Optimisation studies for the synthesis of coumarin photocages with biomedical applications.

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Objective:

To study and optimise synthetic routes to make coumarin based photocages and photocleavable linkers in order to facilitate the sequential delivery of growth factors into stem cells for the treatment of tissue degeneration.

Introduction

Regenerative medicine, the idea that, under certain conditions, tissue can regenerate itself has been around for thousands of years. There is evidence of the Ancient Greeks knowing the liver could spontaneously rebuild itself as is portrayed in the Legend of the Titan god Prometheus and of ancient Egyptians performing skin grafting and other types of xenotransplantation. (Kohlhauser *et al.*)

Finding accessible technologies to tailor and repurpose cells to our liking is essential to treat severe tissue damage or tissue degeneration, so designing systems to grow cells to build custom tissues or even full organs would be ground-breaking.

In this project a chemical approach for a system to direct *in vitro* cell growth has been studied. Its basis lies in the fact that a cell's destiny depends on specific proteins called growth factors, as well as the time at which they are fed to the cell colony and at what concentrations.

Stem cells would be embedded into a hydrogel (a polymeric network holding water in its pores) which would act as a 3D scaffold into which the cells and tissues are free to grow. In order to 'feed' the growth factors into the hydrogel, we aimed to customise the material to make it light-responsive. This way, it would be possible to control what goes 'in' and 'out' of the gel by changing the light irradiated on it **(Figure 1a and 1b)**. This can be achieved by using a series of molecules called photocages which act as a lid that block the entry of growth factors and can be 'opened' and 'closed' with light.

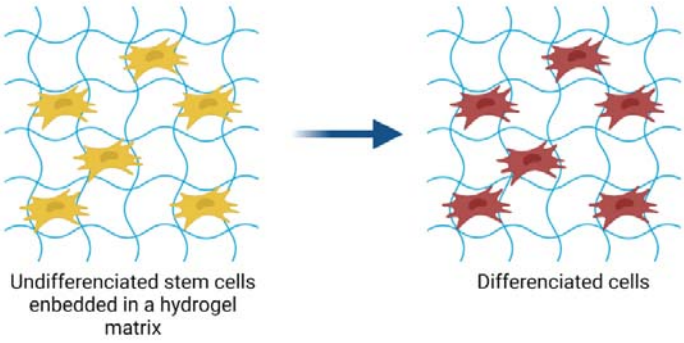


Figure 1a. Stem cells embedded in the hydrogel matrix before and after irradiating with the selected wavelengths of light.

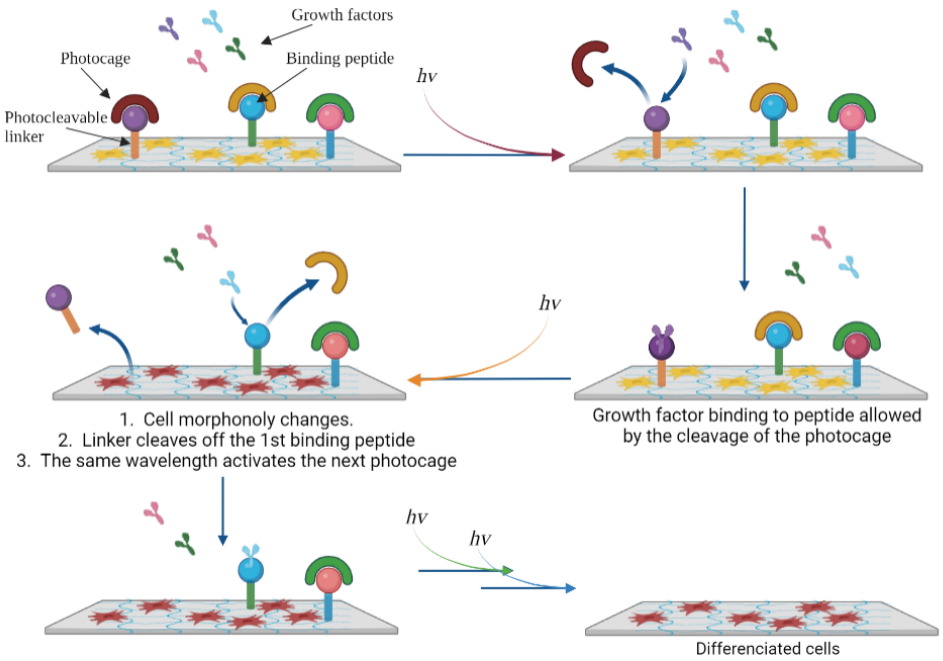


Figure 1b. Process mimicking biological cascades by which irradiating with a wavelength of light aids the binding of a growth factor to a peptide resulting in a change of the cell morphology

Work has been done previously by Broguiere *et al.* to design several photocages and apply them in this way, irradiating the hydrogel system with light and releasing different growth factors at different times. (Broguiere *et al.*)

The aim of this project is to optimise this technology and design a cascade effect by which a growth factor being fed into the hydrogel triggers the release of the next one, mimicking complex biological cascades without constantly feeding the cells. This can be achieved by designing a photocleavable linker (which holds the photocage to the hydrogel) that can be cleaved (closing the lid) with the same wavelength as the one required to activate, or open the lid of the next photocage, allowing the exclusive release of the latter growth factor in the sequence, and so on.

Two molecules were chosen for this study, these being ASM008 (as the photocage) and ASM010 (as a photocleavable linker) (**Figure 2)**. They were chosen due to their responsiveness to light

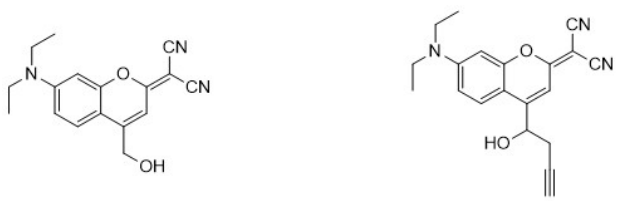
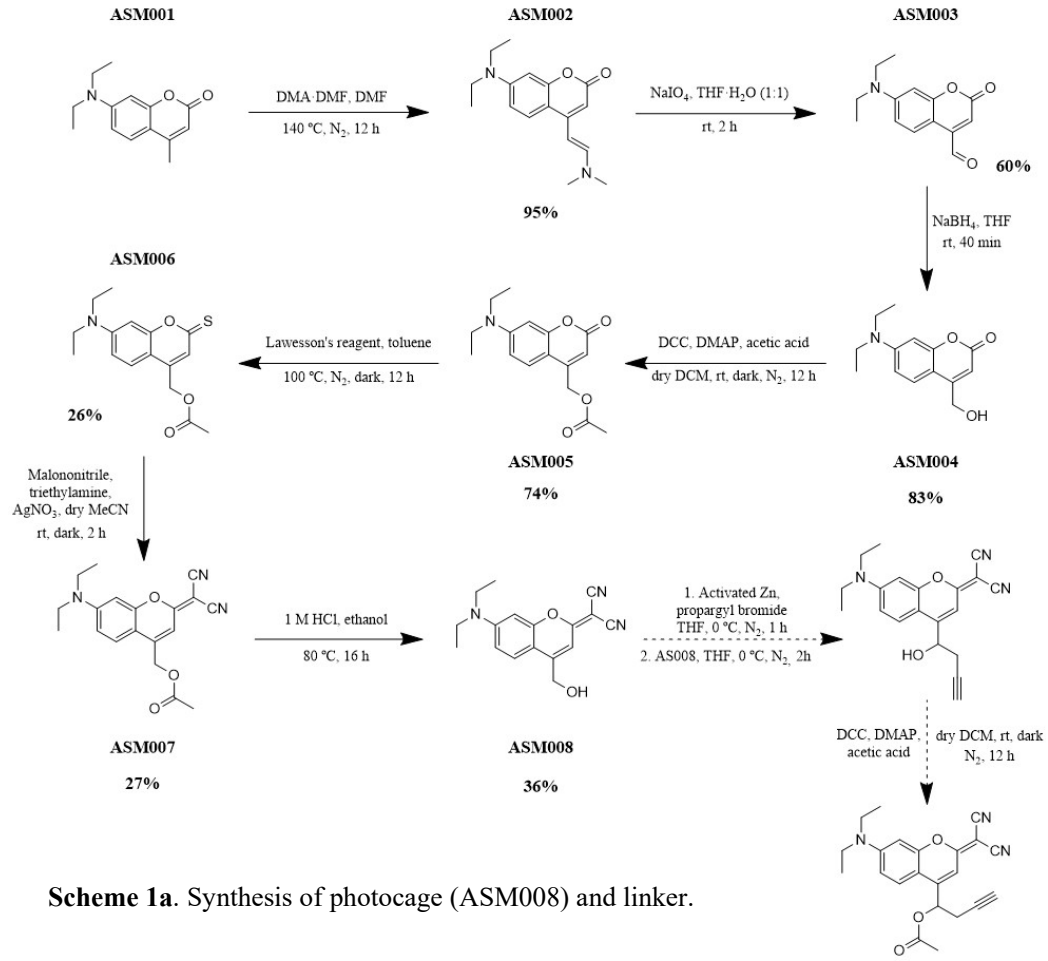


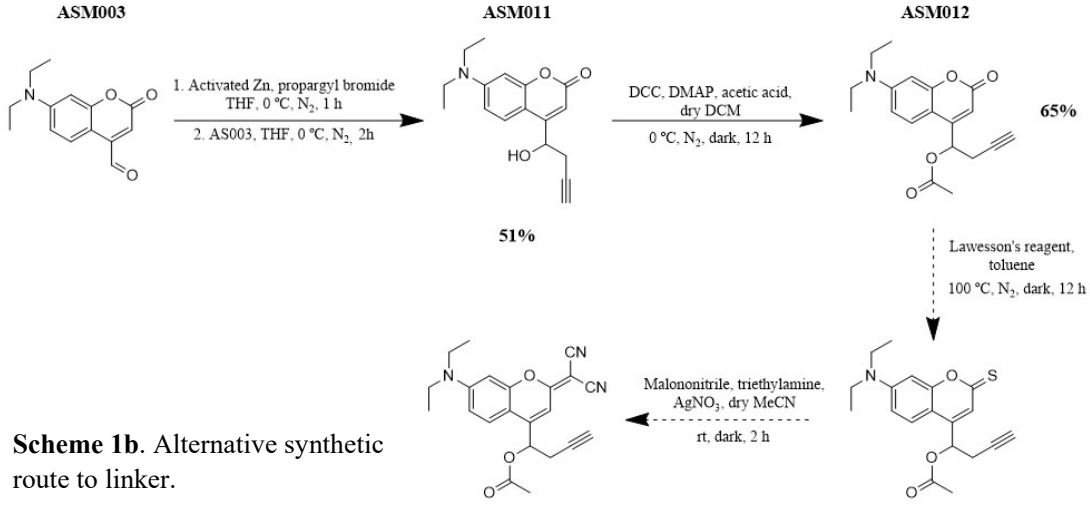
Figure 2. Target molecules of this study

In order to test whether these compounds were photocleavable, they were protected with acetate groups so uncaging experiments could be performed. The synthetic routes followed are presented below in **Schemes 1 and 2**.

Selected synthesis routes:

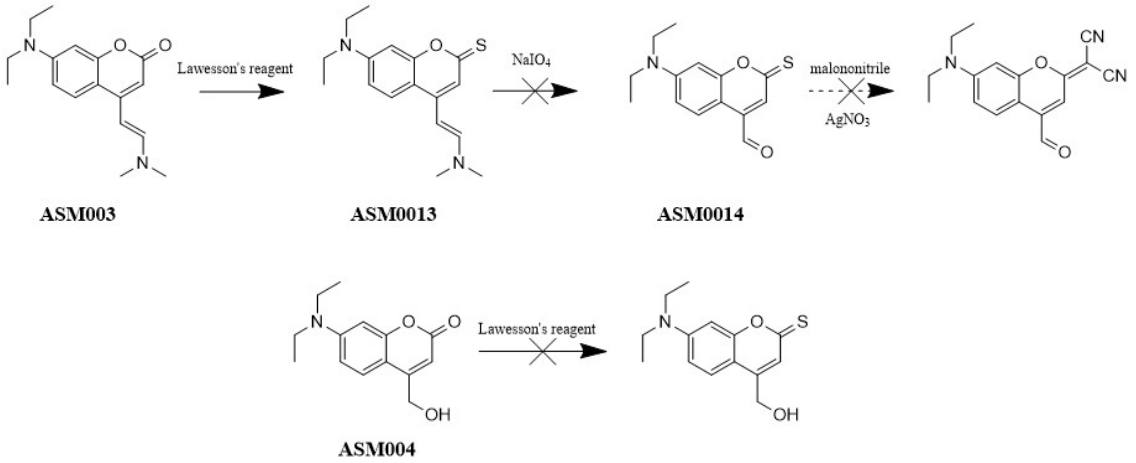


Scheme 1a. Synthesis of photocage (ASM008) and linker.



Scheme 1b. Alternative synthetic route to linker.

Unsuccessful synthetic routes



Scheme 2. Synthetic routes that were unsuccessful.

Materials and Methods:

General considerations:

Thin layer chromatography (TLC) was carried out using aluminium backed sheets coated with 60 F254 silica gel (Merck). Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254, 302, or 366 nm). **Flash column chromatography** was carried out using Geduran Si 60 (40-63 μm) (Merck). Mobile phases are reported as ratios of more polar solvent to less polar solvent. **Basic silica gel** was obtained by making a 'slurry' (*c.a.* 500 mL) with silica gel in DCM and triethylamine (*c.a.* 1 mL), which was then evaporated under reduced pressure until dry.

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR respectively) spectra were recorded on a Jeol ECX-400 (400 MHz) or Bruker AVIIIHD (500 MHz) spectrometer. All chemical shifts are quoted on the δ scale in ppm using residual solvent as the internal standard (^{1}H NMR: $CDCl_3$ = 7.26; MeOD = 3.31; D_2O = 4.69; DMSO-d6 = 2.50 and ^{13}C NMR: $CDCl_3$ = 77.16, MeOD = 49.00, DMSO-d6 = 39.52). Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent, br = broad. **High resolution electrospray ionisation (ESI) mass spectra (HRMS)** were recorded on a Bruker Compact TOF-MS or a Jeol AccuTOF GCx-plus spectrometer. Nominal and exact m/z values are reported in Daltons (Da).

Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrofluorophotometer in a glass fluorescence cuvette with a path length of 1 cm, a sampling interval of 1 nm, and excitation and emission slit widths of 5 nm. **Absorption maxima (υ_{max})** are reported in wavenumbers (cm^{-1}). **UV-Vis spectra** were recorded on a Shimadzu UV-1800 UV spectrophotometer in a glass cuvette, using a 480/30 nm excitation filter and a 580/10 nm emission filter, a pathlength of 1 cm, and a sampling interval of 1 nm.

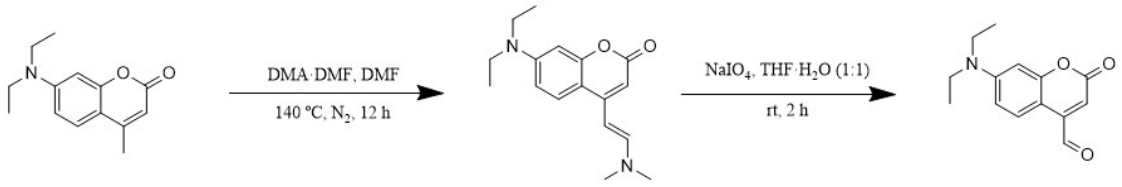
Melting points (m.p.) were recorded on a Gallenkamp melting point apparatus. **Infrared (IR) spectra** were recorded on a Perkin Elmer UATR Two FT-IR spectrometer.

Peptides were synthesised using CEM Liberty Lite Automated Microwave Peptide Synthesiser.

Anhydrous solvents were dried over a PureSolv MD 7 Solvent Purification System. Deionized water was used for chemical reactions. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reagents were purchased from **Sigma-Aldrich** and used as supplied, unless otherwise stated. Brine refers to a saturated solution of sodium chloride. Petrol refers to the fraction of petroleum ether boiling in the range 40-60 °C.

Experimental:

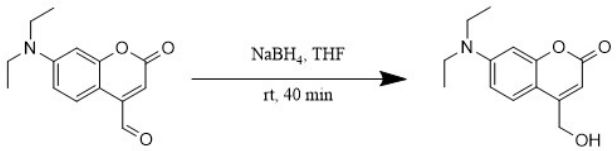
Formation of ASM002 followed by ASM003



7-diethylamino-4-methyl-2*H*-chromen-2-one (10 g, 43 mmol, 1 equiv.) was dissolved in a minimum amount of dry DMF (*c.a.* 15 mL). Then, DMA·DMF (8.6 mL, 64 mmol, 1.5 equiv.) was added to give a dark red/brown solution. The mixture was heated to 140 ºC for 22 h under a nitrogen atmosphere. Once the reaction was complete, the reaction mixture was allowed to cool to room temperature, filtered and washed with acetone (2 x 25 mL). The product was dried with $MgSO_4$, filtered, and evaporated under reduced pressure to obtain a brown solid (10 g, 40.8 mmol, 95%).

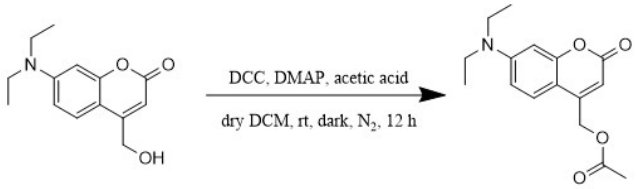
ASM002 (5 g, 17 mmol, 3 equiv.) was dissolved in a mixture of THF·H_2O (500 mL 1:1). Sodium periodate (11.4 g, 52 mmol, 1 equiv.) was added to the solution and the reaction mixture was stirred for 2h. The mixture was filtered under vacuum and the aqueous washed with ethyl acetate (150 mL). The organic layer was washed with saturated sodium bicarbonate solution (300 mL, dried with $MgSO_4$, filtered and evaporated under pressure to obtain a dark black/red oily solid. The residue was purified using flash column chromatography, eluting with 20% ethyl acetate:petrol. Pure fractions were concentrated under reduced pressure to give a crystalline red solid (2.99 g, 12 mmol, 60%). **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.34 (d, *J* = 8.8 Hz, 1H), 7.06 (t, *J* = 1.1 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 6.68 – 6.64 (m, 1H), 5.18 (d, *J* = 1.2 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 5H), 2.19 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 7H).

Formation of ASM004



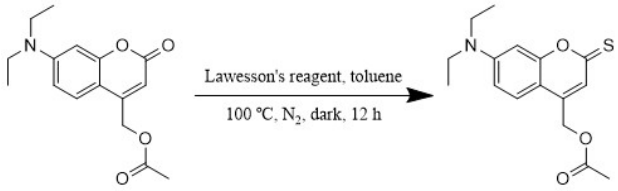
ASM003 (2.99 g, 11.8 mmol, 1equiv) was dissolved in THF (*c.a.* 40 mL). Then, $NaBH_4$ (0.94 g, 24.8 mmol, 2.1 equiv.) was added to the solution and the reaction mixture was stirred for 40 minutes. The reaction was quenched with saturated solution of sodium bicarbonate (150 mL). The organic layer was separated with ethyl acetate (2 x 200 mL), then dried with $MgSO_4$, filtered and evaporated under reduced pressure to obtain a yellow solid (2.43 g, 9.83 mmol, 83.3 %). **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.31 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 9.0 Hz, 1H), 6.59 (s, 1H), 6.16 (t, *J* = 1.3 Hz, 1H), 5.22 (d, *J* = 1.3 Hz, 3H), 3.42 (q, *J* = 7.1 Hz, 6H), 2.22 (d, *J* = 2.8 Hz, 1H), 2.19 (s, 4H), 2.17 (s, 12H), 1.22 (t, *J* = 7.1 Hz, 8H).

Formation of ASM005



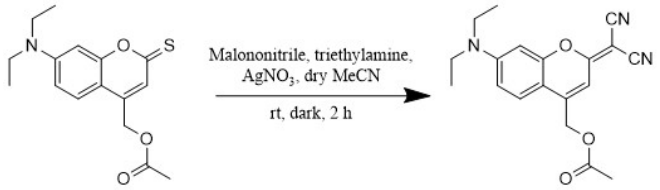
ASM004 (2.43g, 9.83 mmol, 1.18 equiv.), DMAP (1.02 g, 8.35 mmol, 1 equiv.) and acetic acid (555 µL, 1 equiv.) was dissolved in dry DCM (130 mL). The mixture was stirred under a nitrogen atmosphere for 10 minutes. Then, DCC (2.00 g, 9.69 mmol, 1 equiv.) was added and the mixture was stirred for at room temperature for 12 h in the dark and under N_2. The reaction yielded a yellow solid precipitate which was filtered off. The organic layer was extracted from the remaining solution with 1 M HCl and washed with saturated sodium bicarbonate solution. The combined organics were dried with MgSO4, filtered and evaporated under reduced pressure to give yellow needle-like crystals. (2.1 g, 7.26 mmol, 73.9 %). **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.30 (d, *J* = 8.9 Hz, 1H), 6.65 (d, *J* = 8.9 Hz, 1H), 6.56 (s, 1H), 6.14 (t, *J* = 1.3 Hz, 1H), 5.21 (d, *J* = 1.3 Hz, 2H), 3.41 (q, *J* = 7.1 Hz, 4H), 2.23 – 2.13 (m, 4H), 1.20 (t, *J* = 7.1 Hz, 6H).

Formation of ASM006



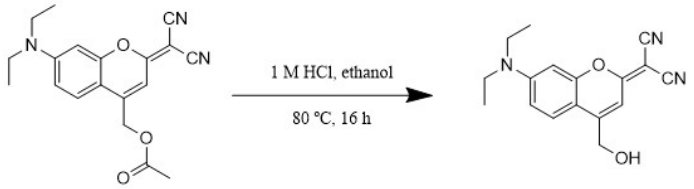
ASM005 (0.51 g, 1.76 mmol, 1.9 equiv.) was dissolved in toluene (12 mL). Then, Lawesson's reagent (0.37 g, 0.91 mmol, 1 equiv.) was added to the solution. The reaction mixture was heated to 100 ºC for 12 h in the dark. Upon completion of the reaction, the aqueous layer was extracted and washed with ethyl acetate. The combined organic layers were dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was purified using column chromatography on basic silica gel, eluting with DCM. Pure fractions were concentrated to yield yellow needle-like crystals (0.14 g, 0.46 mmol, 26%).

Formation of ASM007



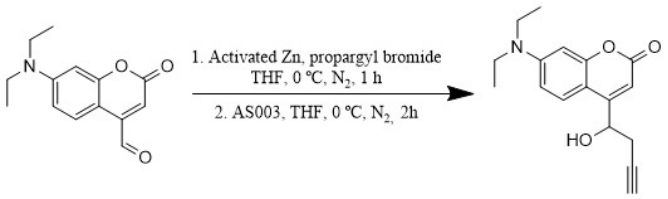
ASM006 (1.92 g, 6.29 mmol, 1 equiv.) was dissolved in dry MeCN (70 mL) and purged with N_2 for 10 min. At the same time and in a second flask, dissolve malononitrile (0.64 g, 9.69 mmol, 1.5 equiv.) and triethylamine (2.54 mL) in dry MeCN (25 mL) and purge in the same way. Add the contents of the first flask to the second flask, taking care to perform the transfer under N_2. The mixture was stirred at room temperature for 2 h in the dark. Then, a solution of $AgNO_3$ (2.36 g, 13.9 mmol, 2.2 equiv.) in a minimum amount of dry MeCN (*c.a.* 15 mL) was purged with nitrogen and added to the reaction mixture which was stirred at room temperature and in the dark for a further 2 hr. Upon completion of the reaction, the aqueous layer was extracted with ethyl acetate and the organic layer was washed with saturated sodium bicarbonate solution. The organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product (1.02 g) was purified using column chromatography on basic silica gel, eluting with DCM and then moving on to 1% methanol in DCM. The pure fractions were concentrated to yield a dark brown solid (0.58 g, 1.72 mmol, 27%). **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.28 (d, *J* = 9.0 Hz, 1H), 6.58 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.52 (d, *J* = 2.6 Hz, 1H), 6.14 (t, *J* = 1.3 Hz, 1H), 5.22 (d, *J* = 1.3 Hz, 1H), 3.60 (s, 1H), 3.43 (dq, *J* = 14.2, 7.1 Hz, 3H), 2.20 (d, *J* = 8.5 Hz, 2H), 1.42 (t, *J* = 7.3 Hz, 0H), 1.21 (t, *J* = 7.1 Hz, 3H).

Formation of ASM008



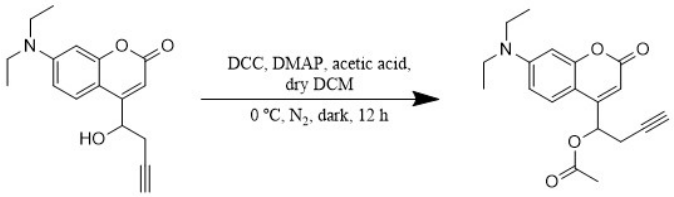
ASM007 (0.16 g, 0.47 mmol, 1 equiv.) was dissolved in ethanol (100 mL). 1 M HCl (1 mL, 1 mmol, 2.1 equiv.) was added and the reaction mixture was heated to 80 ºC for 16 h. The product was extracted with 150 mL ethyl acetate and washed with brine (150 mL). The organic layer was dried with $MgSO_4$, filtered and evaporated under reduced pressure to obtain a dark orange/brown solid (50 mg, 0.17mmol, 36%) **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.34 (dd, *J* = 9.1, 3.6 Hz, 1H), 7.26 (s, 1H), 6.90 (d, *J* = 1.3 Hz, 1H), 6.73 (d, *J* = 1.2 Hz, 0H), 6.66 (dt, *J* = 9.1, 3.0 Hz, 1H), 6.58 (dd, *J* = 4.9, 2.5 Hz, 1H), 5.24 (d, *J* = 1.2 Hz, 1H), 4.88 (d, *J* = 1.3 Hz, 1H), 3.45 (qd, *J* = 7.1, 2.0 Hz, 5H), 2.21 (s, 1H), 1.23 (td, *J* = 7.1, 1.5 Hz, 7H).

Formation of ASM011



Zinc powder (5.24 g, 80 mmol, 20 equiv.) was activated by washing with: 1M HCl (100 mL), H2O (3x100 mL) and 2 x (EtOH (100 mL) then EtOEt (100 mL)). The activated Zn was suspended in THF (25 mL) and purged with N_2. Propargyl bromide (3.9 mL, 34 mmol, 8.5 equiv.) was added dropwise and the mixture was stirred at 0 ºC under N2 for 1 h. Then, ASM003 (1.03 g, 4.2 mmol, 1 equiv.) was dissolved in THF (10 mL) and purged with N_2. It was then added into the reaction mixture which was stirred at 0 ºC, under N_2 for a further 2 h. The reaction was quenched with saturated NH_4Cl solution (200 mL). The product was extracted with ethyl acetate (200 mL) and the aqueous layer was washed with further ethyl acetate (100 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting brown oil was purified with column chromatography, using silica gel and eluting with 100% DCM followed by 10% methanol in DCM. The pure fractions were evaporated under pressure to yield a yellow/orange oil (700 mg, 2.14 mmol, 51%) **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.39 (d, *J* = 9.1 Hz, 1H), 6.57 (dd, *J* = 9.1, 2.7 Hz, 1H), 6.54 – 6.47 (m, 2H), 6.30 (d, *J* = 1.0 Hz, 1H), 3.79 – 3.71 (m, 1H), 3.47 – 3.33 (m, 9H), 2.37 – 2.31 (m, 1H), 2.17 (t, *J* = 2.6 Hz, 1H), 1.89 – 1.80 (m, 2H), 1.26 – 1.14 (m, 13H).

Formation of ASM012



ASM011 (0.35 g, 1.42 mmol, 1 equiv.), DMAP (0.173 g, 1.42 mmol, 1 equiv.) and acetic acid (96.7 µL) were dissolved in dry DCM (25mL). The mixture was stirred under a nitrogen atmosphere for 10 minutes. Then, DCC (0.2992 g, 1.42 mmol, 1 equiv.) was added to the solution which was stirred at 0 ºC under a nitrogen atmosphere and in the dark for 12 h. Then, the reaction mixture was filtered and extracted with 1M HCl (100 mL). The organic layer was washed with saturated sodium bicarbonate solution (100 mL), then dried with MgSO4, filtered and evaporated under reduced pressure to yield an orange/yellow oily solid (0.30 g, 0.92 mmol, 65%). **^{1}H NMR** (400 MHz, CHLOROFORM-D) δ 7.39 (d, *J* = 9.0 Hz, 1H), 6.56 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.48 (d, *J* = 2.6 Hz, 1H), 6.28 (d, *J* = 0.9 Hz, 1H), 5.12 (dd, *J* = 7.6, 4.4 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 1H), 3.39 (q, *J* = 7.1 Hz, 5H), 2.81 (ddd, *J* = 17.0, 4.3, 2.6 Hz, 1H), 2.63 (ddd, *J* = 17.0, 7.6, 2.6 Hz, 1H), 2.16 (s, 5H), 1.21 (dt, *J* = 21.9, 7.1 Hz, 9H). **HRMS:**

m/z (ESI+) calc. for C19H21NO4 $[M+H]^+$: 328.1578 ; Obs.: 328.1546 ; ν_{max}: **(FT-ATR)/cm-1** :3288.58, 2972.71, 2928.32, 1744.78, 1715.67, 1617.71, 1599.75, 1527.59, 1420.92, 1355.48, 1273.08, 1230.31, 1142.86, 1071.45, 826.60, 644.91; **m.p.:** 130-133 °C

One peptide (GAGSP) was synthesised in order to test its binding affinity and ability to allow the photocleaving of the compound. This study was not complete due to difficulties cleaving the peptide off the resin used to synthesise it.

Uncaging experiments:

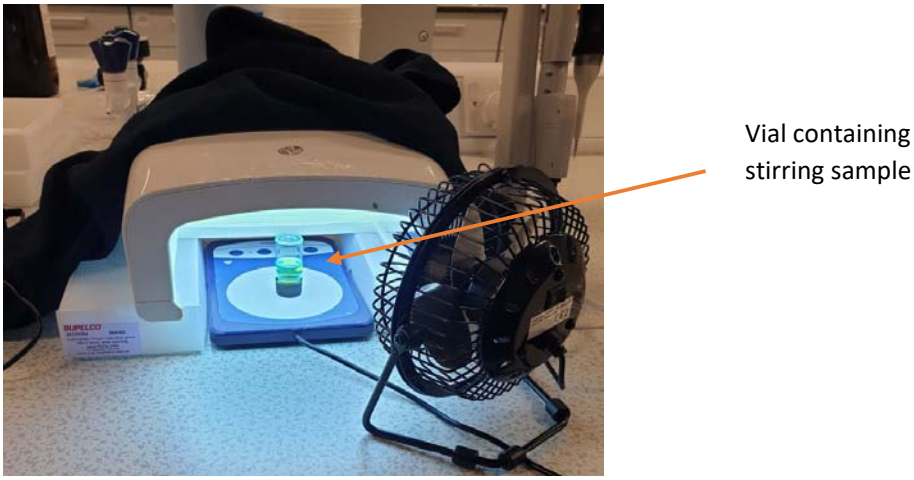
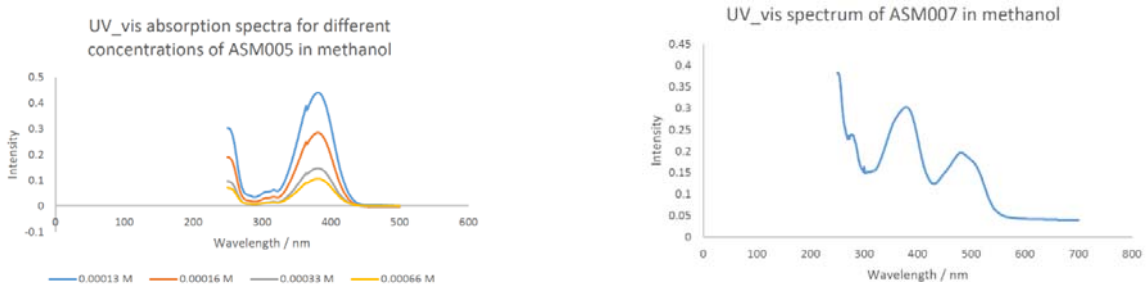


Figure 2. Set up for uncaging studies

The photophysical properties of two synthesised compounds (ASM005 and ASM007) were studied to prove that these compounds could be uncaged with light. The experiment (**Figure 2.**) involved irradiating 5 mg of the compound dissolved in methanol with the maximum absorption wavelength (~350 nm) which was determined with UV_vis spectroscopic studies. (**Graph 1a and 1b**)



Graph 1a (left) Shows the UV_vis absorption spectrum of ASM005 in methanol. **2b (right)** Shows the UV_vis absorption spectrum of ASM007.

Samples were taken from the stirring solution every 30 minutes for a total of 6 hours and these were analysed by LCMS.

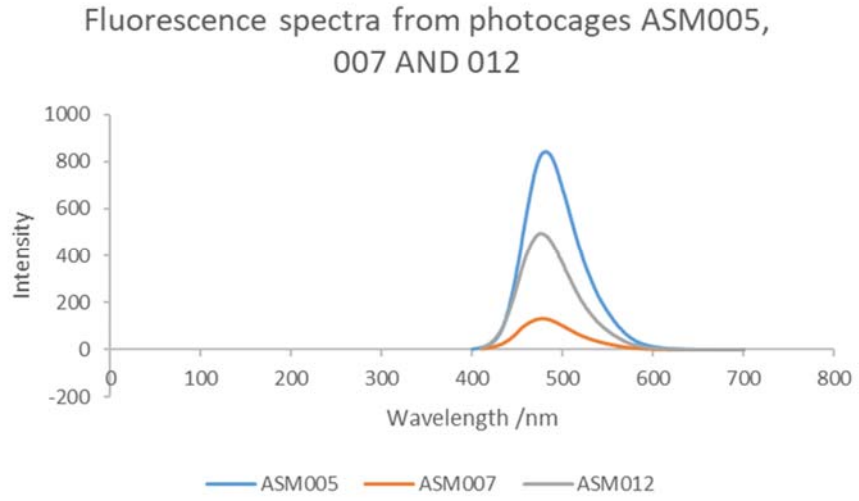
Results and Discussion

Four main synthetic routes were tested but only two gave acceptable yields **(Schemes 1a and 1b)**. The lowest yielding step involved the addition of malononitrile (ASM006 to ASM007). This could have been due to the presence of silver, which was extremely hard to separate from the rest of the reaction mixture as it stuck to the glassware, making flash column chromatography especially challenging.

Although the photocage (ASM008) was obtained, synthesis of the linker could not be achieved on time. Two syntheses are proposed for its formation **(Schemes 1a and 1b, dashed arrows)**. Remarkably, the second route involves less steps and leaves the low-yield malononitrile reaction at the end.

Synthesis routes were designed based on previous reports for similar molecules. However they had to be adjusted to add specific reaction conditions. Smaller scale reactions had to be done to test for the need of a nitrogen atmosphere, ideal temperature or duration of the reaction. The resulting mixtures were assessed for reaction completion and product purity with the use of thin-layer chromatography, mass spectrometry and ^{1}H NMR.

Fluorescence spectra was recorded for 3 compounds with the acetate protecting group (ASM005, ASM007 and ASM012). They all appear to fluoresce at the same wavelength range. This is expected as the extent of the resonance in all 3 molecules is very similar. (**Graph 2**)



Graph 2. Fluorescence spectra of ASM005, ASM007 and ASM012 in methanol.

LCMS results for ASM010 were inconclusive, although this could be due to the impurity of the product. It is likely that the sample contained silver from the previous reaction which would hinder the uncaging. Due to the short duration of this project, the compound was separated by flash column chromatography but the uncaging experiment could not be rerun on time. Nevertheless, LCMS results for the uncaging experiment of ASM005 showed clear signs of uncaging.

Crystal structures were obtained from ASM002, ASM005 and ASM006 but again, due to the short research period, the crystallographic data is currently being awaited upon.

Conclusions

- A novel photocage (ASM012) was synthesised and characterised.
- LCMS data for ASM005 showed proof of uncaging, meaning this family of compounds would be useful for this technology.
- Although the project was not completed in the research period, useful synthetic routes and conclusions can be drawn. For example, routes that involve malononitrile reactions earlier in the synthesis tend to have much lower yields.

Personal reflections

- This project has been a major milestone in my training as a chemist. Not only I have learnt essential hard skills that I will use in the rest of my career (such as the use of a range of purification and analytical techniques) but I have experienced the challenges of doing real, cutting-edge research. This includes organisational skills, interpersonal relationships and ethics, as well as tackling and overcoming unforeseen difficulties and designing alternatives.
- I have found it to be extremely important (in Chemistry) to process data as soon as it is obtained and draw hypotheses from them to either prove or disprove them at the end instead of waiting until all data is collected.

Future Work

Firstly, the synthetic route should be completed as shown with dashed lines in the synthetic schemes. A wider range of photocages should be synthesised and their photophysical properties should be obtained.

Uncaging experiments should be run for all final compounds and repeated at least 3 times to obtain concordant results.

Then, work should be done on peptide synthesis and conjugation of the sequence to a photocage. Uncaging studies should be performed on this to confirm the proof of concept studies previously shown in the results.

The finalised system consisting of photocage, binding peptide, photocleavable linker and hydrogel together with growth factors in solution should then be tested in vitro on live mammalian cells. Work has already been done by my mentor to prepare for these studies.

Acknowledgements

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References

(Figures 1a. and 1b). created with BioRender.com

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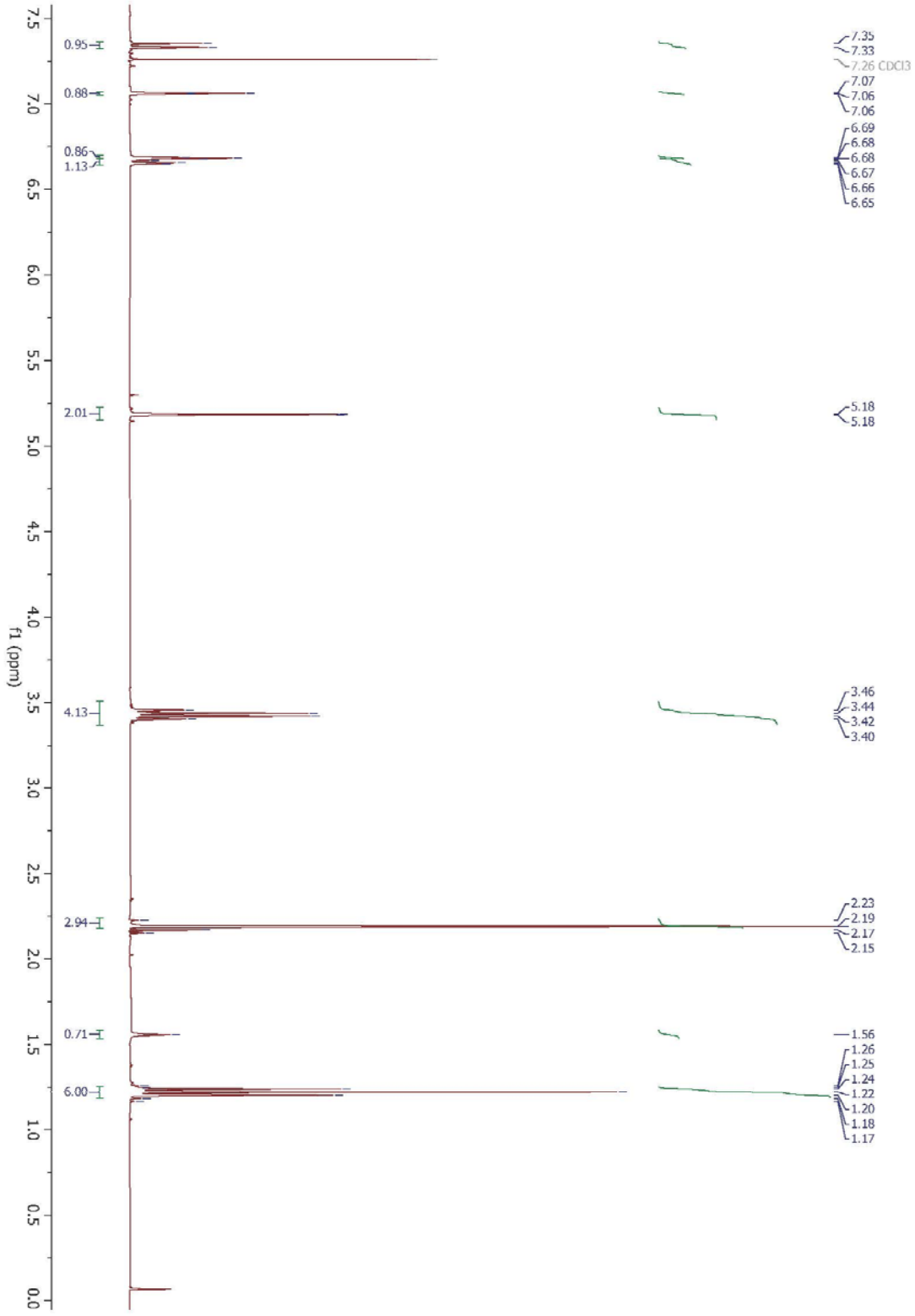
Kohlhauser, M., Luze, H., Nischwitz, S. P., & Kamolz, L. P. (2021). Historical Evolution of Skin Grafting-A Journey through Time. *Medicina (Kaunas, Lithuania)*, *57*(4), 348. https://doi.org/10.3390/medicina57040348

Supporting Information (see below)

ASM003


f1 (ppm)
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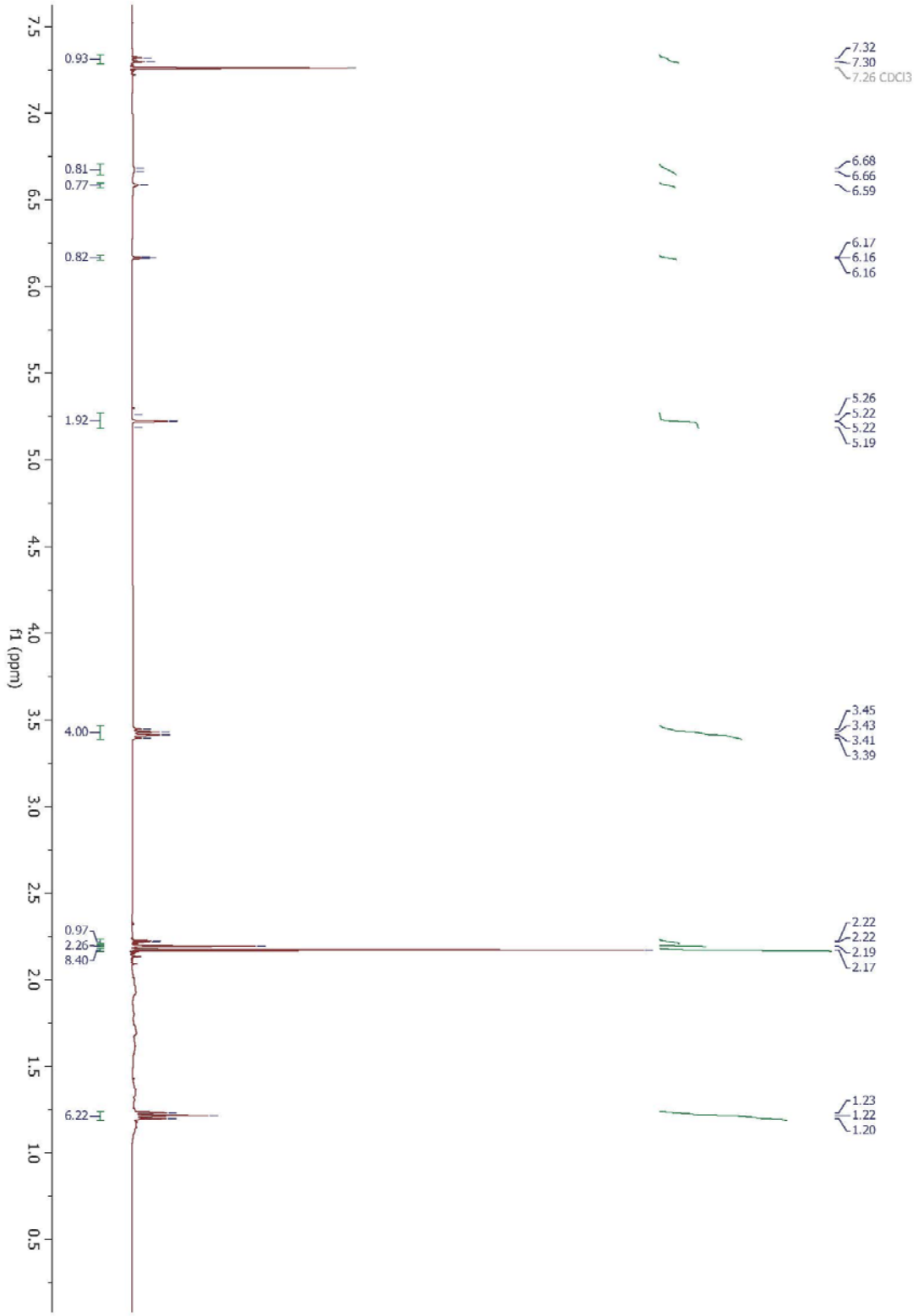
0.88
0.86
1.13
2.01
4.13
2.94
0.71
6.00
7.35
7.33
7.26 CDCl3
7.07
7.06
7.06
6.69
6.68
6.68
6.67
6.66
6.65
5.18
5.18
3.46
3.44
3.42
3.40
2.23
2.19
2.17
2.15
1.56
1.26
1.25
1.24
1.22
1.20
1.18
1.17

ASM004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5.5
5.0
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4.0
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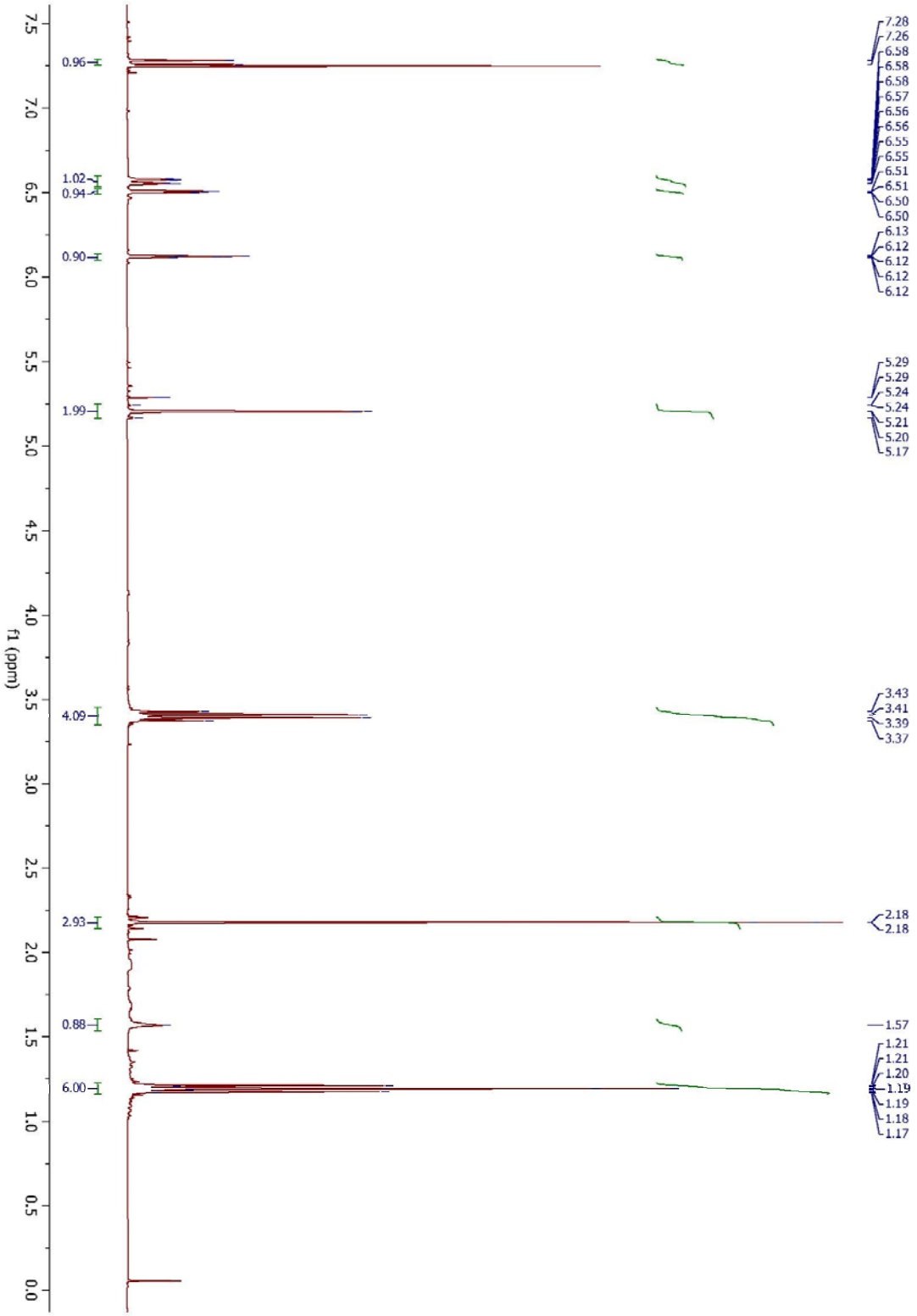
3.0
2.5
2.0
1.5
1.0
0.5
0.93
0.81
0.77
0.82
1.92
4.00
0.97
2.26
8.40
6.22
7.32
7.30
7.26 CDCl3
6.68
6.66
6.59
6.17
6.16
6.16
5.26
5.22
5.22
5.19
3.45
3.43
3.41
3.39
2.22
2.22
2.19
2.17
1.23
1.22
1.20

ASM005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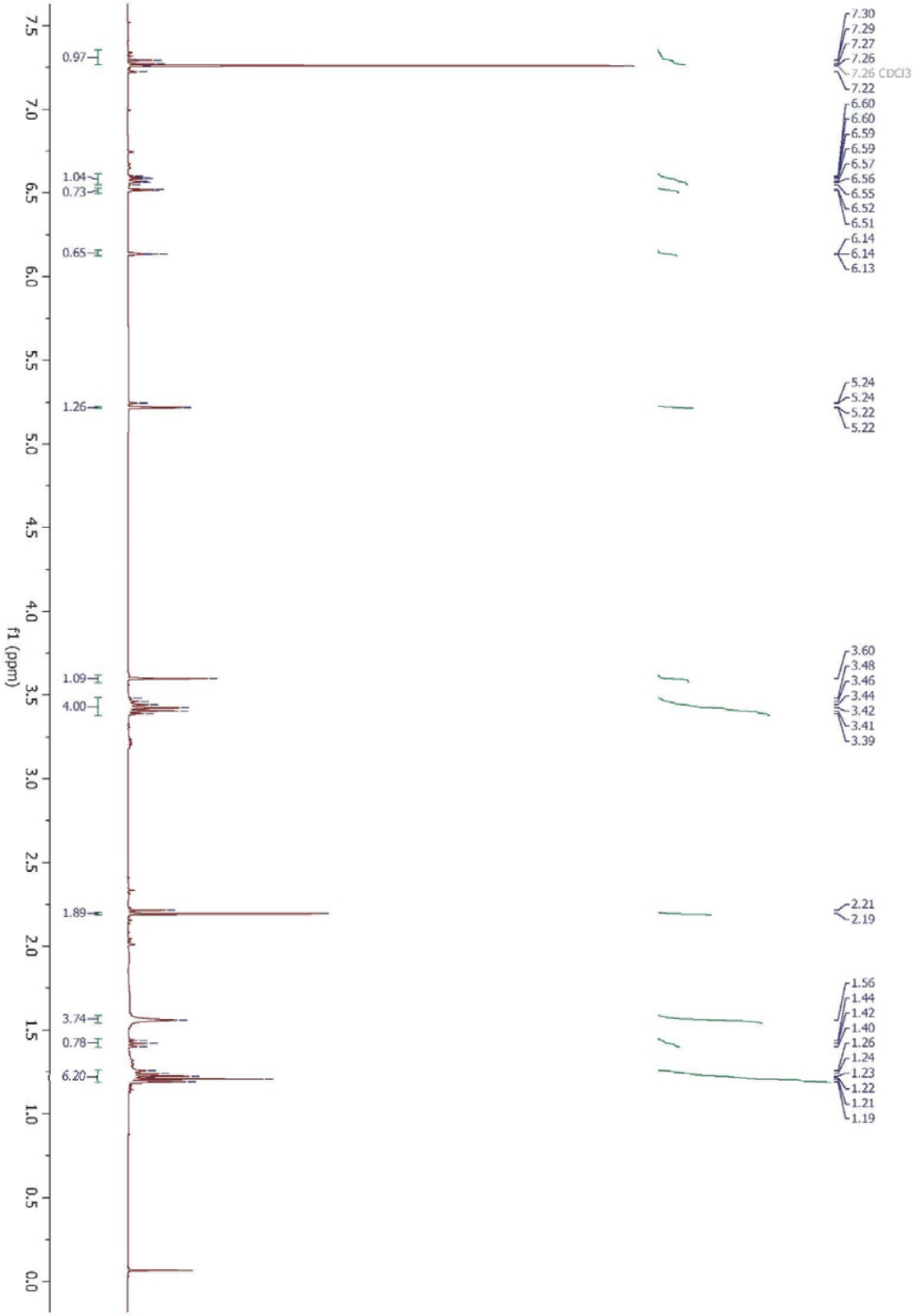
7.26
6.58
6.58
6.58
6.57
6.56
6.56
6.55
6.55
6.51
6.51
6.50
6.50
6.13
6.12
6.12
6.12
6.12
5.29
5.29
5.24
5.24
5.21
5.20
5.17
3.43
3.41
3.39
3.37
2.18
2.18
1.57
1.21
1.21
1.20
1.19
1.19
1.18
1.17

ASM007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1.5
1.0
0.5
0.0
0.97
1.04
0.73
0.65
1.26
1.09
4.00
1.89
3.74
0.78
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7.30
7.29
7.27
7.26
7.26 CDCl3
7.22
6.60
6.60
6.59
6.59
6.57
6.56
6.55
6.52
6.51
6.14
6.14
6.13
5.24
5.24
5.22
5.22
3.60
3.48
3.46
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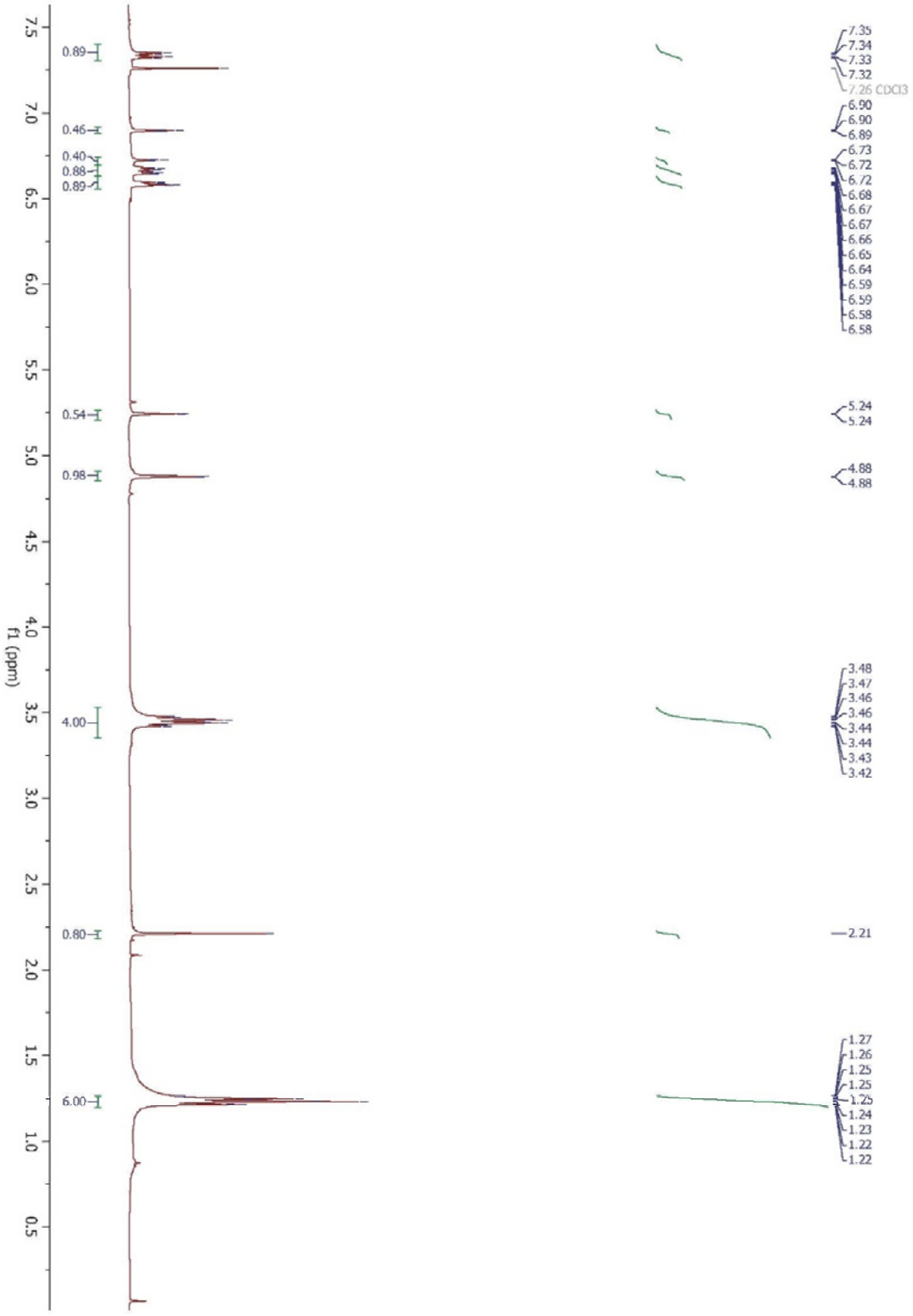
3.42
3.41
3.39
2.21
2.19
1.56
1.44
1.42
1.40
1.26
1.24
1.23
1.22
1.21
1.19

ASM007 - IR

Page 1

ASM008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7.33
7.32
7.26 CDCl3
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6.90
6.89
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6.72
6.72
6.68
6.67
6.67
6.66
6.65
6.64
6.59
6.59
6.58
6.58
5.24
5.24
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3.46
3.46
3.44
3.44
3.43
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1.26
1.25
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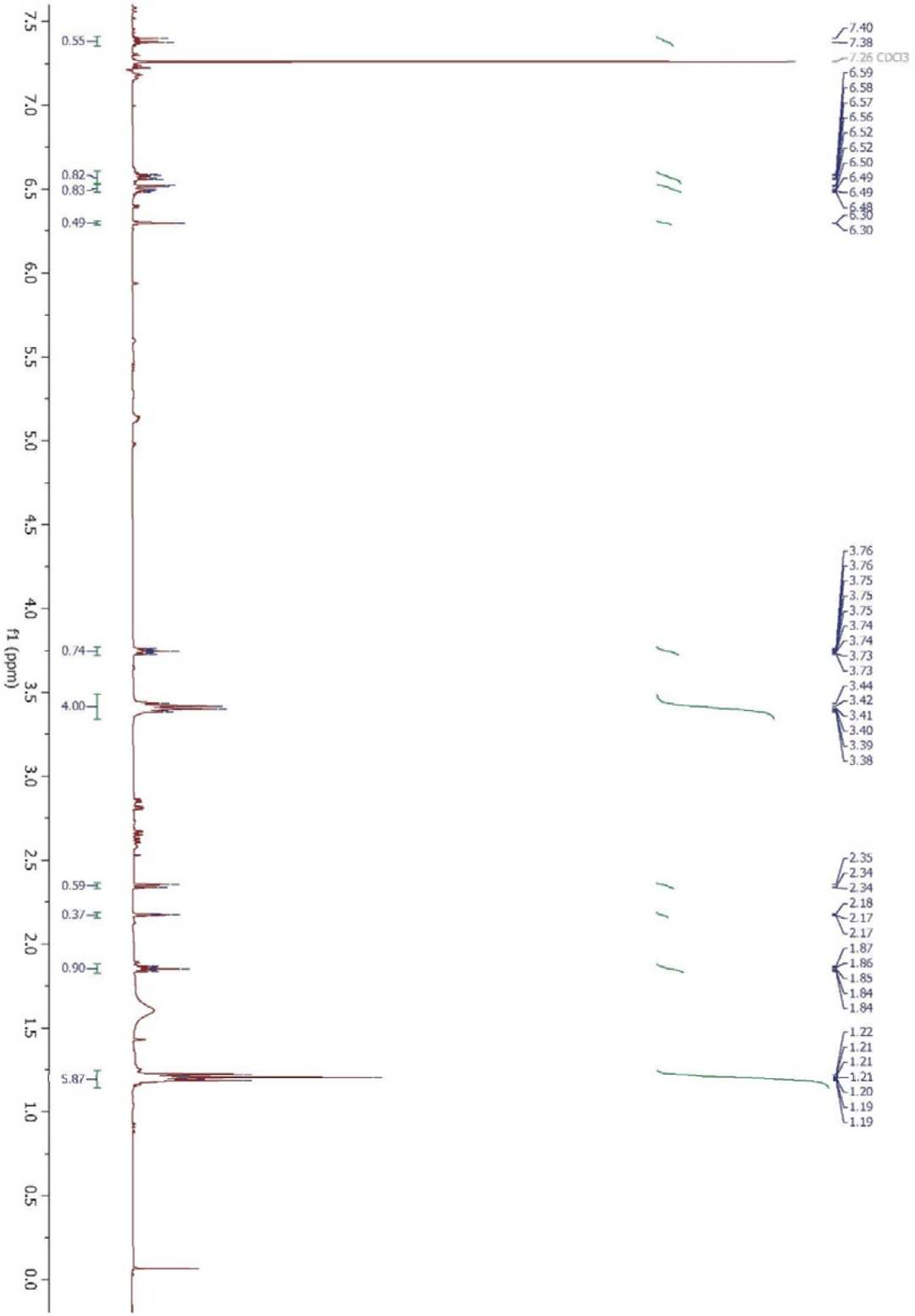
1.24
1.23
1.22
1.22

ASM011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7.38
7.26 CDCl3
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6.58
6.57
6.56
6.52
6.52
6.50
6.49
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3.76
3.75
3.75
3.75
3.74
3.74
3.73
3.73
3.44
3.42
3.41
3.40
3.39
3.38
2.35
2.34
2.34
2.18
2.17
2.17
1.87
1.86
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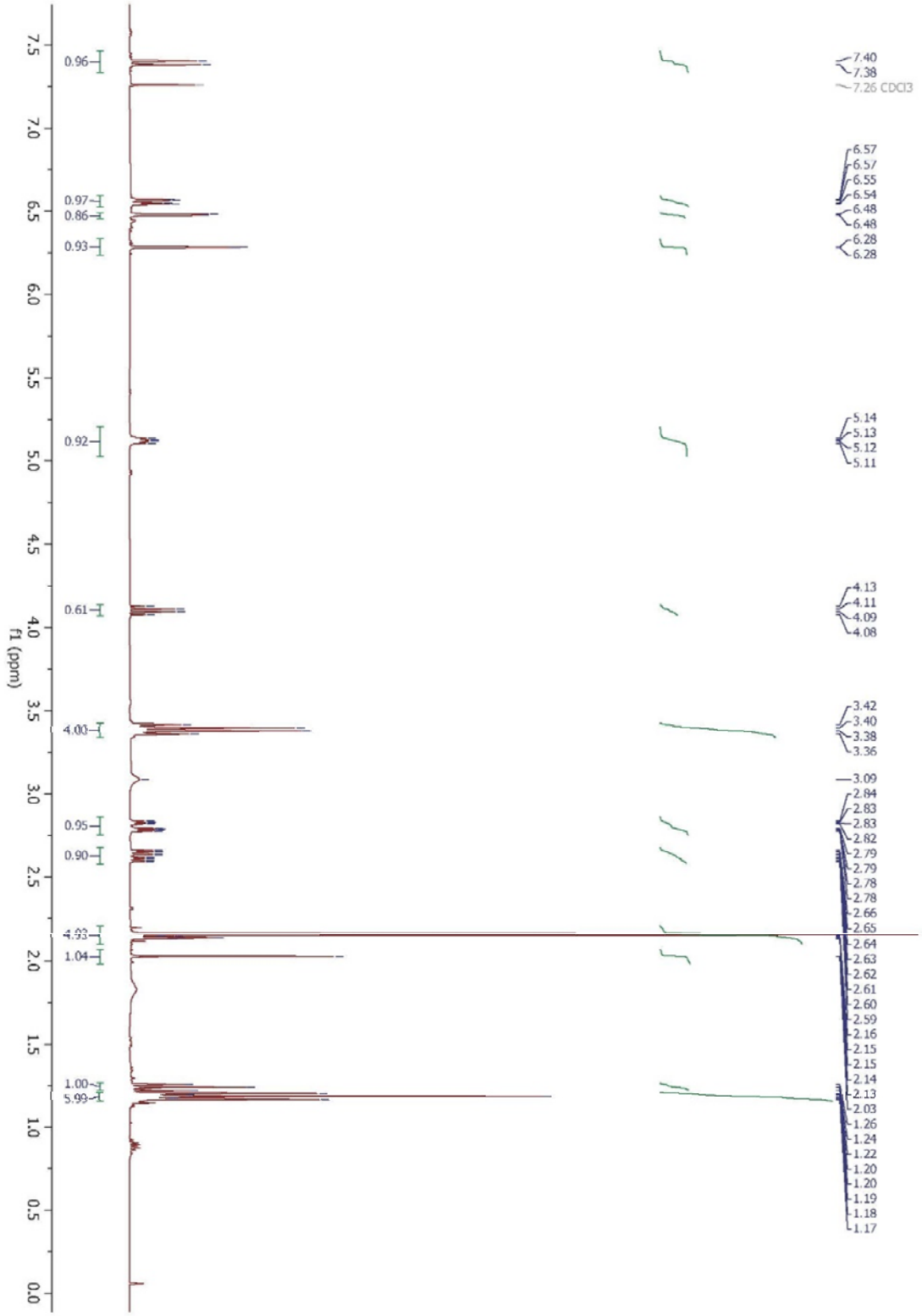
1.21
1.20
1.19
1.19

ASM012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4.93
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1.00
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7.40
7.38
7.26 CDCl3
6.57
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5.12
5.11
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4.11
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4.08
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1.18
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