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1 A Tandem Enzymatic sp^2 -C-Methylation Process: Coupling *In Situ* 2 S-Adenosyl-L-Methionine Formation with Methyl Transfer

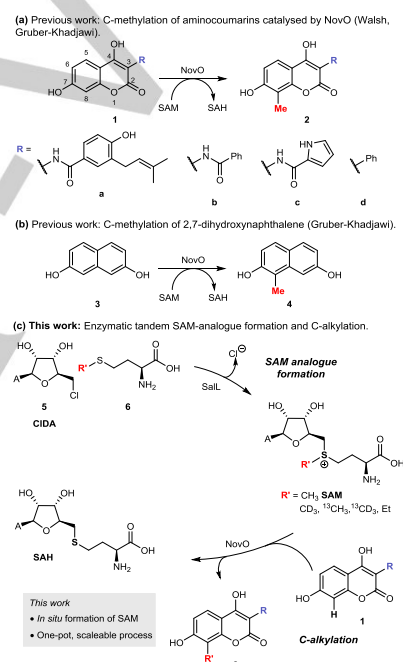
3 Joanna. C. Sadler,^{[a],[b]} Luke D. Humphreys,^{*[a],[c]} Radka Snajdrova,^[a] Glenn. A. Burley^{*[b]}

Abstract: A one-pot, two-step biocatalytic platform for the regiospecific C-methylation and C-ethylation of aromatic substrates is described. The tandem process utilizes SaIL (*Salinospora tropica*) for *in situ* synthesis of S-adenosyl-L-methionine, followed by alkylation of aromatic substrates using the C-methyltransferase NovO (*Streptomyces spheroides*). Application of this methodology is demonstrated by regiospecific methyl, ethyl and isotopically-labelled $^{13}\text{CH}_3$, $^{13}\text{CD}_3$ and CD_3 groups from their corresponding SAM analogues formed *in situ*.

Regiospecific, late-stage methylation is a powerful strategy for tuning the physical and biological properties of small molecules and biomacromolecules.^[1] At present, synthetic methodologies that methylate $\text{C}(sp^2)\text{-H}$ bonds are predominantly limited to transition metal-catalyzed strategies, which require elevated temperatures and the need for additives such as ligands and oxidants.^[2–4] In addition, these methodologies are typically regioselective rather than regiospecific, which further restricts their scope.^[5,6] In contrast, Nature routinely employs S-adenosyl-L-methionine (SAM) dependent methyltransferases (MTs) to methylate at O-, N-, S- and C- sites. Thus, MTs hold considerable potential for the development of a biocatalytic alkylation platform of small molecules.^[7–9]

Of the various MTs available, C-MTs are particularly attractive as they enable C-C bond formation under mild conditions relative to traditional Friedel-Crafts reactions.^[10,11] One C-MT exemplar is NovO, which methylates the C-8 position of **1a** in the biosynthesis of the antibiotic novobiocin to form **2a** (Scheme 1a).^[12] We and others have shown that regiospecific methylation of the 8-position of **1b** is catalyzed by a novel His-Arg motif, which facilitates the deprotonation of the phenolic proton in the 7-position, followed by methylation at position 8 by SAM to form **2b**.^[13,14] NovO is also effective in catalysing Friedel-Crafts alkylations using non-natural SAM analogues to alkylate **1b-d** regiospecifically.^[8,11] Furthermore, NovO accepts non-natural substrates such as **3** to exclusively form **4** (Scheme 1b), which is currently only accessible by this process.^[11]

At present, the broader applicability of small-molecule MTs is hampered by the inherent instability of SAM (942 min at pH 8) as the corresponding methylating agent.^[15,16] Additionally, the synthesis of SAM by chemical methods results in the formation of a diastereomeric mixture, which increases the cost of the process and has the potential for undesirable C-MT inhibition by the unwanted diastereomer.^[17,18]



Scheme 1. Biocatalytic C-methylation of (a) coumarins (**1a-d**) and (b) 2,7-dihydroxynaphthalene (**3**) catalysed by NovO. Both studies require the formation of SAM prior to C-methylation. (c) Development of a one-pot, two-enzyme process involving *in situ* SAM formation (SaIL) and C-methylation (NovO). SAM: S-adenosylmethionine. SAH: S-adenosylhomocysteine.

A powerful strategy that overcomes the need to prepare and isolate SAM and SAM analogues would produce the cofactor *in situ*, followed by alkyl transfer.^[19–23] The enzyme SaIL (*Salinospora tropica*) is known to form SAM from 5'-deoxy-5-chloroadenosine (CIDA, **5**) and methionine (Met, **6**).^[24–28] As CIDA is readily prepared from adenosine in a one-pot process, the use of SaIL offers an inexpensive and atom-efficient alternative to the use of methionine adenosyl transferases, which utilizes expensive ATP as the corresponding adenosyl donor and have been used previously for *in situ* SAM formation.^[21,29–32]

At present, there have not been any reports on the utility of the SaIL-catalysed SAM production in tandem with C-MTs in a one-pot procedure. Herein, we describe such a process for the methylation of small molecule aromatic scaffolds using SaIL and the C-MT NovO (Scheme 1c). Additionally, we show that this

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tandem strategy can be used to transfer ethyl groups and isotopically-labeled methyl groups.^[33–35]

Initial investigations focused on determining the compatibility of Sall and NovO to operate in a one-pot process. The model substrate **1b** was used with an excess of CIDA (**5**, 2 eq.) and Met (**6**, 50 eq.) using an *E. coli* cell-free extract from the overexpression of NovO (**Figure 1a**; **Entry A**). Based on the previously reported kinetic parameters for SAM formation

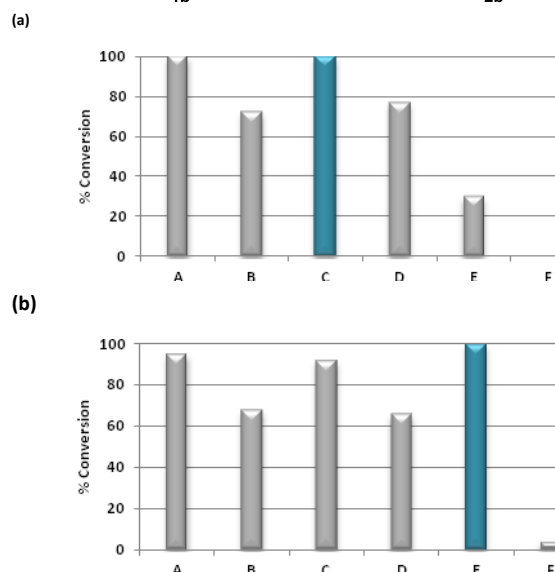
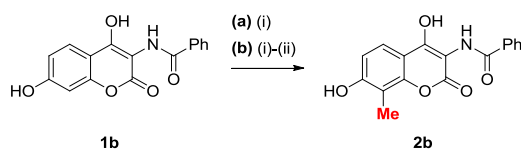


Figure 1. (a) Reaction optimization using substrate **1b**. *E. coli* cell lysates were used and % conversion determined by HPLC (area/area%) after 24 h. *Reagents and conditions:* (i) CIDA, Met, BSA, DTT, NovO cell lysate in 50 mM phosphate buffer, pH 6.5 resuspended at 10 mL/g pellet, Sall cell free extract in 50 mM phosphate buffer, pH 6.8 resuspended at 5 mL/g pellet. **A:** 2 eq. CIDA, 50 eq. Met; **B:** 1 eq. CIDA, 50 eq. Met; **C:** 2 eq. CIDA, 2 eq. Met; **D:** 2 eq. CIDA, 1 eq. Met; **E:** 2 eq. CIDA, no Met added; **F:** No CIDA or Met added. (b) Reaction optimization using purified enzymes. *Reagents and conditions:* (i) Met, Sall, BSA, 50 mM potassium phosphate buffer pH 6.5, CIDA, 37 °C, 8 hours. (ii) NovO, 37 °C, 16 hours. **A:** 2 eq. CIDA, 10 eq. Met, 0.5 μM MTAN added with NovO; **B:** 2 eq. CIDA, 10 eq. Met; 2 μM SAHH added with NovO; **C:** 1.5 eq. CIDA, 1.5 eq. Met, 0.5 μM MTAN added with NovO; **D:** 1.1 eq. CIDA, 1.1 eq. Met, 0.5 μM MTAN added with NovO; **E:** 1.5 eq. CIDA, 2 eq. Met, 1 mM DTT and 0.5 μM MTAN added with NovO; **F:** 1.5 eq. CIDA, no Met added; 0.5 μM MTAN added with NovO. Optimised conditions highlighted in blue.

compared to CIDA formation, low levels of chloride present in the system were not anticipated to affect conversion levels.^[36] No other sources of chloride were added to the system.

This resulted in quantitative conversion of **1b** into the methylated product **2b** in 24 hours. We then optimised the reaction conditions, firstly by minimising the number of equivalents of Met and CIDA relative to substrate **1b** (**Figure 1a** and **Table S1**). When the number of equivalents of CIDA was reduced from 2 to 1.5 eq., a drop off in the formation of **2b** was observed (**Table S1**). Quantitative conversion was optimal with 2 equivalents of Met (**Figure 1a**, **Entry C**). Decreasing the amount of Met further to 1 eq. reduced the conversion of **1b** to **2b** to 77% (**Figure 1a**, **Entry D**). However, when the reaction was run without the addition of Met, 30% methylation of **1b** was observed

(**Figure 1a**, **Entry E**). To determine whether this was due to residual SAM or Met present in the reaction mixture, the reaction was also run in the absence of CIDA (**Figure 1a**, **Entry F**). In this case, no conversion to **2b** was observed, which was indicative of background methylation being caused by residual Met present in the cell lysate and cell free extract (**Table S3**).

To address this issue, purified enzymes were used (**Figure 1b** and **Table S2**). Additionally, methylthioadenosine nucleosidase (MTAN) or SAH hydrolase (SAHH) was added to remove SAH from the reaction mixture, which is a known inhibitor of many SAM dependent MTs.^[18,37,38] Since CIDA inhibits both MTAN^[39] and SAHH^[40], SAM was pre-formed *in situ* before the addition of NovO and MTAN or SAHH. Initially, the reaction was carried out using 2 eq. CIDA and 10 eq. Met with either MTAN or SAHH (**Figure 1b**, **Entries A and B**). Whilst only 68% conversion was achieved with SAHH, nearly quantitative methylation of **1b** was observed using MTAN as an additive. Further optimisation enabled the reduction of the number of equivalents of CIDA from 2.0 to 1.5 without loss of conversion to **2b** when 1 eq. DTT was added.^[41] Carrying out the reaction in the absence of MTAN decreased the conversion by ~40%, confirming the role of MTAN decreasing by-product inhibition caused by SAH. Indeed, an IC₅₀ value of 9.8 μM for CIDA has been reported for MTAN from *E. coli*, which was used in our study.^[42] Finally, only 4% methylation was observed when the reaction was carried out in the absence of Met, which may be due to residual SAM bound in NovO (**Figure 1b** and **Table S2**).

With optimised conditions for a one-pot, tandem SAM formation C-methylation process in hand, we next explored the scalability of the methodology. The tandem process was carried out with Met, three isotopically labelled Met analogues and ethionine; using 20 mg of **1b**, **1c** or **3** in each case. To the best of our knowledge, this is the first time that a tandem process which involved the *in situ* formation of SAM has been used on preparative scale. For the transfer of an unlabelled methyl group, crude *E. coli* cell lysate (NovO) and *E. coli* cell free extract (Sall) was used, whilst purified enzymes were employed for the transfer of isotopically labelled and ethyl groups. Moderate (65%) to excellent (88–100%) levels of conversion were obtained for transfer of CH₃, ¹³CH₃, CD₃ and ¹³CD₃, with **1c** showing quantitative conversion and isolated yields 76–91% in all cases (**Table 1**). High levels of conversion (88–97%) were also obtained for **1b**, although isolated yields were lower due to poor solubility of the corresponding products during work-up. Alkylation of **3** was also successful on this scale to form **4** in high conversions (69–87%) using purified Sall and NovO, whilst 65% of **4** was formed using the cell lysate.

In summary, we have demonstrated a scalable biocatalytic platform for a Friedel-Crafts alkylation using Sall for *in situ* cofactor SAM synthesis from inexpensive starting materials. Furthermore, to the best of our knowledge this is the first example of using Sall for *in situ* cofactor production in tandem with a MT for the site specific C-methylation/alkylation of a small molecule. We envisage that our enzymatic platform could form the basis of a valuable biocatalytic tool for late-stage, regioselective labelling of small molecules.^[33,43–45]

Table 1. Preparative [(20 mg starting material [0.07 mmol **1b** and **1c**; 0.125 mmol **3**)] scale tandem enzymatic alkylation.

Substrate	CH ₃	¹³ CH ₃	CD ₃	¹³ CD ₃	Et
1b	97% (53%) ^[a]	95% (76%)	88% (52%)	90% (47%)	34% (18%) ^[b]
1c	100% (76%) ^[a]	100% (90%)	100% (80%)	100% (75%)	30% (27%) ^[b]
3	65% (46%) ^[a]	94% (83%)	91% (68%)	92% (85%)	7% ^[b]

[a] Reaction carried out using NovO cell lysate and SalL cell free extract and purified MTAN. [b] 32 hour pre-incubation with SalL. *Reagents and conditions:* (i) SalL (0.5 mol%), BSA (1 mg/mL), DTT (1 eq.), 50 mM potassium phosphate buffer pH 6.5, CIDA (**5**, 1.5 eq.), Met or Met analogue (2 eq.), 37 °C, 7 hours (CH₃, ¹³CH₃, CD₃ and ¹³CD₃) or 32 hours (Et). (ii) NovO (4 mol%), MTAN (0.05 mol%), 37 °C, 16 hours. Reactions carried out using 20 mg of substrate. Yields of isolated products in parentheses. Representative chromatograms for each substrate are shown in the Supporting Information.

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Keywords: methyltransferase • alkylation • biocatalytic • S-adenosylmethionine • multi-enzyme reaction

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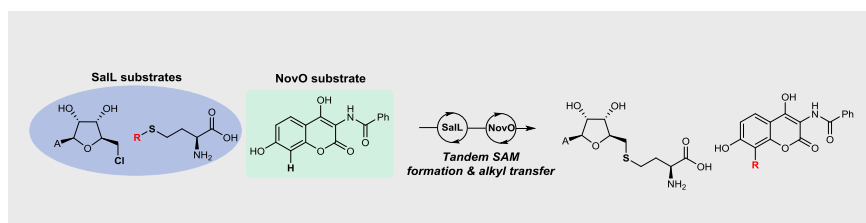
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COMMUNICATION



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Tandem SAM: A one-pot, two-enzyme C-methylation process is described. Linking SAM production using SalL (*S. Tropica*) with the C-methyltransferase NovO (*S. Spheroides*) enables the synthesis a suite of methylated and ethylated coumarin products.

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A One-Pot Enzymatic C-alkylation Cascade of Aromatic Substrates

Supporting Information

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1 Overexpression and purification of SalL, NovO and MTAN

SalL

The plasmid pET26b(+)-SalL (codon optimised for *E. coli*) was transformed into *E. Coli* BL21 DE3 competent cells (Invitrogen) for protein expression. Transformants harbouring the plasmids were grown at 37 °C in LB medium supplemented with 50 µg/ mL kanamycin to provide the seed culture. The seed culture was used to inoculate 1L Overnight Express™ media and grown to an optical density (OD) of 2, then incubated for a further 16 hours at 18 °C. The cells were harvested by centrifugation (4000 g for 20 minutes at 4 °C). To prepare cell free extract for use in the cascade reaction, the cell pellet was resuspended in potassium phosphate buffer (50 mM, pH 6.8) at a volume of ~5 mL/ g of cell pellet. The cells were lysed by sonication on ice (5 minutes, 9.0 sec on, 10.0 sec off), at 40% amplitude. The resulting cell lysate was clarified by centrifugation (20 min, 4000 rpm, 4 °C) and the supernatant stored at -80 °C for further use.

NovO

The plasmid pET26b(+)-NovO (codon optimised for *E. coli*) was transformed into *E. Coli* BL21 DE3 competent cells (Invitrogen) for protein expression. Transformants harbouring the plasmids were grown at 30 °C in LB medium supplemented with 50 µg/ mL kanamycin to provide the seed culture. To 950 mL Magic media in 2.5 L ultra flasks was added 50 mL 'Component B' (5% v/v), 1 mL kanamycin (50 mg/ mL, to a final concentration of 50 µg/ mL) and 20 mL seed culture (2% v/v). The flasks were incubated at 30 °C, 200 rpm and grown to an OD₆₀₀ of ~2 before the temperature was reduced to 18 °C and incubated at 200 rpm overnight. The cells were harvested by centrifugation (4400 rpm, 4 °C, 20 minutes) and stored at -80 °C for further use.

MTAN

The plasmids pET21b(+)-MTAN (codon optimised for *E. coli*) was transformed into *E. Coli* BL21 ((DE3)) competent cells (Invitrogen) for protein expression. Transformants harbouring the plasmids were grown at 37 °C in LB medium supplemented with 100 µg/ mL ampicillin overnight to provide seed culture. Overnight Express (OE) media containing ampicillin (to a final concentration of 100 µg/ mL) was inoculated with seed culture (2% v/v). The flasks were incubated at 30 °C at 200 rpm and grown to an OD of ~2 before lowering the temperature to 18 °C and incubating at 200 rpm overnight. The cells were harvested by centrifugation (4400 rpm, 4 °C, 20 minutes) and were stored at -80 °C for further use.

Purification of SaII

Binding Buffer (Buffer A): 50 mM K Phosphate, 20 mM imidazole, 500 mM Na₂SO₄, pH 7.9.

Elution Buffer (Buffer B): 50 mM K Phosphate, 500 mM imidazole, 500 mM Na₂SO₄, pH 7.9.

The cell pellet harbouring SaII was resuspended in binding buffer (~10 mL/ g) and lysed by sonication (5 min, 0.5 s on, 0.5 s off). The cell lysate was clarified by centrifugation (90 min, 100 000 g, 4 °C) and loaded onto a pre-equilibrated HisTRAP HP 5 mL column at a flow rate of 1 mL / min, collecting the flow through. The column was washed with 25 mM imidazole until UV absorbance was stable. The protein was eluted with a gradient of 0-100% elution buffer over 20 CV to a final concentration of 500 mM imidazole, collecting 2 mL fractions. Fractions containing MTAN were determined by SDS PAGE and pooled and concentrated to 3 mL. The sample was desalted using a PD-10 column (GE healthcare), eluting with 50 mM phosphate buffer, pH 7.5 + 10% glycerol.

Purification of MTAN

Binding Buffer: 50 mM potassium phosphate buffer pH 7.5 + 20 mM imidazole.

Elution buffer: 50 mM potassium phosphate buffer pH 7.5 + 500 mM imidazole.

The cell pellet harbouring MTAN was resuspended in binding buffer (~10 mL/ g) and lysed on ice by sonication (5 min, 0.5 s on, 0.5 s off). The cell lysate was clarified by centrifugation (90 min, 100 000 g,

4 °C) and loaded onto a pre-equilibrated HisTRAP HP 1 mL column at a flow rate of 1 mL/ min, collecting the flow through. The column was washed with 25 mM imidazole until UV absorbance was stable. The protein was eluted with a gradient of 4-50% elution buffer over 20 CV to a final concentration of 250 mM imidazole, collecting 2 mL fractions. Fractions containing MTAN were determined by SDS PAGE, pooled and concentrated to 3 mL. The sample was desalted using a PD-10 column (GE healthcare), eluting with 50 mM HEPES, pH 8 +10% glycerol.

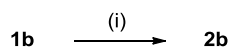
Purification of NovO

Binding Buffer: 50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole pH 8.0

Elution Buffer: 50 mM Tris-HCl, 300 mM NaCl, 500 mM imidazole, pH 8.0

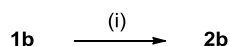
The cell pellet harbouring NovO was resuspended in binding buffer (~10 mL/ g) and lysed on ice by sonication (5 min, 0.5 s on, 0.5 s off, 40% intensity). The cell lysate was clarified by centrifugation (90 min, 100 000 g, 4 °C) and loaded onto a pre-equilibrated HisTRAP HP 5 mL column at a flow rate of 1 mL/ min, collecting the flow through. The column was washed with 25 mM imidazole until UV absorbance was stable. The protein was eluted with a linear gradient of 0-100% elution buffer over 20 CV. Fractions containing NovO or CouO were determined by SDS PAGE and pooled and concentrated to 3 mL. Protein The sample was desalted using a PD-10 column (GE healthcare), eluting with 100 mM Tris-HCl, 150 mM NaCl, 40% glycerol, pH 8.

2 Optimisation of reaction conditions



Entry	Eq. CIDA	Eq. Met	% Conversion
1	5	50	100
2	2	50	100
3	1.5	50	95
4	1	50	72
5	2	5	100
6	2	2	100
7	2	1	77
8	2	-	30
9	-	-	0

Table S1. Optimisation of tandem reaction using *E. coli* cell lysates. % Conversion by area/area% by HPLC reported after 24 h. *Reagents and conditions:* (i) CIDA, Met, BSA, DTT, NovO cell lysate in 50 mM phosphate buffer, pH 6.5 at 10 mL/ g pellet, SalL cell free extract in 50 mM phosphate buffer, pH 6.8 at 5 mL/ g pellet.

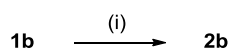


Entry	Eq. ClDA	Eq. L-Met	Additives	Conversion (%)
1	2	10	MTAN	95
2	2	10	SAHH	68
3	2	5	MTAN	100
4	2	2	MTAN	99
5	1.5	1.5	MTAN	92
6	1.5	2	MTAN	98
7	1.1	1.1	MTAN	66
8	1.1	1.5	MTAN	75
9	1.5	2	DTT+MTAN	100
10	1.5	2	-	61
11	1.5	0	DTT+MTAN	4

Table S2. Optimisation of tandem reaction using purified enzymes. % Conversion by area/area% by HPLC reported after 24 h. *Reagents and conditions:* (i) Met, SalL, BSA, 50 mM potassium phosphate buffer pH 6.5, ClDA, 37 °C, 8 hours. (ii) NovO, 37 °C, 16 hours.

3 Investigation into removal of residual Met

A screen of methods to remove Met was carried out (**Table S3**). In each case the conversion of substrate **1b** to methylated substrate **2b** was measured both without and with the addition of 2 eq. Met. The latter was carried out as a positive control to determine whether the enzymes retained activity after each treatment.

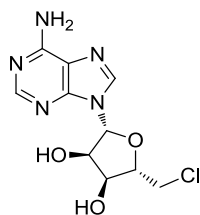


Entry	NovO/ CouO	SalL	- Met	+ Met
			% Conversion NovO	% Conversion NovO
1	Cell lysate	CFE	30	-
2	CFE	CFE	29	-
3	Washed cell lysate	Washed CFE	20	70
4	Filtered CFE	Filtered CFE	9	83
5	Washed cell lysate	Purified	16	98
6	Washed and filtered CFE	Purified	25	92
7	Dialysed CFE	Dialysed CFE	0	55

Table S3. Screening of methods to remove residual Met from tandem reaction system. MTases were diluted at 10 mL/g cell pellet, SalL was diluted at 5 mL/g cell pellet. CFE: cell free extract. Filtered CFE: CFEs were filtered twice using an Amicon Ultra Centrifugal Filter with a 10 KDa MW limit. Washed CFE: cell pellet was washed twice with 50 mM phosphate buffer prior to lysing. Dialysed CFE: CFE dialysed for 16 hours at 4 °C in phosphate buffer using 10 KDa MW limit dialysis tubing. Purified: Enzyme purified by HisTRAP affinity chromatography.

4 Synthesis of CIDA (5)

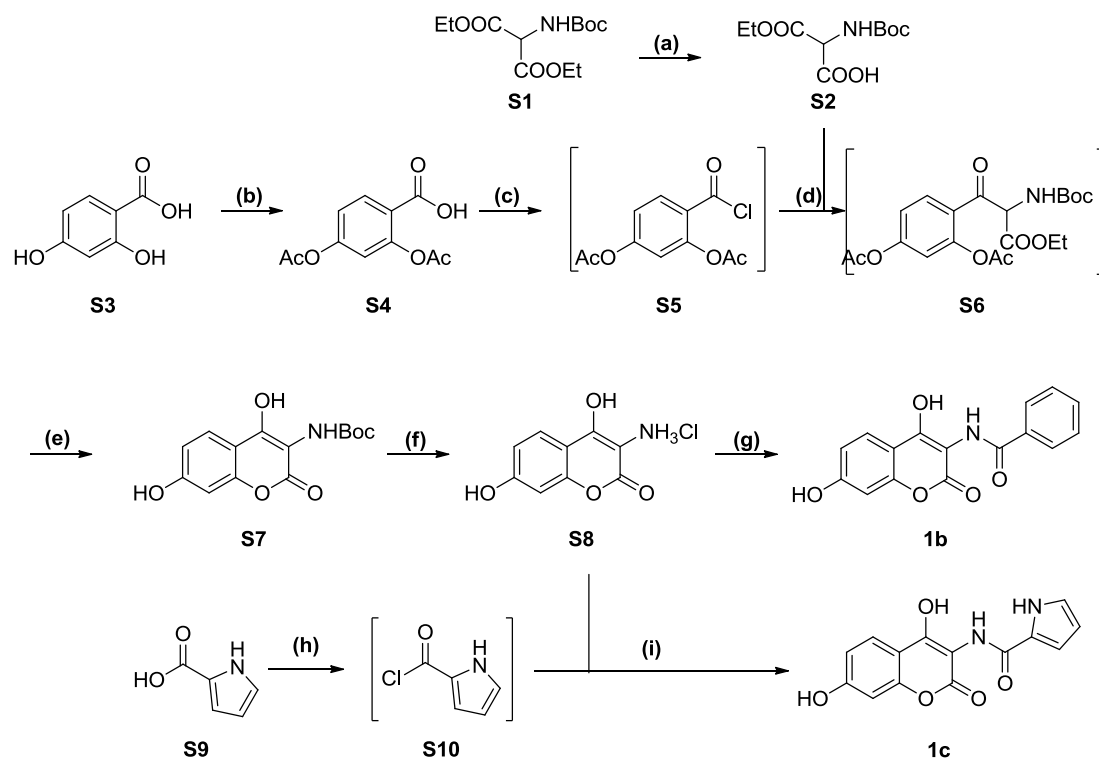
(2*R*,3*R*,4*S*,5*S*)-2-(6-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (5)¹



To an ice cooled suspension of adenosine (1.00 g, 3.74 mmol) in acetonitrile (10 mL) was added pyridine (0.61 mL, 7.48 mmol) and thionyl chloride (1.40 mL, 18.7 mmol) was added dropwise over 5 minutes. The reaction mixture was stirred at 0 °C for 3 hours before being warmed to 22 °C and stirred for 16 hours. The resulting precipitate was filtered and dissolved in water/ methanol (5:1). Aqueous ammonia (25%, 2.00 mL) was added and the reaction mixture stirred at 22 °C for 30 minutes. The solvent was removed under reduced pressure to provide the title compound as a colourless amorphous solid (1.05 g, 98%). ¹H NMR: δ (400 MHz, DMSO-d₆) 8.35 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.31 (s, 2H, NH₂), 5.95 (d, *J*=5.7 Hz, 1H, CH), 5.59 (d, *J*=6.0 Hz, 1H, OH), 5.45 (d, *J*=5.2 Hz, 1H, OH), 4.77 (q, *J*=5.6 Hz, 1H, CH), 4.18-4.32 (m, 1H, CH), 4.03-4.16 (m, 1H, CH), 3.78-4.02 (m, 2H, CH₂). ¹³C NMR: δ (101 MHz, DMSO-d₆) 156.1 (C), 152.7 (CH), 149.4 (C), 139.7 (CH), 119.1 (C), 87.4 (CH), 83.6 (CH), 72.6 (CH), 71.3 (CH), 44.8 (CH₂). **Mpt.:** 72 °C. (lit. 75-80 °C).² **v_{max} (neat):** 3148, 1648, 1601 cm⁻¹. **m/z:** (ES⁺) 286 (M³⁵Cl + H⁺, 100%), 288 (M³⁷Cl + H⁺, 35%). **R_f:** (EtOAc) 0.20.

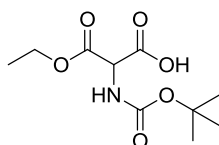
5 Synthesis of aminocoumarin substrates **1b** and **1c**

Aminocoumarin substrates were synthesised by the route outlined by Gruber *et al.* (Scheme S2Scheme S1).³



Scheme S2. Synthetic route to aminocoumarin substrates **1b** and **1c**.³ *Reagents and conditions:* (a) KOH, aq. EtOH, 22 °C. (b) Ac₂O, 4-dimethylaminopyridine (DMAP), NEt₃, THF, 0-22 °C (c) (COCl)₂, *N,N*-dimethylformamide (DMF), PhMe, 40 °C. (d) MgCl₂, NEt₃, THF, 4 °C, then **S4**. (e) aq. NaOH, MeOH, 22 °C. (f) HCl in cyclopentylmeth ether (CPME), *tert*-butylmethyl ether, MeOH, 22 °C. (g) BzCl, NEt₃, EtOAc, 22 °C, then aq. NaOH, MeOH 22 °C. (h) (COCl)₂, *N,N*-dimethylformamide (DMF), PhMe, 40 °C. (i) NEt₃, EtOAc, 22 °C, then aq. NaOH, MeOH 22 °C.

2-((*tert*-butoxycarbonylamino)-3-ethoxy-3-oxopropanoic acid (**S2**)⁴

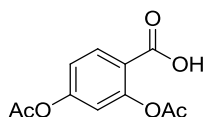


To a solution of diethyl 2-((*tert*-butoxycarbonyl)amino)malonate (**S1**) (10.0 mL, 39.2 mmol) in ethanol (50 mL) was added solid potassium hydroxide (2.25 g, 40.1 mmol). The suspension was stirred at 22 °C for 12 hours before concentrating under reduced pressure. The suspension was redissolved in 1 M

NaHCO₃ (75 mL) and washed with EtOAc (2 x 40 mL). The solution was cooled to 0 °C, acidified with solid KHSO₄ to pH 2 and extracted with EtOAc (3 x 40 mL). The combined organic fractions were washed with brine (20 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure to provide the title compound as an amorphous colourless solid (7.66 g, 77%).

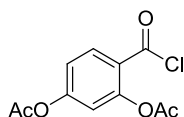
¹H NMR: δ (400 MHz, DMSO-d₆) 7.49 (d, *J*=7.9 Hz, 1H, NH), 4.73 (d, *J*=7.9 Hz, 1H, CH), 4.15 (q, *J*=7.0 Hz, 2H, CH₂), 1.41 (s, 9H, 3xCH₃), 1.21 (t, *J*=7.0 Hz, 3H, CH₃). *COOH signal not detected.* **¹³C NMR:** δ (101 MHz, DMSO-d₆) 167.8 (CO), 167.2 (CO), 156.3 (CO), 78.9 (C), 61.3 (CH₂), 57.6 (CH), 28.1 (CH₃), 13.9 (3xCH₃). **Mpt.:** 84-85 °C (lit.: 93-95 °C [diethyl ether/ hexane]).⁴ **v_{max} (neat):** 3264, 2984, 1756, 1733, 1652 cm⁻¹. **m/z:** (ES⁺) 248 (M+H⁺, 6%), 148 (100%). **R_f:** (EtOAc) 0.53.

2,4-Diacetoxybenzoic acid (S4)⁵



To a solution of 2, 4-dihydroxybenzoic acid (10.0 g, 64.9 mmol) in tetrahydrofuran (100 mL) was added triethylamine (31.7 mL, 227 mmol), 4-dimethylaminopyridine (0.793 g, 6.49 mmol) and acetic anhydride (30.6 mL, 324 mmol) and the reaction was stirred at 22 °C for 4 h. The reaction mixture was acidified with aqueous 1 M HCl to pH 3 and extracted with EtOAc (3 x 100 mL). The combined organic fractions were washed with brine (100 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the resulting solid was recrystallised from EtOAc to provide the title compound as a colourless crystalline solid (12.6 g, 82%). **¹H NMR:** δ (400 MHz, DMSO-d₆) 13.14 (br. s, 1H, COOH), 7.98 (d, *J*=8.6 Hz, 1H, Ar-H), 7.18 (dd, *J*₁=8.6, *J*₂=2.3 Hz, 1H, Ar-H), 7.09 (d, *J*=2.3 Hz, 1H, Ar-H), 2.29 (s, 3H, CH₃), 2.24 (s, 3H, CH₃). **¹³C NMR:** δ (101 MHz, CDCl₃) 169.2 (CO), 168.3 (CO), 168.2 (CO), 155.3 (C), 152.2 (C), 133.5 (C), 119.5 (CH), 119.3 (CH), 117.5 (CH), 21.2 (CH₃), 21.0 (CH₃). **Mpt.:** 135-137 °C (lit. 136-137 °C).⁵ **v_{max} (neat):** 2859, 1773, 1680, 1607 cm⁻¹. **m/z:** (ES⁺) 261 (M+Na⁺, 5%), 179 (100%). **R_f:** (1:1 EtOAc/ heptane) 0.16.

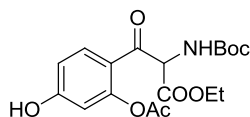
2-(chlorocarbonyl)-5-hydroxyphenyl acetate (S5)



To a suspension of 2,4-diacetoxybenzoic acid (S4) (5.00 g, 21.0 mmol) in anhydrous toluene (50 mL) under a N₂ atmosphere in a flame dried flask at 22 °C was added (COCl)₂ (3.67 mL, 42.0 mmol) and anhydrous DMF (0.16 mL, 2.10 mmol). The reaction mixture was heated to 40 °C and stirred for 2

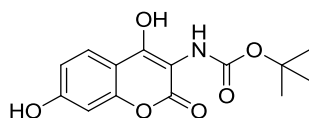
hours. The reaction mixture was concentrated under reduced pressure and the resulting crude residue was used in the next stage of the synthesis without purification.

Ethyl 3-(2-acetoxy-4-hydroxyphenyl)-2-((tert-butoxycarbonyl)amino)-3-oxopropanoate (S6)



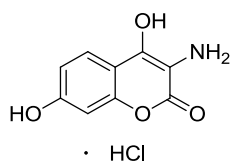
To an ice-cooled solution of 2-(*tert*-butoxycarbonylamino)-3-ethoxy-3-oxopropanoic acid (**S2**) (6.47 g, 26.2 mmol) in anhydrous tetrahydrofuran (50 mL) under an N₂ atmosphere was added triethylamine (18.4 mL, 132 mmol) and anhydrous MgCl₂ (6.79 g, 71.4 mmol). The resulting slurry was stirred vigorously at 4 °C for 2 hours. A solution of the previously prepared crude acid chloride **S5** (theoretical amount 1 eq.) in anhydrous tetrahydrofuran (50 mL) was then added and the resulting suspension stirred for 4 hours. The reaction mixture was quenched with saturated aqueous ammonium chloride (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic fractions were washed with brine (50 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure to provide a brown solid which was used in the next stage of the synthesis without further purification.

tert-Butyl (4,7-dihydroxy-2-oxo-2*H*-chromen-3-yl) carbamate (**S7**)³



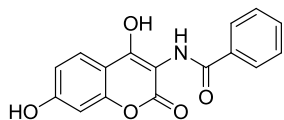
To a solution of crude coupling product (**S6**) (theoretical amount 8.89 g, 21.0 mmol) in methanol (100 mL) was added aqueous 1 M NaOH (105 mL, 105 mmol) and the reaction mixture was stirred at 22 °C for 4 hours. The reaction mixture was acidified with 2 M HCl to pH 3 and extracted with EtOAc (3 x 100 mL). The combined organic fractions were washed with brine (20 mL), dried over MgSO₄ and the solvent removed under reduced pressure. The resulting solid was recrystallised from EtOAc to provide the title compound as a pale orange crystalline solid (3.57 g, 58% over 3 stages). **¹H NMR:** δ (400 MHz, DMSO-*d*₆) 7.79 (br. s, 1H, NH), 7.66 (d, *J*=8.7 Hz, 1H, Ar-H), 6.79 (dd, *J*₁=8.7, *J*₂=2.2 Hz, 1H, Ar-H), 6.70 (d, *J*=2.2 Hz, 1H, Ar-H), 1.44 (s, 9H, 3xCH₃). *OH signals not detected.* **¹³C NMR:** δ (101 MHz, DMSO-*d*₆) 165.4 (CO), 161.2 (CO), 161.2 (C), 157.9 (C), 154.7 (C), 125.0 (CH), 112.8 (CH), 106.3 (C), 101.8 (CH), 79.3 (C), 77.3 (C), 28.2 (3xCH₃). **M.pt.:** 208-210 °C. **v_{max} (neat):** 3370, 1612, 1575 cm⁻¹. **m/z:** (ES⁺) 294 (M+H⁺, 9%), 238 (100%). **R_f:** (1:1 EtOAc/ heptane) 0.38.

3-Amino-4,7-dihydroxy-2H-chromen-2-one hydrochloride (**S8**)³



To an ice cooled solution of *tert*-butyl (4,7-dihydroxy-2-oxo-2H-chromen-3-yl) carbamate (**S7**) (2.50 g, 8.52 mmol) in *tert*-butylmethyl ether (TBME) (50 mL) and methanol (15 mL) was added 3 M HCl in cyclopentylmethyl ether (42.6 mL, 128 mmol) and the mixture stirred at 22 °C for 24 hours. The solvent was removed under reduced pressure and the residue triturated with TBME (10 mL). The suspension was filtered to provide the title compounds as an off-white amorphous solid (1.94 g, 99%). **¹H NMR:** δ (400 MHz, DMSO-*d*₆) 7.78 (d, *J*=8.6 Hz, 1H, Ar-H), 6.75 (dd, *J*₁=8.6, *J*₂=2.3 Hz, 1H, Ar-H), 6.64 (d, *J*=2.3 Hz, 1H, Ar-H). *OH and NH₂ signals not detected.* **¹³C NMR:** δ (101 MHz, DMSO-*d*₆) 161.4 (CO), 160.2 (C), 159.6 (C), 153.8 (C), 125.7 (C), 112.8 (C), 110.3 (CH), 102.3 (CH), 94.3 (CH). **M.pt.:** 240-241 °C. **v_{max} (neat):** 3336, 1710 cm⁻¹. **m/z:** (ES⁺) 194 (M⁺, 100%). **R_f:** (EtOAc) 0.03.

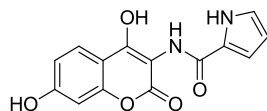
N-(4,7-Dihydroxy-2-oxo-2H-chromen-3-yl) benzamide (**1b**)³



To a solution of 3-amino-4,7-dihydroxy-2H-chromen-2-one hydrochloride (**S8**) (0.50 g, 178 mmol) in EtOAc (25 mL) was added benzoyl chloride (1.26 mL, 10.9 mmol) and triethylamine (3.04 mL, 21.8 mmol). The suspension was stirred at 22 °C for 3 hours before being filtered. The filtrate was concentrated under reduced pressure and the residue dissolved in methanol (25 mL). Aqueous 1 M NaOH (25 mL, 25.0 mmol) was added and the reaction was stirred at 22 °C for 12 hours before acidifying to pH 3 with aqueous 2 M HCl. The reaction mixture was extracted with EtOAc (3 x 50 mL) and the combined organic fractions were washed with brine (50 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude solid washed with EtOAc (10 mL) and filtered to provide the title compound as a pale yellow amorphous solid (0.43 g, 67%). **¹H NMR:** δ (400 MHz, DMSO-*d*₆) 11.82 (br. s, 1H, OH), 10.54 (s, 1H, OH), 9.41 (s, 1H, NH), 8.01 (d, *J*=7.3 Hz, 2H, Ar-H), 7.73 (d, *J*=8.7 Hz, 1H, Ar-H), 7.60 (app. t, *J*=7.3 Hz, 1H, Ar-H), 7.52 (m, 2H, Ar-H), 6.84 (dd, *J*₁=8.7, *J*₂=2.3 Hz, 1H, Ar-H), 6.74 (d, *J*=2.3 Hz, 1H, Ar-H). **¹³C NMR:** δ (101 MHz, DMSO-*d*₆) 166.5 (CO), 161.4 (CO), 160.8 (C), 160.4 (C), 153.5 (C), 133.9 (C), 131.5 (CH), 128.1 (2xCH) 128.0

(2xCH), 125.0 (CH), 112.9 (CH), 108.0 (C), 101.9 (CH), 100.1 (C). **M.pt.:** 283-285 °C (lit.: 283-285 °C). ν_{\max} (neat): 3176, 1665, 1625 cm^{-1} . **m/z:** (ES⁺) 298 (M+H⁺, 100%). **R_f:** (1:1 EtOAc/ heptane) 0.31.

***N*-(4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide (1c)**



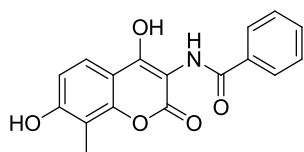
To a suspension of 1H-pyrrole-2-carboxylic acid (968 mg, 8.71 mmol) in anhydrous toluene (50.0 mL) was added oxalyl dichloride (1.525 mL, 17.42 mmol) and *N,N*-dimethylformamide (0.017 mL, 0.218 mmol). The reaction was heated to 65 °C and stirred for 1 hour before cooling to 22 °C and concentrating under reduced pressure. The residue was redissolved in anhydrous dichloromethane (10.00 mL) and added to a solution of 4,7-dihydroxy-2-oxo-2H-chromen-3-aminium chloride (500 mg, 2.18 mmol) and triethylamine (2.43 mL, 17.4 mmol) in dichloromethane (30.0 mL). The reaction was stirred at 22 °C for 1 hour before acidifying to pH 2 with aqueous 2 M HCl and extracting with EtOAc (3x30.0 mL). The combined organic fractions were dried over a hydrophobic frit and concentrated under reduced pressure. The residue was redissolved in methanol (30.0 mL) and sodium hydroxide (10.0 mL, 10.0 mmol) was added. The reaction was stirred at 22 °C for 1 hour before concentrating under reduced pressure and acidifying to pH 5 using aqueous conc. HCl. The mixture was extracted with EtOAc (3x30 mL), the combined organic fractions washed with brine (30 mL) and dried over a hydrophobic frit. The filtrate was concentrated under reduced pressure and the product triturated from MeCN to provide the title compound as an amorphous brown solid (294 mg, 47%). **¹H NMR:** δ (DMSO-*d*₆, 400MHz) 12.00 (br. s., 1H, OH), 11.66 (br. s., 1H, NH), 10.55 (s, 1H, OH), 9.04 (s, 1H, NH), 7.73 (d, *J*=8.7 Hz, 1H, Ar-H), 6.95-7.08 (m, 2H, Ar-H), 6.85 (dd, *J*=8.7, 2.3 Hz, 1H, Ar-H), 6.75 (d, *J*=2.2 Hz, 1H, Ar-H). **¹³C NMR:** δ (101 MHz, DMSO-*d*₆) 161.3 (C), 160.8 (CO), 159.4 (C), 153.2 (C), 125.4 (CH), 125.0 (C), 122.4 (CH), 113.0 (CH), 112.3 (CH), 109.0 (CH), 108.1 (C), 101.9 (CH), 100.4 (C). *One CO signal not detected.* **Mpt.:** decomp. > 290 °C. ν_{\max} (neat): 3355, 1679, 1642, 1530, 1134, 739 cm^{-1} . **m/z:** (ES⁺) 287 (M+H⁺, 37%), 145 (100%). **R_f:** (1:1 EtOAc/ heptane): 0.31.

6 General procedure for the one-pot enzymatic cascade alkylation using *E. coli* cell free extract and *E. coli* cell lysate

To a 50 mL Falcon tube was added substrate **1b**, **1c** or **3** (20.0 mg, 0.125 mmol, as a 100 mM solution in DMSO), **7** (53.6 mg, 0.188 mmol, as a 100 mM solution in DMSO), L-methionine (0.25 mmol, as a 500 mM solution in aqueous 250 mM NaOH) and DTT (19.3 mg, 0.125 mmol, as a 100 mM solution in potassium phosphate buffer). BSA (to a final concentration of 1 mg/ mL) was added before initiating SAM synthesis with SalL cell free extract (15 mL, cell pellet resuspended in 5 mL/ g). The reaction

was incubated at 35 °C, 750 rpm for 2 hours, before adding NovO cell lysate (35 mL, cell pellet resuspended in 10 mL/g) and MTAN (0.5 μM) and incubating the reaction at 35 °C, 750 rpm, for a further 16 hours. The reaction mixture was acidified to pH 5 with aqueous conc. HCl and clarified by centrifugation (20 min, 4000 rpm, 4 °C). The supernatant was extracted 3 times with EtOAc (3x50 mL), each time clarifying the emulsion by centrifugation (10 min, 4000 rpm, 4 °C) and the combined organic fractions purified by mass-directed automated purification to provide the title compounds.

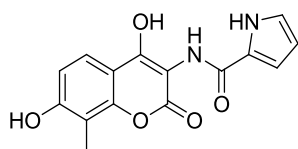
***N*-(4,7-dihydroxy-8-methyl-2-oxo-2H-chromen-3-yl)benzamide³**



97% Conversion by HPLC. Isolated as a colourless solid (11 mg, 53%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.72 (s, 1H, OH), 10.44 (s, 1H, OH), 9.43 (s, 1H, NH), 8.03 (d, *J*=7.3 Hz, 2H, 2xAr-H), 7.57-7.64 (m, 2H, 2xAr-H), 7.50-7.57 (m, 2H, 2xAr-H), 6.91 (d, *J*=8.7 Hz, 1H, Ar-H), 2.20 (s, 3H, CH₃). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 166.5 (CO), 160.8 (CO), 160.4 (C), 159.1 (C), 151.5 (C), 133.9 (C), 131.6 (CH), 128.2 (CH), 128.0 (CH), 121.5 (CH), 111.8 (CH), 110.4 (C), 107.9 (C), 99.9 (C), 8.1 (CH₃). **Mpt.:** Decomp. > 295 °C. **v_{max} (neat):** 3215, 1567, 1534, 1097, 695 cm⁻¹. **¹ m/z:** (ES⁺) 312 (M+H⁺, 100%). **R_f:** (EtOAc) 0.28.

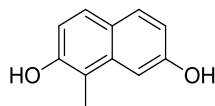
***N*-(4,7-dihydroxy-8-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide³**



100% Conversion by HPLC. Isolated as an amorphous pale yellow solid (16 mg, 76%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.89 (s, 1H, OH), 11.66 (br. s., 1H, NH), 10.41 (s, 1H), 9.03 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H,), 7.04 (br. s., 1H, Ar-H), 6.96 (br. s., 1H, Ar-H), 6.89 (d, *J*=8.7 Hz, 1H), 6.17 (d, *J*=3.0 Hz, 1H), 2.17 (s, 3H, CH₃). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 160.8 (C), 160.8 (CO), 159.5 (C), 159.0 (C), 151.2 (C), 125.4 (C), 122.4 (CH), 121.5 (CH), 112.3 (CH), 111.9 (CH), 110.4 (C), 109.0 (CH), 108.0 (C), 100.1 (C), 8.1 (CH₃). *One CO signal not detected.* **Mpt.:** Decomp. >300 °C. **v_{max} (neat):** 3340, 1584, 1532, 1404, 731 cm⁻¹. **¹ m/z:** (ES⁺) 301 (M+H⁺, 100%). **R_f:** (1:1 EtOAc/heptane) 0.33.

1-methylnaphthalene-2,7-diol³



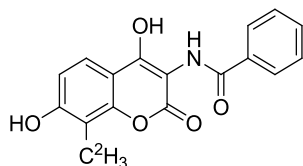
65% Conversion by HPLC. Isolated as a pink solid (10 mg, 46%).

¹H NMR: δ (METHANOL-d₄, 400 MHz) 7.56 (d, $J=8.9$ Hz, 1H, Ar-H), 7.43 (d, $J=8.9$ Hz, 1H, Ar-H), 7.12 (d, $J=2.2$ Hz, 1H, Ar-H), 6.87 (d, $J=8.8$ Hz, 1H, Ar-H), 6.83 (dd, $J=8.8, 2.2$ Hz, 1H, Ar-H), 2.38 (s, 3H, CH₃). **¹³C NMR:** δ (METHANOL-d₄, 101 MHz) 156.8 (C), 153.7 (C), 137.3 (C), 131.0 (CH), 127.9 (CH), 125.3 (C), 115.9 (CH), 115.6 (CH), 106.0 (CH), 10.8 (CH₃). *One C signal not detected.* **Mpt.:** 148.1 °C. **ν_{\max} (neat):** 3221, 1626, 1185, 1098, 822 cm⁻¹. **m/z :** (ES⁺) 173 (M-H⁺, 45%), 347 (100%). **R_f :** (1:1 EtOAc/heptane) 0.28.

7 General procedure for the one-pot enzymatic cascade alkylation using purified enzymes

To a 50 mL Falcon tube was added substrate **1b**, **1c** or **3** (20.0 mg, 0.125 mmol, as a 100 mM solution in DMSO), **7** (53.6 mg, 0.188 mmol, as a 100 mM solution in DMSO), L-methionine (or isotopically labeled analogue, purchased from Sigma Aldrich) or L-ethionine (0.25 mmol, as a 500 mM solution in aqueous 250 mM NaOH), potassium phosphate buffer (50 mM, pH 6.5, 40 mL) and DTT (19.3 mg, 0.125 mmol, as a 100 mM solution in potassium phosphate buffer). BSA (to a final concentration of 1 mg/ mL) was added before initiating SAM or SAM-analogue synthesis with SaIL (5 μ M). The reaction was incubated at 35 °C, 750 rpm for 7 hours (-CH₃, ¹³CH₃, ¹³CD₃, CD₃ transfer) or 32 hours (Et transfer) before adding NovO (40 μ M) and MTAN (0.5 μ M) and incubating the reaction at 35 °C, 750 rpm, for a further 16 hours. The reaction mixture was acidified to pH 5 with aqueous conc. HCl and the solids removed by centrifugation (20 min, 4000 rpm, 4 °C). The supernatant was extracted 3 times with EtOAc (3x50 mL), each time clarifying the emulsion by centrifugation (10 min, 4000 rpm, 4 °C) and the combined organic fractions purified by mass-directed automated purification to provide the title compounds.

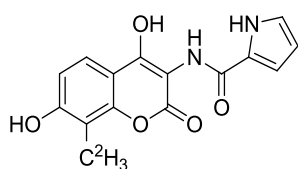
***N*-(4,7-dihydroxy-8-[²H₃]-methyl-2-oxo-2H-chromen-3-yl)benzamide**



88% conversion by HPLC. Product isolated as an amorphous colourless (11 mg, 52%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.72 (s, 1H, OH), 10.43 (s, 1H, OH), 9.42 (s, 1H, NH), 7.98-8.05 (d, $J=3$ Hz, 2H, 2xAr-H), 7.55-7.63 (m, 2H, 2xAr-H), 7.48-7.55 (m, 2H, 2xAr-H), 6.89 (d, $J=8.7$ Hz, 1H, Ar-H). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 166.5 (CO), 160.8 (CO), 160.4 (C), 159.1 (C), 151.5 (C), 133.9 (C), 131.5 (CH), 128.1 (CH), 128.0 (CH), 121.5 (CH), 111.8 (CH), 110.3 (C), 107.9 (C), 99.8 (C). **Mpt.:** Decomp. > 265 °C. **ν_{\max} (neat):** 3214, 1565, 1538 cm⁻¹. **HRMS:** [C₁₇H₁₁²H₃O₅N]⁺ requires m/z 315.1055, found 315.1047. **R_f:** (1:1 EtOAc/heptane) 0.39.

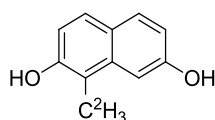
***N*-(4,7-dihydroxy-8-[²H₃]-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide**



100% conversion by HPLC. Product isolated as an amorphous pale yellow solid (17 mg, 80%).

¹H NMR: δ (DMSO-d₆, 400 MHz): 11.89 (s, 1H, OH), 11.65 (br. s, 1H, NH), 10.42 (s, 1H, OH), 9.03 (s, 1H, NH), 7.58 (d, $J=8.6$ Hz, 1H, Ar-H), 7.04 (br. s., 1H, Ar-H), 6.96 (br. s., 1H, Ar-H), 6.89 (d, $J=8.7$ Hz, 1H, Ar-H), 6.17 (d, $J=3.3$ Hz, 1H, Ar-H). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 160.8 (C), 160.8 (CO), 159.5 (C), 159.0 (C), 151.2 (C), 125.4 (C), 122.4 (CH), 121.5 (CH), 112.3 (CH), 111.9 (CH), 109.0 (CH), 108.0 (C), 100.1 (C). *One CO signal and C²H₃ signal not observed.* **Mpt.:** Decomp. >300 °C. **ν_{\max} (neat):** 3343, 1587, 1554, 1400, 728 cm⁻¹. **HRMS:** [C₁₅H₁₀²H₃O₅N₂]⁺ requires m/z 304.1007, found 304.0996. **R_f:** (1:1 EtOAc/heptane) 0.33.

1-(²H₃)-methylnaphthalene-2,7-diol

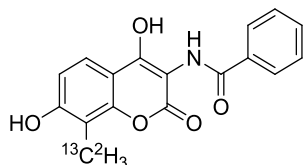


91% conversion by HPLC. Product isolated as a dark pink solid (15 mg, 68%).

¹H NMR: δ (METHANOL-d₄, 400 MHz) 7.56 (d, $J=8.7$ Hz, 1H), 7.43 (d, $J=8.7$ Hz, 1H), 7.12 (d, $J=2.3$ Hz, 1H), 6.86 (d, $J=8.7$ Hz, 1H), 6.84 (dd, $J=8.7, 2.3$ Hz, 1H). **¹³C NMR:** δ (METHANOL-d₄, 101 MHz) 156.8 (C), 153.7 (C), 137.4 (C), 131.0 (CH), 127.9 (CH), 125.3 (C), 115.9 (CH), 115.6 (CH),

106.0 (CH). One C and C²H₃ signals not detected. **Mpt.:** Decomp. >170 °C. **v_{max} (neat):** 3284, 1634, 1188, 1027, 823 cm⁻¹. **HRMS:** [C₁₁H₈²H₃O₂]⁺ requires *m/z* 178.0942, found 178.0935. **R_f:** (1:1 EtOAc/heptane) 0.28.

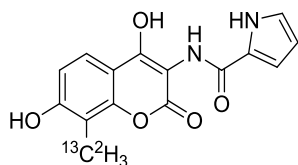
***N*-(4,7-dihydroxy-8-[¹³C²H₃]-methyl-2-oxo-2H-chromen-3-yl)benzamide**



90% conversion by HPLC. Product isolated as an amorphous colourless solid (10 mg, 47%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.72 (br. s., 1H, OH), 10.43 (s, 1H, OH), 9.42 (s, 1H, NH), 8.01 (d, *J*=7.1 Hz, 2H, 2xAr-H), 7.55-7.62 (m, 2H, 2xAr-H), 7.49-7.55 (m, 2H, 2xAr-H), 6.89 (d, *J*=8.7 Hz, 1H, Ar-H). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 166.5 (CO), 160.8 (CO), 160.4 (C), 159.1 (C), 151.5 (C), 133.9 (C), 131.5 (CH), 128.1 (CH), 128.0 (CH), 121.5 (CH), 111.8 (C), 110.1 (C), 107.9 (C), 99.8 (C), 7.3 (spt., *J*=19.7 Hz, ¹³C²H₃). **Mpt.:** Decomp. >300 °C. **v_{max} (neat):** 3219, 1567, 1539, 1139, 695 cm⁻¹. **HRMS:** [C₁₆¹³CH₁₁²H₃O₅N]⁺ requires *m/z* 316.1088, found 316.1079. **R_f:** (1:1 EtOAc/heptane) 0.39.

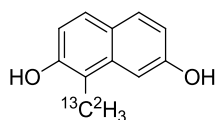
***N*-(4,7-dihydroxy-8-[¹³C²H₃]-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide**



100% conversion by HPLC. Product isolated as a colourless amorphous solid (16 mg, 75%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.89 (s, 1H, OH), 11.66 (br. s., 1H, NH), 10.41 (s, 1H, OH), 9.03 (s, 1H, NH), 7.58 (d, *J*=8.6 Hz, 1H, Ar-H), 7.04 (br. s., 1H, ar-H), 6.96 (br. s., 1H, Ar-H), 6.89 (d, *J*=8.6 Hz, 1H, Ar-H), 6.17 (d, *J*=3.2 Hz, 1H, Ar-H). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 160.8 (C), 160.8 (CO), 159.5 (C), 159.0 (C), 151.2 (C), 125.4 (C), 122.4 (CH), 121.5 (CH), 112.3 (CH), 111.9 (CH), 109.0 (CH), 108.0 (C), 100.1 (C), 7.3 (spt., *J*=19.7 Hz, ¹³C²H₃). One CO signal not observed. **Mpt.:** Decomp. >300 °C. **v_{max} (neat):** 3182, 1574, 1551, 1403, 735 cm⁻¹. **HRMS:** [C₁₄¹³CH₁₀²H₃O₅N₂]⁺ requires *m/z* 305.1041, found 305.1030. **R_f:** (1:1 EtOAc/heptane) 0.33.

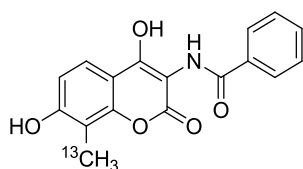
1-(¹³C²H₃)-methyl-naphthalene-2,7-diol



92% conversion by HPLC. Product isolated as a dark pink solid (19 mg, 85%).

¹H NMR: δ (METHANOL-d₄, 400 MHz) 11.89 (s, 1H, OH), 11.66 (br. s., 1H, OH), 10.42 (s, 1H, Ar-H), 9.03 (s, 1H, Ar-H), 7.58 (d, $J=8.6$ Hz, 1H, Ar-H), 7.04 (br. s., 1H), 6.96 (br. s., 1H), 6.89 (d, $J=8.7$ Hz, 1H), 6.17 ppm (d, $J=3.3$ Hz, 1H). **¹³C NMR:** δ (METHANOL-d₄, 101 MHz) 156.8 (C), 153.7 (C), 137.3 (C), 131.0 (CH), 127.9 (CH), 125.3 (d, $J=2.5$ Hz, C), 115.9 (d, $J=1.9$ Hz, CH), 115.6 (CH), 106.0 (d, $J=4.0$ Hz, CH), 10.1 (spt., $J=19.3$ Hz, ¹³C²H₃). *One C signal not detected.* **Mpt.:** Decomp. >170 °C. **ν_{\max} (neat):** 3228, 1627 cm⁻¹. **HRMS:** [C₁₀¹³CH₈²H₃O₂] requires $m/z = 179.0975$, found 179.0970. **R_f:** (1:1 EtOAc/heptane) 0.28.

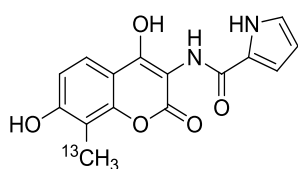
N-(4,7-dihydroxy-8-[¹³C]-methyl-2-oxo-2H-chromen-3-yl)benzamide



95% conversion by HPLC. Product isolated as a dark pink solid (16 mg, 76%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.73 (br. s., 1H, OH), 10.44 (s, 1H, OH), 9.43 (s, 1H, NH), 8.03 (d, $J=7.3$ Hz, 2H, 2xAr-H), 7.57-7.65 (m, 2H, 2xAr-H), 7.50-7.57 (m, 2H, 2xAr-H), 6.91 (d, $J=8.7$ Hz, 1H, Ar-H), 2.19 (d, $J=128.7$ Hz, 3H, ¹³CH₃). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 166.5 (CO), 160.8 (CO), 160.4 (C), 159.1 (C), 151.4 (C), 133.9 (C), 131.5 (CH), 128.1 (CH), 128.0 (CH), 121.5 (CH), 111.8 (CH), 110.6 (C), 107.9 (C), 99.8 (C), 8.1 (¹³CH₃). **Mpt.:** Decomp. >300 °C. **ν_{\max} (neat):** 3203, 1565, 1538, 1096, 695 cm⁻¹. **HRMS:** [C₁₆¹³CH₁₄NO₅]⁺ requires $m/z 313.0867$, found 313.0892. **R_f:** (1:1 EtOAc/heptane) 0.39.

N-(4,7-dihydroxy-8-[¹³C]-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide

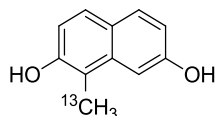


100% conversion by HPLC. Product isolated as an amorphous pale yellow solid (19 mg, 90%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.89 (s, 1H, OH), 11.65 (br. s., 1H, NH), 10.41 (s, 1H, OH), 9.03 (s, 1H, NH), 7.58 (d, $J=8.6$ Hz, 1H, Ar-H), 7.04 (br. s., 1H, Ar-H), 6.96 (d, $J=1.0$ Hz, 1H, Ar-H), 6.89

(d, $J=8.7$ Hz, 1H, Ar-H), 6.17 (d, $J=3.1$ Hz, 1H, Ar-H), 2.17 (d, $J=128.7$ Hz, 3H, $^{13}\text{CH}_3$). **^{13}C NMR:** δ (DMSO-d₆, 101 MHz) 160.8 (C), 160.8 (CO), 159.5 (C), 159.0 (C), 151.2 (C), 125.4 (C), 122.4 (CH), 121.5 (CH), 112.3 (CH), 111.9 (CH), 109.0 (CH), 108.1 (C), 100.1 (C), 8.1 ($^{13}\text{CH}_3$). *One CO signal not observed.* **Mpt.:** Decomp. > 300 °C. **ν_{max} (neat):** 3343, 1587, 1555, 1525, 1401, 729 cm^{-1} . **HRMS:** [$\text{C}_{14}^{13}\text{CH}_{13}\text{N}_2\text{O}_5$]⁺ requires m/z 302.0853, found 302.0840. **R_f:** (1:1 EtOAc/heptane) 0.33.

1-(^{13}C)-methylnaphthalene-2,7-diol



94% conversion by HPLC. Product isolated as a dark pink solid (18 mg, 83%).

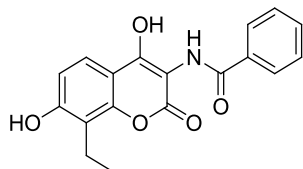
^1H NMR: δ (METHANOL-d₄, 400 MHz) 7.56 (d, $J=8.7$ Hz, 1H), 7.43 (d, $J=8.9$ Hz, 1H), 7.13 (d, $J=2.3$ Hz, 1H), 6.85 (d, $J=8.9$ Hz, 1H), 6.83 (dd, $J=8.7, 2.3$ Hz, 1H), 2.38 (d, $J=126.6$ Hz, 3H, CH_3).

^{13}C NMR: δ (METHANOL-d₄, 101 MHz) 156.8 (C), 153.6 (C), 137.3 (C), 131.0 (CH), 127.9 (CH), 125.3 (d, $J=2.5$ Hz, C), 115.9 (d, $J=1.9$ Hz, CH), 115.6 (CH), 106.0 ([d, $J=4.0$ Hz, C] C), 10.8 ($^{13}\text{CH}_3$).

One C signal not detected. **Mpt.:** Decomp. >170 °C. **ν_{max} (neat):** 3284, 1634, 1185, 1068, 833 cm^{-1} .

HRMS: [$\text{C}_{10}^{13}\text{CH}_{11}\text{O}_2$]⁺ requires m/z 175.0754, found 175.0704. **R_f:** (1:1 EtOAc/heptane) 0.28.

N-(8-ethyl-4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide

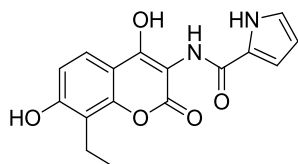


34% conversion by HPLC. Product isolated as an amorphous colourless solid (4 mg, 18%).

^1H NMR: δ (DMSO-d₆, 400 MHz) 11.72 (br. s., 1H, OH), 10.43 (s, 1H, OH), 9.41 (s, 1H, NH), 8.01 (d, $J=7.1$ Hz, 2H, 2xAr-H), 7.56-7.66 (m, 2H, 2xAr-H), 7.47-7.56 (m, 2H, 2xAr-H), 6.90 (d, $J=8.7$ Hz, 1H, Ar-H), 2.74 (q, $J=7.3$ Hz, 2H, CH_2), 1.12 (t, $J=7.4$ Hz, 3H, CH_3). **^{13}C NMR:** δ (DMSO-d₆, 101 MHz) 166.6 (CO), 160.9 (CO), 160.5 (C), 158.8 (C), 151.1 (C), 133.9 (C), 131.5 (CH), 128.1 (CH), 128.0 (CH), 121.8 (CH), 116.6 (C), 112.1 (CH), 108.0 (C), 99.9 (C), 15.8 (CH_2), 13.5 (CH_3).

Mpt.: Decomp. >265 °C. **ν_{max} (neat):** 3331, 1668, 1571, 1543, 1103, 703 cm^{-1} . **HRMS:** [$\text{C}_{18}\text{H}_{16}\text{NO}$]⁺ requires m/z 326.1023, found 326.1012. **R_f:** (1:1 EtOAc/heptane) 0.45.

N-(8-ethyl-4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide



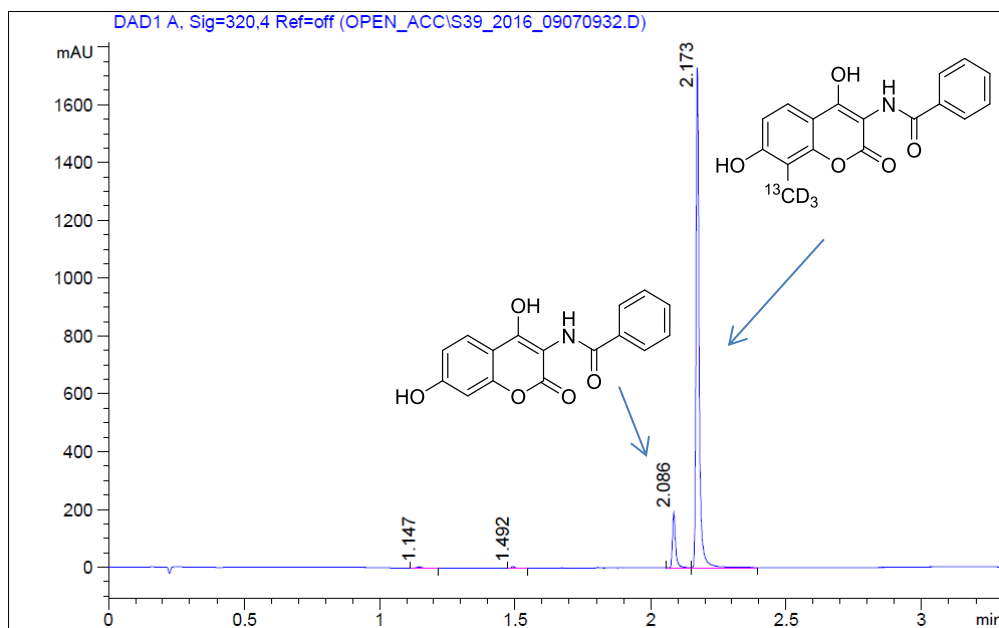
30% conversion by HPLC. Product isolated as an amorphous pale yellow solid (6 mg, 27%).

¹H NMR: δ (DMSO-d₆, 400 MHz) 11.88 (s, 1H, OH), 11.65 (br. s., 1H, NH), 10.40 (s, 1H, OH), 9.02 (s, 1H, NH), 7.58 (d, $J=8.6$ Hz, 1H, Ar-H), 7.04 (br. s., 1H, Ar-H), 6.96 (br. s., 1H, Ar-H), 6.89 (d, $J=8.7$ Hz, 1H, Ar-H), 6.17 (d, $J=3.2$ Hz, 1H, Ar-H), 2.73 (q, $J=7.4$ Hz, 2H, CH₂), 1.11 (t, $J=7.4$ Hz, 3H, CH₃). **¹³C NMR:** δ (DMSO-d₆, 101 MHz) 160.9 (C), 160.8 (CO), 159.6 (C), 158.7 (C), 150.9 (C), 125.4 (C), 122.4 (CH), 121.7 (CH), 116.5 (C), 112.3 (CH), 112.1 (CH), 109.0 (CH), 100.1 (C), 15.8 (CH₂), 13.5 (CH₃). *One CO signal not observed.* **Mpt.:** Decomp. > 260 °C. **ν_{\max} (neat):** 3354, 1576, 1555, 1405, 736 cm⁻¹. **HRMS:** [C₁₆H₁₅N₂O₅]⁺ requires m/z 315.0976, found 315.0965. **R_f:** (1:1 EtOAc:heptane) 0.42 .

8 Representative HPLC chromatograms for calculation of % conversion

Percentage (%) conversion was calculated from the ratio of starting material to product after 24 hours with a detection wavelength of 320 nm (aminocoumarin substrates **1b** and **1c**) or 220 nm (2, 7-dihydroxynaphthalene **3**). For each substrate, a representative chromatogram is shown for the transfer of $^{13}\text{CD}_3$ and Et groups with the peaks for starting material and product labelled in each case.

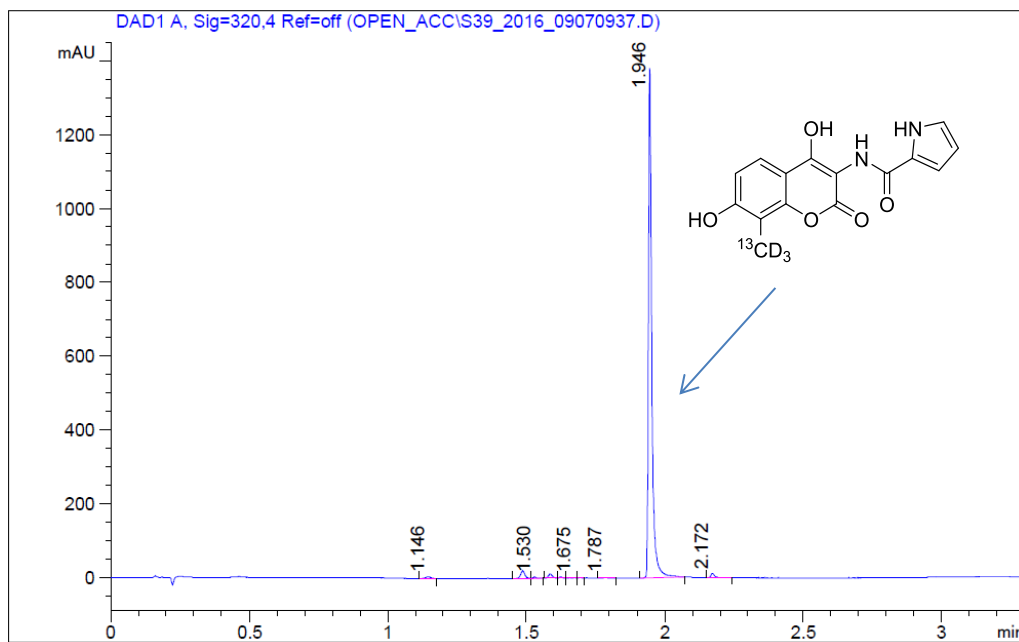
$^{13}\text{CD}_3$ transfer to **1b**



Signal 1: DAD1 A, Sig=320,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.15	0.021	5.094	7.037	0.45
2	1.49	0.012	6.402	5.162	0.33
3	2.09	0.012	191.982	154.083	9.91
4	2.17	0.012	1720.166	1388.353	89.30

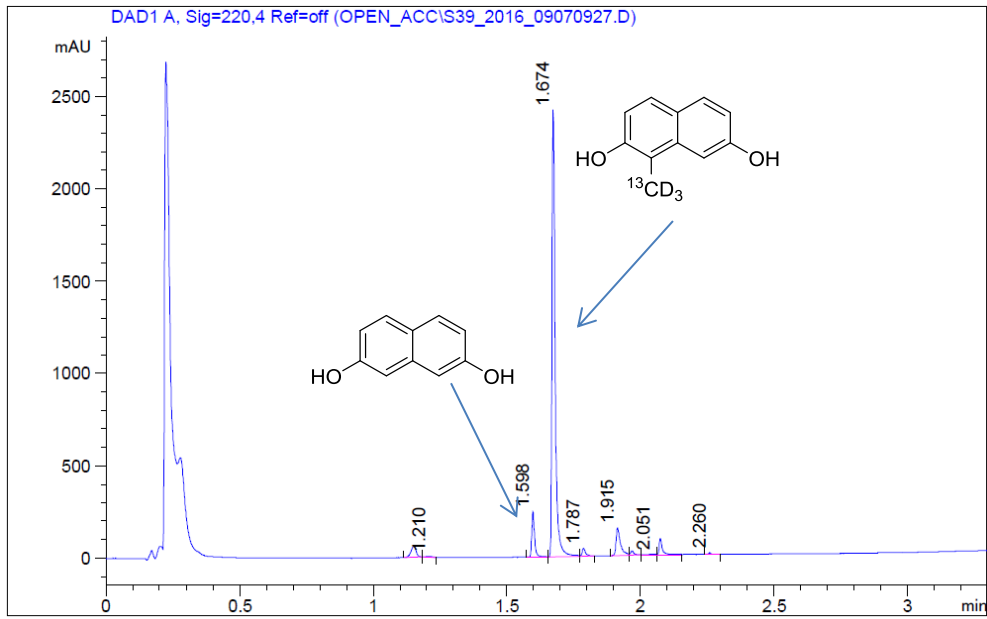
$^{13}\text{CD}_3$ transfer to 1c



Signal 1: DAD1 A, Sig=320,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.15	0.022	4.959	6.870	0.56
2	1.49	0.017	20.292	22.631	1.85
3	1.53	0.013	3.827	3.607	0.29
4	1.59	0.016	12.038	12.877	1.05
5	1.63	0.013	3.555	2.897	0.24
6	1.67	0.022	1.401	2.326	0.19
7	1.70	0.012	2.162	1.839	0.15
8	1.79	0.017	2.437	2.838	0.23
9	1.95	0.013	1372.269	1159.061	94.61
10	2.17	0.013	11.483	10.181	0.83

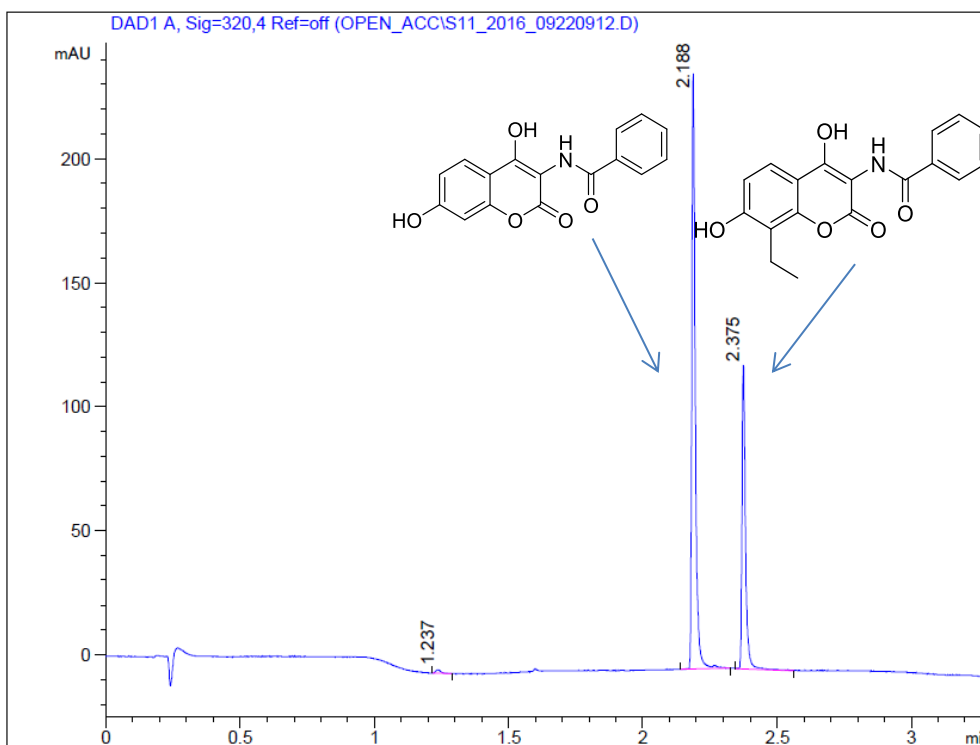
¹³CD₃ transfer to 3



Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.15	0.020	63.079	81.729	3.15
2	1.21	0.021	4.454	6.225	0.24
3	1.60	0.011	243.561	176.713	6.81
4	1.67	0.013	2415.798	2018.958	77.81
5	1.79	0.013	43.905	37.964	1.46
6	1.91	0.017	147.595	170.338	6.56
7	1.97	0.014	22.153	20.253	0.78
8	2.05	0.019	3.614	4.885	0.19
9	2.07	0.012	89.840	71.125	2.74
10	2.26	0.010	9.798	6.692	0.26

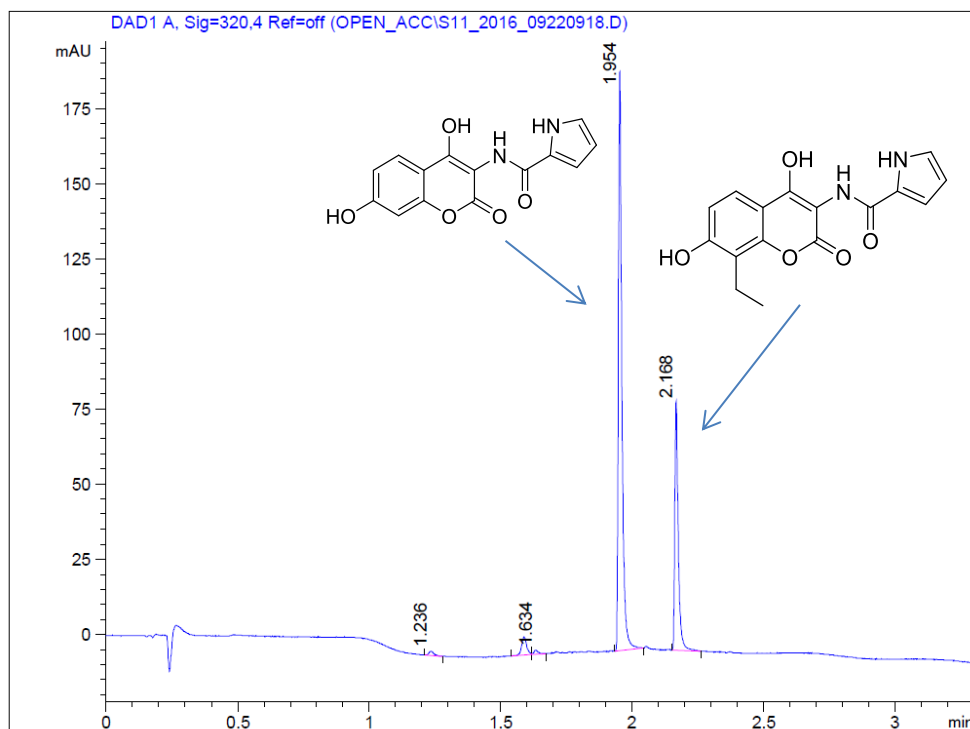
Ethylation of 1b



Signal 1: DAD1 A, Sig=320,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.24	0.021	1.418	2.016	0.62
2	2.19	0.013	238.618	214.508	65.81
3	2.37	0.013	122.030	109.420	33.57

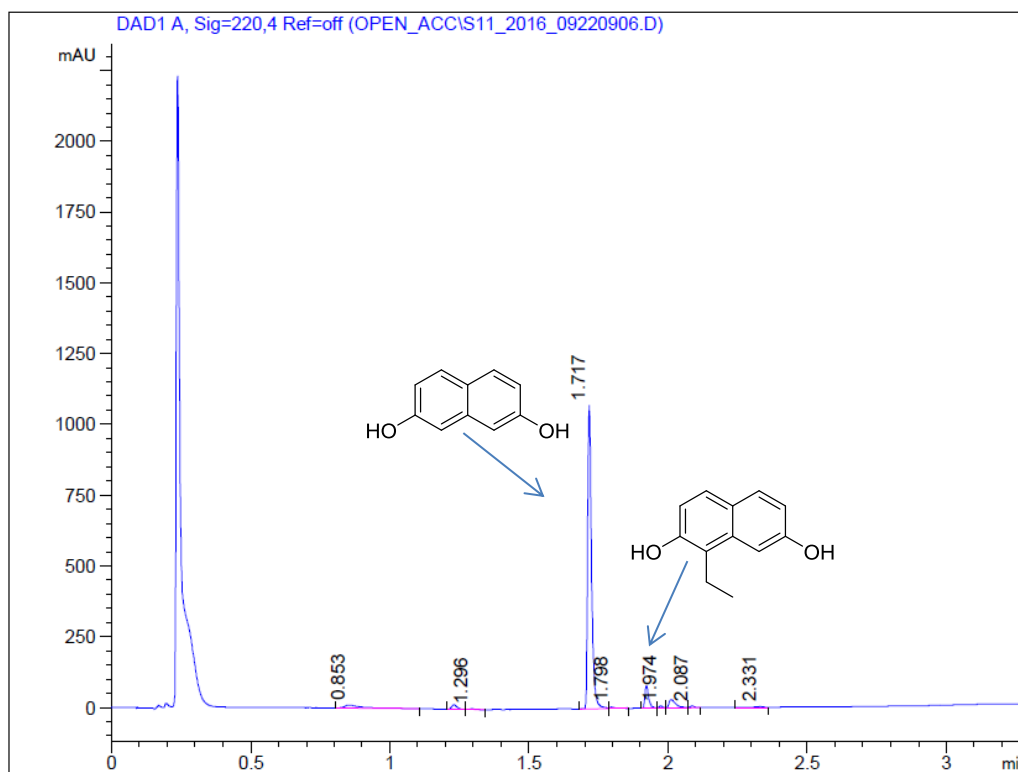
Ethylation of 1c



Signal 1: DAD1 A, Sig=320,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	1.24	0.020	1.437	1.954	0.73
2	1.59	0.021	6.113	8.368	3.13
3	1.63	0.018	1.403	1.791	0.67
4	1.95	0.014	192.549	179.472	67.22
5	2.17	0.014	82.251	75.393	28.24

Ethylation of 3

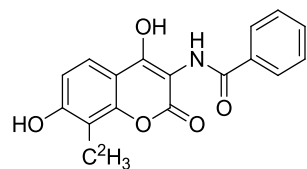


Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RT (min)	Width (min)	Height	Area	Area %
1	0.85	0.062	10.059	45.330	3.61
2	1.23	0.021	16.368	22.365	1.78
3	1.30	0.025	2.241	3.923	0.31
4	1.72	0.015	1072.354	1030.716	82.14
5	1.80	0.021	5.126	7.688	0.61
6	1.92	0.014	79.797	74.962	5.97
7	1.97	0.014	8.008	7.216	0.58
8	2.01	0.024	30.682	50.805	4.05
9	2.09	0.015	7.465	7.292	0.58
10	2.33	0.016	3.847	4.564	0.36

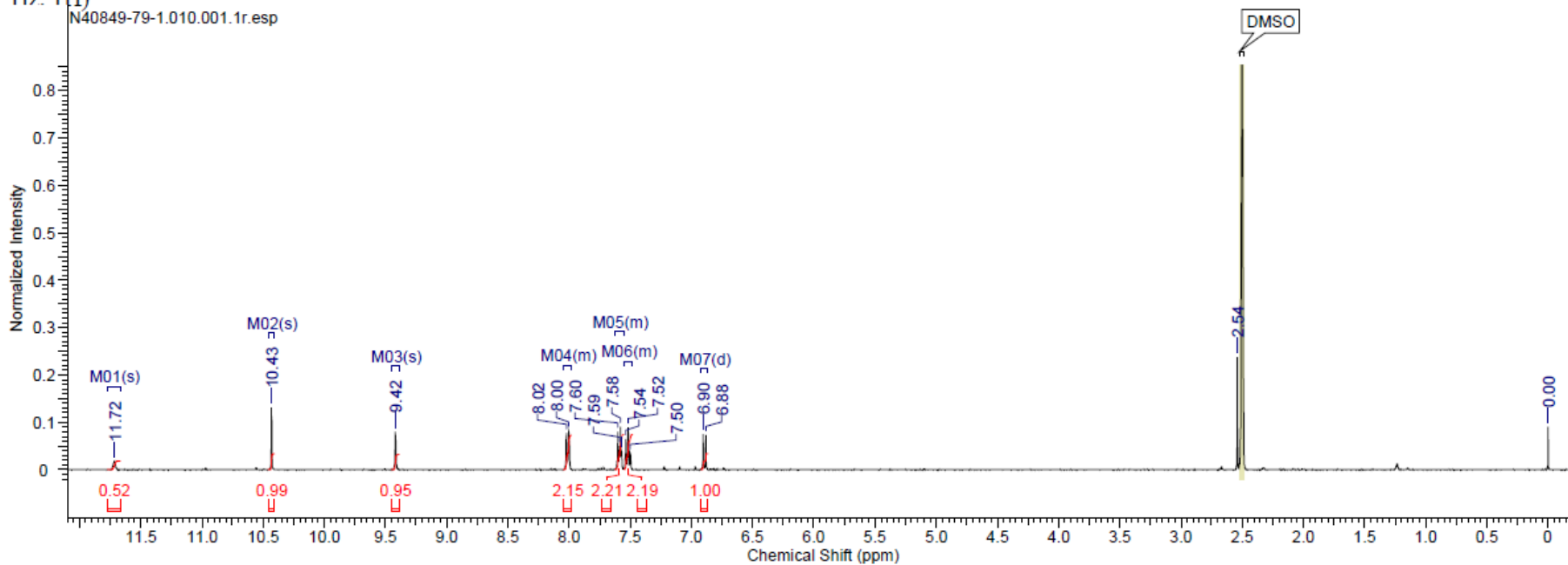
9 NMR spectra of novel compounds

N-(4,7-dihydroxy-8-[²H₃]-methyl-2-oxo-2H-chromen-3-yl)benzamide

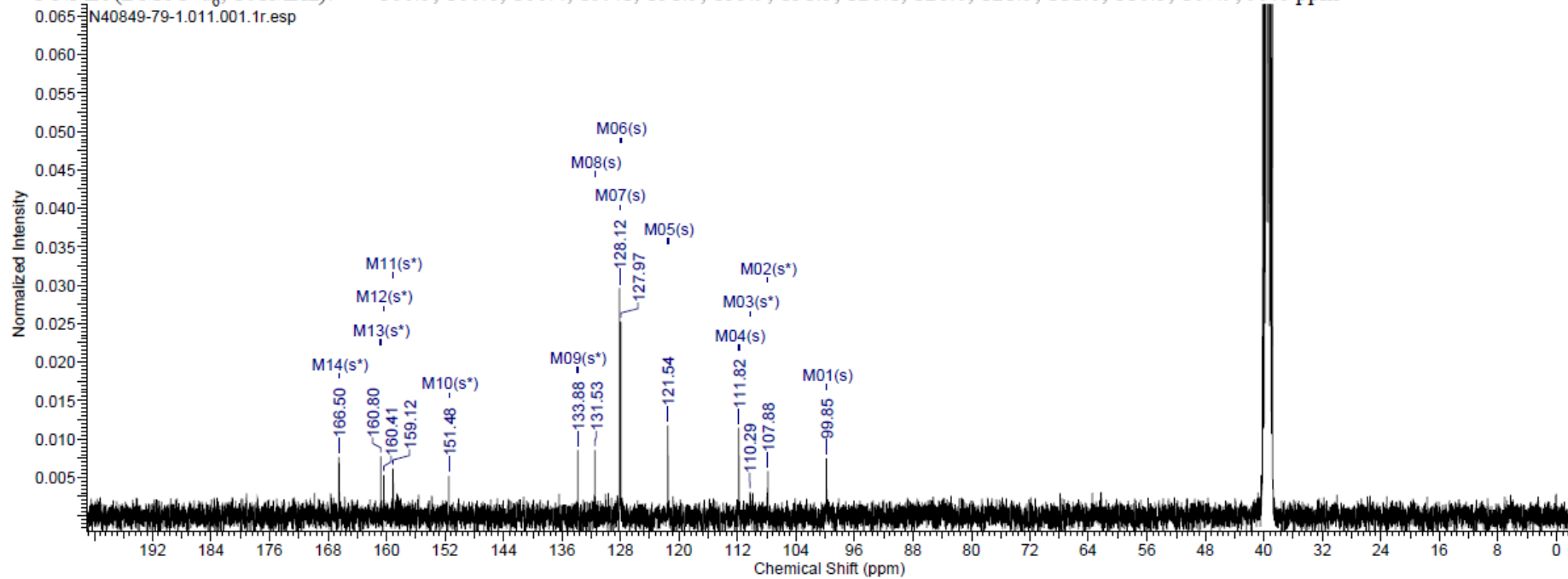


¹H NMR (DMSO-d₆, 400MHz): δ = 11.72 (s, 1H), 10.43 (s, 1H), 9.42 (s, 1H), 7.98-8.05 (m, 2H), 7.55-7.63 (m, 2H), 7.48-7.55 (m, 2H), 6.89 ppm (d, *J*=8.7

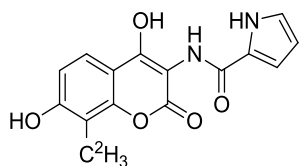
Hz, 1H)



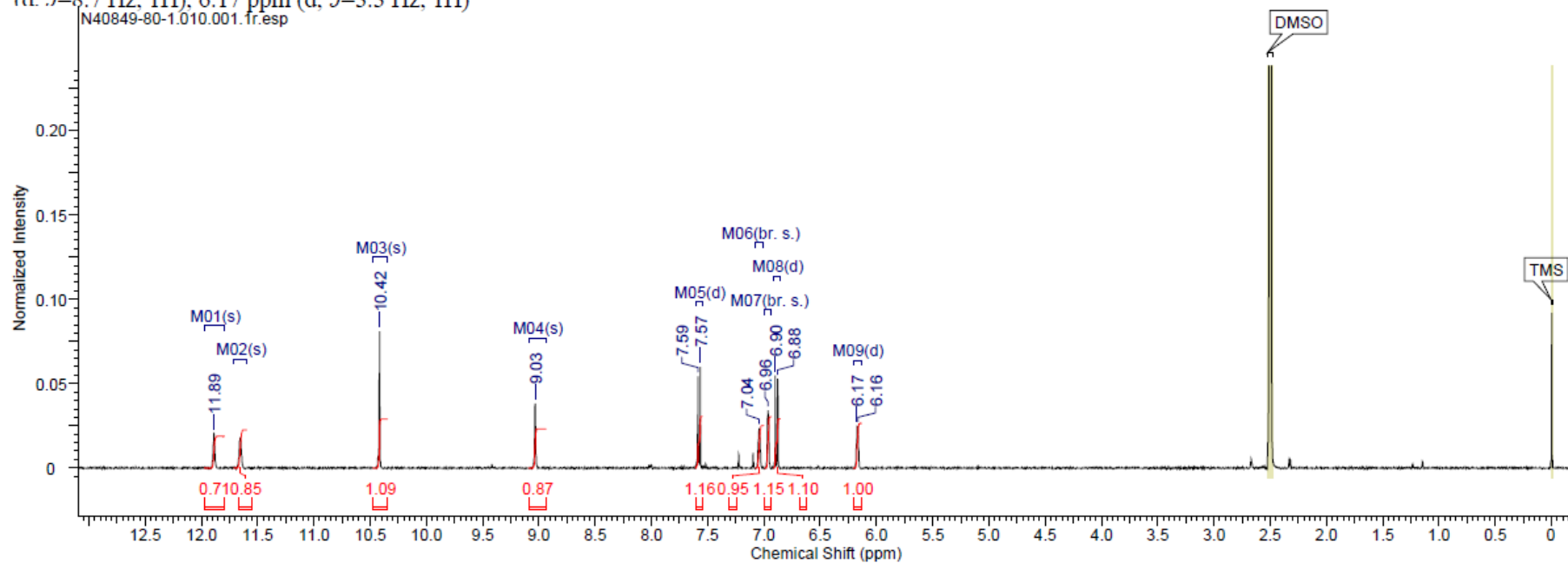
¹³C NMR (DMSO-d₆, 101MHz): δ = 166.5, 160.8, 160.4, 159.1, 151.5, 133.9, 131.5, 128.1, 128.0, 121.5, 111.8, 110.3, 107.9, 99.8 ppm



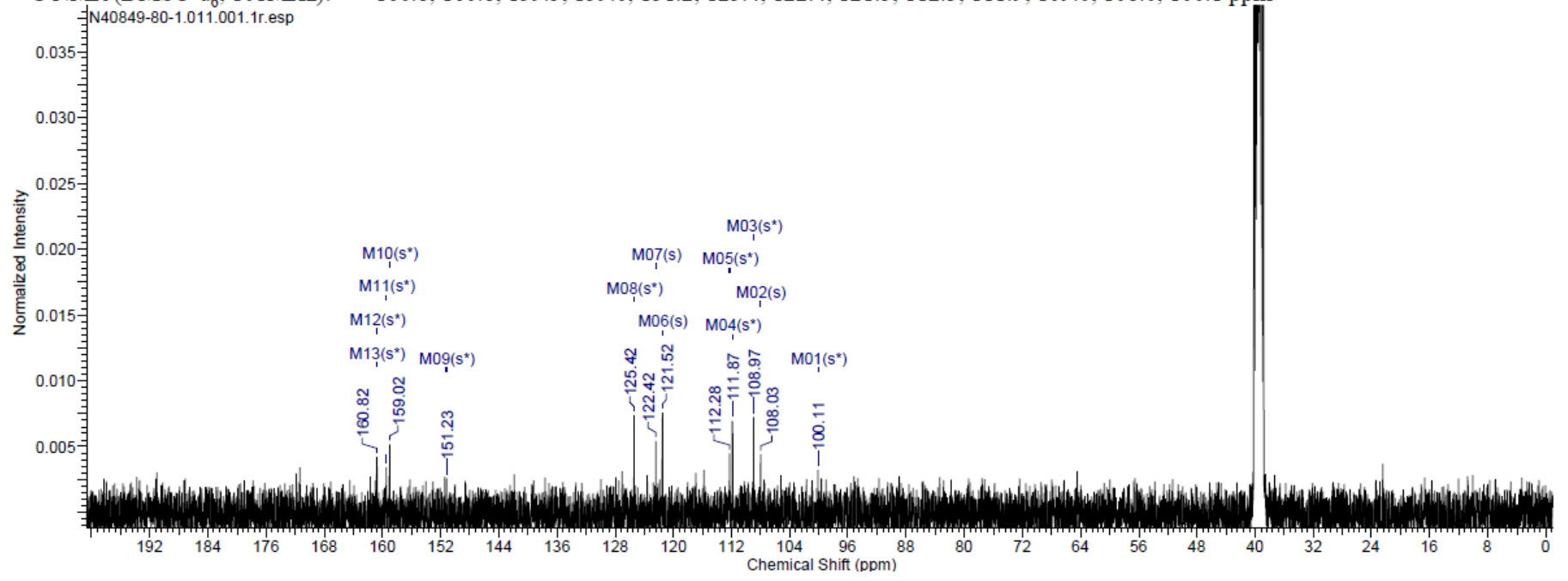
N-(4,7-dihydroxy-8-[²H₃]-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide



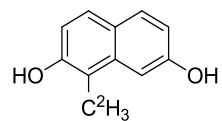
¹H NMR (DMSO-d₆, 400MHz): δ = 11.89 (s, 1H), 11.65 (s, 1H), 10.42 (s, 1H), 9.03 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H), 7.04 (br. s., 1H), 6.96 (br. s., 1H), 6.89 (d, *J*=8.7 Hz, 1H), 6.17 ppm (d, *J*=3.3 Hz, 1H)



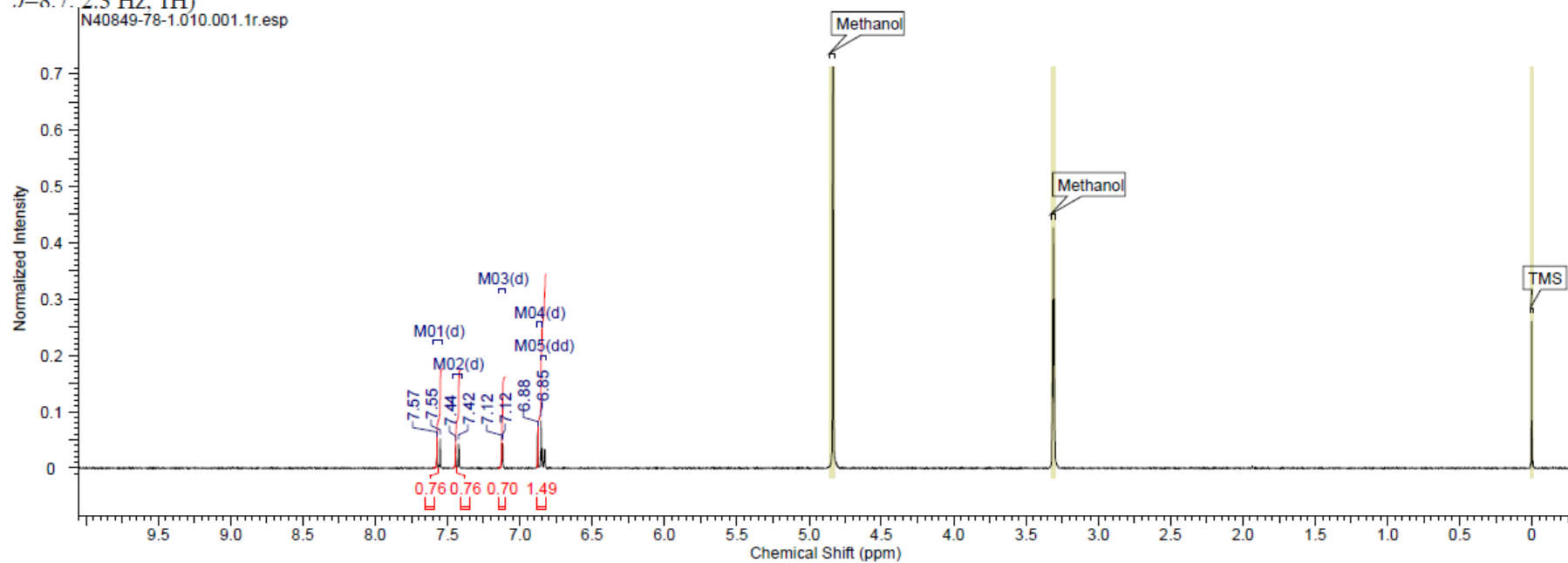
¹³C NMR (DMSO-d₆, 101MHz): δ = 160.8, 160.8, 159.5, 159.0, 151.2, 125.4, 122.4, 121.5, 112.3, 111.9, 109.0, 108.0, 100.1 ppm



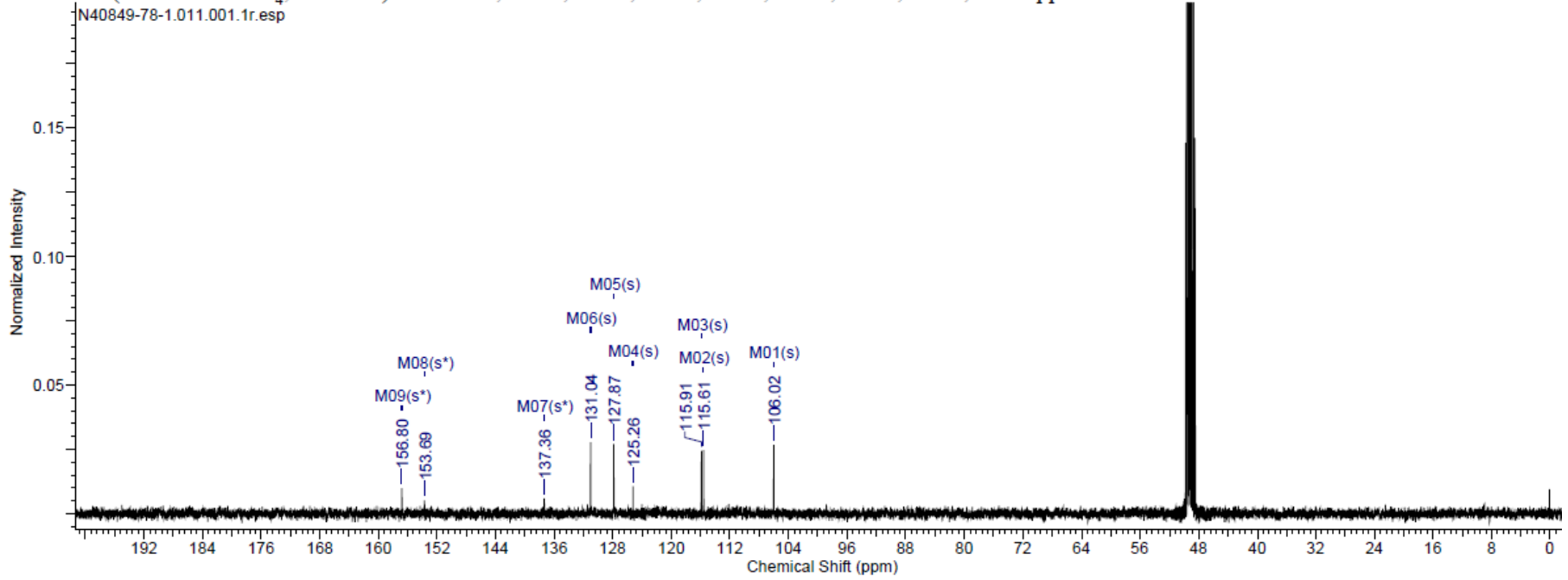
1-(²H₃)-methylnaphthalene-2,7-diol



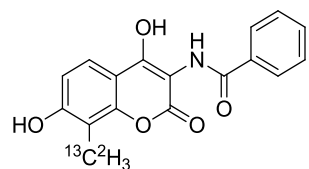
¹H NMR (METHANOL-d₄, 400MHz): δ = 7.56 (d, *J*=8.7 Hz, 1H), 7.43 (d, *J*=8.7 Hz, 1H), 7.12 (d, *J*=2.3 Hz, 1H), 6.86 (d, *J*=8.7 Hz, 1H), 6.84 ppm (dd, *J*=8.7, 2.3 Hz, 1H)



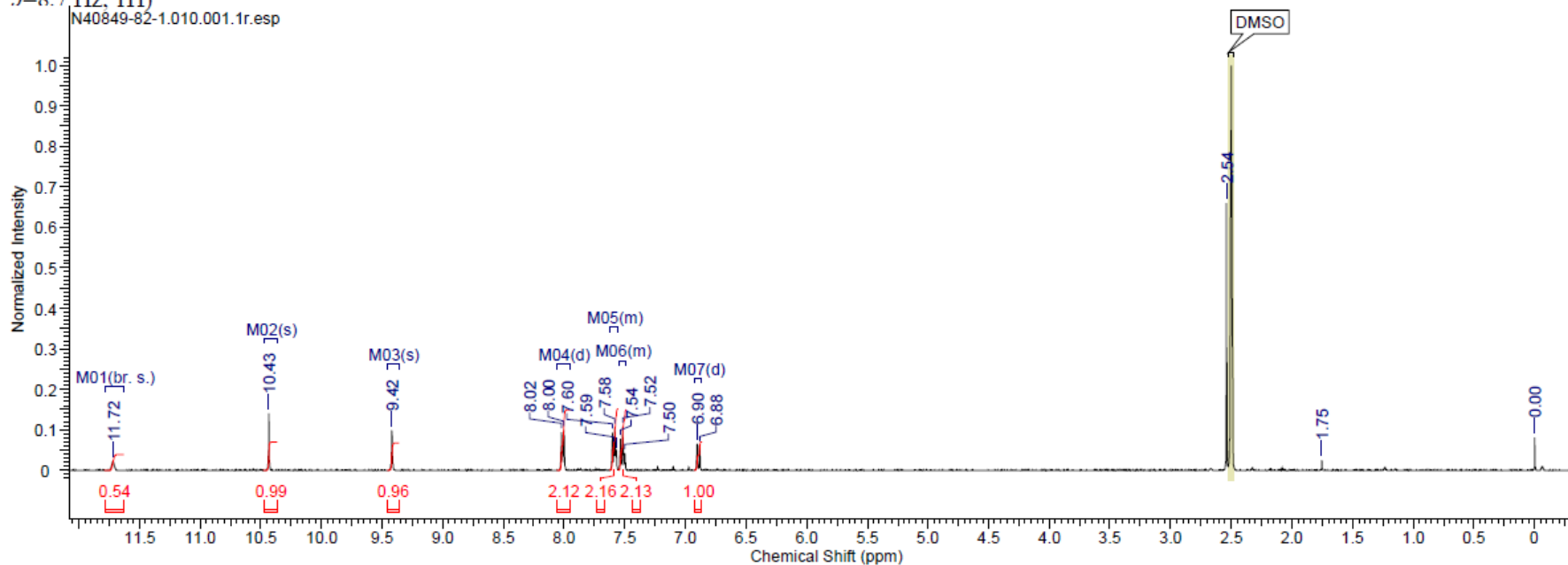
¹³C NMR (METHANOL-d₄, 101MHz): δ = 156.8, 153.7, 137.4, 131.0, 127.9, 125.3, 115.9, 115.6, 106.0 ppm



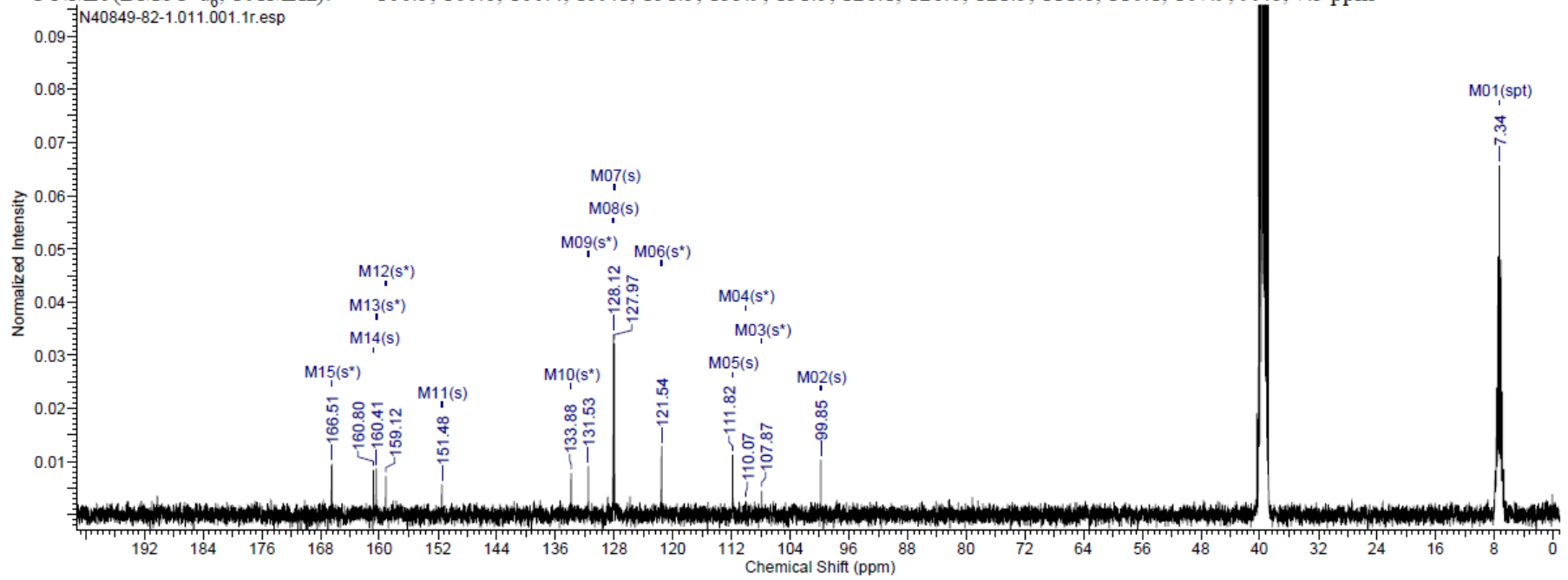
***N*-(4,7-dihydroxy-8-¹³C²H₃)-methyl-2-oxo-2H-chromen-3-yl)benzamide**



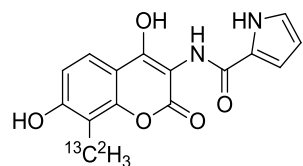
¹H NMR (DMSO-d₆, 400MHz): δ = 11.72 (br. s., 1H), 10.43 (s, 1H), 9.42 (s, 1H), 8.01 (d, *J*=7.1 Hz, 2H), 7.55-7.62 (m, 2H), 7.49-7.55 (m, 2H), 6.89 ppm (d, *J*=8.7 Hz, 1H)



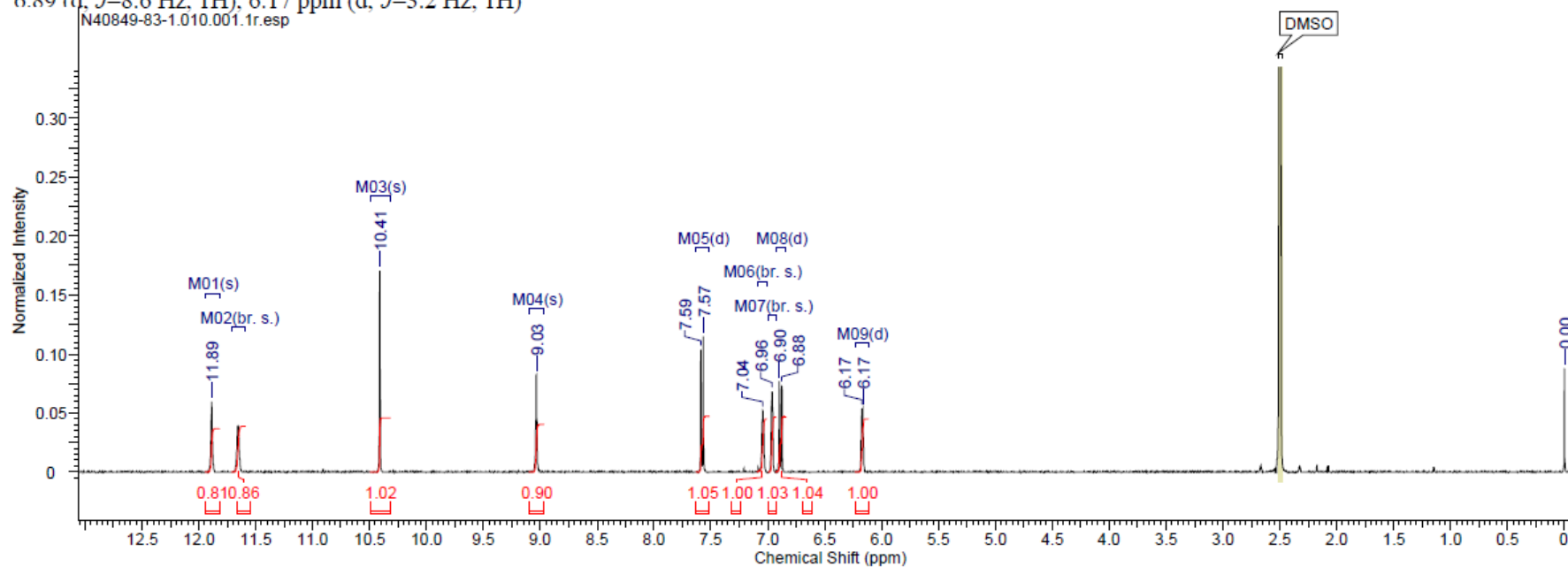
¹³C NMR (DMSO-d₆, 101MHz): δ = 166.5, 160.8, 160.4, 159.1, 151.5, 133.9, 131.5, 128.1, 128.0, 121.5, 111.8, 110.1, 107.9, 99.8, 7.3 ppm



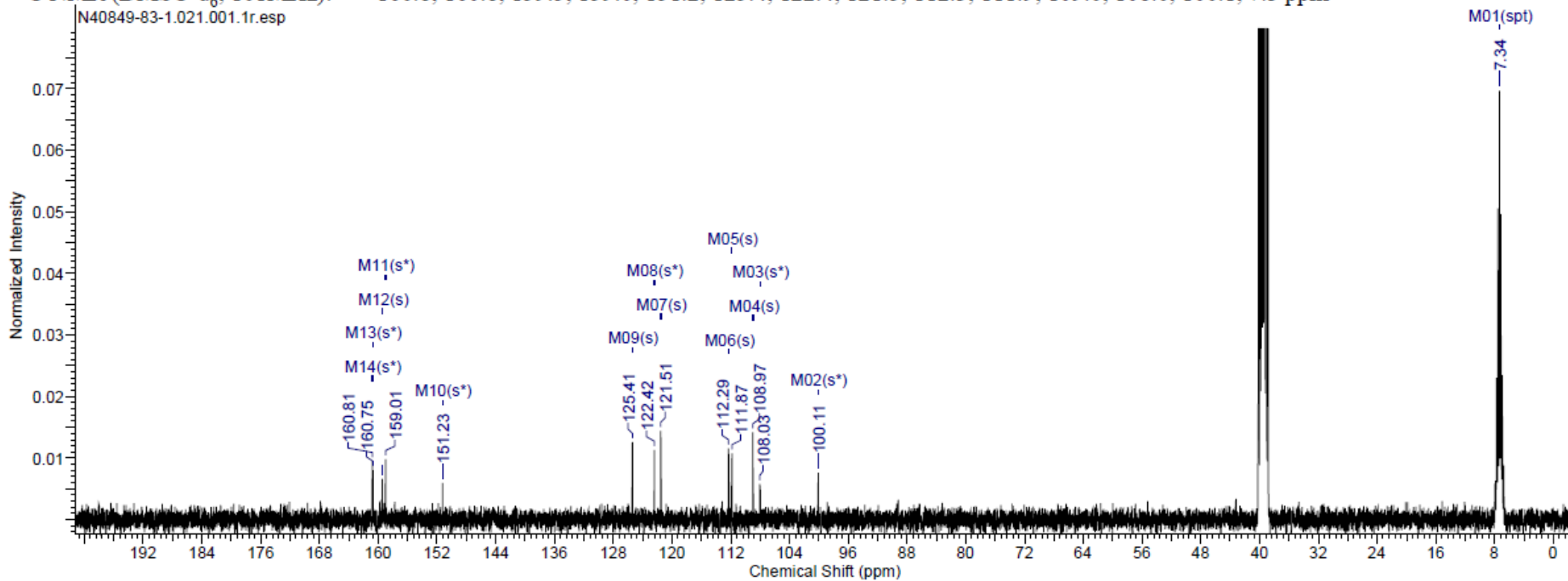
N-(4,7-dihydroxy-8-¹³C²H₃)-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide



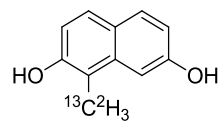
¹H NMR (DMSO-d₆, 400MHz): δ = 11.89 (s, 1H), 11.66 (br. s., 1H), 10.41 (s, 1H), 9.03 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H), 7.04 (br. s., 1H), 6.96 (br. s., 1H), 6.89 (d, *J*=8.6 Hz, 1H), 6.17 ppm (d, *J*=3.2 Hz, 1H)



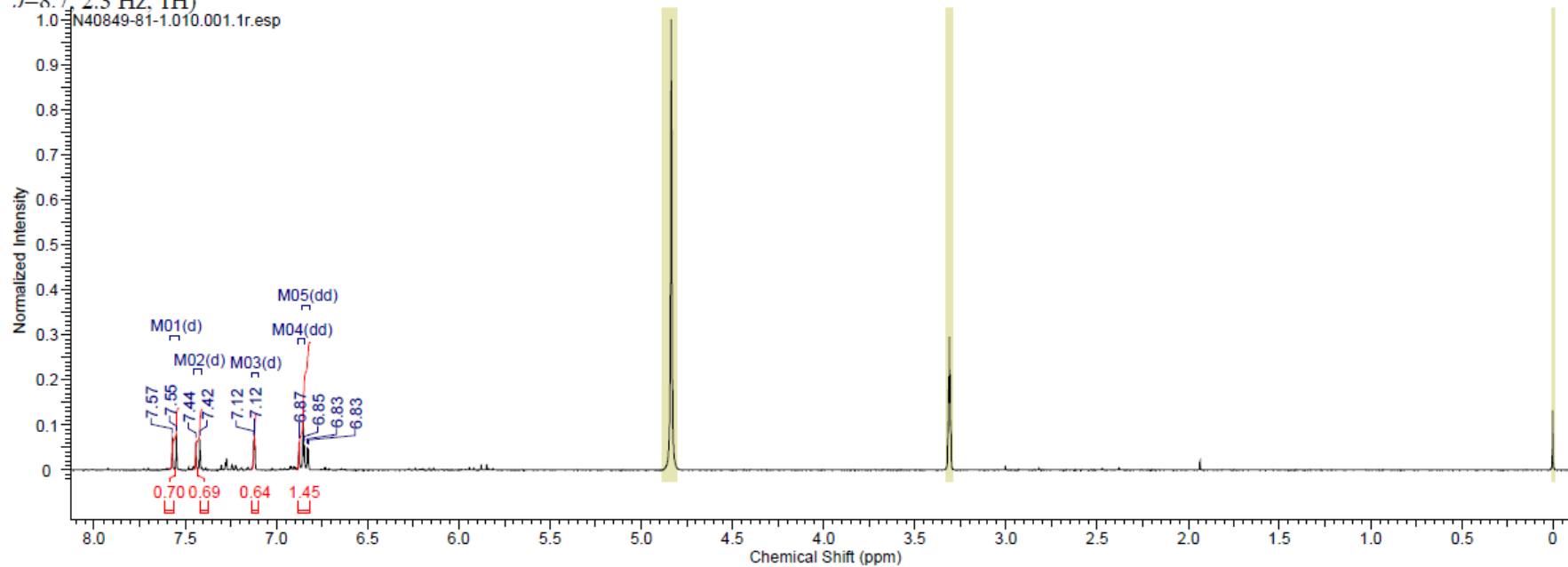
^{13}C NMR (DMSO- d_6 , 101MHz): $\delta = 160.8, 160.8, 159.5, 159.0, 151.2, 125.4, 122.4, 121.5, 112.3, 111.9, 109.0, 108.0, 100.1, 7.3$ ppm



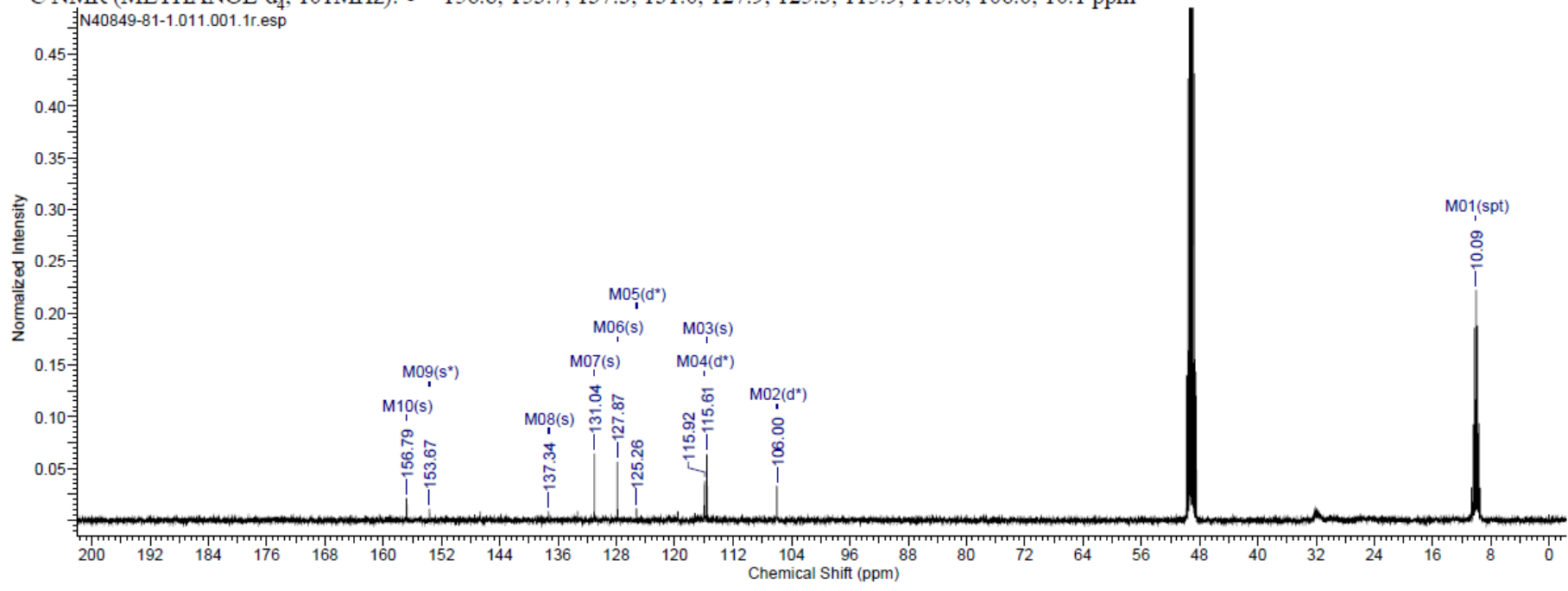
1-($^{13}\text{C}^2\text{H}_3$)-methylnaphthalene-2,7-diol



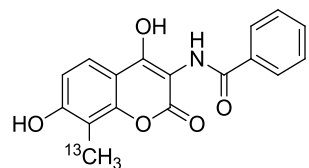
^1H NMR (METHANOL- d_4 , 400MHz): δ = 7.56 (d, $J=8.7$ Hz, 1H), 7.43 (d, $J=8.7$ Hz, 1H), 7.12 (d, $J=2.2$ Hz, 1H), 6.87 (dd, $J=8.7, 1.0$ Hz, 2H), 6.83 ppm (dd, $J=8.7, 2.3$ Hz, 1H)



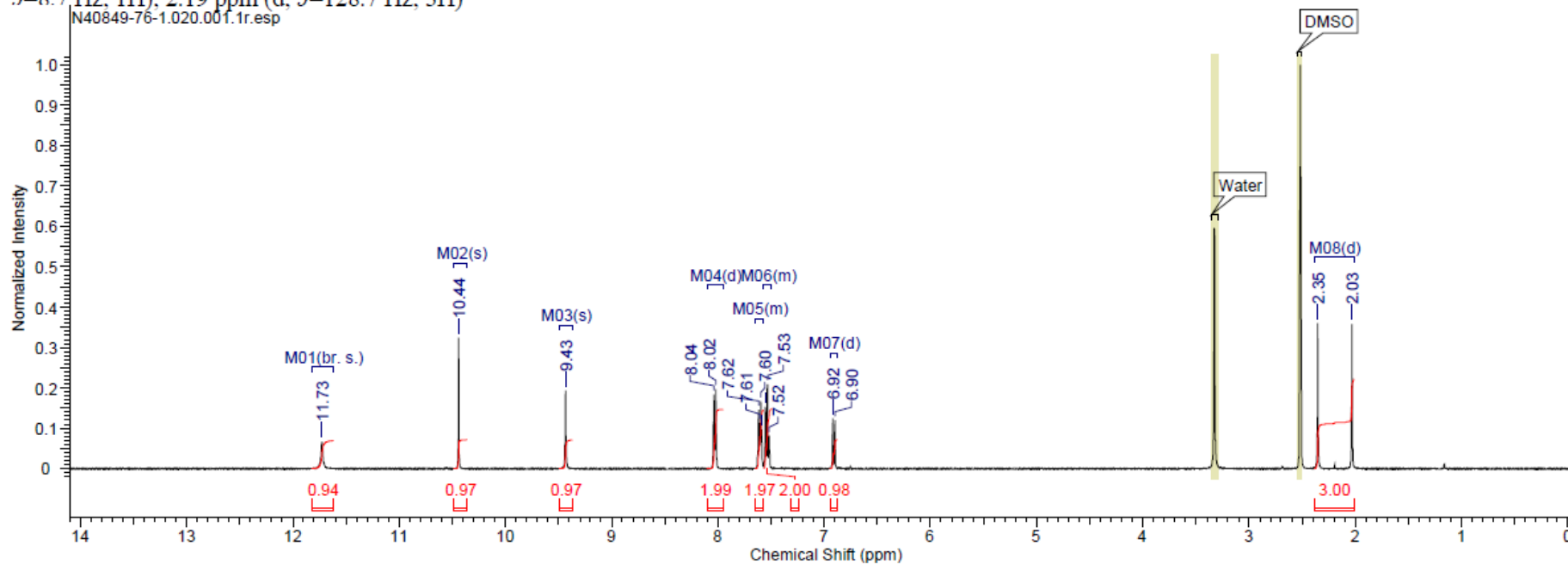
¹³C NMR (METHANOL-d₄, 101MHz): δ = 156.8, 153.7, 137.3, 131.0, 127.9, 125.3, 115.9, 115.6, 106.0, 10.1 ppm



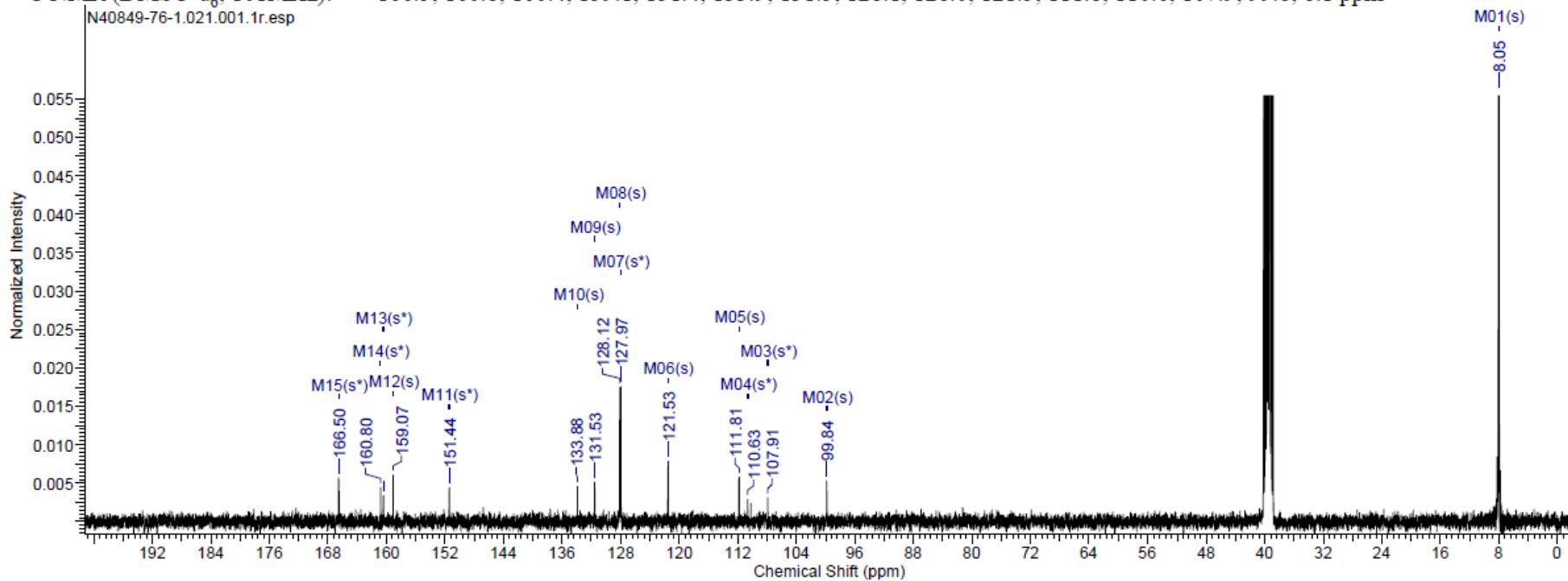
***N*-(4,7-dihydroxy-8-¹³C)-methyl-2-oxo-2H-chromen-3-yl)benzamide**



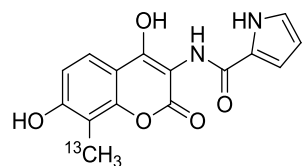
¹H NMR (DMSO-d₆, 400MHz): δ = 11.73 (br. s., 1H), 10.44 (s, 1H), 9.43 (s, 1H), 8.03 (d, *J*=7.3 Hz, 2H), 7.57-7.65 (m, 2H), 7.50-7.57 (m, 2H), 6.91 (d, *J*=8.7 Hz, 1H), 2.19 ppm (d, *J*=128.7 Hz, 3H)



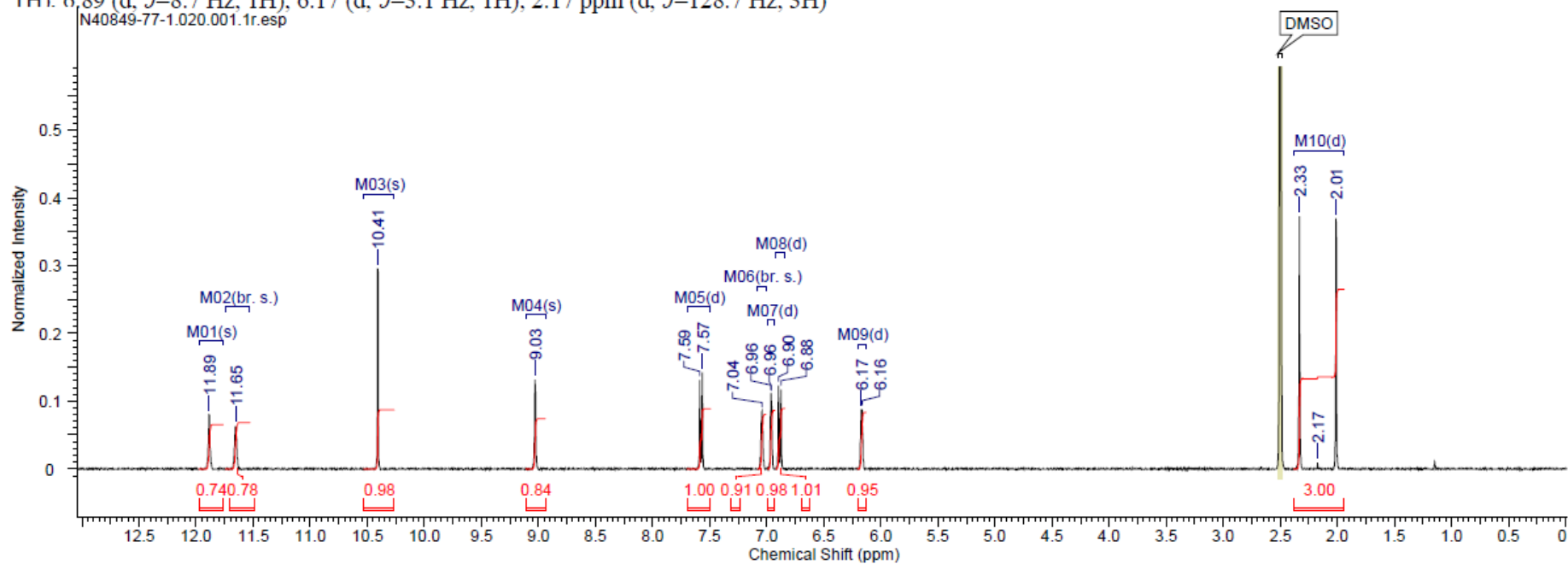
^{13}C NMR (DMSO- d_6 , 101MHz): $\delta = 166.5, 160.8, 160.4, 159.1, 151.4, 133.9, 131.5, 128.1, 128.0, 121.5, 111.8, 110.6, 107.9, 99.8, 8.1$ ppm



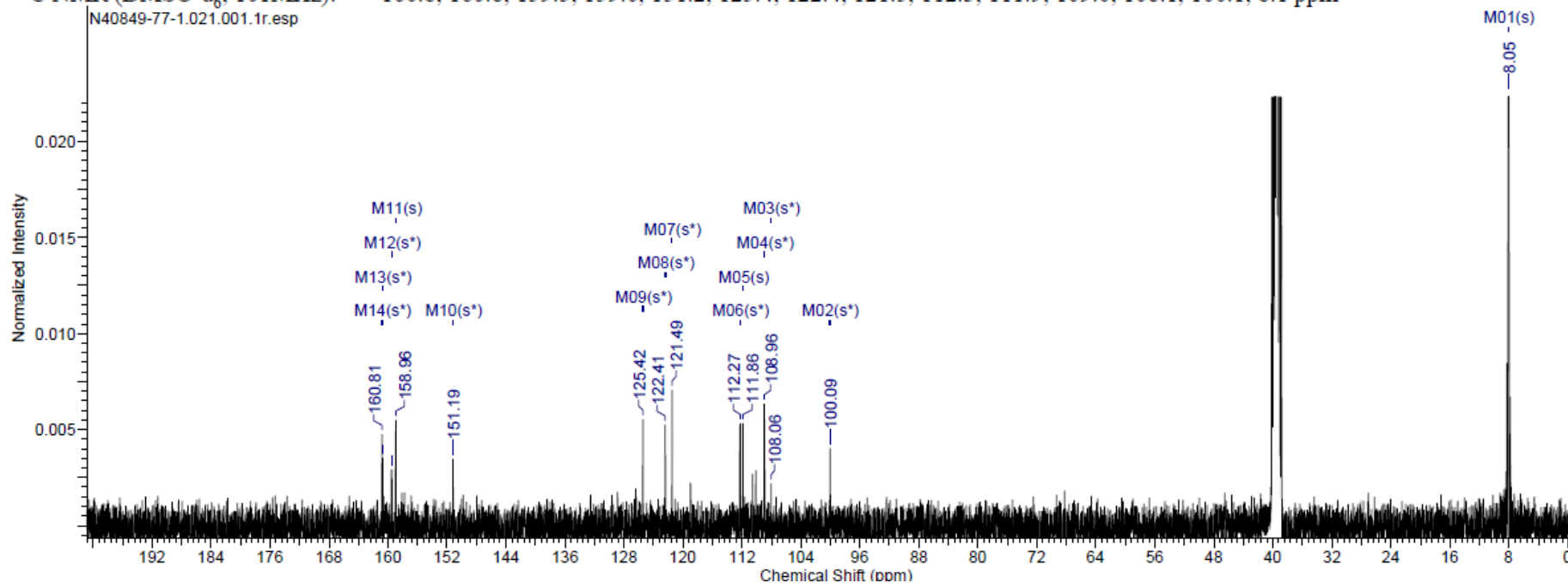
N-(4,7-dihydroxy-8-¹³C)-methyl-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide



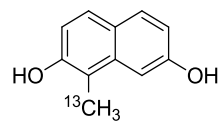
¹H NMR (DMSO-d₆, 400MHz): δ = 11.89 (s, 1H), 11.65 (br. s., 1H), 10.41 (s, 1H), 9.03 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H), 7.04 (br. s., 1H), 6.96 (d, *J*=1.0 Hz, 1H), 6.89 (d, *J*=8.7 Hz, 1H), 6.17 (d, *J*=3.1 Hz, 1H), 2.17 ppm (d, *J*=128.7 Hz, 3H)



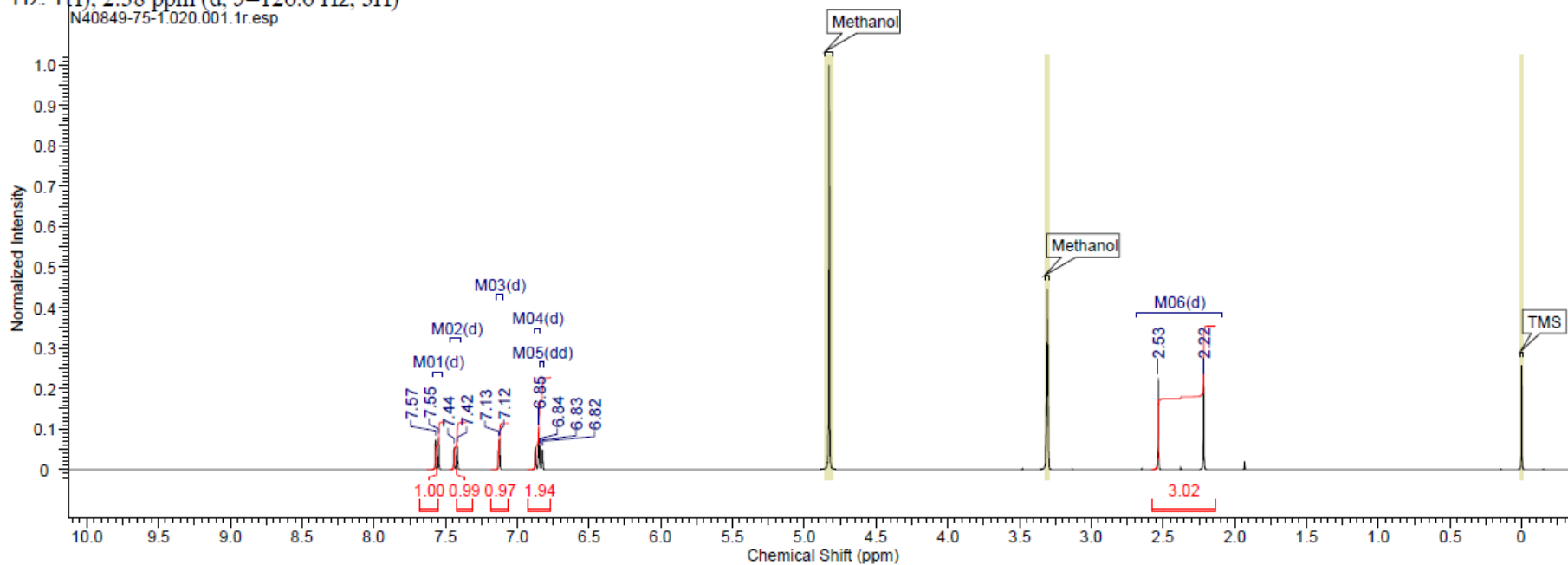
¹³C NMR (DMSO-d₆, 101MHz): δ = 160.8, 160.8, 159.5, 159.0, 151.2, 125.4, 122.4, 121.5, 112.3, 111.9, 109.0, 108.1, 100.1, 8.1 ppm

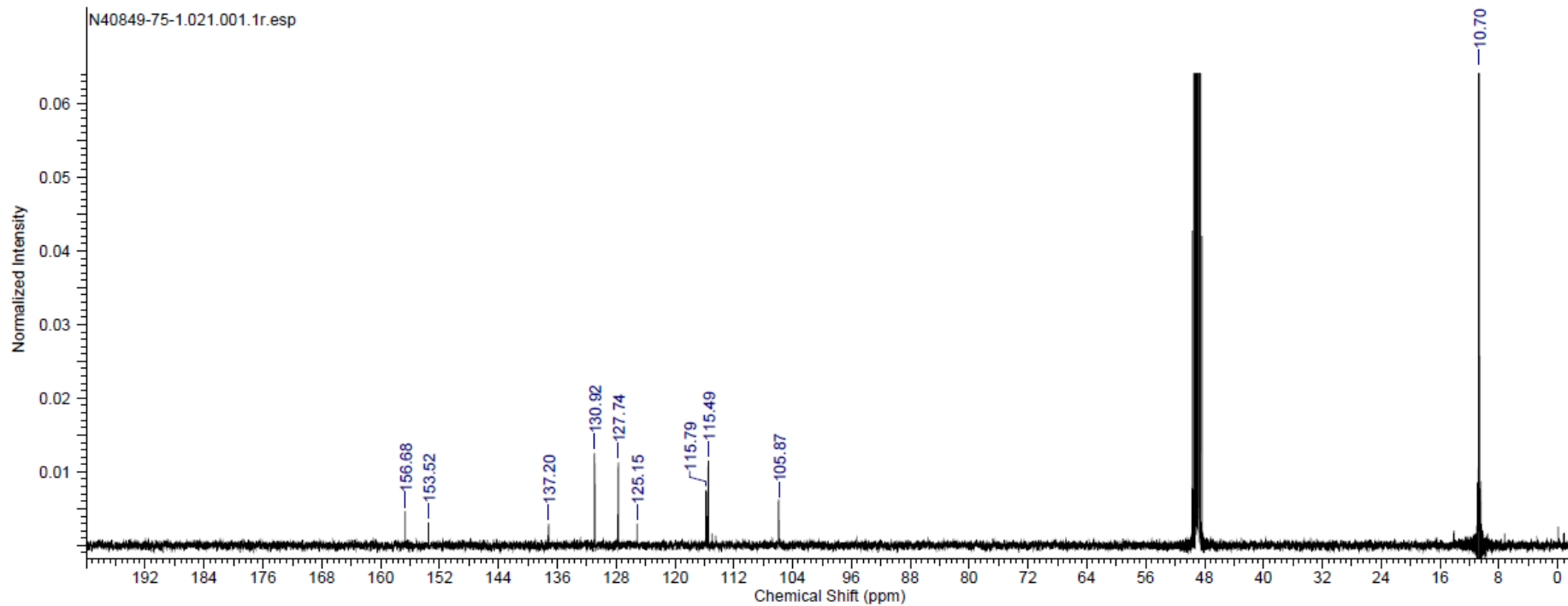


1-(¹³C)-methylnaphthalene-2,7-diol

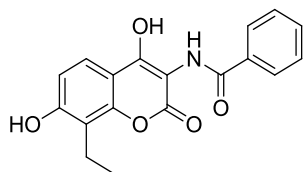


¹H NMR (METHANOL-d₄, 400MHz): δ = 7.56 (d, *J*=8.7 Hz, 1H), 7.43 (d, *J*=8.9 Hz, 1H), 7.13 (d, *J*=2.3 Hz, 1H), 6.85 (d, *J*=8.9 Hz, 1H), 6.83 (dd, *J*=8.7, 2.3 Hz, 1H), 2.38 ppm (d, *J*=126.6 Hz, 3H)

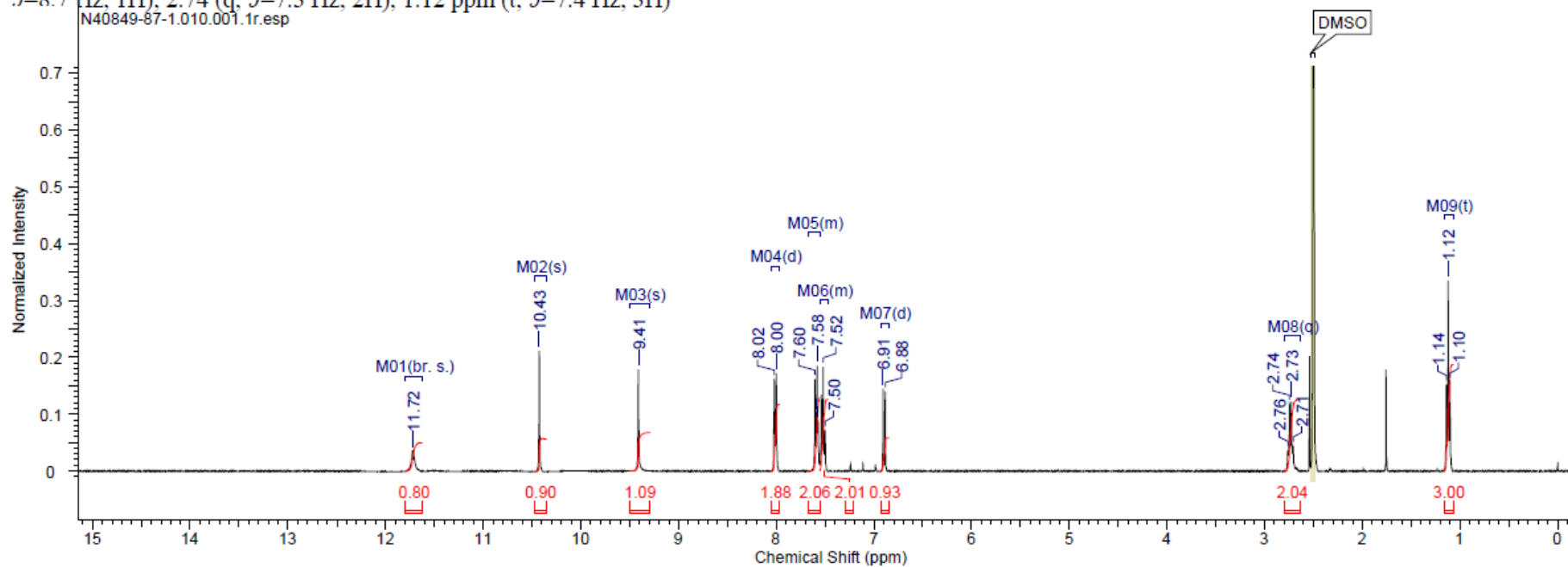




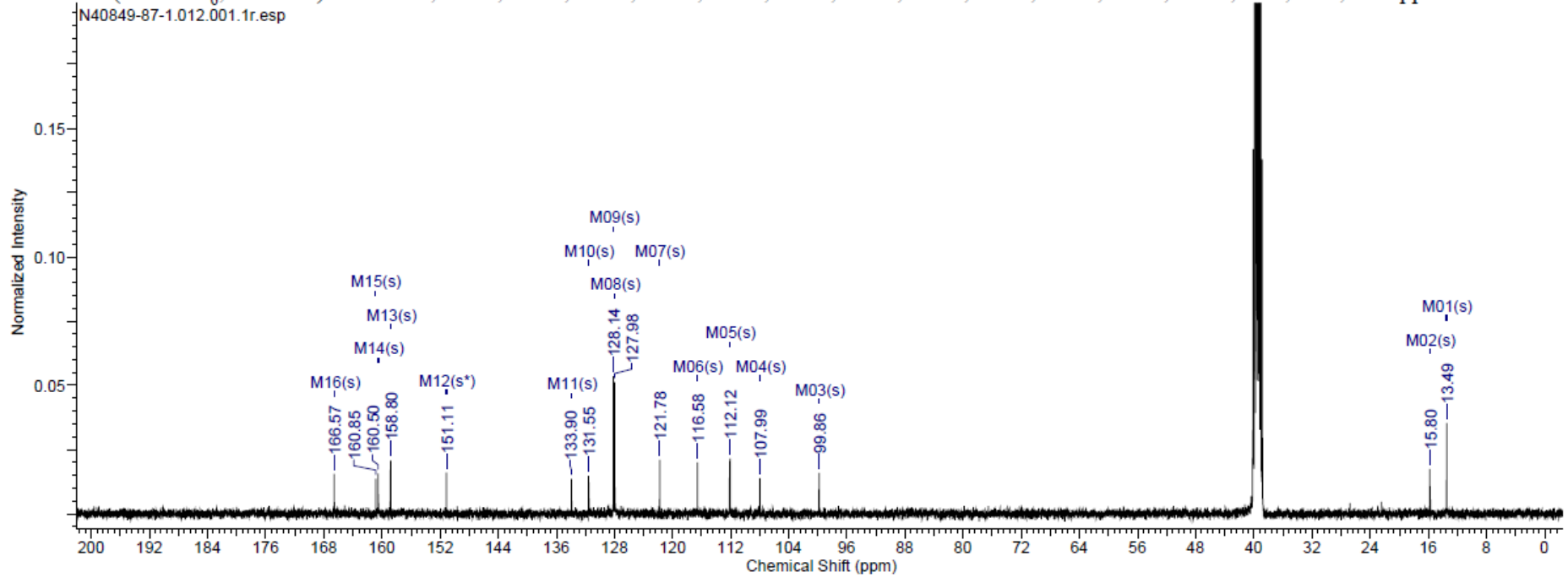
***N*-(8-ethyl-4,7-dihydroxy-2-oxo-2H-chromen-3-yl)benzamide**



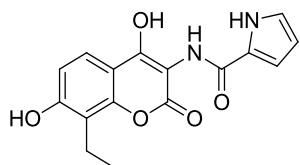
^1H NMR (DMSO- d_6 , 400MHz): δ = 11.72 (br. s., 1H), 10.43 (s, 1H), 9.41 (s, 1H), 8.01 (d, $J=7.1$ Hz, 2H), 7.56-7.66 (m, 2H), 7.47-7.56 (m, 2H), 6.90 (d, $J=8.7$ Hz, 1H), 2.74 (q, $J=7.3$ Hz, 2H), 1.12 ppm (t, $J=7.4$ Hz, 3H)



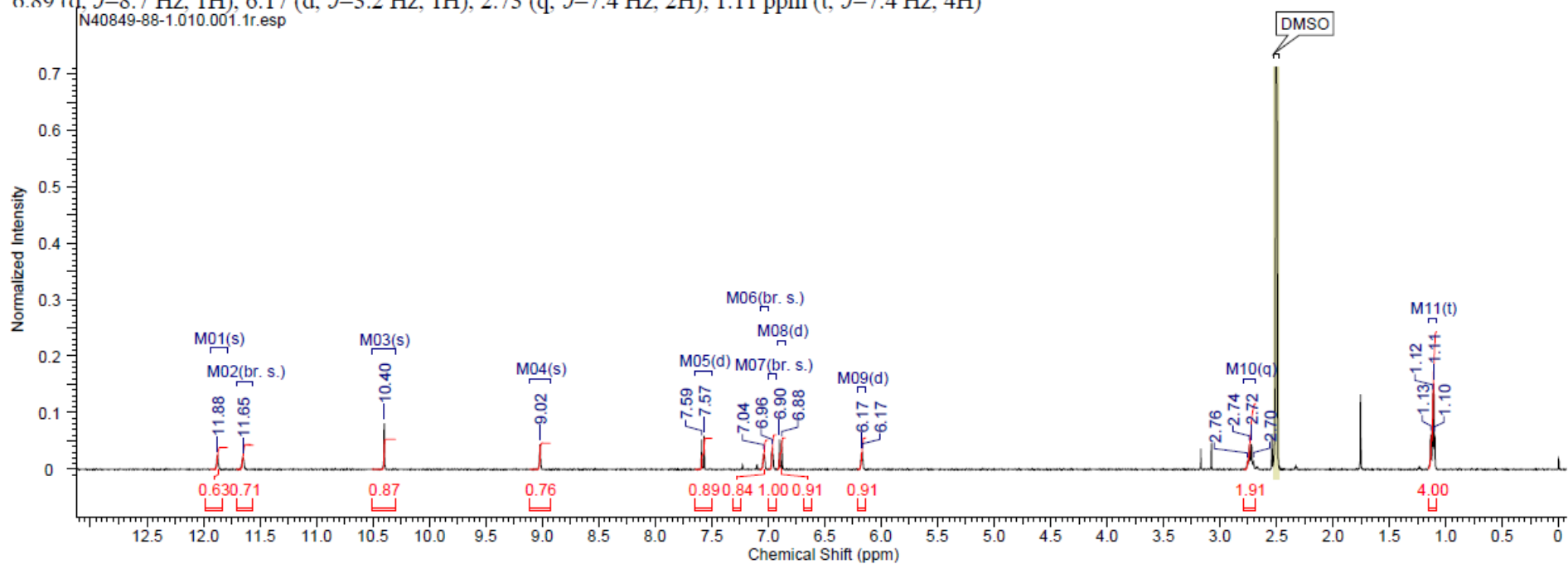
¹³C NMR (DMSO-d₆, 101MHz): δ = 166.6, 160.9, 160.5, 158.8, 151.1, 133.9, 131.5, 128.1, 128.0, 121.8, 116.6, 112.1, 108.0, 99.9, 15.8, 13.5 ppm



N-(8-ethyl-4,7-dihydroxy-2-oxo-2H-chromen-3-yl)-1H-pyrrole-2-carboxamide



^1H NMR (DMSO- d_6 , 400MHz): δ = 11.88 (s, 1H), 11.65 (br. s., 1H), 10.40 (s, 1H), 9.02 (s, 1H), 7.58 (d, $J=8.6$ Hz, 1H), 7.04 (br. s., 1H), 6.96 (br. s., 1H), 6.89 (d, $J=8.7$ Hz, 1H), 6.17 (d, $J=3.2$ Hz, 1H), 2.73 (q, $J=7.4$ Hz, 2H), 1.11 ppm (t, $J=7.4$ Hz, 4H)



^{13}C NMR (DMSO- d_6 , 101MHz): $\delta = 160.9, 160.8, 159.6, 158.7, 150.9, 125.4, 122.4, 121.7, 116.5, 112.3, 112.1, 109.0, 100.1, 15.8, 13.5$ ppm

