# Stereo-Selective Total Synthesis of Calofolic Acid – A: Vasorelaxant Natural Product

A Thesis

submitted to

Indian Institute of Science Education and Research Pune in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

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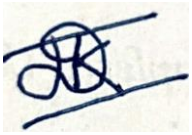
# Certificate

This is to certify that this dissertation entitled “Stereo-Selective Total Synthesis of Calofolic Acid – A: Vasorelaxant Natural Product” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Daksh K. Telang at CSIR - National Chemical Laboratory under the supervision of Dr. Ravindar Kontham, Principal Scientist, Division of Organic Chemistry.



Daksh K. Telang

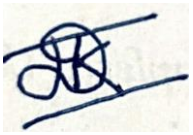
Committee:

Dr. Ravindar Kontham

This thesis is dedicated to my parents and my friends, who have constantly supported me.

# Declaration

I hereby declare that the matter embodied in the report entitled “Stereo-Selective Total Synthesis of Calofolic Acid – A: Vasorelaxant Natural Product” are the results of the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research (IISER) Pune, under the supervision of Dr. Ravindar Kontham and the same has not been submitted elsewhere for any other degree. Wherever others contribute, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussions.



Daksh K. Telang

Date: 15/03/2024

Dr. Ravindar Kontham

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# Abstract

Natural products are the molecules of nature; that is, they are molecules produced by living organisms. These molecules are synthesised by living organisms using the natural biosynthetic pathways. The total synthesis of such molecules using organic reactions has been a challenge since the 1900s.

Calofolic Acids A-F are a class of natural products extracted from the bark of *Calophyllum scriblitifolium*. These compounds are shown to have vasorelaxation activity on phenylephrine-precontracted rat aortic rings. Through experiments, the possible mechanism of activity of calofolic acid A was proposed. Calofolic acid A inhibits the PI3K – AKT – PDE pathway in the VSMCs. This leads to the activation of the cAMP – PKA – MYPT1 – MLC pathway as well as the cAMP – PKA – MYPT1 – HSP 20 pathway. The overall effect of this is the vasorelaxation in the smooth muscle cells in the rat aorta.

Herein, the efforts towards the total synthesis of the vasorelaxant natural product calofolic acid – A are described. Two different routes with different strategies are explored. The first route involved the synthesis of the chromanone core via alkene reduction which had some problems associated with it. However, the second route using a previously described work was successful and the total synthesis is nearing its conclusion.

# Acknowledgments

I would like to express my deepest appreciation to my guide, Dr. Ravindar Kontham, for his invaluable patience and feedback. Dr. Kontham supported me constantly throughout the whole project and gave me a number of suggestions whenever a problem was faced. I also express my gratitude to my expert member Prof. Srinivas Hotha, who gave me very valuable feedback and advice throughout my master's thesis tenure.

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This journey would have been impossible without the constant support of my loving parents who have been by my side throughout. I am also grateful to my friend, Sakshi Kharche who has been a constant source of inspiration for me. I also thank my friends at IISER with whom I have been a part of many sports, like Black Dog F.C. (Vachan SJ, Kumar Ashutosh, Utkarsh Gupta, Namasi, Pradyun Hebbar, Netra, Vaishak Bhat, Arya N, Parijat Banerjee, Subham, Abhisek, Neehar Verma, Shashank Soni, Abhradeep Sarkar, Rakshit Rohan), the IISM TT team (Paritosh Malik, Dhruv Satra, Ameya Mane, Kautik Rehani, Ramya Narayanan, Namrata Arvind, Mukta Londhe) and the IISM football team (Anish Mulchandani, Sanchit Nayak, Moksh Juneja, Ramandeep Singh, Shahin, Siddhesh Sundar, Yuv Agarwal, Varun Mallya).

# Chapter 1 : Introduction

## 1.1 Natural Products

Natural products are the molecules of nature; that is, they are molecules produced by living organisms. These molecules are generally found in cells, tissues, and secretions as metabolites or by-products from biotic organisms such as microorganisms, plants, and animals. The natural biosynthetic pathways occurring in such organisms can lead to the formation of many complex natural products[1].

Natural products have immense use in human life, and they have been exploited by humans for thousands of years. For instance, aspirin and salicylic acid (a metabolite of aspirin), which are the most widely recognised and commonly used medicines globally, originate naturally from salicin, a glycoside found in various species of the plant genera *Salix* and *Populus*[1]. As such, sometimes natural products may have pharmacological activity making them useful as therapeutic agents. Some natural products can also be modified synthetically to improve their potency and therapeutic benefit and, therefore, also act as a starting point for the discovery of drugs[2].

As a result, natural products as a research topic have garnered a lot of academic attention, by both biologists and chemists. They are typically extracted and isolated in quantities adequate for chemical structure elucidation, derivatisation/degradation chemistry, biological testing, and other research purposes[3]. The complex structures of some natural products provide an opportunity for synthetic chemists to discover a synthetic route towards making the natural product in a laboratory setting using basic starting materials, hence leading to the improvement of organic synthesis. Also, understanding the possible biochemical pathway involved in the production of the molecule and deciphering its role in a biological setting helps in improving the understanding of small-molecule mechanisms in biology[4].

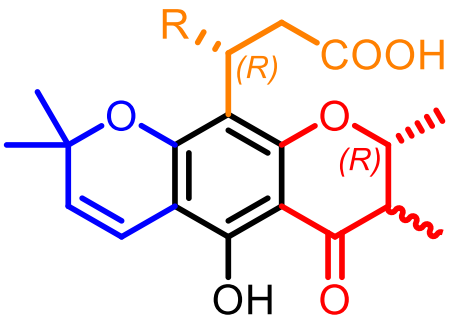
The total synthesis of natural products is a field of research which has been around for more than a century. The birth of this fascinating field of science is marked by the synthesis of urea in 1828 by Friedrich Wöhler. It is regarded as being both a precise science and a fine art and has driven the field of organic synthesis to a greater

improvement. Total synthesis as a field also has a huge impact from the perspective of drug discovery, making it a very exciting field of research[5].

## 1.2 Calofolic Acid A-F: Vasorelaxant Natural Products

*Calophyllum* stands as the largest genus within the Calophyllaceae family, encompassing over 200 species primarily located in tropical regions[6]. Various reports of the plants in this genus exist, which claim that they contain xanthones, flavonoids, terpenoids, and chromanones[7,8].

*Calophyllum Scribilitifolium* is a species of the *Calophyllum* genus which is majorly found in Malaysia and Indonesia[6]. The methanol extracts of the bark of *C.Scribilitifolium* are found to have vasorelaxation activity on the phenylephrine-precontracted rat aortic rings. Using vasorelaxation activity as a guide, the extracts are further separated, and 6 chromanones were discovered; these are the Calofolic Acids A-F (**1**-**6**). Calofolic Acid A is the major constituent found in the extracts[9].




CA-A: R = Et; Me = α (**1**)
CA-B: R = Me; Me = α (**2**)
CA-C: R = Et; Me = β (**3**)
CA-D: R = *n*-Pr; Me = α (**4**)
CA-E: R = *n*-Pr; Me = β (**5**)
CA-F: R = *n*-Bu; Me = α (**6**)

**Figure 1**: Structures of Calofolic Acid A-F

The vasorelaxation activity of calofolic acids A-F are also tested on the rat aorta precontracted with phenylephrine. Dose-dependent vasorelaxation activity by the calofolic acids A-F is observed, as seen in Figure 2. The $IC_{50}$ values for the compounds **1** to **6** are 6.07 μM, 10.3 μM, 10.4 μM, 18.9 μM, 13.8 μM, and 12.1 μM, respectively[9].

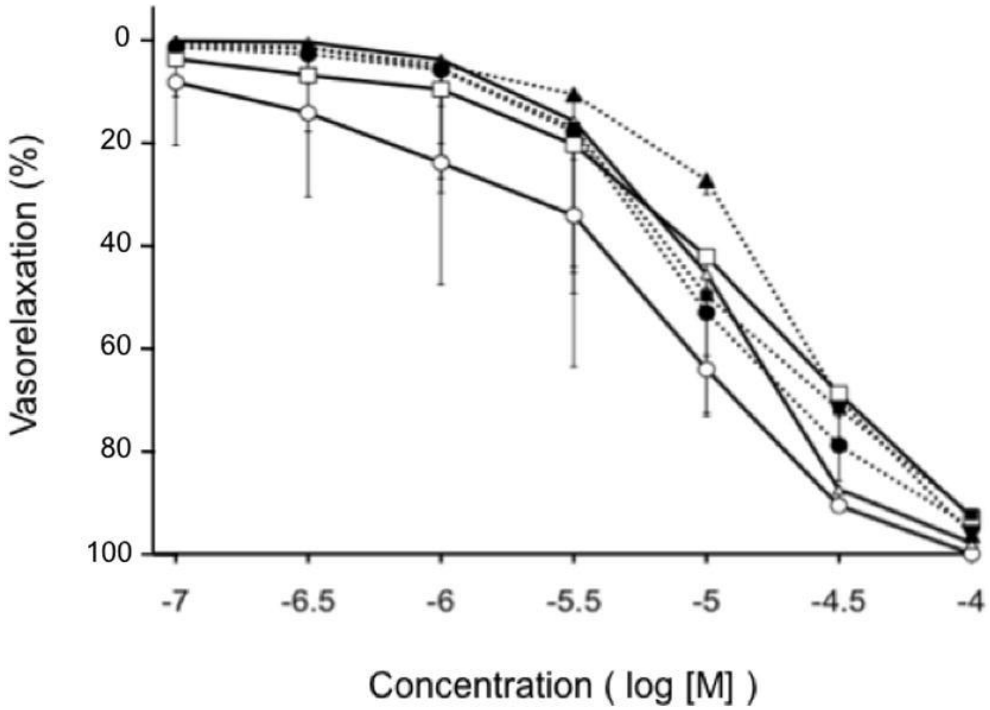



**Figure 2***:* Vasorelaxation activity of Calofolic Acids A-F
**1** - Open circle, **2** - Closed circle, **3** - Open triangle, **4** - Closed triangle, **5** - Open square, **6** - Closed square
(Image credits – Nugroho et al., 2017)

Kaneda et al. discovered the mechanism of vasorelaxation activity of Calofolic Acid – A and reported it in 2022. Calofolic Acid – A induces vasorelaxation on the phenylephrine-precontracted rat aortic rings by mainly acting on the rat vascular smooth muscle cells (VSMCs). Calofolic Acid – A directly inhibits the phosphatidylinositol 3-kinase (PI3K) enzyme activity in a dose-dependent manner. Inhibition of the PI3K enzyme leads to the suppression of the activation of the protein kinase B (AKT). This happens due to the decrease in the phosphorylation at the serine 473 residue of AKT (pAKT Ser473). A decrease in AKT activation, in turn, leads to the inhibition of the cAMP-phosphodiesterase (PDE). PDE is important for the degradation of cAMP into 5'-AMP. Inhibition of PDE leads to the reduction in the degradation levels of cAMP. This leads to the accumulation cAMP in the VSMCs. Increase in the levels of cAMP leads to the activation of cAMP-dependent protein kinase A (PKA). PKA plays a very vital role in vasoconstriction and vasorelaxation and is responsible for the phosphorylation of myosin phosphate targetting subunit 1 (MYPT1) at the serine 695 and the serine 668 residues (pMYPT1 Ser695 and pMYPT1 Ser668). This leads to the increase in the activity of MYPT1. MYPT1 is responsible for the regulation of myosin light chain (MLC) activity. It does so by dephosphorylating pMLC at the serine 19 (MLC Ser19) residue. Increase in the MLC

levels lead to vasorelaxation in the VSMCs. Increase in the PKA activity can also induce smooth muscle relaxation by increasing the phosphorylation in the actin cytoskeleton associated heat shock protein 20 (HSP20). Increase in the pHSP20 levels lead to the inhibition of vasocontraction by inhibiting the formation of pMLC, which leads to vasorelaxation in the VSMCs[10].

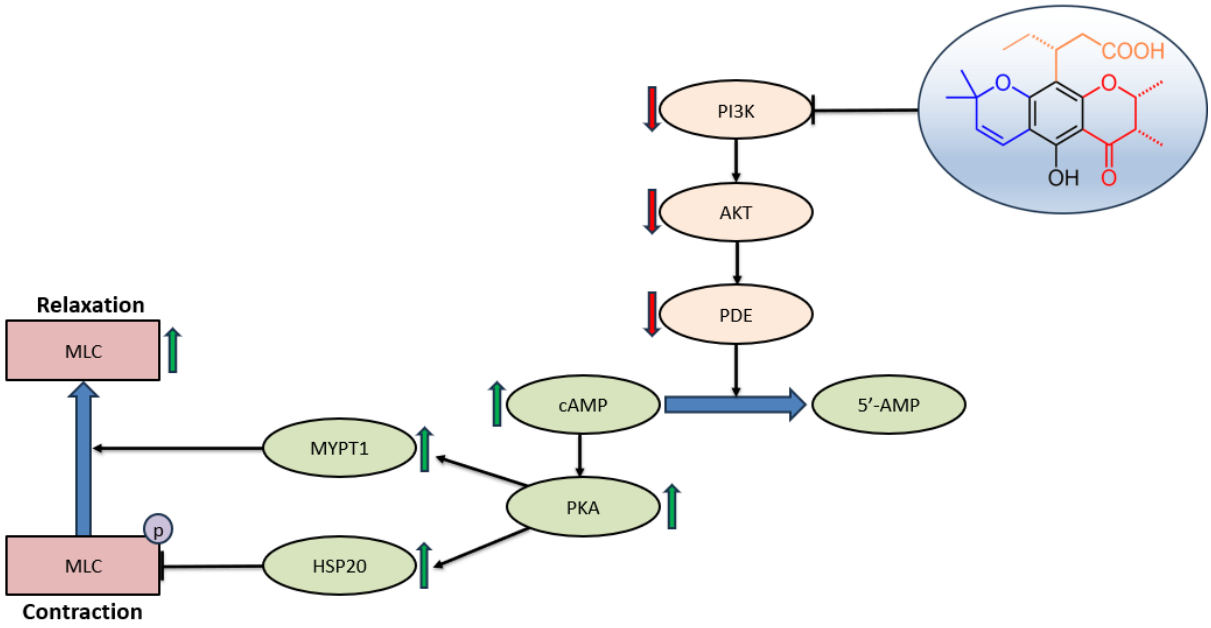



**Figure 3***:* Mechanism of vasorelaxation in the VSMCs by Calofolic Acid - A

## 1.3 Structural Features of Calofolic Acid – A

Calofolic acid – A belongs to the *calophyllum* chromanone class of natural products. It consists of a (2*R*,3*S*)-2,3-dimethyl chroman-4-one core (rings A and B). It also consists of the 2,2-dimethyl-2H-chromene core (rings B and C). The ring B is also attached to the pentenoic acid moiety (chain D) at the third position with a fixed stereochemistry of R.

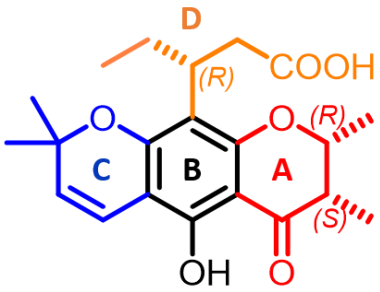



**Figure 4**: Structural features of Calofolic Acid - A

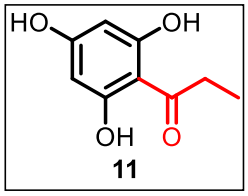
# Chapter 2 : Methods

## 2.1 Experimental Section

1) Synthesis of **1-(2,4,6-trihydroxyphenyl)propan-1-one (11)**:

The reported procedure by Tan et al., 2017 is followed[11].

$AlCl_3$ (8.46 g, 63.44 mmol) is slowly added to a solution of phloroglucinol (**12**) (2.00 g, 15.86 mmol) in $ClCH_2CH_2Cl$:$PhNO_2$ (100 mL) at 0°C. The solution is stirred at 0°C for 10 minutes, after which propionyl chloride (1.66 mL, 19.04 mmol) is added dropwise in an inert atmosphere. The mixture is now heated to 80°C and is stirred at this temperature for 12 hours. The reaction mixture is cooled to room temperature and the reaction is quenched with water (150 mL). Extraction is done using EtOAc (5 x 100 mL). The organic layer is washed with brine and concentrated in vacuo. The crude product is purified via column chromatography (25% EtOAc in Pet Ether) to yield **11** (1.92 g, 10.54 mmol, 66%).



**11**

**Appearance:** Brown solid

**TLC:** $R_f$ value = 0.4 (50% EtOAc in Pet Ether)

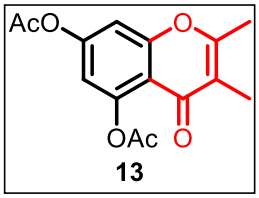
**$^1$H NMR:** (400 MHz, DMSO-$d_6$) δ 12.24 (s, 2H), 10.31 (s, 1H), 5.82 (s, 2H), 3.02 (q, $J$ = 7.2 Hz, 2H), 1.06 (t, $J$ = 7.2 Hz, 3H).

**$^{13}$C NMR:** (101 MHz, DMSO) δ 206.1, 164.9, 164.6, 164.6, 104.1, 95.1, 95.1, 36.8, 9.1.

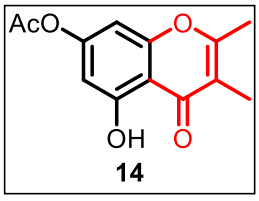
2) Synthesis of **2,3-dimethyl-4-oxo-4H-chromene-5,7-diyl diacetate (13)** and **5-hydroxy-2,3-dimethyl-4-oxo-4H-chromen-7-yl acetate (14)**:

The reported procedure by Rehder and Kepler, 1996 is followed[12].

To a solution of **11** (1.87 g, 10.26 mmol) in acetic anhydride (5.82 mL, 61.59 mmol), sodium acetate (757.86 mg, 9.24 mmol) is added. The solution is refluxed for 14 hours. The solution is then cooled to room temperature and diluted with water (5 mL). The reaction mixture is then extracted with $CH_2Cl_2$ (3 x 15 mL). The organic layer is then washed with water until the aqueous layer has a neutral pH. The organic



**13**



**14**

layer is then dried over $Na_2SO_4$, filtered and concentrated in a vacuum. The crude mixture is purified using column chromatography to afford diacetate **13** (1.61 g, 5.55 mmol, 54%) and monoacetate **14** (0.91 g, 3.67 mmol, 36%).

**13**:

**Appearance**: White solid

**TLC**: $R_f$ value = 0.5 (50% EtOAc in Pet ether)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 7.35 (d, $J$ = 2.2 Hz, 1H), 7.00 (d, $J$ = 2.2 Hz, 1H), 2.39 (s, 3H), 2.32 (s, 3H), 2.31 (s, 3H), 1.88 (s, 3H).

**14**:

**Appearance**: White solid

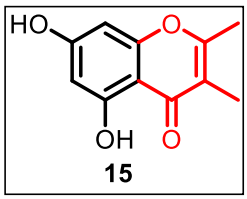
**TLC**: $R_f$ value = 0.7 (50% EtOAc in Pet ether)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 13.04 (s, 1H), 6.86 (d, $J$ = 1.9 Hz, 1H), 6.60 (d, $J$ = 2.0 Hz, 1H), 2.43 (s, 3H), 2.29 (s, 3H), 1.95 (s, 3H).

3) Synthesis of **5,7-dihydroxy-2,3-dimethyl-4H-chromen-4-one (15)**:

The reported procedure by Rehder and Kepler, 1996 is followed[12].

Products **13** and **14** (combined weight of 2.52 g) are taken together, and to it, a 1:1 mixture of saturated $NaHCO_3$ and MeOH (250 mL) is added. The reaction mixture is stirred for 20 hours at room temperature. The reaction mixture is then acidified with 3N HCl solution (50 mL) and the crude mixture is extracted with $CH_2Cl_2$. The next step involves washing the organic layer with brine, followed by drying over $Na_2SO_4$, filtering, and concentrating it under vacuum. The resulting crude mixture is then purified using column chromatography (20% EtOAc in Pet ether) to yield **15** (1.12 g, 5.43 mmol, 53%).



**15**

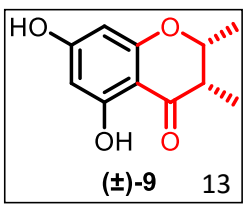
**Appearance:** White crystalline solid

**TLC:** $R_f$ value = 0.6 (50% EtOAc in Pet ether)

**$^1$H NMR**: (400 MHz, DMSO-$d_6$) δ 13.01 (s, 1H), 10.72 (s, 1H), 6.28 (s, 1H), 6.15 (s, 1H), 2.37 (s, 3H), 1.90 (s, 3H).

4) Synthesis of **(±)-5,7-dihydroxy-2,3-dimethylchroman-4-one ((±)-9)**:

A solution of **4** (300 mg, 1.45 mmol) in MeOH (6 mL) is loaded with 10%Pd/C (154.82 mg, 0.14 mmol) catalyst and carefully placed under 1.5 atm $H_2$ atmosphere in a Parr reactor. The mixture is stirred at



**(±)-9**

room temperature for 24 hours. The suspension is then filtered over a celite pad and the pad is washed with MeOH (15 mL). The solution is then concentrated under a vacuum. The crude product is then purified via column chromatography (15% EtOAc in Pet ether) to yield chromanone **(±)-9** (31.27 mg, 0.15 mmol, 10%).

**Appearance:** White solid

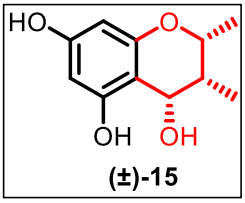
**TLC:** $R_f$ value = 0.5 (30% EtOAc in Pet ether)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 12.12 (s, 1H), 10.77 (s, 1H), 5.86 – 5.85 (m, 2H), 4.62 (qd, *J* = 6.5, 3.3 Hz, 1H), 2.62 (qd, *J* = 7.3, 3.3 Hz, 1H), 1.29 (d, *J* = 6.6 Hz, 3H), 1.05 (d, *J* = 7.3 Hz, 3H).

**$^{13}$C NMR** (101 MHz, DMSO) δ 200.8, 167.0, 164.3, 162.8, 100.7, 96.2, 95.2, 76.2, 43.7, 16.4, 9.8.

5) Attempted synthesis of **(±)-2,3-dimethylchromane-4,5,7-triol ((±)-15)**:

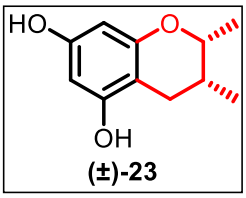
A solution of **(±)-9** (30 mg, 0.14 mmol) in $CH_2Cl_2$ (1 mL) in argon is cooled to -78°C. To this solution, K-selectride (1 M solution in THF, 0.18 mL, 0.18 mmol) is added dropwise, and the resulting solution is stirred for 2 hours at -78°C. Hydrogen peroxide (30% aqueous solution, 1 mL) and 3 N NaOH (1 mL) are added to the reaction mixture at the above temperature. The mixture is then allowed to reach room temperature and is stirred for 5 hours. The phases are then separated, and the aqueous phase is extracted with $CH_2Cl_2$ (4 x 10 mL). The combined organic phase is then washed with brine, dried over $Na_2SO_4$, filtered and concentrated under vacuum. Using TLC, it is observed that there is no conversion in the starting material. $^1$H NMR also confirmed the presence of only starting material.



**(±)-15**

6) Synthesis of **(±)-2,3-dimethylchromane-5,7-diol ((±)-23)**:

A solution of **13** (300 mg, 1.03 mmol) in MeOH (6 mL) is loaded with 10%Pd/C (109.99 mg, 0.10 mmol) catalyst and carefully placed under a 5 atm $H_2$ atmosphere in a Parr reactor. The mixture is stirred at room temperature for 24 hours. The suspension is then filtered over a celite pad and the pad is washed with MeOH (15 mL). The solution is then concentrated under a vacuum. The



**(±)-23**

crude product is then purified via column chromatography (20% EtOAc in Pet ether) to obtain the racemic **(±)-23** (155.53 mg, 0.80 mmol, 77%).

**Appearance:** Colourless syrup

**TLC:** $R_f$ value = 0.7 (50% EtOAc in Pet ether)

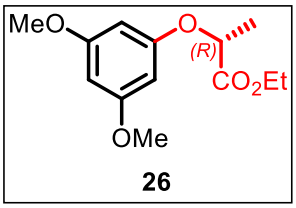
**$^1$H NMR:** (400 MHz, DMSO-*d*$_6$) δ 8.98 (s, 1H), 8.75 (s, 1H), 5.72 (d, *J* = 2.4 Hz, 1H), 5.50 (d, *J* = 2.3 Hz, 1H), 3.93 (qd, *J* = 6.5, 2.5 Hz, 1H), 2.38 (td, *J* = 3.6, 1.8 Hz, 1H), 2.05 (dd, *J* = 16.3, 5.2 Hz, 1H), 1.81 (qd, *J* = 5.7, 2.4 Hz, 1H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.70 (d, *J* = 6.9 Hz, 3H).

**$^{13}$C NMR:** (101 MHz, DMSO) δ 156.8, 156.6, 155.5, 99.4, 95.3, 94.6, 73.8, 29.8, 26.5, 16.9, 14.0.

7) Synthesis of **ethyl-*(R)*-2-(3,5-dimethoxyphenoxy)propanoate (26)**:

The reported procedure by Rama Rao et al., 1994 is followed[13].

A solution of 3,5-dimethoxyphenol (**27**) (3.00 g, 19.46 mmol), ethyl-L-lactate (2.68 mL, 23.35 mmol) and $PPh_3$ (7.66 g, 29.91 mmol) in THF (50 mL) is stirred and cooled to 0°C. To this mixture, diisopropyl azodicarboxylate (DIAD) (5.73 mL, 29.91 mmol) is added dropwise. This reaction mixture is then allowed to warm up to room temperature and is stirred for 36 hours.



**26**

The solvent is then evaporated under a vacuum and the resulting crude reaction mixture is purified via column chromatography (15% EtOAc in Pet ether) to afford compound **26** (4.18 g, 16.44 mmol, 84%).

**Appearance:** Colourless syrup

**TLC:** $R_f$ value = 0.6 (20% EtOAc in Pet ether)

**$^1$H NMR:** (400 MHz, Chloroform-*d*) δ 6.09 (t, *J* = 2.2 Hz, 1H), 6.06 (d, *J* = 2.2 Hz, 2H), 4.70 (q, *J* = 6.8 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.73 (s, 6H), 1.59 (d, *J* = 6.8 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).

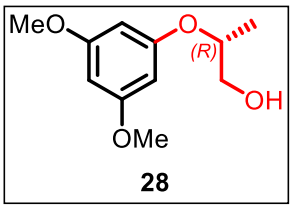
**$^{13}$C NMR:** (101 MHz, $CDCl_3$) δ 172.0, 161.5, 161.5, 159.4, 93.8, 93.8, 93.8, 72.4, 61.2, 55.3, 55.3, 18.4, 14.1.

8) Synthesis of ***(R)*-2-(3,5-dimethoxyphenoxy)propan-1-ol (28)**:

The reported procedure by Rama Rao et al., 1994 is followed[13].

Lithium aluminium hydride ($LiAlH_4$) (1.25 g, 32.88 mmol) is taken in a 2-neck round-bottom flask, and under an inert atmosphere, THF (25 mL) is added with

stirring. To this mixture, compound **26** (4.18 g, 16.44 mmol) dissolved in THF (25 mL) is added slowly at 0°C. The reaction mixture is warmed to room temperature and is allowed to stir for 2 hours. The reaction is then quenched with saturated *aq.* $NH_4Cl$ (50 mL) and extracted with EtOAc (3 x 60 mL). The organic layer is washed with brine, dried over $Na_2SO_4$, filtered and concentrated in a vacuum. The crude mixture is then purified by column chromatography (30% EtOAc in Pet ether) to obtain alcohol **28** (3.32 g, 15.64 mmol, 95%).



**28**

**Appearance:** Colourless syrup

**TLC:** $R_f$ value = 0.3 (30% EtOAc in Pet ether)

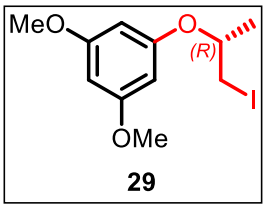
**$^1$H NMR:** (400 MHz, Chloroform-*d*) δ 6.11 (d, *J* = 2.1 Hz, 2H), 6.10 (d, *J* = 2.0 Hz, 1H), 4.45 (pd, *J* = 6.3, 3.7 Hz, 1H), 3.76 (s, 6H), 3.74 – 3.65 (m, 2H), 2.16 (s, 1H), 1.27 (d, *J* = 6.2 Hz, 3H).

**$^{13}$C NMR:** (101 MHz, $CDCl_3$) δ 161.6, 161.6, 159.6, 94.8, 94.8, 93.4, 74.8, 66.2, 55.3, 55.3, 15.8.

9) Synthesis of ***(R)*-1-((1-iodopropan-2-yl)oxy)-3,5-dimethoxybenzene (29)**:

The reported procedure by Rama Rao et al., 1994 is followed[13].

$PPh_3$ (5.67 g, 21.63 mmol) is taken in a round-bottom flask and $CH_2Cl_2$ (20mL) is added to it. $I_2$ crystals (5.49 g, 21.63 mmol) are added to the above mixture in small portions in inert atmosphere and the mixture is stirred for 10 minutes. Imidazole (2.45 g, 36.04 mmol) is then added to the mixture in small portions and stirred for 10 minutes. A solution of alcohol **28** (3.06 g, 14.42 mmol) in $CH_2Cl_2$ (20 mL) is added to the suspension dropwise and the reaction mixture is allowed to stir at room temperature for 4 hours. The reaction is then quenched with saturated *aq.* $Na_2S_2O_3$ (40 mL), and is extracted with $CH_2Cl_2$ (3 x 50 mL). The next step involves washing the organic layer with brine, followed by drying over $Na_2SO_4$, filtering, and concentrating it under vacuum. The resulting crude mixture is then purified using column chromatography (5% EtOAc in Pet ether) to yield the iodo-compound **29** (3.81 g, 11.83 mmol, 82%).



**29**

**Appearance:** Colourless syrup

**TLC:** $R_f$ value = 0.8 (30% EtOAc in Pet ether)

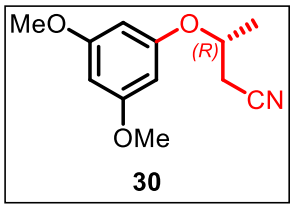
**$^{1}$H NMR:** (400 MHz, Chloroform-*d*) δ 6.02 – 6.00 (m, 1H), 5.99 (d, *J* = 2.2 Hz, 2H), 4.23 (pd, *J* = 6.1, 4.5 Hz, 1H), 3.66 (s, 6H), 3.27 (dd, *J* = 10.2, 4.6 Hz, 1H), 3.17 (dd, *J* = 10.2, 6.4 Hz, 1H), 1.35 (d, *J* = 6.1 Hz, 3H).

**$^{13}$C NMR:** (101 MHz, $CDCl_3$) δ 161.6, 161.6, 159.0, 94.9, 94.9, 93.7, 73.4, 55.4, 55.4, 20.2, 9.7.

10) Synthesis of ***(R)*-3-(3,5-dimethoxyphenoxy)butanenitrile (30)**:

The reported procedure by Rama Rao et al., 1994 is followed[13].

The iodo-compound **29** (2.27 g, 7.05 mmol) is dissolved in DMF (35 mL) and the mixture is kept under stirring. Potassium cyanide (KCN) (1.15 g, 17.62 mmol) is very carefully added to the reaction mixture, and it is stirred for 30 hours. The reaction is then quenched with 0.5 M HCl solution (35 mL). The aqueous layer is then extracted with EtOAc (3 x 40 mL). The next step involves washing the organic layer with ice-cold $H_2O$ and brine, followed by drying over $Na_2SO_4$, filtering, and concentrating it under vacuum. The resulting crude mixture is then purified using column chromatography (20% EtOAc in Pet ether) to afford cyanide **30** (1.26 g, 5.69 mmol, 81%).



**30**

**Appearance:** Colourless syrup

**TLC:** $R_f$ value = 0.5 (30% EtOAc in Pet ether)

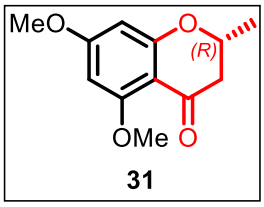
**$^{1}$H NMR:** (400 MHz, Chloroform-*d*) δ 6.12 (t, *J* = 2.2 Hz, 1H), 6.08 (d, *J* = 2.1 Hz, 2H), 4.59 (h, *J* = 5.9 Hz, 1H), 3.76 (s, 6H), 2.68 (d, *J* = 5.5 Hz, 2H), 1.48 (d, *J* = 6.1 Hz, 3H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 161.7, 161.7, 158.4, 116.8, 95.0, 95.0, 94.0, 69.3, 55.4, 55.4, 24.7, 19.5.

11) Synthesis of ***(R)*-5,7-dimethoxy-2-methylchroman-4-one (31)**:

The reported procedure by Rama Rao et al., 1994 is followed[13].

Compound **30** (2.05 g, 9.27 mmol) is dissolved in $Et_2O$ (50 mL) and the solution is kept under stirring. $ZnCl_2$ (2.15 g, 15.75 mmol) is added to this solution. In another two-necked round bottom flask, solid NaCl (100 g) is taken, and to this, concentrated $H_2SO_4$ (250 mL) is added dropwise with the help of an addition funnel. This leads to the generation of gaseous HCl, which is bubbled through the stirred



**31**

solution of **30**, $ZnCl_2$ and $Et_2O$ with the help of a dipping tube. This reaction is run for 2 hours with constant bubbling of gaseous HCl. An orangish-pink solid is formed. The $Et_2O$ is then evaporated in a vacuum and the solid is then dissolved in $H_2O$ (50 mL). The mixture is then refluxed for 2 hours under constant stirring. The solution is then cooled down to room temperature and diluted with $H_2O$ (20 mL). The aqueous layer is extracted with $CH_2Cl_2$ (3 x 50 mL). The organic layer is then washed with saturated *aq.* $NaHCO_3$ and brine solution, dried over $Na_2SO_4$, filtered and concentrated in a vacuum. The crude mixture is then purified using column chromatography (40% EtOAc in Pet ether) to afford the 2-methyl chroman-4-one **31** (1.55 g, 6.97 mmol).

**Appearance:** White solid

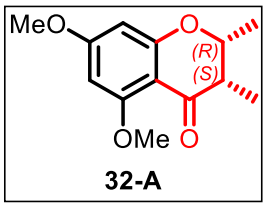
**TLC:** $R_f$ value = 0.2 (50% EtOAc in Pet ether)

**$^1$H NMR:** (400 MHz, Chloroform-*d*) δ 6.07 (d, *J* = 2.3 Hz, 1H), 6.05 (d, *J* = 2.3 Hz, 1H), 4.52 (dddd, *J* = 12.6, 11.1, 6.3, 5.0 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 2.63 – 2.58 (m, 2H), 1.46 (d, *J* = 6.3 Hz, 3H).

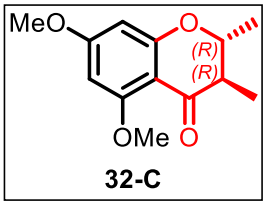
**$^{13}$C NMR:** (101 MHz, $CDCl_3$) δ 189.8, 165.8, 165.1, 162.2, 105.9, 93.3, 92.8, 73.9, 56.1, 55.6, 45.7, 20.8.

12) Synthesis of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A)** and **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C)**:

A solution of compound **31** (230 mg, 1.03 mmol) is dissolved in THF (1.5 mL) and HMPA (0.5 mL) and cooled to -78°C in a chiller with stirring. At this temperature, 1 M solution of LiHMDS in THF (1.35 mL, 1.35 mmol) is added very slowly over 5 minutes. This resulting solution is stirred for 20 minutes. Methyl iodide (93.48 µL, 1.45 mmol) is added to the reaction mixture and is stirred for 24 hours at -78°C. The reaction is then quenched with saturated *aq*. $NH_4Cl$ (2 mL). The aqueous layer is extracted with toluene, and the combined organic layers are washed with $H_2O$ and brine, dried over $Na_2SO_4$, filtered and concentrated in a vacuum. The crude mixture is then purified using column chromatography (**32-A:** 25% EtOAc in Pet ether; **32-C:** 33% EtOAc in Pet ether) to afford the diastereomers **32-A** (60.32 mg, 0.25 mmol, 25%) and **32-C** (90.49 mg, 0.38 mmol, 37%).



**32-A**



**32-C**

**32-A** :-

**Appearance:** White solid

**TLC:** $R_f$ value = 0.5 (50% EtOAc in Pet ether)

**$^1$H NMR**: (400 MHz, Chloroform-*d*) δ 6.05 (d, *J* = 2.7 Hz, 2H), 4.57 (qd, *J* = 6.6, 3.2 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 2.52 (qd, *J* = 7.3, 3.2 Hz, 1H), 1.36 (d, *J* = 6.6 Hz, 3H), 1.13 (d, *J* = 7.3 Hz, 3H).

**$^{13}$C NMR**: (101 MHz, $CDCl_3$) δ 193.0, 164.7, 163.4, 161.5, 103.4, 92.1, 91.8, 75.1, 55.0, 54.5, 45.2, 15.3, 8.3.

**32-C** :-

**Appearance:** White solid

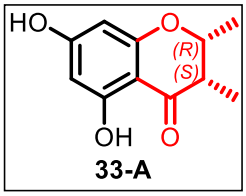
**TLC:** $R_f$ value = 0.4 (50% EtOAc in Pet ether)

**$^1$H NMR**: (400 MHz, Chloroform-*d*) δ 6.03 (s, 2H), 4.20 – 4.12 (m, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 2.44 (dq, *J* = 10.9, 6.9 Hz, 1H), 1.45 (d, *J* = 6.4 Hz, 3H), 1.16 (d, *J* = 7.0 Hz, 3H).

**$^{13}$C NMR**: (101 MHz, $CDCl_3$) δ 191.0, 164.6, 163.4, 161.2, 104.2, 92.0, 91.7, 77.7, 55.0, 54.5, 46.3, 18.6, 9.7.

13) Synthesis of **(2*R*,3*S*)-5,7-dihydroxy-2,3-dimethylchroman-4-one (33-A):**

A solution of **32-A** (60 mg, 0.25 mmol) in $CH_2Cl_2$ (1 mL) is cooled to -78°C with stirring. $BBr_3$ (0.15 mL, 1.52 mmol) is quickly added to the solution. The reaction mixture is then allowed to warm up to room temperature slowly and stirred for 36 hours. The mixture is cooled to 0°C and the reaction is quenched with $H_2O$ (1 mL). $CH_2Cl_2$ is evaporated under a vacuum. The aqueous layer is extracted with EtOAc (3 x 10 mL). The next step involves washing the organic layer with $H_2O$ and brine, followed by drying over $Na_2SO_4$, filtering, and concentrating it under vacuum. The resulting crude mixture is then purified using column chromatography (15% EtOAc in Pet ether) to afford the product **33-A** (7.23 mg, 0.03 mmol, 14%)



**33-A**

**Appearance**: White solid

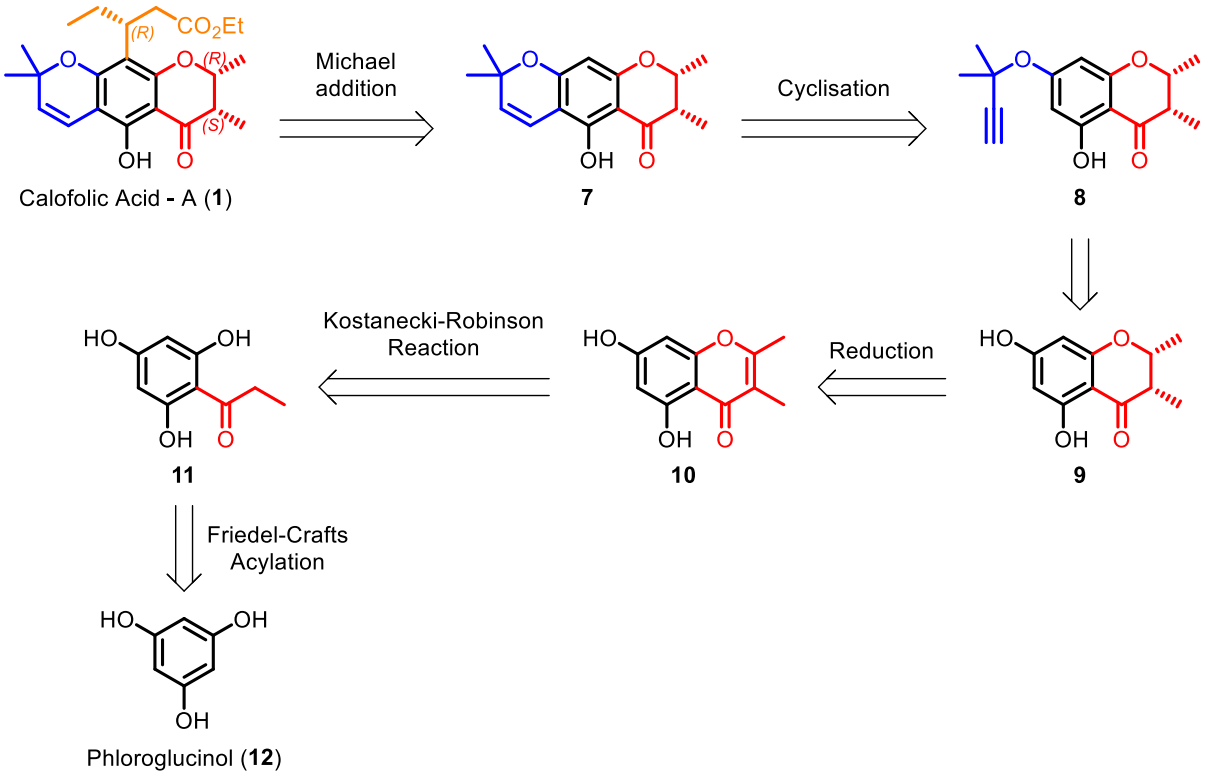
**TLC**: $R_f$ value = 0.7 (50% EtOAc in Pet ether)

**$^1$H NMR**: (400 MHz, DMSO-$d_6$) δ 12.19 (s, 1H), 5.96 (d, *J* = 2.3 Hz, 1H), 5.90 (d, *J* = 2.3 Hz, 1H), 4.18 (dt, *J* = 11.1, 6.2 Hz, 1H), 2.57 (dq, *J* = 11.0, 7.0 Hz, 1H), 1.49 (d, *J* = 6.2 Hz, 3H), 1.21 (d, *J* = 7.0 Hz, 3H).

# Chapter 3 : Results And Discussions

## 3.1 The First Synthetic Route Attempt

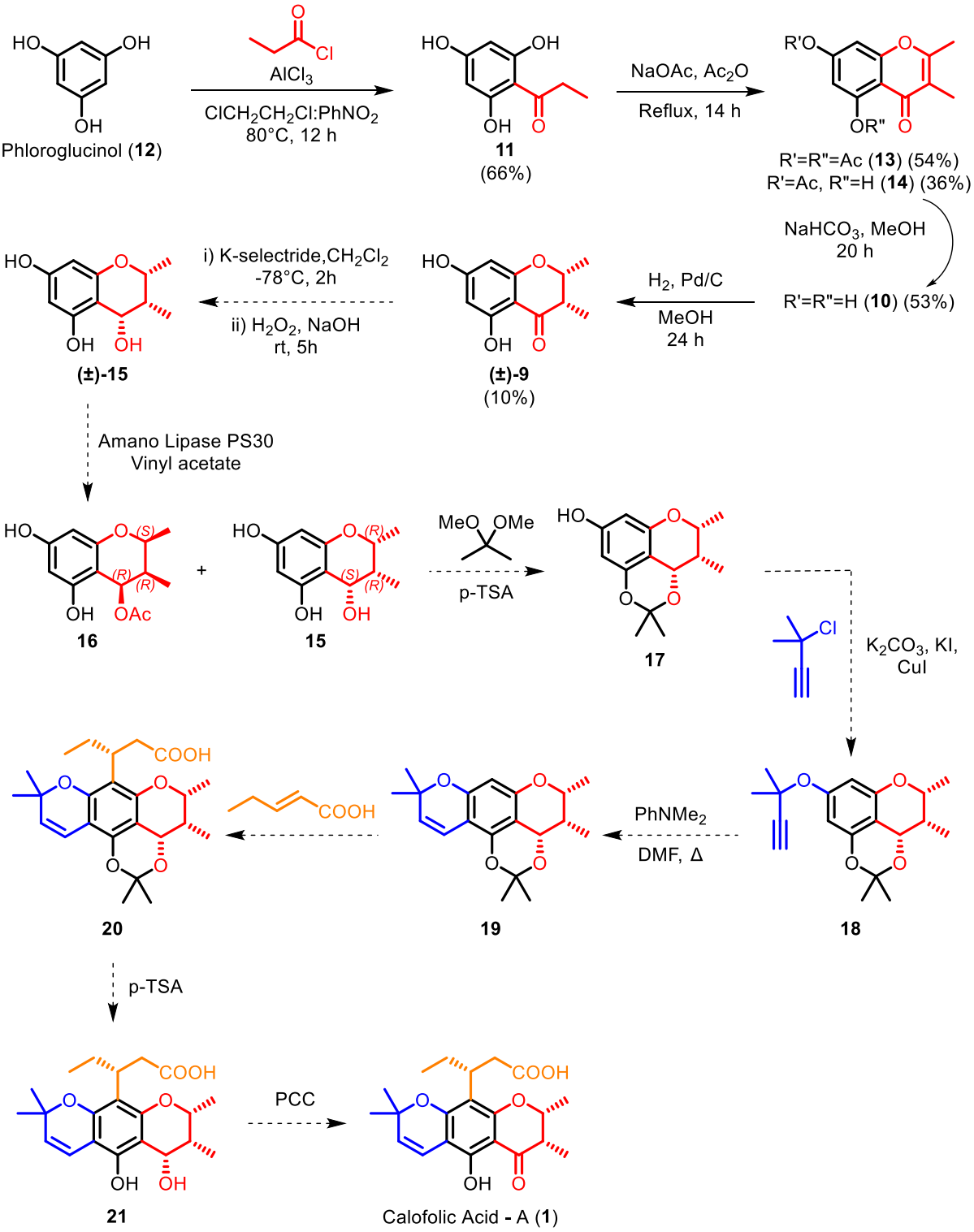
In 1996, Rehder & Kepler reported the synthesis of (±)-Calanolide A. In this report, the synthesis of the chroman-4-one and the 2H-chromene moiety was described[12]. Using this work as an inspiration, the retrosynthetic analysis of Calofolic Acid – A is shown in Scheme 1. The natural product was envisioned to be the Michael addition product of the 2H-chromene derivative **7** and ethyl pent-2-enoate. **7** could be yielded from the cyclisation of propargyl ether **8**, which would be furnished from the chiral chromanone **9** and propargyl chloride. Chromanone **9** could be prepared from chromone **10** via alkene reduction, which could be derived from ketone **11**. **11** could be accessible from the commercially available phloroglucinol **12.**




**Scheme 1**: Retrosynthetic analysis of Calofolic Acid - A

Based on the retrosynthetic scheme, the synthetic route for the total synthesis of calofolic acid – A is designed as shown in Scheme 2. We begin with phloroglucinol (**12**), which will undergo Friedel-Crafts acylation to yield the ketone **11**. The ketone **11** undergoes the Kostanecki-Robinson reaction followed by acetate deprotection to afford **10**, which will be reduced to the racemic chromanone **(±)-9**. Stereospecific

ketone reduction using K-selectride will yield the racemic 4-chromanol **(±)-15**. The desired enantiomer **15** will be resolved through enzymatic resolution, which will then be protected by the acetonide group to yield **17**. Copper(I) iodide-mediated aryl propargyl ether formation will be done to afford **18**, which will undergo cyclisation to

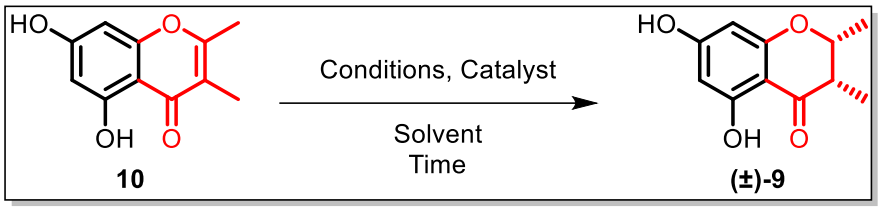



**Scheme 2**: First synthetic route towards the total synthesis of Calofolic Acid - A

form the 2H-chromene derivative **19**. Using the aromatic C-H as a nucleophile, Michael addition will be done on ethyl pent-2-enoate to yield **20**, which will then be followed by acetonide deprotection and oxidation to afford the natural product Calofolic Acid – A (**1**).

The stereoselective total synthesis of calofolic acid – A began with the synthesis of ketone **11** from phloroglucinol. Phloroglucinol was subjected to Friedel-Crafts Acylation using propionyl chloride and $AlCl_3$ to give ketone **11** with a yield of 65%. Ketone **11** underwent the Kostanecki-Robinson reaction using sodium acetate in acetic anhydride to afford the desired 4-chromone moieties, which are the diacetate **13** and monoacetate **14**, with a yield of 54% and 36%, respectively. Subsequent acetate deprotection using sodium bicarbonate afforded the chromone **10** with a 53% yield. (Scheme 2)

With chromone **10** in hand, the double bond reduction to synthesise the racemic chromanone **(±)-9** is investigated (Table 1). Attempts of hydrogenation using 5 mol% and 10 mol% of palladium (10% by weight) supported on carbon as the catalyst in ethyl-acetate were unsuccessful (entries 1 and 2), and the starting material remained unreacted. Upon changing the solvent to methanol, the reaction did not proceed forward (entry 3). However, upon increasing the reaction time to 24 hours, negligible conversion of the starting material was seen (entry 4). In order to increase the conversion, the pressure of the hydrogen atmosphere was increased to 1.5 atmospheres, and the reaction was done in a Parr reactor. It is observed that the reaction does proceed, with a 10% isolated yield of product **(±)-9** (entry 5). The increase in pressure to 5 atm did not improve the yield of the reaction (entry 6). Replacing the palladium catalyst with Wilkinson's catalyst and $PtO_2$ did not lead to the formation of product **(±)-9** (entries 7 and 8). Using ammonium formate as a hydrogen transfer agent in the presence of 10% Pd/C as a catalyst in refluxing methanol for 12 hours also led to negligible conversion, and this conversion did not improve with increasing the reaction time to 24 hours (entries 9 and 10). Using a mixture of triethylamine and formic acid as a hydrogen transfer agent under 10% Pd/C catalyst in N,N-dimethylformamide as the solvent did not lead to any conversion of the starting material (entry 11). With these results, conditions described in entry 5 are used to synthesise chromanone **(±)-9**.



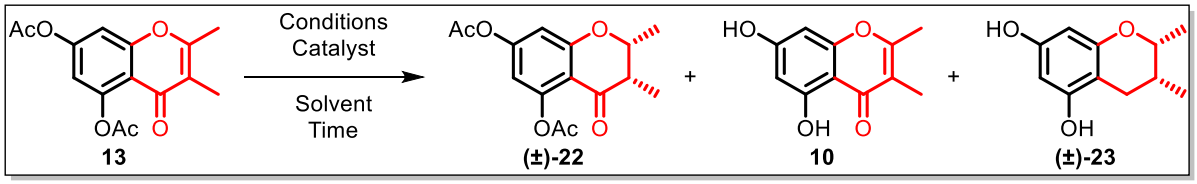

| Entry | Conditions | Catalyst | Solvent | Time | Status of reaction |
|---|---|---|---|---|---|
| 1 | 1 atm $H_2$ | 10% Pd/C (5 mol%) | EtOAc | 12 h | No reaction |
| 2 | 1 atm $H_2$ | 10% Pd/C (10 mol%) | EtOAc | 12 h | No reaction |
| 3 | 1 atm $H_2$ | 10% Pd/C (10 mol%) | MeOH | 12 h | No reaction |
| 4 | 1 atm $H_2$ | 10% Pd/C (10 mol%) | MeOH | 24 h | Negligible conversion |
| 5 | 1.5 atm $H_2$ | 10% Pd/C (10 mol%) | MeOH | 24 h | 10% isolated yield |
| 6 | 5 atm $H_2$ | 10% Pd/C (10 mol%) | MeOH | 24 h | 10% isolated yield |
| 7 | 1 atm $H_2$ | $Rh(PPh_3)_3Cl$ (15 mol%) | Benzene -EtOH | 24 h | No reaction |
| 8 | 5 atm $H_2$ | $PtO_2$ (20 mol%) | MeOH | 24 h | No reaction |
| 9 | $NH_4HCO_2$, reflux | 10% Pd/C (10 mol%) | MeOH | 12 h | Negligible conversion |
| 10 | $NH_4HCO_2$, reflux | 10% Pd/C (10 mol%) | MeOH | 24 h | No improvement compared to 12 h |
| 11 | $Et_3N$ + HCOOH | 10% Pd/C (10 mol%) | DMF | 6 h | No reaction |

**Table 1**: Investigating the reduction of chromone **10** to chromanone ***(±)- 9***

The stereospecific reduction of the racemic chromanone **(±)-9** to afford chromanol **(±)-15** is attempted by using K-selectride. However, no conversion was observed.

Due to the unsatisfactory yields for the reduction of **10** to **(±)-9**, an alternative method is attempted, where the reduction is investigated on the diacetate **13** (Table 2). Attempting hydrogenation at 1.5 atm under 10% Pd/C catalyst in methanol for 24 hours afforded the unexpected product **(±)-23**, where, along with the double bond, it was observed that the ketone was reduced, and the acetate deprotection had also taken place (entry 1). In-situ generation of $H_2$ using ammonium formate under 10%

Pd/C catalyst in methanol for 24 hours led to the deprotection of the acetate group only (entry 2). Using a mixture of triethylamine and formic acid as a hydrogen transfer agent led to no conversion of the starting material (entry 3).




| Entry | Conditions | Catalyst | Solvent & Time | (±)-22 | 10 | (±)-23 |
|---|---|---|---|---|---|---|
| 1 | 5 atm $H_2$ | 10% Pd/C (5 mol%) | MeOH 24 h | - | - | 77% |
| 2 | $NH_4HCO_2$, reflux | 10% Pd/C (10 mol%) | MeOH 24 h | - | 65% | - |
| 3 | $Et_3N$ + HCOOH | 10% Pd/C (10 mol%) | DMF 6 h | - | - | - |

**Table 2***:* Investigating the reduction of the double bond for diacetate **13**

After attempting various conditions, it is found that the yield for the reduction of chromone **10** to chromanone **(±)-9** is poor. Attempting hydrogenation with the diacetate **13** also did not yield the required diacetate-protected chromanone **(±)-22**. These results suggest the need for an alternate synthesis route with a different strategy to access the chromanone core.

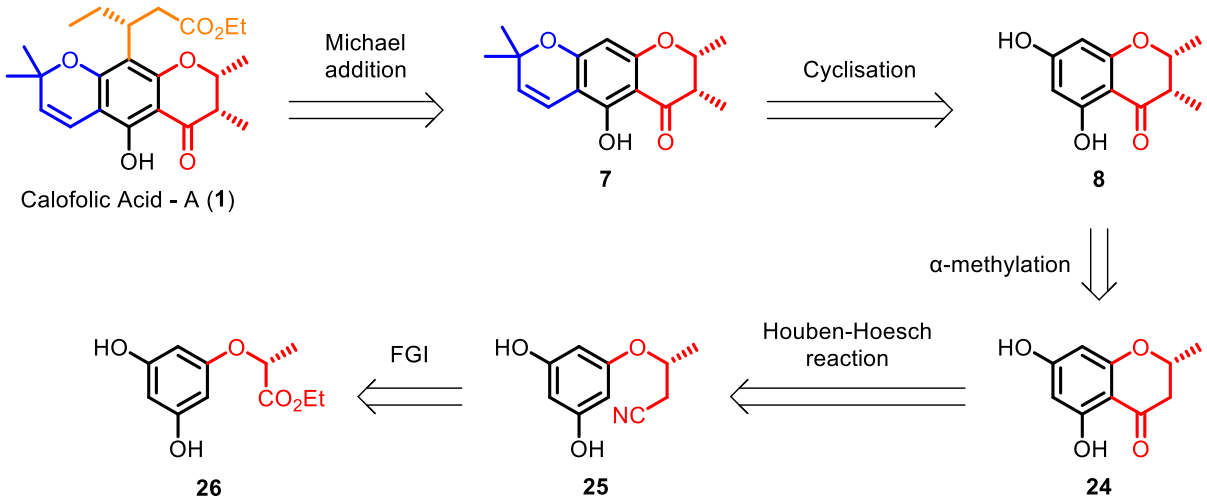
## 3.2 The Second Synthesis Route Attempt

The poor yields for the reduction reactions suggested the need for an alternate synthesis route to access the chromanone core. In 1994, Rama Rao et al. reported a concise synthesis route towards the chiral 2-methyl chroman-4-ones. In this reported route, first, a Mitsunobu reaction is done between 3,5-dimethoxyphenol and ethyl-L-lactate. This is followed by the reduction of the ester to the alcohol. Then, the succeeding steps are the functional group interconversions of the alcohol to the corresponding iodide, followed by the conversion of the iodide to its corresponding

cyanide. The cyclisation of the cyanide is done using the intramolecular Houben-Hoesch reaction to afford the chiral 2-methyl chroman-4-one[13].

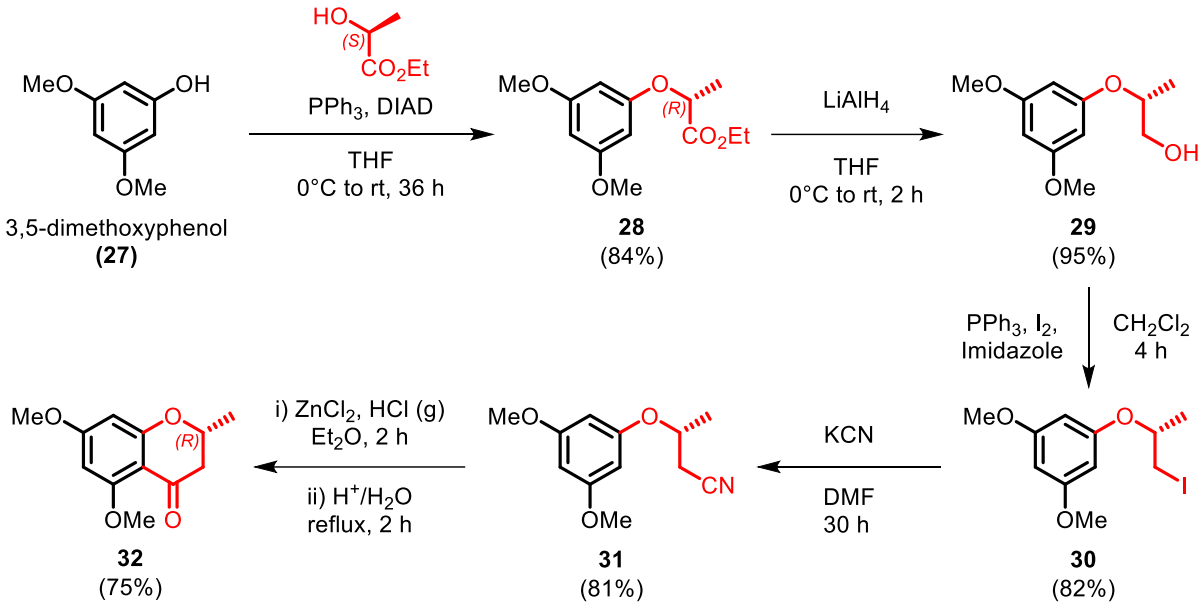
Using the reported route for the synthesis of the chromanone core as the foundation towards the synthesis of calofolic acid – A, a new retrosynthesis for the target molecule is developed (Scheme 3). The retrosynthesis is similar to Scheme 1 till chromanone **9**. Now, **9** is envisioned to be furnished from **24** via methylation at the α-position of the carbonyl group. The chiral 2-methyl chroma-4-one **24** can be accessed via the intramolecular Houben-Hoesch reaction on cyanide **25**, which can be derived from the ester **26** via various functional group interconversions.

With this new retrosynthetic plan, a new approach towards the synthesis of Calofolic Acid – A is planned as shown in Scheme 4. The synthesis begins with the Mitsunobu reaction of 3,5-dimethoxyphenol (**27**) with ethyl-L-lactate using $PPh_3$ and DIAD to give the ester **28** with 84% yield. The ester **28** is reduced using $LiAlH_4$ to afford the corresponding alcohol **29** with a yield of 95%. Iodination of alcohol **29** using solid $I_2$, $PPh_3$, and imidazole afforded the iodo-compound **30** with 82% yield, and subsequent cyanation using potassium cyanide gave the cyanide **31** with a yield of 81%. The



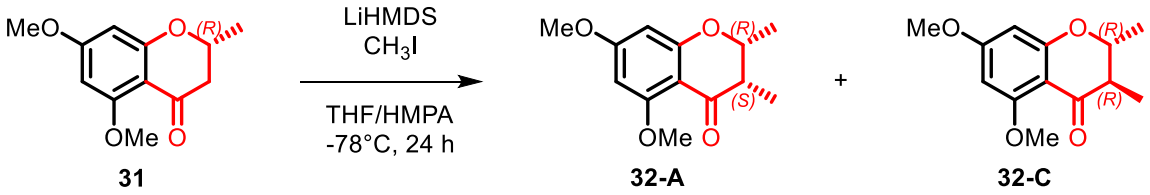

**Scheme 3**: A new retrosynthetic analysis of Calofolic Acid - A

cyanide **31** is subjected to an intramolecular Houben-Hoesch reaction using zinc chloride and bubbling gaseous HCl, followed by acid reflux to afford the 2-methyl chroman-4-one **32** with a 75% yield.




**Scheme 4**: Synthesis of the 2-methyl chroman-4-one (**32**)

The α-methylation of the 2-methyl chroman-4-one **32** to afford the two diastereomers of 2,3-methyl chroman-4-one (**32-A** and **32-C**) is investigated. The reaction was first attempted with the addition of lithium diisopropylamide (LDA, formed in the reaction mixture by the reaction between nBuLi and DIPA) to form the enolate anion, followed by the addition of methyl iodide. However, no reaction was observed in this case. When the reaction was attempted with LiHMDS the formation of the diastereomers is observed. The two diastereomers **32-A** and **32-C** are the precursors for the synthesis of calofolic acid – A and calofolic acid – C respectively. (Scheme 5)




**Scheme 5**: Synthesis of 2,3-methyl chrom-4-one diastereomers **32-A** and **32-C**

The diastereomers are easily separable, and the identification of the diastereomers is done using the $^{1}$H-$^{1}$H NOESY. As can be seen in Figure 5, in the case of the diastereomer **32-A**, the two methyl groups are cis to each other, meaning that the hydrogens at C-2 and C-3 are also cis to each other. Since these two hydrogens

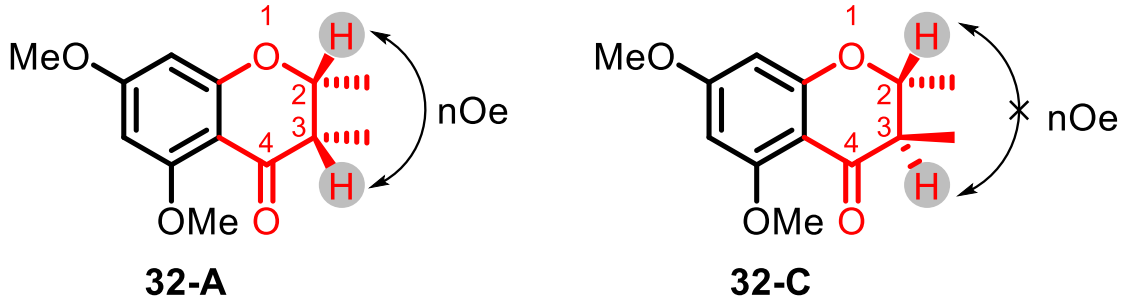
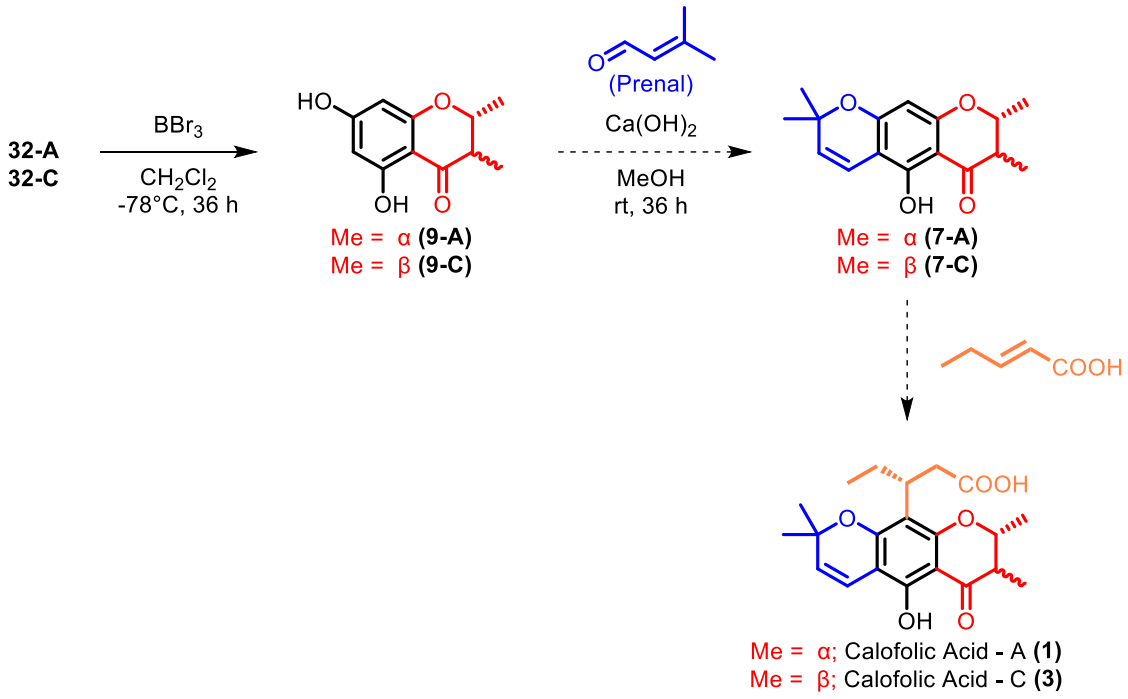



**Figure 5**: nOe correlation between C-2 and C-3 hydrogens in **31-A** and **31-C**

atoms are close to each other in space and have a low internuclear distance between each other, they exhibit the nuclear Overhauser effect (NOE). As a result, in the $^{1}$H-$^{1}$H NOESY spectra, a strong correlation between the hydrogen at C-2 and C-3 is observed. On the other hand, in **32-C**, the C-2 and C-3 hydrogen atoms are trans to each other. As a result, they are further apart from each other in space.




**Scheme 6**: Progress towards the total synthesis of Calofolic Acid - A & C

Hence the correlation between C-2 and C-3 in the $^{1}H$-$^{1}H$ NOESY spectra is significantly weaker.

The diastereomers **32-A** and **32-C** are now taken ahead to complete the total synthesis of calofolic acid – A and calofolic acid – C (Scheme 6). **32-A** is subjected to methoxy-deprotection using $BBr_3$ to afford the chromanone **9-A**. Similarly, **32-C**undergoes methoxy-deprotection to afford the chromanone **9-C**. Now, from here, the synthesis route will be completed via the formation of the 2H-chromene derivatives **7-A** and **7-C** by undergoing cyclisation with prenal using $Ca(OH)_2$. Finally, the stereoselective Michael addition of the 2H-chromene derivatives **7-A** and **7-C** with ethyl pent-2-enoate will afford us the required natural products calofolic acid – A (**1**) and calofolic acid – C (**3**).

# Conclusions

In this work, efforts towards the total synthesis of calofolic acid – A have been made. Two different routes have been studied.

In the first route, Friedel-Crafts acylation followed by the Kostanecki-Robinson cyclisation afforded us the desired chromene. The reduction of the chromene to chrom-4-ane is the key step, and the yield for this reaction turned out to be extremely low.

To overcome this problem, an alternate route for the total synthesis of calofolic acid – A is designed. This route begins with a Mitsunobu reaction to form the ester. The ester undergoes reduction and substitution with iodo and cyanide to afford the cyano compound. The key step is the intramolecular Houben-Hoesch reaction which afford the chroman-4-one core. The α-methylation followed by methoxy deprotection yields the desired core.

The end-game synthesis for calofolic acid – A has been planned, and the efforts to complete the synthesis are currently ongoing.

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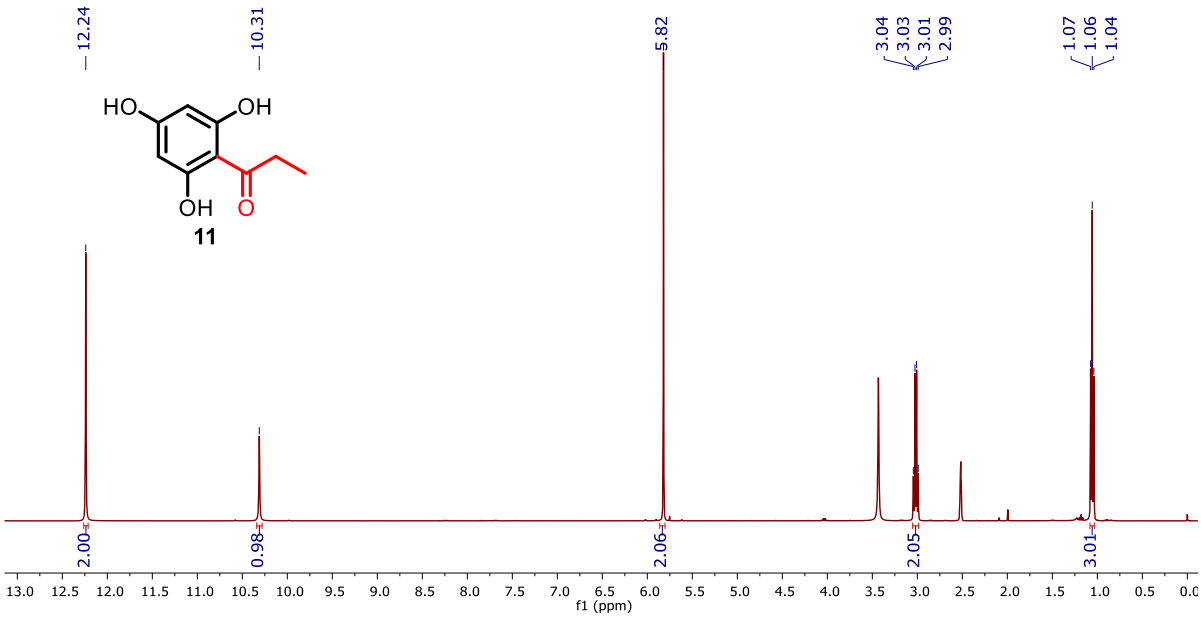
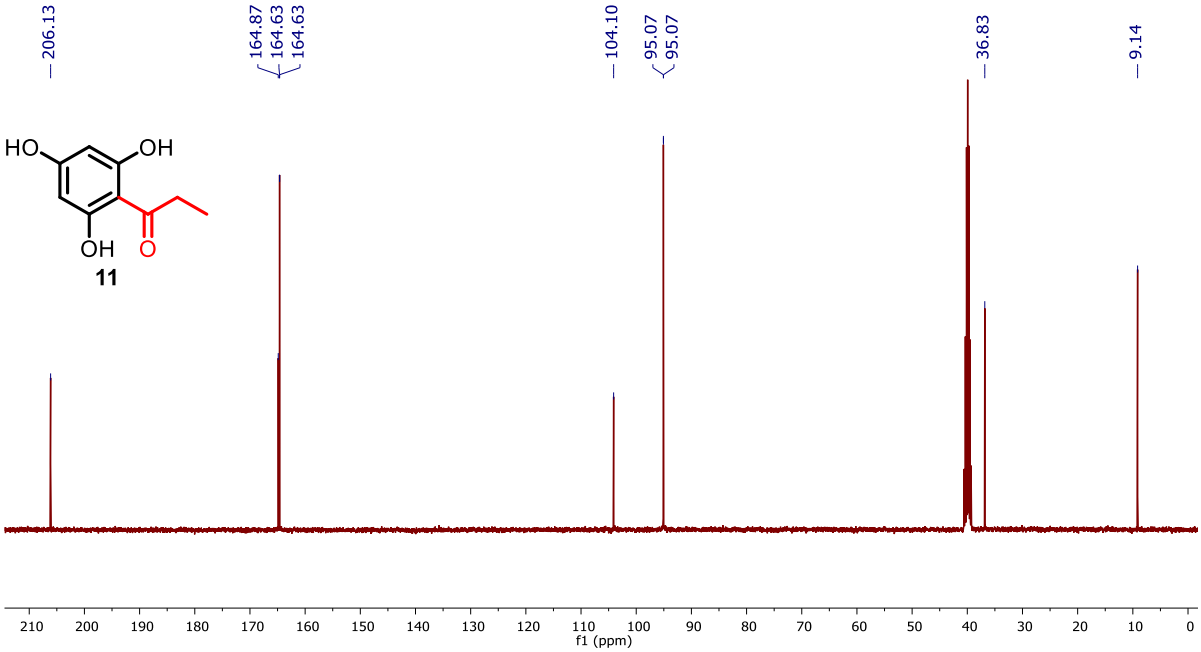
# Appendix – A (NMR Spectral charts)

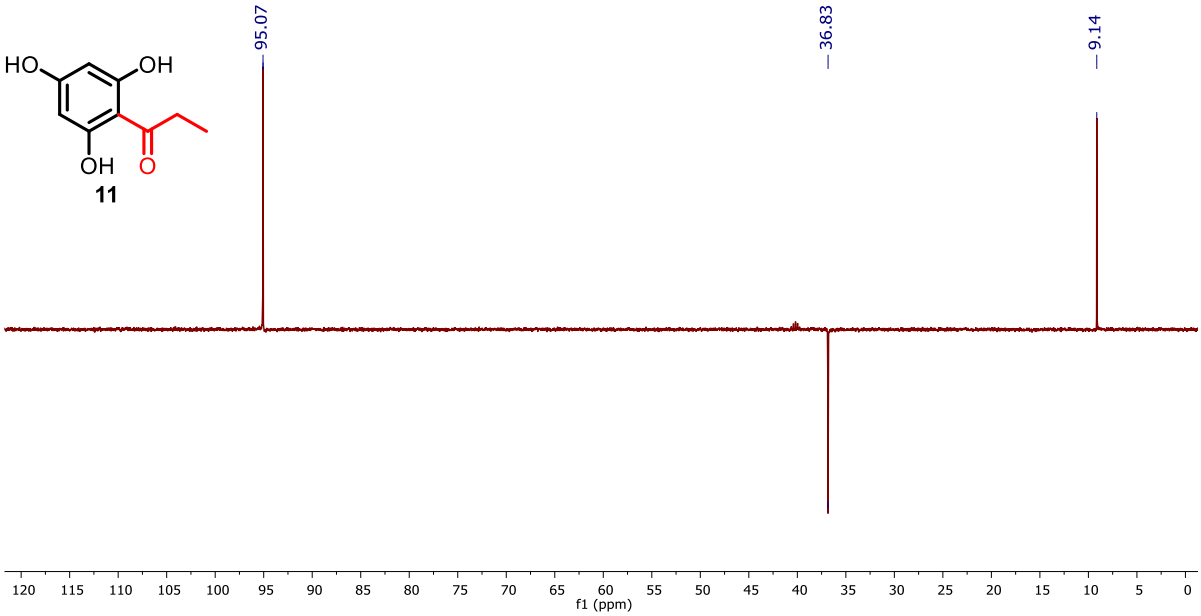
$^{1}$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **1-(2,4,6-trihydroxyphenyl)propan-1-one (11)**



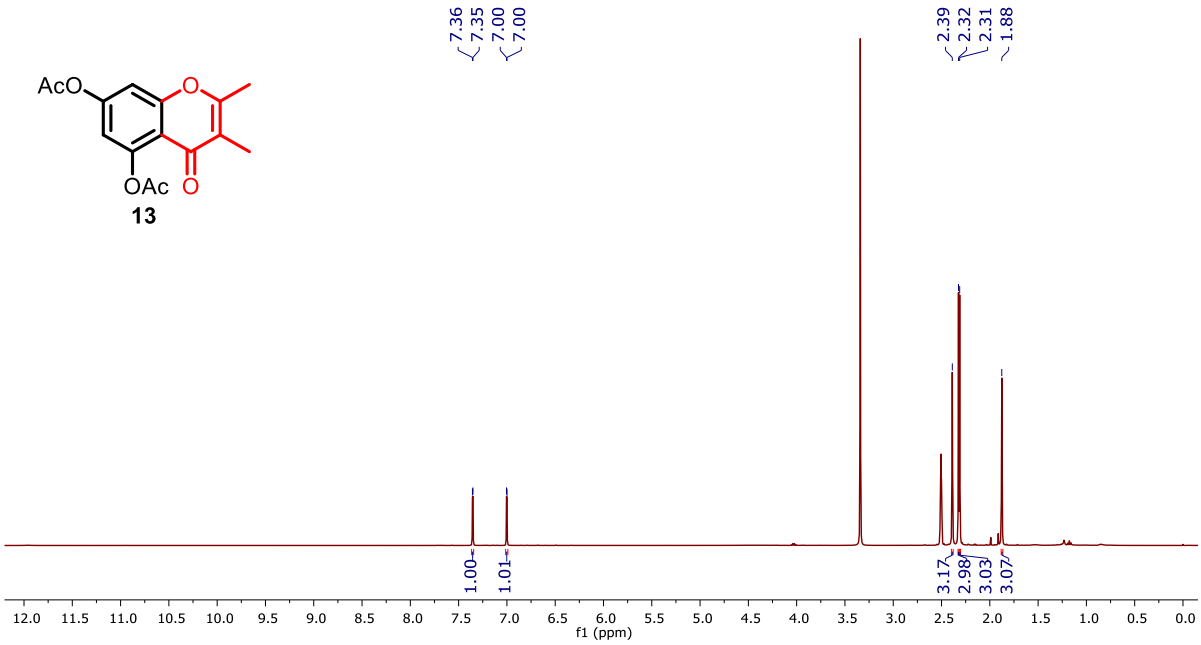

$^{13}$C NMR Spectrum (101 MHz, DMSO-$d_6$) of **1-(2,4,6-trihydroxyphenyl)propan-1-one (11)**



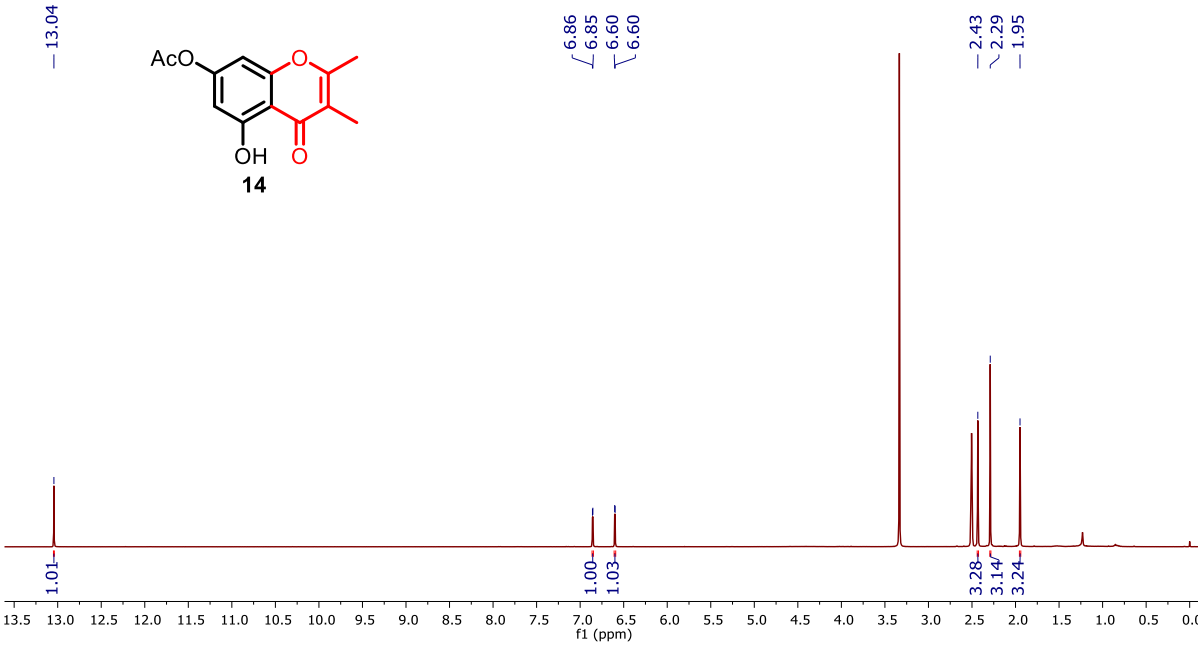
DEPT NMR Spectra (101 MHz, DMSO-$d_6$) of **1-(2,4,6-trihydroxyphenyl)propan-1-one (11)**



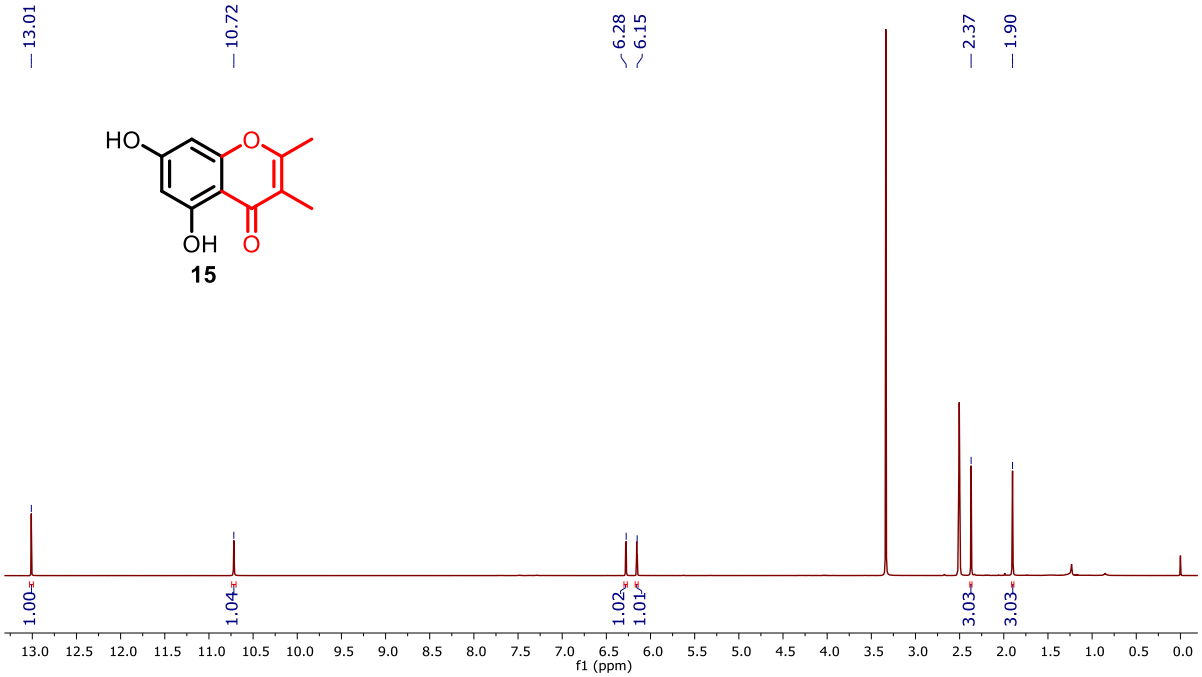

$^1$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **2,3-dimethyl-4-oxo-4H-chromene-5,7-diyl diacetate (13)**



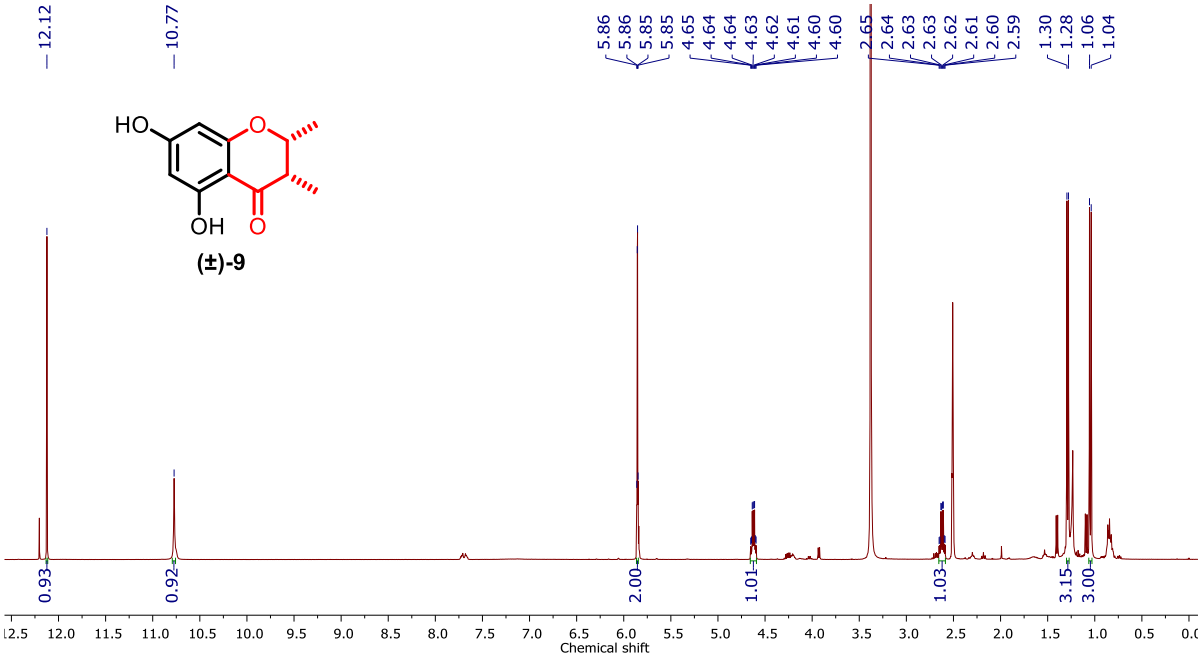
$^1$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **5-hydroxy-2,3-dimethyl-4-oxo-4H-chromen-7-yl acetate (14)**



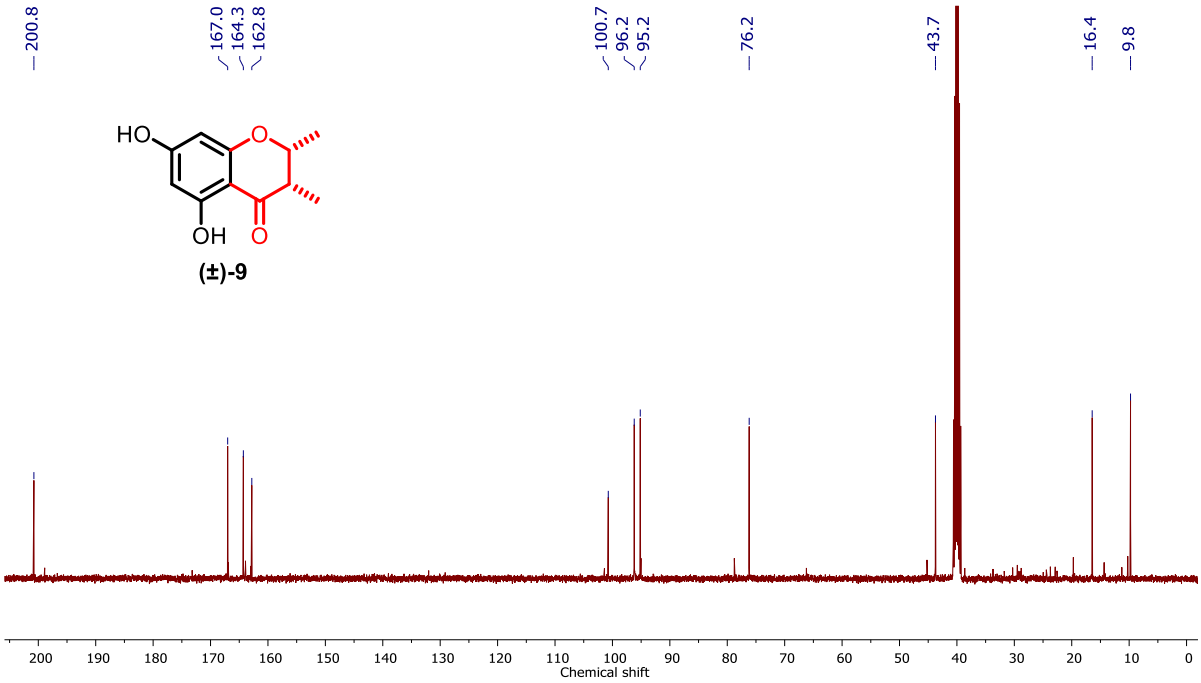

$^1$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **5,7-dihydroxy-2,3-dimethyl-4H-chromen-4-one (15)**



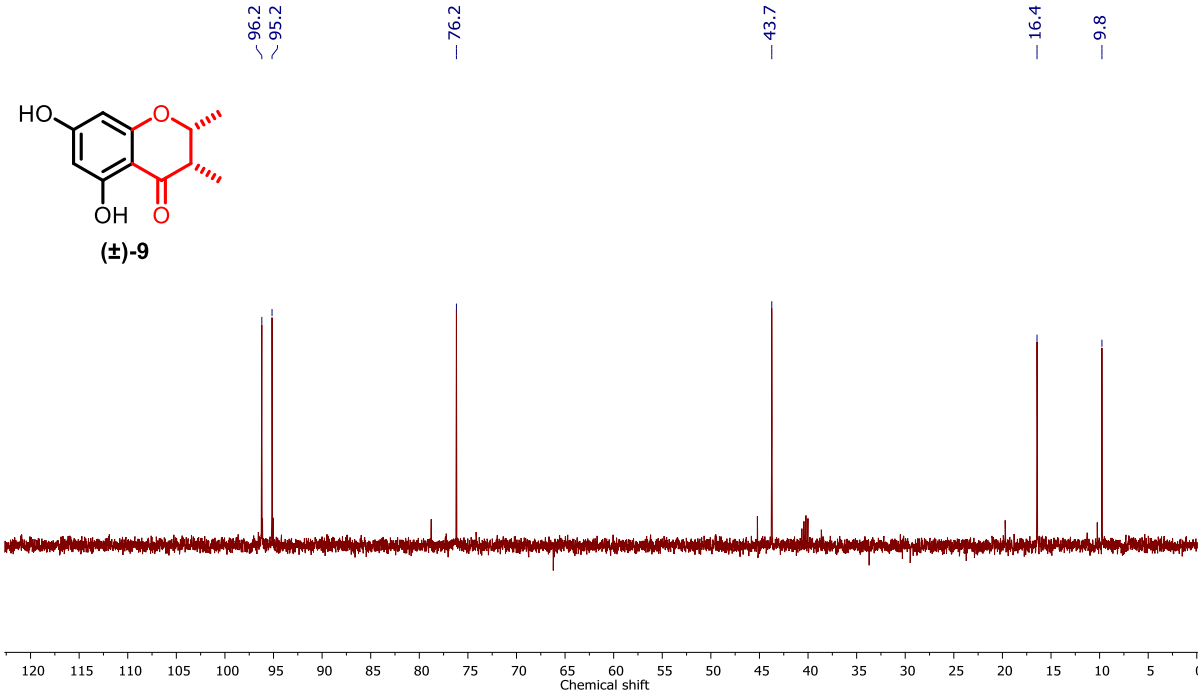
$^{1}$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **(±)-5,7-dihydroxy-2,3-dimethylchroman-4-one ((±)-9)**



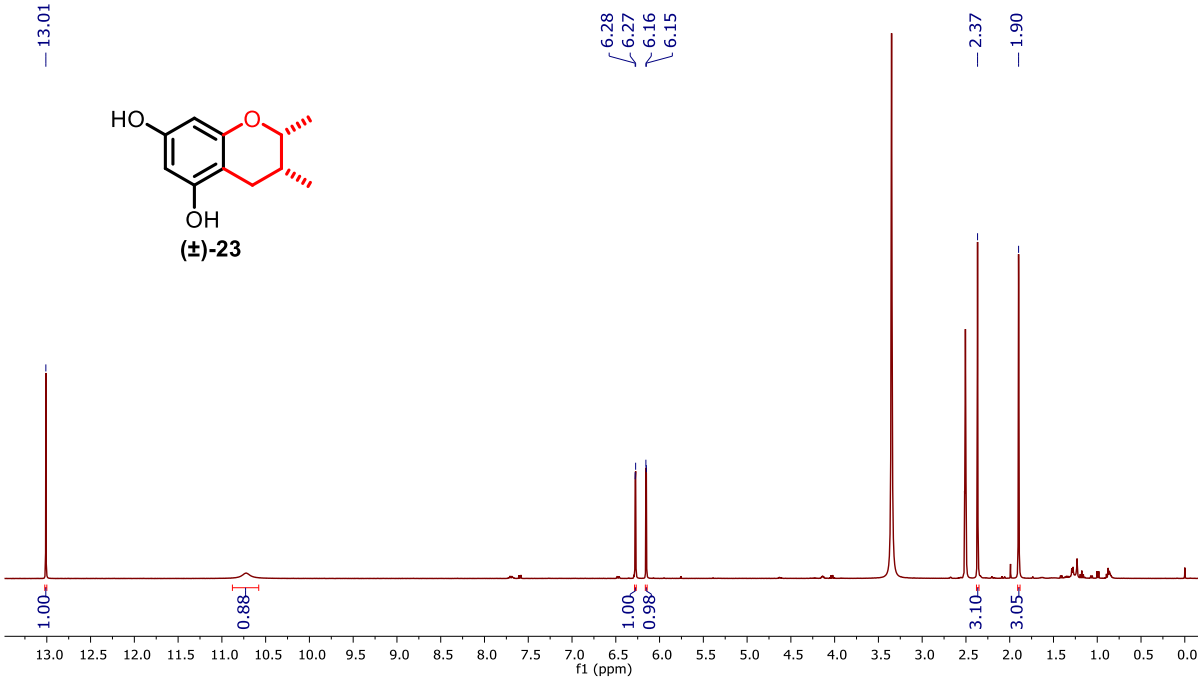

$^{13}$C NMR Spectrum (101 MHz, DMSO-$d_6$) of **(±)-5,7-dihydroxy-2,3-dimethylchroman-4-one ((±)-9)**



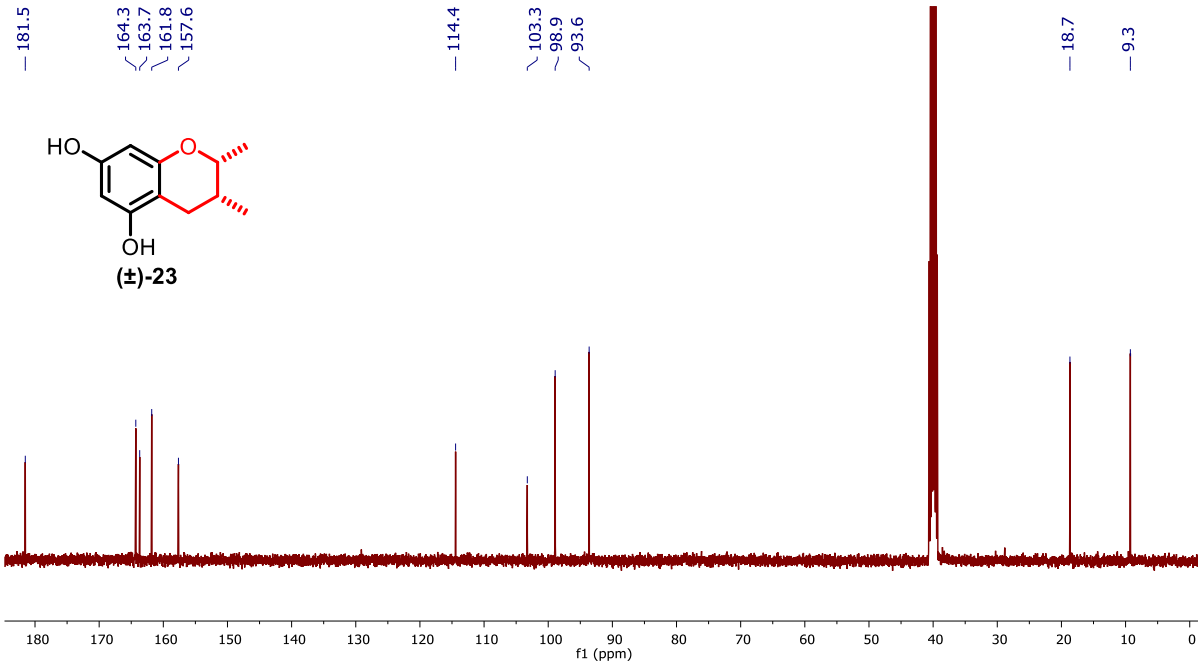
DEPT NMR Spectrum (101 MHz, DMSO-$d_6$) of **(±)-5,7-dihydroxy-2,3-dimethylchroman-4-one ((±)-9)**



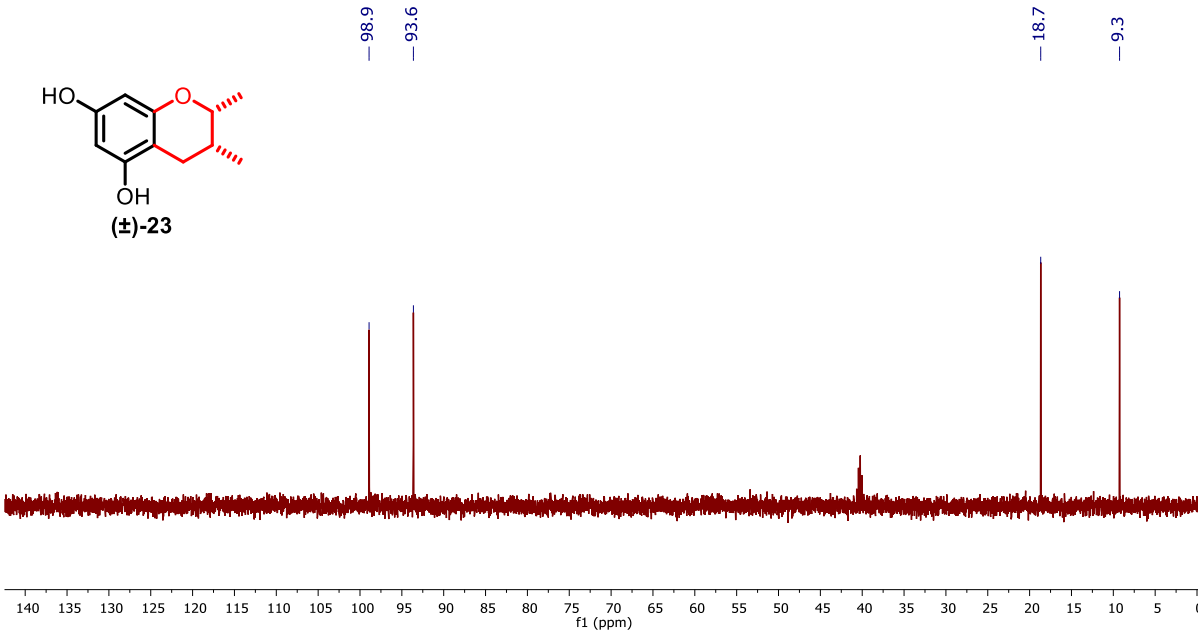

$^1$H NMR Spectrum (400 MHz, DMSO-$d_6$) of **(±)-2,3-dimethylchromane-5,7-diol ((±)-23)**:



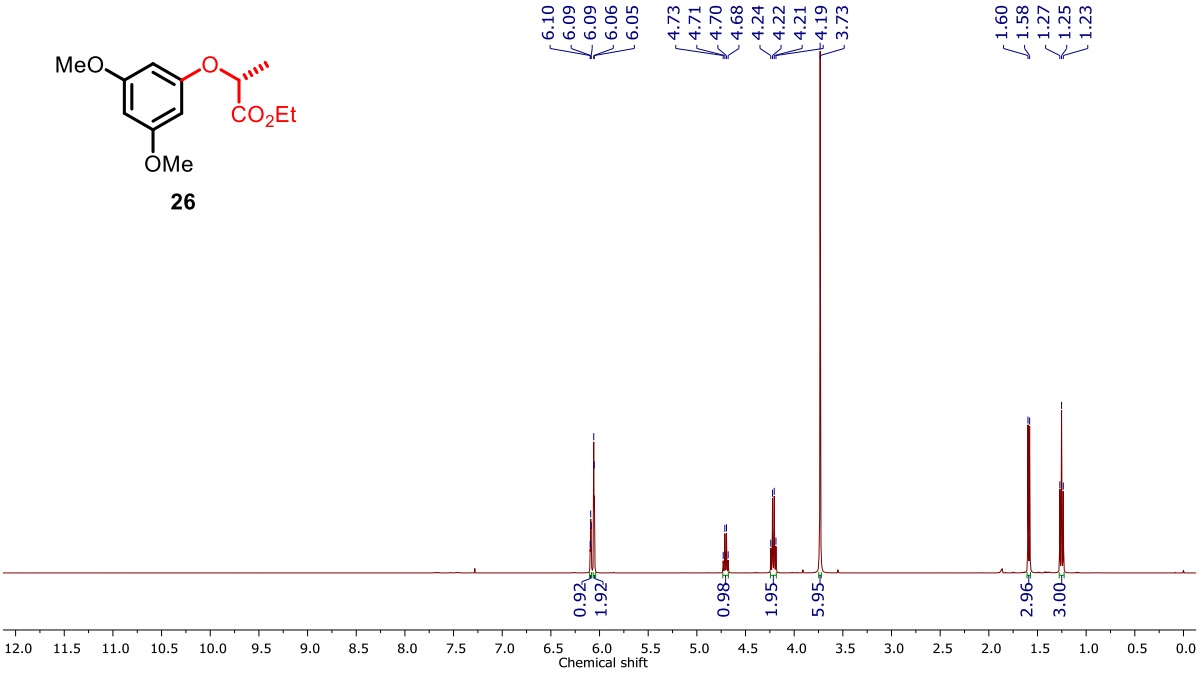
$^{13}$C NMR Spectrum (101 MHz, DMSO-$d_6$) of **(±)-2,3-dimethylchromane-5,7-diol ((±)-23)**:



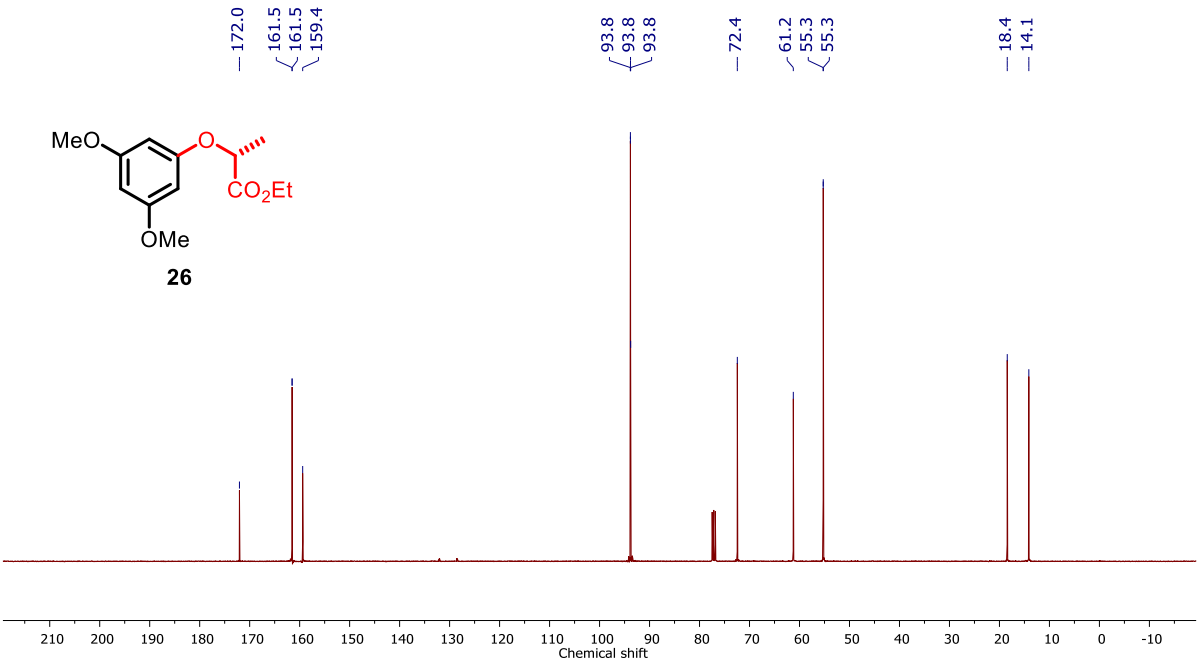

DEPT NMR Spectrum (101 MHz, DMSO-$d_6$) of **(±)-2,3-dimethylchromane-5,7-diol ((±)-23)**:



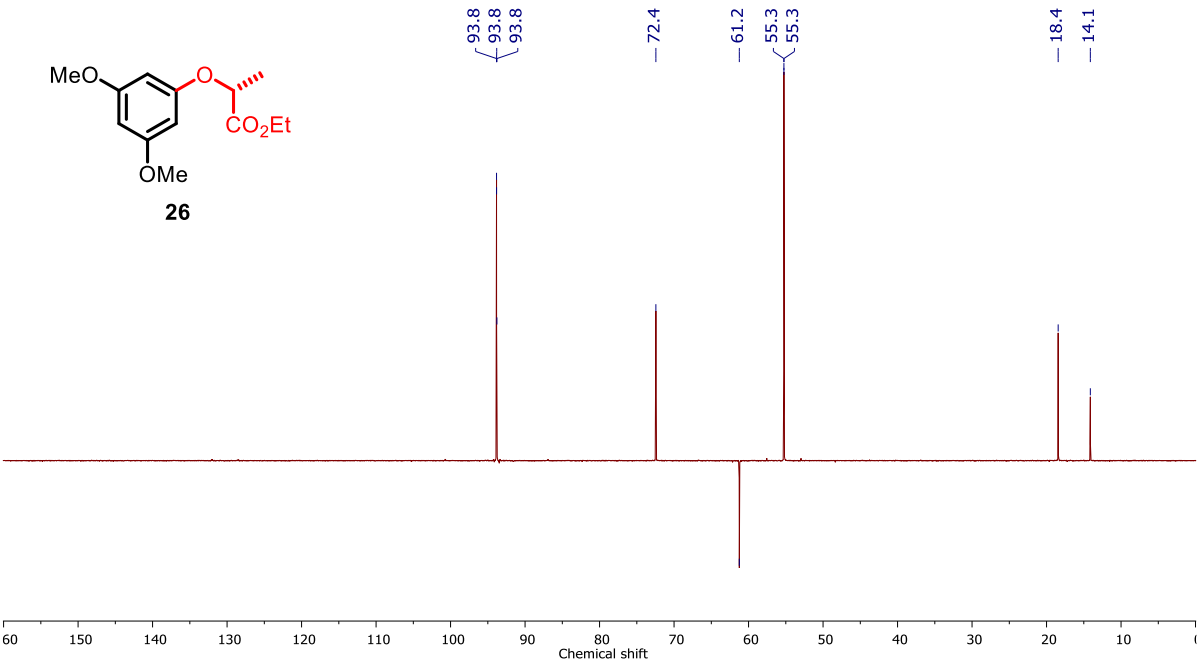
$^{1}$H NMR Spectrum (400 MHz, $CDCl_3$) of **ethyl-(R)-2-(3,5-dimethoxyphenoxy)propanoate (26)**:



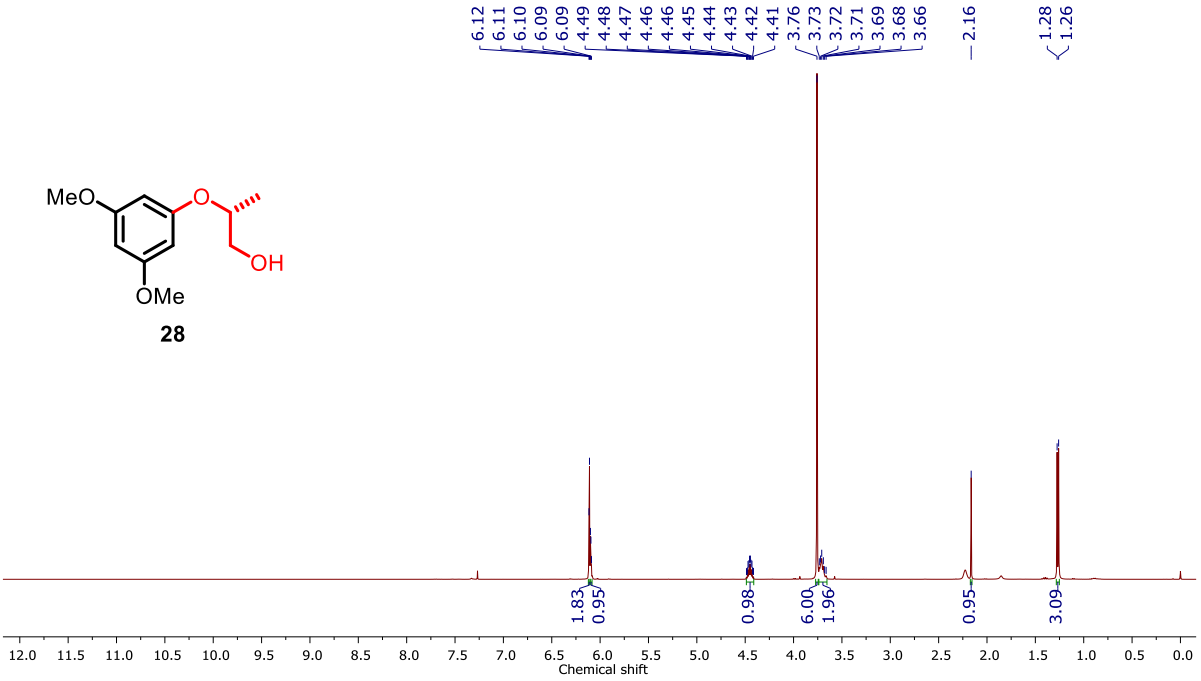

$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **ethyl-(R)-2-(3,5-dimethoxyphenoxy)propanoate (26)**:



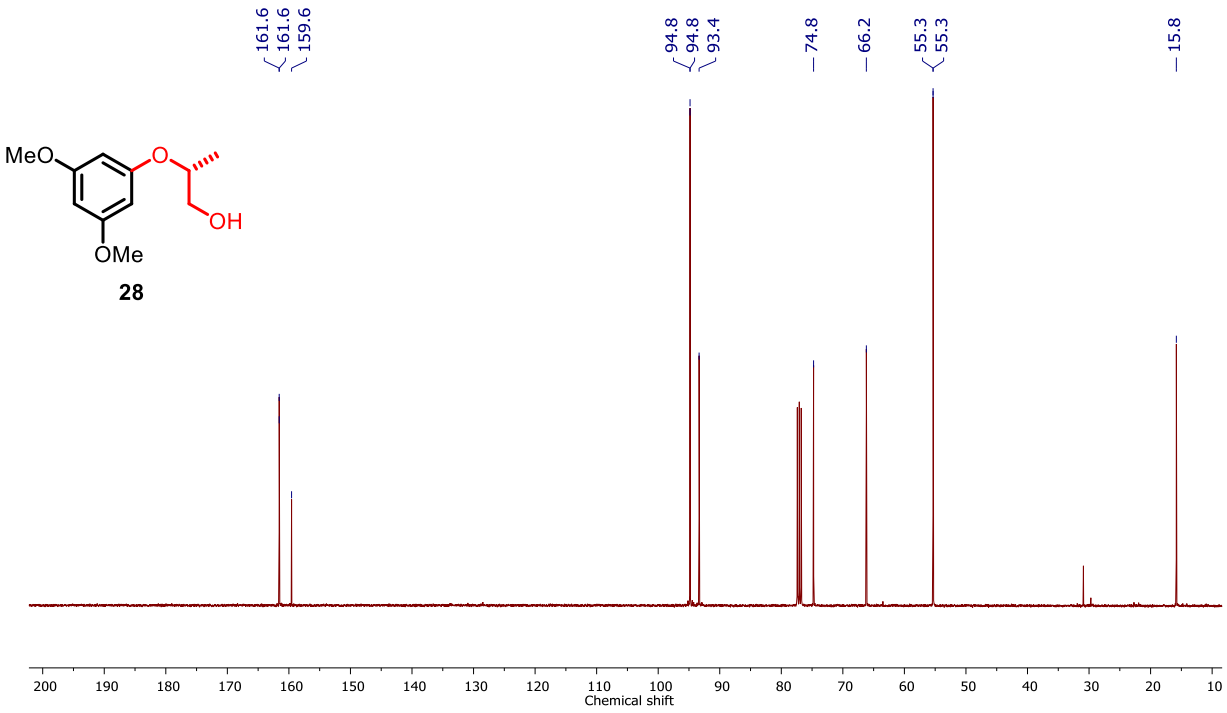
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **ethyl-(R)-2-(3,5-dimethoxyphenoxy)propanoate (26)**:



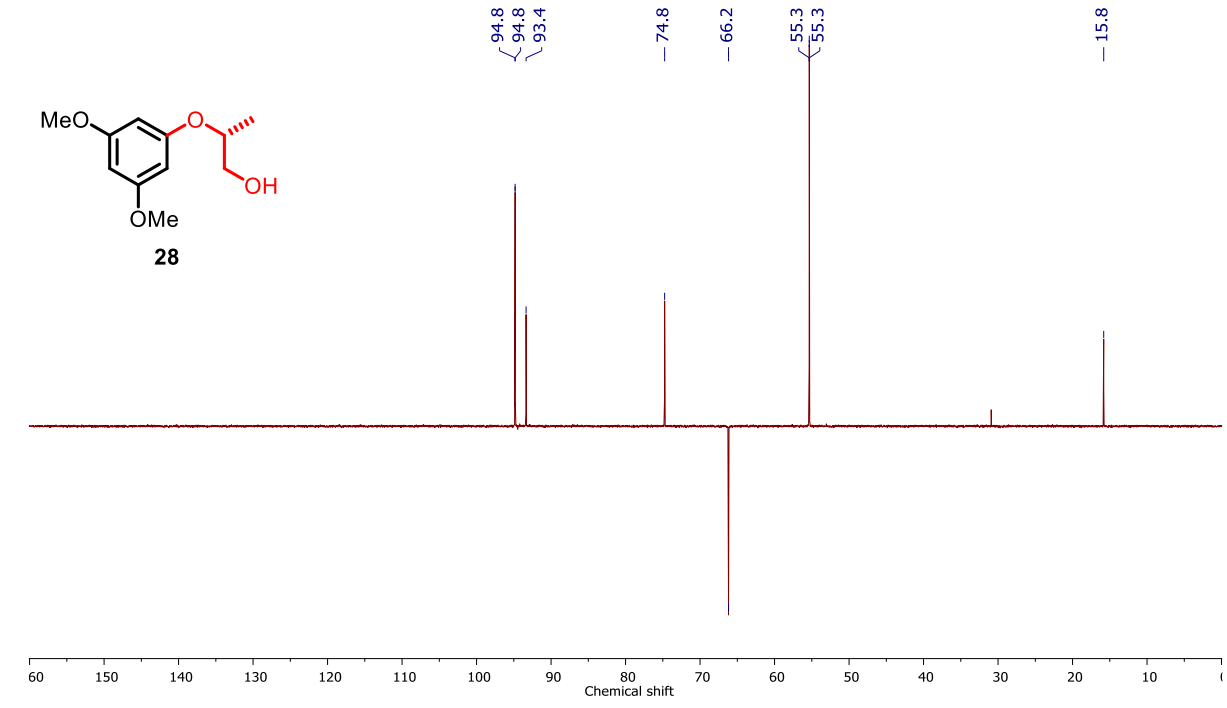

$^1H$ NMR Spectrum (400 MHz, $CDCl_3$) of **(R)-2-(3,5-dimethoxyphenoxy)propan-1-ol (28)**:



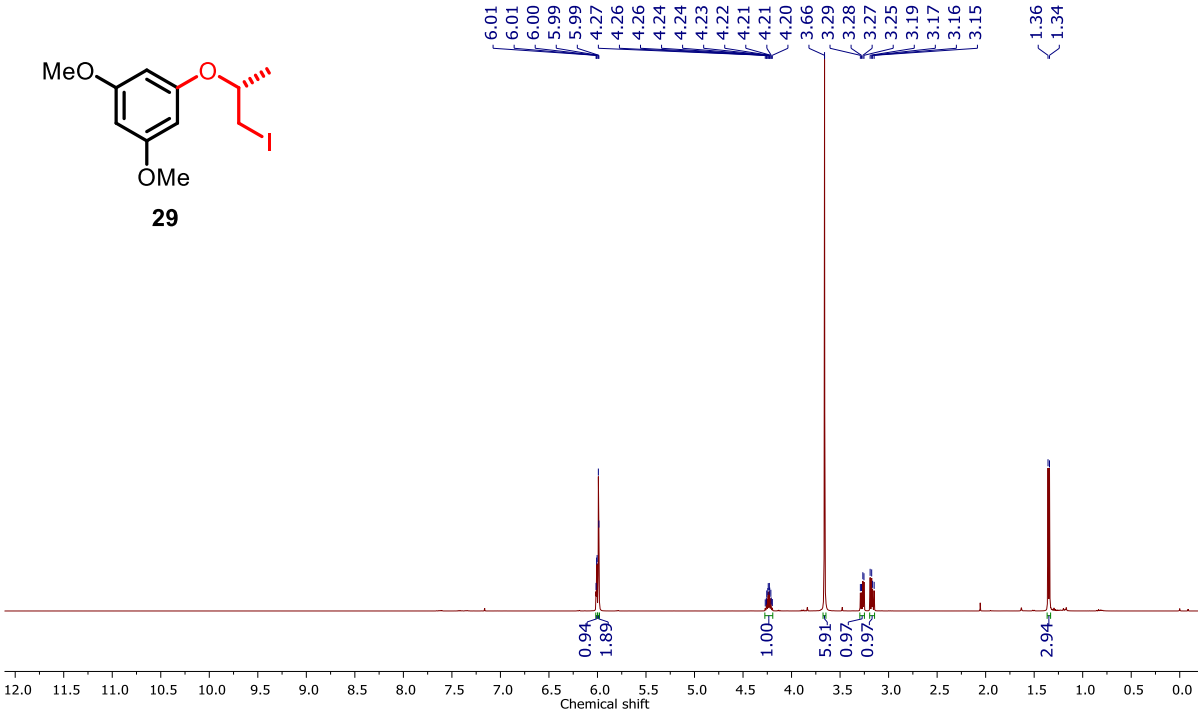
$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-2-(3,5-dimethoxyphenoxy)propan-1-ol (28)**:



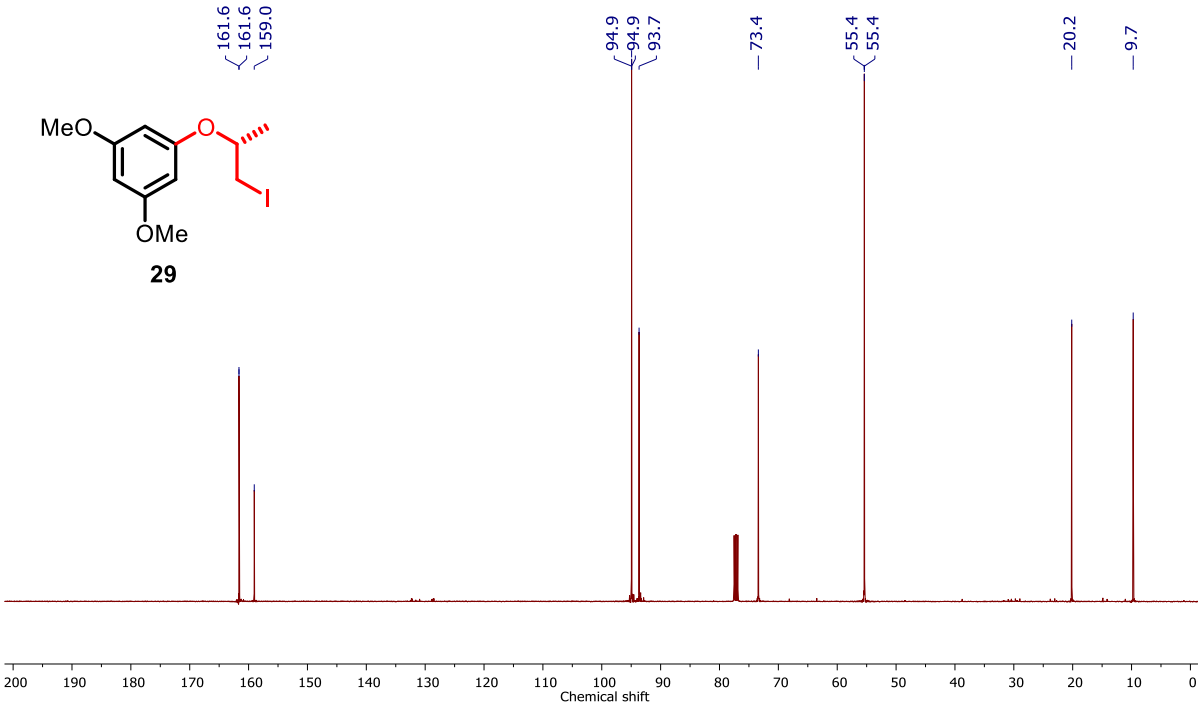

DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-2-(3,5-dimethoxyphenoxy)propan-1-ol (28)**:



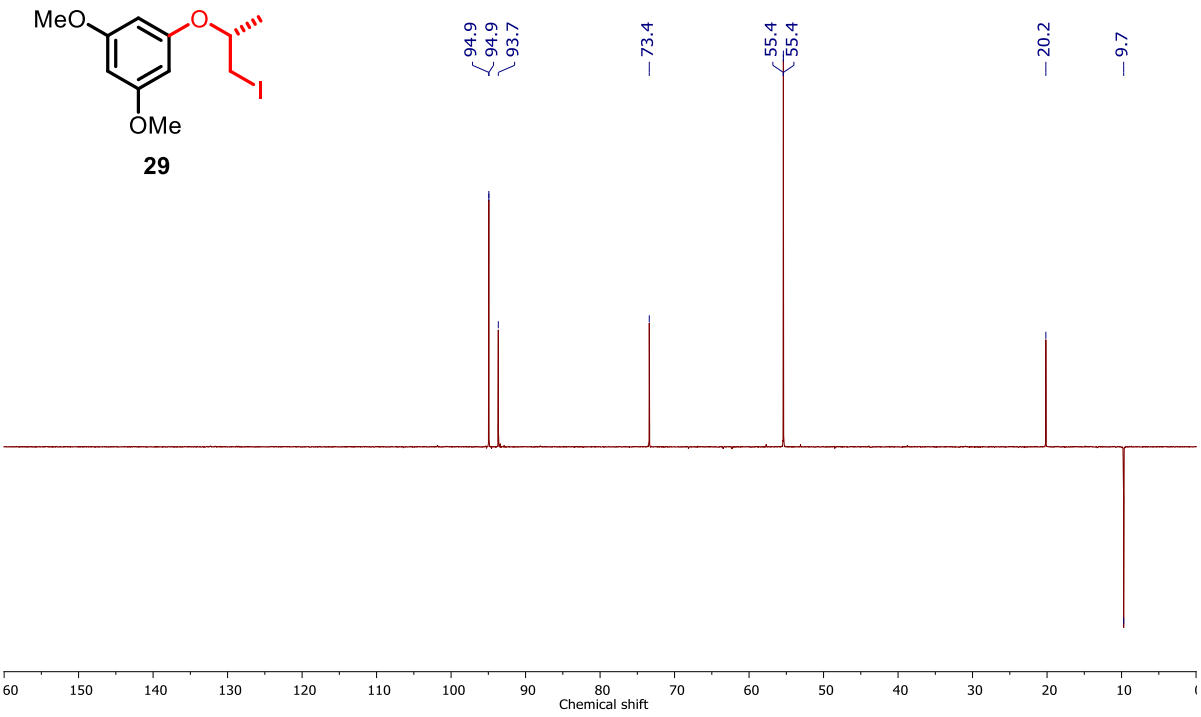
$^{1}$H NMR Spectrum (400 MHz, $CDCl_3$) of **((R)-1-((1-iodopropan-2-yl)oxy)-3,5-dimethoxybenzene (29)**:



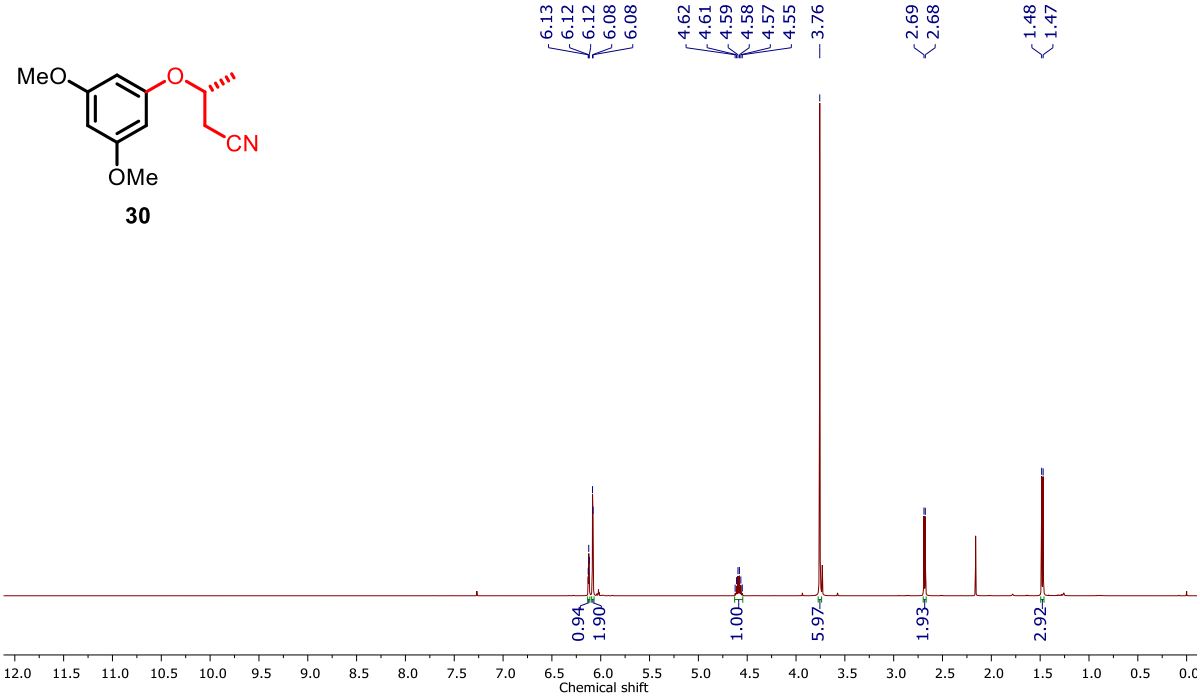

$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **((R)-1-((1-iodopropan-2-yl)oxy)-3,5-dimethoxybenzene (29)**:



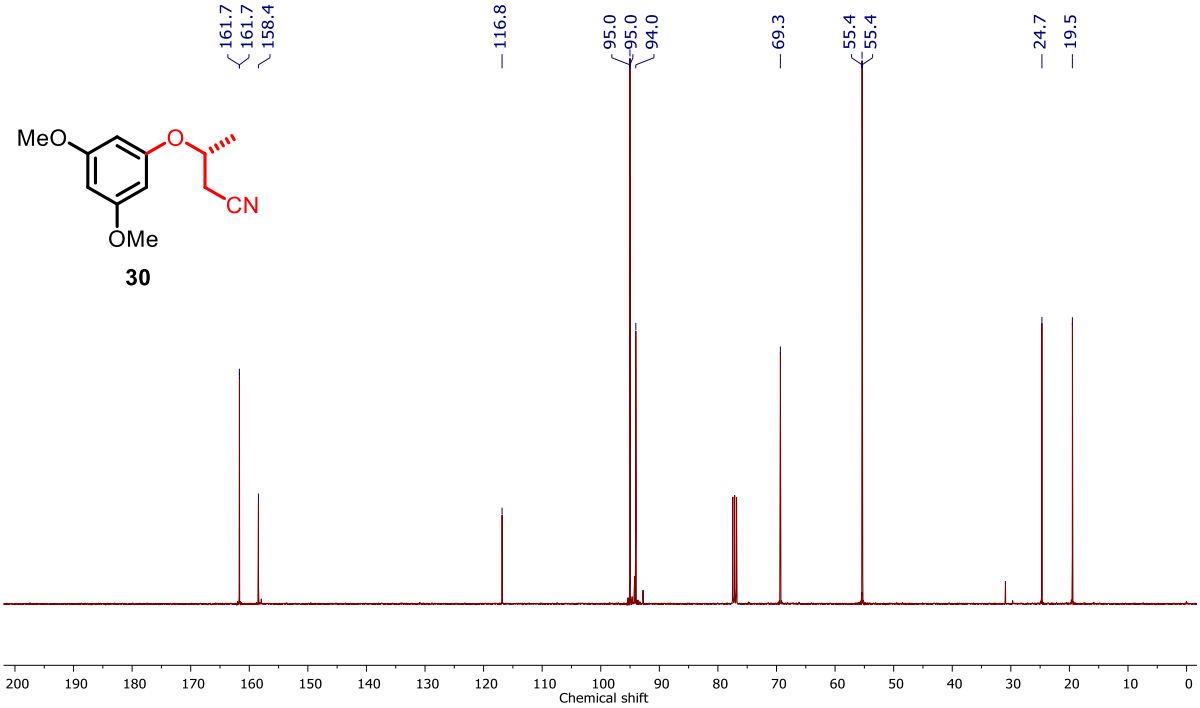
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **((R)-1-((1-iodopropan-2-yl)oxy)-3,5-dimethoxybenzene (29)**:



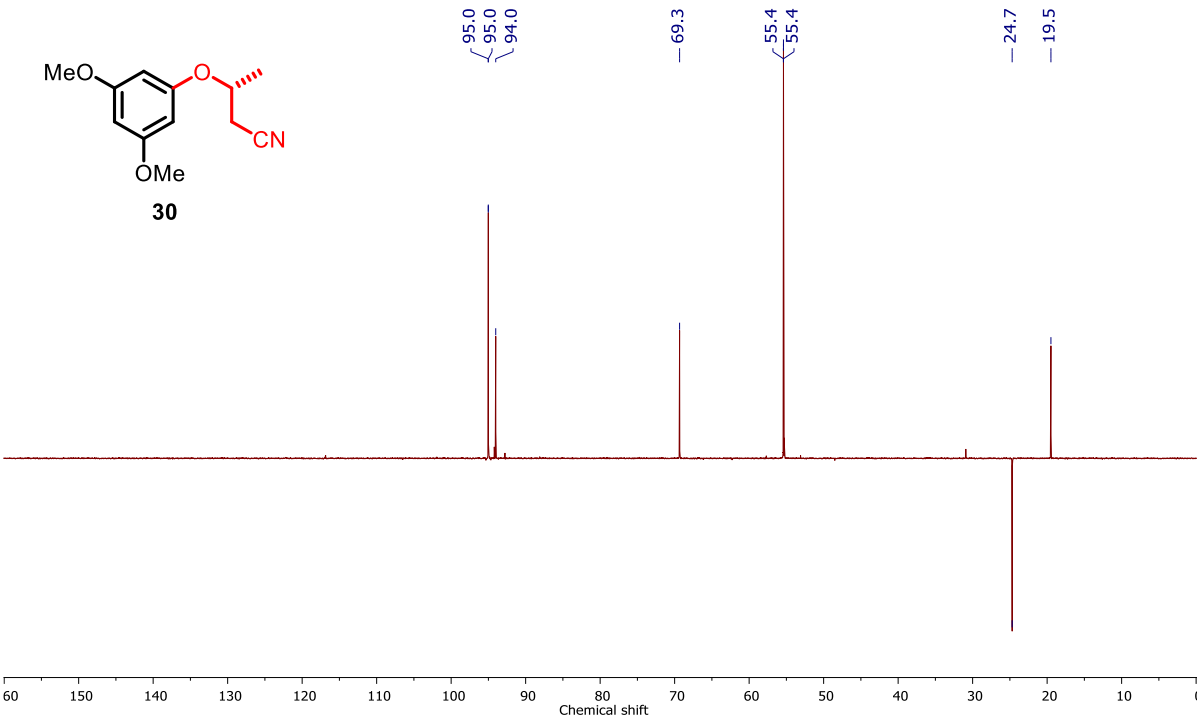

$^1$H NMR Spectrum (400 MHz, $CDCl_3$) of **(R)-3-(3,5-dimethoxyphenoxy)butanenitrile (30)**:



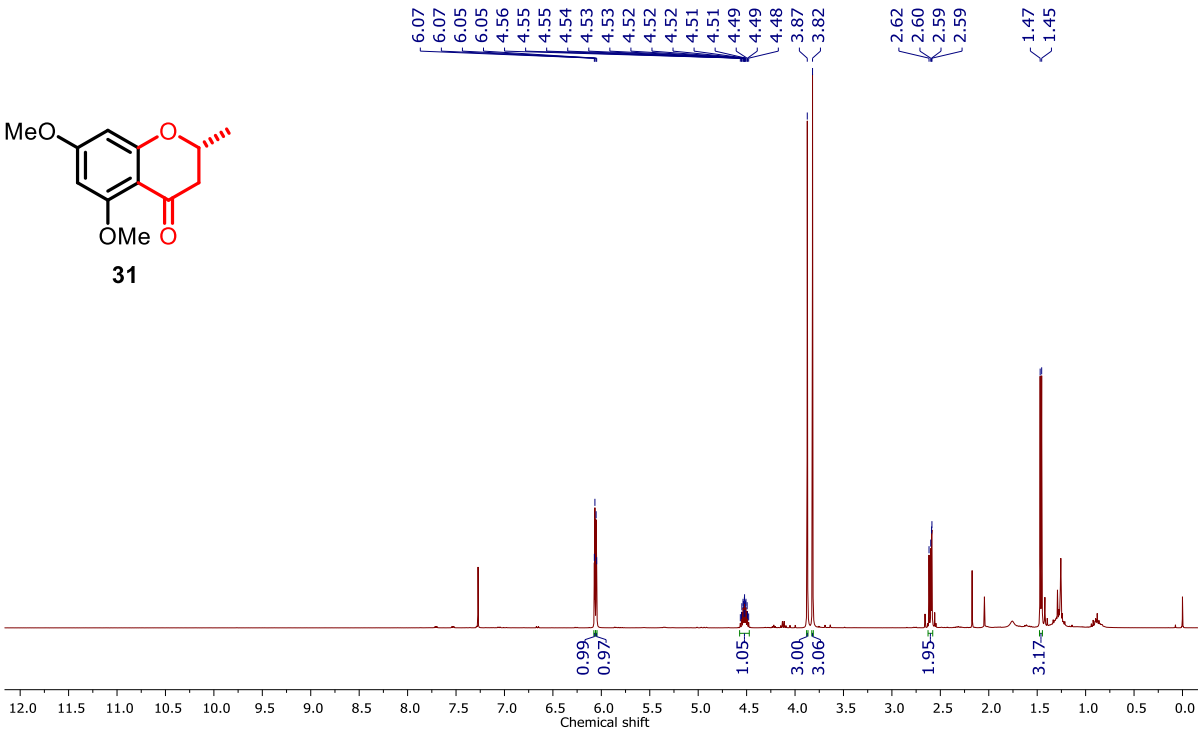
$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-3-(3,5-dimethoxyphenoxy)butanenitrile (30):**



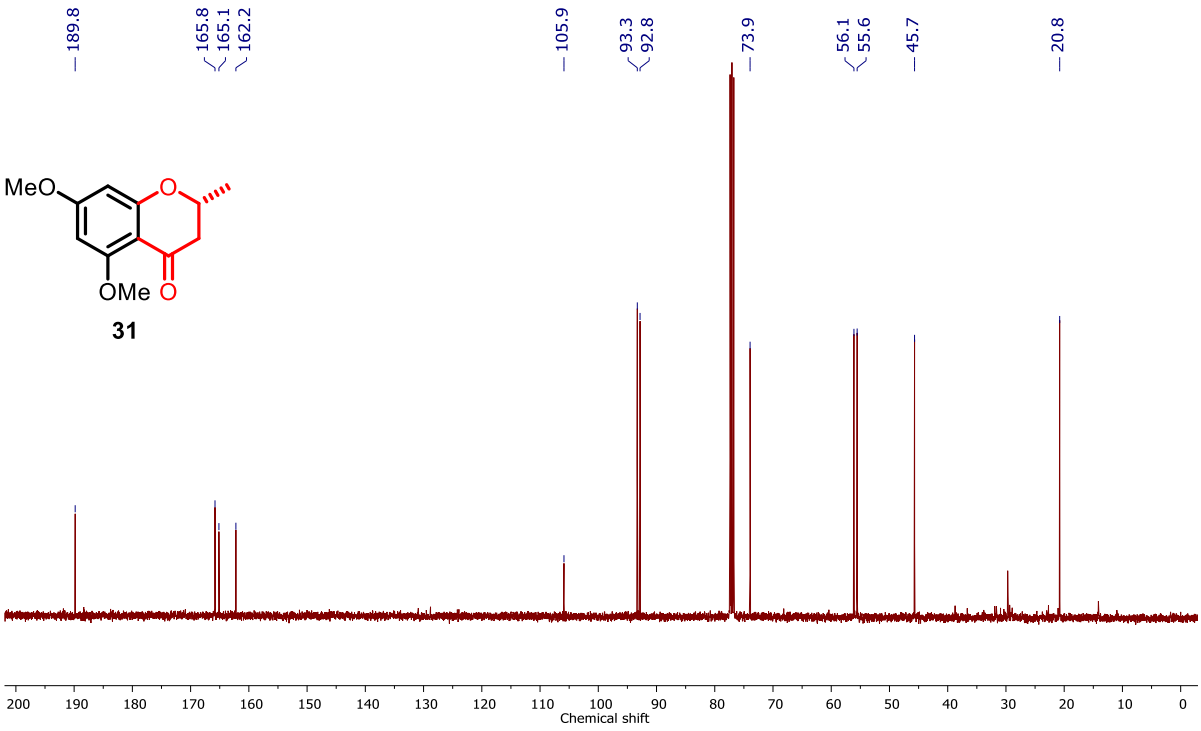

DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-3-(3,5-dimethoxyphenoxy)butanenitrile (30)**:



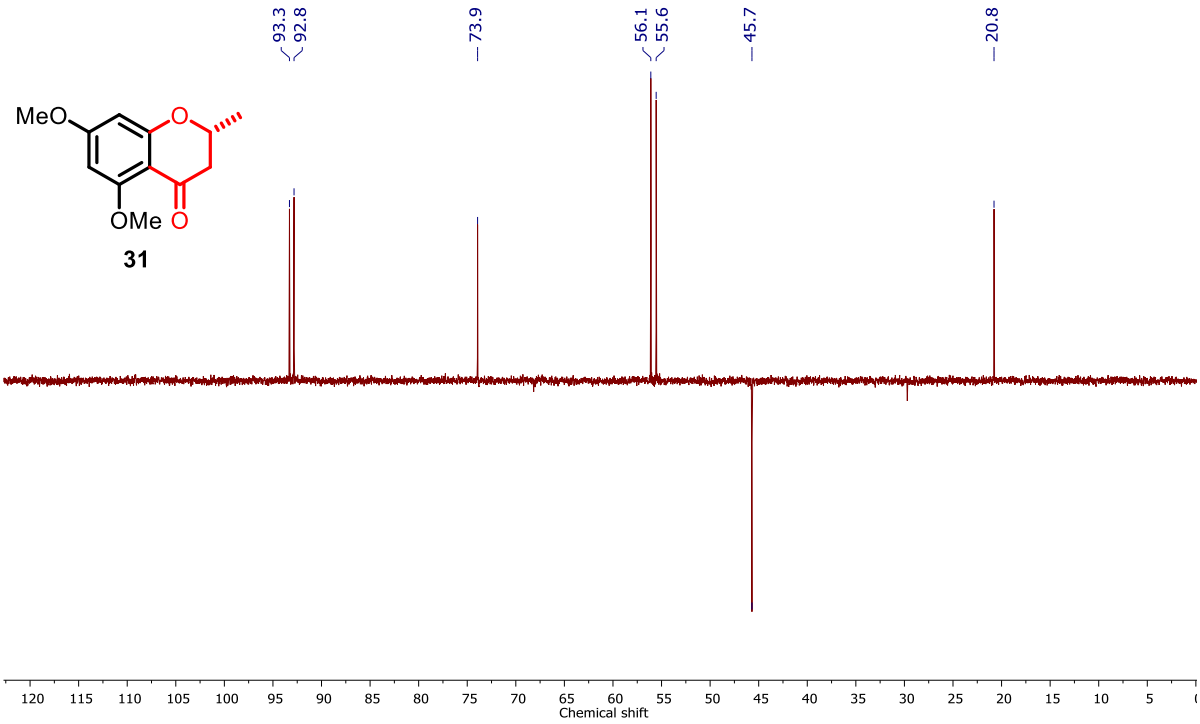
$^{1}$H NMR Spectrum (400 MHz, $CDCl_3$) of **(R)-5,7-dimethoxy-2-methylchroman-4-one (31)**:



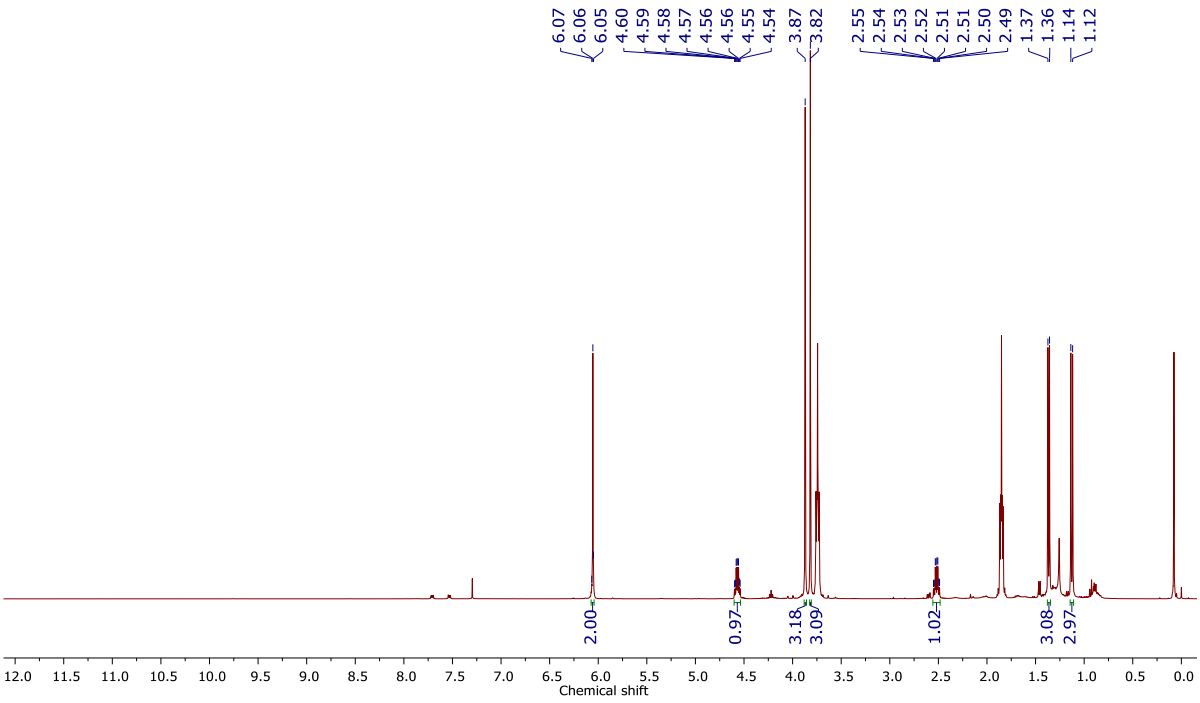

$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-5,7-dimethoxy-2-methylchroman-4-one (31)**:



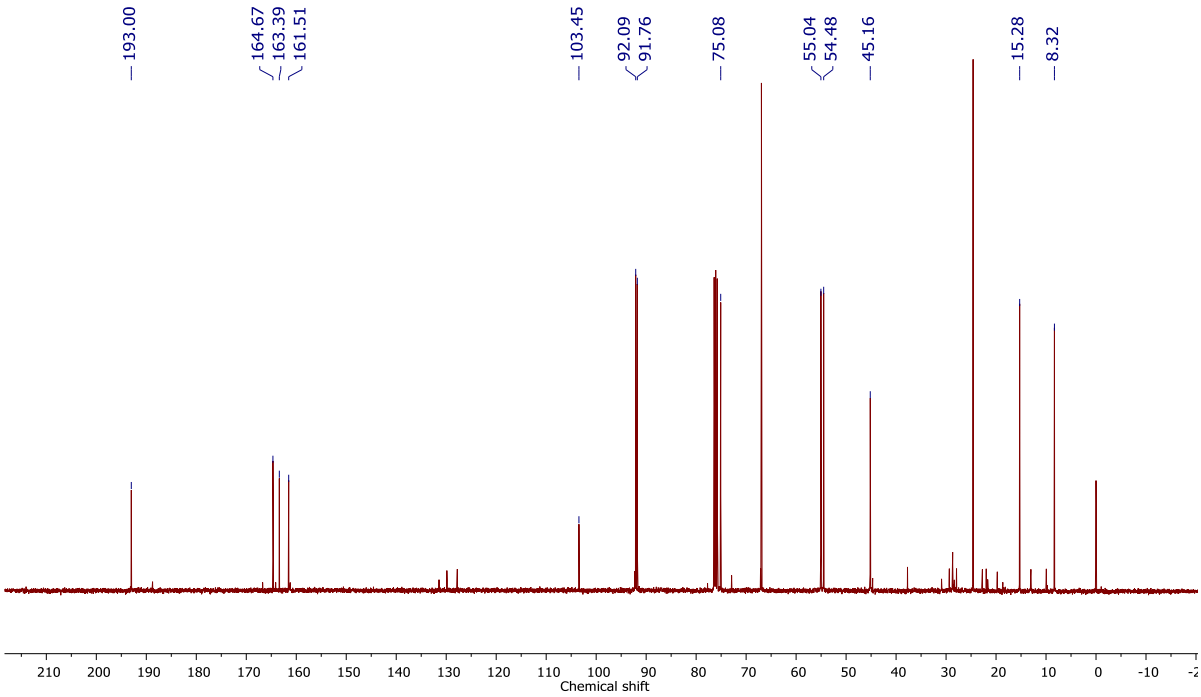
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **(R)-5,7-dimethoxy-2-methylchroman-4-one (31)**:



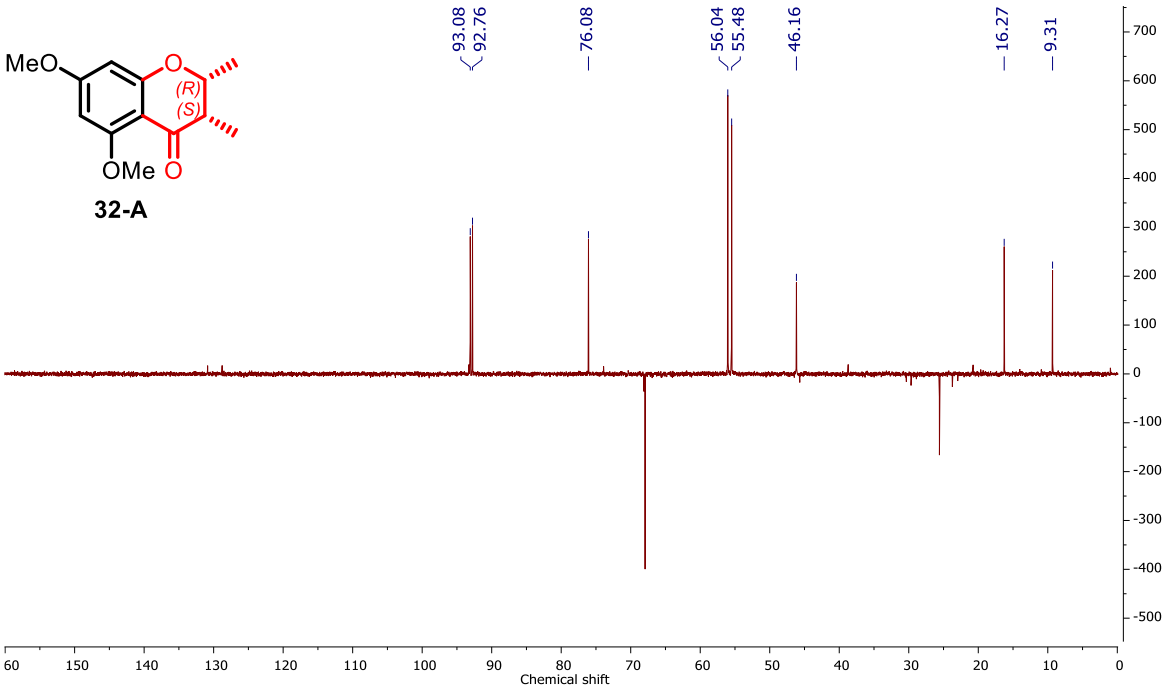

$^1$H NMR Spectrum (400 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



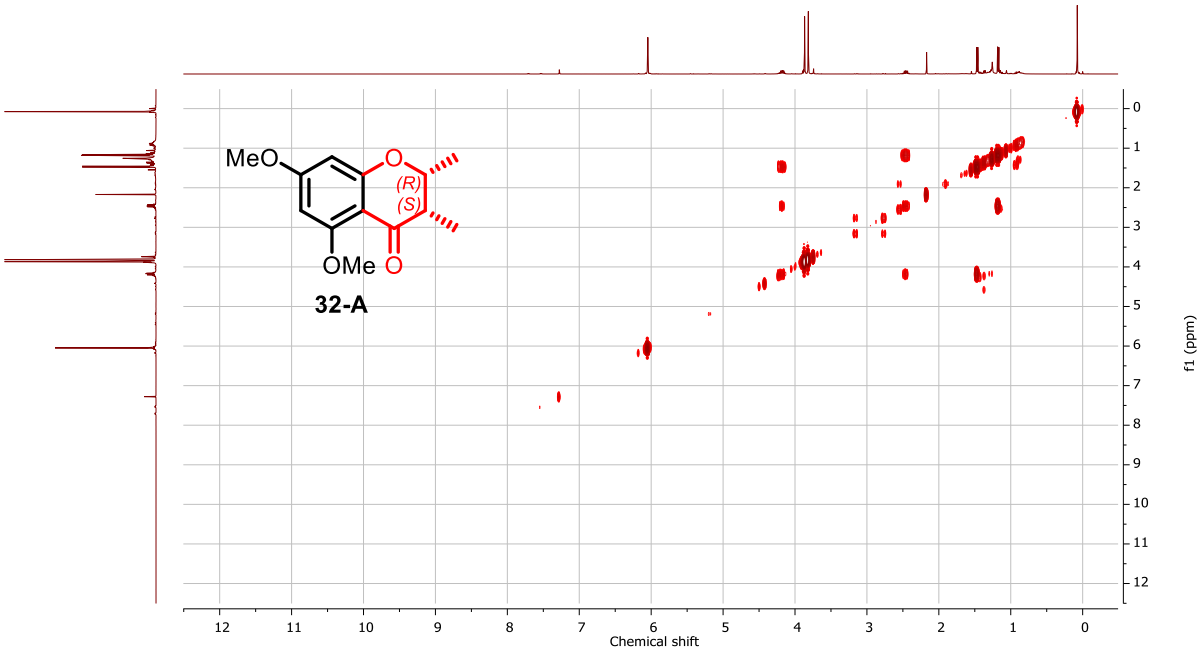
$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



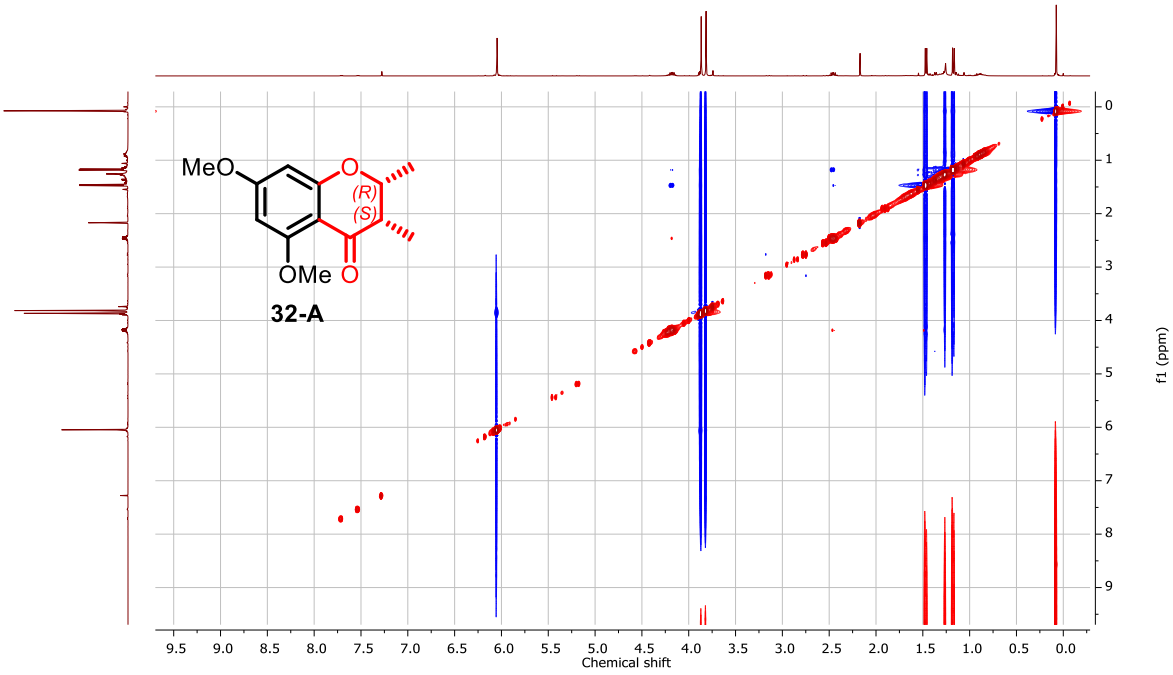

DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



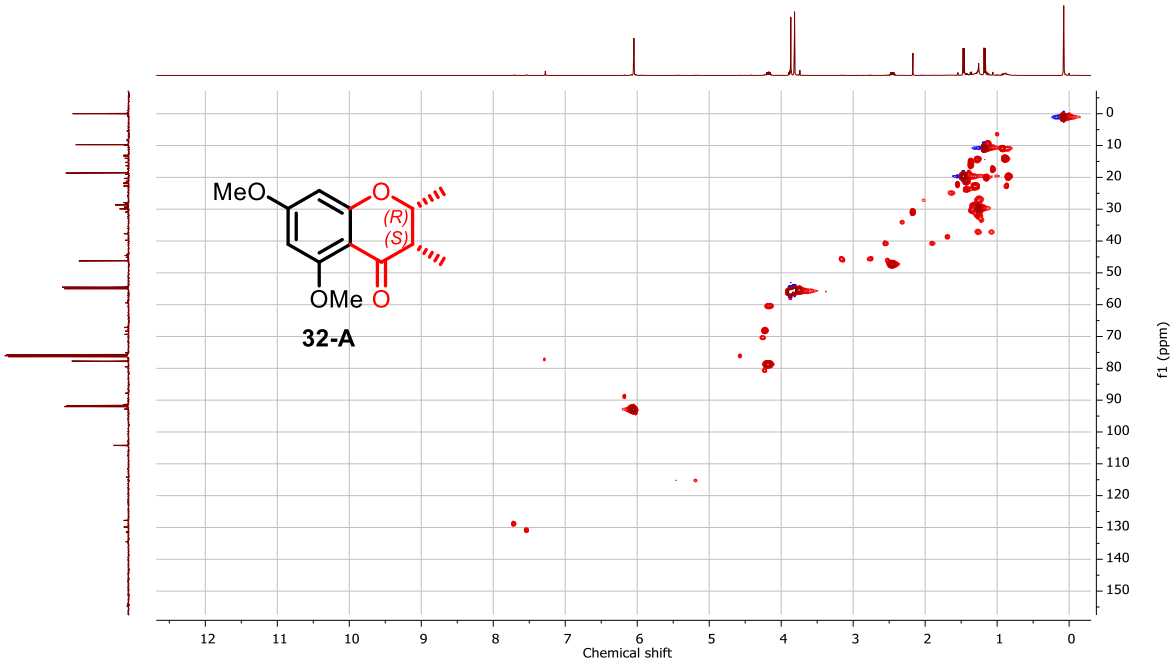
$^{1}$H-$^{1}$H COSY NMR Spectrum (400 MHz, 400 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



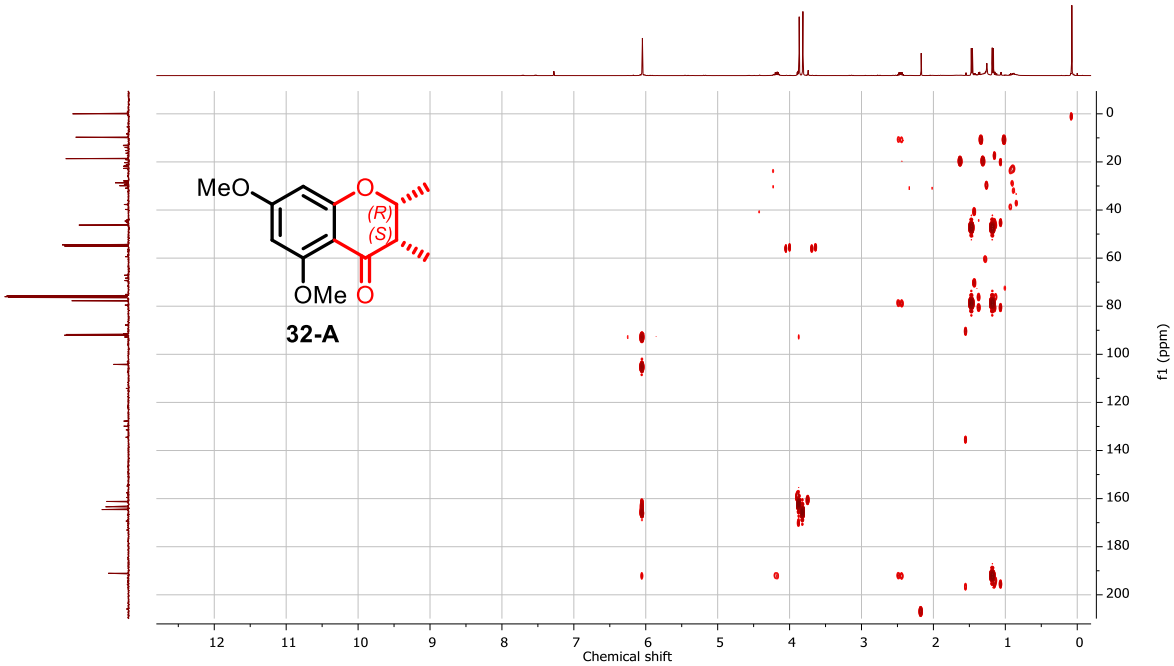

$^{1}$H-$^{1}$H NOESY NMR Spectrum (400 MHz, 400 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



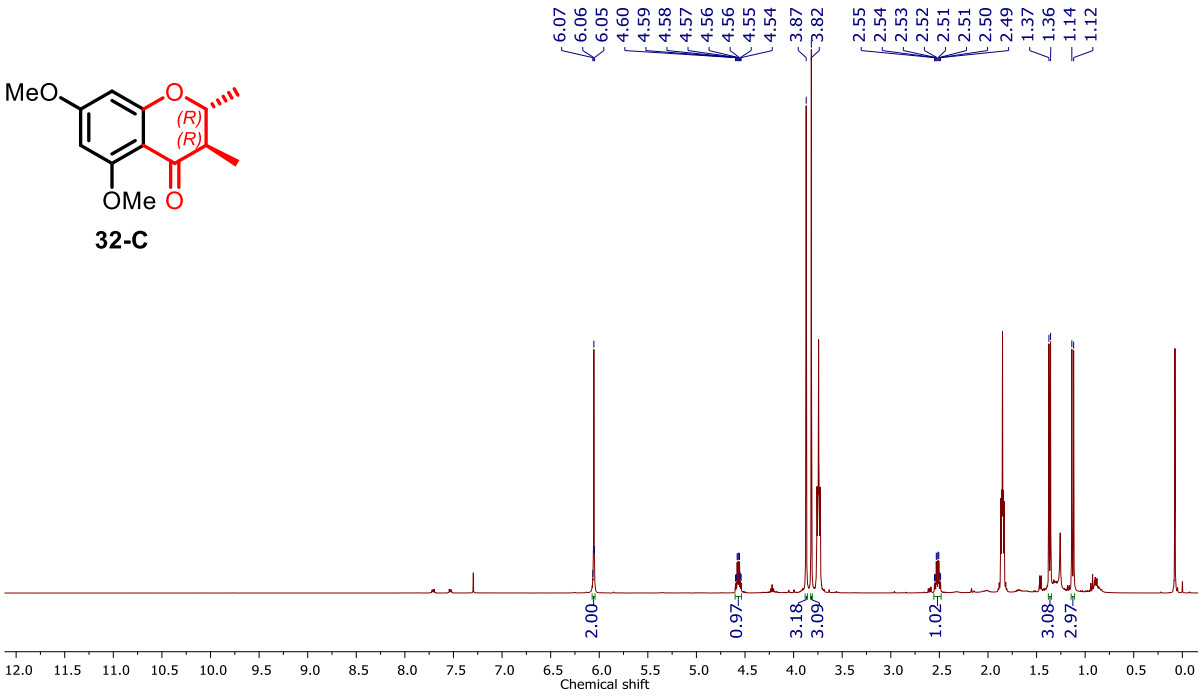
$^{1}H$-$^{13}C$ HSQC NMR Spectrum (400 MHz, 101 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



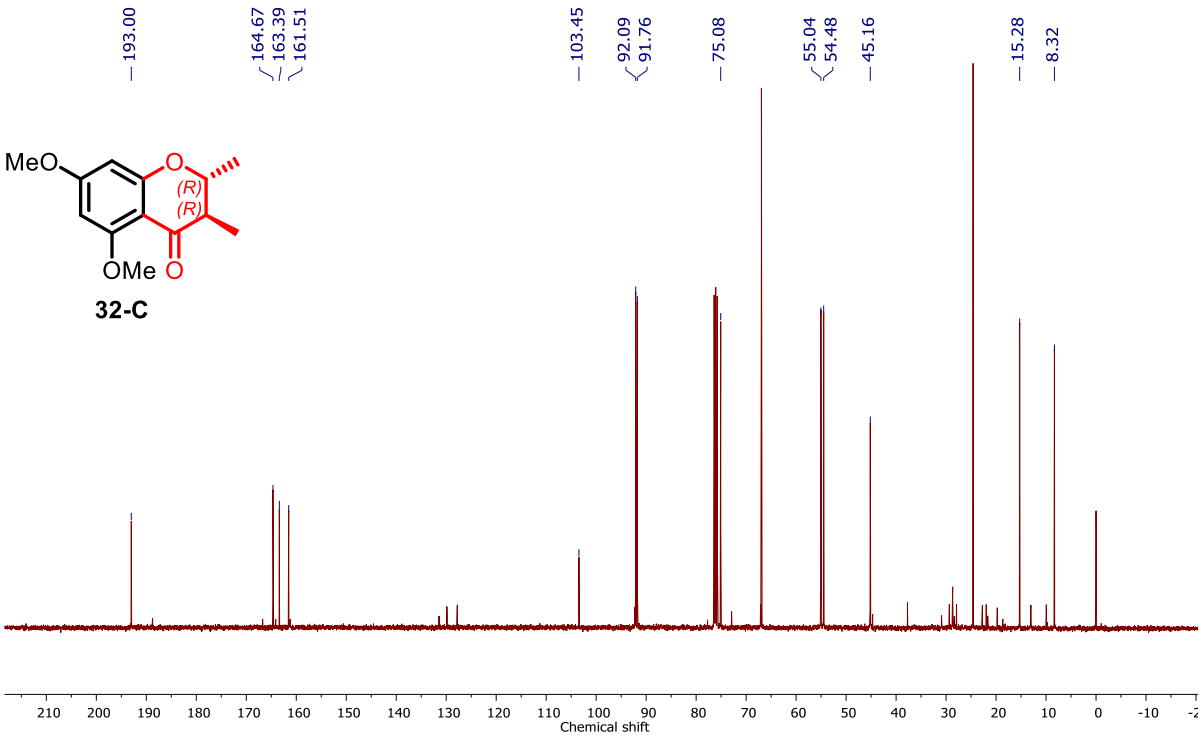

$^{1}H$-$^{13}C$ HMBC NMR Spectrum (400 MHz, 101 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-A):**



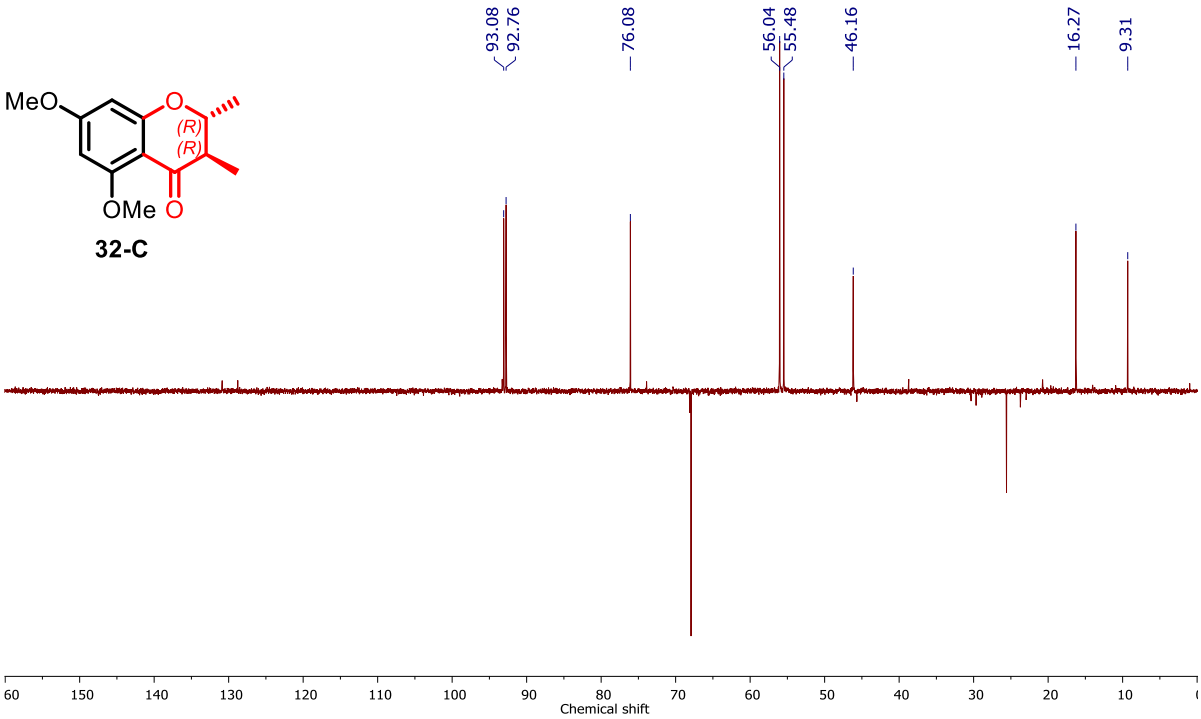
$^{1}$H NMR Spectrum (400 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**



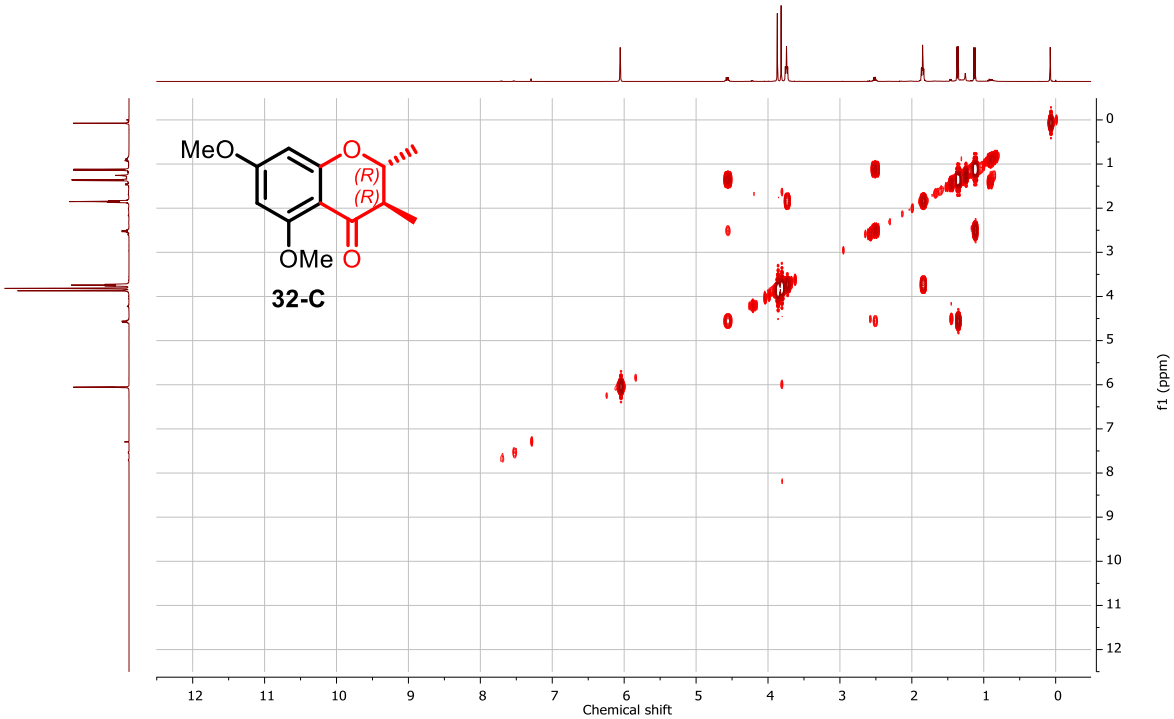

$^{13}$C NMR Spectrum (101 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**



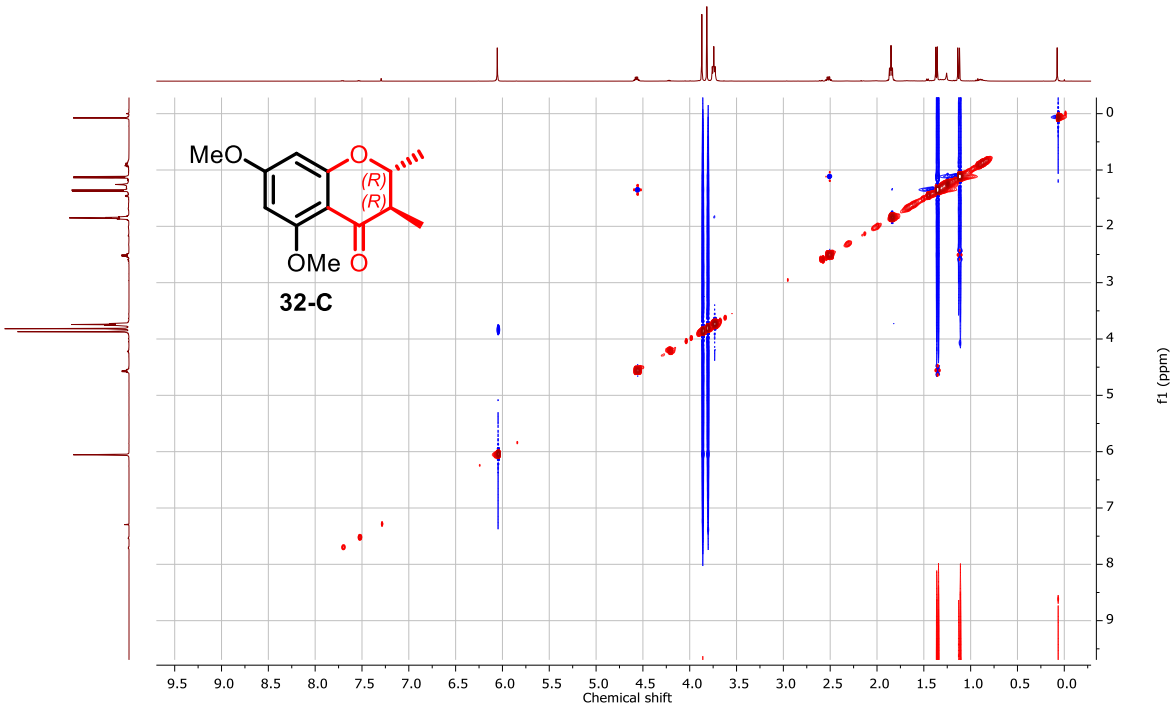
DEPT NMR Spectrum (101 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**



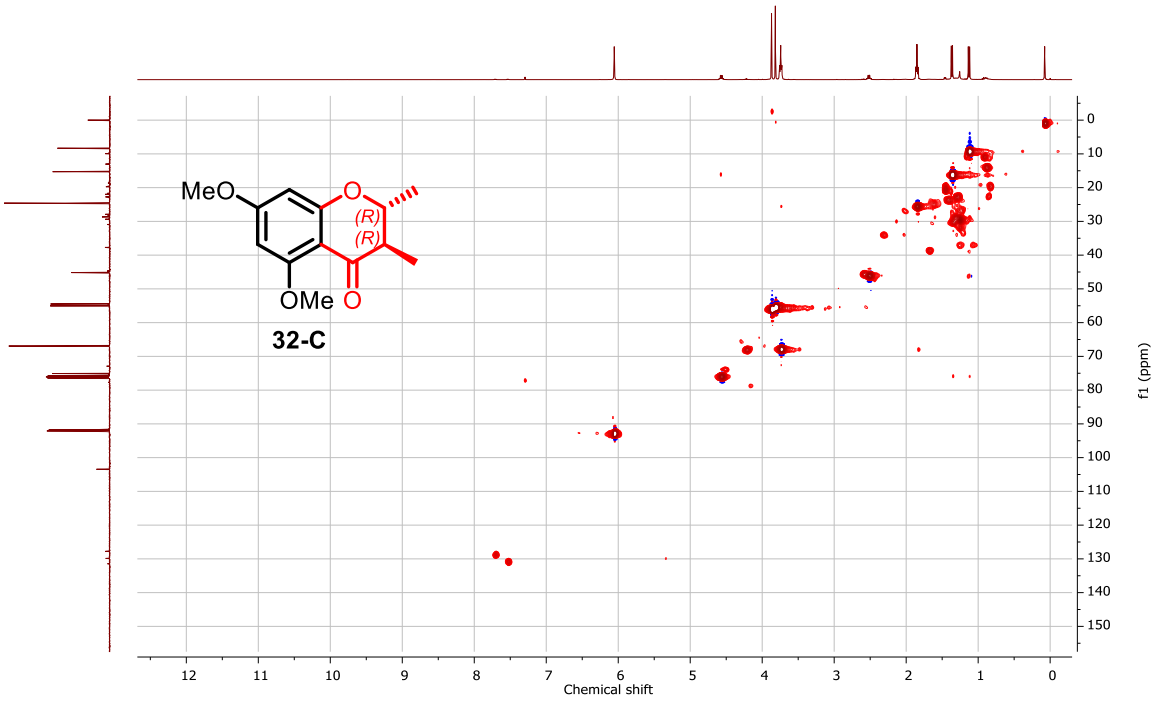

$^1H$-$^1H$ COSY NMR Spectrum (400 MHz, 400 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**



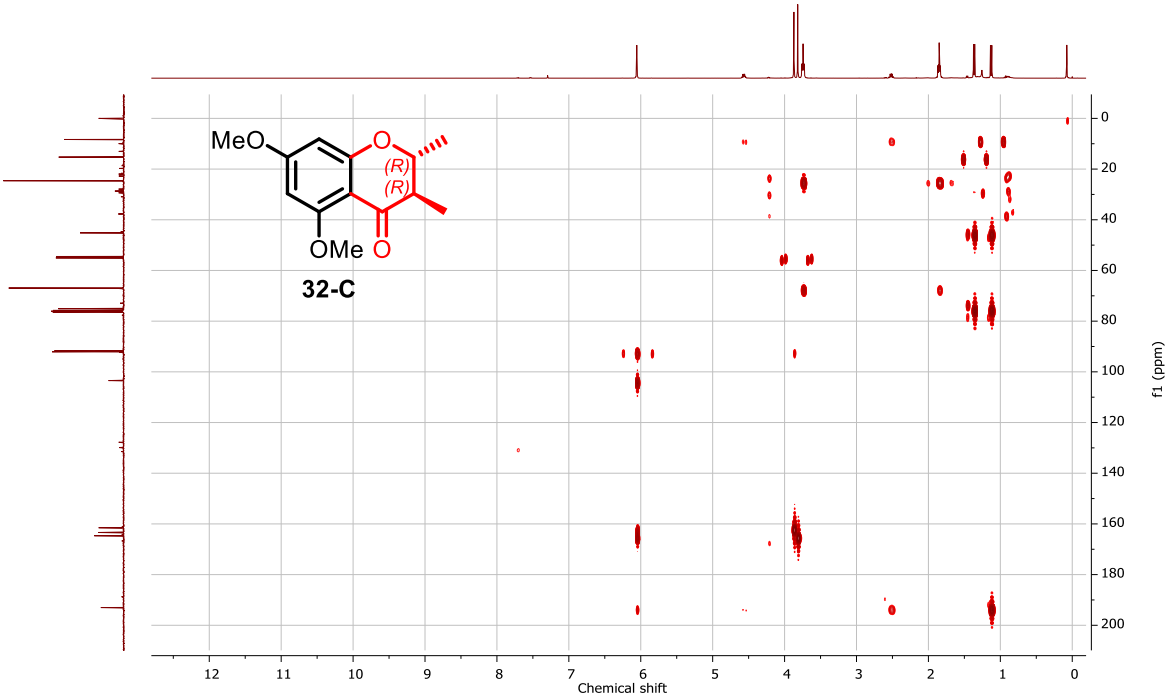
$^{1}$H-$^{1}$H NOESY NMR Spectrum (400 MHz, 400 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**




$^{1}$H-$^{13}$C HSQC NMR Spectrum (400 MHz, 101 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**



$^{1}$H-$^{13}$C HMBC NMR Spectrum (400 MHz, 101 MHz, $CDCl_3$) of **(2*R*,3*R*)-5,7-dimethoxy-2,3-dimethylchroman-4-one (32-C):**




$^{1}$H NMR Spectrum (400 MHz, $CDCl_3$) of **(2*R*,3*S*)-5,7-dihydroxy-2,3-dimethylchroman-4-one (33-A):**