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Solveig Valderhaug

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Doctoral thesis

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Norwegian University of Science and Technology
Thesis for the Degree of
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Abstract

Persistent organic pollutants (POPs) are synthetic chemicals that persist in nature over time and lead to adverse effects towards humans and the environment. Due to their concerning properties, such as persistency, toxicity and bioaccumulation potential, these chemicals must be monitored and restricted to avoid cumulative concentrations in the environment. Consequently, during the Stockholm Convention of 2004, a treaty was signed to reduce or ban the production of POPs. Amongst the currently listed chemicals are a multitude of chlorinated pesticides and insecticides, short-chain chlorinated paraffins (SCCPs, $C_{10\text{-}13}$), polychlorinated biphenyls and perfluorooctanoic acid. After receiving heavy restriction on their production and export, SCCPs were substituted with medium-chain chlorinated paraffins (MCCPs, $C_{14\text{-}17}$) and long-chain chlorinated paraffins (LCCPs, $C_{\geq 18}$). Higher concentrations of MCCPs and LCCPs were subsequently observed in the environment.

In order to enforce the Stockholm Convention, effective methods for monitoring the environmental concentrations of SCCPs are required. Furthermore, monitoring of MCCPs and LCCPs is highly desirable, given their candidate-status for classification as POPs. Analysis of chlorinated paraffins (CPs) is inhibited by both the enormous number of components present in the mixtures and the lack of suitable reference standards.

Through the four publications described herein, we sought to expand the methodology available for the production and analysis of constitutionally defined CPs. Moreover, we sought to synthesise reference standards that were more suitable for CP analysis than those already available. In publications I and II, we described the synthesis of constitutionally defined CP standards, including both non-isotopically enriched reference materials and ^{13}C-labelled internal standards. During publication III we investigated the stereochemistry of the isomer mixture obtained after dichlorination of alkenes. Finally, publication IV encompassed one novel method to calculate the chlorine percentage of both complex and single-chain mixtures, as well as two further methods for calculating the chlorine percentage of single-chain mixtures, inspired by a pre-existing model.

Sammendrag

Persistent organic pollutants (POPs) er en fellesbetegnelse for syntetiske kjemikalier som vedvarer i naturen over lengre perioder og som har uheldige konsekvenser for både mennesker og miljø. Dette er kjemikalier hvor konsentrasjoner i miljøet må overvåkes fordi de har en lang nedbrytningstid som medfører bioakkumulering, samt at de er giftige ved høye nivåer. Stockholmkonvensjonen om POPs ble dermed undertegnet i 2004, slik at produksjon og distribuering av disse kjemikaliene kunne begrenses eller forbys. Blant de listede kjemikaliene finner vi flere forskjellige klorinerte plantevernmidler og insektmidler, short-chain klorinerte parafiner (SCCPs, $C_{10\text{-}13}$), polyklorinert bifenyl og perfluorinert oktansyre. Etter at SCCPs undergikk strenge internasjonale begrensninger på produksjon og distribuering har industrien skiftet disse ut med medium-chain klorinerte parafiner (MCCPs, $C_{14\text{-}17}$) og long-chain klorinerte parafiner (LCCPs, $C_{\geq 18}$). Dette har medført at man observerer høyere konsentrasjoner av disse typene klorinerte parafiner (CPs) i miljøet.

For å håndheve målene som ble satt i Stockholmkonvensjonen er det nødvendig å ha gode metoder for å måle miljøkonsentrasjonene av SCCPs. Videre er det også nødvendig å overvåke konsentrasjonene av MCCPs og LCCPs, siden de har fått kandidatstatus som POPs. Konsentrasjonsanalyse av CPs er derimot en vanskelig oppgave, siden disse blandingene består av en enorm mengde forbindelser og det finnes et begrenset antall egnede referansematerialer.

Gjennom de fire publikasjonene som er beskrevet i denne avhandlingen ønsker vi å utvide den tilgjengelige metodologien for produksjon og analyse av konstitusjonsdefinerte CPs. Videre er det ønsket å tilby en rekke referansestandarder for CP-analyse som er mer egnet enn de som allerede eksisterer i markedet. I publikasjon I og II er syntese av konstitusjonsdefinerte CPs beskrevet, og inkluderer både forbindelser som ikke er isotopmerket og forbindelser som er ^{13}C isotop merket. Stereokjemien for isomerforbindelsene som oppnås ved diklorinering av alkener undersøkes i publikasjon III. I publikasjon IV blir en ny metode for å beregne klorprosenten av komplekse CP-blandinger og blandinger bestående av en bestemt kjedelenge presentert. Videre blir to andre metoder for klorprosentberegning for blandinger av en bestemt kjedelengde beskrevet, hvor begge metodene var inspirert av en tidligere beregningsmodell.

Contents

List of Publications

Publications included in this thesis

I. S. Valderhaug, H. Liu, A. Gorovoy, J. E. Johansen, O. R. Gautun

Synthesis of constitutionally defined chlorinated paraffins as reference standards

Submitted to Results in Chemistry

Contribution: Synthesis and characterisation of all hexachloroalkanes and octachlorotetradecane. Paper drafting, review and editing.

II. (patent) A. Gorovoy, H. Liu, J. Tůma, **S. Valderhaug**, Jonatan Nygren, J. E. Johansen

^{13}C-Labelled chlorinated paraffins and their preparation

Submitted to European Patent Office (Appl. No. EP22162109.7)

Contribution: Chlorination, purification and characterisation of seven ^{13}C-labelled chlorinated paraffins and preparation of Wittig reagents.

III. S. Valderhaug, N. Paškanová, J. Tůma, J. Herciková, V. Eigner, H. Liu, A. Gorovoy, J. E. Johansen, O. R. Gautun

Synthesis, identification, chiral separation and crystal structure of (3R,4R,7S,8S)-3,4,7,8-tetrachlorodecane and its stereoisomers

Submitted to Journal of Molecular Structure

Contribution: Synthesis, purification and crystal growth, as well as gas chromatography and NMR analysis. Paper drafting, review and editing.

IV. S. Valderhaug, H. Liu, A. Gorovoy, J. E. Johansen, L. van Mourik, J. de Boer, O. R. Gautun

Nuclear magnetic resonance as a tool to determine chlorine percentage of chlorinated paraffin mixtures.

Chemosphere **308** 136312 (2022)

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Contribution: Creation of new chlorine percentage calculation method and the two modified methods, NMR processing and analysis, titrations, calculations, validation, accreditation. Paper drafting, review and editing.

Abbreviations and symbols

σ	Standard deviation
μ	Average value
Ac	Acetyl
ANSI	American national standards institute
Atm	Atmosphere
CP	Chlorinated paraffin
DCM	Dichloromethane
DCVC	Dry-column vacuum chromatography
DMSO	Dimethyl sulfoxide
ECNI	Electron capture negative ionisation
EI	Electron impact
ES	Electrospray
Et	Ethyl
FCC	Flash column chromatography
GC	Gas chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IR	Infrared (spectroscopy)
k	Coverage factor for normal distribution
LCCP	Long-chain chlorinated paraffin
MCCP	Medium-chain chlorinated paraffin
M_{Cl}	Elemental mass of chlorine
Me	Methyl
MS	Mass spectrometry
MSD	Mass spectrometry detector
$M_{w,CP}$	Molecular weight of chlorinated paraffin
N	Number of experiments (titrations)
n_{Cl}	Number of chlorines
NCS	*N*-Chlorosuccinimide
NMR	Nuclear magnetic resonance (spectroscopy)
PCA	Polychlorinated alkane
POP	Persistent organic pollutant
Pr	Propyl
SCCP	Short-chain chlorinated paraffin
TBAF	Tetra-*n*-butylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TCICA	Trichloroisocyanuric acid
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
u	Standard uncertainty
U	Expanded uncertainty
UV	Ultraviolet
WCOT	Wall-coated open tubular
$wt\%\ Cl$	Weight percentage of chlorines

Preface

This doctoral thesis was written as a part of the degree *Philosophiae Doctor* (PhD) within chemistry at the Norwegian University of Science and Technology (NTNU). The project was part of an industrial PhD and was conducted in collaboration with Chiron AS. It was written as a collection of articles, including one published article, two submitted articles and one patent. Prior to the discussion of these publications comes an introductory text on the issue of chlorinated paraffin (CP) analysis, the requirement for standards and the background of the chemistry used in the synthesis of new standards. All publications are included as appendices at the end of this thesis. The main supervisor of the project was Ass. Prof. Odd Reidar Gautun (NTNU) and the co-supervisor was Ass. Prof. Huiling Liu (Chiron AS, NTNU).

Aims of the Thesis

The main objective of this thesis was to synthesise new constitutionally defined chlorinated paraffins (CPs) to be marketed as standards by Chiron AS. Included in this objective was finding synthetic methodologies that could be used to synthesise a broad range of CPs, with different chain lengths, number of chlorines and different chlorine substitution patterns. Furthermore, in the synthesis of ^{13}C-labelled CPs, finding methodologies that start from simple starting materials were necessary due to the limited commercial availability of ^{13}C-labelled substrates. All syntheses should also be economically viable (e.g. limited number of steps, affordable substrates), since these products will serve a commercial purpose.

The synthetic methodology can be summarised in three points:

- Synthesis of polyenes with defined chain length and positions of double bonds
- Dichlorination of alkenes (achieved by different chlorination techniques)
- Purification of the final CP products to reach a minimum purity of 98% by mole.

A further goal was to improve or find analytical techniques that could be used to properly define the product distributions of complex CP mixtures, specifically within the field of nuclear magnetic resonance spectroscopy.

1 Introduction

In the introductory section, a short background on chlorinated paraffins (CPs) and standards, scope and strategy of the products and the fundamental chemistry from the synthesis will be described. The intent of this structure is to give the reader an understanding of why more and new types of standards for CPs are needed, and how we intended to obtain these products. Through the next chapters, the different publications will be discussed and will show the achieved results of the PhD project.

1.1 Background

After more than four decades of research, there is still a need for greater understanding of the fate, levels, and toxicity of CPs in the environment.[1-2] One of the main factors that impede progress in this area is an inadequate repertoire of suitable reference standards for analysis.[3] More and improved standards would be beneficial for everything from analytical method validation to targeted research on specific compositions of CPs.

CPs were originally defined, in the chemical industry, as complex mixtures of polychlorinated *n*-alkanes (PCAs).[4] Later, the name has been incorporated to describe everything from enantiomerically pure chlorinated alkanes to complex mixtures found as contaminants in the environment. CP mixtures have a chlorination degree typically between 40 to 70% and a chain length ranging from C_{6-38}.[5-6]

The industrial production of CPs began during the 1920s and has expanded since then.[2] The range of utility of CPs in various industrial applications stems from their large span of physicochemical properties and thermal stability. Among their listed industrial purposes are high-temperature, high-pressure lubricants, flame retardants and additives used in the paint and rubber industry.[7] Consequently, CPs have, over the years, been produced in enormous volume and are considered to be one of the world's most industrially-produced chemicals.[3] That being said, precise records of the scale of their industrial production are scarce. In 1999, an estimated global production of 300,000 tonnes of CPs was reported.[8] Later reports have suggested an annual production of about 1,000,000 tonnes from China alone.[9] The large, open-ended production of CPs, coupled with their environmental persistence, has led to substantial pollution. Furthermore, they have been observed in a multitude of different environmental matrices, including human and animal tissue.[3]

1.1.1 Regulations and the Stockholm convention

Short-chain CPs (SCCP, C_{10-13}) is one of the compound classes that falls under the persistent organic pollutant (POP) definition.[10] The POP definition was first established in 2004, during the Stockholm convention.[11] The Stockholm convention on POPs sought to apply international control measures to compounds showing properties such as persistency, toxicity, bioaccumulation and long-range transport. Part of the control measures was to prohibit, or considerably restrict, the production and usage of POPs, as well as to restrict export and import of these compounds. A significant part of the problem with POPs is their resistance to natural processes such as photolytic, chemical and biological degradation. Thus, they remain unchanged in the environment for long periods of time.

The compounds listed under the original Stockholm treaty were:[11]

- Eight different pesticides or insecticides (e.g. Aldrin, Chlordane),
- Polychlorinated biphenyls (PCBs) and hexachlorobenzene,
- Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.

SCCPs were not added until the 2017 amendment. As a result of the heavy restrictions placed on SCCPs, production shifted towards medium-chain CPs (MCCPs, $C_{14\text{-}17}$) and long-chain CPs (LCCPs, $C_{\geq 18}$). Subsequently, higher concentrations of both MCCPs and LCCPs were observed in the environment,[12] which is concerning due their shared property of lipophilicity.

High lipophilicity can cause a higher uptake- than excretion-rate in organisms.[13] Thus, bioaccumulation of these chemicals is observed and can reach concentrations that may be harmful for the organism. Furthermore, the concentration in lower trophic species is transferred to predators in higher levels of the food-chain.

To be able to put the Stockholm convention treaty into action, it is important to have a thorough monitoring process and routine controls. For CPs, this has proved to be 'easier said than done' and part of this problem is presented in the following sections (Section 1.1.2 and 1.1.3), with a focus on the necessity of CP standards.

1.1.2 CP production: the root of complexity

The analysis of chlorinated paraffins is challenging due to the inherent complexity of the mixtures, as the number of possible isomers may span thousands to hundreds of thousands, depending on the chain length.[4] The number of possible isomers in SCCP mixtures ($C_{10\text{-}13}$) is estimated to be around 6-10,000.[4,6] Expectedly, the case is more complex for MCCPs ($C_{14\text{-}17}$) and LCCPs ($C_{\geq 18}$) and even more so for environmental samples (mixtures of all chain lengths). With the vast number of compounds present in complex CP mixtures, the result from gas chromatography (GC) shows unresolvable broad bands of signals from co-eluting compounds (Figure 1.1.).

The complexity of CPs arises from their industrial production. They are typically produced by direct chlorination of alkane feedstocks (predominantly *n*-alkane isomers) from the petroleum industry.[4-5] Chlorine gas is bubbled through a mixture of paraffins at elevated temperatures and UV-light or catalysts may be used to promote radical chlorination.[14] Manipulation of UV-light intensity, temperature and chlorine gas flow rate will affect the resulting product distribution, allowing the preparation of a product with the desired properties for its industrial purpose. An example of the production plant is shown in Figure 1.1.

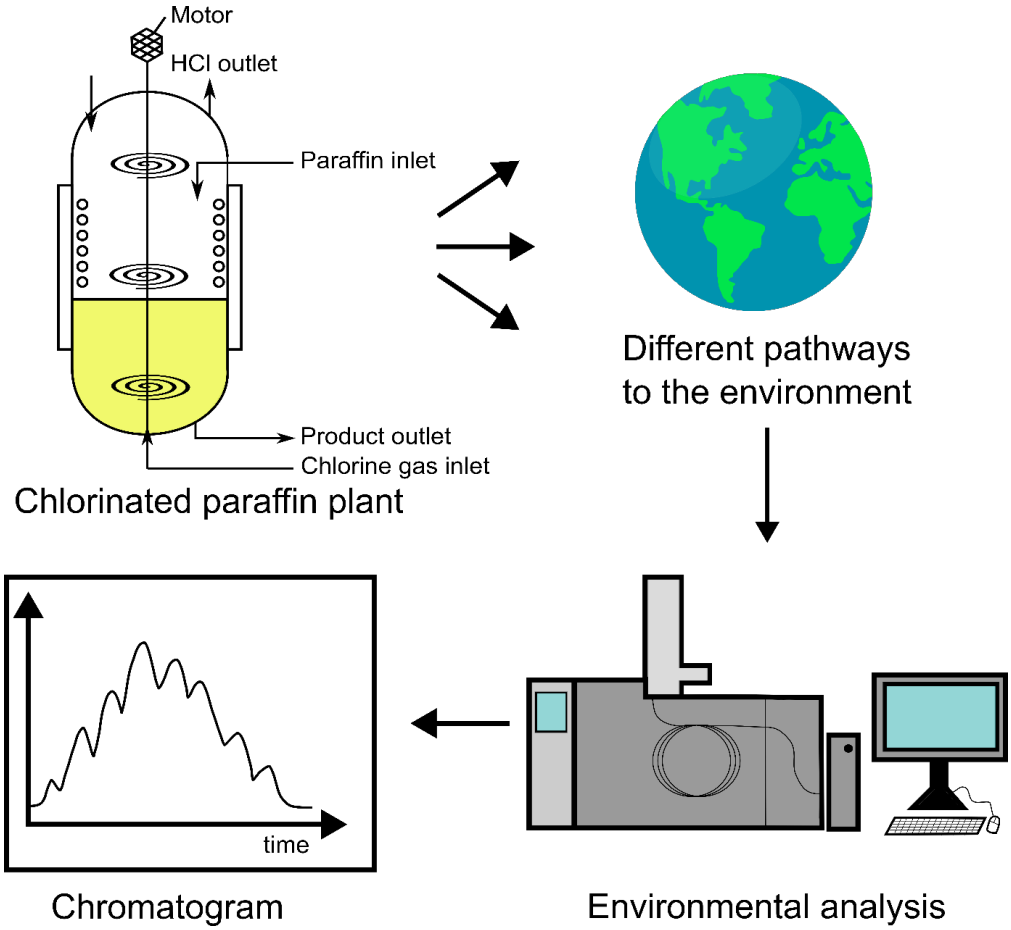


Figure 1.1. Simplified production plant for CPs,[14] which through different exposure pathways (transport, disposal, etc.) ends up in the environment. When analysing complex CP mixtures the typical one-dimensional chromatograms appear as broad unresolved bands, due to the many co-eluting compounds.

The radical chlorination method is not very selective, due to the high reaction rate of propagation and results in a mixture of polychlorinated alkanes with many different substitution patterns.[15] Nevertheless, some selectivity has been observed for radical chlorination, for example a higher relative rate of substitution on secondary over primary carbons, due to radical stability,[16] and effects from previously attached chlorine substituents on the chain propagation process.[17] A chlorine substituent deactivates geminal and vicinal sites for further substitution, likely due to both steric and electronic effects.

1.1.3 The need for new reference standards

When characterising and quantifying CPs in different environmental settings, different reference materials and standards have been used and tested by analytical researchers.[3] Schinkel *et al.* divided the different types of CP standards into four groups:[18]

1. Complex CP mixtures: Industrial/technical mixtures with variable chain length (e.g. 60 wt.%Cl, MCCP)
2. Single-chain CP mixtures: Specific chain length, variable number and position of chlorines (e.g. 60 wt.%Cl, C_{12})
3. Constitutionally defined CPs: Specific chain length, specific number and position of chlorines
4. Isotopically-labelled constitutionally defined CPs

Complex and single-chain CP mixtures

Both complex mixtures and single-chain mixtures have been readily used as quantification standards.[18] Large variation of quantitative measurements have been observed during inter-laboratory studies of the same sample, especially when the participants have utilised different standards.[19-20] With more sophisticated analytical tools available and extended knowledge on CP analysis, the consistency of the quantification results have improved over the last decades.[3] Nevertheless, general laboratories may not have specialist methods available and a good intercomparison is important for more accurate monitoring.[1] Availability of certified matrix reference materials would help to further improve the accuracy of quantification, yet this requires a suitable standard mixture with a consensus amongst laboratories on quantity of CPs.

The most widely applied method of analysis is GC electron capture negative ionisation (ECNI),[1] which relies on monitoring $[M\text{-}Cl]^-$ ions.[4] The high selectivity and sensitivity of this detector makes it popular for CP analysis. It has been observed, for this type of detector, that the response factor is dependent on the chlorination degree.[21] Mixtures with higher chlorine content generally give a higher response and *vice versa*. Additionally, interferences from compounds of the same nominal mass (e.g. other CPs with five more carbons and two fewer chlorines) and matrix effects have been reported.[6,21]

Using a standard that resembles the analyte to a high degree would improve the accuracy of the quantification, yet the composition of CPs in the analyte can differ depending on a multitude of factors (which formulas are manufactured, physicochemical properties and more).[7,22] Since this would require a massive library of complex or single-chain standards, researchers have instead developed methods to account for the difference in response factors. For example, Yuan *et al.*[20] have introduced the concept of congener (C_nCl_m) specific response factors for SCCPs which can be used for the quantification of SCCPs with a fixed number of carbons and chlorines. A different option was reported by Bogdal *et al.*[23] where an algorithm deconvolves the pattern of the CP analytes into a linear combination of the quantification standards. Thus, a combination of standards that best mimic the analyte can be determined. Both of these methods are reliant on having a broad range of standards available for improved accuracy, but the market is inadequate when it comes to standards for MCCPs and LCCPs.[18]

Constitutionally defined CPs

Constitutionally defined CPs have the advantage that their purity can be easily assessed, compared to both complex and single-chain mixtures.[18] High purity of standards is an important feature when it comes to determining a chemical's fate, especially for testing toxicity and biotransformation. Furthermore, they can be used as research chemicals to test different compositional behaviours and compare them to the overall mixture. Fernandes *et al.* tested some constitutionally defined CPs on different detector systems.[3] Their work compared 1,1,1,3,11,12-hexachlorododecane and 1,2,5,6,9,10-hexachlorododecane, which have the same chain length and number of chlorines, however the latter showed a much higher response in general. This indicates that the structure of the individual CPs may also play an important role to the quantitative analysis. Having more such standards available would help unravel further information about the complex CP mixtures. Moreover, availability of constitutionally defined CPs would aid future research within mechanistical, transformation and toxicity studies and could help in the assessment of whether specific CPs are more persistent and toxic than others.

Isotope-labelled standards

The final type of standard that will be discussed is the stable isotope labelled standards.[18] Isotopically labelled standards are used as internal and recovery standards. For a higher accuracy of the recovery rate, it is important to choose standards that behave similarly during the extraction process yet can be distinguished analytically from the compounds in the sample. These properties are often achieved by using an isotope-labelled version of the parent compound, often with ^{2}H, ^{13}C, ^{15}O or ^{18}O replacements.[24] While ^{2}H labelled compounds are generally less expensive to prepare, the deuterium isotope effect induces an undesired higher degree of lipophilicity for these compounds compared to the native (not isotopically enriched) material.[25] Thus, ^{13}C-labelled corresponding compounds are often preferred, since replacing ^{12}C with ^{13}C does not change the physicochemical properties substantially, yet their higher mass allows them to be distinguished from the native sample.[24]

In environmental CP studies, ^{13}C-labelled compounds of non-CPs have often been used as internal standards, such as [$^{13}C_6$]-hexachlorobenzene, [$^{13}C_{10}$]-*trans*-chlordane and [$^{13}C_{10}$]-mirex.[18] Two ^{13}C-labelled CP standards were commercially available prior to this project from Cambridge Isotope Laboratories, Inc.: [$^{13}C_{10}$]-1,5,5,6,6,10-hexachlorodecane and [$^{13}C_{12}$]-1,1,1,3,10,12,12,12-octachlorododecane, however both contained geminal substitution of chlorine, moieties not commonly encountered in industrial CP mixtures.

1.2 Structural considerations for the synthesis of constitutionally defined CP congeners

Since complex CP mixtures span so many compounds, knowing which specific constitutionally defined compounds to synthesise is not straightforward. Several commercially-available, constitutionally defined CPs contain geminal chlorination and a high degree of terminal chlorines. These moieties are disfavoured by the industrial process used for CP synthesis,[18] with geminal chlorination being mainly found in CP mixtures with a very high chlorination degree.[26] It is desirable to produce a set of constitutionally defined CPs that are more representative of the compounds in the environment, yet not much specific literature is published on the subject.

Some structural insights on CP mixtures, predicted or observed, are listed below:

1. Geminal chlorination is less likely to occur and is mainly seen in mixtures with a high degree of chlorination.[26]
2. Terminal chlorines are less likely found in mixtures of lower chlorination degrees.[17,26]
3. High prevalence of chlorination at the third position of the chain.[17]
4. Preference for even substitution along the chain.[3]

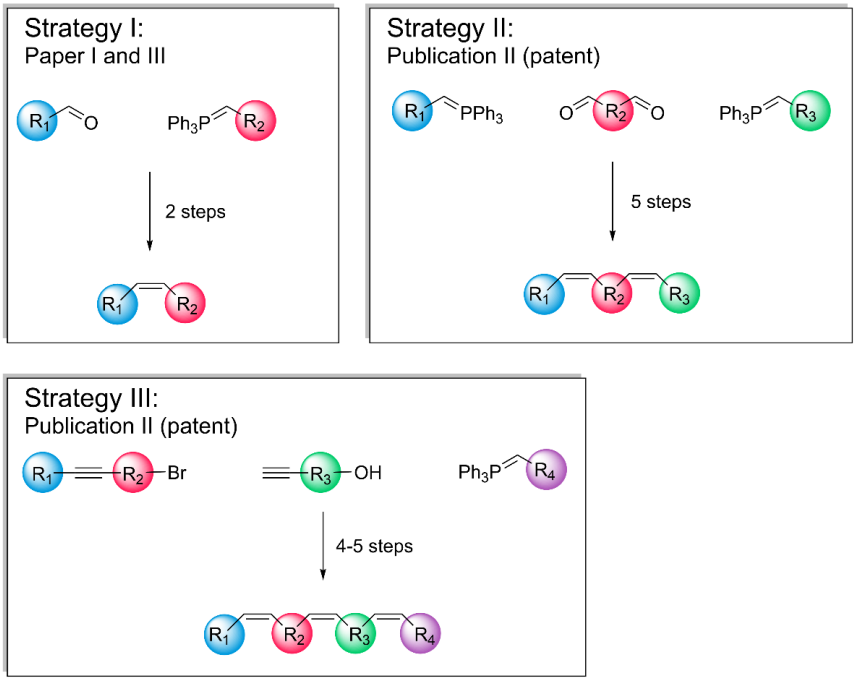
1.3 Synthetic strategy and considerations

To produce standards, a few other considerations were also important, such as the number of steps in the synthesis, price and availability of starting materials and the yield. In addition, a broad range of products was desired to cover a larger span of different physiochemical properties of the CPs.

The route of chlorination that will be utilised in this work is electrophilic chlorination of polyenes. Electrophilic chlorination is an efficient way to add chlorines to a carbon chain. Some polyenes are commercially available, and others can be synthesised using different strategies. The structure of the final CP will be dependent on the polyene chain length and the position of the alkenes.

As a consequence of using electrophilic dichlorination of alkenes, all CPs in this work will have pairwise vicinal chlorines. If an odd number of chlorines is desired in future work, a possible strategy for synthesis could involve a polyenyl alcohol and a subsequent Appel-type chlorination,[27-28] or the strategy proposed by Nikiforov.[29]

A summary of the different polyene synthesis strategies is shown in Scheme 1.1.



Scheme 1.1. Synthetic strategies for the different target CPs reported in this thesis.

Strategy I, used in publication I and III, is a two-step synthesis using either alkenyl bromides (publication I, Section 2) or 1-bromopropane (publication III, Section 3) together with appropriate aldehyde in a Wittig olefination. Since only one double bond is formed during strategy I, this method is reliant on commercially available starting materials with already incorporated double bonds if CPs with more than 2 chlorines are to be obtained. This reliance limits the scope of the "two-step path" and when synthesising a variety of ^{13}C-labelled standards (publication II, Section 3), strategy II and III were deemed preferable (Scheme 1.1).

1.3.1 Wittig olefination

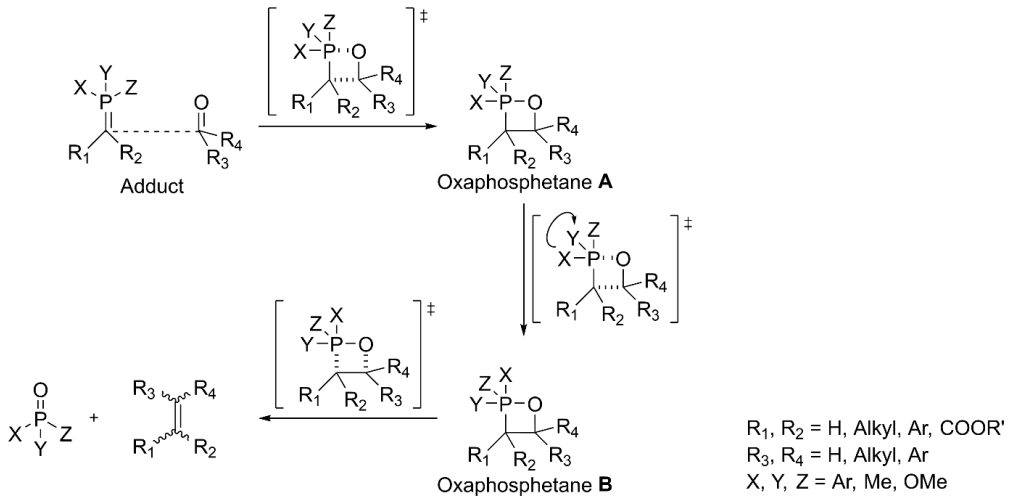
Fundamental to the synthesis of the new constitutionally defined CP standards is the Wittig reaction. The Nobel prize winning reaction was first described by Georg Wittig and Ulrich Schöllkopf in 1954,[30] and has since then been widely used as an alkene-yielding coupling reaction between a phosphonium ylide and either an aldehyde or a ketone. The stereoselectivity of the Wittig reaction depends on the nature of the ylide:[31]

Non-stabilised ylide (alkyl) → *Z* selectivity

Stabilised ylide (ester, ketone) → *E* selectivity

Semi-stabilised ylide (aryl) → Poor *E/Z* selectivity

The theoretical origin of the stereoselectivity is complex and, to this day, debated by scholars.[31-33] While the mechanism of the salt-free Wittig reaction has been extensively discussed in literature,[31,34] the formation of an oxaphosphetane intermediate has been experimentally observed and is generally accepted to occur during the reaction.[33] The general mechanism suggested by Farfán *et al*. is illustrated in Scheme 1.2.[32]



Scheme 1.2. Proposed general mechanism for the salt-free Wittig reaction.[32]

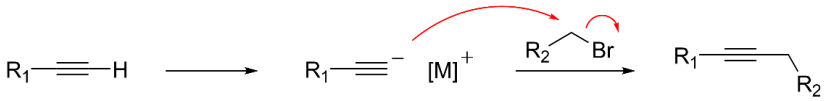
In summary, the mechanism is divided into three parts where the ylide and the carbonyl forms an adduct through long-range contact and after the first transition state forms oxaphosphetane **A**. Although little appears to change from oxaphosphetane **A** to oxaphosphetane **B**, Farfán *et al*. claim that this pseudorotation is crucial for the progress of the reaction, as it causes the C-C and P-O bonds to strengthen and the P-

C and C-O to weaken. Finally, an oxaphosphetane cycloreversion occurs, with the alkene and phosphine oxide being released. The *cis*- selectivity for non-stabilised ylides arises from steric interactions during the formation of oxaphosphetane **A**, while the *trans*-selectivity of stabilised ylides arises from dipole-dipole interactions in the transition state for the formation of oxaphosphetane **A**.[31]

The stereoselectivity of the Wittig reaction is expected to determine which CP diastereomers are produced, as the subsequent dichlorination follows a stereospecific mechanism.[35-37] Thus, CPs of the same constitution, but with different stereochemistry, can be obtained through chlorination of polyenes consisting of either *trans* or *cis*-alkenes. Since the alkyl/alkenyl chain gives the generated ylides a non-stabilised character, a classical Wittig reaction of these compounds is expected follow *cis*-selectivity. Stereoselective synthesis towards *trans*-alkenes can be achieved, for example, by the Schlosser modification of the Wittig reaction,[38] or through a Julia olefination.[39]

1.3.2 Nucleophilic substitution reactions with acetylides

A different way to create a polyene chain, through commercially-available starting materials, is by nucleophilic substitution with acetylides and subsequent partial hydrogenation.[40] The first step of this reaction is the deprotonation of a terminal alkyne with a base. The typical pK_a of terminal alkynes are around pK_a ~26, thus a strong base such as sodium amide, sodium hydride or a Grignard reagent is required. Combining the alkynyl carbanion with a suitable electrophile result in a S_N2 displacement reaction (Scheme 1.3).



Scheme 1.3. Nucleophilic substitution of alkynyl bromide with acetylide.

These reaction work best for primary alkyl halides, as secondary, tertiary or bulky electrophiles are prone to E2 elimination.[40]

1.3.3 Partial reduction of alkynes

The method for reduction of alkenes by hydrogen-gas, in the presence of a metal catalyst, was introduced by Paul Sabatier in 1899,[41] for which he was awarded the Nobel Prize in 1912.[42] The hydrogenation of alkynes under these conditions would lead to a full reduction to alkanes. A selective hydrogenation of alkynes to alkenes was later described by Lindlar.[43] Adding a “poison” to the catalyst system, typically lead oxide and quinoline, led to a catalyst sufficiently deactivated to achieve only the desired partial reduction to the olefin. The product of this reduction is known to be selective towards the *Z*-isomer.

Another *Z*-selective partial reduction of alkynes can be achieved with a nickel boride catalyst.[44] More specifically, the catalyst P-2 nickel, described by Charles A. Brown and Herbert C. Brown, proved to be selective towards the formation of alkenes and thus, could be used for partial hydrogenation.[45] The P-2 catalyst is generated from nickel (II) acetate and sodium borohydride in ethanol.

As mentioned in Section 1.3.1, the stereochemistry of the generated alkenes will give different diastereomer compositions of CPs after the chlorination steps. The work described herein is focussed on *Z*-alkenes. The *E*-alkenes can also be obtained from a partial reduction of alkynes, for example through a dissolving metal reduction with Na/NH_3 as the reducing agent.[40]

1.3.4 Dichlorination

The use of electrophilic halogen sources to warrant vicinal halogenation of double bonds has been a versatile synthetic tool for many decades.[36-37] The dihalo-functionalisation gives stereogenic centres where the outcome of configurational isomers and diastereomers is predictable.

The early vicinal chlorination protocols used molecular chlorine as reagent. Since then, many new reagents have been suggested as a surrogate for chlorination to avoid the handling of molecular chlorine. Among these are:

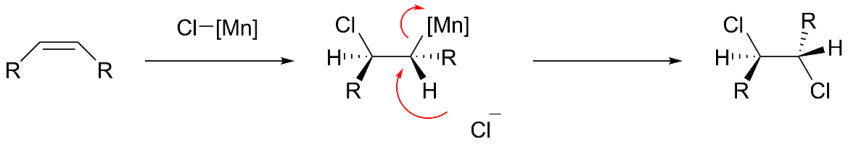
1. Et_4NCl_3[46]
2. $BnEt_3N^+MnO_4^-$/trimethylsilyl chloride[35]
3. Oxone/NaCl[47]
4. H_2O_2/HCl[48]
5. *N*-Chlorosuccinimide/PPh_3[27]
6. SO_2Cl_2[49]
7. $PhICl_2$[50-51]

The first was described by Schlama *et al.* and reacts similar to molecular chlorine,[46] but has the advantage of being a solid (compared to gas), which makes it easier to handle and control stoichiometry. This reagent will, over time, release Cl_2-gas and thus has a somewhat short expiry date. The three following reagents (2.-4.) are examples of oxidative reagents, where the halides are oxidised *in-situ* to allow electrophilic chlorination.[36] In the fifth example, *N*-chlorosuccinimide (NCS) promotes electrophilic chlorination of the alkene and PPh_3 is used to reduce the halenium of NCS to halide, thus following a 1:2 stoichiometry of PPh_3 and NCS.[27] The two last reagents follow a radical chlorination mechanism.[36]

Amongst these, benzyltriethylammonium permanganate ($BnEt_3N^+MnO_4^-$) and trimethylsilyl chloride (TMSCl) was tested as a chlorination reagent (Markó-Maguire reagent), as well as 2:1 NCS/PPh_3 (Yoshimitsu reagent) in publication I (Section 2). Chlorination with chlorine gas was used in publication II (Section 3) and III (Section 4).

Markó's reagent

In the first dichlorination paper by Markó,[52] the use of oxalyl chloride as the halide source was described. Owing to the erratic behaviour of this reagent, it was substituted with trimethylsilyl chloride in subsequent literature.[35] The mechanism was proposed to follow a suprafacial oxidative addition of the manganese complex and chloride to the olefin, followed by an invertive S_N2-type reductive elimination of the manganese complex by a chloride anion.[36] This proposed mechanism is shown in Scheme 1.4.

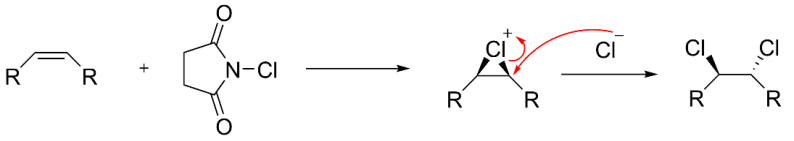


Scheme 1.4. Proposed mechanism for manganese mediated dichlorination.[36]

Suggested by Cresswell *et al.,* there is also a chance that molecular chlorine or a Mioskowski-type chlorinating agent is generated *in-situ*, which may be responsible for the dichlorination.[36,46,53] The exact mechanism of chlorination using the Markó-Maguire protocol is not fully deconvoluted and needs further investigation before being considered conclusive.

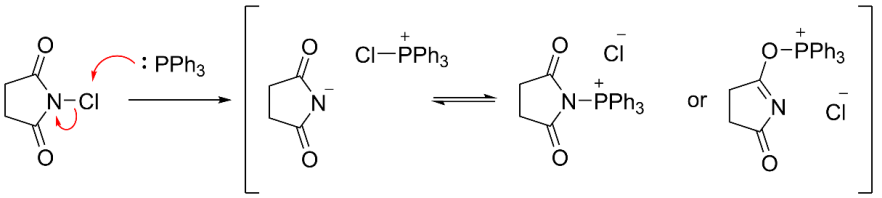
Yoshimitsu's reagent

A different chlorination protocol was proposed by Kamada *et al.*[27] Here, NCS is a source to electrophilic chlorine and the proposed mechanism of chlorine addition is through a chloronium ion intermediate (Scheme 1.5).[54]



Scheme 1.5. Proposed mechanism for chlorination of a Z-alkene through a chloronium intermediate.[54]

The nucleophilic chloride is generated using PPh_3 as a reducing agent (Scheme 1.6).[27,36]



Scheme 1.6. The chlorophosphonium complexes generated from mixing NCS with PPh_3.[27]

Another observation by Kamada *et al*. was that activation of the hydroxy group of alkenyl alcohols with PPh_3 was preferred,[27] resulting in an Appel-type reaction prior to dichlorination. This can be incorporated to straight-chain alkenyl alcohols to directly synthesise CPs with an odd number of chlorines.

2 Publication I: Synthesis of constitutionally defined chlorinated paraffins as reference standards

2.1 Introduction

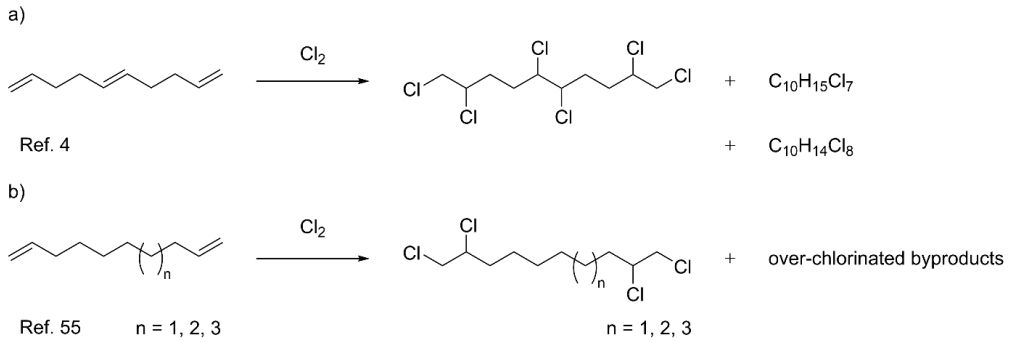
Publication I describe work towards synthesis of native, constitutionally defined CPs to be used as analytical standards. Dichlorination of alkenes is an efficient route to incorporate chlorines in an alkane chain and can achieve many different products, depending on which polyenes are used. Previous research has utilised chlorination of polyenes for the synthesis of constitutionally defined CPs,[4,29,55-56] using chlorine gas as reagent for the reaction.

Due to the added risk of working with gaseous and acutely toxic chemicals, we explored different substitutes for molecular chlorine. To synthesise different native, constitutionally defined CPs, we tested two different chlorination approaches: Markó-Maguire and Yoshimitsu-chlorination.[27,35] These were tested on commercially available **9** (Scheme 2.5) and used in the synthesis of CPs **5a-f** (Scheme 2.4) and **8a-e** (Scheme 2.6), respectively.

2.2 Previous work

The first reported usage and synthesis of a constitutionally defined CP reference standard was described by Tomy *et al.* (Scheme 2.1a).[4] Here, commercial deca-1,5,9-triene was chlorinated to 1,2,5,6,9,10-hexachlorodecane and was used in their analytical research to observe the behaviour of the molecular ion clusters at different ion source conditions on a GC-ECNI-HRMS instrument.

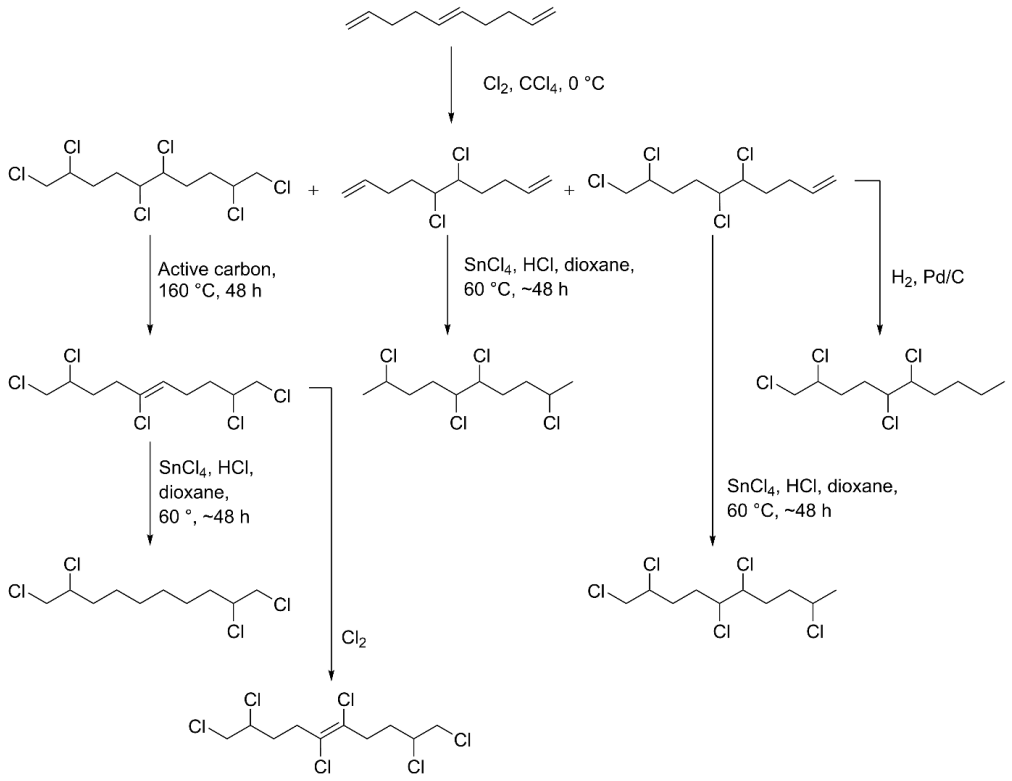
The chlorination was conducted by bubbling molecular chlorine through neat deca-1,5,9-triene at room temperature and with the reaction vessel covered in aluminium foil to exclude light. The same procedure was conducted by Fisk *et al*. to synthesise three new tetrachloroalkanes (Scheme 2.1b).[55]



Scheme 2.1. Synthesis of polychlorinated alkanes by Tomy et al.[4] and Fisk et al.[55]

This chlorination protocol led to product mixtures of the desired product and several over-chlorinated byproducts, built up of the same chlorine substitution, but with one or more additional chlorines attached to unspecified locations in the carbon chain. The over-chlorinated byproducts were thought to arise from the generation of small amounts of chlorine radicals in solution. This could lead to a free radical substitution of hydrogen for chlorine,[55] or possibly a radical allylic halogenation to a double bond prior to dichlorination.[57]

In an effort to avoid the generation of over-chlorinated byproducts, Coelhan used a deficit of molecular chlorine for the chlorination of deca-1,5,9-triene (Scheme 2.2).[56]



Scheme 2.2. Synthesis of polychlorinated alkanes by Coelhan.[56]

Two batches of deca-1,5,9-triene were subjected to molecular chlorine in carbon tetrachloride, using approximately 0.5 and 0.9 equivalents of molecular chlorine per double bond. The crude reaction products were later combined, affording a mixture of single-, double- and triple-dichlorinated products. The different products were separated by column chromatography and were then individually functionalised to different CP products as described in Scheme 2.2. While this method circumvented over-chlorination, the sub-stoichiometric amounts of chlorine led to under-chlorination of the starting material. One of these compounds, together with some different constitutionally defined CPs were used as quantification standards in their subsequent work.[58]

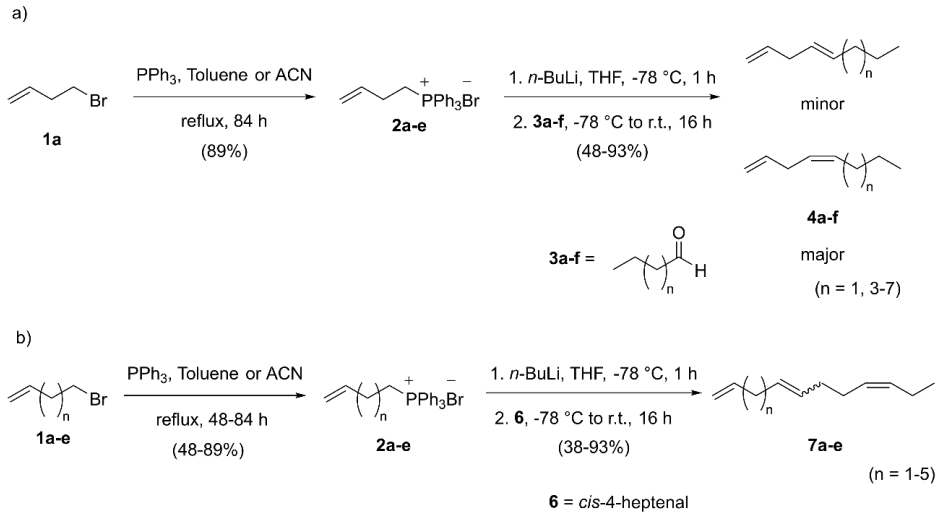
2.3 New compounds and extended synthesis

2.3.1 Polyene synthesis

The work of Tomy,[4] Fisk,[55] and Coelhan[56] (Section 2.2) was based on the chlorination of commercially available alkenes, such as deca-1,5,9-triene. To widen the scope of constitutionally defined CP standards, we sought to synthesise a range of polyene starting materials, containing varying amounts and positions of the double bonds within carbon chains of varying length.

We began by applying the Wittig reaction, using commercially available alkenyl bromide **1a** and saturated aldehydes **3a-f**, to synthesise a range of dienes **4a-f** (see Scheme 2.3a).

Synthesis of the alkenyl phosphonium salt **2a-e**, followed by Wittig olefination with *cis*-4-heptenal **6** provided trienes **7a-e** (Scheme 2.3b).



*Scheme 2.3. Synthesis of polyenes **4a-f** and **7a-e** from alkenyl bromides **1a-e** through a Wittig olefination.*

As expected,[31] the newly-generated olefins were predominantly *cis*-substituted. This was demonstrated by ^{1}H NMR spectroscopy for olefin **7a** (Figure 2.1).

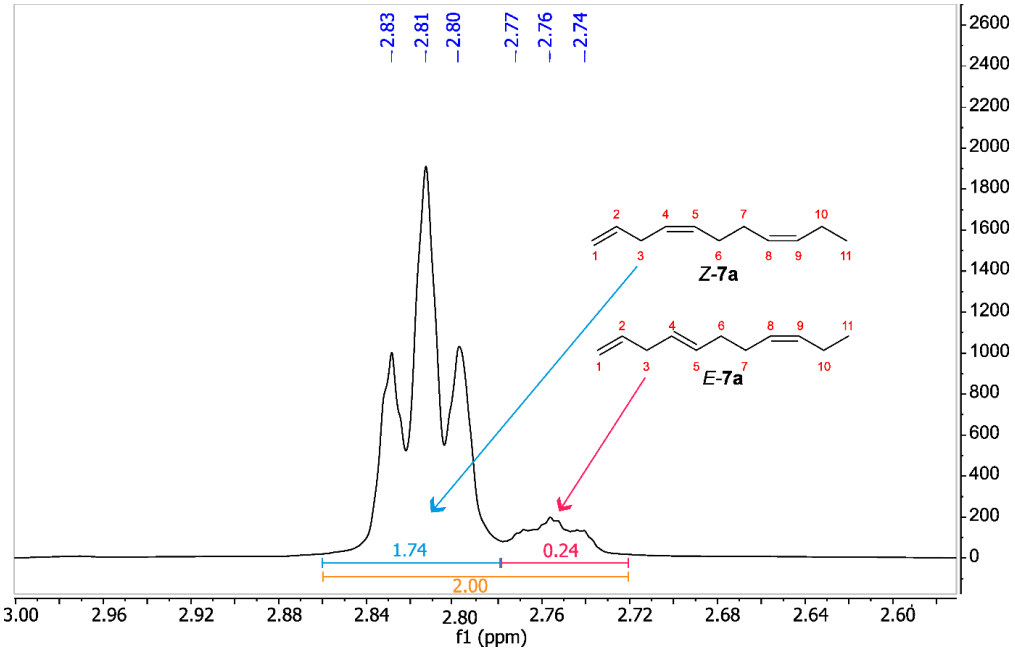


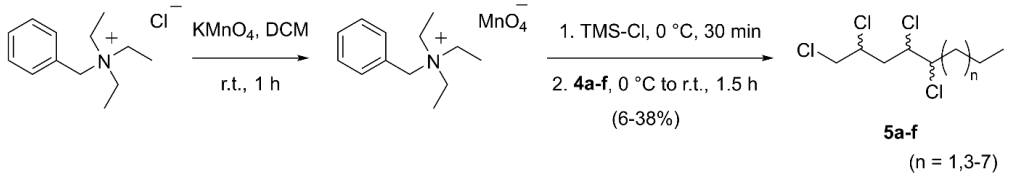
*Figure 2.1. Zoom-in of the ^{1}H NMR spectrum (400 MHz, $CDCl_3$) around the H3-region for **7a**.*

The splitting pattern of the olefinic protons was poorly defined, making the coupling constants difficult to ascertain. Literature showed a slight upfield-shifted value of *E*-H3, compared to *Z*-H3 for compounds with "skipped" double bonds (RCH=CH-C*H*$_2$-CH=CHR).[59-61] With acceptable resolution observed for the *Z*-H3 at δ 2.83 – 2.80 ppm and *E*-H3 signals at δ 2.77 – 2.74 ppm, comparison of the integral values led to a stereoselectivity of 83-95%, in favour of the *cis*- isomers **4a-f** and **7a**.

2.3.2 Chlorination

While the previous research on synthesis of CP standards has revolved around the use of molecular chlorine, alternate reagents were deemed desirable due to its toxicity. Additionally, we desired to avoid the use of carbon tetrachloride as solvent due to its toxic and adverse environmental effects.[62] In Section 1.3.4, potential candidates for molecular chlorine surrogates were described and, from these different reagents, two were tested - $BnEt_3N^+MnO_4^-$/TMSCl (Markó-Maguire reagent) and NCS/PPh_3 (Yoshimitsu's reagent).[27,35]

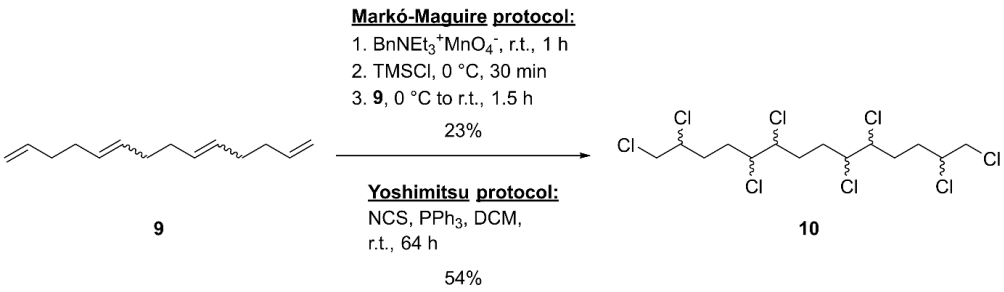
Initially the Markó-Maguire protocol was used to synthesise CPs **5a-f** (Scheme 2.4) and gave yields of 6-38%.



*Scheme 2.4. Markó-Maguire chlorination of dienes **4a-f** to CPs **5a-f**.*

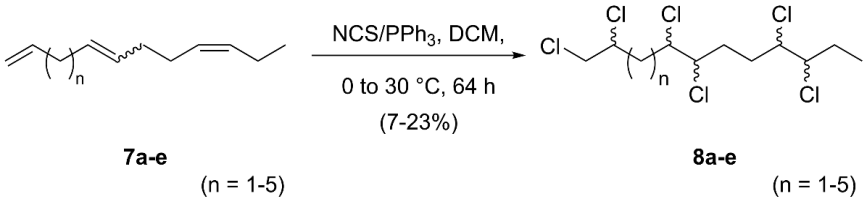
Following the reaction by thin-layer chromatography (TLC) was arduous as the products were troublesome to visualise on TLC, due to their low concentrations compared to the residues of chlorination agents. Furthermore, the initial extraction process proved to be difficult, due to large amounts of intractable inorganic residue. The crude product mixtures obtained after extraction were mixtures of the desired products, the over-chlorinated byproduct (one extra chlorine) and other non-characterised impurities. Although these non-characterised impurities were removable by flash column chromatography, the over-chlorinated byproduct proved to be poorly separable under a range of eluent systems. Thus, the final CP products **5a-f** showed up to 7.5% of over-chlorination by GC-MS analysis, even after several purification attempts.

Due to the difficult purification and the poor associated yields, a new chlorination method (Yoshimitsu) was tested on a tetraene **9** and compared to the Markó-Maguire protocol (Scheme 2.5).



*Scheme 2.5. Chlorination of **9** using Markó's protocol,[35] and Yoshimitsu's protocol.[27]*

The Markó-Maguire protocol gave a sub-optimal yield of 23% for **10**. Subsequent application of the Yoshimitsu protocol led to a much-improved yield of 54%. Thus, the Yoshimitsu protocol was used to synthesise the next series of CPs **8a-e** (Scheme 2.6).



*Scheme 2.6. Yoshimitsu chlorination of polyenes **7a-e**, which afforded CPs **8a-e**.*

The Yoshimitsu protocol generally required 3 equivalents of triphenylphosphine and 6 equivalents of *N*-chlorosuccinimide per alkene unit in the starting material. As with the Markó-Maguire protocol, the low concentration of product, compared to chlorinating agents, made visualisation by TLC difficult. The issue of concentration also led to difficulties in following the reaction by GC-MS. Pleasingly, the absence of inorganic materials simplified the work-up procedure, compared to the Markó-Maguire protocol. Typically, reaction times were considerably longer than with the Markó-Maguire protocol and, in some cases, unreacted double bonds were observable after a reaction time of 64 h. Although a lower amount of over-chlorination was observed by this procedure, it was still present and remained difficult to remove chromatographically.

The hexachloroalkanes **8a-e** were solids and could be recrystallised after column chromatography. Although this was successful in removing the over-chlorinated byproduct, it also gave a substantial reduction in yield. This was attributed to the enrichment of specific diastereomers of the product by recrystallisation, with other diastereomers remaining in the mother liquor with the over-chlorinated byproduct.

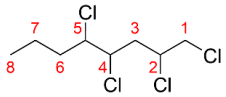
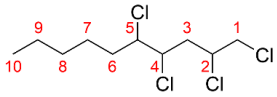
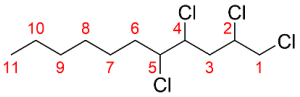
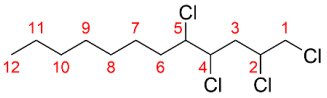
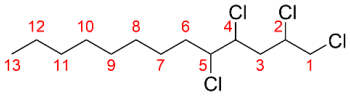
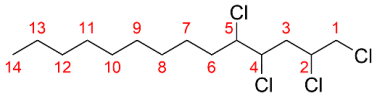
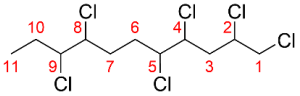
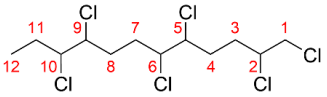
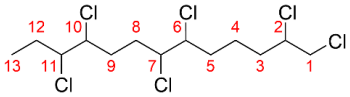
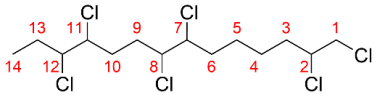
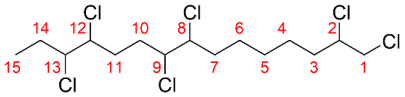
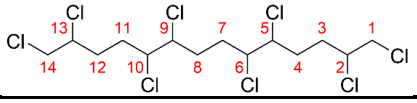
In conclusion, two different series of CPs **5a-f** and **8a-e** were synthesised for the purpose of being used as reference standards. Both the Markó-Maguire and the Yoshimitsu protocol worked for the conversion of polyenes to CPs, yet both suffer from significant drawbacks, such as poor yields and extensive purification.

2.3.3 CP standards

The list of synthesised standards from publication I is listed in Table 2.1. All synthesised standards contain one terminal chlorine, except for octachloroalkane **10** which has two terminal chlorines. The synthesised standards have a range of different

chain-lengths within the SCCP and MCCP definition, as well as a varied chlorine percentage. Generally, the solid CPs **8a-e** and **10**, which could be recrystallised, shows a high purity within the commonly applied purity limits of >98% for reference standards by Chiron. Oily products **5a-f** shows a slightly lower purity due to the difficulty of removing over-chlorinated by column chromatography.

Table 2.1. Overview of the synthesised constitutional CP standards from publication I. Purity was determined from GC-MS. The chlorine percentage is a theoretical value calculated from the molecular formula.

Compound	Structure	Amount [g]	Purity [%]	Chlorine percentage [%Cl]
5a		0.18	95.7	56.3
5b		7.46	97.7	50.6
5c		0.91	95.7	48.2
5d		1.03	97.0	46.0
5e		0.61	97.7	44.0
5f		0.87	98.4	42.2
8a		0.26	99.5	58.6
8b		0.40	98.1	56.4
8c		0.20	>99.9	54.4
8d		0.14	>99.9	52.5
8e		0.29	99.4	50.8
10		0.82	99.8	59.8

Compounds **8a-c** were tested on a two-dimensional GC with a micro-electron capture detector (GC×GC-μECD) together with the previous constitutionally CP standard 1,2,5,6,9,10-hexachlorodecane and the result is shown in Figure 2.2. Compared to 1,2,5,6,9,10-hexachlorodecane, **8a-c** eluted closer to the centre of the SCCP-band, indicating a higher similarity to the CP compounds from that mixture with higher intensity.

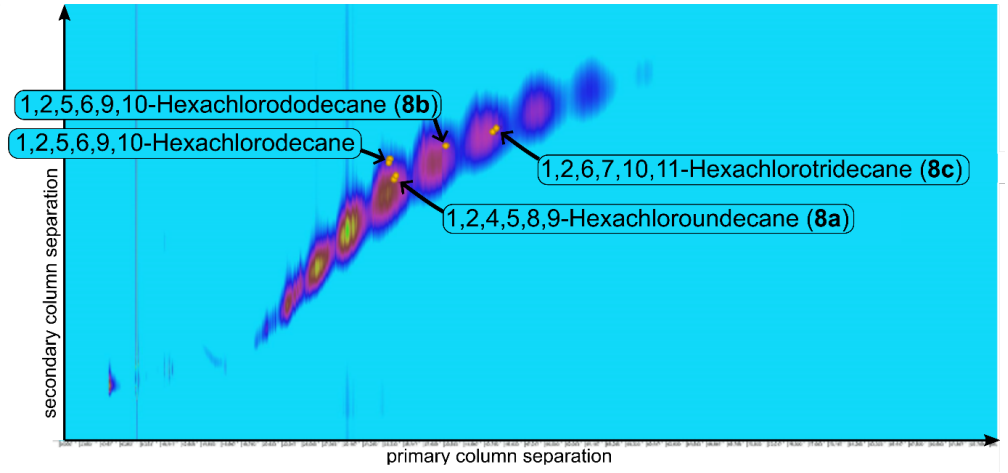


*Figure 2.2. GC×GC-μECD chromatogram of **8a-c** and 1,2,5,6,9,10-hexachlorodecane together with a SCCP mixture (55.5 %Cl, from Dr. Ehrenstorfer).*

3 Publication II (patent): ^{13}C-labelled chlorinated paraffins and their preparation

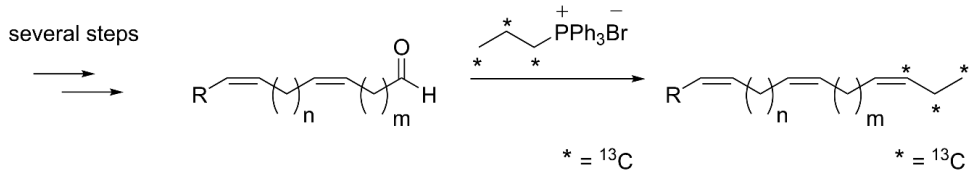
3.1 Introduction

The full scope of the published patent will not be discussed during this section but will rather focus on the preparation of ^{13}C-labelled CP standards where the author was involved in the synthesis.

This section will be divided into four subsections, where the first section describes the synthesis of native (non-isotopically enriched) and labelled Wittig reagents, the second and third describes the two strategies for building up the polyene (strategy II and III, Scheme 1.1) and the final section concerns the electrophilic chlorination.

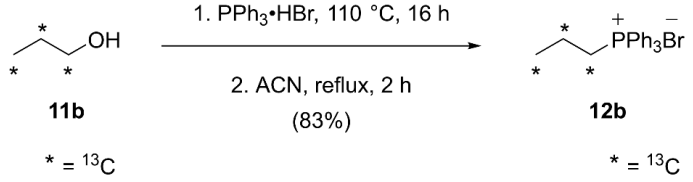
3.2 Phosphonium salts

The synthesis of ^{13}C-labelled CP standards began with the preparation of native and labelled Wittig reagents. A key part of the strategy was to incorporate precious ^{13}C-labelled material into the carbon chain during a late step in the synthetic route to minimise the loss of expensive substrate (Scheme 3.1).



Scheme 3.1. Example of the strategy where ^{13}C-labelled substrate is coupled to the non-isotopically enriched chain at the end of the polyene synthesis.

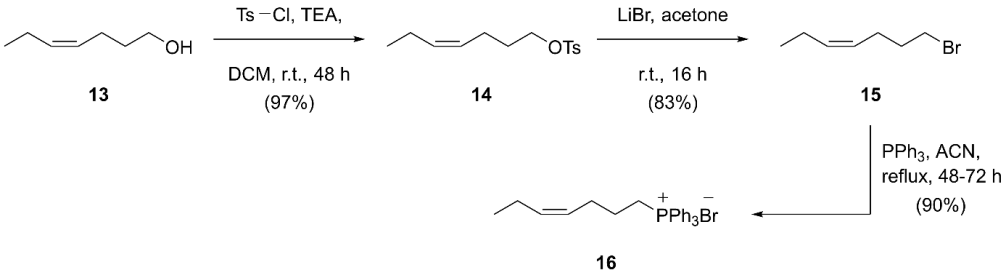
In general, an isotope-labelled internal standard should contain a minimum of three isotope labels to avoid potential interference between the internal standard and naturally-occurring isotopes in the analyte.[24] Considering the necessary number of labelled carbons, together with price and availability of the starting material, 1-propanol-$^{13}C_3$ (**11b**) was chosen as the ^{13}C-labelled building block. It was converted to the corresponding phosphonium salt **12b** *via* a two-step procedure (Scheme 3.2).



*Scheme 3.2. Synthesis of ^{13}C-labelled phosphonium salt **12b** refluxing ^{13}C-labelled propanol **11b** with a triphenylphosphine/hydrobromide complex.*

Upon heating the reagents without solvent, the reaction turned liquid and after 1-2 hours re-solidified, indicating the formation of the Wittig salt. The precipitation of **12b** hindered further stirring of reagents and, in a hope to achieve a higher conversion, the reaction was first left overnight, cooled and later refluxed for two hours in acetonitrile (ACN). Refluxing in acetonitrile caused the solid mass of product to dissolve, facilitating the necessary mixing for completion of the reaction and also making subsequent purification of the reaction mixture more convenient.

The different native phosphonium salts used for the ^{13}C-labelled CP synthesis were **2b**, **2e** and **16a-b**. Compounds **2b** and **2e** (synthesised as described in Scheme 2.3, Section 2.3.1) were used to create CPs **23a-b** (Scheme 3.5a, 3.6a, 3.10) with one terminal chlorine. By converting *cis*-4-heptenol (**13**) to corresponding phosphonium bromide (Scheme 3.3), ^{13}C-labelled CPs **27a-b** (Scheme 3.5b, 3.6b, 3.10) with entirely internal chlorines were achieved.



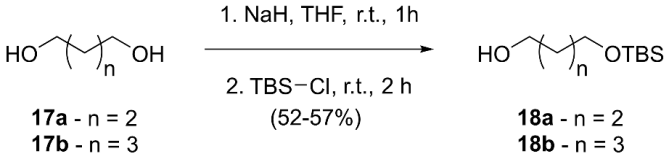
*Scheme 3.3: A three-step synthesis of phosphonium salt **16** from the corresponding alcohol.*

Good overall yield (73%) was achieved for **16** considering the three-step process.

3.3 Swern oxidation and Wittig coupling

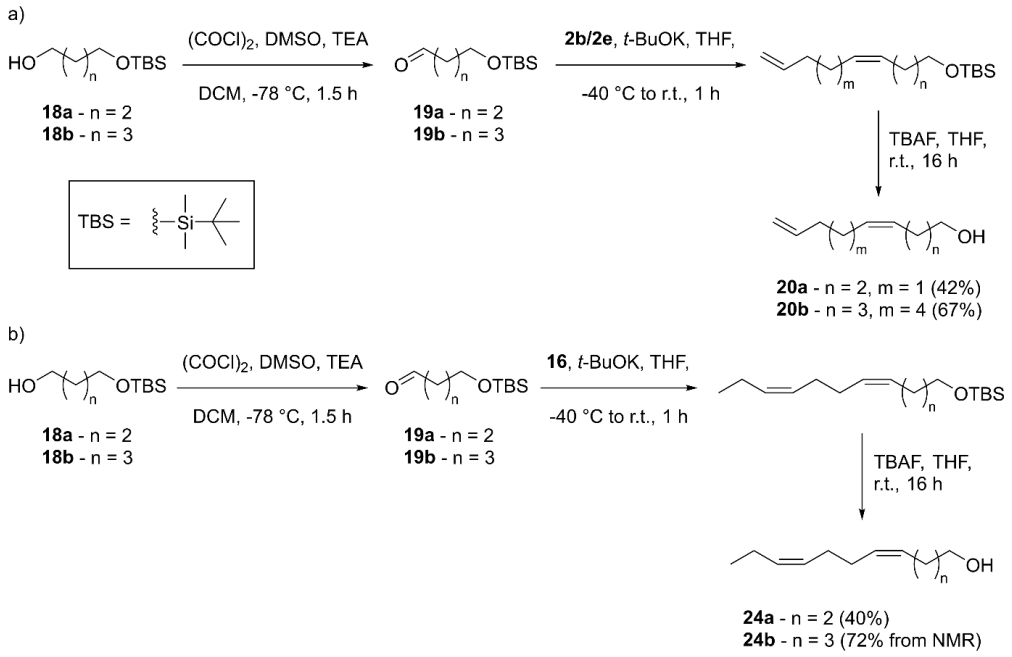
The ^{13}C-labelled trienes **22a-b** and **26a-b** (Scheme 3.5 and 3.6) were achieved using Strategy II (Scheme 1.1). The process involves protection of a diol, followed by a Swern oxidation, Wittig reaction and deprotection to give dienyl alcohols **20a-b** and **24a-b** (Scheme 3.5). The desired trienes were obtained after another Swern oxidation and Wittig reaction. Throughout these syntheses, solvent was generally removed carefully and often not completely. This is due to the volatility of the products and intermediates, or possibly the ability to co-distil during solvent evaporation, where the yields appeared to be lower where a more aggressive solvent removal or purification was conducted. Thus, the yields are calculated over several steps instead of the typical yield over one reaction step.

The synthesis began with the protection of diols **17a-b** with *tert*-butyldimethylsilyl chloride (Scheme 3.4).



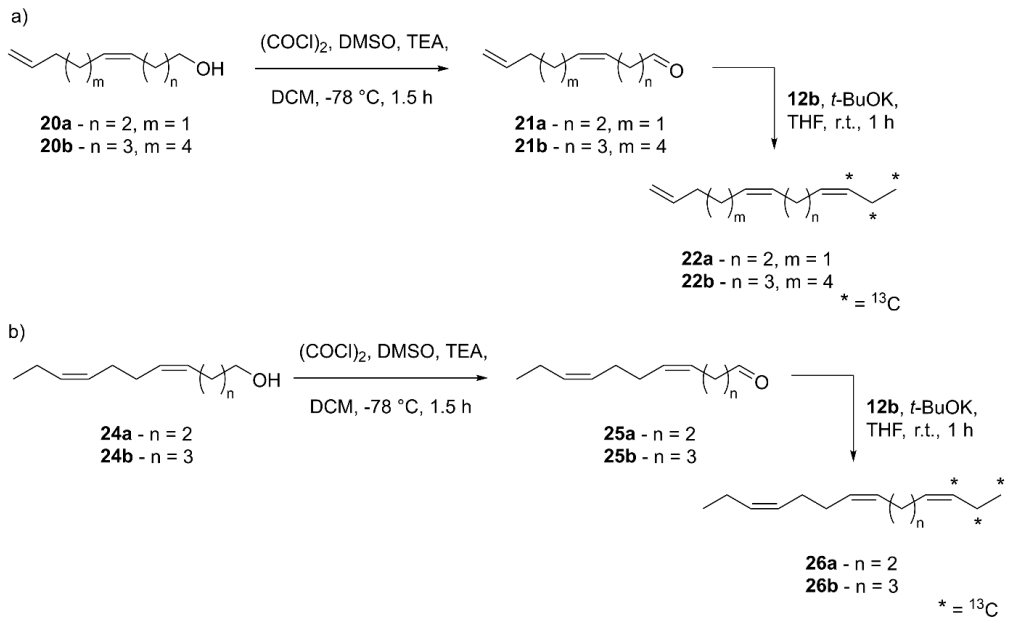
*Scheme 3.4. Mono-protection of diols **17a-b** using NaH and tert-butyldimethylsilyl chloride.*

After protection, a Swern oxidation, Wittig reaction and deprotection process was conducted to obtain **20a-b** (Scheme 3.5a) and **24a-b** (Scheme 3.5b). In the Wittig reaction alkenyl phosphonium salts **2b** and **2e** were used to give dienyl alcohols **20a-b** with one terminal and one internal double bond and **16** were used to give **24a-b** with two internal double bonds.



*Scheme 3.5. Synthesis of dienyl alcohols a) **20a-b** and b) **24a-b**. Yields are given over three steps.*

Following the three-step synthesis from dienyl alcohols **20a-b** (42 – 67%) and **24a-b** (40 – ~72%), another Swern oxidation and Wittig reaction afforded the ^{13}C-labelled polyenes **22a-b** and **26a-b** (Scheme 3.6).

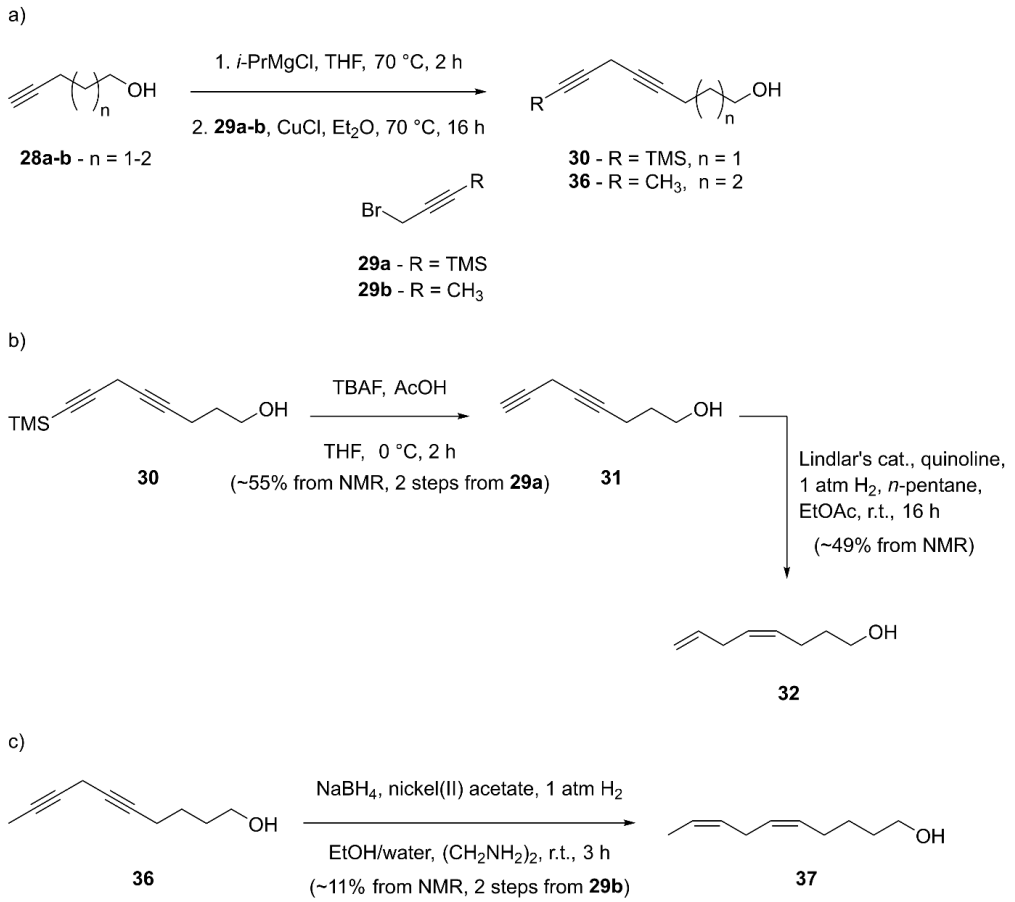


*Scheme 3.6. Synthesis of ^{13}C-labelled trienes a) **22a-b** and b) **26a-b**.*

Due to their expected volatility, the intermediates **22a-b** and **26a-b** were not fully purified and characterised. Instead, they were used directly in the next step, despite the presence of residual solvents. The products were thus obtained as a solution in *n*-pentane after purification and the yields after chlorination were calculated from alcohols **20a-b** and **24a-b** over three steps (see Scheme 3.10).

3.4 Nucleophilic substitution of acetylides, partial reduction, Swern oxidation and Wittig coupling

A different approach to the synthesis of dienyl alcohols was conducted through a deprotonation of terminal alkynyl alcohols **28a-b** with a Grignard reagent and subsequent nucleophilic attack of alkenyl bromides **29a-b** with the acetylide anions to diynyl alcohols **30** and **36** (Scheme 3.7a). After the nucleophilic substitution and removal of trimethylsilyl from **30**, the diynyl alcohols **30** and **36** were partially reduced by two different approaches (Scheme 3.7b and c). The final synthetic step to ^{13}C-labelled trienes followed the same approach as in Section 3.3, where the dienyl alcohol was converted to an aldehyde by a Swern oxidation and yielded trienes **34** and **39** by a Wittig reaction with **12b** (Scheme 3.8).

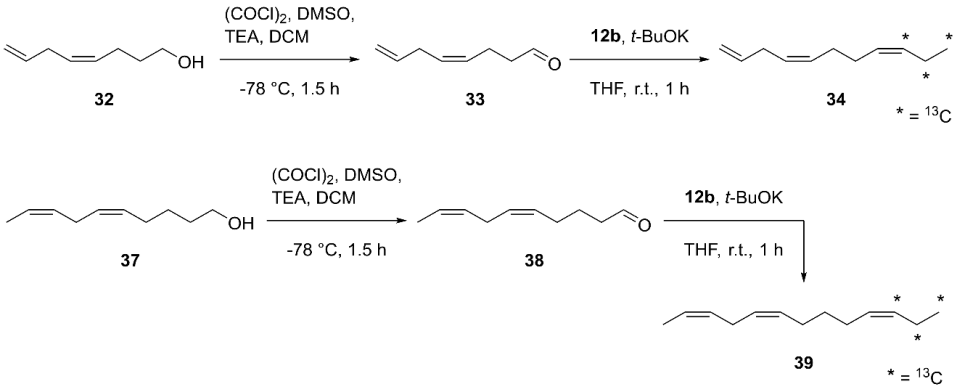


*Scheme 3.7. Synthesis of a) diynyl alcohols **30** and **36**, b) dienyl alcohol **32** and c) dienyl alcohol **34**. Yields are given as an estimate from ^1H NMR and should be considered indicative rather than determinative.*

Lindlar's catalyst was used in the partial hydrogenation of **31** to dienyl alcohol **32** giving an approximate yield of 49% (calculated by ^{1}H NMR). Difficulties tuning the quinoline poisoning and reaction time initially led to a poor conversion of the alkynes after a two-hour reaction time. Leaving the reaction overnight (16 hours) led to full conversion of the triple bonds, but also some reduction of one of the double bonds (22% from GC-MS). The over-reduced byproduct proved difficult to remove and was carried over to the next two steps (Swern oxidation and Wittig reaction). Excess of **28a** was also observed by ^{1}H NMR spectroscopy (apparent dq-splitted signals at δ 4.90-5.00 ppm) in the following reactions. After chlorination these impurities were removed by recrystallisation (see Section 3.6).

Due to the difficulties tuning the reaction conditions for the partial reduction with Lindlar's catalyst, we tested different reagents in the synthesis of **37**. Here, hydrogenation with nickel(II) acetate hydrate and sodium borohydride was utilised giving a yield of roughly 11% over two steps from **29b**. This was a significant drop from the Lindlar reduction; however, no over-reduction was observed. Similar to the synthesis of **32**, excess of the starting material **28b** was difficult to remove and the estimated yields are prone to error.

The dienyl alcohols **32** and **37** were subjected to a Swern oxidation and Wittig reaction with **12b** to afford ^{13}C-labelled trienes **34** and **39** (Scheme 3.8).



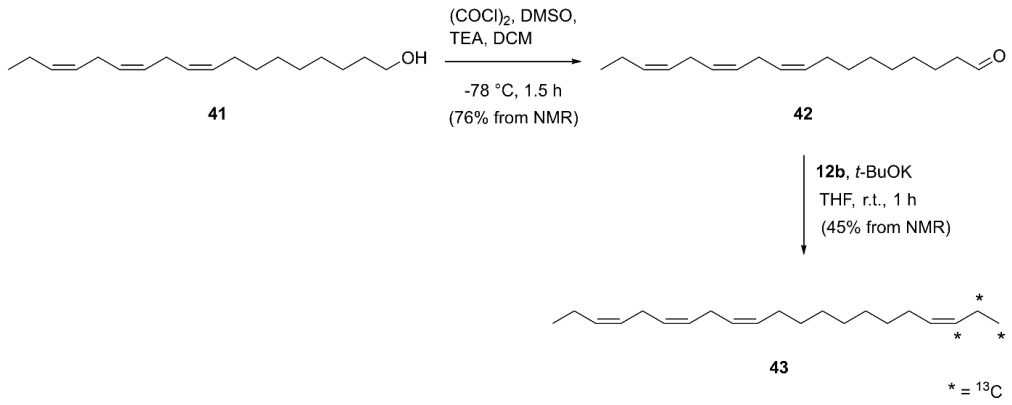
*Scheme 3.8. Swern oxidation and Wittig olefination yielding ^{13}C-labelled trienes **34** and **39**.*

As with the syntheses described in Section 3.3, the intermediates **34** and **39** were volatile, so excessive purification and removal of solvents was judged unwise, due to loss of material. Thus, the crude olefin solutions after FCC, were used directly in the subsequent chlorination reaction and yields were not determined. The total synthesis yields after chlorination are presented in Scheme 3.10.

3.5 Swern oxidation and Wittig coupling of linolenic alcohol

A third option is to use commercially available alkenyl alcohols, although this has a much more limited scope than the previously discussed pathways. Typical eligible commercial starting materials are the alcohols of different unsaturated fatty acids.

Starting from linolenic alcohol (**41**), we synthesised the ^{13}C-labelled tetraene **43** using the same approach as used previously – Swern oxidation and subsequent Wittig olefination (Scheme 3.9).



*Scheme 3.9. Synthesis of ^{13}C-labelled triene **43** from linolenic alcohol **41**. Yields are given as an estimate from ^{1}H NMR and should be considered indicative rather than determinative.*

3.6 Chlorination to ^{13}C-labelled CPs

In the previous publication (Section 2) we reported chlorination of polyenes with the molecular chlorine substitutes reported by Markó *et al.* and Kamada *et al.*[27,35] Both of these protocols gave intractable crude product mixtures, contaminated by byproduct from the chlorinating agent. Henceforth, the use of molecular chlorine was reconsidered. Considering that molecular chlorine is a toxic gaseous chemical which may cause pulmonary irritation, damage or in worst case fatality,[63-64] we sought a method for generation for molecular chlorine that could be conducted safely on a laboratory scale.

The method we used for generating molecular chlorine gas was by adding hydrochloric acid to trichloroisocyanuric acid (TCICA).[65] A experimental set-up and balanced reaction equation is shown in Figure 3.1.

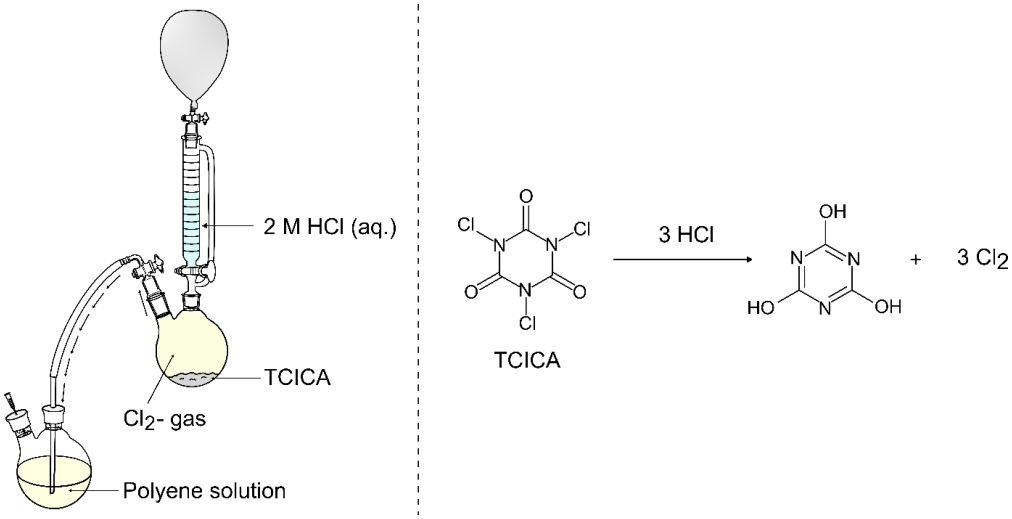
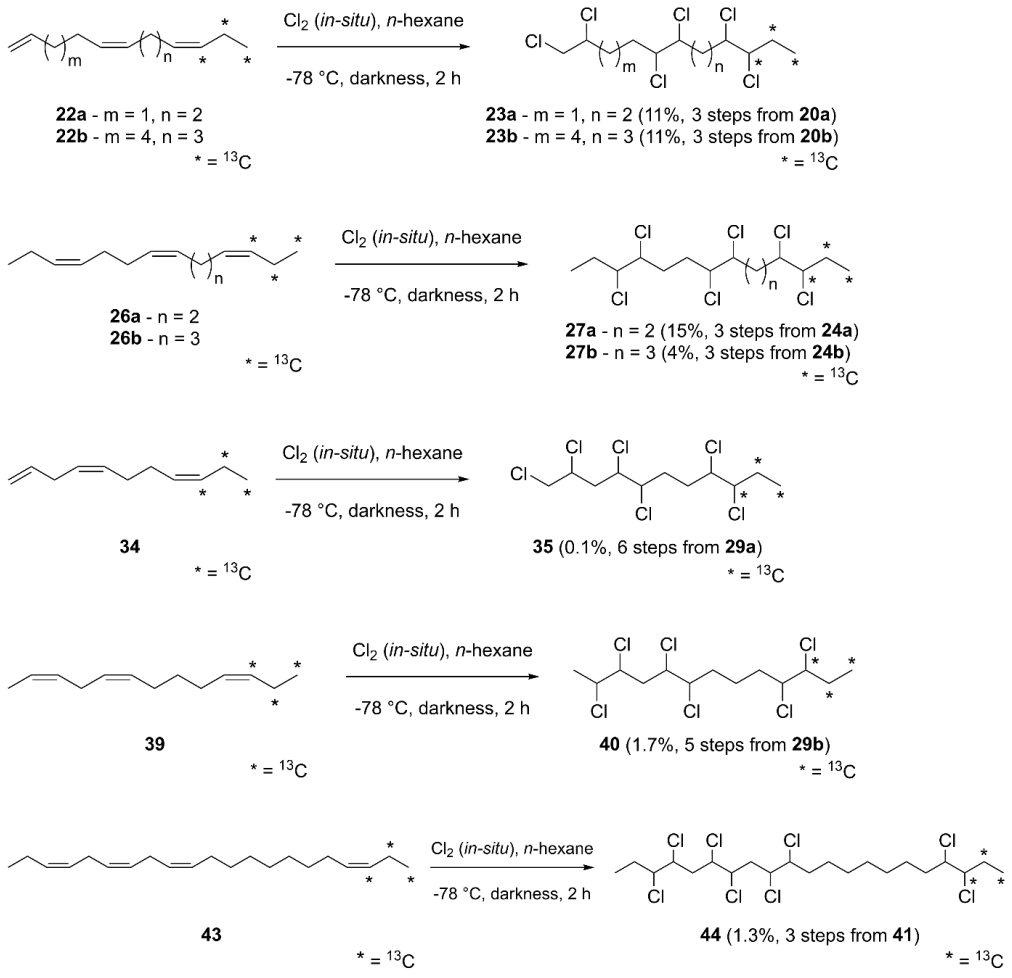


Figure 3.1. Experimental set-up and stoichiometric reaction scheme for the generation of chlorine gas.

A 15% solution of aq. HCl was charged to a separate two-necked flask, connected by a plastic tube to the reaction flask with the polyene in solution. The chlorine gas was pushed over to the reaction vessel by using a positive pressure of argon. After reaction, 1-pentene was added to quench any remaining chlorine gas. Excess chlorine in the "generation"-flask was also quenched after reaction in the same way, thus avoiding release of hazardous chlorine gas.

Each equivalent of TCICA leads to three equivalents of chlorine gas and can theoretically be used to control the equivalents of chlorine with respect to the number of double bonds in the polyene. However, since the precise amount of olefin present in the starting solutions was unknown (Sections 3.3 and 3.4), an excess of chlorine gas was generated and the reaction's progress was followed by the colour of the reaction mixture. The supply line was closed once the reaction solution showed a yellow tint, indicating an excess of Cl_2-gas in solution. If the colour dissipated after reacting over a further 15 min, more chlorine gas was added until the colour persisted. Chlorination of all ^{13}C-labelled polyenes is illustrated in Scheme 3.10.



Scheme 3.10. Chlorination of ^{13}C-labelled polyenes to ^{13}C-labelled CPs.

One of the problems using molecular chlorine is the reported issue of over-chlorination.[4,66] Since it is believed to arise from chlorine radicals, conditions which reduces or inhibits radicals were briefly investigated. All reactions were run at -78 °C and wrapped in aluminium foil to exclude light. Furthermore, DCM was initially used as reaction solvent, but *n*-hexane showed a tendency to give a smaller amount of over-chlorinated byproducts. During the substrate scope testing it was also found that the product CPs often precipitated out of the *n*-hexane solution in a fairly high purity, often with sufficient purity after only one recrystallisation. In the cases where the CPs did not precipitate or there were extensive amounts of product remaining in the reaction solvent, the reaction mixture could be concentrated and yield product after recrystallisation or chromatography and recrystallisation.

Recrystallisation could lead to an enriched mixture, predominantly of a single diastereomer. Repeated recrystallisation could lead to a single diastereomer of the CP. The recrystallisation is on the other hand, prone to loss of product since the products are mixtures of diastereomers and not all may crystallise out as easily. Since we are aiming for constitutionally defined CPs, and not necessarily stereochemically defined, these losses could be considered suboptimal. However, through recrystallisation removed overchlorinated byproducts easily, which was not so easily removed if using FCC.

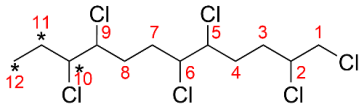
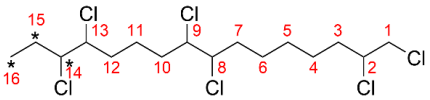
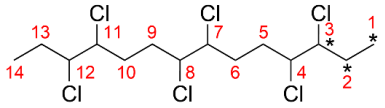
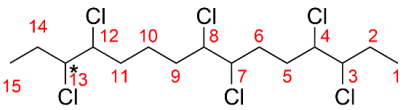
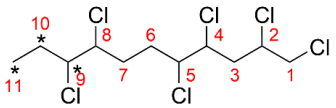
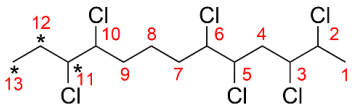
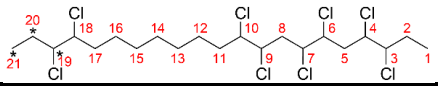
Compared to MnO_4/TMSCl and NCS/PPh_3 methods, tested in publication I (Section 2), this method gave a “clean” conversion of the starting polyenes into the desired CPs, without the presence of byproducts from the chlorinating agents. In many cases, this reduced the amount of purification necessary, as direct recrystallisation of the crude reaction product was sufficient. Impurities from previous reaction steps were removed without complications with the purification protocols.

3.7 ^{13}C-labelled CP products

As a part of this project seven new stable isotope labelled reference standards were synthesised. The products are outlined in Table 3.1.

These new ^{13}C-labelled compounds are answering the need for stable isotope labelled CP standards to be used for environmental analysis. Compared to the previous ^{13}C-labelled CPs, [$^{13}C_{10}$]-1,5,5,6,6,10-hexachlorodecane and [$^{13}C_{12}$]-1,1,1,3,10,12,12,12-octachlorododecane (distributed by Cambridge Isotope Laboratories, Inc.), the new standards are more structural and analytical relevant as none of them contains geminal chlorines. Furthermore, the diversity of chain length, chlorine substitutions and chlorine percentages of the new ^{13}C-labelled CPs gives opportunity for choosing the standard that best matches the physicochemical properties of the CP samples being analysed.

Table 3.1: All ^{13}C-labelled CP reference standards from the patent part of this project. Purity was determined from GC-MS. The chlorine percentage is a theoretical value calculated from the molecular formula.

Compound	Structure	Amount [mg]	Purity [%]	Chlorine percentage [%Cl]
23a		167	99.3	56.0
23b		80	>99.9	48.8
27a		122	>99.9	52.1
27b		45	>99.9	50.4
35		10	>99.9	58.1
40		61	>99.9	54.0
44		29	>99.9	49.3

* = ^{13}C

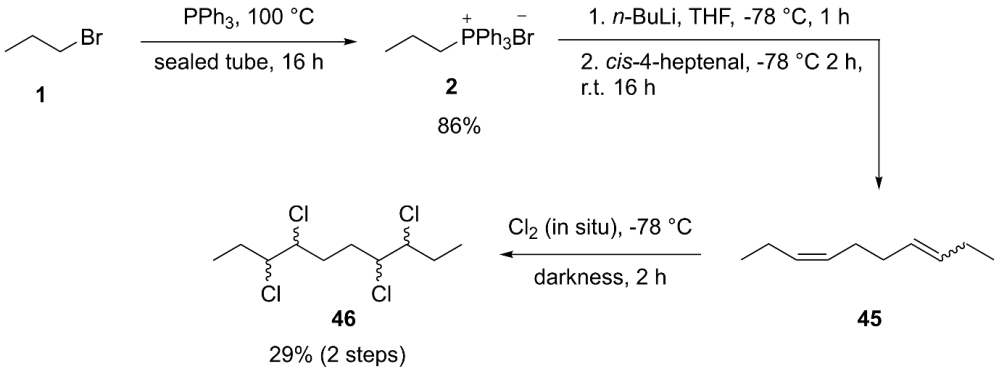
4 Publication III: Synthesis, identification, chiral separation and crystal structure of (3*R*,4*R*,7*S*,8*S*)-3,4,7,8-tetrachlorodecane and its stereoisomers

4.1 Introduction

A new native constitutionally defined CP **46** was synthesised in publication III and its stereochemical composition was investigated. One of the stereoisomers **46a** was isolated through recrystallisation and yielded a product suitable for single crystal X-ray diffraction. This structural confirmation of mesocompound **46a**, coupled with GC-MS, chiral-column supercritical fluid chromatography (SFC) and NMR, provided evidence in favour of *anti*-chlorination of the multiple double bonds from the major precursor obtained through Wittig olefination.

4.2 Stereochemical investigation

CP **46** was synthesised in a three-step procedure, starting from 1-bromopropane, involving phosphonium salt formation, Wittig olefination and subsequent chlorination (Scheme 4.1).

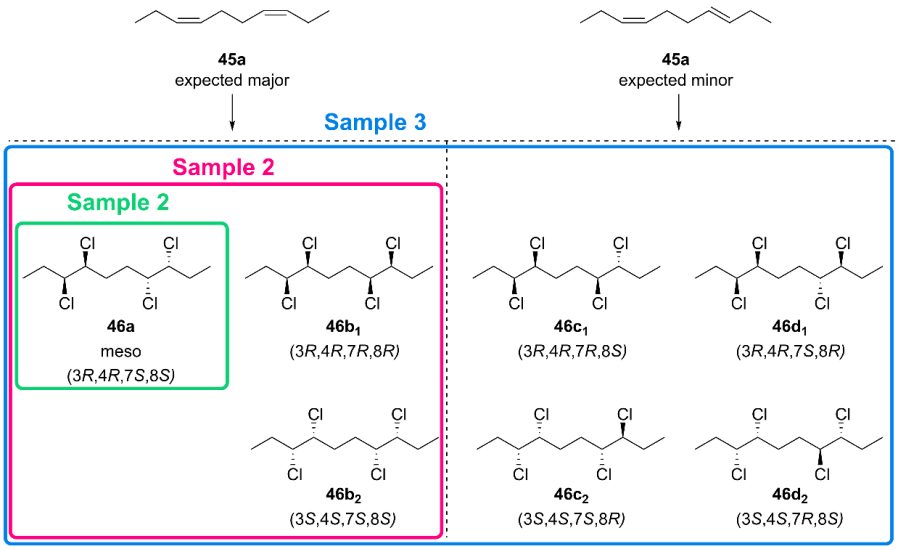


*Scheme 4.1. Synthesis of CP **46** (isomer mixture).*

The Wittig reaction of polyenes **4a-f** and **7a-e** in publication I (Section 2) showed a product mixture of the two stereoisomers, where the created double bond could be in either *Z* or *E*-configuration. While most peaks of the two compounds were unresolved multiplets in the respective ^{1}H NMR spectra, the skipped double bond (RCH=CH-C*H*$_2$-CH=CHR) peak in compounds **4a-f** and **7a** was generally well-resolved. By comparison of this data with known literature,[31] the percentage of *Z*-isomers for these compounds was determined to be 83-95%. Comparable values could be expected for the structurally similar diene **45**, synthesised by the same protocol and following expected selectivity as described in the literature.[31]

For the compounds in publication I, we saw the presence of two major diastereomers of **5a-f** by ^{13}C NMR spectroscopy, as well as some minor peaks, which indicated a stereospecific chlorination of the major precursors *Z*-**4a-f** from the Wittig olefination (Section 2.3.1). Literature dichlorination of alkenes by the Markó-Maguire protocol, Yoshimitsu protocol and molecular chlorine are all expected to follow an *anti*-specific mechanism.[27,35,37] Keeping the *Z*-selectivity of the Wittig reaction and the *anti*-stereospecificity of chlorination in mind, we expected a product mixture consisting

predominantly of two different diastereomers in the synthesis of CP **46**. The expected composition of stereoisomers (based on literature and previous results in publication I) of **46** is presented in Scheme 4.2.



*Scheme 4.2. Expected stereoisomer products of CP **46** based on anti-addition of diene **45**.*

The diastereomer product mixture of **46** was purified as summarised by the schematic shown in Figure 4.1.

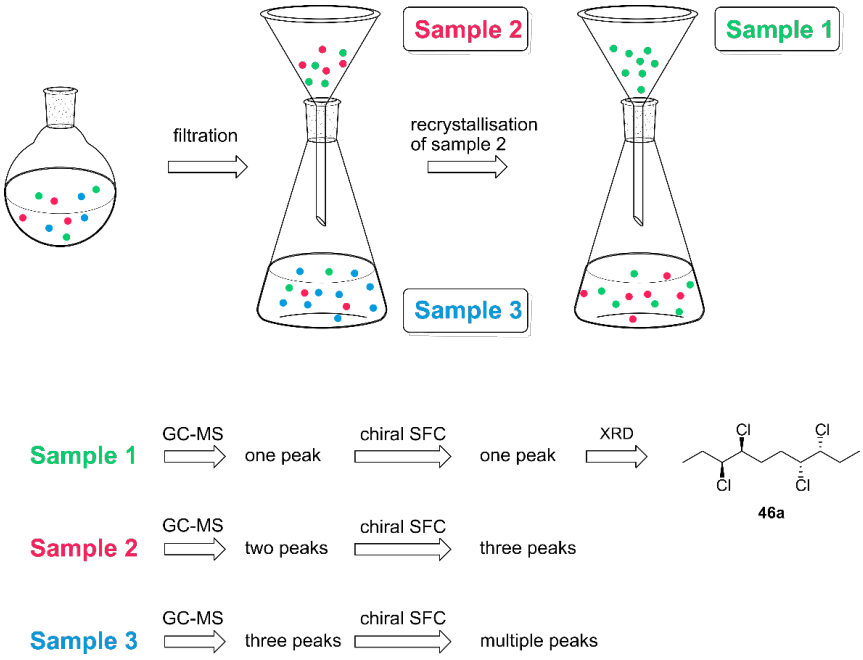


*Figure 4.1. Isolation and enrichment process of the different samples of **46** from the crude reaction mixture and overview of the results gained from the different analyses.*

In the first work-up step, a diastereomeric mixture of **46** (sample 2) was collected by filtration, after having precipitated from the reaction mixture. Sample 2 was obtained in a 21% yield over two steps (from **12a**) and showed the presence of two peaks at t_R = 15.9 (36%) and 16.1 min (64%) by GC-MS, both with a mass-to-charge ratio of m/z = 242.1 ($[M\text{-}HCl]^{+\cdot}$). Recrystallisation of sample 2 from isopropyl alcohol gave a product (sample 1) consisting of only one peak by GC-MS at t_R = 16.1 min. Single-crystal X-ray diffraction analysis gave unambiguous evidence of the presence of **46a** in sample 1. Furthermore, supercritical fluid chromatography (SFC) using a chiral column showed one peak at $t_{\mathbf{46a}}$ = 7.2 min. Evidentially, sample 1 contains only the mesocompound **46a** in high purity.

The chiral SFC analysis of sample 2, with the same conditions as for sample 1, yielded three peaks with relative intensity of 17:66:17 and retention times of $t_{\mathbf{4b1}}$ = 6.0 min, $t_{\mathbf{4a}}$ = 7.2 min, and $t_{\mathbf{4b2}}$ = 8.4 min. These results, when compared with the GC-MS and chiral SFC analysis of sample 1, are consistent with the presence of two different diastereomers **46a** and **46b**, where one of them is an enantiomeric pair $\mathbf{46b_1}$ and $\mathbf{46b_2}$.

Lastly, the mother liquor from the original reaction mixture was concentrated and purified by dry-column vacuum chromatography.[67] This yielded sample 3 which showed four peaks at t_R = 15.6, 15.7, 16.0 and 16.2 min by GC-MS, all with the same mass-to-charge ratio, which were believed to originate from the four different diastereomers **46a-d**. The SFC separation of sample 3 was not fully resolved, showing four major peaks, but likely several more co-eluting peaks.

In conclusion, the evidence obtained through our analyses of the stereoisomers of CP **46** is consistent with the major prevalence of **46a** and **46b**. This is in accordance with the well-documented *Z*-stereoselectivity of the Wittig reaction for non-stabilised ylides and a stereospecific *anti*-dichlorination of the two *Z*-alkene units of **45a**.

5 Publication IV: Nuclear Magnetic Resonance as a tool to determine chlorine percentage of chlorinated paraffin mixtures

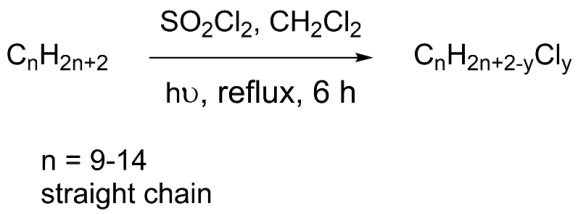
5.1 Introduction

Single-chain CPs are produced through radical chlorination of single *n*-alkanes and are described by their carbon chain length and chlorine percentage.[5,18] Similarly, technical CP mixtures are also produced through radical chlorination but instead, mixed *n*-alkane feedstocks are chlorinated. They are typically reported with carbon chain range (e.g. SCCP, MCCP or LCCP) and chlorine percentage. Despite being characterised by chlorine percentage, it is hard to find information in regard to how this property was determined and is often not stated by distributors. Nevertheless, a few different methods have been reported used for single-chain CP mixtures. In one method the CPs are dechlorinated, converting organic chlorine to inorganic chloride, and the chloride concentration is measured by titration.[68] Another method describes the use of ^{1}H NMR spectroscopy and the result is compared to values from elemental analysis.[26] For technical mixtures, we could not find specific information regarding chlorine percentage determination, but it was suggested it could be determined from the weight of chlorine gas added to the *n*-alkane feedstock in the production plant,[69] or could be obtained through dechlorination and titration.

In this publication, one novel NMR method (method C) for determining chlorine percentages of both single-chain and technical CP mixtures was developed. Two adjusted NMR methods (method A and B) for the chlorine percentage determination of single-chain mixtures based on the method reported by Sprengel *et al*. were also described.[26] All methods were tested on synthesised single-chain mixtures and values were verified by a dechlorination and chloride-titration protocol. The expanded utility of method C made it possible to test it on one technical CP mixture and mixtures of single-chain CPs as well. The purpose of creating the new methods was to obtain a general tool for determining chlorine percentage for single-chain CPs and technical mixtures with an accessible instrumentation. Additionally, it was created to achieve data that are reliable, validated and traceable, as the material is intended to be used as reference standards and in the future as certified reference materials. This section will describe how the new single-chain CP reference standards were produced, the process regarding the chlorine percentage determination and results and finally a general description of the validation. Please refer to the article manuscript and supporting information for the different methods and further details.

5.2 Synthesis

Synthesis of single-chain CPs was conducted as described by Tomy *et al.*,[70] with some minor adjustments. This protocol was used for the synthesis of eleven single-chain CPs (**C_9**, **C_{10A-B}**, **C_{11A-B}**, **C_{12A-B}**, **C_{13A-B}**, **C_{14A-B}**) and will in further work be used to synthesise more MCCPs and LCCPS. The method converts *n*-alkanes to chlorinated paraffins by radical chlorination with sulfuryl chloride, see Scheme 5.1.



Scheme 5.1: Radical chlorination of n-alkane with sulfuryl chloride.

The method is conducted as follows: To a solution of *n*-alkane (1.0 eq.) in dichloromethane (500 mL/10 g *n*-alkane), sulfuryl chloride (7.3 eq.) was added. The reaction mixture was irradiated under UV (400 W Mercury lamp) and refluxed for minimum 6 h. Longer irradiation times were used for longer alkane chains. Excess solvent and sulfuryl chloride were removed by distillation and the residual liquid was purified by flash column chromatography (SiO_2, gradient: 100% petroleum ether to 80% ethyl acetate in petroleum ether). Drying at 60 – 70 °C under high vacuum from 16 h up to several days was carried out to remove excess solvents. This afforded single-chain mixtures $C_{9\text{-}14}$ as viscous oils, ranging from slightly yellow to brown. These mixtures are indexed with A, for example **C_{12A}**.

To achieve higher chlorine percentages the product was subjected to the reaction conditions a second time. Mixtures obtained after the second chlorination round are indexed with B, for example **C_{12B}**.

5.3 Chlorine percentage determination

To determine the chlorine percentages, ^{1}H NMR and Heteronuclear Single Quantum Coherence (HSQC) analysis were conducted for all the different single-chain mixtures and for one technical mixture.

During the spectral processing, the different constitutional regions were individually integrated in method A – C (Figure 5.1c), except for the R'CH_2R and CH_3R areas (R, R' = CP chain) in method A, which was integrated as a joint cluster region (Figure 5.1b). The integration of CP proton regions was based on the different constitutional moieties (Figure 5.1a), identified by HSQC. No geminal chlorination was assumed to occur. Geminal chlorination is typically disfavoured during radical chlorination, yet has been observed as a minor component in CP mixtures, particularly when the chlorine percentage is high.[17,26] It follows that the assumption holds more validity for mixtures of low chlorine content.

Once the area of the different regions in the ^{1}H NMR spectrum has been established, the values can be applied to the equations found in the different methods presented in Section 2.4 of article III. The set of equations for method A and B are both reliant on the chain length of the CP mixture, thus they can only be used for single-chain CP mixtures. Method C, developed in the course of this project, estimates a mean carbon chain length of the CP mixture from the ^{1}H NMR spectrum, which opens the possibility of being used for mixtures of multiple and unknown chain lengths, as well as for single-chain CP mixtures.

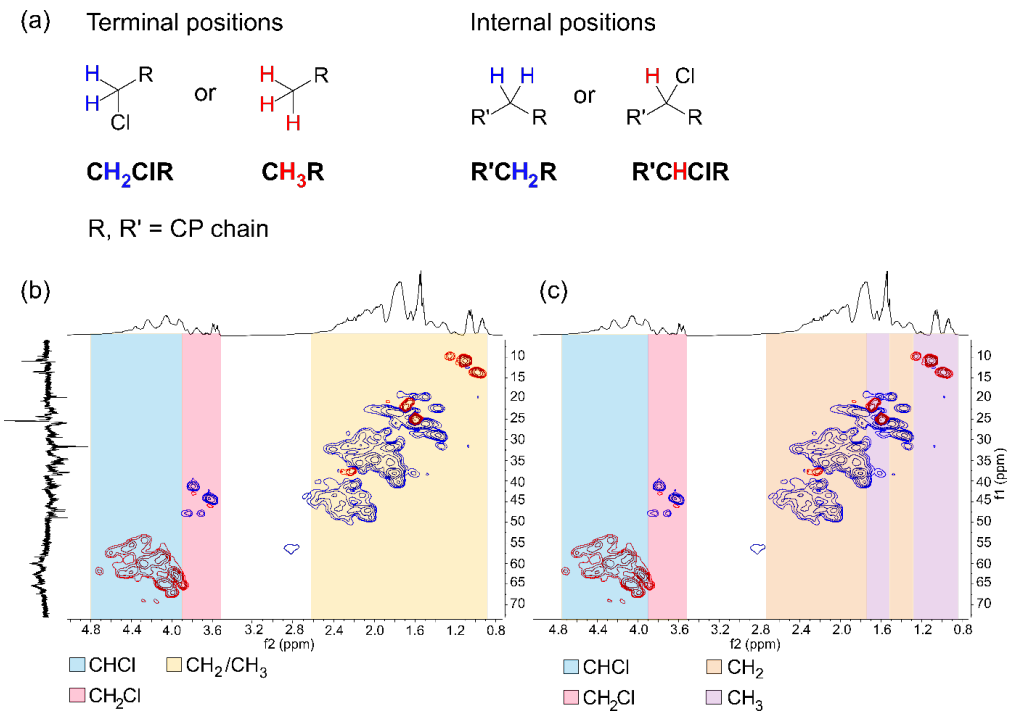


Figure 5.1: a) The possible constitutions in a CP mixture assuming no geminal chlorination and b) and c) ^{1}H-^{13}C HSQC (600 MHz, $CDCl_3$) of single-chain CP mixture ***C_{12A}*** *exemplifying how to separate the integral regions of the ^{1}H NMR spectrum for b) method A and c) method B and C.*

By following the three methods, a chlorine percent estimation was found for our synthesised single-chain CP mixtures (**C_9**, **C_{10A-B}**, **C_{11A-B}**, **C_{12A-B}**, **C_{13A-B}**, **C_{14A-B}**), in addition to two donated single-chain mixtures (**C_{14C}**, **C_{15}**) and one technical mixture (**$C_{tech.}$**). The results are presented in Table 5.1 and are given together with values obtained through titration as a reference. Titration method and the validation of the titration method is described in Section 5.4.

Table 5.1: Results of the chlorine percentage calculation of all single-chain CP mixtures and one purchased technical mixture using ^{1}H NMR methods A-C, as well as the results from titration experiments. The standard error of titration is given with a 99% confidence interval.

Mixture	Chemical formula	Method A [%]	Method B [%]	Method C [%]	Titration[a] [%]
C_9	$C_9H_{20-y}Cl_y$	51.8	47.9	48.6	50.2 ± 0.1
C_{10A}	$C_{10}H_{22-y}Cl_y$	52.8	50.7	52.5	54.4 ± 0.1
C_{10B}	$C_{10}H_{22-y}Cl_y$	60.1	57.6	59.2	62.7 ± 0.3
C_{11A}	$C_{11}H_{24-y}Cl_y$	53.1	52.0	52.1	56.5 ± 0.1
C_{11B}	$C_{11}H_{24-y}Cl_y$	58.3	55.5	57.7	63.9 ± 0.2
C_{12A}	$C_{12}H_{26-y}Cl_y$	53.5	52.0	53.8	54.1 ± 0.7[b]
C_{12B}	$C_{12}H_{26-y}Cl_y$	58.1	54.5	57.3	58.5 ± 0.2[c]
C_{13A}	$C_{13}H_{28-y}Cl_y$	45.6	41.8	43.3	46.8 ± 0.1
C_{13B}	$C_{13}H_{28-y}Cl_y$	57.5	56.3	57.7	60.8 ± 0.1
C_{14A}	$C_{14}H_{30-y}Cl_y$	37.8	36.3	36.5	40.7 ± 0.1
C_{14B}	$C_{14}H_{30-y}Cl_y$	43.3	41.2	42.4	44.9 ± 0.1
C_{14C}	$C_{14}H_{30-y}Cl_y$	59.5	57.6	59.0	60.9 ± 0.1
C_{15}	$C_{15}H_{32-y}Cl_y$	49.6	49.5	48.5	52.3 ± 0.2
$C_{tech.}$	$C_nH_{2n+2-y}Cl_y$	-	-	38.5	40.3 ± 0.7

[a] *Average value of three titrations*

[b] *Average value of nine titrations*

[c] *Average value of six titrations*

Compared to the titration results, all ^{1}H NMR methods gave chlorine percentages on the lower side, which aligns with the fact that neither of the methods accounts for geminal chlorination. The main advantage of using the NMR method over titration is that it is much quicker and easier to perform. Furthermore, the titration is more prone to user error such as differences in end-point reading and weight measurement.

Method B and C are reliant on separation of the constitutional regions for an accurate spectral processing. The effect of integration error of the R'CH_2R and CH_3R region,

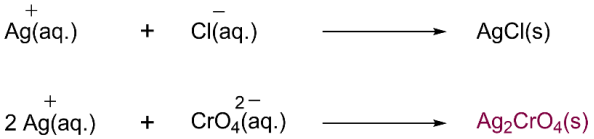
where the most overlap is observed, was illustrated in Fig. 4 Section 3.4 in article III. While both are reliant on the integration, method C was more robust and the chlorine percentage does not change significantly.

The integration error from the division of the R'CH_2R and CH_3R region does not change the chlorine percentage outcome of method A. This is because the total area is adjusted according to a stochastic model of the region and will remain constant as long as the total R'CH_2R and CH_3R area is constant. The stochastic model is based on the assumption that all terminal positions of the CPs in the mixture in inhabited by protons. It follows that the model should perform well with low chlorine percentages, where fewer terminal chlorines are observed, and be less accurate when the chlorine percentage is high.

Of the three different methods presented, method C is the only one that can estimate chlorine percentage for complex mixtures. The two other methods use the chain length directly in the calculations, whereas method C estimates the average chain length of the CPs based on the ^{1}H NMR spectrum. While it would have been interesting to test the method on more technical mixtures, we only had one available for testing at the time. Instead, we mixed different single-chain mixtures and analysed them by ^{1}H NMR and HSQC spectroscopy. The results of these mixtures are shown in Table 3 in article III. Entry 1 was designed to test the method at the extremities of high and low chlorine content, mixing $\mathbf{C_{tech.}}$ (40.3 ± 0.7 %Cl) and $\mathbf{C_{10B}}$ (62.7 ± 0.3 %Cl). The chlorine percentage was estimated to 48.0 %Cl for the mixture, whereas the sum of weight fractions times the chlorine percentages of the individual mixtures gave a value of 49.0 %Cl.

5.4 Titration and Validation

As previously mentioned, the different ^{1}H NMR methods were tested up against a titration method. The titration protocol was validated and accredited (by ANSI national accreditation board) as a result of the work conducted in this publication. The method is described in detail in Section 1 of the SI of paper III. In short summary, the CP mixtures were subjected to a dechlorination reaction (sodium metal in isopropyl alcohol), yielding a solution with inorganic chlorides. The concentration of chloride was measured by a standard Mohr's titration, using K_2CrO_4 as indicator (Scheme 5.2).



Scheme 5.2. Reaction of silver ions with chloride and chromate ions during the titration. The titrand colour changed from yellow to deep red, when all chloride was consumed (titration end-point).

Since the CP mixtures are undefined and does not have a theoretical value for the chlorine percentage, a sample of 1,2,7,8-tetrachlorooctane was used for measuring the accuracy of the titration method and was later used as control for the other titrations. The calculation of the theoretical chlorine percentage ($wt\%\ Cl$) of 1,2,7,8-tetrachlorooctane was conducted using Equation 5.1:

$$wt\%\ Cl = \frac{n_{Cl} * M_{Cl}}{M_{w,CP}} \rightarrow 56.3\ \%Cl$$

Equation 5.1

Where n_{Cl} is the number of chlorines, M_{Cl} is the elemental mass of chlorine and $M_{w,CP}$ is the molecular weight of the CP. The average chlorine percentage, 56.9 %Cl, was compared to the theoretical value and the accuracy over nine titrations was found to be 98.9%.

The uncertainty given in Table 5.1 is the expanded uncertainty (U) calculated from the standard uncertainty (u) with a coverage factor (k). See Equation 5.2.

$$U = k * u$$

Equation 5.2

Assuming a natural distribution of the titration values, a coverage factor of k = 2.58 was set, to find the expanded uncertainty at a 99% confidence interval.[71] The standard uncertainty was determined from the standard deviation (σ) of the chlorine percentage given by Equation 5.3 over nine titrations ($N = 9$).

$$u = \frac{\sigma}{\sqrt{N}}$$

Equation 5.3

The general equation for standard deviation is shown in Equation 5.4.

$$\sigma^2 = \frac{\sum_{i=1}^{N}(\%Cl_j - \mu)^2}{N}$$

Equation 5.4

Where $\%Cl_j$ denotes the different titration values obtained through Mohr's protocol and μ is the average chlorine percentage over all titrations. The accepted value of uncertainty was set to ±2 wt.%Cl. As can be seen in Table 5.1 the expanded uncertainties range between 0.1 – 0.7 %Cl and were well within accepted values.

6 Final Conclusion and Recommendation

Several different strategies to synthesise new constitutionally defined CPs, in high purity, have been investigated during this project. The overall synthetic strategy can be generalised into three steps:

1. Preparation of the unsaturated hydrocarbon chain
2. Dichlorination of alkenes
3. Final purification to obtain high purity standards.

Preparation of unsaturated hydrocarbons

Three different strategies were used for the synthesis of the olefin chains. The shortest reaction path was Strategy I, where dienes **4a-f** and trienes **7a-e** were synthesised through a single Wittig reaction, preceded by the preparation of the Wittig salt. Here, only one double bond was synthesised and the other double bonds were pre-

incorporated from the starting aldehyde and/or the phosphonium salt. While being a conveniently short pathway to olefins, this strategy is heavily reliant on the availability of appropriate starting materials.

In Strategy II and III, non-commercial alcohols with two double bonds were synthesised. These strategies involved several extra steps, but allowed a larger freedom to the structural design of the olefins. Using strategy II, the ^{13}C-labelled polyenes **22a-b** and **26a-b** were obtained. This protocol includes a Swern oxidation of a mono-protected linear diol, followed by a Wittig coupling with an appropriate ylide. After removal of the alcohol protecting group, the dienyl intermediate was oxidised by another Swern oxidation and yielded the desired polyene after a Wittig reaction.

By using Strategy III, ^{13}C-labelled polyenes **34** and **39** were prepared. In this route, diynyl alcohols were synthesised through an acetylide substitution reaction with alkynyl bromide. A partial reduction of the triple bonds was conducted by two different protocols: hydrogenation using Lindlar's catalyst and hydrogenation with P-2 nickel. The former proved difficult to control in terms of conversion: short reaction times gave low conversion of the starting diynyl alcohol, while leaving the reaction for a longer time resulted in over-reduction. The conversion control was improved using the P-2 nickel protocol, but resulted in a lower yield after purification. In conclusion, both reduction protocols are in need of optimisation to achieve better conversion or yields. Nevertheless, both approaches can be used to achieve the desired dienyl alcohols.

Comparing the two strategies II and III, both methods can be used to achieve a broad range of polyenes with different number and position of the double bonds. Both syntheses involve volatile intermediates, which must be handled carefully during the purification steps to avoid evaporation and loss of material. The target olefins are achieved in five steps for Strategy II and four to five steps for Strategy III. The excess of alkynyl bromide in the latter proved difficult to remove with the tested purification protocols and was carried over in the subsequent steps. Fortunately, it could be removed after the chlorination reaction. Depending on the substrate, both strategies (II and III) are applicable for the synthesis of polyenes.

Dichlorination of alkenes and purification

In previous research, CPs have been synthesised from the corresponding *n*-alkanes by using molecular chlorine in either neat conditions or dissolved in carbon tetrachloride. Due to the hazardous nature of the chemicals involved in these protocols, we tested two different chlorination alternatives in the synthesis of CPs **5a-f** and **8a-e**. The Markó-Maguire protocol was used for **5a-f** and converted the olefins to CPs within a few hours. The Yoshimitsu protocol was tested for **8a-e** and often showed the presence of unreacted double bonds, even after 48 hours of reaction time. Both of these methods yielded the desired CPs, but with a large amount of intractable residue from the large amount of reagents required. The purification was further complicated by the presence of under- and over-chlorinated byproducts.

Due to the difficulties with purification using either the Markó-Maguire or Yoshimitsu protocols, the use of molecular chlorine was reconsidered. Molecular chlorine is a highly toxic gas and must be handled safely. In this project, chlorine gas was generated *in-situ* and was bubbled into the reaction solution with appropriate polyene. This

protocol was used to synthesise non-isotope labelled CP **46** and ^{13}C-labelled CPs **23a-b**, **27a-b**, **35**, **40** and **44**.

Using molecular chlorine as chlorinating agent, the reaction is prone to over-chlorination, due to the presence of chlorine radicals in solution. To reduce over-chlorination, the reactions were conducted at -78 °C and in darkness. Furthermore, the use of *n*-hexane facilitated a precipitation of the product from the reaction solution, lowering over-chlorination and simplifying the final purification protocol.

The protocol, using molecular chlorine, is recommended compared to the Markó-Maguire or Yoshimitsu protocols, as this method avoids the large amounts of intractable residues and offers a simplified final purification of solid CP products. A final remark on the purification of CPs is that high purity is difficult to achieve using only column chromatography. Recrystallisation gave compounds in high purity, but this is only possible for solid products. The yield after recrystallisation may vary for different compounds as the reaction conditions result in a diastereomeric mixture of constitutionally defined CPs. Recrystallisation of these mixtures may lead to enrichment of specific diastereomers, at the expense of others.

7 Experimental

This section will describe the experimental detail of the compounds synthesised in publication II (patent) discussed in Section 3.

7.1 General Information

All chemicals were purchased from commercial distributors and were used without further purification. Dry THF was obtained by storing it over 3Å molecular sieves in 20% mass/volume, according to the procedure described by Williams and Lawton.[72] Other dry, septum-sealed solvents were used as supplied. All water-sensitive reactions were conducted with over dried glassware and under argon atmosphere (Ar-atm). Flash column chromatography (FCC) was carried out with silica gel 60 (0.047-0.060 mm, J.T. Baker) and dry column vacuum chromatography (DCVC),[67] was carried out with silica gel 60 (0.015-0.040 mm, Merck).

NMR spectroscopy was performed with a Bruker 400 MHz Avance III HD or a Bruker 600 MHz Avance III HD. Spectral processing was carried out with MestReNova v14.2.1-27684, chemical shifts of NMR signals are listed in ppm using TMS (δ = 0.00) as a reference.

Chromatographic purity was obtained by Agilent 6890N gas chromatograph equipped with Agilent 7683B injector, Agilent DB-5 fused silica WCOT column (30 m × 0.25 mm × 0.25 µm) and a quadrupole mass spectrometry detector (MSD) Agilent 5975B using electron impact (EI) ionisation. The carrier gas used was helium with a flow rate of 1 mL/min. Acquisition was set to full-scan mode.

7.2 General Procedures

Procedure A: Swern oxidation,[73] Wittig reaction[74] and deprotection

DMSO (2.0 eq.) was added to a solution of oxalyl chloride (1.2 eq.) in dry DCM (1 mL/mmol alcohol) under Ar-atm at -78 °C. The mixture was slowly heated to -60 °C and stirred for 5 min. After re-cooling to -78 °C, protected alcohol (1.0 eq.) in dry DCM (1 mL/mmol alcohol) was added slowly. The reaction was warmed to -60 °C and stirred for 5 min. TEA (4.0 eq.) was added at -78 °C and the reaction mixture was stirred for 1 h at -60 °C before it was quenched with water (100 mL). The two layers were separated, and the aqueous layer was extracted with DCM (2 x 150 mL). The combined organic layers were washed with aq. HCl (1 M, 100 mL) and dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by FCC (SiO_2, 10% ether in *n*-pentane, R_f ~0.9) was conducted and aldehyde was used in the next step without further purification.

Potassium *tert*-butoxide (1.1 eq.) in dry THF (1 mL/mmol alcohol from step above) was added to a suspension of Wittig salt (1.2 eq.) in dry THF (4 mL/mmol alcohol) under inert atmosphere at 0 °C. The mixture was stirred for 10 min at 0 °C and 1 h at r.t. After re-cooling to 0 °C, aldehyde in dry THF (1 mL/mmol alcohol) was added to the reaction, which was stirred for 1 h at 0 °C. Sat. aq. NH_4Cl and water (1:1, 100 mL) was used to quench the reaction. Diethyl ether (50 mL) was added, and the two layers separated. The aqueous layer was extracted with diethyl ether (2 x 50 mL) and the pooled organic layers were washed with brine (2 x 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Pentane (~30 mL) was added, the precipitate filtered off and the filtrate concentrated carefully under reduced pressure three times.

The residue after concentration was dissolved in dry THF (4 mL/mmol alcohol) and cooled to 0 °C, before TBAF (1 M, 1.2 eq.) was added. After stirring for 16 at r.t., the reaction was quenched with sat. aq. NH_4Cl and water (1:1, 100 mL) and the two layers were separated. The aqueous layer was extracted with ether (3 x 50 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried over $MgSO_4$, filtered, and carefully concentrated under reduced pressure. Purification by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, stepwise elution diethyl ether/*n*-pentane: 200 mL 75:25 and 200 mL 50:50, R_f ~0.55 (50:50 diethyl ether/*n*-pentane)) and careful concentration under reduced pressure afforded solutions containing the dienyl alcohols.

General procedure B: Swern oxidation[73] and Wittig reaction[74] to trienes

DMSO (2.0 eq.) was added to a solution of oxalyl chloride (1.2 eq.) in dry DCM (8 mL/mmol alcohol) under Ar-atm at -78 °C. The mixture was slowly heated to -60 °C and stirred for 5 min. After re-cooling to -78 °C, protected alcohol (1.0 eq.) in dry DCM (3 mL/mmol alcohol) was added slowly. The reaction was warmed to -60 °C and stirred for 5 min. TEA (4.0 eq.) was added at -78 °C and the reaction mixture was stirred for 1.5 h at -78 °C. The reaction mixture was warmed to r.t. and was stirred for an additional 15 min before it was quenched with water (25 mL). The two layers were separated, and the aqueous layer was extracted with DCM (2 x 50 mL). The combined organic layers were washed with aq. HCl (1 M, 25 mL) and dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by FCC (SiO_2, 10% ether in *n*-pentane, R_f ~0.9) was conducted and aldehyde was used in the next step without further purification.

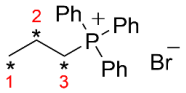
Potassium *tert*-butoxide (12% sol. in THF, 1.1 – 0.6 eq.) was added to a suspension of Wittig salt (1.1 – 0.5 eq.) in dry THF (8 mL/mmol) under Ar-atm at 0 °C. The mixture was stirred for 10 min at 0 °C and 1 h at r.t. After re-cooling to 0 °C, aldehyde in dry THF (2 mL/mmol alcohol) was added to the reaction, which was stirred for 1 h at 0 °C. Sat. aq. NH_4Cl and water (1:1, 50 mL) was used to quench the reaction. Diethyl ether (25 mL) was added, and the two layers separated. The aqueous layer was extracted with diethyl ether (2 x 50 mL) and the pooled organic layers were washed with brine (2 x 50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Pentane (~20 mL) was added, the precipitate filtered off and the filtrate concentrated carefully under reduced pressure three times. Crude triene was purified by FCC (SiO_2, *n*-pentane, R_f ~0.9) and carefully concentrated under reduced pressure.

General procedure C: Chlorination with molecular chlorine

The solution with appropriate crude olefin (full amount from previous step) was diluted with *n*-hexane (50 mL) and cooled to -78 °C. In a different flask 2 M HCl (aq., 5 – 10 mL/g trichloroisocyanuric acid) was dropped over trichloroisocyanuric acid (2 g or ~3 eq.) which was bubbled into the olefin solution in darkness. Extra trichloroisocyanuric acid (~1 g) and 2 M HCl (aq., 5 mL) were added if the reaction mixture did not turn yellow. When the reaction mixture had turned yellow, the solution was assumed saturated with chlorine and the supply was terminated. The reaction was stirred for 2 h at -78 °C in darkness, before it was quenched by addition of 1-pentene (1 – 2 mL) until colourless. Purification is specified under each entry.

7.3 Experimental details

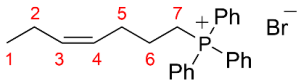
7.3.1 Triphenyl(propyl-$^{13}C_3$)phosphonium bromide (12b)



12b * = ^{13}C

$^{13}C_3$-Propanol (**11b**, 2.02 g, 32 mmol, 1 eq.) and triphenylphosphine hydrobromide (11 g, 32 mmol, 1 eq.) was combined in a pressure vial and heated to 110 °C. After 1 – 2 h the reaction mixture had solidified. It was left for additional 16 h at 110 °C before it was cooled to r.t. The crude solid was dissolved in acetonitrile (10 mL) at 90 – 100 °C and the solution was kept at this temperature for 2 h. Once the solution was cooled, diethyl ether (~5 mL) was added to precipitate out **12b** (6.83 g, 18 mmol) as an off-white solid. The resulting mother liquor was concentrated over celite (~1 g) and purified by DCVC (SiO_2, *d* = 2 cm, *l* = 5 cm, stepwise elution w. EtOAc/MeOH: 2 x 20 mL 100:0, 2 x 20 mL 90:10, 2 x 20 mL 80:20). The resulting product fractions was combined with the previously precipitated product and were concentrated under reduced pressure. This afforded **12b** (10.3 g, 27 mmol, 83%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.97 – 7.92 (m, 3H, p-Ph), 7.88 – 7.78 (m, 12H, o-Ph, m-Ph), 3.75 – 3.46 (m, 2H, H_1), 1.75 – 1.44 (m, 2H, H_2), 1.25 – 0.95 (m, 3H, H_3). ^{1}H NMR spectrum was similar to reported data for native compound,[75] except for extra splitting from one-bond H–^{13}C coupling ($J \approx 125$ Hz) and other H–^{13}C signal splitting.

7.3.2 (*Z*)-Hept-4-en-1-yltriphenylphosphonium bromide (16)



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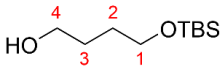
p-Toluenesulfonyl chloride (7.72 g, 41 mmol, 1.1 eq.) in DCM (100 mL) was added to a solution of alcohol **13** (4.22 g, 37 mmol, 1.0 eq.) and TEA (11.3 mL, 81 mmol, 2.2 eq.) in DCM (300 mL) under Ar-atm at r.t. After stirring at r.t. for 48 h, the reaction was quenched with water (100 mL). The two layers were separated, and the aqueous layer was extracted with DCM (3 x 100 mL). The combined organic layers were washed with water (200 mL), 10 wt.% $NaHCO_3$ (200 mL) and brine (200 mL) and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, stepwise elution w. petroleum ether/DCM: 100 mL 70:30, 100 mL 60:40, 100 mL 50:50 and 100 mL 40:60) afforded the tosylate **14** (9.66 g, 36 mmol, 97%).

Lithium bromide (6.43 g, 74 mmol, 2.0 eq.) was added to a solution of **14** (9.66 g, 36 mmol) in acetone (120 mL). After stirring at r.t. for 16 h, the resulting suspension was filtered, and acetone was removed by distillation at atmospheric pressure. The crude was mixed with water and extracted with ether (3 x 50 mL). Combined organic layers were dried over $MgSO_4$ and filtered before ether was removed by distillation at atmospheric pressure. Crude product was purified by Kügelrohr distillation (25 mbar, 100-130 °C) and afforded the alkenyl bromide **15** (5.31 g, 30 mmol, 83%) as a colourless liquid.

Triphenylphosphine (8.21 g, 31 mmol, 1.03 eq.) and acetonitrile (40 mL) was added. The solution was refluxed for 48 h, before it was concentrated under reduced pressure and purified by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, stepwise elution w. EtOAc/MeOH: 200 mL 90:10, 200 mL 80:20). The phosphonium salt **16** (12.0 g, 27 mmol, 90%) was obtained as a white semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 9H), 7.73 – 7.64 (m, 6H), 5.45 – 5.33 (m, 1H), 5.25 – 5.14 (m, 1H), 3.80 – 3.67 (m, 2H), 2.37 (q, *J* = 7.4 Hz, 2H), 1.97 (p, *J* = 7.4 Hz, 2H), 1.68 (app. h, *J* = 7.9 Hz, 2H), 0.86 (app. t, *J* = 7.5 Hz, 3H). Similar to published data.[76]

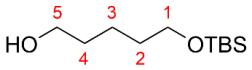
7.3.3 4-((tert-Butyldimethylsilyl)oxy)butan-1-ol (18a)



18a

Sodium hydride (60%, 2.00 g, 50 mmol, 1 eq.) was added in one portion to a solution of butane-1,4-diol (**17a**, 4.51 g, 50 mmol, 1 eq.) in dry THF (100 mL) at r.t. under Ar-atm. After stirring vigorously for 1 h at r.t., *tert*-butyldimethylsilyl chloride (7.54 g, 50 mmol, 1 eq.) was added at r.t. and the mixture was stirred for an additional 2 h at r.t. The reaction mixture was quenched with 10% aq. $NaHCO_3$ (100 mL) and water (10 mL) and the two layers were separated. The aqueous layer was extracted with EtOAc (4 x 50 mL) and the combined organic layers washed with brine (100 mL) and were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, stepwise elution with petroleum ether/DCM: 150 mL 85:15, 150 mL 80:20, 150 mL 60:40 and 150 mL 40:60) followed by Kügelrohr distillation (high vacuum, 150 – 160 °C) afforded **18a** (5.27 g, 26 mmol, 52%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 – 3.53 (m, 4H), 3.25 (br s, 1H), 1.62 – 1.51 (m, 4H), 0.84 (app. s, 9H), 0.01 (app. s, 6H). Similar to reported data.[77]

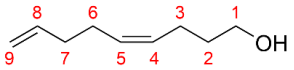
7.3.4 5-((tert-Butyldimethylsilyl)oxy)pentan-1-ol (18b)



18b

Sodium hydride (60%, 2.00 g, 50 mmol, 1 eq.) was added in one portion to a solution of pentane-1,5-diol (**17b**, 5.21 g, 50 mmol, 1 eq.) in dry THF (100 mL) at 0 °C under Ar-atm. After stirring vigorously for 1 h at r.t., *tert*-butyldimethylsilyl chloride (7.51 g, 50 mmol, 1 eq.) was added at 0 °C and the mixture was stirred for an additional 2 h at r.t. The reaction mixture was quenched with 10% aq. $NaHCO_3$ (100 mL) and water (10 mL) and the two layers were separated. The aqueous layer was extracted with EtOAc (4 x 50 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, stepwise elution with petroleum ether/DCM: 150 mL 90:10, 150 mL 80:20 and 150 mL 60:40) followed by Kügelrohr distillation (high vacuum, 120 – 145 °C) afforded **18b** (6.20 g, 28 mmol, 57%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 – 3.53 (m, 4H), 1.63 – 1.52 (m, 4H), 1.45 – 1.37 (m, 2H), 0.90 (app. s, 9H), 0.05 (app. s, 6H). Similar to reported data.[77]

7.3.5 (*Z*)-Nona-4,8-dien-1-ol (20a)

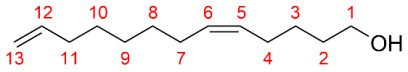


20a

Following general procedure A, aldehyde **19a** was prepared from **18a** (2.04 g, 10 mmol, 1.0 eq.), DMSO (1.42 mL, 20 mmol, 2.0 eq.) and oxalyl chloride (1.04 mL, 12 mmol, 1.2 eq.) in dry DCM (110 mL). Subsequently, TEA (5.6 mL, 40 mmol, 4.0 eq.) was added and after reaction and purification, a solution of **19a** was obtained and used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Dienyl alcohol **20a** was prepared from potassium *tert*-butoxide (1.23 g, 11 mmol, 1.1 eq.), **2b** (4.94 g, 12 mmol, 1.2 eq.) and **19a** in dry THF (60 mL). After reaction and purification, the residue was dissolved in dry THF (40 mL) and TBAF (1 M, 12 mL, 12 mmol, 1.2 eq.) was added. The procedure afforded dienyl alcohol **20a** (584 mg, 4.2 mmol, 42% yield from NMR over three steps from **18a**) as a colourless oil containing residual ether and *n*-pentane. Data for **20a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 – 5.75 (m, 1H), 5.45 – 5.37 (m, 2H), 5.03 (app. dq, *J* = 17.2, 1.7 Hz, 1H), 4.99 – 4.95 (m, 1H), 3.66 (app. t, *J* = 6.5 Hz, 2H), 2.18 – 2.08 (m, 6H), 1.67 – 1.60 (m, 2H). Similar to data for the corresponding *E*-isomer.[78]

7.3.6 (*Z*)-Trideca-5,12-dien-1-ol (20b)

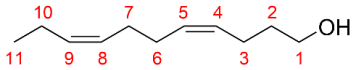


20b

Following general procedure A, aldehyde **19b** was prepared from **18b** (1.09 g, 5 mmol, 1.0 eq.), DMSO (0.71 mL, 10 mmol, 2.0 eq.) and oxalyl chloride (0.52 mL, 12 mmol, 1.2 eq.) in dry DCM (55 mL). Subsequently, TEA (2.8 mL, 40 mmol, 4.0 eq.) was added and after reaction and purification, a solution of aldehyde **19b** was obtained and used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Dienyl alcohol **20b** was prepared from potassium *tert*-butoxide (617 mg, 5.5 mmol, 1.1 eq.), **2e** (2.72 g, 6 mmol, 1.2 eq.) and **19b** in dry THF (35 mL). After reaction and purification, the residue was dissolved in dry THF (20 mL) and TBAF (1 M, 6.0 mL, 6.0 mmol, 1.2 eq.) was added. The procedure afforded dienyl alcohol **20b** (655 mg, 3.3 mmol, 67% yield over three steps from **18a**) as a yellow oil. Data for **20b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (app. ddt, *J* = 17.0, 10.1, 6.6 Hz, 1H), 5.42 – 5.32 (m, 2H), 5.00 (app. dq, *J* = 17.0, 1.7 Hz, 1H), 4.94 (app. ddt, *J* = 10.1, 2.3, 1.3 Hz, 1H), 3.65 (app. t, *J* = 6.6 Hz, 2H), 2.10 – 1.99 (m, 6H), 1.62 – 1.55 (m, 2H), 1.45 – 1.24 (m, 11H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 130.3, 129.4, 114.2, 62.9, 33.8, 32.3, 29.5, 28.8, 28.8, 27.2, 26.9, 25.8. GC-MS (EI): t_R = 22.3 min (99.2%, *m/z* $[M]^+$: 196.2).

7.3.7 (4*Z*,8*Z*)-Undeca-4,8-dien-1-ol (24a)

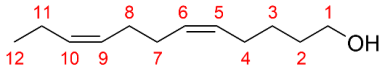


24a

Following general procedure A, aldehyde **19a** was prepared from **18a** (1.09 g, 5 mmol, 1.0 eq.), DMSO (0.71 mL, 10 mmol, 2.0 eq.) and oxalyl chloride (0.52 mL, 12 mmol, 1.2 eq.) in dry DCM (55 mL). Subsequently, TEA (2.8 mL, 40 mmol, 4.0 eq.) was added and after reaction and purification, a solution of **19a** was obtained and used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Dienyl alcohol **24a** was prepared from potassium *tert*-butoxide (617 mg, 5.5 mmol, 1.1 eq.), **16** (2.55 g, 6 mmol, 1.2 eq.) and **19a** in dry THF (35 mL). After reaction and purification, the residue was dissolved in dry THF (20 mL) and TBAF (1 M, 6.0 mL, 6.0 mmol, 1.2 eq.) was added. The procedure afforded dienyl alcohol **24a** (331 mg, 2.0 mmol, 40% yield over three steps from **18a**) as a colourless oil. Data for **24a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 – 5.28 (m, 4H, H_4, H_5, H_8, H_9), 3.65 (t, *J* = 6.6 Hz, 2H, H_1), 2.18 – 2.00 (m, 8H, H_3, H_6, H_7, H_{10}), 1.63 (app. p, *J* = 6.6 Hz, 2H, H_2), 0.96 (app. t, *J* = 7.5 Hz, 3H, H_{11}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.1, 130.0, 129.3, 128.4, 62.5, 32.6, 27.3, 27.1, 23.6, 20.5, 14.3. GC-MS (EI): t_R = 19.97 min (99.7%, *m/z* $[M]^+$: 168.1).

7.3.8 (5*Z*, 9*Z*)-Dodeca-5,9-dien-1-ol (24b)

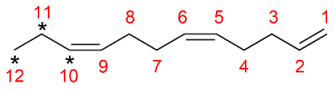


24b

Following general procedure A, aldehyde **19b** was prepared from **18b** (2.18 g, 10 mmol, 1.0 eq.), DMSO (1.42 mL, 20 mmol, 2.0 eq.) and oxalyl chloride (1.04 mL, 12 mmol, 1.2 eq.) in dry DCM (110 mL). Subsequently, TEA (5.6 mL, 40 mmol, 4.0 eq.) was added and after reaction and purification, a solution of **19b** was obtained and used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Dienyl alcohol **24b** was prepared from potassium *tert*-butoxide (1.23 g, 11 mmol, 1.1 eq.), **16** (5.27 g, 12 mmol, 1.2 eq.) and **19b** in dry THF (60 mL). After reaction and purification, the residue was dissolved in dry THF (40 mL) and TBAF (1 M, 12 mL, 12 mmol, 1.2 eq.) was added. The procedure afforded dienyl alcohol **24b** (1.32 g, 7.2 mmol, 72% yield from NMR over three steps from **18a**) as a colourless oil containing residual ether and *n*-pentane. Data for **24b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 – 5.28 (m, 4H, H_5, H_6, H_9, H_{10}), 3.65 (app. t, *J* = 6.6 Hz, 2H, H_1), 2.12 – 2.01 (m, 8H, H_4, H_7, H_8, H_{11}), 1.63 – 1.54 (m, 2H, H_2), 1.47 – 1.39 (m, 2H, H_3), 0.96 (app.t, *J* = 7.5 Hz, 3H, H_{12}). Similar to reported data.[79]

7.3.9 (5*Z*,9*Z*)-Dodeca-1,5,9-triene-10,11,12-$^{13}C_3$ (22a)

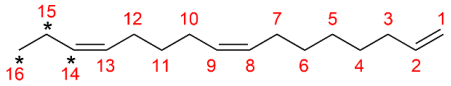


22a * = ^{13}C

Following general procedure B, triene **22a** was prepared by adding alcohol **20a** (0.58 g, 4.1 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.58 mL, 8.2 mmol, 2.0 eq.) and oxalyl chloride (0.53 mL, 5.0 mmol, 1.2 eq.) in dry DCM (25 mL). Subsequently, TEA (2.2 mL, 17 mmol, 4.0 eq.) was added. This afforded aldehyde **21a**, which was used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Potassium *tert*-butoxide sol. (4.25 mL, 4.5 mmol, 1.1 eq.) was added to a suspension of Wittig salt **12b** (1.69 g, 4.4 mmol, 1.05 eq.) in dry THF (20 mL). Aldehyde **21a** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **22a** was obtained in solution with *n*-pentane. Yield over three steps (from **20a** to **23a**) was calculated for final chlorinated product **23a**. Observed presence of **22a** by ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 – 5.76 (m, 1H), 5.68 – 5.49 (m, 0.5H), 5.48 – 5.29 (m, 3H), 5.25 – 5.18 (m, 0.5H), 5.04 (app. dq, *J* = 17.2, 1.6 Hz, 1H), 4.97 (app. ddt, *J* = 10.3, 2.2, 1.1 Hz, 1H), 2.26 – 2.17 (m, 1H), 2.17 – 2.05 (m, 8H), 1.96 – 1.82 (m, 1H), 1.16 – 1.10 (m, app. 1.5H), 0.85 – 0.78 (m, app. 1.5H).

7.3.10 (8*Z*,13*Z*)-Hexadeca-1,8,13-triene-14,15,16-$^{13}C_3$ (22b)

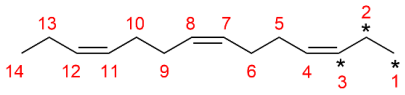


22b * = ^{13}C

Following general procedure B, triene **22b** was prepared by adding alcohol **20b** (0.66 g, 3.4 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.47 mL, 6.7 mmol, 2.0 eq.) and oxalyl chloride (0.34 mL, 4.0 mmol, 1.2 eq.) in dry DCM (25 mL). Subsequently, TEA (1.85 mL, 13 mmol, 4.0 eq.) was added. This afforded aldehyde **21b**, which was used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Potassium *tert*-butoxide sol. (3.41 mL, 3.7 mmol, 1.1 eq.) was added to a suspension of Wittig salt **12b** (1.36 g, 3.5 mmol, 1.05 eq.) in dry THF (20 mL). Aldehyde **21b** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **22b** was obtained in solution with *n*-pentane. Yield over three steps (from **20b** to **23b**) was calculated for final chlorinated product **23b**. Observed presence of **22b** by ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.82 (app. ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.52 – 5.49 (m, 0.5H), 5.44 – 5.29 (m, 3H), 5.27 – 5.24 (m, 0.5H), 5.00 (app. dq, *J* = 17.0, 1.9 Hz, 1H), 4.97 – 4.90 (m, 1H), 2.22 – 2.10 (m, 1H), 2.08 – 2.00 (m, app. 8H), 1.98 – 1.89 (m, 1H), 1.44 – 1.38 (m, 4H), 1.10 – 1.04 (m, 1.5H), 0.87 – 0.84 (m, app. 1.5H). Other ^{1}H NMR peaks were overlapping with residual solvent peaks.

7.3.11 (3*Z*,7*Z*,11*Z*)-Tetradeca-3,7,11-triene-1,2,3-$^{13}C_3$ (26a)



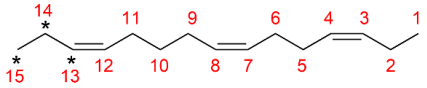
26a

* = ^{13}C

Following general procedure B, triene **26a** was prepared by adding alcohol **24a** (0.33 g, 2.0 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.3 mL, 4.0 mmol, 2.0 eq.) and oxalyl chloride (0.2 mL, 2.3 mmol, 1.2 eq.) in dry DCM (25 mL). Subsequently, TEA (1.1 mL, 7.9 mmol, 4.0 eq.) was added. This afforded aldehyde **25a**, which was used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Potassium *tert*-butoxide sol. (1.7 mL, 2.2 mmol, 1.1 eq.) was added to a suspension of Wittig salt **12b** (0.82 g, 2.1 mmol, 1.05 eq.) in dry THF (20 mL). Aldehyde **25a** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **26a** was obtained in solution with *n*-pentane. Yield over three steps (from **24a** to **27a**) was calculated for final chlorinated product **27a**. Observed presence of **26a** by ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 – 5.55 (m, 0.5H), 5.47 – 5.29 (m, 5H), 5.24 – 5.17 (m, 0.5H), 2.26 – 2.16 (m, 1H), 2.15 – 2.00 (m, 10H), 1.93 – 1.86 (m, 1H), 1.16 – 1.09 (m, 1.5H), 0.84 – 0.78 (m, app. 1.5H).

7.3.12 (3Z,7Z,12Z)-Pentadeca-3,7,12-triene-13,14,15-$^{13}C_3$ (26b)



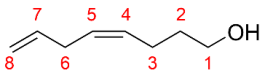
26b

* = ^{13}C

Following general procedure B, triene **26b** was prepared by adding alcohol **24b** (0.48 g, 2.6 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.37 mL, 5.2 mmol, 2.0 eq.) and oxalyl chloride (0.27 mL, 3.1 mmol, 1.2 eq.) in dry DCM (25 mL). Subsequently, TEA (1.5 mL, 11 mmol, 4.0 eq.) was added. This afforded aldehyde **25b**, which was used in the next step without further purification. Yield was assumed quantitative with respect to reagents.

Potassium *tert*-butoxide sol. (2.75 mL, 2.9 mmol, 1.1 eq.) was added to a suspension of Wittig salt **12b** (1.05 g, 2.7 mmol, 1.05 eq.) in dry THF (20 mL). Aldehyde **25b** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **26b** was obtained in solution with *n*-pentane. Yield over three steps (from **24b** to **27b**) was calculated for final chlorinated product **27b**.

7.3.13 (*Z*)-Octa-4,7-dien-1-ol (32)



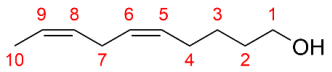
32

A solution of 2-propylmagnesium chloride (2 M, 42.3 mL, 84.6 mmol, 2.5 eq.) in THF was added dropwise to 4-pentyn-1-ol (**28a**, 3.40 g, 40 mmol, 1.3 eq.) in dry THF (60 mL) at 0 °C. The reaction mixture was stirred at 70 °C for 2 h. After cooling to 0 °C, CuCl (0.62 g, 4.6 mmol, 0.15 eq.) was added, followed by 3-bromo-1-(trimethylsilyl)-1-propyne (**29a**, 6.0 g, 31 mmol, 1.0 eq.) in dry diethyl ether (10 mL). The reaction mixture was stirred at 70 °C for 16 h, before it was cooled to r.t. and quenched with sat. aq. NH_4Cl (50 mL). The two layers were separated, and the aqueous layer was extracted with diethyl ether (2 x 60 mL). After combining the organic layers, they were washed with brine (60 mL), dried over $MgSO_4$, filtered, and concentrated carefully *in vacuo*. Crude product was purified by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, 1:1 diethyl ether/*n*-pentane), affording 8-trimethylsilyl-4,7-octadiyn-1-ol (**30**) in a mixture with residual diethyl ether and impurities (total 4.6 g).

To a solution of TBAF (1 M in THF, 33.2 mL, 33.2 mmol) and acetic acid (1.12 g, 19 mmol) in THF (3 mL) was added **30** from previous step at 0 °C. The mixture was stirred for 2 h at 0 °C, before it was concentrated carefully under reduced pressure. Purification by FCC (SiO_2, 95:5 *n*-pentane/diethyl ether) afforded diynyl alcohol **31** (~2.05 g, 17 mmol, 55% yield from NMR over two steps from **29a**) in a solution of diethyl ether.

Quinoline (100 mg, 0.8 mmol) and **31** (~2.05 g, 17 mmol) was added to a suspension of Lindlar's catalyst (100 mg) in *n*-pentane/EtOAc (1:1, 40 mL). The reaction was evacuated and filled with hydrogen gas three times. The reaction mixture was stirred under a hydrogen balloon for 16 h at r.t. and was subsequently filtered through a thin pad of silica gel with *n*-pentane (150 mL) and diethyl ether (150 mL). Partial removal of solvents was conducted under reduced pressure, before the crude was purified by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, 20:80 ether in *n*-pentane). After purification **32** (~ 1.05 g, 8.3 mmol, 49% yield from NMR from **31**) was obtained as a mixture with diethyl ether, *n*-pentane and over-reduced byproduct (22% from GCMS). Observed presence of **32** by ^1H NMR (400 MHz, $CDCl_3$) δ 5.90 – 5.75 (m, 1H), 5.54 – 5.34 (m, app. 2H), 5.05 (app. dq, *J* = 17.0, 1.8 Hz, 1H), 4.99 (app. dq, *J* = 10.1, 1.5 Hz, 1H), 3.66 (app. t, *J* = 6.5 Hz, app. 2H), 2.82 (app. t, *J* = 6.2 Hz, 2H), 2.20 – 2.11 (m, app. 2H), 1.71 – 1.59 (m, app. 2H). Spectral data were similar to reported data using CCl_4 as NMR solvent.[80]

7.3.14 (5*Z*,8*Z*)-Deca-5,8-dien-1-ol (37)

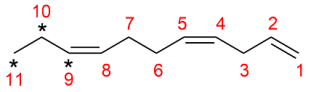


37

A solution of 2-propylmagnesium chloride (2 M, 22.1 mL, 44.2 mmol, 2.5 eq.) in THF was added dropwise to 5-hexyn-1-ol (**28b**, 2.25 g, 23 mmol, 1.3 eq.) in dry THF (40 mL) at 0 °C. The reaction mixture was stirred at 70 °C for 2 h. After cooling to 0 °C, CuCl (0.35 g, 2.6 mmol, 0.15 eq.) was added, followed by 1-bromo-2-butyne (**29b**, 2.35 g, 18 mmol, 1.0 eq.) in dry diethyl ether (10 mL). The reaction mixture was stirred at 70 °C for 16 h, before it was cooled to r.t. and quenched with sat. aq. NH_4Cl (30 mL). The two layers were separated, and the aqueous layer was extracted with diethyl ether (2 x 50 mL). After combining the organic layers, they were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated carefully *in vacuo*. Crude product was purified by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, 1:1 diethyl ether/*n*-pentane), affording 5,8-decadiyn-1-ol (**36**) in a mixture with residual solvents and impurities (total 2.3 g).

Sodium borohydride (1.74 g, 46 mmol, 3 eq.) was added portion wise to a suspension of nickel(II) acetate hydrate (11.45 g, 46 mmol, 3 eq.) in ethanol/water (95:5 wt., 50 mL) at r.t. The resulting black suspension was stirred for 10 min, before ethylenediamine (8.3 g, 9.2 mL, 138 mmol, 9 eq.) was added dropwise. The reaction vessel was evacuated and refilled with hydrogen gas three times. After stirring under a hydrogen balloon for 10 min, **36** (2.3 g, 1.0 eq.) in ethanol/water (95:5 wt., 5 mL) was added and the mixture was stirred for 3 h at r.t. The reaction mixture was filtered through a thin pad of celite, which was washed with diethyl ether (50 mL). The filtrate was washed with water (50 mL) and brine (50 mL) and was dried over $MgSO_4$, filtered, and concentrated carefully under reduced pressure. Crude product was purified by FCC (SiO_2, *d* = 4 cm, *l* = 12 cm, 10:90 ether/*n*-pentane) to afford **37** (~300 mg, 1.9 mmol, 11% yield from NMR over two steps from **29b**) as a solution with residual ether, pentene and 5-hexen-1-ol. Used in next step without further purification. Observed presence of **37** by: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51 – 5.32 (m, app. 4H), 3.65 (app. t, *J* = 6.6 Hz, 2H), 2.79 (app. t, *J* = 6.2 Hz, 2H), 2.13 – 1.99 (m, 3H), 1.69 – 1.50 (m, app. 4H). GC-MS (EI): t_R = 19.4 min (97.5%, *m/z* $[M]^+$: 154.1).

7.3.15 (4*Z*,8*Z*)-undeca-1,4,8-triene-9,10,11-$^{13}C_3$ (34)



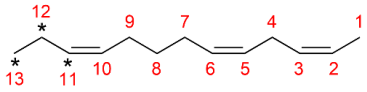
34 * = ^{13}C

Following general procedure B, triene **34** was prepared by adding alcohol **32** (~0.45 g, 3.6 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.51 mL, 7.2 mmol, 2.0 eq.) and oxalyl chloride (0.37 mL, 4.3 mmol, 1.2 eq.) in dry DCM (25 mL). Subsequently, TEA (2.0 mL, 14 mmol, 4.0 eq.) was added. This afforded aldehyde **33**, which was used in the next step without further purification.

Potassium *tert*-butoxide sol. (3.3 mL, 3.6 mmol, 1.0 eq.) was added to a suspension of Wittig salt **12b** (1.20 g, 3.1 mmol, 0.9 eq.) in dry THF (20 mL). Aldehyde **33** in dry

THF (6 mL) was added to the reaction. After reaction and purification, triene **34** was obtained in solution with *n*-pentane and impurities. Yield over five steps (from **29a** to **35**) was calculated for final chlorinated product **35**. Observed presence of **34** by ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 – 5.75* (m, app. 1H), 5.54 – 5.37* (m, app. 3H), 5.10 – 5.01* (m, app. 1H), 5.02 – 4.96* (m, app. 1H), 2.82 (t, *J* = 6.1 Hz, 2H). Other ^{1}H NMR peaks were overlapping with residual solvent peaks. *Peaks are likely in overlap with impurities from excess starting material originating from **28a**.

7.3.16 (2*Z*,5*Z*,10*Z*)-trideca-2,5,10-triene-11,12,13-$^{13}C_3$ (39)

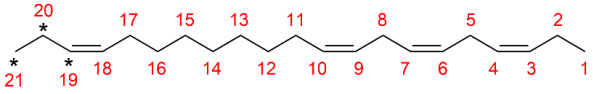


39 * = ^{13}C

Following general procedure B, triene **39** was prepared by adding alcohol **37** (~0.30 g, 1.9 mmol, 1.0 eq.) in dry DCM (10 mL) to a solution of DMSO (0.46 mL, 6.5 mmol, 3.4 eq.) and oxalyl chloride (0.33 mL, 3.8 mmol, 2.0 eq.) in dry DCM (25 mL). Subsequently, TEA (1.5 mL, 13 mmol, 4.0 eq.) was added. This afforded aldehyde **38**, which was used in the next step without further purification.

Potassium *tert*-butoxide sol. (2.7 mL, 2.9 mmol, 1.5 eq.) was added to a suspension of Wittig salt **12b** (1.02 g, 2.6 mmol, 1.4 eq.) in dry THF (20 mL). Aldehyde **38** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **39** was obtained in solution with *n*-pentane and impurities. Yield over four steps (from **29b** to **40**) was calculated for final chlorinated product **40**. Observed presence of **39** by ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 – 5.51* (m, app. 0.5H), 5.49 – 5.26* (m, app. 5H), 5.24 – 5.12* (m, app. 0.5H), 2.79 (app. t, *J* = 6.4 Hz, 2H), 2.25 – 2.13* (m, app. 1H), 2.12 – 1.97* (m, app. 4H), 1.93 – 1.86* (m, app. 1H), 1.16 – 1.08* (m, app. 1.5H), 0.83 – 0.75* (m, app. 1.5H). Other ^{1}H NMR peaks were overlapping with residual solvent peaks. *Peaks are likely in overlap with impurities from excess starting material originating from **28b**.

7.3.17 (3*Z*,6*Z*,9*Z*,18*Z*)-henicosa-3,6,9,18-tetraene-19,20,21-$^{13}C_3$ (43)



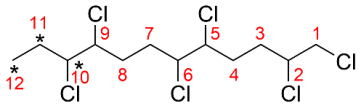
43 * = ^{13}C

Following general procedure B, tetraene **43** was prepared by adding alcohol **41** (1.00 g, 3.8 mmol, 1.0 eq.) in dry DCM to a solution of DMSO (0.54 mL, 7.6 mmol, 2.0 eq.) and oxalyl chloride (0.39 mL, 4.5 mmol, 1.2 eq.) in dry DCM. Subsequently, TEA (2.1 mL, 15 mmol, 4.0 eq.) was added and afforded aldehyde **42** (~0.75 g, 2.9 mmol, 76% from NMR) with residual diethyl ether, which was used in the next step without further purification.

Potassium *tert*-butoxide sol. (2.0 mL, 2.1 mmol, 0.55 eq.) was added to a suspension of Wittig salt **12b** (0.74 g, 1.9 mmol, 0.50 eq.) in dry THF (20 mL). Aldehyde **42** in dry THF (6 mL) was added to the reaction. After reaction and purification, triene **43** (~0.37 g, 1.3 mmol, 45% yield from NMR) was obtained in solution with *n*-pentane.

Yield over three steps (from **41** to **44**) was calculated for final product **44**. Observed presence of **43** by ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 – 5.49 (m, 0.5H), 5.46 – 5.25 (m, 7H), 5.23 – 5.12 (m, 0.5H), 2.82 (app. t, *J* = 5.9 Hz, 4H), 2.25 – 2.15 (m, 1H), 2.14 – 1.98 (m, 6H), 1.93 – 1.84 (m, 1H), 1.12 (m, 1.5H), 0.99 (t, *J* = 7.5 Hz, 3H), 0.81 (m, 1.5H). Other ^{1}H NMR peaks were overlapping with residual solvent peaks.

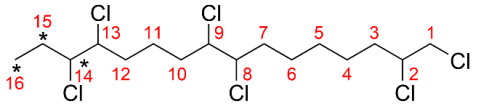
7.3.18 1,2,5,6,9,10-Hexachlorododecane-10,11,12-$^{13}C_3$ (23a)



23a * = ^{13}C

The crude triene **22a** (see Section 6.6.9) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). The resulting suspension was filtered and washed with *n*-hexane, affording a fraction of **23a** (45 mg). The filtrate was concentrated under reduced pressure and recrystallised from *n*-hexane affording another product fraction (122 mg). The resulting solids were combined, affording **23a** (167 mg, 0.44 mmol, 11% yield over three steps from **20a**) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.13 – 3.84 (m, 5H, H_2, $H_{5\text{-}6}$, $H_{9\text{-}10}$), 3.82 (app. ddd, *J* = 11.4, 5.0, 1.7 Hz, 1H, H_1), 3.70 – 3.65 (m, 1H, H_1), 2.43 – 2.22 (m, 3H), 2.23 – 1.85 (m, 6H), 1.84 – 1.68 (m, 1H), 1.08 (app. dtdd, *J* = 126.5, 7.3, 6.1, 4.2 Hz, 3H, H_{12}). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 71.0, 70.7, 67.1 (app. dd, *J* = 37.1, 10.7 Hz), 65.0, 64.9, 64.9, 64.7, 64.0, 60.5, 47.9, 32.7, 32.7, 32.4, 32.4, 32.3, 32.1, 31.4, 31.3, 27.7 (app. ddd, *J* = 37.4, 34.7, 22.6 Hz), 11.4 (app. d, *J* = 34.7 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2960, 2921, 2873, 2850, 1443, 1432, 1280, 1261, 1091, 1025, 960, 813, 798, 738, 664, 590. HRMS (TOF MS ES-): *m/z* calcd for $^{12}C_9{}^{13}C_3H_{19}Cl_6$ [M-H]: 375.9719; found: 375.9715. GC-MS (EI): t_R = 17.3 min (99.3%, *m/z* $[M\text{-}HCl]^{+\cdot}$: 341.0).

7.3.19 1,2,8,9,13,14-Hexachlorohexadecane-14,15,16-$^{13}C_3$ (23b)

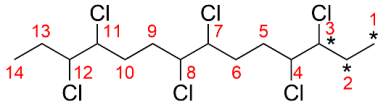


23b * = ^{13}C

The crude triene **22b** (see Section 6.6.10) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). The resulting crude was concentrated under reduced pressure and attempted recrystallised from 5% water in *i*-PrOH but resulted in a sedimented emulsion. A fraction of **23b** (54 mg) crystallised from the *i*-PrOH layer, and another fraction (26 mg) was obtained by recrystallisation of the sedimented oil in 5% water in *i*-PrOH at 4 °C. These two fractions were combined to yield **23b** (80 mg, 0.18 mmol, 11% yield over three steps from **20b**) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.12 – 3.82 (m, 5H, H_2, $H_{8\text{-}9}$, $H_{13\text{-}14}$), 3.78 (app. dd, *J* = 11.4, 5.1 Hz, 1H, H_1), 3.66 (app. dd, *J* = 11.3, 7.6 Hz, 1H, H_1), 2.15 – 1.58 (m, 14H), 1.52 – 1.35 (m, 4H), 1.08 (app. dtdd, *J* = 126.5, 7.3, 6.1, 4.3 Hz, 3H, H_{16}). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 67.2 (app. dd, *J* =

37.4, 7.2 Hz), 65.3, 65.2, 65.0, 65.0, 61.0, 61.0, 48.1, 34.9, 34.8, 34.2, 34.1, 34.0, 33.9, 33.8, 33.6, 28.4, 28.3, 27.7 (app. ddd, *J* = 37.4, 35.2, 18.7 Hz), 26.5, 26.5, 25.6, 25.6, 24.1, 24.1, 24.1, 11.5 (app. d, *J* = 35.1 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2932, 2860, 1456, 1433, 1373, 1261, 1210, 1089, 1029,0803, 727, 660, 638. HRMS (TOF MS ES-): *m/z* calcd for $^{12}C_{13}{}^{13}C_3H_{27}Cl_6$ [M-H]: 432.0345; found: 432.0338. GC-MS (EI): t_R = 20.4 min (>99.9%, *m/z* $[M\text{-}HCl]^{+\cdot}$: 397.0).

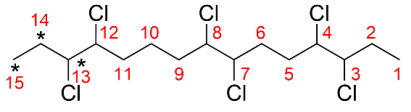
7.3.20 3,4,7,8,11,12-Hexachlorotetradecane-1,2,3-$^{13}C_3$ (27a)



27a * = ^{13}C

The crude triene **26a** (see Section 6.6.11) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). The resulting suspension was concentrated under reduced pressure before it was recrystallised from *n*-hexane. This afforded **27a** (121 mg, 0.30 mmol, 15% yield over three steps from **24a**) as a white solid. ^1H NMR (600 MHz, $CDCl_3$) δ 4.15 – 3.82 (m, 6H, $H_{3\text{-}4}$, $H_{7\text{-}8}$, $H_{11\text{-}12}$), 2.35 – 1.68 (m, 12H, H_2, $H_{5\text{-}6}$, $H_{9\text{-}10}$, H_{13}), 1.22 – 0.95 (m, 3H, H_1), 1.09 (app. td, *J* = 7.2, 4.3 Hz, 3H, H_{14}). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 67.1 (app. dd, J = 37.4, 9.9 Hz), 65.1, 65.0, 65.0, 64.9, 64.1, 64.1, 63.9, 32.4, 32.4, 32.4, 32.3, 31.4, 31.4, 31.3, 27.7 (app. ddd, J = 38.0, 35.2, 22.6 Hz), 11.4 (app. d, J = 35.2 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2963, 2936, 2877, 2850, 1443, 1259, 1085, 1023, 958, 796, 783, 682, 611, 584. HRMS (TOF MS ES-): *m/z* calcd for $^{12}C_{11}{}^{13}C_3H_{23}Cl_6$ [M-H]: 404.0032; found: 404.0032. GC-MS (EI): t_R = 18.2 min (>99.9%, *m/z* $[M\text{-}HCl]^{+\cdot}$: 369.1).

7.3.21 3,4,7,8,12,13-Hexachloropentadecane-13,14,15-$^{13}C_3$ (27b)

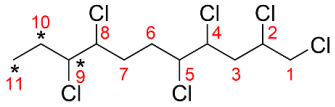


27b * = ^{13}C

The crude triene **26b** (see Section 6.6.12) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). The resulting solution was concentrated under reduced pressure. The crude was dissolved in DCM and concentrated over celite. The celite was added on a dry column (SiO_2) and the product was eluted stepwise with *n*-hexane (15x5 mL) 5% DCM in *n*-hexane (15x5 mL) and finally 10% DCM in *n*-hexane (15x5 mL). Product fractions were pooled and concentrated under reduced pressure. The resulting solids were recrystallised from *n*-hexane and afforded **27b** (45 mg, 0.11 mmol, 4% yield over three steps from **24b**). ^1H NMR (600 MHz, $CDCl_3$) δ 4.15 – 3.81 (m, 6H, $H_{3\text{-}4}$, $H_{7\text{-}8}$, $H_{12\text{-}13}$), 2.32 – 1.66 (m, 14H, H_2, $H_{5\text{-}6}$, $H_{9\text{-}11}$, H_{14}), 1.09 (app. td, *J* = 7.3, 4.1 Hz, 3H, H_1), 1.08 (app. dqd, *J* = 126.5, 7.2, 4.3 Hz, 3H, H_{15}). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 67.2 (app. dd, J = 37.1, 8.0 Hz), 65.1, 65.0, 64.9, 64.9, 64.8, 64.1, 64.1, 64.0, 63.9,

33.9, 33.9, 33.7, 33.7, 33.5, 32.5, 32.4, 32.3, 31.5, 31.5, 31.4, 31.3, 30.1, 29.9, 29.9, 29.6, 28.4, 28.2, 28.1, 27.7 (app. dd, J = 37.4, 35.2, 16.5 Hz), 24.1, 24.1, 24.1, 23.3, 23.1, 11.5 (app. d, J = 35.2 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2963, 1445, 1412, 1258, 1019, 864, 793, 687, 592, 576. HRMS (TOF ES-): *m/z* calcd for $^{12}C_{12}{}^{13}C_3H_{25}Cl_6$ [M-H]: 418.0188; found: 418.0188. GC-MS (EI): t_R = 19.0 min (>99.9%, *m/z* $[M\text{-}HCl]^{+\cdot}$: 383.1).

7.3.22 1,2,4,5,8,9-Hexachloroundecane-9,10,11-$^{13}C_3$ (35)

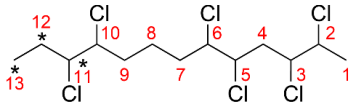


35

* = ^{13}C

The crude triene **34** (see Section 6.6.15) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). The resulting solution was concentrated under reduced pressure. The crude was dissolved in DCM and concentrated over celite. The celite was added on a dry column (SiO_2) and the product was eluted stepwise with *n*-hexane (15x5 mL) 5% DCM in *n*-hexane (15x5 mL) and finally 10% DCM in *n*-hexane (15x5 mL). Product fractions were pooled and concentrated under reduced pressure. The resulting solids were recrystallised from *n*-hexane and part of **35** (5 mg) was collected. Mother liquor was concentrated and another purification by the same column system was performed. Product fractions were pooled and recrystallised from *n*-hexane, and resulting white solids were combined with previous product **35** (10 mg, 0.027 mmol, 0.1% yield over 6 steps from **29a**). ^1H NMR (600 MHz, $CDCl_3$) δ 4.45 (app. dt, *J* = 11.2, 2.2 Hz, 1H, H_2), 4.40 (app. dddd, *J* = 11.5, 7.0, 4.6, 2.1 Hz, 1H, H_4), 4.15 – 3.87 (m, 3H, H_5, $H_{8\text{-}9}$), 3.86 – 3.70 (m, 2H, H_1), 2.70 – 2.50 (m, 1H, H_3), 2.41 – 2.30 (m, 1H, H_3), 2.27 – 1.69 (m, 6H, $H_{6\text{-}7}$, H_{10}), 1.23 – 0.93 (m, 3H, H_{11}). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 69.3, 69.0, 67.2 (app. d, *J* = 37.4, 35.1 Hz), 65.0, 62.0, 57.9, 48.2, 40.8, 32.7, 32.7, 32.3, 32.3, 27.7 (app. dd, *J* = 37.4 Hz), 23.3, 23.0, 11.4 (app. d, *J* = 35.2 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2959, 2926, 2874, 1444, 1431, 1294, 1282, 1257, 1020, 959, 882, 811, 790, 728, 681, 606, 593. HRMS (TOF ES-): *m/z* calcd for $^{12}C_8{}^{13}C_3H_{17}Cl_6$ [M-H]: 358.9461; found: 358.9461. GC-MS (EI): t_R = 16.6 min (>99.9%, *m/z* $[M\text{-}HCl]^{+\cdot}$: 327.1).

7.3.23 2,3,5,6,10,11-Hexachlorotridecane-11,12,13-$^{13}C_3$ (40)



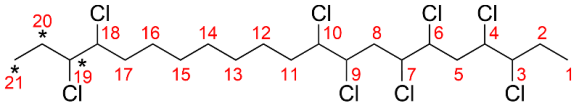
40

* = ^{13}C

The crude triene **39** (see Section 6.6.16) was subjected to general procedure C, using trichloroisocyanuric acid (2 g, 8.61 mmol) and 2 M HCl (aq., 15 mL). Crude product was purified by FCC dry column (SiO_2) and the product was eluted stepwise with 2.5% DCM in *n*-hexane (10x10 mL) and 5% DCM in *n*-hexane (10x10 mL).

Product fractions were pooled, concentrated and recrystallised from *i*-PrOH and water (95:5) and afforded **40** (61 mg, 0.15 mmol, 1.7% yield over 5 steps from **29b**). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.43 (app. dq, *J* = 10.9, 1.8 Hz, 1H, H_3), 4.38 (app. dt, *J* = 10.9, 2.3 Hz, 1H, H_5), 4.28 (app. qd, *J* = 6.6, 2.8 Hz, 1H, H_2), 4.13 – 3.82 (m, 3H, H_6, H_{10-11}), 2.38 (app. dddd, *J* = 14.9, 10.9, 6.5, 2.1 Hz, 1H, H_4), 2.29 (app. dddd, *J* = 14.9, 11.0, 4.9, 2.1 Hz, 1H, H_4), 2.14 – 1.82 (m, 6H, H_7, H_9, H_{12}), 1.80 – 1.67 (m, 2H, H_8), 1.64 (app. d, *J* = 6.7 Hz, 3H, H_1), 1.08 (app. dtdd, *J* = 126.6, 7.2, 6.1, 4.3 Hz, 3H). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 67.4 (app. dd, *J* = 37.4, 5.0 Hz), 65.2, 65.1, 65.1, 65.1, 65.0, 64.8, 64.8, 63.3, 63.3, 62.5, 62.5, 60.0, 40.4, 40.2, 34.6, 34.5, 34.1, 34.0, 30.1, 28.7, 28.6, 28.4, 28.4, 28.0 (app. ddd, *J* = 37.5, 35.3, 14.1 Hz), 24.2, 24.2, 24.2, 24.2, 21.47, 21.46, 11.7 (app. d, *J* = 35.2 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2956, 2934, 2874, 2845, 1458, 1441, 1431, 1382, 1313, 1280, 1248, 1108, 1018, 930, 913, 882, 757, 721, 688, 622, 590, 572. HRMS (TOF ES-): *m/z* calcd for $^{12}C_{10}{}^{13}C_3H_{21}Cl_6$ [M-H]: 386.9774; found: 386.9773. GC-MS (EI): t_R = 10.6 min (>99.9%, *m/z* [M-HCl]$^{+\cdot}$: 355.1).

7.3.24 3,4,6,7,9,10,18,19-Octachlorohenicosane-19,20,21-$^{13}C_3$ (44)



44

* = ^{13}C

The crude triene **43** (~0.37 g, 1.3 mmol) was subjected to general procedure C, using trichloroisocyanuric acid (0.91 g, 3.9 mmol) and 2 M HCl (aq., 10 mL). The crude was recrystallised from *i*-PrOH and water (95:5) and afforded **44** (29 mg, 0.05 mol, 1.3% yield over three steps from **41**) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.44 (app. dtt, *J* = 13.2, 6.4, 2.7 Hz, 4H, H_4, H_{6-7}, H_9), 4.10 – 3.83 (m, 4H, H_3, H_{10}, H_{18-19}), 2.38 (app. dd, *J* = 7.8, 5.2 Hz, 3H, H_5, H_8), 2.13 – 1.67 (m, 9H, H_2, H_5, H_8, H_{11}, H_{17}, H_{20}), 1.62 – 1.31 (m, 10H, H_{12-16}), 1.20 – 0.93 (m, 3H, H_{21}), 1.10 (app. t, *J* = 7.3 Hz, 3H, H_1). ^{13}C NMR* (151 MHz, $CDCl_3$) δ 77.2, 77.0, 76.8, 70.4, 70.2, 68.9, 67.4 (app. d, *J* = 37.4 Hz), 65.4, 65.4, 65.3, 65.2, 65.1, 64.3, 64.1, 63.8, 62.6, 62.2, 61.9, 56.8, 41.4, 41.3, 34.9, 34.4, 29.1, 28.9, 28.8, 28.4, 28.3, 27.8 (app. dd, *J* = 37.7, 35.0 Hz), 27.1, 26.8, 26.6, 26.6, 26.6, 26.5, 23.4, 23.1, 20.4, 20.1, 11.5 (app. d, *J* = 35.2 Hz). *Potential error due to ^{13}C peak overlap, their satellites and general complexity of the stereoisomeric product mixture. ATR-FTIR (cm^{-1}): 2962, 2929, 2875, 2856, 1456, 1419, 1287, 1235, 969, 905. 807, 731, 638, 620, 606, 581, 441. HRMS (TOF ES-): *m/z* calcd for $^{12}C_{18}{}^{13}C_3H_{35}Cl_8$ [M-H]: 570.0348; found: 570.0337. GC-MS (EI): t_R = 33.5 min (>99.9%, *m/z* [M-HCl]$^{+\cdot}$: 535.1).

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Publications

Publication I

Submitted paper

S. Valderhaug, H. Liu, A. Gorovoy, J. E. Johansen, O. R. Gautun

Synthesis of constitutionally defined chlorinated paraffins as reference standards

This paper is submitted for publication and is therefore not included.

Publication II

Submitted patent

A. Gorovoy, H. Liu, J. Tůma, S. Valderhaug, Jonatan Nygren, J. E. Johansen

^{13}C-Labelled chlorinated paraffins and their preparation

This is a submitted patent and is not included.

Publication III

Submitted article

S. Valderhaug, N. Paškanová, J. Tůma, J. Herciková, V. Eigner, H. Liu, A. Gorovoy, J. E. Johansen, O. R. Gautun

Synthesis, identification, chiral separation and crystal structure of (3R,4R,7S,8S)-3,4,7,8-tetrachlorodecane and its stereoisomers

Publication IV

Published paper

S. Valderhaug, H. Liu, A. Gorovoy, J. E. Johansen, L. van Mourik, J. de Boer, O. R. Gautun

Nuclear magnetic resonance as a tool to determine chlorine percentage of chlorinated paraffin mixtures.

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Nuclear magnetic resonance as a tool to determine chlorine percentage of chlorinated paraffin mixtures

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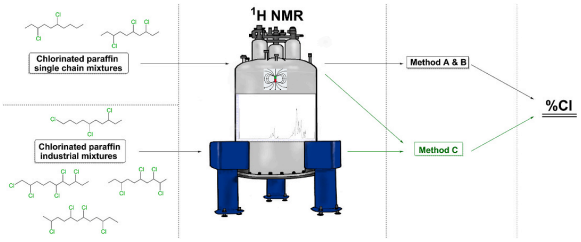
HIGHLIGHTS

- Synthesis of 10 single-chain chlorinated paraffin mixtures.
- Indicative NMR analysis of single poly-chlorinated alkanes and mixtures of diastereomers.
- Chlorine percentage calculations of industrial and single chain chlorinated paraffin mixtures using ^{1}H NMR spectroscopy.

GRAPHICAL ABSTRACT



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ABSTRACT

A new simple method for chlorine percentage calculations (method C), from proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, has been established and applied to an industrial chlorinated paraffin (CP) mixture and 13 single-chain CPs of known carbon chain lengths. Two modified methods (method A and B), originating from the work of Sprengel et al., have been utilized on the same single-chain mixtures. All samples were analysed by ^{1}H NMR and two-dimensional heteronuclear quantum coherence (HSQC) for this purpose. All three methods worked well for medium chlorinated (45–55% Cl) single-chain mixtures of known carbon chain lengths. Method A yielded the best result for mixtures of lower chlorine content (<45% Cl), method C gave better estimations for higher chlorine contents (>55% Cl). Compared to Mohr's titration, method A showed a deviation of 0.7–7.8% (3.6% average), method B 4.1–11.3% (7.0% average) and method C 0.6–11.6% (5.2% average), for all 13 single-chain mixtures. The new method C is the only method that could be applied for determining the chlorine percentage of industrial mixtures of multiple, unknown chain lengths.

1. Introduction

Chlorinated paraffins (CPs) are a class of industrial chemicals used as plasticisers and flame-retardant additives in plastics and rubbers, as high-temperature, high-pressure lubricants in metalworking machinery and several other applications (Glüge et al., 2016; Tomy et al., 1998).

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Produced since early 20th century, technical CPs are described as complex mixtures of polychlorinated *n*-alkanes (i.e., $C_nH_{2n+2-y}Cl_y$), typically with a chlorination degree of 30–70% and chain lengths ranging from 10 to 36 carbons. Their high production volumes (Glüge et al., 2016), resistance to degradation and potential for bioaccumulation and toxicity make CPs of environmental concern. Analysis of CPs has been conducted for decades but, due to the complexity of the mixtures, chromatographic resolution of the constituents remains elusive (van Mourik et al., 2020). The complexity arises from the number of compounds present in the mixtures, with potentially several hundred thousand isomers (Yuan et al., 2020). Due to the challenging analysis, variations in quantitation results have been observed between laboratories, especially if they have insufficient matching of standards and sample (Fernandes, 2022). The variation accentuates the need for representative standards that matches the occurrence profile of the sample. To produce well-defined standards, reliable analysis techniques are of essence.

Nuclear magnetic resonance (NMR) spectroscopy occupies an important role for structural elucidation of molecules (Friebolin, 1998). More recently, NMR spectroscopy has been increasingly applied to the analysis of CP mixtures. Examples include GC-fractionation to attempt to elucidate the composition of enriched samples (van Mourik et al., 2021), or studying two-dimensional spectra of CPs to indicate structural motifs present in mixtures (Sprengel et al., 2019, 2020; Yuan et al., 2020; Fernandes, 2022). Additionally, NMR spectroscopy has been used to estimate the positional selectivity of chlorines in the top one hundred isomers present in a complex mixture, using neural networks from databases of predicted one- and two-dimensional NMR spectra. (Yuan et al., 2020). Furthermore, a chlorine percentage calculation model using ^{1}H NMR spectroscopy has recently been reported for single-chain CP mixtures (Sprengel et al., 2019).

The aim of this study is to investigate ^{1}H NMR as a tool for determination of chlorine percentage in CP mixtures, for both single-chain and industrial mixtures. Thirteen synthesized single-chain CP mixtures ($C_{9\text{-}15}$), one industrial mixture with multiple and unknown chain lengths, and five binary/ternary mixtures of CPs were analysed on one- and two-dimensional NMR. Stereoisomeric mixtures of CPs were also synthesized and analysed, and their analysis were used to indicate structural motifs present in complex mixtures of CPs. The data received from ^{1}H NMR was used for calculation of chlorine percentage by three different calculation models. Two of the models A and B are modified versions of a previous calculation model (Sprengel et al., 2019), and the third C is a new calculation model that also allows chlorine percentage estimation of industrial mixtures of multiple and unknown chain lengths. These were compared against reference values obtained by Mohr's titration for chloride content after dechlorination and in some cases chlorine specific elemental analysis.

2. Materials and methods

2.1. Chemicals

Single chain mixtures of CPs **C_9** to **C_{14B}** has been produced and donated by Chiron AS and single chain mixtures **C_{14c}** and **C_{15}** has been synthesized by Quimica del Cinca and donated by the Chlorinated Paraffin Industry Association (CPIA). Technical CP mixture (**$C_{tech.}$**) has been purchased from FUJIFILM Wako. All single-chain mixtures of CPs have been prepared synthetically and purified by flash column chromatography, excluding any inorganic material, before they were analysed by NMR spectroscopy. The consistency between the analytical results of chlorine percentage additionally indicated that potential halogen contaminants were not present or negligible.

Different mixtures of **$C_{10\text{-}12}$** and **$C_{tech.}$** were weighed out accurately and mixed for chlorine percentage estimation of multicomponent mixtures by NMR method C.

Three stereoisomeric mixtures of 1,2,5,6,9,10-hexachlorododecane (**1**), 1,2,6,7,10,11-hexachlorotridecane (**2**) and 3,4,6,7,10,11-hexachlorotetradecane (**3**) were also prepared by synthetic procedures that are to be published on a later date. Each CP contains compounds of different stereochemistry.

2.2. NMR analysis

Deuterated chloroform with 0.03 v/v% TMS was dried by shaking it with Na_2SO_4 and NaOH, followed by filtration. Compounds **1**, **2** and **3** (15–20 mg) and CP single-chain mixtures (100–150 mg) were dissolved in the deuterated chloroform (0.65 mL) for NMR analysis.

NMR-analysis were performed with either a Bruker 400 MHz Avance III HD equipped with a 5 mm SmartProbe z-gradient probe, or a Bruker 600 MHz Avance III HD equipped with a 5 mm cryogenic CP-TCI z-gradient probe. The resulting data were analysed in ACD/Spectrus processor 2019.2.2 (software). TMS was used as a reference peak (δ 0.00). Data for calculation of chlorine percentage are shown in SI.

2.3. Titration

A detailed procedure for the titrations of chlorinated compounds is described in SI (Section 1). The method was tested up against a control sample of a single CP (1,2,7,8-tetrachlorooctane) with known atomic composition and purity. The control sample was used for method validation of titration, giving a value of 56.9 ± 0.4% Cl (theoretical 56.3% Cl) and 1.1% accuracy through 9 measurements (see SI, Section 1.3).

The general procedure involved converting organic chlorine to inorganic chloride ions by means of sodium in isopropanol followed by a standard Mohr's titration of a known volume of the analyte solution and K_2CrO_4 as indicator (Asinger, 1968; Sezey and Audun, 2019). Silver nitrate was titrated into the chloride solution until formation of a dark red silver chromate precipitate appear (see SI, Section 1.1). The amount of chloride ions in solution was assumed to be equimolar to the number of chlorines in the CP mixture.

2.4. Chlorine percentage calculations from NMR spectroscopy

Three ^{1}H NMR models for chlorine percentage estimations have been applied and investigated in this study, where A and B are modified literature models (Sprengel et al., 2019). The third model C is a new calculation model with expanded opportunities for determining chlorine percentage of industrial mixtures of multiple and unknown chain lengths.The strengths and limitations of each method, when applied to various CP mixtures, will be discussed in the following section.

Method A

Following the ^{1}H NMR literature method (Sprengel et al., 2019), the CH_2 and CH_3 regions are integrated as a joint cluster region (see Fig. 1 (a)) and adjusted by using a stochastic probability model. See Equation (2.1) and Equation (2.2).

$$X_{CH3/CH2} = \frac{A_{CH3/CH2}}{B} \tag{2.1}$$

Where $X_{CH3/CH2}$ is the adjusted area and $A_{CH3/CH2}$ is the total area of CH_2 and CH_3. The B describes the stochastic probability of CH_2 versus CH_3 occurrence in a straight-chain alkane and is given in Equation (2.2), where n denotes number of carbons (chain length) (Sprengel et al., 2019).

$$B = 2^{*}\frac{n-2}{n} + 3^{*}\frac{2}{n} \tag{2.2}$$

This model assumes that all terminal positions in the alkane chains are completely occupied by protons and that chlorination at the end of the chain is negligible. Previous research has shown that terminal chlorination is negligible in CP mixtures of lower chlorine content (Yuan et al., 2020), and method A is therefore expected to give more accurate

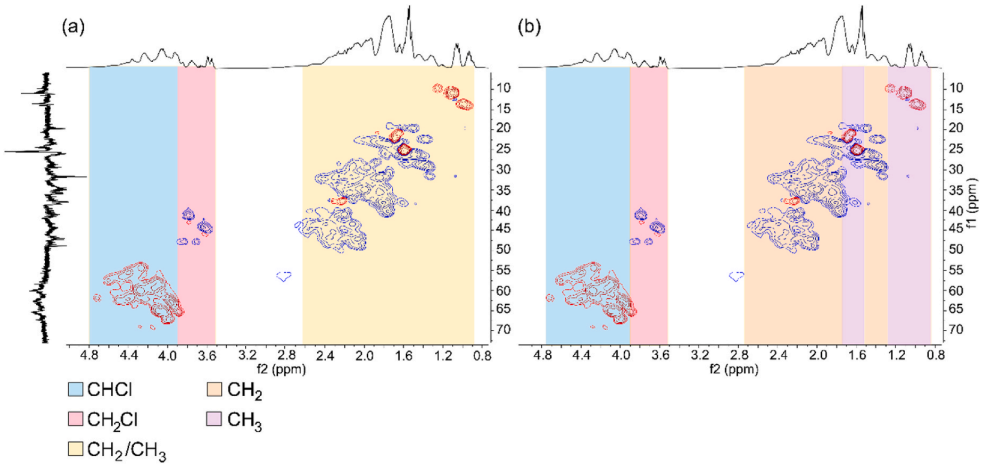


Fig. 1. Separation of the integrated areas used in (a) method A and (b) method B and C, in a ^{1}H NMR spectrum projected onto a 2D HSQC spectrum (600 MHz, $CDCl_3$).

results with lower chlorine content.

One way of minimizing the error caused by a high presence of terminal chlorines is to adjust the CHCl and CH_2Cl region individually (see Fig. 1 (a)). The individual proton integration area ($A_{CH_xCl_y}$) must be adjusted by the positional number of protons (x), as described in Equation (2.3) to give the individual adjusted area ($X_{CH_xCl_y}$).

$$X_{CH_xCl_y} = \frac{A_{CH_xCl_y}}{x} \tag{2.3}$$

The remaining part of calculation A follows the reported model (Sprengel et al., 2019). The relative area (A_{rel,CH_xCl_y}) can hence be calculated from the general formula in Equation (2.4).

$$A_{rel,CH_xCl_y} = \frac{X_{CH_xCl_y}}{\sum X_{CH_xCl_y}} \tag{2.4}$$

An estimation of the number of chlorines (y) present in an average molecule is derived from the general formula of an alkane chain (C_nH_{2n+2}) and is described in Equation (2.5).

$$y = 2n + 2 - n\left(\left(2\frac{n-2}{n} + 3\frac{2}{n}\right)A_{rel,CH3/CH2} + 2A_{rel,CH2Cl} + A_{rel,\ CHCl}\right) \tag{2.5}$$

The number of hydrogens can be expressed from the formula of an alkane chain when the average number of chlorines are known, and together with the chain length of the single-chain mixture, the chlorine percentage (% Cl) can be approximated by the general formula in Equation (2.6), with the atomic weights of carbon (12.011 g/mol), hydrogen (1.078 g/mol) and chlorine (35.453 g/mol) (Meija et al., 2016).

$$\%Cl = \frac{y*M_{Cl}}{n*M_C + (2n+2-y)*M_H + y*M_{Cl}} \times 100\% \tag{2.6}$$

Method B

This ^{1}H NMR method relies upon the ability to separate all the different constitutional regions of the chlorinated alkanes (CH_3, CH_2, CHCl, and CH_2Cl) by chemical shifts (Sprengel et al., 2019). The identity of these regions can be deduced from the ^{1}H NMR spectrum and by assistance from the corresponding 2D-HSQC spectrum, see Fig. 1 (b).

The calculations are essentially the same as in method A, but all constitutional regions are integrated individually and are adjusted by a factor that represents their chemical environment, as described in Equation (2.3). These values are inserted in the equation for relative areas (Equation (2.4)).

The estimation of number of chlorines is described in Equation (2.7).

$$y = 2n + 2 - n\left(3A_{rel(CH3)} + 2A_{rel(CH2)} + 2A_{rel(CH2Cl)} + A_{rel(CHCl)}\right) \tag{2.7}$$

The chlorine percentage is then estimated as before, from Equation (2.6).

Method C

Method C is a new and simple method for chlorine percentage calculation. Unlike the two previous methods, it is also possible to perform a chlorine percentage estimation for mixtures where the chain length or average chain length is not known (industrial/technical mixtures). The constitutional regions (CH_3, CH_2, CHCl, and CH_2Cl) are divided in the same fashion as in method B, see Fig. 1 (b), and the individual adjusted areas are calculated as in Equation (2.3) for all regions.

The number of hydrogens present in the mixture (A_H) is expressed as the sum of integrals of all constitutional regions ($A_{CH_xCl_y}$) in the ^{1}H NMR spectrum, described in Equation (2.8).

$$A_H = \sum A_{CH_xCl_y} \tag{2.8}$$

The amount of chlorines present (A_{Cl}) correlate to the CHCl and CH_2Cl regions and can be calculated by Equation (2.9).

$$A_{Cl} = A_{CHCl} + \frac{A_{CH2Cl}}{2} \tag{2.9}$$

An estimation of the average chain length can be derived from the general equation for straight chain alkanes, as shown in Equation (2.10).

$$n = \frac{A_H + A_{Cl} - 2*SF}{2} \tag{2.10}$$

Where *SF* is a scaling factor to account for incorrect scaling of the integral, much like what is done for a single compound, where a peak corresponding to a known number of protons is set as a reference for the rest of the peaks. *SF* is derived from the possible end positions for an alkane, equal to 6, and the peak clusters that corresponds to end positions. For each CH_2Cl, there must be a Cl population that is half the size of the proton population. The expression for the *SF* is presented in Equation (2.11).

$$SF = \frac{6}{A_{CH3} + A_{CH2Cl} + 0.5A_{CH2Cl}} \tag{2.11}$$

Now that all atoms are 'counted', the chlorine percentage can be calculated from Equation (2.12).

$$\%Cl = \frac{M_{Cl}{}^{*}A_{Cl}}{M_{H}{}^{*}A_{H} + M_{Cl}{}^{*}A_{Cl} + M_{C}{}^{*}n} \quad (2.12)$$

3. Results and discussion

3.1. Indicative NMR analysis

Proton NMR of single chlorinated alkanes can provide some indicative results for proton NMR spectra of complex mixtures (Sprengel et al., 2019; Yuan et al., 2020). Which shifts that are expected to belong to certain motifs can be elucidated by their presence in either the NMR spectra of known single compounds or stereoisomeric mixtures of CPs.

Published NMR data of single isomer CPs or stereoisomeric mixtures are sparse, but a few compounds has been briefly discussed and elucidated in the literature (Beaume, 2005; Coelhan, 2003). There are also NMR data available for short, chlorinated alkanes (chlorobutanes, etc.) in databases (SDBSWeb, 2021). By combining these data, some general trends for the CH_3 shifts become apparent (see Table 1).

Methyl groups with two geminal chlorines in α-position (CH_3–CCl_2-) give proton shifts around 2.2 ppm, protons with one vicinal chlorine in α-position (CH_3–CHCl-) give a shift in the range of 1.5 ppm and isolated CH_3 groups (chlorine in β-position or further) give shifts near 1.0–0.9 ppm. The exact shifts will vary slightly around these generalizations, depending on other specific structural motifs present in complex mixtures. A visualization of this is shown in Figure 17.1 in SI, which also shows how complex the separation of integrals in the CH_2/CH_3 region can be.

A comparison of the $CHCl/CH_2Cl$ region of the three polychlorinated alkanes in Fig. 2 shows a general trend for compounds with vicinal chlorines.

The effect of chlorine on the chemical proton shift in -$(CHCl)_{\alpha'}$-C**H**Cl-$(CH_2)_{\alpha}$-C_{β}-C_{γ}-either at β- or γ-position is demonstrated in Fig. 2. Position H-4 and H-6 in compound **3** has a shift of 4.50–4.20 ppm from chlorines in α′ and β-position, whereas H-7 and H-10 has a shift of 4.10–4.07 ppm due to chlorines in α′ and γ-position. Having only a chlorine in α′, as in position H-3 and H-11 in compound **3**, gives a shift around 4.01–3.97 ppm. Compounds **1** and **2** shows similar proton shifts.

The order of the NMR signals follows the expected inductive substituent effect of an alkane, where the effects are more prominent when the substituents are in closer proximity of the observed proton (Friebolin, 1998). The nearby chlorines cause deshielding of the proton nucleus which experiences an increased magnetic field.

3.2. Chlorine percentage calculations

The results of the ^{1}H NMR chlorine percentage calculations of fourteen different chlorinated paraffin mixtures are listed in Table 2, and values obtained from Mohr titration are displayed as a reference.

Elemental analysis was conducted for $\mathbf{C_{12A}}$ and $\mathbf{C_{tech.}}$ giving values of 53.2% Cl and 41.5% Cl, respectively, for direct measurements of the Cl-atom. Chlorine percentages was also calculated from elemental analysis of the C-, H-, (S-), and N-atoms, assuming no other elements were present, where for $\mathbf{C_{12A}}$ was found 56.2% Cl and for $\mathbf{C_{tech.}}$ 41.3% Cl.

Measurement uncertainty of the NMR methods A, B and C is mainly attributed by operator error (integration error) or sample error (such as inhomogeneity). Impact of integrational error is described below and summarized in Fig. 4, whereas total measurement uncertainty was calculated for one single-chain mixture. The chlorine percentage was estimated by all three methods by four individual samples of the $\mathbf{C_{12A}}$ mixture (for data see SI, Section 7.1) and measurement uncertainty was determined to be 0.4% Cl for method A, 0.2% Cl for method B and 0.7% Cl for method C.

Fig. 3 shows a graphical representation of the experimental results, from Table 2, of the mixtures that were titrated.

The ^{1}H NMR methods A, B and C give similar estimated values, slightly on the lower side of the titration curve. This is expected as ^{1}H NMR will neither account for the carbons with full chlorine occupancy nor potential inorganic chloride contamination.

Compared to the titration approach, the NMR methods are much quicker and easier to perform. There is also a higher probability of user error with titration. Among these, not obtaining full conversion for the dechlorination, inaccurate measurements or equipment, inappropriate conditions, and difference in perception of endpoint. The cost of reagents used for titration may also surpass the cost of an NMR experiment. Finally, whereas NMR analysis is non-destructive, the titration will consume the sample in the dechlorination step.

3.3. Chlorine percentage of binary and ternary CP mixtures

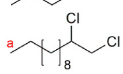
Unlike the two other methods, method C can estimate chlorine percentages for mixtures of different and unknown chain lengths. In Table 3 we present our ^{1}H NMR results with a binary mixture of a low (ca 40% Cl) and high-chlorinated (ca 60% Cl) CPs (Entry 1), a binary mixture of a medium (ca 50% Cl) and high-chlorinated CPs (Entry 2), and three ternary mixtures of CPs (Entries 3–5). Calculations are described in SI, Section 16.

Comparable chlorine percentages were obtained between the sum of individual single-chain CPs (method C^i) and ^{1}H NMR estimations of the mixture (method C). Highest deviation of 1% Cl was observed for the low- and high-chlorinated CP mixture (Entry 1) due to extended overlap in the CH_2/CH_3 region (observed by ^{1}H NMR and HSQC) and less accurate integration.

3.4. Limitation of the models

The three proposed ^{1}H NMR methods all suffer from some

Table 1
^{1}H and ^{13}C NMR ($CDCl_3$) spectral data for CH_3-groups with different methylene chlorine neighbor substitution pattern. Includes 2-chlorobutane (SDBSWeb, 2021), 2, 3-dichlorobutane (SDBSWeb, 2021), 2,2-dichloropropane (SDBSWeb, 2021), and 1,2-dichlorododecane (this work).

Name	Structure	Position	^{1}H NMR shift [ppm]	^{13}C NMR shift [ppm]
2-Chlorobutane		a	~1.5	~25
		b	~1.0	~11
1,2-Dichlorododecane		a	~0.9	~14
2,3-Dichlorobutane		a	~1.6	~20–22
2,2-Dichloropropane		a	~2.2	~39

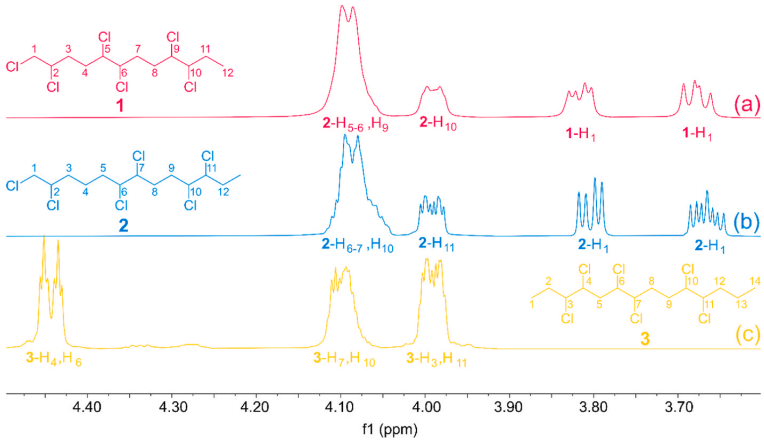


Fig. 2. The chlorinated regions in the ^{1}H NMR spectra (600 MHz, $CDCl_3$) for stereoisomeric mixtures of (a) 1,2,5,6,9,10-hexachlorododecane (**1**), (b) 1,2,6,7,10,11-hexachlorotridecane (**2**) and (c) 3,4,6,7,10,11-hexachlorotetradecane (**3**).

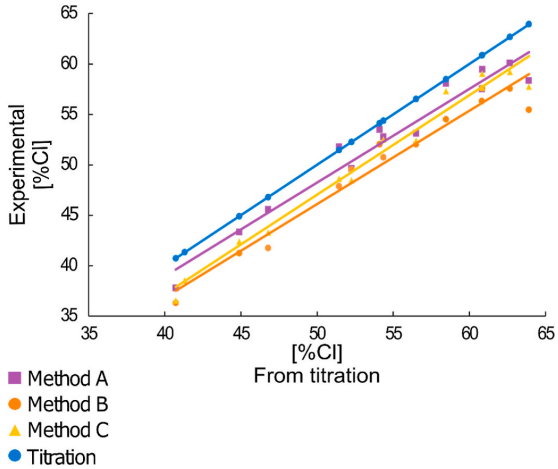


Fig. 3. Graphical representation of the chlorine percentage data for the NMR methods A, B and C compared to the values from titration.

limitations. They are all based upon the assumption that full chlorination of a single carbon (-CCl_2-, -CCl_3) does not take place, as they rely upon integration of the ^{1}H NMR spectra (Sprengel et al., 2019). Hence, all the mixtures are likely to give an underestimation for the chlorine percentages since they do not take constituents that are fully chlorinated into account. This is expected to be more problematic for highly chlorinated mixtures, as they are expected to contain more of fully chlorinated single carbons (Yuan et al., 2020).

Method A gives the highest general chlorine percentage of the three methods (see Fig. 3), which is likely due to the assumption that all ends are populated by hydrogens. The advantage using a stochastic model for the CH_3/CH_2 region is still valid, as the amount of CH_3 with a shift around δ 2.2 ppm increases and is in complete overlap with the CH_2 region. However, the number of terminal chlorines will increase with a higher chlorination degree and the validity of the assumption of full terminal proton occupancy becomes debatable. A higher estimation of the degree of chlorination may look desirable as all methods give an underestimation when -CCl_2-and -CCl_3 is present, nevertheless the results may be "right for the wrong reasons".

Table 2
Chlorination percentage calculation of thirteen single-chain and one technical mixture of CP using the 1H NMR methods A, B and C, and Mohr titration. Standard error of titration is given with a 99% confidence interval.

Mixture	Chemical formula	Method A [%]	Method B [%]	Method C [%]	Titration[a] [%]
C_9	$C_9H_{20-y}Cl_y$	51.8	47.9	48.6	50.2 ± 0.1
C_{10A}	$C_{10}H_{22-y}Cl_y$	52.8	50.7	52.5	54.4 ± 0.1
C_{10B}	$C_{10}H_{22-y}Cl_y$	60.1	57.6	59.2	62.7 ± 0.3
C_{11A}	$C_{11}H_{24-y}Cl_y$	53.1	52.0	52.1	56.5 ± 0.1
C_{11B}	$C_{11}H_{24-y}Cl_y$	58.3	55.5	57.7	63.9 ± 0.2
C_{12A}	$C_{12}H_{26-y}Cl_y$	53.5	52.0	53.8	54.1 ± 0.7[b]
C_{12B}	$C_{12}H_{26-y}Cl_y$	58.1	54.5	57.3	58.5 ± 0.2[c]
C_{13A}	$C_{13}H_{28-y}Cl_y$	45.6	41.8	43.3	46.8 ± 0.1
C_{13B}	$C_{13}H_{28-y}Cl_y$	57.5	56.3	57.7	60.8 ± 0.1
C_{14A}	$C_{14}H_{30-y}Cl_y$	37.8	36.3	36.5	40.7 ± 0.1
C_{14B}	$C_{14}H_{30-y}Cl_y$	43.3	41.2	42.4	44.9 ± 0.1
C_{14C}	$C_{14}H_{30-y}Cl_y$	59.5	57.6	59.0	60.9 ± 0.1
C_{15}	$C_{15}H_{32-y}Cl_y$	49.6	49.5	48.5	52.3 ± 0.2
$C_{tech.}$	$C_nH_{2n+2-y}Cl_y$	–	–	38.5	40.3 ± 0.7

[a] Average value of three titrations.
[b] Average value of nine titrations.
[c] Average value of six titrations.

Model B and C rely on individual integration of the CH_2 and CH_3 region, which in most cases can be readily distinguished. It becomes more complex at the extremities of lower and higher chlorination degrees for the individual single-chain CPs and mixtures of these. For mixtures with lower chlorination percentages the CH_2 region will experience less deshielding from nearby chlorines and move upfield towards the CH_3 region. Conversely, as the chlorination degree becomes higher, the mixtures will contain more CH_3 groups that will overlap with the CH_2 region, see Fig. 17.1 (SI). As a result, mixtures of single-chain and technical CPs of low and high-chlorination degree will give a less accurate integration due to overlap.

Fig. 4 shows a model of how the chlorine percent will vary if the CH_3 area is 0–20% over- and underestimated for the three single-chain mixtures **C_{10B}** (ca 60% Cl), **C_{12A}** (ca 55% Cl) and **C_{14A}** (ca 40%).

Method C is relatively robust in terms of integrational error, while a higher variation can be seen for method B, especially at lower chlorine percentages (Fig. 4 (a)).

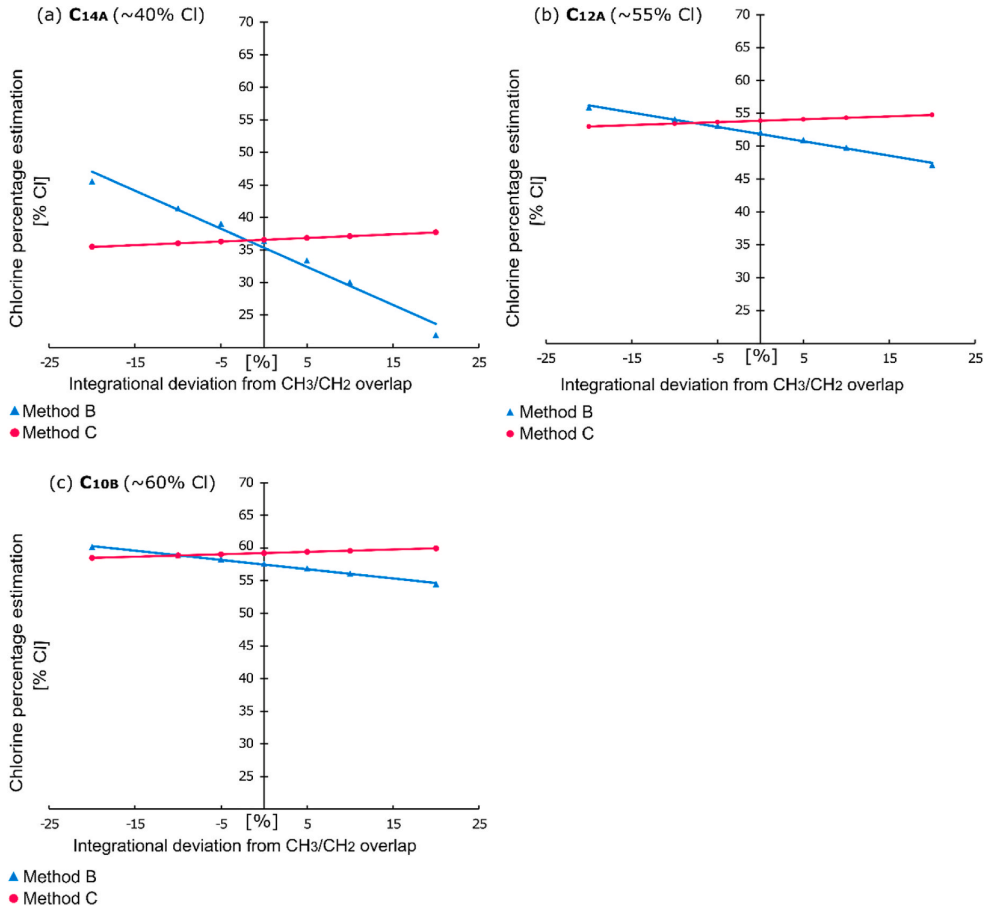


Fig. 4. Variation in the chlorine percentage calculation of NMR method B and C when over- and underestimating the area integration of CH_3 versus the CH_2 of (a) $\mathbf{C_{14A}}$, (b) $\mathbf{C_{12A}}$ and (c) $\mathbf{C_{10B}}$.

Table 3
Chlorination percentage estimation of binary and ternary single-chain CP mixtures from 1H NMR spectroscopy using method C. Sum method Ci ($\%Cl_{mix}$) was calculated using values for the individual single-chain mixtures in Table 2.

Entry	Components	Wt. Fraction	Sum method C[a] [%]	Method C [%]
1	$\mathbf{C_{tech.}}$	0.50	49.0	48.0
	$\mathbf{C_{10B}}$	0.50		
2	$\mathbf{C_{10A}}$	0.49	55.9	55.1
	$\mathbf{C_{10B}}$	0.51		
3	$\mathbf{C_{10B}}$	0.41	55.5	54.4
	$\mathbf{C_{11A}}$	0.28		
	$\mathbf{C_{12A}}$	0.31		
4	$\mathbf{C_{10A}}$	0.36	53.8	53.9
	$\mathbf{C_{11A}}$	0.33		
	$\mathbf{C_{12B}}$	0.30		
5	$\mathbf{C_{10A}}$	0.44	56.8	56.1
	$\mathbf{C_{10B}}$	0.30		
	$\mathbf{C_{11B}}$	0.44		

[a] Calculated from the formula: $\%Cl_{mix} = \sum_n X_n * \%Cl_n$, where X_n is the weight fraction and $\%Cl_n$ is the chlorine percentage for individual single-chain mixture n.

4. Conclusion

A new calculation method from ^{1}H NMR, and two modifications of a previous model, was used to determine the chlorine weight percentage of CP mixtures. Accurate and simple methods for analysis and assessment of mixtures is helpful in the process towards more available standards for CP analysis. The newly developed calculation model (model C) provides an easy and accessible way to determine the chlorine percentages of both single-chain mixtures, as well as technical CP mixtures of multiple, unknown chain lengths.The possible complication of this method, due to overlap between the CH_2 and CH_3 region in the ^{1}H NMR spectrum, was shown to have only a limited effect on the results.

Credit authorship contribution statement

Solveig Valderhaug: Conceptualization, Investigation; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – Original Draft. Huiling Liu: Conceptualization, Project administration; Supervision; Validation; Writing – review & editing. Alexey Gorovoy: Investigation. Jon Eigill Johansen: Funding acquisition; Project administration; Validation. Louise van Mourik: Writing – review & editing. Jacob de Boer: Writing – review & editing. Odd Reidar Gautun: Conceptualization, Supervision; Validation; Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136312.

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Supplementary information

Nuclear magnetic resonance as a tool to determine chlorine percentage of chlorinated paraffin mixtures

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Content

Abbreviations

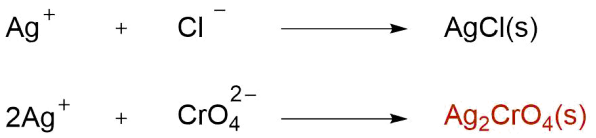
SD	Standard deviance
%RSD	Relative standard deviance
u	Standard uncertainty
U	Expanded uncertainty (at a 99% confidence interval)
Rel U%	Relative uncertainty

1 Titration procedure

1.1 Scope

Dechlorination of chlorinated paraffins (CPs) was conducted by means of sodium metal dispersed in isopropanol.[1,2] There exists several other dehalogenation agents that can be utilized such as trialkyl tin hydrides,[3] but most important is the full conversion to inorganic chlorides. Chloride ion concentration can thus be obtained by standard titration (Mohr, Volhard, etc.).

We used Mohr's titration for the chloride ion concentration determination, where silver nitrate is used as a titrant.[4] When a silver nitrate solution is poured into the chloride solution, a white precipitate of silver chloride is formed. When all chlorines in the solution have precipitated, excess silver ions will form a deep red precipitate together with chromium ions from the indicator (see Scheme 1.1). Endpoint volume is measured when a faint red colour persists in the analyte solution.



Scheme 1.1 Reaction of silver ions with chloride and chromate ions that forms under titration.

The overall titration analysis was accredited by ANSI national accreditation board (ANAB).

1.2 Procedure for dechlorination of CP and Mohr's titration

Indicator: Weigh out ~1.95 g of K_2CrO_4, dissolve and dilute with 100 mL of deionized water.

Accurately weigh out CP (50 mg) and dissolve it in *i*-PrOH (20 mL). Add sodium metal (1.25 g) and reflux for 5 h. Add a little more *i*-PrOH if the solution solidifies. Quench the reaction mixture with 50% aq. *i*-PrOH (5 mL) and follow up by adding deionized water (30 mL).

Let the mixture reach r.t. before adjusting pH to 6.5 - 9 with 10% aq. HNO_3 with a pH meter (add diluted aq. NaOH to readjust if pH goes under desired level). Let the mixture reach r.t. before diluting it with accurately 100 mL deionized water. Transfer the resultant solution into three different flasks with a pipette (3 x 20 mL). Add indicator (1 mL) to each of the three flasks.

To prepare the silver nitrate solution, accurately weigh out neat silver nitrate and dilute to exactly 100 mL. The amount of silver nitrate is adjusted and estimated to fit the burette (aiming for a ~7 mL titration volume fir a 10 mL burette) by Equation 1.1,

$$m_{AgNO_3} = 0.1369 * \%Cl * m_{CP}$$

Equation 1.1

where m_{AgNO_3} is the mass of silver nitrate, %Cl is the estimated chlorine percentage (i.e. from NMR methods) and m_{CP} is the mass of the chlorinated paraffin.

Titrate the flasks with the silver nitrate solution until a slightly red colour persists in the titrand. Record the volumes of amount silver nitrate solution used.

Amount of chloride ions present is calculated from Equation 1.2.

$$n_{Cl-} = 5 * N_{AgNO_3} * V_{AgNO_3}$$

Equation 1.2

Assuming the quantity of chlorides in the solution is equimolar to chlorines in the CP, %Cl can be calculated from Equation 1.3.

$$\%Cl = \frac{35.45 * n_{Cl-}}{m_{CP}} * 100\%$$

Equation 1.3

1.3 Method validation for Mohr's titration for CPs

A control sample of 1,2,7,8-tetrachlorooctane was submitted through the dechlorination/titration procedure at three different days and with three different amounts (25-66 mg, 9 titrations in total) to test the accuracy of %Cl estimations up against the theoretical value from the molecular formula. The %Cl estimations were calculated from Equation 1.3 after titration and the accuracy was measured from the difference between theoretical and average calculated value. More datapoints would give a more accurate representation, however, 9 titrations were considered sufficient for this purpose. Results are shown in Table 1.

Table 1: %Cl results from 9 different titrations of the control sample with calculated variance, standard deviance, uncertainty, and accuracy determination.

Chiron No. **1672.8**	%Cl
Rep 1 (49.1 mg)	56.6
Rep 2 (49.1 mg)	56.6
Rep 3 (49.1 mg)	56.4
Rep 4 (24.8 mg)	57.8
Rep 5 (24.8 mg)	57.6
Rep 6 (24.8 mg)	57.5
Rep 7 (66.3 mg)	56.7
Rep 8 (66.3 mg)	56.5
Rep 9 (66.3 mg)	56.6
Average	56.9
Variance	2.54E-01
SD	0.50
%RSD	0.89
u	0.17
U	0.43
Rel U%	0.76

Accuracy	
Target %Cl	56.3
%Cl result	56.9
Diff. %Cl	0.6
Diff. %	1.1

The mixtures were titrated between 3-9 times and the average value is listed in Table 3.2 (article), together with the uncertainty at ±2,58σ (99% confidence interval) assuming a normal distribution of the titration results. The titration results are shown together with the NMR method results and elemental analysis under each mixtures respective section.

1.3.1 Titration data

1	Value	Uncertainty	Unit
m_{CP}	0.04913	0.00001	g
N_{AgNO3}	0.01967	2.03E-05	mol/L
V_{AgNO3}	0.00798	0.00002	L
n_{Cl-}	0.00078	2.78E-06	mol
%Cl	56.6	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.04913	0.00001	g
N_{AgNO3}	0.01967	2.03E-05	mol/L
V_{AgNO3}	0.00797	0.00002	L
n_{Cl-}	0.00078	2.77E-06	mol
%Cl	56.6	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.04913	0.00001	g
N_{AgNO3}	0.01967	2.03E-05	mol/L
V_{AgNO3}	0.00794	0.00002	L
n_{Cl-}	0.00078	2.77E-06	mol
%Cl	56.4	0.2	%

4	Value	Uncertainty	Unit
m_{CP}	0.02480	0.00001	g
N_{AgNO3}	0.01031	1.06-05	mol/L
V_{AgNO3}	0.00784	0.00002	L
n_{Cl-}	0.00040	1.45E-06	mol
%Cl	57.8	0.2	%

5	Value	Uncertainty	Unit
m_{CP}	0.02480	0.00001	g
N_{AgNO3}	0.01031	1.06-05	mol/L
V_{AgNO3}	0.00781	0.00002	L
n_{Cl-}	0.00040	1.45E-06	mol
%Cl	57.6	0.2	%

6	Value	Uncertainty	Unit
m_{CP}	0.02480	0.00001	g
N_{AgNO3}	0.01031	1.06E-05	mol/L
V_{AgNO3}	0.00780	0.00002	L
n_{Cl-}	0.00040	1.45E-06	mol
%Cl	57.5	0.2	%

7	Value	Uncertainty	Unit
m_{CP}	0.06627	0.00001	g
N_{AgNO3}	0.02645	2.72E-05	mol/L
V_{AgNO3}	0.00801	0.00002	L
n_{Cl-}	0.00106	3.73E-06	mol
%Cl	56.7	0.2	%

8	Value	Uncertainty	Unit
m_{CP}	0.06627	0.00001	g
N_{AgNO3}	0.02645	2.72E-05	mol/L
V_{AgNO3}	0.00799	0.00002	L
n_{Cl-}	0.00106	3.73E-06	mol
%Cl	56.5	0.2	%

9	Value	Uncertainty	Unit
m_{CP}	0.06627	0.00001	g
N_{AgNO3}	0.02645	2.72E-05	mol/L
V_{AgNO3}	0.00800	0.00002	L
n_{Cl-}	0.00106	3.73E-06	mol
%Cl	56.6	0.2	%

2 Chlorine percentage of C_9 mixture

2.1 ^{1}H NMR Methods

Method A

n	9
$A_{CH2/CH3}$	106.50
A_{CHCl}	25.33
A_{CH2Cl}	2.99
B	2.22
$X_{CH2/CH3}$	47.93
X_{CH2Cl}	1.50
A_{sum}	74.75
$A_{rel,CH2/CH3}$	0.64
$A_{rel,CHCl}$	0.34
$A_{rel,CH2Cl}$	0.02
y	3.77
Cl%	51.8

Method B

n	9
A_{CH3} (1.66-1.50, 1.12-0.85 ppm)	46.14
A_{CH2} (2.65-1.66, 1.49-1.23 ppm)	60.36
A_{CHCl} (4.71-3.81 ppm)	25.33
A_{CH2Cl} (3.81-3.50 ppm)	2.99
X_{CH3}	15.38
X_{CH2}	30.18
X_{CH2Cl}	1.50
A_{sum}	72.39
$A_{rel,CH3}$	0.21
$A_{rel,CH2}$	0.42
$A_{rel,CHCl}$	0.35
$A_{rel,CH2Cl}$	0.02
y	3.24
Cl%	47.9

Method C

A_{CH3}	46.14
A_{CH2}	60.36
A_{CHCl}	25.33
A_{CH2Cl}	2.99
A_H	134.82
A_{Cl}	26.83
SF	0.12
2'	16.88
wt_H	135.90
wt_{Cl}	950.95
wt_C	869.34
wt_{tot}	1956.19
Cl%	48.6

2.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.07747	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00914	0.00002	L
n_{Cl-}	0.00112	3.62E-06	mol
%Cl	51.4	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.07747	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00915	0.00002	L
n_{Cl-}	0.00113	3.62E-06	mol
%Cl	51.5	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.07747	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00913	0.00002	L
n_{Cl-}	0.00112	3.62E-06	mol
%Cl	51.4	0.2	%

Results

Description	Calculation
Rep. 1	51.4
Rep. 2	51.5
Rep. 3	51.4
Average	51.45
Variance	2.11E-03
SD	0.05
%RSD	0.09
u	0.03
U	0.07
Rel. U%	0.1

3 Chlorine percentage of C_{10A} mixture

3.1 ^{1}H NMR methods

Method A

n	10
$A_{CH2/CH3}$	1.56
A_{CHCl}	0.43
A_{CH2Cl}	0.18
B	2.2
$X_{CH2/CH3}$	0.71
X_{CH2Cl}	0.09
A_{sum}	1.23
$A_{rel,CH2/CH3}$	0.58
$A_{rel,CHCl}$	0.35
$A_{rel,CH2Cl}$	0.07
y	4.35
Cl%	52.8

Method B

n	10
A_{CH3} (1.68-1.51, 1.18-0.80 ppm)	0.56
A_{CH2} (2.67-1.68, 1.51-1.40 ppm)	1.00
A_{CHCl} (4.74-3.86 ppm)	0.43
A_{CH2Cl} (3.86-3.51 ppm)	0.18
X_{CH3}	0.19
X_{CH2}	0.50
X_{CH2Cl}	0.09
A_{sum}	1.21
$A_{rel,CH3}$	0.16
$A_{rel,CH2}$	0.41
$A_{rel,CHCl}$	0.36
$A_{rel,CH2Cl}$	0.08
y	4.02
Cl%	50.7

Method C

A_{CH3}	0.56
A_{CH2}	1
A_{CHCl}	0.43
A_{CH2Cl}	0.18
A_H	2.17
A_{Cl}	0.52
SF	7.23
2'	0.28
wt_H	2.19
wt_{Cl}	18.43
wt_C	14.49
wt_{tot}	35.11
Cl%	52.5

3.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.05494	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00680	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	54.2	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.05494	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00681	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	54.4	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.05494	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00749	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	54.5	0.2	%

Description	Calculation
Rep. 1	54.2
Rep. 2	54.4
Rep. 3	54.5
Average	54.4
Variance	8.22E-03
SD	0.09
%RSD	0.17
u	0.04
U	0.10
Rel. U%	0.2

4 Chlorine percentage of C_{10B} mixture

4.1 ^{1}H NMR methods

Method A

n	10
$A_{CH2/CH3}$	3.21
A_{CHCl}	1.50
A_{CH2Cl}	0.46
B	2.2
$X_{CH2/CH3}$	1.46
X_{CH2Cl}	0.23
A_{sum}	3.19
$A_{rel,CH2/CH3}$	0.46
$A_{rel,CHCl}$	0.47
$A_{rel,CH2Cl}$	0.07
y	5.79
Cl%	60.1

Method B

n	10
A_{CH3} (2.30-2.19, 1.87-1.50 & 1.27-1.03 ppm)	1.50
A_{CH2} (2.91-2.30 & 2.19-1.87 ppm)	1.71
A_{CHCl} (6.05-5.80 & 5.14-3.98 ppm)	1.50
A_{CH2Cl} (3.98-3.55 ppm)	0.46
X_{CH3}	0.5
X_{CH2}	0.86
X_{CH2Cl}	0.23
A_{sum}	3.09
$A_{rel,CH3}$	0.16
$A_{rel,CH2}$	0.28
$A_{rel,CHCl}$	0.49
$A_{rel,CH2Cl}$	0.08
y	5.24
Cl%	57.6

Method C

A_{CH3}	1.50
A_{CH2}	171
A_{CHCl}	1.50
A_{CH2Cl}	0.46
A_H	5.17
A_{Cl}	1.73
SF	2.740
2'	0.73
wt_H	5.211
wt_{Cl}	61.329
wt_C	37.051
wt_{tot}	103.591
Cl%	59.2

4.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.04717	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00680	0.00002	L
n_{Cl-}	0.00083	3.11E-06	mol
%Cl	62.3	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.04717	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00681	0.00002	L
n_{Cl-}	0.00084	3.11E-06	mol
%Cl	62.8	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.04717	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00742	0.00002	L
n_{Cl-}	0.00084	3.11E-06	mol
%Cl	62.8	0.2	%

Description	Calculation
Rep. 1	62.3
Rep. 2	62.8
Rep. 3	62.8
Average	62.7
Variance	5.74E-02
SD	0.24
%RSD	0.38
u	0.10
U	0.25
Rel. U%	0.4

5 Chlorine percentage of C_{11A} mixture

5.1 ^{1}H NMR methods

Method A

n	11
$A_{CH2/CH3}$	21.93
A_{CHCl}	7.66
A_{CH2Cl}	3.72
B	2.18
$X_{CH2/CH3}$	10.05
X_{CH2Cl}	1.86
A_{sum}	19.57
$A_{rel,CH2/CH3}$	0.51
$A_{rel,CHCl}$	0.39
$A_{rel,CH2Cl}$	0.10
y	5.28
Cl%	55.3

Method B

n	11
A_{CH3} (1.69-1.50, 1.2-0.8 ppm)	5.12
A_{CH2} (2.65-1.69 ppm)	16.81
A_{CHCl} (4.72-3.86 ppm)	7.66
A_{CH2Cl} (3.86-3.50 ppm)	3.72
X_{CH3}	1.71
X_{CH2}	8.41
X_{CH2Cl}	1.86
A_{sum}	19.63
$A_{rel,CH3}$	0.09
$A_{rel,CH2}$	0.43
$A_{rel,CHCl}$	0.39
$A_{rel,CH2Cl}$	0.10
y	5.34
Cl%	55.6

Method C

A_{CH3}	5.12
A_{CH2}	16.81
A_{CHCl}	7.66
A_{CH2Cl}	3.72
A_H	33.31
A_{Cl}	9.52
SF	0.561
2'	3.567
wt_H	33.577
wt_{Cl}	337.484
wt_C	235.776
wt_{tot}	606.837
Cl%	55.6

5.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.04812	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00680	0.00002	L
n_{Cl-}	0.00077	3.04E-06	mol
%Cl	56.5	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.04812	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00681	0.00002	L
n_{Cl-}	0.00077	3.04E-06	mol
%Cl	56.5	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.04812	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00681	0.00002	L
n_{Cl-}	0.00077	3.04E-06	mol
%Cl	56.5	0.2	%

Description	Calculation
Rep. 1	56.5
Rep. 2	56.5
Rep. 3	56.5
Average	56.5
Variance	1,53E-03
SD	0.04
%RSD	0.07
u	0.02
U	0.04
Rel U%	0.1

6 Chlorine percentage of C_{11B} mixture

6.1 ^{1}H NMR methods

Method A

n	11
$A_{CH2/CH3}$	3.51
A_{CHCl}	1.47
A_{CH2Cl}	0.42
B	2.18
$X_{CH2/CH3}$	1.61
X_{CH2Cl}	0.21
A_{sum}	3.29
$A_{rel,CH2/CH3}$	0.49
$A_{rel,CHCl}$	0.45
$A_{rel,CH2Cl}$	0.06
y	5.94
Cl%	58.3

Method B

n	11
A_{CH3} (2.30-2.21, 1.89-1.55 & 1.30-1.03 ppm)	1.55
A_{CH2} (2.90-2.30 & 2.21-1.89 ppm)	1.96
A_{CHCl} (5.16-3.99 ppm)	1.47
A_{CH2Cl} (3.99-3.55 ppm)	0.42
X_{CH3}	0.52
X_{CH2}	0.98
X_{CH2Cl}	0.2
A_{sum}	3.18
$A_{rel,CH3}$	0.16
$A_{rel,CH2}$	0.31
$A_{rel,CHCl}$	0.46
$A_{rel,CH2Cl}$	0.07
y	5.30
Cl%	55.5

Method C

A_{CH3}	1.55
A_{CH2}	1.96
A_{CHCl}	1.47
A_{CH2Cl}	0.42
A_H	5.4
A_{Cl}	1.68
SF	2.75
2'	0.73
wt_H	5.44
wt_{Cl}	59.56
wt_C	38.15
wt_{tot}	103.15
Cl%	57.7

6.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.04572	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00680	0.00002	L
n_{Cl-}	0.00083	3.11E-06	mol
%Cl	64.1	0.3	%

2	Value	Uncertainty	Unit
m_{CP}	0.04572	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00681	0.00002	L
n_{Cl-}	0.00082	3.10E-06	mol
%Cl	64.0	0.3	%

3	Value	Uncertainty	Unit
m_{CP}	0.04572	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00729	0.00002	L
n_{Cl-}	0.00082	3.10E-06	mol
%Cl	63.7	0.3	%

	%Cl
Rep. 1	64.1
Rep. 2	64.0
Rep. 3	63.7
Average	63.9
Variance	3.22E-02
SD	0.18
%RSD	0.28
u	0.07
U	0.19
Rel U%	0.3

7 Chlorine percentage of C_{12A} mixture

7.1 ^{1}H NMR Methods

Method A

n	12
$A_{CH2/CH3}$	52.55
A_{CHCl}	16.28
A_{CH2Cl}	6.62
B	2.17
$X_{CH2/CH3}$	24.25
X_{CH2Cl}	3.31
A_{sum}	43.84
$A_{rel,CH2/CH3}$	0.55
$A_{rel,CHCl}$	0.37
$A_{rel,CH2Cl}$	0.08
y	5.35
Cl%	53.5

Method B

n	12
A_{CH3} (1.69-1.50, 1.2-0.8 ppm)	15.81
A_{CH2} (2.65-1.69 ppm)	36.74
A_{CHCl} (4.75-3.86 ppm)	16.28
A_{CH2Cl} (3.86-3.50 ppm)	6.62
X_{CH3}	5.27
X_{CH2}	18.37
X_{CH2Cl}	3.31
A_{sum}	43.23
$A_{rel,CH3}$	0.12
$A_{rel,CH2}$	0.43
$A_{rel,CHCl}$	0.38
$A_{rel,CH2Cl}$	0.08
y	5.06
Cl%	52.0

Method C

A_{CH3}	15.81
A_{CH2}	36.74
A_{CHCl}	16.28
A_{CH2Cl}	6.62
A_H	75.45
A_{Cl}	19.59
SF	0.23
2'	8.58
wt_H	76.05
wt_{Cl}	694.47
wt_C	519.19
wt_{tot}	1289.71
Cl%	53.8

Measurement uncertainty calculations

Method A	
Description	Calculation
Rep. 1	53.5
Rep. 2	52.8
Rep. 3	52.6
Rep. 4	52.8
Average	52.9
Variance	1.10E-01
SD	0.33
%RSD	0.63
u	0.14
U	0.35
Rel. U%	0.66

Method B	
Description	Calculation
Rep. 1	52.0
Rep. 2	52.1
Rep. 3	52.2
Rep. 4	52.6
Average	52.2
Variance	4.85E-02
SD	0.22
%RSD	0.42
u	0.09
U	0.23
Rel. U%	0.4

Method C	
Description	Calculation
Rep. 1	53.8
Rep. 2	52.5
Rep. 3	52.4
Rep. 4	52.4
Average	52.8
Variance	3.86E-01
SD	0.62
%RSD	1.18
u	0.25
U	0.65
Rel. U%	1.2

7.2 Elemental analysis

Chlorine specific

Entry	Weight (mg)	% Cl
1	20.7120	53.25
2	20.7860	53.46
3	20.7930	53.52

N, C, H, S specific

Entry	Weight (mg)	% N	% C	% H	% S
1	3.6640	-	38.24	5.55	-
2	3.5130	-	38.24	5.53	-
3	3.9240	-	38.22	5.56	-

7.3 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.05077	0.00001	g
N_{AgNO3}	0.02118	0.00002	mol/L
V_{AgNO3}	0.00736	0.00002	L
n_{Cl-}	0.00078	2.92E-06	mol
%Cl	54.4	0.2	%

4	Value	Uncertainty	Unit
m_{CP}	0.03179	0.00001	g
N_{AgNO3}	0.01375	0.00001	mol/L
V_{AgNO3}	0.00699	0.00002	L
n_{Cl-}	0.00048	1.87E-06	mol
%Cl	53.6	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.05077	0.00001	g
N_{AgNO3}	0.02118	0.00002	mol/L
V_{AgNO3}	0.00741	0.00002	L
n_{Cl-}	0.00078	2.93E-06	mol
%Cl	54.8	0.2	%

5	Value	Uncertainty	Unit
m_{CP}	0.03179	0.00001	g
N_{AgNO3}	0.01375	0.00001	mol/L
V_{AgNO3}	0.00698	0.00002	L
n_{Cl-}	0.00048	1.87E-06	mol
%Cl	53.5	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.05077	0.00001	g
N_{AgNO3}	0.02118	0.00002	mol/L
V_{AgNO3}	0.00743	0.00002	L
n_{Cl-}	0.00079	2.93E-06	mol
%Cl	54.9	0.2	%

6	Value	Uncertainty	Unit
m_{CP}	0.03179	0.00001	g
N_{AgNO3}	0.01375	0.00001	mol/L
V_{AgNO3}	0.00697	0.00002	L
n_{Cl-}	0.00048	1.87E-06	mol
%Cl	53.4	0.2	%

Results

Description	Calculation
Rep. 1	54.4
Rep. 2	54.8
Rep. 3	54.9
Rep. 4	53.6
Rep. 5	53.5
Rep. 6	53.4
Average	54.1
Variance	3.80E-01
SD	0.62
%RSD	1.14
u	0.25
U	0.65
Rel. U%	1.2

8 Chlorine percentage of C_{12B} mixture

8.1 ^{1}H NMR methods

Method A

n	12
$A_{CH2/CH3}$	3.62
A_{CHCl}	1.50
A_{CH2Cl}	0.32
B	2.17
$X_{CH2/CH3}$	1.67
X_{CH2Cl}	0.16
A_{sum}	3.33
$A_{rel,CH2/CH3}$	0.50
$A_{rel,CHCl}$	0.45
$A_{rel,CH2Cl}$	0.05
y	6.40
Cl%	58.1

Method B

n	12
A_{CH3} (1.71-1.50, 1.13-1.00 ppm)	1.65
A_{CH2} (2.70-1.71 ppm)	1.97
A_{CHCl} (4.64-3.86 ppm)	1.50
A_{CH2Cl} (3.86-3.50 ppm)	0.32
X_{CH3}	0.55
X_{CH2}	0.99
X_{CH2Cl}	0.16
A_{sum}	3.20
$A_{rel,CH3}$	0.17
$A_{rel,CH2}$	0.31
$A_{rel,CHCl}$	0.47
$A_{rel,CH2Cl}$	0.05
y	5.57
Cl%	54.5

Method C

A_{CH3}	1.65
A_{CH2}	1.97
A_{CHCl}	1.5
A_{CH2Cl}	0.32
A_H	5.44
A_{Cl}	1.66
SF	2.82
2'	0.71
wt_H	5.48
wt_{Cl}	58.85
wt_C	38.37
wt_{tot}	102.70
Cl%	57.3

8.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.03623	0.00001	g
N_{AgNO3}	0.01705	1.76E-05	mol/L
V_{AgNO3}	0.00706	0.00002	L
n_{Cl-}	0.00060	2.33E-06	mol
%Cl	58.9	0.2	%

4	Value	Uncertainty	Unit
m_{CP}	0.07565	0.00001	g
N_{AgNO3}	0.03742	3.85E-05	mol/L
V_{AgNO3}	0.00667	0.00002	L
n_{Cl-}	0.00125	5.03E-06	mol
%Cl	58.5	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.03623	0.00001	g
N_{AgNO3}	0.01705	1.76E-05	mol/L
V_{AgNO3}	0.00701	0.00002	L
n_{Cl-}	0.00060	2.32E-06	mol
%Cl	58.5	0.2	%

5	Value	Uncertainty	Unit
m_{CP}	0.07565	0.00001	g
N_{AgNO3}	0.03742	3.85E-05	mol/L
V_{AgNO3}	0.00666	0.00002	L
n_{Cl-}	0.00125	5.03E-06	mol
%Cl	58.4	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.03623	0.00001	g
N_{AgNO3}	0.01705	1.76E-05	mol/L
V_{AgNO3}	0.00699	0.00002	L
n_{Cl-}	0.00060	2.32E-06	mol
%Cl	58.3	0.2	%

6	Value	Uncertainty	Unit
m_{CP}	0.07565	0.00001	g
N_{AgNO3}	0.03742	3.85E-05	mol/L
V_{AgNO3}	0.00665	0.00002	L
n_{Cl-}	0.00124	5.02E-06	mol
%Cl	58.3	0.2	%

Results

Description	Calculation
Rep. 1	59.0
Rep. 2	58.5
Rep. 3	58.3
Rep. 4	58.5
Rep. 5	58.4
Rep. 6	58.3
Average	58.5
Variance	3.98E-02
SD	0.20
%RSD	0.34
u	0.08
U	0.21
Rel. U%	0.4

9 Chlorine percentage of C_{13A} mixture

9.1 ^{1}H NMR methods

Method A

n	13
$A_{CH2/CH3}$	81.24
A_{CHCl}	15.03
A_{CH2Cl}	1.00
B	2.15
$X_{CH2/CH3}$	37.72
X_{CH2Cl}	0.5
A_{sum}	53.25
$A_{rel,CH2/CH3}$	0.71
$A_{rel,CHCl}$	0.28
$A_{rel,CH2Cl}$	0.01
y	4.25
Cl%	45.6

Method B

n	13
A_{CH3} (1.61-1.49, 1.2-0.8 ppm)	25.19
A_{CH2} (2.60-1.61, 1.49-1.27 ppm)	56.05
A_{CHCl} (4.75-3.81 ppm)	15.03
A_{CH2Cl} (3.81-3.50 ppm)	1.00
X_{CH3}	8.40
X_{CH2}	28.03
X_{CH2Cl}	0.5
A_{sum}	51.96
$A_{rel,CH3}$	0.16
$A_{rel,CH2}$	0.54
$A_{rel,CHCl}$	0.29
$A_{relCH2Cl}$	0.01
y	3.66
Cl%	41.8

Method C

A_{CH3}	25.19
A_{CH2}	56.05
A_{CHCl}	15.03
A_{CH2Cl}	1.00
A_H	97.27
A_{Cl}	15.53
SF	0.23
2'	8.90
wt_H	98.05
wt_{Cl}	550.68
wt_C	623.99
wt_{tot}	1272.72
Cl%	43.3

9.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.06073	0.00001	g
N_{AgNO3}	0.02283	2.35E-05	mol/L
V_{AgNO3}	0.00704	0.00002	L
n_{Cl-}	0.00080	3.11E-06	mol
%Cl	46.9	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.06073	0.00001	g
N_{AgNO3}	0.02283	2.35E-05	mol/L
V_{AgNO3}	0.00701	0.00002	L
n_{Cl-}	0.00080	3.11E-06	mol
%Cl	46.7	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.06073	0.00001	g
N_{AgNO3}	0.02283	2.35E-05	mol/L
V_{AgNO3}	0.00702	0.00002	L
n_{Cl-}	0.00080	3.11E-06	mol
%Cl	46.8	0.2	%

Description	Calculation
Rep. 1	46.9
Rep. 2	46.7
Rep. 3	46.8
Average	46.8
Variance	6.90E-03
SD	0.08
%RSD	0.18
u	0.03
U	0.09
Rel. U%	0.2

10 Chlorine percentage of C_{13B} mixture

10.1 ^{1}H NMR methods

Method A

n	13
$A_{CH2/CH3}$	60.25
A_{CHCl}	25.62
A_{CH2Cl}	8.89
B	2.15
$X_{CH2/CH3}$	27.97
X_{CH2Cl}	4.45
A_{sum}	58.04
$A_{rel,CH2/CH3}$	0.48
$A_{rel,CHCl}$	0.44
$A_{rel,CH2Cl}$	0.08
y	6.78
Cl%	57.5

Method B

n	13
A_{CH3} (2.30-2.21, 1.89-1.55 & 1.30-1.03 ppm)	17.93
A_{CH2} (2.90-2.30 & 2.21-1.89 ppm)	42.32
A_{CHCl} (5.16-3.99 ppm)	25.62
A_{CH2Cl} (3.99-3.55 ppm)	8.89
X_{CH3}	5.98
X_{CH2}	21.16
X_{CH2Cl}	4.45
A_{sum}	57.20
$A_{rel,CH3}$	0.10
$A_{rel,CH2}$	0.37
$A_{rel,CHCl}$	0.45
$A_{rel,CH2Cl}$	0.08
y	6.46
Cl%	56.3

Method C

A_{CH3}	17.93
A_{CH2}	42.32
A_{CHCl}	25.62
A_{CH2Cl}	8.89
A_H	94.76
A_{Cl}	30.07
SF	0.19
2'	10.42
wt_H	95.52
wt_{Cl}	1065.80
wt_C	686.99
wt_{tot}	1848.31
Cl%	57.7

10.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.04785	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00729	0.00002	L
n_{Cl-}	0.00082	3.10E-06	mol
%Cl	60.9	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.04785	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00729	0.00002	L
n_{Cl-}	0.00082	3.10E-06	mol
%Cl	60.9	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.04785	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00728	0.00002	L
n_{Cl-}	0.00082	3.10E-06	mol
%Cl	60.8	0.2	%

Description	Calculation
Rep. 1	60.9
Rep. 2	60.9
Rep. 3	60.8
Average	60.8
Variance	1.55E-03
SD	0.04
%RSD	0.06
u	0.02
U	0.04
Rel. U%	0.1

11 Chlorine percentage of C_{14A} mixture

11.1 ^{1}H NMR methods

Method A

n	14
$A_{CH2/CH3}$	215.97
A_{CHCl}	26.87
A_{CH2Cl}	4.72
B	2.14
$X_{CH2/CH3}$	100.79
X_{CH2Cl}	2.36
A_{sum}	130.02
$A_{rel,CH2/CH3}$	0.78
$A_{rel,CHCl}$	0.21
$A_{rel,CH2Cl}$	0.02
y	3.34
Cl%	37.8

Method B

n	14
A_{CH3} (1.61-1.52, 1.12-0.79 ppm)	49.08
A_{CH2} (2.55-1.61, 1.52-1.20 ppm)	166.89
A_{CHCl} (4.65-3.81 ppm)	26.87
A_{CH2Cl} (3.81-3.50 ppm)	4.72
X_{CH3}	16.36
X_{CH2}	83.45
X_{CH2Cl}	2.36
A_{sum}	129.04
$A_{rel,CH3}$	0.13
$A_{rel,CH2}$	0.65
$A_{rel,CHCl}$	0.21
$A_{rel,CH2Cl}$	0.02
y	3.14
Cl%	36.3

Method C

A_{CH3}	49.08
A_{CH2}	166.89
A_{CHCl}	26.87
A_{CH2Cl}	4.72
A_H	247.56
A_{Cl}	29.23
SF	0.11
2'	18.72
wt_H	249.54
wt_{Cl}	1036.20
wt_C	1549.71
wt_{tot}	2835.45
Cl%	36.5

11.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.06904	0.00001	g
N_{AgNO3}	0.02263	2.33E-05	mol/L
V_{AgNO3}	0.00703	0.00002	L
n_{Cl-}	0.00080	3.08E-06	mol
%Cl	40.8	0.2	%

2	Value	Uncertainty	Unit
	0.06904	0.00001	g
N_{AgNO3}	0.02263	2.33E-05	mol/L
V_{AgNO3}	0.00700	0.00002	L
n_{Cl-}	0.00079	3.08E-06	mol
%Cl	40.7	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.06904	0.00001	g
N_{AgNO3}	0.02263	2.33E-05	mol/L
V_{AgNO3}	0.00700	0.00002	L
n_{Cl-}	0.00079	3.08E-06	mol
%Cl	40.7	0.2	%

Results

Description	Calculation
Rep. 1	40.845
Rep. 2	40.670
Rep. 3	40.670
Average	40.73
Variance	6.75E-03
SD	0.08
%RSD	0.20
u	0.05
U	0.12
Rel. U%	0.3

12 Chlorine percentage of C_{14B} mixture

12.1 ^{1}H NMR method

Method A

n	14
$A_{CH2/CH3}$	52.4
A_{CHCl}	8.84
A_{CH2Cl}	1.78
B	2.14
$X_{CH2/CH3}$	24.45
X_{CH2Cl}	0.89
A_{sum}	34.18
$A_{rel,CH2/CH3}$	0.72
$A_{rel,CHCl}$	0.26
$A_{rel,CH2Cl}$	0.03
y	4.19
Cl%	43.3

Method B

n	14
A_{CH3} (1.59-1.50, 1.12-0.85 ppm)	13.15
A_{CH2} (2.49-1.59, 1.50-1.22 ppm)	39.25
A_{CHCl} (4.65-3.81 ppm)	8.84
A_{CH2Cl} (3.81-3.50 ppm)	1.78
X_{CH3}	4.38
X_{CH2}	19.63
X_{CH2Cl}	0.89
A_{sum}	33.74
$A_{rel,CH3}$	0.13
$A_{rel,CH2}$	0.58
$A_{rel,CHCl}$	0.26
$A_{rel,CH2Cl}$	0.03
y	3.85
Cl%	41.2

Method C

A_{CH3}	13.15
A_{CH2}	39.25
A_{CHCl}	8.84
A_{CH2Cl}	1.78
A_H	63.02
A_{Cl}	9.73
SF	0.38
2'	5.27
wt_H	63.52
wt_{Cl}	344.93
wt_C	405.20
wt_{tot}	813.65
Cl%	42.4

12.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.06346	0.00001	g
N_{AgNO3}	0.02007	2.07E-05	mol/L
V_{AgNO3}	0.00802	0.00002	L
n_{Cl-}	0.00080	2.84E-06	mol
%Cl	45.0	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.06346	0.00001	g
N_{AgNO3}	0.02007	2.07E-05	mol/L
V_{AgNO3}	0.00800	0.00002	L
n_{Cl-}	0.00080	2.83E-06	mol
%Cl	44.9	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.06346	0.00001	g
N_{AgNO3}	0.02007	2.07E-05	mol/L
V_{AgNO3}	0.00800	0.00002	L
n_{Cl-}	0.00080	2.83E-06	mol
%Cl	44.9	0.2	%

Results

Description	Calculation
Rep. 1	45.0
Rep. 2	44.9
Rep. 3	44.9
Average	44.9
Variance	2.79E-03
SD	0.05
%RSD	0.12
u	0.03
U	0.08
Rel. U%	0.2

13 Chlorine percentage of C_{14C} mixture

13.1 ^{1}H NMR method

Method A

n	14
$A_{CH2/CH3}$	4.36
A_{CHCl}	2.15
A_{CH2Cl}	0.49
B	2.14
$X_{CH2/CH3}$	2.04
X_{CH2Cl}	0.25
A_{sum}	4.43
$A_{rel,CH2/CH3}$	0.46
$A_{rel,CHCl}$	0.49
$A_{rel,CH2Cl}$	0.06
y	7.88
Cl%	59.5

Method B

n	14
A_{CH3} (2.30-2.21, 1.89-1.55 & 1.30-1.03 ppm)	1.53
A_{CH2} (2.90-2.30 & 2.21-1.89 ppm)	2.83
A_{CHCl} (5.16-3.99 ppm)	2.15
A_{CH2Cl} (3.99-3.55 ppm)	0.49
X_{CH3}	0.51
X_{CH2}	1.42
X_{CH2Cl}	0.25
A_{sum}	4.32
$A_{rel,CH3}$	0.12
$A_{rel,CH2}$	0.33
$A_{rel,CHCl}$	0.50
$A_{rel,CH2Cl}$	0.06
y	7.32
Cl%	57.6

Method C

A_{CH3}	1.53
A_{CH2}	2.83
A_{CHCl}	2.15
A_{CH2Cl}	0.49
A_H	7
A_{Cl}	2.40
SF	2.65
2'	0.76
wt_H	7.06
wt_{Cl}	84.90
wt_C	51.88
wt_{tot}	143.84
Cl%	59.0

13.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.04896	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00746	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	60.9	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.04896	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00745	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	60.8	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.04896	0.00001	g
N_{AgNO3}	0.02253	2.32E-05	mol/L
V_{AgNO3}	0.00747	0.00002	L
n_{Cl-}	0.00084	3.12E-06	mol
%Cl	60.9	0.2	%

Results

Description	Calculation
Rep. 1	60.9
Rep. 2	60.8
Rep. 3	60.9
Average	60.9
Variance	4.44E-03
SD	0.07
%RSD	0.11
u	0.03
U	0.07
Rel. U%	0.1

14 Chlorine percentage of C_{15} mixture

14.1 ^{1}H NMR method

Method A

n	15
$A_{CH2/CH3}$	20.84
A_{CHCl}	5.14
A_{CH2Cl}	0.97
B	2.13
$X_{CH2/CH3}$	9.77
X_{CH2Cl}	0.49
A_{sum}	15.39
$A_{rel,CH2/CH3}$	0.64
$A_{rel,CHCl}$	0.33
$A_{rel,CH2Cl}$	0.03
y	5.74
Cl%	49.6

Method B

n	15
A_{CH3} (1.66-1.50, 1.12-0.85 ppm)	3.97
A_{CH2} (2.65-1.66, 1.49-1.23 ppm)	16.87
A_{CHCl} (4.71-3.81 ppm)	5.14
A_{CH2Cl} (3.81-3.50 ppm)	0.97
X_{CH3}	1.32
X_{CH2}	8.44
X_{CH2Cl}	0.49
A_{sum}	15.38
$A_{rel,CH3}$	0.09
$A_{rel,CH2}$	0.55
$A_{rel,CHCl}$	0.33
$A_{rel,CH2Cl}$	0.03
y	5.72
Cl%	49.5

Method C

A_{CH3}	3.97
A_{CH2}	16.87
A_{CHCl}	5.14
A_{CH2Cl}	0.97
A_H	26.95
A_{Cl}	5.63
SF	1.11
2'	1.81
wt_H	27.17
wt_{Cl}	199.41
wt_C	184.75
wt_{tot}	411.33
Cl%	48.5

14.2 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.03296	0.00001	g
N_{AgNO3}	0.01346	1.39E-05	mol/L
V_{AgNO3}	0.00723	0.00002	L
n_{Cl-}	0.00049	1.85E-06	mol
%Cl	52.3	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.03296	0.00001	g
N_{AgNO3}	0.01346	1.39E-05	mol/L
V_{AgNO3}	0.00720	0.00002	L
n_{Cl-}	0.00048	1.84E-06	mol
%Cl	52.1	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.03296	0.00001	g
N_{AgNO3}	0.01346	1.39E-05	mol/L
V_{AgNO3}	0.00724	0.00002	L
n_{Cl-}	0.00049	1.85E-06	mol
%Cl	52.4	0.2	%

Results

Description	Calculation
Rep 1	52.3
Rep 2	52.1
Rep 3	52.4
Average	52.3
Variance	1.51E-02
SD	0.12
%RSD	0.24
u	0.07
U	0.18
Rel. U%	0.4

15 Chlorine percentage of technical mixture (Wako)

15.1 ^{1}H NMR method

Method C

A_{CH3} (1.60-1.50, 1.12-0.80 ppm)	6.74
A_{CH2} (2.42-1.60, 1.50-1.15 ppm)	25.54
A_{CHCl} (4.75-3.81 ppm)	4.76
A_{CH2Cl} (3.81-3.50 ppm)	0.26
A_H	37.3
A_{Cl}	4.89
SF	0.84
2'	2.38
wt_H	37.60
wt_{Cl}	173.35
wt_C	239.08
wt_{tot}	450.03
Cl%	38.5

15.2 Elemental analysis

Chlorine specific

Entry	Weight (mg)	% Cl
1	22.7480	41.20
2	19.9610	41.24
3	17.8710	41.34

N, C, H and S specific

Entry	Weight (mg)	% N	% C	% H	% S
1	3.7370	-	50.94	7.73	-
2	3.5340	-	50.96	7.74	-
3	3.5380	-	50.96	7.72	-

15.3 Titration

1	Value	Uncertainty	Unit
m_{CP}	0.07549	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00716	0.00002	L
n_{Cl-}	0.00088	3.37E-06	mol
%Cl	41.4	0.2	%

2	Value	Uncertainty	Unit
m_{CP}	0.07549	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00709	0.00002	L
n_{Cl-}	0.00087	3.36E-06	mol
%Cl	41 .0	0.2	%

3	Value	Uncertainty	Unit
m_{CP}	0.07549	0.00001	g
N_{AgNO3}	0.03075	3.17E-05	mol/L
V_{AgNO3}	0.00721	0.00002	L
n_{Cl-}	0.00089	3.37E-06	mol
%Cl	41.6	0.2	%

Results

Description	Calculation
Rep. 1	41.4
Rep. 2	41.0
Rep. 3	41.6
Average	41.3
Variance	8.08E-02
SD	0.28
%RSD	0.69
u	0.16
U	0.42
Rel. U%	1.0

16 Chlorine percentage of binary and ternary single-chain CP mixtures

Entry	Components	Weight [mg]	Wt. Fraction	Sum method C	Method C
1	C_{10B}	32.37	0.50	49.0	48.0
	$C_{tech.}$	31.75	0.50		
2	C_{10A}	26.16	0.49	55.9	55.1
	C_{10B}	27.04	0.51		
3	C_{10B}	32.74	0.41	55.5	54.4
	C_{11A}	22.79	0.28		
	C_{12A}	25.02	0.31		
4	C_{10A}	32.70	0.36	53.8	53.9
	C_{11A}	29.63	0.33		
	C_{12B}	27.34	0.30		
5	C_{10A}	23.31	0.44	56.8	56.1
	C_{10B}	27.06	0.30		
	C_{11B}	39.13	0.44		

Mixture 1

A_{CH3} (2.30-2.18, 1.60-1.24, 1.11-1.01 ppm)	4.29
A_{CH2} (2.79-2.30, 2.18-1.60 ppm)	6.66
A_{CHCl} (5.14-3.94 ppm)	2.20
A_{CH2Cl} (3.94-3.50 ppm)	1
A_H	14.15
A_{Cl}	2.70
SF	1.04
2'	1.93
Wt. H	14.26
Wt. Cl	95.72
Wt. C	89.59
Wt. tot	199.57
Cl%	48.0

Mixture 2

A_{CH3} (2.28-2.21, 1.73-1.50, 1.14-1.02 ppm)	4.16
A_{CH2} (2.82-2.28, 2.21-1.73 ppm)	6.14
A_{CHCl} (5.19-3.93 ppm)	3.56
A_{CH2Cl} (3.93-3.51 ppm)	1
A_H	14.86
A_{Cl}	4.06
SF	1.06
2'	1.89
Wt. H	14.98
Wt. Cl	143.93
Wt. C	102.29
Wt. tot	261.19
Cl%	55.1

Mixture 3

A_{CH3} (2.28-2.23, 1.76-1.49, 1.14-1.00 ppm)	3.44
A_{CH2} (2.83-2.28, 2.24-1.76 ppm)	5.87
A_{CHCl} (5.18-3.93 ppm)	3.02
A_{CH2Cl} (3.93-3.52 ppm)	1
A_H	13.33
A_{Cl}	3.52
SF	1.21
2'	1.65
Wt. H	13.44
Wt. Cl	124.78
Wt. C	91.30
Wt. tot	229.52
Cl%	54.4

Mixture 4

A_{CH3} (2.28-2.20, 1.75-1.45, 1.15-1.01 ppm)	4.27
A_{CH2} (2.84-2.28, 2.20-1.75 ppm)	6.33
A_{CHCl} (5.15-3.95 ppm)	3.34
A_{CH2Cl} (3.95-3.52 ppm)	1
A_H	14.94
A_{Cl}	3.84
SF	1.04
2'	1.92
Wt. H	15.06
Wt. Cl	136.13
Wt. C	101.22
Wt. tot	252.41
Cl%	53.9

Mixture 5

A_{CH3} (2.32-2.20, 1.74-1.47, 1.14-1.03 ppm)	4.03
A_{CH2} (2.97-2.33, 2.20-1.74 ppm)	4.97
A_{CHCl} (5.16-3.97 ppm)	3.28
A_{CH2Cl} (3.97-3.53 ppm)	1
A_H	13.28
A_{Cl}	3.78
SF	1.08
2'	1.84
Wt. H	13.39
Wt. Cl	134.00
Wt. C	91.38
Wt. tot	238.76
Cl%	56.1

17 Shift regions of the methyl in single-chain CP mixtures from ^{1}H NMR spectroscopy

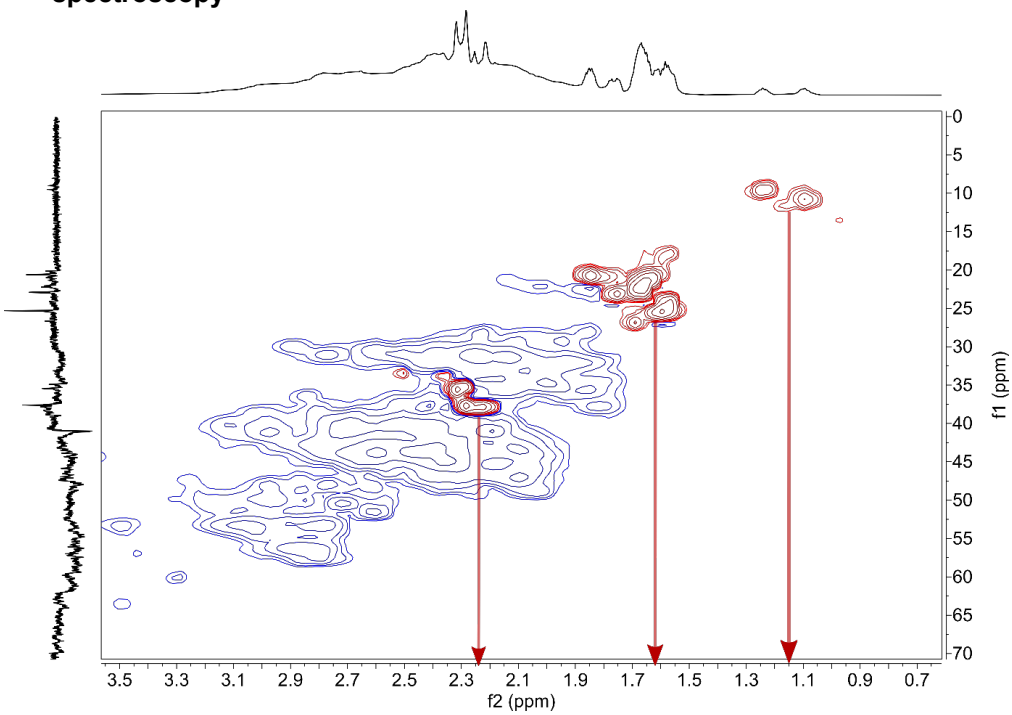
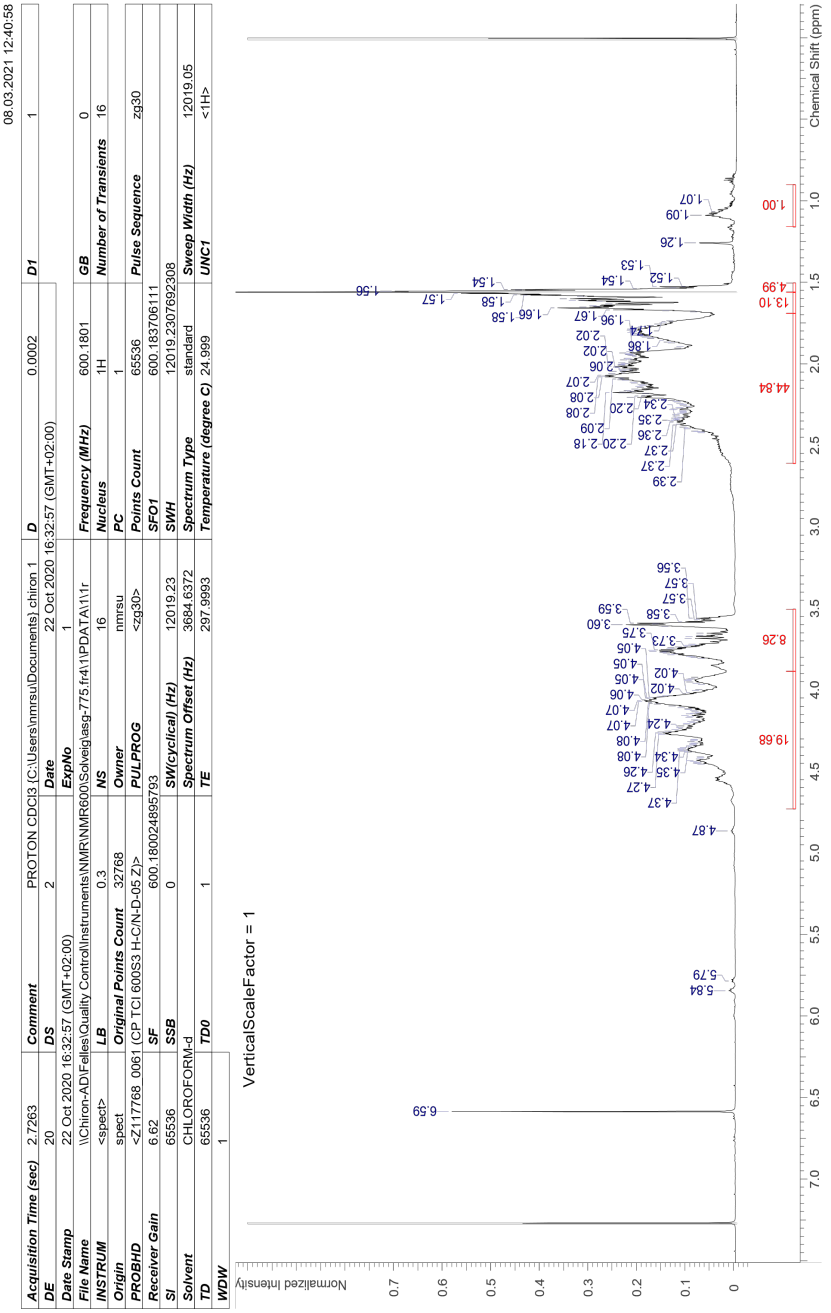


Figure 17.1: *HSQC (600 MHz, $CDCl_3$) of a C_{14} 65 Cl% single-chain mixture to show distributions of CH_3 groups in CP mixtures.*

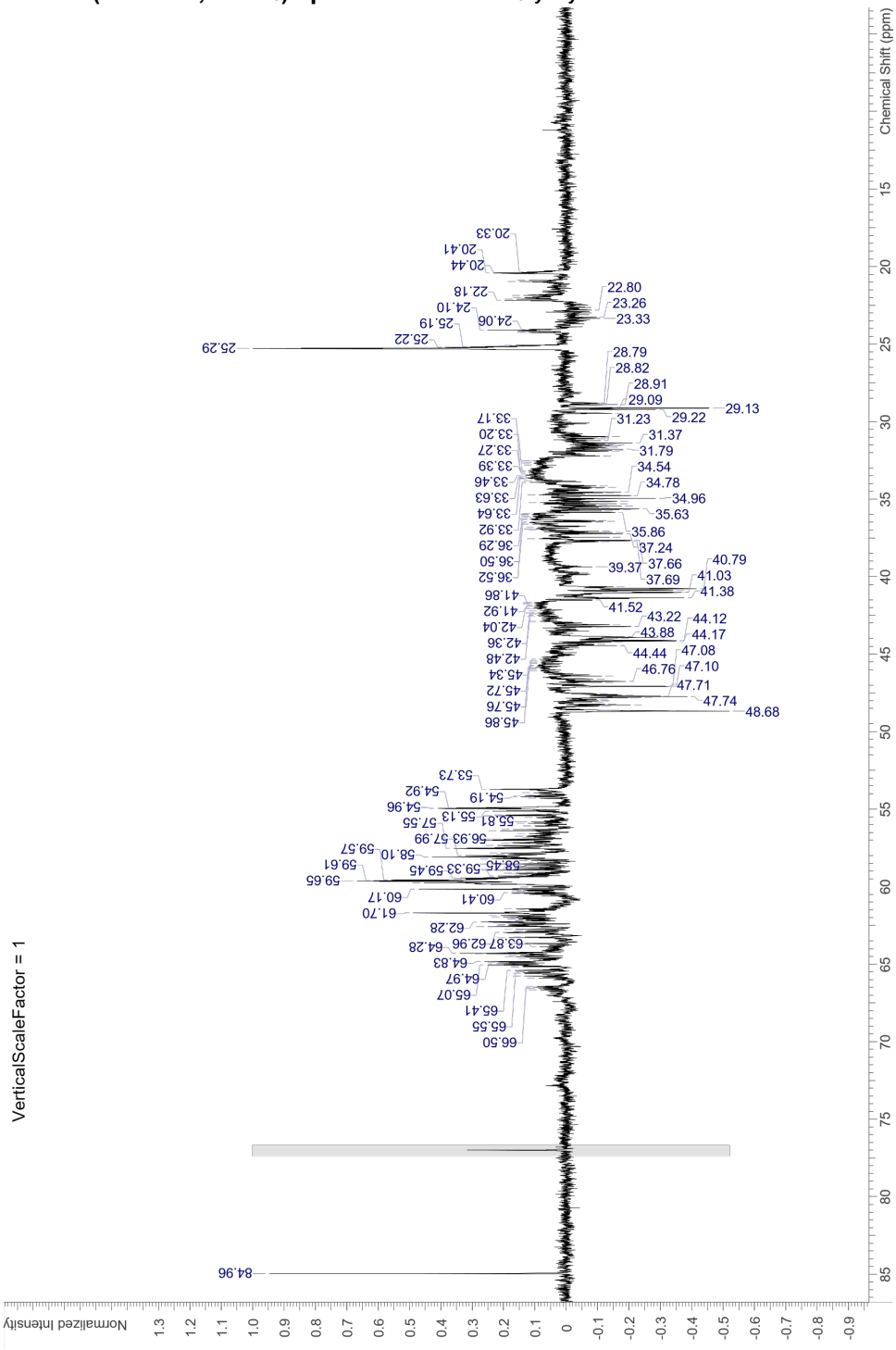
18 ^{1}H NMR, DEPT and HSQC spectra of C_{12} mixture

Spectra of a C_{12} mixture used to show a typical analysis of a single-chain mixture and to show how the integration ranges are generally set for a mixture.

18.1 ^{1}H NMR (600 MHz, $CDCl_3$) spectrum for $C_{12}H_{26-y}Cl_y$



18.2 DEPT (150 MHz, $CDCl_3$) spectrum for $C_{12}H_{26-y}Cl_y$



18.3 HSQC (600 MHz / 150 MHz, $CDCl_3$) spectrum for $C_{12}H_{26-y}Cl_y$

Acquisition Time (sec)	(0.0532, 0.0103)
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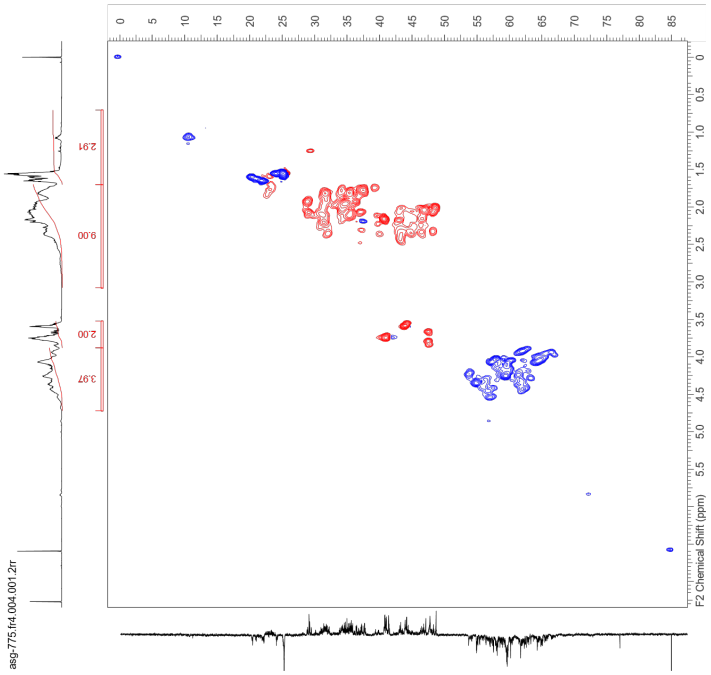
Date	23 Oct 2020 14:45:36
File Name	\\Chiron-AD\Felles\Quality Control\Instruments\NMR\NMR600\Solvia\asg-775,fr4\4\PDATA\1\2rr
Frequency (MHz)	(600.1800, 150.9154)
Nucleus	(1H, 13C)
Number of Transients	8
Origin	spect
Original Points Count	(512, 256)
Owner	nmrsu
Points Count	(1024, 1024)
Pulse Sequence	hsqcedetgpsisp2.3
Solvent	CHLOROFORM-d
Spectrum Type	HSQC-DEPT
Sweep Width (Hz)	(9606.00, 24851.33)
Temperature (degree C)	27.000
Title	echo/antiecho edited HSQC w/sensitivity improvement w/adiabatic bilevel decoupling w/adiabatic refocussing (w/ gradients in back-INEPT w/matched adiabatic sweep) hsqc13C1H NTNU CDCl3 (C:\Users\nmrsu\Documents\) chiron 1



3

19 References

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