



**Progress towards the total synthesis of
Nemamides, Euglenatides and related
analogues**

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Declaration

I hereby declare that any work presented within this document, except where reference to the work of others has been specified, is original and has not been submitted for any other degree or qualification at this, or any other institution.

Tyarna Francesca Smith

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Abstract

Euglenatides A-E and nemamides A and B were isolated from *Euglena gracilis* and *Caenorhabditis elegans* respectively. Both product families contain similar 5-mer cyclic peptide structures, each including a variant of graciline. Graciline is an amino acid containing a polyketide chain with multiple stereogenic centres. Variants include 2-deoxygraciline as well as others possessing modified chain lengths and saturation. Euglenatides A-D were shown to have anti-fungal effects, alongside tumour suppressing output against varying cancer cell lines. Considering the structural similarity, this makes all seven compounds possible therapeutic targets and therefore, the total synthesis of these compound families is desirable.

This thesis focuses on devising a route towards the total synthesis of these two natural product families. Synthetic routes are designed around the key disconnection of the respective graciline variant and the remaining 4-mer linear peptide chain. To allow for generation of all graciline variants, the route was designed to be modified from a late stage intermediate. Approaches outlined within utilise various chiral catalysts and auxiliaries to install the stereogenic centres, and detail initial optimisation strategies towards the synthesis of the late stage intermediate. Initial work using a Brown allylation approach proved to be inefficient, so was redesigned to include other catalytic approaches towards stereo-control. This route utilises a semi-iterative approach which includes an asymmetric aldol directed by a functionalised proline organo-catalyst, and copper catalysed hydroboration for the stereoselective formation of carbon-heteroatom bonds.

Alongside this, the use of model systems to trial the preliminary synthetic steps towards the cyclic peptides is outlined.

Further work is required to complete the total synthesis of nemamides and euglenatides, however, this thesis describes the initial synthetic work, including steps towards graciline and its variants. Preliminary findings suggest that the novel synthetic strategy described herein could lead to successful isolation of the graciline targets with high stereocontrol if substrate specific optimisation of the hydroboration reaction is performed.

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General Experimental Details

Unless stated otherwise, all materials were purchased from commercial sources (Acros, Sigma Aldrich, Alfa Aesar and Fluorochem) and used without any further purification. Reagents requiring further purification were purified *via* standard laboratory procedure dictated by Armarego and Perrin.¹ Anhydrous THF and Et₂O were precured by vertical distillation from an excess of sodium and benzophenone, under a positive argon pressure. Anhydrous DCM was achieved by Vertical distillation from calcium hydride powder under a positive argon pressure. Anhydrous DMF was purchased from Thermo Scientific in AcroSeal[®] bottles. Other anhydrous solvents were obtained by heating over appropriate drying agents and distilling onto activated 4 Å molecular sieves (MS).

Concentration under reduced pressure refers to the use of vacuum-seal rotary evaporators connected to variable vacuum lines to remove solvents from the reaction material. Room temperature (RT) refers to 20-24 °C, where the room temperature failed to meet this reactions were heated to T_{ext} = 24 °C. Denotation of ‘dried over’ refers to adding MgSO₄ until visibly dry The reaction material is achieved or stirring with Na₂SO₄ for 5-10 min, removing solid by filtration and washing with the reaction solvent. Unless otherwise stated any use of water within experimental reports refers to use of distilled water.

Flash column chromatography (FCC) and vacuum aided chromatography (VAC) was performed using silica gel (Sigma Aldrich or Material Harvest, 40-63 µm, 230-400 mesh). Thin layer chromatography (TLC) was performed using aluminium backed Machery-Nagel pre-coated ALGURAM[®] SIL G/UV₂₅₄ TLC plates. Unless stated otherwise, TLC plates were visualised under UV and/or KMnO₄, ninhydrin or phosphomolybdic acid (PMA) stain.

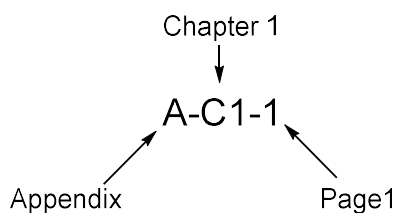
¹H nuclear magnetic resonance spectra (NMR) were recorded at 400 MHz or 500 MHz, ¹³C NMR spectra were recorded at 101 MHz or 126 MHz and ¹⁹F NMR spectra at 470 MHz using Bruker spectrometers. Samples were made up as solutions in appropriate deuterated solvent purchased from Cambridge Isotopes or Sigma Aldrich. Spectra are referenced to the residual solvent signal, as reported by Fulmer *et al.*² Chemical shifts (δ) are given in parts per million (ppm). Multiplicity of peaks is described as follows and where multiplicity cannot be determined from the spectra is referred to as a multiple (m); singlet (s), doublet (d), triplet (t), quartet (q). Where appropriate, NMR spectra assignment was based on 2D NMR data.

Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum Two Fourier Transformed Infrared spectrometer as thin films or solids compressed on a diamond plate. Melting points were attained using a Büchi Melting Point B-545. High resolution mass spectroscopy (HRMS) was performed at the University of East Anglia using AQUITY H-Class PLUS UPLC and Waters SYNAPT AS High Resolution Mass Spectrometer, where samples were ionised with positive and negative electrospray ionisation using 1 $\mu\text{g mL}^{-1}$ solutions in acetonitrile.

Crystal structure acquisition and structural model refining was performed by Dr Benjamin Hofmann using an in-house Rigaku XtaLAB Synergy-S single crystal instrument and Olex2 processing software.

Preface

- Relevant compound structures are numbered per chapter in order of first appearance; where the first number corresponds to its original chapter. When two enantiomers are discussed in text, they are differentiated using R/S after their number or +/- before their number.
- Where a snippet of a final product NMR is given in-text, the corresponding page number for the full spectrum will be provided as below.



- The numbering of compound structures in NMR assignments does not necessarily reflect the numbering of the systematic names.
- The key starting material for each experimental procedure is highlighted in bold.

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List of Abbreviations

2

2-deoxygra: 2-Deoxygraciline.

A

AA: Amino Acid.

Aib: Aminoisobutyric acid.

API: Active Pharmaceutical Ingredient.

Asn: Asparagine.

Asp: Aspartic Acid.

B

Boc: *tert*-Butyl-carbonate.

C

CBS: Corey-Bakshi-Shibata.

CD: Circular Dichroism.

COSY: Correlation Spectroscopy.

CPP: Cell Penetrating Peptide.

CSPS: Classic Solution Peptide Synthesis.

D

d.r.: Diastereomeric ratio.

DBE: Double Bond Energy.

DCVC: Dry Column Vacuum Chromatography.

DEPT: Distortionless Enhancement By Polarization Transfer.

^dIpc₂BH: (+)- α -diisopinocampheylborane.

DNA: Deoxyribonucleic Acid.

Dnv: 4,5-Dihydroxynorvaline.

E

e.e.: Enantiomeric Excess.

F

FCC: Fractional Column Chromatography.

G

Gra: Graciline.

H

HMBC: Heteronuclear Multiple Bond Correlation.

HPLC: High Performance Liquid Chromatography.

HR-LC-MS: High Resolution Liquid Chromatography Mass Spectrometry.

HRMS: High Resolution Mass Spectroscopy.

HSQC: Heteronuclear Single Quantum Coherence.

HWE: Horner-Wadsworth-Emmons.

I

IR: Infrared.

L

LHMDS: lithium bis(trimethylsilyl)amide.

M

M_r: Molecular weight.

MS: Molecular Sieves.

MTBE: ^tButyl methyl ether.

N

NMR: Nuclear Magnetic Resonance.

NOESY: Nuclear Overhauser Effect Spectroscopy.

NP: Natural Product.

NRP: Nonribosomal Protein.

NRPS: Nonribosomal Peptides Synthase.

P

PBDS: Peptide Based Drug Delivery Systems.

PK: Polyketide.

PKS: Polyketide Synthase.

PMA: Phosphomolybdic Acid.

R

ROESY: Rotating-Frame Nuclear Overhauser Effect Correlation Spectroscopy.

RT: Room Temperature. ,

S

SAR: Structure Activity Relationship.

SMD: Small Molecule Drug.

SPPS: Solid Phase Peptide Synthesis.

T

TFA: Trifluoroacetic Acid.

TLC: Thin Layer Chromatography.

TOCSY: Total Correlation Spectroscopy.

U

UV: Ultra Violet.

V

VAC: Vacuum Aided Chromatography.

X

XRD: X-ray diffraction.

W

WT: Wild Type.

Chapter 1: Introduction

1.1. Natural products

1.1.1. The what and the why of natural products

Defining the term ‘natural product’ (NP) is difficult, as it can be used to describe almost all compounds in the natural world which are produced by prokaryotes and eukaryotes. When used in science, it normally refers to complex primary and secondary metabolites of living organisms.³ Nature's great biodiversity, means that while around half a million natural compounds have been isolated and published, there is still a vast well of unexplored species with potentially novel NPs.⁴

Organisms containing NPs have been used in their virgin forms for their remedial effects throughout history. Herbs and plants would be used directly for wound healing or combined and consumed as herbal remedies. Some modern cultures, such as traditional Chinese medicine and Indian Ayurveda, still rely heavily on herbal remedies as a main source of medical treatment.³ Many of these ancient remedies do provide symptom relief and/or overall health improvements and have thus become inspiration for modern medicine.

With the advancement in scientific knowledge and analytical equipment, it is now known that some of these herbal remedies contain specific NPs that are pharmaceutically active. It is now possible to isolate specific compounds within a species, assign a structure and test its activity against multiple different diseases. Isolation of active compounds has allowed for efficient delivery of the desired medicinal properties without interference from other metabolites.⁵ Overall, this has led to reductions in toxicity, higher efficacy, and improved therapeutic profiles.

A key example of this can be seen in the development of morphine derivatives. Opium poppy seeds have been used throughout history to treat conditions such as asthma and stomach ailments, and as analgesics. Morphine (**Fig. 1, 1.1**) was isolated from the poppy plant in the 19th century, which allowed its use as a potent analgesic with reduced negative side-effects. Since the discovery of morphine, many variants on the opioid have been licenced for use in medicine, including semi-synthetic derivative such as hydrocodone (*1.2*) and oxycodone (*1.3*).

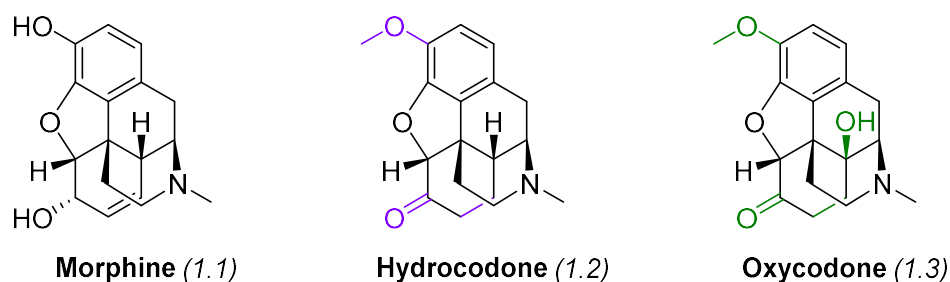


Fig. 1 Chemical structure of morphine and two semi-synthetic analogues, with the structural variations highlighted.

Generally, the natural products that solicit interest have possible applications as modern chemical-based pharmaceuticals.^{6,7} A comprehensive review by Newman *et al.*, showed that almost half of the small molecule drugs (SMD) marketed between 1981 and 2019 were either natural products, derivatives of natural products, or mimics of natural products (**Fig. 2**, segments coloured in blue).^{4,8} They also showed that out of all of the marketed anti-cancer drugs between 1946 and 2019, over half of these came from natural product origins; showing a need for continued mining of new natural products for novel drug design.

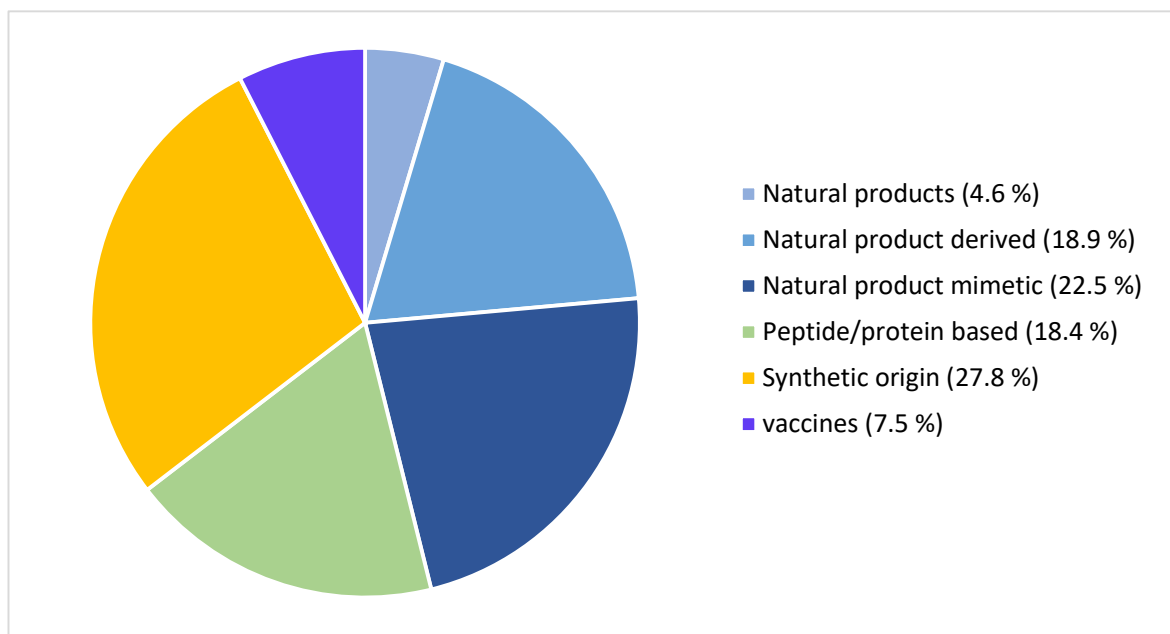


Fig. 2 A pie chart adapted from Newman *et al.*, showing the origins of small molecule drugs marketed between 1981 and 2019.⁸

The beauty of using NPs comes from their structural diversity and unique physico-chemical properties; mainly the increased frequency of sp^3 hybridised carbons aids in the goal to escape the flatlands of drug therapeutics.^{4-6,9,10}

The synthesis of natural products has historically played a pivotal role in synthetic innovation and led to understanding of some of the most fundamental principles in organic chemistry.^{11,12} E.J. Corey said that total synthesis was a ‘never-ending frontier for discovery and never-ending advances in sophistication’ and provides ‘unlimited opportunities for intellectual excitement and satisfaction’.¹³ In the 20th century, strategic design of total synthesis routes was a challenge taken on by many, but in the 21st century the time and expense necessary for total synthesis has reduced its prevalence in chemical academia and industry.¹¹

Within the pharmaceutical sector, the use of computationally aided target orientated synthesis and lead orientated synthesis is taking over drug discovery. With these computational approaches generally starting with known drugs, there is a lack of novel drug scaffolds in discovery. This lack of variability is one factor aiding the rise of drug resistance, low efficacy, and an increase in severe toxic side effects. Isolating NPs is a great way to discover new drug targets and scaffolds, which may show uses in multiple different fields of medicine.¹⁴

1.1.2. Natural product isolation and structural assignment

Natural products can be isolated from almost anything, with plants, bacteria and fungi being the most common source.⁷ Due to the complexity of nature, isolating one small molecule from a plant or microorganism can become a very long, costly, and inefficient process. Isolation begins by homogenisation of starting material, followed by liquid extraction with various solvents (**Fig. 3**). Extracting with various solvents will ensure a maximum number of products are isolated, with different groups of compounds being more soluble in certain solvents. At this point, extracts can be tested against biological assays to see which ones contain active compounds. This initial extract testing is not always carried out. However, if the goal is to find novel drug therapeutics, then it can streamline the full process by aiding an early decision on which extracts are worth spending more resources purifying. The active extracts will contain a large variety of components, so are purified by sequential columns or preparative high performance liquid chromatography (HPLC) to isolate multiple products in quantities normally lower than 100 mg. Purified compounds can then be tested to see if they have any desired medicinal properties. At this point, early spectroscopic data will be compared against a variety of databases to find out which ones are novel. The novel NPs can then be tested against a multitude of biological assays to decide whether they have any desired medicinal properties. Primary assays will generally focus on a very broad range of tests, concentrating on areas of major concern. The common initial test will often include drug resistant bacteria, viral and fungal strains, as well as a variety of cancer cell lines. These initial results show which compounds are worth investing further time and resources in.

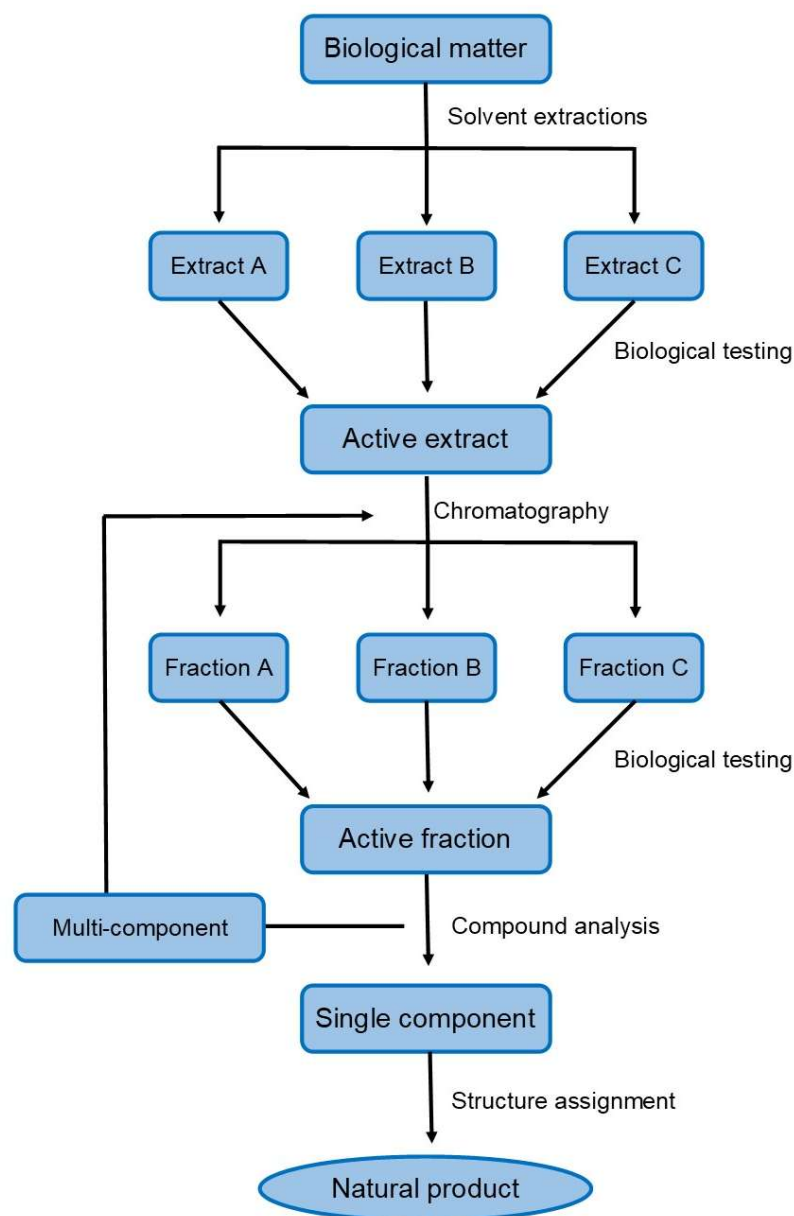


Fig. 3 A brief diagrammatic summary of the process of natural product isolation.

Compounds that show promising results are then needed in much larger quantities to perform secondary testing, which focuses on narrowing the investigations down to specific forms of pathogen or disease. To synthesise the compounds, full characterisation must be carried out and a preliminary structure assigned.

The same features that make NPs useful, also complicate the assignment process.⁶ Typically, assignment will begin with mass spectrometry to give the molecular weight (M_r) and ionisation fragments. From these data, a molecular formula can be determined, and the

double bond equivalent (DBE) can be calculated using **Equation 1**, which can be used to assign the degree of saturation of the NP.



$$\text{DBE} = \text{C} + \text{H}/2 - \text{X}/2 + \text{N}/2$$

Equation 1 Calculation to work out the double bond equivalent of organic compounds, using its known molecular formula, Where X = all halogens.

Next, possible functional groups can be assigned from IR spectroscopy. Alone, these two methods are not very useful, but when combined with NMR spectroscopy a preliminary structure can be assigned.

The proton and carbon environments can be assigned using coupling constants and chemical shifts of the ^1H and ^{13}C NMR spectra, and integration values in the ^1H NMR spectrum. Distortionless enhancement by polarization transfer (DEPT) NMR spectroscopy can be used to easily differentiate primary and tertiary carbons from secondary and quaternary environments. Along with 2D ^1H - ^1H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) NMR experiments, which together with the mass spectrometry ionisation data can be used to generate multiple different fragments of the compound.

Further 2D NMR experiments, such as heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) can be used to piece together the fragments into one preliminary structure. On some occasions, where the NP can be crystallised, x-ray diffraction (XRD) is also used. This can be extremely useful as it can give details on the stereochemistry. However, crystallising isolated natural products can be very challenging, especially when isolated in such small quantities. Therefore, the assignment is generally confirmed by chemical synthesis.

1.1.3. Total synthesis of natural products

The preliminary structure assignment provides an initial target for synthesis. Using this structure, a retrosynthetic analysis can be performed to develop theoretical synthetic pathways.

Due to the complex structure of NPs, the forward synthesis often involves multi-step synthesis to create fragments that are bound together in a convergent synthesis. Sometimes it is not possible to fragment a target in such a way and the synthetic route will consist of one long linear reaction sequence. Generally, a convergent synthesis approach is a better method as it allows for easier adaptations to the route if reactions fail. The presence of multiple stereogenic centres complicates the chemistry and can lead to failures of initial routes. During NP synthesis, the designed route will constantly be adapted based upon failed reactions or isolation of incorrect isomers/enantiomers. This means that it is very hard to put a timeline on NP synthesis.

Structural confirmation is a crucial step in natural product synthesis. Following every step, the product should be analysed to confirm that the desired intermediate has been made. The final molecule will then be submitted for identical analysis to the naturally isolated sample. A comparison of the two sets of data will either confirm or rule out the initial assignment. Upon confirmation of the correct structure, the material can be made in bulk to allow for complete biological testing and structure derivatisation.

It is not uncommon for the synthetic data to not match the wild type data. Assuming that the intermediates have been correctly made at each step, this would suggest that either the final step has failed, or the preliminary structure assignment was incorrect. In the event of a failed reaction, the final step can be altered, or the route modified. If the final step succeeded but the data still did not align, then it can be surmised that the initial assignment was not correct. The similarities and differences in the two sets of data can be noted and then a new structure theorised from this information.

Quite often, the structural mistake involves the configuration of a stereogenic centre, which can often be corrected by epimerization or varying the chiral catalyst used in synthesis. Other times, a structural moiety has been assigned to the wrong point, which would likely require a whole new retrosynthetic design, to produce a regioisomeric product.

Recently the total synthesis of metacridamide B (*1.4*) was reported by Gosh *et al.* (**Fig. 4**).¹⁵ During comparison with the natural data, they stated that the initial elucidated structure was reported incorrectly. They surmised that the configuration at one single position had been erroneously reported, and provided a new proposed structure of metacridamide A (*1.5*) which was confirmed by Yoshida *et al.*, when they carried out the total synthesis of the newly assigned structure.¹⁶ The initial stereochemistry of metacridamide B was assigned through spectroscopic correlations, showing that while analytical tools have improved, errors can still be easily made during the assignment process.

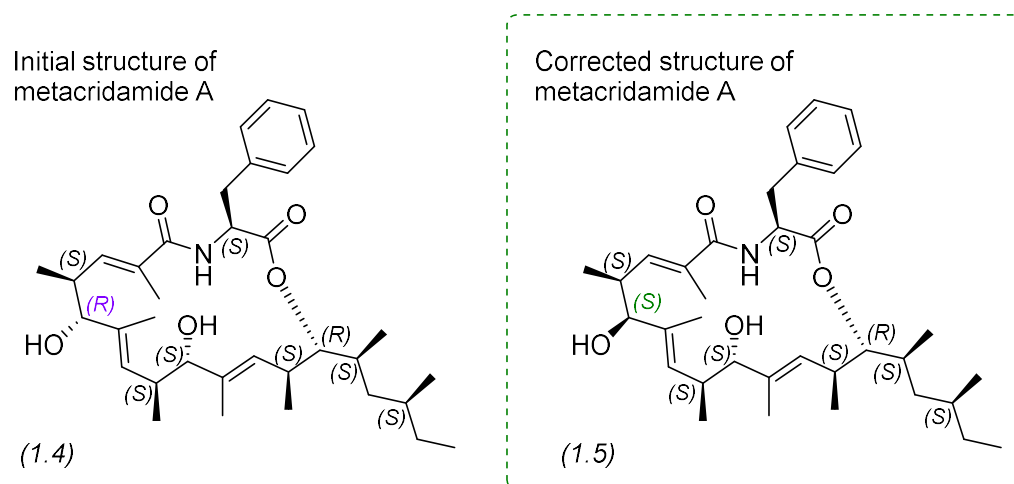


Fig. 4 A comparison of the initial elucidated structure of metacridamide A with the corrected structure confirmed by total synthesis.

Similarly, Hu *et al.* recently published the total synthesis of stemara-13(14)-en-18-ol (*1.6*), revealing that the initial elucidated structure was misassigned by Garbarino and Molinari in 1990 (**Fig. 5**).^{17,18} Tantillo *et al.* utilised computational methods to re-calculate the structure, showing a possible mistake in four of the initial assigned stereocentres and providing a new proposed structure of stemara-13(14)-en-18-ol (*1.7*).¹⁸ Currently their reassignment is theoretical and still needs to be confirmed by total synthesis. Due to the change in multiple stereocentres, the synthetic route may need to be completely reimagined and could take some time before complete confirmation.

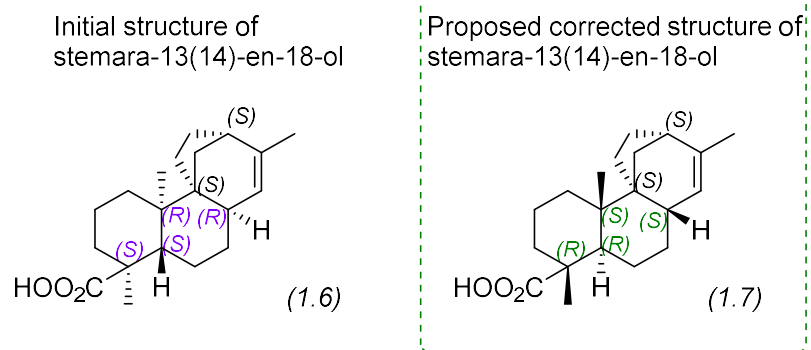


Fig. 5 A comparison of the initial manual elucidated structure of stemara-13(14)-en-18-ol against the computational theoretical structure.

1.2. Natural peptides

1.2.1. Peptides and the human body

Peptides are a chain of amino acids (AA), which the human body uses for signal transduction and as the building blocks of enzymes (**Fig. 6**).¹⁹ There are around 500 known naturally occurring AA, with only 20 of those (a mere 4 %) regularly found within the human proteome (**Fig. 7**).²⁰ All of the proteogenic AA are α -amino acids, meaning the -NH_2 group is attached to the carbon one bond away from the carbonyl. With the exception of the non-chiral glycine (1.12) and the sulfur containing cysteine (1.25), the proteogenic AA are naturally found in the (*S*)-configuration. Even with a limited supply of AA variability, the body can produce millions of different peptides. Peptide chains fold in different ways due to electronic interactions between the side-chain groups causing secondary structures, including α -helices and β -sheets. The peptides can further fold into tertiary structures and/or interact with other peptide chains to form quaternary structures, which are both classified as proteins.

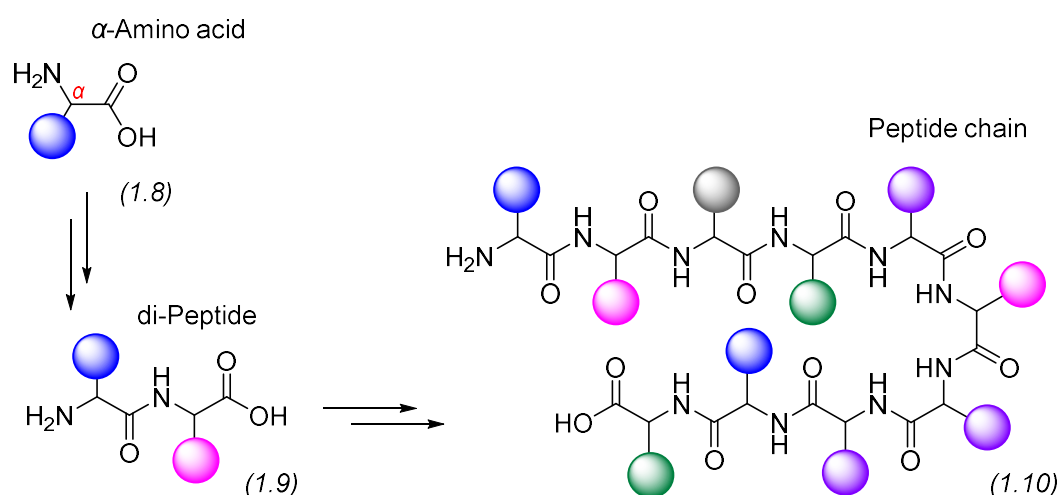


Fig. 6 A generalised structure of an α -amino acid (1.8), which can be coupled to form a di-peptide (1.9) and further coupled either sequentially or modularly to form a desired peptide chain (1.10) with desired side chains indicated as coloured beads.

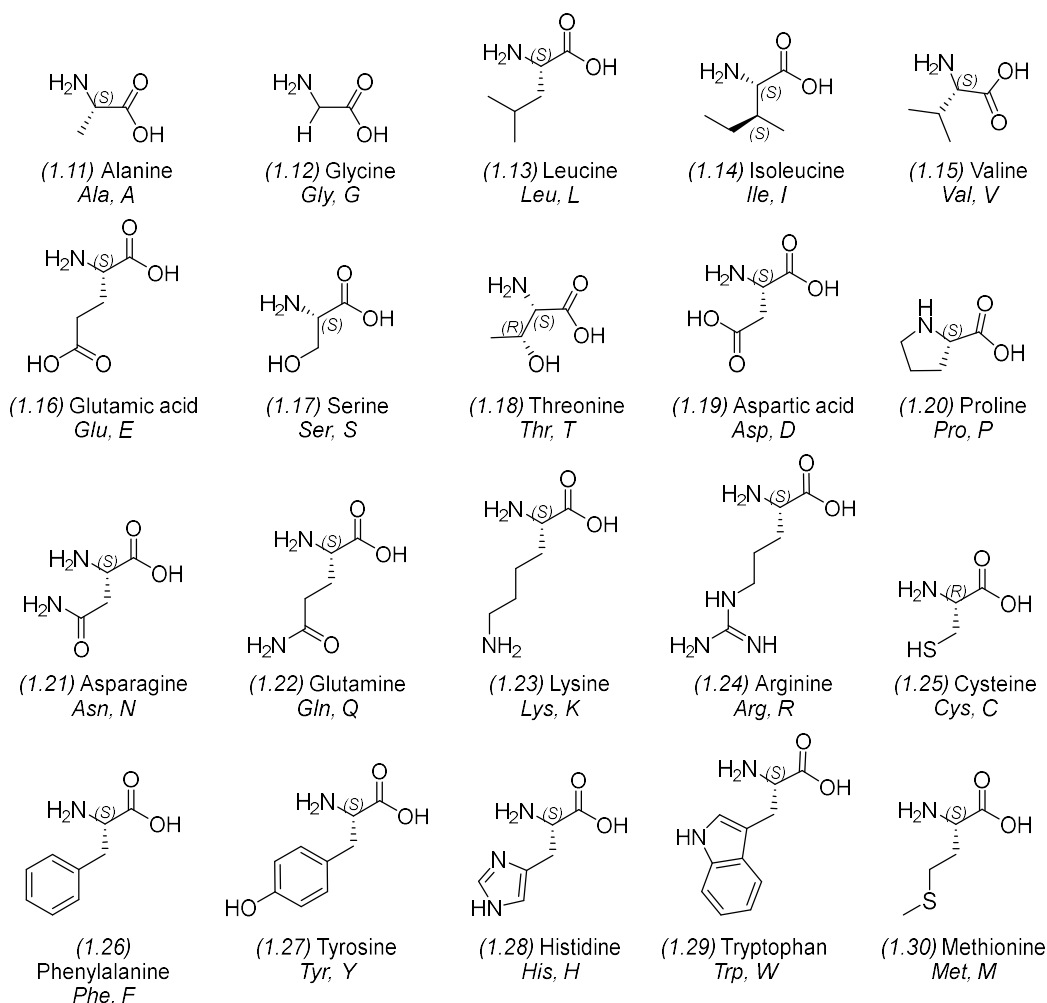


Fig. 7 The main 20 proteogenic amino acid structures alongside their names and standard abbreviations.

During the folding process, protein pockets can form which become key areas for interactions with other bioactive compounds, including disease modulators (**Fig. 8**). Disease modulating proteins are often selected as therapeutic targets for drug discovery. Knowledge about the interactions of specific substrates with these protein pockets allows for understanding of disease progression. More specifically, the ways in which side-chain groups orientate themselves within pockets to recognise and interact with specific disease modulating substrates can allow for the design of targeted therapeutics. These therapeutics can be used to block binding of the disease modulators (**Fig. 8**) alongside enhancing binding of other bioactive compounds.

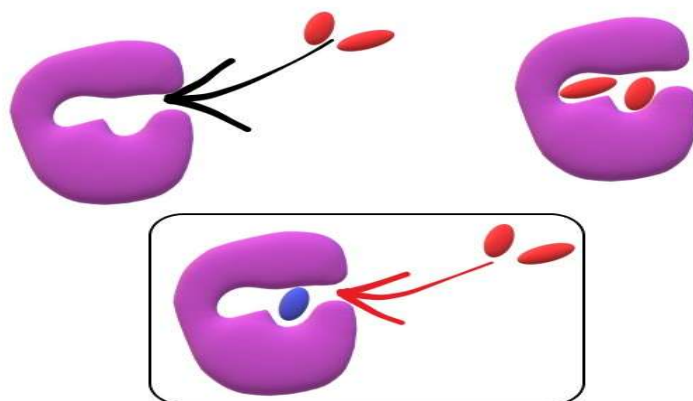


Fig. 8 A cartoon figure of a folded protein with a pocket that allows interactions between bioactive compounds (red). Targeting the pocket with specially designed drugs (blue) changes the accessibility of this protein pocket, which blocks binding of the bioactive compounds.

Natural products that are peptide or protein based offer an exciting avenue for therapeutic intervention, due to their precise physiological recognition. Their natural characteristics lead to high selectivity, efficacy and tolerability, alongside low tissue accumulation and toxicity.²¹ As a result of their low bioavailability and rapid bodily clearance by protease enzymes, unfortunately most of them show poor drug-like qualities.

Formulating a peptide drug for injection administration is the current standard for reducing these problems. The drug is administered directly into the bloodstream, which bypasses the primary metabolism and allows for rapid delivery to the target site. However, oral dosing is much more convenient and cost effective, so improving the drug-like qualities of peptide therapeutics is important.

1.2.3. Cyclic Vs linear peptides

As discussed previously, human peptides are normally linear chains which fold into secondary structures, but many natural product peptides are cyclic. Cyclic peptides are known to be much more stable than their linear counterparts due to their comparative rigidity and lack of *-N* and *-C* termini.²² The structural rigidity also allows for the AA residues to be positioned in specific spatial arrangements which have a reduced likelihood to reorientate into unproductive binding conformations, promoting higher binding affinities and longer lasting binding potentials.²³

Cyclic peptides have been reviewed extensively, one such review by Park *et al.* discusses the superiority of cyclic peptides over their linear counterparts, including their increased cell permeability, higher resistance to proteolytic cleavage and their improved target affinity; and briefly touch on their biomedical applications to increase cell uptake when used as drug delivery systems.²⁴ With cyclic peptides showing improved target specificity and uptake when compared with their linear counterparts, it is clear that cyclisation could be a good technique for optimisation of peptide therapeutics.²⁵

There is a vast amount of research going into the use of cyclic cell penetrating peptides (CPP), not just as lone drugs but also as peptide-based drug-delivery systems (PBDS) to improve translocation of drugs across cell membranes.²⁶ This is done by attaching a known drug to a CPP through a chemical linker (**Fig. 9**), which can be designed to break apart when inside the cell membrane and release the active drug compound. Examples of the success of such compounds have been shown in multiple studies, including one in 2013 by Parang *et al.* where they used a cyclic peptide containing repeating units of tryptophan (W, **Fig. 7, 1.29**) and arginine (R, **Fig. 7, 1.24**), known as [W(RW)₄], bound to doxorubicin through the linkage seen in **Fig. 9**.²⁷ Comparison of the cyclic PBDS (*1.31*) to the linear conjugate and lone doxorubicin showed increased cell uptake within tumour cells. This represents a promising study for improving the cellular delivery of cancer therapeutics, which could help reduce the need for high drug loading in patients and subsequently lower the chance of toxic side-effects of oncodrugs.^{27–29}

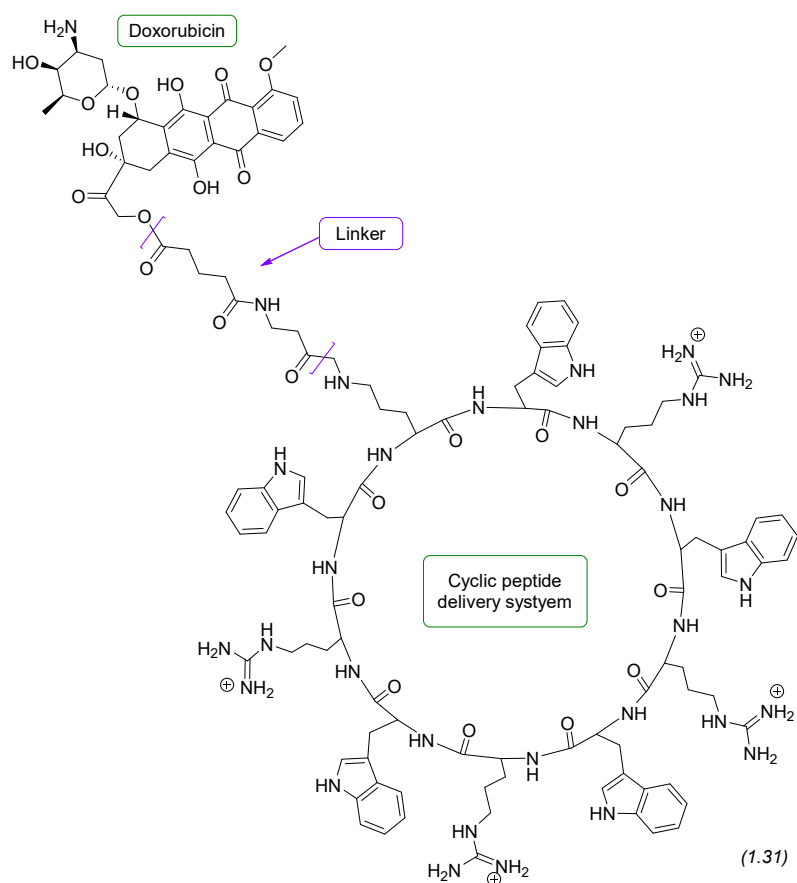


Fig. 9 Peptide based drug delivery system, where doxorubicin, is chemically bound to a cell penetrating peptide using a chemical linker.

1.3. Euglenatides and Nemamides

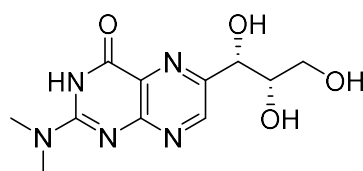
1.3.1. Euglenatides

Euglenatides are a group of cyclic peptide-based natural products isolated from *Euglena gracilis*, which is an abundant freshwater microalga from the genus *Euglenoids*.

Algae refers to a diverse collection of organisms that photosynthesise and/or have plasmids.^{30,31} Eukaryotic algae are a polyphyletic group that have evolved from multiple different phylogenetic lineages and are not related by commonalities in their DNA.^{32–34} The extreme diversity of algal lineage means that quantifying the number of known species is not possible, although it has been said that there could be over one million species.³⁵

Some of the known species release toxic compounds to prevent predation and parasitism, which ensures their ability to rapidly reproduce.³⁶ This has previously proved to be a shortfall in research, as their harmful blooms can be toxic to both humans and local marine life.³³ However, utilising the toxic blooms for their natural properties could be key to finding new solutions to drug resistant strains of bacteria, viruses and fungi. In recent years, some species have been explored, and their metabolites have exhibited antioxidant, anti-inflammatory, anti-melanogenic, antibacterial, and antiviral activities.^{7,37} Considering the vast numbers of algal species, it seems like algae could be a never-ending source of natural metabolites to harvest, and therefore is an opportunistic avenue to explore.

E. gracilis is already commercially grown as a source of lipids, carbohydrates and vitamins in food supplements.³⁸ Until recently, the only secondary metabolite with a reported structure was euglenapterin (**Fig. 10**, 1.32).³⁹ With the complex genome sequencing of *E. gracilis* showing over 30,000 protein-encoding genes, it was assumed that *E. gracilis* has many more useful metabolites that are yet to be discovered and new methodologies were required to isolate these.⁴⁰



(1.32) Euglenapterin

Fig. 10 The chemical structure of euglenapterin, previously the only secondary metabolite isolated and identified from *E. gracilis*.

Recently Aldholmi *et al.* developed a new medium to culture *E. gracilis* which showed an increase in novel metabolites in the crude biomass.³⁸ The initial method used did not yield much more than chlorophyll and xanthophyll, and the addition of small molecule epigenetic factors did not show any improvements. Both of these original culture growth methods used a mixture of amino acids, some of which have previously been shown to inhibit growth of *E. gracilis*.⁴¹ Tryptone and yeast extract were removed as nutrient sources and replaced with glutamic acid as the sole nitrogen source.

Mohammed Aldholmi *et al.* cultured approximately 18 L of *Euglena gracilis* in glutamic acid dense medium, which was lysed and extracted to provide 1.7 g of residue.³⁸ Initial purification by fractional column chromatography provided 187 mg of the compound mixture, which was carried through multiple sequential cycles of semi-prep HPLC to isolate five different NPs, which they termed euglenatides (**Table 1**). With minimal amounts of all five samples, the characterisation effort focused on the highest yielding euglenatide (B) and a comparative analysis of data to characterise the other four compounds.

Euglenatide	A	B	C	D	E
Mass isolated/ mg	2.6	5.7	2.8	2.3	2.4

Table 1 The isolated masses of each of the five euglenatides isolated from *E. gracilis*.

Following the use of IR spectroscopy, HRMS and an assortment of 1D and 2D NMR spectroscopy methods, the structure of euglenatide B was elucidated as a 5-mer cyclic peptide containing an aliphatic chain. Each of the amino acid groups was assigned using a combination of 1D and 2D NMR spectroscopy, suggesting the presence of two asparagine (Asn) residues (**Fig. 11**, *1.21*). Assignment of the configuration of the two Asn residues was not possible *via* XRD, so microscale hydrolysis to the corresponding aspartic acid (Asp) was carried out. The hydrolysate was then subjected to Marfey's analysis, showing the presence of one (*S*)-Asn (*1.21-S*) and one (*R*)-Asn (*1.21-R*). Three non-proteogenic amino acids were also found, including β -aminoisobutyric acid (β Aib, *1.33*), 4,5-dihydroxynorvaline (Dnv, *1.34*) and a novel amino acid. The novel amino acid, which was named graciline (Gra, *1.35*), included four stereogenic centres and a long polyketide containing aliphatic chain (**Fig. 11**).

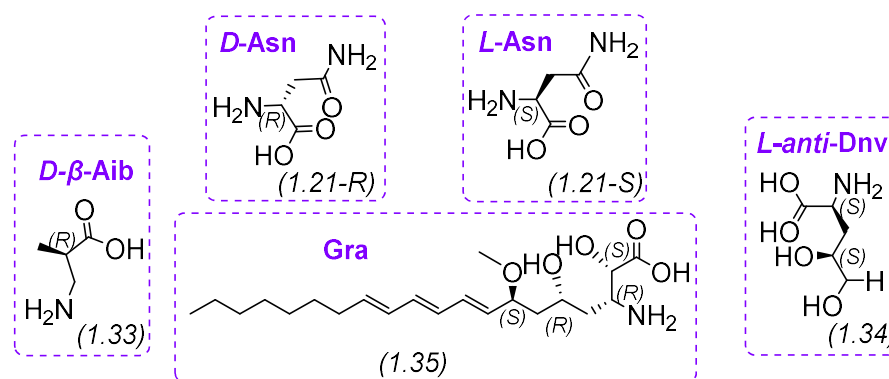


Fig. 11 The 5 amino acids that build up the euglenatide B.

Utilising all the collected data, the order in which the amino acids were coupled together was determined, giving a structural elucidation of euglenatide B (**Fig. 12**, 1.36), which was then used to help determine the structures of A, C, D and E (**Fig. 13**).

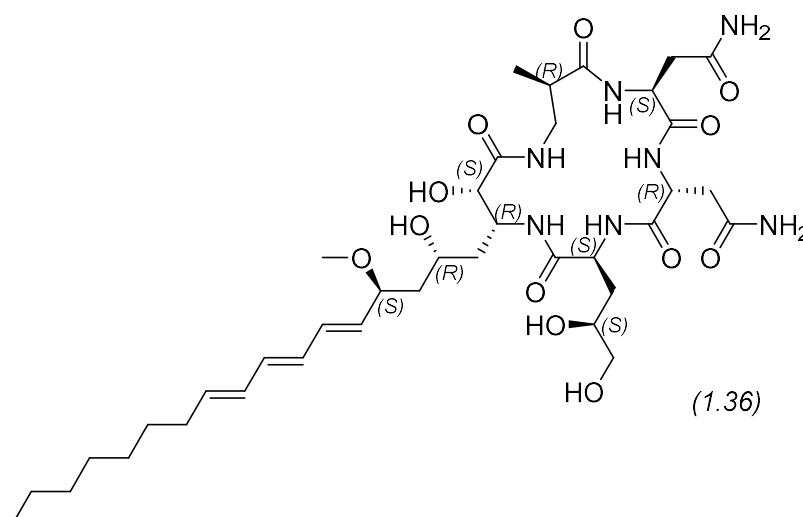


Fig. 12 The proposed structure of euglenatide B.

Euglenatide C (1.37) showed a variation in the Dnv residue (R^2) (**Fig. 13**), switching out the terminal proton for a methyl group with undefined configuration. Euglenatide E (1.38) showed a mono-hydroxy variation in the novel Gra residue (R^1), which was labelled as 2-deoxygraciline (**Fig. 14**, 1.41). Euglenatide A (1.39) and D (1.40) showed more differences from their other family members, with the characterisation data suggesting only one Asn residue, which was characterised as *D*-Asn. In place of the second Asn residue was a second *L-anti*-Dnv residue. Alongside this, euglenatide D contained the 2-deoxygraciline residue (R^1) variation, as in euglenatide E.

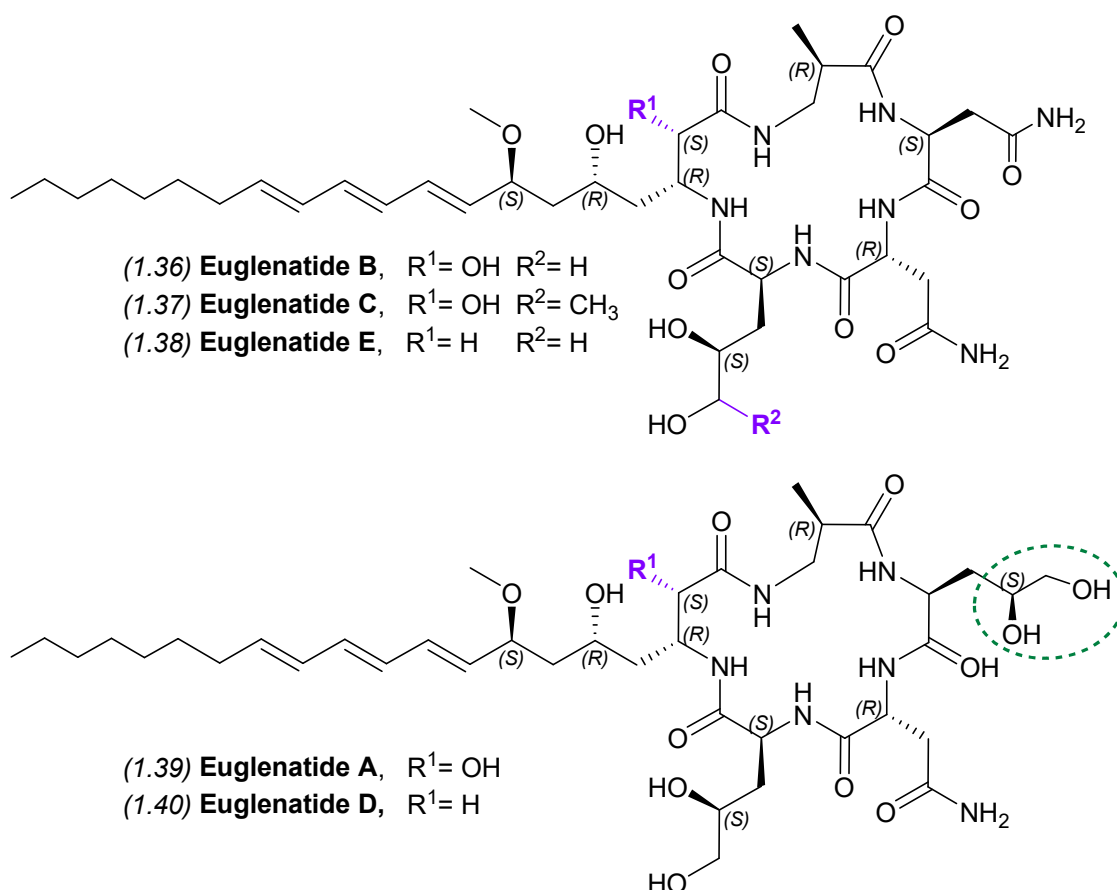


Fig. 13 The general structure of euglenatides B,C and D (top), which contain variations in R^1 and R^2 positions, and euglenatides A and D, showing a second *L-anti*-Dnv residue in replacement of asparagine (green) and variations in the R_1 position (bottom).

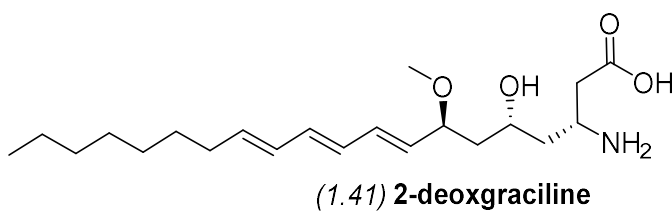


Fig. 14 The chemical structure of 2-deoxygraciline (2-deoxygra), a novel amino acid found in the euglenatide family.

The heteroatom rich external face of the euglenatides and the long aliphatic chain are favourable moieties for cell penetrating systems. Additionally, the variation in side-chains provides multiple sites of variation for optimisation or functionality which could include using them as sites for peptide-drug conjugation. The polyketide chain could also be used as a linker site to attach active drug compounds and improved cell uptake.

With that in mind, euglenatides A-D were tested against various assays, to see if their natural structures hold any useful biological activity. Initial results showed promising power against *Candida*, *Aspergillus* and various human cancer cell lines, however the small amount of each compound isolated (< 6 mg each) was not enough for further testing.³⁸

1.3.2. Nemamides

When compared with NP databanks, the euglenatide scaffold showed large similarities with nemamide A (**Fig. 15**, 1.42), which was isolated from a nematode (*Caenorhabditis elegans*).

⁴² Nemamide A was also characterised as 5-mer cyclic peptide with a polyketide chain. Interestingly, the structural elucidation of the nemamides showed the presence of a non-proteogenic amino acid similar to 2-deoxygraciline (**Fig. 15**, 1.41), with the only difference being a reduced chain length. Alongside nemamide A, Butcher *et al.* isolated a second analogue (nemamide B, 1.43), which varied only in the degree of saturation on the polyketide fragment (**Fig. 16**).

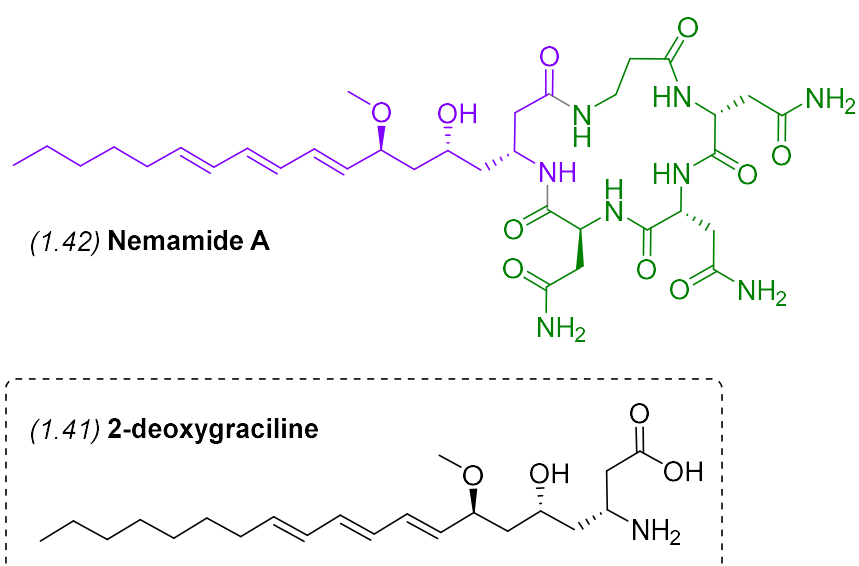


Fig. 15 Reported preliminary structure of nemamide A (1.42), isolated and elucidated by Butcher *et al*, showing a reduced chain length when compared with 2-deoxygraciline (1.41).⁴²

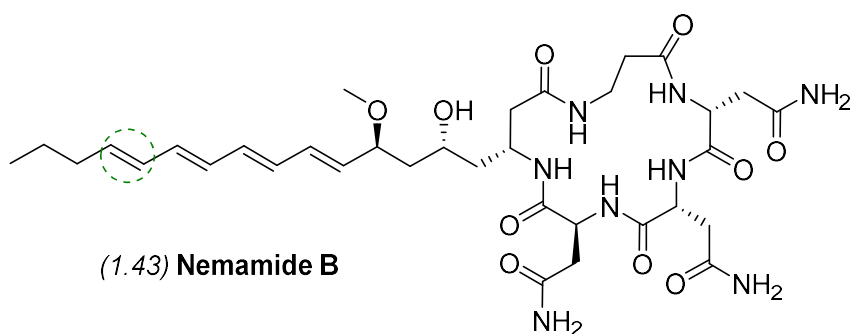


Fig. 16 Reported preliminary structure of nemamide B (1.43), showing the extra alkene in the polyketide chain when compared with nemamide A (1.42).

The structural similarities between nemamides and euglenatides are significant, which is rare considering the differences between the species that they were isolated from. Nemamides A and B we discovered in a worm like nematode called *Caenorhabditis elegans*. *C.elegans* are a species of parasitic protostomia that occupy bacteria heavy habitats, such as decaying organic matter found in compost bins. The worm-like nematodes are classified as bacterivores, which means they have a natural mechanism of safely digesting and processing bacteria. The worms grow up to 1 mm in length and while they have no circulatory or respiratory system, they have a well-defined neuronal system; which is in no way comparable to the single celled *E. gracilis* species.

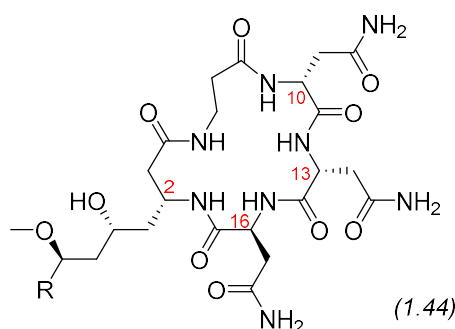
Butcher *et al.* noted that *Caenorhabditis elegans* encodes a hybrid polyketide synthase (PKS) and nonribosomal peptide synthase (NRPS) enzyme and a multi-module NRPS chromosome. These two enzymes are key to the biosynthesis of polyketides (PK) and non-ribosomal peptides (NRP), respectively; both of which are important classes of natural products.⁴³ Due to the general structures of these PK and NRP compounds being relatively easy to chemically alter, many common drug compounds have been derived from NPs within these families.⁴⁴

With the knowledge that *C.elegans* had the capacity to produce a large variety of useful NP based drugs, Butcher *et al.* decided to analyse the products of PKS-1 and NRPS-1, which are the highest coded homologues within the species.⁴² Wild type (WT) worms, PKS-1 mutant and NRPS-1 mutant worms were cultured and extracts from each were analysed by high resolution liquid chromatography mass spectrometry (HR-LC-MS). Two key products were found within the wild-type culture, these were named nemamide A and nemamide B. Nemamides A and B were not seen within the PKS-1 or NRPS-1 mutants, leading the group to believe that these compounds are polyketide nonribosomal peptides and require both PKS-1 and NRPS-1 for their biosynthesis.

To isolate enough material for full structural identification, approximately 50 L of WT worm was cultured, extracted, and purified by sequential column chromatography to isolate ~70 µg of nemamide A and < 70 µg of nemamide B.⁴²

High resolution mass spectrometry revealed the molecular weight of nemamide A and B as 734.40 gmol⁻¹ and 732.38 gmol⁻¹ respectively. The DBE of nemamide A and B were calculated as 11 and 12, indicating that the structure of B holds one more double bond than A (**Table 2**). As the initial ¹H NMR, ¹³C NMR spectroscopy and HRMS characterisation showed almost exact comparable data, the structure of nemamide B was determined from

the assignment of A. Marfey's method was used to determine that nemamide A contained one *L*-Asn and two *D*-Asn. COSY, total correlation spectroscopy (TOCSY), HSQC, HMBC and rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY) were performed and used to assign the complete structure of nemamide A.



	M_r / gmol^{-1}	Chemical formula	DBE	-R
Nemamide A	734.85	$\text{C}_{34}\text{H}_{54}\text{N}_8\text{O}_{10}$	11	$\text{C}_{11}\text{H}_{15}$
Nemamide B	732.84	$\text{C}_{34}\text{H}_{52}\text{N}_8\text{O}_{10}$	12	$\text{C}_{11}\text{H}_{13}$

Table 2 The simplified structure of nemamide A and B, comparing the molecular weight, chemical formula and DBE to determine the difference in the R group.

The stereocentres at C2, C10, C13 and C16 were confirmed by synthesis of three model cyclic peptides and comparison of their NMR spectra with the wild type data (**Fig. 17**). Solid phase synthesis was employed using resin bound H- β -Ala-2-ClTrl and sequential coupling of the protected amino acids.⁴² The linear chain was cleaved from the resin with HFIP and cyclised using dilute amide coupling conditions. Deprotection of functional groups was carried out and the three cyclic models were isolated through multiple rounds of column purification. The *R,R,R,S*-model structure (*1.45*) showed comparative NMR data to the wild type, suggesting that this is the stereochemical arrangement. The full structures have not yet been synthesised and confirmed.

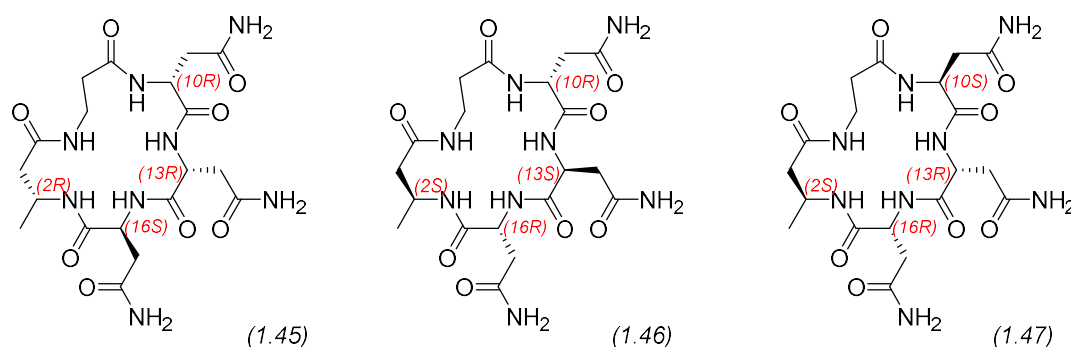


Fig. 17 Model cyclic peptides used to confirm the stereochemistry of nemamide A and B.

The target euglenatides and nemamide compounds all contain derivatives of 2-deoxygra (1.41), which contains three stereogenic centres. The initial characterisation of the nemamide compounds utilised ROESY and ^1H NMR spectroscopy to identify the relative stereochemistry of the alcohol and methoxy group, with ROESY interactions being seen from H_1 to H_3 and H_4 to H_{2a} and H_{2b} (**Fig. 18**).⁴² Using the J values to estimate bond angles with the Karplus curve, the authors suggested that the relative stereochemistry is either *S,R* or *R,S*. Computationally modelled circular dichroism (CD) spectra was used to conclude that the stereochemistry was likely *S*-methoxy-*R*-hydroxy.

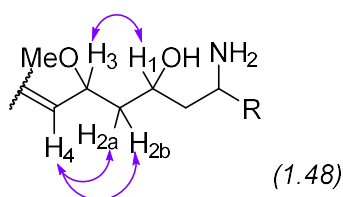


Fig. 18 Proton correlations in the ROESY NMR spectrum of the nemamides showed interactions (indicated in purple) between H_1 and H_3 and H_4 with H_{2a} and H_{2b} .

While no biological testing has been reported on these structures, given their structural similarity to the previously mentioned euglenatides, they may have similar biological activities so present as an interesting opportunity for novel anti-microbial agents.

1.4. Aims and Objectives

Within the nemamide and euglenatide natural product families there are a total of seven known examples, each one varying in the amino acids within the pentapeptide ring. The commonality between all seven compounds lies in the novel β -amino acids graciline (**Fig. 19, 1.35**) and 2-deoxygraciline (**1.41**). At the time of beginning this project, a route towards the synthesis of these amino acids has not been published and the absolute stereochemistry has not been confirmed. This project aimed to develop a route towards the synthesis of 2-deoxygraciline *via* a stepwise asymmetric approach.

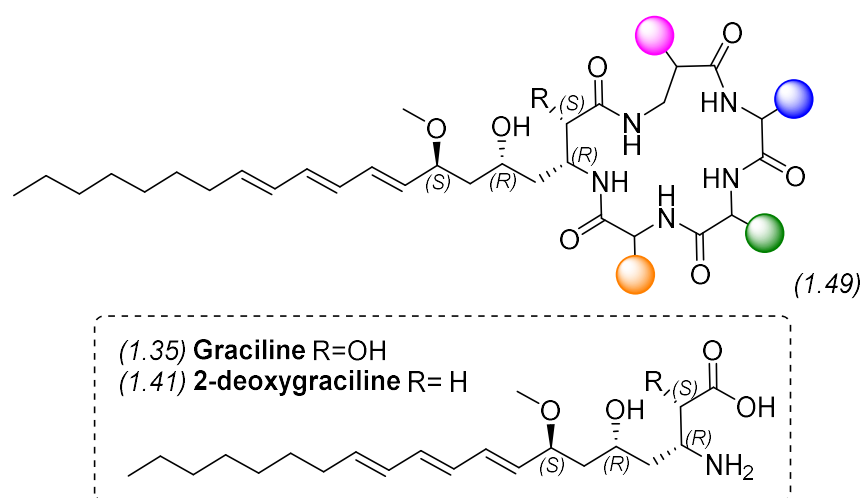


Fig. 19 Skeletal structure of the euglenatide and nemamide families (1.49), where the functional groups of the varying amino acids are depicted in coloured rings, containing the novel graciline (1.35) and 2-deoxygraciline (1.41) β -amino acids.

Some variation can be seen in the chain length and saturation level of the graciline compounds; therefore, an advanced intermediate was chosen that can be utilised in the synthesis of all these natural products. Disconnection at the polyketide chain provided the new target of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50), which can be converted into the different varieties of 2-deoxygraciline (**Fig. 20**). This can be synthesised from 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-ynoic acid (1.51), which may also allow for chain extension. This gives three main synthetic targets.

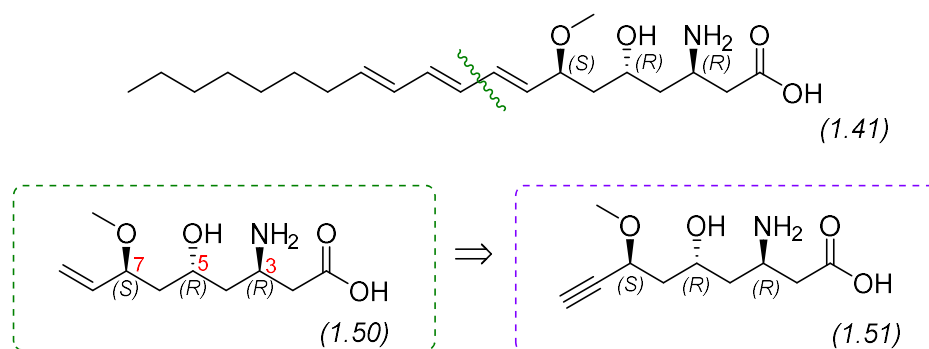


Fig. 20 Disconnection of the polyketide chain in 2-deoxygraciline to form the synthetic target of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid and its advanced intermediate 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-ynoic acid.

Upon completion of 2-deoxygraciline, synthesis of the nemamides can be carried out to confirm that the absolute stereochemistry has been assigned correctly. This requires sequential peptide couplings with protected commercially available amino acids followed by macrocyclisation. Since macrocyclisation can be a challenging process to control, model systems were designed to be used during trials and optimisation of macrocyclisation techniques.

Chapter 2: Model cyclic pentapeptide targets

2.1. Aims and Objectives

This section aimed to optimise protective and coupling strategies for the specific amino acids required in two model target peptides (**Fig. 21**). These two models were designed to be representative of nemamides A and B during reaction optimisation and during future structure activity relationship (SAR) studies.

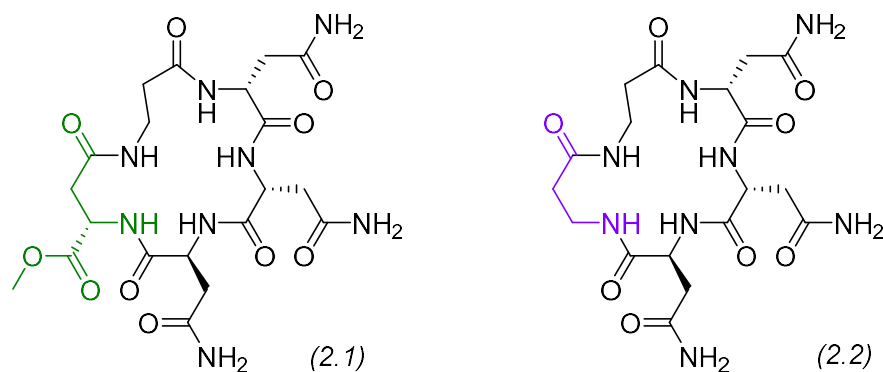


Fig. 21 The two model peptide systems with their amino acid variants indicated.

Synthesis of the model peptides was designed to be representative of the future nemamide route; therefore, it was important to carefully consider where the primary disconnection would take place as this would decide the point at which the macrocyclization would occur in the forward synthesis. Initially, the primary disconnection was considered between 2-deoxygraciline and asparagine (**Fig. 22**, *bond A*). Further consideration of the negative impact of steric interference on macrocyclisation suggested that the functional groups on 2-deoxygra and asparagine might reduce the chance of successful cyclisation. With this in mind, the primary disconnection was moved to between the *N*-terminus of β -alanine and the *C*-terminus of 2-deoxygraciline. (**Fig. 22**, *bond B*).⁴⁵ Since the synthesis of the models was designed to be representative of the nemamide, the primary disconnections were made in the equivalent position within the rings. This means that forward synthesis of model peptides 1 (**Fig. 22**, 2.1) and model peptide 2 (**Fig. 22**, 2.2) should begin from *L*-aspartic acid and β -alanine residues, respectively.

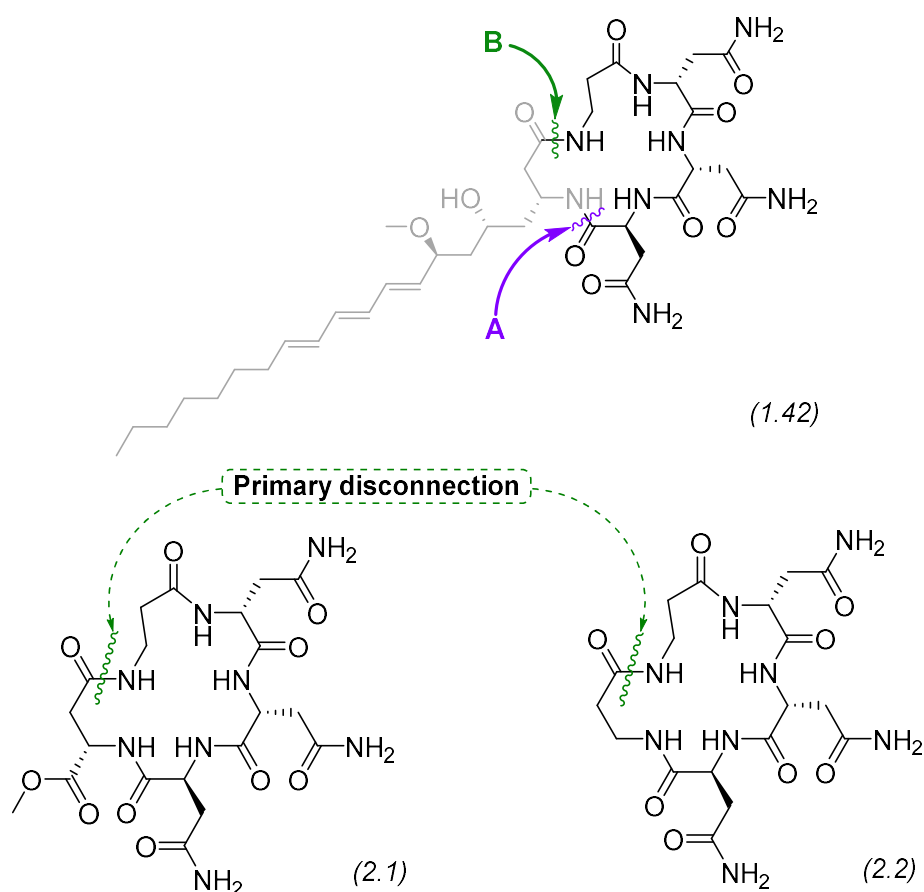
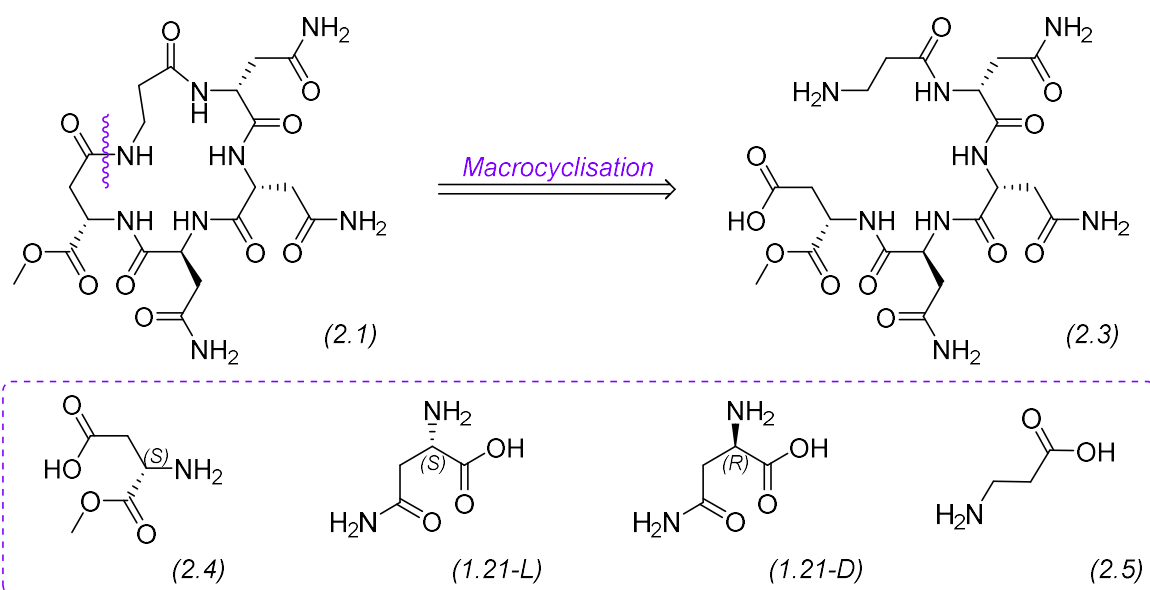


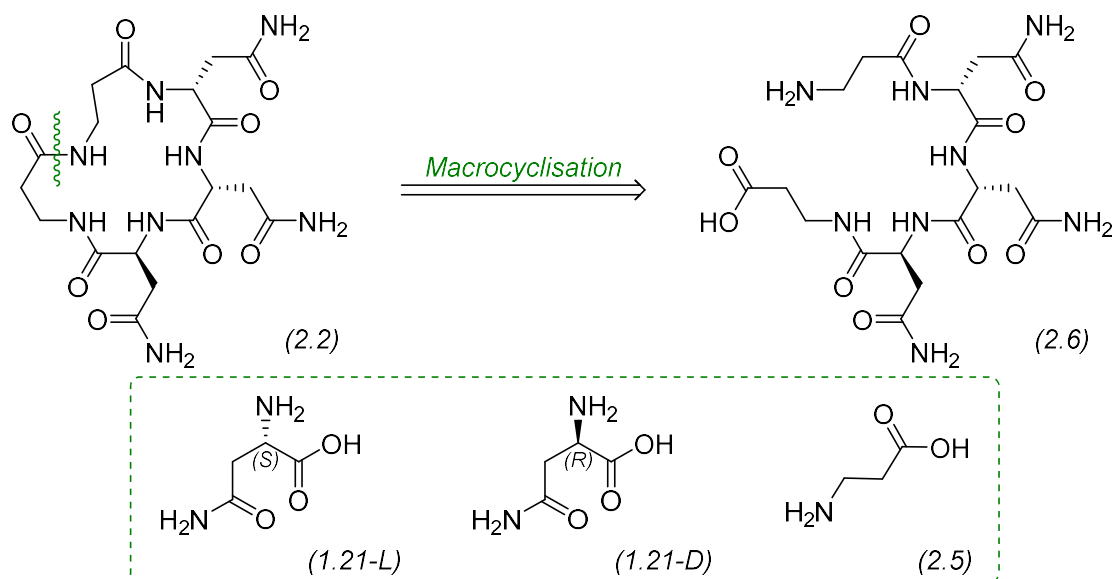
Fig. 22 Primary disconnections of nemamide A (1.42) considered at bond A (purple) and bond B (green). Model peptide 1 (2.1) and model peptide 2 (2.2) with their primary disconnections highlighted in green.

Initial disconnection of model peptide 1 (2.1) residues gave a linear pentapeptide target (Scheme 1, 2.3), which could be synthesised *via* sequential peptide couplings and deprotections. Starting from differentially protected aspartic acid (2.4), which will be coupled with *N*-protected *L*-Asn (1.21-*S*). Deprotection will give a di-peptide which can then be coupled in the same way with *N*-protected *D*-Asn (1.21-*R*). The deprotect and couple sequence will be repeated to add on a second and then β -alanine (2.5), which will give a protected linear pentapeptide. Deprotection of the *C* and *N*-terminals will give the required unprotected linear pentapeptide (2.3), which can be used as a model for macrocyclisation.



Scheme 1 Disconnection of model peptide 1 (2.1) into its linear chain (2.3), which can be made in a series of couplings and deprotections from the protected amino acids.

Similarly, model peptide 2 (2.2) can be broken down into a series of peptide couplings and deprotections to create the linear pentapeptide target (2.6) (**Scheme 2**). For this model, the forward synthesis would begin from C-protected β -alanine (2.5) which can be coupled in the same sequence as in model peptide 1 to give the protected linear pentapeptide. Deprotection of the C and N-terminals will give the unprotected linear pentapeptide (2.6), which can be cyclised to form the target model peptide (2.2).



Scheme 2 Disconnection of model peptide 2 (2.2) into its linear chain (2.6), which can be made in a series of couplings and deprotections from the protected amino acids.

To approach this method of synthesis the necessary amino acids must be protected. This work optimised strategies for the scalable protection of *L*-asparagine, *D*-asparagine and β -alanine with *tert*-butyl carbonate (Boc) (**Fig. 23**). Alongside this, a route to differentially C-terminal protected aspartic acid (2.9) was developed and a practical synthesis was optimised (**Fig. 24**).

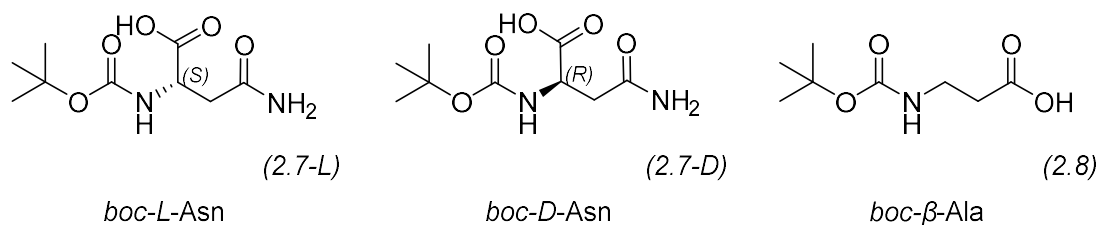


Fig. 23 N-protection of commercially available amino acids with *tert*-butyl carbonate group.

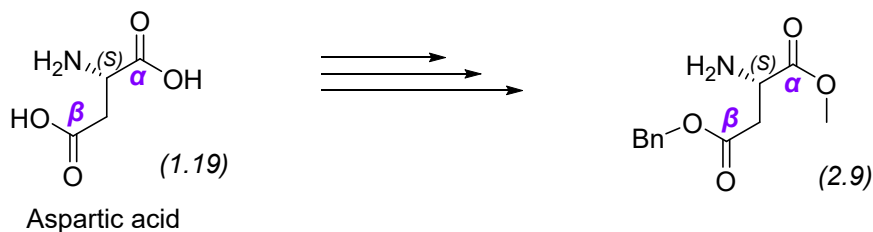


Fig. 24 Commercial aspartic acid (1.19) which is to be differentially protected at the α - and β - carboxylic acids.

2.2. Introduction

Model cyclic pentapeptides (2.10) were designed around the nemamide structures. These models incorporated the same *-L-Asn-D-Asn-D-Asn-β-Ala-* sequence, with the variability being the linker amino acid between *L-Asn* and *β-Ala* (**Fig. 25**, purple). In the natural nemamide compounds this linker amino acid is 2-deoxygraciline, which is a complex non-proteogenic amino acid. Synthetic steps towards 2-deoxygraciline are described in later chapters.

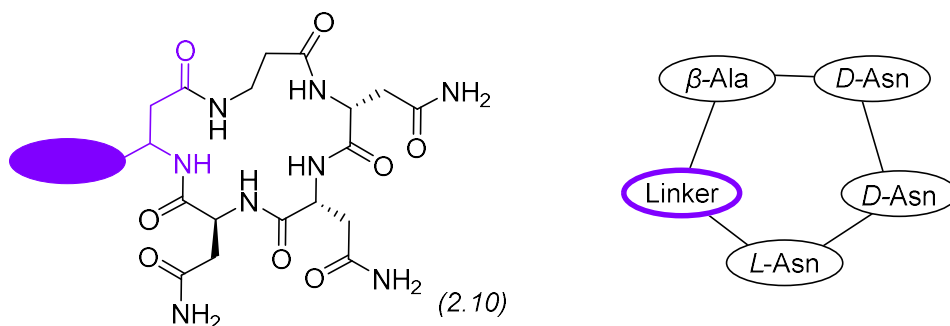
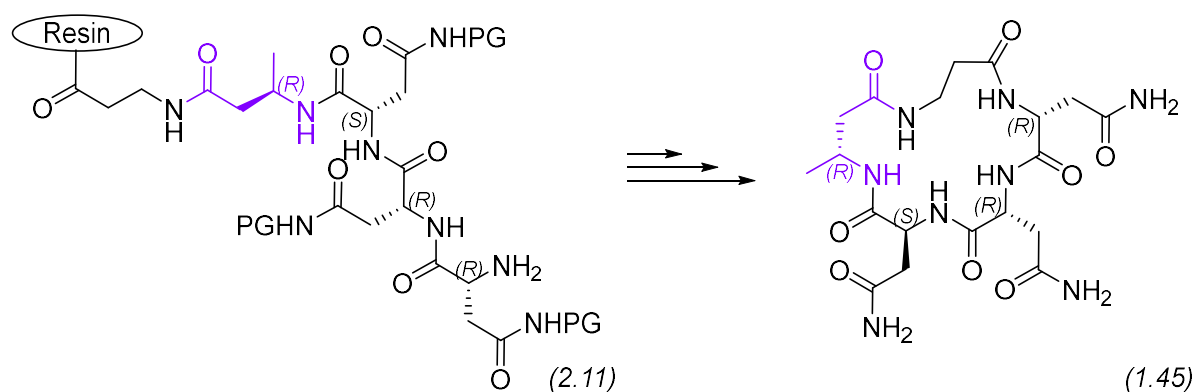


Fig. 25 Generalised structure of the nemamide cyclic peptide models (2.10) with the variable amino acid linker depicted in purple.

Previously, Butcher *et al.* have reported synthesis of a model system (1.45) *via* solid phase peptide coupling (**Scheme 3**).⁴² Utilising a resin bound *β*-alanine, they performed sequential coupling and deprotections to give a linear model (2.11) which saw (3*S*)-aminobutyric acid in the linker position. While this allowed relatively quick and easy synthesis of models to determine the absolute configuration of the nemamide ring structure, this strategy may not be ideal for synthesis of the complete natural products. Graciline and 2-deoxygraciline contain multiple points of functionality, which will each need to be masked with individual protecting groups. Inserting the protected residue early in the chain synthesis provides multiple possible complications, if any of the reaction conditions lead to deprotection of these functional groups. Since solid phase synthesis does not allow for spectral analysis until cleavage from the resin, it will not be possible to determine if any of unwanted deprotection and/or side-reactions had occurred until the final stages. For this reason, it was desirable to find a synthetic method which either installs the graciline in the final stages of chain synthesis or allows for constant structure monitoring throughout the synthesis. This led to models being designed which would be used to trial solution phase peptide synthesis of the cycles.



Scheme 3 Model cyclic peptide (1.45) synthesised by Butcher *et al.* from the resin bound linear chain (2.11), used to determine the absolute configuration of the wild type nemamides.

Model structures were designed to include cheaper commercially available amino acids. This would allow for testing of macrocyclisation techniques without wasting valuable synthetic 2-deoxygraciline. Simplified model structures could also provide valuable data when assessing the SAR of nemamides if the synthetic nemamides show positive pharmaceutical activity in biological assays. Comparison of the full compound and model cyclic peptides in biological assays will help to determine if the complex side-chain of 2-deoxygraciline is required for its biological activity. With this in mind, two model peptides were designed for synthesis.

Model peptide 1 (**Fig. 26**, 2.1) was designed to include an *L*-aspartic acid in place of 2-deoxyGra. The model is a comparative compound to the nemamides, as it contains a smaller functional group which sits on the same face as the polyketide functionality. This will make it be a useful model for macrocyclisation techniques, as any steric interference in the model (2.1) will likely be increased in the complete nemamides. It also provides an external ester which is ideal for further functionalisation. Utilising further peptide couplings or C-C bond formation techniques, current drug compounds and active pharmaceutical ingredients (API) could be bound at this point to investigate the cell penetrating effect of the cyclic peptide for drug delivery (**Fig. 26**, 2.12).

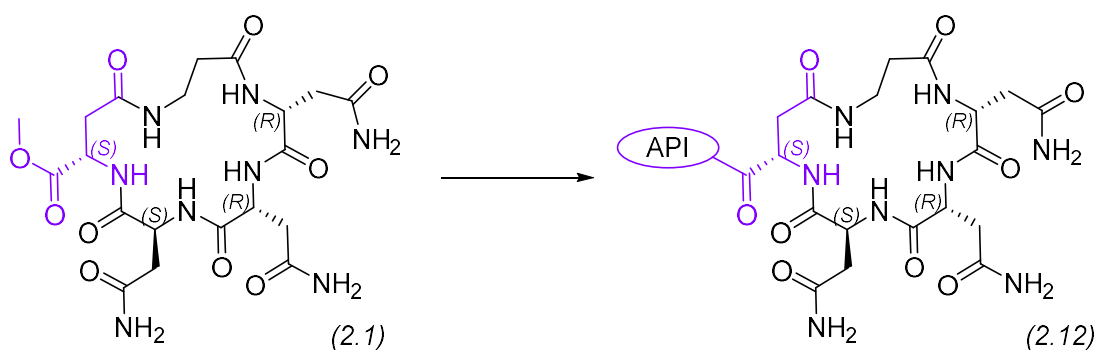


Fig. 26 Model peptide 1 (2.1), utilises an aspartic acid group (purple) in place of 2-deoxyGra to provide a functional sable site for coupling with APIs for PBDS.

Model peptide 2 (**Fig. 27**, 2.2) utilises a second β -alanine in replacement of the 2-deoxygra, which imitates the simplest version of the cyclic system possible. This simplified structure contains no side chain on the replacement AA, which reduces the negative steric effect during macrocyclisation and makes the compound less representative of the nemamides than model 1.⁴⁵ However, this model provides a good comparative structure for SAR studies. Model 2 (2.2) essentially becomes the first phase of an alanine scan, where multiple versions are synthesised replacing one amino acid in each compound with alanine. Utilising alanine eliminates the side-chain and retains the general configuration of the backbone, which makes this test a valued assessment of the biological impact of each amino acid in a structure.⁴⁶ Change in activity, either positive or negative, deems that AA position as important for its biological activity. Replacement of 2-deoxygra with alanine would however impact the ring size as well, and therefore β -alanine was an ideal substitution for this test. Changes in biological activity between this model and the WT or synthesised nemamides would indicate that this position within the peptide is key in their biological activity.

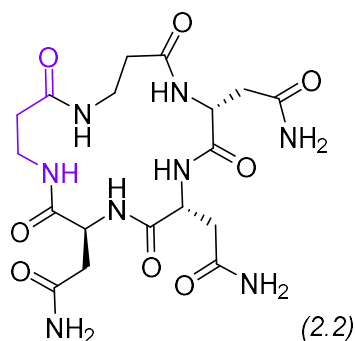


Fig. 27 Model peptide 2 (2.2), utilising β -alanine in place of the 2-deoxygra.

Two major methods of peptide synthesis are classic solutions phase (CSPS) and solid phase (SPPS), the latter is generally the preferred method. SPPS requires binding of a starting amino acid to a polymeric resin/bead, which is then suspended in solution for the sequential addition of amino acids.⁴⁷ After each coupling is performed the excess reagents and byproducts can be washed off the resin loaded peptide negating the need for purification. Upon completion of the desired chain synthesis, the peptide is cleaved and analysed. SPPS however is not an atom economical process. Synthesis requires a large excess of the free amino acid at each coupling stage which can become very costly when using non-proteogenic amino acids.^{48,49}

As previously discussed, it was decided that it would be more useful to perform CSPS in the initial nemamide synthesis. This method follows a classic sequential homogeneous synthesis, which, while more time consuming can be beneficial when synthesising small peptides containing expensive amino acids. Typically, CSPS has a higher performance than SPPS which means that a smaller molar ratio of the two amino acids is required.⁴⁸ CSPS also allows for spectral analysis of the peptide throughout every stage of synthesis, which could be helpful in the confirmation of the natural products absolute stereochemical assignment.

To perform peptide coupling reactions it is important to utilise specific protecting groups to avoid polymerisation byproducts. In each coupling, one amino acid should have its C-terminus protected and the other should have its N-terminus protected (**Fig. 28**). In some cases, it is also necessary to protect the functional side chain of amino acids. Finding the protecting groups is a relatively easy task for most of the proteogenic amino acids due to the existence of extensive reviews on the subject.^{50,51} In many cases, it is possible to purchase the desired protected amino acids, with the common protected proteogenic amino acids often being cheaper to buy than synthesise.

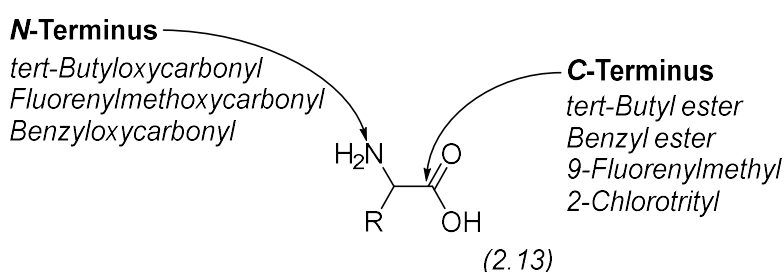
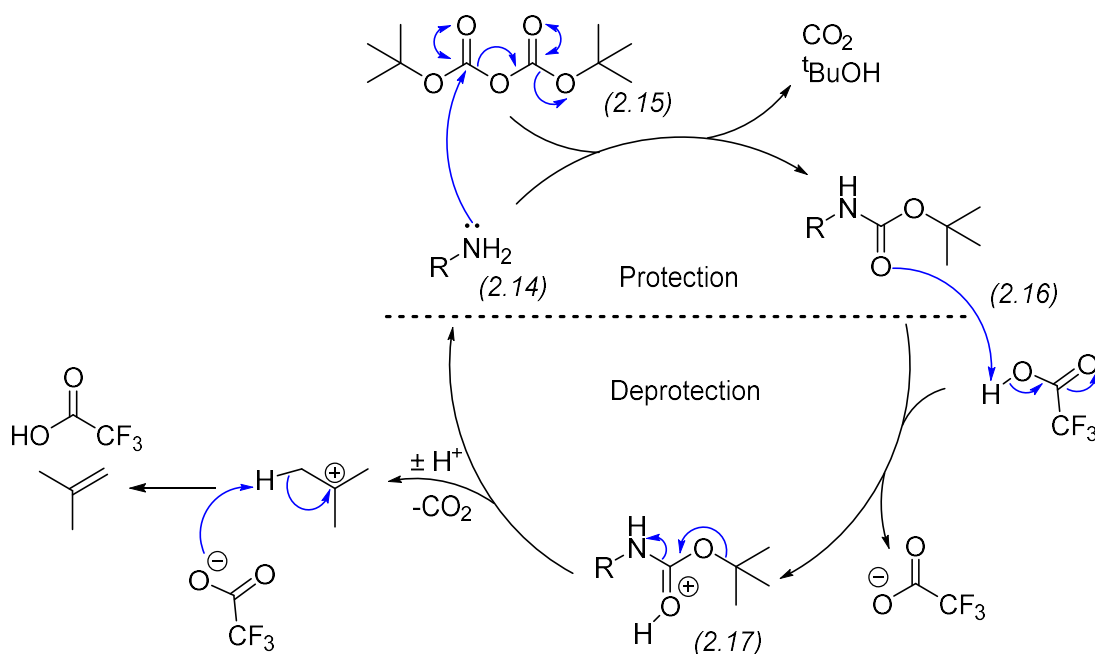


Fig. 28 Examples of common strategies for protecting the *N*-terminus and the *C*-terminus of amino acids.

However, the nemamides and their models are made up of mostly non-proteogenic amino acids. While some of them are commercially available in protected forms, they come with a high price markup compared to non-proteogenic. At the time of route planning, a comparison of prices of Boc-(*R*)-Asn to Boc-(*S*)-Asn showed the non-proteogenic Boc-(*R*)-Asn to be approximately 18 times more expensive than its proteogenic counterpart and can only be purchased in much smaller package sizes. The high cost of purchasing the required protected amino acids was not feasible for this research project and therefore the unprotected AAs were purchased instead. Protection of the AAs in-house also allows for more synthetic flexibility, as the protecting groups can be varied if required.

Initial plans chose to protect the *N*-terminal of each AA with *tert*-butyl carbonyl group. This *tert*-butyl ester group was chosen due its stability towards basic conditions, which means it can withstand the standard coupling reactions utilised in later synthetic steps.⁵⁰ The reaction of an amine (2.14) with Boc anhydride (2.15) releases ^tBuOH and gaseous CO₂, which allows for easy isolation of protected material (2.16) if the reaction reaches 100 % conversion (**Scheme 4**, top). Removal of the group can be easily achieved by acid cleavage, to release carbon dioxide and isobutylene (**Scheme 4**, bottom), both of which are gaseous at room temperature (RT), leading to easy purification of the free amides.



Scheme 4 The mechanism for protecting amines (2.14) with Boc anhydride (2.15) and the deprotection mechanism with trifluoroacetic acid to return the free amine (2.14).

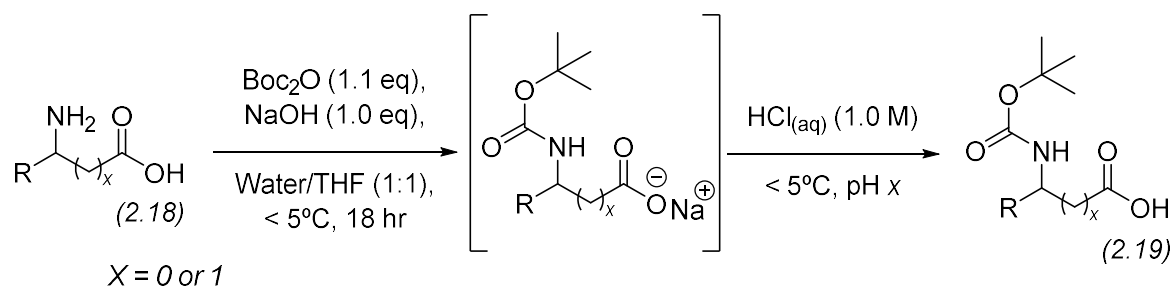
2.3. Results and Discussion

2.3.1. Amino acid protections

2.3.1.1. N-Terminal protections

Literature searches for a general Boc protection of an amino acid gave multiple different approaches, most of which centre around the use of an organic solvent, water and a base.^{52–54} The base that was used varied between different inorganic bases, of which the most favoured was sodium hydroxide. All these methods used THF or 1,4-dioxane as the organic solvent, as both of these provide sufficient dissolution of Boc anhydride and have some miscibility with water.

While these literature approaches all report high yields, following these methods did not get reproducibly high values. Reactions carried out utilising sodium hydroxide in a water/THF mixture gave low and non-reproducible yields, varying between 45–65 % (**Table 3**). TLC monitoring during reactions showed complete conversion and therefore the low yields were likely caused by the accuracy of the neutralisation of the salts and subsequent isolation from the aqueous phase. For the sodium salts of Boc- β -alanine (2.2) and Boc-aspartic acid (2.21), acidification to approximately pH 4–6 was achieved by slow addition of 1M HCl_(aq) and the protected amino acid precipitated out of the solution as a colourless solid. Low yields were initially achieved, but it was found that slow crystallisation over 1–2 hr led to higher product yield (45 and 65 % respectively). For protection of both enantiomers of asparagine, reaching the correct isolation pH was more challenging. The functional side chain contains a primary amide group, which increases its solubility in water, leading to poor yields from crystallisation. By concentrating the aqueous filtrate to around 50 % volume, it was possible to recover more product, however reaction yields never exceeded 49 % yield.



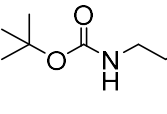
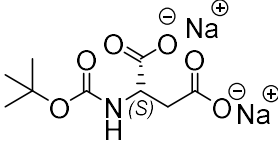
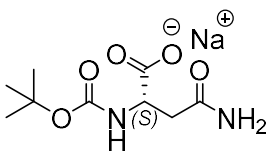
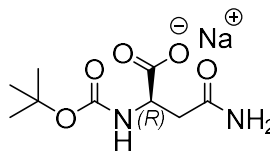
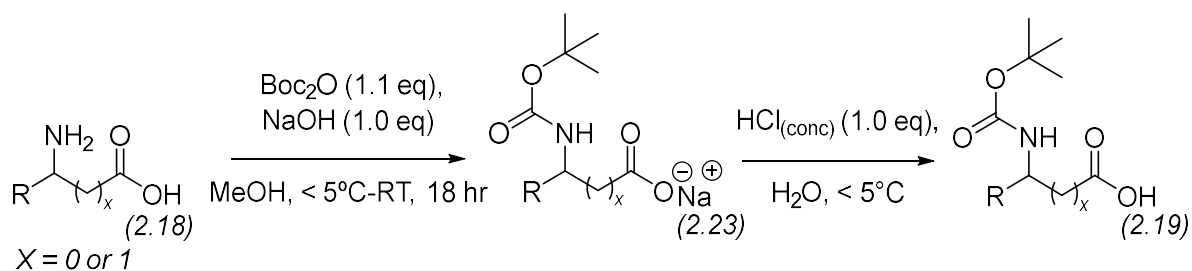
Product	Intermediary salt	Isolation pH (x)	Yield ^(a)
Boc-β-Alanine	 (2.20)	4-6	45 %
Boc-S-Aspartic acid ^(b)	 (2.21-S)	4-6	65 %
Boc-S-Asparagine	 (2.22-S)	5-6	49 %
Boc-R-Asparagine	 (2.22-R)	5-6	47 %

Table 3 Initial yields for the three amino acid protections. ^(a) *Maximum isolated yield.* ^(b) *2.0 eq of sodium hydroxide used in part (1).*

An increase in yields was achieved by utilising methanol as a solvent, instead of water/THF. The methanol was removed after completion of the reaction to isolate the sodium salt, followed by an azeotropic distillation with hexanes was utilised to remove residual methanol and ^tBuOH byproduct. The sodium salt was then dissolved in a minimum amount of water (5-7 volumetric equivalents), cooled to an external temperature of 0 °C and acidified with a known amount of concentrated HCl (1.0 eq). Using this method allowed for a minimum

amount of water to be used, which reduced the loss of material *via* aqueous solubility and increased yields to over 70 % for each amino acid at its maximum run scale (**Table 4**).



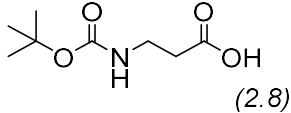
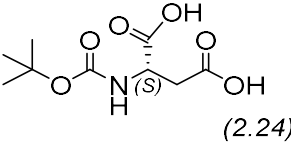
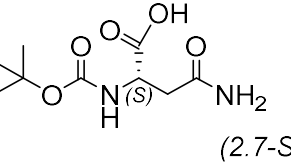
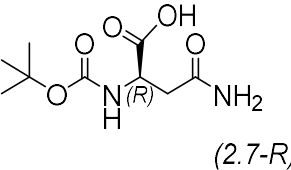
Product	Product structure	Maximum starting material scale/ g	Yield ^(a)
Boc-β-Alanine	 (2.8)	10	95 %
Boc-(<i>S</i>)-Aspartic acid ^(b)	 (2.24)	10	72 %
Boc-(<i>S</i>)-Asparagine	 (2.7-S)	12	82 %
Boc-(<i>R</i>)-Asparagine	 (2.7-R)	5	79 %

Table 4 Final optimised conditions and maximum performed scales for the Boc protection of amino acids. ^(a) Isolated yield on stated maximum scale. ^(b) 2.0 eq of sodium hydroxide used.

2.3.1.2. Differential protection of aspartic acid

The synthetic plan for model peptide 1 utilised aspartic acid (**1.19**) as the C-terminal AA, which required differential protection of the α - and β - carboxylic acid groups (**Fig. 29**). This has previously been achieved during synthesis of the C-terminal gastrin pentapeptide, where the α -acid was protected as a trichlorophenyl ester and the β -acid as a benzyl ester.⁵⁵ Protecting both groups with benzyl substituents, selectively deprotecting one group and subsequently methylating it, left the product with one α -methyl ester and one β -benzyl ester (**2.9**).⁵⁶ The two groups vary in deprotection strategies, which would allow for removal of the β -benzyl ester by Pd/C catalysed hydrogenolysis without effecting the methyl ester. This will allow selective macrocyclisation, forming the desired 5-mer cyclic peptide.

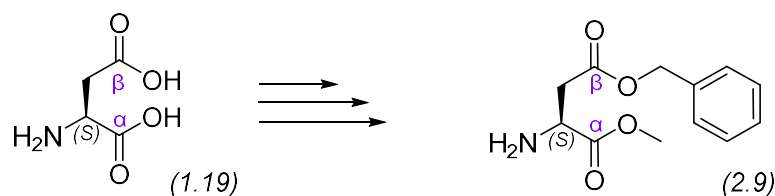
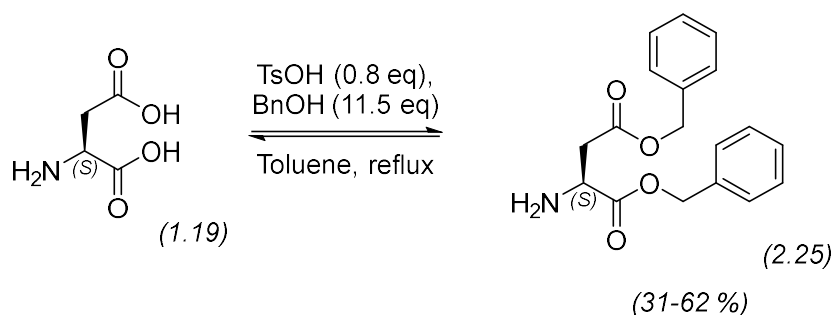


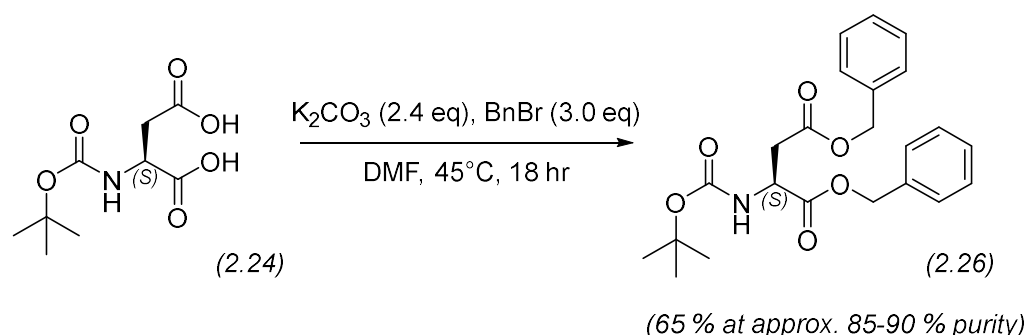
Fig. 29 Desired 4-benzyl 1-methyl (*S*)-aspartate (**2.9**) synthesised by differential protection of α - and β - carboxylic acid groups of aspartic acid (**1.19**).

Prior to the mono deprotection step, it was necessary to synthesise *N*-(*tert*-butoxycarbonyl) (*S*)-aspartic acid dibenzyl ester (**2.25**). Initial attempts utilised benzyl alcohol and TsOH with a Dean-Stark apparatus, to remove the water byproduct and therefore push the equilibrium towards the desired benzyl ester product (**Scheme 5**). The reaction showed very inconsistent conversion rates with non-repeatable reaction times. It is likely that the Dean-Stark apparatus was not removing water sufficiently enough from the reaction to drive the reversible reaction to completion.



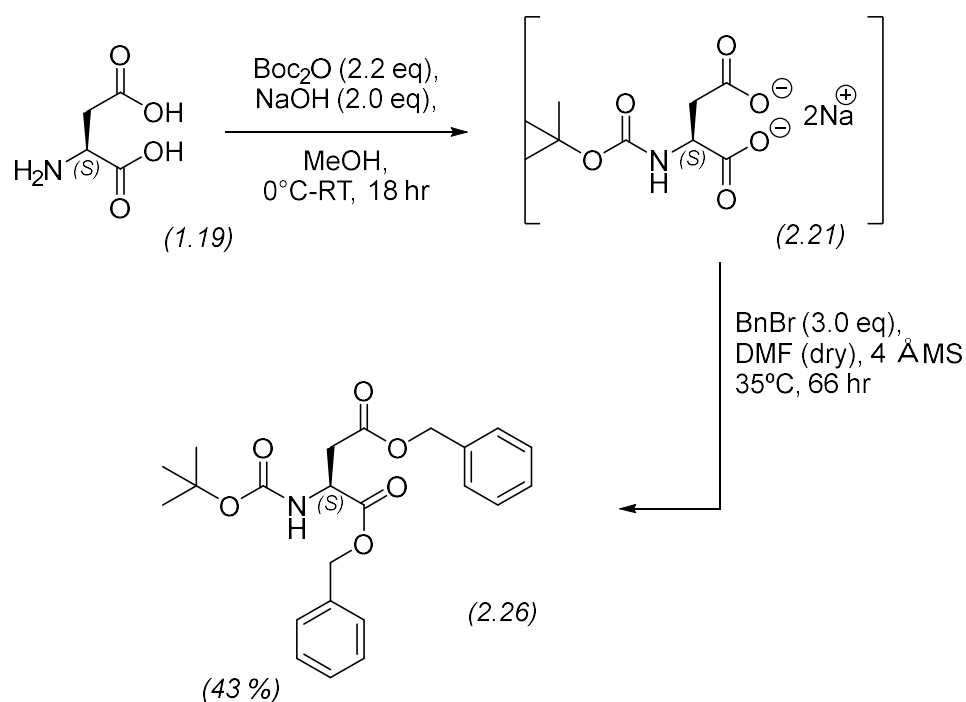
Scheme 5 Initial conditions used for esterification with benzyl alcohol.

Benzyl bromide provided a good alternative to benzyl alcohol, due to the reaction being irreversible (**Scheme 6**). Reactions with benzyl bromide can also lead to *N*-benzylation, therefore were performed on Boc-Asp (2.24). The reaction was performed on a 5.0 g scale showing complete conversion of the di-acid. Analysis of the crude product by ^1H NMR spectroscopy always showed high contamination with benzyl alcohol at around 20-30 %. This impurity was hard to fully remove from the product and required column chromatography followed by trituration with hexanes to provide a 65 % yield, with an estimated purity of 85-90 % by ^1H NMR spectroscopy.



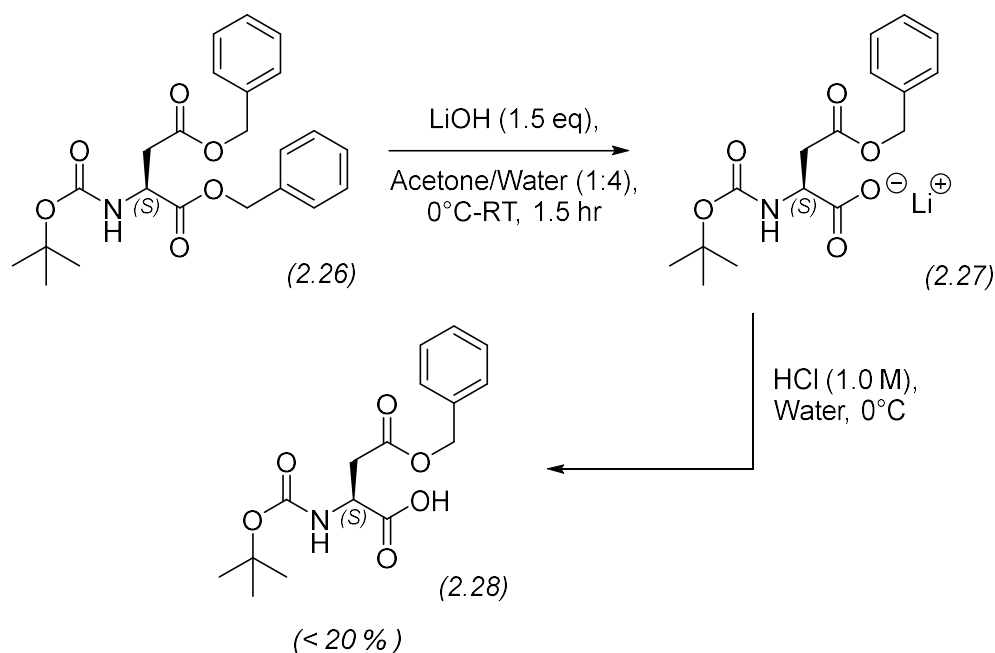
Scheme 6 Revised route to protected aspartic acid (2.26), using benzyl bromide to protect the carboxylic acid groups.

The reaction was further optimised by combining the Boc protection and benzylation into a one-pot style reaction (**Scheme 7**). Instead of neutralising the sodium salt (2.21) formed during the Boc protection, this was dried and azeotropically distilled with hexanes to remove all traces of alcohol. The salt was suspended in DMF and benzyl bromide added. Utilising the salt removed the need for an added base, therefore reducing the requirement for water washes during the work up procedure and was expected to improve the crude product yield. The crude product was purified by sequential trituration with cold hexanes and yielded 43 %. While this yield is lower than the combined two-step yield for the previous method from aspartic acid (~ 46 %), it reduced the solvent requirement and removed the need for column chromatography. Alongside being more environmentally friendly, removing the column chromatography made the reaction much easier to handle on a large scale and reduced the required reaction time. The final purified product (2.26) was estimated at over 95 % purity by ^1H NMR spectroscopy, which was deemed pure enough to continue with synthetic steps. Furthermore, a second crop of material at approximately 60 % purity (contaminated by benzyl alcohol) was isolated from the tritulant, which could be purified by the same sequence of column chromatography and trituration to increase the overall yield.

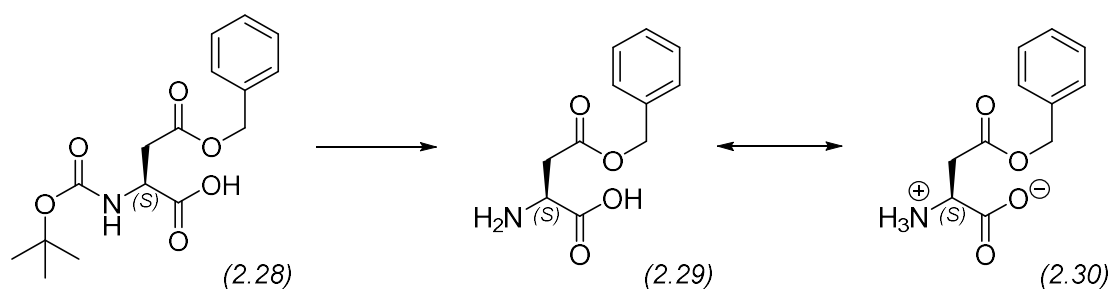


Scheme 7 Final conditions for synthesis of *N*-(*tert*-butyloxycarbonyl) (*S*)-aspartic acid dibenzyl ester (2.26); utilising a one-pot system, where methanol is completely removed from the intermediate salt prior to step 2.

Selective deprotection was achieved by using 1.5 eq of LiOH monohydrate, and careful monitoring by TLC to ensure no deprotection occurred at the β -position (**Scheme 8**). Isolation of the mono-deprotected product was carried out by acidification of the lithium salt in water. A low yield of $< 20\%$ was attained, due to slow crystallisation from water. However, when the mother liquor was left overnight at 5°C , no further material crystallised out of solution. It is possible that extended periods in the acidic environment caused *N*-deprotection and produced a highly water-soluble product (**Scheme 9**, 2.30).



Scheme 8 Initial conditions for isolation of 4-benzyl 1-carboxy *N*-Boc-(*S*)-aspartate (2.28).



Scheme 9 *N*-Deprotection that likely occurs during extended periods in acidic environments, leading to a zwitterionic compound which is highly water soluble.

Literature suggested that the selective hydrolysis occurs on the α -carbonyl position rather than the β .⁵⁶ Comparative analysis of the ^1H NMR spectrum with available literature on the two possible mono-deprotected compounds (**Fig. 30**) confirms that the isolated product of this reaction is the α -deprotected compound. The recorded spectrum and the data published by Aoyama *et. al* on 4-benzyl-*N*-Boc-(*S*)-aspartic acid (2.28) and Perrotta *et. al* on 1-benzyl-*N*-Boc-(*S*)-aspartic acid (2.31) all match quite closely, however, variations are seen in the shifts of the $-\text{CH}$ and $-\text{CH}_2$ peaks.^{57,58} For the β -benzyl compound (2.28) it is reported that the $-\text{CH}_2$ protons present as two doublet of doublets at 3.07 and 2.89 ppm and the $-\text{CH}$ proton is a multiplet at 4.63 ppm.⁵⁷ Whereas, in the α -benzyl compound (2.31) the $-\text{CH}_2$ and the $-\text{CH}$ are reported further upfield at 2.90-2.46 ppm 3.35-3.18 ppm respectively.⁵⁸ The experimental spectra (**Fig. 31**) shows a multiplet at 4.63 ppm and two roofed doublet of

doublets at 3.09 and 2.89 ppm, which closely matches the reported data for of 4-benzyl-*N*-Boc-(*S*)-aspartic acid (2.28), confirming that the deprotection occurred at the α -carbonyl position

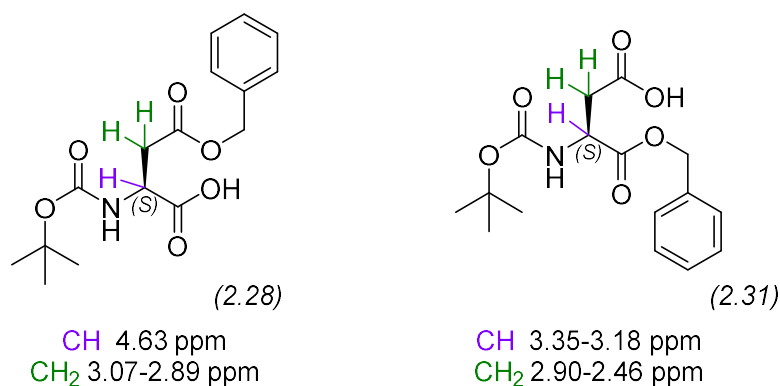


Fig. 30 The structure of 4-benzyl-*N*-Boc-(*S*)-aspartic acid (2.28) and 1-benzyl-*N*-Boc-(*S*)-aspartic acid (2.31) with the reported chemical shifts for the CH and -CH₂ groups in the published ¹H NMR spectrum.

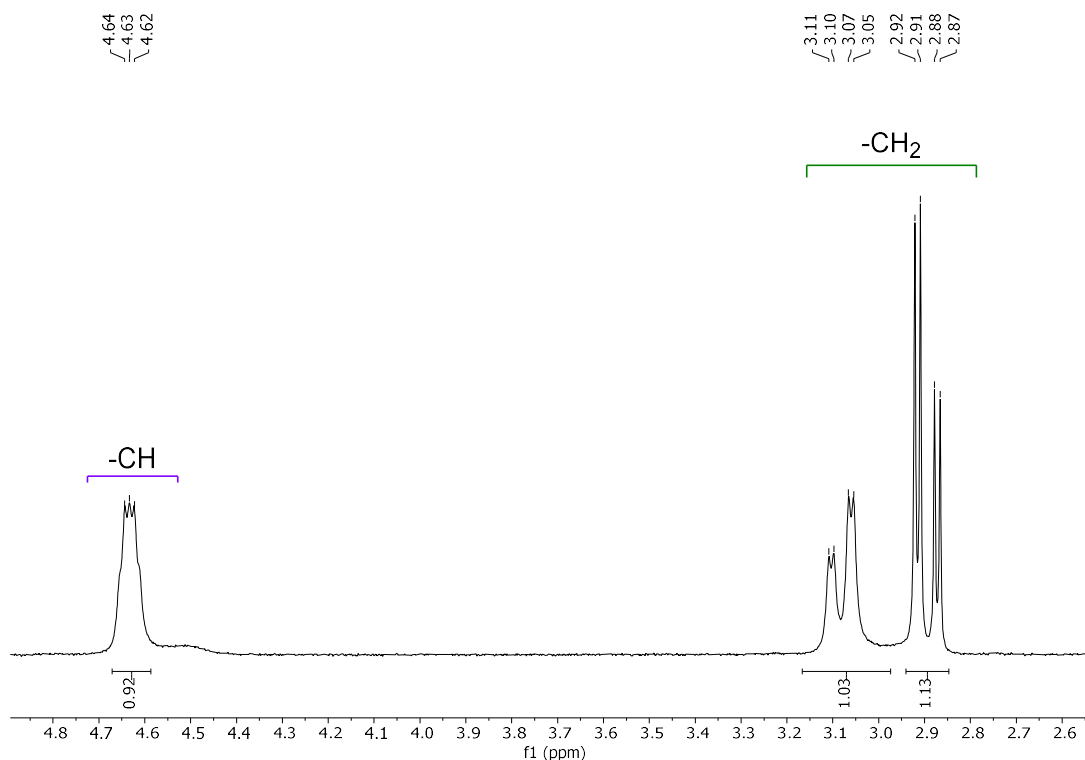
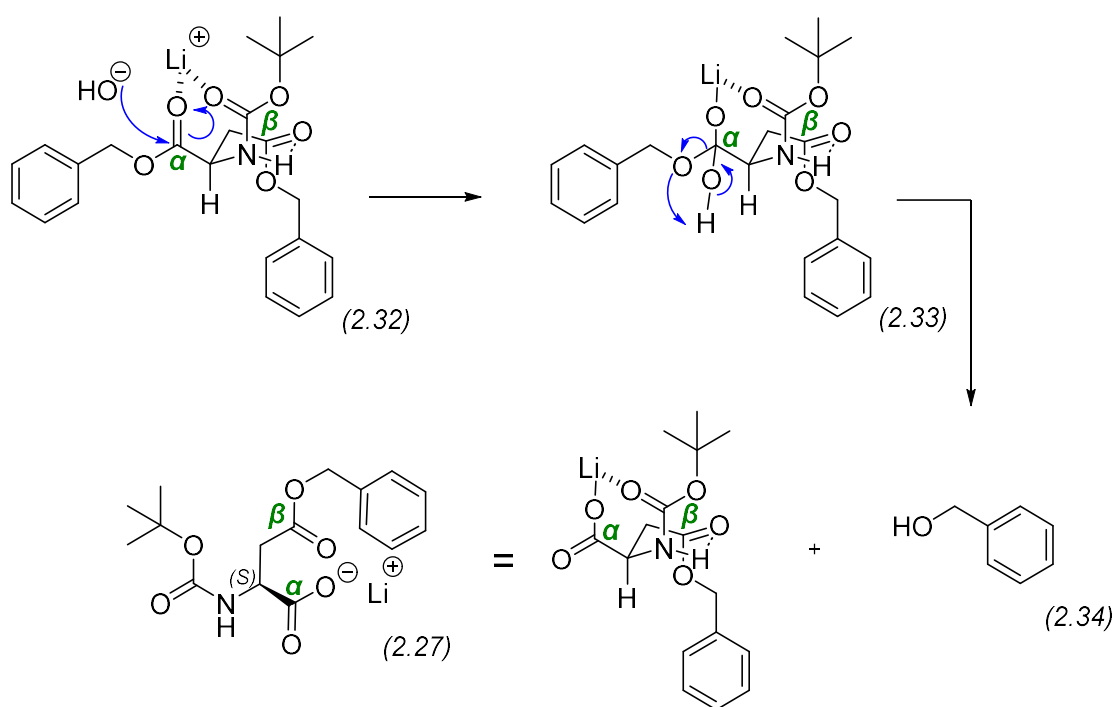


Fig. 31 Section of the ¹H NMR spectrum of the product of mono-deprotection of *N*-(*tert*-butoxycarbonyl) (*S*)-aspartic acid dibenzyl ester with lithium hydroxide, showing the -CH peak at 4.63 ppm and -CH₂ peaks at 3.09 and 2.89 ppm (**A-C2-20**).

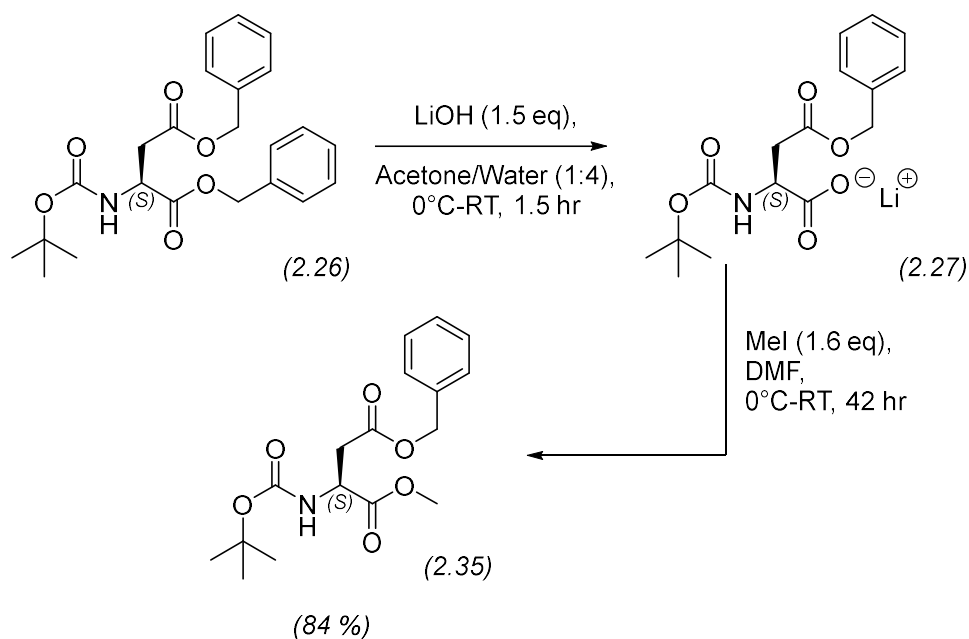
Literature searches revealed no mechanistic explanation for the result, but it is possible that hydrogen bonding between the N-H and the β -carbonyl forming a stable

pseudo-6-membered ring (**Scheme 10**, 2.32). This allows for lithium coordination between the two carbonyls is a pseudo-7-membered ring, allowing for the nucleophilic attack at the α -carbonyl. Lithium coordination at the β -carbonyl within the pseudo-6-membered ring is unlikely to happen and therefore the attack within the ring less electronically favourable. If this hydrogen bonding were to occur between the α -carbonyl it would form a slightly less favourable pseudo-5-membered ring and a subsequent 8 membered ring during co-ordination of the lithium. Both these rings are slightly less favourable and therefore it could be argued that attack at the α -carbonyl is more electronically favourable.



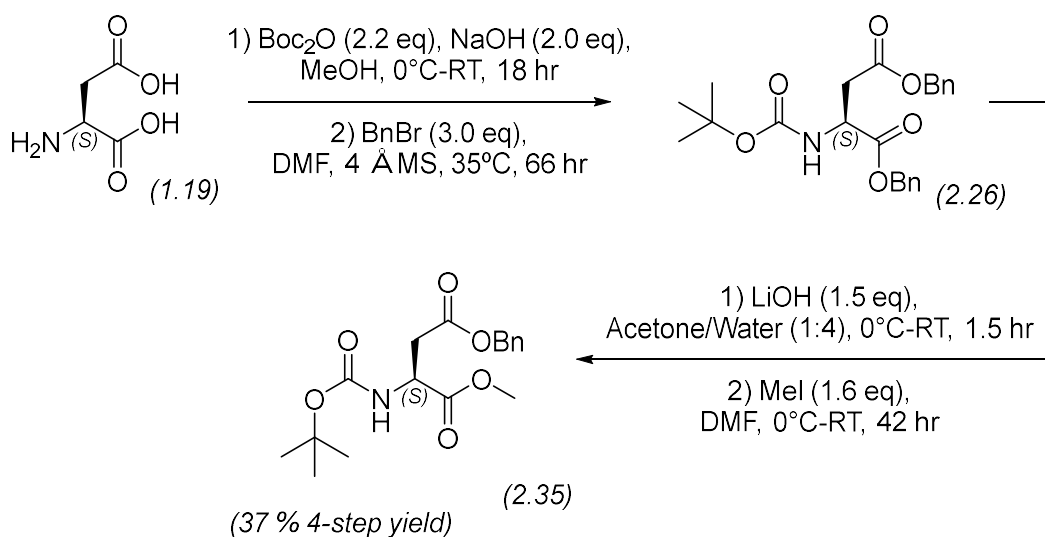
Scheme 10 Proposed mechanism for the selective deprotection of the α -benzyl ester.

The mono-deprotected product was carried forward as a lithium salt (2.27). and subjected to standard methylation conditions using MeI in DMF (**Scheme 11**). The initial reaction was performed with no added base. While some methylated product was observed, full conversion was not achieved, so K_2CO_3 (1.0 eq) was added to drive the reaction towards completion. The purification of the crude product was challenging due to the close polarity of the product and the benzyl alcohol byproduct from the previous step. Sequential washes with NaHCO_3 were carried out to remove the majority of the BnOH, followed by a slow column to remove the remaining BnOH before isolating the product (2.35) in an 84 % yield over the two-steps.



Scheme 11 Conditions used for the selective deprotection at the α -position and direct methylation to produce the desired differentially protected aspartic acid (2.35), isolated in an 84 % yield over two steps.

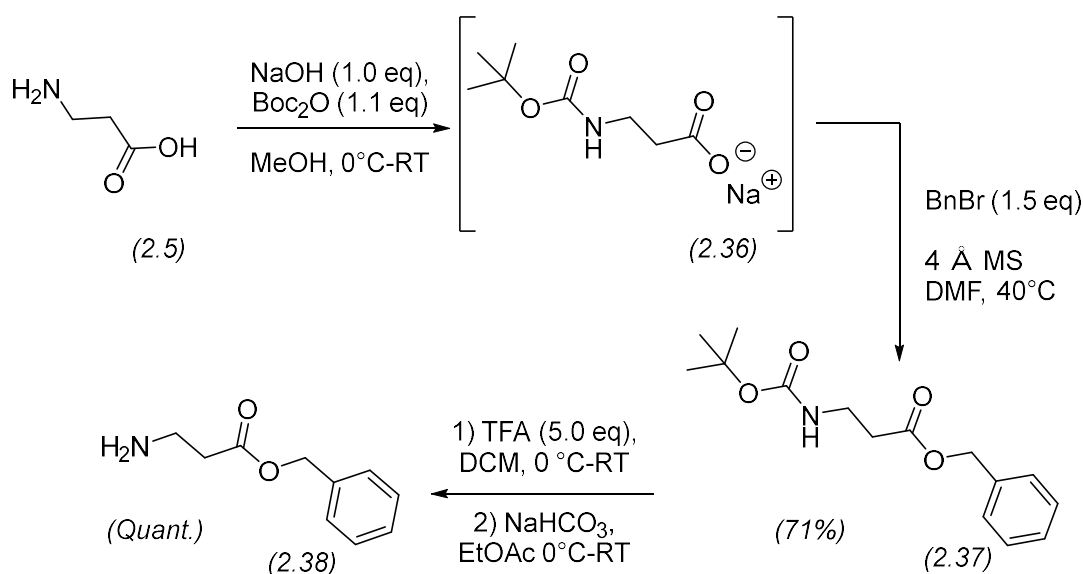
The desired differentially protected product (2.35) was isolated in around 37 % yield over four steps, starting from aspartic acid (1.19) (**Scheme 12**).



Scheme 12 four-step synthetic sequence to achieve differentially protected aspartic acid with an overall 37 % isolation yield.

2.2.1.3. C-terminal protection of β -alanine

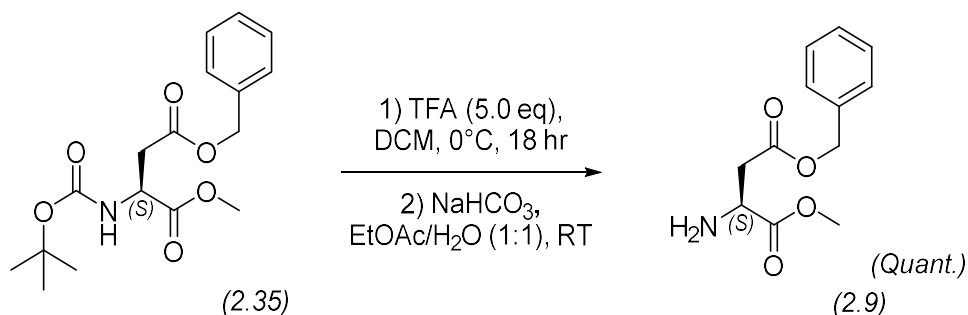
To access model peptide 2, β -alanine (2.5) was chosen as the C-terminus. Protection of the carboxylic acid was performed using a similar method to that used in the previous section. The Boc protection step was achieved utilising methanol as a solvent, creating the Boc protected material as a sodium salt (**Scheme 13**, 2.36). The salt form of the Boc- β -alanine (2.36) was isolated and dried before being protected using benzyl bromide. Prior to the addition of benzyl bromide, the salt was stirred in DMF with activated 4 Å MS for 30 minutes to ensure the reaction was completely free of water. The reaction proceeded with no need for additional base, and was worked up before excess benzyl alcohol was removed by washings with aqueous NaHCO₃ and trituration with cold hexanes to give the product (2.37) in a two-step yield of 71 %. The fully protected amino acid was carried through without further purification. Finally, the N-terminus was deprotected using trifluoroacetic acid (TFA) in DCM to give the C-terminal protected β -alanine (2.38) in an overall three step yield of 71 %.



Scheme 13 Optimised C-terminus protection of β -alanine to yield benzyl β -alaninate.

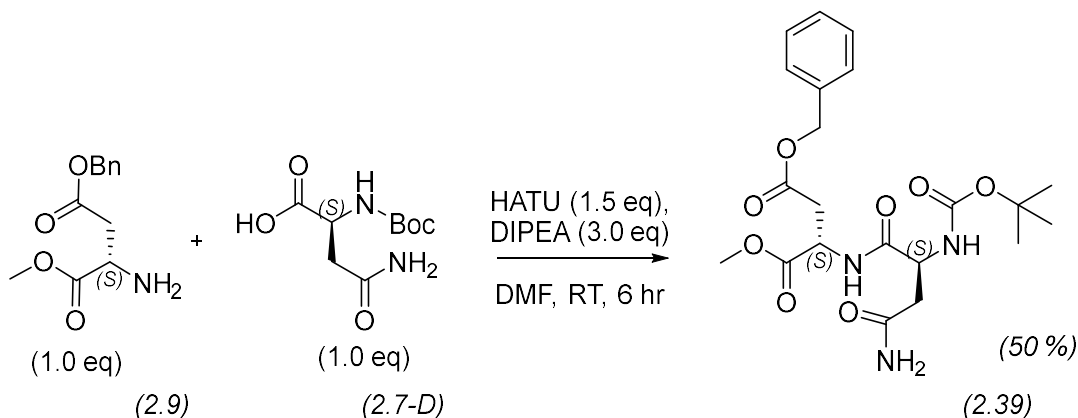
2.3.2. Linear chain synthesis

Beginning from 4-benzyl-1-methyl-*N*-Boc-(*S*)-aspartate (2.35), the *N*-terminus was first deprotected with TFA. A total of 5.0 eq of TFA was added, which ensured complete deprotection (**Scheme 14**). The TFA salt was washed with aqueous NaHCO₃ to free up the amine and excess TFA was removed using azeotropic distillation with hexanes.



Scheme 14 Standardised conditions for deprotection of 4-benzyl-1-methyl-*N*-Boc-(*S*)-aspartate (2.35).

Coupling began by adding Boc-(*S*)-Asn (2.7-*D*) to the pre-prepared 4-benzyl-1-methyl-(*S*)-aspartate (2.9) (**Scheme 15**). Initial attempts utilising HATU, DIPEA and DMF showed a complex mixture of impurities, leading to complicated purification of the crude material and a moderate yield of 50 %.



Scheme 15 Conditions used for the first peptide coupling between Boc-(*S*)-asparagine (2.7-*D*) and 4-benzyl-1-methyl-(*S*)-aspartate (2.9).

Confirmation of formation of the desired product was carried out by analysis of NMR spectra. The ¹H NMR spectrum showed the expected ratios of all proton environments in the product, with a clear 5:9 ratio of aromatic protons (7.35-7.30 ppm) to ^tbutyl protons (1.45 ppm), suggesting a 1:1 ratio of a benzyl containing compound and a Boc containing

compound (**Fig. 32**). Further evidence can be seen by the two pairs of diastereotopic protons between 3.20 and 2.50 ppm. The diastereotopic CH₂ from 4-benzyl-1-methyl-(*S*)-aspartate shows a slight downfield shift from 2.80 ppm to 2.95 ppm, confirming that a reaction has taken place between the two compounds. Final confirmation was provided by HRMS where ESI⁺ provided a value of 452.2218, which is within an expected experimental error of the required value.

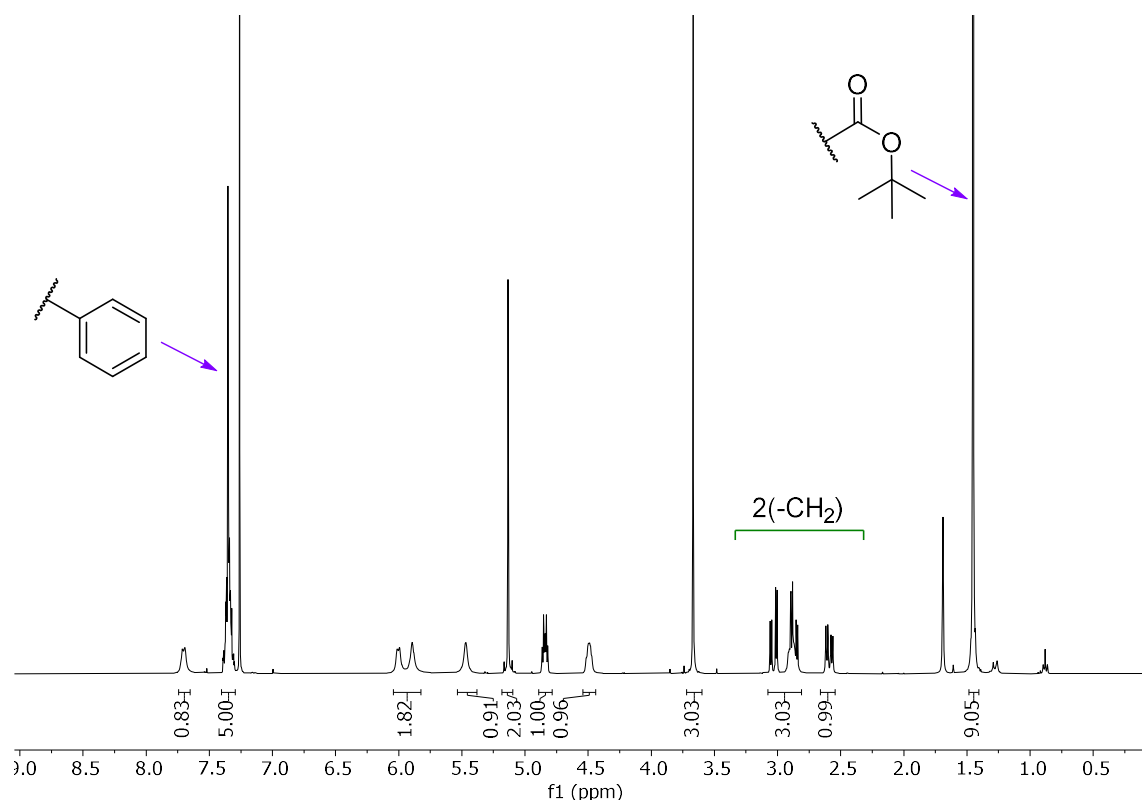
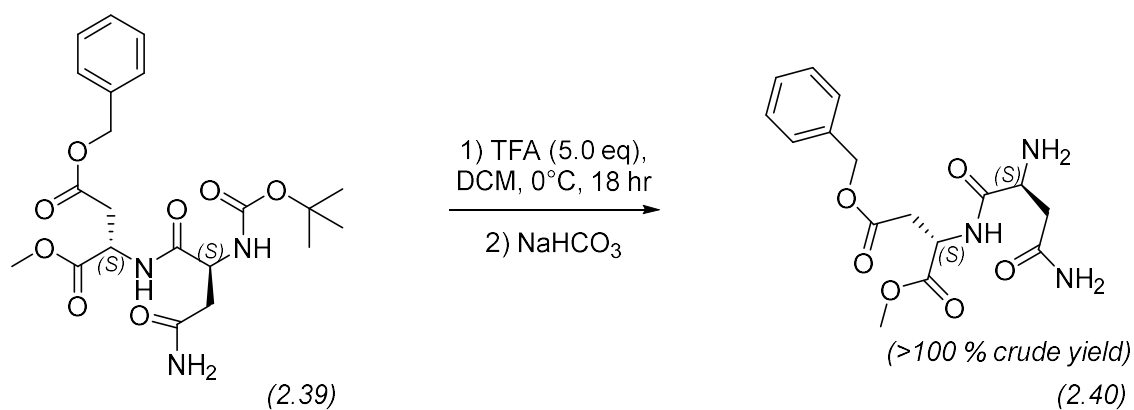


Fig. 32 ¹H NMR spectrum of 4-benzyl-1-methyl (*S*)-aspartyl-*N*-Boc-(*S*)-asparagine in CDCl₃, showing the 5:9 ratio of Boc to aromatic peaks (purple) and the two pairs of diastereotopic protons (green) (**A-C2-26**).

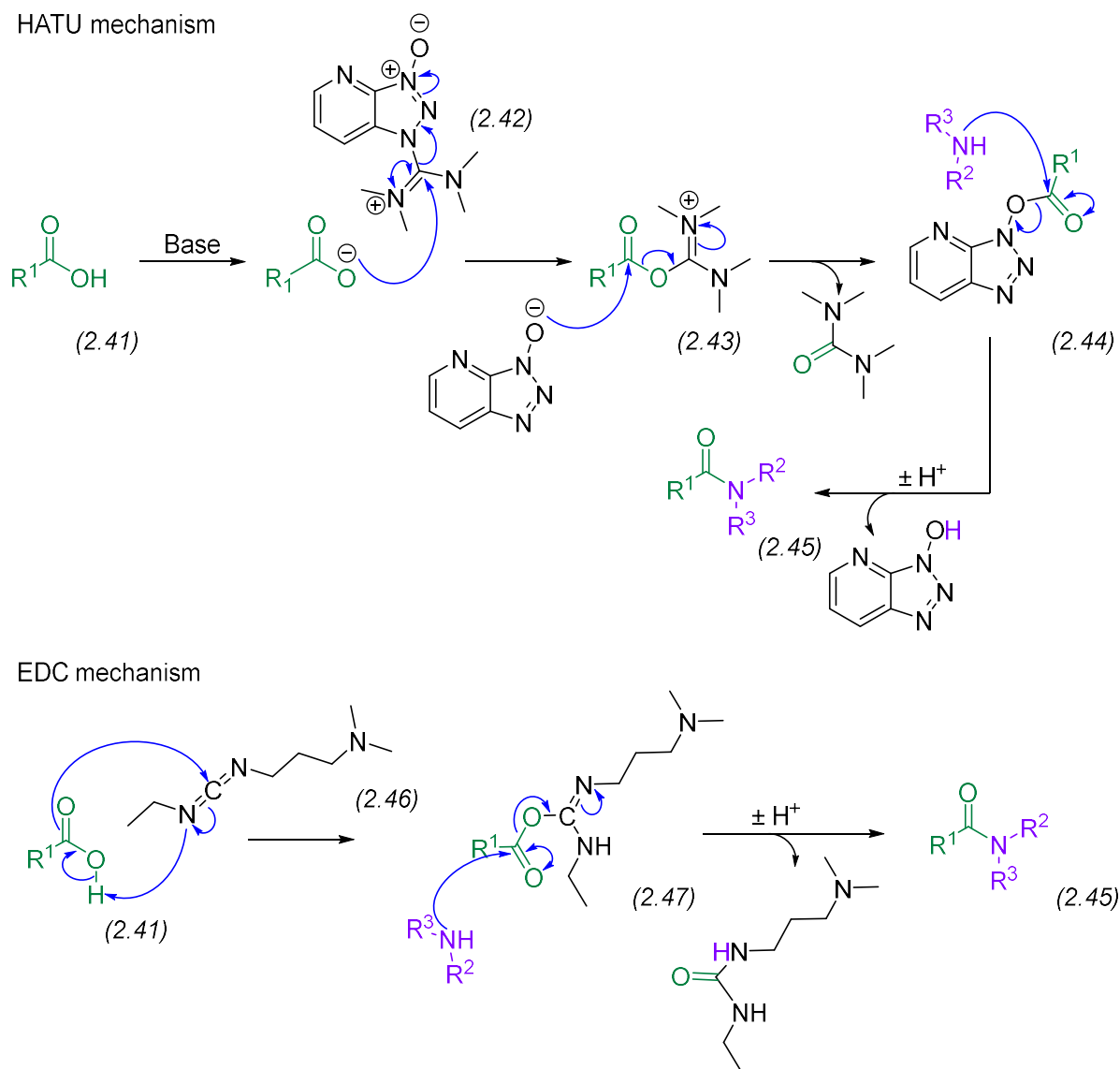
The 2-mer chain was then subjected to Boc deprotection (**Scheme 16**), before carrying out a second coupling with Boc-(*R*)-Asn using the same strategy. Unfortunately, the crude product showed low solubility in available NMR solvents, so full analysis was not carried out. However, formation of the desired product was confirmed by HRMS where ESI⁺ provided a value of 352.1594, which is within an expected experimental error of the required value.



Scheme 16 *N*-deprotection of 4-benzyl-1-methyl (S)-aspartyl-*N*-Boc-(S)-asparagine to form 4-benzyl-1-methyl-(S)-aspartyl-(S)-asparagine.

2.3.3. Optimisation of coupling conditions

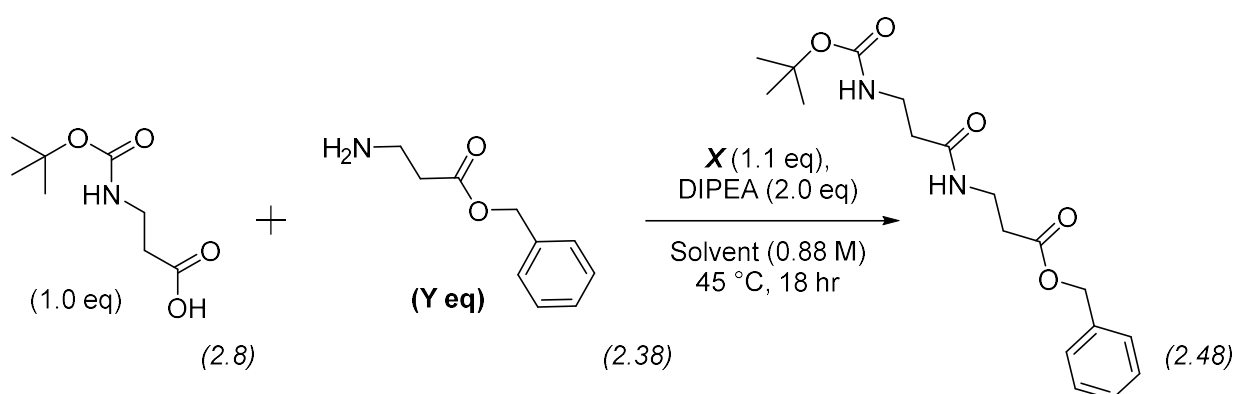
The coupling of the benzyl β -alaninate (2.38) with *N*-(¹butyloxycarbonylamino) propionic acid (2.8) was trialled using two different activating agents (HATU and EDC·HCl). The reaction using HATU (2.42) proceeds through a multi-step mechanism, which gives two side products which can complicate the crude product analysis (**Scheme 17**). Employing EDC (2.46) instead, proceeds through a simpler mechanism, giving one singular side product.



Scheme 17 Generalised mechanism of reaction between a carboxylic acid and amine using HATU (top) and EDC (bottom) as coupling activators.

Due to the ¹H NMR spectra of the crude reactions being complicated by the intermediary structures, carrying out comparable quantitative analysis of the two activating agent was not an easy process. Visual inspection of the ¹H NMR spectra and estimation of the approximate

purity profile was used to determine the optimal conditions for a gram scale trial. For the initial trials, the equivalency of benzyl β -alaninate (2.38) was kept consistent at 1.2 eq to ensure results could be compared. All four initial reactions were performed in parallel and allowed to react for 18 hr at 45 °C. A 100 μ L sample was removed from each vessel *via* syringe and diluted with ethyl acetate. The sample was washed with water, filtered over magnesium sulphate and concentrated. All samples were then dissolved in deuterated chloroform and analysed by ^1H NMR spectroscopy (see **Fig. 33** and **Fig. 34**). Analysis focused on the region between 3.7-2.0 ppm, where four signals were expected corresponding to the four sets of CH_2 protons in the desired product.



	Coupling reagent (X)	Equivalency of Y	solvent	Scale/ g	Purity profile* / %
1a	HATU	1.2	DMF	0.1	89
1b	HATU	1.2	DCM	0.1	66
2a	EDC·HCl	1.2	DMF	0.1	< 60
2b	EDC·HCl	1.2	DCM	0.1	< 75
3a	HATU	1.4	DMF	0.1	92
3b	HATU	1.4	DMF	1.0	91

Table 5 Tested reaction conditions utilised to optimise the coupling conditions, where X is the coupling reagent and Y is the molar equivalency of the free amine in relation to the free acid. *Determined by the molar ratio of product to impurity in the crude ^1H NMR spectrum (see **Fig. 33** and **Fig. 34**).

Visual inspection of the ^1H NMR spectra of the crude reaction mixture of reactions 1a and 1b showed six key peaks within the inspected region, indicating the presence of an impurity (**Fig. 33**). Comparison of the crude NMR spectra with both starting materials indicated that

these peaks did not correspond to remaining starting material. Through comparison with literature data, it was determined that the four peaks indicated in green correspond to the desired product CH_2 peaks and the two extra peaks (purple) were likely remaining activated acid intermediate (2.44).⁵⁹ Since reaction 1a showed a significantly higher purity profile when compared with 1b, it was determined that the HATU coupling proceeds more effectively using DMF as a solvent. However, an approximate 1:9 ratio of intermediate to product indicates that the reaction conditions require further optimisation to reach complete conversion.

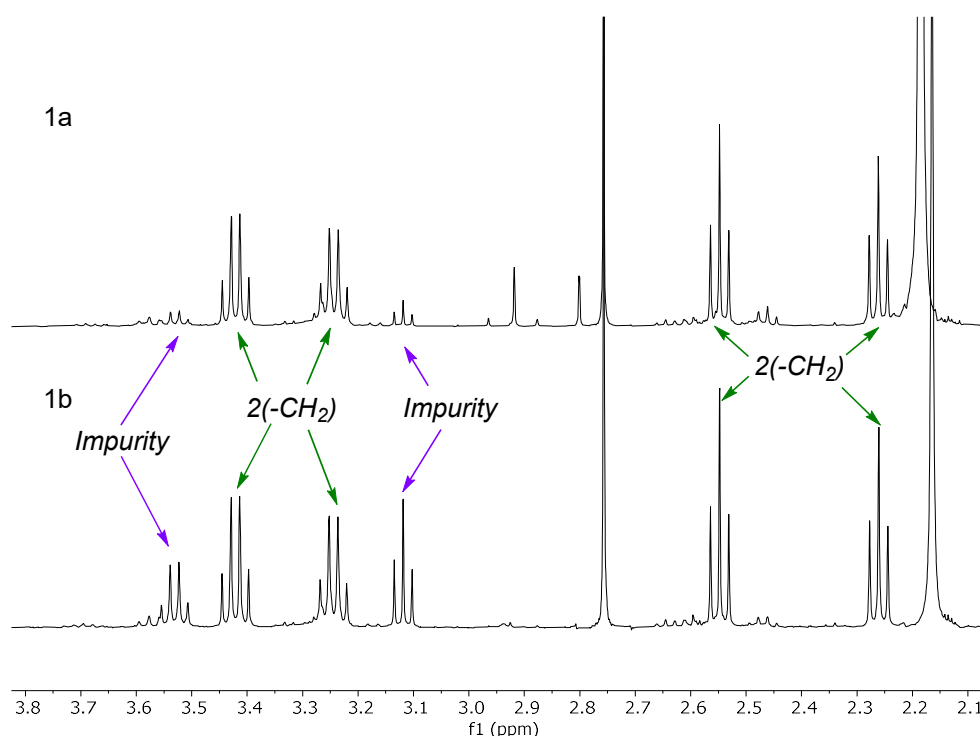


Fig. 33 Comparison of ^1H NMR spectra of crude samples of reaction 1a and 1b in CDCl_3 .

Comparatively, the $\text{EDC} \cdot \text{HCl}$ activated reactions showed a reduced impurity profile in DCM (2b) than in DMF (2a) (**Fig. 34**). Reaction 2b showed a high ratio of impurity to product (approximately 1:3), which was also suspected to be the activated intermediate (2.47). For this reason, it was decided that the HATU conditions used in reaction 1a would be used as a control for the optimisation of coupling conditions.

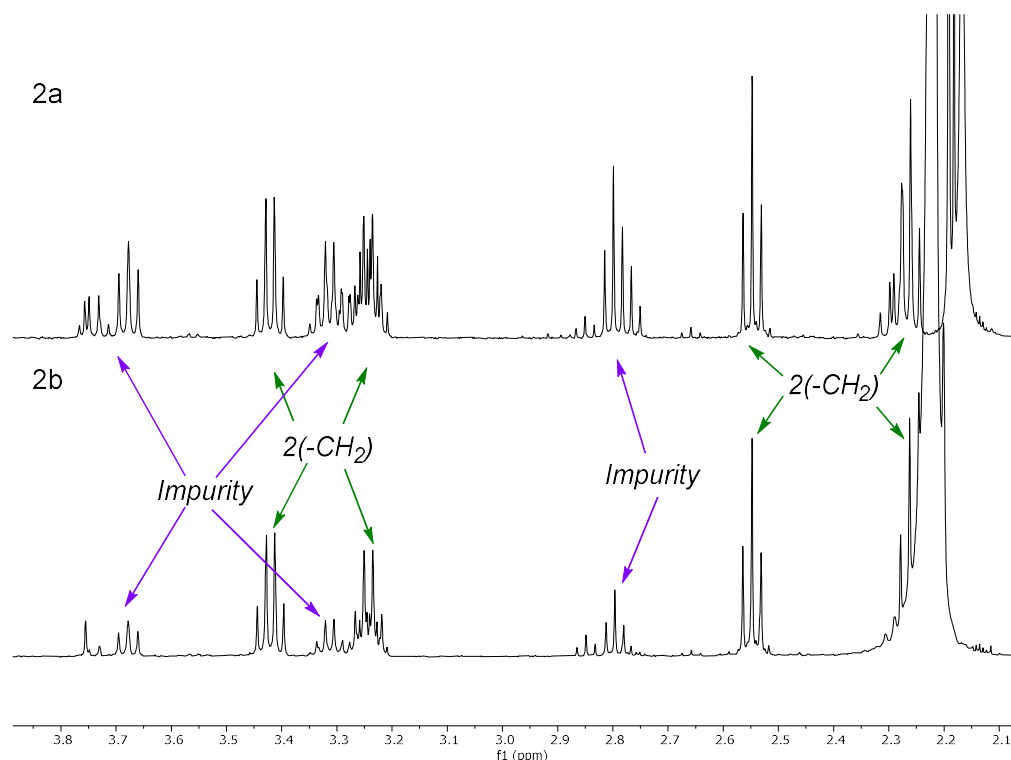
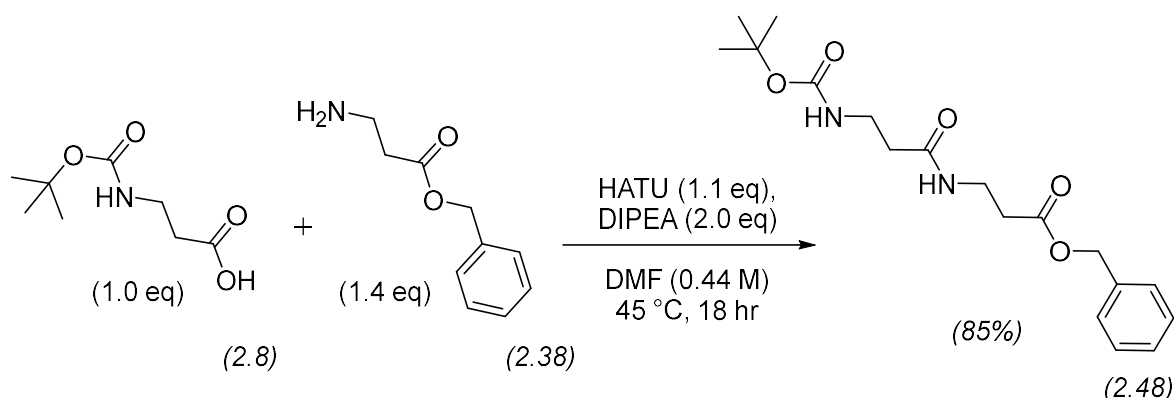


Fig. 34 Comparison of ^1H NMR spectra of crude samples of reaction 2a and 2b in CDCl_3 .

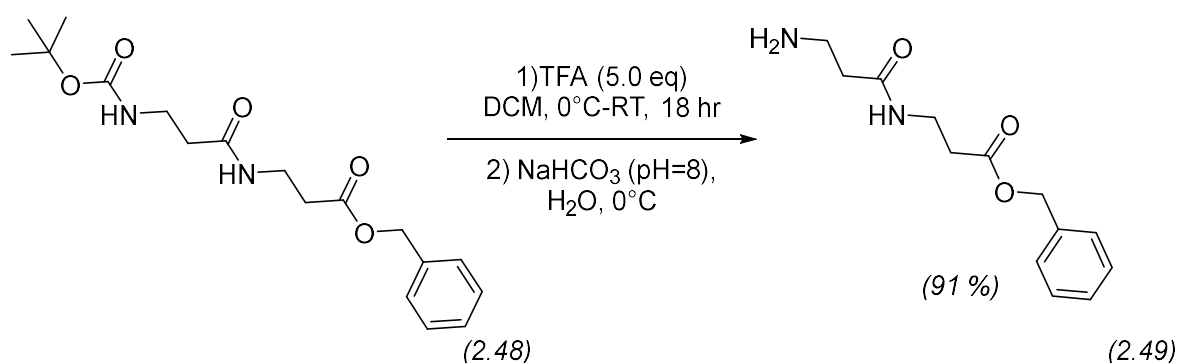
Analysis of the ^1H NMR spectrum of the crude sample of reaction 1a showed probable intermediate, at around 11 %. To drive the reaction towards completion, 3a utilised an increased equivalency of benzyl β -alaninate (2.38), from 1.2 eq to 1.4 eq. This reduced the remaining intermediate in the visual inspection to around 7-8 %. The crude product was isolated, and a sample of pure product was obtained *via* fractional column chromatography. The sample was analysed by NMR spectroscopy, which aligned with reported literature and confirmed the correct assignment of product peaks in the optimisation studies.⁵⁹ These conditions were carried forward into a gram scale test giving a 62 % yield of purified product (2.48).

During purification of the gram scale reaction, it was noted that while minor residual DMF did not affect the column elution, it did cause excess silica to be required for sample dry loading. It was decided that a reduced volume of DMF should be used in future reactions to allow for easier solvent removal prior to purification. This led to the final standard conditions, which were utilised on a 5 g scale to give an 85 % yield of the desired product after purification (**Scheme 18**).



Scheme 18 Optimised conditions for peptide coupling to synthesise benzyl (N-*t*-butyloxycarbonyl)-β-alanyl-β-alaninate (2.48).

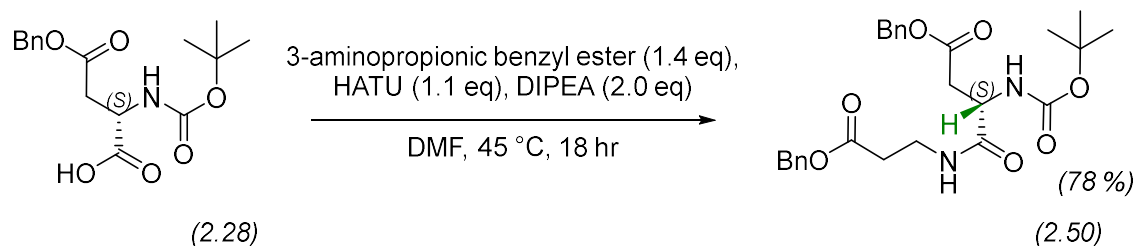
Deprotection of the *N*-terminus was carried out using TFA in DCM to yield benzyl β-alanyl-β-alaninate (2.49), which was stored as a TFA salt until required (**Scheme 19**). The amine was isolated in a 91 % yield by stirring in aqueous sodium bicarbonate solution at 0 °C and extracting with DCM.



Scheme 19 Conditions for *N*-deprotection to provide benzyl β-alanyl-β-alaninate (2.49).

The optimised approach was then used to synthesise benzyl (N-*t*-butyloxycarbonyl)-4-benzyl-(*S*)-aspartyl)-β-alaninate (2.50) by coupling benzyl β-alaninate (2.38) with 4-benzyl-*N*-Boc-(*S*)-aspartic acid (2.28) (**Scheme 20**). The product was isolated with a moderate yield of 78 % and the structure confirmed by NMR spectroscopy and HRMS (**Fig. 35**). The ratio of aromatic (7.46-7.26 ppm) to *t*-butyl (1.40 ppm) signals was approximately 10:9 and a clear shift was seen in the -CH (4.35 ppm, highlighted in green) and three sets of diastereotopic -CH₂ (3.40-2.49 ppm) peaks when compared with the two starting materials. Alongside the identification of the correct mass in the HRMS, this indicated the desired product had been formed. The slight reduced yield of 78 % compared with the optimisation reaction may be

due to some steric hindrance from the protected aspartic acid and could improve with the use of a higher temperature.



Scheme 20 Synthesis of benzyl (*N*-(*t*-butyloxycarbonyl)-4-benzyl-(*S*)-aspartyl)- β -alaninate (2.50) from benzyl β -alaninate (2.38) and benzyl (*N*-(*t*-butyloxycarbonyl)-4-benzyl-(*S*)-aspartyl)- β -alaninate (2.28).

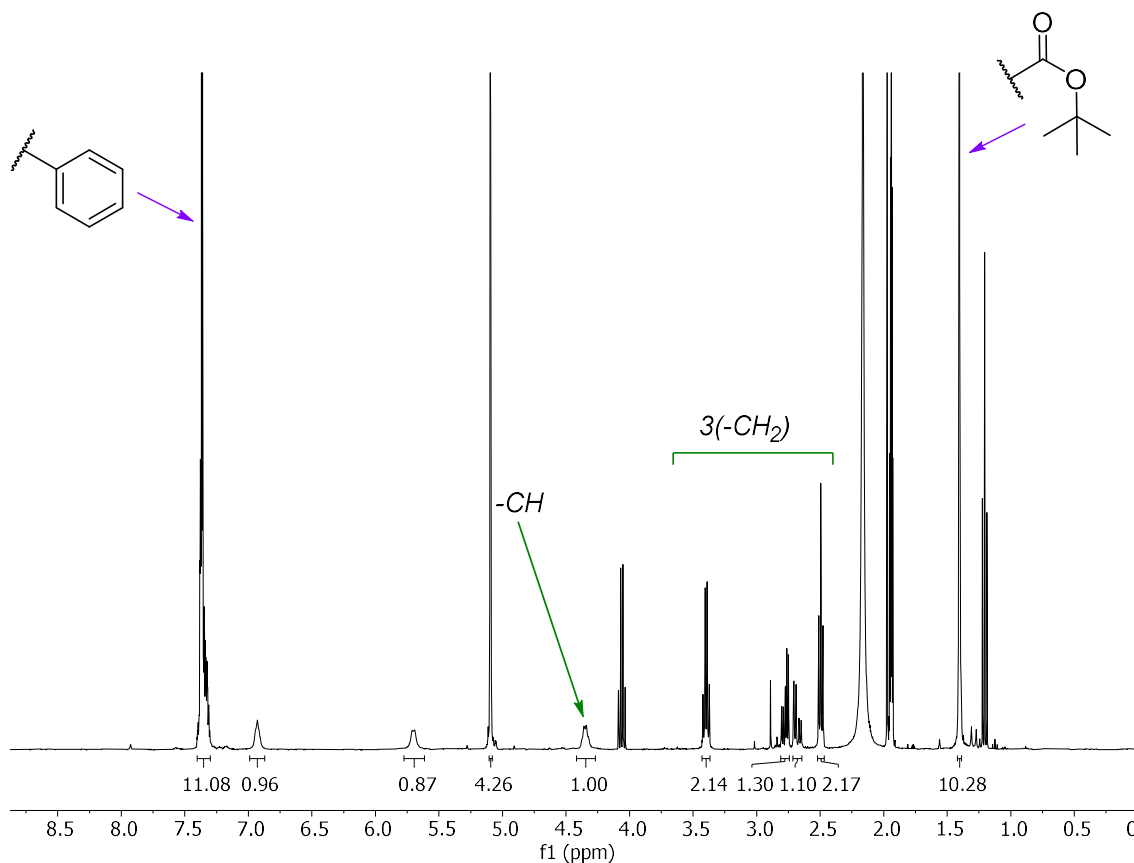
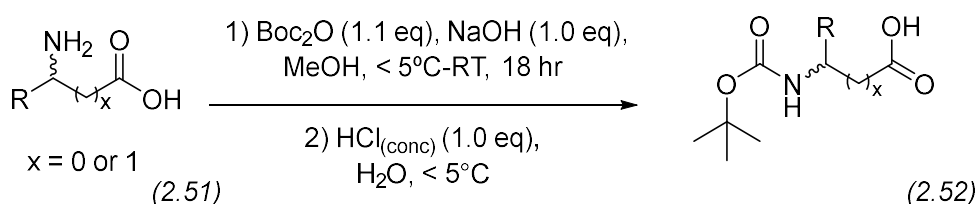


Fig. 35 ^1H NMR spectrum of benzyl (*N*-(*t*-butyloxycarbonyl)-4-benzyl-(*S*)-aspartyl)- β -alaninate (2.50) in CDCl_3 , highlighting the key peaks used to identify the structure (**A-C2-37**).

2.4. Conclusions and Future Work

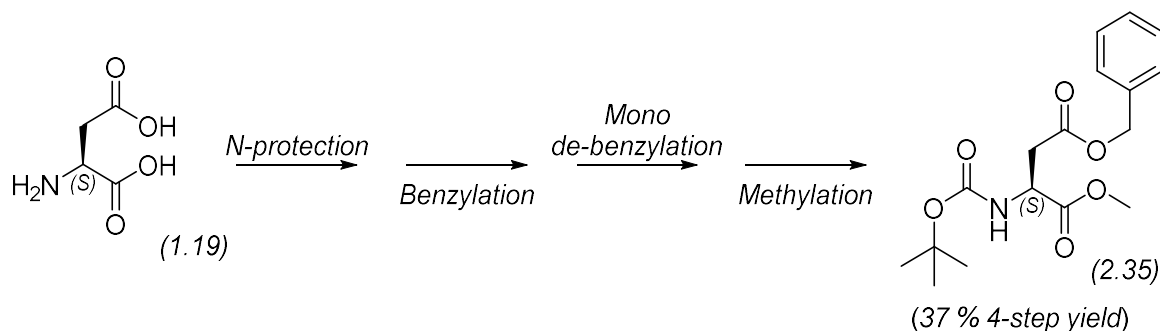
The main goal for this portion of work was to optimise conditions for the synthesis of two model cyclic peptides of the nemamides. This involved finding cheap and efficient methods of protecting amino acids, as well as optimising conditions for solution phase peptide synthesis.

A simple and effective method of *N*-protection of amino acids has been devised and optimised. Using Boc anhydride and sodium hydroxide in methanol provides repeatedly high yields for each of the amino acids (**Scheme 21**). This significantly reduces the cost of synthesis as it allows for purchase of the un-natural amino acids in their un-protected form.



Scheme 21 Optimised method for *N*-protection of amino acids with Boc anhydride.

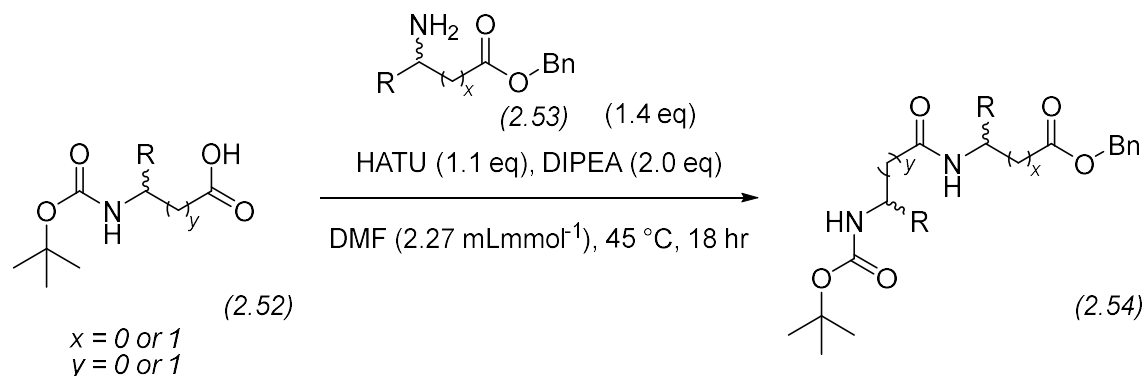
Methods for differentially protecting aspartic acid (*1.19*) have been investigated, providing a simple four step synthesis of 4-benzyl 1-methyl-*N*-Boc-(*S*)-aspartate (*2.35*) from aspartic acid (**Scheme 22**). A moderate yield of 37 % was achieved over the 4-steps, with the loss of yield mostly occurring during the purification of the benzylation step. It may be possible to improve this yield in the future by testing different trituration solvents.



Scheme 22 Four step synthesis towards 4-benzyl 1-methyl-*N*-Boc-(*S*)-aspartate (*2.35*) from aspartic acid (*1.19*), giving an overall 4-step yield of 37 %.

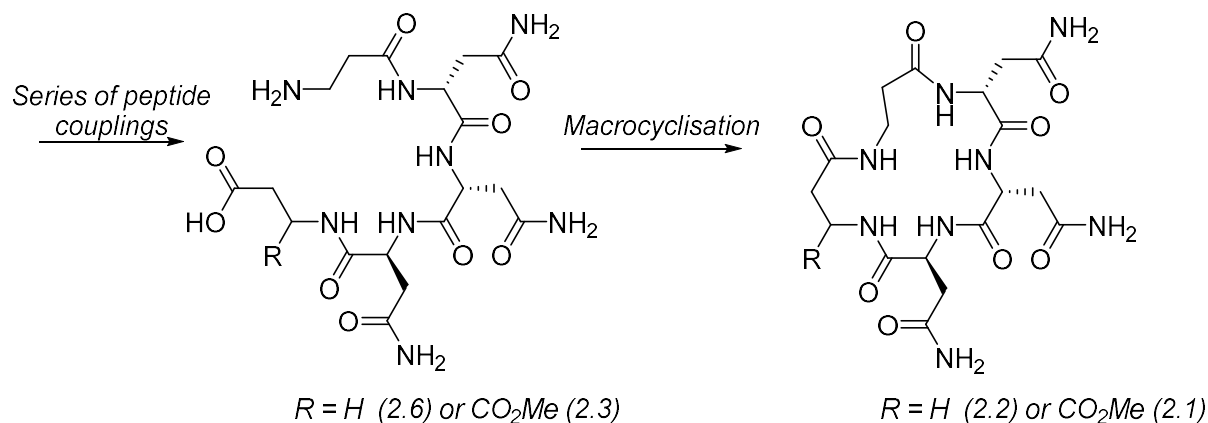
Multiple general coupling conditions have been tested, and a standard reaction protocol has been optimised to use HATU and DIPEA in DMF (**Scheme 23**). This protocol has been tested on two different systems which include two of three types of amino acid in the model

systems. Both reactions showed moderate to high yields. To complete the trial series, these coupling conditions will need to be tested on a reaction containing asparagine.



Scheme 23 Standard conditions for amino acid coupling with HATU and DIPEA in DMF, which have been tested using α - and β -amino acids; where R is the amino acid side chain.

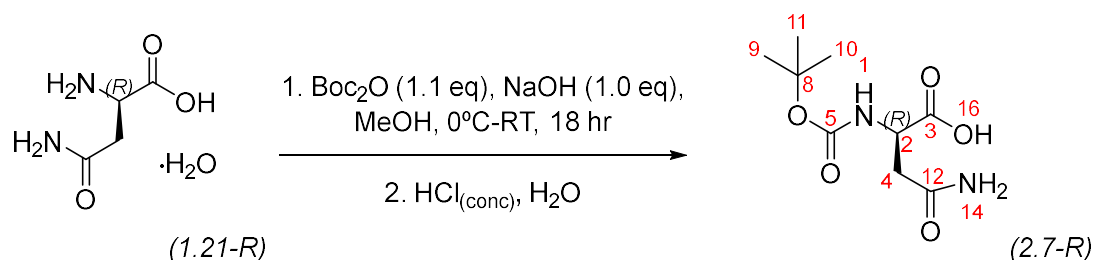
Future work will see these optimised methods utilised in the synthesis of the two model peptides (2.1 and 2.2) (**Scheme 24**). The protected amino acids will be sequentially coupled together to provide the two linear chains (2.3 and 2.6). Macrocyclisation techniques will be trialled and optimised using these two chains, providing an initial method for macrocyclisation of the nemamides.



Scheme 24 Future work will use the optimised approaches to synthesise the model systems in a series of peptide couplings, followed by macrocyclisation.

2.5. Experimental Section

Boc-(*R*)-Asparagine (2.7-*R*)



1.21-*R* (12.00 g, 79.93 mmol, 1.0 eq) was dissolved in methanol (50 mL), cooled to 0 °C and NaOH (3.20 g, 79.93 mmol, 1.0 eq) was added portionwise. Boc₂O (19.19 g, 87.92 mmol, 1.1 eq) was dissolved in MeOH (50 mL) and added dropwise to the solution. Upon completion of addition, the reaction material was allowed to warm to RT and stirred for 18 hr. Solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of water. The reaction material was cooled to 0 °C and HCl (37 % w/v) (6.66 mL, 79.93 mmol, 1.0 eq) was added dropwise. The colourless precipitate was isolated *via* filtration and washed with cold water and hexanes. The product was dried on a high vacuum line to give a colourless solid (15.33 g, 82 % yield).

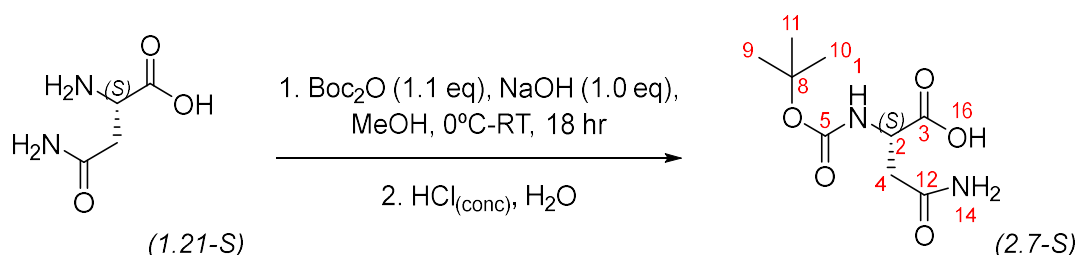
Spectroscopic data is in line with literature.⁶⁰

See A-C2-1 and A-C2-2

¹H NMR (400 MHz, Acetone-*d*₆) δ 7.18 (s, 1H, N(14)-H_a), 6.56 (s, 1H, N(14)-H_b), 6.13 (d, *J* = 8.6 Hz, 1H, N(1)-H), 4.38 (t, *J* = 5.6 Hz, 1H, C(2)-H), 2.85-2.68 (m, 2H, C(4)-H₂), 1.41 (s, 9H, C(9-10)-H₃).

¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.3, 155.9, 78.1, 36.6, 34.7, 28.7

Boc-(S)-Asparagine (2.7-S)



1.21-S (5.00 g, 37.8 mmol, 1.0 eq) was dissolved in methanol (40 mL), cooled to 0 °C and NaOH (1.51 g, 37.8 mmol, 1.0 eq) was added portionwise. Boc_2O (9.07 g, 41.58 mmol, 1.1 eq) was dissolved in MeOH (40 mL) and added dropwise to the solution. Upon completion of addition, the reaction material was allowed to warm to RT and stirred for 18 hr. Solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of water. The reaction material was cooled to 0 °C and HCl (37 % w/v) (3.15 mL, 37.8 mmol, 1.0 eq) was added dropwise. The colourless precipitate was isolated *via* filtration and washed with cold water and hexanes. The product was dried on a high vacuum line to give a colourless solid (6.92 g, 79 % yield)

*Spectroscopic data is in line with literature.*⁶⁰

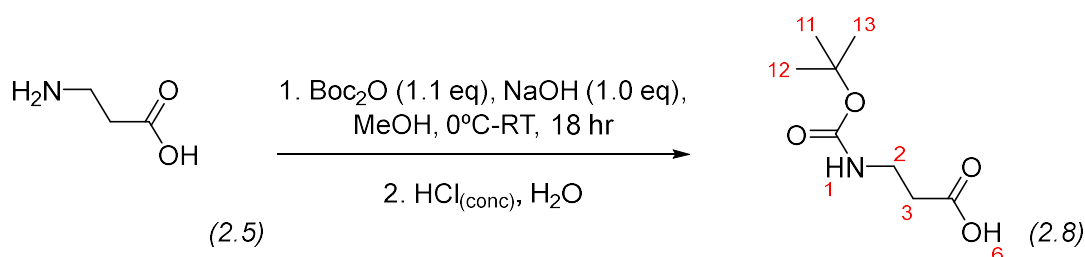
See A-C2-3 to A-C2-5.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.53 (br-s, 1H, O(16)-H), 7.33 (s, 1H, N(14)-H), 6.91 (s, 1H, N(14)-H) 6.87 (d, J = 8.2 Hz, 1H, N(1)-H), 4.26-4.23 (m, 1H, C(2)-H), 2.38-2.47 obscured by solvent peak (m, 2H, C(4)-H₂), 1.37 (s, 9H, C(9-11)-H₃)

^1H NMR (400 MHz, Acetone- d_6) δ 4.36 (t, J = 5.6 Hz, 1H, C(2)-H), 3.02 – 2.53 (m, 2H, C(4)-H₂), 1.41 (s, 9H, C(9-11)-H₃).

^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 173.3, 155.9, 78.1, 36.6, 34.7, 28.7

***N*-(*tert*-Butyloxycarbonylamino) propionic acid (2.8)**



2.5 (10.00 g, 0.112 mol, 1.0 eq) was dissolved in methanol (80 mL), cooled to 0°C and NaOH (4.49 g, 0.112 mol, 1.0 eq) was added portionwise. Boc_2O (26.95 g, 0.124 mol, 1.1 eq) was dissolved in MeOH (80 mL) and added dropwise to the solution. Upon completion of addition, the reaction material was allowed to warm to RT and stirred for 18 hr. Solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of water. The reaction material was cooled to 0°C and HCl (37 % w/v) (9.34 mL, 0.112 mol, 1.0 eq) was added dropwise. The colourless precipitate was isolated *via* filtration and washed with cold water and hexanes. The product was dried on a high vacuum line to give a colourless solid (20.11 g, 95 % yield).

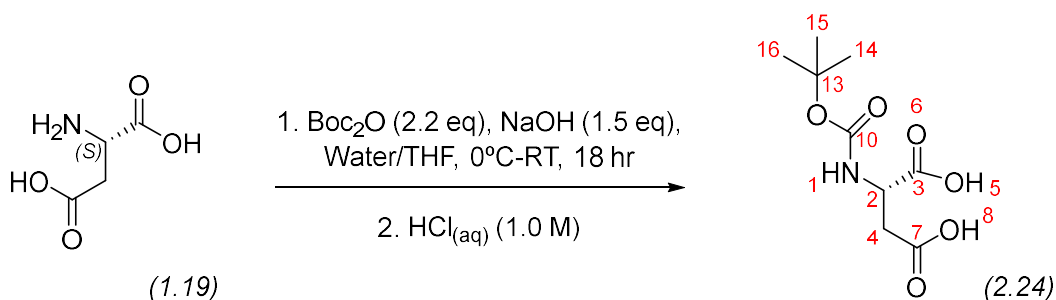
*Spectroscopic data is in line with literature.*⁶¹

See A-C2-6 to A-C2-8

^1H NMR (400 MHz, Acetonitrile- d_3) δ 5.39 (s, 1H, N(1)-H), 3.25 (q, $J = 6.6$ Hz, 2H, C(2)- H_2), 2.44 (t, $J = 6.6$ Hz, 2H, C(3)- H_2), 1.40 (s, 9H, C(11-13)- H_3).

^{13}C NMR (101 MHz, Acetonitrile- d_3) δ 173.3, 156.4, 78.9, 36.7, 34.4, 28.2.

***N*-Boc-(*S*)-Aspartic acid (2.24)**



Sodium hydroxide (4.50 g, 112.7 mmol, 1.5 eq) was added to water (60 mL) and stirred at 0 °C until fully dissolved. **1.19** (10 g, 75.1 mmol, 1.0 eq), was dissolved in THF (60 mL) and added to the solution slowly. Boc₂O (36 g, 165.2 mmol, 2.2 eq) was dissolved in THF (60 mL) and added to the solution dropwise. Upon completion of addition, the reaction material was gradually allowed to warm to RT and stirred for 18 hr. The flask was cooled to 0 °C and HCl (1.0 M, ~1.5 eq) was added dropwise until pH ~ 3-4. The solid was collected by filtration, washed with ice cold water (30 mL) and hexanes (3 × 50 mL). Product was dried on a high vacuum line to give a powdery colourless solid (12.65 g, 72 % yield).

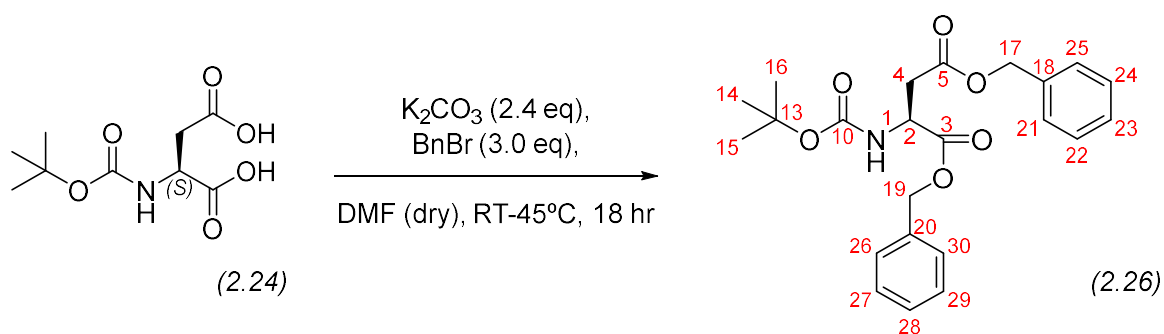
Spectroscopic data is in line with literature.^{62,63}

See A-C2-9 to A-C2-11

¹H NMR (400 MHz, DMSO-*d*₆) δ 12.51 (Br-s, 2H, O(5,8)-H), 7.00 (d, *J* = 8.2 Hz, 1H, N(1)-H), 4.23 (dt, *J* = 8.2, 5.8 Hz, 1H, C(2)-H), 2.57 (m, masked by DMSO-*d*₆, 2H, C(4)-H₂), 1.37 (s, 9H, C(14-16)-H₃)

¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.0, 171.8, 155.2, 78.2, 50.1, 36.3, 28.2

***N*-(*t*-Butyloxycarbonyl) (*S*)-aspartic acid dibenzyl ester (2.26)**



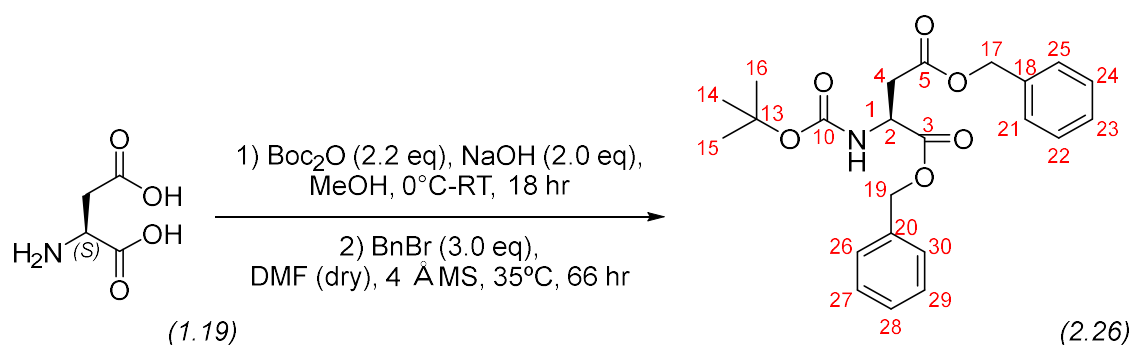
2.24 (5.00 g, 2.1 mmol, 1.0 eq), and K_2CO_3 (7.11 g, 5.1 mmol, 2.4 eq) were charged to a dry flask and sealed under Argon. Anhydrous DMF (20 mL) was charged, and the reaction material was stirred rapidly. BnBr (11.00 g, 6.5 mmol, 3.0 eq) was charged over 5 mins, before heating at 45 °C for 18 hr. Upon confirmation of completion of reaction by TLC (100 % EtOAc), water (30 mL) was charged, and product was extracted with EtOAc (3 × 100 mL). Organic phases were combined, washed water (3 × 100 mL), $NaHCO_3$ (3 × 100 mL) and 1/3 saturated brine solution (3 × 100 mL). The organic phase was dried over Na_2SO_4 , filtered and concentrated under reduced pressure to obtain crude product as a yellow oil (5.7 g, 65 % yield). Crude product was carried through without further purification.

*Spectroscopic data is in line with literature.*⁶⁴

See A-C2-12 to A-C2-15

^1H NMR (400 MHz, Chloroform-*d*) δ 7.44-7.28 (m, 10H, C(21-30)-H), 5.18 (s, 1H, N(1)-H), 5.11 (s, 2H, C(17 or 19)-H₂), 5.09 (s, 2H, C(17 or 19)-H₂), 4.50 (td, J = 7.9, 4.8 Hz, 1H, C(2)-H), 2.93-2.87 (dd, J = 16.3, 7.9, 1H, C(4)-H_a), 2.82-2.74 (dd, J = 16.3, 7.9, 1H, C(4)-H_b), 1.38 (s, 9H, C(14-16)-H₃)

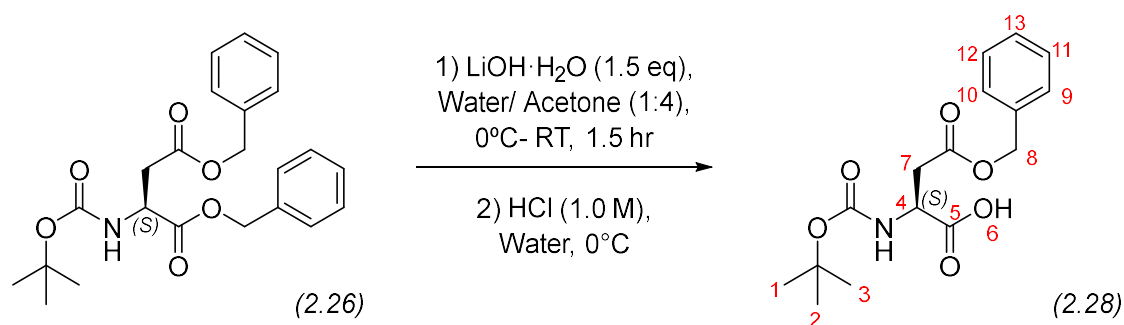
^{13}C NMR (101 MHz, DMSO-*d*₆) δ 171.4, 170.3, 155.7, 136.4, 136.2, 128.8-128.2, 79.0, 66.7, 66.3, 50.7, 36.3, 28.6



1.19 (5.00 g, 37.6 mmol, 1.0 eq) was suspended in methanol (50 mL) and cooled to 0 °C. NaOH (3.00 g, 75.1 mmol, 2.0 eq) was dissolved in methanol (50 mL) and the solution was added dropwise to the cooled suspension. Boc₂O was dissolved in THF (20 mL) and added dropwise to the reaction flask, which was then allowed to warm to RT and stirred for 18 hr. Solvent was concentrated under reduced pressure until around 20 mL of solvent remained, and hexanes (200 mL) was added. The solvent was removed under reduced pressure and azeotropic distillation with hexanes (100 mL) was repeated twice more. The resultant colourless solid was dried on a high vacuum line and dissolved in anhydrous DMF (85 mL). The solution was stirred over activated 4 Å MS for 10 min, BnBr (13.4 mL, 0.113 mol, 3.0 eq) was added and the reaction was heated to 35 °C. The reaction material was stirred for 66 hr before being cooled to RT and filtered over a pad of Celite®. The pad was washed with water (100 mL) and EtOAc (250 mL). The phases of the filtrate were separated, and the aqueous phase was extracted with EtOAc (100 mL × 2). Organic phases were combined and washed with water (150 mL) and 1/3 saturated brine solution (150 mL × 2). The organic phase was dried over MgSO₄, filtered and concentrated. The subsequent oil was triturated with hexanes to give pure product as a colourless solid (6.70 g, 43 % yield).

Spectroscopic data matches those of the previous method.

4-Benzyl-*N*-Boc-(*S*)-aspartic acid (2.28)



2.26 (1.00 g, 2.42 mmol, 1.0 eq) was charged to a flask with acetone (4 mL) and cooled to 0 °C. LiOH·H₂O (152 mg, 3.63 mmol, 1.5 eq) was dissolved in ice cold water (1 mL) and added to the reaction material. The reaction material was allowed to stir for 1.5 hr and concentrated under reduced pressure to obtain the intermediate salt as a viscous colourless compound. Residue was taken up in a minimum volume of water (~3 mL) and cooled to 0 °C. Solution was acidified to pH ~4/5 with HCl (1.0 M) and immediately extracted with EtOAc (6 mL × 3). Organic phases were combined, dried over Na₂SO₄ and concentrated under reduced pressure to give product as a colourless sticky semi-solid (163 mg, 21 % yield).

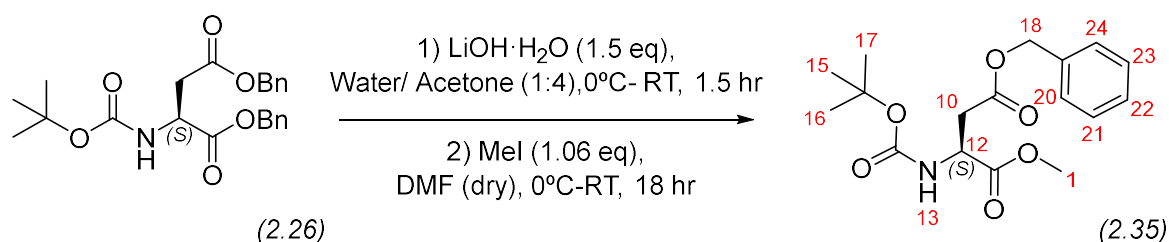
Spectroscopic data is in line with literature.⁵⁷

See A-C2-16 to A-C2-19

¹H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.29 (m, 5H, C(9-13)-H), 5.54 (br d, *J* = 8.3 Hz, 1H, N-H), 5.15 (dd, *J* = 12.3, 2.9 Hz, 2H, C(8)-H₂), 4.74 – 4.54 (m, 1H, C(4)-H), 3.08 (dd, *J* = 17.3, 4.2 Hz, 1H, C(7)-H_a), 2.89 (dd, *J* = 17.3, 4.9 Hz, 1H, C(7)-H_b), 1.45 (s, 9H, C(1-3)-H₃).

¹³C NMR (101 MHz, Chloroform-*d*) δ 175.3, 171.2, 155.8, 135.4, 128.8, 128.6, 128.5, 80.8, 67.2, 50.0, 36.6, 28.4.

4-Benzyl 1-methyl *N*-Boc-(*S*)-aspartate (2.35)



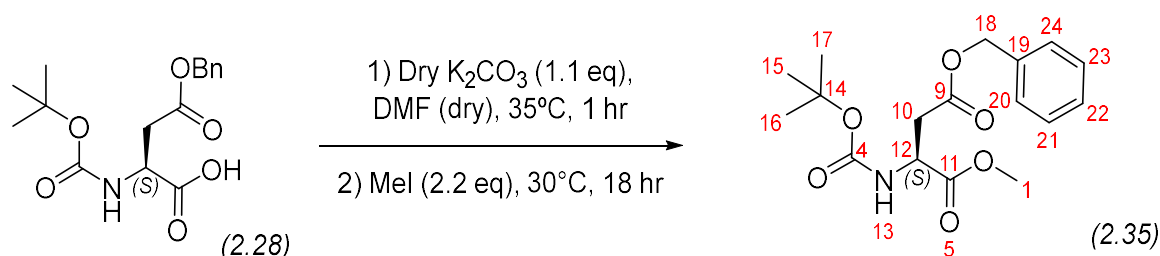
2.26 (2.20 g, 5.33 mmol, 1.0 eq) was charged to a flask with acetone (9 mL) and cooled to 0 °C. LiOH·H₂O (0.33 g, 7.99 mmol, 1.5 eq) was dissolved in ice cold water (2 mL) and added to the reaction material. The reaction was allowed to stir for 1.5 hr before being concentrated under reduced pressure to obtain the intermediate salt as a viscous colourless compound. Anhydrous DMF (7 mL) was charged, and the reaction material was cooled to 0 °C. MeI (0.80 g, 5.65 mmol, 1.06 eq) was added dropwise. The reaction was allowed to stir for 18 hr. Upon confirmation of completion of reaction by TLC (100 % EtOAc), water (30 mL) was charged, and the product was extracted with EtOAc (3 × 100 mL). Organic phases were combined, washed with water (3 × 100 mL), NaHCO₃ (3 × 100 mL) and 1/3 saturated brine solution (3 × 100 mL). The organic phase was dried over Na₂SO₄, filtered and concentrated under reduced pressure to obtain crude product as a yellow oil. Crude product was purified by column chromatography (20-100 % EtOAc in hexanes) to give purified product as a colourless oil (1.5 g, 84 % yield, ~ 90 % purity).

*Spectroscopic data is in line with literature.*⁶⁵

See A-C2-20 to A-C2-23

¹H NMR (400MHz, Chloroform-*d*) δ 7.37-7.26 (m, 5H, C(20-24)-H), 5.47 (br d, *J* = 8.7 Hz, 1H, N(13)-H), 5.13 (d, *J* = 12.3 Hz, 1H, C(18)-H_a), 5.10 (d, *J* = 12.3 Hz, 1H, C(18)-H_b), 4.59 (dt, *J* = 8.7, 4.5 Hz, 1H, C(12)-H), 3.70 (s, 3H, C(1)-H₃), 3.07-3.02 (dd, *J* = 16.8, 4.5 Hz, 1H, C(10)-H_a), 2.90-2.84 (dd, *J* = 16.8, 4.5 Hz, 1H, C(10)-H_b), 1.44 (9H, s, C(15,16,17)-H₃)

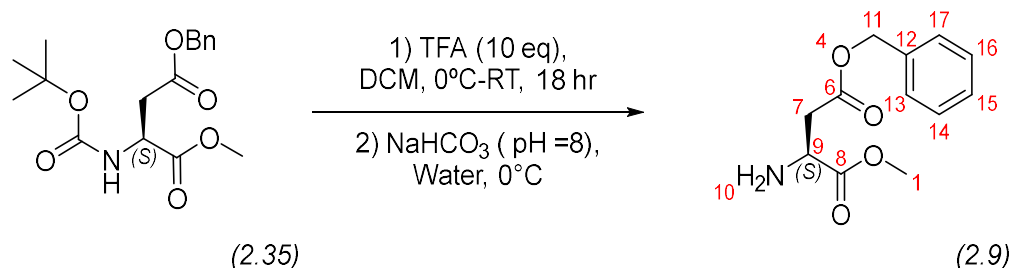
¹³C NMR (101 MHz, Chloroform-*d*) δ 171.6, 170.9, 135.5, 128.8, 128.6, 128.5, 80.3, 67.0, 52.8, 50.1, 37.1, 28.4



2.28 (15.29 g, 0.047 mol, 1.0 eq) was charged to a pre-dried flask under argon with dry K_2CO_3 (7.18 g, 0.052 mol, 1.1 eq). Anhydrous DMF (60 mL) was charged, and the reaction material heated at 35°C for 1 hr. The reaction material was cooled to 30°C and MeI (5.14 mL, 0.104 mol, 2.2 eq) was charged dropwise before being stirred for 18 hr. Upon confirmation of consumption of SM by TLC (100 % EtOAc) the reaction was quenched with water (100 mL) and extracted with ethyl acetate (3×100 mL). The organic extracts were combined, washed with water (3×100 mL) and 1/3 saturated brine solution (3×100 mL). The organic phase was dried over Na_2SO_4 , filtered and concentrated under reduced pressure to yield a pale yellow solid. (15.54 g, 97 % yield).

Spectroscopic data matches that of the previous method.

4-Benzyl 1-methyl (*S*)-aspartate (2.9)



2.35 (10.10 g, 29.9 mmol, 1.0 eq) was dissolved in DCM (70 mL) and cooled to 0 °C. TFA (11.40 mL, 149.9 mmol, 5.0 eq) was mixed with DCM (30 mL) and added dropwise over 2 hr before being stirred at RT for 18 hr. Upon confirmation of completion of reaction by TLC (100 % EtOAc), the reaction was diluted with water (100 mL), cooled to 0 °C and solid NaHCO₃ was charged with vigorous stirring until a consistent basic pH was reached (approx. pH 8). The phases were separated, and the aqueous phase was extracted with DCM (2 × 50 mL). Organic phases were combined, washed with water (3 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure to obtain product as a yellow oil (7.15 g, Quant. yield).

See A-C2-24 to A-C2-25

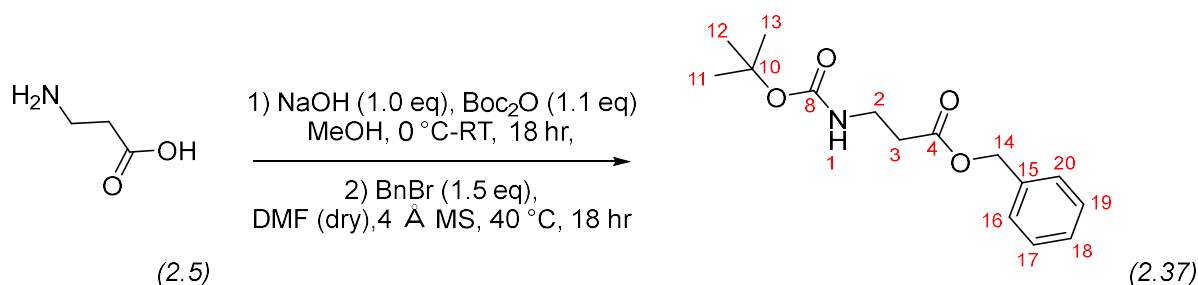
¹H NMR (500 MHz, Chloroform-*d*) δ 7.39 – 7.31 (m, 5H, C(13-17)-H), 5.15 (d, *J* = 12.1 Hz, 1H, C(11)-H_a), 5.12 (d, *J* = 12.1 Hz, 1H, C(11)-H_b), 3.83 (dd, *J* = 7.2, 4.7 Hz, 1H, C(9)-H), 3.69 (s, 3H, C(1)-H₃), 2.90-2.84 (dd, *J* = 4.7, 16.8 Hz, 1H, C(7)-H_a), 2.78-2.72 (dd, *J* = 7.2, 16.8 Hz, 1H, C(7)-H_b) 1.88 (s, 2H, N(10)-H₂).

¹³C NMR (126 MHz, Chloroform-*d*) δ 174.7, 171.1, 135.7, 128.7, 128.5, 128.4, 66.8, 52.4, 51.3, 39.2

IR /cm⁻¹: 3351 (N-H), 1705 (C=O), 1654 (C=O), 1536 (C=C Aromatic), 1153 (C-O)

HRMS ESI⁺ *m/z* calculated for C₁₂H₁₅NO₄ (M + H⁺) 238.1035, Found: 238.1033

***N*-(*t*-Butyloxycarbonylamino) propionic benzyl ester (2.37)**



2.5 (10.00 g, 0.11 mol, 1.0 eq), was charged to a flask with NaOH (4.49 g, 0.11 mol, 1.0 eq) and cooled to 0 °C. MeOH (20 mL) was charged, and the reaction was allowed to stir until complete dissolution occurred. Boc₂O (26.40 g, 0.12 mol, 1.1 eq) was dissolved in MeOH (20 mL) and added dropwise to the reaction over 15 min. The reaction material was allowed to warm to RT and stirred for 18 hr. The solvent was removed under reduced pressure and residual solvent was removed using azeotropic distillation with hexanes, before drying on a high vacuum line.

The salt was dissolved in anhydrous DMF (250 mL) and stirred with 4 Å MS for 20 min. BnBr (28.70 g, 0.17 mol, 1.5 eq) was added dropwise over 5 min, before being heated to 40 °C and stirred for 18 hr. The reaction material was cooled to RT, filtered through a pad of Celite® and washed with EtOAc. The filtrate was washed with water (2 × 200 mL) and brine solution (3 × 200 mL). The organic phase was dried with MgSO₄, filtered and concentrated to obtain product as an orange oil. The oil was subjected to multiple rounds of trituration with hexanes, to give product as a pale yellow oil (22.3 g, 71 % yield).

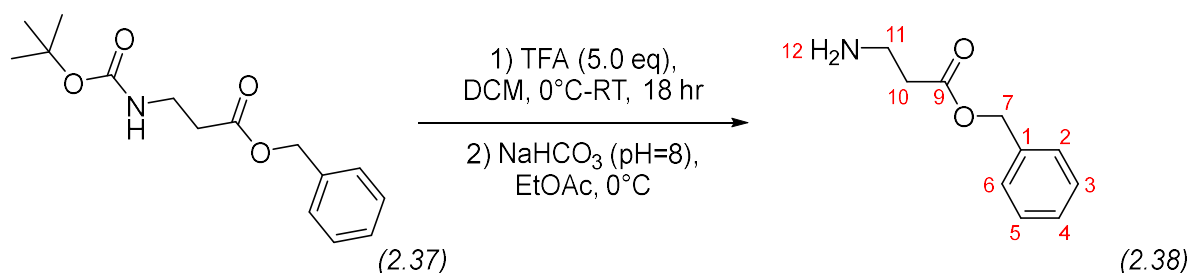
Adapted from literature, spectroscopic data aligns.⁶⁶

See A-C2-26 to A-C2-27

¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.39-7.33 (m, 5H, C(15-19)-H), 5.37 (Br-s, 1H, N(1)-H), 5.10 (s, 2H, C(14)-H₂), 3.29 (q, *J* = 6.6 Hz, 2H, C(2)-H₂), 2.51 (t, *J* = 6.6 Hz, 2H, C(3)-H₂), 1.39 (s, 9H, C(11-13)-H₃)

¹³C NMR (101 MHz, Acetonitrile-*d*₃) δ 172.6, 137.4, 129.5, 129.03, 128.95, 66.9, 37.2, 35.4, 28.5.

Benzyl β -alaninate (2.38)



2.37 (22.2 g, 79.74 mmol, 1.0 eq) was dissolved in DCM (30 mL) and cooled to 0 °C. TFA (45.5 g, 0.39 mol, 5.0 eq) was mixed with DCM (30 mL) and added dropwise to the reaction over 45 min. The reaction material was allowed to warm to RT and stirred for 18 hr. The solvent was removed under reduced pressure. Et₂O was added and the product was extracted with water (3 × 100 mL). The aqueous phase was cooled to 0 °C and EtOAc (100 mL) was added. NaHCO₃ was added portionwise, with vigorous stirring, until pH~ 8. The product was extracted with EtOAc (3 × 100 mL), dried with MgSO₄, filtered and concentrated to obtain a yellow oil which formed a yellow semi-solid upon standing (14.2 g, Quant. yield).

*Spectroscopic data is in line with literature.*⁶⁷

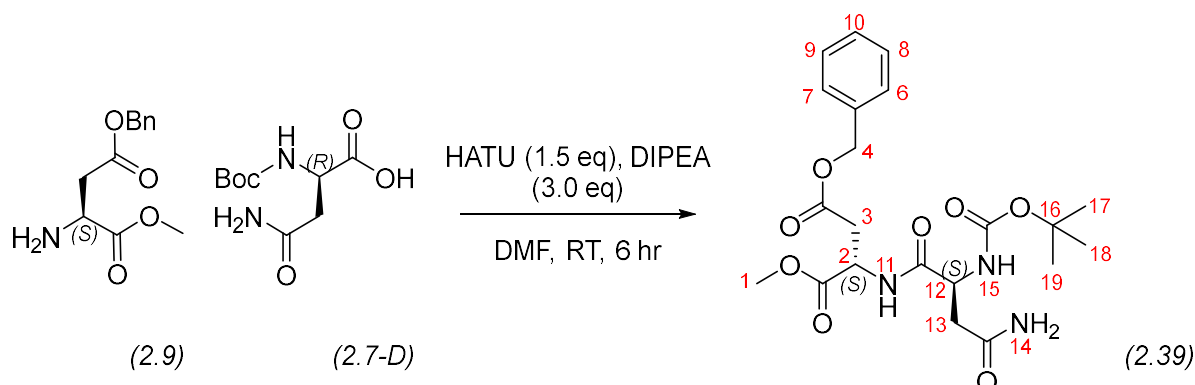
See A-C2-28 to A-C2-30

¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.40-7.33 (m, 5H, C(2-6)-H), 5.14 (s, 2H, C(7)-H₂), 3.21 (t, *J* = 6.6 Hz, 2H, C(10)-H₂), 2.80 (t, *J* = 6.6 Hz, 2H, C(11)-H₂).

¹³C NMR (101 MHz, Acetonitrile-*d*₃) δ 172.04, 136.9 129.5, 129.2, 129.1, 67.5, 36.5, 31.9.

HRMS ESI+ *m/z* calculated for C₁₀H₁₃NO₂ (M + H⁺) 180.0980, Found: 180.1049

4-Benzyl-1-methyl (*S*)-aspartyl-*N*-Boc-*L*-asparagine (2.39)



2.9 (105 mg, 0.45 mmol, 1.0 eq) and **2.7-D** (103 mg, 0.45 mmol, 1.0 eq) were charged to a flask with DIPEA (173 mg, 1.34 mmol, 3.0 eq) and DMF (20 vol). HATU (254 mg, 0.67 mmol, 1.5 eq) was added in 3 portions over 10 min. The reaction material was stirred at RT for 6 hr. Upon confirmation of completion of reaction by TLC (70 % EtOAc in hexanes), the reaction material was diluted with DCM (20 mL), washed with NaHCO₃ (10 mL) and saturated brine solution (10 mL), dried over Na₂SO₄ and filtered. The organic phase was concentrated under reduced pressure and purified by column chromatography (20-100 % EtOAc in hexanes) to obtain product as a colourless oil (100 mg, 50 % yield).

See A-C2-31 to A-C2-32

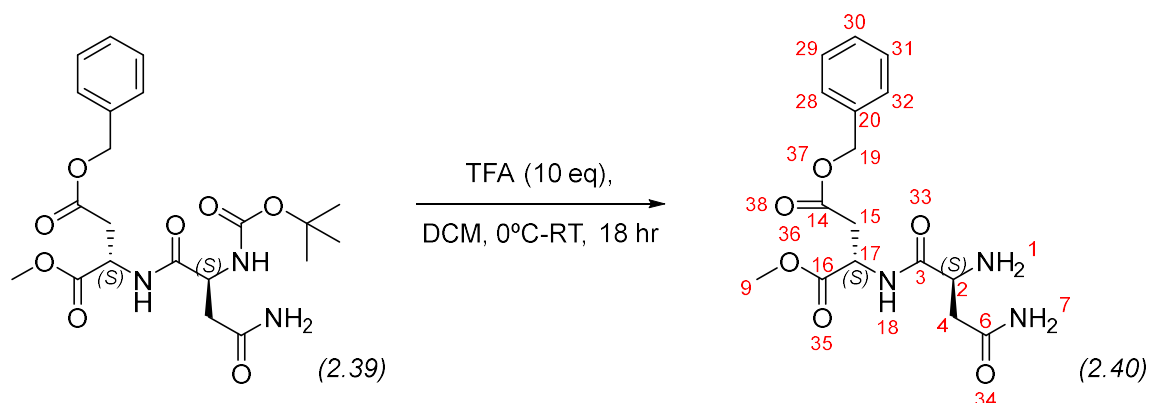
¹H NMR (400 MHz, Chloroform-*d*) δ 7.66 (Br-m, 1H) 7.30-7.35(m, 5H, C(6-10)-H), 6.01 (Br-m, 1H), 5.89 (Br-s, 1H), 5.47 (Br-s, 1H), 5.13 (s, 2H, C(4)-H₂), 4.84 (dt, *J* = 8.3, 4.8 Hz, 1H, C(2 or 12)-H), 4.50 (Br-m, 1H, C(2 or 12)-H), 3.67 (s, 3H, C(1)-H₃), 3.03 (dd, *J* = 16.9, 4.8 Hz, 1H, C(3 or 13)-H_a), 2.94-2.86 (m, 1H, C(3 or 13)-H_b), 2.86 (dd, *J* = 16.9, 5.2 Hz, 1H, m, C(3 or 13)-H_a), 2.04 (dd, *J* = 15.5, 6.1 Hz, 1H, C(3 or 13)-H_b), 1.45 (s, 9H, C(17-18)-H₃)

¹³C NMR (101 MHz, Chloroform-*d*) δ 171.2, 170.8, 170.4, 135.4, 128.6, 128.4, 128.4, 77.2, 66.9, 52.8, 51.5, 48.8, 36.2, 28.3.

IR /cm⁻¹: 3422, 3312, 3204 (N-H stretch), 1739, 1687, 1643 (C=O stretch), 1517 (C=C stretch), 1165 (C-O stretch)

HRMS ESI⁺ *m/z* calculated for C₂₁H₂₉N₃O₈ (M + H⁺) 452.1988, Found: 452.2218

4-Benzyl 1-methyl (*S*)-aspartyl-*L*-asparagine (2.40)

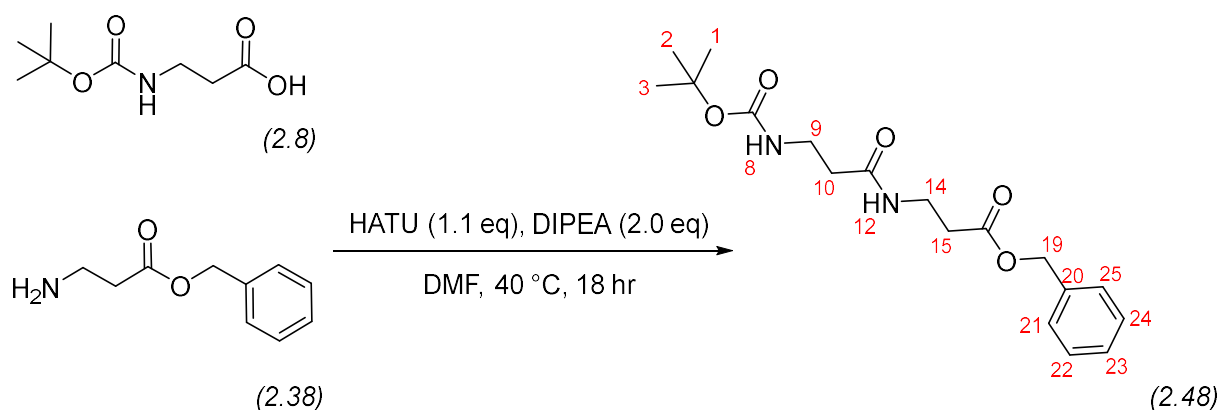


2.39 (100 mg, 0.215 mmol, 1.0 eq) was dissolved in DCM (30 mL) and cooled to 0 °C. TFA (245 mg, 2.15 mmol, 10 eq) was charged dropwise. The reaction material was allowed to slowly warm to RT and stir for 18 hr. Upon confirmation of completion of reaction by TLC (100 % EtOAc), The reaction material was diluted with DCM (30 mL), washed with saturated NaHCO₃ solution (3 × 10 mL) and dried over Na₂SO₄. The organic phase concentrated under reduced pressure to obtain crude product as a colourless oil (100 mg).

HRMS ESI⁺ m/z calculated for C₁₆H₂₁N₃O₆ (M + H⁺) 352.1464, Found: 352.1594

Sample was not determinable by NMR spectroscopy due to low solubility in Chloroform-*d*, Deuterium oxide, Acetone-*d*₆ and Methanol-*d*₄.

Benzyl (N-(⁴butyloxycarbonyl)- β -alanyl- β -alaninate (2.48)



2.8 (5.0 g, 26.4 mmol, 1.0 eq) was stirred in anhydrous DMF (30 mL) under argon. HATU (11.0 g, 29.1 mmol, 1.1 eq) and DIPEA (9.2 mL, 52.9 mmol, 2.0 eq) were added and the reaction was allowed to stir for 30 min. **2.38** (6.6 g, 37.0 mmol, 1.4 eq) was dissolved in anhydrous DMF (30 mL) and added to the reaction, before being heated at 40 °C for 18 hr. The heating was removed, and the reaction was diluted with EtOAc (300 mL) and NaHCO₃ solution (150 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 50 mL). The organics were combined and washed with citric acid solution (10 % w/v, 3 × 75 mL), dried over MgSO₄ and filtered. The solvent was removed under reduced pressure and crude product was purified *via* flash column chromatography (EtOAc:DCM, 1:1) to produce the product as a yellow oil (7.89 g, 85 % yield).

Spectroscopic data is in line with literature.⁵⁹

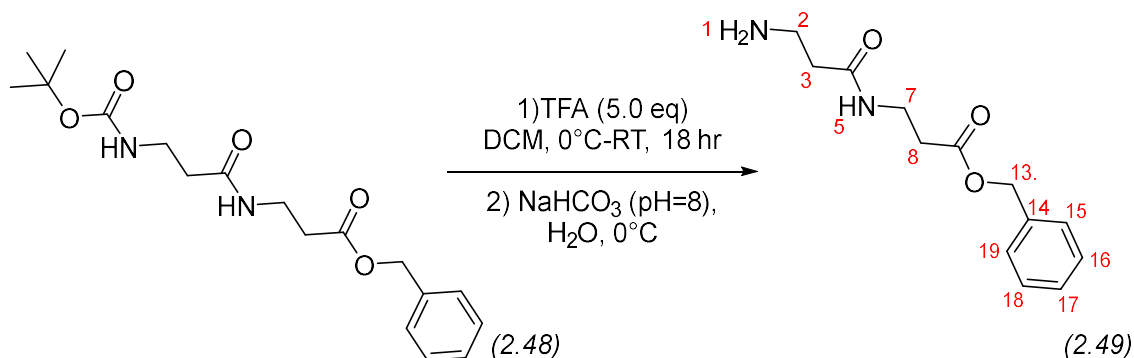
See A-C2-33 to A-C2-36

¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.39-7.33 (m, 5H, C(21-25)-H), 6.55 (br-s, 1H, N(12)-H), 5.41 (s, 1H, N(8)-H), 5.10 (s, 2H, C(19)-H₂), 3.39 (q, *J* = 6.6 Hz, 2H, C(14)-H₂), 3.22 (q, *J* = 6.5 Hz, 2H, C(9)-H₂), 2.52 (t, *J* = 6.6 Hz, 2H, C(15)-H₂), 2.23 (t, *J* = 6.5 Hz, 2H, C(10)-H₂), 1.39 (s, 9H, C(1-3)-H₃).

¹³C NMR (101 MHz, Acetonitrile-*d*₃) δ 172.6, 172.1, 156.7, 137.4, 129.5, 129.03, 128.97, 79.2, 66.9, 37.7, 36.7, 35.8, 34.9, 28.6.

HRMS ESI⁺ *m/z* calculated for C₁₀H₁₃NO₂ (M + Na⁺) 373.1734, Found: 373.1827

Benzyl β -alanyl- β -alaninate (2.49)



2.48 (7.89 g, 22.5 mmol, 1.0 eq) was dissolved in DCM (60 mL) and cooled to 0 °C. TFA (8.67 mL, 0.11 mol, 5.0 eq) was dissolved in DCM (19 mL) and added dropwise to the reaction flask. Upon completion of addition, the reaction was allowed to warm to RT and stirred for 18 hr. Solvent was removed under reduced pressure and the residue was taken up in a minimum volume of water. EtOAc was added and the flask was cooled to 0 °C. NaHCO₃ was added gradually with vigorous stirring, until pH ~ 8. The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 100 mL). The organic phases were combined, washed with saturated NaHCO₃ solution, dried over MgSO₄ and concentrated to give product as an off-white solid (5.12 g, 91 % yield).

See A-C2-37 to A-C2-39

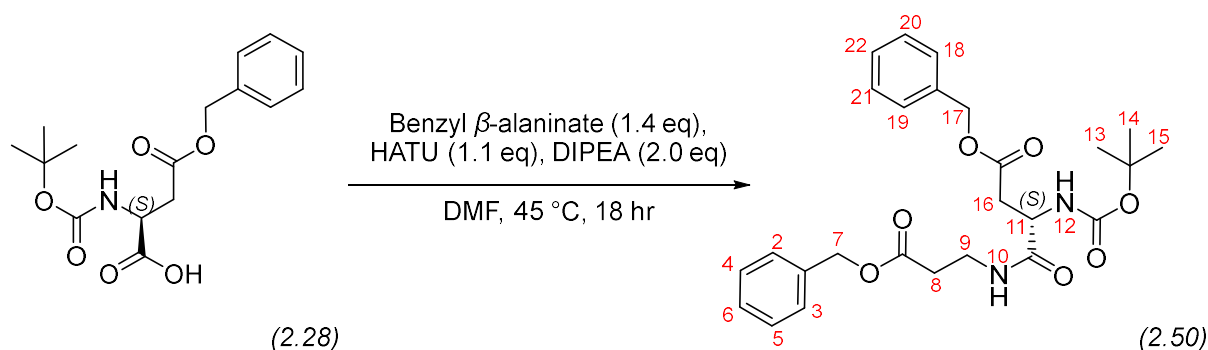
¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.41 (m, 5H, C(15-19)-H), 6.98 (br-s, 1H, N(5)-H) 5.14 (s, 2H, C(13)-H₂), 3.45 (q, *J* = 6.5 Hz, 2H, C(7)-H₂), 2.57 (t, *J* = 6.0 Hz, 2H, C(2)-H₂), 2.57 (t, *J* = 6.5 Hz, 2H, C(8)-H₂), 2.53 (t, *J* = 6.0 Hz, 2H, C(3)-H₂)

¹³C NMR (101 MHz, Chloroform-*d*) δ 172.5, 135.7, 128.7, 128.5, 128.4, 128.4, 66.8, 35.2, 33.9, 32.1, 31.8

IR /cm⁻¹ 3340, 3210 (N-H), 1712, 1649 (C=O), 1540 (C=C Aromatic), 1157 (C-O)

HRMS ESI+ *m/z* calculated for C₁₀H₁₃NO₂ (M + H⁺) 251.1351, Found: 251.1304

Benzyl (*N*-(⁴butyloxycarbonyl)-4-benzyl-(*S*)-aspartyl)- β -alaninate (2.50)



2.28 (0.40 g, 1.20 mmol, 1.0 eq) was dissolved in anhydrous DMF (4 mL). HATU (0.52 g, 1.32 mmol, 1.1 eq) and DIPEA (0.42 mL, 2.4 mmol, 2.0 eq) were charged and the reaction was stirred under argon for 10 min. Benzyl β -alaninate (0.30 g, 1.67 mmol, 1.4 eq) was dissolved in anhydrous DMF (4 mL) and added to the flask. The reaction was heated at 45 °C for 18 hr. Upon confirmation of consumption of KSM, the reaction was cooled to room temperature and was diluted with EtOAc (30 mL) and NaHCO₃ solution (15 mL). The phases were separated, and the aqueous phase was further extracted with EtOAc (2 $\times$ 20 mL). The organic extracts were combined and washed with citric acid solution (10 % w/v, 3 $\times$ 20 mL), dried over MgSO₄ and filtered. The solvent was removed under reduced pressure and crude product was purified *via* flash column chromatography (20 % EtOAc in hexanes) and triturated with cold hexanes to produce pure product as a yellow solid (0.47 g, 78 % yield).

See A-C2-40 to A-C2-42

¹H NMR (400 MHz, Acetonitrile-*d*₃) δ 7.46 – 7.26 (m, 10H, C(2-6 and 18-22)-H), 6.93 (br t, *J* = 5.5 Hz, 1H, N(10)-H), 5.70 (br d, *J* = 7.0 Hz, 1H, N(12)-H), 5.10 (s, 2H, C(7 or 17)-H₂), 5.09 (s, 2H, C(7 or 17)-H₂), 4.35 (m, 1H, C(11)-H), 3.40 (q, *J* = 6.5 Hz, 2H, C(9)-H₂), 2.77 (dd, *J* = 16.3, 5.9 Hz, 1H, C(16)-H_a), 2.68 (dd, *J* = 16.3, 6.8 Hz, 1H, C(16)-H_b), 2.49 (t, *J* = 6.5 Hz, 2H, C(8)-H₂), 1.40 (s, 9H, C(13-15)-H₃).

¹³C NMR (101 MHz, Acetonitrile-*d*₃) δ 172.6, 171.8, 171.6, 137.4, 137.3, 129.53, 129.51, 129.11, 129.10, 129.04, 129.02, 67.2, 67.0, 61.0, 37.2, 35.9, 34.7, 28.5, 21.2, 14.5.

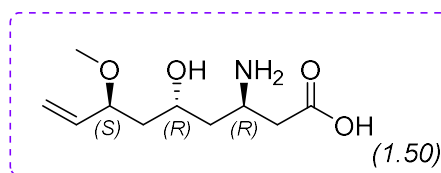
IR /cm⁻¹: 3290, 3212 (N-H stretch), 1721, 1690, 1651 (C=O stretch), 1521 (C=C stretch), 1164 (C-O stretch)

HRMS ESI⁺ *m/z* calculated for C₂₁H₂₉N₃O₈ (M + H⁺) 485.2243, Found: 485.2193

Chapter 3: A Brown allylation approach to 2-deoxygraciline

3.1. Aims and Objectives

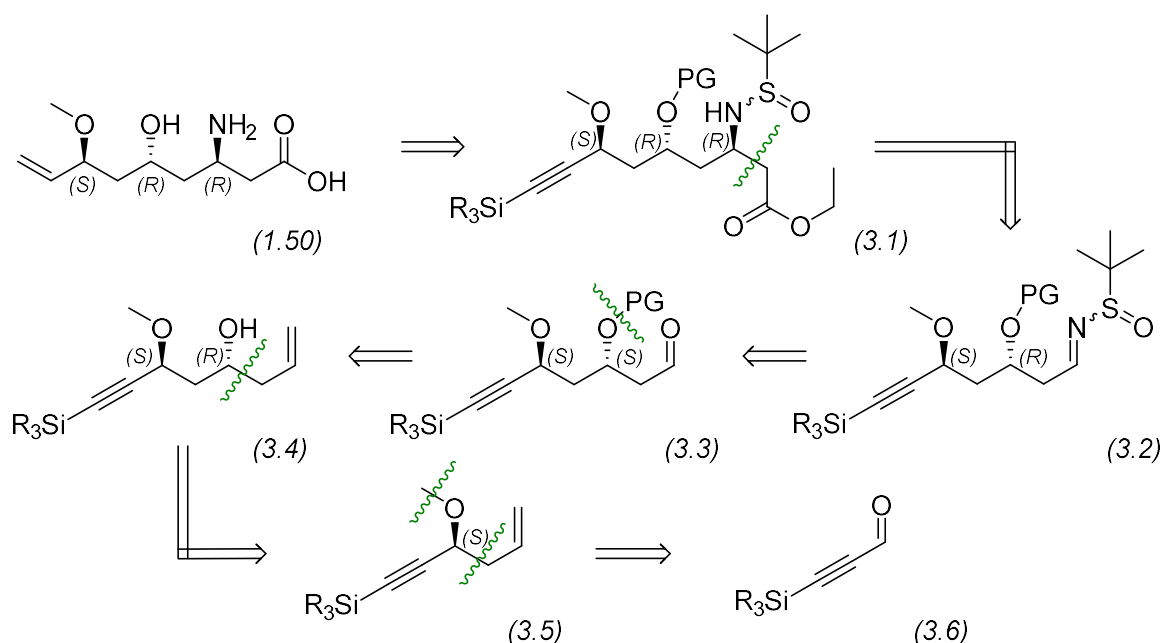
Throughout this chapter a method towards the synthesis of 2-deoxygraciline's advanced intermediate (*1.50*) is detailed (**Fig. 36**). The route aimed to use a semi-iterative Brown allylation and Ellman sulfinamide approach to install the stereogenic centres sequentially.



3(*R*)-Amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid

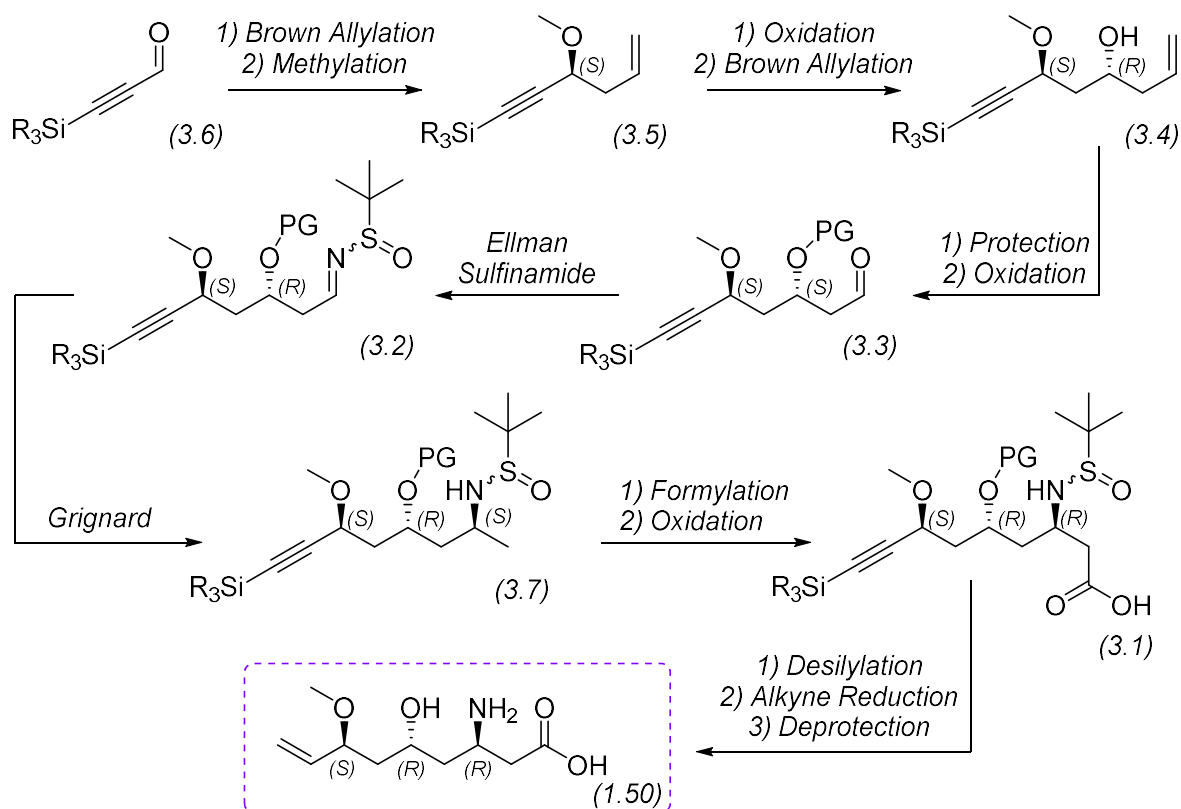
Fig. 36 Target advanced intermediate of 2-deoxygraciline.

The synthetic route was designed based on the disconnection sequence below (**Scheme 25**). Beginning with functional group interconversions to protect the reactive centres, the protected amino acid (*3.1*) accounts for the sulfinamide required for the amine installation. The CH₂-ester is disconnected to form the aldimine (*3.2*), which is accessed from the corresponding aldehyde (*3.3*). This can be synthesised from a terminal alkene (*3.4*), which then leads to an iterative disconnection of the 1,3-hydroxy alkenes (*3.4* and *3.5*), giving a silyl protected propynal (*3.6*) as the key starting material.



Scheme 25 Disconnection of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (*1.50*) from silyl protected propynal (*3.6*), with the key bond disconnections shown in green.

Starting from silyl protected propynal (3.6), a Brown allylation with (-)- α -methoxydiisopinocampheylborane would provide a chiral alcohol, which when methylated forms the first desired stereogenic centre as a 1,3-methoxy alkene (3.5). (Scheme 26).⁶⁸ Subjecting this product to oxidation to form a new aldehyde provides the ideal compound for a second Brown allylation to provide an intermediate (3.4) with the desired 1,3-*anti*-methoxy hydroxy relationship. Protection of the alcohol and the alkene oxidation forms the key starting material (3.3) for an Ellman sulfinamide reaction.⁶⁹ The Ellman sulfinamide approach could be used to synthesise the *N*-^tbutanesulfinyl aldimine (3.2), which has been shown to produce high selectivity and provide highly enantiopure protected amines when a 1,2-addition of methylmagnesium bromide is performed.^{70,71} Utilising this approach provides a product (3.7) with a terminal methyl group, which is converted into the desired acid (3.1) *via* a formylation and oxidation sequence. A desilylation and reduction exposes the terminal alkene, and deprotection of the functional groups provides the target 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50) compound.



Scheme 26 Forward synthesis towards 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid, which utilises a semi-iterative Brown allylation approach and Ellman sulfinamide to install the required stereogenic centres.

3.2 Introduction

α -Methoxydiisopinocampheylborane (**Fig. 37**, **3.10**) is a well-known chiral organoborane, which when reacted with appropriate Grignard reagents, can be used to perform a Brown asymmetric allylation/crotylation.^{72,73} The steric bulk of the pinene groups helps to force high stereocontrol, which makes it a useful tool in total synthesis of natural products including dysoxylactum A and (-)-dactylolide.^{74–76}

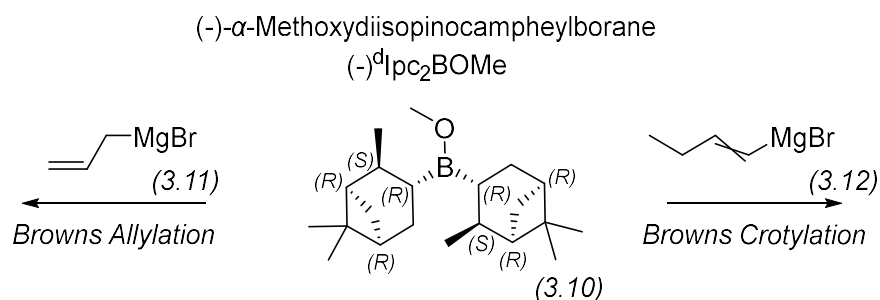
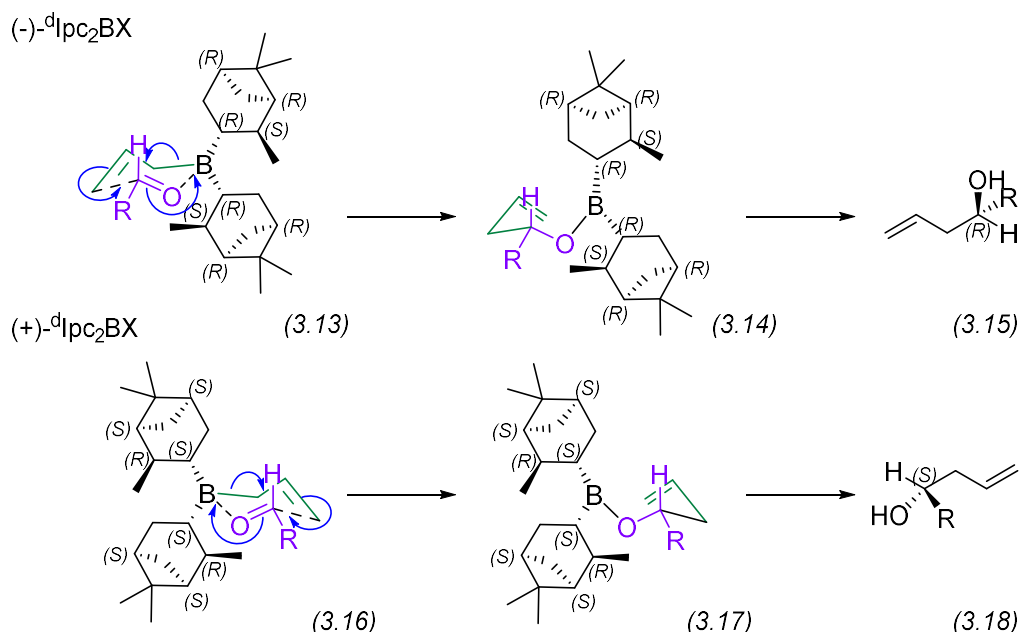


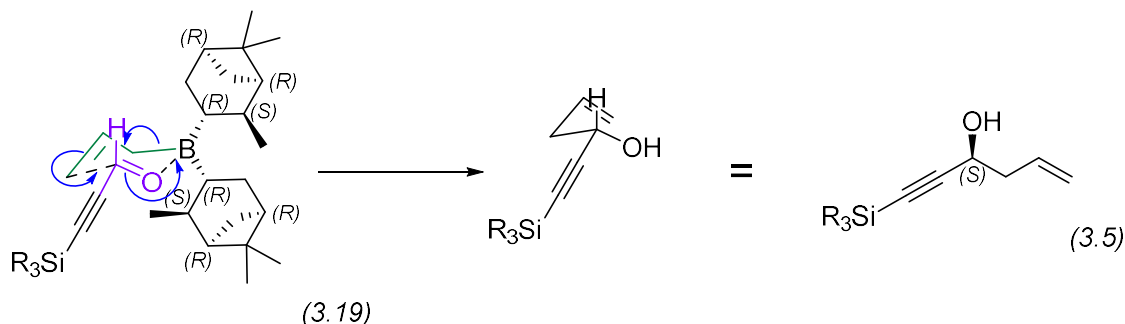
Fig. 37 Structure of (-)- α -methoxydiisopinocampheylborane (**3.10**) and activation with allylmagnesiumbromide (**3.11**) for a use in Brown allylations or crotylmagnesium bromide (**3.12**) for a Brown crotylation.

These reactions proceed through the formation of a six-membered Zimmerman-Traxler style transition state (**Scheme 27**, **3.13** and **3.16**). The carbonyl oxygen co-ordinates to the boron to form the stabilised transition state where the functional group (R) of the aldehyde sits favourably in the equatorial position. The enantiomer of the product is determined by the stereochemistry of the α -pinene. Borylation of (+)- α -pinene makes (-)-^dIpc₂BH and (-)- α -pinene forms (+)-^dIpc₂BH, which will favour co-ordination at opposite faces and therefore upon oxidative cleavage of the auxiliaries will produce opposite enantiomers of the desired alcohol (**3.15** and **3.18**).



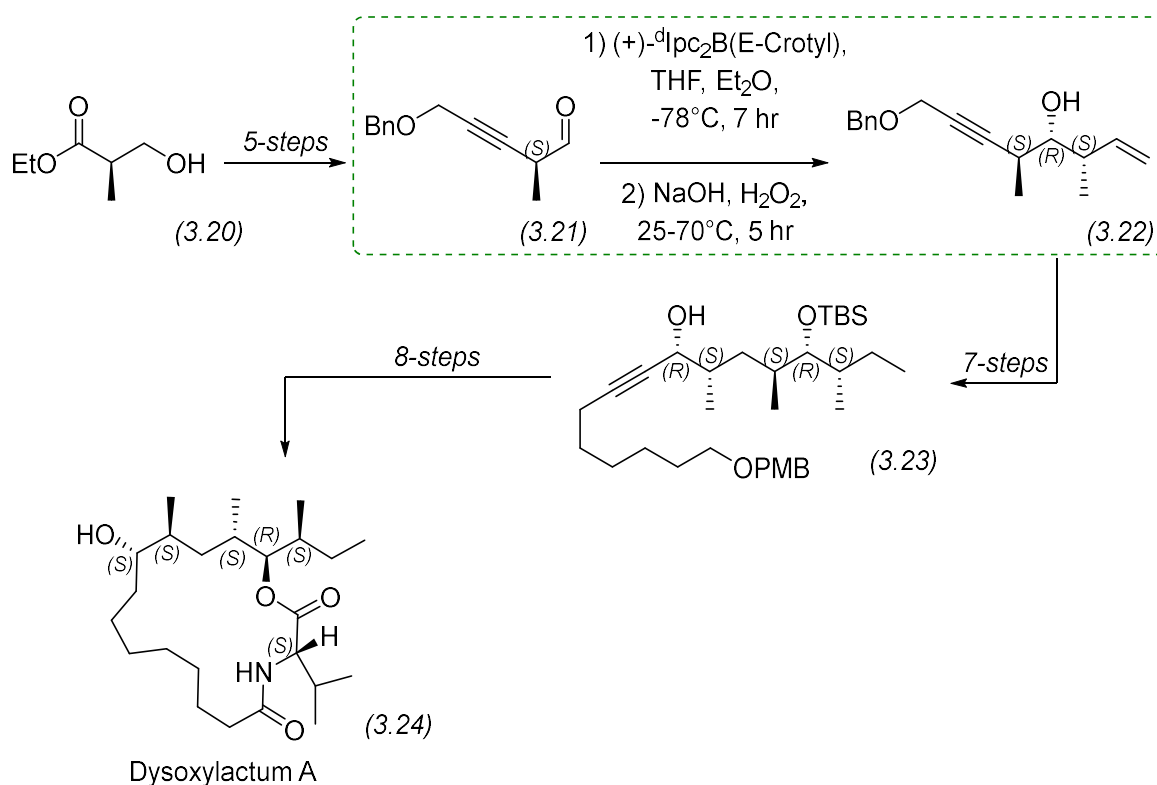
Scheme 27 Method by which the Brown Allylation proceeds, utilising a six-membered transition state to assemble the desired stereochemical configuration.

Literature reports state that the reaction of silyl protected propynal with (+)- $\text{dIpc}_2\text{BAllyl}$ can produce (trimethylsilyl)hex-5-en-yn-3(*R*)-ol with an enantiomeric excess (e.e.) of > 95 %.^{68,77} It is, therefore, likely that the desired (*S*)-(trimethylsilyl)hex-5-en-yn-ol (3.5) can be synthesised in high enantiopurity by using (-)- $\text{dIpc}_2\text{BAllyl}$ (**Scheme 28**).



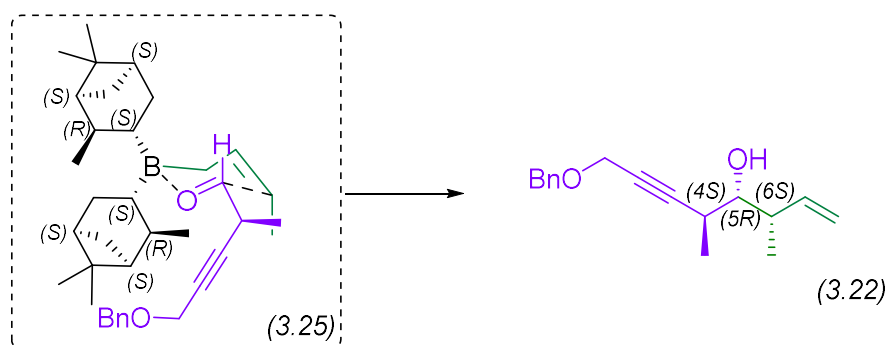
Scheme 28 Proposed use of (-)- $\text{dIpc}_2\text{Ballyl}$ as a chiral auxiliary in a Brown allylation to synthesise (*S*)-(trimethylsilyl)hex-5-en-yn-3-ol (3.5).

Gangathade recently published the total synthesis of dysoxylactum A (**Scheme 29**, 3.24), through a 21 (longest linear chain) step procedure.⁷⁴ A 5-step approach was used to synthesise the chiral aldehyde (3.21) from a commercially available starting material (3.20), which was subjected to a Brown crotylation reaction using (+)- α -diisopinocampheylborane as the chiral auxiliary. Thus produced the intermediary alcohol (3.22) which was subjected to a further 15 steps to form dysoxylactum A (2.24).



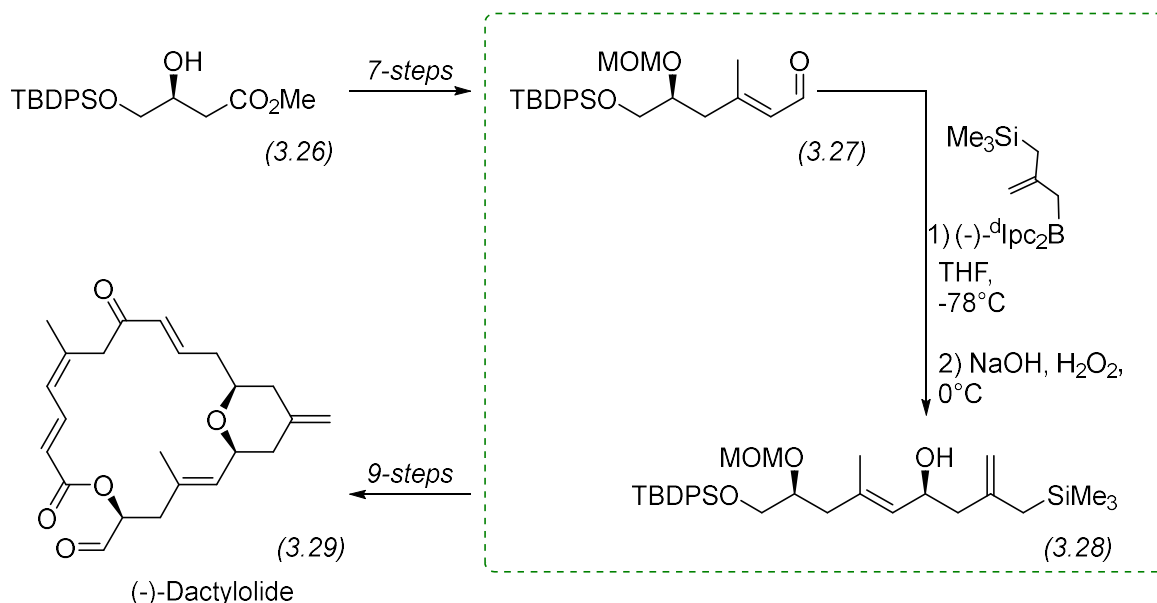
Scheme 29 Synthesis of dysoxylactum A (3.24) using a Brown crotylation reaction (boxed) to install early stereogenic centres with high selectivity.

It was reported that the *syn*-adduct (3.22) was formed, with no trace signs of the *anti*-stereoisomer visible in the NMR spectroscopy analysis.⁷⁴ The overall selectivity for this reaction was determined by two factors. The first of which is the alkene geometry. Use of the *cis* alkene provided the 5,6-*syn* selectivity (**Scheme 30**), whereas the *trans* alkene would produce the 5,6-*anti*-adduct. This means that to gain high selectivity, the alkene used was of high purity, as any trace of the *trans* alkene would have caused a reduction in the diastereomeric ratio. The second factor is the facial selectivity, determined by the auxiliary used. In this reaction, (+)-^dIpc₂B(E-Crotyl) is used to direct the aldehyde so that the existing chain (purple) sits in the equatorial position of the transition state (3.25), and minimise the steric interference from the α -pinene groups. High selectivity of this reaction provided Gangathade with an intermediate containing three of the six stereogenic centres in dysoxylactum A, without the need for complicated separation of diastereomers.⁷⁴



Scheme 30 Transition state (3.25) during the Brown crotylation reaction used to synthesise the intermediary alcohol (3.22) of dysoxylactum A, showing the orientation of the aldehyde to reduce the steric interference from the bulky α -pinene group.

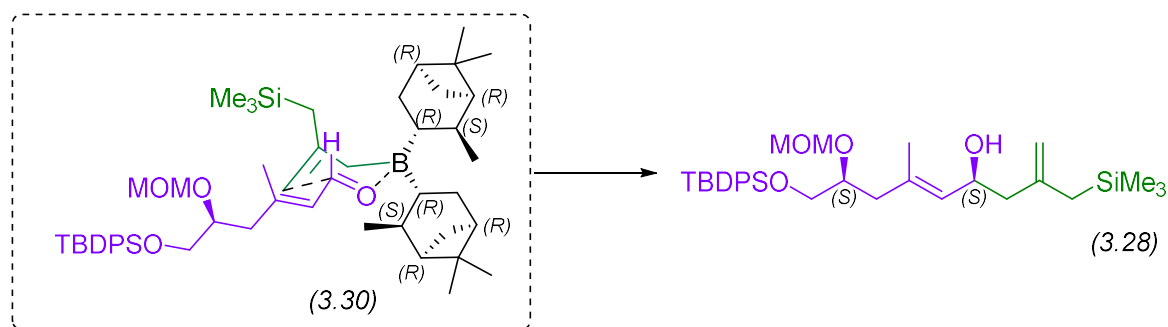
Kadota *et al.* also used a Brown approach within the total synthesis of (-)-dactylolide (**Scheme 31**, 3.29). A seven step procedure was performed to provide the starting aldehyde (3.27), which was subjected to the Brown procedure using a chiral allylic borane derived from (-)- α -methoxydiisopinocampheylborane to produce an advanced intermediate (3.28). A further nine steps yielded synthetic (-)-dactylolide (3.34).



Scheme 31 Synthesis of (-)-dactylolide (3.29) using a Brown allylation reaction (*boxed*) to form the chiral alcohol intermediate (3.28).

Unlike the previously discussed crotylations, this method did see formation of the undesired diastereomer at around 10 %. ⁷⁶ Kadota deemed this to be an acceptable level and choose not to optimise the approach as they were able to easily separate the two diastereomers, while still achieving a high isolated yield of 79 %. It was expected that by using the (-)-^dIpc₂BH

based auxiliary, the facial selectivity of the B-O co-ordination would provide the (*S,S*)-product (3.30) via the favourable transition state (**Scheme 32**, 3.28). This would see to the formation of the desired (*S,S*)-product. However, it seems that there was some substrate interference which led to a reduced facial selectivity. Kadota *et al.* reported that the (*S,S*)-diastereomer was the major product, which suggests that the steric repulsion from the pinene group is more consequential than any control from the substrate's stereogenic centre.



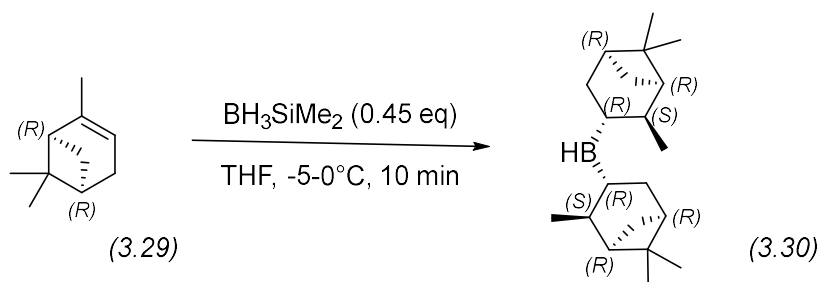
Scheme 32 Two transition states through which the allylation reaction occurs to provide the *S,S*-diastereomer (3.28) as the major product.

While the use of α -methoxydiisopinocampheylborane in natural product synthesis is well established, at the time of starting this project it was not possible to purchase the auxiliary. This is likely due to the known instability of the compound and its synthetic intermediates. Literature searches did not show many modern approaches to the synthesis of (-)- α -methoxydiisopinocampheylborane from (+)- α -pinene, with most referring to the original literature by Brown.^{73,78} Synthesis was carried out using the approaches detailed in this literature.

3.3. Results and Discussion

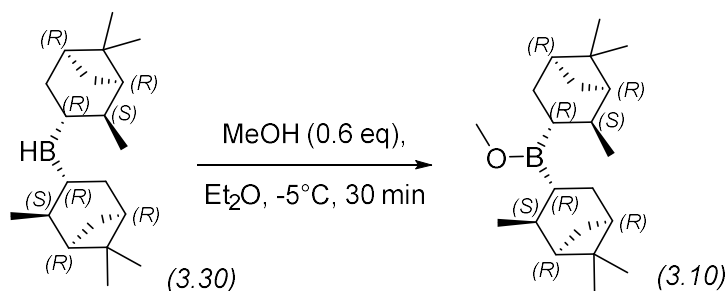
3.3.1. Synthesis of (-)- α -methoxydiisopinocampheylborane

Synthesis of the auxiliary began from (+)- α -pinene (3.29), which was reacted with BH_3SiMe_3 to form (-)- α -diisopinocampheylborane ($^d\text{Ipc}_2\text{BH}$, 3.30) (**Scheme 33**). The product was allowed to crystallise slowly, at 0 °C. Initial attempts to isolate the crystals *via* filtration failed due to the high sensitivity of the organoborane to moisture in the air. Further attempts were made to filter under Schlenk conditions, but it was found that removal of the solvent with a syringe and sequential trituration with cold hexanes led to the lowest loss of yield.



Scheme 33 Synthesis of (-)- α -diisopinocampheylborane (3.30) from α -pinene (3.29).

(-)- $^d\text{Ipc}_2\text{BH}$ (3.30) was dissolved in diethyl ether and reacted with methanol at -5 °C for 30 minutes (**Scheme 34**). Isolation of the product (3.10) was initially attempted by concentration under reduced pressure at 40 °C. However, this led to a low recovery of product. It was theorised that (-)- α -methoxydiisopinocampheylborane (3.10) may degrade into more volatile components when heated. A literature search revealed that some methods used this product as a solution in diethyl ether, further adding to the suspicions of instability.^{79,80} Therefore, to avoid any possible degradation and to produce the highest yield of the desired compound, it was decided that this would be carried through as a solution and conversion was assumed to be 100 %.

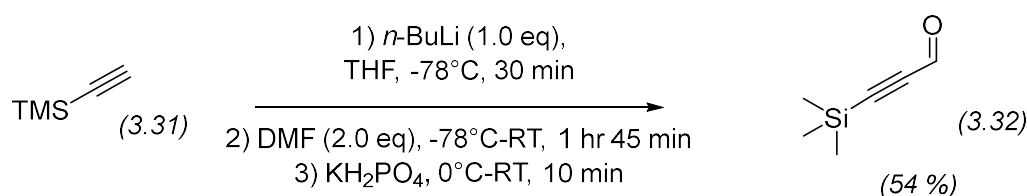


Scheme 34 Synthesis of (-)- α -methoxydiisopinocampheylborane (3.10) from $^d\text{Ipc}_2\text{BH}$ (3.30).

To minimise the stability issues, all reagents and solvents were thoroughly dried and degassed prior to use. The $^d\text{Ipc}_2\text{BH}$ intermediate was dried on a high vacuum line to ensure complete removal of any residual solvent, before being dissolved in fresh anhydrous diethyl ether under Schlenk conditions. Addition of methanol was then expected to form the desired (-)- α -methoxydiisopinocampheylborane. The product was not isolated from the reaction solvent and was sealed in a Schlenk tube under argon until required.

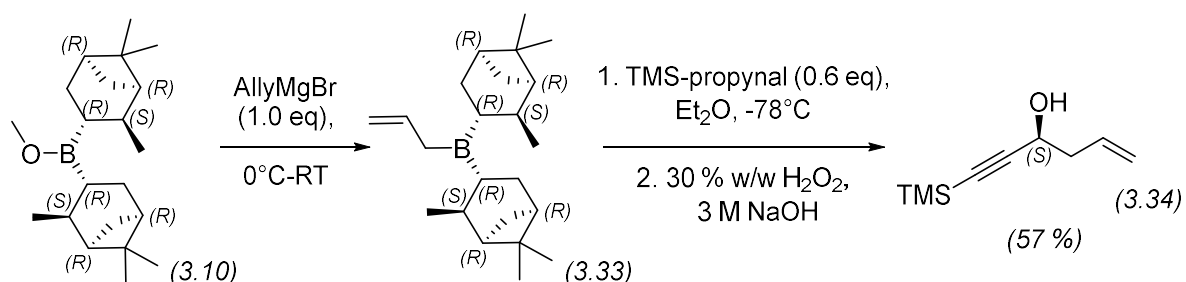
3.3.2. Brown allylation

A formylation reaction was used to synthesise 3-(trimethylsilyl)-2-propynal (3.32) from trimethylsilyl acetylene (3.31) (**Scheme 35**). The terminal alkyne was allowed to stir with $n\text{BuLi}$ for 30 minutes to ensure full deprotonation, then anhydrous DMF was charged, and the reaction was slowly warmed to room temperature. The reaction was monitored until disappearance of the alkyne on TLC, before being worked up and purified by fractional distillation. The product co-distilled by toluene, providing 11.9 g of a 30 wt% solution.



Scheme 35 Synthesis of 3-(trimethylsilyl)-2-propynal (3.32) through a formylation of trimethylsilyl acetylene (3.31).

A previously prepared solution of (-)- α -methoxydiisopinocampheylborane (3.10) in diethyl ether was reacted with allylMgBr at 0°C for 1 hr before 3-(trimethylsilyl)-2-propynal (3.32, 30 wt% solution in toluene) was added (**Scheme 36**). The related literature suggested that this reaction was rapid and therefore an approximate conversion was calculated after 15 minutes using ^1H NMR spectroscopy. This was performed by quenching a sample of the reaction with NaOH solution and H_2O_2 (30 % aqueous solution) and extracting the product into deuterated chloroform (**Fig. 38**). The aldehyde peak was minimal ($< 1\%$), and the reaction was worked up to give the desired (*S*)-(trimethylsilyl)hex-5-en-yn-3-ol (3.34).



Scheme 36 Synthesis of (*S*)-(trimethylsilyl)hex-5-en-yn-3-ol (3.34) from TMS-propynal (3.32), using (-)- α -methoxydiisopinocampheylborane (3.10) to direct a Brown allylation reaction through the six-membered transition states shown.

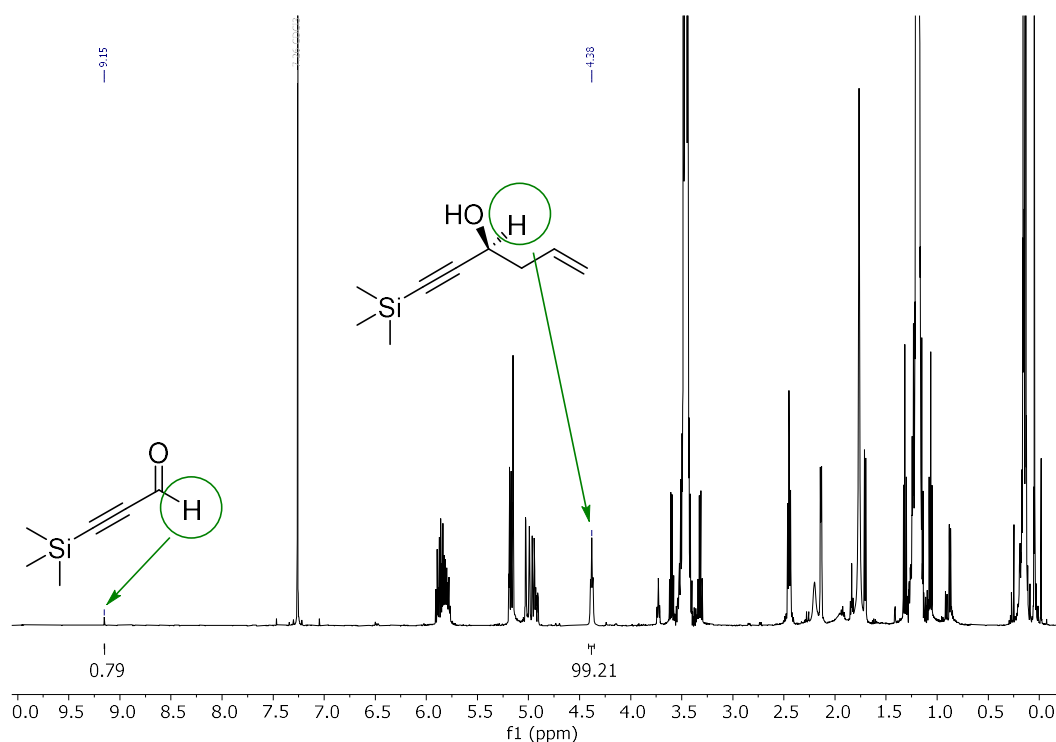
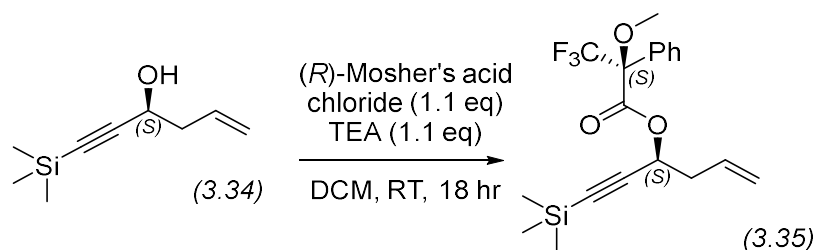


Fig. 38 ^1H NMR spectrum of the crude reaction mixture after 15 minutes, showing less than 1 % remaining of the aldehyde (3.32) starting material.

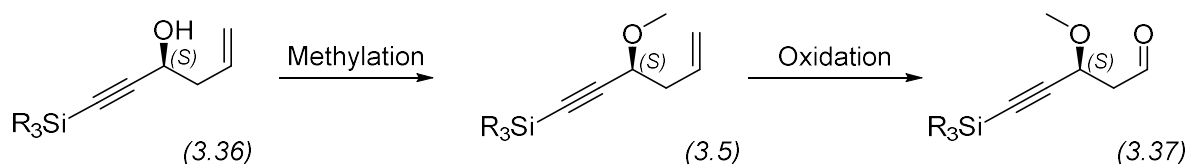
Literature suggests that using (+)- α -methoxydiisopinocampheylborane of high enantiomeric purity ($> 95\%$ e.e) to perform this reaction provides the (*R*)-product with an e.e of $> 95\%$.^{68,72,79–81} A sample of the crude product was retained for future assessment of the enantiomeric excess using Mosher's acid chloride once the opposite enantiomer had been synthesised (**Scheme 37**). However, due to failure of chemistry further down the line, synthesis of (*R*)-(trimethylsilyl)hex-5-en-yn-3-ol was not carried out.



Scheme 37 Conditions for esterification with Mosher's acid chloride, the planned method to assess the reaction selectivity.

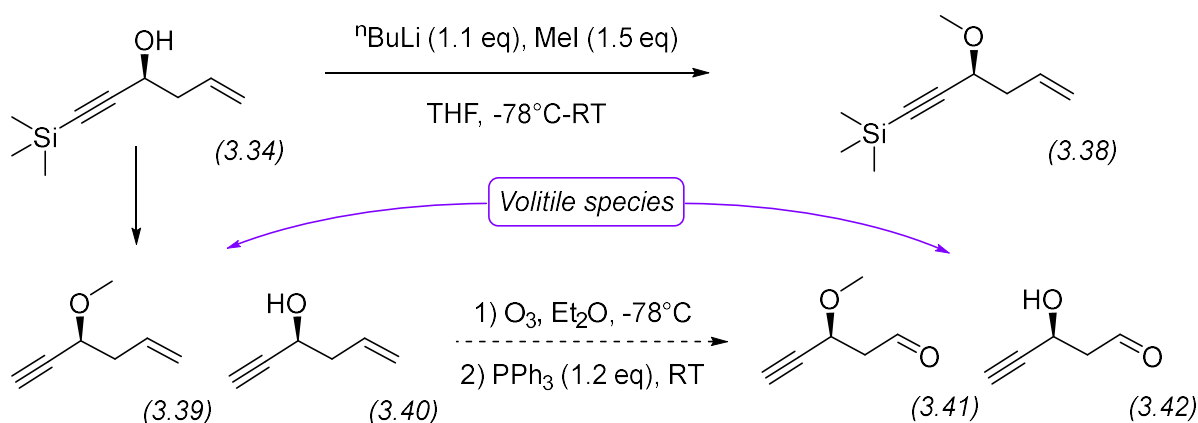
To mitigate any unwanted side reactions, (*S*)-(trimethylsilyl)hex-5-en-yn-3-ol (3.35) was protected prior to alkene oxidation and chain extension (**Scheme 38**). Since the final product is the methoxy derivative, it was decided that methyl group would be installed at this stage

form 3-methoxy-(1-trimethylsilyl)hex-5-en-1-yn (3.5), with the methyl group acting as a protecting group to limit any side reactions possible within the alkene oxidation.



Scheme 38 Next steps in synthesis of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid, requiring a methylation prior to oxidation.

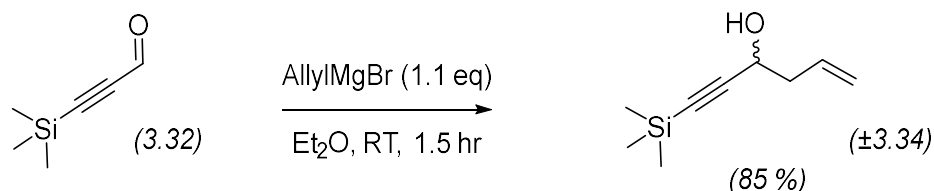
The alcohol (3.36) was initially methylated utilising $^n\text{BuLi}$ and MeI (**Scheme 39**).⁸² The ^1H NMR spectrum showed that the major product of the reaction was not the desired 3-methoxy-(1-trimethylsilyl)hex-5-en-1-yn, and instead showed signs of probable desilylation into 3-methoxy-hex-5-en-1-yn (3.39) alongside the deprotected and unmethylated (*S*)-hex-5-en-yn-3-ol (3.40). This product was carried through as a trial sample while other methylation conditions were researched. The oxidation was monitored by TLC until no starting material was visible, at which point the reaction ozonolysis was halted, reaction warmed to RT and triphenyl phosphine was added. Unfortunately, the volatility of these unprotected alkynes were problematic during the planned ozonolysis reaction. The positive pressure of the ozone, along with a lengthy work up procedure resulted in a low yield and therefore it was not possible to determine if the reaction had succeeded.



Scheme 39 Failed conditions for methylation of the secondary alcohol (3.34), which produced unprotected alkynes (3.39 and 3.40). Subsequent ozonolysis conditions used for an initial trial to transform the alkenes into aldehydes.

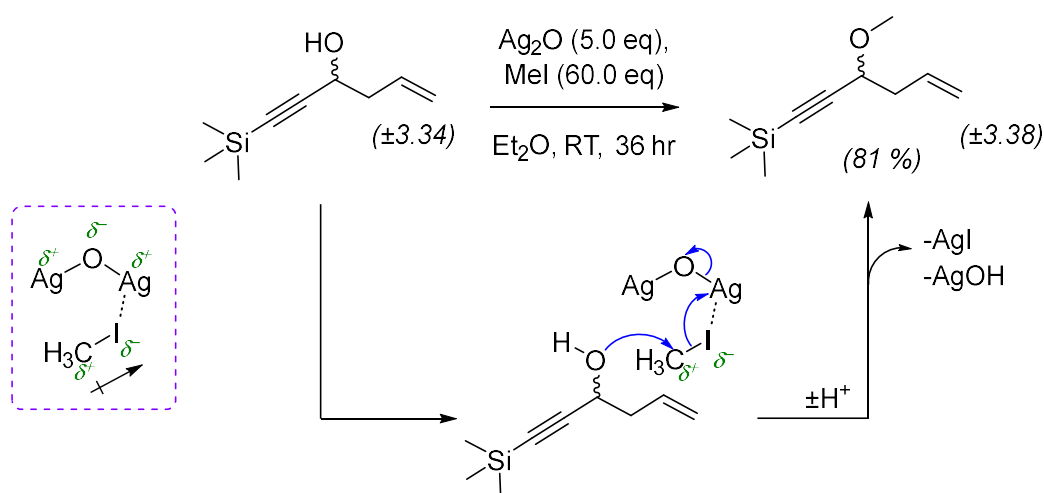
To reduce the cost of reaction optimisation, the testing of methylation conditions was carried out on the racemic alcohol ($\pm$ 3.34). Synthesis of the racemic compound was performed using

a standard Grignard reaction of allylmagnesium bromide with TMS-propynal (3.32) giving the product ($\pm$ 3.34) in an 85 % yield (**Scheme 40**). This product was used to trial an Irvine-Purdie style methylation with silver oxide and methyl iodide.⁸³



Scheme 40 Synthesis of the racemic (trimethylsilyl)hex-5-en-1-yn-3-ol ($\pm$ 3.34) via a Grignard addition.

This method calls for an excess of silver oxide and MeI and proceeds through the coordination of the Lewis acidic silver oxide with the iodide, which increases the dipole moment within the alkyl halide (**Scheme 41**). The methyl group becomes much more electrophilic and therefore a nucleophilic attack from the alcohol ($\pm$ 3.34) becomes possible without initiation with a base. The silver byproducts are removed *via* filtration giving the desired product with no deprotection of the alkyne. The reaction proceeded slowly giving an 81 % isolated yield of the desired ($\pm$)-3-methoxy-(1-trimethylsilyl)hex-5-en-1-yn ($\pm$ 3.38) product.



Scheme 41 Mechanism by which silver oxide mediates the alkylation of ($\pm$)-(trimethylsilyl)hex-5-en-1-yn-3-ol, through induction of a strong dipole moment in the alkyl iodide.

Analysis of the ^1H NMR spectrum showed the expected ratio of peaks for the desired product, revealing that this method proceeded with no desilylation (**Fig. 39, top**). This was

further confirmed by analysing the HMBC spectra, where the trimethyl silyl signal (-C(3-5)-H₃) at 0.17 ppm in the ¹H NMR spectrum shows a correlation to the -C(2) alkyne signal at 91.19 ppm in the ¹³C NMR spectrum (**Fig. 39, bottom**).

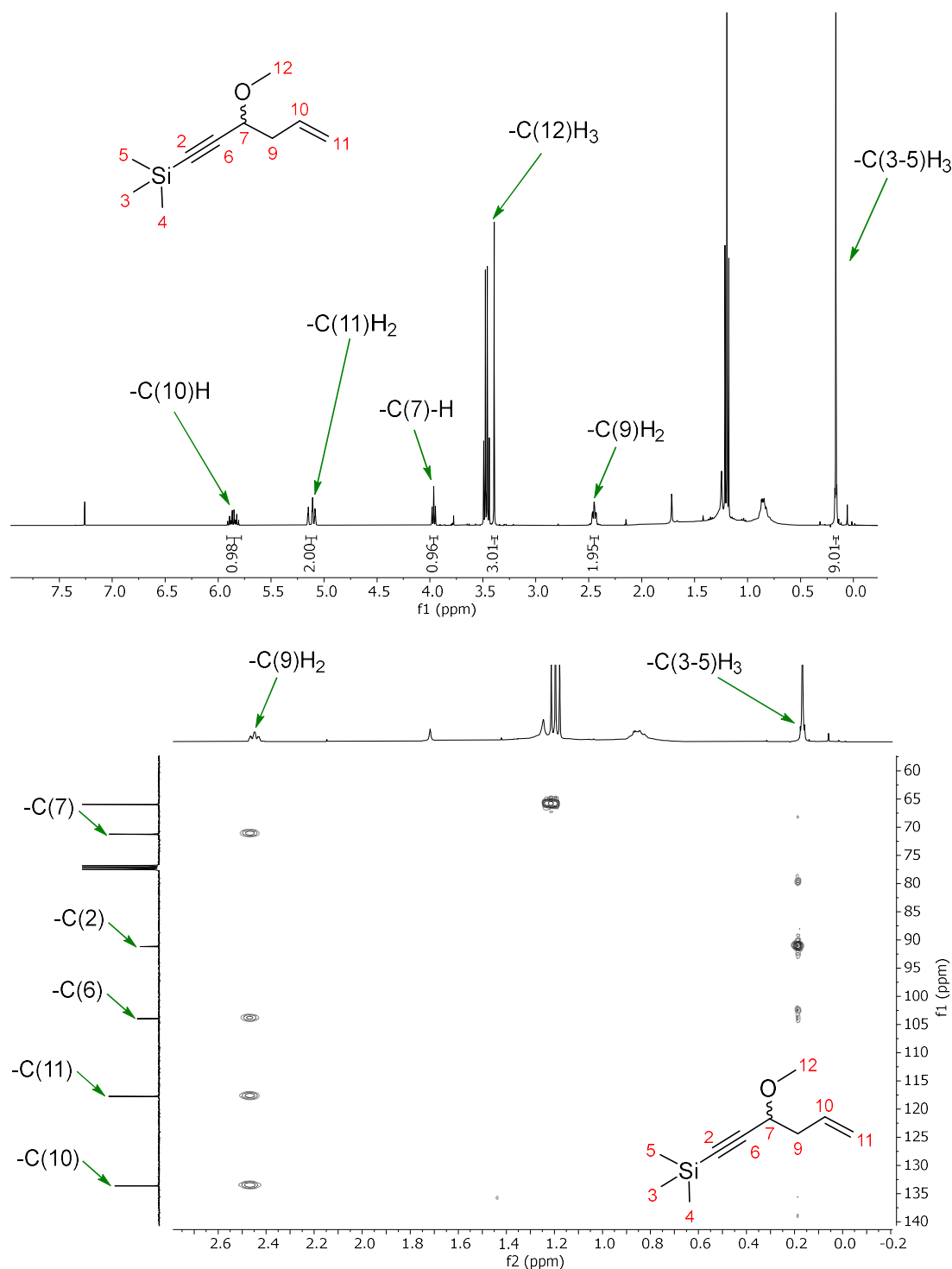
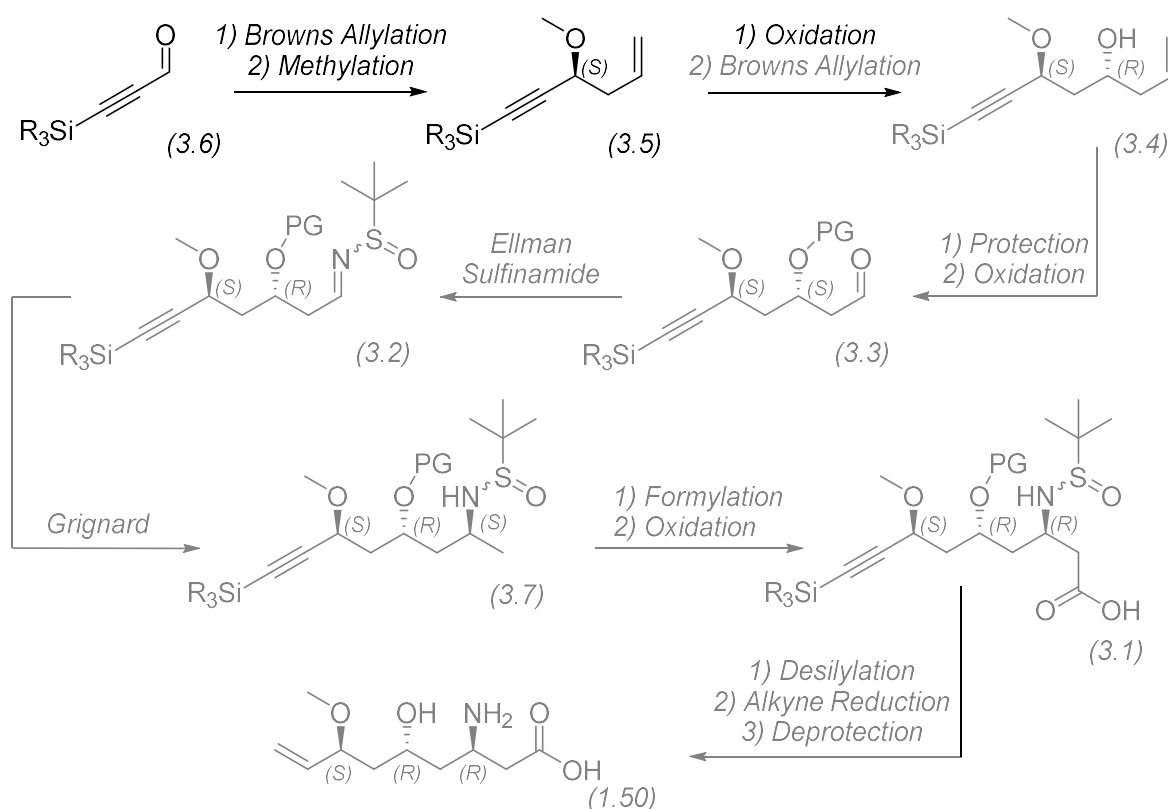


Fig. 39 Assigned ¹H NMR spectrum of (±)-3-methoxy-(1-trimethylsilyl)hex-5-en-1-yn (**A-C3-10**) and the HMBC spectrum showing the correlation between the protons (horizontal) of trimethylsilyl group with some of the key carbons (vertical).

3.4. Conclusions and Future Work

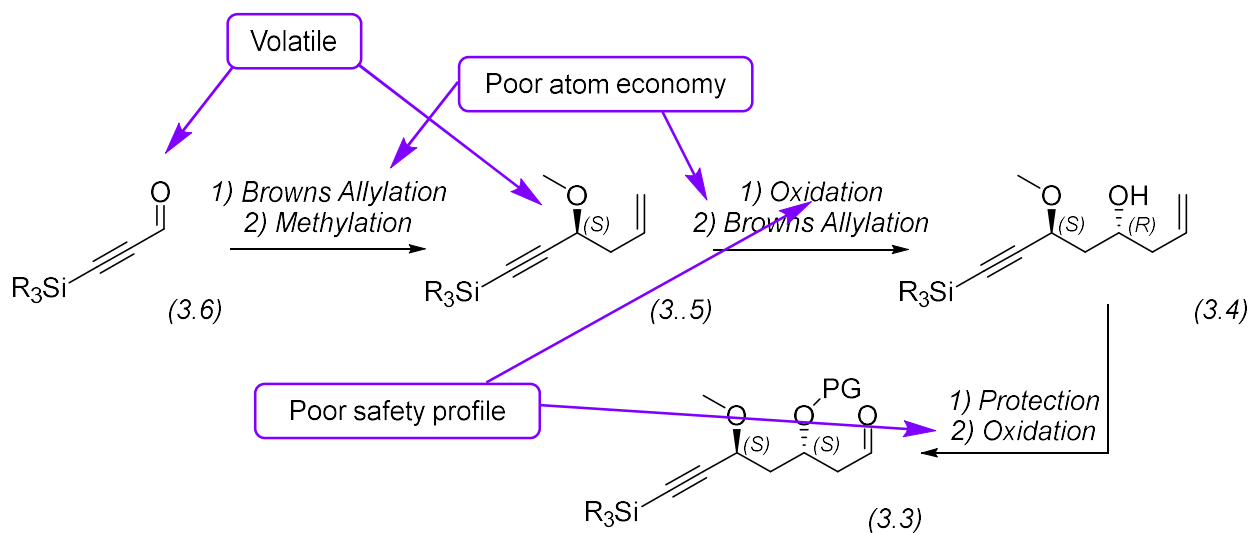
The objective of this work was to synthesise the advanced intermediate of 2-deoxygraciline (1.50) using chiral auxiliaries to induce high enantioselectivity (**Scheme 42**). The method aimed to follow a semi-iterative approach, with a Brown's allylation, protection and oxidation sequence being utilised twice. ^dIpc₂BAllyl was synthesised and utilised as a chiral auxiliary in the first Brown allylation to provide moderate yields. Conditions for the methylation steps were initially ineffective, leading to desilylation of the alkyne. A crude sample was taken forward to initial oxidation trials, but the volatility of the compounds proved to be a problem within ozonolysis. Irvine-Purdie style methylation conditions were trailed and proved to successfully methylate the alcohol with no unwanted side reactions.



Scheme 42 Planned method for synthesis of 3(R)-amino-5(R)-hydroxy-7(S)-methoxy non-8-enoic acid (1.50), showing the un-attempted steps in grey-scale.

Considering the volatility of the early intermediates, continuation of the route would require a change in silyl protecting groups to reduce the compound volatility and the initial Brown allylation would have to be scaled up a lot to produce large quantities of intermediate to mitigate yield losses (**Scheme 43**). This step required a stoichiometric amount of the ^dIpc₂BAllyl auxiliary to proceed and without a method to recover the reagent, the reaction

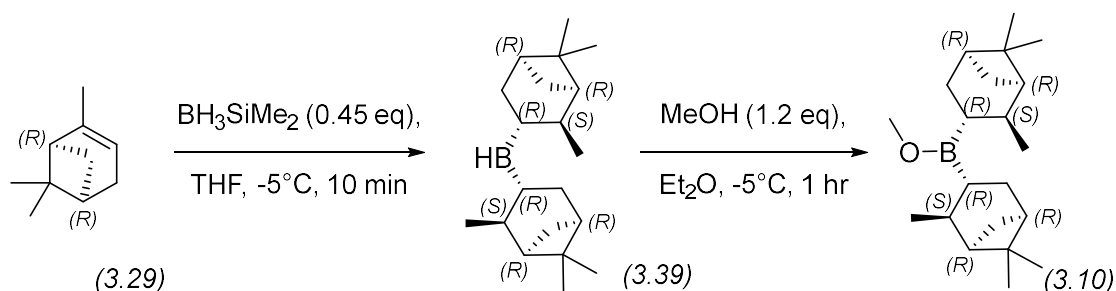
has extremely poor atom economy. It would therefore be a costly route for the scale up required to complete the total synthesis. Alongside this the safety hazards of scaling up an ozonolysis reaction with the equipment available proved to be undesirable. Overall, this sequence was not deemed efficient for the early stages of total synthesis, and no further work was carried out on this approach. A new method for synthesis was planned.



Scheme 43 The downfalls of the early synthetic steps of the planned Brown allylation approach.

3.5. Experimental Section

(-)- α -Methoxydiisopinocampheylborane (3.10)



(+)- α -Pinene (3.29) (2.15 g, 15.8 mmol, 2.4 eq) was charged to a pre-dried Schlenk flask under a slight positive pressure of argon. Anhydrous THF (1 mL) was added, and the flask was cooled to -5°C . BH_3SMe_3 (0.50 g, 0.62 mL, 6.58 mmol, 1.0 eq) was charged dropwise over 10 mins and stirring was stopped immediately. The vessel was sealed under argon and product allowed to crystallise slowly at 0°C before being placed back under an argon positive pressure and the excess solvent removed by syringe. The solid was triturated with cold hexanes ($3 \times 25\text{ mL}$) and the colourless crystals were dried on a high vacuum line for 1 hr. Anhydrous Et_2O (2 mL) was charged, and the reaction was stirred under a slight positive pressure of argon. The reaction flask was cooled to -5°C and methanol (0.23 g, 0.29 mL, 1.2 eq) was added dropwise causing rapid hydrogen release. Upon ceasing of gas evolution (approx. 1 hr), the reaction vessel was sealed and stored at -20°C until required.

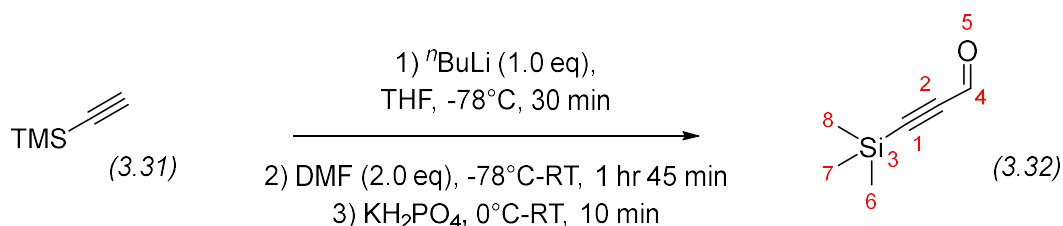
Crude product was not purified further and full analysis not carried out due to product degradation at RT.

*Procedure adapted from literature.*⁷⁸

See A-C3-1

^1H NMR (400 MHz, Methanol- d_4) δ 3.35 (s, 3H), 2.35-2.24 (m, 5H), 2.16-1.95 (m, 3H), 1.94-1.86 (m, 3H), 1.81-1.78 (m, 3H), 1.72-1.61 (m, 5H), 1.25-1.20 (m, 7H), 1.14-1.09 (m, 9H), 1.00-0.90 (m, 11H)

3-(Trimethylsilyl)-prop-2-yn-al (3.32)



TMS-acetylene (3.41) (5.00 g, 54.0 mmol, 1.0 eq) was dissolved in anhydrous THF (15 mL), placed under an argon atmosphere and cooled to -78 °C. *n*BuLi (23 mL, 2.3 M in toluene, 54 mmol, 1.0 eq) was added dropwise over 15 min. The reaction was stirred for 30 min. DMF (7.89 g, 8.36 mL, 108 mmol, 2.0 eq) was charged dropwise over 10 min. The cooling bath was removed, and the reaction was allowed to stir at RT for 1 hr 45 min. The reaction material was poured over a cold (0 °C) solution of KH₂PO₄ (29.4 g, 216 mmol, 4.0 eq) in water (150 mL) and Et₂O (300 mL). The mixture was stirred at 0 °C for 10 min, before the phases were separated. The aqueous phase was extracted with Et₂O (3 × 100 mL), the organic phases were combined and dried over Na₂SO₄. Crude product was purified by distillation under reduced product. Product co-distilled with toluene at 65 °C @ ~35 mbar to give a 30 wt% solution (11.99 g solution, approx. 3.5 g product, 54 % yield.)

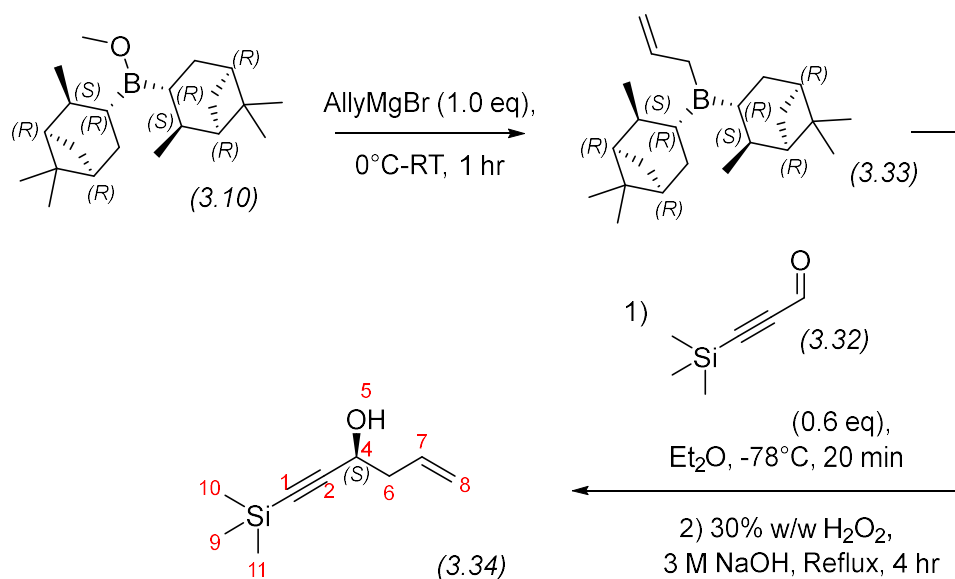
*Spectroscopic data in line with literature.*⁸⁴

See A-C3-2 to A-C3-3

¹H NMR (400 MHz, Chloroform-*d*₃) δ 9.15 (s, 1H, C(4)-H), 0.25 (s, 9H, C(6-8)-H₃).

¹³C NMR (101 MHz, DMSO-*d*₆) δ 177.2, 103.5, 66.3. 0.5

(S)-(Trimethylsilyl)hex-5-en-yn-3-ol (3.34)



3.10 (assumed 2.56 g, 6.58 mmol, 1.0 eq) in a Et₂O solution was cooled to 0 °C under an argon atmosphere. AllylMgBr (0.95 g, 16 wt% solution in Et₂O, 6.58 mmol, 1.0 eq) was added dropwise over 10 min. The cooling bath was removed, and the reaction material was allowed to stir at RT for 1 hr. The vessel was cooled to -78 °C and 3-(trimethylsilyl)-prop-2-yn-al (3.42) (0.49 g, 30 wt% in toluene, 3.94 mmol, 0.6 eq) was added *via* syringe over 10 min. The reaction material was stirred for 20 min before warming to RT and adding a solution of 3 M NaOH and 20 % H₂O₂ (1:2, 10 mL). The reaction was heated to reflux for 4 hr, before being cooled to RT and extracted with EtOAc (3 × 30 mL). Organic phases were combined and dried over Na₂SO₄. Crude product was purified by FCC (2 % EtOAc in hexanes) to give material as a colourless oil (0.37 g, 57 % yield).

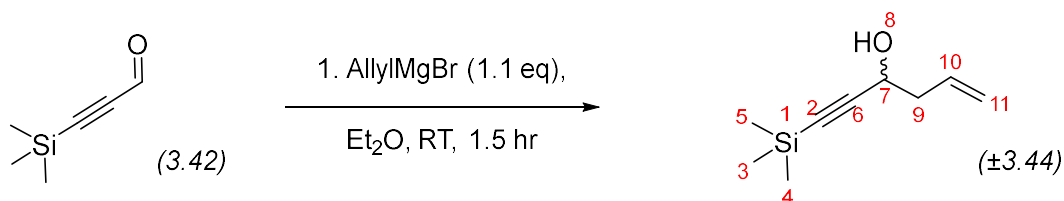
*Spectroscopic data is in line with literature.*⁸⁵

See A-C3-4 to A-C4-7

¹H NMR (400 MHz, Chloroform-*d*₃) δ 5.91-5.81 (m, 1H, C(7)-H), 5.19-5.16 (m, 1H, C(8)-H_a), 5.15-5.14 (m, 1H, C(8)-H_b), 4.38 (apparent q, *J* = 5.9 Hz, 1H, C(4)-H), 2.45 (m, 2H C(6)-H₂), 2.27 (Br-s, 1H, O(5)-H), 0.15 (s, 9H, C(9-11)-H₃)

¹³C NMR (101 MHz, Chloroform-*d*₃) δ 133.1, 119.1, 106.1, 89.9, 62.1, 42.2, -0.3

(±)-(trimethylsilyl)hex-5-en-1-yn-3-ol (±3.34)



3.32 (3 g, 23.8 mmol, 1.0 eq) was dissolved in anhydrous Et₂O (30 mL) and placed under argon. Allylmagnesium bromide (26.18 mL, 1.0 M solution in Et₂O, 26.2 mmol, 1.1 eq) was charged dropwise over 10 mins. The reaction material was allowed to stir for 1.5 hr. Upon confirmation of complete consumption of SM, the reaction material was poured onto saturated ammonium chloride solution (50 mL) and allowed to stir at RT for 10 min. The solution was extracted with Et₂O (50 mL × 2), washed with water (100 mL) and saturated brine solution (100 mL). The extract was dried over MgSO₄ and concentrated under reduced pressure. Crude material was purified by column chromatography (1:19 Et₂O in hexanes) to afford pure product as a pale yellow liquid (3.41 g, 85 % yield).

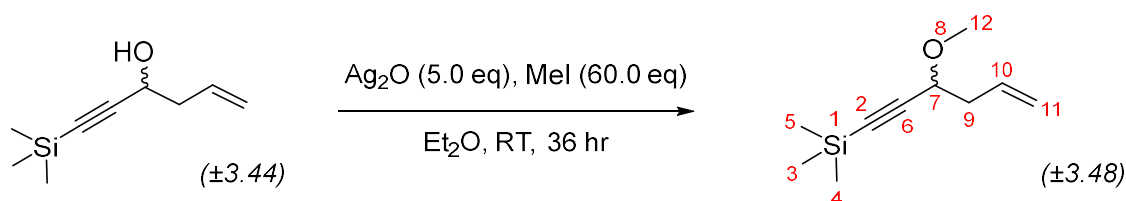
*Spectroscopic data is in line with literature.*⁸⁵

See A-C3-8 to A-C4-9

¹H NMR (400 MHz, Chloroform-*d*₃) δ 5.91-5.81 (m, 1H, C(10)-H), 5.19-5.16 (m, 1H, C(11)-H_a), 5.15-5.14 (t, *J* = 1.2 Hz, 1H, C(11)-H_b), 4.38 (q, *J* = 5.9 Hz, 1H, C(7)-H), 2.45 (m, 2H, C(9)-H₂), 2.27 (br-d, *J* = 7.0 Hz, 1H, O(8)-H), 0.15 (s, 9H, C(3-5)-H₃)

¹³C NMR (100 MHz, Chloroform-*d*₃) δ 133.2, 119.0, 106.2, 89.8, 62.1, 42.2, -0.1.

(±)-3-Methoxy-(1-trimethylsilyl)hex-5-en-1-yn (±3.38)



± **3.34** (1.0 g, 5.94 mmol, 1.0 eq) was dissolved in anhydrous Et_2O . Ag_2O (6.88 g, 29.71 mmol, 5.0 eq) was added and the reaction was placed under a positive pressure of argon. MeI (22 mL, 0.35 mol, 60.0 eq) was charged dropwise over 5 min. The positive argon pressure was removed, and the reaction was allowed to stir at RT for 36 hr. Upon confirmation of completion of reaction, the reaction material was filtered through Celite[®], and the pad was washed with Et_2O (50 mL $\times$ 3). The filtrate was concentrated under reduced pressure and purified *via* fractional column chromatography (0-20 % EtOAc in hexanes) to give product as a pale yellow oil (0.87 g, 81 % yield).

See A-C3-10 to A-C4-13

^1H NMR (400 MHz, Chloroform- d_3) δ 5.89-5.81 (m, 1H, C(10)-H), 5.16-5.08 (m, 2H, C(11)-H₂), 3.95 (t, J = 6.35 Hz, 1H, C(7)-H), 3.39 (s, 3H, C(12)-H₃), 2.47-2.43 (m, 2H, C(9)-H₂), 0.17 (s, 9H, C(3-5)-H₃)

^{13}C NMR (101 MHz, Chloroform- d_3) δ 133.6, 117.7, 103.9, 91.2, 71.2, 67.0, 56.5, 40.1, 0.0

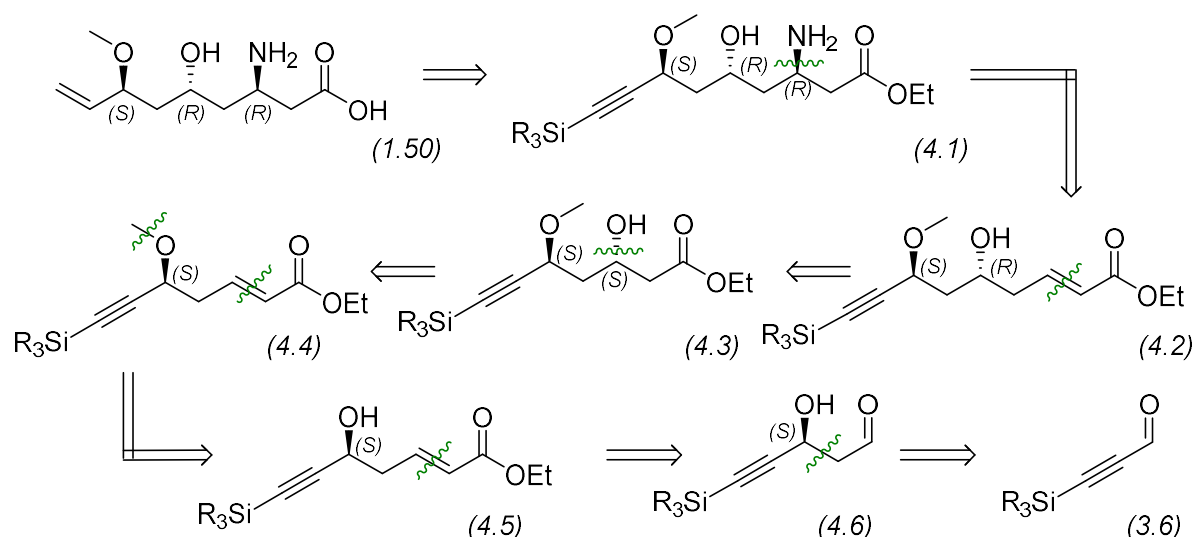
IR: 2958, 2927, 2856 (C-H), 2254 ($\text{C}\equiv\text{C}$), 1712 ($\text{C}=\text{C}$) 1362 (C-H), 1222 (Si-C), 912 (C-H, Alkene)

HRMS ESI⁺ m/z calculated for $\text{C}_{10}\text{H}_{13}\text{NO}_2$ ($\text{M} + \text{H}^+$) 183.1160, Found: 183.1395

Chapter 4: Synthetic route towards 2-deoxygraciline

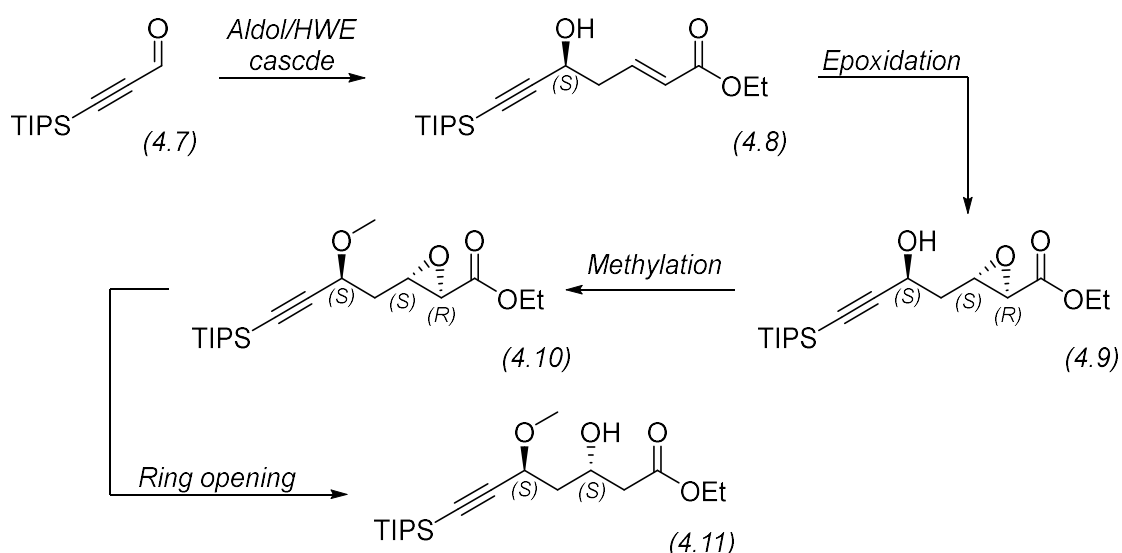
4.1. Aims and Objectives

Since the original methodology detailed in chapter 3 showed flaws in early steps, the new approach centres around completely different chemistry but also aimed to utilise a semi-iterative approach to chain extension. The disconnection sequence from the target 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (**1.50**) begins with functional group interconversions to protect the highly reactive alkene and acid (**Scheme 44**). The amine bond is broken to provide an α,β -unsaturated ester (**4.2**), which can be made in a series of steps from an ester (**4.3**). This alcohol could be synthesised from an alkene (**4.4**), which when demethylated (**4.5**) is one of the key substrates in the asymmetric aldol/HWE cascade route by Hayashi *et.al.*⁸⁶ Therefore, this disconnection can be followed through to reach a silyl protected propynal (**3.6**).



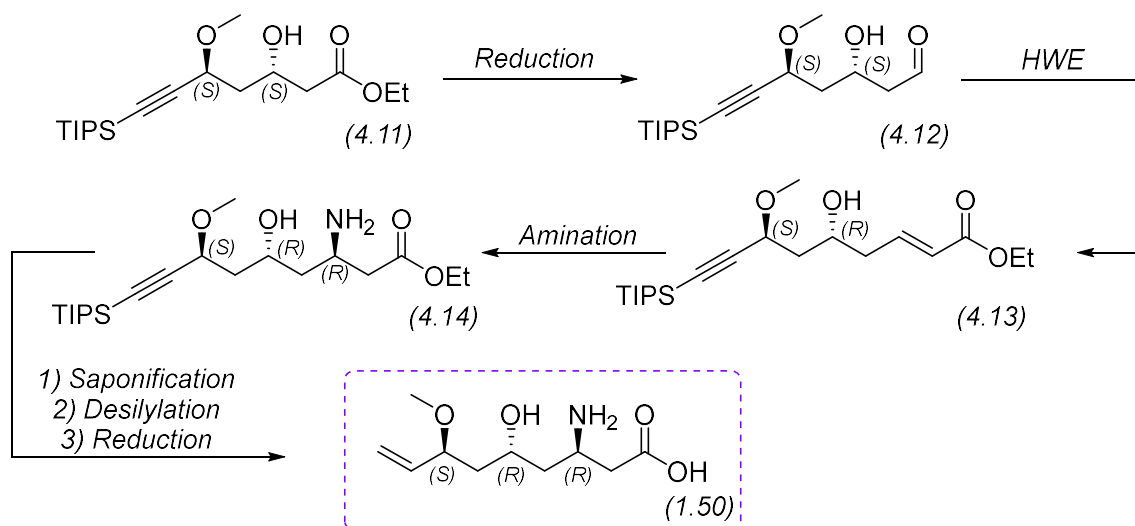
Scheme 44 New disconnection approach of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (**1.50**) from silyl protected propynal (**3.6**), with the key bond disconnections shown in green.

Beginning from the silyl protected propynal (**4.7**), the objective was to synthesise ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**), using an asymmetric aldol/HWE cascade to install the first stereogenic centre under high catalytic control (**Scheme 45**).⁸⁶ The product contains an internal alkene which is a useful reactive centre for a stereoselective epoxidation. Methylation of the alcohol (**4.9**) and subsequent epoxide ring opening provides the *anti*-1,3-methoxy hydroxy intermediate (**4.11**). This planned route heavily follows the published method by Hayashi *et. al.*, with a key difference being the catalyst enantiomer and an additional methylation step.



Scheme 45 First approach to the desired 1,3-methoxy alcohol (4.11), following a very similar approach by Hayashi *et. al.*⁸⁶

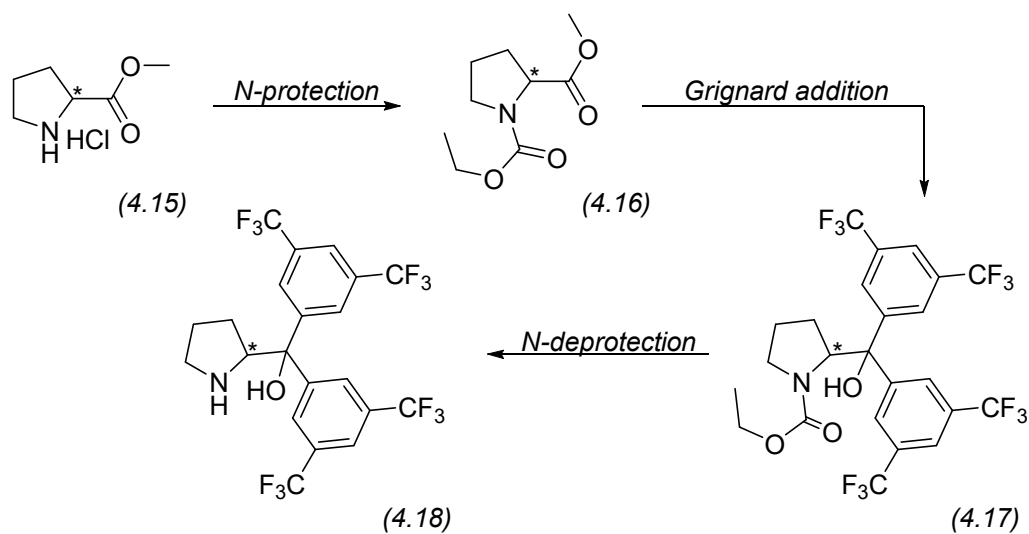
Extension of the 1,3-methoxy alcohol product (4.11) through a reduction and HWE protocol, provided a new α,β -unsaturated ester (4.13). Amination of the alkene through a hydroboration approach provided the protected product (4.14) which is deprotected and reduced to provide 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50).



Scheme 46 Synthetic steps towards the synthesis of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50) from ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (4.11).

Unfortunately, due to the recreational use of diphenyl prolinol as a designer drug, all the major derivatives are not easily purchasable in the United Kingdom. For this reason, the Hayashi-Jørgensen catalyst (4.18) needed to be synthesised in house. This can be synthesised

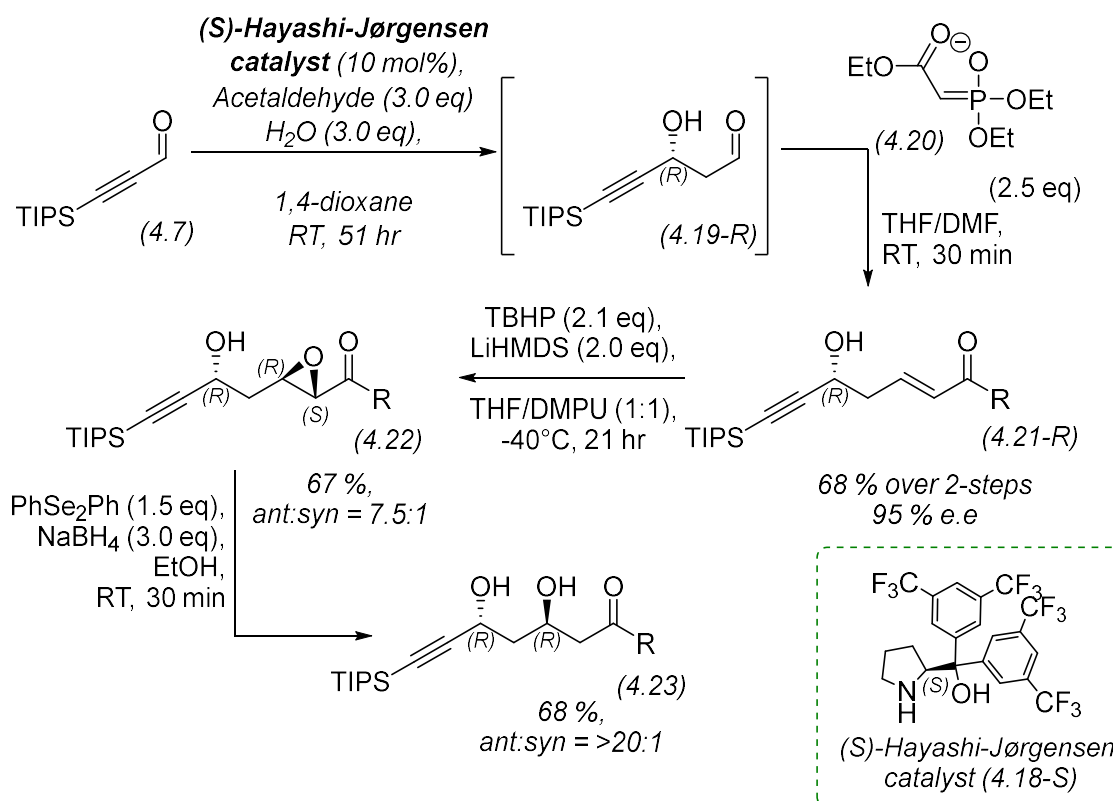
from methyl pyrrolidine-2-carboxylate (**4.15**) by *N*-protection, double Grignard addition and *N*-deprotection (**Scheme 47**).



Scheme 47 Three step synthetic plan towards synthesis of Hayashi-Jørgensen catalyst (**4.18**).

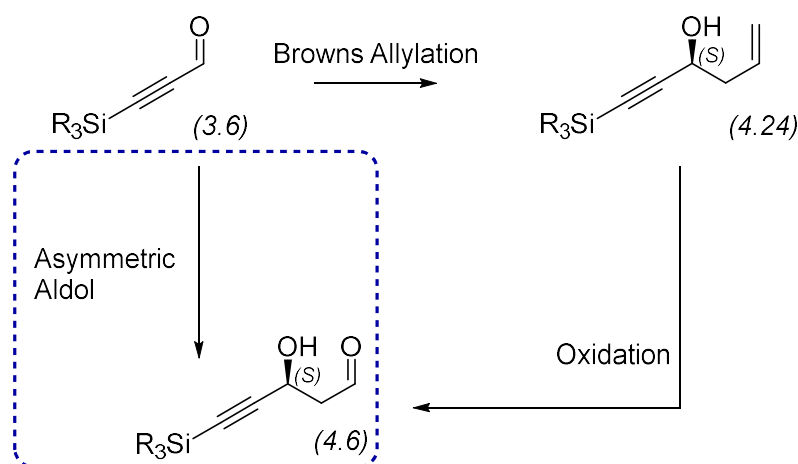
4.2. Introduction

Hayashi *et. al* published a synthetic route towards 1,3-diols, with one of their substrates being an enantiomer of a desired intermediate (4.23) of 2-deoxyGra (**Scheme 48**).⁸⁶ Their method showed similar levels of chiral selectivity when compared to the previous Brown allylation method, with easier to handle reagents and catalytic loading of the chiral auxiliary. This formed the basis for a new route which centred around an asymmetric aldol reaction, directed by a proline-based catalyst.



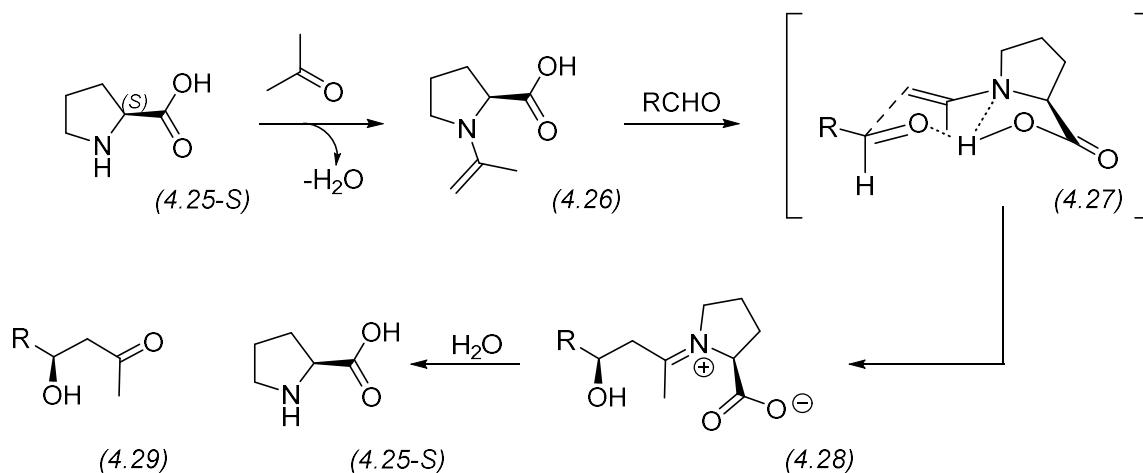
Scheme 48 Synthetic route towards 1,3-diols, published by Hayashi *et. al* through an asymmetric aldol/HWE cascade reaction, followed by a substrate-controlled epoxidation and subsequent ring opening.

This method utilises a similar silyl protected propynal as in the original Brown allylation method to produce 3-hydroxy-(triisopropylsilyl)pentyn-5-al (4.6), a comparable intermediate to the highly volatile 3-hydroxy-(trimethylsilyl)pentyn-5-al synthesised in the previous approach (**Scheme 49**). However, using the Hayashi-Jørgensen catalyst achieves synthesis of this compound in one step and allows utilisation of the intermediary aldehyde in a Horner-Wadsworth-Emmons (HWE) reaction without isolation; whereas the Brown allylation method would have required two steps to reach the same unstable intermediate, which must be isolated and purified for the next stage of synthesis.



Scheme 49 Comparison of the two methods, where the highlighted asymmetric aldol approach utilises a single step to reach to synthesise the same intermediary 1,3-hydroxyl aldehyde (4.6) compound achieved in two steps by the Brown allylation approach.

This method harnesses a well-known property of proline (4.25) derivatives, which allows them to direct asymmetric aldol reactions through an intermediary enamine (4.26) (**Scheme 50**). The enamine is then suspected to assemble into a tricyclic transition state (4.27), where the R group favours being in the pseudo-equatorial position. This creates a highly selective reaction, predominantly forming a single isomer as the product.



Scheme 50 Route by which (S)-proline (4.25) can be used to direct an asymmetric aldol reaction through an enamine and a tricyclic transition state.

Variations on proline have been made and assessed, with the aim to increase the enantiomeric excess of reactions. This has predominantly focused on increasing the steric bulk on the proline derivatives, thereby increasing the favourability of the pseudo-equatorial transition state (**Fig. 40, 4.30**) over the pseudo-axial transition state (**Fig. 40, 4.31**).

Replacing the carboxylic acid group with a tertiary alcohol and introducing aromatic substituents such as in diphenyl prolinol, has been shown to improve this favourability in certain reactions. Diphenyl prolinol (**Fig. 41**, 4.32) is a key intermediate of the Corey-Bakshi-Shibata (CBS) catalyst (**Fig. 41**, 4.33), which is commonly used in organic synthesis to perform reductions and cycloadditions selectively.^{87,88} However, the prolinol derivative itself has also show use as an aid for enantioselective reactions.

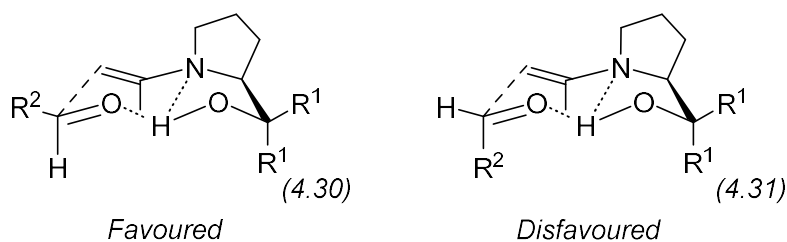


Fig. 40 Tricyclic transition state when prolinol derivative directed asymmetric aldol reaction, showing the more sterically favoured pseudo-equatorial R group on the left and less favoured pseudo-axial R group on the right.

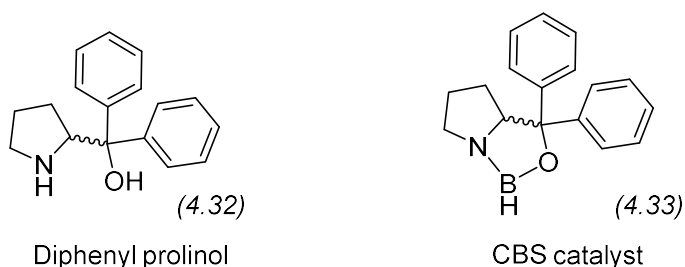
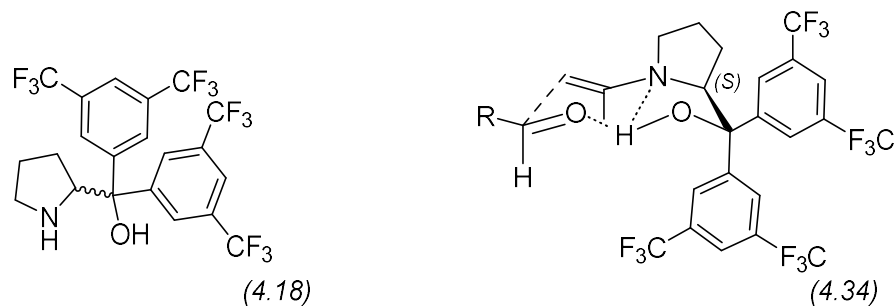


Fig. 41 Structure of CBS catalyst (4.33) and its parent functionalised prolinol, diphenyl prolinol (4.32).

Yujiro Hayashi and his group have shown how valuable this compound is in many different asymmetric reactions including cycloadditions, Michael reactions and aldol reactions.^{89–93} Over the years, the group have tested various aromatic substituents on each of these reactions and shown the value in the diphenyl prolinol derivatives by using them to synthesise various natural products and useful APIs.^{94–96}

The trifluoromethyl derivative, known as Hayashi-Jørgensen catalyst, showed particularly high levels of selectivity when used in sub-stoichiometric amounts to perform enantioselective aldol reactions (**Fig. 42**).⁸⁹ Developing on this, the group utilised this catalyst to synthesise *syn*-1,3-diol compounds and *anti*-1,3-diols, both of which are reported to be highly enantioselective and diastereoselective.^{86,97} Both routes centred around the use of a one pot aldol and HWE or Wittig reacting, with the *syn*-1,3-diols following up with a

domino hemiacetal/oxy-Michael addition. While there are multiple reported methods of producing *syn*-1,3-diols, this approach was reported to be an efficient two-pot approach from readily available, non-chiral compounds. For the synthesis of *anti*-1,3-diols, the group utilised diastereoselective epoxidation and subsequent ring opening, to produce another efficient approach.



Hayashi-Jørgensen catalyst

Fig. 42 The structure of Hayashi-Jørgensen catalyst (4.18), alongside the favoured six-membered transition state (4.34) that (*S*)-Hayashi-Jørgensen would show during an asymmetric aldol reaction.

In the publication of the three-pot synthesis of *anti*-1,3-diols, Hayashi stated that their reasoning for probing this reactivity was to create an efficient, highly enantioselective procedure, which could be performed using abundant and inexpensive reagents.⁸⁶ Upon first reading the paper, it seems as though the group managed to succeed with their goals, showing the results of high to exceptional enantiomeric and diastereomeric excess with moderate to high yields. The reaction was successfully performed on some functionalisable compounds in a two-pot procedure, with most of the reagents being readily available and cheap. The major downfall of the reactions seems to be the price of the catalyst itself; although, it is commonly accepted that expensive catalysts are often needed to yield high enantioselectivity when synthesising chiral compounds.

The reported scope includes compounds that have functionalisable alkynes, halogens and nitro groups.⁸⁶ The inclusion of these groups directs the useful reactivity towards medicinal chemistry, where these are common functionalities used for coupling small molecules to build more complex structures. However, the small scope contains low substrate variation, which does not appropriately show the versatility of the reaction.

Initial tests of these reactions were carried out on *o*-chlorobenzaldehyde (4.35) (Table 6) which gave a moderate yield of 55 % over the two-step process and a high enantiomeric

excess of 98 %. Epoxidation of the alkene then provided a high *anti:syn* ratio of 20:1. Conditions were then tested on unsubstituted benzaldehyde (4.38) and *o*-nitrobenzaldehyde (4.39), which both showed reduced yields during formation of the α,β -unsaturated ester, but similar selectivity at both stages. The group then tested the reactions on *meta* (4.40) and *para* nitrobenzaldehyde (4.41), which also gave very similar results for selectivity.⁸⁶

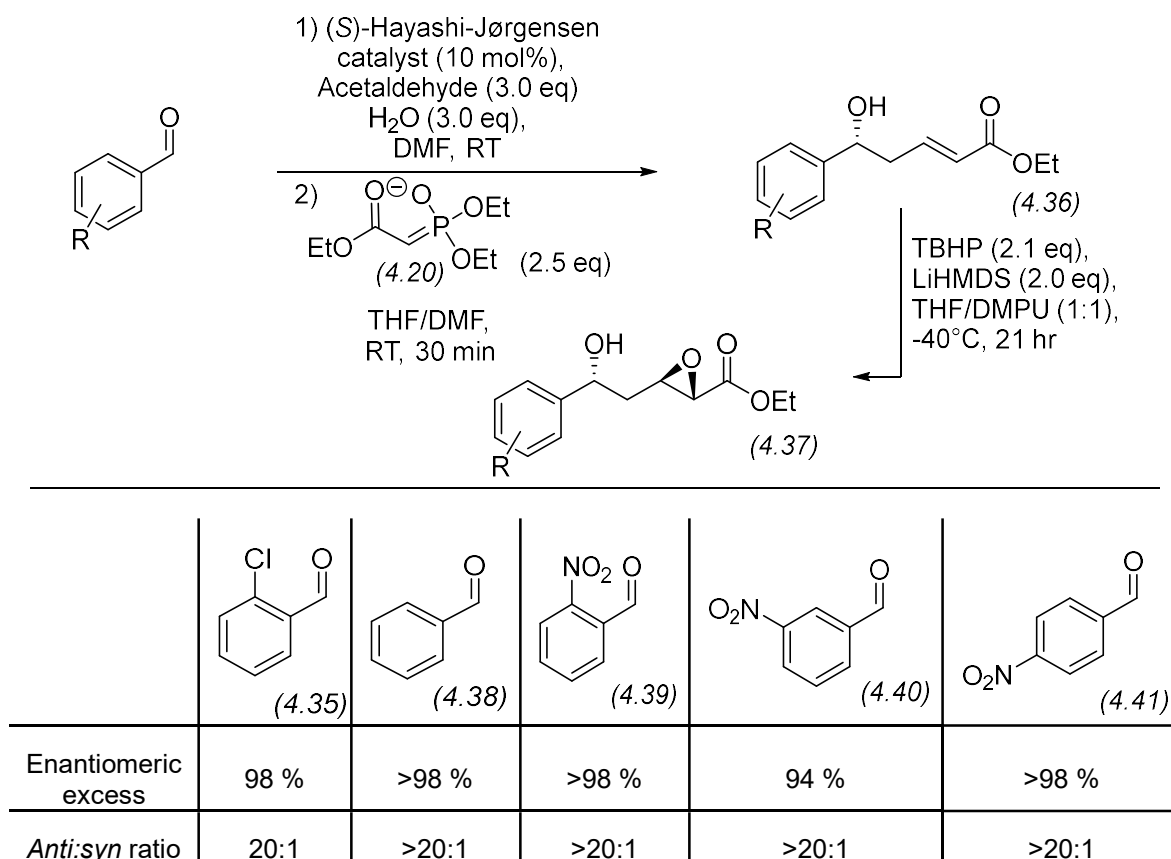
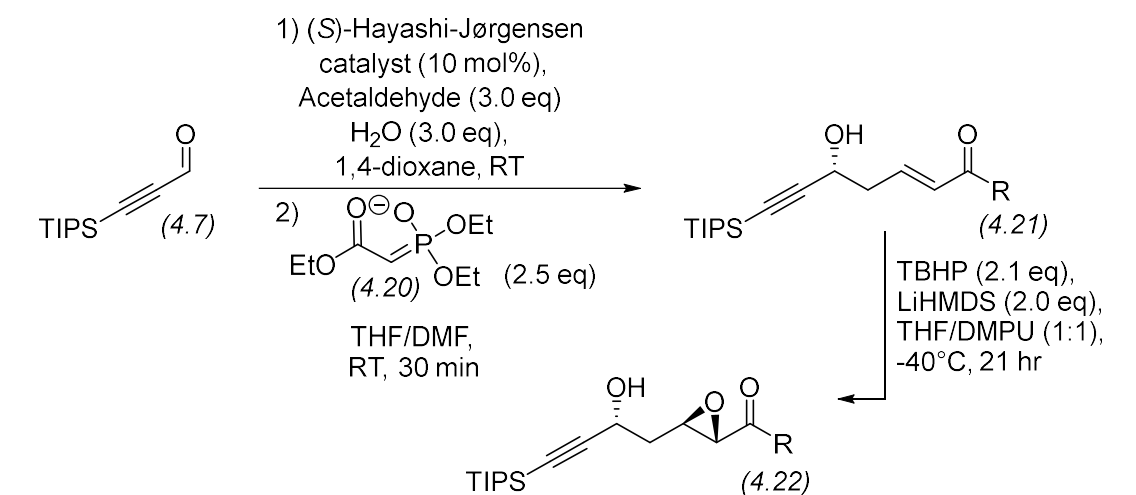


Table 6 Aromatic substrate scope of Hayashi's three-pot diol synthesis, showing the high enantioselectivity of the Aldol/HWE pot and the excellent selectivity of the *anti*-epoxide formation.

The lack of variability in the results between the different positions of the nitro groups might suggest that the substituent electronics do not majorly play a role in the reaction selectivity. As such, it would be easy to assume that the selectivity is mostly controlled by steric factors. To test this theory, they moved on to use alkyne functionalised aldehydes (**Table 7**), in place of the benzaldehyde derivatives. They did see slightly reduced enantioselectivity within the aldol reactions and a large reduction in the selectivity of the epoxidation.⁸⁶



R=	-OEt	-Me
Enantiomeric excess	95 %	94 %
<i>Anti:syn</i> ratio	7.5:1	3.1:1

Table 7 Alkyne containing compounds within the substrate scope of Hayashi's three-pot *anti*-1,3-diol synthesis, showing a reduced selectivity of epoxide formation when compared with the benzene derivative.

While this does suggest that there is a steric element to the selectivity, they could have further backed up this theory with a more variable substrate pool. A good comparison might have included substrates with varying degrees of saturation, such as propionaldehyde (**4.42**), crotonaldehyde (**4.43**) and cyclohexanecarboxaldehyde (**4.44**) (**Fig. 43**). The scope would have also benefitted from studies to extend the chain between the aromatic component and the aldehyde group, such as phenyl acetic aldehyde (**4.45**). As the uses are clearly directed towards medicinal chemistry applications, it would have been ideal to test out more complicated systems, as evidencing its use in late-stage functionalisation is an important factor when demonstrating a reactions useability within drug manufacture. This could have been done by using a multi-functionalised aromatic system such as 4-chloro-3-nitrobenzaldehyde (**4.46**). The α,β -unsaturated ester (**4.47**) which would be produced through the three-pot method would give a product containing 5 functional groups, which could be utilised as coupling or derivatisation points in late stage-functionalisation.

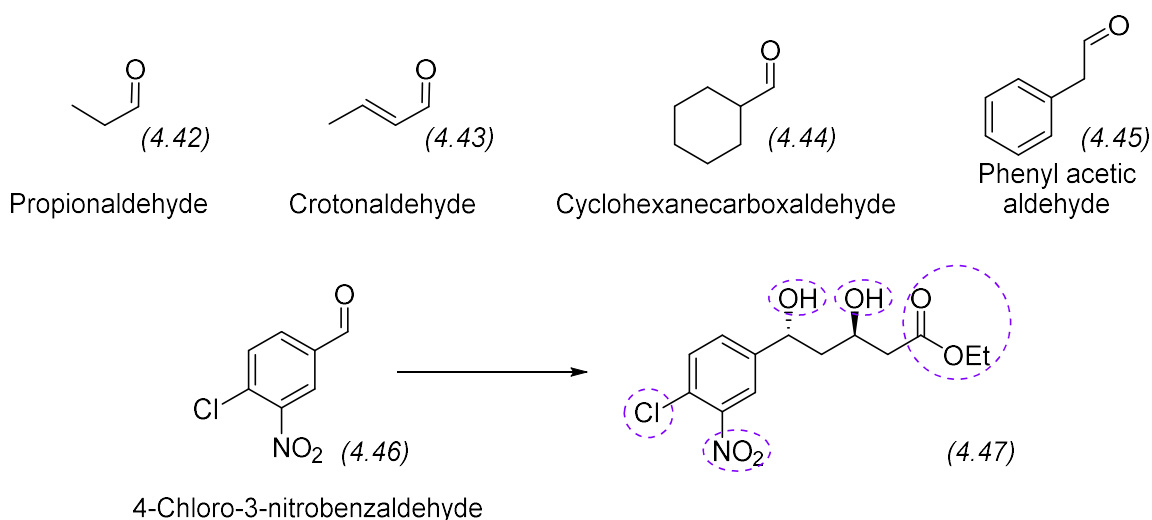


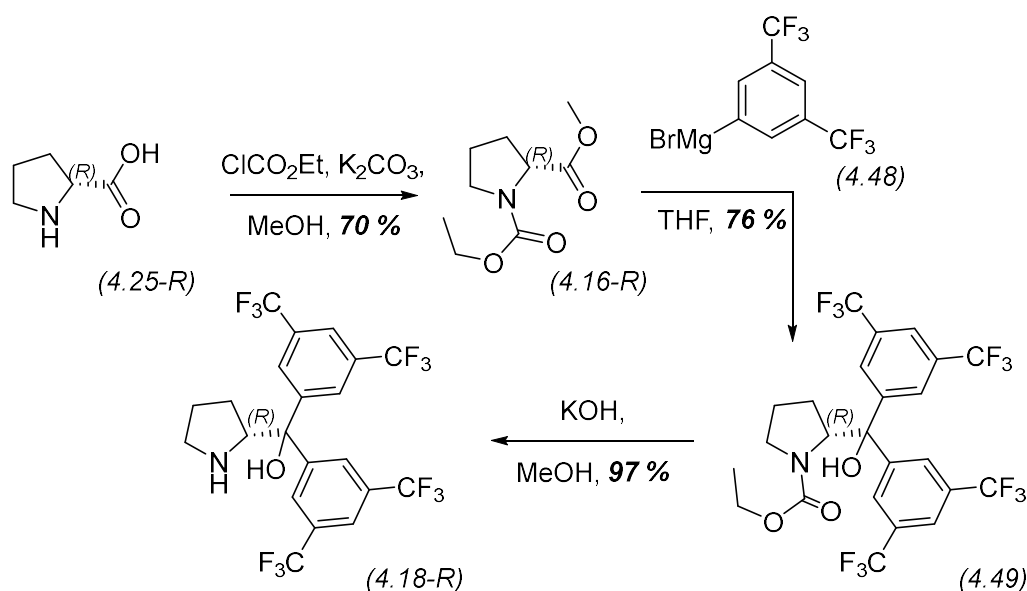
Fig. 43 Some suggested examples to broaden the scope of Hayashi's three-pot *anti*-1,3-diol synthesis, with 4-Chloro-3-nitrobenzaldehyde (4.46) suggested as an early example of its usefulness in medicinal chemistry.

Moving on from the limited variation in the substrate scope, another downfall in the procedure is the need for maintenance of $-40\text{ }^{\circ}\text{C}$ temperatures during the epoxidation.⁸⁶ Some of the published reactions are shown to take over 20 hr, which requires the use of cooling vessels or cold baths. This significantly reduces the accessibility of a reaction due to the expense of the equipment needed to carry out this kind of procedure.

4.3. Results and Discussion

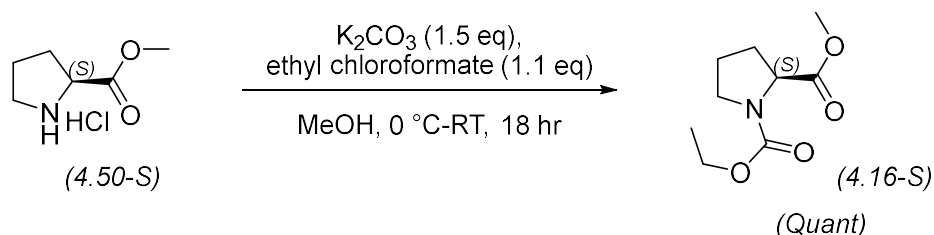
4.3.1. Synthesis of Hayashi-Jørgensen Catalyst

The synthetic route outlined by Shevick *et. al* seemed to provide the simplest access to (*R*)-Hayashi-Jørgensen catalyst (*4.18-R*), with three steps all quoted to be highly yielding (**Scheme 51**).⁹⁸ Unfortunately, the pathway is lacking reagent equivalencies or specific details of reaction conditions. Since the compound closely relates to diphenyl prolinol, specific conditions used in the synthesis of diphenyl prolinol were used alongside this literature route for synthesis of Hayashi-Jørgensen catalyst.^{99–101} The approach comprised of *N*-protection with ethyl chloroformate, Grignard addition to the ester (*4.16-R*), and base mediated hydrolysis of the protecting group.



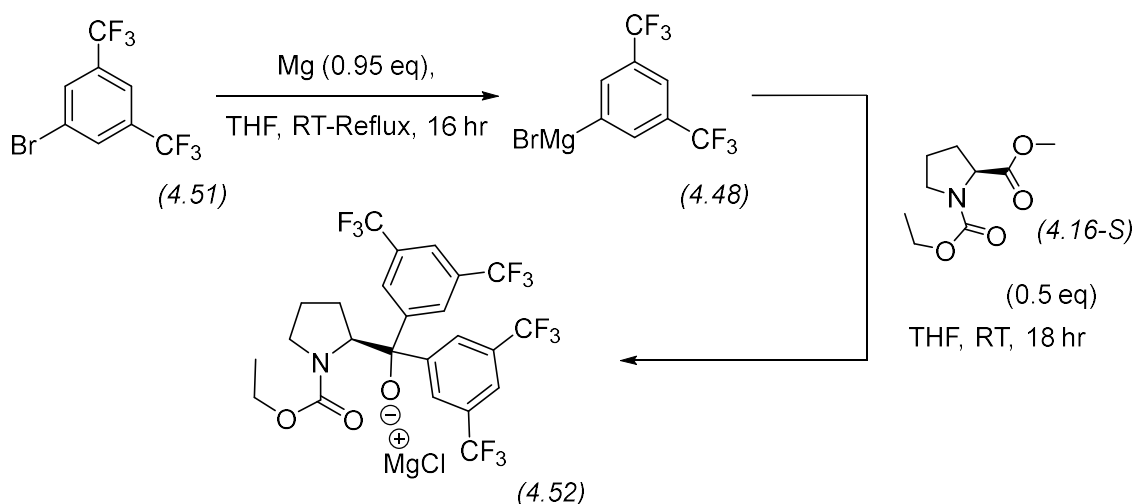
Scheme 51 Synthetic detail provided by Shevick *et. al* for the synthesis of Hayashi-Jørgensen catalyst.⁹⁸

As an initial trial, it was decided that the cheaper (*S*)-proline methyl ester (*4.50-S*) would be used to synthesis the corresponding (*S*)-Hayashi-Jørgensen catalyst (*4.18-S*). *N*-Protection was carried out utilising ethyl chloroformate and K_2CO_3 (**Scheme 52**). The reaction proceeded as expected and was purified by column chromatography to give a quantitative yield of pure product (*4.16-S*).



Scheme 52 Reaction conditions for protection of proline methyl ester (4.50-S).

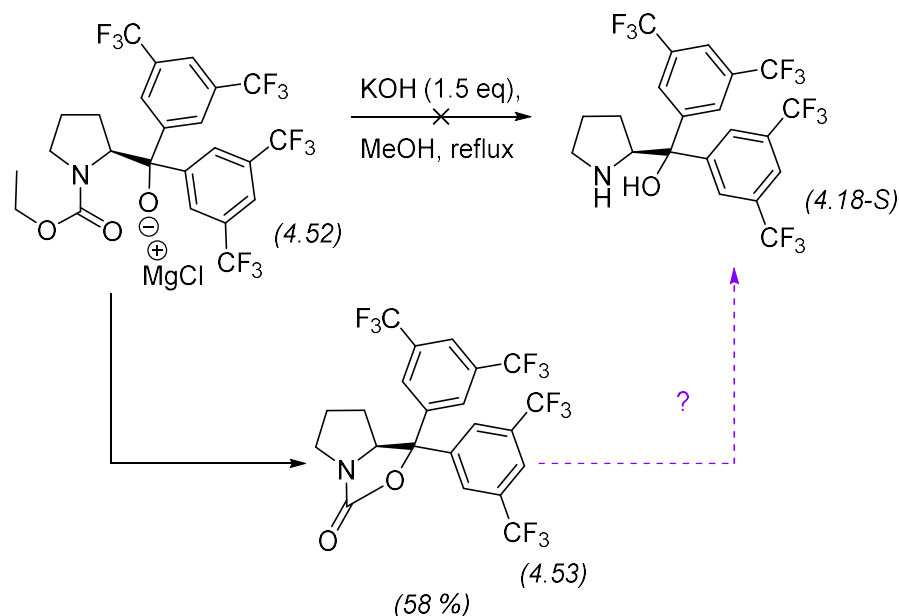
Bis-3,5-trifluoromethylbromobenzene (4.51) was reacted with magnesium to give (bis-3,5-trifluoromethyl)phenylmagnesium bromide (4.48) (**Scheme 53**). The Grignard reagent was carried forward as a solution in THF and assumed to have reached 100 % conversion. The protected proline methyl ester (4.16-S) was added to perform a double Grignard addition, the solvent was removed, and the crude material was carried forward to hydrolysis without an aqueous workup.



Scheme 53 Conditions for formation of the bis-(3,5-trifluoromethyl)phenylmagnesium bromide (4.48), followed by the double Grignard reaction with the protected proline methyl ester (4.16-S).

Hydrolysis was performed by heating at reflux with excess KOH in methanol (**Scheme 54**), which provided an off-white semi-solid which was purified by fractional column chromatography to give a colourless crystalline material. Analysis by ^1H NMR spectroscopy showed a product that almost aligned with the literature data for the desired Hayashi-Jørgensen catalyst. The product was recrystallised from methanol to provide flat needle-like crystals which were analysed by X-ray diffraction to gain insight on the product chirality. The crystal structure provided a Flack parameter was -0.026 (41), which suggests that the desired (*S*)-enantiomer had been made. However, the structure showed a 5,5-bicyclic ring

system instead of the expected Hayashi-Jørgensen catalyst (**Fig. 44**). This suggests that it could be an intermediate of the hydrolysis reaction.



Scheme 54 Intended hydrolysis of the ethyl ester nitrogen protecting group, which showed partial hydrolysis into (*S*)-1,1-bis(3,5-bis(trifluoromethyl)phenyl) tetrahydro-pyrrolo[1,2-*c*]oxazol-3(1*H*)-one (4.53), possibly an intermediate towards the desired product.

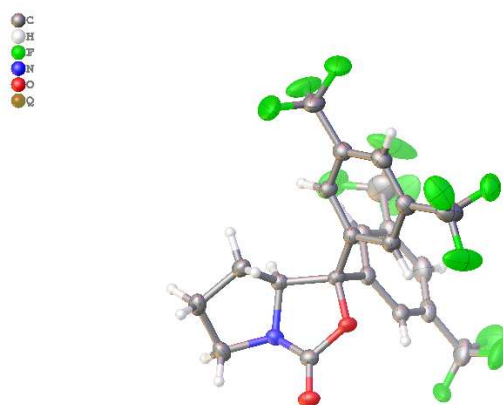
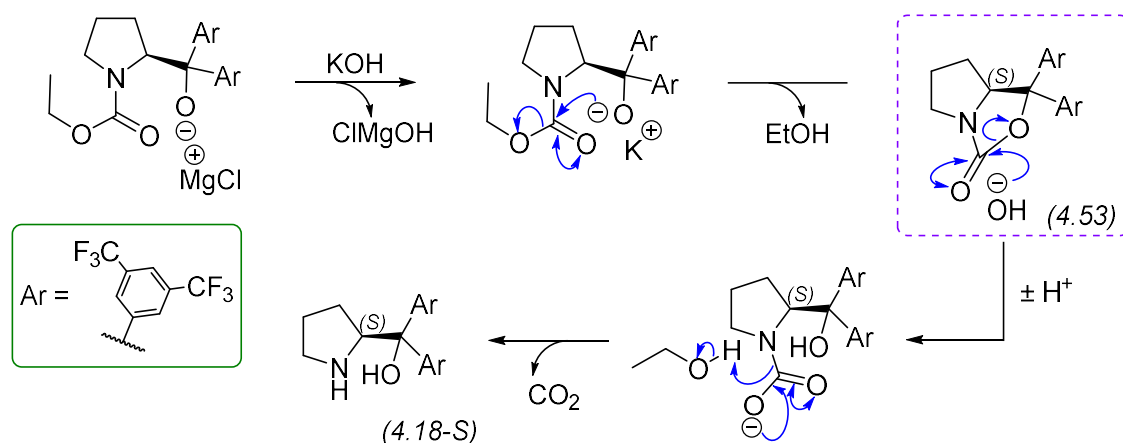


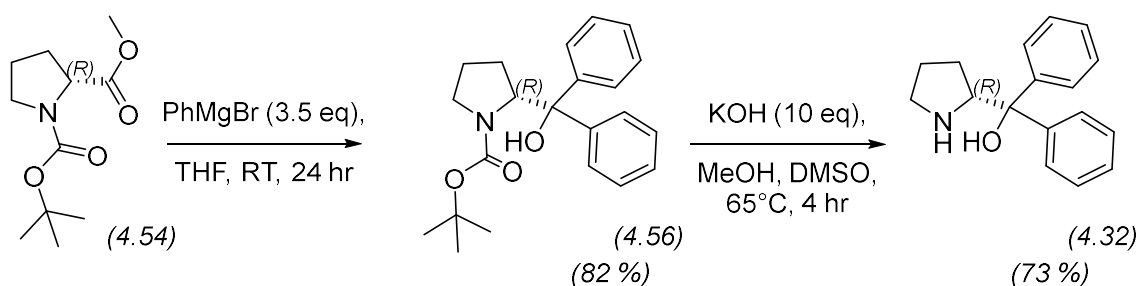
Fig. 44 Structure derived by X-ray diffraction crystal structure analysis of the hydrolysis product as (*S*)-1,1-bis(3,5-bis(trifluoromethyl)phenyl) tetrahydro-pyrrolo[1,2-*c*]oxazol-3(1*H*)-one (4.53).

Isolation of the bicyclic intermediate suggests that when subjected to near-stoichiometric amounts of KOH, the product forms the bicyclic proline derivative (4.53), releasing ethanol as a byproduct (**Scheme 55**). When subjected to excess amounts of KOH, the oxazolone type ring is opened and decarboxylation forms the desired product.



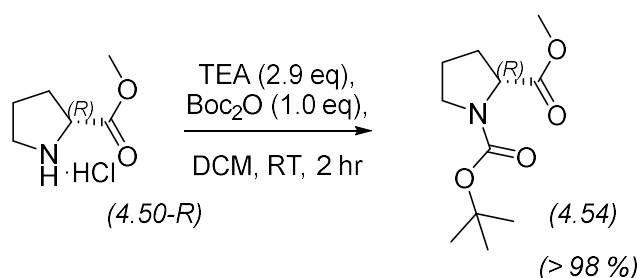
Scheme 55 Suggested mechanism of the KOH deprotection, showing the stable isolated intermediate which under addition of further excess KOH ring opens and decarboxylates to form the desired product.

Further research into synthetic methods highlighted the possibility of utilising a ^tbutyl carbamate protected proline methyl ester (4.54) to synthesise the desired Hayashi-Jørgensen catalyst. Diphenyl prolinol (4.32) has been synthesised from the Boc-protected proline methyl ester using the same Grignard addition and base hydrolysis steps previously described (**Scheme 56**). Schore *et. al* have published detailed methods for this approach, which show high yields at each step.¹⁰¹ Dominic Dennis utilised this approach in synthesis of CBS ligands, and removed the requirements for column purifications at each stage and instead performing slow recrystallisation from methanol.¹⁰² This made the approach more desirable than the previous ethyl ester protected method outlined above. For this reason, the route was altered slightly to utilise the Boc protecting group in synthesis of (*R*)-Hayashi-Jørgensen catalyst.



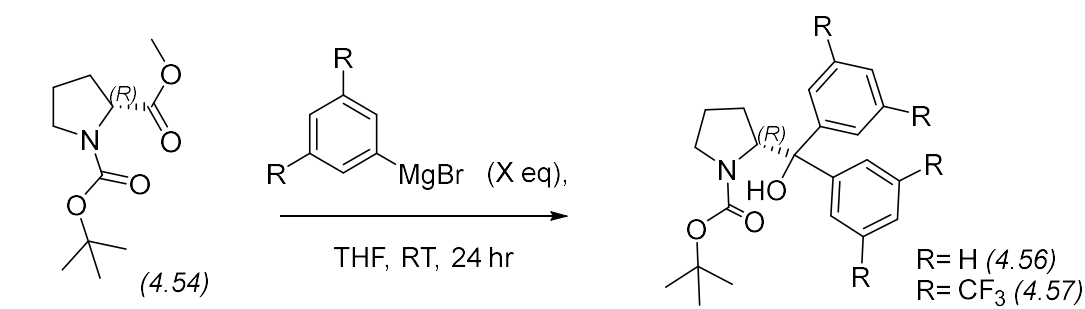
Scheme 56 Synthetic strategy used by Schore *et. al* to make ^tbutyl carbamate protected (*R*)-diphenyl prolinol (4.32), an intermediate towards CBS catalyst.¹⁰¹

To synthesise the Boc-protected starting material, (*R*)-proline methyl ester HCl salt (*4.25-R*) was reacted with Boc anhydride and triethylamine. The protected proline methyl ester was isolated in > 98 % yield (**Scheme 57**).



Scheme 57 *N*-Protection of (*R*)-proline methyl ester as (*R*)-1-*tert*-butyl-2-methyl pyrrolidine-1,2-dicarboxylate (*4.54*).

The original literature procedure indicated a yield of 82 % for the double Grignard reaction using 3.5 equivalents of phenylmagnesium bromide (**Table 8, Entry 1**).¹⁰¹ Optimisation by Dominic Dennis using recrystallisation from methanol, gave a reduction in isolated yield from 82 % to 60 % (*Entry 2*).¹⁰² However, the approach made accessing the intermediate much simpler and quicker, with a possibility to increase the isolated yield with further recrystallisation of the second crop. Since the cost of 3,5-bis(trifluoromethyl)phenylmagnesium bromide (*4.48*) is high, it was decided that the procedure should be further optimised to ascertain whether a reduction in the Grignard reagent equivalency could be made without sacrificing yield. A test run was performed on the synthesis of (*R*)-(diphenylhydroxymethyl)-*N*-Boc-pyrrolidine (*4.32*), using 2.5 equivalents of phenylmagnesium bromide rather than 3.5. Interestingly, this reduction produced an increased yield of 69 % when isolated by recrystallisation. It was decided that a 13 % reduction in isolated yield, when compared with the original literature, was an acceptable sacrifice to reduce the necessary Grignard reagent by 1.0 equivalents. Therefore, this newly optimised protocol was replicated using 3,5-bis(trifluoromethyl)phenylmagnesium bromide. Unlike its phenyl counterpart, it was not possible to recrystallise (*R*)-2-[bis(3,5-bis-trifluoromethylphenyl)hydroxymethyl]-*N*-Boc-pyrrolidine (*4.57*) from methanol. Instead, this was purified by dry column vacuum chromatography (DCVC) to give an isolated yield of 70 %.

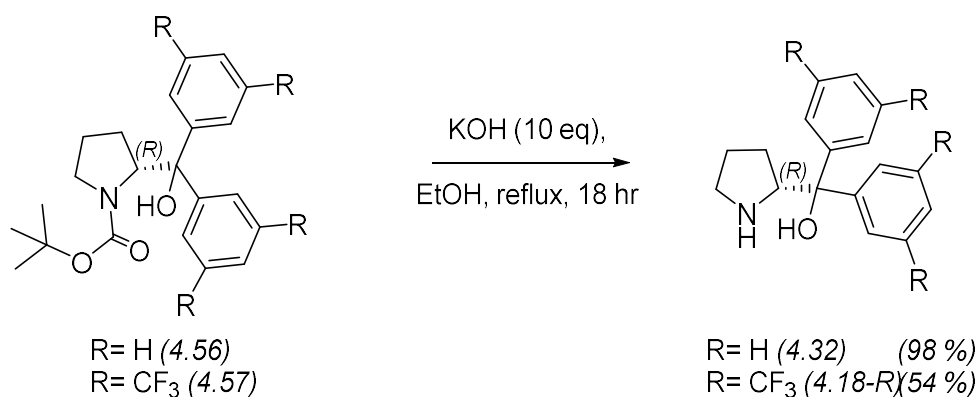


-R	X eq	Purification method	Isolated yield
-H*	3.5	FCC	82 %
-H**	3.5	Recrystallisation	60 %***
-H	2.5	Recrystallisation	69 %***
-CF ₃	2.5	DCVC	70 %

Table 8 Synthesis of Boc protected diphenyl prolinol derivatives. * Indicates the original literature procedure.¹⁰¹ ** Indicates the result optimised within the CJR group.¹⁰² *** First crop recrystallization yield.

The initial purpose of the protecting group change was to improve the purification efficiency, and while DCVC may not be as efficient as recrystallisation, it still improved the ease of purification and greatly reduced the time and solvent required to do so.

Hydrolysis on both Boc-protected proline derivatives was achieved by heating the compound in ethanol with excess KOH (**Scheme 58**). (*R*)-Diphenyl proline (4.32) was isolated in a 98 % yield, without the need for further purification. (*R*)-Hayashi-Jørgensen catalyst (4.18-*R*) was isolated in a quantitative yield, but trace impurities remained. The impurities were assumed to be caused by Wurtz coupling within the Grignard step, so column conditions were carried out as reported in the literature.¹⁰⁰ The product co-eluted with an impurity that was isolated by trituration with neutralised chloroform, providing a final isolated yield of 54 %.



Scheme 58 Hydrolysis of Boc protected intermediate to produce (R)-Hayashi-Jørgensen catalyst (4.18-R) and (R)-diphenyl prolinol (4.32).

Analysis of the isolated impurity *via* ¹H and ¹⁹F NMR spectroscopy showed it to be bis[3,5-bis(trifluoromethyl)phenyl]methanol (4.58), which was confirmed by comparison of spectra to literature data (**Fig. 45**).¹⁰³ This impurity was not witnessed in the crude data, and therefore it seems likely that it formed during the column *via* an acid catalysed pyrrole elimination reaction. Although removal of bis[3,5-bis(trifluoromethyl)phenyl]methanol (4.58) was easily performed by reverse trituration with neutral chloroform, this did result in a significant loss of yield.

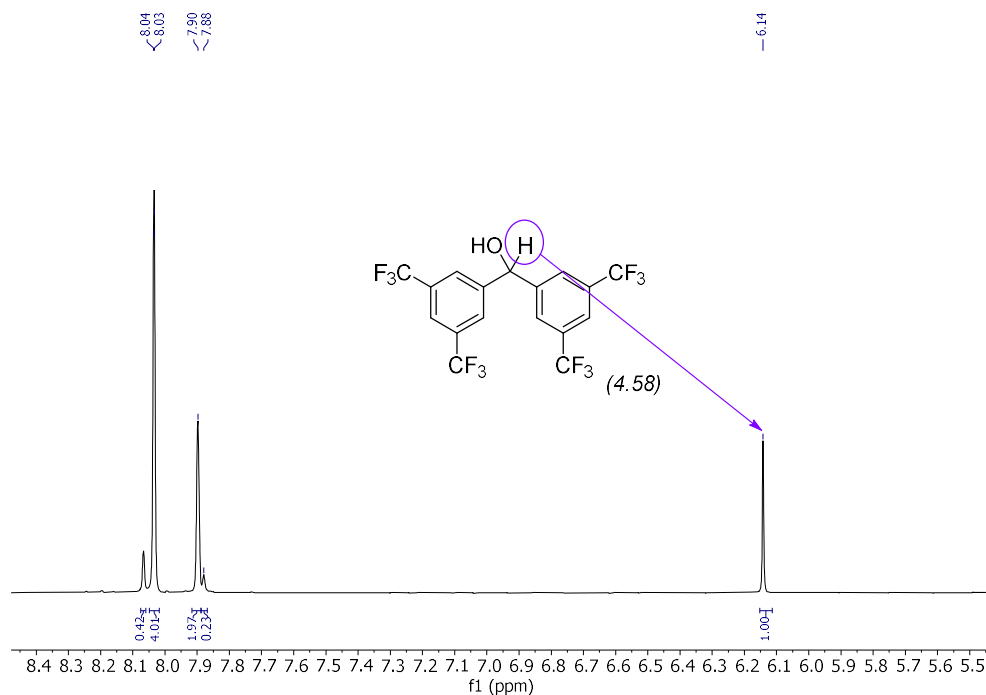
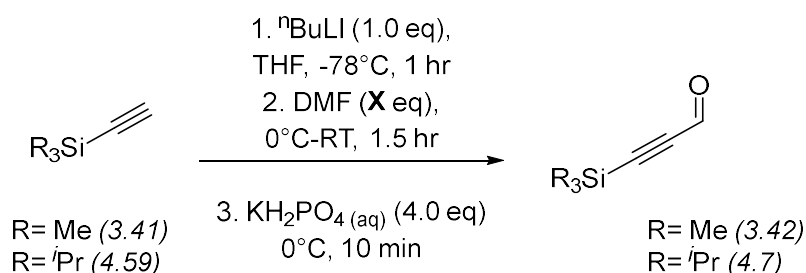


Fig. 45 Proton NMR spectra of the bis[3,5-bis(trifluoromethyl)phenyl]methanol (4.58) impurity, with the benzylic proton (6.14 ppm) and two aromatic environments at 8.03 and 7.90 ppm, for the full spectrum see appendix (A-C4-1).

4.3.2 Asymmetric aldol/ HWE cascade

Synthesis began by formylating silyl protected acetylene (3.41 and 4.59). Initial attempts using the Brown allylation approach (chapter 3) utilises TMS-acetylene (3.41) which was formylated in a 54 % yield using standard techniques with n BuLi and DMF (Table 9, Entry A). The volatility of the product led to challenging isolation from solvents used in the preparation, particularly the excess DMF and the toluene from the n BuLi solution. A simple solution to the volatility problem was a swap to a heavier protecting group. TIPS-acetylene (4.59) was readily available to purchase and increased the molecular weight enough, while also providing an alkyne protection that is simple to remove. Fresh n BuLi was purchased as a solution in hexanes and used in replacement of the toluene solution. This produced the desired 3-(triisopropylsilyl)-prop-2-ynal (4.7) in a 68 % yield on a 1.5 g scale utilising the same conditions as previously (Table 9, Entry B). Further improvements were made during a scale up to 9 g of starting material, where the equivalency of DMF was reduced from 2.0 to 1.2, which reduced the need for purification by distillation, as residual DMF was removed during solvent removal. The product did not require any further purification and was isolated in a quantitative yield (Table 9, Entry C).

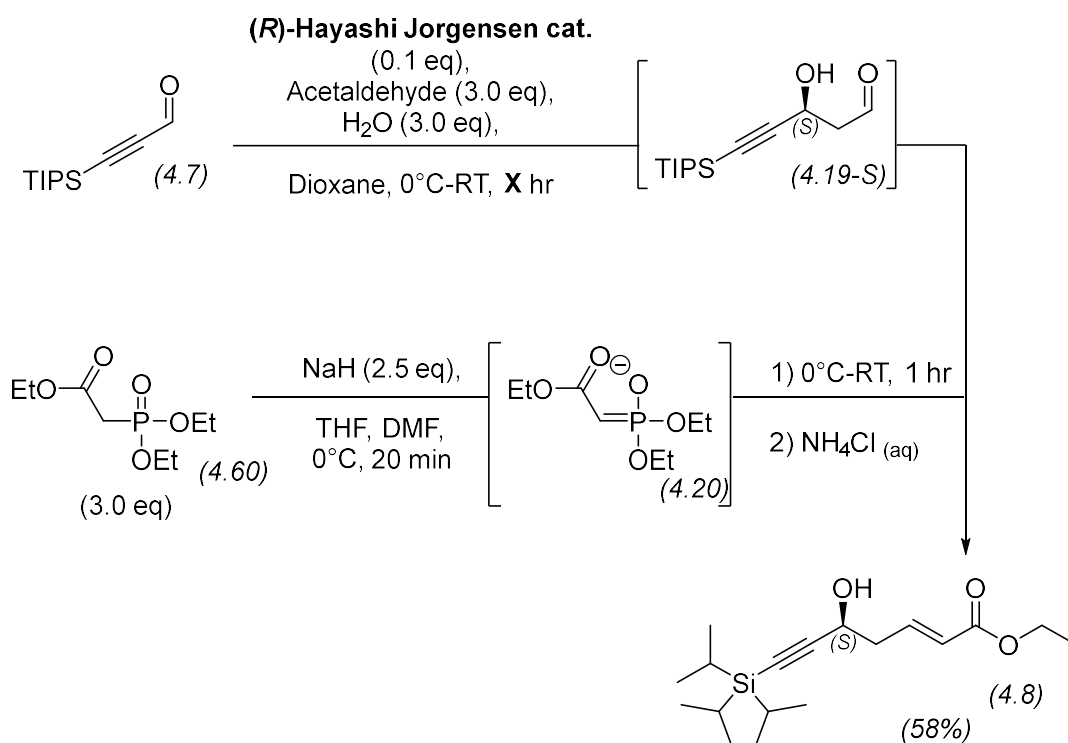


	-R	X	Yield
A	Me	2.0	54 %
B	i Pr	2.0	68 %
C	i Pr	1.2	Quant.

Table 9 Optimisation of the formylation reaction, where [A] is the initial optimised conditions from TMS-acetylene and [B] is the initial conditions for formylation of TIPS-acetylene and [C] optimised conditions for the more stable TIPS-acetylene (4.49).

The product was then subjected to asymmetric aldol conditions using the previously prepared (*R*)-Hayashi-Jørgensen catalyst (4.18-*R*) and acetaldehyde (Scheme 59).⁸⁶ The reaction was monitored by ^1H NMR spectroscopy of small aliquots, until the SM aldehyde

peak at 9.20 ppm had been consumed. This time varied between reaction (**Table 10**), with the smallest scaled reaction taking the longest time to show full conversion. The Horner-Wadworth-Emmons reagent (**4.20**) was freshly prepared by deprotonation of triethyl phosphonoacetate (**4.60**) to form the phosphonate stabilised carbanion. To this was added the prepared aldehyde and ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**) was isolated in 54-60 % yield. The lowest yield was obtained in the smallest sample run, which was due to multiple purifications being required to isolate the product in > 90 % purity. This purification was improved upon in run [B], which allowed fully optimised purification for run [C]. This included two runs of fractional column chromatography to give a 58 % yield of high purity material, with a further fraction of 3.1 g at approximately 60 % purity.

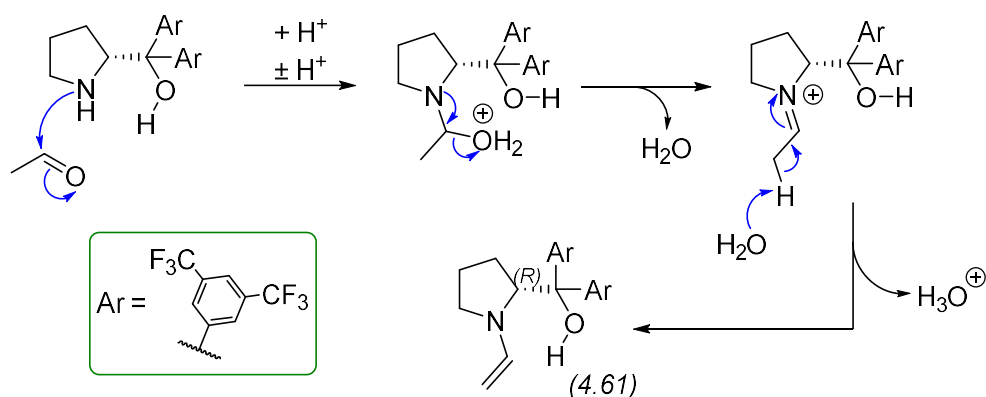


Scheme 59 Synthetic route towards ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**) with an overall 2-step yield of 58-60 %.

Run	Scale	Time (X hr) for Aldol reaction	Isolated yield
[A]	178 mg	92	54 %*
[B]	640 mg	67	60 %
[C]	6.01 g	72	58 %

Table 10 Application of the asymmetric aldol/HWE reaction to synthesise ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**), showing variable reaction times, but relatively consistent yields. *Indicates initial run, not included in the average yield.

The reaction is believed to occur *via* the formation of an enamine (**4.61**) when the catalyst reacts with acetaldehyde (**Scheme 60**). This newly formed enamine is then responsible for inducing the chirality in the aldol reaction.



Scheme 60 Mechanism by which the Hayashi-Jørgensen catalyst reacts with acetaldehyde to form an enamine (**4.61**).

Hydrogen bonding between the hydroxyl proton and proline nitrogen causes a six-membered transition state, which is ordered in a way that the bulky silyl protected alkyne is preferentially directed away from the aromatic groups (**Fig. 46**). In this reaction, the aldehyde favours an equatorial orientation (**4.62**) over the axial resulting in the preferential formation of iminium ion (**4.63**) (**Scheme 61**). Hydrolysis then regenerates the Hayashi-Jørgensen catalyst and produce the desired product in high enantiopurity.

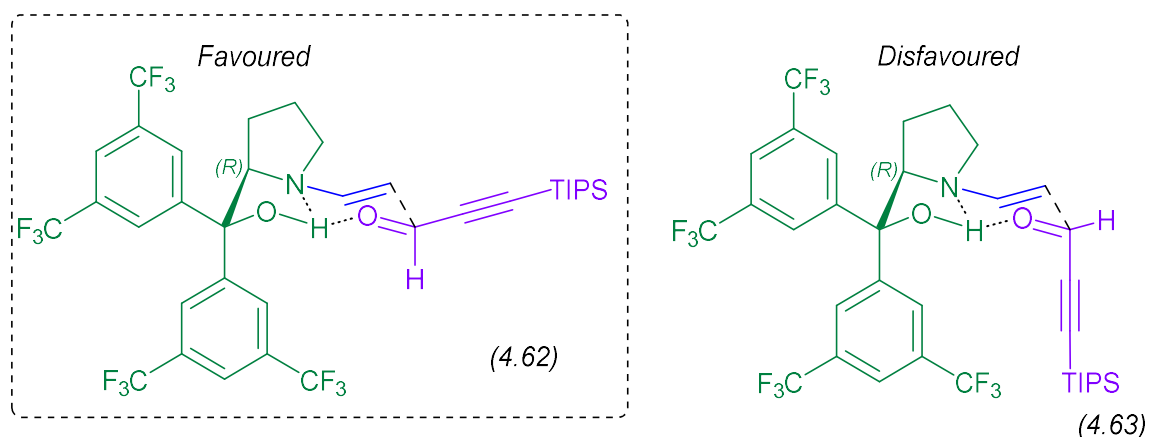
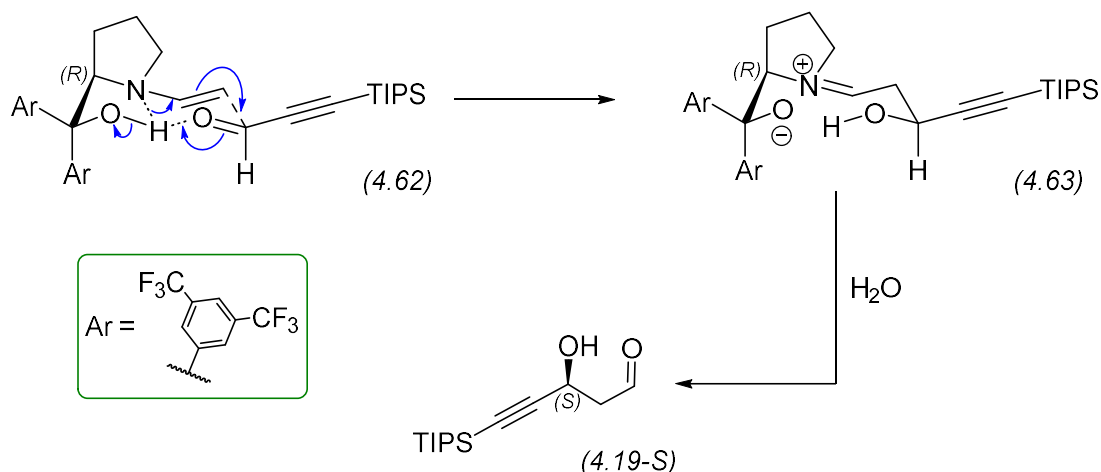
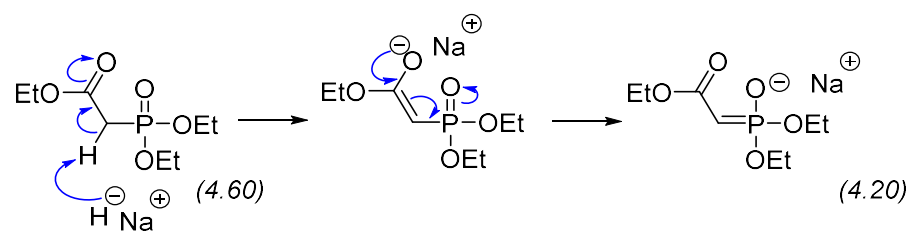


Fig. 46 Comparison of the favoured transition state for the Hayashi-Jørgensen mediated aldol coupling, against the sterically un-favourable transition state.

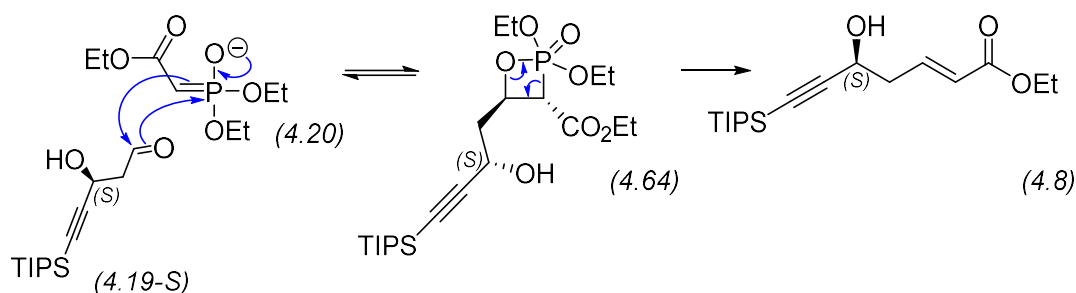


Scheme 61 Formation of the iminium ion intermediate, which results the reaction between Hayashi-Jørgensen enamine reacts and silyl protected propynal to form 3(S)-hydroxy(trimethylsilyl)hexyn-5-al (4.19-S).

The asymmetric aldol was then followed up with a Horner-Wadsworth-Emmons reaction. The ylide (4.20) was synthesised from triethyl phosphonoacetate (4.60), using sodium hydride as a base (**Scheme 62**). To the ylide, was added the aldol reaction mixture. The reaction was complete within an hour and showed complete selectivity of the *E*-alkene, via the *anti*-oxaphosphetane intermediate (**Scheme 63**).

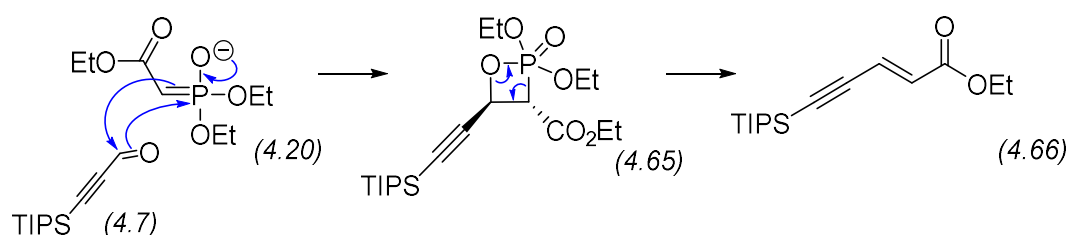


Scheme 62 Mechanism for formation of the HWE ylide from triethyl phosphonoacetate (4.60).



Scheme 63 Mechanism for the Horner-Wadsworth-Emmons reaction between the triethyl phosphonoacetate ylide and 3(*S*)-hydroxy(trimethylsilyl)hexyn-5-al (4.19-*S*), giving an *E*-selective synthesis of ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8).

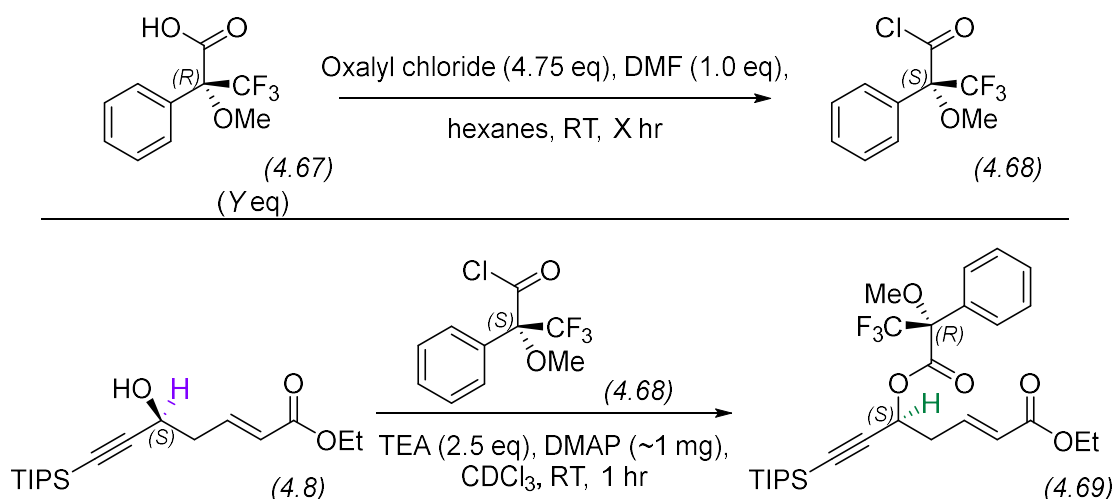
To achieve high purity of the product during the 6.01 g scale up run, fractional column chromatography was carried out twice. The first run acquired two major product containing fractions. Fraction one weighed 6.52 g and appeared to be approximately 85-90 % purity product, which was contaminated with Hayashi-Jørgensen catalyst and an ester byproduct. This is believed to be ethyl (*E*)-5-(triisopropylsilyl)pent-2-en-4-ynoate (4.66) caused by a HWE reaction with small amounts of remaining SM (4.7) (**Scheme 64**). The second fraction weighed 3.1 g and contained approximately 60 % product, with multiple contaminants and was retained for future purification. Fraction one was purified by a second, slower fractional column chromatography. The impurities were slowly bled off the column in 2 % EtOAc in hexanes, before flushing the product out in 20 % EtOAc. Any increase in the eluent polarity caused too much product movement and loss of yield. After this second purification, the overall yield of pure product was 58 %.



Scheme 64 Suspected side-reaction from residual starting material, to give ethyl (*E*)-5-(triisopropylsilyl)pent-2-en-4-ynoate (4.66) as an unwanted byproduct.

Initially, it was hoped that the Hayashi-Jørgensen catalysts could be recovered and recycled for future reactions. As discussed in section 4.3.1 the Hayashi-Jørgensen catalyst appears to be prone to hydrolysis during column chromatography, which meant that without spending time optimising column conditions for recovery, this would not be easily achieved.

To assess the selectivity of the asymmetric aldol product, Mosher's ester analysis was used (**Scheme 65**). This method utilises ^{19}F NMR spectroscopy to assess the diastereomeric ratio (d.r.), *via* reacting a chiral alcohol fully with a single enantiomer of Mosher's acid chloride (4.68).¹⁰⁴ To perform this, a micro-scale synthesis of Mosher's acid chloride from Mosher's acid (4.67) was carried out using the method published by Ward and Ree.¹⁰⁵ Using this approach showed low conversion of the alcohol (4.8) to the ester (4.69) by the ^1H NMR spectroscopy (**Fig. 47**, **Table 11**, [A]). This can be seen by a large excess of the peak highlighted in purple, which corresponds to the proton on the stereogenic centre of the starting material, and only two minor peaks in green corresponding to the highlighted product diastereomers. Since the esterification did not reach completion, this result could not be used to determine the d.r., therefore, the original procedure was modified and a second assessment carried out.



Scheme 65 Synthesis of the Mosher's ester derivative (4.69) of ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8), highlighting the key protons used to determine completion of reaction in the ^1H NMR spectra.

	Run A	Run B
Time for acid chloride synthesis (X)	1 hr	2 hr
Equivalency of Mosher's acid (Y)	1.2	2
Molar conversion of alcohol	22 %	>99 %
d.r. by ^1H NMR	89 : 11	95 : 5
d.r. by ^{19}F NMR	90 : 10	95 : 5

Table 11 Comparison of two different runs of the Mosher's ester analysis, where [A] showed incomplete conversion of the alcohol to the Mosher's ester derivative, and [B] showed complete conversion.

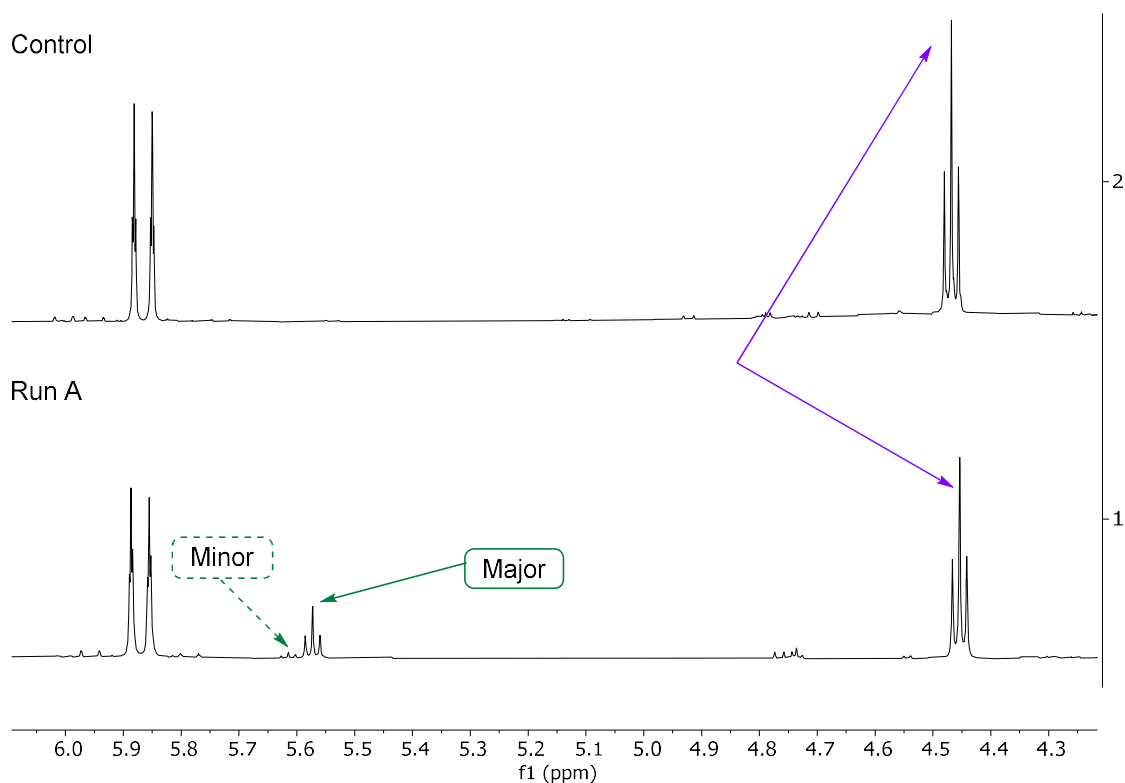


Fig. 47 Mosher's ester analysis of ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**) by ^1H NMR spectroscopy. Showing the control sample (*top*) which contains the alcohol, Mosher's acid and DMAP (**A-C4-36**), and run A (**A-C4-34**) denoted in **Table 11**. The key proton peaks highlighted in purple and green correspond to SM (**4.21-S**) and product (**4.69**) protons referred to in **Scheme 65**.

To improve the conversion, a higher ratio of Mosher's acid chloride (**4.68**) to alcohol was required. This was achieved by making two changes, more time was given for conversion of the Mosher's acid (**4.67**) to Mosher's acid chloride (**4.68**) and the equivalency of acid was increased from 1.2 to 2 (**Table 11**, [B]). Implementing these changes showed complete conversion of the starting material (**4.8**), highlighted by the disappearances of the key proton signal in purple (**Fig. 48**). Integration of the two new proton signals, corresponding to the green chiral proton in the diastereomeric products (**4.69**) (**Scheme 65**) gives a dr of approximately 95:5.

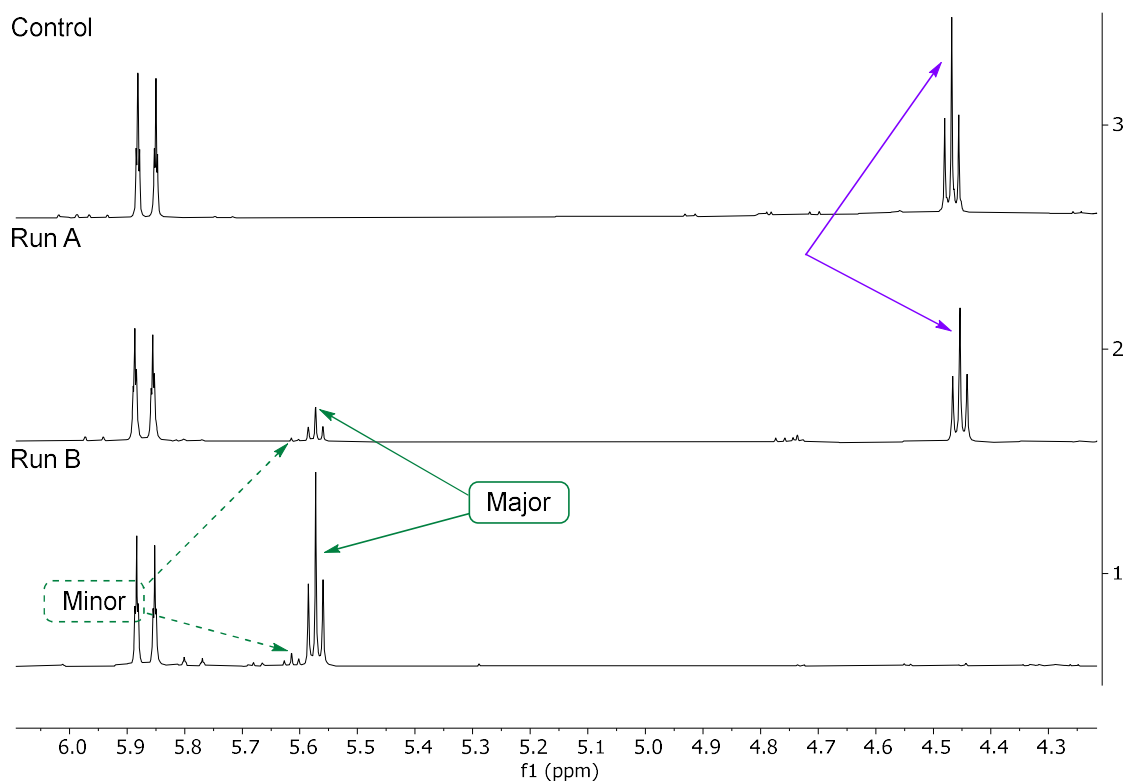


Fig. 48 Mosher's ester analysis of ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**) by ^1H NMR spectroscopy. Showing the control sample (*top*) which contains the alcohol, Mosher's acid and DMAP (**A-C4-36**), run A (**A-C4-34**) and run B (**A-C4-32**) denoted in **Table 11**. The key proton peak highlighted in purple and green correspond to SM and product protons referred to in **Scheme 65**.

Assessment of the ^{19}F NMR spectra confirmed the dr of 95:5, with the two peaks being seen at -71.65 and -71.82 ppm (**Fig. 49**). These were confirmed to be the two diastereomers by comparison with a ^{19}F NMR spectra of the control sample and run [A]. It is clear when comparing the ^1H NMR spectrum alongside the ^{19}F NMR spectrum, that as the ratio of triplet at 5.62 ppm to the triplet at 5.57 ppm reduces in the proton spectra, the ratio of the peak at -71.65 to -71.82 ppm decreases at the same rate. The large signal at -70.65 ppm corresponds to the residual Mosher's acid chloride and smaller peaks between -71.3 and -71.6 ppm can be seen at the same intensity on all spectra, indicating that they likely correspond to minor fluorinated impurities. All these results point to the asymmetric Aldol reaction achieving an enantiomeric excess of approximately 90 %. Confirmation of the enantiomeric excess by chiral HPLC measurement will be required to provide a more accurate value.

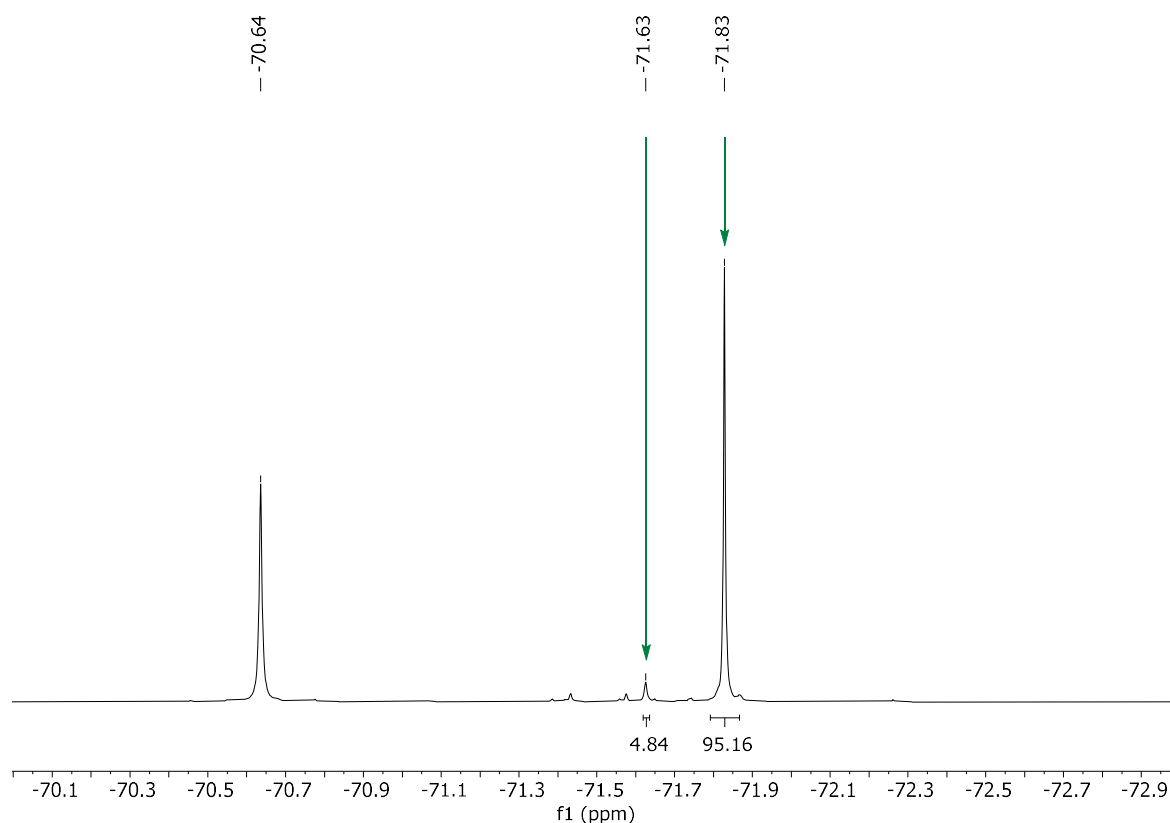
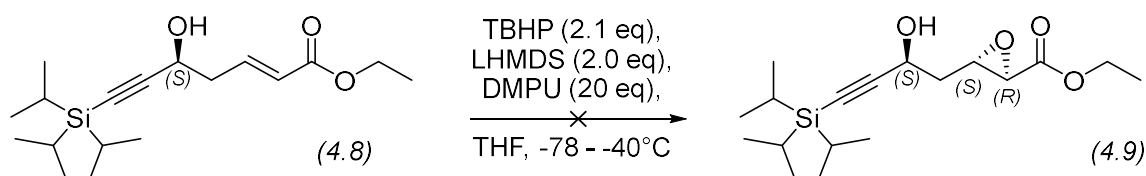


Fig. 49 ^{19}F NMR spectrum of Mosher's ester of ethyl (*S*, *E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.69**) showing the peaks corresponding to the two diastereomeric products in green, at a 95:5 ratio (**A-C4-33**).

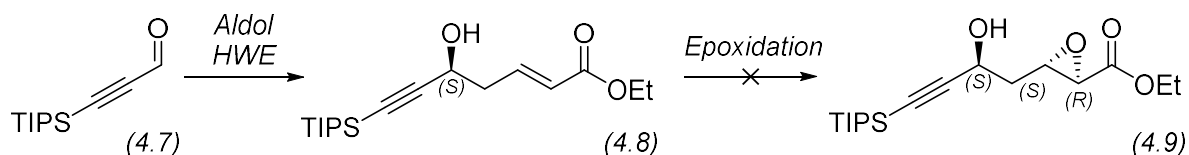
Initial attempts to install the second hydroxy group also followed the procedure found within the paper published by Yujiro Hayashi *et al.*⁸⁶ Utilising lithium bis(trimethylsilyl)amide (LHMDS) and *t*-butyl hydroperoxide (TBHP), epoxidation was performed (**Scheme 66**). While the paper suggested that this could be done selectively to favour the *anti*-product, the synthesis of this compound required maintaining a temperature of -40 °C for 18 hr. Without access to a chiller reaction vessel, this proved challenging to perform. Using the equipment available, the temperature was maintained between -20 and -40 °C for 10 hr. Unfortunately, this did not appear to yield any product and so another approach to installing the next chiral C-O bond was required.



Scheme 66 Conditions published by Hayashi *et al.* for the stereoselective epoxide formation.⁸⁶

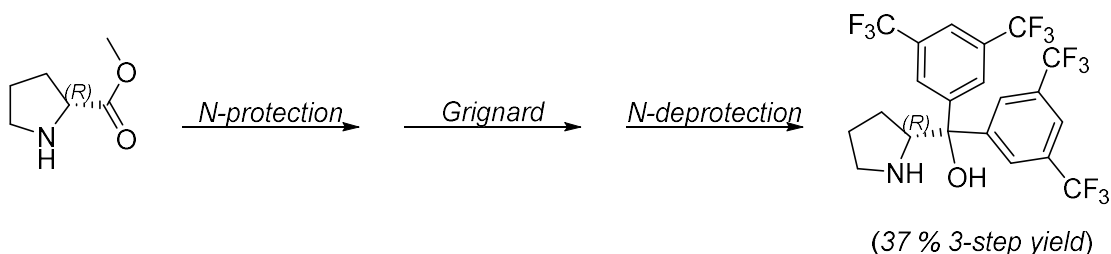
4.4. Conclusions and Future Work

With the previous route having potential major problems on scale up, a new route was designed around an aldol/HWE cascade reaction (**Scheme 67**). The aim was to synthesise ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate using this approach and then utilise the new α,β -unsaturated ester to install the second C-O bond through an epoxidation of the alkene.



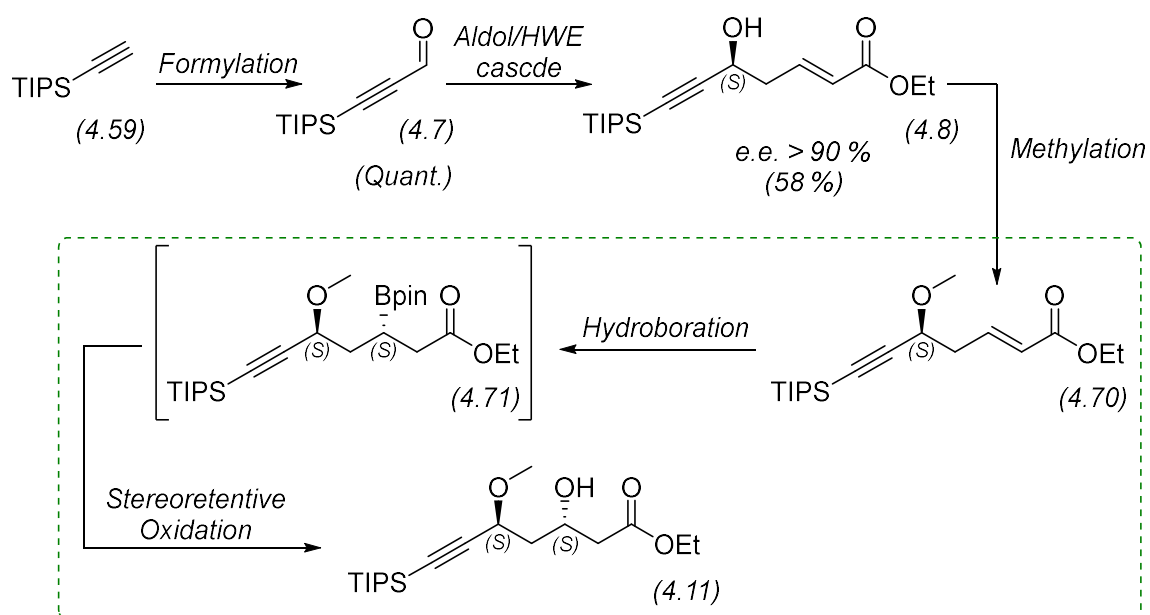
Scheme 67 Planned method for synthesis of the desired diol, showing the un-attempted steps in grey-scale.

The asymmetric aldol approach utilised Hayashi-Jørgensen catalyst, which was synthesised in-house in a 3-step yield of 37 % (**Scheme 68**). The first two steps proceeded with high to moderate yields, while the final step suffered major losses during purification. Further optimisation of this procedure will need to be performed in the future by optimisation of the purification methods, including trials with solvent trituration or FCC with a neutral solid phase.



Scheme 68 3-Step protocol toward synthesis of Hayashi-Jørgensen catalyst, with an overall 37 % yield.

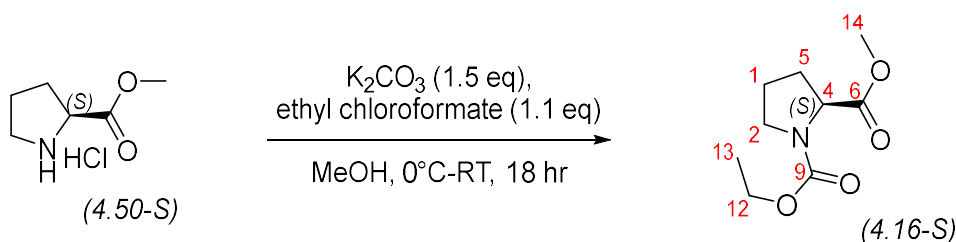
Synthesis of the starting material from TIPS-acetylene has been optimised to give a quantitative yield (**Scheme 69**). This was successfully used in the Aldol/HWE cascade to provide a 58 % of ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate with an enantiomeric excess of approximately 90 %. The protocol for substrate-controlled epoxidation proved to be challenging with the equipment available and therefore, a different approach using a catalyst controlled hydroboration was investigated.



Scheme 69 Successful route towards ethyl 5(S)-methoxy-3(S)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (4.8), with the planned next steps included (boxed).

4.5. Experimental Section

(S)-1-Ethyl-2-methyl-1,2-pyrrolidinedicarboxylate (4.16-S)



4.50-S (5.00 g, 30.19 mmol, 1.0 eq) was dissolved in methanol (80 mL). Pre-dried K₂CO₃ (6.26 g, 45.28 mmol, 1.5 eq) was added and the reaction material was cooled to 0 °C. Ethyl chloroformate (3.60 g, 33.21 mmol, 1.1 eq) was added dropwise. The reaction material was warmed to RT and stirred for 18 hr. Upon confirmation of completion of reaction, the methanol was removed under reduced pressure. The resultant oil was dissolved in DCM (100 mL) and washed with water (2 × 50 mL) and brine (50 mL). The organic phase was dried over Na₂SO₄, filtered and concentrated. The crude product was purified by column chromatography (1:1 EtOAc:hexanes) to give the product as yellow oil (6.68 g, Quant. yield)

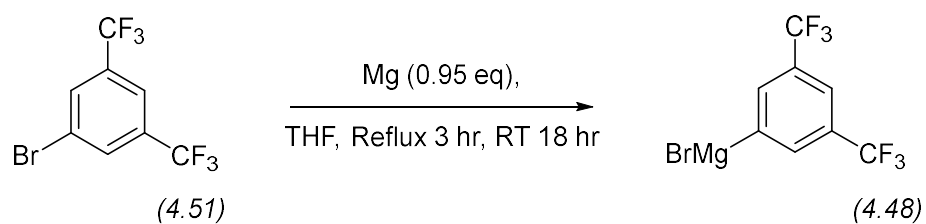
Spectroscopic data is in line with literature.^{100,106}

See A-C4-1 to A-C4-5

¹H NMR (500 MHz, Chloroform-*d*, rotamers approximately 1:1) δ 4.37 (dd, *J*=8.6, 3.7 Hz, 0.5H, C(4)-H), 4.30 (dd, *J*=8.6, 3.7 Hz, 0.5H, C(4)-H), 4.16 (q, *J*=7.3 Hz, 1H, C(12)-H₂), 4.12 (q, *J*=7.3 Hz, 1H, C(12)-H₂), 3.73 (s, 1.5 H, C(14)-H₃), 3.72 (s, 1.5 H, C(14)-H₃), 3.64 – 3.40 (m, 2H, C(1)-H₂), 2.29 – 2.18 (m, 1H, C(5)-H), 2.03 – 1.83 (m, 3H, C(5)-H and C(2)-H₂), 1.27 (t, *J*=7.3 Hz, 1.5H, C(13)-H₃), 1.26 (t, *J*=7.3 Hz, 1.5H, C(13)-H₃)

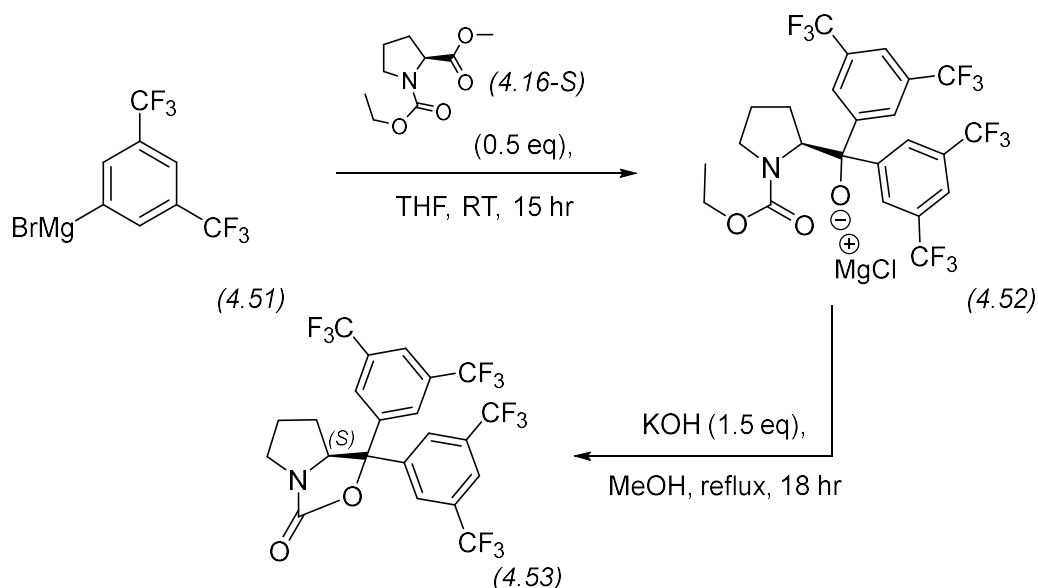
¹³C NMR (126 MHz, Chloroform-*d*, rotamers approximately 1:1) δ 173.5 (C6), 173.4 (C6), 155.4 (C9), 154.8 (C9), 61.6 (C4), 61.5 (C4), 59.2 (C8), 59.0 (C8), 52.3 (C14), 52.24 (C14), 46.9 (C2), 46.5 (C2), 31.0 (C5), 30.0 (C5), 24.4 (C1), 23.7 (C1), 14.8 (C13), 14.7 (C13)

3,5-Bis(trifluoromethyl)phenylmagnesium bromide (4.48)



Magnesium turnings (0.093 g, 3.86 mmol, 0.95 eq) were vigorously stirred in THF under argon for 1 hr. **KSM** (1.00 g, 4.06 mmol, 1.0 eq) was charged dropwise. The reaction material was heated to reflux and stirred for 3 hr, before cooling to RT and stirring for 18 hr. Excess magnesium was removed by filtration under argon. Crude product was not purified and was carried forward as a solution in THF.

(S)-1,1-Bis(3,5-bis(trifluoromethyl)phenyl)tetrahydro-pyrrolo[1,2-c]oxazol-3(1H)-one (4.53)



(S)-1-Ethyl-2-methyl-1,2-pyrrolidinedicarboxylate (*4.16-S*, 0.408 g, 2.03 mmol, 0.5 eq) was dissolved in THF (4 mL) under argon. **4.51** (assumed mass: 1.29 g, 4.06 mmol, 1.0 eq) was added dropwise, allowing the reaction to exotherm. The reaction material was stirred for 18 hr. Upon confirmation of completion of reaction, the solvent was removed under reduced pressure.

The residue was taken up in MeOH (40 mL). Pre-dried KOH (0.342 g, 6.09 mmol, 1.5 eq) was charged, the reaction was heated to reflux and allowed to stir for 18 hr. The reaction material was cooled to RT, filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), washed with water (3 × 25 mL) and dried over Na₂SO₄. The organic phase was filtered and concentrated under reduced pressure. Crude product was purified by column chromatography (0:1 to 1:3 EtOAc : hexanes) to obtain product as a colourless solid (0.65 g, 58 % yield).

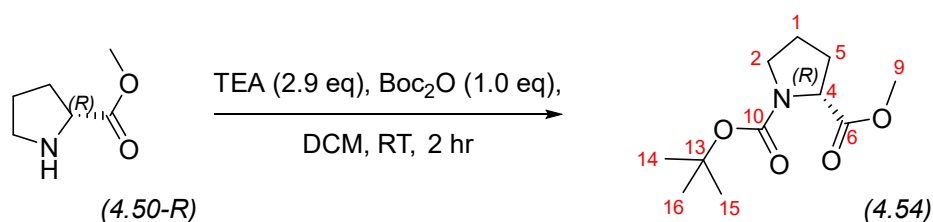
See A-C4-6 to A-C4-11

¹H NMR (400 MHz, Methanol-*d*₄) δ 8.29 (apparent s, 2H), 8.19 (apparent s, 2H), 8.04 (apparent s, 1H), 8.01 (apparent s, 1H), 3.71 (m, 1H), 3.28 – 3.21 (m, obscured by solvent, 2H), 2.15 – 2.04 (m, 1H), 2.04 – 1.94 (m, 1H), 1.93 – 1.80 (m, 1H), 1.04 (m, 1H).

¹³C NMR (126 MHz, Methanol-*d*₄) δ 200.4, 178.0, 161.0, 147.1, 127.2, 85.2, 70.2, 47.4, 29.7, 25.4

¹⁹F NMR (376 MHz, Methanol-*d*₄) δ -64.37, -64.43

(R)-1-*i*-butyl-2-methyl pyrrolidine-1,2-dicarboxylate (4.54)



4.50-*R* (5.00 g, 30.2 mmol, 1.0 eq) was dissolved in DCM (50 mL). Triethylamine (6.00 mL, 43.8 mmol, 1.45 eq) was added to the reaction. Boc₂O (6.59 g, 30.2 mmol, 1.0 eq) was dissolved in DCM (50 mL) and added dropwise to the reaction, followed by the addition of triethylamine (6.0 mL, 43.8 mmol, 1.45 eq). The reaction material was allowed to stir at RT for 2 hr, before being concentrated under reduced pressure. The resultant colourless solid was extracted with EtOAc (3 × 50 mL). The EtOAc was washed with water (2 × 50 mL), dried over MgSO₄ and concentrated under reduced pressure to produce FP as a yellow oil (6.79 g, 98 % yield).

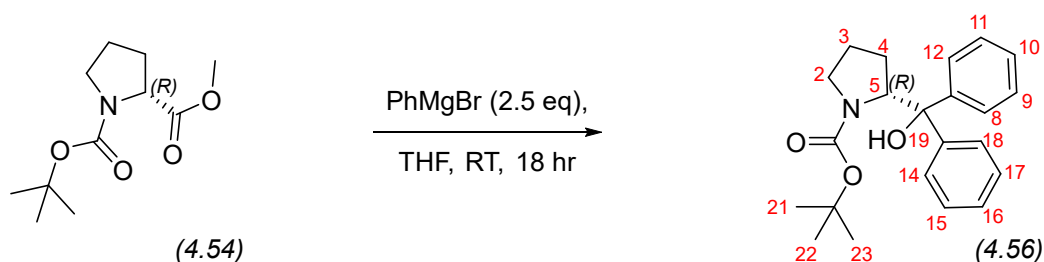
*Spectroscopic data is in line with literature.*¹⁰⁷

See A-C4-12 to A-C4-13

¹H NMR (500 MHz, Chloroform-*d*, rotamers approximately 2:3) δ 4.32 (dd, *J* = 8.8, 3.3 Hz, 0.4H, C(4)-H), 4.22 (dd, *J* = 8.5, 4.1 Hz, 0.6H, C(4)-H), 3.72 (s, 1.2H, (C9)-H₃), 3.72 (s, 1.8H, C(9)-H₃), 3.61 – 3.29 (m, 2H, C(1)-H₂), 2.33 – 2.12 (m, 1H, C(5)-H), 2.01 – 1.77 (m, 3H, C(5)-H and C(2)-H₂), 1.46 (s, 3.6H, C(14-15)-H₃), 1.41 (s, 5.4H, C(14-15)-H₃).

¹³C NMR (126 MHz, Chloroform-*d*, rotamers approximately 2:3) δ 173.9, 173.6, 154.5, 153.9, 80.0, 79.9, 59.2, 58.8, 52.2, 52.0, 46.6, 46.4, 31.0, 30.0, 28.5, 28.4, 24.4, 23.8.

(R)-(Diphenyl hydroxymethyl)-N-Boc-pyrrolidine (4.56)



4.54 (0.50 g, 2.18 mmol, 1.0 eq) was dissolved in anhydrous, degassed THF (5 mL). PhMgBr (1.82 mL, 5.45 mmol, 3.0 M in THF, 2.5 eq) was added to a dry flask under argon and diluted with THF (5 mL), to which was added the solution of **4.54** dropwise over 5 min. The reaction material was allowed to stir for 18 hr. The flask was cooled to -78 °C and water was added dropwise to the reaction material. The resultant slurry was slowly warmed to RT and the solid removed by filtration. The solid was washed with Et₂O (3 × 50 mL), and the filtrate was washed with brine solution (2 × 25 mL). The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The crude product was subjected to a recrystallisation in minimal hot methanol to give pure product as a colourless crystalline solid (0.51 g, 69 % yield).

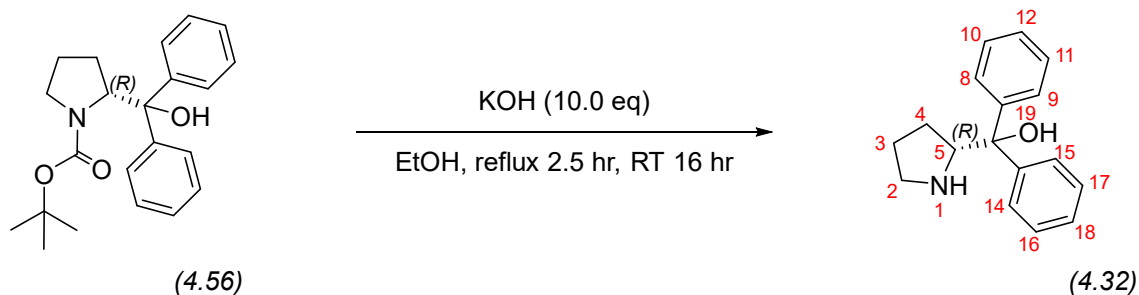
*Spectroscopic data is in line with literature.*¹⁰⁸

See A-C4-14 to A-C4-16

¹H NMR (500 MHz, Chloroform-*d*) δ 7.45 – 7.35 (m, 4H, C(8,12,14,18)-H), 7.35 – 7.13 (m, 6H, C(9-11, 15-17)-H), 6.45 (br-s, 1H, O(19)-H), 4.89 (dd, *J* = 9.0, 3.6 Hz, 1H, C(5)-H), 3.35 (m, 1H, C(2)-H_a), 2.86 (m, 1H, C(2)-H_b), 2.09 (m, 1H, C(3)-H_a), 1.96 – 1.76 (m, 1H, C(3)-H_b), 1.49 – 1.45 (m, 1H, C(4)-H_a), 1.44 (s, 9H, C(21-23)-H₃), 0.77 (m, 1H, C(4)-H_b).

¹³C NMR (126 MHz, Chloroform-*d*) δ 146.6, 143.9, 128.4, 128.0, 127.8, 127.5, 127.2, 127.2, 81.8, 80.8, 65.8, 48.0, 29.9, 28.5, 23.0

(R)-Diphenyl Prolinol (4.32)



4.56 (0.45 g, 1.27 mmol, 1.0 eq) and KOH (0.71 g, 12.7 mmol, 10.0 eq) was suspended in ethanol (6 mL). The reaction was heated to reflux and stirred for 2.5 hr. The reaction material was allowed to cool to RT and stirred for a further 16 hr. Solvent was removed under reduced pressure and residue was taken up in EtOAc (25 mL) and water (25 mL). The phases were separated, aqueous phase extracted with EtOAc (2×25 mL), and the organic phases were combined. The organic phase was washed with water (2×25 mL), dried over Na_2SO_4 and concentrated under reduced pressure to produce a yellow oil which crystallised over time (0.315 g, 98 % yield).

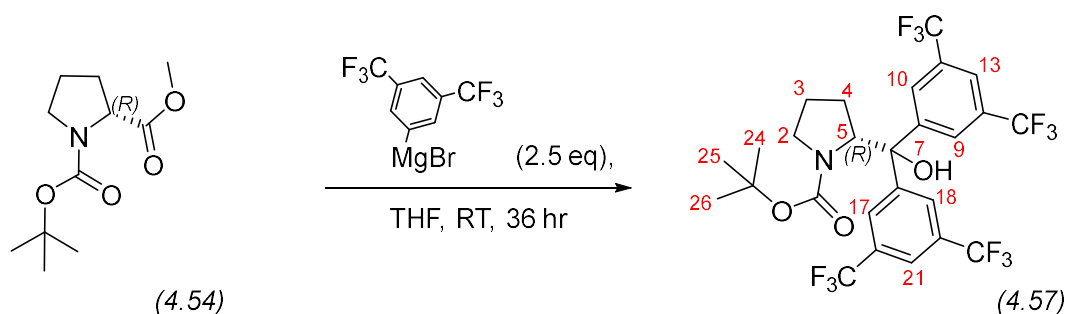
*Spectroscopic data is in line with literature.*⁹⁹

See A-C4-17 to A-C4-18

^1H NMR (400 MHz, Chloroform-*d*) δ 7.62 – 7.45 (m, 4H, C(8,9,14,15)-H), 7.37 – 7.21 (m, 4H, C(10,11,16,17)-H), 7.16 (m, 2H, C(12,18)-H), 4.25 (t, $J = 7.6$ Hz, 1H, C(5)-H), 3.07 – 2.90 (m, 2H, C(2)-H₂), 1.78-1.52 (m, 4H, C(3,4)-H₂).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 148.3, 145.6, 128.4, 128.1, 126.6, 126.5, 126.0, 125.6, 77.2, 64.6, 46.9, 26.4, 25.6.

(R)-2-[Bis(3,5-bis-trifluoromethylphenyl) hydroxymethyl]-N-Boc-pyrrolidine (4.57)



4.54 (2.30 g, 10.0 mmol, 1.0 eq) was dissolved in anhydrous, degassed THF (15 mL) and added dropwise to a solution of 3,5-di(trifluoromethyl)-phenyl magnesium bromide (50 mL, 0.5 M in THF, 25.0 mmol, 2.5 eq) in anhydrous, degassed THF (25 mL). The reaction material was allowed to stir at RT for 36 hr. The flask was cooled to -78 °C and water (50 mL) was added dropwise. The resultant slurry was slowly warmed to RT and extracted with EtOAc (3 × 100 mL). The organic phases were combined and washed with brine (2 × 100 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by DCVC, eluting sequentially with hexanes, DCM and EtOAc to provide product as a yellow-brown oil (4.37 g, 70 % yield).

*Spectroscopic data is in line with literature.*¹⁰⁹

See A-C4-19 to A-C4-21

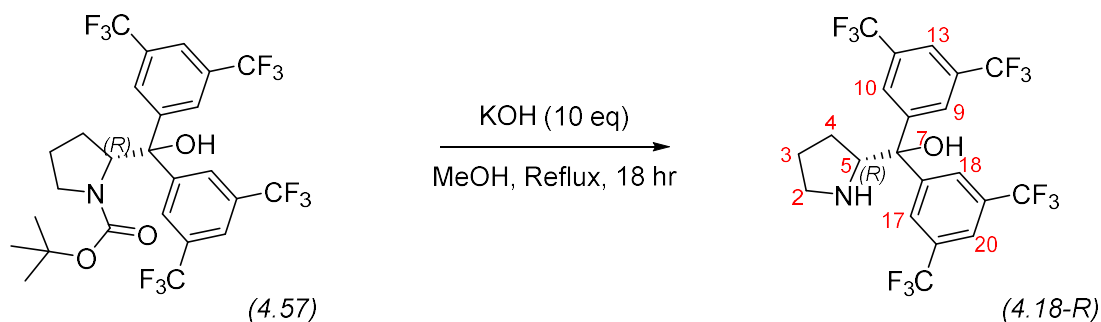
¹H NMR (400 MHz, Chloroform-*d*) δ 7.89 (ap. s, 1H, C(13 or 21)-H), 7.87 (ap. s, 2H, C(17,18 or 9,10)-H), 7.86 (ap. s, 1H, C(13 or 21)-H), 7.81 (ap. s, 2H, C(17,18 or 9,10)-H), 4.82 (dd, *J* = 8.9, 6.0 Hz, 1H, C(5)-H), 3.46 (ddd, *J* = 11.2, 8.2, 6.0 Hz, 1H, C(4)-H_a), 2.84 (dt, *J* = 11.2, 7.2 Hz, C(3)-H_a), 2.13-2.05 (m, 1H, C(4)-H_b), 1.77 (dq, *J* = 13.2, 6.6 Hz, 1H, C(2)-H_a), 1.62 (dtt, *J* = 13.2, 8.2, 6.6 Hz, 1H, C(3)-H_b), 1.40 (s, 9H, C(24-26)-H₃), 1.02 (tt, *J* = 13.2, 7.2 Hz, C(2)-H_b)

¹³C NMR (126 MHz, Chloroform-*d*) δ 147.2, 145.3, 132.0, 131.8, 131.5, 131.3, 128.2, 127.8, 124.5, 124.4, 122.1, 121.9, 82.2, 81.0, 66.7, 48.3, 30.5, 28.3, 28.2, 23.3.

¹⁹F NMR (470 MHz, Chloroform-*d*) δ -62.84, -62.87

(R)-Hayashi-Jørgensen catalyst (4.18-R)

(R)-2-[Bis(3,5-bis-trifluoromethylphenyl) hydroxymethyl] pyrrolidine



KOH (3.88 g, 69.4 mmol, 10 eq) was dissolved in MeOH (60 mL). **4.57** (4.3 g, 6.94 mmol, 1.0 eq) was charged, and the reaction was stirred at RT for 1 hr, before being heated to reflux for 18 hr. Upon completion of reaction, the flask was cooled to RT and the solid precipitate removed by filtration. The solvent was removed under reduced pressure and resultant oil taken up in EtOAc (200 mL). The organic solution was washed with water (2×100 mL) and brine (50 mL), then dried over MgSO_4 . The solvent was removed under reduced pressure and the resultant oil was purified *via* flash column chromatography (5-10 % EtOAc in hexanes). Neutralised chloroform was added to the product, causing precipitation of an impurity, which was removed by filtration. The filtrate was concentrated to give product as a pale yellow solid (1.94 g, 54 % yield).

*Spectroscopic data is in line with literature.*¹⁰⁰

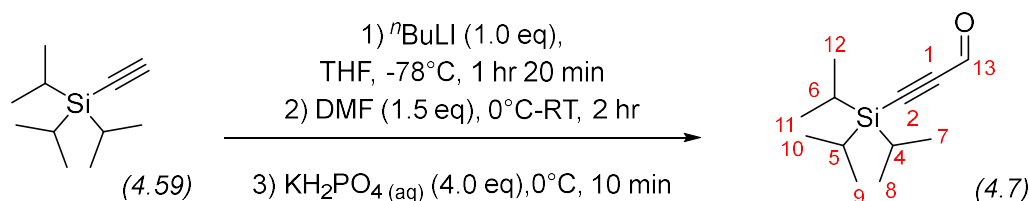
See A-C4-22 to A-C4-26

^1H NMR (500 MHz, Methanol- d_4) δ 8.24 (s, 2H), 8.16 (s, 2H), 7.86 (app. s, 2H), 4.49 (t, J = 7.7 Hz, 1H), 3.03 – 2.85 (m, 2H), 1.82 – 1.71 (m, 2H), 1.67 (m, 1H), 1.55 (m, 1H).

^{13}C NMR (126 MHz, Methanol- d_4) δ 150.6, 149.8, 133.3-132.4 (either 2 quartets or a dq), 129.3, 128.0, 127.6, 125.9, 125.9, 123.8, 123.7, 122.1, 79.0, 65.5, 48.4, 28.0, 27.3.

^{19}F NMR (470 MHz, Methanol- d_4) δ -64.29 (s, 6F), -64.36 (s, 6F).

3-(Triisopropylsilyl)-prop-2-ynal (4.7)



4.59 (9.01 g, 49.0 mmol, 1.0 eq) was dissolved in anhydrous THF (27 mL) and cooled to -78°C . $n\text{BuLi}$ (19.6 mL, 2.5 M in hexanes, 49.0 mmol, 1.0 eq) was charged to the solution dropwise over 5 mins. The reaction material was allowed to stir for 1 hr and 20 min, before being warmed to 0°C . Anhydrous DMF (4.55 mL, 58.8 mmol, 1.2 eq) was added, the reaction was warmed to RT and stirred for a further 2 hr. KH_2PO_4 (26.7 g, 196 mmol, 4.0 eq) was dissolved in water (250 mL) and cooled to 0°C . The reaction material was poured over the aqueous solution and stirred for 10 min. The solution was extracted with Et_2O (300 mL, $100\text{ mL} \times 2$). Organic phases were combined, washed with 1/3 saturated brine solution ($100\text{ mL} \times 2$), dried over MgSO_4 and concentrated to give crude product as a pale-yellow liquid (12.25 g at approx. 82 % weight solution in THF and Et_2O , Quant. yield). Product was not further purified and carried forward as a solution.

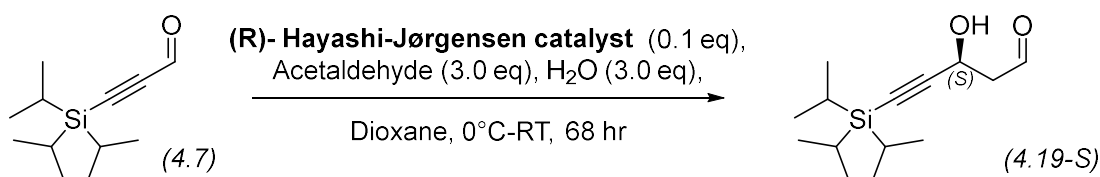
*Spectroscopic data is in line with literature.*¹¹⁰

See A-C4-27 to A-C4-28

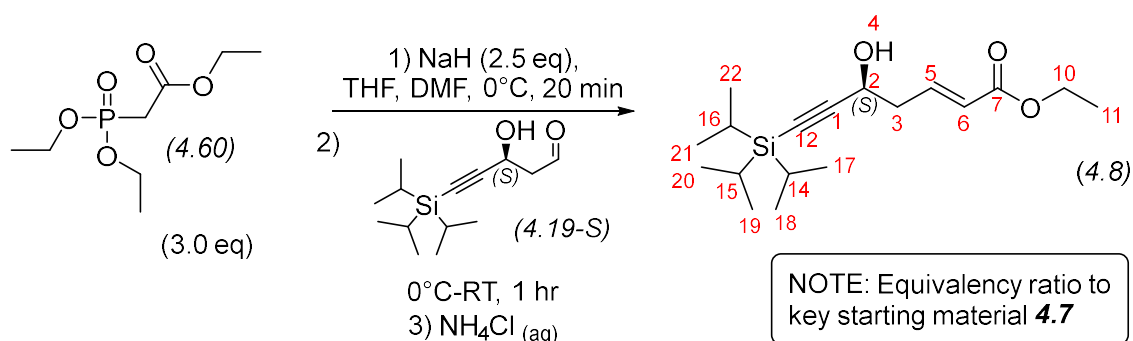
^1H NMR (500 MHz, Chloroform- d_3) δ 9.20 (s, 1H, C(13)-H), 1.11 (m, 21H, C(4-6)-H and C(7-13)-H₃).

^{13}C NMR (126 MHz, Chloroform- d_3) δ 176.7, 104.6, 100.9, 18.6, 11.1

Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8)



4.7 (6.01 g, 28.55 mmol, 1.0 eq), was dissolved in anhydrous dioxane (28.5 mL) and H₂O (1.54 mL, 85.66 mmol, 3.0 eq). (*R*)-Hayashi-Jørgensen catalyst (1.50 g, 2.86 mmol, 10 mol%) was charged and the reaction was cooled to 5 °C. Acetaldehyde (4.8 mL, 85.66 mmol, 3.0 eq) was charged and the reaction was warmed to RT and allowed to stir for 68 hr. Consumption of SM was confirmed by ¹H NMR.



NaH (2.85 g, 71.38 mmol, 60 wt% in mineral oil, 2.5 eq) was washed with anhydrous, pentane (15 mL $\times$ 2), anhydrous, degassed THF (15 mL $\times$ 2), and suspended in anhydrous, degassed THF (47 mL) at 0 °C. Triethyl phosphonoacetate (**4.60**, 17 mL, 85.66 mmol, 3.0 eq) was dissolved in anhydrous DMF (47 mL) and added dropwise to the NaH suspension. The reaction material was allowed to stir at 0 °C until the colourless precipitate dissolved. The solution of prepared aldehyde (**4.19-S**) was added dropwise to the ylide solution. The reaction material was warmed to RT and stirred for 1 hr. NH₄Cl_(aq) (50 mL, saturated sol.) was added dropwise, alongside water (50 mL). The reaction material was extracted with EtOAc (100 mL $\times$ 2). Organic phases were combined, washed with 1/3 saturated brine solution (100 mL $\times$ 2), dried over MgSO₄ and concentrated. Crude product was purified by fractional column chromatography (10-20 % EtOAc in Hexanes) followed by a slower fractional column chromatography of product containing fractions (0-10 % EtOAc in hexanes to yield pure product as a yellow oil (5.4 g, 58 % yield).

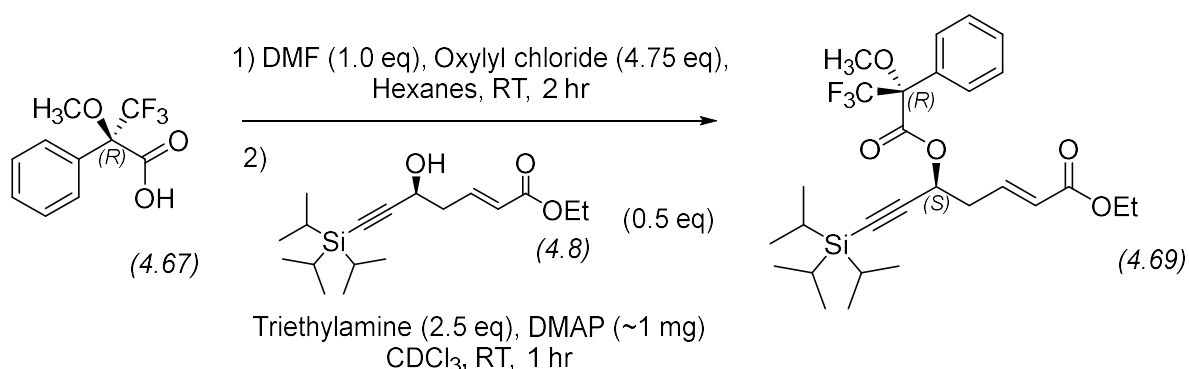
*Spectroscopic data is in line with literature.*⁸⁶

See A-C4-29 to A-C4-31

^1H NMR (500 MHz, Chloroform- d_3) δ 7.01 (dt, $J = 15.7, 7.3$ Hz, 1H, C(5)-H), 5.95 (dt, $J = 15.7, 1.5$ Hz, 1H, C(6)-H), 4.54 (apparent q, $J = 6.0$ Hz, 1H, C(2)-H), 4.18 (q, $J = 7.2$ Hz, 2H, C(10)-H₂), 2.63 (dt, $J = 6.0, 1.5$ Hz, 1H, C(3)-H_a), 2.61 (dt, $J = 6.0, 1.5$ Hz, 1H, C(3)-H_b), 1.90 (d, $J = 5.5$ Hz, 1H, O(4)-H), 1.28 (t, $J = 7.2$ Hz, 3H, C(11)-H₃), 1.12 – 1.02 (m, 21H, C(14-16)-H and C(17-22)-H₃).

^{13}C NMR (126 MHz, Chloroform- d_3) δ 166.2, 143.0, 125.0, 107.2, 87.4, 61.7, 60.4, 40.6, 18.7, 14.4, 11.2.

Ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate Mosher's ester (4.69)



(*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetic acid (4.67, 14.4 mg, 0.0616 mmol, 1.0 eq) was added to a vial with hexanes (0.2 mL) and DMF (4.77 μL, 0.0616 mmol, 1.0 eq). Freshly distilled oxalyl chloride (25.53 μL, 0.293 mmol, 4.75 eq) was added and the mixture stirred for 2 hr. The suspension was filtered through cotton wool, washed through with hexanes (0.5 mL × 2) and the filtrate concentrated under reduced pressure. To the resultant oil was added a solution of ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8, 10 mg, 0.0308 mmol, 0.5 eq) in chloroform-*d*₃ (0.5 mL), triethylamine (13 μL, 0.154 mmol, 2.5 eq) and one small crystal of DMAP. The reaction was sealed and stirred for 1 hr. The reaction was transferred to an NMR tube and analysed by ¹⁹F NMR to provide a dr of 95:5.

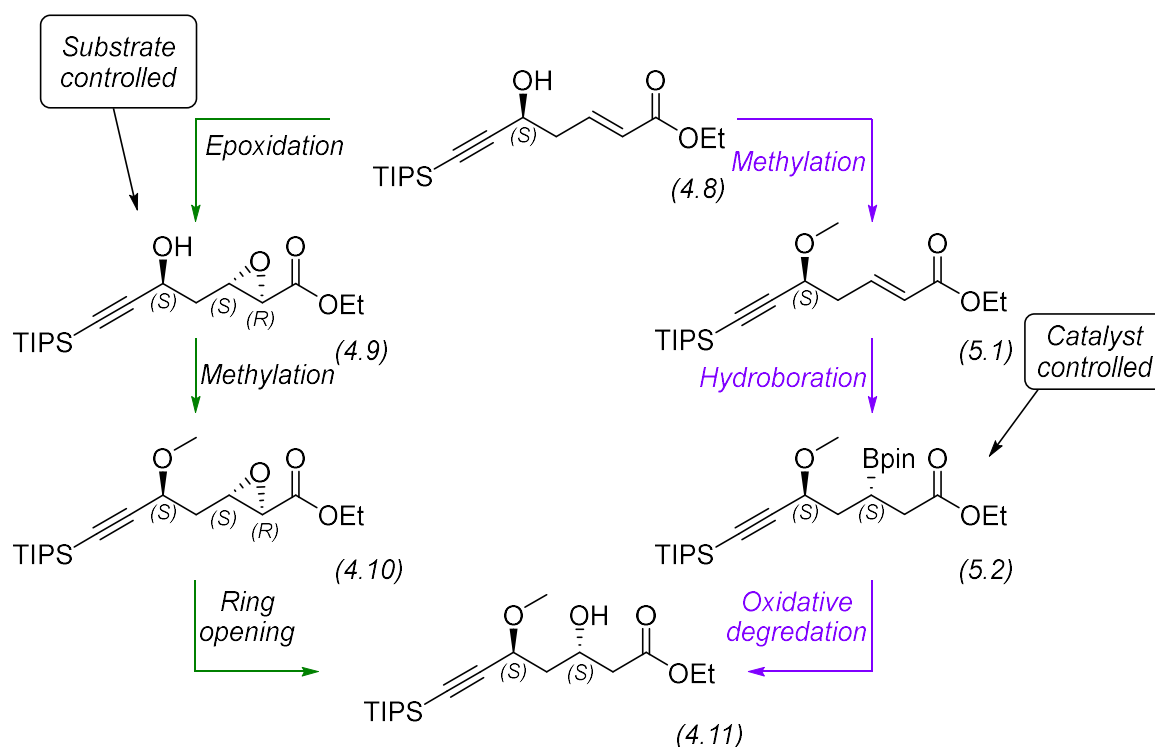
See A-C4-32 to A-C4-33

¹⁹F NMR (470 MHz, Chloroform-*d*₃) δ [*S,S*] -71.82, δ [*R,S*] -71.65

Chapter 5: Hydroboration of α,β -unsaturated esters as a method of forming stereogenic carbon-heteroatom bonds

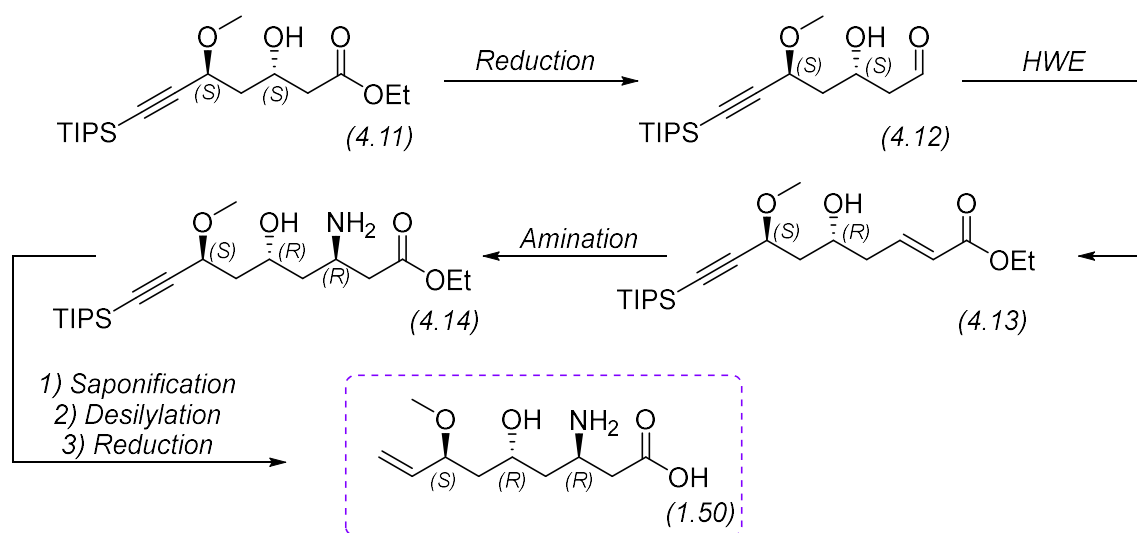
5.1. Aims and Objectives

Hayashi *et. al* demonstrated their method for formation of 1,3-*anti*-diols through a substrate controlled stereoselective epoxidation of α,β -unsaturated esters and subsequent ring opening with a moderate *anti:syn* ratio.⁸⁶ When this method was utilised in chapter 4, it was adapted due to a lack of appropriate equipment to maintain the required reaction temperature and showed no epoxide formation under the reaction conditions (**Scheme 70**). Since the method was reliant on pre-installed stereogenic centre to impart the diastereomeric control which didn't show particularly high *anti* selectivity in the published reports, it was decided that pursuing a new catalyst controlled stereoselective approach would provide better final outcomes than optimising this strategy. Using the same retrosynthetic analysis as detailed in chapter 4, the desired diol (**4.11**) can be synthesised from compound **5.1** via a catalyst controlled stereoselective hydroboration on the α,β -unsaturated ester. This modified approach requires methylation of the alcohol, followed by a catalyst controlled stereoselective hydroboration of the alkene. Subsequent stereo-retentive oxidative degradation accesses the desired 1,3-methoxy alcohol compound.



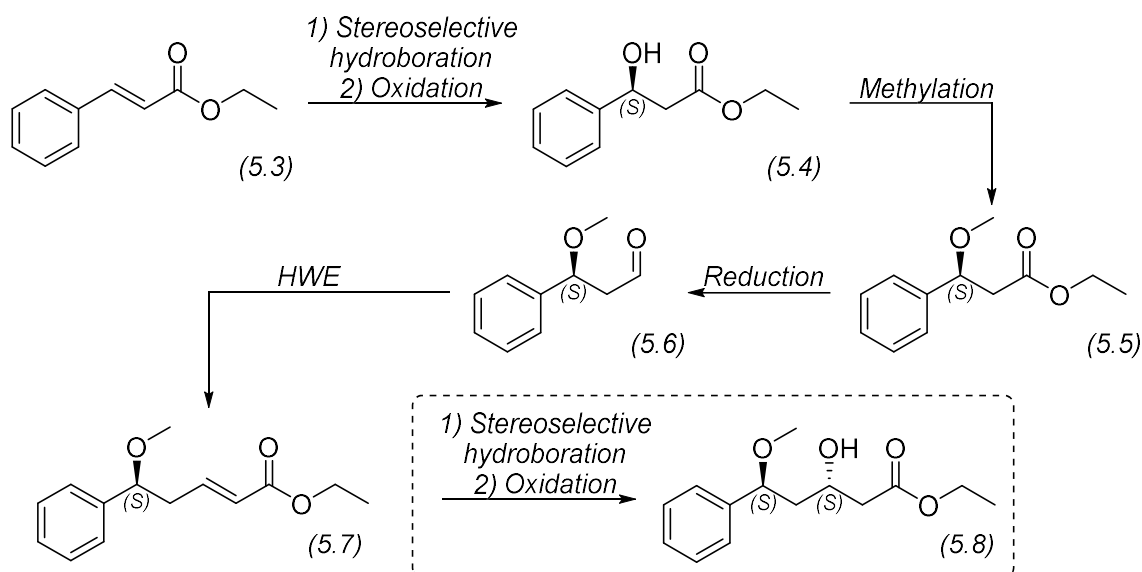
Scheme 70 Synthetic methods towards ethyl 5(S)-methoxy-3(S)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (**4.11**) via substrate controlled stereoselective epoxidation (green) and catalyst controlled, stereoselective hydroboration (purple).

The remaining synthetic pathway follows the planned route in chapter 4, with reduction of the ester to an aldehyde (4.12) and a HWE reaction (Scheme 46). This provides a new α,β -unsaturated ester (4.13), where a stereo controlled amination will be performed to yield a protected version of the desired target (4.14). Saponification of the ester and desilylation and reduction will provide the final target of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50).



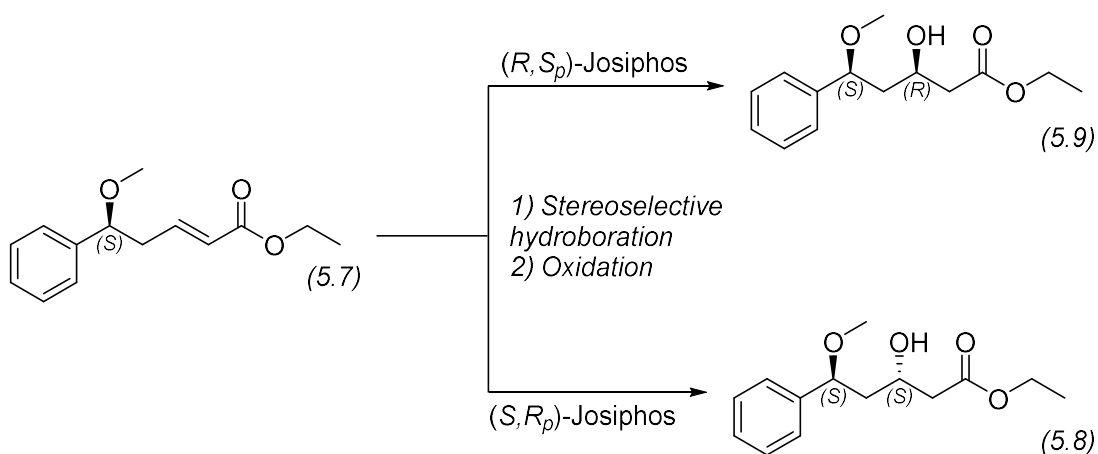
Scheme 71 Synthetic steps towards the synthesis of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50) from ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (4.11).

A model system was designed which allows for testing and optimisation of reaction conditions for the above approach. Ethyl cinnamate (5.3) provides a cheap model system for testing of the pathway towards ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (Scheme 72). First a stereoselective hydroboration and oxidation on ethyl cinnamate provides the chiral alcohol (5.4) which is methylated and reduced to an aldehyde (5.6). This aldehyde is then subjected to a HWE reaction with triethyl phosphonoacetate to produce the model substrate (5.7). Ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7) will then be used to optimise the conditions for stereoselective hydroboration and oxidation.



Scheme 72 Synthetic steps towards a model compound (5.7), which will be used to optimise the reaction conditions for hydroboration of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate.

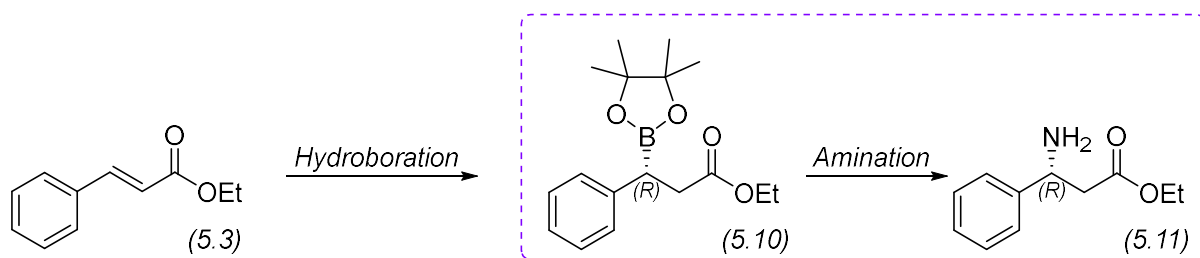
Optimisation of the hydroboration conditions requires the use of both enantiomers of the Josiphos ligated catalysts to synthesise the two diastereomers of ethyl (*S*)-5-methoxy-5-phenyl-3(*)-hydroxy-pentanoate (**Scheme 73**). It was expected that optimised conditions would yield ethyl (*S*)-5-methoxy-5-phenyl-3(*S*)-hydroxy-pentanoate (5.8) as the major isomer, when (*S,R_p*)-Josiphos ligated copper was used



Scheme 73 Optimisation of the stereoselective hydroboration and oxidative degradation reaction sequence using a Josiphos ligated copper catalyst.

Ethyl cinnamate (5.3) will also be used as a model system to trial conditions for a hydroboration/amination cascade (**Scheme 74**). This will utilise the un-optimised standard

borylation conditions and focus on finding specific conditions which will convert the boronic acid derivative into an amide in a stereo-retentive manor, with minimal side-reactions.

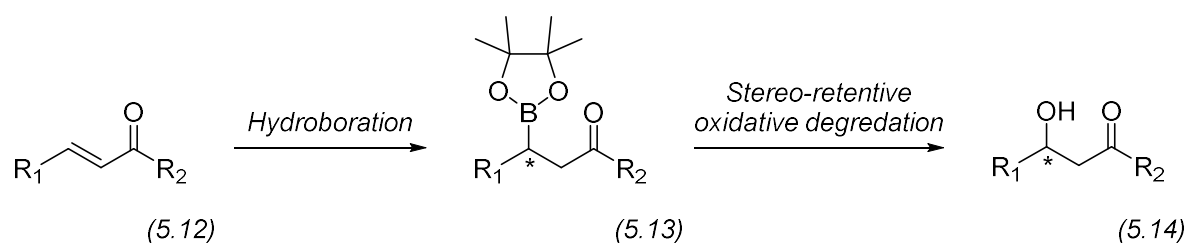


Scheme 74 Model system for trialling and optimising conditions for the stereo-retentive amination of boronic esters, utilising the hydroboration product of ethyl cinnamate as the substrate.

5.2. Introduction

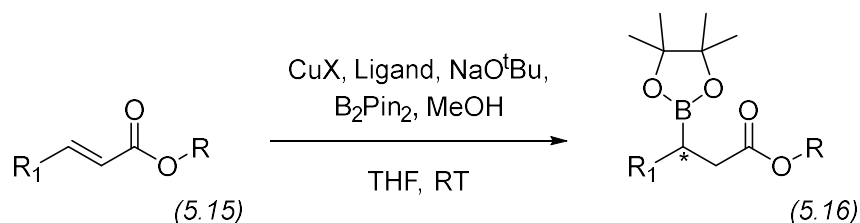
5.2.1 Hydroboration of alkenes

Transition metal catalysed hydroboration of alkenes is a well-studied approach to creating carbon-boron bonds. Platinum, rhodium, copper and nickel based catalysts have been all been extensively reported as efficient for the hydroboration of α,β -unsaturated carbonyl compounds using B_2Pin_2 .^{111–114} Furnishing the catalyst with bulky chiral ligands has progressed these approaches into the area of asymmetric catalysis and is generally reported to have high levels of catalytic control.^{115–118} This stereoselective hydroboration is particularly useful as the C-B bond can be converted into a C-O through a stereo-retentive oxidative degradation, meaning chiral C-O bonds can be formed from α,β -unsaturated carbonyl compounds in two steps (**Scheme 75**).



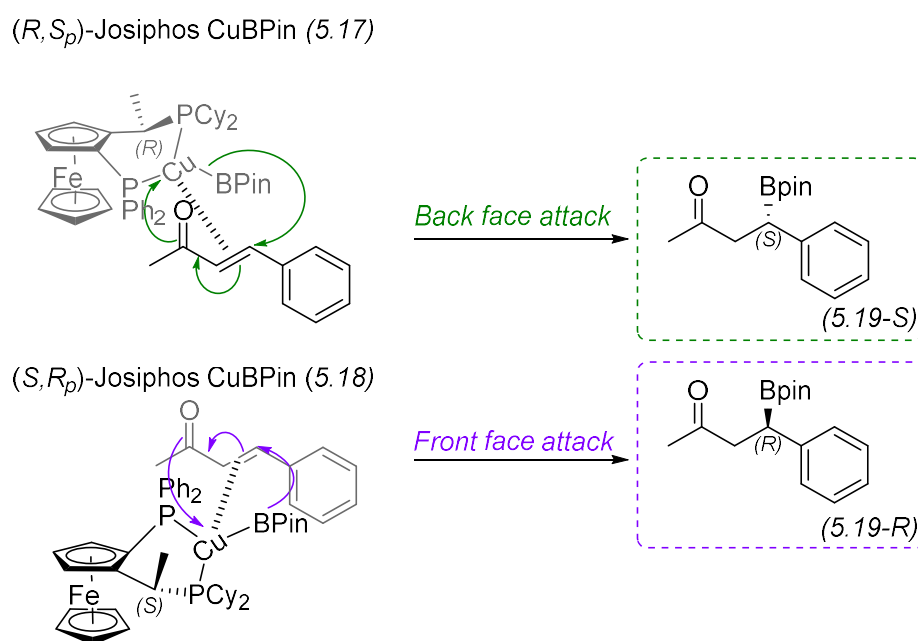
Scheme 75 Two-step synthesis of chiral carbon-oxygen bonds through hydroboration of an α,β -unsaturated carbonyl and subsequent stereo-retentive oxidative degradation.

Over the past 20 years, chemists have been constantly developing new and improved ligands for asymmetric catalysis, many of them working well for this hydroboration chemistry. Many groups, including the Richards group have previously explored different catalysts and ligands for similar compounds containing a α,β -unsaturated ester and found that Josiphos and copper bromide provides reliably high conversion and enantiomeric excess (**Scheme 76**).¹¹⁷



Scheme 76 General conditions for hydroboration of an α,β -unsaturated ester, using a chiral diphosphine ligand and a copper (I) halide to direct stereochemistry.

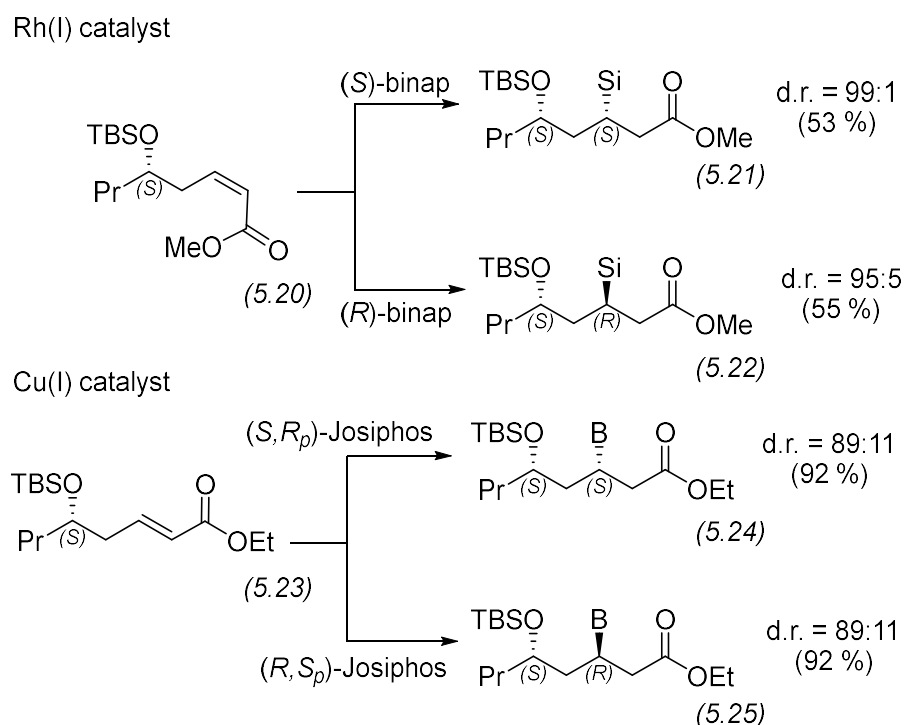
Since the mechanism is believed to occur through co-ordination of the copper catalyst to the alkene, the chirality of the product can be selected by choosing the correct enantiomer of the Josiphos ligand (**Scheme 77**). The steric bulk of the chiral ligand will either direct the copper catalyst to co-ordinate at the front face or back face. When (*R,S_p*)-Josiphos (5.17) was used to direct the hydroboration on *trans*-4-phenyl-3-buten-2-one, the (*S*)-borylated product (5.19-*S*) was formed in majority with moderate e.e. of 71 %.¹¹⁷ This suggests that the ligand directs the catalyst to co-ordinate at the back face, and therefore the Bpin performs a back faced attack of the alkene. Comparatively, the (*S,R_p*)-Josiphos (5.18) catalyst would be expected to co-ordinate on the front face, as it produced the (*R*)-borylated product (5.19-*R*) in majority.



Scheme 77 Ligand directed co-ordination complexes that are believed to determine the chirality of the hydroboration.

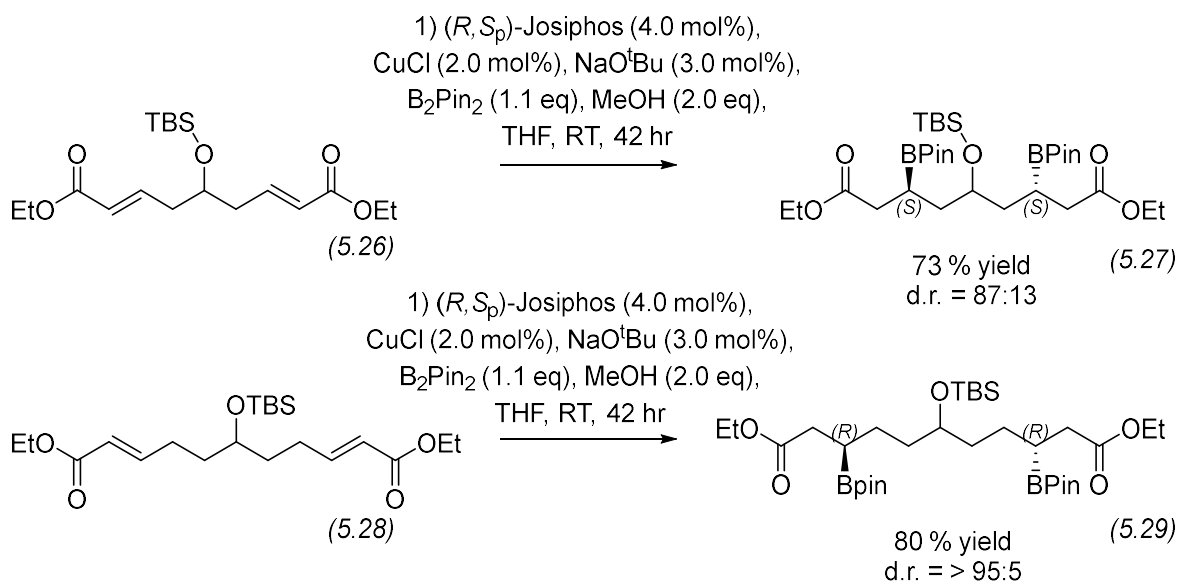
Much of the literature around this hydroboration approach focuses on synthesising compounds with a singular C-O unit, however Hartmann and Oestreich presented a method for desymmetrisation of pro chiral carbinols using hydroboration of α,β -unsaturated ester.¹¹⁹ Within the report, they first outline a comparison of a rhodium catalysed 1,4-addition of silicon with copper catalysed hydroboration to synthesise diastereomeric compounds (**Scheme 78**). The rhodium route showed high selectivity, with the (*R*)-BINP providing 95:5 d.r. and the (*S*)-BINAP providing 99: d.r., indicating a largely catalyst controlled reaction. However, some minor influence of the existing stereogenic centre on the reaction outcome was seen and low yields were achieved. Comparatively, the copper approach showed higher

yields but a less optimal 89:11 dr. There were no sign of matched/mismatched selectivity indicating to the authors that while catalyst control is high, there is imperfect asymmetric control. This is caused by reduced facial selectivity of the catalyst.

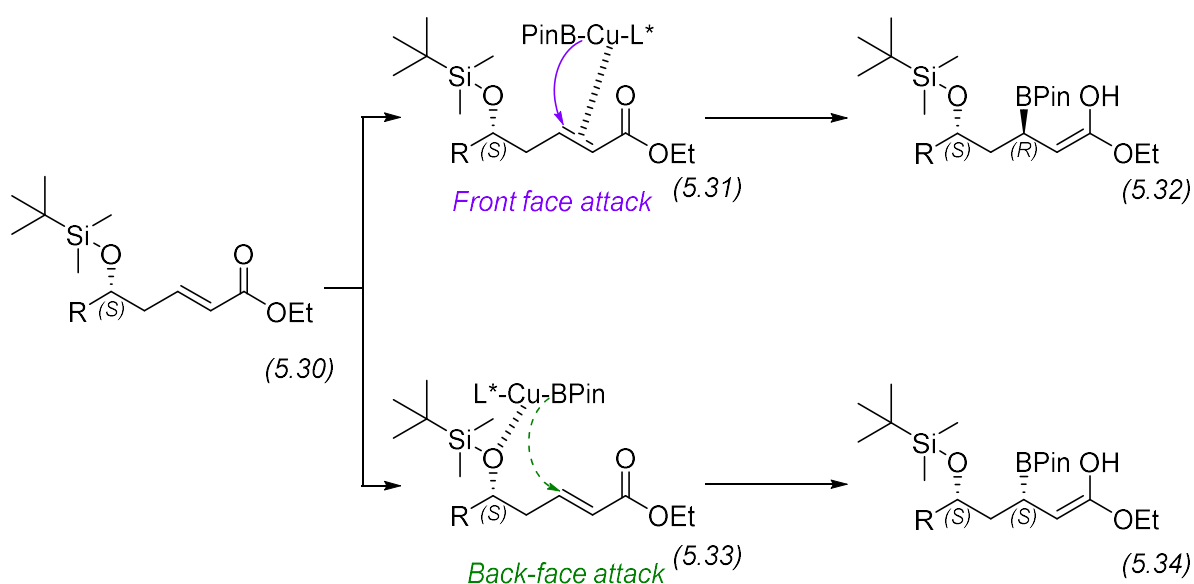


Scheme 78 A comparative study of a rhodium catalysed 1,4-silicon addition into an α,β -unsaturated ester with adjacent chiral carbinol group and copper catalysed hydroboration of a similar an α,β -unsaturated ester.¹¹⁹

Upon applying the methods towards two-directional desymmetrisation, they noted that in the copper catalysed approach the d.r. is improved upon when the carbon count between the alkene and protected alcohol is increased from 1 to 2 (**Scheme 79**).¹¹⁹ This might suggest that the reduced facial selectivity is caused by catalyst co-ordination to the central alcohol. If the copper catalyst were to co-ordinate to the protected alcohol this would lead to the opposing facial selectivity (**Scheme 80**) and formation of the undesired diastereomer. Co-ordination at the alcohol in the extended chain version (**Scheme 79**, 5.28) would have a reduced effect due to positioning the Bpin group further from the alkene and reducing the likelihood of hydroboration on this face.



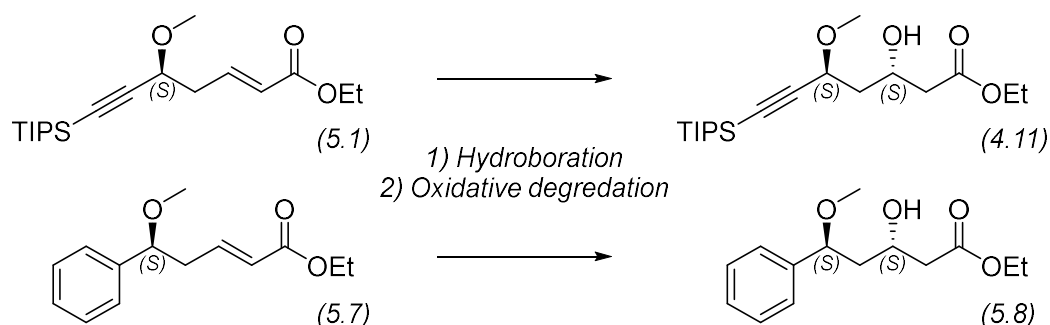
Scheme 79 Two-directional desymmetrisation using the (*S,R*_p)-Josiphos ligand, reported by Oestreich and Hartmann, showing an increased selectivity on the extended chain substrate.¹¹⁹



Scheme 80 Possible copper co-ordination sites, which lead to the imperfect asymmetric induction of the hydroboration approach.

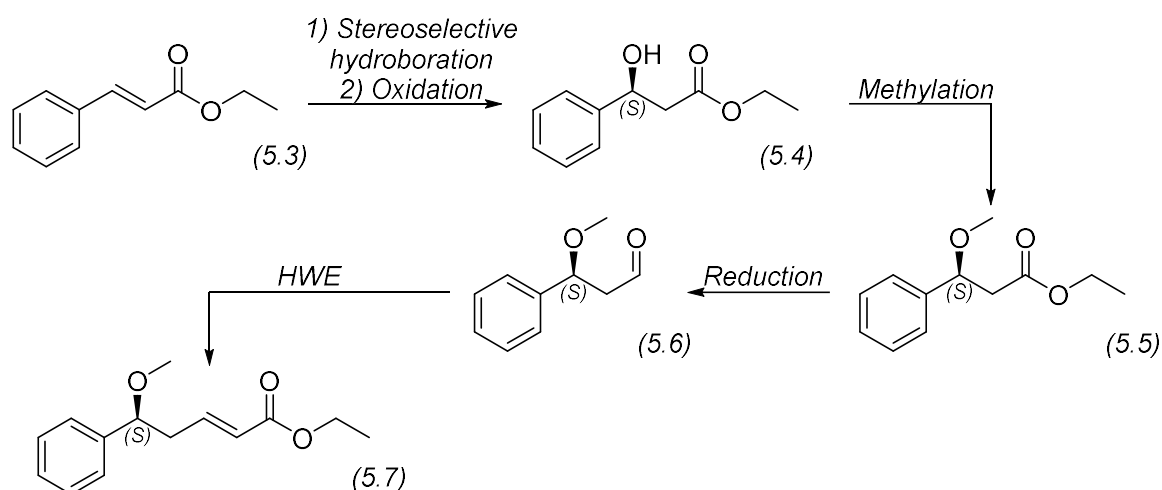
These results by Oestreich and Hartmann suggest that some substrate specific optimisation of the protocol may be required for ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (5.1). Since the starting material for this reaction is costly to make, a model substrate was designed for the initial stages of optimisation (**Scheme 81**). Ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7) was chosen as it contains the same α,β -unsaturated ester as well

as the 1,3-methoxy alkene relationship. Using a phenyl group in placement of the silyl protected alkyne, is not an accurate mimic, but does provide a bulky chromophore that will make analysis of the optimisation reactions easier by HPLC.



Scheme 81 Model reaction which will be used to optimise the reaction conditions for hydroboration and oxidative degradation of ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (5.7).

Accessing ethyl (S,E)-5-methoxy-5-phenyl-pent-2-enoate (5.7) can be achieved from ethyl cinnamate (5.3) through 4 steps, beginning with the hydroboration, oxidative degradation reaction (**Scheme 82**). The β -hydroxy ester (5.4) is methylated, and the ester reduced to give an aldehyde (5.6). Finally, a Horner-Wadsworth-Emmons reaction will provide ethyl (S,E)-5-methoxy-5-phenyl-pent-2-enoate (5.7). This process follows the same approach as the chain extension method planned for later stages of 3(R)-amino-5(R)-hydroxy-7(S)-methoxy non-8-enoic acid synthesis. Therefore, this provides an ideal opportunity to test out and optimise conditions that will be used in the later stages of synthesis.



Scheme 82 Synthetic steps towards synthesis of the model substrate, ethyl (S,E)-5-methoxy-5-phenyl-pent-2-enoate (5.7).

Utilising this model system, it may also be possible to confirm the stereochemical configuration that was reported during structural assignment of the nemamides.⁴² The absolute stereochemical configuration was determined by performing CD analysis and comparing the results with computed data for two triene model compounds (**Fig. 50**). Computational experiments for the (*R,S*)-model triene (**5.35**) showed a positive difference in molar absorptivity ($\Delta\epsilon$) at around 245 nm and a negative $\Delta\epsilon$ at around 275 nm. The (*S,R*)-model triene (**5.36**) had an opposing absorption peak at 245 nm and emission at 275 nm. These were graphed against a measurement of wild type nemamide A, which showed a positive $\Delta\epsilon$ at around 245 nm and a negative $\Delta\epsilon$ in the range of 260-280 nm. Using this computational analysis, it was proposed that the absolute stereochemistry of 2-deoxygraciline was (*S*)-methoxy-(*R*)-hydroxy-(*R*)-amino. At the point of writing, an experimental confirmation of this configuration had not been reported.

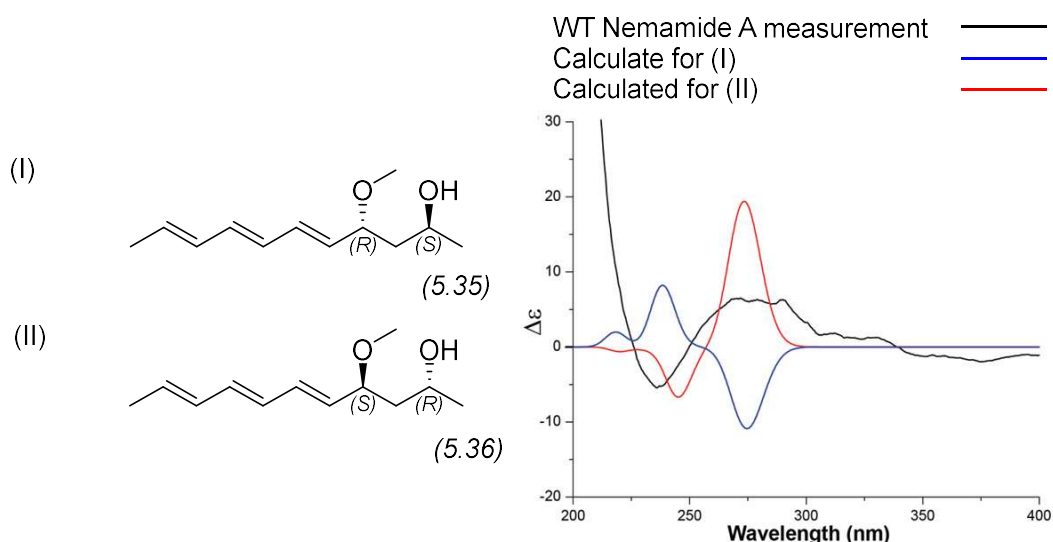
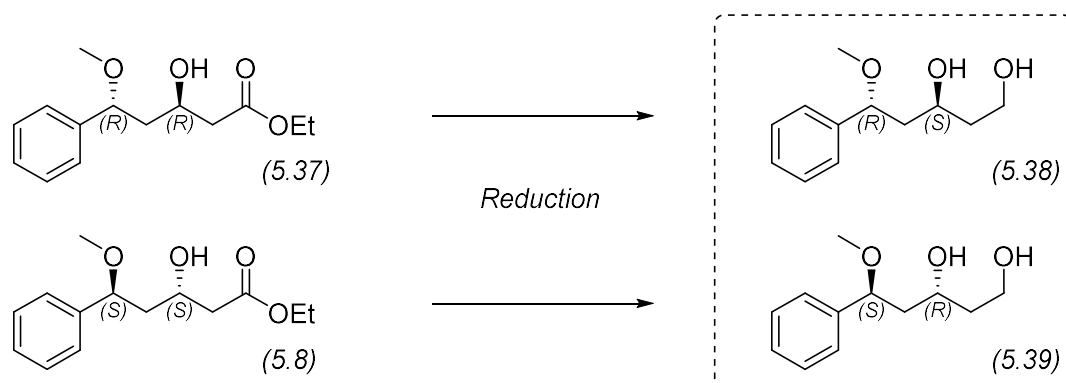


Fig. 50 Model structures used by Butcher *et. al* for computational comparison of CD spectra with the wild type data from nemamide A. Spectral graph is taken from supplementary information (SF-9).⁴²

While the reported model structures differ from the ethyl 5-methoxy-3-hydroxy-5-phenylpentanoate substrates, the λ_{max} of a triene and a phenyl group both lay around 260 nm, so they are expected to have similar effects on the systems spectra (**Scheme 83**). Butcher *et. al* theorise that the Cotton effects in the CD spectra will be mostly caused by the chirality of the methoxy group closest to the chromophore, and therefore having a varied right side should have little effect on the $\Delta\epsilon$. However, some effects on the intensity of the peak may be seen which may require reduction of the ester group to create models with terminal

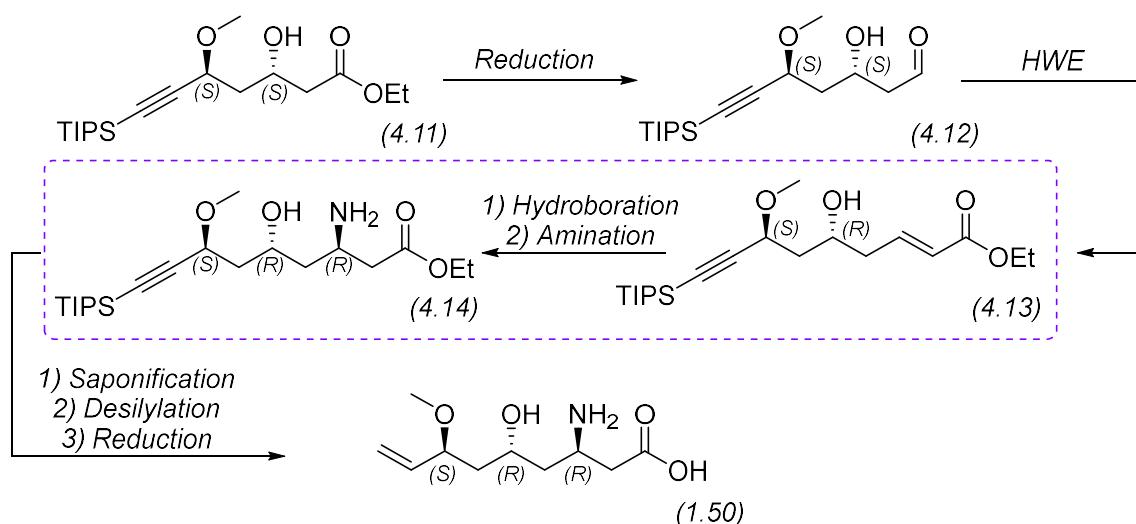
alcohol groups rather than esters. CD spectra analysis of the two synthesised models would give opposing peaks, in line with the related trienes. If the computed data is correct, then the result for ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-5-phenylpentanoate (5.8) and 5(*S*)-methoxy-3(*R*)-hydroxy-5-phenylpentanol (5.39) is expected to show a negative peak at 245 nm and a positive peak at 275 nm. This will provide some experimental confirmation of the reported absolute stereochemical configuration of the 2-deoxygraciline.



Scheme 83 Experimental models for CD studies which can be made from ester reduction of the model substrates.

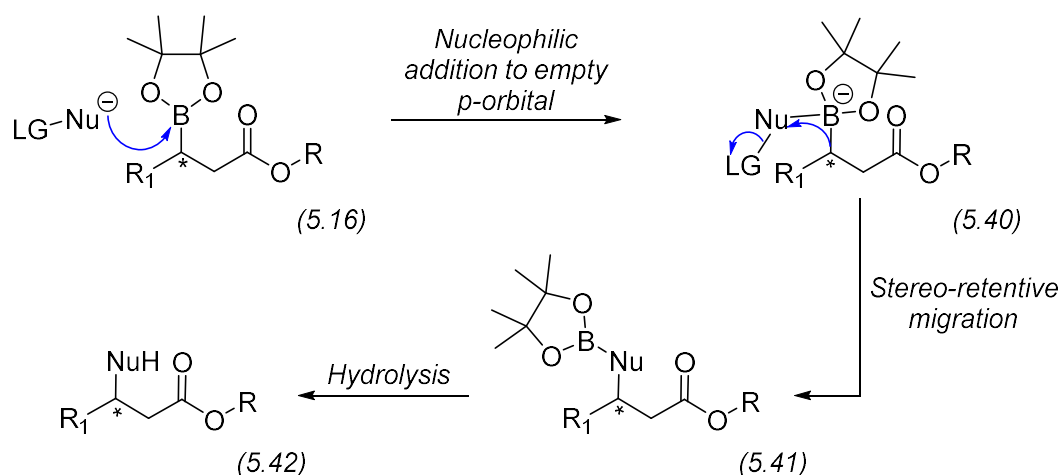
5.2.2. Stereo-selective amination of α,β -unsaturated esters

Once the hydroboration has been optimised and used to synthesise ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (**4.11**), the next steps will require chain extension *via* ester reduction and HWE reaction (**Scheme 84**). This will provide a new α,β -unsaturated ester (**4.13**), which posed the question of whether the amination could be performed using a similar hydroboration approach.



Scheme 84 Later stages planned towards the synthesis of 2-deoxygraciline, featuring a stereo-controlled amination.

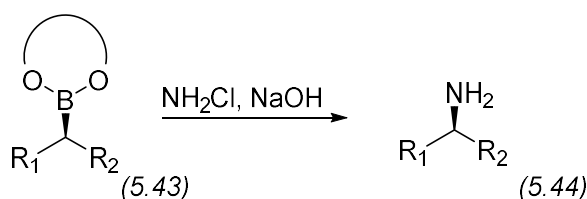
Within the previously discussed hydroboration and oxidative degradation approach, the stereochemistry is installed within the hydroboration and retained during the C-B bond degradation. This is due to the migratory mechanism by which the C-B bond breaks and the C-O bond forms (**Scheme 85**). To set up this bond migration a nucleophile is needed, which can donate into the empty *p*-orbital of the bound Bpin to form the ate complex (**5.40**). This nucleophilic compound must also contain a good leaving group to drive forward the bond migration. Amines are generally considered good nucleophiles, so may be sufficient donors to the empty *p*-orbital, and if bound to a good leaving group then this could provide a perfect opportunity for a semi-iterative hydroboration strategy towards 2-deoxygraciline.



Scheme 85 Mechanism of hydroboration and stereo-retentive migration, requiring a compound with a strong nucleophile and a good leaving group.

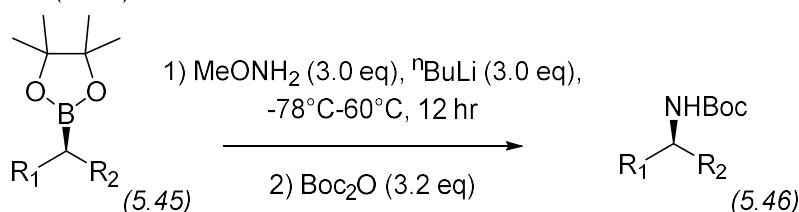
Brown *et. al* published a method in 1987 which utilised chloramine as a nucleophile for the stereo-retentive conversion of boronic acids (5.43) to amines (5.44) (**Scheme 86**).¹²⁰ While the chloride showed to be an effective leaving group for the migration to occur, moderate yields were obtained due to the nitrogen's weak association into the free orbital. This approach has been optimised since, with the aim of increasing the nucleophilicity of the amine compounds to favour the ate complex formation. Morken *et. al* succeeded in producing a more effective method by utilising $\text{MeONH}^+\text{Li}^-$ as the amine source.¹²¹ The methoxy group acts as a leaving group, while the nucleophilicity of the amine is improved by deprotonation to form a lithium salt. Adding Boc anhydride prior to the reaction work up allowed for isolation of the protected chiral amines (5.46), which could be more easily purified than the free amine counterparts. Their approach showed to have moderate to high yields and stereo-retention on a variety of aliphatic and aromatic functionalities.

Brown (1987)



40-60 % yield

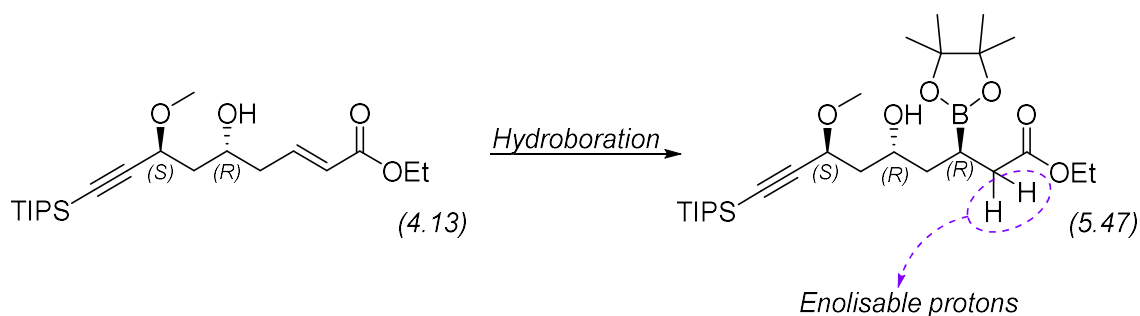
Morken (2012)



70-90 % yield

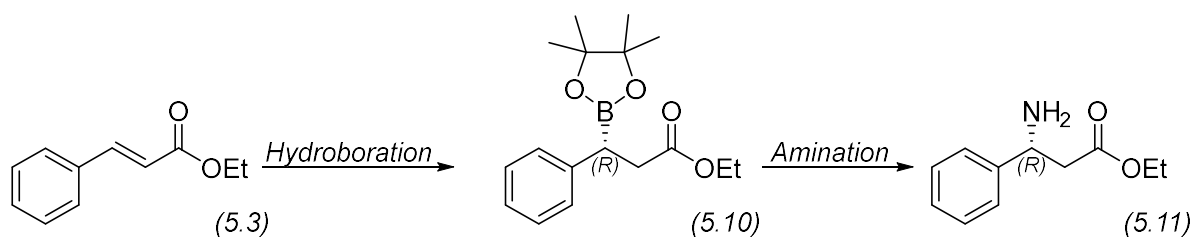
Scheme 86 Previous published routes of stereo-retentive amine formation from boronic esters by Brown and Morken.^{120,121}

A major downfall of Morken's approach is the competitive amination of butyllithium during methoxy amine deprotonation into MeONHLi salt.¹²¹ This meant that low yields were seen when using 1.0 equivalent of the two reagents. Only on use of 3.0 equivalents did they see high optimised yields. While these conditions work well for most of their reported substrate scope, it may pose a problem on ethyl 5(*S*)-methoxy-3(*R*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (**4.13**) to the enolisable proton being highly reactive to base (**Scheme 87**). It is likely that any excess of $t\text{BuLi}$ or the formed lithium salt could cause deprotonation at this site, producing unwanted side products by reacting with the ester and/or reversing the borylation.



Scheme 87 Planned hydroboration of 5(*S*)-methoxy-3(*R*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (**4.13**), giving the boronic acid (**5.47**) product with enolisable protons.

To trial this approach, it was decided that ethyl cinnamate (5.3) will again be used as a model system as it will provide initial data on the interfering reactivity of the acid α -protons. (Scheme 88).



Scheme 88 Model system for testing the hydroboration/amination cascade, utilising ethyl cinnamate as a substrate.

5.3. Results and Discussion

5.3.1. A hydroboration approach

The selectivity of the stereo-selective hydroboration is believed to be caused by copper co-ordination to the alkene, with the orientation of the co-ordination determining which face the Bpin attacks the alkene from. There are two other possible co-ordination sites within ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate which may affect the facial selectivity (**Fig. 51**, 5.48). Possible co-ordination could take place at the alkyne; however, this may be mitigated by the bulky silyl protecting group. Co-ordination to the alcohol is more likely to affect this reaction, and so the alcohol was methylated prior to hydroboration.

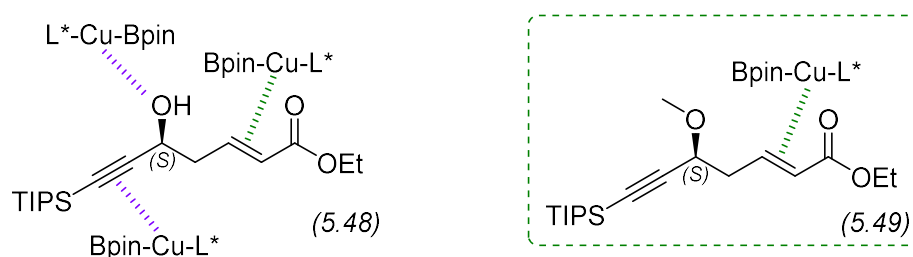
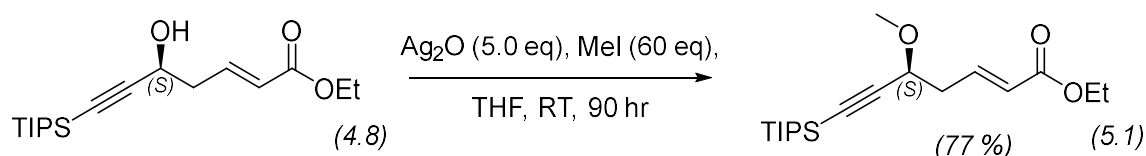


Fig. 51 Copper co-ordination to the alkene, which is believed to cause selectivity of the hydroboration, showing possible interfering co-ordination sites for ethyl (*S, E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate and the expected favourable co-ordination site of the methylated derivative.

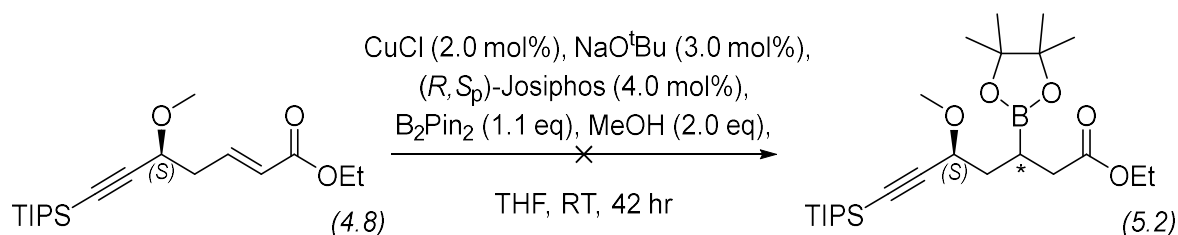
The methylation of ethyl (*S, E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8) was performed using the conditions optimised in chapter 3, providing the methylated product (5.1) in a yield of 77 % (**Scheme 89**).



Scheme 89 Conditions for methylation of ethyl (*S, E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate.

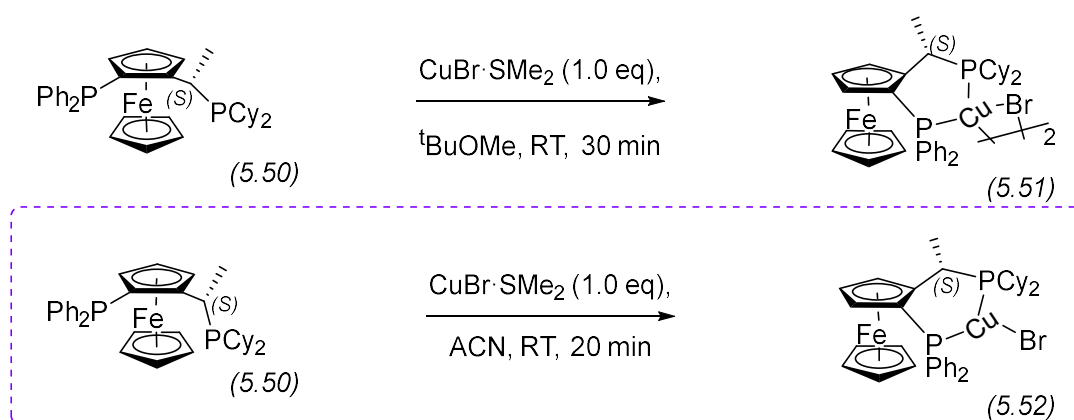
An initial hydroboration was carried out using (*R,S_p*)-Josiphos and conditions published by Hartmann *et. al* (**Scheme 90**).¹¹⁹ The conditions had been optimised and published for use on a similar chiral compound, quoting a dr of 89:11. Unfortunately for this specific compound the reaction did not proceed under these conditions. Since the reaction did not

show any conversion of SM, it was decided the active catalyst was not forming under the conditions and therefore utilising the pre-catalyst would increase the likelihood of success.



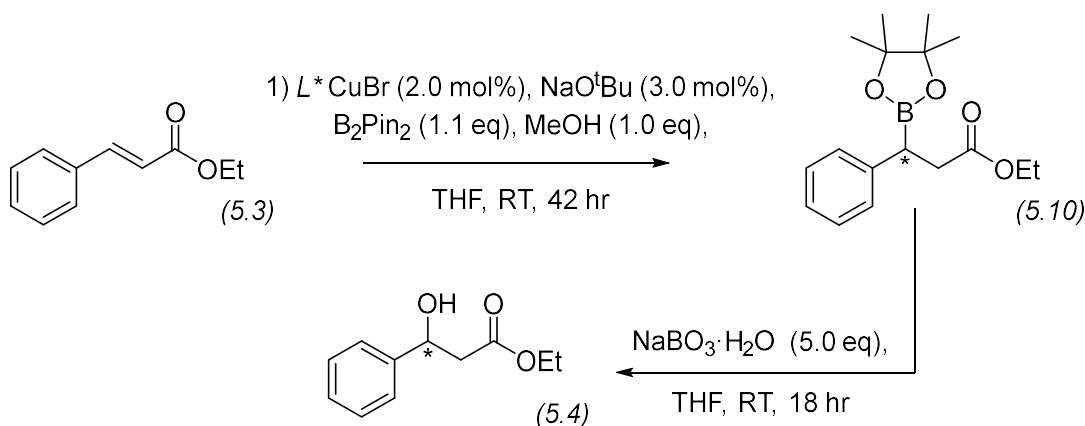
Scheme 90 Initial conditions for hydroboration of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate.

Both enantiomers of Josiphos copper (I) bromide were synthesised following a procedure outlined by Feringa *et. al.*¹²² The publication outlines the simple and effective method towards synthesis of Josiphos copper (I) bromide dimer complexes (5.51) using copper (I) bromide dimethyl sulfide and ^tbutyl methyl ether (MTBE) in around 30 minutes (**Scheme 91**). In addition, the monomer (5.52) was isolated by using acetonitrile as a reaction solvent. During the *in-situ* formation of the catalysts from this pre-catalyst, the dimer is thought to cleave into its monomeric form, so theoretically both these procedures would yield a pre-catalyst that would be functional within this hydroboration approach. Synthesis of the two pre-catalysts was performed under Schlenk conditions utilising acetonitrile as a reaction solvent. Instead of the published 20 minute reaction time, the reaction was allowed to stir over 1 hr, before slow evaporation of the solvent. This allowed for formation of the monomeric Josiphos copper (I) bromide complexes as orange powders in quantitative yields. Using these catalysts a procedure was trialled on the model system



Scheme 91 Literature conditions for synthesis of the Josiphos copper (I) bromide, a pre-catalyst for hydroboration. The highlighted chosen route isolated the monomeric complex upon slow evaporation of the reaction solvent.

Beginning with ethyl cinnamate (5.3), Josiphos ligated copper (I) catalysed hydroboration was carried out (**Scheme 92**). This followed the approach utilised by Arthurs *et al.*, showing full conversion of the alkene to boronic ester in around 42 hrs.¹¹⁷ Both ligand enantiomers were utilised to access the two corresponding boronic esters (**Table 12**). Product chirality and e.e was assumed based on previous literature reports.¹¹⁷ Oxidative degradation of the C-B bonds provided the two chiral alcohols (5.4) in > 90 % yields. Samples of each alcohol were retained for future analysis of the enantiomeric excess.

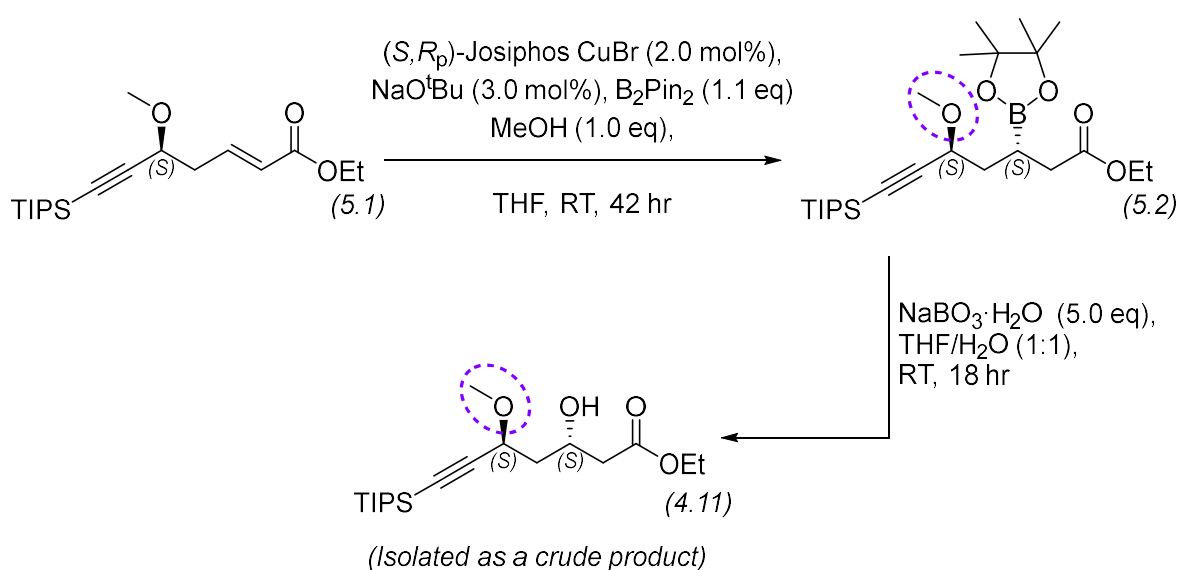


Scheme 92 Hydroboration and oxidative degradation cascade conditions, where L^* refers to the chosen enantiomer of Josiphos.

L^*	Enantiomer of product	Isolated yield (%)
(<i>S,R</i> _p)-Josiphos	(5.4- <i>R</i>)	92
(<i>R,S</i> _p)-Josiphos	(5.4- <i>S</i>)	98

Table 12 Results of the hydroboration and oxidative degradation cascade using the two enantiomers of Josiphos as chiral ligands.

Using the conditions optimised, hydroboration was performed using both (*R,S*_p)-Josiphos copper (I) bromide and (*S,R*_p)-Josiphos copper (I) bromide (**Scheme 93**). The hydroboration was monitored by ¹H NMR spectroscopy and showed complete consumption of starting material after 36 hr at RT. Upon analysis of the hydroboration, the reaction looked to have proceeded with high amounts of diastereomeric control, showing one methoxy peak at 3.38 ppm and a smaller peak at 3.42 ppm (**Fig. 52**). However, the broadness of the peak at 3.38 ppm may indicate that the methoxy peaks for both diastereomers overlay and therefore complete quantification of the diastereomeric ratio cannot be performed by analysis of the ¹H NMR spectroscopy of the borylated intermediate.



Scheme 93 Synthesis of ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (**4.11**), via hydroboration and oxidative degradation.

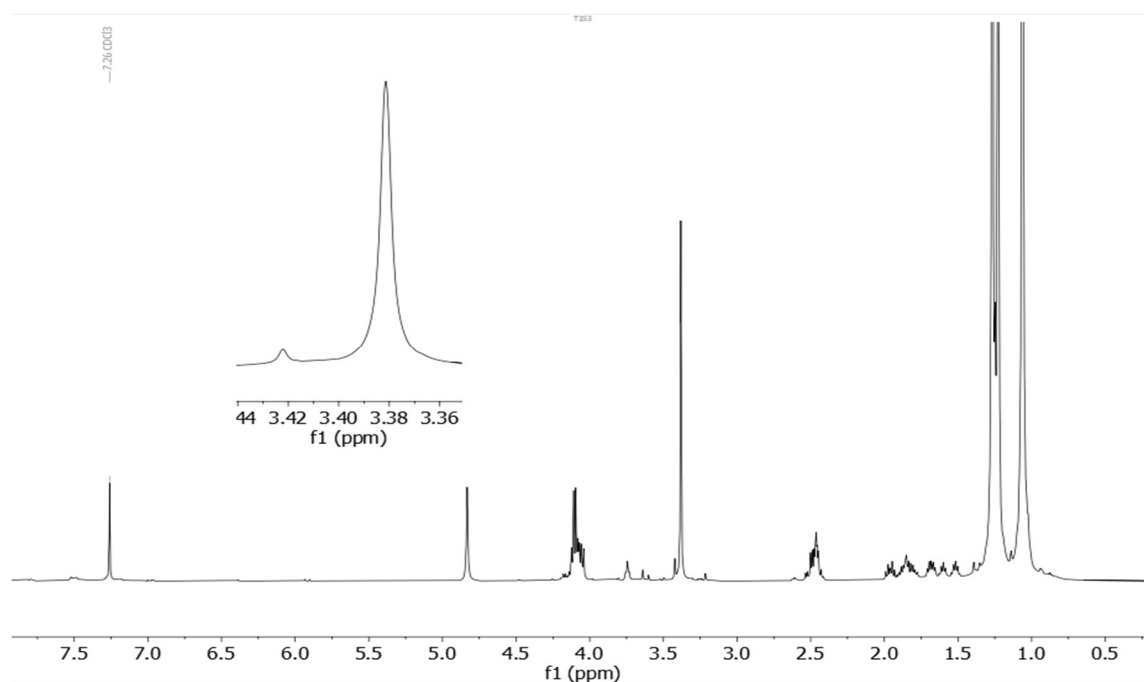


Fig. 52 Analysis of the hydroboration of ethyl (S,E)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**5.1**) using (S,*R*_p)-Josiphos copper (I) bromide by proton NMR spectroscopy.

Analysis after oxidative degradation of the C-B bond, showed two broad singlet peaks for this same methoxy peak, at around 3.45 ppm (**Fig. 53**). Since the oxidative degradation is known to be stereo-retentive, this may indicate a low selectivity of the hydroboration step, which is further evidenced by the clear appearance of duplicate peaks in the 4.45 to 4.10 ppm region. Initial NMR analysis was performed in chloroform-*d*, which showed two overlapping broad singlets for the products methoxy peak (**Fig. 54**). This indicated that the two diastereomers of the products were formed in around 50:50 ratio. A sample of crude reaction was analysed by ¹H NMR spectroscopy in acetonitrile-*d*₃ and THF-*d*₈ to see if this would improve separations and provide distinct peaks for accurate ratio determination. Acetonitrile-*d*₃ proved to broaden the two singlets, leading to worse separation of the two peaks. However, when THF-*d*₈ was used as an NMR solvent, the methoxy peaks of the two diastereomers separated slightly, giving two distinguishable peaks. While there is still some overlap when using THF-*d*₈ as the NMR solvent, this gives the best separation of the two peaks and clearly indicates, that while the ratio is close to 50:50, one of the diastereomers is slightly favoured.

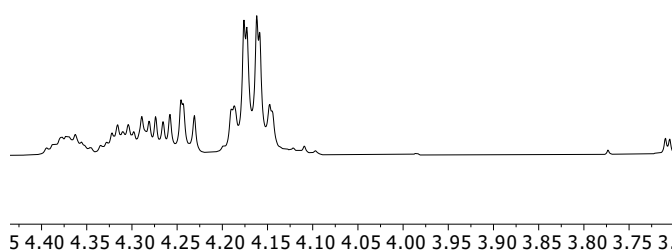


Fig. 53 Analysis of the 3.30 to 4.45 ppm range of the ^1H NMR spectra of the crude product of step 2 in the hydroboration and oxidation of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**5.1**) using (*S,R*_p)-Josiphos copper (I) bromide (**A-C5-5**).

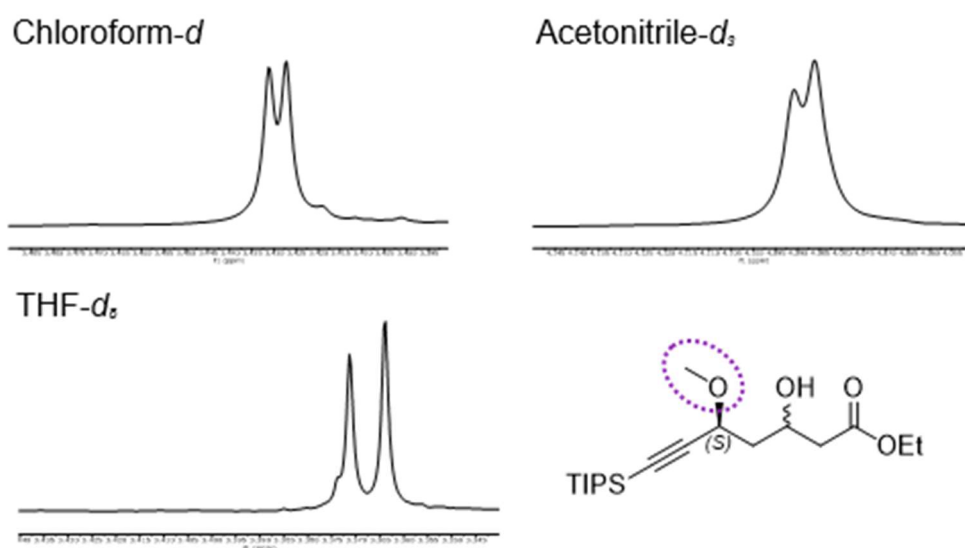


Fig. 54 Analysis of the hydroboration and oxidative degradation of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate by proton NMR spectroscopy, showing poor

differentiation of the two diastereomer peaks in chloroform-*d* and acetonitrile-*d*₃, and mildly improved peak separation in THF-*d*₈.

Since the two diastereomers cannot be accurately distinguished by NMR spectroscopy, it will be important to utilise other methods of analysis to determine the diastereomeric ratios during reaction optimisation. While accurate numbers cannot be provided from the spectra gained, both enantiomers of the catalyst appear to have produced a dr of around 55:45, each slightly favouring opposite diastereomers (**Fig. 55**). Since each catalyst slightly favours different enantiomers, it can be concluded that there is some level of catalytic control within the reaction. However, the overall dr's show poor stereoselectivity for this approach, which may indicate some substrate interference in the facial selectivity of the hydroboration (*as discussed on P147*).

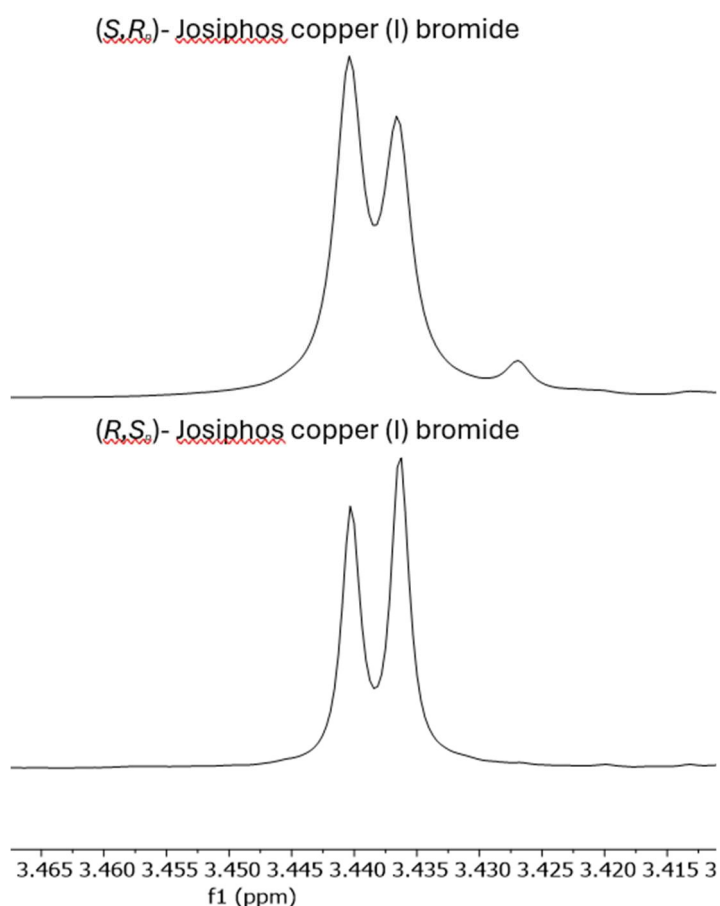
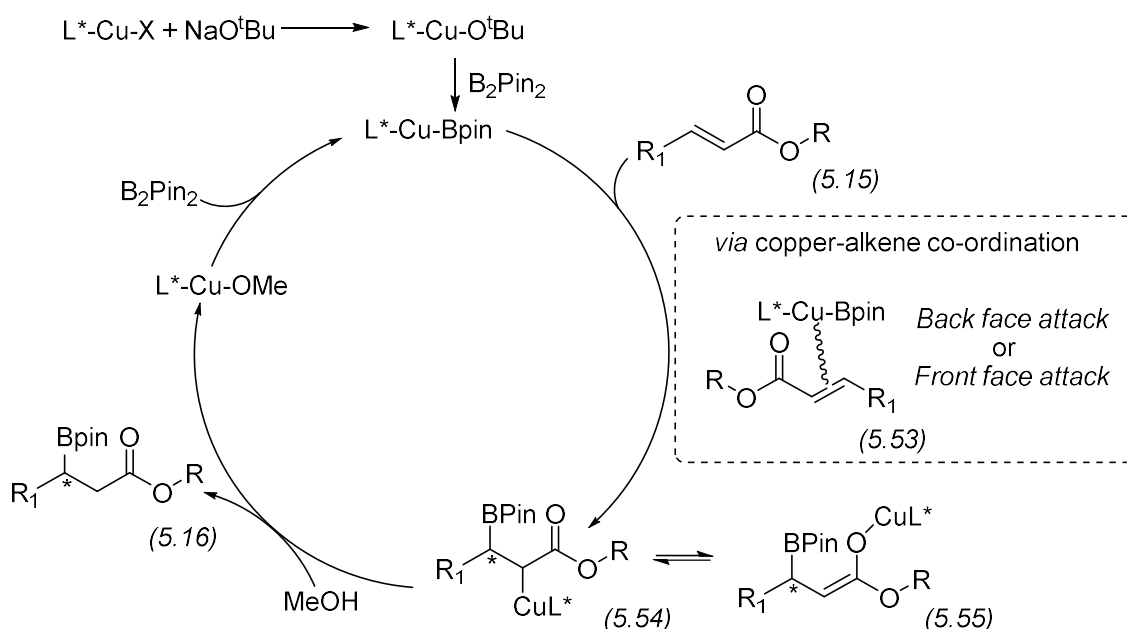


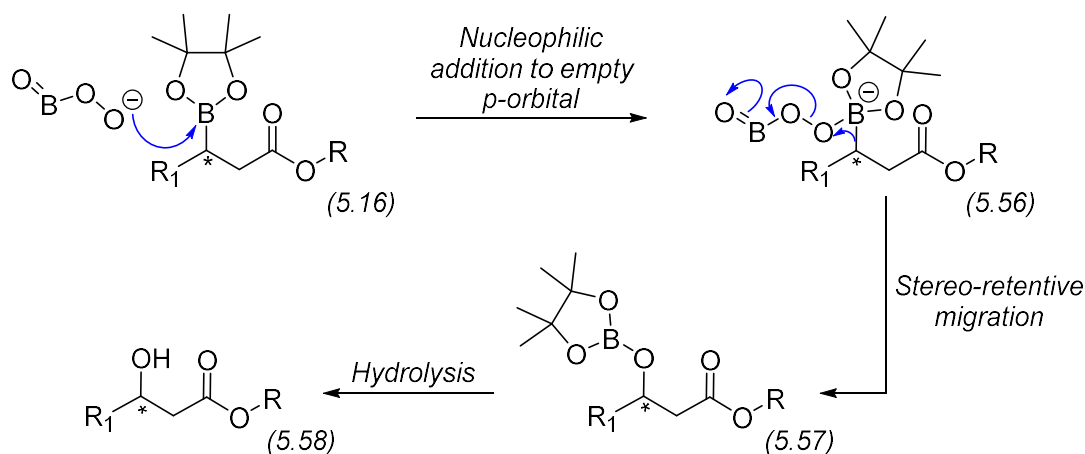
Fig. 55 Comparison of the ¹H NMR spectra of the crude products of hydroboration and oxidative degradation with (S,*R*_p)-Josiphos (**A-C5-5**) and (R,*S*_r)-Josiphos (**A-C5-7**) ligated copper (I) bromide, showing a small amount of catalytic control of the stereoselectivity.

Enantioselectivity of the hydroboration of enones and α,β -unsaturated ester is believed to occur through a diphosphine ligated copper boryl complex (**Scheme 94**, 5.53) which is created through activation of the pre-catalyst by sodium ^tbutoxide.^{113,123} Co-ordination of the copper catalyst to the alkene, allows for a conjugate addition style reaction on the α,β -unsaturated carbonyl. The final compound stereochemistry is determined by the face at which the Bpin attacks the alkene, which is controlled by the chirality of the bulky ligand forcing the copper co-ordination on the most sterically favourable side. The ligated copper species shifts between the α -carbon (5.54) and the carbonyl oxygen (5.55) forming two organocopper intermediates. Methanol provides a proton source and cleaves the copper catalyst to provide the hydroborated compound (5.16) and ligated copper methoxide, which is reactivated by B₂Pin₂.



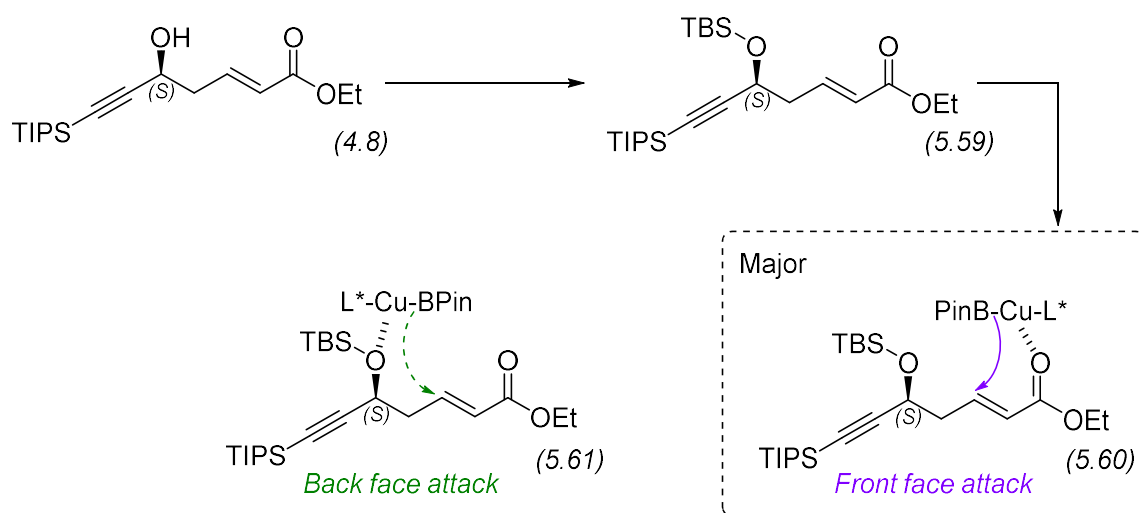
Scheme 94 Mechanism by which the hydroboration of α,β -unsaturated esters with a diphosphine ligated copper catalysts is believed to occur, where L^* represents a chiral diphosphine ligand.

The subsequent oxidative degradation of the C-B bond is stereo-retentive, due to the 1,2-metallate rearrangement (**Scheme 95**). Migration of the C-B σ -bond to the oxygen is driven by the loss of BO_2^- and leads to the formation of the new C-O bond. Subsequent hydrolysis of the Bpin gives the desired alcohol with retained stereochemistry. Since this step should retain the stereochemistry of the C-B bond, it can be deduced that the poor selectivity likely occurs within the hydroboration step.



Scheme 95 Migratory mechanism of oxidative degradation of the C-B bond using sodium perborate.

Since this reaction appears to produce around a 1:1 dr, it can be assumed that the reaction is not under complete catalytic control. As in the previous literature by Oestreich and Hartmann, the asymmetric induction of the catalyst is low due to imperfect facial selectivity of the catalyst.¹¹⁹ The further reduction in selectivity on ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**5.1**) when compared with the published data, may evidence the theory of an interfering co-ordination site on the alcohol. The smaller methyl protecting group may provide more access for co-ordination of the copper catalyst to the oxygen, which would cause the increased formation of the second diastereomer. Given more time, this theory could be tested by protecting ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**4.8**) with TBS to provide a more similar substrate (**5.59**) to the one used by Oestreich and Hartmann and performing the hydroboration reaction under the same conditions (**Scheme 96**). An increased dr, would suggest an increased favourability of the copper-alkene co-ordination which would confirm the theory.



Scheme 96 Proposed method to test the theory of interference by a more electronegative oxygen on the enantioselectivity of the hydroboration reaction.

Should the protecting group change not improve the d.r., then the interference may be coming from a co-ordination to the alkyne group. As the model substrate contains a phenyl group in substitution of the alkyne, this will provide some understanding on this possible interference. An improved d.r. on the model substrate would suggest that the alkyne-copper co-ordination is negatively impacting the selectivity of the reaction (**Fig. 56**). In this case, this model will not be appropriate for optimisation of the hydroboration strategy. However, if the diastereomeric ratio remains low for the same conditions then it provides further confirmation that the copper-oxygen co-ordination is the cause, and initial optimisation can be performed on the model substrate. Hydroboration of alkenes through this method has been utilised on a variety of compounds, generally showing greater than 80 % enantiomeric excess, but the exact conditions required to obtain higher e.e. seem to vary significantly between target species.^{111,113,117–119,122} This provides a large library of conditions which could be used to optimise the reaction, including variations in bases, ligands and temperature.

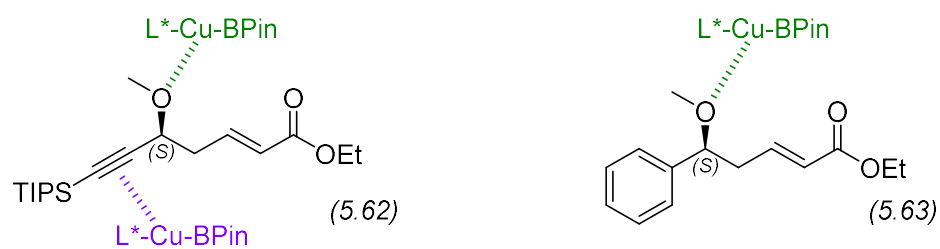


Fig. 56 Two co-ordination sites suspected of causing interference with the facial selectivity of the hydroboration of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate, alongside the model system, containing only one of these co-ordination interference sites.

5.3.2. Synthesis of ethyl (*E*)-5-methoxy-5-phenyl-pent-2-enoate, the model substrate

Successful synthesis of the two enantiomers of ethyl 3-hydroxy-3-phenylpropanoate (5.4) was discussed in the previous section. Once the conditions had been optimised, the synthesis of both enantiomers of ethyl (*E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7) was performed (**Fig. 57**). This planned pathway consists of alcohol methylation, ester reduction and a Horner-Wadsworth-Emmons reaction.

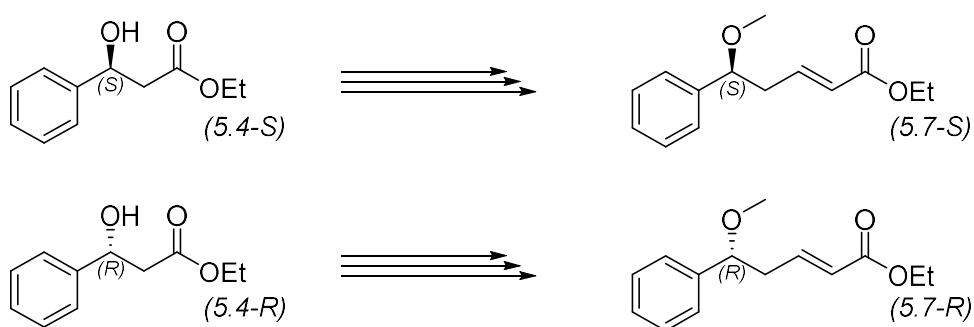
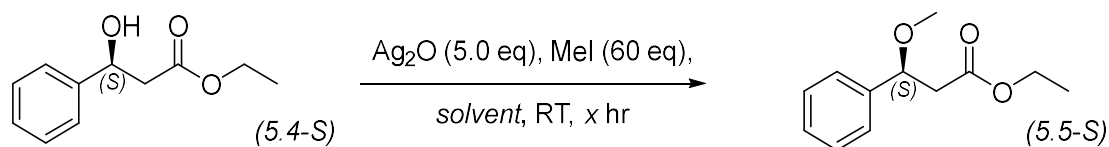


Fig. 57 Synthesis of both enantiomers of ethyl (*E*)-5-methoxy-5-phenyl-pent-2-enoate in a multi-step asymmetric approach, to provide model substrates for hydroboration optimisation.

Methylation was first attempted on ethyl 3(*S*)-hydroxy-3-phenylpropanoate (4.5-*S*), using the same methylation conditions utilised in chapter 5.2.1 (**Scheme 97**). These conditions utilised Ag₂O and methyl iodide in diethyl ether and showed moderate to high yields. The first methylation on ethyl 3(*S*)-hydroxy-3-phenylpropanoate was left to stir for 38 hr showing around 30 % conversion to the methylated derivative (5.5-*S*) (**Table 13**). The same reaction conditions were trialled in THF and toluene, both showing moderate conversions of 35 % and 50 % over 48 hr. To each reaction was added a further portion of Ag₂O (5.0 eq) and MeI (60.0 eq), before being analysed again at 66 hr, showing no further conversion indicating the reaction had stalled.

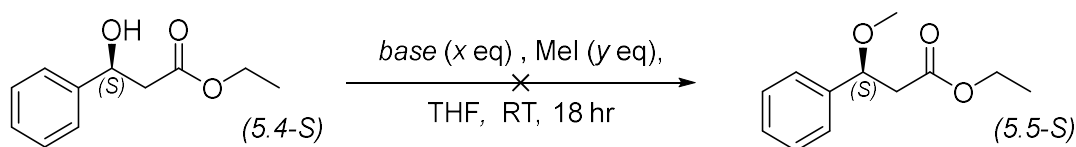


Scheme 97 Standard methylation conditions used during initial optimisation, where the solvent is varied, and x refers to the time when the reaction stalled.

Solvent	x hr	Conversion (%)
Et ₂ O	48	30
THF	66	35
Toluene	66	50

Table 13 Initial reaction conditions used to methylate ethyl 3(S)-hydroxy-3-phenylpropanoate showing the time and conversion at which the reaction appeared to stall. Conversion was estimated using ¹H NMR spectroscopy.

An attempt to use base-mediated methylation was carried out (**Scheme 98**). The reaction was trialled with three different bases. Both KOH and ⁿBuLi returned ethyl cinnamate (5.3) as their primary product, and utilising K₂CO₃, a milder non-nucleophilic base, showed no conversion of the SM to desired product or byproducts (**Table 14**).

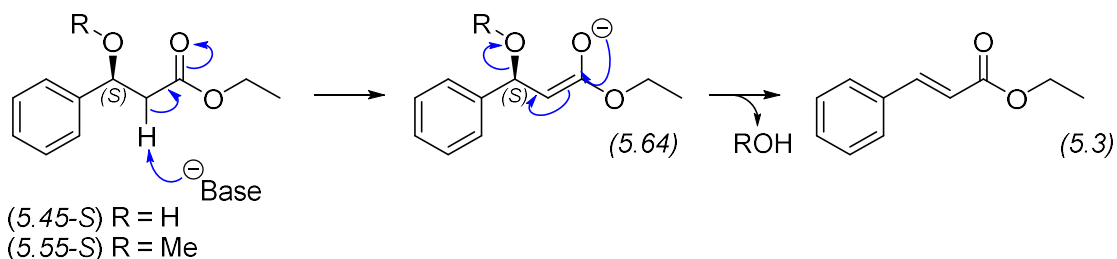


Scheme 98 Failed base mediated methylation conditions.

Base	x eq	y eq	Major product
KOH	1.2	1.6	(5.3)
ⁿ BuLi	1.0	5.0	(5.3)
K ₂ CO ₃	1.2	1.6	(5.4-S)

Table 14 Three attempted base-mediated approaches towards methylation of ethyl 3(S)-hydroxy-3-phenylpropanoate, giving the major products of each trial.

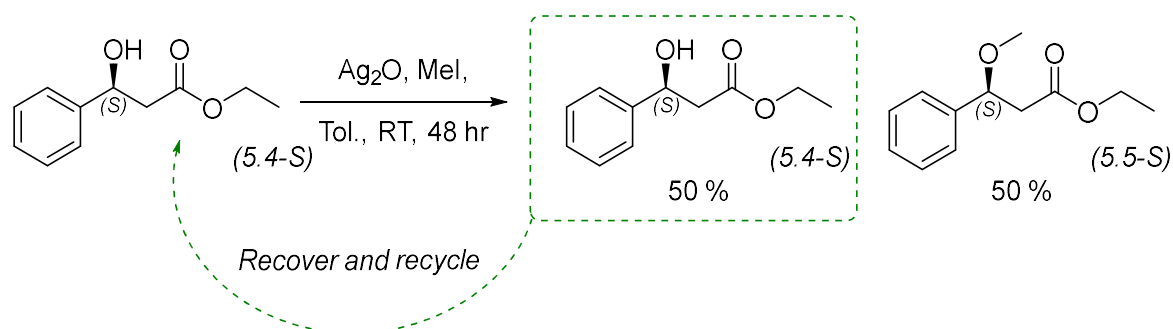
It was expected that some level of dehydroxylation might occur when using both KOH and ⁿBuLi as both the starting material and product contain enolisable protons, which may lead to elimination (**Scheme 99**). It was hoped that deprotonation of the alcohol may be more favourable, however the result suggest that this was not the case.



Scheme 99 Mechanism of the base-mediated elimination, causing the return of ethyl cinnamate.

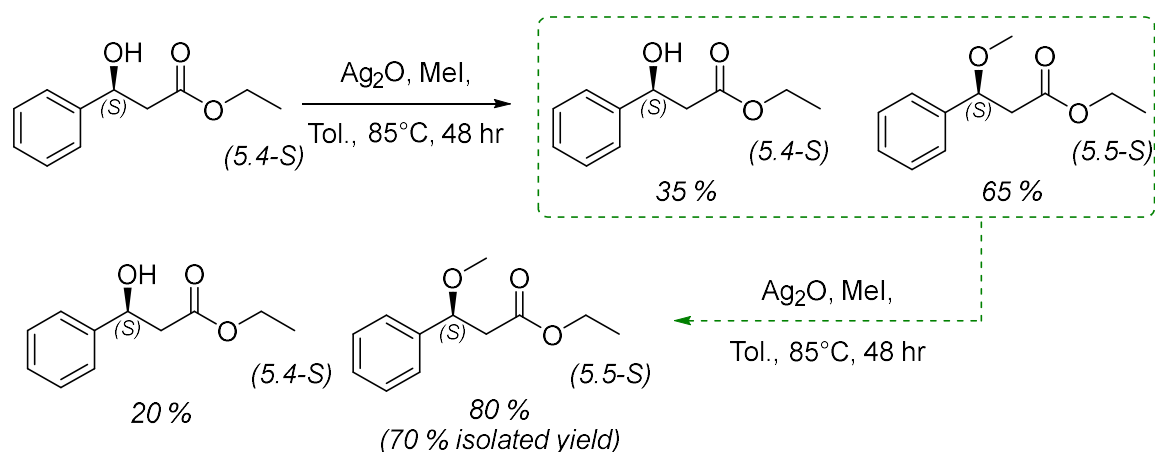
While testing a wider range of weaker bases may have led to conditions that would succeed, they would likely all see some level of degradation into ethyl cinnamate (5.3). The silver

oxide method did not show any degradation of the un-converted starting material and therefore, this could be recovered in purification stages and recycled back into the next run (**Scheme 100**). For this reason, it seemed optimal to continue with the low-conversion silver oxide approach.



Scheme 100 Highest performing methylation conditions, giving around 50 % conversion, with the option to recover and recycle the unreacted starting material into the next round of the reaction.

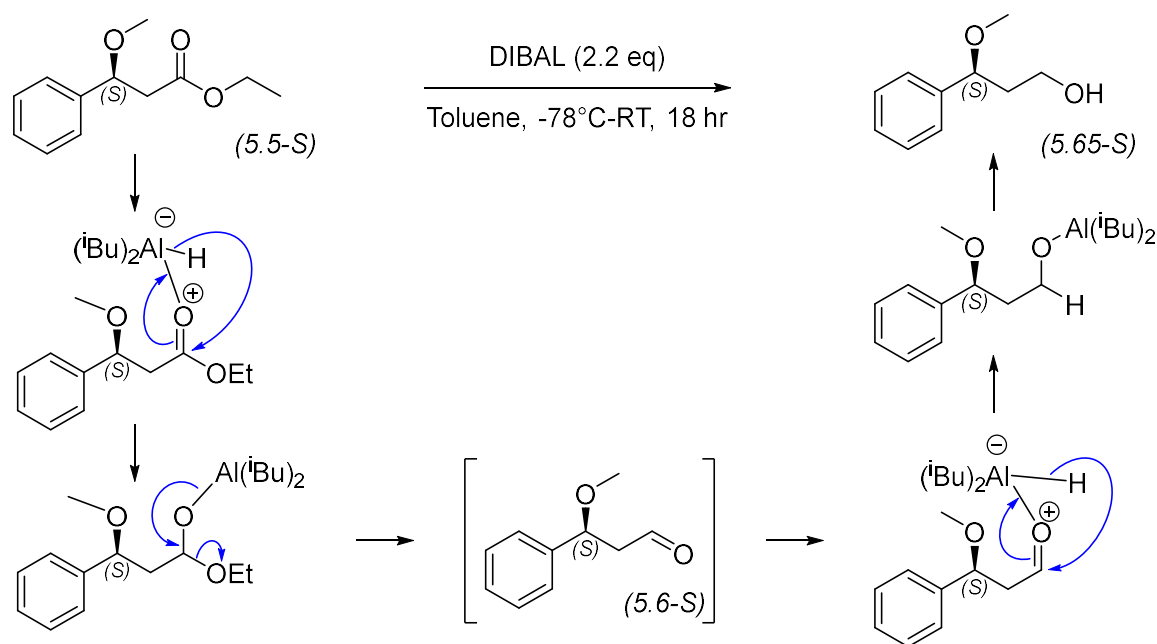
In a final attempt to optimise the procedure, the methylation was performed in a sealed vessel with toluene at elevated temperature (**Scheme 101**). Some increase in conversion was seen, with one reaction reaching 65 % conversion before stalling. The reaction was filtered to remove the metal solids and reduced under concentrated pressure. Since this crude product contained only starting material and product, the crude mixture was taken back up in toluene and fresh reagents added. Some further conversion was seen, leading to around 80 % overall conversion and a 70 % isolation yield of ethyl 3(*S*)-methoxy-3-phenylpropanoate (5.5-S). The same methodology was applied to ethyl 3(*R*)-hydroxy-3-phenylpropanoate to give an isolated yield of 65 %.



Scheme 101 Final method of methylation, where the crude mixture of starting material and product are subjected to a second round of the same conditions, showing increased conversion of the starting material.

When similar conditions were utilised previously on ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8), the reaction could be pushed to complete conversion with addition of more methyl iodide. As the only difference between the two substrates is the phenyl ring and silyl protected alkyne, initial thoughts were that silver co-ordination to the phenyl ring may have reduced the available silver oxide for the reaction to proceed and thereby lowering the conversion. However, utilising over 2.0 eq should mitigate any loss in conversion that may occur from phenyl-silver co-ordination. Further research suggested that some trace amounts of copper remaining from the hydroboration reaction may interact with the silver.¹²⁴ These copper-silver interactions may encourage argentophilic interactions, creating an unreactive metal species. This would explain why adding more silver oxide does not improve the conversion, as it would be poisoned by the argentophilic interactions.

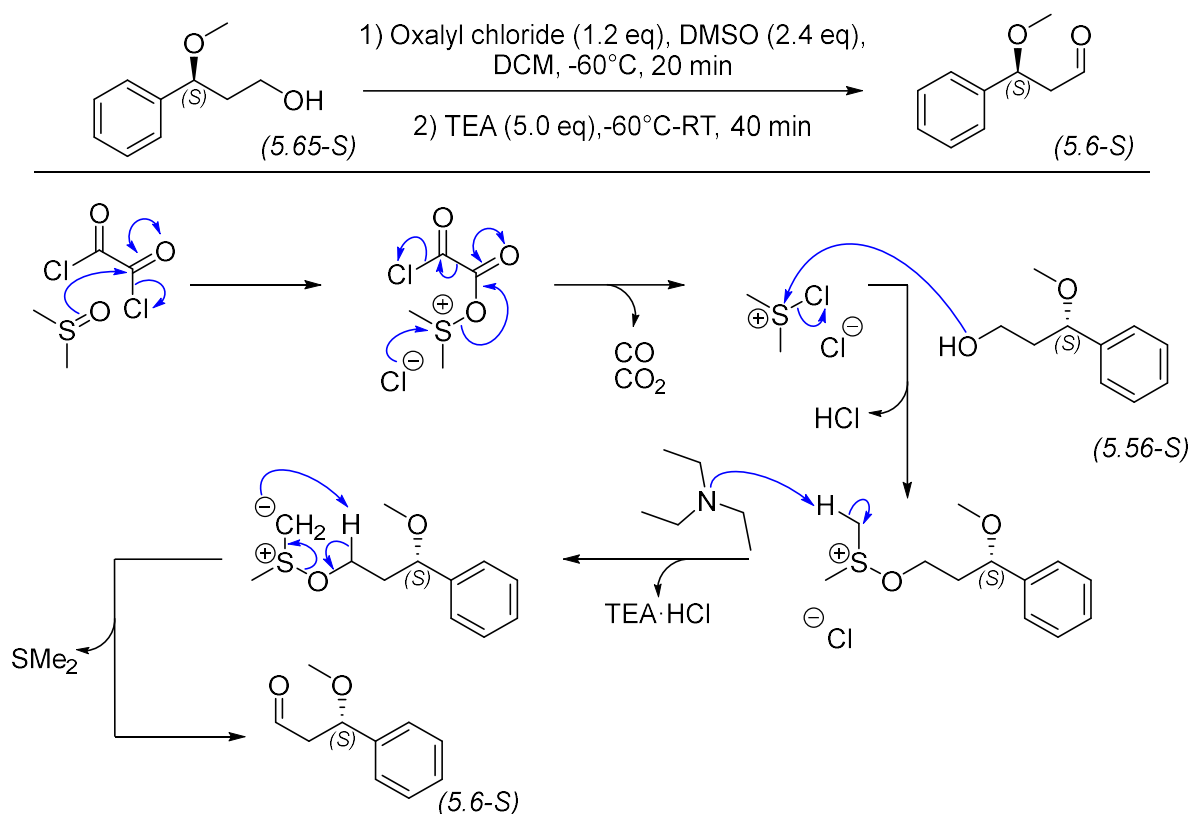
Chain extension of the chiral compounds was planned to include a reduction of the ester functionality and then a HWE reaction using triethyl phosphonoacetate. Both enantiomers of ethyl 3-methoxy-3-phenylpropanoate (5.5) were subjected to DIBAL reduction and monitored until full conversion to the alcohol was seen by ^1H NMR spectroscopy (**Scheme 102**). The *R*- and *S*- alcohols (5.65) were isolated and carried forward crude into Swern oxidations, without obtaining a yield.



Scheme 102 Conditions for complete ester reduction of ethyl 3(*S*)-methoxy-3-phenylpropanoate (5.5-*S*) with DIBAL, and the mechanism of reaction.

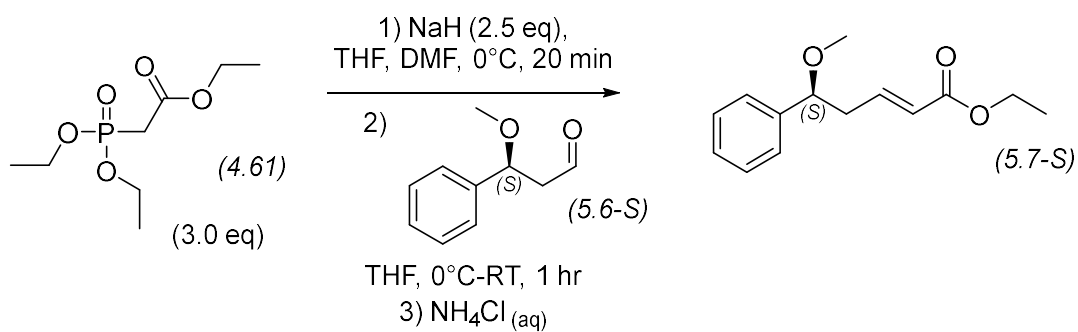
To ensure optimal conditions for the Swern oxidation, all reagents were purified prior to beginning the reaction. Anhydrous DMSO was obtained by storing over activated 4 Å MS for 1 week in an argon sealed Schlenk flask. Purification of TEA and oxalyl chloride was carried out the day prior to the Swern oxidation and the reagents were both stored sealed under argon until required.¹ Oxalyl chloride was flash distilled under a slight positive argon pressure. Triethylamine was stirred over K₂CO₃ for 1 hr and distilled under argon onto activated 4 Å MS.

The Swern oxidation reaction was carried out first on 3(*S*)-methoxy-3-phenylpropanol (5.65-*S*), ensuring the temperature was maintained below -60 °C during each addition (**Scheme 103**). First, the oxalyl chloride was reacted with DMSO for 20 min to ensure complete formation of the active chlorosulfonium ion. The alcohol was added slowly as a solution in DCM and allowed to react for 20 minutes before addition of triethylamine in DCM. At this point, the reaction was maintained -60 °C for 10 minutes and then warmed to RT to stir for a further 30 minutes before being isolated *via* aqueous work up. Excess solvent was removed under reduced pressure, followed by two azeotropic distillations with hexanes (10 mL) to ensure removal of all TEA. While the reaction mixture was concentrating the triethyl phosphonoacetate ylide was prepared using the same methodology discussed in chapter 4.3.2.

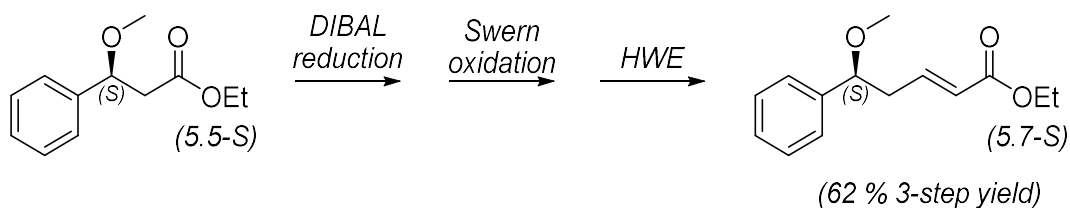


Scheme 103 Conditions utilised for the Swern oxidation of 3(*S*)-methoxy-3-phenylpropanol (**5.65-S**), and the mechanism of oxidation (*bottom*).

The crude aldehyde (**5.6-S**) was taken up in THF and added to the prepared ylide at 0 °C, before being warmed to RT and stirred for 1 hr (**Scheme 104**). Complete disappearance of the aldehyde was seen by TLC analysis in 20 % EtOAc in hexanes and ^1H NMR spectroscopy. The crude material was contaminated with diethyl phosphate and so was purified by column chromatography in 5-30 % EtOAc in hexanes to give an overall 3-step yield of 62 % (**Scheme 105**). For a 3-step cascade, this was deemed a sufficient yield, and no further optimisation was carried out. 3(*R*)-methoxy-3-phenylpropanol (**5.65-R**) was subjected to the same conditions, giving a crude sample of ethyl (*R,E*)-5-methoxy-5-phenylpent-2-enoate (**5.7-R**) which was contaminated with diethyl phosphonate.



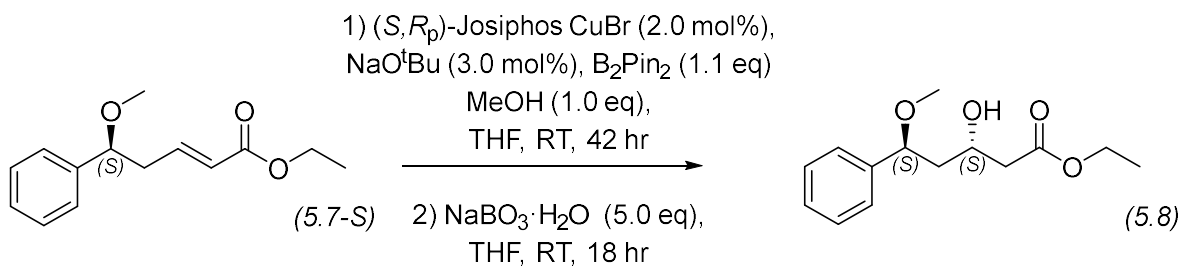
Scheme 104 Reaction conditions for chain extension with a HWE reaction to form the α,β -unsaturated ester.



Scheme 105 3-Step reaction cascade from ethyl 3(S)-methoxy-3-phenylpropanoate (5.5-S) to synthesise the desired model substrate in a 62 % 3-step yield.

5.3.3. Optimisation of the hydroboration strategy

To optimise the conditions for hydroboration, the initial reaction conditions needed to first be trialled on one of the two enantiomers of the model substrate. Since only ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (**5.7-S**) was isolated as a pure compound, the initial control reaction was carried out on this. Using (*S,R*_p)-Josiphos CuBr, the hydroboration was performed as per the conditions discussed in section 5.3.1 (**Scheme 106**).



Scheme 106 Conditions used within the control reaction for optimisation of the hydroboration approach on ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (**5.7-S**).

The hydroboration was monitored by ¹H NMR spectroscopy, which showed complete disappearance of the alkene peaks at 6.94 and 5.84 ppm, indicating full conversion of the alkene after 18 hr of stirring (**Fig. 58**). Unlike with the previous substrate, this boronic ester intermediate showed two distinct methoxy peaks at 3.18 ppm and 3.17 ppm, with a ratio of around 55:45. Further evidence of this can be seen in the peaks in the region of 4.25 to 4.05 ppm, where there is clear indication of duplicate peaks. If these two sets of peaks do correspond to the two diastereomeric products, then the d.r. matches to that of the substrate in chapter 5.3.1, proving this to be a good model substrate for optimisation.

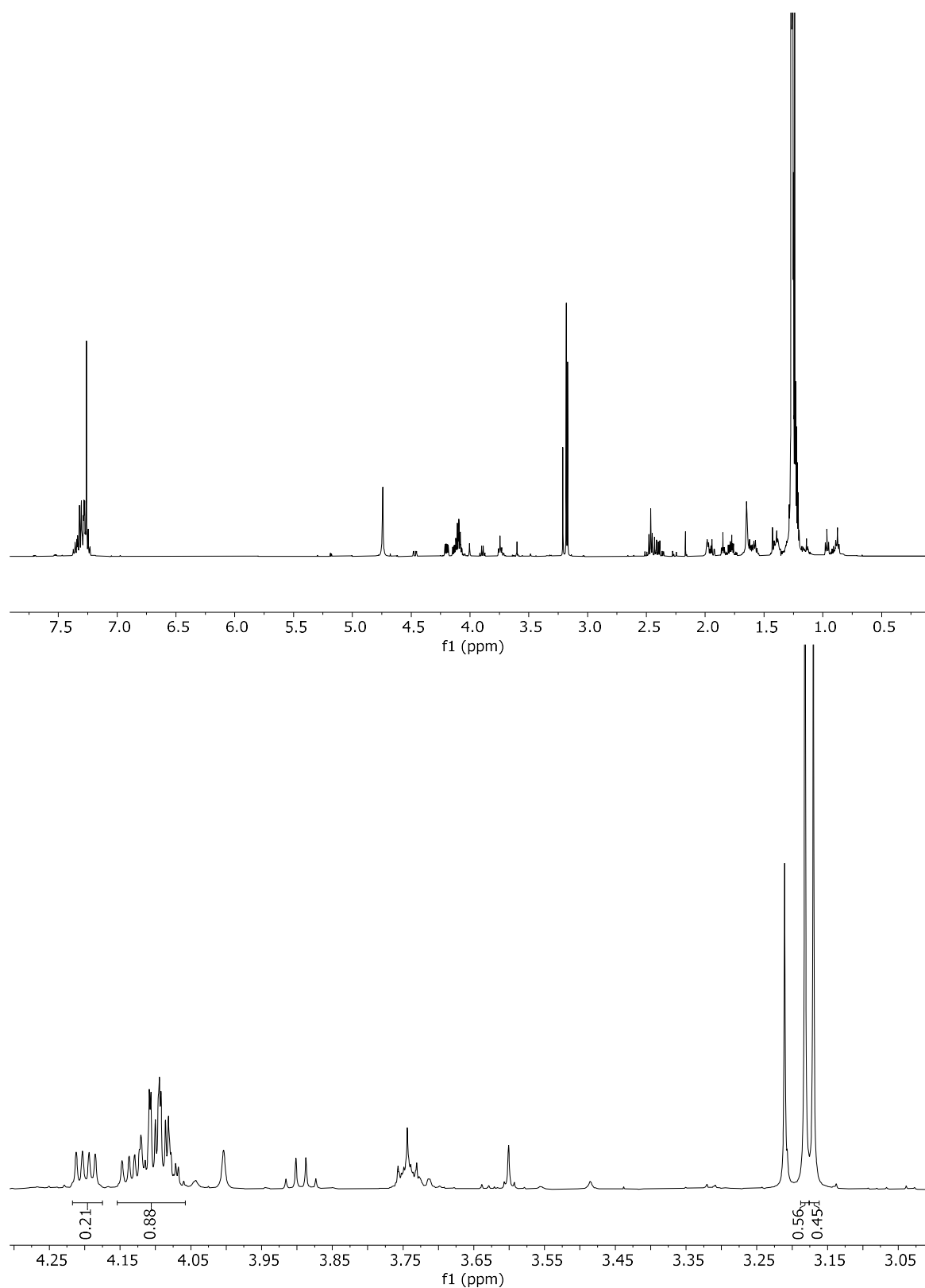


Fig. 58 ^1H NMR spectra of the crude borylation reaction mixture (**A-C5-15**), indicating full consumption of the alkene, by disappearance of the peaks at 6.94 and 5.84 ppm; including an expansion of the region between 4.30 and 3.00 ppm, which shows clear duplication of multiple peaks.

This diastereomeric ratio is less clear in the analysis of the oxidative degradation product, where the region between 3.10 and 3.30 ppm contains four singlet peaks (**Fig. 59**). Some of these peaks will be impurities which will need to be removed to help identify which peaks correspond to the product. However, a visual inspection of the peaks around 4.45 ppm, indicate an almost 1:1 ratio of the two diastereomers. To quantify the d.r. analysis hydroboration on ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (*5.7-S*) must be performed with (*R,S_p*)-Josiphos CuBr, which should form a product with the opposite ratio of peaks. If this is the case, then it confirms that ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (*5.7-S*) is a good model substrate for ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (*5.1*).

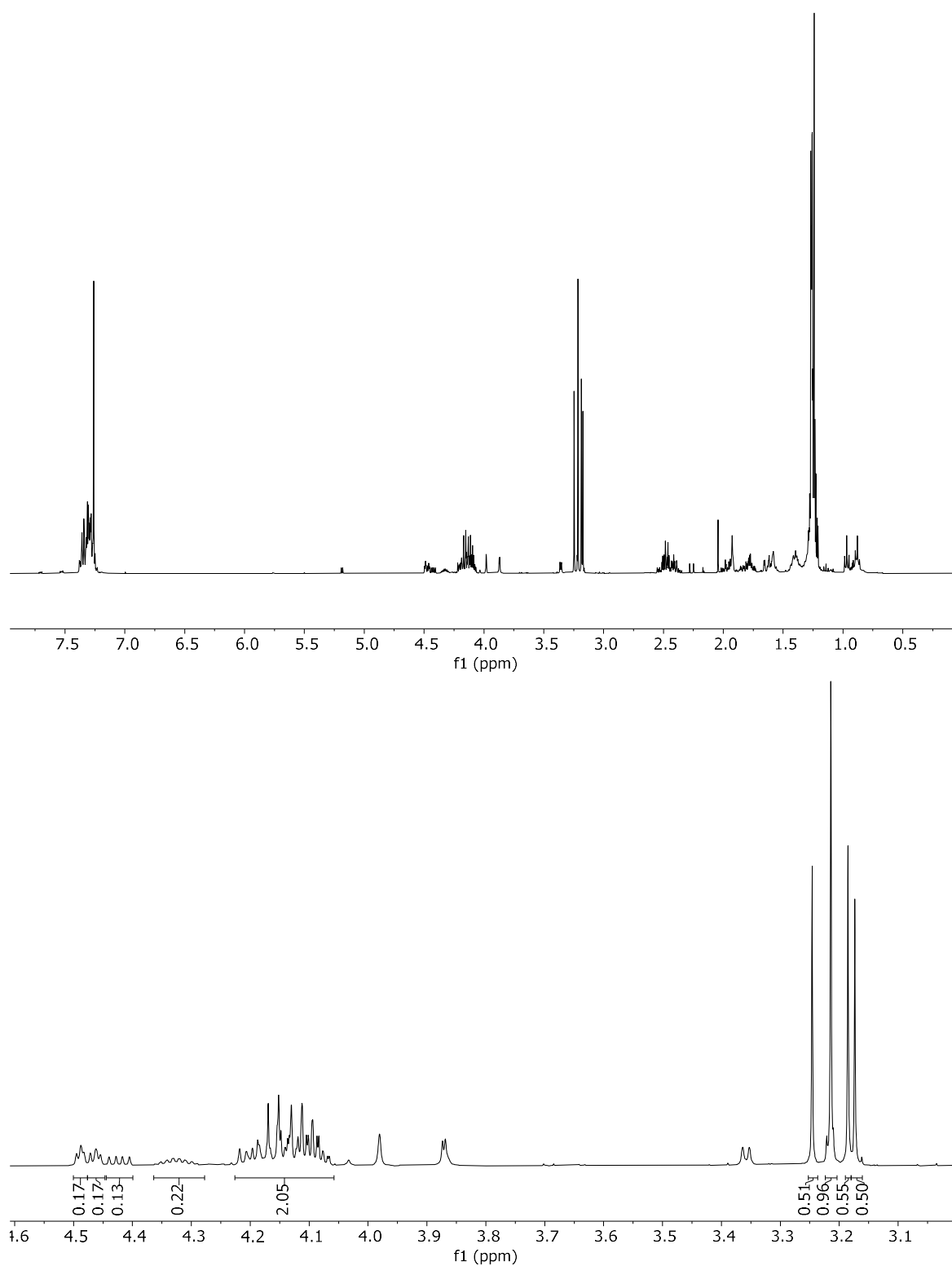
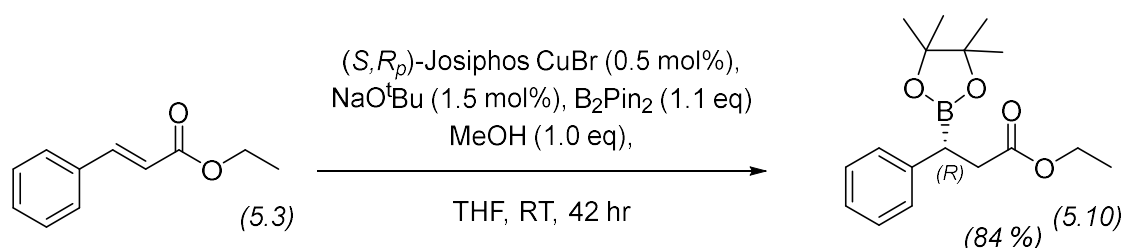


Fig. 59 ^1H NMR spectrum (**A-C5-16**) of the crude reaction mixture of the oxidative degradation product, showing including an expansion of the region between 4.50 and 3.15 ppm, which shows clear duplication of multiple peaks.

5.3.4. Initial attempts towards stereo-retentive amination of β -boronic ester carbonyl compounds

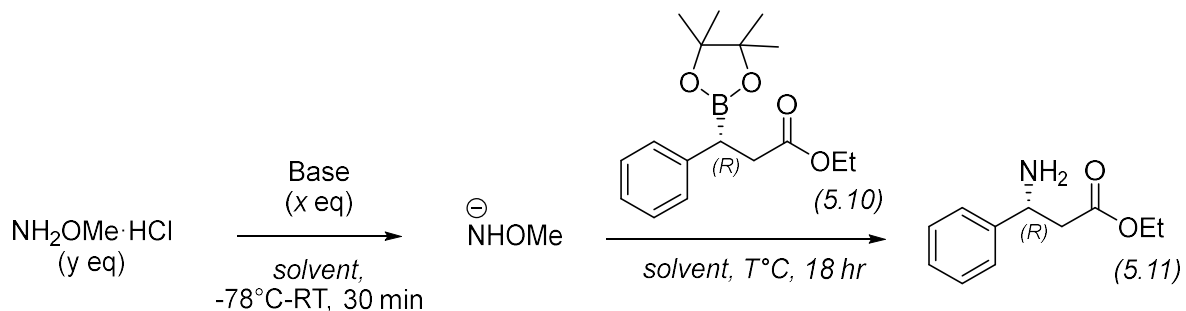
To begin the initial search for reaction conditions for stereo-retentive amination of β -boronic ester carbonyl compounds, hydroboration was carried out on 500 mg of ethyl cinnamate (5.3) (**Scheme 107**). Since stereo-retention was not being measured at this stage, a high and quantified enantiomeric excess was not required, therefore the reaction was performed using a reduced amount of ligated catalyst (0.5 mol%) to reduce the cost of the initial screenings. Ethyl 3-phenyl-3(*R*)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propanoate (5.10-*R*) has previously shown signs of de-borylation when subjected to column chromatography, so the product was not purified. Instead, extra washings were performed with water and brine to ensure full removal of any salts.



Scheme 107 Conditions for hydroboration of ethyl cinnamate, with reduced catalyst loading.

Using this β -boronic ester carbonyl compound, amination was trialled with three different sets of conditions (**Scheme 108**). The first reaction used similar conditions to those outlined by Morken (*entry 1*, **Table 15**).¹²¹ Instead of using 3.0 equivalents of the methoxy amine lithium salt, 2.0 equivalents were made and the boronic ester was added. While starting material could be clearly seen in the ¹H NMR spectrum, it did show some signs of possible amination, with new peaks being seen around 3.2 ppm and 2.4 ppm (**Fig. 60**). This reaction was performed a second time; this time the solvent was removed from the methoxy amine lithium salt, and it was added to the boronic acid as a dried salt (*entry 2*, **Table 15**). The reaction was performed at a reduced temperature and showed very minimal signs of consumption of starting material. This may indicate that higher heat is required to drive the reaction forward or that the salt is unstable and should not be isolated. The third reaction utilised potassium ^tbutoxide as the base for the formation of methoxy amine potassium salt (*entry 3*, **Table 15**). This reaction showed complete conversion of the starting material by

^1H NMR, however, does not show formation of the same peaks as the previous two reactions (Fig. 61).



Scheme 108 General conditions for trial reactions towards stereo-retentive amination of ethyl 3-phenyl-3(*R*)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propanoate.

Entry	Base	<i>x</i> eq	<i>y</i> eq	Solvent	T (°C)
1	$n\text{BuLi}$	2	1	THF	65
2	$n\text{BuLi}$	2	1	THF	50
3	KO^tBu	3	2	Toluene	80

Table 15 Conditions trialled to find initial control reaction conditions for optimisation of stereo-retentive amination of β -boronic ester carbonyl compounds.

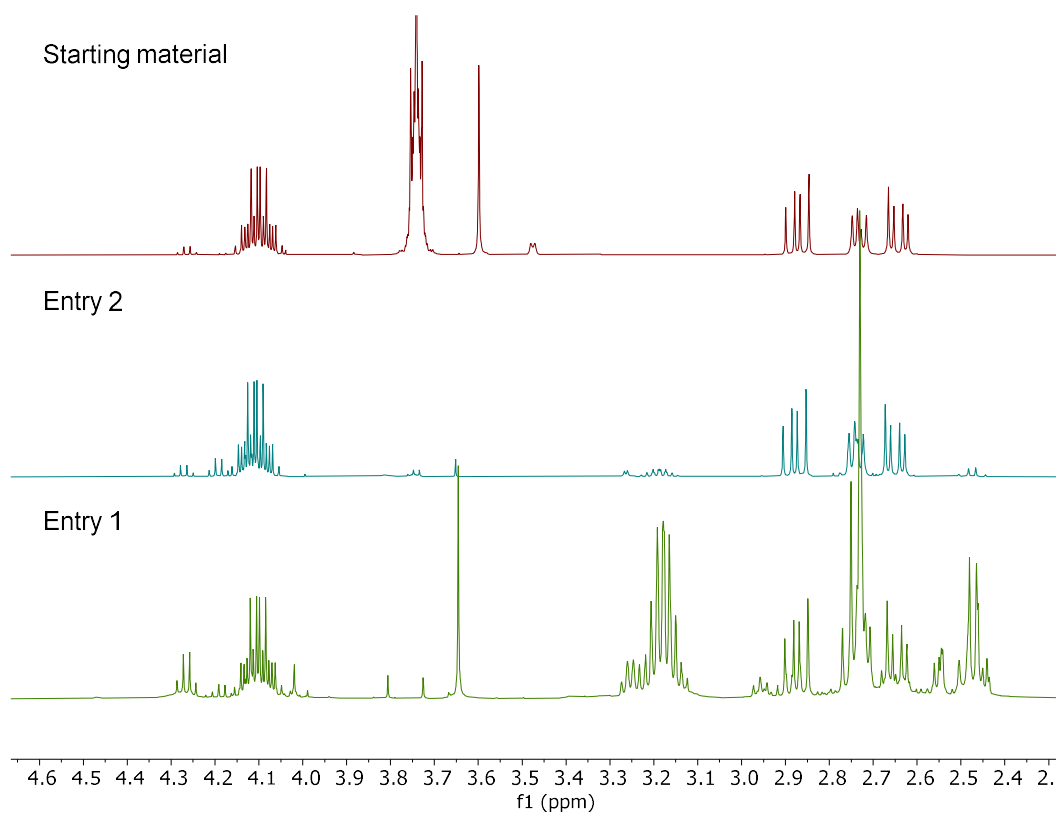


Fig. 60 Comparison of the ^1H NMR spectra of trial reaction 1 (**A-C5-24**) and 2 (**A-C5-25**) and the boronic ester starting material (**A-C5-22**), focussing on the shifts of the diastereotopic protons.

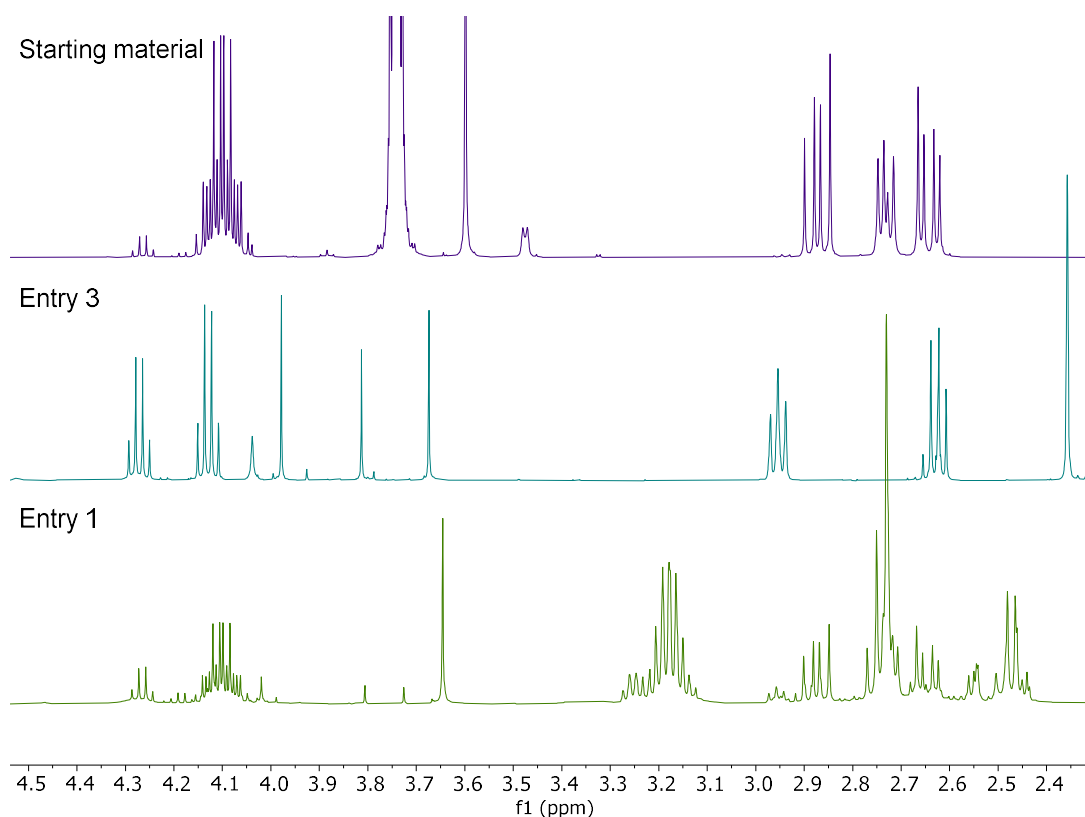
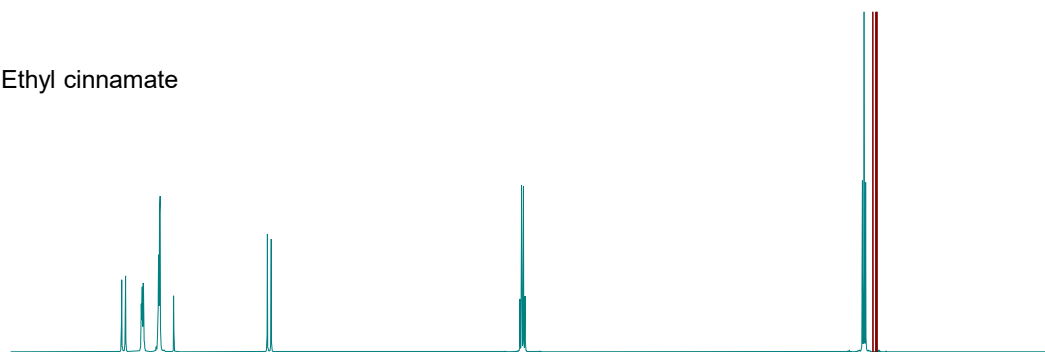


Fig. 61 Comparison of the ^1H NMR spectra of trial reaction 1 (**A-C5-24**) and 3 (**A-C5-26**) and the starting material (**A-C5-22**), focussing on the shifts of the diastereotopic protons.

A comparison of the crude product ^1H NMR spectrum of reaction 3 with ethyl cinnamate (5.3), reveals that these conditions have caused the return of a large amount of ethyl cinnamate (**Fig. 62**). This could have been caused by de-borylation of the starting material or by de-amination of the product, both initiated by enolate formation (**Scheme 109**). From the crude NMR spectrum, it is not possible to determine what the other product is, however a comparison with a report of the ^1H NMR spectrum of the desired product by Sun *et. al* suggests that this is not the desired product.¹²⁵

Ethyl cinnamate



Entry 3

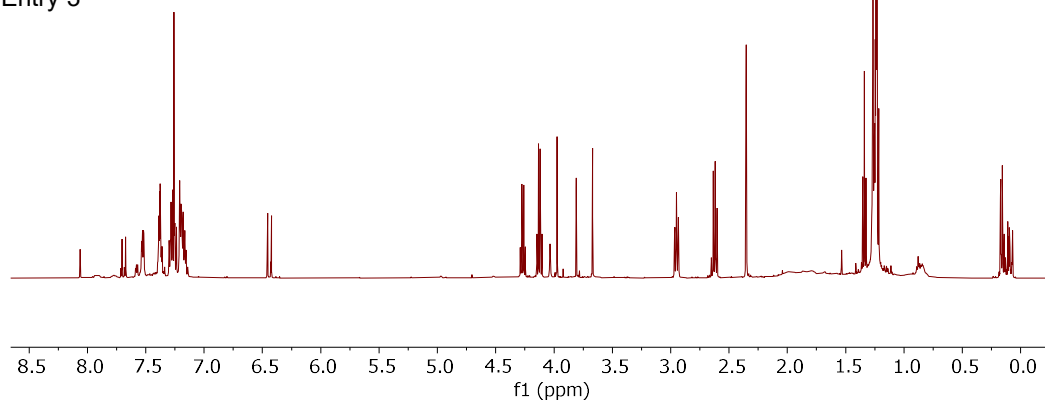
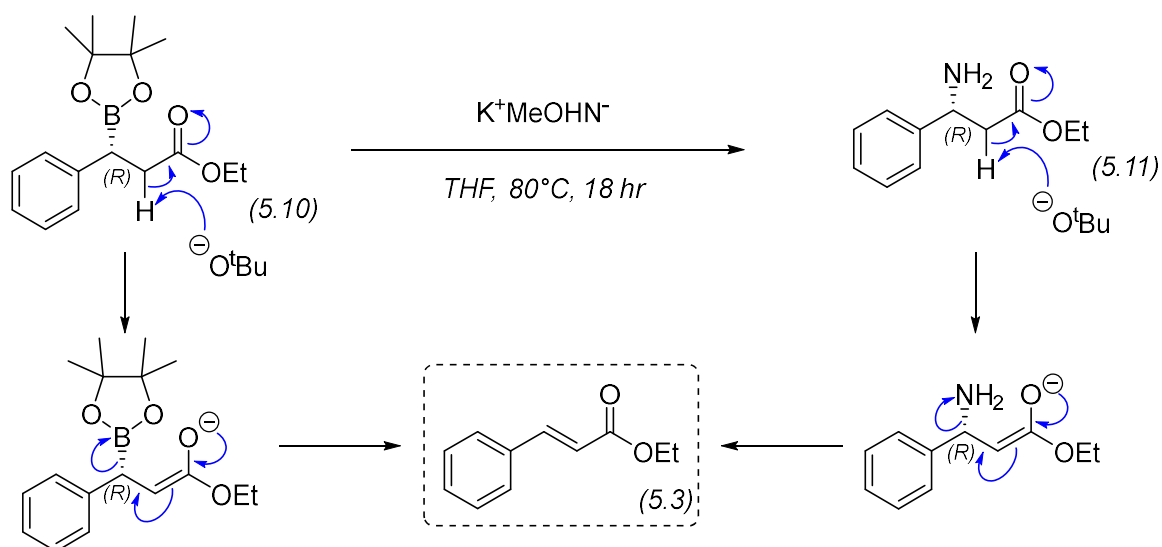


Fig. 62 Comparison of the ^1H NMR spectra of trial reaction 3 (**A-C5-26**) and ethyl cinnamate, showing reformation of the α - β -unsaturated ester as well as an unknown product.

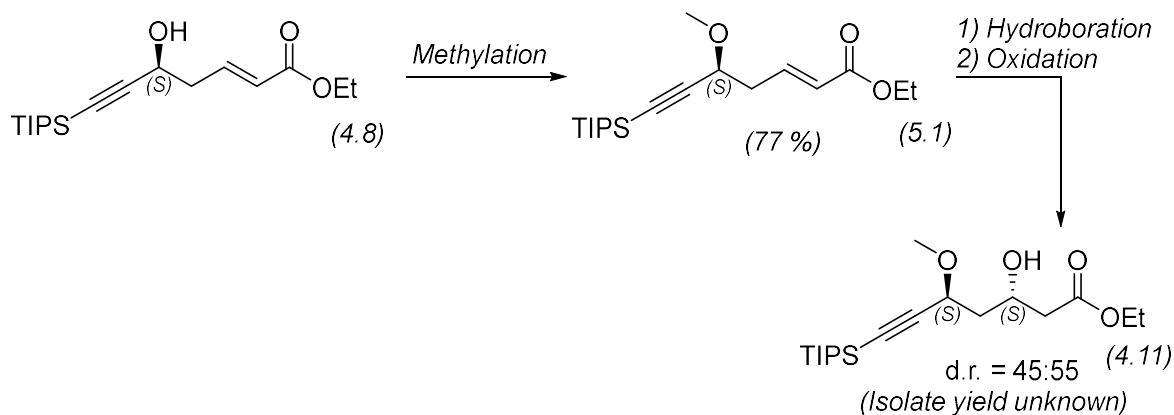


Scheme 109 Trial reaction 3, showing possible de-borylation (*left*) of the starting material (5.10) and de-amination (*right*) of the product (5.11) to return ethyl cinnamate (5.3).

None of the three reactions show particularly promising results, however entries 1 and 2 provide starting points for future reaction optimisation.

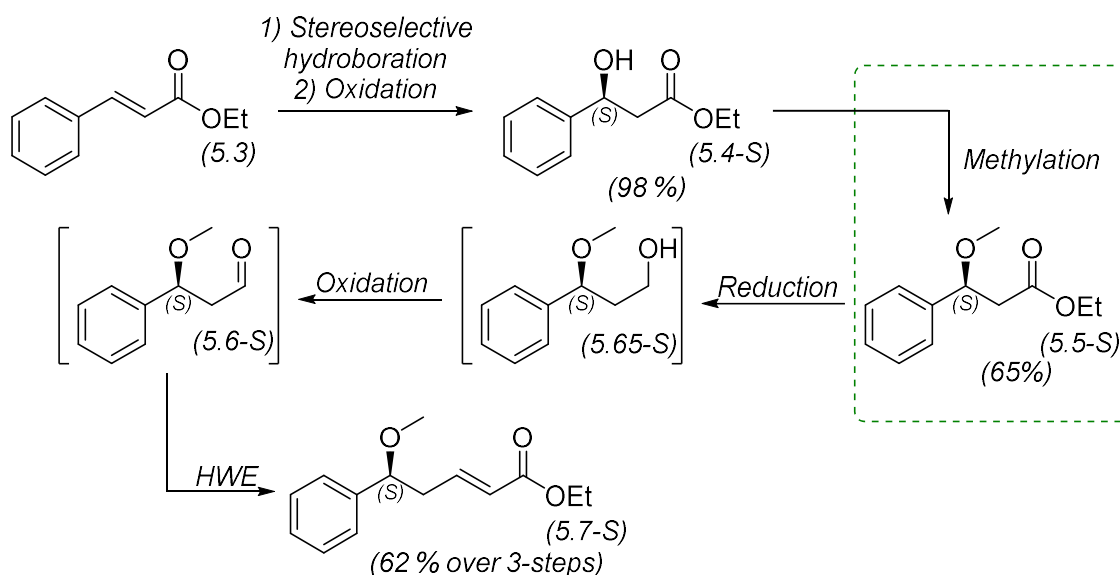
5.4. Conclusions

This approach saw the successful use of previously optimised methylation conditions to give a 77 % yield of ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate, followed by initial attempts towards the hydroboration strategy (**Scheme 110**). This strategy showed high levels of conversion, but low diastereomeric ratios were achieved.



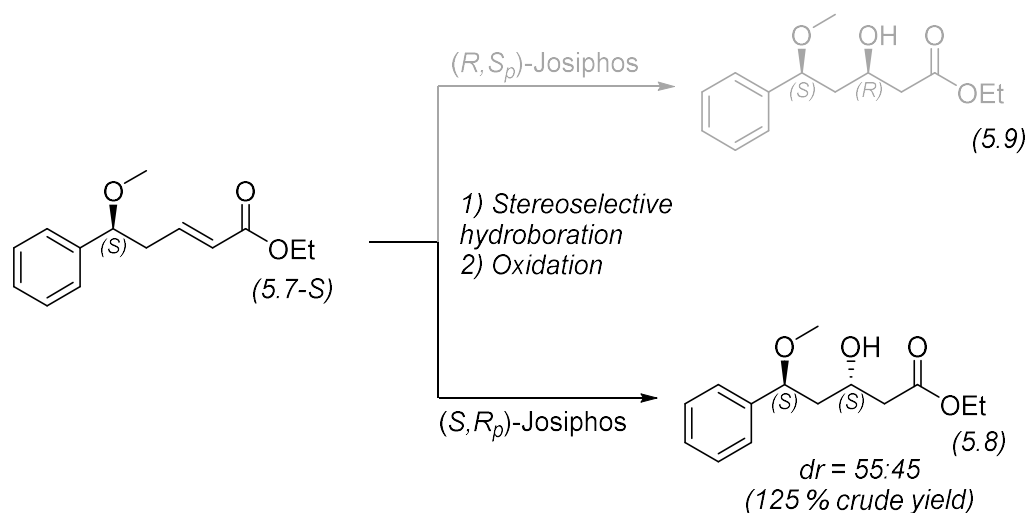
Scheme 110 Formation of ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (4.11) from ethyl (*S,E*)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (4.8) in three steps.

A model (5.7) system was designed and synthesised to further optimise the conditions before continuing the synthetic plan towards 2-deoxygraciline. Synthesis of both enantiomers of the model were performed in 6-steps from ethyl cinnamate (5.3). Hydroboration and oxidative degradation were performed giving a 98 % yield (**Scheme 111**). This was followed by methylation under the previously optimised silver oxide approach. Low yields were obtained, with the highest isolated yield being 70 % for the (*S*)-enantiomer (5.5-*S*). This step may require further optimisation if the substrate is identified as an appropriate model substrate. Reduction to the alcohol (5.65-*S*) and oxidation to the aldehyde (5.6-*S*) were performed without isolating a pure material and were carried forward into a Horner-Wadsworth-Emmons reaction to give a 3-step yield of 62 % for ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7-*S*). Confirmation of synthesis of ethyl (*R,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7-*R*) has been carried out, however, a pure sample has not been obtained. To continue with this approach, a pure sample of ethyl (*R,E*)-5-methoxy-5-phenyl-pent-2-enoate must be obtained and then analysis of the enantiomeric excess will need to be performed, using chiral HPLC to gain accurate measurements.



Scheme 111 Synthetic protocol toward the model substrate from ethyl cinnamate, which requires some optimisation of the methylation approach.

The control hydroboration reaction has been performed on ethyl (*S,E*)-5-methoxy-5-phenylpent-2-enoate (*5.7-S*), using the (*S,R_p*)-Josiphos ligated catalyst (**Scheme 112**). This appeared to give a d.r. of 55:45 by ¹H NMR spectroscopy analysis. The sample was carried through to the oxidative degradation and has been left as a crude sample to be analysed by HPLC for a more accurate determination of the d.r. Currently the d.r. seems to match that of the first attempts on ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (*5.1*), which would suggest that this is a good model system. To confirm this, the reaction should be performed under the exact same conditions, with the opposite ligand enantiomer and comparative analysis carried out. A pure sample of both diastereomers should be obtained to correctly identify the corresponding peaks in the crude analysis of the reactions. If this provides the same d.r., then optimisation of the hydroboration conditions can begin.



Scheme 112 The control hydroboration reaction, which has been performed with the (S, R_p) -Josiphos ligated catalyst but has yet to be performed with the other enantiomer.

Optimisation of the hydroboration conditions should begin with identifying the cause of the low facial selectivity, by testing the same conditions on the TBS protected alcohol (**Fig. 63**, 5.67). If the low facial selectivity is caused by copper co-ordination to the methyl protected alcohol, then this should reduce that and cause an increased d.r. It may then be necessary to test larger protecting groups and/or perform a ligand screen.

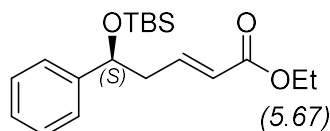
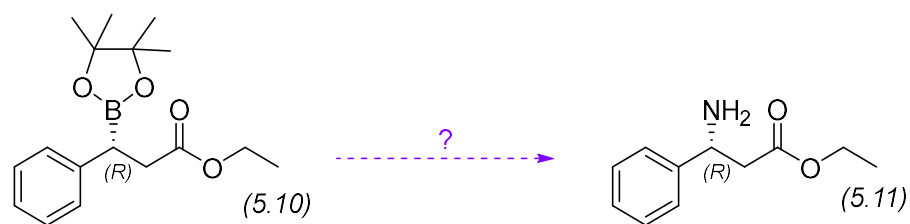


Fig. 63 Ethyl (S, E) -5-(*t*-butyl dimethyl)silyloxy-5-phenyl-pent-2-enoate for use in interference mechanism investigations.

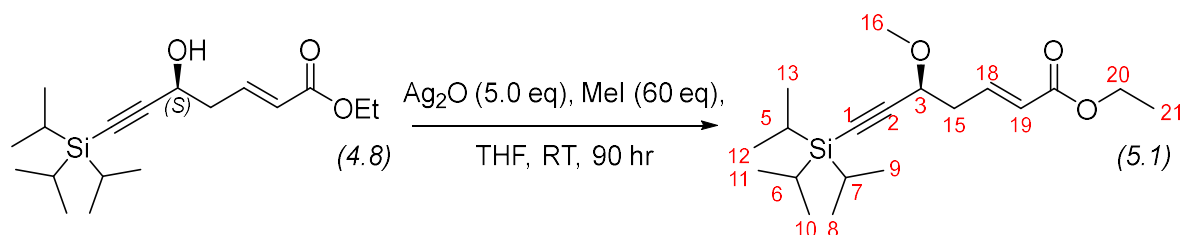
The final goal was to trial some conditions for a hydroboration and amination protocol towards formation of chiral amines from α, β -unsaturated esters (**Scheme 113**). Initial trials were ran using ethyl 3-phenyl-3(*R*)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propanoate (5.10) as a substrate, with none of the test reactions showing obvious promising results. Obtaining a sample of ethyl 3-phenyl-3(*R*)-amino propanoate (5.11) will allow for spiking of reaction NMR samples, to identify if any of these reactions have been successful and if so, which ones should be carried forward into optimisation stages.



Scheme 113 Model system for stereo-retentive amination of α,β -unsaturated esters, successful conditions for which have not yet been identified.

5.5. Experimental Section

Ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**5.1**)



4.8 (1.5 g, 4.62 mmol, 1.0 eq) was dissolved in THF (20 mL). Ag_2O (2.68 g, 11.55 mmol, 2.5 eq) and MeI (13.7 mL, 277.2 mmol, 60.0 eq) was added to the flask. The reaction material was allowed to stir at RT for 90 hr, before being filtered through a Celite® pad. The pad was washed with EtOAc, the filtrate was washed with water (2 × 50 mL), dried over MgSO_4 and concentrated under reduced pressure to yield a yellow oil, which was purified *via* FCC (2–20 % EtOAc in hexanes) to give pure product as a pale-yellow oil (1.21 g, 77 % yield).

See A-C5-1 to A-C5-2

^1H NMR (500 MHz, Chloroform- d_3) δ 6.97 (dt, J = 15.7, 7.2 Hz, 1H, C(18)-H), 5.89 (dt, J = 15.7, 1.5 Hz, 1H, C(19)-H), 4.15 (q, J = 7.2 Hz, 2H, C(20)- H_2), 4.08 (t, J = 6.3 Hz, 1H, C(3)-H), 3.40 (s, 3H, C(16)- H_3), 2.59 (ddt, J = 7.2, 6.3, 1.5 Hz, 2H, C(15)- H_2), 1.25 (t, J = 7.2 Hz, 3H, C(21)- H_3), 1.05 (m, 21H, C(5-7)-H and C(8-13)- H_3).

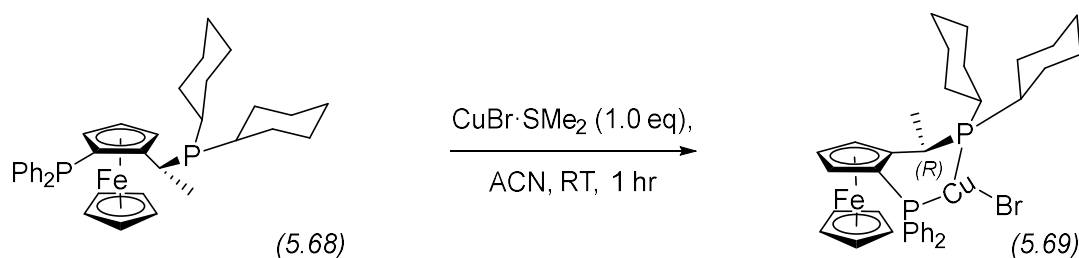
^{13}C NMR (126 MHz, Chloroform- d_3) δ 166.2, 143.5, 124.2, 105.0, 88.2, 70.2, 60.3, 56.4, 38.6, 18.6, 14.3, 11.2.

IR: 2943, 2866 (C-H), 2172 ($\text{C}\equiv\text{C}$), 1721 ($\text{C}=\text{O}$), 1658 ($\text{C}=\text{C}$), 1264.5 (Si-C), 1108.7 (C-O), 882.3 (C-H, Alkene)

HRMS ESI^+ m/z calculated for $\text{C}_{19}\text{H}_{34}\text{O}_3\text{Si}$ ($\text{M} + \text{H}^+$) 339.2311, Found: 339.2403

HRMS ESI^- m/z calculated for $\text{C}_{19}\text{H}_{34}\text{O}_3\text{Si}$ ($\text{M} - ^+\text{CH}_3$) 324.2076, Found: 324.2266

(*R,S*_p)-Josiphos copper (I) bromide (5.69)



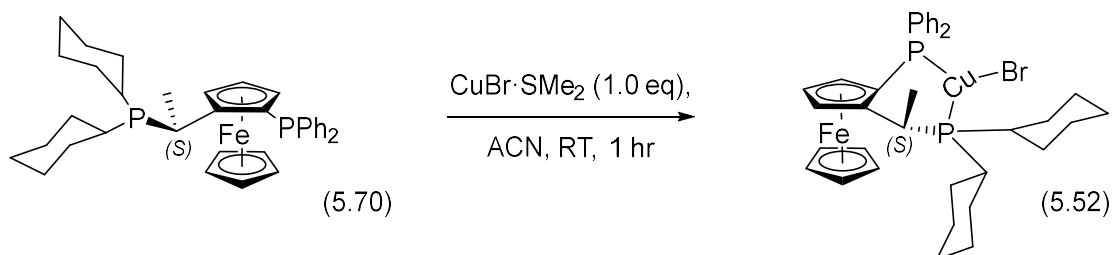
(*R,S*_p)- Josiphos (97.2 mg, 0.16 mmol, 1.0 eq) was charged to a Schlenk tube with CuBr·SMe₂ (34 mg, 0.17 mmol, 1.0 eq). The flask was evacuated and refilled with Argon 3 times before charging anhydrous, degassed acetonitrile (3 mL). The reaction was stirred under argon for 1 hr, before the solvent was removed on the high vacuum line, yielding a fine orange precipitate (118.5 mg, Quant.).

*Spectroscopic data is in line with literature.*¹¹⁷

See A-C5-3

¹H NMR (500 MHz, Chloroform-*d*) δ 8.04-8.00 (m, 2H), 7.49 - 7.48 (m, 3H), 7.28 - 7.23 (m, 5H), 4.52 (br s, 1H), 4.43 (t, *J* = 2.4 Hz, 1H), 4.29 (br s, 1H), 3.79 (s, 5H), 3.49 - 3.45 (m, 1H), 2.08 - 0.80 (22H, m, PCy₂), 1.57 (t, *J* = 7.7 Hz, 3H)

(*S,R*_p)-Josiphos copper (I) bromide (5.52)



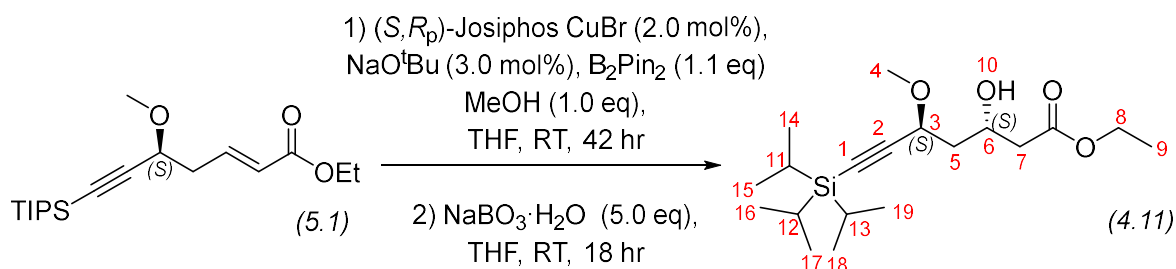
(*S,R*_p)- Josiphos (102 mg, 0.16 mmol, 1.0 eq) was charged to a Schlenk tube with CuBr·SMe₂ (34 mg, 0.17 mmol, 1.0 eq). The flask was evacuated and refilled with Argon 3 times before charging anhydrous, degassed acetonitrile (3 mL). The reaction was stirred under argon for 1 hr, before the solvent was removed on the high vacuum line, to yield a fine orange/brown precipitate (124.8 mg, Quant.).

*Spectroscopic data is in line with literature.*¹¹⁷

See A-C5-4

¹H NMR (500 MHz, Chloroform-*d*) δ 8.04-8.00 (m, 2H), 7.49 - 7.48 (m, 3H), 7.30 - 7.23 (m, 5H), 4.53 (br s, 1H), 4.44 (t, *J* = 2.4 Hz, 1H), 4.29 (br s, 1H), 3.79 (s, 5H), 3.49 - 3.45 (m, 1H), 2.08 - 0.80 (22H, m, PCy₂), 1.57 (t, *J* = 7.8 Hz, 3H)

Ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (4.11)



(*S, R_p*)-Josiphos copper (I) bromide (2.32 mg, 0.00295 mmol, 2.0 mol%) was charged to a Schlenk tube with NaO^tBu (0.43 mg, 0.00444 mmol, 3.0 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (2 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (41.3 mg, 0.163 mmol, 1.1eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.1** (50 mg, 0.148 mmol, 1.0 eq) and methanol (7 μL, 0.163 mmol, 1.0 eq) were charged to the reaction, washing in with THF (2 mL). The reaction was allowed to stir for 42 hr. Completion of reaction was confirmed by ¹H NMR. The reaction material was filtered through Celite[®], and the pad was washed with DCM (10 mL × 3). The filtrate was washed with waster (20 mL × 2) and brine (20 mL × 2) and concentrated under reduced pressure to yield a yellow oil. The oil was redissolved in anhydrous THF (10 mL) and water (5 mL). NaBO₃·H₂O (114 mg, 0.738 mmol, 5.0 eq) was added and the reaction allowed to stir for 18 hr. Water (20 mL) was added and the product was extracted with EtOAc (2 × 20 mL). The organic phases were combined, washed with water (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated crude product as an orange oil, which was analysed without further purification (54 mg, 102 % yield).

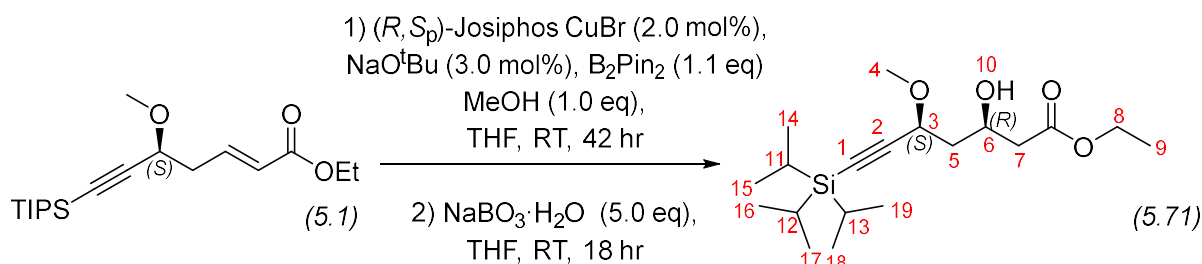
See A-C5-5 to A-C5-6

Approximate d.r. = 45:55

¹H NMR (500 MHz, Chloroform-*d*₃) δ 4.39-4.23 (m, 2H, C(6&3)-H), 4.17 (app. dq, 3H, C(8)-H₂), 3.44 (app. d, 3H, C(4)-H), 3.39 (d, *J* = 2.9 Hz, 0.5H, O(10)-H), 3.26 (d, *J* = 3.5 Hz, 0.5H, O(10)-H), 2.52-2.48 (m, 2H, C(&)-H₂), 2.04 – 1.82 (m, 2H, C(5)-H₂), 1.28-1.26 (t, *J* = 7.0 Hz, 3H, C(9)-H₃), 1.07 (m, 21H, C(11-13)-H and C(14-19)-H₃).

¹³C NMR (126 MHz, Chloroform-*d*₃) δ 172.44, 172.38, 105.7, 105.4, 87.9, 87.6, 70.4, 69.0, 66.8, 65.2, 60.8, 56.6, 56.5, 42.2, 42.2, 41.7, 41.7, 29.8, 25.0, 24.7, 18.8, 18.7, 18.6, 18.6, 18.6, 14.3, 14.3, 11.4, 11.4, 11.4, 11.3

Ethyl 5(*S*)-methoxy-3(*R*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate (5.71)



(*R,S_p*)-Josiphos copper (I) bromide (2.32 mg, 0.00295 mmol, 2.0 mol%) was charged to a Schlenk tube with NaO^tBu (0.43 mg, 0.00444 mmol, 3.0 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (2 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (41.3 mg, 0.163 mmol, 1.1eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.1** (50 mg, 0.148 mmol, 1.0 eq) and methanol (7 μL, 0.163 mmol, 1.0 eq) were charged to the reaction, washing in with THF (2 mL). The reaction was allowed to stir for 42 hr. Completion of reaction was confirmed by ¹H NMR. The reaction material was filtered through Celite[®], and the pad was washed with DCM (10 mL × 3). The filtrate was washed with water (20 mL × 2) and brine (20 mL × 2) and concentrated under reduced pressure to yield a yellow oil. The oil was redissolved in anhydrous THF (10 mL) and water (5 mL). NaBO₃·H₂O (114 mg, 0.738 mmol, 5.0 eq) was added and the reaction allowed to stir for 18 hr. Water (20 mL) was added and the product was extracted with EtOAc (2 × 20 mL). The organic phases were combined, washed with water (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated crude product as an orange oil, which was analysed without further purification (49 mg, 94 % yield).

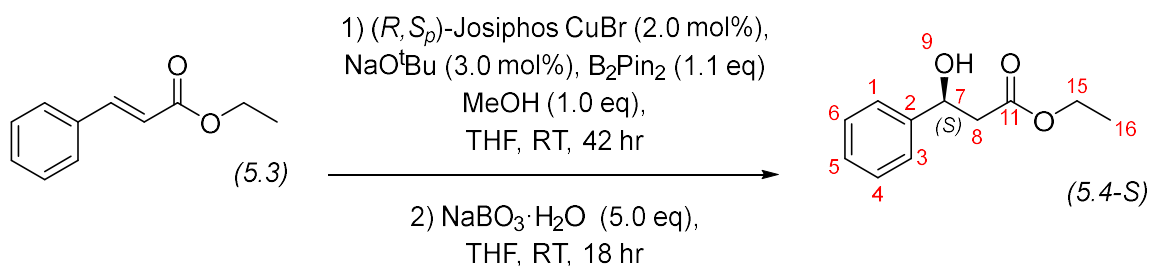
See A-C5-7 to A-C5-8

Approximate d.r. = 55 : 45

¹H NMR (500 MHz, Chloroform-*d*₃) δ 4.39-4.23 (m, 2H, C(6&3)-H), 4.17 (app. dq, 3H, C(8)-H₂), 3.44 (app. d, 3H, C(4)-H), 3.39 (d, *J* = 2.9 Hz, 0.5H, O(10)-H), 3.26 (d, *J* = 3.5 Hz, 0.5H, O(10)-H), 2.52-2.48 (m, 2H, C(&)-H₂), 2.04 – 1.82 (m, 2H, C(5)-H₂), 1.28-1.26 (t, *J* = 7.0 Hz, 3H, C(9)-H₃), 1.07 (m, 21H, C(11-13)-H and C(14-19)-H₃).

¹³C NMR (126 MHz, Chloroform-*d*₃) δ 172.42, 172.37, 105.7, 105.4, 87.8, 87.6, 70.4, 69.0, 66.7, 65.2, 60.8, 56.6, 56.5, 42.2, 42.2, 41.7, 41.7, 29.8, 25.0, 18.8, 18.7, 18.7, 14.3, 11.4, 11.2

Ethyl 3(*S*)-hydroxy-3-phenylpropanoate (5.4-*S*)



(*R,S_p*)-Josiphos CuBr (44.4 mg, 0.056 mmol, 2.0 mol%) was charged to a Schlenk tube with NaO^tBu (8.2 mg, 0.086 mmol, 3.0 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (10 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (793 mg, 3.12 mmol, 1.1 eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.3** (500 mg, 2.84 mmol, 1.0 eq) and Methanol (0.115 mL, 2.84 mmol, 1.0 eq) were charged to the reaction, washing in with THF (5 mL). The reaction was allowed to stir for 42 hr. The reaction material was filtered through Celite[®] and the pad was washed with DCM. The filtrate was washed with water (2 × 20 mL) and brine (2 × 20 mL) and concentrated under reduced pressure to yield a yellow oil. The oil was redissolved in anhydrous THF (10 mL) and water (5 mL). NaBO₃·H₂O (1.12 g, 14.19 mmol, 5.0 eq) was added and the reaction allowed to stir for 18 hr. Water (20 mL) was added and the product was extracted with EtOAc (2 × 20 mL). The organic phases were combined, washed with water (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated crude product as an orange oil, which was carried forward without further purification (541 mg, 98 % yield).

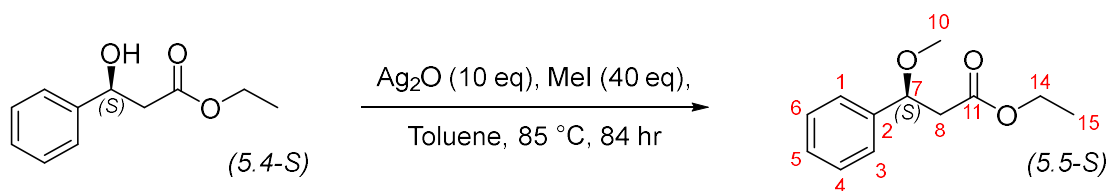
Spectroscopic data is in line with literature.^{126,127}

See A-C5-9 to A-C5-10

¹H NMR (500 MHz, Chloroform-*d*) δ 7.40-7.34 (m, 4H, C(1,3,4,6)-H), 7.31-7.27 (m, 1H, C(5)-H), 5.14 (dt, *J* = 8.9, 3.5 Hz, 1H, C(7)-H), 4.19 (q, *J* = 7.1 Hz, 2H, C(15)-H₂), 3.27 (d, *J* = 3.5 Hz, 1H, O(9)-H), 2.76 (dd, *J* = 16.4, 8.9 Hz, 1H, C(8)-H), 2.71 (dd, *J* = 16.4, 4.0 Hz, 1H, C(8)-H), 1.27 (t, *J* = 7.1 Hz, 3H, C(16)-H₃)

¹³C NMR (126 MHz, Chloroform-*d*) δ 172.5, 142.6, 128.7, 127.9, 125.78, 70.4, 70.0, 43.4, 25.0, 14.3

Ethyl 3(*S*)-methoxy-3-phenylpropanoate (5.5-*S*)



5.4-*S* (406.0 mg, 2.09 mmol, 1.0 eq) was dissolved in anhydrous toluene. Ag_2O (2.42 g, 10.45 mmol, 5.0 eq) and MeI (2.6 mL, 41.8 mmol, 20 eq) were added and the reaction sealed under argon. The reaction material was heated to $T_{\text{ext}} = 85\text{ }^\circ\text{C}$ and stirred for 42 hr, cooled to RT and filtered through Celite[®]. The solvent was removed, and the residue was taken up in anhydrous toluene. Ag_2O (2.42 g, 10.45 mmol, 5.0 eq) and MeI (2.6 mL, 41.8 mmol, 20 eq) were added, and the reaction sealed under argon. The reaction material was heated to $T_{\text{ext}} = 85\text{ }^\circ\text{C}$ and stirred for 42 hr. Upon completion of reaction the solids were removed *via* filtration through Celite[®]. The filtrate was concentrated under reduced pressure and dried under high vacuum. The residue was purified by FCC (10-20 % EtOAc in hexanes) to give pure product as a yellow oil (283 mg, 70 % yield).

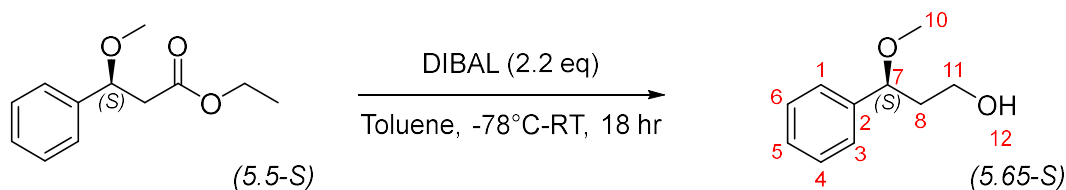
*Spectroscopic data is in line with literature.*¹²⁸

See A-C5-11 to A-C5-12

¹H NMR (500 MHz, Chloroform-*d*) δ 7.33 (m, 5H, C(1, 3-6)-H), 4.63 (dd, 1H, $J = 4.8, 9.3$ Hz, C(7)-H), 4.15 (m, 2H, C(14)-H₂), 3.23 (s, 3H, C(10)-H₃), 2.80 (dd, 1H, $J = 9.2, 15.4$ Hz, C(8)-H₂), 2.57 (dd, 1H, $J = 4.8, 15.4$ Hz, C(8)-H), 1.24 (t, 3H, $J = 7.2$ Hz, C(15)-H₃)

¹³C NMR (126 MHz, Chloroform-*d*) δ 171.1, 140.7, 128.7, 128.1, 126.8, 80.2, 60.7, 57.0, 43.7, 14.3.

3(*S*)-Methoxy-3-phenylpropanol (5.65-*S*)



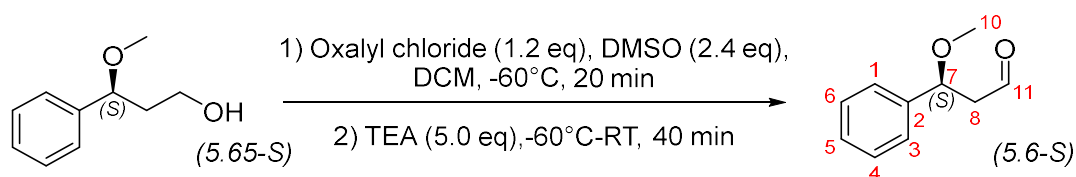
5.5-*S* (168 mg, 0.81 mmol, 1.0 eq) was dissolved in anhydrous, degassed toluene (5 mL) and cooled to -78°C . DIBAL (1.45 mL, 1.2 M in Tol., 1.77 mmol, 2.2 eq) was charged dropwise, while the T_{ext} was maintained at $< -65^{\circ}\text{C}$. The reaction was allowed to slowly warm to RT and stirred for 18 hr. The reaction material was quenched with water and extracted with EtOAc (2×25 mL). The organic phases were combined, washed with water and brine, dried over MgSO_4 and concentrated under reduced pressure to yield a yellow oil.

*Spectroscopic data is in line with literature.*¹²⁹

See A-C5-13

^1H NMR (500 MHz, Chloroform-*d*) δ 7.33 (m, 5H, C(1, 3-6)-H), 4.39 (dd, 1H, $J = 4.1, 9.0$ Hz, C(7)-H), 3.78 (m, 2H, C(11)-H₂), 3.24 (s, 3H, C(10)-H₃), 2.03 (dd, 1H, C(8)-H₂), 1.87 (dd, 1H, C(8)-H₂).

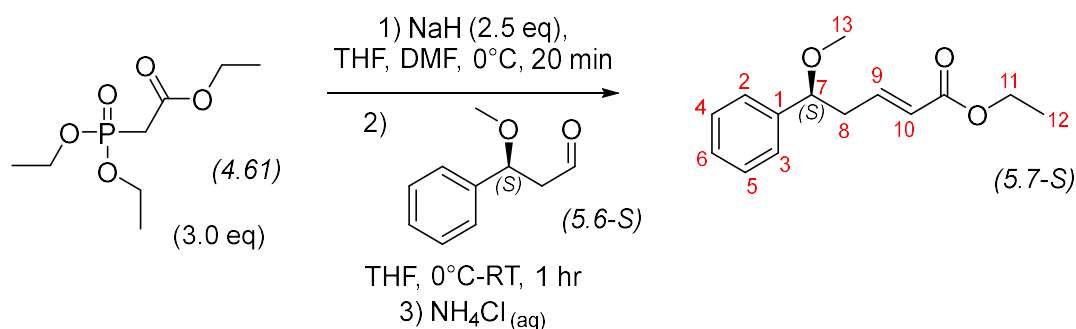
3(*S*)-Methoxy-3-phenylpropanal (5.6-*S*)



Freshly distilled oxalyl chloride (84 μ L, 0.97 mmol, 1.2 eq) was dissolved in anhydrous DCM (0.5 mL) and cooled to -60 °C. DMSO (137 μ L, 1.93 mmol, 2.4 eq) in a solution of anhydrous DCM (0.5 mL) was added dropwise and the reaction material was allowed to stir for 20 min. A solution of **5.65-S** (mass assumed to be 134 mg, 0.81 mmol, 1.0 eq) in anhydrous DCM (0.5 mL) was added dropwise and the reaction material was allowed to stir for a further 20 min. TEA (566 μ L, 4.03 mmol, 5.0 eq) was charged to the reaction material dropwise and the reaction allowed to stir for 10 min before warming to RT and stirring for a further 30 min. The reaction was quenched with water (25 mL), extracted with EtOAc (2 $\times$ 50 mL) and the organic phase was washed with saturated NaHCO₃ solution. The solution was dried over Na₂SO₄ and concentrated to yield a yellow oil.

Due to inherent aldehyde instability, the crude product was carried forward without further purification or analysis.

Ethyl (*S,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7-*S*)



NaH (80.7 mg, 2.02 mmol, 60 wt% in mineral oil, 2.5 eq) was washed with anhydrous, degassed THF (5 mL $\times$ 4), taken up in anhydrous, degassed THF (1.3 mL) and cooled to 0 °C. Triethyl phosphonoacetate (480 μ L, 2.42 mmol, 3.0 eq) was dissolved in anhydrous DMF (1.3 mL) and added dropwise to the NaH solution. The reaction material was allowed to stir at 0 °C until no further effervescence. A solution of **3(*S*)-methoxy-3-phenylpropanal** in THF (1.3 mL) was added dropwise to the ylide solution. The reaction material was warmed to RT and stirred for 1 hr. NH₄Cl_(aq) (3 mL, saturated sol.) was added dropwise, alongside water (10 mL). The reaction material was extracted with EtOAc (25 mL $\times$ 2). Organic phases were combined, washed with 1/3 saturated brine solution (10 mL $\times$ 2), dried over MgSO₄ and concentrated. Crude product was purified by FCC (10-20 % Et₂O in Hexanes) to yield pure product as a yellow oil (117 mg, 62 % 3-step yield from Ethyl 3(*S*)-methoxy-3-phenylpropanoate).

*Spectroscopic data is in line with literature.*¹³⁰

See A-C5-14

¹H NMR (500 MHz, Chloroform-*d*) δ 7.38-7.27 (m, 5H, C(2-6)-H), 6.94 (dt, *J* = 15.6, 7.1 Hz, 1H, C(10)-H), 5.84 (dt, *J* = 15.6, 1.5 Hz, 1H, C(9)-H), 4.24 (dd, *J* = 8.1, 5.0 Hz, 1H, C(7)-H), 4.17 (q, *J* = 7.2 Hz, 2H, C(11)-H₂), 3.22 (s, 3H, C(13)-H₃), 2.72-2.49 (m, 2H, C(8)-H₂), 1.27 (t, *J* = 7.2 Hz, 3H, C(12)-H₃)

Ethyl 5(*S*)-methoxy-3(*S*)-hydroxy-5-phenyl-pentanoate (5.8)

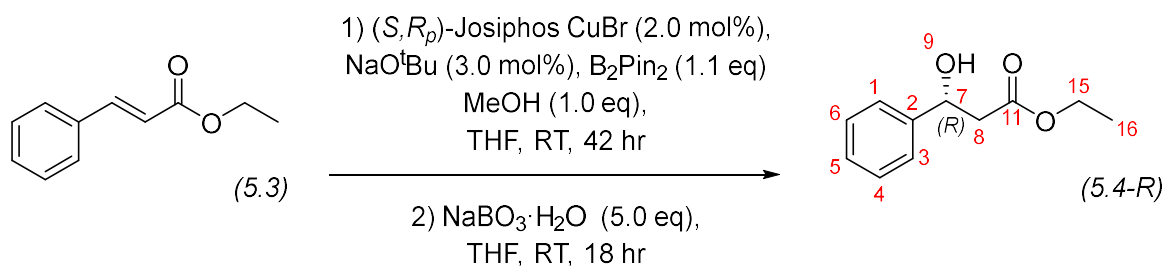


(*S,R_p*)-Josiphos CuBr (7.8 mg, 0.0099 mmol, 2.0 mol%) was charged to a Schlenk tube with NaO^tBu (1.4 mg, 0.015 mmol, 3.0 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (3 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (138 mg, 0.54 mmol, 1.1 eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.7-*S*** (116 mg, 0.495 mmol, 1.0 eq) and Methanol (16 μ L, 0.495 mmol, 1.0 eq) were charged to the reaction, washing in with THF (3 mL). The reaction was allowed to stir for 18 hr. The reaction material was filtered through Celite[®] and the pad was washed with DCM. The filtrate was washed with water (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL) and concentrated under reduced pressure to yield a yellow oil. The oil was redissolved in anhydrous THF (3 mL) and water (1.5 mL). NaBO₃·H₂O (247 mg, 2.48 mmol, 5.0 eq) was added and the reaction allowed to stir for 18 hr. Water (20 mL) was added and the product was extracted with EtOAc (2 $\times$ 20 mL). The organic phases were combined, washed with water (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated crude product as a brown oil (135 mg, 125 % crude yield).

See A-C5-16

¹H NMR (500 MHz, Chloroform-*d*) δ 7.31-7.14 (m, 5H, C(2-6)-H), 4.41-3.99 (m, 4H, C(7)-H, C(9)-H and C(11)-H₂), 3.18-3.08 (m, 3H, C(18)-H₃), 2.47-2.28 (m, 2H, C(8)-H₂ or C(10)-H₂), 1.96-1.66 (m, 2H, C(8)-H₂ or C(10)-H₂), 1.12 (t, *J* = 7.1 Hz, 3H, C(12)-H₃)

Ethyl 3(*R*)-hydroxy-3-phenylpropanoate (5.4-*R*)



(*S,R_p*)-Josiphos CuBr (44.4 mg, 0.056 mmol, 2.0 mol%) was charged to a Schlenk tube with NaO^tBu (8.2 mg, 0.086 mmol, 3.0 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (10 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (793 mg, 3.12 mmol, 1.1 eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.3** (500 mg, 2.84 mmol, 1.0 eq) and Methanol (0.115 mL, 2.84 mmol, 1.0 eq) were charged to the reaction, washing in with THF (5 mL). The reaction was allowed to stir for 42 hr. The reaction material was filtered through Celite[®] and the pad was washed with DCM. The filtrate was washed with water (2 × 20 mL) and brine (2 × 20 mL) and concentrated under reduced pressure to yield a yellow oil. The oil was redissolved in anhydrous THF (10 mL) and water (5 mL). NaBO₃·H₂O (1.12 g, 14.19 mmol, 5.0 eq) was added and the reaction allowed to stir for 18 hr. Water (20 mL) was added and the product was extracted with EtOAc (2 × 20 mL). The organic phases were combined, washed with water (20 mL) and brine (20 mL), dried over MgSO₄ and concentrated crude product was purified by FCC (20 % Et₂O in hexanes) to give a yellow oil (501.4 mg, 92 % yield).

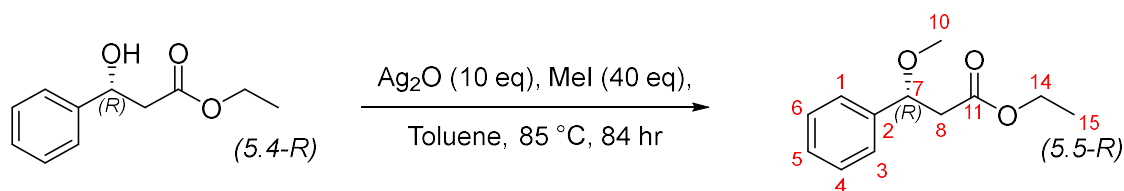
Spectroscopic data is in line with literature.^{126,127}

See A-C5-17 to A-C5-18

¹H NMR (500 MHz, Chloroform-*d*) δ 7.39-7.34 (m, 4H, C(1,3,4,6)-H), 7.31-7.27(m, 1H, C(5)-H), 5.13 (dt, *J* = 9.1, 3.3 Hz, 1H, C(7)-H), 4.19 (q, *J* = 7.2 Hz, 2H, C(15)-H₂), 3.27 (d, *J* = 3.3 Hz, 1H, O(9)-H), 2.76 (dd, *J* = 16.4, 9.1 Hz, 1H, C(8)-H), 2.71 (dd, *J* = 16.4, 4.0 Hz, 1H, C(8)-H), 1.27 (t, *J* = 7.1 Hz, 3H, C(16)-H₃)

¹³C NMR (126 MHz, Chloroform-*d*) δ 172.5, 142.6, 128.7, 127.9, 125.78, 70.4, 70.0, 43.4, 25.0, 14.3

Ethyl 3(*R*)-methoxy-3-phenylpropanoate (5.5-*R*)



5.4-*R* (405.5 mg, 2.09 mmol, 1.0 eq) was dissolved in anhydrous toluene. Ag_2O (2.42 g, 10.44 mmol, 5.0 eq) and MeI (2.6 mL, 41.8 mmol, 20 eq) were added and the reaction sealed under argon. The reaction material was heated to $T_{\text{ext}} = 85\text{ }^\circ\text{C}$ and stirred for 42 hr, cooled to RT and filtered through Celite[®]. The solvent was removed, and the residue was taken up in anhydrous toluene. Ag_2O (2.42 g, 10.44 mmol, 5.0 eq) and MeI (2.6 mL, 41.8 mmol, 20 eq) were added, and the reaction sealed under argon. The reaction material was heated to $T_{\text{ext}} = 85\text{ }^\circ\text{C}$ and stirred for 42 hr. Upon completion of reaction the solids were removed *via* filtration through Celite[®]. The filtrate was concentrated under reduced pressure and dried under high vacuum. The residue was purified by FCC (10-20 % EtOAc in hexanes) to give pure product as a yellow oil (301 mg, 65 % yield).

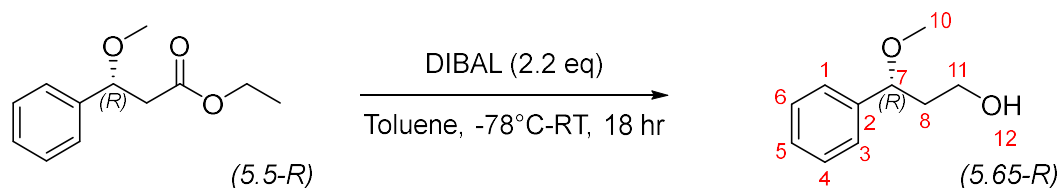
*Spectroscopic data is in line with literature.*¹²⁸

See A-C5-19 to A-C5-20

^1H NMR (500 MHz, Chloroform-*d*) δ 7.33 (m, 5H, C(1, 3-6)-H), 4.63 (dd, 1H, $J = 4.8, 9.2$ Hz, C(7)-H), 4.15 (m, 2H, C(14)-H₂), 3.23 (s, 3H, C(10)-H₃), 2.80 (dd, 1H, $J = 9.2, 15.4$ Hz, C(8)-H₂), 2.57 (dd, 1H, $J = 4.8, 15.4$ Hz, C(8)-H), 1.24 (t, 3H, $J = 7.2$ Hz, C(15)-H₃)

^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.1, 140.7, 128.7, 128.1, 126.8, 80.2, 60.7, 57.0, 43.7, 14.3.

3(*R*)-Methoxy-3-phenylpropanol (5.65-*R*)



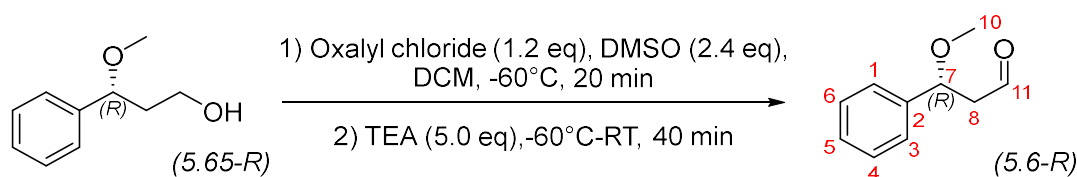
5.5-*R* (210 mg, 1.01 mmol, 1.0 eq) was dissolved in anhydrous, degassed toluene (5 mL) and cooled to -78 °C. DIBAL 1.7 mL, 1.2 M in Tol., 2.02 mmol, 2.0 eq) was charged dropwise, while the T_{ext} was maintained at < - 65 °C. The reaction was allowed to slowly warm to RT and stirred for 18 hr. The reaction material was quenched with water and extracted with EtOAc (2 × 25 mL). The organic phases were combined, washed with water and brine, dried over MgSO₄ and concentrated under reduced pressure to yield a yellow oil.

*Spectroscopic data is in line with literature.*¹²⁹

See A-C5-21

¹H NMR (500 MHz, Chloroform-*d*) δ 7.33 (m, 5H, C(1, 3-6)-H), 4.39 (dd, 1H, J = 4.1, 9.0 Hz, C(7)-H), 3.78 (m, 2H, C(11)-H₂), 3.24 (s, 3H, C(10)-H₃), 2.03 (m, 1H, C(8)-H₂), 1.87 (m, 1H, C(8)-H₂).

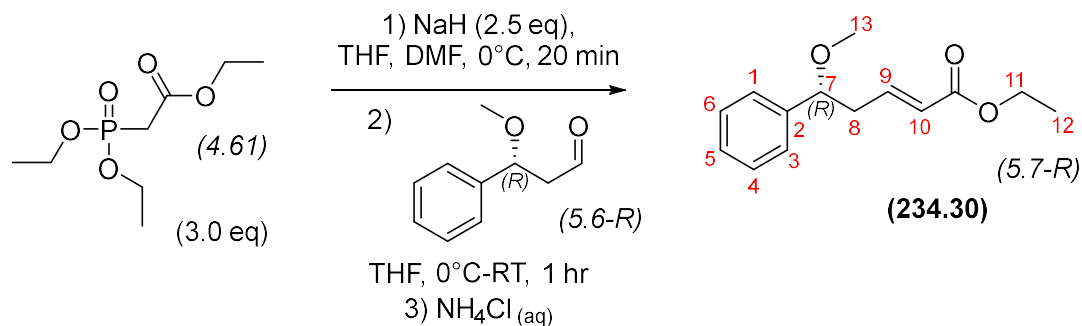
3(*R*)-Methoxy-3-phenylpropanal (5.6-*R*)



Freshly distilled oxalyl chloride (105 μ l, 1.21 mmol, 1.2 eq) was dissolved in anhydrous DCM (0.5 mL) and cooled to -60 °C. DMSO (172 μ L, 2.42 mmol, 2.4 eq) in a solution of anhydrous DCM (0.5 mL) was added dropwise and the reaction material was allowed to stir for 20 min. A solution of **5.65-*R*** (167 mg, 1.01 mmol, 1.0 eq) in anhydrous DCM (0.5 mL) was added dropwise and the reaction was allowed to stir for a further 20 min. TEA (708 μ L, 5.04 mmol, 5.0 eq) was charged to the reaction dropwise and the reaction allowed to stir for 10 min before warming to RT and stirring for a further 30 min. The reaction was quenched with water (25 mL), extracted with EtOAc (2 $\times$ 50 mL) and the organic phase was washed with saturated NaHCO₃ solution. The solution was dried over Na₂SO₄ and concentrated to yield a yellow oil.

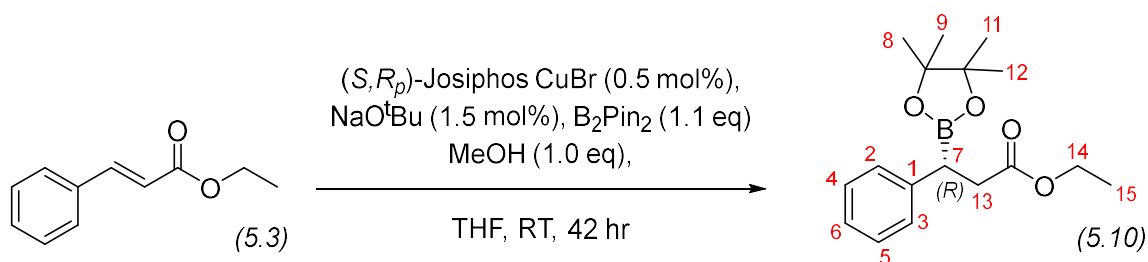
Due to inherent aldehyde instability, the crude product was carried forward without further purification or analysis.

Ethyl (*R,E*)-5-methoxy-5-phenyl-pent-2-enoate (5.7-*R*)



NaH (100 mg, 2.52 mmol, 60 wt% in mineral oil, 2.5 eq) was washed with anhydrous, degassed THF (5 mL $\times$ 4), taken up in anhydrous, degassed THF (1.3 mL) and cooled to 0 °C. Triethyl phosphonoacetate (600 μ L, 3.052 mmol, 3.0 eq) was dissolved in anhydrous DMF (1.3 mL) and added dropwise to the NaH solution. The reaction material was allowed to stir at 0 °C until no further effervescence. A solution of **3(*R*)-methoxy-3-phenylpropanal** in THF (1.3 mL) was added dropwise to the ylide solution. The reaction material was warmed to RT and stirred for 1 hr. NH₄Cl_(aq) (3 mL, saturated sol.) was added dropwise, alongside water (10 mL). The reaction material was extracted with EtOAc (25 mL $\times$ 2). Organic phases were combined, washed with 1/3 saturated brine solution (10 mL $\times$ 2), dried over MgSO₄ and concentrated to give crude product as a yellow oil (324 mg) which has not been purified.

Ethyl 3-phenyl-3(R)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) propanoate (5.10)



(S,R_p)-Josiphos CuBr (11.1 mg, 0.014 mmol, 0.5 mol%) was charged to a Schlenk tube with NaO^tBu (4.09 mg, 0.043 mmol, 1.5 mol%) and the flask was evacuated and refilled with argon three times. Degassed, anhydrous THF (10 mL) was charged, and the reaction material was allowed to stir for 5 min. B₂Pin₂ (793 mg, 3.12 mmol, 1.1 eq) was charged and the reaction allowed to stir for a further 5 minutes. **5.3** (500 mg, 2.84 mmol, 1.0 eq) and Methanol (0.115 mL, 2.84 mmol, 1.0 eq) were charged to the reaction, washing in with THF (5 mL). The reaction was allowed to stir for 42 hr. The reaction material was filtered through Celite[®] and the pad was washed with DCM. The filtrate was washed with water (4 × 20 mL) and brine (4 × 20 mL) and concentrated under reduced pressure to yield a yellow oil (734 mg, 84 % yield).

*Spectroscopic data is in line with literature.*¹³¹

See A-C5-22

¹H NMR (500 MHz, Chloroform-*d*) δ 7.26-7.20 (m, 4H, C(2-5)-H), 7.16-7.12 (m, 1H, C(6)-H), 4.10 (m, 2H, C(14)-H₂), 2.87 (dd, *J* = 16.3, 10.1 Hz, 1H, C(13)-H), 2.74 (dd, *J* = 10.1, 6.2 Hz, 2H, C(7)-H), 2.64 (dd, *J* = 16.3, 6.2 Hz, 1H, C(13)-H), 1.21 (t, *J* = 7.0 Hz, 3H, C(15)-H₃), 1.21 (s, 6H, C(8-9)-H₃ or C(10-11)-H₃), 1.16 (s, 6H, C(8-9)-H₃ or C(10-11)-H₃)

Chapter 6: Conclusions and future work

The overall goal of total synthesis of Nemamides and Euglenatides was not achievable in the timeframe of this project, therefore, the aim of this project was to design a route towards the compounds and make synthetic progress towards the first target in the series. This was carried out by breaking the molecule into two target compounds; the cyclic pentapeptide and the polyketide bound unnatural amino acid (**Fig. 64**). Synthetic methods of macrocyclisation are to be trialled on the model cyclic systems, so a simple and scalable route towards the linear chain was devised. A retrosynthetic analysis was carried out on the target unnatural amino acid, which provided two asymmetric approaches towards its synthesis. Unexpected challenges were faced within the synthesis of each target; however, significant steps have been made towards successful synthesis of these three target compounds.

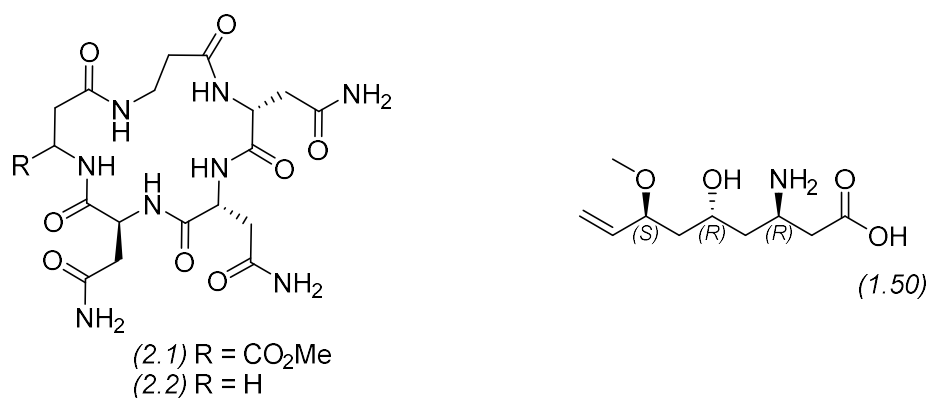
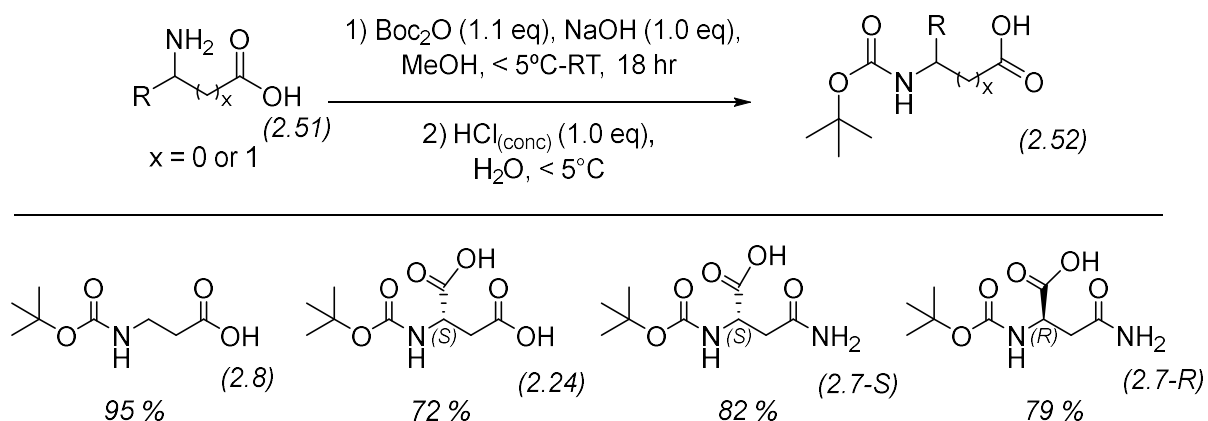
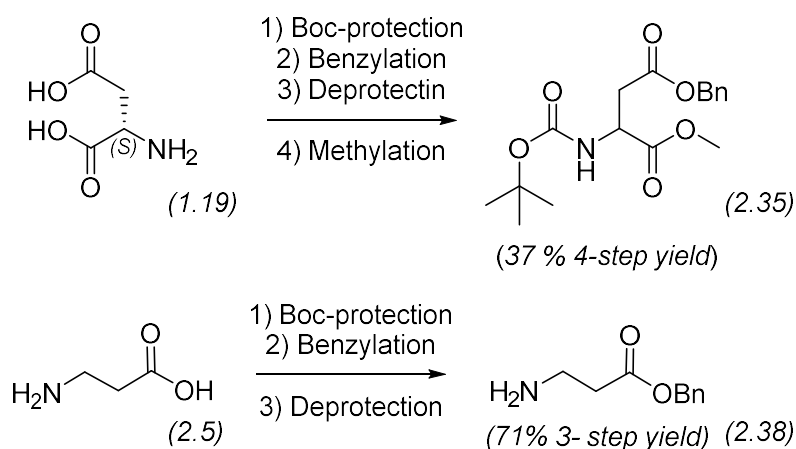


Fig. 64 Model cyclic pentapeptide targets (2.1 and 2.2) and the target advanced intermediate of 2-deoxygraciline (1.50).

Cheap and efficient strategies towards the *N*-protection of amino acids with Boc anhydride have been optimised, allowing easy access to the necessary protected amino acids with high yields (**Scheme 114**). Multiple methods were attempted towards differential *C*-protection of aspartic acid; one of which provided the desired compound in 4 simple steps, requiring purification at only two of these stages (**Scheme 115**). *C*-Terminal protection of β -alanine was achieved using the optimised benzylation protocol, providing protected versions of the two linker AAs for the synthesis of the peptide models.

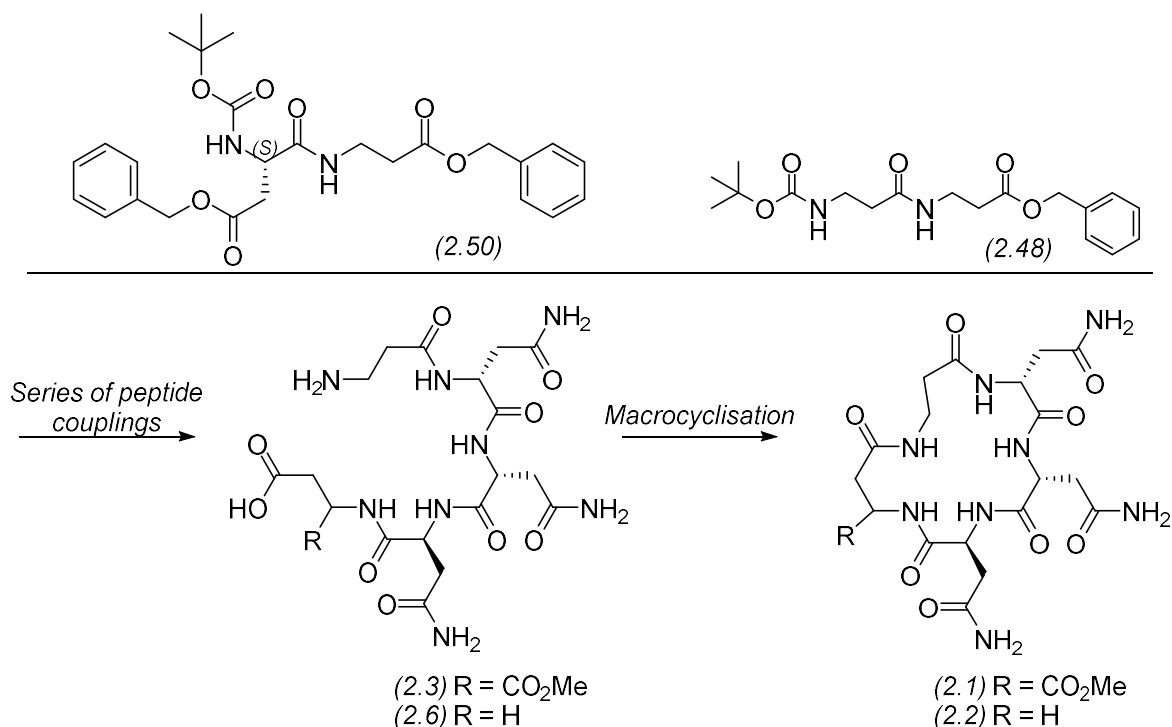


Scheme 114 Optimised method of N-protection with Boc anhydride.



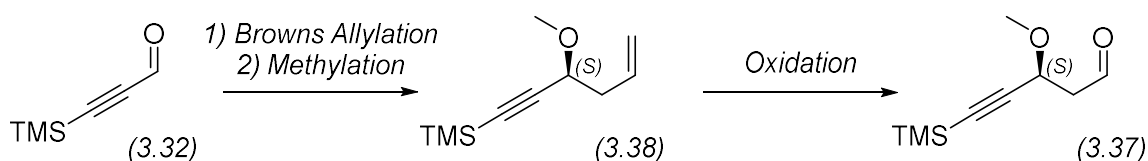
Scheme 115 Optimised synthetic steps towards the C-protection of the two linker AAs in the model systems.

Having accessed the correctly protected amino acids, coupling strategies were trialled by making two dipeptide systems. Future work on these targets will see the synthesis of the two linear model peptides using the optimised reaction conditions for dipeptide synthesis (**Scheme 116**). These products will then be used to test macrocyclisation conditions, creating the two target model peptide systems.



Scheme 116 Two dipeptides synthesised in peptide coupling trials and the future goal to synthesise the linear pentapeptides to trial macrocyclisation techniques.

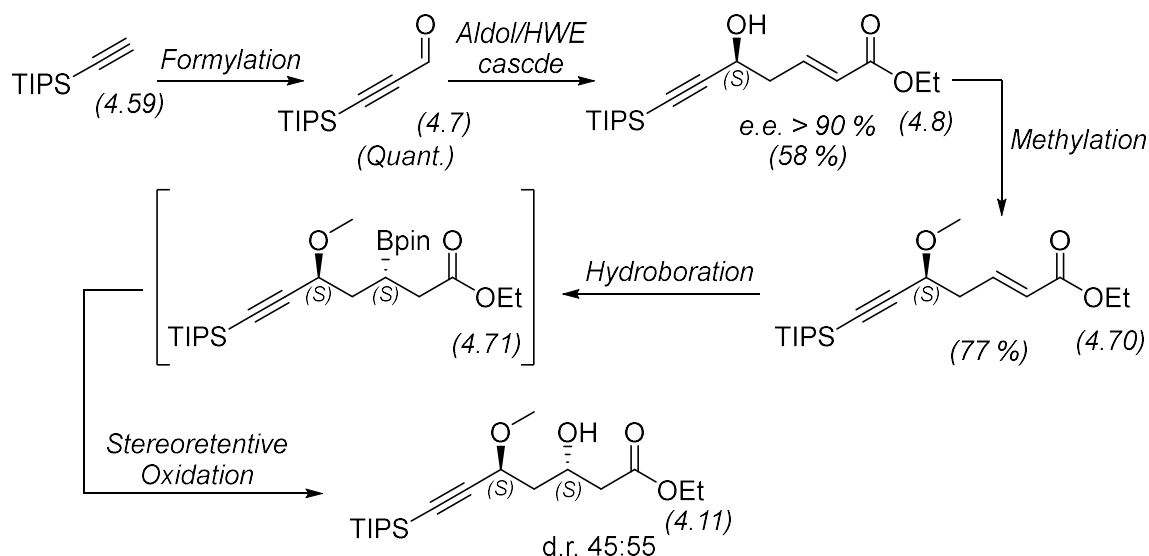
Significant efforts were made towards a step-wise asymmetric synthesis of 2-deoxygraciline. An initial route utilising Brown allylation generally showed a poor scale up profile due to issues with reagent volatility, reaction safety and poor atom economy (**Scheme 117**). While efforts could be made to improve upon some of these pitfalls, these problems will lead to an inefficient route and so no future work will be carried out on this method.



Scheme 117 First steps in the initial synthetic plan towards 2-deoxygraciline.

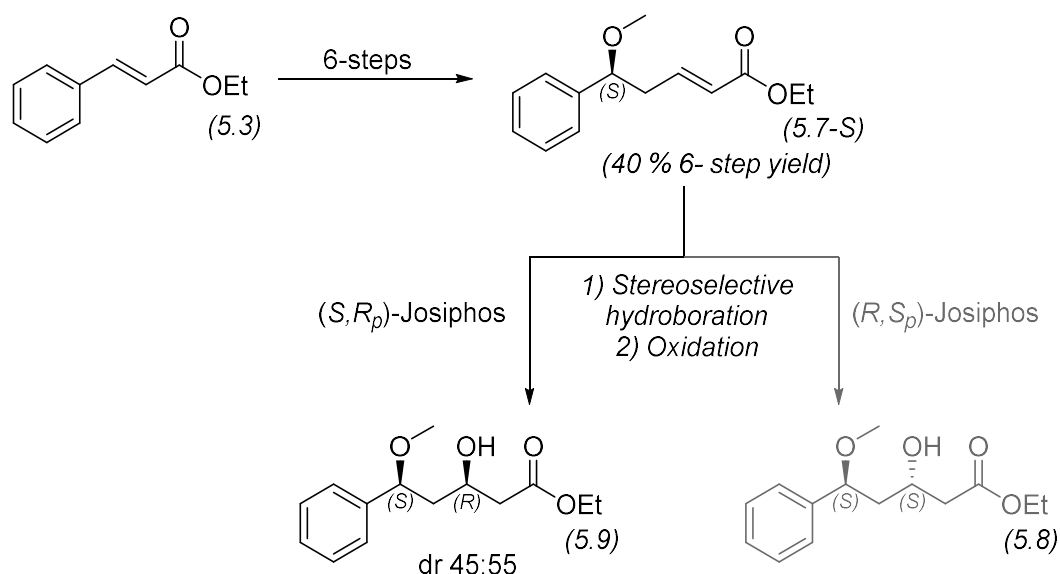
A more efficient approach was designed, and early synthetic stages were successfully carried out; beginning with formylation of a simple and cheap protected alkyne (**Scheme 118**). The aldehyde was then subjected to an asymmetric aldol reaction, followed by a HWE to synthesise the chiral α,β -unsaturated ester with an e.e. of > 90 %. Methylation of the alcohol was carried out using MeI and silver oxide, giving the key intermediate. A method to install the second point of chirality was devised using a catalyst controlled hydroboration and

stereo-retentive oxidative degradation. This method provided the 1,3-methoxy diol with a low diastereomeric ratio, requiring optimisation.



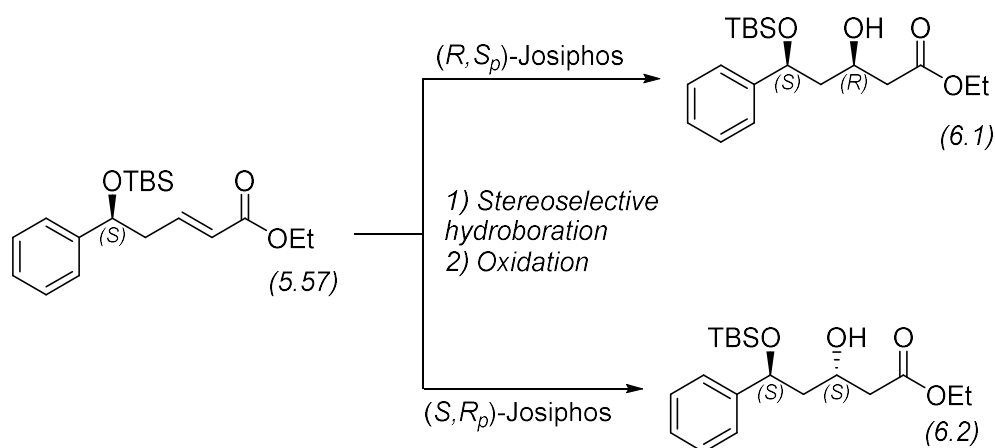
Scheme 118 Initial steps of the synthetic protocol towards 2-deoxygraciline.

Work was carried out to design and synthesise a model system which could be used to optimise the facial selectivity of the hydroboration and improve this diastereomeric ratio. The model was successfully synthesised in six steps, and a control reaction was performed (**Scheme 119**). Initial analysis of the crude reaction product showed very similar selectivity as with ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate, which identifies this to be a good model system for optimisation of the approach. Future work will focus on isolation of the opposite diastereomer to confirm correct peak assignment has been made, thereby confirming the diastereomeric ratio.

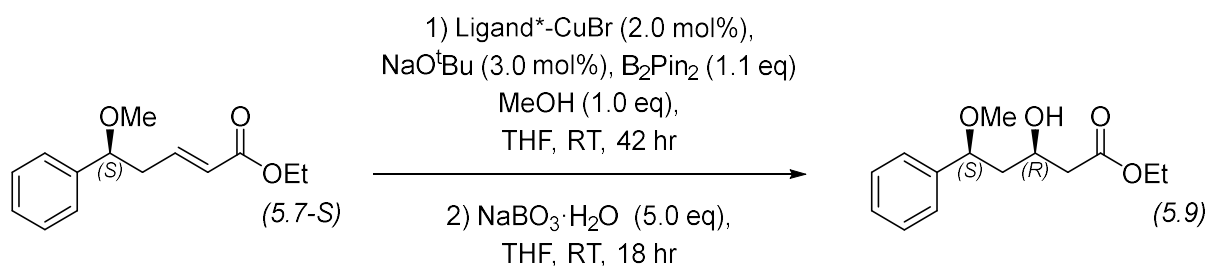


Scheme 119 The model system for hydroboration optimisation, synthesised in a 40 % 6- step yield from ethyl cinnamate; with the initial control reaction giving a 45:55 d.r.

The mechanism of chiral selectivity will then be probed by synthesis of the TBS protected derivative, and performing the same control hydroboration conditions (**Scheme 120**). This will confirm whether the alcohol is interfering with the catalyst's facial selectivity by copper-oxygen co-ordination. Optimisation of the hydroboration approach will then be carried out, focusing first on a ligand screen (**Scheme 121**). If a ligand is not found that significantly increases the d.r., then it will be necessary to consider other alcohol protecting groups or employ a different metal catalyst.

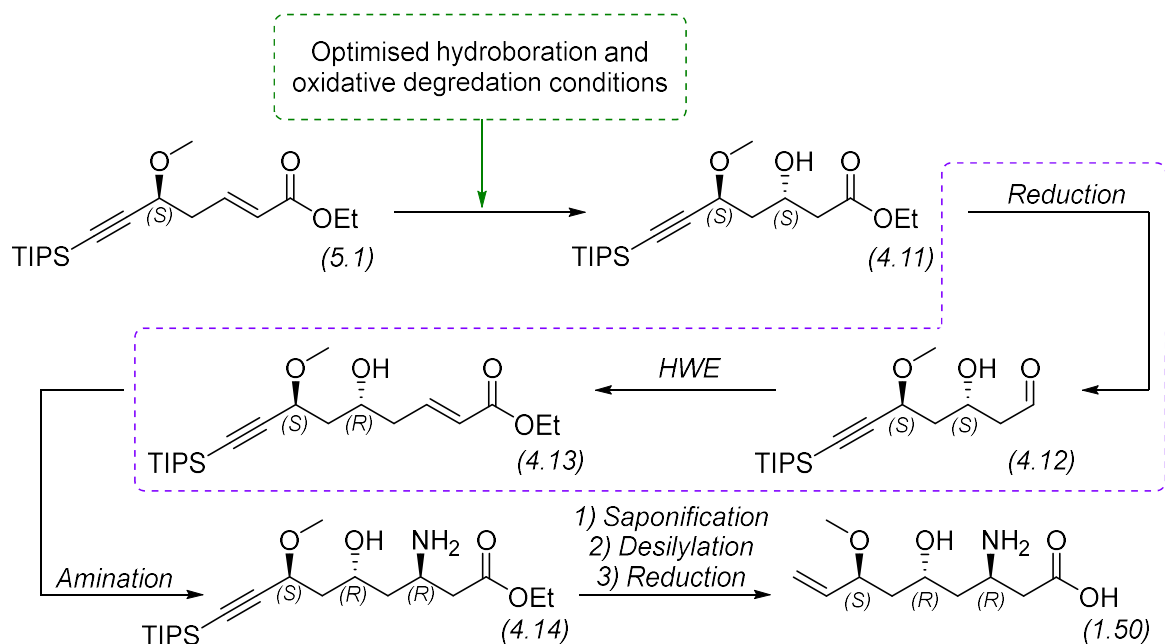


Scheme 120 Method for probing the interference mechanism of facial selectivity of the hydroboration approach, by using a bulkier protecting group to block copper-alcohol co-ordination.



Scheme 121 Control reaction for a ligand screen to optimise the facial selectivity of the hydroboration approach.

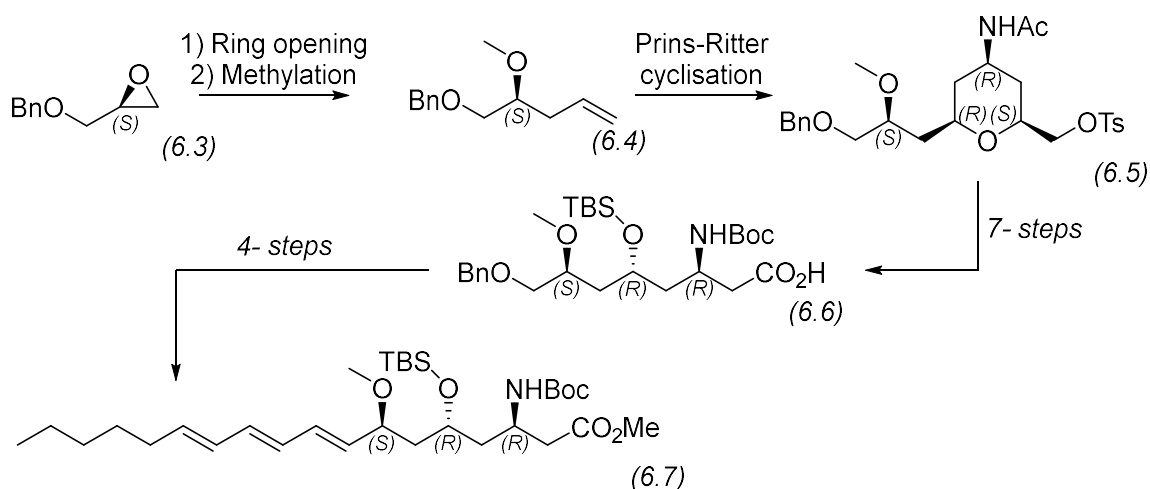
To complete the synthesis of 2-deoxygraciline, this optimised method will need to be used on ethyl (*S,E*)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate (**Scheme 122**). Then, the chain extension method detailed in chapter 6, *via* ester reduction and HWE, will provide a new α,β -unsaturated ester. Stereoselective amination conditions can be employed to provide a key intermediate in the synthesis of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid, one of the desired late-stage intermediates.



Scheme 122 Future work to utilise the optimised hydroboration strategy and chain extension approach in the synthesis of 3(*R*)-amino-5(*R*)-hydroxy-7(*S*)-methoxy non-8-enoic acid (1.50).

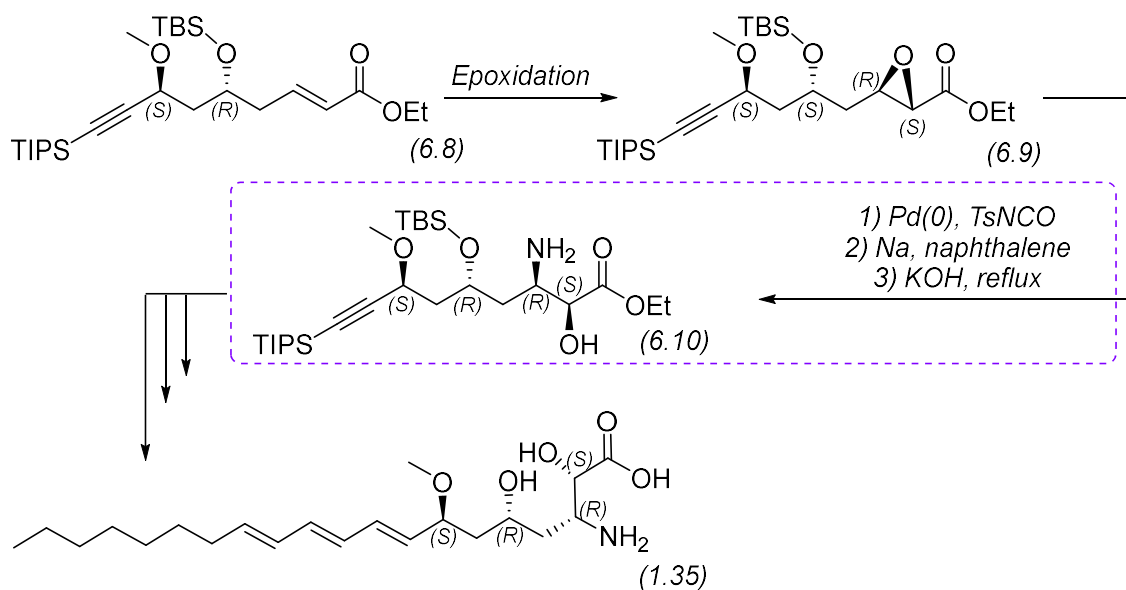
During the final stages of this project a stereoselective synthesis of 2-deoxygraciline was published by Goswami *et. al* (**Scheme 123**).¹³² While their route was successful in providing a protected version of 2-deoxygraciline, it relied on pre-determined substrate chirality to install all three stereogenic centres. Beginning from the commercially available chiral

epoxide, a regioselective ring opening and methylation was performed to provide the methoxy functionality. Then a Prins-Ritter cyclization was used to install the hydroxy and amine functionality, followed by a further 11 steps to give the protected product.



Scheme 123 Approach towards synthesis of protected 2-deoxygraciline by Goswami *et. al*, indicating the key steps to install chirality.¹³²

Comparatively, the synthetic method devised in this thesis utilises catalytically controlled enantioselective reactions, which presents possibilities for simple differentiation of each of the stereogenic centres without the need for a new synthetic method. Using the semi-iterative approach also provides a late-stage opportunity to divert the synthesis towards graciline, by using the α,β -unsaturated ester to form an epoxide (**Scheme 124**).^{133,134} Subsequent ring opening can be performed with a nucleophilic nitrogen compound, in a retentive or inversive manor. The reported configuration of graciline will require a stereo-retentive opening, which could be carried out using the 3- step approach published by Somfai *et. al*.¹³⁵



Scheme 124 Late stage diversion of the synthetic approach to form graciline, using an alkene epoxidation and stereo-retentive ring opening with a nucleophilic nitrogen.

Overall, there is still much work to be done to perfect this route towards 2-deoxygraciline and graciline. However, a divergent synthesis to both compounds has been designed, which can be continued once optimisation of the hydroboration approach has been achieved.

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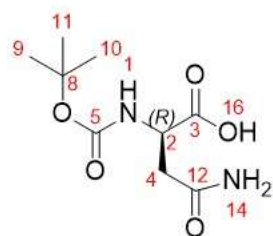
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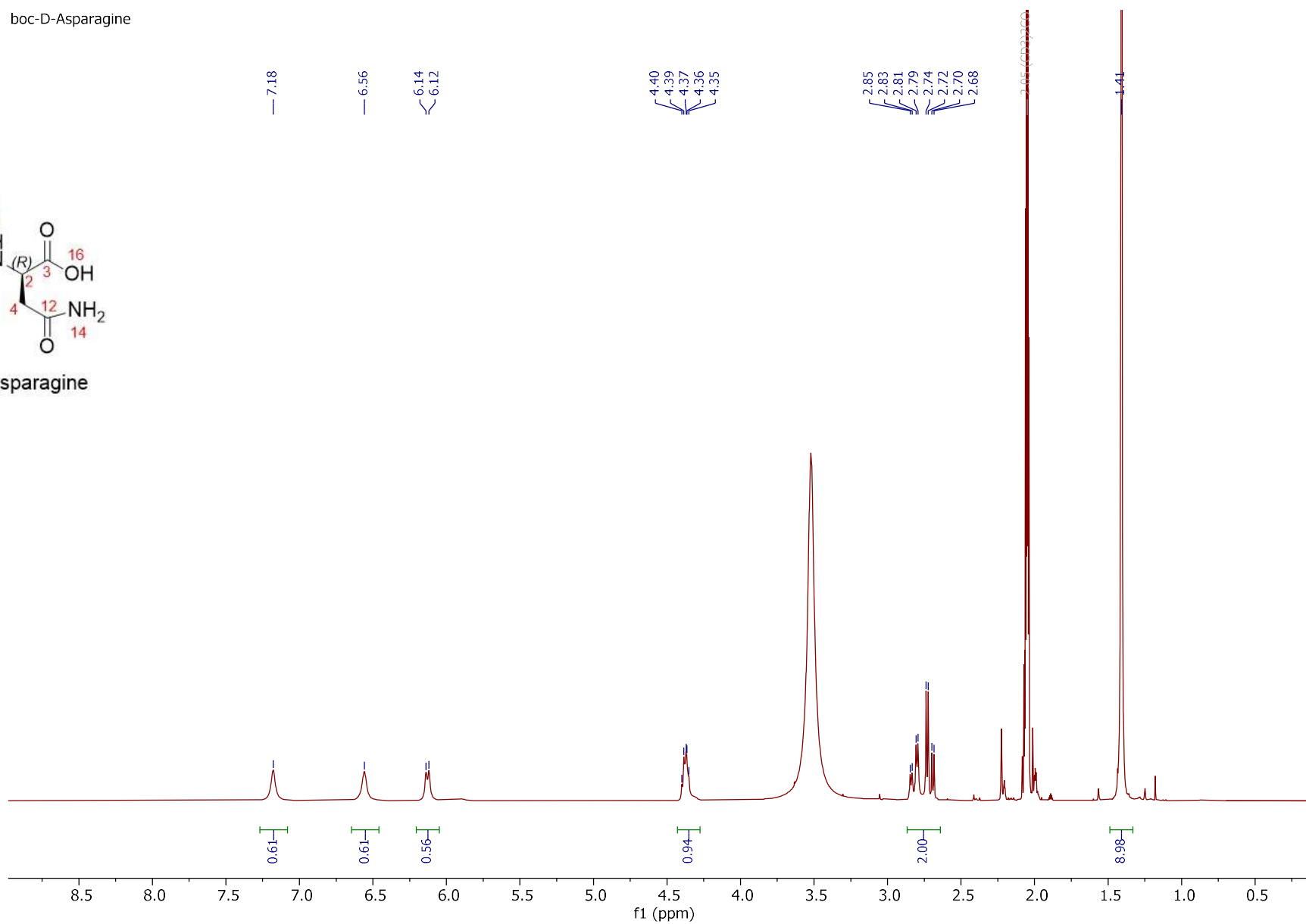
Appendix

A-C2

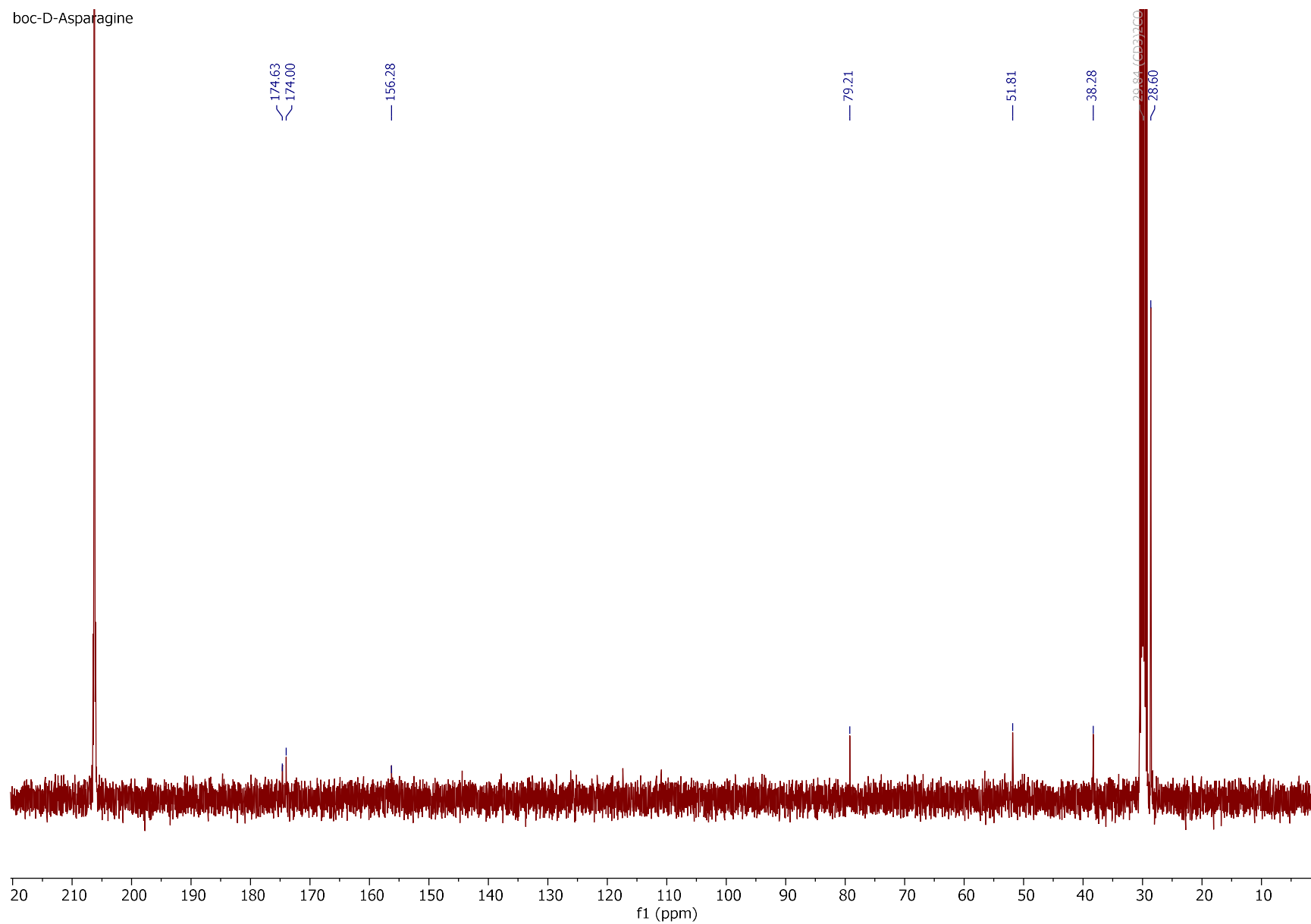
boc-D-Asparagine



boc-D-Asparagine

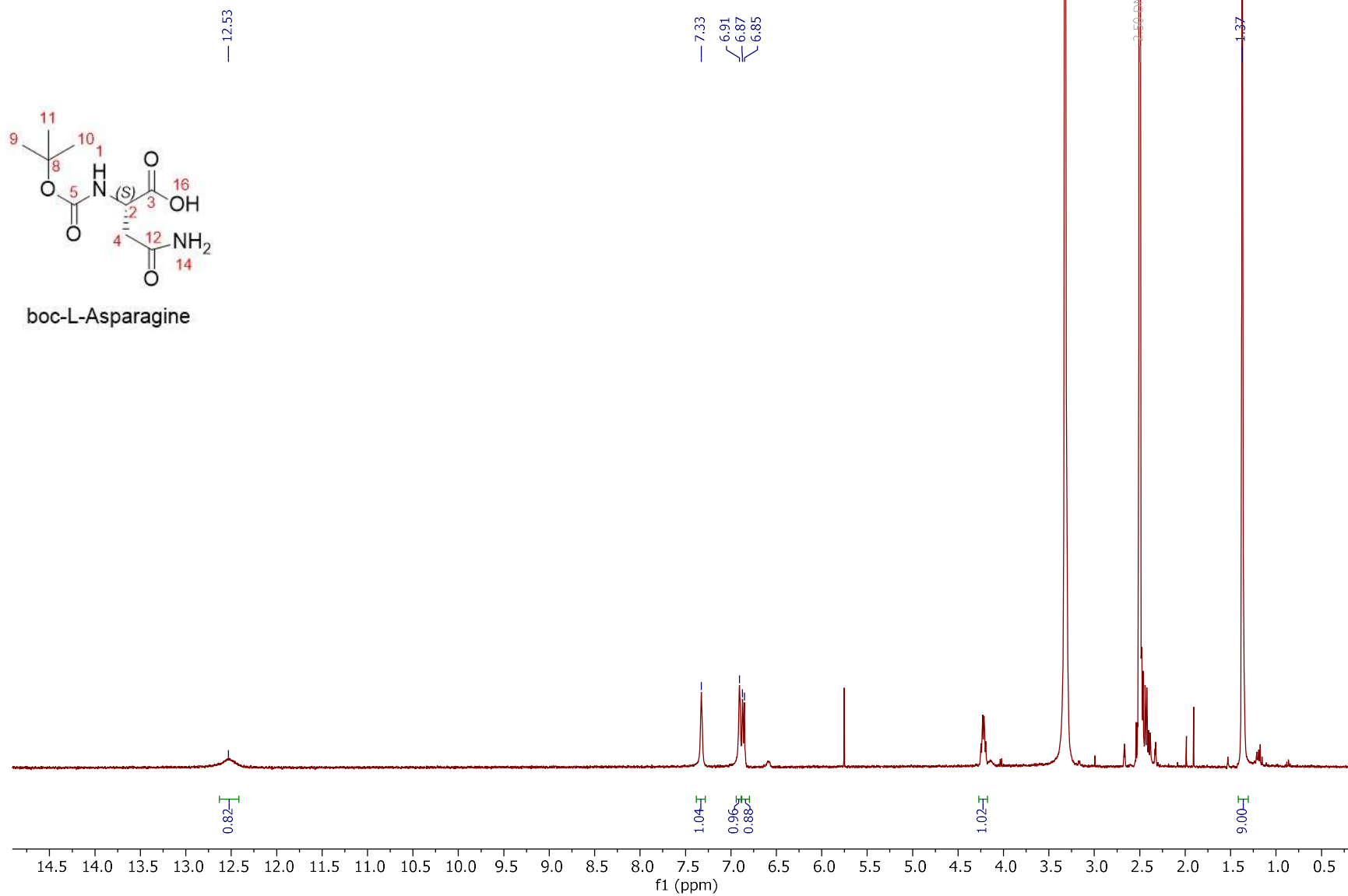


A-C2-1



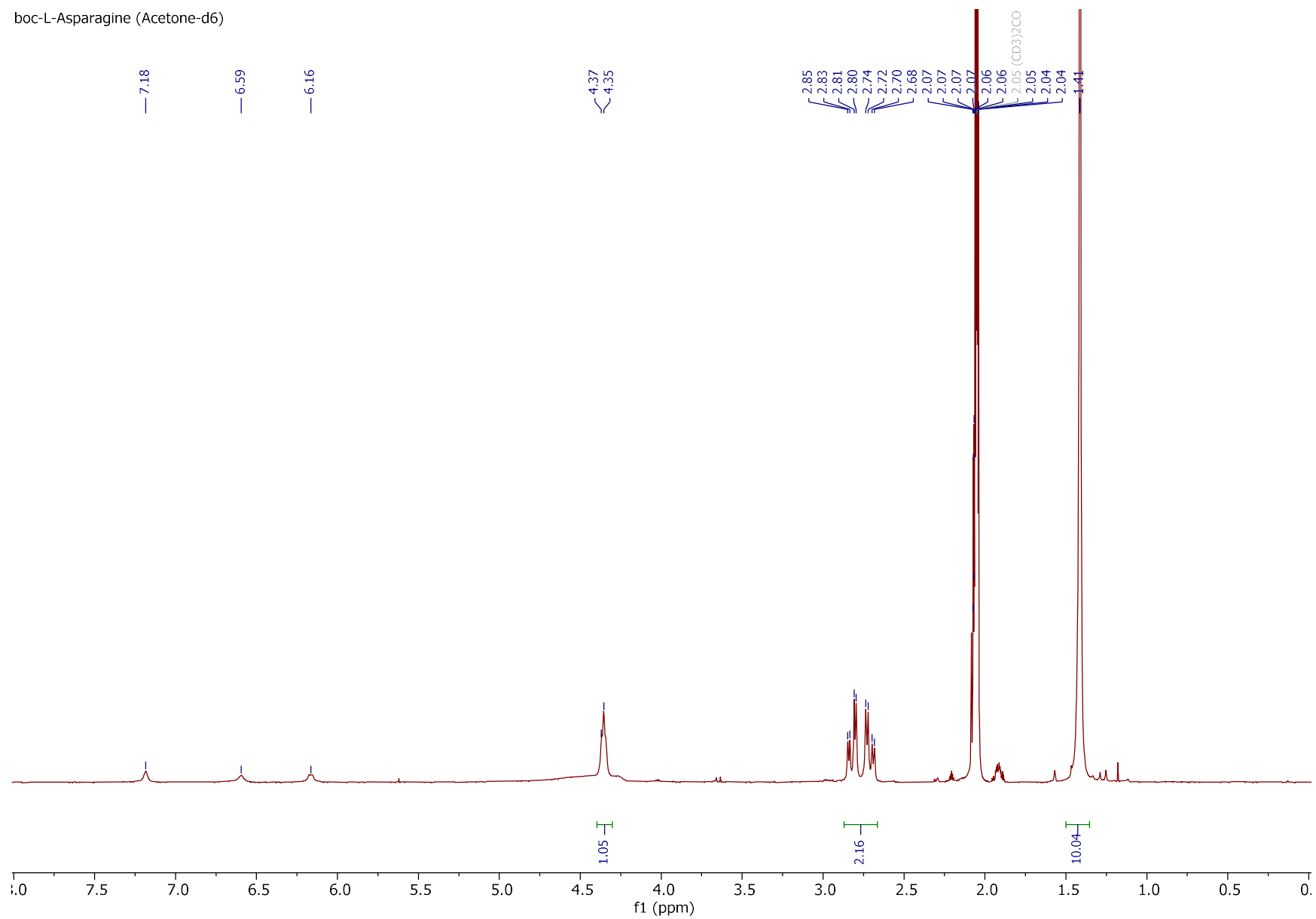
A-C2-3

boc-L-Asparagine (DMSO-d6)



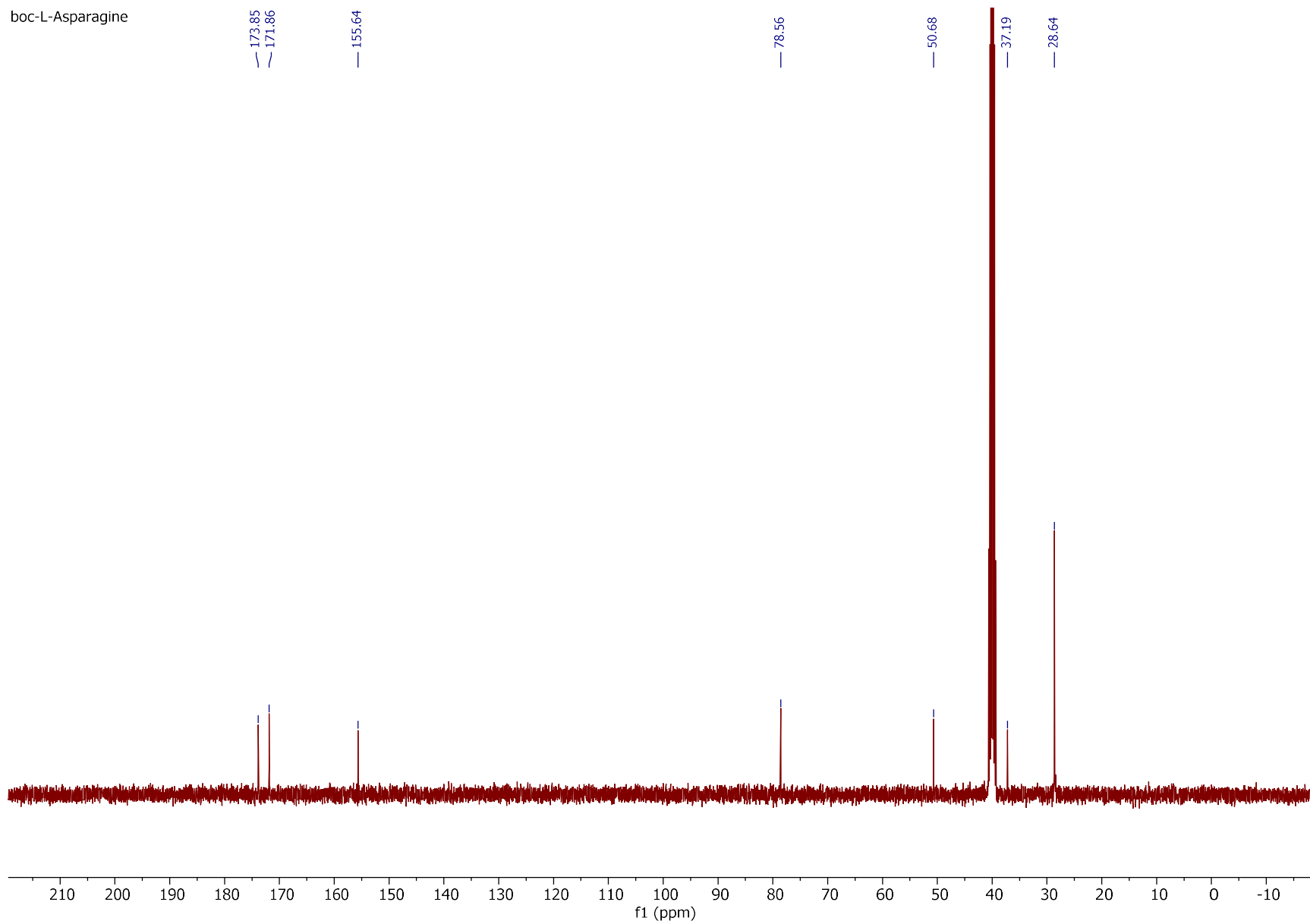
A-C2-4

boc-L-Asparagine (Acetone-d6)



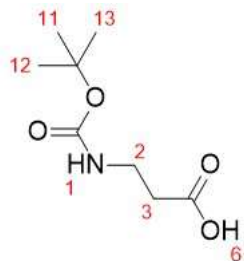
A-C2-5

boc-L-Asparagine

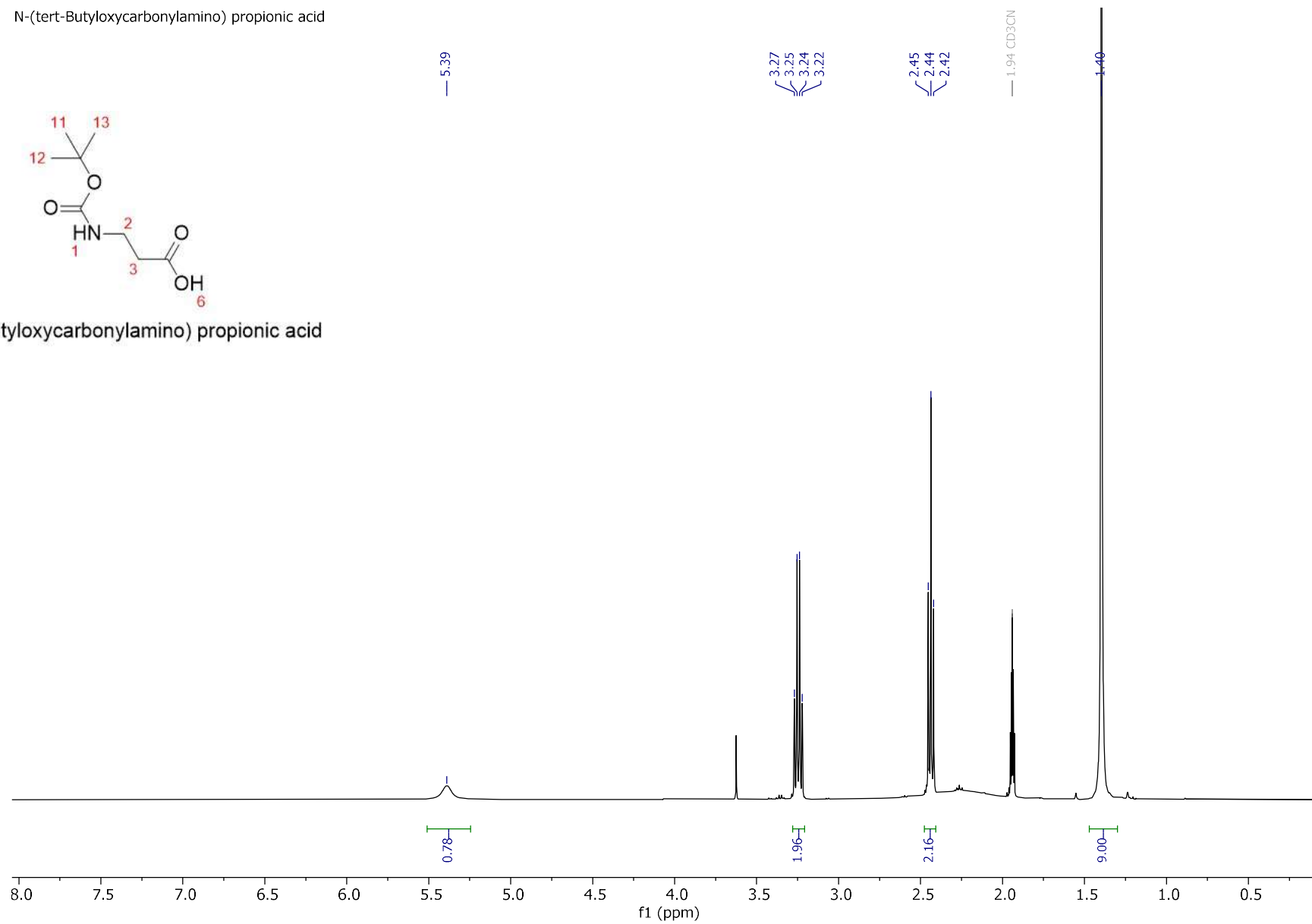


A-C2-6

N-(tert-Butyloxycarbonylamino) propionic acid

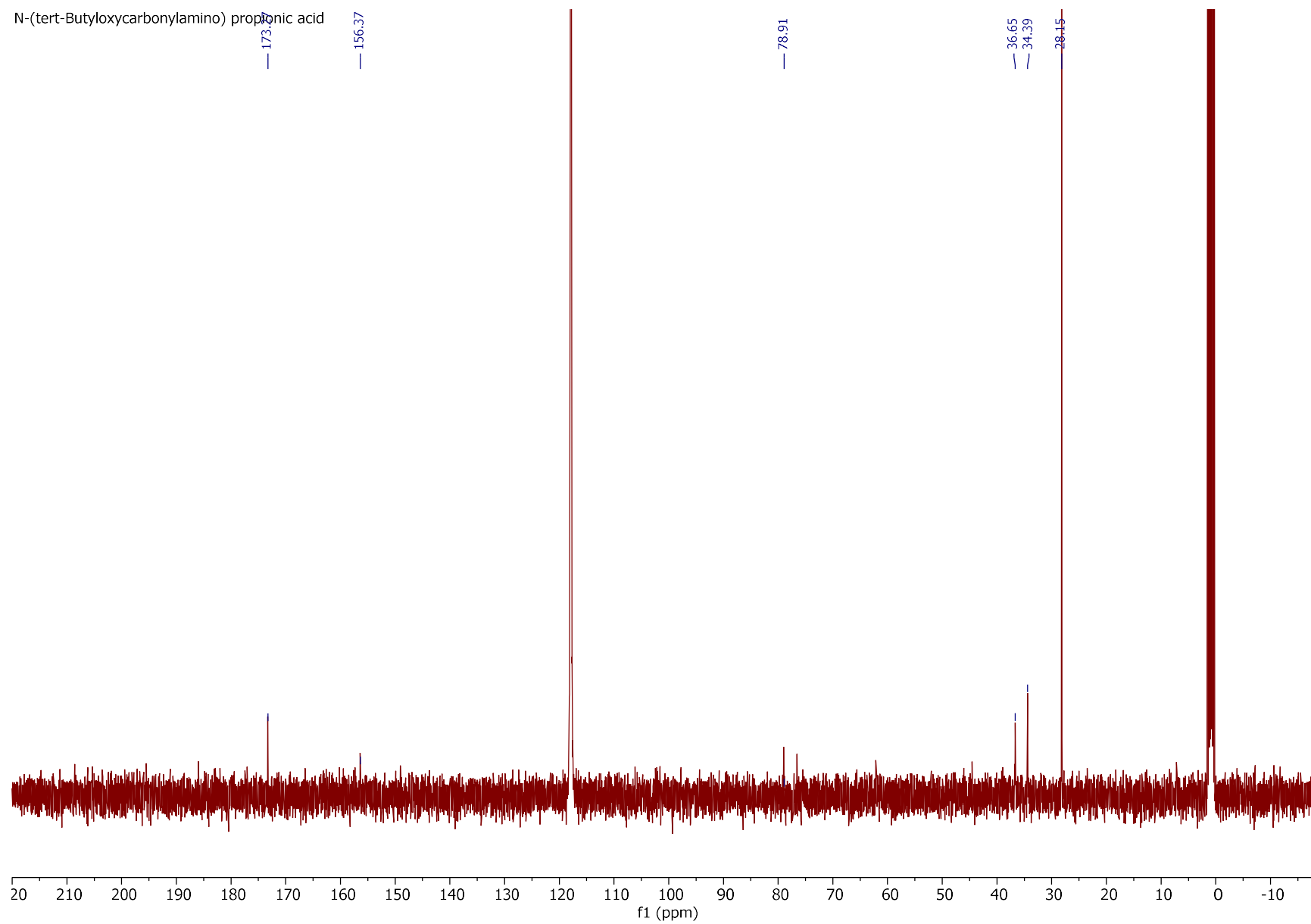


N-(tert-Butyloxycarbonylamino) propionic acid

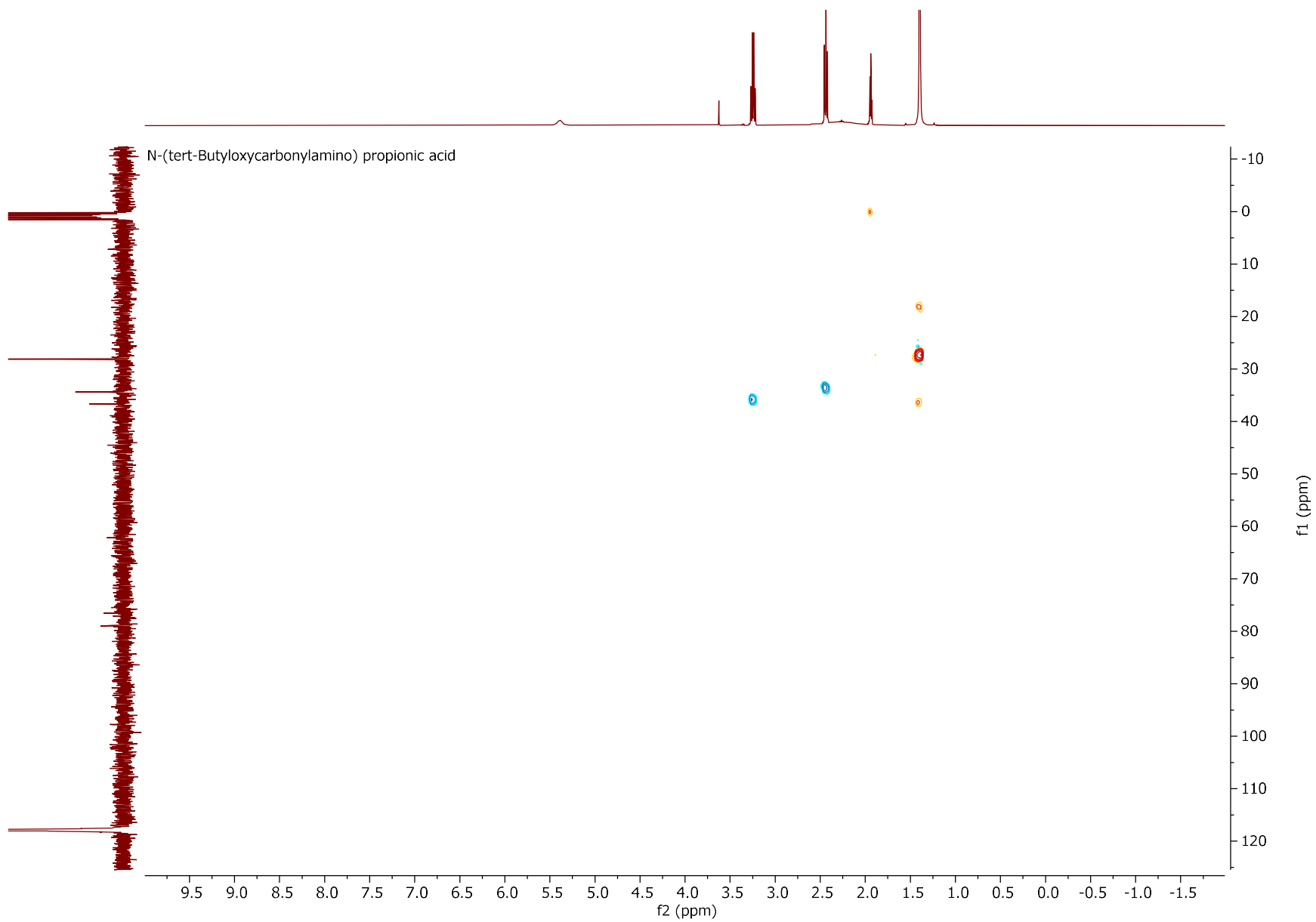


A-C2-8

N-(tert-Butyloxycarbonylamino) propionic acid

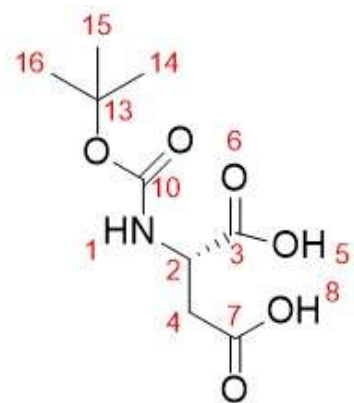


A-C2-9

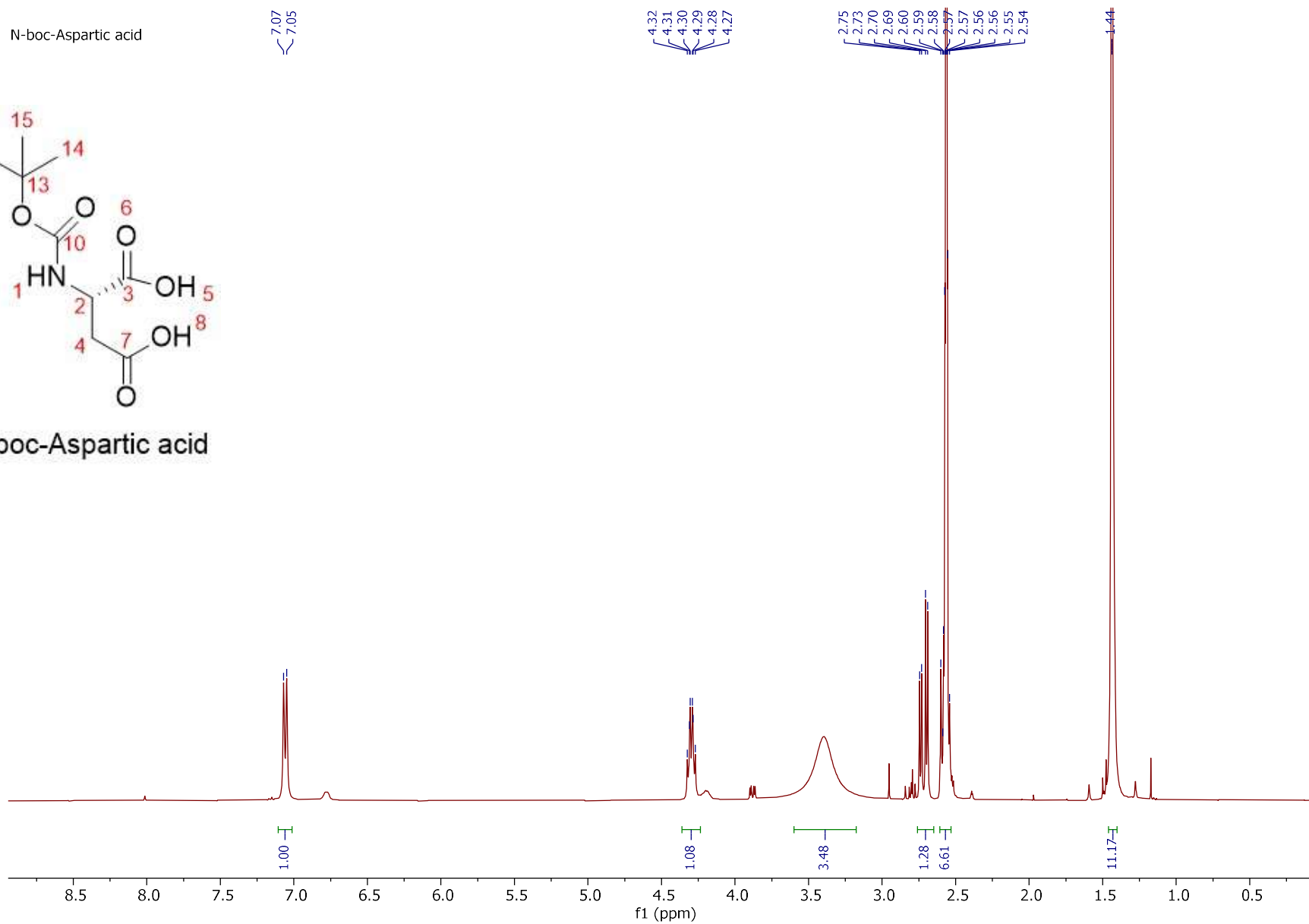


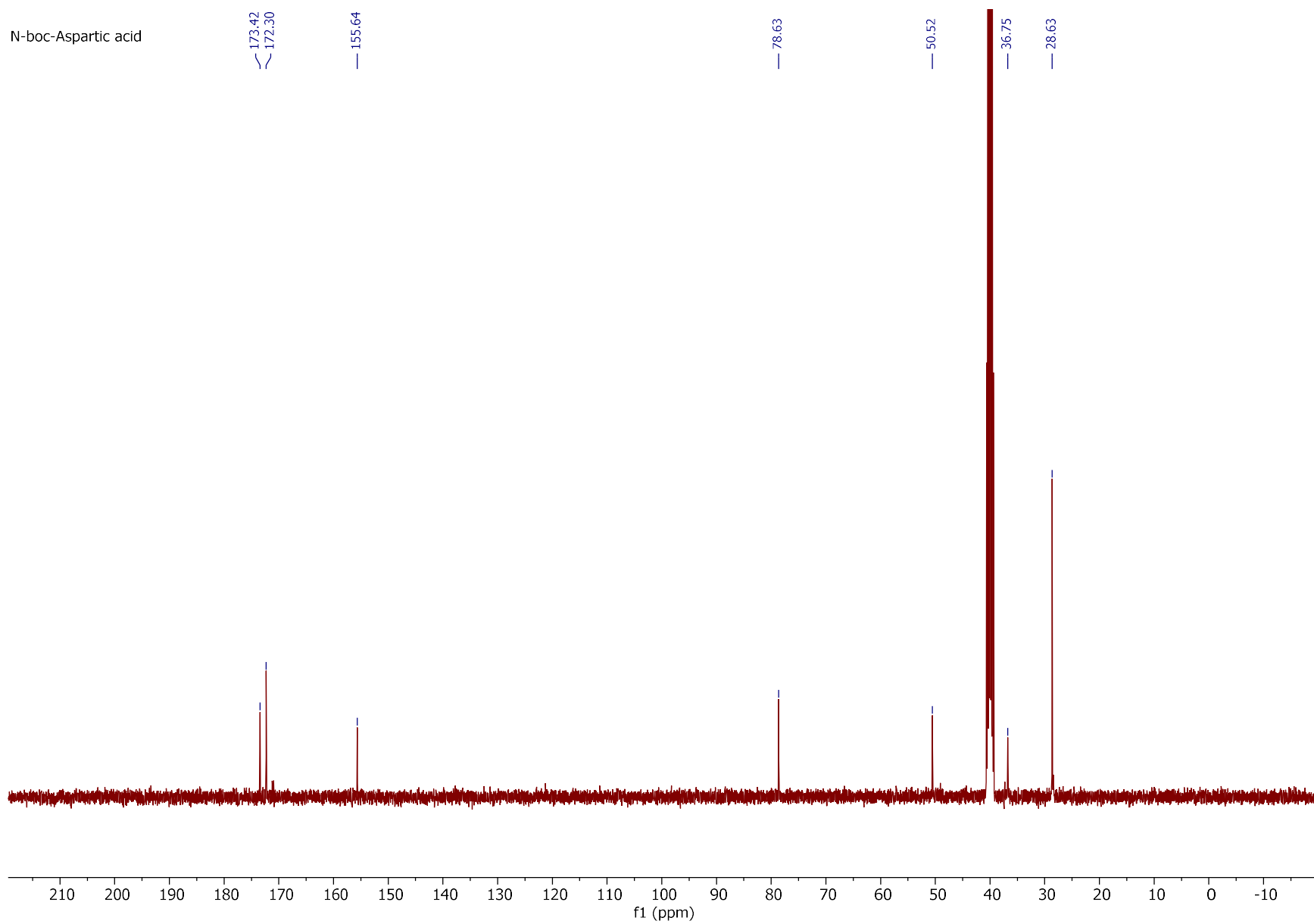
A-C2-10

N-boc-Aspartic acid

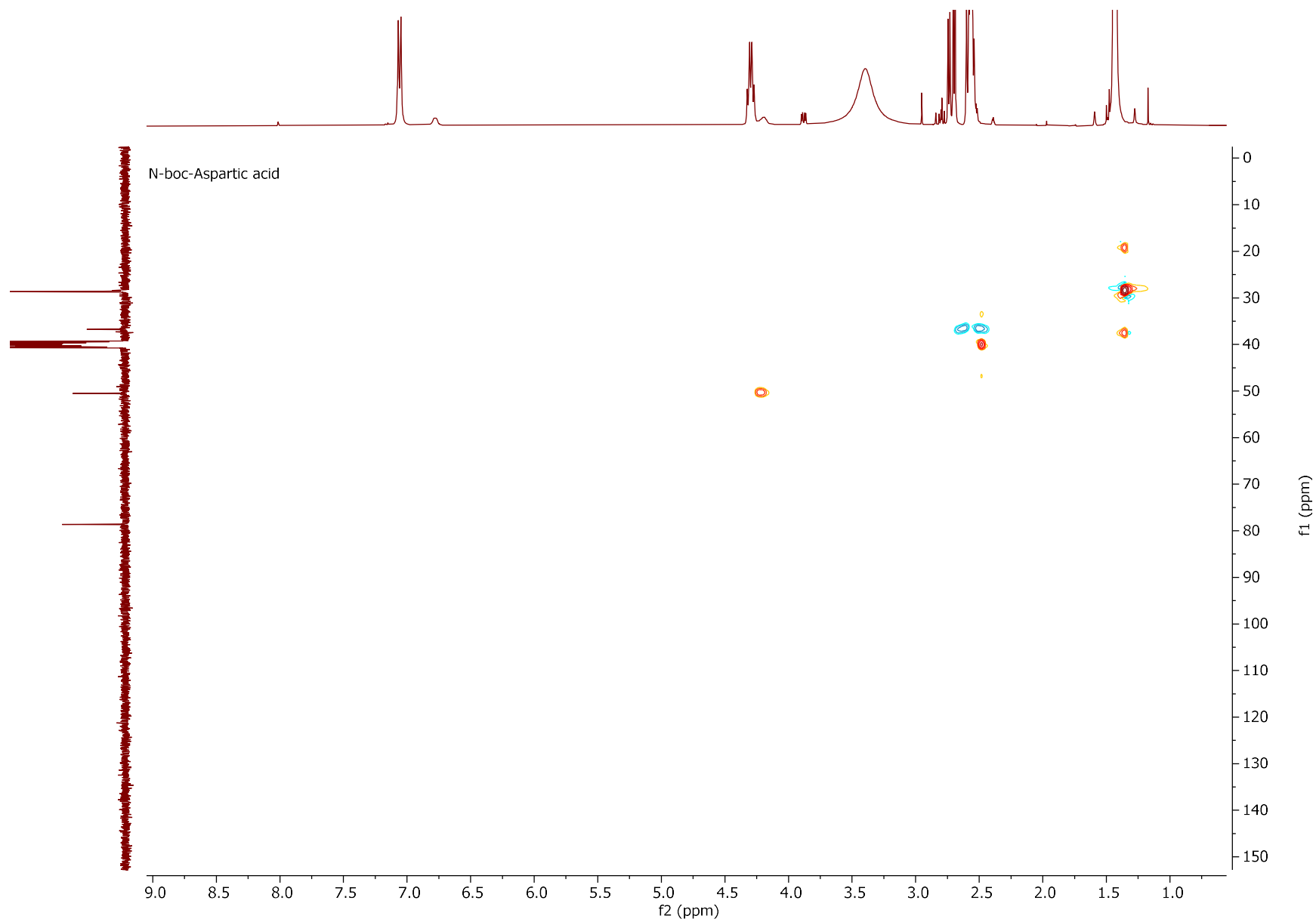


N-boc-Aspartic acid



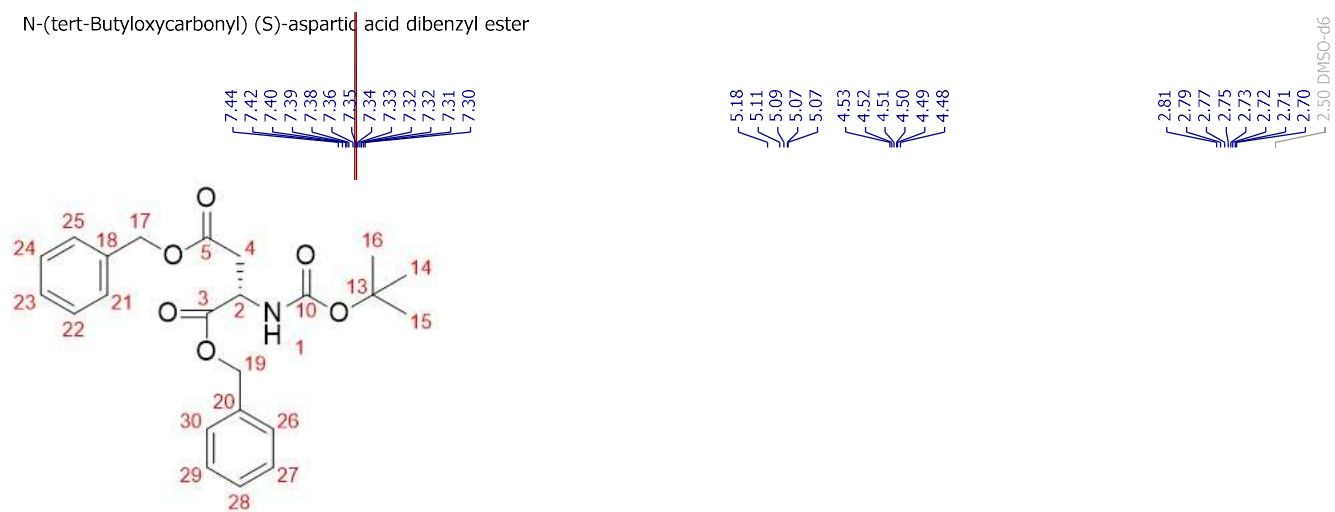


A-C2-13

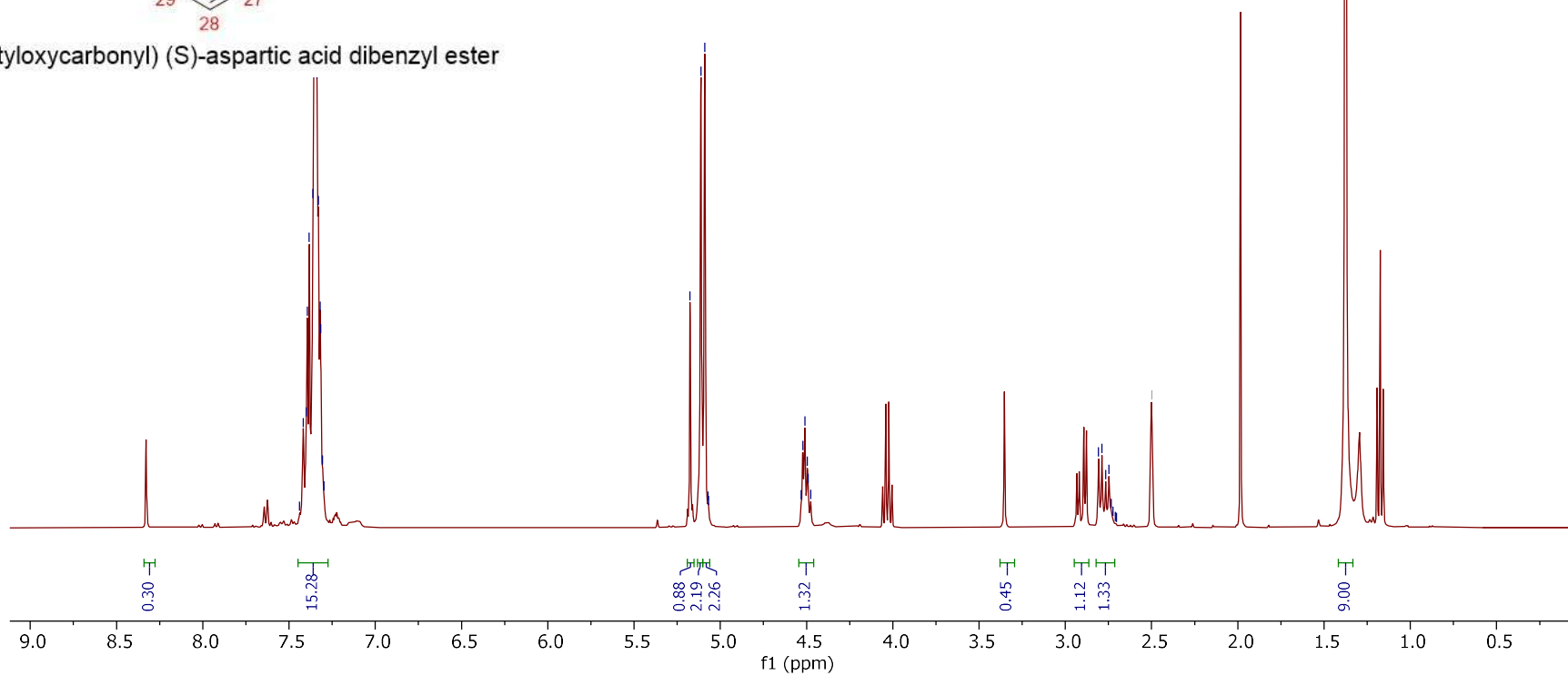


A-C2-14

N-(tert-Butyloxycarbonyl) (S)-aspartic acid dibenzyl ester

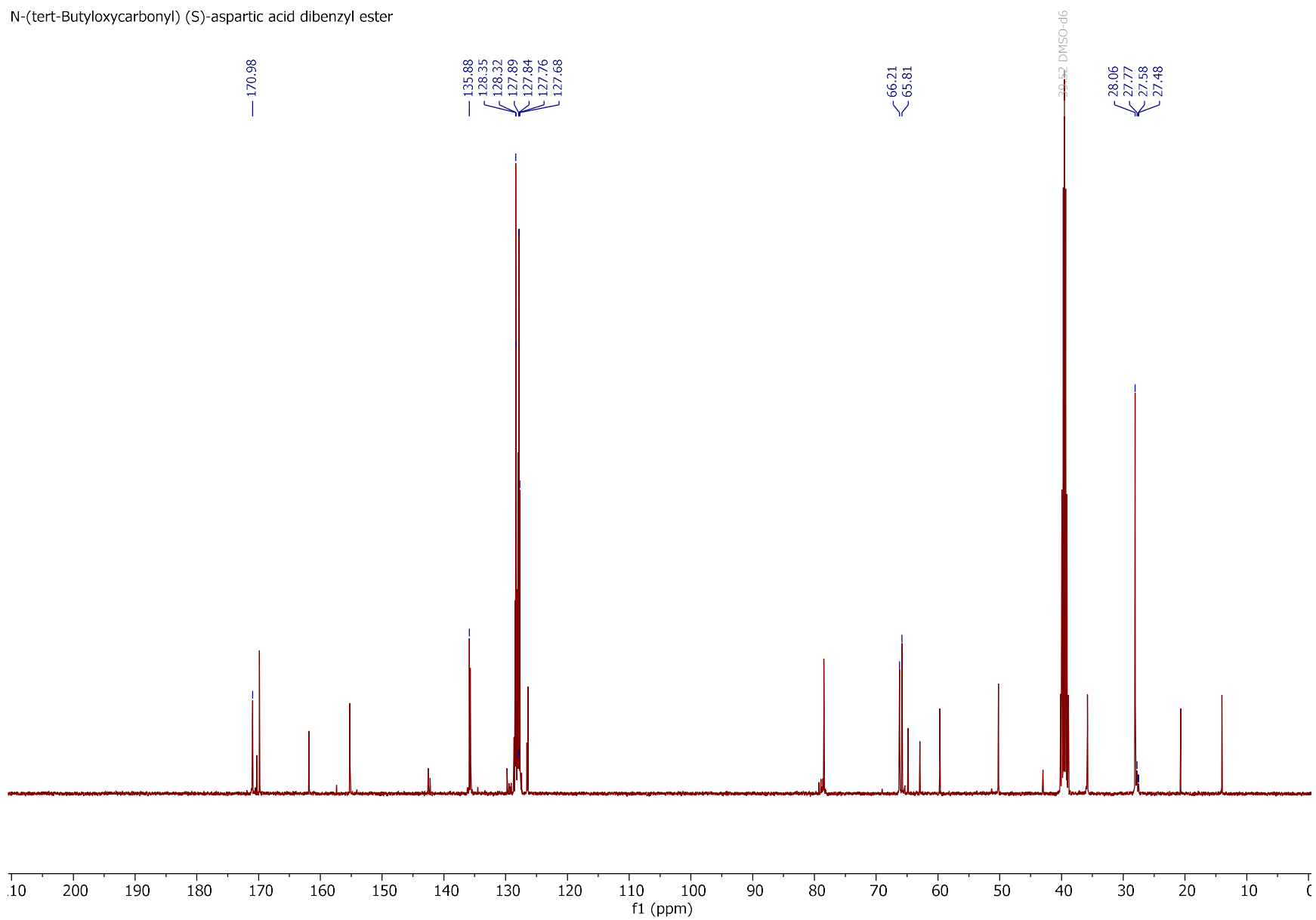


N-(tert-Butyloxycarbonyl) (S)-aspartic acid dibenzyl ester

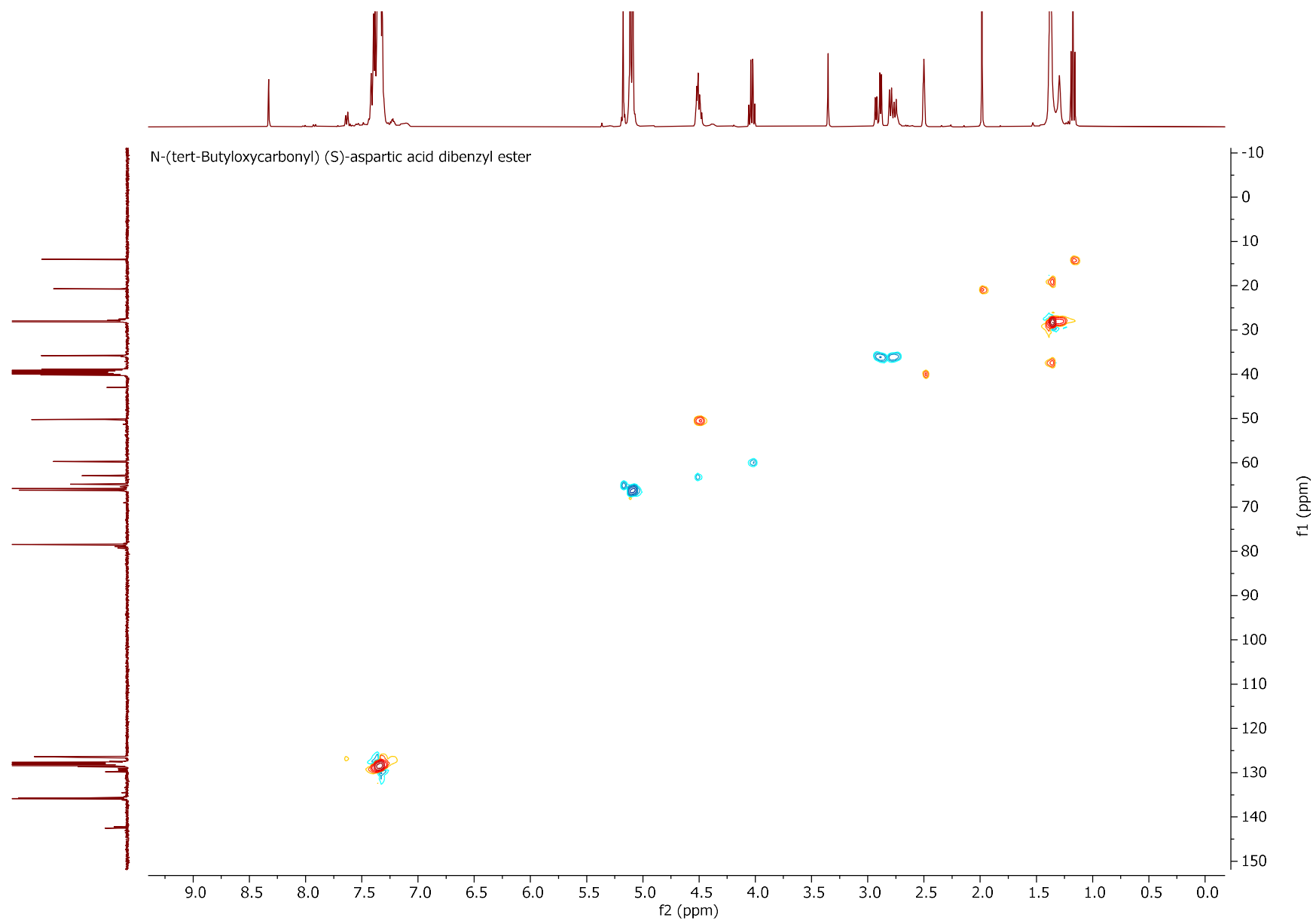


A-C2-16

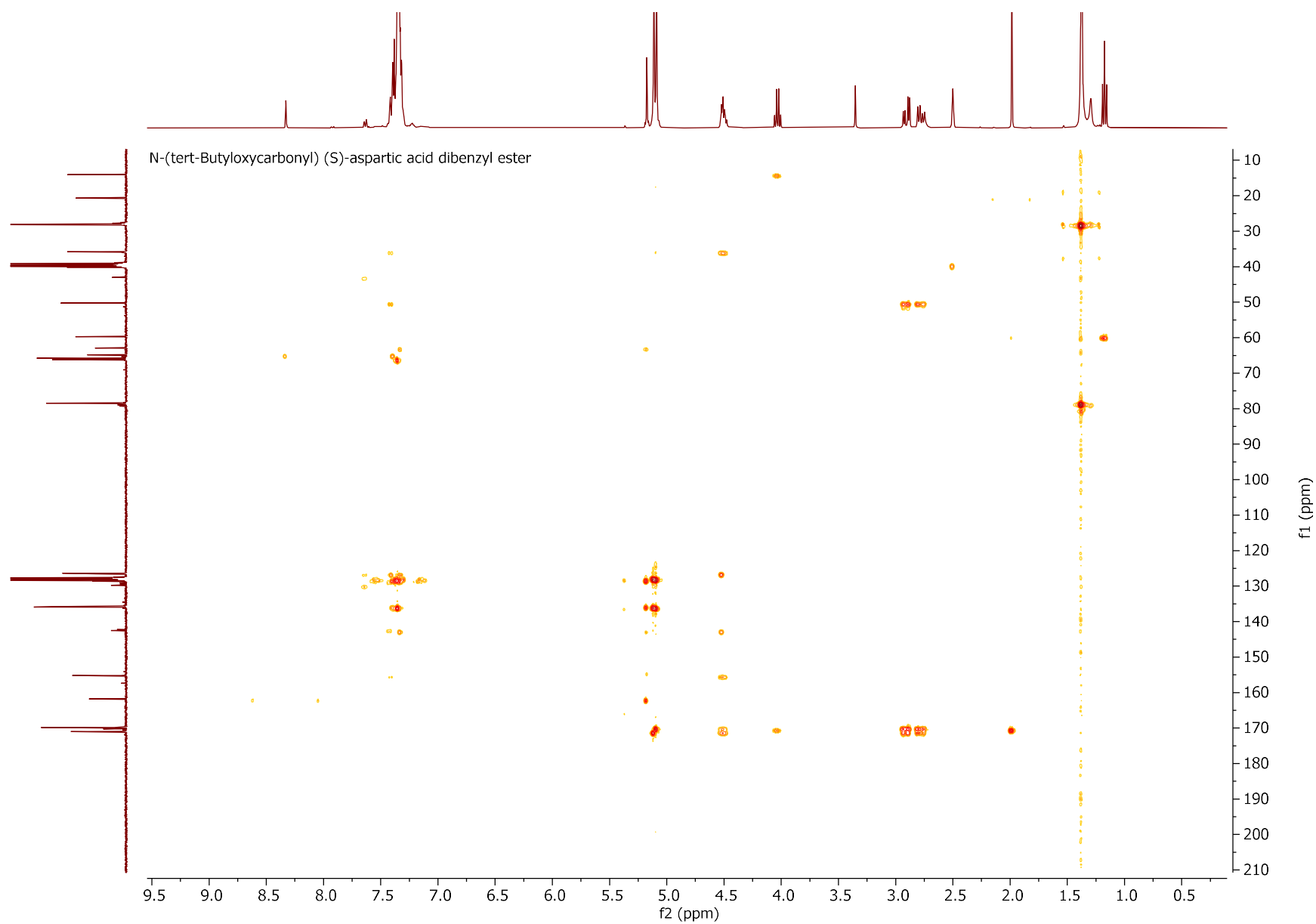
N-(tert-Butyloxycarbonyl) (S)-aspartic acid dibenzyl ester



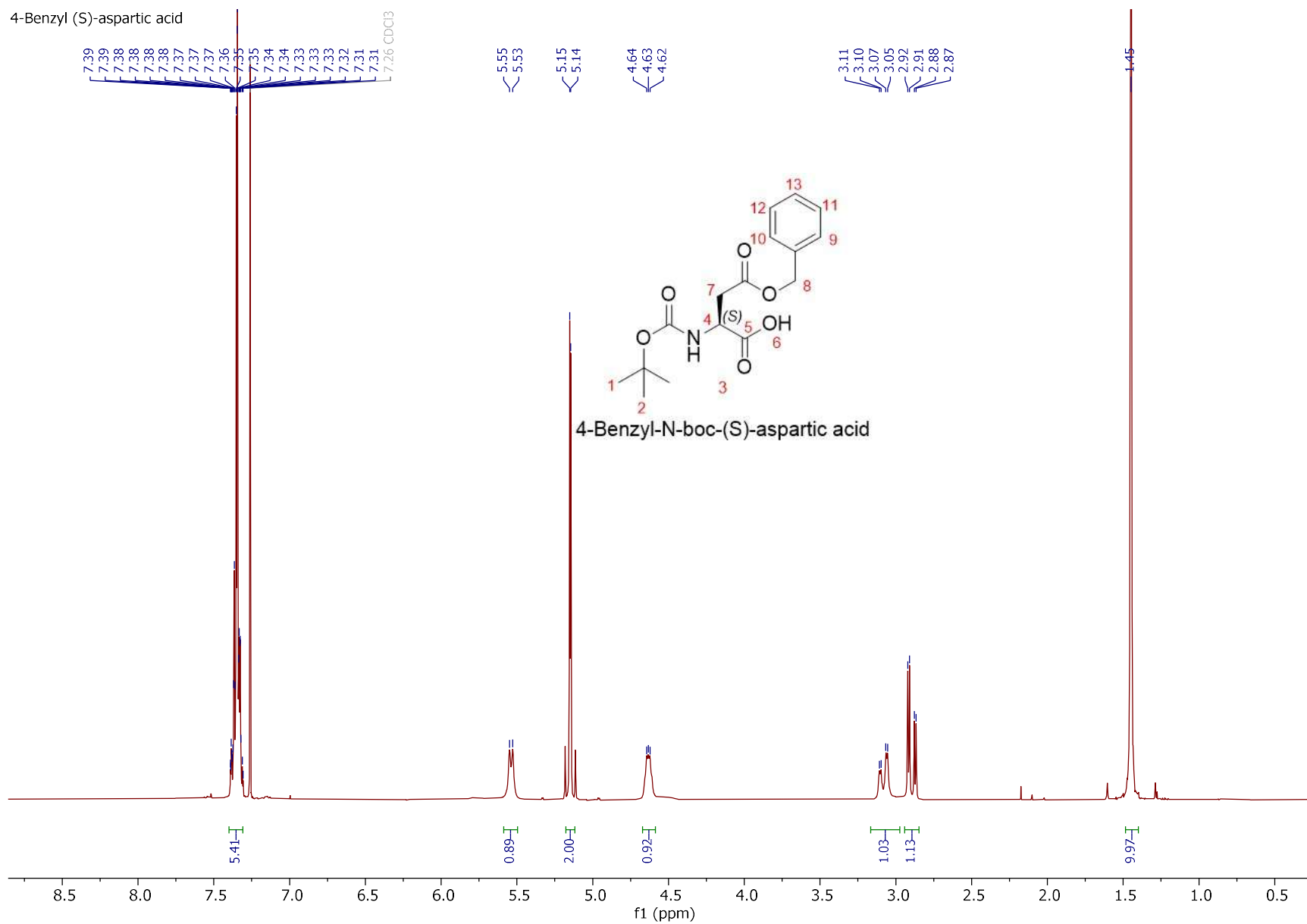
A-C2-17



A-C2-18

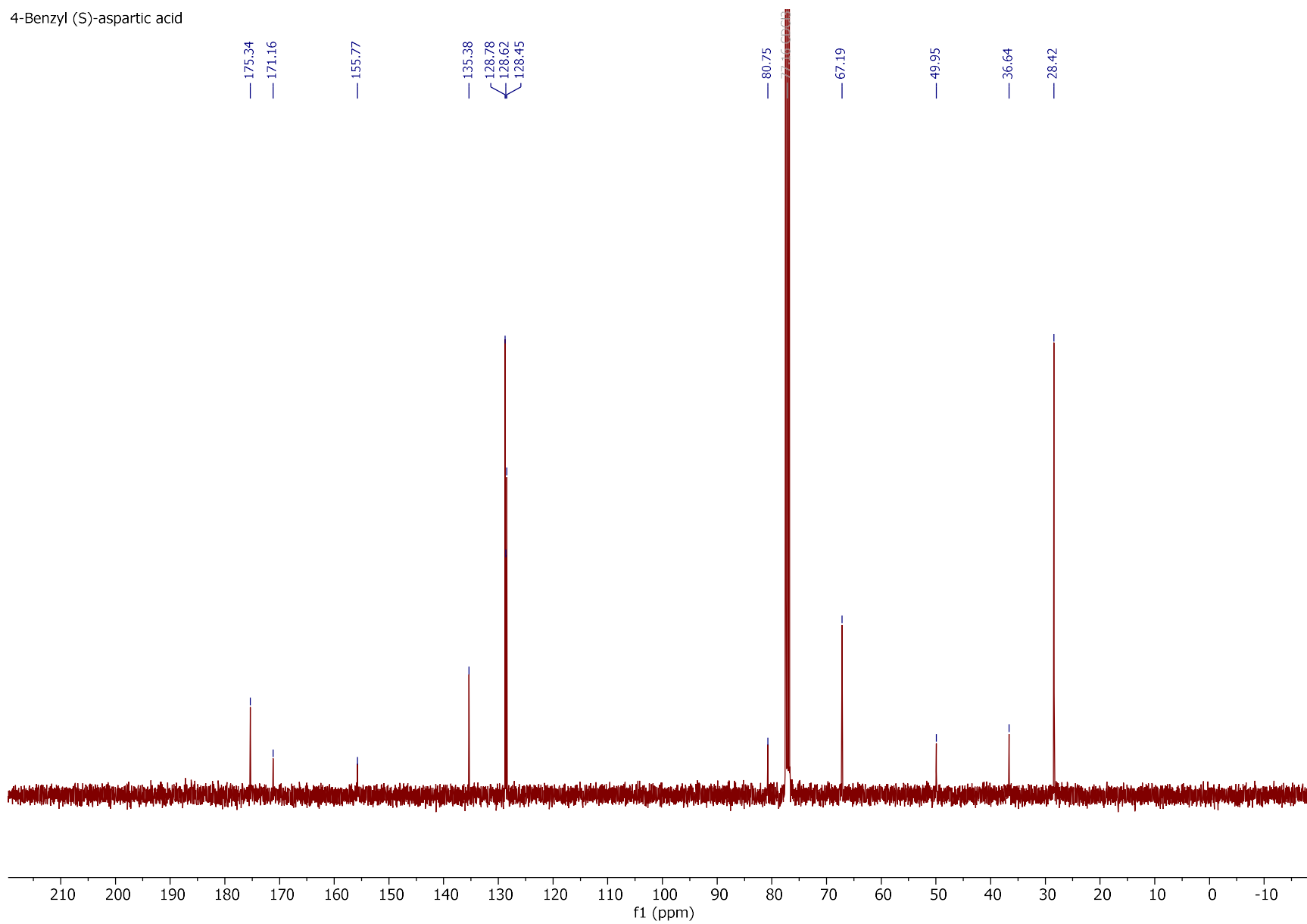


A-C2-19

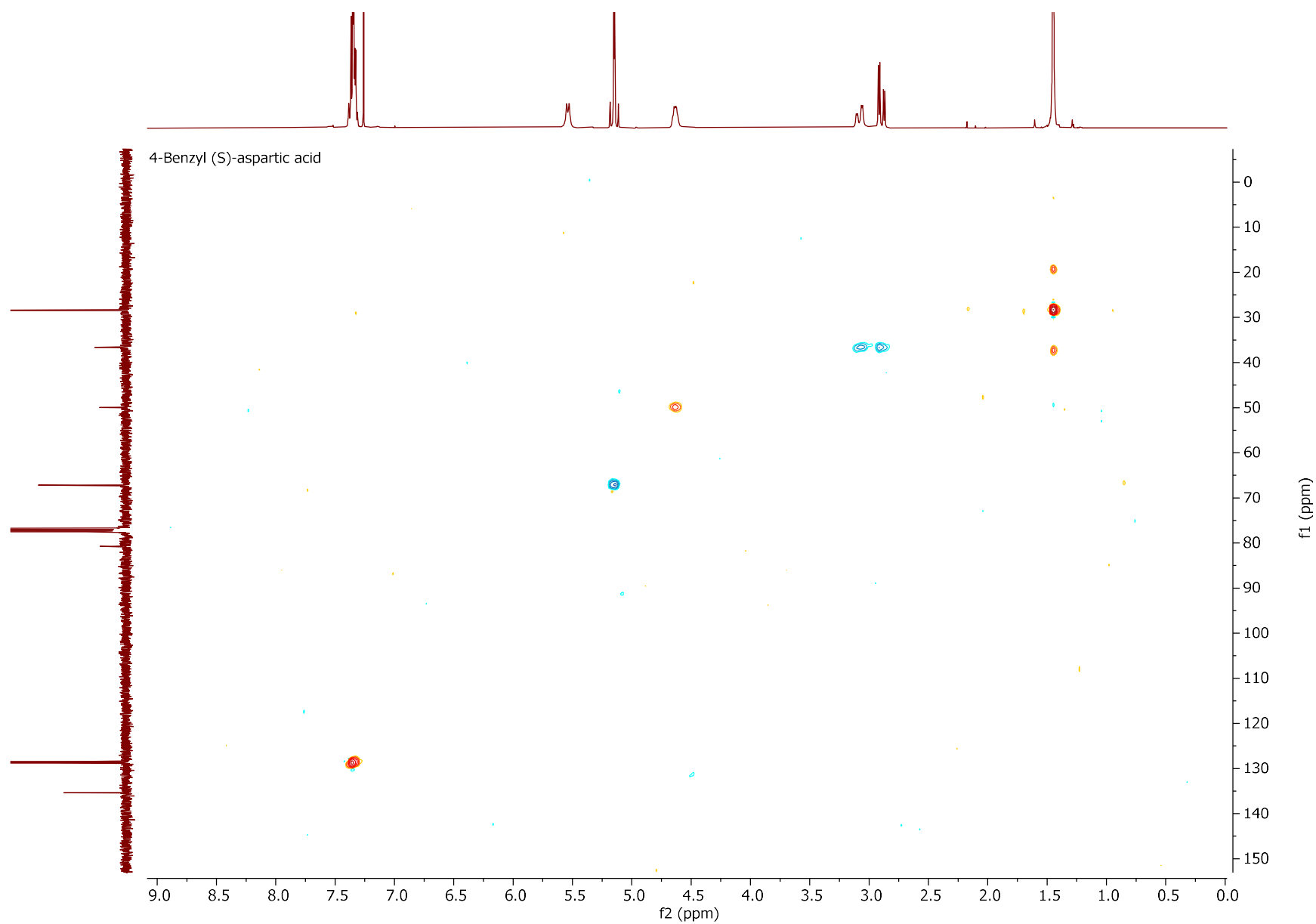


A-C2-21

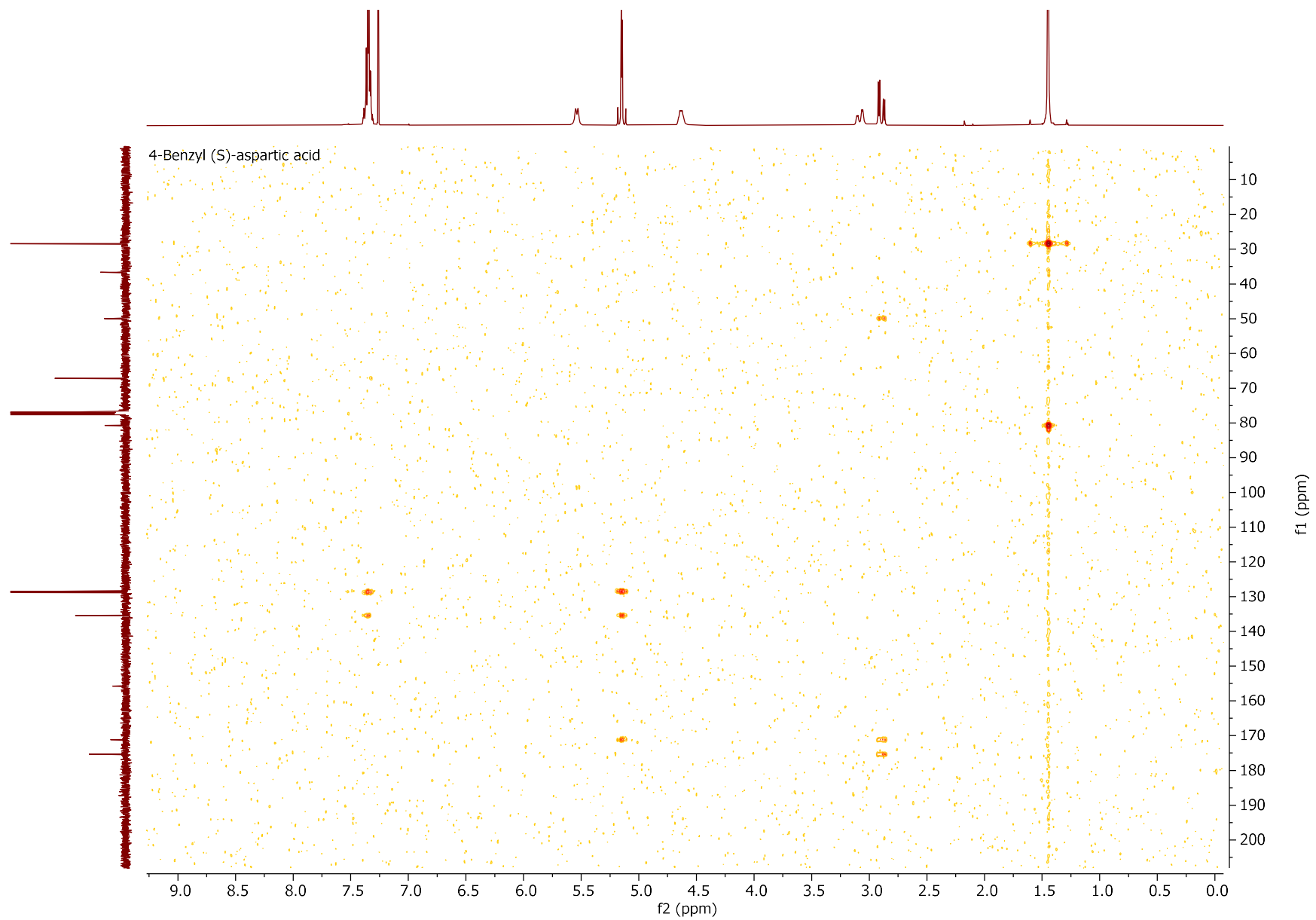
4-Benzyl (S)-aspartic acid



A-C2-22



A-C2-23



A-C2-24

4-Benzyl 1-methyl N-boc-(S)-aspartate

7.37
7.35
7.35
7.34
7.26
7.26 CDCl₃

5.48
5.46

5.13
5.12

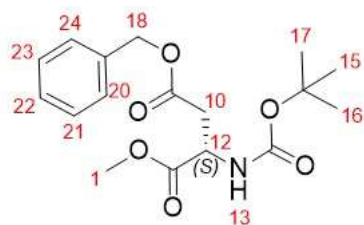
4.61
4.60
4.59
4.58
4.57

3.70

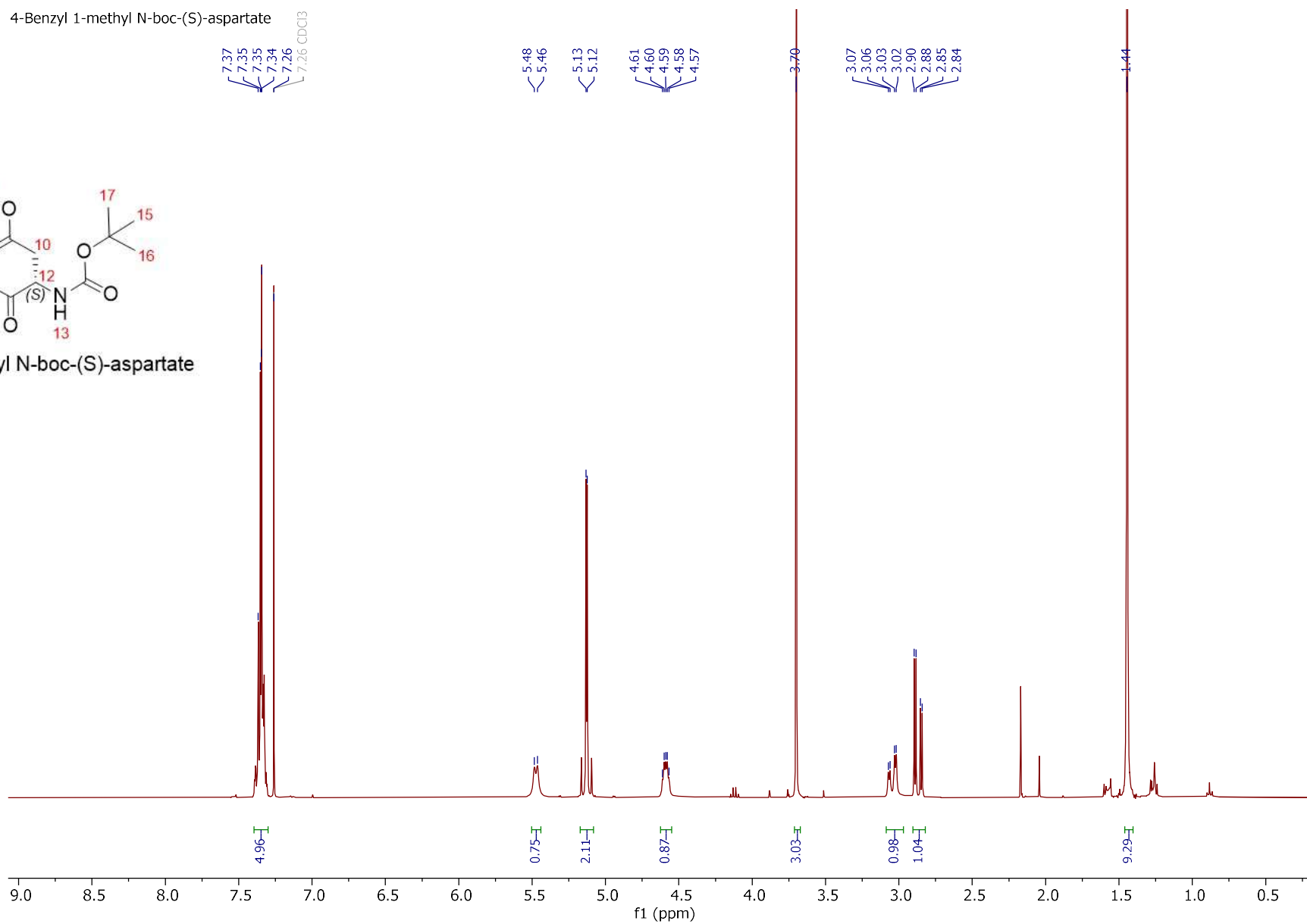
3.07
3.06
3.03
3.02

2.90
2.88
2.85
2.84

1.44

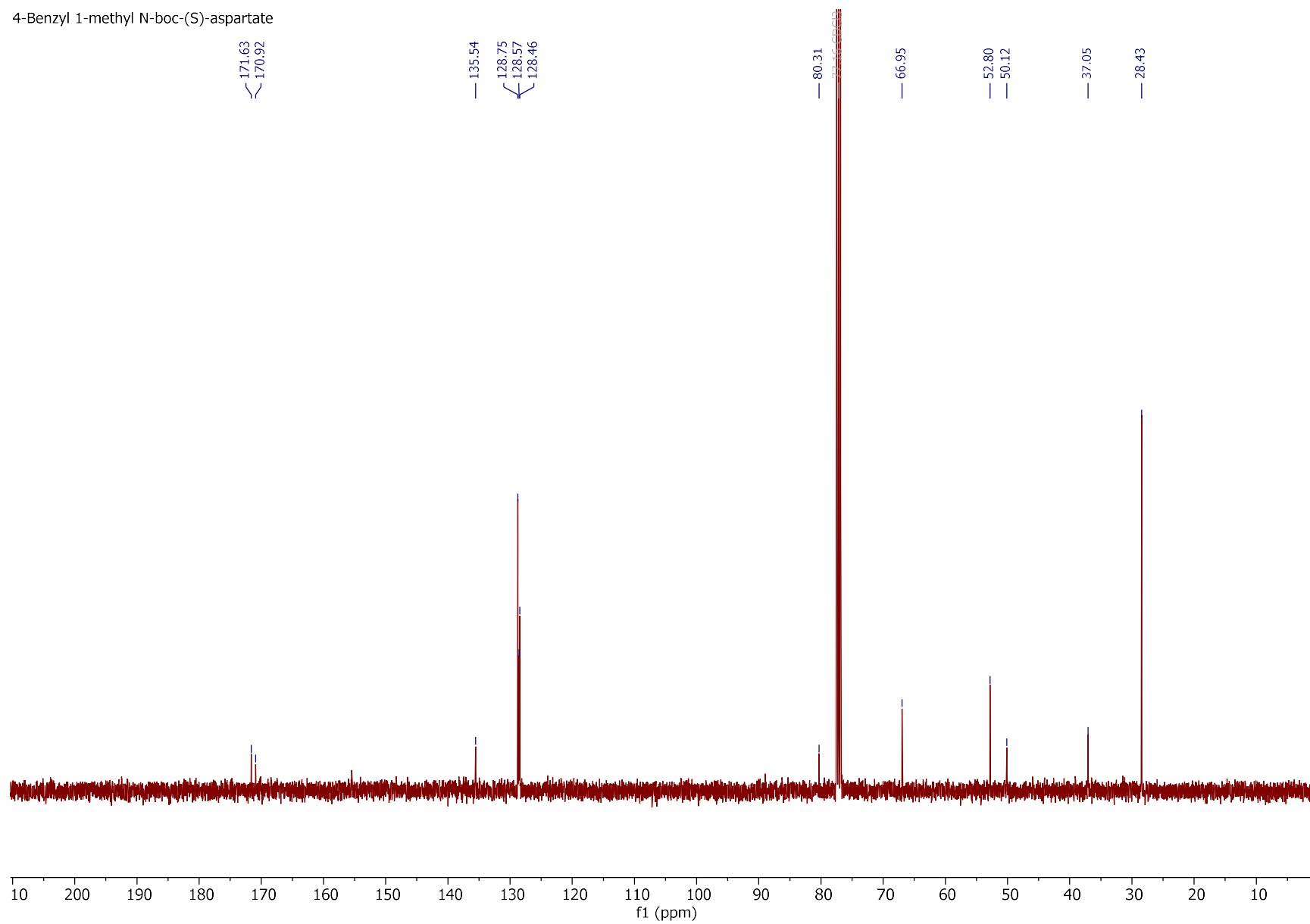


4-Benzyl 1-methyl N-boc-(S)-aspartate

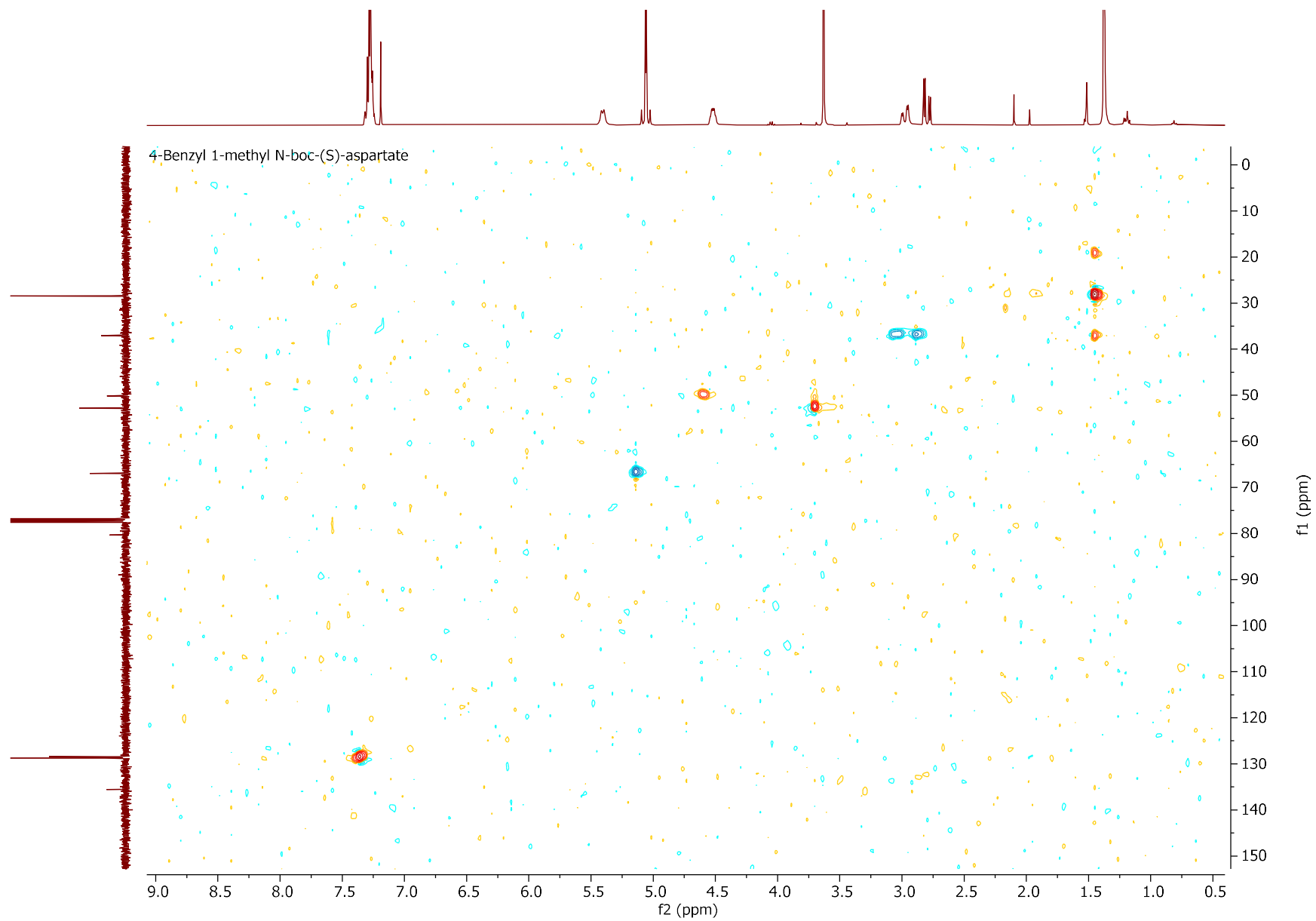


A-C2-25

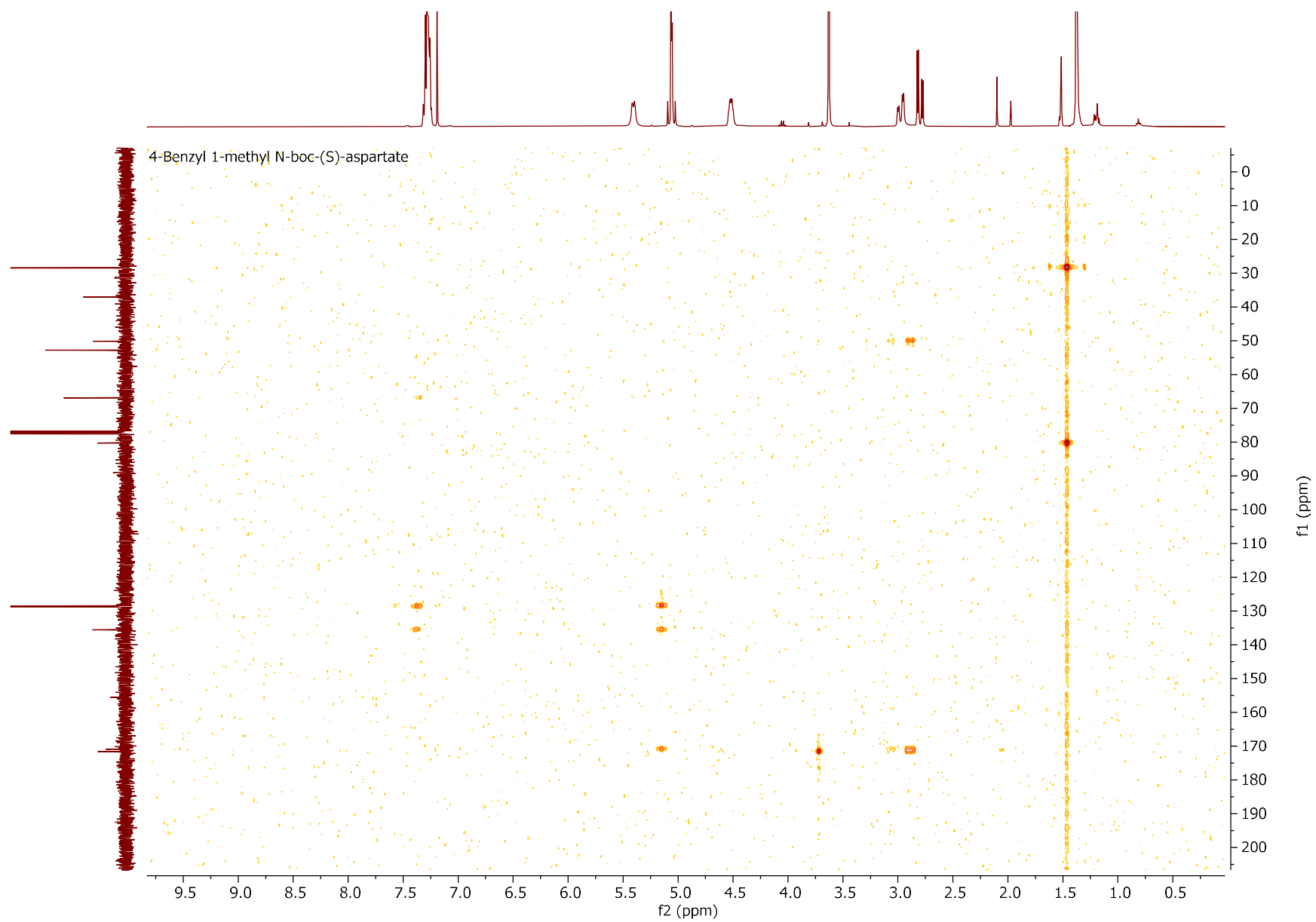
4-Benzyl 1-methyl N-boc-(S)-aspartate



A-C2-26

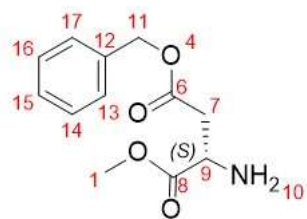


A-C2-27

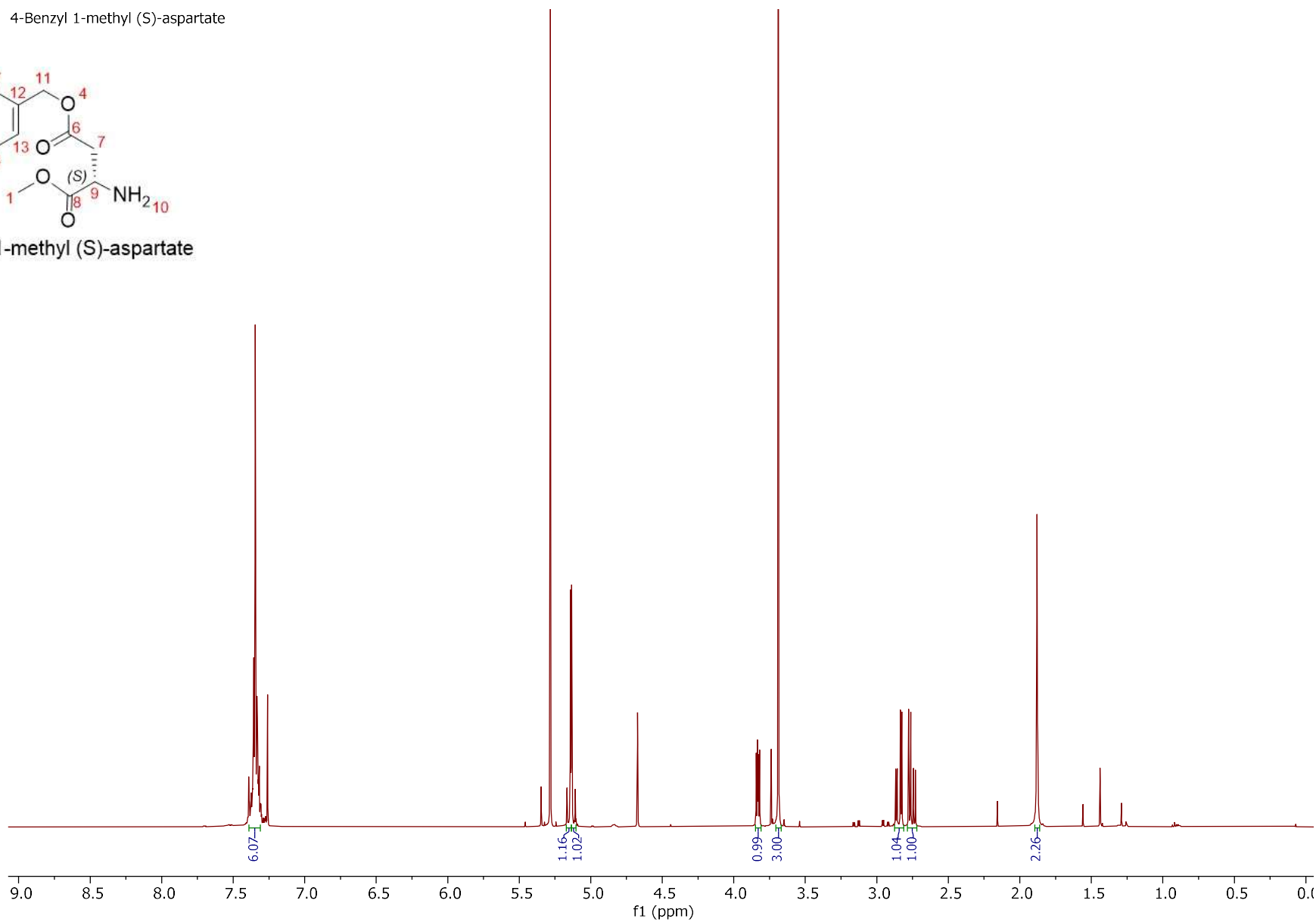


A-C2-28

4-Benzyl 1-methyl (S)-aspartate

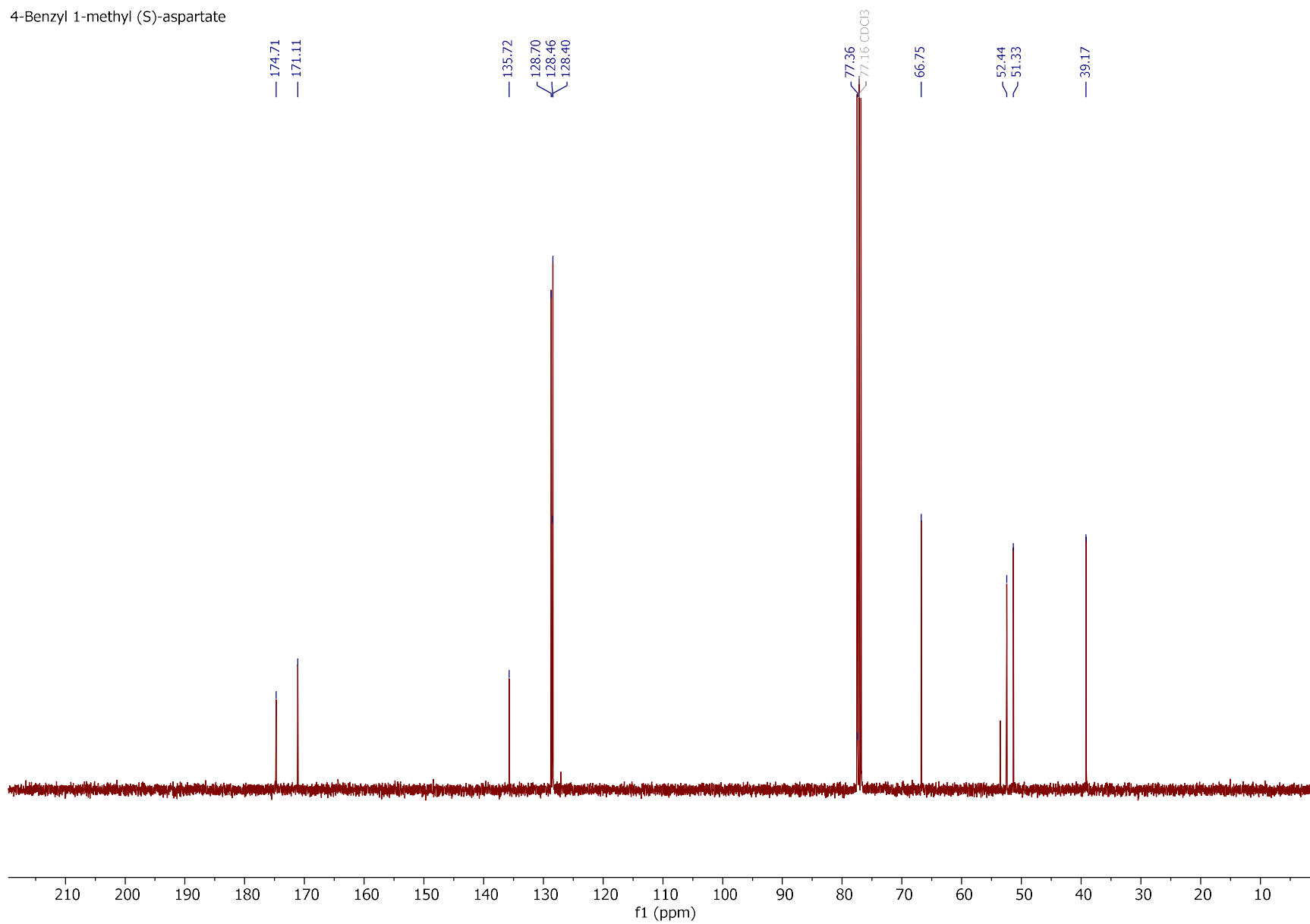


4-Benzyl 1-methyl (S)-aspartate



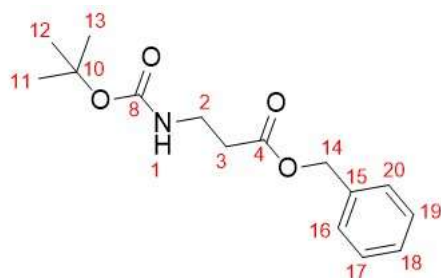
A-C2-30

4-Benzyl 1-methyl (S)-aspartate

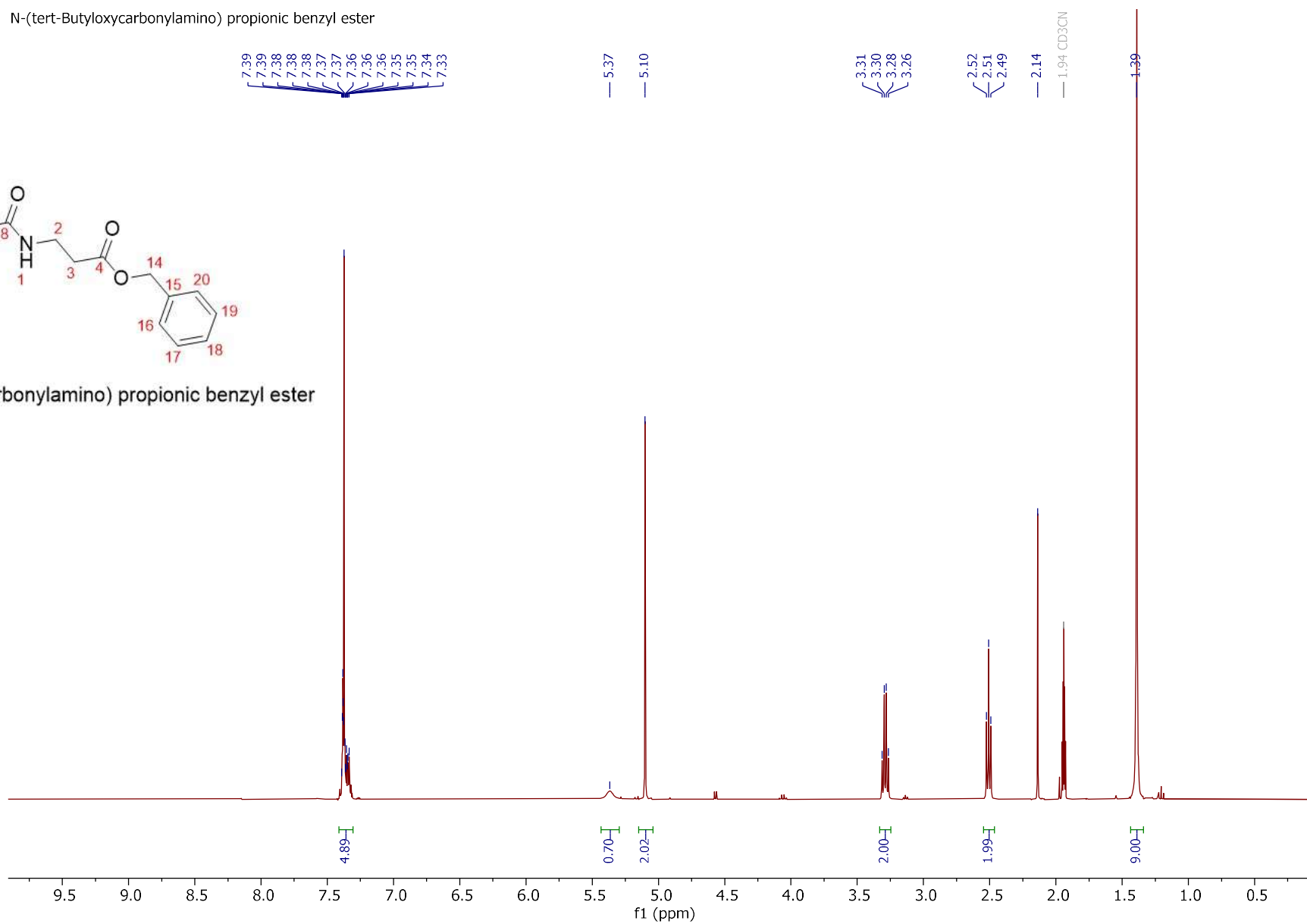


A-C2-31

N-(tert-Butyloxycarbonylamino) propionic benzyl ester

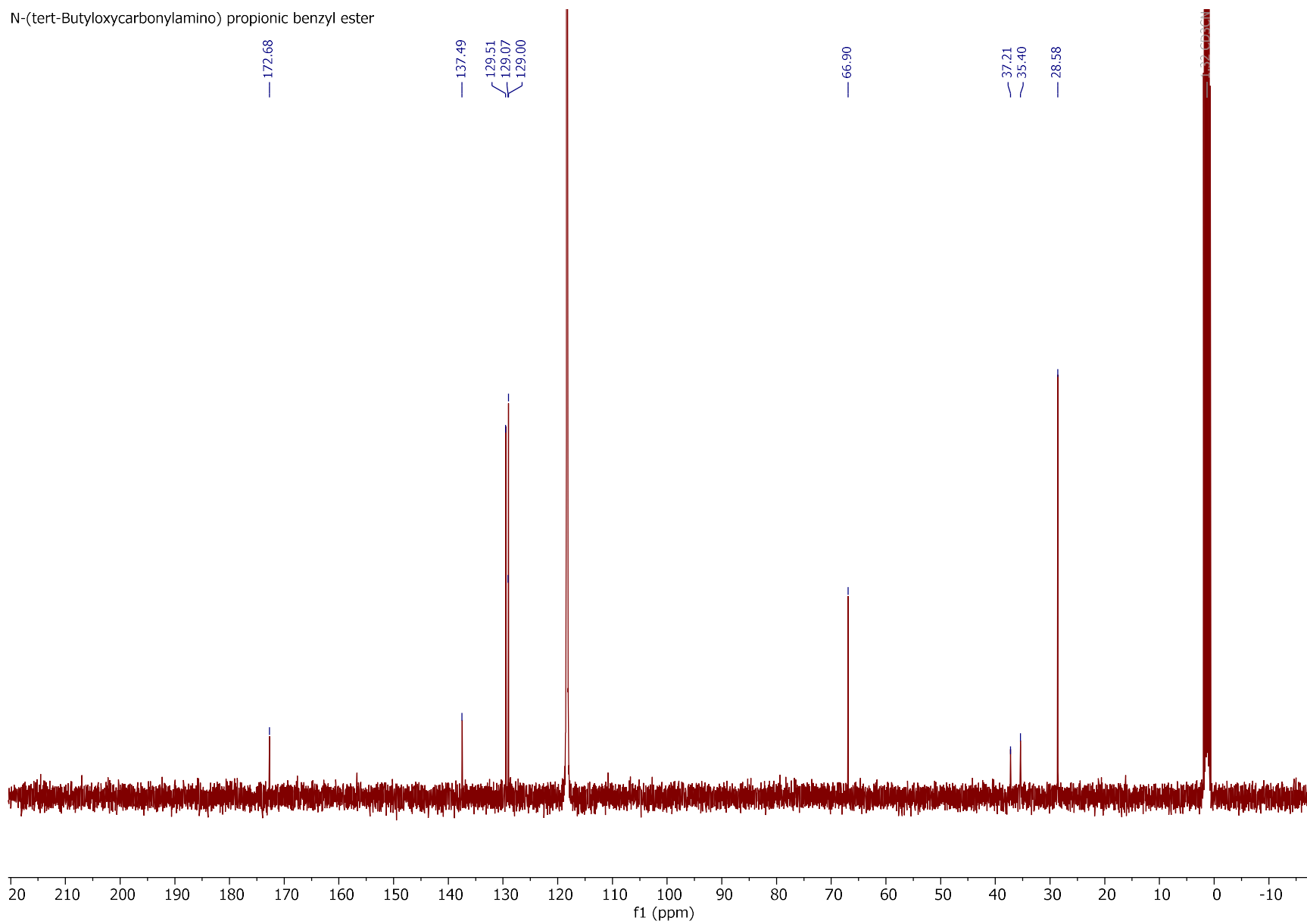


N-(tert-Butyloxycarbonylamino) propionic benzyl ester



A-C2-32

N-(tert-Butyloxycarbonylamino) propionic benzyl ester



A-C2-33

3-Aminopropionic benzyl ester

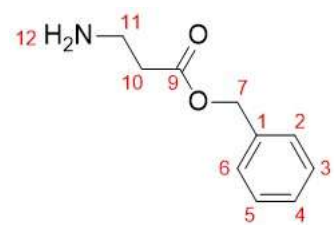
7.40
7.39
7.38
7.38
7.37
7.36
7.35
7.35
7.33

5.14

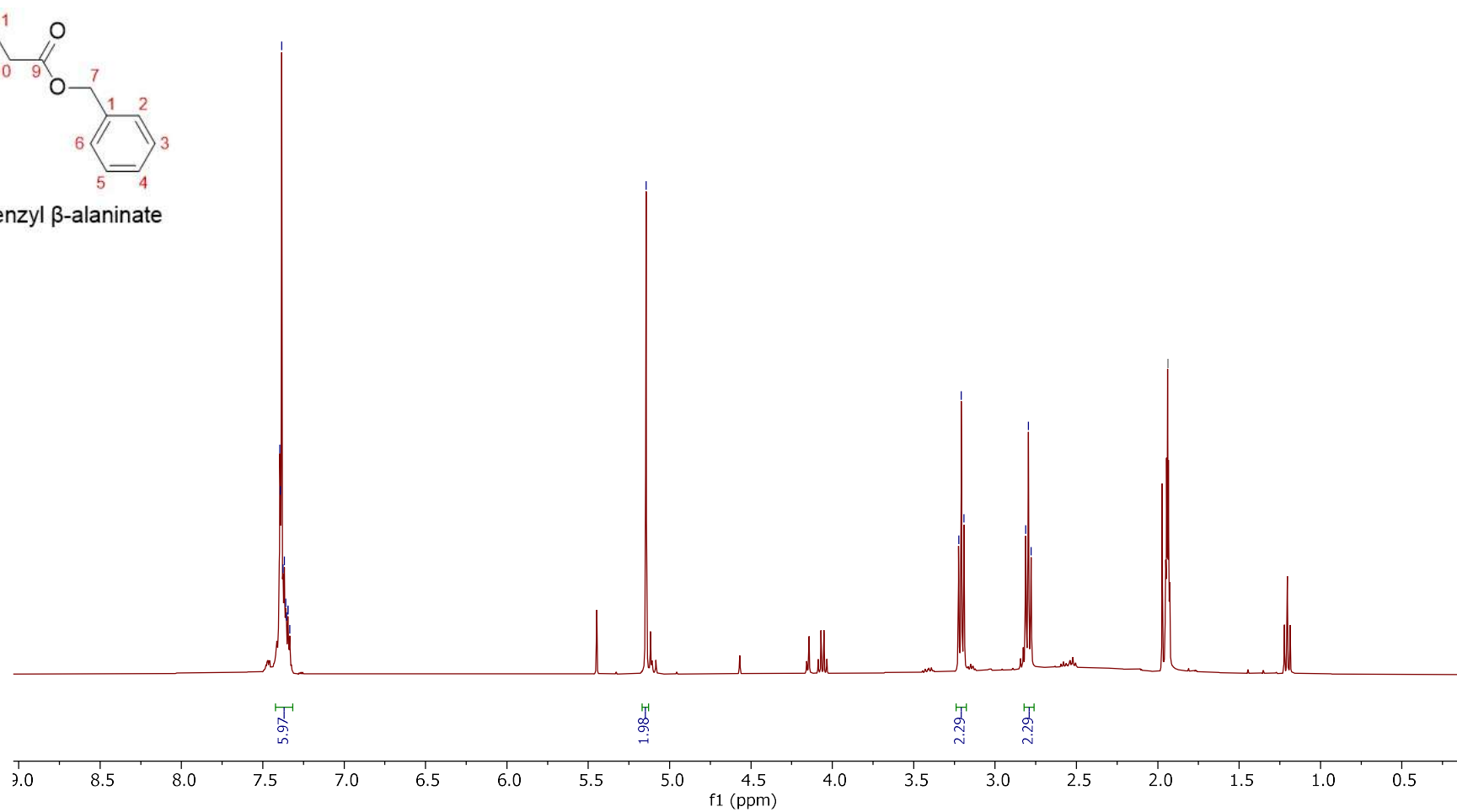
3.22
3.21
3.19

2.81
2.80
2.78

1.94 CD3CN

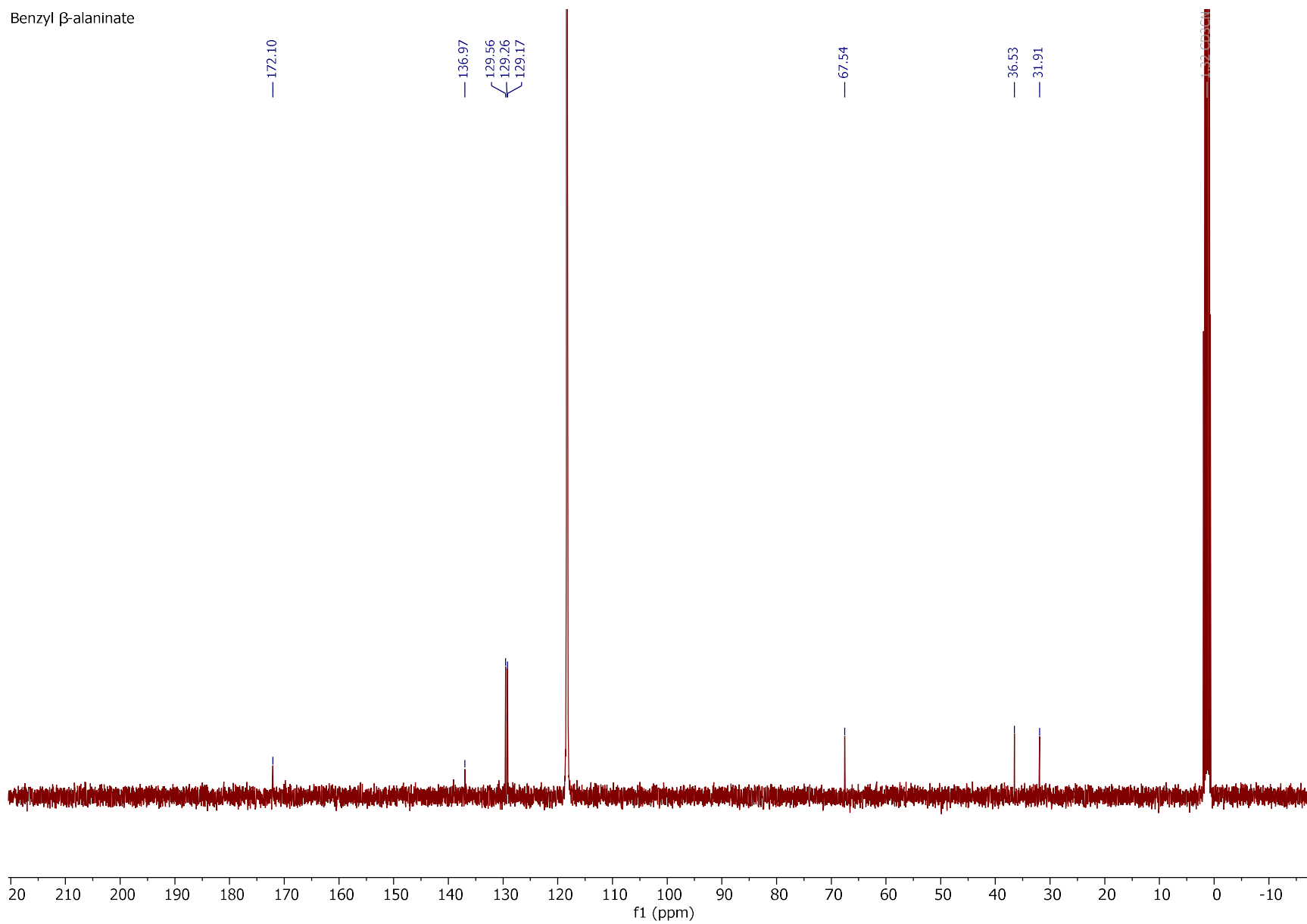


Benzyl β -alaninate

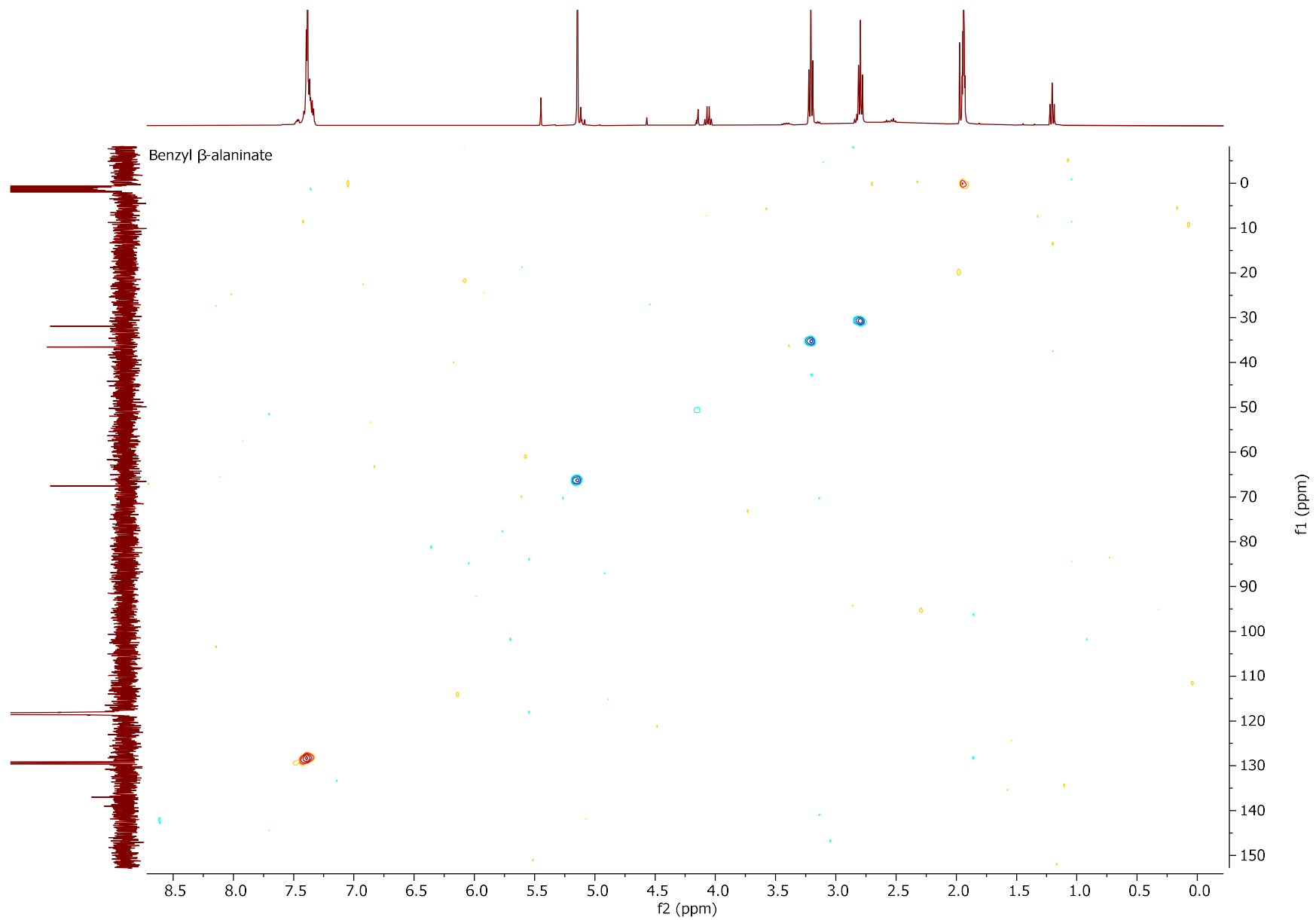


A-C2-34

Benzyl β -alaninate

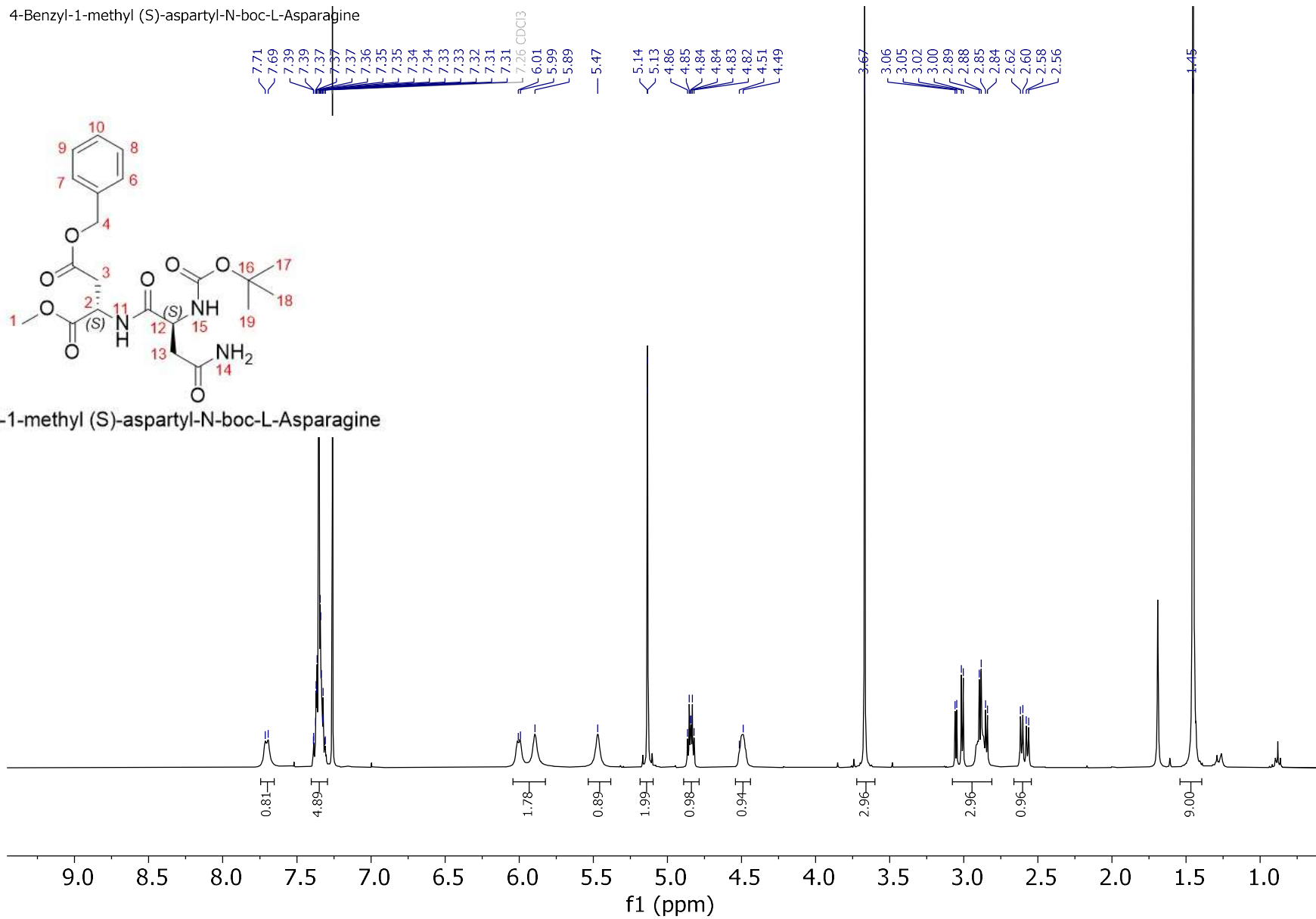


A-C2-35



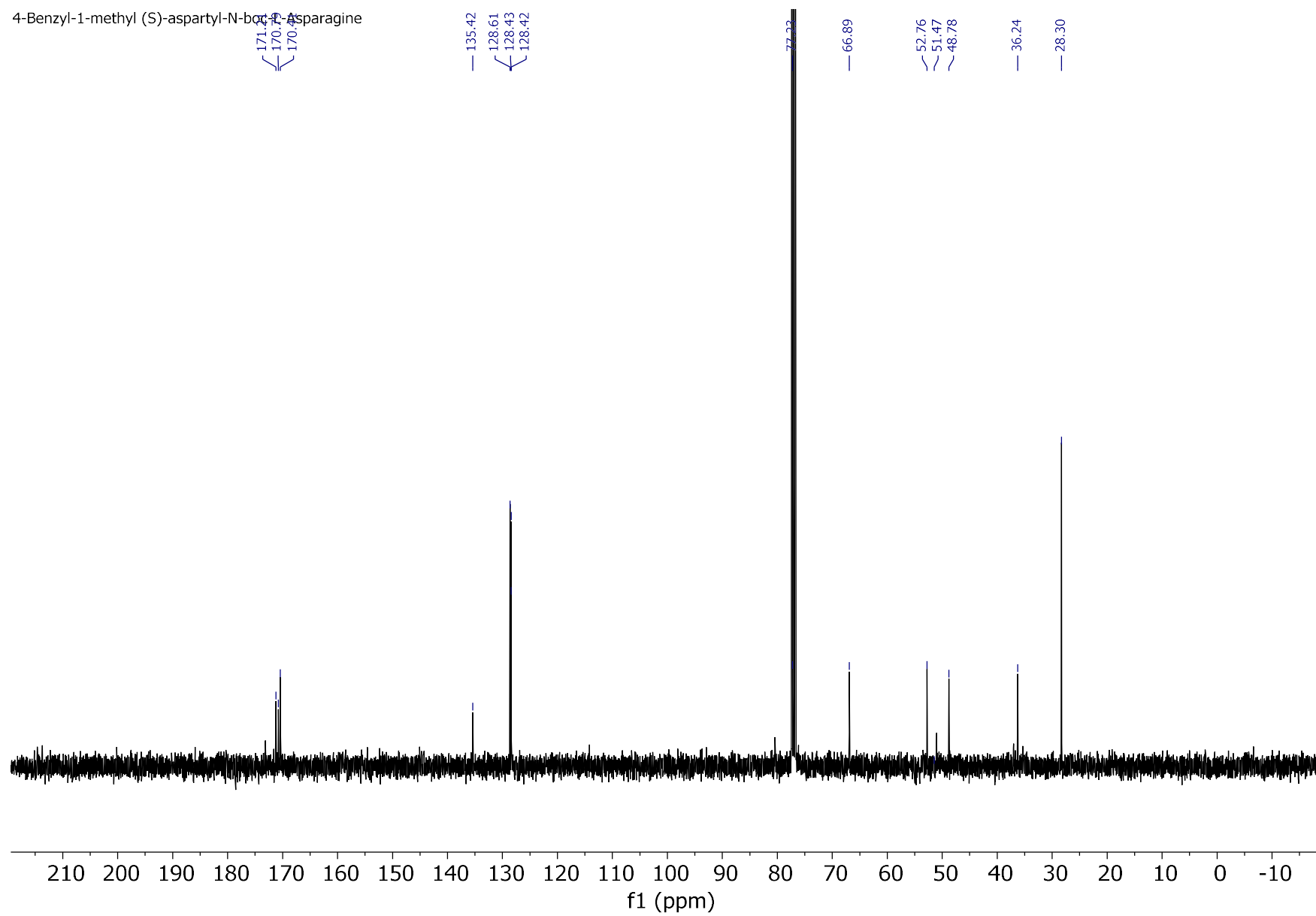
A-C2-36

4-Benzyl-1-methyl (S)-aspartyl-N-boc-L-Asparagine



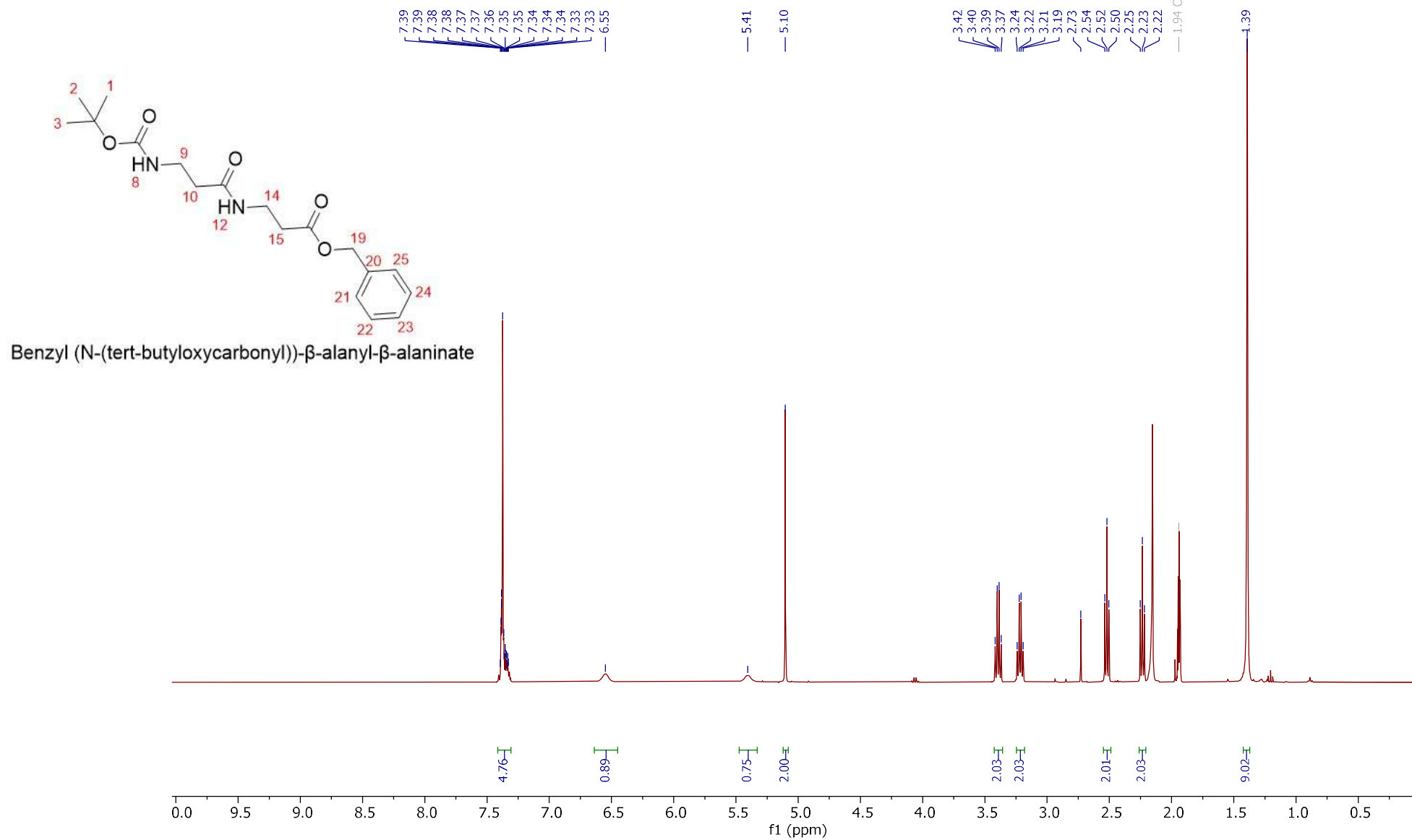
4-Benzyl-1-methyl (S)-aspartyl-N-boc-L-Asparagine

4-Benzyl-1-methyl (S)-aspartyl-N-benzyloxycarbonyl-L-asparagine



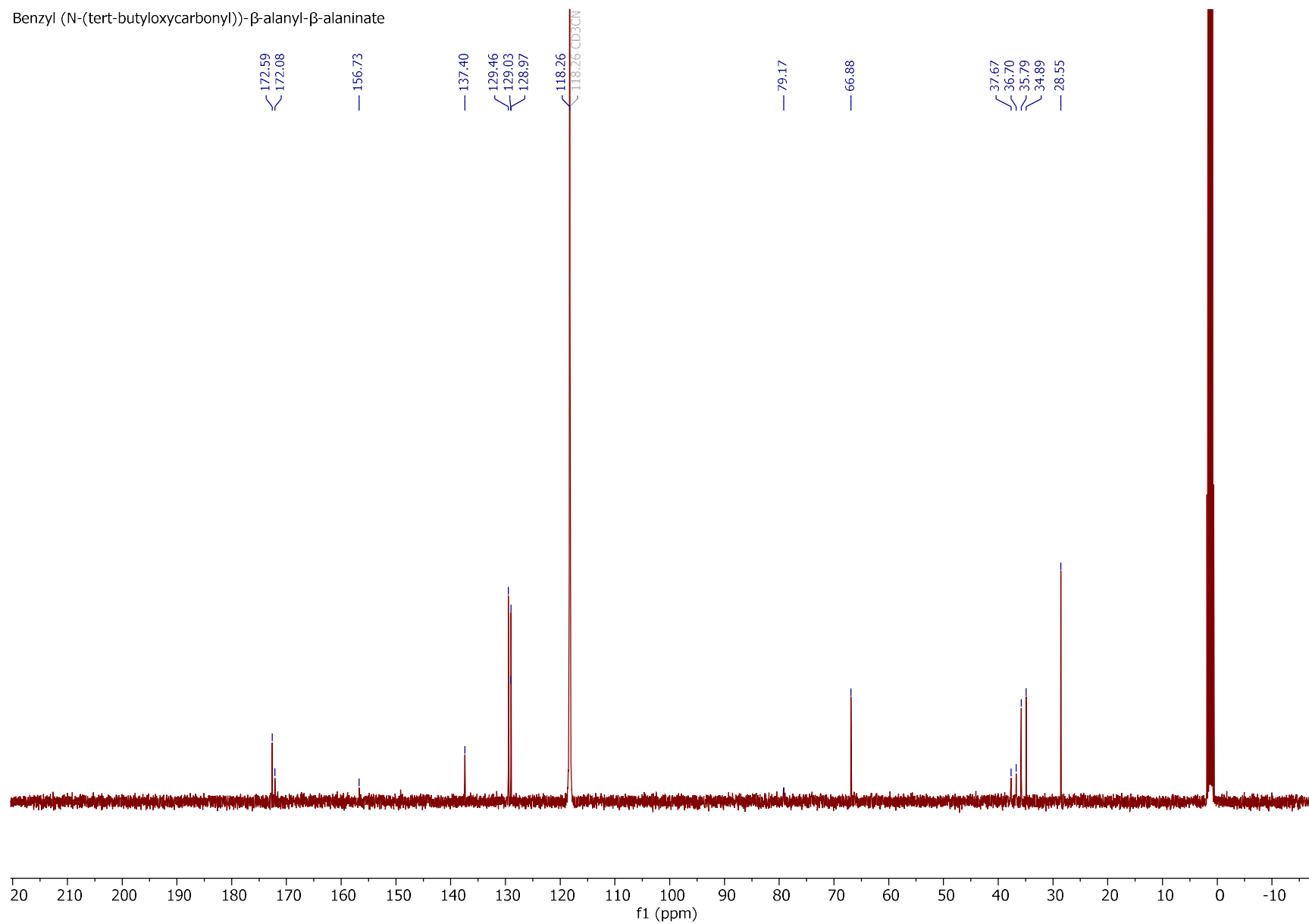
A-C2-38

Benzyl (N-(tert-butyloxycarbonyl))-β-alanyl-β-alaninate

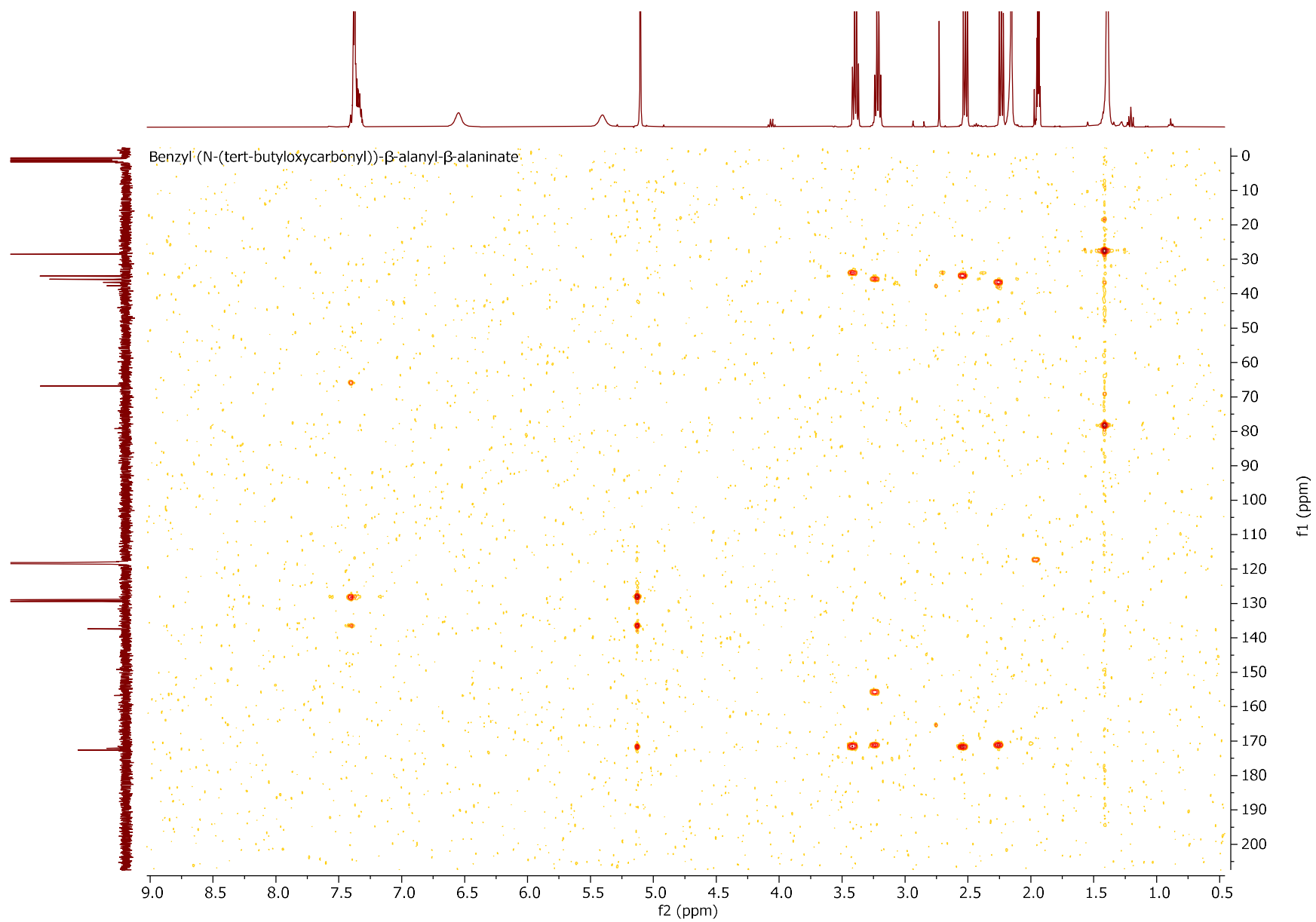


A-C2-39

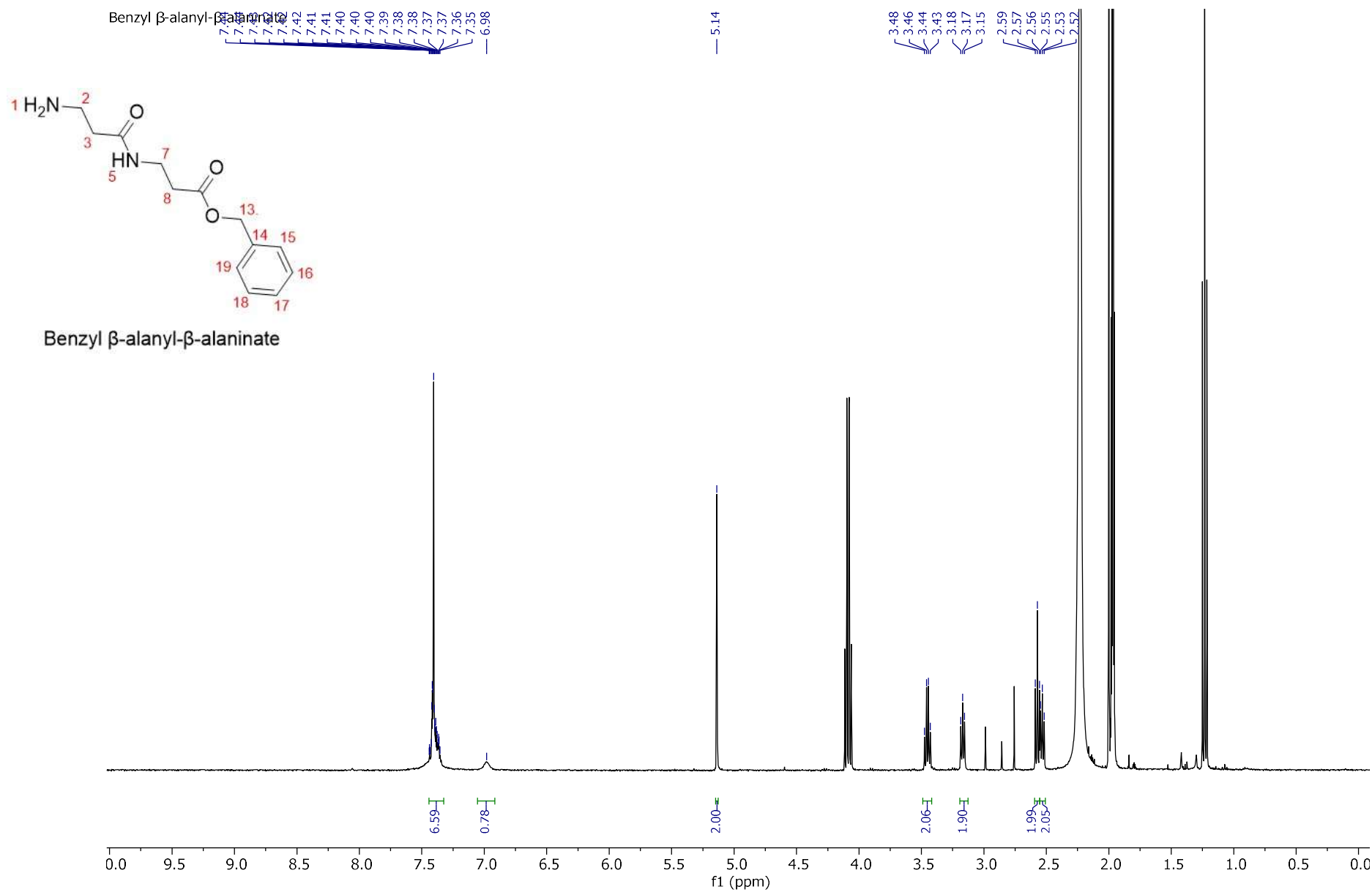
Benzyl (N-(tert-butyloxycarbonyl))-β-alanyl-β-alaninate



A-C2-40

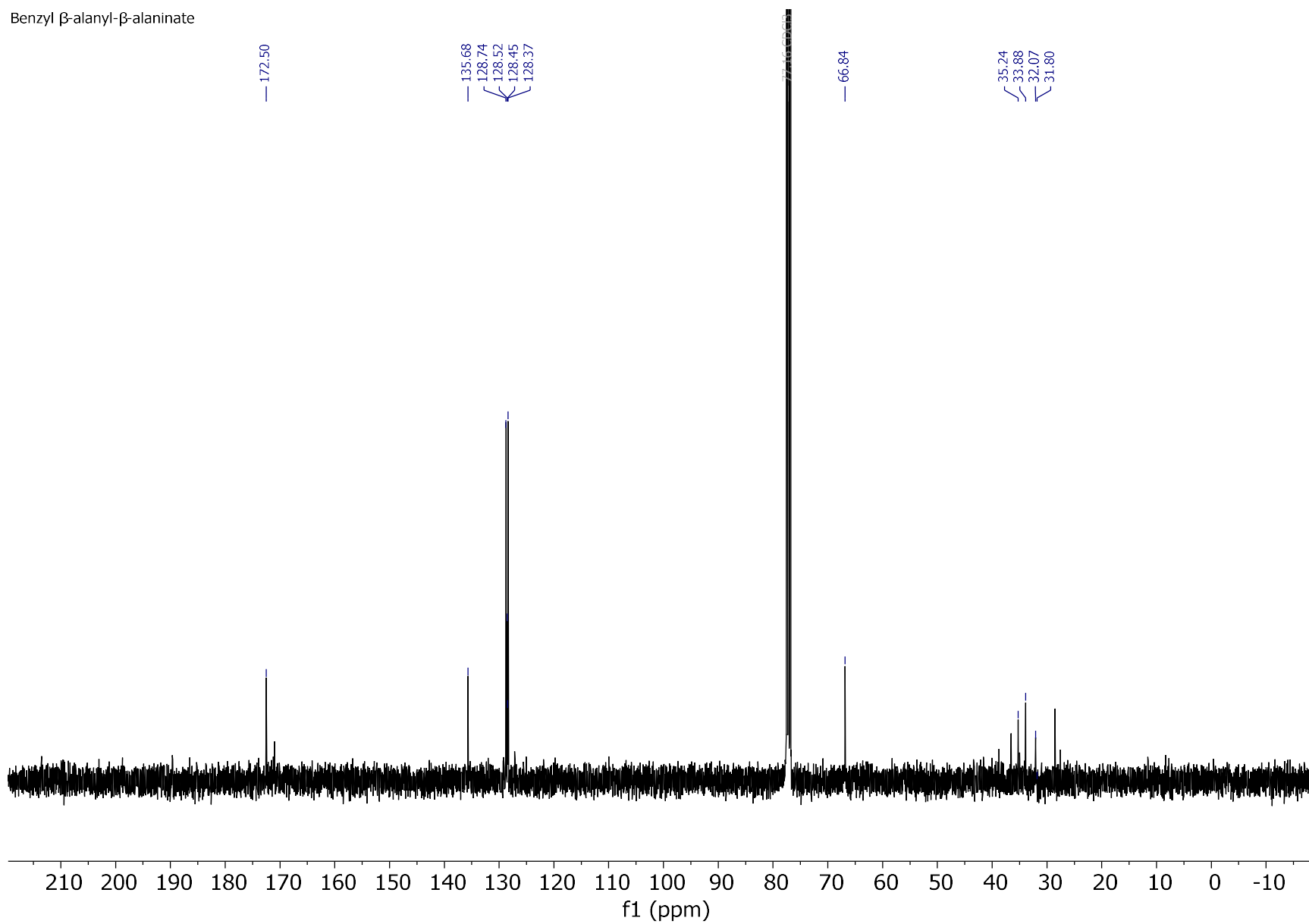


A-C2-42

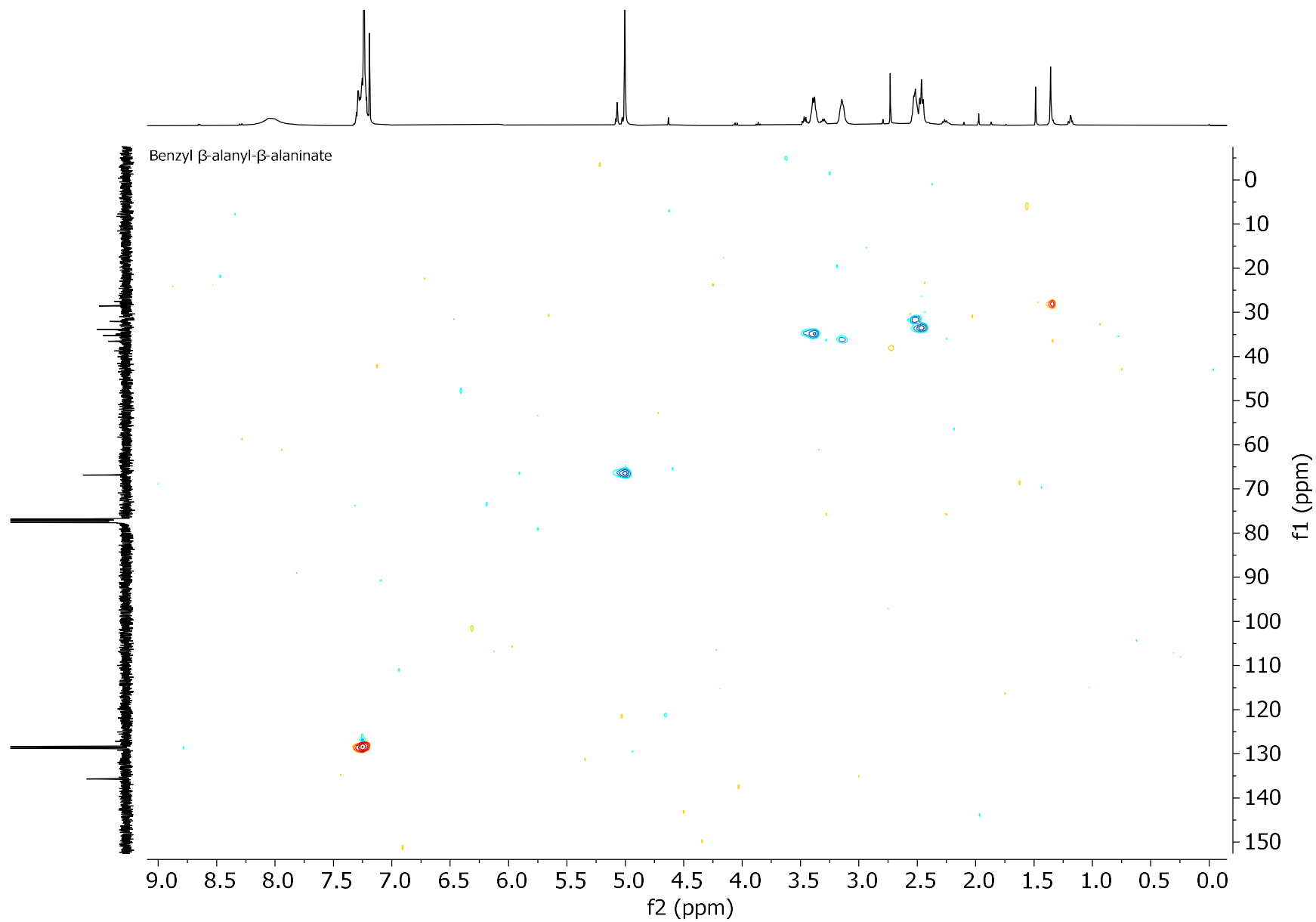


A-C2-43

Benzyl β -alanyl- β -alaninate

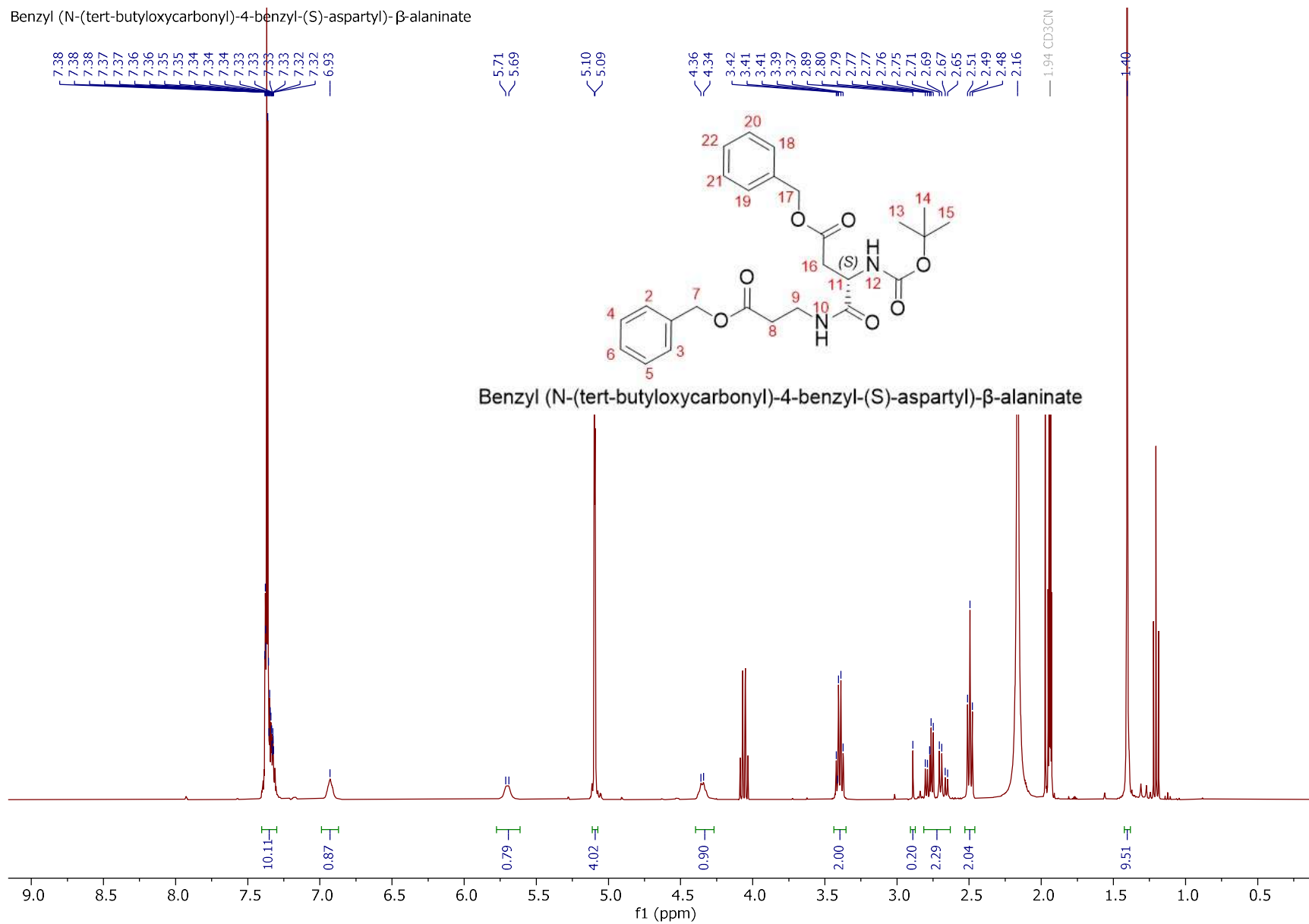


A-C2-44



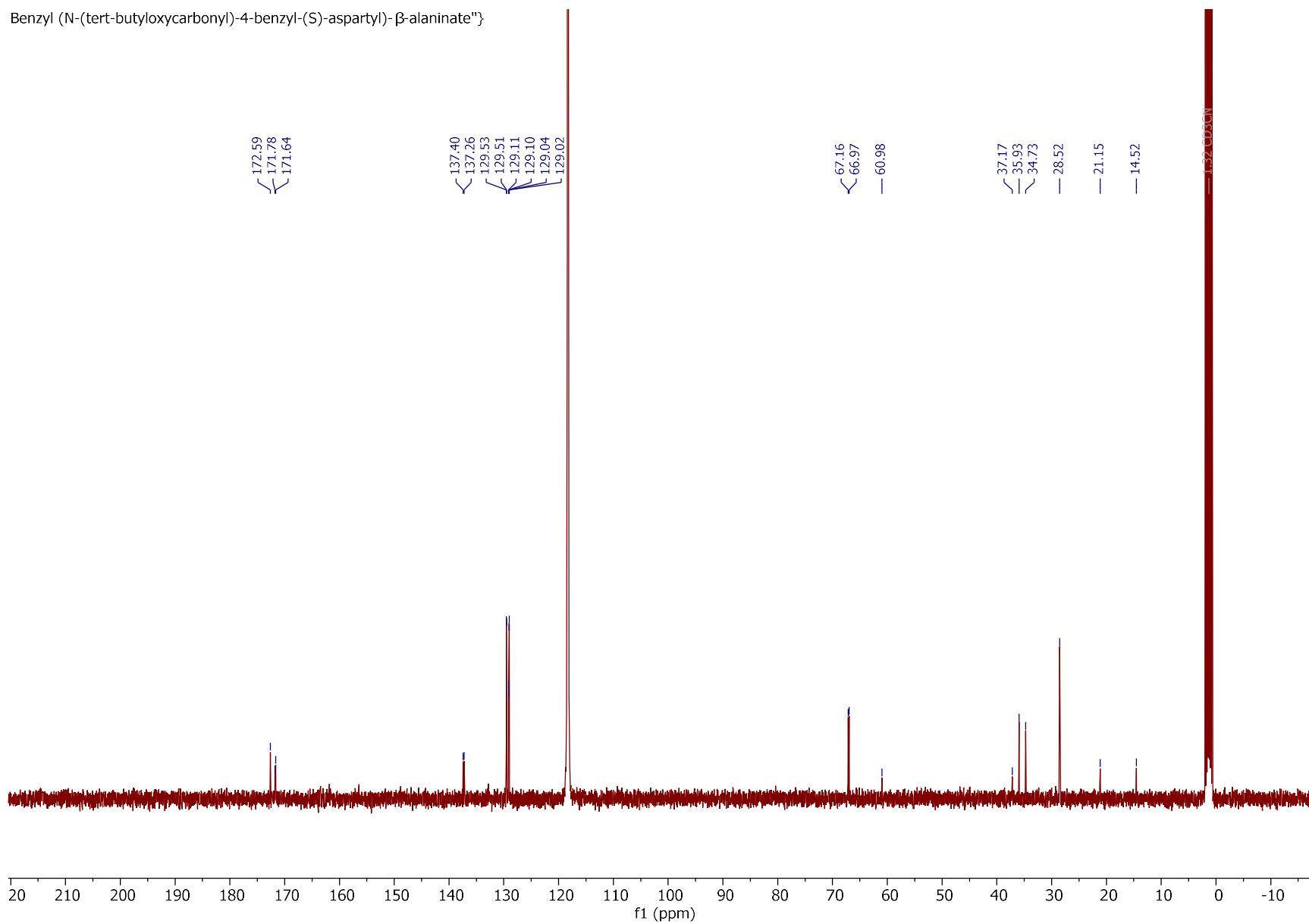
A-C2-45

Benzyl (N-(tert-butyloxycarbonyl)-4-benzyl-(S)-aspartyl)- β -alaninate

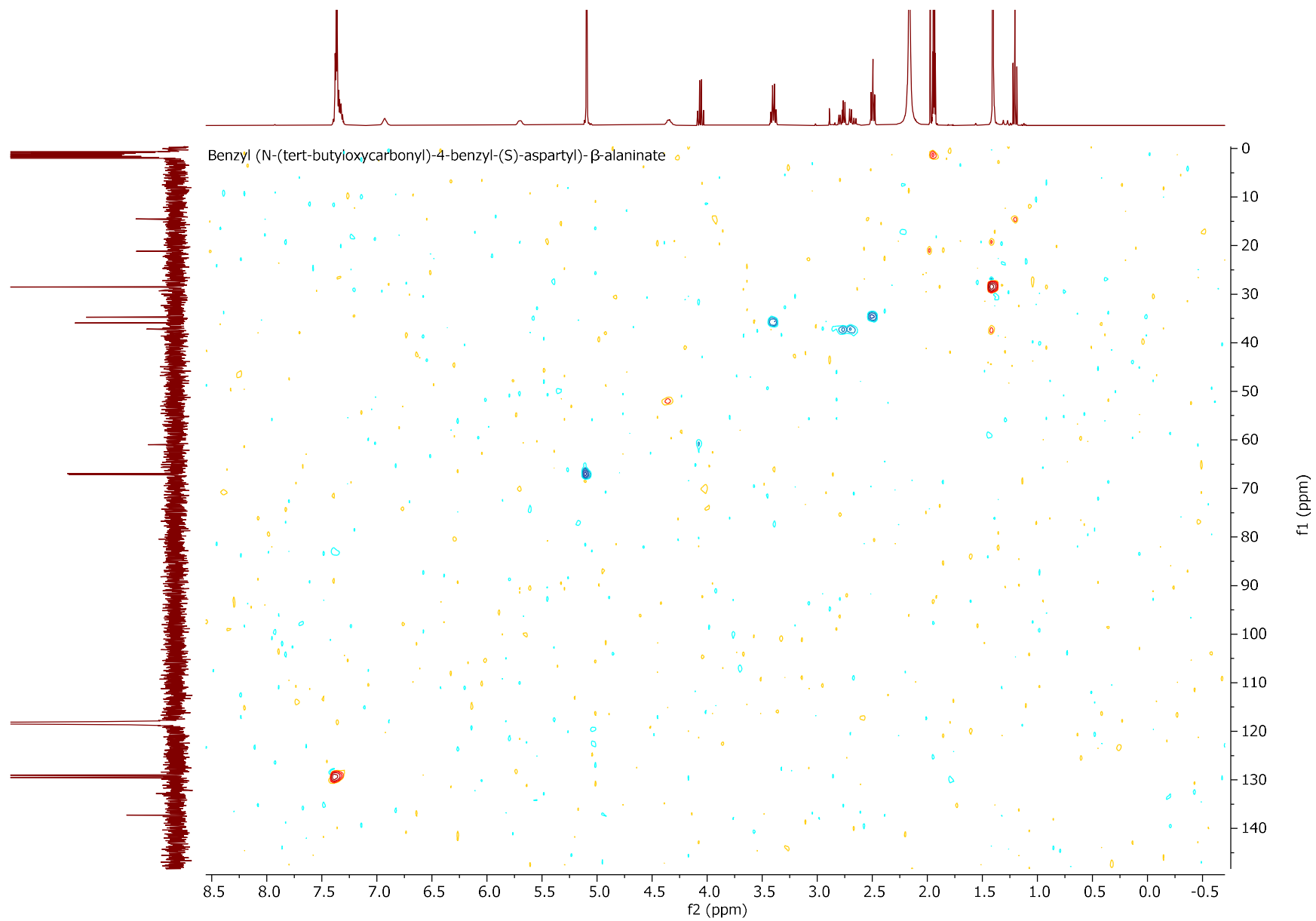


A-C2-46

Benzyl (N-(tert-butyloxycarbonyl)-4-benzyl-(S)-aspartyl)- β -alaninate"}
CC(C)(C)OC(=O)N[C@@H](Cc1ccccc1)C[C@@H](Cc2ccccc2)C(=O)OCC(=O)[O-]



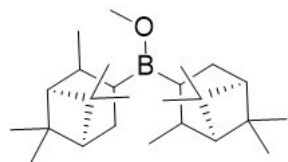
A-C2-47



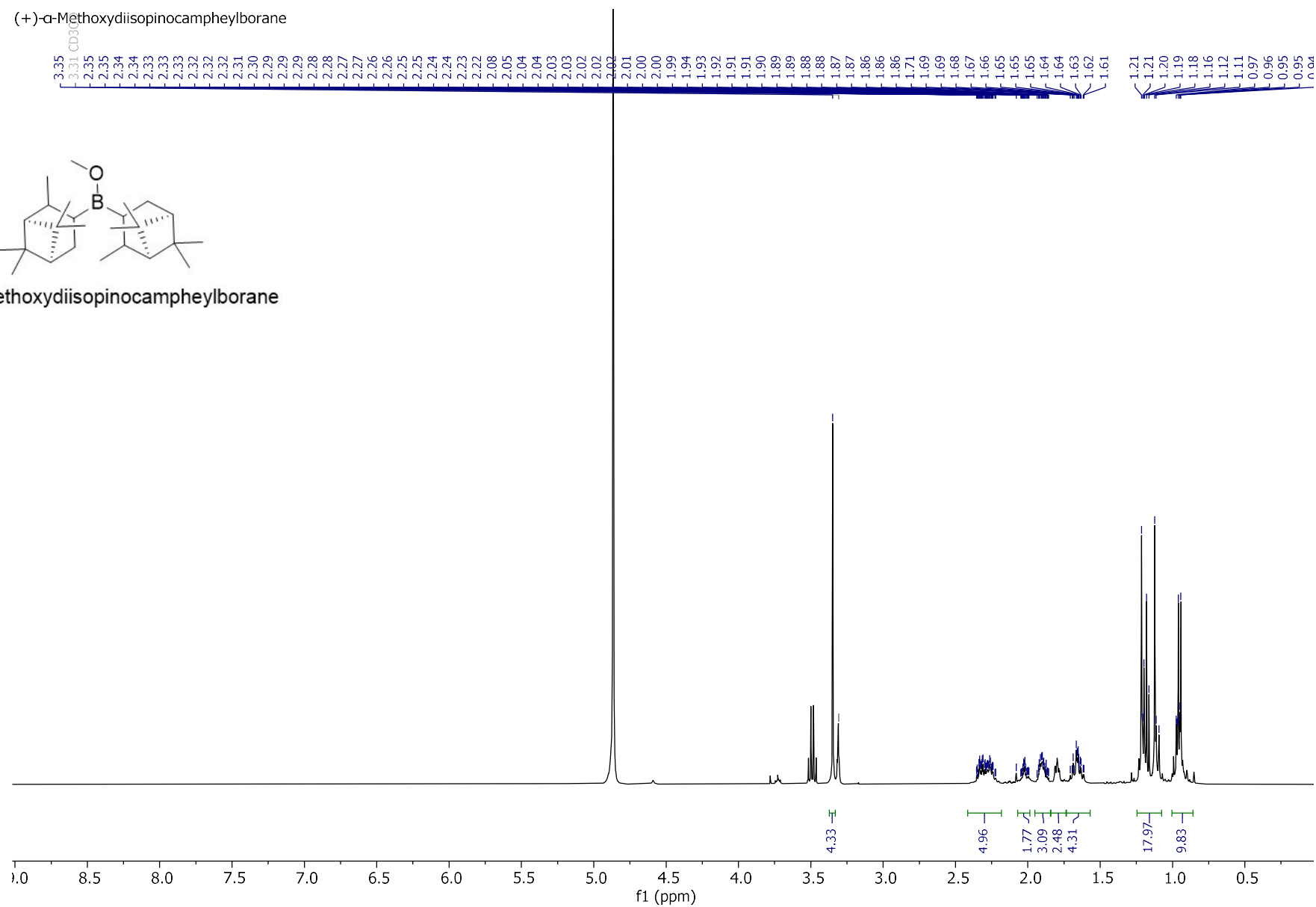
A-C2-48

A-C3

(+)- α -Methoxydiisopinocampheylborane



(+)- α -Methoxydiisopinocampheylborane

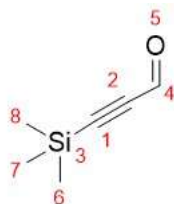


A-C3-1

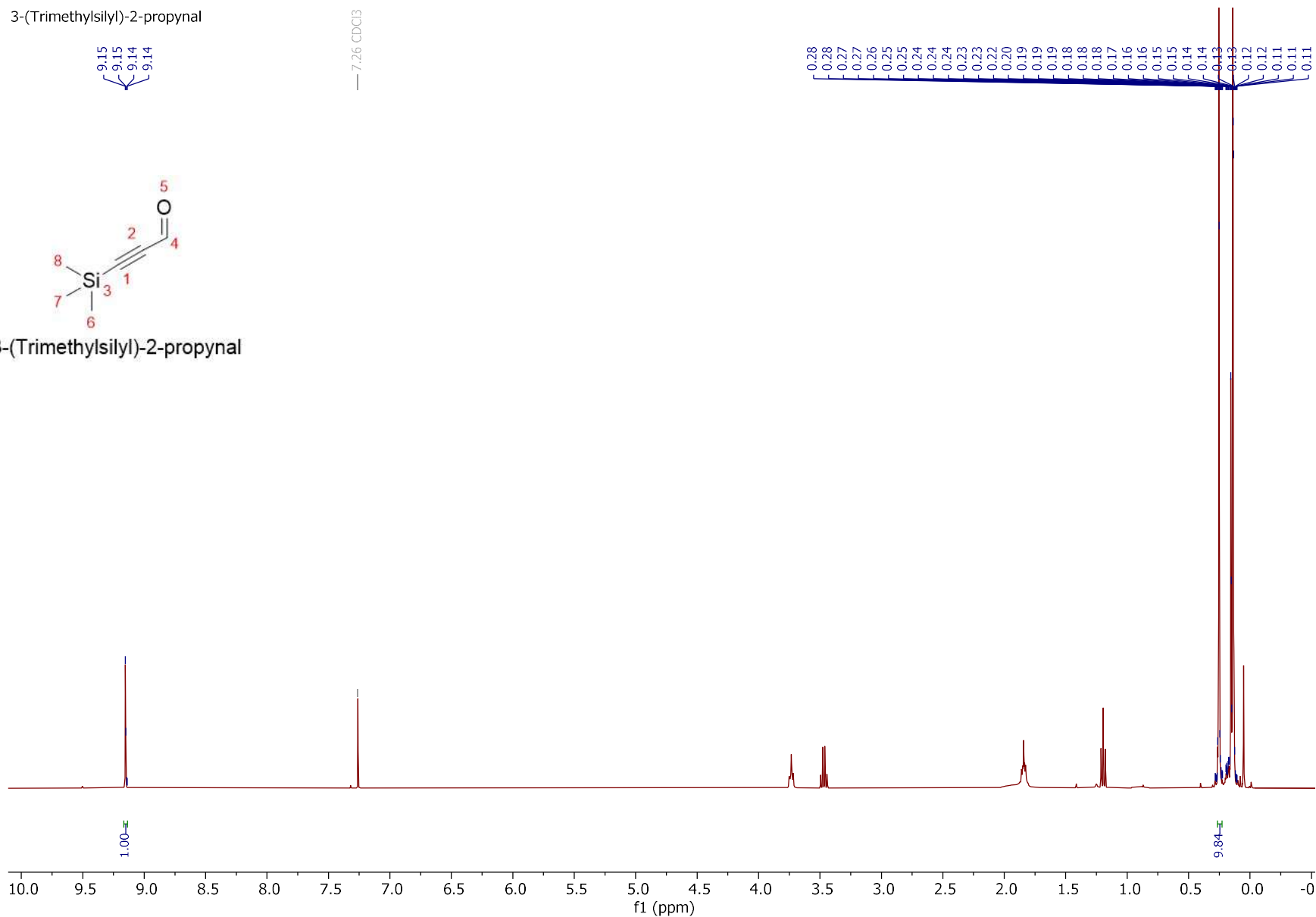
3-(Trimethylsilyl)-2-propynal

9.15
9.15
9.14
9.14

— 7.26 CDCl₃

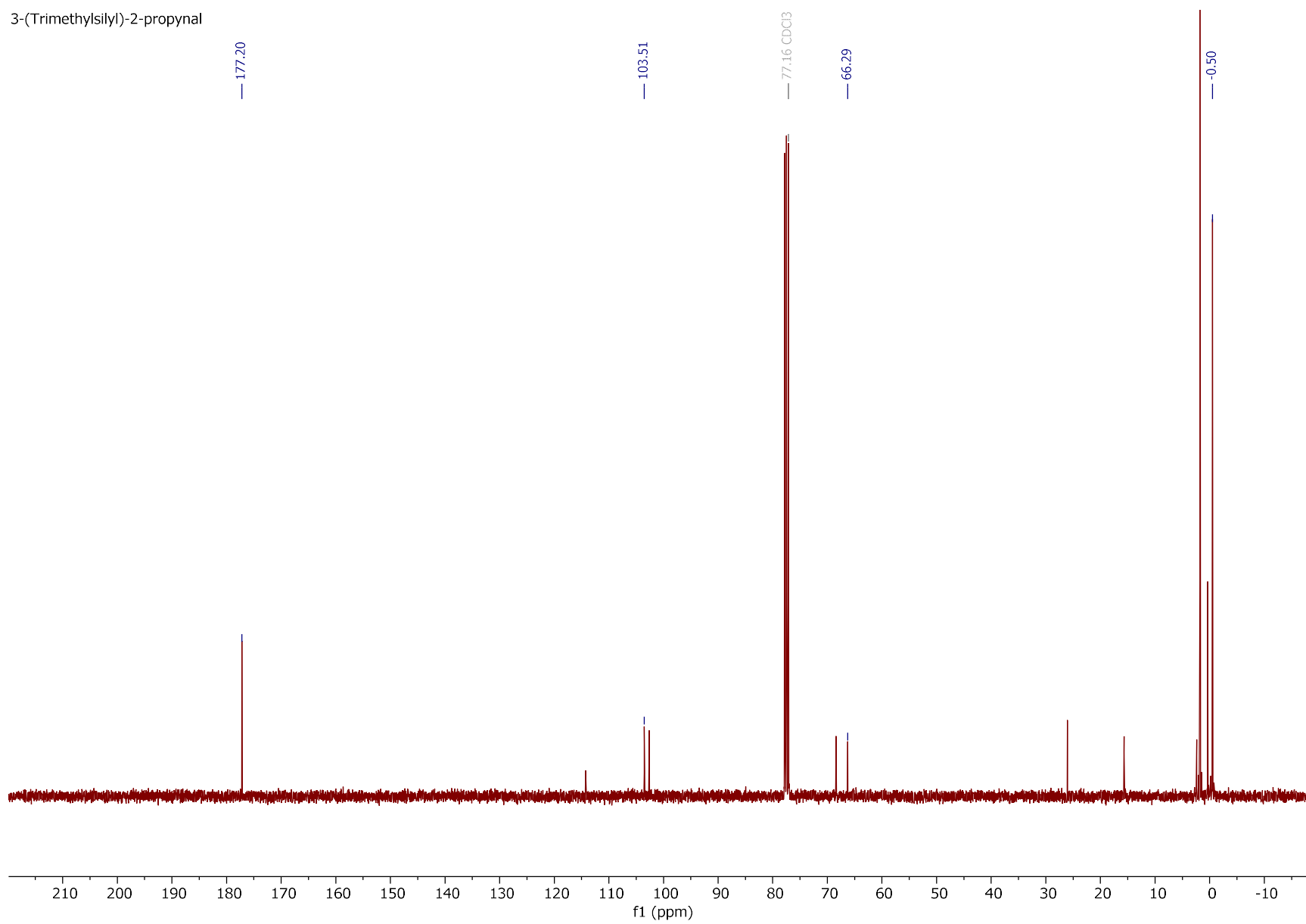


3-(Trimethylsilyl)-2-propynal



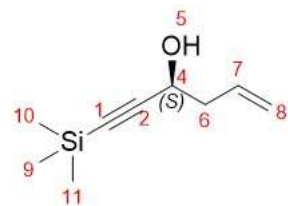
A-C3-2

3-(Trimethylsilyl)-2-propynal

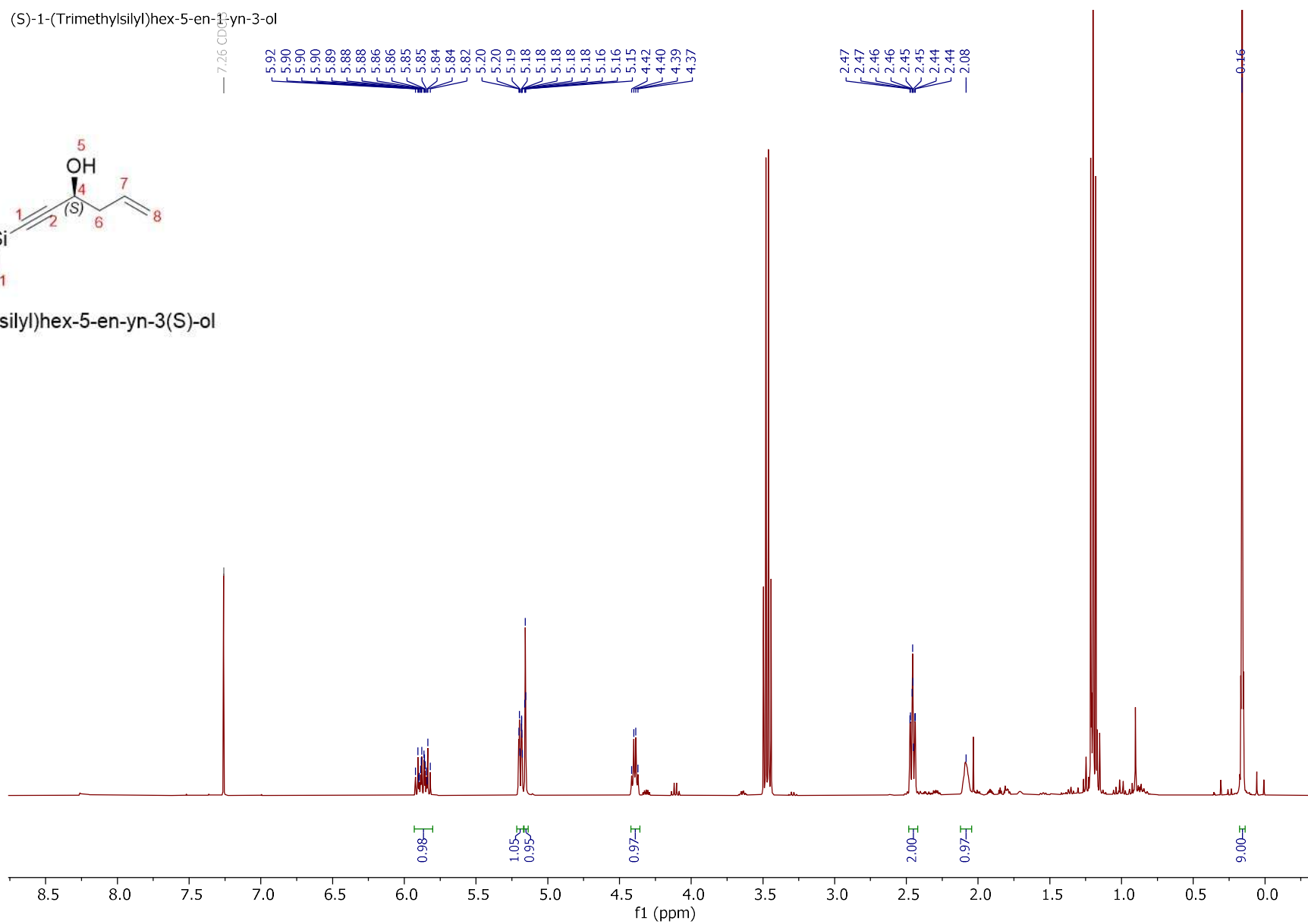


A-C3-3

(S)-1-(Trimethylsilyl)hex-5-en-1-yn-3-ol

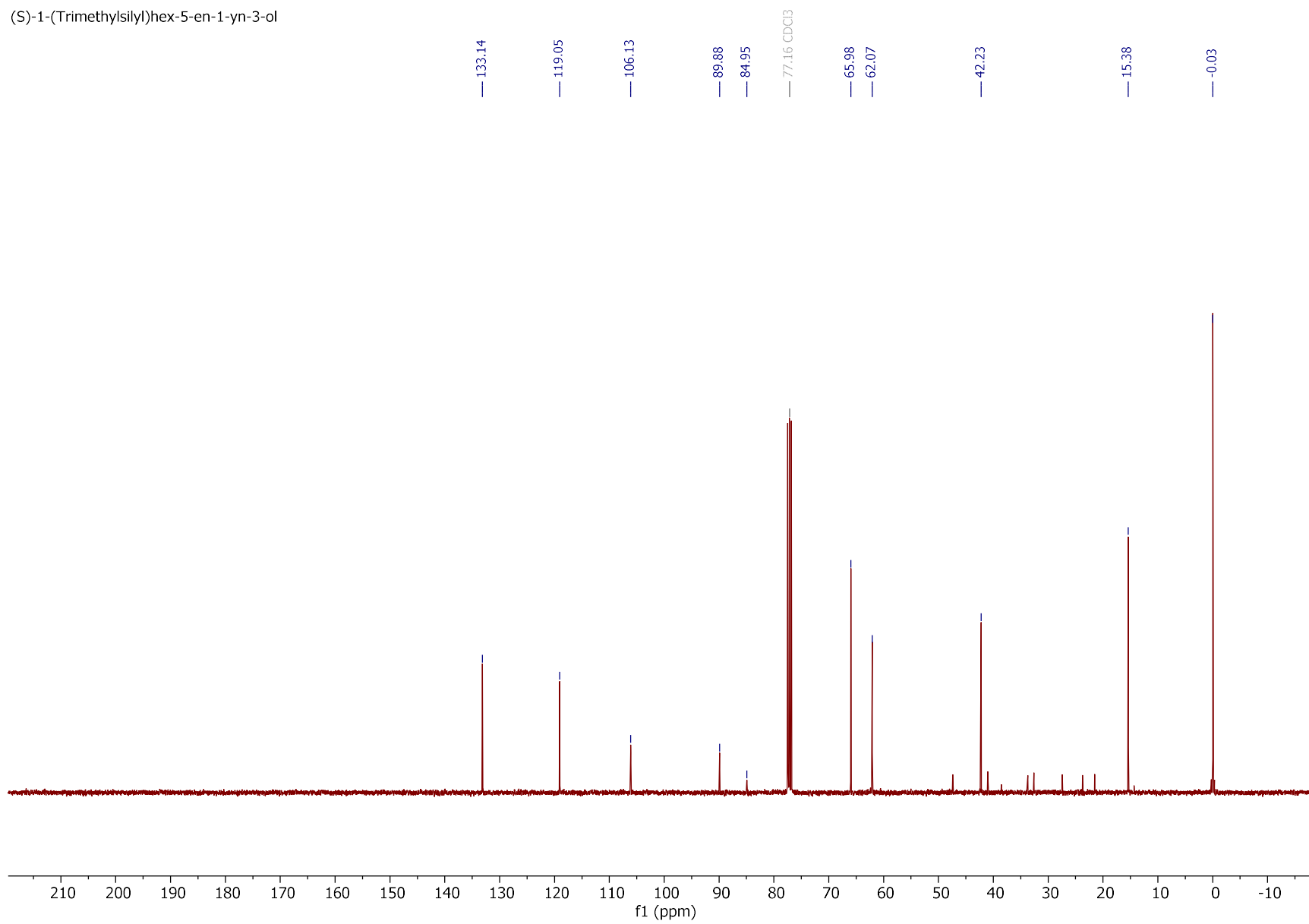


(Trimethylsilyl)hex-5-en-yn-3(S)-ol

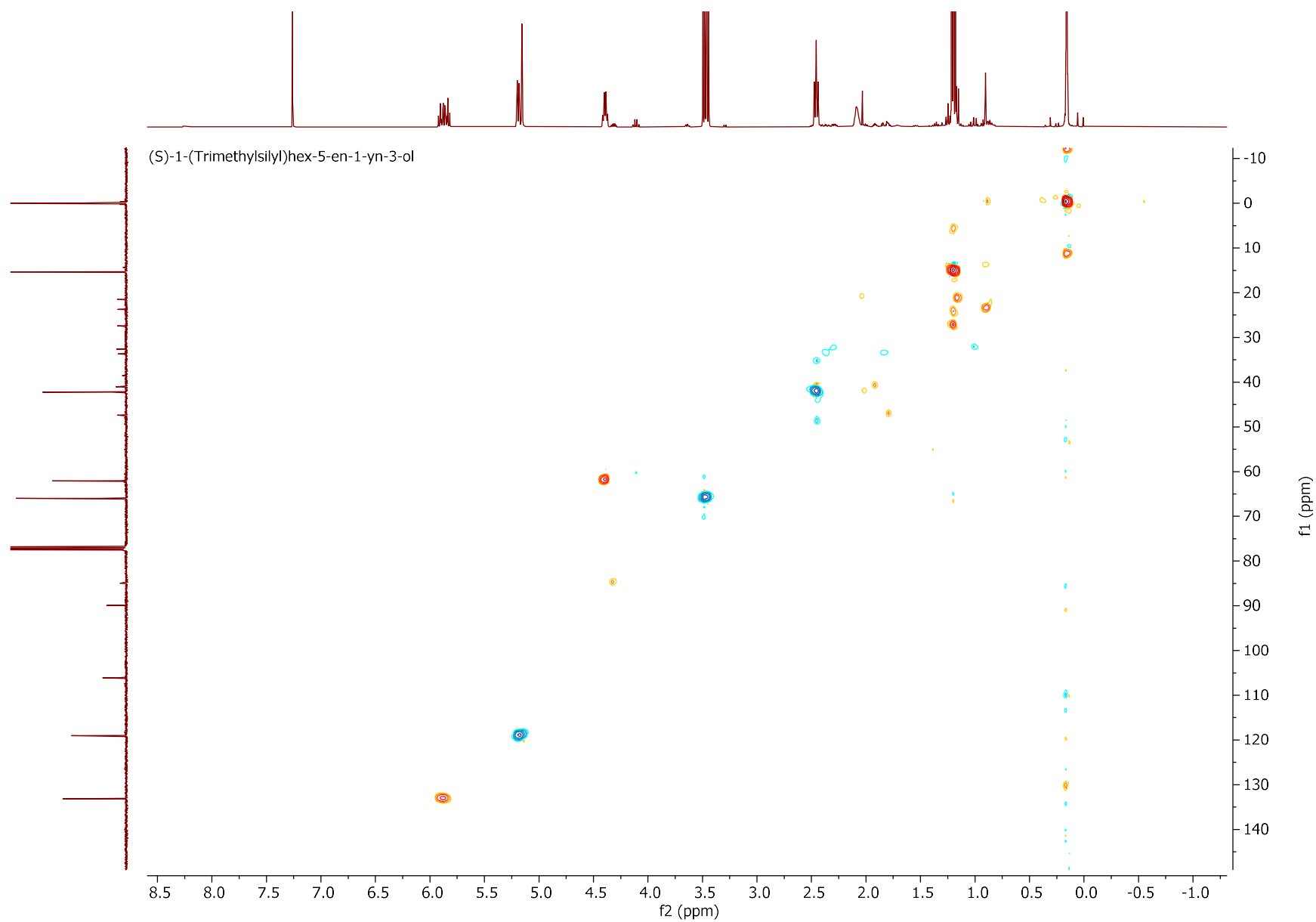


A-C3-4

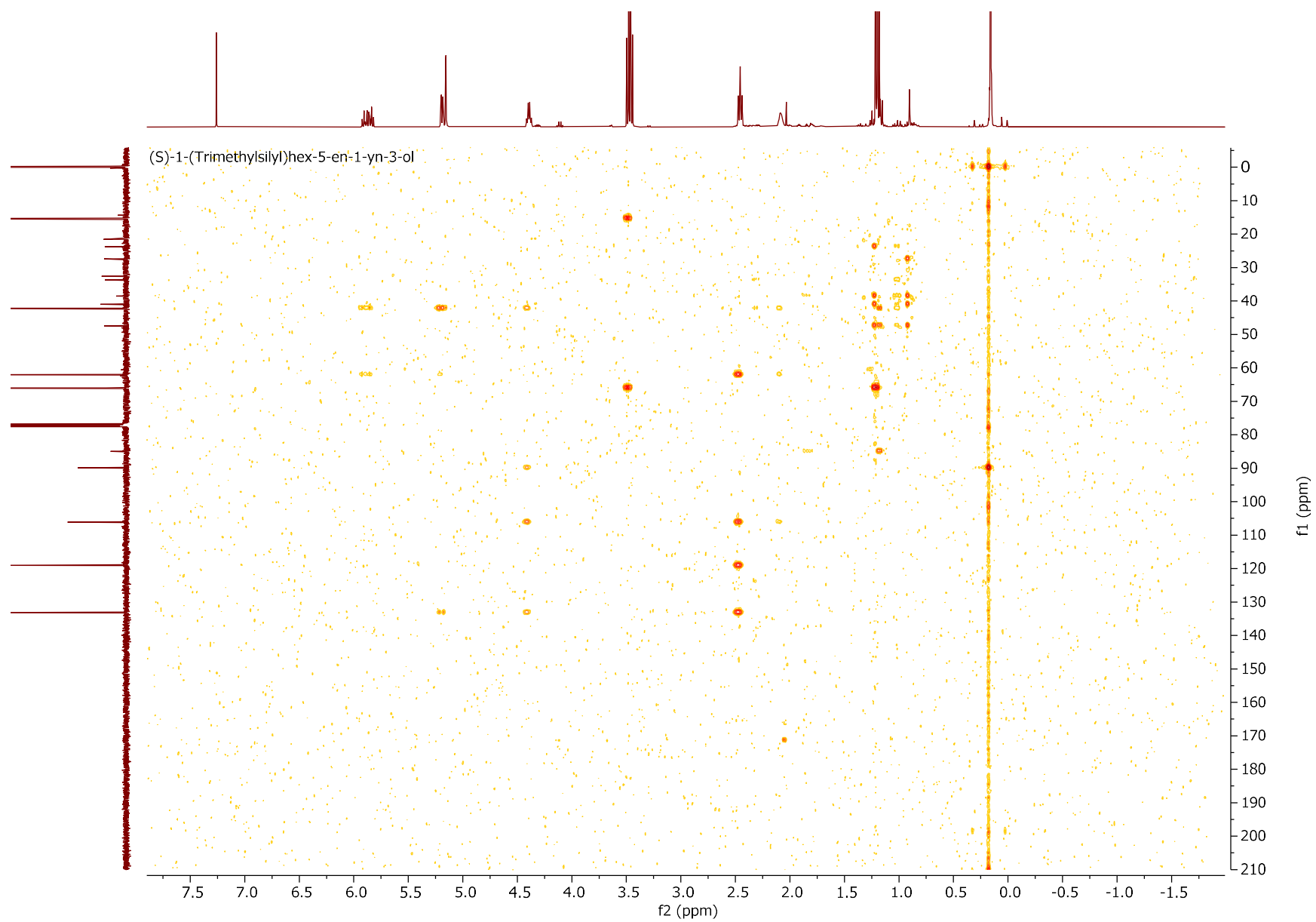
(S)-1-(Trimethylsilyl)hex-5-en-1-yn-3-ol



A-C3-5

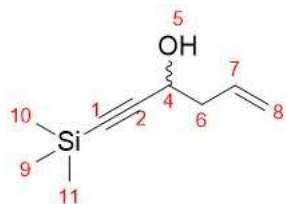


A-C3-6

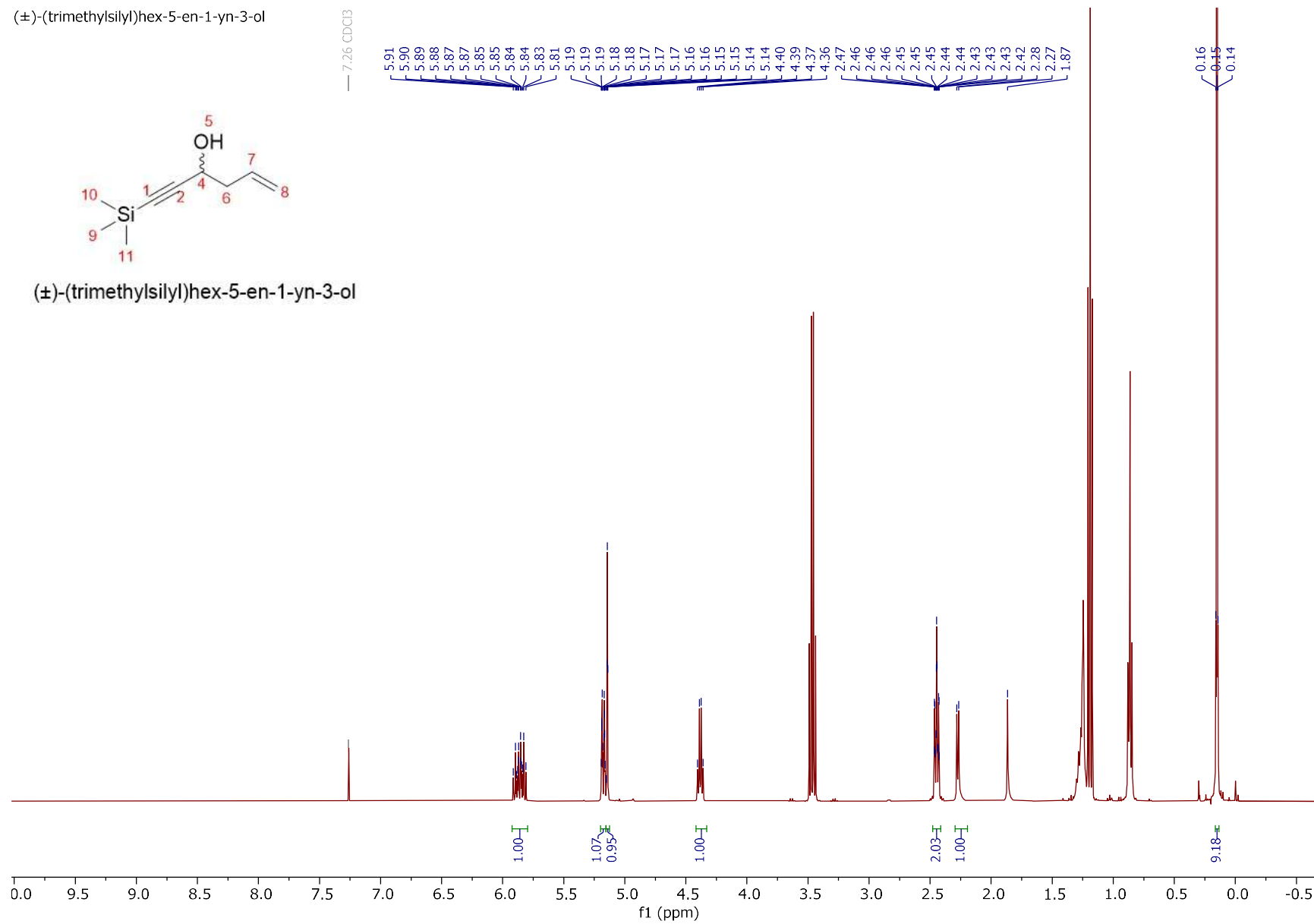


A-C3-7

(±)-(trimethylsilyl)hex-5-en-1-yn-3-ol

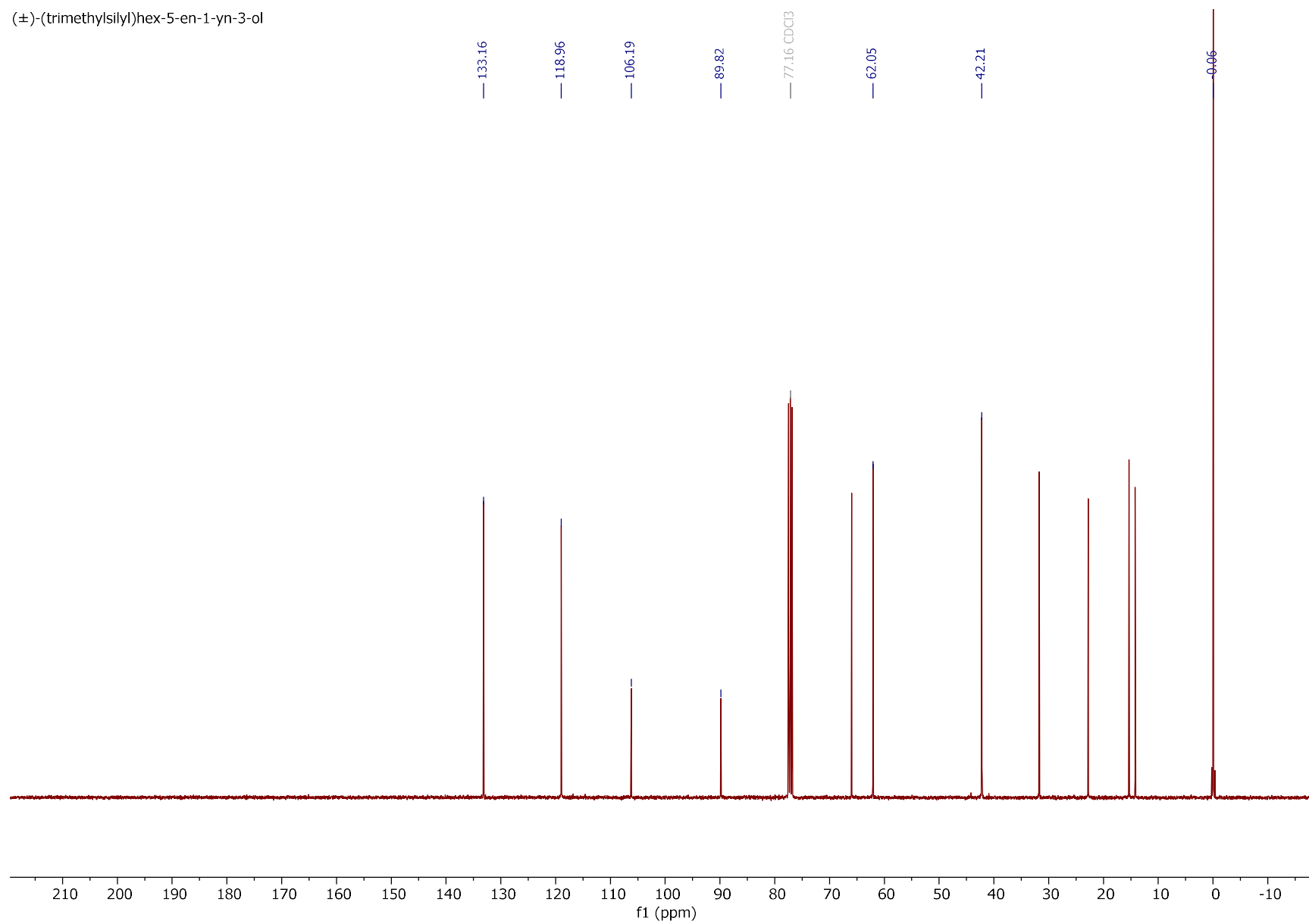


(±)-(trimethylsilyl)hex-5-en-1-yn-3-ol



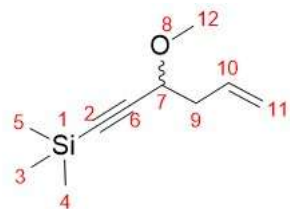
A-C3-8

(±)-(trimethylsilyl)hex-5-en-1-yn-3-ol

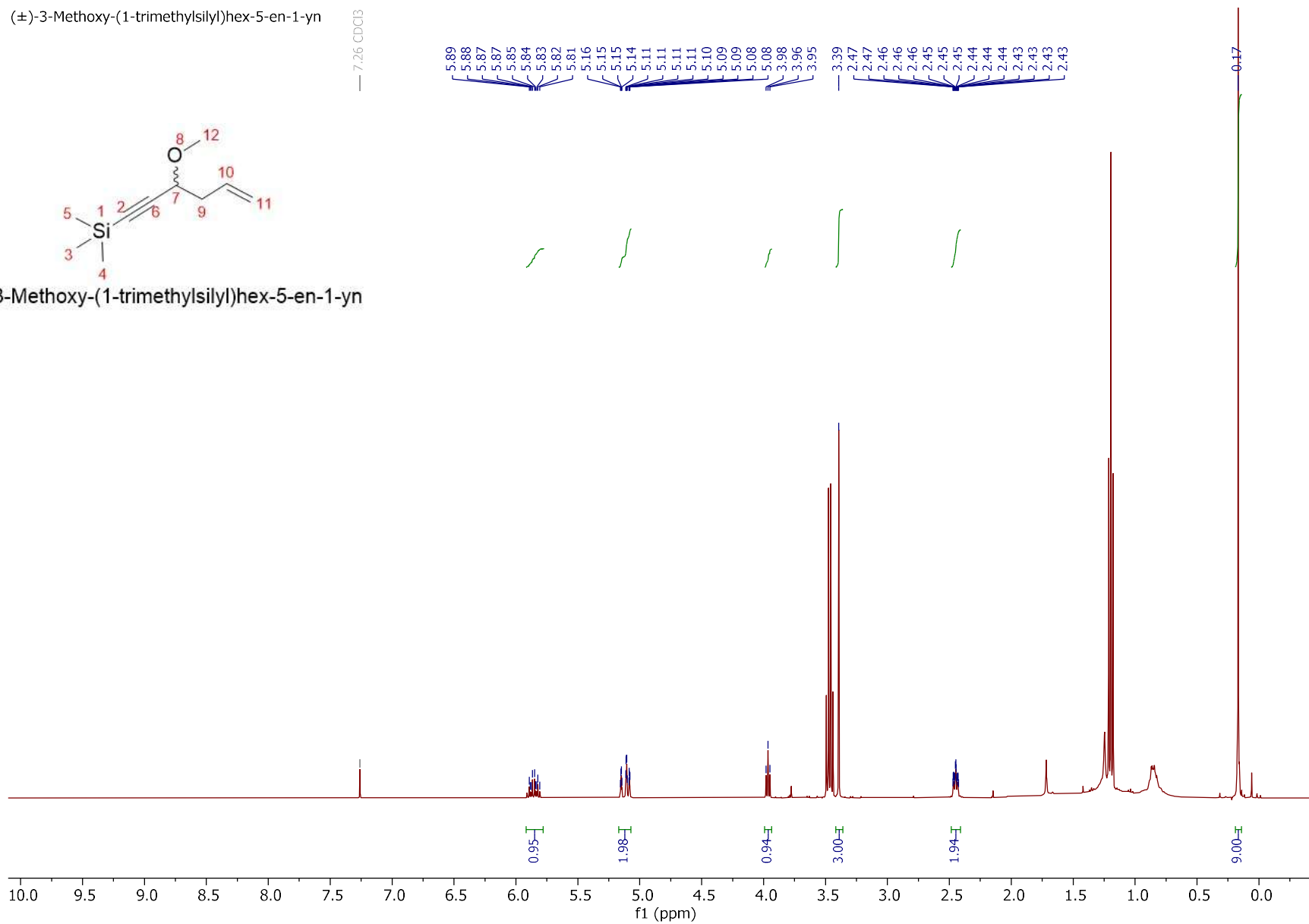


A-C3-9

(±)-3-Methoxy-(1-trimethylsilyl)hex-5-en-1-yn

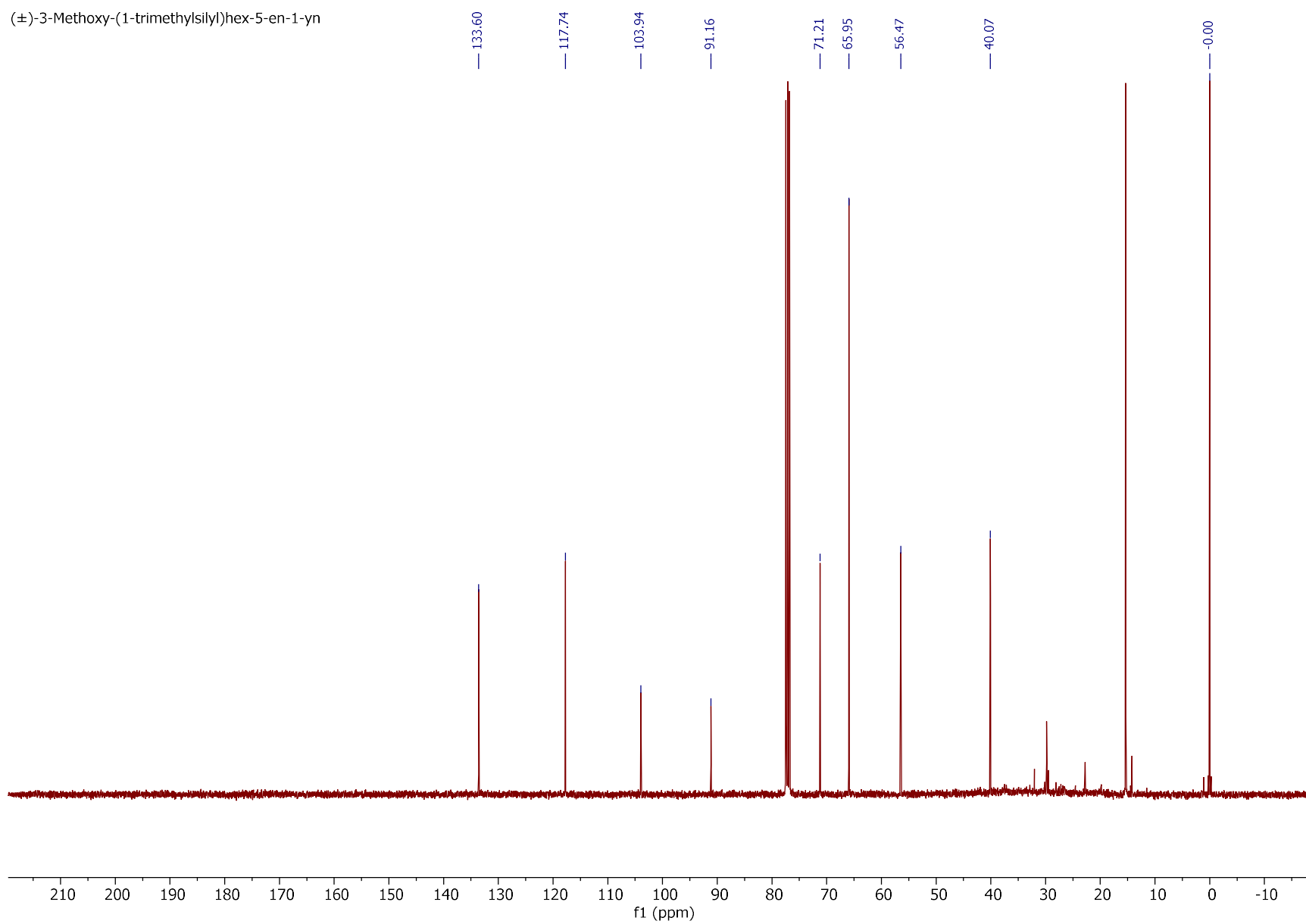


(±)-3-Methoxy-(1-trimethylsilyl)hex-5-en-1-yn

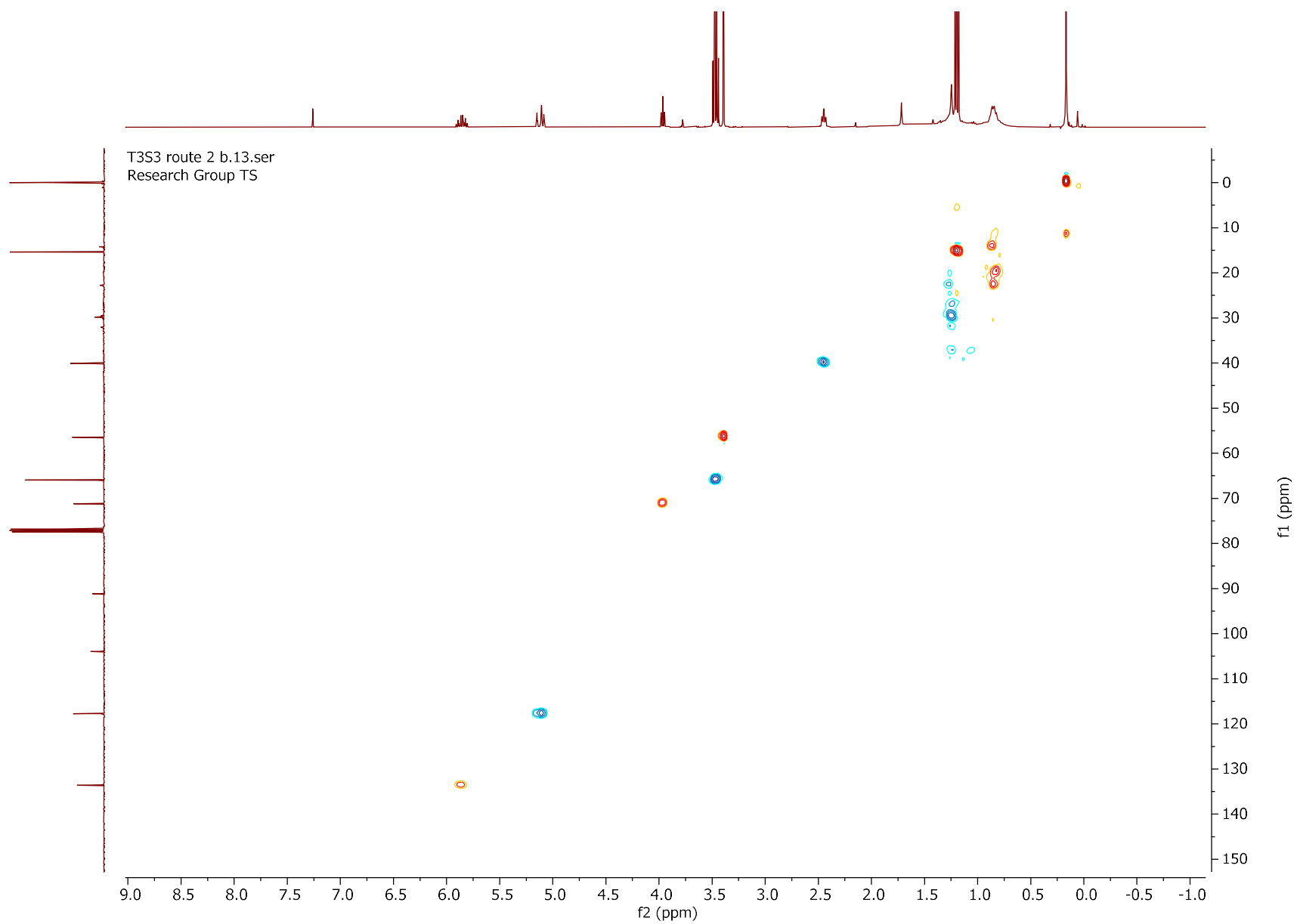


A-C3-10

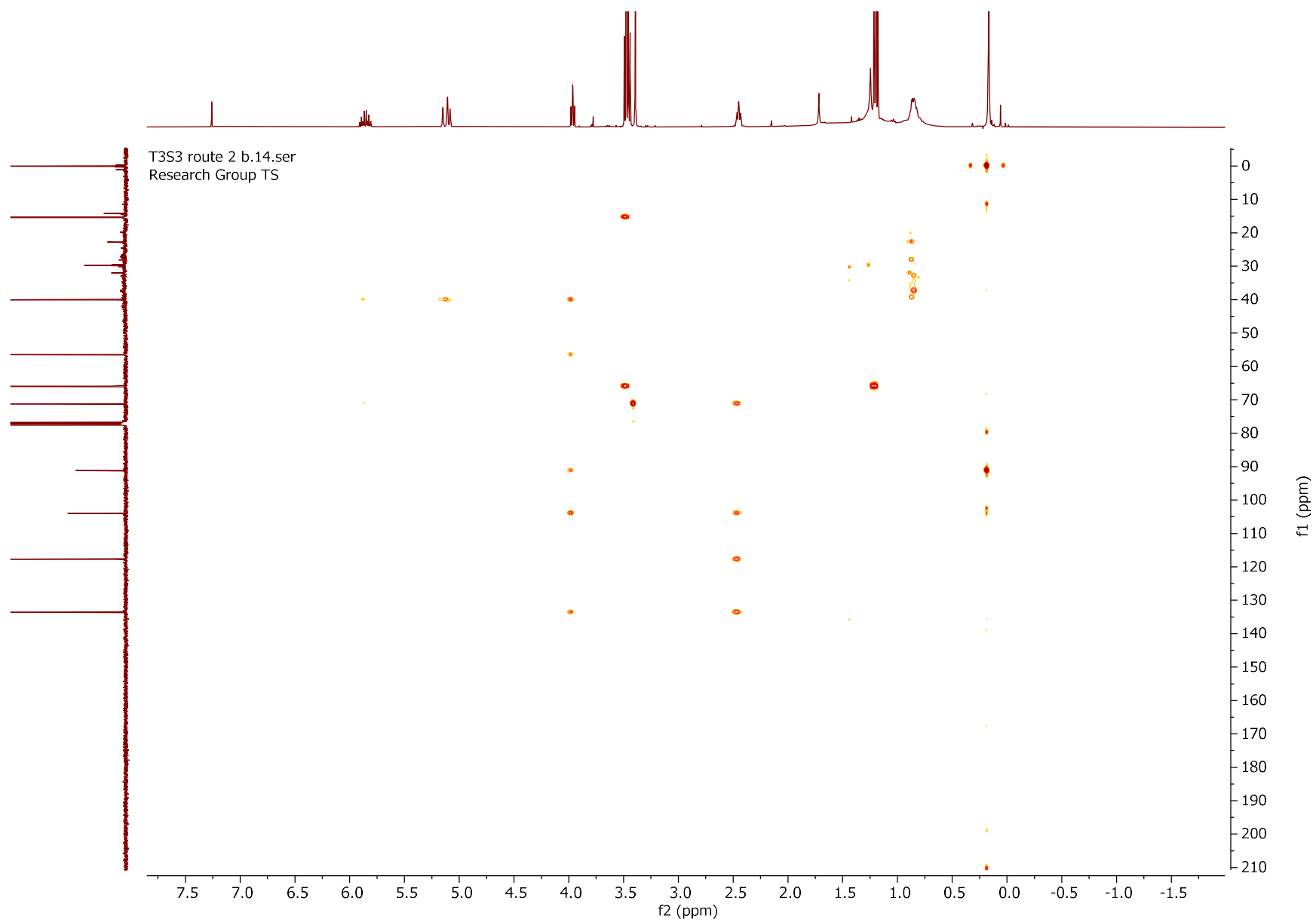
(±)-3-Methoxy-(1-trimethylsilyl)hex-5-en-1-yn



A-C3-11



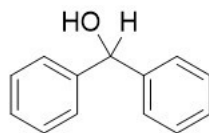
A-C3-12



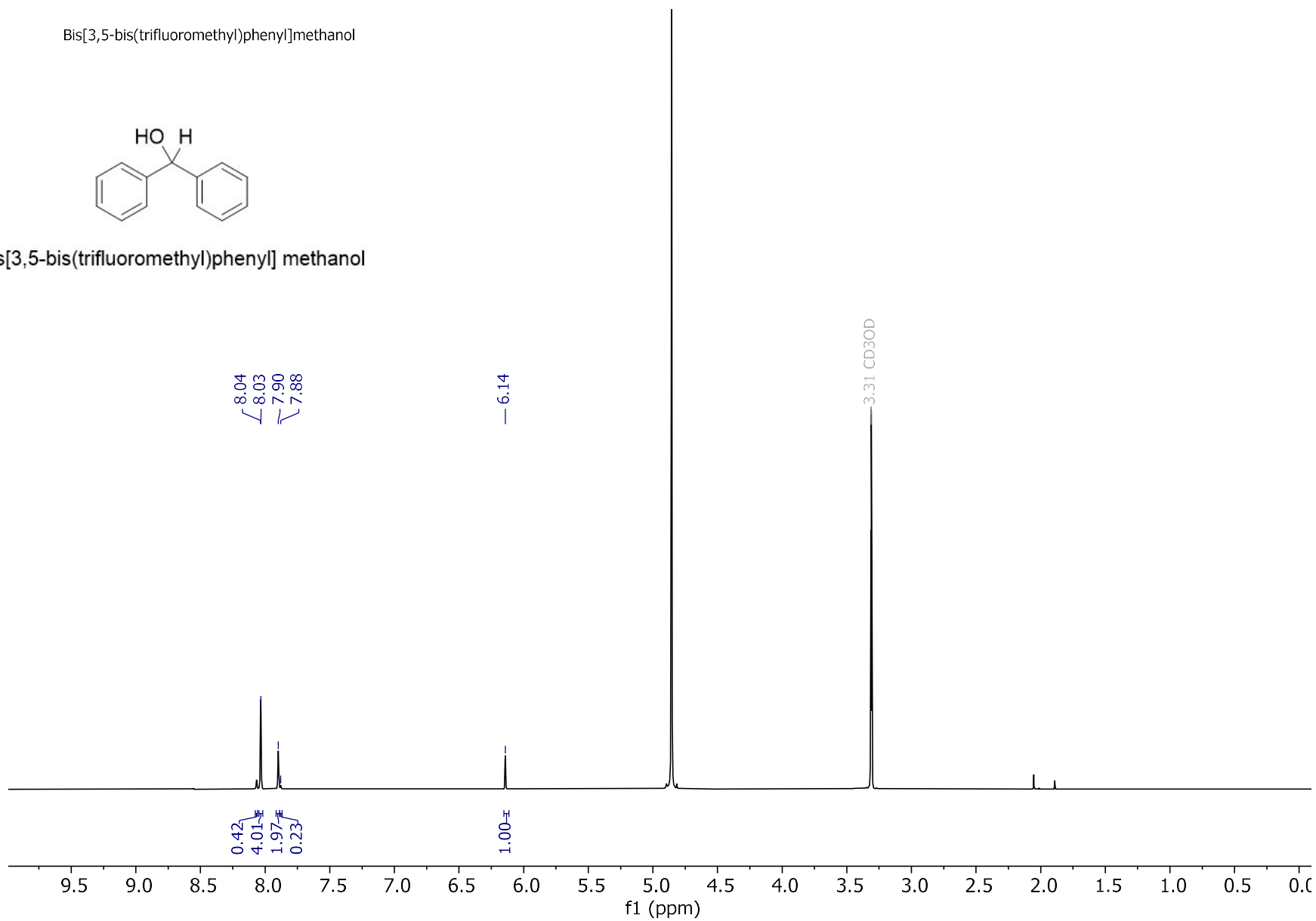
A-C3-13

A-C4

Bis[3,5-bis(trifluoromethyl)phenyl]methanol

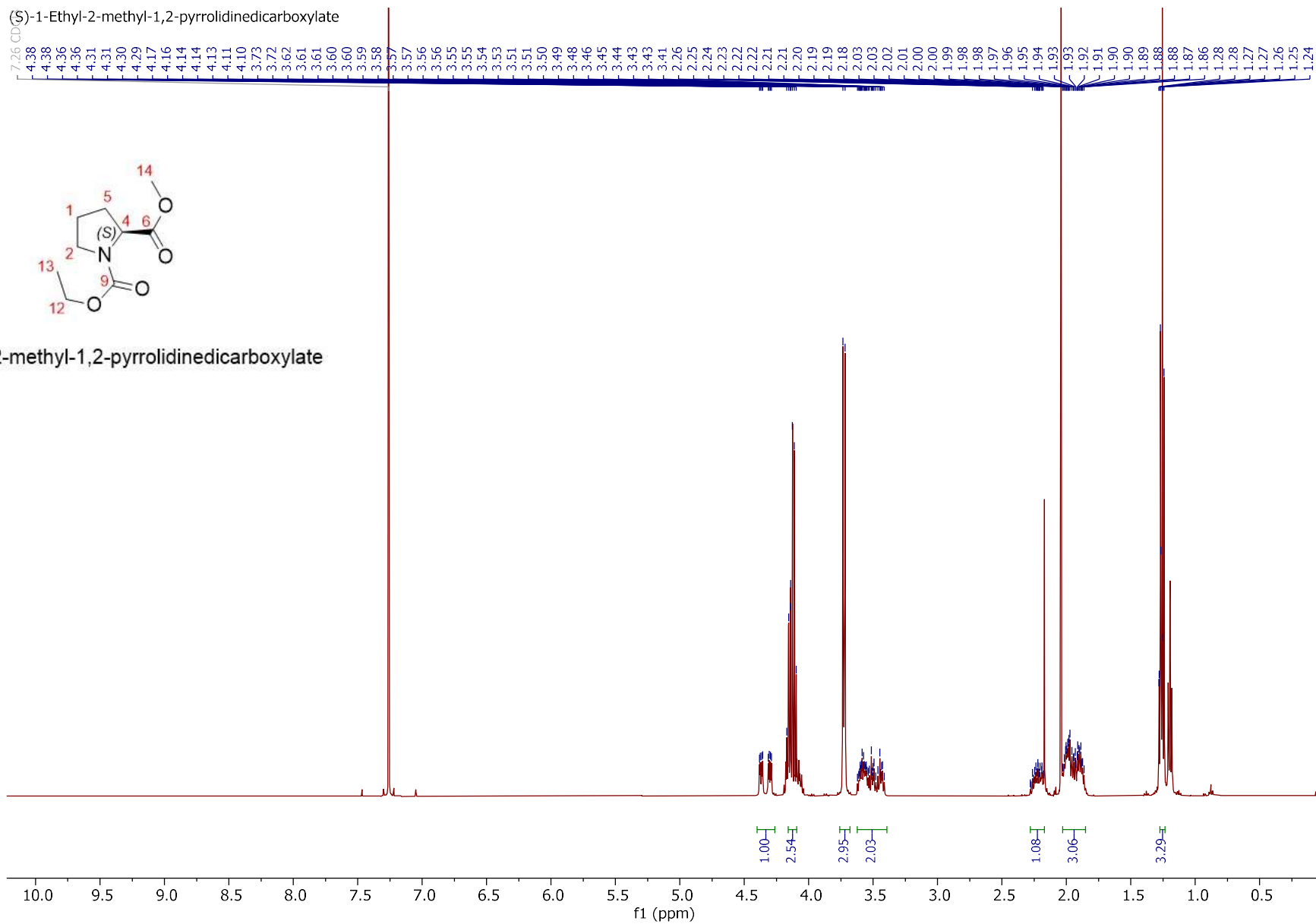


Bis[3,5-bis(trifluoromethyl)phenyl] methanol



A-C4-1

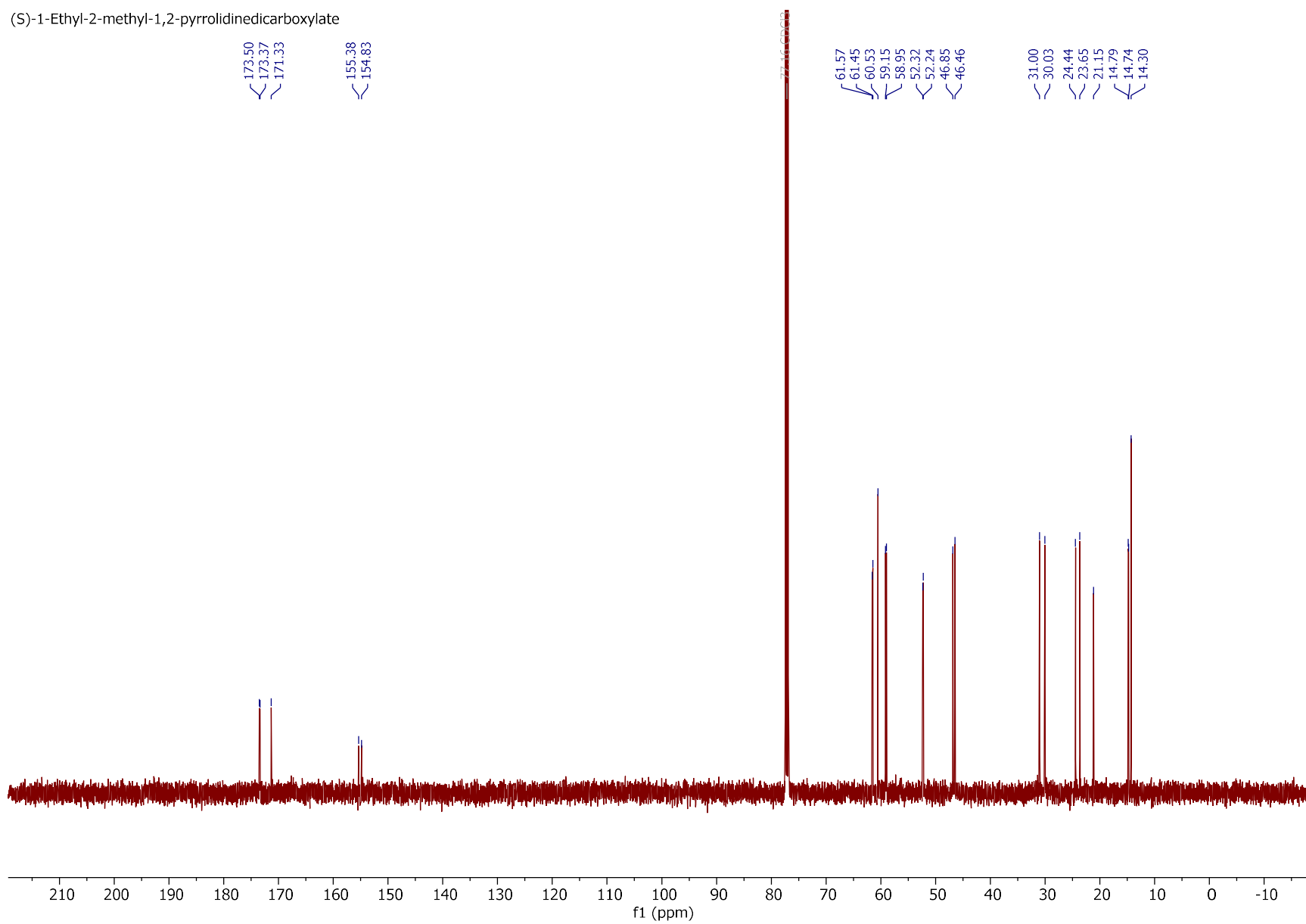
(S)-1-Ethyl-2-methyl-1,2-pyrrolidinedicarboxylate



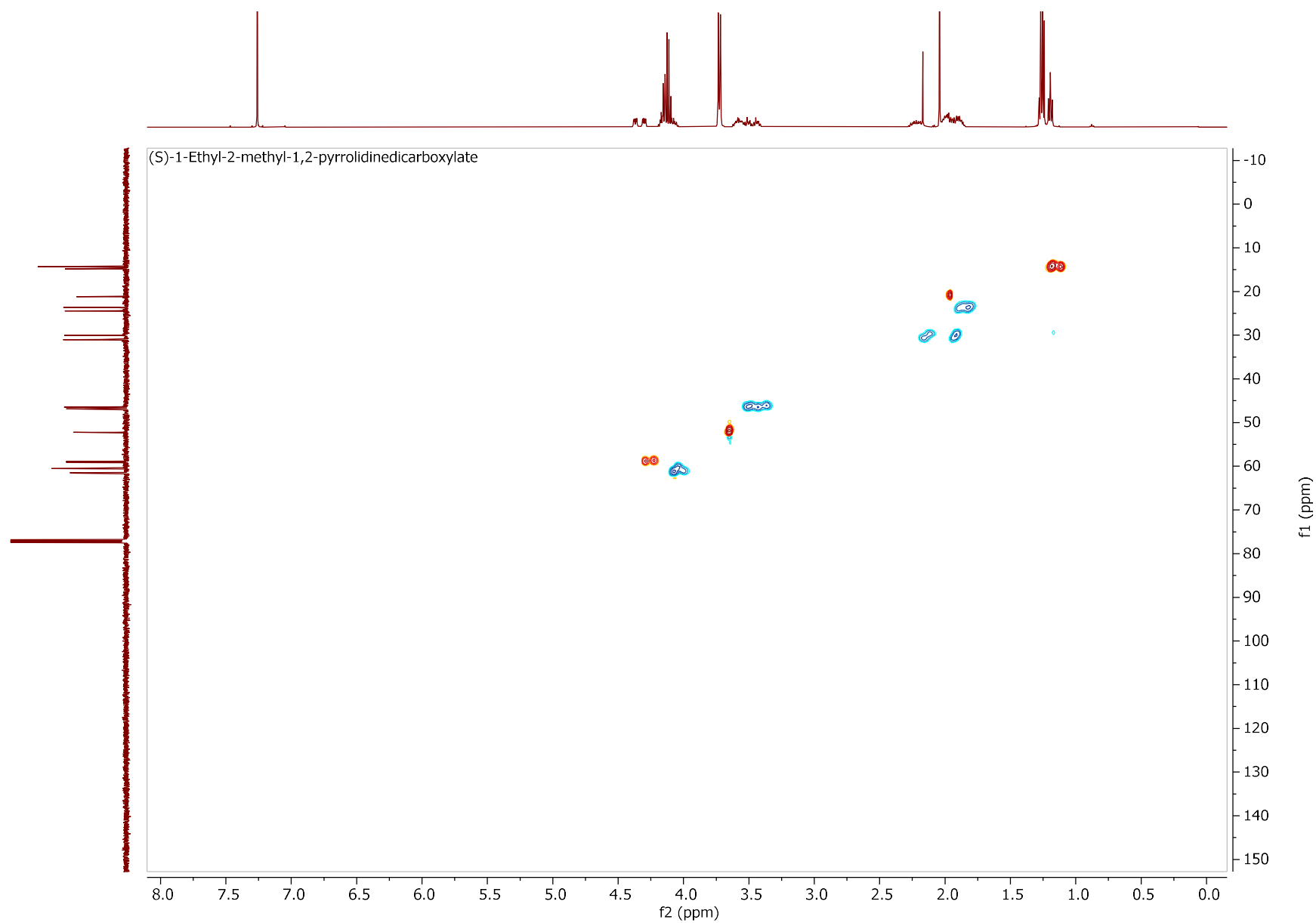
(S)-1-Ethyl-2-methyl-1,2-pyrrolidinedicarboxylate

A-C4-2

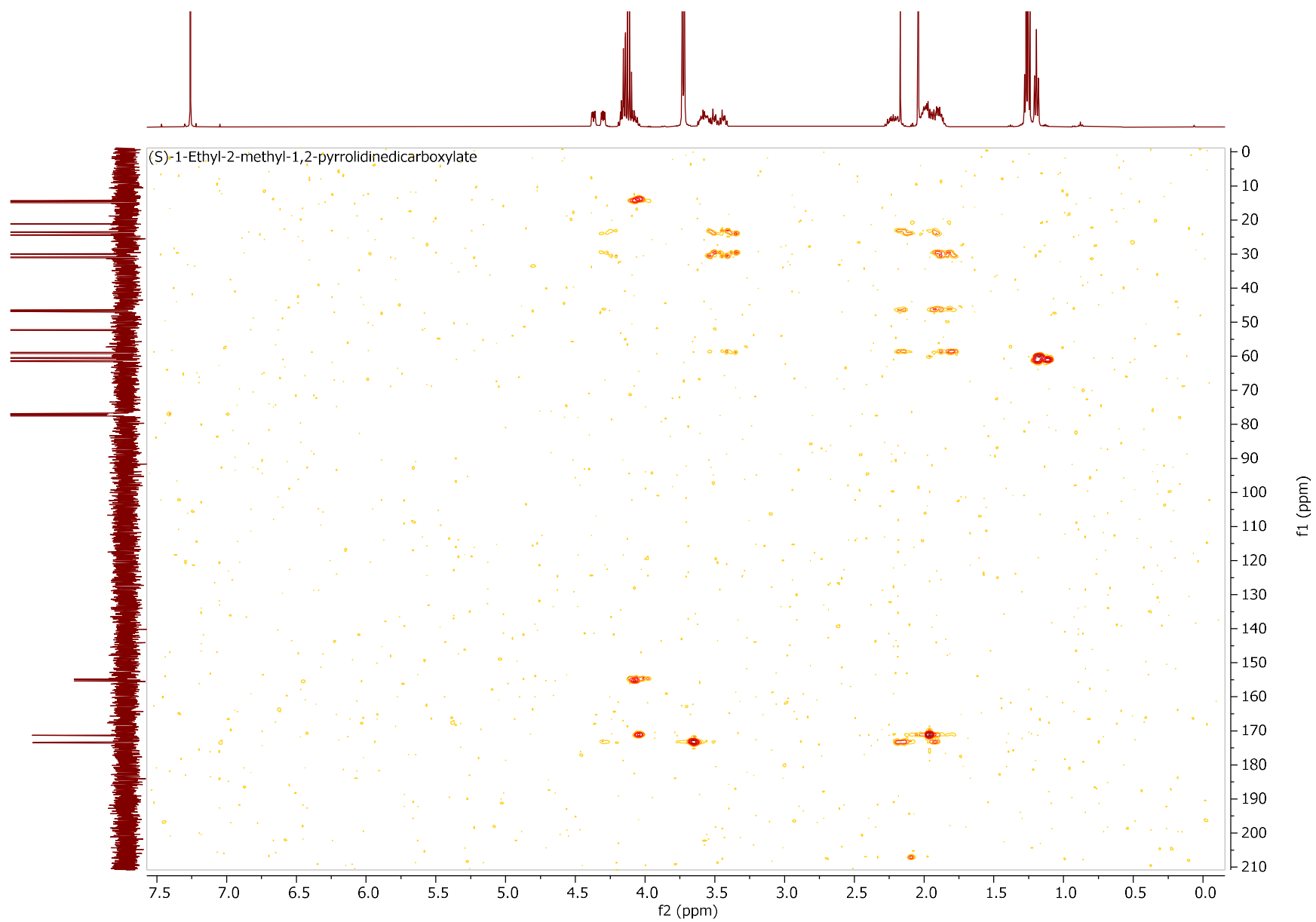
(S)-1-Ethyl-2-methyl-1,2-pyrrolidinedicarboxylate



A-C4-3

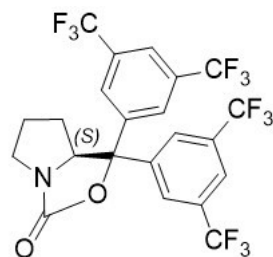


A-C4-4

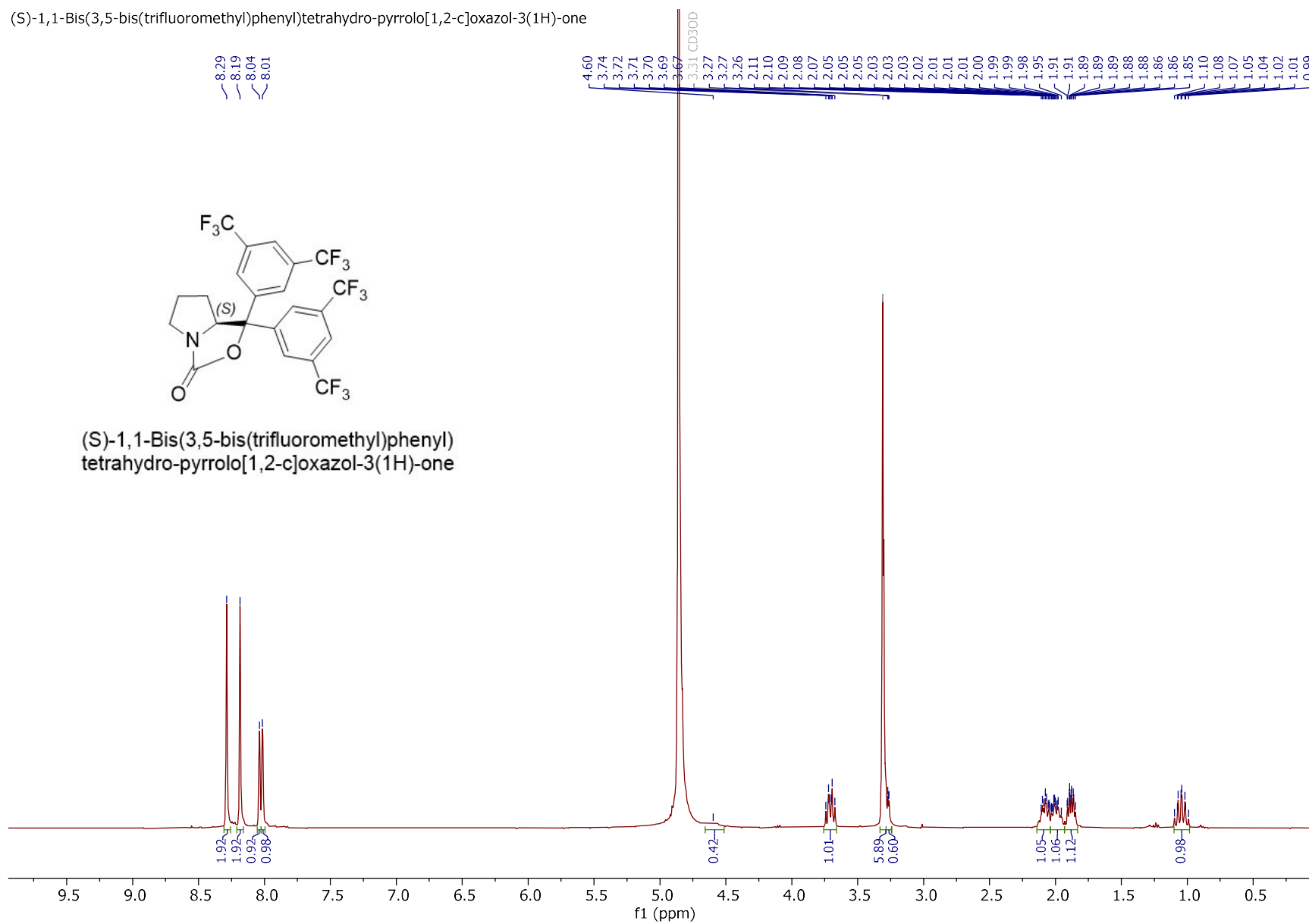


A-C4-5

(S)-1,1-Bis(3,5-bis(trifluoromethyl)phenyl)tetrahydro-pyrrolo[1,2-c]oxazol-3(1H)-one

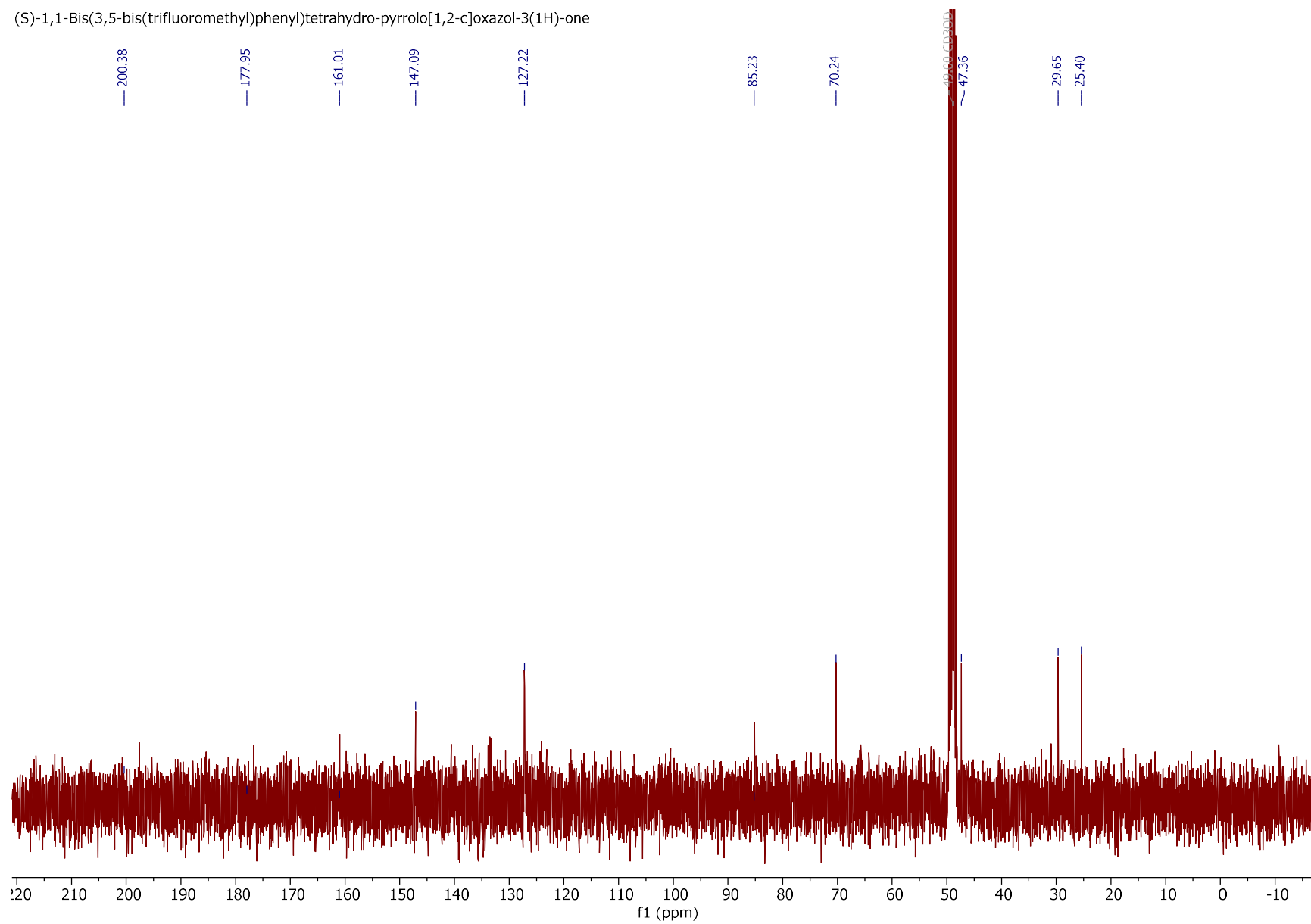


(S)-1,1-Bis(3,5-bis(trifluoromethyl)phenyl)
tetrahydro-pyrrolo[1,2-c]oxazol-3(1H)-one



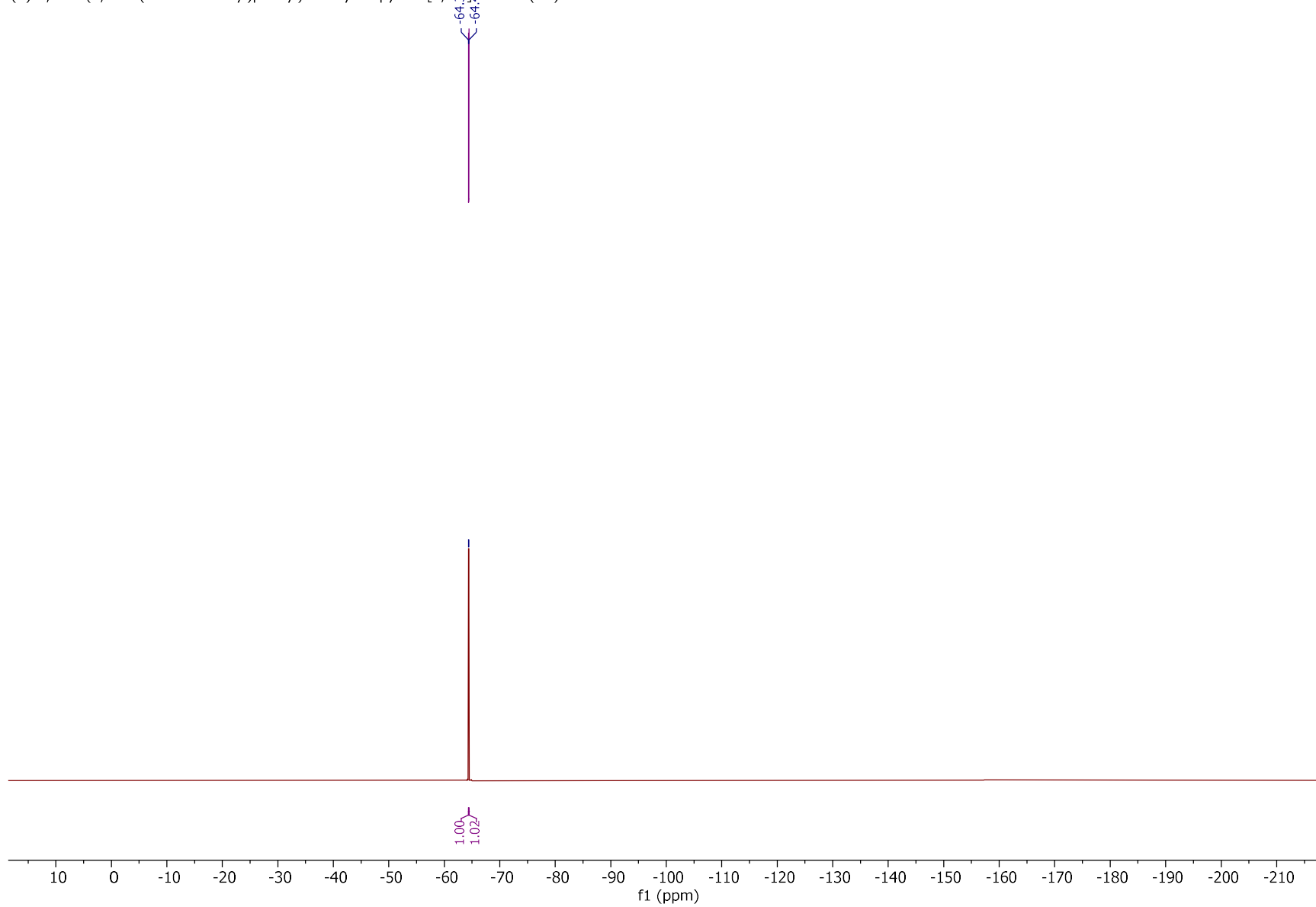
A-C4-6

(S)-1,1-Bis(3,5-bis(trifluoromethyl)phenyl)tetrahydro-pyrrolo[1,2-c]oxazol-3(1H)-one

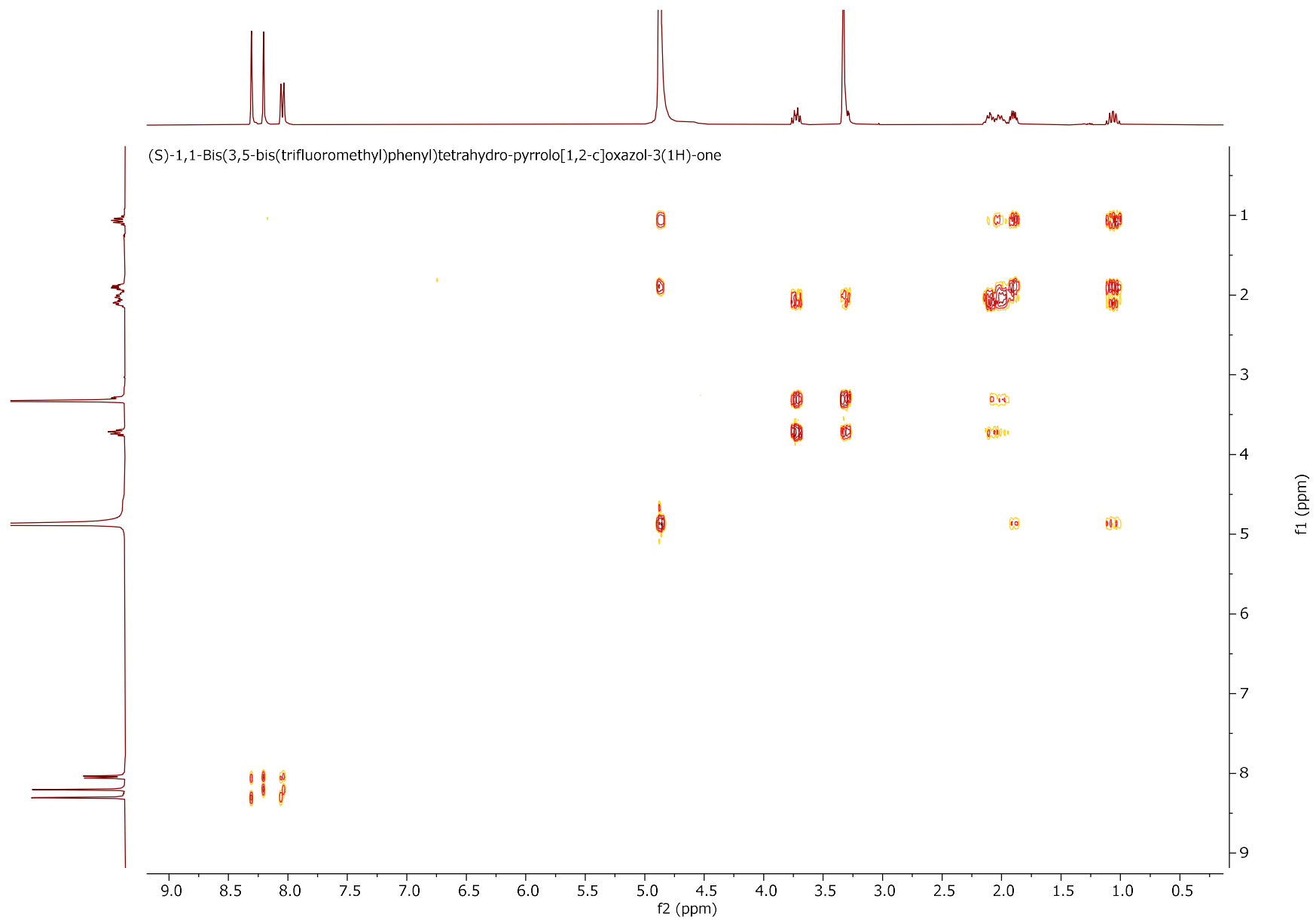


A-C4-7

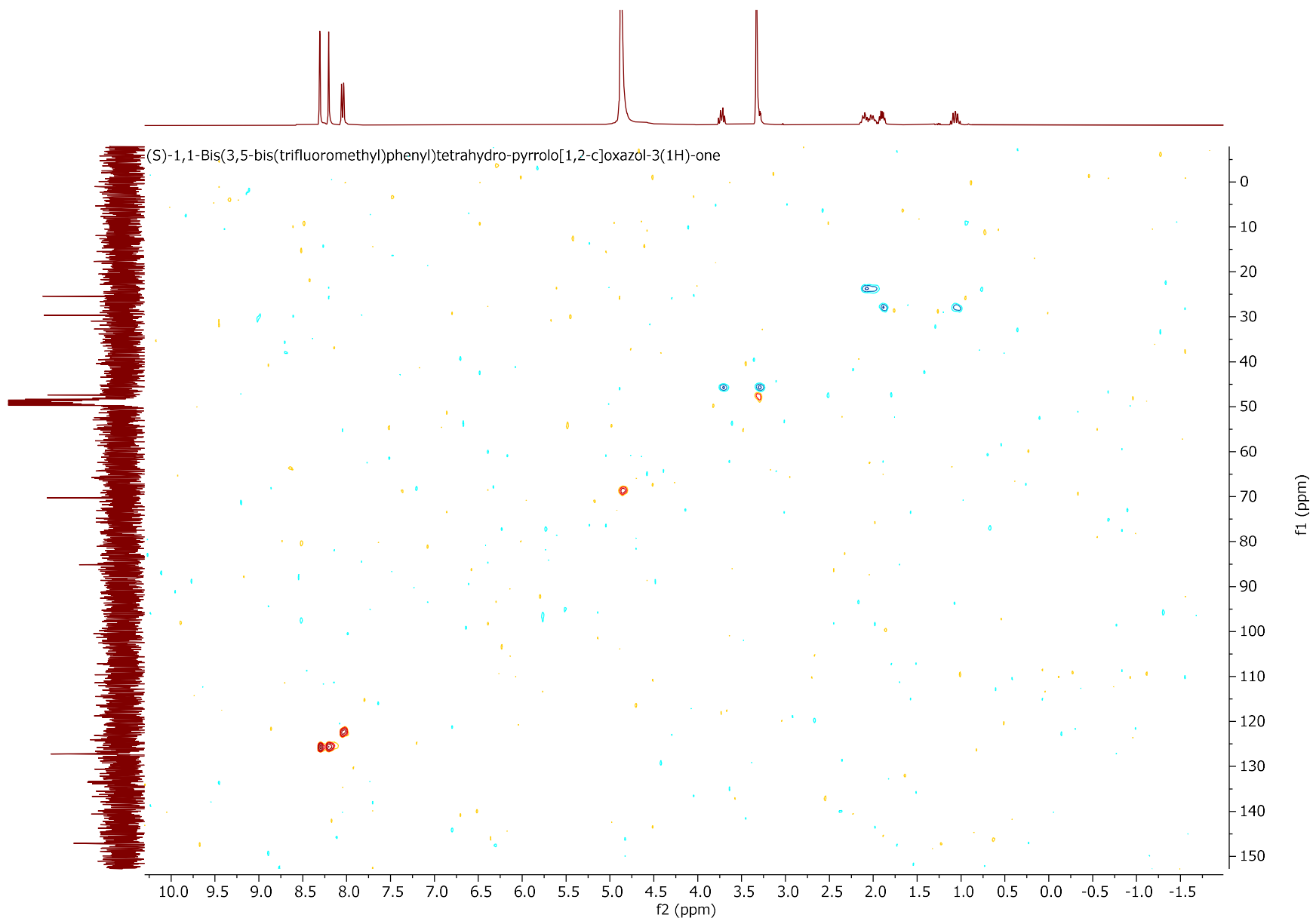
(S)-1,1-Bis(3,5-bis(trifluoromethyl)phenyl)tetrahydro-pyrrolo[1,2-a]oxazol-3(1H)-one



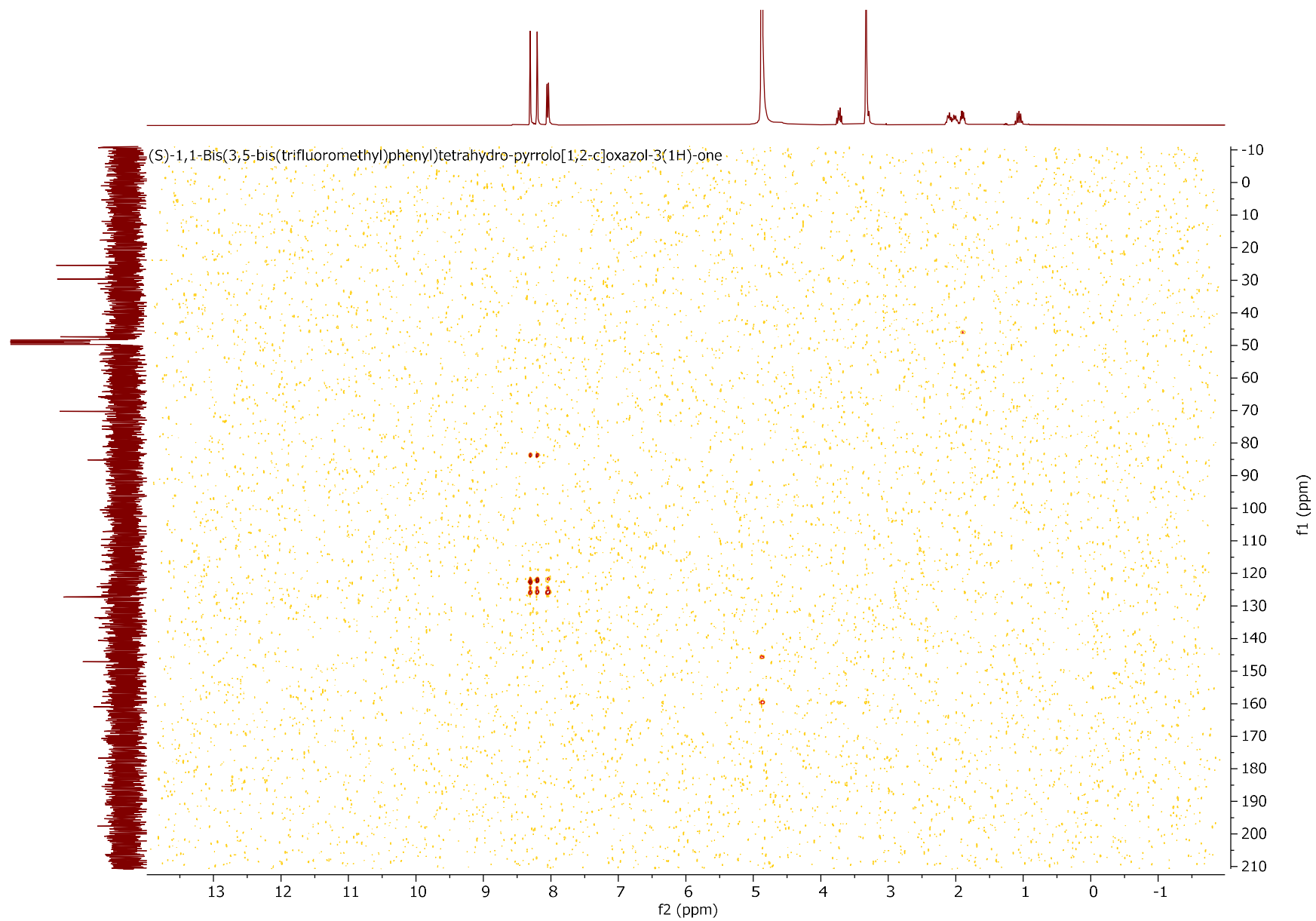
A-C4-8



A-C4-9

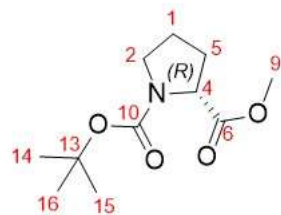
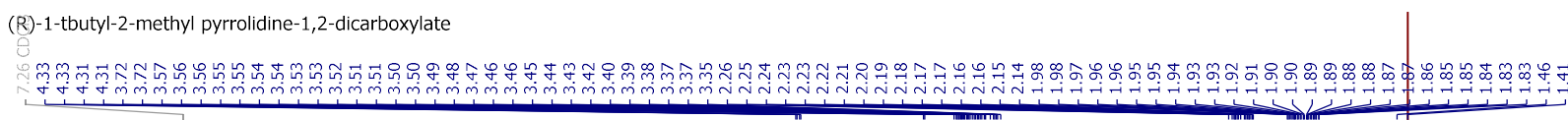


A-C4-10

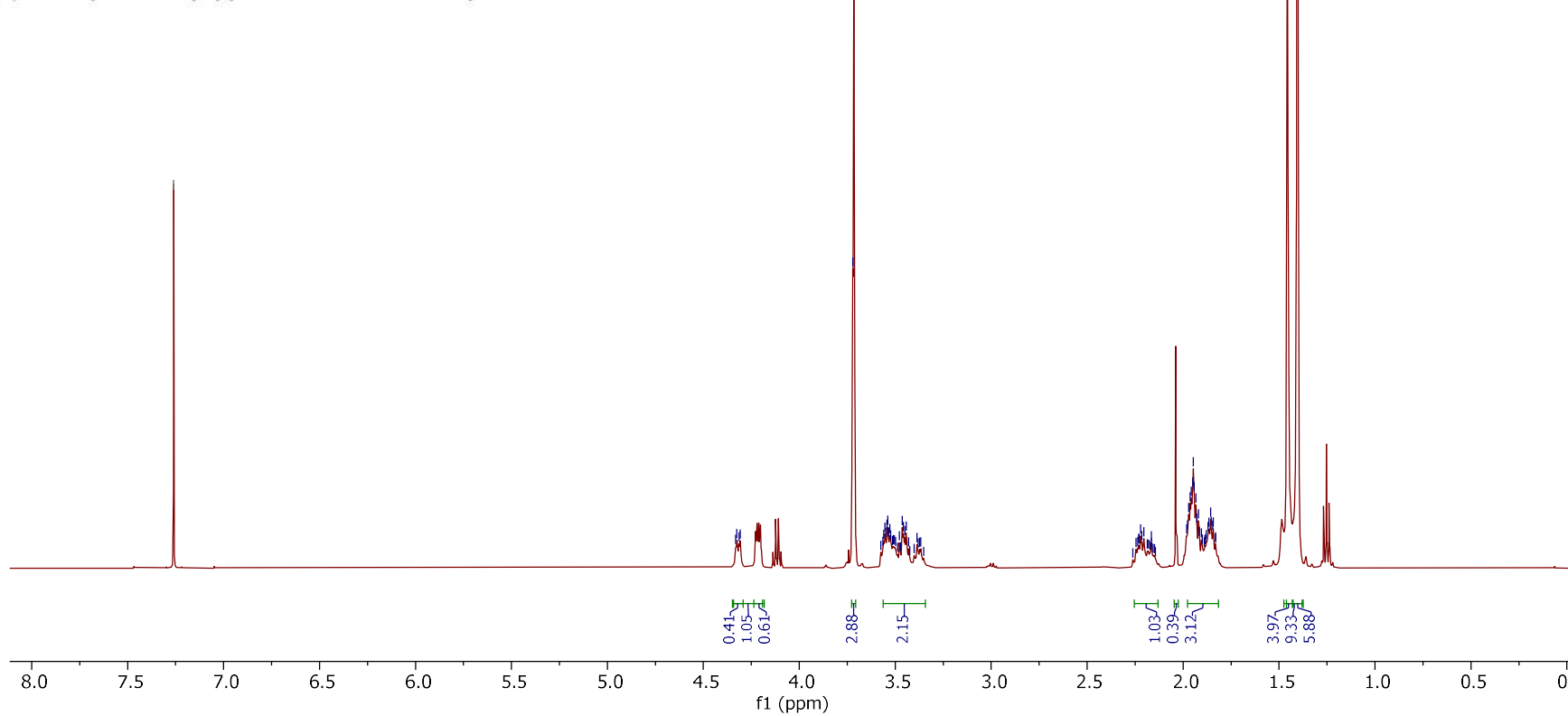


A-C4-11

(R)-1-*t*-butyl-2-methyl pyrrolidine-1,2-dicarboxylate

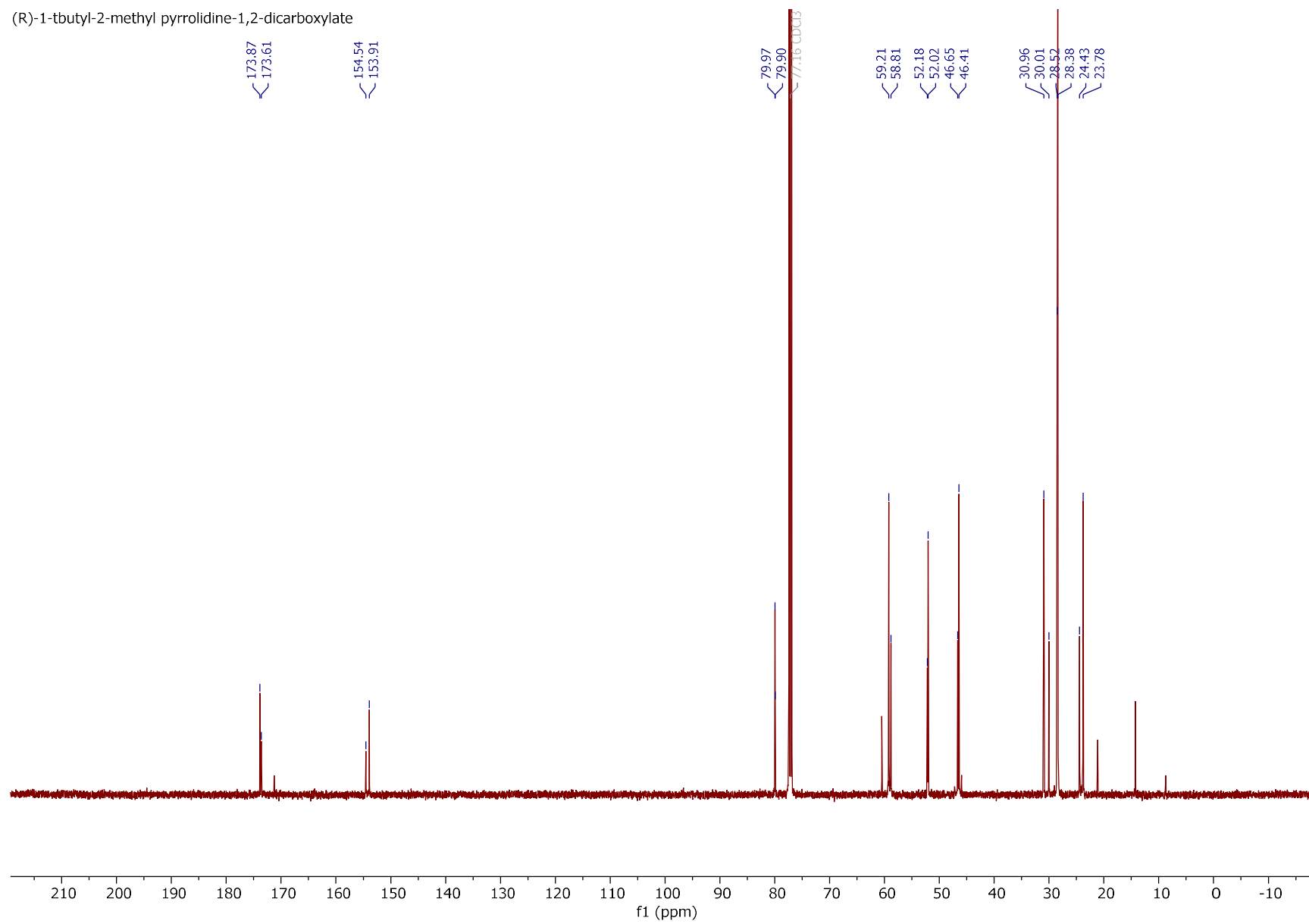


(R)-1-*t*-butyl-2-methyl pyrrolidine-1,2-dicarboxylate



A-C4-12

(R)-1-*t*-butyl-2-methyl pyrrolidine-1,2-dicarboxylate



A-C4-13

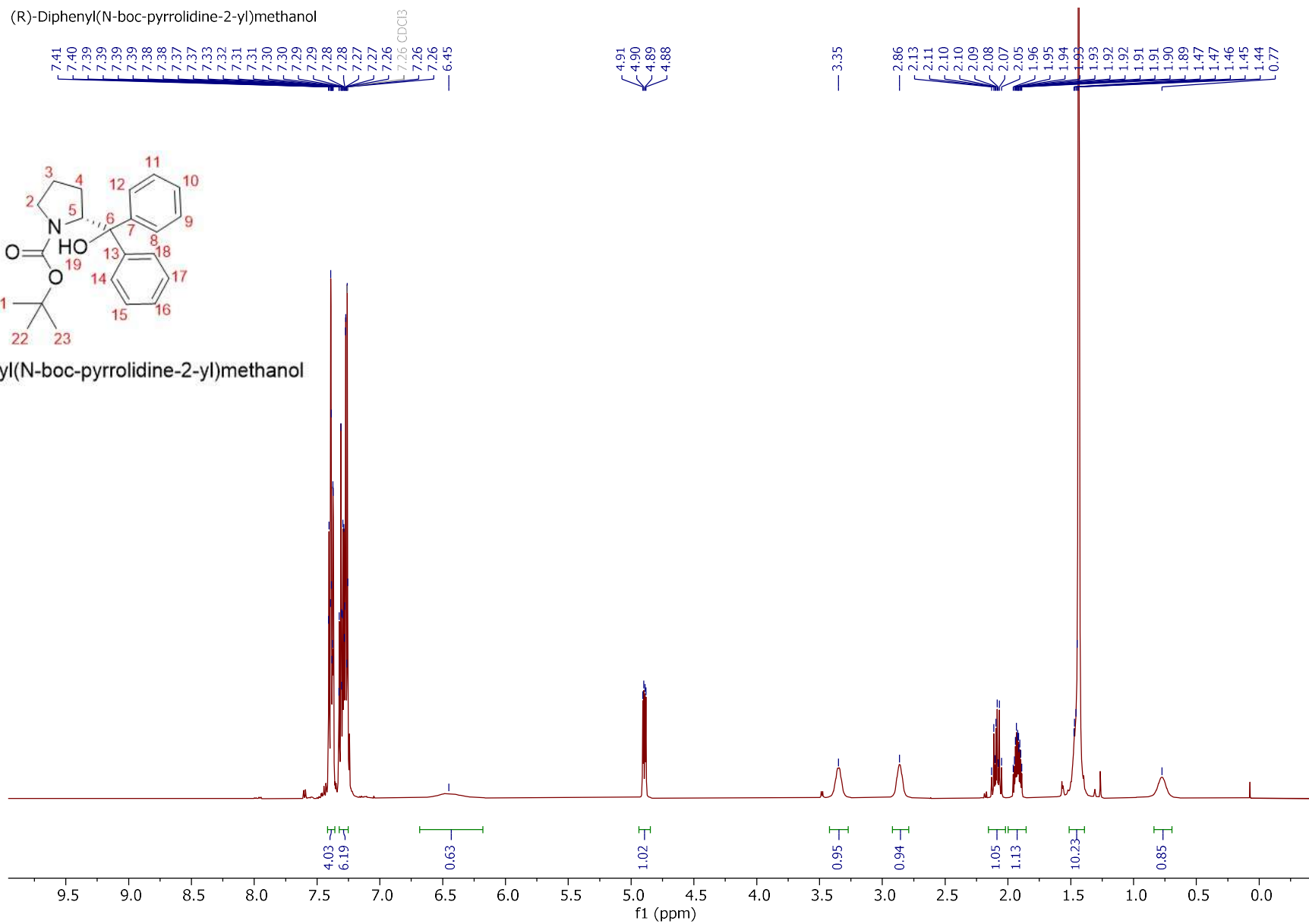
(R)-Diphenyl(N-boc-pyrrolidine-2-yl)methanol

7.41
7.40
7.39
7.39
7.39
7.38
7.38
7.37
7.37
7.33
7.32
7.31
7.31
7.30
7.30
7.29
7.29
7.28
7.28
7.27
7.27
7.26
7.26
7.26
6.45

CDCl₃

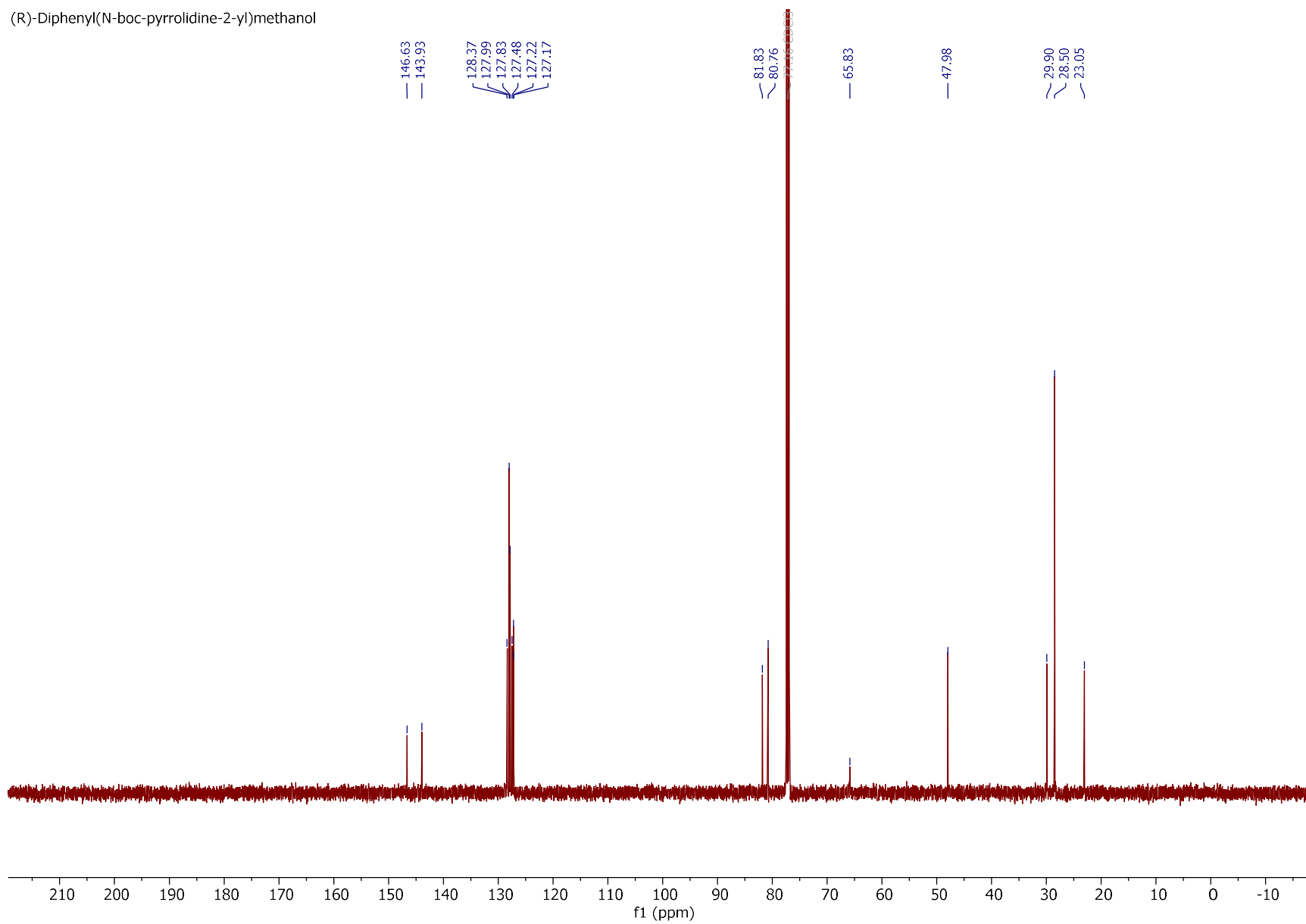


(R)-Diphenyl(N-boc-pyrrolidine-2-yl)methanol

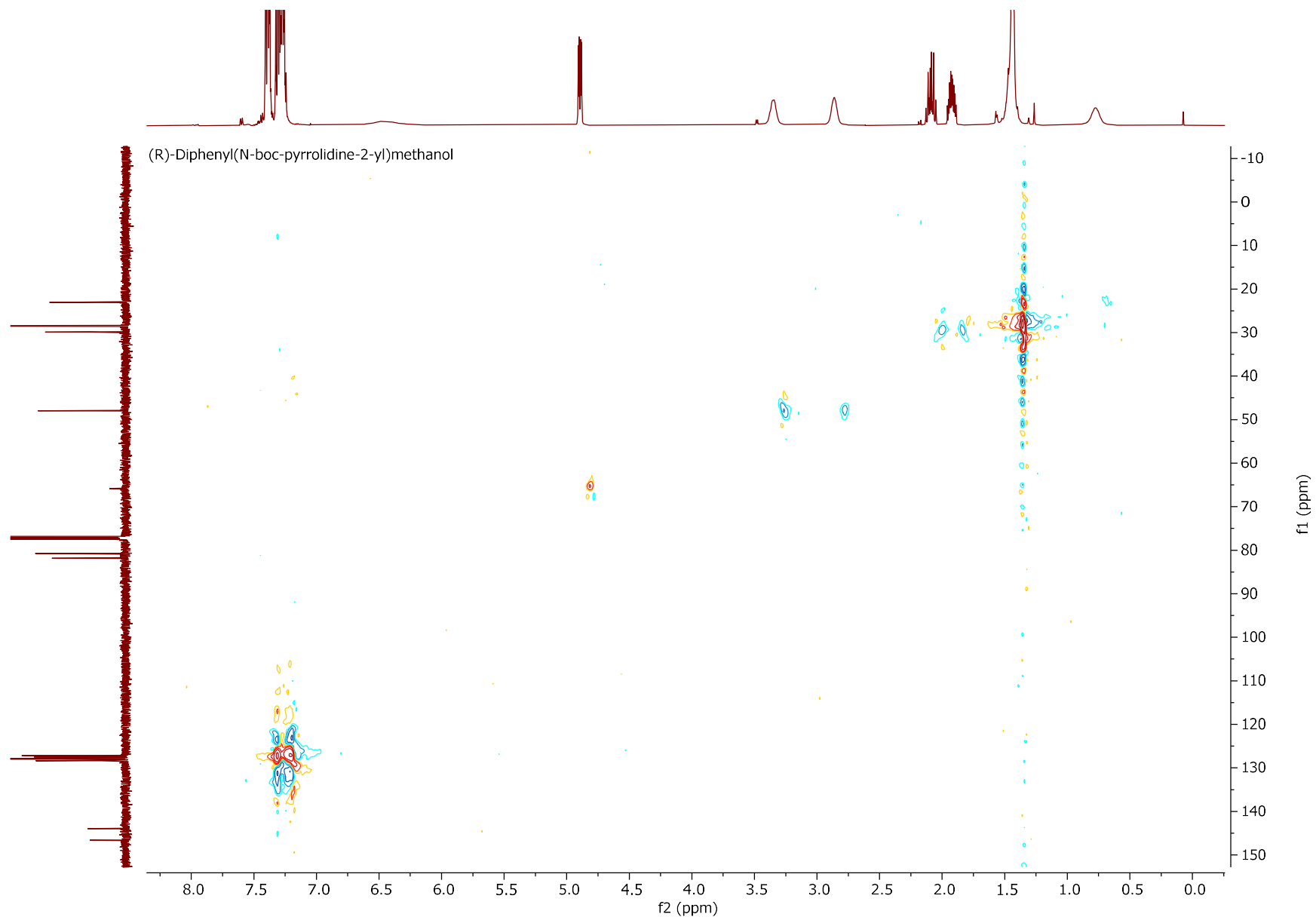


A-C4-14

(R)-Diphenyl(N-boc-pyrrolidine-2-yl)methanol

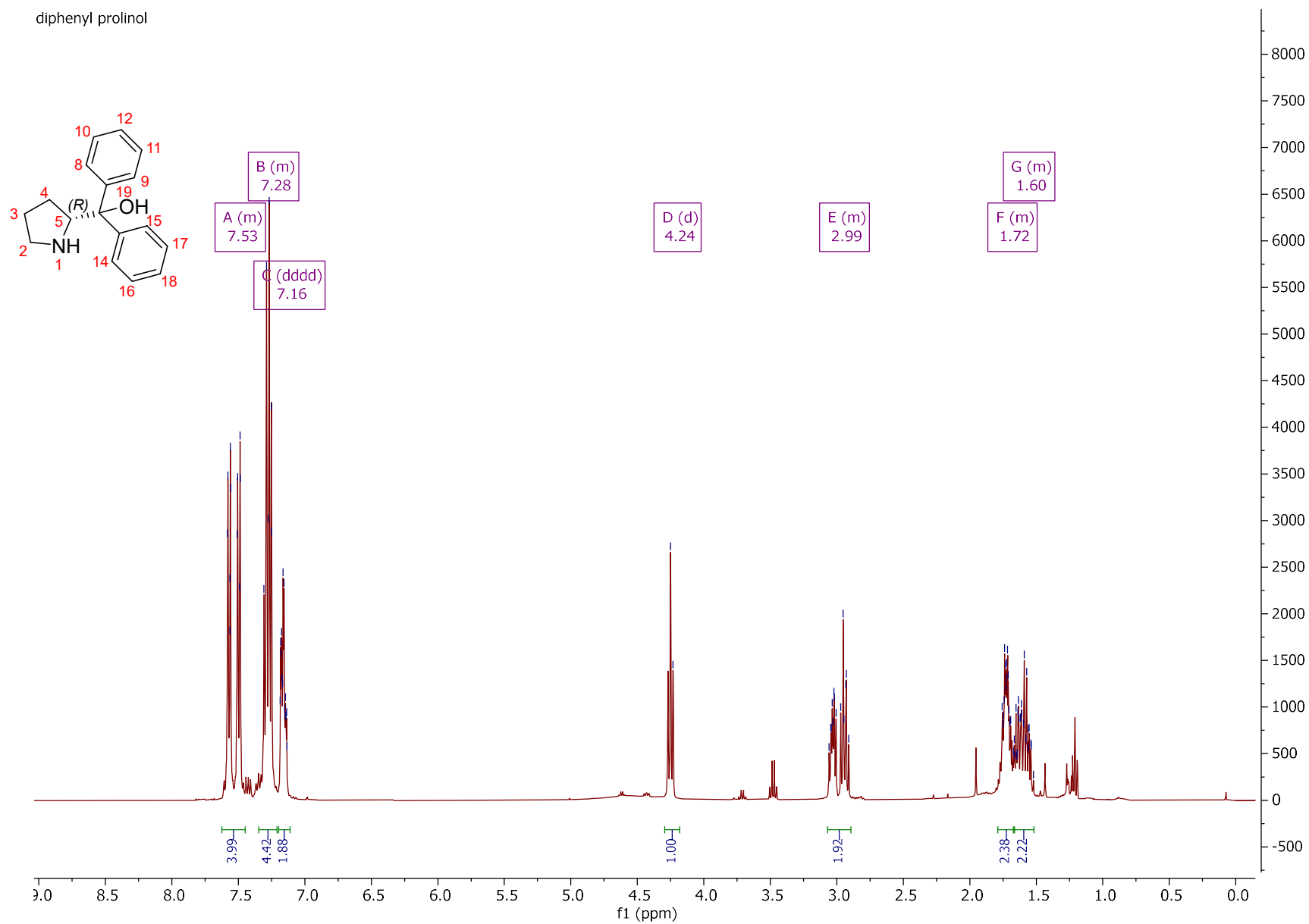


A-C4-15



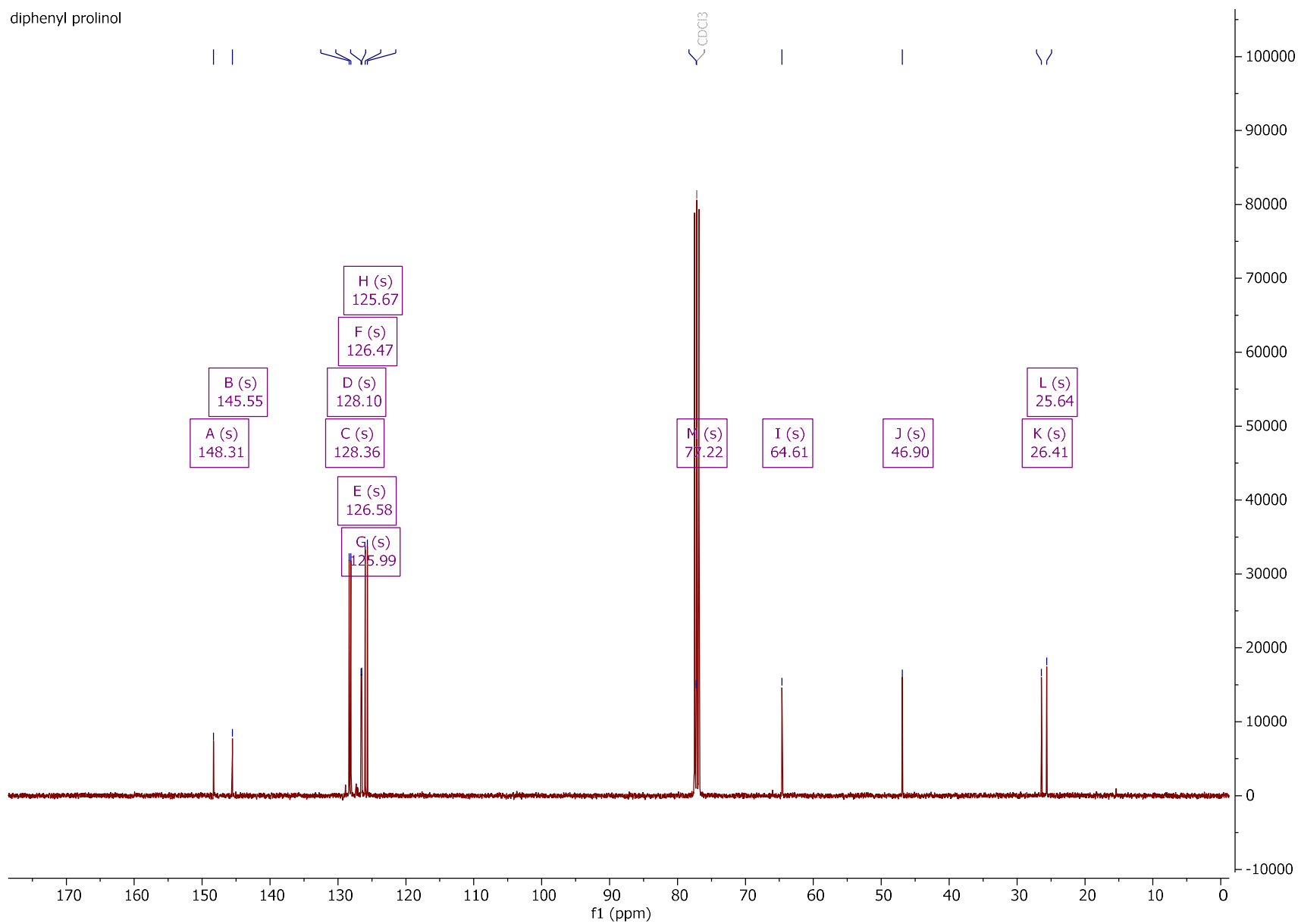
A-C4-16

diphenyl prolinol



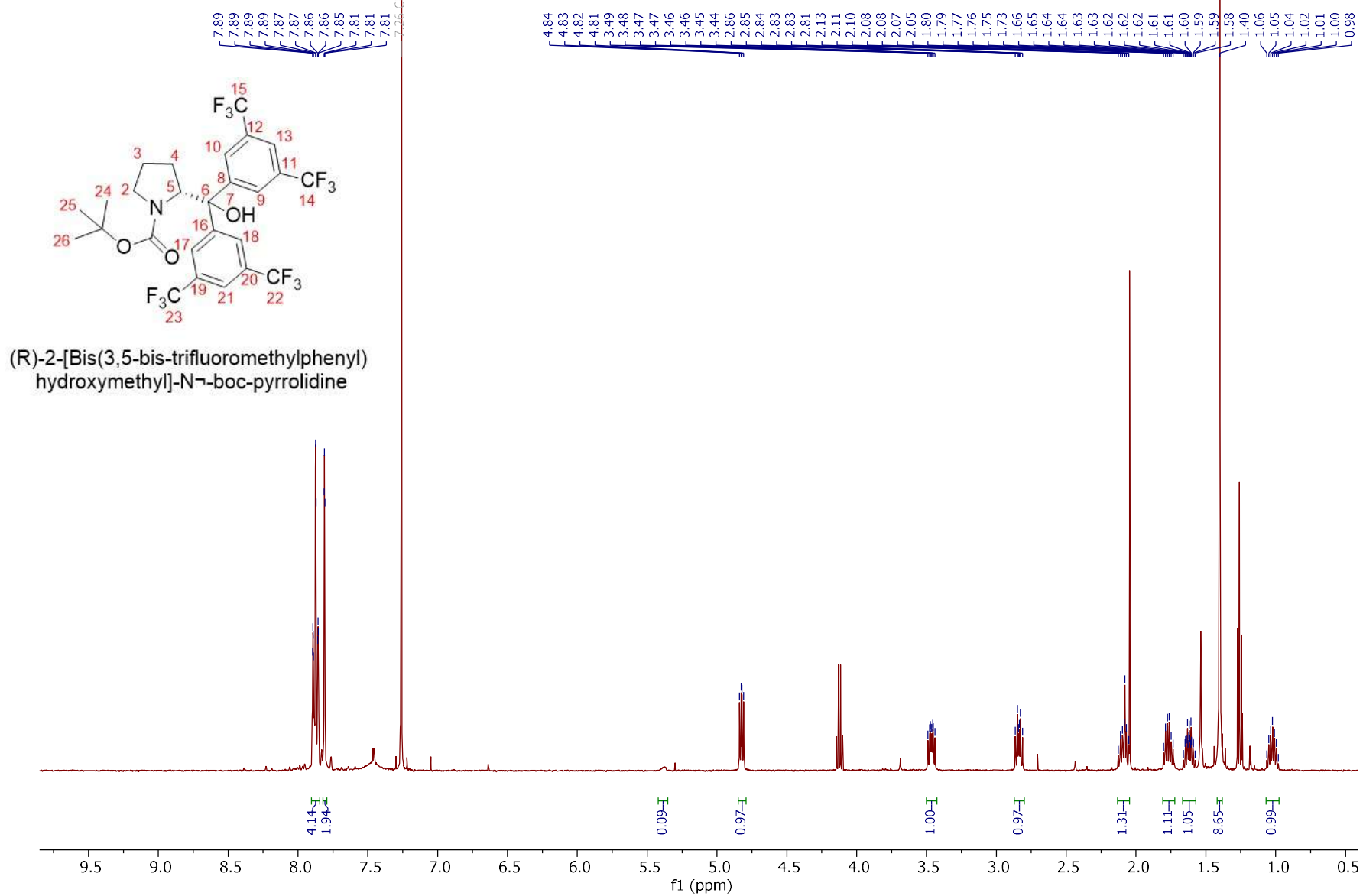
A-C4-17

diphenyl prolinol



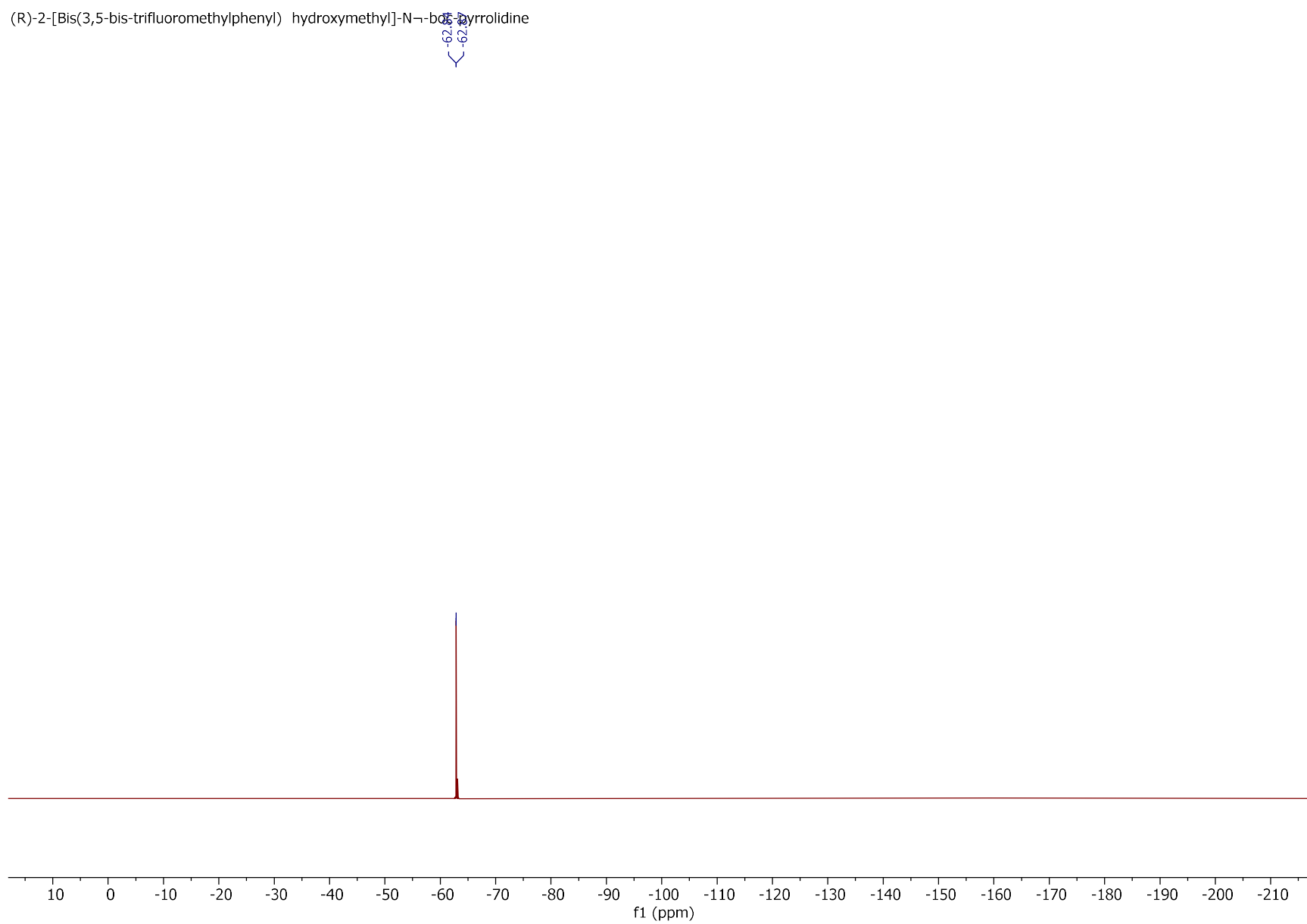
A-C4-18

(R)-2-[Bis(3,5-bis-trifluoromethylphenyl) hydroxymethyl]-N- γ -boc-pyrrolidine



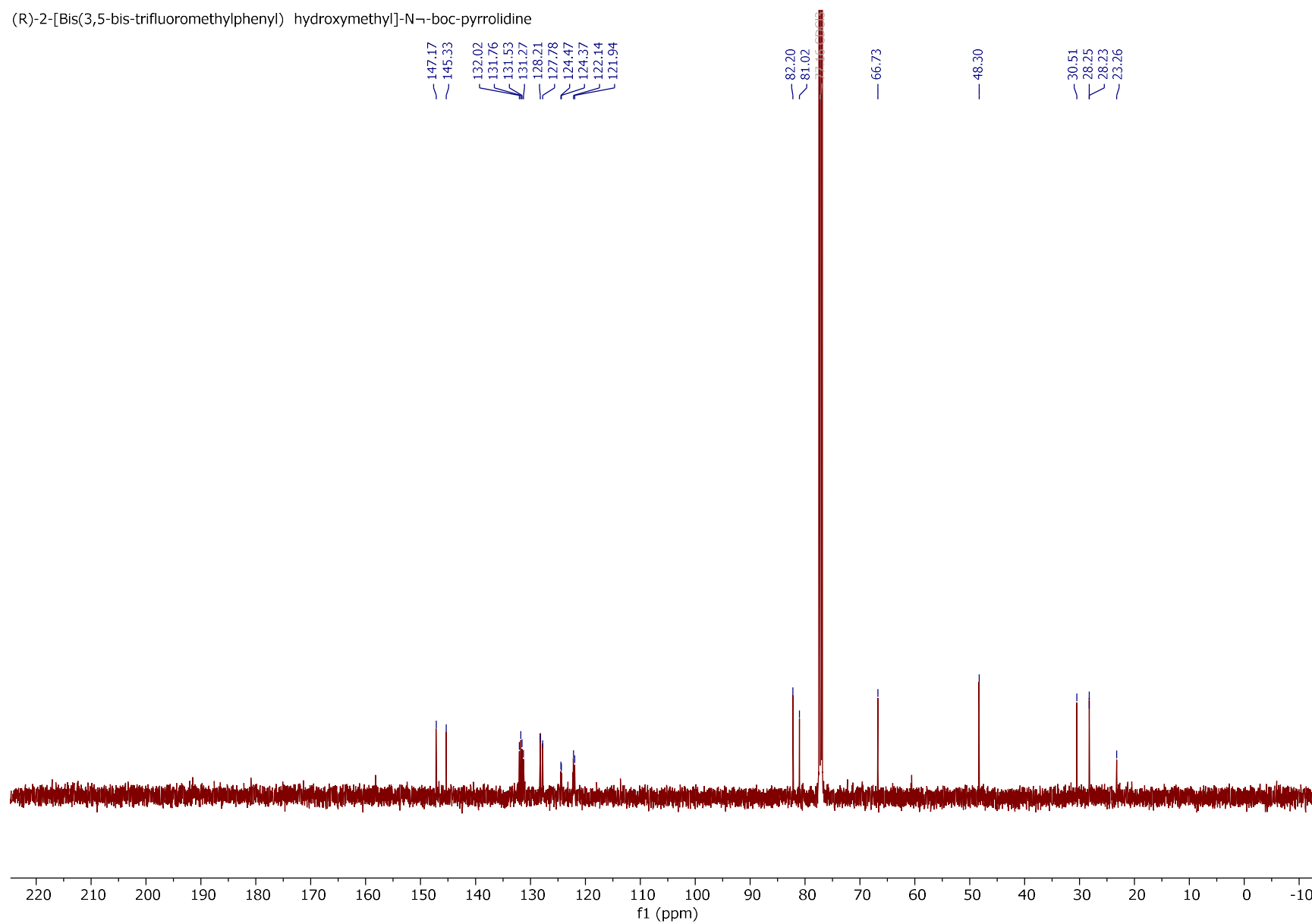
A-C4-19

(R)-2-[Bis(3,5-bis-trifluoromethylphenyl) hydroxymethyl]-N- α -pyrrolidine

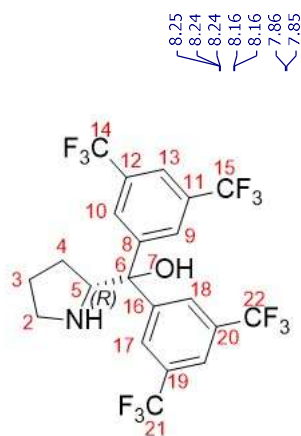


A-C4-20

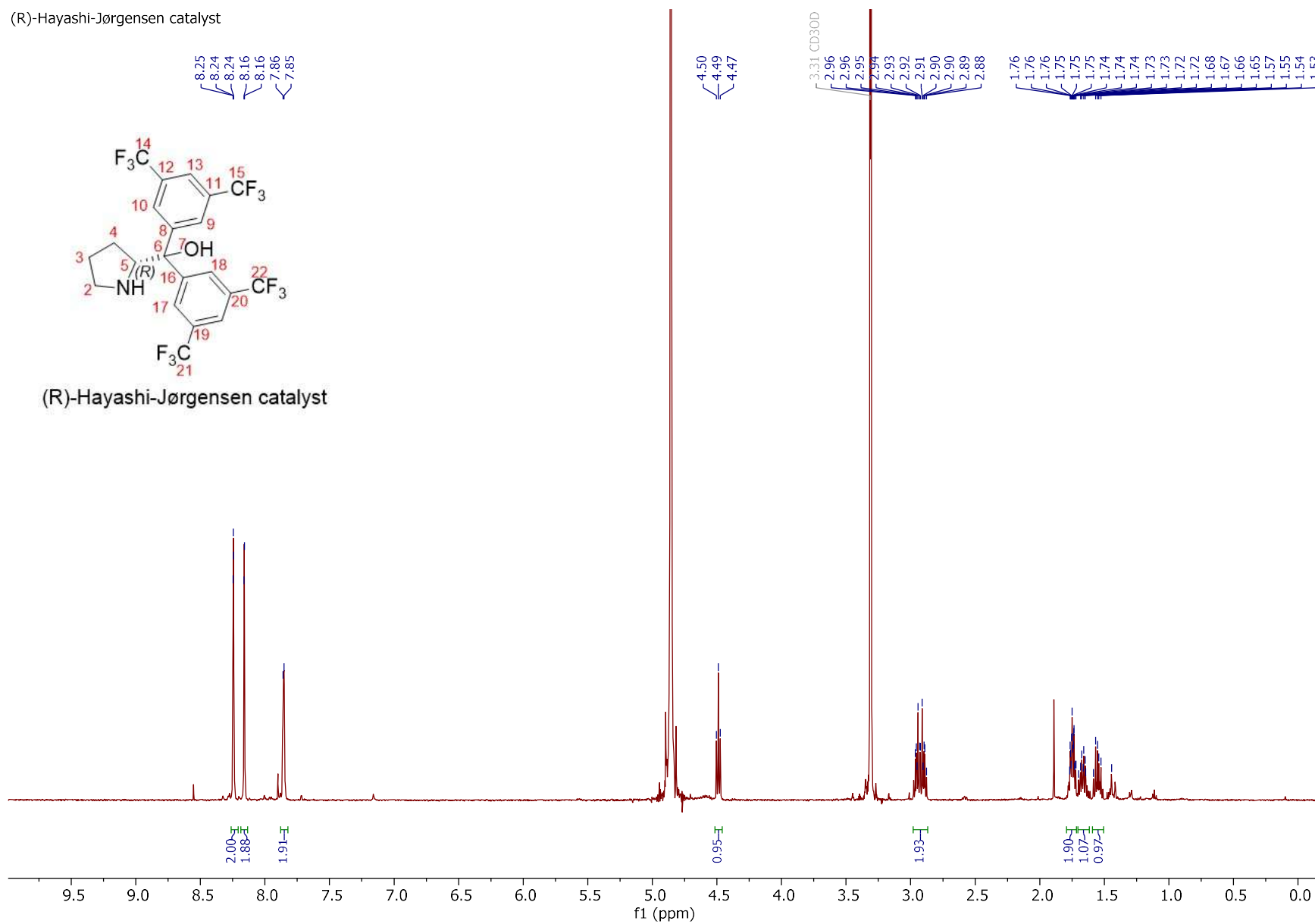
(R)-2-[Bis(3,5-bis-trifluoromethylphenyl) hydroxymethyl]-N- α -boc-pyrrolidine



(R)-Hayashi-Jørgensen catalyst

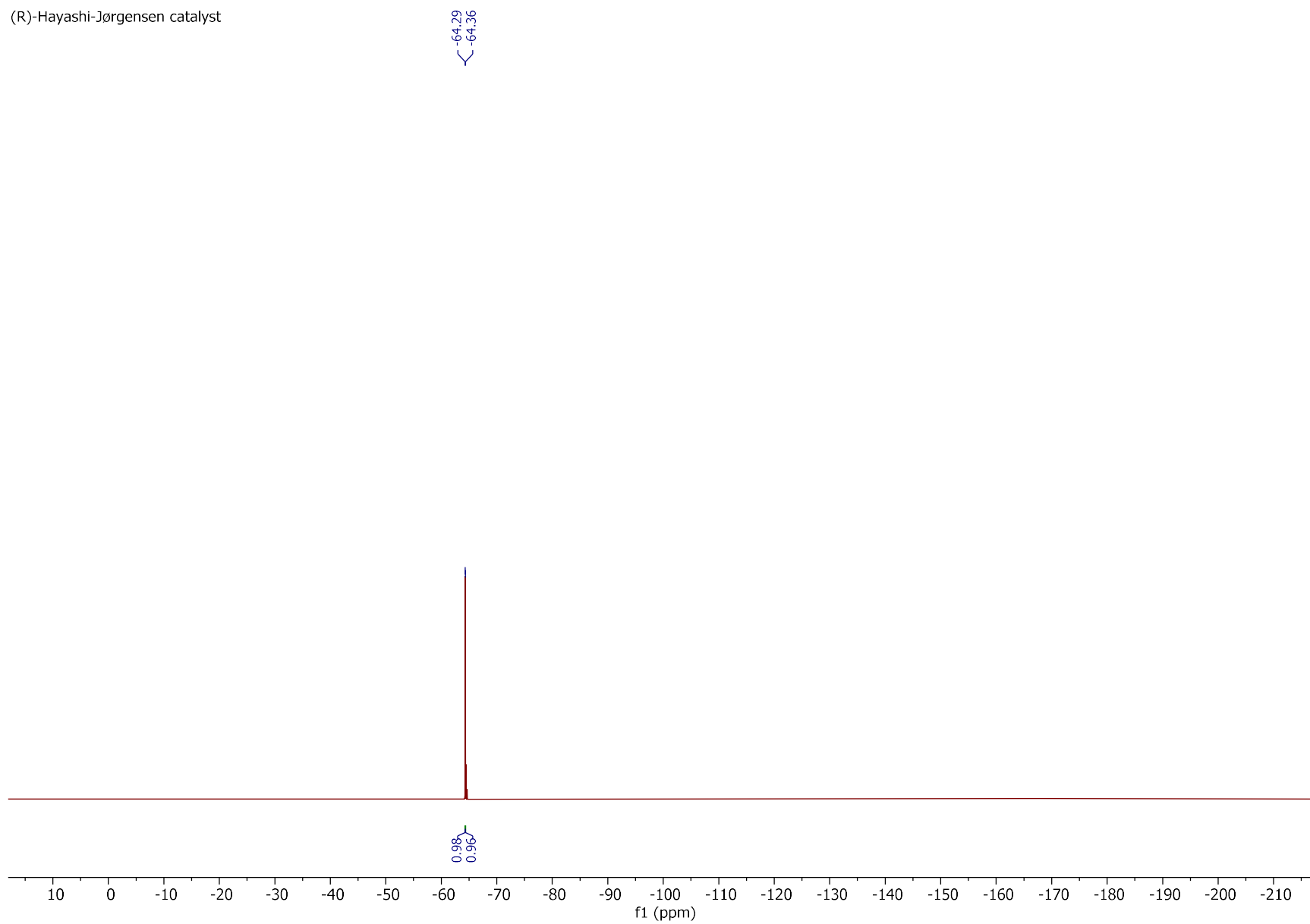


(R)-Hayashi-Jørgensen catalyst



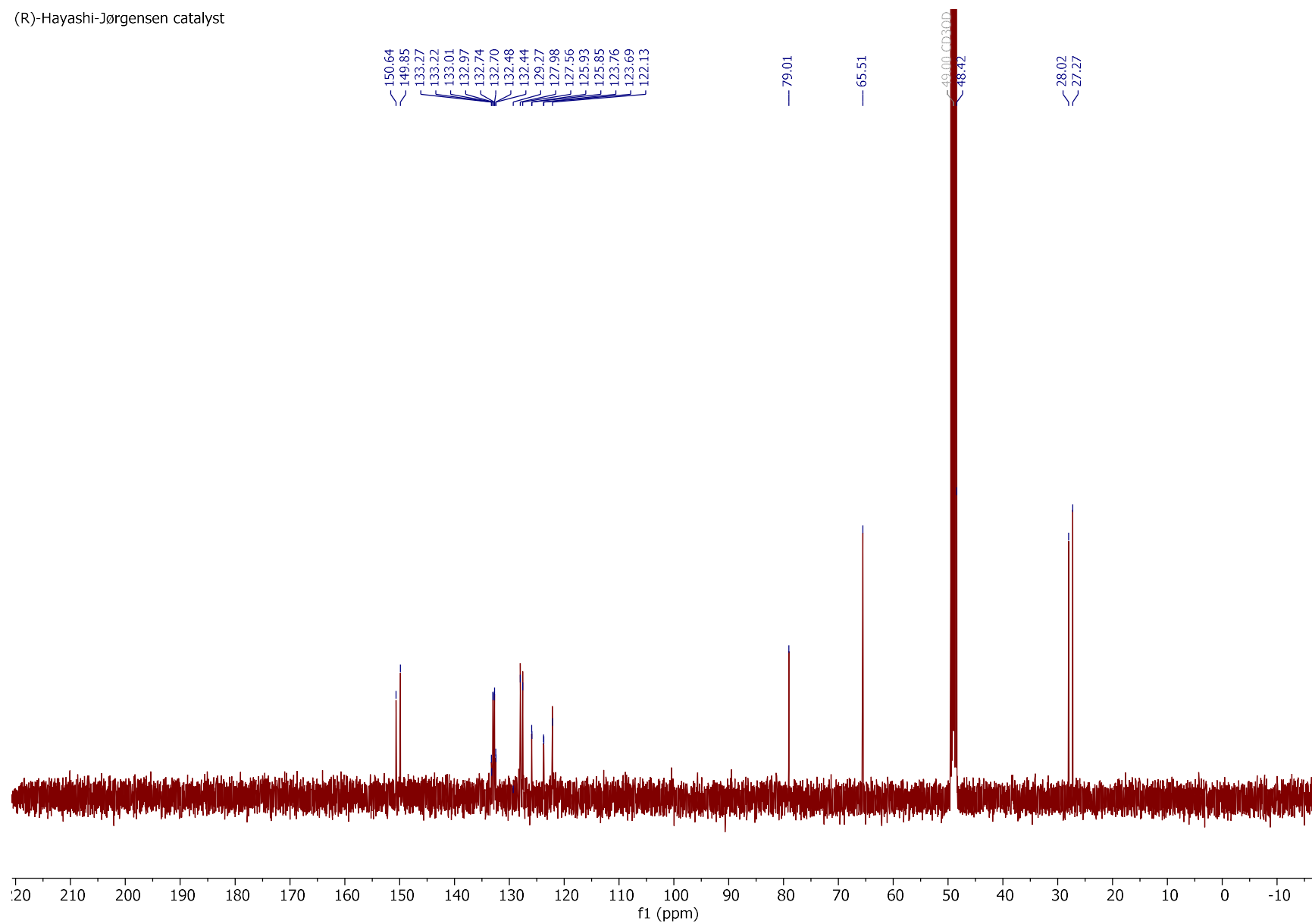
A-C4-22

(R)-Hayashi-Jørgensen catalyst

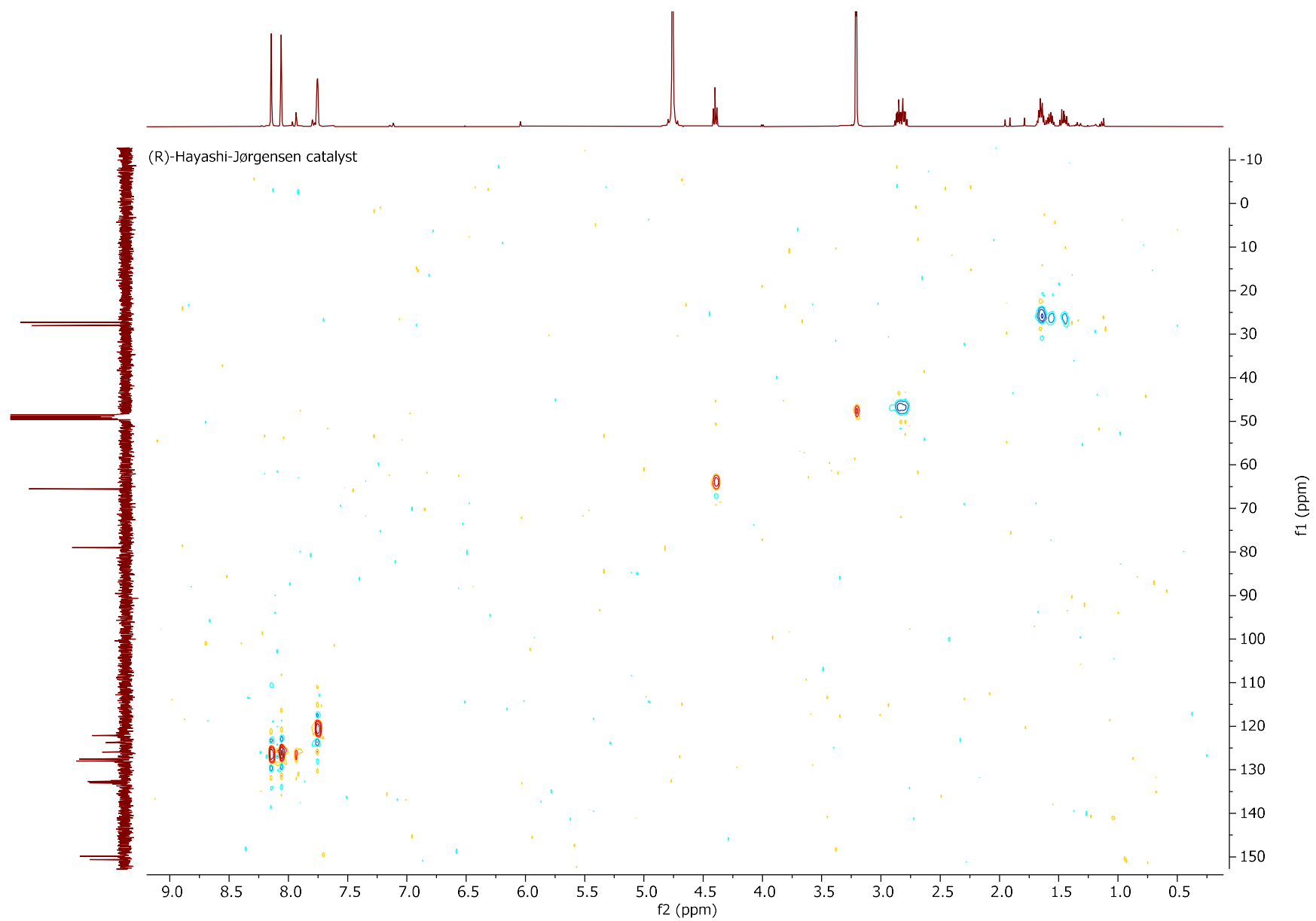


A-C4-23

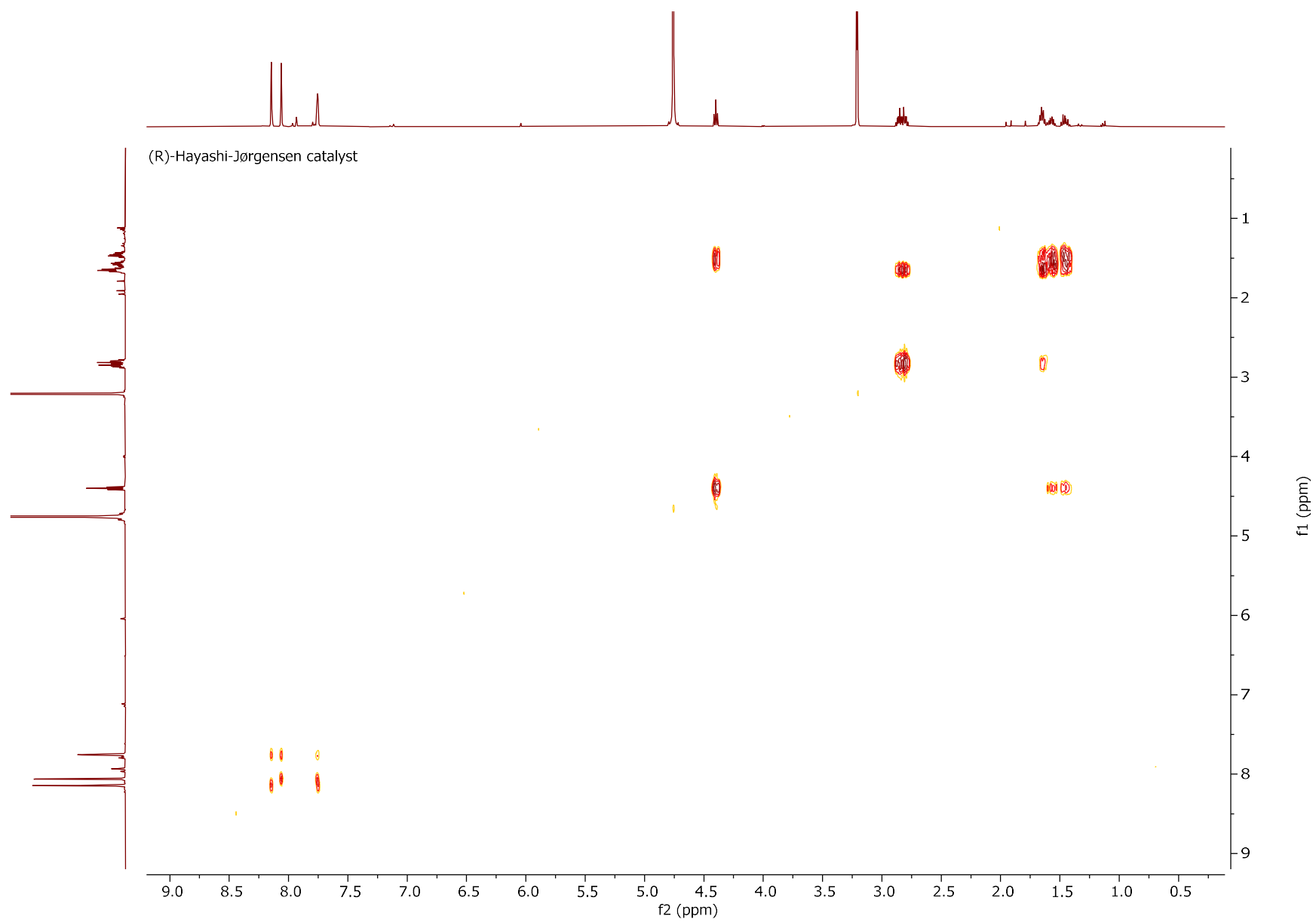
(R)-Hayashi-Jørgensen catalyst



A-C4-24



A-C4-25



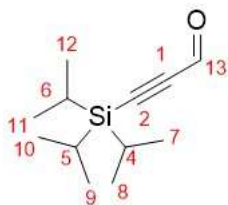
A-C4-26

3-(Triisopropylsilyl)-prop-2-ynal

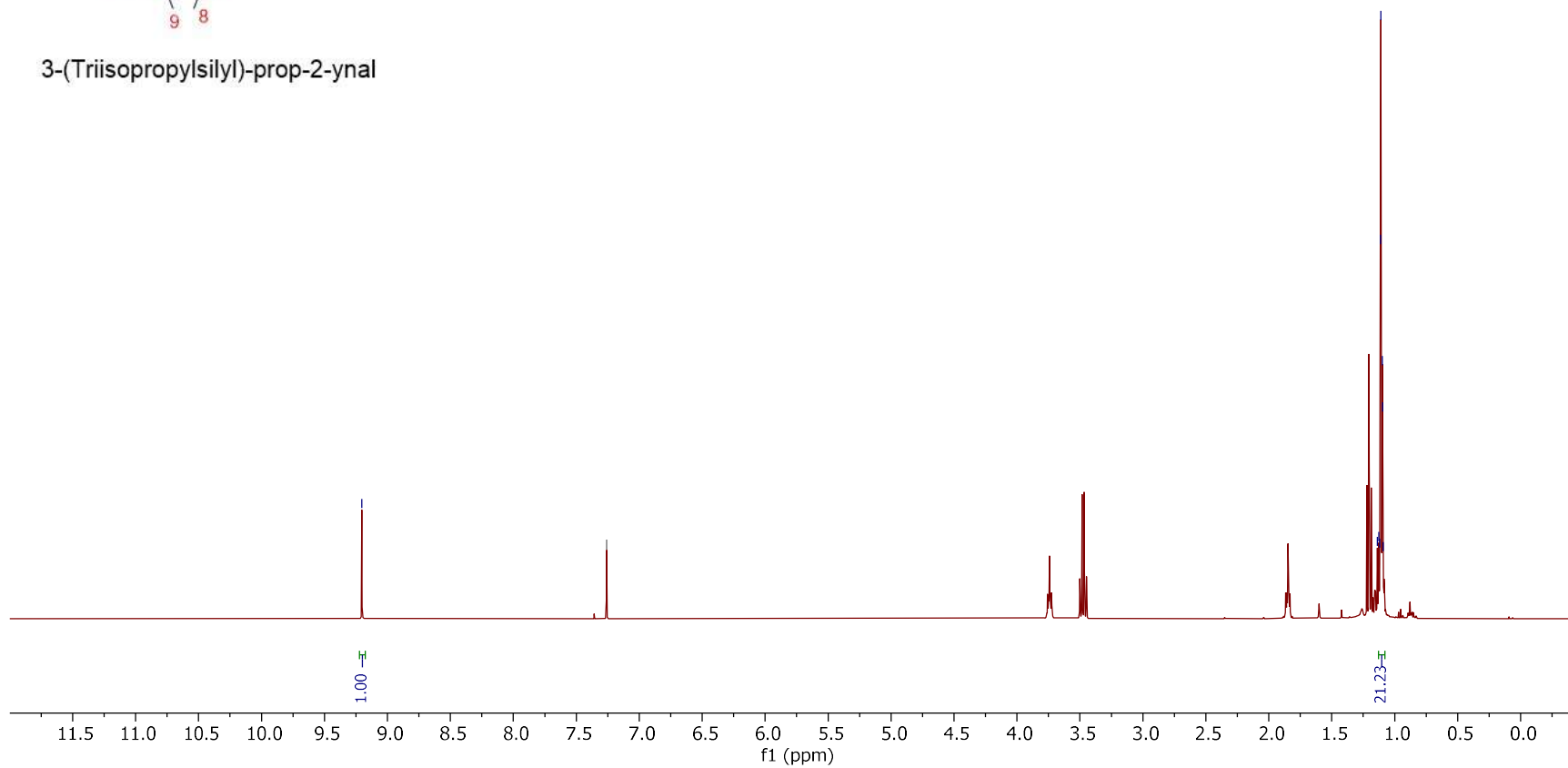
— 9.20

— 7.26 CDCl₃

1.14
1.13
1.12
1.11
1.11
1.10
1.10
1.09

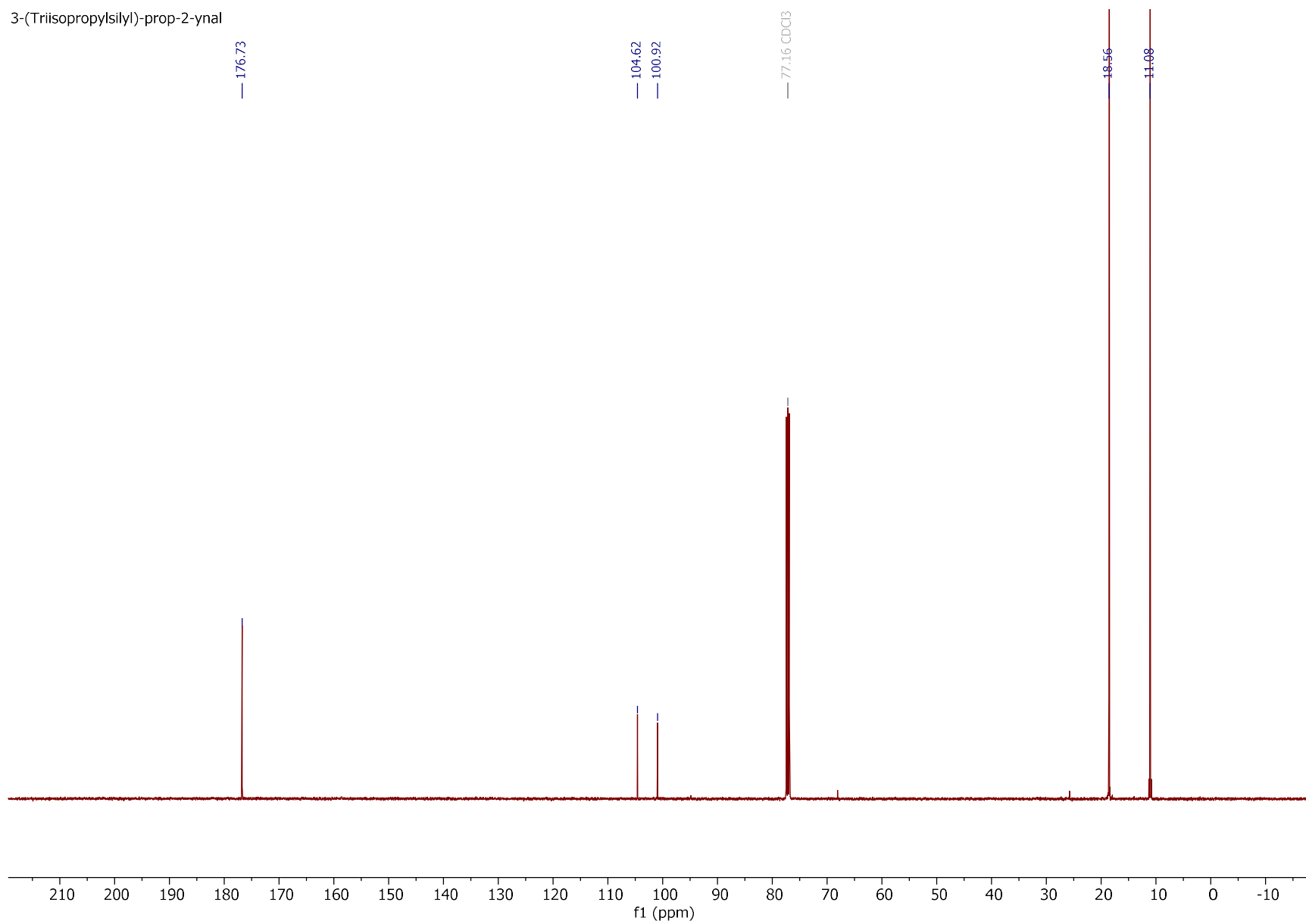


3-(Triisopropylsilyl)-prop-2-ynal



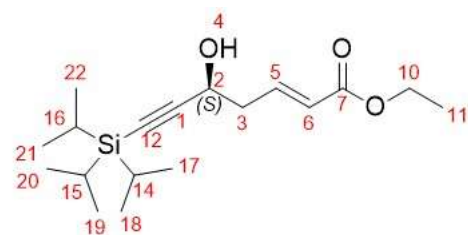
A-C4-27

3-(Triisopropylsilyl)-prop-2-ynal

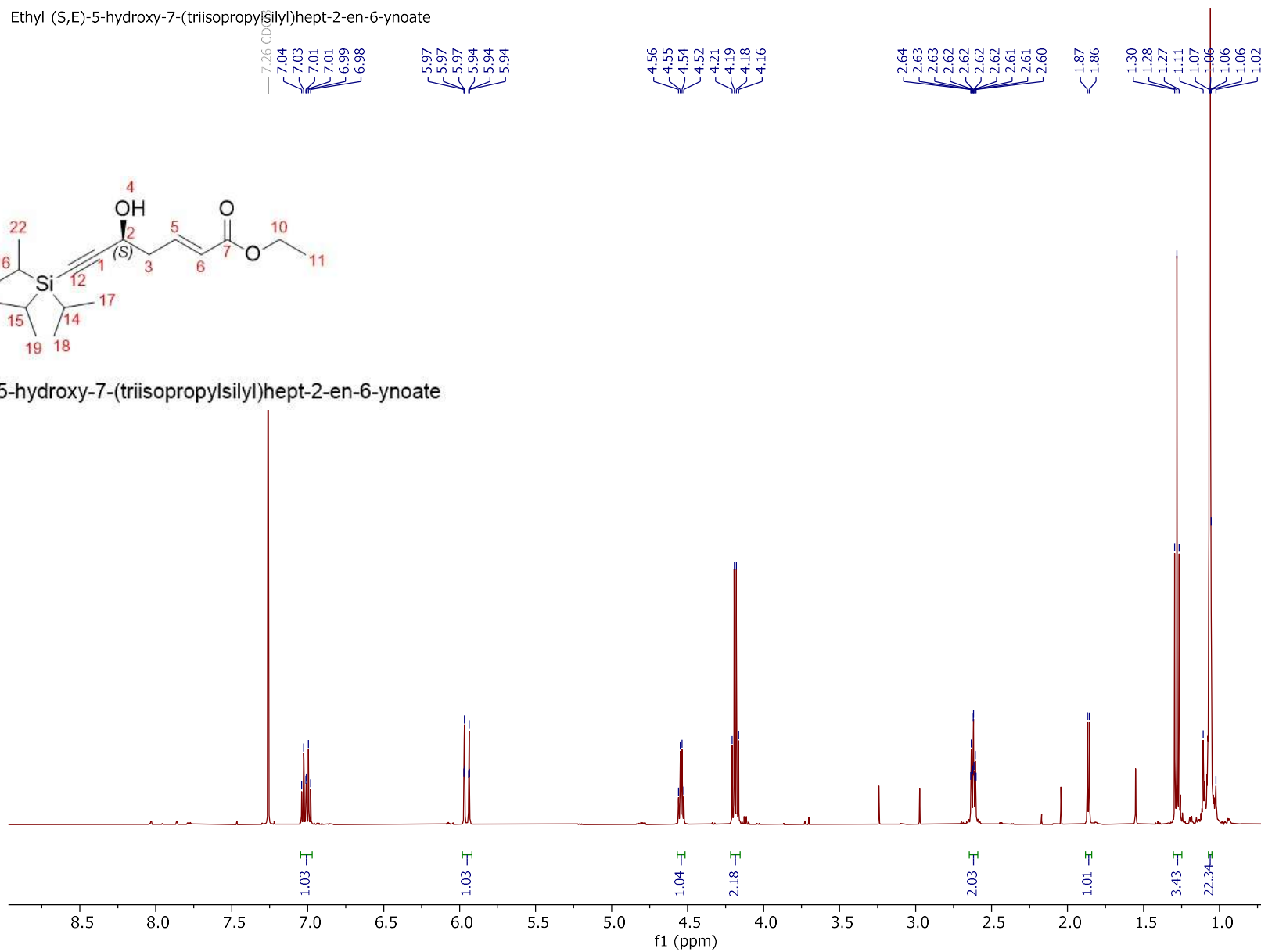


A-C4-28

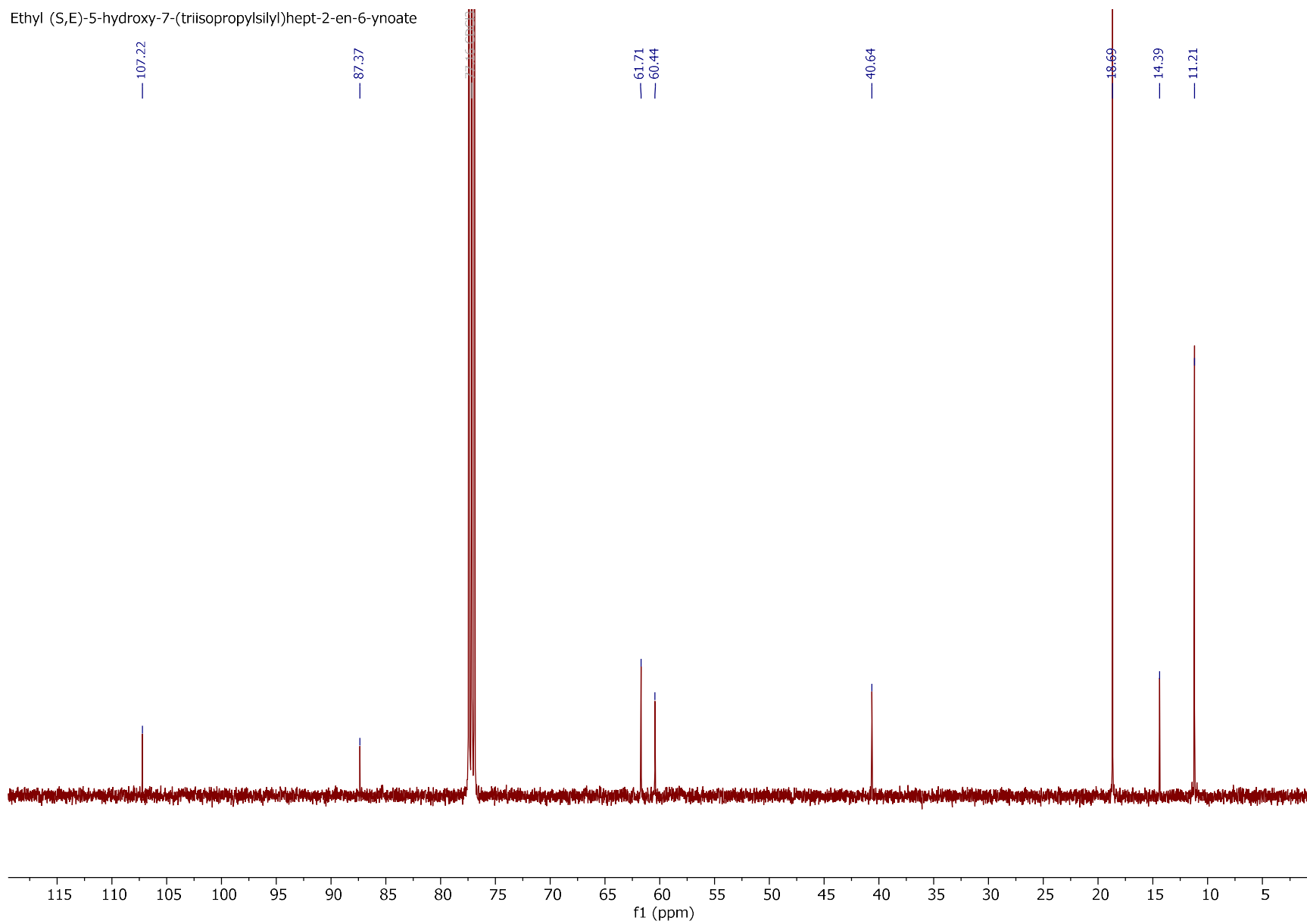
Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate



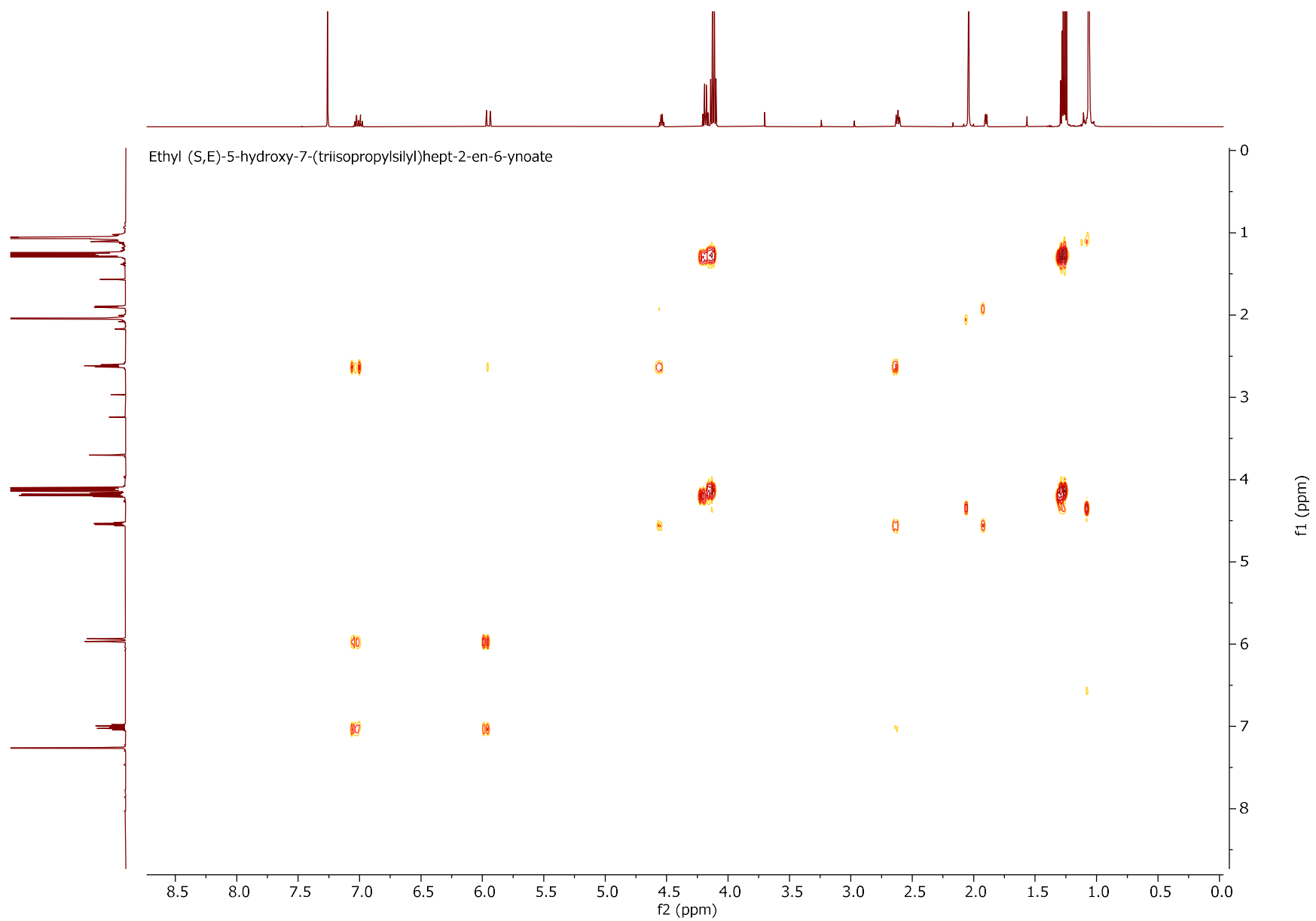
Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate



Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate

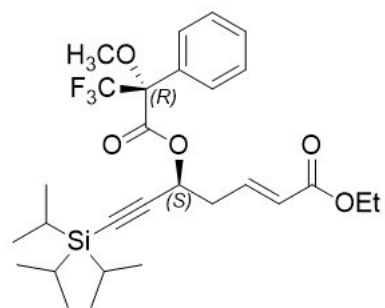


A-C4-30

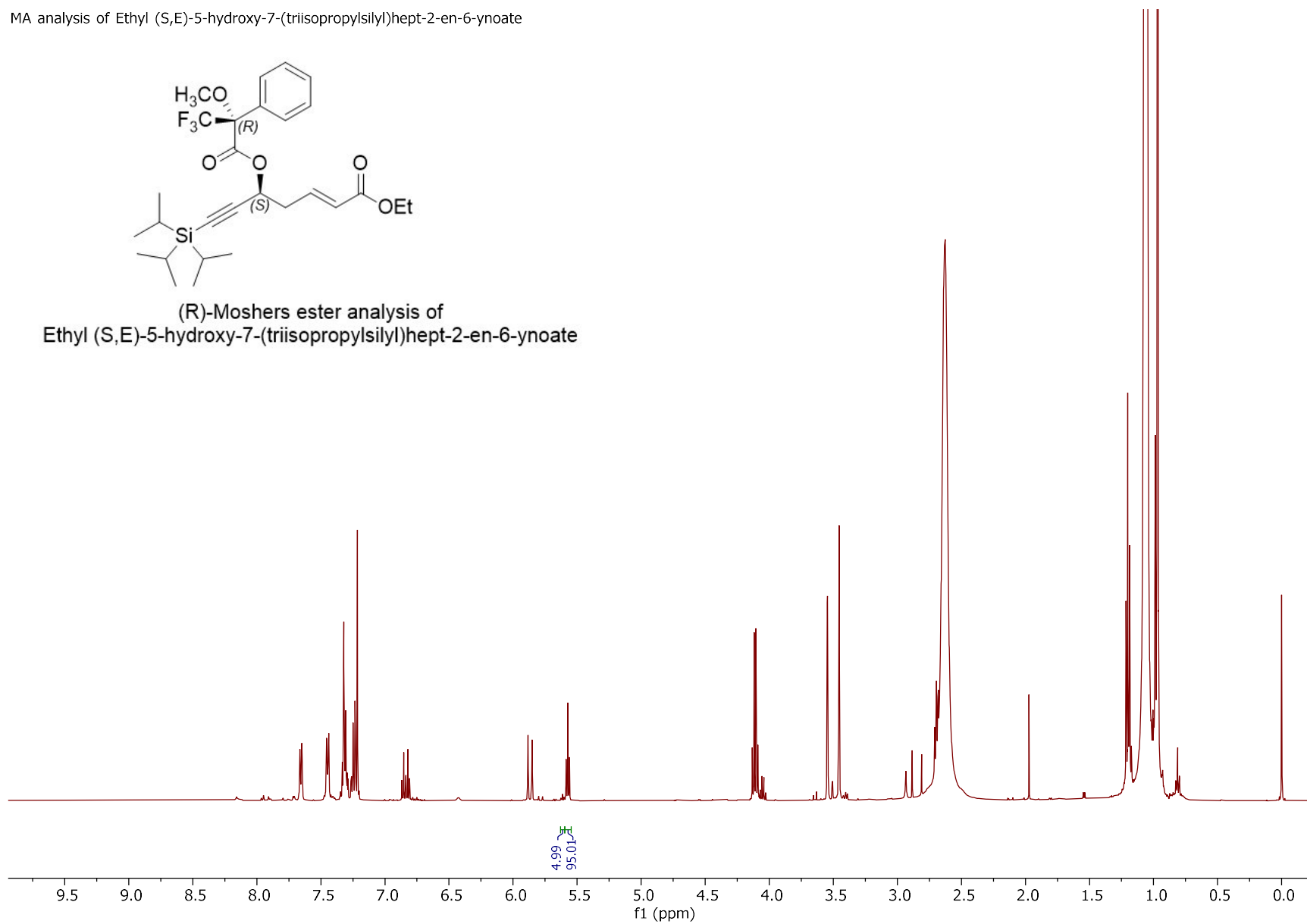


A-C4-31

MA analysis of Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate

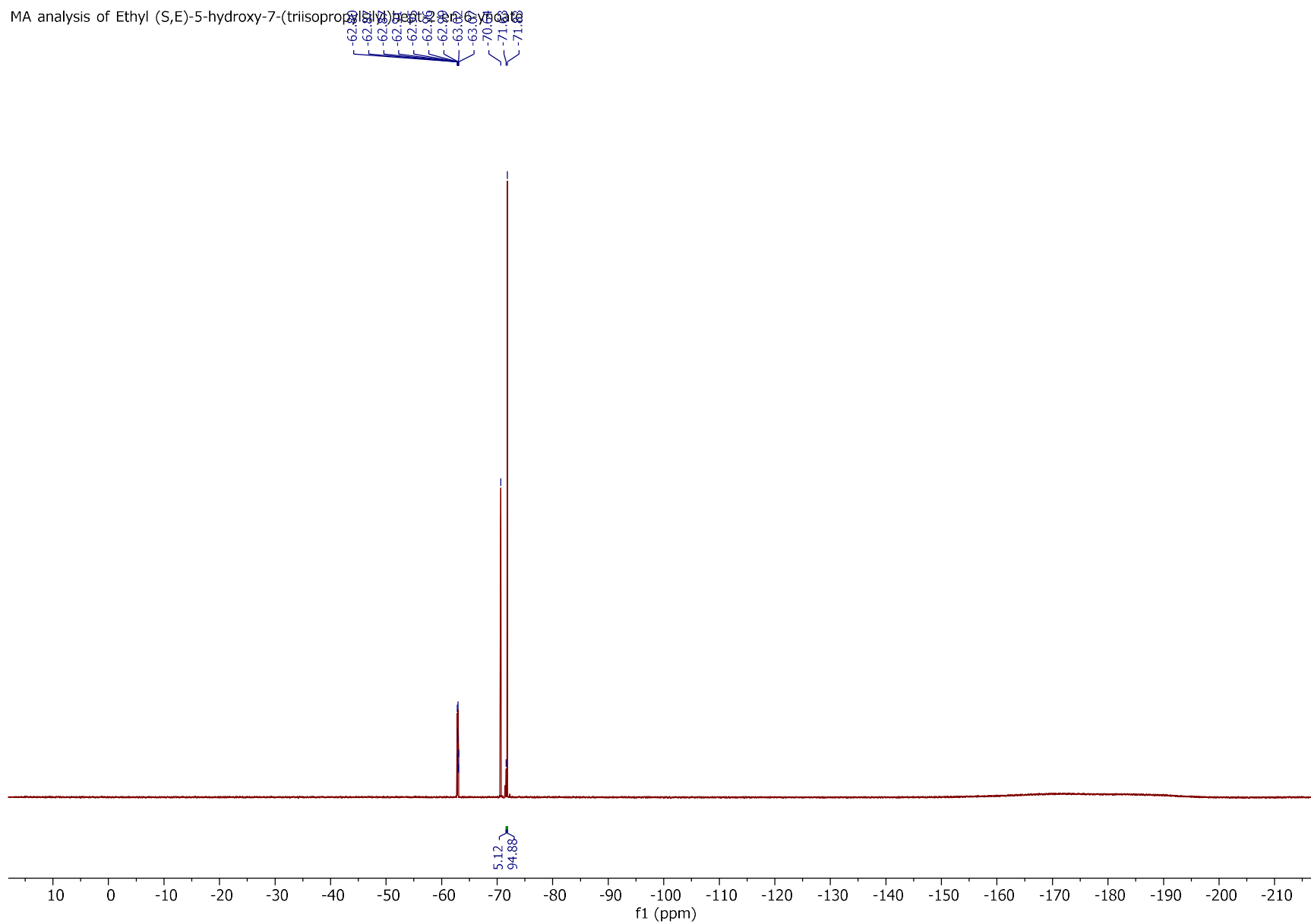


(R)-Mosher's ester analysis of
Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-en-6-ynoate



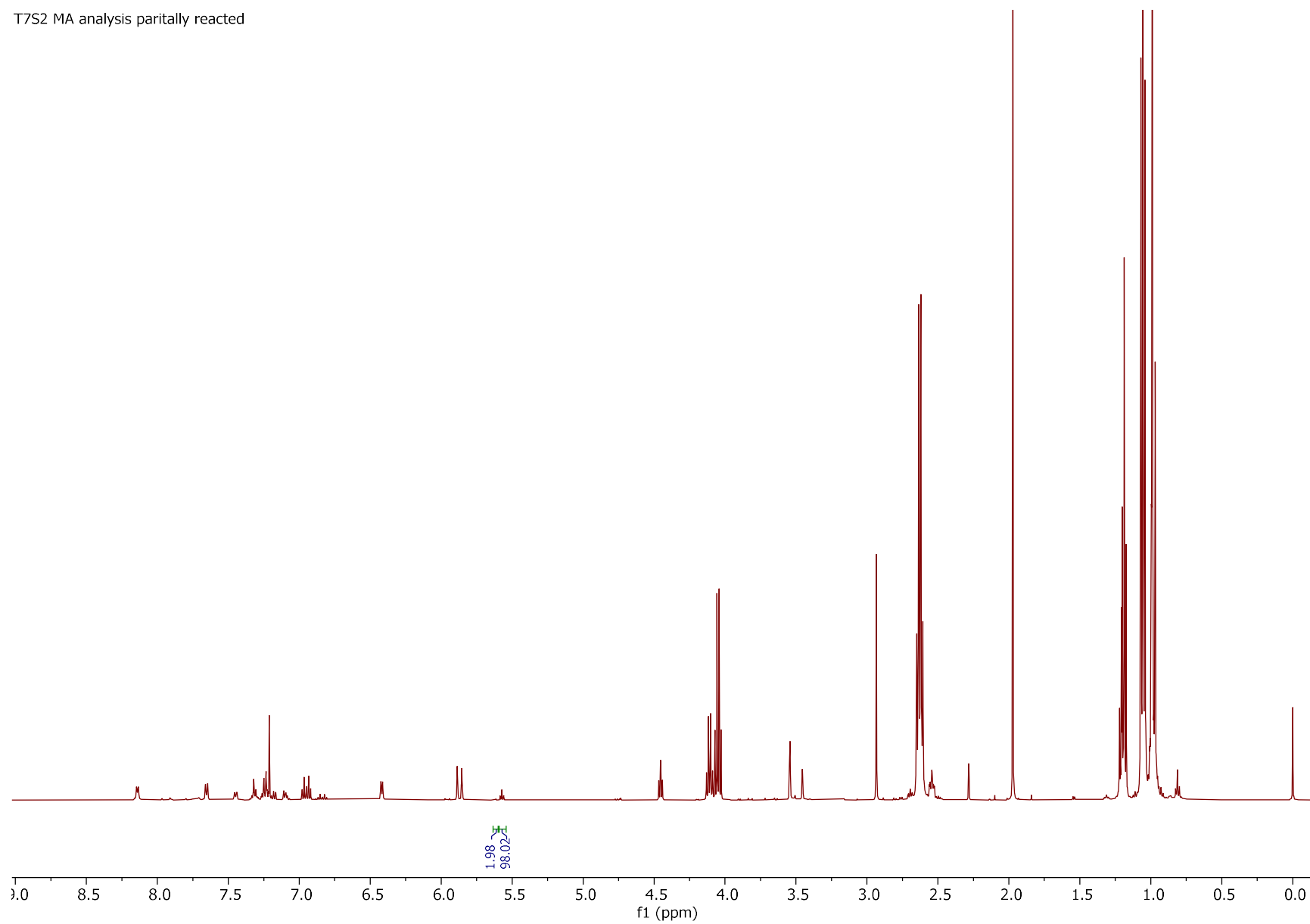
A-C4-32

MA analysis of Ethyl (S,E)-5-hydroxy-7-(triisopropylsilyl)hept-2-enoate



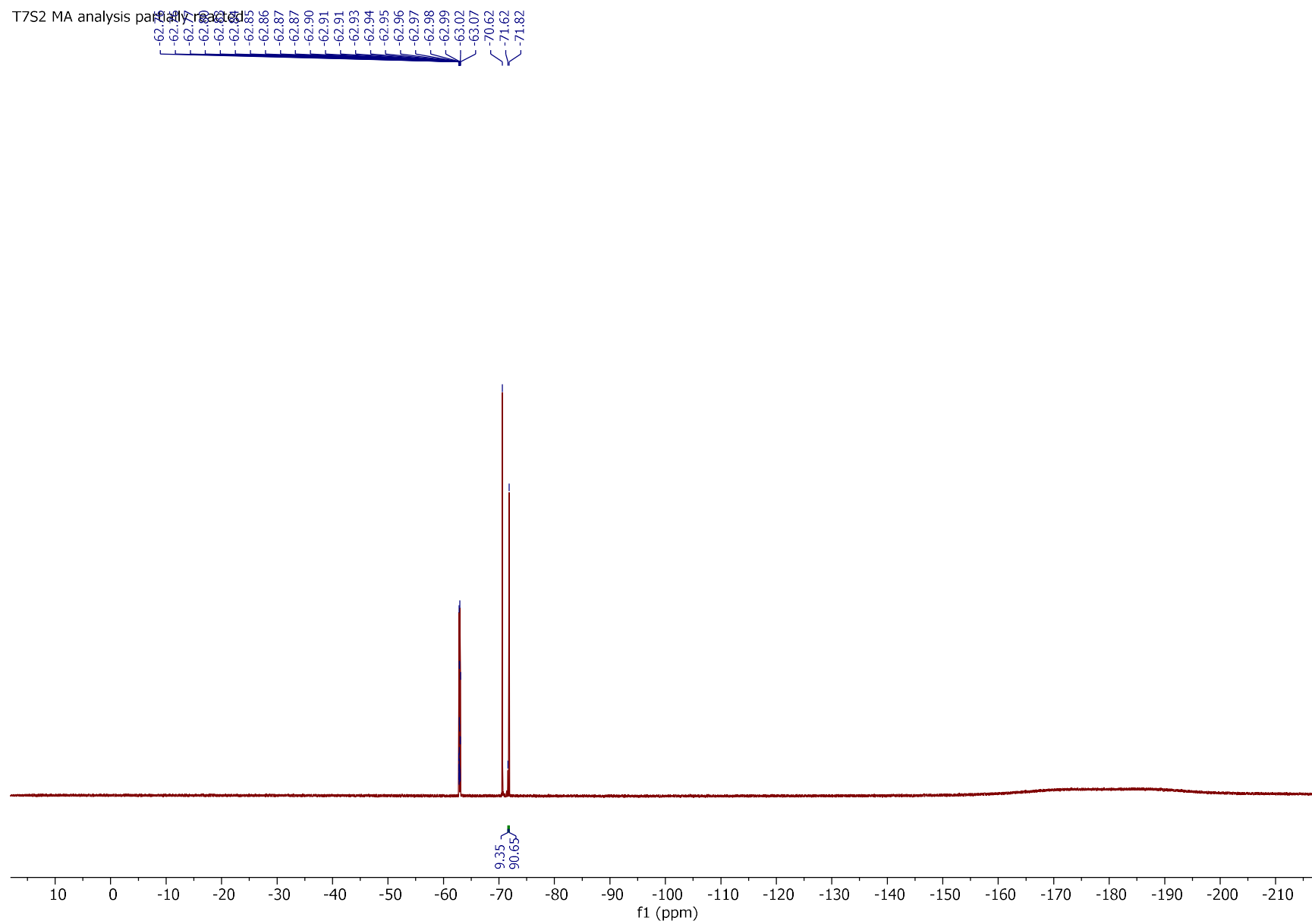
A-C4-33

T7S2 MA analysis paritally reacted



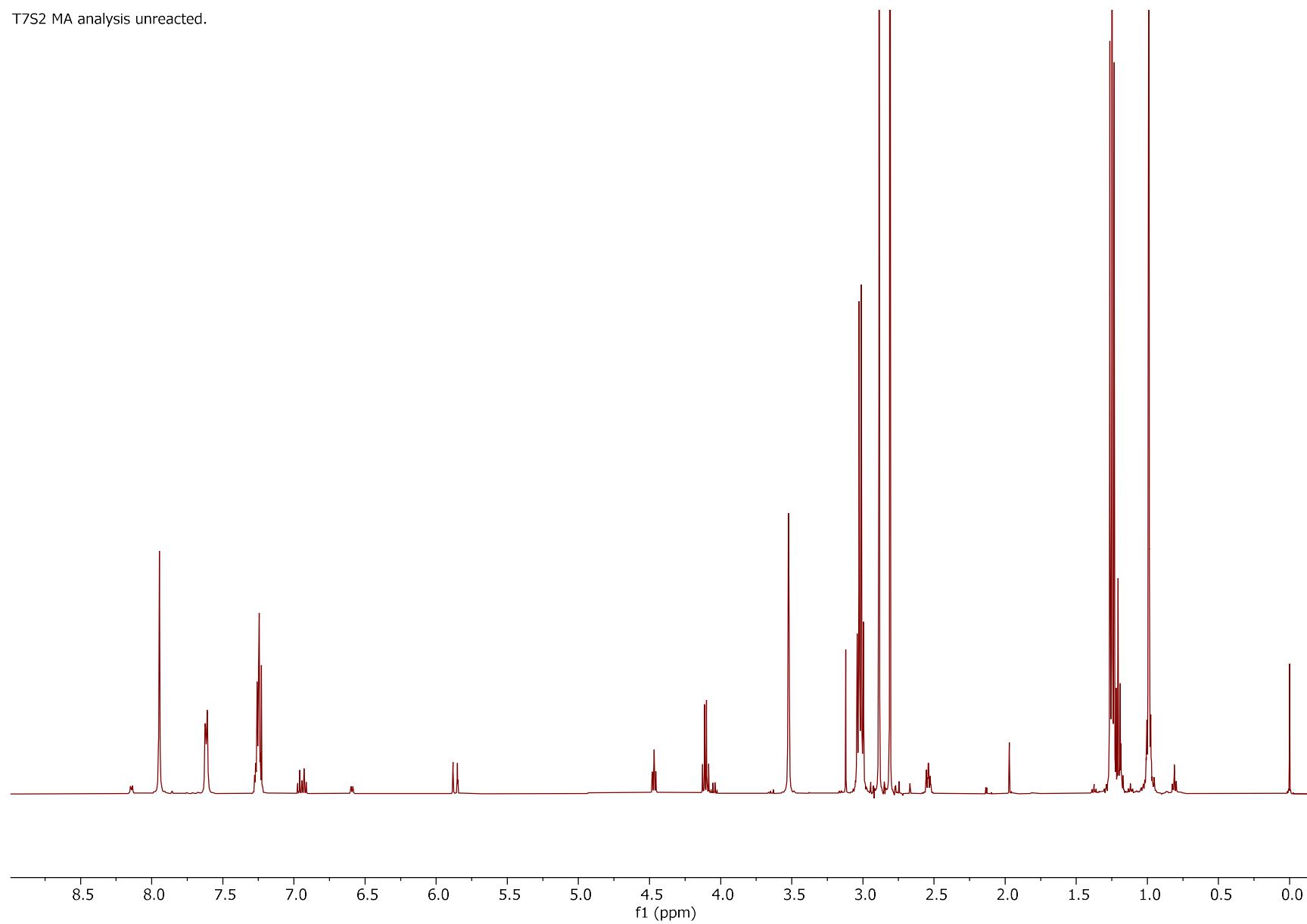
A-C4-34

T7S2 MA analysis partially reacted



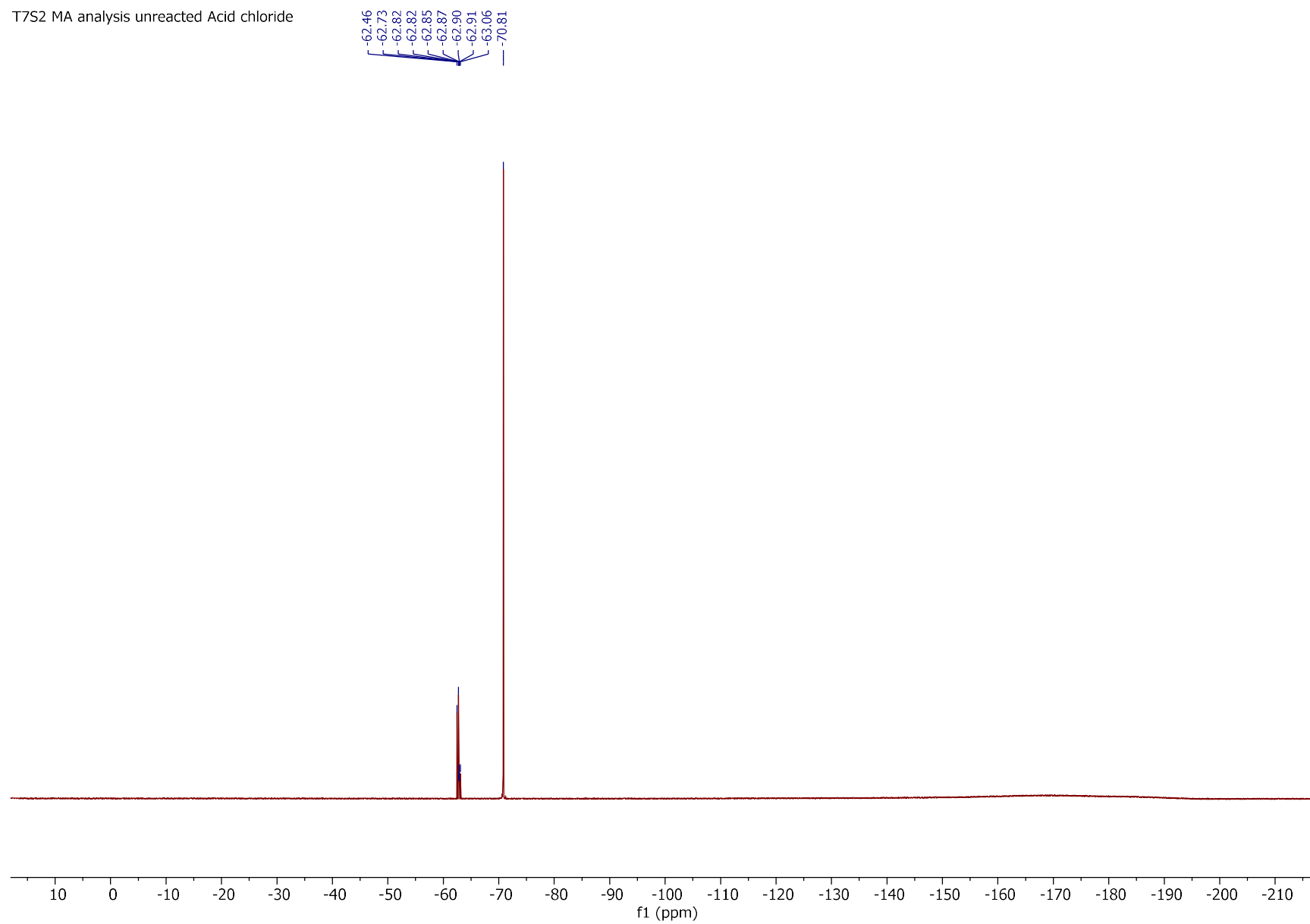
A-C4-35

T7S2 MA analysis unreacted.



A-C4-36

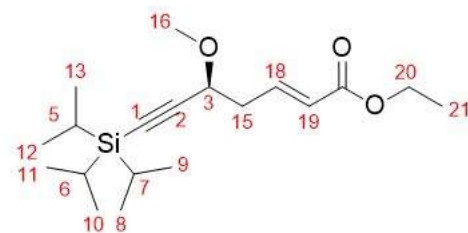
T7S2 MA analysis unreacted Acid chloride



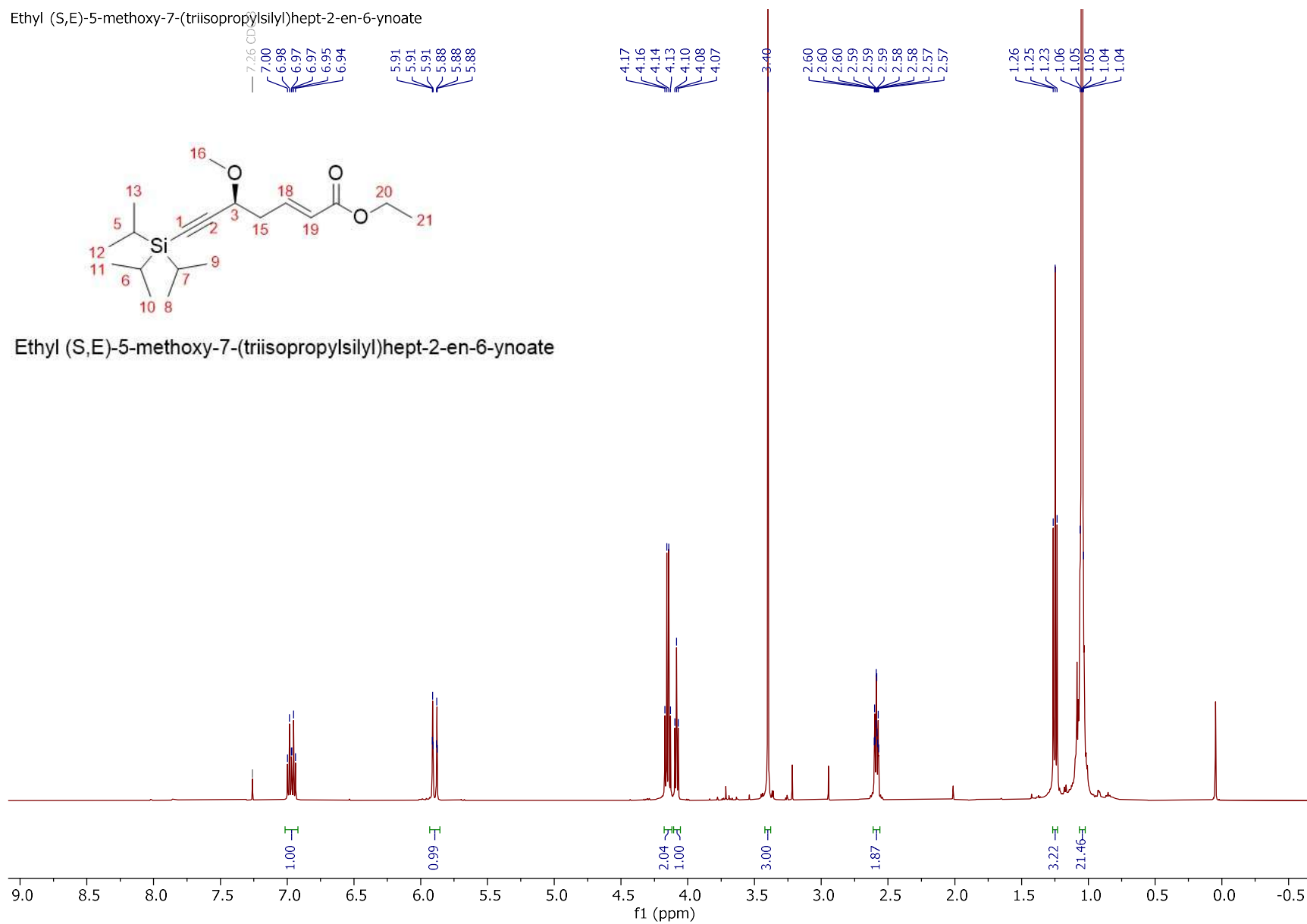
A-C4-37

A-C5

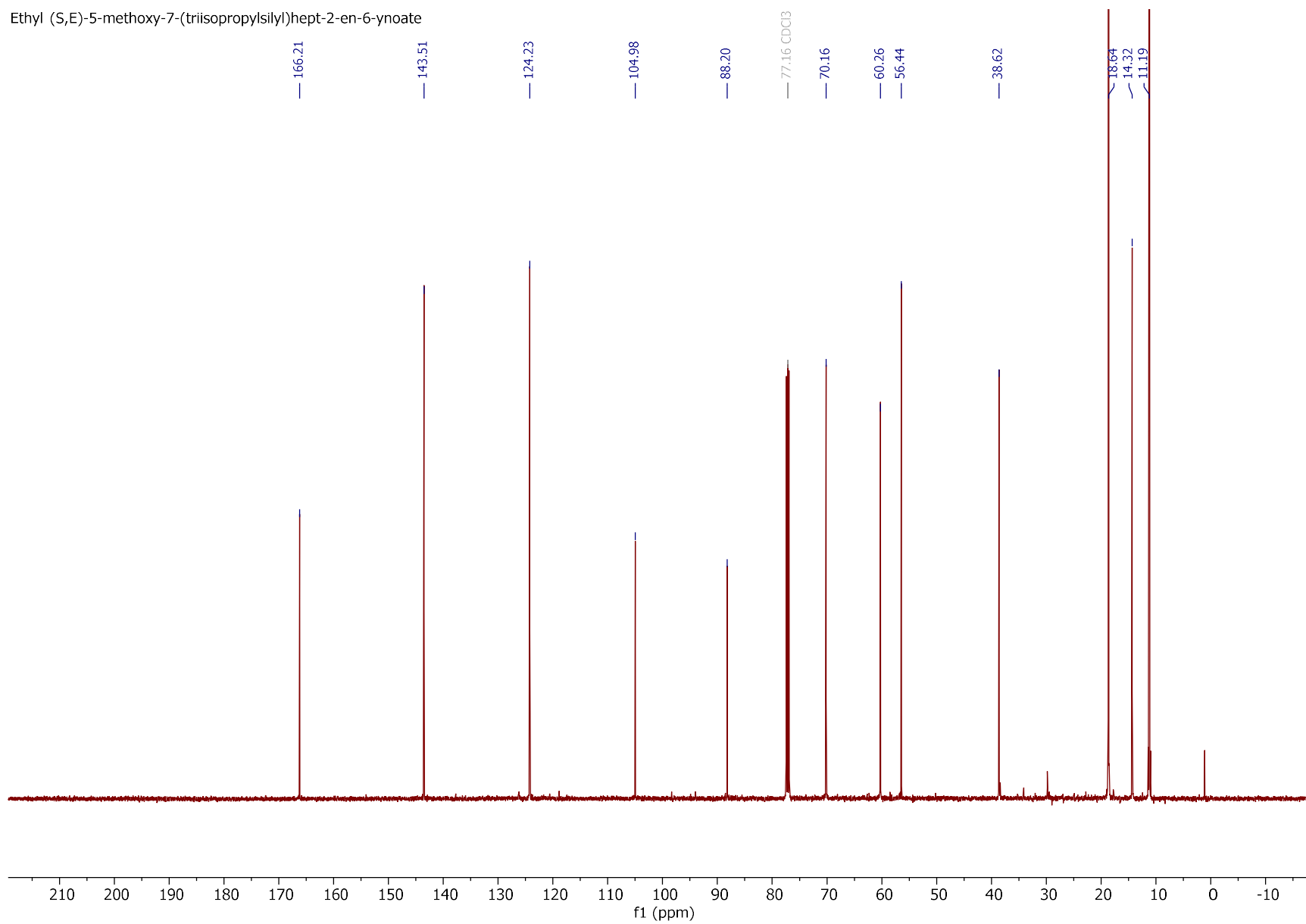
Ethyl (S,E)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate



Ethyl (S,E)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate

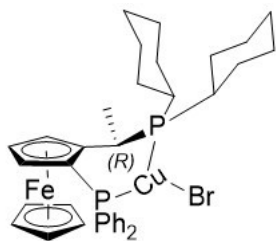


Ethyl (S,E)-5-methoxy-7-(triisopropylsilyl)hept-2-en-6-ynoate

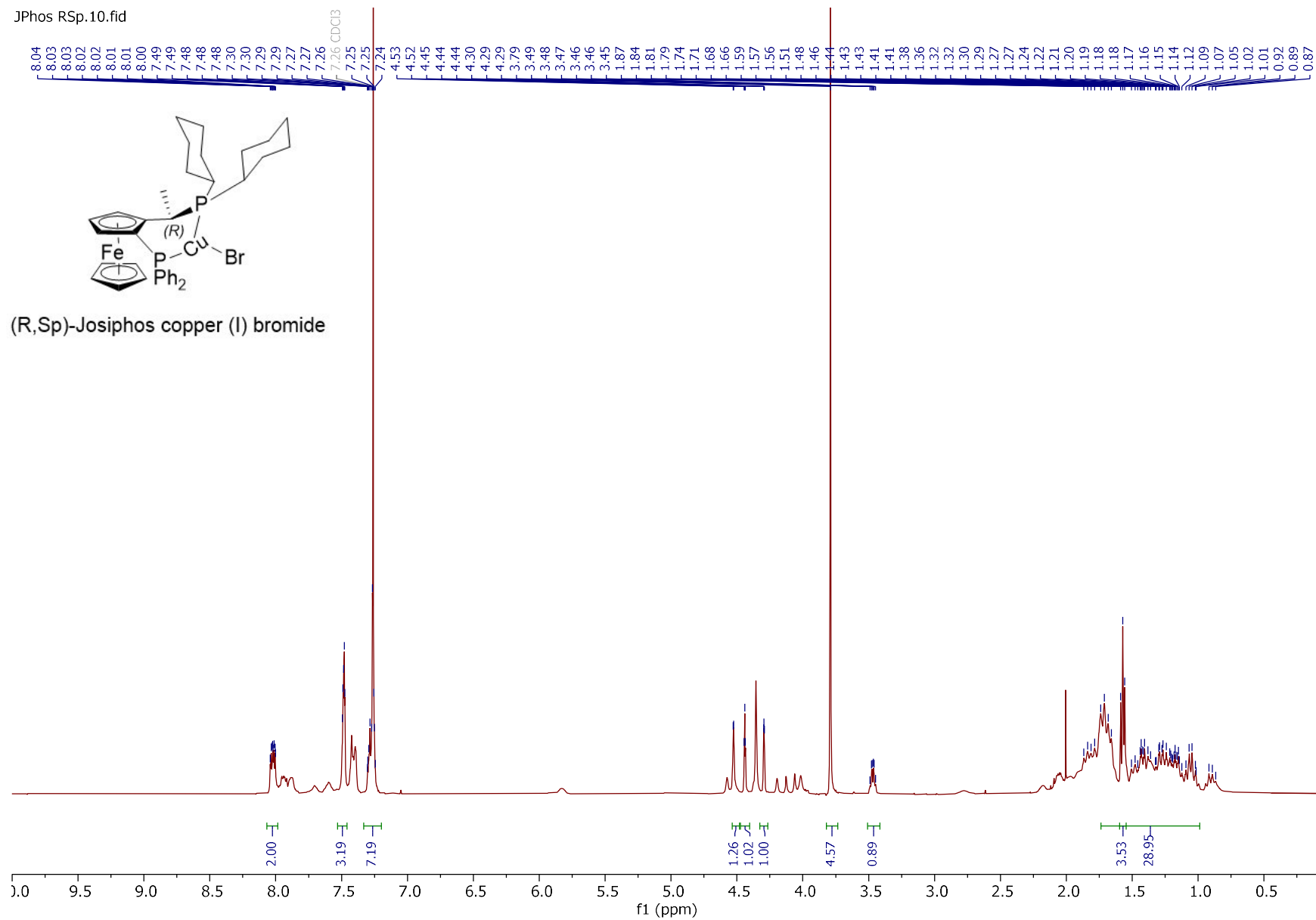


A-C5-2

JPhos RSp.10.fid

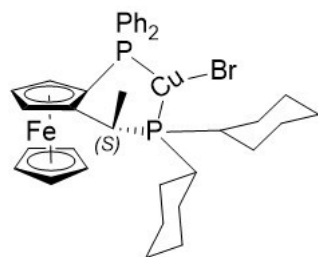


(R,Sp)-Josiphos copper (I) bromide

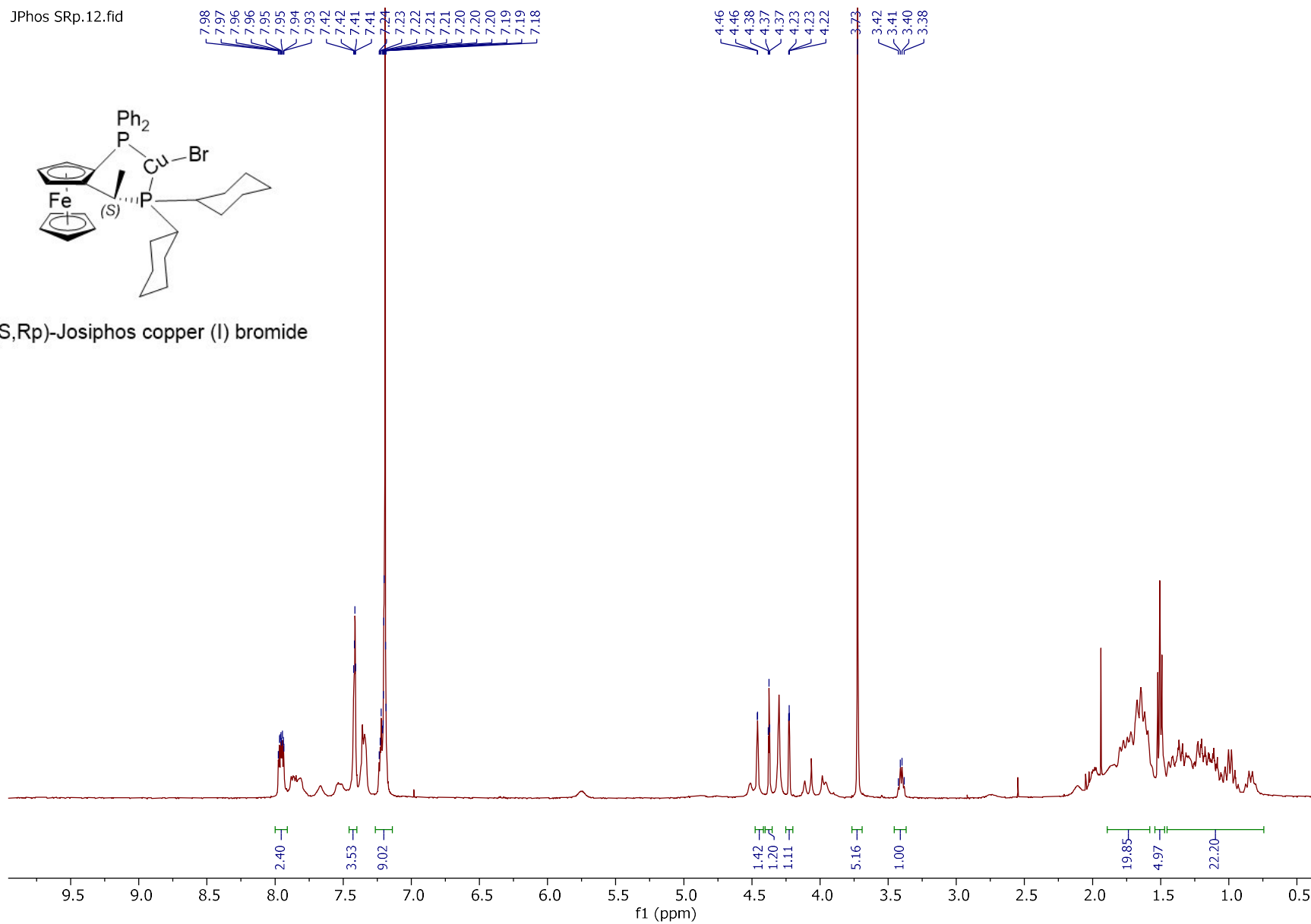


A-C5-3

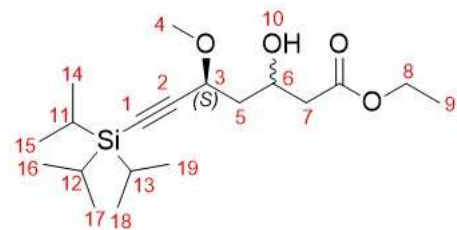
JPhos SRp.12.fid



(S,Rp)-Josiphos copper (I) bromide

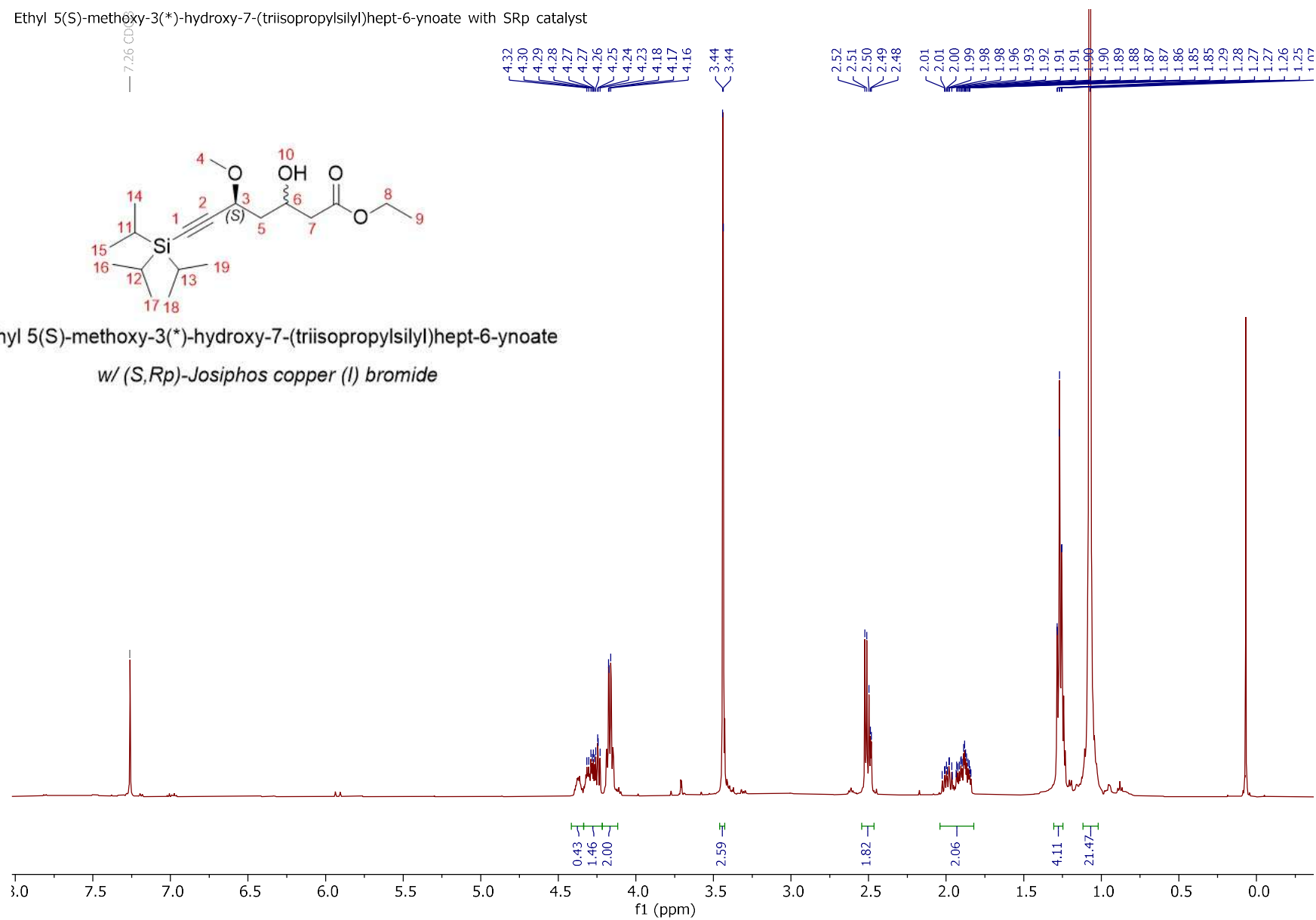


Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate with SRp catalyst



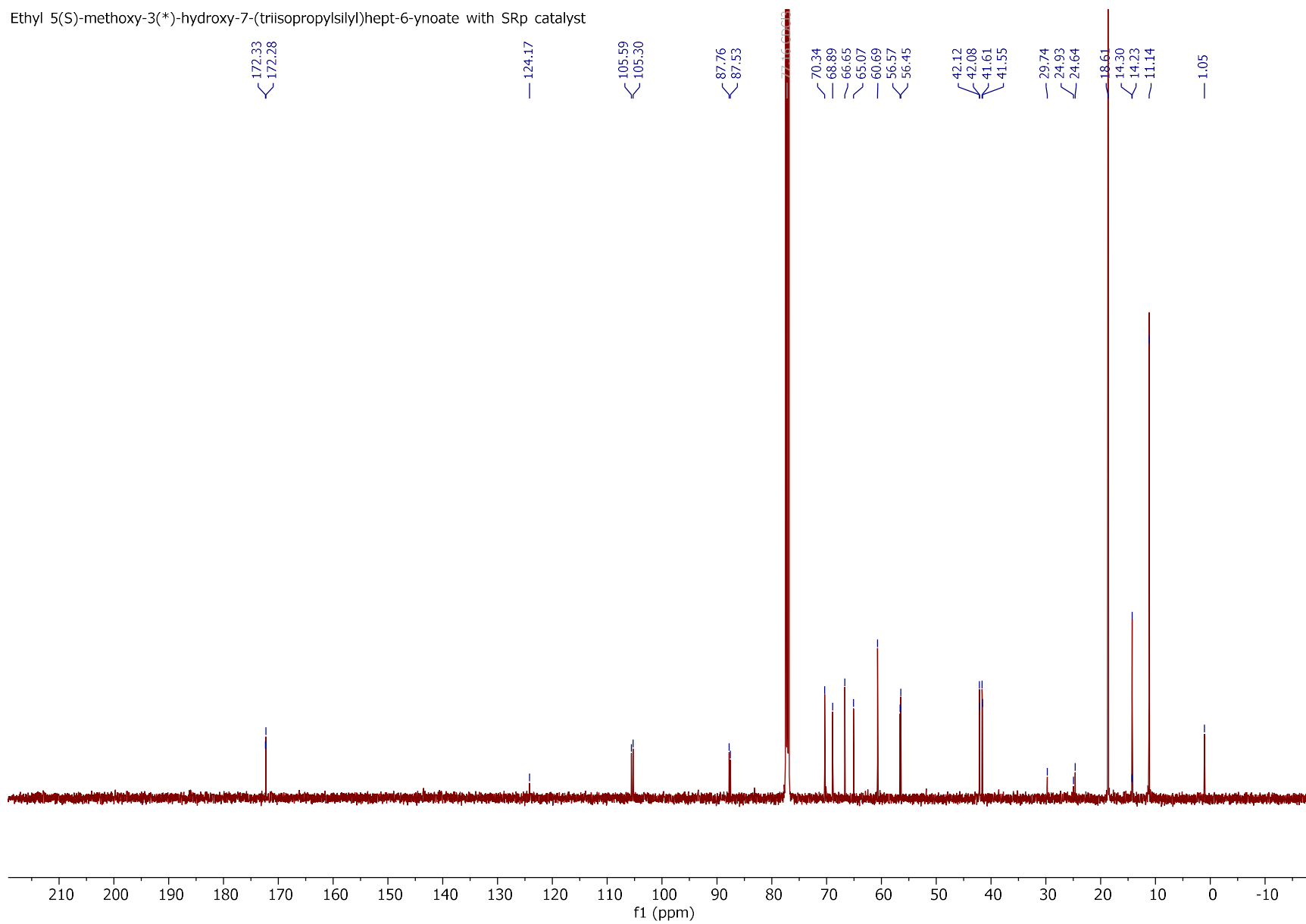
Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate

w/ (*S,Rp*)-Josiphos copper (I) bromide



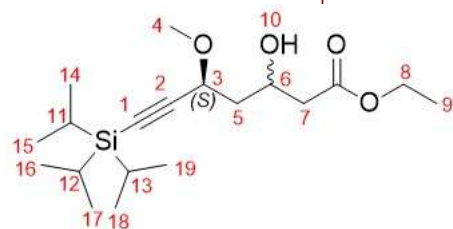
A-C5-5

Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate with SRp catalyst



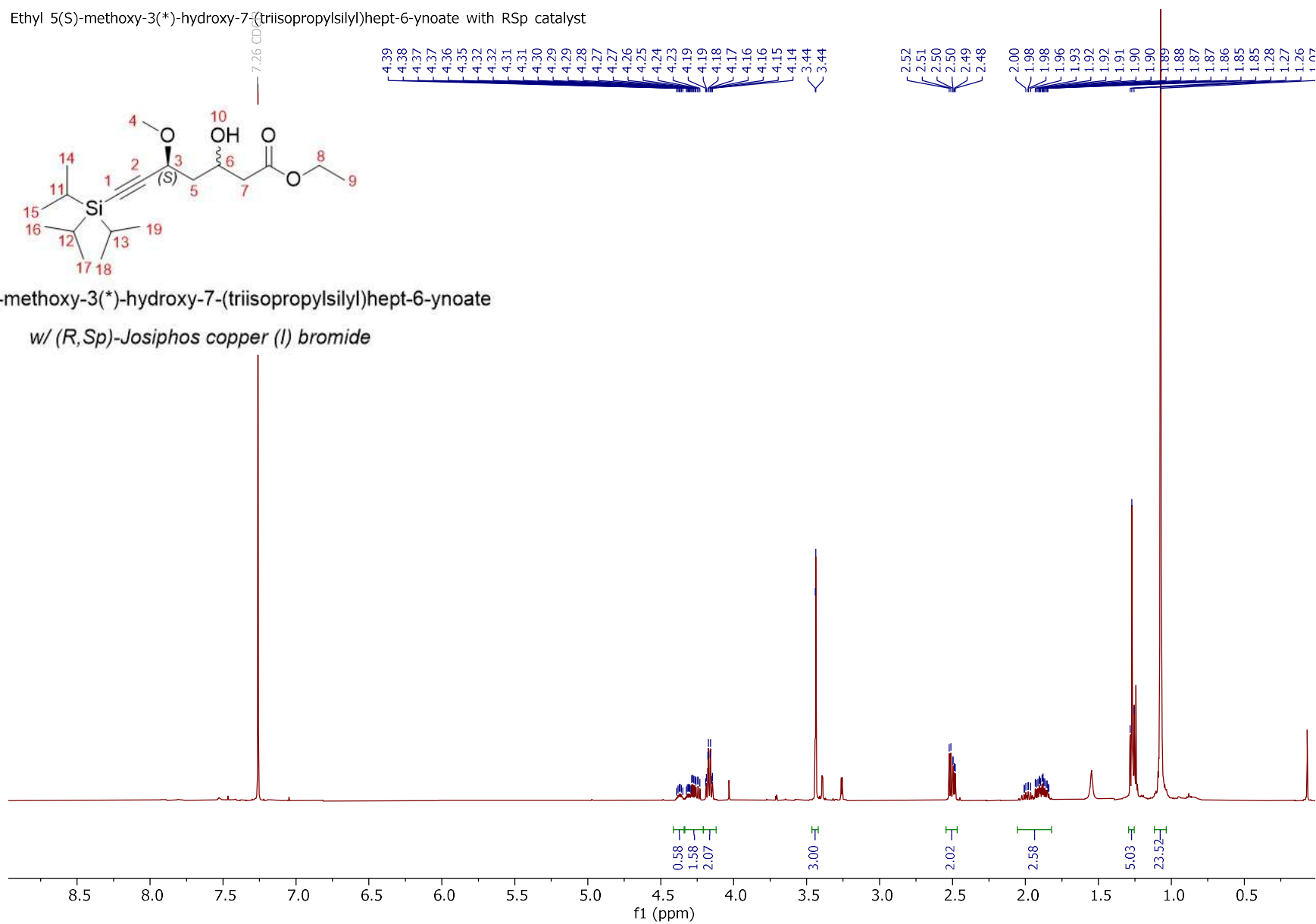
A-C5-6

Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate with RSp catalyst



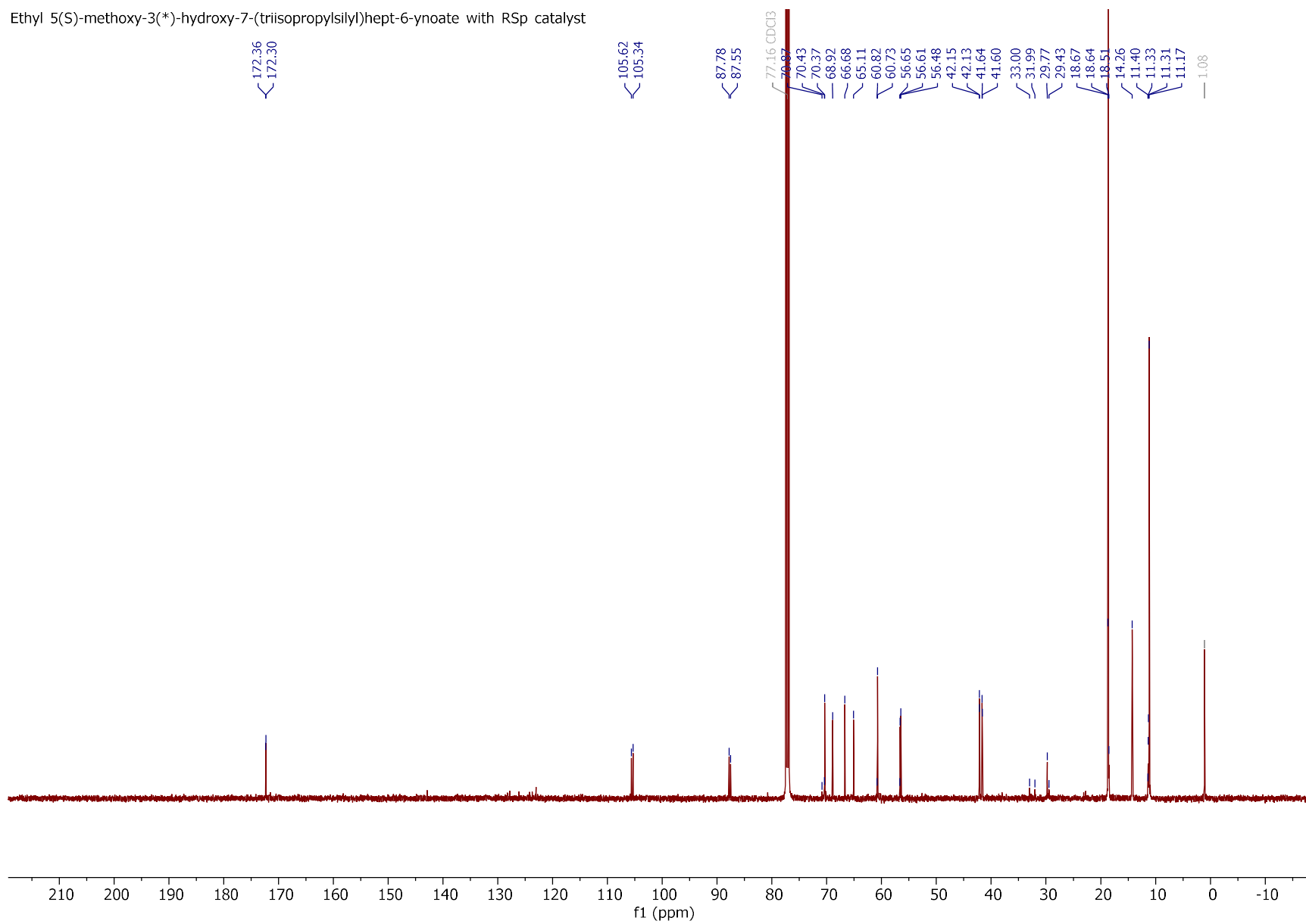
Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate

w/ (R,Sp)-Josiphos copper (I) bromide



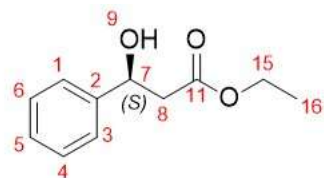
A-C5-7

Ethyl 5(S)-methoxy-3(*)-hydroxy-7-(triisopropylsilyl)hept-6-ynoate with RSp catalyst

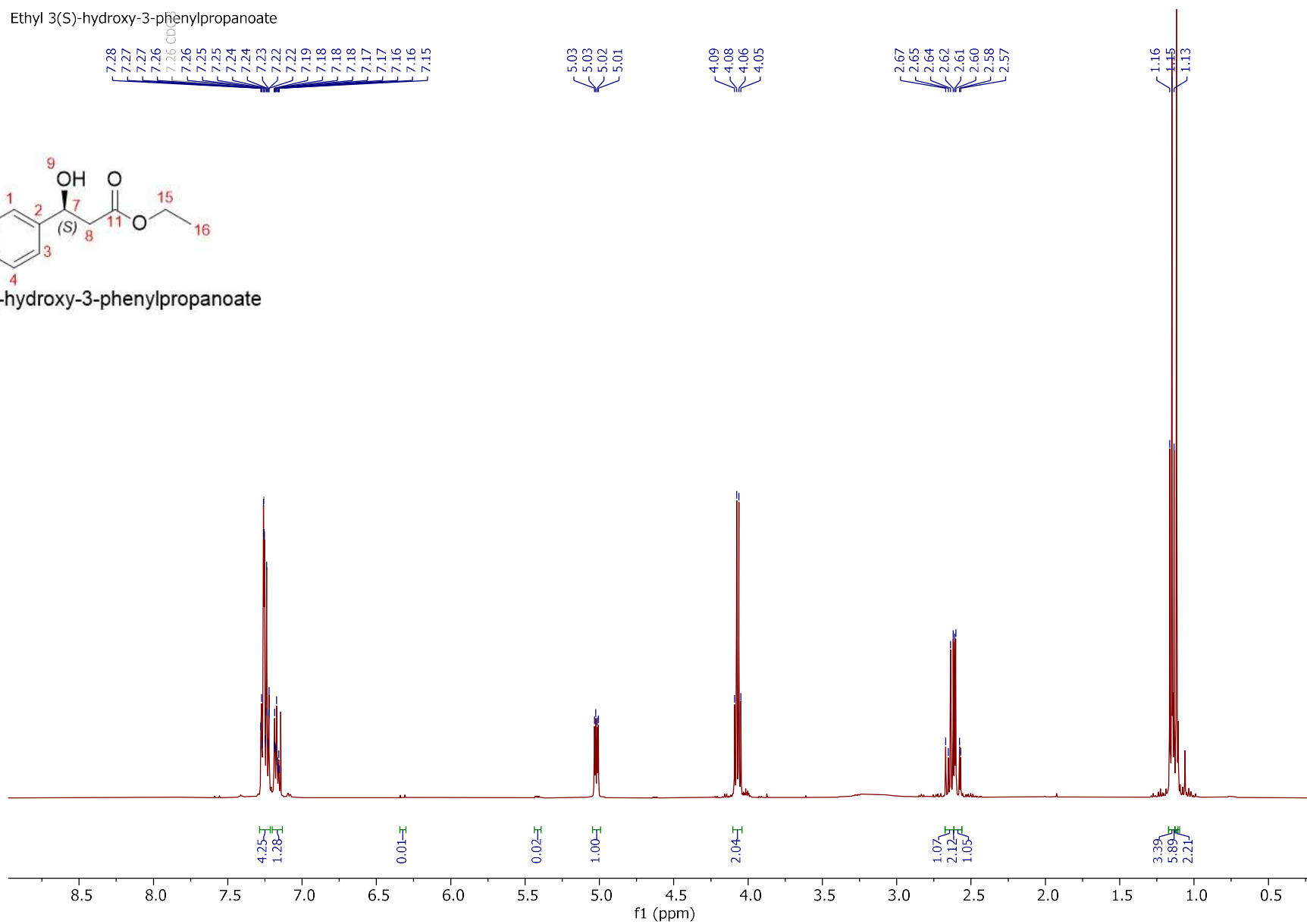


A-C5-8

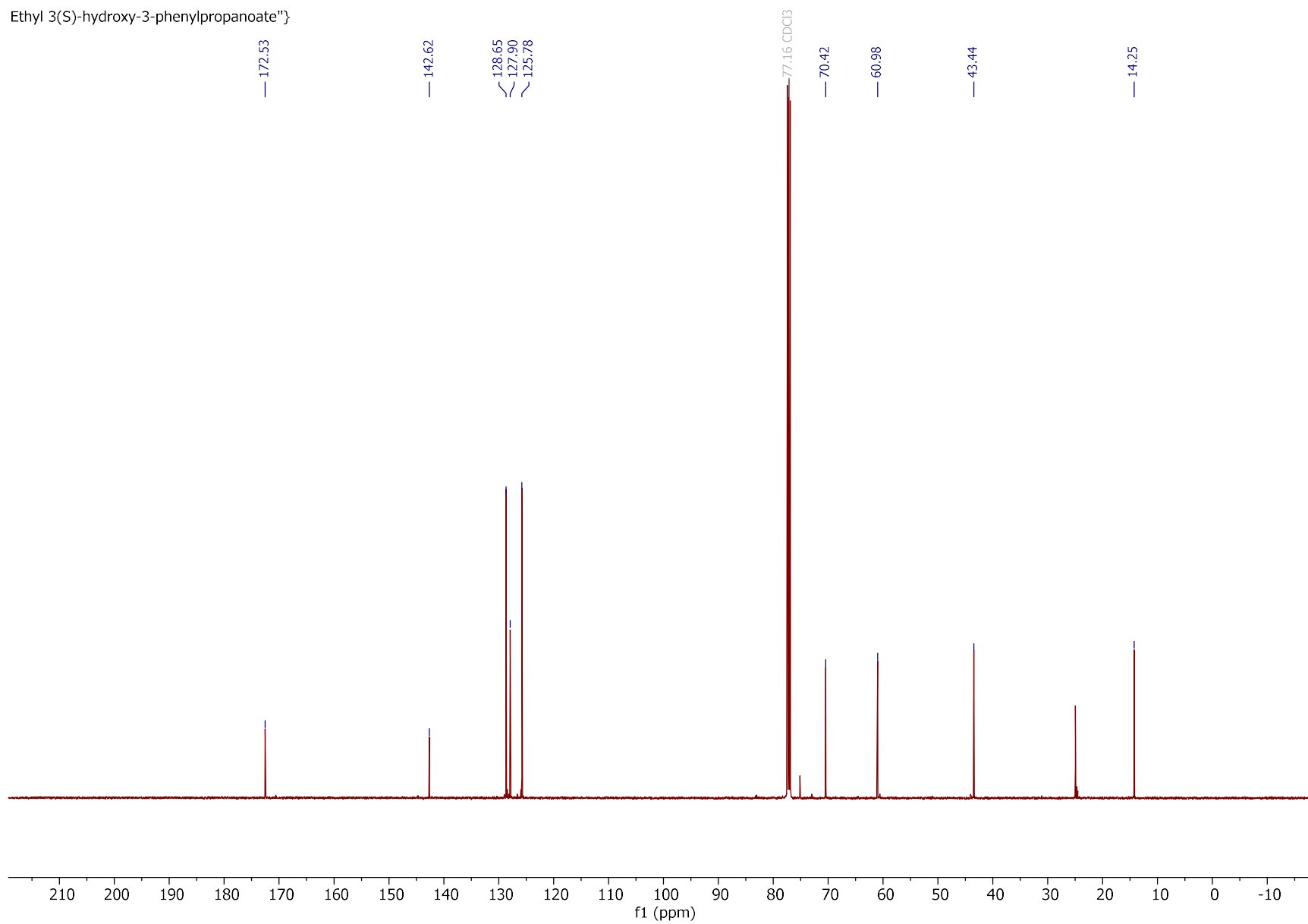
Ethyl 3(S)-hydroxy-3-phenylpropanoate



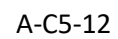
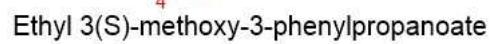
Ethyl 3(S)-hydroxy-3-phenylpropanoate



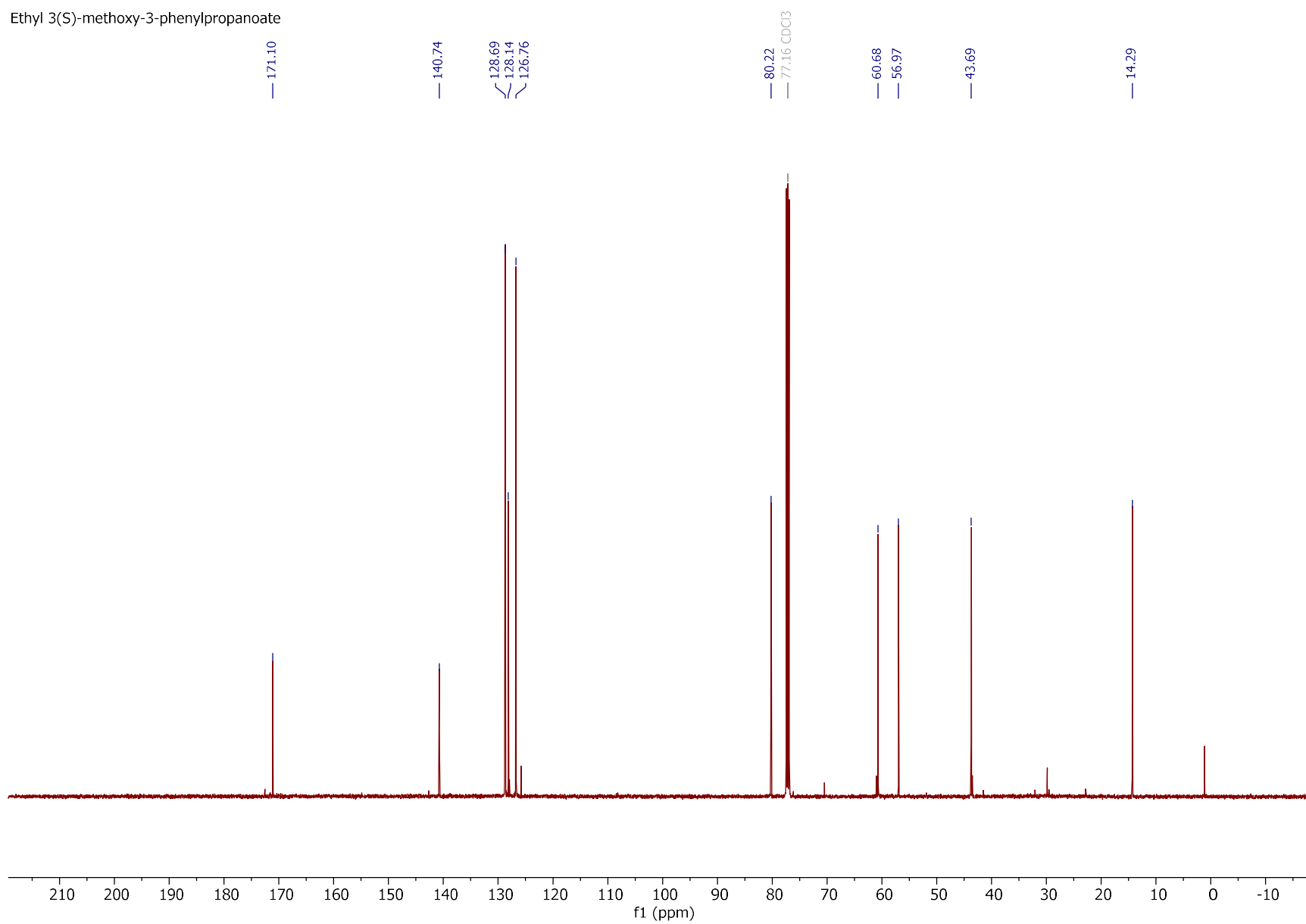
Ethyl 3(S)-hydroxy-3-phenylpropanoate"}
CCOC(=O)C(O)(c1ccccc1)CO



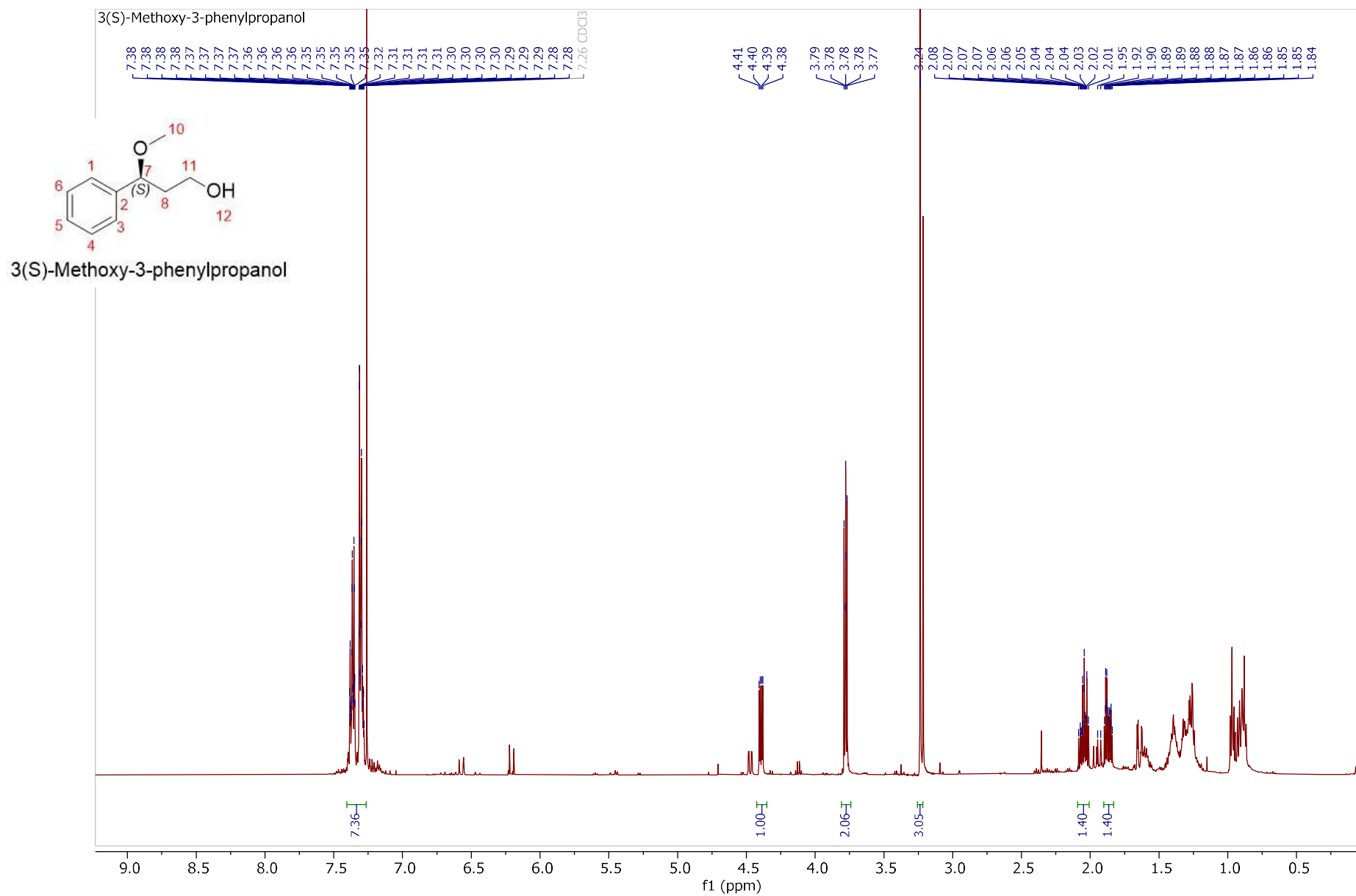
A-C5-10



Ethyl 3(S)-methoxy-3-phenylpropanoate

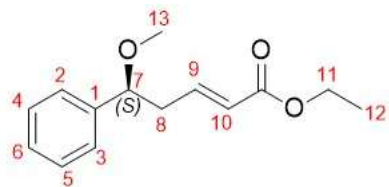


A-C5-13

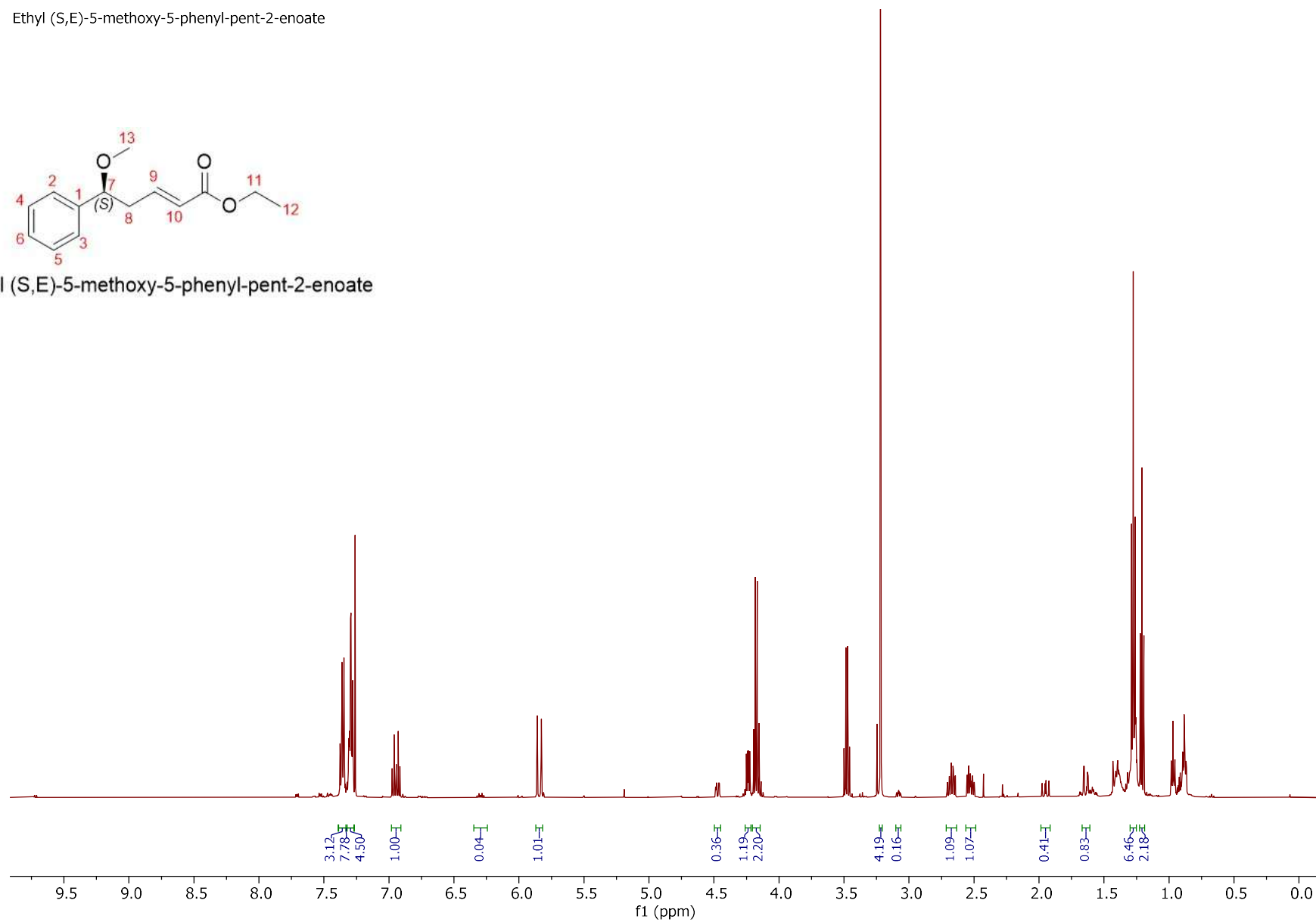


A-C5-14

Ethyl (S,E)-5-methoxy-5-phenyl-pent-2-enoate

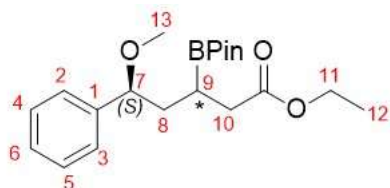


Ethyl (S,E)-5-methoxy-5-phenyl-pent-2-enoate

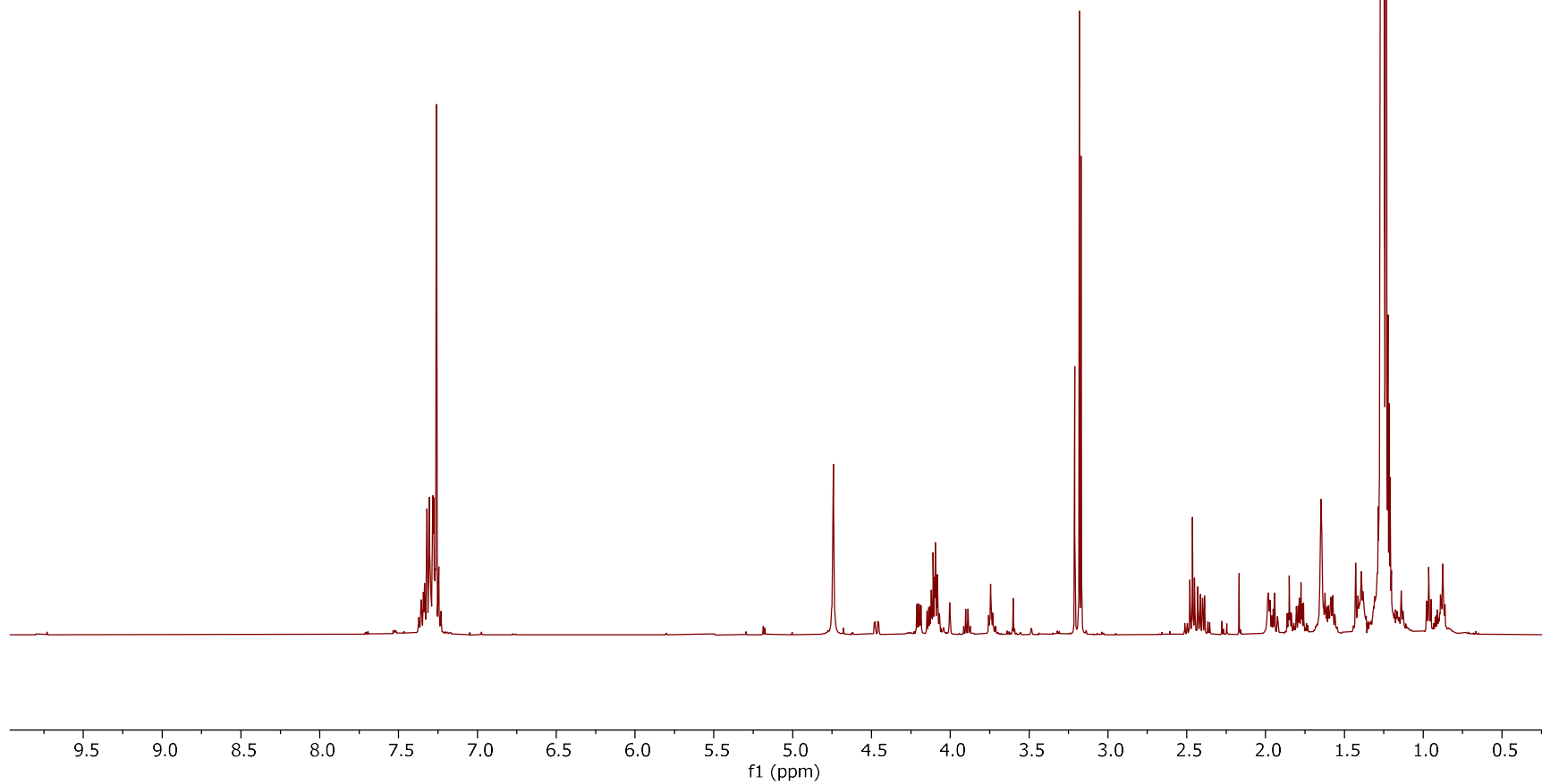


A-C5-16

Boronic ester intermediate for Ethyl 5(S)-methoxy-3(*)-hydroxy-5-phenyl-pentanoate

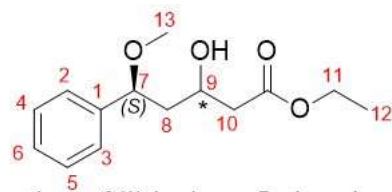


Ethyl 5(S)-methoxy-3(*)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)
5-phenyl-pentanoate

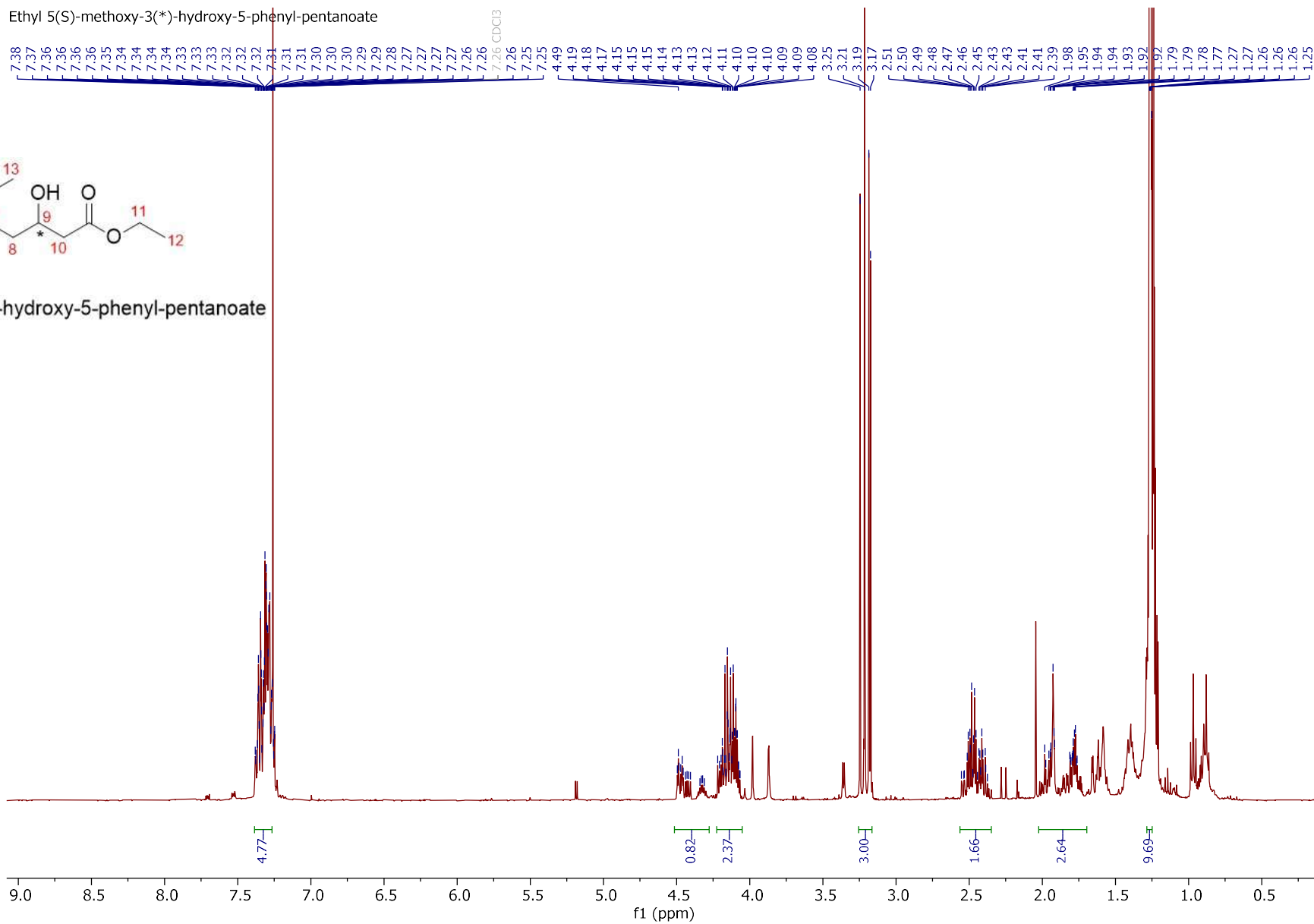


A-C5-18

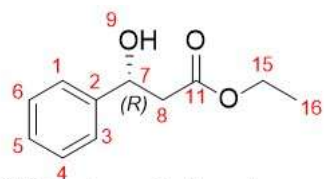
Ethyl 5(S)-methoxy-3(*)-hydroxy-5-phenyl-pentanoate



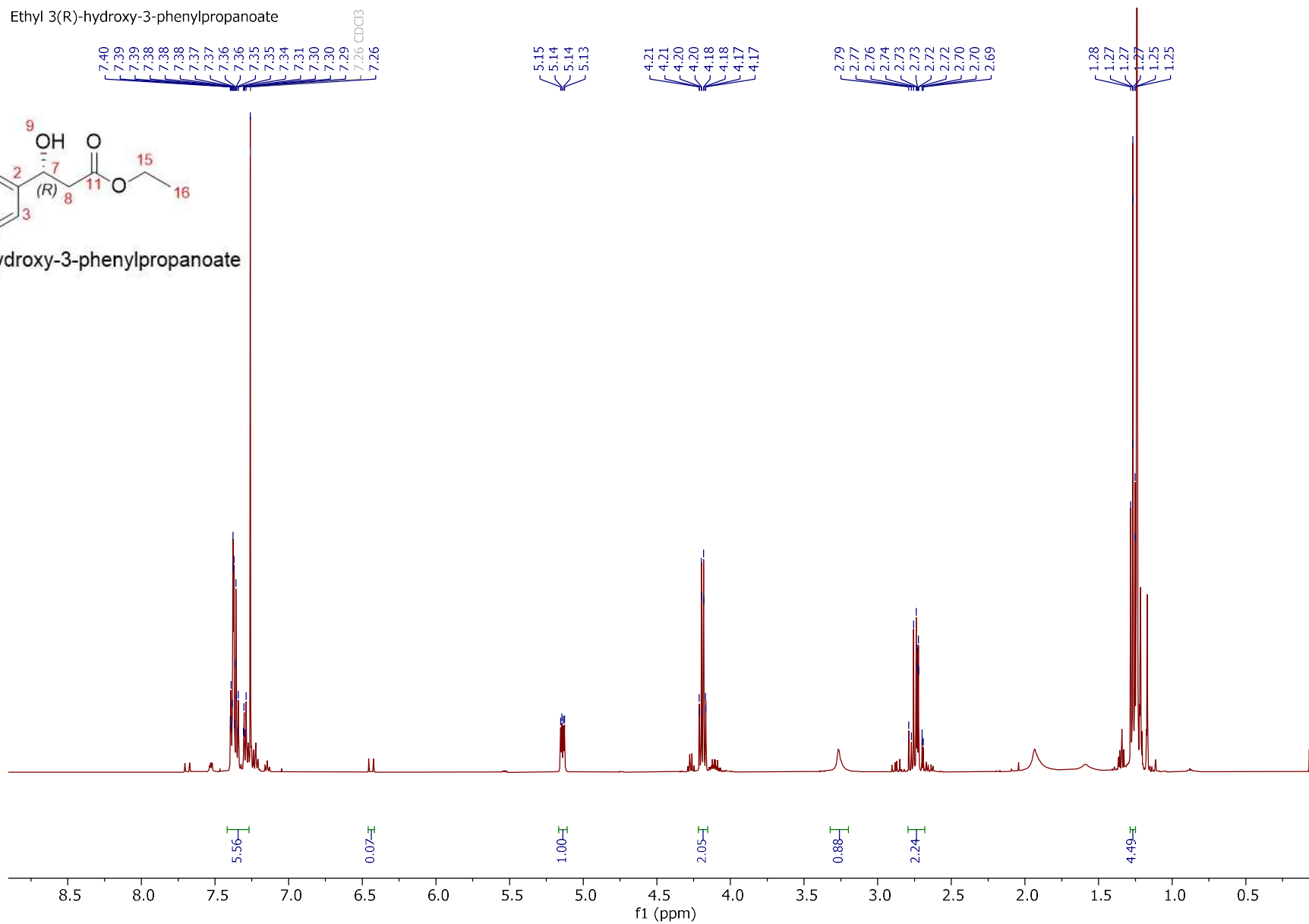
Ethyl 5(S)-methoxy-3(*)-hydroxy-5-phenyl-pentanoate



Ethyl 3(R)-hydroxy-3-phenylpropanoate

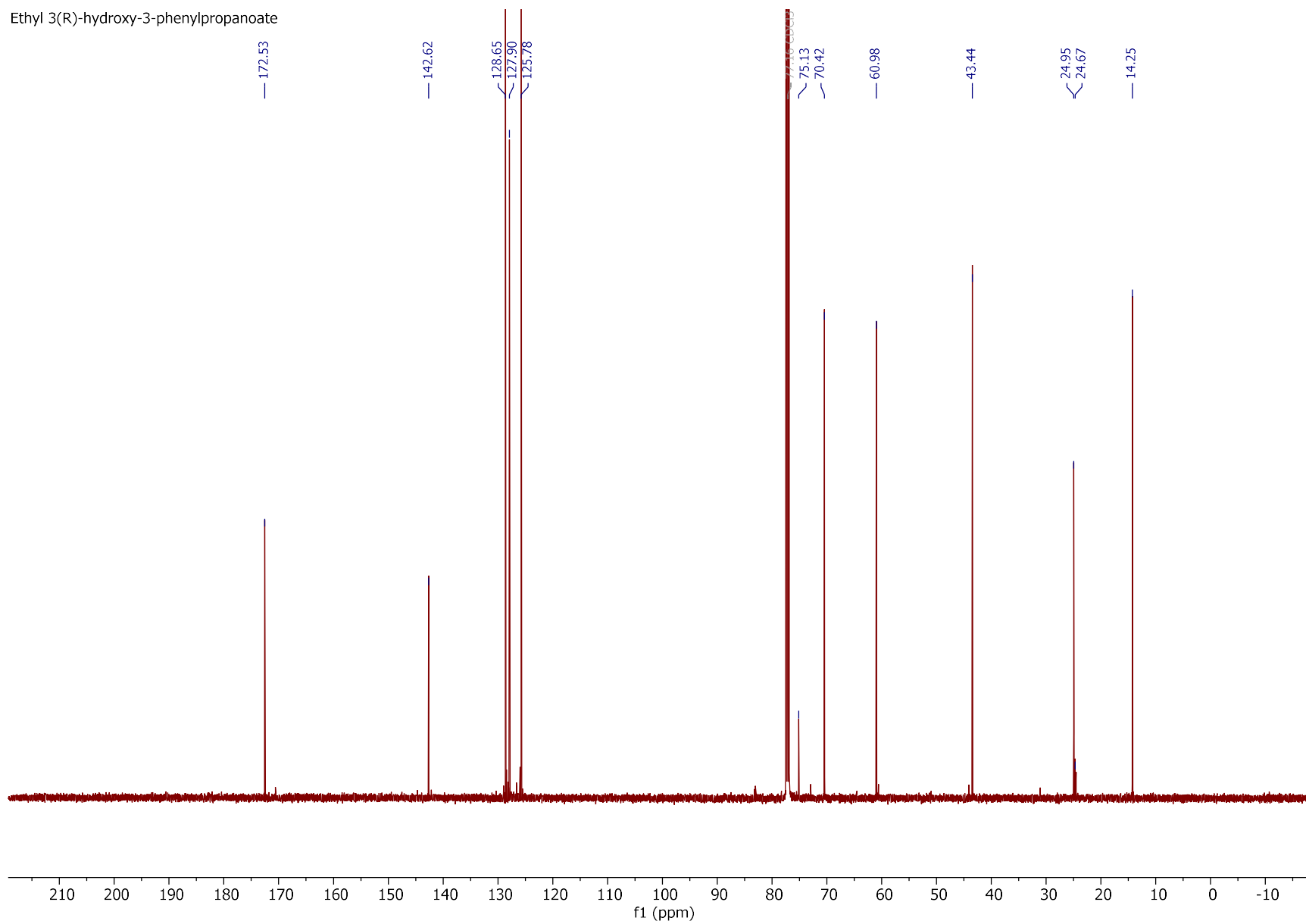


Ethyl 3(R)-hydroxy-3-phenylpropanoate



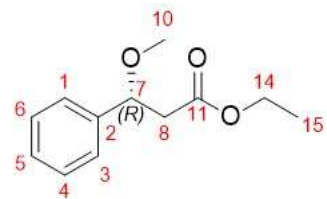
A-C5-20

Ethyl 3(R)-hydroxy-3-phenylpropanoate

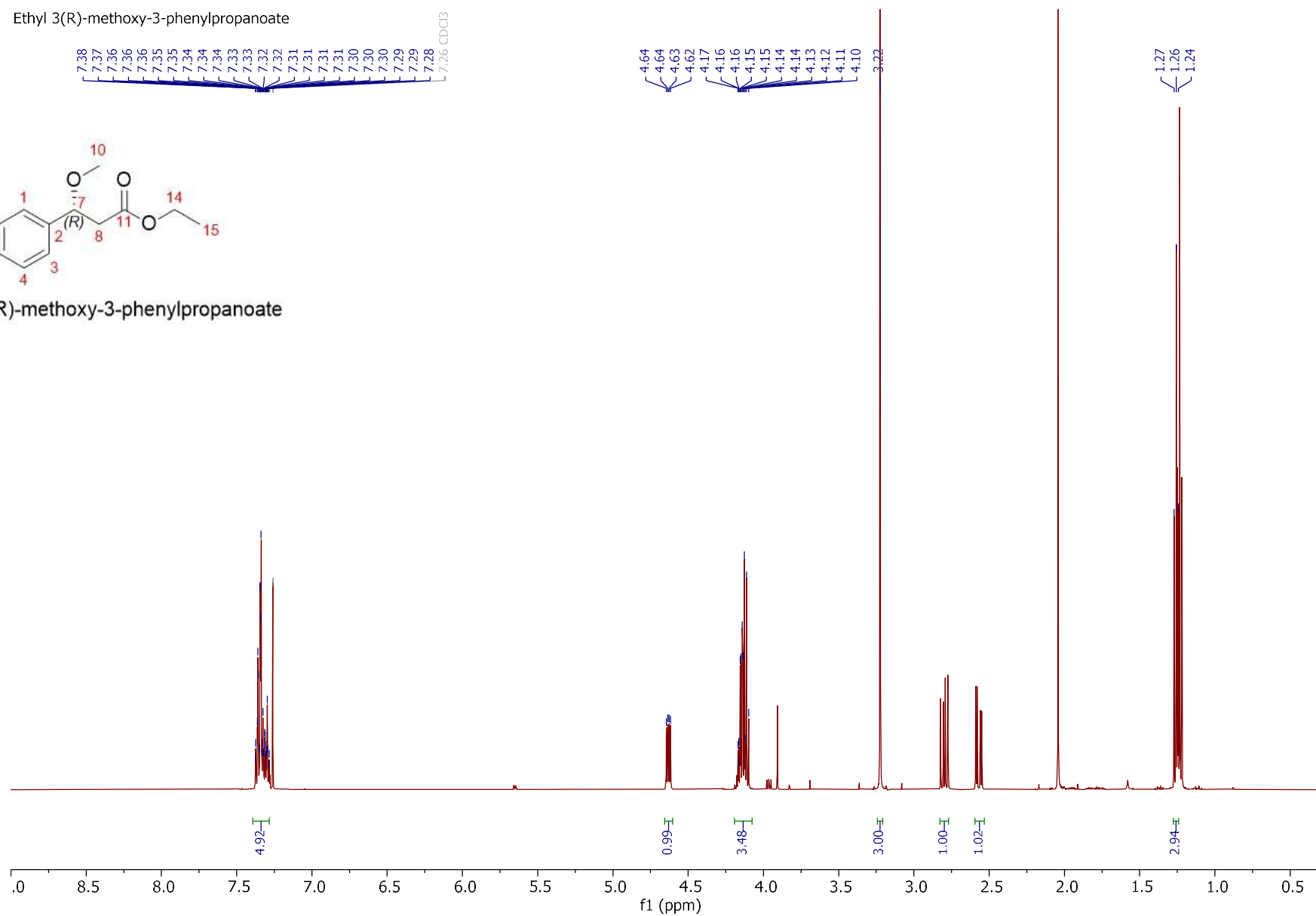


A-C5-21

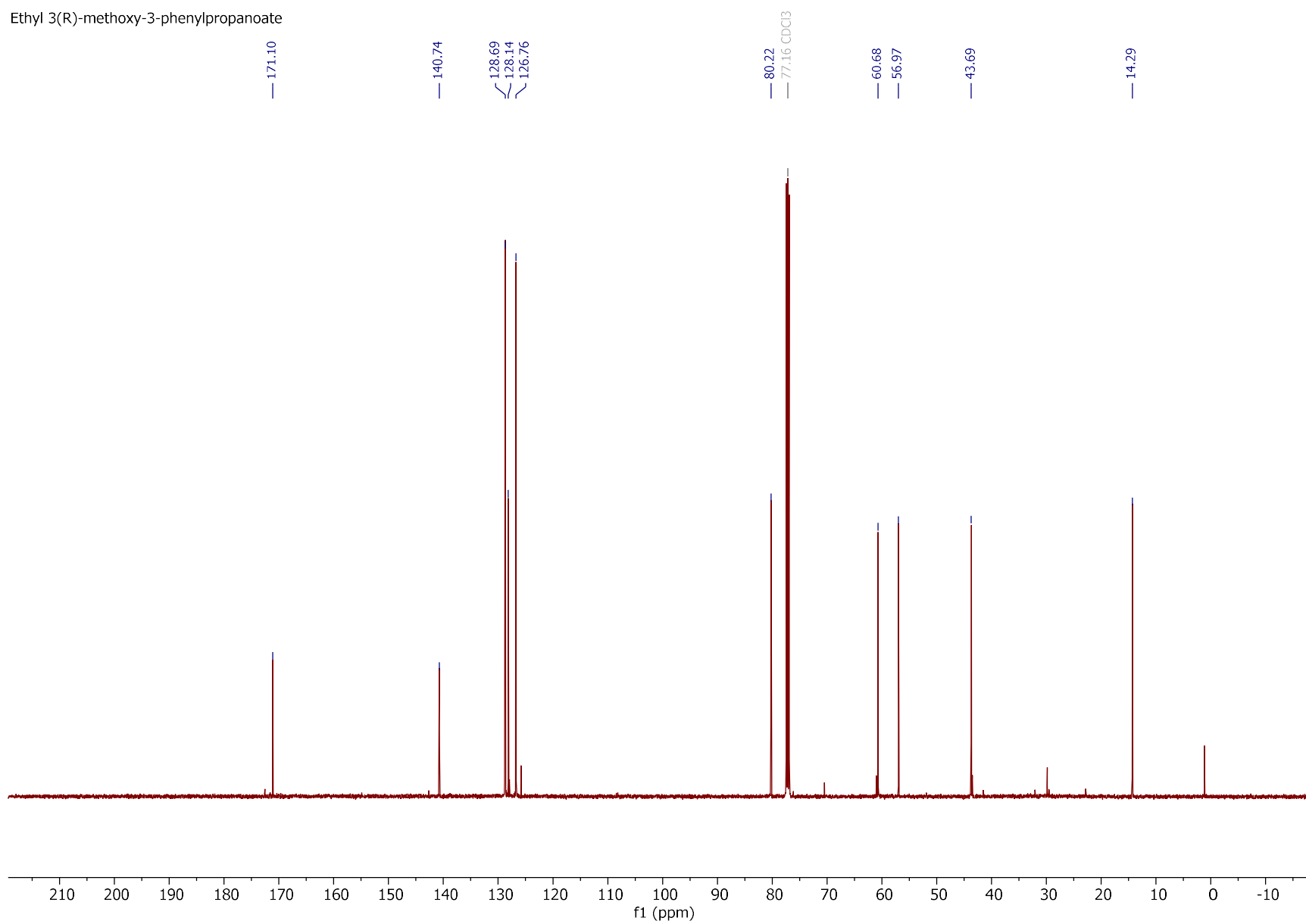
Ethyl 3(R)-methoxy-3-phenylpropanoate



Ethyl 3(R)-methoxy-3-phenylpropanoate

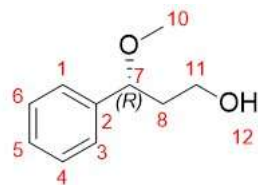


Ethyl 3(R)-methoxy-3-phenylpropanoate

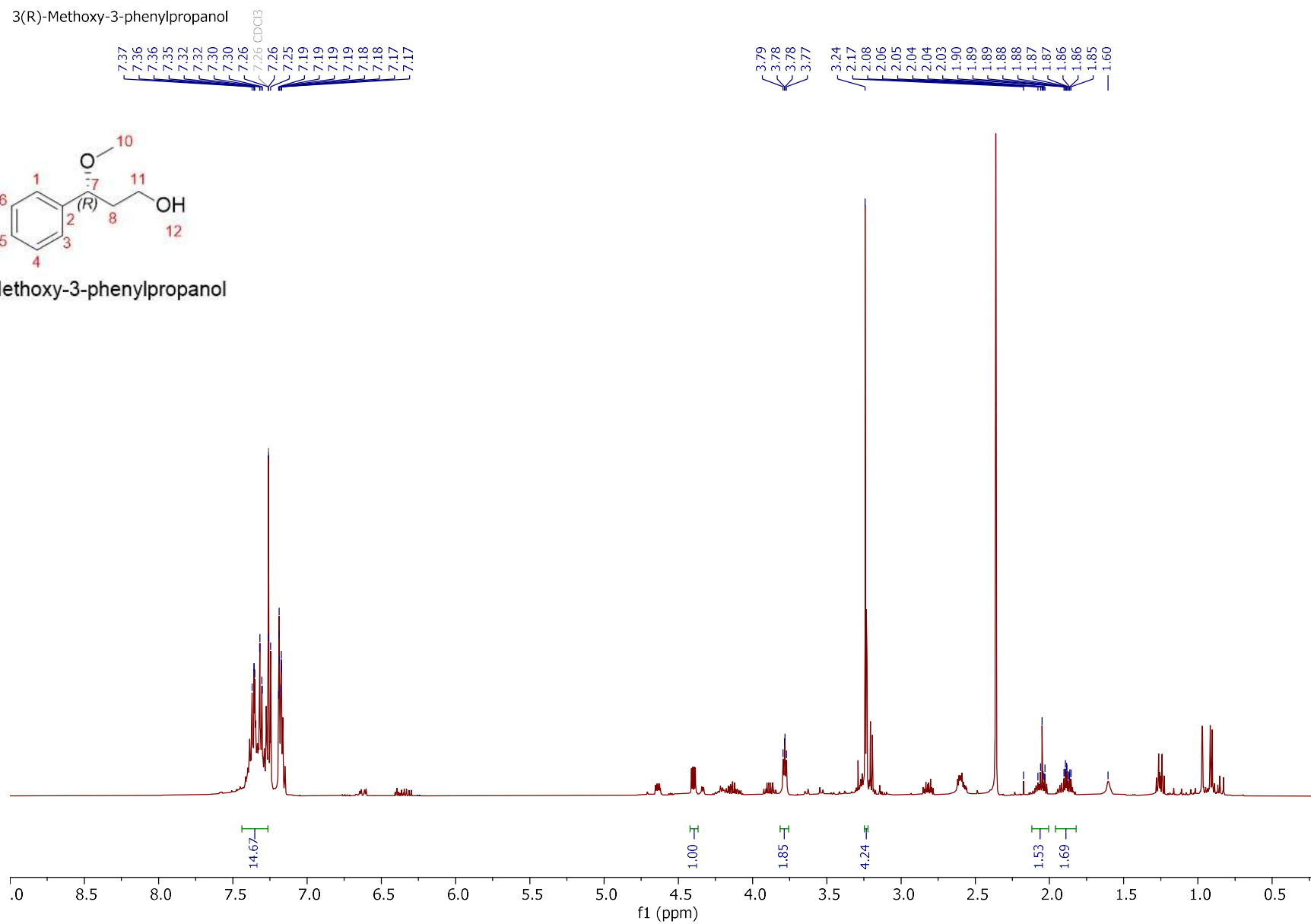


A-C5-23

3(R)-Methoxy-3-phenylpropanol



3(R)-Methoxy-3-phenylpropanol



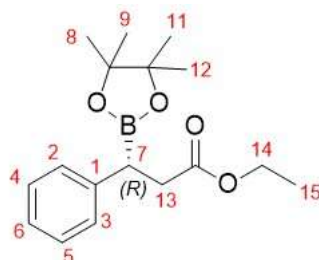
Ethyl 5(S)(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)
5-phenyl-propanoate

7.36
7.27
7.27
7.27
7.26
7.26
7.26
7.25
7.24
7.24
7.24
7.23
7.22
7.22
7.21
7.21
7.20
7.16
7.16
7.15
7.15
7.14
7.14
7.13
7.13

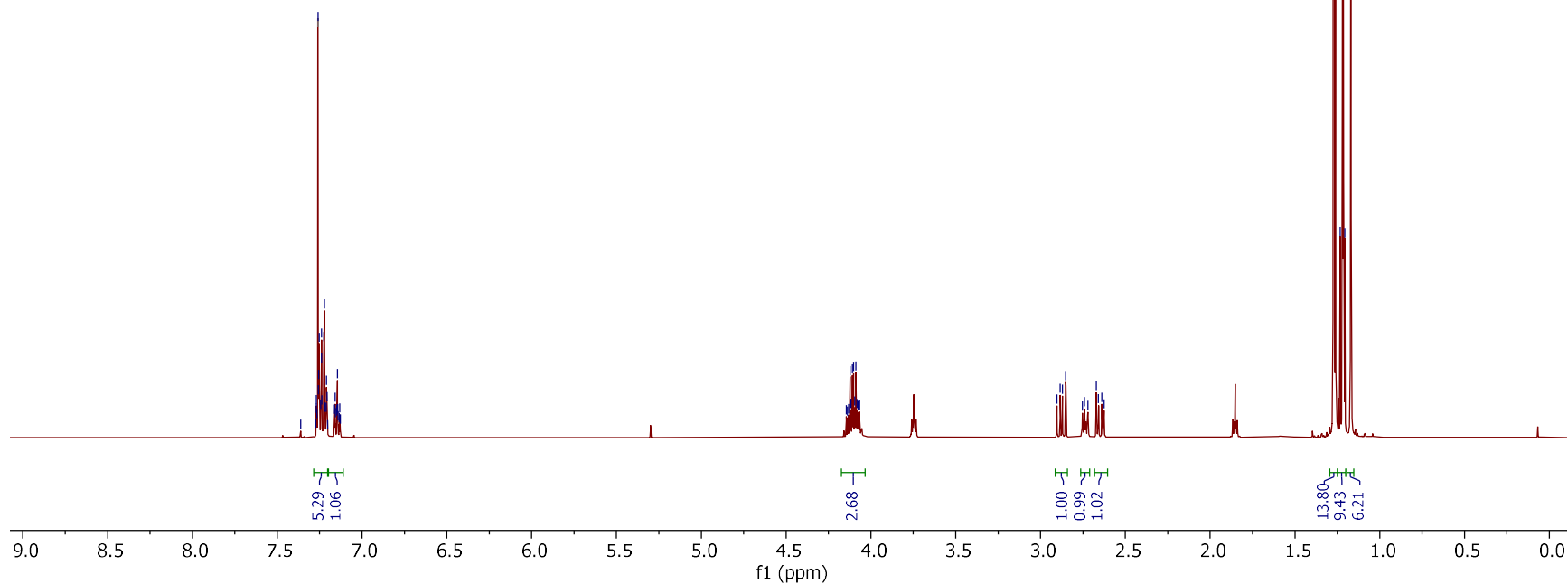
4.14
4.14
4.13
4.12
4.12
4.11
4.10
4.09
4.08
4.07

2.90
2.88
2.87
2.85
2.75
2.74
2.72
2.67
2.66
2.64
2.63

1.23
1.22
1.22
1.21
1.17

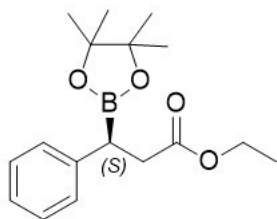


Ethyl 3-phenyl-3(R)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)
propanoate

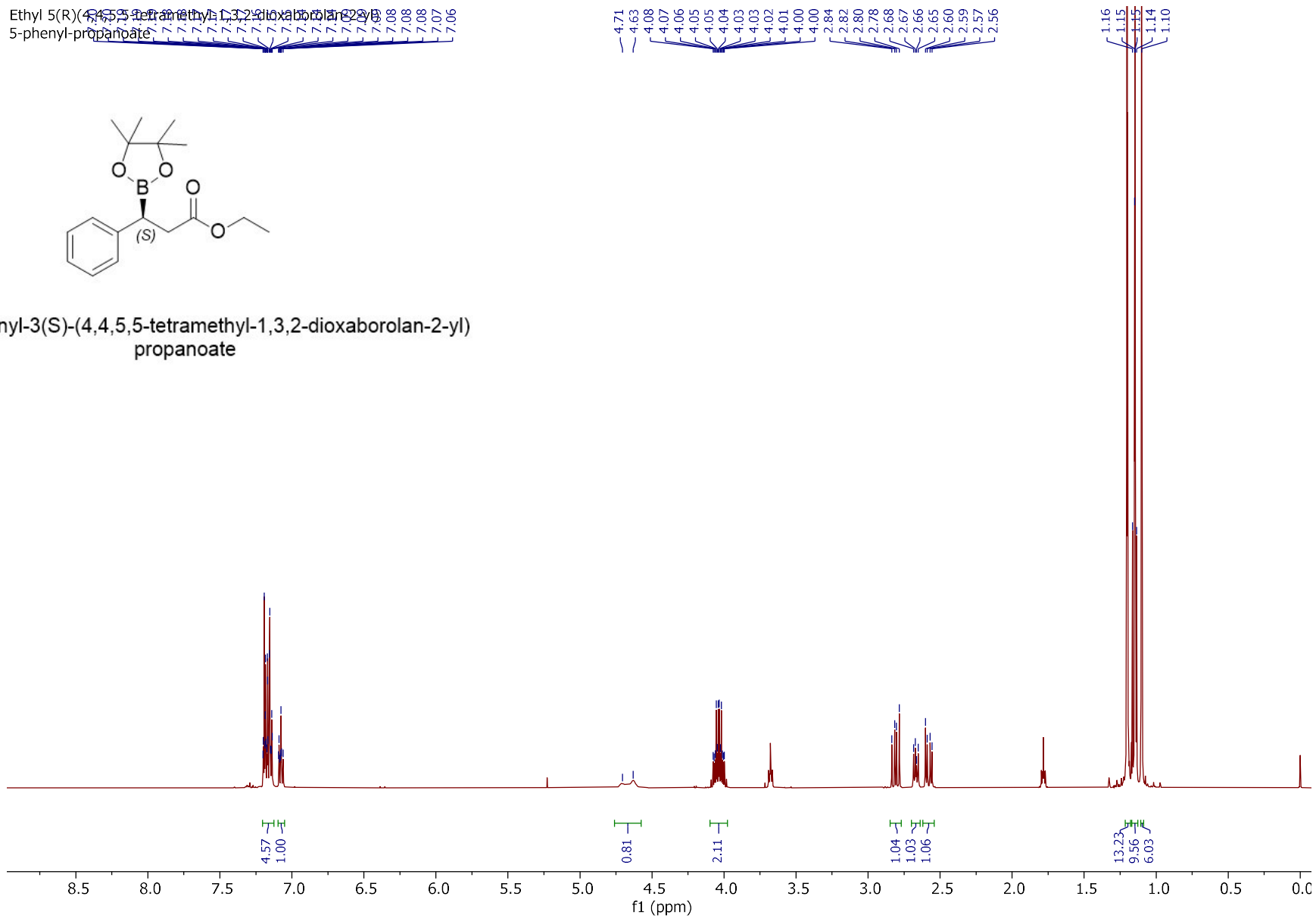


A-C5-25

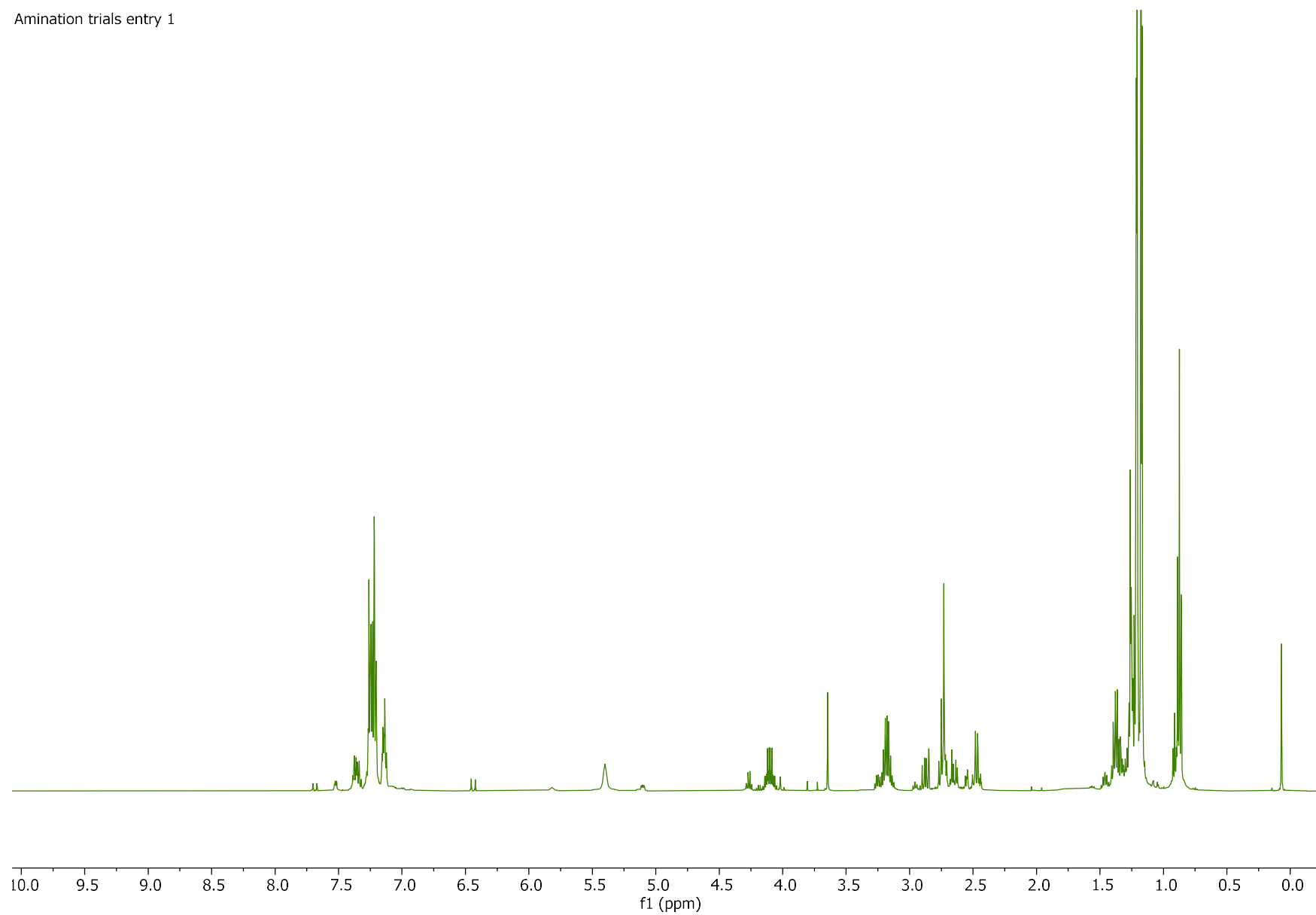
Ethyl 5(R)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-phenyl-propanoate



Ethyl 3-phenyl-3(S)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propanoate

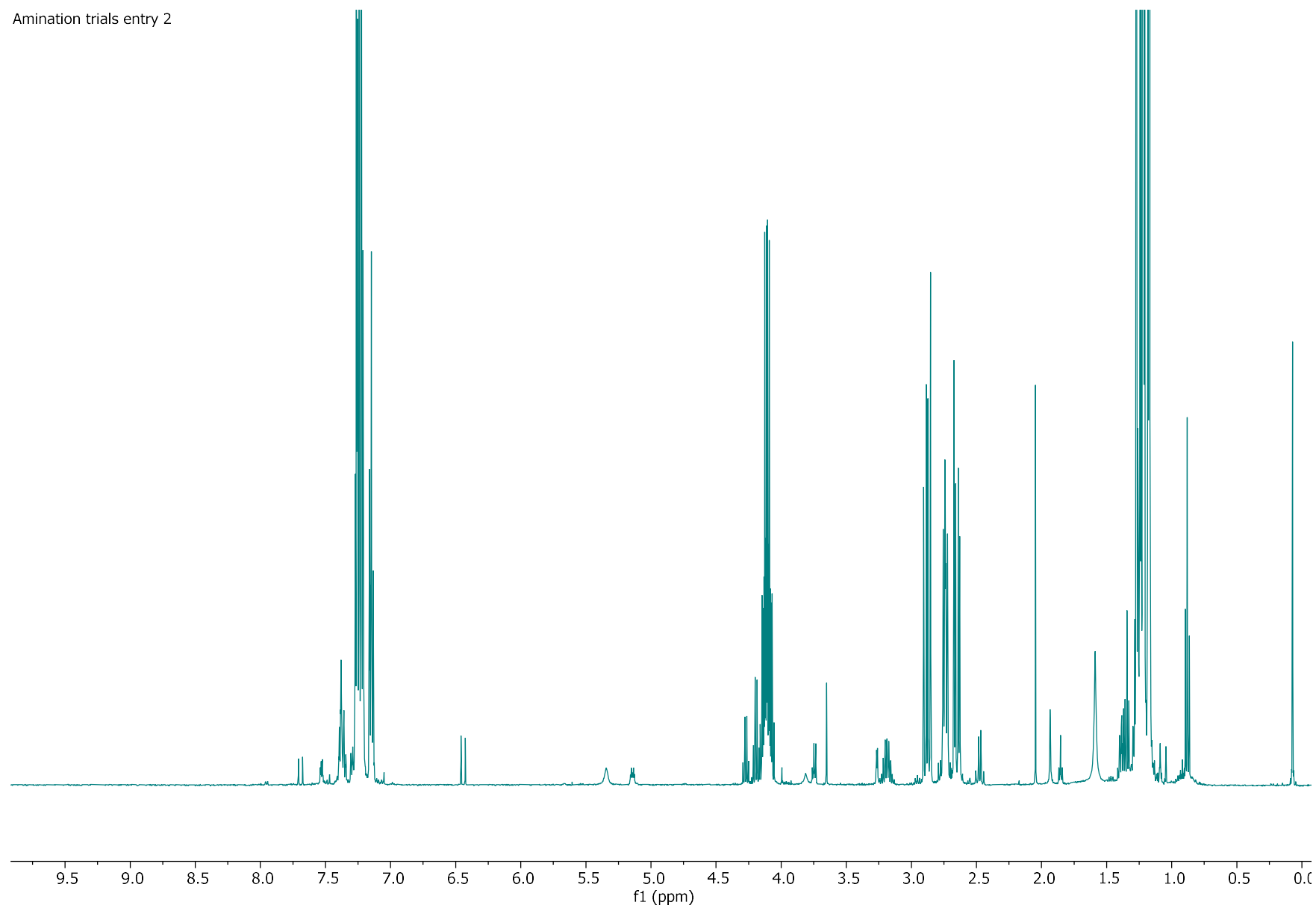


Amination trials entry 1



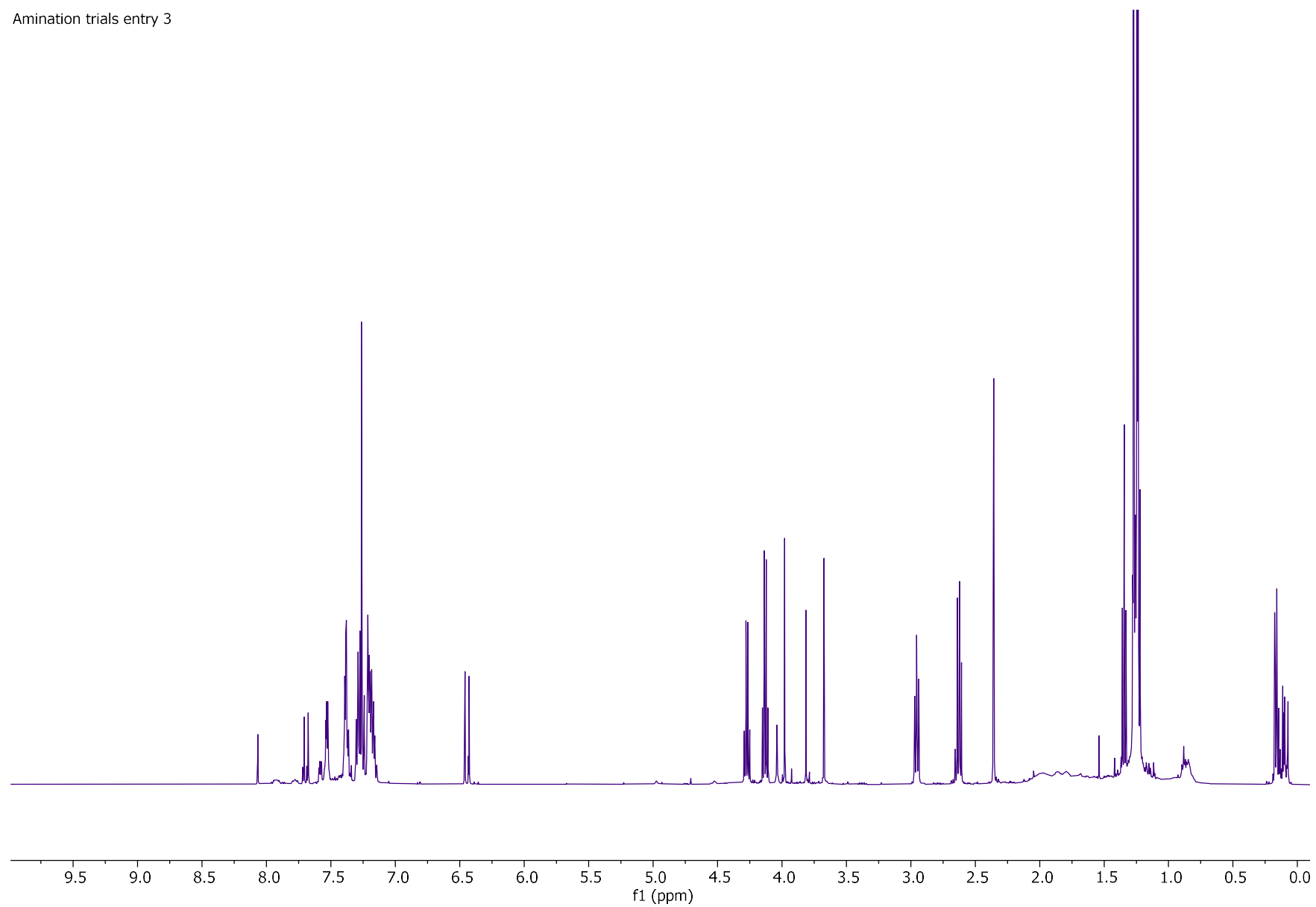
A-C5-27

Amination trials entry 2



A-C5-28

Amination trials entry 3



A-C5-29

