

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**STRUCTURAL INVESTIGATIONS ON ARTIFICIAL SWEET MOLECULES
BY NMR SPECTROSCOPY AND COMPUTATIONAL METHODS**

M.Sc. THESIS

Birgöl SAĞLAM

Department of Chemistry

Chemistry Programme

JANUARY 2014

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Thesis Advisor: Assoc. Prof. Nurcan Ş. TÜZÜN

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**YAPAY TATLANTIRICI BİLEŞİKLERİN NMR SPEKTROSKOPİSİ VE
HESAPSAL YÖNTEMLERLE YAPISAL ANALİZİ**

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To my family,

FOREWORD

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ABBREVIATIONS

1D, 2D	: One- and two- dimensional
¹H	: Proton
B3LYP	: Becke style three parameter functional in combination with Lee-Yang-Parr correlation functional
CD	: Cyclodextrin
CIS	: Complexation induced chemical shifts
CSGT	: Continuous set of gauge transformations
DFT	: Density functional theory
DMSO	: Dimethylsulfoxide
DQF-COSY	: Double quantum filtered correlation spectroscopy
FID	: Free induction decay
GIAO	: Gauge-including atomic orbital
HF	: Hartree-Fock
HSQC	: Heteronuclear single quantum coherence
Hz	: Hertz
k_{ex}	: Exchange rate
MM	: Molecular mechanics
MMFF	: Merck molecular force field
NHDC	: Neohesperidin dihydrochalcone
NMR	: Nuclear magnetic resonance
NOE	: Nuclear overhauser effect
NOESY	: Nuclear overhauser and exchange spectroscopy
ppb	: part per billion (10 ⁻⁹)
ppm	: part per million (10 ⁻⁶)
PCM	: Polarizable continuum model
ROESY	: Rotating-frame overhauser enhancement spectroscopy
TOCSY	: Total correlation spectroscopy
s	: Second
δ	: Chemical shift
Δδ	: Chemical shift difference
Å°	: Angström

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STRUCTURAL INVESTIGATIONS ON ARTIFICIAL SWEET MOLECULES BY NMR SPECTROSCOPY AND COMPUTATIONAL METHODS

SUMMARY

Intensification of the sugar related diseases like diabetes, obesity at alarming rates results in the hunt for new non-caloric substances. Sweet taste plays a vital role for human life and, thereby, for medical concerns. Although sweet taste is important in human life, the formation of sweet taste is not known completely. The generation of sweet taste in the body is different from other taste perception mechanisms.

Nevertheless, scientific studies of sweet taste were carried out as early as the beginning of 20th century. A widely accepted study accomplished by Shallenberger and Agreer indicates that there is a hydrogen bond interaction between the sweetener and the receptor. There should be AH-B unit on the sweet molecule and complementary BH-A unit on the sweet taste receptor and the distance between the units should be *ca.* 2.5 up to 4 Å. Other theories trying to explain sweet taste formation include this hydrogen bonding interaction as well. The discovery of sweet taste receptor cannot give the ultimate answer on the generation of sweet taste. It is known that structural behavior of sweeteners is important on the taste and there is a transient interaction occurring during the formation of sweet taste.

This thesis is composed of experimental and computational parts. Nuclear magnetic resonance (NMR) implemented as the experimental technique in the current study is unusual and different from traditional NMR methods. Experimental method is based on contemplating the hydrogen bonding properties of artificial sweet molecules. NMR parameters such as chemical shift (δ), temperature coefficient ($d\delta/dT$), exchange rate (k_{ex}), coupling constant (J) and chemical shift difference ($\Delta\delta$) can be used to get valuable information about dynamic properties of the selected compounds. Furthermore, complexation with cyclodextrin molecules was also studied to shed light on molecular behaviors of sweet molecules in complexation mechanisms. Computational methodology was used to examine the contribution of conformers to the attainable NMR properties. At the first step of NMR calculations, a reasonable optimized geometry is needed. Monte Carlo method with MMFF calculations were carried out to obtain plausible conformers for the subject molecule. Consequently, single point energy calculations were performed with DFT method and B3LYP/6-31G* level of theory for accurate energy calculation. In order to reduce the computational cost, conformers that have relative energies larger than 7 kcal mol⁻¹ were omitted. Further geometry optimizations were carried out using DFT method at the B3LYP/6-31G* and B3LYP/6-311+G** levels of theories. After obtaining reasonable geometries, NMR calculations were performed with DFT method, employing B3LYP functional and 6-311+G** basis set.

At first glance, experimental and computational results indicate that there is no common molecular property for the selected artificial sweeteners. Complexation studies indicate that the size of sweetener plays a significant role and the temperature dependence does not change significantly upon complexation.

High percent errors were obtained especially for proton chemical shifts, since the computational studies were carried out in the gas phase. Moreover, employing a Boltzmann distribution in the calculations of partial effects of conformers does not affect on the accuracy of the calculations.

This thesis has been produced as a part of TUBITAK project entitled “Why are some molecules sweet? – NMR Investigations on Structure-Taste Relationship as Revealed by the Interactions between Sweetener and Water Molecules and Sweetener-Cyclodextrin Complexes” (Grant No: 109T606). This project covers structural analyses of both natural and artificial sweet molecules by NMR spectroscopy and computational methods.

YAPAY TATLANDIRICI BİLEŞİKLERİN NMR SPEKTROSKOPİSİ VE HESAPSAL YÖNTEMLERLE YAPISAL ANALİZİ

ÖZET

Tatlandırıcılara bağlı olarak artan obezite, diyabet gibi rahatsızlıklar kalorisiz veya diğer bir ifade ile şeker içermeyen yapay tatlandırıcıların sentezlenmesini tetiklemektedir. Sağlık problemlerine bağlı olarak yapılan diyetlerde bu yapay tatlandırıcılar elzem olarak kullanılmakla beraber diyet endüstrisinde de kendilerine yer bulmaktadırlar. Yaygın kullanımlarına rağmen yapay tatlandırıcıların güvenliği sentezlendiği günden bu yana hep tartışma konusu olmuştur. 1879 yılında sentezlenen ilk yapay tatlandırıcı olan sakarinin güvenli bir tatlandırıcı olup olmadığı 2000 yılına kadar süregelen tartışmalara konu olmuştur. Tatlılık hissinin insan hayatındaki önemi göz ardı edilemeyecek kadar büyüktür. İnsan hayatında önemli bir yere sahip olmasında karşın bu hissin nasıl oluştuğu henüz tam anlamıyla bilinmemektedir. Tatlandırıcı moleküller ile algılayıcı reseptörler arasındaki etkileşim klasik sinyal oluşumundan farklıdır. Klasik sinyal oluşumunda tetikleyici moleküller ile algılayıcı reseptörler arasında kompleks oluşumu gözlenirken tatlılık hissi oluşumunda bu özellik kompleks oluşumu şeklinde değil geçici etkileşimler olarak kendini göstermektedir. Tatlılık hissinin oluşumunun geçici etkileşimler şeklinde olması tatlılık hissinin araştırma konusu olmasını bir hayli gecikmiştir ve ancak 20. yüzyılın başlarında ilk çalışmalar gerçekleştirilmiştir. Yaygın olarak kabul edilen bir çalışmaya göre tatlılık hissinin, tatlandırıcı moleküller üzerinde yapısal olarak özel gruplar aracılığı ile oluştuğu ifade edilmiştir. Bu çalışmadan sonra bilim insanları tarafından en çok kabul gören çalışma Shallenberger ve Agree tarafından gerçekleştirilmiştir. Shallenberger ve Agree'nin yaptığı çalışmaya göre tatlandırıcı moleküller ile reseptör arasında hidrojen bağı oluşumu söz konusudur ve tatlandırıcı molekül ile reseptör arası uzaklık 2.5 ile 4 Å arasında olmalıdır. Tatlandırıcı moleküller üzerinde hidrojen bağı oluşturacak AH-B birimi var iken reseptör üzerinde de bu birime karşı gelecek BH-A birimi mevcuttur. Daha sonra yapılan çalışmalar bu hidrojen bağı temeli üzerine kurulmuş olup tatlandırıcı moleküller üzerine farklı yapısal grupların tat hissine katkıda bulundukları belirtilmiştir. Tatlandırıcıların çok çeşitli kimyasal yapılara sahip olmalarından dolayı tatlılık hissinin oluşumunu sağlayan reseptör ancak 2001 yılında keşfedilebilmiştir. Yapılan çalışmalarda bütün tatlandırıcı moleküllerin T1R2_T1R3 heterodimerini aktive ettiği ortaya çıkmıştır. Reseptörler ve tatlandırıcı moleküller üzerinde spesifik etkileşimler olduğu bilinse de tatlılık hissinin tam olarak nasıl oluştuğu bilinmemektedir. Bununla beraber tatlılık hissinin oluşumunda tatlandırıcı moleküllerin yapısal özelliklerinin etkili olduğu bilinmektedir. Ayrıca hidrojen bağı oluşumu reseptör keşfinden önceki bütün tatlılık hissinin oluşumunda yapılan çalışmalarda ortak nokta olmuştur. Bu tez kapsamında bir grup yapay tatlandırıcı incelenmiştir. analizi yapılan yapay tatlandırıcılar asesülfam potasyum, sodyum siklamat, sakarin, aspartam, neotam, isomalt, sukraloz ve neohesperidin dihidroçalkondur.

Tez içerisinde izlenen yöntem deneysel metot ve hesapsal metot olarak iki kısımdan oluşmaktadır. Deneysel yöntemde nükleer manyetik rezonans (NMR) spektroskopisi tekniği kullanılmıştır. Hidrojen bağının daha önce çalışılan modellerde ortak özellik olmasından yola çıkılarak yapay tatlandırıcı moleküllerin su ile etkileşimlerinin incelenmesi NMR metodunun temelini oluşturmaktadır. Tezde kullanılan NMR metodu kimyasal kayma (δ), sıcaklık değişim sabiti ($d\delta/dT$), hidroksit protonlarının ortamdaki su molekülleri ile yer değişim hız sabiti (k_{ex}), etkileşim sabiti (J) ve kimyasal kayma farkı ($\Delta\delta$) gibi parametrelerden yararlanılarak dinamik özelliklerin açıklanmasına olanak sağlamaktadır. Teze konu olan yapılarda NMR parameterleri ile hidroksit protonlarının su molekülleri ile etkileşimleri, molekül içi ve moleküller arası hidrojen bağı eğilimleri incelenmiştir. Deneysel kısımda moleküllerin sulu ortamdaki davranışlarına ek olarak siklodekstrinler ile birlikte inklüzyon kompleks oluşum eğilimleri incelenmiştir. Siklodekstrinler glikopiranoz birimlerinden oluşan oligosakkaritlerdir ve tez kapsamında sırasıyla 6, 7 ve 8 glikopiranoz biriminden oluşan α -, β -, γ - siklodekstrinleri kullanılmıştır. Siklodekstrinler kesik koni biçiminde olup koninin iç kısmı hidrofobik dış kısmı ise yapısındaki hidroksit protonlarının yönlendirilmesi ile birlikte hidrofilik yapıya sahiptir. Inklüzyon kompleksleri misafir molekülün hidrofobik/hidrofilik özelliğine bağlı olarak oluşmaktadır. Molekül üzerinde daha hidrofobik kısım koninin hidrofobik olan iç kısmına yerleşmeyi tercih ederken molekül üzerinde daha hidrofilik özelliğe sahip olan bölümü ve yüklü gruplar solvent içerisinde kesik koni biçimindeki yapının büyük kenarında kalmayı tercih eder. Inklüzyon kompleksleri oluşumunda herhangi bir kovalent bağ oluşmaz veya kırılmaz. Ayrıca kompleks oluşturan moleküller ile kompleks oluşumuna katılmayan moleküller arasında bir denge mevcuttur. Kompleks oluşum analizinde daha önce hesaplanan NMR parametrelerine ek olarak CIS (kompleks oluşum kimyasal kayma farkı) değerleri hesaplanmıştır. CIS değerlerinin pozitif veya negatif olması kompleks oluşumu ile ilgili önemli bilgiler vermektedir. Kompleks oluşumu tatlandırıcı moleküllerin siklodekstrinler ile nasıl bir etkileşim içerisinde olduğu tatlandırıcı molekülün büyüklüğü göz önüne alınarak incelenmiştir. Hesapsal yöntem yapıya katkısı olabilecek konformerlerin analizini gerçekleştirmek için uygulanmıştır. Bu amaçla yapılan NMR hesaplamaları iki aşamadan oluşmaktadır. Birinci aşama molekül için uygun konformerlerin elde edilmesi, ikinci aşama da izotropik perdeleme sabitlerinin hesaplanmasıdır. Uygun konformerlerin elde edilmesi çeşitli basamaklardan oluşmaktadır. Öncelikle molekül için geçerli olabilecek konformerler Monte Carlo ve MMFF metodu ile elde edilmiştir. Elde edilen konformerlere daha hassas bir yöntem olan DFT metodu B3LYP fonksiyoneli ve 6-31G* baz seti ile tek nokta enerji hesabı yapılmıştır. Elde edilen konformerlerden yüksek enerjili olanların NMR hesaplamalarına katkıları az olacağından bu basamakta konformerlerin elimine edilmesi gerekmektedir. Bağlı enerjileri 7 kcal mol⁻¹ değerinden büyük olan konformerler optimizasyon ve NMR hesaplamalarına katılmamıştır. Yüksek enerjili konformerlerin çıkartılmasından sonra konformerlere optimizasyon işlemi uygulanır. Optimizasyon hesaplama süresini olumlu etkilemesini sağlamak amacıyla iki basamakta gerçekleştirilmiştir. İlk basamakta DFT/B3LYP/6-31G* metodu, ikinci basamakta da DFT/B3LYP/6-311+G** metodu ile konformerlerin optimizasyonu gerçekleştirilmiştir. Optimizasyon basamağından sonra konformerler için NMR hesaplamaları gerçekleştirilmiştir. NMR hesaplamaları GIAO metodu ile birlikte DFT/B3LYP/6-311+G** metodu kullanılarak gerçekleştirilmiştir. NMR hesaplamaları sonucunda izotropik perdeleme sabitleri elde edilmiştir. NMR hesaplamaları ile aynı metotta hesaplanan TMS molekülüne ait izotropik perdeleme sabitlerinden yararlanılarak

konformerler için kimyasal kayma değerleri elde edilmiştir. Kimyasal kayma değerleri üç farklı metotla incelenmiştir. İlk olarak enerji dahil edilmeden bütün konformerlerden elde edilen kimyasal kayma değerlerinin ortalaması alınmıştır. İkinci olarak konformerlerinin enerjilerinin de göz önüne alındığı Boltzmann dağılımı ile yine konformerlerden elde edilen kimyasal kayma değerlerinin ortalaması alınmıştır. Üçüncü yöntemde ise sistematik hataları elimine etmek amacıyla lineer regresyon analizi uygulanmıştır. Lineer regresyon analizi için deneysel kimyasal kayma değerlerine karşı hesaplanan ortalama kimyasal kayma değerleri grafiğe çizilmiştir. Grafikten elde edilen eğim ve kayım değerlerinden düzenleme sabiti oluşturulur ve bu düzenleme sabiti hesaplanan ortalama kimyasal kayma değerlerine uygulanarak düzeltilmiş kimyasal kayma değerleri elde edilmiştir. Üç yöntemle elde edilen kimyasal kayma değerleri deneysel kimyasal kayma değerleri ile karşılaştırılmıştır. İncelenen tatlandırıcılar üzerinde yapılan NMR hesaplamalarına ek olarak daha iyi sonuç elde edebilmek amacıyla fonksiyonel farkını ve çözücü etkisini incelemek için çeşitli hesaplamalar gerçekleştirilmiştir. Fonksiyonel farkını incelemek için siklamat ve isomalt kimyasal kayma değerleri literatürde önerilen B3LYP ve PBE0 fonksiyonelleri ve 6-311+G(2d,p) baz seti kullanılarak hesaplanmıştır. Çözücü etkisini incelemek için ise siklamat molekülüne ait kimyasal kayma değerleri su içerisinde PCM metodu kullanılarak hesaplanmıştır. Kullanılan NMR metodu ile tatlandırıcı moleküller arasında ortak sayılabilecek bir sonuca varılamamıştır. Kompleks oluşum çalışmalarında yapısal özelliklerin yanında moleküllerin büyüklüğünün hangi siklodekstrin ile etkileşime gireceğinde etkili bir faktör olduğu söylenebilir. Ayrıca kompleks oluşum analizlerinde yer değiştirebilir protonların sıcaklık değişim sabitlerinin önemli ölçüde değişmediği gözlemlenmiştir. Hesapsal çalışmaların sonucunda hata oranlarının özellikle proton kimyasal kayma değerleri için yüksek olduğu görülmektedir. Bu durum hesapsal çalışmaların gaz fazında deneysel yöntemin de sulu bir ortamda gerçekleştirilmesinden ortaya çıkmıştır. Fakat bununla beraber gaz fazında elde edilen yüksek yüzde hataların sulu ortamda molekülde konformasyonel bir değişimden ileri geldiği düşünülebilir. Kimyasal kayma değerleri üç farklı yöntem ile elde edilmiştir. Bu yöntemlerden Boltzmann dağılımı ile elde edilen kimyasal kayma değerleri yalnızca sukraloz için deneysel değerlere daha yakın sonuç vermiştir. Bu durum da -263 K' de konformerlerde çok büyük değişim göstermediği belirli konformerlerde sabit kaldığı şeklinde yorumlanabilir. Fonksiyonel farkını incelemek için yapılan çalışmada PBE0 ve B3LYP fonksiyonellerinin karbon ve proton atomları için farklı eğilimlerde sonuçlar verdiği fakat lineer regresyon analizi sonucunda iki fonksiyonel için hata oranlarının birbirine çok yakın olduğu tespit edilmiştir. Çözücü etkisinin incelenmesi için yapılan hesaplamada hata miktarının gaz fazına göre iyileştiği görülmüştür. Tez kapsamında yapılan çalışmalar "Bazı moleküller neden tatlı? – Tatlandırıcı Moleküllerinin Su Molekülleri ile Etkileşimlerinin ve Siklodekstrin Kompleksleri Olarak Özelliklerinin Yapı-Tat İlişkisi Açısından NMR Spektroskopisi ile İncelenmesi" isimli hem yapay hem de doğal tatlandırıcıları içeren TÜBİTAK projesinin (Proje No: 109T606) yapay tatlandırıcıları içeren bölümünü kapsamaktadır.

1. INTRODUCTION

1.1 Artificial Sweet Molecules

The sense of taste is important for humans as well as other animals. The sense of taste provides valuable information about the quality and the nutritional value of food [1]. The human taste can be categorized into five different classes: sweet, bitter salty, sour and umami [2]. The most significant one from the five different types of taste is definitely sweet taste due to both anthropological and medical reasons. Sugar related diseases like diabetes, obesity have increased lately [3]. Medicinal concern triggers to design low calorie and safer artificial sweeteners [4]. However, there are always doubts about how healthy these artificial sweeteners are [5]. For example saccharin is the first artificial sweetener that was found in 1879 and several papers have been published about its safety until 2000 [6-8]. Although there are many concerns about the safety of non-nutritive sweeteners, there is a dramatical increase in the use of non-nutritive sweeteners, generally in diet studies area [9,10]. Beside the safety of sweeteners, there still exists a subtle question of what makes these molecules taste sweet. There are still deficiencies about figuring out the molecular basis of sweet taste. Researchers have performed number of studies to understand the sweetness sensation up till now [1,11-15]. Moreover, receptor models have been used to examine the formation of sweet taste [11]. Molecules that give the sensation of sweet taste vary in size as well as chemical nature *i.e.* sugars, amino acids, peptides, proteins, olefinic alcohols, nitroanilines, saccharin, chloroform and many other organic compounds [4]. Although the formation of sweet taste is not completely known, concerning the sweet taste perception there are some specific findings on chemical structures. Aminoacids that belong to D- series are usually sweet tasting while L-series are usually bitter or tasteless [16,17]. The sweetness intensity is different for D- and L- monosaccharides. Some monosaccharides belonging to L- series are sweeter than D-monosaccharides [18].

1.2 Sweet Taste Models

The sweet taste mechanism is different from other taste mechanisms and the related receptor could be discovered only in recent times, although there exists studies carried out starting from the beginning of 20th century. The most renowned study conducted by G. Cohn indicates that, for the formation of sweet taste, there should be special structural groups on the molecule [19]. After this foundation, scientists continuously studied to clarify the related mechanisms. Some of these researches have focused on glucophores that activate the sweet taste mechanism, while others have concentrated on preparing models of common physical properties for sweeteners [20-23]. The first fairly accepted method was accomplished by Schallenberger and Agree. This study stated that all sweeteners have a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) and the distance between these two units should be between 2.5 and 4 Å. Moreover, there should be complimentary B-HA unit to AH-B unit on the receptor [24]. In addition to this theory, another study was carried out by Kier *et al*, in which it has been stated that there should be an additional X component responsible for hydrophobic interactions with the receptor [21]. Another study contributing to this theory was carried out by Nofre and Tinti, where they have reported that there should be at least six functional groups to AH-B-X unit and these functional groups account for the high affinity and secondary interactions [25]. Although, there exist many studies in the literature, the structure-activity relationship of sweet taste signalling is still unknown. In all the studies mentioned, hydrogen bonding between sweetener and the receptor was a common but undiscovered point.

Since the chemical structure of proteins are far larger than regular sweeteners, the discovery of sweet proteins persuade that there may be different receptors responsible for the formation of sweet taste [26,27]. The first sweet taste receptor was discovered in 2001 and the discovery of sweet receptor has clarified that all sweeteners activates T1R2_T1R3 heterodimer [28]. After the discovery of T1R2_T1R3 heterodimer, it is observed that there are some specific interactions between receptor and sweeteners. Another study states that molecules of different sizes activates different parts of the receptor [29]. Furthermore, it is found out that there should be at least three binding sites on the receptor and this finding indicated different chemical structures and different sizes of the sweeteners [30]. Recent

studies have shown that the carbohydrate sweeteners activate only one single site of the receptor [31,32].

1.3 Aim of the Thesis

Human sweet taste receptor has been discovered, however, the discovery *per se* cannot entirely answer the question of what makes the molecules taste sweet. On the other hand, hydrogen bonding properties seem to be a common feature for the models developed for the sweet taste. Moreover, it is known that sweet taste formation is different from other senses in human body. The sense of sweet taste is a result of transient interactions with sweeteners and the receptor, whereas the other senses occur by the complexation of receptors and the molecules that trigger the related sense. The transient interactions and the variety of sweeteners complicate the elucidation of the evidences for the formation of sweet taste.

Increase in health problems due to high sugar intake prompts researcher to synthesize new "non-caloric" or "sugar free" sweeteners. Generally non-nutritive sweeteners are 3000-13000 time sweet than sugar (sucrose) [9]. This thesis is composed of experimental and computational studies on a set of artificial sweeteners shown in Figure 1.1. Nuclear magnetic resonance (NMR) spectroscopy was used as the experimental technique by which the dynamic properties of sweeteners can be revealed. Outcomes of the employed NMR method *i.e.* chemical shift (δ), temperature coefficient ($d\delta/dT$), coupling constant (J), exchange rate (k_{ex}) and chemical shift differences ($\Delta\delta$) can be used to acquire information about hydrogen bonding properties of the sweet molecules. Moreover, the interactions between artificial sweeteners and cyclodextrins are also studied for the examination of common behaviors of sweeteners in more complicated complexation environments. Cyclodextrins' torus like structure allows to form inclusion complexes with organic substances in hydrophobic cavity. Complexation induced chemical shift (CIS) differences turns into a useful parameter that gives valuable information about the complexation with cyclodextrins. The measurable data that can be obtained from NMR spectroscopy can be considered as an average result of the ensemble of every conformers in solution. Computational studies were carried out for detailed conformational analysis and the comparison of averaged results of conformers with experimental data.

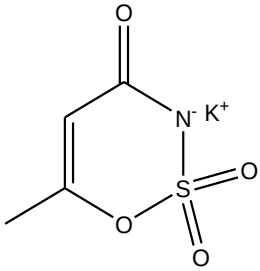
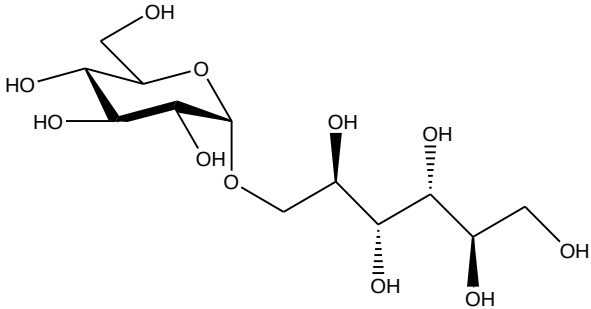
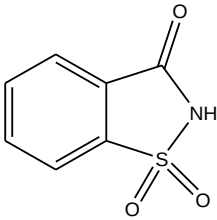
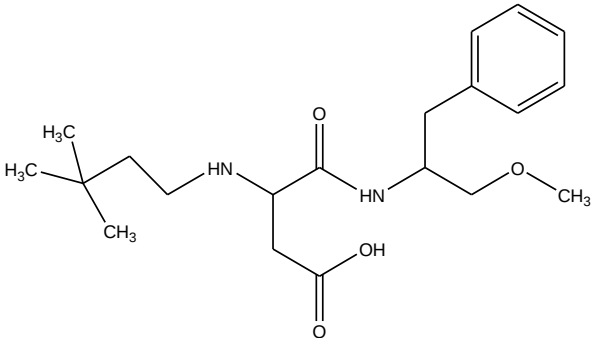
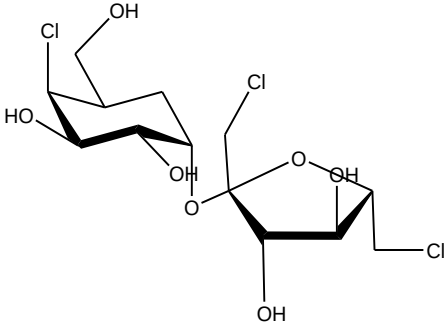
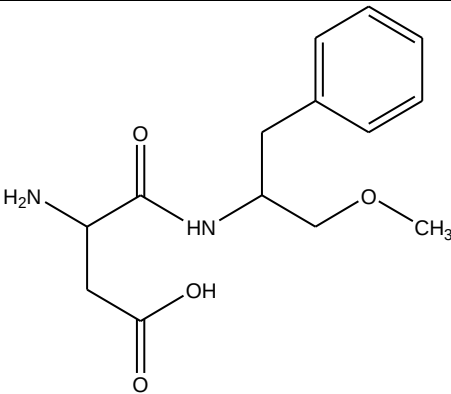
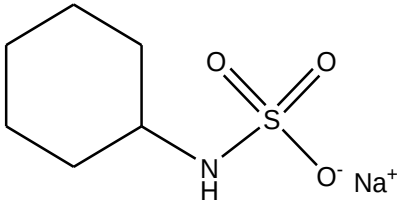
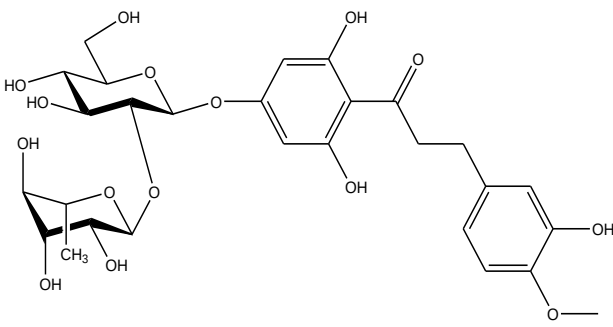
 Acesulfame potassium	 Isomalt
 Saccharin	 Neotame
 Sucralose	 Aspartame
 Sodium cyclamate	 Neohesperidin dihydrochalcone (NHDC)

Figure 1.1 : Investigated artificial sweeteners.

2. METHODOLOGY

2.1 Theory Behind the Methodology

2.1.1 Nuclear magnetic resonance spectroscopy

In the current study, the experimental structural analyses have been carried out with NMR spectroscopy. NMR spectroscopy is a powerful method used for the determination of the structure and the dynamic properties of the molecule. NMR spectroscopy relies on the magnetic properties of the NMR-active atomic nuclei. When a nucleus with non-zero spin quantum number is placed into external field it splits into different energy levels. Applied magnetic field is usually in the range of radio wave frequency. The number of energy levels is determined with $2I+1$ where I is the spin quantum number. These energy levels depend not only on the applied magnetic field but also on the magnetic moment of the nuclei. NMR spectroscopy can be coarsely described as measuring the resonance frequency between the energy levels in the applied magnetic field. The resonance frequency called as Larmor frequency can be formulated as in (2.1)

$$\nu_0 = \gamma B_0 / 2 \quad (2.1)$$

where ν_0 is the Larmor frequency, γ is the gyromagnetic ratio and B_0 is the strength of the applied magnetic field. Every nuclei in different magnetic environments gives different frequencies. The resonant frequency in time domain *i.e.* Free Induction Decay (FID), is then processed using so-called Fourier transformation in order to obtain the NMR spectrum of frequency.

In addition to 1D experiments, 2D experiments can also be used to obtain structural information on organic molecules. Heteronuclear or homonuclear interactions throughout bond or spatial correlations can be analyzed using specially designed 2D experiments.

2.1.2 Importance and the use of exchangeable protons

Exchangeable protons are common in almost all natural sweeteners and usually these exchangeable protons are in hydroxyl groups. The chemical shifts of aliphatic protons are close to each other, in return, hampering the assignment, as well as possible information in the form of coupling constants. The dispersion of hydroxy protons' chemical shifts is also small, however, they can be assigned easier than aliphatic protons since hydroxy proton signals are not complicated and overlapping as aliphatic protons. Furthermore, both chemical shifts and NMR parameters of hydroxy protons give valuable information about the structural analyses as well as dynamic properties of the molecules in question.

In traditional NMR spectroscopic methods deuterated solvents are used to avoid huge proton signal arising from solvent. Moreover, NMR spectra acquired with deuterated solvents become simpler, deprived of all exchangeable proton signals. In this study, nevertheless, non-deuterated solvent is used on purpose for the analysis of exchangeable protons.

NMR methods have been carried out for the determination of structural investigation of carbohydrates and sweeteners [33-35]. Several NMR studies show that NMR experiments can be performed for structural determination of exchangeable hydroxy protons [36-47]. NMR parameters that can be useful for structural investigation are chemical shifts (δ), temperature coefficient ($d\delta/dT$), exchange rate (k_{ex}) coupling constants (J) and chemical shift differences ($\Delta\delta$). Furthermore, in complexation studies complexation induced chemical shifts (CIS) is another parameter calculated for hydroxy protons in order to better understand the relationship between host and guest molecules.

2.1.3 Computational methods

Computational chemistry allows analyses of various properties of different types of molecules like polymers, drugs, organic and inorganic substances by using computer skills. This discipline can be a very fast method by using high computing power.

Within the scope of present work, NMR calculations involve two steps: First, the optimum structure of the molecule should be calculated. To find the plausible geometries, the structures are optimized with a computational methodology (molecular mechanics, semi-empirical, ab-initio or DFT methods). In some of the

studies in the literature, rather than taking a single structure, a set of conformers are considered. The NMR spectroscopic analysis are carried out on each conformers of subject molecule and then the spectroscopic results can be calculated by averaging results from whole possible conformers of the molecule. Comparison of calculated results with the experimental NMR data allowed one to account on the success of the computational method. In some studies, computational calculations of NMR properties assisted structural elucidations as well.

2.1.4 Molecular mechanics

Molecular mechanics (MM) uses only nuclear motions during the calculation process of the structure and energy of molecule. In MM calculations, electrons are not considered explicitly, instead, it is presumed that, if the positions of nuclei are known, electrons are supposed to be integrated into the nuclei. This presumption is relied on Born-Oppenheimer approximation which states that nuclei are much heavier than electrons and their motion is negligible compared to electrons. Thus, nuclear movements can be calculated separately from electrons.

Geometries and energies for medium to large-sized molecules are usually calculated by using MM. Since MM is fast, it needs low computational cost, can be accurate and it yields reasonable starting geometries for quantum mechanical calculations [48]. Molecular mechanics can be applied only to the ground state of molecules such as inorganics, organics, oligonucleotides, peptides, saccharides, metallo-organic and inorganics [49]. Since molecular mechanics calculation needs low computational cost, it is generally used for conformational analysis [50].

Molecular mechanics considers atoms as spheres and bonds as spring and calculates the potential energy of a molecule by using so-called “force field”, which is a set of functions and constants (2.2).

$$E_{\text{total}} = E_r + E_\theta + E_\varphi + E_{\text{nb}} + (\text{specific terms}) \quad (2.2)$$

The total energy (E_{total}) is calculated as the sum of energies associated with bond stretching (E_r), bond angle bending (E_θ), bond torsion (E_φ), non-bond interactions (E_{nb}) and specific terms such as hydrogen bond for biochemical systems [49].

Merck Molecular Forced Field (MMFF) is the MM computational method used in the present study. MMFF is expressed as the sum of energies regarding bond stretching, angle bending, out-of-plane bending, torsion interactions, van der Waals

interactions, and electrostatic interactions as valence terms; and stretch bending interactions as a cross term in the energy expression [51].

MMFF is used to calculate energies of the conformers that are acquired from Monte Carlo method [52]. Monte Carlo method uses random numbers to generate new conformers and then an elimination process is used to generate acceptable conformers [48].

2.1.5 Density functional theory

Density functional theory (DFT) [53-56], which was proposed by Hohenberg and Kohn, utilizes the electron density $\rho(r)$ to determine ground state energy and molecular properties. Conversely to wavefunction, $\rho(r)$ is coming from physical properties of all molecules.

A functional is depicted as a function of a function and energy is a functional of $\rho(r)$. $\rho(r)$ is a function of three spatial coordinates that are the -x,-y, -z positions of electron (2.3). The determination of $\rho(r)$ does not depend on the number of electrons, on the other hand, an increase in number of electrons results in complicated calculations of the wavefunction,

$$\rho(r) = f(x, y, z) \quad (2.3)$$

where f is a function. $\rho(r)$ is called as functional since it is a function of another function. Hohenberg-Kohn Theorem states that the ground state energy is at the minimum value if electron density is correctly calculated. Moreover, it was proposed that by utilizing electron density functional at ground state, any molecular property can be further calculated [53].

According to Kohn-Sham Theory [54], total ground state energy was depicted as in (2.4)

$$E = E_r + E_v + E_j + E_{xc} \quad (2.4)$$

where E_r is the kinetic term, E_v is the potential energy of the nuclear-electron attraction and nuclear-nuclear repulsion term, E_j is the electron-electron repulsion term and E_{xc} is the exchange-correlation repulsion term.

DFT calculations can be categorized in three methods *i.e.* local density approximation (LDA), gradient corrected (GC) and hybrid methods. The density of the molecule is the same in every part of the molecule with LDA, whose success is

limited. However, GC methods take into account of the difference of electron density throughout the molecule. Hybrid method is a combination of *ab initio* methods (specifically Hartree-Fock methods) with some of the improvements of DFT mathematical expressions. In computational chemistry hybrid methods are used more commonly, especially B3LYP (Becke style three parameter functional in combination with Lee-Yang-Parr correlation functional) method [57] is the most common hybrid functional that is used by theoretical chemists. Also for conformational analysis of carbohydrates, B3LYP functional is widely employed [58].

DFT methods deal with electron density in three dimensional variable as N^3 where N is the number of basis functions, whereas *ab initio* methods scale as N^4 . As a result, DFT methods need lower computational cost and also give more accurate results than low level *ab initio* methods like Hartree-Fock (HF) [59-61]. Since Coulomb-type electrons are not considered in HF methods, some systematical errors can appear during calculation of energy for low energy conformers [58,62]. For geometry and energy calculation of carbohydrates the usage of B3LYP functional with 6-311+G** basis set is recommended, since it has been reported to give fairly adequate results in such systems [58,63].

2.1.6 Theoretical NMR studies

Theoretical NMR studies have been widely used to clarify experimental ambiguities confronted occasionally for the structural analyses of some molecules, or most commonly it is used for comparing computational outcomes with experimental results. ^{31}P , ^{15}N , ^1H , ^{17}O and ^{13}C are the most widely used NMR active nuclei in calculations of NMR chemical shifts [64]. An acceptable geometry needs to be acquired prior to NMR calculations. NMR calculations generally do not need large basis sets. Basis sets with diffuse functions with B3LYP method are recommended since this methodology has been reported to give accurate results by applying scaling [65]. Several NMR methods are used to calculate NMR parameters. Among these methods, two methods *i.e.* gauge-including atomic orbital (GIAO) [66] and the continuous set of gauge transformations (CSGT) [67] are the most widely used ones. In comparison with other methods, GIAO method can be used with smaller basis sets, thus, it is the most preferred method for NMR calculations [68,69]. Same basis sets for NMR calculations and geometry optimizations should not necessarily be the

same [70]. Isotropic shielding constants are obtained from NMR calculations. To acquire NMR chemical shifts, a suitable reference should be used and subtraction of the isotropic shielding constant from the reference isotropic shielding constant gives the chemical shift of the atom. There may be some systematical errors in the results of calculations. One of the widely used method from avoiding systematical error is linear regression method [71]. A plot of experimental chemical shift versus calculated isotropic chemical shift or experimental chemical shift versus calculated chemical shift is formed. By using a scaling factor, which is generated from slope and intercept, systematical errors can be eliminated from calculated chemical shifts [70].

2.2 Experimental Methodology

2.2.1 Sample preparation

The NMR method employed in the current study is based on the observation of hydroxy protons. Hydroxy protons can be made visible by using H₂O instead of deuterated water as the solvent. On the other hand, if solvent is H₂O, exchange between hydroxy protons of the subject molecules and water is faster than what can be observed by NMR time scale at around room temperature. Thus, exchange should be lowered by removing impurities, pH adjustment, and lowering the temperature. Ionic impurities can catalyze the exchange between water and hydroxy protons. Cleaning process is performed by cleaning NMR tubes with (i) acetone (ii) methanol (iii) water step by step. pH values of NMR samples should adjusted to sustain a value between 6.5-6.9 [72]. Lowering temperature also decreases the exchange, therefore, k_{ex} values [37,73-75]. If not stated otherwise, the temperature was adjusted to -10°C (263 K) for all the NMR experiments of the present study. Addition of %15 acetone-d₆ of volume ratio into the aqueous solution is used to avoid freezing. Acetone-d₆ was used also as the source of reference signal for frequency calibration. Carbon and proton chemical shifts of acetone were used as the reference values of 32.32 and 2.204 ppm, respectively. NMR samples were prepared within the concentration range of 14 to 21 mM.

2.2.2 NMR experiments

1D ^1H and ^{13}C experiments and also 2D experiments for examining ^1H - ^1H correlations (DQF-COSY, TOCSY, NOESY, ROESY) were performed on a Varian VNMR-600 MHz spectrometer. Also for observation of ^1H - ^{13}C correlations HSQC experiments were carried out on a Varian Unity Inova 300 MHz spectrometer. HSQC experiment of saccharin was carried out on Varian 800 MHz spectrometer.

The observation of hydroxy protons can only be done by using water suppression techniques since ordinary water (H_2O) is the solvent for all the samples. Pulse sequences of the combination of WATERGATE [63] and DPGFSE [76] were optimized and used for suppressing huge water signal.

2.2.3 Processing of raw NMR data

Raw NMR data, that is called FID (Free Induction Decay), is processed by MestReNova program for 1D experiments, whereas processing of 2D experiments were carried out by nmrPipe program. Analysis of 2D experiment like peak listing, volume calculations for exchange rate was done by using Sparky software. Processing of FIDs were carried out with executive nmrPipe scripts which consist of several functions to apply for each experiment. An example of nmrPipe script is given in Table A.1.

2.2.4 Assignment

Assignment of artificial sweeteners are done by using 1D ^{13}C -APT and ^1H spectra, for ^1H - ^1H through bond correlation DQF-COSY and TOCSY spectra, for ^1H - ^1H through space interactions and hydroxy protons NOESY and ROESY spectra, for ^{13}C - ^1H through bond correlation HSQC spectra. If assignment of signals cannot be accomplished by only 1D experiments, 2D spectra are used to clarify the assignment.

2.2.5 Temperature coefficient

Temperature coefficient ($d\delta/dT$) is an NMR parameter that indicates temperature dependency for a chemical shift. For the NMR method employed in the current study, chemical shifts of hydroxy protons are obtained from 1D spectra recorded at an temperature interval of 263 K to 283 K, employing 5 K steps (Figure 2.1). Temperature coefficient is then calculated from the slopes of the graphs where temperature versus chemical shift of hydroxy protons are plotted (Figure 2.2).

Hydroxy proton, which is included in hydrogen bonding, should have low temperature coefficient because anisotropy would be less efficient for the proton involved in hydrogen bonding property [38]. For the protons involved in strong hydrogen bonding has temperature coefficient lower than 3 ppb deg^{-1} for saccharides in DMSO solutions [40]. For an aqueous solution temperature coefficient of trisaccharides have been obtained as low as 4 ppb deg^{-1} [44].

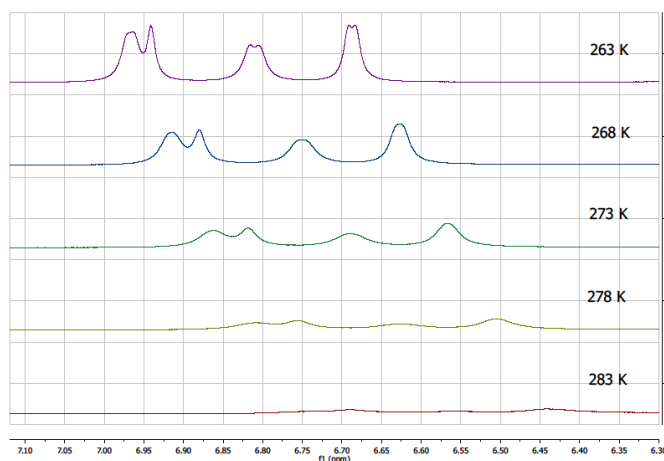


Figure 2.1 : Temperature dependence of sucralose.

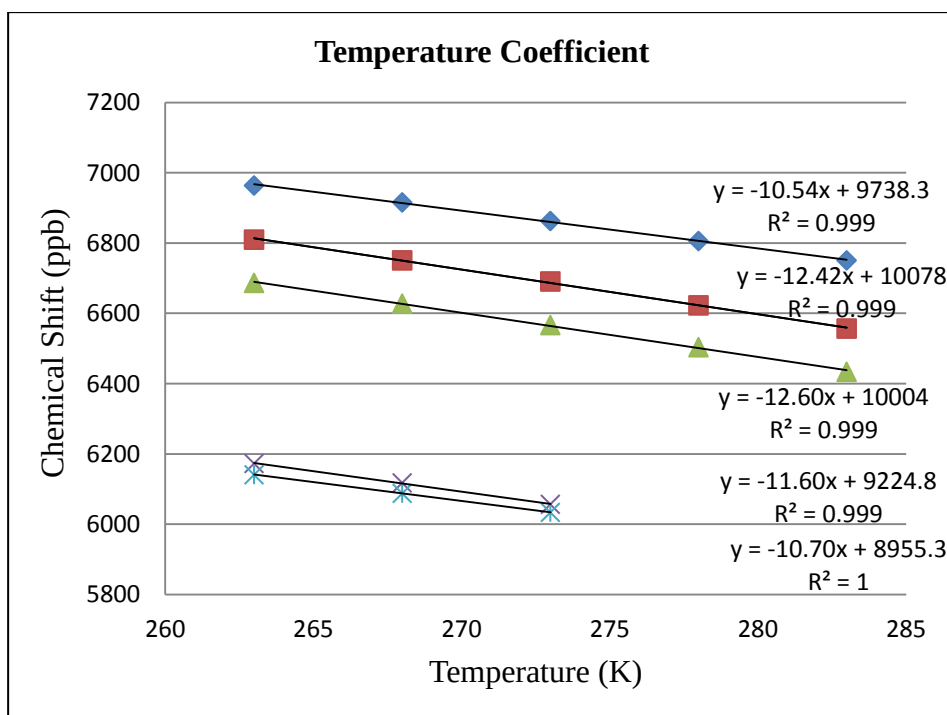


Figure 2.2 : Temperature coefficient graph of sucralose.

2.2.6 Exchange rates

Exchange rate similar to temperature coefficient is another NMR parameter that can be used to obtain information about hydrogen bonding properties for hydroxy protons. Exchange rate can be described as the number of protons transfer between hydroxyl group and bulk water in unit time (s^{-1}).

For the first step of calculation of exchange rates for a hydroxy proton, NOE volumes of diagonal hydroxy proton peaks and cross peaks with water are calculated in NOESY spectra of different mixing times. Exchange rate of hydroxy protons can be calculated as the rate of the initial build-up rates of exchange peaks over the volume of diagonal peaks at zero mixing time [36, 43-45, 73-78]. The diagonal peak volume at zero mixing time experiment is extrapolated from the data points from 3 ms to 18 ms at intervals of 3 ms. (Figure 2.3).

The processing of NOESY spectra at -10°C with mixing time 3 ms, 6 ms, 9 ms, 12 ms, 15 ms and 18 ms are performed on nmrPipe software with same processing parameters in each spectra. NOE volume calculations of diagonal hydroxy peaks and cross peaks with water are performed with Sparky program.

Overlapping of hydroxy proton signals or overlapping with water signal hinder the calculation of NOE volumes, hence k_{ex} . Therefore, exchange rate of some hydroxy protons cannot be calculated for the artificial sweet molecules isomalt and sucralose. Ionic residues in the NMR samples or elevated temperature can catalyze the exchange progress with water. Thus, exchange rate values can only be compared for a single specific sample. Low exchange rate values may indicate a hydrogen bond for a hydroxy proton since hydroxy proton prefers to be involved in hydrogen bond rather than exchange proton with water.

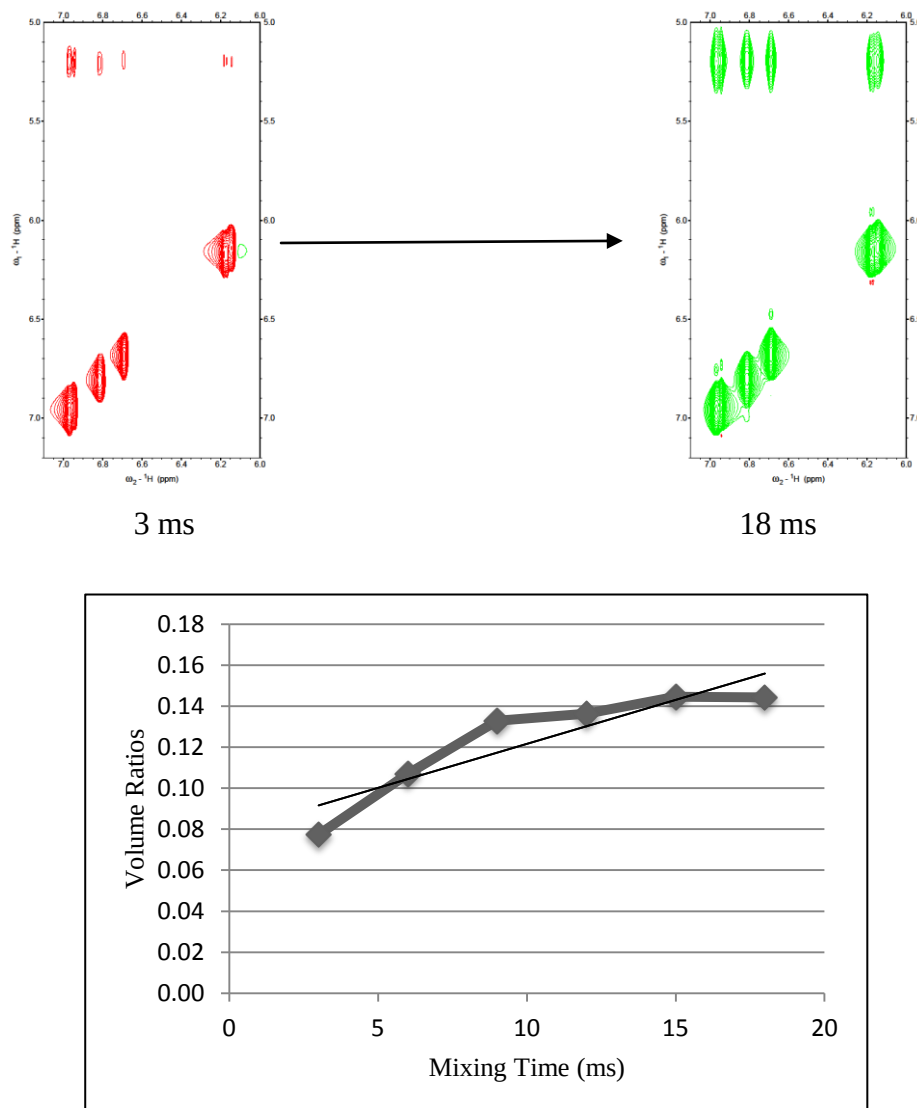


Figure 2.3 : Calculation of k_{ex} for sucralose.

2.2.7 Coupling constants

For the structure determination, coupling constant can be used to get information about conformational behavior. Couplings are not dependent on the strength of magnetic field and originate from transfer of magnetization between nuclei attached with chemical bond. Vicinal ($^3J_{HH}$) and geminal ($^2J_{HH}$) coupling constants are analyzed in the current study. Geminal couplings occur for the protons that are attached to same carbon and it has negative value due to the orientation of the nuclear magnetic moments of protons [79]. Vicinal couplings occur for the protons related through three bond configuration and it is correlated with the dihedral angle of the protons. Dihedral angle is the angle between H-C-C-H for $^3J_{HC-CH}$ and H-C-O-

H for $^3J_{\text{HO-CH}}$ coupling constants and the symbol of the dihedral angle is φ . By using vicinal coupling constants dihedral angle can be predicted by Karplus equation (2.5). Relationship of $^3J_{\text{HC-CH}}$ and dihedral angle can be seen in Karplus-Conroy curve derived from Karplus equation (Figure 2.4).

$$J(\varphi) = A \cos^2 \varphi + B \cos \varphi + C \quad (2.5)$$

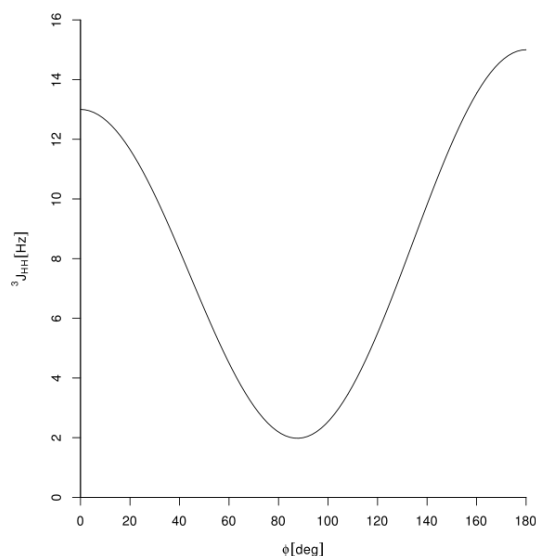


Figure 2.4 : Karplus-Conroy curve.

According to modified Karplus equation (2.6) for hydroxy protons it can be inferred that vicinal coupling of a hydroxy proton is in the range of 5.5 ± 0.5 Hz, implying free rotation around C-O bond [80].

$$^3J_{\text{HO-CH}} = 10.4 \cos^2 \varphi - 1.5 \cos \varphi + 0.2 \quad (2.6)$$

Hydroxy proton that is included in hydrogen bonding does not prefer to rotate freely around HO-CH bond. Thus, coupling constants measured out of this range should be considered as for the hydroxy protons participating in hydrogen bonding interactions. Due to fast exchange, the signal of a hydroxy proton becomes broad, therefore, the corresponding coupling constant cannot be measured. Overlapping is another challenge to measure coupling constants.

Coupling constants were measured with MestReNova software by using 1D proton spectra with the tool of multiplet analyzer. For measuring the coupling constants Gaussian window functions were used. Moreover, spin simulation technique was used to obtain the coupling constants for saccharin due to very complicated and higher order spectra. Spin simulation technique can be used to reach the experimental

spectra by simulating the model spin system of the attributed chemical shifts and couplings.

2.2.8 Chemical shift differences

Chemical shift differences are calculated by subtracting the hydroxy proton chemical shift of the subject molecule from its corresponding (usually monomeric) sub-structural unit. Chemical shift differences can be used to get structural information as previously reported [75]. Absolute large upfield chemical shift differences have been explained by the proximity of hydroxy proton to the ring oxygen or another hydroxyl group. Followed by this reasoning, it has been shown that hydroxy protons having large $\Delta\delta$ values have reduced interaction with water [36,43-45]. On the other hand, positive large chemical shift difference can be a sign of stable hydrogen bond for a hydroxy proton [47].

For the artificial sweeteners that are examined in the present work, different monomeric or rather sub-units were used to calculate the chemical shift differences. For instance, neotame $\Delta\delta$ values were calculated by using aspartame as a sub-unit, whereas for isomalt α -D-glucopyronside and mannitol were used for the same purpose [36,81]. Chemical shift differences for sucralose were obtained by subtracting sucralose chemical shifts from sucrose chemical shifts [81]. For the calculation of NHDC chemical shift differences the chemical shifts of α -D-glucopyronside for O(18)H and rhamnose for O(23)H and O(25)H were needed as monomer units. Although α -D-glucopyronside chemical shifts were obtained with the same NMR method [36], the data for the hydroxy protons of rhamnose were not available. Thus, for NHDC, O(23)H and O(25)H chemical shift differences could not be calculated.

2.2.9 Complexation studies

NMR samples of complexation studies were prepared in volume ratio of 1:1 with 25 mM α -, β -, or γ -cyclodextrin and 25 mM of the artificial sweetener. This gives 1:1 molar ratio complexes of 12.5 mM. 1D ^1H and 2D TOCSY, NOESY and ROESY spectra were used for the assignments of both cyclodextrins and the artificial sweeteners. Assignment of saccharin was accomplished using HSQC spectra as well. In addition to HSQC spectra, spin simulation technique was used to obtain chemical shifts and coupling constants of protons. Processing of 2D spectra was performed

using nmrPipe program and for the assignment procedure MestReNova and Sparky programs were used to take into account 1D and 2D spectra of artificial sweeteners in aqueous solution, respectively. It was possible to calculate the temperature coefficients and coupling constants, whereas NOE signal volumes were not suitable for the calculation of exchange rates. Thus exchange rates cannot be calculated for the complexation studies. Chemical shift changes upon complexation (CIS) are calculated for the inclusion complexes of artificial sweeteners. It is the difference between hydroxy proton chemical shift in the complex and hydroxy protons chemical shift of the molecule in aqueous solution. If the calculated CIS values refer to significant shielding or deshielding effect for guest molecule, it can be informative about the structural properties of the molecules forming the complexes [30].

The experimental results of the α -, β -, and γ -cyclodextrins have been previously reported and CIS values were calculated according to results in the related article [36].

2.2.10 Cyclodextrins

Cyclodextrins (CDs) are oligosaccharides that consist of α -(1,4)-linked glucopyranosyl units. α -, β - and γ - CDs, the most common cyclodextrins, are composed of six, seven and eight α -(1,4)-linked glucopyranosyl units, respectively. CDs have distinctive torus like structures and a hydrophobic cavity suitable for the inclusion of organic substances [83]. Inclusion complexes are automatically formed depending on hydrophobicity of the guest molecules. The more hydrophobic part of the guest molecule is positioned into the cavity, while the more hydrophilic and often charged group of the guest molecule remains into the solvent, just outside of wider opening of the cavity. These findings are derived from both thermodynamic and NMR studies [83]. During the formation of inclusion complexes, covalent bonds are not broken or formed and there is an equilibrium of the complexed molecules with uncomplexed molecules in solution [36]. Most CDs' applications are in medicine, catalysis, or in food chemistry, separation and sensor technology, where they usually involve structural characterization of supramolecular complexes. CDs are extensively used incorporated into NMR spectroscopy applications. In the literature, more than 1000 papers have been published on the NMR experiments of CDs [82].

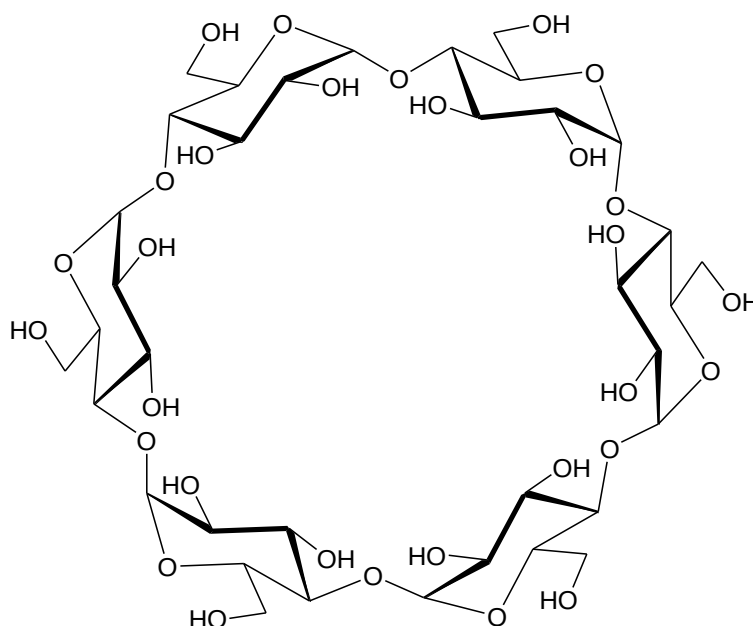


Figure 2.5 : Representative structure of cyclodextrins.

2.3 Computational Methodology

Monte Carlo method included in Spartan 04 [84] package software program was used to obtain low energy conformers and energy minimization was done by using MMFF methodology. After conformer distribution, for the structures with many rotatable bonds, the maximum number of conformer is limited to 100. Then, single point energy calculations were performed by using DFT method with B3LYP functional and 6-31G* basis set to obtain an energy/stability order. Elimination among 100 conformers is needed since significant contribution to chemical shifts should originate from lower energy conformers. Conformers that have relative energies larger than 7 kcal mol⁻¹ were eliminated. Elimination of conformers is based on hydrogen bonding energies. By using B3LYP functional and 6-31G* basis set, each additional hydrogen bond lowers the energy by almost 2.8 kcal mol⁻¹, thus 7 kcal mol⁻¹ is in accord with *ca.* 2.5 hydrogen bond.

After elimination of high energy conformers, geometry optimizations were performed with B3LYP/6-31G* and by B3LYP/ 6-311+G* successively in two steps. Geometry optimizations were carried out with Spartan 04 package program. However, due to the software related errors during geometry optimizations, for neotame, sucralose,

and NHDC, the second steps of geometry optimizations were performed using Gaussian09 package program [84,85].

All chemical shift calculations were carried out on Gaussian09 package program [85] with GIAO method and B3LYP/6-311+G** level of theory *in vacuo*. Reference chemical shifts were calculated for TMS with the same level of theory. Calculated and experimental chemical shifts were averaged for the protons that were experimentally indistinguishable. All the energies are electronic energies throughout the text unless otherwise stated.

Averaged chemical shifts were calculated for systems with a set of conformers obtained after elimination by 7.0 kcal/mol threshold energy. Moreover, Boltzmann distribution was used to obtain average chemical shifts for examining the contribution of conformers to the calculated chemical shifts. Boltzman averaged chemical shifts were calculated using the equations (2.7) and (2.8);

$$\frac{P_i}{P_j} = e^{(G_i - G_j)/k_B T} \quad (2.7)$$

$$\delta = \frac{\sum_i \delta_i e^{-G_i/RT}}{\sum_j e^{-G_j/RT}} \quad (2.8)$$

where P_i/P_j is the abundance ratio of conformer i and j, G_i and G_j are the free energies calculated for conformer i and j respectively; δ is the calculated chemical shift, k_B is the Boltzmann constant, T is the temperature and R is the molar gas constant [86,87].

Linear regression method was used to eliminate the systematic errors of averaged chemical shifts [64,70]. For linear regression method, computed chemical shifts versus experimental chemical shifts are plotted. Scaled chemical shifts are calculated with a scaling factor acquired from the graphs. Scaled chemical shifts are computed by using equations (2.9) and (2.10);

$$\delta_{\text{Calculated Chemical Shift}} = a + b \delta_{\text{Experimental chemical shift}} \quad (2.9)$$

$$\delta_{\text{Scaled Chemical Shift}} = (\delta_{\text{Calculated Chemical Shift}} - b)/a \quad (2.10)$$

where a is the slope and b is the intercept of the graph of computed chemical shift versus experimental chemical shifts.

Error% is calculated by using the equation **(2.11)**;

$$\text{Error\%} = \frac{(\delta_{\text{Calculated Chemical Shift}} - \delta_{\text{Experimental Chemical Shift}})}{\delta_{\text{Experimental Chemical Shift}}} * 100 \quad \textbf{(2.11)}$$

The numbering in Figure 2.6 will be referred to throughout the thesis.

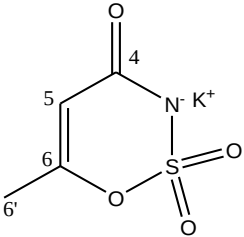
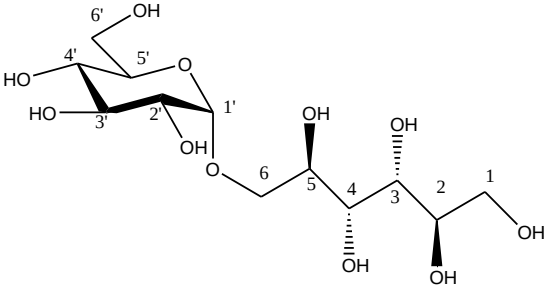
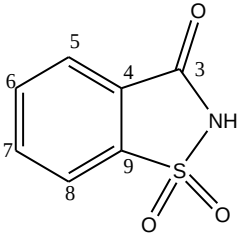
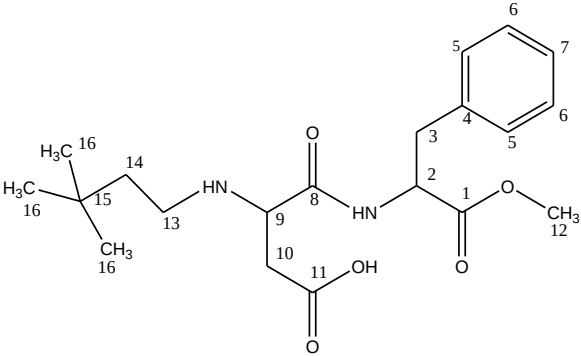
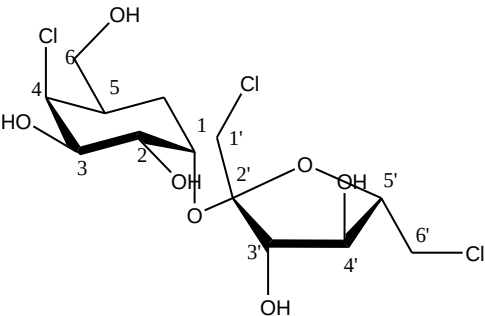
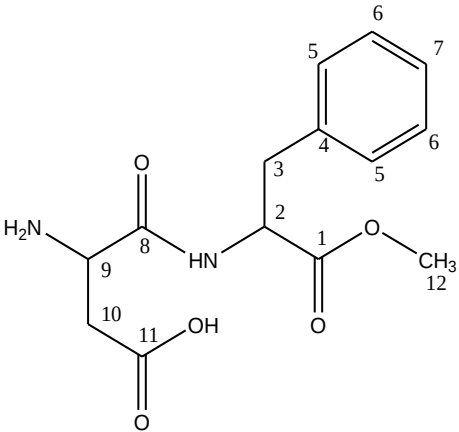
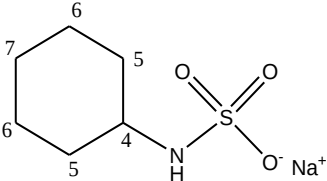
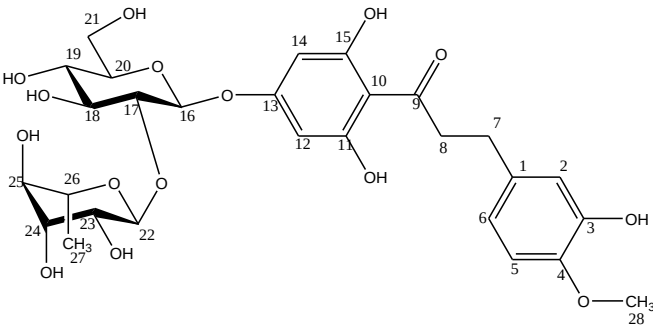
 Acesulfame potassium	 Isomalt
 Saccharin	 Neotame
 Sucralose	 Aspartame
 Sodium cyclamate	 NHDC

Figure 2.6 : Atom numberings in investigated sweeteners.

3. RESULTS AND DISCUSSION

3.1 Experimental Results

3.1.1 NMR spectroscopy

Temperature coefficients and coupling constants are the parameters that can be compared in different molecules, while exchange rates with water cannot because different ionic contents of different samples effect the exchange rates. Besides, when calculating the NOE peak volumes, different processing parameter for different samples may change the exchange rate. Assignments and NMR parameters of eight artificial sweeteners are given in Table 3.1 from Table 3.8 and aliphatic coupling constants are listed in Table A26 to Table A33. The NMR spectra of the artificial sweeteners are represented in Figure A.1 to A.16.

3.1.2 Assignments and chemical shifts

Acesulfame potassium is a charged sweetener and has a rigid structure. This sweetener does not have any exchangeable protons. Assignment of acesulfame is done for carbon and protons in the structure. Sodium cyclamate is a charged sweetener like acesulfame potassium and has only one exchangeable proton, which could not be assigned. The assignment of sodium cyclamate could be performed for its cyclohexane ring. Electronegative atoms on the structure affect the most C(4)H proton and carbon chemical shifts. Saccharin has rigid structure and one exchangeable NH proton that could not be assigned. Assignment of saccharin is hard to solve due to proximity of the proton and the carbon signals. Thus, spin simulation technique is used to clear up the ambiguities in the assignment. Along with spin simulation, in the assignment of carbon chemical signals, also a HSQC spectrum of 800 MHz Varian NMR spectrometer is used. Aspartame is a methylester of dipeptide that consists of aspartate and phenylalanine. Aspartame and neotame have similar structures. The difference between the structures of these two sweeteners is an additional 3,3-dimethylbutyl group on neotame. Assignment of these two sweeteners

are similar due to their similar magnetic properties. Some chemical shifts of NHDC could not be assigned because of overlapping of signals.

3.1.3 Temperature coefficients

Temperature coefficients give the most accurate results among all NMR parameters attained, because the process of calculation of the temperature coefficient is unequivocal and results in low experimental and analytical errors. Analysis of temperature coefficient should be done by using magnitude of $d\delta/dT$ values, because the negative sign of temperature coefficient comes from the graph of chemical shift versus temperature. Absolute values of temperature coefficients show dependence of chemical shift to temperature. Smaller temperature coefficients are obtained for protons that are involved in hydrogen bonding [38]. Protons that have temperature coefficients lower than 3 ppb deg^{-1} was considered as involved in strong hydrogen bonding for saccharides in DMSO [40]. In previous studies for disaccharides, trisaccharides and Lewis b tetrasaccharide $d\delta/dT$ values vary between -7.3 to $-13.0 \text{ ppb deg}^{-1}$, -6.2 to $-12.8 \text{ ppb deg}^{-1}$ and -6.9 to $-12.3 \text{ ppb deg}^{-1}$, all respectively [44,45,88].

Acesulfame potassium does not have any exchangeable proton, whereas sodium cyclamate, saccharin have only one exchangeable proton, NH, that cannot be assigned. Therefore, for these sweeteners temperature coefficients could not be calculated.

Aspartame possesses two exchangeable protons that are bonded to nitrogen atom. One of them (N(2)H) is highly flexible due to fast exchange; this proton gives broad signal. It is not possible to calculate temperature coefficient for the broad signal, because this peak disappears at even higher temperatures. The other exchangeable proton (N(1)H) gives sharp signal and its temperature coefficient is $-4.20 \text{ ppb deg}^{-1}$ like N(1)H proton in neotame which has the temperature coefficient $-4.70 \text{ ppb deg}^{-1}$. These $d\delta/dT$ values are smaller values than in previous work that has been done in aqueous solution. This can be considered as a sign for hydrogen bonding [44,45,88]. Due to overlapping signals of hydroxy protons in isomalt, temperature coefficients can only be calculated for O(1)H, O(2')H and O(6')H, -11.28 , -10.96 and $-12.06 \text{ ppb deg}^{-1}$, all respectively. These $d\delta/dT$ values are larger than 3 ppb deg^{-1} . This can be considered as an indication for an exchangeable proton as involved in hydrogen bonding.

Temperature coefficients are calculated for all hydroxy protons in sucralose and varies between -10.54 and -12.60 ppb deg⁻¹. These values indicate that exchangeable protons of sucralose show high temperature dependency.

dδ/dT values of NHDC hydroxy protons are -7.9, -11.2 and -5.9 ppb deg⁻¹ for O(18)H, O(23)H and O(25)H, all respectively. In these dδ/dT values, O(23)H has higher temperature dependence than other exchangeable protons. O(18)H and O(25)H have slightly smaller dδ/dT values than O(23)H.

3.1.4 Exchange rate

Exchange rate is a parameter that is calculated using the NOE volumes from diagonal peak of hydroxy protons and cross peaks between hydroxy protons and water as mentioned in Section 2.2.6. Exchange rates are only comparable within a set of exchangeable protons of a single sample. Exchange rate of a proton involved in hydrogen bonding should be lower than other protons that are not involved in hydrogen bonding.

Since NH protons cannot be assigned for sodium cyclamate and saccharin, the rate of exchange with water could not be calculated for these sweeteners. Exchangeable protons of aspartame and neotame do not give any positive exchange rate values. Rate of exchange with water can be calculated for isomalt, sucralose and NHDC hydroxy protons.

Overlapping signals obstruct the calculation of the rate of exchange with waters. For isomalt, exchange rates of O(1)H, O(5)H, O(6')H are calculated and the values are 5.43, 10.20 and 2.52, respectively. In general, hydroxymethyl groups exchange protons with water easily due to close relation with bulk water, causing bigger exchange rate values for hydroxymethyl protons. Interestingly isomalt O(6')H has the smallest exchange rate in isomalt hydroxy protons.

Exchange rates of only O(3)H and O(3')H hydroxy protons could be calculated for sucralose due to overlapping and k_{ex} values are 2.72 s⁻¹ and 4.29 s⁻¹ respectively. O(3)H has a relatively smaller exchange rate value than O(3')H.

Exchange rate is calculated only for NHDC hydroxy protons that can be assigned. In these rates, O(25)H has the smallest value of 3.98 s⁻¹ whereas other k_{ex} values are 7.59 and 6.20 s⁻¹ that belong to O(18)H and O(23)H, respectively. With

consideration of temperature coefficients of NHDC hydroxy protons, it can be stated that O(25)H may be involved in hydrogen bonding whereas O(23)H is not.

3.1.5 Vicinal coupling constants

It is mentioned in Section 2.2.7 that according to the Karplus equation modified for hydroxy protons, vicinal coupling constant of ($^3J_{\text{HO-CH}}$) 5.5 ± 0.5 Hz states a free rotation of the hydroxy group around the C-O bond [80]. Rotational restriction around C-O bond may originate from being involved in hydrogen bonding. Fast exchange with water molecule causes broad and weak peaks, which hampers the calculation of vicinal coupling constants. Beside fast exchange, overlapping in hydroxy peaks as well as benzene peaks obstruct the measurement of $^3J_{\text{HO-CH}}$.

Acesulfame potassium, sodium cyclamate and saccharin do not carry any exchangeable protons that could be assigned. $^3J_{\text{HO-CH}}$ cannot be measured for hydroxy protons of NHDC due to broad signals of that protons.

Aspartame and neotame have N(1)H protons that have the coupling constant 7.9 Hz and 7.6 Hz respectively. These coupling constants are not in the range for free rotation around in C-O bond. Coupling constants, as well as temperature coefficients for these exchangeable protons indicate a hydrogen bonding. These hydrogen bond may be an intramolecular hydrogen bond considering electronegative atoms in these structures.

Coupling constants can be measured for only one hydroxy proton of isomalt. It is the C(2')H having the $^3J_{\text{CH-OH}}$ value of 5.3 Hz. According to Karplus equation it leads to free rotation around C(2')-O(2') bond. Considering also the temperature coefficients of this hydroxy proton, it seems not being involved in any hydrogen bonding interactions.

Three coupling constants can be measured for sucralose that belong to O(3)H', O(2)H and O(3)H. The $^3J_{\text{H-OH}}$ value of O(2)H is 8.1 indicating somewhat restricted rotation. However, temperature coefficient of O(2)H is fairly large value to be a sign for hydrogen bonding interaction.

3.1.6 Chemical Shift Differences

Chemical shift differences could be calculated for neotame, isomalt, sucralose and NHDC.

For the exchangeable proton of neotame, the $\Delta\delta$ value is 0.174 ppm. Hydroxy protons that are involved in hydrogen bond has positive large chemical shift differences [75]. All the parameters that are calculated for this proton; temperature coefficients, vicinal coupling constant and chemical shift difference, indicate that this proton can be involved in hydrogen bonding. There are electronegative atoms in the structure of neotame that may form an intramolecular hydrogen bond with the N(1)H proton. Moreover, this proton gives the same tendency with the N(1)H proton of aspartame, but the chemical shift difference is a fairly big value that may indicate a conformational change due to the addition of 3,3-dimethyl group to the aspartame.

Isomalt chemical shift differences vary between $|0.016|$ to $|1.189|$ ppm. O(1)H has a $\Delta\delta$ value of 0.297 ppm, exchange rate of 5.43 s^{-1} and $d\delta/dT$ value of $-11.28\text{ ppb deg}^{-1}$. For this hydroxy proton, $\Delta\delta$ value is downfield shifted and the exchange rate is an average value in all hydroxy protons of isomalt. This may be a sign for hydrogen bonding. On the other hand, temperature coefficient is large to be considered as being involved in hydrogen bonding. Since temperature coefficient gives the most accurate results, it can be said that O(1)H is not involved in hydrogen bonding. Chemical shift difference, exchange rate, temperature coefficient and vicinal coupling constant of O(2')H is -0.051 ppm , 10.20 s^{-1} , $-10.96\text{ ppb deg}^{-1}$ and 5.3 Hz , respectively. All of these parameters indicate that O(2')H does not get involved in hydrogen bonding. Chemical shift difference, exchange rate and temperature coefficient for O(6')H are -0.410 ppm , 2.52 s^{-1} and $-12.06\text{ ppb deg}^{-1}$, respectively. Since it is a terminal hydroxy proton, it gives broad signal due to fast exchange. Thus, vicinal coupling constant for this hydroxy proton could not be calculated. Negative chemical shift differences come from lack of relation with water molecules [75]. Exchange rate of O(6')H is the smallest value in isomalt hydroxy protons. This may indicate hydrogen bonding, however, chemical shift difference and temperature coefficient give conflicting results compared to exchange rate. Since the most sensitive calculation in these parameters is exchange rate, it can be concluded that O(6')H is not a member of any hydrogen bonding.

Sucralose chemical shift differences vary between $|0.072|$ to $|0.352|$ ppm. Chemical shift difference, temperature coefficient and vicinal coupling constant for O(2)H are -0.072 ppm, -11.60 ppb deg^{-1} and 8.1 Hz, respectively. Vicinal coupling constant indicates restricted rotation in contrast to chemical shift difference and temperature coefficient indicating no hydrogen bond. Chemical shift difference, exchange rate, temperature coefficient and vicinal coupling constant for O(3)H are 0.311 ppm, 2.72 s^{-1} , -12.60 ppb deg^{-1} and 4.7 Hz, respectively. Although O(3)H has the smallest exchange rate, downfield shift with respect to its monomeric unit, larger temperature coefficient (>3 ppb deg^{-1}) and scalar coupling value indicating free rotation can be considered that this hydroxy proton does not get involved in hydrogen bonding. Although O(6)H has larger positive chemical shift difference, 0.238 ppm, its temperature coefficient is -10.70 ppb deg^{-1} . It may be said that this proton is not involved in hydrogen bonding. Chemical shift difference, exchange rate, temperature coefficient and vicinal coupling constant for O(3')H are 0.352 ppm, 4.29 s^{-1} , -12.42 ppb deg^{-1} and 6.4 Hz, respectively. O(3')H has downfield chemical shift, exchange rate larger than O(3)H, larger temperature coefficient and slightly larger coupling constant value that refers to free rotation. It can be concluded with all these parameters that O(3')H does not get involved in hydrogen bonding.

Chemical shift difference could only be calculated for O(18)H as 0.029 ppm for NHDC. Since the chemical shifts of one of the monomer units of NHDC, *i.e.* rhamnose, were not available, chemical shift differences of O(23)H and O(25)H could not be calculated. Together with the other NMR parameters of this hydroxy proton, it can be concluded that O(18)H of NHDC is not included in hydrogen bonding.

Table 3.1 : ^1H and ^{13}C chemical shifts (δ) of acesulfame potassium.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3\text{J}_{\text{CH-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(5)H	103.748	5.666	-	-	-	-
C(4)H	174.967	-	-	-	-	-
C(6)H	166.894	-	-	-	-	-
C(6')H	21.904	2.120	-	-	-	-

Table 3.2 : ^1H and ^{13}C chemical shifts (δ) of sodium cyclamate.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3\text{J}_{\text{CH-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(4)H	56.011	3.062	-	-	-	-
C(5)Ha	35.963	1.216	-	-	-	-
C(5)He	35.963	1.994	-	-	-	-
C(6)Ha	27.429	1.284 ^a	-	-	-	-
C(6)He	27.429	1.730	-	-	-	-
C(7)Ha	27.660	1.158 ^a	-	-	-	-
C(7)He	27.660	1.591	-	-	-	-

^a Chemical shifts are obtained from HSQC spectrum.**Table 3.3 :** ^1H and ^{13}C chemical shifts (δ) of saccharin.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3\text{J}_{\text{CH-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(3)	175.187	-	-	-	-	-
C(4)	135.204	-	-	-	-	-
C(5)H	126.515	7.823	-	-	-	-
C(6)H	136.752	7.817	-	-	-	-
C(7)H	136.241	7.819	-	-	-	-
C(8)H	123.154	7.888	-	-	-	-
C(9)	144.468	-	-	-	-	-

^a Chemical shifts are obtained from spin simulation technique.

Table 3.4 : ^1H and ^{13}C chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J) and temperature coefficients ($d\delta/dT$) of aspartame.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3J_{\text{CH-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(2)H	57.393	4.751	-	-	-	-
C(3)Ha	39.858	3.267	-	-	-	-
C(3)Hb	39.858	3.067	-	-	-	-
C(5)H	131.995	7.284	-	-	-	-
C(6)H	131.643	7.384	-	-	-	-
C(7)H	130.096	7.325	-	-	-	-
C(9)H	53.393	4.174	-	-	-	-
C(10)Ha	39.188	2.663	-	-	-	-
C(10)Hb	39.188	2.774	-	-	-	-
C(12)H	55.762	3.750	-	-	-	-
N(1)H	-	8.967	7.9	-4.20	-	-
N(2)H	-	8.236	^a	^a	-	-

^a Parameters that cannot be obtained/calculated from spectra.

Table 3.5 : ^1H and ^{13}C chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and chemical shift differences ($\Delta\delta$) of neotame.

Atom	^{13}C δ (ppm) ^a	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(2)H	56.838	4.825	-	-	-	-
C(3)Ha	39.257	2.957	-	-	-	-
C(3)Hb	39.257	3.357	-	-	-	-
C(5)H	131.750	7.298	-	-	-	-
C(6)H	131.437	7.387	-	-	-	-
C(7)H	130.015	7.323	-	-	-	-
C(9)H	60.294	4.078	-	-	-	-
C(10)Ha	39.243	2.642	-	-	-	-
C(10)Hb	39.243	2.735	-	-	-	-
C(12)H	55.600	3.790	-	-	-	-
C(13)Ha	46.290	2.597	-	-	-	-
C(13)Hb	46.290	2.856	-	-	-	-
C(14)Ha	41.576	1.557	-	-	-	-
C(14)Hb	41.576	1.488	-	-	-	-
C(16)H	30.811	0.879	-	-	-	-
N(1)H	-	9.141	7.6	-4.70	-	0.174

^a Chemical shifts are obtained from HSQC spectrum.

^b Parameters that cannot be obtained/calculated from spectra.

Table 3.6 : ^1H and ^{13}C chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$), exchange rates (k_{ex}) and chemical shift differences ($\Delta\delta$) of isomalt.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(1)H	65.176	3.559 ^a	-	-	-	-
C(2)H	76.780 ^a	4.167 ^a	-	-	-	-
C(3)H	77.758	4.149	-	-	-	-
C(4)H	77.135	4.234	-	-	-	-
C(5)H	81.705	3.978	-	-	-	-
C(6)Ha	70.462	3.695	-	-	-	-
C(6)Hb	70.462	3.889	-	-	-	-
C(1')H	101.039	4.980	-	-	-	-
C(2')H	72.426	3.579 ^a	-	-	-	-
C(3')H	75.952	3.769 ^a	-	-	-	-
C(4')H	74.322	3.420	-	-	-	-
C(5')H	74.868	3.751 ^a	-	-	-	-
C(6')Ha	63.419	3.778 ^a	-	-	-	-
C(6')Hb	63.419	3.878 ^a	-	-	-	-
O(1)H		6.095	^d	-11.28	5.43	0.297
O(2)H		6.789	^d	^d	^d	1.189
O(3)H		6.437 ^b	^d	^c	^c	0.563
O(4)H		6.805	^d	^d	^d	0.931
O(5)H		6.430	^d	^c	^c	0.830
O(2')H		6.318	5.3	-10.96	10.20	-0.051
O(3')H		6.430	^d	^c	^c	0.030
O(4')H		6.430	^d	^c	^c	0.016
O(6')H		6.004	^d	-12.06	2.52	-0.410

^a Chemical shifts are obtained from HSQC spectrum.

^b Chemical shifts are obtained from TOCSY spectrum.

^c Hydroxy protons that have the chemical shifts at 6.430 ppm are overlapped. Thus the temperature coefficient and the rate of exchange for that protons cannot be calculated.

^d Parameters that cannot be obtained/calculated from spectra.

Table 3.7 : ^1H and ^{13}C chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$), exchange rates (k_{ex}) and chemical shift differences ($\Delta\delta$) of sucralose.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(1')H	45.891	3.786 ^a	-	-	-	-
C(2')H	106.148	-	-	-	-	-
C(3')H	78.429	4.450 ^a	-	-	-	-
C(4')H	78.067	4.130	-	-	-	-
C(5')H	84.303	4.077	-	-	-	-
C(6')Ha	47.589	3.891	-	-	-	-
C(6')Hb	47.589	3.964	-	-	-	-
C(1)H	95.351	5.473	-	-	-	-
C(2)H	70.358	3.961	-	-	-	-
C(3)H	70.860	4.208	-	-	-	-
C(4)H	65.682	4.525	-	-	-	-
C(5)H	73.716	4.437 ^a	-	-	-	-
C(6)H	64.325	3.779 ^a	-	-	-	-
O(3')H		6.810	6.4	-12.42 ^b	4.29	0.352
O(4')H		6.964	^d	-10.54 ^b	^d	0.193
O(2)H		6.173	8.1	-11.60 ^c	^d	-0.072
O(3)H		6.687	4.7	-12.60	2.72	0.311
O(6)H		6.141	^d	-10.70 ^c	^d	0.238

^a Chemical shifts are obtained from HSQC spectrum.

^b Chemical shifts at 283K are overlapped

^c Chemical shifts at 278K and 283K cannot be obtained. Thus the temperature coefficients are calculated with the spectra at 263K, 268K and 278K.

^d Parameters that cannot be obtained/calculated from spectra.

Table 3.8 : ^1H and ^{13}C chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$), exchange rates (k_{ex}) and chemical shift differences ($\Delta\delta$) of NHDC.

Atom	^{13}C δ (ppm)	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	k_{ex} (s^{-1}) 3-18ms	$\Delta\delta$ (ppm)
C(2)H	122.718	6.798	-	-	-	-
C(5)H	114.589	6.933	-	-	-	-
C(6)H	122.817	6.792 ^a	-	-	-	-
C(7)H	31.712	2.725	-	-	-	-
C(8)Ha	47.891	3.079	-	-	-	-
C(8)Hb	47.891	3.201	-	-	-	-
C(12)H	-	5.915	-	-	-	-
C(14)H	-	5.915	-	-	-	-
C(16)H	99.569	4.790	-	-	-	-
C(17)H	74.640	3.506 ^a	-	-	-	-
C(18)H	71.608	3.456	-	-	-	-
C(19)H	80.343	3.574	-	-	-	-
C(20)H	78.564	3.551	-	-	-	-
C(21)Ha	63.078	3.738 ^a	-	-	-	-
C(21)Hb	63.078	3.912 ^a	-	-	-	-
C(22)H	97.562	5.950	-	-	-	-
C(23)H	72.851	4.065	-	-	-	-
C(24)H	72.613	3.731 ^a	-	-	-	-
C(25)H	79.137	3.512 ^a	-	-	-	-
C(26)H	71.327	3.932 ^a	-	-	-	-
C(27)H	19.670	1.367	-	-	-	-
C(28)H	58.011	3.840	-	-	-	-
O(18)H	-	6.485	^b	-7.90	7.56	0.029
O(23)H	-	6.242	^b	-11.20	6.63	-
O(25)H	-	6.689	^b	-5.90	3.98	-

^a Chemical shifts are obtained from HSQC spectrum.

^b Parameters that cannot be obtained/calculated from spectra.

3.1.7 Complexation studies

Assignments and NMR parameters of CD inclusion complexes with artificial sweeteners are given from Table A.2 to Table A.25 and aliphatic coupling constants are listed in Table A.34 to Table A.57.

Acesulfame potassium: For analysis of complexation studies complexation induced shifts (CIS) are generally larger for hydroxy protons than aliphatic protons. For acesulfame potassium and CDs samples larger CIS values are calculated for O(6)H

protons. The largest CIS value of acesulfame potassium and CDs belongs to β -CD O(6)H with -0.051 ppm. Vicinal coupling constant for hydroxy protons could be calculated for O(2)H of CDs and the sequence of the coupling constants is β -CD < γ -CD < α -CD. Coupling constant values are relatively smaller than single CD complexes. In single CD vicinal coupling of O(2)H indicate restricted rotation however for complexes of β - and γ -CDs coupling constant refers to free rotation. There is not any significant change in terms of temperature coefficients for hydroxy protons of CDs. Considering CIS values and change in vicinal coupling constants there may be transient interactions between acesulfame potassium and CDs. Larger CIS values for O(6)H and variation for coupling constant for O(2)H acesulfame potassium may interact with the both sides of CDs.

Sodium cyclamate: For complexation studies for sodium cyclamate CIS values are larger for O(2)H and O(6)H than O(3)H of CDs. However generally difference in chemical shift are smaller for aliphatic protons, cyclohexane protons of sodium cyclamate exhibit unusual behavior. The most affected protons are axial proton of C(5) and equatorial proton of C(7). The addition of sodium cyclamate to CDs change the chemical shifts of both sodium cyclamate and the cyclodextrin protons more in α - and β -CDs than γ -CD. Despite that CIS values are smaller in γ -CD vicinal coupling constant changes the most in this complex. Although coupling constant of CD O(2)H indicates free rotation for complex, in single CD vicinal coupling of O(2)H refers to restricted rotation. There is not any significant change in terms of temperature coefficients for hydroxy protons of CDs. All NMR parameters and CIS values may indicate that there is an interaction between sodium cyclamate and CD and these interactions may be intense for specific atoms. Although CIS value is smaller for O(6)H than O(2)H, the vicinal coupling of O(2)H is not affected in the addition of sodium cyclamate to α -CD. For β -CD inclusion complex form with sodium cyclamate CIS value of O(6)H of CD is distinctively larger than other protons. These results may indicate that sodium cyclamate prefer to be in interaction with both sides of the torus. For γ -CD inclusion complex the situation is different than the other CD complexes. In γ -CD inclusion complex there is not any distinctive change for hydroxy protons of CD. There is significant change of coupling constant for O(2)H. In this inclusion complex large CIS values for axial protons of C(5) and C(7) on sodium cyclamate indicate that there may be interactions on specific atoms.

Saccharin: CIS values are calculated between |0.000| and |0.091| ppm for the complexation studies of saccharin. Examination of CIS values represent that larger values are acquired in β -CD inclusion complex. Besides larger CIS values are obtained for O(6)H in terms of hydroxy protons. Saccharin protons give different results in three complexes. For α -CD complex saccharin protons are not affected significantly however there is little change in chemical shifts for β -cyclodextrin inclusion complex. C(5)H and C(8)H protons of saccharin are affected the most in γ -CD sample. Coupling constant of O(2)H proton has shown the biggest change for β -CD complex that refers to free rotation. There is not any significant change in terms of temperature coefficients of hydroxy protons for CDs. The results for saccharin and CDs indicate that saccharin may not interact with α -CD whereas there may be an interaction with β - and γ -CDs. CIS values and coupling constants of β -CD state there may be interactions in both sides of the torus. On the other hand interactions may probably be stronger in the smaller rim of the γ -CD inclusion complex. Moreover, distinctive differences in chemical shifts of saccharin C(5)H and C(8)H indicate that specific interactions may occur with saccharin and γ -CD. Results in three complex forms of saccharin may indicate that saccharin is bigger than the cavity of α -CD while saccharin affect the both sides of β -CD that shows it may be immersed into the cavity. On the other hand specific interactions take places for the inclusion complex form of γ -CD with saccharin.

Aspartame: CIS values vary between |0.000| and |0.053| ppm for the complexation studies of aspartame. Addition of aspartame to α -CD is not affected the chemical shifts of aspartame and α -CD whereas CIS values are relatively bigger for β - and γ -CD inclusion complexes. The largest CIS values of each complex are N(1)H protons of aspartame for α -CD and β -CD 0.018 and 0.053 ppm, relatively. On the other hand the biggest CIS value belongs to C(9)H of aspartame with 0.032 ppm in γ -CD complex. Vicinal coupling constant of N(1)H proton does not change significantly while there is dramatic changes for CD O(2)H proton. The most dramatic change belongs to β -CD O(2)H with the value of 3.4 Hz which indicates limited rotation. Generally the coupling constant of cyclodextrin O(2)H refers to restricted rotation but the value varies between 7-8 Hz. The vicinal coupling constant of this proton in α -CD inclusion complex indicates free rotation. As the other artificial sweeteners there is not any dramatic change for temperature coefficients. Results of α -CD inclusion complex show that there is not distinctive changes except the CIS value for N(1)H

and the vicinal coupling constant of O(2)H. These consequences indicate that the interactions between α -CD and aspartame are transient and condense relatively for particular atoms. Outcomes for β - and γ -CD inclusion complexes are different from α -CD inclusion complex. In these two complex forms chemical shifts are affected both exchangeable protons and aliphatic protons. Especially for C(3)H_b and C(9)H protons are altered the most. While the chemical shift of O(6)H is the most affected among hydroxy protons, CIS values of other hydroxy protons of CDs are larger values than aliphatic protons. Consideration of the CIS values and the coupling constants of O(2)H proton in β - and γ -CDs may indicate that aspartame has interactions with both sides of the torus. Moreover consideration of CIS values for these CDs may state that interactions between aspartame and β -CD are stronger than γ -CD inclusion complex.

Noetame: CIS values vary between |0.000| and |0.036| ppm for the complexation studies of neotame. Among the CDs, CIS values of α -CD inclusion complex are smaller than β - and γ -CDs. The assignment of O(6)H protons of three CDs can not be done. These situations may indicate that interactions may occur in the addition of neotame to CDs from the smaller rim of the torus. Among all exchangeable protons coupling constant could be calculated for neotame N(1)H proton and the variation between the single molecule and the inclusion complex is 1 Hz. Another important result with the complexation study of neotame is the vicinal coupling constant for O(2)H of CDs can not be measured. These results may indicate that interactions are also occurred between host and guest molecule from the bigger side of the rim. Consideration of whole results may indicate that interactions between neotame and CDs are stronger for β - and γ -CDs than α -CD as in the complexation studies of aspartame. Moreover CIS values of C(3)H_a and C(13)H_a, that are aliphatic protons of neotame, are larger values in β - and γ -CDs which may indicate that interactions are stronger for specific atoms.

Isomalt: CIS values of the complexation studies of isomalt vary between |0.000| and |0.051| ppm. The largest CIS value among all complexes belongs to C(4')H proton in the complex form of α -CD and isomalt. CIS values of α -CD inclusion complex are smaller than other complexes. The largest CIS value may indicate that there are specific and transient interactions with isomalt and α -CD. CIS values are larger for β -CD inclusion complex than α -CD form. Moreover, vicinal coupling constant of O(2)H of β -CD cannot be measured. This situation may indicate that there is

transient interaction with β -CD as well. CIS values of the complexation studies for γ -CD and isomalt are close to β -CD inclusion complex and vicinal coupling constant of O(2')H of isomalt can not be measured. This may indicate that there is a conformational change in isomalt upon the complexation. Furthermore vicinal coupling constant for aliphatic protons are calculated for C(3)H, C(4)H and C(4')H protons of isomalt in all three inclusion complex. These coupling constants strengthen the probability of interactions between isomalt and CDs.

Sucralose: CIS values are calculated between |0.000| and |0.034| ppm for the complexation studies of sucralose. The largest CIS value belongs to β -CD O(6)H proton. CIS values of hydroxy protons are larger than aliphatic protons and among three inclusion complexes CIS values are closer to each other. There is not significant change for vicinal coupling constants of sucralose hydroxy protons on the other hand coupling constant of CD O(2)H is affected in the inclusion complexes of β - and γ -CDs. For these two complex forms coupling constants refer to free rotation. This situation may indicate that there are interactions between sucralose and all CDs. Vicinal coupling constant is measured for C(4')H of sucralose β - and γ -CD inclusion complexes. For single sucralose molecule in aqueous solution this coupling constant cannot be measured. The vicinal coupling constant of C(4')H may indicates that that there is stronger specific interaction between sucralose and β - and γ -CDs besides transient interactions.

NHDC: CIS values of the complexation studies of NHDC vary between |0.000| and |0.235| ppm. The largest CIS value among selected artificial sweeteners is obtained for C(22)H of NHDC which is an aliphatic proton. Due to overlapping of signals, some of the NHDC protons can not be assigned. Moreover, the vicinal coupling constant for CDs O(2)H cannot be measured. This situation indicates that there may be interactions between NHDC and CDs. It is not possible to say that these interactions are stronger for specific atoms on the molecule, since the proton assignment can not be completed. On the other hand, larger CIS values and the vicinal coupling constant that can not be measured indicate that there is an interaction with CDs and NHDC.

3.2 Computational Results

3.2.1 Comparison of computational results with experimental results : Error discussion

The NMR shifts of the investigated artificial sweeteners are tabulated in Tables 3.9-3.27 and the energies of conformers are tabulated in Tables A.58- A.63. The graph of experimental vs. calculated chemical shifts and three-dimensional structures of investigated sweeteners are represented in Figures 3.1-3.27. The hydrogen bonding feature cannot be examined since the NMR calculations could be carried in gas phase. Thus, computational results enabled us to analyze the non-exchangeable protons in the molecule.

Acesulfame potassium: Acesulfame potassium, which has a rigid structure, is a negatively charged molecule. The sulfur atom in the molecule complicates the quantum mechanical calculations. Computational studies are performed on the anionic structure without its counterion. At the first step of the calculation Monte Carlo conformer search is performed with MMFF method. Only one conformation is obtained for acesulfame due to its stable structure. Three-dimensional structure of acesulfame is represented in Figure 3.1.

¹³C Calculation: Four different kinds of carbon atoms exist in acesulfame. The largest %error is calculated for C(6') carbon atom. Other carbon atoms have low percent errors. Linear regression method is used to diminish the error for carbons. The graph of linear regression method show that the mean percent error is highly low. Thus, scaled chemical shifts are in good agreement with experimental chemical shifts.

¹H Calculation: Although carbon chemical shifts are close to the experimental chemical shifts, proton calculation results have higher percent errors. However, it should not be forgotten that the range of proton chemical shifts is much smaller than carbon chemical shifts which causes the relatively bigger percent errors. There are two different types of protons in the molecule. One of the protons is bonded to carbon which is involved in double bond whereas other protons are methyl protons. Percent error for methyl protons is bigger than the other proton. Since there are two kinds of protons linear regression method cannot be used for acesulfame protons.

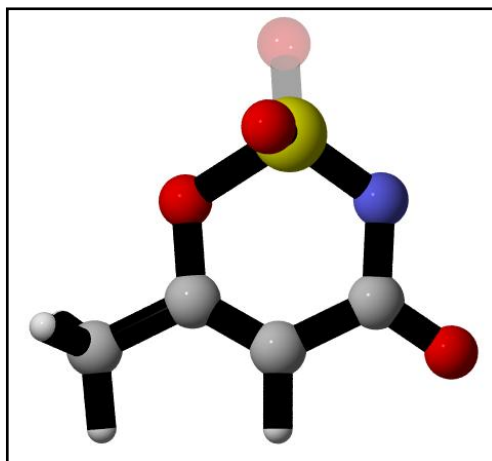


Figure 3.1 : Three-dimensional structure of acesulfame.

Table 3.9 : Experimental chemical shifts, calculated chemical shifts and errors of acesulfame.

Assignment Label	Experimental Chemical Shift (ppm)	Calculated Chemical Shift (ppm)	Error A ^a	Scaled Chemical Shifts	Error S ^b
C(4)	174.967	172.697	1.30	173.856	0.63
C(5)	103.748	104.486	0.71	104.047	0.29
C(6)	166.894	166.874	0.01	167.897	0.60
C(6')	21.904	24.036	9.73	21.712	0.88
C(5)H	5.666	4.871	14.03	-	-
C(6')H	2.120	1.627	23.24	-	-
¹³ C			2.94		0.60
¹ H			18.64		-

^a Percent error of calculated chemical shifts.

^b Percent error of scaled chemical shifts.

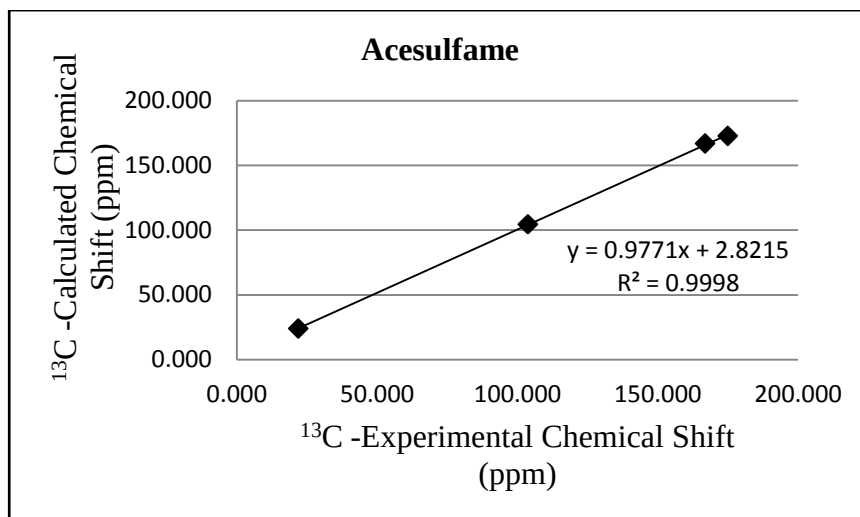


Figure 3.2 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^{13}C chemical shifts of acesulfame.

Sodium cyclamate: Sodium cyclamate is a charged molecule as acesulfame potassium. At the first step of the calculation which is the Monte Carlo method after energy minimization with MMFF three conformations are acquired for cyclamate. The conformations of three conformers depend on the position of $-\text{NH}-\text{SO}_3$ group with respect to the cyclohexane ring. This group is equatorial to the cyclohexane ring in two conformers whereas axial in one conformer. Chemical shifts are calculated via three different ways: First, chemical shifts are calculated by averaging the shift of a certain atom from all conformers. In the second method shifts are calculated according to their energies with Boltzmann distribution. In the third method, chemical shifts are obtained by using linear regression method and named as “scaled chemical shifts”. Three-dimensional structure of cyclamate that has the lowest energy is represented in Figure 3.3.

^{13}C Calculation: The largest percent error belongs to C(7') carbon atom. It is surprising because this atom is far from the electronegative atoms in the molecule. Minimum percent error among cyclamate carbons is obtained for C(4) which is the nearest atom to the $-\text{NH}-\text{SO}_3$ group. The mean percent errors of averaged shifts and Boltzmann averaged shifts give almost similar values. Thus, it is not possible to conclude which method is better. On the other hand percent error that is obtained with linear regression method is better than the other two methods. Error % is decreased for carbon atoms with linear regression method except for C(5) which has almost the same error with the first method.

¹H Calculation: Protons in the cyclamate molecule are methylene protons except C(4)H and one is on axial position and the other is on equatorial position. The mean value for both experimental and calculated methylene chemical shift are used for cyclamate. The largest error is obtained for C(5)H proton by using averaged chemical shifts however Boltzmann distribution method have revealed the chemical shifts closer to the experimental data. Chemical shift that are calculated with Boltzmann distribution are closer to the experimental chemical shifts except for C(4)H. On the other hand, linear regression method gives the best chemical shift for C(4)H. Among three ways for obtaining chemical shifts Boltzmann averaged chemical shifts have relatively smaller the mean percent error.

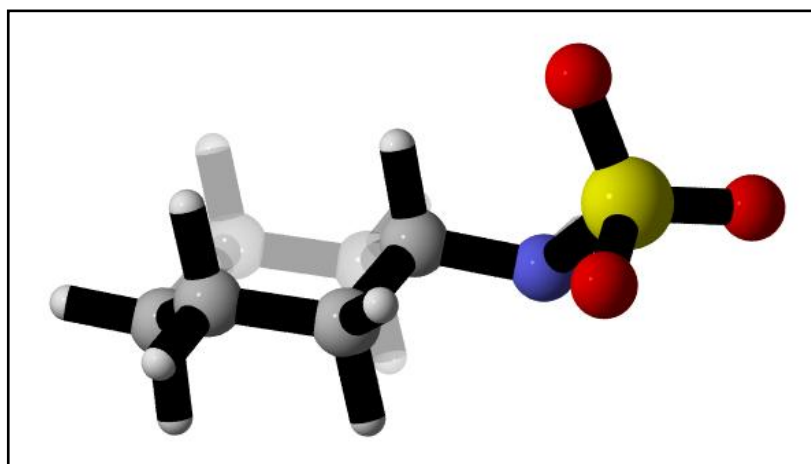


Figure 3.3 : Three-dimensional structure of cyclamate conformer with the lowest energy.

Table 3.10 : Experimental chemical shifts, calculated chemical shifts and errors of cyclamate.

Assignment label	Experimental Chemical Shifts (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(4)	56.011	58.660	4.73	57.077	1.9	56.498	0.87
C(5)	35.963	37.286	3.68	38.079	5.88	34.276	4.69
C(6)	27.429	30.053	9.57	29.744	8.44	26.757	2.45
C(7)	27.660	32.718	18.28	32.975	19.21	29.527	6.75
C(4)H	3.062	3.132	2.29	3.386	10.58	3.044	0.59
C(5)H	1.605	1.813	12.96	1.593	0.75	1.772	10.43
C(6)H	1.507	1.478	1.92	1.488	1.26	1.449	3.82
C(7)H	1.375	1.306	5.02	1.306	5.02	1.284	6.64
¹³ C			9.07		8.86		3.69
¹ H			5.55		4.40		5.37

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

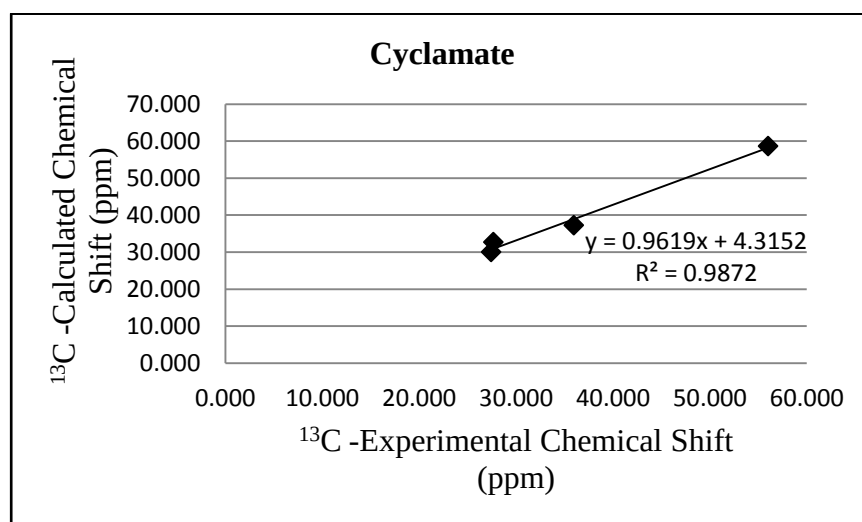


Figure 3.4 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹³C chemical shifts of cyclamate.

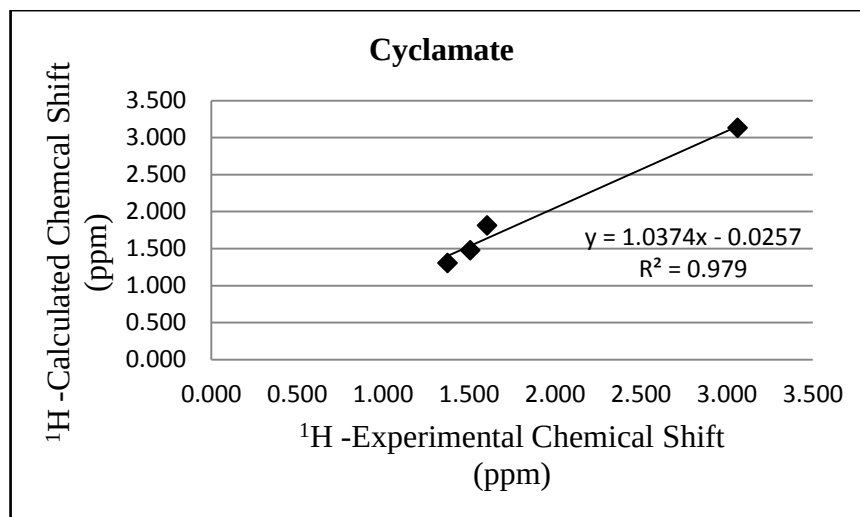


Figure 3.5 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹H chemical shifts of cyclamate.

Saccharin: Saccharin has a rigid structure like acesulfame potassium and its structure is almost planar, thus there is only one possible conformation. Three-dimensional structure of saccharin is represented in Figure 3.6.

¹³C Calculation: All of the carbon atoms in saccharin molecule is sp² hybridized. One of these carbons is carbonyl carbon whereas others are included in benzene ring. The largest error is 7.26% for C(3) carbon which is the carbonyl carbon atom. The second largest error is calculated for C(9) with the value of %5.53. This error may arise from sulfur atom being close to the carbon. Scaled chemical shifts are calculated although the coefficient determination (R²) is not good. There is not improvement for carbon percent errors with linear scaling since R² value is not reliable.

¹H Calculation: Protons that are calculated in saccharin are benzene protons and the electronegative atoms, that can affect calculations, are far away from these protons. The largest error is obtained for C(5)H and the errors for other protons are fairly smaller than C(5)H. R² value obtained from linear regression is not an acceptable value. Thus, systematic errors cannot be excluded with linear regression method for saccharin.

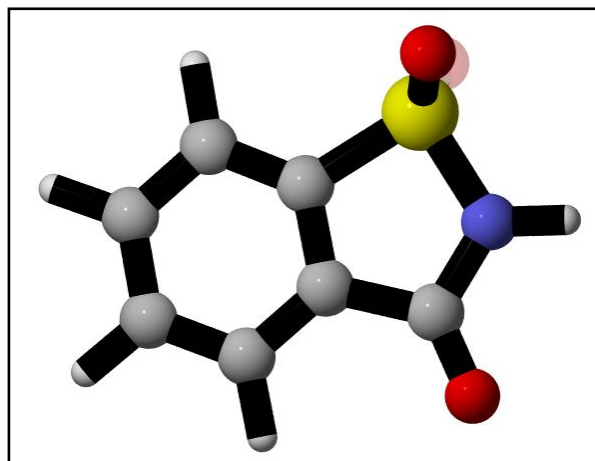


Figure 3.6 : Three-dimensional structure of saccharin.

Table 3.11 : Experimental chemical shifts, calculated chemical shifts and errors of saccharin.

Assignment label	Experimental Chemical Shift (ppm)	Calculated Chemical Shifts (ppm)	Error A ^a	Scaled Chemical Shifts (ppm)	Error S ^b
C(3)	175.187	162.463	7.26	170.967	2.41
C(4)	135.204	133.330	1.39	128.904	4.66
C(5)	126.515	131.799	4.18	126.693	0.14
C(6)	136.752	138.960	1.61	137.033	0.21
C(7)	136.241	140.209	2.91	138.836	1.90
C(8)	123.154	126.162	2.44	118.555	3.73
C(9)	144.468	152.455	5.53	156.517	8.34
C(5)H	7.823	8.233	5.24	7.647	2.25
C(6)H	7.817	7.830	0.16	7.915	1.26
C(7)H	7.819	7.878	0.76	7.883	0.82
C(8)H	7.888	7.852	0.46	7.901	0.16
¹³ C			3.62		3.06
¹ H			1.66		1.12

^a Percent error of calculated chemical shifts.

^b Percent error of scaled chemical shifts.

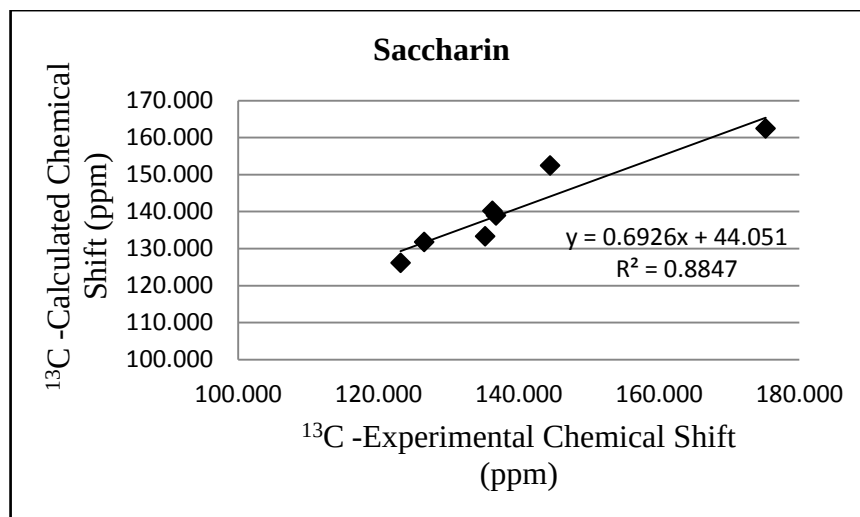


Figure 3.7 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^{13}C chemical shifts of saccharin.

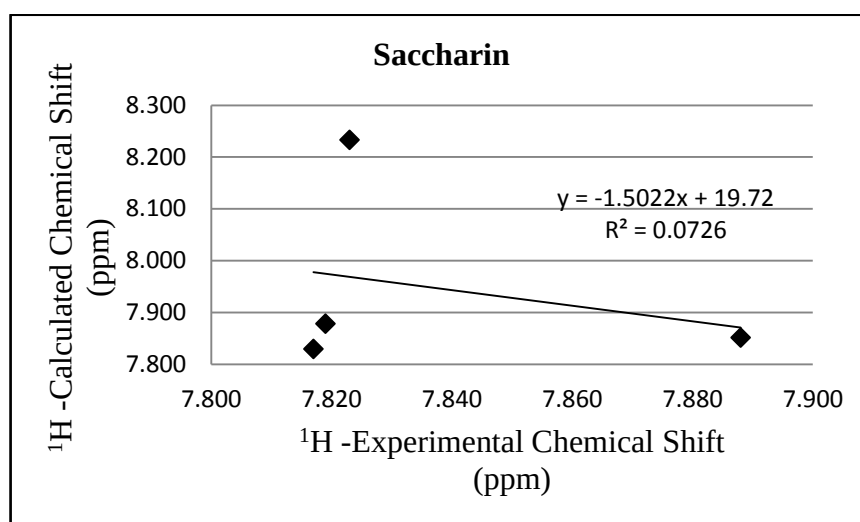


Figure 3.8 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of saccharin.

Aspartame: Calculation for aspartame is different from the sweeteners that are discussed so far since it has so many rotatable bonds. In the first step of calculation the number of conformers are reduced to 100 which reduces to 50 after eliminating the ones from the single point calculations with energies larger than 7 kcal mol^{-1} . In the last step of calculation sequence NMR calculations are done successfully for 47 conformers of aspartame. Three-dimensional structure of aspartame that has the lowest energy is represented in Figure 3.9.

^{13}C Calculation: Error of averaged chemical shifts vary between 1.67% and 12.46%. The highest error belongs to C(10) carbon atom. This high error may arise from the neighboring COOH group. The average error calculated with Boltzmann distribution

is close to the mean error of the first method. Linear regression method is used for aspartame and R squared is the best among selected artificial sweeteners. Linear regression method gives the best average error % for carbon atoms in aspartame.

¹H Calculation: The largest error is calculated for C(9)H in all three methods. C(9)H is bonded to the same carbon atom with -NH₂ group which can exchange protons with water easily. High error may arise from a conformational change due to this exchange because NMR calculations are done in gas phase whereas experimental chemical shifts are obtained in aqueous solution. C(10)H proton has larger error with the method of Boltzmann distribution. R² which is acquired from the graph of ¹H calculated shift vs. ¹H experimental shift indicates that calculated chemical shifts are in good agreement with the experimental results. The correction with linear regression method is used to scale chemical shifts and this method diminishes the error for aspartame protons. The average error shows that averaged chemical shifts and linear scaling give better results than Boltzmann averaged chemical shifts. Furthermore, linear regression method is used for aspartame protons except C(9)H which has the largest error in all methods.

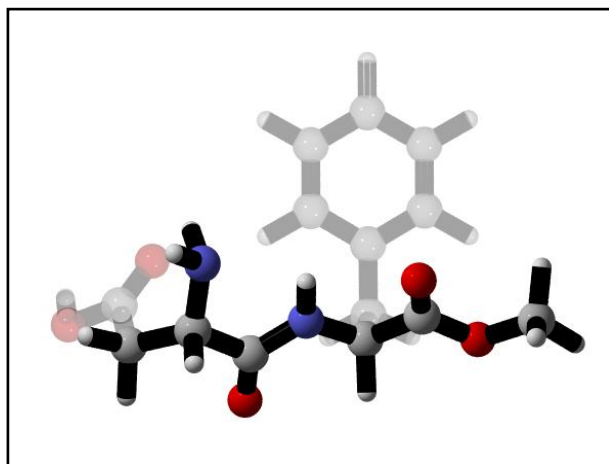


Figure 3.9 : Three-dimensional structure of aspartame conformer with the lowest energy.

Table 3.12 : Experimental chemical shifts, calculated chemical shifts and errors of aspartame.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(2)	57.393	60.375	5.20	60.212	4.91	57.170	0.39
C(3)	39.858	43.717	9.68	43.433	8.97	40.481	1.56
C(5)	131.995	135.882	2.95	136.278	3.25	132.821	0.63
C(6)	131.643	134.62	2.26	134.342	2.05	131.557	0.07
C(7)	130.096	132.962	2.20	132.879	2.14	129.896	0.15
C(9)	53.393	58.168	8.94	57.718	8.10	54.959	2.93
C(10)	39.188	44.071	12.46	42.275	7.88	40.835	4.20
C(12)	55.762	54.830	1.67	54.664	1.97	51.615	7.44
C(2)H	4.701	4.851	3.19	4.889	4.00	4.898	4.19
C(3)H	3.167	3.170	0.08	3.187	0.63	3.326	5.02
C(5)H	7.284	7.452	2.31	7.481	2.70	7.330	0.63
C(6)H	7.384	7.511	1.72	7.5	1.57	7.385	0.01
C(7)H	7.325	7.458	1.81	7.451	1.72	7.335	0.14
C(9)H	4.174	3.505	16.02	3.325	20.34	3.640	12.80
C(10)H	2.719	2.594	4.58	3.132	15.21	2.788	2.55
C(12)H	3.750	3.678	1.92	3.717	0.88	3.801	1.37
¹³ C			5.67		4.91		2.17
¹ H			3.95		5.88		3.34

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

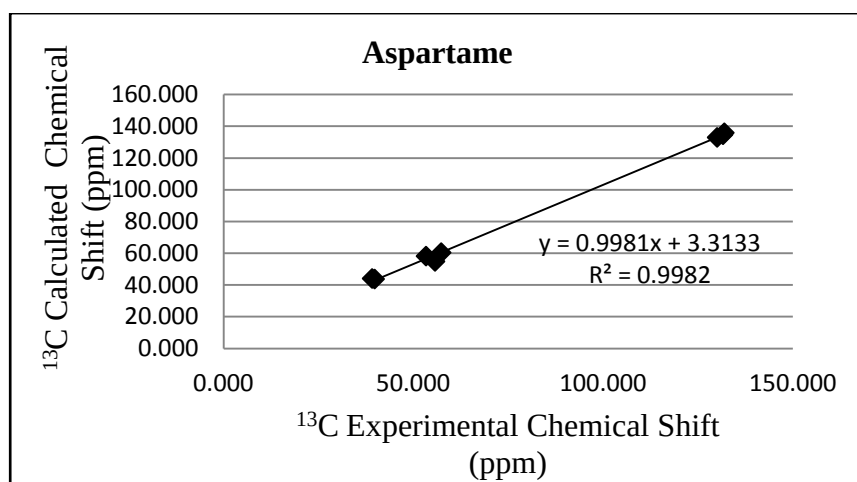


Figure 3.10 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^{13}C chemical shifts of aspartame.

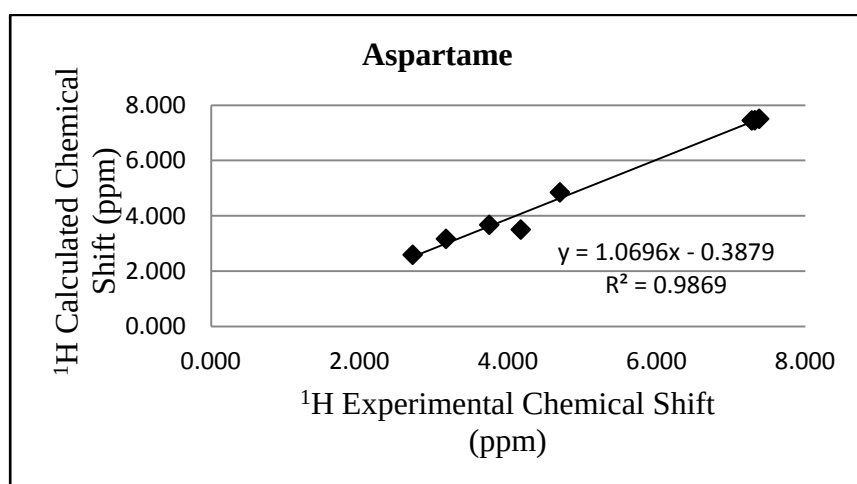


Figure 3.11 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of aspartame.

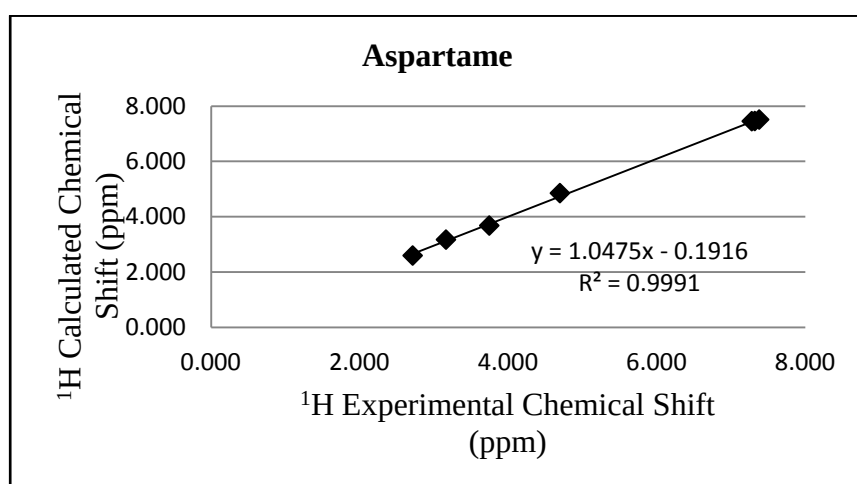


Figure 3.12 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of aspartame (C(9)H omitted).

Table 3.13 : Experimental chemical shifts, calculated chemical shifts and errors of aspartame (C(9)H omitted).

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Scaled Chemical Shifts (ppm)	Error S ^b
C(2)H	4.701	4.851	3.19	4.814	2.4
C(3)H	3.167	3.17	0.08	3.209	1.32
C(5)H	7.284	7.452	2.31	7.297	0.18
C(6)H	7.384	7.511	1.72	7.353	0.42
C(7)H	7.325	7.458	1.81	7.303	0.3
C(10)H	2.719	2.594	4.58	2.659	2.18
C(12)H	3.75	3.678	1.92	3.694	1.49

^a Percent error of averaged chemical shifts.

^b Percent error of scaled chemical shifts.

Neotame: Neotame has 100 conformers in the first step of calculation, like aspartame. After taking out the conformers that have the relative energies higher than 7 kcal mol⁻¹ number of neotame conformers decreases to 45. Geometry optimizations and NMR calculations are performed for these 45 conformers. Three-dimensional structure of neotame that has the lowest energy is represented in Figure 3.13.

¹³C Calculation: Error of averaged carbon chemical shifts vary between 1.12% and 16.69%. The largest error is calculated for C(14) atom. The average errors indicate that calculated chemical shifts are in agreement with experimental results. Linear regression method is used for neotame carbons and R square is 0.9974 indicating the harmony of calculated chemical shifts with the experimental results. Correction derived from linear regression is used for averaged chemical shifts and errors decrease significantly for neotame carbons except C(12) and C(16) that are hydroxymethyl and methyl group, respectively.

¹H Calculation: Error of averaged proton chemical shifts vary between 1.16% and 17.02%. The largest error of neotame protons belongs to C(9)H. This proton is attached to the same carbon atom with -NH₂ group which can exchange protons with water easily as in aspartame. Second largest error among neotame protons is calculated for C(14)H. The high error may be due to conformational changes on the molecule in aqueous solution. Linear regression method is used for neotame protons

and R square is 0.9907 which is the best result among all artificial sweeteners. When the average error is considered, three methods gives similar results. Eliminating C(9)H data further improves R square value to 0.9979.

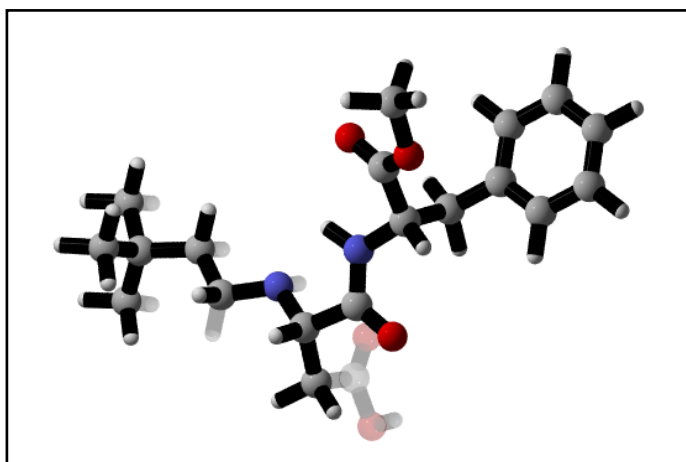


Figure 3.13 : Three-dimensional structure of neotame conformer with the lowest energy.

Table 3.14 : Experimental chemical shifts, calculated chemical shifts and errors of neotame (C(9)H omitted).

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Scaled Chemical Shifts (ppm)	Error S ^b
C(2)H	4.825	4.630	4.04	4.637	3.91
C(3)H	3.157	3.284	4.01	3.324	5.28
C(5)H	7.298	7.383	1.16	7.321	0.32
C(6)H	7.387	7.492	1.42	7.427	0.55
C(7)H	7.323	7.441	1.61	7.378	0.75
C(10)H	2.689	2.549	5.19	2.607	3.02
C(12)H	3.790	3.694	2.53	3.724	1.75
C(13)H	2.727	2.671	2.04	2.726	0.01
C(14)H	1.523	1.330	12.64	1.419	6.82
C(16)H	0.879	0.934	6.26	1.032	17.46

^a Percent error of averaged chemical shifts.

^b Percent error of scaled chemical shifts.

Table 3.15 : Experimental chemical shifts, calculated chemical shifts and errors of neotame.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(2)	56.838	61.183	7.64	60.743	6.87	58.411	2.77
C(3)	39.257	42.687	8.74	44.328	12.92	40.086	2.11
C(5)	131.750	135.891	3.14	135.907	3.16	132.431	0.52
C(6)	131.437	134.573	2.39	134.536	2.36	131.125	0.24
C(7)	130.015	132.85	2.18	132.963	2.27	129.418	0.46
C(9)	60.294	64.068	6.26	63.836	5.87	61.270	1.62
C(10)	39.243	39.958	1.82	38.413	2.12	37.382	4.74
C(12)	55.600	54.816	1.41	54.627	1.75	52.103	6.29
C(13)	46.290	49.017	5.89	49.213	6.31	46.357	0.15
C(14)	41.576	48.514	16.69	49.002	17.86	45.859	10.30
C(16)	30.811	31.157	1.12	31.011	0.65	28.662	6.97
C(2)H	4.825	4.630	4.04	4.831	0.12	4.696	2.68
C(3)H	3.157	3.284	4.01	3.143	0.44	3.384	7.19
C(5)H	7.298	7.383	1.16	7.430	1.81	7.378	1.10
C(6)H	7.387	7.492	1.42	7.504	1.58	7.484	1.32
C(7)H	7.323	7.441	1.61	7.456	1.82	7.435	1.52
C(9)H	4.078	3.384	17.02	3.096	24.08	3.482	14.62
C(10)H	2.689	2.549	5.19	2.751	2.32	2.668	0.75
C(12)H	3.790	3.694	2.53	3.596	5.12	3.784	0.16
C(13)H	2.727	2.671	2.04	2.637	3.28	2.787	2.23
C(14)H	1.523	1.330	12.64	1.387	8.90	1.481	2.74
C(16)H	0.879	0.934	6.26	0.943	7.28	1.095	24.57
¹³ C			5.21		5.65		3.29
¹ H			5.27		5.16		5.35

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

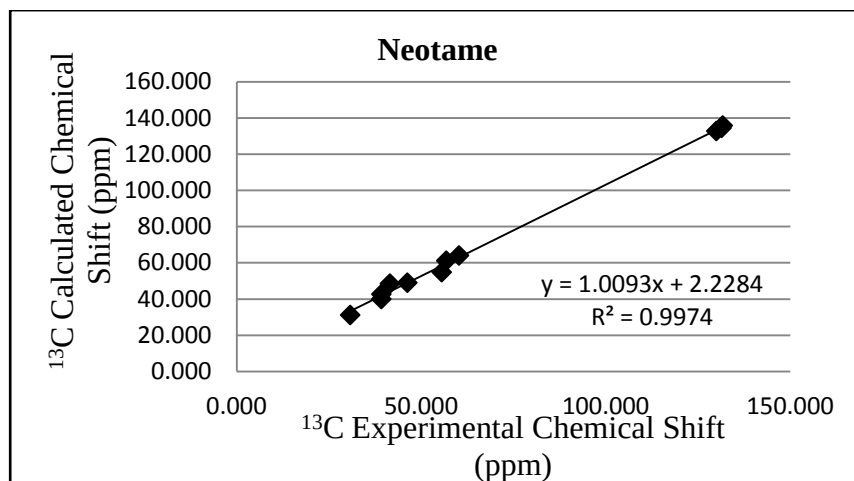


Figure 3.14 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^{13}C chemical shifts of neotame.

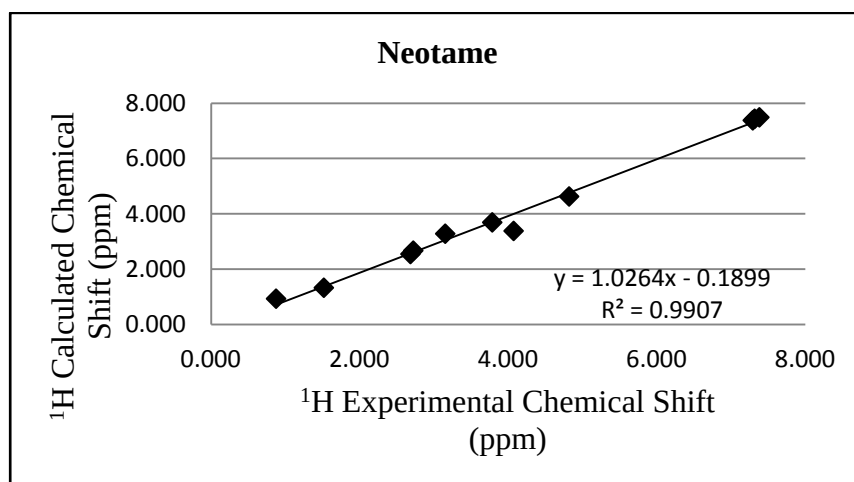


Figure 3.15 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of neotame.

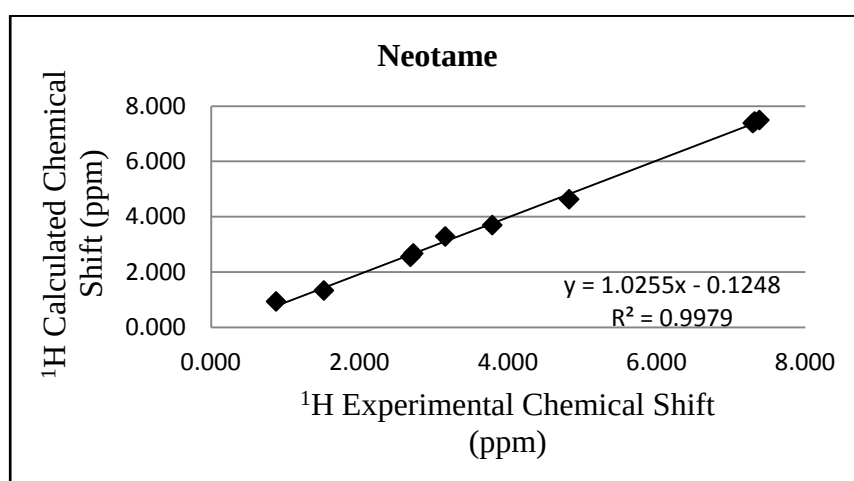


Figure 3.16 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of neotame (C(9)H omitted).

Isomalt: After elimination of the conformers that have relative energies larger than 7 kcal mol⁻¹, isomalt have 9 conformers. Geometry optimizations and NMR calculations are performed for these 9 conformers. Three-dimensional structure of isomalt that has the lowest energy is represented in Figure 3.17.

¹³C Calculation: The difference between calculated chemical shifts and experimental data is bigger for C(6') atom. The average error of averaged chemical shift and Boltzmann averaged chemical shifts are almost the same showing that contributions to average estimation based on energy is not important. The average error of linear regression method is better than other two methods. Thus, it can be said that linear regression diminishes systematic errors for isomalt carbons.

¹H Calculation: The largest error of averaged chemical shifts for isomalt protons is calculated for C(2)H. This may indicate conformational changes on the molecule including C(2)H. The conformational change for C(2)H may originate from O(2)H is involved in hydrogen bonding. Some NMR parameters of O(2)H cannot be calculated experimentally whereas chemical shift difference is calculated as 1.189 ppm and it is a relatively big value. Consideration of experimental and calculation results of these groups strengthen that O(2)H may be involved in hydrogen bonding. The second largest error belongs to C(1)H proton which is the average value of methylene protons. The error may arise from averaging of two protons since those protons may be exposed to different environments. The graph of ¹H calculated vs. ¹H experimental chemical shifts of isomalt indicates that calculated chemical shifts are not in agreement with experimental chemical shifts. Scaling does not refine the trend further.

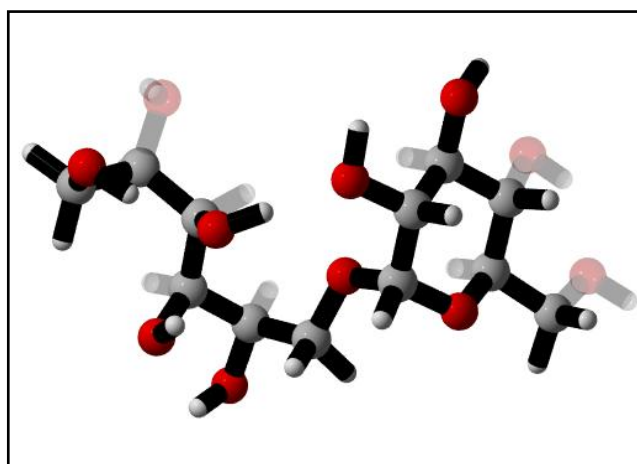


Figure 3.17 : Three-dimensional structure of isomalt conformer with the lowest energy.

Table 3.16: Experimental chemical shifts, calculated chemical shifts and errors of isomalt.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(1)	65.176	68.327	4.83	67.545	3.63	63.628	2.38
C(2)	76.78	78.988	2.88	79.514	3.56	75.531	1.63
C(3)	77.758	79.461	2.19	79.219	1.88	76.060	2.18
C(4)	77.135	81.188	5.26	81.196	5.26	77.988	1.11
C(5)	81.705	76.578	6.27	77.321	5.37	72.841	10.85
C(6)	70.462	76.581	8.68	76.608	8.72	72.844	3.38
C(1')	101.039	105.105	4.02	105.905	4.82	104.693	3.62
C(2')	72.426	77.896	7.55	77.95	7.63	74.312	2.60
C(3')	75.952	80.868	6.47	80.919	6.54	77.631	2.21
C(4')	74.322	79.075	6.40	79.464	6.92	75.629	1.76
C(5')	74.868	77.437	3.43	76.832	2.62	73.800	1.43
C(6')	63.419	70.538	11.22	70.699	11.48	66.096	4.22
C(1)H	3.559	3.988	12.05	3.979	11.8	4.125	15.89
C(2)H	4.167	3.498	16.06	3.382	18.84	3.315	20.45
C(3)H	4.149	3.890	6.24	3.959	4.57	3.963	4.49
C(4)H	4.234	3.899	7.90	3.859	8.86	3.978	6.06
C(5)H	3.978	3.955	0.58	3.93	1.20	4.070	2.31
C(6)H	3.792	4.041	6.57	3.984	5.06	4.212	11.08
C(1')H	4.980	4.743	4.76	4.734	4.94	5.372	7.88
C(2')H	3.579	3.504	2.10	3.445	3.74	3.325	7.10
C(3')H	3.769	3.665	2.76	3.631	3.66	3.591	4.73
C(4')H	3.420	3.421	0.03	3.490	2.05	3.188	6.80
C(5')H	3.751	3.877	3.36	3.798	1.25	3.941	5.07
C(6')H	3.828	3.989	4.19	3.993	4.31	4.125	7.77
¹³ C			5.77		5.70		3.11
¹ H			5.55		5.86		8.30

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

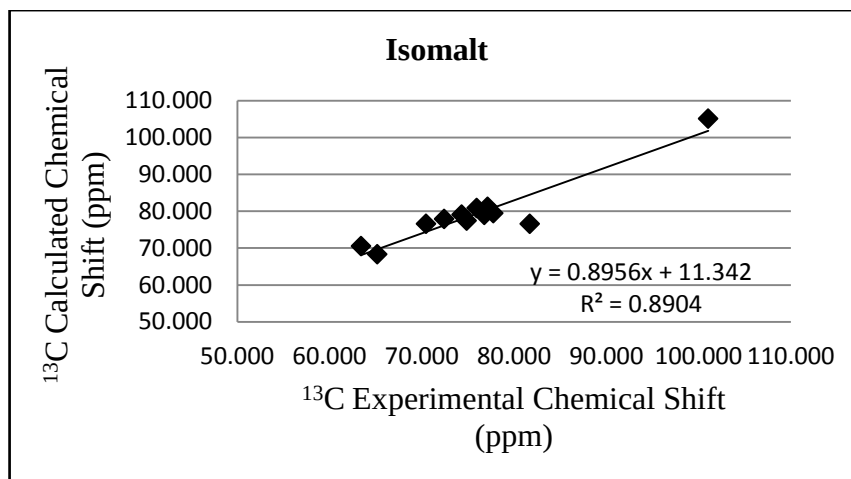


Figure 3.18 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹³C chemical shifts of isomalt.

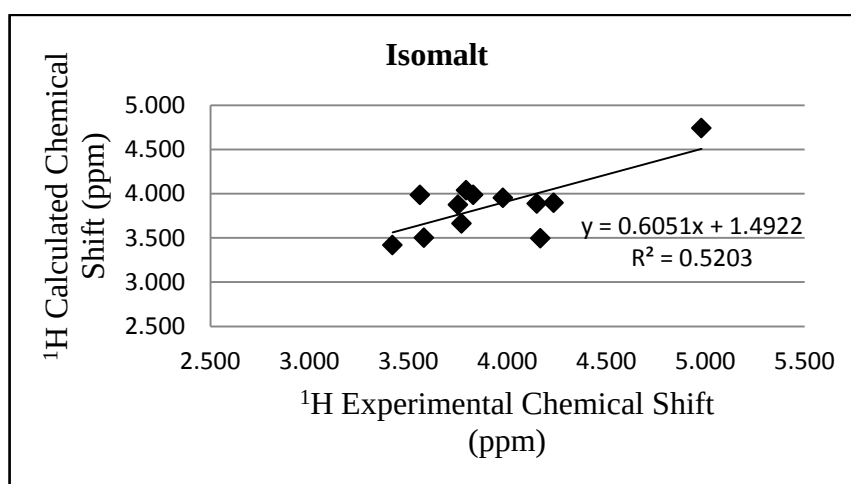


Figure 3.19 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹H chemical shifts of isomalt.

Sucralose: After the elimination of the conformers that have relative energies larger than 7 kcal mol⁻¹, sucralose had 60 conformers for geometry optimization and NMR calculations are performed on these conformers. Three-dimensional structure of sucralose that has the lowest energy is represented in Figure 3.20.

¹³C Calculation: Larger errors of sucralose carbons are calculated for C(1'), C(6') and C(4) atoms. Common feature for these atoms is that they are bonded to chlorine atom and causes heavy atom effect which is the strong deshielding of chemical shifts of carbon atoms bonded to halogens or other third row elements [67]. Among the three methods, linear regression method decreases the error for these three atoms. Consideration of the average errors, linear regression method gives the best results for sucralose carbon atoms among three methods.

¹H Calculation: Calculated chemical shifts of protons are in agreement with all three methods. Calculated percent errors of proton chemical shifts of sucralose are under 6%. However error is lower than 6%, surprisingly R square is 0.9374 for sucralose. The average of errors for three methods are almost the same.

Table 3.17 : Experimental chemical shifts, calculated chemical shifts and errors of sucralose.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(1')	45.891	56.450	23.01	54.059	17.8	48.402	5.47
C(2')	106.148	112.172	5.68	112.354	5.85	109.360	3.03
C(3')	78.429	86.465	10.25	84.567	7.83	81.237	3.58
C(4')	78.067	83.721	7.24	80.966	3.71	78.235	0.22
C(5')	84.303	87.086	3.30	86.524	2.63	81.917	2.83
C(6')	47.589	55.600	16.83	55.306	16.22	47.472	0.25
C(1)	95.351	97.479	2.23	98.271	3.06	93.286	2.17
C(2)	70.358	75.072	6.7	75.176	6.85	68.774	2.25
C(3)	70.86	75.867	7.07	74.900	5.70	69.643	1.72
C(4)	65.682	75.09	14.32	77.549	18.07	68.793	4.74
C(5)	73.716	78.961	7.12	77.079	4.56	73.028	0.93
C(6)	64.325	67.606	5.10	67.988	5.69	60.606	5.78
C(1')H	3.786	3.778	0.21	3.787	0.03	3.833	1.24
C(3')H	4.449	4.211	5.34	4.38	1.54	4.222	5.10
C(4')H	4.130	4.184	1.32	4.236	2.56	4.198	1.65
C(5')H	4.077	4.025	1.29	4.159	2.00	4.055	0.54
C(6')H	3.928	3.844	2.14	3.880	1.21	3.892	0.91
C(1)H	5.471	5.768	5.42	5.583	2.04	5.622	2.76
C(2)H	3.967	3.981	0.34	3.862	2.65	4.015	1.22
C(3)H	4.208	4.051	3.74	4.154	1.27	4.078	3.08
C(4)H	4.525	4.544	0.42	4.523	0.05	4.522	0.07
C(5)H	4.437	4.338	2.23	4.549	2.52	4.336	2.27
C(6)H	3.779	3.946	4.42	3.839	1.59	3.984	5.42
¹³ C			9.07		8.16		2.75
¹ H			2.44		1.59		2.21

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

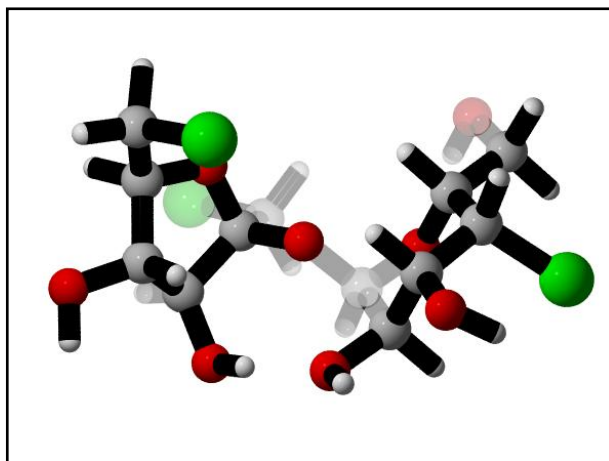


Figure 3.20 : Three-dimensional structure of sucralose conformer with the lowest energy.

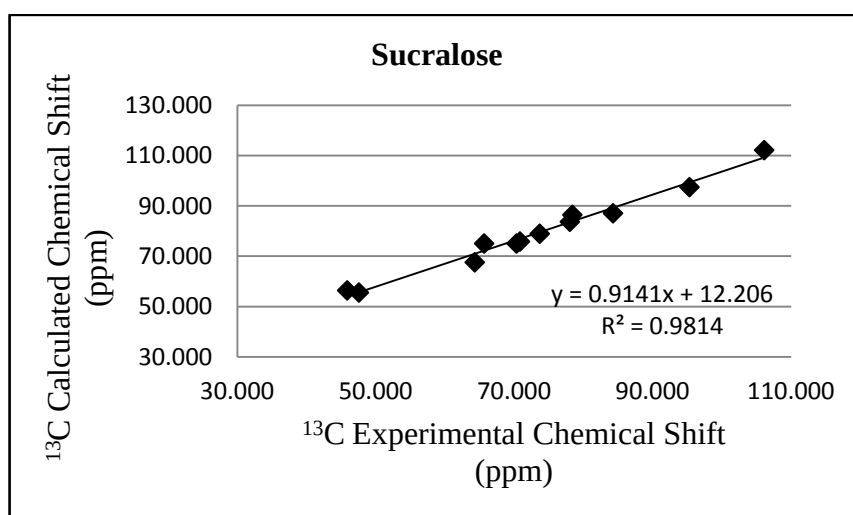


Figure 3.21 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹³C chemical shifts of sucralose.

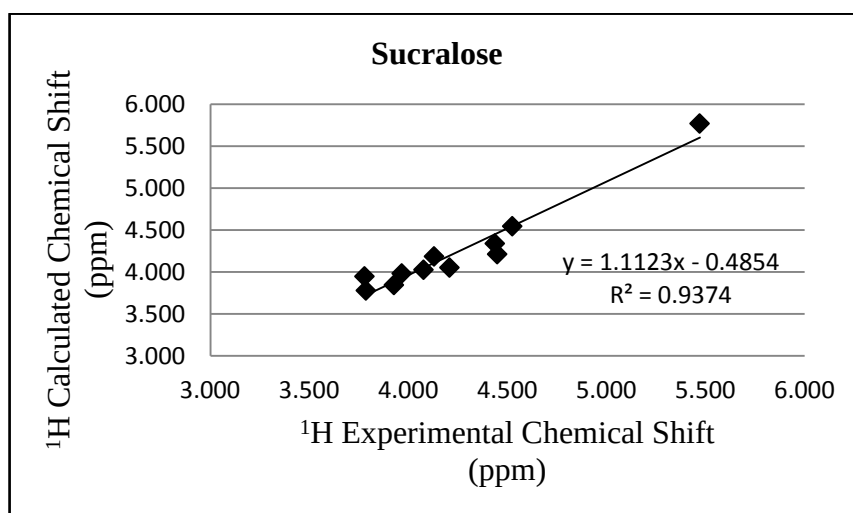


Figure 3.22 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹H chemical shifts of sucralose.

NHDC: For the first step of calculation maximum number of conformers are acquired for NHDC. After elimination of relative energies bigger than 7 kcal mol⁻¹, 9 conformers are obtained. Three-dimensional structure of sucralose that has the lowest energy is represented in Figure 3.23.

¹³C Calculation: Error of averaged carbon chemical shifts vary between 0.51% and 24.89% for NHDC. The largest error among three method belongs to C(7) atom. Three ways of calculation chemical shifts have the similar mean error. Errors of C(27) and C(28) carbons increase with linear regression method significantly. These carbons are methyl and methoxy carbons relatively.

¹H Calculation: The largest error among NHDC protons is calculated for C(8)H. Moreover larger errors of C(7), C(7)H, and C(8)H indicate that there may be conformational change for this atoms on the molecule. Besides C(7)H and C(8)H, C(17)H has also large error indicating that there may be interactions or stacked conformation between glucose and rhamnose monomers. With the average errors, it is not possible to say which method is better for NHDC protons. On the other hand Boltzmann averaged chemical shifts have relatively higher percent error.

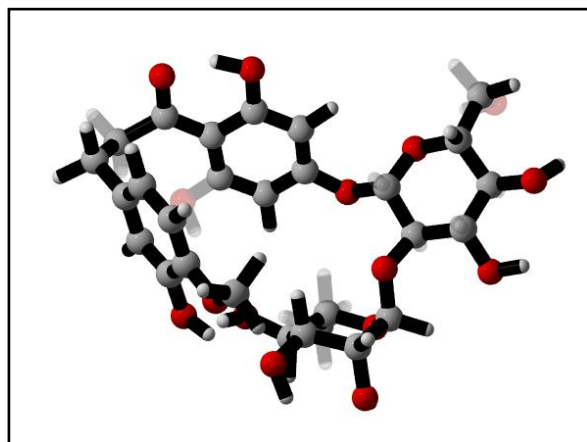


Figure 3.23 : Three-dimensional structure of NHDC conformer with the lowest energy.

Table 3.18 : Experimental chemical shifts, calculated chemical shifts and errors of NHDC.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(2)	122.718	121.465	1.02	120.971	1.42	118.973	3.05
C(5)	114.589	114.006	0.51	112.149	2.13	111.439	2.75
C(6)	122.817	125.638	2.30	126.490	2.99	123.187	0.30
C(7)	31.712	39.605	24.89	40.036	26.25	36.294	14.45
C(8)	47.891	46.023	3.90	44.258	7.59	42.776	10.68
C(16)	99.569	104.325	4.78	107.405	7.87	101.661	2.10
C(17)	74.640	80.154	7.39	77.700	4.10	77.249	3.49
C(18)	71.608	83.554	16.68	83.581	16.72	80.683	12.67
C(19)	80.343	78.049	2.86	77.316	3.77	75.123	6.50
C(20)	78.564	79.619	1.34	79.193	0.80	76.708	2.36
C(21)	63.078	68.687	8.89	68.969	9.34	65.667	4.10
C(22)	97.562	105.192	7.82	104.119	6.72	102.537	5.10
C(24)	72.613	76.977	6.01	76.578	5.46	74.040	1.96
C(25)	79.137	80.991	2.34	82.324	4.03	78.094	1.32
C(26)	71.327	74.037	3.80	73.635	3.24	71.070	0.36
C(27)	19.670	19.388	1.43	19.405	1.35	15.875	19.29
C(28)	58.011	56.557	2.51	55.820	3.78	53.416	7.92
C(2)H	6.798	7.227	6.31	7.400	8.86	7.440	9.44
C(5)H	6.933	6.484	6.48	6.446	7.02	6.639	4.24
C(6)H	6.792	6.684	1.59	6.749	0.63	6.855	0.92
C(7)H	2.725	2.998	10.02	3.009	10.42	2.883	5.81
C(8)H	3.140	3.619	15.25	3.720	18.47	3.552	13.14
C(12)H	5.915	5.813	1.73	6.063	2.51	5.916	0.02
C(14)H	5.915	5.690	3.81	5.857	0.98	5.784	2.22
C(16)H	4.790	4.740	1.05	4.700	1.88	4.760	0.62
C(17)H	3.506	3.883	10.76	3.970	13.24	3.837	9.44
C(18)H	3.456	3.663	5.98	3.760	8.80	3.600	4.16
C(19)H	3.574	3.673	2.77	3.753	5.01	3.611	1.03
C(20)H	3.551	3.320	6.52	3.371	5.07	3.230	9.03
C(21)H	3.825	3.936	2.89	4.003	4.64	3.893	1.79
C(22)H	5.950	5.521	7.21	5.744	3.46	5.602	5.86
C(23)H	4.065	3.764	7.41	3.652	10.16	3.709	8.77

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

Table 3.18 (continued) : Experimental chemical shifts, calculated chemical shifts and errors of NHDC.

Assignment label	Experimental Chemical Shift (ppm)	Averaged Chemical Shifts (ppm)	Error A ^a	Boltzmann Averaged Chemical Shifts (ppm)	Error B ^b	Scaled Chemical Shifts (ppm)	Error S ^c
C(24)H	3.731	3.517	5.74	3.351	10.19	3.443	7.73
C(25)H	3.512	3.456	1.60	3.376	3.88	3.377	3.85
C(26)H	3.932	4.313	9.69	4.736	20.44	4.300	9.36
C(27)H	1.367	1.449	5.99	1.451	6.11	1.215	11.15
C(28)H	3.840	3.727	2.94	3.702	3.60	3.669	4.46
¹³ C			5.78		6.26		5.55
¹ H			5.79		7.27		5.65

^a Percent error of averaged chemical shifts.

^b Percent error of Boltzmann averaged chemical shifts.

^c Percent error of scaled chemical shifts.

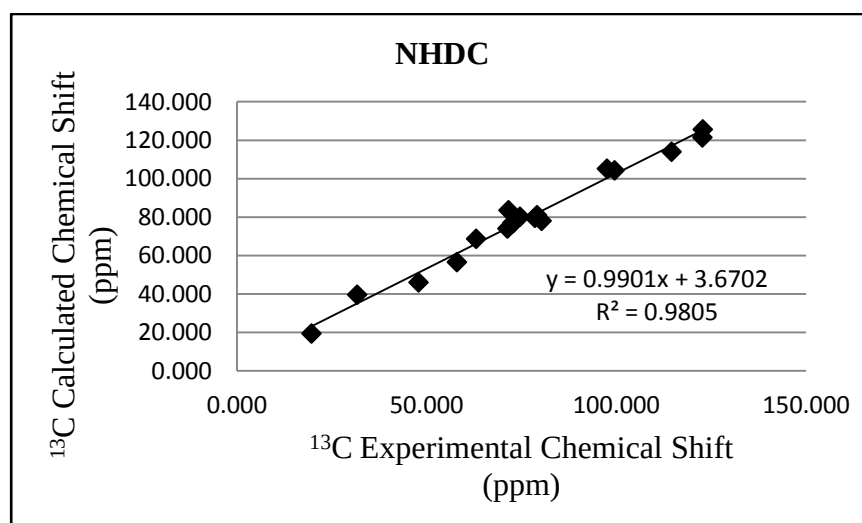


Figure 3.24 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹³C chemical shifts of NHDC.

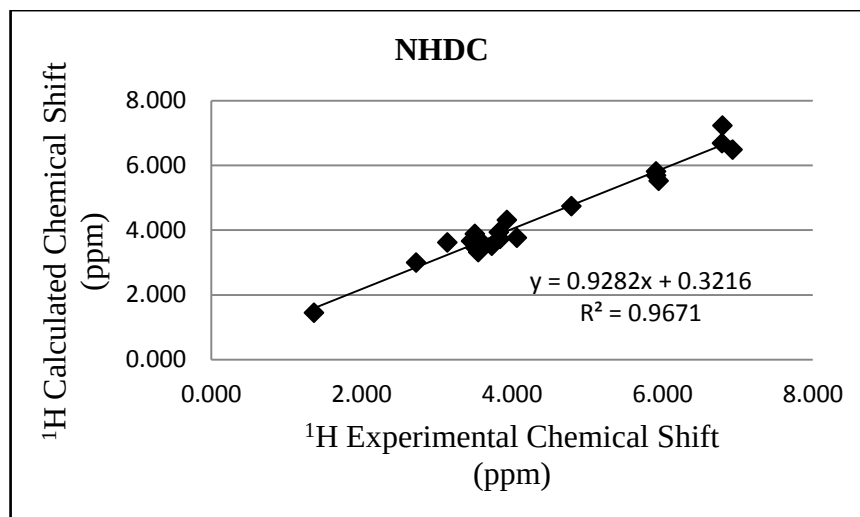


Figure 3.25 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ¹H chemical shifts of NHDC.

The average errors % are calculated for whole artificial sweeteners in three ways. Boltzmann averaged errors % are calculated except acesulfame and saccharin since these sweeteners have only one conformer.

¹³C calculation: The average errors of averaged and Boltzmann averaged chemical shifts are 6.03% and 6.40%, respectively. Moreover, linear regression analysis have been applied to whole data extracted from artificial sweeteners. The average error from linear regression method is calculated as 4.17%. This result indicates that systematic results are diminished for carbons by linear regression method.

¹H calculation: The mean error calculated from averaged chemical shifts is 5.07% and from Boltzmann averaged chemical shifts is 5.29%. Linear regression method gives the mean error 5.06% for protons. The percent error of three was are almost same that means there is no superiority between these methods. The highest percent error is obtained for acesulfame among the investigated molecules. R² value of linear regression is significantly change to 0.981 however it has only two protons.

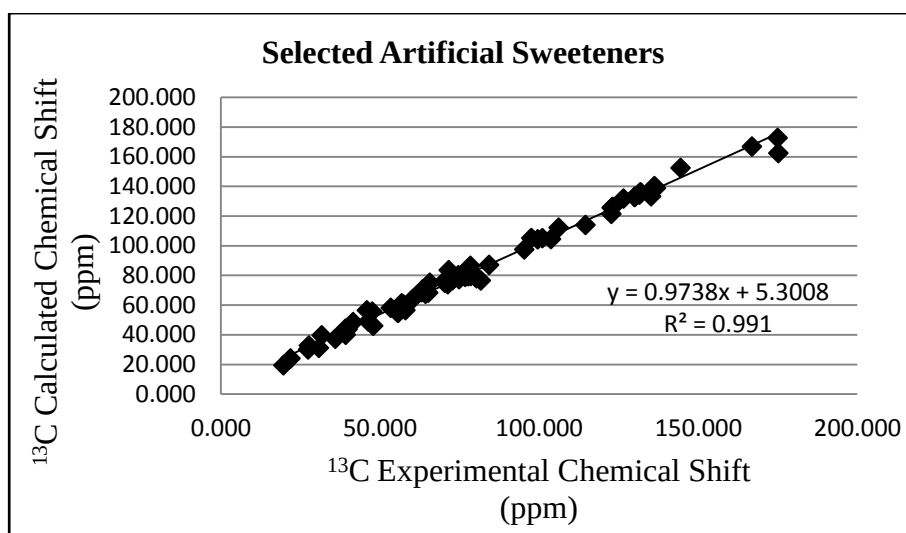


Figure 3.26 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^{13}C chemical shifts of selected artificial sweeteners.

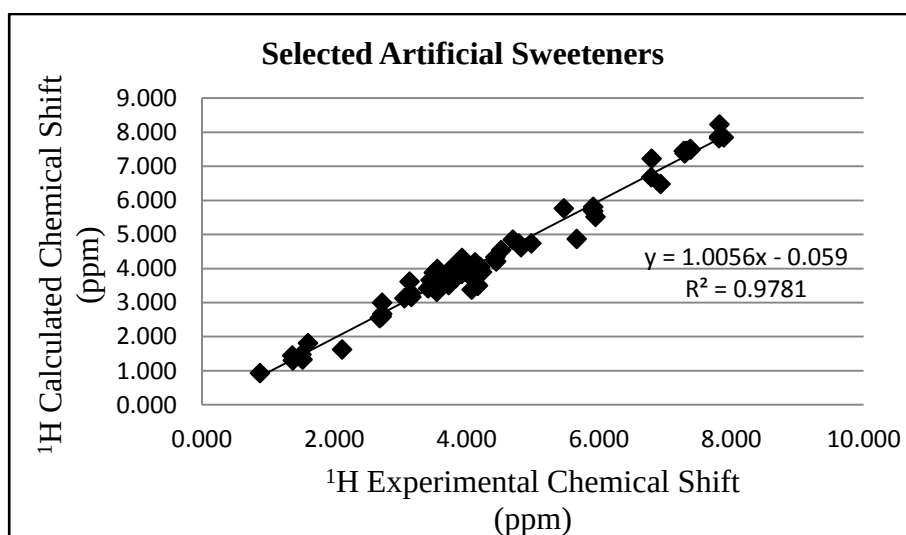


Figure 3.27 : Graph of calculated (B3LYP/6-311+G**) vs. experimental ^1H chemical shifts of selected artificial sweeteners.

3.2.2 Level of theory calculations

The recommended level of theories are B3LYP or PBE0 functionals with medium to large sized basis sets for NMR chemical shift calculations. It has been indicated that the difference between methods diminishes significantly by using linear regression. However, it has been stated that PBE0 functional provide modest improvement over B3LYP functional as well. It has been also reported that increasing the basis set did not have a significant effect once reliable geometries of the system is acquired [70]. Thus, the level of theory calculations are carried out with 6-311+G(2d,p) basis set and PBE0 and B3LYP functionals for cyclamate and isomalt. The results of calculations in terms of error % are tabulated in Table 3.43. Errors % of carbon

chemical shifts obtained with PBE0 functional are better than B3LYP functional for averaged and Boltzmann averaged results. On the other hand proton chemical shifts' errors indicate that B3LYP functional gives better results than PBE0 functional. Although there is a small increment of the percent error for isomalt protons, errors % of cyclamate protons are increased significantly with PBE0 functional. Although percent errors of proton and carbon chemical shifts give different tendencies for the two functionals, linear regression method gives almost the same percent error for cyclamate and isomalt. That means the differences in the percent error for proton and carbon originate from systematic errors and it can be concluded that there is no superiority of linear regression between B3LYP and PBE0 functional for the two sweeteners.

Table 3.19 : Level of theory calculations of cyclamate and isomalt.

		Error % (Averaged Chemical Shifts)		Error % (Boltzmann Averaged Chemical Shifts)		Error % (Scaled Chemical Shifts)	
		¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H
Cyclamate	B3LYP	11.07	7.78	10.95	6.71	3.66	5.33
	PBE0	8.61	15.11	8.72	14.06	3.53	5.32
Isomalt	B3LYP	6.82	5.89	6.40	5.35	3.12	8.06
	PBE0	3.43	7.27	3.81	7.09	3.12	7.74

3.2.3 Solvent effect

NMR calculations of cyclamate was performed to examine the solvent effect on chemical shifts. PCM (Polarizable Continuum Model) was used as implicit solvent method with UFF (Universal Force Field) cavity model [89-91]. Solvent is treated as a polarizable continuum with a dielectric constant by using implicit solvent models. PCM method is recommended with the same method is employed to geometry optimization of the system. Furthermore, linear regression analysis was reported to improve the accuracy by reducing systematic error [70].

The mean errors % of cyclamate solvent and gas phase with three ways are listed in Table 3.44. The mean errors % are decreased significantly for both protons and carbons in the three ways of calculation NMR chemical shifts by using PCM method. Especially with first and second methods there is a significant improvement for error

% of carbon atoms. Although there are only four protons after averaging of the calculated and experimental chemical shifts for the similar protons, linear regression method is employed and this method provides further improvement for carbon and proton errors %. The results are obtained from cyclamate PCM method show that solvent calculation may improve the results of NMR chemical shifts for other systems.

Table 3.20 : The calculated errors of cyclamate in gas phase and in water with PCM methodology.

		Error % (Averaged Chemical Shifts)		Error % (Boltzmann Averaged Chemical Shifts)		Error % (Scaled Chemical Shifts)	
		¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H
Cyclamate	Gas phase	9.07	5.55	8.86	4.40	3.69	5.37
	PCM	4.75	4.25	4.75	3.93	2.58	1.00

4. CONCLUSION AND RECOMMENDATIONS

Structural analysis on the selected artificial sweeteners *i.e.* acesulfame potassium, sodium cyclamate, saccharin, aspartame, neotame, isomalt, sucralose and NHDC was performed employing NMR spectroscopy and computational methods.

The investigated artificial sweeteners show a common property of being sweet although they have completely different structures both chemically and physically. In the experimental part, a common feature that could lead to sweet taste, in terms of NMR parameters have been sought. Within the level of in-depth analysis of the NMR data, no common structural feature could be withdrawn from the experimental results. However, there is enough evidence that this possibility of finding a common feature relating the structures of the artificial sweeteners and water through amphiphilic interactions cannot be excluded provided that a further scrutiny on the data is performed.

Complexation studies indicate that interactions between sweeteners and CDs depend not only on hydrophobicity but also the size of the guest molecule. Acesulfame potassium, sodium cyclamate and saccharin interact strongly with β -CD while specific interactions are observed with γ -CD. On the other hand, there are strong interactions with β - and γ -CDs with aspartame, neotame and sucralose. Complexation studies have also demonstrated that temperature dependence of exchangeable protons is not altered significantly with addition of sweeteners to the CDs. Chemical shifts of sweeteners were calculated and compared with the experimental results. The calculated NMR chemical shifts have not shown systematic errors. The errors in shifts are rather random. When the % errors of calculated proton shifts are examined, a common structural feature could not be observed. This has also been reported in the literature [70]. Among the three ways of percent error calculations, none of the methods have shown superiority above the others. Although Boltzmann distribution averaged chemical shifts have been recommended in the literature, the method has only improved the calculated results for cyclamate. This result may indicate that the energy contributions of conformers are not significantly important at 263 K since at this temperature minimization of free

rotation around the rotatable bonds hampers a certain conformation to dominate rather than a distribution of conformations.

Computational studies were carried out on gas phase and some of the protons revealed results with relatively high percent error. This may be due to specific solute-solvent interactions taking place in a real system which could not be accounted for in our gas phase calculations. The solvent calculations on cyclamate has improved the results significantly. Although the number of considered protons is not high in the cyclamate system, the refinement with the solvent is promising in improving the results further for the other systems as well.

In the literature, it has been reported that increasing the basis set did not have a significant effect once reliable geometries of the system is obtained. Calculations with the PBE functional have provided a modest improvement over B3LYP [70]. However there are only two systems are used in the thesis, the results give different tendencies from previous study. After linear regression method is employed B3LYP and PBE0 level of theories give almost the same error % to the systems.

Since the structural investigations on sweet molecules in aqueous solution do not provide direct verification for any common feature, interactions between sweet receptors i.e. T1R2 and T1R3 and sweeteners can be further studied by various other NMR techniques such as Saturation-Transfer Difference (STD) spectroscopy. In terms of computation, explicit or implicit solvent methods is suggested to obtain refined chemical shifts. Molecular Dynamics (MD) calculations may be performed to create a solvation shell around the investigated molecules which then could be used for NMR calculations.

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APPENDICES

APPENDIX A: Spectra and tables for investigated sweeteners.

APPENDIX A

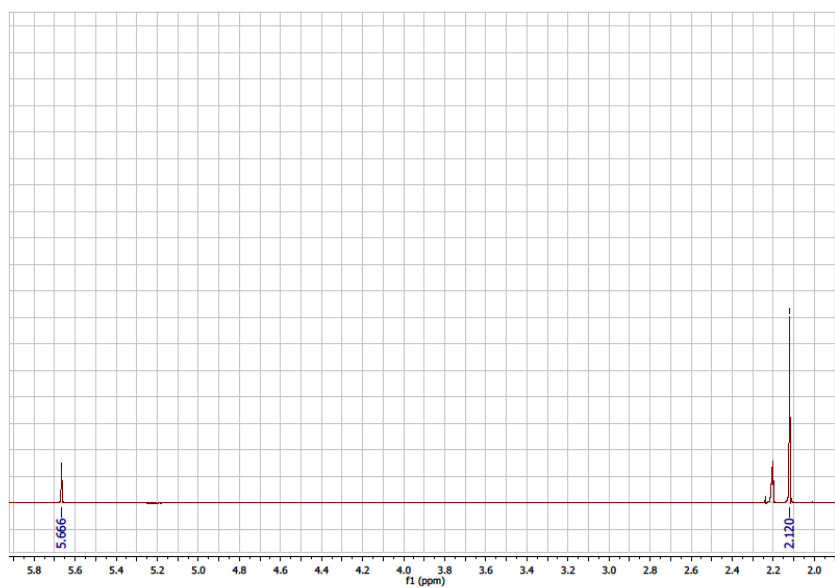
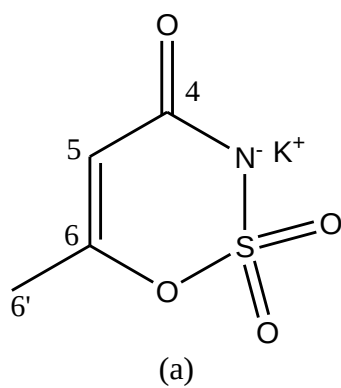
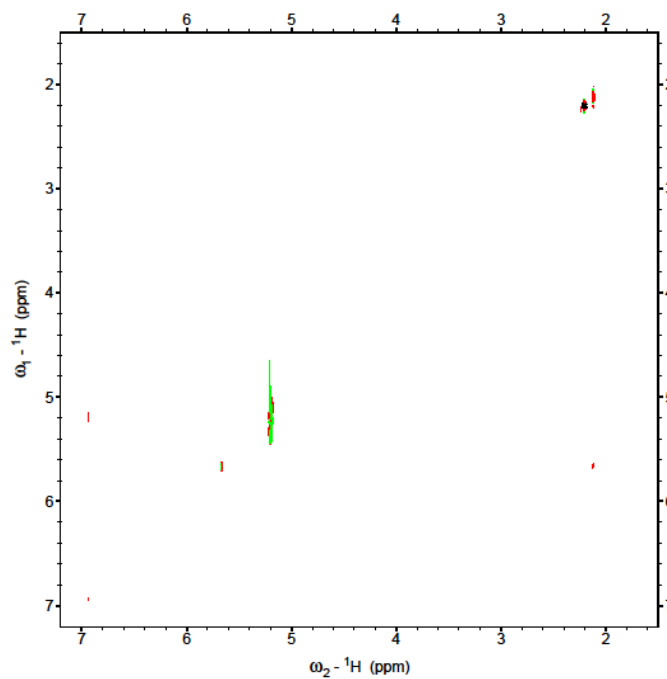
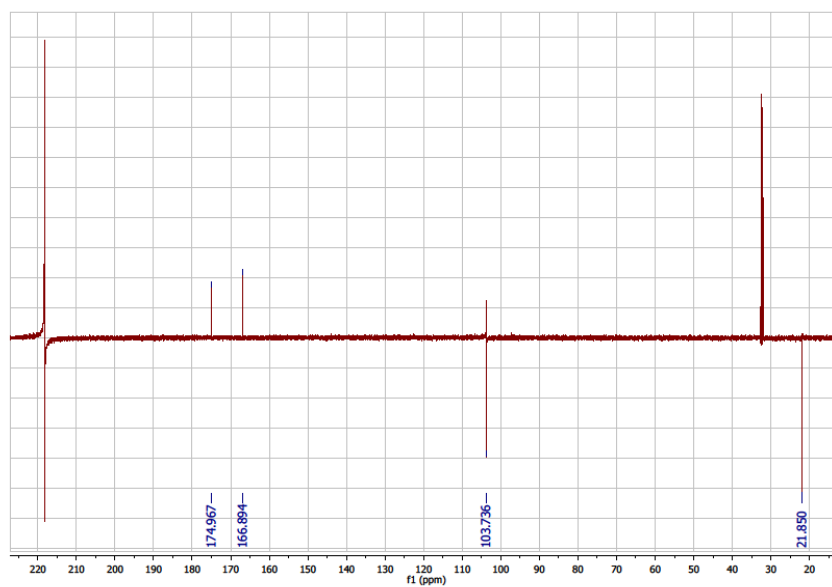


Figure A.1 : Spectra of acesulfame potassium (a)Structure. (b)¹H spectrum.

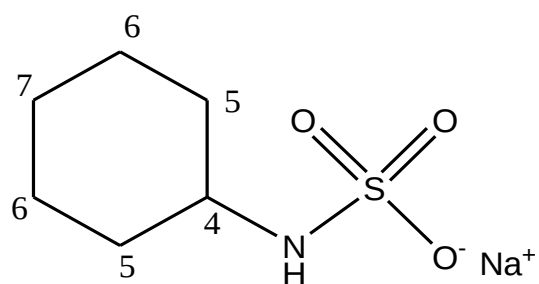


(a)

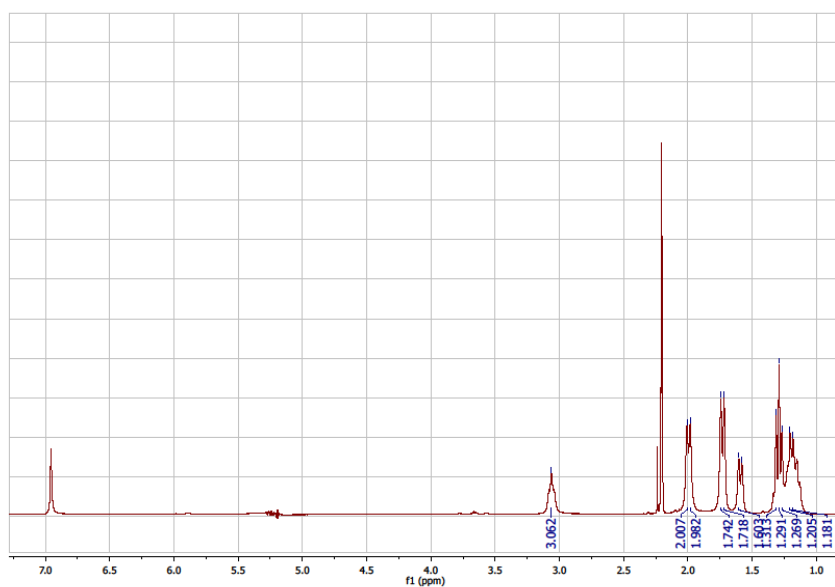


(b)

Figure A.2 : Spectra of acesulfame potassium (a)TOCSY spectrum. (b) ^{13}C spectrum.



(a)



(b)

Figure A.3 : Spectra of sodium cyclamate (a)Structure. (b) ^1H spectrum.

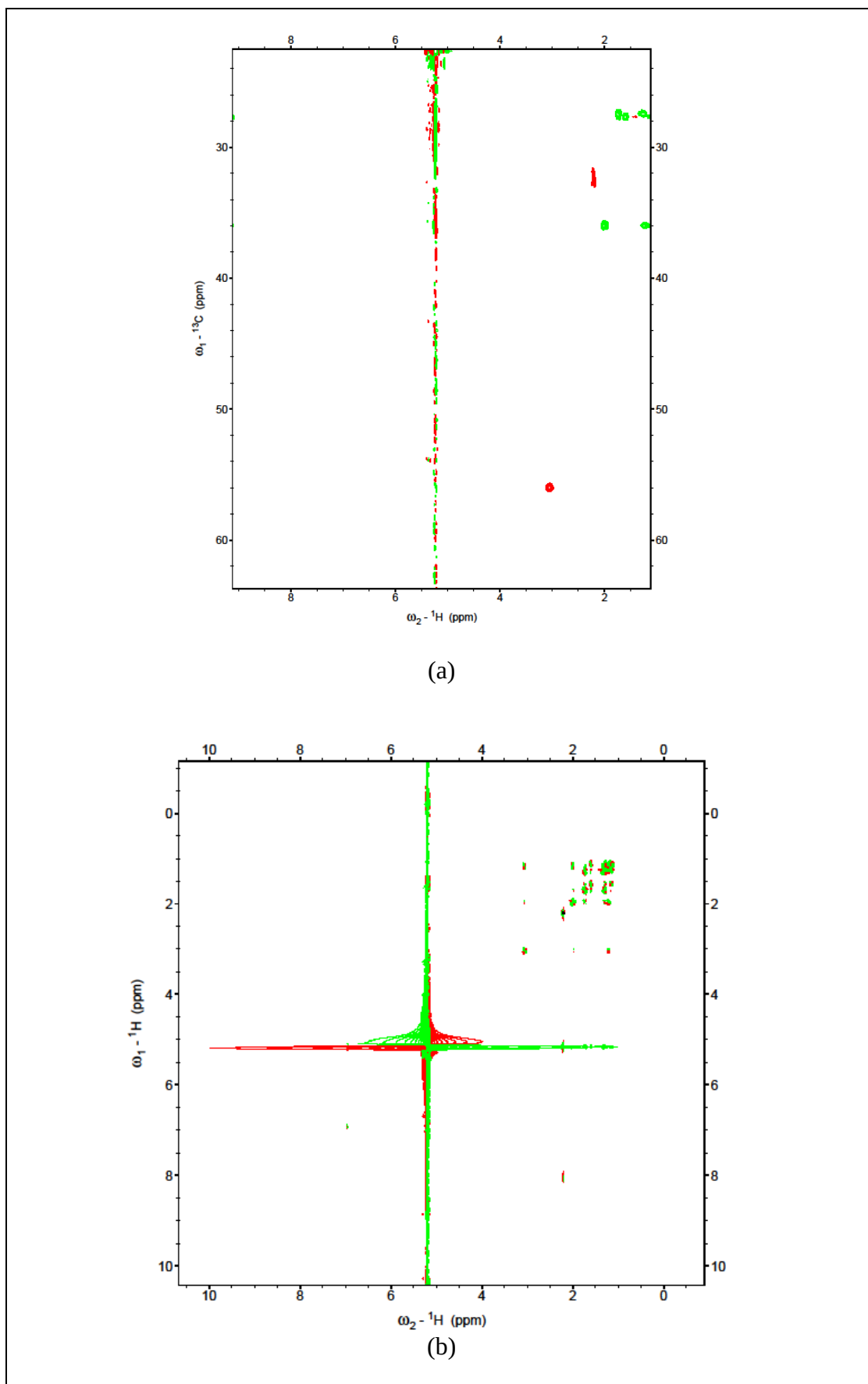
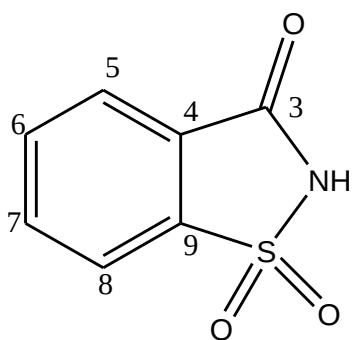
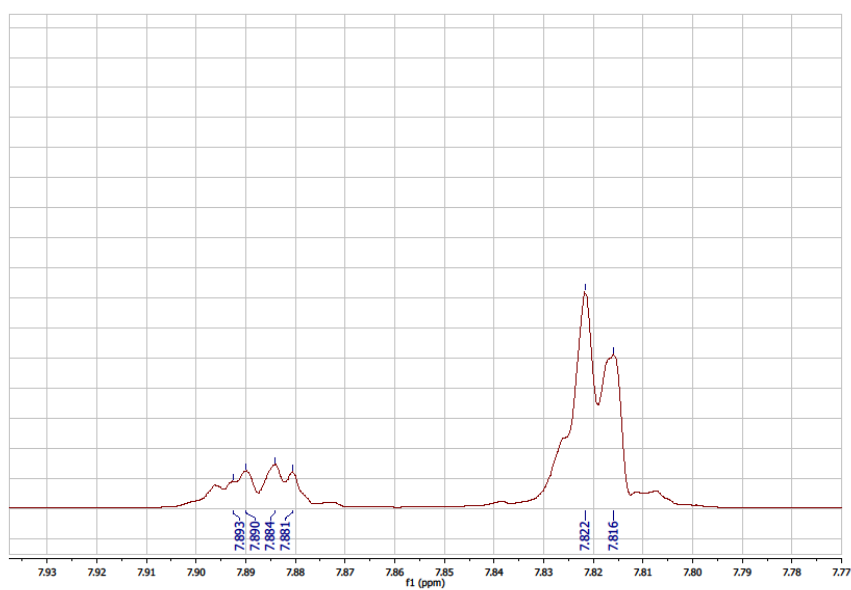


Figure A.4 : Spectra of sodium cyclamate (a)HSQC spectrum. (b)DQF-COSY spectrum.



(a)



(b)

Figure A.5 : Spectra of saccharin (a)Structure. (b) ^1H spectrum.

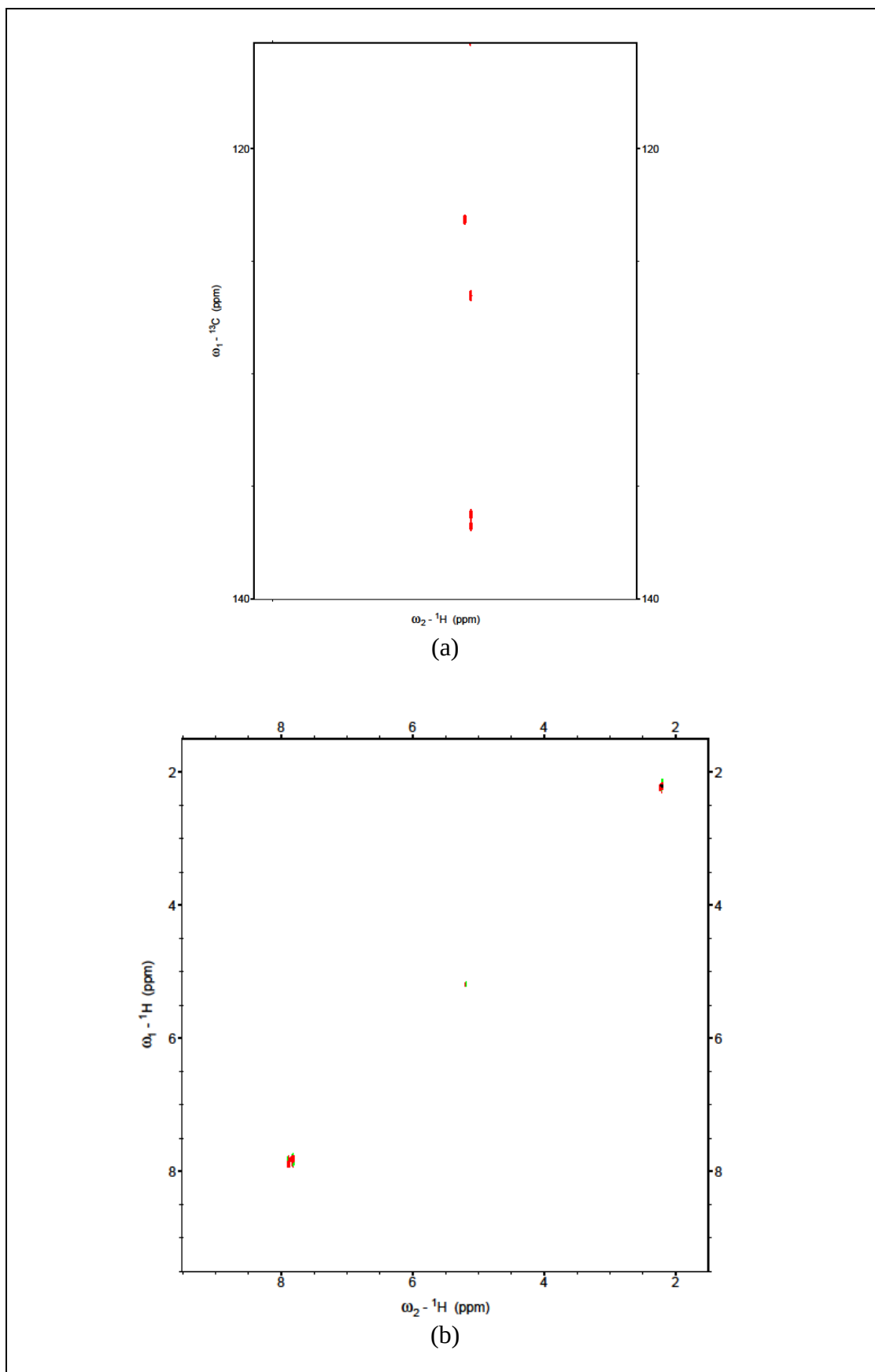
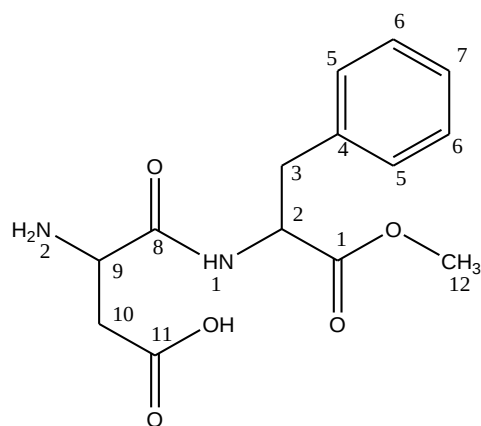
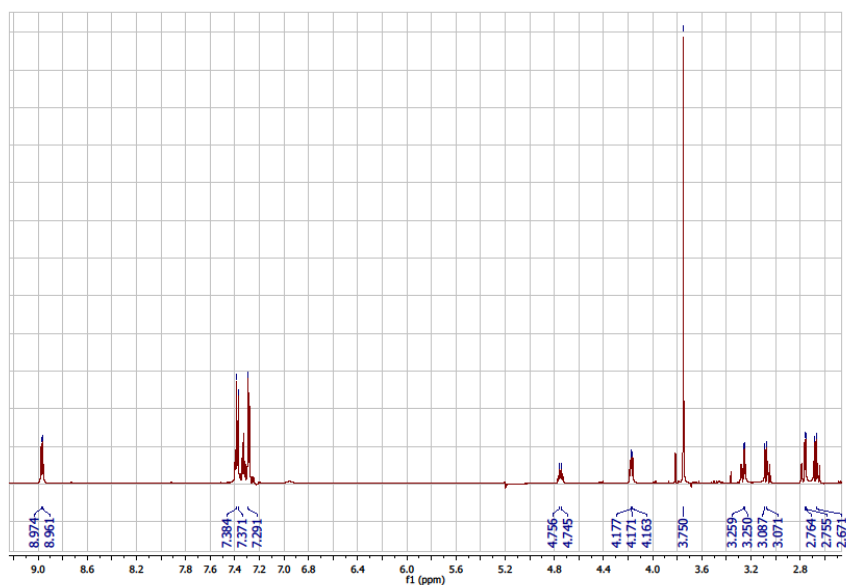


Figure A.6 : Spectra of saccharin (a)HSQC spectrum. (b)NOESY spectrum.



(a)



(b)

Figure A.7 : Spectra of aspartame (a)Structure. (b)¹H spectrum.

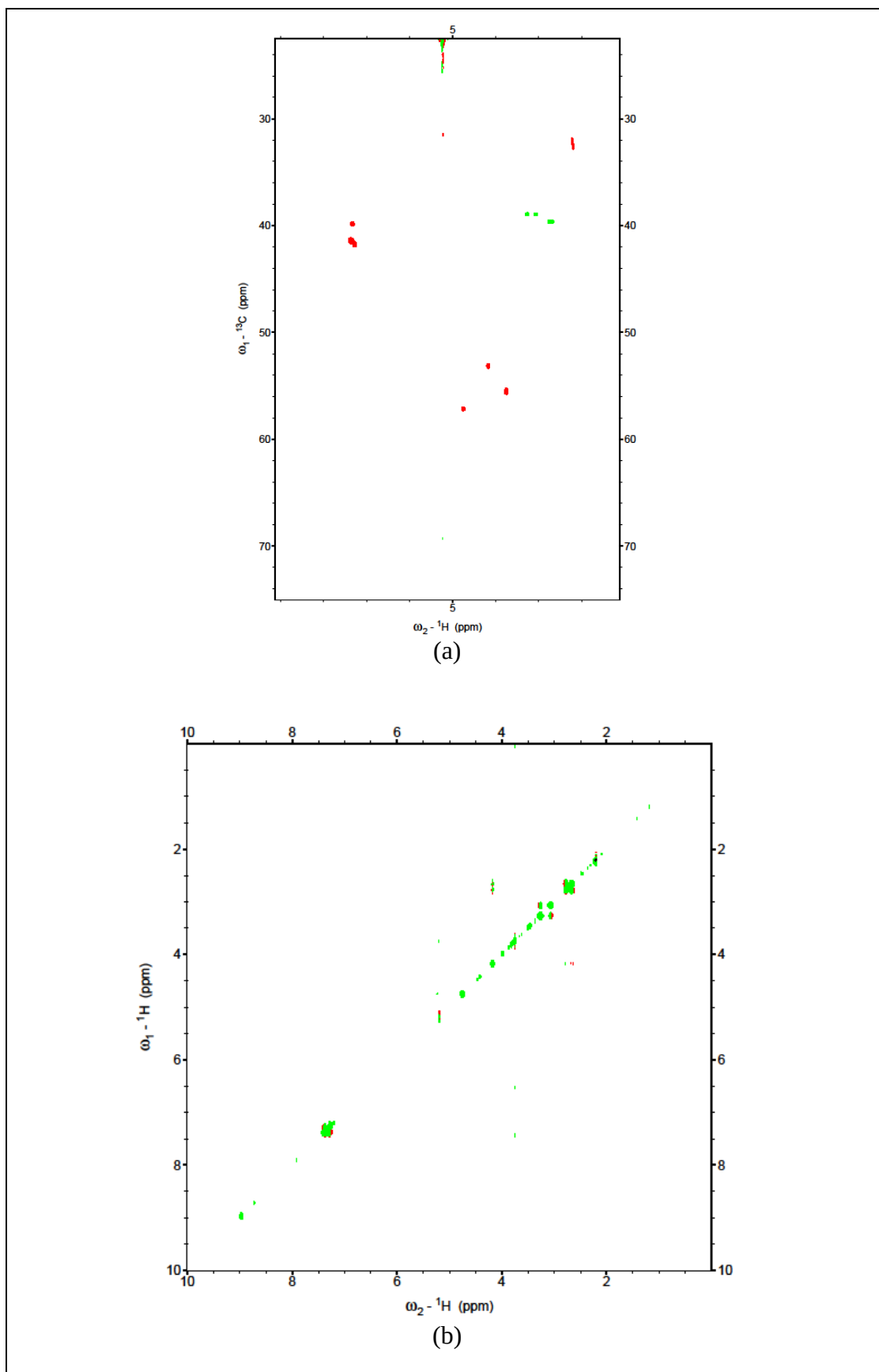
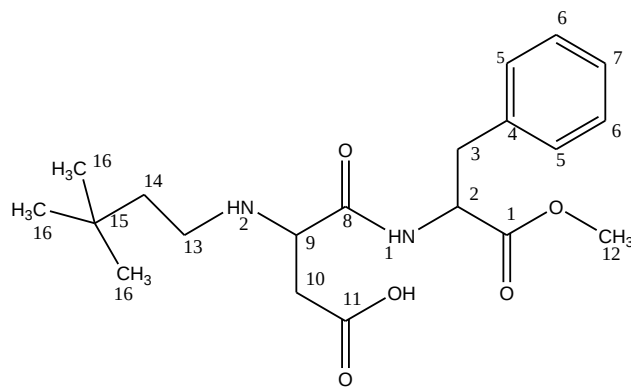
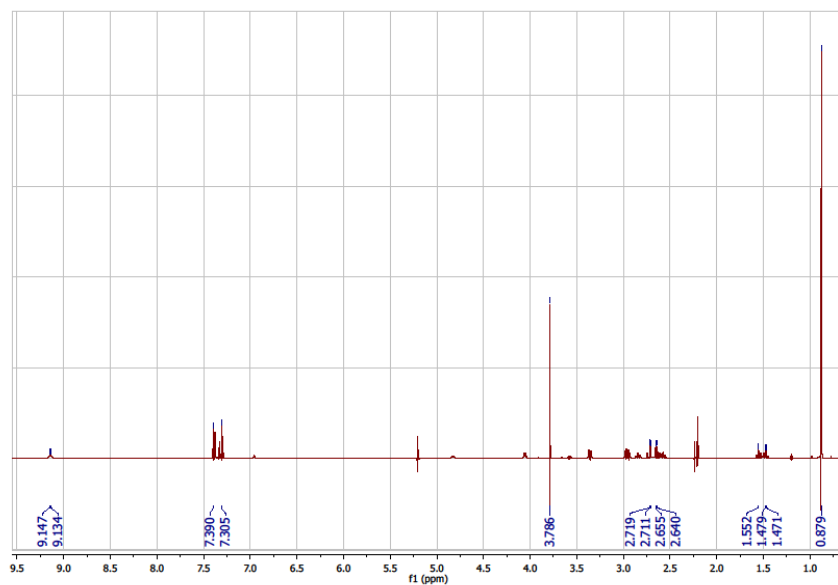


Figure A.8 : Spectra of aspartame (a)HSQC spectrum. (b)NOESY spectrum.



(a)



(b)

Figure A.9 : Spectra of neotame (a)Structure. (b)¹H spectrum.

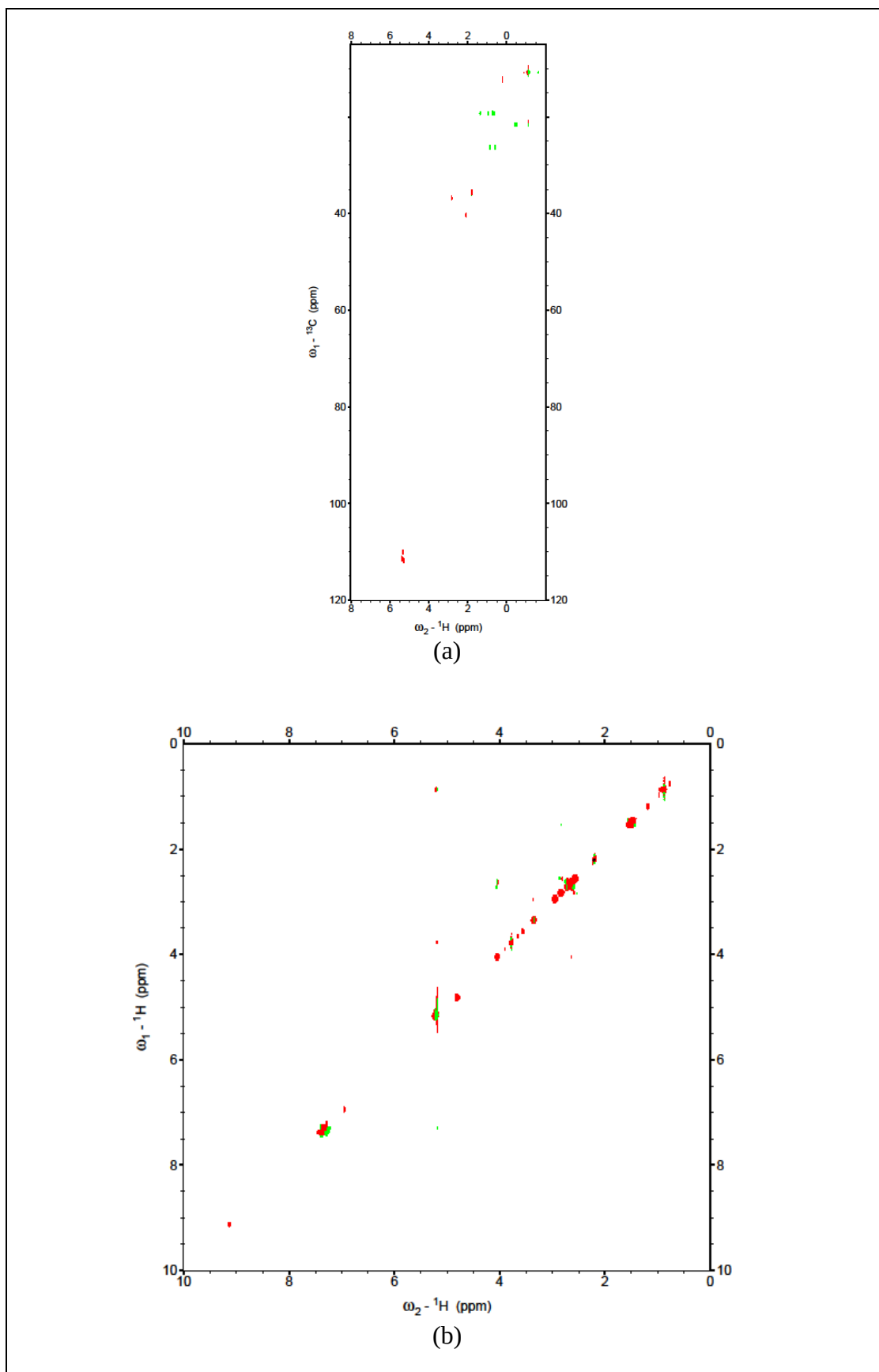


Figure A.10 : Spectra of neotame (a)HSQC spectrum. (b)NOESY spectrum.

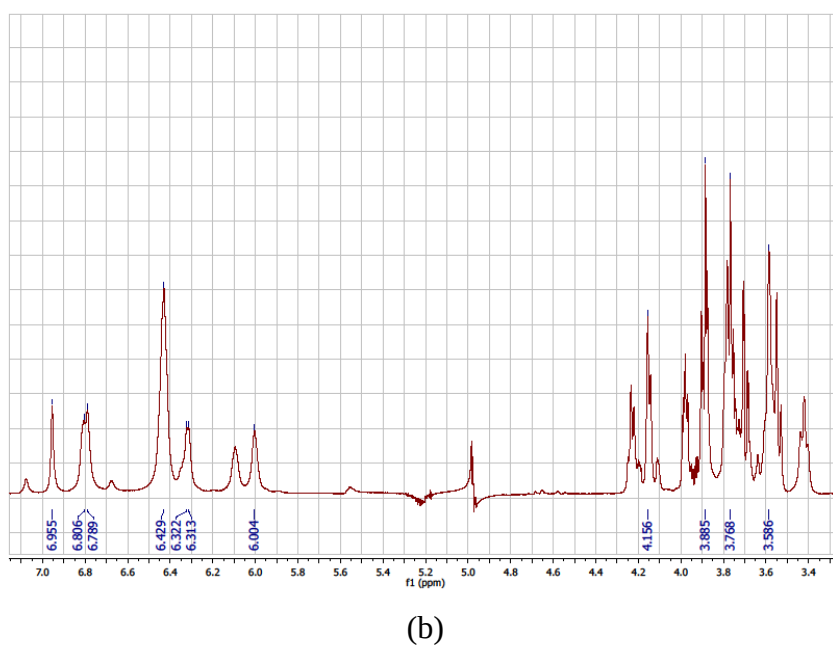
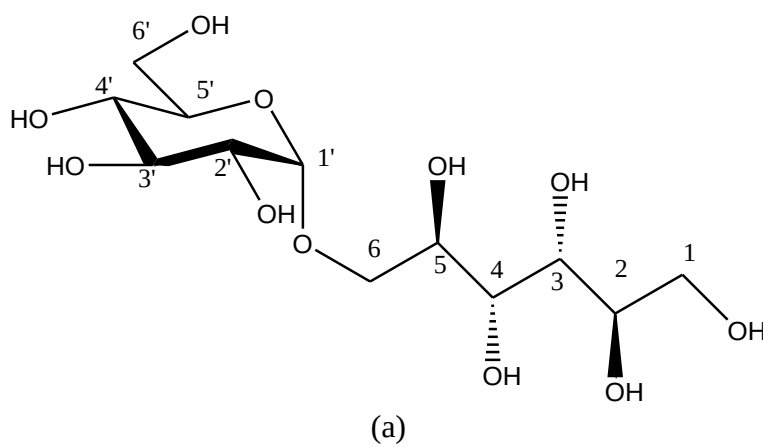


Figure A.11 : Spectra of isomalt (a)Structure. (b) ^1H spectrum.

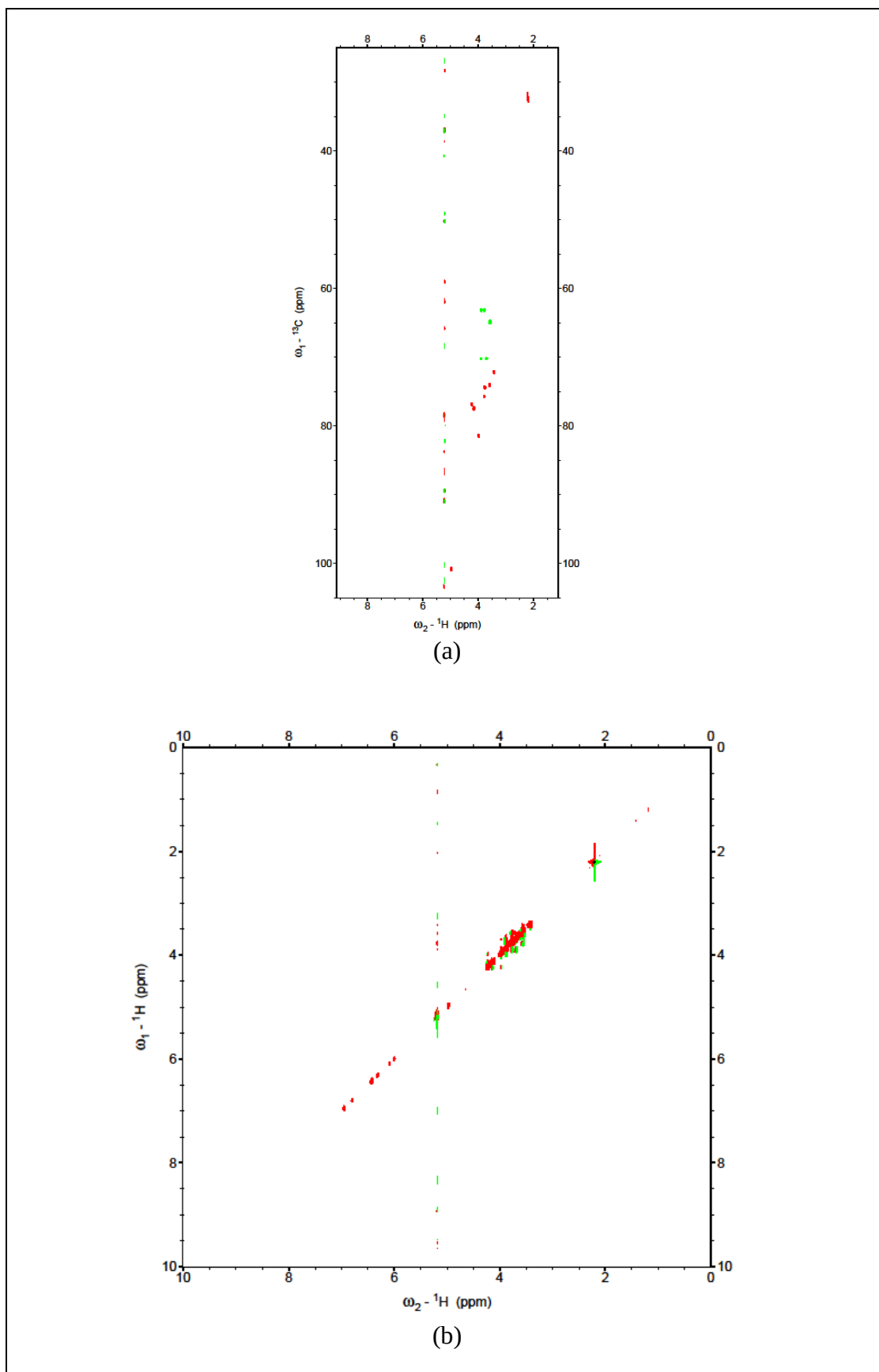
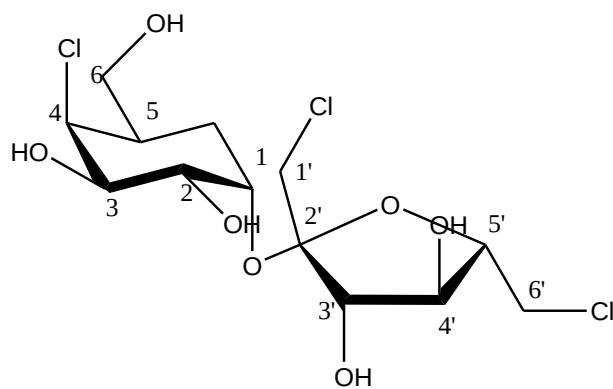
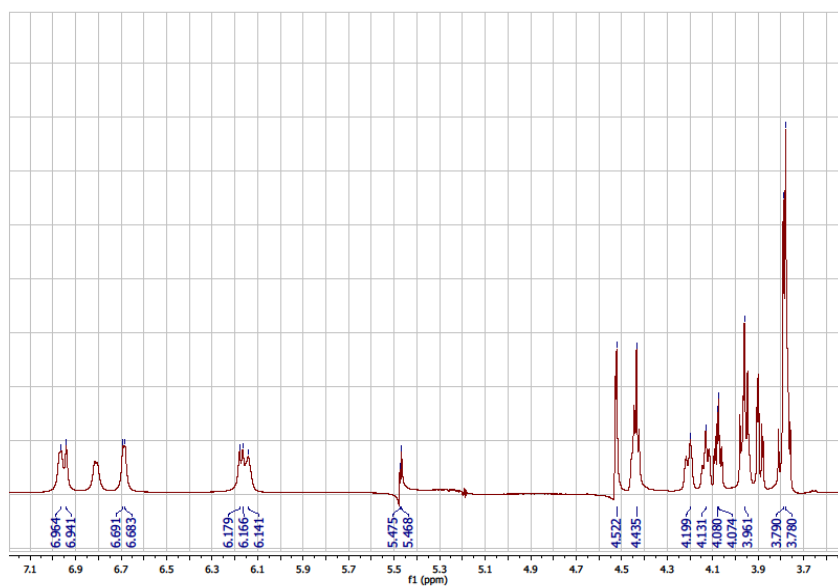


Figure A.12 : Spectra of isomalt (a)HSQC spectrum. (b)NOESY spectrum.



(a)



(b)

Figure A.13 : Spectra of sucralose (a)Structure. (b) ^1H spectrum.

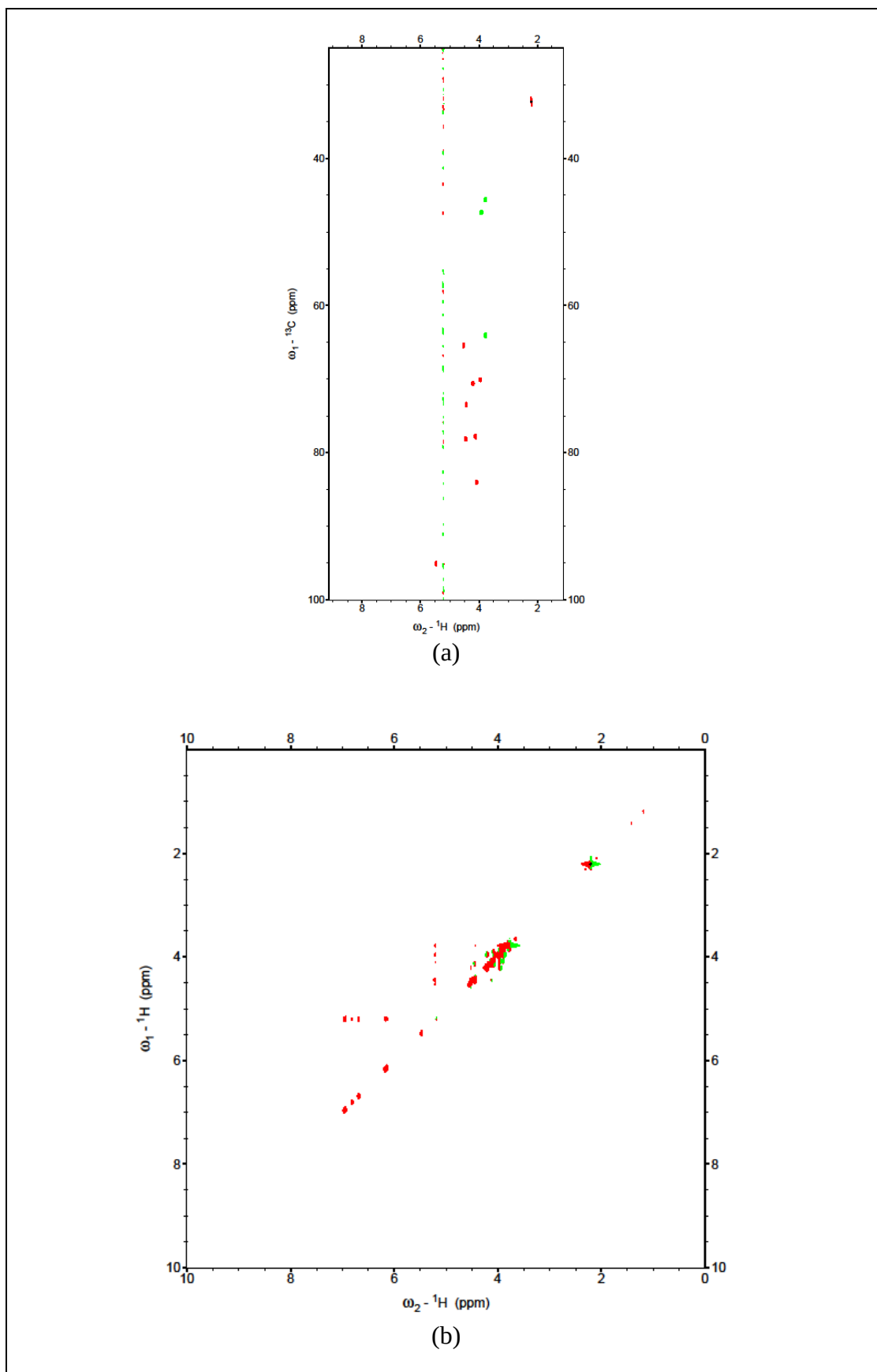
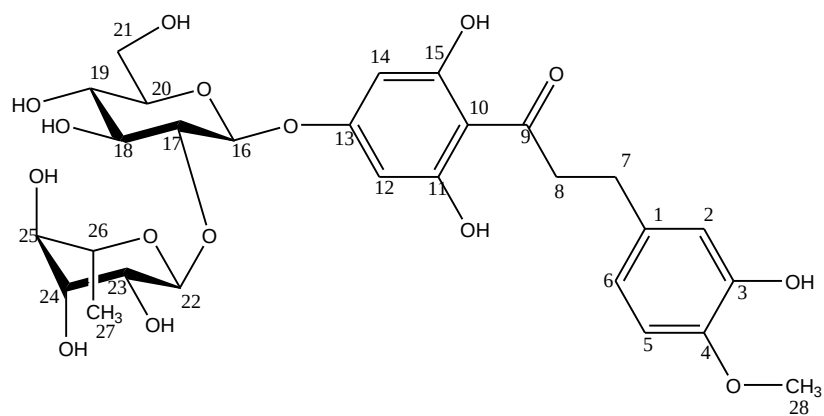
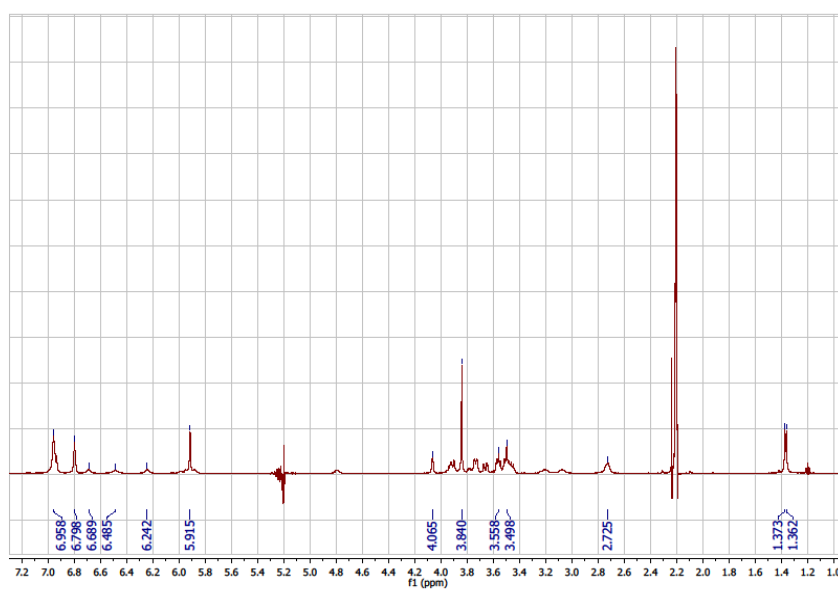


Figure A.14 : Spectra of sucralose (a)HSQC spectrum. (b)NOESY spectrum.



(a)



(b)

Figure A.15 : Spectra of NHDC (a)Structure. (b) ^1H spectrum.

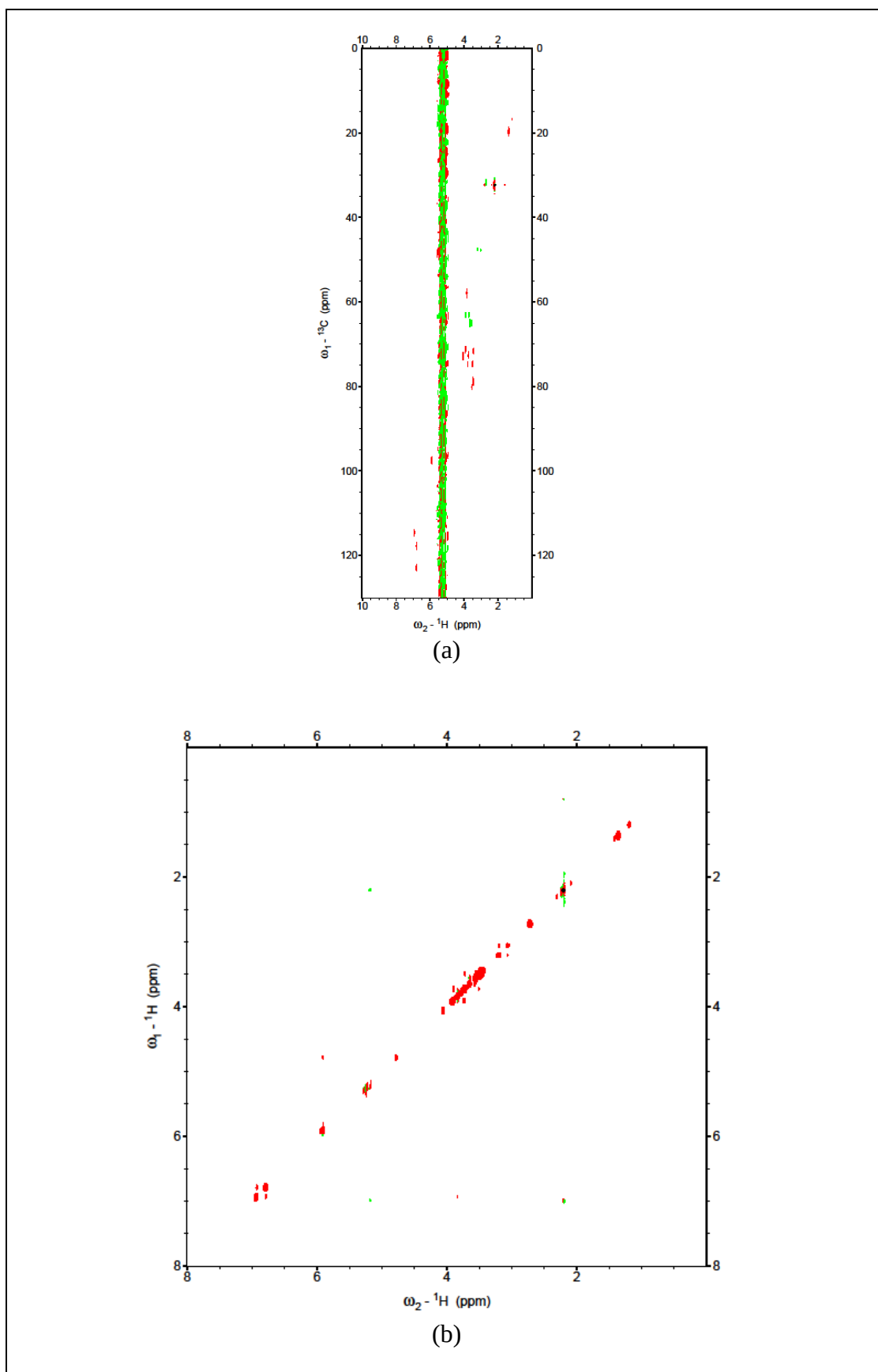


Figure A.16 : Spectra of NHDC (a) HSQC spectrum. (b)NOESY spectrum.

Table A.1 : Script used for processing of NOESY spectra.

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nmrPipe -fn ZF -size 4096 \
nmrPipe -fn FT \
nmrPipe -fn PS -p0 -1.0 -p1 -14.4 -di -verb \
nmrPipe -fn TP \
nmrPipe -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5 \
nmrPipe -fn ZF -size 1024 \
nmrPipe -fn FT \
nmrPipe -fn PS -p0 -134.4 -p1 4.7 -di -verb \
nmrPipe -fn TP \
nmrPipe -fn POLY -auto -xn 50% \
nmrPipe -fn POLY -auto -x1 50% \
-ov -out noesy.ft2

Table A.2 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of acesulfame potassium/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.063	-	-	0.001
C(2)H	3.644 ^a	-	-	-0.003
C(3)H	3.937	-	-	0.003
C(4)H	3.622	-	-	0.004
C(5)H	3.824	-	-	0.006
C(6)H	3.907 ^b	-	-	0.008
O(2)H	6.199	6.2	-7.28	-0.021
O(3)H	6.558	^c	-8.54	-0.011
O(6)H	6.066	^c	-12.20	-0.026
Acesulfame potassium				
C(4)H	-	-	-	-
C(5)H	5.664	-	-	-0.002
C(6)H	-	-	-	-
C(6')H	2.121	-	-	0.001

^a Chemical shifts are obtained from TOCSY spectrum.^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.^c Parameters that cannot be obtained/calculated from spectra.

Table A.3 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of acesulfame potassium/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.071	-	-	-0.001
C(2)H	3.655	-	-	0.001
C(3)H	3.912	-	-	0.008
C(4)H	3.608	-	-	0.004
C(5)H	3.800	-	-	0.021
C(6)H	3.887 ^{a,b}	-	-	-0.002
O(2)H	6.377	5.8	-6.86	-0.023
O(3)H	6.694	^c	-8.26	-0.024
O(6)H	6.015	^c	-11.86	-0.051
Acesulfame potassium				
C(4)H	-	-	-	-
C(5)H	5.658	-	-	-0.008
C(6)H	-	-	-	-
C(6')H	2.122	-	-	0.002

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.4 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of acesulfame potassium/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.111	-	-	0.003
C(2)H	3.663	-	-	-0.006
C(3)H	3.920	-	-	0.007
C(4)H	3.610	-	-	0.001
C(5)H	3.840	-	-	0.012
C(6)H	3.885 ^a	-	-	0.003
O(2)H	6.412	5.9	-7.10	-0.023
O(3)H	6.703	^b	-8.28	-0.012
O(6)H	5.974	^b	-12.08	-0.031
Acesulfame potassium				
C(4)H	-	-	-	-
C(5)H	5.652	-	-	-0.014
C(6)H	-	-	-	-
C(6')H	2.121	-	-	0.001

^a For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^b Parameters that cannot be obtained/calculated from spectra.

Table A.5 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sodium cyclamate/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.063	-	-	0.001
C(2)H	3.646	-	-	-0.001
C(3)H	3.940	-	-	0.006
C(4)H	3.618	-	-	0.000
C(5)H	3.812	-	-	-0.006
C(6)H	3.906 ^b	-	-	0.007
O(2)H	6.174	6.8	-7.06	-0.046
O(3)H	6.562	^c	-8.38	-0.007
O(6)H	6.060	^c	-11.98	-0.032
Sodium cyclamate				
C(4)H	3.072	-	-	0.010
C(5)Ha	1.213 ^a	-	-	-0.003
C(5)He	2.019	-	-	0.025
C(6)Ha	1.303 ^a	-	-	0.019
C(6)He	1.746	-	-	0.016
C(7)Ha	1.183 ^a	-	-	0.025
C(7)He	1.605	-	-	0.014

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.6 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sodium cyclamate/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.074	-	-	0.002
C(2)H	3.650	-	-	-0.004
C(3)H	3.917	-	-	0.013
C(4)H	3.603	-	-	-0.001
C(5)H	3.795	-	-	0.016
C(6)H	3.899 ^b	-	-	0.010
O(2)H	6.352	6.6	-6.90	-0.048
O(3)H	6.726	^c	-8.32	0.008
O(6)H	5.993	^c	-11.62	-0.073
Sodium cyclamate				
C(4)H	3.076	-	-	0.014
C(5)Ha	1.213 ^a	-	-	-0.003
C(5)He	2.017	-	-	0.023
C(6)Ha	1.303 ^a	-	-	0.019
C(6)He	1.750	-	-	0.020
C(7)Ha	1.184 ^a	-	-	0.026
C(7)He	1.604	-	-	0.013

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.7 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sodium cyclamate/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.113	-	-	0.005
C(2)H	3.664	-	-	-0.005
C(3)H	3.920	-	-	0.007
C(4)H	3.610	-	-	0.001
C(5)H	3.838	-	-	0.010
C(6)H	3.888 ^b	-	-	0.006
O(2)H	6.416	4.9	-7.14	-0.019
O(3)H	6.698	^c	-8.06	-0.017
O(6)H	5.979	^c	-12.12	-0.026
Sodium cyclamate				
C(4)H	3.062	-	-	0.000
C(5)Ha	1.195 ^a	-	-	-0.021
C(5)He	1.999	-	-	0.005
C(6)Ha	1.291 ^a	-	-	0.007
C(6)He	1.731	-	-	0.001
C(7)Ha	1.179 ^a	-	-	0.021
C(7)He	1.592	-	-	0.001

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.8 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of saccharin/ α -CD inclusion complex.

Atom	^{13}C δ (ppm) ^b	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD					
C(1)H	104.282	5.064	-	-	0.002
C(2)H	74.336	3.646 ^a	-	-	-0.001
C(3)H	76.140	3.935	-	-	0.001
C(4)H	83.833	3.617	-	-	-0.001
C(5)H	74.535	3.822 ^a	-	-	0.004
C(6)H	62.812	3.908 ^{a,c}	-	-	0.009
O(2)H	-	6.218	6.8	-7.84	-0.002
O(3)H	-	6.568	^d	-8.88	-0.001
O(6)H	-	6.085	^d	-12.52	-0.007
Saccharin					
C(5)H	126.250	7.825	-	-	0.002
C(6)H	136.488	7.819	-	-	0.002
C(7)H	135.962	7.821	-	-	0.002
C(8)H	122.894	7.888	-	-	0.000

^a Chemical shifts are obtained from HSQC spectrum.

^b ^{13}C chemical shifts are obtained from HSQC spectrum.

^c For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^d Parameters that cannot be obtained/calculated from spectra.

Table A.9 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of saccharin/ β -CD inclusion complex.

Atom	^{13}C δ (ppm) ^b	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD					
C(1)H	104.707	5.057	-	-	-0.015
C(2)H	74.725	3.647 ^a	-	-	-0.007
C(3)H	75.970	3.869 ^a	-	-	-0.035
C(4)H	83.448	3.604	-	-	0.000
C(5)H	74.254	3.739	-	-	-0.040
C(6)H	62.535	3.868 ^{a,c}	-	-	-0.021
O(2)H	-	6.379	5.5	-6.70	-0.021
O(3)H	-	6.700	^d	-8.62	-0.018
O(6)H	-	5.975	^d	-10.86	-0.091
Saccharin					
C(5)H	126.241	7.840	-	-	0.016
C(6)H	136.389	7.831	-	-	0.014
C(7)H	135.908	7.836	-	-	0.017
C(8)H	122.777	7.880	-	-	-0.008

^a Chemical shifts are obtained from HSQC spectrum.

^b ^{13}C chemical shifts are obtained from HSQC spectrum.

^c For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^d Parameters that cannot be obtained/calculated from spectra.

Table A.10 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of saccharin/ γ -CD inclusion complex.

Atom	^{13}C δ (ppm) ^b	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD					
C(1)H	104.679	5.100	-	-	-0.008
C(2)H	75.033	3.661 ^a	-	-	-0.008
C(3)H	75.688	3.906	-	-	-0.007
C(4)H	83.137	3.605	-	-	-0.004
C(5)H	74.266	3.820	-	-	-0.008
C(6)H	62.636	3.881 ^{a,c}	-	-	-0.001
O(2)H	-	6.406	6.4	-7.04	-0.029
O(3)H	-	6.714	^d	-8.80	-0.001
O(6)H	-	5.959	^d	-11.88	-0.046
Saccharin					
C(5)H	126.259	7.776	-	-	-0.047
C(6)H	136.318	7.804	-	-	-0.013
C(7)H	135.856	7.816	-	-	-0.003
C(8)H	122.803	7.837	-	-	-0.051

^a Chemical shifts are obtained from HSQC spectrum.

^b ^{13}C chemical shifts are obtained from HSQC spectrum.

^c For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^d Parameters that cannot be obtained/calculated from spectra.

Table A.11 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of aspartame/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.063	-	-	0.001
C(2)H	3.653	-	-	0.006
C(3)H	3.932	-	-	-0.002
C(4)H	3.616	-	-	-0.002
C(5)H	3.823	-	-	0.005
C(6)H	3.910 ^a	-	-	0.011
O(2)H	6.217	5.7	-7.62	-0.003
O(3)H	6.560	^b	-8.54	-0.009
O(6)H	6.084	^b	^b	-0.008
Aspartame				
C(2)H	4.753	-	-	0.002
C(3)Ha	3.276	-	-	0.009
C(3)Hb	3.071	-	-	0.004
C(5)H	7.292	-	-	0.008
C(6)H	7.393	-	-	0.009
C(7)H	7.327	-	-	0.002
C(9)H	4.181	-	-	0.007
C(10)Ha	2.779	-	-	0.005
C(10)Hb	2.666	-	-	0.003
C(12)H	3.752	-	-	0.002
N(1)H	8.968	7.9	-4.18	0.001
N(2)H	8.254	^b	^b	0.018

^aFor each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^b Parameters that cannot be obtained/calculated from spectra.

Table A.12 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of aspartame/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.081	-	-	0.009
C(2)H	3.670	-	-	0.016
C(3)H	3.923	-	-	0.019
C(4)H	3.618	-	-	0.014
C(5)H	3.740 ^a	-	-	-0.039
C(6)H	3.882 ^b	-	-	-0.007
O(2)H	6.412	3.4	-7.60	0.012
O(3)H	6.743	^c	-9.16	0.025
O(6)H	6.040	^c	^c	-0.026
Aspartame				
C(2)H	4.753	-	-	0.002
C(3)Ha	3.259	-	-	-0.008
C(3)Hb	3.102	-	-	0.035
C(5)H	7.286	-	-	0.002
C(6)H	7.390	-	-	0.006
C(7)H	7.336	-	-	0.011
C(9)H	4.208	-	-	0.034
C(10)Ha	2.793	-	-	0.019
C(10)Hb	2.682	-	-	0.019
C(12)H	3.749	-	-	-0.001
N(1)H	9.020	7.8	-4.86	0.053
N(2)H	8.261	^c	^c	0.025

^a Chemical shifts are obtained from NOESY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.13 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of aspartame/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.103	-	-	-0.005
C(2)H	3.663	-	-	-0.006
C(3)H	3.905	-	-	-0.008
C(4)H	3.605	-	-	-0.004
C(5)H	3.819	-	-	-0.009
C(6)H	3.887 ^a	-	-	0.005
O(2)H	6.424	4.5	-7.36	-0.011
O(3)H	6.718	^b	-8.58	0.003
O(6)H	5.986	^b	^b	-0.019
Aspartame				
C(2)H	4.762	-	-	0.011
C(3)Ha	3.245	-	-	-0.022
C(3)Hb	3.096	-	-	0.029
C(5)H	7.273	-	-	-0.011
C(6)H	7.382	-	-	-0.002
C(7)H	7.325	-	-	0.000
C(9)H	4.206	-	-	0.032
C(10)Ha	2.791	-	-	0.017
C(10)Hb	2.678	-	-	0.015
C(12)H	3.738	-	-	-0.012
N(1)H	8.969	7.7	-4.00	0.002
N(2)H	8.267	^b	^b	0.031

^aFor each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^b Parameters that cannot be obtained/calculated from spectra.

Table A.14 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of neotame/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.066	-	-	0.004
C(2)H	3.638 ^a	-	-	-0.009
C(3)H	3.938	-	-	0.004
C(4)H	3.619	-	-	0.001
C(5)H	3.810 ^a	-	-	-0.008
C(6)H	3.909 ^b	-	-	0.010
O(2)H	6.220	^c	-7.64	0.000
O(3)H	6.560	^c	-9.12	-0.009
O(6)H	^c	^c	^c	^c
Neotame				
C(2)H	4.827	-	-	0.002
C(3)Ha	2.960	-	-	0.003
C(3)Hb	3.361	-	-	0.004
C(5)H	7.303	-	-	0.005
C(6)H	7.394	-	-	0.007
C(7)H	7.329	-	-	0.006
C(9)H	4.078	-	-	0.000
C(10)Ha	2.648	-	-	0.006
C(10)Hb	2.743	-	-	0.008
C(12)H	3.788	-	-	-0.002
C(13)Ha	2.595	-	-	-0.002
C(13)Hb	2.857	-	-	0.001
C(14)Ha	1.560	-	-	0.003
C(14)Hb	1.490	-	-	0.002
C(16)H	0.887	-	-	0.008
N(1)H	9.150	8.6	-4.83	0.009

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.15 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of neotame/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.084	-	-	0.012
C(2)H	3.672	-	-	0.018
C(3)H	3.919	-	-	0.015
C(4)H	3.617	-	-	0.013
C(5)H	3.756 ^a	-	-	-0.023
C(6)H	3.891 ^b	-	-	0.002
O(2)H	6.414	^c	-7.22	0.014
O(3)H	6.714	^c	-8.58	-0.004
O(6)H	^c	^c	^c	^c
Neotame				
C(2)H	4.829	-	-	0.004
C(3)Ha	2.982	-	-	0.025
C(3)Hb	3.355	-	-	-0.002
C(5)H	7.307	-	-	0.009
C(6)H	7.399	-	-	0.012
C(7)H	7.336	-	-	0.013
C(9)H	4.093	-	-	0.015
C(10)Ha	2.654	-	-	0.012
C(10)Hb	2.746	-	-	0.011
C(12)H	3.785	-	-	-0.005
C(13)Ha	2.621 ^a	-	-	0.024
C(13)Hb	2.863 ^a	-	-	0.007
C(14)Ha	1.566	-	-	0.009
C(14)Hb	1.506	-	-	0.018
C(16)H	0.893	-	-	0.014
N(1)H	9.156	8.6	-4.44	0.015

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.16 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of neotame/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.109	-	-	0.001
C(2)H	3.666	-	-	-0.003
C(3)H	3.910	-	-	-0.003
C(4)H	3.608	-	-	-0.001
C(5)H	3.819	-	-	-0.009
C(6)H	3.886 ^b	-	-	0.004
O(2)H	6.422	^c	-7.34	-0.013
O(3)H	6.700	^c	-8.46	-0.015
O(6)H	^c	^c	^c	^c
Neotame				
C(2)H	4.839	-	-	0.014
C(3)Ha	2.978	-	-	0.021
C(3)Hb	3.348	-	-	-0.009
C(5)H	7.300 ^a	-	-	0.002
C(6)H	7.388	-	-	0.001
C(7)H	7.320	-	-	-0.003
C(9)H	4.095	-	-	0.017
C(10)Ha	2.654	-	-	0.012
C(10)Hb	2.749	-	-	0.014
C(12)H	3.780	-	-	-0.010
C(13)Ha	2.633 ^a	-	-	0.036
C(13)Hb	2.866	-	-	0.010
C(14)Ha	1.563	-	-	0.006
C(14)Hb	1.495	-	-	0.007
C(16)H	0.887	-	-	0.008
N(1)H	9.153	8.5	-4.38	0.012

^a Chemical shifts are obtained from TOCSY spectrum.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.17 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of isomalt/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.063	-	-	0.001
C(2)H	3.645 ^a	-	-	-0.002
C(3)H	3.933	-	-	-0.001
C(4)H	3.616	-	-	-0.002
C(5)H	3.820	-	-	0.002
C(6)H	3.910 ^d	-	-	0.011
O(2)H	6.211	7.4	-7.52	-0.009
O(3)H	6.559	^e	-8.44	-0.010
O(6)H	6.075	^e	-12.26	-0.017
Isomalt				
C(1)H	3.564 ^a	-	-	0.005
C(2)H	^c	-	-	^c
C(3)H	4.147	-	-	-0.002
C(4)H	4.227	-	-	-0.007
C(5)H	3.976	-	-	-0.002
C(6)Ha	3.692	-	-	-0.003
C(6)Hb	3.885 ^a	-	-	-0.004
C(1')H	4.976	-	-	-0.004
C(2')H	3.573 ^b	-	-	-0.006
C(3')H	3.778 ^a	-	-	0.009
C(4')H	3.471	-	-	0.051
C(5')H	3.757 ^a	-	-	0.006
C(6')Ha	3.767 ^b	-	-	-0.011
C(6')Hb	3.881 ^b	-	-	0.003
O(1)H	6.089 ^a	^e	^e	-0.006
O(2)H	6.774	^e	-10.44	-0.015
O(3)H	6.432 ^a	^e	^e	-0.005
O(4)H	6.799	^e	-10.24	-0.006
O(5)H	6.420	^e	^e	-0.010
O(2')H	6.306	^e	-11.7	-0.012
O(3')H	6.420	^e	^e	-0.010
O(4')H	6.420	^e	^e	-0.010
O(6')H	5.990	^e	-12.18	-0.014

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Since peaks are overlapped with CD signals they cannot be detected.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.18 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of isomalt/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.072	-	-	0.000
C(2)H	3.657	-	-	0.003
C(3)H	3.904	-	-	0.000
C(4)H	3.602	-	-	-0.002
C(5)H	3.773 ^a	-	-	-0.006
C(6)H	3.889 ^{a,d}	-	-	0.000
O(2)H	6.386 ^b	^e	^e	-0.014
O(3)H	6.696	^e	-8.16	-0.022
O(6)H	6.033	^e	-12.28	-0.033
Isomalt				
C(1)H	3.565 ^a	-	-	0.006
C(2)H	^c	-	-	^c
C(3)H	4.148	-	-	-0.001
C(4)H	4.228	-	-	-0.006
C(5)H	3.977	-	-	-0.001
C(6)Ha	3.692	-	-	-0.003
C(6)Hb	3.884 ^a	-	-	-0.005
C(1')H	4.977	-	-	-0.003
C(2')H	3.578 ^a	-	-	-0.001
C(3')H	3.780 ^a	-	-	0.011
C(4')H	3.417	-	-	-0.003
C(5')H	3.755 ^a	-	-	0.004
C(6')Ha	3.778 ^a	-	-	0.000
C(6')Hb	^c	-	-	^c
O(1)H	6.079	^e	-11.0	-0.016
O(2)H	6.766	^e	-10.42	-0.023
O(3)H	6.420 ^a	^e	^e	-0.017
O(4)H	6.792	^e	-10.32	-0.013
O(5)H	6.410 ^a	^e	n/a	-0.020
O(2')H	6.301	5.9	-11.34	-0.017
O(3')H	6.410 ^a	^e	^e	-0.020
O(4')H	6.410 ^a	^e	^e	-0.020
O(6')H	5.984	^e	-11.88	-0.020

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Since peaks are overlapped with CD signals they cannot be detected.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.19 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of isomalt/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.110	-	-	0.002
C(2)H	3.667 ^a	-	-	-0.002
C(3)H	3.918	-	-	0.005
C(4)H	3.610	-	-	0.001
C(5)H	3.836	-	-	0.008
C(6)H	3.887 ^{b,d}	-	-	0.005
O(2)H	6.420	6.3	-7.26	-0.015
O(3)H	6.697	^e	-8.14	-0.018
O(6)H	5.983	^e	-12.26	-0.022
Isomalt				
C(1)H	3.567	-	-	0.008
C(2)H	^c	-	-	^c
C(3)H	4.147	-	-	-0.002
C(4)H	4.227	-	-	-0.007
C(5)H	3.977	-	-	-0.001
C(6)Ha	3.694 ^a	-	-	-0.001
C(6)Hb	3.884 ^a	-	-	-0.005
C(1')H	4.977	-	-	-0.003
C(2')H	3.587 ^a	-	-	0.008
C(3')H	3.778 ^a	-	-	0.009
C(4')H	3.417	-	-	-0.003
C(5')H	3.758 ^a	-	-	0.007
C(6')Ha	3.771 ^a	-	-	-0.007
C(6')Hb	3.883 ^a	-	-	0.005
O(1)H	6.084	^e	-11.30	-0.011
O(2)H	6.770	^e	-10.60	-0.019
O(3)H	6.423 ^a	^e	n/a	-0.014
O(4)H	6.796	^e	-10.46	-0.009
O(5)H	6.418 ^a	^e	n/a	-0.012
O(2')H	6.304	3.8	-11.50	-0.014
O(3')H	6.418 ^a	^e	^e	-0.012
O(4')H	6.418 ^a	^e	^e	-0.012
O(6')H	^c	^c	^c	^c

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Since peaks are overlapped with CD signals they cannot be detected.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.20 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sucralose/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.064	-	-	0.002
C(2)H	3.647 ^a	-	-	0.000
C(3)H	3.935	-	-	0.001
C(4)H	3.624	-	-	0.006
C(5)H	3.814	-	-	-0.004
C(6)H	3.908 ^{a,d}	-	-	0.009
O(2)H	6.194	6.7	-7.06	-0.026
O(3)H	6.545	^e	-8.16	-0.024
O(6)H	6.062	^e	-12.02	-0.030
Sucralose				
C(1')H	3.767 ^{b,c}	-	-	-0.019
C(3')H	4.449 ^a	-	-	-0.001
C(4')H	4.126	-	-	-0.004
C(5')H	4.065	-	-	-0.012
C(6')Ha	3.896 ^b	-	-	0.005
C(6')Hb	3.972 ^a	-	-	0.008
C(1)H	5.469	-	-	-0.004
C(2)H	3.951 ^a	-	-	-0.010
C(3)H	4.208	-	-	0.000
C(4)H	4.525	-	-	0.000
C(5)H	4.426 ^a	-	-	-0.011
C(6)H	3.778 ^{b,c}	-	-	-0.001
O(3')H	6.798	5.9	-12.08	-0.012
O(4')H	6.957	^e	-10.34	-0.007
O(2)H	6.159	7.8	-10.96	-0.014
O(3)H	6.669	^e	-11.94	-0.018
O(6)H	6.121	^e	-10.46	-0.020

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Chemical shifts are overlapped signals in NOESY spectrum.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.21 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sucralose/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.071	-	-	-0.001
C(2)H	3.656	-	-	0.002
C(3)H	3.093	-	-	-0.811
C(4)H	3.603	-	-	-0.001
C(5)H	3.777 ^b	-	-	-0.002
C(6)H	3.891 ^d	-	-	0.002
O(2)H	6.385	5.9	-7.04	-0.015
O(3)H	6.692	^e	-8.08	-0.026
O(6)H	6.032	^e	-12.24	-0.034
Sucralose				
C(1')H	3.766 ^{b,c}	-	-	-0.020
C(3')H	4.450 ^a	-	-	0.000
C(4')H	4.130	-	-	0.000
C(5')H	4.072	-	-	-0.005
C(6')Ha	3.895 ^a	-	-	0.004
C(6')Hb	3.971 ^a	-	-	0.007
C(1)H	5.470	-	-	-0.003
C(2)H	3.956 ^a	-	-	-0.005
C(3)H	4.207	-	-	-0.001
C(4)H	4.524	-	-	-0.001
C(5)H	4.426 ^b	-	-	-0.011
C(6)H	3.784 ^{b,c}	-	-	0.005
O(3')H	6.801	6.3	-12.28	-0.009
O(4')H	6.957	^e	-10.38	-0.007
O(2)H	6.164	7.8	-11.12	-0.009
O(3)H	6.669	^e	-11.86	-0.018
O(6)H	6.133	^e	-10.96	-0.008

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Chemical shifts are overlapped signals in NOESY spectrum.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.22 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of sucralose/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.111	-	-	0.003
C(2)H	3.665	-	-	-0.004
C(3)H	3.919	-	-	0.006
C(4)H	3.609	-	-	0.000
C(5)H	3.834	-	-	0.006
C(6)H	3.888 ^{a,d}	-	-	0.006
O(2)H	6.413	5.1	-7.12	-0.022
O(3)H	6.689	^e	-7.96	-0.026
O(6)H	5.974	^e	-12.04	-0.031
Sucralose				
C(1')H	3.768 ^{b,c}	-	-	-0.018
C(3')H	4.452 ^a	-	-	0.002
C(4')H	4.130	-	-	0.000
C(5')H	4.069	-	-	-0.008
C(6')Ha	3.896 ^a	-	-	0.005
C(6')Hb	3.966	-	-	0.002
C(1)H	5.468	-	-	-0.005
C(2)H	3.964	-	-	0.003
C(3)H	4.208	-	-	0.000
C(4)H	4.526	-	-	0.001
C(5)H	4.415 ^b	-	-	-0.022
C(6)H	3.784 ^{b,c}	-	-	0.005
O(3')H	6.791	5.7	-11.94	-0.019
O(4')H	6.939 ^b	^e	-10.26	-0.025
O(2)H	6.156	7.8	-11.18	-0.017
O(3)H	6.666	^e	-11.80	-0.021
O(6)H	6.125	^e	-10.86	-0.016

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Chemical shifts are overlapped signals in NOESY spectrum.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.23 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of NHDC/ α -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
α -CD				
C(1)H	5.063	-	-	0.001
C(2)H	3.652	-	-	0.005
C(3)H	3.934	-	-	0.000
C(4)H	3.617	-	-	-0.001
C(5)H	3.822	-	-	0.004
C(6)H	3.910 ^b	-	-	0.011
O(2)H	6.215	^c	-7.52	-0.005
O(3)H	6.563	^c	-8.56	-0.006
O(6)H	6.078	^c	-12.42	-0.014
NHDC				
C(2)H	6.806	-	-	0.008
C(5)H	6.928	-	-	-0.005
C(6)H	6.974	-	-	0.182
C(7)H	2.805	-	-	0.080
C(8)Ha	3.212	-	-	0.133
C(8)Hb	3.302	-	-	0.101
C(12)H	^a	-	-	^a
C(14)H	^a	-	-	^a
C(16)H	^a	-	-	^a
C(17)H	^a	-	-	^a
C(18)H	^a	-	-	^a
C(19)H	^a	-	-	^a
C(20)H	^a	-	-	^a
C(21)Ha	^a	-	-	^a
C(21)Hb	^a	-	-	^a
C(22)H	^a	-	-	^a
C(23)H	4.065	-	-	0.000
C(24)H	^a	-	-	^a
C(25)H	^a	-	-	^a
C(26)H	^a	-	-	^a
C(27)H	1.351	-	-	-0.016
C(28)H	^a	-	-	^a
O(18)H	^a	^c	^c	^a
O(23)H	^a	^c	^c	^a
O(25)H	^a	^c	^c	^a

^a Since peaks are overlapped with CD signals they cannot be detected.

^b For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^c Parameters that cannot be obtained/calculated from spectra.

Table A.24 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of NHDC/ β -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
β -CD				
C(1)H	5.069	-	-	-0.003
C(2)H	3.656	-	-	0.002
C(3)H	3.923	-	-	0.019
C(4)H	3.620	-	-	0.016
C(5)H	3.723 ^b	-	-	-0.056
C(6)H	3.901 ^d	-	-	0.012
O(2)H	6.368	^e	-7.26	-0.032
O(3)H	6.683	^e	-8.76	-0.035
O(6)H	6.051	^e	-12.48	-0.015
NHDC				
C(2)H	6.786	-	-	-0.012
C(5)H	6.924	-	-	-0.009
C(6)H	6.687 ^b	-	-	-0.105
C(7)H	2.871 ^b	-	-	0.146
C(8)Ha	3.204	-	-	0.125
C(8)Hb	3.339	-	-	0.138
C(12)H	^c	-	-	^c
C(14)H	^c	-	-	^c
C(16)H	^c	-	-	^c
C(17)H	^c	-	-	^c
C(18)H	3.457 ^b	-	-	0.001
C(19)H	^c	-	-	^c
C(20)H	^c	-	-	^c
C(21)Ha	^c	-	-	^c
C(21)Hb	^c	-	-	^c
C(22)H	6.185	-	-	0.235
C(23)H	4.083	-	-	0.018
C(24)H	3.727 ^b	-	-	-0.004
C(25)H	^c	-	-	^c
C(26)H	3.921 ^b	-	-	-0.011
C(27)H	1.392	-	-	0.025
C(28)H	3.830 ^a	-	-	-0.010
O(18)H	6.432 ^b	^e	^e	-0.053
O(23)H	6.251	^e	^e	0.009
O(25)H	6.689 ^b	^e	^e	0.000

^a Chemical shifts are obtained from TOCSY spectrum.

^b Chemical shifts are obtained from NOESY spectrum.

^c Since peaks are overlapped with CD signals they cannot be detected.

^d For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons.

^e Parameters that cannot be obtained/calculated from spectra.

Table A.25 : ^1H chemical shifts (δ), $^3J_{\text{CH-OH}}$ coupling constants (J), temperature coefficients ($d\delta/dT$) and complexation induced chemical shift differences (CIS) of NHDC/ γ -CD inclusion complex.

Atom	^1H δ (ppm)	$^3J_{\text{H-OH}}$ (Hz)	$d\delta/dT$ (ppb/K)	CIS (ppm)
γ -CD				
C(1)H	5.099	-	-	-0.009
C(2)H	3.662	-	-	-0.007
C(3)H	3.900	-	-	-0.013
C(4)H	3.604	-	-	-0.005
C(5)H	3.808	-	-	-0.020
C(6)H	3.868 ^{a,c}	-	-	-0.014
O(2)H	6.414	^d	-7.34	-0.021
O(3)H	6.687	^d	-8.00	-0.028
O(6)H	5.988	^d	-12.52	-0.017
NHDC				
C(2)H	6.678 ^a	-	-	-0.120
C(5)H	6.822 ^a	-	-	-0.111
C(6)H	6.535 ^a	-	-	-0.257
C(7)H	2.812 ^a	-	-	0.087
C(8)Ha	^b	-	-	^b
C(8)Hb	3.274 ^a	-	-	0.073
C(12)H	^b	-	-	^b
C(14)H	^b	-	-	^b
C(16)H	^b	-	-	^b
C(17)H	^b	-	-	^b
C(18)H	^b	-	-	^b
C(19)H	^b	-	-	^b
C(20)H	^b	-	-	^b
C(21)Ha	^b	-	-	^b
C(21)Hb	^b	-	-	^b
C(22)H	6.055 ^a	-	-	0.105
C(23)H	4.101	-	-	0.036
C(24)H	^b	-	-	^b
C(25)H	3.495 ^a	-	-	-0.017
C(26)H	3.930 ^a	-	-	-0.002
C(27)H	1.378	-	-	0.011
C(28)H	3.832 ^a	-	-	-0.008
O(18)H	^b	^d	^d	^b
O(23)H	6.237 ^a	^d	^d	-0.005
O(25)H	^b	^d	^d	^b

^a Chemical shifts are obtained from NOESY spectrum.

^b Since peaks are overlapped with CD signals they cannot be detected.

^c For each CD molecule, the chemical shifts for the two C(6)Hs protons were measured from the joint signals of both C(6)H' and C(6)H'' protons

^d Parameters that cannot be obtained/calculated from spectra.

Table A.26 : Coupling constants of acesulfame potassium.

Group	J_{H-H} (Hz)
C(5)H-C(6')H	0.8

Table A.27 : Coupling constants of sodium cyclamate.

Group	J_{H-H} (Hz)
C(4)H-C(5)Ha	11.3
C(5)Ha-C(5)He	-14.8
C(6)Ha-C(6)He	-14.4
C(7)Ha-C(7)He	-14.3

Table A.28 : Coupling constants of saccharin.

Group	$^3J_{H-H}$ (Hz) ^a
C(5)H-C(6)H	9.0
C(5)H-C(7)H	1.2
C(5)H-C(8)H	0.6
C(6)H-C(7)H	8.0
C(6)H-C(8)H	1.5
C(7)H-C(8)H	7.5

^a Coupling constants are obtained from spin simulation technique.**Table A.29 :** Coupling constants of aspartame.

Group	J_{H-H} (Hz)
C(2)H-C(3)Ha	9.4
C(2)H-C(3)Hb	5.9
C(3)Ha-C(3)Hb	-14.3
C(5)H-C(6)H	7.7
C(6)H-C(7)H	7.4
C(9)H-C(10)Ha	8.6
C(9)H-C(10)Hb	5.2
C(10)Ha-C(10)Hb	-17.6

Table A.30 : Coupling constants of neotame.

Group	J_{H-H} (Hz)
C(2)H-C(3)Ha	10.7
C(2)H-C(3)Hb	4.7
C(3)Ha-C(3)Hb	-13.9
C(5)H-C(6)H	7.6
C(6)H-C(7)H	7.5
C(9)H-C(10)Ha	8.8
C(9)H-C(10)Hb	4.8
C(10)Ha-C(10)Hb	-17.4
C(13)Ha-C(13)Hb	-12.0
C(13)Ha-C(14)Hb	4.9
C(13)Hb-C(14)Ha	5.2
C(14)Ha-C(14)Hb	-12.5

Table A.31 : Coupling constants of isomalt.

Group	J_{H-H} (Hz)
C(3)H-C(4)H	8.2
C(4)H-C(5)H	8.2
C(5)H-C(6)Ha	2.7
C(5)H-C(6)Hb	5.7
C(6)Ha-C(6)Hb	-11.4
C(1')H-C(2')H	3.8
C(3')H-C(4')H	10.8
C(4')H-C(5')H	10.5

Table A.32 : Coupling constants of sucralose.

Group	J_{H-H} (Hz)
C(4')H-C(5')H	8.4
C(5')H-C(6')Ha	3.5
C(1)H-C(2)H	4.0
C(2)H-C(3)H	10.9
C(4)H-C(5)H	3.6

Table A.33 : Coupling constants of NHDC.

Group	J_{H-H} (Hz)
C(2)H-C(6)H	1.8 ^a
C(5)H-C(6)H	8.5 ^a
C(7)H-C(8)Ha	7.6 ^a
C(7)H-C(8)Hb	7.8 ^a
C(8)Ha-C(8)Hb	-16.6 ^a
C(17)H-C(18)H	9.3
C(19)H-C(20)H	6.9
C(20)H-C(21)Hb	7.8
C(26)H-C(27)H	6.4

^a Chemical shifts are calculated with strong apodization 0.20 Hz<LB<1.76 Hz, -0.30Hz<GB<-0.40Hz.

Table A.34 : Coupling constants of acesulfame potassium/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	2.8
C(3)H-C(4)H	10.2
C(4)H-C(5)H	9.8
Acesulfame potassium	
C(5)-C(6')H	0.7

Table A.35 : Coupling constants of acesulfame potassium/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	2.8
C(3)H-C(4)H	9.8
C(4)H-C(5)H	10.0
Acesulfame potassium	
C(5)-C(6')H	0.8

Table A.36 : Coupling constants of acesulfame potassium/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(2)H-C(3)H	11.2
C(3)H-C(4)H	10.0
C(4)H-C(5)H	10.1
Acesulfame potassium	
C(5)-C(6')H	0.8

Table A.37 : Coupling constants of sodium cyclamate/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	2.8
C(2)H-C(3)H	11.1
C(3)H-C(4)H	9.8
C(4)H-C(5)H	9.6
Sodium cyclamate	
C(5)Ha-C(5)He	-13.8
C(6)Ha-C(6)He	-14.3
C(7)Ha-C(7)He	-13.8

Table A.38 : Coupling constants of sodium cyclamate/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(3)H-C(4)H	9.8
C(4)H-C(5)H	9.9
Sodium cyclamate	
C(5)Ha-C(5)He	-13.4
C(6)Ha-C(6)He	-14.0
C(7)Ha-C(7)He	-13.7

Table A.39 : Coupling constants of sodium cyclamate/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(3)H-C(4)H	9.9
C(4)H-C(5)H	10.0
Sodium cyclamate	
C(5)Ha-C(5)He	-13.8
C(6)Ha-C(6)He	-13.6
C(7)Ha-C(7)He	-14.4

Table A.40 : Coupling constants of saccharin/ α -CD inclusion complex.

Group	J_{H-H} (Hz) ^a
α -CD	
C(1)H-C(2)H	2.4
C(3)H-C(4)H	10.0
C(4)H-C(5)H	9.6
Saccharin	
C(5)H-C(6)H	9.0
C(5)H-C(7)H	1.2
C(5)H-C(8)H	0.6
C(6)H-C(7)H	8.0
C(6)H-C(8)H	1.5
C(7)H-C(8)H	7.5

^a Coupling constants belongs to saccharin are obtained from spin simulation technique.

Table A.41 : Coupling constants of saccharin/ β -CD inclusion complex.

Group	J_{H-H} (Hz) ^a
β -CD	
C(1)H-C(2)H	2.6
C(3)H-C(4)H	9.7
C(4)H-C(5)H	9.9
Saccharin	
C(5)H-C(6)H	7.9
C(5)H-C(7)H	1.2
C(5)H-C(8)H	0.1
C(6)H-C(7)H	6.8
C(6)H-C(8)H	0.8
C(7)H-C(8)H	8.3

^a Coupling constants belongs to saccharin are obtained from spin simulation technique.

Table A.42 : Coupling constants of saccharin/ γ -CD inclusion complex.

Group	J_{H-H} (Hz) ^a
γ -CD	
C(1)H-C(2)H	2.5
C(3)H-C(4)H	10.0
C(4)H-C(5)H	10.2
Saccharin	
C(5)H-C(6)H	7.8
C(5)H-C(7)H	1.0
C(5)H-C(8)H	0.7
C(6)H-C(7)H	7.6
C(6)H-C(8)H	0.9
C(7)H-C(8)H	7.6

^a Coupling constants belongs to saccharin are obtained from spin simulation technique.

Table A.43 : Coupling constants of aspartame/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	2.6
C(3)H-C(4)H	9.9
C(4)H-C(5)H	9.6
Aspartame	
C(2)H-C(3)Ha	9.4
C(2)H-C(3)Hb	5.6
C(3)Ha-C(3)Hb	-14.3
C(5)H-C(6)H	7.5
C(6)H-C(7)H	7.4
C(9)H-C(10)Ha	8.5
C(9)H-C(10)Hb	5.1
C(10)Ha-C(10)Hb	-17.6

Table A.44 : Coupling constants of aspartame/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	2.6
C(2)H-C(3)H	10.3
C(3)H-C(4)H	9.9
C(4)H-C(5)H	9.6
Aspartame	
C(2)H-C(3)Ha	9.1
C(2)H-C(3)Hb	6.1
C(3)Ha-C(3)Hb	-14.1
C(5)H-C(6)H	7.4
C(6)H-C(7)H	7.3
C(9)H-C(10)Ha	8.1
C(9)H-C(10)Hb	5.6
C(10)Ha-C(10)Hb	-17.6

Table A.45 : Coupling constants of aspartame/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(2)H-C(3)H	10.3
C(3)H-C(4)H	10.0
C(4)H-C(5)H	10.2
Aspartame	
C(2)H-C(3)Ha	8.8
C(2)H-C(3)Hb	6.2
C(3)Ha-C(3)Hb	-14.1
C(5)H-C(6)H	7.4
C(6)H-C(7)H	7.4
C(9)H-C(10)Ha	8.6
C(9)H-C(10)Hb	5.2
C(10)Ha-C(10)Hb	-17.6

Table A.46 : Coupling constants of neotame/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	4.9
C(2)H-C(3)H	10.1
C(3)H-C(4)H	9.8
C(4)H-C(5)H	9.7
Neotame	
C(2)H-C(3)Ha	11.0
C(2)H-C(3)Hb	4.9
C(3)Ha-C(3)Hb	-14.5
C(5)H-C(6)H	7.5
C(6)H-C(7)H	7.5
C(9)H-C(10)Ha	9.3
C(9)H-C(10)Hb	4.9
C(10)Ha-C(10)Hb	-17.5
C(13)Ha-C(14)Hb	5.2
C(13)Hb-C(14)Ha	5.5
C(14)Ha-C(14)Hb	-12.7

Table A.47 : Coupling constants of neotame/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	4.8
C(2)H-C(3)H	10.4
C(3)H-C(4)H	9.9
C(4)H-C(5)H	9.7
Neotame	
C(2)H-C(3)Ha	10.9
C(2)H-C(3)Hb	4.6
C(3)Ha-C(3)Hb	-14.5
C(5)H-C(6)H	7.6
C(6)H-C(7)H	7.4
C(9)H-C(10)Ha	9.1
C(9)H-C(10)Hb	4.9
C(10)Ha-C(10)Hb	-17.5
C(13)Ha-C(14)Hb	5.2
C(13)Hb-C(14)Ha	5.6
C(14)Ha-C(14)Hb	-12.7

Table A.48 : Coupling constants of neotame/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(1)H-C(2)H	6.9
C(2)H-C(3)H	10.8
C(3)H-C(4)H	10.0
C(4)H-C(5)H	9.9
Neotame	
C(2)H-C(3)Ha	10.6
C(2)H-C(3)Hb	4.9
C(3)Ha-C(3)Hb	-14.6
C(5)H-C(6)H	7.5
C(6)H-C(7)H	7.4
C(9)H-C(10)Ha	9.4
C(9)H-C(10)Hb	4.9
C(10)Ha-C(10)Hb	-17.5
C(13)Ha-C(14)Hb	5.0
C(13)Hb-C(14)Ha	5.5
C(14)Ha-C(14)Hb	-12.7

Table A.49 : Coupling constants of isomalt/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	3.2
C(2)H-C(3)H	10.7
C(3)H-C(4)H	9.8
C(4)H-C(5)H	9.4
Isomalt	
C(3)H-C(4)H	8.1
C(4)H-C(5)H	9.7
C(5)H-C(6)Ha	2.7
C(5)H-C(6)Hb	5.6
C(6)Ha-C(6)Hb	-11.5
C(1')H-C(2')H	3.7
C(3')H-C(4')H	10.0
C(4')H-C(5')H	9.9
C(3)H	5.2
C(4)H	6.4
C(4')H	6.0

Table A.50 : Coupling constants of isomalt/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	3.3
C(3)H-C(4)H	10.0
C(4)H-C(5)H	9.7
Isomalt	
C(3)H-C(4)H	7.8
C(4)H-C(5)H	9.9
C(5)H-C(6)Ha	2.8
C(5)H-C(6)Hb	5.7
C(6)Ha-C(6)Hb	-11.6
C(1')H-C(2')H	3.7
C(3')H-C(4')H	10.1
C(4')H-C(5')H	9.9
C(3)H	3.8
C(4)H	7.5
C(4')H	5.5

Table A.51 : Coupling constants of isomalt/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(1)H-C(2)H	3.4
C(2)H-C(3)H	10.9
C(3)H-C(4)H	10.2
C(4)H-C(5)H	10.4
Isomalt	
C(3)H-C(4)H	8.1
C(4)H-C(5)H	10.4
C(5)H-C(6)Ha	2.7
C(1')H-C(2')H	3.7
C(3')H-C(4')H	9.8
C(4')H-C(5')H	9.8
C(3)H	5.1
C(4)H	6.6
C(4')H	6.4

Table A.52 : Coupling constants of sucralose/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	2.9
C(3)H-C(4)H	9.1
C(4)H-C(5)H	10.0
Sucralose	
C(4')H-C(5')H	8.4
C(5')H-C(6')Ha	3.4
C(1)H-C(2)H	3.8
C(2)H-C(3)H	10.3
C(4)H-C(5)H	3.2

Table A.53 : Coupling constants of sucralose/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	2.5 ^a
C(3)H-C(4)H	9.3
C(4)H-C(5)H	9.5
Sucralose	
C(4')H-C(5')H	8.4
C(5')H-C(6')Ha	3.6
C(5')H-C(6')Hb	8.1
C(1)H-C(2)H	3.9
C(4)H-C(5)H	3.9
C(4')H	3.7

^aCoupling constants are obtained with strong apodization LB=1.00 Hz, GB=-0.50 Hz.

Table A.54 : Coupling constants of sucralose/ γ -CD inclusion complex.

Group	J_{H-H} (Hz)
γ -CD	
C(3)H-C(4)H	9.8
C(4)H-C(5)H	9.7
Sucralose	
C(3')H-C(4')H	9.0
C(4')H-C(5')H	8.5
C(5')H-C(6')Ha	3.4
C(5')H-C(6')Hb	8.6
C(6')Ha-C(6')Hb	-11.7
C(1)H-C(2)H	3.7
C(4)H-C(5)H	3.0
C(4')H	3.7

Table A.55 : Coupling constants of NHDC/ α -CD inclusion complex.

Group	J_{H-H} (Hz)
α -CD	
C(1)H-C(2)H	2.7
C(3)H-C(4)H	9.5
C(4)H-C(5)H	9.3
NHDC	
C(26)H-C(27)H	5.4 ^a

^aCoupling constants are obtained with strong apodization LB=0.90 Hz, GB=-0.40 Hz.

Table A.56 : Coupling constants of NHDC/ β -CD inclusion complex.

Group	J_{H-H} (Hz)
β -CD	
C(1)H-C(2)H	2.7
C(2)H-C(3)H	10.7
C(3)H-C(4)H	9.4
C(4)H-C(5)H	10.1
NHDC	
C(26)H-C(27)H	5.8

Table A.57 : Coupling constants of NHDC/ γ -CD inclusion complex.

Group	J _{H-H} (Hz)
γ -CD	
C(2)H-C(3)H	10.1
C(3)H-C(4)H	9.8
C(4)H-C(5)H	10.0
NHDC	
C(26)H-C(27)H	5.5

Table A.58 : Calculated (B3LYP/6-311+G**) electronic energies for sodium cyclamate conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
1	-573975.25	0.60
2	-573975.85	0.00
3	-573973.36	2.49

Table A.59 : Calculated (B3LYP/6-311+G**) electronic energies for aspartame conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)	Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
2	-646527.01	2.36	43	-646529.36	0.00
5	-646524.88	4.48	46	-646526.55	2.81
7	-646527.10	2.27	52	-646528.77	0.59
9	-646526.68	2.69	54	-646526.79	2.57
10	-646527.40	1.97	55	-646527.72	1.64
11	-646528.71	0.65	56	-646527.77	1.59
12	-646528.52	0.85	57	-646524.20	5.17
13	-646525.45	3.91	60	-646527.27	2.09
15	-646526.86	2.50	65	-646527.47	1.89
16	-646526.66	2.70	67	-646527.83	1.53
17	-646528.18	1.18	68	-646527.80	1.56
19	-646526.27	3.09	70	-646527.13	2.24
20	-646526.65	2.72	75	-646528.98	0.39
21	-646528.15	1.21	77	-646525.70	3.66
22	-646526.91	2.45	79	-646525.62	3.74
24	-646524.27	5.09	81	-646526.50	2.87
25	-646525.33	4.03	83	-646526.14	3.23
26	-646525.04	4.33	85	-646526.53	2.84
28	-646526.37	2.99	88	-646527.86	1.51
31	-646526.13	3.23	89	-646526.54	2.83
33	-646526.42	2.95	90	-646525.05	4.32
34	-646524.73	4.64	92	-646526.58	2.79
35	-646525.94	3.42	98	-646527.56	1.81
37	-646523.90	5.47			

Table A.60: Calculated (B3LYP/6-311+G**) electronic energies for neotame conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)	Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
1	-794577.99	2.04	32	-794577.94	2.10
2	-794576.31	3.73	33	-794577.94	2.10
4	-794576.31	3.73	36	-794577.34	2.69
5	-794576.57	3.46	37	-794576.22	3.82
6	-794578.15	1.89	40	-794576.78	3.26
7	-794579.98	0.05	42	-794578.27	1.76
9	-794576.15	3.88	43	-794577.21	2.82
12	-794576.30	3.73	45	-794576.48	3.56
13	-794578.95	1.09	46	-794577.22	2.82
14	-794579.79	0.25	48	-794576.47	3.56
15	-794578.15	1.89	49	-794578.27	1.76
16	-794578.15	1.89	51	-794573.75	6.29
17	-794578.13	1.91	53	-794580.04	0.00
18	-794578.13	1.91	58	-794578.72	1.32
19	-794577.91	2.13	63	-794576.88	3.15
20	-794577.94	2.10	68	-794579.22	0.82
21	-794576.59	3.45	69	-794575.36	4.68
22	-794578.00	2.04	78	-794577.37	2.66
24	-794578.95	1.09	79	-794576.24	3.80
25	-794576.23	3.81	83	-794576.37	3.66
27	-794575.76	4.28	85	-794576.76	3.28
28	-794574.80	5.23	90	-794579.80	0.23
29	-794575.36	4.67			

Table A.61 : Calculated (B3LYP/6-311+G**) electronic energies for isomalt conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
7	-815487.53	0.02
8	-815485.14	2.40
16	-815487.46	0.09
27	-815487.55	0.00
34	-815486.94	0.61
46	-815487.05	0.49
71	-815484.50	3.05
84	-815487.18	0.37
93	-815483.06	4.49

Table A.62 : Calculated (B3LYP/6-311+G**) electronic energies for sucralose conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)	Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
1	-1538322.61	1.57	42	-1538317.86	6.31
2	-1538322.75	1.43	45	-1538322.59	1.58
4	-1538321.26	2.91	46	-1538321.12	3.06
5	-1538320.12	4.05	47	-1538319.02	5.16
6	-1538321.72	2.45	49	-1538320.35	3.82
7	-1538319.97	4.20	54	-1538318.81	5.36
8	-1538322.01	2.17	56	-1538321.44	2.73
9	-1538319.34	4.84	57	-1538321.83	2.34
10	-1538319.83	4.34	58	-1538320.48	3.70
11	-1538321.87	2.30	60	-1538321.24	2.93
12	-1538320.49	3.68	62	-1538319.19	4.98
13	-1538324.17	0.00	65	-1538318.33	5.84
14	-1538320.37	3.80	67	-1538318.04	6.13
16	-1538318.38	5.79	68	-1538322.95	1.22
17	-1538318.96	5.21	70	-1538320.27	3.90
19	-1538318.13	6.05	72	-1538317.61	6.56
20	-1538321.31	2.86	73	-1538320.17	4.00
21	-1538320.42	3.75	74	-1538319.26	4.91
22	-1538317.83	6.34	75	-1538322.30	1.88
23	-1538317.64	6.53	77	-1538320.63	3.55
24	-1538323.42	0.76	78	-1538319.84	4.33
26	-1538320.13	4.04	79	-1538323.13	1.04
27	-1538319.94	4.24	83	-1538319.06	5.12
28	-1538319.50	4.67	90	-1538322.93	1.25
29	-1538320.25	3.92	91	-1538321.29	2.88
32	-1538320.96	3.22	92	-1538319.77	4.40
35	-1538323.67	0.50	93	-1538318.39	5.79
37	-1538318.40	5.78	94	-1538319.94	4.24
38	-1538321.83	2.34	97	-1538320.55	3.62
39	-1538321.98	2.19	99	-1538318.17	6.01

Table A.63 : Calculated (B3LYP/6-311+G**) electronic energies for NHDC conformers.

Conformer	Energy (kcal/mol)	Relative E. (kcal/mol)
1	-1391587.16	0.12
7	-1391584.67	2.61
13	-1391587.28	0.00
14	-1391583.30	3.98
22	-1391583.29	3.99
30	-1391582.95	4.33
31	-1391584.22	3.06
54	-1391583.84	3.44
79	-1391583.69	3.59

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

- Sağlam, B., Tuzun, N. Ş., Bekiroğlu, S. and Oruc, G., 2012: Sweet Taste Investigations on Aspartame and Neotame Using NMR Spectroscopy and Computational Methods, 4th EuCheMS Congress, August 26-30, 2012, Prague, Czech Republic.