Supporting Information for

Rapid and efficient microwave-assisted synthesis of highly sulfated organic scaffolds

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CONTENTS

GENERAL METHODS

All reactions sensitive to air or moisture were carried out under nitrogen atmosphere in oven-dried glassware. All reagent solutions unless otherwise noted were handled under an inert nitrogen atmosphere using syringe techniques. Anhydrous dichloromethane and acetonitrile were purchased from Sigma-Aldrich and Acros Organics, respectively, and were used without further drying. Trimethylamine-sulfur trioxide and pyridine-sulfur trioxide complexes were purchased from Alfa-Aesar and Fluka, respectively. All other reagents/chemicals were purchased from Sigma-Aldrich and were used as supplied. Analytical thin-layer chromatography (TLC) was performed using UNIPLATE^{TM} silica gel GHLF 250 μm pre-coated plates (ANALTECH, Newark, DE) that were analyzed by fluorescence (254 nm). Column chromatography was performed using silica gel (40-60 μm, 60 Å, Silicycle, Quebec, Canada) and the indicated technical grade solvents. After chromatography, solvents were evaporated using a Büchi rotary evaporator, followed by further treatment under high vacuum.

Microwave-based sulfation reactions were performed using a CEM-Discover (Matthews, NC) synthesizer in sealed reaction vessels (7 mL). The stirring parameter in the microwave synthesizer was set to “Hi-speed”. The reaction mixture was ramped to 100 or 120 ºC using the following power-temperature steps: 1) 50 W, r.t. – 80 ºC, and 2) 10 W, 80-100/120 ºC. The reaction vessel was simultaneously cooled using nitrogen (45 psi) to maintain the set temperature.

Sephadex G10 chromatography (de-salting) and SP Sephadex-Na chromatography (cation exchange) were performed using Flex columns (KIMBLE/KONTES, Vineland, NJ) of dimensions 170 × 1.5 cm and 75 × 1.5 cm, respectively. Cation exchange was performed with 30-fold excess of sodium ion equivalents. Samples were chromatographed at a controlled flow rate of 0.5 mL/min with water as eluent. Five mL fractions were collected and analyzed by RP-HPLC or capillary electrophoresis (see below).

^{1}H-NMR and ^{13}C-NMR were recorded on Varian Mercury-300 MHz or Varian Inova-400 MHz spectrometers in $CDCl_3$, DMSO-d6, CD_3OD or CD_3COCD_3. All signals are reported in ppm with the internal chloroform, DMSO, CD_3OD and CD_3COCD_3 signals at 7.26, 2.50, 3.31 and 2.05 ppm, respectively, as standards. The data is being reported as: chemical shifts (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet or unresolved, br = broad signal), coupling constant(s) (Hz), and integration.

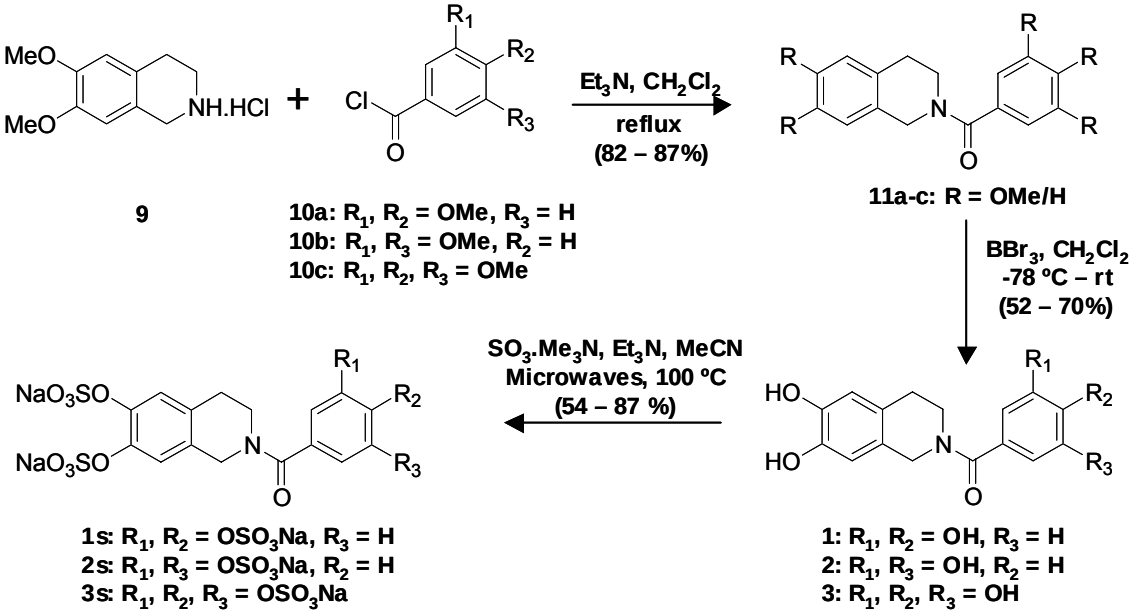
ESI mass spectra were recorded using a Micromass ZMD 4000 single quadrupole spectrometer. Samples were dissolved in methanol or methanol-acetonitrile (1:1) and infused at a rate 10 μL/min. Mass scans were obtained in the range 100-1300 amu at a scan rate of 400 amu/s. Ionization conditions were optimized for each compound to maximize ionization of the parent ion. The capillary voltage was varied between 3.0 and 4.5 V, while the cone voltage usually ranged from 20 to 80 V. For all experiments, the extractor voltage was set to 4.0, the Rf lens voltage to 0.1 V, the source block temperature to 100 ºC and the desolvation temperature to 120 ºC.

Capillary electrophoresis using a Beckmann PACE/ MDQ unit was performed to test completion of sulfation reaction and assess isolated product purity. An uncoated fused silica capillary of 50 μm internal diameter and 32.5 cm effective length to the detector window was used. Samples were typically injected under a pressure of 0.5-1 psi for 5 s and detected spectrophotometrically using a 254 nm filter. Electrophoresis was performed under reverse polarity conditions at 25 °C and a constant voltage of 10 kV using 20 mM sodium phosphate, pH 2.7 or 4.3.

HPLC analysis was carried out on a Shimadzu chromatography system using Waters Atlantis dC18 column (5μ, 4.6 × 250 mm). The mobile phase consisted of a 100 mM sodium chloride-acetonitrile mixture(7:3 v/v) run at a constant flow rate of 0.5 mL/min. Analysis was carried out using a uv-vis detector at 254 nm.

EXPERIMENTAL PROCEDURES AND SPECTRAL DATA

SCHEME I



Synthesis of amides **11a** -**c**: To a stirred suspension of amine **9** (2g, 8.7 mmol) and triethylamine (6.1 mL, 43.5 mmol) in dichloromethane (40 mL) at 0 ºC, was added acid chloride **10a-c** (9.14 mmol, 1.05 equiv.). The reaction mixture was allowed to warm to room temperature and refluxed. After 4 hrs, the reaction mixture diluted with dichloromethane (50 mL), washed with 0.5 N HCl (3 × 50 mL) and potassium carbonate (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to obtain a colorless oil (82 **-** 87 %). **11a:** ^{1}H-NMR (300 MHz, CD_3COCD_3): δ 6.59 – 6.90 (m, 5H), 4.71 (br, 2H), 3.64 – 3.84 (m, 14 H), 2.87 (br, 2H); ESI (+ve) *m/z* calcd for $C_{20}H_{23}NO_5$ [$(M+H)^+$] 358.17, found 358.3; **11b:** ^{1}H NMR (300 MHz, CD_3COCD_3) δ 6.87 – 6.92 (m, 3H), 6.60 (s, 2H), 4.5 (s, 2H, CH_2), 3.63 – 3.72 (m, 14 H), 2.67 (t, *J* = 5.7 Hz, 2H); ESI (+ve) *m/z* calcd for $C_{20}H_{23}NO_5$ [$(M+H)^+$] 358.17, found 358.1; **11c:** ^{1}H-NMR (300 MHz, CD_3COCD_3):

δ 6.77 – 6.79 (m, 4H), 4.68 (s, 2H), 3.77 – 3.88 (m, 17 H), 2. 84 (t, *J* = 5.7 Hz, 2H); ESI (+ve) *m/z* calcd for $C_{21}H_{25}NO_6$ [$(M+H)^+$] 388.18, found 388.1

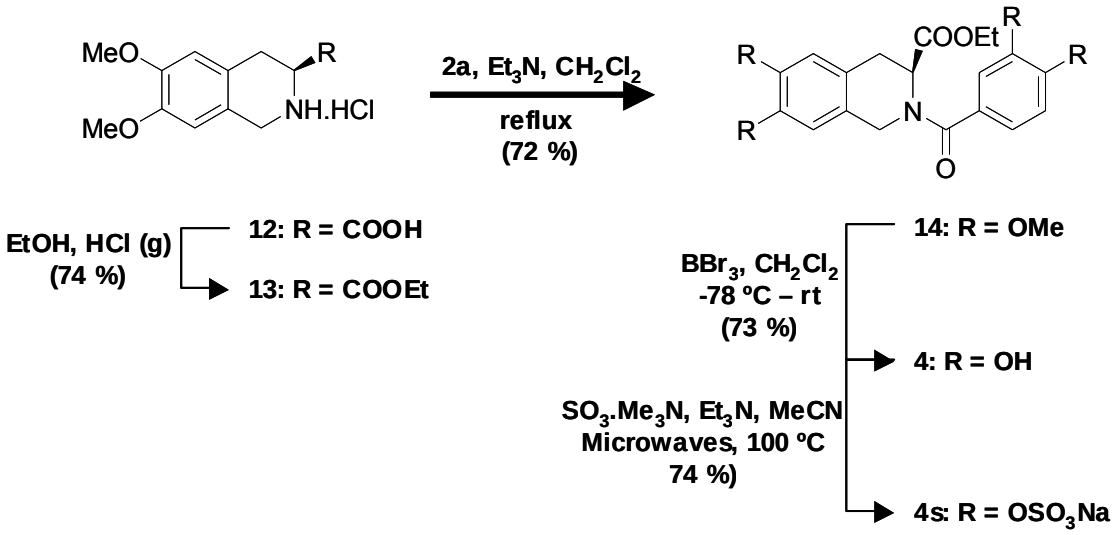
Polyphenols **1-3**: To a stirred solution of the amide (7.0 – 7.5 mmol) in dichloromethane (80 mL) at -78 ºC, was added BBr_3 (36 – 42 mL of 1M solution in CH_2Cl_2, 1.2 equiv per OMe group) under N_2 over 15 minutes. After stirring for 12 hrs at rt, the reaction was quenched at 0 °C with MeOH (10 mL) and water (10 mL). The reaction mixture was partitioned between EtOAc (220 mL) and 2N HCl (50 mL). The aqueous layer was diluted with brine (50 mL) and washed with EtOAc (6 x 50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel chromatography (Hexanes/EtOAc = 1:1, 1:4, 1:4.5, 0:1) to give a yellow solid (52 - 70%). **1:** ^{1}H NMR (300 MHz, CD_3OD) δ: 6.30 – 6.61 (m, 5H, isomers I & II), 4.66 (s, 2H, isomer I), 4.44 (s, 2H, isomer II), 3.88 (t, *J* = 6.0 Hz, 2H, isomer II), 3.62 (t, *J* = 6.0 Hz, 2H, isomer I), 2.80 (t, *J* = 6.0 Hz, 2H, isomer II), 2.73 (t, *J* = 6.0 Hz, 2H, isomer I); ESI (+ve) *m/z* calcd for $C_{16}H_{15}NO_5$ [$(M+H)^+$] 302.10, found 302.2, ESI (-ve) calcd for $C_{16}H_{15}NO_5$ [$(M-H)^-$] 300.09, found 300.07; **2:** 1H NMR (300 MHz, CD_3OD): δ 6.40 - 6.91 (m, 5H), 4.63 (br 2H), 3.68 – 3.86 (m, 2H), 2.77 (s, 2H); ESI (+ve) calcd *m/z* for $C_{16}H_{15}NO_5$ [$(M+H)^+$] 302.10, found 302.16, ESI (-ve) calcd for $C_{16}H_{15}NO_5$ [$(M-H)^-$] 300.09, found 300.1; **3:** ^{1}H NMR (400 MHz, CD_3OD): δ 6.54 (s, 2H), 6.44 (s, 2H), 4.48 – 4.56 (m, 2H), 3.62 – 3.78 (m, 2H), 2.72 (s, 2H); ESI (+ve) *m/z* calcd for $C_{16}H_{15}NO_6$ [$(M+H)^+$] 318.10, found 318.0, ESI (-ve) calcd for $C_{16}H_{15}NO_6$ [$(M-H)^-$] 316.08, found 316.0

Per-sulfates **1s and 2s**: To a stirred solution of the poly-alcohol (20 mg, 0.066 mmol) in MeCN (1 mL) at rt, Et_3N (0.4 mL, 2.9 mmol) and $Me_3N.SO_3$ (220 mg, 1.6 mmol) was added. The reaction vessel was sealed and micro-waved for 20 minutes at 100 ºC. The reaction was repeated for 4 times and the reaction mixture was pooled together. The MeCN layer was decanted and pooled, while the residue was washed with MeCN (5 mL) and centrifuged. The combined MeCN layers were concentrated *in vacuo*. Water (5 mL) was added to the residue and stirred for 10 min. The water layer was concentrated to approximately 2 mL, loaded onto a Sephadex G10 column (~ 160 cm) and chromatographed using water as eluent. Fractions were combined based on RP-HPLC profiles, concentrated and re-loaded onto a SP Sephadex C25 column for sodium exchange. Appropriate fractions were pooled, concentrated *in vacuo* and lyophilized to obtain a white powder (84 - 87 %). **1s:** ^{1}H NMR (DMSO, 400 MHz) δ: 7.29 – 7.30 (m, 2H), 6.94 – 6.97 (m, 3H), 4.58 (s, 2H, isomer I), 4.48 (s, 2H, isomer II), 3.58 (s, 2H, isomer II), 3.50 (s, 2H, isomer I), 2.66 (br, 2H, isomer I & II); ESI (-ve) *m/z* calcd for $C_{16}H_{11}NNa_4O_{17}S_4$ [(M-Na)$^-$] 685.86, found 686.1; **2s:** ^{1}H NMR (DMSO, 400 MHz) δ: 7.65 (d, *J* = 2.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.29 (s, 2H), 6.99 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.54 (s, 2H), 3.70 (br, 2H), 2.69 (t, *J* = 4.8 Hz, 2H); ESI (-ve) *m/z* calcd for $C_{16}H_{11}NNa_4O_{17}S_4$ [(M-Na)$^-$] 685.86, found 686.0

Per-sulfate **3s**: To a stirred solution of the poly-alcohol (20 mg, 0.063 mmol) in MeCN (1.3 mL) at rt, Et_3N (0.5 mL, 3.6 mmol) and $Me_3N.SO_3$ (390 mg, 2.8 mmol) was added. The reaction vessel was sealed and microwaved for 30

minutes at 100 ºC. The reaction was repeated for 4 times for scale up. The product **3s** (121 mg, 54 %) was isolated according to the above procedure for **1s** and **2s**. **3s:** ^{1}H NMR (DMSO, 400 MHz) δ: 7.37 (s, 2H), 7.29 (s, 2H), 4.54 (s, 2H), 3.53 (s, 2H), 2.68 (s, 2H); ESI (-ve) *m/z* calcd for $C_{16}H_{10}NNa_5O_{21}S_5$ [(M-Na)$^-$] 803.79, found 804.1

SCHEME II



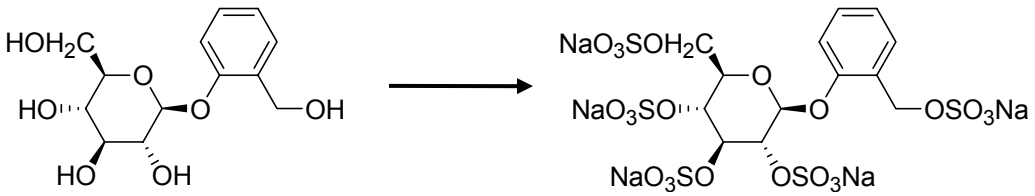
Ester **13** — To a stirred solution of amino acid **12** (2 g, 7.32 mmol) in EtOH (50 mL) at rt, HCl (g) was passed for 2 minutes and the reaction was refluxed. After 24 hrs, the reaction was brought to rt and the solvent was evaporated off. Ethyl acetate (50 mL) was added to the residue and extracted with 5 % K_2CO_3 solution (3 x 25 mL) and water (2 x 20 mL). The organic layer was dried (Na_2SO_4) and evaporated to obtain a colorless oil (1.4 g, 72 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.59 (s, 1H), 6.51 (s, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 4.03 (s, 2H), 3.84

(s, 3H), 3.82 (s, 3H), 3.68 (dd, *J* = 10.4, 4.4 Hz, 1H), 2.98 (dd, *J* = 16, 4.4 Hz, 1H), 2.86 (dd, *J* = 15.6, 10 Hz, 1H), 1.29 (t, *J* = 7.2 Hz, 3H)

Amide **14:** The procedure for the synthesis of amides **11a-c** was used to prepare **14** (72%) ^{1}H NMR (DMSO, 400 MHz) δ: 6.69 – 7.03 (m, 5H), 4.81 – 5.14 (m, 1H, isomers I - III), 4.27 – 4.57 (m, 2H, isomers I – III), 3.98 – 4.04 (m, 2H, isomers I – III), 3.62 – 3.78 (m, 12H, isomers I – III), 3.09 – 3.32 (m, 2H, isomers I – III), 1.00 – 1.11 (m, 3H, isomers I – III); ESI (+ve) *m/z* calcd for $C_{23}H_{27}NO_7$ $[(M+H)^+]$ 430.18, found 430.4

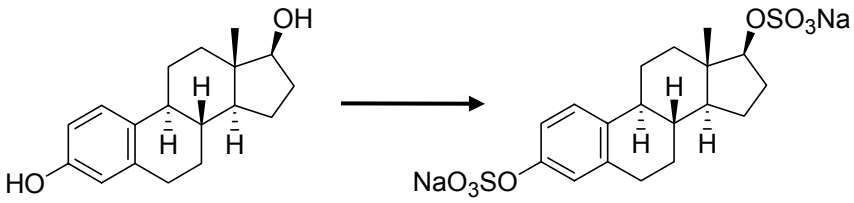
Poly-phenol **4**: The procedure for the synthesis of polyphenols **1-3** was to prepare **4** from amide **14** (73%). ^{1}H NMR (DMSO, 400 MHz) δ: 8.78 – 9.35 (m), 6.35 – 6.81 (m, 5H), 4.69 – 4.99 (m, 1H, isomers I - III), 4.17 – 4.43 (m, 2H, isomers I – III), 3.50 – 3.57 (m, 2H, isomers I – III), 2.89 – 3.02 (m, 2H, isomers I – III), 1.00 – 1.11 (m, 3H, isomers I – III)

Per-sulfate **4s**: The procedure for the synthesis of poly-sulfates **1s** and **2s** was used to prepare **4s** from polyphenol **4** (74%). ^{1}H NMR (DMSO, 400 MHz): δ 6.96 – 7.70 (m, 5H), 4.83 – 5.16 (m, 1H isomers I - III), 4.18 – 4.44 (m, 2H, isomers I – III), 3.55 – 3.61 (m, 2H, isomers I – III), 2.99 – 3.13 (m, 2H, isomers I – III), 1.08 – 1.16 (m, 3H, isomers I – III); ESI (-ve) *m/z* calcd for $C_{19}H_{15}NNa_4O_{19}S_4$ $[(M-Na)^-]$ 757.88, found 757.5



5 **5s**

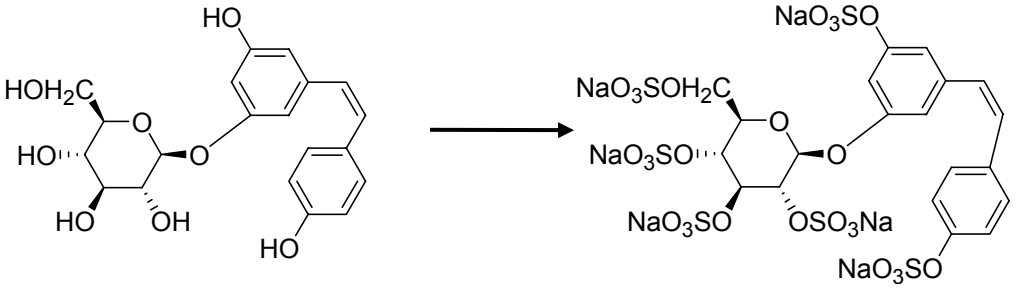
Salicin per-sulfate **5s**: To a stirred suspension of salicin (**5**, 20 mg, 0.07 mmol) in MeCN (1 mL) at rt, pyridine (0.3 mL, 3.8 mmol) was added to form a clear solution. $C_5H_5N.SO_3$ (500 mg, 3.14 mmol) was added and the reaction vessel was sealed and micro-waved for 30 minutes at 100 ºC. The reaction was repeated for 4 times. The reaction mixture was quenched with methanol (2 mL), pooled and concentrated *in vacuo*. The residue was taken up in water (1 mL) and chromatographed following the protocol established for **1s** to obtain **5s** (236.8 mg, 84 %). ^{1}H NMR (DMSO, 400 MHz) δ: 7.34 (d, 1H, *J*=8.0 Hz), 7.16 (t, 1H, *J*=8.0 Hz), 6.95 (t, 1H, *J*=8.0 Hz), 6.89 (d, 1H, *J*=8.0 Hz), 5.24 (d, 1H, *J* = 8.0 Hz), 4.86 (s, 2H), 4.62 (d, 2H, *J*=4.0 Hz), 4.35 (m, 1H), 4.21–4.27 (m, 1H), 3.93–3.99 (m, 1H), 3.75 (m, 1H); ESI (-ve) *m/z* calcd for $C_{16}H_{10}NNa_5O_{21}S_5$ [(M-Na)$^-$] 772.81, found 772.7



6 **6s**

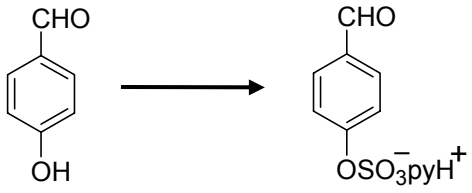
Estradiol-3,17β-disulfate **6s**: The procedure for the synthesis of salicin per-sulfate **5s** was used to prepare **6s** from estradiol (94%). ^{1}H NMR (DMSO, 400

MHz) δ: 7.1 (d, *J* = 8.4 Hz, 1H), 6.81 – 6.83 (m, 2H), 4.02 (t, *J* = 8.0 Hz, 1H), 2.71 – 2.74 (m, 2H), 2.21 – 2.25 (m, 1H), 2.08 – 2.12 (m, 1H), 1.87 – 2.00 (m, 2H), 1.75 – 1.78 (m, 1H), 1.48 – 1.60 (m, 2H), 1.09 – 1.34 (m, 6H), 0.66 (s, 3H); ESI (-ve) *m/z* calcd for $C_{18}H_{22}Na_2O_8S_2$ [(M-Na)$^-$] 453.07, found 453.1



7 **7s**

7s: The procedure for the synthesis of salicin per-sulfate **5s** was essentially used to prepare **7s** from stilbene **7**, except that 12 equiv. SO_3.py complex per –OH group was used and the reaction vessel was irradiated for 10 min at 120 ºC (72%). ^{1}H NMR (DMSO, 400 MHz) δ: 7.56 (s, 1H), 7.54 (s, 1H), 6.99 – 7.10 (m, 7H), 6.61 (s, 1H), 5.36 (s, 1H), 4.63 (s, 1H), 4.59 (s, 1H), 4.39 (s, 1H), 4.14-4.18 (m, 1H), 4.08 (d, *J* = 8.8 Hz, 1H) 3.81 (t, *J* = 10 Hz, 1H); ESI (-ve) *m/z* calcd for $C_{20}H_{16}Na_6O_{26}S_6$ [(M-Na)$^-$] 978.77, found 979.0



8 **8s**

***p*-Hydroxy benzaldehyde *O*-sulfate 8s**: The procedure for the synthesis of salicin per-sulfate **8** was essentially used to prepare **8s** from aldehyde **8**, except that the reaction vessel was irradiated for 10 min at 120 ºC following which the product with pyridinium cation was directly isolated by lyophilization after G10 chromatography (97%,). ^{1}H NMR (DMSO, 400 MHz) δ: 9.76 (s, 1H), 8.94 – 8.96 (m, 2H), 8.62 – 8.68 (m, 1H), 8.09 – 8.14 (m, 2H), 7.72 – 7.75 (m, 2H), 6.92 – 6.95 (m, 2H); ESI (-ve) *m/z* calcd for $C_{12}H_{11}NO_5S$ $[(M\text{-}pyH^{+})^{-}]$ 200.99, found 200.8

CAPILLARY ELECTROPHEROGRAMS