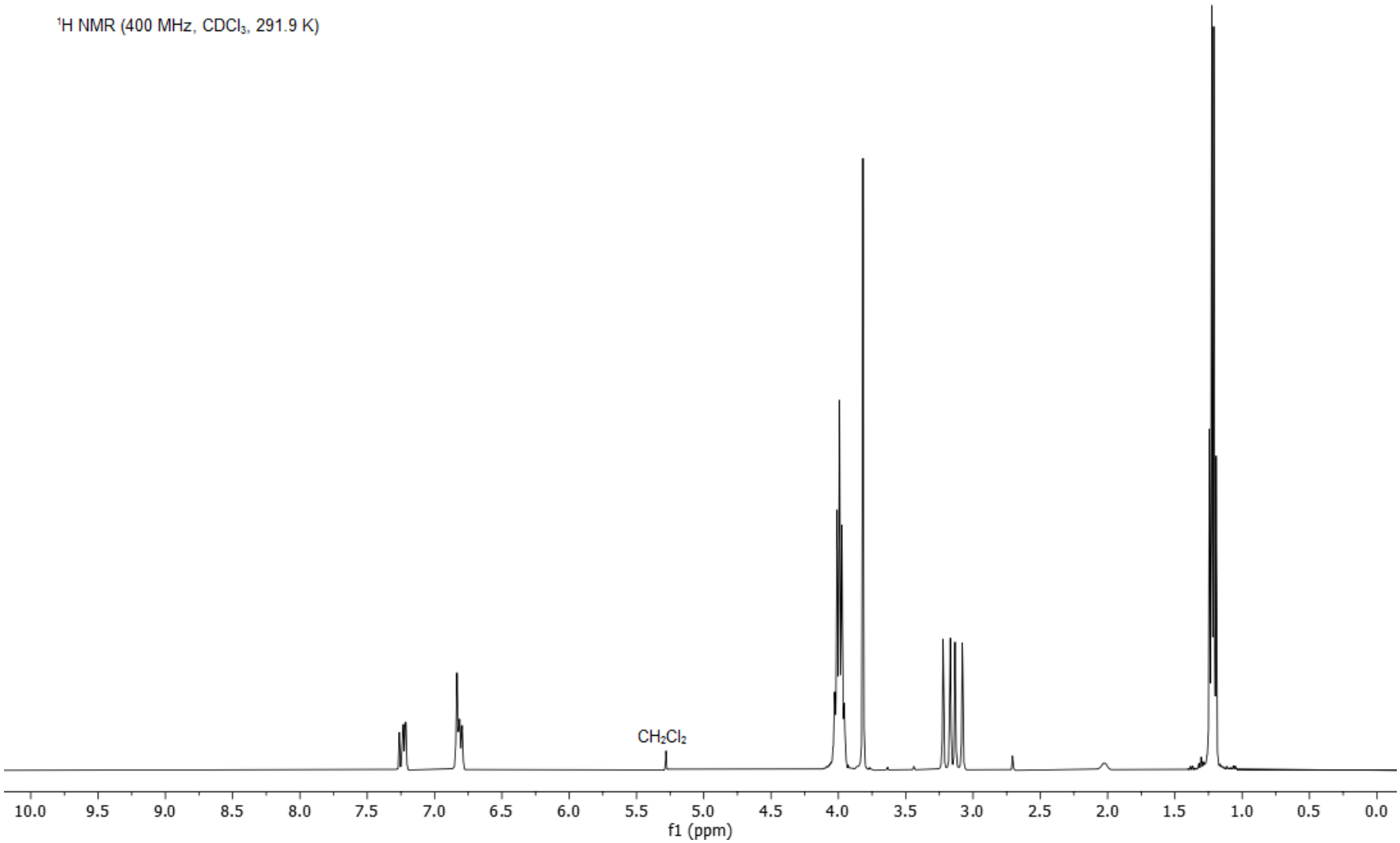
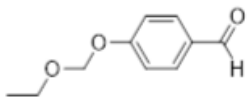
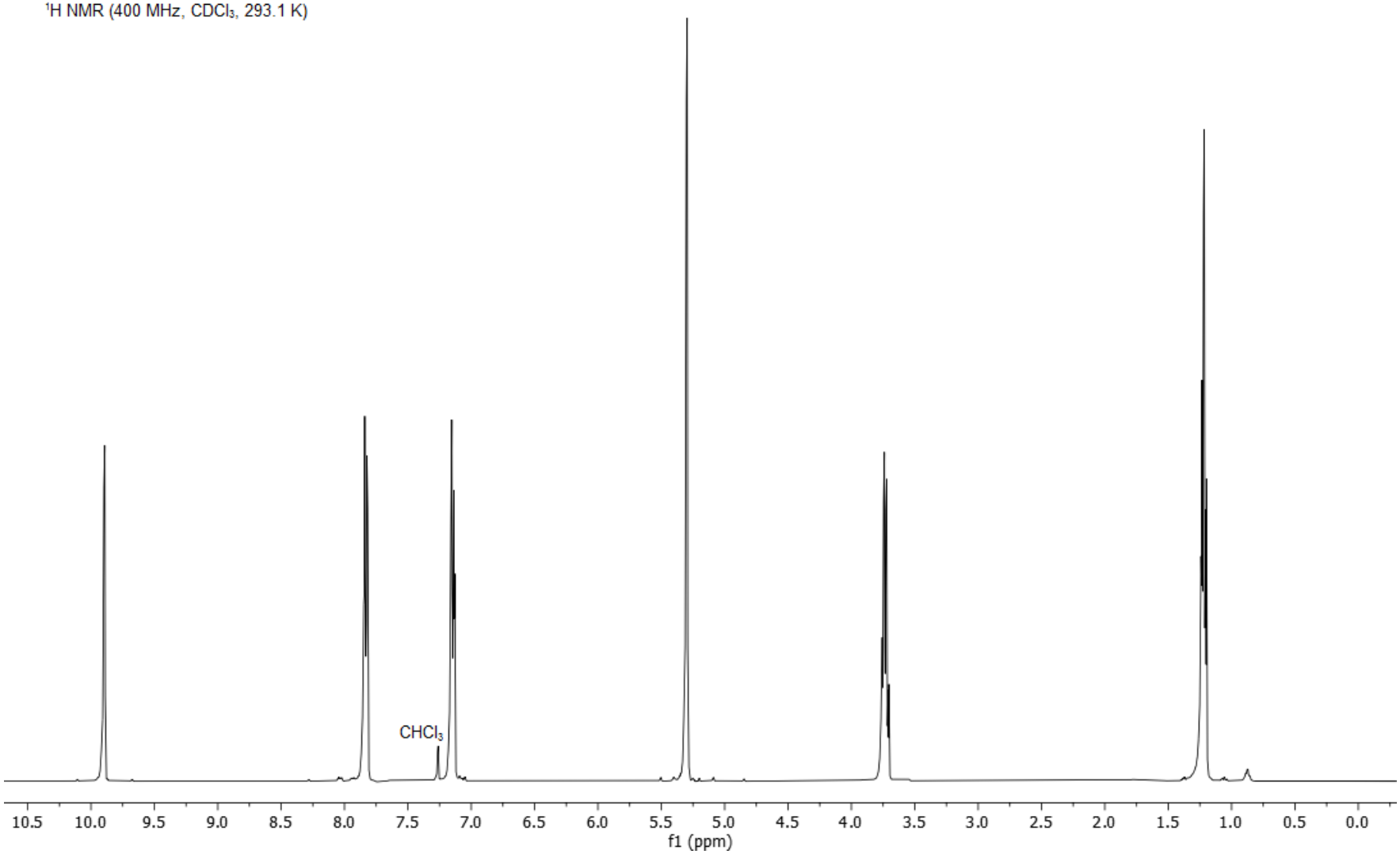
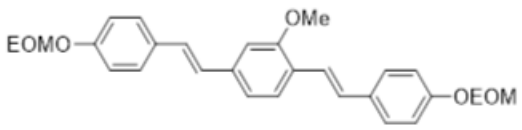


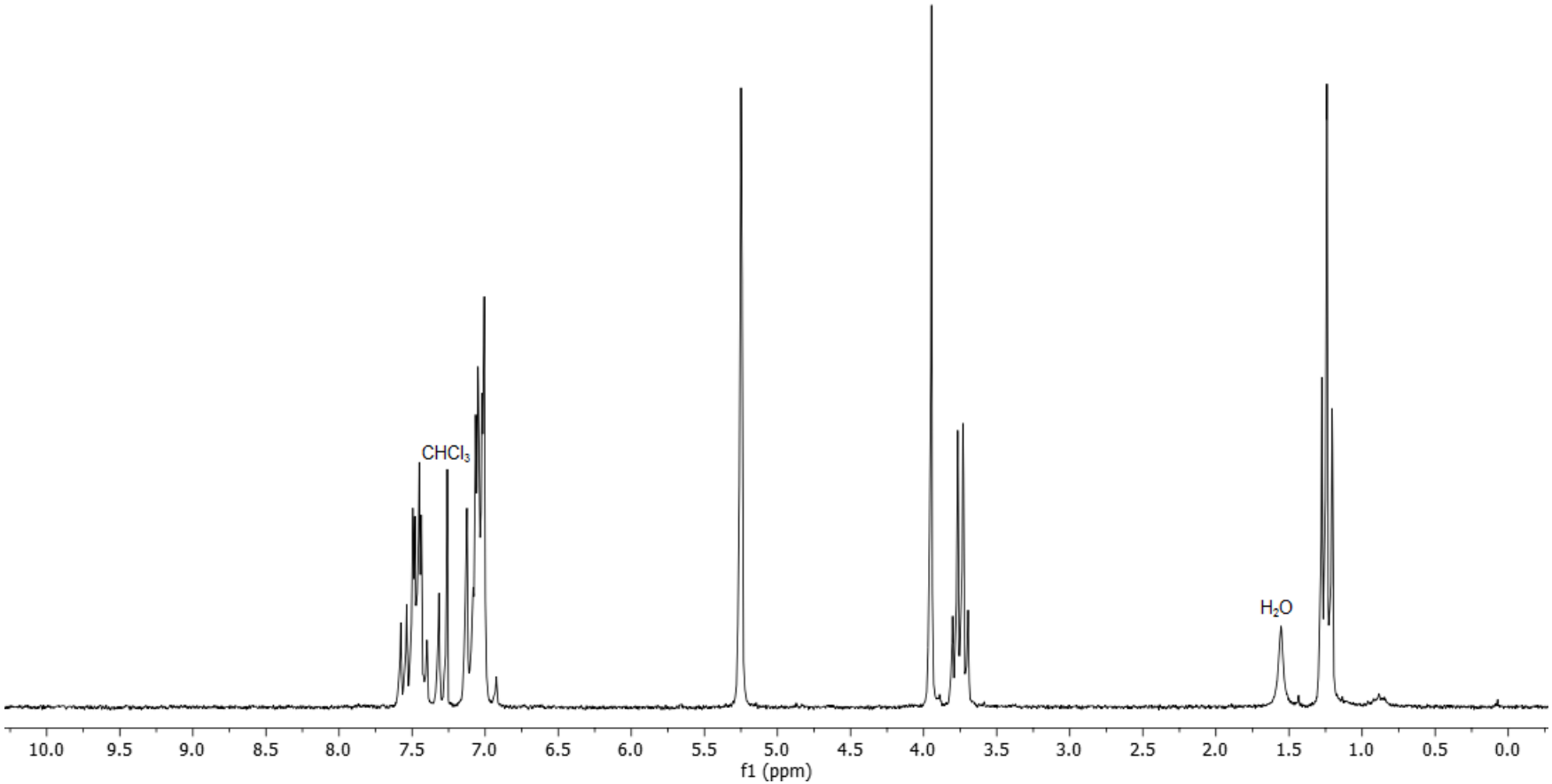


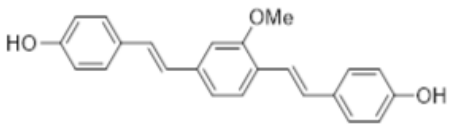
^{1}H NMR (400 MHz, $CDCl_3$, 293.1 K)



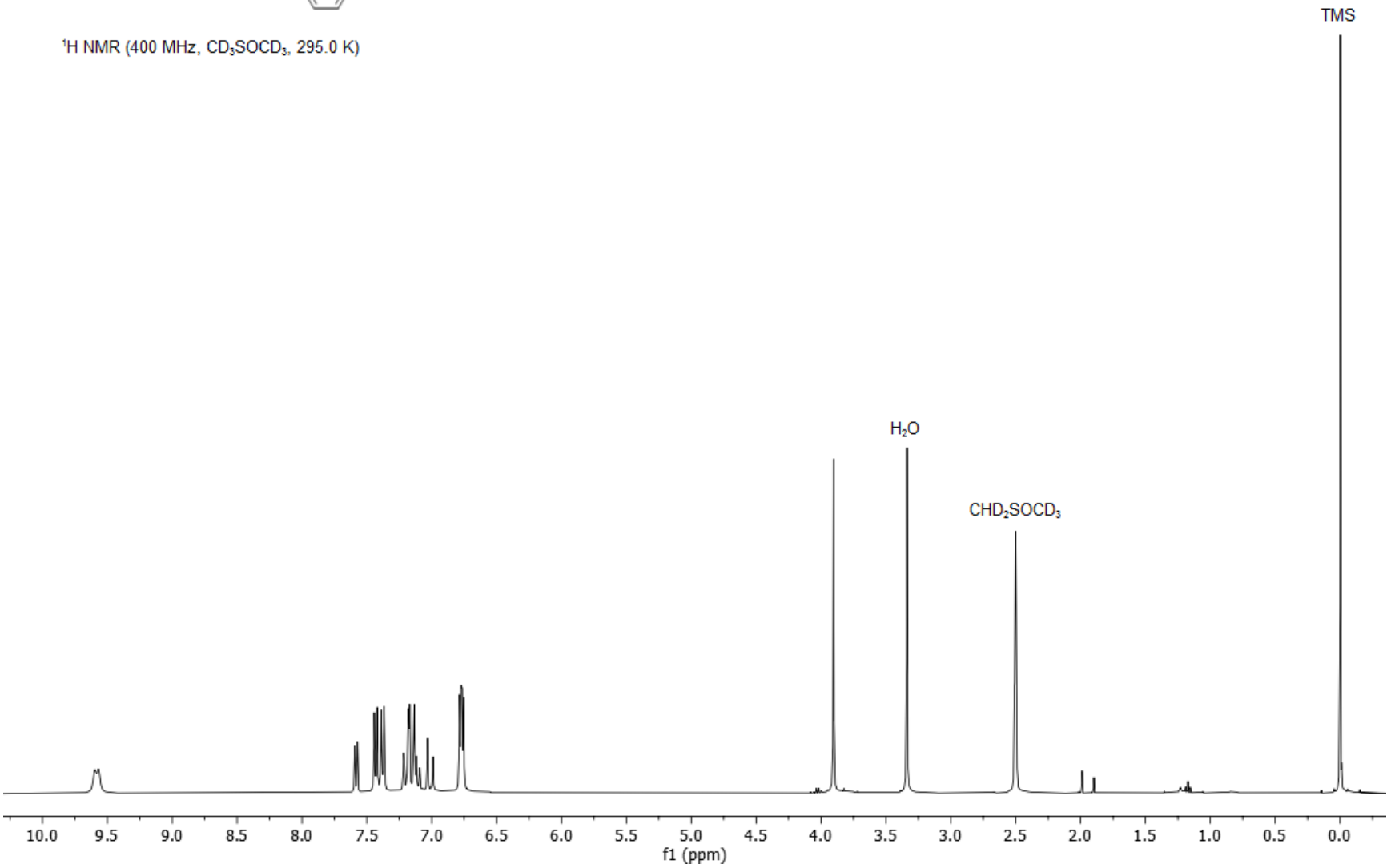


^{1}H NMR (200 MHz, $CDCl_3$, 298.2 K)





^{1}H NMR (400 MHz, CD_3SOCD_3, 295.0 K)

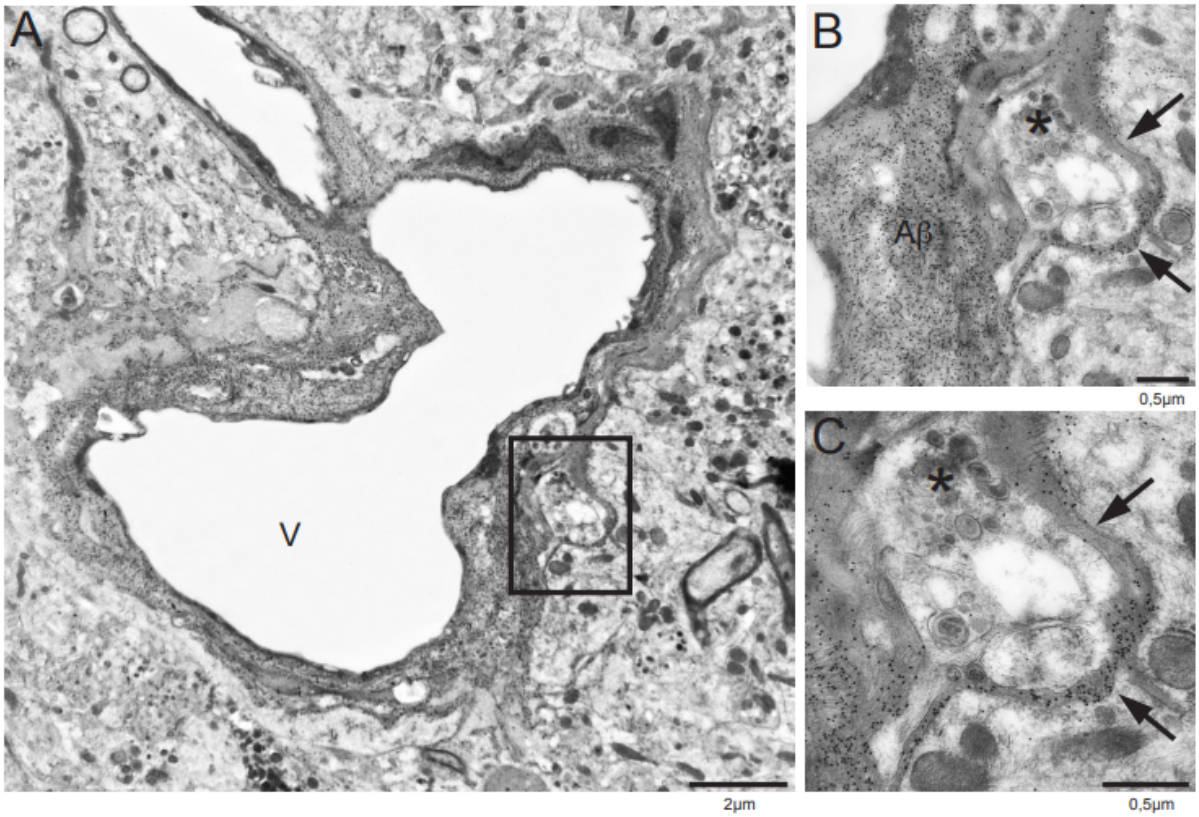


Supplementary Table 1. qPCR results demonstrated with p-values

Gene	Mean copy number/ng of total RNA; Wild types	Mean copy number/ng of total RNA; Tg-ArcSwe	p
Hif1a	144 ± 13	55 ± 3.4	0.000*
Vegfa	221 ± 31	184 ± 13	0.195
Vegfb transcript variant 1	166 ± 16	186 ± 8.3	0.234
Vegfr1/Flt1 transcript variant 1	1169 ± 109	919 ± 81	0.130
Vegfr1/Flt1 transcript variant 2	144 ± 8,7	143 ± 25	0.645
Vegfr2/Kdr transcript variant 1	380 ± 35	304 ± 36	0.161
Vegfr2/Kdr transcript variant 2	350 ± 36	234 ± 16	0.038*
Angpt1	13.8 ± 1.7	18.6 ± 1.6	0.038*
Angpt2	5.1 ± 0.71	8.0 ± 1.3	0.05*
Tie1	139 ± 14.9	144 ± 12.5	0.80
Tie2	3081 ± 368	4794 ± 425	0.015*

The table shows mean copy number reported with SEM. Significant p-values (≤ 0.05) are marked with asterisk.

Supplementary Figure 1. Cortical vessel with CAA



A) The electron micrograph demonstrates a cross section of a cortical vessel with severe CAA. The Aβ fibrils are marked with gold particles (using antibody $Aβ_{x-40}$) and can be seen around the entire perimeter of the vessel. Necrotic tissue surrounds the vessel. The frame in panel A is enlarged in panel B and C. Scale bar 2 µm. B, C) A swollen pathological pericyte (asterisk). The arrows point to gold particles marking Aβ in the basal membrane. Scale bar 0.5 µm. V, vessel lumen.