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# Regioselective syntheses of [$^{13}$C]$_4$-labelled sodium 1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate and sodium 2-carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [$^{13}$C]$_4$-maleic anhydride†

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**The entitled monohydrolysis products, also known as $\alpha$-ethylhexyl and $\beta$-ethylhexyl sulfosuccinate (EHSS), of the surfactant diisooctyl sulfosuccinate (DOSS) were synthesized in stable isotope-labelled form from [$^{13}$C]$_4$-maleic anhydride. Sodium [$^{13}$C]$_4$-1-carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate ($\alpha$-EHSS) was prepared by the method of Larpent by reaction of 2-ethylhexan-1-ol with [$^{13}$C]$_4$-maleic anhydride followed by regioselective conjugate addition of sodium bisulfite to the resulting monoester (38% overall yield). The regiochemical outcome of bisulfite addition was confirmed by a combination of $^{13}$C/$^{13}$C (incredible natural abundance double quantum transfer) and $^{1}$H/$^{13}$C (heteronuclear multiple-bond correlation (HMBC)) NMR spectral correlation experiments. Sodium [$^{13}$C]$_4$-2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate ($\beta$-EHSS) was prepared in four steps by reaction of 4-methoxybenzyl alcohol with [$^{13}$C]$_4$-maleic anhydride, regioselective sodium bisulfite addition, *N,N*′-dicyclohexylcarbodiimide-mediated esterification with 2-ethylhexan-1-ol, and *p*-methoxybenzyl ester deprotection with trifluoroacetic acid (13% overall yield). The regiochemical outcome of the second synthesis was confirmed by a combination of $^{1}J_{CC}$ scalar coupling constant analysis and $^{1}$H/$^{13}$C (HMBC) NMR spectral correlation. The materials prepared are required as internal standards for the liquid chromatography–mass spectrometry (LC-MS)/MS trace analysis of the degradation products of DOSS, the anionic surfactant found in Corexit, the oil dispersant used during emergency response efforts connected to the Deepwater Horizon oil spill of April 2010.**

**Keywords:** sulfosuccinate surfactants; DOSS; EHSS; INADEQUATE

## Introduction

To mitigate the environmental impact of the massive oil spill that resulted from a high-pressure methane explosion aboard the Deepwater Horizon drilling platform on 20 April 2010, exceptionally large quantities of Corexit 9500 and 9527 oil dispersants were applied to the Gulf of Mexico (4.1 million litres to surface and 2.9 million litres subsurface).[1] Details of the composition of these proprietary formulations were later released by the US Environmental Protection Agency (EPA) to facilitate the development of analytical tools to track the fate of the oil dispersants.[2] Accordingly, it is now known that Corexit 9500 and Corexit 9527 contain diisooctyl sulfosuccinate (DOSS, **1**) among three other surfactant ingredients (Figure 1). DOSS is of widespread use in a variety of consumer products[3]; however, comparatively, little is known about its biodegradation in aquatic environments, a critical consideration when one contemplates the ultimate environmental legacy of the Deepwater Horizon oil spill to the Gulf of Mexico. Using model microorganisms, Hales discovered that biodegradative ester hydrolysis of di-*n*-alkyl sulfosuccinates occurs under aerobic or anaerobic conditions to yield both possible monoester regioisomers.[4] More recently, an EPA group established that ethylhexyl sulfosuccinate (EHSS) monoesters are likewise obtained from the branched sulfosuccinate diester DOSS (**1**) during its biodegradation by oil-degrading microorganisms found in the Gulf of Mexico.[5]

As part of an ongoing effort to develop a trace analysis for surfactants and their degradation products associated with Corexit oil dispersants in Gulf of Mexico seawater field and laboratory microcosm samples,[6] we required access to stable isotope mass spectrometric standards of $\alpha$-EHSS (**2**) and $\beta$-EHSS (**3**) for quantification of liquid chromatography–mass spectrometry (LC-MS)/MS experiments. Herein, we report regioselective syntheses of quadruply labelled stable isotope

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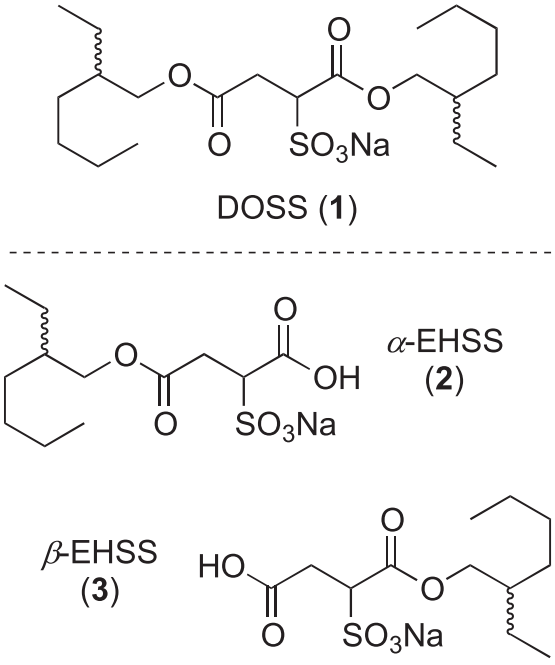
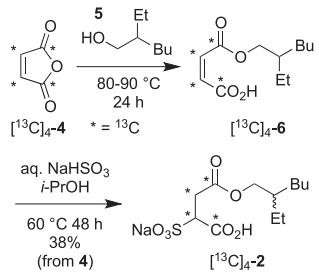



**Figure 1.** Sodium diisooctyl sulfosuccinate (DOSS, **1**), a significant component of Corexit oil dispersants 9500 and 9527, and its monoester derivatives $\alpha$-ethylhexyl sulfosuccinate ($\alpha$-EHSS, **2**) and $\beta$-ethylhexyl sulfosuccinate ($\beta$-EHSS, **3**).

mass spectrometric standards for $\alpha$-EHSS ([$^{13}$C]$_4$-**2**) and $\beta$-EHSS ([$^{13}$C]$_4$-**3**) from [$^{13}$C]$_4$-maleic anhydride (**4**) and prove the regiochemistry of both compounds by $^{13}$C/$^{13}$C and $^{1}$H/$^{13}$C NMR spectral correlation experiments.

## Results and discussion

Larpent and co-workers have described the regioselective synthesis of $\alpha$-type alkyl sulfosuccinate monoesters (e.g. **2**) by addition of sodium bisulfite to maleic acid monoesters obtained by alcoholysis of maleic anhydride (**4**).[7] Given the commercial availability of [$^{13}$C]$_4$-maleic anhydride, we elected to apply this straightforward approach to access our first target [$^{13}$C]$_4$-**2**. The Larpent procedure was modified slightly to function on the small scale necessitated by the expense of [$^{13}$C]$_4$-**4** and gave [$^{13}$C]$_4$-**2** as indicated in a 38% overall yield (Scheme 1).[8] The high level of $^{13}$C-atom enrichment in [$^{13}$C]$_4$-**2** afforded us the opportunity to unambiguously establish the location of the sulfonate group in relation to the ester side chain by a combined use of two 2D NMR spectral correlation experiments: the rarely used incredible natural abundance double quantum transfer (INADEQUATE) $^{13}$C/$^{13}$C correlation experiment[9] and a standard




**Scheme 1.** Synthesis of sodium [$^{13}$C]$_4$-1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate ([$^{13}$C]$_4$-**2**).

2-3 bond heteronuclear multiple-bond correlation (HMBC) $^{1}$H/$^{13}$C correlation experiment[10] (Figure 2A). The first technique enabled differentiation of the close proximity in frequency C1 (directly bonded to sulfonate bearing methine) and C4 (distal from sulfonate bearing methine) carboxyl atom resonances, while the second method served to indicate the close proximity of C4 to the ester alkyl chain. This determination complements and lies in agreement with the less secure regiochemical assignment for **2** made by Larpent *et al.* using an argument based on $^{1}$H/$^{13}$C scalar couplings and the strong dependence of a free acid carboxyl $^{13}$C-atom chemical shift value on changes to pH.[7]

Attention was next focused on the synthesis of the second target, [$^{13}$C]$_4$-$\beta$-EHSS (**3**). The $\beta$-type of sulfosuccinate monoester has previously been obtained by saponification of DOSS; however, this procedure leads to $\beta$-EHSS in low purity.[11] To improve on the DOSS-based approach, we elected to access [$^{13}$C]$_4$-**3** in a controlled manner by selective deprotection of a regiodefined mixed sulfosuccinic diester [$^{13}$C]$_4$-**8** (Scheme 2). The $\alpha$-type sulfosuccinate monoester [$^{13}$C]$_4$-**7** was first prepared by analogy to [$^{13}$C]$_4$-**2** via opening of [$^{13}$C]$_4$-maleic anhydride with *p*-methoxybenzyl (PMB) alcohol followed by regioselective addition of sodium bisulfite as before. Extensive hydrolysis of the sensitive PMB ester occurred during the sulfonation step, and [$^{13}$C]$_4$-**7** was obtained as a ca. 1:1 mixture with [$^{13}$C]$_4$-sulfosuccinic acid (refer to the Experimental section for details). The mixture of monoester [$^{13}$C]$_4$-**7** and [$^{13}$C]$_4$-sulfosuccinic acid was further converted to a chromatographically inseparable mixture of diesters [$^{13}$C]$_4$-**8** and [$^{13}$C]$_4$-DOSS (**1**) by *N,N'*-dicyclohexylcarbodiimide (DCC)-mediated coupling with 2-ethylhexan-1-ol (**5**).[7] Treatment of the mixture of diesters with trifluoroacetic acid resulted in selective removal of only the PMB moiety from [$^{13}$C]$_4$-**8** leaving [$^{13}$C]$_4$-DOSS (**1**) intact. The lipophilic component ([$^{13}$C]$_4$-**1**) was then easily removed from the desired polar target molecule by a simple trituration with $Et_2O$ that left behind $\beta$-EHSS [$^{13}$C]$_4$-**3** as a colourless amorphous powder in a 13% overall yield from [$^{13}$C]$_4$-maleic anhydride.[8] In this case, the $^{13}$C NMR signals arising from the $^{13}$C-atom-enriched sulfosuccinate moiety showed fully resolved multiplets, and pairwise mapping of the well-differentiated individual $^{1}J_{CC}$ scalar coupling constants enabled an unambiguous assignment of C1 and C4 carboxyl atom resonances without recourse to an INADEQUATE experiment (Figure 2B). As before, an HMBC experiment then served to locate the point of attachment of the ester side chain to the sulfosuccinate region, and in this manner, the compound prepared was confirmed to possess the desired $\beta$-type regiochemistry in line with expectation.

## Experimental

All commercially available reagents were used as received unless otherwise noted. Preparative chromatographic separations were performed on silica gel 60 (35–75 μm), and reactions were followed by thin layer chromatography analysis using silica gel 60 plates (2–25 μm) with fluorescent indicator (254 nm) and visualized with UV or phosphomolybdic acid. Infrared (IR) spectra were recorded in Fourier transform mode using KBr discs for solids, while oils were supported between NaCl plates (neat). $^{1}$H and $^{13}$C NMR spectra were recorded in Fourier transform mode at the field strength specified and from the indicated deuterated solvents in standard 5-mm diameter tubes. Chemical shift in ppm is quoted relative to residual solvent signals calibrated as follows: $CDCl_3$ $\delta_H$ ($CHCl_3$) = 7.26 ppm, $\delta_C$ = 77.2 ppm; $(CD_3)_2SO$ $\delta_H$ ($CD_3SOCHD_2$) = 2.50 ppm, $\delta_C$ = 39.5 ppm; $CD_3OD$ $\delta_H$ ($CHD_2OD$) = 3.31 ppm,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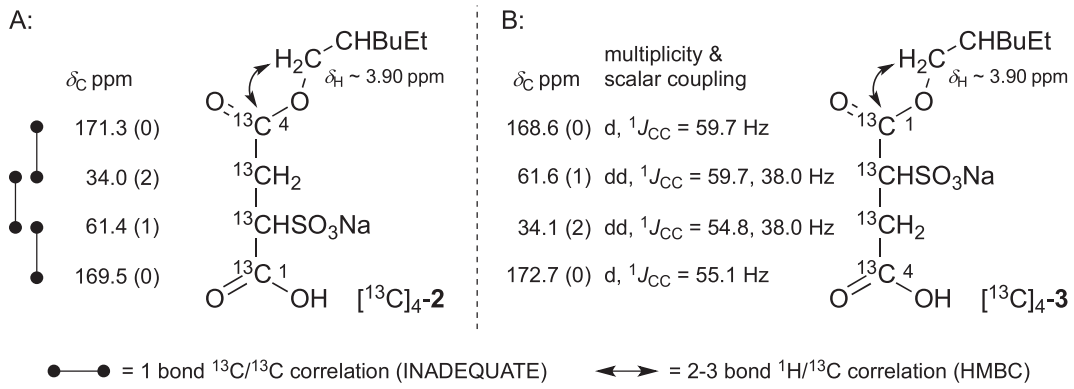
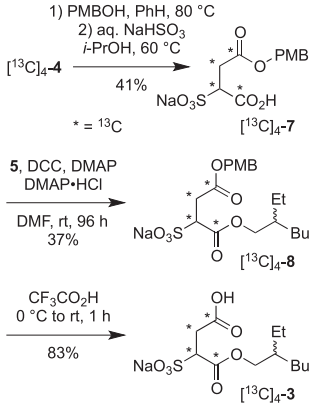



**Figure 2.** Assignment of the sulfosuccinate-related $^{13}$C NMR signals (175 MHz, $d_6$-DMSO) for [$^{13}$C]$_4$-**2** and [$^{13}$C]$_4$-**3** by one bond $^{13}$C/$^{13}$C correlation (INADEQUATE or $^1J_{CC}$ scalar coupling) and identification of ester side-chain attachment point by three bond $^1$H/$^{13}$C correlation (HMBC). Numbers in parentheses following $^{13}$C NMR chemical shift values indicate the number of attached hydrogen atoms.




**Scheme 2.** Synthesis of sodium [$^{13}$C]$_4$-2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate ([$^{13}$C]$_4$-**3**). PMB = *p*-methoxybenzyl.

$\delta_C$ = 49.0 ppm; and $(CD_3)_2CO$ $\delta_H$ ($CD_3COCHD_2$) = 2.05 ppm, $\delta_C$ = 29.8 ppm. Multiplicities in the $^1$H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Numbers in parentheses following carbon atom chemical shifts refer to the number of attached hydrogen atoms as revealed by distortionless enhancement of polarization transfer or heteronuclear single quantum correlation techniques. Low (MS) and high resolution mass spectra (HRMS) were obtained using either electron impact (EI) or electrospray (ES) ionization techniques. Ion mass/charge (*m/z*) ratios are reported as values in atomic mass units.

## Sodium [$^{13}$C]$_4$-1-carboxy-2-(2-ethylhexyloxycarbonyl) ethanesulfonate ([$^{13}$C]$_4$-2)

A 3.0-mL glass vial was charged with 2-ethylhexan-1-ol (**5**, 86 μL, *d* = 0.833, 71.5 mg, 0.55 mmol) and [$^{13}$C]$_4$-maleic anhydride (**4**, 51 mg, 0.50 mmol) then heated to 80–90 °C (oil bath) and sealed with a screw cap. After heating for 24 h, $^1$H NMR (700 MHz, $d_6$-acetone) spectral analysis indicated a molar ratio of monoester* [$^{13}$C]$_4$-**6** : diester : alcohol **5** of 14:1:1. The mixture was allowed to cool to room temperature (rt), dissolved in *i*-PrOH (3.0 mL) and transferred to a 50-mL round-bottomed (RB) flask provided with a magnetic stir bar. The flask was flushed with Ar and the contents treated dropwise with a freshly prepared solution of aqueous $NaHSO_3$ (2.3 mL, 0.44 M, 1.01 mmol; sparged with Ar for 30 min before use). The resulting mixture was stirred for 48 h at 60°C (oil bath) under Ar. After this time, the reaction mixture was allowed to cool and concentrated *in vacuo*. The residue was triturated with MeOH–$H_2O$ (4:1, 5 × 5 mL) to leave behind inorganic matter. The methanolic triturant was concentrated *in vacuo*, and the residue was triturated with $Et_2O$ (6 × 5 mL) to remove less polar contaminants; this time, the triturant being the discard and the residual powder the retained material. The powder was further purified by column chromatography ($SiO_2$, eluting with 0.5% $CF_3CO_2H$/19.5% MeOH in EtOAc) followed by a final trituration with EtOAc (4 × 1 mL) to afford pure [$^{13}$C]$_4$-**2** (64 mg, 0.190 mmol, 38% yield from [$^{13}$C]$_4$-**4**) as a colourless residual amorphous solid. Data for [$^{13}$C]$_4$-**2**: IR (KBr) 3528, 2959, 2931, 1677, 1557, 1372, 1224, 1154, 1035, 859, and 643 cm$^{-1}$; $^1$H NMR (700 MHz, $d_6$-DMSO) δ 12.11 (1H, br s), 3.93–3.87 (2H, m), 3.61 (1H, dm, $^1J_{CH}$ = 138.4 Hz), 2.84 (1H, dm, $^1J_{CH}$ = 128.4 Hz), 2.73 (1H, dm, $^1J_{CH}$ = 130.0 Hz), 1.50 (1H, septet, *J* = 5.9 Hz), 1.33–1.21 (8H, m), 0.86 (3H, t, *J* = 6.9 Hz), and 0.83 (3H, td, *J* = 7.4, 1.1 Hz) ppm; $^{13}$C NMR (175 MHz, $d_6$-DMSO) δ 171.3 (0, d, $^1J_{CC}$ = 58.4 Hz), 169.5 (0, d, $^1J_{CC}$ = 56.4 Hz), 65.9 (2), 61.4 (1, dd, $^1J_{CC}$ = 56.3, 38.2 Hz), 38.1 (1), 34.0 (2, dd, $^1J_{CC}$ = 58.4, 38.2 Hz), 29.7 (2), 28.3 (2), 23.2 (2), 22.4 (2), 13.9 (3), and 10.8 (3) ppm; MS (ES+) *m/z* 359 (M + Na)$^+$; and HRMS (ES+) *m/z* 359.0943 (calcd. for $^{12}C_8{}^{13}C_4H_{21}Na_2O_7S$: 359.0938).

*Data for intermediate compound [$^{13}$C]$_4$-**6**: IR (KBr) 3043, 2960, 2931, 1690, 1668, 1463, 1396, 1380, 1200, 1160, and 803 cm$^{-1}$; $^1$H NMR (700 MHz, $CDCl_3$) δ 6.44 (1H, dtm, *J* = 166.8, 14.0 Hz), 6.37 (1H, dt, *J* = 167.5, 13.0 Hz), 4.22–4.16 (2H, m), 1.65 (1H, septet, *J* = 6.1 Hz), 1.40–1.35 (2H, m), 1.34–1.24 (6H, m), 0.90 (3H, t, *J* = 7.5 Hz), and 0.89 (3H, t, *J* = 7.0 Hz) ppm; $^{13}$C NMR (175 MHz, $CDCl_3$) δ 168.1 (0, d, $^1J_{CC}$ = 72.1 Hz), 165.1 (0, d, $^1J_{CC}$ = 67.4 Hz), 136.4 (1, t, $^1J_{CC}$ = 67.6 Hz), 129.6 (1, t, $^1J_{CC}$ = 69.8 Hz), 69.6 (2), 38.7 (1), 30.4 (2), 29.0 (2), 23.8 (2), 23.1 (2), 14.2 (3), and 11.1 (3) ppm; MS (EI+) *m/z* 233 (39%, M + H)$^+$, 121 (100), 112 (22), 104 (52), 83 (22), 70 (57); and HRMS (EI+) *m/z* 233.1570 (calcd. for $^{12}C_8{}^{13}C_4H_{21}O_4$: 233.1574).

## Sodium [$^{13}$C]$_4$-1-carboxy-2-(4-methoxybenzyloxycarbonyl) ethanesulfonate ([$^{13}$C]$_4$-7)

A solution of [$^{13}$C]$_4$-maleic anhydride ([$^{13}$C]$_4$-**4**, 51 mg, 0.50 mmol) and *p*-methoxybenzyl alcohol (PMBOH, 62 μL, d = 1.113, 69 mg, 0.50 mmol) in PhH (2.0 mL) was stirred at a gentle reflux for 21 h under Ar and then concentrated *in vacuo*. $^1$H NMR (700 MHz, $d_6$-acetone) spectral analysis confirmed predominant conversion of the anhydride to the desired monoester (diester : monoester : maleic anhydride : maleic acid = 6:85:6:3). The residue was dissolved in *i*-PrOH (3.0 mL) and transferred to a 20-mL thick-walled glass reaction vessel (a 'sealed-tube' apparatus) equipped with a magnetic stir bar. The vessel was flushed with Ar, and the contents were treated dropwise with a freshly prepared solution of aq. $NaHSO_3$ (2.3 mL, 0.44 M, 1.01 mmol; sparged with Ar for 30 min before use). The reaction vessel was then partially submerged in a 60°C oil bath, sealed with a Teflon screw stopper, and the contents stirred vigorously for 48 h. After this time, the reaction mixture was allowed to cool, and the stopper was removed cautiously. The mixture was concentrated *in vacuo*, and the residue was triturated with MeOH–$H_2O$ (4:1, 5 × 5 mL) to leave behind inorganic matter. The methanolic triturant was concentrated *in vacuo*, and the residue was dried azeotropically by repeated addition of

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PhH (5 × 10 mL) followed each time by concentration. To remove less polar contaminants, the residue was triterated with $Et_2O$ (4 × 5 mL); this time, the triturant being the discard and the residual powder the retained material. Analysis of the resulting amorphous powder (115 mg, dry weight) by $^1$H NMR spectroscopy revealed it to be a 1:1 molar mixture of [$^{13}$C]$_4$-**7** and [$^{13}$C]$_4$-sulfosuccinate (i.e. 61 wt% in [$^{13}$C]$_4$-**7**, 70 mg, 0.203 mmol, 41% yield from [$^{13}$C]$_4$-**4**) that was used without further purification in the next step. NMR spectral data for [$^{13}$C]$_4$-**7** (from a mixture with [$^{13}$C]$_4$-sulfosuccinate): $^1$H NMR (700 MHz, $CD_3OD$-$D_2O$, 2:1) δ 7.32 (2H, d, $J$ = 8.5 Hz), 6.95 (2H, d, $J$ = 8.6 Hz), 5.08 (1H, d, $J$ = 12.5 Hz), 5.06 (1H, d, $J$ = 13.2 Hz), 4.06 (1H, dm, $^1J_{CH}$ = 136.4 Hz), 3.82 (3H, s), and 3.35–2.88 (2H, m) ppm; $^{13}$C NMR (175 MHz, $CD_3OD$-$D_2O$, 2:1) δ 173.6 (0, d, $^1J_{CC}$ = 58.3 Hz), 172.5–171.6 (0, m), 131.6 (2C, 1), 115.4 (2C, 1), 66.8 (2), 64.4–63.3 (1, m), 56.5 (3), and 35.3 (2, dd, $^1J_{CC}$ = 58.5, 37.8 Hz) ppm.

### Sodium [$^{13}$C]$_4$-1-(2-ethylhexyloxycarbonyl)-2-(4-methoxybenzyloxycarbonyl)-ethanesulfonate ([$^{13}$C]$_4$-**8**)

A 100-mL RB flask equipped with a magnetic stir bar was charged with the mixture of [$^{13}$C]$_4$-**7** and [$^{13}$C]$_4$-sulfosuccinate obtained previously (115 mg, 1:1 molar ratio, 0.203 mmol [$^{13}$C]$_4$-**7**), 2-ethylhexan-1-ol (**5**, 86 μL, $d$ = 0.833, 72 mg, 0.551 mmol), 4-(dimethylamino)pyridine (DMAP, 122 mg, 1.00 mmol), and 4-(dimethylamino)pyridinium chloride (DMAP•HCl, 119 mg, 0.751 mmol). The flask was flushed with Ar gas and, then, the contents dissolved in anhydrous dimethylformamide (DMF) (6.0 mL) with stirring (30 min). DCC (185 mg, 0.898 mmol) in anhydrous DMF (1.0 mL) was added dropwise, and the resulting mixture was allowed to stir for 96 h at rt. After this time, the mixture was diluted with EtOAc (75 mL), filtered, and the filtrate was concentrated *in vacuo*. The residue was triturated with three portions of EtOAc (25 mL then 2 × 7 mL), and the combined triturant was concentrated *in vacuo*. The residue (244 mg) was subjected to column chromatography ($SiO_2$, eluting with 0–5% MeOH in EtOAc) to afford a co-eluting mixture of [$^{13}$C]$_4$-**8**, [$^{13}$C]$_4$-DOSS (**1**), and DMAP. To remove the DMAP, the mixture was dissolved in EtOAc (35 mL), washed with aq. 1.0 M HCl (2 × 1.50 mL), $H_2O$ (1 mL), and brine (3 × 1.0 mL), then dried ($Na_2SO_4$) and concentrated *in vacuo*. This treatment afforded 94 mg of a pure two component mixture of [$^{13}$C]$_4$-**8** (36 wt.%, eff. 34 mg, 0.075 mmol, 37%) and [$^{13}$C]$_4$-DOSS (**1**) as a colourless paste that was used directly in the next transformation. Data for [$^{13}$C]$_4$-**8** (from a mixture with [$^{13}$C]$_4$-**1**): $^1$H NMR (700 MHz, $CDCl_3$) δ 7.24 (2H, d, $J$ = 8.1 Hz), 6.83 (2H, d, $J$ = 8.2 Hz), 5.02 (2H, s), 4.40 (1H, br d, $^1J_{CH}$ = 139.9 Hz), 4.12–3.90 (2H, m), 3.77 (3H, s), 3.18 (2H, br d, $^1J_{CH}$ = 132.0 Hz), 1.53–1.45 (1H, m), 1.40–1.20 (8H, m), and 0.90–0.82 (6H, m) ppm; $^{13}$C NMR (175 MHz, $CDCl_3$) δ 171.8 (0, d, $^1J_{CC}$ = 58.7 Hz), 170.0 (0, d, $^1J_{CC}$ = 58.9 Hz), 159.7 (0), 130.2 (2C, 1), 128.0 (0), 114.0 (2C, 1), 69.0 (2), 66.8 (2), 61.2 (1, dd, $^1J_{CC}$ = 59.1, 36.4 Hz), 55.4 (3), 38.5 (1), 33.2 (2, dd, $^1J_{CC}$ = 59.0, 36.7 Hz), 30.1 (2), 28.9 (2), 23.4 (2), 23.1 (2), 14.2 (3), and 10.9 (3) ppm; MS (ES+) *m/z* 479 $(M + Na)^+$; and HRMS (ES+) *m/z* 479.1524 (calcd. for $^{12}C_{16}^{13}C_4H_{29}Na_2O_8S$: 479.1513).

### Sodium [$^{13}$C]$_4$-2-carboxy-1-(2-ethylhexyloxycarbonyl) ethanesulfonate ([$^{13}$C]$_4$-**3**)

A stirred solution of a portion of the two component mixture of [$^{13}$C]$_4$-**8** and [$^{13}$C]$_4$-DOSS (**1**) obtained previously (68 mg, 36 wt.% in [$^{13}$C]$_4$-**8**, eff. 24.5 mg, 0.054 mmol) in $CH_2Cl_2$ (1.5 mL) at 0°C was treated dropwise with trifluoroacetic acid (0.10 mL) during 1 min. The resulting mixture was allowed to warm to rt during 1 h and then concentrated *in vacuo*. The residue was treated with $Et_2O$ (5 mL) and $H_2O$ (0.1 mL), shaken, and concentrated. The resulting solids were triturated with excess pentane (17 mL), the triturant was separated by filtration, and the filter cake was washed with further pentane (3 mL). The combined pentane filtrate and washings (containing [$^{13}$C]$_4$-**3** and [$^{13}$C]$_4$-**1** but now free of any minor inorganic impurities) were concentrated *in vacuo*. To remove a majority of [$^{13}$C]$_4$-**1** from [$^{13}$C]$_4$-**3**, the residue was now triturated with $Et_2O$ (3 × 5 mL); this time, the triturant being discarded and the residual powder retained. Analysis of this final colourless amorphous solid material (16 mg, dry weight) by $^1$H NMR spectroscopy revealed it to be the desired labelled β-EHSS (>94 wt.% in [$^{13}$C]$_4$-**3**, eff. 15 mg, 0.045 mmol, 83% yield) associated now with only a minor residual amount of [$^{13}$C]$_4$-**1** (<6 wt.%). Data for [$^{13}$C]$_4$-**3**: IR (KBr) 2927, 1679, 1463, 1400, 1262, 1219, 1199, 1153, 1054, 898, 789, 729, 669, and 645 $cm^{-1}$; $^1$H NMR (700 MHz, $d_6$-DMSO) δ 12.2 (1H, br s, OH), 3.93–3.83 (2H, m), 3.59 (1H, br d, $^1J_{CH}$ = 139.0 Hz), 2.85 (1H, br d, $^1J_{CH}$ = 132.5 Hz), 2.72 (1H, br d, $^1J_{CH}$ = 131.0 Hz), 1.52–1.48 (1H, m), 1.37–1.20 (8H, m), 0.86 (3H, t, $J$ = 6.4 Hz), and 0.83 (3H, tm, $J$ = 7.2 Hz) ppm; $^{13}$C NMR (175 MHz, $d_6$-DMSO) δ 172.7 (0, d, $^1J_{CC}$ = 55.1 Hz), 168.6 (0, d, $^1J_{CC}$ = 59.7 Hz), 66.0 (2, d, $^2J_{CC}$ = 7.2 Hz), 61.6 (1, dd, $^1J_{CC}$ = 59.9, 38.0 Hz), 38.1 (1), 34.1 (2, dd, $^1J_{CC}$ = 54.8, 38.0 Hz), 29.5 (2), 28.3 (2), 22.9 (2), 22.4 (2), 13.9 (3), and 10.7 (3) ppm; MS (ES+) *m/z* 359 $(M + Na)^+$; and HRMS (ES+) *m/z* 359.0926 (calcd. for $^{12}C_8^{13}C_4H_{21}Na_2O_7S$: 359.0938).

## Conclusion

Regioselective syntheses of stable isotope-labelled α-EHSS ([$^{13}$C]$_4$-**2**) and β-EHSS ([$^{13}$C]$_4$-**3**) have been successfully realized. The ultimate regiochemical outcome of each synthesis was unequivocally established by the combined action of $^{13}$C/$^{13}$C and $^1$H/$^{13}$C NMR spectral correlation experiments that were facilitated by the high level of $^{13}$C-atom enrichment. The stable isotope-labelled materials described herein will prove useful as LC-MS/MS standards for the trace analysis of DOSS and its degradation products in laboratory microcosms and in Gulf of Mexico field samples collected as a result of emergency response efforts connected to the Deepwater Horizon oil spill.

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## Conflict of interest

The authors did not report any conflict of interest.

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